

Supplemental Materials: STAT5a/b Deficiency Delays, but does not Prevent, Prolactin-Driven Prostate Tumorigenesis in Mice

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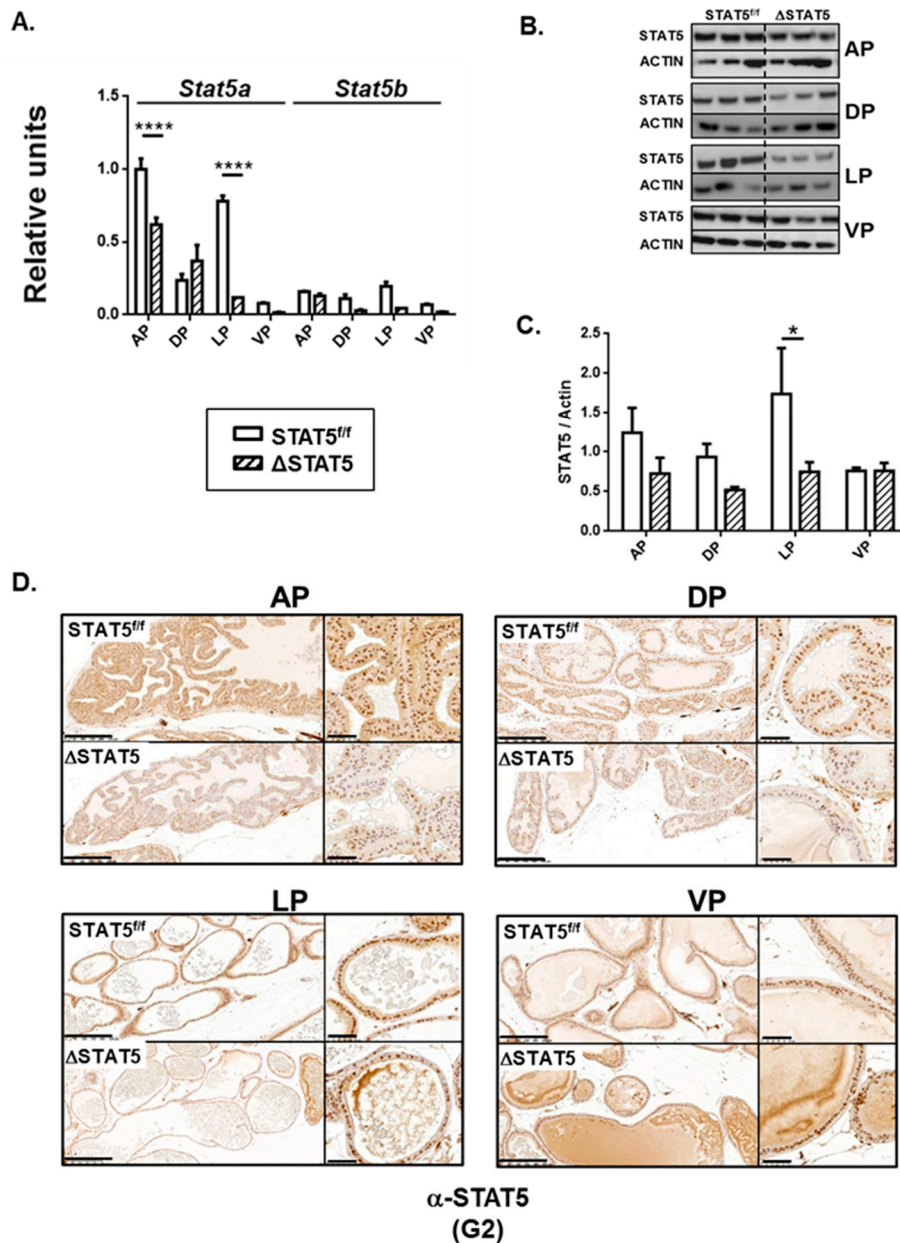


Figure S1. Lobe-specific pattern of STAT5 deletion in 6 month-old Δ STAT5 mice. **A.** Lobe-specific expression of *STAT5A* and *STAT5B* mRNA in *STAT5^{fl/fl}* and Δ STAT5 mice as determined by RT-qPCR. **B–C.** Lobe-specific expression of STAT5 protein in *STAT5^{fl/fl}* and Δ STAT5 mice as determined by immunoblot (each lane corresponds to a different animal). Quantification of STAT5 expression (STAT5/ACTIN) was performed by densitometry (C). **D.** Immunohistochemical analysis of STAT5 expression in anterior (AP), dorsal (DP), lateral (LP) and ventral (VP) prostates of *STAT5^{fl/fl}* and Δ STAT5 mice, as indicated. *Statistics:* Stars denote significant differences in a repeated-measures two-way ANOVA with Sidak's multiple comparisons. Size bars: 250 μ m in large images and 50 μ m in insets. * $p < 0.05$; **** $p < 0.0001$.

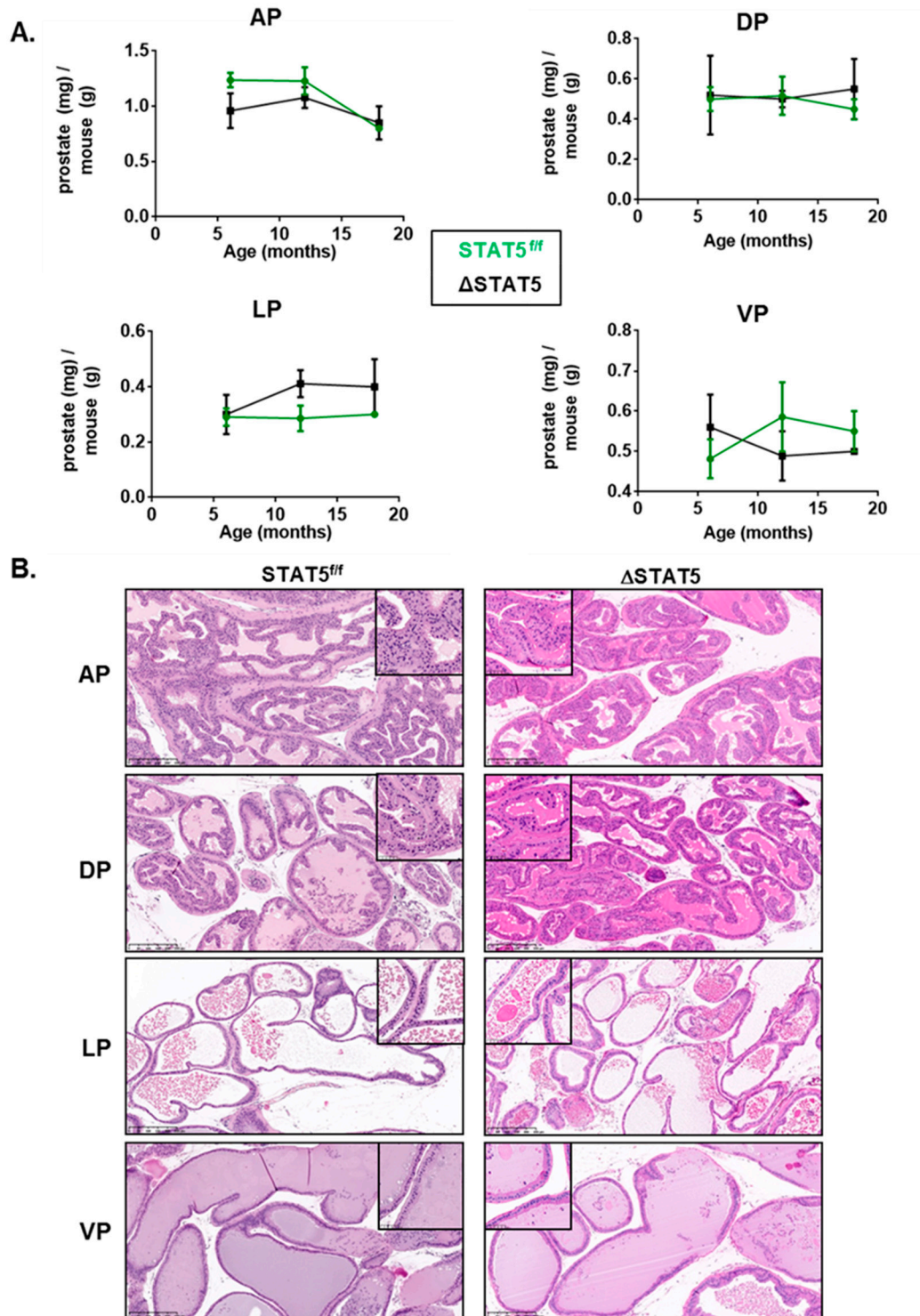


Figure S2. STAT5 deletion has no detectable effect on the prostate tissue. **A.** The prostate weight in 6, 12 and 18 month-old STAT5^{fl/fl} and ΔSTAT5 mice is expressed as the ratio of total prostate normalized to the weight of corresponding animal. There was no genotype-dependent difference between age-matched mice. **B.** Histological analysis (hematoxylin counterstaining) of the four prostate lobes from 6 month-old STAT5^{fl/fl} and ΔSTAT5 mice showing no obvious sign of abnormalities. Size bars: 250 μm in large images and 50 μm in insets.

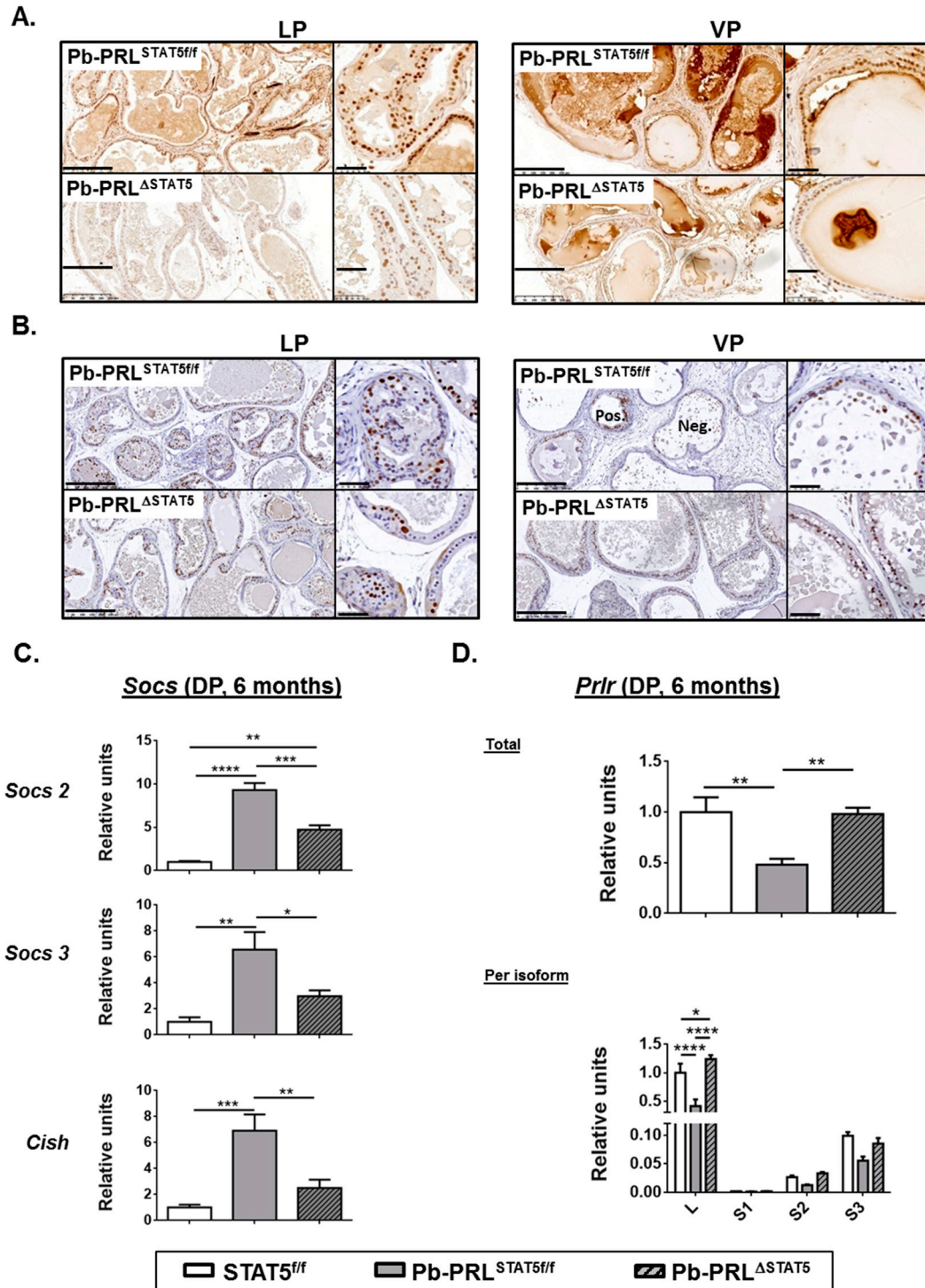


Figure S3. Lobe-specific pattern of STAT5 deletion of 6 month-old $Pb-PRL^{\Delta STAT5}$ mice (complementary to Figure 1). **A–B.** Immunohistochemical analysis of STAT5 expression (A) and phosphorylation (B) in lateral (LP) and ventral (VP) prostates of $Pb-PRL^{STAT5^{fl/fl}}$ and $Pb-PRL^{\Delta STAT5}$ mice, as indicated (see main Figure 1 for details). **C–D.** The mRNA expression of SOCS genes (C) and *PRLR* (D) was monitored in dorsal prostates of 6 month-old $STAT5^{fl/fl}$, $Pb-PRL^{STAT5^{fl/fl}}$ and $Pb-PRL^{\Delta STAT5}$ using RT-qPCR. Statistics: Stars denote significant differences in a repeated-measures one-way ANOVA with Tukey’s multiple comparisons (C) or two-way ANOVA with Sidak’s multiple comparisons (D). Size bars: 250 μm in large images and 50 μm in insets. Size bars: 250 μm in large images and 50 μm in insets. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

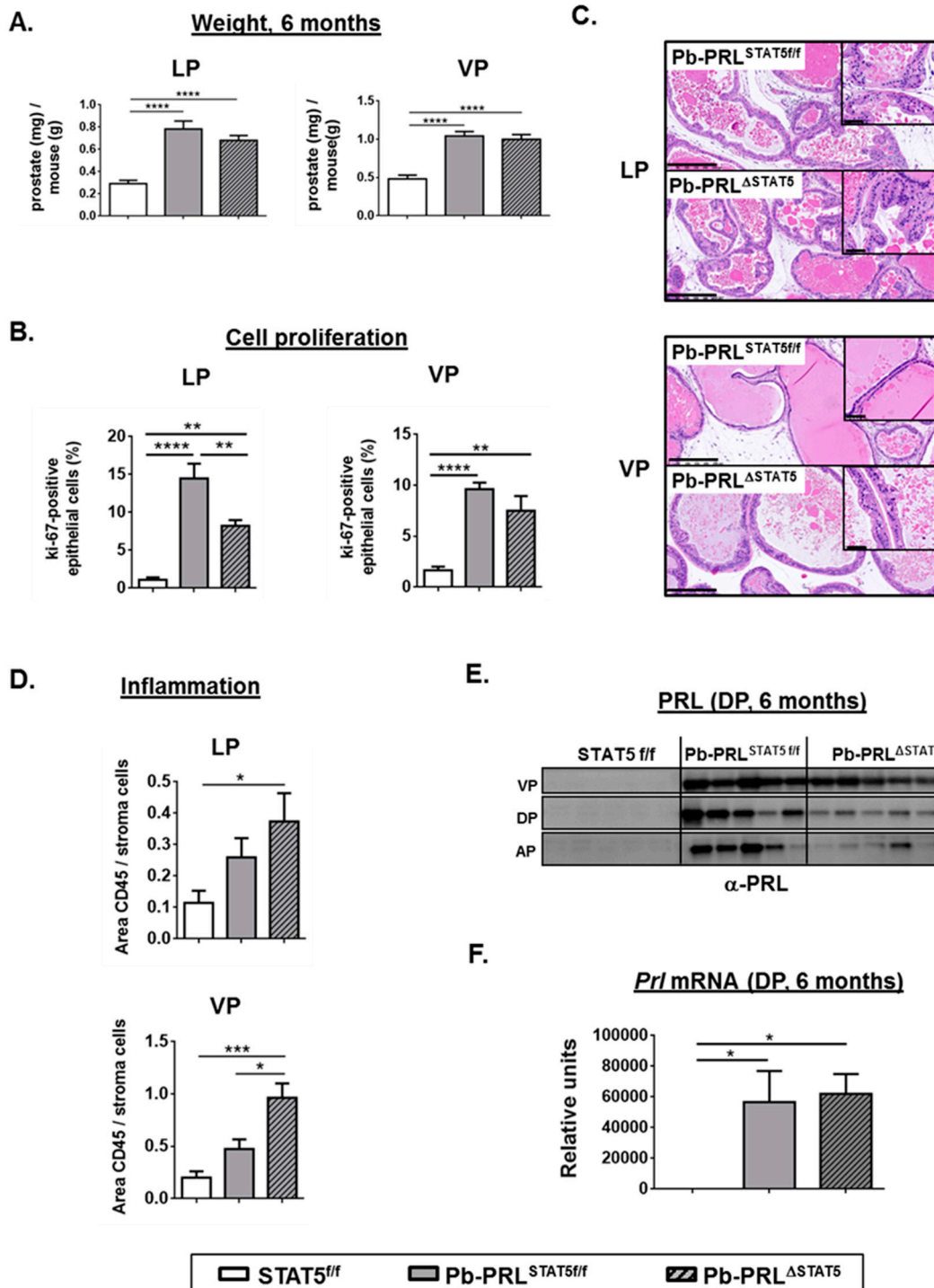
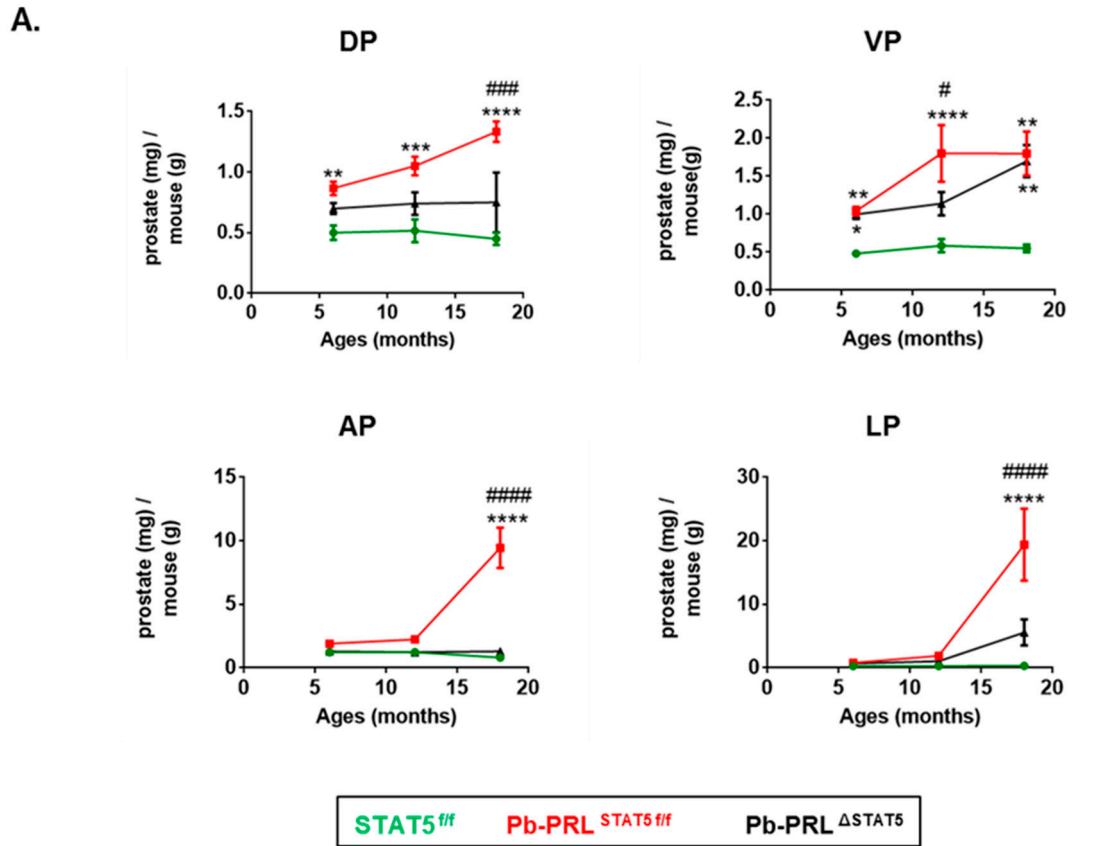


Figure S4. Lobe-specific effects of STAT5 deletion of 6 month-old Pb-PRL^{ΔSTAT5} mice (complementary to Figure 4). **A.** The weight of lateral (LP) and ventral (VP) prostate lobes in 6 month-old STAT5^{fl/fl}, Pb-PRL^{STAT5^{fl/fl}} and Pb-PRL^{ΔSTAT5} mice is expressed as the ratio of lobe weight normalized to the weight of corresponding animal. **B.** The proliferation index of lateral and ventral prostate was determined in the three genotypes (see Figure 4 for details). **C.** Histological analysis (hematoxylin counterstaining) of lateral and ventral lobes of Pb-PRL^{STAT5^{fl/fl}} and Pb-PRL^{ΔSTAT5} mice showing similar hyperplasia in both genotypes. **D.** Inflammation was identified using CD45 immunostaining. The degree of inflammation was quantified using Calopix software and is represented as the ratio of CD45+ area *versus* stroma area. **E,F.** Expression of rat *PRL* transgene was monitored using immunoblot (D) and RT-qPCR (E) in the three genotypes. Stars denote significant differences in a repeated-measures one-way ANOVA with Tukey’s multiple comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



B.

12 months

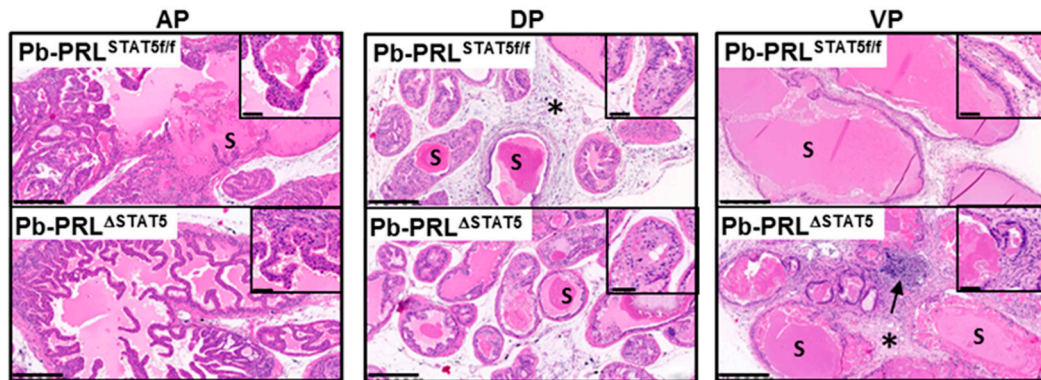


Figure S5. STAT5 deletion does not prevent prostate tumor progression in aged Pb-PRL mice (complementary to Figure 5). **A.** The prostate weight in 6, 12 and 18 month-old STAT5^{f/f}, Pb-PRL^{STAT5^{f/f}} and Pb-PRL^{ΔSTAT5} mice is expressed as the ratio of total prostate weight normalized to the weight of corresponding animal. Symbols: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (versus STAT5^{f/f}); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ (versus Pb-PRL^{ΔSTAT5}). **B.** Histological analysis (hematoxylin counterstaining) of the anterior (AP), dorsal (DP) and ventral (VP) prostate lobes from 12 month-old Pb-PRL^{STAT5^{f/f}} and Pb-PRL^{ΔSTAT5} mice showing similar abnormalities including PINs, increased stromal density (stars), inflammation (arrows) and dense eosinophilic secretions (S). Statistics: Symbols (*: vs age-matched STAT5^{f/f}; #: versus age-matched Pb-PRL^{ΔSTAT5}) denote significant differences in a repeated-measures two-way ANOVA with Tukey's multiple comparisons. Size bars: 250 μm in large images and 50 μm in insets.

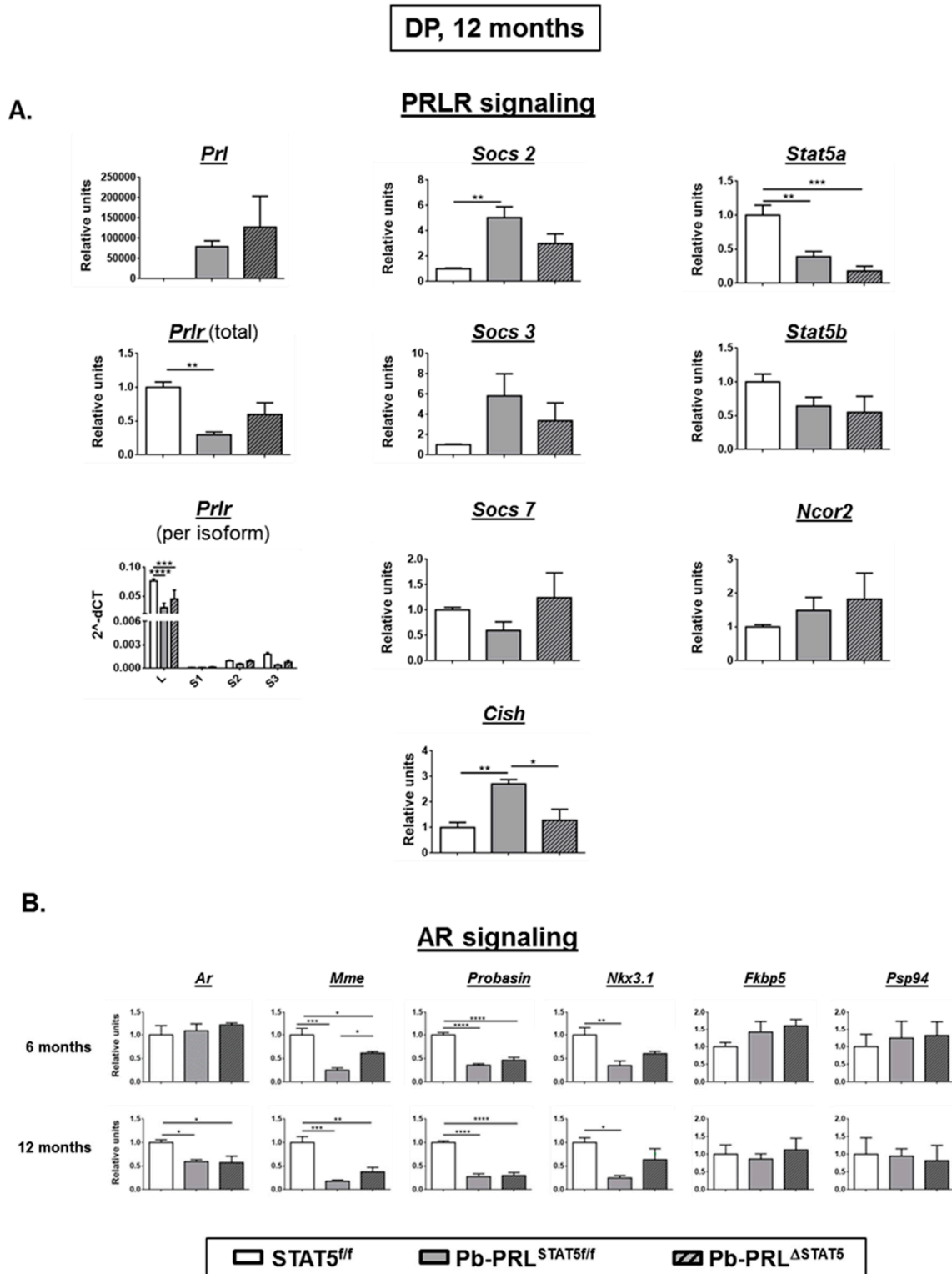


Figure S6. Characterization of PRLR and AR signaling pathways in dorsal prostate of 12 month-old STAT5^{fl/fl}, Pb-PRL^{STAT5fl/fl} and Pb-PRL^{ΔSTAT5} mice (complementary to Figure 6). **A.** The expression of various actors of the PRLR pathway as determined by RT-qPCR was analyzed in dorsal prostates of 12 month-old STAT5^{fl/fl}, Pb-PRL^{STAT5fl/fl} and Pb-PRL^{ΔSTAT5} mice. **B.** The expression of AR and of target genes of the AR pathway as determined by RT-qPCR was analyzed in dorsal prostates of 6 and 12 month-old STAT5^{fl/fl}, Pb-PRL^{STAT5fl/fl} and Pb-PRL^{ΔSTAT5} mice, as indicated. In both panels data are expressed as fold-induction versus STAT5^{fl/fl} samples. Statistics: Stars denote significant differences in a repeated-measures one-way ANOVA with Tukey's multiple comparisons. Except for PRLR (per isoform), stars denote significant differences in a repeated-measures two-way ANOVA with Tukey's multiple comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Breeding scheme

Italics : mouse genotypes
Bold : name of mice used in this study

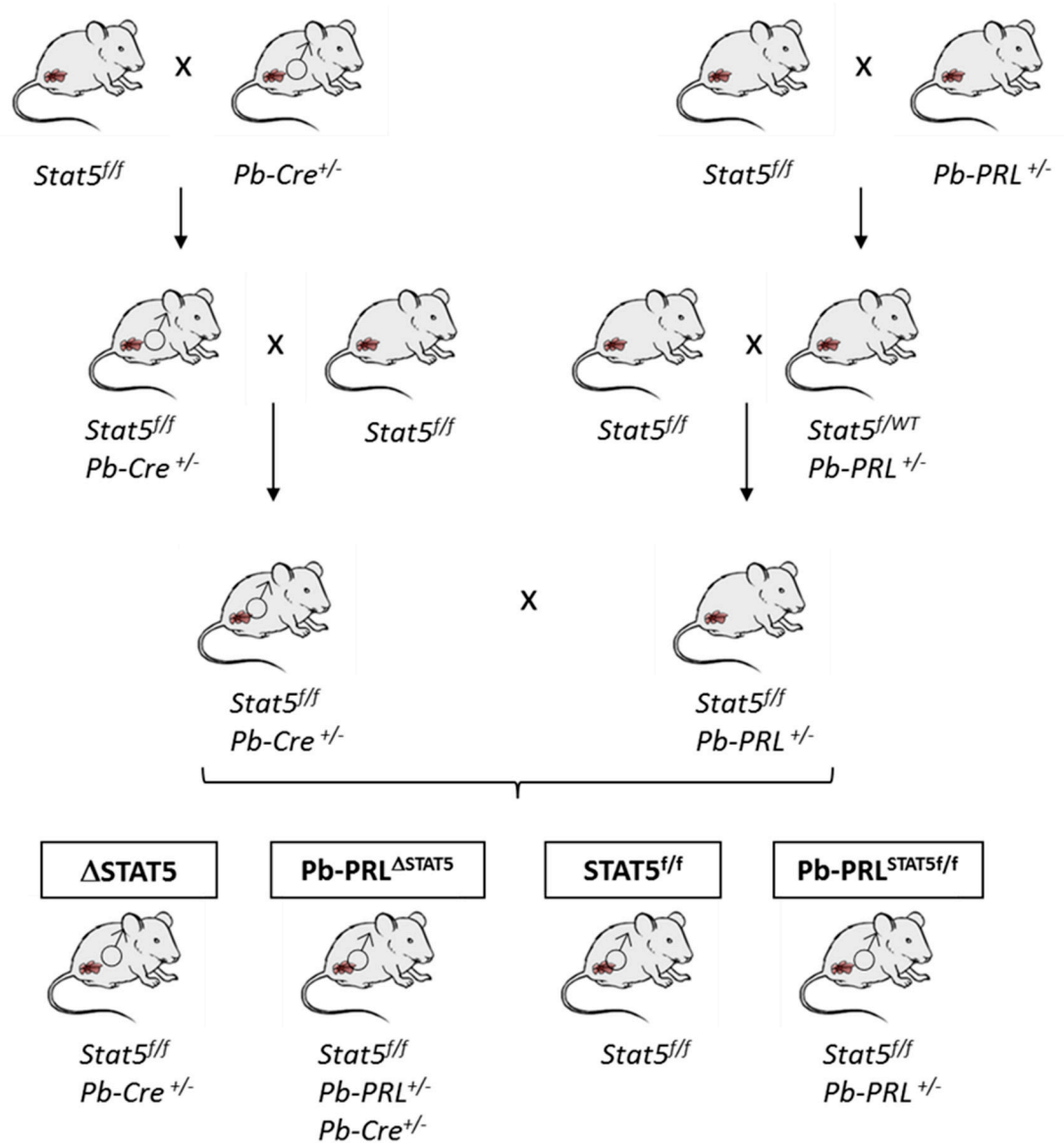


Figure S7. Breeding scheme.

Western-blot corresponding to Fig. 1B

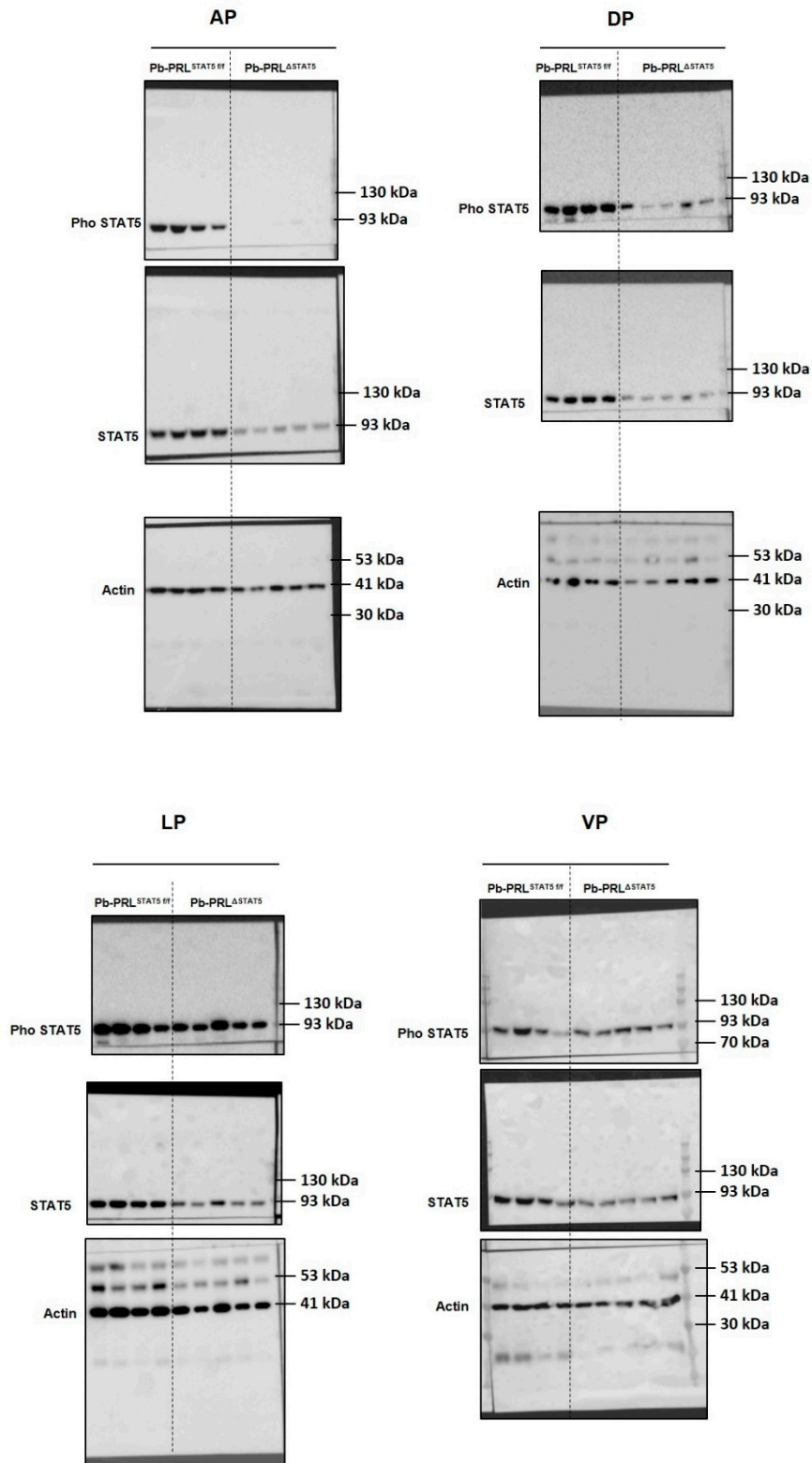


Figure S8. Cont.

Western-blot corresponding to Fig. 4A

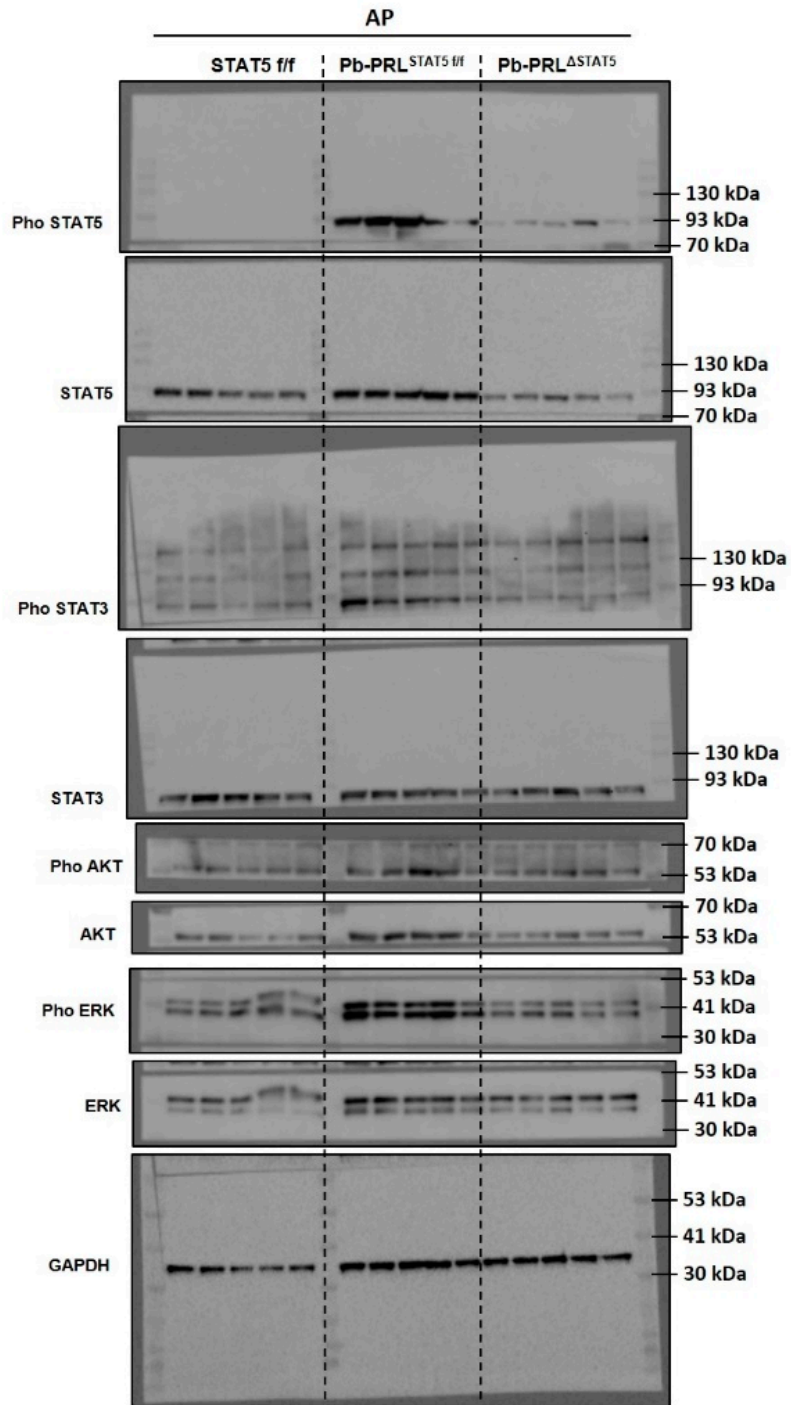


Figure S8. Cont.

Western-blots corresponding to Fig. 4B

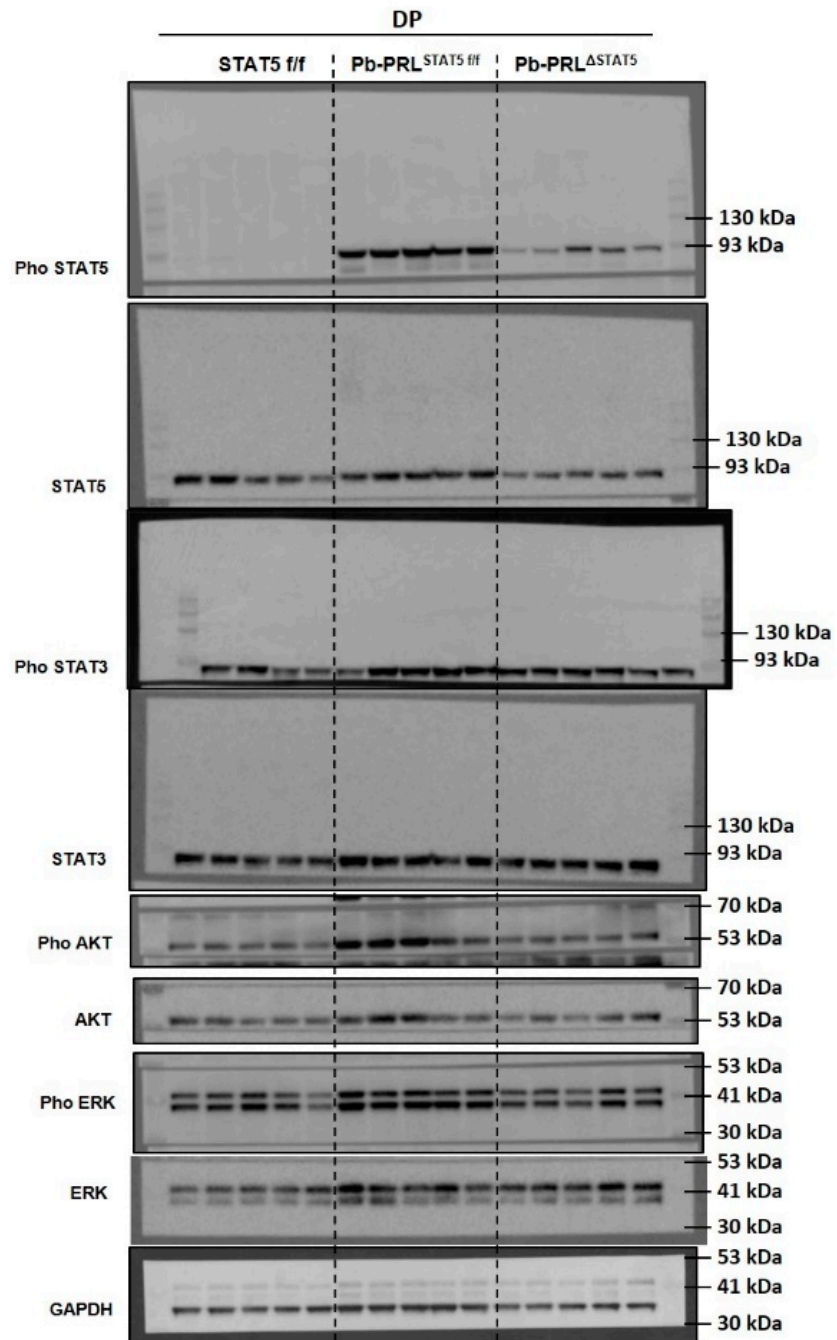


Figure S8. Cont.

Western-blot corresponding to Fig. 4C

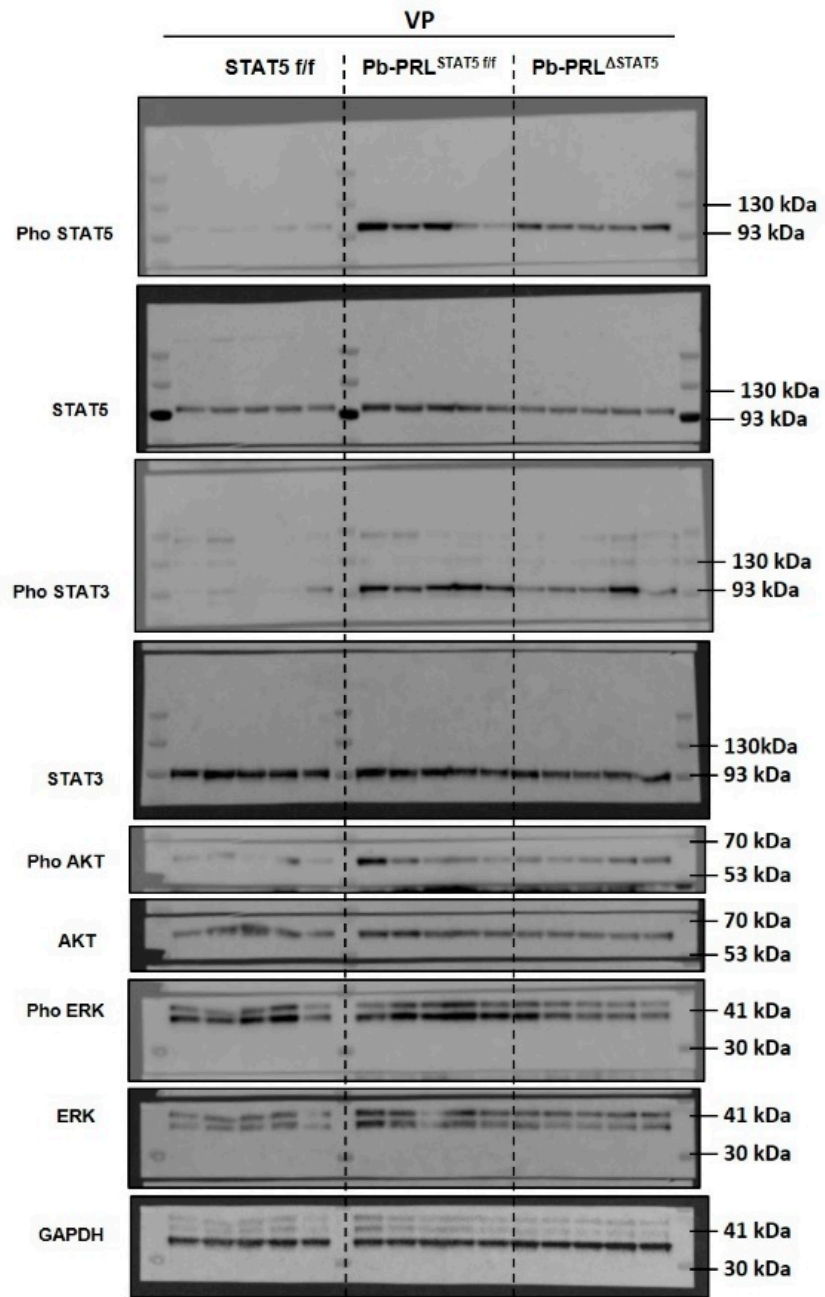


Figure S8. Cont.

Western-blots corresponding to Fig. 6A

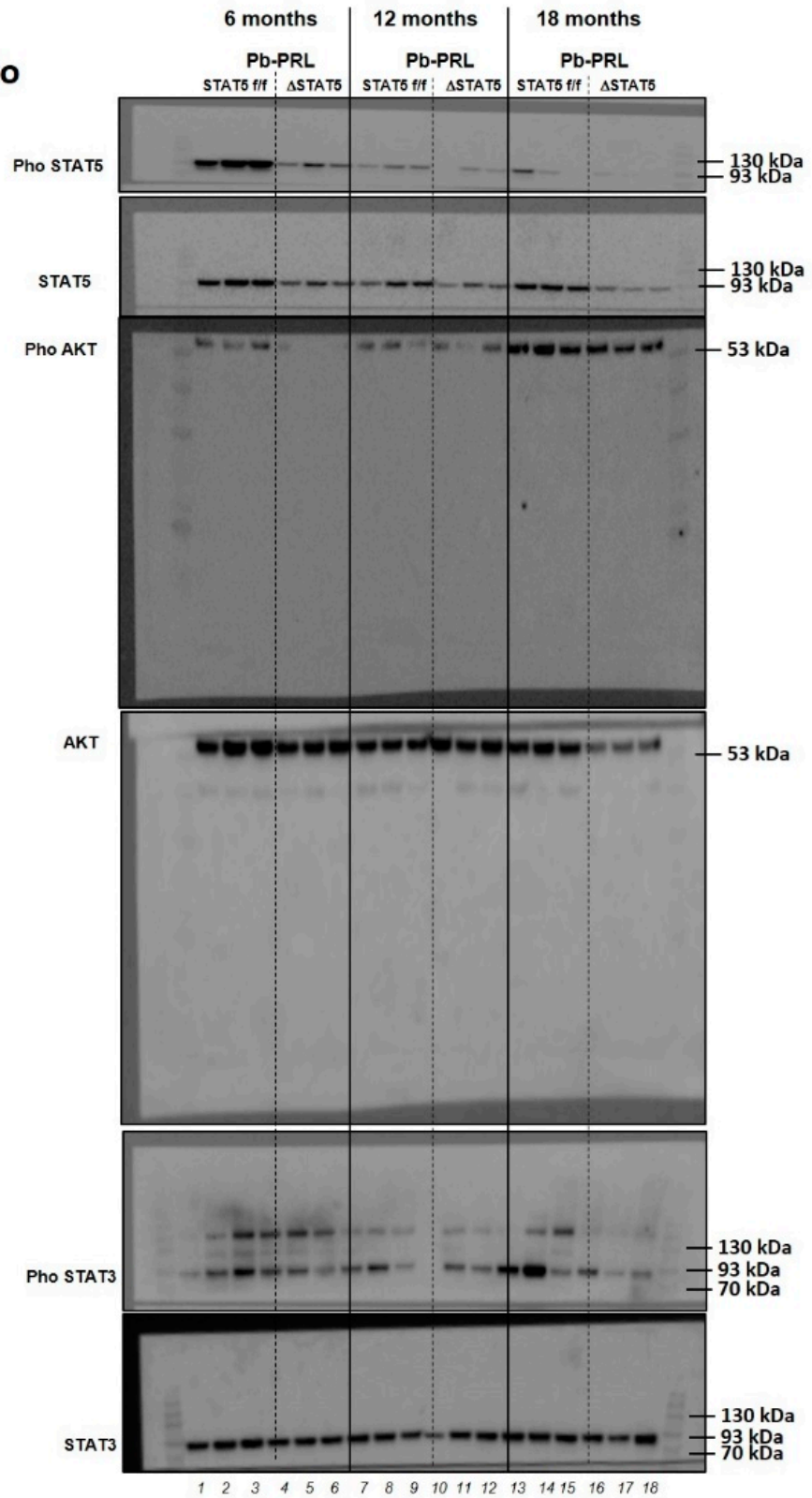


Figure S8. Cont.

**Western-blot
corresponding to
Fig. 6A (continued)**

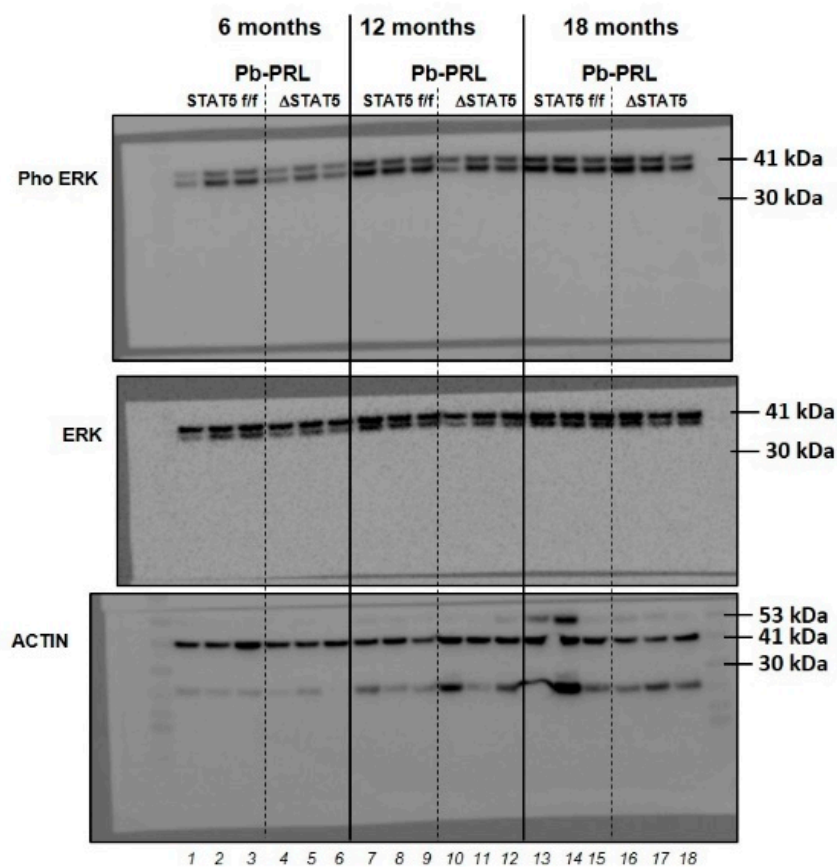


Figure S8. Cont.

Western-blots corresponding to Fig. S1B

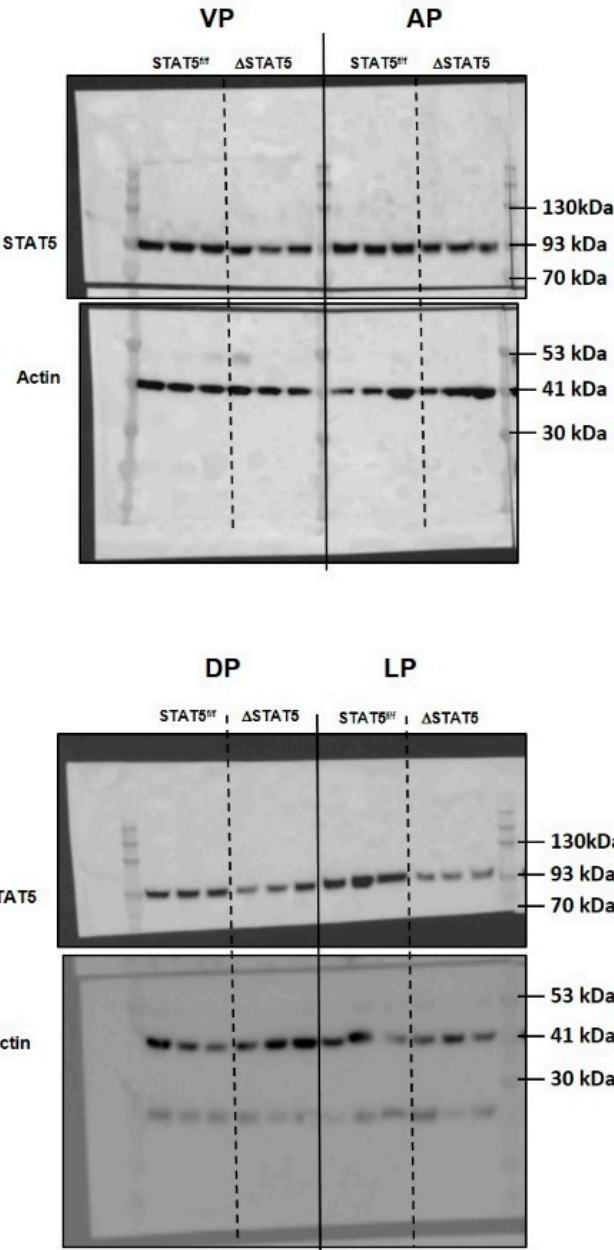


Figure S8. Cont.

Western-blot corresponding to Fig. S4E

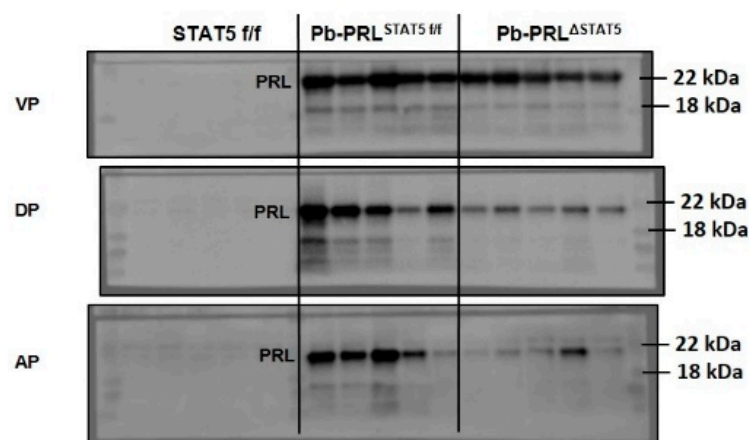


Figure S8. Whole Western blot membranes corresponding to representative images shown in main and supplemental Figures, as indicated (continued on the next pages)

Table S1. Antibody references and conditions of use.

Target	Ref./Clone	Provider	Dilution
STAT5	Sc 835/C-17	Santa cruz	Blot: 1/2,000
STAT5b	Sc 1656/G2 *	Santa cruz	IHC: 1/200
pSTAT5	9359/C11C5	Cell Signaling	Blot: 1/1000 IHC: 1/300
STAT3	12640/D3Z2G	Cell Signaling	Blot: 1/1000
pSTAT3	9131S	Cell Signaling	Blot: 1/1000
Erk1/2	9102	Cell Signaling	Blot: 1/3000
pErk1/2	4370/D13.14.4E	Cell Signaling	Blot: 1/1000
Akt	9272	Cell Signaling	Blot: 1/1000
pAkt	9271S	Cell Signaling	Blot: 1/1000
CD45	sc-53665/30-F11	Santa Cruz	IHC: 1/150
Ki-67	RM9106/SP6	Thermo Scientific	IHC: 1/300
GAPDH	2118S/14C10	Cell Signaling	Blot: 1/1000
Actin	A5316	Sigma	Blot: 1/5000
PRL	Anti-mPRL-IC AFP879151	National hormone & peptide program (Dr. Parlow)	Blot: 1/1000

* maps amino acids 750-779 that are common to both STAT5 isoforms.

Table S2. Primers used for qPCR.

Gene	Name	5'-3' sequence
<i>Cyclophilin</i>	Cyclophilin A-R	TTGCTGGTCTTGCCATTCCT
	Cyclophilin A-R	CAGGTCCTGGCATCTTGTC
<i>Probasin</i>	Mouse F	GCATGTGCTAGGCGTCTCC
	Mouse R	GTTCTCAATGGTGAGCCTTCAT
<i>Stat5a</i>	Mouse F	CATTGCTTGGAAAGTTTGACTCTC
	Mouse R	CACGTAGATAAGGTAGTTCAGGTC
<i>Stat5b</i>	Mouse F	GCACCTTCAGATCAACCAAAC
	Mouse R	CAGCTGGGCAAACCTGAG
<i>Socs 1</i>	Mouse F	ATTCCACTCCTACCTCTCCAT
	Mouse R	CAGAAAAATGAAGCCAGAGACC
<i>Socs 2</i>	Mouse F	GCGCGAGCTCAGTCAAAC
	Mouse R	CTGGAGCCTCTTTTAATTTCTCTTT
<i>Socs3</i>	Mouse F	GAGATTTGCTTCGGGACTA
	Mouse R	GGAAACTTGCTGTGGGTGA
<i>Cish</i>	Mouse F	GCTCCTTTCTCCTTATCC
	Mouse R	CCGCCAATTTGCTCCA
<i>Ncor2</i>	Mouse F	TGGAACCCGGCACCGC
	Mouse R	GGTAGGGTAGACCCCTT
<i>Prlr</i> (all isoforms)	Mouse F	ATAAAAGGATTTGATACTCATCTGCTAGAG
<i>Prlr L</i>	Mouse R	TGTCATCCACTTCCAAGAACTCC
<i>Prlr S1</i>	Mouse R	CATAAAAACCTCAGTTGTTGGAATCTTCA
<i>Prlr S2</i>	Mouse R	GGAAAAAGACATGGCAGAAACC
<i>Prlr S3</i>	Mouse R	AGTCCCCTTCATTGTCCAGTTT
<i>Ar</i>	Mouse F	GAGAGAGGCAGCTTGTGCAT
	Mouse R	TACTGAATGACCGCCATCTG
<i>Nkx3.1</i>	Mouse F	ATGCTTAGGGTAGCGGAGC
	Mouse R	TGCGGATGCTGAGTGTGTC
<i>Fkbp5</i>	Mouse F	TTTGAAGATTCAGGCGTTATCCG
	Mouse R	GGTGGACTTTTACCGTTGCTC
<i>Mme</i>	Mouse F	GGTTGAAACGCAACGTCATTC
	Mouse R	CTGCACTGCTACTATGTCTTCAG
<i>Psp94</i>	Mouse F	TGGTGATAGCATCCAAAGCA
	Mouse R	GCTTGTACCATCAGCATCC
<i>Probasin</i>	Mouse F	GCATGTGCTAGGCGTCTCC
	Mouse R	GTTCTCAATGGTGAGCCTTCAT

