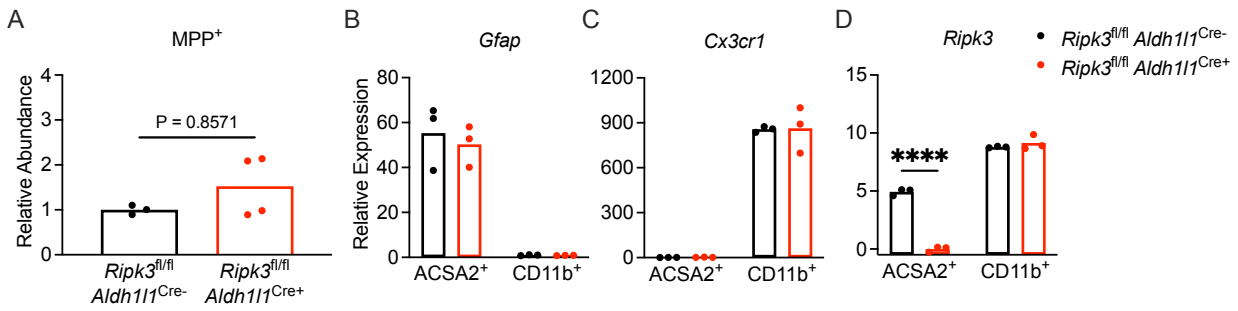
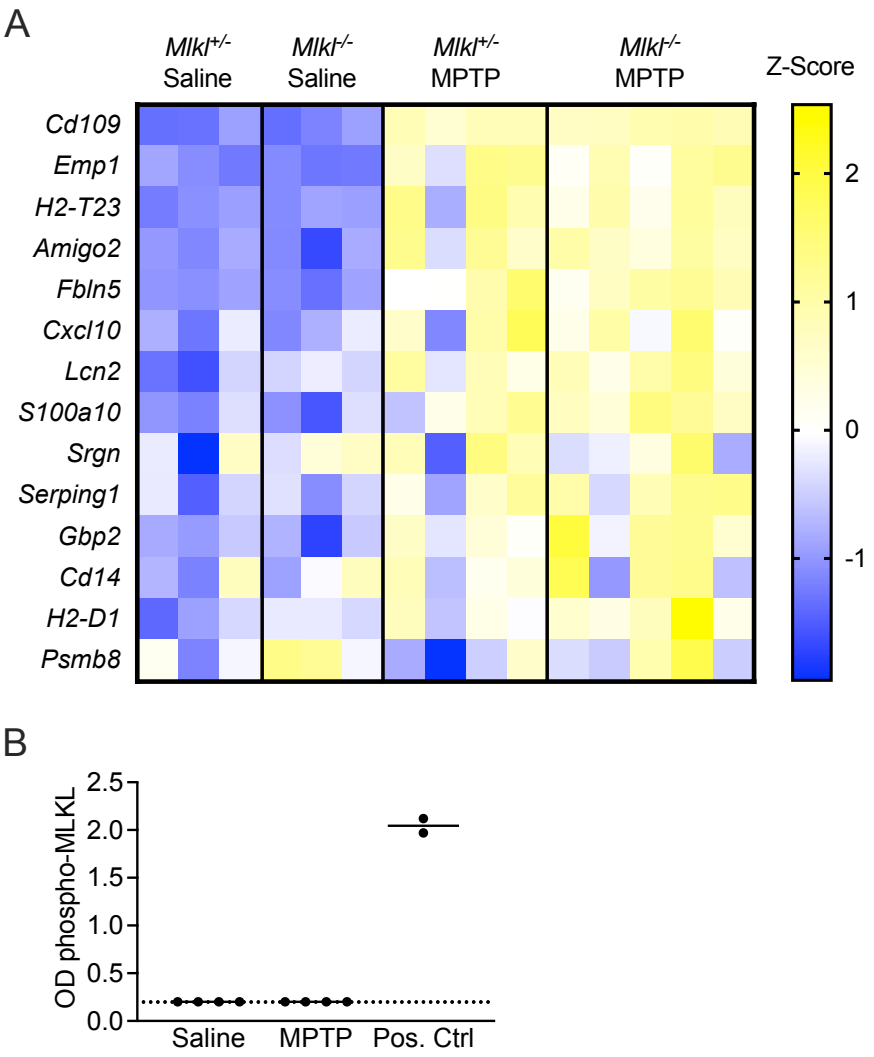
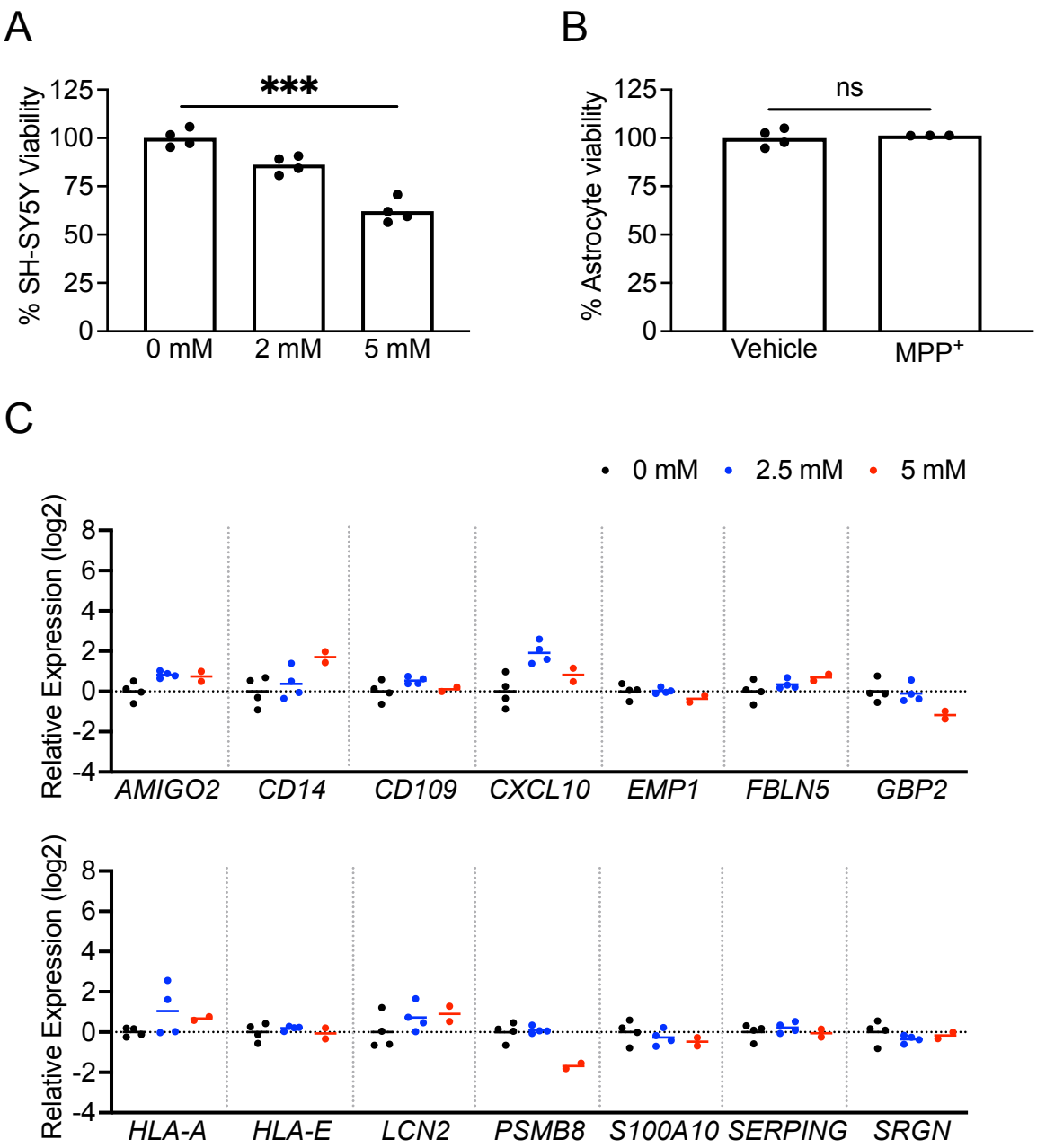


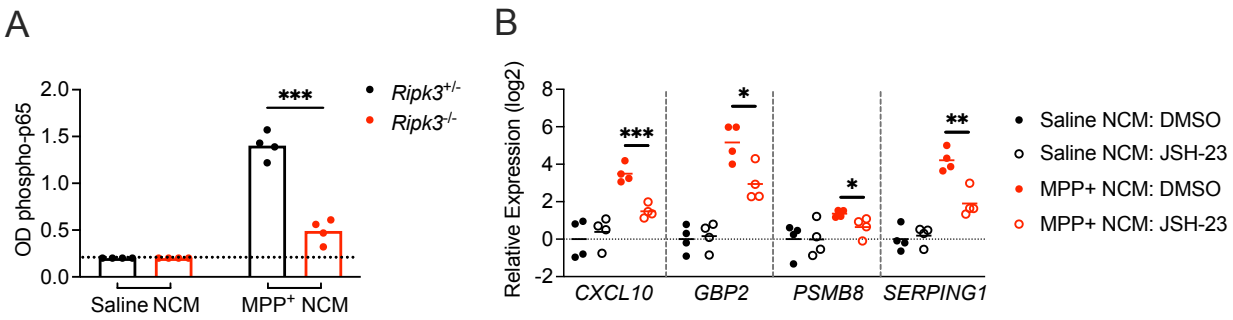
Supplemental Figure 1. ***Deletion of astrocytic Ripk3 does not impact MPTP metabolism in vivo.*** **(A)** LC-MS analysis of MPP^+ abundance in midbrain homogenates of mice of indicated genotypes 90 minutes following intraperitoneal MPTP injection. (B-C) ACSA2+ (astrocytes) and CD11b+ (microglia) cells were isolated from brains of mice of indicated genotypes via magnetic activated cell sorting (MACS). Purity was confirmed via qRT-PCR detection of cell type specific genes (*Gfap* for astrocytes; *Cx3cr1* for microglia) **(B-C)**. **(D)** qRT-PCR detection of *Ripk3* in sorted cell populations. ****p<0.0001.



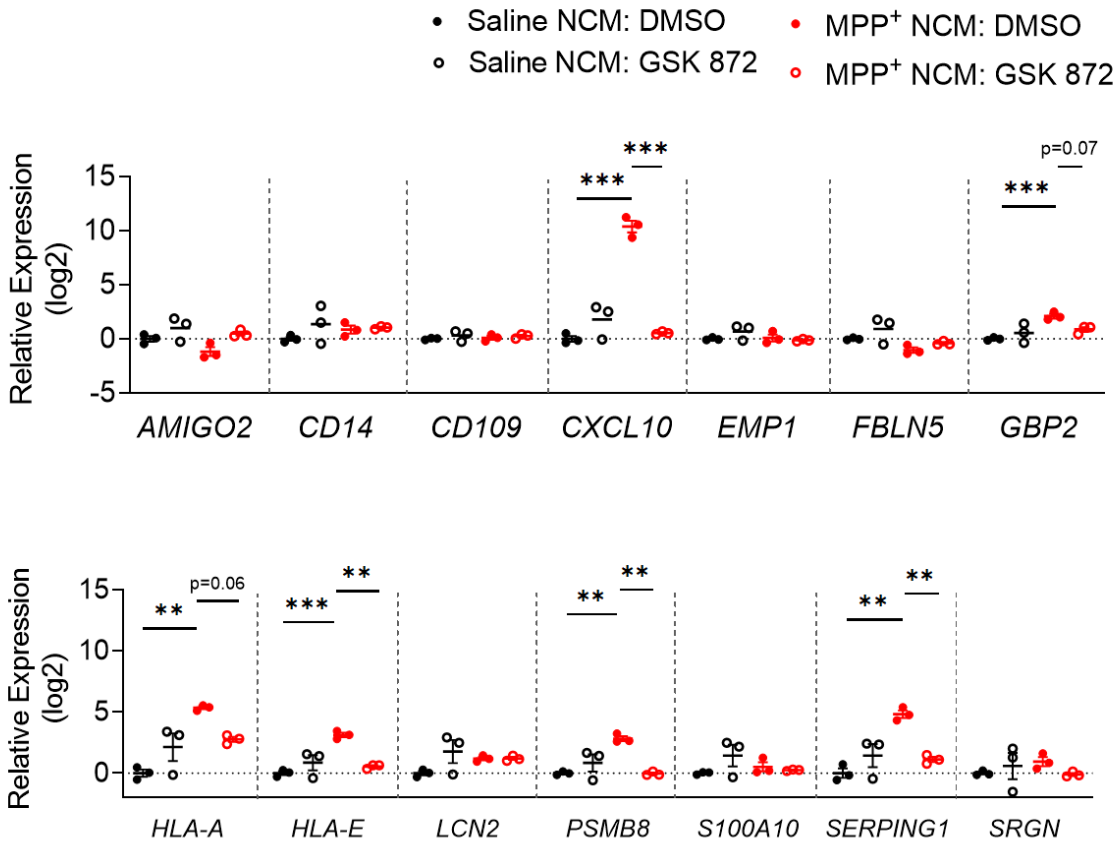
Supplemental Figure 2. ***Genes associated with astrocyte activation are not impacted by loss of MLKL in the MPTP model.*** A) qRT-PCR analysis of indicated genes in midbrain homogenates of *Mlkl* knockout and heterozygous littermate control animals 3 days post treatment with MPTP or saline. B) ELISA detection of phospho-MLKL (ser-345) in midbrain homogenates of WT mice 3 days post treatment with MPTP or saline. Positive control represents calyculin-treated HT29 cell lysate provided in kit.



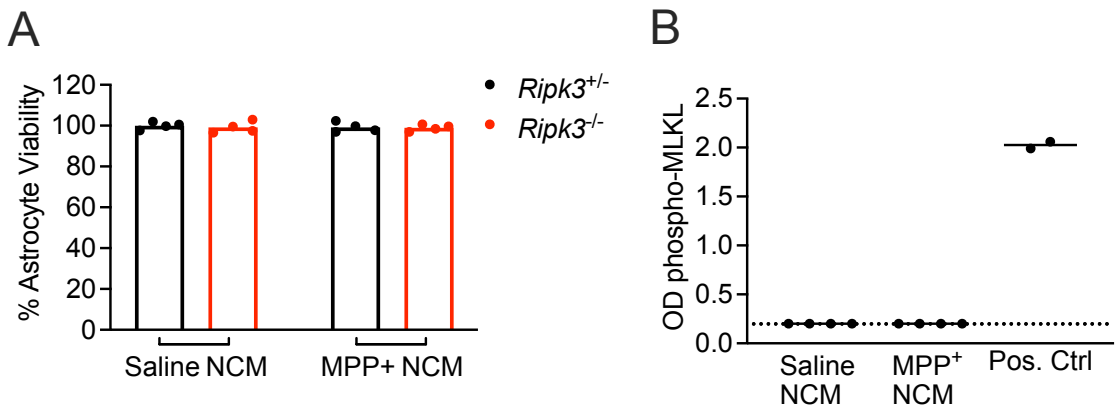
Supplemental Figure 3. ***MPP⁺ induces death in SH-SY5Y cells, but does not induce death or transcriptional activation in astrocytes.*** **(A-B)** Cell Titer Glo analysis of viability in SH-SY5Y **(A)** or primary human midbrain astrocyte **(B)** cultures treated with 2 or 5 mM **(A)** or 2.5 mM **(B)** MPP⁺ for 24h. **(C)** qRT-PCR analysis of indicated genes in primary human midbrain astrocytes treated with 2.5 mM MPP⁺ for 24h. *** p<0.001.



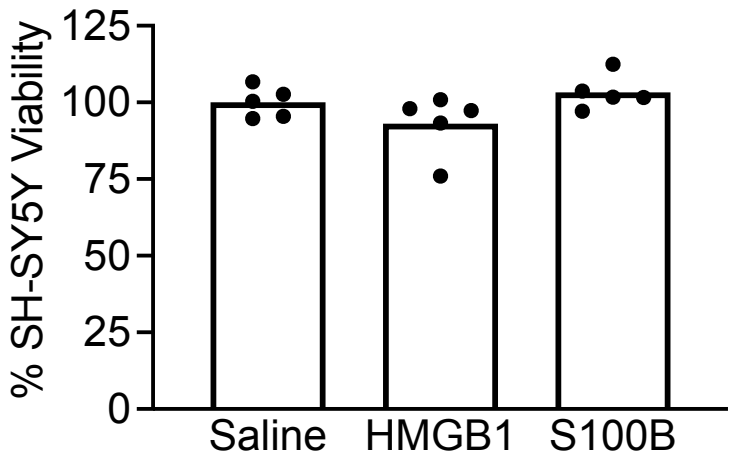
Supplemental Figure 4. ***MPP+ NCM induces RIPK3-dependent NFkB activation and NFkB-mediated gene expression.*** A) Primary human midbrain astrocytes of indicated genotypes were treated with NCM and phospho-p65 was detected via ELISA 4h post treatment. B) qRT-PCR analysis of indicated genes in primary human midbrain astrocytes treated for 24h with indicated conditions. *p<0.05, **p < 0.01, ***p < 0.001.



Supplemental Figure 5. ***Astrocytes maintain transcriptional activation for at least 24 hours following removal of MPP+ NCM.*** qRT-PCR analysis of indicated genes in primary human midbrain astrocytes treated for 24h with indicated conditions, followed by washing, addition of fresh culture medium (with no treatment), and an additional 24h incubation before harvest. *p<0.05, **p < 0.01, ***p < 0.001.



Supplemental Figure 6. ***MPP+ NCM does not induce cell death or phosphorylation of MLKL in primary midbrain astrocytes.*** A) Primary murine midbrain astrocytes of indicated genotypes were treated with NCM and viability was assessed after 24h via Cell Titer Glo. B) ELISA detection of phospho-MLKL (ser-345) in midbrain astrocyte cultures 24h post treatment with NCM. Positive control represents calyculin-treated HT29 cell lysate provided in kit.



Supplemental Figure 7. ***Recombinant DAMPs are not intrinsically toxic to SH-SY5Y cells.*** **(A-B)** Cell Titer Glo analysis of viability in SH-SY5Y treated with indicated DAMP ligand for 24 hours.

Supplemental Table 1.

Primer sequences for genotyping

Target	Note	Primer Sequence (5'-3')	Product size
Aldh1l1-Cre/ERT2	Internal control Forward	CTGTCCCTGTATGCCTCTGG	415bp
	Internal control reverse	AGATGGAGAAAGGACTAGGCTACA	
	Transgene Forward	CTTCAACAGGTGCCTTCCA	198bp
	Transgene Reverse	GGCAAACGGACAGAAGCA	
Mlkl$^{-/-}$	MLKL_001	TATGACCATGGCAACTCACG	WT 498bp KO 158bp
	MLKL_002	ACCATCTCCCCAAACTGTGA	
	MLKL_003	TCCTTCCAGCACCTCGTAAT	
Nestin-Cre	WT Forward	TTGCTAAAGCGCTACATAGGA	WT 246bp Cre 150bp
	Common Reverse	GCCTTATTGTGGAAGGACTG	
	Transgene Forward	CCTTCCTGAAGCAGTAGAGCA	
Ripk3$^{-/-}$	RIP3_001	CGCTTTAGAAGCCTTCAGGTTGAC	WT 733bp KO 485bp
	RIP3_002	GCAGGCTCTGGTGACAAGATTCATGG	
	RIP3_003	CCAGAGGCCACTTGTGTAGCG	
Ripk3$^{fl/fl}$	R3FL_001	ACGATGTCTTCTGTCAAGTTATG	WT 300bp LoxP 334bp
	R3FL_002	CAGTTCTTCACGGCTCAC	
	R3FL_003	TCTGGTAAGGAGGGTCAC	
Ripk3-2xFV$^{fl/fl}$	ROSA Forward	AGCACTTGCTCTCCCAAAGTC	346bp
	ROSA Reverse	CCGACAAAACCGAAAATCTGTGGG	
	Transgene Forward	CGCTTTAGAAGCCTTCAGGTTGAC	349bp
	Transgene Reverse	GCAGGCTCTGGTGACAAGATTCATGG	

Supplemental Table 2.

Primer sequences for qRT-PCR studies

Target	Forward	Reverse
18S hu	AGAAACGGCTACCACATCCA	CCCTCCAATGGATCCTCGTT
18s ms	CTTAGAGGGACAAGTGGCG	ACGCTGAGCCAGTCAGTGTA
AMIGO2 hu	CTTCAGCGTTTGGAGGGCT	CAGGGAACAGTCACAGACAAAT
Amigo2 ms	GAGGCGACCATAATGTCGTT	GCATCCAACAGTCCGATTCT
CCL2 hu	GCAGCAAGTGTCCCAAAGAA	CTGGGGAAAGCTAGGGGAAA
CD109 hu	CAGGAATGTGGACTCTGGGT	CTTTCGGACATGTGGACTGC
CD109 ms	CACAGTCGGGAGCCCTAAAG	GCAGCGATTTCGATGTCCAC
CD14 hu	CCGCTGTGTAGGAAAGAAGC	GCAGCGGAAATCTTCATCGT
CD14 ms	GGACTGATCTCAGCCCTCTG	GCTTCAGCCCAGTGAAAGAC
CXCL10 hu	GTGGCATTCAAGGAGTACCTC	TGATGGCCTTCGATTCTGGATT
Cxcl10 ms	CCCACGTGTTGAGATCATTG	CACTGGGTAAAGGGGAGTGA
EMP1 hu	CCAGTACACCAGCAGAGGAA	AACAGTAGCGATGTGGACCA
Emp1 ms	GAGACACTGGCCAGAAAAGC	TAAAAGGCAAGGGAATGCAC
FBLN5 hu	TCGCCAGTCAGGACAGTGT	AGTAGGGGTTCGAGTAGGGC
Fbln5 ms	CTTCAGATGCAAGCAACAA	AGGCAGTGTCAGAGGCCTTA
GBP2 hu	CTATCTGCAATTACGCAGCCT	TGTTCTGGCTTCTTGGGATGA
Gbp2 ms	GGGGTCACTGTCTGACCACT	GGGAAACCTGGGATGAGATT
HLA-A hu	GACCAGGAGACACGGAATGTG	CCTCGTTCAAGGCGATGTAATC
HLA-E hu	TTCCGAGTGAATCTGCGGAC	GTCGTAGGCGAACTGTTCATAC
H2-D1 ms	TCCGAGATTGTAAAGCGTGAAGA	ACAGGGCAGTGCAGGGATAG
H2-T23 ms	GGACCGCGAATGACATAGC	GCACCTCAGGGTGACTTCAT
LCN2 hu	GAAGTGTGACTACTGGATCAGGA	ACCACTCGGACGAGGTAACT
Lcn2 ms	CCAGTTCGCCATGGTATTTT	CACACTCACCACCCATTCAG
PSMB8 hu	GGTCCTACATTAGTGCCTTACGG	CGCAGATAGTACAGCCTGCATT
Psmb8 ms	CAGTCCTGAAGAGGCCTACG	CACTTTCACCCAACCGTCTT
S100A10 hu	ATGAAGGACCTGGACCAGTG	GCAGATTCCTTAAGCGACCC
S100a10 ms	CCTCTGGCTGTGGACAAAAT	CTGCTCACAAGAAGCAGTGG
SERPING1 hu	GGGATGCTTTGGTAGATTTCTCC	GAGGATGCTCTCCAGGTTTGT
Serping1 ms	ACAGCCCCCTCTGAATTCTT	GGATGCTCTCCAAGTTGCTC
SRGN hu	GGACTACTCTGGATCAGGCTT	CAAGAGACCTAAGGTTGTCATGG
Srgn ms	GCAAGGTTATCCTGCTCGGA	TGGGAGGGCCGATGTTATTG

Supplemental Table 3.

Flow cytometry antibodies		
CD11b	BioLegend	Clone M1/70
CD45.2	BioLegend	Clone 104
CD80	BioLegend	Clone 16-10A1
F4/80	BioLegend	Clone BM8
MHC-II	BioLegend	Clone M5/114.15.2
Zombie NIR	BioLegend	#423105
BioTracker CSFE	Sigma-Aldrich	SCT110
Immunofluorescence antibodies		
Rat anti-GFAP	Invitrogen	13-0300
Rabbit anti-IBA-1	Wako Chemicals	1919741
Chicken anti-tyrosine hydroxylase	Aves Labs	TYH
Rabbit anti-tyrosine hydroxylase	Abcam	Ab112
Mouse anti-SMI32	BioLegend	801701
Chemicals, peptides, and recombinant proteins		
Human BDNF	Sigma-Aldrich	B3795
GSK872	Millipore Sigma	530389
FPS-ZM1	Sigma-Aldrich	55030
Retinoic acid	Sigma-Aldrich	R2625
Cyclohexamide	Sigma-Aldrich	66-81-9
Recombinant HMGB1	R&D Systems	1690-HMB-050
Recombinant mouse S100β	Novus Biologicals	NBP2-53070
B/B Homodimerizer	Takara USA lnc.	AP20187
MPP+ iodide	Sigma-Aldrich	D048-100MG
JSH-23	Selleck Chem	S7351
Anti-HMGB1 nAb	Arigobio	ARG66714