Supplemental information for

Myelin repair stimulated by CNS-selective thyroid hormone action

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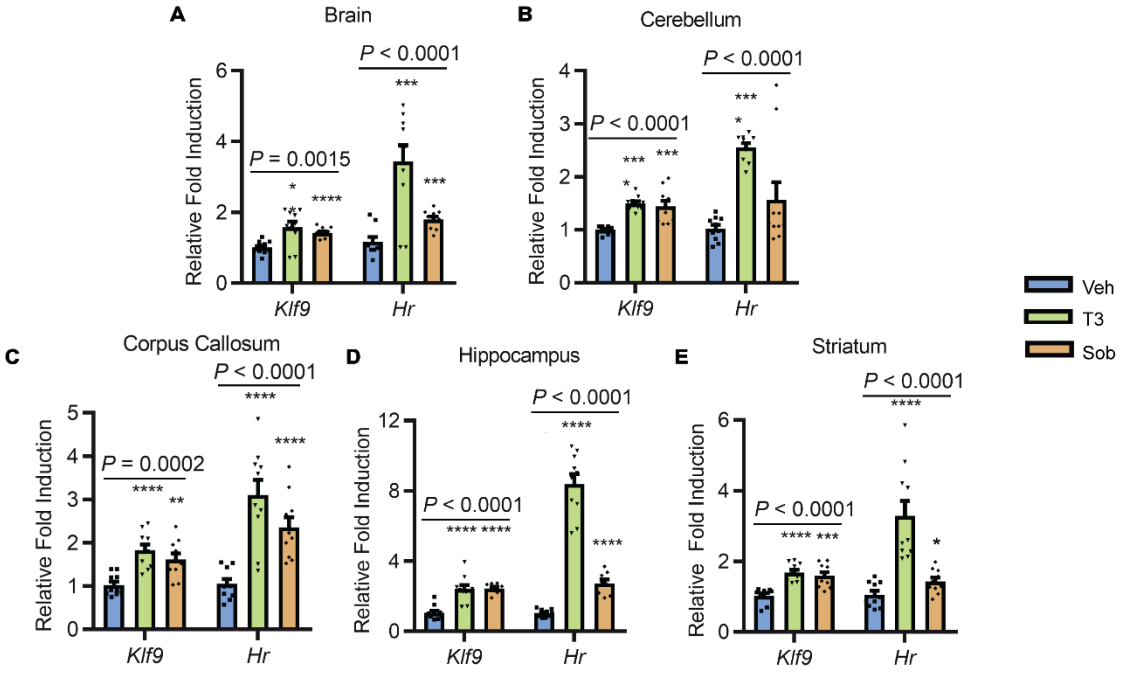
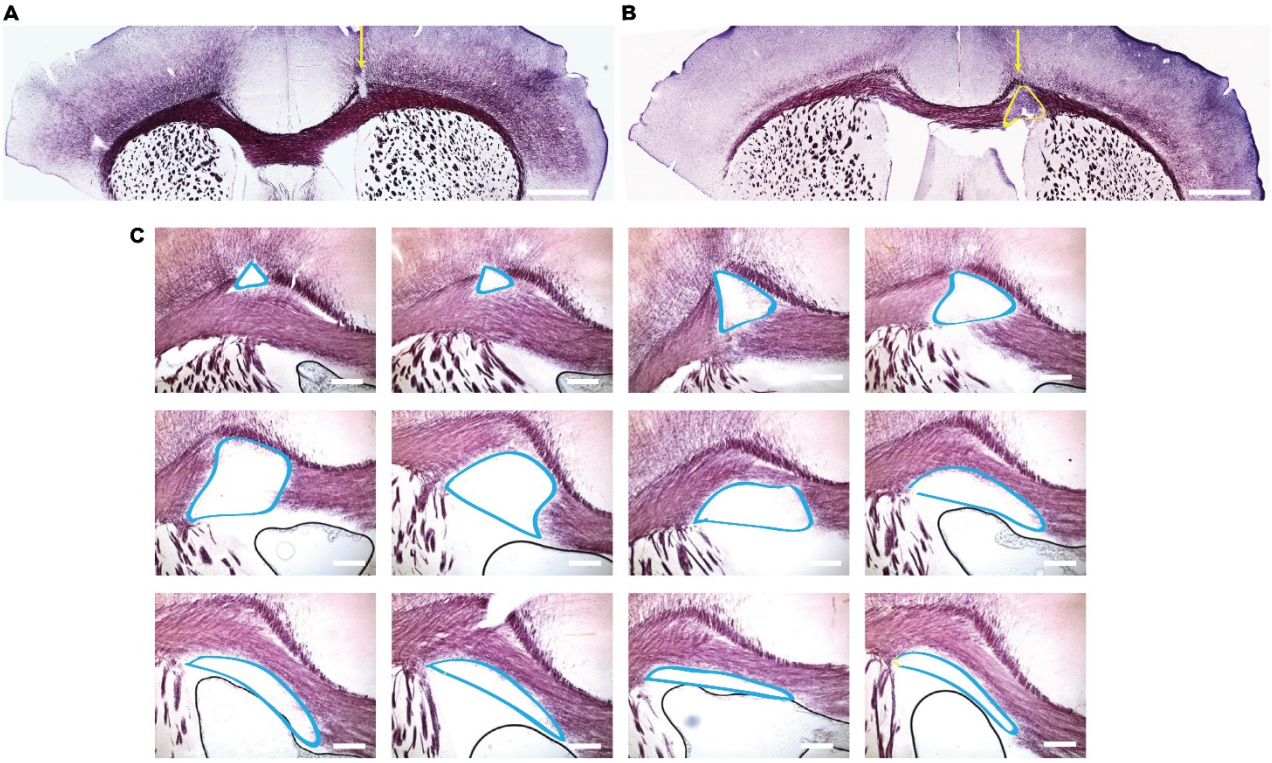
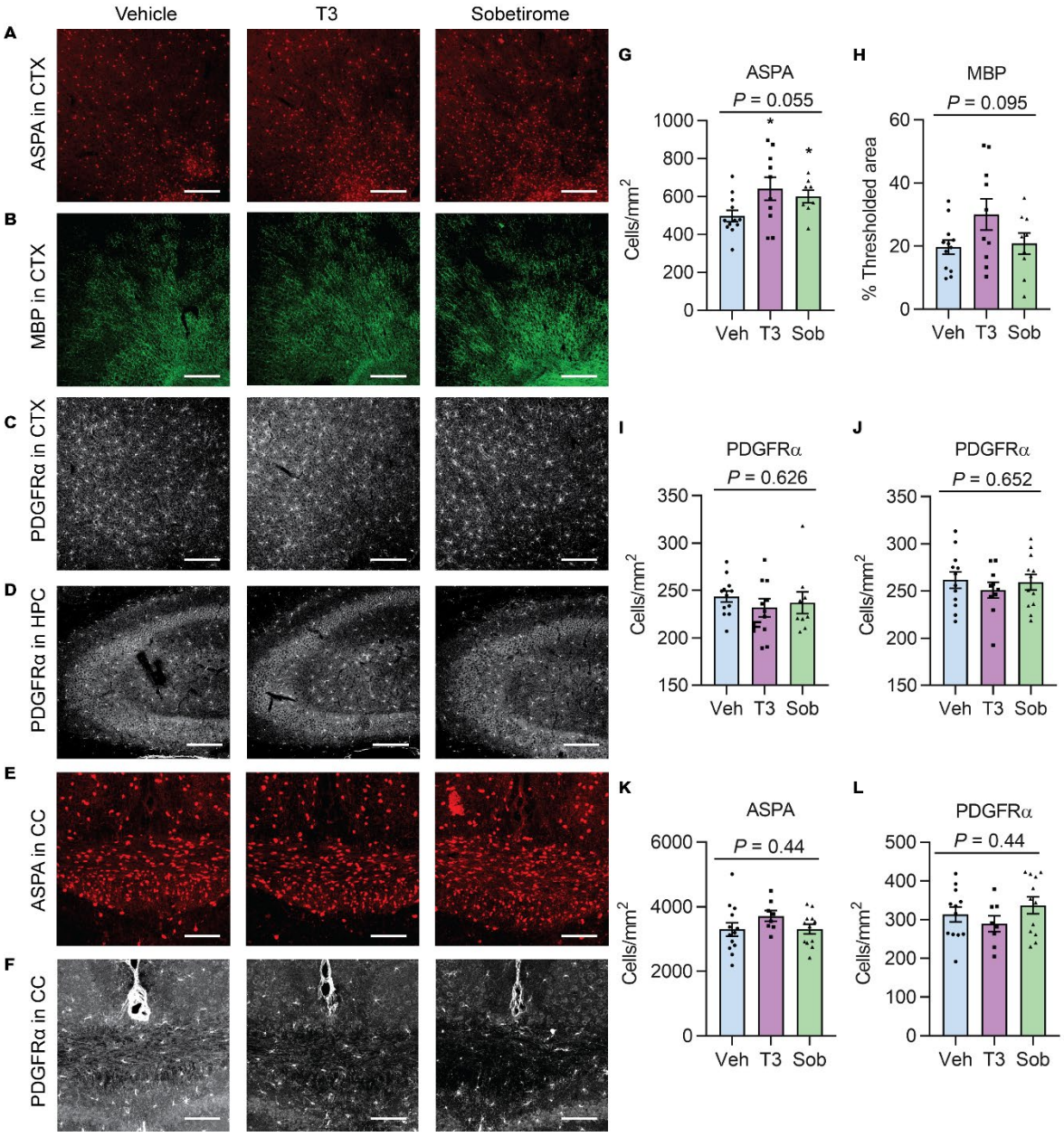


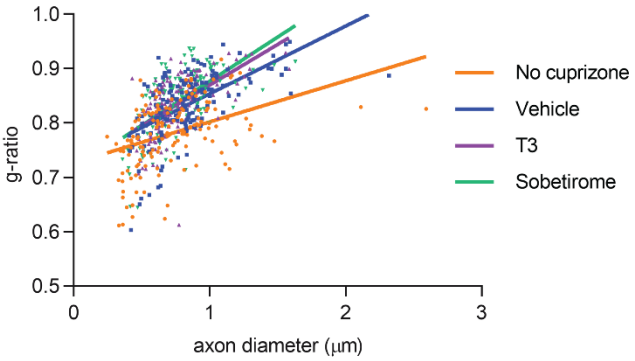
Supplemental Figure 1. Sobetirome upregulates thyroid hormone responsive genes in the brain. Mice were treated for 7 days with daily i.p. injections of vehicle, T3 (1 mg/kg) or sobetirome (1 mg/kg). Whole brains were isolated or regions (cerebellum, corpus callosum, hippocampus, or striatum) were microdissected. (**A**-**E**) Transcript levels of *Klf9* and *Hr* were measured using qPCR with technical duplicates. Statistical significance was determined by one-way ANOVA across all groups (P value in figure) followed by a two-tailed, unpaired t-test for comparisons between vehicle and treatment denoted with asterisks (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001). All graphs show mean ± SEM.



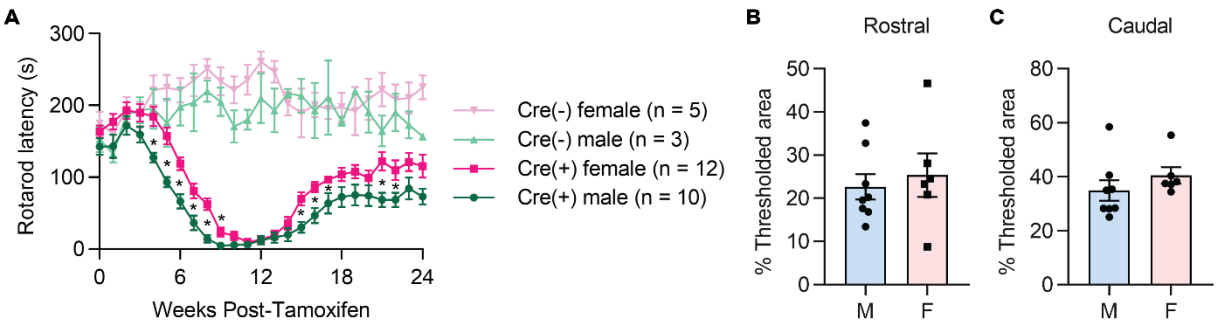
Supplemental Figure 2. Lysolecithin lesion volumes are quantified through serial sectioning. (**A** and **B**) Whole brain sections in the rostral corpus callosum are shown after stereotactic injection of PBS (left) or 2% lysolecithin in PBS (right). Injection path is indicated with a yellow arrow and lesion in **B** is outlined in yellow. Scale bars represent 1 mm. (**C**) Sequential BlackGold staining of a representative lysolecithin lesion is shown from rostral to caudal. Sequential sections through the lesion were stained (50 µm free-floating sections) and the lesion volume from the first 12 sections containing lesion was measured to estimate a lesion volume for each mouse. Lesions are outlines in blue. Scale bars represent 100 µm.



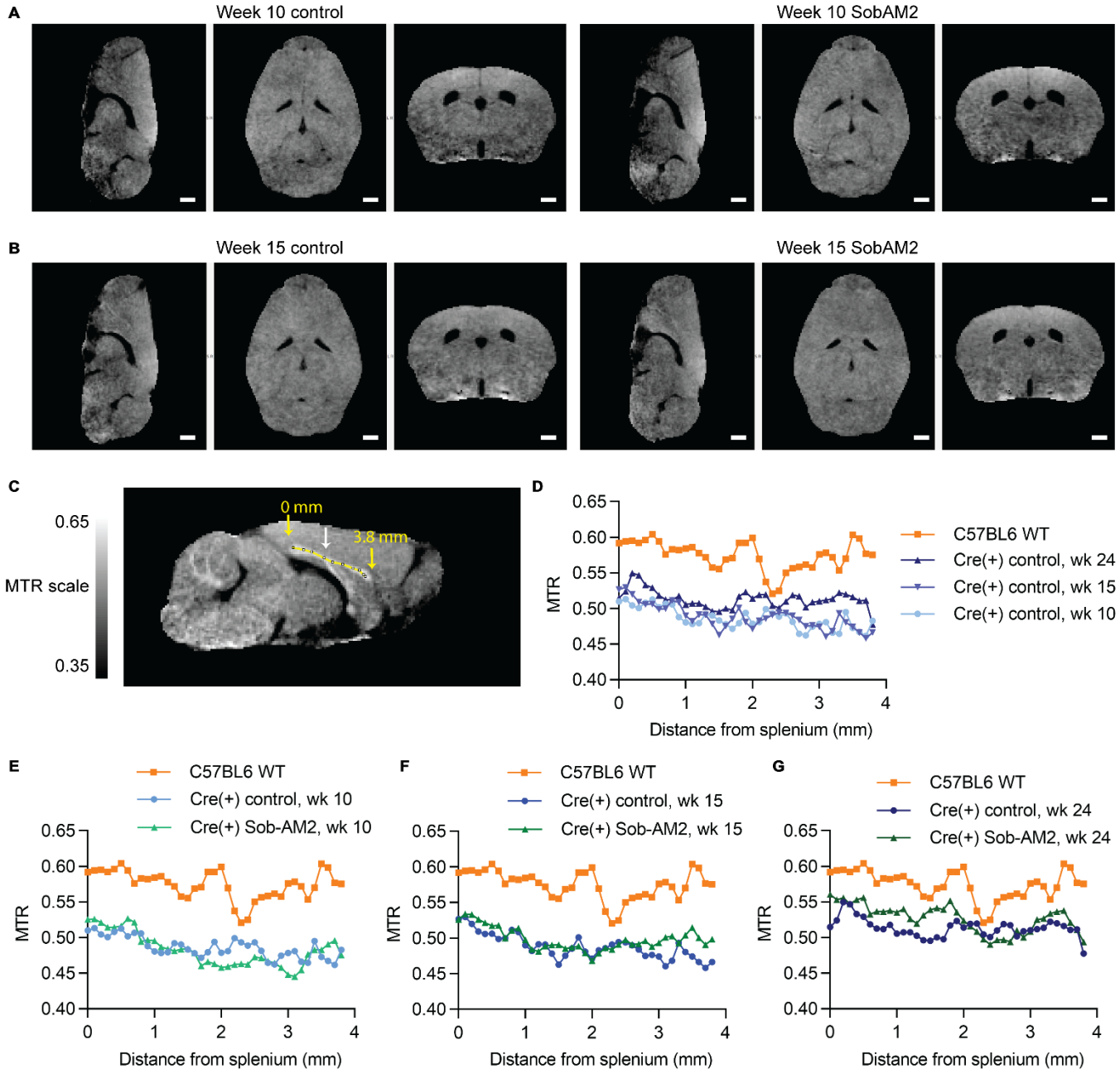
Supplemental Figure 3. T3 and sobetirome treatment in the cuprizone model increase oligodendrocyte density in the cortex. Mice received 12 weeks of 0.2% cuprizone chow followed by 3 weeks of daily i.p. injections with vehicle, T3 (1 mg/kg), or sobetirome (1 mg/kg). Representative images include (**A**) ASPA staining of oligodendrocytes in cortex (CTX), (**B**) MBP (myelin basic protein) staining in CTX, (**C**) PDGFRα staining of oligodendrocyte progenitor cells (OPCs) in CTX, (**D**) PDGFRα staining (white) in hippocampus (HPC), (**E**) ASPA staining in the corpus callosum (CC), and (**F**) PDGFRα staining in CC. Quantification of images include (**G**) ASPA-positive oligodendrocytes, (**H**) MBP staining by threshold analysis, (**I**) PDGFRα-positive OPCs in CTX, (**J**) PDGFRα-positive OPCs in HPC, (**K**) Aspa-positive oligodendrocytes in CC, and (**L**) PDGFRα-positive OPCs in CC. Data represent the following groups: for ASPA and PDGFRα, Veh (n = 6), T3 (n = 5), and Sob (n = 6); for MBP, Veh (n = 6), T3 (n = 4), and Sob (n = 5). Two images were quantified for each animal. Statistical significance was determined by one-way ANOVA followed by a two-tailed, unpaired student t-test for comparisons between vehicle and treatment (*P ≤ 0.05). For **A-D**, the scale bar represents 200 µm and for **E-F,** the scale bar represents 100 µm. All graphs show mean ± SEM.



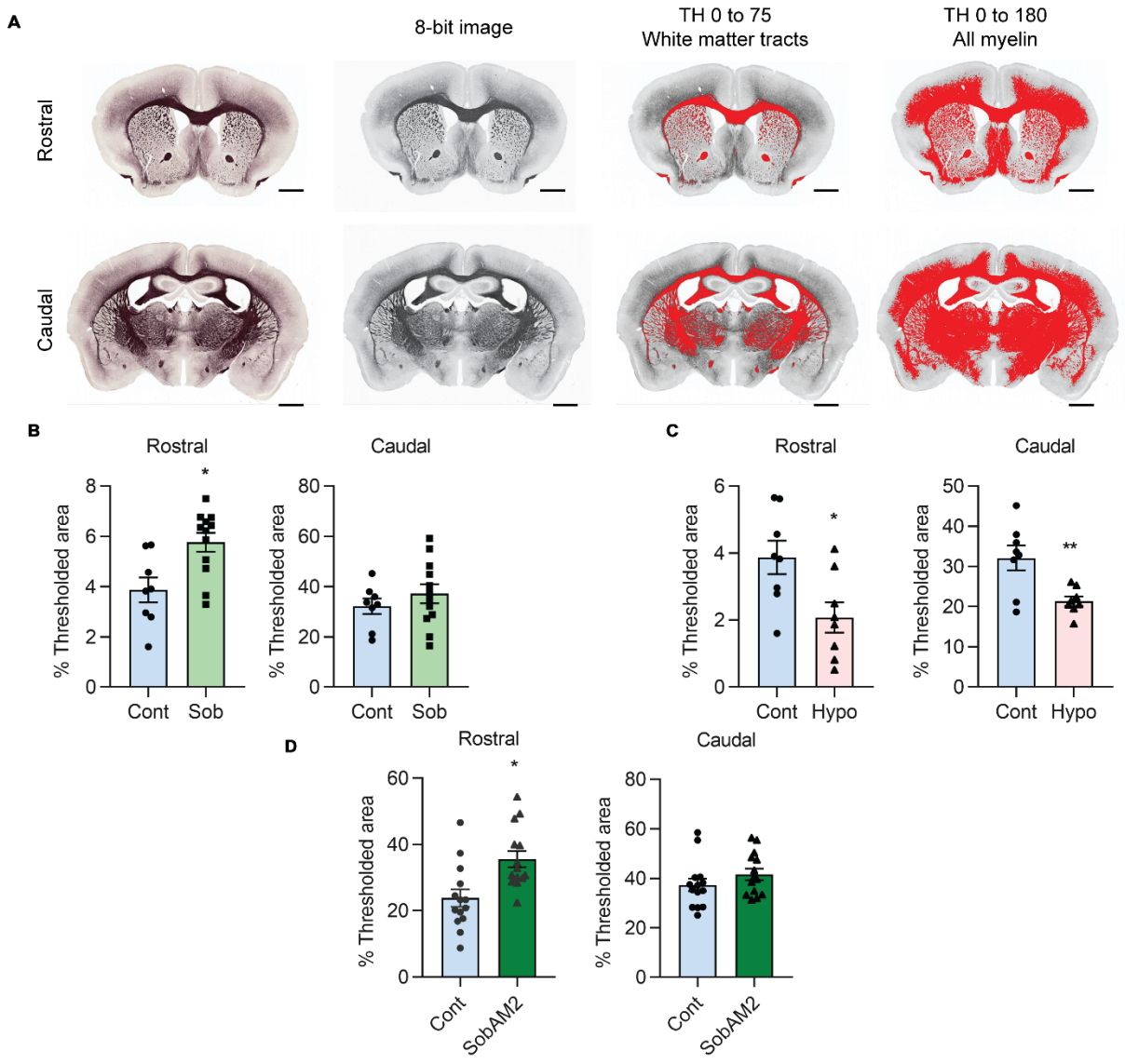
Supplemental Figure 4. G-ratios of myelinated axons in corpus callosum are significantly higher after cuprizone administration. The g-ratios were determined from at least 150 myelinated axons in the corpus callosum from mice in the cuprizone experiment. G-ratios were calculated by determining the ratio of the inner axon diameter to the total myelin diameter. Mice treated with cuprizone for 12 weeks followed by 3 weeks of daily IP injections with vehicle, T3 (1 mg/kg), or sobetirome (1 mg/kg) are compared to naïve mice that did not receive cuprizone.



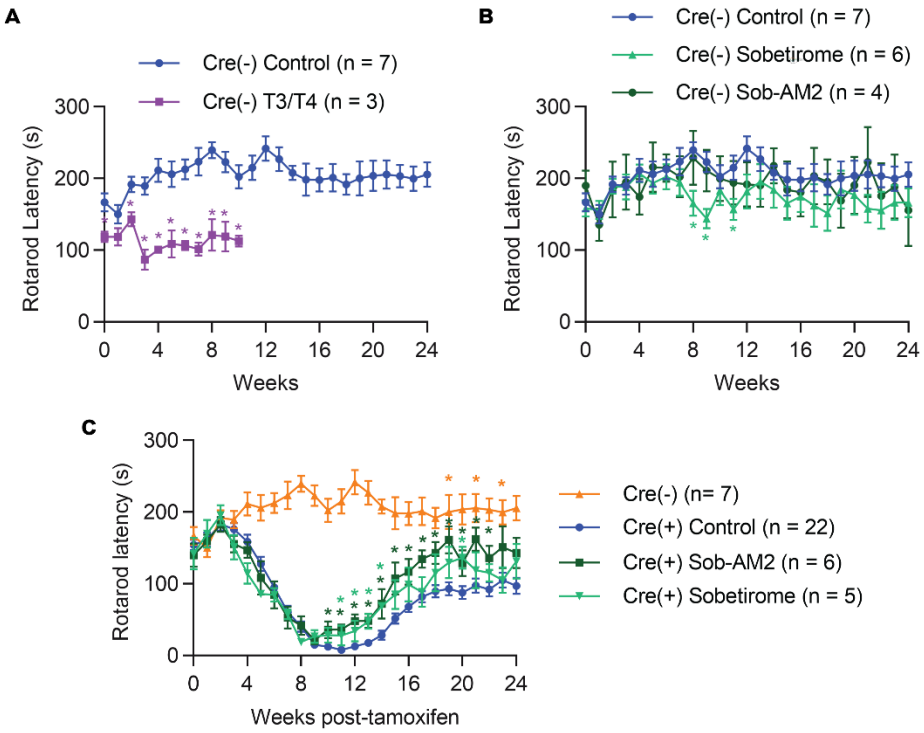
Supplemental Figure 5. Male and female iCKO-*Myrf* mice show differences in rotarod performance, but not in myelin content. (**A**) Rotarod testing was performed weekly and consisted of three trials for each mouse with step-wise speed increases from 8 rpm to 40 rpm over 5 minutes as described in the methods. Statistical significance was determined by a two-tailed, unpaired t-test comparing male and female Cre positive mice (*P ≤ 0.05). Each t-test is performed independently. (**B** and **C**) Quantification of BlackGold images was performed by threshold analysis for white matter tracts (n = 4 for male and n = 3 for female, two images per animal). No statistically significant differences (two-tailed, unpaired t-test) were observed between male and female cohorts in rostral (*P = 0.63) or caudal (*P = 0.30) regions. All graphs show mean ± SEM.



Supplemental Figure 6. Magnetization transfer ratio (MTR) analysis of iCKO-*Myrf* mice imaged *in vivo* shows increased signal in mice treated with Sob-AM2 at 24 weeks. (**A** and **B**) Sagittal, horizontal, and coronal MTR maps of control or Sob-AM2 treated mice iCKO-*Myrf* mice at 10 weeks (**A**) and 15 weeks (**B**) after tamoxifen injection. Scale bars represent 1 mm. (**C**) The MTR values along the corpus callosum (caudal to rostral) starting at the splenium (yellow arrow, 0 mm) are plotted in **D-G**. White arrow indicates the isthmus/body of the corpus callosum in which the largest difference is observed between control and Sob-AM2 treatment. (**D**) MTR plot along corpus callosum of control iCKO-*Myrf* mice at weeks 10, 15, and 24 compared to wild type. (**E-G**) MTR plot along corpus callosum comparing control and Sob-AM2 treated iCKO-*Myrf* mice with wild type at week 10 (**E**), week 15 (**F**), and week 24 (**G**). All iCKO-*Myrf* maps from control and Sob-AM2 groups are averaged (n = 3 for each), and wild type data from C57BL6 (n = 5) was previously published (34).



Supplemental Figure 7. BlackGold threshold analysis shows increases in gray matter myelin with Sob and Sob-AM2 treatment. (**A**) Threshold analysis methodology of brains from iCKO-*Myrf* stained with BlackGold is depicted. A representative brain from a normally myelinated Cre negative mouse is shown. Color images were converted to 8-bit, and two thresholds were set: a limited threshold that included only the primary white matter tracts such as the corpus callous, and an inclusive threshold that encompassed BlackGold staining in gray matter such as the cortex. Scale bars represent 1 mm. (**B-D**) Inclusive threshold analysis of all myelin staining was performed comparing BlackGold staining in the control group with (**B**) sobetirome, (**C**) hypothyroidism, or (**D**) Sob-AM2. Two sections were quantified for each animal. Statistical significance was determined by a two-tailed, unpaired t-test comparing vehicle to each treatment group (*P ≤ 0.05, **P ≤ 0.01).



Supplemental Figure 8. Rotarod analysis of Cre negative mice shows motor effects of treatment in the absence of demyelination. Rotarod testing was performed weekly and consisted of three trials for each mouse with step-wise speed increases from 8 rpm to 40 rpm over 5 minutes as described in the methods. (**A**) Comparison of rotarod performances from Cre negative mice administered control chow or T3/T4 chow. (**B**) Comparison of rotarod performances from Cre negative mice administered control, sobetirome, or Sob-AM2 chow. (**C**) Direct comparison of rotarod performances from Cre negative and Cre positive mice administered control chow, and Cre positive mice administered chow containing sobetirome or Sob-AM2. Statistical significance was determined by a two-tailed, unpaired t-test comparing control to treatment (*P ≤ 0.05). Each t-test is performed independently, and all graphs show mean ± SEM. This data is also in Fig. 4 and 8. Orange asterisks indicate weeks in which the Sob-AM2 treated mice are *not* significantly different from unaffected Cre negative mice (*P > 0.10).

Supplemental Table 1. MTR values from the isthmus/body of the corpus callosum of individual mice at weeks 10, 15, and 24. The maximum ΔMTR for each mouse was calculated from the difference between week 24 and week 10 or 15 with the lowest MTR (highlighted in blue).

Mouse ID	**336**	**367**	**387**	**328**	**393**	**394**
Treatment	control	control	control	Sob-AM2	Sob-AM2	Sob-AM2
Week 10	0.487	0.493	0.483	0.469	0.474	0.503
Week 15	0.480	0.496	0.487	0.492	0.500	0.472
Week 24	0.507	0.501	0.511	0.519	0.523	0.518
Max ΔMTR	0.027	0.009	0.029	0.050	0.049	0.046

Mean ± SEM		control	0.022 ± 0.011		Sob-AM2	0.048 ± 0.001