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Supplemental information

A systemic cell stress signal confers neuronal resilience toward oxidative stress in a Hedgehog-dependent manner

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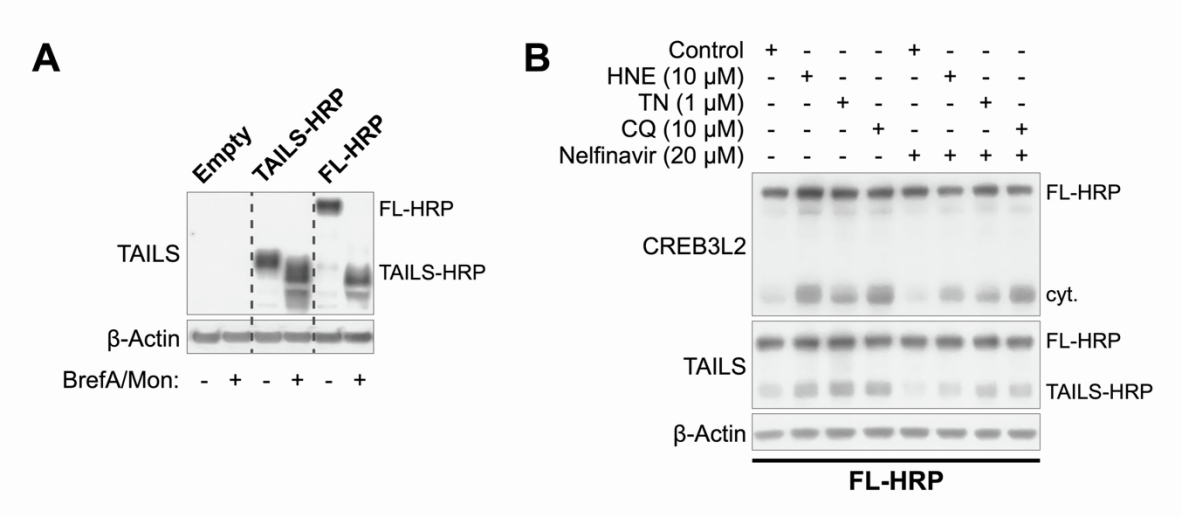

Figure S1. TAILS is generated and extracellularly released through the secretory pathway in response to stress, related to Figure 2.

(A) Western blot of TAILS in HEK293T cells expressing FL- or TAILS-HRP constructs. Secretory pathways were inhibited by treatment with brefeldin A and monensin to display accumulation of TAILS-HRP subjected to the secretory pathway.

(B) Western blot of TAILS in HEK293T cells expressing FL- or TAILS-HRP constructs treated with oxidative (HNE; 10 μM), ER (TN; 2 μM), and lysosomal (CQ, chloroquine; 10 μM) stress inducers.

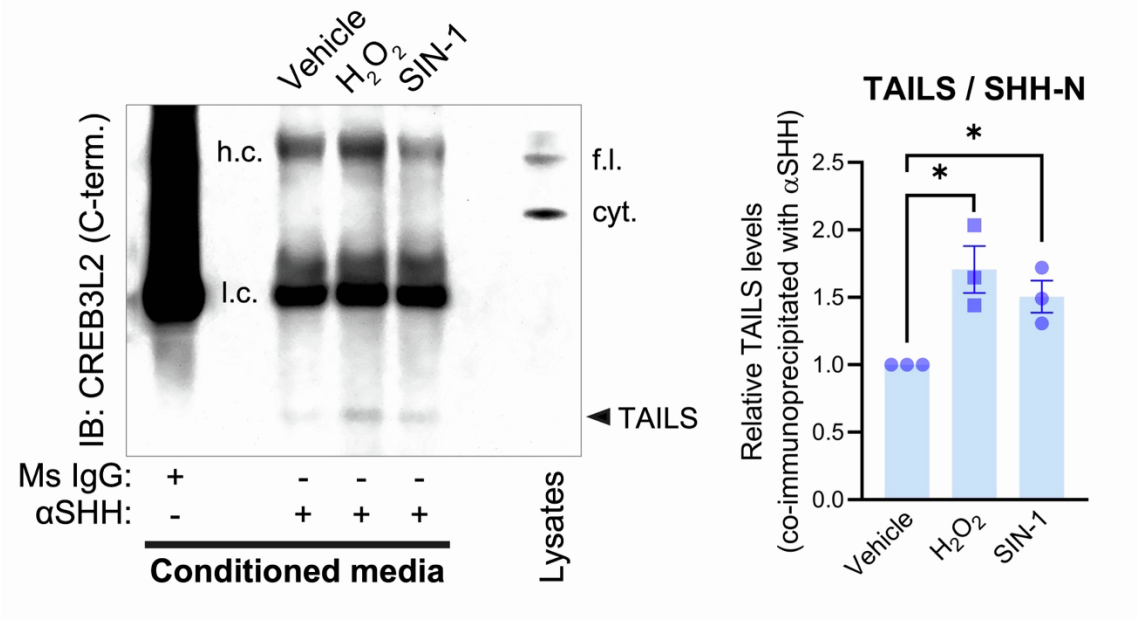

Figure S2. TAILS proteins bind SHH and are induced by oxidative stress in neurons, related to Figure 3.

Extracellular SHH proteins from co-cultures of primary cortical and hippocampal neurons were immunoprecipitated from condition media and the SHH-bound TAILS were detected with a CREB3L2 antibody specific for the C-terminus. Oxidative stress was induced by 6-h treatment of hydrogen peroxidase (200 μM) or SIN-1 (200 μM) in neurons. Neuronal lysate was prepared from the vehicle-treated condition in parallel as control. h.c., heavy chain; l.c., light chain; f.l., full-length; cyt., cytosolic. Means ± SEM (n=3 biological replicates). One-Way ANOVA, Tukey's multiple comparisons test. *, $P<0.05$.

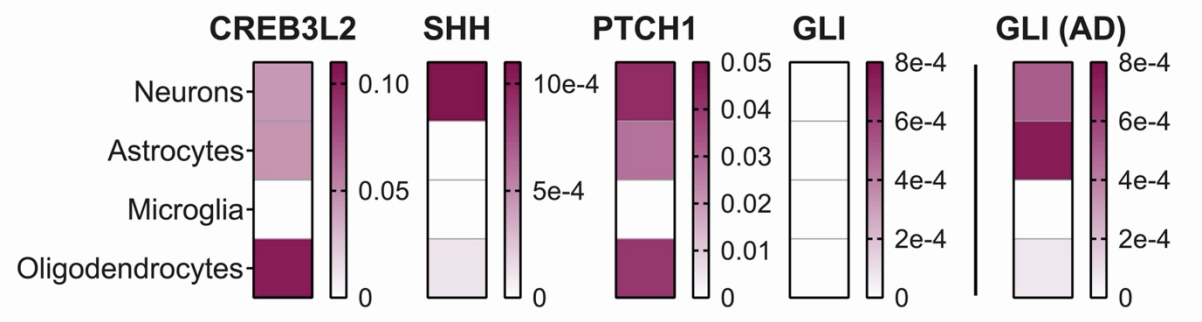

Figure S3. Single-cell resolution transcriptomic profiles of CREB3L2 and hedgehog pathway genes in human hippocampus, related to Figure 3.

Post-mortem non-demented and Alzheimer's disease (AD) brains were profiled at single-cell resolution (Yang et al., 2022). Average normalized counts of raw sequencing data (GSE163577) were acquired via https://twc-stanford.shinyapps.io/human_bbb.

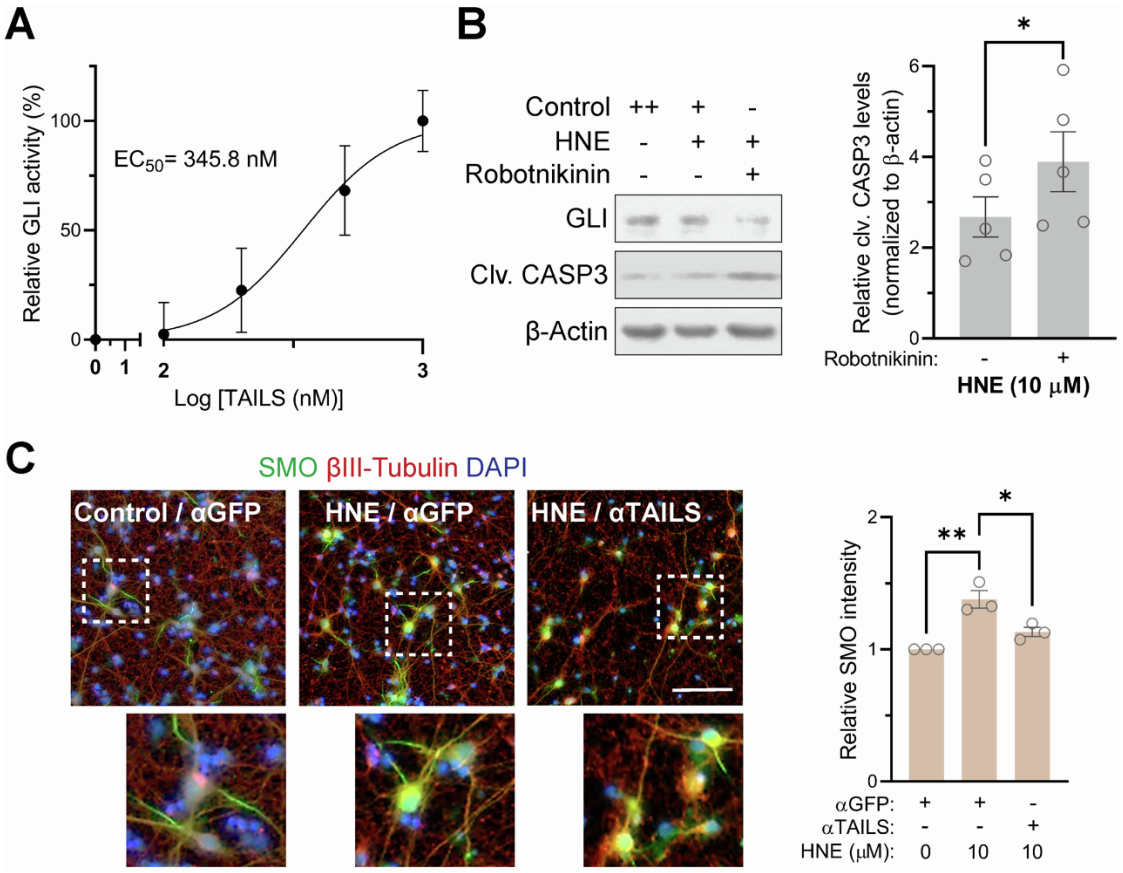

Figure S4. TAILS facilitates SHH signaling and blockade of its signaling abolishes TAILS-induced resilience in neurons, related to Figure 3.

(A) Dose-response curve of GLI activity in 7xGLI-GFP expressing Neuro2a cells treated with recombinant TAILS proteins. Non-linear modeling was used to determine EC_{50} (345.8 nM) of GLI activity in cells under treatment with varying concentrations of recombinant TAILS. Mean ± SEM (n=8 replicates).

(B) Western blot analysis of cleaved CASP3 in HNE-stressed hippocampal neurons treated with or without a SHH inhibitor robotnikinin (20 μM). Quantification of cleaved CASP3. Mean ± SEM (n=5 biological replicates). Paired t-test, one-tailed. *, *P*=0.0223.

(C) Immunofluorescence detection of SMO in hippocampal neurons treated with TAILS or GFP antibody (0.2 μg/mL). Quantification of SMO fluorescence intensity. Means ± SEM (n=3 biological replicates). One-way ANOVA, Tukey's multiple comparisons test. *, *P*=0.0159; **, *P*=0.002.

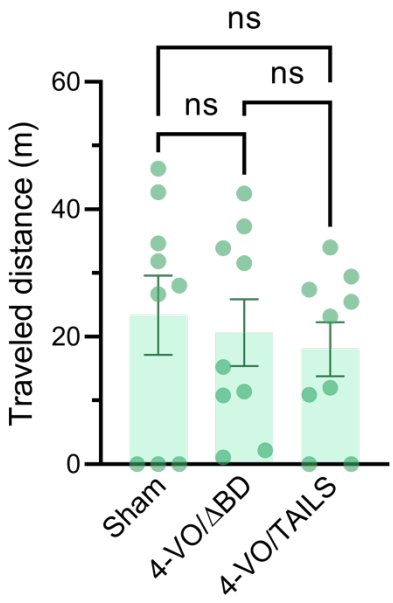

Figure S5. 4-VO surgery or injection of recombinant protein did not affect animal locomotion during the novel object recognition task, related to Figure 6.

Behavioral assessment of cognitive deficits in animals subjected for 4-VO or sham operation, administered with TAILS or ΔBD (100 ng μl^{-1}; 3 µl per hemisphere) through intrahippocampal injection in both hemispheres. Measurement of locomotion activity during the NOR task shows that animals subjected for 4-VO or sham operation have no locomotion deficits. One-way ANOVA, Tukey's multiple comparisons test.

Classification	Neuropathological Diagnosis	Sample	Age	Gender	Cold PMI[a]	Frozen PMI[b]	NIA-Reagan Consensus	Braak NFT	CERAD Plaque[c]
Non-AD	Encephalopathy of hypoxic-ischemic type, acute	148	66	Male	10:25	21:55	Not eligible	I	Not eligible
Non-AD	No diagnosis	5382	62	Male		5:24	Not eligible	I	Not eligible
Non-AD	No diagnosis	346	84	Male	10:00	14:10	Low	II	A
Non-AD	No diagnosis	3799	89+	Female	2:55	14:09	Not eligible	III	Not eligible
Alzheimer's disease	Possible AD	4051	89+	Male	3:10	16:55	Intermediate	V	B
Alzheimer's disease	Possible AD	4857	89+	Male		12:40	Intermediate	III	B
Alzheimer's disease	Possible AD	4871	86	Female	1:50	23:45	Intermediate	V	B
Alzheimer's disease	Probable AD	4389	86	Male			Intermediate	V	C
Alzheimer's disease	Encephalopathy, degenerative, moderate consistent with probable AD	4897	89+	Female	0:15	18:35	Intermediate	V	C
Alzheimer's disease	AD neuropathologic changes	5226	89+	Female	2:40	10:50	Intermediate	VI	C
Alzheimer's disease	Dementia	4856	89+	Female	0:45	16:30	High	V	C

Table S1. Neuropathological evaluation of post-mortem human specimens of dorsolateral prefrontal cortex tissues derived from Brodmann area (BA) 9/10, related to Figure 2D.

[a] Cold PMI: Post-mortem interval calculated from the reported time of death to the time the patient was brought into the cold room.

[b] Frozen PMI: Post-mortem interval calculated from the reported time of death to the time the brain was processed.

[c] Neuropathological classification follows ABC criteria.

* Cases are listed in the same order as in Figure 2D.