Cell Reports, Volume *31*

Supplemental Information

GDE2-Dependent Activation of Canonical Wnt Signaling in Neurons Regulates Oligodendrocyte Maturation

Bo-Ran Choi, Clinton Cave, Chan Hyun Na, and Shanthini Sockanathan

Figure S1

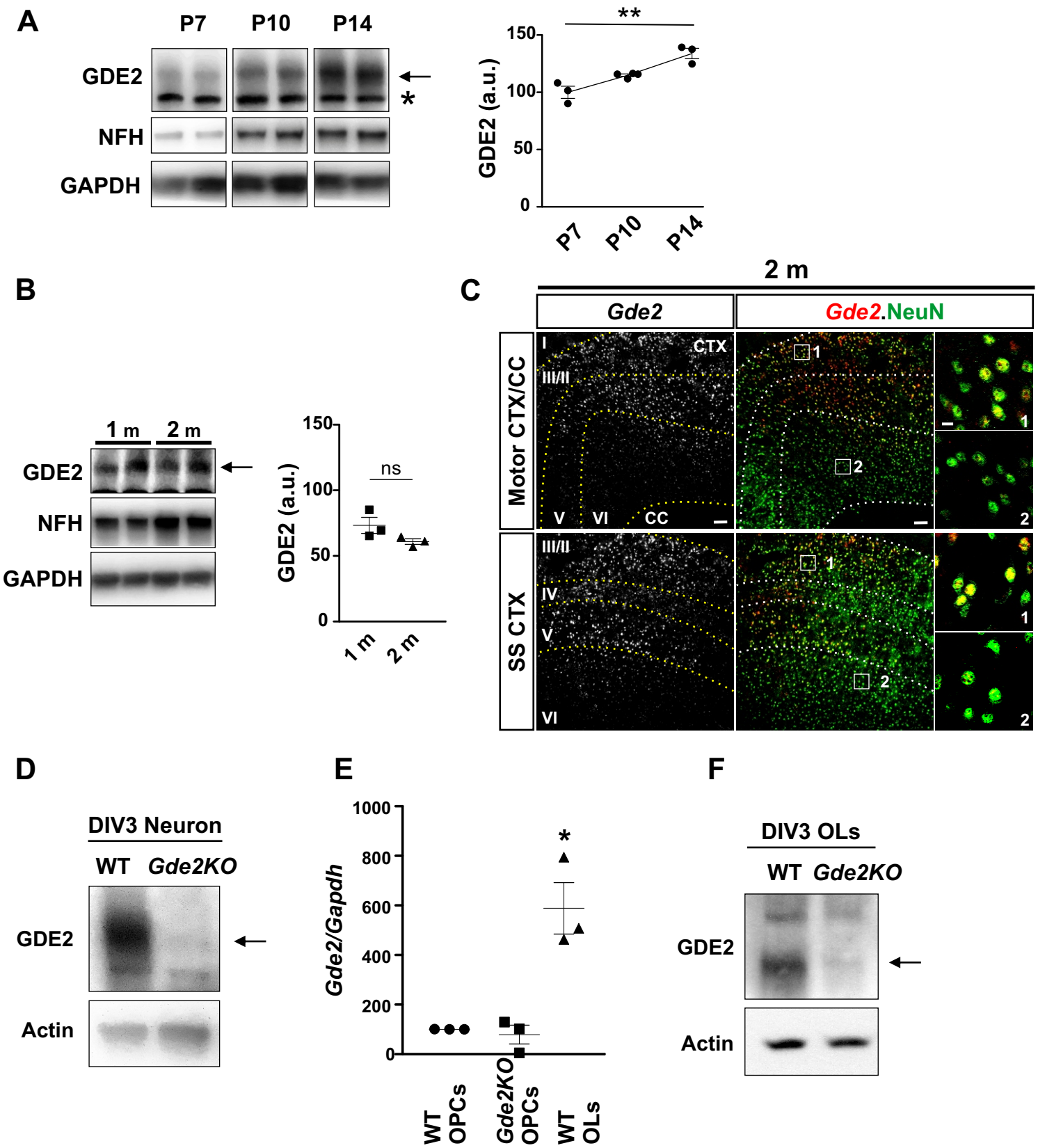

A
P7
P10
P14
GDE2
NFH
GAPDH
*
**
GDE2 (a.u.)
150
100
50
0
P7
P10
P14
B
1 m
2 m
GDE2
NFH
GAPDH
ns
GDE2 (a.u.)
150
100
50
0
1 m
2 m
C
2 m
Gde2
Gde2.NeuN
Motor CTX/CC
SS CTX
I
III/II
CTX
V
VI
CC
IV
1
2
D
DIV3 Neuron
WT
Gde2KO
GDE2
Actin
E
Gde2/Gapdh
1000
800
600
400
200
0
*
WT OPCs
Gde2KO OPCs
WT OLs
F
DIV3 OLs
WT
Gde2KO
GDE2
Actin

Figure S1. Related to Figure 1. GDE2 is expressed in neurons and OLs.

(A) Western blot of cortical extracts from WT animals at postnatal day 7 (P7), P10 and P14. GAPDH is a loading control, NFH is expressed in axons and provides a readout of neurons in brain tissue. Arrow indicates GDE2. Asterisk indicates a nonspecific band. Graph quantifying Western data from P7-P14. a.u. = arbitrary units. **p = 0.0013. n = 3 P7, 4 P10, 3 P14, 1-way ANOVA. (B) Western blot and quantification of GDE2 protein expression at 1 and 2 months (m) of age. ns p = 0.182. n = 3 for each timepoint, two-tailed unpaired t-test. (C) Cortical coronal sections showing *Gde2* transcript distribution. CC: corpus callosum, SS: somatosensory cortex. Cortical layers are marked by dotted lines. Boxed areas 1 and 2 are magnified in right panels. Scale bar: 100 µm, insets 10 µm (D) Western blot of DIV3 cortical neuronal cultures. Arrow marks GDE2. Actin is a loading control. (E) qPCR of *Gde2* transcripts normalized to *Gapdh* mRNA. *p = 0.0021. n = 3 sets of WT, *Gde2KO* OPCs, and WT OLs, 1-way ANOVA. (F) Western blot shows GDE2 is expressed in WT OLs (marked by arrow). Actin is a loading control. All graphs: Mean ± sem.

Figure S2

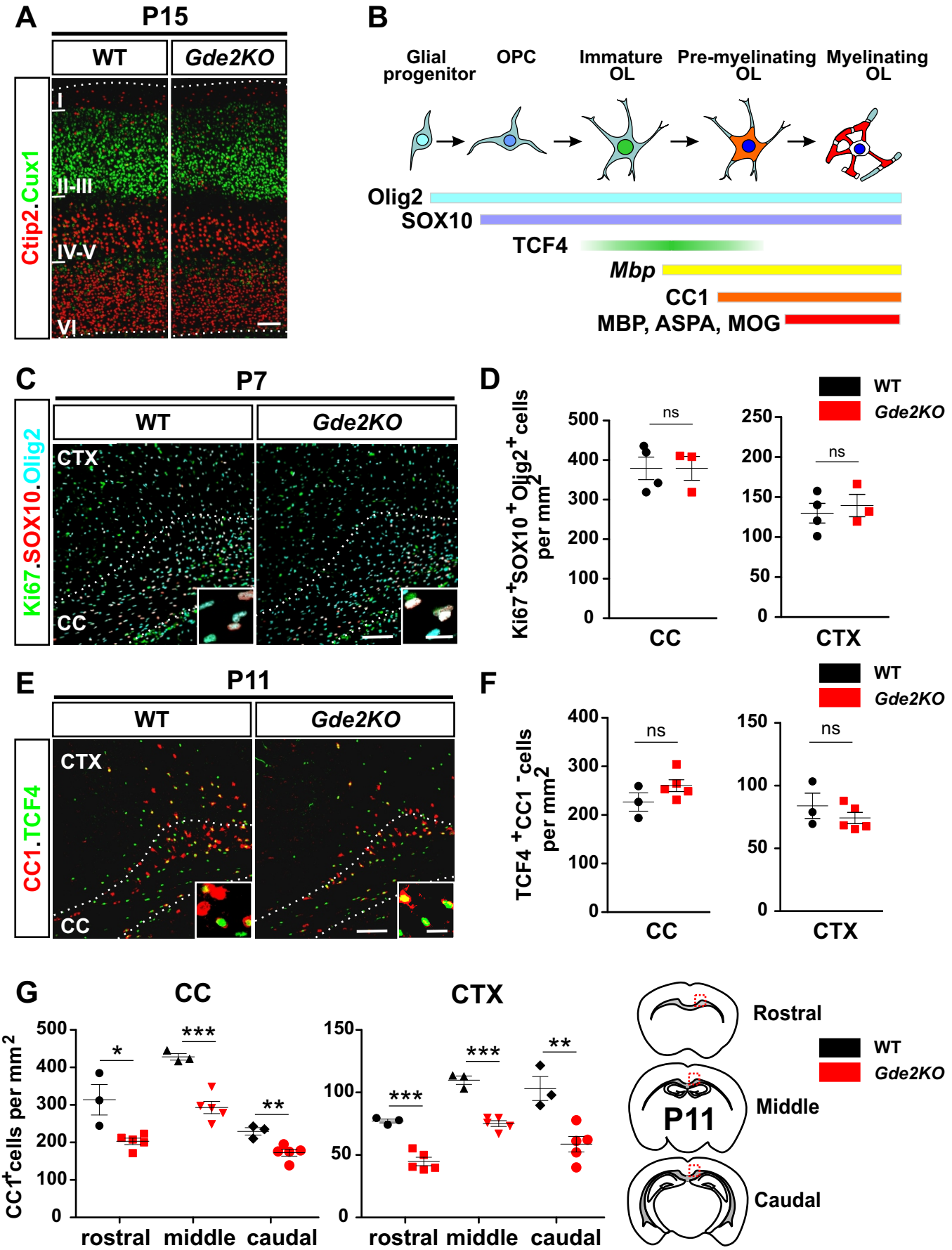

A
P15
WT
Gde2KO
Ctip2.Cux1
I
II-III
IV-V
VI
B
Glial progenitor
OPC
Immature OL
Pre-myelinating OL
Myelinating OL
Olig2
SOX10
TCF4
Mbp
CC1
MBP, ASPA, MOG
C
P7
WT
Gde2KO
Ki67.SOX10.Olig2
CTX
CC
D
Ki67+SOX10+Olig2+ cells per mm2
ns
CC
CTX
WT
Gde2KO
E
P11
WT
Gde2KO
CC1.TCF4
CTX
CC
F
TCF4+CC1- cells per mm2
ns
CC
CTX
WT
Gde2KO
G
CC
CTX
CC1+cells per mm2
rostral
middle
caudal
Rostral
Middle
Caudal
P11
WT
Gde2KO

Figure S2. Related to Figure 2. GDE2 loss impairs OL maturation.

(A) Coronal section of P15 mouse cortex, hatched lines mark cortical boundaries. Cortical layers I-VI are marked. (B) Schematic showing the progression of OL maturation coincident with marker expression. (C) Coronal section of P7 mouse cortex (CTX) and corpus callosum (CC). Hatched line marks boundary between CTX and CC. Inset box shows magnified image of proliferating OPCs (white, Ki67+Sox10+Olig2+). (D) Graphs quantifying the number of proliferating OPCs (Ki67+Sox10+Olig2+) CC ns p = 0.9981 and CTX ns p = 0.6352, n = 4 WT, 3 *Gde2KO*. Two tailed unpaired Student's t-test. (E) Coronal section of P11 mouse CTX and CC. Hatched line demarcates the CC. Inset box shows magnified image of immature (TCF4+CC1-) and mature OLs (CC1+). (F) Graphs quantifying the number of immature OLs (TCF4+CC1-). CC ns p = 0.2306 and CTX ns p = 0.8021, n = 3 WT, 5 *Gde2KO*. Two tailed unpaired Student's t-test. (G) Graphs quantifying number of CC1+ cells in boxed areas in rostral, middle and caudal regions of mouse P11 CC and CTX as shown in schematic. Data for middle regions are the same as in Figure 2B and are reproduced here for comparison purposes. CC: *p rostral = 0.013 ***p middle = 0.0007 **p caudal = 0.0072; CTX: *p rostral = 0.0004 ***p middle = 0.0035 **p caudal = 0.0063. n = 3 WT 5 *Gde2KO*. Two tailed unpaired Student's t-test. All graphs: Mean ± sem. Scale bars: (A, C, E) 100 µm, insets (C, E) 5 µm.

Figure S3

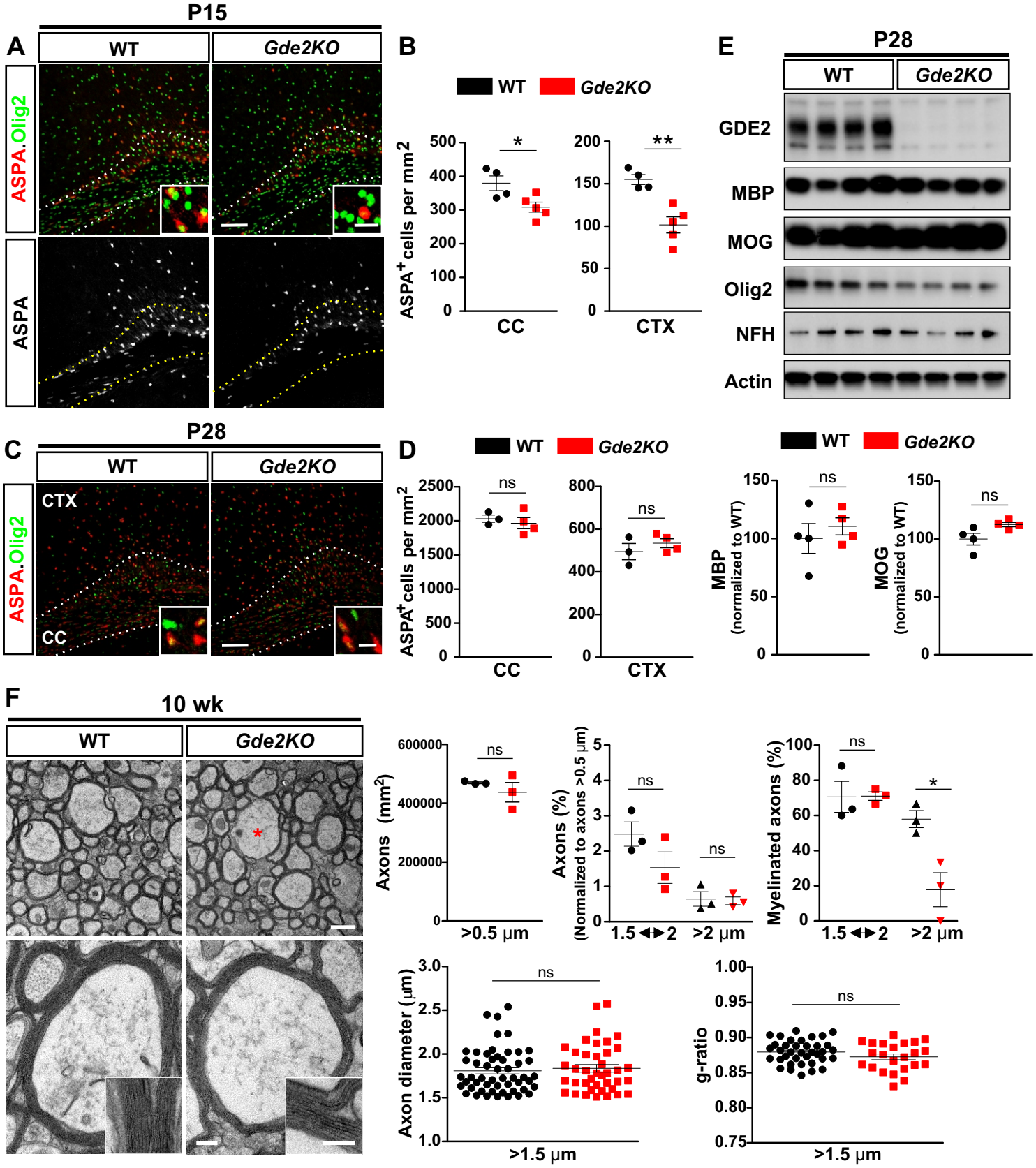

A
P15
WT
Gde2KO
ASPA.Olig2
ASPA
B
WT
Gde2KO
ASPA+ cells per mm2
500
400
300
200
100
0
*
CC
200
150
100
50
0
**
CTX
E
P28
WT
Gde2KO
GDE2
MBP
MOG
Olig2
NFH
Actin
C
P28
WT
Gde2KO
CTX
CC
ASPA.Olig2
D
WT
Gde2KO
ASPA+ cells per mm2
2500
2000
1500
1000
500
0
ns
CC
800
600
400
200
0
ns
CTX
WT
Gde2KO
MBP
(normalized to WT)
150
100
50
0
ns
MOG
(normalized to WT)
150
100
50
0
ns
F
10 wk
WT
Gde2KO
*
Axons (mm2)
600000
400000
200000
0
ns
>0.5 µm
Axons (%)
(Normalized to axons >0.5 µm)
5
4
3
2
1
0
ns
1.5 ◄►2
ns
>2 µm
Myelinated axons (%)
100
80
60
40
20
0
ns
1.5 ◄►2
*
>2 µm
Axon diameter (µm)
3.0
2.5
2.0
1.5
1.0
ns
>1.5 µm
g-ratio
1.00
0.95
0.90
0.85
0.80
0.75
ns
>1.5 µm

Figure S3. Related to Figure 2. *Gde2* KOs show recovery of myelin protein but have decreased myelination of large-diameter axons.

(A, C) Coronal sections of mouse cortex (CTX) and corpus callosum (CC). Hatched lines delineate the CC. Insets show high magnification of mature OLs. Scale bars: 100 µm insets: 10 µm in A and C. (B, D) Graphs quantifying numbers of ASPA+ OLs in CC and CTX. (B) CC *p = 0.0275 and CTX **p = 0.0028, n = 4 WT, 5 *Gde2KO* (D) CC ns p = 0.539 and CTX ns p = 0.4373, n = 3 WT, 4 *Gde2KO*. (E) Western blot of cortical extracts from P28 animals. Graphs quantifying myelin associated proteins MBP ns p = 0.5198, MOG ns p = 0.1035 n = 4 WT, 4 *Gde2KO.* (F) Representative TEM images of 10 week WT and *Gde2KO* animals. * marks exemplar unmyelinated larger-diameter axon in *Gde2KO* condition. Scale bar: (Top) 1 µm, (Bottom) 200 nm, Inset 100 nm. Graphs quantifying axon numbers (ns p = 0.3905) and the percentage of axons with diameters between 1.5 and 2µm (ns p = 0.1649) and larger than 2µm (ns p = 0.8323). Although the percentage of myelinated axons between 1.5 and 2µm is equivalent between WT and *Gde2KO* animals (ns p = 0.9667), the percentage of myelinated axons with diameters larger than 2µm is dramatically reduced (*p = 0.0206) n = 3 WT, 3 *Gde2KO.* Diameters of axons greater than 0.5µm (ns p = 0.5977) and g-ratios of myelinated axons with diameters larger than 1.5µm are unchanged between WT and *Gde2KO* animals (ns p = 0.156). Each point refers to individual myelinated axons from 3 WT and 3 *Gde2KO*. All graphs Mean ± sem, two tailed unpaired t-test.

Figure S4

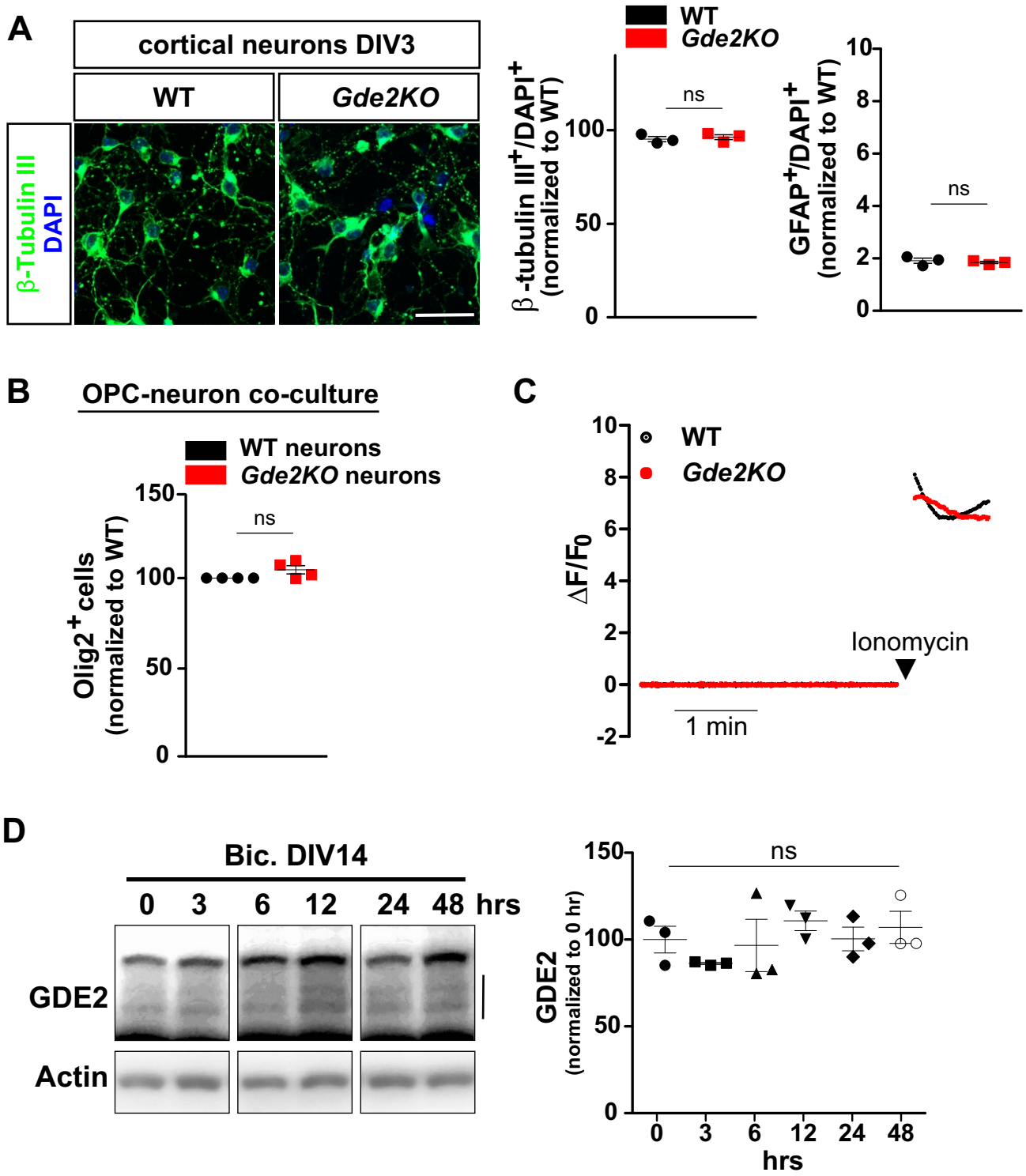
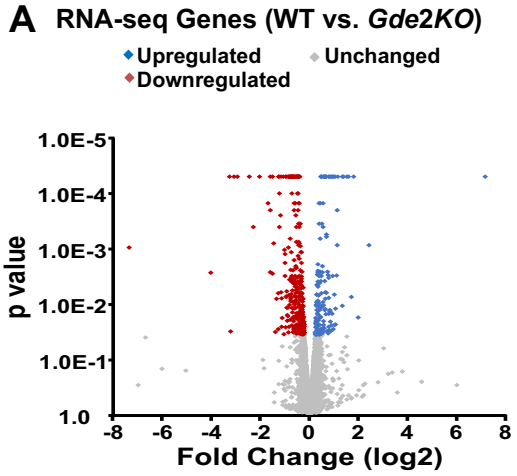
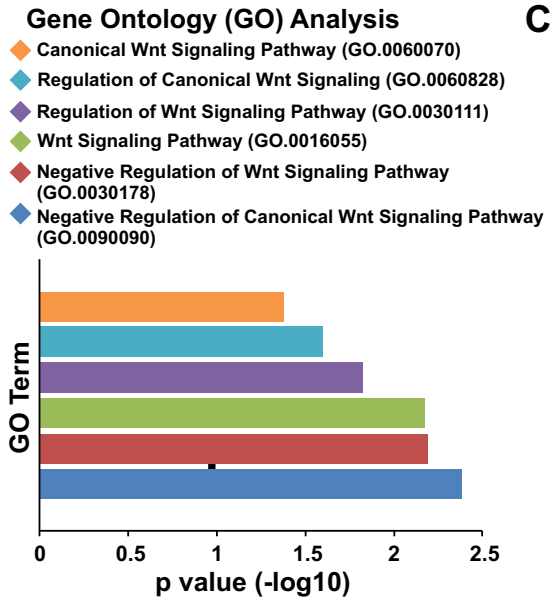

A
cortical neurons DIV3
WT
Gde2KO
β-Tubulin III
DAPI
WT
Gde2KO
ns
β-tubulin III+/DAPI+ (normalized to WT)
100
50
0
ns
GFAP+/DAPI+ (normalized to WT)
10
8
6
4
2
0
B
OPC-neuron co-culture
WT neurons
Gde2KO neurons
ns
Olig2+cells (normalized to WT)
150
100
50
0
C
WT
Gde2KO
ΔF/F0
10
8
6
4
2
0
-2
Ionomycin
1 min
D
Bic. DIV14
0 3 6 12 24 48 hrs
GDE2
Actin
ns
GDE2 (normalized to 0 hr)
150
100
50
0
0 3 6 12 24 48
hrs

Figure S4. Related to Figure 4. Characterization of neuron-OPC co-cultures.

(A) Immunocytochemical staining of DIV3 cortical neuronal cultures. Scale bar: 50 μm. Graphs quantifying percentage neurons (β-tubulin III+) and astrocytes in DIV3 WT and *Gde2KO* cortical neuronal cultures (β-tubulin III+ ns p = 0.6243; GFAP+ ns p = 0.6093; n = 3 WT 3 *Gde2KO*). (B) Graph quantifying the number of Olig2+ cells after neuron-OPC co-culture (ns p = 0.1235, n = 4 WT neuron-WT OPC co-cultures, 4 *Gde2KO* neuron-WT OPC co-cultures). Two tailed unpaired Student's t-test. All graphs: Mean ± sem. (C) Graph of fluorescence changes (Δ F/F0) in DIV3 WT and *Gde2KO* neurons loaded with the calcium indicator Fluo-4 over a 3.5 minute period. Each data point represents mean value of $\Delta F/F_0$ from at least 11 recordings per group at a given time. Arrowhead marks the time of Ionomycin addition, which permeabilizes the membrane and acts as a positive control. (D) Western blot of DIV14 cortical neurons treated with bicuculine for specified times. Bar denotes GDE2. Graph quantifying GDE2 protein levels show no change in expression after stimulation. 1 way ANOVA ns p = 0.4692, n = 3 for each timepoint.

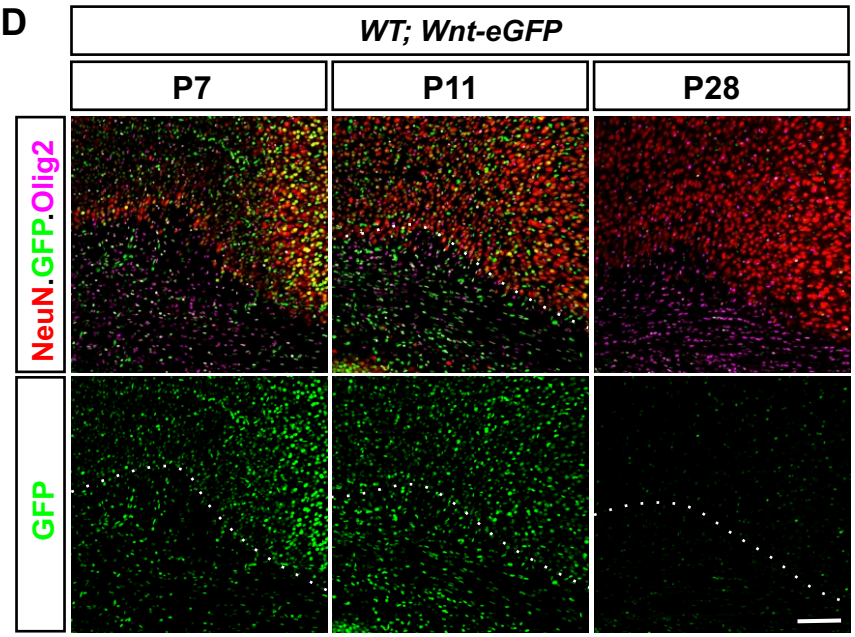
Figure S5

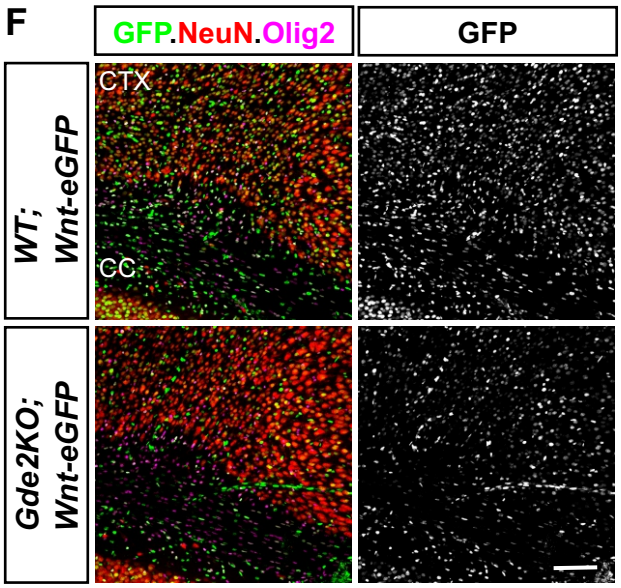


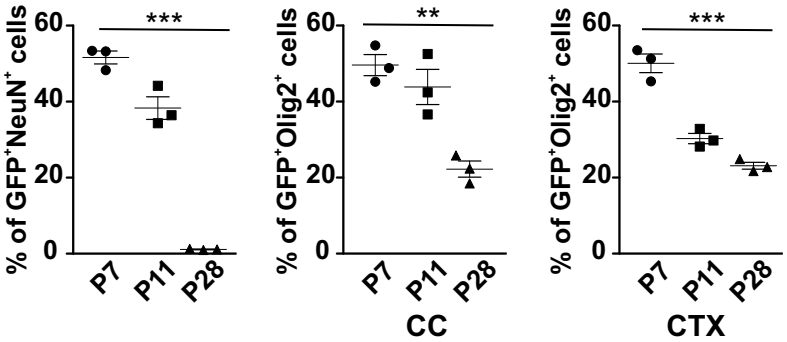


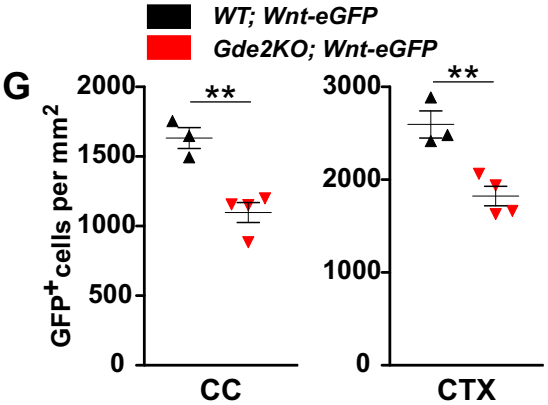
C

Gene name	log2(FC)	p value
Mitf	-2.03989	0.00005
Pttg1	-0.685154	0.00005
Sox9	-0.440899	0.00005
Vegfc	-0.678135	0.00245
Islr	-0.387588	0.0037
Axin2	-0.353966	0.024
Cd44	-0.314482	0.0263









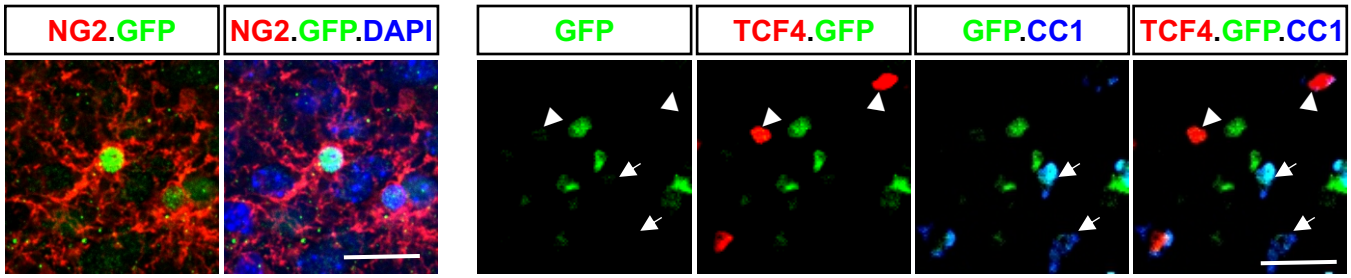


Figure S5. Related to Figure 5. Canonical Wnt signaling is reduced when GDE2 is disrupted. (A) Volcano plot showing differentially expressed genes between WT and *Gde2KO* spinal cord. (B) Gene ontology analysis using p-value (< 0.05) highlights Wnt signaling pathways are disrupted in absence of GDE2. (C) List of known Wnt target genes that are altered in *Gde2KO* condition. (D) Analysis of Wnt-reporter animals (*Wnt-eGFP*) show that canonical Wnt signaling (eGFP) is high at P7 and P11 but is minimal at P28. Hatched line marks the boundary between cortex and corpus callosum. (E) Graphs quantifying the percentage of reporter gene expression in *Wnt-eGFP* mice at P7, P11 and P28 in neurons (NeuN+) and oligodendroglia (Olig2+) in corpus callosum (CC) and cortex (CTX). GFP+NeuN+ ***p < 0.0001, GFP+Olig2+ CC **p = 0.0027, GFP+Olig2+ CTX ***p <0.0001. n = 3 P7, 3 P11, 3 P28, 1-way ANOVA. Data for P11 are the same as presented in Figure 4F and 4H (WT) and are included here for comparison purposes. (F) Coronal sections of P11 *WT; Wnt-eGFP* and *Gde2KO;Wnt-eGFP* animals (G) Graphs quantifying GFP+ cells in CC and CTX. CC **p = 0.004, CTX **p = 0.007, n = 3 *WT; Wnt-eGFP*, 4 *Gde2KO;Wnt-eGFP*, two-tailed unpaired t-test. (H) Representative image of P11 cortex of *Wnt-eGFP* mice. Arrowheads mark TCF4+CC1- immature OLs, arrows mark mature CC1+ OLs; both populations do not co-express eGFP. All graphs: Mean ± sem. Scale bar: (D, F) 100 µm, (H) 20µm.

Figure S6

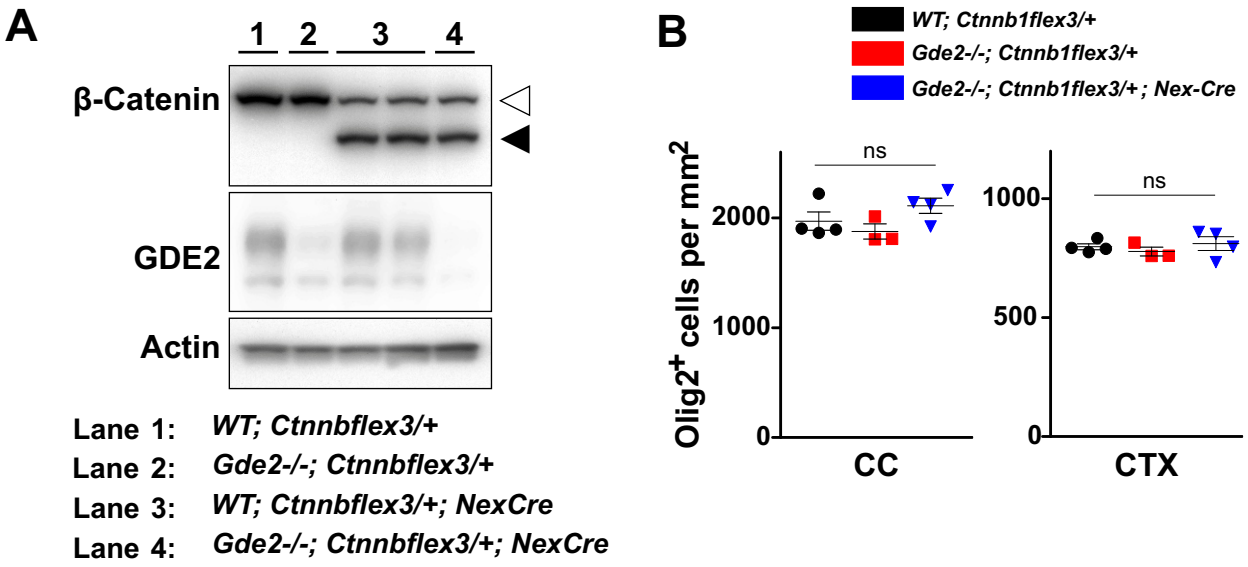
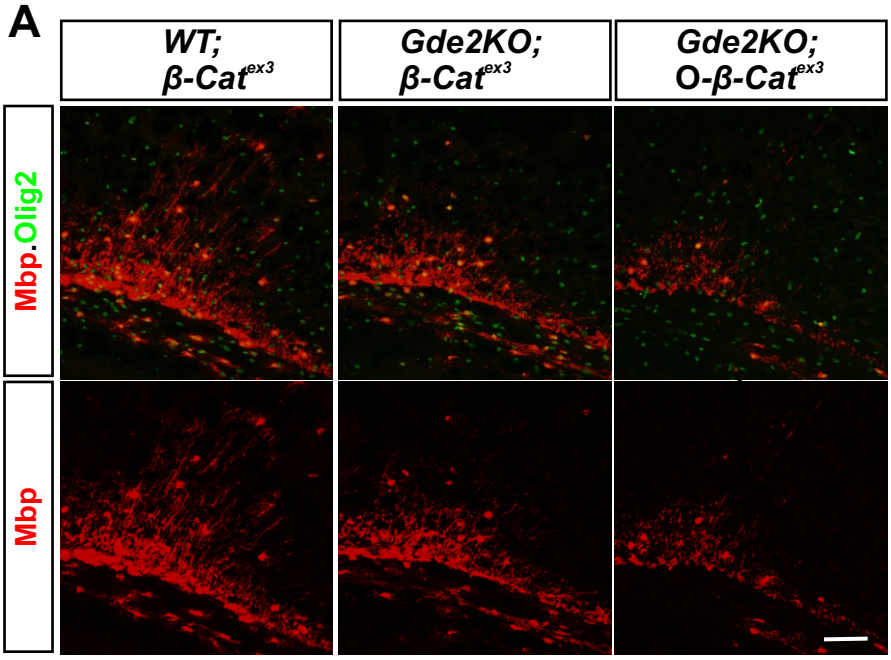
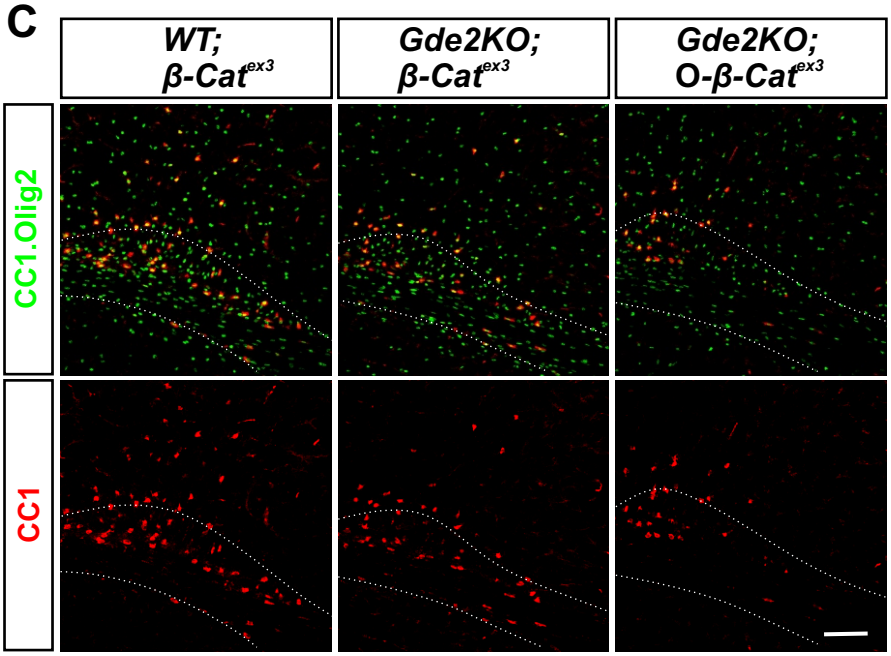

A
1
2
3
4
β-Catenin
GDE2
Actin
Lane 1: WT; Ctnnbflex3/+
Lane 2: Gde2-/-; Ctnnbflex3/+
Lane 3: WT; Ctnnbflex3/+; NexCre
Lane 4: Gde2-/-; Ctnnbflex3/+; NexCre
B
WT; Ctnnb1flex3/+
Gde2-/-; Ctnnb1flex3/+
Gde2-/-; Ctnnb1flex3/+ ; Nex-Cre
Olig2+ cells per mm2
ns
2000
1000
0
CC
ns
1000
500
0
CTX

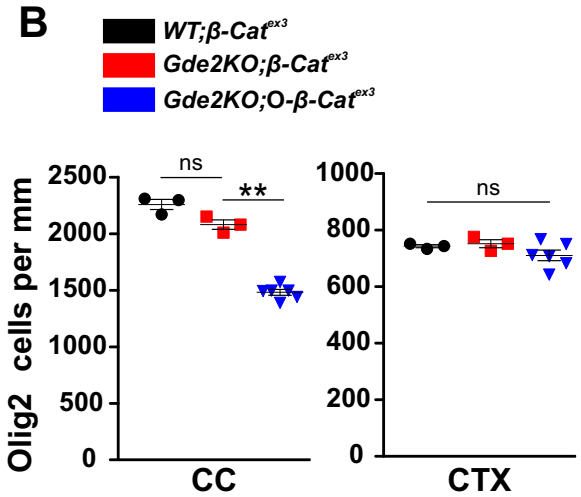
Figure S6. Related to Figure 6. Genetic stabilization of β-catenin in neurons does not change total Olig2+ cells.

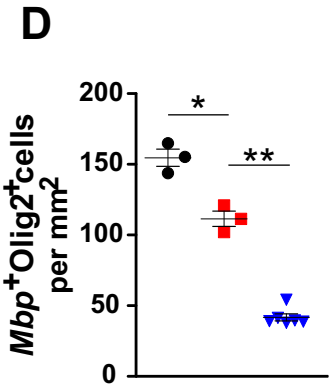
(A) Western blot of P14 cortical extracts. Open arrowhead marks WT β-catenin; black arrowhead marks β-catenin deleted for exon 3. Actin is used as a loading control. (B) Graphs quantifying the number of Olig2+ cells in P11 corpus callosum (CC) and cortex (CTX). ns CC p = 0.1608 (1-way ANOVA/Bonferroni's multiple comparison test), ns CTX p = 0.6109 (1-way ANOVA/Bonferroni's multiple comparison test). n = 4 *WT;β-cat*^{ex3}, 3 *Gde2KO;β-cat*^{ex3}; 4 *Gde2KO;*N-*β-cat*^{ex3}. All graphs: Mean ± sem.

Figure S7


A
WT; β-Cat ex3
Gde2KO; β-Cat ex3
Gde2KO; O-β-Cat ex3
Mbp.Olig2
Mbp


C
WT; β-Cat ex3
Gde2KO; β-Cat ex3
Gde2KO; O-β-Cat ex3
CC1.Olig2
CC1


B
WT;β-Cat ex3
Gde2KO;β-Cat ex3
Gde2KO;O-β-Cat ex3
Olig2 cells per mm
2500
2000
1500
1000
500
0
ns
**
CC
1000
800
600
400
200
0
ns
CTX


D
Mbp+Olig2+cells per mm2
200
150
100
50
0
*
**

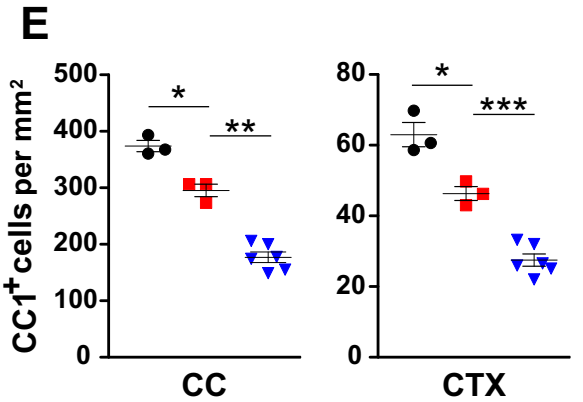

E
CC1+cells per mm2
500
400
300
200
100
0
*
**
CC
80
60
40
20
0
*
***
CTX

Figure S7. Related to Figure 5. Stabilization of β-catenin in OPCs does not rescue *Gde2KO* OL maturation.

(A, C) Coronal sections of P11 mouse cortex (CTX) and corpus callosum (CC). Hatched lines in panel C outlines CC boundaries. (B) Graphs quantifying the number of Olig2+ cells in CC and CTX. CC ns p = 0.0601, **p = 0.0011, two-tailed unpaired t- test; CTX ns p = 0.1235, 1-way ANOVA Bonferroni's multiple comparison test, all 3 genotypes. n = 3 *WT;β-cat*^{ex3}, 3 *Gde2KO;β-cat*^{ex3}, 6 *Gde2KO;*O-*β-cat*^{ex3}. (D) Graph quantifying the number of MBP+Olig2+ cells *p = 0.0132, **p = 0.0073, n = 3 *WT;β-cat*^{ex3}, 3 *Gde2KO;β-cat*^{ex3}, 6 *Gde2KO;*O-*β-cat*^{ex3}. Two tailed unpaired Student's t-test. (E) Graphs quantifying number of CC1+ OLs. CC *p = 0.0132, **p = 0.0012; CTX *p = 0.0242, ***p = 0.0008, n = 3 *WT;β-cat*^{ex3}, 3 *Gde2KO;β-cat*^{ex3}, 6 *Gde2KO;*O-*β-cat*^{ex3}. Two tailed unpaired Student's t-test. All graphs: Mean ± sem. Scale bars: 100 μm.

Figure S8

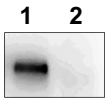
A

Protein name	Gene name	KO/WT ratio
Glypican-1;Secreted glypican-1	*Gpc1*	0.82
Glypican-2;Secreted glypican-2	*Gpc2*	0.91
Semaphorin-7A	*Sema7a*	0.94
Cadherin-13	*Cdh13*	0.95
Contactin-2 (a.k.a. TAG-1)	*Cntn2*	1.05
Neural cell adhesion molecule 1	*Ncam1*	1.11
RGM domain family member B	*Rgmb*	1.22
Lipoprotein lipase	*Lpl*	1.23
Repulsive guidance molecule A	*Rgma*	1.25
Contactin-1	*Cntn1*	1.25
Neural cell adhesion molecule 2	*Ncam2*	1.40

B

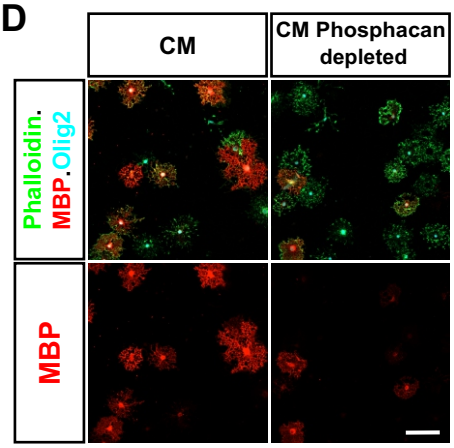
Protein name	*Gene name*	KO/WT ratio
Carboxypeptidase B2	*Cpb2*	0.33
Phosphatidylethanolamine-binding protein 1	*Pebp1*	0.43
Receptor-type tyrosine-protein phosphatase zeta	*Ptprz1*	0.44
Gamma-glutamyl hydrolase	*Ggh*	0.47
Follistatin-related protein 5	*Fstl5*	0.50
Glucose-6-phosphate isomerase	*Gpi*	0.51
ProSAAS	*Pcsk1n*	0.53
Noelin	*Olfm1*	0.53
Glia-derived nexin	*Serpine2*	0.54
Complement C5	*C5*	0.57
Collagen alpha-1(I) chain	*Col1a1*	3.98

C

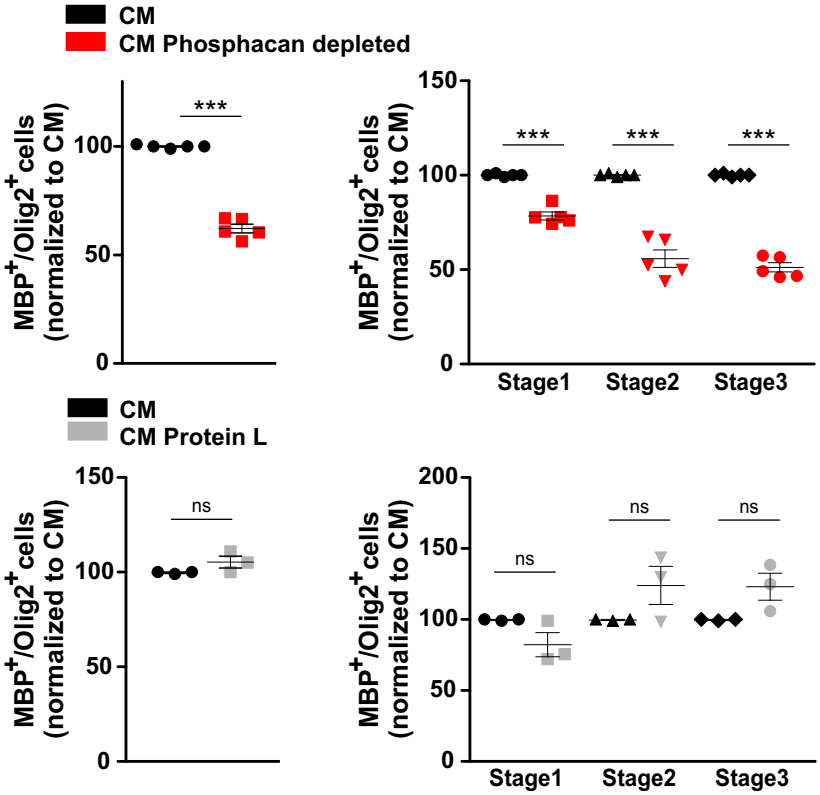


1 CM
2 CM Phosphacan depleted

D



E



F

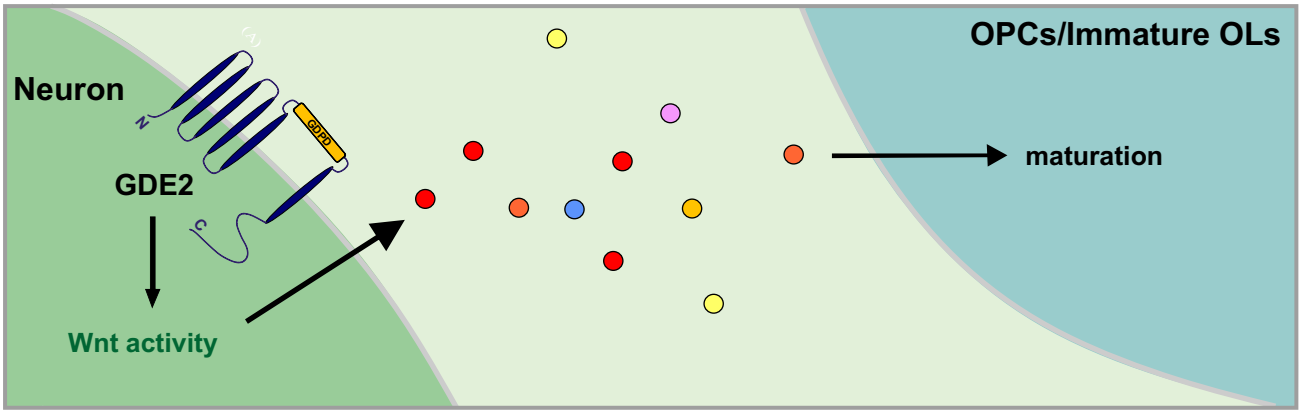


Figure S8. Related to Figure 7. Candidate mechanisms for GDE2-dependent OL maturation. (A) List of GPI-anchored proteins identified in WT and *Gde2KO* CM by mass spectrometry. (B) List of secreted and extracellular matrix proteins identified in WT and *Gde2KO* CM by mass spectrometry. RPTPzeta (phosphacan) is highlighted in red. (C) Western blot of WT neuronal CM showing effective depletion of phosphacan using neuronal phosphacan antibodies conjugated to protein-L (D) Representative images of WT OPCs after culturing with WT neuronal CM and WT neuronal CM depleted for phosphacan. (E) Graphs quantifying the percentage of MBP+Olig2+ OLs (normalized to WT CM) in WT OPC cultures grown with WT neuronal CM or phosphacan depleted WT neuronal CM. Top panel ***$p<0.0001$, n = 5 WT CM, 5 Phosphacan depleted CM, two tailed unpaired t-test. All 3 stages of OL maturation are also affected (2-way ANOVA ***$p < 0.0001$; Bonferroni correction Stage 1 ***$p <0.001$; Stage 2 ***$p <0.001$; Stage 3 ***$p <0.001$; n = 5 WT CM, 5 Phosphacan depleted CM. No change in OPC maturation is observed between WT neuronal CM or WT neuronal CM preincubated with protein L alone. Bottom panel ns $p = 0.1456$, n = 3 WT CM, 3 Protein L incubated CM, two tailed unpaired t-test. Similarly, stages of OL maturation are unchanged (2-way ANOVA ns $p = 0.1262$; Bonferroni correction Stage 1 ns $p >0.05$; Stage 2 ns $p >0.05$; Stage 3 ns $p >0.05$; n = 5 WT CM, 5 Phosphacan depleted CM.(F) Model for GDE2 regulation of OL maturation. GDE2 stimulates canonical Wnt signaling in neurons. Wnt activation leads to release of neuronally-derived factors such as phosphacan, which act on neighboring OPCs or immature OLs to promote their maturation into myelinating oligodendrocytes.

Table S1: Cell counts for in vitro cultures. Refers to Figure 4, Figure 7, and Figure S8.

Fig 4

B/F			D/F			D		
DIV3+4_coculture	WT	*Gde2KO*	DIV3+4 CM	WT	*Gde2KO*	DIV3 CM	WT	*Gde2KO*
Olig2+	2959	3030	Olig2+	1251	1126	Olig2+	730	907
MBP_stage 1	159	89	MBP_stage 1	251	194	MBP_stage 1	50	60
MBP_stage 2	116	43	MBP_stage 2	219	147	MBP_stage 2	71	51
MBP_Stage 3	53	18	MBP_Stage 3	62	30	MBP_Stage 3	48	27
total MBP+	328	152	total MBP+	532	371	total MBP+	169	138

Fig 7B

	Gde2KO;β-Cat^{ex3} CM	*Gde2KO;* N-*β-Cat*^{ex3} CM
Olig2+	936	894
MBP_stage 1	65	100
MBP_stage 2	26	43
MBP_Stage 3	17	28
total MBP+	108	171

Fig S8E

	CM	CM phsophacan depleted		CM	CM protein L
Olig2+	2189	2815	Olig2+	1007	869
MBP_stage 1	290	306	MBP_stage 1	132	97
MBP_stage 2	377	278	MBP_stage 2	132	140
MBP_Stage 3	189	138	MBP_Stage 3	61	67
total MBP+	856	722	total MBP+	325	304

Table S3: Related to Supplemental Figure 8. List of proteins showing > 40% differential enrichment in *Gde2KO* CM.

Number of altered protein expression: 149

Protein IDs	Protein names	Gene names	Sequence coverage KO [%]	Sequence coverage WT [%]	LFQ intensity KO	LFQ intensity WT	Ratio (KO/WT)
A8DUK4	beta-globin	*Hbbt1*	60.9	58.2	3.67E+06	3.93E+08	0.0093385
Q80ZV4	Cadherin-4	*Cdh4*	3.6	3.6	3.59E+05	6.73E+06	0.0533103
P35979	60S ribosomal protein L12	*Olfm1*	9.7	9.7	3.50E+05	3.35E+06	0.104707
Q3TLP8	Ras-related C3 botulinum toxin substrate 1	*Rac1*	9.45	12.3	1.22E+06	1.16E+07	0.1055619
P58283	E3 ubiquitin-protein ligase RNF216	*Rnf216*	0.8	0.8	7.20E+05	5.81E+06	0.1241085
Q6ZWQ9	Myosin regulatory light chain 12B	*Myl12a*	17.4	9.3	2.92E+06	2.11E+07	0.1384735
P48678	Prelamin-A/C	*Lmna*	1.8	0.75	7.71E+04	3.94E+05	0.1955637
F6VYE2	Zinc finger MYM-type protein 4	*Zmym4*	0.7	0.7	3.81E+07	1.28E+08	0.2985482
G5E866	Splicing factor 3B subunit 1	*Sf3b1*	0.35	1.1	6.29E+05	2.08E+06	0.3018324
Q99JI6	Ras-related protein Rap-1b	*Rap1b*	11.15	11.15	2.20E+06	7.24E+06	0.304105
P62897	Cytochrome c, somatic	*Cycs*	17.15	17.15	1.47E+06	4.78E+06	0.3074461
P32233	Developmentally-regulated GTP-binding protein 1	*Drg1*	4.4	4.4	4.55E+05	1.37E+06	0.3315903
Q2KIG3	Carboxypeptidase B2	*Cpb2*	2.6	2.15	2.81E+05	8.34E+05	0.3367496
P19253	60S ribosomal protein L13a	*Rpl13a*	8.1	8.1	1.20E+06	3.52E+06	0.3409558
P97350	Plakophilin-1	*Pkp1*	2.25	2.25	2.43E+06	6.66E+06	0.3647232
P62242	40S ribosomal protein S8	*Rps8*	8.9	7.2	7.11E+05	1.75E+06	0.4058655
Q3UHN9	Bifunctional heparan sulfate N-deacetylase	*Ndst1*	0.8	1.6	9.53E+05	2.35E+06	0.4063519
A0A087WPL5	ATP-dependent RNA helicase A	*Dhx9*	0.6	0.85	4.55E+05	1.11E+06	0.4094201
Q8QZY9	Splicing factor 3B subunit 4	*Sf3b4*	1.65	3.3	2.16E+06	5.03E+06	0.4288
P70296	Phosphatidylethanolamine-binding protein 1	*Pebp1*	32.6	29.15	4.56E+07	1.05E+08	0.4363098
Q62189	U1 small nuclear ribonucleoprotein A	*Snrpa*	9.55	9.55	7.22E+06	1.64E+07	0.4395122
B9EKR1	Receptor-type tyrosine-protein phosphatase zeta	*Ptprz1*	7.05	6.8	1.08E+08	2.43E+08	0.445375
Q9DBG3	AP-2 complex subunit beta;AP complex subunit beta	*Ap2b1*	2	1.3	2.06E+06	4.55E+06	0.4524362
Q60648	Ganglioside GM2 activator	*Gm2a*	8.8	4.4	1.39E+06	2.98E+06	0.4666868
Q9CXV9	DCN1-like protein 5	*Dcun1d5*	6.3	6.3	3.76E+07	8.06E+07	0.466781
Q9Z0L8	Gamma-glutamyl hydrolase	*Ggh*	12.9	12.9	6.49E+06	1.37E+07	0.4751144
P80317	T-complex protein 1 subunit zeta	*Cct6a*	4.35	2.85	1.13E+06	2.35E+06	0.4792104
B1AX58	Plastin-3	*Pls3*	0.7	1.4	8.46E+05	1.76E+06	0.4811927
Q9R1P3	Proteasome subunit beta type-2	*Psmb2*	6.2	9.95	8.15E+06	1.65E+07	0.4936686
Q8R093	Uridine phosphorylase	*Upp2*	4.2	2.1	5.18E+07	1.03E+08	0.5007006
Q8BFR2	Follistatin-related protein 5	*Fstl5*	8	9.8	1.04E+07	2.08E+07	0.5017444
Q9EST1	Gasdermin-A	*Gsdma*	1	2.6	1.80E+06	3.50E+06	0.5148759
Q91V64	Isochorismatase domain-containing protein 1	*Isoc1*	5.2	10.4	1.33E+06	2.58E+06	0.5164249
P06745	Glucose-6-phosphate isomerase	*Gpi*	5.2	5.2	9.57E+05	1.84E+06	0.518811
Q9CQU0	Thioredoxin domain-containing protein 12	*Txndc12*	6.45	4.4	1.93E+06	3.68E+06	0.5235417
Q08331	Calretinin	*Calb2*	11.8	11.8	3.97E+06	7.51E+06	0.5290136
Q9QXV0	ProSAAS	*Pcsk1n*	12.2	10.5	9.84E+06	1.85E+07	0.53233
A3KGE4	Noelin	*Olfm1*	4.3	4.3	7.73E+05	1.44E+06	0.5356937
Q3TKX1	V-type proton ATPase subunit S1	*Atp6ap1*	14.2	14.2	1.28E+07	2.37E+07	0.5403885
Q9DAY9	Nucleophosmin	*Npm1*	4.1	14	1.17E+07	2.15E+07	0.5426547
Q07235	Glia-derived nexin	*Serpine2*	13.35	13.35	5.49E+06	1.00E+07	0.5480371
Q9JJU8	SH3 domain-binding glutamic acid-rich-like protein	*Sh3bgrl*	25	18	7.48E+06	1.36E+07	0.548844
P61164	Alpha-centractin	*Actr1a*	2.95	7.85	1.46E+06	2.65E+06	0.550069
P61358	60S ribosomal protein L27	*Rpl27*	7.35	10.65	2.31E+06	4.20E+06	0.5504141
O09061	Proteasome subunit beta type-1	*Psmb1*	14.15	18.3	1.62E+07	2.92E+07	0.5534894
Q5RKN9	F-actin-capping protein subunit alpha-1	*Capza1*	13.6	13.6	4.36E+06	7.85E+06	0.5548677
E9PYH2	Cytosolic acyl coenzyme A thioester hydrolase	*Acot7*	8.3	6.75	6.44E+06	1.15E+07	0.559416
P24369	Peptidyl-prolyl cis-trans isomerase B	*Ppib*	18.05	16	2.04E+07	3.62E+07	0.5644408
Q9D1C8	Vacuolar protein sorting-associated protein 28 homolog	*Vps28*	2.75	5.5	7.42E+05	1.31E+06	0.5652669
Q3TX55	Actin-related protein 2/3 complex subunit 4	*Arpc4*	20.25	17.9	1.39E+07	2.44E+07	0.569117
P62702	40S ribosomal protein S4	*Gm15013*	5.1	8.1	7.23E+06	1.27E+07	0.569801
S4R1N6	40S ribosomal protein S18	*Rps18*	22.9	18.7	1.08E+07	1.88E+07	0.5729743
P06684	Complement C5	*C5*	1.05	1.05	2.07E+06	3.60E+06	0.5752787
D3Z6E4	Enolase;Gamma-enolase	*Eno2*	6.35	7.9	4.46E+05	7.64E+05	0.5833966
P50247	Adenosylhomocysteinase	*Ahcy*	9	9	5.44E+06	9.19E+06	0.5925307
P20029	78 kDa glucose-regulated protein	*Hspa5*	7.25	9.4	2.20E+06	3.68E+06	0.5991105
P55821	Stathmin-2	*Stmn2*	14.5	14.5	2.67E+07	1.91E+07	1.4007286
O35136	Neural cell adhesion molecule 2	*Ncam2*	3.5	3.5	2.09E+06	1.49E+06	1.4047028
Q60994	Adiponectin	*Adipoq*	10.75	10.75	7.19E+07	5.10E+07	1.4090303
Q60864	Stress-induced-phosphoprotein 1	*Stip1*	2.9	2.9	5.38E+06	3.81E+06	1.4108147
D3YYE1	Acidic leucine-rich nuclear phosphoprotein 32 family member A	*Anp32a*	13.9	17.2	1.19E+07	8.38E+06	1.4150217
Q8BGQ7	Alanine--tRNA ligase, cytoplasmic	*Aars*	0.9	0.9	1.01E+06	7.09E+05	1.4222222
Q93092	Transaldolase	*Taldo1*	14	11.5	1.43E+08	9.97E+07	1.4302839
Q99J36	THUMP domain-containing protein 1	*Thumpd1*	8.45	7.3	2.46E+06	1.72E+06	1.434379
Q99PT1	Rho GDP-dissociation inhibitor 1	*Arhgdia*	29.9	30.15	8.03E+07	5.58E+07	1.4393366
Q61598	Rab GDP dissociation inhibitor beta	*Gdi2*	17.75	16.95	5.91E+07	4.07E+07	1.4504449
A2A4I8	Amine oxidase	*Aoc3*	1.9	1.9	1.04E+08	7.17E+07	1.45059
P08030	Adenine phosphoribosyltransferase	*Aprt*	10	7.2	4.33E+06	2.98E+06	1.4560516
P21460	Cystatin-C	*Cst3*	27.5	27.5	5.77E+08	3.93E+08	1.4677356
P35441	Thrombospondin-1	*Thbs1*	4.8	5	6.15E+06	4.14E+06	1.4860469
P04444	Hemoglobin subunit beta-H1	*Hbb-bh1*	21.1	21.1	7.56E+06	5.08E+06	1.4896048
P02104	Hemoglobin subunit epsilon-Y2	*Hbb-y*	40.15	39.15	2.39E+08	1.60E+08	1.4902499
Q8CBG6	6-phosphogluconolactonase	*Pgls*	7.2	10.9	4.86E+06	3.26E+06	1.4918735
P63158	High mobility group protein B1	*Hmgb1*	24.3	24	5.03E+07	3.37E+07	1.4921339
Q564E2	L-lactate dehydrogenase	*Ldha*	32.5	32.5	1.43E+08	9.51E+07	1.5027012
P34022	Ran-specific GTPase-activating protein	*Ranbp1*	8.1	10.8	5.39E+06	3.58E+06	1.5047218
P62869	Transcription elongation factor B polypeptide 2	*Tceb2*	26.25	22	7.90E+06	5.24E+06	1.5070908
O70251	Elongation factor 1-beta	*Eef1b2*	11.7	15.2	6.12E+06	4.03E+06	1.5189003
Q9WU60	Attractin	*Atrn*	0.55	0.55	2.72E+06	1.78E+06	1.5249867

A2AI62	Hephaestin	*Heph*	0.9	0.9	6.04E+07	3.94E+07	1.5319845
Q5XJF6	Ribosomal protein	*Rpl10a*	12.4	12.4	1.26E+07	8.16E+06	1.5469304
Q9WTP6	Adenylate kinase 2	*Ak2*	6.9	4.2	6.54E+05	4.19E+05	1.560639
P21619	Lamin-B2	*Lmnb2*	2.3	1.15	8.17E+05	5.23E+05	1.5621844
P10126	Elongation factor 1-alpha 1	*Eef1a1*	15.35	12.75	2.10E+09	1.33E+09	1.5713376
Q5SXR6	Clathrin heavy chain;Clathrin heavy chain 1	*Cltc*	10.55	10.15	6.69E+07	4.26E+07	1.5719838
Q9CQI6	Coactosin-like protein	*Cotl1*	14.45	16.55	1.03E+08	6.50E+07	1.5812476
Q6P1J1	Dihydropyrimidinase-related protein 1	*Crmp1*	6.15	6.15	5.37E+06	3.38E+06	1.5860785
B2M1R7	Poly(rC)-binding protein 2	*Pcbp2*	14.25	13.85	1.76E+07	1.11E+07	1.5870965
Q9DCD0	6-phosphogluconate dehydrogenase, decarboxylating	*Pgd*	5.8	5.8	1.23E+07	7.69E+06	1.6018917
Q8BHZ0	Protein FAM49A	*Fam49a*	8.5	6.5	7.84E+05	4.87E+05	1.6099238
P35585	AP-1 complex subunit mu-1	*Ap1m1*	5.8	4.05	1.90E+06	1.17E+06	1.627294
Q61171	Peroxiredoxin-2	*Prdx2*	25	25	5.06E+08	3.10E+08	1.6326191
F6ZBL2	E3 ubiquitin-protein ligase MIB1	*Mib1*	2.5	2.5	1.95E+09	1.18E+09	1.6437232
Q3U3V1	Coagulation factor X	*F10*	1.8	1.8	3.78E+06	2.28E+06	1.6583261
Q8BTU6	Eukaryotic initiation factor 4A-II	*Eif4a2*	24.2	21.65	9.47E+06	5.57E+06	1.6980858
P43277	Histone H1.3	*Hist1h1d*	18.55	18.55	2.84E+08	1.67E+08	1.6982031
Q91WU0	Carboxylic ester hydrolase	*Ces1f*	2.1	2.1	6.60E+07	3.84E+07	1.7195086
E0CZ27	Histone H3	*H3f3a*	15.55	19.3	5.70E+08	3.30E+08	1.7248706
E9Q6B6	C-Jun-amino-terminal kinase-interacting protein 3	*Mapk8ip3*	1.4	1.4	3.23E+07	1.86E+07	1.7333405
Q8BIZ0	Protocadherin-20	*Pcdh20*	0.7	0.7	6.59E+05	3.79E+05	1.7368837
P29788	Vitronectin	*Vtn*	3.8	3.8	2.38E+06	1.35E+06	1.7573225
Q9CQV8	14-3-3 protein beta/alpha	*Ywhab*	20.9	23.15	4.20E+07	2.38E+07	1.7635641
P17897	Lysozyme C-1	*Lyz1*	8.1	8.1	3.90E+06	2.19E+06	1.7755069
P97333	Neuropilin-1	*Nrp1*	2.3	3.1	3.55E+06	1.99E+06	1.7817533
G3X9I4	Aly/REF export factor 2	*Alyref2*	5	5	1.75E+07	9.73E+06	1.8022802
P63260	Actin	*Actg1*	47.6	44.65	3.53E+06	1.94E+06	1.8262027
P62082	40S ribosomal protein S7	*Rps7*	2.1	2.1	7.19E+06	3.94E+06	1.8266545
H7BX52	Cullin-2	*Cul2*	17.8	17.8	5.21E+06	2.85E+06	1.8277042
Q9R1P1	Proteasome subunit beta type-3	*Psmb3*	6.8	3.4	6.64E+06	3.63E+06	1.8284916
F6W8R9	Nesprin-2	*Syne2*	1.1	0.55	7.38E+06	4.00E+06	1.843401
Q8VEK3	Heterogeneous nuclear ribonucleoprotein U	*Hnrnpu*	4.65	4.65	5.36E+06	2.89E+06	1.8557495
A2A813	Protein deglycase DJ-1	*Park7*	9.1	7.7	7.71E+06	4.15E+06	1.8572581
G3UZR0	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2	*Ddah2*	16.55	19.7	3.48E+06	1.87E+06	1.8595037
P2604	Moesin	*Msn*	4.75	4.75	3.57E+06	1.91E+06	1.8641588
Q9JMG7	Hepatoma-derived growth factor-related protein 3	*Hdgfrp3*	8.2	8.2	2.53E+06	1.34E+06	1.8807877
D3Z7E6	Platelet-activating factor acetylhydrolase IB subunit gamma	*Pafah1b3*	13.9	16.4	8.71E+06	4.61E+06	1.8873094
Q5SW88	Ras-related protein Rab-1A	*Rab1*	8.9	12.4	1.80E+07	9.38E+06	1.9214568
Q61233	Plastin-2	*Lcp1*	9	6.45	1.52E+07	7.75E+06	1.9644182
D3Z315	Coatomer subunit epsilon	*Cope*	7.3	9.9	1.54E+06	7.76E+05	1.9872323
P26645	Myristoylated alanine-rich C-kinase substrate	*Marcks*	16.2	8.1	5.31E+06	2.52E+06	2.1103271
Q62084	Protein phosphatase 1 regulatory subunit 14B	*Ppp1r14b*	17	17.35	8.34E+06	3.89E+06	2.1448012
G3UY42	Polyadenylate-binding protein 2	*Pabpn1*	8.5	8.5	2.45E+06	1.12E+06	2.1813408
B7FAV1	Filamin-A	*Flna*	1	0.3	2.64E+06	1.19E+06	2.217373
Q9CQI3	Glia maturation factor beta	*Gmfb*	14.6	14.6	1.14E+07	5.01E+06	2.267461
H7BXC3	Triosephosphate isomerase	*Tpi1*	12.9	12.6	4.71E+06	2.07E+06	2.2687512
Q60865	Caprin-1	*Caprin1*	3.3	3.3	2.74E+07	1.20E+07	2.2891093
F6Q2E3	26S protease regulatory subunit 6A	*Psmc3*	7.9	3.95	6.35E+05	2.72E+05	2.3318521
F6VRI6	Ubiquitin-like modifier-activating enzyme 5	*Uba5*	16.2	8.1	5.56E+05	2.33E+05	2.383202
P60867	40S ribosomal protein S20	*Rps20*	14.25	9.65	1.46E+07	6.10E+06	2.3875143
Q14AA6	GTP-binding nuclear protein Ran	*Ran*	9.7	14.8	5.11E+07	2.13E+07	2.3989767
P68134	Actin, alpha skeletal muscle	*Acta1*	26.4	23.45	8.55E+07	3.55E+07	2.4108667
P35700	Peroxiredoxin-1	*Prdx1*	17.75	20.15	6.77E+07	2.75E+07	2.4657958
P02089	Hemoglobin subunit beta-2	*Hbb-b2*	46.9	46.6	1.84E+08	7.40E+07	2.4912119
Q04750	DNA topoisomerase 1	*Top1*	2.1	2.1	1.98E+06	7.37E+05	2.6873618
Q543K9	Purine nucleoside phosphorylase	*Pnp;Pnp2*	6.6	3.3	7.72E+05	2.84E+05	2.7204358
Q8CGP4	Histone H2A	*Hist1h2aa*	21.4	21.4	4.88E+08	1.74E+08	2.8048444
P83917	Chromobox protein homolog 1	*Cbx1*	9.2	7.55	1.22E+06	4.26E+05	2.8537753
O35864	COP9 signalosome complex subunit 5	*Cops5*	14.3	14.3	4.69E+06	1.63E+06	2.8804678
Q9CR00	26S proteasome non-ATPase regulatory subunit 9	*Psmd9*	5.1	2.55	4.20E+06	1.36E+06	3.0804593
P00015	Cytochrome c, testis-specific	*Cyct*	24.3	17.65	1.89E+07	5.88E+06	3.2051827
P28658	Ataxin-10	*Atxn10*	1.45	1.45	8.12E+05	2.29E+05	3.5437179
P20357	Microtubule-associated protein 2	*Map2*	1.75	1.4	4.00E+06	1.13E+06	3.5489953
P61750	ADP-ribosylation factor 4	*Arf4*	11.7	11.7	9.87E+06	2.49E+06	3.955426
P11087	Collagen alpha-1(I) chain	*Col1a1*	6.15	3.95	1.68E+07	4.21E+06	3.9850303
P97379	Ras GTPase-activating protein-binding protein 2	*G3bp2*	12	12	3.87E+06	9.04E+05	4.278474
D3Z7K0	Ubiquitin thioesterase OTUB1	*Otub1*	18.65	14.9	2.72E+07	5.13E+06	5.3105051
P27048	Small nuclear ribonucleoprotein-associated protein B	*Snrpb*	8.25	3.25	1.68E+06	2.01E+05	8.3721104
P35396	Peroxisome proliferator-activated receptor delta	*Ppard*	1.6	1.6	1.39E+08	1.00E+07	13.877689
P07309	Transthyretin	*Ttr*	11.9	5.1	7.35E+07	1.55E+06	47.408315