Anti-hemojuvelin antibody corrects anemia caused by inappropriately high hepcidin levels

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doi:10.3324/haematol.2015.140772

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Materials and Methods

Rat ACD model

Twelve week old healthy Sprague Dawley female rats were injected intraperitoneally (ip) with 15 μg/rhamnose/g peptidoglycan-polysaccharide (PGPS-10) (BD Biosciences) as described previously [10]. Three weeks after the injection, anemia ensued and the antibody treatment started. Antibodies were administered iv into the tail vein at 20 mg/kg once per week for 4 weeks. Rats were sacrificed one week after the last dose. Hb was measured by taking no more than 500 μl of blood (n=16-18/group). Rats that did not develop anemia were excluded from the study.

Mouse inflammatory high hepcidin model

Inflammation was induced in 14-16 week old healthy wild-type C57BL/6J female mice by ip injection with 200 μl diluted heat-killed Brucela abortus antigen (HKBA) (c.c.pro GmbH (Germany)) as described previously[11] and 30 min later treated iv with 20mg/kg h5F9-AM8. 6 hours after HKBA injection mice were sacrificed and livers snap frozen (n=6/group). Mice that did not develop anemia were excluded from the study.

Mouse non-inflammatory high hepcidin model

20μl of fresh blood from 14 week-old homozygous ($Tmprss6^{-/-}$) and heterozygous ($Tmprss6^{+/-}$) male and female mice was used to determine Hb levels (Day 0). Next day, mice were injected iv with 20mg/kg mAbs. Every 2 weeks Hb was measured. At necropsy bone marrow (BM) was collected, fixed and semi-quantitative histopathology performed (n=5-6/ group).

Hematological analysis

Complete blood counts (CBC) were measured using the scilVet ABC blood analyzer (scilVet, Germany).

RNA extraction and *Hamp* mRNA analysis

Total RNA was extracted from frozen tissue using TRIzol and 1µg was used for cDNA synthesis using Superscript II transcriptase according to standard protocols. Liver *Hamp* mRNA expression was analyzed using the mouse specific primers for *Hamp*: 5'-ATACCAATGCAGAAGAGAAGG-3', 5'-AACAGATACCACACTGGGAA-3' and *Tubulin5* (*mTubb5*): 5'- GGGAAATCGTGCACATCCA-3', 5'-ATGCCATGTTCATCGCTTATCA-3' and SYBR green master mix.

Binding studies

Nunc-Immuno™ MicroWell™ 96 well solid plates (Sigma-Aldrich) were coated with 2.5µg/ml recombinant human (rh)BMP-6 (RnD Systems), rhBMP-4 (RnD Systems) or Neogenin (RnD Systems) and incubated for 1hour. The plates were blocked using 3% BSA and incubated with appropriate antibodies for 90 minutes. Biotinylated anti-human HJV (RnD Systems) was added for 90 minutes. 50µl of streptavidin POD (Roche) were added and after 1hour of incubation, the reaction was ended by immuno Pure TMB Substrat Kit (Pierce) solution were added. The reaction was stopped using 50µl 2.5M H_2SO_4 solution. The plates were washed three times between all steps using PBST (0.02% Tween-20) and all incubation steps were carried out in a humidity chamber.

Luciferase reporter assay

The BMP-responsive element (BRE) based on the BRE promoter sequence of Id1 [11] was cloned into a basic luciferase reporter vector pGL4.27 [luc2P/minP/Hygro] (Promega) to generate a BRE luciferase reporter construct. The HJV BMP reporter assay was established by cotransfection of 293HEK cells with the BMP reporter and HJV expression plasmids. Transfected cells were treated with serial dilutions of h5F9.23 or h5F9-AM8, and incubated overnight at 37°C, 5% CO_2. Luciferase activity was measured by adding One-Glo luciferase reagent (Promega) to cells and reading the plate in a Topcount reader. IC50 values were calculated by the GraphPad Prism 5 software using Four Parameter Logistic non-linear regression curve-fitting model.

Statistical analysis

Data are presented as mean ± SEM. $P<0.05$ was considered statistically significant. For mouse experiments, unpaired 2-tailed Student's t-tests were performed using Graph PadPrism software. For the cross-over experiment P values were calculated using mixed effects model fitting change from baseline by timepoint and using baseline as a covariate. Rat ACD experiments were analyzed using mixed model and by ANOVA.

Study approval

Approval for the use of animals was granted according to the European Molecular Biology Laboratory animal protection guidelines.

Supplementary Table 1. **The monoclonal RGMa/c antibodies inhibit the interaction of HJV with the BMP ligands (BMP-4 and BMP-6) and Neogenin in a binding assay.** ++ = very strong competition in the low nanomolar range, + = strong competition with an approx. ten-fold difference to ++. Firefly luciferase crystalizible fragment antibody heavy chain (FL–fc), Neogenin Histag (Neo-HIS), bone morphogenetic protein (BMP), hemojuvelin (HJV).

mAb	Compete with RGMa-FL-fc for binding to Neo-HIS, n=4	Compete with HJV for binding to BMP-2, n=4	Compete with HJV for binding to BMP-4, n=4	Compete with HJV for binding to BMP-6, n=4	Compete with HJV for binding to Neo-HIS, n=3	Compete with RGMa-FL-fc for binding to BMP-6, n=3
h5F9.23	++	++	++	++	++	+
h5F9-AM8	++	++	++	++	++	+

Supplementary Table 2: Complete blood count (CBC) indices for rats injected with saline or PGPS and treated with 20mg/kg hIgG or RGMa/c mAbs (h5F9.23, h5F9-AM8) for four weeks. Experimental design is shown in Figure 2A. Table shows Mean ± SEM. Peptidoglycan polysaccharide (PGPS).

	Treatment	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
week 0	Saline/hIgG	7.72 ± 0.22	6.13 ± 0.06	14.87 ± 0.13	37.37 ± 0.36	60.92 ± 0.34	24.22 ± 0.10	39.78 ± 0.15	773 ± 19
	PGPS/hIgG	8.43 ± 0.19	6.16 ± 0.09	14.6 ± 0.14	37.67 ± 0.36	61.25 ± 0.56	23.86 ± 0.30	39.01 ± 0.23	750 ± 18
	PGPS/h5F9.23	7.42 ± 0.19	6.05 ± 0.06	14.31 ± 0.09	36.92 ± 0.33	60.89 ± 0.42	23.68 ± 0.19	38.79 ± 0.16	825 ± 17
	PGPS/h5F9-AM8	6.80 ± 0.39	6.04 ± 0.1	14.47 ± 0.15	37.34 ± 0.36	62.1 ± 0.53	24.04 ± 0.22	38.89 ± 0.22	724 ± 14
week 3	Saline/hIgG	7.14 ± 0.14	5.81 ± 1.03	15.45 ± 0.17	34.02 ± 6.63	58.43 ± 0.93	26.71 ± 3.84	45.81 ± 7.36	598 ± 137
	PGPS/hIgG	7.77 ± 1.12	4.89 ± 0.89	11.94 ± 0.48	30.71 ± 2.72	57.89 ± 0.11	22.66 ± 1.13	39.14 ± 1.89	1032 ± 154
	PGPS/h5F9.23	6.67 ± 0.12	5.87 ± 0.18	11.7 ± 0.50	32.74 ± 1.36	55.67 ± 1.09	19.92 ± 0.47	35.74 ± 0.26	1184 ± 109
	PGPS/h5F9-AM8	6.45 ± 0.06	5.55 ± 0.21	11.75 ± 0.47	32.07 ± 1.40	57.7 ± 1.38	21.21 ± 0.51	36.7 ± 0.22	1041 ± 83
week 4	Saline/hIgG	6.99 ± 0.42	5.37 ± 1.27	14.65 ± 0.75	31.55 ± 7.63	58.79 ± 0.46	30.73 ± 9,73	52.38 ± 16.93	620 ± 178
	PGPS/hIgG	3.85 ± 0.76	5.66 ± 0.18	12.37 ± 0.25	32.71 ±1.01	57.68 ± 0.18	21.88 ± 1.00	37.9 ± 1.81	937 ± 64
	PGPS/h5F9.23	5.15 ± 0.45	5.91 ± 0.18	12.34 ± 0.16	33.38 ± 1.60	56.32 ± 1.2	21.17 ± 0.4	37.57 ± 1.49	996 ± 71
	PGPS/h5F9-AM8	4.53 ± 0.23	5.72 ± 0.3	12.39 ± 0.34	33.47 ± 0.96	58.32 ± 1.43	21.58 ± 1.86	36.83 ± 2.26	1029 ± 29
week 5	Saline/hIgG	5.24 ± 2.46	7.12 ± 0.34	15.62 ± 0.04	41.86 ± 1.79	58.97 ± 0.22	21.18 ± 1.86	35.96 ± 2.99	674 ± 68
	PGPS/hIgG	3.76 ± 0.76	5.96 ± 0.26	12.34 ± 0.83	33.78 ± 0.79	56.48 ± 1.1	20.26 ± 0.83	35.99 ± 2.1	1155 ± 34
	PGPS/h5F9.23	8.11 ± 0.62	6.59 ± 0.49	13.05 ± 0.27	37.47 ± 3.83	56.68 ± 1.57	19.84 ± 1.08	35.12 ± 2.87	1130 ± 40
	PGPS/h5F9-AM8	9,44 ± 0.39	6.72 ± 0.31	13.9 ± 0.47	39.54 ± 1.99	58.75 ± 0.32	20.77 ± 0.91	35.37 ± 1.69	982 ± 111
week 6	Saline/hIgG	7.243 ± 1.45	7.21 ± 0.54	15.45 ± 0.26	42.52 ± 2.84	59.2 ± 0.28	21.52 ± 1.2	36.41 ± 1.79	649 ± 162
	PGPS/hIgG	9.47 ± 0.7	6.49 ± 0.08	12.92 ± 0.34	36.58 ± 0.11	56.04 ± 0.53	19.84 ± 0.31	35.39 ± 0.94	1065 ± 63
	PGPS/h5F9.23	9.2 ± 0.93	6.78 ± 0.01	13.59 ± 0.17	37.79 ± 1.71	55.53 ± 0.86	19.99 ± 0.27	35.98 ± 1.01	962 ± 108
	PGPS/h5F9-AM8	9.64 ± 0.75	6.59 ± 0.25	13.83 ± 0.03	37.98 ± 1.66	57.38 ± 0.37	20.57 ± 0.39	35.75 ± 0.91	1083 ± 35
week 7	Saline/hIgG	7.78 ± 0.38	7.12 ± 0.24	15.1 ± 0.09	42.29 ± 1.43	59.53 ± 0.02	21.34 ± 0.92	35.85 ± 1.54	764 ± 31
	PGPS/IgG	9.86 ± 0.55	6.59 ± 0.02	12.74 ± 0.42	36.85 ± 0.41	55.74 ± 0.83	19.26 ± 0.62	34. 62 ± 1.63	975 ± 106
	PGPS/h5F9.23	6.92 ± 0.44	6.65 ± 0.23	12.96 ± 0.16	37.03 ± 1.43	55.57 ± 0.06	19.46 ± 0.93	35.01± 1.77	1079 ± 76
	PGPS/h5F9-AM8	6.95 ± 0.13	6.66 ± 0.25	13.04 ± 0.17	39.07 ± 1.87	58.52 ± 0.83	19.55 ± 0.51	33.46 ±1.25	962 ± 199

Supplementary Table 3: CBC and RBC indices in Tmprss6-/- and Tmprss6+/- mice treated with 20mg/kg h5F9-AM8 or hIgG.
Detailed experimental schematic is in Figure 3B. Data are shown as Mean ± SEM.

Week 0	WBC	LYM	LYM%	MON	MON%	GRA	GRA%	EOS%	RBC	HGB	HCT	MCV	MCH	MCHC	RWD	PLT
Tmprss6 +/- hIgG	12.49 ± 0.51	10.04 ± 0.29	81.06 ± 1.45	0.48 ± 0.05	4.16 ± 0.17	1.96 ± 0.2	14.78 ± 1.3	1.04 ± 0.3	10.51 ± 0.10	16.37 ± 0.24	53.29 ± 0.65	50.8 ± 0.37	15.58 ± 0.17	30.72 ± 0.12	12.22 ± 0.27	1427 ± 116
Tmprss6 +/- h5F9-AM8	12.65 ± 0.32	10.26 ± 0.30	81.34 ± 0.73	0.52 ± 0.02	4.38 ± 0.17	1.88 ± 0.07	14.28 ± 0.63	1.14 ± 0.26	11.2 ± 0.27	17.3 ± 0.38	56 ± 1.3	50 ± 0.31	15.46 ± 0.25	30.92 ± 0.41	12.2 ± 0.11	1349 ± 86
Tmprss6 -/- hIgG	17.02 ± 1.75	12.74 ± 1.09	75.54 ± 1.45	0.94 ± 0.19	5.7 ± 0.56	3.32 ± 0.48	18.76 ± 1.03	2.26 ± 1.17	11.43 ± 0.22	13.57 ± 0.43	43.54 ± 1.4	38 ± 0.45	11.86 ± 0.16	31.16 ± 0.14	11.02 0.64	1906 ± 164
Tmprss6 -/- h5F9-AM8	16.73 ± 1.002	14.1 ± 0.75	84.68 1.7	0.6 ± 0.05	3.82 ± 0.24	2.04 ± 0.38	11.5 ± 1.69	146 ± 0.58	1145 ± 0.04	13.46 ± 0.21	42.15 ± 0.28	36.8 ± 0.2	11.76 ± 0.2	3193 ± 0.45	9,838 ± 0.20	2168 ± 36
Week 2																
Tmprss6 +/- hIgG	20.22 ± 1.93	14.7 ± 1.5	73.24 ± 3.9	0.86 ± 0.13	4.56 ± 0.57	4.66 ± 0.79	22.2 ± 3.38	3.5 ± 1.77	11.25 ± 0.19	17.85 ± 0.65	56.93 ± 1.2	50.6 ± 0.4	15.85 ± 0.40	31.34 ± 0.74	12.15 ± 0.17	1174 ± 277
Tmprss6 +/- h5F9-AM8	18.02 ± 4.16	13.58 ± 2.3	78.52 ± 3.47	0.7 ± 0.23	3.92 ± 0.31	3.76 ± 1.64	17.56 ± 3.2	2.9 ± 1.9	10.93 ± 0.13	16.99 ± 0.27	55.74 ± 0.7	51.2 ± 0.2	15.55 ± 0.10	30.48 ± 0.23	12.79 ± 1.15	1217 ± 131
Tmprss6 -/- hIgG	17.84 ± 0.31	14.8 ± 0.42	83.2 ± 1.4	0.58 ± 0.04	3.58 ± 0.24	2.44 ± 0.22	13.22 ± 1.23	0.9 ± 0.19	11.47 ± 0.09	13.58 ± 0.17	43.38 ± 0.71	37.6 ± 0.4	11.85 ± 0.09	31.32 ± 0.21	10. 61 ± 0.32	2290 ± 81
Tmprss6 -/- h5F9-AM8	18.82 ± 3.7	14.78 ± 2.4	80.44 ± 2.4	0.74 ± 0.19	4.02 ± 0.21	3.28 ± 1.17	15.54 ± 2.23	2.12 ± 1.3	11.79 ± 0.12	16.12 ± 0.25	51.92 ± 0.55	44.2 ± 0.37	13.67 ± 0.19	31.04 ± 0.21	14.43 ± 0.17	2017 ± 186
Week 4																
Tmprss6 +/- hIgG	12.03 ± 1.1	9.56 ± 0.82	79.92 ± 1.2	0.48 ± 0.08	4.4 ± 0.3	1.98 ± 0.23	15.68 ± 0.93	0.98 ± 0.07	11 ± 0.06	16.46 ± 0.21	54.4 ± 0.58	49.4 ± 0.4	14.97 ± 0.11	30.26 ± 0.14	12.29 ± 0.13	1503 ± 101
Tmprss6 +/- h5F9-AM8	12.32 ± 1.16	9.76 ± 0.9	79.66 ± 0.96	0.5 ± 0.08	4.3 ± 0.21	2.06 ± 0.21	16.04 ± 0.81	1.12 ± 0.12	10.89 ± 0.21	16.64 ± 0.3	53.8 ± 1.04	49.4 ± 0.25	15.29 ± 0.25	30.95 ± 0.46	12.43 ± 0.15	1311 ± 62
Tmprss6 -/- hIgG	16.24 ± 1.33	12.84 ± 1.13	79.18 ± 1.18	0.64 ± 0.05	4.32 ± 0.27	2.76 ± 0.25	16.5 ± 0.9	1.34 ± 0.32	10.13 ± 0.28	12.46 ± -/23	37.36 ± 1.06	37 ± 0.31	12.31 ± 0.25	33.4 ± 0.722	9.69 ± 0.21	2880 ± 98
Tmprss6 -/- h5F9-AM8	13.38 ± 0.6	10.56 ± 0.37	79.52 ± 1.79	0.52 ± 0.07	4.2 ± 0.35	2.32 ± 0.28	16.28 ± 1.54	1.16 ± 0.29	10.97 ± 0.12	14.66 ± 0.16	47.11 ± 0.38	43 ± 0.31	13.37 ± 0.13	31.12 ± 0.22	17.27 ± 0.42	1914 ± 61
Week 6																
Tmprss6 +/- hIgG	18.19 ± 3.1	13.46 ± 2.3	74.58 ± 3.17	0.7 ± 0.14	4.06 ± 0.12	4.02 ± 0.8	21.36 ± 3.1	4.7 ± 2.38	11.28 ± 0.15	16.85 ± 0.35	55.48 ± 0.82	49.2 ± 0.2	14.94 ± 0.15	30.36 ± 0.26	12.47 ± 0.26	1269 ± 214
Tmprss6 +/- h5F9-AM8	18.02 ± 2.7	18.02 ± 2.7	12.95 ± 1.73	73.28 ± 3.18	0.675 ± 0.11	4.1 ± 0.14	4.4 ± 1.06	22.63 ± 3.11	532 ± 2.84	17.34 ± 0.57	55.8 ± 1.02	48.5 ± 0.29	15.14 ± 0.33	31.06 ± 0.62	12.39 ± 0.31	1140 ± 289
Tmprss6 -/- hIgG	16.11 ± 1.47	12.23 ± 1.04	76.77 ± 4.1	0.575 ± 0.08	4.07 ± 0.35	3.32 ± 0.86	19.15 ± 3.74	3.25 ± 2.12	9.48 ± 0.21	12.18 ± 0.57	35.28 ± 0.39	37.5 ± 0.5	12.85 ± 0.56	34.55 ± 1.80	9.89 ± 0.31	3077 ± 226
Tmprss6 -/- h5F9-AM8	18.49 ± 3.14	13.66 ± 2.08	75.28 ± 3.6	0.74 ± 0.19	4.14 ± 0.31	4.12 ± 1.08	20.58 ± 3.38	3.9 ± 1.9	11.01 ± 0.15	13.83 ± 0.36	44.57 ± 0.93	40.6 ± 0.25	12.57 ± 0.16	31.03 ± 0.21	14.14 ± 0.47	2054 ± 440
Week 8																
Tmprss6 +/- hIgG	16.33 ± 2.34	12.02 ± 1.08	75.64 ± 3.21	0.58 ± 0.13	3.72 ± 0.26	3.72 ± 1.14	20.64 ± 2.98	2.96 ± 1.89	11.12 ± 0.20	16.34 ± 0.27	54.64 ± 1.07	49.2 ± 0.2	14.7 ± 0.17	29.91 ± 0.28	12.56 ± 0.22	1463 ± 174
Tmprss6 +/- h5F9-AM8	16.79 ± 2.16	11.2 ± 0.92	69.04 ± 4.15	0.78 ± 0.18	4.05 ± 0.58	4.82 ± 1.15	26.38 ± 3.7	7.06 ± 1.85	16.61 ± 2.43	16.76 ± 0.42	71.8 ± 10.8	48.6 ± 0.5	10.9 ± 1.4	22.51 ± 3.13	13.14 ± 0.27	917 ± 231
Tmprss6 -/- hIgG	12.64 ± 0.62	10.45 ±0.60	82.7 ± 1.47	0.4 ± 0.04	3.6 ± 0.36	1.77 ± 0.14	13.7 ± 1.17	1.05 ± 0.16	12.64 ± 0.16	12.6 ± 0.45	51.56 ± 1.53	40.75 ± 1.11	9.97 ± 0.40	24.56 ± 1.51	13.28 ± 1.48	3456 ± 295
Tmprss6 -/- h5F9-AM8	17.69 ± 3.35	14.06 ± 2.45	80.42 ±1.67	0.64 ± 0.14	3.86 ± 0.31	2.96 ± 0.80	15.72 ± 1.40	1.5 ± 0.61	12.04 ± 0.14	13.1 ± 0.22	47.87 ± 0.84	39.8 ± 0.37	10.88 ± 0.09	27.37 ± 0.18	12.59 ± 0.61	2631 ± 162
Cross over																
Week 10																
Tmprss6 +/- hIgG	13.28 ± 1.18	9.93 ± 0.38	77.38 ± 5.54	0.8 ± 0.25	5.83 ± 1.21	2.55 ± 0.82	16.78 ± 4.36	1.7 ± 0.72	11.12 ± 0.17	16.01 ± 0.32	53.49 ± 1.02	47.83 ± 0.31	14.4 ± 0.19	29.93 ± 0.29	13.13 ± 0.19	1521 ± 59
Tmprss6 +/- h5F9-AM8	11.86 ± 0.17	9.8 ± 0.91	82.92 ± 1.83	0.48 ± 0.02	4.38 ± 0.21	1.6 ± 0.2	12.7 ± 1.71	2.28 ± 0.85	11.01 ± 0.15	15.8 ± 0.27	53.34 ± 0.9	48.4 ± 0.4	14.35 ±0.09	29.63 ± 0.11	12.98 ± 0.13	1525 ± 150
Tmprss6 -/- hIgG	15.62 ± 1.47	13.48 ± 1.25	86.9 ± 0.32	0.48 ± 0.06	3.36 ± 0.09	1.66 ± 0.18	9.74 ± 0.25	1.22 ± 0.18	10.72 ± 0.21	12.98 ± 0.26	39.77 ± 1.04	37.2 ± 0.37	12.11 ± 0.18	32.69 ± 0.76	9.732 ± 0.38	2858 ± 397
Tmprss6 -/- h5F9-AM8	19.3 ± 1.8	16.34 ± 1.17	85.64 ± 2.31	0.62 ± 0.12	3.32 ± 0.3	2.32 ± 0.62	11.04 ± 2.04	1.54 ± 0.87	9.67 ± 0.54	12.2 ± 0.62	36.23 ± 2.4	37.4 ± 0.75	12.63 ± 0.12	33.84 ± 0.67	10.13 ± 0.67	3357 ± 440
Week 12																
Tmprss6 +/- hIgG	12.23 ± 0.5	9.37 ± 0.44	77.35 ± 3.08	0.47 ± 0.09	4.18 ± 0.56	2.4 ± 0.34	18.47 ± 2.53	0.93 ± 0.06	11.28 ± 0.28	16.68 ± 0.46	54.51 ± 1.6	48.17 ± 0.31	14.79 ± 0.11	30.62 ± 0.12	13.2 ± 0.25	1363 ± 250
Tmprss6 +/- h5F9-AM8	12.94 ± 1.24	10.18 ± 0.96	79.02 ± 0.78	0.5 ± 0.06	4.08 ± 0.2	2.26 ± 0.25	16.9 ± 0.61	1.16 ± 0.24	11.12 ± 0.15	16.95 ± 0.18	55.39 ± 0.51	49.8 ± 0.37	15.24 ± 0.15	30.59 ± 0.06	13.5 ± 0.16	1558 ± 100
Tmprss6 -/- hIgG	10.86 ± 2.0	9.14 ± 1.8	83.4 ± 1.9	0.3 ± 0.04	3.44 ± 0.35	1.42 ± 0.16	13.16 ± 1.55	1.3 ± 0.3	9.96 ± 0.54	12.49 ± 0.64	36.97 ± 2.5	36.8 ± 0.58	12.54 ± 0.15	33.95 ± 0.84	10.09 ± 0.32	2905 ± 383
Tmprss6 -/- h5F9-AM8	22.1 ± 2.9	16.36 ± 1.7	76.44 ± 2.5	0.96 ± 0.18	4.52 ± 0.2	4.38 ± 1.0	19.04 ± 2.33	1.97 ± 1.24	10.78 ± 0.42	16.66 ± 0.71	50.41 ± 2.3	46.8 ± 0.58	15.45 ± 0.25	33.1 ± 0.65	16.93 ± 0.54	2576 ± 441
Week 14																
Tmprss6 +/- hIgG	13.3 ± 2.8	11 ± 0.6	69.86 ± 2.4	0.88 ± 0.16	5.62 ± 0.68	4.08 ± 0.57	24.52 ± 0.18	3.64 ± 1.33	10.85 ± 0.14	15.89 ± 0.27	52.14 ± 0.72	48 ± 0.26	14.65 ± 0.21	30.47 ± 0.31	13.18 ± 0.27	1494 ± 105
Tmprss6 +/- h5F9-AM8	14.82 ± 1.3	11.62 ± 1.23	78.14 ± 2.03	0.64 ± 0.04	4.54 ± 0.37	2.56 ± 0.13	17.32 ± 1.8	1.6 ± 0.47	10.48 ± 0.14	16.18 ± 0.34	51.9 ± 0.69	49.6 ± 0.4	15.45 ± 0.34	31.2 ± 0.61	12.94 ± 0.16	1574 ± 109
Tmprss6 -/- hIgG	13.8 ± 2.07	11.96 ± 1.7	87.6 ± 1.71	0.32 ± 0.07	2.76 ± 0.27	1.52 ± 0.35	9.64 ± 1.46	0.64 ± 0.09	9.58 ± 0.14	11.94 ± 0.11	35.97 ± 0.90	37.4 ± 0.5	12.47 ± 0.12	33.25 ± 0.65	10.53 ± 0.45	2725 ± 428
Tmprss6 -/- h5F9-AM8	16.48 ± 1.32	12 ± 0.69	73.84 ± 3.19	0.76 ± 0.13	4.88 ± 0.54	3.74 ± 0.7	21.28 ± 2.9	3.44 ± 1.93	10.37 ± 0.32	15.13 ± 0.56	45.67 ± 1.75	44 ± 0.54	14.59 ± 0.16	33.13 ± 0.14	17.09 ± 0.64	2250 ± 437