

## **Supplementary Materials:**

Supplementary Fig 1-3, Supplementary Table S1

## **Supplementary Figure Legends**

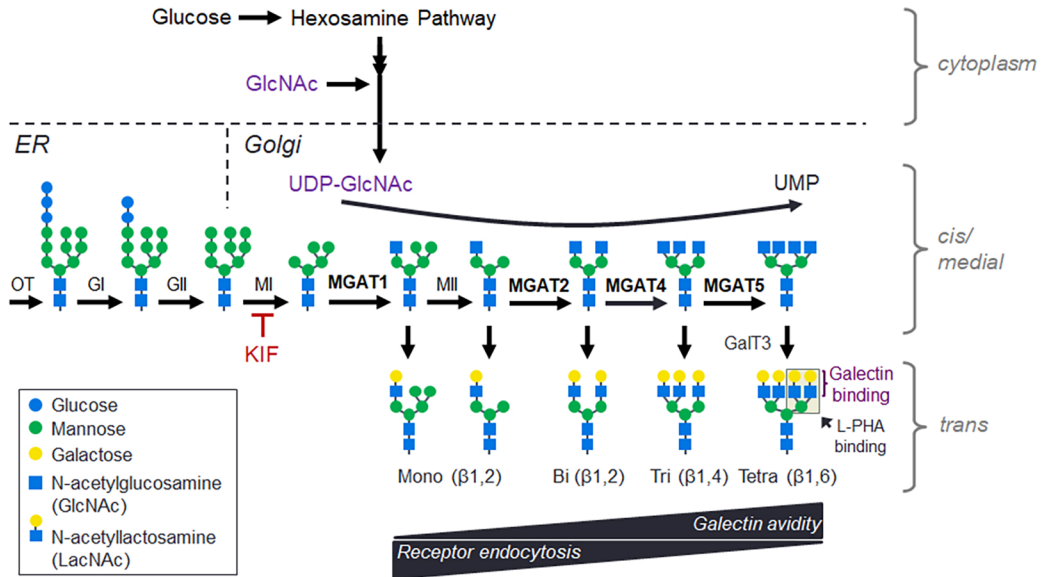
**Supplementary Figure 1. GlcNAc promotes oligodendrogenesis from precursor cells. (A)** UDP-GlcNAc, which is synthesized *de novo* from glucose or salvaged from GlcNAc, is the donor substrate utilized by the Mgat branching enzymes. **(B)** Immunofluorescence microscopy of E12.5 NSC's from CD1 mice cultured in growth media (FGF+EGF)  $\pm$  GlcNAc for 48 hours. **(C-G)** Flow cytometry of C57BL/6 mouse E12.5 NSC's of the indicated genotypes treated in either growth media (FGF+EGF, C), in differentiation media (FGF+PDGF-AA (10ng/mL)) (E-G) or as indicated **(D)** with/without GlcNAc 80 mM (C-E), kifunensine for 48hrs (F) or doxycycline pre-treatment for 8 days (G). Data are 3 technical replicates per group and representative of 2 experiments. P-values are by one-way ANOVA with Sidaks's multiple comparison test. ( \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001).

**Supplementary Figure 2. GlcNAc and N-glycan branching promote primary myelination. (A)** Flow cytometry of brains from PL/J pups whose mothers were treated with GlcNAc (1mg/mL) in drinking water from P5 or E12.5 to P8 (N=5,5 and 2, 11; one sided t-test). **(B)** *Mgat1<sup>f/f</sup>Plp1-cre/ERTc<sup>+</sup>* mice were injected with or without tamoxifen (75mg/kg) daily for 3 days and brains analyzed by flow cytometry. Data is representative of 3 mice per group. **(C)** *Mgat1<sup>f/f</sup>Plp1-cre/ERTc<sup>+</sup>* mice (10 weeks old) were treated with tamoxifen at week 0 and 4, sacrificed at week 8 and cerebellums were analyzed by immunofluorescence microscopy for MBP and myelin (fluoromyelin) (N=5 (2 male, 3 female),8 (6 male, 2 female); one sided t-test). Data points represents average fluorescence of area depicted from 2 slices per mouse. **(D-E)** The indicated mice (10 weeks old) were treated with tamoxifen at week 0 and then sacrificed at week 2 and brains were analyzed by immunofluorescence microscopy for MBP and myelin (fluoromyelin) (N=8 (4 male, 4 female), 6 (4 male, 2 female (D), N=8 (4 male, 4 female), 4 (1 male, 3 female) (E), one sided t-test. Data points represent average fluorescence of area highlighted in red in 2 slices per mouse.

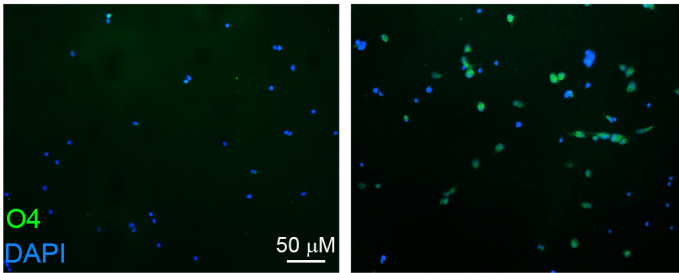
**Supplementary Figure 3. Oral GlcNAc promotes oligodendrogenesis and re-myelination while limiting axonal injury.** (A) Per mouse averages of g-ratio, total axons, myelinated axons, paranodes, and dystrophic axons as well number of degenerating axons from electron micrographs of the medial CC of the *Mgat5<sup>+/-</sup>* mice from Fig. 3G (N=3,3 (2 male and 1 female per group)) with data obtained blind to treatment conditions and averaged from 2 fields (g-ratio) or 6 fields per mouse (105  $\mu\text{m}/\text{field}$ ). Degenerating axons highlighted by red arrows and paranodes denoted by blue asterisks. Mice were treated  $\pm$  GlcNAc for 4 weeks after 5 weeks of cuprizone. P-value by one-sided t-test and chi-square test. Scale bar = 1 $\mu\text{m}$  (B) Electron micrographs of 10-week old *Mgat5<sup>+/+</sup>* and *Mgat5<sup>+/-</sup>* littermates were analyzed for number of myelinated axons per field (N=12, 12 fields; 6 fields per mouse, 105  $\mu\text{m}/\text{field}$ , one-sided t-test) and g-ratio (N=839 axons over 6 fields). Scale bar = 1 $\mu\text{m}$ . (C) Fluoromyelin staining of medial CC of *Mgat5<sup>+/-</sup>* mice who were treated  $\pm$  GlcNAc for 6 weeks after 5 weeks of cuprizone starting at 10 weeks of age (N=3 (3 male), 6 (4 male, 2 female); one sided t-test). Data points represent average of 4 slices per mouse.

# Supplementary Figure 1

A



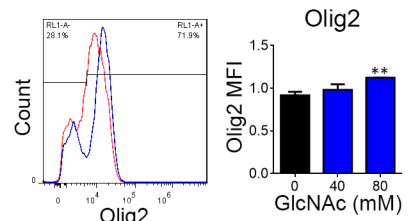
B



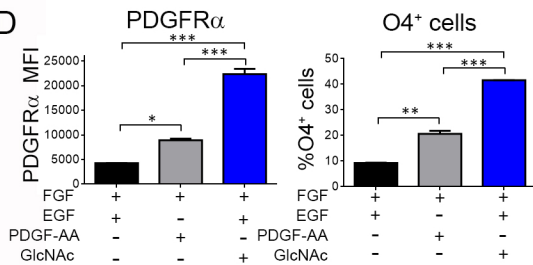
GlcNAc (mM) 0

80

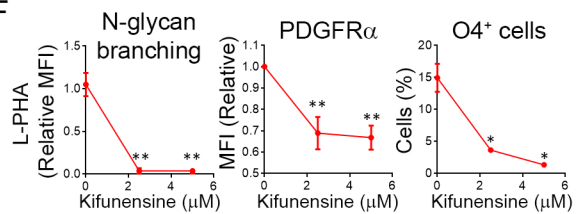
C



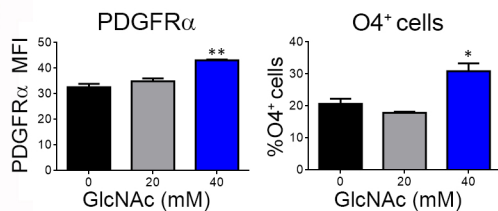
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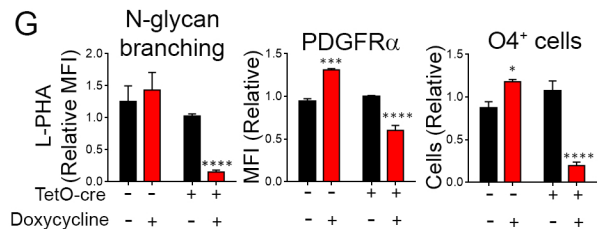
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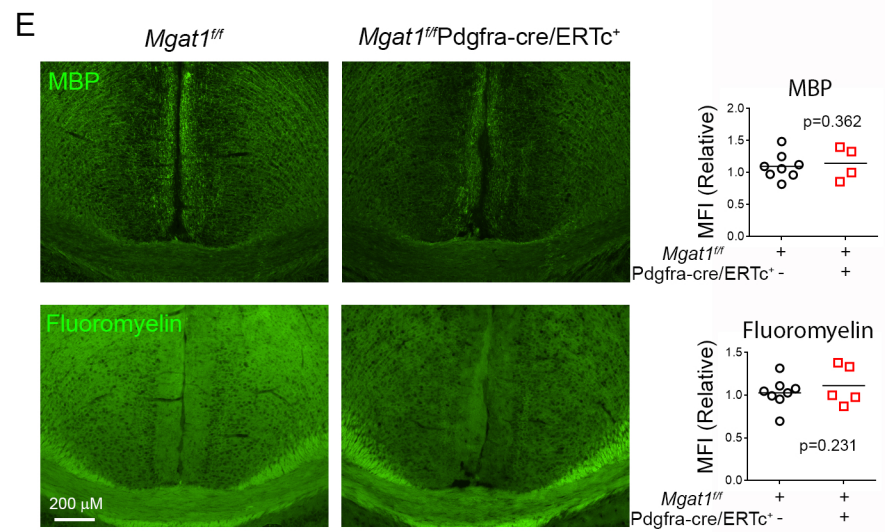
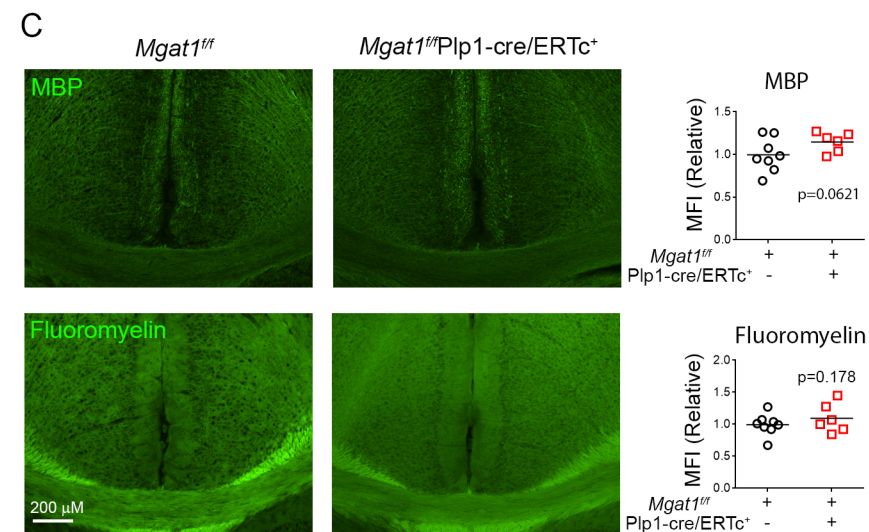
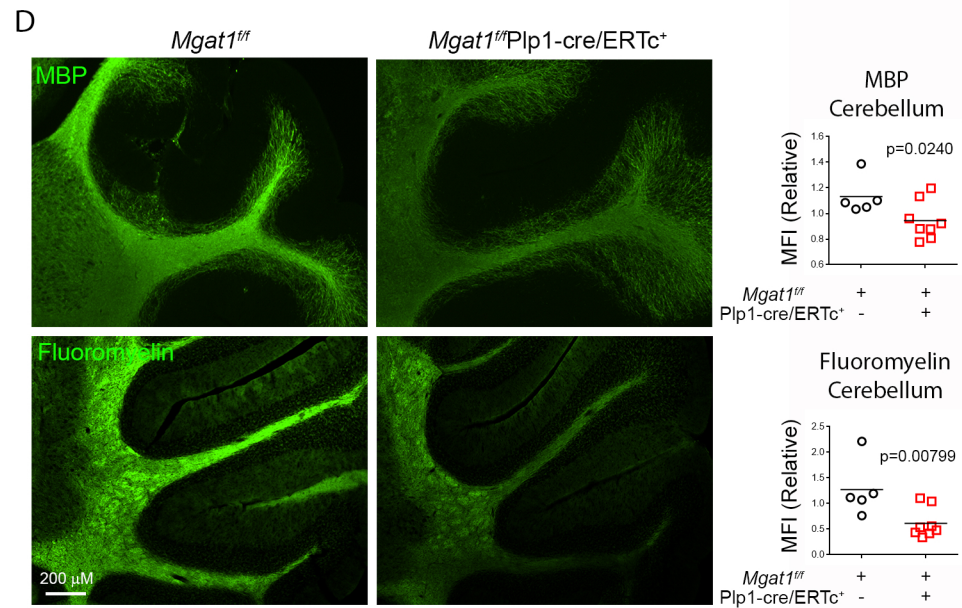
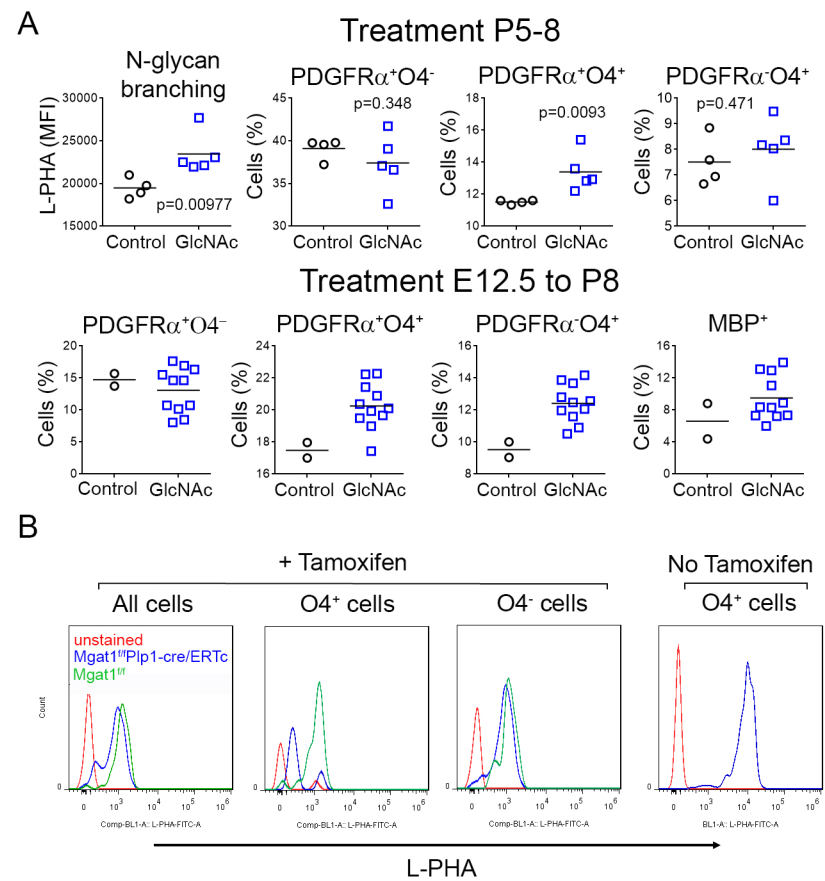
E



G

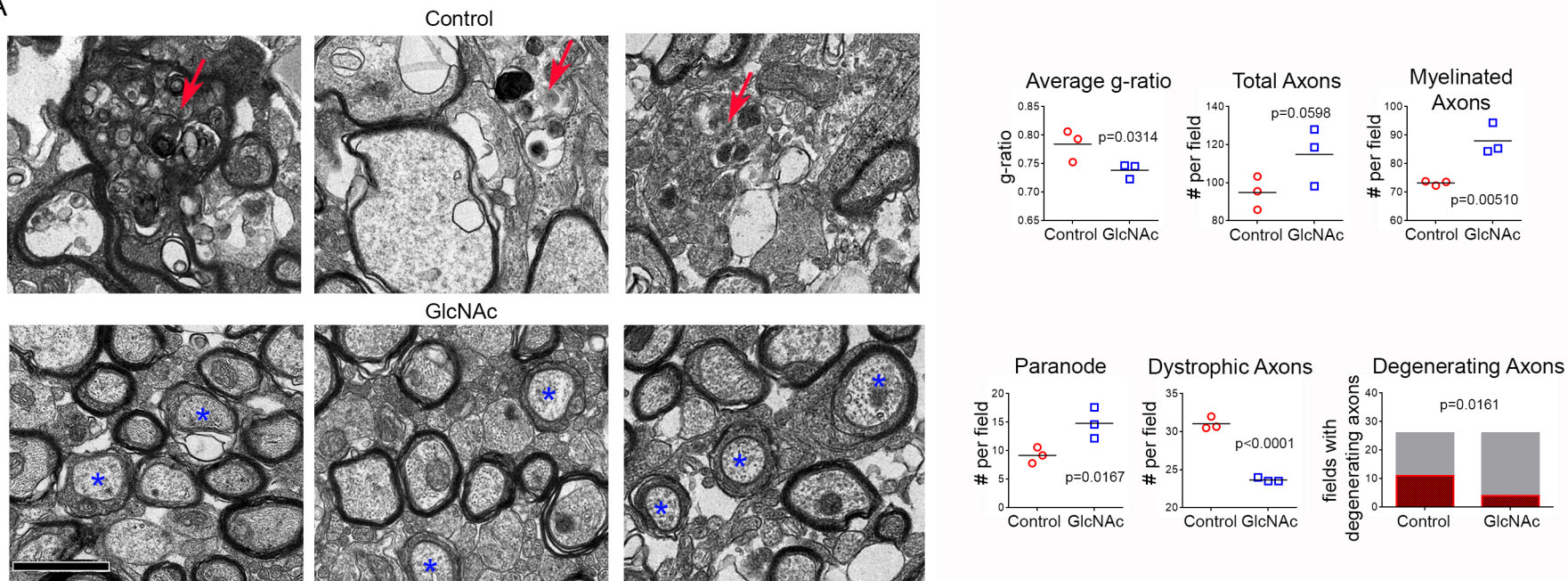


Supplementary Figure 2

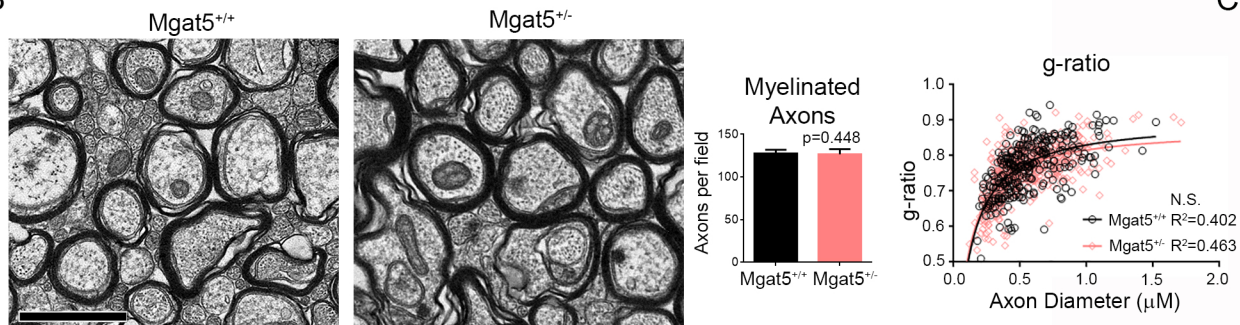


# Supplementary Figure 3

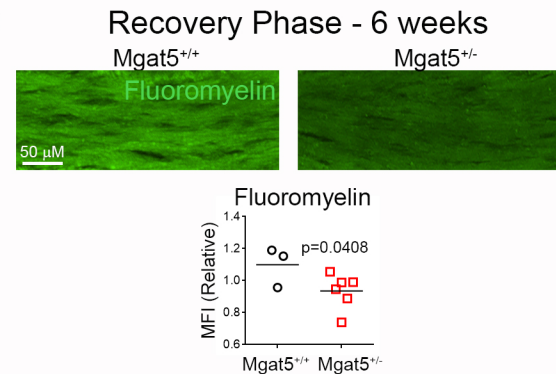
## A



## B



## C



**Supplementary Data Table 1 – MS cohort**

<b>MS patients</b>	<i>n</i>	<b>180</b>
<i>RRMS</i>	<i>n</i>	<i>125</i>
<i>PPMS</i>	<i>n</i>	<i>23</i>
<i>SPMS</i>	<i>n</i>	<i>32</i>
<b>Age (years)</b>	Mean±SD	<b>42.7±9.4</b>
<b>Sex (M/F)</b>	<i>n/n</i>	<b>73/107</b>
<b>Time since diagnosis (months)</b>	Mean±SD	<b>118.2±87.6</b>
<b>EDSS</b>	Median (Min - Max)	<b>3.0 (0.0-8.0)</b>
<b>T2LC</b>	Median (Min - Max)	<b>29 (0 – 162)</b>
<b>T2LV (ml)</b>	Median (Min - Max)	<b>2790 (0 – 54,900)</b>
<b>CELC</b>	Median (Min - Max)	<b>0 (0 – 6)</b>

Abbreviations: MS, multiple sclerosis; RRMS, relapsing-remitting MS; PPMS, primary progressive MS; SPMS, secondary progressive MS; SD, standard deviation; EDSS, Expanded Disability Status Scale; T2LC, T2w lesion count; T2LV, T2w lesion volume; CELC, contrast enhancing lesion count.