Supplementary Information for

SR9009 has REV-ERB-independent effects on cell proliferation and metabolism

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Figs. S1 to S5

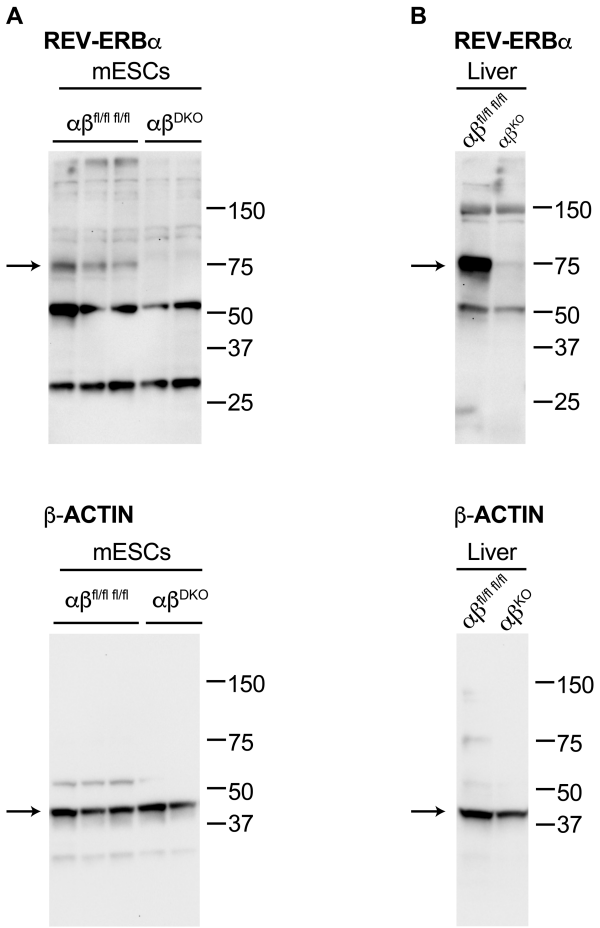

Fig. S1. Cre-mediated recombination of *Rev-erbα* allele does not lead to altered protein forms. Full immunoblots for REV-ERBα and β-ACTIN from **(*A*)** double floxed control (n=3) vs DKO (n=2) mouse ES cells (mESCs) and **(*B*)** double floxed control vs DKO liver. Arrows denote the full-length protein.

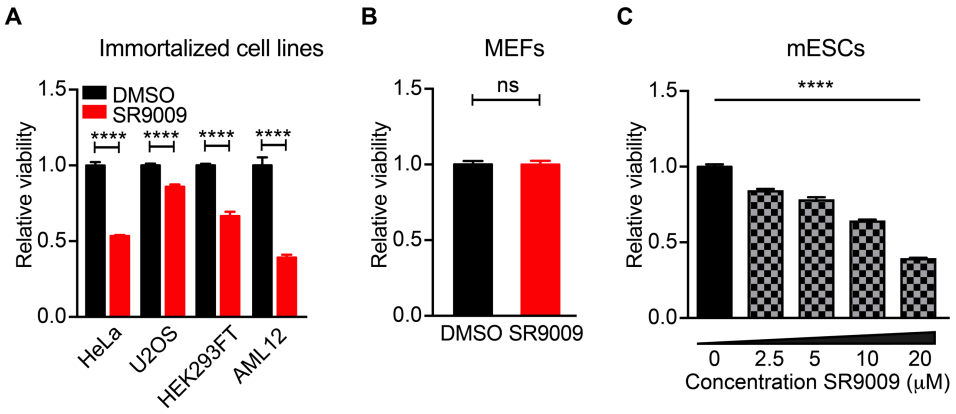

Fig. S2. SR9009 reduces cell viability of immortalized cell lines and mESCs. (*A*) Relative viability of 4 immortalized cell lines (n=8/condition) treated for 2 days with 10μM SR9009. **(*B*)** Relative viability of mouse embryonic fibroblasts (MEFs) (n=12/condition) treated for 2 days with 10μM SR9009, ****$P < 0.0001$, by two-sided Student's *t*-test. ns, not significant. **(*C*)** Dose-response curve showing relative viability of wild type mESCs treated with varying concentrations of SR9009 for 2 days (n=16/condition), ****$P < 0.0001$, by one-way ANOVA. Data are presented as mean ±SEM.

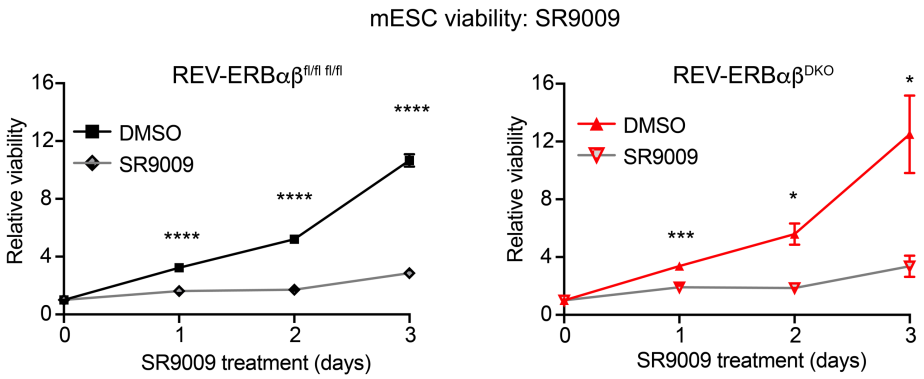

Fig. S3. Time course of reduced mESC viability by SR9009. Relative viability of double floxed control (n=5/condition) vs DKO (n=4/condition), treated with 10μM SR9009 for 3 days. **P* < 0.05, ****P* < 0.005, *****P* < 0.0005, by two-sided Student's *t*-test. Reduced mESC viability is apparent after 1 day. Data are presented as mean ±SEM.

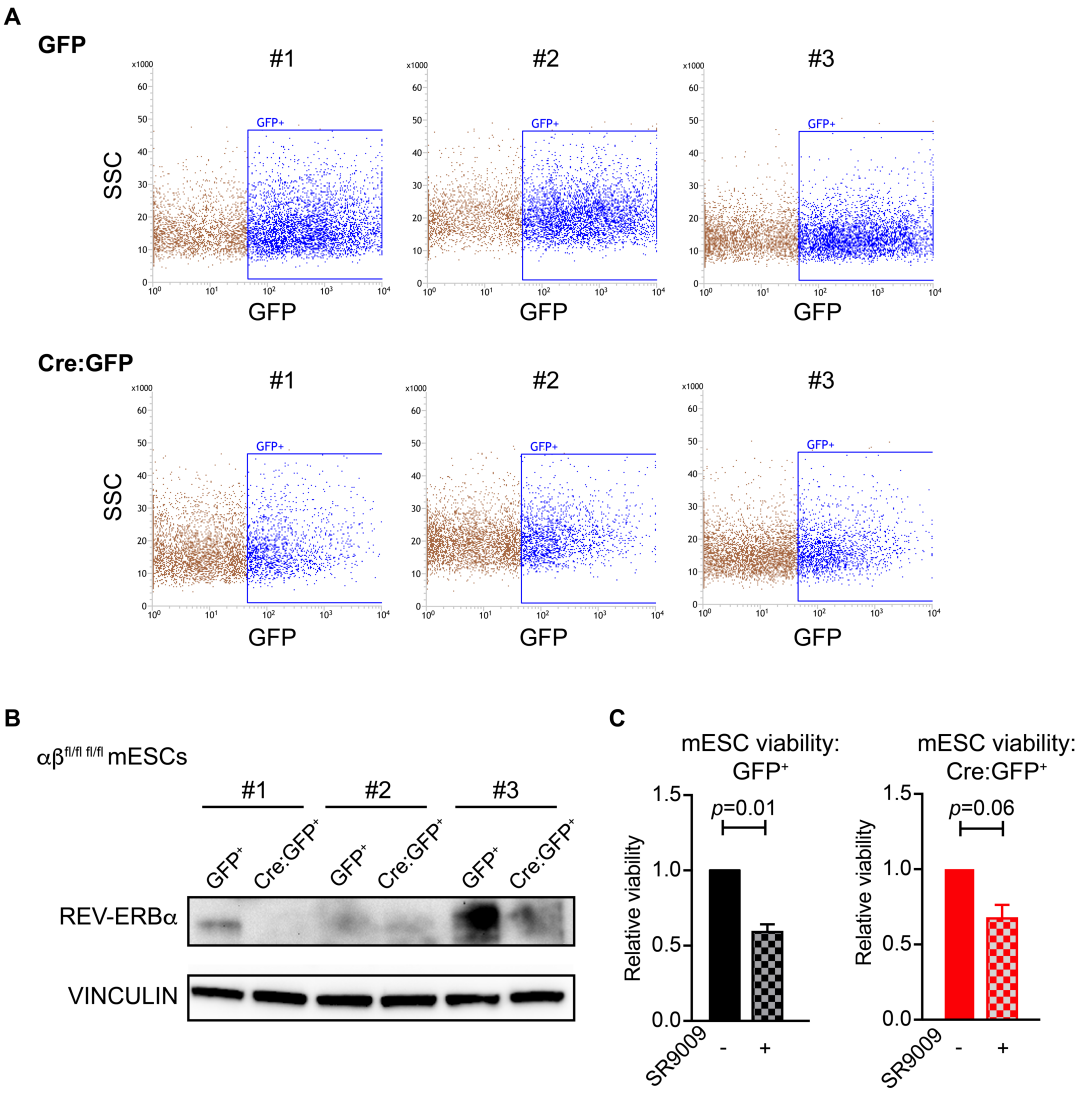

Fig. S4. Decreased mESC viability after SR9009 treatment is not due to long term culture of REV-ERB-depleted mESCs. **(*A*)** Gating of GFP$^+$-sorted REV-ERBα/β double floxed mESCs transfected with GFP (control) vs Cre:GFP overexpressing plasmids. **(*B*)** Immunoblot for REV-ERBα on the sorted cells from (*A*). **(*C*)** Relative viability of sorted control (GFP$^+$) (n=3 clones/condition) and DKO (Cre:GFP$^+$) (n=3/condition) mESCs, treated with 10μM SR9009 for 2 days. *P*-values are determined by two-sided Student's *t*-test. Data are presented as mean ±SEM.

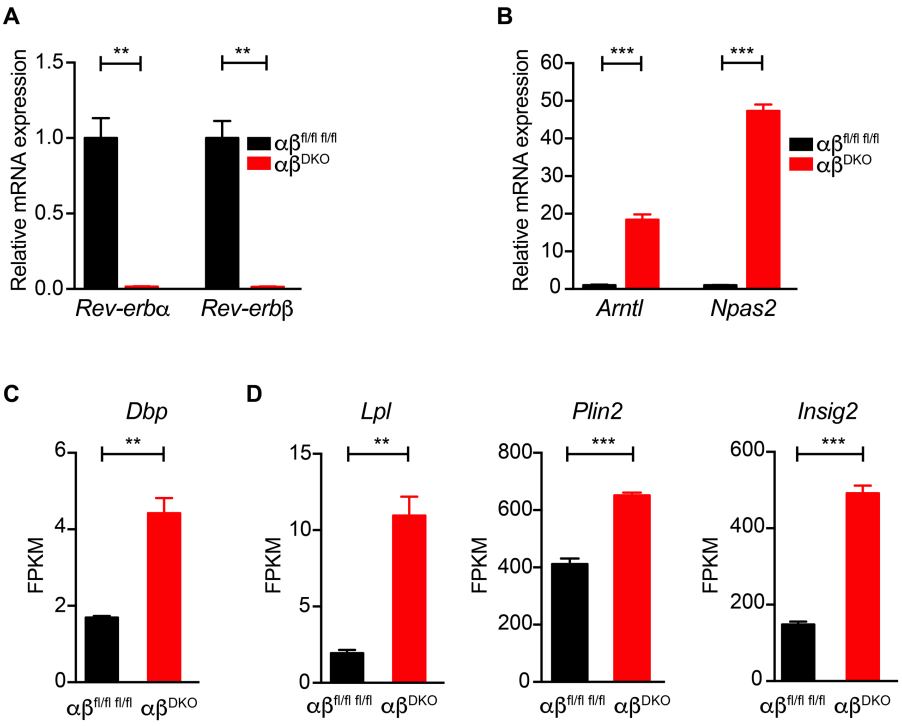

Fig. S5. Molecular characterization of REV-ERBα/β DKO hepatocytes culture ex vivo. (*A*) Relative *Rev-erbα/β* and **(*B*)** canonical REV-ERB clock target gene (*Arntl* and *Npas2*) mRNA expression in double floxed control vs DKO hepatocytes, measured by qRT-PCR (n=3 per genotype). **(*C*)** Fragments per kilobase of exon per million reads mapped (FPKM) for clock output (*Dbp)* and for **(*D*)** metabolic REV-ERB target genes (*Lpl*, *Plin2* and *Insig2*) genes in double floxed control vs DKO hepatocytes, measured by RNA-sequencing. ***P* < 0.005, ****P* < 0.0005, by two-sided Student's *t*-test. Data are presented as mean ±SEM.