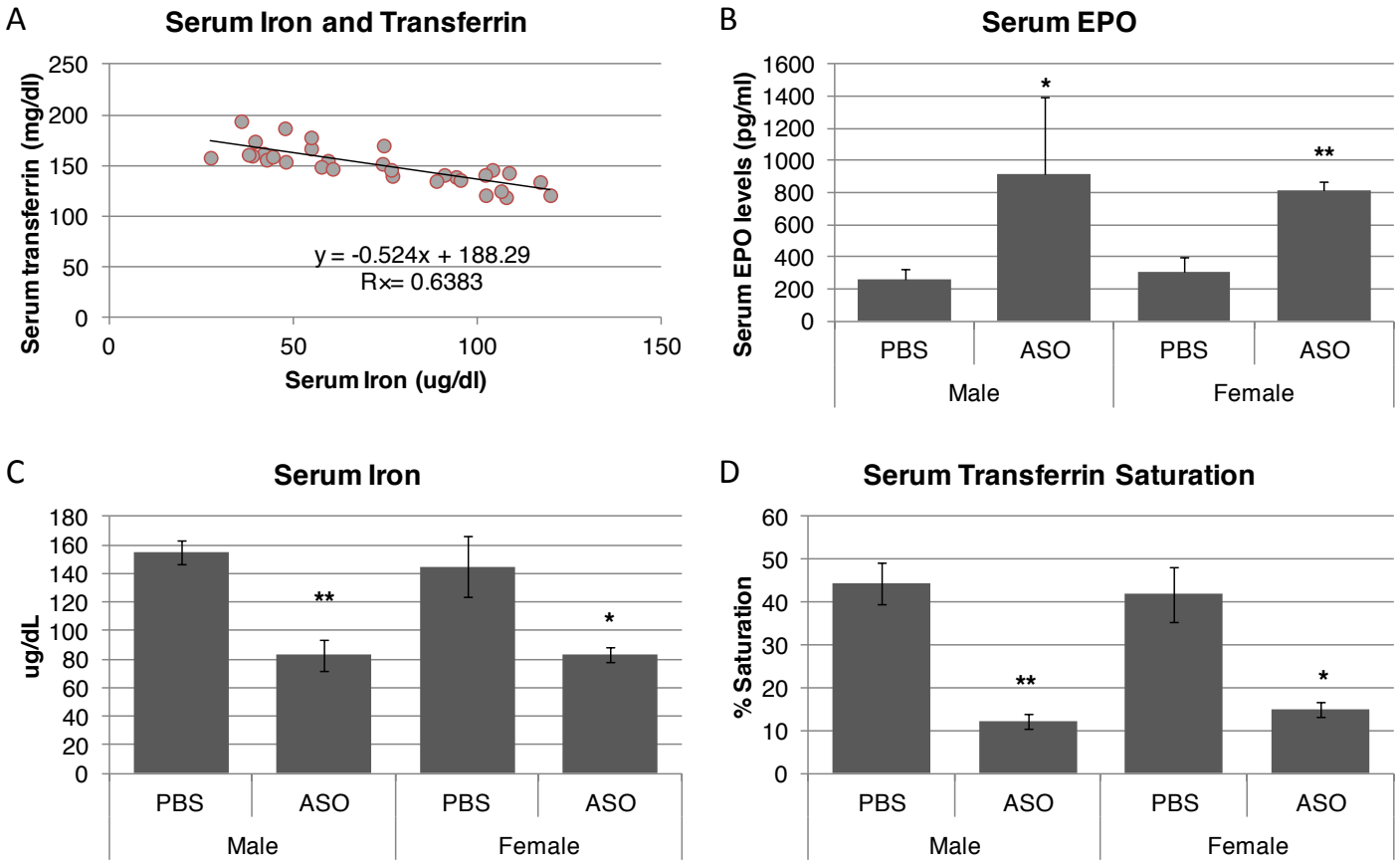
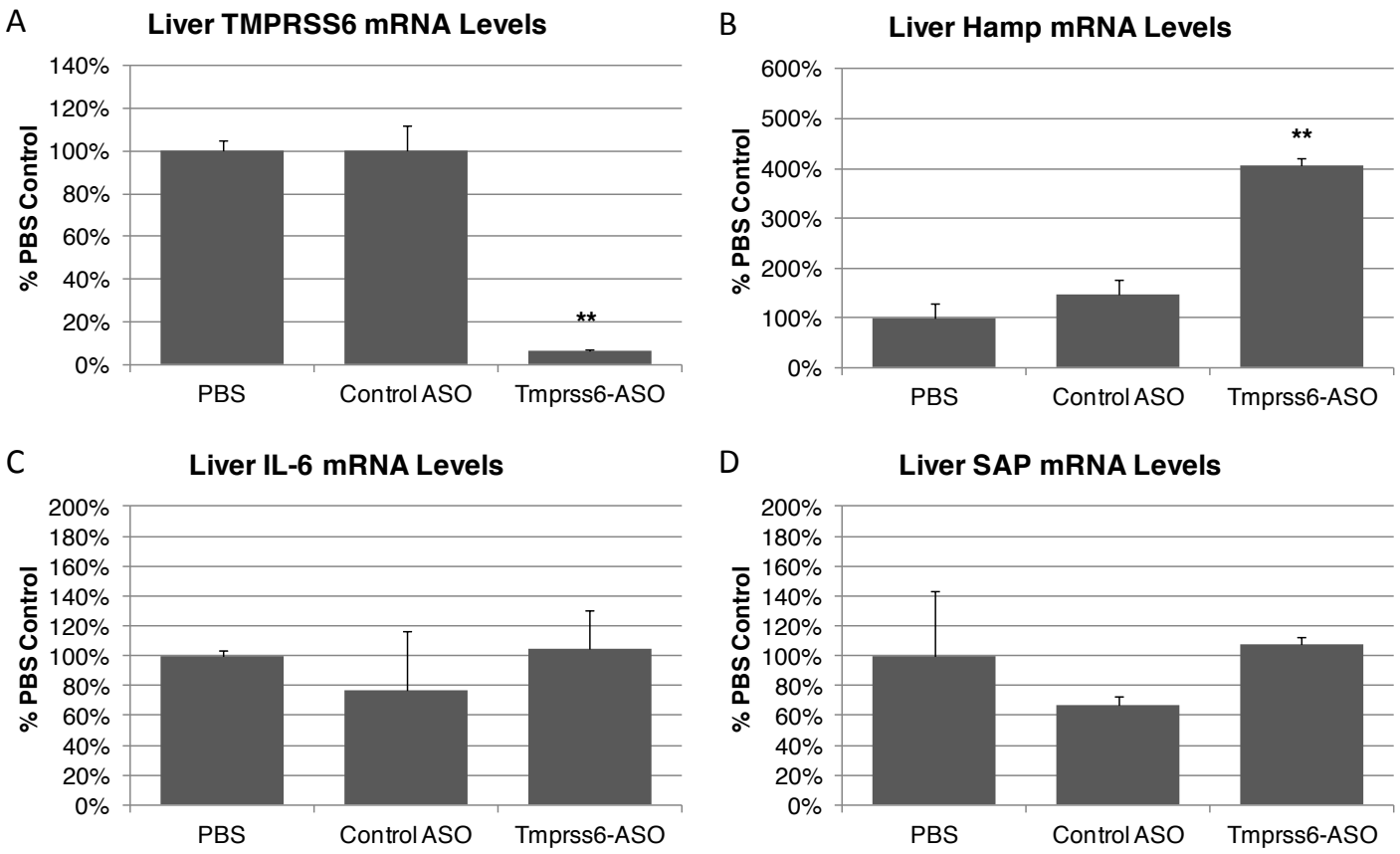
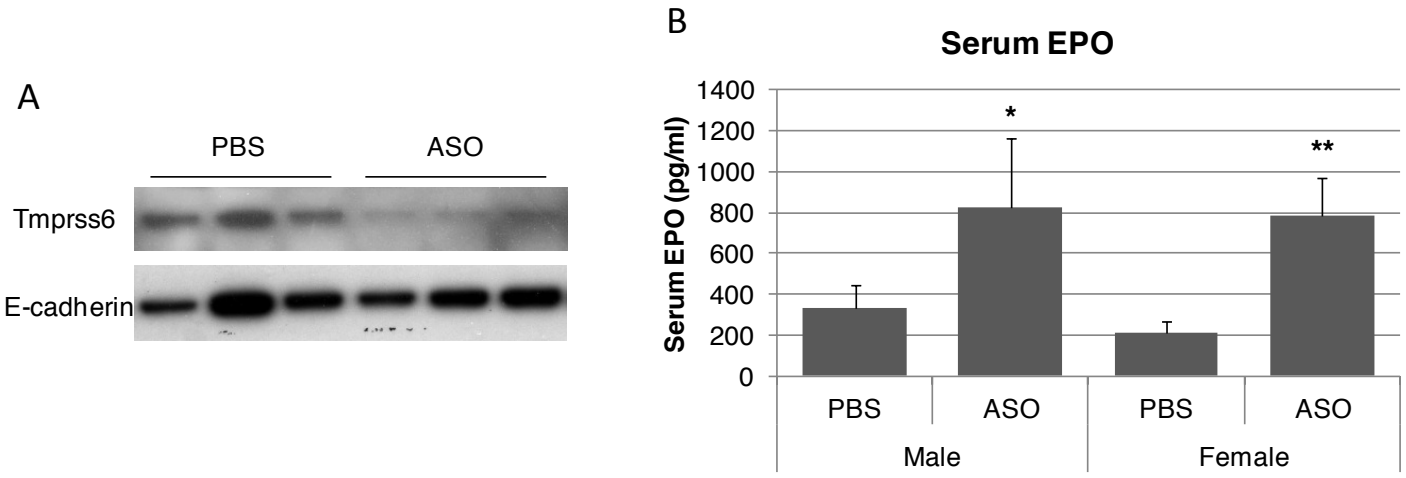


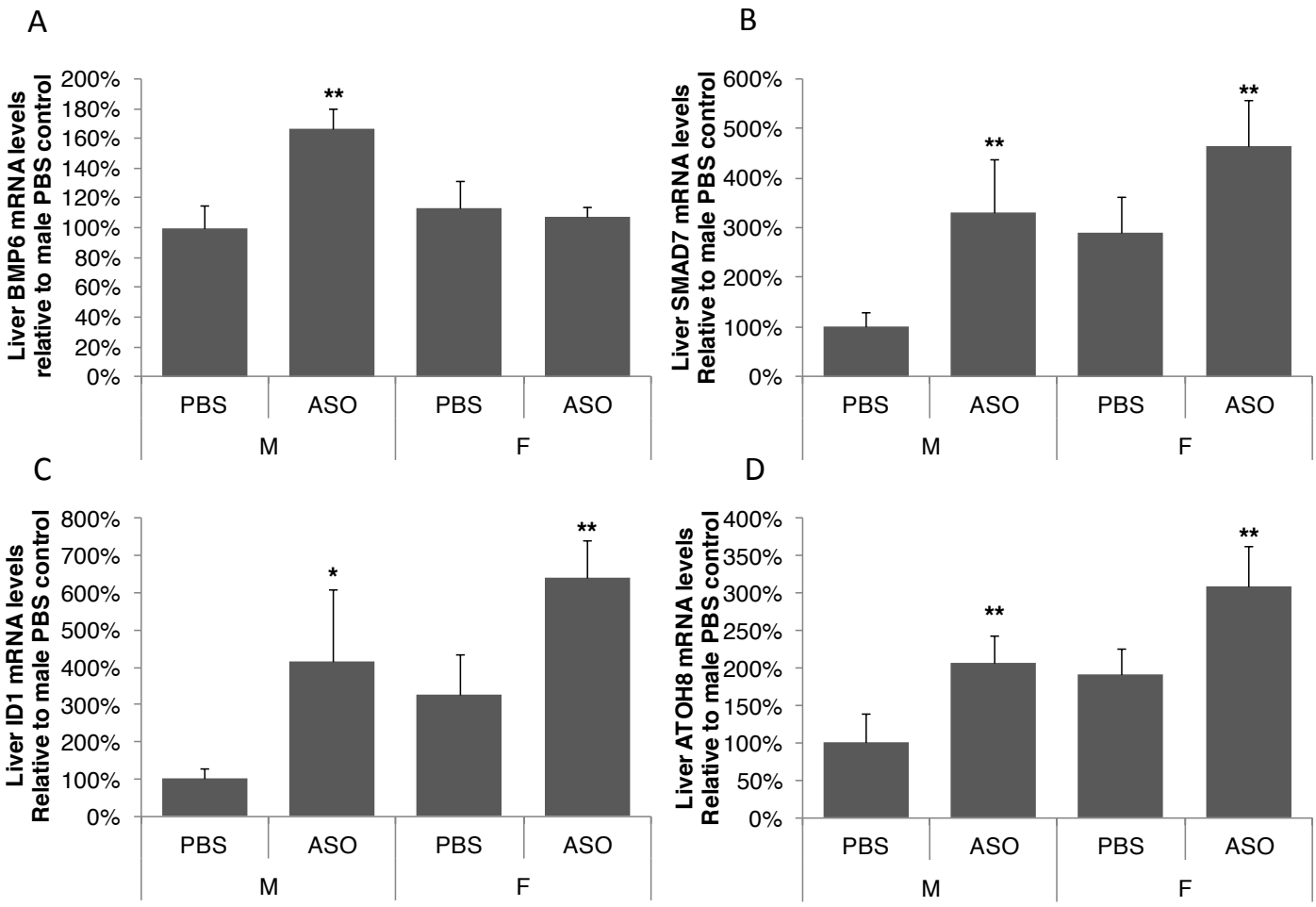
Supplementary Figure 1. (**A**) Inverse relationship between serum iron and serum transferrin levels. Six-week old C57BL/c male mice were treated for 4 weeks with the indicated doses in mg/kg/week of *Tmprss6*-ASO via subcutaneous injection as indicated in Figure 1. Serum iron and transferrin levels were plotted as dots. (**B**) EPO elevation, (**C**) Serum iron reduction and (**D**) serum transferrin saturation in both male and female WT mice after 6 week treatment with 100mg/kg/week. Results represent mean ± standard deviation (N=4); *: $P<0.05$, **: $P<0.01$ by Student's t-test.



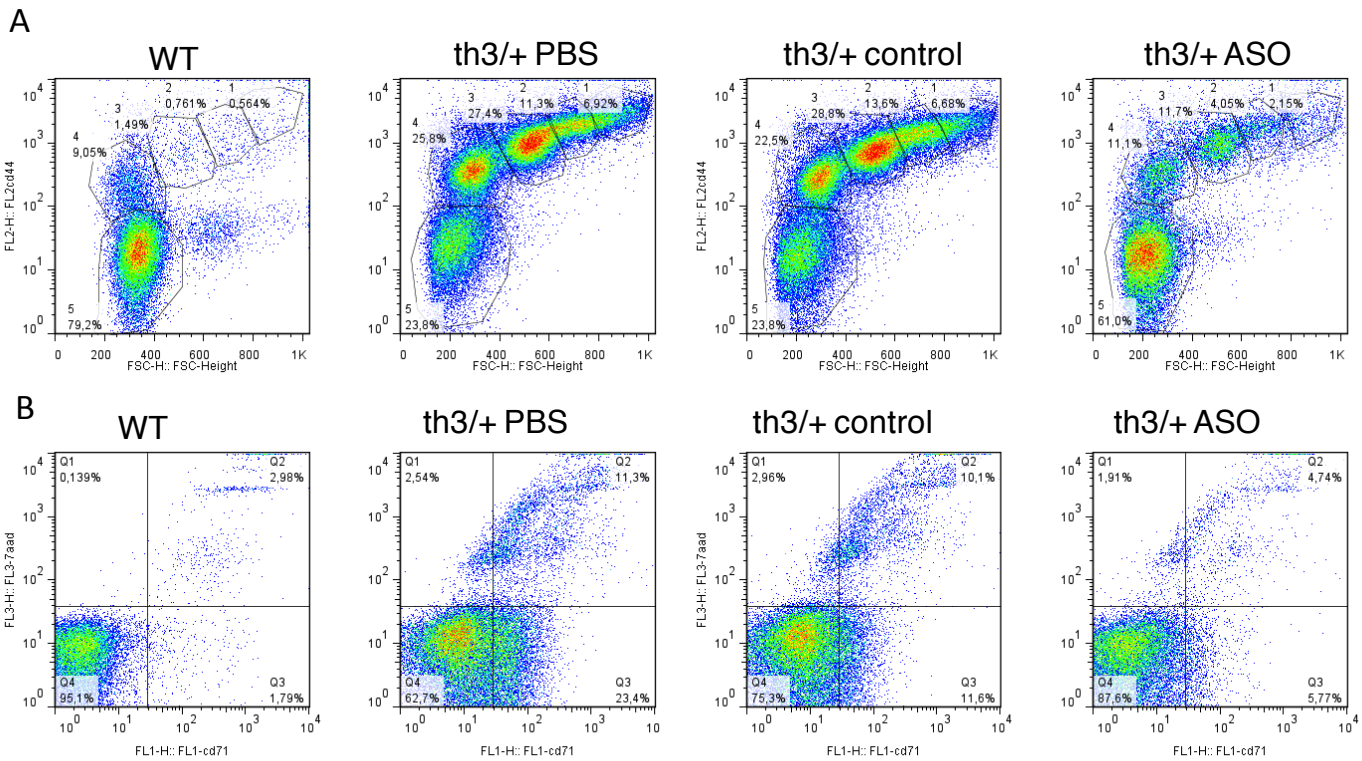
Supplementary Figure 2. *Tmprss6*-ASO or control ASO did not induce significant inflammatory response. (**A**) Control ASO treatment did not change *Tmprss6* mRNA in liver. (**B**) Control ASO treatment did not significantly change *Hamp* mRNA in liver, (**C**) Liver IL-6 and (**D**) SAP did not change significantly in C57BL/6 mice after 6 week treatment with 100mg/kg/week. Results represent mean ± standard deviation (N=3); *: $P<0.05$, **: $P<0.01$ by Student's t-test when ASO treatment group was compared with PBS group.



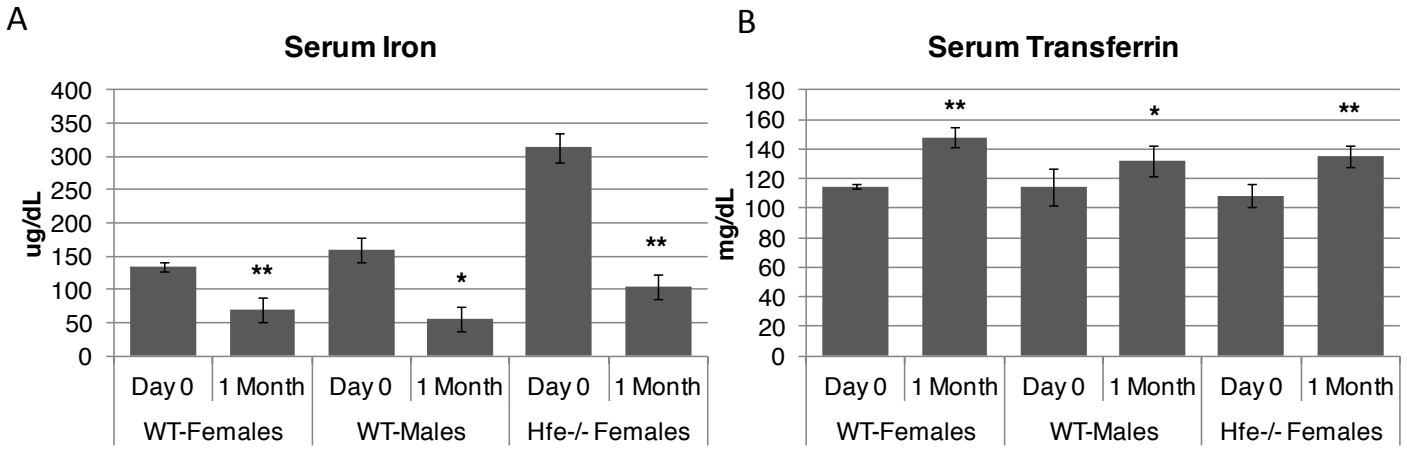
Supplementary Figure 3. Liver Tmprss6 protein reduction and serum EPO elevation after *Tmprss6*-ASO treatment. (**A**) Tmprss6 Western blot. Hfe-/- mice were treated with PBS or 100mg/kg/week Tmprss6-ASO for 6 weeks. Liver membrane prep from snap-frozen liver tissues was prepared as described (Enns et al. PMID: 22893705) and separated using 4-12% NuPAGE Novex gel (Invitrogen) under reducing conditions. Western blotting was achieved via anti-TMPRSS6 (Sigma) and anti-E-cadherin (Cell Signaling) antibodies. (**B**) Serum EPO was analyzed with ELISA. Results represent mean ± standard deviation (N=3); *: $P<0.05$, **: $P<0.01$ by Student's t-test when ASO treatment group was compared with PBS group.



Supplementary Figure 4. *Tmprss6*-ASO treatment affect gene expression involved in erythropoiesis. (**A**) *Bmp6*, (**B**) *Smad7*, (**C**) *Id1* and (**D**) *Atoh8* expression were increased after *Tmprss6*-ASO treatment. Liver mRNA was analyzed after 6-week treatment with 100mg/kg/week in th3/+ mice. Results represent mean ± standard deviation (N=3); *: $P<0.05$, **: $P<0.01$ by Student's t-test when ASO treatment group was compared with PBS group of the same gender.



Supplementary Figure 5. (A) Representative Facs analysis to discriminate different stages of erythroid differentiation in the spleen using the Ter119, CD71 and CD44 antibodies in the spleen of th3/+ mice. This assay allows the separation of erythroid cells into distinct populations corresponding to proerythroblasts (fraction I), basophilic (II), polychromatic (III), orthochromatic cells, and reticulocytes (IV), and mature RBC (V). **(B)** Representative Facs analysis to determine apoptosis using the CD71 antibody and 7-AAD in the spleen of th3/+ mice.



Supplementary Figure 6. (**A**) Serum iron reduction and (**B**) serum transferrin elevation in both male and female WT mice, and female *Hfe*-/- mice after one month on a low iron diet (4ppi). Results represent mean ± standard deviation (N=4 for WT female mice, N=3 for WT male and *Hfe*-/- mice)); *: $P<0.05$, **: $P<0.01$ by Student's t-test compared with baseline.

Strain	gender	treatment	RBC (10^6/µL)	HGB (g/dl)	HCT (%)	MCV (fL)	MCH (pg)	RETICULOCYTE (10^9 cells/L)
WT	M	PBS	9.9 ± 0.4	14.9 ± 0.6	48.2 ± 1.1	48.5 ± 1.3	14.9 ± 0.4	331.0 ± 101.3
		ASO	9.1 ± 0.1**	11.3 ± 0.6**	38.0 ± 1.6**	41.5 ± 1.7**	12.4 ± 0.6**	281.0 ± 8.0
	F	PBS	9.3 ± 0.4	14.0 ± 0.3	46.1 ± 1.2	50.0 ± 1.4	15.1 ± 0.5	273.3 ± 76.4
		ASO	9.3 ± 0.3	12.3 ± 0.4**	41.0 ± 1.8*	44.3 ± 0.5**	13.2 ± 0.2**	288.3 ± 35.2
Hfe -/-	M	PBS	9.4 ± 0.4	15.2 ± 0.5	49.7 ± 1.7	53.0 ± 1.2	16.3 ± 0.2	355.5 ± 59.5
		ASO	8.6 ± 0.2*	12.4 ± 0.4**	40.1 ± 0.9**	46.8 ± 0.5**	14.5 ± 0.4**	302.3 ± 99.6
	F	PBS	9.4 ± 0.4	15.7 ± 0.8	50.5 ± 2.7	53.8 ± 1.0	16.7 ± 0.2	397.8 ± 67.0
		ASO	8.9 ± 0.5	13.5 ± 0.5*	44.3 ± 1.6*	50.0 ± 0.8**	15.2 ± 0.8*	567.8 ± 128.7*

Supplementary table 1. CBC values in WT and *Hfe*-/- mice after treatment with *Tmprss6*-ASO or PBS control. 8-week old C57BL/6 and *Hfe*-/- mice were treated with 100mg/kg/week for 6 weeks before analysis. CBC was analyzed at IDEXX. Results represent mean ± standard deviation; N=4 for each group. *: $P<0.05$, **: $P<0.01$ by Student's t-test when ASO treatment group was compared with PBS group.

Time point		D0			D21			D42		
RBC (X 10^6 cells/uL)	PBS	7.30	±	0.31	7.72	±	0.57	8.12	±	0.62
	control ASO	7.63	±	0.23	7.69	±	0.12	8.03	±	0.11
	Tmprss6-ASO	7.71	±	0.68	8.79	±	0.72	10.20	±	0.82*
HGB (g/dL)	PBS	7.40	±	0.28	7.60	±	0.57	7.80	±	0.28
	control ASO	7.20	±	0.33	7.10	±	0.20	7.20	±	0.00
	Tmprss6-ASO	6.93	±	0.46	7.73	±	0.46	8.53	±	0.23**
HCT (%)	PBS	29.80	±	0.28	29.20	±	0.00	31.60	±	0.00
	control ASO	28.60	±	2.37	27.30	±	1.19	28.60	±	0.69
	Tmprss6-ASO	27.07	±	0.61	29.33	±	0.83*	32.80	±	1.83*
RETIC (x 10^9 cells/L)	PBS	2138	±	573	2069	±	16	1818	±	100
	control ASO	1720	±	513	1634	±	317	1454	±	196
	Tmprss6-ASO	1303	±	360	839	±	154**	426	±	155**
MCV (fL)	PBS	40.65	±	2.19	37.70	±	2.69	39.10	±	2.97
	control ASO	37.40	±	1.88	35.35	±	1.05	35.85	±	1.25
	Tmprss6-ASO	35.13	±	2.40	33.53	±	1.88	32.23	±	0.96**
MCH (pg)	PBS	10.05	±	0.07	9.80	±	0.14	9.65	±	0.78
	control ASO	9.43	±	0.05	9.35	±	0.26	8.95	±	0.21
	Tmprss6-ASO	9.10	±	0.26	8.70	±	0.20**	8.43	±	0.42*
RDW (%)	PBS	35.30	±	4.24	35.10	±	2.97	32.75	±	2.05
	control ASO	37.03	±	0.59	37.20	±	1.79	36.65	±	1.20
	Tmprss6-ASO	35.90	±	5.19	28.50	±	2.77**	24.43	±	2.30**

Supplementary table 2. CBC values in *Th3/+* mice after treatment with PBS, control ASO or *Tmprss6*-ASO PBS in th3/+ mice. Mice were treated with 100mg/kg/week for 6 weeks before analysis. Results represent mean ± standard deviation; N=3 for each group. *: $P<0.05$, **: $P<0.01$ by Student's t-test when *Tmprss6*-ASO treatment group was compared with control ASO treatment group.

Name	Forward Sequence	Reverse Sequence	Probe Sequence
mIL6	CCTACCCCAATTTCCAATGCT	GAATTGGATGGTCTTGGTCCTTA	CCTAACAGATAAGCTGGAGTCACAGAAGGAGTGG
mSAP	ATTTTGAATTGGCAGGCTCTTAA	GCCATCTGATGTCCATGAGGTT	ATGGCTACGTAGTCATCAGGCCCCG
mATOH8	CGCCTCAACGGAGATCAAAG	CGAAGGCTGCGCTGATG	AGACCCGGAGGCTTCTGGCCAAC
mBMP6	CCAAGTCTTGCAGGAGCATCA	AATTCCAGCCAACCTTCTTCTG	ACTCTGACCTATTTTTGTTGGACACCCGG
mID1	CAGTGGGTAGAGGGTTTGATCAA	AACCCCCTCCCCAAAGTCT	AGAGCCTCACCCTCTCCACCTTTCAGC
mSMAD7	CCATCAAGGCTTTTGACTATGAGA	CCATGGTTGCTGCATGAACT	CTACAGCCTGCAGCGGCCCAA

Supplementary table 3. Additional Q-RT-PCR primers and probes used in this study.