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

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Short Title: Tissue specific action of TSEC

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

Abstract

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

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

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

1. Introduction

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

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

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

Thus, the consequences of the co-administration of estrogens and SERM at the molecular, cellular and whole body levels are undoubtedly highly complex. Here, our goal was to explore the tissue-specific effects of BZA combined with CE *in vivo* in mice comparing in two major 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

2. Materials and Methods

2.1. Mice

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

2.2. Histological analysis

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

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

2.3. Analysis of mRNA levels by qPCR

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

2.4. Statistical analysis

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

3. RESULTS

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

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

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

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

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

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

No difference in body weight was observed between ovariectomized untreated mice (mean = 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,

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

4. DISCUSSION

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

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

Finally, Mauvais-Jarvis and coworkers recently reported that BZA and TSEC exhibits estrogen-mimetic action with regard to glucose and energy homeostasis (Kim et al., 2014). Interestingly, they reported that lipid oxidation and increased energy expenditure promoted by CE, BZA and TSEC involved different pathways as revealed by different target gene pattern 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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Figure 1: Effect of acute administration of E2 and CE alone or in combination with BZA on uterus

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

Figure 2: Effect of acute administration of E2 and CE alone or in combination with BZA on transcriptional regulation in the liver

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

A

B

C

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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

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

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. (A) Data obtained from 96.96 Dynamic Arrays were used to generate a cluster diagram of the significant gene

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

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: *** $p < 0.001$).

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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	CTGTCCGGCTGCGCAAGTGTT	AACATTCTCCCTCCTCGGCGGT	Lifr	AAGAGCATTGCTTCCCTCGCA	TGTTGGCGTGGTAACGAGCAT
Ramp3	TGTTGTTGCTGCTTTGTGGTGA	CTCCAGCATCCCTGTCTCGTT	Il17ra	GGTGGGATCTGTCAATCGTGCT	CTACGGGCAAGATGCCATTGA
Mad2l1	GAGAGGCAGGGAGGACAGCTT	CCTCGTTTCAGGCACCAACAAA	Tsen54	GTATCTGCTGGAGTGTGGTTCGAT	CATAGCCCAGTCTCTTCAGGTGG
Gadd45g	AGTCTGAATGTGGACCTGACA	GCAGAACGCCTGAATCAACGTG	Lsyna1	TTCACGGTGCCCCGCTGGGT	CGCGCACAGGTGCGAAGGAGG
Inhbb	TTTGCAGAGACAGATGGCCTCG	GCCTGCACCACGAATAGGTTCT	Synpo	TGCGGCATCTGGAGAAGGTTG	GTTTGGTGAGGACTGCTGGGT
Prtn3	AGTCCACTCCATGAAGAATGCCA	TATGCCGGGAACACAACGTG	Itpk1	CCAGGGGGCCCTGCTCCTCAA	GCGGAGCTGTGCAGGAAGCGAG
Lcn2	TCTGTACCTGAGGATACCTGTGCAT	ATGTCACTTCCATCCTGGTCA	Acot1l	AAGCCGGGGGTGGCCTGGTT	GGTCACGCTGCCCACGCACC
p21	CAGGCGCAGATCCACAGCGA	GCACACTTTGCTCCTGTGCGGA	Spata2l	GCTGCCTTGAGAAAAGGAGTT	ACCATTAGCGGTGGTTGTCC
Slc34a2	TGTACTGAGCAGCGCCTTCC	CCAGTCCAGCCACAGGATTAGAC	Dsc2	TGTATCAAACACCGAGGCTCCTG	TGCCCTCCACTTTTGACTCCAG
IGFBP1	AGGGATGTCTCACTGTTTGCT	GCCCTGCCAACGAGAACTCTA	Ehhadh	GCCAGTGGGAGATTTAAAGCCA	TGCCATCAGTCCAACGTGAATCA
Gdf15	TGGGACCCCAATCTCACCTCT	AGCCGAGAGGACTCGAACTCA	Agpat9	GCCATGGTTAAGGCTTGCTCTC	ATTGTTGATGCAAGTACCTTCTGGG
Crybb3	CTCCAGAACAACCTGCTCCCA	AAGGTGGGCTCCATCCAAGT	Pgpep1	CTCCAGTCCCTCAGCCAGGAT	ACAGTATGCTCCCCAAAAGGCG
Lsyna1	TTCACGGTGCCCCGCTGGGT	CGCGCACAGGTGCGAAGGAGG	Baiap2l1	TGCTTCTACCTTCAGAGCGCC	GCAAAGGGGTTTTCTCCACTGA
Srgap3	TCTTTAACGGCAGTATGGAAGCGT	TCCCCTGCTGCTGGAGTCCATA	Tgm2	TCATACAGGGGATCGGAAAG	TGGAGAATCCCGAAATCAAC
Elov13	AGACGACCACTATGAGAAATGAGCTT	GTCTGCAAAATCGAAATGGACACAT	Peo1	TGCAGACAGCATCCATTTTCGG	AAAGCGATTCTTGACACCTGC
Ly6d	TCCTCCTTGCTTGGCTGTGG	AGAAGTTGGACGGGCGACCT	C2cd2l	TTGGGTGCGAGCCTTGAATGAG	TGGTGGGAGTTGGGGTATCTCTT
Marco	TCTGCCTACAAGACCTGGGAC	GGAATCCAGGGATTGCAGGTGT	Caskin2	GCGCAGAAGCACCAGTGCCCA	GCGGCCGGGAGAGACCTCA
Slc3a1	AATCTCAACAGCGTATCTGAAGTC	AAGGACAGTGACAAGGATGGG	Spon1	TGTGTGATTCTGAAGGCCAGC	GTCCGTCACCCCATCAAGTGT
Cyp8B1	AAGACATCCCCGTGCTTGGC	TTTTGTGCCCTGGCTGGGTC	Etnk2	CGGGCTGTGCTACGAGTATGT	TGGCGTGGATGGTGTGAATCT
SHP	AAGGGCACGATCCTCTTCAA	CTGTTGCAGGTGTGCGATGT	Inf2	CCTCAAGGAACCAAGAAGAGTTTG	AGGCAACACATCCAGCCGAT
Corin	GGGACCTGAGTGACGAGCAAAA	GGCTGTGACAAGAGCAGTTGA	Dopey2	CGCAGATCAAAGGGGATGAGAAAT	CCTCCTGCAGGTCTGGTATGGA
FGF21	GCCATGGGCTTCAGACTGGTA	TTGCCAACAGCCAGATGGAGC	Upp2	TCTCTGCTATCTGCCACCG	TCTTCGTCCATGCCTTCCAGG
Hmgcr	TTCACGAGAAAGCTCTAGGACCAG	TGCCTTGTGATTGGAGTTGGCA	Sulf2	ACTCAGAGCGGCCAGTGAAC	TGTGGAGCAGTTTGCCTCTCTC
Ms4a4a	GGCTGCTTCGAACCAAACCTT	TGTCGACCGGTGAAATACCCC	Rad51l1	CGCAACCTGGTGGACCTGTAA	ACGGCTTATGAGTTAAAGACACGA
Trim2	ACAGGCAGTTTGAGGTCCC	TCCATTGGAGCCAAACTTCAACAT	Mn1	AGAAGGCCAAGTCCAGAACC	CCTTGTTGGTGGGGTGGTCAT
Ggct	GTGTGGGGAGTGGTATGGAGAA	CGTCATCAGGTAACCTTCGGCA	Ngfr	CTCCATCTTGGCTGCTGTGGT	CCGGCTGTTGGCTCCTTGTTT
Insig2	GTGGCGGTGTTCTGTTGGGTATAA	GTGCAGCCAGTGTGAGGGAAA	Zfp276	CAGTGACCTTTCTGAGGGAGAC	GCTTCTCTGCCTTCGCTCTTCT
Smtnl2	CATGCCACCTTTTCGCTGTCC	ATCCGTTGGCTGGACCTGCAT	Pcp4l1	CCGGGATGAGCGAGCTTAACA	CTCTGGCGCTGTCAAGTCAAT
Stat5a	CTTCTGGGACATGGCGTCAACC	ACACAGCTCCAGAACACGTATGACC	PPARα	TGCCTTCCCTGTGAACGTGACG	TGTTGGATGGATGTGGCCAGG
Ptgds	CACAGAGGAGGACATTGTTTTCC	ACTGACTTCTCTCACCTGCGTTT	Clec2h	GGAGACACAGTGGAGATGATTCTGA	CAGCTCACAGATGAATGCTGCAA
Cyp17a1	CATCCACACAAGGCTAACA	CAGTGCCCCAGAGATTGATGA	Sucnr1	TGACATTCCCAAGCAGTCCAA	ACAGAAGCCGACAGCAGAATG
Aqp8	GGCTCATCATTGCTACCTTGGG	AGCGACAGCAGGGTTGAAG	Coq10b	GCTGAGAGTGCAGAAGGGAGCGG	TGGCGTTTTAGCCAGGTCTTCCT
Flrt1	ACATGGGGCCAAGGATGTGAG	TGACCCACCCAACCTGGAGT	Car1	GGTTGGCTGGACCAACCTTCA	CTGAGCACACCGTGGATGGAA
Cyp17	ATTTACCTTCGGAGCTGGT	AGGGCAGCTGTTGTGCATCT	Fmo2	CAAGAGTTTTCAAAGGCTTGTTAGC	TCCAGGTAGTCAACGTAGTTGGT
Psen2	ACTGGCAACGAGACTGGAA	AACACAGCAAGCAGCAGGAGG	Upp2	TTCCTGCTATCTGCCACCG	TCTTCGTCCATGCCTTCCAGG
Map3k13	CTGGAGATGCGCGAGAAGGA	ATTGCGGTGGATGATTGGACGG	Nr1d1	GGAGGAGCCACTAGAGCCAATG	GAGGCCATCAACCTCCAGTT
Dusp8	GCGCCTTCAGCAGGGAAAAGTG	CTGGCATCTCGTGTGCTCTGGT	Aqp4	TACCTGGTCACACTCCAGT	CCAGCGGTGAGGTTTCCATGA
Traf3ip2	GAGGACGAGCATGGCTTACATACT	CGGCACATGCTCCTCTTGG	Cyp2c55	GCCAGGGTCAAATACTTCTGGGTT	CAATGTGCCCCATGCAGCTA

Supplementary Table 2

Supplementary Table 3				Fold induction (compared to PLB)			
Regulated by	Gene	Gene –associated functions (when available)	References	E2	E2 + BZA 10	E2 + BZA 2	BZA 10
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
	Synpo	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
E2 , (E2 + BZA10) and (E2 +BZA 2)	Cco10b	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
	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	1.36
	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
	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
E2, (E2 + BZA 2) and BZA 10	Ikzf4	regulation of transcription, DNA-templated	<i>Palierne et al., Mol endo , 2016</i>	1.88	1.32	2.76	1.82
	Fb1	Cilium Assembly	<i>Palierne et al., Mol endo , 2016</i>	1.61	1.19	1.95	1.70
E2, (E2 + BZA 10) and BZA 10	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

				Fold induction (compared to PLB)			
Regulated by	Gene	Gene –associated functions (when available)	References	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>Palierne et al., Mol endo , 2016</i>	41.62	8.21	7.03	6.68
	Gdf15	<i>TGF-beta signaling pathway</i>	<i>Palierne et al., Mol endo , 2016</i>	12.06	4.03	5.72	4.19
	Il17ra	Cytokine Signaling in Immune system	<i>Palierne et al., Mol endo , 2016</i>	5.38	2.76	1.54	1.85
	Gpcpd1	Metabolism of lipids and lipoproteins, Glycerophospholipid metabolism	<i>Palierne et al., Mol endo , 2016</i>	4.27	2.03	1.82	2.14
	Spata2l	<i>nd</i>	<i>Palierne et al., Mol endo , 2016</i>	3.39	1.92	2.07	1.68
	Synpo	Protein-protein interactions in the podocyte	<i>Palierne et al., Mol endo , 2016</i>	2.98	1.55	1.55	1.67
	Pcp4l1	<i>nd</i>	<i>Palierne et al., Mol endo , 2016</i>	2.24	2.37	1.97	2.16
CE 10, CE 3, (CE 10 + BZA)	Lifr	Immune System	<i>Palierne et al., Mol endo , 2016</i>	8.96	1.56	1.85	0.88
	Map3k13	Insulin Signaling	<i>Palierne et al., Mol endo , 2016</i>	6.58	1.99	1.92	1.38
	Erc1	NF-kappa B signaling pathway	<i>Palierne et al., Mol endo , 2016</i>	1.88	1.65	1.92	0.91
CE 10 and (CE 10 + BZA)	Ngfr	Cytokine-cytokine receptor interaction	<i>Palierne et al., Mol endo , 2016</i>	3.53	1.64	1.05	0.98
	Zfp276	regulation of transcription, DNA-templated	<i>Palierne 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>Palierne 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>Palierne et al., Mol endo , 2016</i>	9.38	1.1	2.43	1.08
	Smtnl2	<i>nd</i>	<i>Palierne et al., Mol endo , 2016</i>	3.04	1.19	1.74	1
	Stat5a	Immune System	<i>Palierne et al., Mol endo , 2016</i>	2.51	1.24	1.54	1.35
	Peo1	Mitochondrial biogenesis	<i>Palierne et al., Mol endo , 2016</i>	2.35	1.34	1.58	1.5
	Etnk2	Glycerophospholipid metabolism	<i>Palierne et al., Mol endo , 2016</i>	2.27	1.5	1.64	1.38
	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
CE 10 and (CE 3 + BZA)	Srgap3	PDGF signaling pathway	<i>Palierne et al., Mol endo , 2016</i>	2.1	1.39	0.87	1.78
	Itpk1	Metabolic pathways, Inositol phosphate metabolism, Phosphatidylinositol signaling system	<i>Palierne et al., Mol endo , 2016</i>	1.91	1.15	1.31	1.66
	Mad2l1	Cell Cycle	<i>Palierne 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>Palierne 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>Palierne 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>Palierne et al., Mol endo , 2016</i>	3.17	0.87	1.33	1.38
	Coq10b	Respiratory electron transport, ATP synthesis by chemiosmotic coupling	<i>Palierne et al., Mol endo , 2016</i>	2.86	1.46	1.36	1.17
	Dsc2	Formation of the cornified envelope, Keratinization	<i>Palierne et al., Mol endo , 2016</i>	2.46	1.12	1.33	1.4
	Rad51l1	DNA Repair, organism-specific biosystem	<i>Palierne et al., Mol endo , 2016</i>	2.06	1.07	1.41	1.01
	Psen2	Notch signaling pathway	<i>Palierne et al., Mol endo , 2016</i>	2.06	1.04	1.09	0.79
	Tsen54	tRNA processing in the nucleus	<i>Palierne et al., Mol endo , 2016</i>	2.03	1	1.23	0.93
	Pgpep1	Proteolysis	<i>Palierne et al., Mol endo , 2016</i>	2.00	1.05	1.21	1.09
	Baia2l1	Signaling by FGFR3	<i>Palierne et al., Mol endo , 2016</i>	1.82	1.07	0.83	1.11
	Upp2	Metabolic pathways, Drug metabolism - other enzymes	<i>Palierne et al., Mol endo , 2016</i>	1.81	1.28	1.14	1.43
	Insig2	Regulation of cholesterol biosynthesis by SREBP	<i>Palierne 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>Palierne et al., Mol endo , 2016</i>	1.68	1.21	0.99	1.08
	Cyp17	Metabolism of steroid hormones	<i>Palierne et al., Mol endo , 2016</i>	1.63	1.16	1.3	1.1
	Tgm2	Huntington's disease	<i>Palierne 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>Palierne et al., Mol endo , 2016</i>	1.58	1.15	1.4	1.2
	Mn1	Regulation of transcription, DNA-templated	<i>Palierne 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
	Insig2	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
	Sulf2	Liver regeneration, metabolic process, bone development, kidney development	<i>Palieme et al., Mol endo , 2016</i>	0.94	1.43	1.51
BZA and (BZA + CE)	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>Palierne 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>Palierne 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>Palierne 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>Palierne 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