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1 **Molecular basis for nuclear accumulation and targeting of the inhibitor of apoptosis**

2 **BIRC2**

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27

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29 **Abstract**

30 The inhibitor of apoptosis protein (IAP) BIRC2 regulates fundamental cell death and survival
31 signaling pathways. Aberrant activity of BIRC2 is associated with immunological diseases,
32 inflammation, and cancer. Unlike other IAPs, BIRC2 can localize to and function in the nucleus
33 through unknown mechanisms. Here, we show that BIRC2 accumulates in the nucleus via
34 binding of its second and third BIR domains, BIRC2_{BIR2} and BIRC2_{BIR3}, to histone H3 tail and
35 report the crystal structure of the BIRC2_{BIR3}:H3 complex. RNA-seq analysis of cells in which the
36 *BIRC2* gene is knocked out by CRISPR/Cas9 reveals that the genes involved in the interferon
37 and defense response signaling and the cell cycle regulation are most affected by depletion of
38 BIRC2. Overexpression of BIRC2 delays DNA damage repair and recovery of the cell cycle
39 progression and extends the etoposide-induced G2/M cell cycle arrest. We describe the
40 structural mechanism for targeting of BIRC2_{BIR3} by a potent but biochemically uncharacterized
41 small molecule inhibitor LCL161 and demonstrate that LCL161 disrupts the association of
42 endogenous BIRC2 with H3 and readily stimulates cell death in cancer cells. We further show
43 that LCL161 mediates degradation of BIRC2 in HIV-1 infected human CD4+ T cells. Our
44 findings provide mechanistic insights into the nuclear accumulation of and blocking BIRC2.

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51 **Introduction**

52 Human inhibitor of apoptosis (IAP) proteins are major regulators of apoptotic pathways and are
53 implicated in inflammatory and innate immune signaling. Aberrant activities particularly of
54 cellular IAPs (cIAPs) – cIAP1, also known as BIRC2, and cIAP2 – have been associated with
55 cancer, inflammation and immunopathogenesis¹⁻³. Acute or latent human immunodeficiency
56 virus type 1 (HIV-1) infection increases the expression of IAPs⁴ leading to the inhibition of HIV-1
57 transcription⁵⁻⁷. cIAPs contain three baculovirus IAP repeats (BIRs), a ubiquitin associating
58 (UBA) domain, a CARD domain, and a RING finger (Fig. 1a). The second and third BIR repeats
59 of cIAPs form complexes with caspases, whereas the RING finger of cIAPs functions as an E3
60 ubiquitin ligase, which mediates autoubiquitination and ubiquitination of protein substrates⁸⁻¹⁰.
61 The anti-apoptotic activity of cIAPs is countered by IAP antagonists, such as second
62 mitochondria-derived activator of apoptosis (Smac)¹¹⁻¹³. The upregulation of IAPs has been
63 shown to reduce autophagy through the ubiquitination of BECN1, inhibit apoptosis through the
64 antagonization, ubiquitination and neddylation of caspases, and increase the ubiquitination of
65 RIPK1^{1,2,9,14}. The latter promotes pro-survival NF- κ B signaling while also inhibiting the assembly
66 of distinct RIPK1 complexes that regulate cell death. Beyond the regulation of apoptosis, cIAPs
67 were found to promote cell proliferation, migration and invasion and mediate the cell cycle,
68 chromosome segregation in mitosis, DNA damage response and transcription¹⁵⁻²⁰.

69 Unlike other IAP family members that are found primarily in the cytosolic fraction, BIRC2
70 can localize to the cell nucleus through an unknown mechanism^{21,22}. Furthermore, because
71 BIRC2 is overexpressed in response to HIV-1 infection and in various cancers, it has emerged
72 as an attractive therapeutic target²³⁻²⁸. Among many cIAP targeting small molecule compounds
73 developed in the past few years, LCL161, designed to mimic Smac, has received considerable
74 attention²⁵. LCL161 is characterized by favorable pharmacological properties and minor toxicity
75 and is currently being investigated in many pre-clinical and phase I and phase II clinical
76 studies^{25,26}. It shows potent anti-cancer activity in at least two dozen tumor types and sensitizes

77 cancer cells to conventional chemotherapy with cisplatin and vincristine^{25,26}, yet our
78 understanding of the biochemical mechanisms underlying the LCL161 effect is lacking.

79 Here, we show that BIRC2 engages with chromatin through binding to histone H3 tail
80 and elucidate the mechanistic details and biological consequences of this engagement. We also
81 describe the structural basis for targeting of BIRC2 by LCL161 and highlight the molecular
82 mechanism by which LCL161 triggers cell death in cancerous and HIV-1-infected T cells.

83

84 **Results and Discussion**

85 **BIRC2 recognizes histone tails**

86 The association of BIRC2 with chromatin was originally observed in salt gradient fractionation of
87 the isolated nuclei from SW480 cells and proximity ligation assay (PLA). Endogenous BIRC2
88 was detected in the nuclear fraction containing 0.3-0.5 M NaCl (Fig. 1b), and it was bound to
89 histone H3 in PLA (Fig. 1c, d). The histone binding function of BIRC2 was substantiated by
90 pulldown experiments using BIR domains of BIRC2 and histone peptides (Fig. 1e). The third
91 BIR domain (BIRC2_{BIR3}) bound to the N-terminus of histone H3 (residues 1-22 of H3) but did not
92 recognize other histones. The second BIR domain (BIRC2_{BIR2}) associated mostly with H3 and to
93 a lesser extent with the N-termini of H4 and H2A, whereas the first BIR domain (BIRC2_{BIR1}) did
94 not show histone binding activity. The histone selectivity, i.e. the strong binding to H3 and
95 weaker binding to H4 and H2A, was maintained in the construct containing all three BIRs
96 (BIRC2_{3BIR}), which represented a unique histone reader capable of recognizing all but H2B tails.
97 To characterize the binding of BIRs in detail, we produced ¹⁵N-labelled domains and monitored
98 their interactions with the H3₁₋₁₂ (residues 1-12 of H3) peptide by NMR spectroscopy (Fig. 1f).
99 ¹H,¹⁵N HSQC (heteronuclear single quantum coherence) spectra of BIRs were recorded while
100 the H3 peptide was added stepwise to the NMR samples. Large chemical shift perturbations
101 (CSPs) in the spectra of BIRC2_{BIR3} and BIRC2_{BIR2} were induced by the peptide and indicated
102 formation of the BIR-H3 complexes. CSPs were in the slow exchange regime on the NMR

103 timescale for BIRC2_{BIR3} and the intermediate exchange regime for BIRC2_{BIR2} and suggested
104 tight binding to both domains. In agreement, a 400 nM binding affinity of BIRC2_{BIR3} for the H3₁₋₁₂
105 peptide and a 720 nM binding affinity of BIRC2_{BIR2} for the H3₁₋₁₂ peptide was measured in
106 fluorescence assays (Fig. 1g, h and Suppl. Fig. 1). These K_d values were in the range of binding
107 affinities of other histone binding modules²⁹⁻³¹, supporting the finding that BIRs are epigenetic
108 readers with selectivity for H3.

109

110 **Molecular mechanism for the BIRC2_{BIR3} association with H3**

111 To gain insight into the molecular basis for the recognition of the H3 tail, we co-crystallized
112 BIRC2_{BIR3} and H3₁₋₁₂ peptide and obtained a 1.8 Å resolution crystal structure of the
113 BIRC2_{BIR3}:H3 complex (Fig. 1i-k and Suppl. Table 1). In the complex, BIRC2_{BIR3} folds into a
114 compact globular structure consisting of five short α -helices, a three-stranded anti-parallel β -
115 sheet, and a zinc-binding cluster (Fig. 1i). The histone peptide is bound in a groove formed by
116 the β 3 strand, α 3 helix and the β 3- α 3 loop. Analysis of the structure reveals an extensive
117 network of hydrogen bonding, hydrophobic and electrostatic contacts that stabilize the complex.
118 Ala1 of the H3 peptide occupies a highly negatively charged pocket lined by the side chains of
119 E317, D320 and E325 of the protein (Fig. 1i, j). The N-terminal amino group of Ala1 of H3 is
120 restrained through the hydrogen bonds with the carboxyl group of D320 and the backbone
121 carbonyl group of C315 (Fig. 1k). The backbone carbonyl of Ala1 of H3 forms a hydrogen bond
122 with the indolyl nitrogen atom of W329, whereas the backbone amino and carbonyl groups of
123 Arg2 of H3 are involved in hydrogen bonding contacts with the backbone carbonyl and amino
124 groups of R314. The sidechain ϵ -amino moiety of Lys4 donates a hydrogen bond to the carboxyl
125 group of D303, and the backbone amino group of Lys4 is hydrogen bonded to the carbonyl
126 oxygen atom of G312.

127 Overlay of the structures of BIRC2_{BIR3} in complex with H3, Smac and caspase-9 reveals
128 that these ligands occupy the same binding site of BIRC2_{BIR3} (Suppl. Fig. 2). The position of the
129 backbone and even various side chain atoms of the three bound to BIRC2_{BIR3} sequences –
130 ARTK of H3, AVPI of Smac, and ATPF of caspase-9 – is remarkably similar, despite the fact
131 that only the first alanine residue is conserved. The deletion of Ala1/Arg2 in the histone H3
132 peptide (H₃₋₃₃) notably reduced binding of BIRC2_{BIR2}, BIRC2_{BIR3} and BIRC2_{3BIR} (Fig. 2a).
133 Posttranslational modifications (PTMs), commonly found in histone H3, including methylation,
134 acetylation and crotonylation of lysine residues, did not alter binding of BIRs, but methylation of
135 Arg2 reduced binding of BIRC2_{BIR3} to H3 (Suppl. Fig. 3). Yet, the most notable PTM-induced
136 effect was caused by phosphorylation of Thr3, which abrogated binding of BIRC2_{BIR3} but only
137 slightly reduced binding of BIRC2_{BIR2}, as seen in pull down assays and NMR titration
138 experiments (Fig. 2b-d). The structure of the BIRC2_{BIR3}-H3 complex provides an explanation to
139 the observed sensitivity of BIRC2_{BIR3}. Thr3 is in close proximity to the negatively charged Ala1-
140 binding pocket, therefore the presence of the negatively charged phosphate group should lead
141 to electrostatic repulsion. Additionally, phosphorylation of Thr3 is unfavorable due to steric
142 clashes with the bulky side chain of W329, the position of which is fixed as it forms the hydrogen
143 bond with the carbonyl group of Ala1 (Fig. 1k). In contrast, BIRC2_{BIR2} contains less bulky H243
144 in place of W329, which may tolerate Thr3 phosphorylation, especially if it is at least partially
145 protonated.

146

147 **BIRs mediate histone and DNA binding functions of BIRC2**

148 The importance of caging Ala1 of H3 for the interaction of BIRC2_{BIR3} and BIRC2_{BIR2} with the
149 histone tail was corroborated by mutagenesis. We generated D320A and E325A mutants of
150 BIRC2_{BIR3} and the corresponding D234A and E239A mutants of BIRC2_{BIR2} and tested them by
151 NMR (Fig. 2e-g) and pulldown assays (Fig. 2h, i). As shown in Fig. 2e-i, substitution of the
152 negatively charged residues with an alanine abrogated binding of individual BIRC2_{BIR2} and

153 BIRC2_{BIR3} to H3 and H3T3ph, however, mutations in both domains (D234A/D320A) concurrently
154 were needed to disrupt binding of the triple BIRC2_{3BIR} (Fig. 2i). Notably, the BIRC2_{BIR1} sequence
155 contains positively charged lysine residues in place of E317 and E325 in BIRC2_{BIR3} (Fig. 2j),
156 which likely accounts for the inability of BIRC2_{BIR1} to recognize H3 (Fig. 1e, f). As genetic
157 mutations within BIRC2_{BIR2} and BIRC2_{BIR3} have been identified in various cancers, we also
158 generated a set of cancer-relevant mutants and examined their histone binding activities in
159 pulldown assays (Suppl. Fig. 4). The cancer relevant mutations in BIRC2_{BIR3}, including L313F,
160 W316R and D320N, eliminated binding to H3, and the BIRC2_{BIR2} H243W mutant lost its ability to
161 tolerate Thr3 phosphorylation, pointing to a potential role of the BIR-H3 interactions in
162 oncogenesis.

163 A number of chromatin-binding proteins have been shown to associate with both histone
164 tails and DNA³². We tested whether BIRC2 has a DNA binding function using electrophoretic
165 mobility shift assays (EMSA). Increasing amounts of BIRC2_{BIR1}, BIRC2_{BIR2} and BIRC2_{BIR3} were
166 incubated with 601 DNA, and the reaction mixtures were resolved on a native polyacrylamide
167 gel (Fig. 2k-m). A shift of the free 601 DNA indicated formation of the BIRC2_{BIR1}:DNA complex,
168 whereas no DNA binding activity was detected for BIRC2_{BIR2} and BIRC2_{BIR3}. These data imply
169 that BIRC2 can bivalently engage with chromatin through binding of BIRC2_{BIR2} and BIRC2_{BIR3} to
170 H3 and binding of BIRC2_{BIR1} to DNA.

171

172 **BIRC2 regulates the cell defense and cell cycle signaling**

173 To identify the genes regulated by BIRC2, we knocked out endogenous BIRC2 in the MCF-7
174 breast cancer cell line using CRISPR/Cas9 and two sgRNAs targeting BIRC2 (Suppl. Fig. 5)
175 and extracted total RNA for next generation sequencing. RNA-seq analysis identified 526 genes
176 that were upregulated and 443 genes downregulated in the cells expressing BIRC2 sgRNAs
177 (Fig. 3a and Suppl. Table 2). Gene Ontology (GO) analysis revealed that the genes involved in
178 the interferon and defense response signaling pathways and the cell cycle regulation were most

179 affected by the depletion of BIRC2 (Fig. 3b and Suppl. Table 3). In support of GO analysis, in
180 the interferon- γ (IFN- γ) stimulated *ex-vivo* differentiated normal peripheral blood monocytes,
181 inhibition of IAPs with the XIAP inhibitor embelin led to an increase of interleukin-6 (IL-6), as
182 observed in the cytokines antibody array (Fig. 3c). We also investigated DNA damage response
183 and the cell cycle regulation in BIRC2-overexpressing HeLa cells (Fig. 3d-f). Cells were briefly
184 treated with a low concentration of etoposide until the DNA damage marker γ H2AX was
185 detected (Fig. 3e, f) and then cultured in a drug-free medium to allow for DNA repair and the
186 restoration of the normal cell cycle. Overexpression of BIRC2 delayed the DNA damage repair
187 judging by the persistent level of γ H2AX in western blot and flow cytometry assays, even 6 h
188 after the drug removal (Fig. 3e, f). The delay in the DNA damage repair was further
189 corroborated by a flow cytometry analysis of the cell cycle (Fig. 3g). Overexpression of BIRC2
190 delayed the recovery of the cell cycle progression and extended the etoposide-induced G2/M
191 cell cycle arrest (Fig. 3g, h).

192

193 **LCL161 binds to BIRC2_{BIR3} and BIRC2_{BIR2} but not to BIRC2_{BIR1}**

194 Among several Smac mimetic compounds developed in the past few years, LCL161 (Fig. 4a)
195 displays one of the most promising anti-proliferation and bioavailability properties^{25,26,33}. While
196 therapeutic potency of LCL161 has been established in pre-clinical and clinical studies, little is
197 known about its binding mechanism. To better understand the molecular basis underlying its
198 inhibitory activity, we investigated how LCL161 interacts with each BIR domain of BIRC2.
199 LCL161 was titrated into NMR samples of individual ¹⁵N-labelled BIRC2_{BIR1}, BIRC2_{BIR2} and
200 BIRC2_{BIR3} while ¹H,¹⁵N HSQC spectra were recorded, processed and overlaid (Fig. 4b-d).
201 LCL161 caused CSPs in the spectra of BIRC2_{BIR3} and BIRC2_{BIR2} but not in the spectrum of
202 BIRC2_{BIR1}, revealing that BIRC2_{BIR3} and BIRC2_{BIR2} but not BIRC2_{BIR1} are direct targets of the
203 compound. The K_d values, measured by tryptophan fluorescence, were found to be 490 nM for
204 BIRC2_{BIR3} and 2.2 μ M for BIRC2_{BIR2} (Fig. 4e, f).

205 The mechanistic details of the interaction with LCL161 were obtained from the crystal
206 structure of the BIRC2_{BIR3}:LCL161 complex (Fig. 4g, h and Suppl. Table 1). The complex is
207 stabilized by a large set of hydrogen bonds. These include contacts between the side chain
208 carboxyl groups of D320 and E325 and the methylamino group of LCL161 and between the
209 carbonyl group of alanine in LCL161 and the indolyl moiety of W329. The amino group and the
210 carbonyl group of cyclohexylglycine in LCL161 are hydrogen bonded with the backbone
211 carbonyl group and the amino group of R314, respectively. Additionally, the side chain
212 guanidino group of R314 is near the *p*-fluorobenzoyl group in LCL161, whereas the thiazole ring
213 of LCL161 is in close proximity to G312. LCL161 lays in the histone H3-binding site, ideally
214 tracing the geometry of the H3 peptide (Fig. 4i, j), and overlays with GDC0152, another BIRC2
215 inhibitor (Suppl. Fig. 6).

216

217 **LCL161 disrupts binding of BIRC2 to H3 and stimulates cell death**

218 The impact of LCL161 on endogenous BIRC2 was tested in colon adenocarcinoma SW480
219 cells. As shown in Figures 5a and 5b, treatment of the cells with LCL161 disrupted the
220 association of BIRC2 with histone H3 in PLA (Fig. 5a, b). This result can be attributed to the
221 binding of LCL161 to BIRC2_{BIR3} and BIRC2_{BIR2}, as incubation of the isolated GST-tagged
222 BIRC2_{BIR3} and BIRC2_{BIR2} with LCL161 abolished binding of BIRC2_{BIR3} to the H3 peptide and
223 decreased binding of BIRC2_{BIR2} in pulldown experiments (Fig. 5c). Western blot analysis of
224 BIRC2 following the treatment of SW480 cells with LCL161 for 1 hr showed that LCL161
225 induces BIRC2 degradation (Fig. 5d). LCL161 treatment of IFN- γ stimulated *ex-vivo*
226 differentiated normal human peripheral blood monocytes led to an increase in IL-6 and RANTES
227 expression, supporting the cytokines antibody arrays data (Fig. 3c) and BIRC2 degradation (Fig.
228 5e-g).

229 To explore the effect of LCL161 on pro-survival function of BIRC2, we monitored cell
230 survival following treatment with LCL161, tumor necrosis factor α (TNF α), or both in a

231 colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. Consistent with its
232 ability to divert tumor necrosis factor receptor 1 (TNFR1) signaling toward cell death pathways,
233 LCL161 sensitized murine embryonic fibroblasts (MEFs) and human glioma LN18 cells to killing
234 by TNF α (Fig. 5h, i).

235

236 **LCL-161 mediates BIRCs degradation in HIV-1-infected CD4+ T cells**

237 The mechanism by which LCL161 promotes cell death was examined in *in vitro* HIV-1 infected
238 human CD4+ T cells (HIV-T_{CM}). As identified previously, these HIV-T_{CM} have increased
239 expression of BIRC2 and BIRC3 (cIAP2), the tumor necrosis factor (TNF) receptor superfamily
240 (TNFRSF) death receptor FAS, and both membrane-bound and soluble FAS ligand (FASLG)
241 compared with T_{CM} (Fig. 5j)^{34,35} and the majority express the lineage markers CD45RO, CD62L,
242 CCR7 and CD27 while not expressing high levels of activation (HLA-DR and CD25) or cellular
243 proliferation (MKI67) markers after 30 d culture with IL-7 (Suppl. Fig. 7). Under physiologic
244 conditions, the interaction of TNFRSF with its cognate ligand leads to receptor trimerization and
245 the recruitment of FADD and caspase-8 to form a prominent death inducing signaling complex
246 (DISC). IAPs can inhibit this effect through the post-translational ubiquitination of RIPK1 which
247 activates canonical NF- κ B pro-survival signaling pathways^{36,37}. In cells with high expression of
248 FAS and FASLG, loss of IAPs can result in the deubiquitination and autophosphorylation of
249 RIPK1 at Ser¹⁶⁶ that activates and increases RIPK1 recruitment to the FAS DISC alongside
250 FADD, caspase-8 and cFLIP isoforms^{38,39}. As the activation of FAS-induced apoptosis is known
251 to be inhibited by IAPs, and FAS, FASLG, BIRC2, and BIRC3 are upregulated in HIV-T_{CM}, we
252 treated HIV-T_{CM} and uninfected T_{CM} with LCL161. LCL161 induced the rapid degradation of
253 BIRC2 and BIRC3 in both HIV-T_{CM} and the uninfected cells (Fig. 5k). This correlated with the
254 deubiquitination and Ser¹⁶⁶ phosphorylation, and thus activation of RIPK1 (Fig. 5k, l). We then
255 examined the FAS DISC using co-immunoprecipitation for FAS. We readily detected the
256 association of FADD, pro-caspase-8, activated caspase-8, RIPK1 (total, Ser¹⁶⁶ phosphorylated,

257 and the cleaved form [p39]), cFLIP_S, and the p43 fragment of cFLIP_L with FAS in HIV-T_{CM} after
258 LCL161 treatment (Fig. 5k), whereas FADD, cFLIP_S, and Ser¹⁶⁶ phosphorylated RIPK1 did not
259 associate with FAS in the LCL161 treated T_{CM}.

260 The formation of the FAS DISC in HIV-T_{CM} correlated with the cleavage of caspase-3,
261 the proteolysis of poly(ADP-ribose) polymerase 1 (PARP1) (Fig. 5m), an increase in cytoplasmic
262 histone-associated DNA fragments (mono- and oligonucleosomes) (Fig. 5n), the translocation of
263 phosphatidylserine from the inner to the outer leaflet (extracellular side) of the plasma
264 membrane, permeabilization of the plasma membrane (Suppl. Fig. 8), and an increase in lactate
265 dehydrogenase release from HIV-T_{CM} (Fig. 5n) but not in the uninfected cells, indicating that the
266 cleavage of pro-caspase-8 within the FAS DISC resulted in the execution of apoptosis in the
267 HIV-T_{CM}. This suggests that although RIPK1 was cleaved and cFLIP_S was recruited to the FAS
268 DISC, it was insufficient to prevent LCL161-mediated cell death⁴⁰. In agreement with previous
269 studies, a specific increase in HIV-T_{CM} cell death was not accompanied by an increase in HIV-1
270 p24 antigen release from infected cells (Suppl. Fig. 7c-d)^{6,41}. To confirm a role for the LCL161-
271 mediated degradation of BIRC2 in the observed cell death, we performed RNAi for *BIRC2*
272 (Suppl. Fig. 9a-b), which resulted in the death of HIV-T_{CM} but not T_{CM} (Suppl. Fig. 9c).
273 Collectively, these data demonstrate that the presence of BIRC2 inhibits FAS-mediated cell
274 death of HIV-T_{CM}, and that the LCL161-mediated degradation of BIRC2 in HIV-T_{CM} leads to the
275 assembly of the FAS DISC and subsequent cell death.

276 In conclusion, in this study we elucidate a mechanism by which BIRC2 localizes to the
277 cell nucleus. We found that BIRC2 engages with chromatin in a multivalent manner: while its
278 BIRC2_{BIR2} and BIRC2_{BIR3} domains robustly recognize histone H3 tail, BIRC2_{BIR1} binds to DNA.
279 We show that the nuclear pool of BIRC2 regulates a large set of the genes involved in the
280 interferon and defense response signaling pathways and the cell cycle regulation. In support,
281 immunoprecipitation and flow cytometry assays demonstrate that BIRC2 negatively regulates
282 the DNA damage repair and the recovery of the cell cycle progression and extends the

283 etoposide-induced G2/M cell cycle arrest. The crystal structure of BIRC2_{BIR3} in complex with a
284 small molecule inhibitor LCL161 along with binding affinities of BIRC2_{BIR2} and BIRC2_{BIR3} toward
285 LCL161 provide mechanistic insights into the inhibition of BIRC2, which is instrumental in the
286 development of a new generation of Smac-derived inhibitors of IAPs with the goal to kill cancer
287 and HIV-infected cells.

288

289 **Data availability**

290 Coordinates and structure factors have been deposited in the Protein Data Bank under
291 accession numbers 7TRL and 7TRM. All other relevant data supporting the key findings of this
292 study are available within the article, its Supplementary Information or from the corresponding
293 authors upon reasonable request. Source Data are provided with this paper.

294

295 **Code availability**

296 This paper does not report original code.

297

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310

311 **Author contributions**

312 A.H.T., Y.Y., S.C., G.R.C., B.J.K., H.X., J.C., M.A.M., A.Z. and T.A.H. performed experiments
313 and together with H.W., C.J.H., S.A.S., L.D., X.S. and T.G.K. analyzed the data. A.H.T. and
314 T.G.K. wrote the manuscript with input from all authors.

315

316 **Conflict of interest**

317 The authors declare no competing interests.

318

319 **Online Methods**

320 **Cell culture**

321 Human HeLa and SW480 cells were purchased from ATCC and cultured in Dulbecco's modified
322 Eagle medium (DMEM) (Cellgro) supplemented with 10% fetal bovine serum (FBS) (Sigma), 2
323 mM L-glutamine and 100 U ml⁻¹ penicillin/streptomycin. Human MCF-7 and LN18 cells and
324 murine embryonic fibroblasts were cultured in DMEM with high glucose (Invitrogen; CA, USA)
325 supplemented with 10% FBS (Sigma or Scientifix Life; VIC, Australia) and cells maintained at
326 37 °C in air supplemented with 5% CO₂. HIV-1 was prepared and infectivity calculated as
327 previously described³⁵. Whole blood was drawn from HIV-1-seronegative healthy male and
328 female volunteers, ages between 18 and 65 years, at UC San Diego Health Sciences using
329 protocols approved by the Human Research Protections Program of the University of California
330 San Diego in accordance with the requirements of the Code of Federal Regulations on the
331 Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56). All volunteers gave written
332 informed consent prior to their participation, all samples were de-identified, and donors
333 remained anonymous. Peripheral blood mononuclear cells (PBMC) were isolated from whole

334 blood by density gradient centrifugation over Ficoll-Paque Plus (GE Healthcare) and HIV-1
335 infected, resting CD4+ T cells (HIV-T_{CM}) were prepared from these PBMC and characterized as
336 previously described³⁵. Briefly, CD4+ T cells were isolated from PBMC using the CD4+ T cell
337 isolation kit (Miltenyi Biotec, Cat# 130-096-533) and incubated for 48 h in GM (RPMI 1640
338 supplemented with 10% [vol/vol] heat-inactivated FBS, 50 μM 2-sulfanyethan-1-ol [both Sigma],
339 100 μM non-essential amino acids, 1 mM sodium pyruvate, 0.1 mg/mL streptomycin, 100 U/mL
340 penicillin [all Gibco]) supplemented with 29 nM CCL19 (R&D Systems) before infection with 0.4
341 multiplicity of infection HIV-1_{NL4-3} (obtained from Malcolm Martin via the NIH HIV Reagent
342 Program and prepared and titered as previously described³⁵ for 3 h. Cells were washed then
343 plated at 5×10^5 cells/mL in GM supplemented with 250 ng/mL staphylococcal enterotoxin B
344 (Sigma) and 25 U/mL IL-2 (Roche) and cultured for 3 d in 5% CO₂ at 37 °C. Cells were then
345 washed and resuspended at 5×10^5 cells/mL in GM supplemented with 25 U/mL IL-2 and
346 cultured at 5% CO₂ at 37 °C. Every 2-3 days, cell concentration was adjusted back to 5×10^5
347 cells/mL with 25 U/mL IL-2-containing GM and supernatant HIV-1 p24 release was monitored
348 using the Perkin Elmer Alliance HIV p24 antigen ELISA. After 12 days memory CD4+ T cells
349 were isolated by negative selection using a memory CD4+ T cell isolation kit (Miltenyi Biotec,
350 Cat# 130-091-893). Memory CD4+ T cells were then cultured in RPMI 1640 supplemented with
351 10% (vol/vol) heat-inactivated FBS, 0.1 mg/mL streptomycin, 100 U/mL penicillin, and 1 ng/mL
352 IL-7 (R&D Systems) for 30 d at 37 °C, 5% CO₂. Phenotyping and phytohemagglutinin-M (PHA;
353 Sigma, Cat# L8902) inducible virus was assessed by flow cytometry, and quantification of HIV-1
354 integrated DNA was performed using a previously described nested Alu-LTR quantitative PCR
355 (qPCR) assay³⁵. LCL161 was obtained from Selleck Chemicals. Cell cytotoxicity was calculated
356 using a lactate dehydrogenase (LDH) release assay according to the manufacturer's protocol
357 (Takara Bio).

358

359 **Protein expression and purification**

360 The human BIRC2 BIR domains BIR1 (aa 37-123), BIR2 (aa 175-260) and BIR3 (aa 261-346)
361 were cloned into pGEX 6p-1 vectors. All constructs were expressed in *Escherichia coli*
362 BL21(DE3) RIL cells in ¹⁵N-labeled M9 minimal media or LB media supplemented with 50 μM
363 ZnCl₂. Cultures were grown to an OD₆₀₀ of 0.8 and induced with 0.5 mM IPTG for 18 hours at
364 16 °C. Bacteria were harvested by centrifugation and lysed by sonication. GST-tagged proteins
365 were purified on Pierce glutathione agarose beads (Thermo Scientific) using first 20 mM Tris-
366 HCl (pH 7.5), 500 mM NaCl, 0.5% Triton X-100 and 2 mM DTT buffer, and then 20 mM Tris-HCl
367 (pH 7.5), 150 mM NaCl, and 2 mM DTT buffer. The GST-tag was cleaved with PreScission
368 protease overnight at 4 °C. Proteins were further purified by size exclusion chromatography
369 (SEC) and concentrated in Millipore concentrators (Millipore). For crystallographic studies of
370 BIR3 and LCL-161, 20 mM Bis-Tris Propane (pH 7.5), 150 mM NaCl and 2 mM DTT was used
371 in SEC.

372

373 **Chromatin fractionation**

374 SW480 cells were lysed in 10 mM HEPES pH 7.8 buffer, supplemented with 10 mM KCl, 1.5
375 mM MgCl₂, 0.34 M sucrose, 0.2% (v/v) NP-40, 10% (v/v) glycerol and protease inhibitors
376 (Complete protease inhibitor cocktail tablet, Merck, 11697498001), and lysates were
377 centrifuged. The nuclear fraction (pellet) was suspended in 3 mM EDTA buffer, supplemented
378 with 0.2 EGTA and protease inhibitors, and centrifuged to separate supernatant (nucleoplasm)
379 from the pellet. The pellet was successively subjected to gradient salt extraction with increasing
380 concentrations of NaCl (0.1 M to 0.7 M) in 50 mM Tris buffer supplemented with 0.05% NP-40.
381 Proteins of nucleoplasm and chromatin-associated fractions were then quantified and subjected
382 to western blot analysis.

383

384 **PLA**

385 SW480 cells grown on No. 1.5 coverslips were fixed using 4% PFA in phosphate-buffered saline
386 for 10 min at room temperature. Cells were permeabilized by chilled methanol at -20 °C for 10
387 min. The PLA was carried out using Duolink protocol and anti-BIRC2 (Atlas Antibodies,
388 HPA005513), anti-histone H3 (Cell Signaling Technologies, 14269), anti-rabbit plus (Sigma-
389 Aldrich, DUO92002) and anti-mouse minus (Sigma-Aldrich, DUO92004) PLA probes and PLA
390 detection kit orange (Sigma-Aldrich, DUO92007) in the presence or absence of 10 µM LCL161.
391 Microscopy images were acquired using a Zeiss Axio Imager 2 (Zeiss GmbH) equipped with
392 ×20 0.46NA, ×40 0.6NA, and ×63 1.45 NA Plan Apochromatic objectives, and an mRM4 CCD
393 camera. Illumination was provided by an HXP120 metal halide lamp (Zeiss GmbH). Filter sets
394 used were 49 HE (DAPI; 488049-0000), 38 HE (Alexa488, 489038-0000) and 43 HE (Alexa568,
395 489043-0000). Image analysis was carried out using the ICY software
396 (<http://icy.bioimageanalysis.org>), on 10 randomly taken fields (based on DAPI). Nuclei were
397 segmented using the Active Contour plugin on DAPI signal; PLA were detected using the Spot
398 Detector plugin. Fluorescence signals were measured in maximal projections of unmodified
399 images. Western blotting of endogenous BIRC2 was performed before and after the treatment
400 of SW480 cells with 10 µM LCL161 for 1 hour. SW480 cells were lysed in RIPA buffer (50 mM
401 Tris HCl pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, supplemented
402 with a protease and phosphatase inhibitor cocktail). Anti BIRC2 and β-actin peroxidase
403 conjugated antibodies were from R&D system (Cat# AF8181) and Sigma-Aldrich (Cat# 3854).

404

405 **IFN-γ stimulation**

406 Human peripheral blood monocytes were obtained from healthy donors with informed consent,
407 purified and differentiated *ex vivo* into macrophages as previously described⁴². Macrophages
408 were then stimulated by 100 U/ml IFN-γ for 24 hrs with or without 10 µM embelin (Sigma-
409 Aldrich) or 10 µM LCL161 in RPMI 1640 medium (Lonza), 10% fetal calf serum (Lonza). The
410 supernatants were analyzed for the cytokines content by an antibody array (RayBiotech)

411 following the manufacturer protocol. The cytokines arrays were performed in duplicate. The
412 arrays include: positive control, positive control, negative control, negative control, GCSF, GM-
413 CSF, GRO, GRO- α , IL-1 α , IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IFN- γ , MCP-1,
414 MCP-3, MIG, RANTES, TGF- β , TNF- α , TNF- β , and positive control. IL-6 and RANTES
415 expression were quantified by RT-qPCR as previously described⁴². Western blotting of
416 endogenous BIRC2 was performed before and after the treatment of macrophages with 10 μ M
417 LCL161 for 24 hours as above.

418

419 **DNA damage assay**

420 HeLa cells were transfected with the pCI or pCI-BIRC2 vectors using JetPEI (Plyplus
421 transfection, Ozyme). Cells were then treated with 1 μ M etoposide for 3 hours, washed and
422 cultured for 0 to 48 hours in a drug-free medium. DNA damage was evaluated by western-blot
423 and immunofluorescence analysis of γ H2AX using anti-phospho-H2AX (Ser139) (Millipore #
424 DAM1546024, Merckmillipore). For immunofluorescence, cells were fixed using 4% PFA in PBS
425 for 10 min, washed, permeabilized for 10 min with 100% methanol at -20°C and washed again.
426 Nonspecific binding was blocked by 3% bovine serum albumin (BSA) for 1 hr. Samples were
427 then incubated overnight with anti- γ H2AX, washed four times, incubated with anti-rabbit Alexa
428 Fluor 660 for 1 hr at room temperature, rinsed in water and analyzed on BD KSR II flow
429 cytometer (BD Biosciences) (excitation: 488 nm laser light, detection: bandpass filter 620/20).
430 For the cell cycle analysis, cells were fixed in cold 70% ethanol for 30 min, washed, treated with
431 ribonuclease and stain with propidium iodide solution (25 μ g/mL) prior to flow cytometry analysis
432 (excitation: 488 nm laser light, detection: bandpass filter 620/20).

433

434 **NMR experiments**

435 NMR experiments were performed at 298K on a Varian INOVA 600 MHz spectrometer
436 equipped with a cryogenic probe. The NMR samples contained 0.1 mM uniformly ¹⁵N-labeled

437 wild-type or mutated BIR domains of BIRC2 in 20 mM Tris-HCl (pH 7) buffer supplemented with
438 150 mM NaCl, 5 mM DTT and 10% D₂O. Binding was characterized by monitoring chemical
439 shift changes in ¹H,¹⁵N HSQC spectra of the proteins induced by the addition of histone
440 peptides (aa 1-12 of H3, synthesized by Synpeptides) or LCL-161.

441

442 **Fluorescence spectroscopy**

443 Spectra were recorded at 25 °C on a Fluoromax-3 spectrofluorometer (HORIBA). The samples
444 containing 1 μM BIR domains of BIRC2 (in 20 mM Tris (pH 6.8), 150 mM NaCl and 1 mM DTT
445 buffer) and progressively increasing concentrations of peptides or LCL161 were excited at 295
446 nm. Emission spectra were recorded between 330 and 360 nm with a 0.5 nm step size and a 1
447 s integration time. The K_d values were determined using a nonlinear least-squares analysis and
448 the equation:

$$449 \quad \Delta I = \frac{\Delta I_{max} \left(([L] + [P] + K_d) - \sqrt{([L] + [P] + K_d)^2 - 4[P][L]} \right)}{2[P]}$$

450

451 where [L] is concentration of the peptide or inhibitor, [P] is concentration of the protein, ΔI is the
452 observed change of signal intensity, and ΔI_{max} is the difference in signal intensity of the free and
453 bound states of the protein. K_d values were averaged over three separate experiments, and
454 error was calculated as the standard deviation between the runs.

455

456 **X-ray crystallography**

457 The BIR3 domain of human BIRC2 (residues 261-346) was concentrated to 10 mg/ml and
458 incubated on ice in a 1:1.2 molar ratio with either the H3 peptide (aa 1-12) or LCL-161 for 1 h.
459 Crystals of the BIRC2_{BIR3}:H3 complex were grown using the sitting-drop vapor diffusion method
460 at 18 °C by mixing equal volumes of protein solution with well solution composed of 0.1 M SPG
461 buffer (2:7:7 molar ratio of succinic acid:sodium dihydrogen phosphate: glycine) pH 7.0,

462 supplemented with 25% PEG 1,500. Crystals of the BIRC2_{BIR3}:LCL161 complex were grown at
463 4 °C using the sitting-drop vapor diffusion method in 0.1 M Bis-Tris Propane (pH 7.5), 0.2 M
464 Sodium Citrate, and 20% PEG3350. X-ray diffraction data were collected from single crystals on
465 the CU Anschutz Medical Campus X-ray crystallography core facility Rigaku Micromax 007
466 high-frequency microfocus X-ray generator equipped with a Pilatus 200K 2D area detector. The
467 phase solutions for the BIRC2_{BIR3}:H3 (Phenix) and BIRC2_{BIR3}:LCL161 (CCP4) complexes were
468 obtained by molecular replacement using the BIRC2_{BIR3} structure (PDB ID: 3D9T with the
469 peptide, solvent and the zinc ion removed) as a search model. Indexing and scaling was
470 completed using HKL3000. Manual model building was performed using Coot⁴³, and the
471 structure was refined using Phenix⁴⁴. The final structure was verified on the PDB validation
472 server. The X-ray diffraction and structure refinement statistics are summarized in
473 Supplementary Table 1.

474

475 **Peptide pulldown assays**

476 1 µg biotinylated histone peptides (synthesized by CPC scientific) with different modifications
477 were incubated with 1 µg GST-fused BIRC2 BIR domains in binding buffer (50 mM Tris-HCl
478 pH7.5, 300 mM NaCl, 0.1% NP-40, and 1 mM PMSF) overnight with rotation at 4 °C.
479 Streptavidin magnetic beads (Pierce) were added to the mixture, and the mixture was incubated
480 for 1 h with rotation at 4 °C. The beads were then washed three times using a magnetic stand
481 and the bound proteins were analyzed using SDS-PAGE and Western blotting. Anti GST
482 antibodies were from Santa Cruz (Cat# sc-459, AB_631586).

483

484 **RNA-seq**

485 Total RNAs were extracted from MCF7 cells by RNeasy Plus Mini Kit (QIAGEN, Cat. 74136).
486 Illumina NovaSeq 6000 were used for generating polyA-plus 50 bp pair-end reads. Fastq reads
487 were mapped to hg38 human genome by HISAT2 (v2.1.0)⁴⁵ with -k 1. DEGs were calculated by

488 edgeR (v3.16.5)⁴⁶ with Exact test model (FDR<0.05). GO term enrichment was done by DAVID
489 6.8⁴⁷. Anti GAPDH antibodies were from Santa Cruz (sc-32233, RRID:AB_627679), and anti
490 BIRC2 antibodies were from Abcam (Cat# ab108361, RRID:AB_10862855).

491

492 **EMSA**

493 EMSAs were performed by mixing increasing amounts of BIR domains of BIRC2 with 0.25 pmol
494 of 601 DNA/lane in 25 mM Tris-HCl pH 7.5, 150 mM NaCl and 0.5 mM
495 ethylenediaminetetraacetic acid (EDTA) in a 10 μ L reaction volume. Reaction mixtures were
496 incubated at room temperature for 10 min (2 μ l of loading dye was added to each sample) and
497 loaded onto a 5% native polyacrylamide gel. Electrophoresis was performed in 0.2x Tris-borate-
498 EDTA (TBE) at 80-100 V on ice. The gels were stained with SYBR Gold (Thermo Fisher Sci)
499 and visualized by Blue LED (UltraThin LED Illuminator-GelCompany).

500

501 **MTT assays**

502 Two thousand MEF or LN18 cells were seeded into wells of 96 well plates. Equal volumes of
503 media containing LCL161 and/or TNF α were added to the cells and incubated for 24 h.
504 Tetrazolium (MTT) reduction by viable cells to produce formazan crystals was quantitated as a
505 measure of survival⁴⁸.

506

507 **Flow cytometry**

508 1×10^5 CD4+ T cells were harvested, washed in phosphate buffered saline (PBS)
509 supplemented with 1 mg/mL bovine serum albumin and 100 μ g/mL NaN₃ (FACS buffer) and
510 stained with the following fluorescently conjugated antibodies in FACS buffer for 30 mins at 4
511 $^{\circ}$ C: allophycocyanin (APC)-eFluor 780 conjugated anti-CD3 (Cat# 47-0036-41,
512 RRID:AB_10718679) or isotype control IgG (Cat# 47-4714-80, RRID:AB_1271993), eFluor 450
513 conjugated anti-CD4 (Cat# 48-0047-41, RRID:AB_1603232) or isotype control IgG (Cat# 48-

514 4714-80, RRID:AB_1271995), Super Bright 600 conjugated anti-CD25 (Cat# 63-0259-42,
515 RRID:AB_2637187) or isotype control IgG (Cat# 63-4714-80, RRID:AB_2637447), peridinin
516 chlorophyll protein (PerCp)-cyanine (Cy) 5.5 conjugated anti-CD25 (Cat# 45-0251-80,
517 RRID:AB_914323) or isotype control IgG (Cat# 45-4301-80, RRID: AB_906256), Super Bright
518 645 conjugated anti-CD27 (Cat# 64-0279-42, RRID:AB_2688222) or isotype control IgG (64-
519 4714-80, RRID:AB_2665350), phycoerythrin (PE)-Cy 7 conjugated anti-CD45RO (Cat# 25-
520 0457-42, RRID:AB_10718534) or isotype control IgG (25-4724-81, RRID:AB_470203), APC
521 conjugated anti-CD45RO (Cat# 17-0457-41, RRID:AB_1907398) or isotype control IgG (17-
522 4724-81, RRID:AB_470188), APC conjugated anti-CD45RA (Cat# 17-0458-42,
523 RRID:AB_1907398) or isotype control IgG (17-4732-81, RRID:AB_763656), fluorescein
524 isothiocyanate (FITC) conjugated anti-CD62L (Cat# 11-0629-41, RRID:AB_10669578) or
525 isotype control IgG (Cat# 11-4714-81, RRID:AB_470021), PE conjugated anti-CCR7 (Cat# 12-
526 1979-41, RRID:AB_10667886) or isotype control IgG Cat# 12-4321-80, RRID:AB_1834380),
527 and PE-Cy5.5 conjugated anti-HLA-DR (Cat# MHLDR18, RRID:AB_10372966) or isotype
528 control IgG (Cat# 35-4732-80, RRID:AB_11218699) (all from Invitrogen). Cells were then
529 washed in FACS buffer, then fixed and permeabilized using BD Cytotfix/Cytoperm (BD
530 Biosciences) for 30 mins at 4 °C followed by staining with APC conjugated anti-MKI67 (Cat# 17-
531 5699-41, RRID:AB_2573217) or isotype control IgG (Cat# 17-4714-81, RRID:AB_763650) (both
532 from Invitrogen) or PE-conjugated anti-HIV-1 core antigen (Beckman Coulter, Cat# 6604667,
533 RRID:AB_1575989) or isotype control IgG (Invitrogen, Cat# 12-4714-42, RRID:AB_1944423) for
534 30 mins at 4 °C. Cells were then washed in FACS buffer before blinded acquisition using a BD
535 Biosciences Aria II SORP flow cytometer using 405 nm laser excitation with a 605/40 BP filter to
536 detect Super Bright 600 and a 662/15 BP filter to detect Super Bright 645, a 488 nm laser
537 excitation with a 530/30 BP filter to detect FITC, a 585/15 BP filter to detect PE, a 680/60 BP
538 filter to detect PE-Cy5.5, and a 705/70 BP filter to detect PerCp-Cy5.5, a 561 nm yellow/green
539 laser and a 660/20 BP filter to detect APC and a 780/60 BP filter to detect PE-Cy7, and a 633

540 nm red laser with a 450/40 BP filter to detect eFluor 450 and a 780/60 BP filter to detect APC-
541 eFluor 780. Analysis was performed using FlowJo v. 10 (BD Biosciences) using FMO and INC
542 to determine gating strategy.

543 For cell death flow cytometry analysis of CD4⁺ T cells, 1×10^5 CD4⁺ T cells were
544 harvested and stained with Alexa Fluor 488-conjugated annexin V (Cat# A13201; Thermo
545 Fisher) and 1 µg/mL propidium iodide (Cat# P3566; Thermo Fisher) in 10 mM HEPES, 140 mM
546 NaCl, 2.5 mM CaCl₂, pH 7.4 staining for 15 mins at 4 °C. Samples were then diluted 1:4 in 10
547 mM HEPES, 140 mM NaCl, 2.5 mM CaCl₂, pH 7.4 before blinded acquisition using a BD
548 FACSCalibur flow cytometer (BD Biosciences) using the 488 nm blue laser for excitation and
549 530/30 BP filter for detection of Alexa Fluor 488 emission and 670/LP filter for propidium iodide.
550 Analysis was performed using FlowJo v. 10.

551

552 **LDH assay**

553 To assess the extent of cell death, lactate dehydrogenase (LDH) activity of supernatants was
554 measured using a mixture of diaphorase/NAD⁺ and 3-(4-iodophenyl)-2-(4-nitrophenyl)-5-phenyl-
555 2H-tetrazol-3-ium chloride/sodium 2-hydroxypropanoate according to the manufacturer's
556 protocol (Takara Bio). Staurosporine (Sigma-Aldrich Cat# S6942) was used as a control.

557

558 **Cell death detection ELISA**

559 To assess the qualitative and quantitative determination of cytoplasmic histone-associated DNA
560 fragments (mono- and oligonucleosomes) after LCL161 or staurosporine (positive control)
561 treatment, the Cell Death Detection ELISA was used according to the manufacturer's protocol.
562 Enrichment factor is calculated using the following equation when mU is absorbance [10^{-3}]:

563

564
$$\text{Enrichment factor} = \frac{\text{mU of the sample (dead/dying cells)}}{\text{mU of the corresponding control (viable cells)}}$$

565

566 **siRNA transfection**

567 CD4⁺ T cells were transfected with Mission *BIRC2* esiRNA (ID# EHU002711) or control (Cat#
568 EHUEGFP) siRNA (siNS) using lipofectamine RNAiMAX transfection reagent (all Invitrogen) in
569 Opti-MEM (Gibco) according to the manufacturer's instructions. 48 h later, cells were analyzed
570 for target gene silencing by western blot. Transfection efficiency was assessed with BLOCK-iT
571 Alexa Fluor Red fluorescent control (Cat# 14750100, Invitrogen) using a BD FACSCalibur flow
572 cytometer with the 488 nm blue laser for excitation and a 585/42 BP filter.

573

574 **Western blotting**

575 Cell lysis, co-immunoprecipitation, and western blotting were performed as previously
576 described^{35,49}. Briefly, cell lysates were prepared using 20 mM HEPES, 150 mM NaCl, and 1
577 mM EDTA supplemented with 1% Triton X-100 and 1% Thermo Scientific Halt protease and
578 phosphatase inhibitor cocktail. Co-immunoprecipitation was performed using the Pierce Co-
579 Immunoprecipitation Kit with 50 µg cell lysates and either 50 µg anti-FAS IgG1 (Cat# 8023,
580 RRID:AB_10860778) with a mouse IgG1 (Cat# 5415, RRID:AB_10829607) as a negative
581 control, or anti-RIPK1 IgG XP (Cat# 3493, RRID:AB_2305314) with a rabbit IgG XP (Cat# 3900,
582 RRID:AB_1550038) as a negative control (all from Cell Signaling) according to the
583 manufacturer's directions. Cell lysates and immunoprecipitates were resolved using 2-[bis(2-
584 hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol buffered polyacrylamide gels,
585 transferred to 0.2 µm polyvinylidene difluoride membranes, probed with primary antibodies
586 overnight at 4 °C, followed by detection using alkaline phosphatase tagged secondary
587 antibodies (Invitrogen) and 0.25 mM CDP-Star supplemented with 5% Nitro-Block II (both
588 Applied Biosystems). The primary antibodies raised against the following were used in western
589 blots: *BIRC2* (Cat# 7065, RRID:AB_10890862), *BIRC3* (Cat# 3130, RRID:AB_10693298),
590 cFLIP (Cat# 56343, RRID:AB_2799508), CASP3 (Cat# 14220, RRID:AB_2798429), CASP8

591 (Cat# 9496, RRID:AB_561381), FADD (Cat# 2782, RRID:AB_2100484), FAS (Cat# 4233,
592 RRID:AB_2100359), FASLG (Cat# 4273, RRID:AB_2100652), PARP1 (Cat# 9532,
593 RRID:AB_659884), RIPK1 (Cat# 3493, RRID:AB_2305314), Phospho-RIPK1-Ser166 (Cat#
594 44590, RRID:AB_2799268) from Cell Signaling Technologies, and ACTB (Sigma Cat# A2228,
595 RRID:AB_476697).

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726 **Figure legends**

727 **Figure 1. BIRC2 recognizes histone tails.** (a) BIRC2 domain architecture. (b) Western blot
728 analysis of the salt gradient chromatin fractionation of SW480 nuclei. (c) Representative images
729 of PLA (pink) between BIRC2 and histone H3 in SW480 cells. The nuclei are stained with DAPI
730 (blue). (d) Quantification of the PLA signal in (c) with at least 75 cells analyzed per condition.
731 Statistical analysis was performed using Wilcoxon test. (e) Western blot analysis of pull-downs
732 of the GST-tagged BIR domains of BIRC2 with the indicated histone peptides. RBP2-PHD,
733 control. (f) Superimposed ^1H , ^{15}N HSQC spectra of ^{15}N -labeled BIR domains of BIRC2, collected
734 while the H3₁₋₁₂ peptide was titrated in the NMR samples. Spectra are color coded according to
735 the protein:peptide molar ratio. (g, h) Representative binding curves used to determine K_d s by
736 tryptophan fluorescence. The K_d values were averaged over three separate experiments, with
737 error calculated as standard deviation between the runs. (i) Electrostatic surface potential of
738 BIRC2_{BIR3} is colored blue and red for the positive charge and the negative charge, respectively.
739 The bound H3 peptide is shown as yellow sticks. (j, k) A ribbon diagram of the BIRC2_{BIR3} (green)
740 in complex with the histone H3 peptide (yellow). Dashed lines represent hydrogen bonds. The
741 zinc ion is shown as a grey sphere.

742

743 **Figure 2. BIRs mediate histone and DNA binding functions of BIRC2.** (a, b) Western blot
744 analysis of pull-downs of the GST-tagged BIR domains of BIRC2 with the indicated histone
745 peptides. RBP2-PHD, control. (c, d) Overlays of ^1H , ^{15}N HSQC spectra of the BIR domains of
746 BIRC2 collected before (black) and after addition of the indicated peptides. Spectra are color
747 coded according to the protein:peptide molar ratio. (e-g) Overlays of ^1H , ^{15}N HSQC spectra of
748 the mutated BIRC2 BIR domains collected before (black) and after addition of the indicated
749 peptides. Spectra are color coded according to the protein:peptide molar ratio. (h, i) Western
750 blot analysis of pull-downs of the GST-tagged mutated BIRC2 BIR domains with the indicated
751 histone peptides. (j) Alignment of a fragment of the amino acid sequences of the BIRC2 BIR

752 domains. Identical residues are highlighted by red boxes and moderately conserved residues
753 are colored red. BIRC2_{BIR3} residues are labeled. (k-m) EMSAs of 147 bp 601 DNA in the
754 presence of increasing amounts of the indicated BIR domains of BIRC2. DNA:protein ratio is
755 shown below gel images.

756

757 **Figure 3. BIRC2 mediates the cell defense and cell cycle signaling.** (a) Heatmap of
758 common differentially expressed genes (DEGs) in MCF7 cells treated with BIRC2 sgRNAs or
759 the control GFP sgRNA. Genes are shown as Z-score log₂ CPM (counts per million reads
760 mapped). Up-regulated genes are shown in red and down-regulated genes in blue. (b) Top
761 ranked Gene Ontology (GO) biological process terms of up (red) and down (blue) regulated
762 genes of DEGs as in (a) in BIRC2 KO cells. The full lists are shown in Suppl. Tables 2 and 3. (c)
763 A cytokines antibody array with supernatants derived from human macrophages obtained by *ex-*
764 *vivo* differentiation of normal blood monocytes and stimulated with IFN- γ for 24 h in the
765 presence of 10 μ M embelin or vehicle as control. (d-f) HeLa cells transfected with either BIRC2-
766 encoding of empty vector were untreated (NT) or treated with 1 μ M etoposide (eto) for 3 h (0),
767 then washed and incubated for 2 to 45 h in a drug-free medium. Western blot analysis of BIRC2
768 and the loading control HSC70 is shown in (d). Western blot analysis of γ H2AX, a mark of DNA
769 damage, in untreated cells (NT) or etoposide treated cells that were washed and incubated for
770 additional 2 or 6 h in an etoposide-free medium is shown in (e). Flow cytometry analysis of
771 γ H2AX in untreated cells (NT, grey histograms) or etoposide treated cells that were washed and
772 incubated for additional 2 or 6 h in an etoposide-free medium (eto, red histograms) is shown in
773 (f). The percentage of cells expressing γ H2AX is indicated. (g) Flow cytometry analysis of the
774 cell cycle in etoposide treated HeLa cells that were incubated for indicated time in an etoposide-
775 free medium. DNA was stained with Propidium iodide. (h) % of cells in G2/M. Data represent
776 mean \pm SEM from three independent biological replicates for 24, 30 and 48 hrs and two for 45
777 hrs (the unpaired t-test per row in GraphPad Prism).

778

779 **Figure 4. LCL161 binds to BIRC2_{BIR2} and BIRC2_{BIR3}.** (a) Chemical structure of LCL161. (b-d)
780 Superimposed ¹H,¹⁵N HSQC spectra of ¹⁵N-labeled BIRC2_{BIR1}, BIRC2_{BIR2} and BIRC2_{BIR3},
781 collected while LCL161 was titrated in the NMR samples. Spectra are color coded according to
782 the protein:inhibitor molar ratio. (e, f) Representative binding curves used to determine K_ds by
783 tryptophan fluorescence. The K_d values were averaged over three separate experiments, with
784 error calculated as standard deviation between the runs. (g) Electrostatic surface potential of
785 BIRC2_{BIR3} is colored blue and red for the positive charge and the negative charge, respectively.
786 The bound LCL161 compound is shown as green sticks. (h) A ribbon diagram of the BIRC2_{BIR3}
787 (wheat) in complex with LCL161 (green). Dashed lines represent hydrogen bonds. The zinc ion
788 is shown as a grey sphere. (i) Structural overlay of BIRC2_{BIR3} bound to LCL161 (green) and the
789 H3 peptide (yellow). (j) Superimposed LCL161 (green) and the H3 peptide (yellow) bound to
790 BIRC2_{BIR3}.

791

792 **Figure 5. LCL161 disrupts binding of BIRC2 to H3 and induces cell death.** (a)
793 Representative images of the PLA between BIRC2 and histone H3 in SW480 cells treated with
794 or without 10 μM LCL161 for 1 h. The nuclei were stained with DAPI (blue). (b) Quantification of
795 the PLA signal in (a) with at least 75 cells analyzed per condition. Statistical analysis was
796 performed using Wilcoxon test. (c) Western blot analysis of peptide pull-downs of GST-tagged
797 BIRC2_{BIR3} with the histone H3 peptide (aa 1-22) in the presence of increasing concentrations of
798 LCL161. (d) Western blot analysis of BIRC2 in SW480 cells treated with or without 10 μM
799 LCL161 for 1 h. β-Actin was used as loading control. (e) Western blot analysis of BIRC2 in
800 human macrophages obtained by *ex-vivo* differentiation of normal blood monocytes and
801 stimulated or non-stimulated with IFN-γ for 24 h in the presence or absence of 10 μM LCL161.
802 β-Actin was used as loading control. (f, g) RT-qPCR analysis of IL-6 (f) and RANTES (g) mRNA

803 expression levels in human macrophages stimulated or non-stimulated with IFN- γ for 24 h in the
804 presence or absence of 10 μ M LCL161. Data represent mean \pm SD of four independent
805 experiments (the paired t-test in GraphPad Prism). * $P < 0.05$; ** $P < 0.01$. (h, i) MTT assay
806 showing survival of murine MEF (h) or human glioma LN18 cells (i) incubated for 24 h in media
807 containing the indicated concentrations of LCL161 and/or TNF α . Data represent mean \pm SEM
808 from three independent biological replicates. (j) Representative western blots of FAS,
809 membrane-bound FASLG (mFASLG), soluble FASLG (sFASLG), BIRC2, and BIRC3 in
810 uninfected CD4 $^{+}$ T cells (T_{CM}) and HIV-1-infected CD4 $^{+}$ T cells (HIV- T_{CM}). $n = 4$. (k) T_{CM} and
811 HIV- T_{CM} were treated with 100 nM LCL161 for 4 h. FAS was immunoprecipitated using anti-FAS
812 with a mouse IgG1 (α -IgG as a negative control). Immunoprecipitates and cell lysates were
813 analyzed by western blot using the indicated antibodies. $n = 4$. (l) T_{CM} and HIV- T_{CM} were treated
814 with 100 nM LCL161 for 5 min. RIPK1 was immunoprecipitated using anti-RIPK1 with a rabbit
815 IgG (α -IgG) as a negative control, and endogenous RIPK1 ubiquitination detected by western
816 blotting using an RIPK1 antibody. $n = 4$. (m) T_{CM} and HIV- T_{CM} were treated with 100 nM LCL161
817 for 4 h. Cell lysates were analyzed by western blot using the indicated antibodies. $n = 4$. (n) T_{CM}
818 and HIV- T_{CM} were treated with LCL161 or 2 μ M staurosporine (STS) for 4 h. *Top*, aliquots of
819 supernatants were spectrophotometrically tested for LDH as a measure of cell death. *Bottom*,
820 cells were harvested and cytoplasmic fractions assayed for cytoplasmic histone-associated
821 DNA fragments (mono- and oligonucleosomes) by ELISA (see Methods for calculation of
822 enrichment factor). $n = 4$.

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825

826 **Supplementary Table 2.** CPM values for all expressed genes.

827 **Supplementary Table 3.** Gene Ontology biological process terms of DEGs in BIRC2 KO cells.

Figure 1

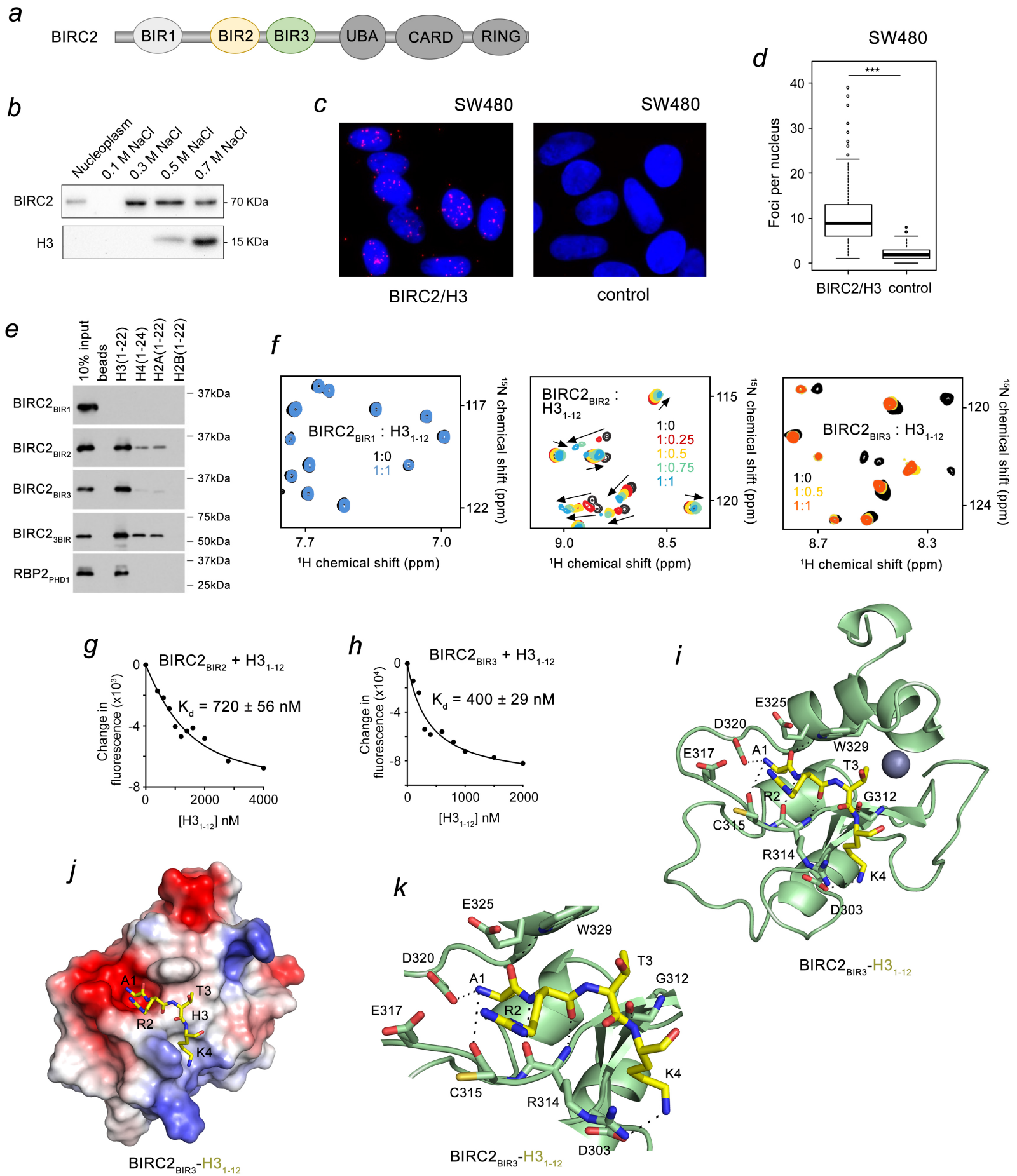


Figure 2

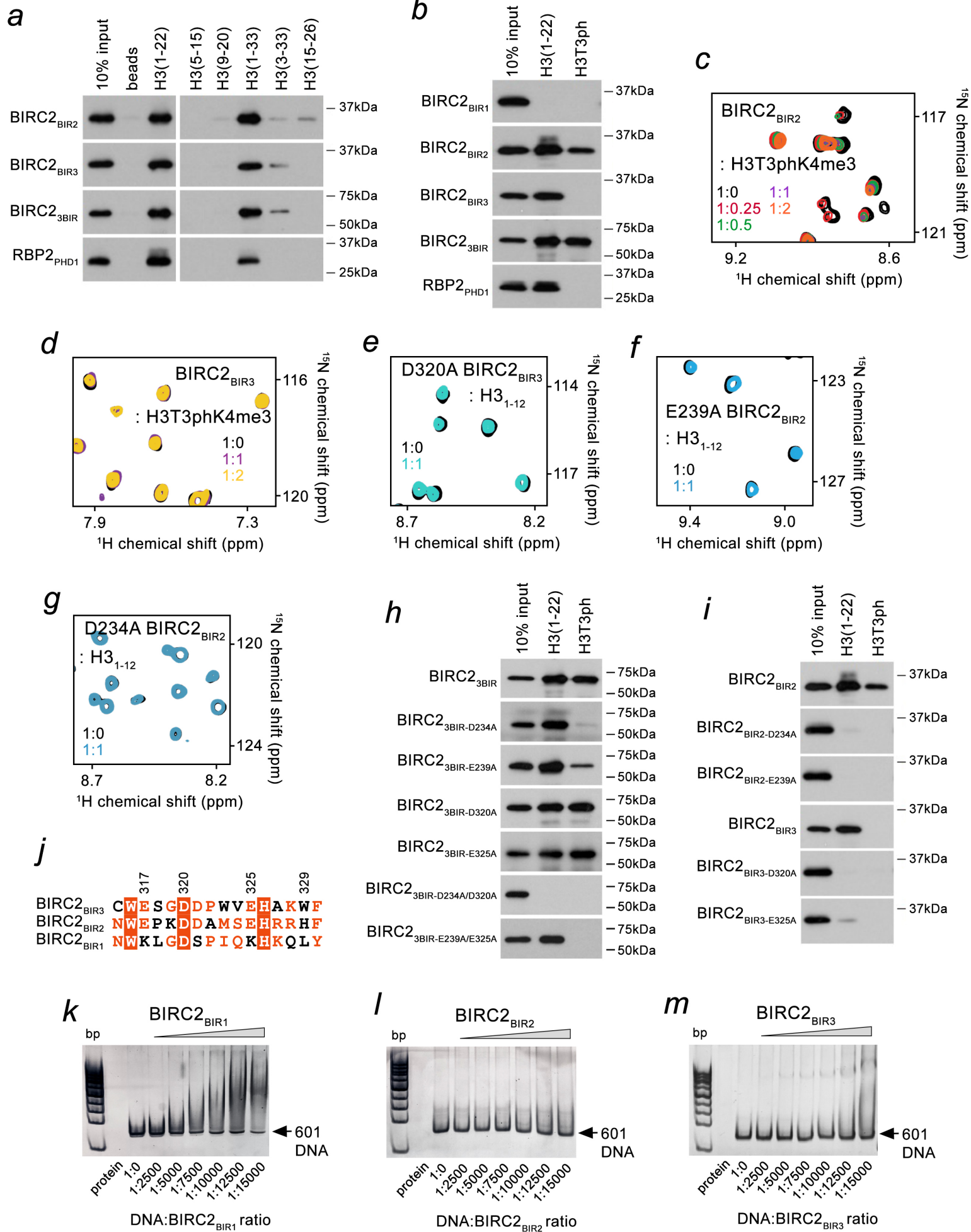


Figure 3

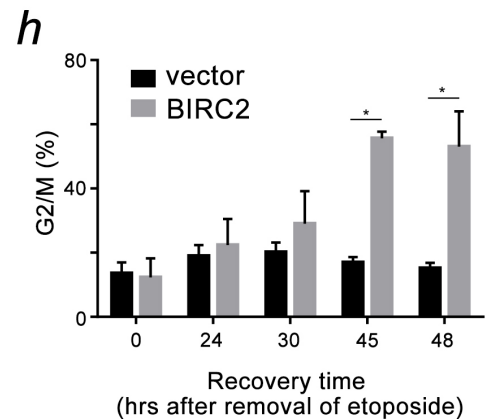
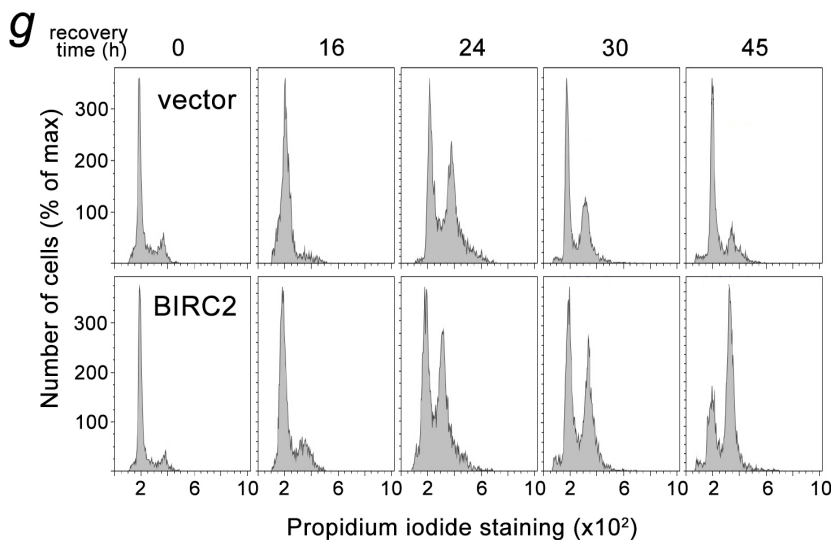
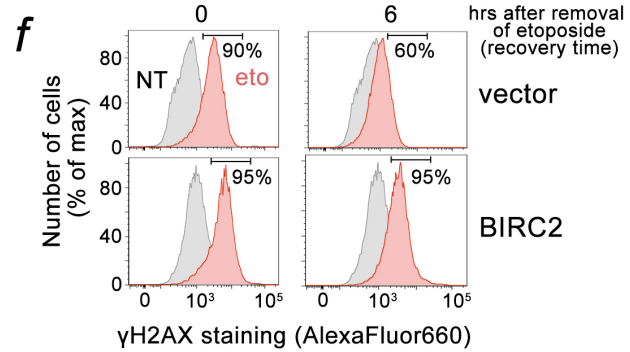
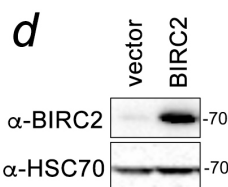
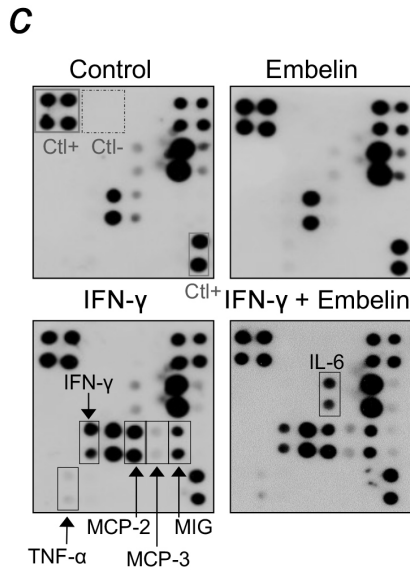
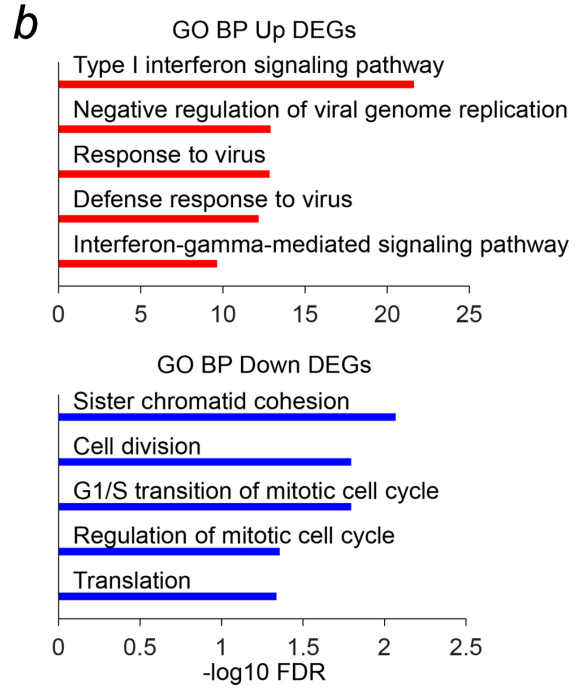
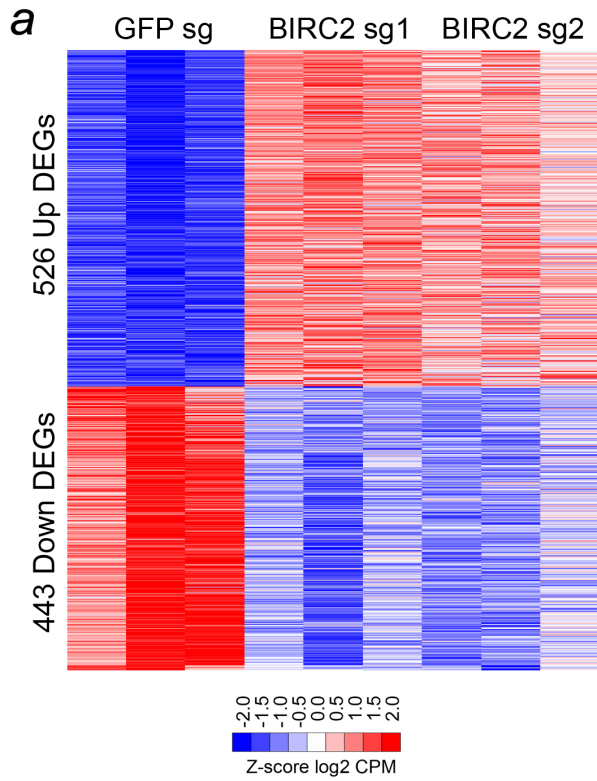


Figure 4

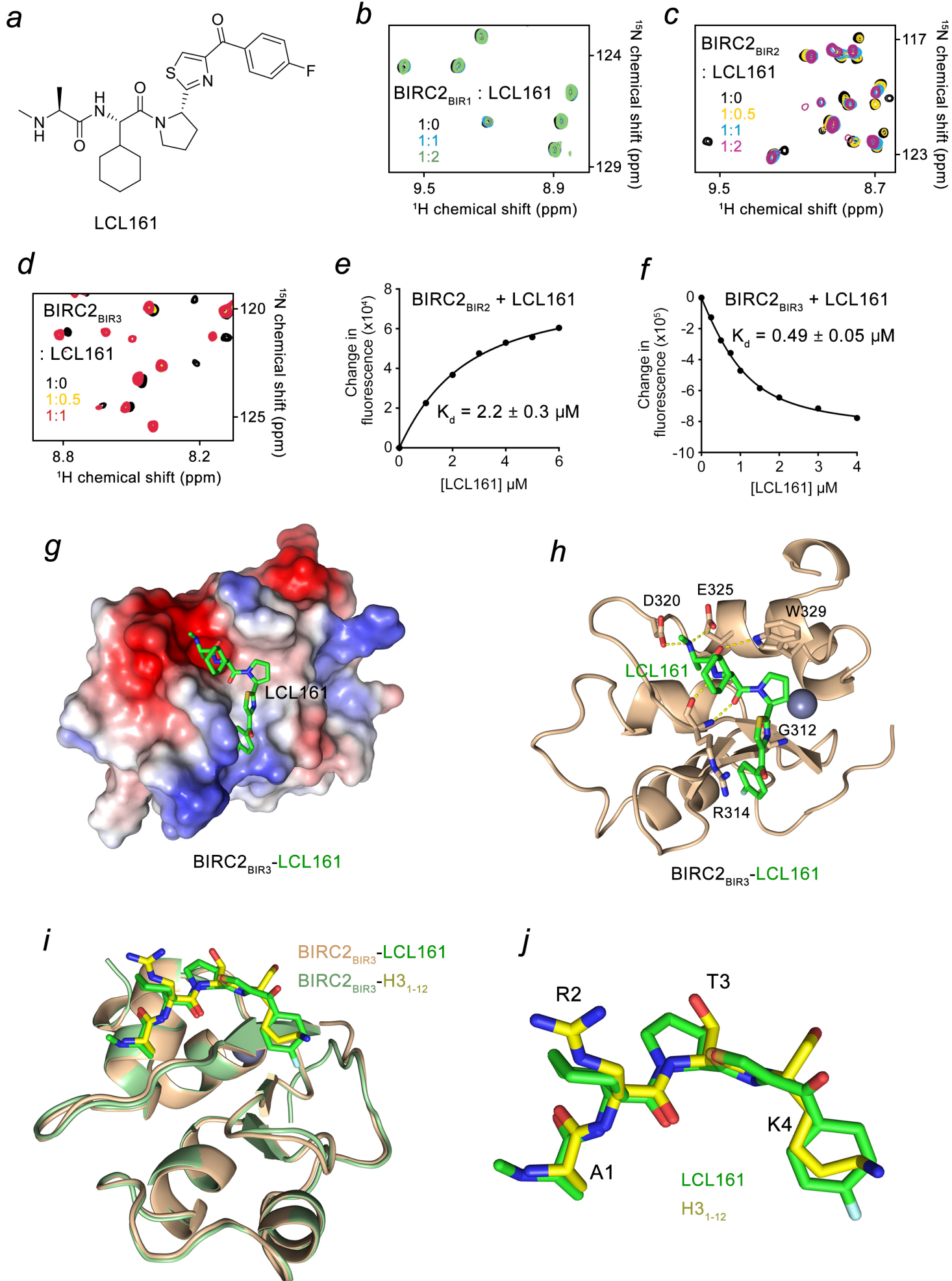


Figure 5

