Supplementary - SOCS2 regulation of growth hormone signaling requires a canonical interaction with phosphotyrosine

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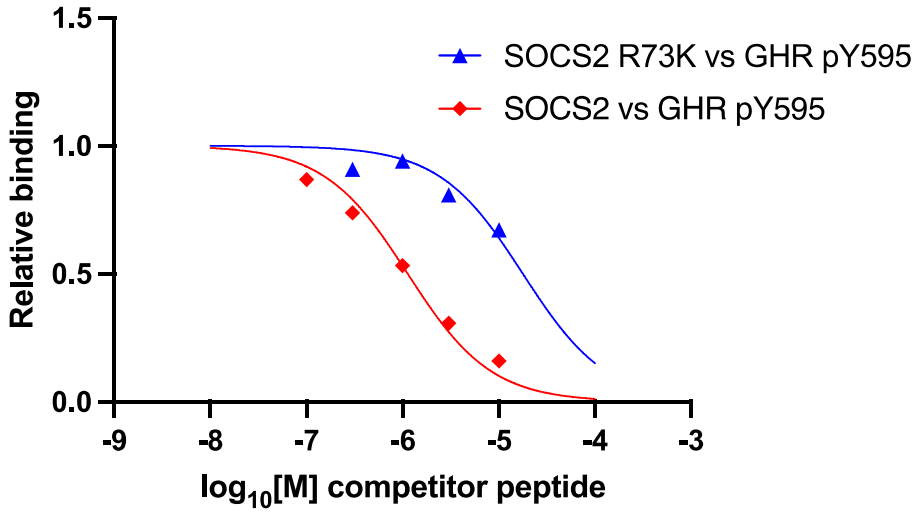
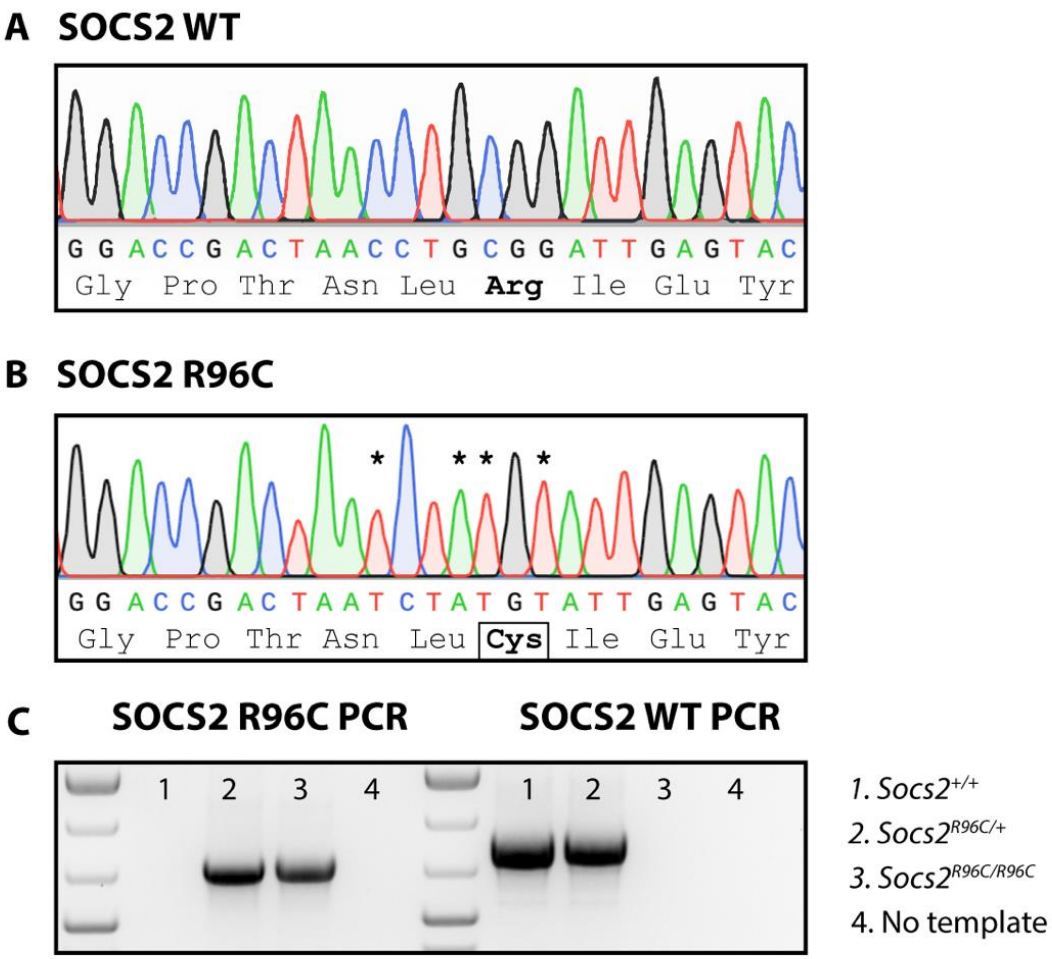
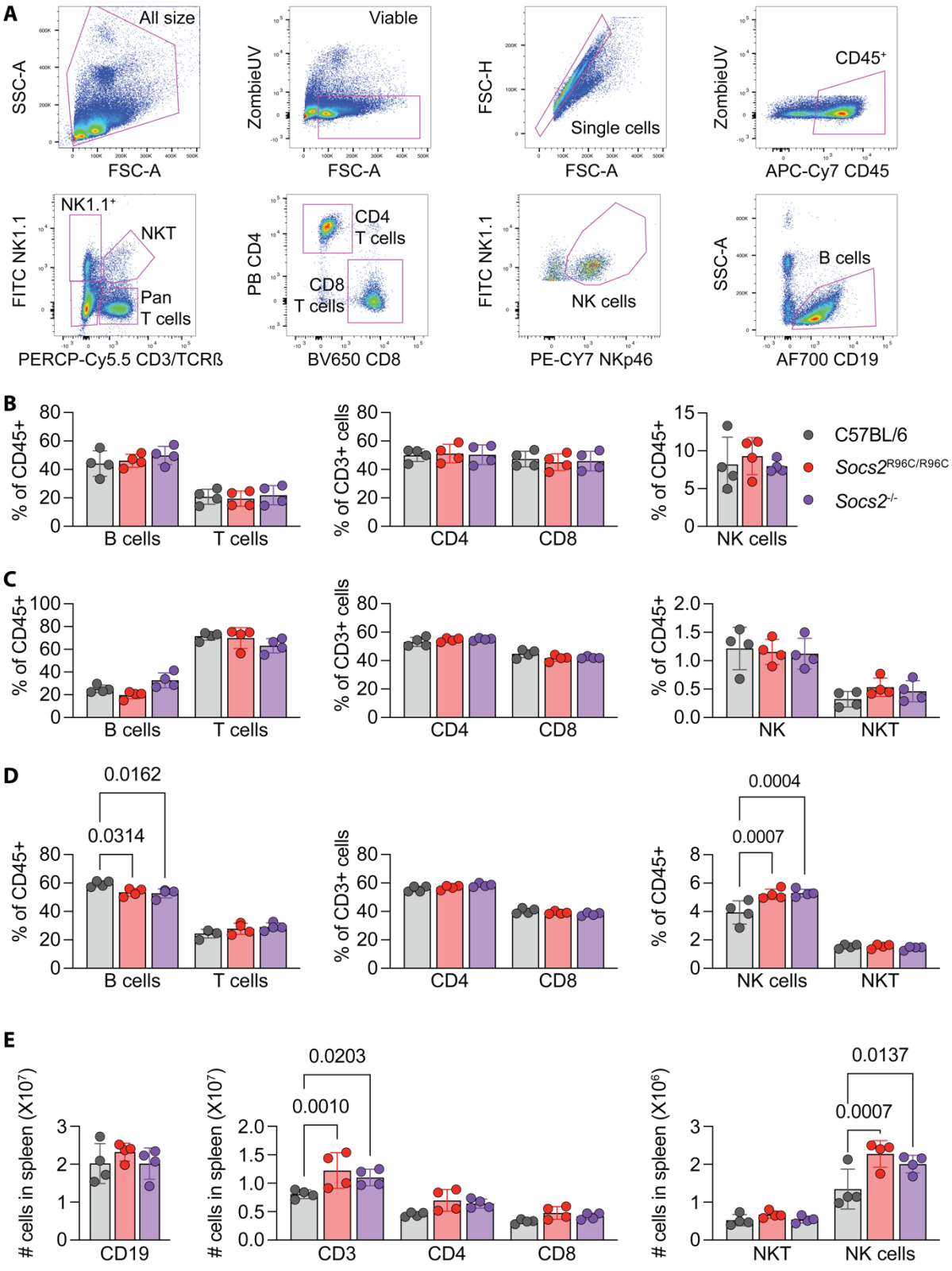


Supplementary Figure 1. Mutation of Arg73 to Lys in the SOCS2-SH2 domain results in reduced binding to phosphopeptide. A competitive surface plasmon resonance (SPR) assay was used to assess the impact of the R73K mutation. SOCS2 bound to a phosphopeptide derived from GHR pY595 with an IC_{50} 1.1 ± 0.05 μM. The SOCS2-R73K mutation reduced binding to GHR pY595, IC_{50} 26.7 ± 1.0 μM. IC_{50} values are mean ± S.D., derived from three independent experiments.

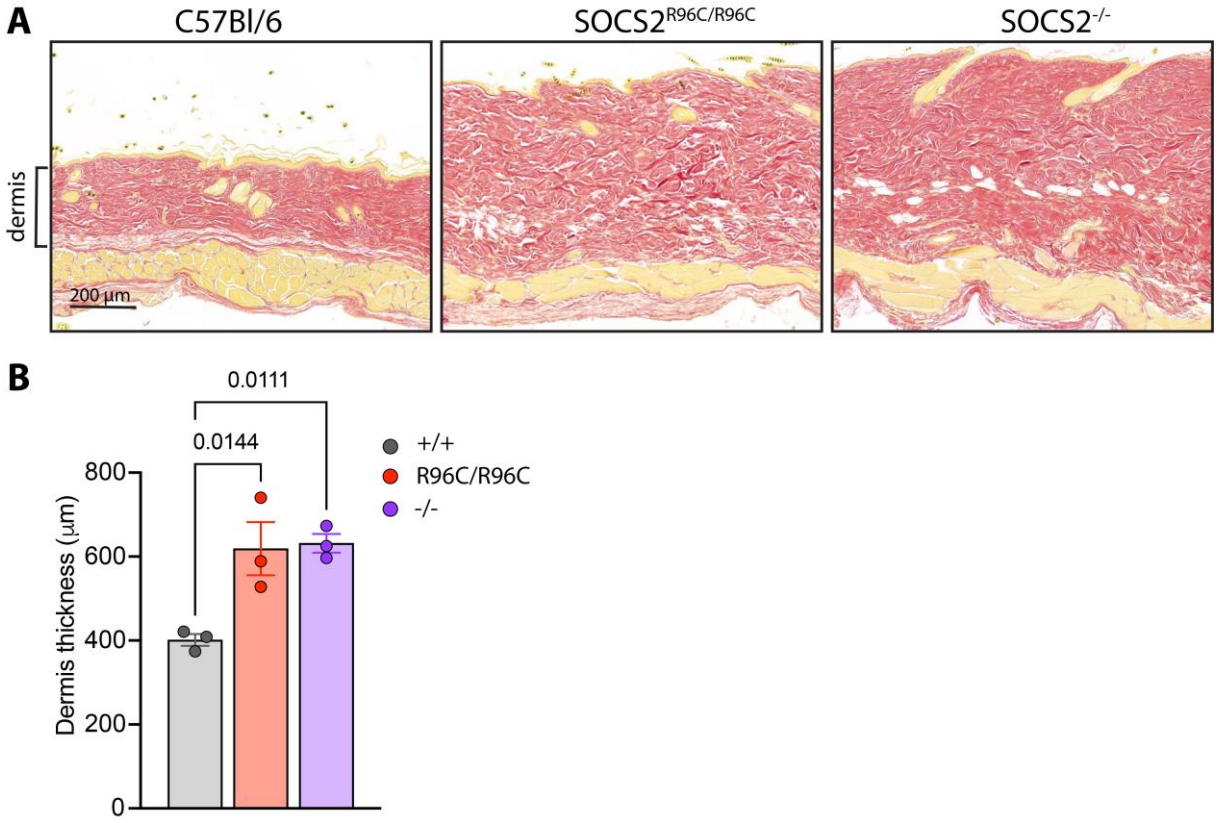


Supplementary Figure 2. Genotyping confirmation of the correct sequence change in the *Socs2*^{R96C} mouse. Sanger sequencing chromatograms confirming **(A)** the WT *Socs2* sequence and **(B)** the mutated *Socs2*^{R96C} sequence (homozygous mutant allele). *Highlights the CRISPR targeted base residue changes, two of which are synonymous and were introduced to enable primer specificity for standard gDNA genotyping. **(C)** Example 2% agarose gel confirming genotyping specificity by PCR. Primer sequences are available in **Supplementary Table 1**.

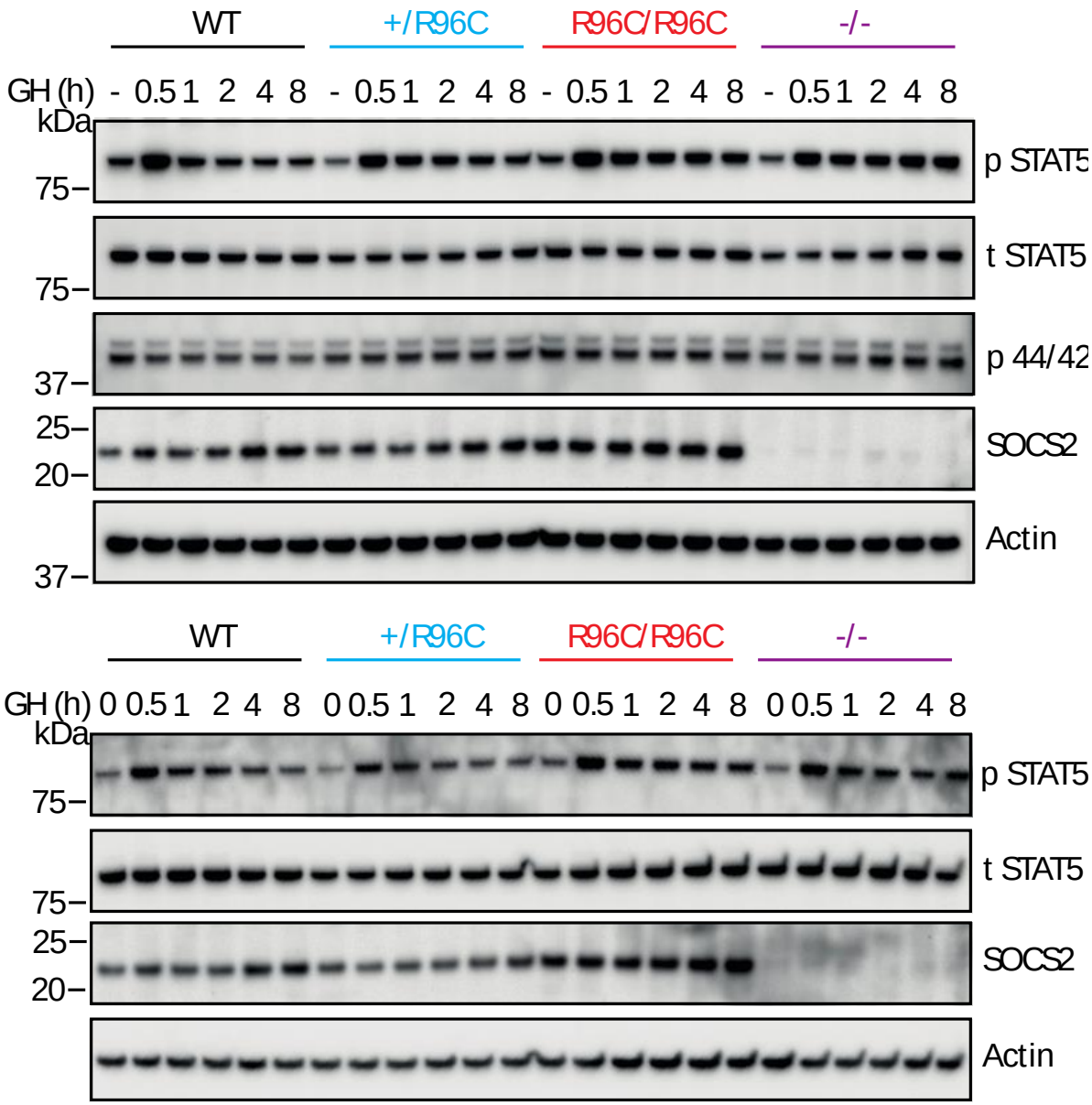


Supplementary Figure 3. Homozygous *Socs2*$^{R96C/R96C}$ and *Socs2*$^{-/-}$ mice display increased numbers of splenic NK cells. Single cell suspension peripheral blood mononuclear cells (PBMCs), splenocytes and lymphocytes from 8-12-week-old control C57BL/6 (black),

Socs2$^{R96C/R96C}$ (red) and *Socs2*$^{-/-}$ (purple) mice were stained with fluorescently conjugated antibodies to various immune markers and analysed by flow cytometry. **(A)** Example gating for the analysis of viable leukocytes (ZombieUV^{-}CD45^{+}), NKT (CD3^{+}TCRß$^{+}$NK1.1^{+}), Pan T cells (CD3^{+}TCRß$^{+}$NK1.1^{-}), CD4 T cells (CD3^{+}TCRß$^{+}$NK1.1^{-}CD4^{+}), CD8 T cells (CD3^{+}TCRß$^{+}$NK1.1^{-}CD8^{+}), Pan B cells (CD3^{-}TCRß$^{-}$NK1.1^{-}CD19^{+}) and NK cells (CD3^{-}TCRß$^{-}$NK1.1^{+}NKp46^{+}). Percentage of various immune populations in the **(B)** blood, **(C)** axial and inguinal lymph nodes, and **(D)** spleen. **(E)** Enumeration of immune populations in the spleen using counting beads (123count eBeads). **(B-E)** Each dot represents cells from an individual mouse. Significance determined by two-way ANOVA with Sidak's multiple comparisons test.



Supplementary Figure 4. Homozygous *Socs2*$^{R96C/R96C}$ and *Socs2*$^{-/-}$ mice display thickening of the skin. (A) Van Giessen-stained dorsal skin section from 10-week-old male WT, *Socs2*$^{R96C/R96C}$ and *Socs2*$^{-/-}$ mice. *Socs2* mutant and null mice show increased collagen deposits and a thickened dermis. Scale bar = 200 µm. Representative images of 3 mice of each genotype. **(B)** Quantification of dermis thickness. n=3 mice/genotype. Data were analyzed using a one-way ANOVA.



Supplementary Figure 5. *Socs2*$^{R96C/R96C}$ and *Socs2*$^{-/-}$ MEFs display prolonged growth hormone signal activation. *Socs2*$^{+/+}$, *Socs2*$^{R96C/+}$, *Socs2*$^{R96C/R96C}$ and *Socs2*$^{-/-}$ MEFs were treated with 50 ng/mL of GH, lysed and analysed by immunoblotting with antibodies to the indicated proteins. P: phosphorylated, T: total. Two additional independent experiments related to **Figure 4.**

Supplementary Table 1. *Socs2*^{R96C} genotyping primers.

	Allele	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	PCR Product (bp)
Standard genotyping	*Socs2*^{R96C}	AGCTTTCCACTTTGTCCCCTA	ATCTGAATTTCCCATCTTGGTACTCAATACAT	766
	Socs2$^{+/+}$	GCTGGACCGACTAACCTGC	AGCATGGTCAGCTTAACGGAA	901
NGS* genotyping		GTGACCTATGAACTCAGGAGTCTGACTGTTAATGAAGCCAAAGAG	CTGAGACTTGCACATCGCAGCGTGAACAGTCCCATTCCGTG	290

*Next generation sequencing