Supplement Table 1. The dynamics of BSEP transactivation before, during and after pregnancy

Mouse Tag	Pre-mating	Day -6	Day -2	Day -1	Day 0	Day +1	Day +6	Day +11
5	48.1[a]	243	12.15	5.06	0.597	73.6	206	255
6	255	170	91.5	0.316	96.9	30.1	101	79.6
7	395	118	40.1	2.23	7.76	2.230	145	65.5
8	42.4	46.9	N/A[b]	N/A	55.1	0.629	44.1	51.4
9	93.5	319	16.2	4.88	401	453	284	287
10	81.9	546	27.3	85.6	0.524	138	208	308
12	134	320	3.12	29.9	165	33.1	381	32.7
13	196	157	78.5	72.7	0.743	25.3	163	84.5
17	46.7	107	53.5	4.65	45	67.5	N/A	N/A
21	138	86.1	43.05	17	0.322	21.2	76.7	N/A
25	1120	1090	54.5	212	0.423	998	1280	839
27	81	296	248	49.5	1.23	72.5	129	205
31	743	116	58	20.1	3.22	11.1	128	147
34	65	179	28.9	1.27	0.0245	139	133	58.5
40	94.3	402	146	0.27	92	N/A	321	256

Notes: [a] total photon/second ($x10^6$). [b] N/A data was not collected due to technical issues or mouse well-being.

Supplement Table 2. Serum E2 levels in mice before, during and after pregnancy

Mouse Tag	Pre-mating	Day -15	Day -10	Day -5	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2
5	4.4[a]	7.0	N/A[b]	5.0	12.0	13.0	N/A	16.0	16.0	N/A
6	10.0	6.0	8.0	8.0	19.4	17.5	13.0	11.5	17.5	11.0
7	10.6	10.0	14.0	9.5	12.0	20.4	20.0	13.0	13.0	12.0
8	12.0	6.6	9.2	10.0	12.0	21.0	16.0	23.5	15.0	14.4
9	6.0	7.0	8.0	4.0	12.0	11.0	14.4	8.2	12.6	15.0
10	11.0	5.0	31.7	4.4	6.2	11.3	11.6	8.2	6.5	8.0
12	3.8	10.3	11.1	22.6	20.0	22.0	22.6	18.5	41.0	14.2
13	7.4	11.6	N/A	16.8	26.3	24.0	19.2	6.8	8.0	7.0
21	13.0	10.3	13.6	12.5	16.8	24.0	6.4	33.0	12.0	8.6
25	1.2	15.6	2.0	38.0	41.3	33.0	43.0	28.9	10.4	10.0
27	12.0	7.5	17.5	14.0	11.6	14.0	13.5	11.0	8.0	17.0
31	14.0	6.0	25.0	6.0	10.3	10.0	46.0	12.2	9.6	9.0
34	13.6	1.8	15.2	17.3	15.0	49.0	12.2	41.0	8.4	10.4
40	N/A	3.5	10.5	8.5	10.0	47.0	17.0	37.0	14.6	18.0

Notes: [a] serum E2 concentration (pg/ml). [b] N/A data was not collected due to technical issues or mouse well-being.

Supplement Figure Legends

Supplement Fig. 1. Effects of E2 on ERa expression in Huh 7 cells. (A) Huh 7 cells were transfected with pcDNA5 vector or ERa, followed by treatment with E2 (10nM) or vehicle EtOH (0.1%) for 24h. ERa mRNA levels were quantified with real-time PCR. The mRNA levels from cells treated with EtOH were defined as 1. The Ct values were given on the top of the bars. (B) ERa protein levels were detected with Western blot. The protein levels from cells treated with EtOH were defined as 1. GAPDH was used as an internal standard to normalize mRNA and protein levels of ERa. * $p<0.05$ in Student's t-test.

Supplement Fig. 2. Hepatic expression of ERa in mice before, during and after pregnancy. Liver tissues were collected from 5 non-pregnant mice, 5 pregnant mice at gestation day 18 and 5 mice 7 days post-delivery. (A) ERa mRNA levels were quantified with real-time PCR. The mRNA level from non-pregnant mice was defined as 1. The Ct values were given on the top of the bars. (B) ERa protein levels were detected with Western blot. GAPDH was used as an internal standard to normalize mRNA and protein levels of ERa. No statistic differences were detected with one-way ANOVA analysis.

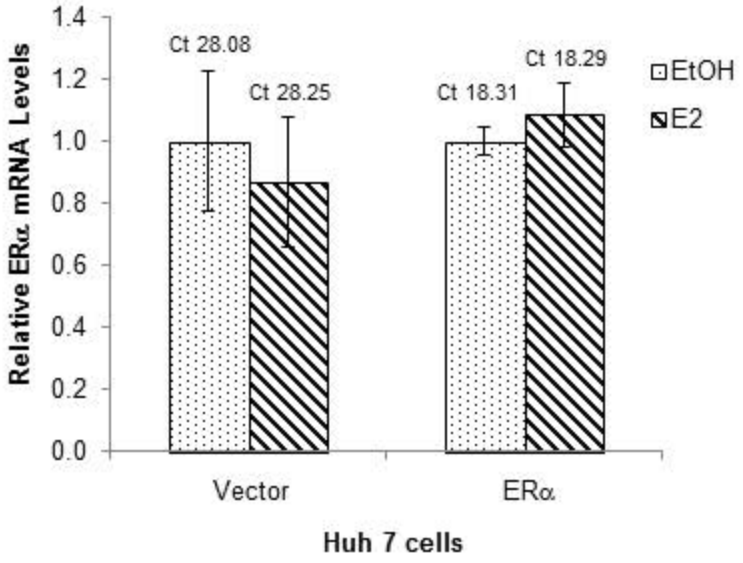
Supplement Fig. 3. Mutations in ligand and DNA binding domain abolished ERα's functions in its classical transactivation pathway. Huh 7 cells were transfected with pTK-2xERE and either ERα, ERα-LBD-Mut or ERα-DBD-Mut, followed by treatment with E2 (10nM) or vehicle EtOH (0.1%). Luciferase activities were detected by the dual luciferase assays. Data were presented as means ± SD of at least three repeated experiments. * $p<0.05$ in Student's t-test.

Supplement Fig. 4. FXR and ERα proteins fused with eGFP fragment remained functional. (A) Huh 7 cells were transfected with human BSEP promoter reporter phBSEP(-2.6kb), ERα and either FXR or eGFPn-FXR. (B) Huh 7 cells were transfected with phBSEP(-2.6kb), FXR and either ERα or ERα-eGFPc. The transfected cells were treated with CDCA (10μM), CDCA and E2 (10nM) or DMSO, followed by detection of luciferase activities by dual luciferase assay. Data were presented as means ± SD of at least three repeated experiments. * $p<0.05$ in Student's test.

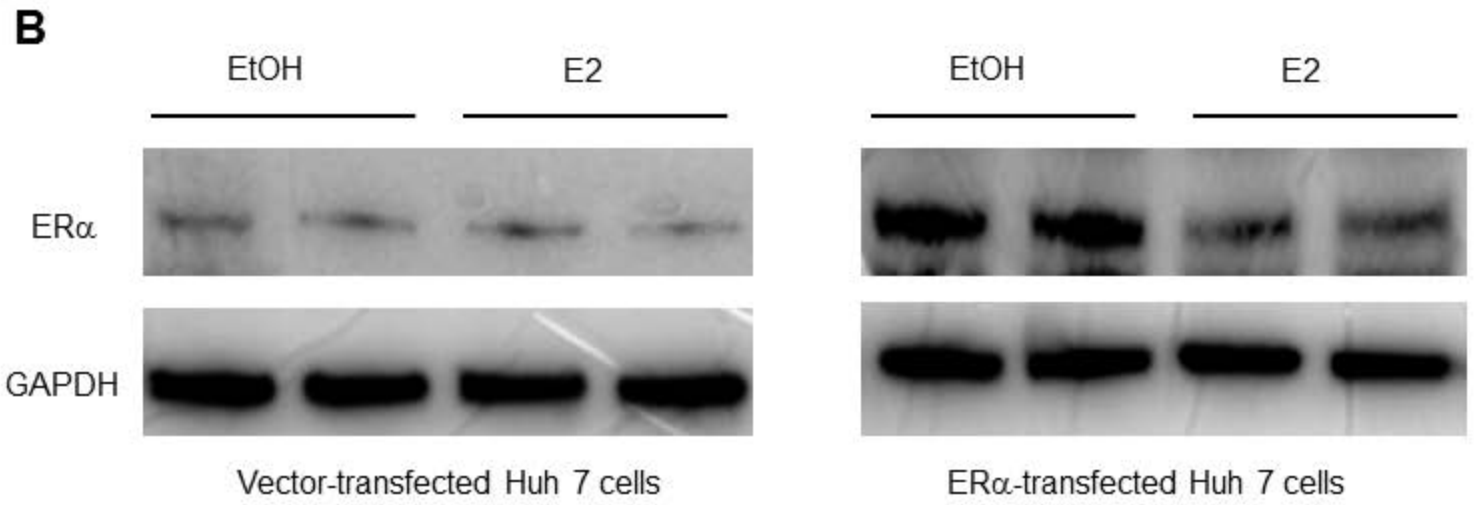
Supplement Fig. 5. The effects of pregnancy on hepatic expression of SHP in mice. Liver tissues were collected from 5 non-pregnant mice, 5 pregnant mice at gestation day 18 and 5 mice 7 days post-delivery. (A) SHP mRNA levels were quantified with real-time PCR. The mRNA level from non-pregnant mice was defined as 1. (B) SHP protein levels were detected with Western blot. GAPDH was used as an internal standard to normalize mRNA and protein levels of SHP. One-way ANOVA was applied to analyze data, followed by Tukey post-hoc test for multiple comparisons. ** $p<0.01$.

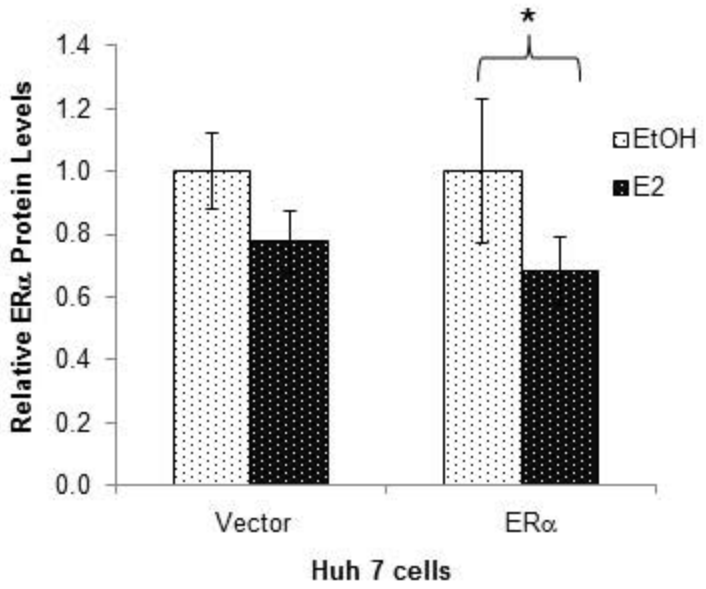
Supplement Fig. 1

A



Supplement Fig. 1





Supplement Fig. 2

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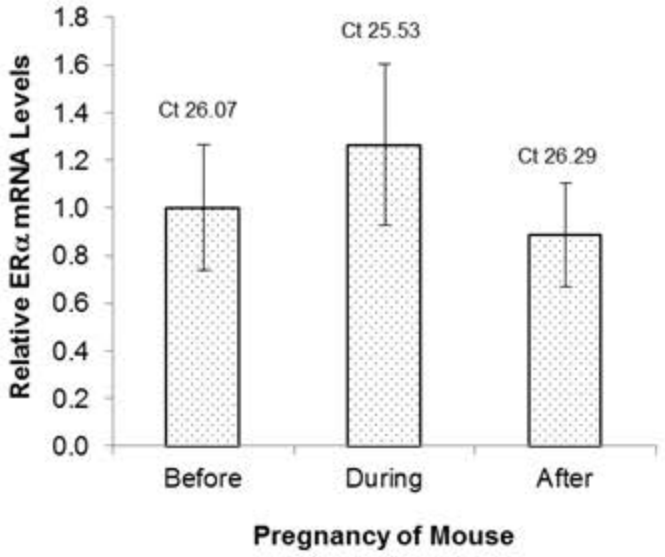

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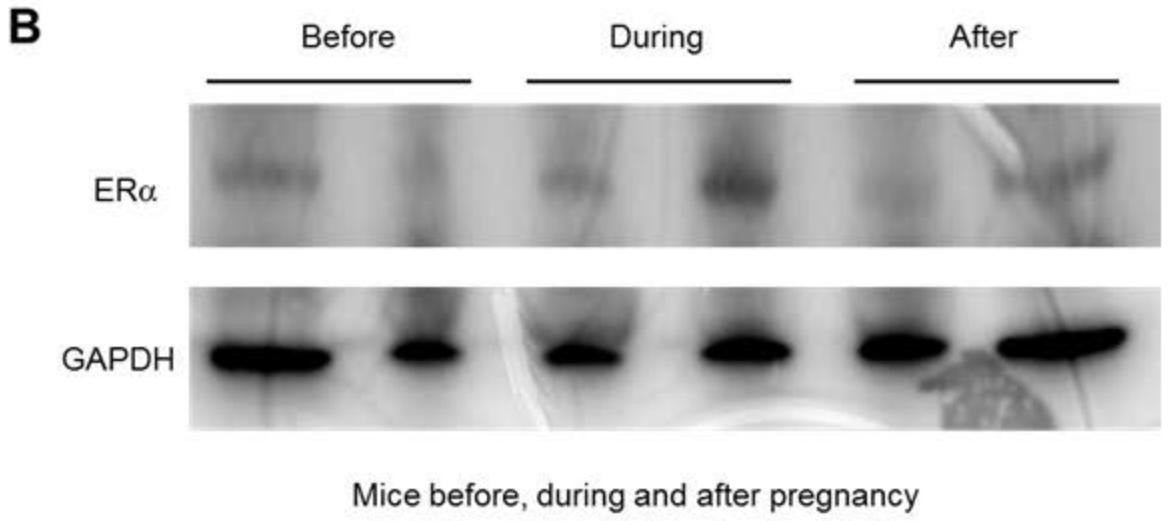

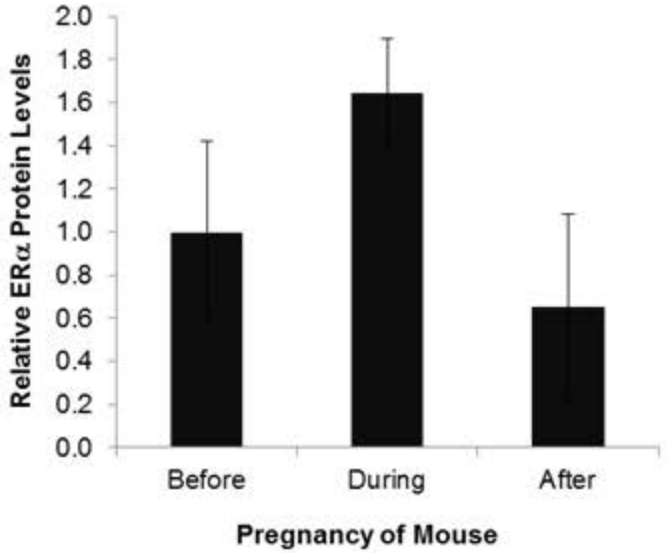

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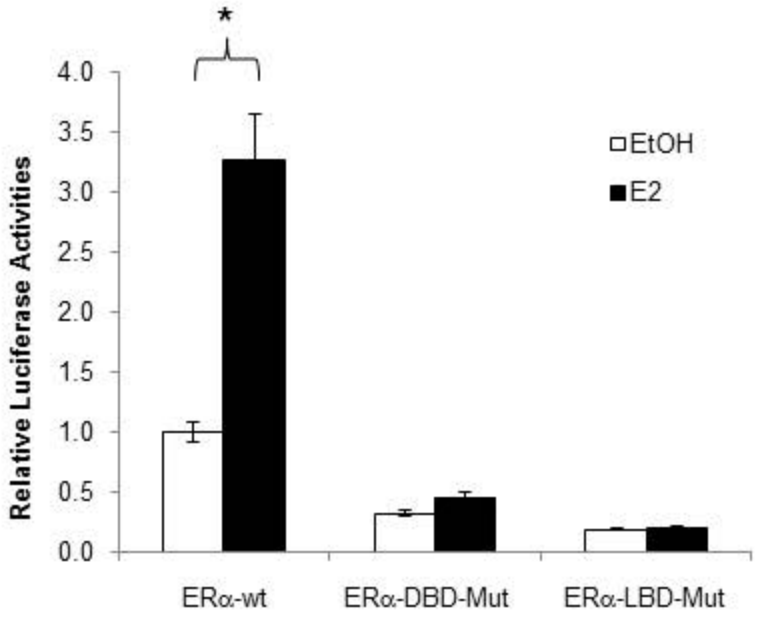

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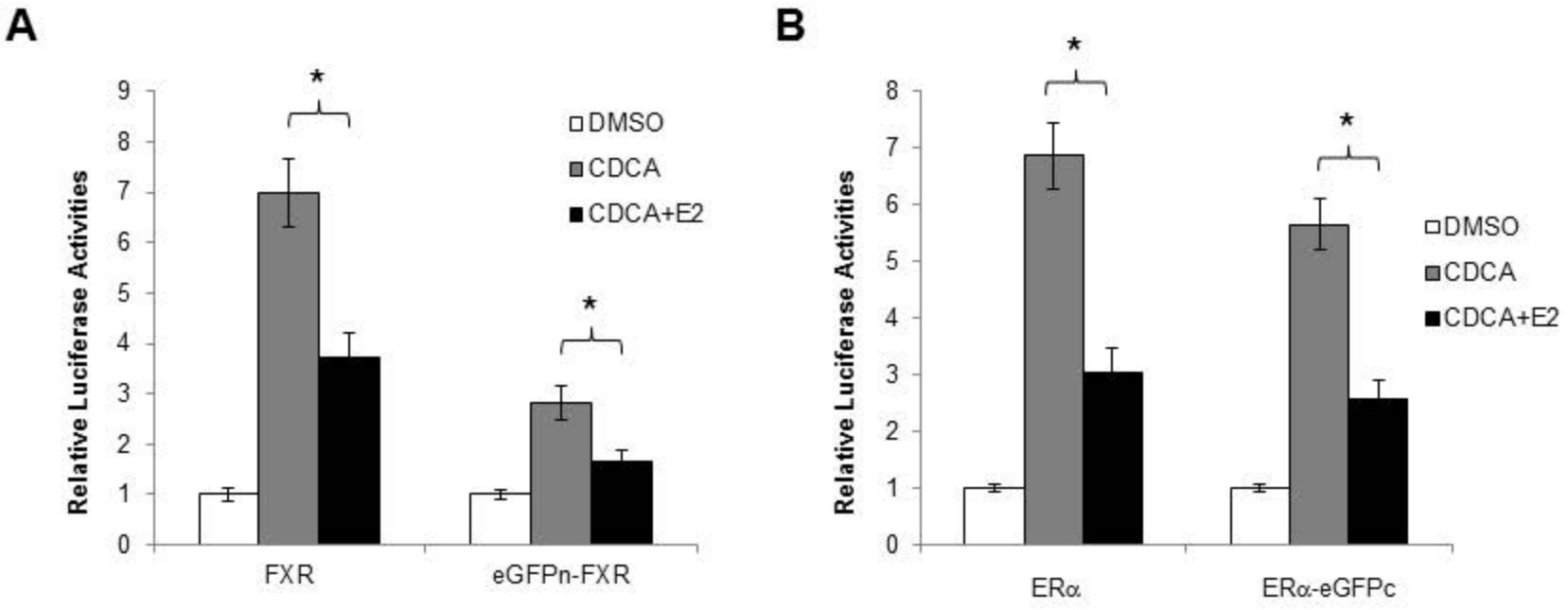

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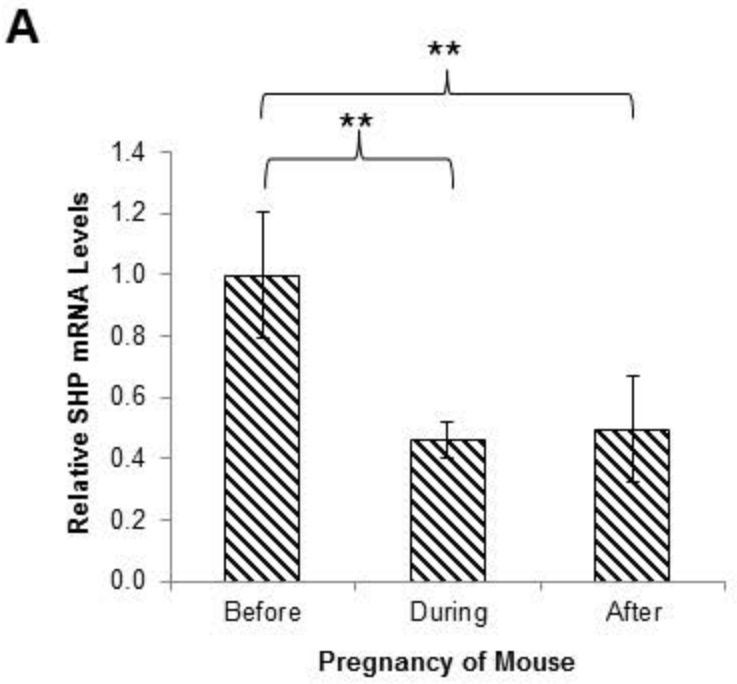

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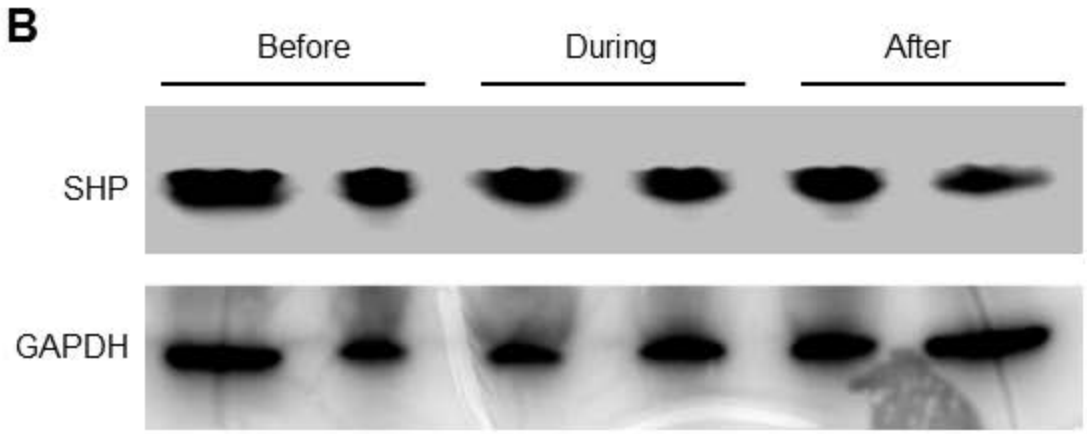

Mice before, during and after pregnancy