Effect of estradiol on LTD in ERα (+/–), ERβKO and ERβ (+/–) In ERα hetero knockout (+/-) mice, estradiol-induced change in LTD was not observed (Fig S1). The EPSP (excitatory postsynaptic potential) amplitude at 60 min was approx. 80 % in both the presence and absence of 10 nM estradiol. No effect of estradiol in ERα (+/-) mice is probably due to decreased expression level of ERα to approximately 50 % of that in wild mice as revealed by western blot analysis (Fig. S2). This result in ERα (+/-) mice was very different from the case of ERα homo knockout (–/–) mice where estradiol suppressed LTD by elevating EPSP from 78% to 90% (Fig. 5). On the other hand, ERβ hetero knockout (+/-) mice showed nearly the same enhancement of estradiol-induced LTD as that in wild type mice (Fig S1). Although ERβ (+/-) mice is expected to have reduced expression of ERβ, application of 10 nM estradiol did not change LTD (Fig S1).

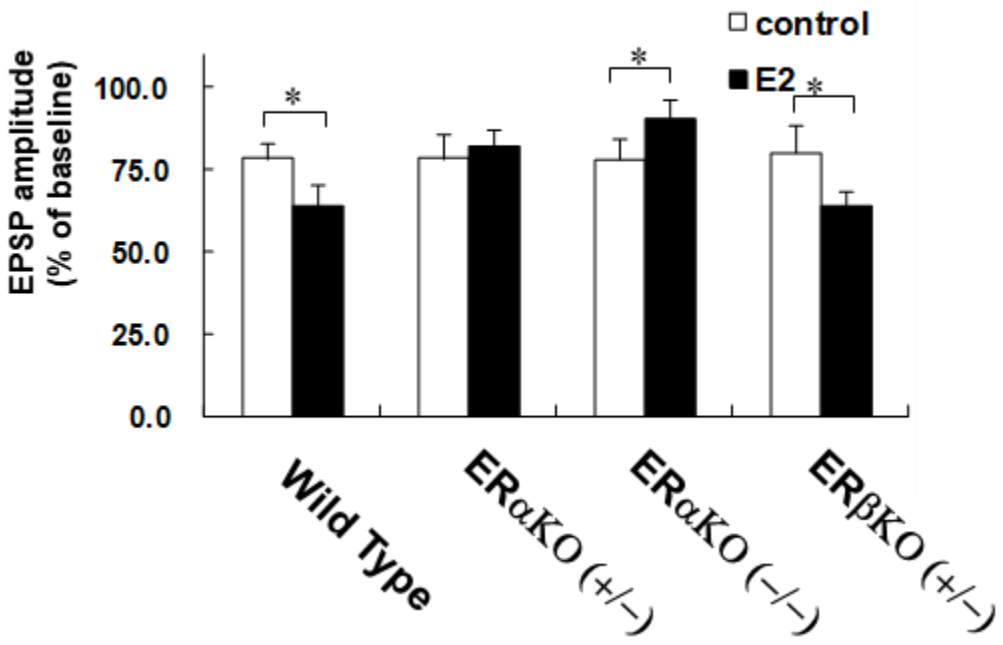

Figure S1 Modulation of LTD by 10 nM estradiol in ER hetero knockdown mice. From left to right, wild type, ERαKO (+/-), ERαKO (-/-) and ERβKO (+/-) mice without estradiol (E2) (Cont) or with 10 nM E2 (E2). Vertical axis indicates amplitude of EPSP at 60 min after NMDA stimulation. Here, 100% refers to the EPSP value at t=−40 min prior to NMDA stimulation, irrespective of the test condition. LTD was induced by 30 μM NMDA perfusion at time t=0-3 min. *p<0.05.

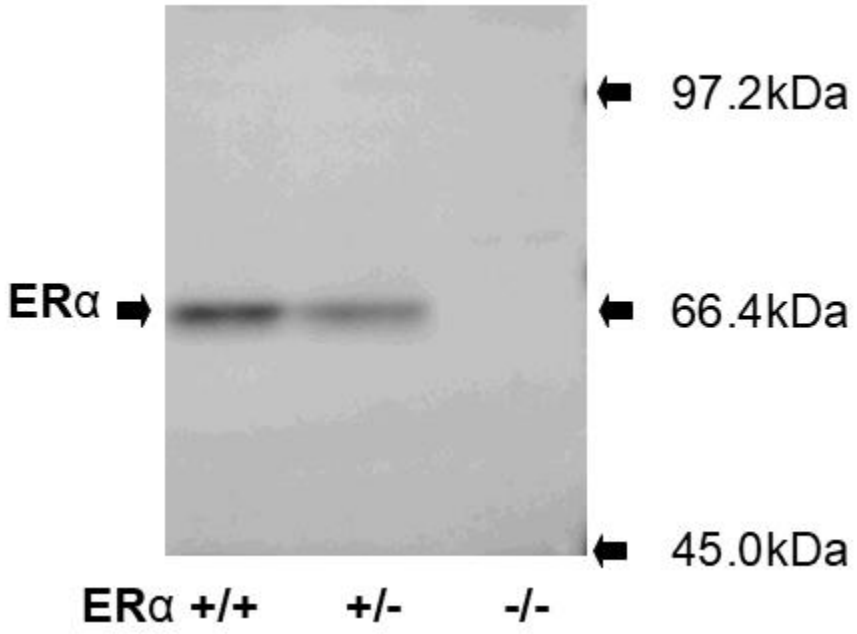

Figure S2 Staining of ERα with RC-19 in the hippocampus of wild type, ERαKO (+/-) and ERαKO (-/-) mice from left to right. The amount of protein applied to each lane was 20 μg.

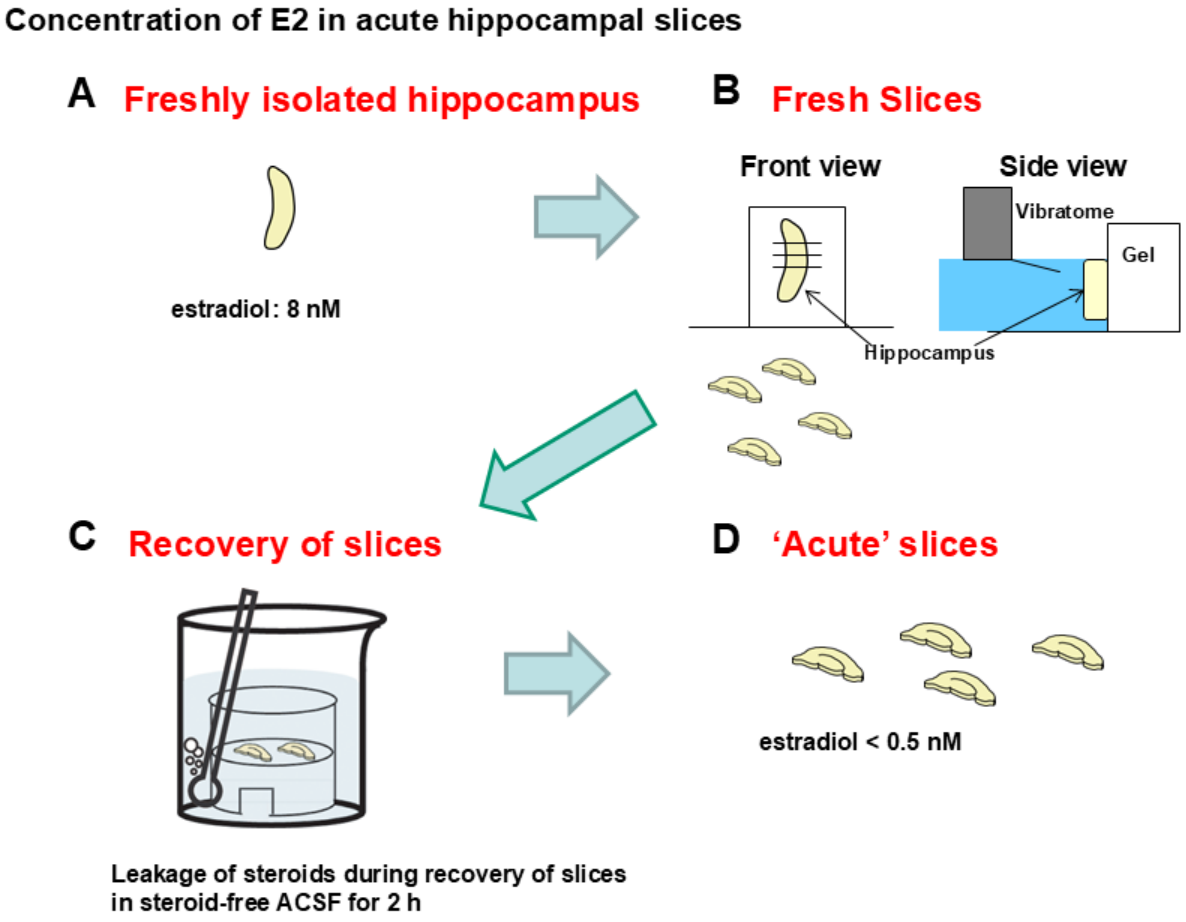

Figure S3 Model illustration for concentration of estradiol in isolated acute hippocampal slices determined by mass-spectrometric analysis. (A) Freshly isolated hippocampus contains approx. 8 nM estradiol. (B) Preparation of hippocampal slices. (C) For preparation of widely used 'acute' slices, we incubated slices for recovery in steroid-free ACSF for 2 h, resulting in leakage of steroids. (D) The acute hippocampal slices contain less than 0.5 nM estradiol.