Proteomic Analysis and Biochemical Correlates of Mitochondrial Dysfunction following Low-Intensity Primary Blast Exposure

Supplemental Information

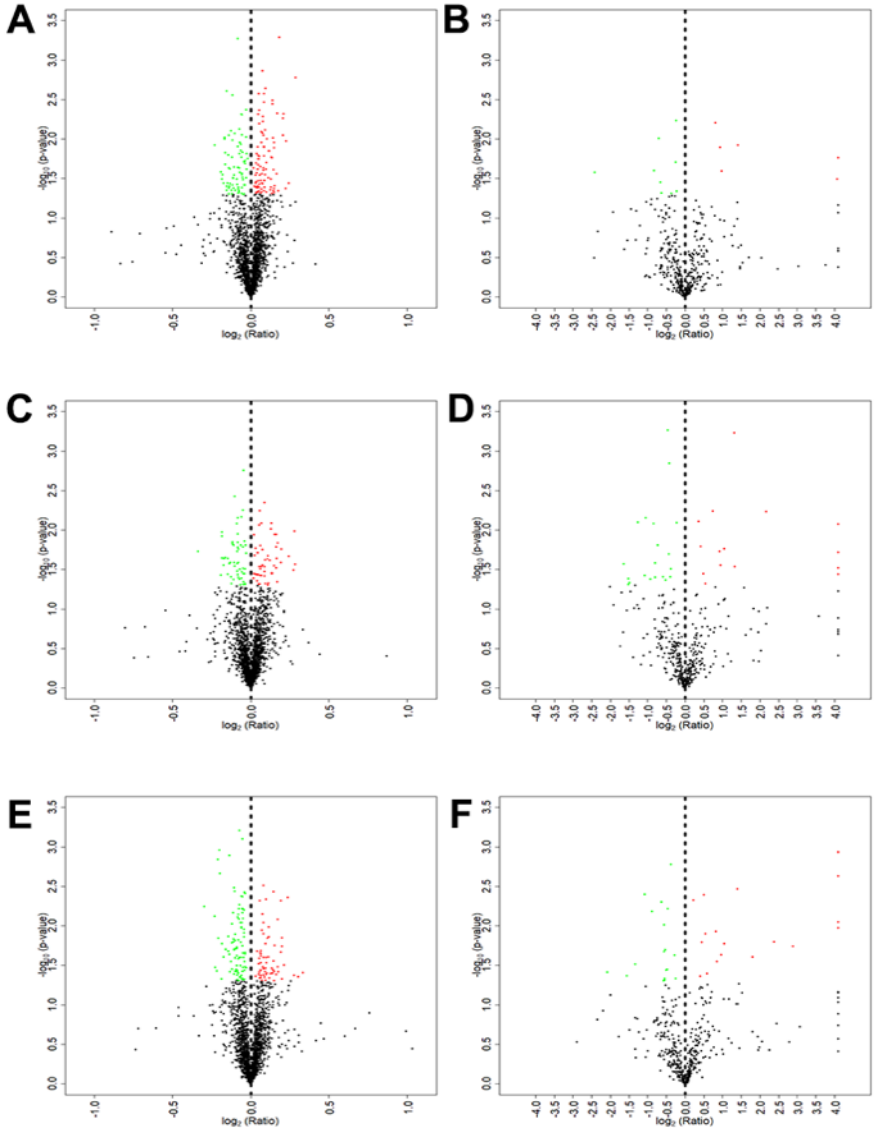
Supplemental Method

Fluorescence Immunohistochemistry and Quantitative Analyses

The fluorescent staining and imaging process was followed as previously described[1]. Mouse brains were dissected and cut into serial coronal sections of 40-mm thickness by a vibratome (VT1200S, Leica Microsystems, Inc., Bannockbum, IL) after transcardiac perfusion fixation with 4% paraformaldehyde. Fixed coronal sections were permeabilized with 1% Triton X-100 in phosphate-buffered saline (PBS) incubated with protein markers (MnSOD (ab13534 Abcam, 1:200); Drp1 (ab184247 Abcam, 1:500); Mitofusin 2 (cst9482 Cell Signaling Technology, 1:100)) overnight. Then, the sections were incubated with fluorophore-conjugated secondary antibodies (1:200, goat anti-rabbit IgG-Alexa488, A21441; Invitrogen, San Diego, CA), and then counter-stained with nuclear DNA dye Hoechst 33342 (1:1500). Fluorescence photomicrographs of the areas of interest were captured by a Leica DMI 6000B automated epifluorescence microscope (Leica Microsystems Inc., Buffalo Grove, IL). Stereological sections from bregma -1.70 to -1.94 mm, were used for quantification of MnSOD, Drp1, and Mitofusin2 expression levels in cerebral cortex regions. The region of interest was analyzed in a double blind manner using ImageJ software. Sections from all mice in different groups were stained in a single batch. The mean percentage of the area for each region from each mouse was compiled for statistical analysis.

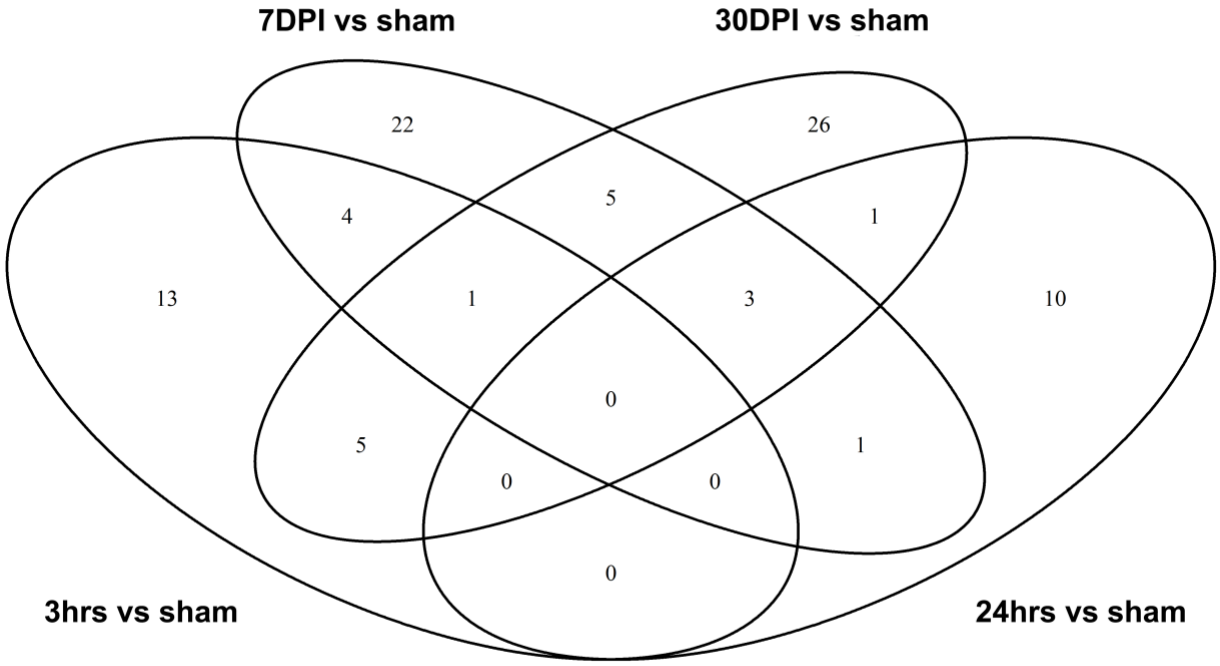
Supplemental Figures and Figure Legends

Supplemental Figure S1. Low-intensity blast induced alterations of protein expressions at different time points.



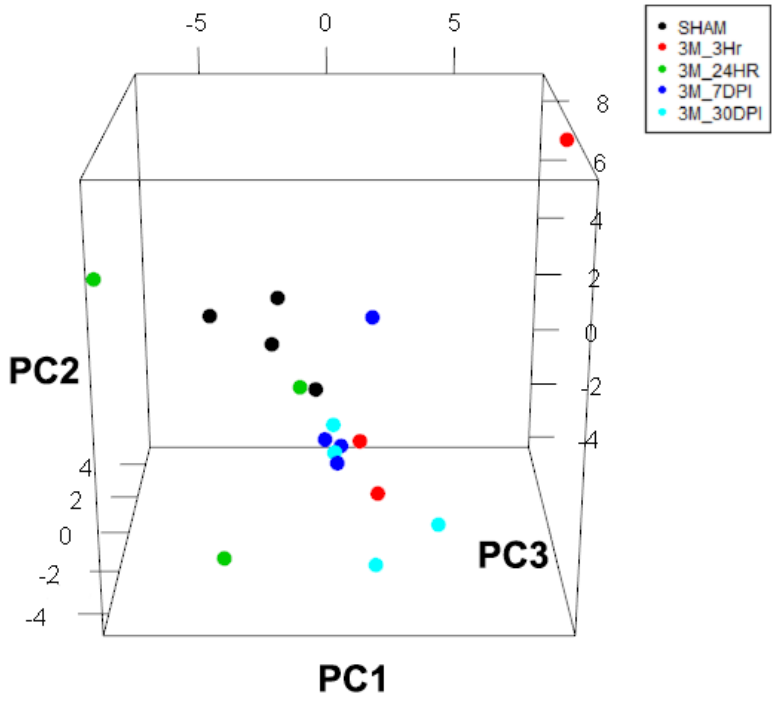
(A-B) Statistically differential expression of 182 global-proteins (A) and 15 phospho-proteins (B) at 24 hrs time point. (C-D) Differentially expression of 108 global-proteins (C) and 36 phospho-proteins (D) at 7 DPI time point. (E-F) Differentially expression of 173 global-proteins (E) and 41 phospho-proteins (F) at 30 DPI time point. Volcano plots were generated by R statistical software. Dashed lines indicate the thresholds of $\log_2$ (ratio/fold change) and $-\log_{10}$ (P). The identified proteins are displayed as dots in red (up-regulation with significant changes), green (down-regulation with significant changes), or black color (no significant change).

Supplemental Figure S2. Presentation of differentially expressed phospho-proteins by Venn diagram.



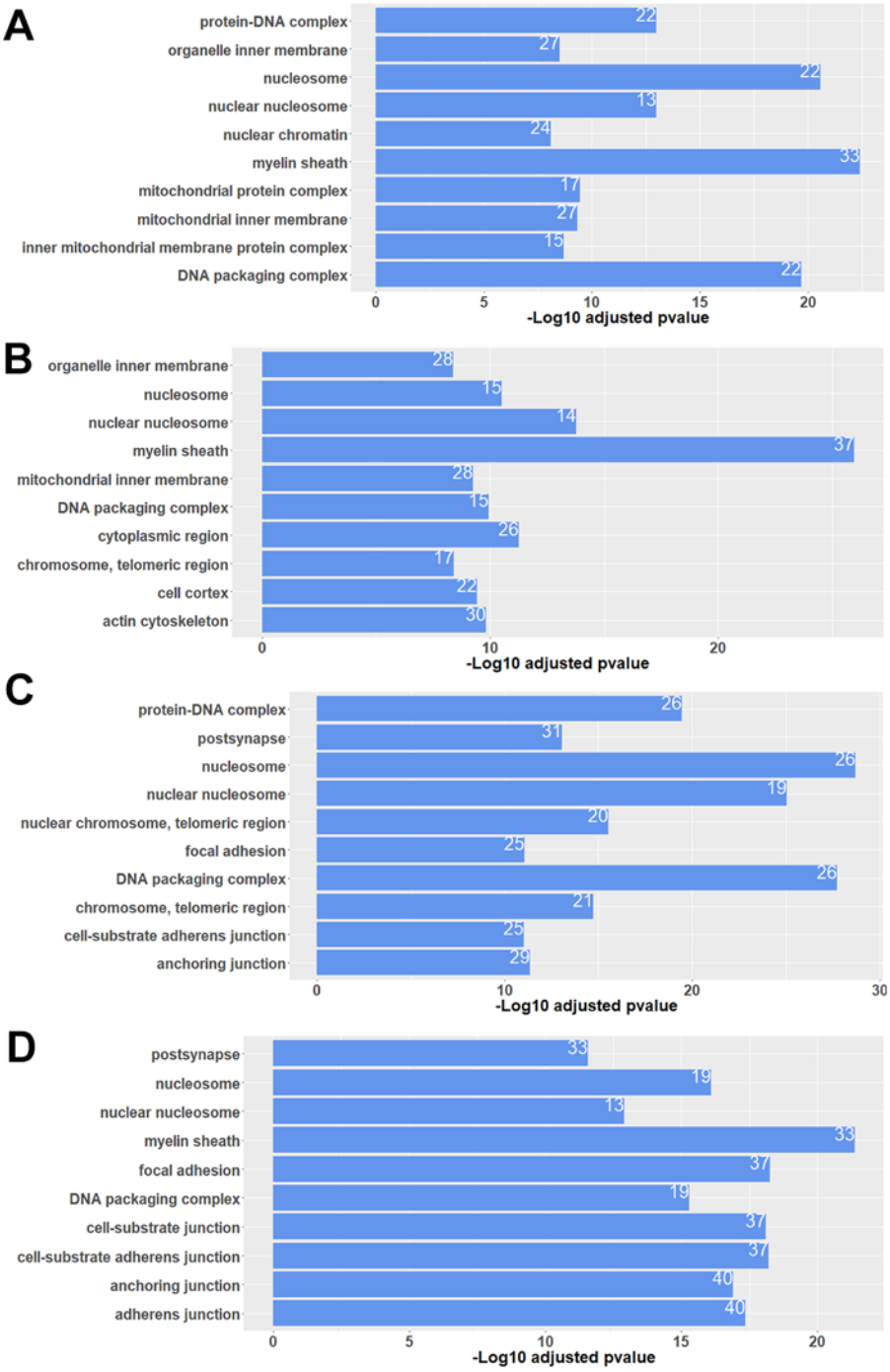
Venn diagram showing the common and distinct differentially expressed phospho-proteins among groups. The overlap portions indicate the common proteins in same region.

Supplemental Figure S3. Strong clustering for identified phospho-proteins using principle component analysis.



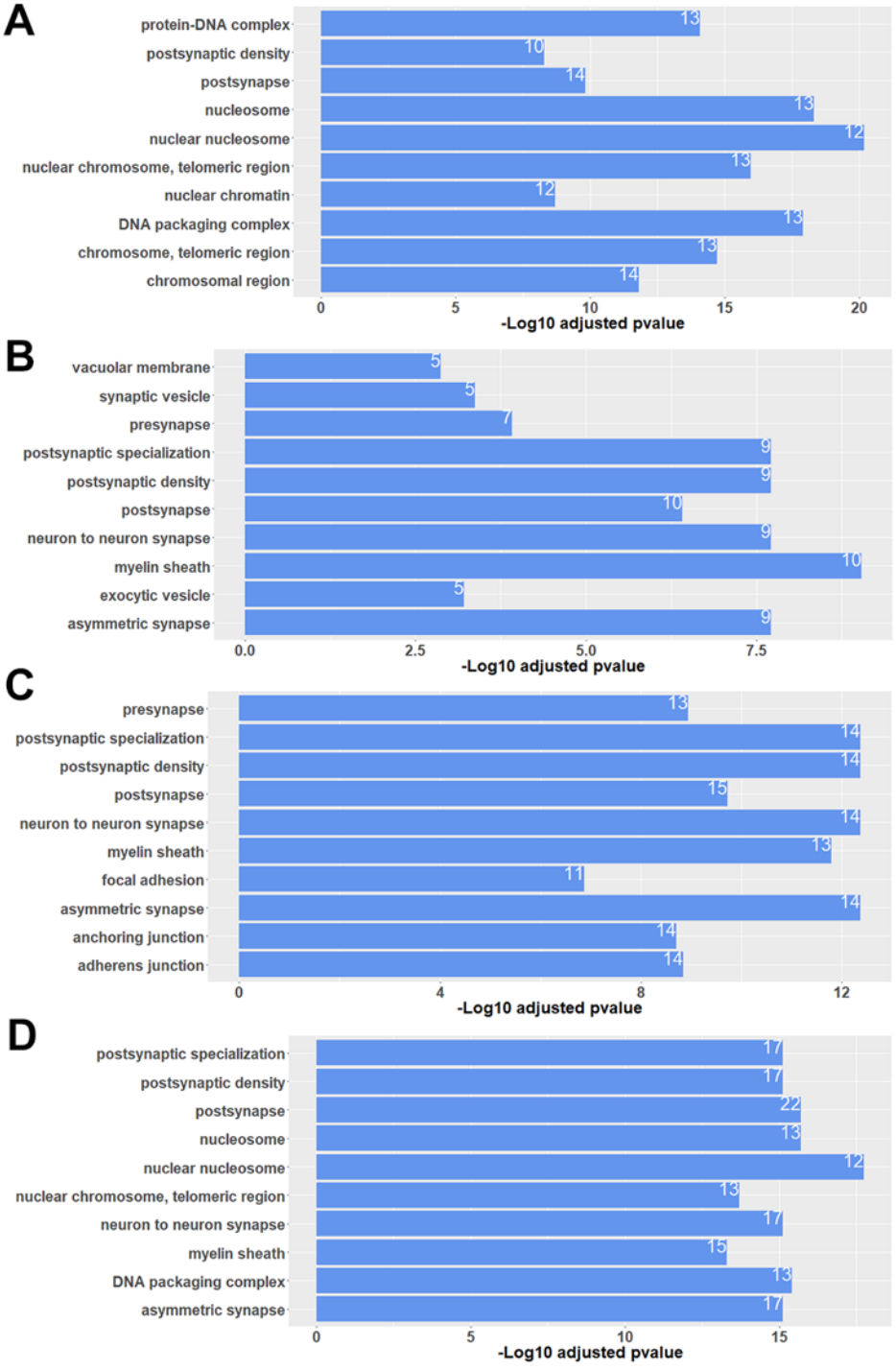
Principle component analysis showed that those differentially expressed phospho-proteins effectively separated blast at 3 hrs (red), 24 hrs (green), 7 DPI (dark blue), and 30 DPI (light blue) from sham control (black).

Supplemental Figure S4. Top 10 GO cellular components for global-proteome after blast.



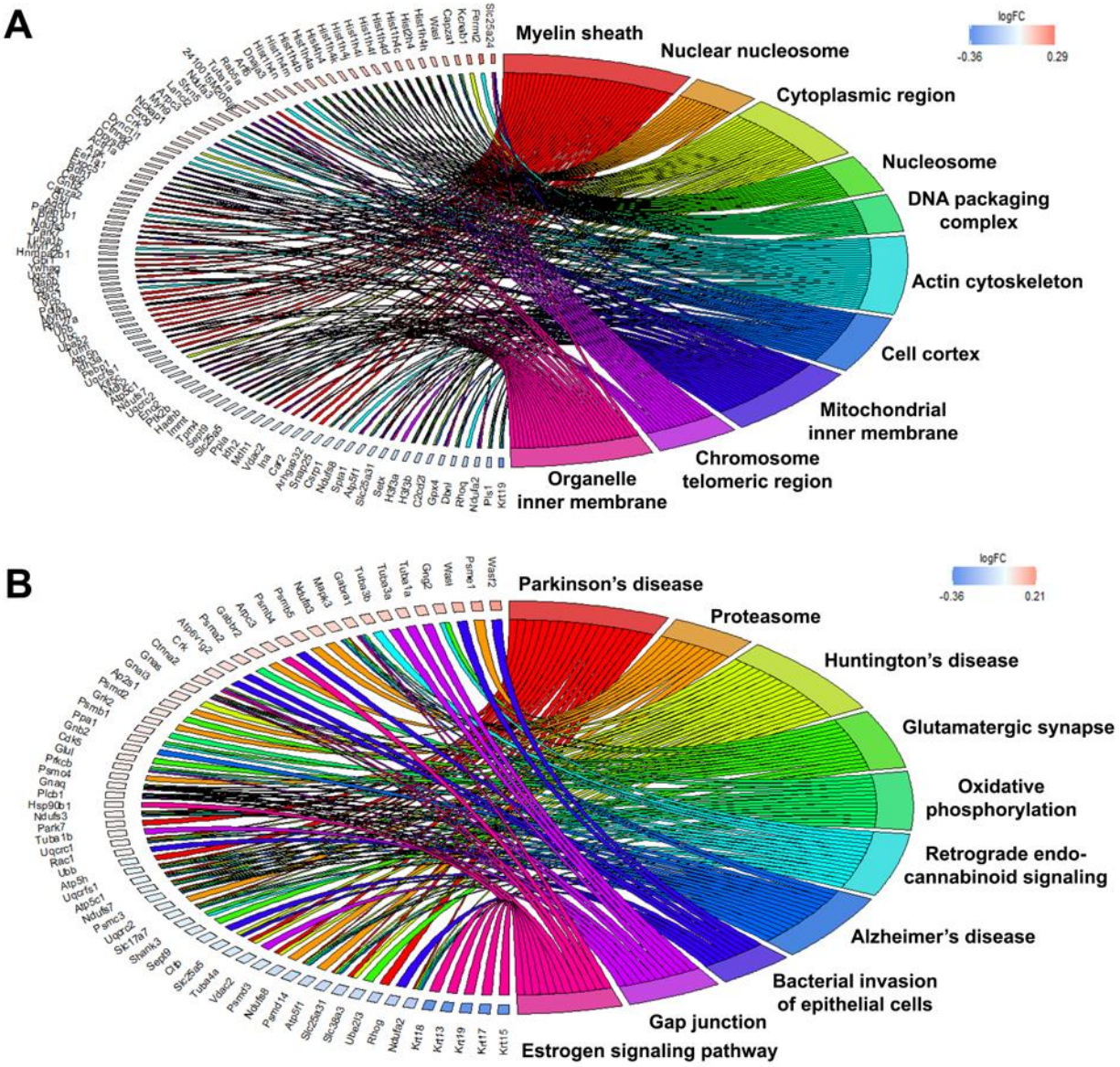
GO ontology analysis of cellular components of global-proteome at 3 hrs (A), 24 hrs (B), 7 DPI (C), and 30 DPI (D) time point after blast. Note: the number in the bar graph indicates the number of differentially expressed proteins enriched in its corresponding specific cellular component.

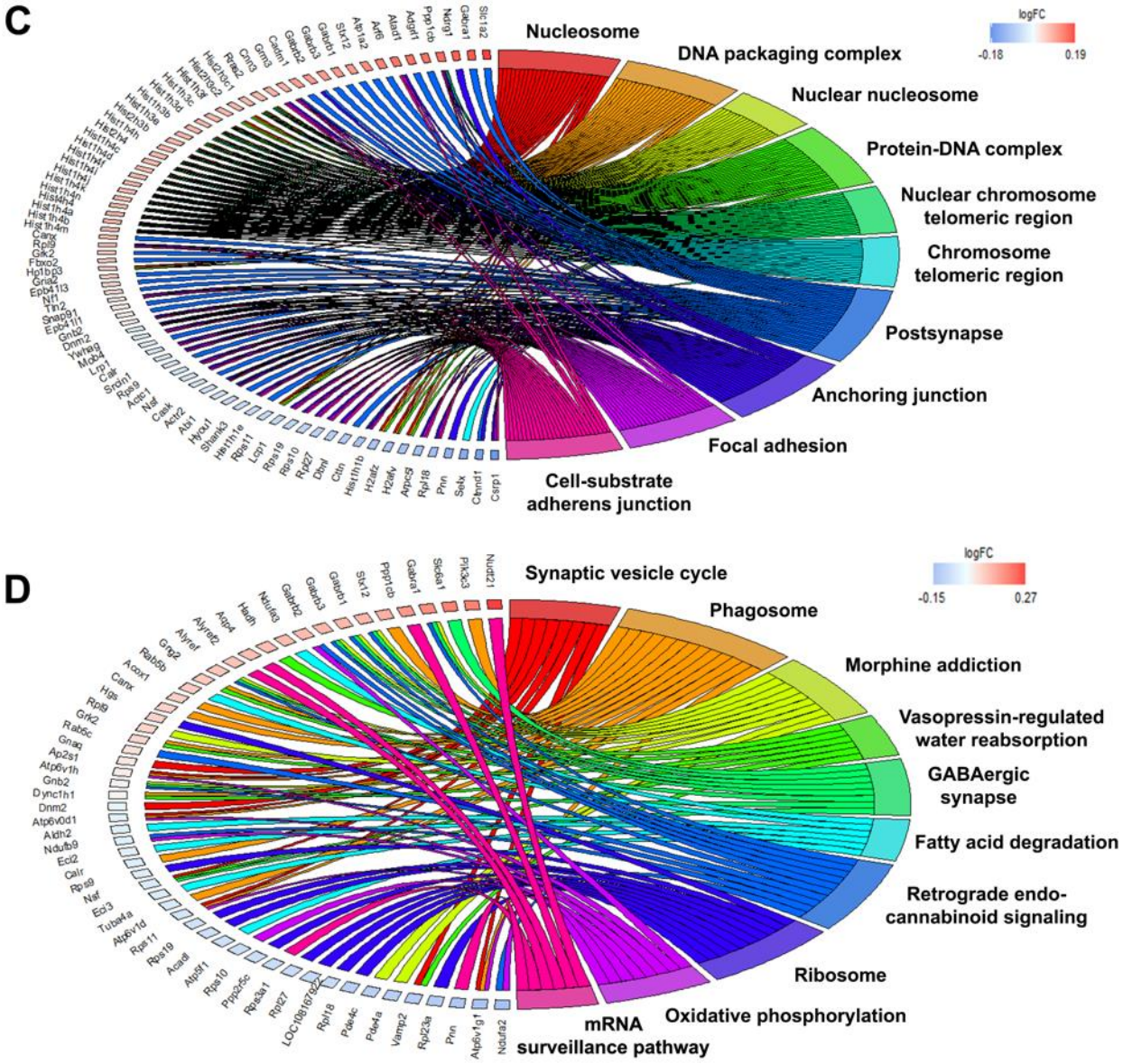
Supplemental Figure S5. Top 10 GO cellular components for phospho-proteome after blast.

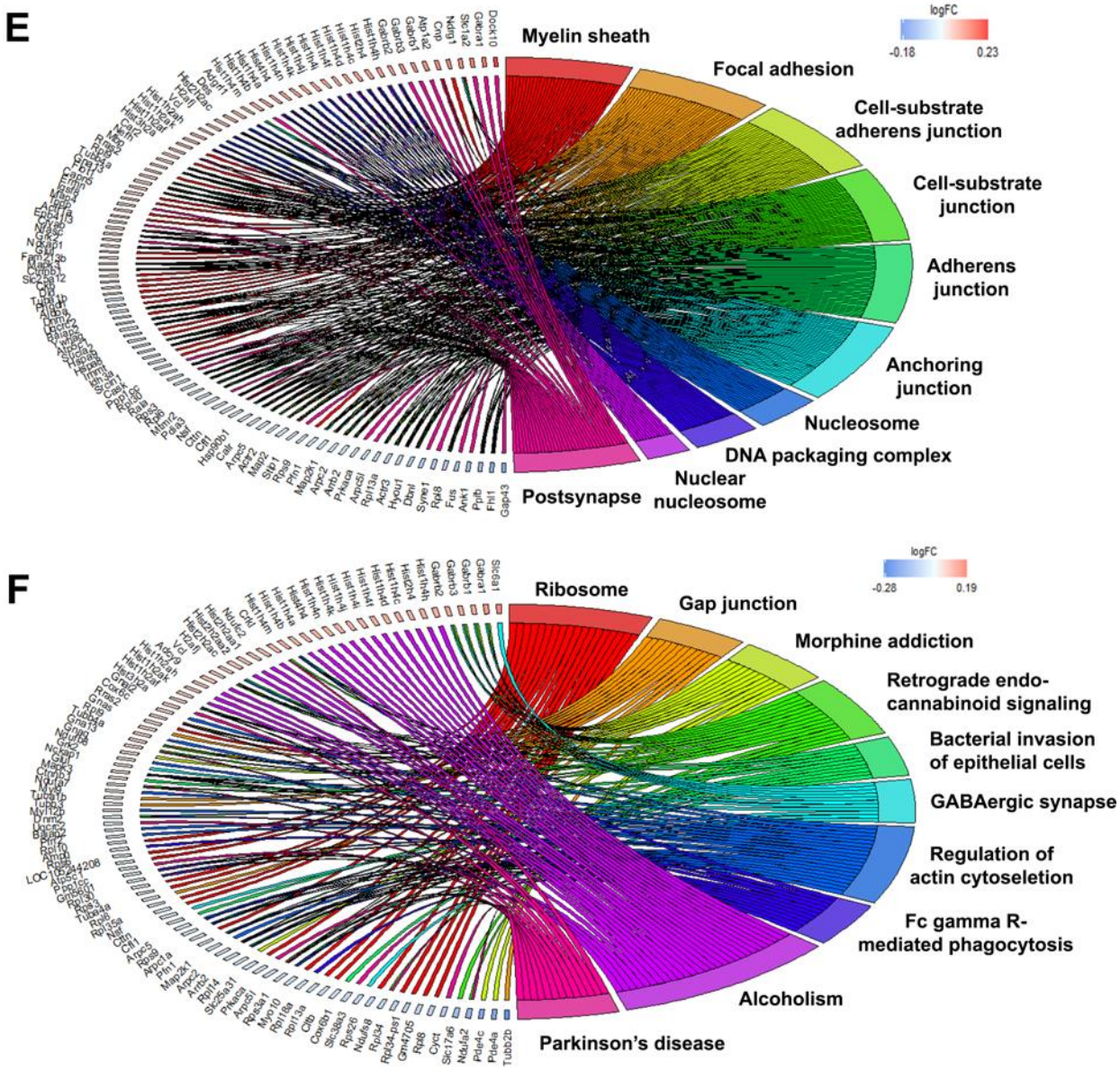


GO ontology analysis of cellular components of phospho-proteome at 3 hrs (A), 24 hrs (B), 7 DPI (C), and 30 DPI (D) time point after blast. Note: the number in the bar graph indicates the number of differentially expressed proteins enriched in its corresponding specific cellular component.

Supplemental Figure S6. GO cellular components and signaling pathways and enriched in global proteome at 24 hrs, 7 DPI, and 30 DPI after blast injury.

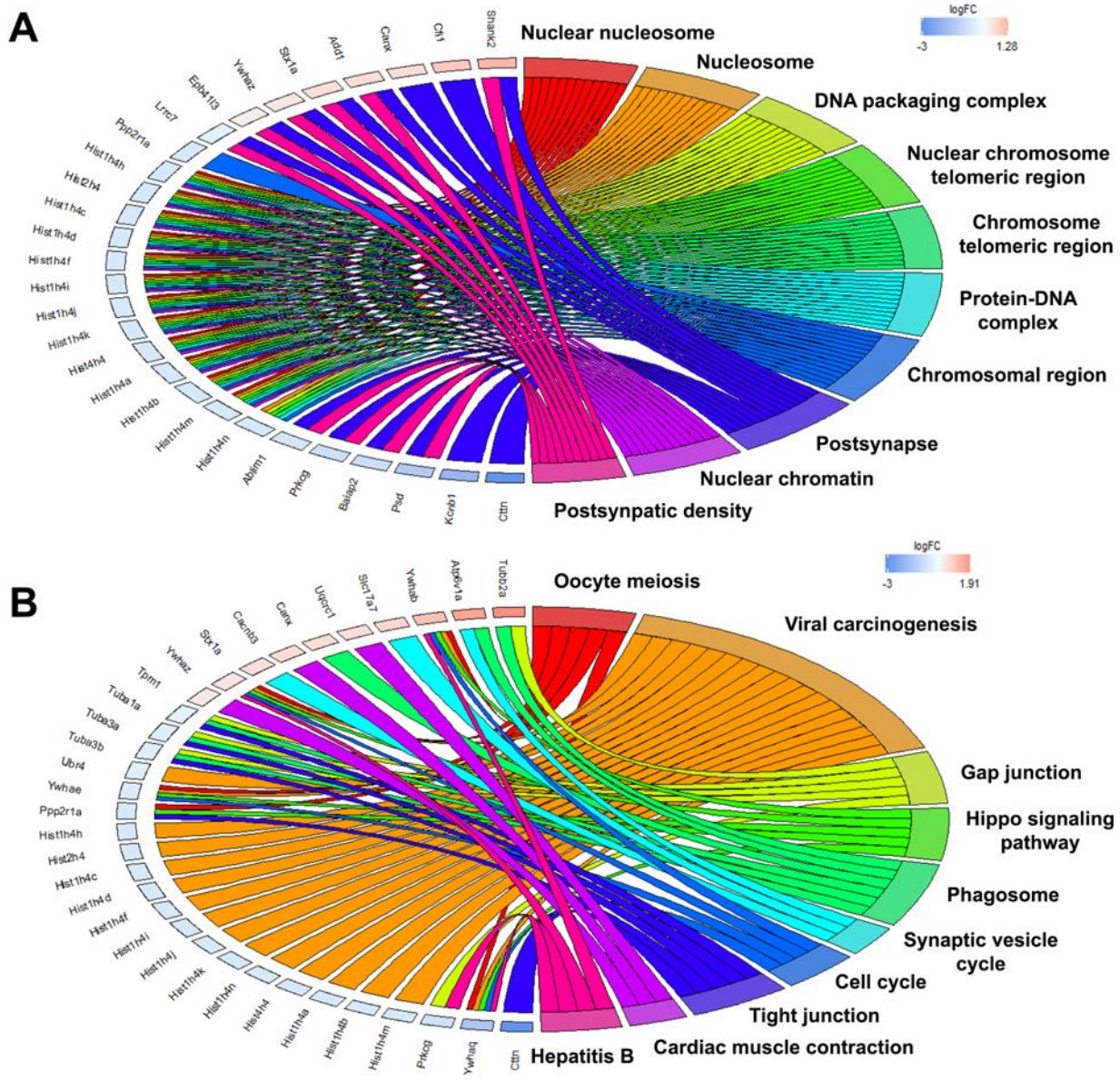


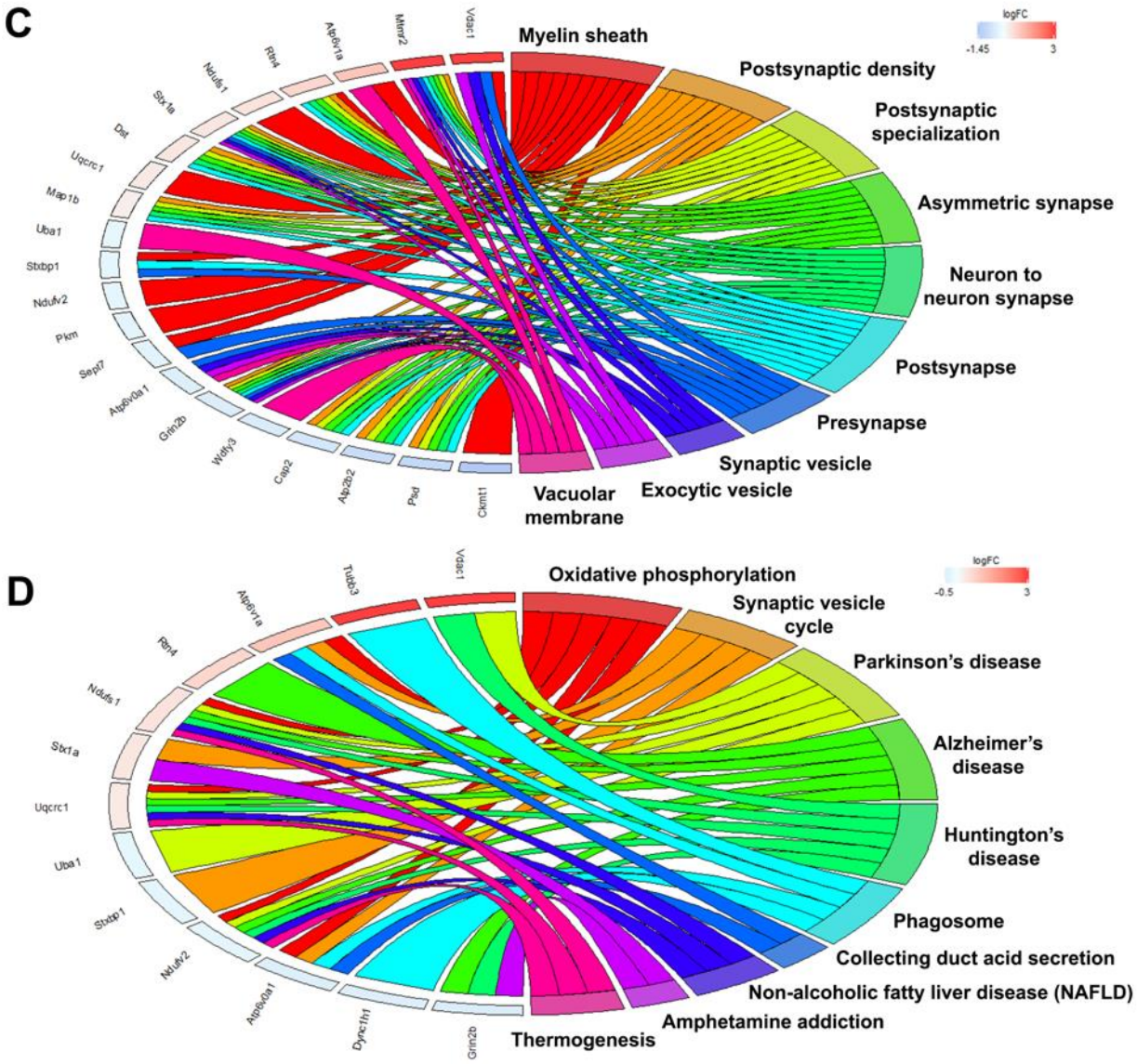

C
logFC
-0.18
0.19
Nucleosome
DNA packaging complex
Nuclear nucleosome
Protein-DNA complex
Nuclear chromosome telomeric region
Chromosome telomeric region
Postsynapse
Anchoring junction
Focal adhesion
Cell-substrate adherens junction
D
logFC
-0.15
0.27
Synaptic vesicle cycle
Phagosome
Morphine addiction
Vasopressin-regulated water reabsorption
GABAergic synapse
Fatty acid degradation
Retrograde endo-cannabinoid signaling
Ribosome
Oxidative phosphorylation
mRNA surveillance pathway

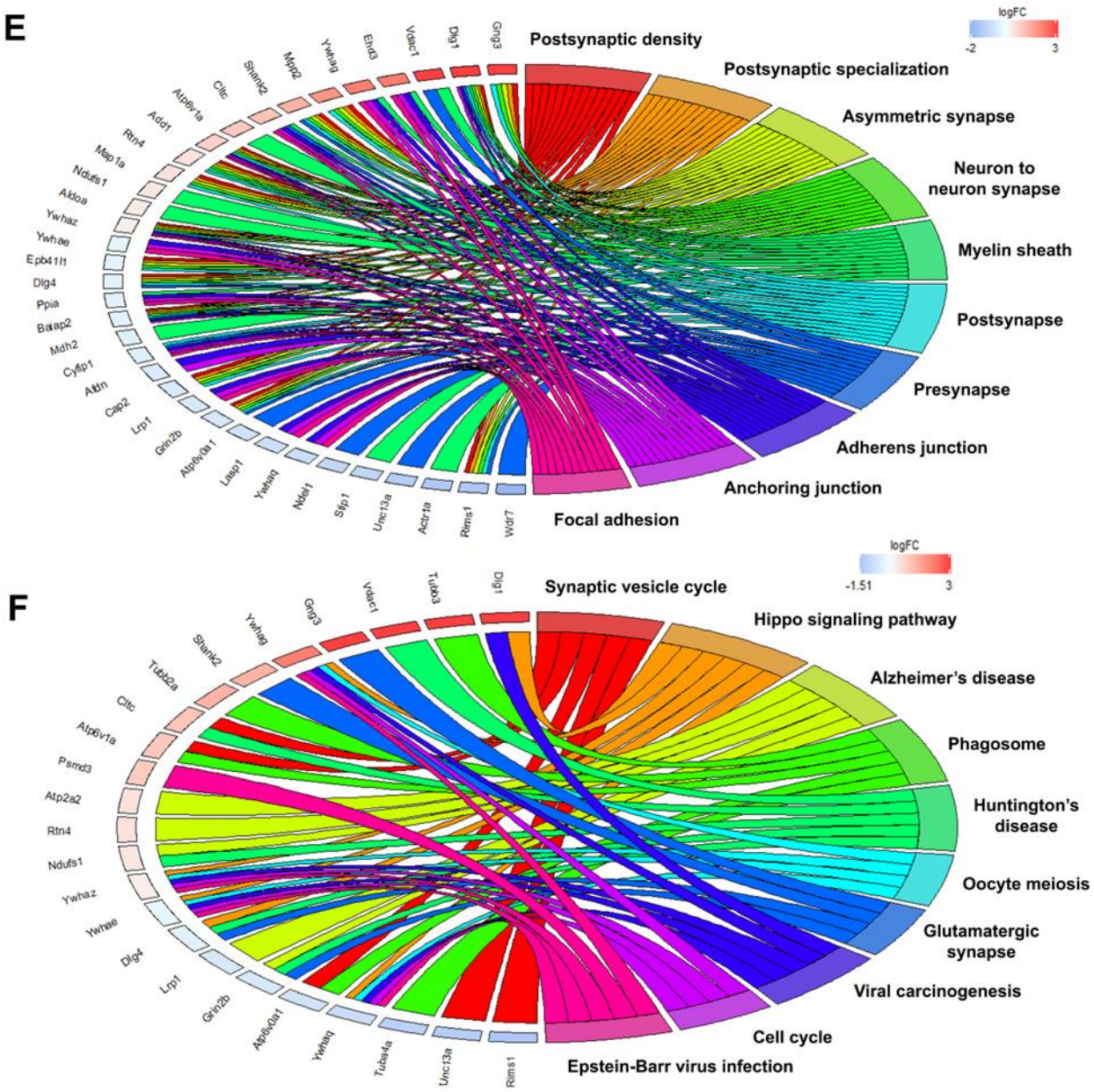


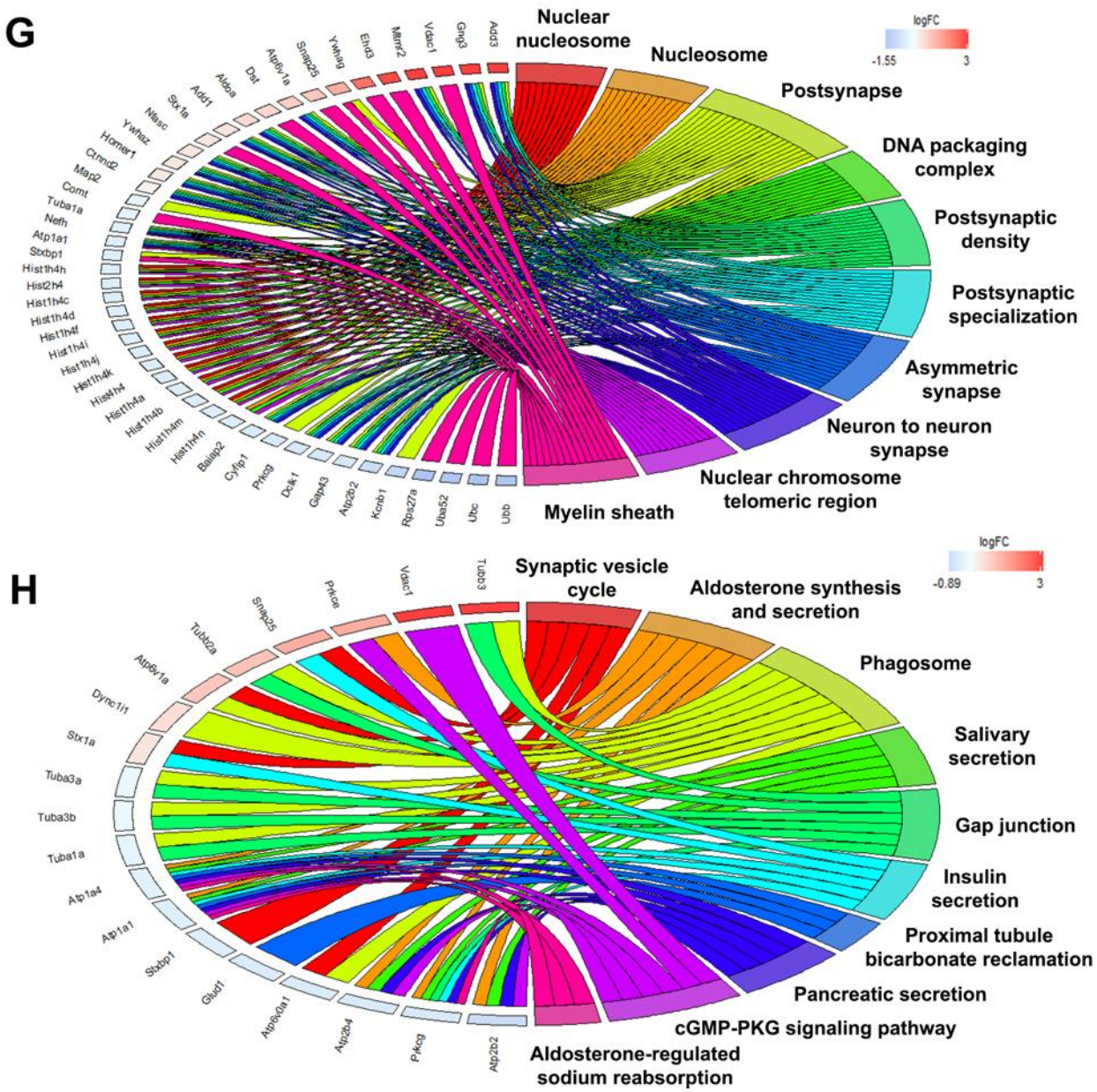
Global proteins enriched in the top shared cellular components at 24 hrs (A), 7 DPI (C), and 30 DPI (E) time points. Global proteins enriched in the top shared functional pathways at 24 hrs (B), 7 DPI (D), and 30 DPI (F) time points.

Supplemental Figure S7. GO cellular components and signaling pathways and enriched in phospho proteome at 3 hrs, 24 hrs, 7 DPI, and 30 DPI after blast injury.



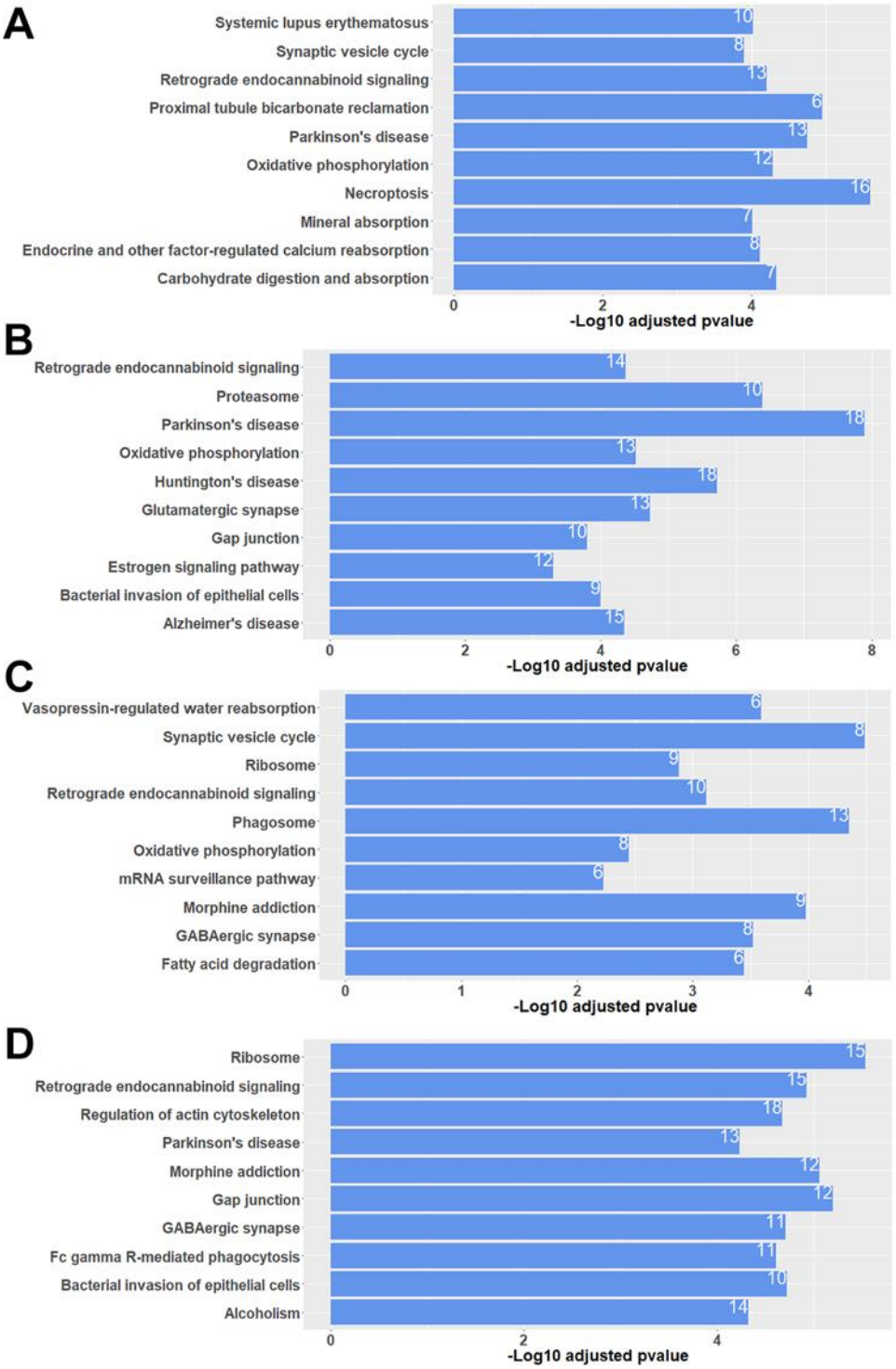

C
logFC
-1.45
3
Myelin sheath
Postsynaptic density
Postsynaptic specialization
Asymmetric synapse
Neuron to neuron synapse
Postsynapse
Presynapse
Synaptic vesicle
Exocytic vesicle
Vacuolar membrane
Vdac1
Mbnl2
Atp6v1a
Rtn4
Ndufs1
Stx1a
Dat
Uqcrc1
Map1b
Uba1
Stxbp1
Ndufv2
Pkm
Sept7
Atp6v0a1
Grin2b
Wdfy3
Cap2
Atp2b2
Psd
Ckmt1
D
logFC
-0.5
3
Oxidative phosphorylation
Synaptic vesicle cycle
Parkinson's disease
Alzheimer's disease
Huntington's disease
Phagosome
Collecting duct acid secretion
Non-alcoholic fatty liver disease (NAFLD)
Amphetamine addiction
Thermogenesis
Vdac1
Tubb3
Atp6v1a
Rtn4
Ndufs1
Stx1a
Uqcrc1
Uba1
Stxbp1
Ndufv2
Atp6v0a1
Dync1h1
Grin2b


E
logFC
-2
3
Postsynaptic density
Postsynaptic specialization
Asymmetric synapse
Neuron to neuron synapse
Myelin sheath
Postsynapse
Presynapse
Adherens junction
Anchoring junction
Focal adhesion
Gng3
Dlg1
Vdac1
Ehd3
Ywhag
Mprp2
Shank2
Cltc
Atp6v1a
Add1
Rtn4
Map1a
Ndufs1
Aldoa
Ywhaz
Ywhae
Epb41l1
Dlg4
Ppia
Baiap2
Mdh2
Cyfip1
Atln
Cap2
Lrp1
Grin2b
Atp6v0a1
Lasp1
Ywhaq
Ndel1
Sfp1
Unc13a
Actr1a
Rims1
Wdr7
F
logFC
-1.51
3
Synaptic vesicle cycle
Hippo signaling pathway
Alzheimer's disease
Phagosome
Huntington's disease
Oocyte meiosis
Glutamatergic synapse
Viral carcinogenesis
Cell cycle
Epstein-Barr virus infection
Dlg1
Tubb3
Vdac1
Gng3
Ywhag
Shank2
Tubb2a
Cltc
Atp6v1a
Psmd3
Atp2a2
Rtn4
Ndufs1
Ywhaz
Ywhae
Dlg4
Lrp1
Grin2b
Atp6v0a1
Ywhaq
Tuba4a
Unc13a
Rims1



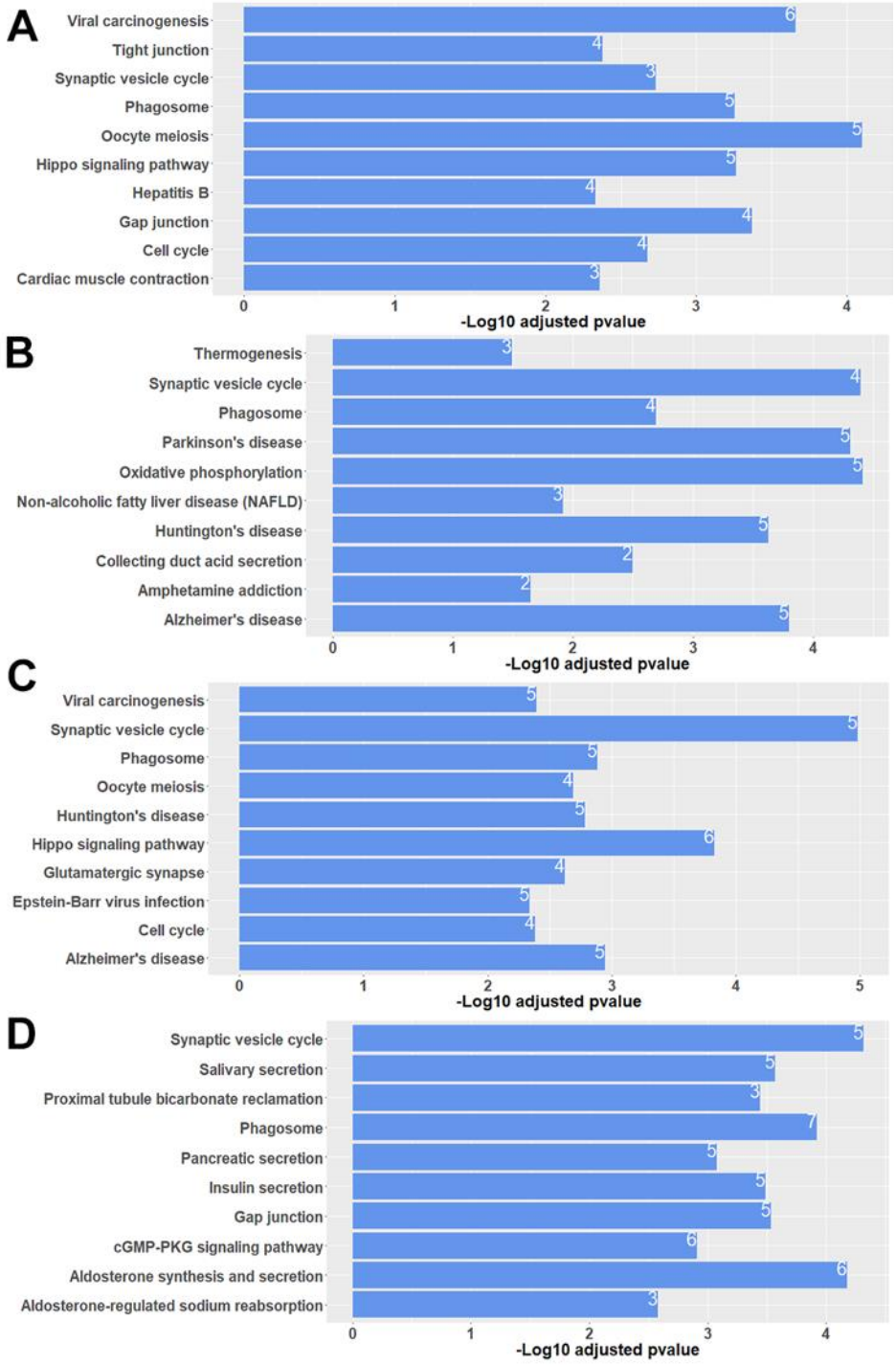
proteins enriched in the top shared cellular components at 3 hrs (A), 24 hrs (C), 7 DPI (E), and 30 DPI (G) time points. Phospho proteins enriched in the top shared functional pathways at 3 hrs (B), 24 hrs (D), 7 DPI (F), and 30 DPI (H) time points.

Supplemental Figure S8. Top 10 canonical pathways information for global-proteome after blast.



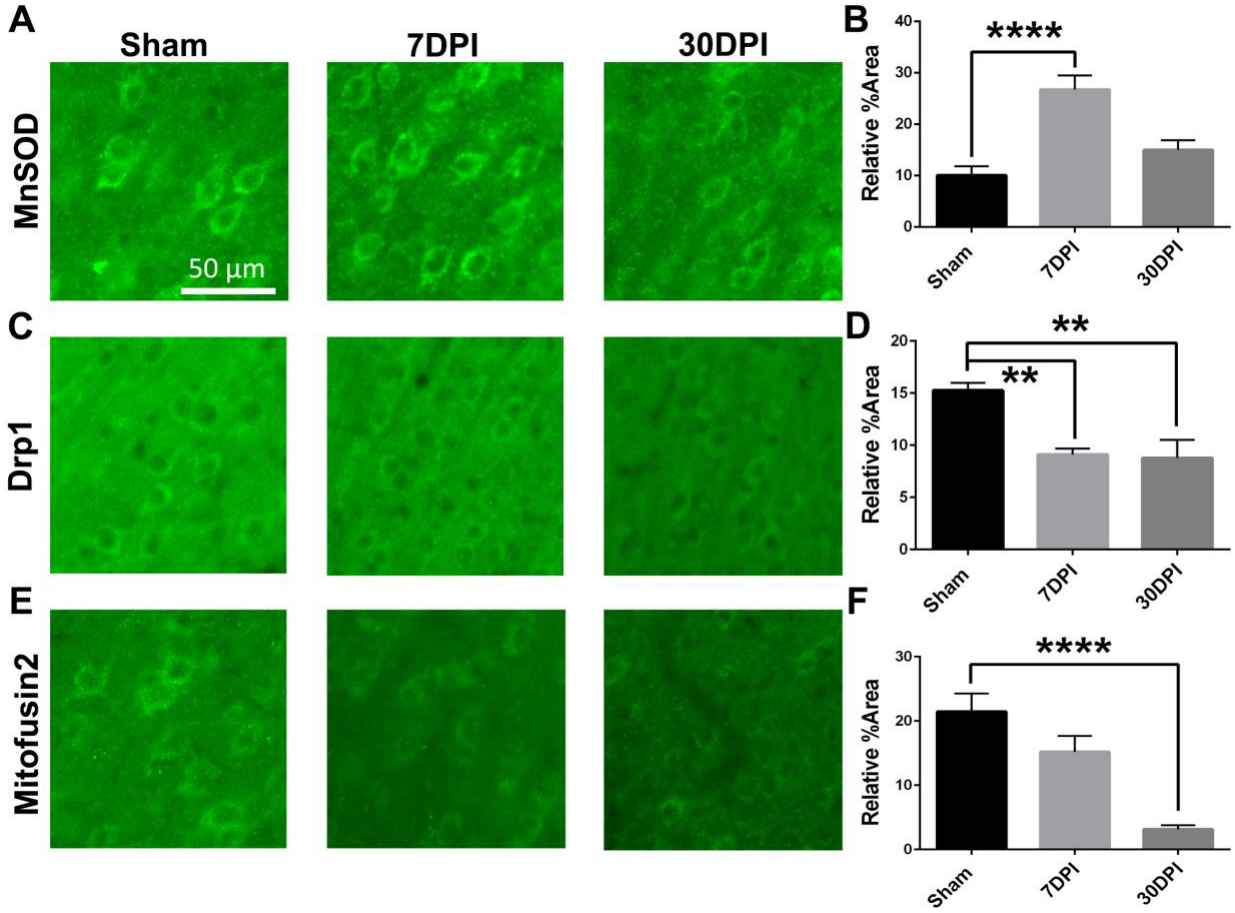
KEGG analysis of differentially expressed proteins at the global level from mouse brains at 3 hrs (A), 24 hrs (B), 7 DPI (C), and 30 DPI (D) after blast injury. Note: the number in the bar graph indicates the number of differentially expressed proteins enriched in its corresponding specific canonical signaling pathway.

Supplemental Figure S9. Top 10 canonical pathways information for phospho-proteome after blast.



KEGG analysis of differentially expressed proteins at the phospho level from mouse brains at 3 hrs (A), 24 hrs (B), 7 DPI (C), and 30 DPI (D) after blast injury. Note: the number in the bar graph indicates the number of differentially expressed proteins enriched in its corresponding specific canonical signaling pathway.

Supplemental Figure S10. Expression levels of mitochondrial markers of oxidative stress, fission and fusion were changed after blast.



Representative images of staining were shown. (A-B) Significant increase of MnSOD staining in cortex at 7 DPI after blast as compared to sham. (C-D) Significant decreases of Drp1 staining in cortex at 7 and 30 DPI after blast as compared to sham. (E-F) Significant decrease of Mitofusin2 staining in cortex both at 30 DPI after blast as compared to sham. One-way ANOVA: $P<0.05$; Fisher's LSD test for all comparisons between two groups; n=3 in each group.

Supplemental Tables

Supplemental Table S1. The list of all identified proteins in global-proteome analysis after blast. Please see the attached excel file deposited to the http://calla.rnet.missouri.edu/mass_spectrometry/; (username: gulabm706; password: SGs$6108).

Supplemental Table S2. Differentially expressed protein identification in global-proteome analysis. Please see the attached excel file deposited to the http://calla.rnet.missouri.edu/mass_spectrometry/; (username: gulabm706; password: SGs$6108).

Supplemental Table S3. The list of all identified proteins in phospho-proteome analysis after blast. Please see the attached excel file deposited to the http://calla.rnet.missouri.edu/mass_spectrometry/; (username: gulabm706; password: SGs$6108).

Supplemental Table S4. Differentially expressed phosphorylated protein identification in phospho-proteome analysis. Please see the attached excel file deposited to the http://calla.rnet.missouri.edu/mass_spectrometry/; (username: gulabm706; password: SGs$6108).

Supplemental Table S5. Summary of PCA.

Importance of Components	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	3.368636 86	3.146021 55	2.649467 37	2.539721 92	2.347995 25	2.240123 31	1.99392 26	1.940327 16
Proportion of Variance	0.16415	0.14317	0.10154	0.0933	0.07975	0.07259	0.05751	0.05446
Cumulative Proportion	0.16415	0.30732	0.40886	0.50217	0.58192	0.65451	0.71202	0.76648

PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17	PC18
1.81462 685	1.72179 068	1.49318 375	1.42347 336	1.25293 83	1.19744 132	1.13153 271	1.02454 647	0.54431 378	1.81E-15
0.04763	0.04288	0.03225	0.02931	0.02271	0.02074	0.01852	0.01518	0.00429	0
0.81411	0.857	0.88925	0.91856	0.94127	0.96201	0.98053	0.99571	1	1

Principal components were ranked based on the proportion of variance they accounted in the dataset.

Supplemental Table S6. Canonical pathways information for global-proteome at different time points after blast. Please see the attached excel file deposited to the http://calla.rnet.missouri.edu/mass_spectrometry/; (username: gulabm706; password: SGs$6108).

Supplemental Table S7. Canonical pathways information for phospho-proteome at different time points after blast. Please see the attached excel file deposited to the http://calla.rnet.missouri.edu/mass_spectrometry/; (username: gulabm706; password: SGs$6108).

Supplemental Table S8. Cross comparison of the canonical pathways among groups by IPA analysis. Please see the attached excel file deposited to the http://calla.rnet.missouri.edu/mass_spectrometry/; (username: gulabm706; password: SGs$6108).

Supplemental Table S9. KEGG annotation of canonical pathways from phospho-proteome associated with oxidative stress and mitochondrial dysfunction after blast injury.

Canonical pathway		**Differentially expressed proteins**			
ID	**Name**	**3hrs**	**24hrs**	**7DPI**	**30DPI**
mmu00190	Oxidative phosphorylation	Uqcrc1	Atp6v1a, Ndufv2	Atp6v1a, Atp6v0a1	Atp6v1a
		3.80E-05	4.68E-02	8.71E-02	2.11E-02
mmu04137	Mitophagy	NA	NA	NA	Ubb
		1.00E+00	3.62E-01	1.00E+00	1.00E+00
mmu04142	Lysosome	NA	Atp6v0a1	Atp6v0a1	NA
		3.52E-01	2.18E-01	1.00E+00	1.35E-01
mmu04145	Phagosome	NA	Atp6v1a	Tubb3, Atp6v1a	Atp6v1a
		2.00E-03	1.16E-04	5.44E-04	1.29E-03
mmu04150	mTOR signaling pathway	NA	Atp6v1a	Atp6v1a	Atp6v1a
		4.08E-01	2.85E-01	2.62E-02	5.44E-01
mmu04210	Apoptosis	Tuba1a, Tuba3a	NA	NA	Tuba1a
		1.00E+00	2.72E-01	1.29E-01	5.32E-01

(Note: P values are presented directly under each pathway at different time points. NA: no differentially expressed protein is identified)

Supplemental Table S10. KEGG annotation of canonical pathways from global-proteome associated with myelin sheath and axonal injury after blast injury.

Canonical pathway		**Differentially expressed proteins**			
ID	**Name**	**3hrs**	**24hrs**	**7DPI**	**30DPI**
mmu00190	Oxidative phosphorylation	Uqcrc1	Atp6v1a, Ndufv2	Atp6v1a, Atp6v0a1	Atp6v1a
		3.80E-05	4.68E-02	8.71E-02	2.11E-02
	Mitophagy	NA	NA	NA	Ubb

mmu04137		1.00E+00	3.62E-01	1.00E+00	1.00E+00
mmu04142	Lysosome	NA	Atp6v0a1	Atp6v0a1	NA
		3.52E-01	2.18E-01	1.00E+00	1.35E-01
mmu04145	Phagosome	NA	Atp6v1a	Tubb3, Atp6v1a	Atp6v1a
		2.00E-03	1.16E-04	5.44E-04	1.29E-03
mmu04150	mTOR signaling pathway	NA	Atp6v1a	Atp6v1a	Atp6v1a
		4.08E-01	2.85E-01	2.62E-02	5.44E-01
mmu04210	Apoptosis	Tuba1a, Tuba3a	NA	NA	Tuba1a
		1.00E+00	2.72E-01	1.29E-01	5.32E-01

Supplemental Table S11. KEGG annotation of canonical pathways from global-proteome associated with synaptic dysregulation after blast injury.

Canonical pathway		**Differentially expressed proteins**			
ID	**Name**	**3hrs**	**24hrs**	**7DPI**	**30DPI**
mmu04721	Synaptic vesicle cycle	NA	Stxbp1, Atp6v1a	Atp6v1a, Atp6v0a1	Stxbp1, Atp6v1a
		3.96E-05	4.75E-05	1.84E-03	1.02E-05
mmu04727	GABAergic synapse	Prkcg	NA	Gng3	Prkcg, Gng3
		1.00E+00	1.05E-01	4.44E-02	3.35E-01
mmu04724	Glutamatergic synapse	NA	NA	Grin2b	NA
		3.17E-01	4.18E-02	1.14E-02	2.37E-03
mmu04720	Long-term potentiation	NA	NA	Grin2b	NA
		2.07E-01	3.77E-01	2.51E-01	2.93E-01
mmu04730	Long-term depression	Prkcg	NA	NA	Prkcg
		1.00E+00	3.65E-01	3.09E-02	1.00E+00

References:

1. Song, H., Konan, L.M., Cui, J., Johnson, C.E., Langenderfer, M., Grant, D., Ndam, T., Simonyi, A., White, T., Demirci, U., Mott, D.R., Schwer, D., Hubler, G.K., Cernak, I., DePalma, R.G. and Gu, Z. (2018). Ultrastructural brain abnormalities and associated behavioral changes in mice after low-intensity blast exposure. Behav Brain Res 347, 148-157.