

## SUPPLEMENTARY INFORMATION

### **FcR $\gamma$ -dependent immune activation initiates astrogliosis during the asymptomatic phase of Sandhoff disease model mice**

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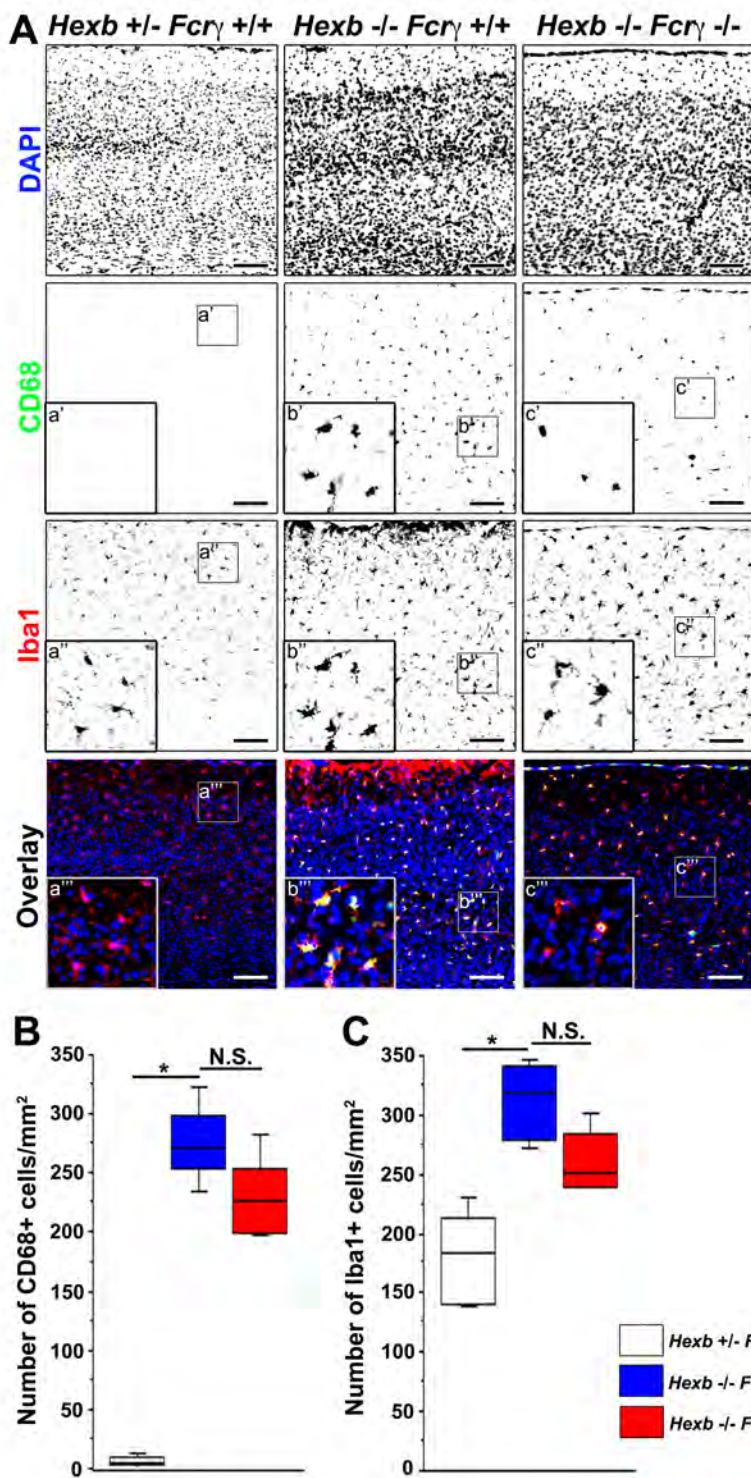
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Table S1. Top 50 up-regulated genes in cortices of 16-week-old *Hexb*<sup>-/-</sup> mice (n=4, *P*<0.05).

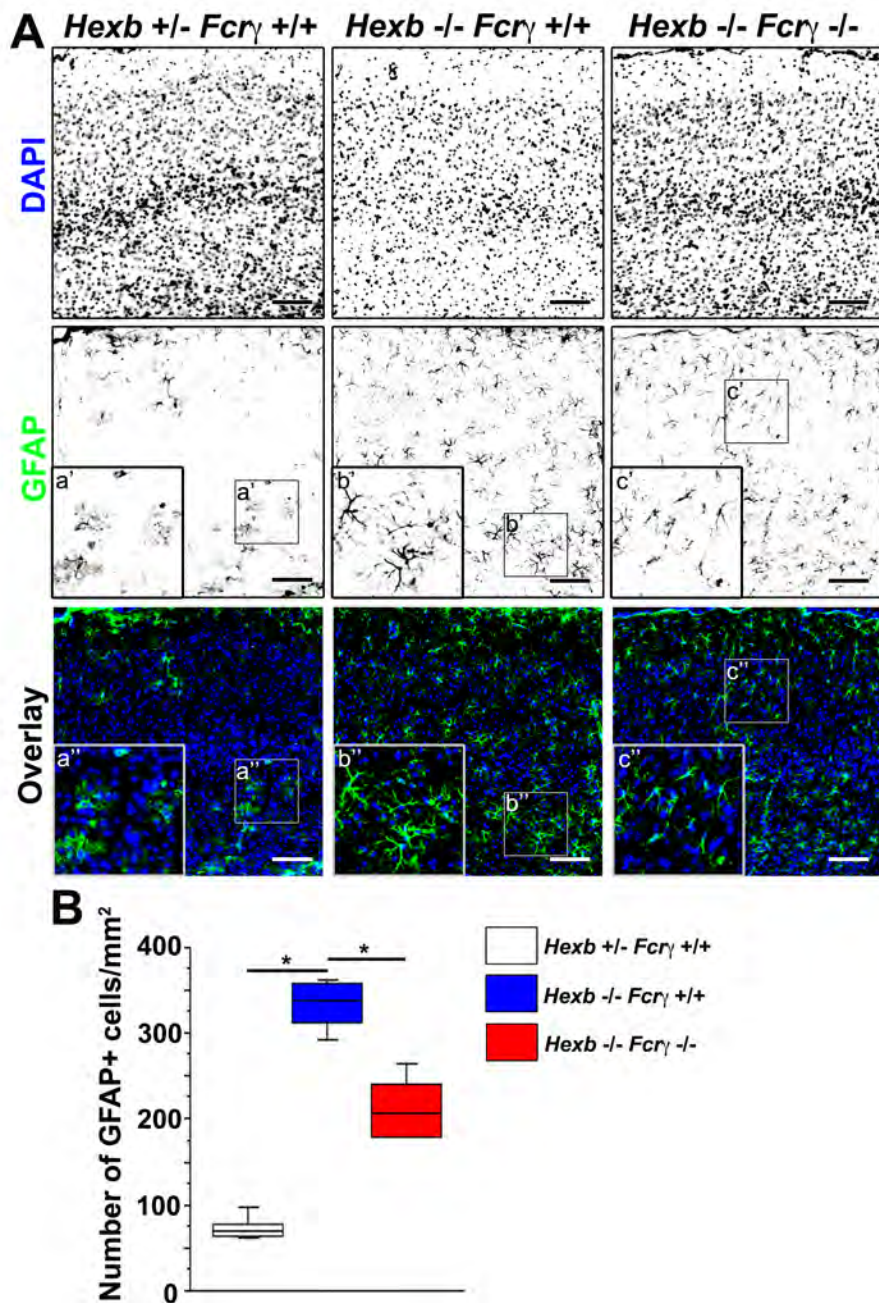
Gene accession No.	Gene symbol	Gene name	Fold change
NM_017372	<i>Lyz2</i>	Lysozyme 2	3.59
NM_011337	<i>Ccl3</i>	Chemokine (C-C motif) ligand 3	3.43
NM_001081957	<i>Wfdc17</i>	WAP four-disulfide core domain 17	3.07
NM_021274	<i>Cxcl10</i>	Chemokine (C-X-C motif) ligand 10	2.87
NM_009977	<i>Cst7</i>	Cystatin F	2.58
NM_009853	<i>Cd68</i>	CD68 antigen	2.48
NM_010821	<i>Mpeg1</i>	Macrophage expressed gene 1	2.41
NM_009779	<i>C3ar1</i>	Complement component 3a receptor 1	2.32
NM_009780	<i>C4b</i>	Complement component 4B	2.30
NM_010501	<i>Ifit3</i>	Interferon-induced protein with tetratricopeptide repeats 3	2.00
NM_013489	<i>Cd84</i>	CD84 antigen	1.98
NM_011662	<i>Tyrobp</i>	TYRO protein tyrosine kinase binding protein	1.96
NM_030691	<i>Igsf6</i>	Immunoglobulin superfamily, member 6	1.92
NM_030720	<i>Gpr84</i>	G protein-coupled receptor 84	1.91
NM_001077189	<i>Fcgr2b</i>	Fc receptor, IgG, low affinity IIb	1.90
NM_031254	<i>Trem2</i>	Triggering receptor expressed on myeloid cells 2	1.87
NM_010277	<i>Gfap</i>	Glial fibrillary acidic protein	1.86
NM_001111058	<i>Cd33</i>	CD33 antigen	1.85
NM_011150	<i>Lgals3bp</i>	Lectin, galactoside-binding, soluble, 3 binding protein	1.84
NM_010130	<i>Emr1</i>	EGF-like module containing, mucin-like, hormone receptor-like sequence 1	1.82
NM_008534	<i>Ly9</i>	Lymphocyte antigen 9	1.76
NM_010554	<i>Il1a</i>	Interleukin 1 alpha	1.69
NM_001166409	<i>Rbm3</i>	RNA binding motif protein 3	1.68
NM_019549	<i>Plek</i>	Pleckstrin	1.67
NM_001267695	<i>Ctss</i>	Cathepsin S	1.65
NM_010185	<i>Fcer1g</i>	Fc receptor, IgE, high affinity I, gamma polypeptide	1.65
NM_023065	<i>Ifi30</i>	Interferon gamma inducible protein 30	1.64
NM_139142	<i>Slc6a20a</i>	Solute carrier family 6 member 20A	1.64
NM_008220	<i>Hbb-bt</i>	Hemoglobin, beta adult t chain	1.64
NM_007806	<i>Cyba</i>	Cytochrome b-245, alpha polypeptide	1.63
NM_007572	<i>C1qa</i>	Complement component 1, q subcomponent, alpha polypeptide	1.63
NM_020008	<i>Clec7a</i>	C-type lectin domain family 7, member a	1.63
NM_010188	<i>Fcgr3</i>	Fc receptor, IgG, low affinity III	1.62
NM_008331	<i>Ifit1</i>	Interferon-induced protein with tetratricopeptide repeats 1	1.60
NM_007574	<i>C1qc</i>	Complement component 1, q subcomponent, C chain	1.60
AK006938	<i>1700072H12Rik</i>	1700072H12 product:hypothetical protein	1.61
NM_001083955	<i>Hba-a2</i>	Hemoglobin alpha, adult chain 2	1.60
NM_013590	<i>Lyz1</i>	Lysozyme 1	1.60
NM_134158	<i>AF251705</i>	cDNA sequence AF251705	1.58
NM_021334	<i>Itgax</i>	Integrin alpha X	1.58
NM_008218	<i>Hba-a1</i>	Hemoglobin alpha, adult chain 1	1.57
NM_007649	<i>Cd48</i>	CD48 antigen	1.56
NM_001042489	<i>Hvcn1</i>	Hydrogen voltage-gated channel 1	1.55
NM_027836	<i>Ms4a7</i>	Membrane-spanning 4-domains, subfamily A, member 7	1.55
NM_008479	<i>Lag3</i>	Lymphocyte-activation gene 3	1.54
NM_009777	<i>C1qb</i>	Complement component 1, q subcomponent, beta polypeptide	1.53
NM_011332	<i>Ccl17</i>	Chemokine (C-C motif) ligand 17	1.52
NM_025378	<i>Ifitm3</i>	Interferon induced transmembrane protein 3	1.52
NM_001204910	<i>AI607873</i>	Expressed sequence AI607873	1.52
NM_013706	<i>Cd52</i>	CD52 antigen	1.52

Table S2. Top 50 down-regulated genes in cortices of 16-week-old *Hexb*<sup>-/-</sup> mice (n=4, *P*<0.05).

Gene accession No.	Gene symbol	Gene name	Fold change
NR_004414	<i>Rnu2-10</i>	<i>U2 small nuclear RNA 10</i>	1.73
NR_029412	<i>Snora16a</i>	<i>Small nucleolar RNA, H/ACA box 16A</i>	1.71
NM_023842	<i>Dsp</i>	<i>Desmoplakin</i>	1.68
NM_177068	<i>Olfml2b</i>	<i>Olfactomedin-like 2B</i>	1.68
NM_138304	<i>Calml4</i>	<i>Calmodulin-like 4</i>	1.67
NR_002905	<i>Snora74a</i>	<i>Small nucleolar RNA, H/ACA box 74A</i>	1.66
NM_026956	<i>Cd209f</i>	<i>CD209f antigen</i>	1.65
XM_003086154	<i>Vwa3b</i>	<i>Von Willebrand factor A domain containing 3B</i>	1.64
NR_045188	<i>St18</i>	<i>Suppression of tumorigenicity 18</i>	1.63
NM_029972	<i>Ernm</i>	<i>Ermin, ERM-like protein</i>	1.58
NR_004439	<i>Rprl2</i>	<i>Ribonuclease P RNA-like 2</i>	1.54
NM_028390	<i>Anln</i>	<i>Anillin, actin binding protein</i>	1.54
NM_146257	<i>Slc29a4</i>	<i>Solute carrier family 29, member 4</i>	1.53
NM_001081078	<i>Lct</i>	<i>Lactase</i>	1.52
NM_009026	<i>Rasd1</i>	<i>RAS, dexamethasone-induced 1</i>	1.51
NR_033336	<i>Snora23</i>	<i>Small nucleolar RNA, H/ACA box 23</i>	1.51
NM_008937	<i>Prox1</i>	<i>Prospero homeobox 1</i>	1.51
NM_011674	<i>Ugt8a</i>	<i>UDP galactosyltransferase 8A</i>	1.49
NM_001252552	<i>Folr1</i>	<i>Folate receptor 1</i>	1.47
NR_026942	<i>E330013P04Rik</i>	<i>RIKEN cDNA E330013P04 gene</i>	1.46
NM_133238	<i>Cd209a</i>	<i>CD209a antigen</i>	1.44
NR_028078	<i>Snora21</i>	<i>Small nucleolar RNA, H/ACA box 21</i>	1.44
NR_046306	<i>DQ267102</i>	<i>SnoRNA DQ267102</i>	1.44
NM_001100182	<i>Cyp2j12</i>	<i>Cytochrome P450, family 2, subfamily j, polypeptide 12</i>	1.42
NM_153156	<i>Stoml3</i>	<i>Stomatin (Epb7.2)-like 3</i>	1.42
NM_178685	<i>Pcdh20</i>	<i>Protocadherin 20</i>	1.42
NM_015743	<i>Nr4a3</i>	<i>Nuclear receptor subfamily 4, group A, member 3</i>	1.41
NM_178086	<i>Fa2h</i>	<i>Fatty acid 2-hydroxylase</i>	1.41
NM_010762	<i>Mal</i>	<i>Myelin and lymphocyte protein, T cell differentiation protein</i>	1.41
NM_080726	<i>Rem2</i>	<i>Rad and gem related GTP binding protein 2</i>	1.41
NM_024413	<i>Plekhf1</i>	<i>Pleckstrin homology domain containing, family F (with FYVE domain) member 1</i>	1.40
NR_046144	<i>n-R5s136</i>	<i>Nuclear encoded rRNA 5S 136</i>	1.39
NM_011177	<i>Klk6</i>	<i>Kallikrein related-peptidase 6</i>	1.39
NR_004434	<i>Rprl1</i>	<i>Ribonuclease P RNA-like 1</i>	1.39
NM_008037	<i>Fosl2</i>	<i>Fos-like antigen 2</i>	1.39
NR_004412	<i>Rnu1b1</i>	<i>U1b1 small nuclear RNA</i>	1.38
NM_153520	<i>Opalin</i>	<i>Oligodendrocytic myelin paranodal and inner loop protein</i>	1.38
NM_010153	<i>ErbB3</i>	<i>V-erb-b2 erythroblastic leukemia viral oncogene homolog 3</i>	1.37
NM_177624	<i>Sntn</i>	<i>Sentan, cilia apical structure protein</i>	1.37
NM_026731	<i>Ppp1r14a</i>	<i>Protein phosphatase 1, regulatory (inhibitor) subunit 14A</i>	1.37
NM_001033349	<i>Gm410</i>	<i>Predicted gene 410</i>	1.35
NM_001013374	<i>Lman2l</i>	<i>Lectin, mannose-binding 2-like</i>	1.35
NR_001460	<i>Rmrp</i>	<i>RNA component of mitochondrial RNAase P</i>	1.35
NM_010338	<i>Gpr37</i>	<i>G protein-coupled receptor 37</i>	1.34
NM_009398	<i>Tnfaip6</i>	<i>Tumor necrosis factor alpha induced protein 6</i>	1.34
NM_029001	<i>Elovl7</i>	<i>ELOVL family member 7, elongation of long chain fatty acids</i>	1.34
NM_025821	<i>Carhsp1</i>	<i>Calcium regulated heat stable protein 1</i>	1.34
NM_001042715	<i>Ccdc135</i>	<i>Coiled-coil domain containing 135</i>	1.34
NM_198102	<i>Tra2a</i>	<i>Transformer 2 alpha homolog</i>	1.33
NM_001161767	<i>Galnt6</i>	<i>UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 6</i>	1.33

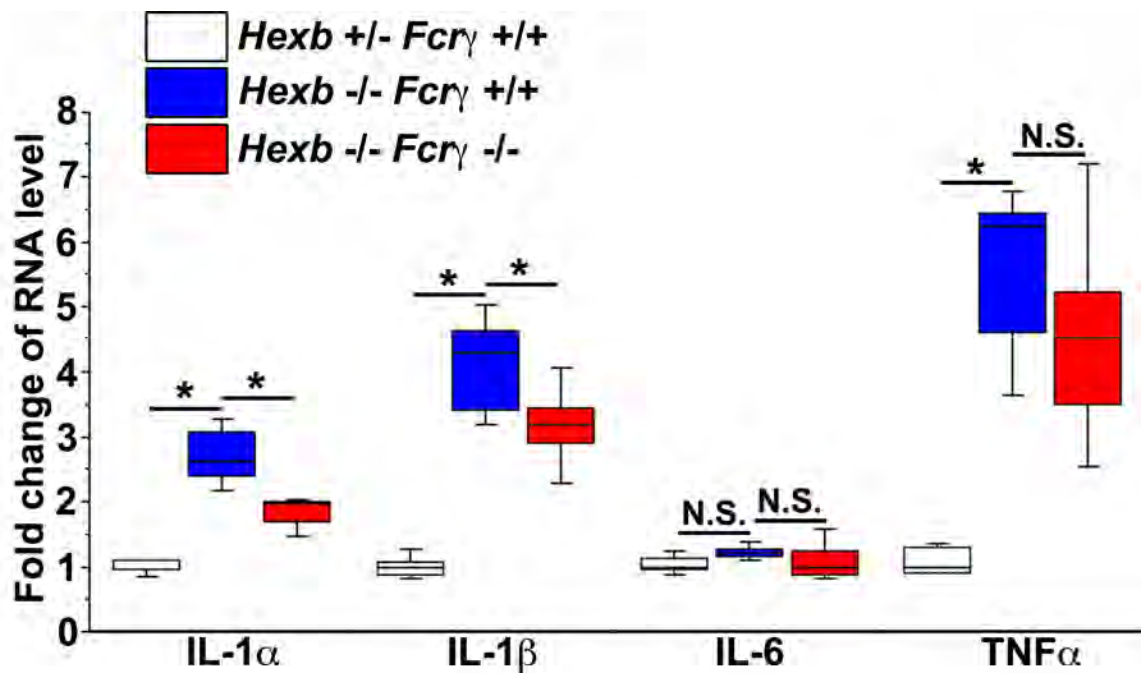


**Figure S1.** Reduction in microglial activity in cortices of *Hexb*<sup>-/-</sup> *Fcγ*<sup>-/-</sup> mice at 16 weeks. *A*, Immunostaining of coronal sections for CD68 (green) and Iba1 (red) in the cerebral cortices of *Hexb*<sup>+/-</sup> *Fcγ*<sup>+/+</sup>, *Hexb*<sup>-/-</sup> *Fcγ*<sup>+/+</sup>, and *Hexb*<sup>-/-</sup> *Fcγ*<sup>-/-</sup> mice at 16 weeks. Blue represents DAPI staining. Insets (a–c) show magnified views of the boxed regions. Scale bar, 100  $\mu$ m. *B* and *C*, Quantitative analysis for the number of CD68+ (*B*) and Iba1+ (*C*) cell immune signals in the cerebral cortices of *Hexb*<sup>+/-</sup> *Fcγ*<sup>+/+</sup>, *Hexb*<sup>-/-</sup> *Fcγ*<sup>+/+</sup>, and *Hexb*<sup>-/-</sup> *Fcγ*<sup>-/-</sup> mice at 16 weeks. Boxes, 25<sup>th</sup>–75<sup>th</sup> percentile with the median indicated; bars, 10<sup>th</sup> and 90<sup>th</sup> percentiles. Analyzed using a Kruskal–Wallis test (nonparametric ANOVA) followed by a Dunn’s post hoc test ( $n=5$ ). N.S.: difference not significant ( $P>0.05$ ),  $*P<0.05$ .

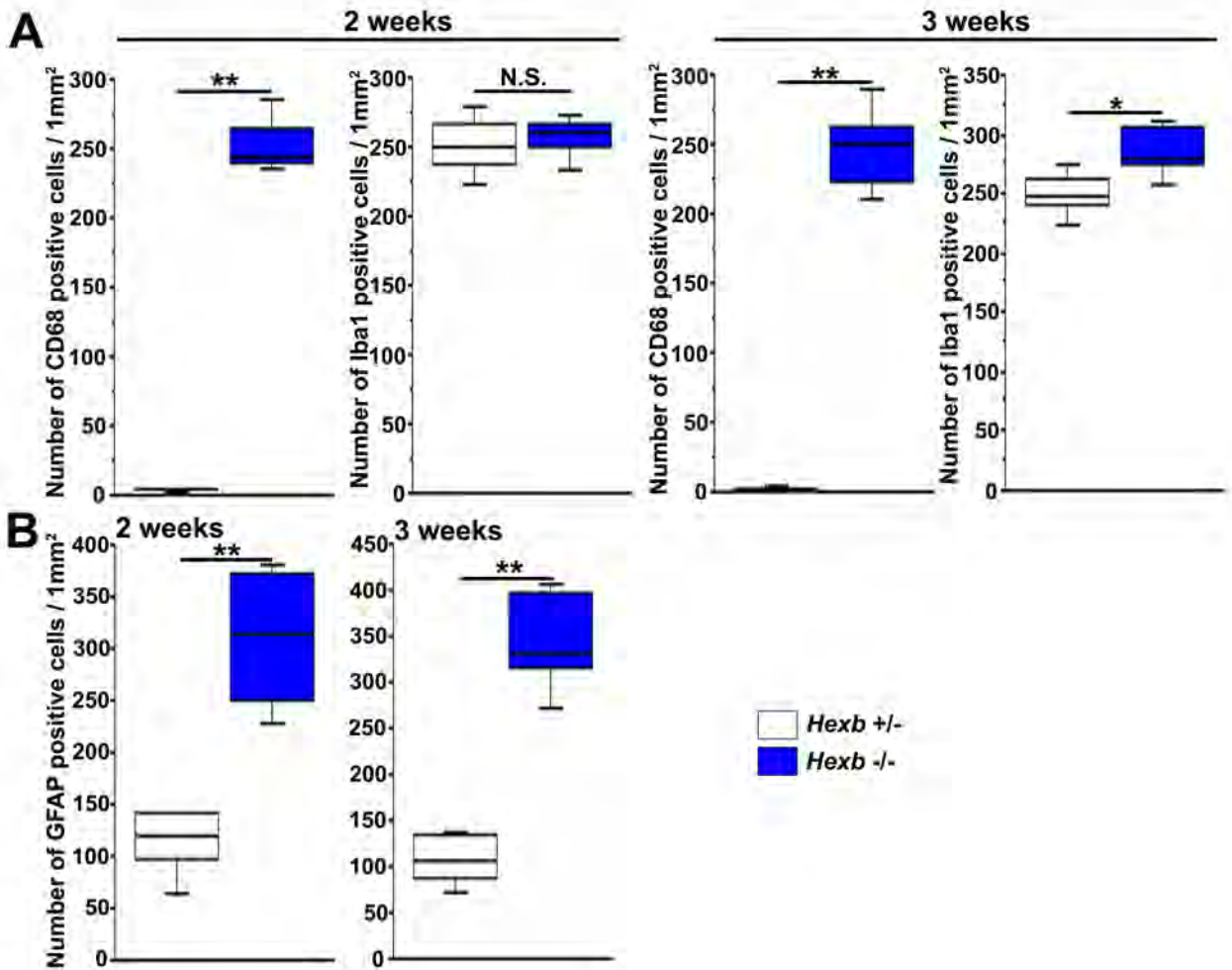


**Figure S2.** Reduction in reactive astrogliosis in cortices of *Hexb*<sup>-/-</sup> *FcRγ*<sup>-/-</sup> mice at 16 weeks. *A*, Immunostaining of coronal sections for GFAP (green) in the cerebral cortices of *Hexb*<sup>+/-</sup> *FcRγ*<sup>+/+</sup>, *Hexb*<sup>-/-</sup> *FcRγ*<sup>+/+</sup>, and *Hexb*<sup>-/-</sup> *FcRγ*<sup>-/-</sup> mice at 16 weeks. Blue represents DAPI staining. Insets (a–c) show magnified views of the boxed regions. Scale bar, 100 μm. *B*, Quantitative analysis for the number of GFAP+ cell immune signals in the cerebral cortices of *Hexb*<sup>+/-</sup> *FcRγ*<sup>+/+</sup>, *Hexb*<sup>-/-</sup> *FcRγ*<sup>+/+</sup>, and *Hexb*<sup>-/-</sup> *FcRγ*<sup>-/-</sup> mice at 16 weeks. Boxes, 25<sup>th</sup>–75<sup>th</sup> percentile with the median indicated; bars, 10<sup>th</sup> and 90<sup>th</sup> percentiles. Analyzed using a Kruskal–Wallis test (nonparametric ANOVA) followed by a Dunn’s post hoc test (n=5). \**P*<0.05.

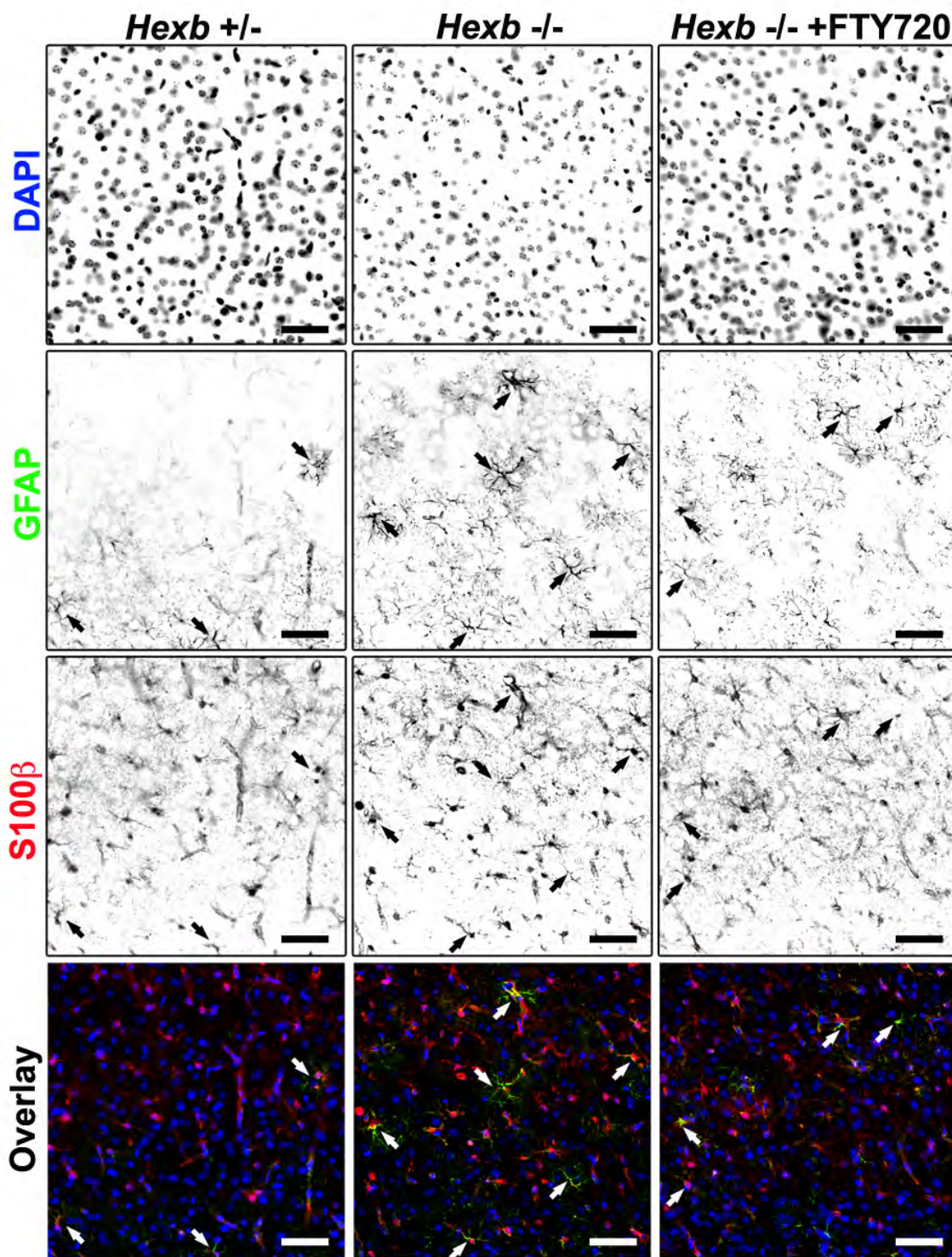




**Figure S3.** mRNA expression levels were measured by real-time PCR. Expression levels were standardized by those of 18S ribosomal RNA. The values show relative gene expression levels in the cerebral cortices of *Hexb*<sup>+/-</sup> *FcR $\gamma$* <sup>+/+</sup> (open box), *Hexb*<sup>-/-</sup> *FcR $\gamma$* <sup>+/+</sup> (blue box), and *Hexb*<sup>-/-</sup> *FcR $\gamma$* <sup>-/-</sup> (red box) mice at 16 weeks. Boxes, 25<sup>th</sup>–75<sup>th</sup> percentile with the median indicated; bars, 10<sup>th</sup> and 90<sup>th</sup> percentiles. Analyzed using a Kruskal–Wallis test (nonparametric ANOVA) followed by a Dunn’s post hoc test (n=5). N.S.: difference not significant ( $P>0.05$ ), \* $P<0.05$ .



**Figure S4.** Quantitative analysis for microglial activation and astrogliosis in the cerebral cortices of *Hexb*<sup>+/-</sup> and *Hexb*<sup>-/-</sup> mice during development from 2 weeks to 3 weeks. *A*, Quantitative analysis for the number of CD68<sup>+</sup> and Iba1<sup>+</sup> cell immune signals. Median values of CD68-positive cells per 1-mm<sup>2</sup> section were 4.0 (3.5–5.0) and 244 (239–272) in 2-week-old *Hexb*<sup>+/-</sup> and *Hexb*<sup>-/-</sup> mice; 1.0 (0.5–3.0) and 251 (218–271) in 3-week-old *Hexb*<sup>+/-</sup> and *Hexb*<sup>-/-</sup> mice. Median values of Iba1-positive cells per 1-mm<sup>2</sup> section were 250 (233–271) and 260 (244–269) in 2-week-old *Hexb*<sup>+/-</sup> and *Hexb*<sup>-/-</sup> mice; 247 (235–266) and 280 (269–307) in 3-week-old *Hexb*<sup>+/-</sup> and *Hexb*<sup>-/-</sup> mice. *B*, Quantitative analysis for the number of GFAP<sup>+</sup> cell immune signals. Median values of GFAP-positive cells per 1-mm<sup>2</sup> section were 120 (87–141) and 314 (243–376) in 2-week-old *Hexb*<sup>+/-</sup> and *Hexb*<sup>-/-</sup> mice; 106 (84–136) and 332 (301–400) in 3-week-old *Hexb*<sup>+/-</sup> and *Hexb*<sup>-/-</sup> mice. Boxes, 25<sup>th</sup>–75<sup>th</sup> percentile with the median indicated; bars, 10<sup>th</sup> and 90<sup>th</sup> percentiles. Analyzed using the Mann-Whitney *U*-test (n=5). \**P*<0.05, \*\**P*<0.01. N.S.: difference not significant (*P*>0.05).



**Figure S5.** Astroglial activation in brains of 4-week-old *Hexb*<sup>-/-</sup> mice. Immunostaining of coronal sections for GFAP (green) and S100β (red) in the cerebral cortices of *Hexb*<sup>+/-</sup>, *Hexb*<sup>-/-</sup> and FTY720-treated *Hexb*<sup>-/-</sup> mice at 4 weeks. Arrows indicate GFAP / S100β double-positive cells. Blue represents DAPI staining. Scale bar, 50 μm.