PMP22 antisense oligonucleotides reverse clinical, pathological, and molecular features of Charcot-Marie-Tooth disease type 1A

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List of Supplementary Materials

Supplemental methods:

Behavioral assessments and electrophysiology

Behavioral assessments and electrophysiology were performed by an investigator who was blinded to treatment and genotypes. Tests were performed in the following order - rotarod, grip strength, then electrophysiology; one test per day. Mice were dosed with ASOs after all assessments were completed to avoid stress.

The rotarod test was performed as described (*1*). Briefly, mice were trained on a rotating rod (Ugo Basile, Varese, Italy) which accelerated from 2 to 30 rpm in 3 minutes. Two training sessions were given, followed by 1 h rest, and then two trial sessions. Latency to fall was recorded and averaged over two testing trials.

Hind limb grip strength was assessed using the Grip Strength Meter (Columbus Instruments, Columbus, OH). Briefly, the mouse was lifted such that the hind paws were at the same height as the metal grid which was attached to a force transducer meter. Grip was visually checked to assure that it was symmetric and tight, and that both paws were on the grid. The mouse was gently and slowly pulled away at a constant speed until its grasp was broken. Force was recorded by the meter and was averaged over three trials.

Electrophysiology was done as described with slight modifications (*2*). Briefly, mice were anesthetized under 3% isoflurane in 100% oxygen, and maintained under 2% isoflurane during the recordings. Temperature was maintained at 37 ºC using a heating pad. The left sciatic nerve was studied using Viking Quest NCS/EMG Portable EMG machine (Natus Neurology). Disposable mono-polar 27G needle electrodes (Natus Medical Inc., San Carlos, CA, catalog # 019-476200) were used for both stimulating and recording. For measurement of nerve

conduction velocity, stimulating cathodes were placed at sciatic notch (proximal) and 10 mm distal to the sciatic notch, while recording electrodes were placed sub-dermally on the muscle belly of the tibialis anterior muscle, and a ground electrode was placed at the animal's back, near the midline. Single pulses of 0.1 ms duration were delivered. Motor nerve conduction velocities (MNCVs) were calculated over the 10 mm segment. For measuring the amplitude of the compound motor action potential (CMAP), supramaximal pulses of 0.1 ms duration were delivered and CMAP amplitudes (peak to peak) were recorded.

References

1. H. T. Tran, C. H. Chung, M. Iba, B. Zhang, J. Q. Trojanowski, K. C. Luk, V. M. Lee, Alpha-synuclein immunotherapy blocks uptake and templated propagation of misfolded alpha-synuclein and neurodegeneration. *Cell Rep* **7**, 2054-2065 (2014).
2. C. Verhamme, R. H. King, A. L. Ten Asbroek, J. R. Muddle, M. Nourallah, R. Wolterman, F. Baas, I. N. van Schaik, Myelin and Axon Pathology in a Long-Term Study of PMP22-Overexpressing Mice. *J Neuropathol Exp Neurol* **70**, 386-398 (2011).

Figure S1

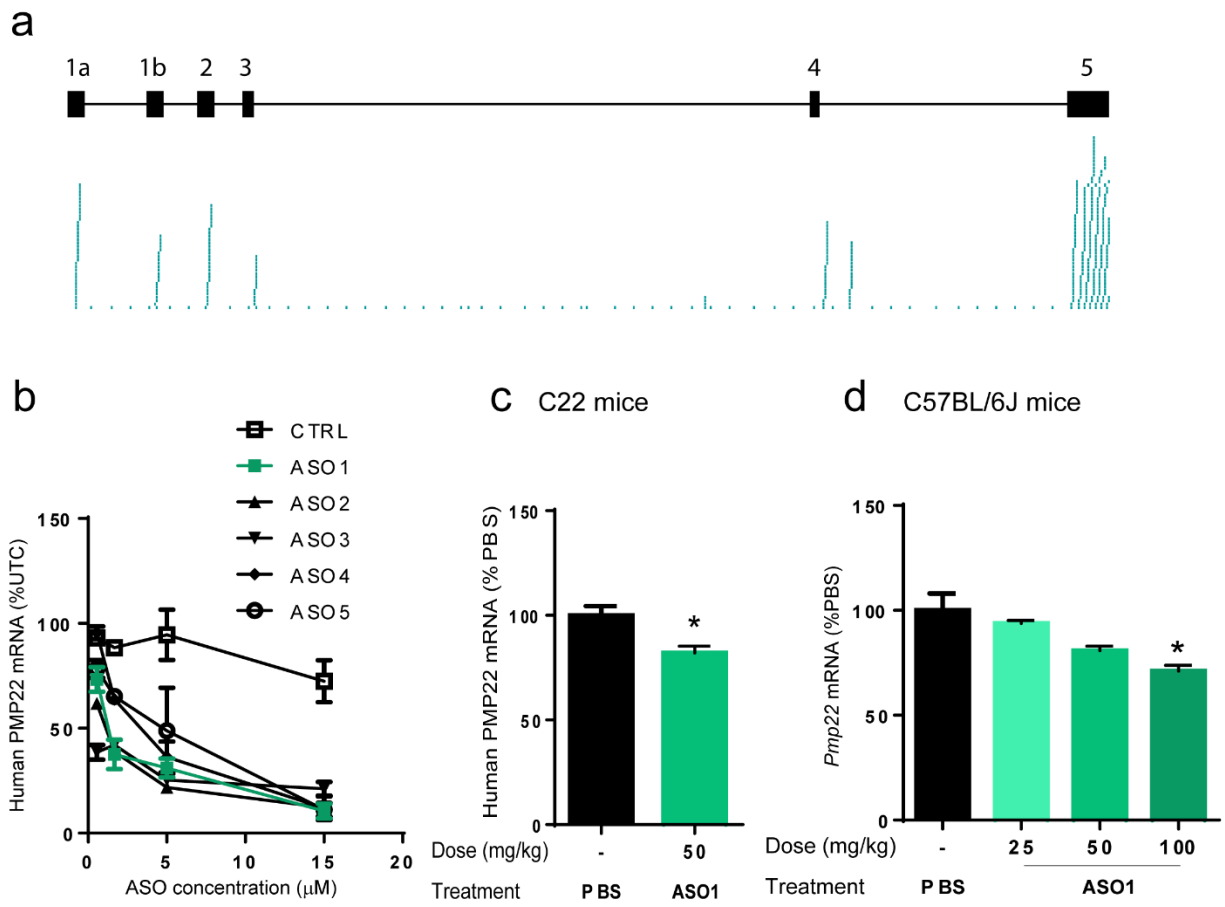

Figure S1. Identification of human PMP22 ASO1. (A) Schematic of human PMP22 gene and locations of approximate 500 ASOs screened in K-562 cells. **(B)** Dose-response confirmation of top 5 ASOs in K-562 cells. **(C)** Relative human PMP22 mRNA expression in sciatic nerve of PBS vs 50 mg/kg of ASO1 in C22 mice after 2 wks. Student's t-test, * $p < 0.05$. **(D)** Relative mouse *Pmp22* mRNA expression in sciatic nerves of PBS, or ASO1 at 25, 50, or 100 mg/kg for 2 wks in C56BL/6J mice. One-way ANOVA with Dunnet's post-test against PBS group, * $p < 0.05$.

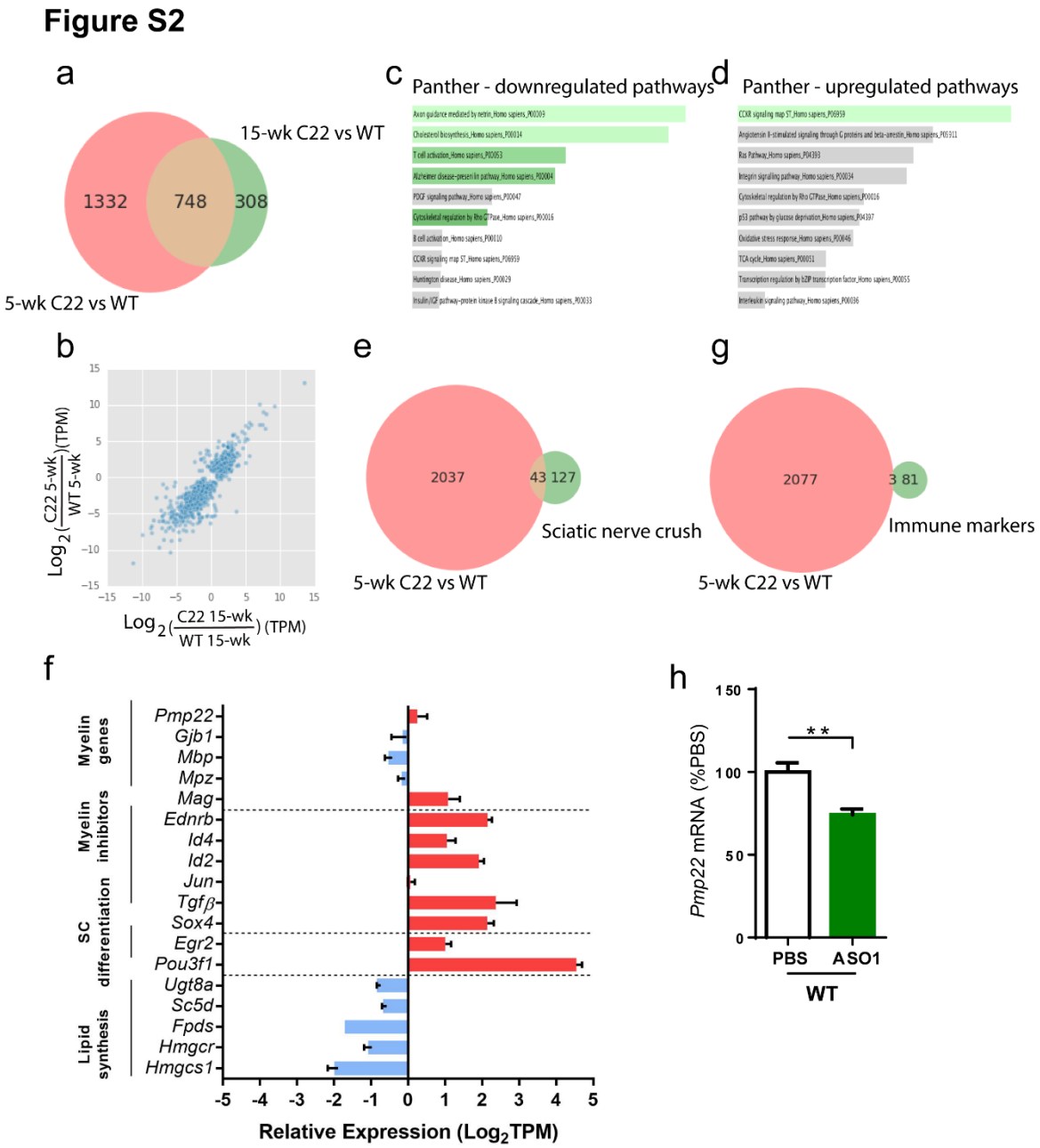

Figure S2. Transcriptomic characterization of WT and C22 littermates at 5-wk and 15-wk of age. (A) Venn diagram represent number of DEGs in 5-wk old WT vs C22 vs 15-wk old WT vs C22 mice. **(B)** Fold change coherence analysis of DEGs in 5-wk old WT vs C22 vs 15-wk old WT vs C22 mice. **(C-D)** Panther pathway analyses of down- and up-regulated genes in 5-wk C22 compared to WT littermates. **(E)** Venn diagram represent overlapped genes identified in 5-wk old WT vs C22 and those from sciatic nerve crush. **(F)** Relative expression (Log_2 TPM) of subset of genes up- (red) and down-regulated (blue) in C22 compared to WT littermates. **(G)** Venn diagram represent overlapped genes identified in 5-wk old WT vs C22 and immune markers. **(H)** qRT-PCR of mouse *Pmp22* mRNA in sciatic nerve of WT mice treated with PBS or 100 mg/kg of ASO1 for 9 wks. Student's t-test, * $p < 0.05$.

Figure S3

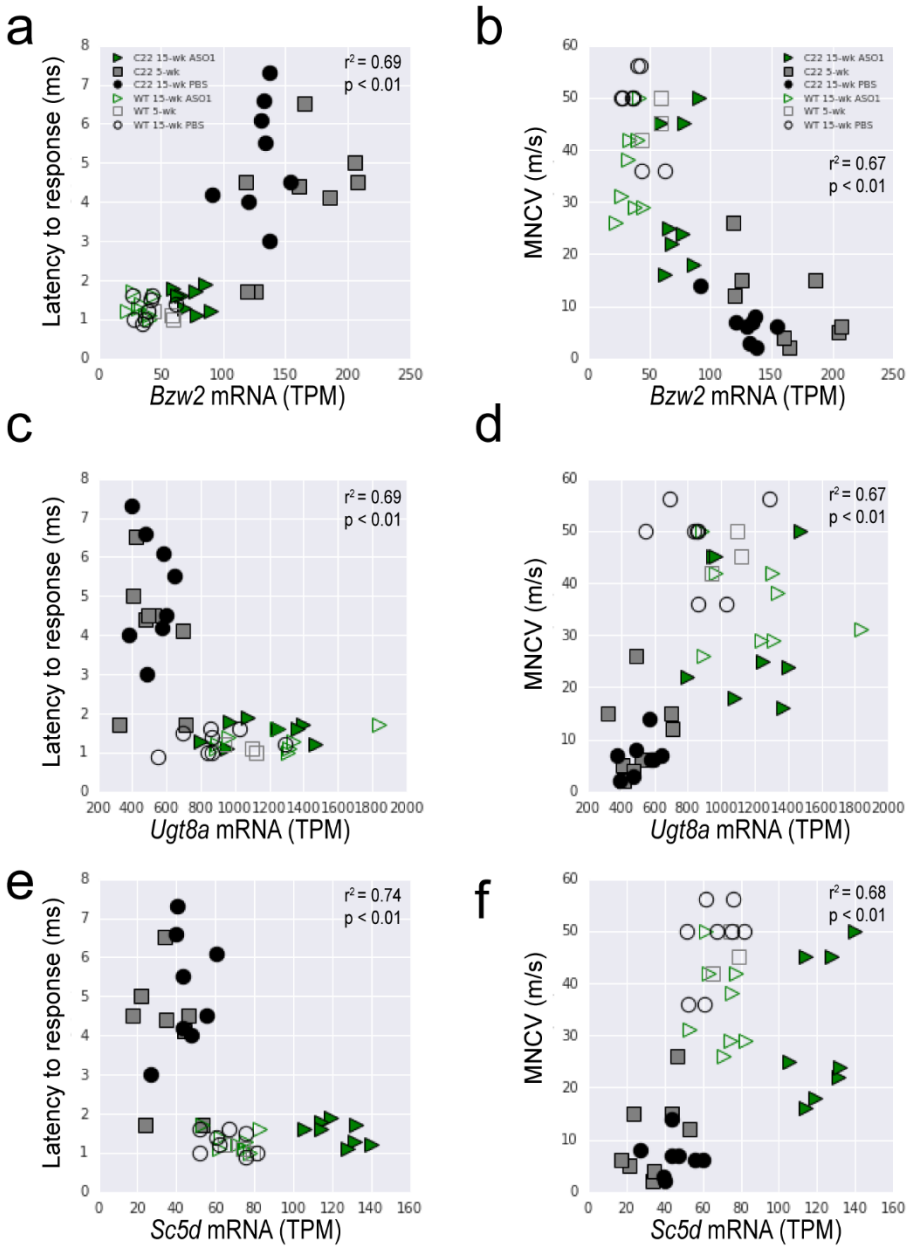

Figure S3. Correlation analyses of electrophysiological measures and mRNA expression of *Bzw2*, *Ugt8a*, and *Sc5d*. **(A)** Correlation analyses of latency to response (ms) vs *Bzw2* mRNA expression (TPM), Pearson $r^2 = 0.69$, $p < 0.01$, **(C)** *Ugt8a*, Pearson $r^2 = 0.69$, $p < 0.01$, and **(E)** *Sc5d*, Pearson $r^2 = 0.74$, $p < 0.01$. **(B)** Correlation analyses of MNCV (m/s) vs *Bzw2* mRNA expression (TPM), Pearson $r^2 = 0.67$, $p < 0.01$, **(D)** *Ugt8a*, Pearson $r^2 = 0.67$, $p < 0.01$, and **(F)** *Sc5d*, Pearson $r^2 = 0.68$, $p < 0.01$.

Figure S4

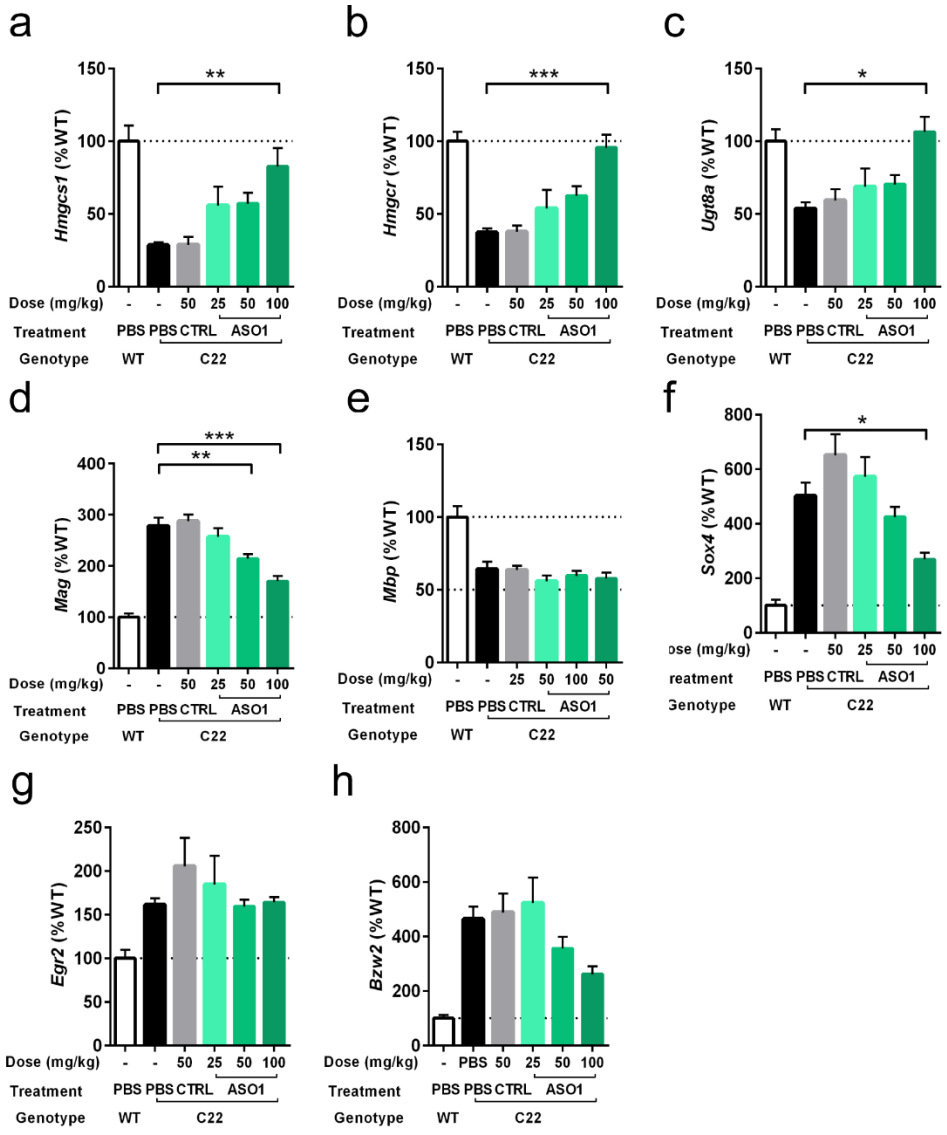

Figure S4. qRT-PCR confirmation in C22 mice. qRT-PCR confirmation of relative expression of lipid biosynthetic genes **(A-C)**, myelin-related genes **(D-E)**, transcriptional factors **(F-G)** and repressor **(H)** in C22 mice treated with PBS, or 50 mg/kg of CTRL, or 25, 50, or 100 mg/kg of ASO1 for 9 wks, N = 8 per group. WT littermates treated with PBS were included as controls, N = 8 per group. One-way ANOVA with Dunnett's post-test was used to compared between C22 with PBS- and ASO1-treated groups, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S5

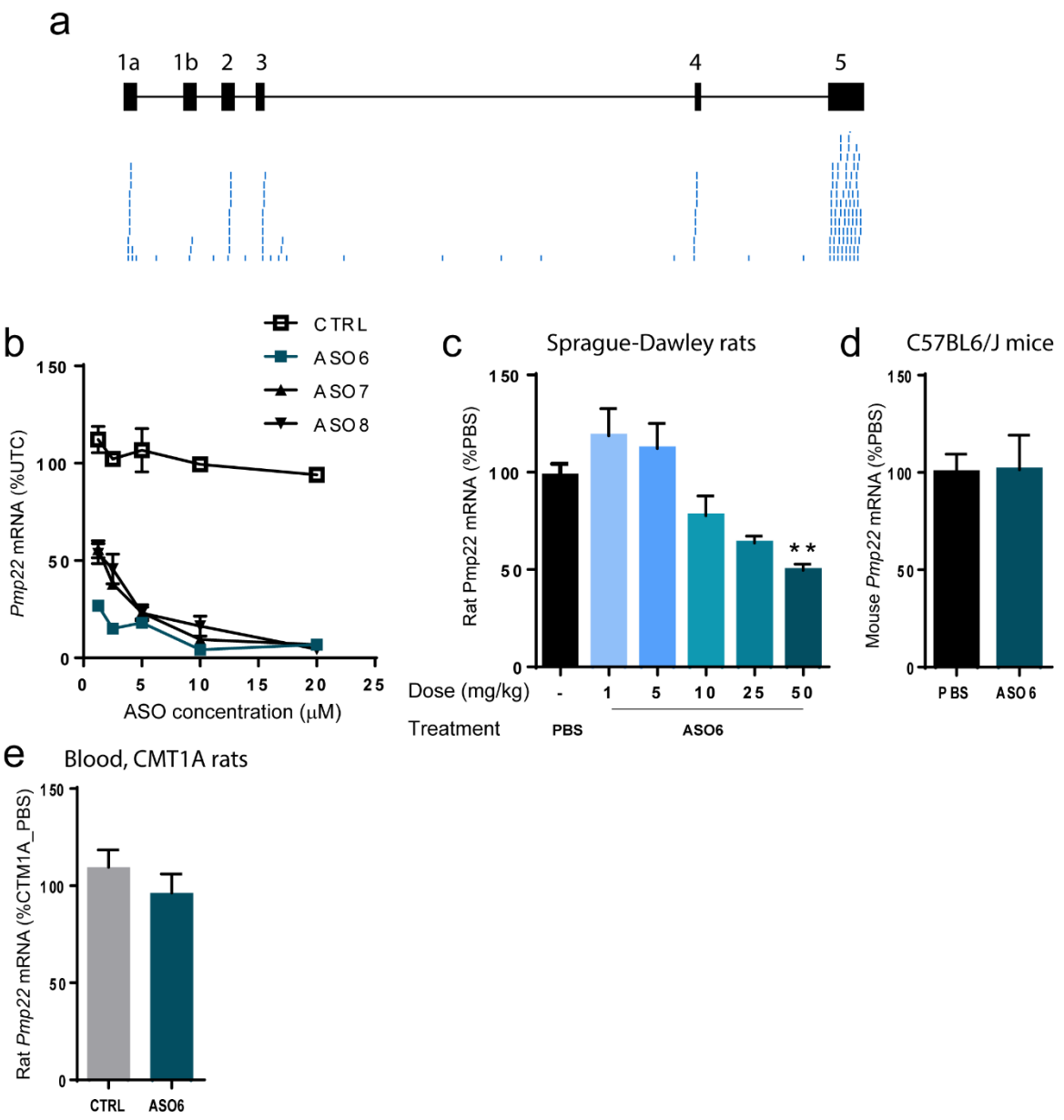

Figure S5. Identification of rat *Pmp22* ASO6 and *Pmp22* mRNA level in the blood of CMT1A rats. (A) Schematic of rat *Pmp22* gene and locations of approximate 500 ASOs screened in RT-DPT462 cells. **(B)** Dose-response confirmation of top e ASOs in RT-DPT462 cells **(C)** Relative rat *Pmp22* mRNA expression in sciatic nerve of adult Sprague-Dawley rats treated with PBS, or 1, 5, 10, 25, or 50 mg/kg of ASO6 for 4 wks One-way ANOVA with Dunnett's post-test was used to compared between PBS- and ASO-treated groups, ** $p < 0.01$. **(D)** Relative mouse *Pmp22* mRNA expression in sciatic nerves of PBS or 50 mg/kg ASO6 treated C56BL/6J mice for 4 wks. **(E)** Relative rat *Pmp22* mRNA expression in blood of CTRL- vs ASO6-treated CMT1A rats for 12 wks.

Gene	Log_2 TPM (C22 5-wk / WT 5-wk)	Log_{10} p-value	Log_2 TPM (C22 15-wk / WT 15-wk)	Log_{10} p-value	Log_2 TPM (C22_ASO1 15-wk / C22_PBS 15-wk)	Log_{10} p-value
Ugt8a	-1.18	-3.80	-0.69	-2.73	1.13	-3.42
Fdps	-2.00	-100.00	-1.66	-9.40	1.17	-2.65
Rspo1	-2.72	-8.44	-4.22	1.00	2.01	-2.00
Piwil4	5.21	-15.95	4.89	-15.95	-1.66	-5.96
Bpnt1	2.14	-2.64	0.22	-0.71	-1.29	-1.92
Wasf1	3.49	-3.95	2.42	-2.94	-1.37	-4.47
Cuedc2	2.76	-13.29	3.55	-6.35	-1.82	-12.46
Oaf	3.09	-4.20	2.38	-3.56	-0.87	-3.18
Cpsf2	0.54	-1.58	0.20	-0.59	-0.68	-2.96
Gm37034	16.24	-5.16	15.52	-7.74	-1.47	-2.54
Gnpda1	2.02	-1.71	1.44	-4.08	-1.09	-3.57
Nup93	1.18	-5.30	0.85	-2.51	-0.41	-2.46
Retnla	-1.05	-0.57	-1.63	-3.86	1.85	-2.33
Ppp2r2c	4.63	-15.95	5.01	-4.44	-2.31	-11.47
Gm12840	13.18	-1.18	13.70	-3.43	2.62	-2.63
Sc5d	-1.12	-2.18	-0.57	-1.70	1.50	-16.16
Plekha1	0.87	-1.50	1.20	-1.75	-0.62	-3.36
Slc22a2	-3.84	1.00	-1.98	-2.69	1.91	-2.61
Taf9b	3.38	-6.80	2.28	-3.29	-1.31	-2.96
Mtmr4	0.89	-1.96	1.00	-3.00	-0.67	-3.46
Fmo2	-2.60	-12.68	-1.60	-2.43	1.21	-1.51
Gm42503	14.72	-3.70	14.46	-4.50	1.38	-2.58
Rps12-ps3	1.55	-3.07	1.17	-3.64	-0.52	-6.09
Arih1	-0.21	-0.79	-0.51	-3.64	-0.65	-5.83
Gnptab	2.02	-2.29	1.24	-1.94	-0.92	-7.86
Ier5	1.37	-5.44	1.40	-10.76	-0.65	-3.25
Hspa8	1.27	-2.28	1.07	-3.92	-0.64	-3.71
Jun	0.97	-5.77	0.09	-1.36	-1.21	-10.96
Zfp972	1.40	-1.94	0.68	-0.75	2.09	-2.45
Psmd3	1.21	-5.01	1.67	-15.95	-0.53	-1.68

Emid1	3.41	-15.26	3.60	-8.79	-1.17	-5.67
Gtf3c2	0.35	-0.45	0.47	-3.62	-0.26	-1.71
Tnfrsf21	4.09	-5.70	2.51	-2.10	-1.14	-12.17
Col5a1	0.67	-2.96	0.57	-1.02	-0.87	-7.80
Ngfr	1.72	-12.53	0.76	-1.20	-1.31	-2.55
Tnfrsf25	3.24	-3.98	3.07	-3.32	-0.82	-2.09
Eif4g2	0.84	-2.59	-0.13	-0.67	-0.42	-2.09
Dusp16	2.16	-15.95	1.34	-2.40	-1.21	-7.07
Gypc	3.61	-14.10	3.32	-11.74	-0.89	-2.71
Gm26809	18.81	-9.05	18.36	-13.94	2.14	-2.52
Bzw2	1.46	-3.94	1.79	-11.99	-0.87	-7.01
Olfml2b	1.33	-1.60	0.79	-1.46	-1.19	-7.24
Olfml2a	3.22	-15.95	2.36	-9.41	-1.21	-4.51
Btf3l4	0.83	-2.44	0.24	-1.07	-0.67	-2.81
Mpzl1	3.88	-5.26	2.80	-5.92	-1.77	-6.84
Gm14033	12.72	-1.59	12.34	-2.31	-1.63	1.00
Gnb1	1.76	-2.46	1.96	-4.85	-0.69	-2.74
Mthfd1l	1.74	-2.53	2.19	-5.34	-1.11	-2.09
Gm9945	2.98	-5.23	2.82	-4.33	-1.56	-6.15
Eif4a1	2.63	-4.11	1.67	-2.22	-1.02	-4.68
Efhd1	1.09	-4.32	1.06	-2.02	-1.18	-2.56
Ttyh1	1.69	-7.36	1.61	-4.88	-1.05	-6.47
Sh3pxd2b	1.76	-2.49	1.73	-4.92	-1.24	-2.67
Msln	-0.89	-1.41	-1.60	-1.87	2.22	-1.46
Csrp2	3.75	-15.95	2.82	-7.54	-1.76	-12.54
Shfm1	0.56	-1.33	-0.08	-0.38	-1.03	-4.99
Sox4	2.33	-2.42	2.39	-6.35	-1.46	-6.62
Gm29331	16.17	-10.59	15.60	-8.08	-1.16	-3.12
Snx16	2.61	-11.02	2.45	-10.36	-1.11	-4.34
Pou3f1	4.18	-8.11	4.42	-11.64	-0.82	-2.21
Kcnj10	2.89	-5.29	2.35	-2.62	-1.80	-10.15
Cnn3	1.56	-8.83	0.56	-1.98	-0.82	-2.25
Zfp276	1.67	-11.05	2.02	-14.63	-0.73	-2.72
Serinc5	2.89	-3.91	2.63	-3.12	-1.44	-13.80
Gria3	0.82	-1.36	0.75	-2.82	-0.91	-2.04
Txndc16	0.67	-0.69	1.61	-3.55	-0.75	-4.21

Bhlhe22	3.63	-7.08	3.05	-6.94	-1.08	-9.49
Ezr	1.47	-5.56	1.63	-3.42	-0.95	-2.71
Pcolce2	-1.30	-1.54	-0.37	-1.07	-1.70	1.00
Padi2	2.22	-20.63	2.33	-3.32	-1.34	-14.77
Marveld3	13.57	-4.73	12.84	-2.94	-2.14	1.00
Ahnak2	0.80	-2.22	0.45	-1.28	-0.99	-3.77
Chst2	-1.60	-5.03	-0.45	-1.34	0.92	-2.17
Cxcl14	5.74	-7.02	3.62	-6.35	-2.43	-100.00
Deb1	2.85	-3.58	2.19	-2.99	-1.33	-4.96
Acot1	1.42	-3.13	0.27	-1.05	-1.19	-1.95

Table S1. List of 76 genes modulated by ASO treatment in C22 mice.

Oligonucleotide	Sequence	Targets
ASO1	ATCTTCAATCAACAGC	human *PMP22* and mouse *Pmp22*
ASO6	TTGGTTTCGCAGAGGG	rat *Pmp22*
CRTL	GGCCAATACGCCGTCA	NA

Table S2. Sequences of ASOs used. Underlined residues indicates constrained ethyl modified bases, while the rest are deoxyribonucleotides.

Target	Sequence or assay catalogue number
Mouse *Pmp22*	F: 5'-CCGCAGCACAGCTGTCTTT-3' R: 5'-AGCAGATTAGCCTCAGGCACAA-3' P: 5'-Fam-CCAGCAACCCAGTGGACGCACC-Tamra-3'
Rat *Pmp22*	F: 5'-AGGGAGCTCCACCAGAGAACA-3' R: 5'-GCTTGGATTTCTTTGCAATCG-3' P: 5'-Fam-CTGTGAGCATCCGCTGTCCTGCG-Tamra-3'
Human *PMP22*	F: 5'-CTCCTCCTGTTGCTGAGTATC-3' R: 5'-GCTACAGTTCTGCCAGAGA-3' P: 5'-Fam-CAGTTGCGTGTCCATTGCCCA-Tamra-3'
Rat *Mpz*	F: 5'- GTCCAGTGAATGGGTCTCAGATG-3' R: 5'- CTTGGCATAGTGGAAGATTGAAA-3' P: 5'-Fam-TTTACCTGGCGCTACCAGCCTG-Tamra-3'
Mouse/rat *Pou3f1*	Mm.PT.58.33607006.g (IDT, San Jose, CA)
Mouse *Mpz*	Mm.PT.58.5771188 (IDT, San Jose, CA)
Mouse *Sox4*	Mm.PT.58.32478304.g (IDT, San Jose, CA)
Mouse *Sc5d*	Mm.PT.58.33540072 (IDT, San Jose, CA)
Rat *Sc5dl*	Rn.PT.58.12179010 (IDT, San Jose, CA)
Mouse *Ugt8a*	Mm.PT.58.31646155 (IDT, San Jose, CA)
Mouse Mag	Mm.PT.58.13580215 (IDT, San Jose, CA)
Mouse Mbp	Mm0166402_m1 (Thermo Fisher Scientific, Grand Island, NY)

Mouse Egr2	Mm.PT.56a.30480551 (IDT, San Jose, CA)
Rat *Utg8*	Rn.PT.58.35975515 (IDT, San Jose, CA)
Mouse *Id2*	Mm.PT.58.42755760.g (IDT, San Jose, CA)
Rat *Id2*	Rn.PT.58.35551013 (IDT, San Jose, CA)
Mouse *Bzw2*	Mm.PT.56a.14131067 (IDT, San Jose, CA)
Rat *Bzw2*	Rn.PT.58.1027770 (IDT, San Jose, CA)
Mouse *Hmgcs1*	Mm.PT.58.11038920 (IDT, San Jose, CA)
Mouse *Hmgcr*	Mm.PT.58.31538611 (IDT, San Jose, CA)

Table S3. Detail of PCR primer/probes.