The erythroid function of transferrin receptor 2 revealed by *Tmprss6* inactivation in different models of transferrin receptor 2 knockout mice

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AN and RMP contributed equally to this work.

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLES

**Supplemental Table 1. Oligonucleotide primers (Applied Biosystem) used for qRT-PCR by TaqMan**

Name	Id
Hprt1	Mm01318743_m1
Hamp	Mm00519025_m1
Bmp6	Mm01332882_m1
Id1	Mm00775963_g1
Tfr1	Mm00441941_m1

Supplemental Table 2. Evaluation of statistical significant differences through 2-tailed t-test analysis

	WBC	RBC	Ret	Hb	Hct	MCV	MCH	TS	LIC	Tfr1	SIC	EPO	Bmp6	Hamp	Hamp/LIC	Id1	Bmp6/LIC
Tmprss6$^{+/-}$ vs wt	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tmprss6$^{-/-}$ vs wt	ns	**	*	**	**	**	**	*	*	*	ns	*	ns	*	*	*	ns
Tfr2$^{-/-}$ vs wt	ns	ns	ns	*	*	ns	ns	*	*	ns	ns	ns	*	ns	*	ns	ns
Tmprss6$^{+/-}$ Tfr2$^{-/-}$ vs wt	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tmprss6$^{-/-}$ Tfr2$^{-/-}$ vs wt	ns	**	*	**	*	**	**	*	*	*	ns	*	ns	*	*	*	ns
Tfr2^{LCKO} vs wt	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	*	ns	*	ns	ns
Tmprss6$^{+/-}$ Tfr2^{LCKO} vs wt	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns	ns	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs wt	ns	*	*	**	**	**	**	**	*	*	ns	*	ns	**	*	*	ns
Tmprss6$^{-/-}$ vs Tmprss6$^{+/-}$	ns	*	**	**	**	**	**	*	*	ns	ns	*	ns	*	*	*	ns
Tfr2$^{-/-}$ vs Tmprss6$^{+/-}$	ns	ns	ns	*	ns	**	**	*	*	*	ns	ns	*	ns	*	ns	ns
Tmprss6$^{+/-}$ Tfr2$^{-/-}$ vs Tmprss6$^{+/-}$	ns	ns	ns	*	ns	**	**	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
Tmprss6$^{-/-}$ Tfr2$^{-/-}$ vs Tmprss6$^{+/-}$	ns	**	**	**	*	**	**	*	*	*	ns	*	ns	ns	*	*	ns
Tfr2^{LCKO} vs Tmprss6$^{+/-}$	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	*	ns	*	ns	ns
Tmprss6$^{+/-}$ Tfr2^{LCKO} vs Tmprss6$^{+/-}$	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns	ns	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs Tmprss6$^{+/-}$	ns	*	**	**	**	**	**	**	ns	ns	ns	*	ns	*	*	*	ns
Tfr2$^{-/-}$ vs Tmprss6$^{-/-}$	ns	*	*	**	**	**	**	**	**	*	ns	*	*	*	**	*	ns
Tmprss6$^{+/-}$ Tfr2$^{-/-}$ vs Tmprss6$^{-/-}$	ns	*	*	**	**	**	**	*	*	ns	ns	*	ns	*	*	*	ns
Tmprss6$^{-/-}$ Tfr2$^{-/-}$ vs Tmprss6$^{-/-}$	ns	*	*	ns	ns	*	*	ns	ns	ns	ns	ns	ns	*	*	*	ns
Tfr2^{LCKO} vs Tmprss6$^{-/-}$	ns	*	*	**	**	**	**	**	**	*	ns	**	*	**	**	*	ns
Tmprss6$^{+/-}$ Tfr2^{LCKO} vs Tmprss6$^{-/-}$	ns	*	*	**	**	**	**	*	*	ns	ns	**	*	*	*	*	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs Tmprss6$^{-/-}$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tmprss6$^{+/-}$ Tfr2$^{-/-}$ vs Tfr2$^{-/-}$	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	*	ns	*	ns	ns
Tmprss6$^{-/-}$ Tfr2$^{-/-}$ vs Tfr2$^{-/-}$	ns	**	*	**	*	**	**	*	*	*	ns	*	*	*	*	*	ns
Tfr2^{LCKO} vs Tfr2$^{-/-}$	ns	ns	ns	*	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tmprss6$^{+/-}$ Tfr2^{LCKO} vs Tfr2$^{-/-}$	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs Tfr2$^{-/-}$	ns	*	*	**	**	**	**	**	**	*	ns	*	*	**	*	*	ns
Tmprss6$^{-/-}$ Tfr2$^{-/-}$ vs Tmprss6$^{+/-}$ Tfr2$^{-/-}$	ns	**	*	**	*	**	**	*	*	ns	ns	*	ns	ns	*	*	ns
Tfr2^{LCKO} vs Tmprss6$^{+/-}$ Tfr2$^{-/-}$	ns	ns	ns	*	ns	ns	*	ns	*	ns	ns	ns	*	ns	*	ns	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs Tmprss6$^{+/-}$ Tfr2$^{-/-}$	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs Tmprss6$^{+/-}$ Tfr2$^{-/-}$	ns	*	*	**	**	**	**	*	*	ns	ns	*	ns	*	*	*	ns
Tfr2^{LCKO} vs Tmprss6$^{-/-}$ Tfr2$^{-/-}$	ns	**	**	**	**	**	**	**	**	*	ns	*	*	*	*	*	ns
Tmprss6$^{+/-}$ Tfr2^{LCKO} vs Tmprss6$^{-/-}$ Tfr2$^{-/-}$	ns	**	**	**	**	**	**	**	*	ns	ns	*	*	ns	*	*	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs Tmprss6$^{-/-}$ Tfr2$^{-/-}$	ns	*	ns	ns	*	*	*	ns	ns	ns	ns	ns	ns	*	*	*	ns
Tmprss6$^{+/-}$ Tfr2^{LCKO} vs Tfr2^{LCKO}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs Tfr2^{LCKO}	ns	*	**	**	**	**	**	**	**	*	ns	*	*	**	*	*	ns
Tmprss6$^{-/-}$ Tfr2^{LCKO} vs Tmprss6$^{+/-}$ Tfr2^{LCKO}	ns	ns	**	**	**	**	**	**	**	ns	ns	ns	*	**	*	*	ns

Complete list of the P-values referred to Table 1 and Figure 1, 2 and supplemental Figure 1. Asterisks refer to a statistically significant difference. *$P<0.05$; **$P<0.005$; ns= not significant. WBC= White Blood Cells; RBC= Red Blood Cells; Ret= reticulocytes; Hb= Hemoglobin; Hct= Hematocrit; MCV= Mean Corpuscolar Volume; MCH= Mean Corpuscolar Hemoglobin; TS= Transferrin Saturation; LIC= Liver Iron Content; *Tfr1*= mRNA expression of Transferrin Receptor 1; SIC= Spleen Iron Content; Epo= erythropoietin; *Bmp6*= mRNA expression of Bone Morphogenetic Protein 6; *Hamp*= mRNA expression of Hepcidin; *Hamp*/LIC= Hepcidin/Liver Iron Content ratio; *Id1*= mRNA expression of Inhibitor of DNA binding 1; *Bmp6*/LIC= Bone Morphogenetic Protein 6/Liver Iron Content ratio.

SUPPLEMENTAL FIGURE 1
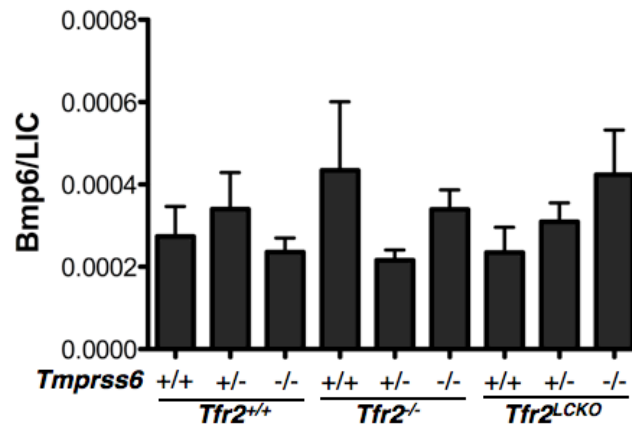
LEGEND TO SUPPLEMENTAL FIGURE 1

Normalization of *Bmp6* mRNA on the Liver Iron Content (LIC)

In the figure is graphed the hepatic expression of *Bmp6* normalized on the levels of Liver Iron Content (LIC). Mean Values of 3-6 animals for genotype are graphed and error bars indicate standard deviation.