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1 **The antagonist properties of Bazedoxifene after acute treatment are shifted to**
2 **stimulatory action after chronic exposure in the liver but not in the uterus.**

3
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22
23 **Short Title:** Tissue specific action of TSEC

24 **Keywords:** estrogen receptor (ER), Bazedoxifene, tissue-selective estrogen complex (TSEC),
25 hormone therapy, menopause, liver

26 **Abstract**

27 A promising alternative to conventional hormone therapy for postmenopausal symptoms is
28 treatment combining Bazedoxifene (BZA), a third-generation selective estrogen receptor
29 modulator (SERM), and conjugated equine estrogen (CE). This combination is also known as
30 a tissue-selective estrogen complex (TSEC). Understanding the tissue-specific actions of
31 SERMs and the TSEC remains a major challenge to try to predict their clinical effects.

32 The aim of this study was to compare acute *versus* chronic treatment with BZA, CE or
33 CE+BZA in two major targets of estrogens, the uterus and the liver. In these two tissues,
34 acute treatment with CE, but not with BZA, induced similar gene expression change than the
35 most important endogenous estrogen, 17- β estradiol (E2). Acute induction of gene expression
36 by E2 or by CE was antagonized by the addition of BZA. Concomitantly, BZA alone or in
37 combination with E2 or CE induced a partial degradation of ER α protein after acute exposure.
38 In uterus, chronic treatment of BZA alone had no impact on tissue weight gain or on epithelial
39 cell proliferation, and also antagonized CE-effect in uterus, thereby mimicking the acute
40 effect. By contrast, in the liver, chronic BZA and CE+BZA elicited agonistic transcriptional
41 effects similar to those of CE alone. In addition, at variance to BZA acute effect, no change in
42 ER α protein abundance was observed after chronic treatment in this tissue.

43 These experimental *in vivo* data highlight a new aspect of the time-dependent tissue-specific
44 action of BZA or TSEC, *i.e.* they can act acutely as antagonists but become agonists after
45 chronic treatment. This shift was observed in liver tissue, but not in proliferative sex target
46 such as the uterus.

47

48 1. Introduction

49 The decline of estrogen levels after menopause is associated with bone loss, hot flushes and
50 vulvar–vaginal atrophy, affecting sexual function, relationships, and quality of life. Estrogen–
51 progestin therapy was previously considered as the standard for managing moderate to
52 severely bothersome symptoms associated with menopause but this drug combination is now
53 controversial due to the increased risk of breast cancer and thromboembolism (Anderson et
54 al., 2004, Chlebowski et al., 2003, Rossouw et al., 2002). The combination of conjugated
55 estrogen (CE) with Bazedoxifene (BZA) is known as the tissue-selective estrogen complex
56 (TSEC). It was designed to minimize the undesirable effects of hormone therapy on breast
57 tissue yet allow the beneficial effects of estrogen on other estrogen-target tissues, thus
58 suppressing climacteric symptoms and preventing osteoporosis (Mirkin and Komm, 2013).
59 This drug combination was developed by Pfizer and has been marketed in the US for two
60 years as Duavee[®] (CE, 0.45 mg / BZA, 20 mg). It has also been approved by the European
61 Medicines Agency (EMA) (Komm and Mirkin, 2013). The efficacy and safety of CE+BZA in
62 postmenopausal women, and the impact of this drug combination on quality of life was
63 evaluated through a series of five pivotal phase 3 randomized, double-blinded, multicentered,
64 active and/or placebo-controlled studies, called the Selective estrogens, Menopause And
65 Response to Therapy (SMART) trials (Kagan et al., 2010, Mirkin and Komm, 2013, Pickar et
66 al., 2009, Pinkerton et al., 2009, Umland et al., 2016). From these trials, CE+BZA was found
67 to be associated with significant benefits such as a reduction in the frequency and severity of
68 vasomotor symptoms, the prevention of bone loss, improved sleep and better menopause-
69 specific quality of life, whilst also providing a reasonable level of protection against
70 endometrial hyperplasia. Additionally, CE+BZA exhibited promising effects on breast tissue
71 since it did not increase mammographic breast density (Goldberg and Fidler, 2015, Lobo et
72 al., 2009, Pinkerton et al., 2014, Pinkerton et al., 2010, Pinkerton et al., 2009, Ronkin et al.,

73 2005, Stovall et al., 2011). The SMART trials also suggested that CE+BZA treatment was
74 associated with similar rates of coronary heart disease, stroke, venous thromboembolism and
75 menorrhagia as placebo-treated patients (Skouby et al., 2015), although definitive conclusions
76 cannot yet be made on these aspects due to the total number of women enrolled in these trials
77 and the trial durations.

78 The molecular mechanisms underlying TSEC action are far from fully understood. CE is a
79 mixture of different natural estrogens and BZA is one of a new generation of selective
80 estrogen receptor modulators (SERM) that demonstrate selective binding to the estrogen
81 receptors (ER) over other members of the nuclear receptors family (Alio Del Barrio et al.,
82 2017). SERMs are thought to be ER agonists or antagonists depending on the tissue involved.
83 Contrary to the action of 17- β -estradiol (E2) or CE that allow ER to recruit coactivators,
84 leading to an enhanced transcription of target genes, SERM is reported to rather provoke the
85 mobilization of corepressors to the ERs (Shang and Brown, 2002). This differential
86 recruitment of ER coregulators by estrogens *versus* SERMs can be attributed, at least in part,
87 to the different ER conformational changes induced by the binding of these ligands (Alio Del
88 Barrio et al., 2017). In addition, TSEC was shown to cause ER α degradation *via* the ubiquitin
89 proteasome system in the breast and uterus, which was at least in part responsible for the
90 suppression of ER α -mediated transcription (Han et al., 2016). However, this ER α modulation
91 appears to be cell- and tissue-specific since BZA treatment was able to exert an estrogen-
92 mimetic action on bone (Palacios et al., 2015) and on the metabolism contributing to
93 beneficial glucidic and lipidic effects (Barrera et al., 2014, Kim et al., 2014).

94 Thus, the consequences of the co-administration of estrogens and SERM at the molecular,
95 cellular and whole body levels are undoubtedly highly complex. Here, our goal was to explore
96 the tissue-specific effects of BZA combined with CE *in vivo* in mice comparing in two major
97 targets of estrogens, the uterus and the liver (Ahlbory-Dieker et al., 2009, Boverhof et al.,

98 2004, Gao et al., 2008, Gordon et al., 2014, Handgraaf et al., 2013, Kim et al., 2014,
99 Kobayashi et al., 2013, Palierne et al., 2016, Pedram et al., 2013). Our results indicate that the
100 effects of BZA treatment varies according to the duration of treatment (acute *versus* chronic
101 administration) and the tissue, with the responses observed in the liver being dramatically
102 different from those observed in the uterus.
103

104 2. **Materials and Methods**

105 2.1. **Mice**

106 Female C57BL/6J mice were purchased from Charles River Laboratories. All procedures
107 were performed in accordance with the principles established by the National Institute of
108 Medical Research and were approved by the local Ethical Committee of Animal Care. Mice
109 were anesthetized by intraperitoneal injection of ketamine (25 mg/kg) and xylazine (10
110 mg/kg) and ovariectomized at 4 weeks of age. To study the chronic effects of CE, BZA and
111 BZA+CE, 2 weeks after ovariectomy mice were implanted with subcutaneous osmotic
112 minipumps (Alzet; Alza, Palo Alto, CA) that released CE (3 mg/kg/day) or BZA (10
113 mg/kg/day) alone or in combination diluted in a solution of hydroxypropyl- β -cyclodextrin –
114 with a 0.63 degree of substitution - in HEPES buffer for a 3 week period. For acute treatment,
115 ovariectomized mice were orally administered with vehicle (PBS containing 0.5%
116 methylcellulose, 2% Tween 80 and 5% DMSO), CE (3 or 10 mg/kg) or E2 (1 mg/kg) alone or
117 in combination with BZA (10 mg/kg). These dose have been chosen based on previous studies
118 (Kim et al., 2014, Barrera et al., 2014, Della Torre et al., 2011, Fontana et al., 2014, Naqvi et
119 al., 2014, Oliva et al., 2013, Peano et al., 2009, Song et al., 2012) (Supplementary Table 1)
120 and in particular on those realized by Peano *et al.* in which uterus weight was used as final
121 endpoint to determine the minimal dose of CE (i.e 3 mg/kg/day) inducing uterine hypertrophy
122 and that of BZA (i.e 10 mg/kg/day) antagonizing this effect.

123 2.2. **Histological analysis**

124 Paraffin-embedded transverse sections (4 μ m) from formalin-fixed uterine specimens was
125 stained as previously described (Abot et al., 2013) with anti-Ki-67 (RM-9106; Thermo-
126 scientific) and anti-ER α antibodies (MC-20, Santa Cruz Biotechnology, Santa Cruz,
127 California). Sections were examined after numerization using a NanoZoomer Digital
128 Pathology®. To examine the proliferative effects of each treatment, the ratio of Ki-67–

129 positive epithelial cell/total cell number was evaluated from two microscopic fields of
130 measurement at x20 magnification for each uterine section.

131 **2.3. Analysis of mRNA levels by qPCR**

132 Tissues were homogenized using a Precellys tissue homogenizer (Bertin Technology, Cedex,
133 France), and total RNA from tissues was extracted using the TRIzol reagent (Invitrogen,
134 Carlsbad, CA). A total of 500 ng to 1 µg (depending on the tissue) was reverse transcribed for
135 10 minutes at 25°C and 2 hours at 37°C in a 20 µL final volume using the High Capacity
136 cDNA Reverse Transcriptase Kit (Applied Biosystems, Villebon sur Yvette, France). We
137 evaluated genes expression levels in liver by high throughput qPCR, using 6.5 ng cDNA from
138 each sample in 96.96 Dynamic Arrays analyzed in the microfluidic BioMark system
139 (Fluidigm Corporation, CA, USA). For gene expression in uterus, qPCR was performed using
140 SsoFast EvaGreen Supermix (Bio-Rad) on a StepOne instrument (Applied Biosystems).
141 Primers (Supplementary Table 2) were validated by testing PCR efficiency using standard
142 curves ($95\% \leq \text{efficiency} \leq 105\%$). Gene expression was quantified using the comparative C_T
143 method. Genes' functional annotations were retrieved from different databases. Kegg and
144 Reactome were obtained under the webgestalt platform
145 (<http://www.webgestalt.org/option.php>) and wikipathways directly from their website
146 (<http://www.wikipathways.org/>). Genes with no clear annotations were then manually curated
147 in the Biosystem database from the ncbi gene portal (<https://www.ncbi.nlm.nih.gov/gene>).
148 Genes involved in the regulation of metabolic pathway in at least one of these databases are
149 highlighted in yellow in the supplementary tables of results (Supplementary Tables 3 to 5).

150 **2.4. Statistical analysis**

151 Results are expressed as means \pm SEM. Statistical analyses were performed using graph pad.
152 1-way ANOVA was used to determine significance. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

153 3. **RESULTS**

154 **3.1. Similar inhibitory action of BZA after acute treatment in the uterus and the**
155 **liver.**

156 To determine the relative effects of TSEC after acute treatment (4 hours), we first used 2
157 doses of CE (3 or 10 mg/kg) and the expression profile of selected genes known to be
158 regulated by E2 was evaluated in the uterus (Abot et al., 2013). We found that acute CE
159 treatment (3 mg/kg) induced a weak transcriptional response whereas a higher dose of CE (10
160 mg/kg) was able to induce gene expression changes to a similar extent as E2 (1 mg/kg)
161 (Figure 1A). The addition of BZA to E2 or to CE significantly attenuated these effects, while
162 BZA alone had no effect. Analysis of protein levels by Western blot indicated that BZA alone
163 or in combination with E2 or CE markedly down-regulated the amounts of ER α protein
164 (Figures 1B and 1C). In contrast, none of these treatments had a significant impact of on ER α
165 mRNA levels (Figure 1D).

166 Then, we analyzed the transcriptional response of liver to these acute treatments (Tables 3 and
167 4) analyzing a set of genes described to be regulated by estrogens or selective ER modulators
168 exposure in the liver (Palierne et al., 2016, Boverhof et al., 2004, Gordon et al., 2014, Kim et
169 al., 2014, Pedram et al., 2013, Ahlbory-Dieker et al., 2009, Kobayashi et al., 2013). As
170 observed in the uterus, a dose of 3 mg/kg CE was less efficient than 10 mg/kg CE to induce
171 an optimal transcriptional response. Only around 40% of the genes induced by 10 mg/kg CE
172 or 1 mg/kg E2 were induced by 3 mg/kg CE (Figures 2A and 2B). Co-administration of BZA
173 strongly attenuated the transcriptional induction observed with E2 (Figure 2A and
174 Supplementary Table 3) or CE (Figure 2B and Supplementary Table 4). Acute BZA
175 treatment, both alone and when combined with estrogens, elicited a clear-cut down-regulation
176 of ER α at the protein level (Figures 3A and 3B), but not at the level of mRNA (Figure 3C).

177 Altogether, these results demonstrated that acute BZA treatment is able to exert similar
178 antagonistic action on the transcriptional modulations induced by E2 or CE in both uterus and
179 liver. Concomitantly, we observed reduced amounts of ER α protein in response to BZA in
180 these two tissues, an effect that occurred in a post-transcriptional manner as no differences in
181 ER α mRNA expression were observed following drug treatment.

182 **3.2. Chronic BZA treatment exerts inhibitory action on uterus and stimulatory effect** 183 **on liver.**

184 To determine the relative effects of TSEC after a chronic treatment of the animals, 4-week-old
185 ovariectomized female mice were treated with placebo, BZA (10 mg/kg/day), CE (3
186 mg/kg/day) or CE+BZA for 3 weeks, as previously described (Barrera et al., 2014, Della
187 Torre et al., 2011, Fontana et al., 2014, Kim et al., 2014, Naqvi et al., 2014, Oliva et al., 2013,
188 Peano et al., 2009, Song et al., 2012) (Supplementary Table 1). Analysis of the uterine wet
189 weight confirmed that BZA antagonized the uterotrophic effect of CE (Figure 4A).
190 Accordingly, epithelial proliferation was significantly decreased in CE+BZA-treated mice
191 compared to CE-treated mice, as indicated by the immunohistochemical detection of the Ki-
192 67 antigen (Figures 4B and 4C). After chronic exposure, large changes in uterine cellular
193 populations induced by CE treatment did not allow for a relevant comparison of protein
194 expression in tissue homogenates by Western blotting. Thus, ER α protein levels were
195 evaluated by immunohistochemistry (Figure 4D) which is essentially qualitative rather than
196 quantitative, and no obvious effect of BZA or CE+BZA on ER α abundance was apparent.
197 Altogether, the analysis of different parameters including uterine weight and epithelial
198 proliferation clearly shows that BZA antagonizes the action of CE and E2 in the uterus.

199 No difference in body weight was observed between ovariectomized untreated mice (mean =
200 22.4 ± 2.1 g, n = 20), CE-treated mice (23.8 ± 0.5 g, n = 16), BZA-treated mice (21.8 ± 1.6 g,

201 n = 23) and CE+BZA-treated mice (21.4 ± 1.2 g, n = 22), showing that CE, BZA or CE+BZA
202 administrated subcutaneously for 3 weeks had no detectable impact on weight gain. Although
203 some genes were found to be regulated by either CE or BZA, the majority of the genes
204 studied were regulated by both CE and BZA (Figure 5 and Supplementary Table 5). Overall,
205 chronic BZA and CE+BZA elicited agonistic effects similar to those of CE alone (Figure 5).
206 For example, whereas BZA antagonizes CE-induced transcriptional activation of gene such as
207 p21, Trim2, Psen2, Pgep1 or Inf2 after acute exposure (Supplementary Table 4), these genes
208 are induced by CE+BZA treatment after chronic exposure (Supplementary Table 5A). Genes
209 described to be involved in the regulation of metabolic pathways do not correspond to a
210 specific regulatory profile in response to estrogens or BZA and are found in all the analyzed
211 cases (i.e regulated by estrogens only, BZA only or after combination of BZA + CE)
212 (Supplementary Table 5). In addition, chronic CE administration increased ER α protein and
213 mRNA abundance in the liver, whereas BZA alone or CE+BZA had no effect compared to
214 vehicle (Figures 6). Altogether, these data highlight a new aspect of the action of BZA on
215 liver tissue, where it can have antagonistic effects after acute treatment and agonistic effects
216 after chronic exposure.

217

218 4. DISCUSSION

219 The approval of Duavee[®] CE (0.45mg)/BZA(20mg) was based on Selective Estrogen,
220 Menopause, and Response to Therapy (SMART) trials that aimed to evaluate CE/BZA's
221 safety and efficacy in the management of moderate-to-severe vasomotor symptoms, bone
222 mineral density, endometrial hyperplasia, moderate-to-severe vulvar/vaginal atrophy, and
223 overall safety (Kagan et al., 2010, Mirkin and Komm, 2013, Pickar et al., 2009, Pinkerton et
224 al., 2009, Umland et al., 2016). In parallel, preclinical studies in animal models reported
225 beneficial effect on metabolic parameters (Barrera et al., 2014, Kim et al., 2014) and have

226 shown that, when combined with CE, BZA attenuates CE-induced uterine and mammary
227 gland cell proliferation (Kim et al., 2014, Barrera et al., 2014, Peano et al., 2009, Song et al.,
228 2012). However, assessing the relative effects of estrogens or SERMS *in vivo* in rodents is
229 complicated by the fact that contrary to human, mice do not express the sex hormone binding
230 globulin (SHBG) which modulates the bioactivity of sex steroids by limiting their diffusion
231 into target tissues. Based on the uterine weight as endpoint, dose response study determinates
232 CE (3mg/kg/day) and BZA (10 mg/kg/day) as reference doses (minimum fully effective
233 agonist and antagonist doses respectively) for combination studies with SERMS (Peano et al.,
234 2009). Our results confirm that BZA (10 mg) antagonizes CE (3 mg) on uterus weight and
235 proliferative response after chronic treatment in uterus and also show that BZA inhibits the
236 CE-mediated transcriptional gene regulations (10 mg) after acute treatment extending this
237 particular mechanism of action of BZA on sex targets. Furthermore, in cellular models of
238 breast cancer, BZA was shown to function as a pure ER α antagonist and effectively inhibited
239 the growth of both tamoxifen-sensitive and tamoxifen-resistant breast tumor xenografts
240 (Wardell et al., 2013). The antiestrogens that are currently available fall into two general
241 classes, acting as selective estrogen receptor modulators (SERMs) and/or selective estrogen
242 receptor down-regulators (SERD). We found that, after acute treatment, BZA led to a
243 decrease in ER α protein levels in both uterus and liver without impacting ER α mRNA levels.
244 Indeed, BZA has been described to induce a unique conformational change in ER α that results
245 in its proteosomal degradation, although this property was shown to be dispensable for its
246 antagonistic activity (Wardell et al., 2013). ER α immunodetection generated similar signals in
247 tissues from untreated, CE-, BZA- and CE+BZA-treated ovariectomized mice, suggesting that
248 BZA behaves as a SERM rather than a SERD after chronic treatment. Interestingly, a very
249 specific profile of BZA was previously reported in biochemical and cell-based assays, as its
250 combination with CE displayed different properties from those observed following the

251 combination of CE with other SERMs (Berrodin et al., 2009, Chang et al., 2010, Wardell et
252 al., 2012). Indeed, *in vitro*, whereas raloxifene and lasofoxifene completely inhibited the CE-
253 mediated recruitment of all cofactor peptides by ER, BZA inhibited the CE-mediated
254 recruitment of only some of the evaluated peptides. Furthermore, in MCF-7 breast cancer
255 cells, the combination of BZA with CE did not totally abrogate the transcriptional response of
256 a subset of CE-responsive genes which were, in contrast, completely antagonized by
257 raloxifene and lasofoxifene. Interestingly, the CE-regulated genes antagonized by all three
258 SERMs were involved in cell cycle regulation and cell-to-cell signaling (Berrodin et al., 2009,
259 Chang et al., 2010). Subsequent studies of differential gene regulation by TSEC, estrogen or
260 the SERM alone highlighted the importance of the promoter context on the activity of these
261 ER ligands (Wardell et al., 2012). However, these studies only questioned the short term
262 action of CE, BZA or TSEC using *in vitro* models.

263 Here, we used an *in vivo* model to show that BZA acts as an antagonist after acute treatment
264 in the liver, but displays agonistic effects in the liver following chronic exposure. This
265 underlines the importance of the time- and tissue-specific action of BZA, which induces a
266 very similar action to that of CE or E2 following chronic treatment. Our transcriptional
267 analyses also revealed a group of genes for which CE and BZA cooperatively regulated their
268 expression under chronic treatment. This means that the effect of CE+BZA cannot be simply
269 extrapolated from its short-term action and suggests that additional pathways facilitating
270 combined agonist/antagonist action must exist. It was previously proposed that, in addition to
271 the competitive inhibition of BZA on CE, BZA and CE could also cooperatively activate gene
272 transcription with one ER monomer bound to CE and the other one occupied by BZA (Liu et
273 al., 2013). High-throughput sequencing of chromatin immunoprecipitation experiments
274 (ChIP-seq) performed on livers from mice subjected to acute treatment with E2 led us to
275 recently show that the ER α cistrome (all of the ER binding sites in the genome) in mouse

276 liver has unique properties (Palierne et al., 2016). In order to acquire novel insights into the
277 specific mechanisms involved in the shift of action of BZA from antagonism to agonism, it
278 would therefore be of major interest to compare the liver cistrome of ER α in response to acute
279 and chronic treatment with CE, BZA and CE+BZA. These experiments would question: *i)*
280 whether ER α bound to CE or BZA targets different/specific chromatin regions, which would
281 explain the difference in gene regulation; *ii)* whether these cistromes vary according to the
282 acute or chronic administration of these hormones, in relation to differences in gene
283 regulation; and *iii)* whether the specific properties of the E2-bound ER α cistrome are
284 conserved using these treatments. Interestingly, the cistrome of ER α bound to 4-
285 hydroxytamoxifen (OHT), another SERM, has been determined in MCF-7 cells and was
286 found to be comparable with that of E2-bound ER α (with 60-75% similarity) (Hurtado et al.,
287 2011), although this SERM had no agonist activity on gene transcription. It was therefore
288 proposed that, in these cells, OHT-bound ER α is able to target the same sequences on
289 chromatin (although with less efficiency than E2-bound ER α) but as an inactive complex or
290 repressive form since OHT-bound ER α is able to recruit corepressors (Shang and Brown,
291 2002). Hence, the shift of the action of BZA from antagonism to agonism may have a limited
292 impact on the cistrome of ER α and instead reflect a corepressor to coactivator exchange. The
293 mass spectrometry analysis of proteins associated with ER on chromatin (ChIP-MS) when
294 bound to these different ligands (Mohammed et al., 2013) would also generate important
295 knowledge to further decipher the mechanisms of action of TSEC.

296 Finally, Mauvais-Jarvis and coworkers recently reported that BZA and TSEC exhibits
297 estrogen-mimetic action with regard to glucose and energy homeostasis (Kim et al., 2014).
298 Interestingly, they reported that lipid oxidation and increased energy expenditure promoted by
299 CE, BZA and TSEC involved different pathways as revealed by different target gene pattern
300 in the liver for these ER α ligands. Thus, the shift reported here from antagonist to agonist

301 activities of BZA in the liver, and potentially in other tissues, have undoubtedly contributed to
302 the metabolic benefit of BZA alone or in TSEC that mimic those provided by estrogens in
303 female mice on high fat diet.

304 In conclusion, these observations provide novel insights into the mechanisms underlying BZA
305 action which can not only act as an agonist or antagonist in a tissue-specific fashion, but can
306 also, in a given tissue such as the liver, be acutely antagonistic and chronically agonistic. This
307 could help to explain, among other reasons, the reported beneficial effects of TSEC such as its
308 favorable effects on glucidic and lipidic metabolism (Barrera et al., 2014, Kim et al., 2014).

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323

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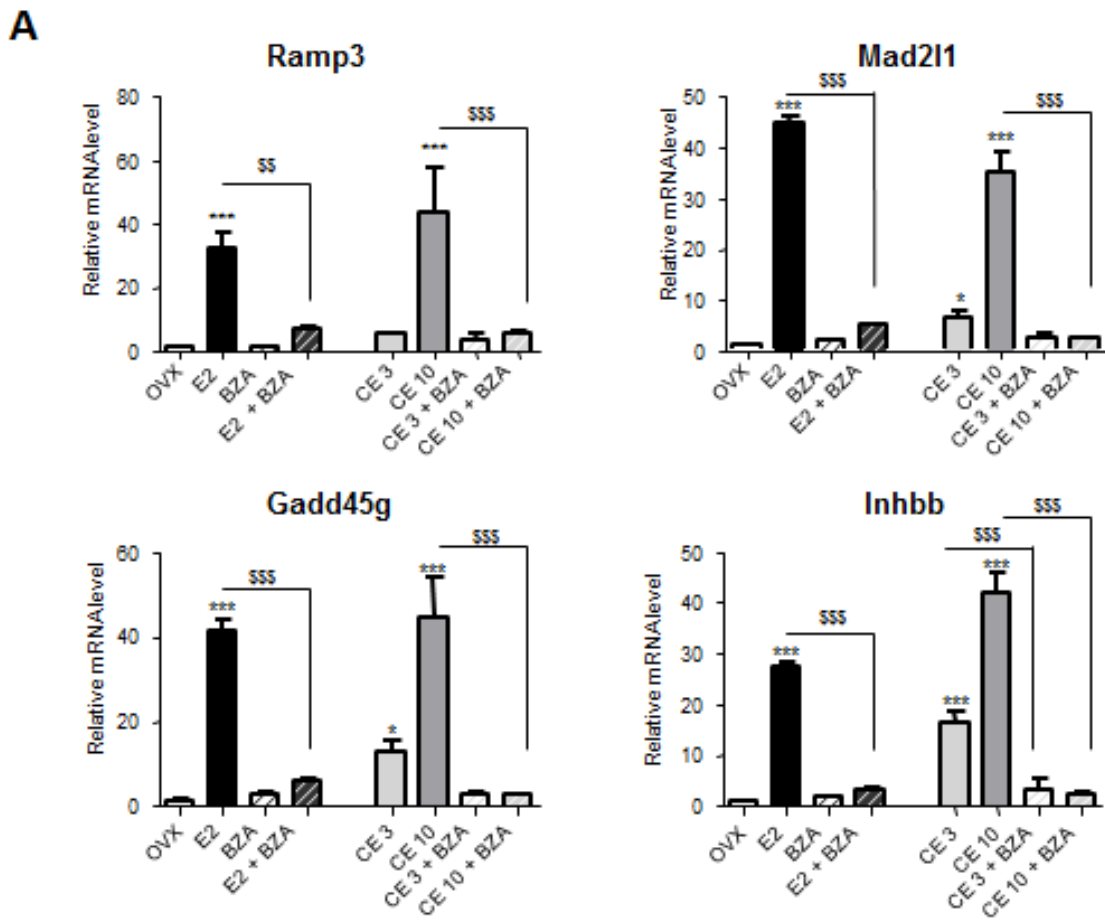
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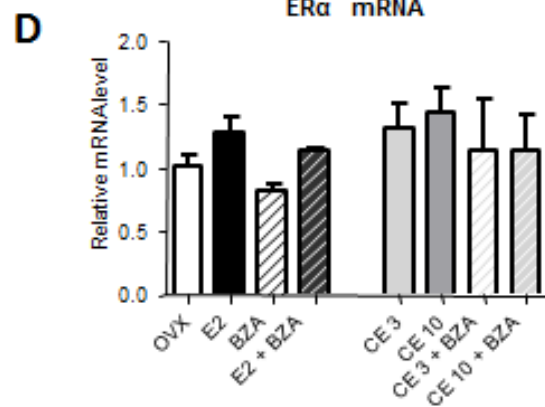
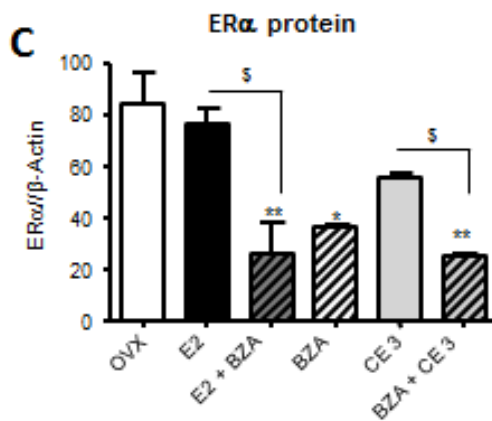
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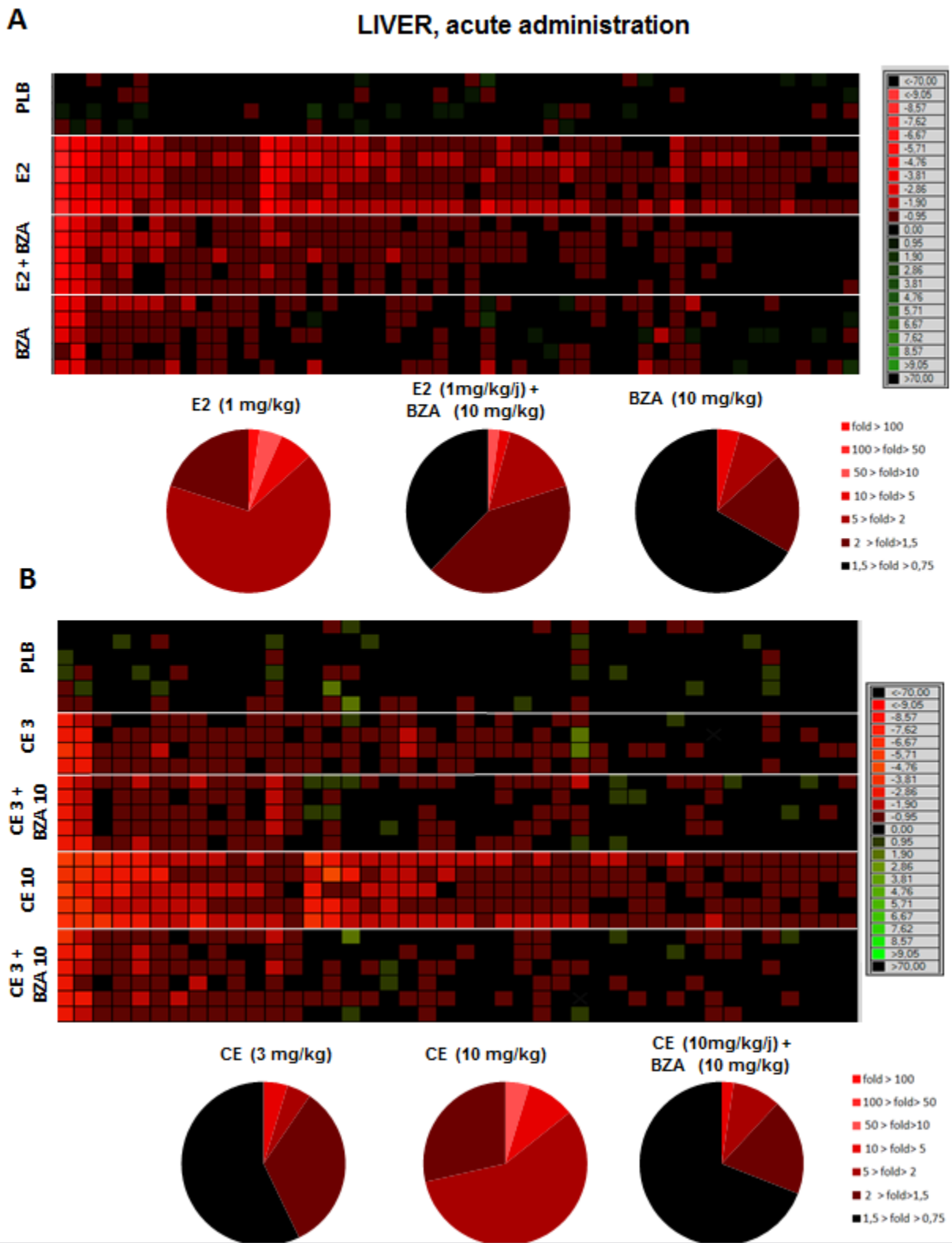
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480 **Figure 1: Effect of acute administration of E2 and CE alone or in combination with BZA**
481 **on uterus**

482 Four-week-old ovariectomized mice received an oral administration of vehicle (PBS
483 containing 0.5% methylcellulose, 2% Tween 80 and 5% DMSO), 17- β estradiol (E2, 1
484 mg/kg), conjugated equine estrogen (CE, 3 or 10 mg/kg) alone or in combination with
485 Bazedoxifene (BZA, 10 mg/kg). (A) Quantification of mRNA levels of the indicated genes,
486 (B) representative Western blot and (C) quantification of ER α protein levels and (D)
487 quantification of ER α mRNA levels in the uteri from these mice. Results are expressed as
488 means \pm SEM. To test the respective roles of each treatment, one-way ANOVA and a
489 Bonferroni's multiple comparison test were performed.

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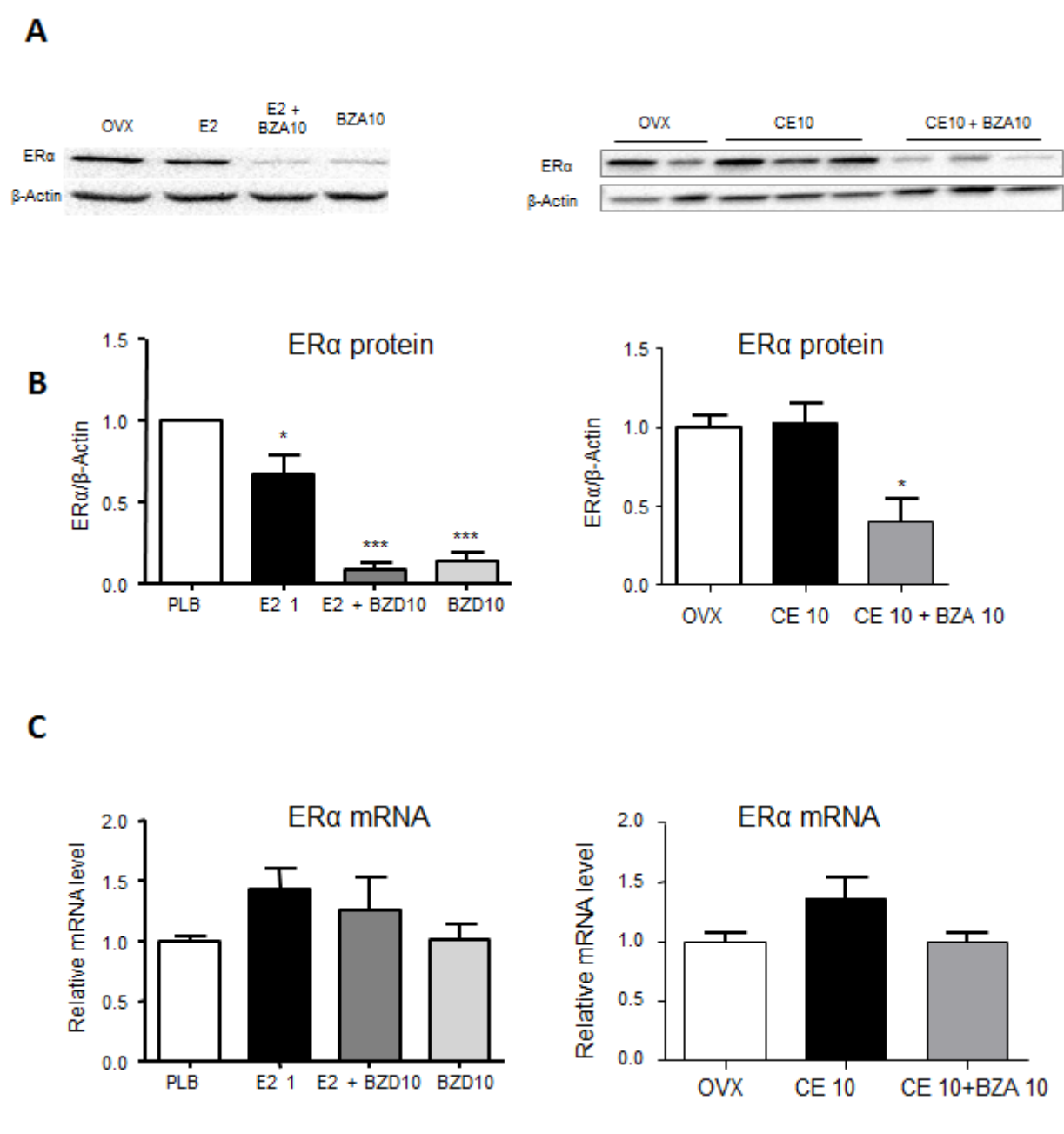
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492 **Figure 2: Effect of acute administration of E2 and CE alone or in combination with BZA**

493 **on transcriptional regulation in the liver**

494 Four-week-old ovariectomized mice received an oral administration of vehicle (PBS
495 containing 0.5% methylcellulose, 2% Tween 80 and 5% DMSO), 17- β estradiol (E2, 1
496 mg/kg), conjugated equine estrogen (CE, 3 or 10 mg/kg) alone or in combination with
497 Bazedoxifene (BZA, 10 mg/kg). **(A)** Data obtained from 96.96 Dynamic Arrays were used to
498 generate a cluster diagram of the significant gene expression changes. Each vertical line
499 represents a single gene. Each horizontal line represents an individual sample. **(B)** Pie chart
500 displaying the distribution of significantly induced genes according to the value of the fold
501 change. Genes that were up-regulated at least 1.5 fold relative to placebo are in red and the
502 color intensity indicates the degree of variation in expression. Genes whose expression was
503 not affected by treatments appear in black.

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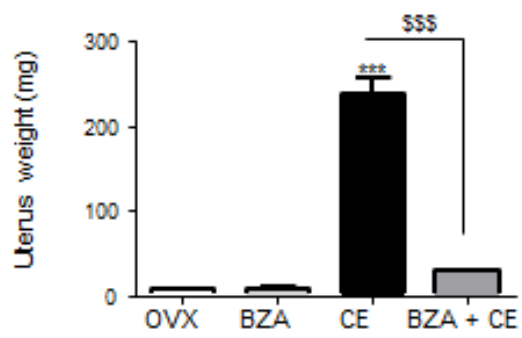
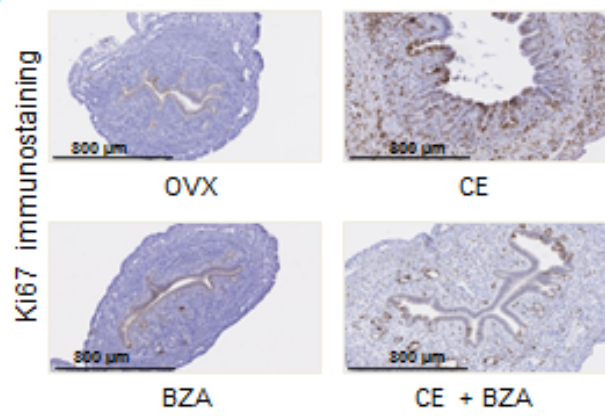
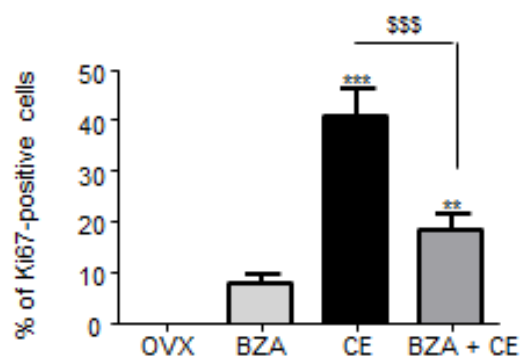
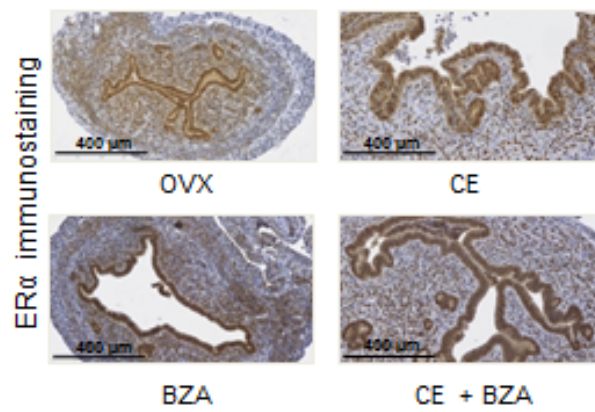


505

506 **Figure 3: Effect of acute administration of E2 and CE alone or in combination with BZA**
 507 **on ERα expression in the liver**

508 Four-week-old ovariectomized mice received an oral administration of vehicle (PBS
 509 containing 0.5% methylcellulose, 2% Tween 80 and 5% DMSO), 17-β estradiol (E2, 1
 510 mg/kg), conjugated equine estrogen (CE, 10 mg/kg) alone or in combination with
 511 Bazedoxifene (BZA, 10 mg/kg). **(A)** Representative Western blots of ERα protein levels and
 512 **(B)** quantification of ERα protein expression. **(C)** Quantification of ERα mRNA levels by

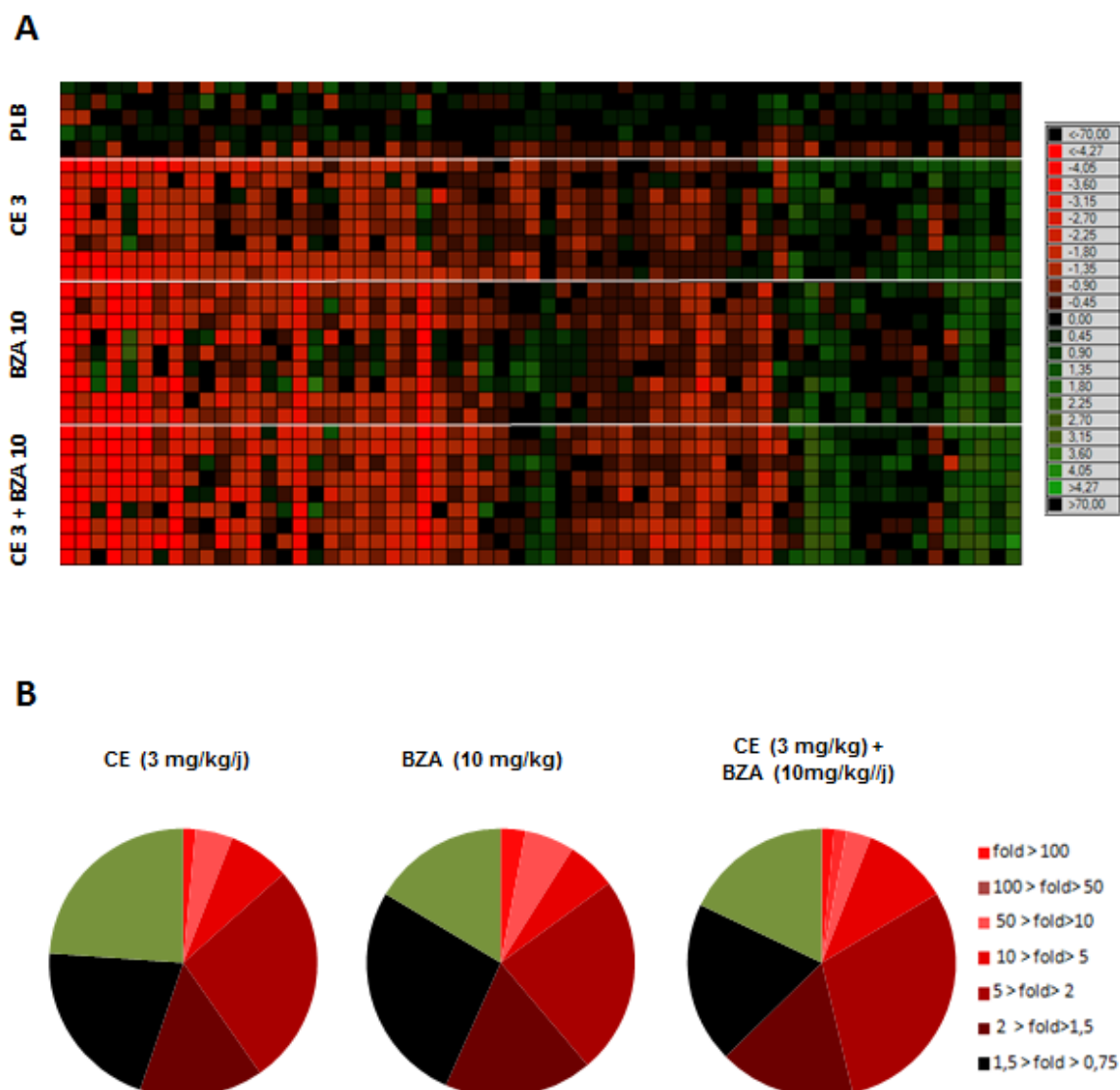
513 qPCR. Results are expressed as means \pm SEM. To compare the effects of each treatment, one-
514 way ANOVA and a Bonferroni's multiple comparison test were performed (treatment *versus*
515 placebo: *** $p < 0.001$).

A**B****C****D**

517 **Figure 4: Effect of chronic administration of E2 and CE alone or in combination with**
518 **BZA on uterus**

519 Four-week-old ovariectomized C57Bl/6J mice were treated with BZA (10 mg/kg/day), CE (3
520 mg/kg/day) alone or in combination (BZA+CE) during 3 weeks. **(A)** Uterus weight, **(B)**
521 representative Ki-67 immunostaining picture **(C)** and quantification in transverse uterus
522 sections (scale bar = 800 μ m). Results are expressed as means \pm SEM. To test the respective
523 roles of each treatment, one-way ANOVA and a Bonferroni's multiple comparison test were
524 performed. **(D)** Detection of ER α by immunochemistry in transverse uterus sections (scale
525 bar =400 μ m).

LIVER, chronic administration



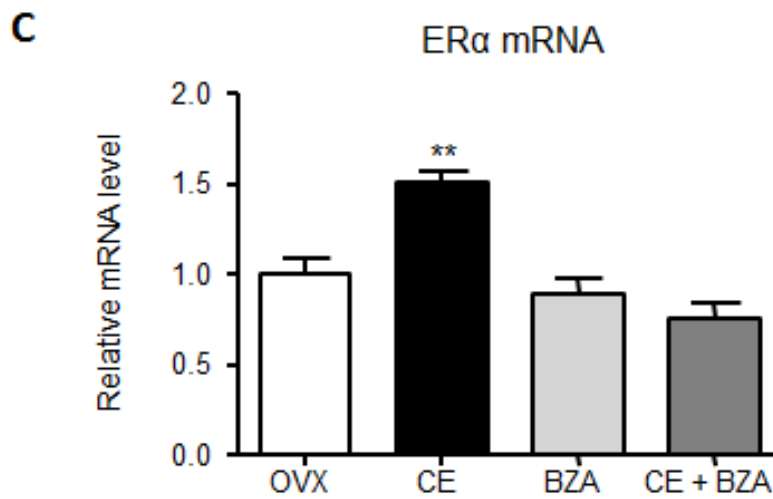
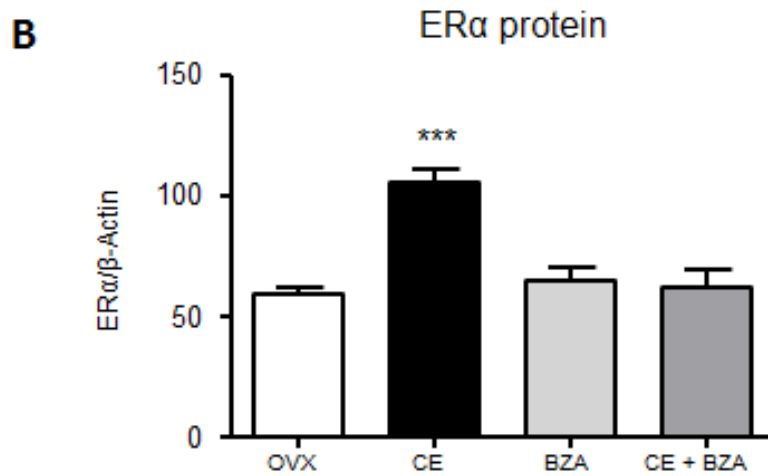
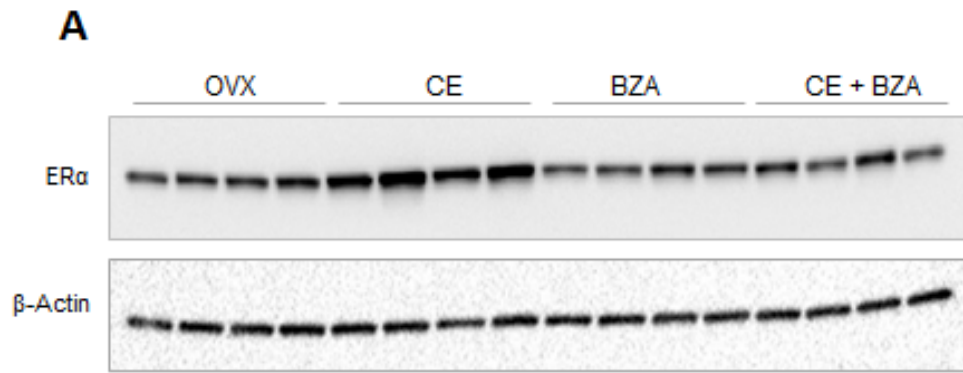
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527 **Figure 5: Effect Effect of chronic administration of E2 and CE alone or in combination**
 528 **with BZA on transcriptional regulation in the liver**

529 Four-week-old ovariectomized C57Bl/6J mice were chronically treated (3 weeks) with BZA
 530 (10 mg/kg/day), CE (3 mg/kg/day) alone or in combination (BZA+CE). Gene regulation was
 531 then analyzed using extracted liver RNA by 96.96 Dynamic Arrays. (A) Data obtained from
 532 96.96 Dynamic Arrays were used to generate a cluster diagram of the significant gene

533 expression changes. Each vertical line represents a single gene. Each horizontal line
534 represents an individual sample. **(B)** Pie chart displaying the distribution of significantly
535 induced genes according to the value of the fold change. Genes that were up-regulated at least
536 1.5 fold relative to placebo are in red, whereas down-regulated genes are in green. The color
537 intensity indicates the degree of variation in expression. Genes whose expression was not
538 affected by treatments appear in black.

539



540

541 **Figure 6: Effect of chronic administration of E2 and CE alone or in combination with**

542 **BZA on ERα expression in the liver**

543 Four-week-old ovariectomized C57Bl/6J mice were chronically treated (3 weeks) with BZA
544 (10 mg/kg/day), CE (3 mg/kg/day) alone or in combination (BZA+CE). **(A)** Representative
545 Western blots of ER α protein levels and **(B)** quantification of ER α protein expression. **(C)**
546 Quantification of ER α mRNA levels by qPCR. Results are expressed as means \pm SEM. To
547 compare the effects of each treatment, one-way ANOVA and a Bonferroni's multiple
548 comparison test were performed (treatment *versus* placebo: *** p<0.001).

549

Supplementary Table 1: Summary of BZA and CE doses used to study TSEC effect *in vivo* in mice with corresponding references

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Supplementary Table 2 : List of specific primers used in the study**Supplementary Table 3: Effect of the acute administration of E2 and BZA alone or in combination on liver mRNA levels.**

Four-week-old ovariectomized mice received an oral administration of vehicle (PBS containing 0.5% methylcellulose 2% Tween 80 and 5% DMSO), E2 (10 mg/kg) and/or BZA (2 or 10 mg/kg). Gene regulation was then analyzed using extracted liver RNA by 96.96 Dynamic Arrays. Significant fold gene regulation (>1.5 fold) are red highlighted. Gene involved in the regulation of metabolic pathway are yellow highlighted. *nd*= not determined

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Supplementary Table 4: Effect of the acute administration of CE (10 mg/kg) and BZA (10mg/kg) alone or in combination on liver mRNA levels.

Four-week-old ovariectomized mice received an oral administration of vehicle (PBS containing 0.5% methylcellulose 2% Tween 80 and 5% DMSO), CE (10 mg/kg) and/or BZA (10 mg/kg). Gene regulation was then analyzed using extracted liver RNA by 96.96 Dynamic Arrays. Significant fold gene regulation (>1.5 fold) are red highlighted. Genes involved in the regulation of metabolic pathway are yellow highlighted. *nd* = not determined

- Palierne G, Fabre A, Solinhac R, Le Peron C, Avner S, Lenfant F, Fontaine C, Salbert G, Flouriot G, Arnal JF, Metivier R. Changes in gene expression and estrogen receptor cistrome in mouse liver upon acute e2 treatment. *Molecular endocrinology*. 2016;30:709-732
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Supplementary Table 5: Effect of chronic administration of CE (10 mg/kg) and BZA (10mg/kg) alone or in combination on liver mRNA levels.

Four-week-old ovariectomized C57Bl/6J mice were chronically treated (3 weeks) with BZA (10 mg/kg/day), CE (3 mg/kg/day) alone or in combination (BZA+CE). Gene regulation was then analyzed using extracted liver RNA by 96.96 Dynamic Arrays. Significant fold gene regulation induced by the different treatments are reported in the Table. (A) Red highlight represents up-(>1.5 fold) regulated genes and (B) Green highlight down-(<0.65 fold) regulated genes. Genes involved in the regulation of metabolic pathway are yellow highlighted. *nd* = not determined

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CE dose (mg/kg/day)	BZA dose (mg/kg/day)	Duration	References
2.5	3	8 weeks	Kim <i>et al.</i> Mol metabolism (2014)
5	3	6 to 12 weeks	Barrera <i>et al.</i> AJP endo (2014)
3	0.08-0.4-2 or 10	2 weeks	Peano <i>et al.</i> Endocrinology (2009)
3	10	3 weeks	Fontana <i>et al.</i> Neuroendocrinology (2014)
3	2	4 weeks	Song <i>et al.</i> Endocrinology (2012)
3	1-2-3 or 5	8 weeks	Naqvi <i>et al.</i> Biol reproduction (2014)
3	10	3 weeks	Oliva et al. Reproductive science (2013)
3	10	3 weeks	Della-Torre <i>et al.</i> Endocrinology (2011)

Gene symbol	Reverse	Forward	Gene symbol	Reverse	Forward
ERα	CTGTCGGCTGCGCAAGTGTT	AACATTCTCCCTCCTCGGCGGT	Lifr	AAGAGCATTGCTTCCTCGCA	TGTTGGCGTGGTAAACGAGCAT
Ramp3	TGTTGTTGCTGCTTTGTGGTGA	CTCCAGCATCCCTGTCTGTT	Il17ra	GGTGGGATCTGTATCGTGCT	CTACGGGCAAGATGCCATTGA
Mad2l1	GAGAGGCAGGGAGGACAGCTT	CCTCGTTTCAGGCACCACAAA	Tsen54	GTATCTGCTGGAGTGTGTTTCGAT	CATAGCCCCAGTCTCTTCAGGTGG
Gadd45g	AGTCTGAATGTGGACCCTGACA	GCAGAACGCCTGAATCAACGTG	Lsyna1	TTCACGGTGCCCGCTGGGT	CGCGCACAGGTGCGAAGGAGG
Inhbb	TTTGACAGACAGATGGCCTCG	GCCTGCACCACGAATAGTTCT	Synpo	TGCGGCATCTGGAGAAGGTTG	GTTTGGTGAGGACTGCTGGGT
Prtn3	AGTCCACTCCATGAAGAATGCCA	TATGCCGGGAACACAACGTG	Itpk1	CCAGGGGGCCCTGCTCCTCAA	GCGGAGCTGTGCAGGAAGCGAG
Lcn2	TCTGTACTGAGGATACTGTGCAT	ATGTACCTCCATCCTGGTCA	Acot1l	AAGCCGGGGGTGGCCTGGTT	GGTCACGCTGCCACGCACC
p21	CAGGCGCAGATCCACAGCGA	GCACACTTGTCTGTGCGGA	Spata2l	GCTGCCTGGAGAAAGGAGTT	ACCATTAGCGGTGGTTGTCC
Slc34a2	TGTAAGTACGAGCAGCCTTCC	CCAGTCCAGCCACAGGATTAGAC	Dsc2	TGTATCAAACACCAGGCTCCTG	TGCCCTCCACTTTTGACTCCAG
IGFBP1	AGGGATGTCTCACACTGTTTGCT	GCCCTGCCAACGAGAAGTCTA	Ehhadh	GCCAGTGGGAGATTTAAAGCCA	TGCCATCAGTCCAAGTGAATCA
Gdf15	TGGGACCCCAATCTCACCTCT	AGCCGAGAGGACTCGAAGTCA	Agpat9	GCCATGGTTAAGGCTTGTCTCT	ATTGTTGATGCAAGTACCTTCTGGG
Crybb3	CTCCAGAACAACCTGCTCCCA	AAGGTGGGCTCCATCCAAGT	Pgpep1	CTCCAGTCCCTACAGCCAGGAT	ACAGATGCTCCCAAAAGGGC
Lsyna1	TTCACGGTGCCCGCTGGGT	CGCGCACAGGTGCGAAGGAGG	Baiap2l1	TGCTTCTACCTTCAGAGCGCC	GCAAAGGGGTTTTCTCCACTGA
Srgap3	TCTTTAACGGCAGTATGGAAGCGT	TCCCTGCTGCTGGAGTCCATA	Tgm2	TCATACAGGGGATCGGAAAG	TGGAGAATCCCGAAATCAAC
Elov13	AGACGACCACTATGAGAAATGAGCTT	GTCTGCAAATCGAAATGGACACAT	Peo1	TGCAGACAGCATCCATTTTCGG	AAAGCGATTCTTGACACCTGC
Ly6d	TCCTCCTTGTCTTGGCTGTGG	AGAAGTTGGACGGGCAGACCT	C2cd2l	TTGGGTGCGAGCCTTGAATGAG	TGGTGGGAGTTGGGGTATCTCTT
Marco	TCTGCCTACAAGACCTGGGAC	GGAATCCAGGGATTGCAGGTGT	Caskin2	GCGCAGAAGCACCAGTGCCCA	GCGGCCGGGAGAGACCTCA
Slc3a1	AATCTCAACAGCGTATCTGAAGTC	AAGGACAGTGACAAGGATGGG	Spon1	TGTGTGATTCTGAAGGCCAGC	GTCCGTCACCCATCAAGTGT
Cyp8B1	AAGACATCCCCGTGCTGGC	TTTTGTGCCCTGGCTGGGTCT	Etnk2	CGGGCTGTGCTACGAGTATGT	TGGCGTGGATGGTGTGAATCT
SHP	AAGGGCACGATCCTCTTCAA	CTGTTGCAGGTGTGCGATGT	Inf2	CCTCAAGGAACCAAGAAGAGTTTG	AGGCAACACATCCAGCCGAT
Corin	GGGACCTGAGTGACGAGCAAAA	GGCTGTGACAAGAGCAGTTGA	Dopey2	CGCAGATCAAAGGGGATGAGAAGT	CCTCTGCAGGTCTGGTATGGA
FGF21	GCCATGGGCTTCAGACTGGTA	TTGCCAAGCCAGATGGAGC	Upp2	TTCCTGCTACTGCCCCACCG	TCTCTGCATGCTTCCAGG
Hmgcr	TTCACGAAAGCTCTAGGACCAG	TGCCTTGTGATTGGAGTTGGCA	Sulf2	ACTCAGAGCGCCAGTGAAC	TGTGGAGCAGTTTGCCTCTCTC
Ms4a4a	GGCTGCTTCGAACCAACCTT	TGTCGACCGGTGAAATACCCC	Rad51l1	CGCAACCTGGTGGACCTGTAA	ACGGCTTATGAGTTAAAGACACGA
Trim2	ACAGGCAGTTTGAGGTCCTCC	TCCATTGGAGCCAAACTTCAACAT	Mn1	AGAAGGCCAAGTCCAGAACC	CCTTGTGGTGGGGTGGTCAT
Ggct	GTGTGGGGAGTGGTATGGAGAA	CGTCATCAGGTAACCTCGGCA	Ngfr	CTCCATCTTGGCTGCTGTGGT	CCGGCTGTTGGCTCCTTGTTT
Insig2	GTGGCGGTGTTCTGTTGGTATAA	GTGCAGCCAGTGTGAGGGAAA	Zfp276	CAGTGACCTTCTGAGGGAGAC	GCTTCTGCCTTCGCTCTTCT
Smtnl2	CATGCCACCTTTTCGCTGTCC	ATCCGTTGGCTGGACCTGCAT	Pcp4l1	CCGGGATGAGCGAGCTTAACA	CTCTGGCGCTGTGAGTCAAT
Stat5a	CTTCTGGGACATGGCGTCAACC	ACACAGCTCCAGAACAGTATGACC	PPARα	TGCCTTCCCTGTGAAGTACGAC	TGTTGGATGGATGTGGCCAGG
Ptgds	CACAGAGGAGGACATTGTTTTCC	ACTGACTTCTCTCACCTGCGTTT	Clec2h	GGAGACACAGTGGAGATGATTCTGA	CAGCTCACAGATGAATGCTGCAA
Cyp17a1	CATCCACACAAGGCTAACA	CAGTGCCCAAGAGATTGATGA	Sucnr1	TGACATTTCCAAGCAGTCCAA	ACAGAAGCCGACAGCAGAATG
Aqp8	GGCTCATCATTGCTACCTTGGG	AGCGACAGCAGGGTTGAAG	Coq10b	GCTGAGAGTGACAAGGGAGCGG	TGGCGTTTTAGCCAGGTCTTCT
Flrt1	ACATGGGGCCAAGGATGTGAG	TGACCCACCAACCTGGAGT	Car1	GGTTGGCTGGACCAACCTTCA	CTGAGCACACCGTGGATGGAA
Cyp17	ATTTACCTTCGGAGCTGGT	AGGGCAGCTGTTTGTCACT	Fmo2	CAAGAGTTTTCAAAGGCTTGTGTAGC	TCCAGGTAGTCAACGTAGTTGGT
Psen2	ACTGGCAACGAGACTGGAA	AACACAGCAAGCAGCAGGAGG	Upp2	TTCCTGCTATCTGCCACCG	TCTTCGTCATGCCTTCCAGG
Map3k13	CTGGAGATGCGCGAGAAGGA	ATTCCGGTGGATGATTGGACGG	Nr1d1	GGAGGAGCCACTAGAGCCAATG	GAGGCCATCAACCTCCAGTT
Dusp8	GCGCCTCAGCAGGGAAAAGTG	CTGGCATCTGTTGTCTCTGGT	Aqp4	TACCTGGTCAACCTCCAGT	CCAGCGGTGAGGTTTCCATGA
Traf3ip2	GAGGACGAGCATGGCTTACATACT	CGGCACATGCTCCTTCTGG	Cyp2c55	GCCAGGGTCAAATACTTCTGGGT	CAATGTGCCCCATGCAGCTA

Supplementary Table 3				Fold induction (compared to PLB)			
				E2	E2 + BZA 10	E2 + BZA 2	BZA 10
Regulated by	Gene	Gene –associated functions (when available)	References				
E2, BZA 10, (E2 + BZA10) and (E2 +BZA 2)	Slc34a2	Type II Na+/Pi cotransporters, Transmembrane transport, Metabolism of proteins	<i>Palierne et al., Mol endo , 2016</i>	124.67	42.93	82.25	7.62
	Gdf15	TGF-beta signaling pathway	<i>Palierne et al., Mol endo , 2016</i>	15.59	6.65	10.27	6.91
	Corin	Myometrial Relaxation and Contraction Pathways	<i>Palierne et al., Mol endo , 2016</i>	6.86	2.86	4.31	1.52
	Map3k13	Insulin Signaling	<i>Palierne et al., Mol endo , 2016</i>	5.08	2.46	3.31	2.28
	Il17ra	Cytokine Signaling in Immune system	<i>Palierne et al., Mol endo , 2016</i>	4.83	3.33	5.22	2.31
	Shp	Bile secretion, organism-specific biosystem	<i>Gao et al. Mol endo, 2008</i>	3.26	1.67	2.66	2.08
	Gpcpd1	Metabolism of lipids and lipoproteins, Glycerophospholipid metabolism	<i>Palierne et al., Mol endo , 2016</i>	3.19	2.42	2.92	1.98
	Synp	Protein-protein interactions in the podocyte	<i>Palierne et al., Mol endo , 2016</i>	3.07	1.99	2.72	2.20
	Spata2l	nd	<i>Palierne et al., Mol endo , 2016</i>	2.69	2.02	2.27	1.59
	Ehhadh	Metabolism of lipids and lipoproteins, Mitochondrial LC-Fatty Acid Beta-Oxidation	<i>Palierne et al., Mol endo , 2016</i>	2.65	1.50	1.98	1.54
	Agpat9	Metabolism of lipids and lipoproteins, Phospholipid, Fatty acid, Triacylglycerol metabolism	<i>Palierne et al., Mol endo , 2016</i>	2.61	1.94	2.72	1.61
Zfp276	Regulation of transcription, DNA-templated	<i>Palierne et al., Mol endo , 2016</i>	2.47	1.84	2.03	1.51	
Ccq10b	Respiratory electron transport, ATP synthesis, and heat production by uncoupling proteins	<i>Palierne et al., Mol endo , 2016</i>	2.20	1.78	2.19	1.59	
E2, (E2 + BZA10) and (E2 +BZA 2)	Flrt1	Downstream signaling of activated FGFR1	<i>Palierne et al., Mol endo , 2016</i>	24.14	2.92	3.82	1.27
	Lifr	Immune System	<i>Palierne et al., Mol endo , 2016</i>	6.74	2.06	2.24	1.49
	Mad2l1	Cell Cycle	<i>Palierne et al., Mol endo , 2016</i>	4.76	1.90	2.45	1.33
	p21	Cell Cycle	<i>Ahlbory-Dieker et al. Mol endo, 2009</i>	4.03	1.89	7.65	1.46
	Ngfr	Cytokine-cytokine receptor interaction, Circadian rythm related genes	<i>Palierne et al., Mol endo , 2016</i>	3.57	1.99	2.40	1.33
	Tsen54	tRNA processing in the nucleus	<i>Palierne et al., Mol endo , 2016</i>	3.51	1.58	1.67	0.99
	Lsyna1	nd	<i>Palierne et al., Mol endo , 2016</i>	3.34	1.52	1.60	0.82
	Itpk1	Metabolic pathways, Inositol phosphate metabolism, Phosphatidylinositol signaling system	<i>Palierne et al., Mol endo , 2016</i>	3.04	1.83	2.16	1.20
	Acot1l	Metabolic pathways, Biosynthesis of unsaturated fatty acids	<i>Palierne et al., Mol endo , 2016</i>	2.70	1.88	1.94	1.36
	Peo1	Mitochondrial biogenesis	<i>Palierne et al., Mol endo , 2016</i>	2.47	1.57	1.64	1.43
	Caskin2	nd	<i>Palierne et al., Mol endo , 2016</i>	2.29	1.53	2.20	1.40
	Spon1	Metabolism of proteins, O-glycosylation of TSR domain-containing proteins	<i>Ahlbory-Dieker et al. Mol endo, 2009</i>	2.28	1.77	2.07	1.33
	Insig2	Regulation of cholesterol biosynthesis by SREBP	<i>Palierne et al., Mol endo , 2016</i>	2.13	1.60	2.19	0.93
	Upp2	Metabolic pathways, Drug metabolism - other enzymes	<i>Palierne et al., Mol endo , 2016</i>	1.67	1.76	1.86	1.35
	E2 and (E2 + BZA 2)	Dusp8	MAPK signaling pathway, Oxidative stress response	<i>Palierne et al., Mol endo , 2016</i>	3.08	1.18	3.77
Stat5a		Immune System	<i>Palierne et al., Mol endo , 2016</i>	3.00	1.42	1.58	1.27
Baiap21l		Signaling by FGFR3	<i>Palierne et al., Mol endo , 2016</i>	2.55	1.35	2.05	1.22
Tgm2		Huntington's disease	<i>Palierne et al., Mol endo , 2016</i>	2.55	1.27	1.72	1.01
Srgap3		PDGF signaling pathway	<i>Palierne et al., Mol endo , 2016</i>	2.42	1.19	1.75	0.84
IGFBP1		Factors and pathways affecting insulin-like growth factor (IGF1)-Akt signaling	<i>Boverhof et al. Carcinogenesis, 2014</i>	2.32	1.31	2.90	0.92
C2cd2l		Positive regulation of insulin secretion involved in cellular response to glucose stimulus	<i>Palierne et al., Mol endo , 2016</i>	2.32	1.47	1.60	1.36
Etnk2		Glycerophospholipid metabolism	<i>Palierne et al., Mol endo , 2016</i>	1.87	1.46	1.82	1.33
Dopey2		Golgi to endosome transport	<i>Palierne et al., Mol endo , 2016</i>	1.68	1.19	1.54	1.10
E2, (E2 + BZA 2) and BZA 10	Sulf2	Liver regeneration, metabolic process, bone development, kidney development	<i>Palierne et al., Mol endo , 2016</i>	1.56	1.27	1.50	1.17
	Ikzf4	regulation of transcription, DNA-templated	<i>Palierne et al., Mol endo , 2016</i>	1.88	1.32	2.76	1.82
E2, (E2 + BZA 10) and BZA 10	Fbf1	Cilium Assembly	<i>Palierne et al., Mol endo , 2016</i>	1.61	1.19	1.95	1.70
	Smtnl2	nd	<i>Palierne et al., Mol endo , 2016</i>	4.18	1.83	1.49	1.56
E2 and BZA 10	Nox4	Cellular responses to stress	<i>Palierne et al., Mol endo , 2016</i>	1.50	1.26	1.01	1.61
E2 only	Dsc2	Formation of the cornified envelope, Keratinization	<i>Palierne et al., Mol endo , 2016</i>	2.68	1.41	1.44	1.10
	Pgpep1	proteolysis	<i>Palierne et al., Mol endo , 2016</i>	2.57	1.35	1.33	1.17
	Psen2	Notch signaling pathway	<i>Palierne et al., Mol endo , 2016</i>	2.18	1.05	1.29	0.91
	Traf3ip2	IL17 signaling pathway	<i>Palierne et al., Mol endo , 2016</i>	2.14	0.89	1.10	0.84
	Inf2	protein-protein interactions in the podocyte	<i>Palierne et al., Mol endo , 2016</i>	1.84	1.09	1.32	1.10
	Trim2	Interferon gamma signaling	<i>Ahlbory-Dieker et al. Mol endo, 2009</i>	1.81	1.10	1.29	0.98
	Mn1	regulation of transcription, DNA-templated	<i>Palierne et al., Mol endo , 2016</i>	1.56	1.07	1.20	1.09
	Cyr61	protein-protein interactions in the podocyte	<i>Boverhof et al. Carcinogenesis, 2004</i>	1.55	0.91	0.84	0.96
	Ets2	Cellular Senescence, Cellular responses to stress	<i>Boverhof et al. Carcinogenesis, 2004</i>	1.52	1.00	1.35	1.11
Rad51l1	DNA Repair, organism-specific biosystem	<i>Palierne et al., Mol endo , 2016</i>	1.50	1.26	1.38	0.95	

Supplementary Table 4

Regulated by	Gene	Gene –associated functions (when available)	References	Fold induction (compared to PLB)			
				CE 10	CE 10 + BZA	CE 3	CE 3 + BZA
CE 10, C3 3 (CE 10 + BZA), and (CE 3 + BZA)	Slc34a2	Metabolism of proteins, Transmembrane transport of small molecules, Type II Na+/Pi cotransporters	<i>Palieme et al., Mol endo , 2016</i>	41.62	8.21	7.03	6.68
	Gdf15	<i>TGF-beta signaling pathway</i>	<i>Palieme et al., Mol endo , 2016</i>	12.06	4.03	5.72	4.19
	Il17ra	Cytokine Signaling in Immune system	<i>Palieme et al., Mol endo , 2016</i>	5.38	2.76	1.54	1.85
	Gpcpd1	Metabolism of lipids and lipoproteins, Glycerophospholipid metabolism	<i>Palieme et al., Mol endo , 2016</i>	4.27	2.03	1.82	2.14
	Spata2l	<i>nd</i>	<i>Palieme et al., Mol endo , 2016</i>	3.39	1.92	2.07	1.68
	Synpo	Protein-protein interactions in the podocyte	<i>Palieme et al., Mol endo , 2016</i>	2.98	1.55	1.55	1.67
CE 10, CE 3, (CE 10 + BZA)	Pcp4l1	<i>nd</i>	<i>Palieme et al., Mol endo , 2016</i>	2.24	2.37	1.97	2.16
	Lifr	Immune System	<i>Palieme et al., Mol endo , 2016</i>	8.96	1.56	1.85	0.88
	Map3k13	Insulin Signaling	<i>Palieme et al., Mol endo , 2016</i>	6.58	1.99	1.92	1.38
CE 10 and (CE 10 + BZA)	Erc1	NF-kappa B signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	1.88	1.65	1.92	0.91
	Ngfr	Cytokine-cytokine receptor interaction	<i>Palieme et al., Mol endo , 2016</i>	3.53	1.64	1.05	0.98
	Zfp276	regulation of transcription, DNA-templated	<i>Palieme et al., Mol endo , 2016</i>	2.67	1.53	1.07	0.74
	C2cd2l	positive regulation of insulin secretion involved in cellular response to glucose stimulus	<i>Palieme et al., Mol endo , 2016</i>	2.45	1.51	1.19	1.2
CE 10 and CE 3	Flrt1	Downstream signaling of activated FGFR1	<i>Palieme et al., Mol endo , 2016</i>	9.38	1.1	2.43	1.08
	Smtnl2	<i>nd</i>	<i>Palieme et al., Mol endo , 2016</i>	3.04	1.19	1.74	1
	Stat5a	Immune System	<i>Palieme et al., Mol endo , 2016</i>	2.51	1.24	1.54	1.35
	Peo1	Mitochondrial biogenesis	<i>Palieme et al., Mol endo , 2016</i>	2.35	1.34	1.58	1.5
	Etnk2	Glycerophospholipid metabolism	<i>Palieme et al., Mol endo , 2016</i>	2.27	1.5	1.64	1.38
CE 10 and (CE 3 + BZA)	IGFBP1	Factors and pathways affecting insulin-like growth factor (IGF1)-Akt signaling	<i>Boverhof et al. Carcinogenesis, 2004</i>	3.43	0.81	1.49	1.51
	Srgap3	PDGF signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	2.1	1.39	0.87	1.78
	ltpk1	Metabolic pathways, Inositol phosphate metabolism, Phosphatidylinositol signaling system	<i>Palieme et al., Mol endo , 2016</i>	1.91	1.15	1.31	1.66
	Mad2l1	Cell Cycle	<i>Palieme et al., Mol endo , 2016</i>	1.75	1.35	1.07	1.54
CE 10, CE 3 and CE 3 + BZA	Lsyna1	<i>nd</i>	<i>Palieme et al., Mol endo , 2016</i>	3.42	1.19	1.54	1.56
	Spon1	Metabolism of proteins, O-glycosylation of TSR domain-containing proteins	<i>Ahlbory-Dieker et al. Mol endo , 2009</i>	2.54	1.3	1.61	1.51
	Caskin2	<i>nd</i>	<i>Palieme et al., Mol endo , 2016</i>	2.29	1.37	1.65	2.77
Only CE 10	p21	Cell Cycle	<i>Ahlbory-Dieker et al. Mol endo , 2009</i>	4.75	1.21	1.46	1.41
	Traf3ip2	IL17 signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	3.17	0.87	1.33	1.38
	Coq10b	Respiratory electron transport, ATP synthesis by chemiosmotic coupling	<i>Palieme et al., Mol endo , 2016</i>	2.86	1.46	1.36	1.17
	Dsc2	Formation of the cornified envelope, Keratinization	<i>Palieme et al., Mol endo , 2016</i>	2.46	1.12	1.33	1.4
	Rad51l1	DNA Repair, organism-specific biosystem	<i>Palieme et al., Mol endo , 2016</i>	2.06	1.07	1.41	1.01
	Psen2	Notch signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	2.06	1.04	1.09	0.79
	Tsen54	tRNA processing in the nucleus	<i>Palieme et al., Mol endo , 2016</i>	2.03	1	1.23	0.93
	Pgpep1	Proteolysis	<i>Palieme et al., Mol endo , 2016</i>	2.00	1.05	1.21	1.09
	Baiaip2l1	Signaling by FGFR3	<i>Palieme et al., Mol endo , 2016</i>	1.82	1.07	0.83	1.11
	Upp2	Metabolic pathways, Drug metabolism - other enzymes	<i>Palieme et al., Mol endo , 2016</i>	1.81	1.28	1.14	1.43
	Insig2	Regulation of cholesterol biosynthesis by SREBP	<i>Palieme et al., Mol endo , 2016</i>	1.68	1.34	1.04	0.94
	Agpat9	Metabolism of lipids and lipoproteins, Phospholipid metabolism, Fatty acid, triacylglycerol metabolism	<i>Palieme et al., Mol endo , 2016</i>	1.68	1.21	0.99	1.08
	Cyp17	Metabolism of steroid hormones	<i>Palieme et al., Mol endo , 2016</i>	1.63	1.16	1.3	1.1
	Tgm2	Huntington's disease	<i>Palieme et al., Mol endo , 2016</i>	1.63	0.9	1.1	0.82
	Trim2	Interferon gamma signaling	<i>Ahlbory-Dieker et al. Mol endo , 2009</i>	1.6	1.02	1.27	0.93
	Inf2	Protein-protein interactions in the podocyte	<i>Palieme et al., Mol endo , 2016</i>	1.58	1.15	1.4	1.2
	Mn1	Regulation of transcription, DNA-templated	<i>Palieme et al., Mol endo , 2016</i>	1.53	1.2	1.22	1.04

Supplementary Table 5A

				Fold induction (compared to PLB)		
Regulated by	Gene	Gene –associated functions (when available)	References	CE	BZA	CE + BZA
CE, BZA and (BZA + CE)	Prtn3	Signaling by Interleukins	<i>Pedram et al., Science Signaling, 2013</i>	371.64	352.72	281.47
	Lcn2	Innate Immune System	<i>Pedram et al., Science Signaling, 2013</i>	40.63	8.38	6.25
	p21	Cell Cycle	<i>Ahlbory-Dieker et al. Mol endo, 2009</i>	17.59	8.64	8.18
	Slc34a2	Metabolism of proteins, Transmembrane transport of small molecules, Type II Na+/Pi cotransporters	<i>Palieme et al., Mol endo , 2016</i>	10.09	105.48	50.83
	IGFBP1	Factors and pathways affecting insulin-like growth factor (IGF1)-Akt signaling	<i>Boverhof et al. Carcinogenesis, 2004</i>	9.60	12.19	7.55
	Gdf15	TGF-beta signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	7.63	17.65	22.35
	Crybb3	Structural constituent of eye lens	<i>Palieme et al., Mol endo , 2016</i>	6.17	2.35	2.82
	Lsyna1	nd	<i>Palieme et al., Mol endo , 2016</i>	6.12	8.26	7.60
	Srgap3	PDGF signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	5.42	2.42	2.94
	Elovl3	Metabolism of lipids and lipoproteins, Fatty acid, triacylglycerol, and ketone body metabolism	<i>Palieme et al., Mol endo , 2016</i>	4.11	1.87	3.95
	Ly6d	Metabolism of proteins	<i>Palieme et al., Mol endo , 2016</i>	4.06	5.48	1.89
	Marco	Phagosome	<i>Pedram et al., Science Signaling, 2013</i>	3.95	3.17	5.08
	Slc3a1	Transmembrane transport of small molecules	<i>Pedram et al., Science Signaling, 2013</i>	3.57	3.40	5.39
	Cyp8B1	Nuclear receptors in lipid metabolism and toxicity, PPAR signaling pathway	<i>Pedram et al., Science Signaling, 2013</i>	3.57	2.88	1.54
	SHP	Bile secretion	<i>Kim et al, Mol Medecine, 2014</i>	3.41	3.10	4.87
	Corin	Muscle contraction	<i>Palieme et al., Mol endo , 2016</i>	3.28	14.16	9.75
	FGF21	Regulation of Actin Cytoskeleton	<i>Kim et al, Mol metab, 2014</i>	3.23	2.67	2.52
	Ms4a4a	nd	<i>Pedram et al., Science Signaling, 2013</i>	3.00	2.11	3.66
	Trim2	Interferon gamma signaling	<i>Ahlbory-Dieker et al. Mol endo, 2009</i>	2.83	2.31	2.28
	Ggct	Metabolism, Glutathione metabolism	<i>Palieme et al., Mol endo , 2016</i>	2.66	4.01	3.68
	Insiq2	Regulation of cholesterol biosynthesis by SREBP	<i>Palieme et al., Mol endo , 2016</i>	2.63	1.97	2.83
	Smtnl2	nd	<i>Palieme et al., Mol endo , 2016</i>	2.49	4.41	4.78
	Stat5a	Immune System	<i>Palieme et al., Mol endo , 2016</i>	2.48	2.10	2.03
	Ptgds	Metabolic pathways, Metabolism of lipids and lipoproteins, Arachidonic acid metabolism	<i>Gao et al. , Mol endo 2008</i>	2.47	12.48	16.93
	Flrt1	Downstream signaling of activated FGFR1	<i>Palieme et al., Mol endo , 2016</i>	1.99	2.64	3.17
	Psen2	Notch signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	1.98	1.61	1.74
Map3k13	Insulin Signaling	<i>Palieme et al., Mol endo , 2016</i>	1.88	2.05	3.44	
Dusp8	MAPK signaling pathway, Oxidative stress response	<i>Palieme et al., Mol endo , 2016</i>	1.62	1.73	1.85	
Ngfr	Cytokine-cytokine receptor interaction, Circadian rythm related genes	<i>Palieme et al., Mol endo , 2016</i>	1.60	1.50	1.88	
CE only	Hmgcr	Metabolic pathways, Metabolism of lipids and lipoproteins, Cholesterol biosynthesis, Bile secretion	<i>Palieme et al., Mol endo , 2016</i>	3.20	1.47	1.18
	Aqp8	Bile secretion	<i>Palieme et al., Mol endo , 2016</i>	2.31	1.04	0.91
	Esr1	Estrogen signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	1.66	0.90	0.76
	Traf3ip2	IL17 signaling pathway	<i>Palieme et al., Mol endo , 2016</i>	1.62	1.37	1.30
	Mn1	Regulation of transcription, DNA-templated	<i>Palieme et al., Mol endo , 2016</i>	1.61	1.07	1.44
BZA only (BZA + CE) only	Peo1	Mitochondrial biogenesis	<i>Palieme et al., Mol endo , 2016</i>	1.16	1.67	1.34
	Pgpep1	Proteolysis	<i>Palieme et al., Mol endo , 2016</i>	1.39	1.32	1.66
	Spon1	Metabolism of proteins, O-glycosylation of TSR domain-containing proteins	<i>Ahlbory-Dieker et al. Mol endo, 2009</i>	1.04	1.41	2.63
BZA and (BZA + CE)	Sulf2	Liver regeneration, metabolic process, bone development, kidney development	<i>Palieme et al., Mol endo , 2016</i>	0.94	1.43	1.51
	Caskin2	nd	<i>Palieme et al., Mol endo , 2016</i>	1.49	1.95	2.00
	Tsen54	tRNA processing in the nucleus	<i>Palieme et al., Mol endo , 2016</i>	1.45	1.67	1.54
	Spata2l	nd	<i>Palieme et al., Mol endo , 2016</i>	1.43	2.22	1.91
	Il17ra	Cytokine Signaling in Immune system	<i>Palieme et al., Mol endo , 2016</i>	1.37	3.01	3.14
	Inf2	Protein-protein interactions in the podocyte	<i>Palieme et al., Mol endo , 2016</i>	1.29	1.57	2.44
	PCK1	Glycolysis and Gluconeogenesis	<i>Kim et al, Mol metab, 2014</i>	1.16	1.82	1.82
	Etnk2	Metabolism of lipids and lipoproteins, Phospholipid metabolism, Glycerophospholipid metabolism	<i>Palieme et al., Mol endo , 2016</i>	1.06	1.77	1.73
Pcp4l1	nd	<i>Palieme et al., Mol endo , 2016</i>	0.60	4.14	4.66	
CE and (BZA+CE)	Cyp17a1	Metabolism of lipids and lipoproteins, Steroid hormone biosynthesis	<i>Pedram et al., Science Signaling, 2013</i>	2.47	1.19	2.46

Supplementary Table 5B

	Gene	Gene –associated functions (when available)	References	CE	BZA	CE + BZA
CE and BZA	Aqp4	Bile secretion	<i>Palieme et al., Mol endo , 2016</i>	0.35	0.57	0.85
BZA and BZA + CE	Aacs	Metabolism of lipids and lipoproteins, Metabolism of lipids and lipoproteins	<i>Pedram et al., Science Signaling, 2013</i>	0.86	0.47	0.19
CE + BZA only	Cyp2B10	Nuclear receptors in lipid metabolism and toxicity	<i>Boverhof et al. Carcinogenesis, 2004</i>	0.75	1.12	0.53
	Srebp1c	AMPK signaling pathway	<i>Handgraaf et al, Diabetes, 2013</i>	0.72	0.68	0.47
BZA only	Foxa2	Liver steatosis AOP	<i>Gordon, Mol endo 2014</i>	0.94	0.54	0.80
CE only	Zfp276	regulation of transcription, DNA-templated	<i>Palieme et al., Mol endo , 2016</i>	0.63	1.11	0.71
	PPARa	Nuclear Receptors in Lipid Metabolism and Toxicity, Steatosis AOP	<i>Kim et al, Mol Medecine, 2014</i>	0.59	0.69	0.67
	Coq10b	Respiratory electron transport, ATP synthesis by chemiosmotic coupling	<i>Palieme et al., Mol endo , 2016</i>	0.47	1.04	0.66
	Car1	Nitrogen metabolism	<i>Pedram et al., Science Signaling, 2013</i>	0.46	0.66	0.84
CE, BZA and BZA + CE	Clec2h	Immune System	<i>Pedram et al., Science Signaling, 2013</i>	0.52	0.38	0.45
	Sucnr1	cAMP signaling pathway, Signaling by GPCR	<i>Pedram et al., Science Signaling, 2013</i>	0.47	0.25	0.26
	Fmo2	Metabolism	<i>Pedram et al., Science Signaling, 2013</i>	0.45	0.39	0.27
	Upp2	Metabolic pathways, Drug metabolism - other enzymes	<i>Palieme et al., Mol endo , 2016</i>	0.40	0.61	0.36
	Cyp2c55	Metabolic pathways, Steroid hormone biosynthesis, Arachidonic acid metabolism, Linoleic acid metabolism	<i>Kobayashi et al, J endocrinol, 2013</i>	0.32	0.33	0.25