1 Supplementary Information Figures

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1 Figure S1: Food consumption of 5d and 30d survival cohorts



- 1 Supplemental Fig. S1: (A, B) Food consumption in male and female mice from 5d survival cohort. (C, D) Food
- 2 consumption in male and female mice from 30d survival cohort. (E, F) Total food consumption over 5d
- 3 PLX3397/vehicle treatment in cohort 1 (5d survival) and cohort 2 (30d survival) in male and female mice. (G, H)
- 4 Food consumption pooled from cohort 1 and cohort 2. (I, J) Total food consumption pooled from cohort 1 and
- 5 cohort 2. Values from cages (two animals per cage) and mean ± SEM are shown, each scatter is a cage. Significant
- 6 differences are highlighted, two-way ANOVA followed by Holm Šídák's multiple comparisons test. *p<0.05. Full
- 7 statistics are reported in Suppl. Table S2-3.
- 8

9 Figure S2: Body weight of 5d (cohort 1) and 30d survival (cohort 2)



Supplemental Fig. S2: (A, B) Body weight in male and female mice from cohort 1 (5d survival) and cohort 2 (30d survival). Values from individual animals and mean ± SEM are shown, each scatter is a mouse. Significant differences are highlighted, two-way ANOVA followed by Holm Šídák`s multiple comparisons test. *p<0.05, **p<0.01. Full statistics are reported in Suppl. Table S3-3.</p>

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- 1 Figure S3: Schemes showing brain sections at different Bregma levels collected by serial cryo-
- 2 sectioning.





Fig. S3: Schemes showing brain sections at different Bregma levels collected by serial cryosectioning from +0.64
mm to -2.86 mm. Slight deviations from the depicted Bregma levels are due to image availability. Red rectangles
indicate regions of interests that were processed for qPCR, Western Blot, or RNA-sequencing from TBI and sham
animals (lesions not shown). Images were taken from the Mouse Brain in Stereotaxic Coordinates 3rd Edition
Franklin & Paxinos.

1 Figure S4: Immunohistochemistry of M/M at 5 dpi



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Fig. S4: (A) Anti-Iba1 immunostaining showing that PLX3397 treatment attenuated M/M accumulation at 5 dpi.
(B) Histogram showing that Iba1⁺ M/M were reduced approximately 50% by PLX3397 treatment in both male
and females at 5 dpi (TBI: n=6, per group, equal sex ratios). Significant differences are highlighted, Student's
unpaired two-tailed t test. Values from individual animals and mean ± SEM are shown. *p<0.05, **p<0.01. Scale:
50 μm (A), 1mm (C). Full statistics are reported in Suppl. Table S6.



1 Figure S5: Immunohistochemistry of M/M and astrocytes at 30 dpi

Fig. S5: (A) Anti-CD68/anti-GFAP double-immunostaining showing repopulation of M/M and sustained astrogliosis at 30 dpi. (B, C) Histograms show the percentage area of CD68⁺ microglia/macrophages. The area of CD68⁺ microglia/macrophages was increased in TBI PLX3397 females vs. vehicle females (TBI: n=6, sham: n=4, per group, equal sex ratios, two-way ANOVA followed by Holm Šídák`s multiple comparisons test). Values from individual animals and mean \pm SEM are shown. Asterisks indicate PLX3397 vs. vehicle, **p<0.01. Crosses indicate TBI vs. sham, ####p<0.0001, ns=not significant. Scale: 100 μ m (A). Full statistics are reported in Suppl. Table S7.

1 Figure S6: Heatmap of leading edge differentially expressed genes in TBI mice treated with PLX3397

2 vs. vehicle



GSEA Leading Edge genes

TITLE	SCORE
UB and zona pellucida like domains 1	1.0406524
cyl-CoA wax alcohol acyltransferase 2	0.7762267
UB1 mitotic checkpoint serine/threonine kinase	0.76527816
ANCD2 opposite strand	0.70289135
amily with sequence similarity 83 member C	0.68971896
orkhead box D2	0.68935114
bromosome 11 open reading frame 16	0.0783307
-aminocyclopropane-1-carboxylate synthase homolog (inactive) like	0.6547417
pase H	0.6507007
omplement C1r subcomponent like	0.63184565
locking protein 2	0.6306571
hisa like 2B	0.6275896
TPase H+ transporting V1 subunit C2	0.6264042
IIIa and flagella associated protein 73	0.6166859
ctodysplasio A2 recentor	0.606794
otassium two pore domain channel subfamily K member 7	0.597321
protein-coupled receptor 160	0.58900946
eratin 15	0.5836542
otassium inwardly rectifying channel subfamily J member 5	0.57574457
AIS18 binding protein 1	0.5718744
polipoprotein C3	0.56824815
arboxylesterase 1	0.56725043
RP2 binding protein	0.5647875
ell division cycle 25C	0.56081975
ollectrin, amino acid transport regulator	0.5606576
omplement C5	0.5540898
mall integral membrane protein 5	0.5482738
iliary associated calcium binding coiled-coil 1	0.5476003
esus expressed metallotnionein like protein	0.5472343
inetochore associated 1	0.54701966
vnuclein gamma	0.54506016
adial spoke head 14 homolog	0.5442772
eucine rich repeat containing 71	0.54169005
ilia and flagella associated protein 157	0.537231
rylformamidase	0.5354919
polipoprotein A2	0.53428584
ilia and flagella associated protein 126	0.52842134
amma-butyrobetame nyuroxylase 1	0.5274559
vsozvme e2	0.5261924
rokineticin receptor 2	0.52575994
eft-right determination factor 2	0.52539474
amily with sequence similarity 166 member B	0.5240629
AM47E-STBD1 readthrough	0.52292717
hondrolectin	0.51988906
Incomper protein 438	0.5186722
inc finger and BTB domain containing 32	-0.7754126
ona pellucida glycoprotein 3	-0.6681483
ymogen granule protein 16B	-0.6582309
hromosome 20 open reading frame 202	-0.6389216
hospholipase A2 group IIF	-0.62882733
ructose-bisphosphatase 2	-0.61040837
nyosin binding protein C1	-0.6083/996
artase	-0.597616
em nuclear organelle associated protein 4	-0.58609533
IF interleukin 6 family cytokine	-0.57896525
roplakin 1B	-0.5783265
olute carrier family 23 member 4, pseudogene	-0.5734411
alcium voltage-gated channel auxiliary subunit gamma 1	-0.56803983
polipoprotein F dharian Giaratain, coupled receptor D1	-0.56694543
ASH domain containing 5	-0.56277865
erilipin 4	-0.553751
eucine rich alpha-2-glycoprotein 1	-0.5508636
nterferon alpha inducible protein 27 like 2	-0.5253784
chlafen family member 13	-0.5248041
nterferon regulatory factor 7	-0.5242461
iesmoprakin Inc.45 myosin chanerone B	-0.51935667
uclear RNA export factor 2B	-0.510377/5
eat shock protein family A (Hsp70) member 1B	-0.50898105
rotocadherin beta 2	-0.50350374
mall integral membrane protein 22	-0.5022136
adical S-adenosyl methionine domain containing 2	-0.49085754
ytokine like 1	-0.48846218
actase like	-0.48834452
Oraxes 1, ONCA1 A COMPLEX SUDURI: OU class 2 homeobox associating factor 1	-0.4863127
RB2 related adaptor protein 2	-0.48571894
ryptase alpha/beta 1	-0.48557717
lucagon like peptide 2 receptor	-0.48524523
JDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 4	-0.4842957
HC adaptor protein 3	-0.48294863
H2 domain containing 4B	-0.4761375
denyiate cyclase 1 ransmembrane protein 1320	-0.47550845
D101 molecule	-0.47388000
-5' oligoadenylate synthetase like 2, pseudogene	-0.4707648
IPHS1 adhesion molecule, nephrin	-0.4698216
alectin 3 binding protein	-0.4682616
HD finger protein 11	-0.46717808
protein-coupled receptor 63	-0.46640754
evenin hinding protoin II	A 1000000
nyosin binding protein H iwi like RNA-mediated gene silencing 2	-0.4657669

1	Fig. S6: Heatmap of leading edge differentially expressed genes in TBI mice treated with PLX3397 vs. vehicle.
2	Mice were treated with the CSF1R inhibitor PLX3397 (n = 5 male, 5 female) or vehicle (n = 5 male, 5 female) for
3	five days after TBI. Brain tissue of the ipsilateral upper quadrant was collected at 30 dpi and analysed per RNAseq.
4	Gene set enrichment analysis was done with an input of 16970 valid genes using GSEA 4.1.0 (<u>https://www.gsea-</u>
5	msigdb.org/gsea/index.jsp), and 14980 gene were successfully ranked according to fold regulation and P-value.
6	Leading edge 50 upregulated and 50 downregulated genes are presented in red to blue colour showing high to
7	low relative expression.
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1 Figure S7: Gene enrichment plots of GSEA gene sets in TBI mice treated with PLX3397 vs. vehicle



1	Fig. S7: Gene enrichment plots of GSEA gene sets in TBI mice treated with PLX3397 versus vehicle. Mice were
2	treated as described in Suppl. Fig. S2 (above), and gene set enrichment analysis was run with GSEA 4.1.0 for the
3	gene sets KEGG, GOcc, GOmf and GObp (GO, gene ontology; cc, cellular component; mf, molecular function; bp,
4	biological process). Enrichment plots show the enrichment score of a given gene in the set. The green line
5	represents the running ES as the analysis goes down the ranked list. The value at the peak is the final ES. Genes
6	enriched in vehicle treated mice are depicted as positive ES (red), genes enriched in PLX3397 treated mice are
7	depicted as negative ES in blue.
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1 Figure S8: Exemplary heatmaps of gene sets enriched in TBI mice treated with PLX3397





Fig. S8: Exemplary heatmaps of genes enriched in TBI mice treated with PLX3397. Mice were treated as described
in Suppl. Fig.2 (above). Gene sets of synapse, postsynaptic membrane, synaptic vesicle exocytosis, axonal
membrane, spine development and similar synaptic terms were enriched in PLX3397-treated TBI mice relative
to vehicle-treated TBI mice. Two exemplary heat maps are presented. The colour code ranges from high
enrichment score (ES) in dark red to low ES in dark blue.

1 Figure S9: Exemplary heatmaps of genes enriched in TBI mice treated with vehicle

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Fig. S9: Exemplary heatmaps of gene sets enriched in TBI mice treated with vehicle. Mice were treated as described in Suppl. Fig. S2 (above). Gene sets of ribosome, ribosomal subunit, cytoplasmic translation were enriched in vehicle treated mice and terms of oxidoreductase activity, oxidative phosphorylation or electron transfer were enriched in vehicle treated mice. Two exemplary heat maps are presented. The colour code ranges from high enrichment score (ES) in dark red to low ES in dark blue.

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1 Figure S10: Scatter plot and heatmap of top regulated genes according to p-value/q-value



Hierarchical clustering, Euclidean distance metrics, ttest significant genes

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- 1 Fig.S10: Scatter plot and heatmap of top regulated genes according to p-value/q-value. (A) Scatter plot of top 50
- 2 regulated genes according to FDR adjusted p-value (q-value) sorted for abundance. The data are log2
- 3 transformed normalized reads. (B) Heatmap showing the relative expression vs. the mean of PLX3397-treated
- 4 mice (left column) of Student's t test significant regulated genes in PLX3397-treated TBI mice vs. vehicle-treated
- TBI mice. Genes and mice were clustered according to Euclidean distance metrics. The colour scale ranges from
 minus 3.5-fold SD to plus 3.5-fold SD. The upper dendrogram shows two clusters reflecting the treatment groups.







tissue of the ipsilateral upper quadrant was collected 30 days after CCI and subjected to RNAseq analysis. (A): Volcano plots of differential gene expression in PLX-treated female versus PLX-treated male mice The X-axis shows the log2 difference (i.e. fold difference). Genes higher in PLX-treated female mice are positive, genes lower in females are negative. The Y-axis shows the negative logarithm of the P-value. Top 150 candidate according to P-value are highlighted in red. (B): In analogy to A, the Volcano plots shows gene expression in vehicle treated female versus vehicle-treated male mice. The same genes were marked in red as in A, but are mostly hidden because they were not regulated. Few regulated red genes are X-chromosome genes. Y-chromosome genes were excluded. (C): Exemplary scatter plots of candidate genes which were upregulated in PLX3397 treated female mice over PLX-treated male mice. The genes were filtered according to fold difference (Log2 difference ≥1.3 for PLX-female > PLX male) and are sorted left to right according to abundance (normalized reads). (D): Genes as in C comparing vehicle treated female versus vehicle treated male mice.

1 Figure S12: Exemplary heatmap of genes enriched in PLX3397-treated female vs. male TBI mice

M-PLX_S1 M-PLX_S11 M-PLX_S12 W_PLX_S12	M-PLA 317 M-PLA 518 F-PLA 523 F-PLA 528 F-PLA 528 F-PLA 529	SampleNa:	me	
		Ddy 3m	DDV2V	DEAD-boy balicase 2 V-linked [Source:HCMC Symbol: Acc:HCMC: 2500]
		Eif2s3y	EIF2S3	eukaryotic translation initiation factor 2 suburit gamma [Source:HGNC Symbol:Acc:HGNC:3267]
		Kdm5d	KDM5D	lysine demethylase 5D [Source:HGNC Symbol:Acc:HGNC:11115]
		Ut.y	UTTY	"ubiquitously transcribed tetratricopeptide repeat containing, Y-linked [Source:HGNC.Symbol:Acc:HGNC:12638]"
		Plekha6	PLEKHGE	Tencine File repeat containing 32 [Source:now. Sympositic Containing Containi
		Prss46	PRSS46P	"serine protease 46, pseudogene [Source:HGNC Symbol:Acc:HGNC:37325]"
		Ttc30a2	TTC30A	tetratricopertide repeat domain 30A [Source:HGNC.Symbol:Acc:HGNC:25853]
		Fredos	KIKKI	RAILIRFEID RI [Source:HGMC Symbol:Acc:HGMC:E371]
		Upk3a	ШРКЗА	uroplakin 3A [Source:HGNC Symbol;Acc:HGNC:12580]
		114	<u>11.4</u>	interlepkin 4 [Source:HGMC_Symbol:Acc:HGMC:6014]
		Upk2 Hmcn2	UPK2 HMCN2	uroplakin 2. [Source:HGNC Symbol:Acc:HGNC:12579]
		Pfn3	PFN3	profilin 3 [Source:HONC Symbol:Acc:HONC:18627]
		Hpx	HPX	hemopexin [Source:HGNC_Symbol;Acc:HGNC:5171]
		Histlh2hr	PLECHG4	pieckstrin homology and kholekk domain containing 64 [Source:HGNU: Symbol;Acc:HGNU::25011]
		Gabrr2	GABER2	gamma-aminobutyric acid type & receptor subunit rbo2 [Source:HGNC Symbol;Acc:HGNC:4091]
		Ifitm5	IFITMS	interferon induced transmembrane protein 5 [Source:HGMC_Symbol;Acc:HGMC_16644]
		Histlb2ad	H21C4	<u>netainehomocysteine S-methylitansterase / Source-How Symbol:Acc:HuwL:H4Hj</u>
		BC016548		
		Fpr1	FPR1 CDDTA	formyl peptide receptor 1 [Source:HGNC.Symbol:Acc:HGNC.3266]
		Scon	SCGN	Laria unprimate region transcript 4 Lanuterinum, aymoni (ACCHUML: 14383) "secretagodin, EP-hand calcium binding protein [Source:HOMC Symbol: Arc:HOMC (16941)"
		Cdr1	CDR1	cerebellar degeneration related protein 1 [Source:HGNC Symbol:Acc:HGNC:1798]
		AT463170	MID 1 1 2	mulas reactor subfacily is a new I women 2 [Source (USV Contelline 1000, 2000]
		Fgf2os	Juri La	nortea terefora ann am tá t-àraid t memort a fann ceinnar tàmaoitaceinnar;taval
		Lpin3	LPIN3	lipin 3 [Source:HGNC Symbol:Acc:HGNC:14451]
		Zfp473	ZNF473	zinc_finger_protein_473_[Source:HGNC_Symbol;Acc:HGNC:23239]
		Hsf2bp	HSF2BP	protamine _source:mum. symmolizaccinum:5949] heat.shock_transcription_factor_2_binding_protein_[Source:HGNC_Symbol:Acc:HGNC:5226]
		Hspalb	HSPAIB	heat shock protein family A (Hsp70) member 1B [Source:HGNC.Symbol;Acc:HGNC:5233]
		Chil5	CHIA	chitinase acidic [Source:HGNC_Symbol;Acc:HGNC:17432]
		Tmem30c	TMEM30CP	"transmembrane protein 30C, pseudogene [Source:HGNC Symbol:Acc:HGNC:30443]"
		Ciart	CIART	circadian associated repressor of transcription [Source:HGNC Symbol:Acc:HGNC:25200]
		Syt.14	SYTL4	synaptotagmin like 4 [Source:HGNC Symbol:Acc:HGNC:15588]
		Pou4f1	POU4F1	Auan meraliopepiloase comarto si fource: HGNC symbol: Accelor(: HGNC: HG
		Hif3a	HIF3A	bypoxia inducible factor 3 subunit alpha [Source:HGNC Symbol:Acc:HGNC:15825]
		Zfp54	1CPD7	and Col binding densin containing 7 [Senses HONC Symbols Loss HONCs 17715]
		Ccdc38	CCDC38	acyletox binning domain containing is [Source:HewC. Symbol:Acc:HewC:17713]
		Lta	LTA	lymphotoxin_alpha_[Source:HGWC_Symbol;Acc:HGWC:6709]
		Plin4	PLIN4	perilipin 4 [Source:HGNC Symbol;Acc:HGNC:29393]
		Pla2g4c	PLA2G4C	phospholipase A2 group IVC [Source:HGNC Symbol;Acc:HGNC:9037]
		Xist	XIST	X inactive specific transcript [Source:HGWC_Symbol;Acc:HGWC:12810]
		I.gals3	LGALS3	matrix metallopeptidase 12 [Source:Huku, Symbol;acc:Huku:758]
		Gdf3	GDF3	growth differentiation factor 3 [Source:HGNC Symbol:Acc:HGNC:4218]
		Stah2	STAB2	stabilin 2 [Source: HGNC Symbol: Acc: HGNC: 18629]
		Col3al	COL3A1	Clander L2 domains, nuclear [Source:HewL Sympol;Acc:HewL:HAS4]
		Acp5	ACP5	"acid phosphatase 5, tartrate resistant [Source:HGNC Symbol:Acc:HGNC:124]"
		Cd36	CD36	CD36 molecule [Source:HGMC Symbol;Acc:HGMC:1663]
		Fabp4	FABP4	fatty acid binding protein 4 [Source:HGNC Symbol:Acc:HGNC:3559]
		Ccdc192	CCDC192	coiled-coil domain containing 192 [Source:HGNC Symbol:Acc:HGNC:49566]
		Kcnj15	RCNJ15	HUBICAN LADUTCEINGNE SYMBOLIACCINENCIATZAL DOLASSIUM INVARIALU RECITEVING CHARDEL SUBFAMILY J member 15 [Source:HCNC Symbol:Mcc.HCNC:6251]
		Prlr	PRLR	prolactin receptor [Source:HGNC Symbol:Acc:HGNC:9446]
		Mfap4	MFAP4	microfibril associated protein 4 [Source:HGWC Symbol:Acc:HGWC:7035]
		Kng2	KNG1	giulatione perusidase a lautreinum, agnotizaceinum, 4555] kininoren l [Source:HGNC: Symbol:Acce:HGNC:45383]
		Sdc1	SDC1	syndecán l [Šource:HGNC Symbol:Ácc:HGNC:10658]
		Scara5	SCARA5	scavenger receptor class A member 5 [Source:HGNC Symbol;Acc:HGNC:228701]
		Collal	COLIAI	collagen type I alpha i chain [Source:HGWL Symbol:Acc:HGWL:2191]
		Sfrp4	SFRP4	secreted frizzled related protein 4 [Source:ĤGWC Symbol:Acc:HGWC:10778]
		Ltbp2	LTBP2	latent transforming growth factor beta hinding protein 2 [Source:HGNC Symbol; Acc:HGNC:6715]
		Lox11	LOXI.1	LD2W molecule [Source:HEWL Symbol;Acc:HEWL:LD33]
		Kcnn4	KCNN4	potassium calcium-activated channel subfamily N member 4 [Source:HGNC Symbol;Acc:HGNC:6293]
		Colla2	COL1A2	collagen type I alpha 2 chain [Source:HGNC Symbol;Acc:HGNC:2198]
		Lyz1	LYZ	lysozyme [Source:HGNC_Symbol:Acc:HGNC:6740]
		Wfdc17	ANOS1	añosmín l [Source:HGNC Śymbol;Acc:HGNC:6211]
		Ubaly	CVCD2	C.V.C. matif. shamphing resentant 2. [Source: HCNC. Sumhall. Los. HCNC. 47.403
		Sec1413	SEC14L3	SECIA like lipid binding (Source:HOMC Symbol:Acc:HOMC:HOMC)
		Atp6v0d2	ATP6VOD2	ATPase H+ transporting VO subunit d2 [Source:HGNC Symbol:Acc:HGNC:18266]
		Pira2	LILER5 THEN71	leukocyte immunoglobulin like receptor B5 [Source:HGNC.Symbol;Acc:HGNC:6609]
		Cpz	CPZ	carboxymentate principal (1 paurice:http://www.ice.indoi.org/10/1/2014)
		Ct.sk	CTSK	cathepsin K [Source:HGNC Symbol:Acc:HGNC:2536]
		Rnf186	CERVI.	ring ringer protein 186 [Source:HGNC:Symbol:Acc:HGNC:S278]
		Angpt.14	ANGPTL4	and on it in like 4 [Source:HGMC.Symbol:Acc:HGMC:L6039]
		Clec12a	CLEC12A	C-type lectin domain family 12 member A [Source:HGNC Symbol;Acc:HGNC:31713]
		Clecila	SEDINCA	C-type lectin domain containing 11A [Source:HGNC.Symbol;Acc:HGNC:10576]
		Lox	LOX	Just avidase [Source:HOR. Symbol:Acc:HOR. 6664]
		Shisa3	SHISA3	shisa family member 3 [Source:HGNC Symbol:Acc:HGNC:25159]
		Fbinl Strafi	FRINI	TIDUIII [SOURCE:HGNC: Symbol;Acc:HGNC:3600]
		Sla2	SLA2	Src like adaptor 2 [Source:HGNC Symbol:Acc:HGNC:17329]

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3 Fig.S12: Exemplary heatmaps of Top 50 genes enriched in PLX3397-treated female vs. male TBI mice. Mice were

4 treated as described in Suppl. Fig.2 (above). The colour code ranges from high enrichment score (ES) in dark red

5 to low ES in dark blue.