

Supplemental Materials

Figure S1

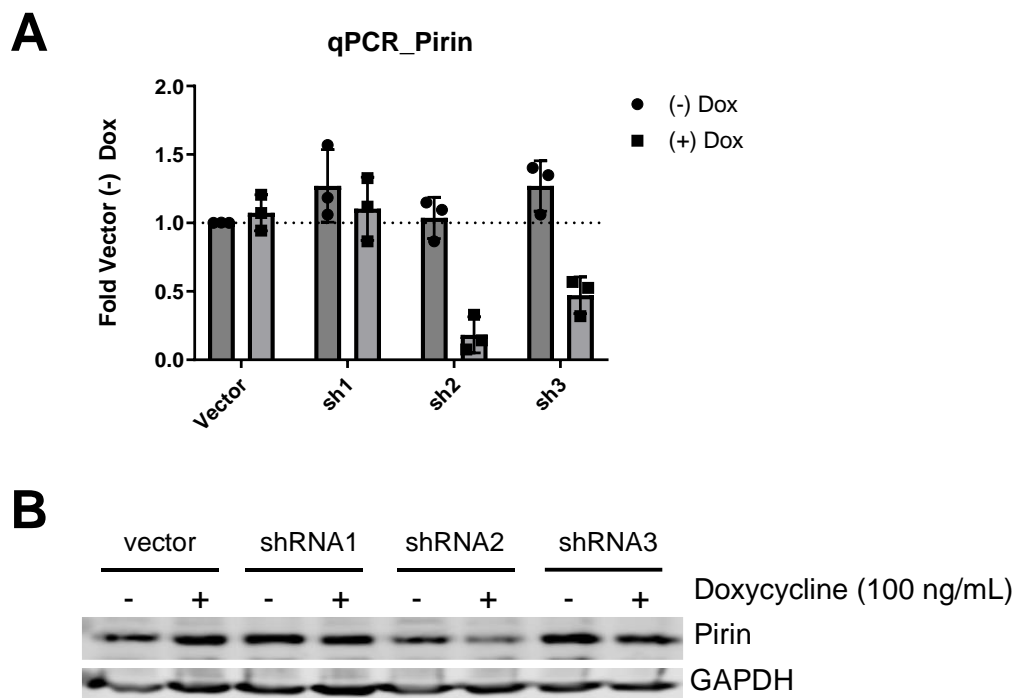


Figure S1. Generation of knockdown pirin cell lines. (A) qPCR and (B) Westernblot for pirin expression from 3T3 cells stably transfected with different silencing RNAs for Pirin.

Figure S2

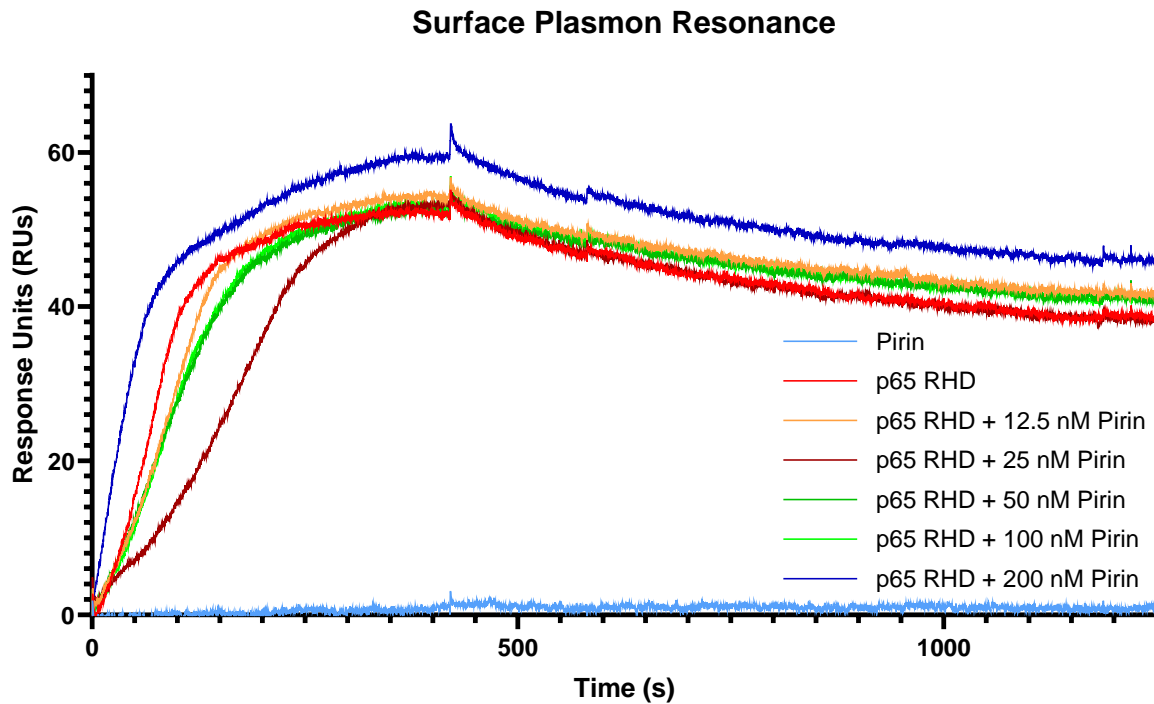


Figure S2. Surface Plasmon Resonance of p65RHR and pirin. Biotinylated IκB DNA was bound to Channel A on a Streptavidin Chip. Non-specific DNA was bound to Channel B. P65RHR alone (50 nM) or with different concentrations of Pirin (12.5 – 200 nM), or pirin alone, were run over the chip. Signal from channel B was subtracted from Channel A to correct for non-specific binding. Graph is representative of 3 different experiments.

Figure S3

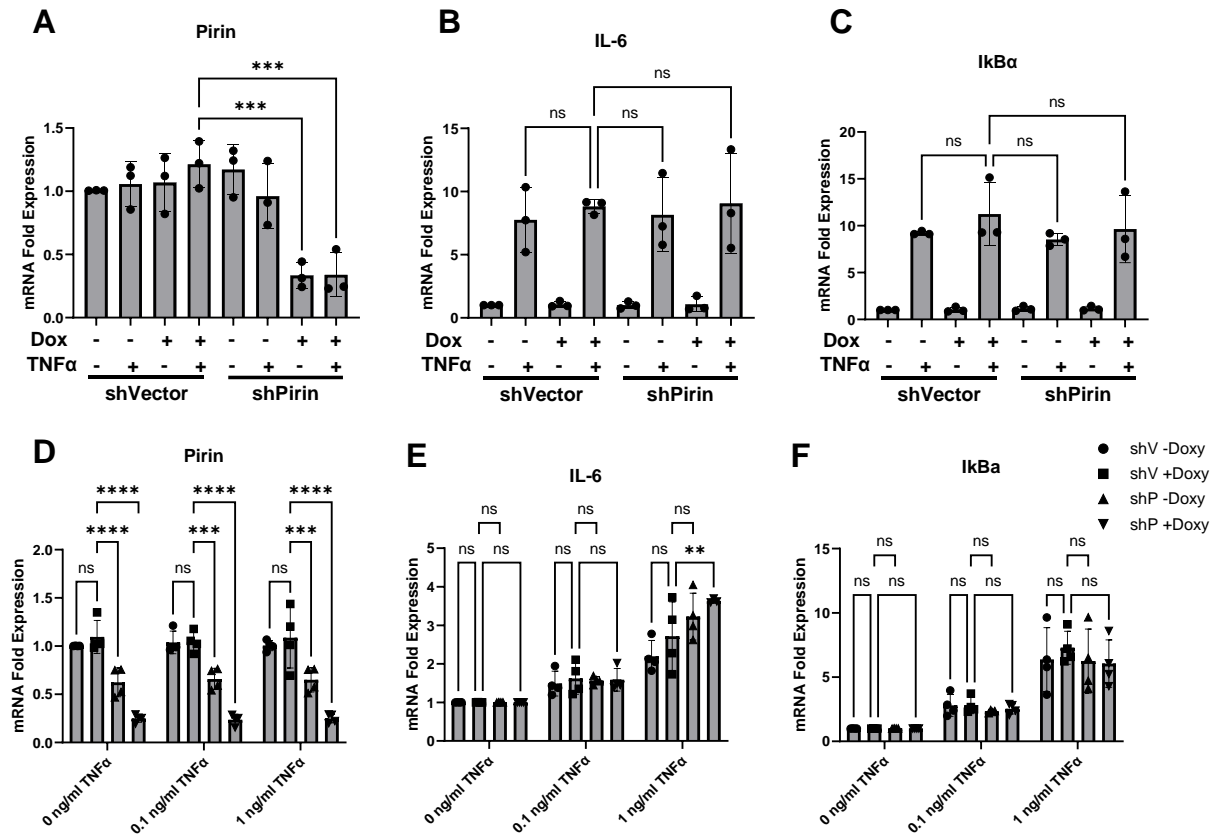


Figure S3. Expression of NF-κB response genes. (A) Full dataset shown for Figure 3. Cells were treated with 10 ng/ml TNFα. (B) shVector and shPirin cells treated with different concentrations of TNFα. Data are mean ± SD from 3 independent experiments each with 3 technical replicates. Statistical analysis was done by One-way ANOVA (A-C) or two-way ANOVA with Dunnetts (D-E) in GraphPad Prism 10 with significance values of * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

Figure S4

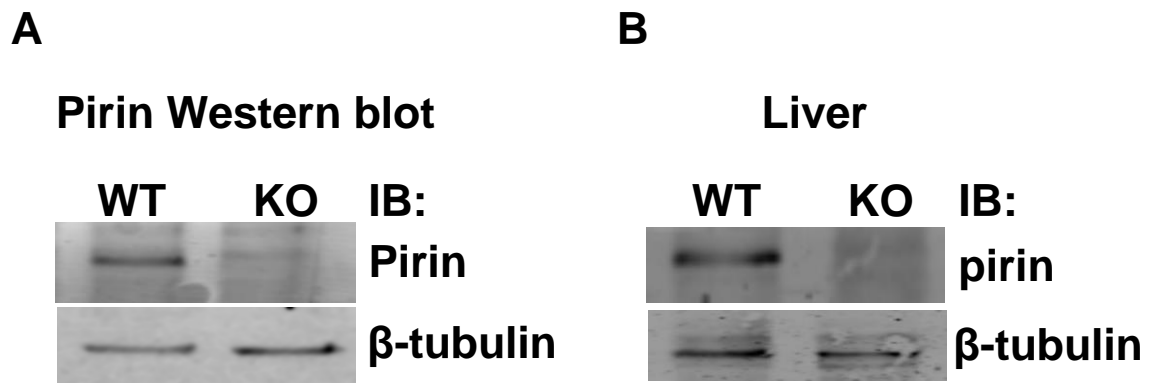


Figure S4. Pirin knockout mice show global knockout of Pirin. Westernblots show pirin protein expression in fibroblasts (A) and liver (B) of pirin knockout mice. Beta-tubulin is used as loading control.

Figure S5

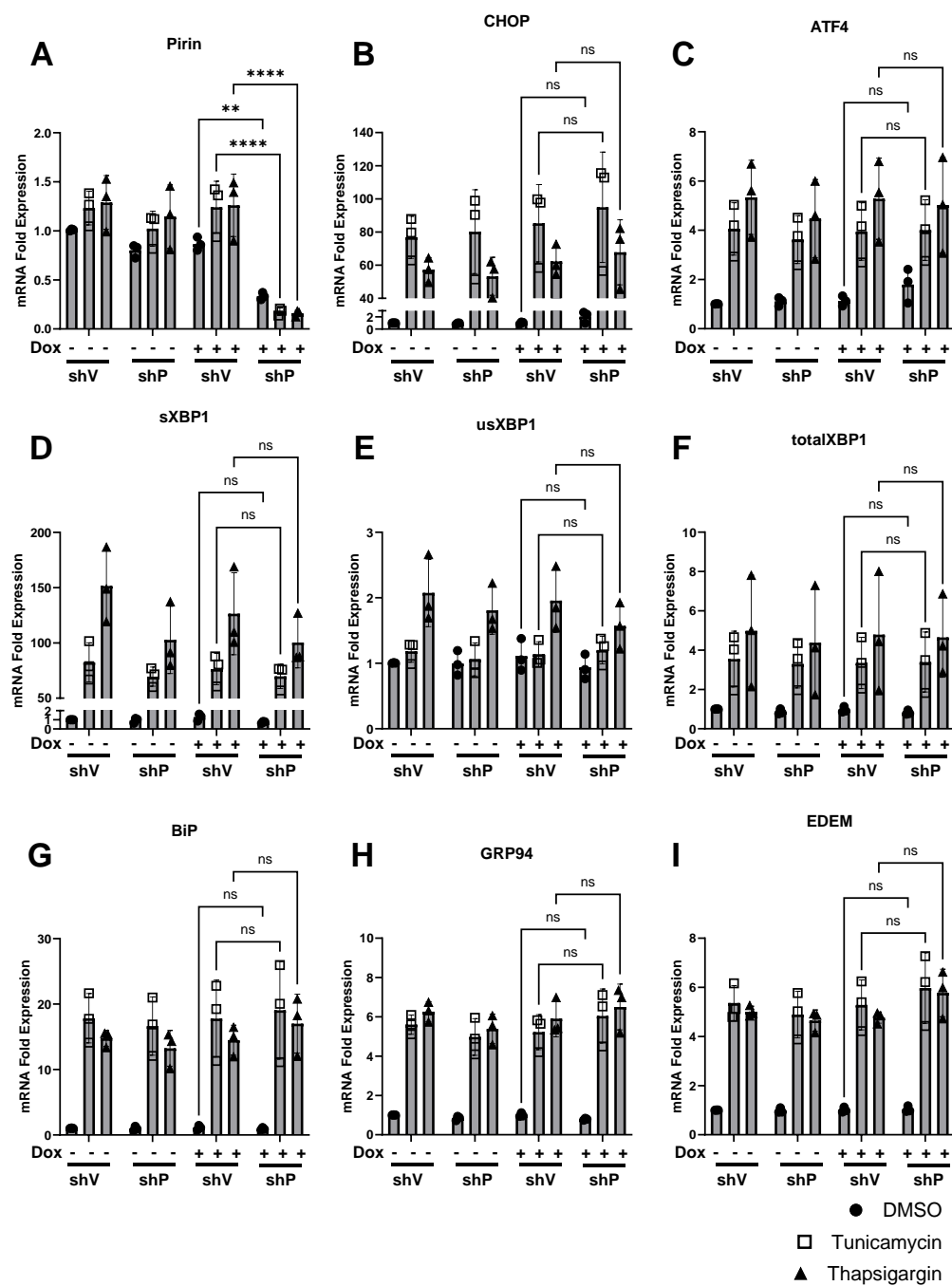


Figure S5. Expression of ER stress genes in pirin knockdown cells (shP+Dox) and control cells (shVector-Dox, shVector+Dox, shP-Dox) measured through qRT-PCR. Full

data for Figure 5. ER stress in cells was induced for 5 hours with Tunicamycin or Thapsigargin before RNA was isolated. Pirin knockdown in shP+Dox cells was confirmed through qRT-PCR (A). Statistical analysis was done by One-way ANOVA in GraphPad Prism 10 with significance values of * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$. Error bars show standard deviation. Genes tested include CHOP (B), ATF4 (C), sXBP1 (D), usXBP1 (E), totalXBP1 (F), BiP (G), GRP94 (H), and EDEM (I).

Figure S6

Offspring of Breeding Wildtype Males and Heterozygous Females

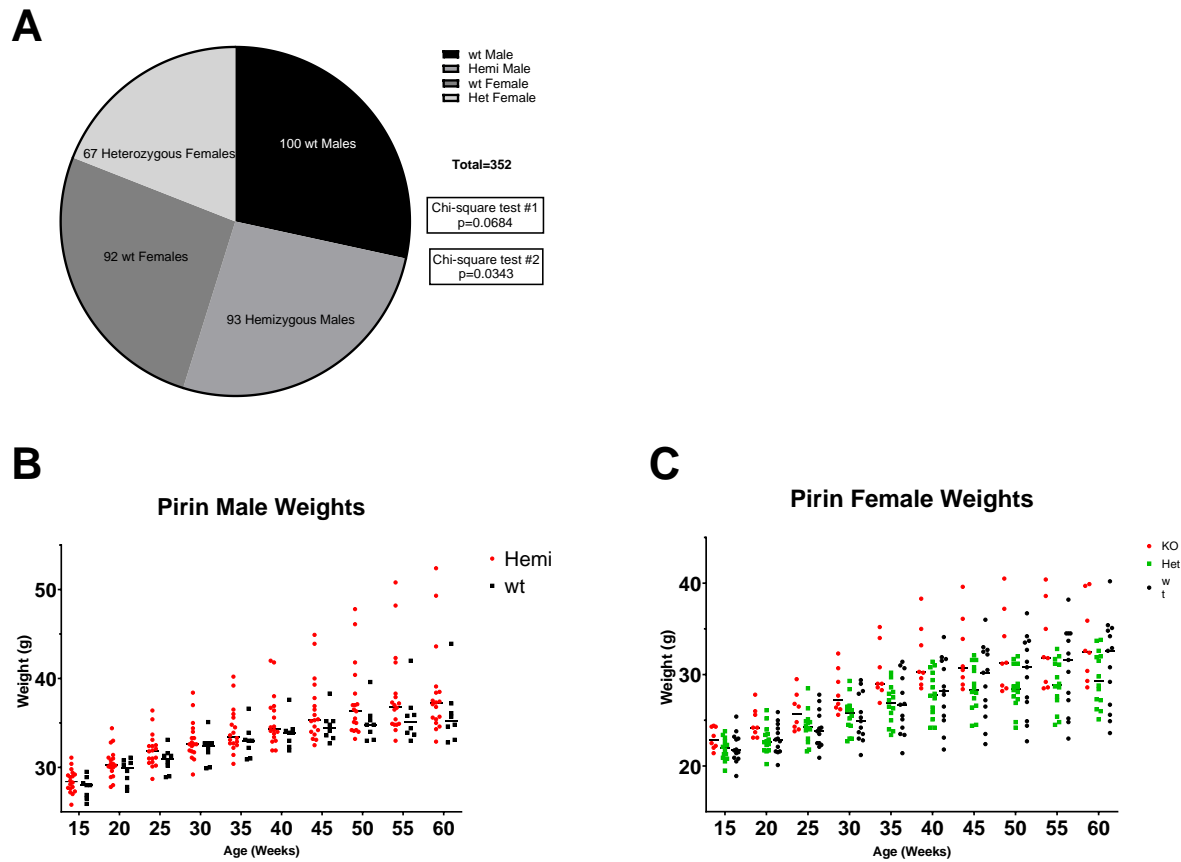


Figure S6. Breeding outcomes of Pirin knockout mice.