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3 **APP DEFICIENCY AND HTRA2 MODULATES PrP^c**
4 **PROTEOSTASIS IN HUMAN CANCER CELLS**

5

6 Denis S.F. BIARD^{1,3*}, Rafika JARRAY¹, Nicolas REBERGUE³, François LETEURTRE², and
7 Dulce PAPY-GARCIA³

8

9 ¹ CEA. Institut de Biologie François Jacob (IBFJ), SEPIA, Université Paris-Saclay, F-
10 92265 Fontenay-aux-Roses, France

11 ² CEA. Institut de Biologie François Jacob (IBFJ), SREIT, Laboratoire de Cancérologie
12 Expérimentale Université Paris-Saclay, F-92265 Fontenay-aux-Roses, France,

13 ³ Université Paris Est Créteil (UPEC), Glycobiology, Cell Growth and Tissue Repair
14 Research Unit (Gly-CRRET), F-94010 Creteil, France

15 * Correspondence: denis.biard@cea.fr

16

17 Highlights :

- 18
- 19 • PrP^c /APP imbalance affects cancer cell survival in a 3D tissue like environment.
 - 20 • APP gene silencing enhances cytoplasmic PrP^c protein accumulation
 - 21 • Impaired APP expression induces HTRA2 and destabilizes lipid raft-associated
22 protein partners (e.g. CD24)
 - 23 • HTRA2 up- or down-expression dysregulates PRNP
 - 24 • HTRA2 is a new modulator for PrP^c proteostasis in human cancer cells.

Biard et al. APP deficiency and HTRA2 modulates PrP^c proteostasis in human cancer cells

25 1. Abstract

26 Cellular protein homeostasis (proteostasis) requires an accurate balance between protein
27 biosynthesis, folding, and degradation, and its instability is causally related to human diseases
28 and cancers. Here, we created numerous engineered cancer cell lines targeting APP (amyloid β
29 precursor protein) and/or PRNP (cellular prion) genes and we showed that APP knocking-down
30 impaired PRNP mRNA level and vice versa, suggesting a link between their gene regulation.
31 PRNP^{KD}, APP^{KD} and PRNP^{KD}/APP^{KD} HeLa cells encountered major difficulties to grow in a
32 3D tissue-like environment. Unexpectedly, we found a cytoplasmic accumulation of the PrP^c
33 protein without PRNP gene up regulation, in both APP^{KD} and APP^{KO} HeLa cells. Interestingly,
34 APP and/or PRNP gene ablation enhanced the chaperone/serine protease HTRA2 gene
35 expression, which is a protein processing quality factor involved in Alzheimer's disease.
36 Importantly, HTRA2 gene silencing decreased PRNP mRNA level and lowered PrP^c protein
37 amounts, and conversely, HTRA2 overexpression increased PRNP gene regulation and
38 enhanced membrane-anchored and cytoplasmic PrP^c fractions. PrP^c, APP and HTRA2
39 destabilized membrane-associated CD24 protein, suggesting changes in the lipid raft structure.
40 Our data show for the first time that APP and the dual chaperone/serine protease HTRA2
41 protein could modulate PrP^c proteostasis hampering cancer cell behavior.

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45 **Keywords:** PRNP; PrP^c; APP; HTRA2; CD24; cancer cells; proteostasis; pEBVsiRNA; RNA
46 interference; CRISPR-Cas9;

47

48 2. Introduction

49 The prion protein (PRNP gene) and the amyloid β precursor protein (APP gene) have been
50 extensively studied in neural functions and pathologies. While the APP and the normal cellular
51 PrP (PrP^c) proteins are distributed all over the organism, independently of their roles in
52 neurodegeneration, their relationships remain still elusive in cancer cells. APP has been shown
53 to be upregulated in some cancers such as breast cancers by promoting cell proliferation and
54 motility [1,2]. Besides, an overexpression of PrP^c have been reported in numerous tumors,
55 related to cancer cell migration, invasion and metastasis, and also associated with poor
56 prognosis [3–5]. A putative role of APP and PrP^c protein in cell mobility and cancer spreading
57 could arise from their localization on lipid rafts (*lipid raft-like microdomains*) [6], which allows
58 numerous interactions with components of the extracellular matrix [6,7]. As pivotal scaffold
59 proteins, they also allow the transduction of intracellular signals. For this reason, we sought to
60 bring insights into the implication of both endogenous PrP^c and APP proteins on growth and
61 progression of cancer cells.

62 Originally, the PrP^c protein got considerable attention for its crucial involvement in
63 neurodegenerative pathogenesis (e.g. Creutzfeldt-Jakob disease) since it became the center of
64 the protein conformational hypothesis in neurodegeneration through its conversion from a
65 normal alpha helix structure to an abnormal β -sheet enriched form, called the scrapie PrP^{Sc}
66 form [8]. However, while this conversion occurs in brain, PrP^c is ubiquitously expressed in all
67 known mammals, where it is mostly located at the cell surface tightly tethered to the plasma
68 membrane through a glycosyl-phosphatidylinositol (GPI) anchor [9]. Indeed, at the cell
69 membrane, the localization of PrP^c on lipid rafts allows its interaction with numerous
70 neighbouring proteins including receptors, signalling molecules and components of the
71 extracellular matrix, such as glycosaminoglycans (GAGs) [10,11]. The cellular functions of
72 PrP^c, while still debated, include cell adhesion and mobility, signaling, and differentiation
73 [4,12]. Interestingly, some of these functions have also been conferred to APP, another
74 ubiquitous protein expressed in the central nervous system and in peripheric organs and tissues.
75 The APP protein is a transmembrane protein with a large extracellular ectodomain, an
76 hydrophobic transmembrane domain and an intracellular cytoplasmic tail that contains the
77 regulatory AICD domain (amyloid precursor protein intracellular domain) [13,14], reported to
78 act as a transcriptional enhancer [15]. APP alternative splicing generate ten isoforms from
79 which APP₆₇₅, APP₇₅₁ and APP₇₇₀ are the most abundant ones. APP₆₇₅ is predominantly

80 expressed in neurons while APP₇₅₁ and APP₇₇₀ are found in non-neuronal cells. APP is involved
81 in the pathogenesis of Alzheimer disease (AD) through an abnormal accumulation of the
82 amyloid- β peptide (A β) in several brain regions, including the hippocampus and cortex.

83 Synthesis and maturation of PrP^c and APP seems to be fine-tuned and common components in
84 their interactomes could participate to both processes. Several reports have brought evidences
85 of functional links between PrP^c and APP in AD [11,16–18]. For instance, PrP^c is a key neuronal
86 receptor for β -amyloid oligomers [19] in that the N-terminal PrP^c fragment (amino acids 95 to
87 113) can trap oligomeric A β [20]. PrP^c could also lowered the amyloidogenic processing of
88 APP by inhibiting its β secretase cleavage, reducing A β formation [11]. Interestingly, during
89 this process PrP^c has to be localized in the cholesterol and glycosphingolipid-rich lipid rafts,
90 where it interacts with BACE1 (β -site APP cleaving enzyme) via GAG side chains in the
91 proteoglycans coreceptors. As APP, PrP^c is proteolytically cleaved from the cell surface by the
92 disintegrin and metalloprotease 10 (ADAM10), which reduces cellular binding and toxicity of
93 amyloid- β oligomers [21]. Therefore, both physical and functional relationships between PrP^c
94 and APP can be proposed to contribute not only to the regulation of each of their own normal
95 biological functions but also to the fine-tuned regulation of cell growth in a tissue environment.
96 Because PrP^c and APP (whole protein *vs* A β fragment) are aggregation-prone molecules, it is
97 conceivable that their cytoplasmic accumulation could affect various stress events such as the
98 endoplasmic reticulum unfolded protein response (UPRER) [22,23].

99 Cellular proteostasis requires an accurate balance between protein synthesis, folding,
100 conformational maintenance, and degradation. During evolution, highly sophisticated pathways
101 have been conserved to finely monitor the fate of unfolded or aggregated proteins through the
102 proteasome or autophagy pathways, always in cooperation with an adaptive network of
103 molecular chaperones that recognize misfolded proteins [24,25]. In addition to UPRER-
104 associated quality control (QC) factors, mitochondria possess an array of endogenous
105 chaperones and proteases that maintain mitochondrial protein homeostasis. Among the
106 mitochondrial UPR (UPRmt) players, there is HTRA2, a member of the HTRA family (High-
107 Temperature Requirement serine protease A). HTRA proteins are homo-oligomeric and ATP-
108 independent serine proteases, highly conserved during evolution in different organisms, from
109 bacteria to human [26,27]. In human, heat shock HTRA proteins encompass four paralogs
110 (HTRA1 - 4) displaying serine protease and molecular chaperone activities [28]. They
111 participate to regulation of apoptosis, autophagy, cell signaling, and aging and they are involved
112 in neurodegenerative disorders and mitochondrial dysfunctions, as observed in the Cockayne's

113 syndrome or Alzheimer's disease [27,29–32]. After various stresses, such as heat shock,
114 hypoxia, ischemia/reperfusion injury or heart failure, these proteins can switch their function
115 from chaperones (for refolding proteins) to proteases (for hydrolyzing proteins) to efficiently
116 reduce the amount of damaged or unfolded proteins in order to eliminate or refold them, or to
117 trigger apoptosis [33–35].

118 HTRA2, previously named as OMI, is the member of the HTRA family which is located in the
119 mitochondrial intermembrane space [36]. HTRA2 is released from the mitochondria to the
120 cytoplasm during stress or apoptosis [26]. A strong positive association between HTRA2 and
121 AD has been highlighted in that AD patient brain extracts, which displayed a higher cytosolic
122 distribution of HTRA2 protein and a significant enhanced activity [30]. Furthermore, APP
123 appeared to be directly cleaved by HTRA2 in mitochondria, and APP interacted with HTRA2
124 through its A β region [37]. A fraction of HTRA2 localizes in the cytosolic side of ER
125 membranes tethered with the immature form of APP prompting its degradation [38]. Moreover,
126 aberrant HTRA2 function have been shown to lead to neurodegeneration and parkinsonian
127 phenotypes. Hence, HTRA2 appears to be a regulatory APP-interacting chaperone protease. To
128 our knowledge, nothing is known about the relationship between HTRA2 and PrP^c in cancer
129 cells.

130 Here, based on evidence reported by observational studies with a battery of cancer cell lines
131 and engineered cells, we described for the first time a link between APP, HTRA2 and PrP^c. We
132 show that silencing APP in HeLa cells results in enhanced HTRA2 protein expression and
133 cytoplasmic PrP^c accumulation and that HTRA2 up- and down-regulation affects PRNP gene
134 expression. Hence, impaired APP and HTRA2 can disrupt the PrP^c proteostasis, contributing to
135 hamper cancer cell growth in a tissue-like environment, probably through change in the lipid
136 rafts composition.

137 **3. Results**

138 *3.1 Great diversity of PrP^c protein expression profiles*

139 As APP and PrP^c are both expressed in numerous cells and tissues, we sought to determine
140 whether these two proteins could coordinately work to influence the complex regulation of
141 tumor cell growth. To first investigate this possibility, we cultivated in parallel 33 human tumor
142 cell lines from diverse tissue origins (Table S1). By using three different anti-PrP^c antibodies
143 (SAF32, SHA31 and BAR233), we showed that the protein was expressed in most cell lines,

144 although at different levels and sub-cellular locations (Figure 1a and S1a). It was noticeable
145 that PrP^c levels and localizations were dependent on the cell proliferation state (Figure S2). In
146 epithelial cells, PrP^c was mainly localized on the cytoplasmic membrane, in particular in colon
147 carcinoma cells (e.g. HCT-116, HT-29, SW480, RKO) but also in HeLa cells. In other cell
148 lines, PrP^c staining was diffuse and localized into intense foci, closely to the nuclear
149 compartment (e.g. MIA PaCa-2, H1299, SK-N-AS, and T98G cells). These dense fluorescent
150 foci might correspond to the rough endoplasmic reticulum and the Golgi apparatus, where
151 processed and misprocessed PrP^c protein could accumulate [39]. We also observed significant
152 variations in the location of the PrP^c protein depending on tissue origin (e.g. neuroblastoma vs
153 glioblastoma) (Figure 1a and S1a). For instance, neuroblastoma L-A-N-1 and SH-SY5Y cells
154 exhibited a nearly undetectable PrP^c protein level. On the other side, APP protein was difficult
155 to visualize at the cell membrane possibly because only 10% of nascent APP reaches the
156 cytoplasmic membrane by immunocytochemical (ICC) staining [14]. Accordingly, APP was
157 mostly observed inside the cytoplasmic compartment, likely in the *trans*-Golgi network, where
158 APP is processed, as observed in T98G, MDA-MB-231 and MIA Paca-2 cells (Figure 1d).

159 Western blot analyses confirmed the wide range of PrP^c protein expression with both
160 quantitative and qualitative differences, suggesting complex cell dependent PrP^c production and
161 post-translational maturation, as evidenced by three independent experiments (Figure 1b, 1c
162 and S1b). T98G, H1299, MDA-MB-231, MIA PaCa-2, and SK-MEL-37 cells were among cells
163 that constitutively overexpressed PrP^c.

164 Together, these results suggest that depending on the cell in which it is expressed, PrP^c follows
165 different cellular paths with different post-translational maturation and levels of protein
166 expression.

167 *3.2 High diversity and relationship between of PRNP and APP* 168 *gene expressions*

169 To confirm the variability of PrP^c expression at the transcript level in the different cells and to
170 assess a possible correlation with APP expression, PRNP and APP transcripts were analysed
171 by means of RT qPCR in using RPLPO as an internal control. Figure S3a and b show the
172 PRNP/RPLPO and APP/RPLPO ratios. We observed a wide range of endogenous PRNP gene
173 expression unrelated to tissue of origin (Figure S3a), with elevated PRNP gene expressions in
174 most mutated TP53 cell lines as compared to cells harboring a wild-type TP53 genotype. As
175 expected, among a same tissue origin, different cell lines displayed a wide diversity of PRNP

176 expression, as evidenced in glioblastoma cells (T98G 308%, Malme-3M 158% and U87-MG
177 84%). It was noteworthy that neuroblastoma cells expressed nearly undetectable PRNP mRNA
178 contents. A correlation between PRNP mRNA and PrP^c protein contents was observed in most
179 cases (Figure 1 vs S3a). In parallel, we also detected a large range of APP gene expression
180 levels albeit lower than that observed for PRNP (Figure S3b). Only lymphoma cells failed to
181 express detectable APP mRNA. Interestingly, PRNP and APP genes expression was not
182 restricted to brain-derived cells, since three melanoma cell lines were among cells that
183 constitutively overexpressed PRNP, and the pancreatic MIA PaCa-2 cells were found among
184 APP expressing cells we found.

185 Thus, we calculated the RQ factor to compare the relative expression of PRNP and APP in each
186 cell lines using HeLa cells as calibrator. Hence, we could classify our cell lines in three
187 categories (Figure 1e): (i) cells with high APP gene expression but low PRNP mRNA level
188 (↑APP, ↓ PRNP), (ii) cells displaying an equilibrium between both genes, and (iii) cells
189 exhibiting high PRNP gene expression but reduced APP mRNA content (PRNP ↑, ↓ APP).
190 These data support the existence of a link between PRNP and APP gene expressions.

191 *3.3 Relation between PRNP and APP gene expressions*

192 To study the link between PRNP and APP gene expression we silenced APP, PRNP, and for
193 the first time, PRNP and APP simultaneously in different cell lines by using replicative
194 pEBVsiRNA vectors. Each cell line was transfected with two pEBVsiRNA plasmids per gene
195 in at least three independent experiments. We observed a dramatic decrease in cell survival for
196 most of the PRNP^{KD} and APP^{KD} cell lines (Figure S4a, b & c). Only HeLa^{KD} and RKO^{KD} cells
197 exhibited moderate effects on their growth potential in 2D culture. It was noteworthy that
198 simultaneous PRNP and APP gene silencing failed to trigger synthetic lethality in middle term
199 experiments. However, with longer times in 2D culture, double PRNP^{KD}/APP^{KD} cells displayed
200 a decreased growth potential (Figure S4d). Together, these results show that efficient disruption
201 of PrP^c and/or APP expression strongly affects growth in most PRNP^{KD} and APP^{KD} cancer cells
202 grown in 2D cultures.

203 Thereafter, we focused on interest on MDA-MB-231^{KD}, HCT-116^{KD} and HeLa^{KD} cells because
204 they exhibited different TP53 genotypes. RT qPCR analyses showed that, several weeks after
205 transfection, silencing of the PRNP gene in MDA-MB-231 cells (3% of remaining PRNP
206 mRNA as compared to control) induced a lowered APP transcription (57%; Figure 2a). In
207 opposition, HCT-116 and HeLa PRNP^{KD} cells displayed an increased APP mRNA content

208 (167% and 140% respectively as compared to control) (Figure 2b and c). Interestingly, APP
209 shutting down always decreased PRNP transcription at 64%, 49% and 74% in MDA-MB-231,
210 HCT-116 and HeLa cells respectively (Figure 2a, b and d). These results were consistent with
211 a link between APP and PRNP gene expressions, which was cell dependent. It was noticeable
212 that TP53 deficiency triggered a reduced amount of APP mRNA in MDA-MB-231 cells (65%
213 as compared to control) and an enhanced amount in HCT-116 cells (185%) (Figure 2a and b).
214 Because HCT-116 cells harbor a wild type TP53 genotype, our result supported the idea that
215 activation of wild type TP53 could repress APP gene expression, as previously reported [40].

216 To confirm these findings, reversion experiments were performed in HeLa^{KD} cells. The use of
217 replicative pEBVsiRNA vectors allowed us to reverse the imposed gene silencing after
218 selection pressure withdrawal, which allows a slow disappearance of pEBV plasmids with
219 phenotypic reversion [29,41]. Reversed PRNP^{KD} (REV_P) and APP^{KD} (REV_A) HeLa cells
220 recovered a normal PRNP and APP mRNA content as compared to control (Figure 2d). It was
221 noteworthy that reversed PRNP^{KD}/APP^{KD} (REV_{P/A}) cells maintained very high expression
222 levels of both PRNP and APP genes even 40 days post-reversion but not latter. This observation
223 outlines the existence of an adaptive response that might take place during the gene silencing
224 period possibly to bypass the loss of PrP^c and APP proteins without success, as previously
225 described [29].

226 *3.4 PRNP and APP gene silencing impaired cell growth in 3D* 227 *culture*

228 To explore the cellular phenotypes induced by PRNP and/or APP silencing we focused on
229 HeLa^{KD} cells because they endured the loss of PRNP and/or APP expression very well in 2D
230 culture albeit exhibiting stable gene silencing, as evidenced by RT PCR (Figure 3a). Firstly, we
231 observed that PRNP^{KD} HeLa cells acquired a fibroblastic-like appearance during the transient
232 period following transfection (14 days) as evidenced by quantitative ImageJ analysis using
233 circularity and roudness shape indicators (Figure 3b and S5c). This suggested a regulation of
234 cell adhesion cytoskeleton dynamics, as previously reported [4]. As control, we established
235 CD24 and CD44 deficient HeLa cells (respectively CD24^{KD} and CD44^{KD} cells). The GPI-
236 anchored CD24 protein is a heavily glycosylated protein with diverse physiological functions,
237 including cell adhesion, spreading, and cell-cell and cell-matrix interactions [42]. CD44 is a
238 hyaluronic acid receptor and a stem cell marker, also associated to lipid rafts and tightly related
239 to CD24 during tumor development [43]. Change in cell morphology was also observed in

240 CD24^{KD} cells. After about 20 days, PRNP^{KD} and CD24^{KD} HeLa cells always recovered their
241 epithelial shape, as tumor cell plasticity was transient and reversible [44]. In opposition,
242 CD44^{KD}, APP^{KD} and PRNP^{KD}/APP^{KD} HeLa cells showed no change in cell morphology. Hence,
243 PrP^c and CD24 are likely necessary to maintain a stable morphology

244 Because the PrP^c protein and a fraction of APP are facing the outside of the cell, we carried out
245 three dimensional cell cultures, in either soft agar or collagen type I. Soft agar is a stringent
246 assay for studying malignant potential in anchorage independent condition [45], and collagen
247 matrices mimic a tissue-like environment [46,47]. In soft agar, PRNP^{KD} and APP^{KD} HeLa cells
248 exhibited a tremendous decrease in colony formation as compared to control cells, which was
249 resumed in reverted clones (Figure 3c), suggesting a direct link between growth without
250 anchorage and expression of both PrP^c and APP. Importantly, PrP^c and/or APP deficient cells
251 were unable to colonize collagen type I matrices (Figure 3d). This was confirmed with different
252 HeLa^{KD} clones through independent experiments (Figure S5a). As a positive control, we used
253 TP53^{KD} HeLa cells, which displayed enhanced colony formation capabilities. Together, these
254 results light up that loss of PrP^c and/or APP in HeLa cells strongly disrupt cell growth in 3D
255 culture, especially in collagen type I matrices in which cell proliferation, invasion and survival
256 are dependent of cell-microenvironment interactions.

257 *3.5 Stable PRNP^{KD} and/or APP^{KD} altered membrane-anchored* 258 *CD24 content*

259 Because PrP^c and APP co-localized on lipid raft microdomains on the plasma membrane [48],
260 we assumed that their impaired expression could affected lipid raft stability, hampering 3D cell
261 growth. It has been suggested that GPI anchored proteins, such as CD24, act as gate-keepers
262 for lipid rafts and thus they represent selective markers of raft stability [49,50]. Thus; we
263 quantified the cell membrane-anchored CD24 in living HeLa^{KD} cells by means of flow
264 cytometry. On one hand it was noteworthy to observe that APP^{KD} cells displayed a tremendous
265 decrease in the membrane-anchored CD24, as evidenced by median fluorescence intensities, in
266 either nearly confluent (21% and 53% respectively for clones 1c and 2c, compared to the
267 control) or proliferating cells (clone 1c: 21%, clone 2c: 33%) (Figure 4a and S5d). These APP^{KD}
268 cells also showed a moderate increase in the content of membrane-anchored CD44.
269 Interestingly, these cells failed to change their membrane-associated PrP^c content (Figure 4a
270 and S5d); as confirmed by ICC with short integration times (Figure S6a). On the other hand,
271 PRNP^{KD} and PRNP^{KD}/APP^{KD} cells showed nearly undetectable membrane-anchored PrP^c

272 (between 0% to 4%), with no significant CD24 change in PRNP^{KD} cells, but with an enhanced
273 membrane-associated CD24 in either confluent (clone 1c: 615%, clone 2c: 505%) and
274 proliferating (clone 1c: 190%, clone 2c: 180%) PRNP^{KD}/APP^{KD} cells (Figure 4a and S5d).
275 Thereafter, we analyzed the PrP^c protein content in the KD clones by Western blot analyses
276 (Figure 4b) and ICC stainings (Figure 4c). Strikingly, we observed a cytoplasmic PrP^c
277 accumulation in APP^{KD} HeLa cells without significant enhanced PRNP transcription, as
278 aforementioned. These data lent to support that APP and PRNP/APP deficiencies disrupted
279 lipid rafts in an opposite way.

280 3.6 HTRA2 monitored PRNP gene expression

281 Lipid rafts are master regulators of signal transduction, and changes in their composition could
282 lead to misregulated processes triggering various stresses [7]. Because APP^{KD} HeLa cells
283 accumulated intracellular PrP^c protein without upregulation of the PRNP gene, we thought that
284 a defect of the QC proteins could be involved. We thus focused our interest on HTRA2, known
285 to directly interact with APP to cleave it. We first investigate the correlation between HTRA2
286 and PrP^c proteins in different cancer cell lines. It appeared that cells which overexpressed PrP^c
287 also exhibited enhanced HTRA2 levels, such as MIA Paca2 or SKMEL-37 cells (Figure 5). We
288 then silenced HTRA2 gene in HeLa cells with two pEBVsiHTRA2 plasmids displaying either
289 high (HTRA2^{KD1}) or low (HTRA2^{KD2}) inhibition efficiencies. We found that silencing the
290 HTRA2 gene with the highly efficient plasmid (HTRA2^{KD1} cells) lowered the PRNP gene
291 expression at a greater extent than with its less efficient counterpart (Figure 6b). Moreover, both
292 HTRA2^{KD1} cells and HTRA2 knock out cells (HTRA2^{KO}) displayed a lowered PrP^c protein
293 content as well as a decreased amount of CD44 (Figure 6c and S7). Accordingly, flow
294 cytometry analyses revealed that the membrane-associated PrP^c mean fluorescence intensity
295 fell down to 33% (day 9 after transfection) and 50% (day 11) in HTRA2^{KD1} cells as compared
296 to control (Figure 6d). We also found a tremendous decrease of the GPI-anchored CD24 protein
297 amounts, reaching 17% and 33% respectively at day 9 and 11 after HTRA2 knocking down
298 (Figure 6d). These results highlighted that HTRA2 deficiency could induce major changes in
299 the lipid raft composition and directly affect PrP^c protein content.

300 In parallel, we found that both APP^{KD} and PRNP^{KD} cells displayed an enhanced HTRA2 protein
301 level few days after transfection (day 14; Figure 6a) or in stable clones (Figure 7a and 7b).
302 Moreover, in two independent experiments, we detected moderate up-regulation of the HTRA2
303 gene transcription in PRNP^{KD}, APP^{KD} and PRNP^{KD}/APP^{KD} HeLa clones (Figure 7c and S5e).
304 To confirm results obtained with stable KD HeLa cells, we established knock out HeLa

305 (HeLa^{KO}) cells. As expected, APP^{KO} cells also showed a higher level of the intracellular PrP^c
306 protein compared to control (Figure 7d). Besides, PRNP^{KO}, APP^{KO} and PRNP^{KO}/APP^{KO} cells
307 displayed an enhanced HTRA2 protein content, as found in their KD counterparts. These results
308 further confirm the crosstalk between APP, HTRA2 and PrP^c.

309 Because silenced HTRA2 gene expression lowered the PrP^c content, overexpression
310 experiments were undertaken. Very high expression of ectopic PrP^c in HeLa cells did not affect
311 cell viability (data not shown), nor HTRA2 protein and mRNA levels (Figure 8a and 8b).
312 Consequently, HTRA2 up regulation in APP^{KD} cells was not due to PrP^c protein accumulation.
313 Instead, HTRA2 overexpression triggered intracellular PrP^c accumulation (Figure 7a and 7c)
314 and enhanced PRNP transcription (Figure 8b). Besides, 10 and 14 days after transfection,
315 HTRA2⁺ cells exhibited elevated membrane-associated PrP^c amount with 150% (day 11) or
316 225% (day 14) as compared to control cells carrying an empty vector (Figure 8d and 8e). In
317 PRNP⁺ cells, we also detected an enhanced intracellular CD44 accumulation without change at
318 the membrane level (Figure 8a and 8d). It was also noticeable that PRNP⁺, HTRA2⁺ and
319 HTRA3⁺ cells displayed an enhanced APP transcription (Figure 8b). Furthermore, HTRA2⁺
320 cells showed an increased amount of cytoplasmic (Figure 8a) and membrane-bound CD44
321 (213% at day 11 and 338% at day 14) (Figure 8d and e) and a lowered membrane-anchored
322 CD24 (22% at day 11 and 8% at day 14). Again, this suggested that altered HTRA2 expression
323 induces major changes in lipid raft composition. Besides, it was noteworthy that in contrast to
324 PrP^c in PRNP⁺ cells, most of ectopic CD24 protein in CD24⁺ cells localized at the plasma
325 membrane (4674%), lowering membrane-anchored PrP^c (59%) and CD44 (65%) protein levels
326 (Figure 8d). It can then be assumed that overexpressed PrP^c exhibited both preferential
327 intracellular accumulation and extracellular export in HeLa cells. These data reinforced the idea
328 of a tight relationship between HTRA2, PRNP and APP.

329 **4. Discussion**

330 For a long time, PrP^c and APP proteins have been studied for their involvement in
331 neurodegenerative diseases through a common molecular mechanism characterized by the
332 aggregation of specific host proteins, which became abnormal and toxic. This is the prion-like
333 concept, which underlies the propagation of toxic protein aggregates from cell to cell [51,52].
334 This concept is currently considered in the propagation of several protein aggregates including
335 the prion protein (PrP) in prion diseases, α -synuclein, β -amyloid and/or MAPT
336 (microtubule-associated protein tau) in other neurodegenerative diseases such as Parkinson's

337 and Alzheimer's diseases [53]. However, a general consensus on the exact biological role of
338 PrP^c and APP outside the nervous system remains debated, in particular in cancer cells. Herein,
339 using various tumor cell lines and engineered MBA-MB-231, HCT-116 and HeLa cells, we
340 highlighted a link between endogenous PRNP and APP gene expressions.

341 Under physiological conditions, constitutive PrP^c protein is subjected to sequential and complex
342 proteolytic processing generating several polypeptides and harboring either protective or
343 deleterious roles [54,55]. Accordingly, our results on PrP^c immunoblotting show profiles in
344 agreement with the complexity of newly synthesized PrP^c fragments, as previously described
345 [56]. The PrP^c expression and protein localization variability in each cell line strongly suggests
346 differences in the protein maturation process, which can greatly influence the biological
347 functions of PrP^c, especially when these polypeptides can move to different subcellular
348 locations. PrP^c and APP proteins are mainly localized in outer plasma membrane lipid rafts
349 (*lipid raft-like microdomains*), which play an essential role in tumor cell adhesion and migration
350 [7]. However, the GPI-anchored PrP^c protein is a mobile element, shifting from different
351 membrane domains to internal organelles. Hence, PrP^c constantly undergoes endocytic cycles
352 between the plasma membrane, the trans-Golgi compartment, and ultimately the ER to be
353 recycled back to the plasma membrane [57,58]. Thus, the internalized PrP^c can reach membrane
354 structures close to the nucleus [59]. This was observed in various cell lines, such as
355 MIA PaCa-2, H1299, SK-N-AS, and T98G, where the endogenous PrP^c was mainly localized
356 closely to the nuclear compartment, presumably in the rough ER and the Golgi apparatus, where
357 processed and misprocessed PrP^c protein could accumulate [39]. This retrograde transport may
358 lead to an accumulation of PrP^c in the ER. Indeed, a member of the Ras superfamily of small
359 GTPase (e.g. Rab6a) stimulates this PrP delocalization and increases PrP^{Res} production in prion-
360 infected neuroblastoma N2a cells [60]. Hence, we assume that the loss of membrane-associated
361 APP in the APP^{KD} and APP^{KO} cells might enhance this process. Because the cellular PrP^c is
362 handled by the ER-associated degradation (ERAD)-proteasome pathway [61], it is conceivable
363 to expect UPRER and UPRmt protein recruitment. For the first time, it was here shown that
364 HTRA2, a UPRmt factor, could participate to the global UPR response leading to intracellular
365 PrP^c protein accumulation, highlighting a role of HTRA2 in PrP^c proteostasis.

366 The involvement of HTRA2 in the PrP^c proteostasis was observed in cells exhibited an impaired
367 APP expression, such as APP^{KD} and APP^{KO} HeLa cells, leading to the intracellular
368 accumulation of PrP^c in its trafficking, probably through a retrograde mechanism [60,61]. The
369 interplay between HTRA2 and PrP^c was confirmed in HTRA2^{KD} and HTRA2^{KO} HeLa cells,

370 where HTRA2 deficiency reduced both PRNP transcription and PrP^c protein contents. In
371 opposition, HTRA2 overexpression enhanced PRNP mRNA levels and PrP^c protein amounts.
372 We also showed that, among our set of tumor cells, MIA Paca2 and SKMEL-37 cells exhibited
373 a very high endogenous PrP^c protein content as well as an elevated HTRA2 protein level.

374 The member of the HTRA family are stress sensors and regulators of UPR to manage the fate
375 of misfolded, aggregated, and mislocated proteins. Especially, HTRA1 can cleave proteins
376 associated with AD, such as tau (MAPT), APP, and APOE4 [62–64]. In this way, the
377 mitochondrial HTRA2 member participates to the QC pathway as an adaptive dual chaperone
378 protease [35]. Herein, we had postulated that HTRA2 acted in coordination with UPRER factors
379 to manage PrP^c, leading to the accumulation of aggregation-prone PrP molecules in the cytosol,
380 as observed after proteasome dysfunction and ER stress [22,23,61]. Indeed, different studies
381 have already shown that some UPRER factors, such as ATF6A, spliced XBP1 (sXBP1) or
382 CREB3/Luman, were able to enhance PRNP gene expression, suggesting a protective role of
383 PrP^c in the global response to misfolded proteins [65]. For instance, ATF6A and sXBP1
384 transactivated PRNP gene expression through ER stress response elements (ERSE) motifs
385 stretch on the human PRNP promoter [66]. Similarly, GRP78/BIP (also termed HSPA5),
386 another master regulator of ER homeostasis and a key component for sensing ER stress, is also
387 an ER chaperone of the cellular PrP^c [23,67]. Accordingly, GRP78/BIP was shown to modulate
388 the propagation of the pathogenic and infectious form of the prion protein PrP^{Sc}, reducing prion
389 pathogenesis [68]. Moreover, in murine model of scrapie, [69]GRP58 (also termed
390 PDIA3/ERP57), another ER molecular chaperone, showed to interact with the lectin chaperone
391 calnexin and to modulate the folding of mutant PrP protein through its disulfide isomerase
392 activity [69]. These compelling data highlight the tight regulation of PrP^c by key building blocks
393 of UPRER. In HeLa cells, the PrP^c protein has also been associated with a UPRmt-related
394 chaperone, namely HSP60 [70]. Interestingly, both HSP60 and HTRA2 expressions were
395 upregulated in A β 25-35-treated human neuroblastoma SH-SY5Y cells and in APP^{sw}/PS1dE9
396 transgenic mice [71], indicating that these two UPRmt chaperones participate to the PrP^c
397 proteostasis, particularly during an abnormal state for APP metabolism.

398 On another hand, in APP^{KD} and PRNP^{KD}/APP^{KD} HeLa cells we observed tremendous changes
399 in membrane-associated CD24, which are usually concentrated on plasma membrane lipid rafts.
400 As the detection of lipid rafts is very tricky [72,73], the fate of GPI-anchored CD24 in living
401 cells can reveal their stability [6,74]. Our results suggested that impaired APP and/or PRNP
402 gene expression disrupt lipid rafts composition. Better results were obtained in HeLa cells

403 displaying impaired HTRA2 gene expression. As lipid rafts articulate a set of synergistic
404 signaling factors and their composition plays a key role in normal PrP^c functions [75],
405 disruption of these lipid rafts should alter cellular PrP^c signaling. On another hand, CD24
406 expression in intact lipid rafts has been tightly correlated with tumor invasion and metastasis
407 [42,76]. Hence, we observed a hampered growth of APP^{KD} and/or PRNP^{KD} HeLa cells in 3D-
408 culture, reinforcing the idea that intact lipid rafts are essential for cell growth in collagen
409 matrices [47].

410 To conclude, our observational data revealed that HTRA2 modify the PRNP gene expression
411 and alters the subcellular localization of PrP^c, particularly in APP deficient cells. The HTRA
412 family encompasses mitochondrial, cytoplasmic and extracellular members. It is noteworthy
413 that HTRA1 and HTRA3 are secreted into the intracellular space where they degrade various
414 substrates of the ECM, playing a major role in extracellular matrix homeostasis. For instance,
415 HTRA1 degrade fibronectin, type II collagen, biglycan, clusterin, vitronectin, aggrecan, decorin
416 or fibromodulin [62,77–79]. Interestingly, Kaur et al. [80] showed that staurosporine-treated
417 HUVEC cells released HTRA2 in the culture medium. An emerging question is now to known
418 whether these three members of the HTRA family may play a role in the behavior of
419 extracellular PrP^c and its spreading.

420 **5. Materials and Methods**

421 *5.1. Cell culture and survival assays*

422 Cell origins: MCF7, MDA-MB-231, MDA-MB-436, MIA PaCa2, PANC-1, HeLa, T47D,
423 ZR-75-1, BE(2)-C (ATCC); SK-MEL-37 (Dr R. Chammas, Sao Paulo University, Brazil);
424 SK-MEL-5, SK-MEL-28, Malme-3M (Dr C Gazin, CNRS-CEA, Evry, France), RKO (Dr M.F.
425 Poupon, CNRS, Paris, France); HCT-116 (Dr B. Bugler, CNRS Toulouse, France); SK-N-AS,
426 SH-SY5Y, LA-N-1; LA-N-5, KELLY, T98G (Theranexus, CEA Fontenay aux Roses, France);
427 U-87 MG, U-251 MG (Dr L. Gauthier, CEA, Fontenay aux Roses, France); K562, RAMOS,
428 BL2 (Dr I. Délic, CEA Fontenay aux Roses, France); HOS, H1299 (Dr P. May, CNRS Villejuif,
429 France), HEK293 (Dr A. Delaunay, CEA, Saclay) Main features of the cells were indicated in
430 the Table S1. Parental cell lines were always cultivated in parallel.

431 Cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented
432 with 10% foetal calf serum (FCS), 100 U/mL penicillin / 100 µg/mL streptomycin / 2 mM
433 glutamin, 10 mM HEPES (Sigma products), under 5% CO₂. Transfection experiments were

434 performed with either JetPrime (Ozyme) or FugeneHD (Promega) reagents following
435 manufacturer's recommendations. As control of transfection we used a pEBV vector over-
436 expressing the YFP protein. 24h after transfection, cells were cultured in the presence of
437 hygromycin B at relevant concentrations (Table S1). Transfected cells were plated in 6 cm
438 diameter dishes to follow cell growth. Depending on the cell type, cells were counted 2 to 4
439 weeks later. Transfection experiments were repeated at least three times, and more than ten
440 times for HeLa cells. Average numbers of survival cells from each experiment were plotted (+/-
441 SD). For long term survival, cells were trypsinized, counted and plated at 5,000 cells per dish
442 in 6-well dishes for an additional period in culture. Three wells per condition were counted and
443 experiments were reproduced twice in different independent transfection experiments. For very
444 long-term cultures (more than one month) numerous knock down (KD) clones were established
445 in independent transfection experiments (e.g. for HeLa cells). Clones were picked up randomly,
446 propagated and analyzed. Gene silencing efficiency was constantly assessed.

447 5.2. *pEBVsiRNA and overexpressing pEBV vectors*

448 pEBVsiRNA plasmids and establishment of stable knock down cells have been extensively
449 described [41,81,82]. Cloning was performed using our replicative pEBV₂siRNA LacZ' vectors
450 for either single or double gene silencing. For dual expression of two shRNAs, transcription
451 cartridges were fitted side by side in the same orientation (in tandem). These vectors carried the
452 H1 RNA polymerase III promoter (RNA pol III) to drive the transcription of short hairpin RNA
453 (shRNA), which gives rise to siRNA like molecules *in vivo*. Because the crucial step in the
454 quality of RNA interference is the unfailing recognition of the cognate mRNA by the siRNA
455 sequence, we used the DSIR program which includes an exact similarity search algorithm for
456 potential off-target detection [83]. For each gene, 3 to 5 vectors were built and only 2 of them
457 were selected for efficient gene silencing without side effects [82]. Control cells (termed as
458 CTL) were obtained by transfection with the vector pBD650, which carries an inefficient
459 shRNA [41]. All plasmids, target sequences, and predicted efficiencies are presented in
460 Table S2. CRISPR-Cas9 cell lines were established using new home-made plasmids carrying
461 one or two H1-sgRNA transcription cassette and allowing a very high level of gene silencing
462 (manuscript in preparation). These cells were analysed at the DNA genomic level and thereafter
463 by RT PCR and ICC staining. For expression of ectopic proteins, we used our well-validated
464 pEBV plasmids [84]. Transcription was driven by either a CMV or a CAG promoter. Name and
465 main features of these plasmids are mentioned in Table S3. All plasmids have been checked by
466 sequencing (Eurofins Genomics).

467 5.3. *Soft agar assay for colony formation*

468 Soft agar assay was performed in six-well plate using a 2 ml basal layer of 0.63% bacto-agar
469 (Becton Dickinson) in complemented DMEM. Cells were trypsinized, counted and plated at
470 6,000 cells per well in 0.42% bacto-agar in complemented DMEM medium. Depending on the
471 cell lines, after 3 to 4 weeks, the colonies were stained with 0.4% crystal violet overnight at
472 37°C and counted. Images were captured using Argimed software (Microvision instruments,
473 France) and quantified using NHI ImageJ software. Only particles ranging from 30 to 1,000
474 000 pixels were counted. Each experiment was repeated at least twice with two replicates each.
475 Average numbers of colonies from each experiment were plotted.

476 5.4. *Culture in a tissue-like environment (collagen matrices)*

477 Cell growth in a tissue-like environment has been adapted from study on *in vitro* reconstructed
478 skin [85]. Briefly, ice-cold collagen type I isolated from rat tail tendons (4 mg/ml) was mixed
479 with Hank's balanced saline solution and neutralised with NaOH. Complemented DMEM
480 containing 3,000 cells per gel was added and cell-populated collagens were poured in
481 independent wells of 24-well plates. For each cell lines, three wells were seeded. Collagen gels
482 were allowed to polymerize for 1-2 hours in a humid incubator and complemented DMEM was
483 added onto each well. Culture proceeded for two weeks. Collagen specimens were fixed with
484 4% buffered formol (VWR) for 10 min and stained with Crystal Violet (0.004%, Sigma)
485 overnight. Analyse was performed with a binocular microscope and an indirect microscope.
486 Experiments were reproduced 4 times with different pools of cells.

487 5.5. *Western blot and immunostaining staining*

488 Procedures were described elsewhere [41]. For PrP^c, we used the following monoclonal
489 antibodies: SAF32, BAR233, and SHA31 (CEA). We also used antibodies against APP (clone
490 22C11, Affymetrix), α -tubulin (clone B-5-1-2, Sigma), APE-1/Ref-1 (H-6 sc-55498, Santa
491 Cruz), TP53 (DO-7, Tébu), HTRA2 (GTX54154, GeneTex), SOD2 (ABIN204760, Antibodies-
492 online), and CD44 (clone EPR1013Y, Abcam). ICC stainings were analysed with a Leica
493 DM6000 microscope. For ICC stainings and for each experiments, more than 1,500 individual
494 cells were circled in different fields and analysed. To evaluate change in cell shape, 300 cells
495 were quantified in at least three fields per sample using ImageJ shape indicators (circularity and
496 roundness).

497 *5.6. RNA extraction and quantitative RT qPCR*

498 RNA was extracted with NucleoSpinRNA II kit (Macherey-Nagel, France) and quantified using
499 Nanodrop (ND-1000 spectrophotometer). For RT experiments, 1 µg of each RNA sample was
500 reverse-transcribed into cDNA using iScript^cDNA Synthesis Kit (Bio-Rad, USA) with 20 µL
501 of random hexamer primers, oligo-dT mixes and incubated at 25°C for 5 min, then at 42°C for
502 30 min and at 85°C for 5 min. Relative gene expression level of each target gene in cells was
503 determined by real-time quantitative PCR (RT-qPCR). Ribosomal protein RPLP0 was used as
504 a reference gene. Primer sequences were mentioned in the Table S4. Two microliters of cDNA
505 (1:5 diluted) and 0.3 µM of primers were mixed with components from the IQTM SYBR[®] Green
506 Supermix kit (Biorad, France) in a final volume of 10 µL. Reactions in triplicate were carried
507 out in the MiniOpticon real-time PCR machine (Biorad, France) under the following conditions:
508 initial denaturation at 95°C for 3 min and then 45 cycles of denaturation at 95°C for 10 sec,
509 annealing/extension at 60°C for 30 sec. Melting curves were obtained to examine the purity of
510 amplified products. Absolute quantitative data and C_q values were obtained by analysis with
511 BioRad MFX Software 2.0 by second derivative method. Normalization of data was done with
512 RPLP0 as reference gene and HeLa cells as calibrator. We used the following equation: [copy
513 numbers of target gene in a sample/copy numbers of RPLP0 in a sample)]/[copy numbers of
514 target gene in HeLa cells/copy numbers of RPLP0 in HeLa cells]. To reveal a correlation
515 between PRNP and APP mRNA expression (Figure 1e), we calculated the RQ factor using the
516 following equation: $RQ = 2^{(Cq\ RPLP0 - Cq\ target)}$, C_q target being C_q APP or C_q
517 PRNP.

518 Alternatively, total RNA was isolated from cells using a TRIzol[®] Reagent according to the
519 manufacturer's recommendations (ThermoFisher). Extracted RNAs were treated with
520 recombinant rDNase (New England Biolabs) for 15 min at 37°C and deactivated at 75°C for
521 10 min. RT was performed in 1 µg of RNA that was reverse transcribed with the GoScriptTM
522 Reverse Transcription Mix kit (Promega). PCR reactions were performed with the OneTaq[®]
523 Quick-load 2X Master Mix. Primers sequences were indicated in the Table S4 and the level of
524 ribosomal protein RPLP0 mRNA was used as internal control. PCR reactions were carried out
525 as followed: initial denaturation (98°C 30 sec), 26 cycles of denaturation (98°C 10 sec) -
526 annealing (64-70°C depending of the primer's T_M, 30 sec) - extension (72°C 30 sec). cDNAs
527 were loaded onto 2% agarose gels and analyzed with a U:Genius device (Syngene).

528 5.7. *Flow cytometry analysis*

529 Living cells were recovered and stained with the antibodies against CD24 (clone ML5), CD44
530 (clone C26), and CD230 (PrP, clone 4D5) from eBioscience. Isotypic controls were from BD
531 Biosciences (San Jose, CA, USA). Cell immunolabelling was performed in PBS 1% BSA
532 according to manufacturer's recommendations. Cells were analyzed on a SORP LSR-II
533 analyzer (Configuration: 488 nm, 561 nm, 405 nm, 355 nm, and 635 nm). For each experiment,
534 10,000 to 20,000 cells were analyzed. Gating strategy was performed following exclusion of
535 debris and cellular aggregates and live/dead discrimination. The positive populations were
536 identified using an isotypic control to determine the negative populations. Data were treated
537 with FlowJo v7.6.1 software (Tree Star). $\Delta\Delta\text{MFI}$ (median fluorescence intensity) and
538 percentage expression as compared to control were calculated.

539 5.8. *Statistical analysis*

540 ICC stainings and Western blots were quantified using ImageJ v1.49o (NIH, USA) statistical
541 analysis was carried out with GraphPad Prism v5 (GraphPad Software, La Jolla, CA, USA).
542 The same software was used to design graphs. Values were subjected to a Shapiro-Wilk
543 normality test. When data did not show a normal distribution, they were compared by a Kruskal-
544 Wallis test with a Dunns (compare all pairs of columns) post test. Differences were considered
545 statistically significant for p values < 0.05.

546 **6. Legends to Figures**

547 Figure 1: **Quantitative and qualitative diversity for PrP^c and APP protein content.** Cells
548 were plated ($9 \cdot 10^5$ cells per 6 cm diameter dishes) and cultivated at the same time. Cells were
549 recovered 2 days later for protein analyses. **(a)** ICC stainings were performed with a pool of 2
550 antibodies against PrP^c, SAF32 (epitope amino acids 79-92) and SHA31 (aa 148-159), this
551 allows detection of all putative PrP^c fragments. Antibodies epitope location is depicted in Fig.
552 S1c. Cells were counterstained with DAPI to visualize nuclei (scale bar 25 μm). On the right
553 of the panel (a) overlapping PrP^c/DAPI stainings are shown for HeLa, H1299 and MIA PaCa2
554 cells. **(b)** Western blot analysis of PrP^c was performed with 5 μg of protein per slot or **(c)** 10^5
555 cell equivalents. Proteins were loaded and separated on a 12% SDS-polyacrylamide gel.
556 Western blots b) and c) represented two independent culture experiments. Western blot were
557 quantified in using ImageJ. Color code corresponds to the TP53 genotype of each cell lines:

558 green = wild-type, blue = null, red = mutated, black = wild-type with viral protein or
 559 transcription-inactive p53 β variant. **(d)** ICC staining against APP using the monoclonal 22C11
 560 antibody and counterstained with DAPI (scale bar 25 μ m). On the right of the panel (d)
 561 overlapping APP/DAPI stainings were showed for HeLa, H1299 and MIA PaCa2 cells.
 562 **Relationship between endogenous PRNP and APP gene expressions in parental cell lines.**
 563 **(e)** In parallel to protein analyses, mRNA was quantified by RT qPCR, and ordered in function
 564 of their relative quantification. Experiments were reproduced twice.

565 Figure 2: Relationship between PRNP and APP gene expression. RT qPCR analysis of PRNP
 566 and APP in stable (a) MDA-MB-231 or (b) HCT-116 cells. Reversion experiment in HeLa^{KD}
 567 cells. Stable HeLa^{KD} clones were maintained in culture for 20 and 40 additional days with (c)
 568 or without (d) hygromycin B to revert gene silencing. At indicated time, PRNP and APP mRNA
 569 were analysed by RT qPCR. _{REV}P means reversed PRNP^{KD}, _{REV}A means reversed APP^{KD},
 570 _{REV}P/A means reversed PRNP^{KD}/APP^{KD}. Primers used are indicated in Table S4.

571 Figure 3: **PRNP and/or APP gene silencing impairs 3D cell growth.** **(a)** Characterization of
 572 stable HeLa^{KD} cells by RT PCR; primers used are indicated in Table S4. **(b)** PRNP^{KD} and
 573 CD24^{KD} cells acquired a fibroblastic-like appearance. Illustrative bright field observations of
 574 HeLa^{KD} cells 14 days after transfection are shown (scale bar: 50 μ m). At least 3 fields
 575 corresponding to 300 cells per cell line were quantified with ImageJ for shape indicators
 576 (circularity and roundness, mean +/- SEM), and statistical analysis was performed using
 577 GraphPad Prism v5 right), n=2. **(c) Culture in soft agar.** HeLa^{KD} cells were cultivated in
 578 presence or not of hygromycin B for 2 weeks and thereafter 5,000 HeLa cells were seeded in
 579 soft agar (+/- hygromycin B) for 18 additional days. Each point represents the mean of 3 wells
 580 +/- SD. **(d) Culture in a tissue-like environment.** 3,000 stable HeLa^{KD} clones were embedded
 581 into collagen matrices (three wells per point) and cultivated for 17 days. Collagen gels were
 582 analysed with a binocular microscope or an indirect microscope, as indicated in M&M.
 583 Growing colonies were counted. Indicated values correspond to mean +/-SD. Experiments has
 584 been reproduced four times.

585 Figure 4: **Altered membrane-anchored CD24 content in PRNP and APP deficient stable**
 586 **HeLa cells.** **(a)** Membrane-associated CD24, CD44, and PrP^c proteins were analysed by flow
 587 cytometry in living stable HeLa^{KD} clones 57 days after transfection. 200,000 cells were plated
 588 in 6 cm diameter dishes and analyzed 6 days later at about 95% of confluence. Percentages
 589 correspond to the mean fluorescence intensity as compared to control HeLa cells carrying the
 590 pBD650 plasmid. The Table underneath the panel (a) represents the mean +/-SEM of three

591 independent flow cytometry experiments performed with different clones and/or different
 592 culture conditions. **Cytoplasmic accumulation of PrP^c in APP^{KD} HeLa clones (b)**
 593 Representative Western blot were quantified with ImageJ (mean +/- SEM, n = 3). (c)
 594 Representative PrP^c stainings in HeLa^{KD} cells (scale bar = 25 μm). 1,237 individual cells in 4
 595 to 7 fields per sample were analysed with ImageJ and GraphPad Prism (mean +/- SEM, n = 3).

596 **Figure 5: HTRA2 and PrP^c co-expression.** (a) ICC detection of HTRA2 in four tumor-derived
 597 cells cultivated at the same time. Cells were cultivated as indicated in legend of Fig. 1, and
 598 stained for PrP^c (SHA31 + SAF32 antibodies) or HTRA2. They were counterstained with DAPI
 599 to visualize nuclei (scale bar 25 μm). (b) RT PCR analysis of PRNP and HTRA2 expression in
 600 10 cell lines cultivated in parallel (n=2). Primer used are described in Table S4. (c) Western
 601 blot analysis of HTRA2 in 5 cell lines.

602 **Figure 6: HTRA2 down regulation lowers PRNP gene expression.** (a) Representative PrP^c
 603 (SHA31 + SAF32 antibodies), APP or HTRA2 stainings 14 days after transfection of HeLa^{KD}
 604 cells. Cells were counterstained with DAPI to visualize nuclei (scale bar 25 μm). 1,637
 605 individual cells in 4 to 5 fields per sample were analysed with ImageJ and GraphPad Prism
 606 (mean +/- SEM, n=3). (b) RT qPCR analysis of HTRA2 and PRNP gene expression in 26 days
 607 old HTRA2^{KD} HeLa cell populations. Reactions were carried out in triplicate and values were
 608 normalized with control BD650 HeLa cells (mean +/- SEM). Cells were transfected with
 609 pEBVsiHTRA2 plasmids displaying a high (HTRA2^{KD1}, pBD3115 vector) or moderate
 610 (HTRA^{KD2}, pBD3116 vector) gene silencing efficiency. For HTRA2 mRNA quantification, 2
 611 sets of primers were used: primers 1 (pr1) detecting either total HTRA2 (short and long HTRA2
 612 mRNA chains) and primers 2 (pr2) detecting only long chain. Primers are described in Table
 613 S4. (c) Western blot analysis of the HTRA2 protein content in stable knock down (HTRA2^{KD1})
 614 and knock out (HTRA2^{KO}) HeLa clones. Protein equivalent to 10⁵ cells were loaded onto a 10%
 615 PAGE. PrP^c was revealed with both SHA31 and SAF32 antibodies. (d) Flow cytometry analysis
 616 of membrane-associated CD24, CD44, and PrP^c in living HeLa^{KD} cells 9 or 11 days after
 617 transfection in 2 independent culture experiments. Percentages correspond to the mean
 618 fluorescence intensity as compared to control. The loss of membrane-anchored PrP^c was
 619 revealed after PrP^c stainings in HTRA2^{KD1} cells as compared to CTL (scale bar 25 μm).

620 **Figure. 7: Intracellular accumulation of PrP^c in APP^{KD} and APP^{KO} HeLa cells.**
 621 Characterization of different HeLa^{KD} clones by: (a) Representative ICC stainings of HeLa^{KD}
 622 clones stained for PrP^c (SHA31 + SAF32 antibodies), or HTRA2, and counterstained with
 623 DAPI to visualize nuclei (scale bar 25 μm). 1,632 individual cells in 4 to 7 fields per sample

624 were analysed with ImageJ and GraphPad Prism (mean \pm SEM, n = 3). **(b)** Western blot was
625 performed with protein extracted from 10^5 cells and were loaded onto a 10% PAGE. **(c)** For
626 RT PCR, the used primers are indicated in Table S4. **(d)** CRISPRCas9-engineered HeLa^{KO} cells
627 were analysed by ICC stainings. Cells were stained and analysed as mentioned in panel a (scale
628 bar 25 μ m).

629 **Figure. 8: Overexpression of HTRA2 enhances PrP^c protein content.** **(a)** Representative
630 stainings of PrP^c (SHA31 + SAF32 antibodies), HTRA2 and CD44 performed 10 days after
631 transfection of PRNP or HTRA2 CDS in HeLa cells. Cells were counterstained with DAPI to
632 visualize nuclei (scale bar 25 μ m). 1,399 individual cells in 5 to 8 fields per sample were
633 analysed with ImageJ and GraphPad Prism (mean \pm SEM). **(b)** RT PCR analysis of HeLa cells,
634 52 days after transfection with either PRNP, APP695, HTRA2 or HTRA3 CDS. Primers used
635 are indicated in Table S4. **(c)** Western blot analysis of HTRA2 and PrP^c (SHA31 antibody) in
636 HeLa cells silenced for HTRA2 (KD1 and KO) and overexpressing HTRA2 (HTRA2+). Cells
637 were transfected with two different pEBVCAG-HTRA2 plasmids (1 means pBD3357; 2 means
638 pBD3696). Proteins extracted from 10^5 cells were loaded onto a 10% PAGE. *: unspecific band.
639 Plasmids were described in Table S3. **Altered expression of membrane-associated CD24,
640 CD44 and PrP^c in living HTRA2+ HeLa cells.** **(d)** 11 and **(e)** 14 days after transfection, living
641 HeLa cells were stained for CD24, CD44 and PrP^c and analysed by flow cytometry as indicated
642 in M&M. PRNP+ and CD24+ cells are used as controls. Panels d and e represent two
643 independent transfection experiments. Percentages correspond to the $\Delta\Delta$ MFI as compared to
644 control.

645

646

7. Tables (supplementary data)

647 Table S1: Human tumor cell lines, TP53 genotype and hygromycin B resistance

Name	Origin	TP53 genotype (1)	Hygromycin B ⁽²⁾
HeLa	Cervical adenocarcinoma	Wild type + E6	250 µg/ml
UACC62	Melanoma	Wild type	NT ⁽³⁾
Malme-3M	Melanoma	Wild type	NT
SK-MEL-5	Melanoma	Wild type but aberrant p21	125 µg/ml
SK-MEL-28	Melanoma	pLeu145Arg	125 µg/ml
SK-MEL-37	Melanoma	pArg175His	125 µg/ml
MDA-MB-231	Breast adenocarcinoma	pArg280Lys	250 µg/ml
MDA-MB-436	Breast adenocarcinoma	p204Ins7c	250 µg/ml
MCF-7	Breast adenocarcinoma	Wild type	125 µg/ml
ZR-75-1	Breast carcinoma	Wild type	125 µg/ml
T-47D	Breast carcinoma	pLeu194Phe	125 µg/ml
RKO	Colon carcinoma	Wild type	500 µg/ml
Caco2 TC7	Colorectal carcinoma	pGlu204Stop	500 µg/ml
HCT-116	Colorectal carcinoma	Wild type	125 µg/ml
HT-29	Colorectal adenocarcinoma	pArg273His	NT
SW-480	Colorectal adenocarcinoma	Pro306Ser / Arg273His	125 µg/ml
SK-N-AS	Neuroblastoma (bone Marrow Metastasis)	truncated TP53β	125 µg/ml
BE(2)-C	Neuroblastoma (clone of SK-N-BE(2))	Cys135Phe	NT
SH-SY5Y	Neuroblastoma (bone Marrow Metastasis)	Wild type + TP53β	125 µg/ml
LA-N-1	Neuroblastoma (bone Marrow Metastasis)	pCys182Stop	125 µg/ml
LA-N-5	Neuroblastoma (bone Marrow Metastasis)	Wild type	NT
KELLY	Neuroblastoma	Wild type	NT
T98G	Glioblastomamultiforme	pMet237Ile	125 µg/ml
U-87 MG	Glioblastomamultiforme	Wild type	500 µg/ml
SF-763	Glioblastomamultiforme	pArg158Leu	NT
K562	Chronic myelogenous leukemia	pGln136Stop	NT
RAMOS	Burkitt's lymphoma	pIle254Asp	NT
BL2	EBV-negative Burkitt's lymphoma	Wild type	NT
HOS	Osteosarcoma	pArg156Pro	125 µg/ml
H1299	Non-Small Cell Lung Cancer (carcinoma)	TP53-null (deleted)	250 µg/ml
PANC-1	Pancreatic adenocarcinoma	pArg273His	125 µg/ml
MIA PaCa2	Pancreatic adenocarcinoma	pArg248Trp	250 µg/ml
HaCaT	Immortalized keratinocyte	pHis179Tyr / Arg282Trp	NT
HEK293	Immortalized embryonic kidney cells	Wild type + E1b	125 µg/ml

648 (1) Data from “The TP53 web site” (http://p53.free.fr/Database/Cancer_cell_lines/p53_cell_lines.html)
649 except for SK-MEL-28 and SK-MEL-37 [86], SK-MEL-5 [87], U-251 MG [88], ZR-75-1 [89],
650 SH-SY5Y and SK-N-AS [90], BE(2)-C which is a subclone SK-N-BE(2) [90], KELLY [91]
651 and SF763 [92]. (2) Best selection conditions for establishing stable populations. (3) NT: not
652 tested for selection.

653

654 Table S2: pEBVsiRNA, target sequences and predicted efficiencies

Gene	N° vector	siRNA target	Corrected scores*	Alias	Plasmid name **
CTL	pBD650	***		CTL	pEBVsiCTL
PRNP (NM_000311)	pBD2619	5'-GGACCGTTACTATCGTGAA-3'	89,40%	PRNP ^{KD1}	pEBVsiPrP-438
	pBD2620	5'-GCGTCAATATCACAATCAA-3'	96,60%	PRNP ^{KD2}	pEBVsiPrP-536
APP (NM_000484)	pBD2678	5'-CGGTGTTGTCATAGCGACA	76,70%	APP ^{KD1}	pEBVsiAPP-2124
	pBD2679	5'-GACAGACAGCACACCCTAA-3'	81,10%	APP ^{KD2}	pEBVsiAPP-1510
PRNP + APP	pBD2747	siPRNP ^{KD1} + siAPP ^{KD2}		P ^{KD1} / A ^{KD2}	pEBVsiPrP-438-siAPP-1510
	pBD2749	siPRNP ^{KD1} + siAPP ^{KD1}		P ^{KD1} / A ^{KD1}	pEBVsiPrP-438-siAPP-2124
TP53 (NM_000546)	pBD1167	5'-GCTTCGAGATGTTCCGAGA-3'	88,50%	TP53 ^{KD}	pEBVsiP53-1010
HTRA2 (NM_013247)	pBD3115	GAATAGATCACCAAGAGTA	82,20%	HTRA2 ^{KD1}	pEBVsiHTRA2-1376 (3'UTR)
	pBD3116	GCTAAGCGGCGACACGTAT	87,70%	HTRA2 ^{KD2}	pEBVsiHTRA2-630
CD24 (NM_0013230)	pBD2506	CCAAGAAACGCTTCTAAA	92,50%	CD24 ^{KD}	pEBV2siCD24-25 (3'UTR)
CD44 (NM_000610)	pBD2957	GCAGATCGATTGAATATA	95,70%	CD44 ^{KD2}	pEBV2siCD44-60
MAPT (NM_005910) (Tau)	pBD3005	GCTACACCATGCACCAAGA	92,50%	Tau ^{KD}	pEBV2siTau-83

655 *: Corrected scores as described by DSIR algorithm [83]. **: position of the first nucleotide on
656 the mRNA, « 1 » being A of ATG; *** control sequence (see M&M)

657

658 Table S3: overexpressing pEBV plasmids with cloning primers

Gene	N° vector	Transgene *	Plasmid Name	CDS origin	primer PCR (Forward) **	primer PCR (Reverse) **
CTL	pBD3532	without	pEBVCAG	none	--	--
PRNP+ (NM_000311)	pBD2607	PrP ^c (aa 1-253)	pEBVCAG-PrP ^c	CEA	primer BD11_314F: ataccggtctagaATGGCGAACCTTG GCTGCTGGATGCTG	primer BD11_315R: atgtcgacgcccgcTCATCCCAC TATCAGGAAGATGAGGAA
APP695+ (NM_201414)	pBD3125	APP (aa 1-695)	pEBVCAG-PrP ^c	CEA	primer BD12_450F: ataccggtctagaATGCTGCCCGGTT TGGCACTGCTCCTG	primer BD12_456R: aatgtcgacgcccgcCTAGTTCTG CATCTGCTCAAAGAACTT
HTRA2+ (NM_013247)	pBD3357	HTRA2 (aa 1-459)	pEBVCAG-HTRA2	Dharmacon, Clone ID_3508944	primer BD15_805F: ataccggtctagaATGGCTGCGCCGA GGGCGGGGCGGGGT	primer BD15_806R: atgtcgacgcccgcTCATTCTGTG ACCTCAGGGGTCACATA
HTRA2+ (NM_013247)	pBD3696	HTRA2 (aa 1-459)	pEBVCAG-HTRA2 (Kozak sequence)	Dharmacon, Clone ID_3508944	primer BD15_1054F: ataccggtctagaccaccATGGCTGC GCCGAGGGCGGGGCGGGGT	primer BD15_806R: atgtcgacgcccgcTCATTCTGTG ACCTCAGGGGTCACATA
HTRA3+ (NM_053044)	pBD3188	HTRA3 (aa 1-454)	pEBVCAG-HTRA3	Thermo Scientist, Clone ID_5588608	primer BD14_723F: aataccggtctagaATGCAGGCGCGA GCGCTGCTCCTGGCC	primer BD14_724R: aatgtcgacgcccgcTCACATGAC CACCTCAGGTGCGATGCT
CD24+ (NM_0013230)	pBD2610	CD24 (aa 1-80)	pEBVCMV-HTRA2	Thermo Scientist, Clone ID_5591617	primer BD11_316F: ataccggtctagaATGGGCAGAGCA ATGGTGGCCAGGCTC	primer BD11_317R: atgtcgacgcccgcTTAAGAGTA GAGATGCAGAAGAGAGAG

659 *: Transgene with protein length (amino acids). **: Cloning primers. Lowercase letters:
660 sequences with restriction enzyme sites; uppercase letters: 5' (Forward) and 3' (Reverse)
661 overlapping CDS sequences.

662

663 Table S4: Primers used for PCR

Gene	Method	primer nb *	Forward primer (5'-3')	Reverse primer (5'-3')
RPLP0 (NM_053275)	qPCR & PCR	366F-367R 162 bps (exons 5-6)	CATTGCCCATGTGAAGTC	GCTCCCACTTTGTCTCCAGT
PRNP (NM_000311)	qPCR	330F-331R 126 bps (exon 2)	CCGAGACCGACGTTAAGATG	TCACAGGTGGAGAGGAGAAG
PRNP _{endo} (NM_000311)	PCR	1169F-331R 730 bps (exons 2)	GCAGTCATTATGGCGAACCT	TCACAGGTGGAGAGGAGAAG
PRNP _{total} (NM_000311)	PCR	2048F-2049R 222 bps (exons 2)	ATGGAACACTGGGGGCAG	TTACTCGGCTTGTCCACTGACTG
APP (NM_000484)	qPCR & PCR	380F-381R, 147 bps (exons 5-6)	CGGAGCAGACACAGACTATG	TCCTCAGCCTCTCTCTCTAC
APP _{endo} (NM_000484)	PCR	1177F-381R 791 bps (exons 1-6)	CGCAGGGTCGCGATGCTGCC	TCCTCAGCCTCTCTCTCTAC
APP _{total} (NM_000484)	PCR	2128F-2129R 467 bps (exons 2-5)	CTGTGGCAGACTGAACATGC	TTTCTTCAGCCAGTGGGCAA
TP53 (NM_000546)	qPCR & PCR	235F-236R 235 bps (exons 8/9-11)	GGAGCACTAAGCGAGCACTG	ATGGCGGGAGGTAGACTGAC
CD24 (NM_0013230)	PCR	1128F-264R 172 bps (exon 2-3)	GGGACATGGGCAGAGCAAT	TTGGTGGTGGCATTAGTTGG
CD44 (NM_000610)	PCR	2071F-567R 392 bps (exons 1-4/5)	TCCGTTTCGCTCCGGACACCA	GTGGAGCTGAAGCATTGAAG
HTRA2 (NM_013247)	qPCR PCR primers1	873F-874R 102 bps (exons 1-2)	GCAGATGTGGTGGAGAAGAC	TCCTGAGCCGTTTCGAGATAG
HTRA2 (NM_013247)	qPCR PCR primers2	612F-613R 135 bps (exons 2-3)	ACGCTGAGGATTCAGACTAAGG	AACAATGCCGGATGTGATCG
HTRA2 (NM_013247)	PCR	2109F-2110R 695 bps (exons 1-7)	TGTGGTGGAGAAGACAGCAC	AGCTTGGTTCTCGAAGCTGT
HTRA2 (NM_013247)	PCR	2077F-1013R 640 bps (exons 1-2)	GCTGATGGCTGCGCCGAGGG	GCTTAGCAGTCTCACACGGA
HTRA1 (NM_002775)	PCR	1032F-1033R 162 bps (exons 1-2)	CCAACACCTACGCCAACCTG	CCACCACGTCCGCGATAAAG

664 *: Primer numbers (Forward and Reverse) with names, amplicon lengths and positions on
665 genomic DNA (exons).

666 8. Supplementary Figures

667 Figure S1: **Analysis of PrP^c in a set of human tumor cell lines.** Cells were plated (9.10⁵ cells
668 per 6 cm diameter dishes) and cultivated at the same time. Proteins were analysed by (a) ICC
669 staining with three different antibodies BAR233, SHA31, and SAF32 (magnification x100),
670 and (b) Western blot with 105 cell equivalents per slot. Proteins were loaded and separated on
671 a 12% SDS-polyacrylamide gel. (c) Scheme of the PrP^c protein with structural domains, sites
672 of endogenous cleavages and location of epitopes

673 Figure S2: **Endogenous PrP^c protein expression in growing or confluent HeLa, T98G, and**
674 **MIA PaCa2 cells.** Cells were plated at 6.10⁵ cells / 6 cm Ø dishes and fixed 1, 2 or 3 days later.
675 Cells were stained with (a) SAF32 or (b) BAR233 antibodies against PrP^c and counterstained
676 with DAPI (nuclei) (magnification x50).

677 Figure S3: **Wide range of endogenous PRNP and APP gene expression in numerous tumor**
678 **cell lines.** Cells were cultivated as indicated in legend of Figure 1. RT qPCR analyses were
679 carried out for PRNP (a) or APP (b) gene expressions. Values were normalized with an
Biard et al. APP deficiency and HTRA2 modulates PrP^c proteostasis in human cancer cells

680 endogenous control (RPLP0) giving rise to $[C_{q_{\text{sample}}}/C_{q_{\text{RPLP0}}}]$ and percentages (+/-SD) were
 681 determined with HeLa cells as calibrator. Experiments were carried out twice. It was
 682 noteworthy that skin cells expressed highest PRNP mRNA levels, including SK-MEL-28
 683 (2361%), SK-MEL-37 (764%), UACC62 (408%), and HaCaT (372%). Conversely,
 684 neuroblastoma cells were among those that expressed very low or nearly undetectable PRNP
 685 mRNA (LA-N-1 0%, SH-SY5Y 1% and KELLY 10%). Moreover, the three tested lymphoma
 686 cell lines exhibited rather low PRNP mRNA content (BL 2 32%, K562 29% and RAMOS 1%).
 687 Among cells exhibiting higher APP mRNA contents we found MIA PaCa2 (425%), SH-SY5Y
 688 (313%), and LA-N-5 (273%). As observed for PRNP, lymphoma cells failed to express
 689 detectable APP mRNA, (BL2, K562 and RAMOS 0%). Color code for the TP53 genotype: see
 690 the legend of Figure 1.

691 Figure S4: Hampered 2D cell growth after PRNP and/or APP knocking down.

692 In order to validate our RNAi technology in all tested cell lines we always assessed the
 693 efficiency of gene silencing using an efficient plasmid targeting the TP53 gene (pEBV_{sip53}).
 694 19 over 33 cell lines were selected in which we could imposed a robust TP53 gene silencing as
 695 a technical control. After transfection and hygromycin B selection (Table 1S), cells were
 696 recovered two to 4 weeks later depending on their proliferation rate and they were counted.
 697 Values represent the mean of three to ten independent transfection experiments. 100%
 698 corresponds to cells transfected with a control plasmid (pBD650). We observed a dramatic
 699 decrease in cell survival for most of the PRNP^{KD} and APP^{KD} cell lines. In particular, HOS,
 700 MDA-MB-436, U87-MG, U251-MG, H1299, and the 3 SK-MEL cell lines exhibited major
 701 difficulties to proliferate while other cells, such as T98G, HCT-116 or MDA-MB-231, showed
 702 elevated cell death although stable knock down cells could be propagated in culture, despite of
 703 difficulties. A third set of cells, comprising HeLa and RKO, exhibited only moderate effects on
 704 their growth potential in 2D culture albeit exhibiting very efficient gene silencing. (a) Breast
 705 cancer cells. (b) Brain cancer cells. (c) Other cancer cells. (d) Prolonged cell survival for HeLa
 706 cells. Cells were grown for 14, 28 and 45 days, trypsinized and counted. It was noteworthy that
 707 HeLa TP53^{KD} cells elicited a growth potential that increased with time in culture.

708 Figure S5: **PRNP and/or APP gene silencing abrogated 3D cell growth.** (a) 80 days after
 709 transfection, 3,000 HeLa^{KD} cells were embedded into collagen I matrices and allowed to grow
 710 for additional 10 days. Two KD clones per gene were plated in 3 different wells. One
 711 representative field is shown. (b) In two independent experiments at days 51 or 80 days after
 712 transfection, growing HeLa^{KD} colonies embedded into collagen matrices were counted as

713 indicated in M&M. Values correspond to means +/- SD. (c) Fibroblastic-like appearance of
714 PRNP^{KD} and CD24^{KD} HeLa cells 8 days after transfection. Two representative bright fields of
715 HeLa^{KD} cells are shown, and 300 cells per clone were analysed for shape change in at least
716 three fields, as described in the legend of Figure 3b and in M&M (mean +/-SEM and statistical
717 values). (d) Membrane-anchored CD24, CD44 and PrP^c in proliferating living HeLa^{KD} cells
718 were analyzed 77 days after transfection by flow cytometry. (e) HTRA2 transcript levels in five
719 HeLa^{KD} clones were analyzed by RT PCR.

720 Figure S6 (a) **Stable APP^{KD} HeLa cells overexpress cytoplasmic PrP^c**. Two antibodies were
721 used (SHA31 and SAF32) to detect PrP^c by ICC. Cells were counterstained with DAPI
722 (magnification x50). (b) **Cytoplasmic accumulation of ectopic PrP^c in PRNP+ HeLa cells**
723 **assessed 10 days after transfection**. Cells were transfected with the pBD2607 plasmid and
724 fixed 10 days later. Two antibodies were used (SHA31 and SAF32) to detect PrP^c. Cells were
725 counterstained with DAPI (magnification x100).

726 Figure S7: **Decreased amounts of PrP^c and CD44 proteins in HTRA2^{KD} HeLa cells 14 days**
727 **after transfection**. Cells were stained with two antibodies against PrP^c (SHA31 and SAF32)
728 and counterstained with DAPI (magnification x100).

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740 10. Authors' contributions

741 B.D.S.F. conceived and designed experiments. B.D.S.F. created and validated KD and KO
742 cells, performed 2D and 3D-culture in collagen type I experiments, ICC stainings and

743 qualitative RT PCR. R.J. realized RT qPCR experiments & analysis, and soft agar assays. N.R.
 744 performed Western blots. F.L. carried out flow cytometry analyses. B.D.S.F. drafted the
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751 **13. References**

- 752 [1] S. Lim, B.K. Yoo, H.-S. Kim, H.L. Gilmore, Y. Lee, H. Lee, S.-J. Kim, J. Letterio, H.
 753 Lee, Amyloid- β precursor protein promotes cell proliferation and motility of advanced
 754 breast cancer, *BMC Cancer*. 14 (2014) 928. <https://doi.org/10.1186/1471-2407-14-928>.
- 755 [2] X. Wu, S. Chen, C. Lu, Amyloid precursor protein promotes the migration and invasion
 756 of breast cancer cells by regulating the MAPK signaling pathway, *Int. J. Mol. Med.* 45
 757 (2020) 162–174. <https://doi.org/10.3892/ijmm.2019.4404>.
- 758 [3] M. Mehrpour, P. Codogno, Prion protein: From physiology to cancer biology, *Cancer*
 759 *Letters*. 290 (2010) 1–23. <https://doi.org/10.1016/j.canlet.2009.07.009>.
- 760 [4] M.B. Prado, M.I. Melo Escobar, R.N. Alves, B.P. Coelho, C.F. de L. Fernandes, J.M.
 761 Boccacino, R.P. Iglesia, M.H. Lopes, Prion Protein at the Leading Edge: Its Role in Cell
 762 Motility, *Int J Mol Sci*. 21 (2020). <https://doi.org/10.3390/ijms21186677>.
- 763 [5] L. Ryskalin, F. Biagioni, C.L. Busceti, M.A. Giambelluca, L. Morelli, A. Frati, F. Fornai,
 764 The Role of Cellular Prion Protein in Promoting Stemness and Differentiation in Cancer,
 765 *Cancers (Basel)*. 13 (2021). <https://doi.org/10.3390/cancers13020170>.
- 766 [6] K. Simons, M.J. Gerl, Revitalizing membrane rafts: new tools and insights, *Nat. Rev. Mol.*
 767 *Cell Biol.* 11 (2010) 688–699. <https://doi.org/10.1038/nrm2977>.
- 768 [7] J.D. Greenlee, T. Subramanian, K. Liu, M.R. King, Rafting Down the Metastatic Cascade:
 769 The Role of Lipid Rafts in Cancer Metastasis, Cell Death, and Clinical Outcomes, *Cancer*
 770 *Res.* 81 (2021) 5–17. <https://doi.org/10.1158/0008-5472.CAN-20-2199>.
- 771 [8] C.I. Lasmézas, J.P. Deslys, O. Robain, A. Jaegly, V. Beringue, J.M. Peyrin, J.G. Fournier,
 772 J.J. Hauw, J. Rossier, D. Dormont, Transmission of the BSE agent to mice in the absence
 773 of detectable abnormal prion protein, *Science*. 275 (1997) 402–405.
- 774 [9] R. Linden, The Biological Function of the Prion Protein: A Cell Surface Scaffold of
 775 Signaling Modules, *Front. Mol. Neurosci.* 10 (2017).
 776 <https://doi.org/10.3389/fnmol.2017.00077>.

- 777 [10] R.G. Warner, C. Hundt, S. Weiss, J.E. Turnbull, Identification of the heparan sulfate
778 binding sites in the cellular prion protein, *J. Biol. Chem.* 277 (2002) 18421–18430.
779 <https://doi.org/10.1074/jbc.M110406200>.
- 780 [11] E.T. Parkin, N.T. Watt, I. Hussain, E.A. Eckman, C.B. Eckman, J.C. Manson, H.N.
781 Baybutt, A.J. Turner, N.M. Hooper, Cellular prion protein regulates beta-secretase
782 cleavage of the Alzheimer's amyloid precursor protein, *Proc. Natl. Acad. Sci. U.S.A.* 104
783 (2007) 11062–11067. <https://doi.org/10.1073/pnas.0609621104>.
- 784 [12] L. Gasperini, G. Legname, Prion protein and aging, *Front. Cell Dev. Biol.* 2 (2014).
785 <https://doi.org/10.3389/fcell.2014.00044>.
- 786 [13] U.C. Müller, H. Zheng, Physiological functions of APP family proteins, *Cold Spring Harb*
787 *Perspect Med.* 2 (2012) a006288. <https://doi.org/10.1101/cshperspect.a006288>.
- 788 [14] X. Wang, X. Zhou, G. Li, Y. Zhang, Y. Wu, W. Song, Modifications and Trafficking of
789 APP in the Pathogenesis of Alzheimer's Disease, *Front Mol Neurosci.* 10 (2017) 294.
790 <https://doi.org/10.3389/fnmol.2017.00294>.
- 791 [15] H. Zhang, Q. Ma, Y. Zhang, H. Xu, Proteolytic processing of Alzheimer's β -amyloid
792 precursor protein, *J. Neurochem.* 120 Suppl 1 (2012) 9–21. <https://doi.org/10.1111/j.1471-4159.2011.07519.x>.
- 794 [16] D.A. Gimbel, H.B. Nygaard, E.E. Coffey, E.C. Gunther, J. Laurén, Z.A. Gimbel, S.M.
795 Strittmatter, Memory impairment in transgenic Alzheimer mice requires cellular prion
796 protein, *J. Neurosci.* 30 (2010) 6367–6374. <https://doi.org/10.1523/JNEUROSCI.0395-10.2010>.
- 798 [17] H.H. Griffiths, I.J. Whitehouse, H. Baybutt, D. Brown, K.A.B. Kellett, C.D. Jackson, A.J.
799 Turner, P. Piccardo, J.C. Manson, N.M. Hooper, Prion protein interacts with BACE1
800 protein and differentially regulates its activity toward wild type and Swedish mutant
801 amyloid precursor protein, *J. Biol. Chem.* 286 (2011) 33489–33500.
802 <https://doi.org/10.1074/jbc.M111.278556>.
- 803 [18] J. Laurén, D.A. Gimbel, H.B. Nygaard, J.W. Gilbert, S.M. Strittmatter, Cellular prion
804 protein mediates impairment of synaptic plasticity by amyloid-beta oligomers, *Nature.*
805 457 (2009) 1128–1132. <https://doi.org/10.1038/nature07761>.
- 806 [19] S.V. Salazar, S.M. Strittmatter, Cellular prion protein as a receptor for amyloid- β
807 oligomers in Alzheimer's disease, *Biochem. Biophys. Res. Commun.* 483 (2017) 1143–
808 1147. <https://doi.org/10.1016/j.bbrc.2016.09.062>.
- 809 [20] F. Meslin, A. Hamäi, P. Gao, A. Jalil, N. Cahuzac, S. Chouaib, M. Mehrpour, Silencing
810 of prion protein sensitizes breast adriamycin-resistant carcinoma cells to TRAIL-mediated
811 cell death, *Cancer Res.* 67 (2007) 10910–10919. <https://doi.org/10.1158/0008-5472.CAN-07-0512>.
- 813 [21] H.H. Jarosz-Griffiths, N.J. Corbett, H.A. Rowland, K. Fisher, A.C. Jones, J. Baron, G.J.
814 Howell, S.A. Cowley, S. Chintawar, M.Z. Cader, K.A.B. Kellett, N.M. Hooper,
815 Proteolytic shedding of the prion protein via activation of metallopeptidase ADAM10
816 reduces cellular binding and toxicity of amyloid- β oligomers, *J. Biol. Chem.* 294 (2019)
817 7085–7097. <https://doi.org/10.1074/jbc.RA118.005364>.
- 818 [22] S.F. Godsave, P.J. Peters, H. Wille, Subcellular distribution of the prion protein in sickness
819 and in health, *Virus Res.* 207 (2015) 136–145.
820 <https://doi.org/10.1016/j.virusres.2015.02.004>.

- 821 [23] M. Nunziante, K. Ackermann, K. Dietrich, H. Wolf, L. Gädtke, S. Gilch, I. Vorberg, M.
822 Groschup, H.M. Schätzl, Proteasomal dysfunction and endoplasmic reticulum stress
823 enhance trafficking of prion protein aggregates through the secretory pathway and
824 increase accumulation of pathologic prion protein, *J. Biol. Chem.* 286 (2011) 33942–
825 33953. <https://doi.org/10.1074/jbc.M111.272617>.
- 826 [24] B. Chen, M. Retzlaff, T. Roos, J. Frydman, Cellular Strategies of Protein Quality Control,
827 *Cold Spring Harb Perspect Biol.* 3 (2011). <https://doi.org/10.1101/cshperspect.a004374>.
- 828 [25] C.L. Klaips, G.G. Jayaraj, F.U. Hartl, Pathways of cellular proteostasis in aging and
829 disease, *J. Cell Biol.* 217 (2018) 51–63. <https://doi.org/10.1083/jcb.201709072>.
- 830 [26] T. Clausen, C. Southan, M. Ehrmann, The HtrA family of proteases: implications for
831 protein composition and cell fate, *Mol. Cell.* 10 (2002) 443–455.
832 [https://doi.org/10.1016/s1097-2765\(02\)00658-5](https://doi.org/10.1016/s1097-2765(02)00658-5).
- 833 [27] J. Skorko-Glonek, D. Zurawa-Janicka, T. Koper, M. Jarzab, D. Figaj, P. Glaza, B.
834 Lipinska, HtrA protease family as therapeutic targets, *Curr. Pharm. Des.* 19 (2013) 977–
835 1009. <https://doi.org/10.2174/1381612811319060003>.
- 836 [28] T. Clausen, M. Kaiser, R. Huber, M. Ehrmann, HTRA proteases: regulated proteolysis in
837 protein quality control, *Nat. Rev. Mol. Cell Biol.* 12 (2011) 152–162.
838 <https://doi.org/10.1038/nrm3065>.
- 839 [29] L. Chatre, D.S.F. Biard, A. Sarasin, M. Ricchetti, Reversal of mitochondrial defects with
840 CSB-dependent serine protease inhibitors in patient cells of the progeroid Cockayne
841 syndrome, *Proc. Natl. Acad. Sci. U.S.A.* 112 (2015) E2910-2919.
842 <https://doi.org/10.1073/pnas.1422264112>.
- 843 [30] T. Darreh-Shori, S. Rezaeianyazdi, E. Lana, S. Mitra, A. Gellerbring, A. Karami, N.
844 Bogdanovic, C.U. Lithner, B. Winblad, H. Behbahani, Increased Active OMI/HTRA2
845 Serine Protease Displays a Positive Correlation with Cholinergic Alterations in the
846 Alzheimer’s Disease Brain, *Mol. Neurobiol.* 56 (2019) 4601–4619.
847 <https://doi.org/10.1007/s12035-018-1383-3>.
- 848 [31] S. Kang, T. Fernandes-Alnemri, E.S. Alnemri, A novel role for the mitochondrial
849 HTRA2/OMI protease in aging, *Autophagy.* 9 (2013) 420–421.
850 <https://doi.org/10.4161/auto.22920>.
- 851 [32] N. Moiso, K. Klupsch, V. Fedele, P. East, S. Sharma, A. Renton, H. Plun-Favreau, R.E.
852 Edwards, P. Teismann, M.D. Esposti, A.D. Morrison, N.W. Wood, J. Downward, L.M.
853 Martins, Mitochondrial dysfunction triggered by loss of HtrA2 results in the activation of
854 a brain-specific transcriptional stress response, *Cell Death Differ.* 16 (2009) 449–464.
855 <https://doi.org/10.1038/cdd.2008.166>.
- 856 [33] Z. Chen, L. Huang, A. Tso, S. Wang, X. Fang, K. Ouyang, Z. Han, Mitochondrial
857 Chaperones and Proteases in Cardiomyocytes and Heart Failure, *Front Mol Biosci.* 8
858 (2021) 630332. <https://doi.org/10.3389/fmolb.2021.630332>.
- 859 [34] C. Spiess, A. Beil, M. Ehrmann, A temperature-dependent switch from chaperone to
860 protease in a widely conserved heat shock protein, *Cell.* 97 (1999) 339–347.
861 [https://doi.org/10.1016/s0092-8674\(00\)80743-6](https://doi.org/10.1016/s0092-8674(00)80743-6).
- 862 [35] Y. Toyama, R.W. Harkness, T.Y.T. Lee, J.T. Maynes, L.E. Kay, Oligomeric assembly
863 regulating mitochondrial HtrA2 function as examined by methyl-TROSY NMR, *Proc Natl*
864 *Acad Sci U S A.* 118 (2021) e2025022118. <https://doi.org/10.1073/pnas.2025022118>.

- 865 [36] H.-G. Goo, M.K. Jung, S.S. Han, H. Rhim, S. Kang, HtrA2/Omi deficiency causes damage
866 and mutation of mitochondrial DNA, *Biochim Biophys Acta.* 1833 (2013) 1866–1875.
867 <https://doi.org/10.1016/j.bbamcr.2013.03.016>.
- 868 [37] H.-J. Park, S.-S. Kim, Y.-M. Seong, K.-H. Kim, H.G. Goo, E.J. Yoon, D.S. Min, S. Kang,
869 H. Rhim, Beta-amyloid precursor protein is a direct cleavage target of HtrA2 serine
870 protease. Implications for the physiological function of HtrA2 in the mitochondria, *J. Biol.*
871 *Chem.* 281 (2006) 34277–34287. <https://doi.org/10.1074/jbc.M603443200>.
- 872 [38] H.J. Huttunen, S.Y. Gu enette, C. Peach, C. Greco, W. Xia, D.Y. Kim, C. Barren, R.E.
873 Tanzi, D.M. Kovacs, HtrA2 regulates beta-amyloid precursor protein (APP) metabolism
874 through endoplasmic reticulum-associated degradation, *J. Biol. Chem.* 282 (2007) 28285–
875 28295. <https://doi.org/10.1074/jbc.M702951200>.
- 876 [39] D.A. Harris, *Cellular Biology of Prion Diseases*, *Clin Microbiol Rev.* 12 (1999) 429–444.
- 877 [40] A. Cuesta, A. Zambrano, M. Royo, A. Pascual, The tumour suppressor p53 regulates the
878 expression of amyloid precursor protein (APP), *Biochem. J.* 418 (2009) 643–650.
879 <https://doi.org/10.1042/BJ20081793>.
- 880 [41] D.S.F. Biard, E. Despras, A. Sarasin, J.F. Angulo, Development of new EBV-based
881 vectors for stable expression of small interfering RNA to mimic human syndromes:
882 application to NER gene silencing, *Mol. Cancer Res.* 3 (2005) 519–529.
883 <https://doi.org/10.1158/1541-7786.MCR-05-0044>.
- 884 [42] P. Baumann, W. Thiele, N. Cremers, S. Muppala, J. Krachulec, M. Diefenbacher, O.
885 Kassel, G. Mudduluru, H. Allgayer, M. Frame, J.P. Sleeman, CD24 interacts with and
886 promotes the activity of c-src within lipid rafts in breast cancer cells, thereby increasing
887 integrin-dependent adhesion, *Cell Mol Life Sci.* 69 (2012) 435–448.
888 <https://doi.org/10.1007/s00018-011-0756-9>.
- 889 [43] A. Jaggupilli, E. Elkord, Significance of CD44 and CD24 as cancer stem cell markers: an
890 enduring ambiguity, *Clin Dev Immunol.* 2012 (2012) 708036.
891 <https://doi.org/10.1155/2012/708036>.
- 892 [44] Y. Chao, Q. Wu, M. Acquafondata, R. Dhir, A. Wells, Partial mesenchymal to epithelial
893 reverting transition in breast and prostate cancer metastases, *Cancer Microenviron.* 5
894 (2012) 19–28. <https://doi.org/10.1007/s12307-011-0085-4>.
- 895 [45] M.G. Dodson, J. Slota, C. Lange, E. Major, Distinction of the phenotypes of in vitro
896 anchorage-independent soft-agar growth and in vivo tumorigenicity in the nude mouse,
897 *Cancer Res.* 41 (1981) 1441–1446.
- 898 [46] J. Sapudom, T. Pompe, Biomimetic tumor microenvironments based on collagen matrices,
899 *Biomater Sci.* 6 (2018) 2009–2024. <https://doi.org/10.1039/c8bm00303c>.
- 900 [47] K.M. Yamada, E. Cukierman, Modeling Tissue Morphogenesis and Cancer in 3D, *Cell.*
901 130 (2007) 601–610. <https://doi.org/10.1016/j.cell.2007.08.006>.
- 902 [48] D.R. Taylor, N.M. Hooper, The prion protein and lipid rafts, *Mol. Membr. Biol.* 23 (2006)
903 89–99. <https://doi.org/10.1080/09687860500449994>.
- 904 [49] S. Patschan, H. Li, S. Brodsky, D. Sullivan, D.A. De Angelis, D. Patschan, M.S.
905 Goligorsky, Probing lipid rafts with proximity imaging: actions of proatherogenic stimuli,
906 *Am. J. Physiol. Heart Circ. Physiol.* 290 (2006) H2210-2219.
907 <https://doi.org/10.1152/ajpheart.01112.2005>.

- 908 [50] S. Runz, C.T. Mierke, S. Joumaa, J. Behrens, B. Fabry, P. Altevogt, CD24 induces
909 localization of beta1 integrin to lipid raft domains, *Biochem Biophys Res Commun.* 365
910 (2008) 35–41. <https://doi.org/10.1016/j.bbrc.2007.10.139>.
- 911 [51] C. Scheckel, A. Aguzzi, Prions, prionoids and protein misfolding disorders, *Nat. Rev.*
912 *Genet.* 19 (2018) 405–418. <https://doi.org/10.1038/s41576-018-0011-4>.
- 913 [52] B.E. Stopschinski, M.I. Diamond, The prion model for progression and diversity of
914 neurodegenerative diseases, *The Lancet Neurology.* 16 (2017) 323–332.
915 [https://doi.org/10.1016/S1474-4422\(17\)30037-6](https://doi.org/10.1016/S1474-4422(17)30037-6).
- 916 [53] M. Goedert, NEURODEGENERATION. Alzheimer's and Parkinson's diseases: The
917 prion concept in relation to assembled A β , tau, and α -synuclein, *Science.* 349 (2015)
918 1255555. <https://doi.org/10.1126/science.1255555>.
- 919 [54] M.-A. Wulf, A. Senatore, A. Aguzzi, The biological function of the cellular prion protein:
920 an update, *BMC Biol.* 15 (2017) 34. <https://doi.org/10.1186/s12915-017-0375-5>.
- 921 [55] M. Béland, J. Motard, A. Barbarin, X. Roucou, PrP(C) homodimerization stimulates the
922 production of PrP(C) cleaved fragments PrP^{Sc}1 and PrP^{Sc}2, *J Neurosci.* 32 (2012) 13255–
923 13263. <https://doi.org/10.1523/JNEUROSCI.2236-12.2012>.
- 924 [56] A. Orsi, L. Fioriti, R. Chiesa, R. Sitia, Conditions of endoplasmic reticulum stress favor
925 the accumulation of cytosolic prion protein, *J. Biol. Chem.* 281 (2006) 30431–30438.
926 <https://doi.org/10.1074/jbc.M605320200>.
- 927 [57] V. Mattei, P. Matarrese, T. Garofalo, A. Tinari, L. Gambardella, L. Ciarlo, V. Manganelli,
928 V. Tasciotti, R. Misasi, W. Malorni, M. Sorice, Recruitment of cellular prion protein to
929 mitochondrial raft-like microdomains contributes to apoptosis execution, *Mol Biol Cell.*
930 22 (2011) 4842–4853. <https://doi.org/10.1091/mbc.E11-04-0348>.
- 931 [58] R.N. Alves, R.P. Iglesia, M.B. Prado, M.I. Melo Escobar, J.M. Boccacino, C.F. de L.
932 Fernandes, B.P. Coelho, A.C. Fortes, M.H. Lopes, A New Take on Prion Protein
933 Dynamics in Cellular Trafficking, *Int J Mol Sci.* 21 (2020) E7763.
934 <https://doi.org/10.3390/ijms21207763>.
- 935 [59] K.S. Lee, A.C. Magalhães, S.M. Zanata, R.R. Brentani, V.R. Martins, M.A. Prado,
936 Internalization of mammalian fluorescent cellular prion protein and N-terminal deletion
937 mutants in living cells, *J Neurochem.* 79 (2001) 79–87. <https://doi.org/10.1046/j.1471-4159.2001.00529.x>.
- 939 [60] F. Béranger, A. Mangé, B. Goud, S. Lehmann, Stimulation of PrP(C) retrograde transport
940 toward the endoplasmic reticulum increases accumulation of PrP(Sc) in prion-infected
941 cells, *J Biol Chem.* 277 (2002) 38972–38977. <https://doi.org/10.1074/jbc.M205110200>.
- 942 [61] J. Ma, S. Lindquist, Wild-type PrP and a mutant associated with prion disease are subject
943 to retrograde transport and proteasome degradation, *Proc Natl Acad Sci U S A.* 98 (2001)
944 14955–14960. <https://doi.org/10.1073/pnas.011578098>.
- 945 [62] A. Tennstaedt, S. Pöpsel, L. Truebestein, P. Hauske, A. Brockmann, N. Schmidt, I. Irle,
946 B. Sacca, C.M. Niemeyer, R. Brandt, H. Ksiezak-Reding, A.L. Tirniceriu, R. Egensperger,
947 A. Baldi, L. Dehmelt, M. Kaiser, R. Huber, T. Clausen, M. Ehrmann, Human high
948 temperature requirement serine protease A1 (HTRA1) degrades tau protein aggregates, *J*
949 *Biol Chem.* 287 (2012) 20931–20941. <https://doi.org/10.1074/jbc.M111.316232>.
- 950 [63] Q. Chu, J.K. Diedrich, J.M. Vaughan, C.J. Donaldson, M.F. Nunn, K.-F. Lee, A.
951 Saghatelian, Htra1 Proteolysis of ApoE In Vitro Is Allele Selective, *J Am Chem Soc.* 138
952 (2016) 9473–9478. <https://doi.org/10.1021/jacs.6b03463>.

- 953 [64] S. Grau, A. Baldi, R. Bussani, X. Tian, R. Stefanescu, M. Przybylski, P. Richards, S.A.
 954 Jones, V. Shridhar, T. Clausen, M. Ehrmann, Implications of the serine protease HtrA1 in
 955 amyloid precursor protein processing, *Proc Natl Acad Sci U S A.* 102 (2005) 6021–6026.
 956 <https://doi.org/10.1073/pnas.0501823102>.
- 957 [65] M.-A. Déry, A.C. LeBlanc, Luman contributes to brefeldin A-induced prion protein gene
 958 expression by interacting with the ERSE26 element, *Sci Rep.* 7 (2017) 42285.
 959 <https://doi.org/10.1038/srep42285>.
- 960 [66] M.-A. Déry, J. Jodoin, J. Ursini-Siegel, O. Aleynikova, C. Ferrario, S. Hassan, M. Basik,
 961 A.C. LeBlanc, Endoplasmic reticulum stress induces PRNP prion protein gene expression
 962 in breast cancer, *Breast Cancer Res.* 15 (2013) R22. <https://doi.org/10.1186/bcr3398>.
- 963 [67] T. Jin, Y. Gu, G. Zanusso, M. Sy, A. Kumar, M. Cohen, P. Gambetti, N. Singh, The
 964 chaperone protein BiP binds to a mutant prion protein and mediates its degradation by the
 965 proteasome, *J Biol Chem.* 275 (2000) 38699–38704.
 966 <https://doi.org/10.1074/jbc.M005543200>.
- 967 [68] K.-W. Park, G. Eun Kim, R. Morales, F. Moda, I. Moreno-Gonzalez, L. Concha-
 968 Marambio, A.S. Lee, C. Hetz, C. Soto, The Endoplasmic Reticulum Chaperone
 969 GRP78/BiP Modulates Prion Propagation in vitro and in vivo, *Sci Rep.* 7 (2017) 44723.
 970 <https://doi.org/10.1038/srep44723>.
- 971 [69] C. Hetz, M. Russelakis-Carneiro, S. Wälchli, S. Carboni, E. Vial-Knecht, K. Maundrell,
 972 J. Castilla, C. Soto, The disulfide isomerase Grp58 is a protective factor against prion
 973 neurotoxicity, *J Neurosci.* 25 (2005) 2793–2802.
 974 <https://doi.org/10.1523/JNEUROSCI.4090-04.2005>.
- 975 [70] F. Edenhofer, R. Rieger, M. Famulok, W. Wendler, S. Weiss, E.L. Winnacker, Prion
 976 protein PrPc interacts with molecular chaperones of the Hsp60 family, *J Virol.* 70 (1996)
 977 4724–4728. <https://doi.org/10.1128/JVI.70.7.4724-4728.1996>.
- 978 [71] Y. Shen, M. Ding, Z. Xie, X. Liu, H. Yang, S. Jin, S. Xu, Z. Zhu, Y. Wang, D. Wang, L.
 979 Xu, X. Zhou, P. Wang, J. Bi, Activation of Mitochondrial Unfolded Protein Response in
 980 SHSY5Y Expressing APP Cells and APP/PS1 Mice, *Front Cell Neurosci.* 13 (2019) 568.
 981 <https://doi.org/10.3389/fncel.2019.00568>.
- 982 [72] A.S. Klymchenko, R. Kreder, Fluorescent probes for lipid rafts: from model membranes
 983 to living cells, *Chem. Biol.* 21 (2014) 97–113.
 984 <https://doi.org/10.1016/j.chembiol.2013.11.009>.
- 985 [73] I. Levental, K.R. Levental, F.A. Heberle, Lipid Rafts: Controversies Resolved, Mysteries
 986 Remain, *Trends Cell Biol.* 30 (2020) 341–353. <https://doi.org/10.1016/j.tcb.2020.01.009>.
- 987 [74] P. Sharma, R. Varma, R.C. Sarasij, null Ira, K. Gousset, G. Krishnamoorthy, M. Rao, S.
 988 Mayor, Nanoscale organization of multiple GPI-anchored proteins in living cell
 989 membranes, *Cell.* 116 (2004) 577–589. [https://doi.org/10.1016/s0092-8674\(04\)00167-9](https://doi.org/10.1016/s0092-8674(04)00167-9).
- 990 [75] V. Lewis, N.M. Hooper, The role of lipid rafts in prion protein biology, *Front Biosci*
 991 (Landmark Ed). 16 (2011) 151–168. <https://doi.org/10.2741/3681>.
- 992 [76] C.T. Mierke, N. Bretz, P. Altevogt, Contractile forces contribute to increased
 993 glycosylphosphatidylinositol-anchored receptor CD24-facilitated cancer cell invasion, *J.*
 994 *Biol. Chem.* 286 (2011) 34858–34871. <https://doi.org/10.1074/jbc.M111.245183>.
- 995 [77] E. An, S. Sen, S.K. Park, H. Gordish-Dressman, Y. Hathout, Identification of novel
 996 substrates for the serine protease HTRA1 in the human RPE secretome, *Invest Ophthalmol*
 997 *Vis Sci.* 51 (2010) 3379–3386. <https://doi.org/10.1167/iovs.09-4853>.

- 998 [78] S. Grau, P.J. Richards, B. Kerr, C. Hughes, B. Caterson, A.S. Williams, U. Junker, S.A.
999 Jones, T. Clausen, M. Ehrmann, The role of human HtrA1 in arthritic disease, *J Biol*
1000 *Chem.* 281 (2006) 6124–6129. <https://doi.org/10.1074/jbc.M500361200>.
- 1001 [79] A. Tsuchiya, M. Yano, J. Tocharus, H. Kojima, M. Fukumoto, M. Kawaichi, C. Oka,
1002 Expression of mouse HtrA1 serine protease in normal bone and cartilage and its
1003 upregulation in joint cartilage damaged by experimental arthritis, *Bone.* 37 (2005) 323–
1004 336. <https://doi.org/10.1016/j.bone.2005.03.015>.
- 1005 [80] G. Kaur, D. Stallmann, N. Schanze, R. Laumann, L.A. Heger, J. Steinfurt, P. Stachon, K.
1006 Peter, C. Bode, M. Moser, I. Ahrens, D. Duerschmied, M. Hortmann, Extracellular HtrA2
1007 Induces Apoptosis in Human Umbilical Vein Endothelial Cells, *Int J Mol Sci.* 20 (2019)
1008 E5446. <https://doi.org/10.3390/ijms20215446>.
- 1009 [81] D.S. Biard, New Insight on Entangled DNA Repair Pathways: Stable Silenced Human
1010 Cells for Unraveling the DDR Jigsaw, *DNA Repair - On the Pathways to Fixing DNA*
1011 *Damage and Errors.* (2011). <https://doi.org/10.5772/23681>.
- 1012 [82] D.S.F. Biard, Untangling the relationships between DNA repair pathways by silencing
1013 more than 20 DNA repair genes in human stable clones, *Nucleic Acids Res.* 35 (2007)
1014 3535–3550. <https://doi.org/10.1093/nar/gkm195>.
- 1015 [83] J.-P. Vert, N. Foveau, C. Lajaunie, Y. Vandenbrouck, An accurate and interpretable model
1016 for siRNA efficacy prediction, *BMC Bioinformatics.* 7 (2006) 520.
1017 <https://doi.org/10.1186/1471-2105-7-520>.
- 1018 [84] D.S. Biard, P. Kannouche, C. Lannuzel-Drogou, P. Mauffrey, F. Apiou, J.F. Angulo,
1019 Ectopic expression of (Mm)Kin17 protein inhibits cell proliferation of human tumor-
1020 derived cells, *Exp. Cell Res.* 250 (1999) 499–509. <https://doi.org/10.1006/excr.1999.4515>.
- 1021 [85] D.S. Biard, Y. Saintigny, M. Maratrat, M.C. Vozenin, M. Martin, F. Daburon, J.F. Angulo,
1022 Differential expression of the HsKin17 protein during differentiation of in vitro
1023 reconstructed human skin, *Arch. Dermatol. Res.* 289 (1997) 448–456.
1024 <https://doi.org/10.1007/s004030050220>.
- 1025 [86] Z. Ji, C.N. Njauw, M. Taylor, V. Neel, K.T. Flaherty, H. Tsao, p53 rescue through HDM2
1026 antagonism suppresses melanoma growth and potentiates MEK inhibition, *J. Invest.*
1027 *Dermatol.* 132 (2012) 356–364. <https://doi.org/10.1038/jid.2011.313>.
- 1028 [87] L. Girnita, A. Girnita, B. Brodin, Y. Xie, G. Nilsson, A. Dricu, J. Lundeberg, J. Wejde, A.
1029 Bartolazzi, K.G. Wiman, O. Larsson, Increased expression of insulin-like growth factor I
1030 receptor in malignant cells expressing aberrant p53: functional impact, *Cancer Res.* 60
1031 (2000) 5278–5283.
- 1032 [88] J. Ikeda, M. Tada, N. Ishii, H. Saya, K. Tsuchiya, K. Okaichi, K. Mishima, Y. Sawamura,
1033 G. Fulci, T.J. Liu, E.G. Van Meir, Restoration of endogenous wild-type p53 activity in a
1034 glioblastoma cell line with intrinsic temperature-sensitive p53 induces growth arrest but
1035 not apoptosis, *Int. J. Cancer.* 94 (2001) 35–43. <https://doi.org/10.1002/ijc.1431>.
- 1036 [89] A. Brekman, K.E. Singh, A. Polotskaia, N. Kundu, J. Bargonetti, A p53-independent role
1037 of Mdm2 in estrogen-mediated activation of breast cancer cell proliferation, *Breast Cancer*
1038 *Res.* 13 (2011) R3. <https://doi.org/10.1186/bcr2804>.
- 1039 [90] D. Goldschneider, E. Horvilleur, L.-F. Plassa, M. Guillaud-Bataille, K. Million, E.
1040 Wittmer-Dupret, G. Danglot, H. de Thé, J. Bénard, E. May, S. Douc-Rasy, Expression of
1041 C-terminal deleted p53 isoforms in neuroblastoma, *Nucleic Acids Res.* 34 (2006) 5603–
1042 5612. <https://doi.org/10.1093/nar/gkl619>.

- 1043 [91] E.A. Afanasyeva, P. Mestdagh, C. Kumps, J. Vandesompele, V. Ehemann, J. Theissen,
1044 M. Fischer, M. Zapatka, B. Brors, L. Savelyeva, V. Sagulenko, F. Speleman, M. Schwab,
1045 F. Westermann, MicroRNA miR-885-5p targets CDK2 and MCM5, activates p53 and
1046 inhibits proliferation and survival, *Cell Death Differ.* 18 (2011) 974–984.
1047 <https://doi.org/10.1038/cdd.2010.164>.
- 1048 [92] B. Georger, G. Vassal, P. Opolon, C.M.F. Dirven, J. Morizet, L. Laudani, J. Grill, G.
1049 Giaccone, W.P. Vandertop, W.R. Gerritsen, V.W. van Beusechem, Oncolytic activity of
1050 p53-expressing conditionally replicative adenovirus AdDelta24-p53 against human
1051 malignant glioma, *Cancer Res.* 64 (2004) 5753–5759. [https://doi.org/10.1158/0008-](https://doi.org/10.1158/0008-5472.CAN-04-0499)
1052 [5472.CAN-04-0499](https://doi.org/10.1158/0008-5472.CAN-04-0499).
- 1053