Supplementary Table 1

Human Forward and Reverse Primers for conventional and Real-time quantitative PCR

mRNA	Primer/Probe Sequence	Amplicon length	NCBI Accession #
Conventional PCR			
ESR1	F: 5'-TGCCTTGTTGGATGCTGAGC-3' R: 5'-AGCATCTCCAGCAGCAGGTCATAG-3'	Exon7 inclusion 681 bp (ERα66/46) Exon7 exclusion 497 bp (ERΔ7)	NM_001122742
	F: 5'-TTCTATAGCATAAGAAGACAG-3' R: 5'-GCCTGAAGCATAGTCATTGC-3'	Exon1 inclusion 830 bp (ERα66/Δ7) Exon1 exclusion 308 bp (ERα46)	
RT-qPCR			
ERα66/46	F: 5'-ATCCTCTCCCACATCAGGCA-3' R: 5'-ATGAAGTAGAGCCCGCAGTG-3'	199 bp	NM_000125
ERΔ7	F: 5'-AATGTGTAGAGGGCATGGTGG-3' R: 5'-CCATGCCTTTGTTACAGAATTAAGC-3'	137 bp	XM_006715374
hnRNPG	F: 5'-TGGAAGAGGAGGAAGTGGAGG-3' R: 5'-GGTCCCCTGGAAGAACTCAT-3'	105 bp	NM_002139
RPLP0	F: 5'-GTGCTGATGGGCAAGAACAC-3' R: 5'-TGGTGAACACAAAGCCCACA-3'	118 bp	NM_001002
Human ERα exon 2 end labeled probe	Probe: 5'-CCAGGGTGGCAGAGAAAGAT-3'	Used as probe for 830 bp and 308 bp ERα66/46 RTPCR southern Supplementary Figure 2C.	NM_001122742

Supplementary Figure Legends:

Supplementary Figure 1: Immunolocalization of ERα and ERβ in the pregnant human uterus. A representative uterine section from pregnant non-laboring women (36 weeks gestation) shows (A) ERα immunostaining in both the endometrium (arrows) and myometrium (arrow heads) while (B) ERβ staining is limited to the endometrium.

Supplementary Figure 2: Structure of the human ERα isoforms produced from the ESR1 gene. (A) The mRNA coding sequences (CDS) of 3 *ERα* isoforms found in the myometrium are shown. *ERα66* mRNA is the full-length isoform containing exons 1-8 and translation is initiated from the first AUG in exon 1. *ERα46* mRNA is an exon 1 skip isoform (translation starts at an AUG in exon 2), while *ERΔ7* is an exon 7 skip variant of *ERα* causing translation to be truncated due to a frameshift and a premature stop codon in exon 8 (denoted by *). (B) The full length ERα (ERα66, 595 a.a.) protein structure showing the functional domains: NTD- amino terminal domain, DBD- DNA binding domain, D- hinge region, LBD- ligand binding domain and F domain is located towards the C-terminal end. The two activation function domains AF-1 (hormone-independent) and AF-2 (hormone-dependent) regulate the transcriptional activity of ERα66. ERα46 contains amino acids corresponding to residues numbered 174-595 in ERα66 and lacks the AF-1 domain. ERΔ7 (a 51 KD protein) has a truncated LBD (E*) with variant-specific 10 amino acid sequences (456-466) and lacks the AF-2 domain. (C) RT-PCR analysis of preterm non-laboring (PTNL) and term non-laboring (TNL) human myometrium utilizing primers that spanned exon E1 and exon 2 produced two bands, a 830 bp representing the *ERα66/ERΔ7* and a 308 bp lacked exon 1 and was consistent with

ERα46. An RT-PCR southern was performed using a p32 end labeled oligonucleotide probe with sequence homology to exon 2. Both bands hybridized to the probe as shown in autoradiographs. Sequencing confirmed southern analysis. (D) RT-PCR analysis of preterm human myometrium utilizing primers that spanned exon 4 through exon 8 of the *ESR1* mRNA reveal the presence of two bands, a 681 bp representing the *ERα66/ERα46* isoforms and a 497 bp product which when sequenced was shown to lack exon 7 corresponding to *ERΔ7*.

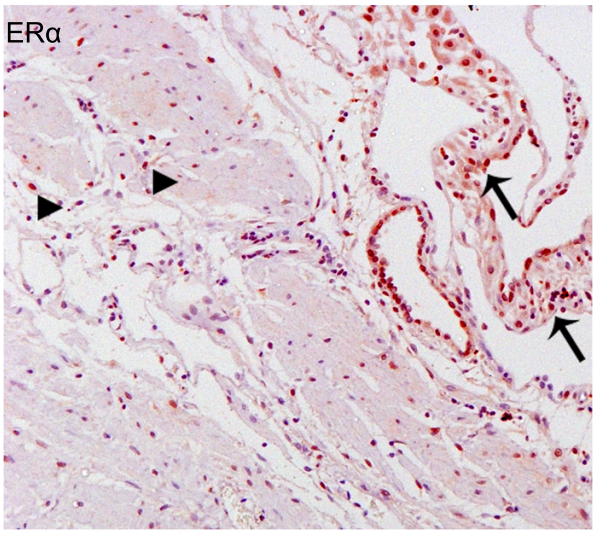
Supplementary Figure 3: siRNA knockdown of ERα66 and ERα46 depletes the levels of GJA1 in vitro. HTERT-HM cells were transfected with ERα siRNA targeting the exon 7 of ESR1 mRNA which targets *ERα66* and *ERα46* for knockdown but not *ERΔ7*. Cytosolic and nuclear fractions were isolated and analyzed for ERα66, ERα46 and GJA1 proteins. siRNA knockdown of ERα66 and ERα46 led to the downregulation of GJA1. A representative blot from three different experiments is shown.

Supplementary Figure 4: Doxycycline dose dependence of ERΔ7 expression and nuclear localization of ERΔ7. (A) Doxycycline inducible expression of functional ERΔ7. ERΔ7 was transiently over-expressed in hTERT-HM^{Tet3G} cells with a doxycycline (Dox) inducible recombinant lentivirus and treated with varying concentrations of Dox (0 to 1000 ng/ml) for 48 hr. Increased expression of ERΔ7 (nuclear fraction) and down-regulation of GJA1 (cytoplasmic fraction) in a Dox responsive/ERΔ7 concentration dependent manner was observed. GAPDH and NCOA3 are cytoplasmic and nuclear loading controls. (B) Nuclear localization of ERΔ7 in HEK293T cells. Cells were co-

transfected with recombinant ERΔ7 plasmid (pLVX-TREG-mCherry-ERΔ7; 2µg) along with transactivator rtTA plasmid (pLVX-EF1a-Tet3G; 2µg) and cultured in the presence or absence of DOX (500 ng/ml) for 48hr. Cytosolic (Cyto) and nuclear (Nuc) fractions were isolated and analyzed for ERΔ7 expression. Western blot shows abundant nuclear localization of ERΔ7 in HEK293T cells. GAPDH and NCOA3 are cytoplasmic and nuclear loading controls. A representative blot from three different experiments is shown.

Supplementary Figure 1

A



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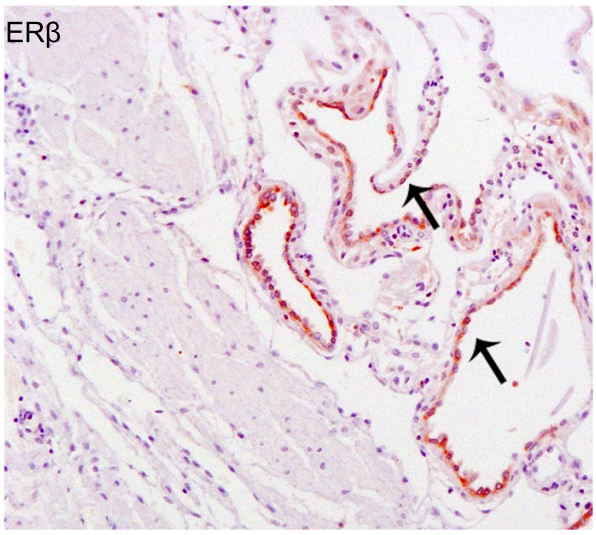
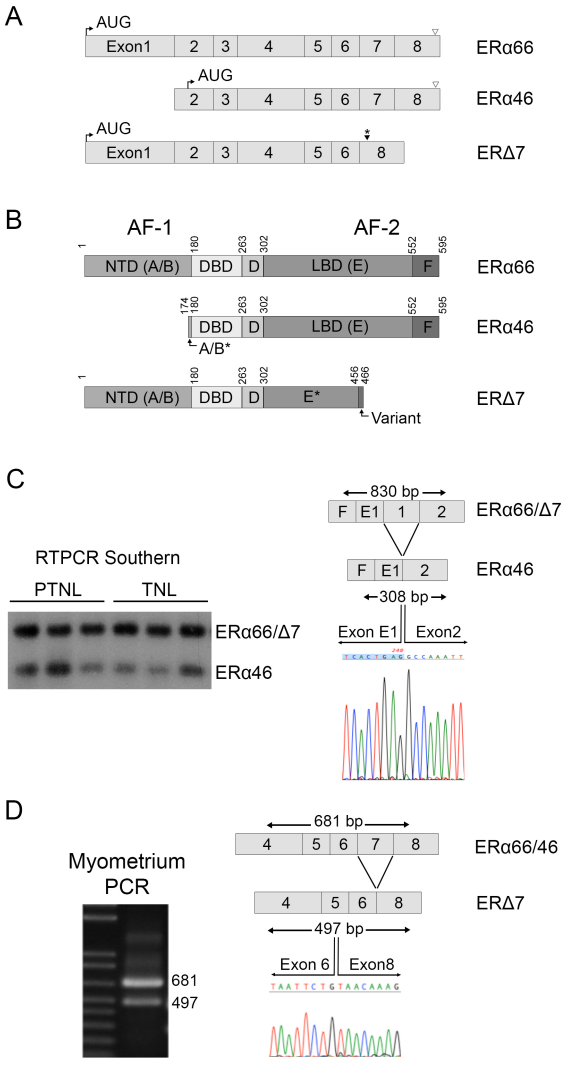
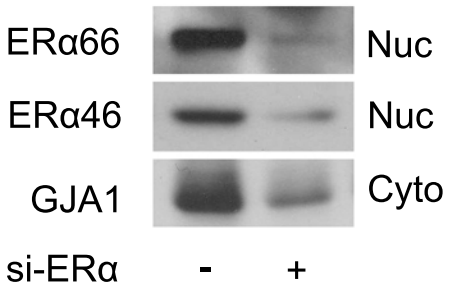


Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4