Supplementary 1

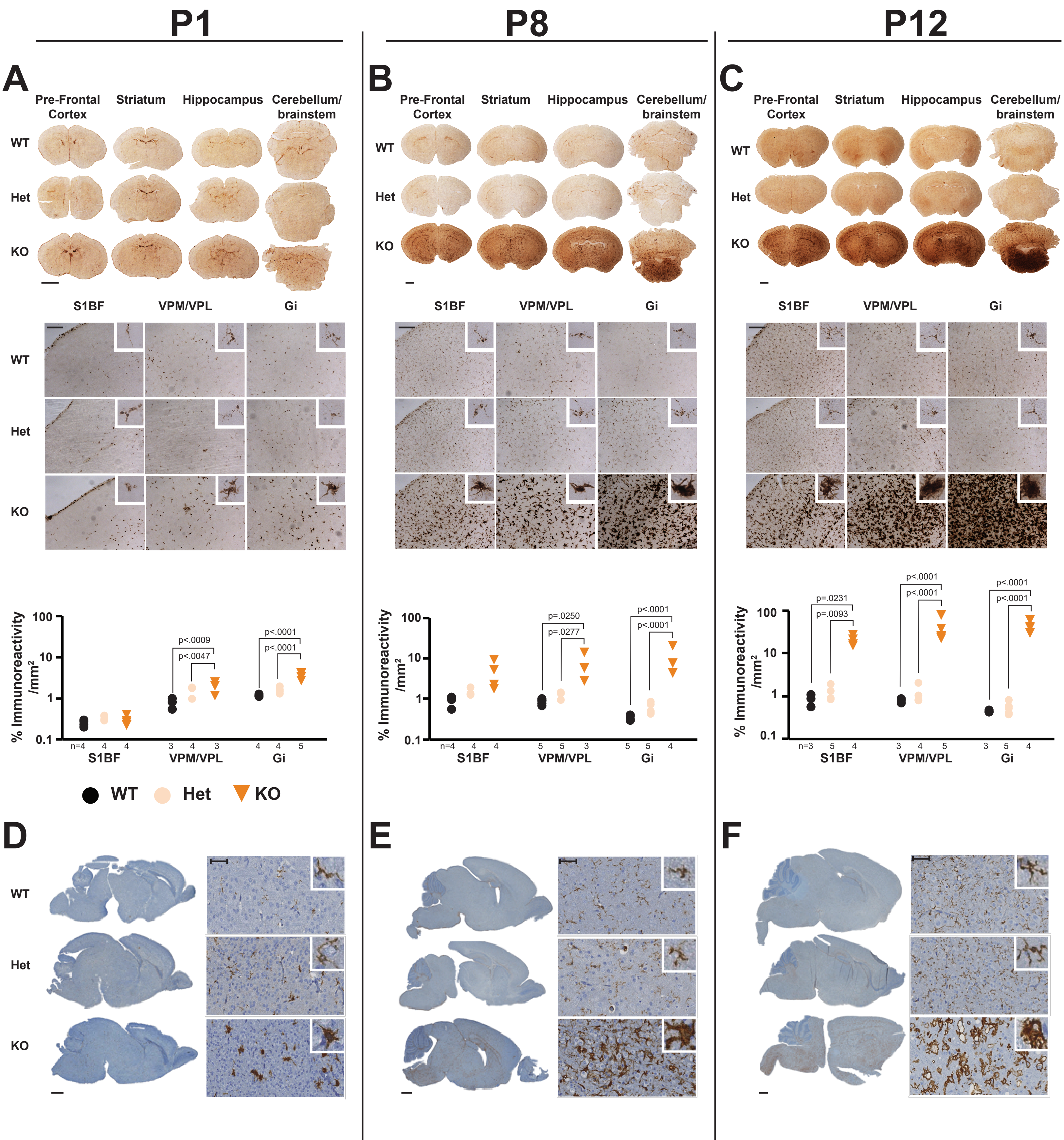

Fig. S1. Microglia-mediated inflammation in nGD mice.
CD68 immunohistochemistry and quantification on brain sections from wildtype, heterozygous and knockout mice at P1 (A), P8 (B) and P12 (C). Whole brain scale bar = 1mm; high magnification sections scale bar = 0.25mm, (2-way ANOVA, Tukey's multiple comparison). Scale bars = 0.25mm. (D) Iba1 immunohistochemistry at P1, at P8 (E), and P12 (F) in sagittal paraffin embedded sections. Whole brain scale bar = 0.5mm; high magnification sections scale bar = 40µm. Numbers of mice are stated under each group. n.s. = not significant

Supplementary 2

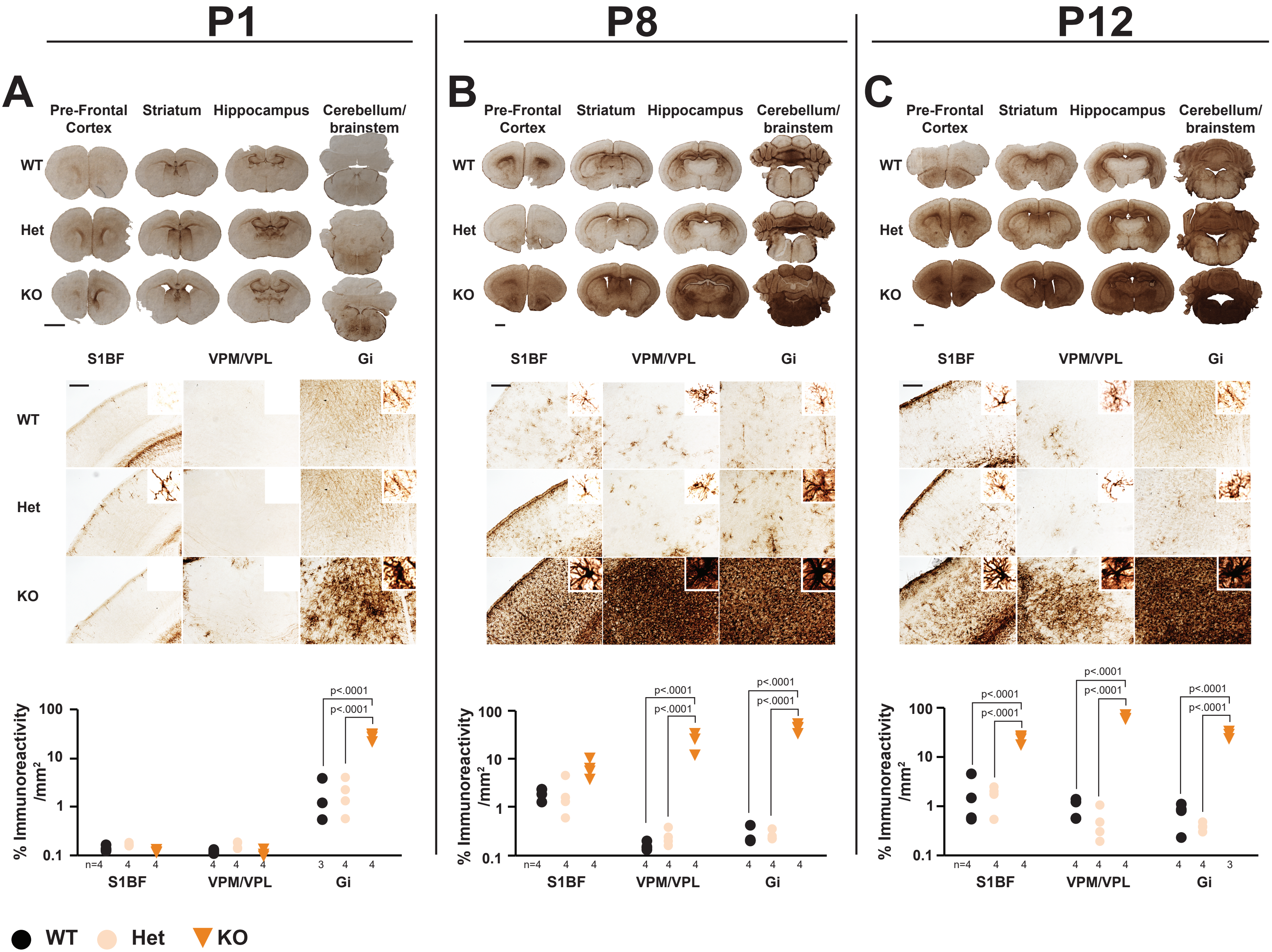

Fig. S2. Astrogliosis in nGD mice.
GFAP immunohistochemistry and quantification on brain sections from wildtype, heterozygous and knockout mice at P1 (A), P8 (B) and P12 (C). Whole brain scale bar = 1mm; high magnification sections scale bar = 0.25mm (2-way ANOVA, Tukey's multiple comparison, n=4-5 mice). Scale bars = 0.25mm. Numbers of mice are stated under each group. n.s. = not significant

Supplementary 3

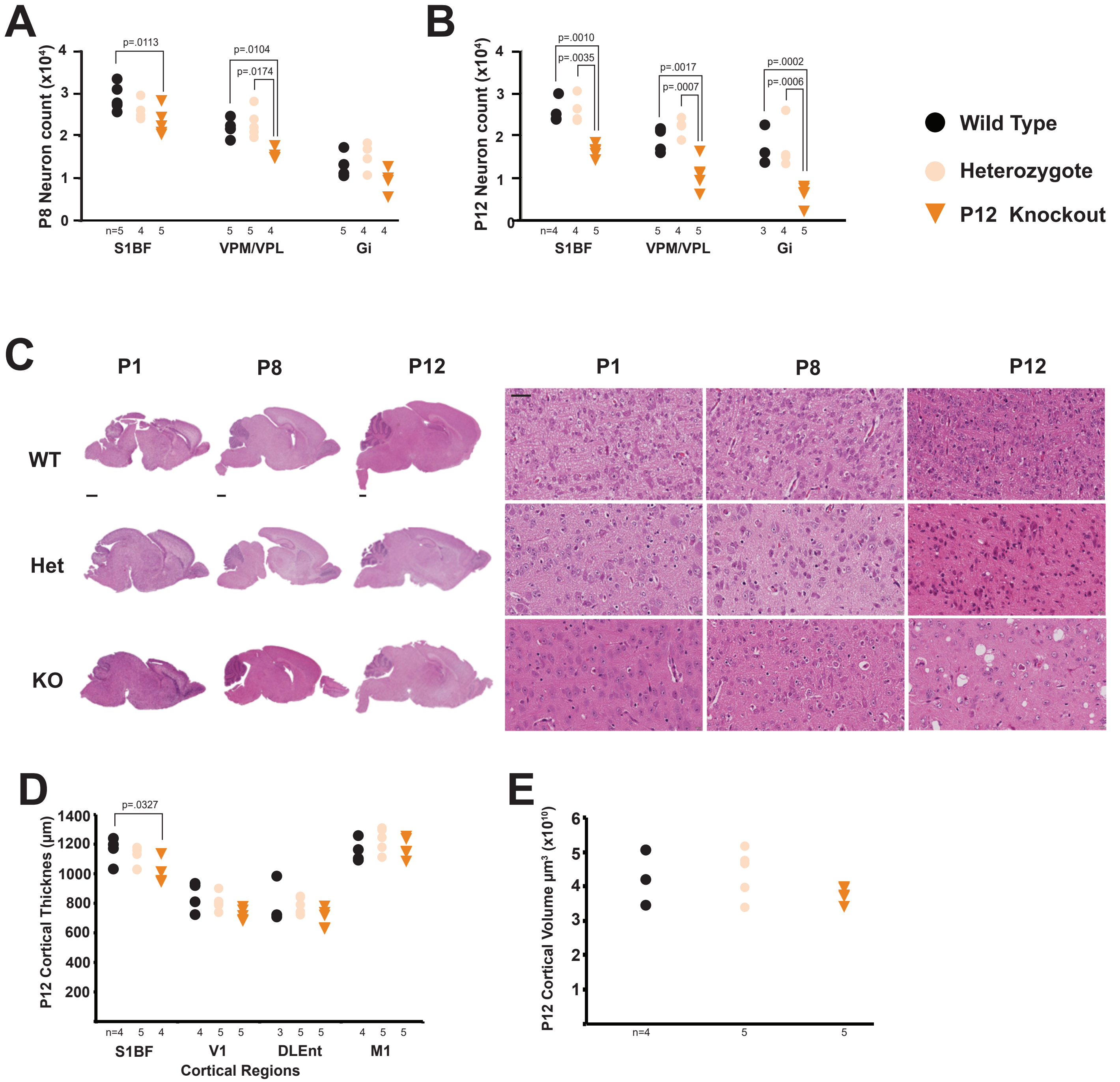

Fig. S3. Histological and stereological analysis of nGD mouse brains.
Stereological counts of neurons in brain sections from (A) P8 and (B) P12 knockouts, heterozygous and wildtypes (2-way ANOVA, Tukey's multiple comparison). (C) Haematoxylin and eosin staining of P1, P8 and P12 brains. Whole brain scale bar = 0.5mm; high magnification sections scale bar = 40µm. (D) Cortical thickness and (E) cortical volume measurements from P12 mice (Students one-tailed t-test). Numbers of mice are stated under each group. n.s. = not significant

Supplementary 4

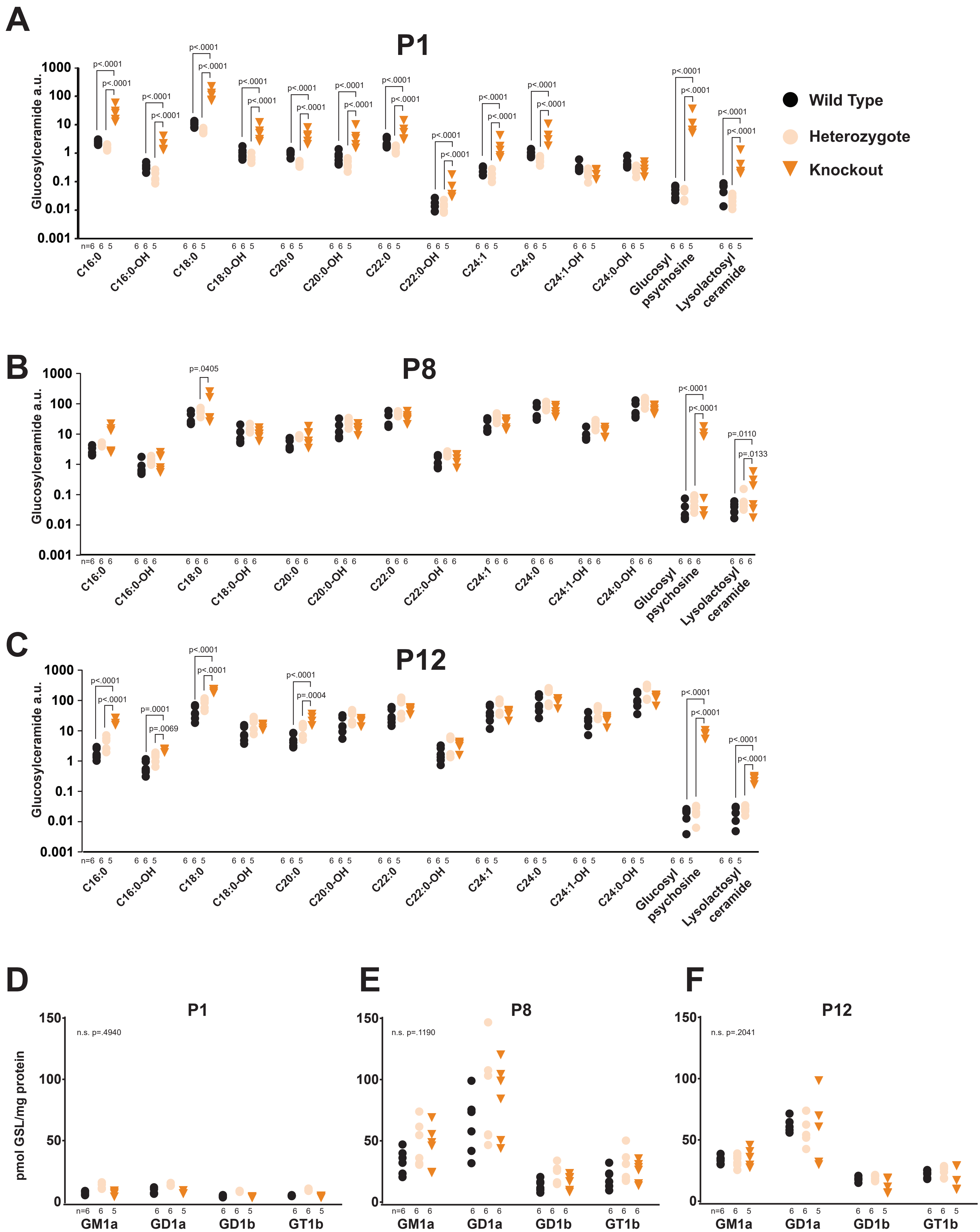

Fig. S4. Metabolite and glycosphingolipid profile analysis of nGD mouse brains.
(A) Mass spectrometry of P1, (B) P8 and (C) P12 brains from knockout, heterozygous and wildtype mice (2-way ANOVA on log-transformed data, n=5 vs. 6 mice; Bonferroni's multiple comparison). The glycosphingolipid profiles of GM1a, GD1a, GD1b and GT1b were measured in (D) P1, (E) P8 and (F) P12 brains (2-way ANOVA on log-transformed data, n=5 vs. 6 mice; Bonferroni's multiple comparison). Numbers of mice are stated under each group. n.s. = not significant

Supplementary 5

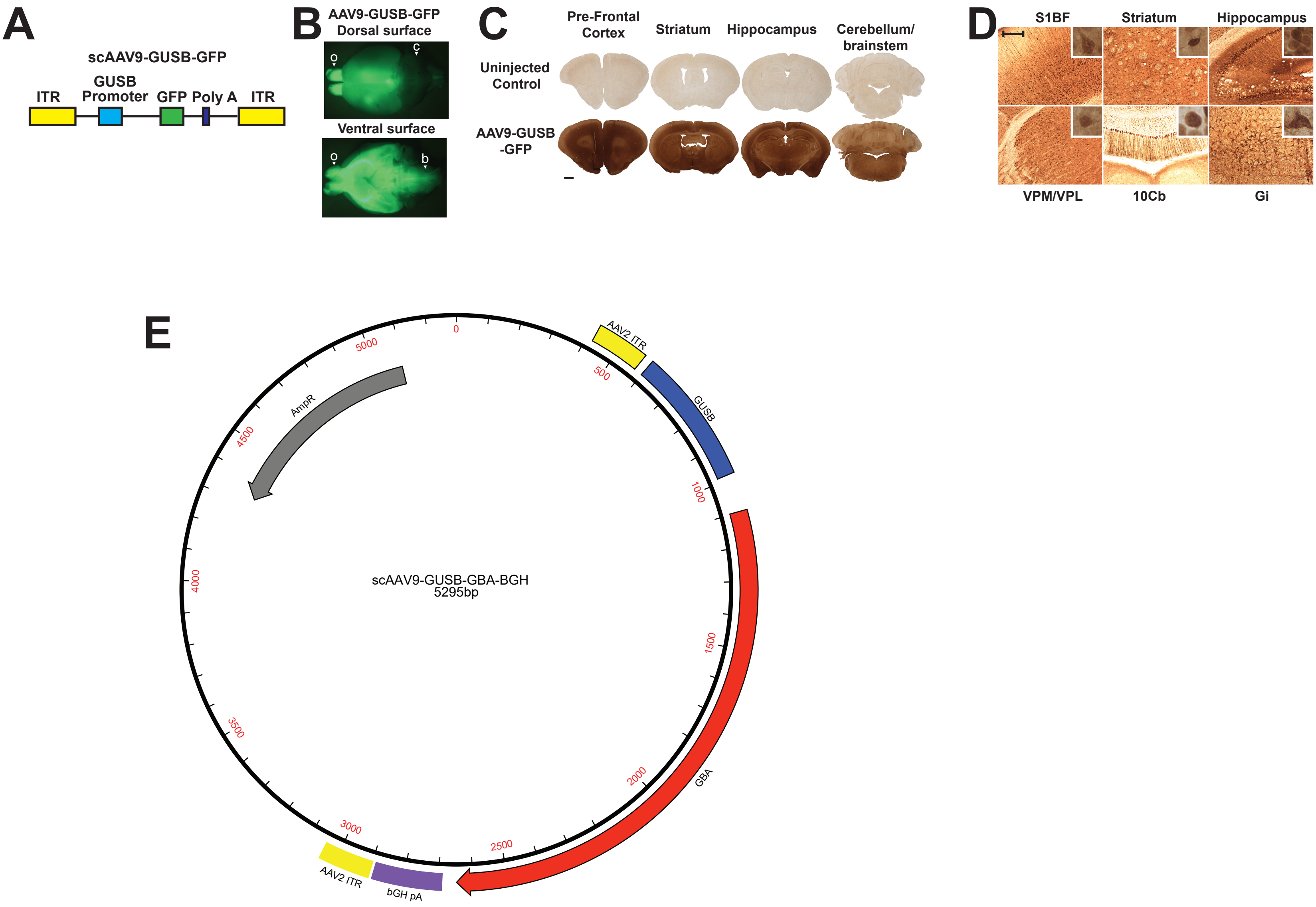

Fig. S5. Fetal gene delivery marker gene studies in wild type mice.
(A) An illustration of the self-complementary genome configuration of AAV9-GUSB-GFP. The expression cassette contained the beta-glucuronidase promoter driving expression of the GFP gene followed by a polyadenylation signal. The expression cassette was flanked by the viral inverted terminal repeats. (B) Green fluorescent protein in administered brains (o-olfactory bulbs, b-brainstem, c – cerebellum). Low (C) and high (D) magnification images following anti-GFP immunohistochemistry of free-floating brain sections. Whole brain scale bar = 1mm; high magnification sections scale bar = 0.25mm. Representative of 3 administered and unadministered mice replicates. (E) Plasmid map of AAV9-GUSB-GBA.

Supplementary 6

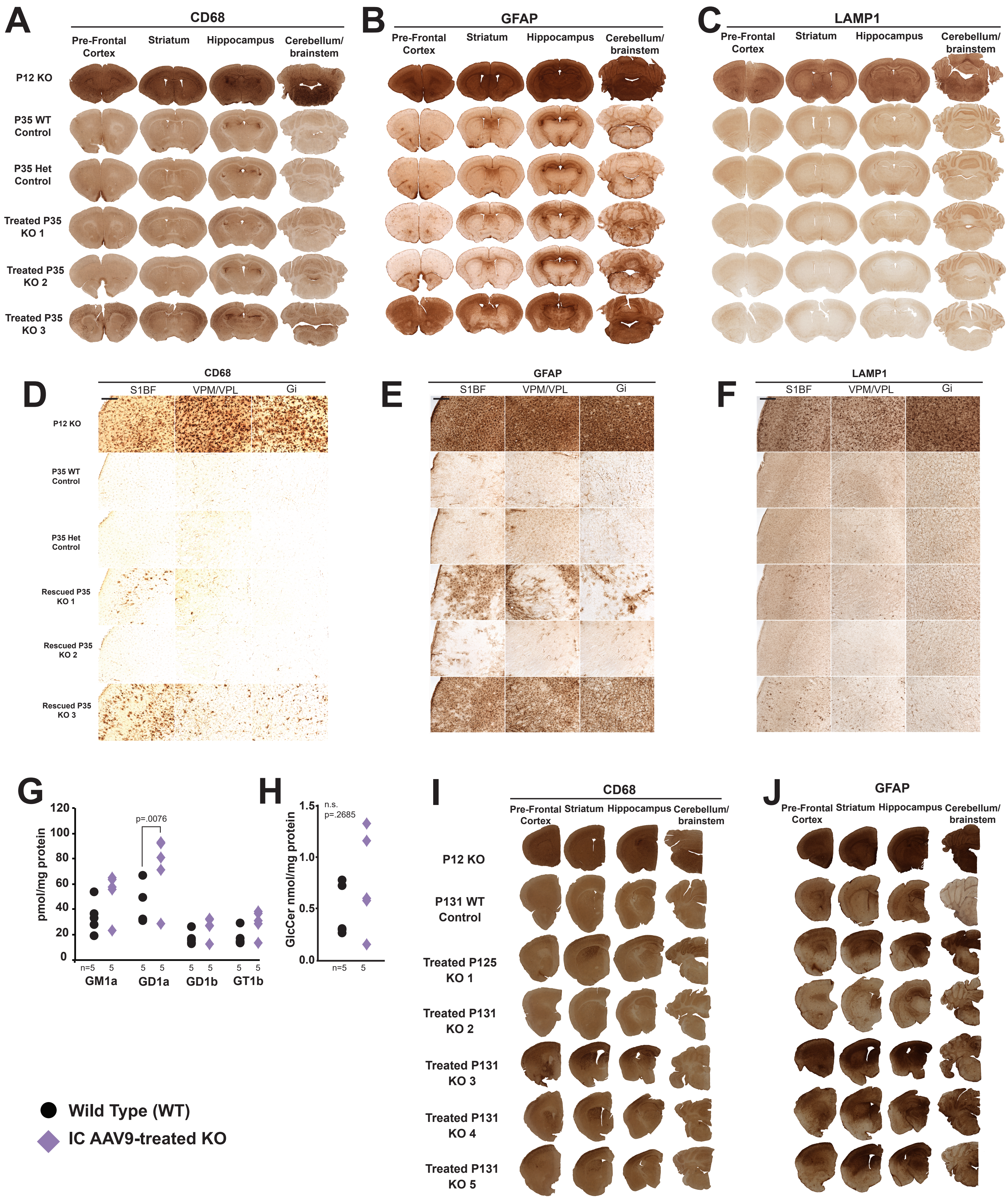

Fig. S6. Fetal gene therapy of nGD mice neuropathology.

(A and D) CD68, (B and E) GFAP and (C and F) LAMP1 immunostaining on brain sections of P12 untreated knockouts, P35 wildtypes and heterozygous and gene therapy treated knockouts. Scale bars = 0.25mm. Representative of 3 mice replicates per experimental cohort. (G) Analysis of GSLs in treated mice and control wild types, (2-way ANOVA on log-transformed data; Bonferroni's multiple comparison). (H) Glucosylceramide levels in the brains of treated knockouts and wildtypes from long term studies (Students one-tailed t-test, n=5 mice). (I) CD68 and (J) GFAP immunostaining on brain sections from long term treated knockouts and wild type mice (all replicates shown). Numbers of mice are stated under each group. n.s. = not significant

Supplementary 7

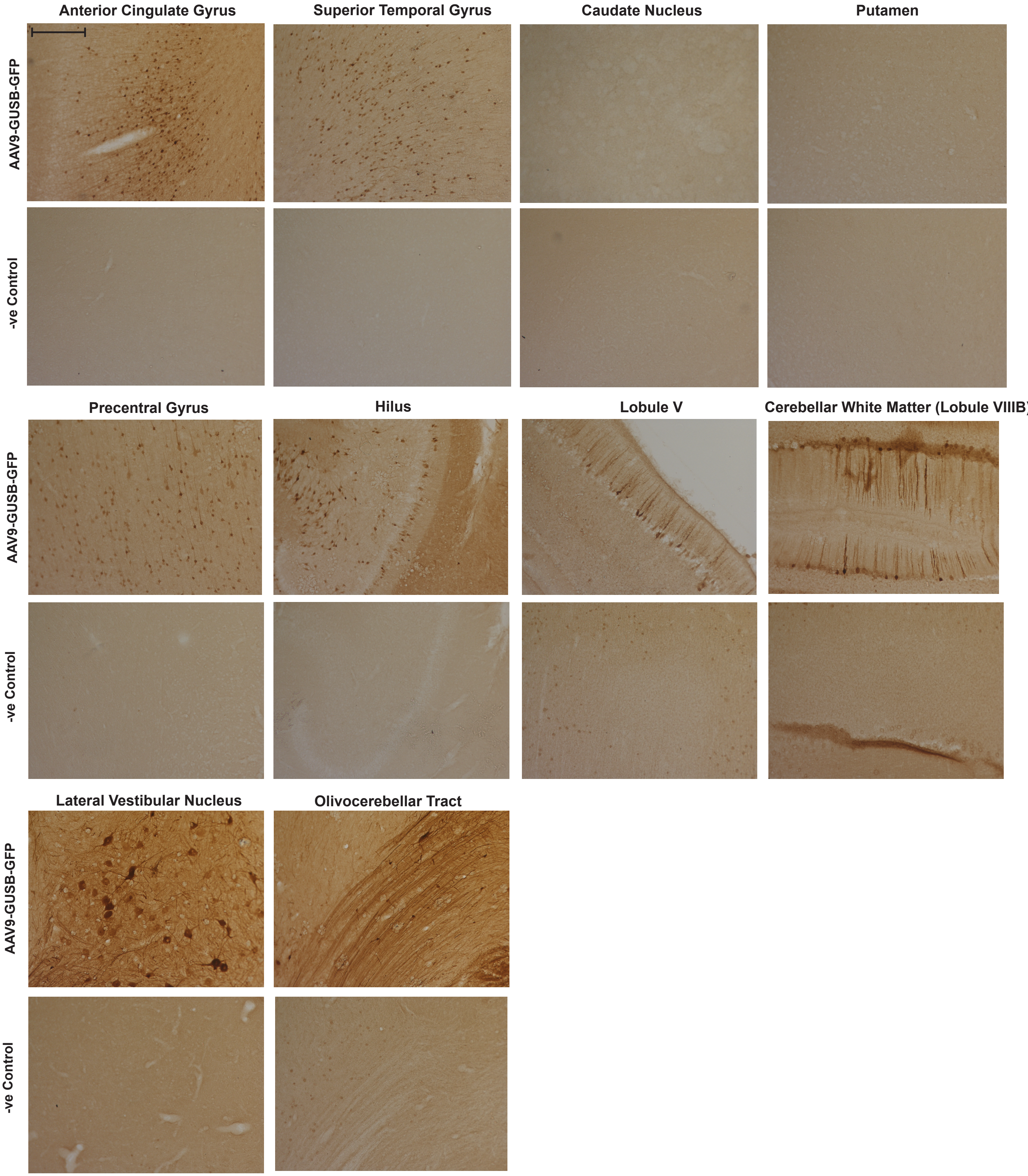

Fig. S7. Fetal gene delivery marker gene studies in macaques.
GFP immunohistochemistry to detect cells transduced with scAAV9-GUSB-GFP following ultrasound-guided intracerebroventricular injection of to the fetal macaque. Scale bar = 0.25mm. Representative of two replicates.

Supplementary 8

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGC
GTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGA
GAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGC
TGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCGAGCTCGGTACCTCGCGAATGCATCTAGAGGCCACTC
CCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA
GTGGCCAACTCCATCACTAGGGGTTCCTGGAGGGGTGGAGTCGTGACAGATCTGAATTCCTGCTGGGAAAAGCAAGTGGAGGTGCTCCTTGAAGAAACAGGGGGATCCC
ACCGATCTCAGGGGTTCTGTTCTGGCCTGCGGCCCTGGATCGTCCAGCCTGGGTCGGGGTGGGGAGCAGACCTCGCCCTTATCGGCTGGGGCTGAGGGTGAGGGTCCCG
TTTCCCCAAAGGCCTAGCCTGGGGTTCCAGCCACAAGCCCTACCGGGCAGCGCCCGGCCCCGCCCCTCCAGGCCTGGCACTCGTCCTCAACCAAGATGGCGCGGATGGC
TTCAGGCGCATCACGACACCGGCGCGTCACGCGACCCGCCCTACGGGCACCTCCCGCGCTTTTCTTAGCGCCGCAGACGGTGGCCGAGCGGGGGACCGGGAAGCATGG
CCCGGGCTGCAGCTCTAAGGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTCTCTCTTTTAGATTCCAACCTTTGGAACTCAATTCAGCCA
CCATGGCTGGCAGTCTTACAGGTCTCCTGCTCCTGCAAGCTGTCTCTTGGGCTTCTGGGGCCAGGCCCTGTATCCCCAAATCCTTTGGATACTCATCTGTGGTGTGTGTTTG
TAATGCCACTTATTGTGATAGCTTTGACCCCCCCACCTTTCCTGCACTGGGCACCTTTTCAAGGTATGAATCTACCAGGTCTGGGAGGAGGATGGAGCTGAGTATGGGGCC
CATCCAAGCAAACCATACTGGCACTGGCTTGCTGCTGACACTGCAACCTGAACAGAAGTTCCAGAAAGTGAAGGGCTTTGGAGGAGCCATGACTGATGCTGCTGCCCTC
AATATTTTGGCCCTGAGCCCCCCTGCTCAGAATCTCCTTTTGAAATCATACTTCTCTGAGGAGGGAATTGGATACAATATCATCAGGGTGCCAATGGCCTCATGTGACTTTAG
TATTAGGACTTACACCTATGCTGATACCCCTGATGATTTCCAGCTGCATAACTTCTCATTGCCTGAGGAGGATACCAAATTGAAGATCCCACTCATTCACAGGGCCCTGCAAC
TGGCTCAGAGACCAGTGTCATTGCTGGCCTCCCCCTGGACCTCCCCAACTTGGCTCAAAACCAATGGGGCTGTCAATGGTAAGGGCTCTCTTAAGGGGCAGCCTGGAGAC
ATTTACCATCAGACCTGGGCCAGGTATTTTGTGAAGTTCCTGGATGCTTATGCTGAGCACAAATTGCAATTTTGGGCTGTTACAGCTGAGAATGAACCCTCTGCAGGACTG
CTGTCTGGCTATCCTTTCCAGTGCCTGGGCTTTACCCCTGAGCATCAGAGGGATTTCATTGCCAGGGACCTGGGACCTACTCTTGCCAATAGCACACACCATAATGTGAGG
CTTCTGATGCTTGATGACCAGAGACTTCTGCTGCCACACTGGGCCAAGGTTGTCCTGACAGATCCTGAGGCTGCCAAGTATGTTCATGGGATTGCTGTGCACTGGTATCTG
GACTTCCTTGCTCCAGCTAAGGCCACCCTGGGAGAAACACACAGGTTGTTTCCCAATACAATGCTTTTTGCATCAGAGGCCTGTGTGGGCAGTAAATTTTGGGAGCAGTC
TGTTAGGCTGGGGAGCTGGGATAGAGGAATGCAATACTCCCATTCTATCATCACCAATCTGCTCTACCATGTGGTGGGGTGGACTGACTGGAACCTTGCCCTTAACCCTGA
GGGTGGCCCCAATTGGGTCAGGAATTTTGTGGATAGTCCCATCATTGTGGATATCACCAAGGACACATTCTATAAGCAACCAATGTTCTATCACCTGGGTCACTTTAGTAAGT
TTATCCCTGAGGGGTCCCAGAGGGTGGGACTGGTGGCTTCCCAGAAGAATGATCTGGATGCTGTGGCCCTGATGCACCCTGATGGCAGTGCTGTGGTTGTTGTTCTCAATA
GAAGCTCTAAAGATGTGCCCTTGACCATCAAAGATCCAGCTGTGGGATTTCTGGAAACAATTTCCCCTGGTTATAGCATCCACACTTACCTTTGGAGAAGGCAGTGAAAAT
GAAGGCCTGATAATTGCACCACCAGGCCTGATAGGCCCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTC
CCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAA
GACAATAGCAGGCATGCACTAGTCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCA
GTGAGCGAGCGAGCGCGCAGAGAGGGATCTAGATATCGGATCCCGGGCCCGTCGACTGCAGAGGCCTGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTG
TGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCAC
TGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACT
GACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAG
CAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAG
GTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTC
CCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGAC
CGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGC
GGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAG
CTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTAC
GGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCA
ATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTC
GTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCC
GGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAAC
GTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCA
AAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCC
GTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCAC
ATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCA
ACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACT
CATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTT
CCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Figure S8. Sequence of scAAV9-GUSB-GBA

Supplementary Table 1

CD68

	1	8	12
Cortex			
Frontal Association	-/+	++	+++
Motor	+	++	+++
Sensory	-/+	++	++++
Piriform	-/+	++	+++
Auditory	-/+	++	+++
Visual	-/+	++	+++
CEnt	-/+	++	+++
Basal Ganglia			
Caudate Putamen	-	++	++
Globus Pallidus	-	++	+++
Substantia Nigra	-	++	+++
Hippocampus			
CA1	-	+	+++
CA2/CA3	-	+	+++
Dentate Gyrus	-	-	-
Dorsal Subiculum	-	+	+++
Hypothalamus			
Anterior Hypothlamic Area	+	++	+++
Lateral Nucleus	+	++	+++/++++
Medial Pre-Optic Nucleus	+	++/+++	+++
Ventromedial Nucleus	+/-	++	+++
Mammillary Nucleus	+/-	++	+++
Thalamic Nuclei			
Anteroventral	+	++	++++
Anterodorsal	+	++	++++
Paraventricular	-	++	++/+++
Reticular	-	++	+++
Ventral Post Medial/ Ventral Post Lateral	+	+++	++++
Mediodorsal	+/-	++	+++/++++
Habenular	-	+	++
Laterodorsal Posterior	+/-	++	+++

	1	8	12
Midbrain			
Red Nucleus	-	++	+++
Superficial Area of Superior Colliculus (SC)	-	+	+
Intermediate and Deep Layers of SC	-	+/++	++
Periaqueductal Gray	-	+/++	++
Inferior Colliculas	-	++	+++
Pons & Medulla			
Reticulotegmental Nucleus of Pons	+/++	+++	++++
Pontine Reticular Nucleus	+/++	+++	++++
Ventral Cochlear Nucleus	+/-	++	+++
8 Nerve	-	-	+
Facial Nucleus	-	-	+
Vestibular Nucleus	-	+	++
External Cuneate	-	++	+++
LRt	+	+++	++++
Parvicellular Reticular Nucleus (PCRt)	+	+++	++++
Motor Trigeminal Nucleus	-	++	+++
Motor Root of the Trigeminal Nerve	+	+++	++++
Peripheral Sensory Trigeminal Nucleus	-	++	+++
SP5	-	++	+++
Dorsal Motor Nucleus Of Vagus	-	+	+++
Hypoglossal Nucleus	+	++	++++
Gigantocellular Nucleus	++	+++	++++

Table S1. Scoring of CD68 immunohistochemistry in the nGD mouse model.
Discrete areas of the brain in knockout, wild type and heterozygous mice were scored at P1, P8 and P12. A scale of - to ++++ was used, where - = no activated microglia visible and ++++ was area densely populated with activated microglia.

Supplementary Table 2

GFAP

	1	8	12
Cortex			
Frontal Association	-	++	++
Orbital	-	++	++
Motor	-/+	+++	+++
Sensory	-/+	+++	+++
Piriform	-/+	++	+++/++++
Auditory	-/+	++	+++
Visual	-/+	++	+++
CEnt	-/+	++	+++
Basal Ganglia			
Caudate Putamen	-	+/++	++
Globus Pallidus	-	+/++	++/+++
Substantia Nigra	-/+	++	+++
Hippocampus			
CA1	+	++/+++	+++
CA2/CA3	+	++/+++	+++
Dentate Gyrus	-	++/+++	+++
Dorsal Subiculum	-	++/+++	+++
Hypothalamus			
Anterior Hypothlamic Area	+	++/+++	+++
Medial Pre-Optic Nucleus	-/+	++	+++
Ventromedial Nucleus	+	++	+++
Mammillary Nucleus	-	++	+++
Thalamic Nuclei			
Anteroventral	++	+++	+++/++++
Anterodorsal	++	+++	+++/++++
Paraventricular	++	+++	+++/+++ +
Ventral Post Medial/ Ventral Post Lateral	+	+++/++++	++++
Mediodorsal	+	++	+++
Habenular	+	++	++/+++
Laterodorsal	+	++	+++
Posterior	-/+	++	+++

	1	8	12
Midbrain			
Red Nucleus	-/+	++	+++
Superficial Area of Superior Colliculus (SC)	-/+	++	+++
Intermediate and Deep Layers of SC	-/+	++	+++
Periaqueductal Gray	-	++	+++
Inferior Colliculas	-	++	+++
Pons & Medulla			
Reticulotegmental Nucleus of Pons	++	++	++++
Pontine Reticular Nucleus	+	++/+++	++++
Ventral Cochlear Nucleus	+	++	+++
8 Nerve	+	+++	++++
Facial Nucleus	+	+++	++++
Vestibular Nucleus	-	+++	++++
External Cuneate	-	+++	++++
LRt	+	+++	++++
Parvicellular Reticular Nucleus (PCRt)	+	+++	++++
Motor Trigeminal Nucleus	+	+++	++++
Motor Root of the	-/+	+++	++++
Trigeminal Nerve	-/+	+++	++++
Peripheral Sensory	-/+	+++	++++
Trigeminal Nucleus	-/+	+++	++++
SP5	+	+++	++++
Dorsal Motor Nucleus Of Vagus	++	+++	++++
Hypoglossal Nucleus	++/+++	+++	++++
Gigantocellular Nucleus	++/+++	+++	++++

Table S2. Scoring of GFAP immunohistochemistry in the nGD mouse model.
Discrete areas of the brain in knockout, wild type and heterozygous mice were scored at P1, P8 and P12. A scale of - to ++++ was used, where - = no activated astrocytes visible and ++++ was area densely populated with activated astrocytes.

Supplementary Table 3

Fold change in glycosphingolipids, KO/WT

	P1	P8	P12
C16:0	21	4	13
C16:0-OH	12	2	4
C18:0	22	4	6
C18:0-OH	10	1	2
C20:0	8	2	5
C20:0-OH	10	1	1
C22:0	5	1	2
C22:0-OH	7	1	2
C24:1	13	1	1
C24:0	8	1	1
C24:1-OH	1	1	1
C24:0-OH	1	1	1
Glucosylpsychosine	524	216	485
Lysolactosyl ceramide	15	6	14

Table S3. Fold change in glycosphingolipids.

Supplementary Table 4

Glycosphingolipid MRM transitions

Glycospingolipid name	Precusor m/z	Fragment m/z	Cone (V)	Collision (V)
glucosylpsychosine	462.4	264.3	74	16
lyso-lactosyl ceramide	624.6	264.4	18	20
C16:0 glucosylceramide	722.6	560.6	130	40
d4C16:0 glucosylceramide	726.6	564.7	130	38
C16:0-OH glucosylceramide	738.6	264.3	130	32
C18:0 glucosylceramide	750.6	588.6	130	38
C18:0-OH glucosylceramide	766.6	264.3	130	32
C20:0 glucosylceramide	778.6	616.7	132	42
C20:0-OH glucosylceramide	794.6	264.3	132	36
C22:0 glucosylceramide	806.7	644.7	130	38
C22:0-OH glucosylceramide	820.7	658.8	130	52
C24:1 glucosylceramide	832.7	670.7	130	52
C24:0 glucosylceramide	834.7	672.7	130	42
C24:1-OH glucosylceramide	848.7	686.7	130	42
C24:0-OH glucosylceramide	850.8	688.9	130	42

Table S4. Details of multiple reaction monitoring (MRM).

Movie S1. Treated knockout mouse with two knockout pups
Treated knockout male and female mice were bred at ten weeks of age. The dam gave birth to two knockout pups which exhibited classic nGD phenotype at 14 days of age.

Movie S2. Behavior of treated knockouts versus age-matched controls
Treated knockouts (lower panel of four) exhibited hyperkinesis and stereotypic circling phenotype compared with age-matched controls (upper panel of five).