SUPPLEMENTARY FIGURES

Supplementary Figure 1 Expression of the dominant negative mutant ERα, ERα∆250-260, or the Gαi binding domain blocking peptide, ERα peptide 251-260, does not alter nuclear ER function in endothelial cells. BAEC were cotransfected with 3ERE-Luc luciferase reporter plasmid and either sham plasmid (control), plasmid encoding the dominant-negative mutant receptor ERα∆250-260 that prevents non-nuclear ER activation of eNOS, or plasmid that expresses a peptide consisting of amino acids 251-260 of the receptor (ERα peptide 251-260) that blocks ERα-Gαi interaction. Reporter activity was then determined 24h later in response to 48h treatment with vehicle (Basal) or 10^{-8}M E_2. Values are mean±SEM, n=4, * p<0.05 vs. basal.

Supplementary Figure 2 EDC does not activate ER-mediated gene transcription in vivo. (**A**) Optical imaging is shown of the bioluminescence emitted from a representative ERE-luc reporter mouse treated with vehicle (control), E_2 or EDC via osmotid minipump for 24 or 48h. (**B**) Summary data, values are mean±SEM, n=6, (control is set at 1) * p<0.05 vs. Control.

Supplementary Figure 3 Tissue distribution of EDC. Mice received ^{3}H-labeled EDC as a single IP injection of 6 µg of E_2 equivalents, and tissues were harvested 2, 24 and 72h later. EDC abundance was quantified by liquid scintillation counting, and is expressed as % injected dose/gram of tissue. Values are means for two mice at each timepoint.

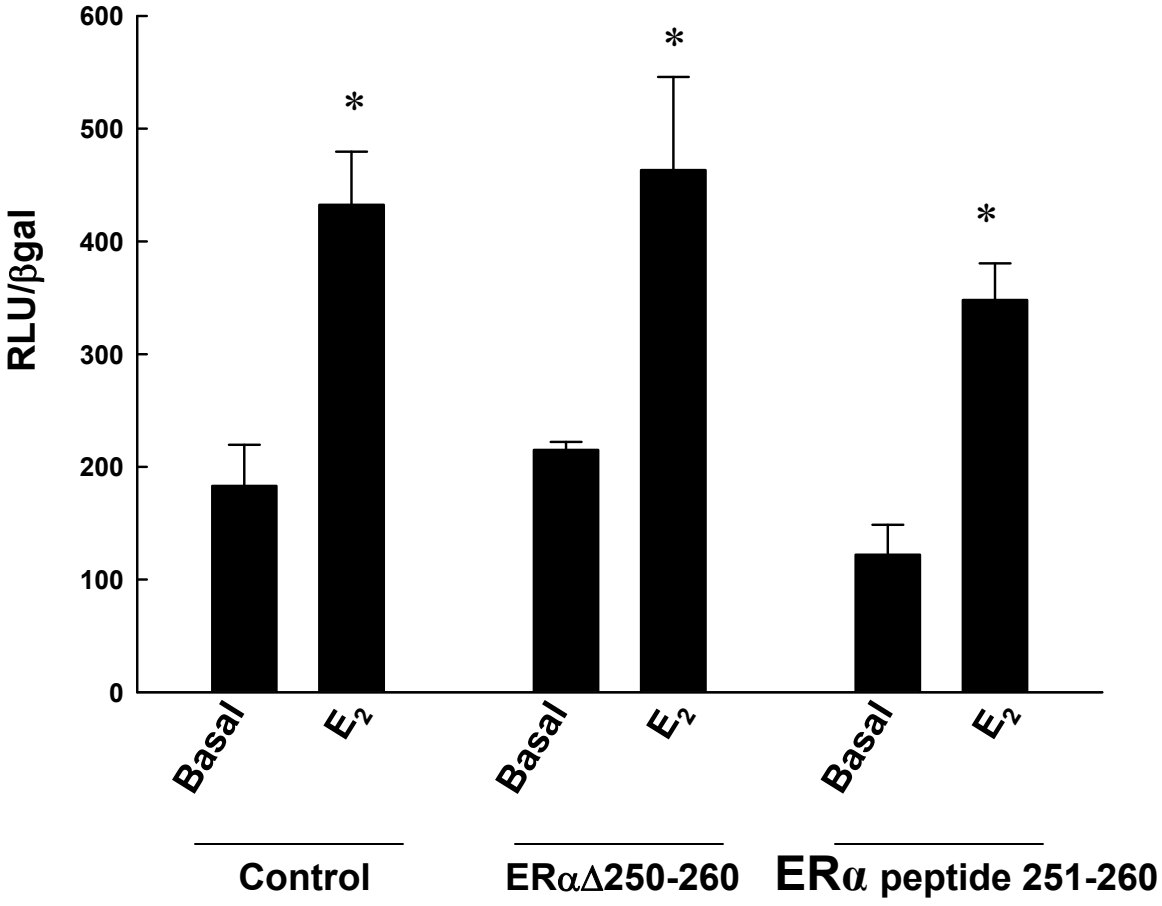
Supplementary Figure 4 Carotid artery reendothelialization mediated by nongenomic ER activation requires ERα, and residues within the A/B domain of ERα are dispensible for this process. (**A**) The intimal surfaces of Evans blue-stained arteries from dendrimer (Dend) or EDC-treated *ERα*$^{+/+}$ versus *ERα*$^{-/-}$ littermates are shown (upper panel). Area

of denudation was quantified and expressed in arbitrary units (lower panel). In **A**, values are mean±SEM, n=6-8, *p<0.05 vs dendrimer. Similar experiments were performed in vehicle versus E_2 or dendrimer versus EDC-treated wild-type mice (**B**) and in comparably-treated *ERα-Neo* knockout mice expressing a truncated 55 kDa ERα (**C**). In **B** and **C**, values are mean±SEM, n=7-12, * p<0.05 vs. vehicle or dendrimer.

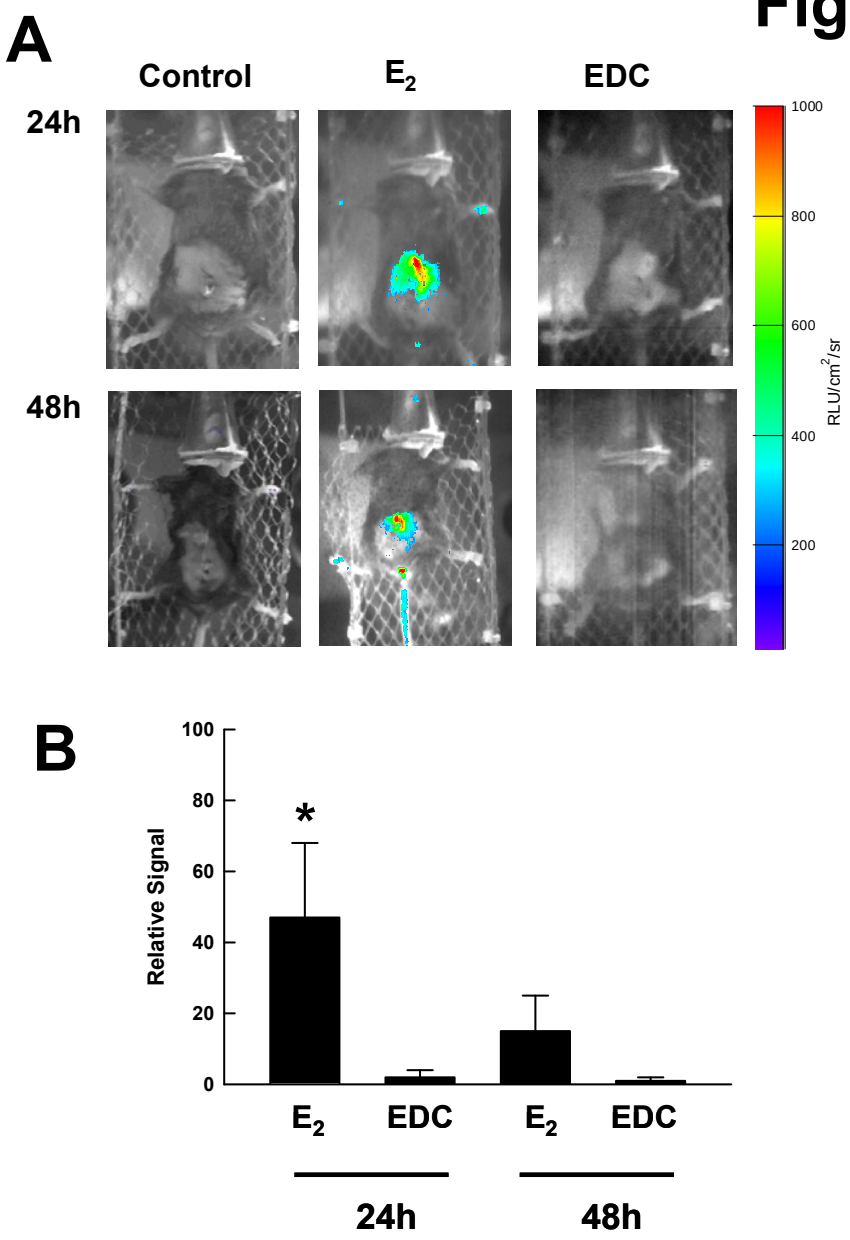
Supplementary Figure 5 Non-nuclear ER signaling does not promote endometrial cell growth. (**A**) ERK phosphorylation: Ishikawa cells were treated for 0-15 min with DMSO (vehicle control),10^{-8}M E_2, dendrimer, or EDC (10^{-8}M E_2 equivalents), and ERK phosphorylation was evaluated by immunoblot analyses for phospho-ERK (pERK) and total ERK (tERK). Summary data are shown in **B**, in which values are mean±SEM, n=3, *p<0.05 vs. time 0. (**C**) Cell growth: Cells were treated daily with the reagents in **A**, and cell number was quantified every 24h. 1% FBS was used as a positive control. Values are mean±SEM for cell number expressed as % of plated cells, n=3, * p<0.05 vs. DMSO.

Supplementary Figure 6 Pertussis toxin attenuates the carotid vascular conductance response to acetylcholine (Ach). Ovariectomized, E_2-treated mice were administered the B-oligomer of pertussis toxin (open symbol) or intact pertussis toxin (PTX, closed symbol), and 3 d later were instrumented for measurements of carotid artery vascular conductance. Responses to varying doses of Ach were tested in random order. Values are mean±SEM, n=5, *p<0.05 vs B-oligomer.

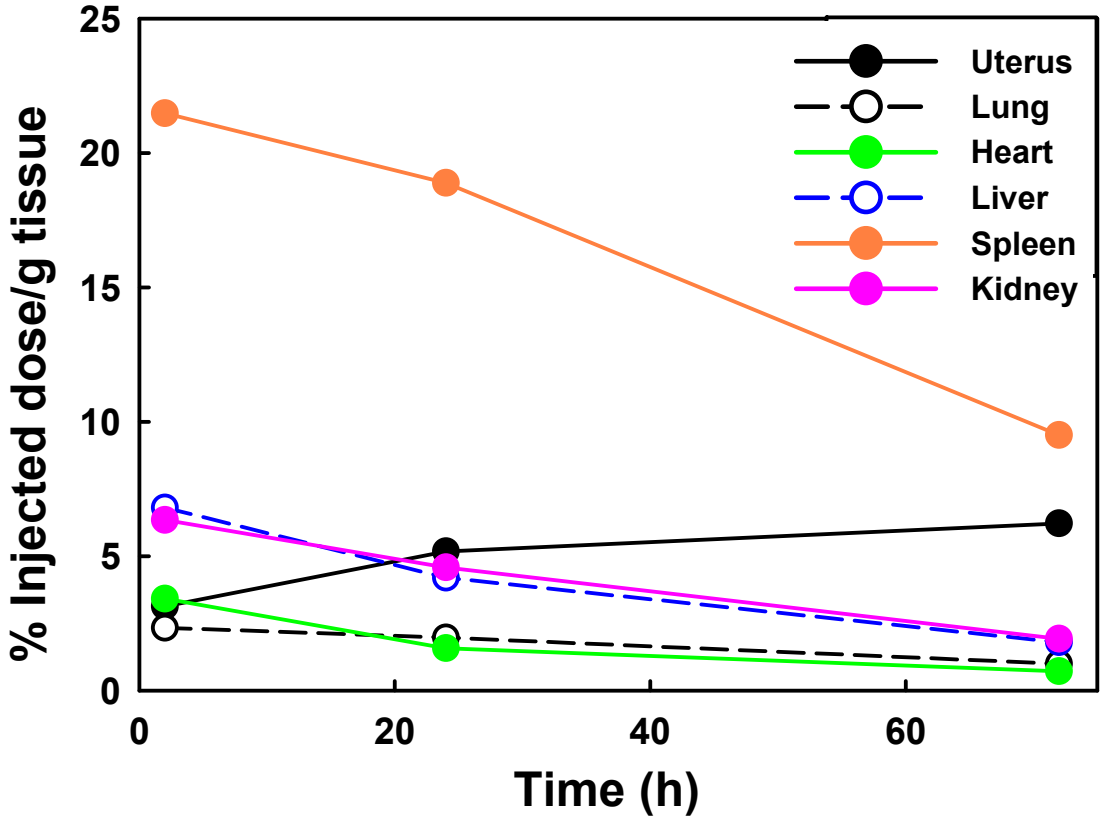
Supplementary Fig. 1



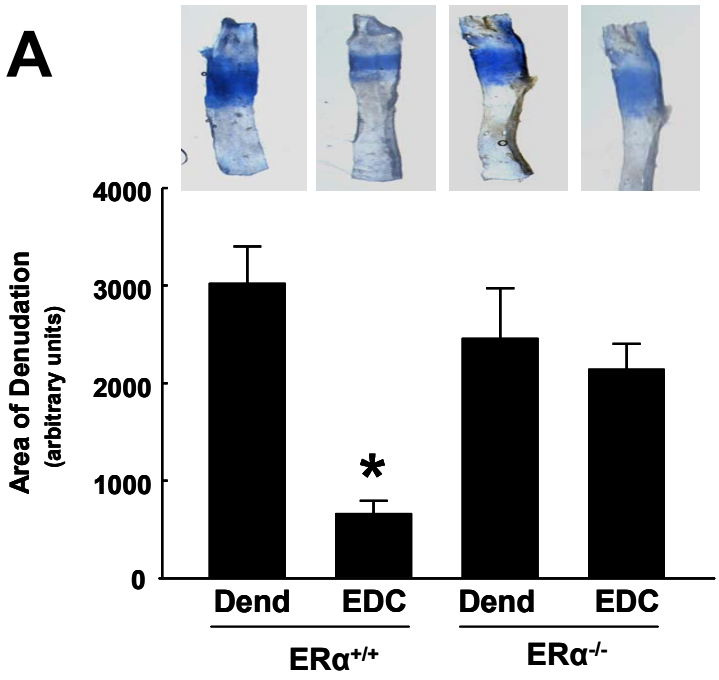
Supplementary Fig. 2

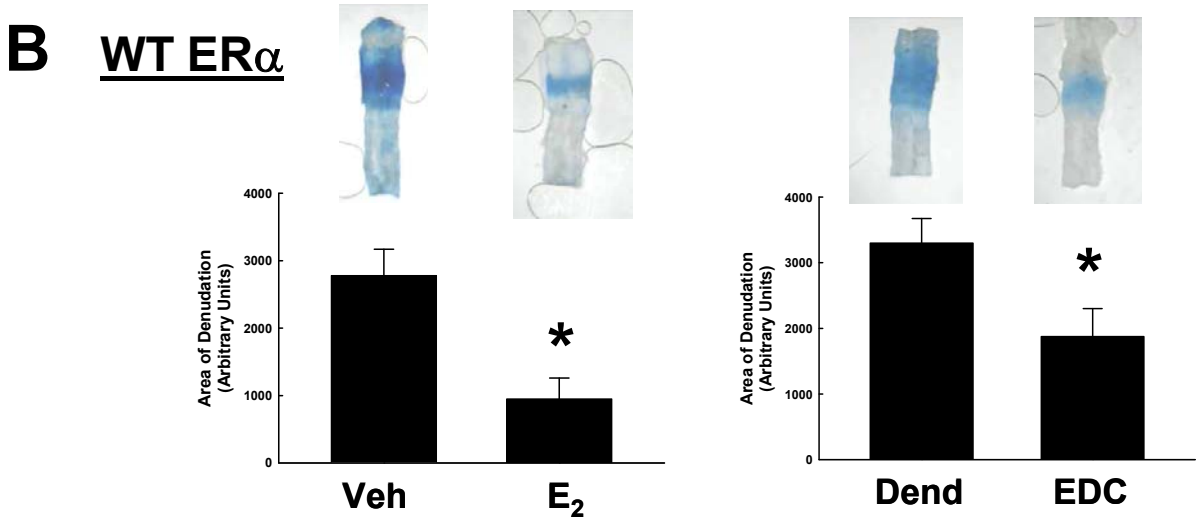


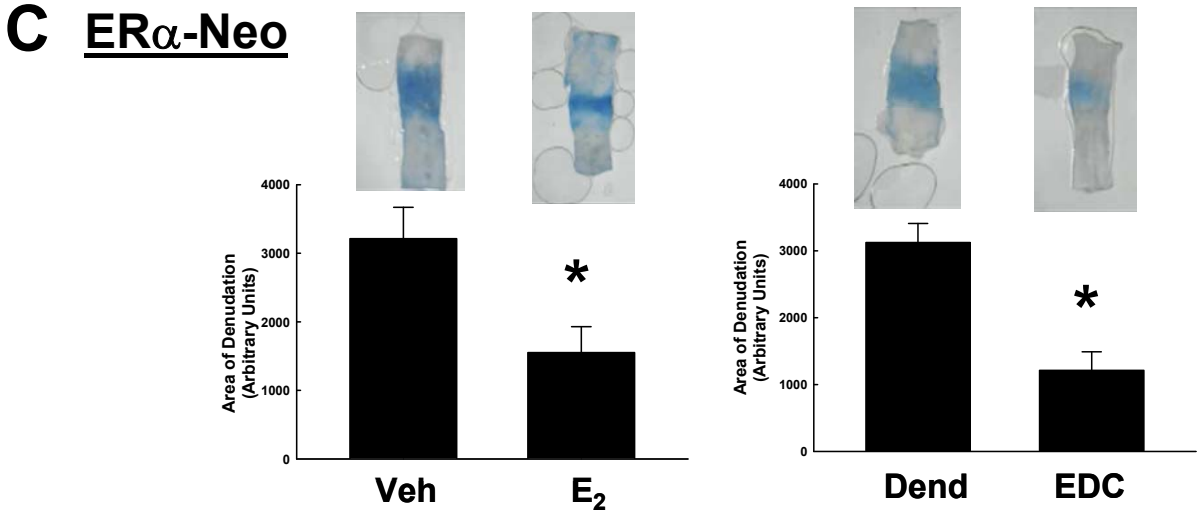
Supplementary Fig. 3



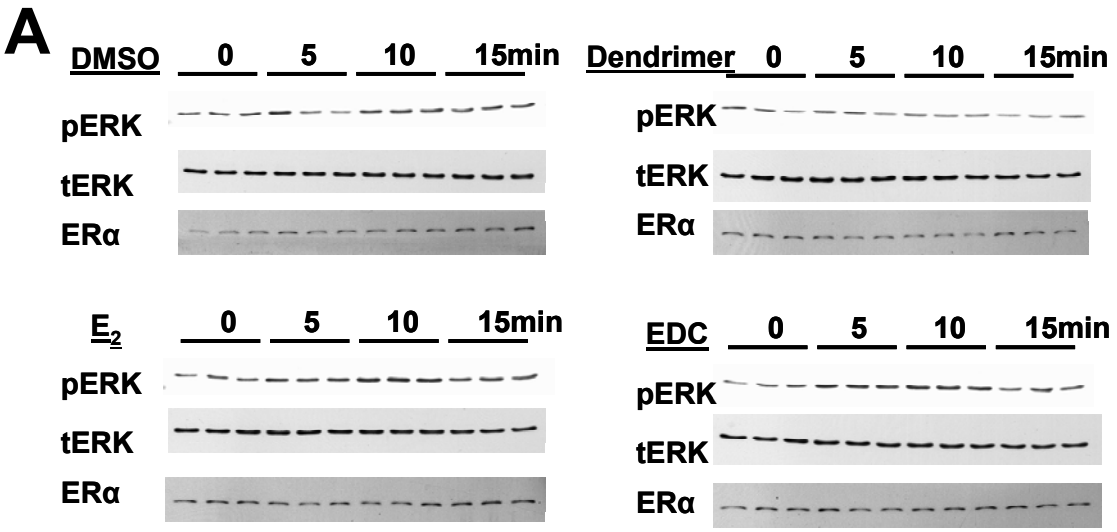
Supplementary Fig. 4

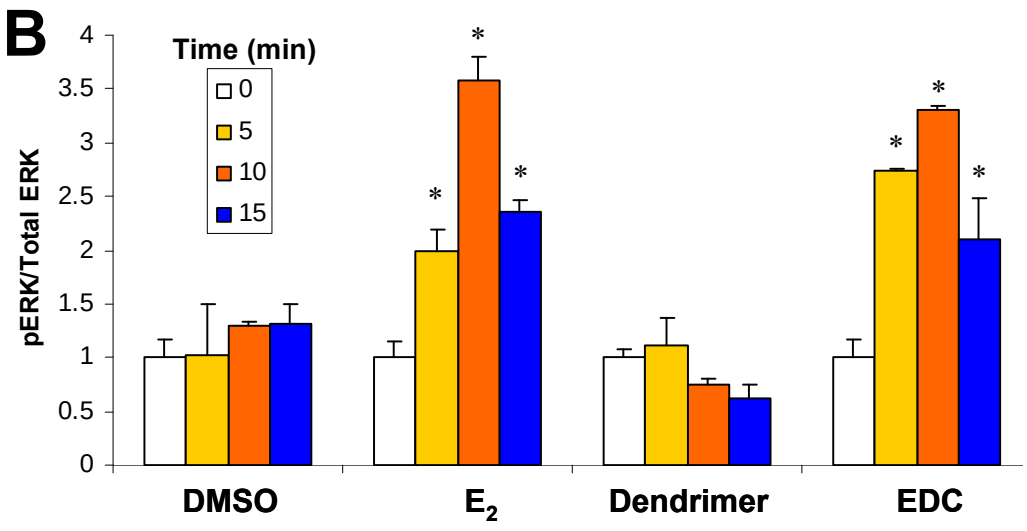


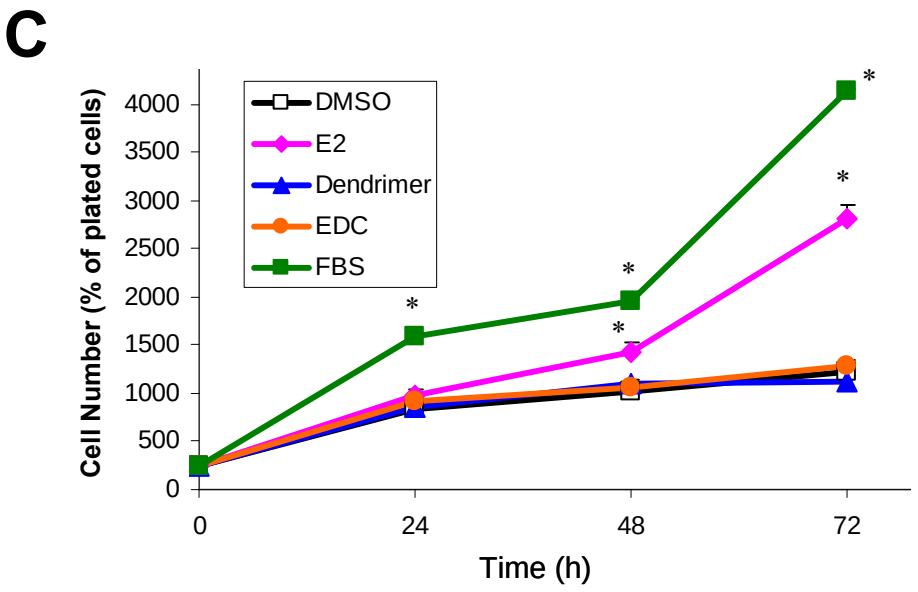




Supplementary Fig. 5







Supplementary Fig. 6