Supplemental Material

Microglia Activation in Niemann-Pick Disease, type C1 is Amendable to Therapeutic Intervention

Cougnoux et al.

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Supplementary Table 1. Selected differentially expressed genes

Gene	Log2FC	pAdj	Gene	Log2FC	pAdj	Gene	Log2FC	pAdj
Cytokines			**CXC Ligands & Receptors**			**Phagosome processing, maturation and signal transduction**		
Il1a	-0.44	4.19E-02	Cxcl10	2.96	5.30E-09			
Il1b	2.25	6.47E-21	Cxcl11	3.86	1.22E-14			
Il1r1	2.27	1.50E-07	Cxcl14	1.95	3.30E-05	*Pla2g4a*	-0.70	4.12E-09
Il1rn	3.18	1.37E-13	Cxcl16	1.09	9.15E-09	*Pld2*	-0.17	6.51E-01
Il2rg	1.88	2.39E-04	Cxcr4	2.63	1.45E-08	*Cnn2*	-1.60	1.02E-06
Tnf	1.18	2.87E-03	Cx3cr1	-0.77	7.84E-11	*Pld1*	-1.03	2.90E-06
Tgfa	0.68	9.30E-05				*Axl*	2.70	1.73E-94
Tgfb1	-0.45	1.08E-03				*Clic4*	1.21	4.36E-08
Tgfb2	1.57	4.17E-03	**Phagocytosis associated receptors**			*Csk*	-0.44	2.00E-03
Spp1	3.01	9.20E-32	*Cd14*	-0.74	4.35E-05	*Lyn*	-0.30	4.47E-02
			Cd36	0.87	4.02E-02	*Mapk14*	-0.64	1.11E-06
CC Ligands & Receptors			*Cd163*	-2.10	2.20E-06	*Mertk*	-0.66	3.03E-06
Ccl3	2.27	8.39E-25	*Clec12a*	1.54	7.59E-05	*Msn*	0.81	4.32E-08
Ccl4	1.45	1.59E-11	*Clec4a2*	-1.21	9.84E-08	*Pros1*	-0.31	3.93E-02
Ccl5	3.02	8.80E-12	*Clec5a*	-0.46	7.58E-03	*Pten*	-0.51	1.72E-03
Ccr1	-2.60	6.37E-12	*Clec7a*	1.93	4.80E-20	*Rac1*	0.45	4.23E-03
Ccr3	-1.49	1.22E-02	*Colec12*	0.83	1.85E-16	*Rac2*	0.48	2.26E-02
Ccr5	-1.54	4.63E-16	*Fcer1g*	0.57	6.34E-03	*Syk*	-0.30	8.00E-02
Ccr6	-1.30	6.81E-18	*Itgam*	-0.36	1.71E-03	*Vav1*	-0.47	2.68E-05
			Itgav	-0.98	7.98E-14			
Purinoreceptors			*Pecam1*	-1.14	8.05E-04	**Neurotoxic markers**		
P2rx1	-1.28	1.61E-05	*Tlr2*	0.74	1.06E-04	*Cybb*	1.53	1.44E-08
P2rx4	0.57	1.22E-04	*Tlr3*	-0.77	1.23E-03	*Optn*	2.02	3.94E-10
P2rx7	-0.30	1.46E-02	*Tlr4*	-0.76	1.57E-06	*Mmp12*	1.34	2.69E-03
P2ry12	-0.91	6.87E-17	*Tlr5*	-1.96	2.18E-10	*Pde2a*	3.15	8.67E-29
P2ry13	-1.05	2.07E-10	*Tlr9*	-0.53	3.16E-03	*Pde3b*	-0.60	8.20E-05
P2ry2	1.45	1.56E-02				*Hmgb1*	0.40	2.38E-02
P2ry6	-0.55	7.93E-04	**Recognition & engulfment**					
			Anxa1	1.21	2.14E-02	**Neuroprotective markers**		
Innate immunity markers			*C3*	3.62	2.60E-64	*Igf1*	3.36	4.96E-27
Fcer1g	0.57	6.34E-03	*Cd44*	1.73	1.35E-05	*Igf2*	1.36	1.41E-02
Fcgr1	-0.77	4.69E-06	*Cd47*	-0.63	9.55E-03	*Igf2bp2*	1.31	2.14E-04
Fcgr2b	0.33	2.80E-02	*Csf1*	2.44	6.18E-51	*Igf2r*	1.60	2.66E-04
Fcgr3	-0.09	7.46E-01	*Elmo1*	-0.77	7.67E-12	*Ttr*	1.37	1.99E-02
Fcgr4	0.96	1.93E-02	*Mif*	1.76	7.95E-09	*Cd22*	3.87	1.15E-93
Fcrl1	1.43	4.18E-03	*Tgm2*	-0.72	3.35E-07	*Vegfb*	1.33	6.63E-13
Ifnar1	0.02	9.36E-01	*Tnf*	1.18	2.87E-03	*Sod1*	0.71	2.57E-02
Ifnar2	-0.11	6.02E-01				*Sod2*	0.79	7.99E-04
Ifngr1	-0.86	1.45E-10				*Sod3*	1.89	1.76E-04
Ifitm3	2.15	2.82E-12				*Bcl2*	0.88	6.42E-03

Supplementary Table 1. Selected differentially expressed genes (continued)

Gene	Log2FC	pAdj		Gene	Log2FC	pAdj
Other antioxidants				**HIF1 pathway**		
Gsr	1.05	1.84E-03		*Hif1a*	1.28	1.39E-24
Sod1	0.71	2.57E-02		*Anxa2*	1.40	1.12E-02
Sod2	0.79	7.99E-04		*Btg1*	-0.49	4.93E-03
Sod3	1.89	1.76E-04		*Egr1*	-1.76	1.29E-22
Srxn1	2.19	2.21E-08		*Hmox1*	0.98	1.98E-04
Cyba	0.62	2.00E-02		*Lox*	4.19	9.83E-26
Scd1	1.08	3.03E-02		*Plau*	-0.62	1.09E-03
				Aldoa	1.38	1.55E-12
Other oxidative stress response genes				*F10*	1.65	4.62E-03
				Mif	1.76	7.95E-09
Als2	0.59	4.95E-02		*Ldha*	1.04	1.07E-09
Apoe	2.43	3.97E-55		*Pdk1*	0.30	2.80E-01
Hmox1	0.98	1.98E-04		*Pfkfb3*	0.51	6.08E-03
Prnp	1.68	2.07E-05		*Pfkfb4*	-0.48	2.58E-02
Vim	1.91	5.49E-12		*Pfkl*	0.56	1.53E-03
				Pfkp	1.59	4.17E-06
Peroxidases				*Pgam1*	1.31	5.41E-10
Gpx3	2.88	7.30E-09		*Pgk1*	1.11	3.18E-05
Gstk1	1.00	4.48E-02		*Tpi1*	1.29	6.20E-09
Prdx1	1.18	4.37E-08		*Bnip3*	1.28	2.20E-07
Prdx4	0.64	3.77E-02		*Bnip3l*	0.32	3.60E-02
Prdx5	0.67	4.50E-03		*Pim1*	1.19	1.62E-02
Prdx6	0.87	5.11E-03		*Ccng2*	-0.92	2.33E-12
Cat	0.71	1.52E-07		*Mxi1*	0.43	4.69E-03
Ctsb	1.81	7.98E-45		*Txnip*	-0.96	5.76E-10
				Fos	-1.23	5.67E-04
Glycolysis				*Rbpj*	0.64	4.13E-03
Eno2	1.74	2.94E-07		*Vdac1*	0.44	9.17E-02
Aldoc	1.33	1.20E-03		*Ctsa*	0.65	8.66E-06
Igf1	3.36	4.96E-27		*Dnajc5*	0.37	2.75E-02
Hif1a	1.28	1.39E-24		*Eif4ebp1*	0.78	1.83E-02
Tpi1	1.29	6.20E-09		*Lgals3*	2.74	2.51E-16
Pkm	1.49	3.95E-10		*Map3k1*	0.39	8.22E-04
Gpi1	1.03	2.00E-05				
Gapdh	1.14	6.64E-08				
Pgk1	1.11	3.18E-05				
Eno11	0.64	2.85E-01				

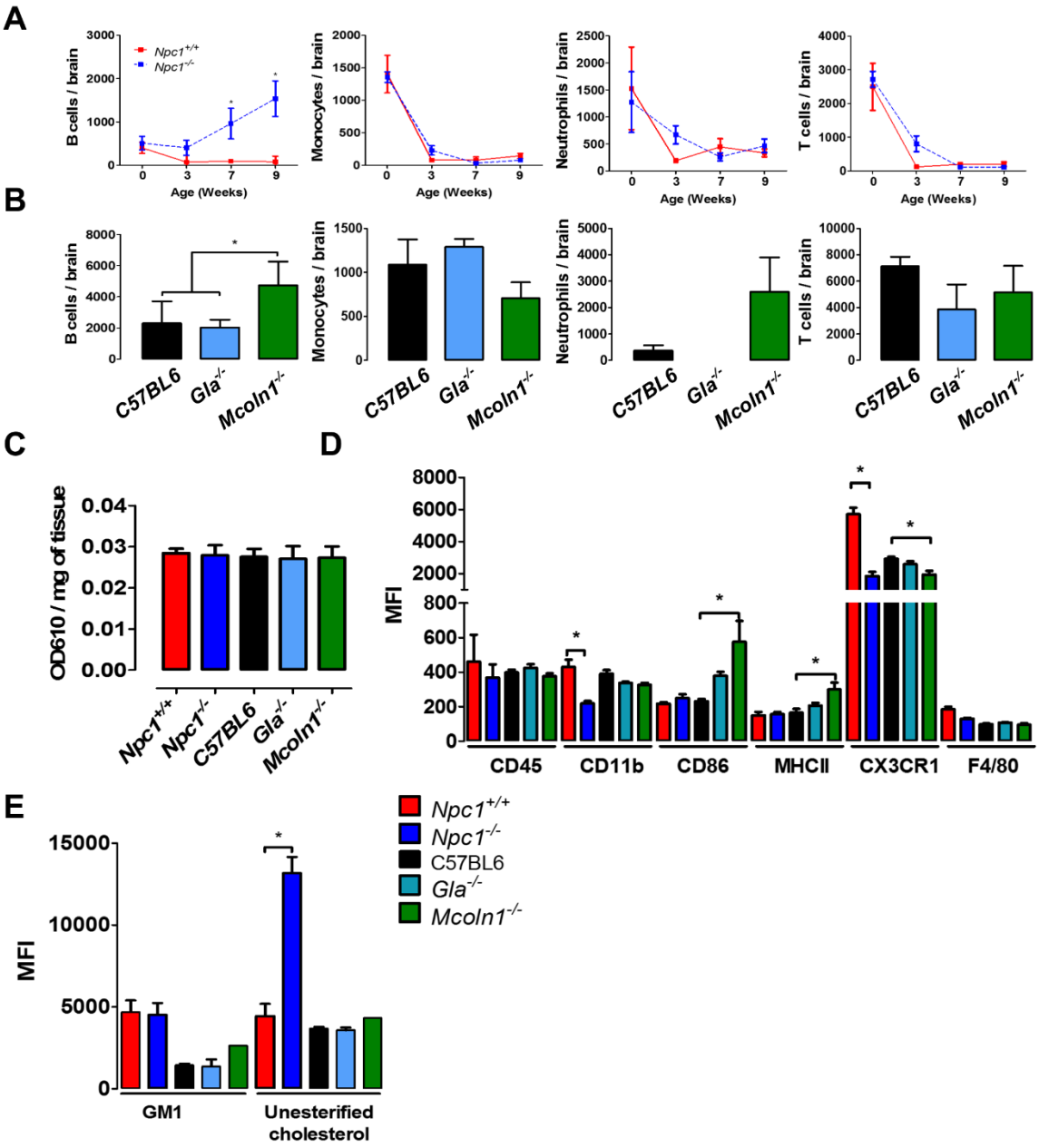

Figure S1. FACS analysis of peripheral immune cell infiltration into brain tissue of NPC1 mice and comparison to other lysosomal storage disorders. (**A**) Peripheral immune cell infiltration was evaluated in BALB/C *Npc1*$^{+/+}$ (red) and *Npc1*$^{-/-}$ (dashed blue) brain tissue at birth, 3-, 7- and 9-weeks of age by FACS analysis of surface markers of B-cells (CD19+, CD45+, MHCII+), Monocytes (Ly6C^{hi}, CD45+, CD11b+), Neutrophils (CD45+, Ly6G+, CD11b+) and T-

cells (CD3e+, CD45+, MHCII-). Data are from n≥6 mice. (**B**) Number of B-cells, monocytes, neutrophils and T-cells in brain tissue from 4-month-old control C57BL/6, *Gla*$^{-/-}$ and *Mcoln1*$^{-/-}$ mice. Data are from n≥3 for each genotype. (**C**) Evans blue uptake measured at 610 nm normalized to tissue mass (mg) in 9-week-old BALB/C *Npc1*$^{+/+}$ or *Npc1*$^{-/-}$ and 4-month-old C57BL6 control, *Gla*$^{-/-}$ and *Mcoln1*$^{-/-}$ mice. No significant differences were observed, n≥6. (**D**) Microglia surface marker expression as mean fluorescence intensity from 7-week- old *Npc1*$^{+/+}$ or *Npc1*$^{-/-}$ and 4-month-old C57BL6, *Gla*$^{-/-}$ or *Mcoln1*$^{-/-}$ mice. Data are from n≥6. (**E**) GM1 and unesterified cholesterol accumulation quantified by staining isolated microglial cells with Alexa488-labeled cholera toxin and filipin, respectively, n≥6.

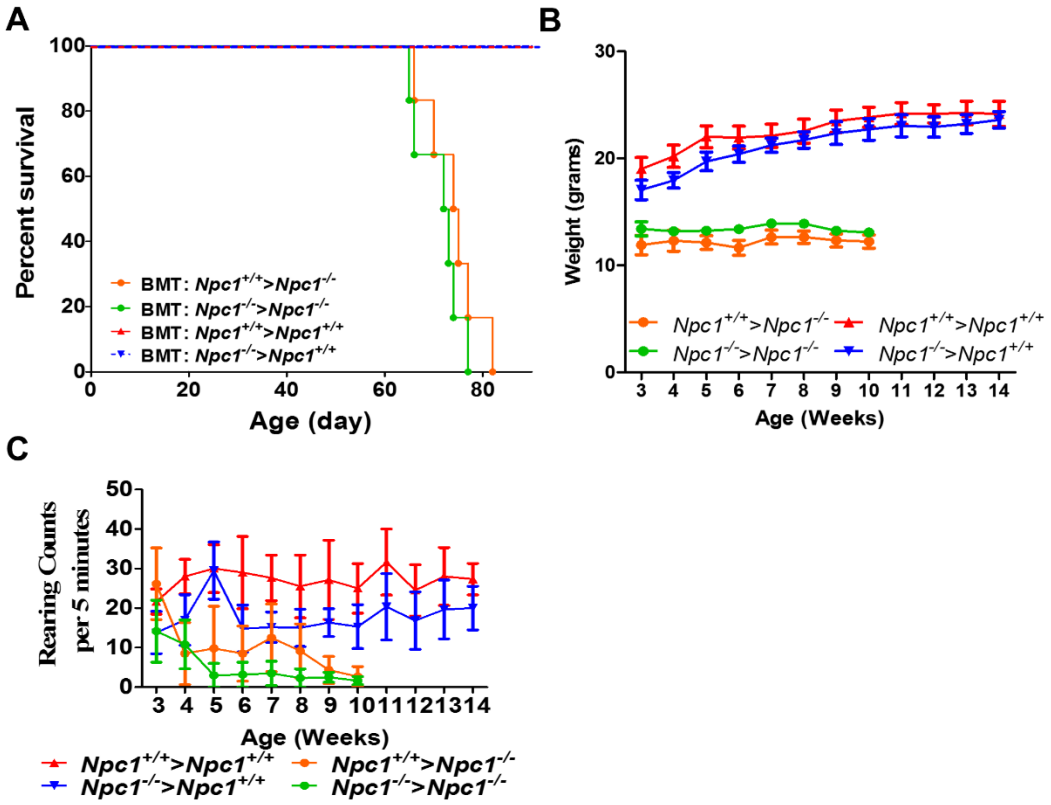

Figure S2. Reciprocal bone marrow transplantation in *Npc1* mutant and control mice. (**A**) Survival of *Npc1*$^{-/-}$ mice lethally irradiated and transplanted with bone marrow from either *Npc1*$^{+/+}$ (orange) or *Npc1*$^{-/-}$ (green) donors (n=6). Transplantation of *Npc1*$^{-/-}$ derived bone marrow into lethally irradiated *Npc1*$^{+/+}$ mice had no appreciable effect on survival. Mice were followed for 10 weeks after engraftment. (**B**) Growth curve of *Npc1*$^{+/+}$ or *Npc1*$^{-/-}$ lethally irradiated and transplanted with bone marrow from either *Npc1*$^{+/+}$ or *Npc1*$^{-/-}$ donors (n=6/group). (**C**) Rearing count per 5 min in *Npc1*$^{+/+}$ or *Npc1*$^{-/-}$ lethally irradiated and then transplanted with bone marrow from either *Npc1*$^{+/+}$ or *Npc1*$^{-/-}$ donors. n=6.

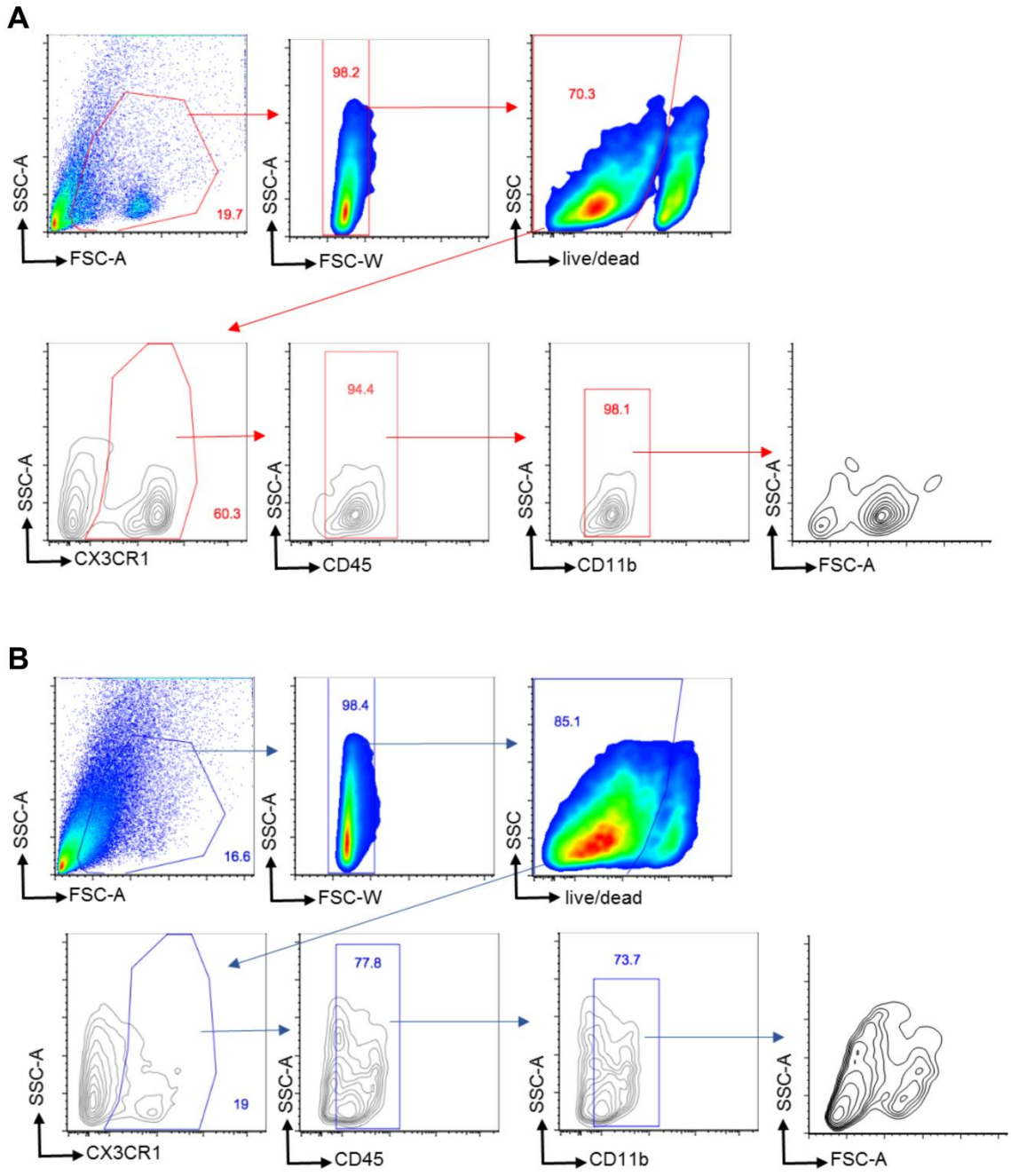

Figure S3. Gating strategy for FACS analysis. Representative FACS plots for 7-week old *Npc1*$^{+/+}$ (**A**) or *Npc1*$^{-/-}$ (**B**) mice.

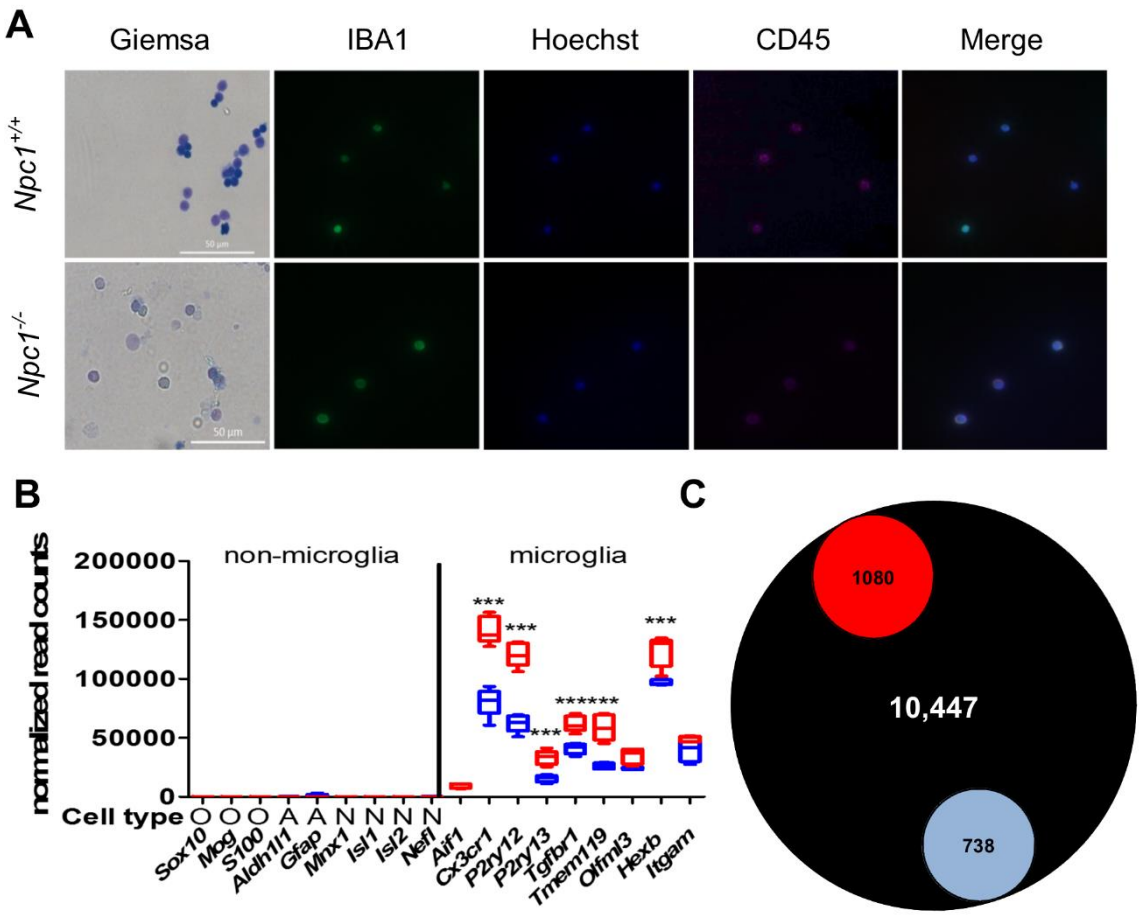

Figure S4. Microglia purification for transcriptome analysis. A) Microglia preparation stained with Giemsa (left panel). Purified control and Npc1 mutant microglia stained with IBA1, Hoechst and CD45 (right panels). CD45 is a marker of myeloid cells and all CD45 positive cells were IBA1 positive indicating significant enrichment for microglia. (**B**) Expression of non-microglia (O= oligodendrocytes: *Sox10*, *S100*, *Mog*; A= astrocytes: *Aldh1l1*, *Gfap*; N= Neurons: *Mnx1*, *Isl1*, *Isl2*, *Nefl*) and microglia expressed genes was also used to confirm enrichment of the microglia preparation. Normalized reads count in the samples from *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ microglia. (**C**) Venn diagram of genes with total number of genes with >100 read count in at least on group (10447) and the subset of genes with significantly ($p<0.05$) increased (red, 1080) or decreased (blue, 738) expression in *Npc1*$^{-/-}$ relative to *Npc1*$^{+/+}$ microglia from 7-week-old mice.

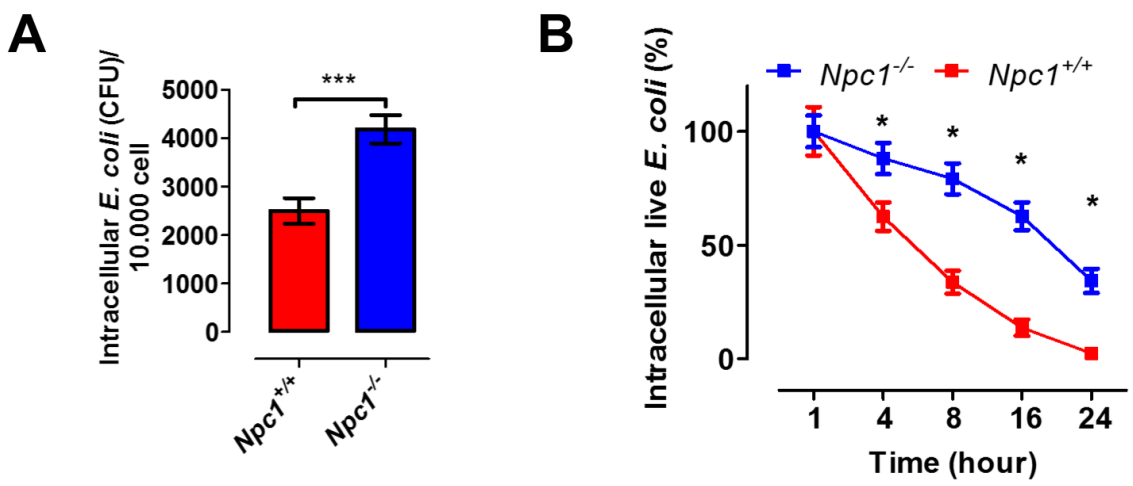

Figure S5. Phagocytosis and killing of live *Escherichia coli* by *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ microglia. (**A**) Number of internalized *E. coli* bacterial (colony forming units) after incubation for one hour with *Npc1*$^{+/+}$ (red) and *Npc1*$^{-/-}$ (blue) microglia. (**B**) Kinetic analysis of bacterial killing by *Npc1*$^{+/+}$ (red) and *Npc1*$^{-/-}$ (blue) microglia. 100% represents the number of internalized *E. coli* CFU present after one hour of coincubation, n≥5.

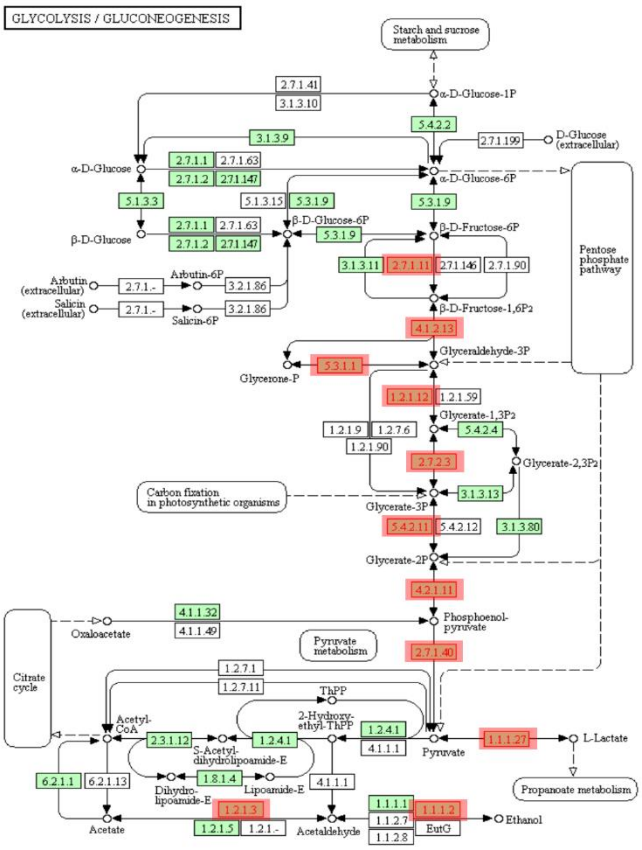

Figure S6. Differential expression of glycolysis associated genes. KEGG pathway of the glycolytic metabolism, genes with significantly ($p<0.01$) increased expression in *Npc1*$^{-/-}$ microglia are highlighted in red. See also Table S1.

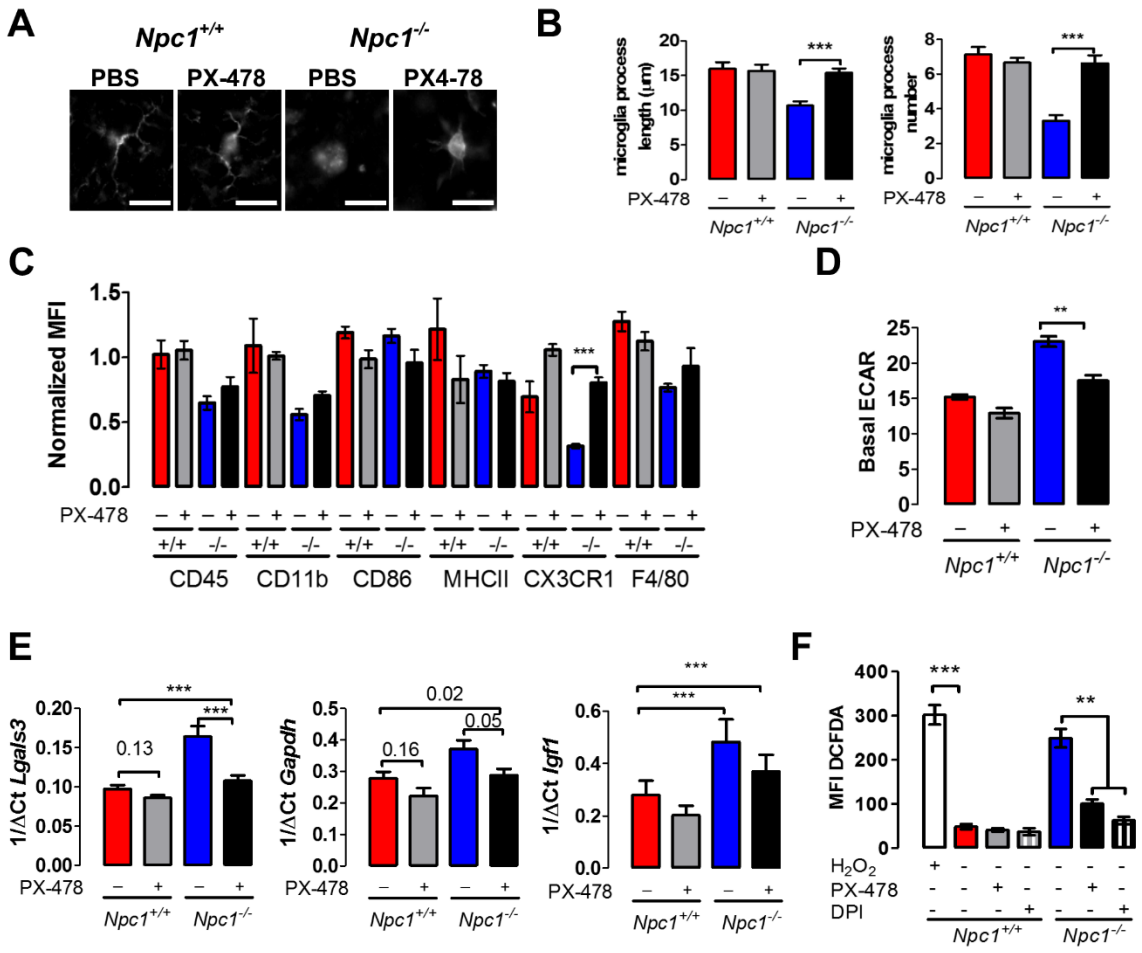

Figure S7. Effect of PX-478 inhibition of HIF1α in Npc1 mutant mice. (**A**) Representative IBA1 staining demonstrating a less activated microglial morphology in *Npc1*$^{-/-}$ mice treated with PX-478. (**B**) Consistent with the observed morphological change, PX-478 treatment increased both number and length of microglial processes in 7-week old *Npc1*$^{-/-}$ mice, n≥6. (**C**) Microglia surface marker expression expressed as mean fluorescence intensity (MFI) for 7-week old *Npc1*$^{+/+}$ or *Npc1*$^{-/-}$ treated with either PBS or PX-478. (**D**) Basal ECAR level measured by Seahorse assay was consistent with a decrease toward normal glycolytic metabolism in *Npc1*$^{-/-}$ microglia from PX-478 treated mice. (**E**) RT-qPCR expression level of HIF1α target genes: *Lgals3, Gapdh* and *Igf1* in *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ microglia. Treatment with PX-478 decreased expression toward normal for all

three target genes in *Npc1*$^{-/-}$ mice. n=5/group. (**F**) Total ROS production was evaluated using DCFDA staining. *Npc1*$^{-/-}$ microglia from PX-478 treated mice showed decreased DCFDA staining consistent with decreased ROS production. Treatment of control microglia with 1μM H_2O_2 served as a positive control for the DCFDA assay. Diphenyleneiodonium chloride (DPI) at 1μM was used to inhibit mitochondrial ROS production. Results from 3 independent experiments all with at least 3 samples. Each sample included 10000 cells.

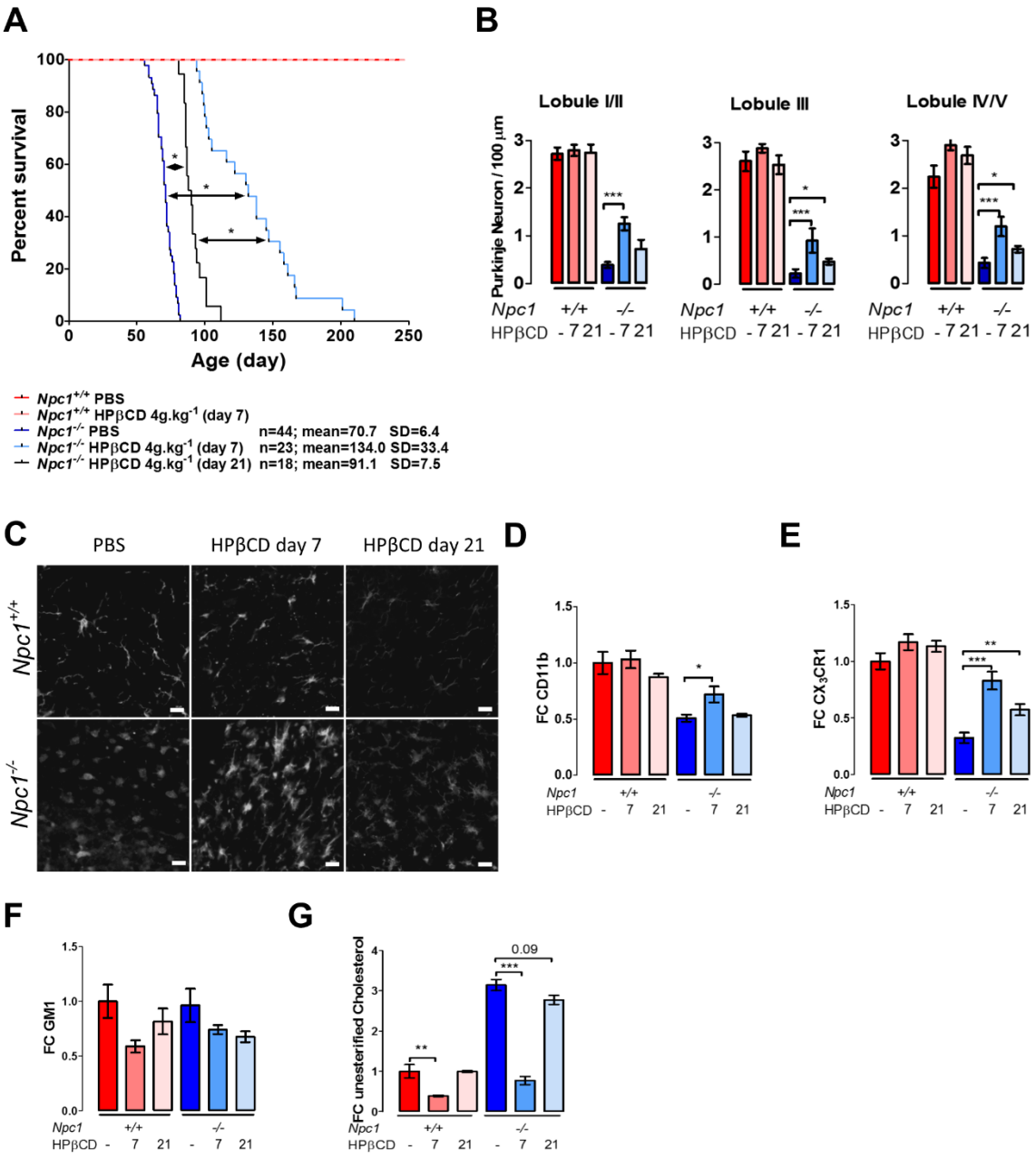

Figure S8. Effect of HPβCD treatment on *Npc1*$^{-/-}$ microglia. (**A**) Survival of *Npc1*$^{-/-}$ mice treated with 4000mg.kg^{-1} HPβCD starting on day of life 7 (light blue) or day of life 21 (black) and then treated three times a week. Control PBS treatment is in dark blue. Survival of control *Npc1*$^{+/+}$ day of life 7 HPβCD treated (red) and untreated (orange) *Npc1*$^{+/+}$ mice is also shown. (**B**) Quantification of cerebellar Purkinje neuron density per 100 µm in lobules I/II, III and IV/V at 7

weeks of age. *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice treated with PBS or 4000mg.kg^{-1} HPβCD starting at either day of life 7 or 21 and then administered twice a week after weaning. (**C**) Representative IBA1 staining showing restoration of the ramified microglial morphology in HPβCD treated *Npc1*$^{-/-}$ mice. (**D**) CD11b surface expression was only increased toward normal in *Npc1*$^{-/-}$ mice treated with HPβCD starting at DOL7. (**E**) CX_3CR1 surface expression was increased toward normal in *Npc1*$^{-/-}$ mice treated with HPβCD starting at both DOL 7 and 21. Storage of intracellular GM1 (**F**) was not different from control at this age (7-weeks) nor was it changed by treatment with HPβCD. In contrast, unesterified cholesterol storage was markedly and significantly ($p<0.001$) decreased in *Npc1*$^{-/-}$ microglia when HPβCD treatment was initiated on DOL 7. We only observed a minimal trend ($p=0.09$) toward decreased unesterified cholesterol when HPβCD treatment was initiated on DOL 21.