

789 **Online Supplemental Material**

790 **Supplemental Figure 1:** **A)** Design of the knockin construct used to construct the mice.
791 **B)** amino acid sequence of the cytoplasmic domain of wild type and mutant CD28
792 proteins. The proximal and distal motifs are boxed. **C)** Southern blot analysis of DNA
793 extracted from mice heterozygous for the mutant CD28-Y170F allele prior to removal of
794 the Neo cassette with Cre-recombinase. The DNA was digested with Bgl2 and probed
795 with the 5' external probe. **D)** DNA was isolated from mice of the indicated genotypes
796 and exon IV amplified by PCR. The product was then digested with BspH1 to confirm
797 the presence of the mutation. **E)** PCR analysis of DNA isolated from post-Cre mice of
798 each genotype. Using primers p1 and p2 (illustrated in panel a), the post cre allele can be
799 distinguished from the endogenous CD28 allele due to the remaining small amount of
800 foreign DNA.

