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Supplementary Materials for

Cross-talk between GABAergic postsynapse and microglia regulate synapse loss after brain ischemia

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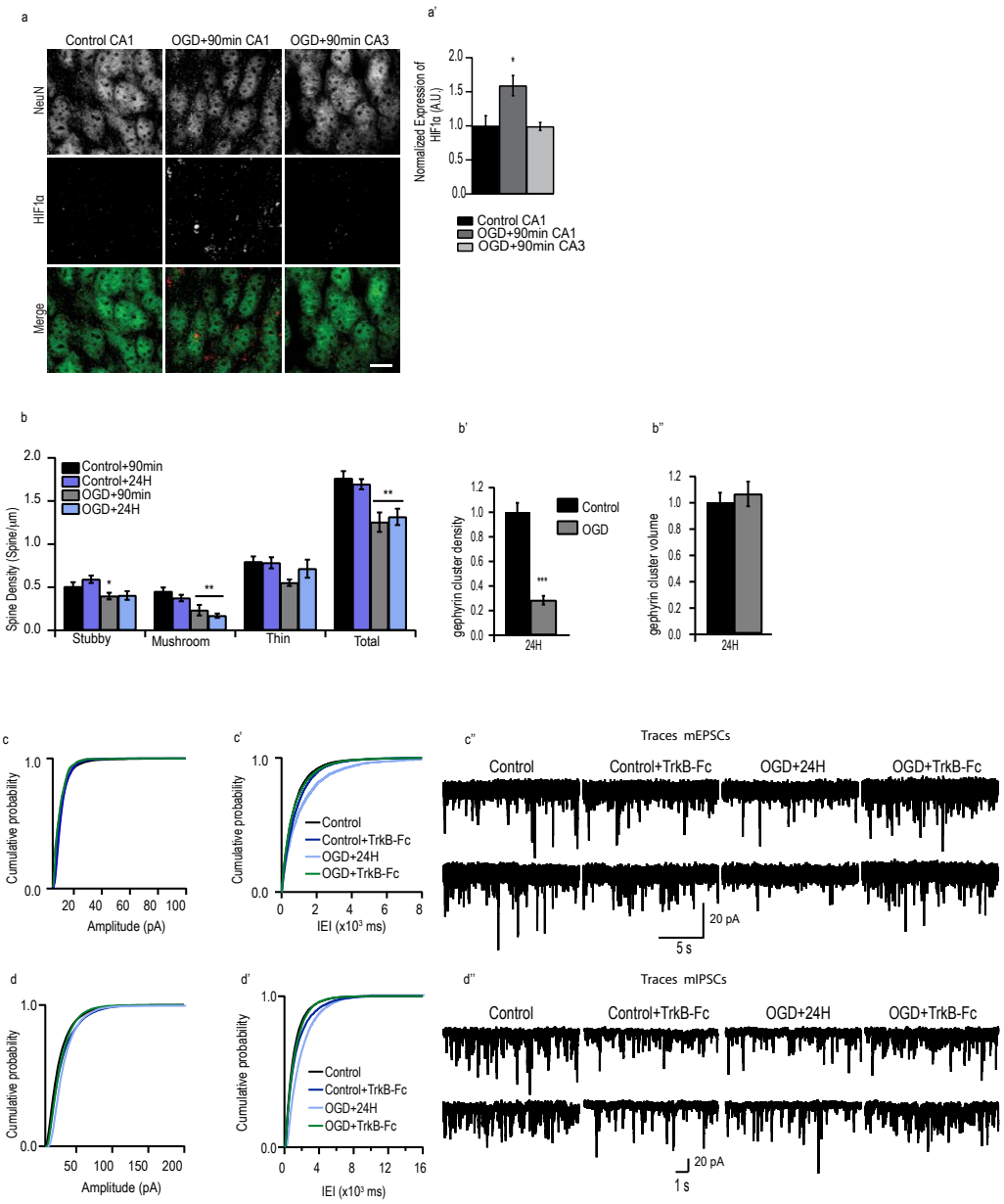
This PDF file includes:

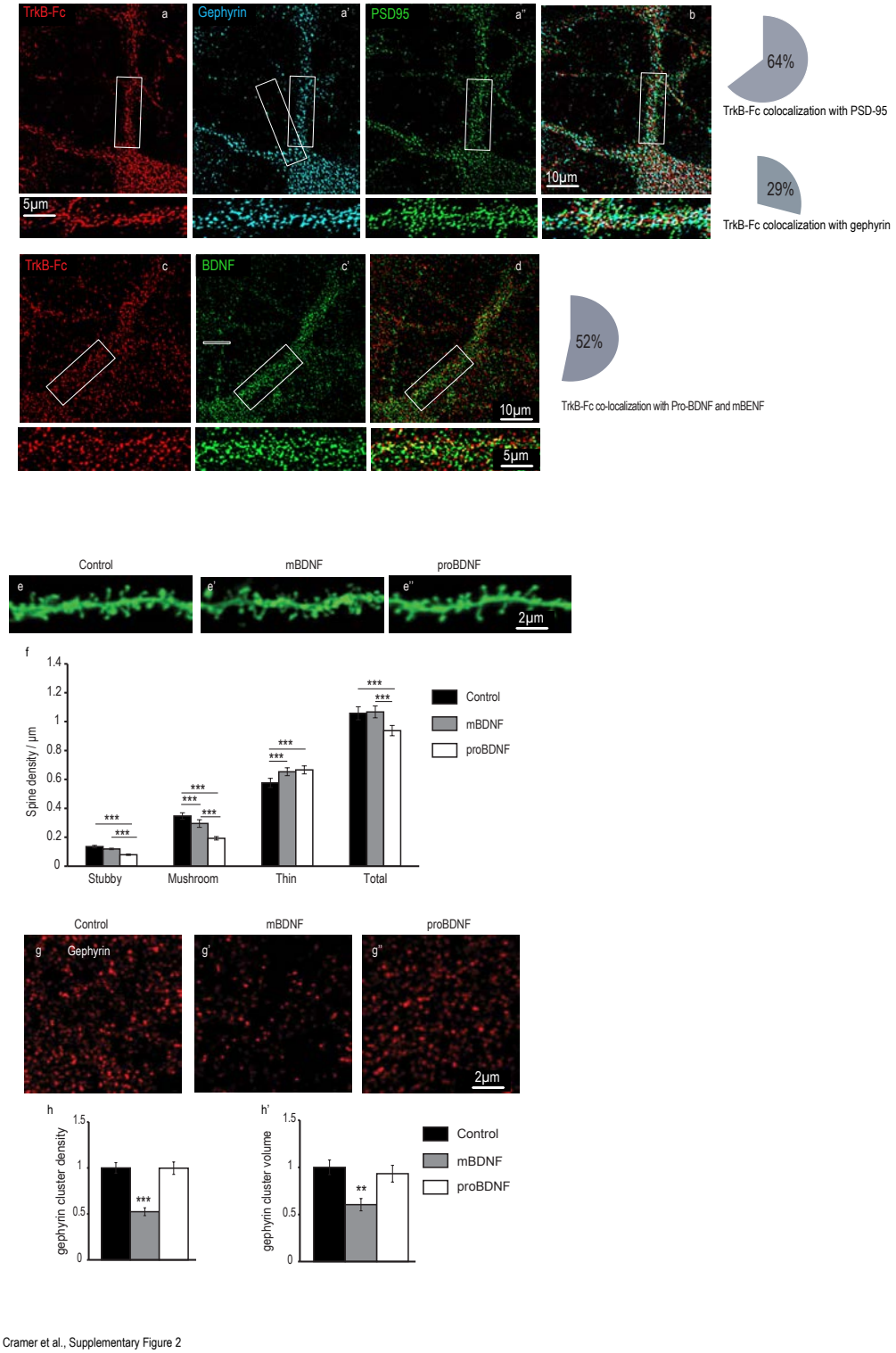
Fig. S1.



Cramer et al., Supplementary Figure 1

OGD causes glutamatergic and GABAergic synapse loss via BDNF signaling. **(a)** HIF1α expression in CA1 pyramidal neurons at 90min after OGD in hippocampal slice culture. **(a')** Quantification of HIF1α expression in CA1 pyramidal neurons. **(b)** Quantification of dendritic spines at 90 min and 24 h after OGD. The spines were categorized into stubby, mushroom and long thin subtypes (*$p < 0.05$ and **$p < 0.01$, two-tailed independent Student's t-test). Total spine density (spines/μm of dendrite). **(b')** Quantification of gephyrin cluster density 24 h after OGD (consisting of five 512x512 pixel z-planes each; ***$p < 0.0001$, two-tailed independent Student's t-test). **(b'')** Quantification of the total gephyrin cluster volume at 24 h after OGD (***$p < 0.0001$, two-tailed independent Student's t-test). **(c)** Cumulative probability histogram of mEPSC amplitude in TrkB-Fc treated neurons at 24 h post OGD ($p < 0.05$ Kolmogorov-Smirnov test). **(c')** Cumulative probability histogram of mEPSC IEIs ($p < 0.05$, Kolmogorov-Smirnov test). **(c'')** Example traces of AMPA-mediated mEPSC recordings after TrkB-Fc treatment and OGD. **(d)** Cumulative probability histogram of mIPSC amplitudes in TrkB-Fc treated neurons at 24 h post OGD ($p < 0.05$, Kolmogorov-Smirnov test). **(d')** Cumulative probability histogram of mIPSC IEIs ($p < 0.05$, Kolmogorov-Smirnov test). **(d'')** Example trace of mIPSCs after TrkB-Fc treatment and OGD .

Fig. S2

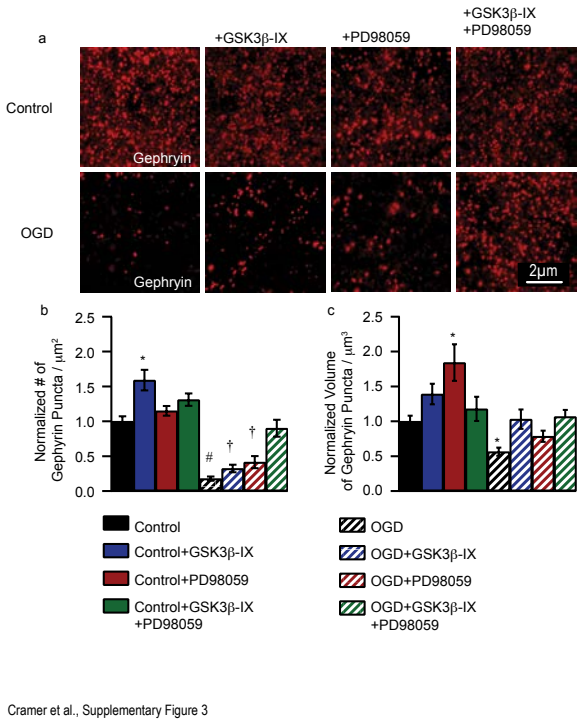


Cramer et al., Supplementary Figure 2

proBDNF and mBDNF act on glutamatergic and GABAergic synapses respectively. (a-a'') Representative image of primary hippocampal neuron (15DIV) treated with TrkB-Fc and co-stained for gephyrin and PSD-95. **(b)** Composite image showing colocalization of TrkB-Fc-

gephyrin or TrkB-Fc- PSD-95. High magnification dendrite segment is shown in the panel below. Quantification shows 64% colocalization of PSD-95 with TrkB-Fc and 29% colocalization with gephyrin (n=15). **(c-c')** Representative image of primary hippocampal neuron treated with TrkB-Fc and co-stained for BDNF. **(d)** Composite image showing colocalization of TrkB-Fc and BDNF. Quantification shows 52% colocalization of PSD-95 with TrkB-Fc and BDNF (n=15). **(e-e'')** Representative image of a dendrite segment of CA1 area from hippocampal slice culture treated with proBDNF or mBDNF. **(f)** Quantification of different spine subtypes or total spines on pyramidal neuron after treatment with proBDNF or mBDNF. *p< 0.05, **p< 0.001, ***p < 0.0001, Two-way ANOVA with Bonferroni multiple comparison test (n=19). **(g-g'')** Representative image of CA1 *Stratum Radiatum* gephyrin staining from hippocampal slice culture treated with proBDNF or mBDNF. **(h)** Quantification for gephyrin puncta density /μm^2 after proBDNF or mBDNF treatment for 90min. **(i)** Quantification for gephyrin puncta volume / μm^3 after proBDNF or mBDNF treatment for 90min. ***p<0.001, One-way ANOVA followed by Bonferroni multiple comparison test (n=12). Data shown as mean ± SEM.

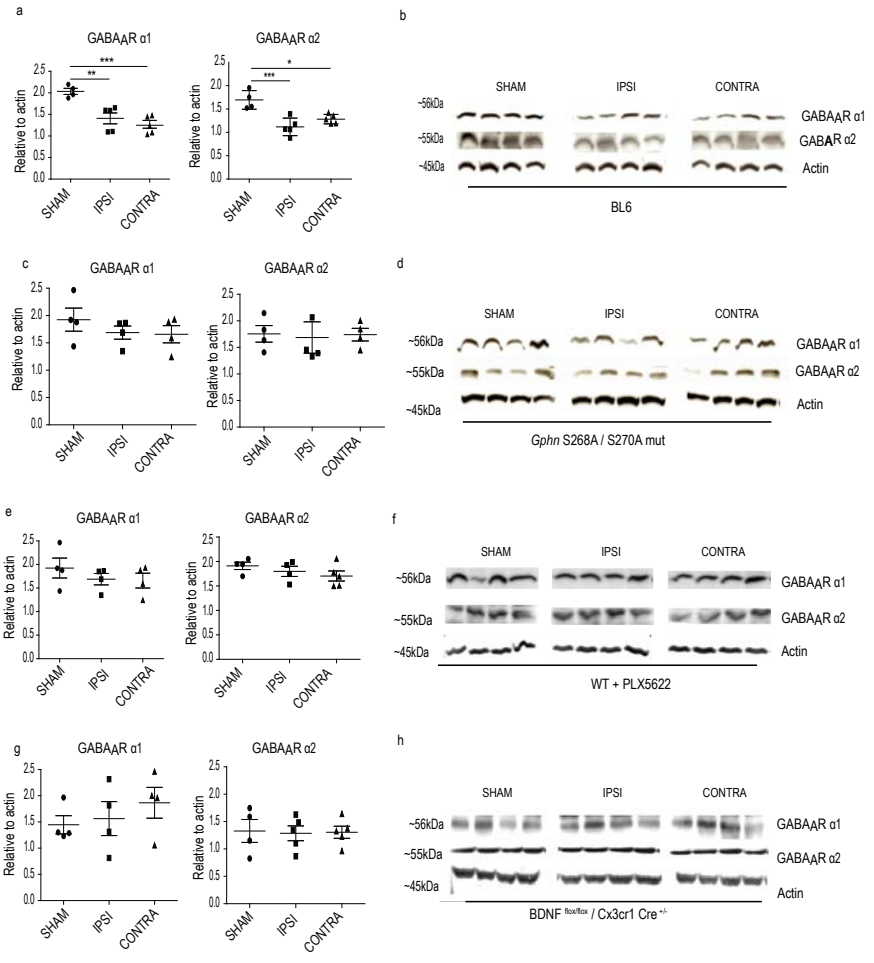
Fig. S3



Cramer et al., Supplementary Figure 3

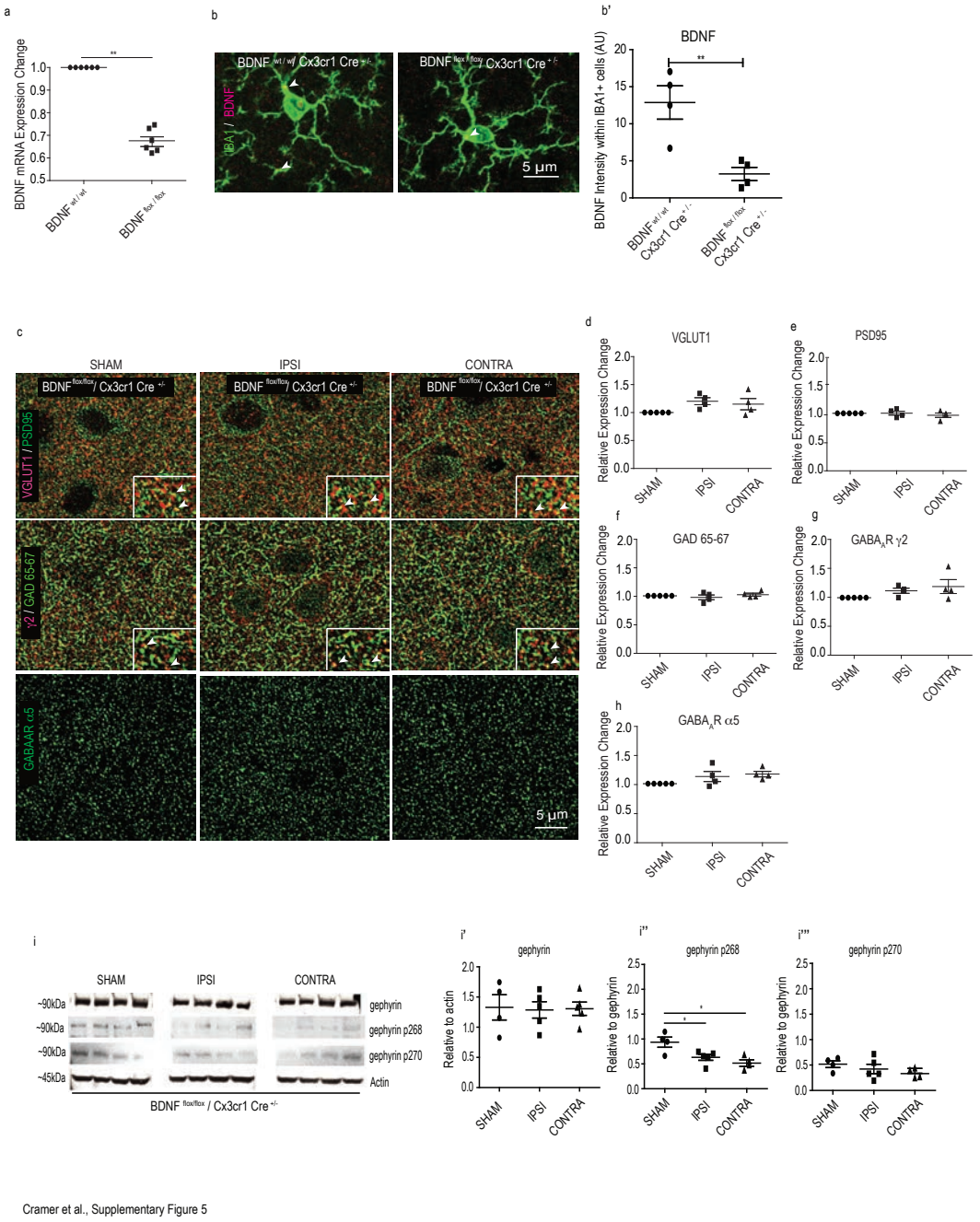
ERK1/2 and GSK3β signaling pathways are activated after OGD to downregulate gephyrin clustering. (a) Representative image showing gephyrin clustering in CA1 *Stratum Radiatum* area of hippocampus in control or OGD treated slice cultures treated with GSK3β inhibitor GSK3β-IX, or MEK inhibitor PD98059 or GSK3β-IX + PD98059. **(b)** Quantification of gephyrin puncta density / μm^3. **(c)** Quantification of gephyrin puncta volume / μm^2. (n=4).

Fig. S4



α1 and α2 GABA$_A$R expression levels in different mouse lines after OGD. **(a-b)** WB analysis and quantification for α1 and α2 GABA$_A$R in BL6 WT mice at 24 h after MCAO. **(c-d)** WB analysis and quantification for α1 and α2 GABA$_A$R in *Gphn*S268A/S270A mutant mice at 24 h after MCAO. **(e-f)** WB analysis and quantification for α1 and α2 GABA$_A$R in BL6 WT mice treated with PLX5622 at 24 h after MCAO. **(g-h)** WB analysis and quantification for α1 and α2 GABA$_A$R in BDNF$^{flox/flox}$ / Cx3Cr1$^{CreERT2+/-}$ mice at 24 h after MCAO. Quantification expressed as mean ± s.d. (N=4); *P<0.05; **P<0.01; ***P<=0.001 (One-way ANOVA, Bonferroni multiple comparison test).

Fig. S5

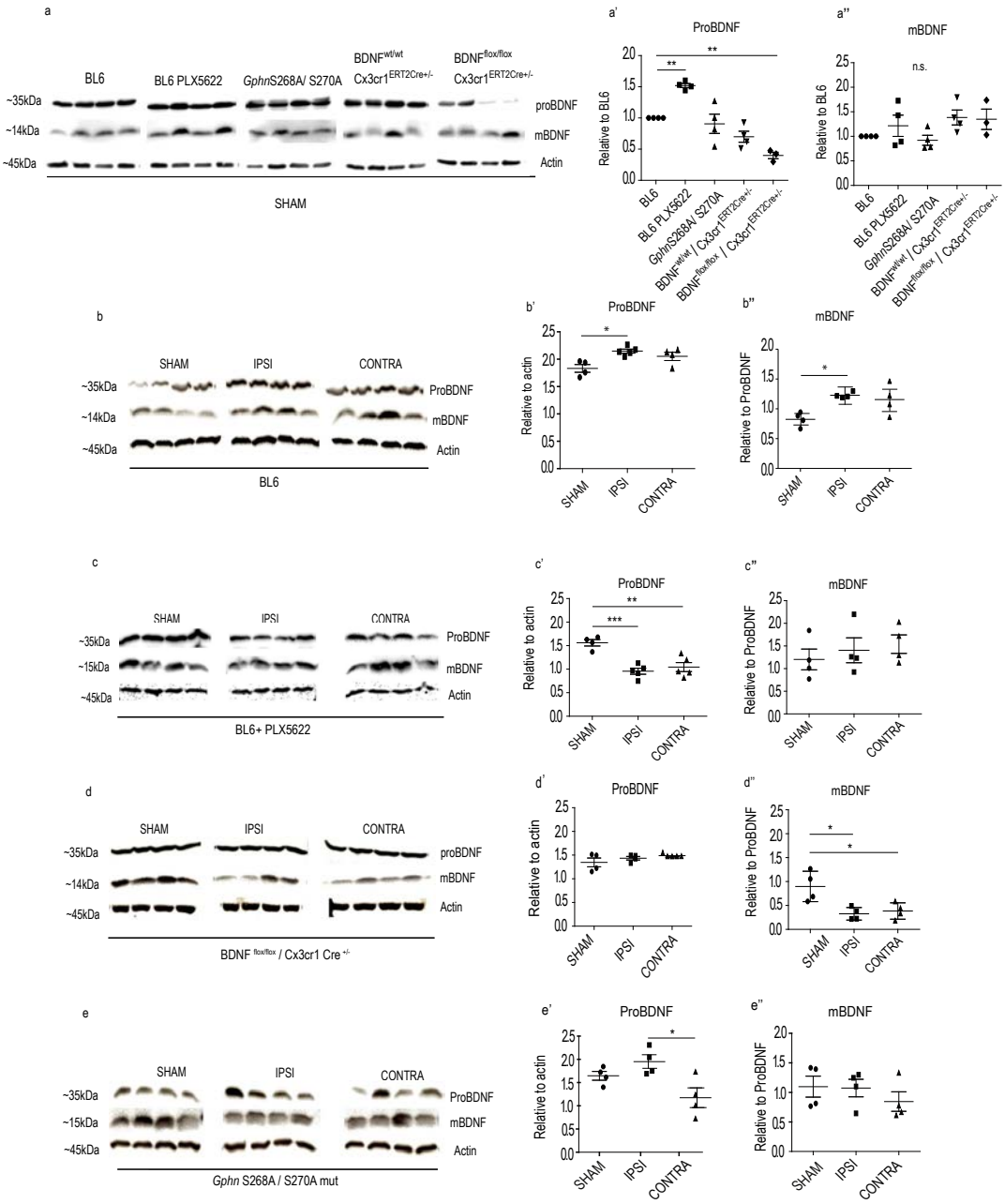


Cramer et al., Supplementary Figure 5

Bdnf deletion from microglia stabilize synapses after MCAO. **(a)** Quantitative RT-PCR for *Bdnf* mRNA changes in cultured microglia from $BDNF^{wt/wt}$ / $CX3CR1^{CreERT2+/-}$ and $BDNF^{flox/flox}$ / $CX3CR1^{CreERT2+/-}$ mice. **(b)** Example images of BDNF protein expression within microglia from $BDNF^{wt/wt}$ / $CX3CR1^{CreERT2+/-}$ and $BDNF^{flox/flox}$ / $CX3CR1^{CreERT2+/-}$ mice. **(b')** Quantification of BDNF intensity within IBAI cells in $BDNF^{wt/wt}$ / $CX3CR1^{CreERT2+/-}$ and $BDNF^{flox/flox}$ / $CX3CR1^{CreERT2+/-}$ mice. **(c)** Example images from $BDNF^{flox/flox}$ / $CX3CR1^{CreERT2+/-}$ sham and MCAO mice showing VGLUT1/PSD95 (column 1), $GABA_AR$ γ2/ GAD65-67 (column 2), $GABA_AR$ α5 (column 3). **(d-e)** Quantification of VGLUT1, PSD95 expression change in MCAO

animals relative to sham. **(f-h)** Quantification of GAD65-67, $GABA_AR$ γ2 and $GABA_AR$ α5 expression changes in MCAO animals compared to sham. **(i)** WB analysis for total gephyrin, phospho-gephyrin S268 and S270 in $BDNF^{flox/flox}$ / $CX3CR1^{CreERT2+/-}$ sham and MCAO mice. **(i'-i''')** Quantification of total gephyrin, phospho gephyrin S268 and S270. Morphology analysis shown as mean ± s.d. (n=4 animals); P=0.55 (Two-way ANOVA, Bonferroni multiple comparison test; F=(2,9)=8.7).

Fig. S6



Cramer et al., Supplementary Figure 6

BDNF expression difference between mouse lines at baseline and after MCAO. **(a-a'')** WB analysis for proBDNF and mBDNF from parietal cortex L2/3 in BL6 WT mice, BL6 WT mice treated with PLX5622, *Gphn*S268A/S270A mutant mice, $BDNF^{wt/wt}$ / $Cx3cr1^{ERT2Cre+/-}$ and $BDNF^{flox/flox}$ / $Cx3cr1^{ERT2Cre+/-}$. **(b)** WB analysis of cortex L2/3 in WT mice for changes in proBDNF and mBDNF levels 24 h following MCAO. **(b'-b'')** Quantification for proBDNF (One-way ANOVA, Bonferroni multiple comparison test; $F(2,10)=7.3$; $P=0.01$) and mBDNF (One-way ANOVA, Bonferroni multiple comparison test; $F(2,9)=6.8$; $P=0.015$) from cortex of ipsi- and corresponding contra-lateral hemispheres in WT mice 24 h post MCAO. **(c)** WB analysis of cortex L2/3 in BL6 WT mice treated with PLX5622 for changes in proBDNF and mBDNF levels 24 h post MCAO. **(c'-c'')** Quantification for proBDNF (One-way ANOVA, Brown-Forsythe test; $F(2,11)=15.8$; $P=0.0006$) and mBDNF from ipsi- and corresponding contra-lateral hemispheres in BL6 WT mice treated with PLX5622. **(d)** WB analysis of cortex L2/3 in $BDNF^{flox/flox}$ / $CX3CR1^{CreERT2+/-}$ for proBDNF and mBDNF levels 24 h following MCAO. **(d'-d'')** Quantification for proBDNF and mBDNF (One-way ANOVA, Brown-Forsythe test; $F(2,9)=8.044$; $P=0.0099$) from ipsi- and corresponding contra-lateral hemispheres in $BDNF^{flox/flox}$ / $CX3CR1^{CreERT2+/-}$ mice. **(e)** WB analysis of cortex L2/3 in *Gphn*S268A/S270A mutant mice for proBDNF (One-way ANOVA, Brown-Forsythe test; $F(2,9)=6.1$; $P=0.02$) and mBDNF levels 24 h following MCAO. **(e'-e'')** Quantification for proBDNF and mBDNF from ipsi- and corresponding contra-lateral hemispheres in *Gphn*S268A/S270A mutant mice.