

Supplementary Figure S1. Low dose of E2 failed to completely inhibit uterine epithelial apoptosis. After daily injection of 200ng E2 from P1 to P5, apoptotic cells was still detected in the uterine epithelium by TUNEL assay on P5 (A). Immunofluorence of active caspase-9 and -3 revealed that both proteins were present on P5 (B, C). Bars: 50µm in A-C.





Supplementary Figure S2. Real-time RT-PCR showed that lower doses of E2 only slightly induced Birc1 gene expression. 50 and 200ng of daily E2 injection barely induced *Birc1a* and *1e* on P5 (A and C), and had no effect on *Birc1b* expression (B).



Supplementary Figure S3. Bcl2 level is not changed upon DES exposure at P5. A,B) Immunohistochemistry shows that Bcl2 is universally expressed in the uterus and DES treatment causes no noticeable change in Bcl2 leve. C,D) Corresponding slides stained without primary Antibody as negative controls. Bars: 50µm in A-D.



Supplementary Figure S4. Ovarian hormone treatment in ovariectomized mouse up-regulates Birc1 gene expression in uterine epithelium. A-D) Immunohistochemistry staining of Birc1s in the uterus shows that Birc1 proteins are induced in both luminal and glandular epithelium by E2, P4 or the combination of two after eight hours. E) Real-time RT-PCR of Birc1a, 1b and 1e after hormone treatment. Birc1a is reduced 10 times by E2, 25 times by P4, but only 9 times by the combination of both. Birc1b expression is doubled by either E2 or P4, but up-regulated over 6 times by the combination of both. Birc1e expression is induced two-fold by E2, five-fold by P4 and around 4-fold by combination treatment. Bars: 50mm in A-D



Supplementary Figure S5. Birc1 expression during estrous cycle. A) RT-PCR analysis of Birc1 genes showed that expression Birc1a and 1b is lower at Diestrous phase, but higher at the rest of the cycle. Birc1e expression is consistent throughout the cycle. Birc1f expression is lower at diestrous and estrous phase, but higher at proestrous and metestrous cycle. B) Immunofluorescence with Birc1 antibody revealed lower overall Birc1 protein level at diestrous phase, but higher at the other phases. The expression is more prominent in the luminal and glandular epithelia. Bars: 50µm in B-E.

 Table S1. Primers used for RT-PCR and real-time RT-PCR

| Gene Name | Accession Number | Primers | Product Size (bp) |
|----------------------------|---------------------|--|-------------------------|
| Birc1a | NM_008670 | 5'TGCCTTGGCAGTCCTTATTT3' 5'TGCTGCTGTGAGTGACAATG3' | 400 |
| Birc1b | NM_010872 | 5'ggaaagacaccctcagtcca3' 5'gagggcatcagatcccatta3' | 319 |
| Bircle | NM_010870 | 5'aaagtatgttgggccctcct3' 5'gtcagctgcagccatgataa3' | 403 |
| Birc1f | NM_010871 | 5'CCTGGAGTAAAAGATGCTCCTG3' 5'TGCTTGCATGAGTGTGTGTG3' | 407 |
| β-actin | NM_007393 | 5'agccatgtacgtagccatcc3' 5'ctctcagctgtggtggtgaa3' | 228 |
| Caspase-3 | NM_009810 | 5'gtgggactgatgaggagatg3' 5'acctgatgtcgaagttgagg3' | 482 |
| Caspase-9 | NM_015733 | 5'TCCCCACAGATCAAGTCTCC3' 5'CTCTTGGCCCTGAGAGTCAC3' | 651 |
| <i>Birc1a</i> real-time | NM_008670 | 5'CGCAGTCTCCTGGTTAGCAC3' 5'AGTGAGAAGGCAGCAAGCAG3' | 106 |
| <i>Birc1b</i> real-time | NM_010872 | 5'gcaggggggatcaagagtttg3' 5'accaccatgtggttgctgag3' | 107 |
| <i>Birc1e</i> real-time | NM_010870 | 5'atcatggctgcagctgactc3' 5'cccattggcaatagatgcac3' | 104 |
| β -actin real-time | NM_007393 | 5'CTAAGGCCAACCGTGAAAAG3' 5'CCATCACAATGCCTGTGGTA3' | 126 |

Table S2. Restriction Enzymes and RNA polymerases used to generate sense or anti-sense probes for in situ hybridization

| Probe Name | Restriction Enzyme | RNA polymerase | Probe Size (nt) |
|------------|---------------------------------|------------------------------|--------------------|
| Bircla | anti-sense: NotI sense: PstI | anti-sense: T3 sense: T7 | ~400 |
| Birc1b | anti-sense: ApaI sense: PstI | anti-sense: Sp6 sense: T7 | ~320 |
| Bircle | anti-sense: NcoI sense: SpeI | anti-sense: Sp6 sense: T7 | ~400 |