

1 **Supplemental Figure 1. OVX decreases uterine wet weight and increases body weight**

2 (A) Serum levels of mice treated with 27HC for 28 days. Data (n=4-5) is the mean±SEM. \*\* p<0.01  
3 compared to placebo-treated mice (unpaired 2-tailed t-test on raw data). (B) Uterine wet weight and  
4 weight gain (over the 28 day study) were assessed at the conclusion of the study. Mice underwent sham  
5 or OVX surgery, with the OVX mice assigned to either placebo (OVX P) or E2 (OVX E) treatment. Data  
6 (n=4-10) is the mean±SEM. \*\*\*p<0.001, \*p<0.05 compared to the sham-operated *Cyp7b1*<sup>+/+</sup> group,  
7 ^^p<0.001 compared to the sham-operated *Cyp7b1*<sup>-/-</sup> group (one-way ANOVA with SNK). (C) Uterine  
8 weight of intact *Cyp7b1*<sup>-/-</sup> mice treated with placebo or E2. Data (n=4-5) is the mean±SEM.

9

10 **Supplemental Figure 2. Role of ER subtypes in 27HC mediated bone pathology**

11 (A) Primary calvarial pre-OBs have increased ER $\alpha$  mRNA expression compared to ER $\beta$ . (B) The  
12 selective ER $\beta$  ligands (ERB-041 and DPN) do not activate alkaline phosphatase activity in primary pre-  
13 OBs to the same extent as 27HC or E2 (see Fig. 7 for details). (C) ER $\alpha$ , ER $\beta$ , and Cathepsin K mRNA  
14 expression throughout osteocalstogenesis in RAW 264.7 cells is depicted. Cells were grown in  
15 differentiation media containing RANKL and mCSF. Cathepsin K is included as a positive control of  
16 osteoclastogenesis. Data are presented as the normalized expression relative to cyclophilin and shown as  
17 the mean±SEM.

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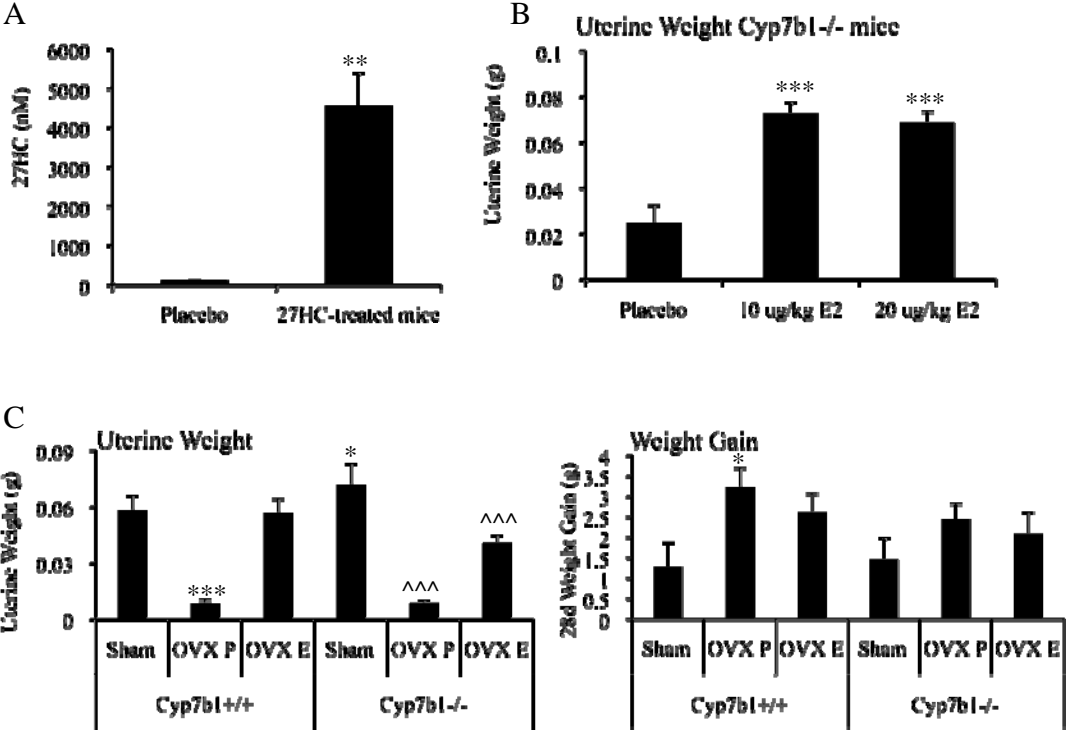
19 **Supplemental Figure 3. Gene expression analysis**

20 The calvaria from each mouse was harvested, flash frozen in liquid N<sub>2</sub>, and stored at -80 C. RNA was  
21 isolated using Trizol extraction followed by DNase treatment. cDNA prepared from isolated RNA was  
22 subjected to qRT-PCR analysis for analysis of target gene expression. Data are presented as the  
23 normalized expression relative to cyclophilin and shown as the mean±SEM.

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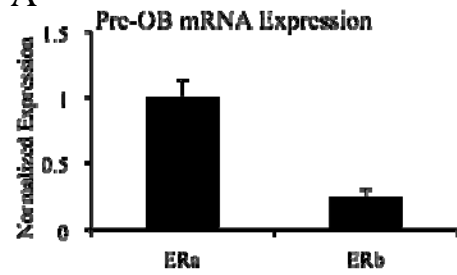
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# Supplemental Figure 1

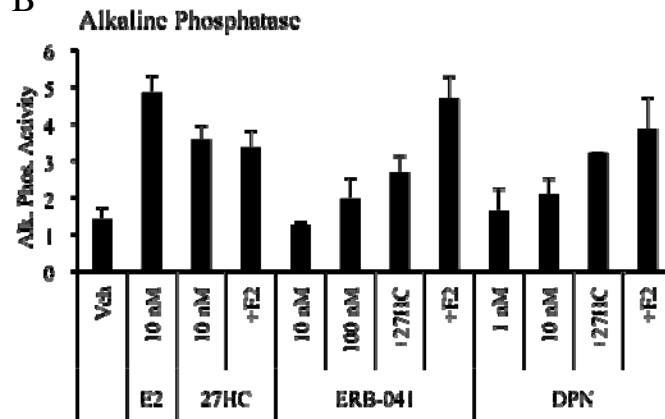


## Supplemental Figure 2

A

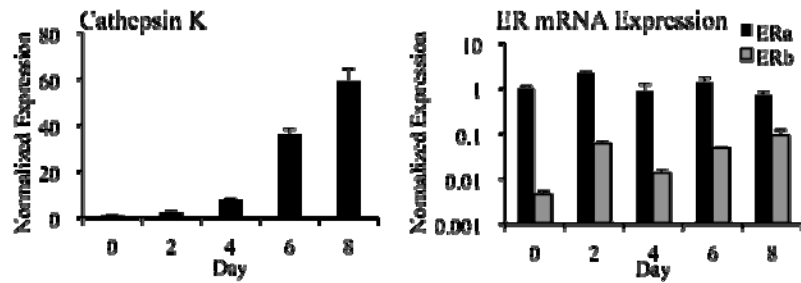


B



C

RAW264.7 Osteoclastogenesis



### Supplemental Figure 3

