Supporting Information

Wong et al. 10.1073/pnas.0914501107

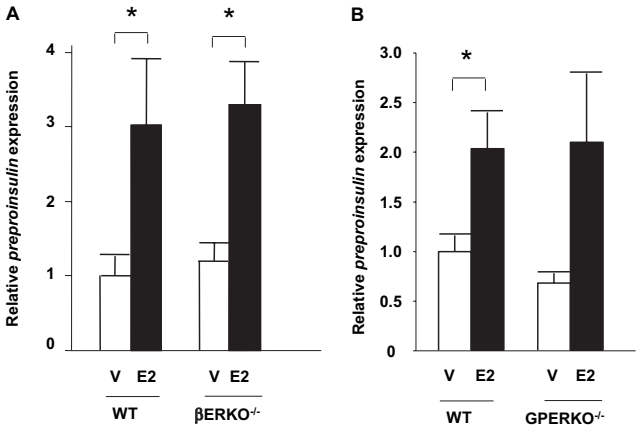

Fig. S1. Relative *preproinsulin* expression in βERKO$^{-/-}$ islets (*A*) and GPERKO$^{-/-}$ islets (*B*) after E2 (10^{-8} M) treatment in vitro (n = 3–12 mice/group). Results are expressed as mean ± SEM.

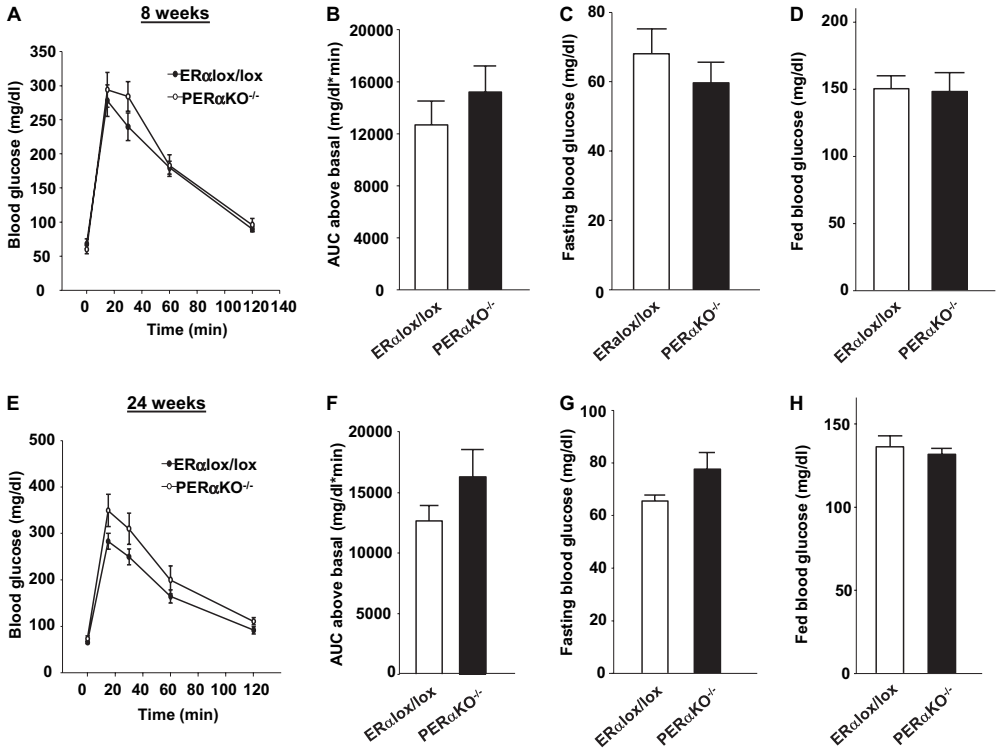

Fig. S2. Metabolic characterization of PERαKO$^{-/-}$ mice. (*A–D*) Glucose tolerance (*A*) and corresponding area under the curve (*B*), fasting blood glucose (*C*), and fed blood glucose (*D*) at 8 wk in PERαKO$^{-/-}$ and ERαlox/lox mice. (*E–H*) Glucose tolerance (*E*) and corresponding area under the curve (*F*), fasting blood glucose (*G*), and fed blood glucose (*H*) at 24 wk in PERαKO$^{-/-}$ and ERαlox/lox mice (n = 6–20/group). Results are expressed as mean ± SEM.

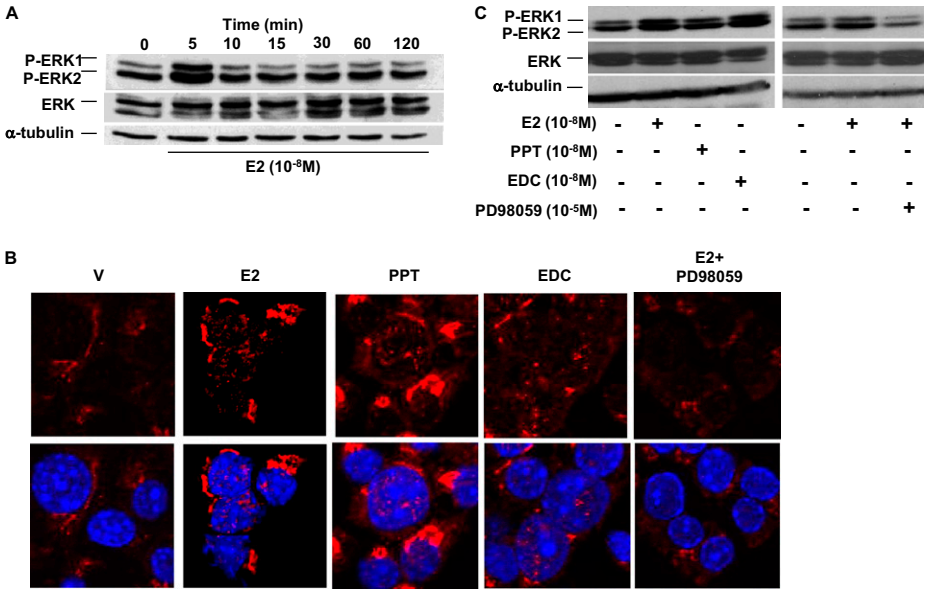

Fig. S3. ERα-mediated ERK1/2 phosphorylation and nuclear translocation. (*A* and *C*) MIN6 cells were treated with E2 (10^{-8} M) as indicated (*A*) or with E2 (10^{-8} M), EDC (10^{-8} M), PPT (10^{-8} M), or PD 98059 (10^{-5} M) (*C*) for 5 min. Phosphorylation of ERK1/2 was determined by Western blot analysis with an antibody against phosphorylated ERK1/2. (*B*) Localization of phosphorylated ERK1/2 in MIN6 cells after treatment with E2 (10^{-8} M), PPT (10^{-8} M), EDC (10^{-8} M), or E2 + PD98059 (10^{-5} M) for 5 min.

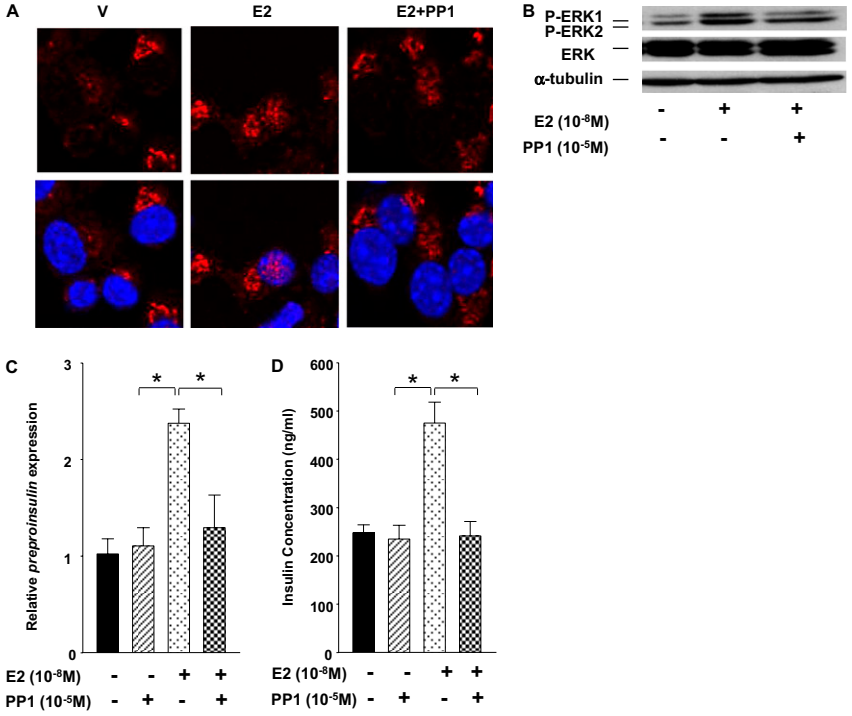

Fig. S4. Inhibition of E2-induced ERK1/2 phosphorylation by PP1. (*A*) Localization of phosphorylated ERK1/2 in MIN6 cells treated with either E2 (10^{-8} M) or E2 + PP1 (10^{-5} M). (*B*) Inhibition of E2-induced ERK1/2 phosphorylation by PP1 in MIN6 cells. Cells were treated with E2 (10^{-8} M) or PP1 (10^{-5} M) for 5 min before Western blot analysis. (*C* and *D*) Inhibition of E2-induced preproinsulin gene expression (*C*) and rise in insulin concentration (*D*) by PP1 (10^{-5} M) in WT islets. Mouse islets were treated with PP1 for 48 h before measurements of *preproinsulin* expression and insulin concentration. Results are expressed as mean ± SEM.

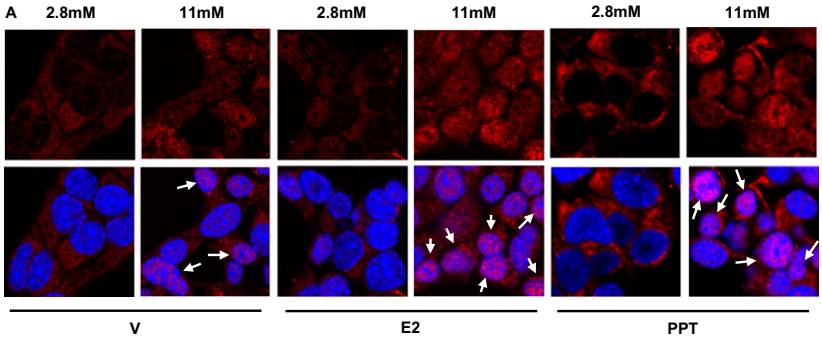

Fig. S5. ERα-induced NeuroD1 nuclear localization. NeuroD1 predominant nuclear localization in the presence of E2 (10^{-8} M) or PPT (10^{-8} M) at 2.8 mM and 11 mM glucose in MIN6 cells (n = 3–6/group). Representative images of four independent experiments are shown. Arrows indicate cells with predominant nuclear localization of NeuroD1.

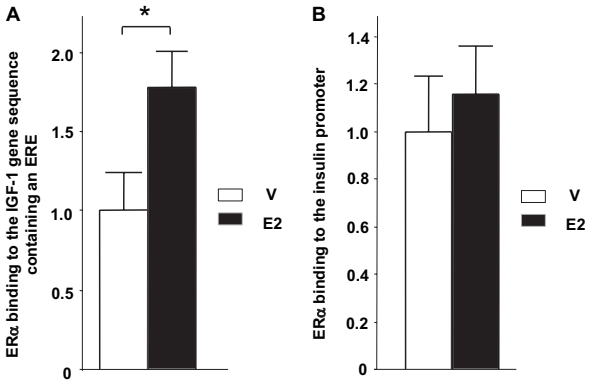

Fig. S6. E2-induced *preproinsulin* transcription does not involve direct binding of ERα to the insulin promoter. ChIP showing the recruitment of ERα to the IGF1 gene sequence containing an ERE (*A*) or insulin promoter (*B*) after 24-h treatment with E2 (10^{-8} M) or vehicle (V) at 11 mM glucose in MIN6 cells. After immunoprecipitation of ERα (HC20; Santa Cruz Biotechnology), real-time qPCR amplification of the IGF1 gene sequence containing an ERE and insulin promoter were performed using Sybr Green (BioRad), as described in *Materials and Methods*. Results were normalized to vehicle 11 mM glucose and represent mean ± SEM (n = 4–5/group). *$P < 0.05$.

Table S1. PCR primer sequences

Gene	Forward primer	Reverse primer	Ref.
mERα	5′-TTGCCCGATAACAATAACAT-3′	5′-GGCATTACCACTTCTCCTGGGAGTCT-3′	(1)
β-actin	5′-AGGTCATCACTATTGGCAAC-3′	5′-ACTCATCGTACTCCTGCTTG-3′	(2)
mInsulin promoter	5′-GAAGGTCTCACCTTCTGG-3′	5′-GGGGGTTACTGGATGCC-3′	(3)
mIGF-1	5′GCAGATAGAGCCTGCGCAATGGA-3′	5′-GGCTGCTGATTTTCCCCATCGCT-3′	(4)

1. Dupont S, et al. (2000) Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. *Development* 127: 4277–4291.
2. Hong YH, et al. (2005) Acetate and propionate short-chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology* 146:5092–5099.
3. Andrali SS, Qian Q, Ozcan S (2007) Glucose mediates the translocation of NeuroD1 by O-linked glycosylation. *J Biol Chem* 282:15589–15596.
4. Hewitt SC, Li Y, Li L, Korach KS (2010) Estrogen-mediated regulation of Igf1 transcription and uterine growth involves direct binding of estrogen receptor alpha to estrogen-responsive elements. *J Biol Chem* 285:2676–2685.