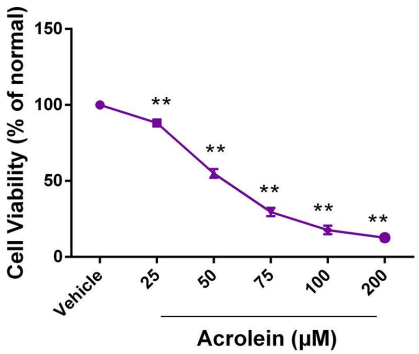
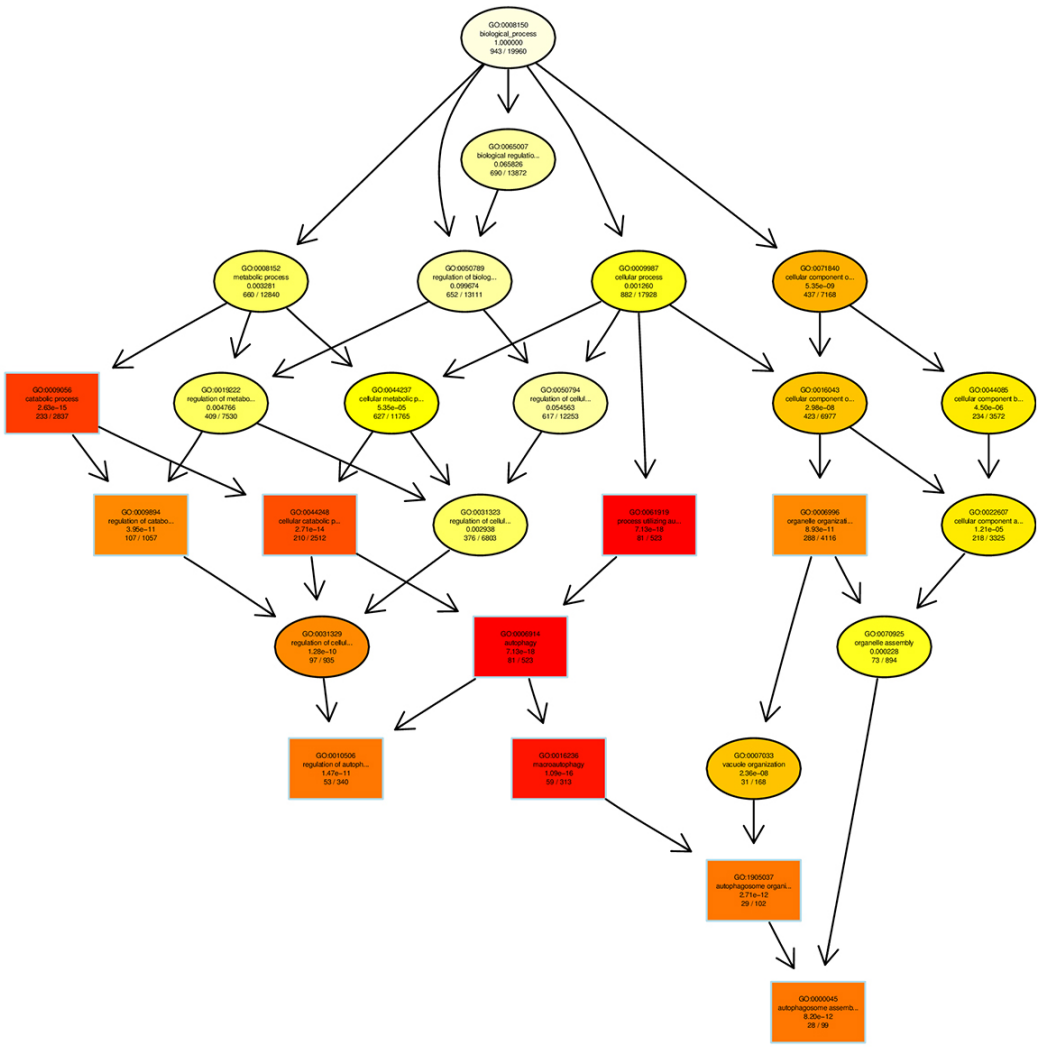
Supplementary Materials

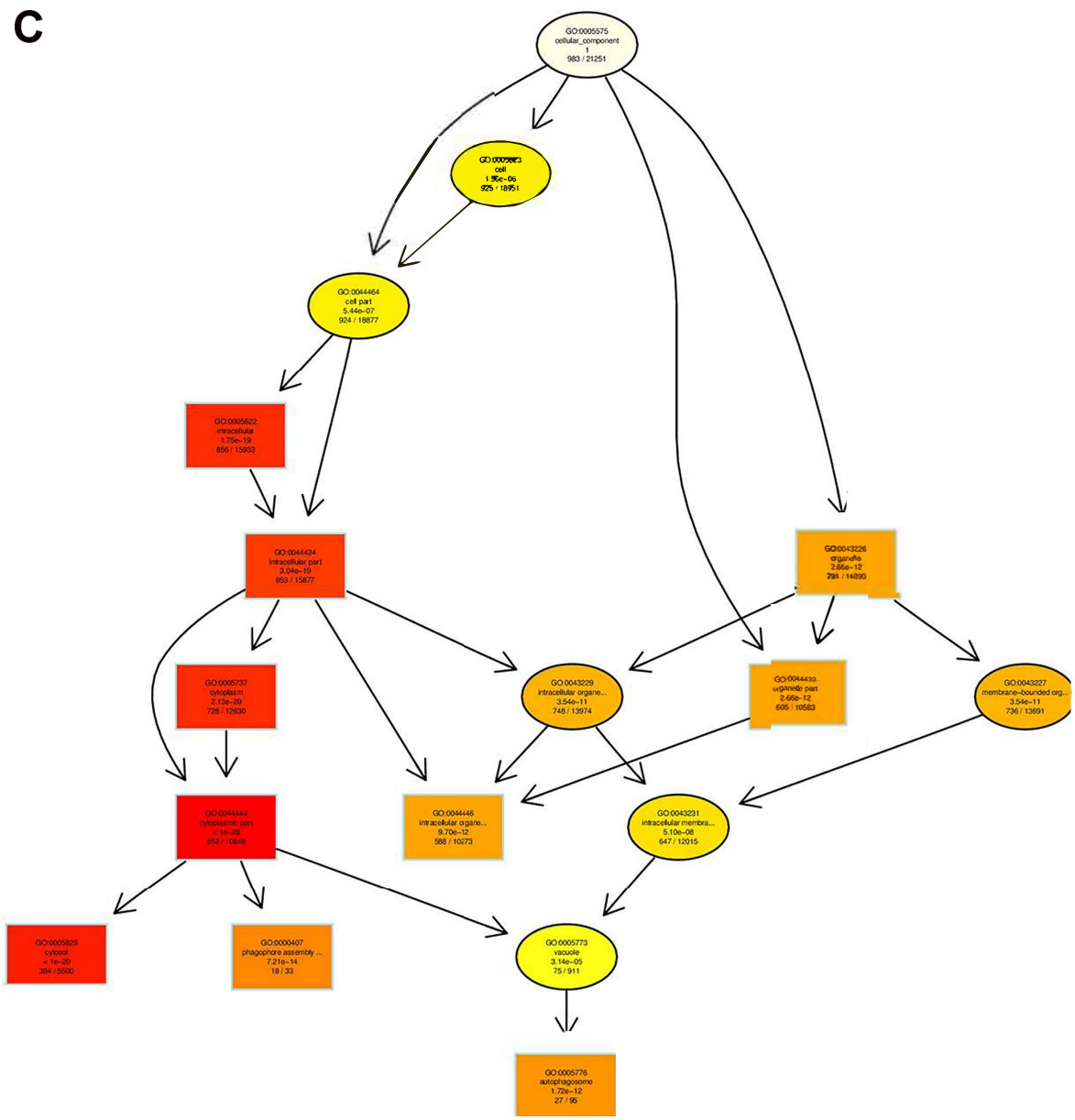
A



B



C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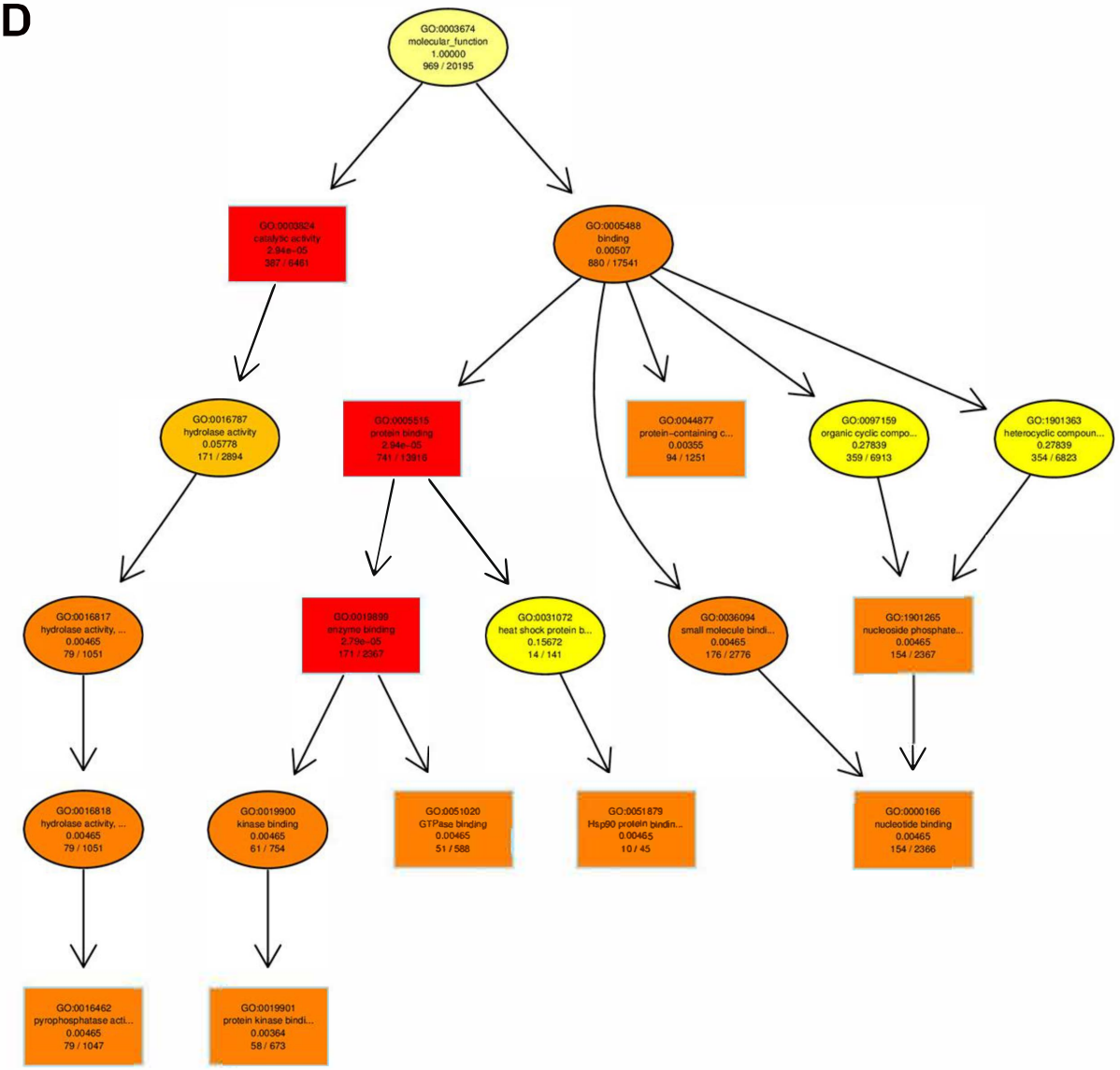
1
983 / 21251
GO:0005623
cell
1.95e-06
925 / 18951
GO:0044464
cell part
5.44e-07
924 / 18877
GO:0005622
intracellular
1.75e-19
856 / 15933
GO:0044424
intracellular part
3.04e-19
853 / 15877
GO:0043226
organelle
2.65e-12
791 / 14890
GO:0005737
cytoplasm
2.13e-20
728 / 12630
GO:0043229
intracellular organe...
3.54e-11
748 / 13974
GO:0044422
organelle part
2.66e-12
605 / 10583
GO:0043227
membrane-bounded org...
3.54e-11
736 / 13691
GO:0044444
cytoplasmic part
< 1e-20
652 / 10648
GO:0044446
intracellular organe...
9.70e-12
588 / 10273
GO:0043231
intracellular membra...
5.10e-08
647 / 12015
GO:0005829
cytosol
< 1e-20
394 / 5500
GO:0000407
phagophore assembly ...
7.21e-14
18 / 33
GO:0005773
vacuole
3.14e-05
75 / 911
GO:0005776
autophagosome
1.72e-12
27 / 95

D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molecular_function
1.00000
969 / 20195
GO:0003824
catalytic activity
2.94e-05
387 / 6461
GO:0005488
binding
0.00507
880 / 17541
GO:0016787
hydrolase activity
0.05778
171 / 2894
GO:0005515
protein binding
2.94e-05
741 / 13916
GO:0044877
protein-containing c...
0.00355
94 / 1251
GO:0097159
organic cyclic compo...
0.27839
359 / 6913
GO:1901363
heterocyclic compoun...
0.27839
354 / 6823
GO:0016817
hydrolase activity, ...
0.00465
79 / 1051
GO:0019899
enzyme binding
2.79e-05
171 / 2367
GO:0031072
heat shock protein b...
0.15672
14 / 141
GO:0036094
small molecule bindi...
0.00465
176 / 2776
GO:1901265
nucleoside phosphate...
0.00465
154 / 2367
GO:0016818
hydrolase activity, ...
0.00465
79 / 1051
GO:0019900
kinase binding
0.00465
61 / 754
GO:0051020
GTPase binding
0.00465
51 / 588
GO:0051879
Hsp90 protein bindin...
0.00465
10 / 45
GO:0000166
nucleotide binding
0.00465
154 / 2366
GO:0016462
pyrophosphatase acti...
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79 / 1047
GO:0019901
protein kinase bindi...
0.00364
58 / 673

E

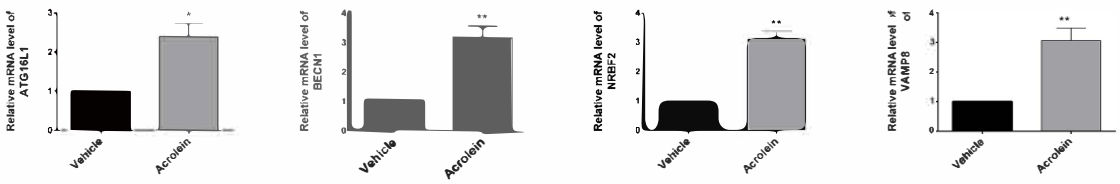

Relative mRNA level of ATG16L1
Vehicle
Acrolein
Relative mRNA level of BECN1
Vehicle
Acrolein
Relative mRNA level of NRBF2
Vehicle
Acrolein
Vehicle
Acrolein

Fig S1 mRNA-Seq analysis showing that acrolein activates autophagy. **A** CCK8 assays of cell viability in HUVECs treated with acrolein (25, 50, 75, 100, and 200 μmol/L) and vehicle for 6 h. **B–D** GO and pathway analysis of biological process (**B**), cellular components (**C**), and molecular function (**D**). **E** qPCR validates the changes in autophagy-related gene expression. Values are represented as the mean ± SEM, **P* <0.05 and ***P* <0.01 *vs* vehicle group.

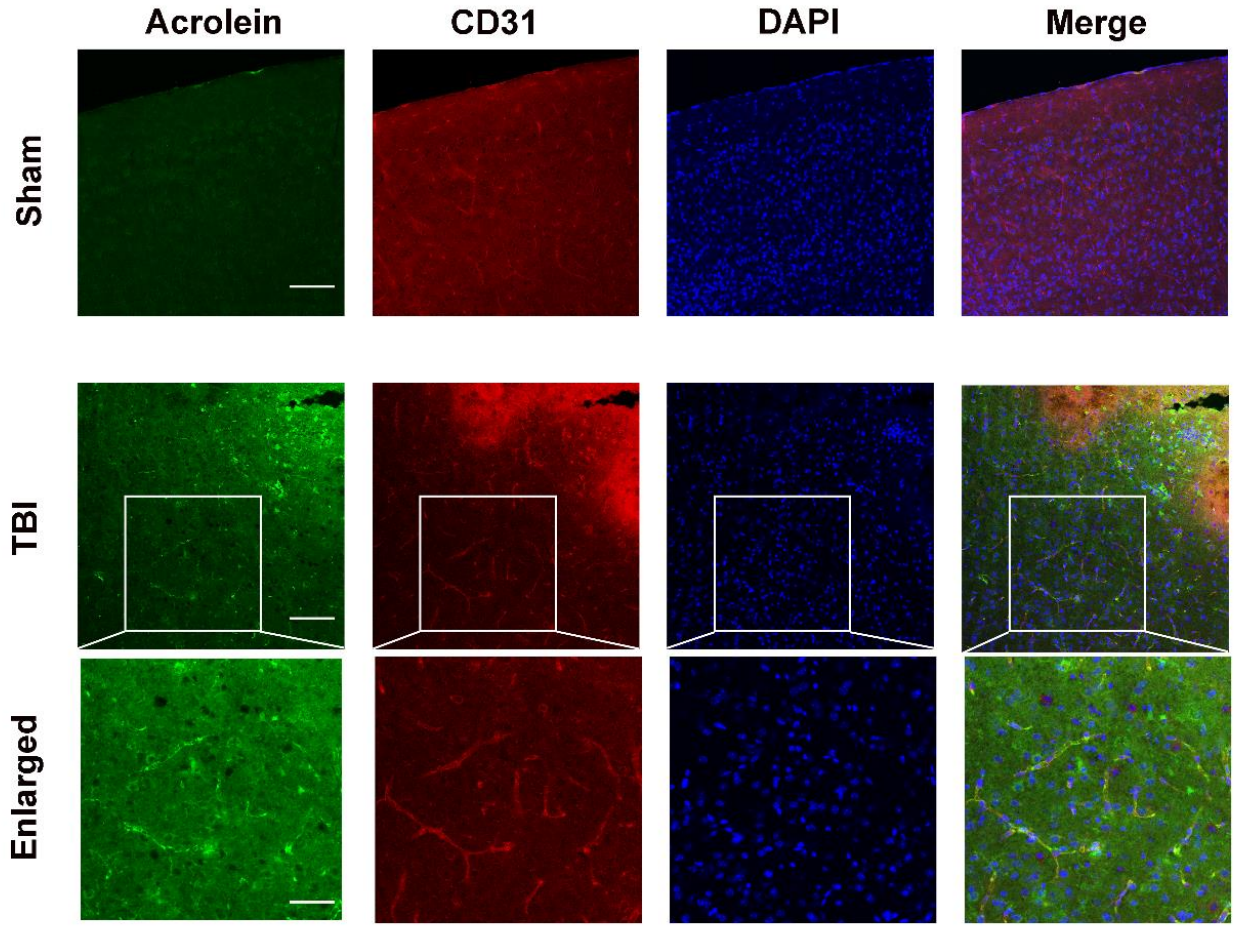


Fig. S2 Representative images of immunofluorescence staining for acrolein (green) and CD31 (red) in the perilesional cortex 24 h after TBI (scale bars, 100 μm; enlarged, 50 μm).

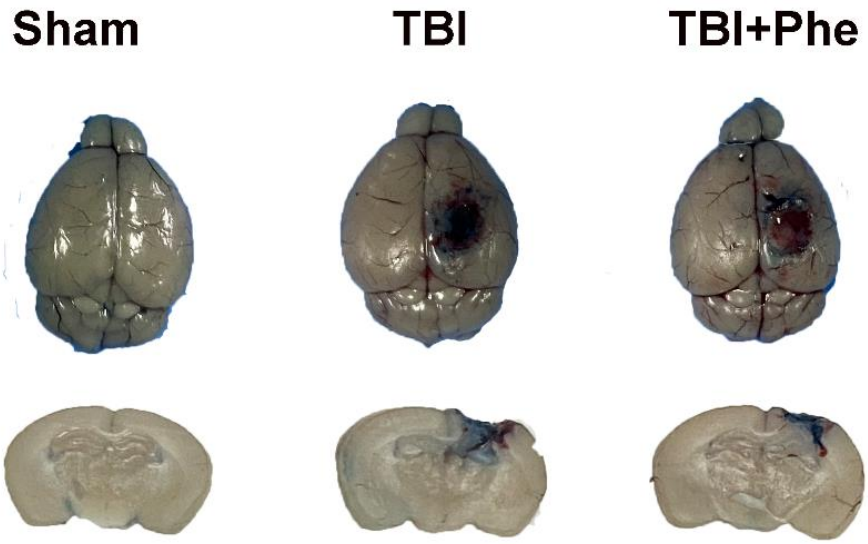
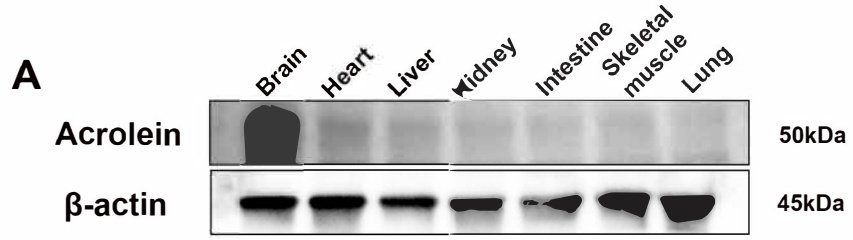
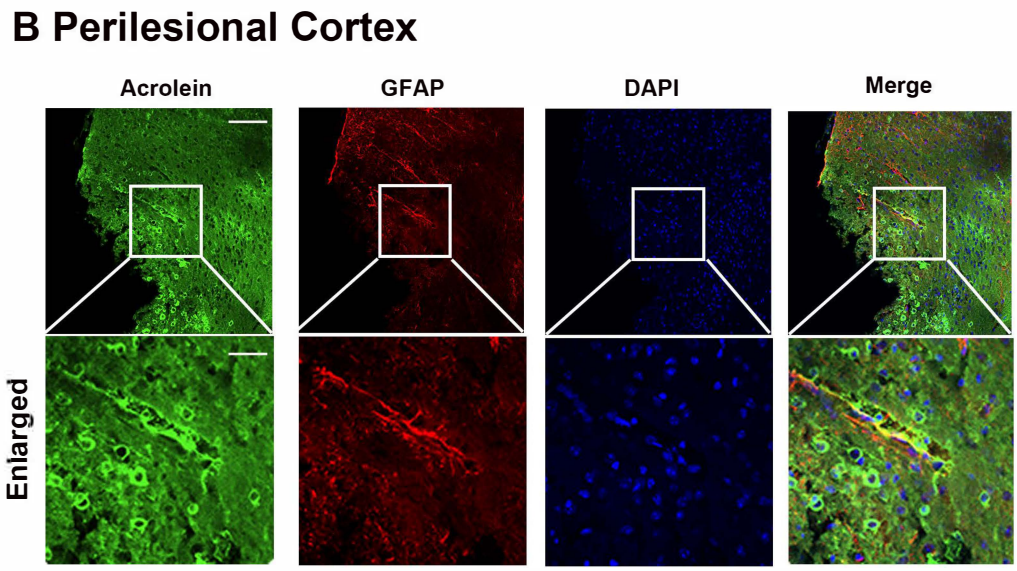


Fig. S3 Representative images of the dorsal surface and coronal section showing Evans blue extravasation.


A
Brain
Heart
Liver
Kidney
Intestine
Skeletal muscle
Lung
Acrolein
β-actin
50kDa
45kDa


B Perilesional Cortex
Acrolein
GFAP
DAPI
Merge
Enlarged

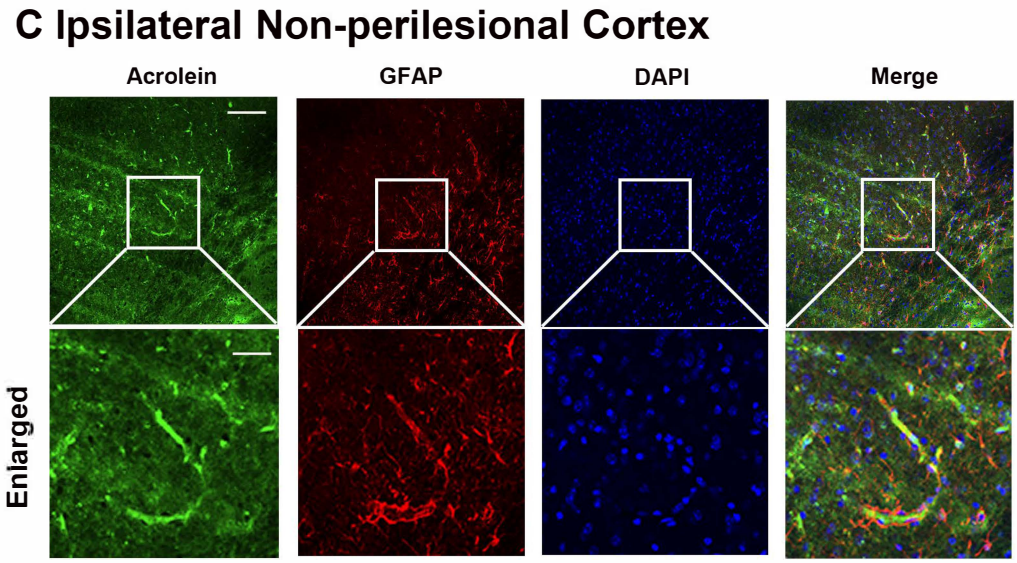

C Ipsilateral Non-perilesional Cortex
Acrolein
GFAP
DAPI
Merge
Enlarged

Fig. S4 A Western blots of acrolein in the brain, heart, liver, kidney, intestine, skeletal muscle, and lung after TBI. **B, C** Representative images of double immunofluorescence staining for acrolein (green) and GFAP (red) in perilesional cortex (**B**) and ipsilateral non-perilesional cortex (**C**) (scale bars, 100 μm; enlarged, 30 μm).

Table S1 Sequences of primers used in qRT-PCR

Name	Forward primer (5′–3′)	Reverse primer (5′–3′)
Beclin-1	GGGCTCCCGAGGGATGG	GCTGTTGGCACTTTCTGTGG
ATG16L1	TGGGGAGTTAGCTCAACTGGTG	AAGAGACAGAGCGTCTCCCA
Vamp8	GGAAGCCACATCTGAGCACT	AGCCCACTCTAAGGACCCAA
NRBF2	AAGGCTGCAGCATATCTTTCTGA	TCTCAGGCAGGCATTTCTCTG