Supporting Information

Extending the Calpain-Cathepsin Hypothesis to the Neurovasculature: Protection of Brain Endothelial Cells and Mice from Neurotrauma

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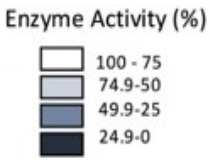
Table S1. Enzyme inhibition by compounds **1**-**5** at 1µM and 10µM against calpain-1 (CAPN1), cathepsin B (CTSB), cathepsin K (CTSK) and papain. Data represents Mean ± SD of at least n=6 replicates.



	1	2	3	4	5	
CAPN1	9.8 ± 5.62%	15.73 ± 3.35	-7.1 ± 3.09%	14.8 ± 3.07%	3.3 ± 3.02%	1uM
CTSB	87.6 ± 1.89%	105.6 ± 9.73%	88.6 ± 12.26%	98.2 ± 5.38%	74.0 ± 9.54%	
CTSK	92.6 ± 2.77%	94.4 ± 12.52%	86.5 ± 4.31%	91.7 ± 6.05%	89.6 ± 7.49%	
Papain	97.1 ± 9.58%	100.2 ± 6.68%	84.8 ± 5.45%	97.27 ± 14.6%	91.7 ± 7.30%	
CAPN1	0.78 ± 3.99%	5.8 ± 6.68%	11.2 ± 4.65%	-4.5 ± 2.1%	-5.1 ± 2.93%	10uM
CTSB	41.4 ± 2.30%	44.9 ± 4.8%	34.4 ± 3.14%	62.8 ± 6.05%	34.3 ± 4.18%	
CTSK	48.2 ± 4.68%	71.6 ± 2.80%	45.34 ± 1.19%	76.6 ± 7.03%	50.4 ± 3.03%	
Papain	67.7 ± 5.13%	84.4 ± 15.79%	90.83 ± 16.6%	99.6 ± 7.12%	72.8 ± 3.8%	

Table S2. Primers used in all rt-qPCR experiments

Gene Symbol	Gene Name	UniGene ID	Product Number	Probe
ACTB	Actin, beta	Mm.328431	Mm01205647_g1	VIC
Cldn5	Claudin 5	Mm.22768	Mm00727012_s1	FAM
HPRT	Hypoxanthine guanine phosphoribosyl transferase	Mm.299381	Mm03024075_m 1	VIC
Nos3	Nitric oxide synthase 3, endothelial cell	Mm.258415	Mm00435217_m 1	FAM
Ocln	Occludin	Mm.4807	Mm00500912_m 1	FAM
Tjp1	Tight Junction Protein 1 (ZO-1)	Mm.4342	Mm01320638_m 1	FAM

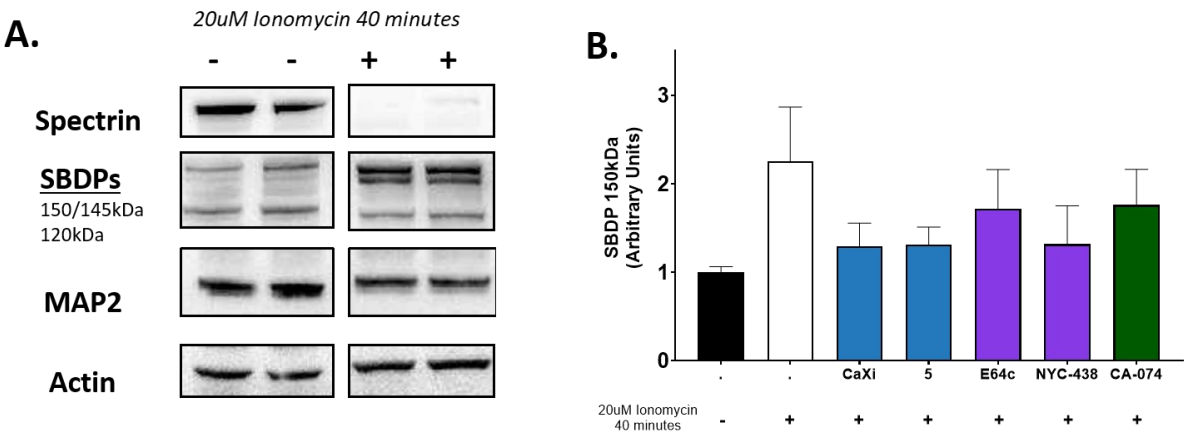


Figure S1. Inhibitory efficacy of compounds on proteolysis *in vitro*. **(A)** Representative immunoblot of HT22 cells treated for 40 minutes with 20µM ionomycin or vehicle control probed with Spectrin, MAP2 and Actin Abs. (**B**) Quantitative analysis of SBDP150kDa from western blots of HT22 cells treated with OGD-R and 10µM calpain/cathepsin inhibitors or vehicle control, compared to nontreated cells. Data represents mean ± SEM of at least n=3 duplicates of cell passages. All protein was normalized to the housekeeping protein, actin. Equal protein amounts were loaded in all lanes of immunoblots.

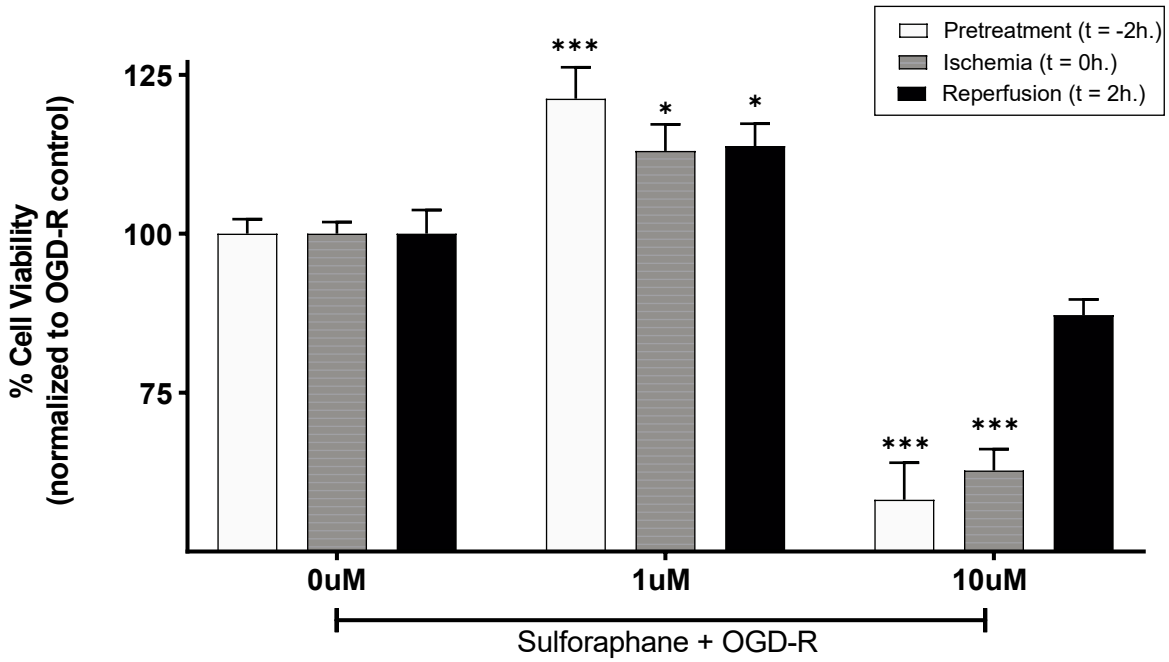


Figure S2. Sulforaphane is a positive and negative control in the OGD-R assay. OGD-R of SH-SY5Y cells treated with 1 or 10µM at pre-treatment *(t*=-2h.), ischemia (*t*=0h.) or reperfusion (*t*=-2) treatment paradigms. Data represents mean ± SEM of at least n=6 replicates analyzed by one-way ANOVA with Dunnett's or Tukey's multi-comparison analysis

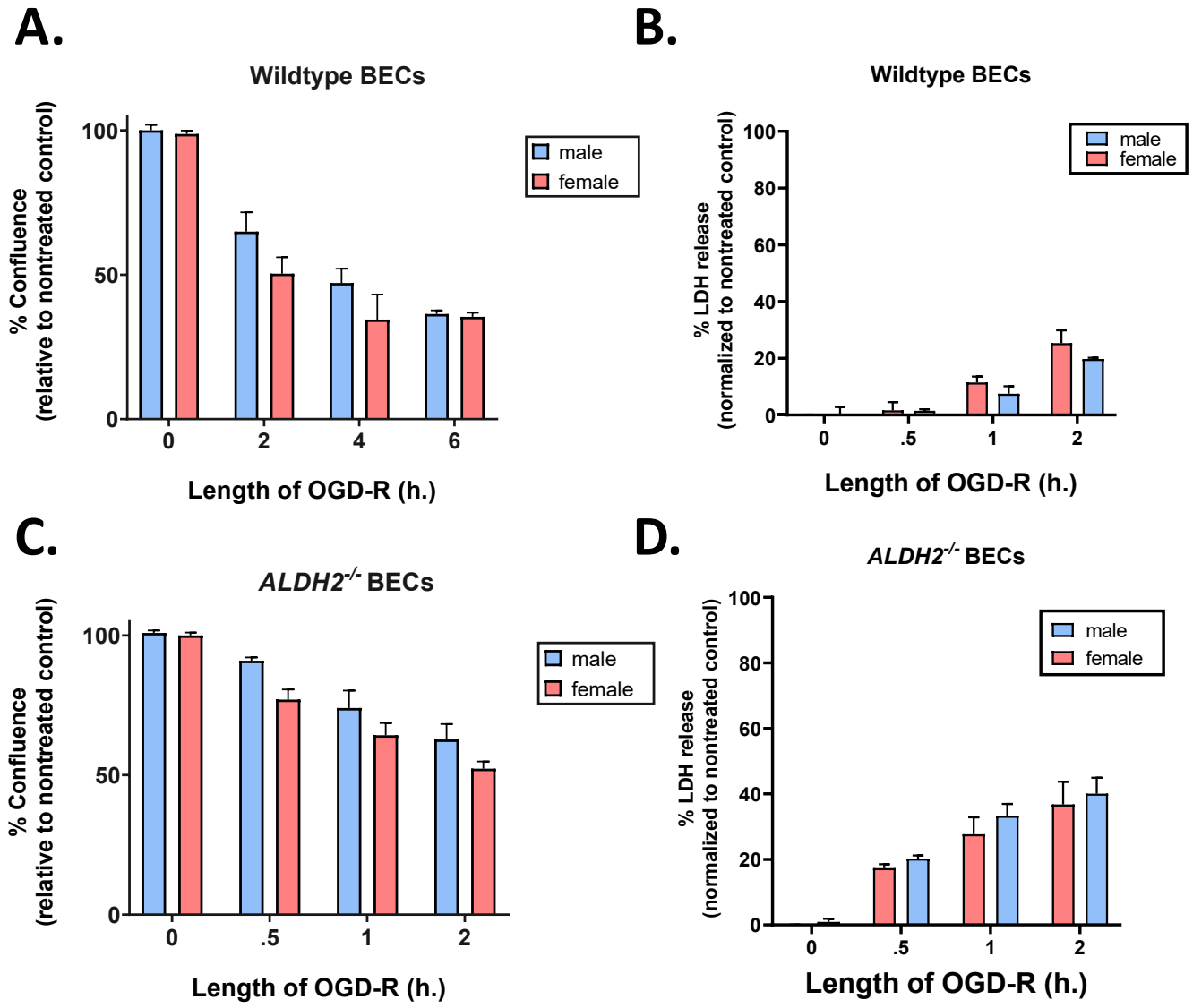


Figure S3. BECs from male and female mice show no significant sex difference in WT or *ALDH2*$^{-/-}$ BECs. **(A** and **B)** MTS and LDH, respectively of WT BECs isolated from female or male mice. (**C** and **D**) MTS and LDH, respectively, of *ALDH2*$^{-/-}$ BECs isolated from female or male mice. (**E** and **F**) Quantitative analysis of immunoblots probed with ZO-1 (**E**) or occludin (**F**) of WT or *ALDH2*$^{-/-}$ BECs isolated from female or male mice. Data represents mean ± SEM of at least n=3 replicates in duplicates of cell passages. All protein was normalized to the housekeeping protein, actin. Equal protein amounts were loaded in all lanes.

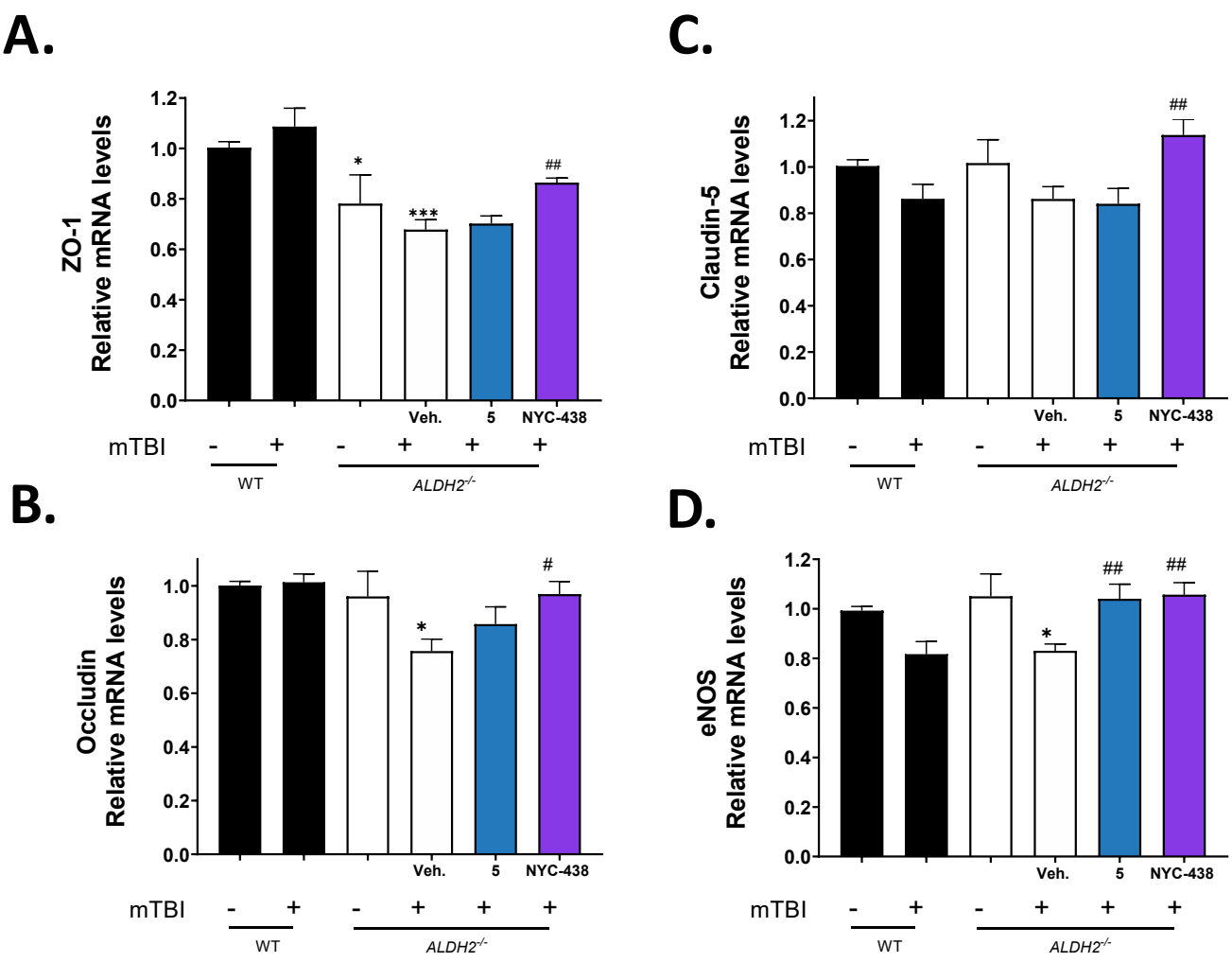


Figure S4. Minimal Transcriptional Changes of tight junction proteins. Levels of mRNA in ipsilateral hemispheres of WT and *ALDH2*$^{-/-}$ 24 hours post-mTBI or control animals with 10mg/kg i.p of ***5*** or ***NYC-438***: mRNA levels of ZO-1 (**A**) Occludin (**B**), Claudin-5 (**C**), and eNOS (**D**) quantified by rt-qPCR. Data represents mean ± SEM of n=7-10 animals analyzed by One-Way ANOVA with Tukey's multi-comparison analysis