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Potential Role of Estrogen Receptor Beta as a Tumor Suppressor of Epithelial Ovarian Cancer

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36 **Abstract**

37 Ovarian cancer is the gynecological cancer exhibiting the highest morbidity and improvement of
38 treatments is still required. Previous studies have shown that Estrogen-receptor beta (ER β) levels
39 decreased along with ovarian carcinogenesis. Here, we present evidence that reintroduction of
40 ER β in BG-1 epithelial ovarian cancer cells, which express ER α , leads *in vitro* to a decrease of
41 basal and estradiol-promoted cell proliferation. ER β reduced the frequency of cells in S phase and
42 increased the one of cells in G2/M phase. At the molecular level, we found that ER β downregulated
43 total retinoblastoma (Rb), phosphorylated Rb and phospho-AKT cellular content as well as cyclins
44 D1 and A2. In addition, ER β had a direct effect on ER α , by strongly inhibiting its expression and
45 activity, which could explain part of the anti-proliferative action of ER β . By developing a novel
46 preclinical model of ovarian cancer based on a luminescent orthotopic xenograft in athymic Nude
47 mice, we further revealed that ER β expression reduces tumor growth and the presence of tumor
48 cells in sites of metastasis, hence resulting in improved survival of mice. Altogether, these findings
49 unveil a potential tumor-suppressor role of ER β in ovarian carcinogenesis, which could be of
50 potential clinical relevance for the selection of the most appropriate treatment for patients.

51

52

53 **Introduction**

54 The single epithelial cell layer that surrounds ovaries is currently believed to be one of the sources
55 of preneoplastic lesions leading rise to epithelial ovarian tumors, which represent the vast majority
56 of ovarian cancers [1]. Epithelial ovarian cancer (EOC) is the seventh most common cancer.
57 However, it remains the fourth most deadly one because it is difficult to diagnose at early stages
58 and, hence, to treat [2]. Either classified on morphological categories (i.e., serous, mucinous,
59 endometrioid, and clear cells) based on histological criteria and resemblance to epithelial
60 components of the normal reproductive tract, or more recently, classified as low- or high-grade
61 tumors [2], EOC is a complex disease for which the etiology is poorly understood. Novel markers
62 and targets for therapies are thus urgently needed.

63 Ovary is the main organ of production of estrogens, which mainly impact on the growth,
64 differentiation and function of reproductive tissues [3]. Through their mitogenic action, estrogens
65 play roles in ovarian carcinogenesis. Several studies have highlighted an increased risk of ovarian
66 cancer in patients receiving long-term estrogen replacement therapy [4,5,6,7], while patients treated
67 with oral contraception combining estrogens and progestins showed a reduced risk of developing an
68 ovarian cancer [8,9]. Estrogen action is mediated by two receptors, ER α and ER β , two transcription
69 factors of a large family of nuclear receptors [10,11]. About 40 to 60% of ovarian cancers express
70 ER α [12], but it is intriguing to notice that only a small proportion of them will benefit from anti-
71 estrogen therapy [13]. The role of ER β in the ovarian biology remains poorly understood, but it
72 seems to be different from that of ER α [14]. *ER β* knock-out animals (β ERKO) are subfertile,
73 producing fewer litters and pups upon superovulation induction [15,16]. The ovaries of β ERKO
74 animals contain fewer large antral follicles and corpus luteum compared to wild-type littermates,
75 which is concomitant with lower levels of estradiol produced [17] and a reduced expression of key

76 genes involved in ovary function such as aromatase (*Cyp19a1*), LH receptor (*Lhcgr*), and
77 prostaglandin synthase 2 (*Ptgs2*) [18].

78 Several studies have unraveled a potential role for ER β in EOC. In particular, ER β levels
79 were lower in ovarian tumors compared to normal tissues [19,20,21,22]. Moreover, the loss of ER β
80 expression could correlate with a shorter overall survival of ovarian cancer patients [23]. ER β levels
81 are also associated with metastatic lymph node status [24]. A polymorphism (rs127572) of the *ER β*
82 gene has also been identified recently and shown to be associated with an increased risk of
83 developing an ovarian cancer [25]. However, it is still unknown whether this polymorphism affects
84 the expression of ER β . The intracellular location of ER β in tumor cells seems to be important.
85 Indeed, a recent study has shown that ER β was localized in the cytoplasm of tumor cells, while it
86 was mainly nuclear in normal epithelial cells [26]. In addition, cytoplasmic expression of ER β was
87 correlated to a poor outcome for patients with advanced serous ovarian cancer [14]. These findings,
88 combined with the aforementioned clinical correlations between ER β and patient survival, lead us to
89 hypothesize that ER β is a critical factor in ovarian tumor progression and to delineate the precise
90 contribution of this receptor in the molecular pathways underlying EOC carcinogenesis.

91 For this purpose, we used BG-1 cells as a cellular model and took advantage of an orthotopic
92 xenograft mouse model we have developed. BG-1 cell line is a human EOC cell line derived from a
93 solid primary tumor tissue from a patient with stage III, poorly differentiated ovarian
94 adenocarcinoma [27]. These cells express ER α and are sensitive to estrogens in terms of
95 proliferation [21,28]. . Experimental models of ovarian carcinogenesis are essential to understand
96 the molecular mechanisms involved in the development of the disease but also to evaluate the
97 efficacy of novel therapeutic drugs [29]. Several models have been developed, including different
98 xenograft and transgenic models, none being fully satisfactory. The xenograft models that are
99 currently used are either intraperitoneal, subcutaneously or orthotopically intrabursal in the ovary.

100 Only few reports describe orthotopic xenograft. Nevertheless, orthotopic cell implantation can be
101 perceived as more physiological, as the cancer cells are directly inoculated in the ovarian
102 environment and can lead to metastasis. Therefore, to investigate the role of ER β in EOC
103 carcinogenesis, we chose to take advantage of an orthotopic xenograft mouse model based on the
104 use of luciferase (Luc)-expressing human epithelial ovarian cancer BG-1 cells.

105 We show here that reintroduction of ER β in BG-1 cells using an adenovirus leads *in vitro* to
106 an inhibition of both basal and estradiol-induced cell proliferation. ER β exerts its anti-proliferative
107 action through a reduction of the frequency of cells in S phase, an increase of cells in G2 phase,
108 along with an altered expression of cell cycle regulators. At the molecular level, ER β was able to
109 repress the expression, the activity and the signaling of ER α , and thus to block its proliferative
110 action. Moreover, ER β was able to strongly reduce the development of orthotopic ovarian xenograft
111 as well as the presence of tumor cells the sites of metastasis, leading to an increased survival of the
112 mice. Altogether, these findings support a role for ER β as tumor-suppressor in EOC carcinogenesis.

113

114

115 **Results**

116 Previous reports have shown that ER β is weakly expressed in EOC tissues and derived cell lines
117 compared to normal tissue [11]. We took advantage of the human EOC cell line BG-1, which
118 expresses endogenous levels of ER α and is sensitive to estrogens [27]. Here, we first confirm that
119 BG-1 cells display low steady-state levels of ER β products, i.e. mRNA and proteins (Fig. 1A, B).
120 The next step towards assessing the role of ER β in ovarian carcinogenesis was to restore its
121 expression in ovarian cancer cells. Thus, BG-1 cells were infected with a backbone (Ad5) or human
122 *ER β* (Ad β) encoding adenovirus [30,31]. *ER β* overexpression was indeed obtained in Ad β -infected
123 cells as validated by real-time PCR and Western blot analyses (Fig. 1A, B). Interestingly, ER β
124 levels were strongly down-regulated by estradiol (E2) both at RNA and protein levels. Moreover, in
125 the absence of ER β , ER α levels were also down-regulated by E2, although at a lesser extent. When
126 ER β was introduced in the cells, the basal expression level of ER α in the absence of E2 was reduced
127 and by half. The presence of ER β strongly diminished ER α levels in the presence of E2 (more than
128 15 fold decrease in 3 h), suggesting that ER β enhances the degradation of ER α . This is likely due to
129 proteasome-dependent degradation of ER α and ER β proteins [32]. The effects of ER β
130 overexpression on estrogen responsiveness were then investigated. We analyzed the ability of ER β
131 to transactivate a synthetic luciferase reporter sensitive to estrogens in BG-1 cells. Endogenous ER α
132 was able to activate the reporter in the presence of E2 (Fig. 1C). ER β strongly repressed the activity
133 of ER α in response to E2, suggesting that in the presence of ER α , ER β behaves rather as a repressor
134 than an activator of estrogen signaling. To ensure of the functionality of the receptor produced, we
135 also checked the activity of ER β in the EOC cell line PEO14 [33] that is reported to express low
136 levels of ER α (Fig. 1E) and hence does not respond to estrogens [34]. Both ER α and ER β were able
137 to stimulate an estrogen-sensitive reporter upon E2 exposure, even though ER β was a little bit less
138 active than ER α (Fig. 1D). Therefore, in the absence of ER α , ER β retains the ability to transactivate
139 estrogen signaling pathways. When ER α and ER β were coexpressed in PEO-14 cells, the activity of

140 the reporter in the presence of E2 was similar to the one of ER α alone. This suggests that in the ER-
141 negative PEO-14 cell line, ER β cannot affect ER α activity. Western blot experiments in PEO-14
142 cells show that when ER α and ER β were expressed separately, their levels were strongly decreased
143 in the presence of E2 (Fig. 1E). When ER α and ER β were coexpressed, the degradation of ER α in
144 the presence of E2 was slightly increased but not in the proportion seen in BG-1 cells. For ER β , the
145 coexpression of ER α enhanced slightly the degradation of ER β . Overall, these data suggest that ER α
146 levels are not regulated in the same manner in BG-1 and PEO-14 cells, which could explain why
147 ER α activity is not drastically reduced by the presence of ER β in PEO-14 in comparison to BG-1
148 cells. .

149 We next studied the effects of ER β expression on cancer cell proliferation. BG-1 cells
150 infected with Ad5 or Ad β adenoviruses were grown *in vitro* in the absence or the presence of E2. As
151 expected, E2 stimulated the proliferation of control BG-1 cells (Fig. 2A). Interestingly, ER β
152 repressed by 50% both the basal and E2-dependent proliferation of BG-1 cells. We also observed
153 anti-proliferative action of ER β in ER α - and ER β -negative PEO14 cells. However, this inhibition
154 was not affected by E2 (Supplemental Fig. S2). While the *in vitro* effects of ER β have been
155 observed so far, very little is known of its *in vivo* action in ovarian cancer models. As a first
156 approach to assess this point, we used a model of subcutaneous injection. To enable a sensitive,
157 dynamic and early follow-up of tumor growth, we stably transfected BG-1 cells with a constitutive
158 Luc reporter. Ovariectomized athymic Nude mice were implanted a pellet of cholesterol or E2 and
159 injected with BG-1 cells infected with Ad5 or Ad β adenoviruses. BG-1 cells formed tumors only in
160 the presence of E2 (Fig. 2B). ER β expression significantly reduced by 70% the E2-promoted growth
161 of BG-1 cells, thus supporting a potential anti-proliferative role for ER β in EOC.

162 We then explored the mechanisms responsible for the anti-tumoral action of ER β . Cell cycle
163 distribution was compared by flow cytometry in control and ER β -expressing BG-1 cells (Fig. 3A).
164 We did not detect any SubG0 peak, suggesting that no apoptosis occurred. ER β expression did not

165 modify the proportion of BG-1 cells in G0-G1 phase, but it strongly reduced that of cells in S phase.
166 We also found that *ERβ* expression triggered an increased number of cells in G2-M phase of cell
167 cycle. The expression of cell cycle markers was next monitored by Western blot analyses (Fig. 3B).
168 Several cell cycle regulators such as Cyclins A and D1, AKT and Rb are reported to be regulated by
169 estrogens [35]. We observed that phosphorylation of AKT was increased upon E2 treatment in Ad5-
170 infected BG-1 cells (Fig. 3B). *ERβ* expression led to a decrease in phospho-AKT content in both
171 vehicle- and E2-treated cells and this occurred at 3, 6 and 24h. *ERβ* caused similar changes in Cyclin
172 D1, total Rb and phosphorylated Rb expression. Indeed, the levels of cyclin D1, total Rb and
173 phosphorylated Rb were up-regulated upon E2 treatment in Ad5 infected cells and this induction
174 was reduced by *ERβ*. Cyclin A2 displayed a late induction 24h after E2 treatment and this was
175 reduced by the presence of *ERβ*.

176 To further explore the *in vivo* role of *ERβ* in ovarian carcinogenesis, we set up a more
177 physiologic model of orthotopic implantation of tumor cells in the ovary. Ad5- or Adβ-infected BG-
178 1-Luc cells were injected in the left ovary and tumor growth was monitored by bioluminescence. As
179 shown in Fig. 4A, *ERβ* expression significantly prevented tumor growth. When euthanized at day 28
180 post-injection, control mice displayed a clear increase of peritoneal volume and of tumor volume in
181 the left ovary (Fig. 4B). In sharp contrast, the volume of both peritoneum and ovary appeared much
182 reduced in mice injected with *ERβ*-expressing BG-1 cells. We next determined whether the
183 metastatic process was affected by *ERβ* expression. To achieve this, the lung, liver and contralateral
184 right ovary were collected. The extent of tumor cells present in these organs was estimated by
185 measuring the Luc activity in our orthotopic model. Luciferase activity was detected in the lung,
186 liver and contralateral ovary from mice injected with Ad5-infected BG-1 cells, (Fig. 5A).
187 Interestingly, *ERβ* expression strongly reduced the presence of tumor cells and potentially
188 metastasis to all these organs, suggesting that *ERβ* exerts a dual role on the tumor growth and
189 dissemination. This was confirmed by *in vitro* wound healing experiments showing that *ERβ*

190 expression decreases the motility of BG-1 cells (data not shown). Since ER β impacted on both
191 tumor growth and cell dissemination, we wondered whether this could enable to improve mice
192 survival. Mice were followed-up for 80 days and daily checked for any sign of morbidity. *ER β*
193 expression significantly delayed death as two months after injection with Ad β -infected BG-1 cells,
194 50% of mice still survived (Fig. 5B). This is in strong contrast with the 100% death of control mice,
195 which occurs in less than two months.

196

197 **Discussion**

198 We report here that the introduction of ER β in ovarian cancer cells displaying endogenous levels of
199 ER α leads to a strong inhibition of *in vivo* growth and cell dissemination, mediated through the
200 control of ER α expression and signaling.

201 *In vitro* proliferation experiments show a clear inhibition of the E2-dependent proliferation
202 by ER β in the ER α -positive ovarian cancer cell line BG-1. This is the first demonstration of an anti-
203 proliferative action of ER β in an ER α -positive ovarian cancer cell line. Moreover, ER β could also
204 inhibit the growth of the ER α -negative ovarian cancer cell line PEO-14. These results are in
205 agreement with our previous findings obtained with ER α -negative cell lines from breast [31] and
206 prostate [30] cancer cells and with another study performed in ER α -negative SKOV3 EOC-derived
207 cell line [36]. In cells devoid of estrogen receptors, it is likely that restoration of ER α cannot enable
208 a stimulation of proliferation upon E2 treatment, since it triggers a different program of
209 transcriptional regulation, compared to cells expressing naturally ER α , as previously showed in
210 breast cancer cells [37]. Indeed, we observed that endogenous ER α levels in BG-1 cells were
211 strongly reduced by the presence of ER β , whereas ER β affected less ER α levels in PEO-14 cells.
212 Moreover, ER β could inhibit completely ER α activity in BG-1 cells but not in PEO-14 cells. This
213 could be due to the fact that in BG-1 cells, endogenous ER α is under the control of its own
214 promoter, whereas in PEO-14 cells, exogenous ER α is controlled by a viral promoter. Moreover,
215 one cannot exclude that the cofactors required for ER α and ER β activity in the two cell types are
216 different, which accounts for their differential activity in the BG-1 and PEO-14 cells.

217 The novelty of our study is to have extended these data to two *in vivo* models. If clinical evidences
218 based on ER β levels in normal tissue and cancer suggest that this receptor could act as a potential
219 tumor suppressor, so far, no preclinical proof has been brought to confirm this hypothesis. We first
220 used a classical subcutaneous model, to answer to this question and more precisely to determine if
221 estrogen were required or not. BG-1 *in vivo* growth was clearly dependent on the presence of

222 estradiol and ER β could counteract tumor growth. We also used orthotopic implantation of
223 bioluminescent cells in the ovary. In agreement with *in vitro* experiments, we observed a strong
224 reduction of tumor growth when BG-1 cells express ER β .

225 Cell cycle analysis demonstrated that ER β inhibited cell proliferation, by decreasing the
226 proportion of cells in S phase and increasing the proportion of cells in G2-M phase. This situation is
227 similar to that reported in breast cancer cells [38]. At the molecular level, ER β could decrease the
228 phosphorylation of Akt and Rb. In addition, ER β also reduced the expression of cyclin D1 and
229 cyclin A. Cyclin D1 interacts with Cyclin-Dependent Kinase-4 and -6, which leads to the
230 phosphorylation of Rb and the dissociation of Rb/E2F complex, which causes the progression
231 through the G1 [35]. Cyclin A interacts with Cyclin-Dependent Kinase-2 to promote the transition
232 from the S to G2 phase [39]. Moreover, the phosphorylation of AKT favors G1/S transition by
233 blocking the transcriptional activity of Foxo factors, which regulate Cyclin D1 and p21 expression
234 [40]. Based on these findings, we propose that ER β leads to a decrease in AKT phosphorylation,
235 which in turn results in a decreased expression of Cyclin D1 and phosphorylation of Rb.

236 To explain how ER β can affect cell proliferation, we have investigated whether it could
237 directly modify ER α expression and signaling in BG-1 cells. It is indeed interesting to notice that
238 ER β has also profound effects of ER α levels as it diminished by about 15 fold ER α expression in
239 3h. Although the exact molecular mechanisms accounting for the negative effect of ER β on ER α in
240 BG-1 cells remains to be elucidated, the extent and rate of degradation of ER α is certainly critical
241 for its activity and these parameters change if ER β is present or not. Indeed, in parental BG-1 cells,
242 expressing only ER α , the receptor is also subjected to E2-dependent degradation. This is likely due
243 to proteasomal degradation of the receptor as shown in a number of cell types by previous studies
244 [41,42]. Paradoxically, the E2-dependent proteasomal degradation of ER α is also required for its full
245 activity through the regulation of the interactions of the receptors with its coactivators [42] and the
246 cycling of ER α on the promoter of its target genes [43]. The situation appears different when ER β is

247 coexpressed with ER α in BG-1 cells. Indeed, we report that ER β could reduce more rapidly and
248 strongly the expression of ER α in BG-1 cells in the presence of E2, compared to cells in which only
249 ER α is present. This is in agreement with what is observed in the ER α -positive breast cancer cell
250 line T47-D transfected with ER β [44]. This suggests that ER β could trigger a rapid proteasomal
251 degradation of ER α in the presence of E2. If we suppose that ER α and ER β form heterodimers as
252 previously described [45,46], ER β might increase the degradation of ER α present in the complex, by
253 altering its conformation and preventing it from being active. Consequently, ER α would be less
254 efficient to activate its target genes and could not play its proliferative role.

255 Indeed, in addition to its effects on ER α expression, we cannot exclude that ER β also
256 reduces ER α activity, as shown by transfection of an estrogen-responsive reporter in BG-1 cells.
257 This is in agreement with a previous study performed in an ER α -positive breast cancer cell lines in
258 which ER β was transfected, suggesting that ER α and ER β have distinct roles [47]. In turn, this could
259 affect the E2-dependent signaling of ER α , which could not regulate the levels of the activity of key
260 target genes involved in the proliferation, such as AKT, Rb, cyclin D1 or cyclin A2. Moreover,
261 other studies have reported a negative action of ER β on ER α signaling [44,48]. In particular, ER β
262 has been shown to alter the recruitment of AP-1 complexes to ER α target genes [44], which is
263 known to act in synergy with ER α . Once heterodimerized with ER α , ER β could also modify the
264 ability of ER α to interact with coactivators. A previous study has also suggested a Ying Yang action
265 of ER β *in vivo*, which is a repressor of ER α signaling in the presence of ER α , but can also replace
266 ER α in the absence of ER α [48]. The down-regulation of ER α expression by ER β may not be the
267 only mechanism of growth inhibition by ER β , as ER β can also reduce cell growth of ER α -negative
268 PEO-14 cells. It is possible that ER β directly affect factors involved in cell proliferation, such as
269 transcriptional coregulators, cell cycle regulators but also growth factors.

270 We report that ER β could not only reduce the growth of the primary tumor, but could also
271 decrease the extent of metastasis, or at least the presence of tumor cells in different organs. We have

272 previously shown that ER β could inhibit *in vitro* cancer cell invasion [31], which certainly accounts
273 for the decreased dissemination observed. However, we cannot completely exclude the possibility
274 that the reduced growth of the primary tumor leads also to a decreased metastasis. Whatever the
275 effect exerted by ER β , this reduction of metastasis certainly explains the increased survival of the
276 mice implanted with ER β cells.

277 Taken together, our findings provide evidence for a scenario in which ER β acts as a potential
278 tumor-suppressor and represents a potential target for future therapies of EOC. To our knowledge,
279 this is the first *in vivo* demonstration of anti-proliferative and -metastatic actions of ER β in a
280 preclinical orthotopic model of ovarian carcinogenesis. Our results linking ER β and the metastasis
281 process are in complete agreement with clinical studies revealing that ER β is not expressed in
282 metastatic forms of ovarian cancers [20] and the loss of its expression correlates with a shorter
283 overall survival [23]. Here, we further unravel that ER β directly impacts on ER α expression, cell
284 cycle and invasive properties of cancer cells. The reasons accounting for the weak expression of
285 ER β in ovarian cancer remain elusive. Recent reports suggest possible epigenetic modifications
286 leading to *ER β* silencing, as treatment of ovarian cancer cells with DNA methyltransferase or
287 histone deacetylase inhibitors could restore its expression [49,50,51]. Another hypothesis would be
288 a preferential degradation of ER β protein by the proteasome, resulting in low levels of this receptor
289 in cancer cells [32]. These could be the tracks to explore in the future for controlling ER β
290 expression and developing novel therapies in ovarian cancer.

291

292

293 **Materials and Methods**

294 **Tumor cell line**

295 The human ovarian cell lines BG-1 (ER α -positive cells) [52] or PEO14 [33] were obtained
296 from Dr. P Pujol [52] and grown in DMEM supplemented with 10% FCS and gentamycin at 37°C
297 in a humidified atmosphere containing 5% CO₂. To wean the cells off steroids, they were cultured in
298 phenol red-free DMEM-F12 supplemented with 10% charcoal dextran-treated FCS (CDFCS) for 4
299 days.

300 The stably transfected BG-1-luc cell line was obtained after transfection with the plasmid CMV-
301 LUC-Neo encoding the luciferase reporter under the control of CMV promoter. Transfected cells
302 were then selected by G418 at a concentration of 0.5 mg/ml. Luminescent clones were identified
303 using photon-counting camera (NightOWL II LB 983 from Berthold, France) by addition of
304 luciferin in the growth medium, and the most responsive clones were isolated.

305 **RNA extraction and real-time PCR**

306 Total RNA was isolated with TRIzol reagent (Invitrogen) as described by the manufacturer.
307 Reverse transcription was performed using random primers and Superscript II enzyme (Invitrogen).
308 Real-time PCR quantification was then performed using a SYBR Green approach (Light Cycler;
309 Roche), as previously described [53]. For each sample, *ER α* and *ER β* mRNA levels were
310 normalized with *RS9* mRNA levels. The sequences of the oligonucleotides used were previously
311 described [49].

312 **Recombinant adenovirus construction, propagation and infection.** The non-recombinant
313 adenovirus Ad5, and the adenovirus encoding ER α (Ad α) or ER β (Ad β) used in this study have
314 been previously described [31]. BG-1 cells were infected overnight at a multiplicity of infection
315 (MOI) of 25 and PEO-14 at a MOI of 100 in DMEM/F12 10% CDFCS.

316 **Constructs and Transient transfection.** The ERE-TK-LUC construct consists of two ERE in
317 tandem upstream of TK promoters [49]. 3.10⁵ of steroid-weaned cells were plated in 12-well plates

318 in phenol red-free DMEM-F12, and supplemented with 10% CDFCS 24 h before transfection.
319 Cells were infected with Ad5, Ad α or Ad β viruses as mentioned above. Transfections were
320 performed using lipofectamine according to the manufacturer's recommendations, using 2 μ g of the
321 luciferase reporter, along with 0.5 μ g of the internal reference reporter plasmid (CMV-Gal) per
322 well. After 6 h incubation, the medium was removed and the cells were placed into a fresh medium
323 supplemented with a control vehicle (ethanol) or E2. Twenty-four hours later, cells were harvested
324 and assayed for luciferase activity using a Centro LB960 Berthold luminometer. β -galactosidase
325 was determined as previously described [49].

326 **Protein extracts and Western blots**

327 BG-1 cells were cultured for 4 days in CDFCS. Cells were treated for 24h with 4 mM thymidine
328 before adenoviral infection with Ad5 or Ad β viruses. Cells were harvested in Tris-glycerol buffer
329 (Tris-HCl 50mM, EDTA 1.5mM, 10% glycerol) supplemented with protease inhibitor cocktail
330 (Roche) and phosphatase inhibitors, and were then sonicated. 30 μ g of protein extracts were
331 subjected to SDS-PAGE protein samples Western blot analyses were done using ER α (Santa Cruz,
332 ref SC-543), ER β [31], Cyclin D1 (Cell Signaling, ref 2926), Akt (Cell Signaling, ref 9372), p-AKT
333 (Cell Signaling, ref 9271), Cyclin A2 (Sigma-Aldrich, ref C0244), p-Rb (Cell Signaling, ref 9308),
334 total Rb (Cell Signaling, ref 9309) and β -actin (Santa Cruz, ref: SC-1615) antibodies.
335 Immunoreactivity was detected with Millipore ECL system. Actin was used as a loading control.

336 **Proliferation assay.**

337 20000 BG-1 or PEO14 cells were plated in 24-well plates and grown in the presence of control
338 vehicle ethanol or 10^{-8} M estradiol for 4-days. Cells were then collected their proliferation was
339 quantified by counting the cells on a cell counter.

340 **Flow cytometry**

341 1×10^6 BG-1 were collected 24h after infection with Ad5 or Ad β adenoviruses. Cells were
342 resuspended in 75% ethanol and fixed for 12 min. After centrifugation, cells were incubated in PBS

343 containing 40 µg/ml propidium iodide and 100 µg/ml RNase for half an hour at 37°C. Cell cycle
344 analysis was performed on an Epics-XL flow cytometer (Beckman Coulter, Fullerton, CA, USA)
345 and analyzed with Modfit software (Verity Software, Topsham, ME, USA).

346 **Animal xenografts**

347 Female Nu/Foxn1 athymic nude mice, 7 weeks old were obtained from Harlan. Mice were
348 acclimatized for 1 week before the experiment, and were kept under pathogen-free conditions in
349 laminar-flow boxes (5 mice/cage) maintained under standard conditions (22±2 °C, 45±10% relative
350 humidity, 12 h light/12 h dark cycle each day, standard diet and water ad libitum). All experiments
351 were performed in accordance with the French guidelines for experimental animal studies and
352 declared to ethical committee (Comité d'Ethique pour l'Expérimentation Animale Languedoc
353 Roussillon (CEEA-LR)) (Permit No. obtained for this study: CEEA-LR-11014). All efforts were
354 made to minimize suffering.

355 When indicated, before cell implantation, a silicone tube (silastic) filled with a solid mixture
356 of E2 and cholesterol as a carrier (1:10) was implanted subcutaneously (sc) in the interscapular
357 region of ovariectomized mice as previously described [54]. Two days later, 5.10⁶ BG-1 cells
358 prepared in 75 µl serum-free culture medium, combined with phenol red free Matrigel (1:1, v/v, BD
359 Biosciences) were sc grafted on both flanks of these mice. Alternatively, 1.10⁶ BG-1 cells prepared
360 in 20 µl serum-free culture medium combined with matrigel (2:1, v/v) were orthotopically grafted in
361 the left ovary surgically exposed of anaesthetized mice.

362 ***In vivo* bioluminescent imaging BG-1 cells**

363 To measure luciferase activity, mice were first sedated by isoflurane gas anesthesia system
364 (T.E.M., Bordeaux, France). Mice were then injected intraperitoneally with 125 mg/kg body weight
365 of luciferin (sodium salt; Promega) in aqueous solution. Luminescence was measured using
366 NightOWL II LB 981 CCD camera and integrated for a 5-min period. The signal intensities from
367 regions of interest (ROI) were obtained and data were expressed as photon (Ph/s). Background was

368 defined from a region of the same size placed in a non-luminescent area nearby the animal and then
369 subtracted from the measured luminescent signal intensity. The correlation of luciferase signal with
370 tumor volume and weight was demonstrated (Supplemental Fig. S1).

371 **Tissues extracts luciferase activity**

372 Lysates from tissue samples were prepared in ceramic beads-containing tubes (Lysing matrix, MP
373 Biomedicals), by disruption in luciferase lysis buffer (25mM Tris Phosphate pH7.8, 2 mM DTT, 2
374 mM EDTA, 10% Glycerol, 1% Triton X100, 1mg/ml BSA). The samples were subjected to two
375 oscillations at 7,000 r/min for 15 seconds. The lysates were then centrifuged at 10,000 rpm for 30
376 min at 4°C, and the supernatant was saved and assayed. 10 µl of the supernatant were loaded onto
377 96-well white opaque tissue plates (Lumitrac 200), and luciferase activity was measured as
378 previously described [49].

379

380

381

382 **References**

383 1. Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC (2001) Ovarian surface epithelium: biology, endocrinology,
384 and pathology. *Endocr Rev* 22: 255-288.

385 2. Cho KR, Shih Ie M (2009) Ovarian cancer. *Annu Rev Pathol* 4: 287-313.

386 3. Rosenfeld CS, Wagner JS, Roberts RM, Lubahn DB (2001) Intraovarian actions of oestrogen. *Reproduction* 122:
387 215-226.

388 4. Beral V, Bull D, Green J, Reeves G (2007) Ovarian cancer and hormone replacement therapy in the Million Women
389 Study. *Lancet* 369: 1703-1710.

390 5. Glud E, Kjaer SK, Thomsen BL, Hogdall C, Christensen L, et al. (2004) Hormone therapy and the impact of estrogen
391 intake on the risk of ovarian cancer. *Arch Intern Med* 164: 2253-2259.

392 6. Lacey JV, Jr., Brinton LA, Leitzmann MF, Mouw T, Hollenbeck A, et al. (2006) Menopausal hormone therapy and
393 ovarian cancer risk in the National Institutes of Health-AARP Diet and Health Study Cohort. *J Natl Cancer Inst*
394 98: 1397-1405.

395 7. Rossing MA, Cushing-Haugen KL, Wicklund KG, Doherty JA, Weiss NS (2007) Menopausal hormone therapy and
396 risk of epithelial ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 16: 2548-2556.

397 8. Beral V, Hannaford P, Kay C (1988) Oral contraceptive use and malignancies of the genital tract. Results from the
398 Royal College of General Practitioners' Oral Contraception Study. *Lancet* 2: 1331-1335.

399 9. Deligeoroglou E, Michailidis E, Creasas G (2003) Oral contraceptives and reproductive system cancer. *Ann N Y*
400 *Acad Sci* 997: 199-208.

401 10. Katzenellenbogen BS, Katzenellenbogen JA (2000) Estrogen receptor alpha and estrogen receptor beta: regulation
402 by selective estrogen receptor modulators and importance in breast cancer. *Breast Cancer Res* 2: 335-344.

403 11. Bardin A, Boulle N, Lazennec G, Vignon F, Pujol P (2004) Loss of ERbeta expression as a common step in
404 estrogen-dependent tumor progression. *Endocr Relat Cancer* 11: 537-551.

405 12. Greenlee RT, Murray T, Bolden S, Wingo PA (2000) Cancer statistics, 2000. *CA Cancer J Clin* 50: 7-33.

406 13. Hatch KD, Beecham JB, Blessing JA, Creasman WT (1991) Responsiveness of patients with advanced ovarian
407 carcinoma to tamoxifen. A Gynecologic Oncology Group study of second-line therapy in 105 patients. *Cancer*
408 68: 269-271.

409 14. Drummond AE, Fuller PJ (2010) The importance of ERbeta signalling in the ovary. *J Endocrinol* 205: 15-23.

410 15. Krege JH, Hodgins JB, Couse JF, Enmark E, Warner M, et al. (1998) Generation and reproductive phenotypes of
411 mice lacking estrogen receptor beta. *PNAS* 95: 15677-15682.

412 16. Couse JF, Curtis Hewitt S, Korach KS (2000) Receptor null mice reveal contrasting roles for estrogen receptor alpha
413 and beta in reproductive tissues. *J Steroid Biochem Mol Biol* 74: 287-296.

414 17. Couse JF, Yates MM, Deroo BJ, Korach KS (2005) Estrogen Receptor- β Is Critical to Granulosa Cell
415 Differentiation and the Ovulatory Response to Gonadotropins. *Endocrinology* 146: 3247-3262.

416 18. Emmen JM, Couse JF, Elmore SA, Yates MM, Kissling GE, et al. (2005) In vitro growth and ovulation of follicles
417 from ovaries of estrogen receptor (ER) α and ER β null mice indicate a role for ER β in
418 follicular maturation. *Endocrinology* 146: 2817-2826.

419 19. Pujol P, Rey JM, Nirde P, Roger P, Gastaldi M, et al. (1998) Differential expression of estrogen receptor-alpha and -
420 beta messenger RNAs as a potential marker of ovarian carcinogenesis. *Cancer Res* 58: 5367-5373.

421 20. Rutherford T, Brown WD, Sapi E, Aschkenazi S, Munoz A, et al. (2000) Absence of estrogen receptor-beta
422 expression in metastatic ovarian cancer. *Obstet Gynecol* 96: 417-421.

423 21. Lazennec G (2006) Estrogen receptor beta, a possible tumor suppressor involved in ovarian carcinogenesis. *Cancer*
424 *Lett* 231: 151-157.

425 22. Chan KK, Wei N, Liu SS, Xiao-Yun L, Cheung AN, et al. (2008) Estrogen receptor subtypes in ovarian cancer: a
426 clinical correlation. *Obstet Gynecol* 111: 144-151.

427 23. Halon A, Nowak-Markwitz E, Maciejczyk A, Pudelko M, Gansukh T, et al. (2011) Loss of estrogen receptor beta
428 expression correlates with shorter overall survival and lack of clinical response to chemotherapy in ovarian
429 cancer patients. *Anticancer Res* 31: 711-718.

430 24. Burges A, Bruning A, Dannenmann C, Blankenstein T, Jeschke U, et al. (2010) Prognostic significance of estrogen
431 receptor alpha and beta expression in human serous carcinomas of the ovary. *Arch Gynecol Obstet* 281: 511-
432 517.

433 25. Lurie G, Wilkens LR, Thompson PJ, Shvetsov YB, Matsuno RK, et al. (2011) Estrogen Receptor Beta rs1271572
434 Polymorphism and Invasive Ovarian Carcinoma Risk: Pooled Analysis within the Ovarian Cancer Association
435 Consortium. *PLoS One* 6: e20703.

436 26. De Stefano I, Zannoni GF, Prisco MG, Fagotti A, Tortorella L, et al. (2011) Cytoplasmic expression of estrogen
437 receptor beta (ERbeta) predicts poor clinical outcome in advanced serous ovarian cancer. *Gynecol Oncol* 122:
438 573-579.

- 439 27. Geisinger KR, Kute TE, Pettenati MJ, Welander CE, Dennard Y, et al. (1989) Characterization of a human ovarian
440 carcinoma cell line with estrogen and progesterone receptors. *Cancer* 63: 280-288.
- 441 28. Baldwin WS, Curtis SW, Cauthen CA, Risinger JI, Korach KS, et al. (1998) Bg-1 ovarian cell line - an alternative
442 model for examining estrogen-dependent growth in vitro. *In Vitro Cellular & Developmental Biology Animal*
443 34: 649-654.
- 444 29. Shaw TJ, Senterman MK, Dawson K, Crane CA, Vanderhyden BC (2004) Characterization of intraperitoneal,
445 orthotopic, and metastatic xenograft models of human ovarian cancer. *Mol Ther* 10: 1032-1042.
- 446 30. Cheng J, Lee EJ, Madison LD, Lazennec G (2004) Expression of estrogen receptor beta in prostate carcinoma cells
447 inhibits invasion and proliferation and triggers apoptosis. *FEBS Lett* 566: 169-172.
- 448 31. Lazennec G, Bresson D, Lucas A, Chauveau C, Vignon F (2001) ER beta inhibits proliferation and invasion of
449 breast cancer cells. *Endocrinology* 142: 4120-4130.
- 450 32. Picard N, Charbonneau C, Sanchez M, Licznar A, Busson M, et al. (2008) Phosphorylation of Activation Function-1
451 Regulates Proteasome-Dependent Nuclear Mobility and E6-Associated Protein Ubiquitin Ligase Recruitment
452 to the Estrogen Receptor {beta}. *Mol Endocrinol* 22: 317-330.
- 453 33. Langdon SP, Lawrie SS, Hay FG, Hawkes MM, McDonald A, et al. (1988) Characterization and properties of nine
454 human ovarian adenocarcinoma cell lines. *Cancer Res* 48: 6166-6172.
- 455 34. Freund A, Chauveau C, Brouillet JP, Lucas A, Lacroix M, et al. (2003) IL-8 expression and its possible relationship
456 with estrogen-receptor-negative status of breast cancer cells. *Oncogene* 22: 256-265.
- 457 35. Foster JS, Henley DC, Ahamed S, Wimalasena J (2001) Estrogens and cell-cycle regulation in breast cancer. *Trends*
458 *Endocrinol Metab* 12: 320-327.
- 459 36. Treeck O, Pfeiler G, Mitter D, Lattrich C, Piendl G, et al. (2007) Estrogen receptor {beta}1 exerts antitumoral
460 effects on SK-OV-3 ovarian cancer cells. *J Endocrinol* 193: 421-433.
- 461 37. Licznar A, Caporali S, Lucas A, Weisz A, Vignon F, et al. (2003) Identification of genes involved in growth
462 inhibition of breast cancer cells transduced with estrogen receptor. *FEBS Lett* 553: 445-450.
- 463 38. Paruthiyil S, Parmar H, Kerekatte V, Cunha GR, Firestone GL, et al. (2004) Estrogen receptor beta inhibits human
464 breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. *Cancer Res* 64: 423-428.
- 465 39. Johnson DG, Walker CL (1999) Cyclins and cell cycle checkpoints. *Annu Rev Pharmacol Toxicol* 39: 295-312.
- 466 40. Burgering BM, Medema RH (2003) Decisions on life and death: FOXO Forkhead transcription factors are in
467 command when PKB/Akt is off duty. *J Leukoc Biol* 73: 689-701.
- 468 41. Alarid ET, Bakopoulos N, Solodin N (1999) Proteasome-mediated proteolysis of estrogen receptor: a novel
469 component in autologous down-regulation. *Mol Endocrinol* 13: 1522-1534.
- 470 42. Lonard DM, Nawaz Z, Smith CL, O'Malley BW (2000) The 26S proteasome is required for estrogen receptor-alpha
471 and coactivator turnover and for efficient estrogen receptor-alpha transactivation. *Mol Cell* 5: 939-948.
- 472 43. Reid G, Hubner MR, Metivier R, Brand H, Denger S, et al. (2003) Cyclic, proteasome-mediated turnover of
473 unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling. *Mol*
474 *Cell* 11: 695-707.
- 475 44. Matthews J, Wihlen B, Tujague M, Wan J, Strom A, et al. (2006) Estrogen receptor (ER) beta modulates ERalpha-
476 mediated transcriptional activation by altering the recruitment of c-Fos and c-Jun to estrogen-responsive
477 promoters. *Mol Endocrinol* 20: 534-543.
- 478 45. Pettersson K, Grandien K, Kuiper GG, Gustafsson JA (1997) Mouse estrogen receptor beta forms estrogen response
479 element-binding heterodimers with estrogen receptor alpha. *Mol Endocrinol* 11: 1486-1496.
- 480 46. Cowley SM, Hoare S, Mosselman S, Parker MG (1997) Estrogen receptors alpha and beta form heterodimers on
481 DNA. *J Biol Chem* 272: 19858-19862.
- 482 47. Williams C, Edvardsson K, Lewandowski SA, Strom A, Gustafsson JA (2008) A genome-wide study of the
483 repressive effects of estrogen receptor beta on estrogen receptor alpha signaling in breast cancer cells.
484 *Oncogene* 27: 1019-1032.
- 485 48. Lindberg MK, Moverare S, Skrtic S, Gao H, Dahlman-Wright K, et al. (2003) Estrogen Receptor (ER)-{beta}
486 Reduces ER{alpha}-Regulated Gene Transcription, Supporting a "Ying Yang" Relationship between
487 ER{alpha} and ER{beta} in Mice. *Mol Endocrinol* 17: 203-208.
- 488 49. Duong V, Licznar A, Margueron R, Bouille N, Busson M, et al. (2006) ERalpha and ERbeta expression and
489 transcriptional activity are differentially regulated by HDAC inhibitors. *Oncogene* 25: 1799-1806.
- 490 50. Yap OW, Bhat G, Liu L, Tollefsbol TO (2009) Epigenetic modifications of the Estrogen receptor beta gene in
491 epithelial ovarian cancer cells. *Anticancer Res* 29: 139-144.
- 492 51. Suzuki F, Akahira J, Miura I, Suzuki T, Ito K, et al. (2008) Loss of estrogen receptor beta isoform expression and its
493 correlation with aberrant DNA methylation of the 5'-untranslated region in human epithelial ovarian carcinoma.
494 *Cancer Sci* 99: 2365-2372.
- 495 52. Moll F, Katsaros D, Lazennec G, Hellio N, Roger P, et al. (2002) Estrogen induction and overexpression of fibulin-
496 1C mRNA in ovarian cancer cells. *Oncogene* 21: 1097-1107.
- 497 53. Lucas A, Kremer EJ, Hemmi S, Luis J, Vignon F, et al. (2003) Comparative transductions of breast cancer cells by
498 three DNA viruses. *Biochem Biophys Res Commun* 309: 1011-1016.

499 54. Pillon A, Servant N, Vignon F, Balaguer P, Nicolas JC (2005) In vivo bioluminescence imaging to evaluate
500 estrogenic activities of endocrine disrupters. *Anal Biochem* 340: 295-302.
501
502

503 **Figure legends**

504

505 **Figure 1: Expression levels and transcriptional activity of ER β in BG-1.** **A.** BG-1 cells were
506 infected with Ad5 or Ad β adenoviruses and treated for 24h with control vehicle ethanol (Control) or
507 E2 10⁻⁸M (E2). The expression of ER β and rS9 reference gene was measured by real-time PCR.
508 Results represent the mean \pm SD of ER β expression normalized by rS9 of 3 independent
509 experiments. Measurements of Ad β and Ad β + E2 groups were compared by unpaired Student's *t*
510 test. ** $p < 0.001$. **B.** Proteins were extracted from cells infected in the same conditions as in A and
511 treated for 0, 3, 6 or 24h with E2 10⁻⁸M (E2). Levels of ER α and ER β were analyzed by western
512 blot. Actin was used as a loading control. The upper band of ER β blot labeled with a star
513 corresponds to aspecific staining. **C.** Transcriptional activity of ER β . BG-1 cells were infected with
514 Ad5 or Ad β adenoviruses and transfected with ERE2-TK-Luc reporter along with β -galactosidase
515 reporter. The cells were treated with ethanol as vehicle (Control) or E2 (10⁻⁸M) for 24h. Results
516 show relative Luc activities (% of values of Ad5-infected cells without E2) \pm SD after normalization
517 with β -gal activity (3 independent experiments). Measurements of Ad5+E2 and Ad β + E2 groups
518 were compared by unpaired Student's *t* test. * $p < 0.05$. **D.** PEO14 cells were infected with Ad5,
519 Ad α , Ad β or the combination of Ad α and Ad β adenovirus and transfected with ERE2-TK-LUC
520 reporter along with β -galactosidase reporter. Cells were treated with control vehicle (Control) or E2
521 (10⁻⁸M) for 24h. Results show relative luciferase activities (% of values of Ad5 infected cells
522 without E2) \pm SD after normalization with β -gal activity (3 independent experiments).
523 Measurements of Ad α , Ad β and Ad α + β groups were compared by unpaired Student's *t* test. *** $p <$
524 0.001. NS: non significant. **E.** Proteins from PEO14 cells infected in the same conditions as in D
525 and treated with vehicle ethanol (Control) or E2 10⁻⁸M (E2) for 3, 6 or 24h were analyzed by
526 western blot with antibodies against ER α and ER β . Actin was used as a loading control. The upper
527 band labeled with a star in right panel corresponds to aspecific staining.

528

529 **Figure 2: ER β is a negative regulator of BG- cell growth.** A. The growth of BG-1 cells,
530 expressing or not ER β , was monitored *in vitro* using a cell counter. BG-1 cells were plated in 24-
531 well plates and cultured in the presence of vehicle or E2 (10⁻⁸M). Proliferation is expressed as % of
532 control cells grown at day 0. Data represent the mean \pm SD from triplicates. Measurements of Ad5 +
533 E2 and Ad β + E2 groups were compared by unpaired Student's *t* test. ** p<0.001. B. ER β inhibits
534 tumor growth in a bioluminescent subcutaneous mouse model. BG-1 cells stably expressing Luc and
535 infected with Ad5 or Ad β adenoviruses were injected subcutaneously in ovariectomized female
536 Nude mice. Luciferase activity was monitored for 25 days. Results are expressed in photons/s (Ph/s)
537 and represent the mean \pm SD from 8 animals. Measurements of Ad5 + E2 and Ad β + E2 groups
538 were compared by unpaired Student's *t* test. * p<0.05.

539
540 **Figure 3: ER β disturbs cell cycle of BG-1 cells and regulators.** A. BG-1 cells were collected 24h
541 after infection with Ad5 or Ad β adenoviruses and analyzed for cell cycle distribution. Results
542 represent the mean \pm SEM of 3 experiments. Ad5 and Ad β groups were compared by unpaired
543 Student's *t* test. ** p<0.001. B. BG-1 cells were infected with Ad5 or Ad β viruses. After infection,
544 cells were treated for 3, 6 or 24h with vehicle (ethanol, C) or 10⁻⁸M E2. 30 μ g of protein extracts
545 were used for Western blot. β -actin was used as a loading control. Unless specified, the ratio of
546 target proteins over β -actin is indicated below the gels. Representative of 2 experiments.

547
548 **Figure 4: ER β inhibits tumor growth in an orthotopic xenograft mouse model.** A. BG-1-Luc
549 cells infected with Ad5 or Ad β adenoviruses were injected in the left ovary of Nude mice. Luc
550 activity was monitored for 35 days as described in Fig. 1C. Results are expressed in photons/s (Ph/s)
551 and represent one representative experiment corresponding to the average \pm SD of at least 5 animals
552 per group. Mann-Whitney test was used for comparison. * p<0.05 and ** p<0.01. A representative

553 image of day 35 is shown. **B.** Representative pictures of whole animals and genital tract of animals
554 euthanized at day 35 are displayed.

555

556 **Figure 5: ER β reduces metastasis and improves survival.** **A.** Nude mice injected with BG-1-Luc
557 cells in the same conditions as in Fig. 4. were euthanized 28 days after injection. Lung, liver and
558 right contralateral ovary were taken and Luc activity was assayed as mentioned above. Results are
559 expressed as Photons/s/mg of proteins and represent the mean of 5 animals \pm SEM. Mann-Whitney
560 test was used for comparison. * $p < 0.05$ and ** $p < 0.01$. **B.** Kaplan-meier survival curve. 10 mice
561 were orthotopically xenografted with BG-1 cells stably expressing Luc, infected with Ad5 or Ad β
562 adenoviruses, followed-up daily for the development of respiratory distress, limb paralysis and
563 weight loss, and euthanized immediately if noted. P value is the one obtained in log-rank tests.

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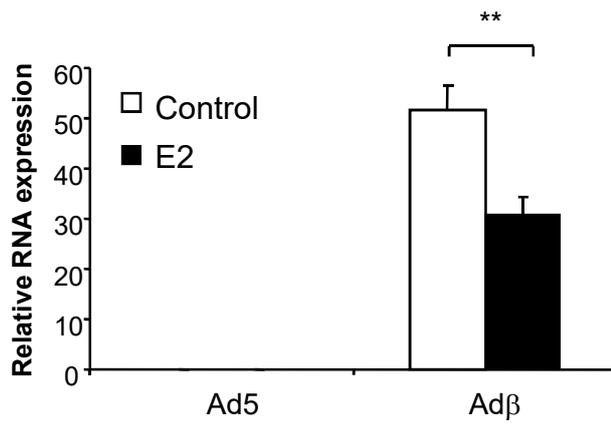
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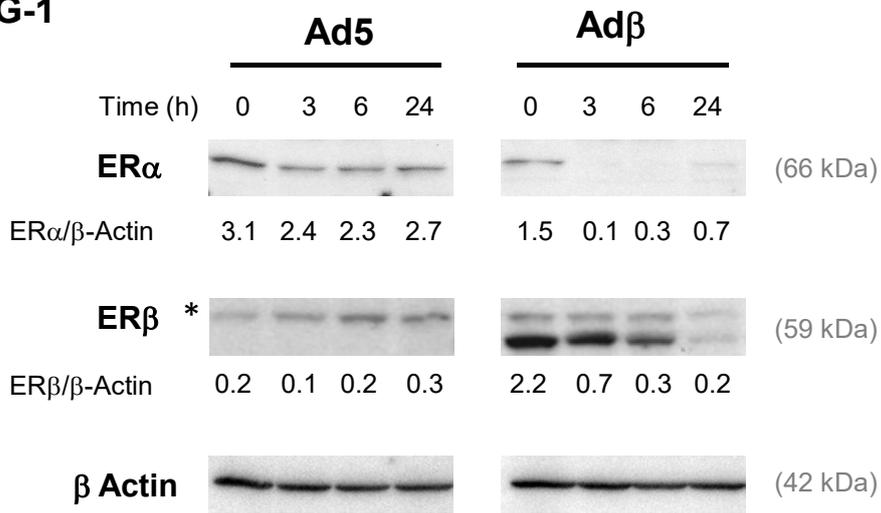
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A BG-1



B BG-1



C BG-1

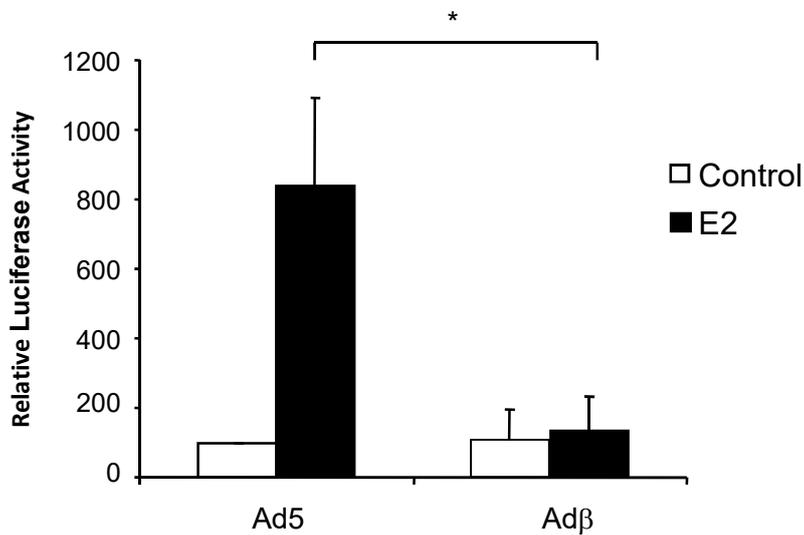


Figure 1

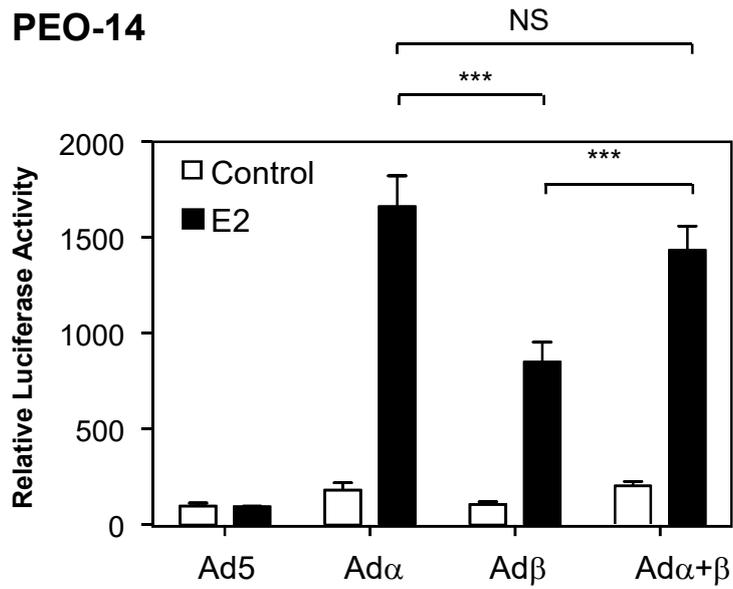
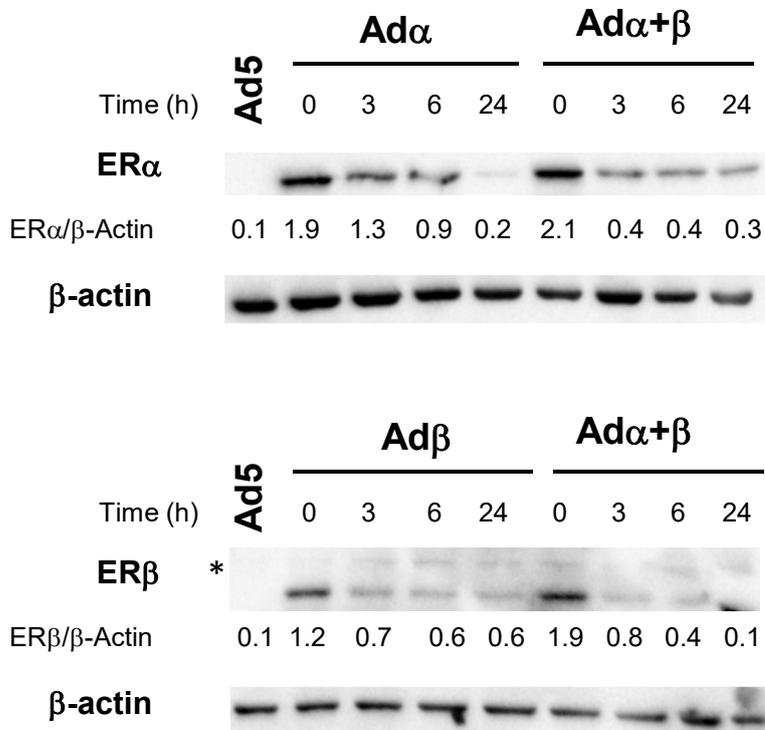
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Figure 1

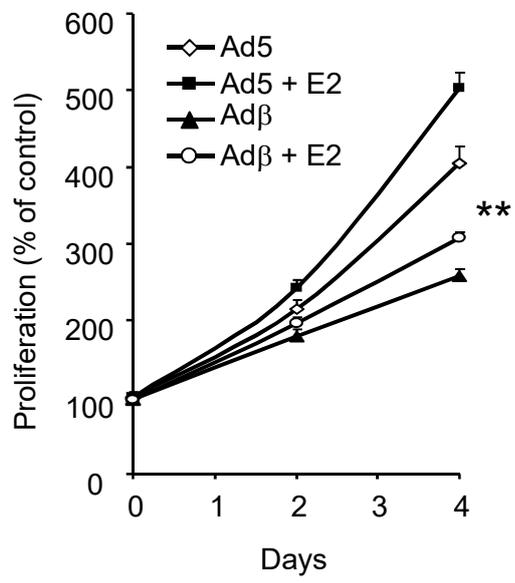
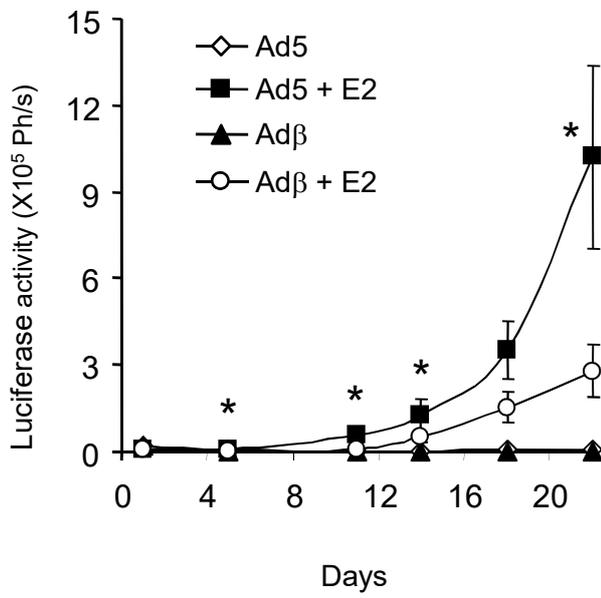
A**B**

Figure 2

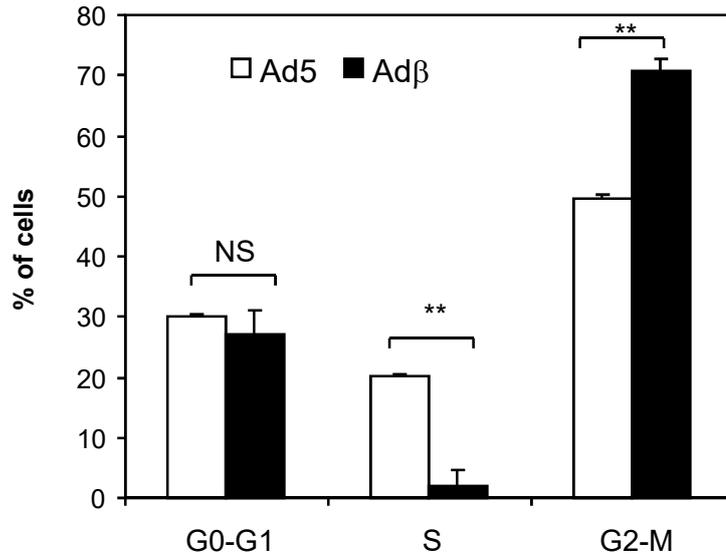
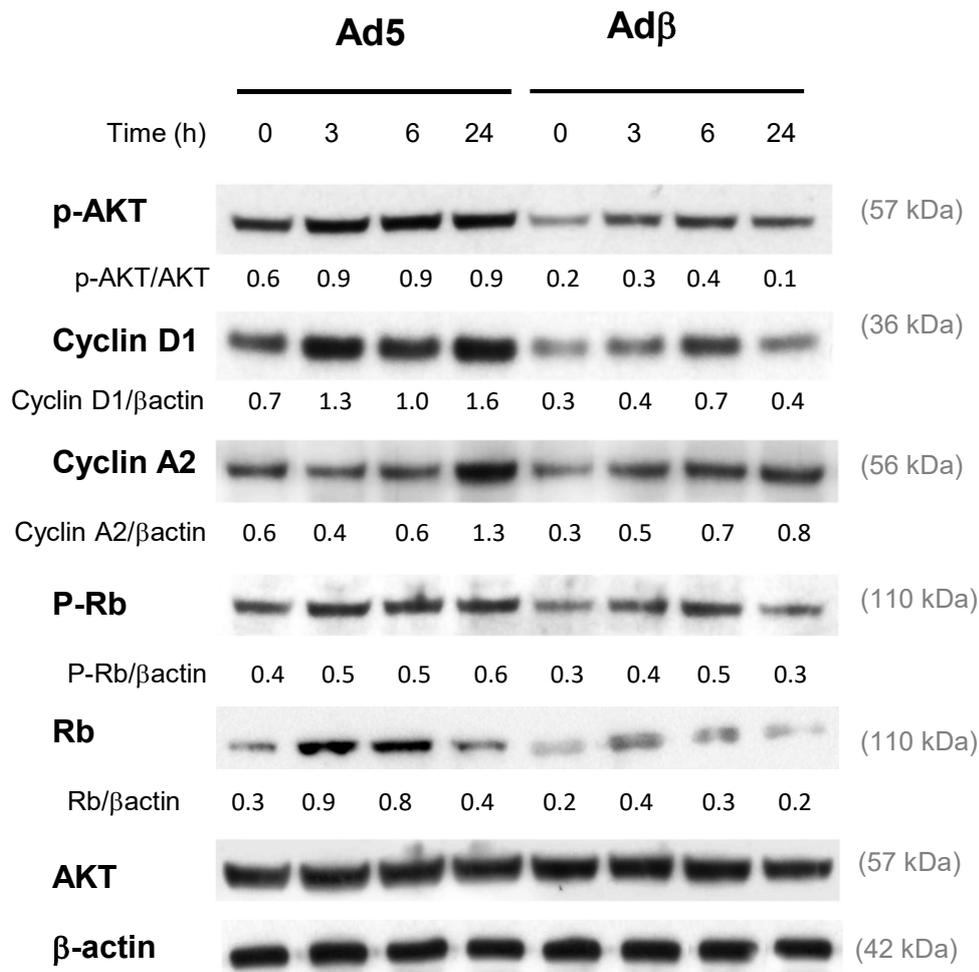
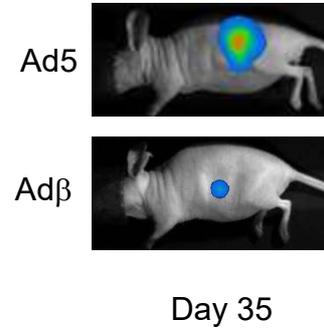
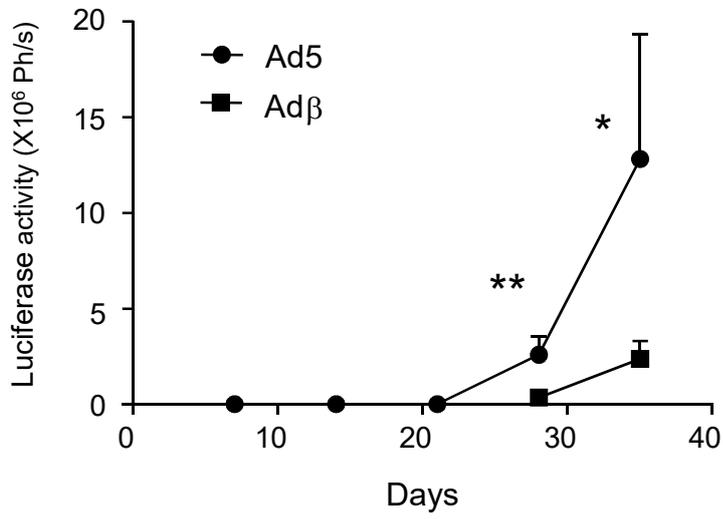
A**B**

Figure 3

A



B

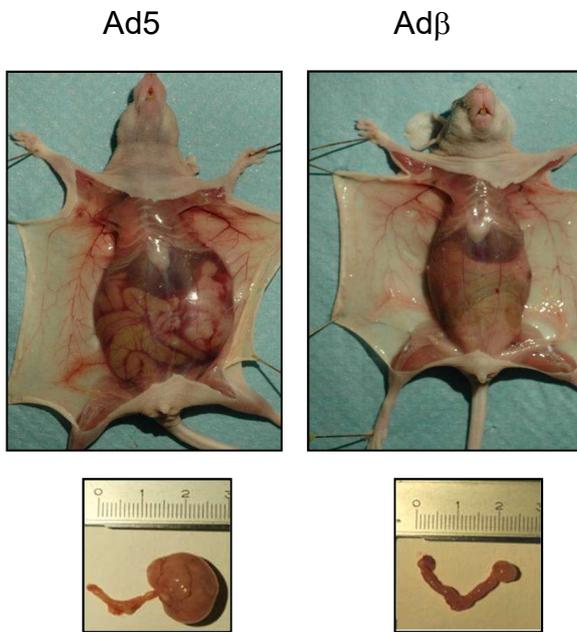


Figure 4

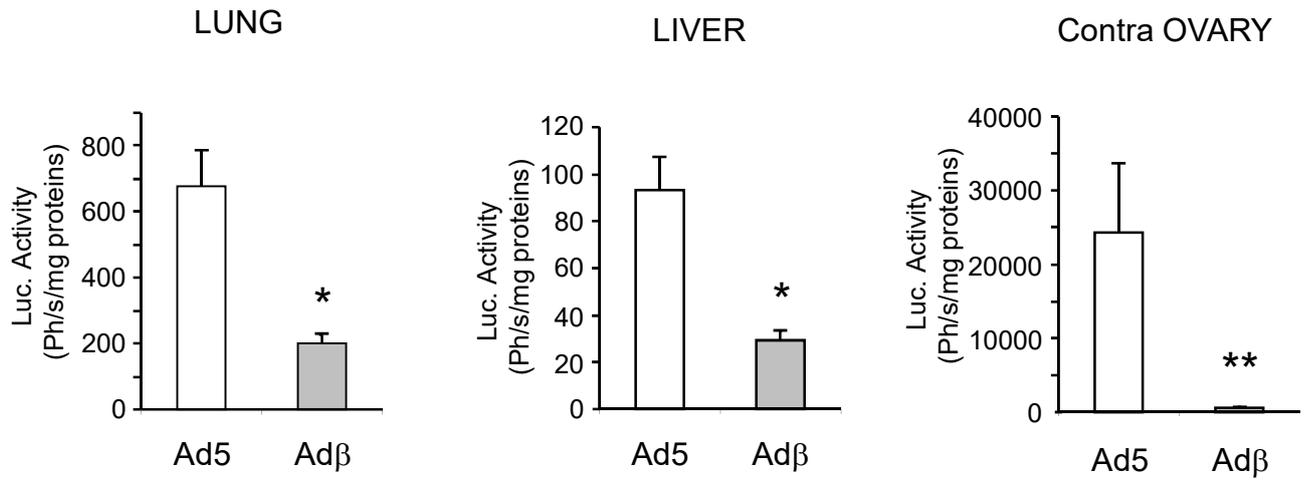
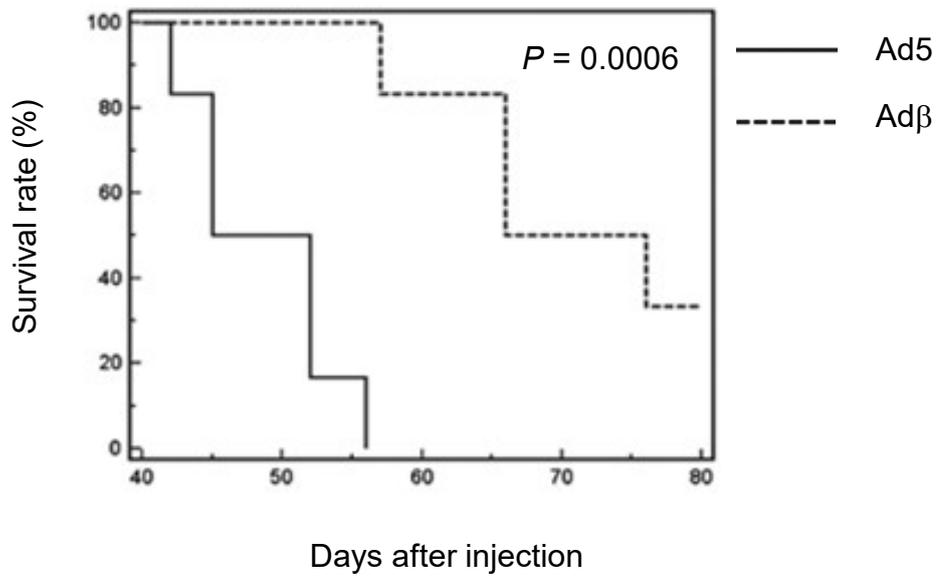
A**B**

Figure 5

Supplemental figure legends

Figure S1: In vivo monitoring of orthotopically injected BG-1-luc cells in the left ovary.

Cells were injected into the bursal membrane of the left ovary and animals were monitored by bioluminescence. At day 25, animals were euthanized, and bioluminescence, the volume and weight of the ovary were measured. Correlation of the volume of the tumor (left panel) or weight (right panel) with the luciferase is shown.

Figure S2: In vitro growth of PEO14 cells expressing or not ER β . In vitro growth was monitored by counting the cells on a cell counter after 4 days of proliferation. PEO14 cells were infected with Ad5, Ad α or Ad β virus and cultured in the presence of control vehicle ethanol (Control) or E2 (10⁻⁸M). Proliferation is expressed as fold of control cells grown at day 4. Data represent the mean \pm SD from triplicates. Measurements of Ad α and Ad β groups were compared to Ad5 by unpaired Student's t test. Only Ad β groups were significantly different from Ad5 groups.

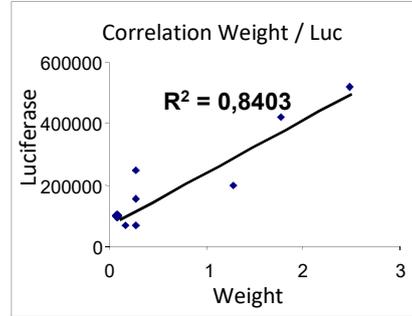
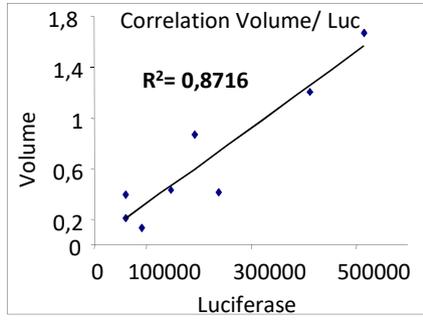


Figure S1

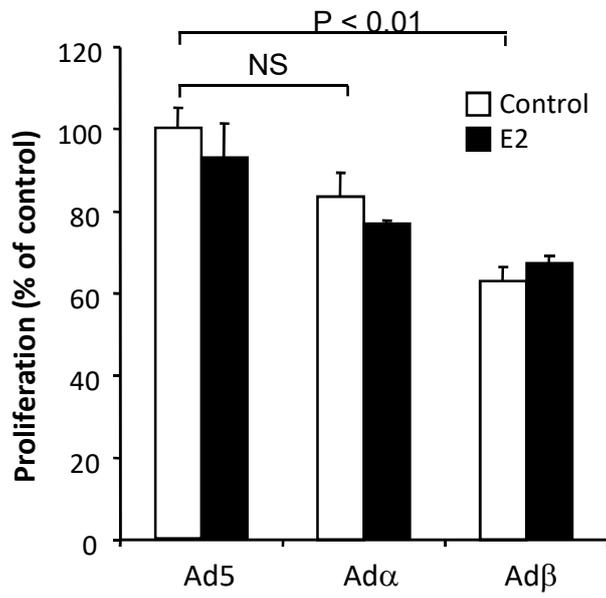


Figure S2