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1 **UBAP2L drives scaffold assembly of nuclear pore complexes** 2 **at the intact nuclear envelope**

3 4 **Scaffold assembly of the nuclear pore complex**

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33 **Abstract**

34 Assembly of macromolecular complexes at correct cellular sites is crucial for cell function.
35 Nuclear pore complexes (NPCs) are large cylindrical assemblies with eightfold rotational
36 symmetry, built through hierarchical binding of nucleoporins (Nups) forming distinct
37 subcomplexes. Here, we uncover a direct role of ubiquitin-associated protein 2-like (UBAP2L)
38 in the biogenesis of properly organized and functional NPCs at the intact nuclear envelope
39 (NE) in human cells. UBAP2L localizes to the nuclear pores and drives the formation of the
40 Y-complex, an essential scaffold component of the NPC, and its localization to the NE.
41 UBAP2L facilitates the interaction of the Y-complex with POM121 and Nup153, the critical
42 upstream factors in a well-defined sequential order of Nups assembly onto NE during
43 interphase. Timely localization of the cytoplasmic Nup transport factor fragile X-related
44 protein 1 (FXR1) to the NE and its interaction with the Y-complex are likewise dependent on
45 UBAP2L. Thus, this NPC biogenesis mechanism integrates the cytoplasmic and the nuclear
46 NPC assembly signals and ensures efficient nuclear transport, adaptation to nutrient stress and
47 cellular proliferative capacity, highlighting the importance of NPC homeostasis at the intact
48 nuclear envelope.

49

50 **Teaser**

51 Liao et al. show how UBAP2L drives the assembly of the scaffold elements into symmetrical
52 and functional NPCs at the nuclear envelope in human cells.

53

54 **Introduction**

55 Nuclear pore complexes (NPCs) are among the largest and the most intricate multiprotein
56 assemblies in eukaryotic cells. They constitute the sole communication gates between the
57 nucleus and the cytoplasm thereby ensuring cellular function and survival. NPCs are inserted

58 in the nuclear envelope (NE), a double membrane structure surrounding the cell nucleus, and
59 mediate the transport of proteins and RNAs between the two cellular compartments (Hampoelz
60 *et al*, 2019; Knockenhauer & Schwartz, 2016). Multiple copies of around 30 different
61 nucleoporins (Nups) are the building protein units of the NPCs. Nups initially form various
62 sub-complexes which can subsequently co-assemble, following a hierarchical principle, into
63 functional NPCs (Onischenko *et al*, 2020). The mature NPCs contain a scaffold that surrounds
64 and anchors the Nups with disordered domains forming the inner passage channel (so called
65 Phenylalanine-Glycine repeat Nups or FG-Nups), as well as two asymmetric complex
66 components, the cytoplasmic filaments facing the cytoplasmic side of the NE and the nuclear
67 basket pointing towards the inside of the nucleus. How these architectural elements of the NPC
68 are assembled at the intact NE represents an intriguing and unresolved biological question.
69 Previous studies using biochemical and high-resolution structural techniques revealed the
70 eightfold rotational symmetry as a characteristic feature of the NPC three-dimensional
71 organization (Beck & Hurt, 2017; Grossman *et al*, 2012; Hampoelz *et al*, 2019; Knockenhauer
72 & Schwartz, 2016; Lin & Hoelz, 2019). One of the main components of the NPC scaffold is
73 the evolutionarily conserved Y-complex (also known as Nup107-160 complex) forming the
74 cytoplasmic and the nuclear rings that encompass the inner ring of the NPC (von Appen *et al*,
75 2015). In metazoans, the Y-complex is composed of Nup133, Nup107, Nup96 and Sec13,
76 Nup160, Nup37, Elys, Nup85, Seh1 (also named Seh11) and Nup43 and it is critical for NPC
77 assembly (Doucet *et al*, 2010; Walther *et al*, 2003). Interestingly, FG-Nups can also build the
78 links with the structural scaffold elements and contribute to the biogenesis of the NPC in yeast
79 (Onischenko *et al*, 2017). In metazoan cells, NPCs are formed concomitantly with the
80 reassembly of the NE during mitotic exit but the interphase pathway also exists where NPCs
81 can be formed *de novo* and are inserted into the intact NE through an inside-out mechanism
82 (Otsuka *et al*, 2016). Nup153 and POM121 are the critical upstream components in a well-

83 defined sequential order of Nups assembly onto the interphase nuclei (Otsuka *et al*, 2016;
84 Weberruss & Antonin, 2016). In addition, fragile X-related protein 1 (FXR1) can interact with
85 cytoplasmic Y-complex Nups and facilitate their localization to the NE during interphase
86 through a microtubule- and dynein-dependent mechanism, contributing to the NPC
87 homeostasis (Agote-Aran *et al*, 2020; Agote-Arán *et al*, 2021; Holzer & Antonin, 2020).
88 However, the crosstalk between the nuclear (POM121, Nup153) and the cytoplasmic (FXR1)
89 determinants of the NPC assembly during interphase and the pathways governing the formation
90 of the essential NPC sub-complexes (such as the Y-complex) at the intact NE, remained
91 unexplored. Likewise, it remained unknown what are the signaling pathways defining the
92 oligomerization state of these scaffold elements and ultimately the assembly of the eightfold-
93 symmetrical NPC. Here, we uncover a molecular mechanism based on UBAP2L protein which
94 links the cytoplasmic and the nuclear NPC assembly signals and by which human cells can
95 build the scaffold elements into functional NPCs at the NE during interphase, thereby ensuring
96 cellular function and survival.

97

98 **Results**

99 **UBAP2L localizes to the NPCs and interacts with Nups and NPC assembly factors.**

100 NPC assembly during interphase is particularly active as cells grow during early G1 phase
101 where an increase in NPC biogenesis has been observed immediately after NE reformation
102 (Dultz & Ellenberg, 2010; Rampello *et al*, 2020). The number of NPCs can be also modulated
103 in response to cellular needs, for instance during differentiation processes or in carcinogenesis
104 when the density of NPCs and nucleocytoplasmic trafficking augment dramatically (Kau *et al*,
105 2004). UBAP2L (also known as NICE-4) has been associated with the development of various
106 types of cancer (Chai *et al*, 2016; He *et al*, 2018; Li & Huang, 2014; Ye *et al*, 2017; Zhao *et*
107 *al*, 2015; Guerber *et al*, 2022), however, the cellular mechanisms underlying its oncogenic

108 potential remain currently unknown. In search for additional biological functions of UBAP2L,
109 we analyzed its subcellular localization using immunofluorescence microscopy and the
110 antibody specifically recognizing endogenous UBAP2L protein. Consistent with published
111 findings (Cirillo *et al*, 2020; Youn *et al*, 2018; Huang *et al*, 2020a; Maeda *et al*, 2016),
112 UBAP2L localized to stress granules (SGs) upon exposure to stress by sodium arsenite, but a
113 weak UBAP2L signal was also found in the nucleus (Fig. 1A) as demonstrated previously
114 (Asano-Inami *et al*, 2023). Likewise, in cells not treated with sodium arsenite, we observed a
115 fraction of endogenous (Fig. 1A) as well as ectopically expressed GFP- (Fig. 1B) and Flag-tag
116 (Fig. 1C) UBAP2L protein to be localized at the NE during interphase. Moreover, UBAP2L
117 was able to accumulate in the nucleus upon treatment with the Leptomycin B (inhibitor of
118 nuclear export factor Exportin 1) similar to the dual specificity protein kinase MPS1 (also
119 known as TTK) which is known to shuttle between nucleus and cytoplasm in interphase cells
120 (Jia *et al*, 2015) (Fig. S1, A to C). These results indicate that UBAP2L also shuttles between
121 these two compartments. Cellular fractionation experiments and western blotting confirmed
122 that a fraction of UBAP2L could be found in the nucleus in interphase (Fig. S1D), in
123 accordance with our published findings (Guerber *et al*, 2023). NE localization of endogenous
124 UBAP2L was detected in early prophase, late telophase and in G1 cells (Fig. S2A), suggesting
125 the role of this protein at the sealed nuclear envelope.

126 To dissect the nuclear UBAP2L localization more precisely, we used multi-color ratiometric
127 single molecule localization microscopy with a dichroic image splitter (splitSMLM) analysis
128 (Andronov *et al*, 2022, 2021). The splitSMLM analysis revealed that UBAP2L is localized at
129 the NPCs embedded in the NE, where it was found both in the central channel labelled by
130 Nup62 and surrounding the nuclear and cytoplasmic rings labelled by Nup96 of the NPCs (Fig.
131 1, D to F). Interestingly, fluorescence intensity quantifications indicated that UBAP2L is
132 frequently localized at the side of the Nup96-positive nuclear ring (Fig. 1F). Given that the

133 used super-resolution technique makes it possible to obtain fluorescence images with a
134 resolution in the 20 nm range (Andronov *et al*, 2022), our results suggest that UBAP2L co-
135 localizes with several Nups and building elements of the NPCs at the NE.
136 These observations prompted us to analyze any possible interactions of UBAP2L with the Nups
137 and the NPC-assembling factors. As expected, immunoprecipitations (IPs) of ectopically
138 expressed GFP-Nup85 in HeLa cells demonstrated an interaction with endogenous Y-complex
139 Nups Nup133 and SEC13 (Doucet *et al*, 2010; Walther *et al*, 2003), with POM121 and Nup153,
140 responsible for targeting Y-complexes to the NE (Otsuka *et al*, 2016; Weberruss & Antonin,
141 2016) and with the cytoplasmic Nup transporter FXR1 (Agote-Aran *et al*, 2020). GFP-Nup85
142 also co-immunoprecipitated with endogenous UBAP2L in this analysis (Fig. 2A). In addition,
143 endogenous UBAP2L interacted with FXR1, FXR2 and FMRP (Fig. 2B) as previously shown
144 (Huang *et al*, 2020a; Marmor-Kollet *et al*, 2020; Sanders *et al*, 2020) and with some FG-Nups
145 (detected by the monoclonal antibody mAb414) that are known to contribute to the biogenesis
146 of the NPC in yeast (Onischenko *et al*, 2017) (Fig. 2B). Since the mAb414 is known to interact
147 primarily with Nup62, as well as with Nup358, Nup214, and Nup153 (Davis & Blobel, 1987),
148 it appears that UBAP2L may preferentially interact with Nup214 (Fig. 2B). Finally, ectopically
149 expressed GFP-FXR1 interacted with Y-complex Nups and with UBAP2L (Fig. 2C). Taken
150 together, the interaction of UBAP2L with Y-complex Nups as well as with the nuclear and
151 cytoplasmic NPC assembly factors suggests a possible function of UBAP2L on Nups assembly
152 and/or on NPC biogenesis.

153

154 **UBAP2L regulates Nups localization.**

155 To understand if UBAP2L regulates Nups assembly, we generated two clonal HeLa cell lines
156 with CRISPR/Cas9-mediated deletion of the *UBAP2L* gene which were recently characterized
157 (Guerber *et al*, 2023). As expected (Cirillo *et al*, 2020; Huang *et al*, 2020a; Youn *et al*, 2018),

158 deletion of UBAP2L inhibited formation of SGs upon stress (Fig. S1E) and abolished nuclear
159 localization of endogenous UBAP2L (Fig. S2B), confirming the specificity of UBAP2L
160 antibodies. Relative to isogenic control cell line (wild type, (WT)), both UBAP2L knock-out
161 (KO) cell lines revealed accumulation of foci containing Nups (Nup133, FG-Nups and
162 RanBP2) as well as Importin- β and Exportin-1 in the cytoplasm but did not show defects in the
163 localization of the NPC basket component Nup153 (Fig. 3, A to E). UBAP2L KO cells also
164 displayed cytoplasmic granules containing both Importin- β and Nup133 (Fig. 3A) and
165 likewise, RanBP2-containing granules co-localized with FG-Nups labelled by mAb414 (Fig.
166 3A). Such accumulation of cytoplasmic Nups strongly resembles the cellular phenotypes
167 observed upon downregulation of the factors required for the assembly of NPCs at the NE such
168 as FXR1 (Agote-Aran *et al*, 2020). We were unable to detect any changes in protein levels of
169 several Nups as well as in Exportin-1 and Lamin A and B1 (Fig. 3F) in the whole cell extracts
170 but deletion of UBAP2L led to reduced NE intensity of FG-Nups (Fig. 3, G and H).
171 Fractionation experiments confirmed moderately reduced levels of Nups in the nucleus and an
172 increased pool of cytoplasmic Nups upon deletion of UBAP2L (Fig. 3I), suggesting that
173 UBAP2L does not regulate total protein levels of Nups but rather their localization to the NE
174 during interphase. Owing to the fact that UBAP2L deletion can delay mitotic exit (Guerber *et*
175 *al*, 2023), which could theoretically influence the length of G1 phase and, indirectly, the
176 localization of Nups, we have arrested cells in G1 using lovastatin, which inhibits proteasome
177 leading to the accumulation of p21 and p27 (Rao *et al*, 1999). Deletion of UBAP2L in G1-
178 arrested cells led to accumulation of cytoplasmic Nup-containing granules, reduced NE
179 intensity of FG-Nups (Fig. S2, C to F) without affecting the nuclear size (Fig. S2E). The same
180 results were obtained in G0/G1-arrested cells using Psoralidin, which was suggested to
181 transcriptionally regulate cdk inhibitors (Gulappa *et al*, 2013) (Fig. S2, G to J). Lovastatin led
182 to a decrease in nuclear size (Fig. S2F) as previously demonstrated (Iida *et al*, 2022) relative

183 to Psoralidin (Fig. S2J) and to untreated HeLa cells (Fig. S5E) (Guerber *et al*, 2023) but no
184 significant differences could be detected between WT and UBAP2L KO cells upon both
185 treatments and under untreated conditions (Fig. S2, F and J, and Fig. S5E), which is in
186 accordance with our recent published findings (Guerber *et al*, 2023). These results suggest that
187 UBAP2L may regulate Nups without affecting the size of the nucleus and possibly the length
188 of G1 phase. Our results demonstrate that UBAP2L localizes to the NE and the NPCs, interacts
189 with Nups and regulates their localization and it may be involved in the assembly of the
190 cytoplasmic Nups at the NE during interphase.

191

192 **UBAP2L regulates localization of Nups in interphase but not in postmitotic cells.**

193 Two distinct pathways of NPC assembly at the NE have been described during the cell cycle
194 in higher eukaryotic cells (Weberruss & Antonin, 2016). In the postmitotic pathway, NPC
195 assembly occurs on segregated chromosomes, while during interphase, both Nup153 and
196 POM121 drive *de novo* assembly of NPCs into an enclosed NE (D'Angelo *et al*, 2006; Doucet
197 *et al*, 2010; Vollmer *et al*, 2015), which can be facilitated by FXR1 and microtubule-dependent
198 transport of cytoplasmic Nups towards NE (Agote-Aran *et al*, 2020; Agote-Arán *et al*, 2021;
199 Holzer & Antonin, 2020). Given the strong interaction of UBAP2L with FXR1 (Fig. 2, B and
200 C), we hypothesized that UBAP2L may selectively affect Nups assembly during interphase.
201 Indeed, accumulation of Nup-containing cytoplasmic granules could be first observed during
202 late telophase, early G1 as well as in phospho-Rb-positive cells (which is present in mid-late
203 G1, S and G2 phases) and but not during anaphase and early telophase stages (Fig. 4, A to F).
204 FG-Nups assembled normally on segregating chromosomes in anaphase and on decondensing
205 chromatin during early telophase (Fig. 4G) upon deletion of UBAP2L but reduced NE levels
206 of FG-Nups were observed in early G1 and in phospho-Rb-positive cells in the absence of
207 UBAP2L (Fig. 4, H and I). The percentage of cells in mid-late G1, S and G2 phases was not

208 affected by UBAP2L deletion (Fig. 4J), further suggesting that the progression through
209 interphase occurred normally in UBAP2L KO cells. Our findings suggest that UBAP2L drives
210 Nups localization to NE during interphase but not in postmitotic cells.

211

212 **UBAP2L mediates the assembly of the NPC scaffold elements and the biogenesis of NPCs.**

213 Our data demonstrate that UBAP2L deletion leads to decreased Nup levels at the NE and to
214 the formation of Nup-containing granules in the cytoplasm. However, can UBAP2L also
215 regulate the assembly of functional NPCs at the NE? The splitSMLM analysis revealed that
216 deletion of UBAP2L decreased the density of the NPCs at the NE (Fig. 5, A and B) and
217 confirmed the presence of RanBP2 and FG-Nups cytoplasmic assemblies (Fig. S3A), which
218 often displayed linear-like organization with symmetrical RanBP2 distribution (Fig. S3A),
219 contrary to the non-symmetrical distribution at the cytoplasmic site of the NE (Fig. 5A).

220 Overexpression of Flag-tagged version of UBAP2L in interphase HeLa cells was also sufficient
221 to moderately increase the density of NPCs at the NE (Fig. S3B and Fig. 5C), suggesting that
222 UBAP2L might be required for NPC biogenesis onto intact NE. Flag-UBAP2L also
223 occasionally co-localized with the cytoplasmic FG-Nups assemblies (Fig. S3B). The alignment
224 and segmentation analysis of Nup133 or RanBP2 particles was performed as described
225 previously (Andronov *et al*, 2022) and further suggested that the structure of the NE-localized
226 NPCs was slightly altered upon deletion of UBAP2L (Fig. 5, A and D). Relative to WT,
227 UBAP2L KO cells showed moderately increased percentage of NPCs with a 4-fold rotational
228 symmetrical arrangement of the scaffold spokes, while the number of NPC structures with 5 to
229 8-fold symmetrical organization was slightly decreased upon UBAP2L deletion (Fig. 5, A and
230 D). Two clonal U2OS cell lines with CRISPR/Cas9-mediated deletion of *UBAP2L* gene with
231 stably integrated Nup96-GFP (Nup96-GFP knock-in (KI)) (Fig. S4, A and B) likewise showed

232 the accumulation of cytoplasmic Nup-containing granules (Nup96-GFP and FG-Nups) (Fig.
233 S4, C to E) and reduced density of the NPCs at the NE (Fig. 5, E and F).

234 Consistent with the observed role of UBAP2L in the biogenesis of mature NPCs, deletion of
235 UBAP2L in HeLa cells reduced the interaction of GFP-Nup85 with other components of the
236 Y-complex, Nup133 and SEC13 in both unsynchronized (Fig. 5G) and in G1/S-synchronized
237 cells (Fig. S4F) as well as decreased the interaction of GFP-Nup85 with the two Nups, Nup153
238 and POM121 (Fig. 5G), involved in the assembly of the NPCs at the enclosed NE through the
239 interphase pathway (Funakoshi *et al*, 2011; Vollmer *et al*, 2015). Immunoprecipitated (IP)
240 Nup96-GFP in U2OS cells also demonstrated reduced interaction of Y-complex components
241 Nup85 and SEC13 and inhibition of Nup96-GFP binding to Nup153 and POM121 in the
242 absence of UBAP2L (Fig. 5H). Interestingly, the interaction of endogenous Nup85 with other
243 components of the Y-complex appeared moderately increased in G1/S cells relative to cells
244 arrested in prometaphase using Eg5 inhibitor STLC (Fig. S4G), suggesting that Y-complex
245 assembly may also take place during interphase. In addition, the interaction of the cytoplasmic
246 Nup transporter factor FXR1 with both GFP-Nup85 and Nup96-GFP was reduced in the
247 absence of UBAP2L (Fig. 5, G and H) and UBAP2L deletion inhibited the binding of
248 immunoprecipitated GFP-FXR1 with Nup85, SEC13 and with the components of the dynein
249 complex dynactin p150^{Glued} and BICD2 (Fig. S4H) that work with FXR1 to transport Nups
250 along microtubules towards NE during interphase (Agote-Aran *et al*, 2020). Collectively, these
251 results demonstrate that UBAP2L is critically involved in the biogenesis of NPCs at the NE
252 during interphase likely through the regulation of the assembly of the NPC scaffold elements
253 from the cytoplasmic Nups and by facilitating the interaction of the Y-complex with both the
254 nuclear (Nup153, POM121) as well as with the cytoplasmic (FXR1, dynein complex) NPC
255 assembly signals.

256

257 **UBAP2L regulates localization of the Nup transporting factor FXR1.**

258 What is the molecular, UBAP2L-dependent mechanism fueling the assembly of cytoplasmic
259 Nups into Y-complex? The cellular phenotypes on cytoplasmic Nups observed upon deletion
260 of UBAP2L strongly resemble downregulation of the fragile X-related proteins (FXRPs)
261 (FXR1, FXR2 and FMRP) which drive transport and spatial assembly of the cytoplasmic Nups
262 to the NE in human cells during early interphase (Agote-Aran *et al*, 2020; Agote-Arán *et al*,
263 2021). The fact that UBAP2L not only facilitated the interaction of the Y-complex with
264 Nup153 and POM121 but also with FXR1 and the dynein complex (Fig. 5, G and H, and Fig.
265 S4H) and that FXRPs strongly interacted with UBAP2L (Fig. 2, B and C) prompted us to
266 analyze the dynamics of FXRPs in UBAP2L-deficient cells in more detail. Interestingly,
267 deletion of UBAP2L led to changes in the localization of FXR1 protein. In contrast to WT cells
268 where FXR1 was localized at the NE and diffusely in the cytoplasm, as reported previously
269 (Agote-Aran *et al*, 2020), both UBAP2L KO cell lines displayed reduced NE localization of
270 FXR1 and formation of cytoplasmic FXR1-containing granules (Fig. S5, A and C) in addition
271 to FG-Nups-containing granules, which did not co-localize with FXR1 in the cytoplasm (Fig.
272 S5, A and B). Both UBAP2L KO cell lines also showed irregular nuclear shape (Fig. S5D) but
273 no changes in the nuclear size (Fig. S5E) could be observed, in accordance with our previous
274 findings (Guerber *et al*, 2023). Fractionation experiments confirmed moderately reduced levels
275 of FXR1 in the nucleus upon deletion of UBAP2L both in G1-synchronized (Fig. S5F) and in
276 unsynchronized interphase cells (Fig. S5G), similar to Nups and to the nuclear transport factor
277 Ran (Fig. S5, F and G). The same phenotype was observed for FMRP (Fig. S6, A and B), and
278 UBAP2L deletion did not appear to affect protein levels of any of the three FXRPs (Fig. S6C).
279 Downregulation of endogenous UBAP2L using specific siRNAs confirmed the cellular
280 phenotypes of UBAP2L KO cells and displayed accumulation of FXR1 foci, cytoplasmic
281 Nups-containing granules and irregular nuclear shape as also observed upon depletion of FXR1

282 and in contrast to control cells (Fig. S6, D to G). These results suggest that FXR1 cytoplasmic
283 granules are not the result of any possible compensation effects due to stable deletion of
284 UBAP2L in KO cells. Since UBAP2L was previously demonstrated to contribute to the
285 assembly of SGs (Cirillo *et al*, 2020; Huang *et al*, 2020a; Youn *et al*, 2018) and FXRPs and
286 Nups are able to localize to these protein assemblies (Huang *et al*, 2020a; Zhang *et al*, 2018),
287 we aimed to understand if observed phenotypes could be linked to cellular stress signaling. As
288 expected (Cirillo *et al*, 2020; Huang *et al*, 2020a; Youn *et al*, 2018), deletion of UBAP2L
289 inhibited formation of SGs (Fig. S1E) upon stress but the SG components G3BP1 and TIA-1
290 did not localize to FXR1-containing granules under normal growing conditions in UBAP2L
291 KO cells (Fig. S6, H and I), suggesting that FXR1 foci are distinct from SGs. Our findings
292 indicate that UBAP2L-mediated regulation of Nups might be independent of UBAP2L's
293 function on SGs. Importantly, UBAP2L not only facilitates the interaction of FXRPs with the
294 scaffold Nups but also helps to localize FXRPs to the NE thereby fueling the assembly of Nups
295 from the cytoplasm to the nucleus.

296

297 **Arginines within the RGG domain of UBAP2L mediate the function of UBAP2L on Nups**
298 **and FXRPs.**

299 To dissect the molecular basis of the UBAP2L-FXR1-Nup pathway and to understand if the
300 function of UBAP2L on cytoplasmic Nups and on FXRPs is specific, we performed rescue
301 experiments. In contrast to GFP, ectopic expression of GFP-UBAP2L efficiently rescued Nup-
302 and FXR1-granules as well as the irregular nuclei phenotypes in both UBAP2L KO cell lines
303 (Fig. S7, A to E). GFP-UBAP2L protein fragment encompassing 98-430 aa was required (Fig.
304 S8, A and B) and sufficient (Fig. S8C) for the interaction with FXR1 in the
305 immunoprecipitation experiments. Interestingly, the 98-430 aa protein fragment of UBAP2L
306 contains the RGG domain (Fig. S8A) which often engages in interactions with mRNAs and

307 mediates UBAP2L's function in protein translation and RNA stability (Luo *et al*, 2020).
308 Surprisingly, GFP-tagged UBAP2L (Fig. S8D) and endogenous UBAP2L (Fig. S8E) interacted
309 with endogenous FXR1 and FMRP despite the absence of RNAs after RNase A treatment,
310 suggesting that the role of UBAP2L on FXRPs-Nups pathway may be, to a large extent, RNA-
311 independent. The arginines present in the RGG domains were previously demonstrated to
312 regulate localization of other proteins also in an RNA-independent manner (Thandapani *et al*,
313 2013) and to be asymmetrically di-methylated (ADMA) by the protein arginine
314 methyltransferase PRMT1 (Huang *et al*, 2020a; Maeda *et al*, 2016). Indeed, Flag-tagged mutant
315 form of UBAP2L, where all 19 arginines were exchanged to alanines (UBAP2L R131-190A),
316 did not interact with endogenous PRMT1 and showed reduced ADMA signal as expected (Fig.
317 6A). The UBAP2L R131-190A mutant also did not bind to Nups and FXR1 (Fig. 6A),
318 suggesting the role of arginines within the RGG domain of UBAP2L in Nups assembly. The
319 GFP-UBAP2L protein fragment encompassing 98-430 aa could rescue localization defects of
320 Nups and FXR1 in UBAP2L KO cells, in a manner similar to the full length UBAP2L protein
321 (Fig. S9, A to D) but the UBAP2L R131-190A mutant was unable to restore the FXR1 and
322 Nups localization defects and irregular nuclear shape in UBAP2L KO cells (Fig. 6, B to E).
323 We conclude that the function of UBAP2L on the regulation of FXRPs and Nups localization
324 may be mediated through the arginines present within its RGG domain.

325

326 **UBAP2L regulates localization of FXR1 to the NE.**

327 How can the function of UBAP2L in NPC biogenesis and in ensuring the interaction of FXR1
328 with Nups be linked to the observed subcellular localization of FXR1? And why, and when can
329 FXRPs form cytoplasmic assemblies in the absence of UBAP2L? Although UBAP2L regulates
330 some factors involved in mitotic exit (Guerber *et al*, 2023; Maeda *et al*, 2016), the localization
331 defects of Nups upon UBAP2L deletion could be also observed in cells arrested in G1 phase

332 (Fig. S2, C to J). In addition, inhibition of Polo-like kinase 1 (PLK1) activity, the downstream
333 target of UBAP2L during mitosis, was reported to rescue the mitotic defects observed in the
334 absence of UBAP2L (Guerber *et al*, 2023) but it could not reverse the Nup localization defects
335 in the same experimental setting (Fig. S10, A and B), arguing that UBAP2L-dependent
336 regulation of Nups assembly could be largely uncoupled from the role of UBAP2L in mitotic
337 progression.

338 Importantly, the increased numbers of FXR1-containing foci were also observed in UBAP2L
339 KO late telophase cells when compared to the corresponding WT cells synchronized in the
340 same cell cycle stage (Fig. S10, C and D). The average size of the FXR1-containing granules
341 was likewise increased in late telophase synchronized UBAP2L KO relative to WT cells (0.346
342 and 0.218 μm^2 , respectively) (Fig. S10, C and E). Reduced NE localization of FXR1 and
343 formation of cytoplasmic granules were observed in early and mid-late G1, S and in G2 phases
344 in UBAP2L KO relative to WT cells (Fig. S10, F to I). In addition, endogenous UBAP2L could
345 interact with endogenous FXR1 and FMRP in asynchronous cells as well as in cells
346 synchronized during mitosis and in interphase (Fig. S11A). Interestingly, the effect of UBAP2L
347 deletion on the percentage of FXR1 granules-containing cells, the number of granules per cell
348 and the size of FXR1 granules was the most evident in early G1 compared to other cell cycle
349 stages analyzed (Fig. S10, F to I), in line with our findings suggesting that UBAP2L
350 preferentially regulates Nups localization to NE during early G1 (Fig. 4, A to I). The fact that
351 FXR1-containing granules are also observed in the WT late telophase cells, although to a lesser
352 extent as compared to UBAP2L KO cells (Fig. S10, C to E), suggests that these structures do
353 not form *de novo* upon deletion of UBAP2L but may originate from some similar assemblies
354 existing before mitotic exit.

355 For this reason, we analyzed FXR1 and FMRP localization during mitosis in wild-type cells
356 synchronized in prometaphase-like stage using Nocodazole or Eg5 inhibitor STLC.

357 Interestingly, wild-type mitotic cells displayed strong accumulation of granules containing
358 both FXR1 and FMRP (Fig. 7A). Time-lapse analysis using live video spinning disk
359 microscopy of cells expressing GFP-FXR1 revealed its dynamic localization during mitotic
360 progression and confirmed the presence of GFP-FXR1-containing granules in control mitotic
361 cells (Fig. 7, B to D) where GFP-FXR1 granules could be observed first during late prophase
362 and throughout prometaphase, metaphase and anaphase stages. Interestingly, unlike in control
363 cells where GFP-FXR1 mitotic granules spread out in the vicinity of the NE concomitant with
364 the nuclei reformation during mitotic exit, in UBAP2L-deleted cells, these granules remained
365 in the cytoplasm, surrounding the nucleus and GFP-FXR1 localization at the NE appeared to
366 be reduced (Fig. 7, B to D). Accordingly, both the number as well as the average size of FXR1-
367 containing granules were increased in dividing UBAP2L-deficient cells relative to WT cells
368 (Fig. 7, C and D). These results suggest that UBAP2L may remodel FXR1 protein assemblies
369 present in mitotic cells to restrict and ensure their timely localization to the vicinity of the NE
370 after completion of mitosis, where it could interact with Nups and transport them towards NE
371 allowing for the formation of mature NPCs during early interphase. Indeed, endogenous
372 UBAP2L and FXR1 can localize to NE and occasionally co-localize in the cytoplasmic
373 assemblies in the proximity of NE in early interphasic cells (Fig. 7E). In addition, Flag-tagged
374 WT, but not the R131-190A mutant form of UBAP2L, frequently co-localized to FXR1-
375 containing granules in the proximity of NE in late telophase cells (Fig. 7F) and WT but not
376 R131-190A mutant UBAP2L was able to disperse endogenous FXR1-containing mitotic
377 granules (Fig. S11, B to D). Similar observations were made when either the full length or the
378 98-430 aa UBAP2L-fragment fused to GFP, but not GFP alone, were expressed in STLC-
379 synchronized mitotic cells (Fig. S11, E to G), suggesting that UBAP2L may chaperone and/or
380 remodel FXR1 to ensure its interaction with Nups and their timely localization to the NE. The
381 exact molecular mechanism underlying UBAP2L-mediated remodeling of FXR1 will have to

382 be investigated in the future but it is interesting to note that DNAJB6, a molecular chaperone
383 of the heat shock protein network, which was demonstrated to prevent aggregation of Nups and
384 promote their NE assembly during interphase (Kuiper *et al*, 2022) could also interact with
385 endogenous UBAPL2 in our hands (Fig. S11H), further corroborating the role of UBAP2L in
386 the assembly of cytoplasmic Nups. Collectively, our results identify UBAP2L as an important
387 component of the FXRPs-Nups pathway that drives assembly of NPCs during early interphase
388 by regulating the localization of FXR1 and Nups to the NE during early G1.

389

390 **UBAP2L-mediated biogenesis of NPCs ensures nuclear transport, adaptation to nutrient**
391 **stress and cellular proliferation.**

392 Next, it was important to understand the physiological relevance and functional implications
393 of the UBAP2L-mediated assembly of NPCs at the NE. One of the main functions of the NPCs
394 is the regulation of the nucleocytoplasmic transport across the NE. Our data so far demonstrated
395 that deletion of UBAP2L leads to the cytoplasmic sequestration of FG-Nups (Fig. 3, A and G),
396 which constitute the selective permeability barrier of NPCs as well as of Importin- β and
397 Exportin-1 (Fig. 3, A and C, and E), the essential components of the nucleocytoplasmic
398 transport system (Pemberton & Paschal, 2005). UBAP2L KO cells also display a reduced
399 number of NPCs at the intact NE (Fig. 5, A and B, E and F). To understand if these Nups and
400 NPCs defects in UBAP2L-deficient cells affect the function of nuclear pores, we measured the
401 rates of nucleocytoplasmic transport of an ectopic import/export reporter plasmid XRGG-GFP
402 that shuttles to the nucleus (accumulating in the nucleoli) when induced with dexamethasone
403 as previously described (Agote-Aran *et al*, 2020; Love *et al*, 1998). Deletion of UBAP2L
404 decreased the rates of XRGG-GFP nuclear import (Fig. 8, A and B) and its nuclear export (Fig.
405 8, C and D) relative to WT cells, suggesting that UBAP2L is important for the transport
406 function of NPCs. To corroborate these observations using a marker which does not localize at

407 specific structures, we analyzed the gradient of endogenous Ran, a guanine nucleotide
408 triphosphatase, as shown in previously (Coyne *et al*, 2020; Zhang *et al*, 2015). Most of Ran
409 protein is actively imported to the nucleus with help of transport factors, a process that requires
410 Ran binding to GDP (Ribbeck *et al*, 1998; Smith *et al*, 2002, 1998). Therefore, we analyzed
411 the nuclear-cytoplasmic (N/C) distribution of Ran and observed that UBAP2L deletion led to
412 significant reduction in the N/C ratio of Ran (Fig. 8, E and F). Together, with our analysis in
413 living cells, and with the reduced nuclear levels of Ran in fractionation experiments (Fig. 3I,
414 and Fig. S5, F and G), these results suggest that UBAP2L may facilitate the nucleocytoplasmic
415 transport across the NE.

416 Interestingly, in the live video analysis we observed that UBAP2L-deficient cells displaying
417 strong transport defects may undergo cellular death (Fig. 8C) in accordance with the previous
418 reports demonstrating an essential role of transport across NE for cell viability (Hamada *et al*,
419 2011). Colony formation assays showed that the long-term proliferation capacity of both
420 UBAP2L KO cell lines was reduced relative to WT cells (Fig. S12, A to E) in agreement with
421 our published study (Guerber *et al*, 2023) and propidium iodide labelling and flow cytometry
422 indicated reduced viability of UBAP2L KO cells (Fig. S12, F and G). Future studies will have
423 to address whether UBAP2L-dependent regulation of NPC assembly can directly promote cell
424 survival or if the effects of UBAP2L deletion on NPC function and viability are circumstantial.

425 Because the Y-complex can selectively affect survival and proliferation of cancer cells in
426 response to presence of nutrients (such as high serum and growth factors) (Sakuma *et al*, 2020)
427 and changes in nutrient availability can lead to NPC reorganization (clustering) in fission yeast
428 (Varberg *et al*, 2021), we studied how UBAP2L-dependent biogenesis of NPCs can be affected
429 by nutrient deprivation in human cells.

430 Nutrient deprivation further potentiated inhibition of cell viability in UBAP2L-dependent
431 manner (Fig. S12, F and G) and led to reduced NE levels of Nups and accumulation of Nup-

432 containing granules (Fig. 9, A to C). Interestingly, NE localization and protein levels of
433 UBAP2L were moderately reduced upon nutrient deprivation (Fig. 9, A and D, and E) but the
434 total protein levels of several tested Nups were unaffected under nutrient poor conditions (Fig.
435 9E).

436 Nutrient stress could also lead to reduced density of NPCs at the NE, a phenotype which could
437 be partially rescued by overexpression of GFP-UBAP2L (Fig. 9, F and G), suggesting that
438 presence of UBAP2L is important for NPC biogenesis also under nutrient stress conditions.
439 Finally, nutrient deprivation could induce the formation of the cytoplasmic Nup granules,
440 which were rescued by Flag-UBAP2L overexpression also upon inhibition of active protein
441 translation (using cycloheximide) (Fig. 9, H and I), suggesting that UBAP2L-mediated NPC
442 formation under nutrient stress conditions is independent of production of new proteins at least
443 during early interphase. The possible regulation of NPC biogenesis by UBAP2L in response to
444 nutrient poor conditions or upon induction of autophagy will have to be investigated in future.
445 Taken together, our data are consistent with the hypothesis that the role of UBAP2L in NPC
446 biogenesis at the NE is important for nuclear transport and adaptation to nutrient stress.

447

448 **Discussion**

449 NPCs are large (approximately 100 nm wide and 40 nm high) eightfold symmetrical assemblies
450 composed of more than 550 copies of around 30 different Nups. Nups assemble into
451 biochemically stable subcomplexes that form eight identical protomer units, traditionally
452 named “spokes” which are radially arranged around the central channel or the “central
453 transporter”. Although deviations from typical eightfold rotational symmetry have been
454 observed in *Xenopus* oocytes (Hinshaw & Milligan, 2003) and NPCs can dilate their inner ring
455 by moving the spokes away from each other (Mosalaganti *et al*, 2018), the identity of the
456 molecular pathways defining the NPC structural organization remains unknown.

457 Our data suggest a model (Fig. 10) how UBAP2L ensures assembly of the NPC scaffold
458 elements into mature NPCs at the intact NE in human cells during early G1. On one hand,
459 UBAP2L localizes to the NE and to NPCs and drives the formation of the Y-complex and its
460 interaction with Nup153 and POM121, which are known to be crucial for the Y-complex
461 recruitment to the NE during interphase (Funakoshi *et al*, 2011; Vollmer *et al*, 2015). On the
462 other hand, UBAP2L can remodel or “chaperone” FXRP proteins to restrict their timely
463 localization to the NE and their interaction with the Y-complex. Thus, UBAP2L can link and
464 integrate the nuclear and the cytoplasmic NPC upstream assembly signals during interphase.
465 Since FXRPs were shown to transport cytoplasmic Y-complex Nups towards the NE through
466 a microtubule-based mechanism (Agote-Aran *et al*, 2020), their interaction with UBAP2L may
467 fuel the assembly of Y-complexes and biogenesis of new NPCs with fresh Nups by bringing
468 them to interact with Nup153 and POM121. We speculate that this dual function of UBAP2L
469 may bring together all required components at close vicinity of the NE, to ensure proper
470 assembly of NPCs and their cellular function during early interphase (Fig. 10). Although our
471 data are consistent with the role of UBAP2L in the biogenesis of new NPCs at the NE during
472 early G1, at present, we cannot rigorously exclude the possibility that UBAP2L may also
473 regulate a repair mechanism ensuring maintenance of the structural organization of the existing
474 NPCs through its function on the Y-complex.

475 The Y-complex named after its Y-shaped structure (Siniosoglou *et al*, 2000) is an essential
476 component of the scaffold forming the cytoplasmic and the nuclear rings, respectively that
477 encompass the inner ring of the NPC (von Appen *et al*, 2015). These sub-complexes
478 oligomerize head to tail in a double-ring arrangement in each cytoplasmic and nuclear outer
479 rings adding to 32 Y-complexes present in the human NPC (Bui *et al*, 2013). The molecular
480 mechanisms governing the spatiotemporal assembly of the Y-complex and its oligomerization
481 state into organized NPC scaffold remain uncharacterized. Our findings provide some insights

482 into this biological riddle and identify UBAP2L as an important factor ensuring correct
483 architectural organization of the NPC through the regulation of the formation of the Y-complex
484 in human cells. Indeed, immunoprecipitation of Nup85 (Fig. 5G) and Nup96 (Fig. 5H) revealed
485 interaction with other Y-complex Nups but this binding was reduced upon UBAP2L deletion.
486 It remains to be determined if the oligomerization status of the Y-complex and its interaction
487 with other NPC structural elements can be also regulated by UBAP2L.
488 In particular, the NPC cytoplasmic filaments component RanBP2 (also known as Nup358) was
489 shown to wrap around the stems of Y-complexes to stabilize the scaffold (Huang *et al.*, 2020b;
490 von Appen *et al.*, 2015). Similarly, the inner ring elements Nup188 and/or Nup205 were shown
491 to contact Y-complex rings in vertebrate cells (Bui *et al.*, 2013; Huang *et al.*, 2020b; Kosinski
492 *et al.*, 2016; Lin *et al.*, 2016). Future studies should provide further molecular insights into
493 UBAP2L-mediated NPC assembly process and explain why the structure of the NPC visualized
494 in our hands by super-resolution microscopy appears to be altered in the absence of UBAP2L
495 (Fig. 5, A and D). Indeed, UBAP2L KO displayed moderately reduced number of NPC
496 assemblies with mature symmetrical organization (Fig. 5, A and D) and the density of NPCs at
497 the NE was decreased upon deletion of UBAP2L (Fig. 5, A and B, E and F). Because pre-pore
498 structures observed by electron microscopy display eightfold arrangement already during early
499 steps of NPC assembly by an inside-out extrusion mechanism at the intact NE (Otsuka *et al.*,
500 2016), our results suggest that UBAP2L may act during initial steps of nuclear pore formation,
501 prior to the described extrusion process. In agreement with this assumption, our data show that
502 a portion of UBAP2L protein shuttles to the nucleus (Fig. S1, A to D) and that it can both
503 localize to the NE (Fig. 1, A to C, and Fig. S2, A and B) and to the NPCs at the NE (Fig. 1, D
504 to F) as well as it can interact with several Nups (Fig. 2, A to C, and Fig. 6A).
505 Moreover, the splitSMLM analysis suggested that UBAP2L appears to be more frequently
506 localized at the nuclear ring labelled by Nup96 (Fig. 1, D and F) relative to the cytoplasmic

507 ring, suggesting that UBAP2L may be transported through the existing mature NPCs to help
508 the assembly of new pre-pore structures from the nuclear side. Future studies using electron
509 microscopy (EM) approaches could shed some light on the presence of NPC assembly
510 intermediates and on their structural organization in UBAP2L KO cells. Interestingly,
511 interphase NPC assembly is initiated by the upstream Nups POM121 and Nup153 (Funakoshi
512 *et al*, 2011; Vollmer *et al*, 2015) which recruit the Y-complex to the NE and their interaction
513 with Y-complex is reduced in the absence of UBAP2L (Fig. 5, G and H). Thus, UBAP2L may
514 not only regulate the formation of the Y-complex but also its timely recruitment to the NE
515 through the binding to Nup153 and POM121.

516 Even though Nup153 and POM121 represent established upstream signals for the NPC
517 biogenesis during interphase but not in the post-mitotic pathway, it was important to confirm
518 that regulation of Nups by UBAP2L does not occur during mitotic exit, in particular in view of
519 our recent analysis of the function of UBAP2L on PLK1 (Guerber *et al*, 2023). Indeed, we
520 could also observe Nup defects in cells arrested in G1 phase (Fig. S2, C and D, G and H) and
521 PLK1 inhibition could not reverse the Nup localization defects (Fig. S10, A and B), arguing
522 that UBAP2L-dependent regulation of Nups could be largely uncoupled from its role in mitotic
523 progression. Importantly, the described Nup localization defects upon deletion of UBAP2L
524 could be first observed during late telophase/early G1 and throughout interphase but not in cells
525 undergoing anaphase and early telophase (Fig. 4, A to F). In line with these observations, the
526 regulation of FXR1 localization by UBAP2L appeared to be the most evident in early G1
527 compared to other cell cycle stages analyzed (Fig. S10, F to I). Thus, UBAP2L may regulate
528 NPC assembly solely during interphase but not during mitotic exit.

529 A third pathway of the NPC assembly has been described in cells with rapid cell cycles and is
530 based on the existence of the cytoplasmic stacks of double membranes, termed annulate
531 lamellae (AL), which are structures containing partly assembled NPCs embedded in the

532 endoplasmic reticulum (ER) membrane sheets, a feature associated with disturbances in NPC
533 biogenesis (Hampoez *et al*, 2016). AL can be inserted *en bloc* into the expanding NE in fly
534 embryos (Hampoez *et al*, 2016) and in higher eukaryotic cells, AL-based NPC assembly may
535 represent an intermediate step in the postmitotic pathway (Ren *et al*, 2019). Interestingly, the
536 splitSMLM analysis occasionally revealed the presence of linearly organized cytoplasmic
537 assemblies of Nups in the absence of UBAP2L where RanBP2 was distributed symmetrically
538 (Fig. S3A) and the cytoplasmic Nup foci induced by UBAP2L deletion did not contain Nup153
539 (Fig. 3A). These two observations are similar to the reported features of AL-NPC (Hampoez
540 *et al*, 2016) and could indicate that UBAP2L may, at least partially, contribute to the assembly
541 of AL-NPC which will require further experimental efforts in the future.

542 Importantly, the biological significance of the UBAP2L-mediated assembly of the NPCs at the
543 intact NE during early interphase is documented by defects in nucleocytoplasmic transport
544 (Fig. 8) as well as by reduced proliferation capacity (Fig. S12, A to G) observed in UBAP2L-
545 deficient cells. Although future studies will need to address whether UBAP2L-dependent
546 regulation of NPC assembly can directly promote cell survival, UBAP2L was suggested to act
547 as an oncogene (Chai *et al*, 2016; He *et al*, 2018; Li & Huang, 2014; Ye *et al*, 2017; Zhao *et*
548 *al*, 2015; Guerber *et al*, 2022), and one could speculate that the role of UBAP2L in NPC
549 biogenesis may explain, at least to some extent, the oncogenic potential of UBAP2L. This role
550 of UBAP2L might be further regulated to meet differential demands on NPC functionality
551 which may operate during changing cellular conditions such as stress or nutrient availability.

552 Interestingly, deletion of Y-complex Nups can selectively affect survival and proliferation of
553 colon cancer cells in response to presence of nutrients (Sakuma *et al*, 2020) and UBAP2L is
554 sufficient to restore the NPC density after nutrient deprivation (Fig. 9), suggesting that
555 UBAP2L-Nup pathway plays an important role under nutrient stress conditions which have

556 been previously implicated in the regulation of NPC numbers in fission yeast (Varberg *et al*,
557 2021).

558 Taken together, our findings identify a molecular pathway driving biogenesis of mature and
559 functional NPCs through spatiotemporal assembly of the Y-complex at the intact envelope in
560 proliferating human cells. Our data further suggest a detailed molecular mechanism fueling the
561 assembly of cytoplasmic Nups into Y-complex at the NE through a regulation of FXR1 protein
562 by UBAP2L. UBAP2L strongly interacts with FXR1 (Fig. 2, B and C, and Fig. 6A, and Fig.
563 S8, B to D) and regulates its localization to the NE (Fig. 7, and Fig. S5, A to G) and its
564 interaction with the Y-complex (Fig. 5, G and H, and Fig. S4H) and the components of the
565 dynein complex dynactin p150^{Glued} and BICD2 (Fig. 5, G and H, and Fig. S4H). Therefore, it
566 can be speculated that UBAP2L may remodel cytoplasmic FXR1-assemblies found in mitotic
567 cells specifically during early interphase and to promote the reported transport function of
568 FXR1 by a minus-end directed microtubule-based mechanism towards NE (Agote-Aran *et al*,
569 2020). Thus, cells deficient for UBAP2L display both the cytoplasmic protein assemblies
570 containing FXR1 or Nups, as a likely result of their assembly defects at the NE. How UBAP2L
571 can execute its “chaperone-like” function on either FXR1 or Y-complex Nups and what are the
572 regulatory mechanisms upstream of UBAP2L that may restrict its role to early interphase stage
573 remain the subjects of future investigations. Interestingly, our data demonstrate that a region
574 comprising 19 arginines present within the RGG domain may mediate UBAP2L’s function on
575 FXR1 and Nups (Fig. 6). Globally, this mechanism appears to operate in an RNA-independent
576 manner (Fig. S8, D and E), however, at this stage of analysis it cannot be excluded that specific
577 RNAs might be involved in the UBAP2L-dependent regulation of Nups and FXR1. Consistent
578 with a previous report (Huang *et al*, 2020a), mutation of the 19 arginines to alanines also led
579 to loss of the asymmetric dimethylarginine (ADMA) signal (Fig. 6A). This raises an intriguing
580 possibility, to be analyzed in the future, that ADMA or other arginine modifications of

581 UBAP2L may regulate its function on Nups and their assembly into functional NPCs at the NE
582 during early interphase.
583

584 **Materials and Methods**

585 **Antibodies**

586 The following primary antibodies were used in this study: rabbit anti-UBAP2L (1-430 aa)
587 (Antibody facility IGBMC), mouse anti-FMRP (Antibody facility IGBMC), mouse anti-
588 FXR1+2 (Antibody facility IGBMC), mouse monoclonal anti-GFP (Antibody facility
589 IGBMC), mouse monoclonal anti- β -Actin (Sigma, A2228), rabbit polyclonal anti-GAPDH
590 (Sigma, G9545), mouse monoclonal anti- α -Tubulin (Sigma, T9026), mouse monoclonal anti-
591 FLAG® M2 (Sigma, F1804), rabbit polyclonal anti-FLAG® (Sigma, F7425), rabbit polyclonal
592 anti-FXR1 (Sigma, HPA018246), rabbit polyclonal anti-Lamin A (C-terminal) (Sigma,
593 L1293), rabbit monoclonal anti-Nup98 (C39A3) (Cell Signaling Technology, 2598), rabbit
594 polyclonal anti-PRMT1 (A33) (Cell Signaling Technology, 2449), rabbit polyclonal anti-
595 Tubulin (Abcam, ab18251), rabbit polyclonal anti-GFP (Abcam, ab290), rabbit polyclonal
596 anti-FMRP (Abcam, ab17722), rabbit polyclonal anti-UBAP2L (Abcam, ab138309), rabbit
597 monoclonal anti-Nup133 (Abcam, ab155990), mouse monoclonal anti-Nuclear Pore Complex
598 Proteins (mAb414) (Abcam, ab24609), rabbit polyclonal anti-Nup153 (Abcam, ab84872),
599 rabbit polyclonal anti-Nup188 (Abcam, ab86601), rabbit polyclonal anti-RanBP2 (Abcam,
600 ab64276), rabbit polyclonal anti-Lamin B1 (Abcam, ab16048), rabbit monoclonal anti-
601 NTF97/Importin beta (Abcam, ab2811), mouse monoclonal anti-Cyclin B1 (G-11) (Santa Cruz
602 Biotechnology, sc-166757), mouse monoclonal anti-Cyclin E (HE12) (Santa Cruz
603 Biotechnology, sc-247), mouse monoclonal anti-Nup133 (E-6) (Santa Cruz Biotechnology, sc-
604 376763 AF488), mouse monoclonal anti-TIA-1 (Santa Cruz Biotechnology, sc-166247),
605 mouse monoclonal anti-FXR1 (Millipore, 03-176), rabbit polyclonal anti-dimethyl-Arginine,
606 asymmetric (ASYM24) (Millipore, 07-414), mouse monoclonal anti-Mps1 (Millipore, 05-
607 682), rabbit polyclonal anti-G3BP1 (GeneTex, GTX112191), rabbit polyclonal anti-POM121
608 (GeneTex, GTX102128), rabbit polyclonal anti-Cyclin B1 (GeneTex, GTX100911), rabbit

609 polyclonal anti-FXR2 (Proteintech, 12552-1-AP), mouse monoclonal anti-Nucleoporin p62
610 (BD Biosciences, 610497), mouse monoclonal anti-Ran (BD Biosciences, 610340), rabbit
611 monoclonal anti-SEC13 (R&D systems, MAB9055), rabbit polyclonal anti-UBAP2L (1025-
612 1087 aa) (Bethyl, A300-534A), rabbit polyclonal anti-Nup85 (Bethyl, A303-977A), rabbit
613 polyclonal anti-Nup160 (Bethyl, A301-790A), rabbit polyclonal anti-CRM1/Exportin 1
614 (Novus, NB100-79802) and rat monoclonal anti-GFP (3H9) (ChromoTek, 3h9-100), rabbit
615 polyclonal anti-LC3B (Novus biological, NB100-2331), guinea pig polyclonal anti-p62
616 (Progen, GP62-C), rabbit monoclonal anti-DNAJB6 (Abcam, ab198995), rabbit monoclonal
617 anti-BiCD2 (Sigma, HPA023013), mouse monoclonal anti-p150^{Glued} (BD biosciences,
618 610473).

619 Secondary antibodies used were the following: goat polyclonal anti-Mouse CF680 (Sigma,
620 SAB4600199), goat polyclonal anti-Chicken CF660C (Sigma, SAB4600458), goat polyclonal
621 anti-Mouse AF647 (Thermo Fisher Scientific, A-21236), goat polyclonal anti-Mouse AF568
622 (Thermo Fisher Scientific, A-11031), goat polyclonal anti-Mouse AF555 (Thermo Fisher
623 Scientific, A-11029), goat polyclonal anti-Mouse AF488 (Thermo Fisher Scientific, A-21424),
624 goat polyclonal anti-Rabbit AF647 (Thermo Fisher Scientific, A-21245), goat polyclonal anti-
625 Rabbit AF568 (Thermo Fisher Scientific, A-11036), goat polyclonal anti-Rabbit AF555
626 (Thermo Fisher Scientific, A-21429), goat polyclonal anti-Rabbit AF488 (Thermo Fisher
627 Scientific, A-11034), goat Anti-Mouse IgG antibody (HRP) (GeneTex, GTX213111-01), goat
628 Anti-Mouse IgG antibody (HRP) (GeneTex, GTX213110-01) and goat Anti-Rat IgG antibody
629 (HRP) (Cell Signaling Technology, 7077S).

630

631 **Generation of UBAP2L KO cell lines**

632 UBAP2L knock-out (KO) in HeLa cells were described previously (Guerber *et al*, 2023).
633 UBAP2L KO in Nup96-GFP knock-in (KI) U2OS (CLS Cell Line Service, 300174; a generous

634 gift of Arnaud Poterszman, IGBMC) cell lines were generated using CRISPR/Cas9 genome
635 editing system as described previously (Jerabkova *et al*, 2020). Two guide RNAs (gRNA) were
636 designed using the online software Benchling (<https://www.benchling.com/>), 5'-
637 TGGCCAGACGGAATCCAATG-3' and 5'-GTGGTGGGCCACCAAGACGG-3', and cloned
638 into pX330-P2A-EGFP/RFP (Zhang *et al*, 2017) through ligation using T4 ligase (New
639 England Biolabs). Nup96-GFP KI U2OS cells were transfected using X-tremeGENE™ 9 DNA
640 Transfection Reagent (Roche), and 24h after transfection, GFP and RFP double positive cells
641 were collected by FACS (BD FACS Aria II), cultured for 2 days and seeded with FACS into
642 96-well plates. Obtained UBAP2L KO single-cell clones were validated by Western blot and
643 sequencing of PCR-amplified targeted fragment by Sanger sequencing (GATC). The following
644 primers were used for PCR amplification: 5'-TGCTGAGTGGAGAATGGTTA-3' (forward)
645 and 5'-AGACTGGTGGCAGTTGGTAG-3' (reverse). Primers used for cloning and
646 sequencing are described in Table S1.

647

648 **Cell culture**

649 All cell lines were cultured at 37°C in 5% CO₂ humidified incubator. HeLa (Kyoto) and its
650 derived UBAP2L KO cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM)
651 (4.5 g/L glucose) supplemented with 10% fetal calf serum (FCS), 1% Penicillin + 1%
652 Streptomycin. U2OS were cultured in DMEM (1 g/L glucose) supplemented with 10% FCS +
653 Gentamicin 40 µg/mL. Nup96-GFP KI U2OS and its derived UBAP2L KO cell lines were
654 cultured in DMEM (1 g/L glucose) supplemented with 10% FCS, Non-Essential Amino Acids
655 + Sodium Pyruvate 1 mM + Gentamicin 40 µg/mL.

656

657 **Cell cycle synchronization treatments**

658 Cells were synchronized in different stages of cell cycle by double thymidine block and release
659 (DTBR) protocol. Briefly, cells were treated with 2 mM thymidine for 16h, washed out (three
660 times with warm thymidine-free medium), then released in fresh thymidine-free culture
661 medium for 8h, treated with 2 mM thymidine for 16h again, washed out, and then released in
662 fresh thymidine-free culture medium for different time periods (0, 3, 6, 8, 9, 10 and 12h). 0h
663 time point corresponds to G1/S phase, approximately 8h to 9h to mitotic peak, 10h to mitotic
664 exit and 12h to early G1 phase. Cells were synchronized in G1 phase using lovastatin for 16h
665 at 10 μ M final concentration and in G0/G1 using Psoralidin (3,9-Dihydroxy-2-
666 prenylcoumestan) for 24h at 5 μ M final concentration. Cells were synchronized in
667 prometaphase using Nocodazole for 16h at 100 ng/ml, STLC for 16h at 5 μ M, and monastrol
668 for 16h at 100 μ M final concentration.

669

670 **Plasmids**

671 All pEGFP-C1-UBAP2L wild type (WT) (NCBI, NM_014847.4), pEGFP-C1-UBAP2L UBA
672 (1-97 aa), pEGFP-C1-UBAP2L Δ UBA (Δ 1-97 aa), pEGFP-C1-UBAP2L 98-430 aa, pEGFP-
673 C1-UBAP2L 1-430 aa, pEGFP-C1-UBAP2L Δ 1-429 aa, pEGFP-C1-UBAP2L Δ (UBA+RGG)
674 (Δ 1-195 aa) and pEGFP-C1-FXR1 WT (NCBI, NM_001013438.3) plasmids were generated
675 by Stephane Schmucker (IGBMC). pcDNA3.1-Flag-N-UBAP2L WT (NCBI, NM_014847.4)
676 was generated by Evanthia Pangou (IGBMC). Primers used for cloning are described in Table
677 S1. pEGFP-C1 was purchased from Clontech. pcDNA3.1-Flag-N was obtained from IGBMC
678 cloning facility, and pcDNA3.1-Flag-UBAP2L R131-190A was a generous gift of Zhenguo
679 Chen (Southern Medical University, P. R. China) (Huang *et al*, 2020a). pEGFP-C1-Nup85 was
680 kindly provided by Valérie Doye (Institut Jacques Monod, Paris), and pXRGG-GFP was kindly
681 provided by Jan M. van Deursen (Hamada *et al*, 2011; Love *et al*, 1998).

682

683 **Plasmid and siRNA transfections**

684 Lipofectamine 2000 (Invitrogen), jetPEI-DNA transfection reagent (Polyplus-transfection) and
685 X-tremeGENE™ 9 DNA Transfection Reagent (Roche) were used to perform plasmid
686 transient transfection according to the manufacturer's instructions. Lipofectamine RNAiMAX
687 (Invitrogen) was used to deliver siRNAs for gene knock-down (KD) according to the
688 manufacturer's instructions at a final concentration of 20 to 40 nM siRNA. The following
689 siRNA oligonucleotides were used: Non-targeting individual siRNA-2 5'-
690 UAAGGCUAUGAAGAGAUAC-3' (Dharmacon), UBAP2L siRNA 5'-
691 CAACACAGCAGCACGUUAU-3' (Dharmacon) and FXR1 siRNA-1 5'-
692 AAACGGAAUCUGAGCGUAA-3' (Dharmacon).

693

694 **Protein preparation and Western blotting**

695 Cells were collected by centrifugation at 200 g for 4 min at 4 °C and washed twice with cold
696 phosphate buffered saline (PBS), and cell lysates for Western blot were prepared using 1X
697 RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM
698 EGTA, 2 mM Sodium pyrophosphate, 1 mM Na₃VO₄ and 1 mM NaF) supplemented with
699 protease inhibitor cocktail (Roche) and incubated on ice for 30 min. After centrifugation at 16
700 000 g for 15 min at 4 °C, cleared supernatant was transferred to the new clean Eppendorf tubes
701 and total protein concentration was measured using Bradford assay by Bio-Rad Protein Assay
702 kit (Bio-Rad). Nuclear and cytoplasmic proteins were prepared using the NE-PER nuclear and
703 cytoplasmic extraction reagent kit (Thermo Scientific™, 78833). Protein samples were boiled
704 for 8 min at 95 °C in 1X Laemmli buffer (LB) with β-Mercaptoethanol (BioRad, 1610747),
705 resolved on 10% polyacrylamide gels or pre-cast 4-12% Bis-Tris gradient gels (Thermo
706 Scientific, NW04120BOX) or pre-cast NuPAGE™ 3-8% Tris-Acetate gradient Gels (Thermo
707 Scientific, EA0378BOX) and transferred to a polyvinylidene difluoride (PVDF) membrane

708 (Millipore, IPFL00010) using semi-dry transfer unit (Amersham) or wet transfer modules
709 (BIO-RAD Mini-PROTEAN® Tetra System). Membranes were blocked in 5% non-fat milk
710 powder, 5% bovine serum albumin (BSA, Millipore, 160069), or 5% non-fat milk powder
711 mixed with 3% BSA and resuspended in TBS-T (Tris-buffered saline-T: 25 mM Tris-HCl, pH
712 7.5, 150 mM NaCl 0.05% Tween) for 1h at room temperature, followed by incubation with
713 antibodies diluted in TBS-T 5% BSA/5% milk. All incubations with primary antibodies were
714 performed for overnight at 4°C. TBS-T was used for washing the membranes. Membranes were
715 developed using SuperSignal West Pico (Pierce, Ref. 34580) or Luminata Forte Western HRP
716 substrate (Merck Millipore, Ref. WBLUF0500).

717

718 **Immunoprecipitations**

719 Cell lysates for immunoprecipitations (IP) were prepared using 1X RIPA buffer supplemented
720 with protease inhibitor cocktail and incubated on ice for 1h. When indicated, 1X RIPA buffer
721 was supplemented with with RNase A or Benzonase. After centrifugation at 16 000 g for 15
722 min at 4 °C, cleared supernatant was transferred to the new clean Eppendorf tubes. Lysates
723 were equilibrated to volume and concentration.

724 For endogenous IP experiments, IgG and target specific antibodies as well as protein G
725 sepharose 4 Fast Flow beads (GE Healthcare Life Sciences) were used. Samples were
726 incubated with the IgG and target specific antibodies overnight at 4 °C with rotation. Beads
727 were blocked with 3% BSA diluted in 1X RIPA buffer and incubated for 2h at 4 °C with
728 rotation. Next, the IgG/ specific antibodies-samples and blocked beads were incubated in 1.5
729 ml Eppendorf tubes to a final volume of 1 ml 4h at 4 °C with rotation. The incubated IgG/
730 specific antibodies-samples-beads were washed with washing buffer (25 mM Tris-HCl pH 7.5,
731 300 mM NaCl, 0.5% Triton X-100, 0.5 mM EDTA, 0.5 mM EGTA, 1 mM Sodium
732 pyrophosphate, 0.5 mM Na₃VO₄ and 0.5 mM NaF) or TBS-T supplemented with protease

733 inhibitor cocktail 4 to 6 times for 10 min each at 4°C with rotation. Beads were pelleted by
734 centrifugation at 200 g for 3 min at 4 °C. The washed beads were directly eluted in 2X LB with
735 β -Mercaptoethanol and boiled for 12 min at 95 °C for Western blot.

736 For GFP-IP/Flag-IP experiments, GFP-Trap A agarose beads (Chromotek) or Flag beads
737 (Sigma) were used. Cells expressing GFP- or Flag-tagged plasmids for at least 24 h were used
738 to isolate proteins using 1X RIPA buffer supplemented with protease inhibitor cocktail. Beads
739 were blocked with 3% BSA diluted in 1X RIPA buffer and incubated for 2h at 4 °C with
740 rotation. Samples were incubated with the blocked beads for 2h or overnight at 4 °C with
741 rotation, and the beads were washed and boiled as for endogenous IP.

742

743 **Immunofluorescence**

744 Cells grown on glass coverslips (Menzel-Glaser) were washed twice in PBS and then fixed
745 with 4% paraformaldehyde (PFA, Electron Microscopy Sciences 15710) in PBS for 15 min at
746 room temperature, washed 3 times for 5 min in PBS and permeabilized with 0.5% NP-40
747 (Sigma) in PBS for 5 min. Cells were washed 3 times for 5 min in PBS and blocked with 3%
748 BSA in PBS-Triton 0.01% (Triton X-100, Sigma, T8787) for 1h. Cells were subsequently
749 incubated with primary antibodies in blocking buffer (3% BSA in PBS-Triton 0.01%) for 1h
750 at room temperature, washed 3 times for 8 min in PBS-Triton 0.01% with rocking and
751 incubated with secondary antibodies in blocking buffer for 1h at room temperature in the dark.
752 After incubation, cells were washed 3 times for 8 min in PBS-Triton 0.01% with rocking in the
753 dark and glass coverslips were mounted on glass slides using Mowiol containing 0.75 μ g/ml
754 DAPI (Calbiochem) and imaged with a 100x or 63x objective using Zeiss epifluorescence
755 microscope. For mitotic cells immunofluorescence, cells were collected from dishes with cell
756 scrapers, centrifuged on Thermo Scientific Shandon Cytospin 4 Cytocentrifuge for 5 min at
757 1000 rpm and fixed immediately with 4% PFA for 15 min at room temperature.

758 For nucleoporins (Nups) immunofluorescence, cells grown on glass coverslips were washed
759 twice in PBS and then fixed with 1% PFA in PBS for 10 min at room temperature, washed 3
760 times for 5 min in PBS and permeabilized with 0.1% Triton X-100 and 0.02% SDS
761 (Euromedex, EU0660) in PBS for 5 min. After permeabilization, cells were washed 3 times for
762 5 min in PBS and blocked with 3% BSA in PBS-Triton 0.01% for 1h at room temperature or
763 overnight at 4 °C. Cells were subsequently incubated with primary antibodies in blocking
764 buffer (3% BSA in PBS-T) for 1h at room temperature, washed 3 times for 8 min with rocking
765 in blocking buffer and then incubated with secondary antibodies in blocking buffer for 1h at
766 room temperature in the dark. After incubation, cells were washed 3 times for 8 min with
767 rocking in blocking buffer in the dark and then permeabilized again with 0.1% Triton X-100
768 and 0.02% SDS in PBS for 1 min and post-fixed for 10 min with 1% PFA in PBS at room
769 temperature in the dark. Then coverslips were washed twice in PBS for 5 min and mounted on
770 glass slides using Mowiol containing 0.75 µg/ml DAPI.

771 An adapted protocol was used for the experiments presented in Fig. S2A as described
772 previously (Guerber *et al*, 2023). After the appropriate synchronization using DTBR, the
773 cytoplasm was extracted from the cells to remove the large cytoplasmic fraction of UBAP2L
774 by incubating the coverslips in cold 0,01% Triton X-100 for 90sec. 4% PFA was immediately
775 added to the coverslips after the pre-extraction and the standard IF protocol was followed.

776

777 **Sample preparation for single molecule localization microscopy**

778 For super-resolution single molecule localization microscopy (splitSMLM), cells were plated
779 on 35 mm glass bottom dish with 14 mm micro-well #1.5 cover glass (Cellvis). Cells were
780 washed twice with PBS (2 ml/well) and then fixed with 1% PFA in PBS for 15 min at room
781 temperature, washed 3 times for 5 min in PBS (store samples submerged in PBS at 4 °C until
782 use) and permeabilized with 0.1% Triton X-100 (Tx) in PBS (PBS/Tx) for 15 min. Cells were

783 blocked with 3% BSA in 0.1% PBS/Tx (PBS/Tx/B) for 1h and then incubated with primary
784 antibodies (optimal working concentration of primary antibody is 2 $\mu\text{g}/\text{ml}$) in PBS/Tx/B (200
785 $\mu\text{l}/\text{well}$) for overnight at 4 $^{\circ}\text{C}$ in wet chamber. After incubation, cells were washed 3 times for
786 8 min with rocking in PBS/Tx/B and subsequently incubated with secondary antibodies
787 (optimal working concentration of secondary antibody is 4 $\mu\text{g}/\text{ml}$) in PBS/Tx/B (200 $\mu\text{l}/\text{well}$)
788 for 2h at room temperature in the dark. Immediately after, cells were washed 3 times for 8 min
789 with rocking in PBS/Tx and post-fixed for 10 min with 1% PFA at room temperature in the
790 dark, then cells were washed twice in PBS and kept in PBS in the dark.

791 The samples were imaged in a water-based buffer that contained 200 U/ml glucose oxidase,
792 1000 U/ml catalase, 10% w/v glucose, 200 mM Tris-HCl pH 8.0, 10 mM NaCl and 50 mM
793 MEA. 2 mM cyclooctatetraene was added to the buffer for multi-color imaging (Andronov *et*
794 *al*, 2022). The mixture of 4 kU/ml glucose oxidase (G2133, Sigma) and 20 kU/ml catalase
795 (C1345, Sigma) was stored at -20 $^{\circ}\text{C}$ in an aqueous buffer containing 25 mM KCl, 4 mM TCEP,
796 50% v/v glycerol and 22 mM Tris-HCl pH 7.0. MEA-HCl (30080, Sigma) was stored at a
797 concentration of 1M in H₂O at -20 $^{\circ}\text{C}$. Cyclooctatetraene (138924, Sigma) was stored at 200
798 mM in dimethyl sulfoxide at -20 $^{\circ}\text{C}$. The samples were mounted immediately prior to imaging
799 filling the cavity of the glass-bottom petri dishes with \sim 200 μl of the imaging buffer and placing
800 a clean coverslip on top of it, which allowed imaging for \geq 8 hours without degradation of the
801 buffer. After imaging, the samples were washed once with PBS and kept in PBS at +4 $^{\circ}\text{C}$.

802

803 **Single molecule localization microscopy**

804 The SMLM experiments were performed on a splitSMLM system (Andronov *et al*, 2022) that
805 consisted of a Leica DMI6000B microscope; an HCX PL APO 160x/1.43 Oil CORR TIRF
806 PIFOC objective; a 642 nm 500 mW fiber laser (MBP Communication Inc.) for fluorescence
807 excitation and a 405 nm 50 mW diode laser (Coherent Inc.) for reactivation of fluorophores.

808 The sample was illuminated through a Semrock FF545/650-Di01 dichroic mirror and the
809 fluorescence was filtered with Semrock BLP01-532R and Chroma ZET635NF emission filters.
810 For single-color imaging that was used for estimation of the NPC density at the NE, the
811 fluorescence was additionally filtered with a Semrock BLP01-635R-25 long-pass filter and was
812 projected onto an Andor iXon+ (DU-897D-C00-#BV) EMCCD camera.

813 For multi-color imaging, the fluorescence was split into two channels with a Chroma
814 T690LPXXR dichroic mirror inside an Optosplit II (Cairn Research) image splitter. The short-
815 wavelength channel was additionally filtered with a Chroma ET685/70m bandpass filter and
816 both channels were projected side-by-side onto an Andor iXon Ultra 897 (DU-897U-CS0-#BV)
817 EMCCD camera.

818 The SMLM acquisitions began with a pumping phase, during which the sample was
819 illuminated with the 642 nm laser but the fluorescence was not recorded due to a very high
820 density of fluorophores in a bright state. When the density dropped to a level that allowed
821 observation of individual molecules, the images started to be recorded. Pumping and imaging
822 were performed at 30-50% of maximal power of the 642 nm laser. When the density of
823 fluorophores in the bright state dropped further due to photobleaching, the sample started to be
824 illuminated with the 405 nm laser for reactivation of fluorophores. The intensity of the 405 nm
825 laser was increased gradually to account for the photobleaching. For estimation of the NPC
826 density, to increase speed, the pumping and imaging were performed at 100% laser power and
827 the acquisitions were stopped after about 2 min of imaging.

828

829 **Processing of single molecule localization microscopy data**

830 The fitting of single-molecule localizations was done in the Leica LAS X software with the
831 “direct fit” method. For single-color imaging, the obtained localization tables were corrected
832 for drift and reconstructed as 2D histograms with a pixel size of 15 nm in SharpViSu (Andronov

833 *et al*, 2016). For multi-color imaging, the localizations were first unmixed in SplitViSu
834 (Andronov *et al*, 2022). Next, they were corrected for drift and for relocalizations in SharpViSu,
835 and reconstructed as 2D histograms with a pixel size of 5 nm.

836 For quantification of the rotational symmetry of the NPCs, individual NPCs were picked
837 manually on the NE of each imaged cell. Only particles that are in focus and in correct “top
838 view” orientation were selected. For the analysis, the localizations within a radius of 130 nm
839 from the manually picked center of each NPC were used. The obtained particles were aligned
840 in `smlm_datafusion2d` with random rotation of every particle by $n \cdot 45^\circ$, $n = [0, 7]$, after each
841 alignment iteration (Heydarian *et al*, 2018). The aligned particles were then converted to polar
842 coordinates and localizations with radii from 50 to 70 nm were kept for further analysis. A sine
843 function with a period of $\pi/4$ was fitted to the polar angle distribution of the sum of all aligned
844 particles. The localizations were split into eight sectors using the minima of the sine function
845 as the edges of the sectors. The number of localizations within each sector was calculated for
846 each NPC. For a given NPC, a sector was considered occupied if the number of localizations
847 within it was higher than the half of the mean number of localizations per sector for this NPC.
848 The quantified number of subunits of an NPC is the number of the occupied sectors.

849 The axial and radial profiles of the NPCs were obtained as described previously (Andronov *et*
850 *al*, 2022). For radial profiles, the localizations of co-imaged proteins were transformed using
851 the alignment parameters of Nup96 after 8-fold alignment in `smlm_datafusion2d` (Heydarian
852 *et al*, 2018). For the “side view” profile, the axial profiles of individual particles were calculated
853 in Fiji (Schindelin *et al*, 2012), averaging through the whole thickness of the NPC. The axial
854 profiles of Nup96 particles were fitted with a sum of two Gaussians in Matlab. Nup96 particles
855 and co-imaged proteins were aligned using this fit of Nup96.

856

857 **Live-cell imaging**

858 For FXR1 mitotic granules assay, WT and UBAP2L KO HeLa cells expressing GFP-FXR1
859 were grown on 35/10 mm 4 compartment glass bottom dishes (Greiner Bio-One, 627871) and
860 synchronized by double thymidine block, released for 8h and analyzed by Nikon PFS spinning
861 disk (63× objective) for 9h. Z-stacks (7 μm range, 1 μm step) were acquired every 5 min and
862 movies were made with maximum intensity projection images for every time point shown at
863 speed of 7 frames per second. Image quantification analysis was performed using ImageJ
864 software.

865 For protein import and export assay, WT and UBAP2L KO HeLa cells were grown on 8-well
866 Chambered Coverglass w/non-removable wells (Thermo Fisher Scientific, 155411PK) and
867 transfected with the reporter plasmid XRGG-GFP for 30h, and incubated with full media with
868 SiR-DNA 1:1500 and Verapamil 1:1000 for at least 1h before filming. Then SiR-DNA and
869 Verapamil were kept with media and cells were incubated in media with 0.01 μM
870 dexamethasone. Dexamethasone-induced nuclear import of XRGG-GFP was recorded by
871 Leica CSU-W1 spinning disk (63X objective) for 129 min (1 acquisition every 1 min, 12 μm
872 range, 3 μm step). For nuclear export, dexamethasone was washed out at 129 min time point
873 with warm dexamethasone-free medium, cells were incubated with full media with SiR-DNA
874 1:6000 and Verapamil 1:4000 and nuclear export of XRGG-GFP was recorded for 170 min (1
875 acquisition every 1 min, 12 μm range, 3 μm step). Image quantification analysis was performed
876 using ImageJ software.

877

878 **Nuclear envelope intensity analysis of nucleoporins**

879 A CellProfiler software pipeline was previously generated by Arantxa Agote-Aran (Agote-
880 Aran *et al*, 2020) that automatically recognizes cell nuclei based on the DAPI fluorescent image.
881 A threshold of nuclei size was applied to the pictures to exclude too small or too big nuclei and
882 nuclei edges were enhanced using the Prewitt edge-finding method. This allowed identification

883 and measurement of the nuclei area, form factor and nuclear mean intensity of desired channels.

884 The parameters' measurements of the software were exported to an Excel file and statistically

885 analysed. At least 200 cells from three different biological replicates were measured.

886

887 **Colony formation assay**

888 500 WT and UBAP2L KO HeLa cells were seeded per well in 6-well plates and incubated at

889 37 °C in 5% CO₂ for 7 days until colonies formed. Cells were washed with 1X PBS, fixed with

890 4% PFA and stained with 0,1% Crystal Violet for 30 min. The number of colonies was first

891 manually counted and then automatically quantified with Fiji software.

892

893 **Flow cytometry**

894 For cell death analysis, HeLa cells were spun down and resuspended in cold PBS supplemented

895 with 50 µg/ml propidium iodide (PI) (Sigma-Aldrich, Ref. P4170). PI positive cells were

896 analyzed by BD FACS Celesta™ Flow Cytometer.

897

898 **Experimental design, data acquisition and statistical analysis**

899 All experiments were done in a strictly double-blind manner. At least three independent

900 biological replicates were performed for each experiment (unless otherwise indicated) and

901 image quantifications were carried out in a blinded manner. Curves and graphs were made

902 using GraphPad Prism and Adobe Illustrator software. Data was analyzed using one-sample

903 two-tailed T-test or two sample two-tailed T-test (two-group comparison or folds increase

904 relative to the control, respectively). A p-value less than 0.05 (typically ≤ 0.05) was considered

905 statistically significant and stars were assigned as follows: *P < 0.05, **P < 0.01, ***P < 0.001,

906 ****P < 0.0001. In all graphs, results were shown as mean \pm SD, and details for each graph

907 were listed in the corresponding figures' legends.

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1103 in vitro. *Int J Mol Med* 36: 1012–1018
- 1104
1105

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1122

1123 **Author contributions**

1124 Y.L., and L.A. designed and performed experiments and helped writing the manuscript. X.L.,
1125 J.L., L.G., L.L., A.A.A., E.P., L.R., C.K., M.Q., S.S., and L.C. performed experiments. Z.Z.
1126 helped performing experiments. D.R., M.G., and B.P.K. helped designing the experiments and
1127 supervising. I.S. supervised the project, designed experiments and wrote the manuscript with
1128 input from all authors.

1129

1130 **Competing interests**

1131 The authors declare no competing financial interests.

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1133 **Data and materials availability**

1134 All data needed to evaluate the conclusions in the paper are present in the paper and/or the

1135 Supplementary Materials.

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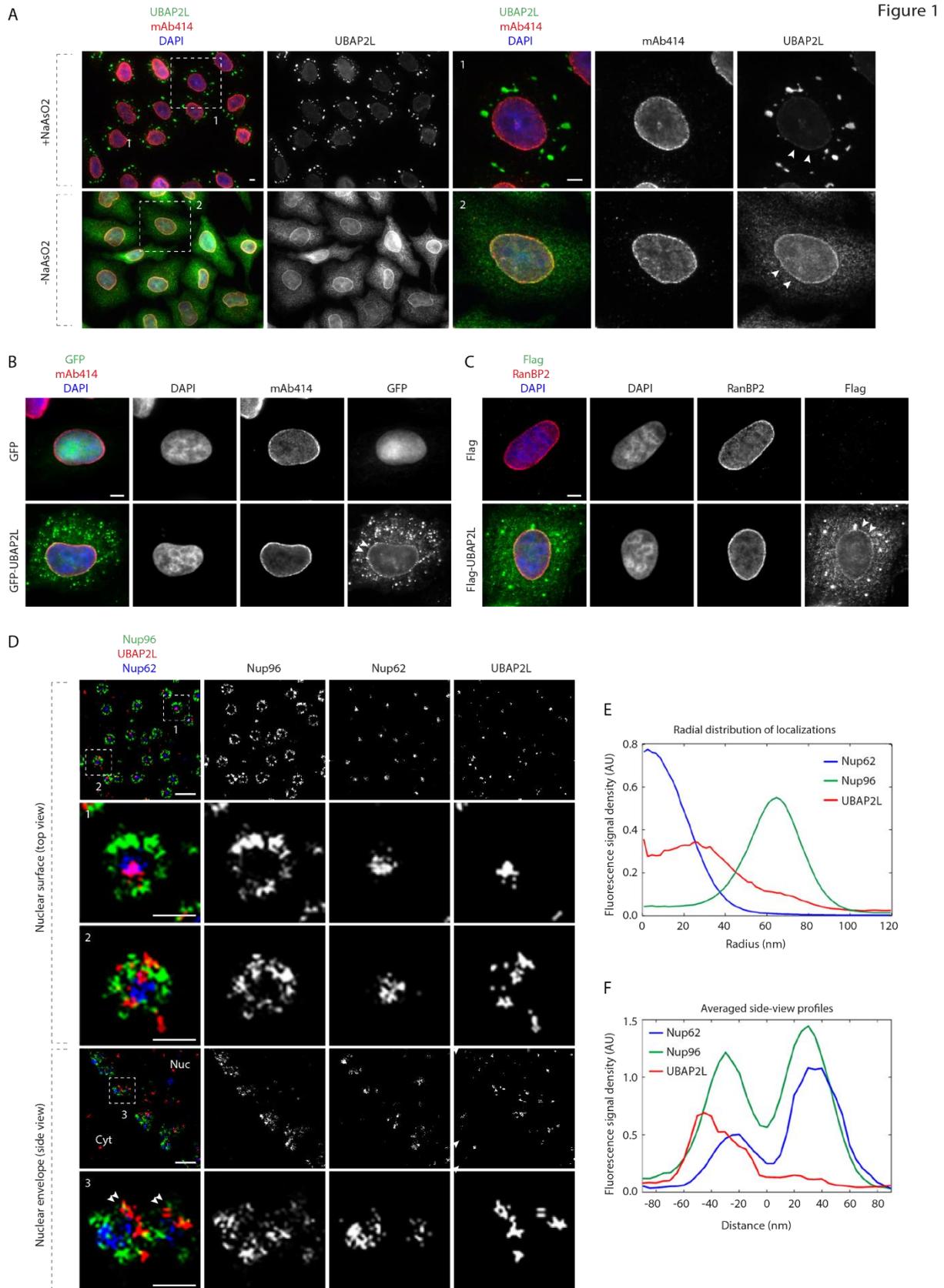
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1155 **Figures**

1156



1157

1158 **Fig. 1. UBAP2L localizes to the NE and NPCs.**

1159 **(A)** Representative images of the localization of UBAP2L and nucleoporins (Nups) in HeLa
1160 cells with/without NaAsO₂ treatment shown by immunofluorescence microscopy with
1161 UBAP2L and mAb414 antibodies. Nuclei were stained with DAPI. The arrowheads indicate
1162 the nuclear envelope (NE) localization of endogenous UBAP2L. The magnified framed regions
1163 are shown in the corresponding numbered panels. Scale bars, 5 μ m.

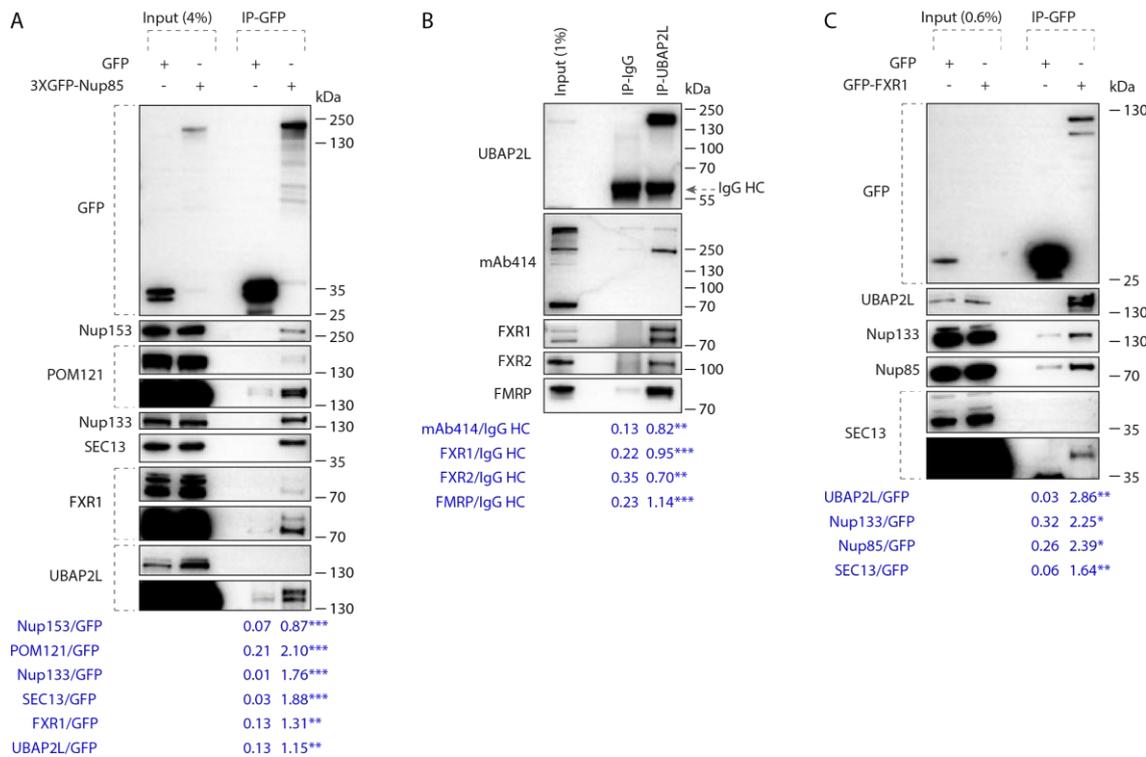
1164 **(B)** Representative immunofluorescence images depicting the localization of UBAP2L and
1165 Nups (mAb414) in HeLa cells expressing GFP alone or GFP-UBAP2L. The arrowheads
1166 indicate the NE localization of GFP-tagged UBAP2L. Scale bar, 5 μ m.

1167 **(C)** Representative immunofluorescence images depicting the localization of UBAP2L and
1168 Nups (RanBP2) in HeLa cells expressing Flag alone or Flag-UBAP2L. The arrowheads
1169 indicate the NE localization of Flag-tagged UBAP2L. Scale bar, 5 μ m.

1170 **(D to F)** Representative super-resolution immunofluorescence images of Nup96-GFP Knock-
1171 in (KI) U2OS cells acquired using multi-color single molecule localization microscopy with a
1172 dichroic image splitter (splitSMLM) show NPCs on the nuclear surface (top view) and in the
1173 cross section of the NE (side view). Nup96 signal labels the cytoplasmic and nuclear ring of
1174 the NPC and the localization of the central channel NPC component is analyzed by Nup62
1175 antibody. Nuclear (Nuc) and cytoplasmic (Cyt) side of the NE are indicated in the side view.
1176 The magnified framed regions are shown in the corresponding numbered panels. Note that
1177 UBAP2L can localize to both structures within the NPCs (framed regions 1 and 2 in the top
1178 view) and is found preferentially at the nuclear ring labelled with Nup96 (double arrowheads
1179 in framed region 3 in the side view). Scale bars, 300 and 100 nm, respectively **(D)**. Radial
1180 distribution of localizations of Nup62, Nup96 and UBAP2L in **(D)** was obtained by averaging
1181 1932 NPC particles **(E)**. Averaged “side view” profiles of Nup62, Nup96 and UBAP2L in **(D)**
1182 were obtained by alignment of 83 individual NPCs **(F)**.

1183

Figure 2



1184

1185 **Fig. 2. UBAP2L interacts with Nups and NPC assembly factors.**

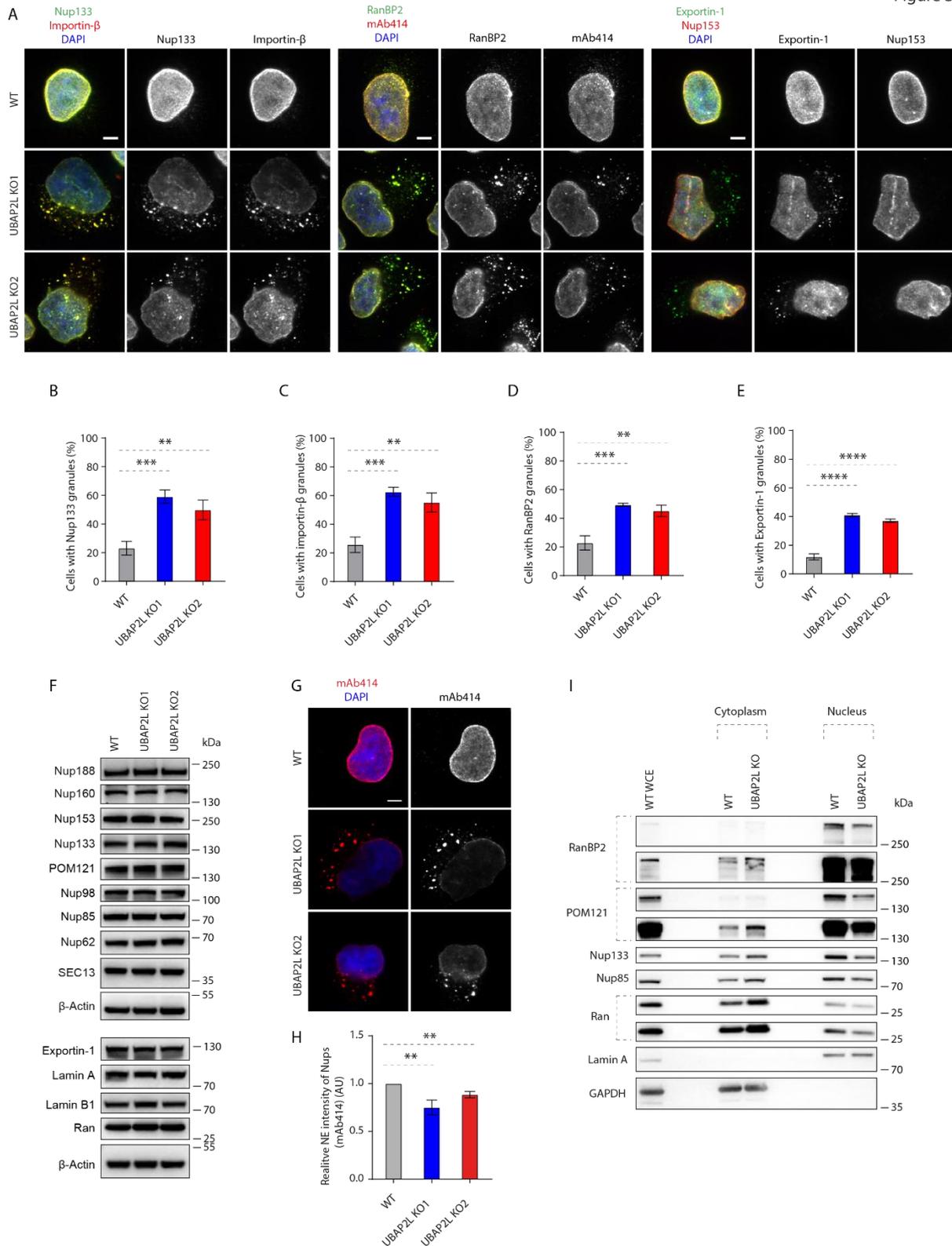
1186 (A) HeLa cells lysates expressing GFP alone or 3XGFP-Nup85 for 27h were
 1187 immunoprecipitated using agarose GFP-Trap A beads (GFP-IP), analyzed by Western blot and
 1188 signal intensities were quantified (shown a mean value, **P < 0.01, ***P < 0.001; N = 3).
 1189 Molecular weight markers are indicated in kilodalton (kDa).

1190 (B) HeLa cells lysates were immunoprecipitation using UBAP2L antibody or IgG, analyzed
 1191 by Western blot and signal intensities were quantified (shown a mean value, **P < 0.01, ***P
 1192 < 0.001; N = 3). The arrow indicates the band corresponding to the IgG heavy chain (HC).

1193 (C) Lysates of HeLa cells expressing GFP alone or GFP-FXR1 for 27h were
 1194 immunoprecipitated using agarose GFP-Trap A beads (GFP-IP), analyzed by Western blot and
 1195 signal intensities were quantified (shown a mean value, *P < 0.05, **P < 0.01; N = 3).

1196

Figure 3



1197

1198 **Fig. 3. UBAP2L regulates Nups localization.**

1199 (A to E) Representative immunofluorescence images depicting the localization of Nups and

1200 NPC-associated factors in wild type (WT) and UBAP2L Knock-out (KO) HeLa cells

1201 synchronized in interphase by double thymidine block and release (DTBR) at 12h (**A**). Nuclei
1202 were stained with DAPI. The percentage of cells with the cytoplasmic granules containing
1203 Nup133 (**B**), Importin- β (**C**), RanBP2 (**D**) and Exportin-1 (**E**) in (**A**) were quantified. At least
1204 200 cells per condition were analyzed (mean \pm SD, **P < 0.01, ***P < 0.001, ****P < 0.0001,
1205 two-tailed t-test, $N = 3$). Scale bars, 5 μ m.

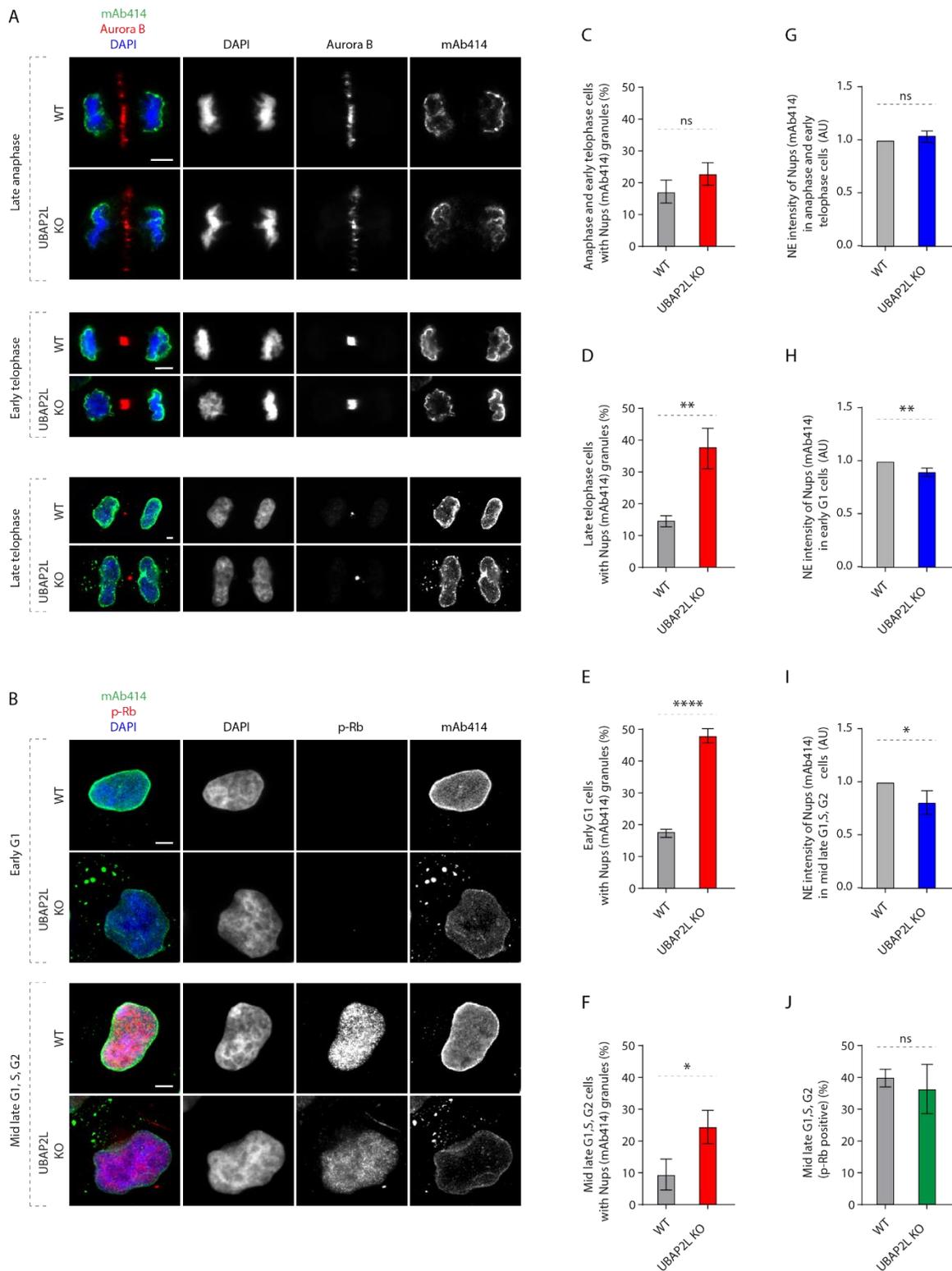
1206 (**F**) The protein levels of Nups and NPC-associated factors in WT and UBAP2L KO HeLa cells
1207 synchronized in interphase by DTBR at 12h were analyzed by Western blot.

1208 (**G and H**) Representative immunofluorescence images of FG-Nups (mAb414) at the NE in
1209 WT and UBAP2L KO HeLa cells in interphase cells synchronized by DTBR at 12h (**G**). Nuclei
1210 were stained with DAPI. The NE intensity of Nups (mAb414) in (**G**) was quantified (**H**). At
1211 least 150 cells per condition were analyzed (mean \pm SD, **P < 0.01, two-tailed t-test, $N = 3$).
1212 Scale bar, 5 μ m.

1213 (**I**) The nuclear and cytoplasmic protein levels of Nups and NPC transport-associated factors
1214 in WT and UBAP2L KO HeLa cells synchronized in the G1/S transition phase by thymidine
1215 18h were analyzed by Western blot. WCE indicates whole cell extract.

1216

Figure 4



1217

1218 **Fig. 4. UBAP2L regulates localization of Nups in interphase but not in postmitotic cells.**

1219 (A and B) Representative immunofluorescence images depicting the localization of Nups

1220 (mAb414) in WT and UBAP2L KO HeLa cells in different cell cycle stages. Mitotic cells were

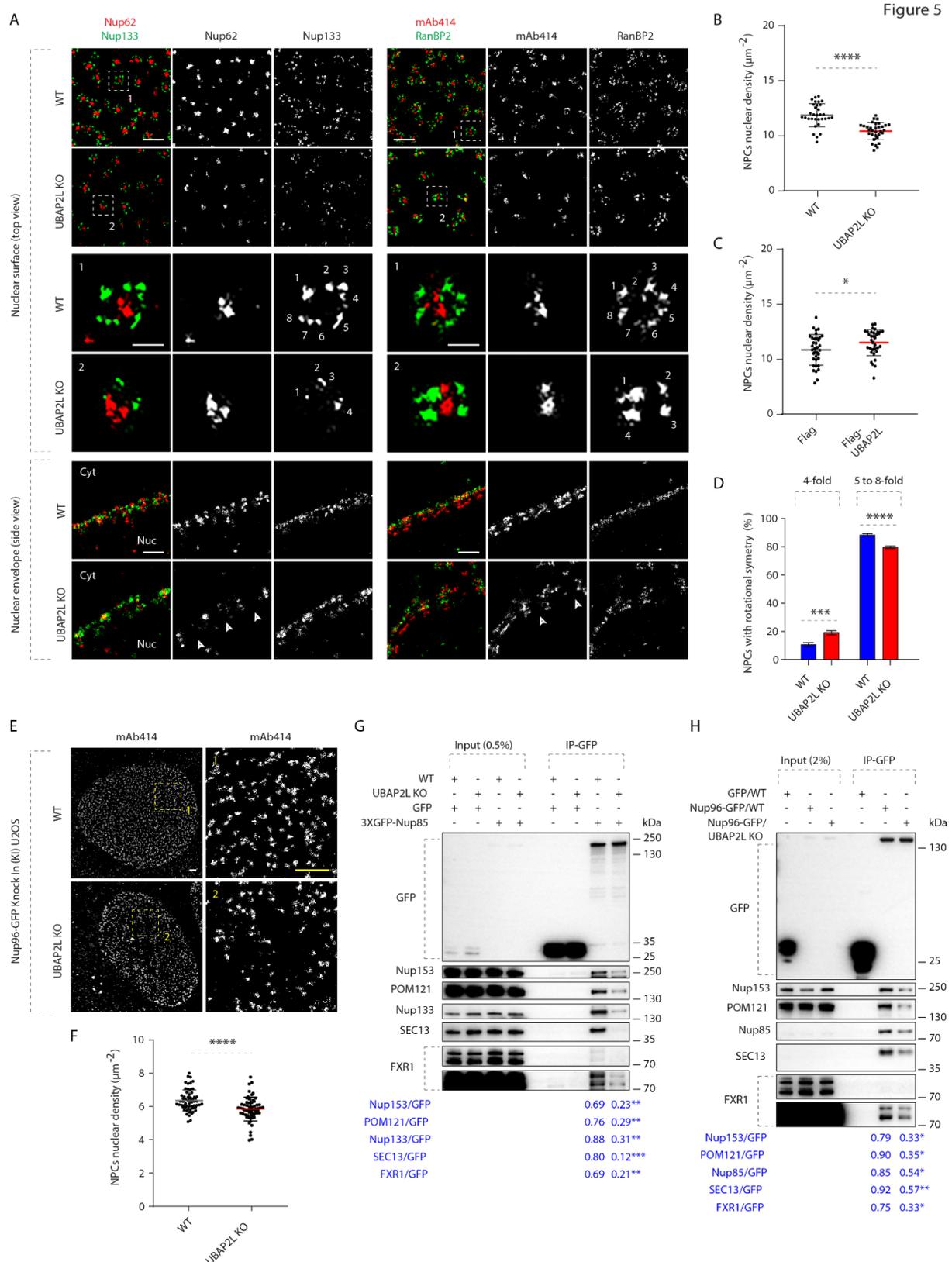
1221 labeled by Aurora B (**A**) while p-Rb was used to distinguish between early G1 (p-Rb-negative
1222 cells) and mid-late G1, S and G2 (p-Rb-positive cells) stages (**B**). Nuclei were stained with
1223 DAPI. Scale bars, 5 μ m.

1224 (**C to F**) The percentage of cells with the cytoplasmic granules of Nups (mAb414) in anaphase
1225 and early telophase (**C**), late telophase (**D**), early G1 (**E**) and mid-late G1, S, G2 (**F**) in (**A, B**)
1226 were quantified. At least 150 cells per condition were analyzed (mean \pm SD, ns, non-significant,
1227 *P < 0.05, **P < 0.01, ****P < 0.0001, two-tailed t-test, $N = 3$).

1228 (**G to I**) The NE intensity of Nups (mAb414) in anaphase and early telophase cells (**G**), early
1229 G1 cells (**H**) and mid-late G1, S, G2 cells (**I**) in (**A, B**) were quantified. At least 100 cells per
1230 condition were analyzed (mean \pm SD, ns, non-significant, *P < 0.05, **P < 0.01, two-tailed t-
1231 test, $N = 3$).

1232 (**J**) The percentage of p-Rb-positive cells in (**B**) was quantified. At least 150 cells per condition
1233 were analyzed (mean \pm SD, ns, non-significant, two-tailed t-test, $N = 3$).

1234



1235

1236 **Fig. 5. UBAP2L mediates the assembly of the NPC scaffold elements and the biogenesis**

1237 **of NPCs.**

1238 **(A)** Representative splitSMLM images depicting several NPC components on the nuclear
1239 surface (top view) and in the cross section of the NE (side view) in WT and UBAP2L KO HeLa
1240 cells synchronized in early interphase by DTBR at 12h. Nup133 signal labels the cytoplasmic
1241 and nuclear rings of the NPC, the localization of the central channel is visualized by Nup62
1242 and mAb414 antibodies and cytoplasmic filaments are labeled by RanBP2. The magnified
1243 framed regions are shown in the corresponding numbered panels. Nuclear (Nuc) and
1244 cytoplasmic (Cyt) side of the NE are indicated in the side view. The arrowheads indicate the
1245 disrupted localization of Nup62 or mAb414 at NE in UBAP2L KO HeLa cells and the numbers
1246 point to the individual identified spokes of the NPC. Scale bars, 300 and 100 nm, respectively.

1247 **(B and C)** The nuclear density of NPCs (mAb414 and RanBP2) in cells shown in (A) was
1248 quantified **(B)** (mean \pm SD, ****P < 0.0001, two-tailed t-test; counted 32 cells per cell line).
1249 The nuclear density of NPCs (mAb414) in HeLa cells expressing Flag alone or Flag-UBAP2L
1250 for 35h and synchronized in interphase by DTBR at 12h was quantified **(C)** (mean \pm SD, *P <
1251 0.05, two-tailed t-test; counted 36 cells for Flag and 33 cells for Flag-UBAP2L). The
1252 corresponding representative images are shown in the Fig. S3B.

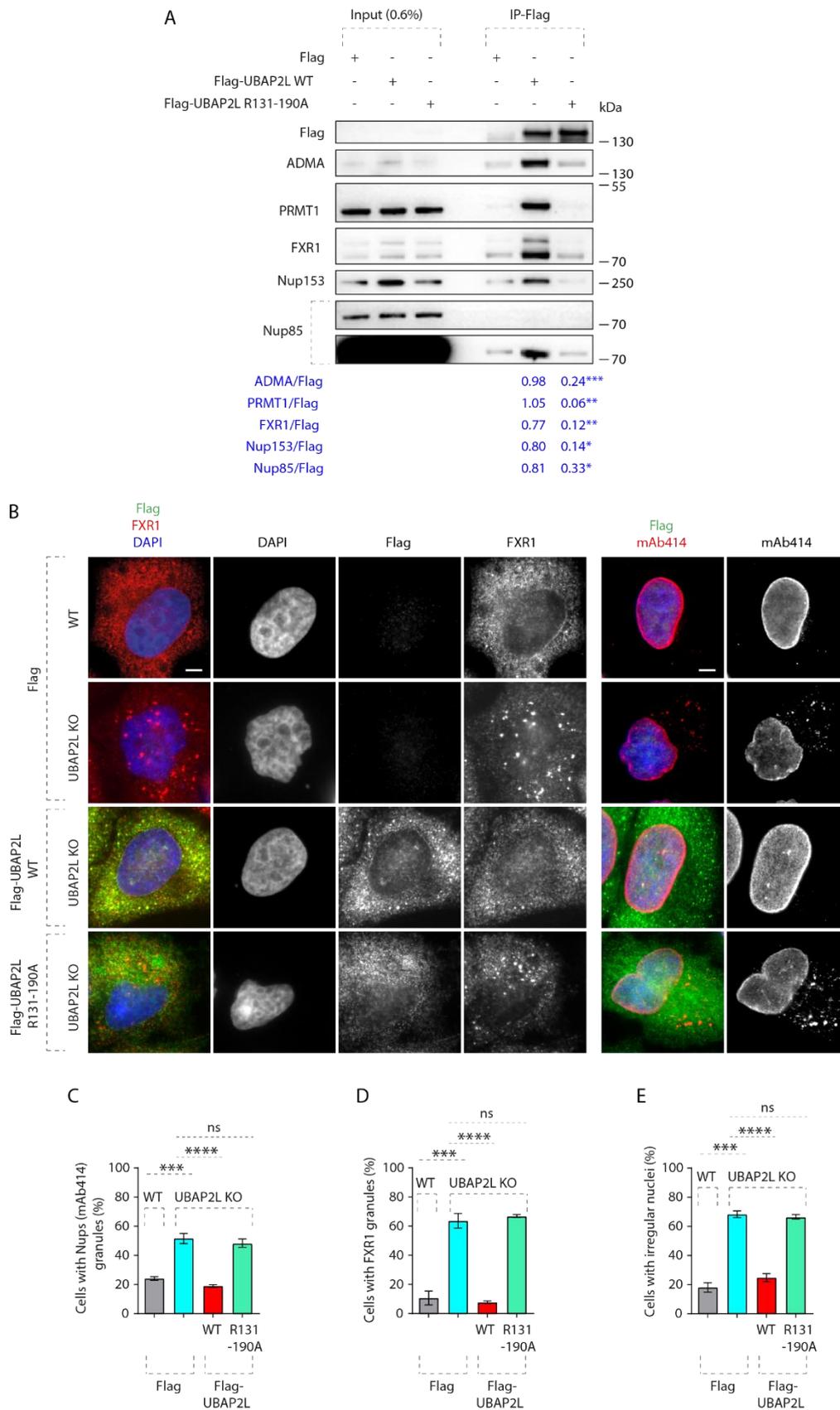
1253 **(D)** The 8-fold rotational symmetry of NPCs in **(A)** was quantified by alignment of Nup133
1254 particles and segmentation analysis (mean \pm SD, ***P < 0.001, ****P < 0.0001, two-tailed t-
1255 test; counted 851 NPCs for WT HeLa cell line and 559 NPCs for UBAP2L KO HeLa cell line).

1256 **(E and F)** Representative SMLM immunofluorescence images of FG-Nups (mAb414) at the
1257 nuclear surface in Nup96-GFP KI U2OS WT and UBAP2L KO cells in interphase cells
1258 synchronized by DTBR at 12h **(E)**. The nuclear density of NPCs (mAb414) in cells shown in
1259 **(E)** was quantified in **(F)** (mean \pm SD, ****P < 0.0001, two-tailed t-test; counted 60 cells per
1260 cell line). Scale bars, 1 μ m.

1261 **(G and H)** Lysates of interphase WT and UBAP2L KO HeLa cells expressing GFP alone or
1262 3XGFP-Nup85 for 27h were immunoprecipitated using agarose GFP-Trap A beads (GFP-IP),

1263 analyzed by Western blot and signal intensities were quantified (shown a mean value, **P <
1264 0.01, ***P < 0.001; $N = 3$) (**G**). Lysates of interphase U2OS cells expressing GFP alone for
1265 27h and Nup96-GFP KI U2OS WT and UBAP2L KO cells were immunoprecipitated using
1266 agarose GFP-Trap A beads (GFP-IP), analyzed by Western blot and signal intensities were
1267 quantified (shown a mean value, *P < 0.05, **P < 0.01; $N = 3$) (**H**).
1268

Figure 6

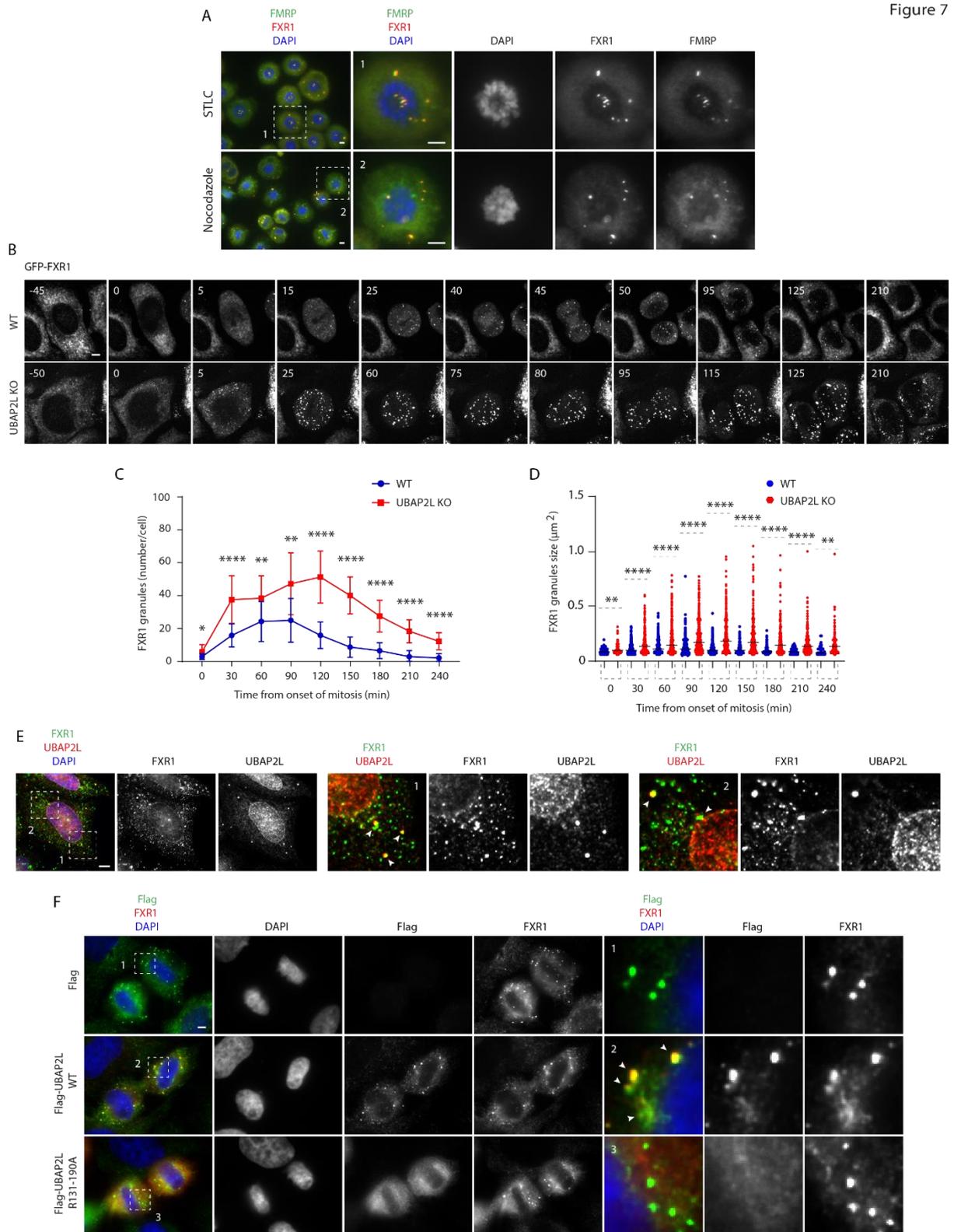


1270 **Fig. 6. Arginines within the RGG domain of UBAP2L mediate the function of UBAP2L**
1271 **on Nups and FXRPs.**

1272 (A) Lysates of interphase HeLa cells expressing Flag alone, Flag-UBAP2L WT or mutated
1273 Flag-UBAP2L version where 19 arginines located in the RGG domain were replaced by
1274 alanines (R131-190A) for 27h were immunoprecipitated using Flag beads (Flag-IP), analyzed
1275 by Western blot and signal intensities were quantified (shown a mean value, *P < 0.05, **P <
1276 0.01, ***P < 0.001; N = 3).

1277 (B to E) Representative immunofluorescence images depicting nuclear shape and localization
1278 of FXR1 and Nups (mAb414) in WT and UBAP2L KO HeLa cells expressing Flag alone or
1279 Flag-UBAP2L (WT or R131-190A) for 60h and synchronized in interphase by DTBR at 12h
1280 (B). Nuclei were stained with DAPI. The percentage of cells with the cytoplasmic granules of
1281 Nups (mAb414) (C) and of FXR1 (D) and irregular nuclei (E) shown in (B) were quantified.
1282 At least 200 cells per condition were analyzed (mean ± SD, ns: not significant, ***P < 0.001,
1283 ****P < 0.0001, two-tailed t-test, N = 3). Scale bars, 5 μm.

1284



1285

1286 **Fig. 7. UBAP2L remodels FXR1-protein assemblies in the cytoplasm and drives**

1287 **localization of FXR1 to the NE.**

1288 **(A)** Representative immunofluorescence images depicting the localization of FXR1 and FMRP
1289 in HeLa cells synchronized in prometaphase using STCL 16h or nocodazole 16h.
1290 Chromosomes were stained with DAPI. Scale bars, 5 μm .

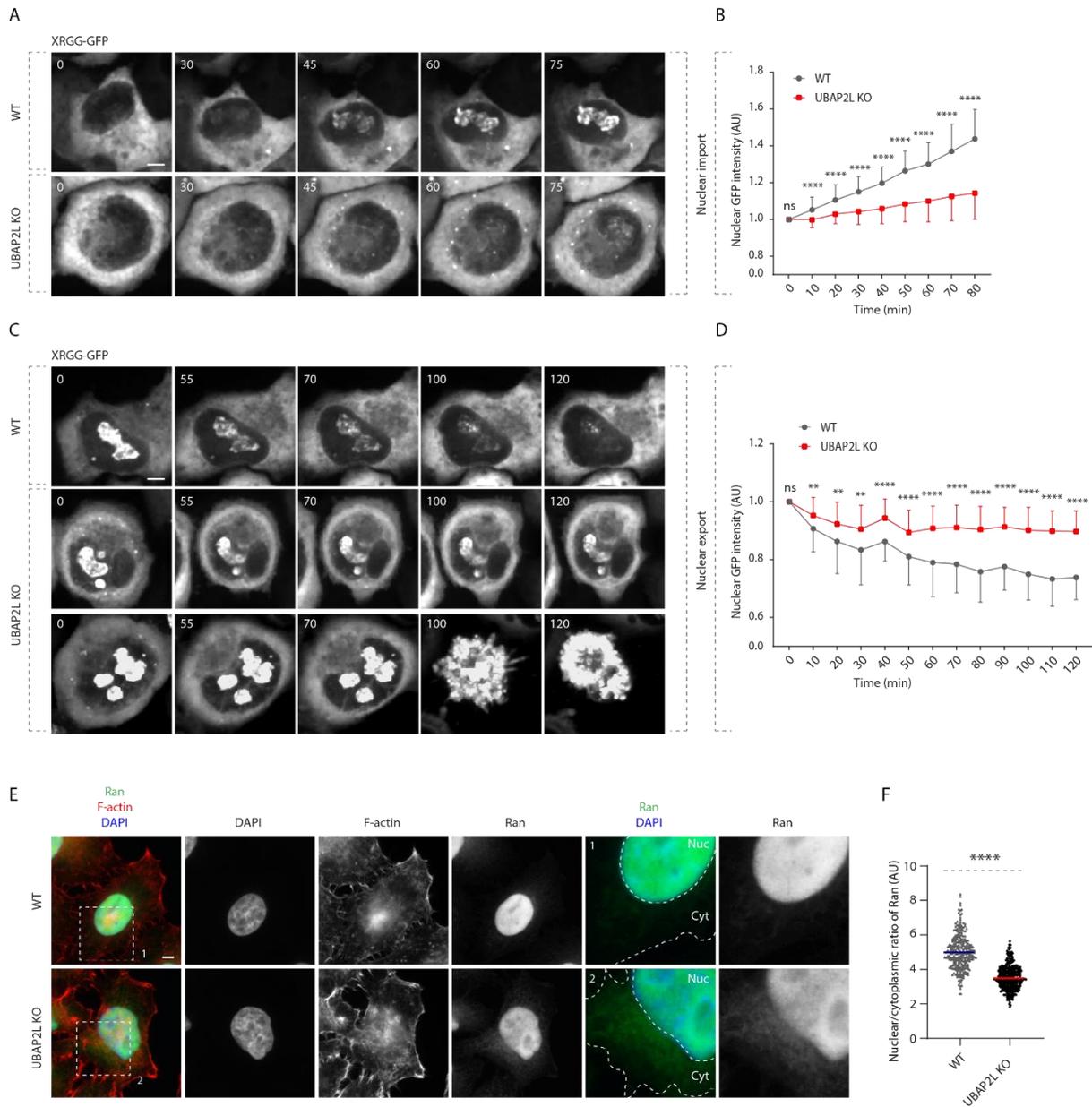
1291 **(B to D)** WT and UBAP2L KO HeLa cells expressing GFP-FXR1 were synchronized by
1292 DTBR and analyzed by live video spinning disk confocal microscopy. The selected
1293 representative frames of the movies are depicted and time is shown in minutes. Time point 0
1294 indicates mitotic entry during prophase **(B)**. GFP-FXR1 granules number (number/cell) shown
1295 in **(B)** at indicated times during mitotic progression were quantified **(C)**. GFP-FXR1 granules
1296 sizes (granule $\geq 0.061 \mu\text{m}^2$) shown in **(B)** at indicated times during mitotic progression were
1297 quantified **(D)**. 16 WT and 11 UBAP2L KO HeLa cells were counted, respectively. Scale bar,
1298 5 μm .

1299 **(E)** Representative immunofluorescence images depicting the cytoplasmic and NE localization
1300 of endogenous UBAP2L and FXR1 in interphase HeLa cells. Nuclei were stained with DAPI.
1301 The magnified framed regions are shown in the corresponding numbered panels. The arrows
1302 indicate co-localization of UBAP2L and FXR1 foci in the cytoplasm. Scale bar, 5 μm .

1303 **(F)** Representative immunofluorescence images depicting the localization of FXR1, Flag alone
1304 and Flag-UBAP2L (WT or R131-190A) in late telophase in HeLa cells. Nuclei were stained
1305 with DAPI. The magnified framed regions are shown in the corresponding numbered panels.
1306 Note that Flag-UBAP2L WT but not Flag alone and Flag-UBAP2L R131-190A, is localized
1307 to FXR1 containing granules in proximity of NE. Scale bar, 5 μm .

1308

Figure 8

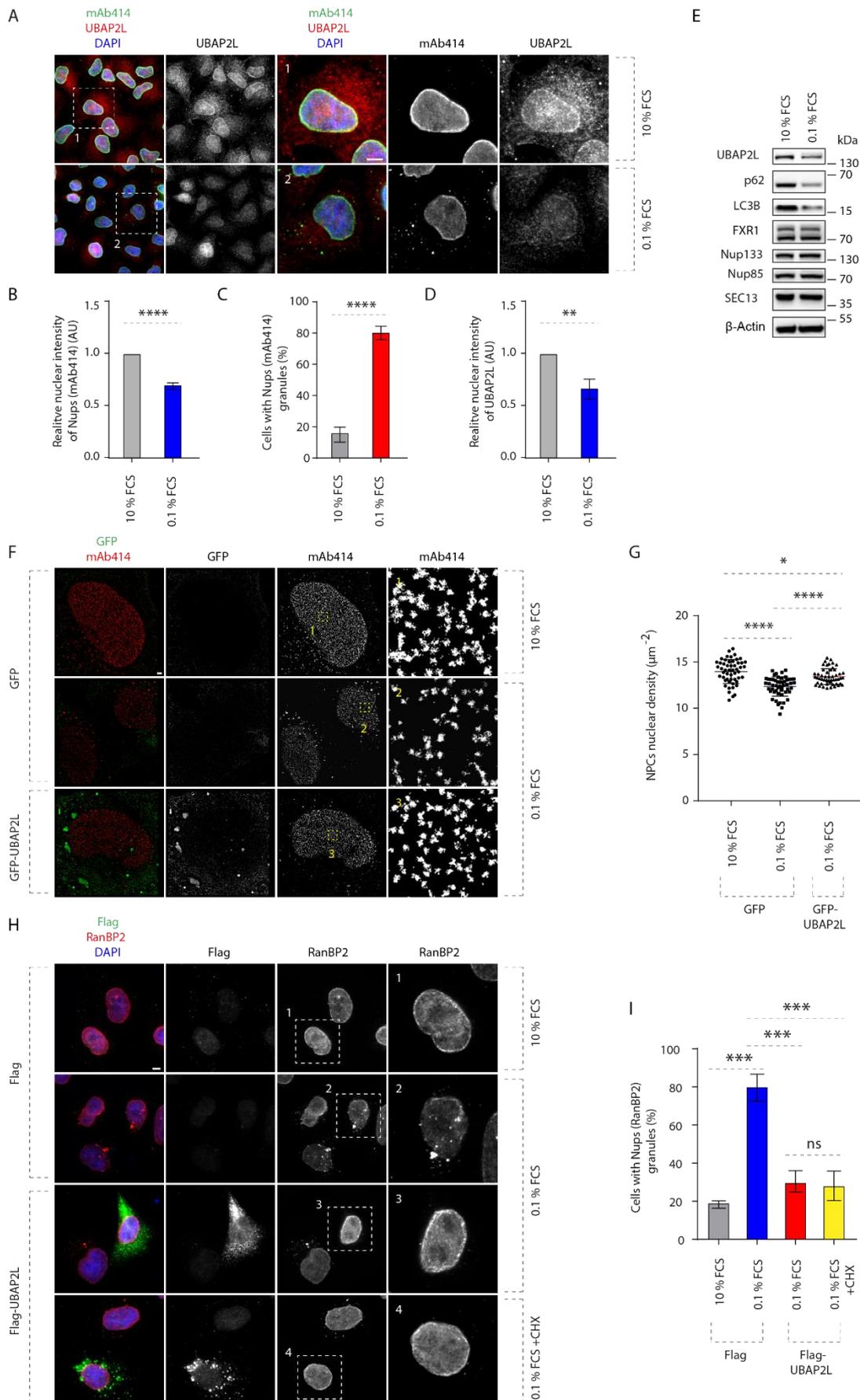


1309

1310 **Fig. 8. UBAP2L regulates nucleocytoplasmic transport.**

1311 (A to D) WT and UBAP2L KO HeLa cells expressing reporter plasmid XRGG-GFP for 30h
 1312 were analyzed by live video spinning disk confocal microscopy. The selected representative
 1313 frames of the movies are depicted and time is shown in minutes. Time point 0 in top panel
 1314 (nuclear import of XRGG-GFP) indicates that dexamethasone (0.01 μ M) was added, while
 1315 time point 0 in bottom panel (nuclear export of XRGG-GFP) indicates that dexamethasone was
 1316 washed out (A, B). The arrowheads indicate dead cells in UBAP2L KO cells. The nuclear

1317 intensity (fold change) of XRGG-GFP (to DNA labeled by SiR-DNA probe) in top panel
1318 (nuclear import) (**C**) and in bottom panel (nuclear export) (**D**) shown in (**A, B**) were quantified.
1319 At least 10 cells per condition were analyzed (mean \pm SD, ns: not significant, **P < 0.01,
1320 ****P < 0.0001, two-tailed t-test, $N = 3$). Scale bars, 5 μ m.
1321 (**E and F**) Representative immunofluorescence images depicting the nuclear (Nuc) and
1322 cytoplasmic (Cyt) localization of Ran in asynchronously proliferating WT and UBAP2L KO
1323 HeLa cells (**E**). Nuclei were stained with DAPI. Actin filaments (also known as F-actin) were
1324 stained with phalloidin. The magnified framed regions are shown in the corresponding
1325 numbered panels. The nuclear (Nuc)-to-cytoplasmic (Cyt) ratio of Ran shown in (**E**) was
1326 quantified (**F**) (mean \pm SD, ****P < 0.0001, two-tailed t-test; counted 277 cells for WT and
1327 306 cells for UBAP2L KO). Scale bars, 5 μ m.
1328

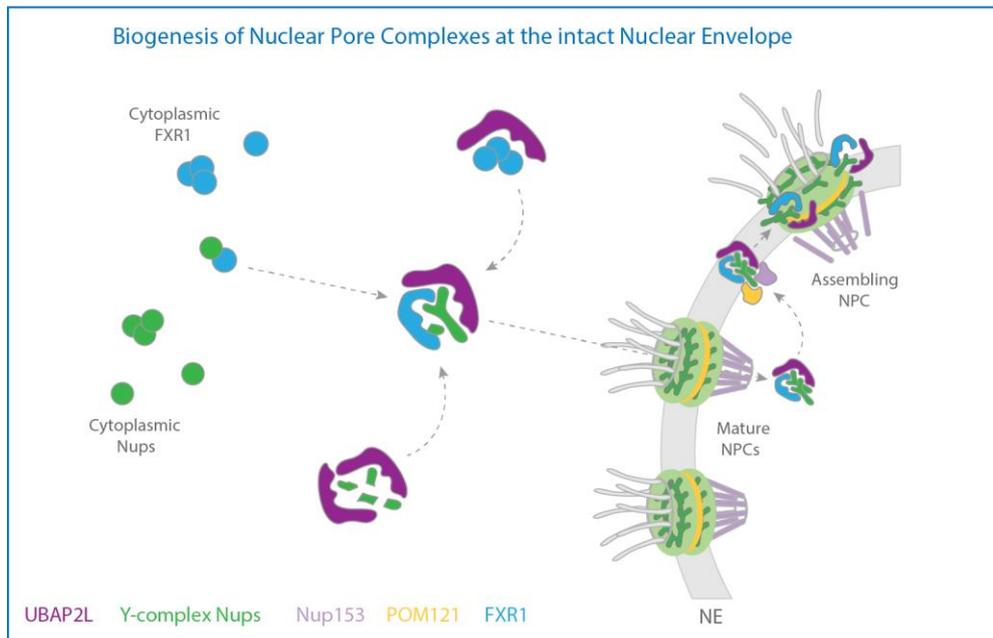


1329

1330 **Fig. 9. UBAP2L ensures NPC biogenesis upon nutrient stress.**

1331 **(A to D)** Representative immunofluorescence images depicting the localization of UBAP2L
1332 and Nups (mAb414) in HeLa cells cultured in the indicated concentrations of serum for 72h
1333 **(A)**. Nuclei were stained with DAPI. Scale bars, 5 μ m. The nuclear intensity of Nups (mAb414)
1334 **(B)** the percentage of cells with the cytoplasmic granules of Nups (mAb414) **(C)** and nuclear
1335 intensity of UBAP2L **(D)** shown in **(A)** were quantified. At least 150 cells per condition were
1336 analyzed (mean \pm SD, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-tailed t-test, $N = 3$).
1337 **(E)** The protein levels of UBAP2L, Nups, FXR1 and other indicated factors in HeLa cells
1338 cultured in the indicated concentrations of serum for 72h were analyzed by Western blot.
1339 **(F and G)** Representative SMLM immunofluorescence images of FG-Nups (mAb414) at the
1340 nuclear surface in interphase HeLa cells expressing GFP alone or GFP-UBAP2L WT for 48h
1341 cultured in the indicated concentrations of serum for 72h **(F)**. The magnified framed regions
1342 are shown in the corresponding numbered panels. The nuclear density of NPCs (mAb414) in
1343 cells shown in **(F)** was quantified **(G)** (mean \pm SD, *P < 0.05, ****P < 0.0001, two-tailed t-
1344 test; counted 51 cells per cell line). Scale bar, 1 μ m.
1345 **(H and I)** Representative immunofluorescence images depicting the localization of RanBP2 in
1346 HeLa cells expressing Flag alone or Flag-UBAP2L for 30h cultured in the indicated
1347 concentrations of serum for 72h **(H)**. Note that Cycloheximide (CHX) was used at a
1348 concentration of 0.1 mg/ml for 8h prior to sample collection. The magnified framed regions
1349 are shown in the corresponding numbered panels. Nuclei were stained with DAPI. The
1350 percentage of cells with the cytoplasmic granules containing RanBP2 shown in **(H)** was
1351 quantified **(I)**. At least 200 cells per condition were analyzed (mean \pm SD, ns: not significant,
1352 ***P < 0.001, two-tailed t-test, $N = 3$). Scale bar, 5 μ m.
1353

Figure 10



1354

1355 **Fig. 10. Hypothetical model how UBAP2L regulates the biogenesis of NPCs at the intact**
1356 **nuclear envelope.**

1357 In the proximity of the nuclear envelope, UBAP2L (dark purple) interacts with cytoplasmic Y-
1358 complex nucleoporins (Nups) (green) and drives the formation of Y-complex. UBAP2L also
1359 interacts with the transporting factor of Nups in the cytoplasm, FXR1 (blue) and restricts its
1360 localization to NE during early G1 phase and ensures its interaction with Nups to fuel assembly
1361 and/or repair of NPCs. UBAP2L mediates the interaction of Y-complex Nups with Nup153
1362 (light purple) and POM121 (yellow), which facilitates the assembly of functional and mature
1363 NPCs during interphase. This NPC biogenesis mechanism integrates the cytoplasmic and the
1364 nuclear NPC assembly signals and ensures efficient nuclear transport, adaptation to nutrient
1365 stress and cellular proliferative capacity, highlighting the importance of NPC homeostasis at
1366 the intact nuclear envelope.

1367

1368 **Supplementary Materials**

1369 This PDF file includes:

1370 Figs. S1 to S12

1371 Tables S1 and S2

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Supplementary Materials for

1389

1390 **UBAP2L drives scaffold assembly of nuclear pore complexes at the intact**

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nuclear envelope

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Yongrong Liao et al.

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Corresponding author: Izabela Sumara, sumara@igbmc.fr

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Figs. S1 to S12

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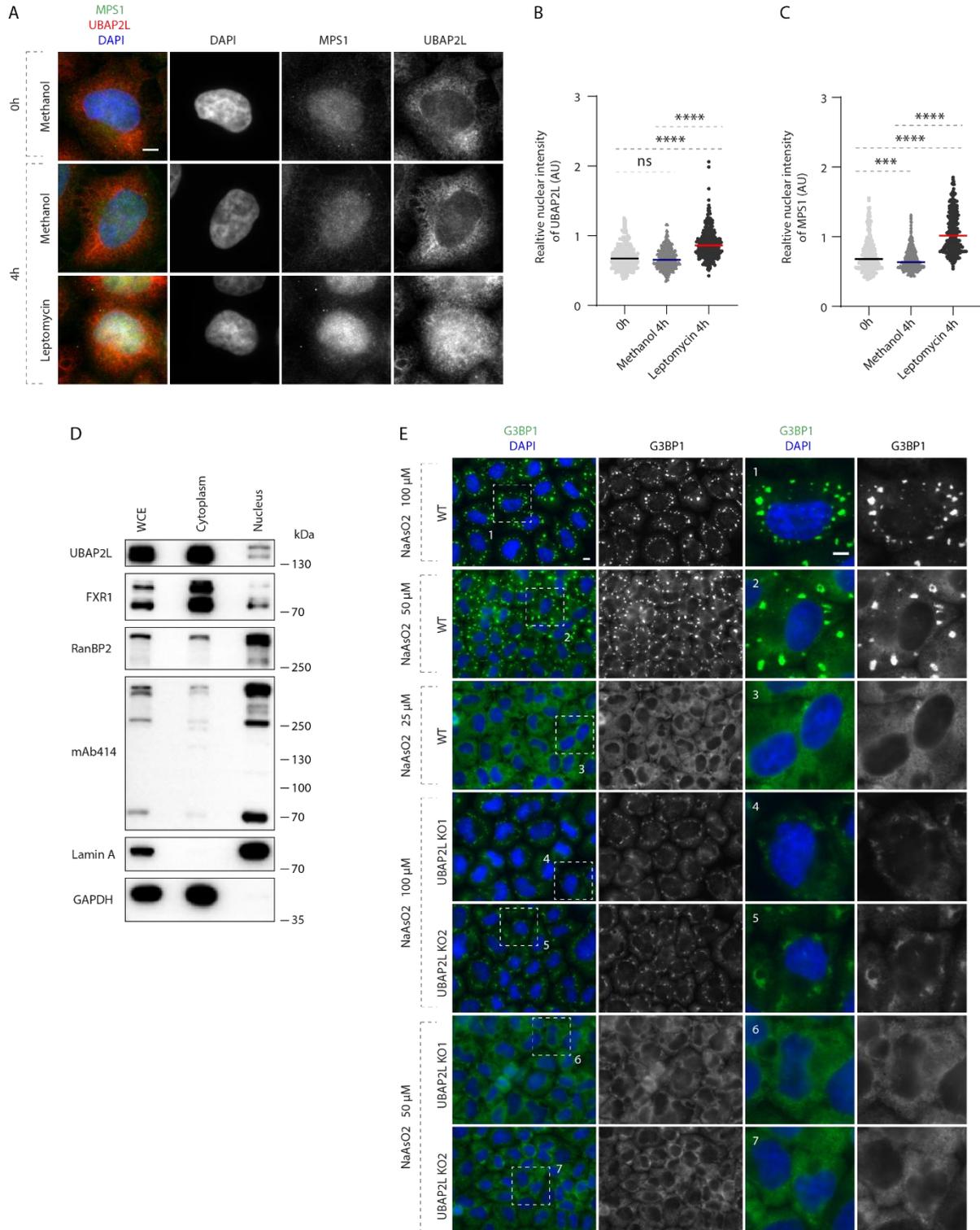
Tables S1 and S2

1399

1400 **Supplemental figures**

1401

Supplementary Figure 1



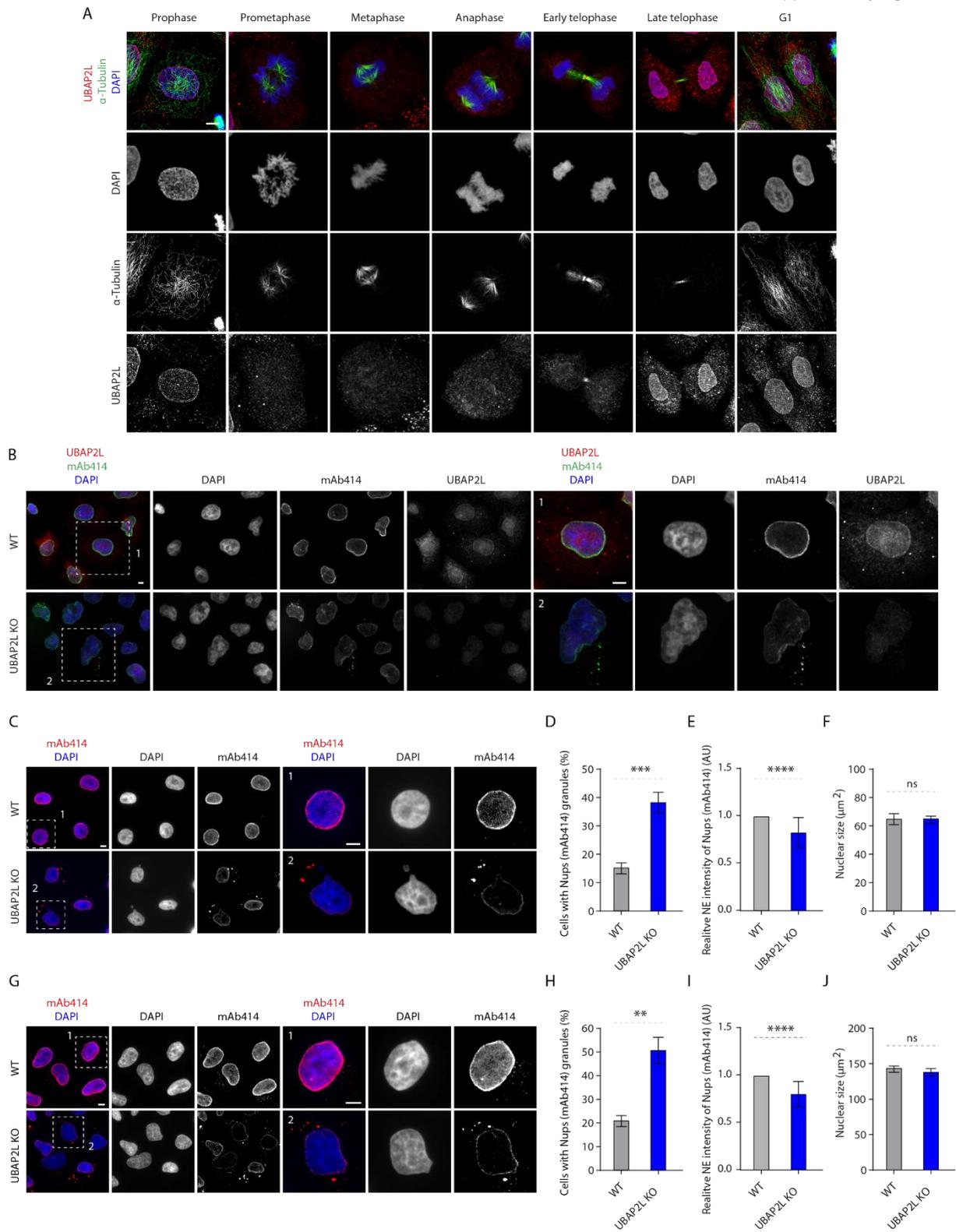
1402

1403 **Fig. S1. UBAP2L shuttles between cytoplasm and nucleus.**

1404 **(A to C)** Representative immunofluorescence images depicting the cytoplasmic and nuclear
1405 localization of UBAP2L and MPS1 (also known as protein kinase TTK) after treatment with
1406 the Leptomycin B (inhibitor of nuclear export factor Exportin 1) (100 ng/ml) for 4h **(A)**. Nuclei
1407 were stained with DAPI. The relative nuclear intensity (AU) of UBAP2L **(B)** and MPS1 **(C)**
1408 shown in **(A)** was quantified. At least 150 cells per condition were analyzed (mean \pm SD, ns:
1409 not significant, ***P < 0.001, ****P < 0.0001; two-tailed t-test, $N = 3$). Scale bar, 5 μ m.
1410 **(D)** Protein levels of UBAP2L, FXR1 and Nups were analyzed by Western blot in the whole
1411 cell extract (WCE) and in nuclear and cytoplasmic fractions of HeLa cells.
1412 **(E)** Representative immunofluorescence images of WT and UBAP2L KO HeLa cells depicting
1413 formation of stress granules (SGs) labelled by G3BP1 at indicated arsenite concentrations. The
1414 magnified framed regions are shown in the corresponding numbered panels. Nuclei were
1415 stained with DAPI. Scale bars, 5 μ m.
1416

1417

Supplementary Figure 2



1418

1419 **Fig. S2. Localization of UBAP2L during cell cycle progression.**

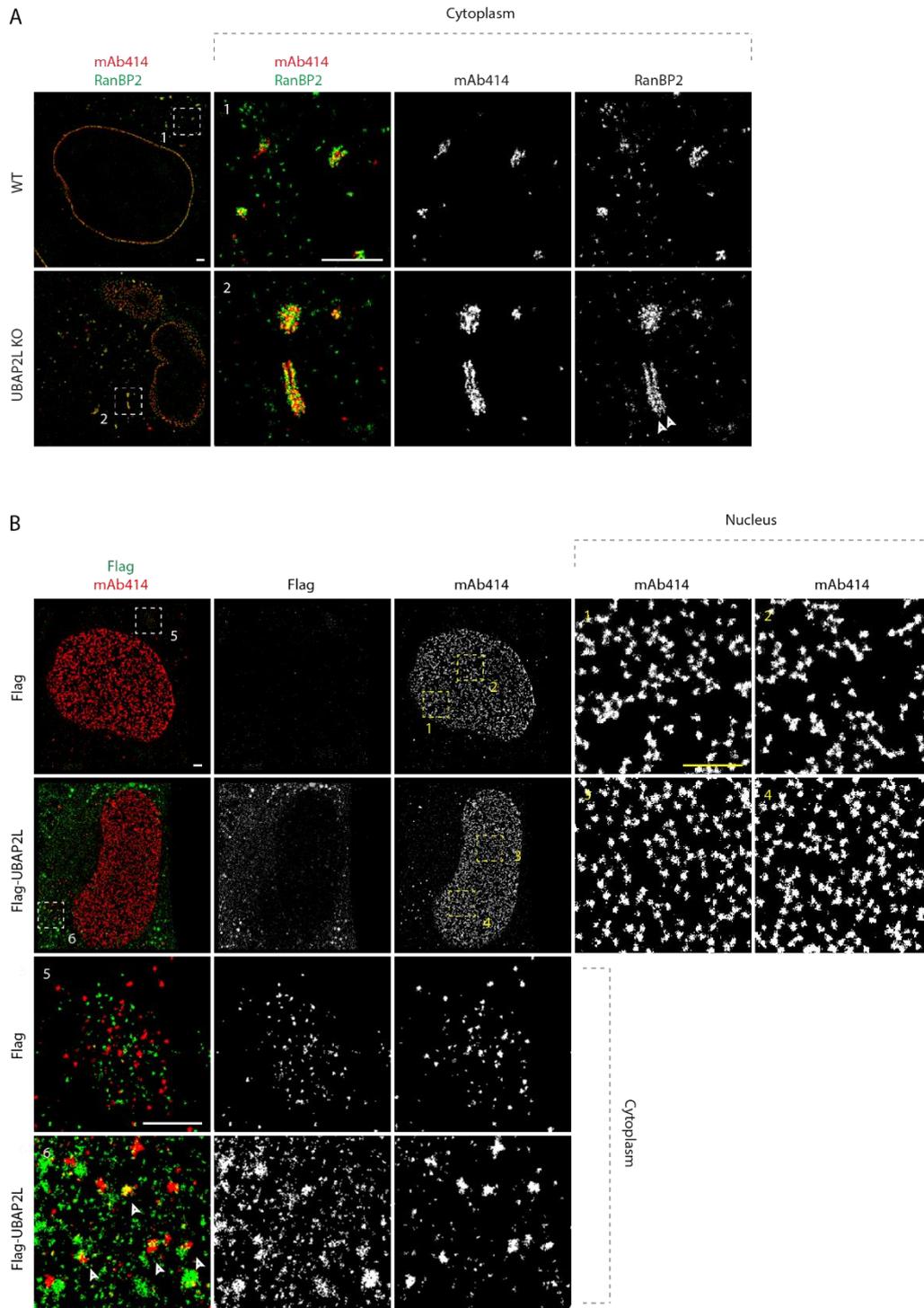
1420 **(A)** Representative immunofluorescence images depicting the localization of UBAP2L in
1421 HeLa cells after chemical pre-extraction of the cytoplasm using 0,01% of Triton X-100 for
1422 90sec in indicated cell cycle stages and visualized by UBAP2L antibody. Nuclei and
1423 chromosomes were stained with DAPI. Scale bar, 5 μ m.

1424 **(B)** Representative immunofluorescence images depicting the localization of nucleoporins
1425 (Nups) and UBAP2L in asynchronously proliferating wild type (WT) and UBAP2L Knock-out
1426 (KO) HeLa cells visualized by mAb414 and UBAP2L antibodies. Nuclei were stained with
1427 DAPI. The magnified framed regions are shown in the corresponding numbered panels. Note
1428 that UBAP2L signal is absent in UBAP2L-deleted cells. Scale bars, 5 μ m.

1429 **(C to F)** Representative immunofluorescence images depicting the localization and NE
1430 intensity of Nups (mAb414) and nuclear size in WT and UBAP2L KO HeLa cells synchronized
1431 in G1 phase by lovastatin (10 μ M) for 16h **(C)**. The magnified framed regions are shown in the
1432 corresponding numbered panels. Scale bars, 5 μ m. The cells with Nups (mAb414) granules
1433 **(D)**, the NE intensity of Nups (mAb414) **(E)** and the nuclear size **(F)** shown in **(C)** were
1434 quantified. At least 150 cells per condition were analyzed (mean \pm SD, ns: not significant, ****P
1435 < 0.001, *****P < 0.0001, two-tailed t-test, $N = 3$).

1436 **(G to J)** Representative immunofluorescence images depicting the localization and NE
1437 intensity of Nups (mAb414) and nuclear size in WT and UBAP2L KO HeLa cells synchronized
1438 in G0/G1 phase by Psoralidin (5 μ M) for 24h **(G)**. The magnified framed regions are shown in
1439 the corresponding numbered panels. Scale bars, 5 μ m. The cells with Nups (mAb414) granules
1440 **(H)**, the NE intensity of Nups (mAb414) **(I)** and the nuclear size **(J)** shown in **(G)** were
1441 quantified. At least 200 cells per condition were analyzed (mean \pm SD, ns: not significant, **P
1442 < 0.01, *****P < 0.0001, two-tailed t-test, $N = 3$).

1443



1444

1445 **Fig. S3. UBAP2L may inhibit formation of cytoplasmic annulate lamellae (AL) or AL-**
1446 **like Nup assemblies.**

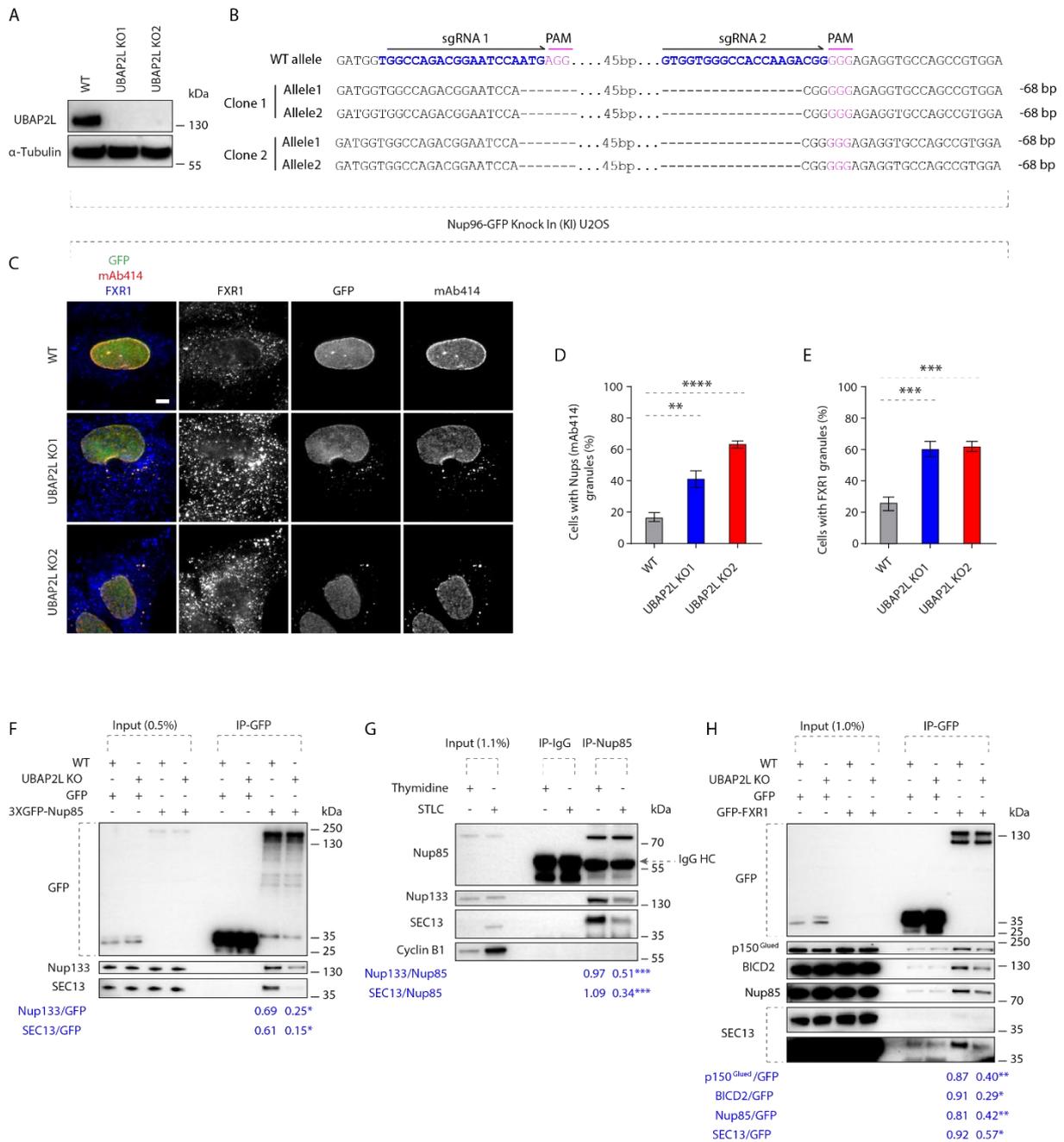
1447 (A) Representative splitSMLM immunofluorescence images depicting the localization of NPC

1448 components corresponding to central channel (FG-Nups labeled by mAb414) and cytoplasmic

1449 filaments (RanBP2) at NE and in the cytoplasm in WT and UBAP2L KO HeLa cells
1450 synchronized in interphase by DTBR at 12h. Note that unlike at the NE where RanBP2 can
1451 localize exclusively to the cytoplasmic side of the NPCs (Fig. 5A), deletion of UBAP2L leads
1452 to the accumulation of the Nup assemblies in the cytoplasm with a symmetric distribution of
1453 RanBP2. The magnified framed regions are shown in the corresponding numbered panels.
1454 Scale bars, 1000 and 300 nm, respectively.

1455 **(B)** Representative SMLM immunofluorescence images of FG-Nups (mAb414) at the nuclear
1456 surface in interphase HeLa cells expressing Flag alone or Flag-UBAP2L for 35h and
1457 synchronized by DTBR at 12h. The magnified framed regions are shown in the corresponding
1458 numbered panels and corresponding quantification is shown in Fig. 5C. The arrowheads
1459 indicate the cytoplasmic colocalization of UBAP2L and FG-Nups. Scale bars, 1000 nm.

1460



1461

1462 **Fig. S4. UBAP2L regulates the interaction between FXR1 and Y-complex Nups.**

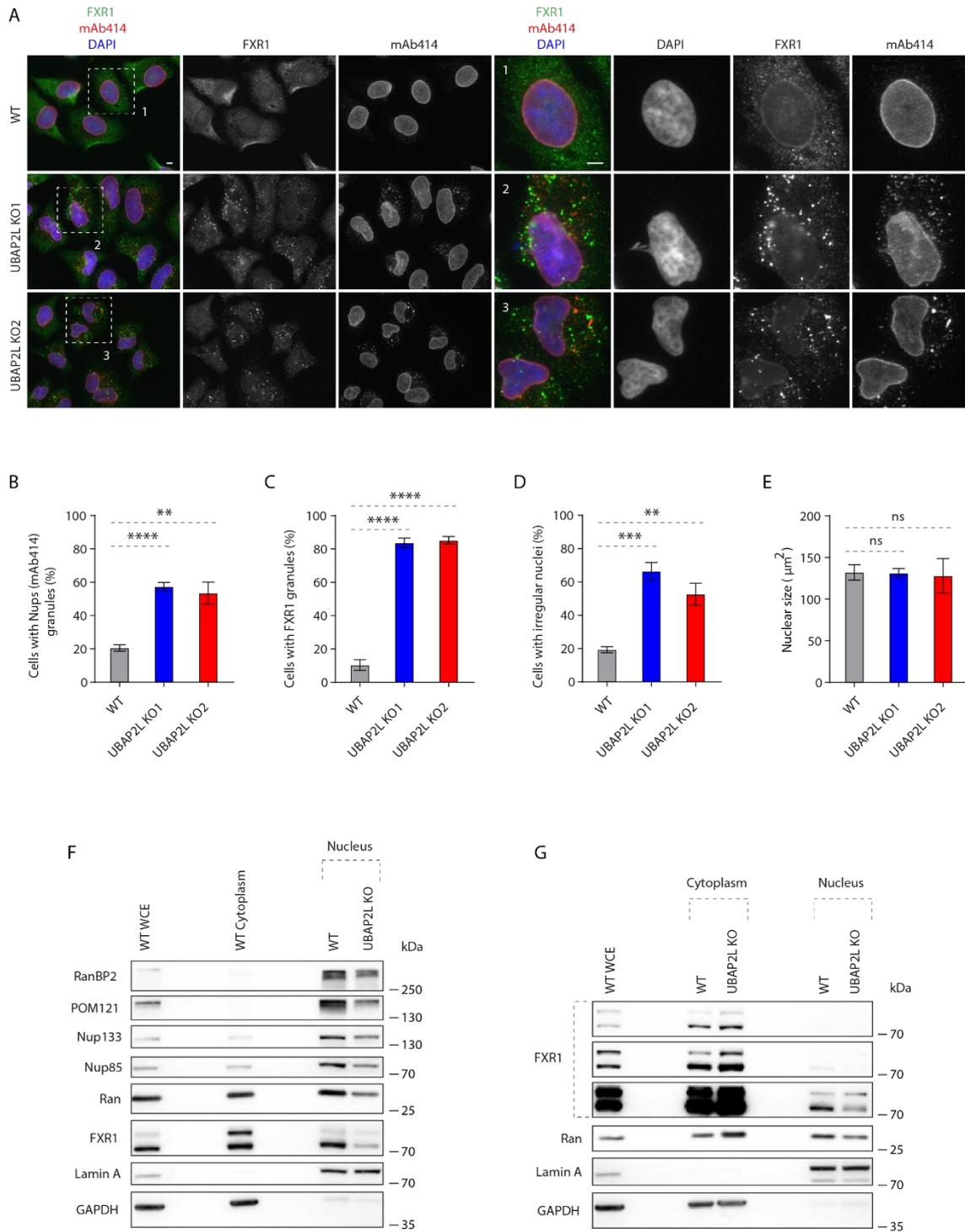
1463 (A and B) Validation of CRISPR/Cas9-mediated UBAP2L KO Nup96-GFP KI U2OS cell
 1464 clones by Western blot (A) and Sanger sequencing (B).

1465 (C to E) Representative immunofluorescence images of the localization of Nups (GFP-Nup96
 1466 and mAb414) and FXR1 in WT and in two UBAP2L KO Nup96-GFP KI U2OS clonal cell
 1467 lines in interphase cells synchronized by DTBR at 15h (C). Nuclei were stained with DAPI.

1468 The percentage of cells with cytoplasmic granules of Nups (mAb414) **(D)** and of FXR1 **(E)**
1469 shown in **(C)** were quantified. At least 200 cells per condition were analyzed (mean \pm SD, **P
1470 < 0.01 , ***P < 0.001 , ****P < 0.0001 , two-tailed t-test, $N = 3$). Scale bar, 5 μ m.
1471 **(F)** Lysates of WT and UBAP2L KO HeLa cells expressing GFP alone or 3XGFP-Nup85 for
1472 27h and synchronized in G1/S phase by Thymidine 16h were immunoprecipitated using
1473 agarose GFP-Trap A beads (GFP-IP), analyzed by Western blot and signal intensities were
1474 quantified (shown a mean value, *P < 0.05 ; $N = 3$).
1475 **(G)** HeLa cells lysates of cells synchronized in interphase (Thymidine 16h) and of cells
1476 synchronized in mitosis (STLC 16h) were immunoprecipitated using Nup85 antibody or IgG,
1477 analyzed by Western blot and signal intensities were quantified (shown a mean value, ****P $<$
1478 0.001; $N = 3$).
1479 **(H)** Lysates of interphase WT and UBAP2L KO HeLa cells expressing GFP alone or GFP-
1480 FXR1 for 27h were immunoprecipitated using agarose GFP-Trap A beads (GFP-IP), analyzed
1481 by Western blot and signal intensities were (shown a mean value, *P < 0.05 , **P < 0.01 ; $N =$
1482 3).
1483

1484

Supplementary Figure 5



1485

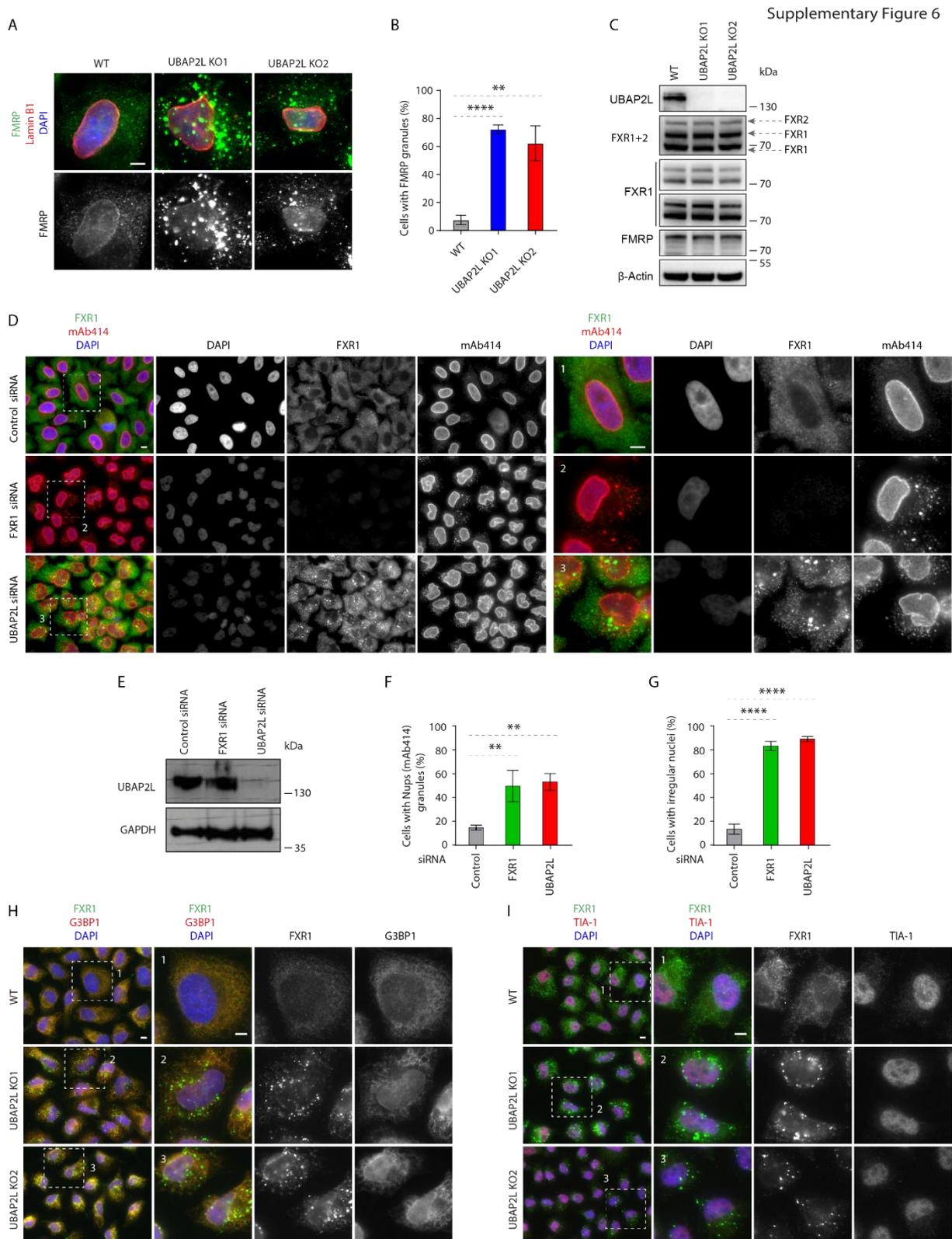
1486 **Fig. S5. UBAP2L regulates localization of Nups and FXR1.**

1487 **(A to E)** Representative immunofluorescence images depicting the nuclear shape and
1488 localization of Nups (mAb414) and FXR1 in WT and UBAP2L KO HeLa cells in interphase
1489 cells synchronized by DTBR at 12h **(A)**. Nuclei were stained with DAPI. The percentage of
1490 cells with cytoplasmic granules of Nups (mAb414) **(B)** and of FXR1 **(C)** and irregular nuclei
1491 **(D)** and the nuclear size **(E)** shown in **(A)** were quantified. At least 250 cells per condition were
1492 analyzed (mean \pm SD, ns, non-significant, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-
1493 tailed t-test, $N = 3$). The magnified framed regions are shown in the corresponding numbered
1494 panels. Scale bars, 5 μ m. The magnified framed regions are shown in the corresponding
1495 numbered panels. Scale bars, 5 μ m.

1496 **(F)** The nuclear and cytoplasmic protein levels of Nups and NPC transport-associated factors
1497 in WT and UBAP2L KO HeLa cells synchronized as in **(A)** were analyzed by Western blot.
1498 WCE indicates whole cell extract.

1499 **(G)** The nuclear and cytoplasmic protein levels of Nups and NPC transport-associated factors
1500 in in asynchronously proliferating WT and UBAP2L KO HeLa cells were analyzed by Western
1501 blot. WCE indicates whole cell extract.

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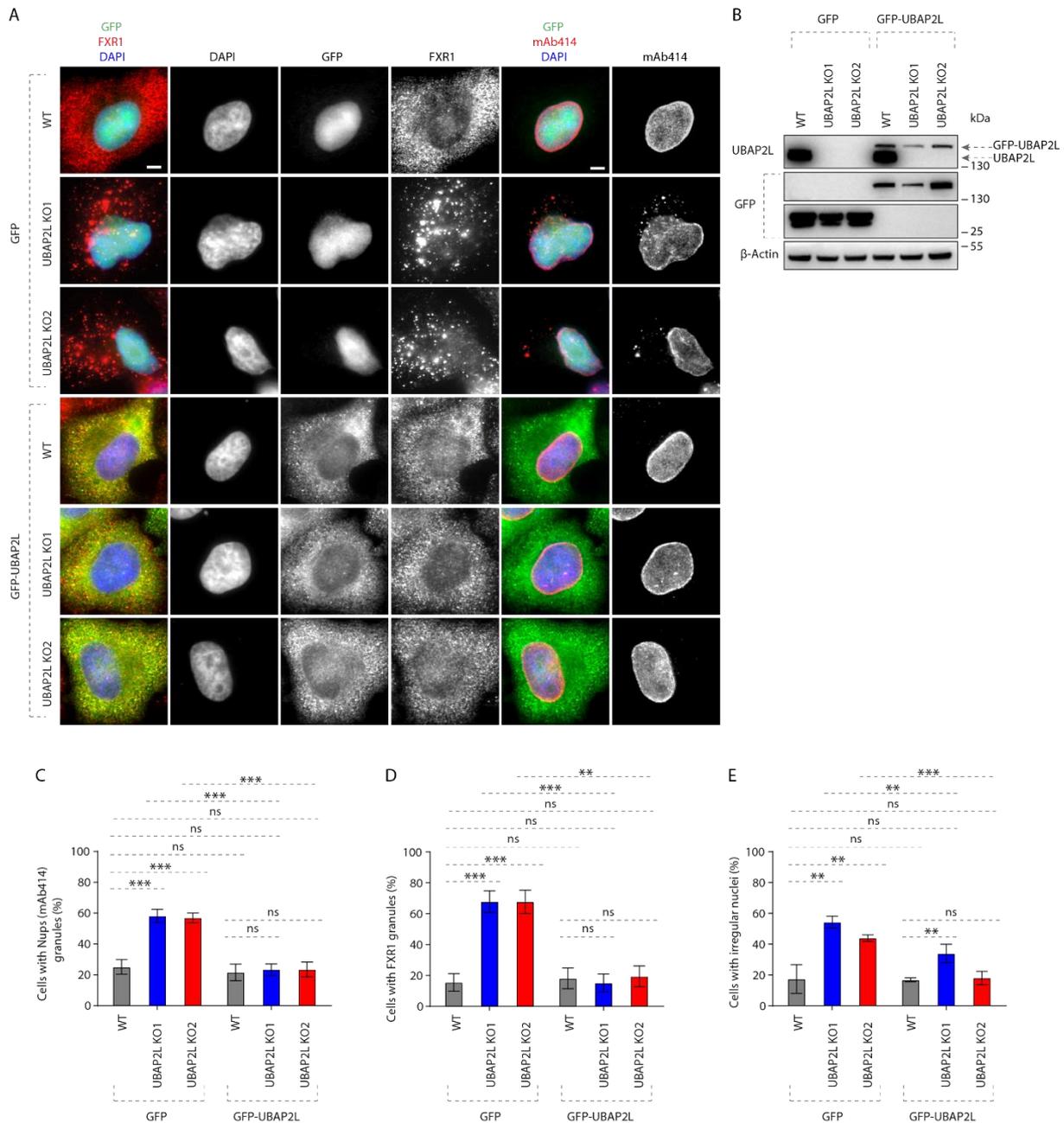
1504 **Fig. S6. UBAP2L regulates FXRP proteins in the cytoplasm.**

1505 (A and B) Representative immunofluorescence images depicting the localization of FMRP and

1506 Lamin B1 in WT and UBAP2L KO HeLa cells synchronized in interphase by DTBR at 12h

1507 (A). Nuclei were stained with DAPI. The percentage of cells with the cytoplasmic granules
1508 containing FMRP shown in (A) was quantified (B). At least 200 cells per condition were
1509 analyzed (mean \pm SD, $**P < 0.01$, $****P < 0.0001$, two-tailed *t*-test, $N = 3$). Scale bar, 5 μ m.
1510 (C) The protein levels of FXRP proteins in WT and UBAP2L KO HeLa cells synchronized in
1511 interphase by DTBR at 12h were analyzed by Western blot.
1512 (D to G) Representative immunofluorescence images depicting localization of FXR1 and Nups
1513 (mAb414) and the nuclear shape in the HeLa cells treated with indicated siRNAs and
1514 synchronized in interphase by DTBR at 12h (D). Nuclei were stained with DAPI. The
1515 magnified framed regions are shown in the corresponding numbered panels. UBAP2L protein
1516 levels in (D) were analyzed by Western blot (E). The percentage of cells with the cytoplasmic
1517 granules of Nups (mAb414) (F) and irregular nuclei (G) shown in (D) were quantified. At least
1518 200 cells per condition were analyzed (mean \pm SD, $**P < 0.01$, $****P < 0.0001$, two-tailed *t*-
1519 test, $N = 3$). Scale bars, 5 μ m.
1520 (H and I) Representative immunofluorescence images of WT and UBAP2L KO HeLa cells
1521 synchronized in interphase by DTBR at 12h under non-stress conditions depicting localization
1522 of FXR1 (H, I), G3BP1 (H) and TIA-1 (I). Nuclei were stained with DAPI. Note that FXR1-
1523 containing granules present in non-stressed UBAP2L KO HeLa cells do not co-localize with
1524 stress granule (SG) components. The magnified framed regions are shown in the corresponding
1525 numbered panels. Scale bars, 5 μ m.
1526

Supplementary Figure 7



1527

1528 **Fig. S7. UBAP2L specifically regulates localization of Nups and FXR1 and nuclear shape.**

1529 (A and B) Representative immunofluorescence images depicting the nuclear shape and

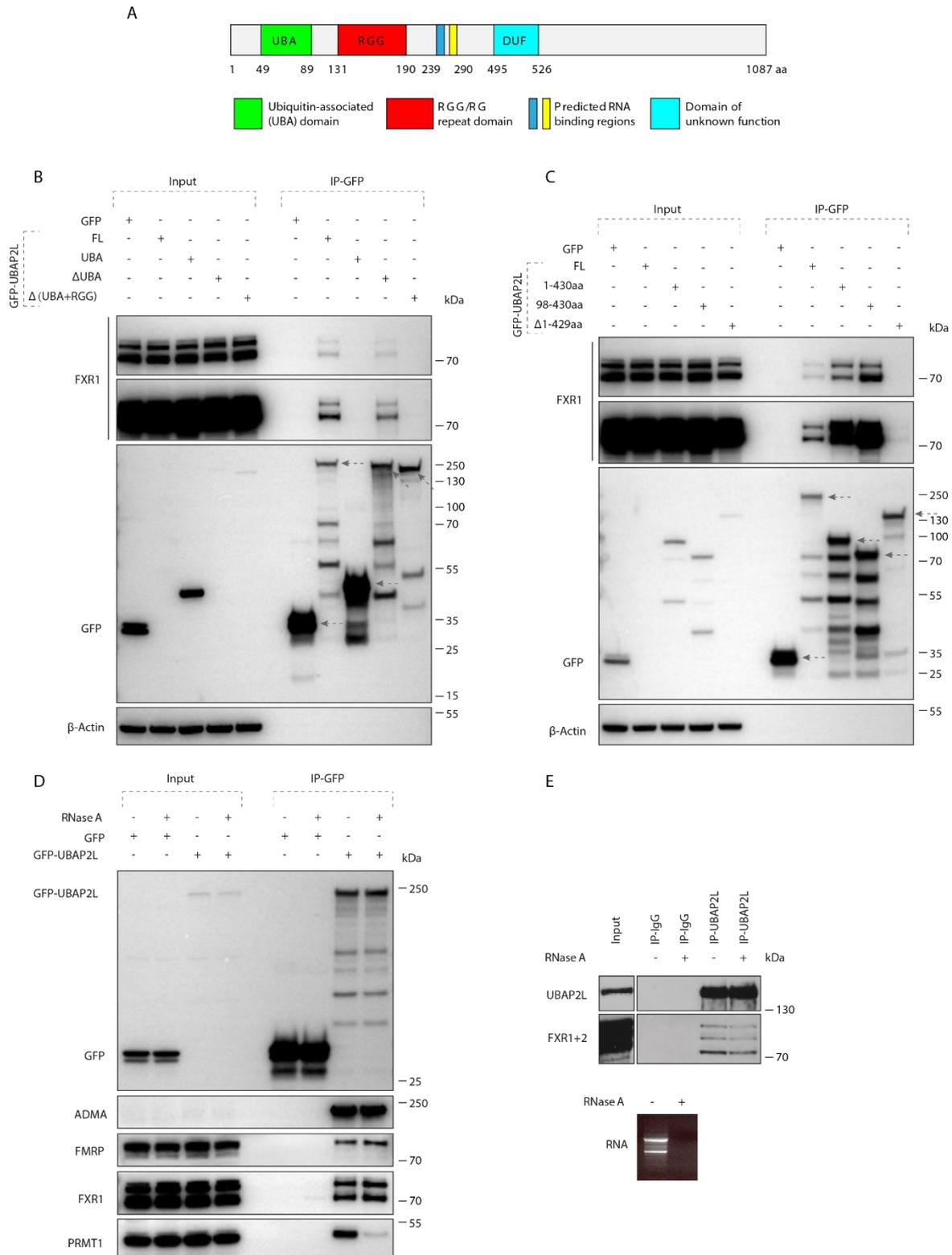
1530 localization of FXR1 and Nups (mAb414) in WT and UBAP2L KO HeLa cells expressing GFP

1531 alone or GFP-UBAP2L for 60h and synchronized in interphase by DTBR at 12h (A). Nuclei

1532 were stained with DAPI. Note that ectopic expression of GFP-UBAP2L but not GFP can rescue

1533 the nuclear and localization phenotypes in both UBAP2L KO HeLa cell lines. Scale bars, 5

1534 μm . The protein levels of endogenous UBAP2L, GFP and GFP-UBAP2L of cells shown in **(A)**
1535 were analyzed by Western blot **(B)**.
1536 **(C to E)** The percentage of cells with the cytoplasmic granules of Nups (mAb414) **(C)** and of
1537 FXR1 **(D)** and irregular nuclei **(E)** shown in **(A)** were quantified. At least 200 cells per
1538 condition were analyzed (mean \pm SD, ns: not significant, **P < 0.01, ***P < 0.001, two-tailed
1539 *t*-test, *N* = 3).
1540



1541

1542 **Fig. S8. Arginines within the RGG domain of UBAP2L are required for the interaction**

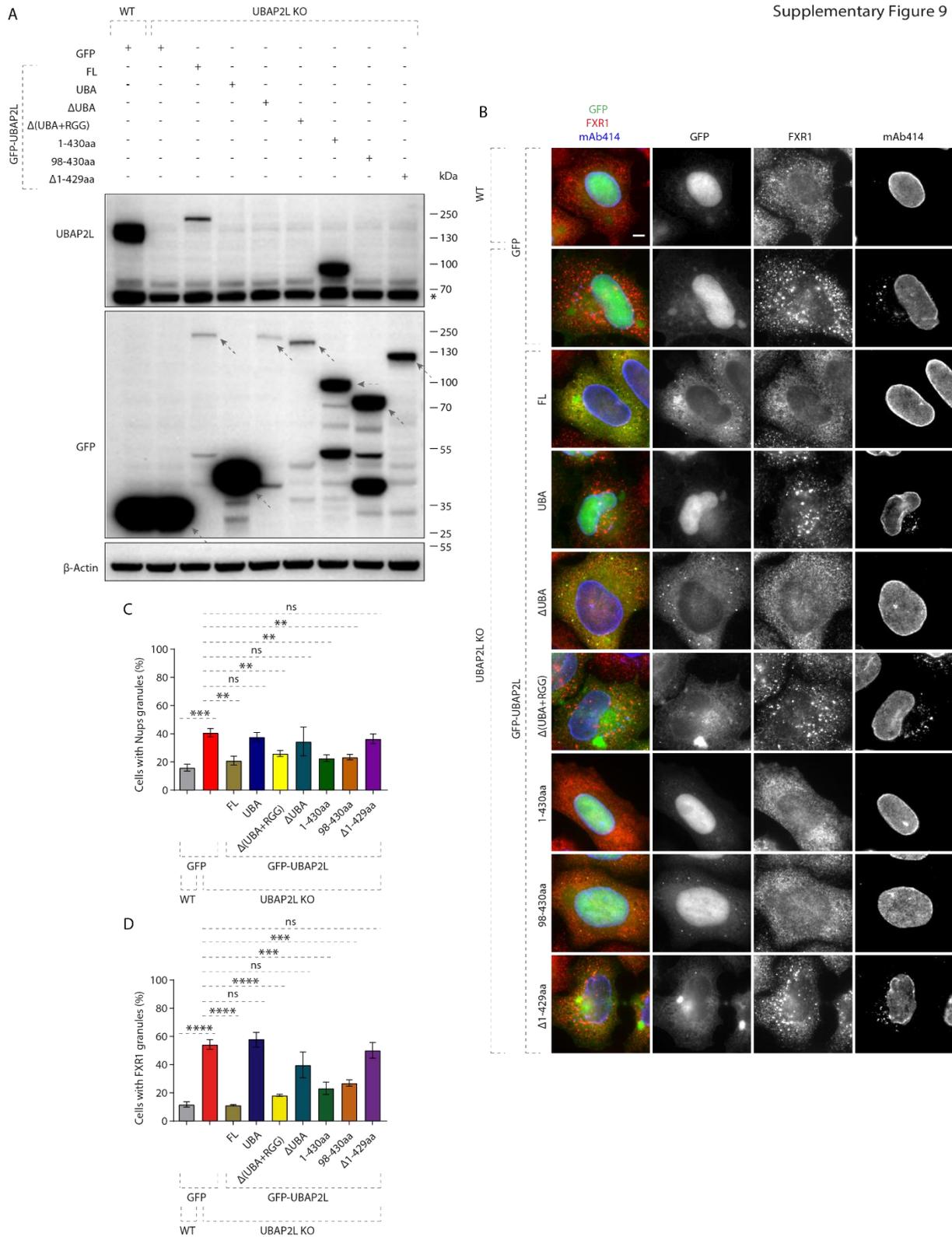
1543 **with FXR1.**

1544 **(A)** Domain organization of UBAP2L depicting UBA domain, RGG/RG repeat domain, two
1545 predicted RNA binding regions and the domain of unknown function (DUF).

1546 **(B and C)** Lysates of HeLa cells expressing GFP alone or GFP-UBAP2L-derived constructs
1547 (full length FL, UBA, Δ UBA or Δ (UBA+RGG) fragments) for 27h were immunoprecipitated
1548 using agarose GFP-Trap A beads (GFP-IP) and analyzed by Western blot **(B)**. Lysates of HeLa
1549 cells expressing GFP alone or several GFP-UBAP2L-derived constructs (FL, 1-430 aa, 98-430
1550 aa or Δ 1-429 aa fragments) for 27h were immunoprecipitated using agarose GFP-Trap A beads
1551 (GFP-IP) and analyzed by Western blot **(C)**. The arrows indicate the bands corresponding to
1552 the expressed GFP proteins while the remaining bands are non-specific.

1553 **(D and E)** Interphase HeLa cells expressing GFP alone or GFP-UBAP2L for 27h and cell
1554 lysates were treated with RNase A, immunoprecipitated using agarose GFP-Trap A beads
1555 (GFP-IP) and analyzed by Western blot. Note that RNase treatment can abolish interaction
1556 with PRMT1 but not with FXRPs **(D)**. Immunoprecipitations from cell lysates of HeLa cells
1557 treated with RNase A using UBAP2L antibody or IgG were analyzed by Western blot.
1558 Efficiency of the RNase treatment was confirmed by imaging of mRNAs by agarose gel
1559 electrophoresis and ethidium bromide staining **(E)**.

1560



1561

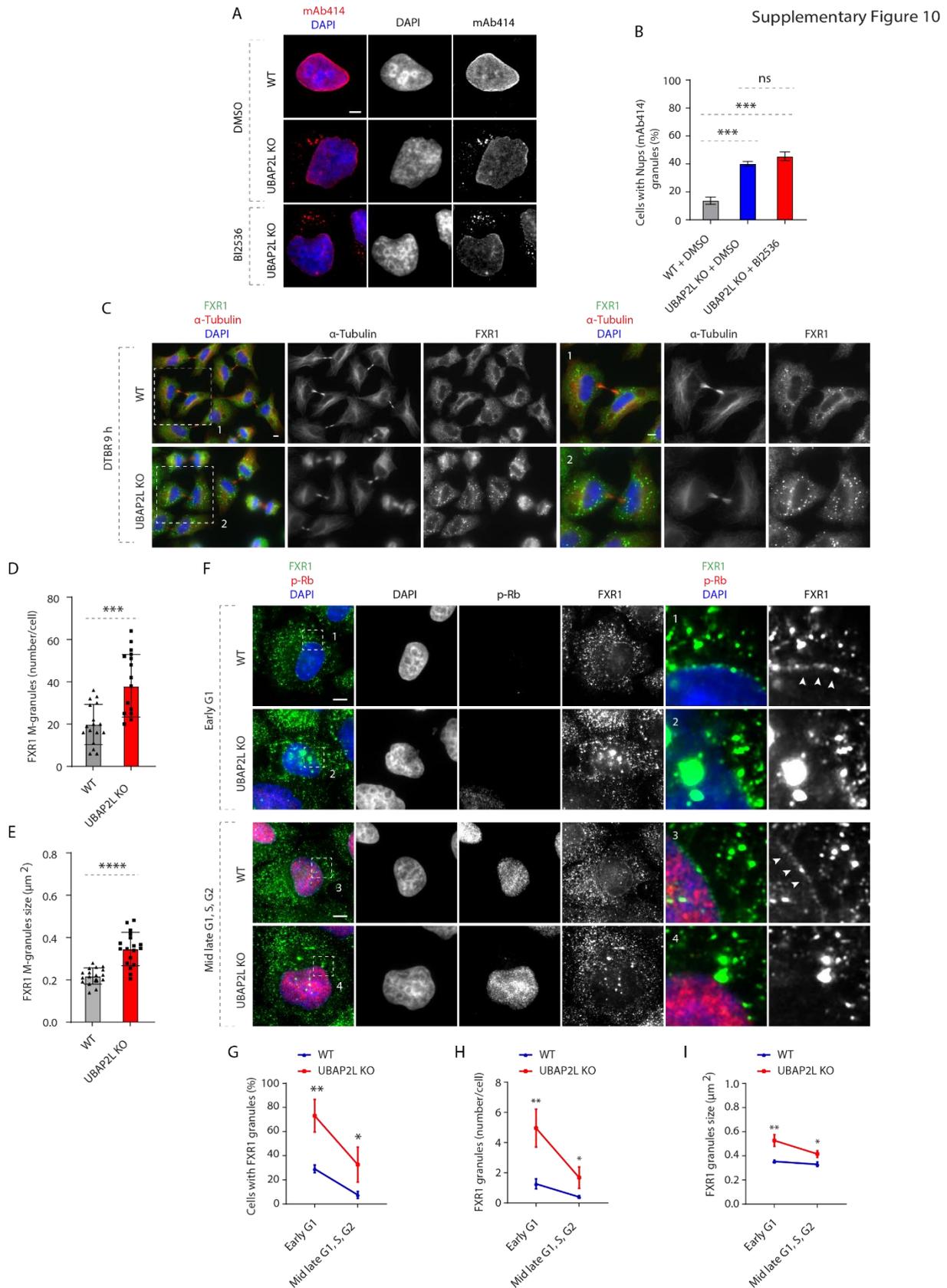
1562 **Fig. S9. 98-430 aa fragment of UBAP2L protein is required for the the function of**

1563 **UBAP2L on Nups and FXR1.**

1564 (A) The protein levels of endogenous UBAP2L, GFP and GFP-UBAP2L-derived versions (FL,
1565 UBA, ΔUBA, Δ(UBA+RGG), 1-430 aa, 98-430 aa or Δ1-429 aa) of cells shown in (B) were
1566 analyzed by Western blot. The arrows indicate the bands corresponding to the expressed GFP
1567 proteins while the remaining faster migrating bands are either non-specific or degradation
1568 products.

1569 (B to D) Representative immunofluorescence images depicting localization of FXR1 and Nups
1570 (mAb414) in WT and UBAP2L KO HeLa cells expressing GFP alone or GFP-UBAP2L-
1571 derived fragments (FL, UBA, ΔUBA, Δ(UBA+RGG), 1-430 aa, 98-430 aa or Δ1-429 aa) for
1572 60h and synchronized in interphase by DTBR at 12h (B). Note that the UBAP2L 98-430 aa
1573 protein fragment containing the RGG domain is required for the function of UBAP2L on Nups.
1574 The percentage of cells with the cytoplasmic granules of Nups (mAb414) (C) and of FXR1 (D)
1575 shown in (B) were quantified. At least 200 cells per condition were analyzed (mean ± SD, ns:
1576 not significant, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-tailed *t*-test, *N* = 3). Scale bar,
1577 5 μm.

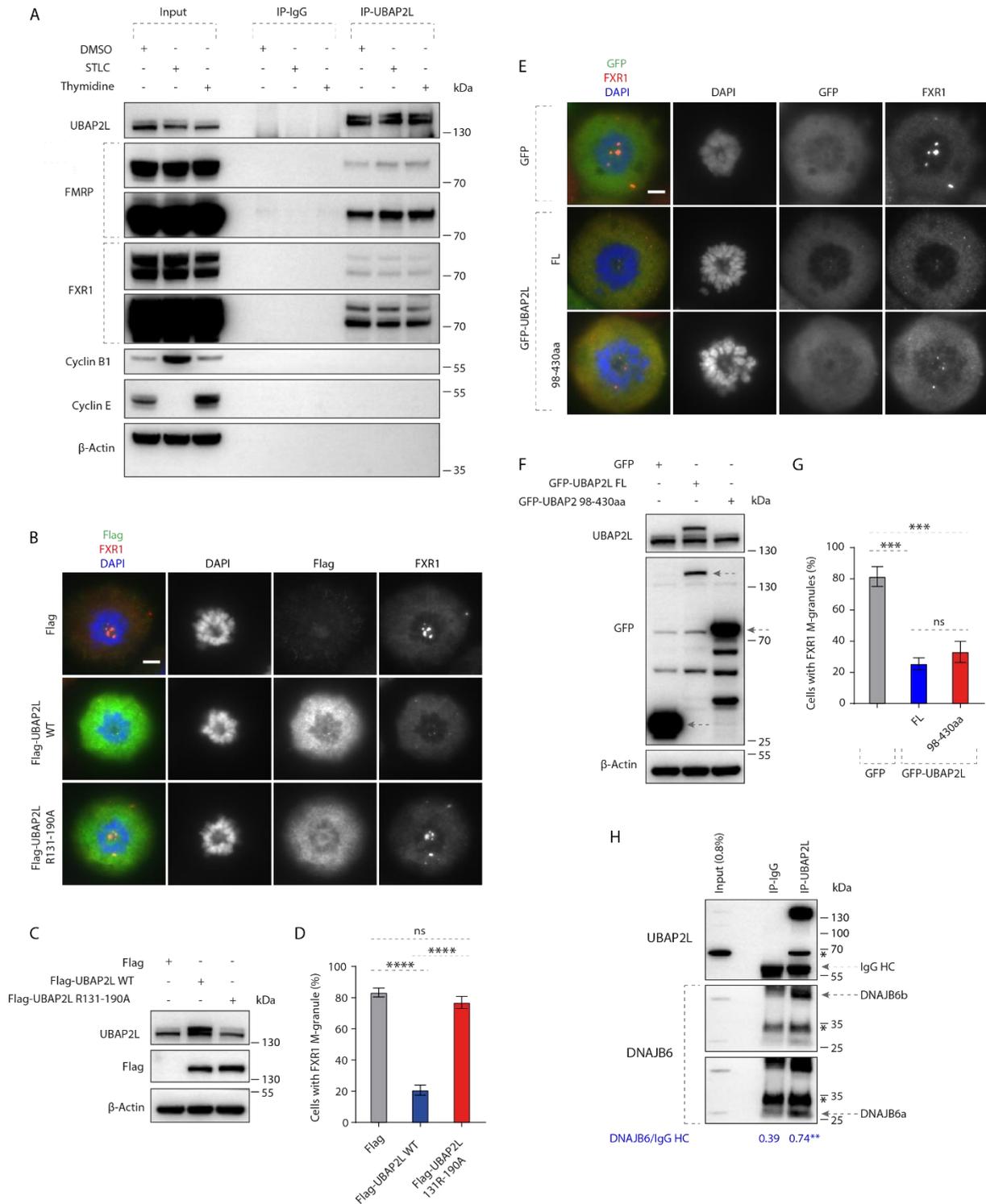
1578



1579

1580 **Fig. S10. UBAP2L drives localization of FXR1 to the NE during early G1.**

1581 **(A and B)** Representative immunofluorescence images depicting the localization of Nups
1582 (mAb414) in WT and UBAP2L KO HeLa cells synchronized in interphase by double
1583 thymidine block and release (DTBR) at 12h **(A)**. PLK1 inhibitor BI 2536 (or solvent control)
1584 was used at a concentration of 100 nM for 45 min prior to sample collection. Nuclei were
1585 stained with DAPI. The percentage of cells with the cytoplasmic granules containing Nups
1586 (mAb414) shown in **(A)** was quantified **(B)**. At least 150 cells per condition were analyzed
1587 (mean \pm SD, ns: not significant, ***P < 0.001, two-tailed t-test, $N = 3$). Scale bar, 5 μ m.
1588 **(C to E)** Representative immunofluorescence images depicting the localization of FXR1 in
1589 WT and UBAP2L KO HeLa cells synchronized by DTBR 9h in late telophase **(C)**. Nuclei were
1590 stained with DAPI. The magnified framed regions are shown in the corresponding numbered
1591 panels. Scale bars, 5 μ m. The number of FXR1 granule per cell (number/cell) **(D)** and the size
1592 of FXR1 granules (granule $\geq 0.105 \mu\text{m}^2$) **(E)** shown in **(C)** were quantified. 17 WT and 18
1593 UBAP2L KO HeLa cells were counted, respectively.
1594 **(F to I)** Representative immunofluorescence images depicting the localization of FXR1 in
1595 different cell cycle stages in asynchronously proliferating WT and UBAP2L KO HeLa cells
1596 **(F)**. p-Rb was used to distinguish between early G1 (p-Rb-negative cells) and mid-late G1, S
1597 and G2 (p-Rb-positive cells) stages. Nuclei were stained with DAPI. The arrowheads indicate
1598 the nuclear envelope (NE) localization of endogenous FXR1. Scale bars, 5 μ m. The percentage
1599 of cells with the cytoplasmic FXR1 granules **(G)**, the number of FXR1 granule per cell
1600 (number/cell) **(H)** and the size of FXR1 granules (granule $\geq 0.2109 \mu\text{m}^2$) **(I)** shown in **(F)** were
1601 quantified. At least 200 cells per condition were analyzed (mean \pm SD, ns: not significant, *P
1602 < 0.05, **P < 0.01, two-tailed t-test, $N = 3$).
1603



1604

1605 **Fig. S11. UBAP2L can dissolve FXR1-containing mitotic foci.**

1606 (A) Immunoprecipitations from HeLa cells lysates of asynchronously proliferating cells

1607 (DMSO 16h), cells synchronized in mitosis (STLC 16h) or in interphase (thymidine 16h) using

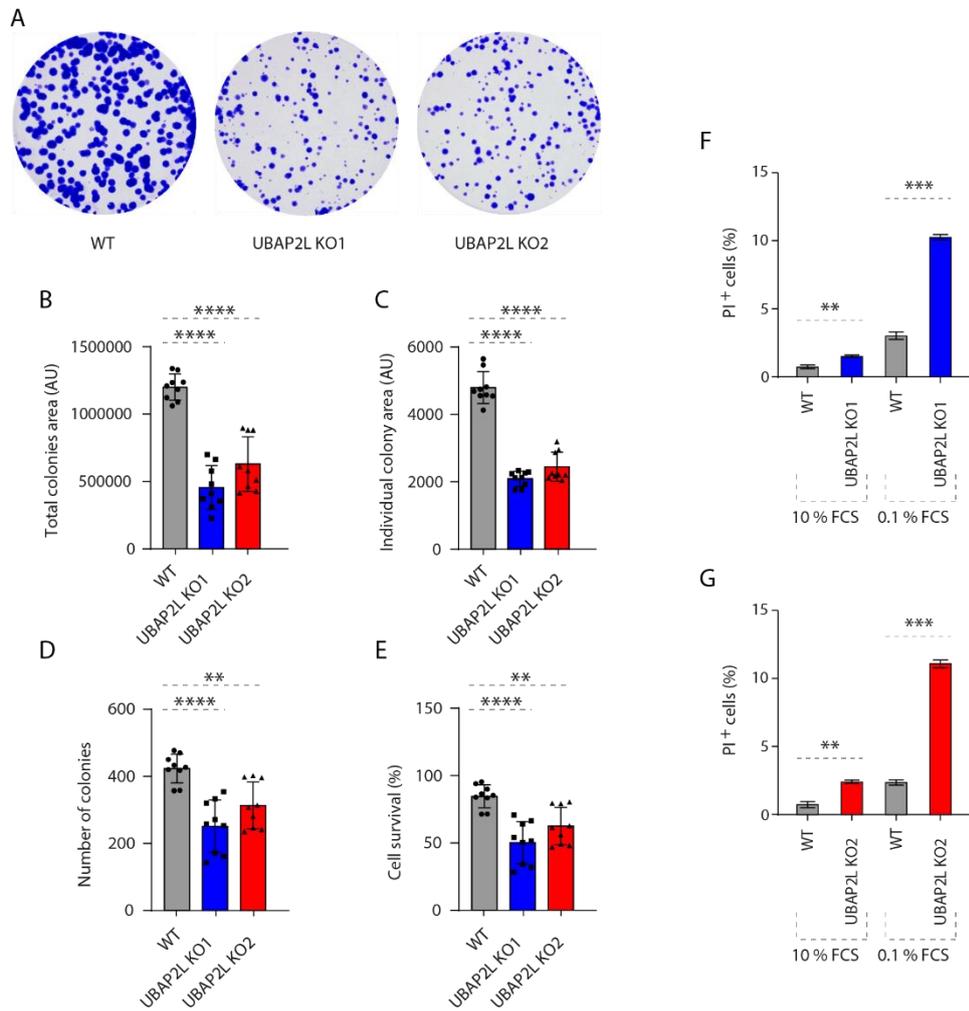
1608 UBAP2L antibody or IgG were analyzed by Western blot.

1609 **(B to D)** HeLa cells expressing Flag, Flag-UBAP2L WT or Flag-UBAP2L R131-190A for 27h
1610 were synchronized in prometaphase using STCL for 16h and representative
1611 immunofluorescence images depicting localization of FXR1 are shown in **(B)**. Chromosomes
1612 were stained with DAPI. The protein levels of Flag-UBAP2L and endogenous UBAP2L in **(B)**
1613 were analyzed by Western blot **(C)**. The percentage of cells with FXR1-granules shown in **(B)**
1614 were quantified **(D)**. At least 200 cells per condition were analyzed (mean \pm SD, ns: not
1615 significant, ****P < 0.0001, two-tailed *t*-test, *N* = 3). Scale bar, 5 μ m.

1616 **(E to G)** Representative immunofluorescence images depicting the localization of FXR1 in
1617 HeLa cells expressing GFP, GFP-UBAP2L FL or GFP-UBAP2L 98-430aa for 27h
1618 synchronized in prometaphase using STCL for 16h **(E)**. Chromosomes were stained with
1619 DAPI. The protein levels of GFP-UBAP2L and endogenous UBAP2L in **(E)** were analyzed by
1620 Western blot **(F)**. The percentage of cells with FXR1-granules shown in **(E)** was quantified
1621 **(G)**. At least 200 cells per condition were analyzed (mean \pm SD, ns: not significant, ***P <
1622 0.001, two-tailed *t*-test, *N* = 3). Scale bar, 5 μ m.

1623 **(H)** HeLa cells lysates were immunoprecipitated from using UBAP2L antibody or IgG,
1624 analyzed by Western blot and signal intensities were quantified (shown a mean value, **P <
1625 0.01; *N* = 3). The arrows indicate the bands corresponding to the IgG heavy chain (HC) and to
1626 DNAJB6a and b, respectively . * Indicates non-specific bands.

1627



1628

1629 **Fig. S12. UBAP2L regulates long-term proliferation capacity of HeLa cells and ensures**
 1630 **survival of HeLa cells upon nutrient stress.**

1631 **(A to E)** Representative images of colony formation assays of WT and UBAP2L KO HeLa
 1632 cells maintained in culture for 7 days **(A)**. Total colony area **(B)**, individual colony area **(C)**,
 1633 average number of colonies **(D)** and cell survival **(E)** of cells shown in **(A)** were quantified
 1634 using the Fiji software (mean \pm SD, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001;
 1635 two-tailed t-test, N = 3).

1636 **(F and G)** The percentage of propidium Iodide (PI)-positive cells in WT and UBAP2L KO
 1637 HeLa cells cultured in the indicated concentrations of serum for 72h were quantified by

1638 fluorescence activated cell sorting (FACS) (mean \pm SD, **P < 0.01, ***P < 0.001, two-tailed
1639 t-test, $N = 3$).

1640 **Supplementary tables**

1641

1642 **Table S1** describes the cloning primers used in the study

1643 **Table S2** describes other reagents and resources including bacterial stains, cell lines,

1644 chemicals, cDNAs and software used in the study

1645

1646 **Table S1 Cloning primers**

Cloning of hUBAP2L in pEGFP-C1	
pEGFP-C1-hUBAP2L-WT-Fwd	ttattaCTCGAGCCATGATGACATCGGTGGG CACTAACCG
pEGFP-C1-hUBAP2L-WT-Rvs	attattGAATTCTCAGTTGGCCCCCAGCTGT AGC
pEGFP-C1-hUBAP2L-UBA (1-97 aa)-Fwd	ttattaCTCGAGCCATGATGACATCGGTGGG CACTAACCG
pEGFP-C1-hUBAP2L-UBA (1-97 aa)-Rvs	tatataGAATTCTcaCTCCCAGGAATGCGTGTC TGG
pEGFP-C1-hUBAP2L-98-430 aa-Fwd	ttattaCTCGAGCCatgGTCGGGAAGAAGAAG GGAGTC
pEGFP-C1-hUBAP2L-98-430 aa-Rvs	tatataGAATTCTcaGGTTGAAGATGGGGTAA AAGCC
pEGFP-C1-hUBAP2L-1-430 aa-Fwd	ttattaCTCGAGCCATGATGACATCGGTGGG CACTAACCG
pEGFP-C1-hUBAP2L-1-430 aa-Rvs	tatataGAATTCTcaGGTTGAAGATGGGGTAA AAGCC
pEGFP-C1-hUBAP2L-ΔUBA-Fwd	ttattaCTCGAGCCatgGTCGGGAAGAAGAAG GGAGTC
pEGFP-C1-hUBAP2L-ΔUBA-Rvs	attattGAATTCTCAGTTGGCCCCCAGCTGT AGC
pEGFP-C1-hUBAP2L-Δ(UBA+RGG)-Fwd	ttattaCTCGAGCCatgGGAACCTTTAACCCAG CTG
pEGFP-C1-hUBAP2L-Δ(UBA+RGG)-Rvs	attattGAATTCTCAGTTGGCCCCCAGCTGT AGC
pEGFP-C1-hUBAP2L-Δ1-429 aa-Fwd	tatattCTCGAGCCatgATGGAGGTGTTCTTC AGGAG
pEGFP-C1-hUBAP2L-Δ1-429 aa-Rvs	attattGAATTCTCAGTTGGCCCCCAGCTGT AGC
Cloning of hFXR1 in pEGFP-C1	
pEGFP-C1-hFXR1-WT-Fwd	ttattaCTCGAGCCATGGCGGAGCTGACGGT GGAGG
pEGFP-C1-hFXR1-WT-Rvs	tattatGAATTCTTATGAAACACCATTCAGGA CTGC
Cloning of hUBAP2L in pcDNA3.1-Flag-N	
pcDNA3.1-Flag-N-hUBAP2L-WT-Fwd	tttGAATTCTTATGACATCGGTGGGCACTA ACC
pcDNA3.1-Flag-N-hUBAP2L-WT-Rvs	tttCTCGAGTCAGTTGGCCCCCAGC
Cloning of hUBAP2L KO sgRNAs in pX330-P2A-EGFP/RFP	

hUBAP2L KO exon5 sgRNA-1-Fwd	caccGTGGCCAGACGGAATCCAATG
hUBAP2L KO exon5 sgRNA-1-Rvs	aaacCATTGGATTCCGTCTGGCCAC
hUBAP2L KO exon5 sgRNA-2-Fwd	caccGGTGGTGGGCCACCAAGACGG
hUBAP2L KO exon5 sgRNA-2-Rvs	aaacCCGTCTTGGTGGCCACCACC
U6-CRISP/Cas9-promoter sequencing primer	gggcctatttcccatgattc
Sequencing of UBAP2L KO clones - cloning of genomic DNA in pUC57	
hUBAP2L KO exon5-DNA sequencing-Fwd	CGAATGCATCTAGATATCGGATCCCTGCT GAGTGGAGAATGGTTA
hUBAP2L KO exon5-DNA sequencing-Rvs	GCCTCTGCAGTCGACGGCCCGGGAGAC TGGTGGCAGTTGGTAG

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1648 **Table S2 Reagents and resources**

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial strains		
DH5alpha Competent <i>E. coli</i>	NEW ENGLAND BioLabs	Cat# C2987I
Chemicals and Peptides		
Thymidine	Sigma-Aldrich	Cat# T1895-5G
Nocodazole	Sigma-Aldrich	Cat# M-1404
Monastrol	Sigma-Aldrich	Cat# M8515
4',6-Diamidino-2-phenylindole dihydrochloride (DAPI)	Sigma-Aldrich	Cat# D8417
MG132	Tocris Bioscience	Cat# 1748
STLC (S-Trityl-L-cysteine)	Enzo Life Sciences	Cat# ALX-105-011-M500
MOWIOL 4-88 Reagent	Millipore	Cat# 475904-M
jetPEI®-DNA transfection reagent	Polyplus transfection	Cat# 101-01N
SiR-DNA	Spirochrom	Cat# SC007
Lipofectamine™ 2000 Transfection Reagent	Invitrogen	Cat# 11668019
Lipofectamine™ RNAiMAX Transfection Reagent	Invitrogen	Cat# 13778150
X-tremeGENE™ 9 DNA Transfection Reagent	Roche	Cat# 6365787001
Dexamethasone	Sigma-Aldrich	Cat# D8833
T4 DNA Ligase	New England Biolabs	Cat# M0202T
Exonuclease III	Takara	Cat# 2170B
Cycloheximide	Sigma-Aldrich	Cat# C4859
Glucose oxidase	Sigma-Aldrich	Cat# G2133
Cyclooctatetraene	Sigma-Aldrich	Cat# 138924
Catalase	Sigma-Aldrich	Cat# C1345
Leptomycin B	Abcam	Cat# ab120501
Lovastatin	Sigma-Aldrich	Cat# 75330-75-5
Psoralidin	Sigma-Aldrich	Cat# 18642-23-4
Benzonase® Nuclease	Millipore	Cat# 70746
RNAse A	Thermo Fisher Scientific	Cat# EN0531
Propidium iodide (PI)	Sigma-Aldrich	Cat# P4170
Phalloidin 488	Thermo Fisher Scientific	Cat# A12379
Cell Lines		
Human: HeLa (Kyoto)	ATCC	Cat# CCL-2
Human: HeLa UBAP2L KO	This study	N/A

Human: U2OS bone osteosarcoma	ATCC	Cat# HTB-96
Human: Nup96-GFP KI U2OS	Arnaud Poterszman (IGBMC)	N/A
Human: Nup96-GFP KI U2OS UBAP2L KO	This study	N/A
Oligonucleotides		
siRNA: Non-targeting siGENOME	Dharmacon	Cat# D-001210-02-05
siRNA: FXR1 individual	Dharmacon	Cat# J-012011-06-0005
siRNA: UBAP2L individual	Dharmacon	Cat# J-021220-09-0002
Primers used for Cloning and Sequencing are described in Table S1	This study	N/A
Recombinant DNA		
pcDNA3.1-Flag-N	This study	N/A
pcDNA3.1-Flag-N-UBAP2L WT	This study	N/A
pcDNA3.1-Flag-UBAP2L R131-190A	(Huang <i>et al</i> , 2020)	N/A
pEGFP-C1	Clontech	Cat# 6084-1
pEGFP-C1-UBAP2L WT	This study	N/A
pEGFP-C1-UBAP2L UBA	This study	N/A
pEGFP-C1-UBAP2L Δ UBA	This study	N/A
pEGFP-C1-UBAP2L 98-430aa	This study	N/A
pEGFP-C1-UBAP2L 1-430aa	This study	N/A
pEGFP-C1-UBAP2L Δ 1-429aa	This study	N/A
pEGFP-C1-UBAP2L Δ (Δ UBA+RGG)	This study	N/A
pEGFP-C1-FXR1 WT	This study	N/A
pEGFP-C1-Nup85 WT	Valérie Doye (Institut Jacques Monod, Paris)	N/A
pXRGG-GFP	(Hamada <i>et al</i> , 2011; Love <i>et al</i> , 1998)	N/A
pUC57	Thermo	Cat# SD0171
pX330-P2A-EGFP	(Zhang <i>et al</i> , 2017)	N/A
pX330-P2A-RFP	(Zhang <i>et al</i> , 2017)	N/A
Software and Algorithms		
CRISPR/Cas9 Guide RNA Design	Benchling	https://www.benchling.com/
Fiji Image Analysis	ImageJ	https://imagej.net/Fiji
Prism	GraphPad	N/A
Illustrator	Adobe	N/A
MATLAB	Mathworks	N/A

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