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Supplementary Information for

Primary cilia safeguard cortical neurons in neonatal mouse forebrain from environmental stress-induced dendritic degeneration

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Figure S1

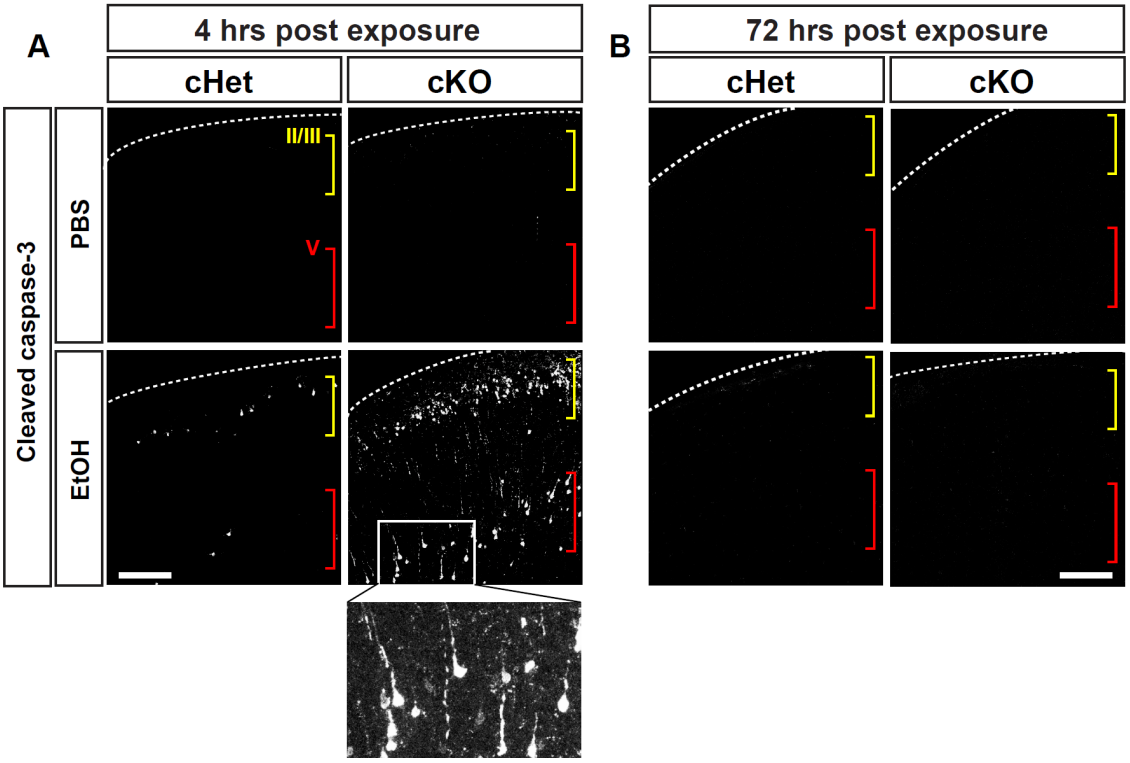

Fig. S1. Caspase-3 activation 4 and 72 hours post exposure to EtOH

(A, B) Immunohistochemistry for cleaved Caspase-3 in M1 for the indicated conditions 4 (A) and 72 (B) hours post exposure. Moderate and highly augmented levels of Caspase-3 activation were observed in EtOH-exposed cHet and cKO mice, respectively, 4 hours after exposure. Caspase-3 activation diminished by 72 hours. The boxed area in A is magnified to better visualize Caspase-3 activation in soma and dendrites. Yellow and red brackets indicate layers II/III and V, respectively. Bar = 200 μm.

Figure S2

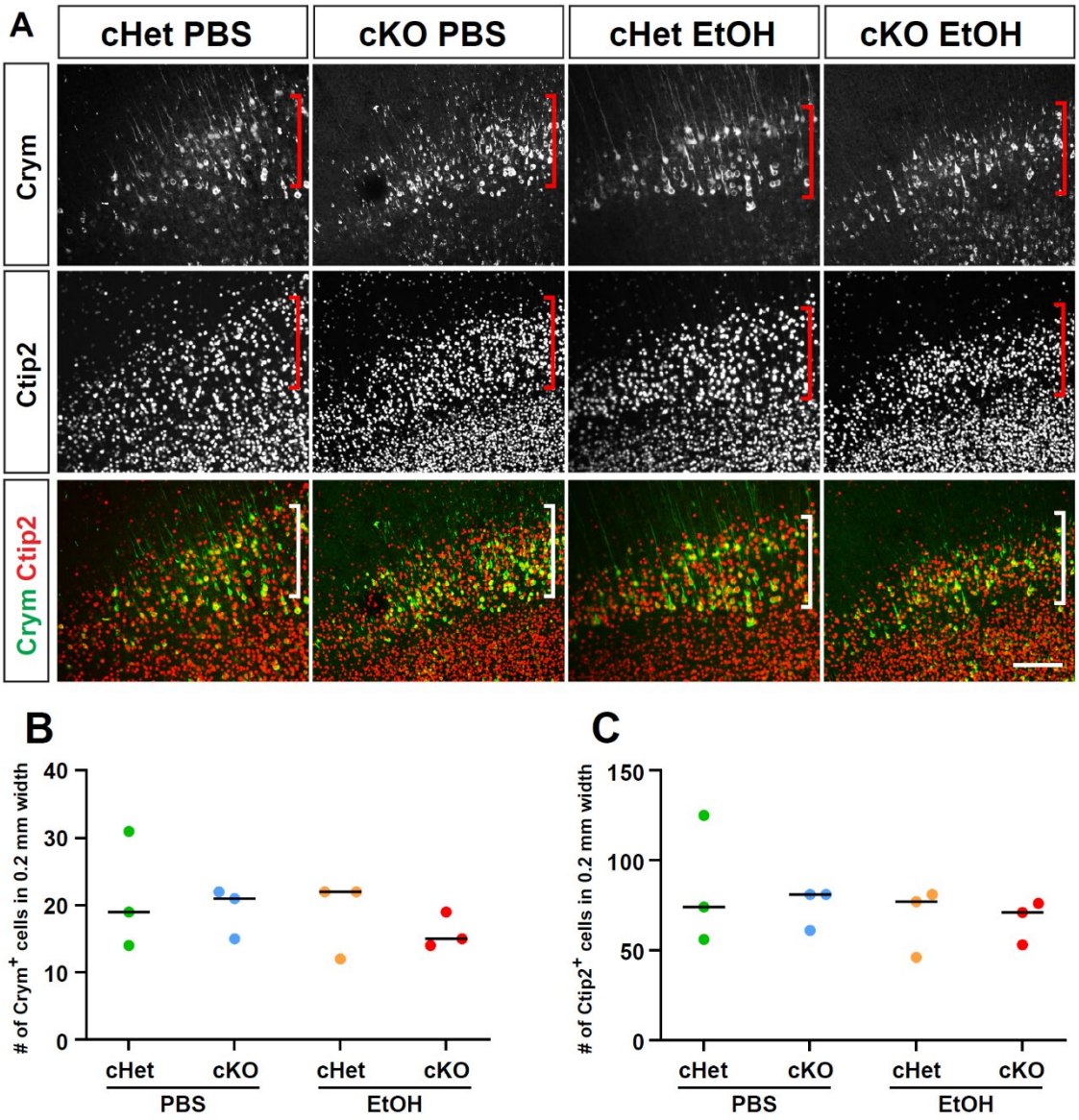

Fig. S2. No significant loss of Crym-positive or Ctip2-positive cortical neurons in layer V by EtOH exposure and/or loss of cilia

(A) Immunohistochemistry for Crym (green or white) and Ctip2 (red or white) at P21 for the indicated conditions. Brackets indicate layer V. Bar = 200 μm. (B, C) Quantification of Crym- (B) and Ctip2-positive neurons (C) in layer V in M1. The line indicates the mean.

Figure S3

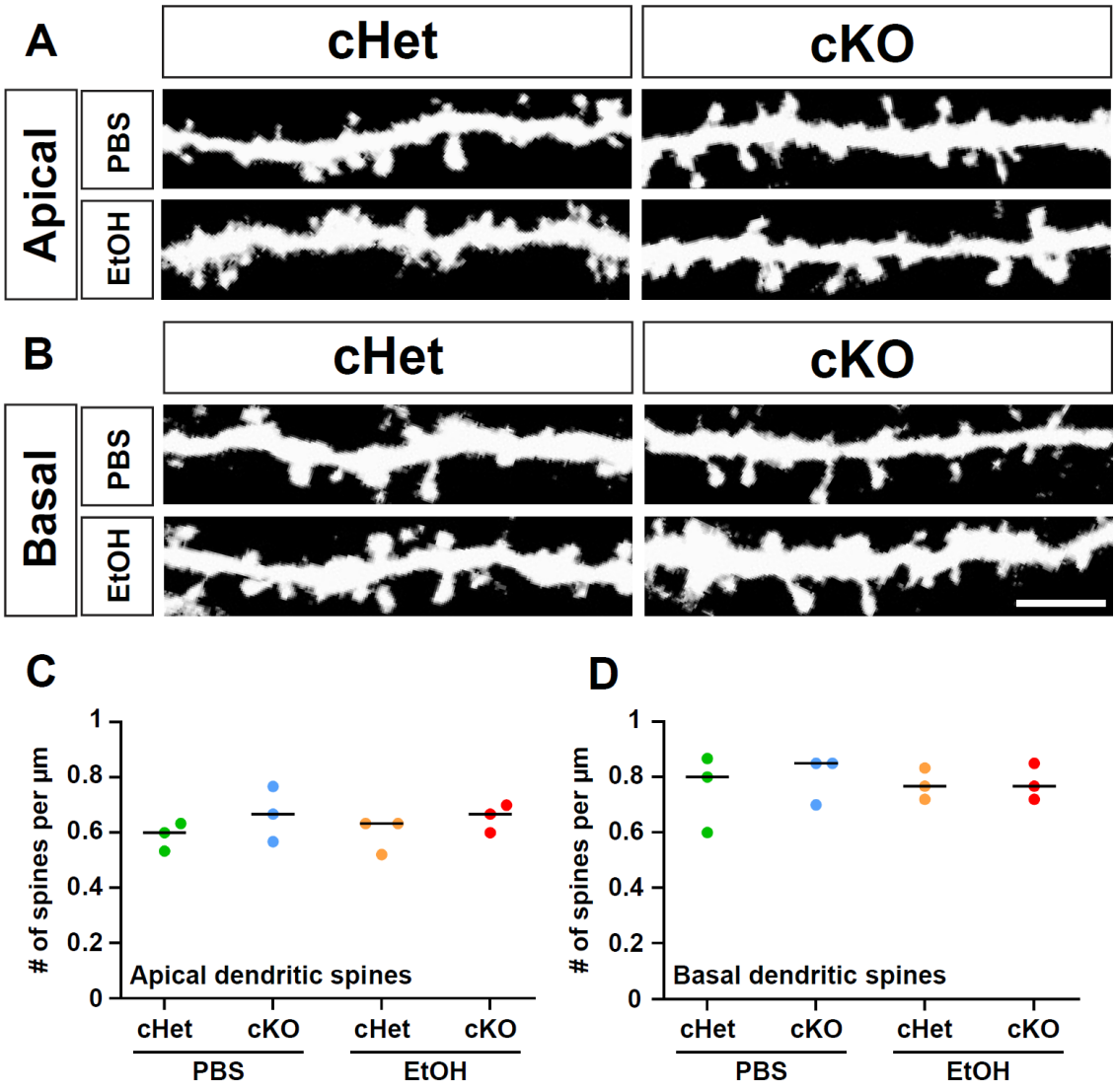

Fig. S3. No significant abnormalities in the dendritic spines of Thy1-YFP-positive cortical neurons by EtOH exposure and/or loss of cilia

(A, B) Representative images of spines on apical (A) and basal (B) dendrites of Thy1-YFP-positive layer V neurons in M1 for the indicated conditions at P21. Bar = 5 μm. (C, D) Quantification of spines on apical and basal dendrites of Thy1-YFP-positive neurons. The line indicates the mean.

Figure S4

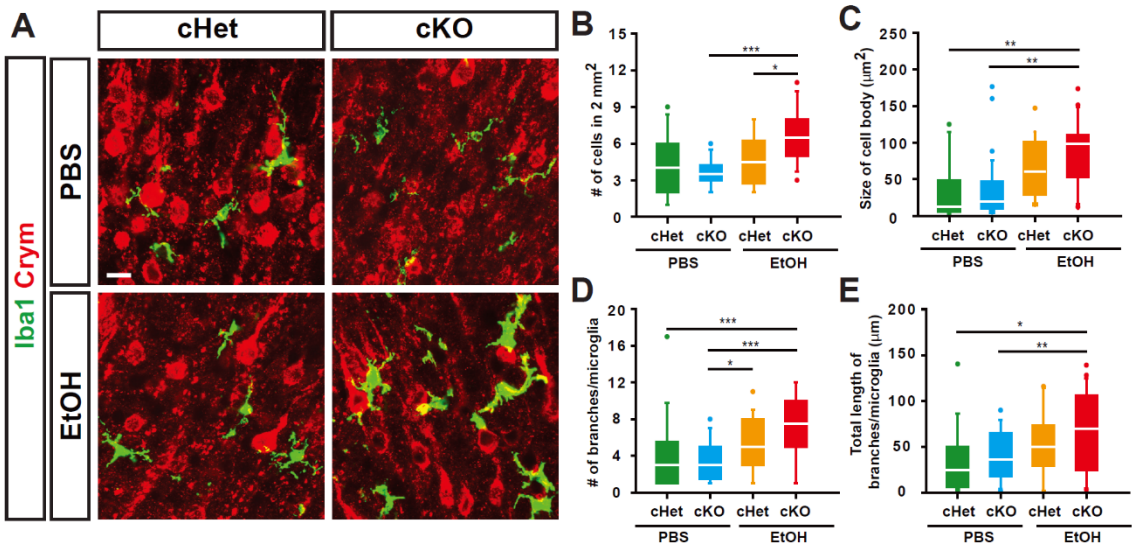

Fig. S4. Morphology of microglia in the cortex of cKO mice exposed to EtOH

(A) Iba1-positive microglia (green) are detected adjacent to Crym-positive layer V pyramidal neurons (red) for the indicated conditions 24 hours after exposure. Microglia with a large, ameboid shape are observed in EtOH-exposed cKO mice. Bar = 10 μm. (B-E) Measurements of cell number (B), soma size (C), number of branches per cell (D), and total length of branches (E) per cell indicate significant increase in the size and morphological complexity of microglia in cKO mice exposed to EtOH. *P < 0.05, **P < 0.01, ***P < 0.001 by post hoc Tukey or simple main effect test following two-way ANOVA.

Table S1. F and P values of two-way ANOVA analyses

Fig. #	Exposure/Treatment		Genotype		Interaction	
	F	P	F	P	F	P
1E	F (1,12) = 2.917	0.1133	F (1,12) = 0.0229	0.8822	F (1,12) = 0.102	0.7552
1F	F (1,12) = 50.89	<0.0001	F (1,12) = 44.88	<0.0001	F (1,12) = 43.99	<0.0001
1H	F (1,12) = 6.981	0.0215	F (1,12) = 3.875	0.0725	F (1,12) = 3.904	0.0716
1I	F (1,12) = 19.92	0.0008	F (1,12) = 14.98	0.0022	F (1,12) = 14.84	0.0023
2B	F (1,36) = 0.510	0.4800	F (1,36) = 3.907	0.0558	F (1,36) = 0.871	0.3568
2D (Total)	F (1,56) = 5.215	0.0262	F (1,56) = 13.25	0.0006	F (1,56) = 2.540	0.1166
2D (Apical)	F (1,56) = 3.826	0.0555	F (1,56) = 9.289	0.0035	F (1,56) = 3.826	0.0555
2D (Basal)	F (1,56) = 4.152	0.0463	F (1,56) = 10.93	0.0017	F (1,56) = 0.851	0.3601
2E (1st Apical)	F (1,56) = 1.333	0.2531	F (1,56) = 0.333	0.5660	F (1,56) = 0.333	0.5660
2E (2nd Apical)	F (1,56) = 1.510	0.2243	F (1,56) = 1.510	0.2243	F (1,56) = 2.109	0.1520
2E (3rd Apical)	F (1,56) = 4.206	0.0450	F (1,56) = 12.22	0.0009	F (1,56) = 4.206	0.0450
2E (1st Basal)	F (1,56) = 4.029	0.0496	F (1,56) = 5.401	0.0238	F (1,56) = 0.011	0.9162
2E (2nd Basal)	F (1,56) = 3.659	0.0609	F (1,56) = 10.61	0.0019	F (1,56) = 0.965	0.3302
2E (3rd Basal)	F (1,56) = 2.164	0.1469	F (1,56) = 9.513	0.0032	F (1,56) = 2.164	0.1469
2F (1st Apical)	F (1,44) = 0.028	0.8677	F (1,44) = 0.5307	0.4702	F (1,44) = 0.134	0.7165
2F (2nd Apical)	F (1,44) = 0.011	0.9153	F (1,44) = 6.779	0.0125	F (1,44) = 0.010	0.9226
2F (3rd Apical)	F (1,44) = 3.604	0.0642	F (1,44) = 10.17	0.0026	F (1,44) = 4.971	0.0309
2F (1st Basal)	F (1,44) = 0.343	0.561	F (1,44) = 0.283	0.5974	F (1,44) = 0.002	0.9618
2F (2nd Basal)	F (1,44) = 0.481	0.4916	F (1,44) = 3.904	0.0545	F (1,44) = 0.555	0.4602
2F (3rd Basal)	F (1,44) = 0.622	0.4345	F (1,44) = 4.285	0.0444	F (1,44) = 0.346	0.5597
2H	F (1,12) = 12.55	0.0040	F (1,12) = 9.350	0.0099	F (1,12) = 10.04	0.0081
2I	F (1,12) = 121.3	<0.0001	F (1,12) = 78.44	<0.0001	F (1,12) = 78.78	<0.0001
2J	F (1,12) = 15.37	0.0020	F (1,12) = 4.373	0.0584	F (1,12) = 4.315	0.0599

2K	F (1,12) = 23.90	0.0004	F (1,12) = 4.109	0.0655	F (1,12) = 4.285	0.0607
3E	F (1,12) = 9.566	0.0093	F (1,12) = 1.585	0.2319	F (1,12) = 2.071	0.1757
4C	F (1,12) = 44.26	<0.0001	F (1,12) = 32.89	<0.0001	F (1,12) = 27.84	0.0002
4F	F (1,12) = 12.66	0.0039	F (1,12) = 10.64	0.0068	F (1,12) = 9.996	0.0082
4G	F (1,12) = 14.21	0.0027	F (1,12) = 15.50	0.0020	F (1,12) = 14.15	0.0027
4I	F (1,36) = 0.100	0.7541	F (1,36) = 0.015	0.9040	F (1,36) = 0.642	0.4282
4J (Total)	F (1,56) = 1.221	0.2739	F (1,56) = 4.387	0.0407	F (1,56) = 6.039	0.0171
4J (Apical)	F (1,56) = 0.020	0.8888	F (1,56) = 3.332	0.0733	F (1,56) = 1.080	0.3032
4J (Basal)	F (1,56) = 2.454	0.1228	F (1,56) = 2.454	0.1228	F (1,56) = 8.009	0.0064
4K (1st Apical)	F (1,56) = 0.123	0.7273	F (1,56) = 0.123	0.7273	F (1,56) = 0.123	0.7273
4K (2nd Apical)	F (1,56) = 0.073	0.7879	F (1,56) = 1.827	0.1819	F (1,56) = 1.372	0.2464
4K (3rd Apical)	F (1,56) = 0.090	0.7655	F (1,56) = 2.884	0.0950	F (1,56) = 0.693	0.4087
4K (1st Basal)	F (1,56) = 0.128	0.7217	F (1,56) = 2.406	0.1265	F (1,56) = 4.612	0.0361
4K (2nd Basal)	F (1,56) = 2.704	0.1057	F (1,56) = 0.920	0.3415	F (1,56) = 4.808	0.0325
4K (3rd Basal)	F (1,56) = 4.334	0.0419	F (1,56) = 2.048	0.1579	F (1,56) = 8.193	0.0059
4L (1st Apical)	F (1,56) = 0.051	0.8217	F (1,56) = 0.390	0.5348	F (1,56) = 0.272	0.6043
4L (2nd Apical)	F (1,56) = 0.338	0.5635	F (1,56) = 1.895	0.1742	F (1,56) = 0.459	0.5010
4L (3rd Apical)	F (1,56) = 0.094	0.7597	F (1,56) = 3.102	0.0836	F (1,56) = 1.578	0.2143
4L (1st Basal)	F (1,56) = 0.092	0.7633	F (1,56) = 1.314	0.2565	F (1,56) = 3.833	0.0552
4L (2nd Basal)	F (1,56) = 3.898	0.0533	F (1,56) = 0.680	0.4131	F (1,56) = 0.942	0.3360
4L (3rd Basal)	F (1,56) = 2.176	0.1458	F (1,56) = 0.300	0.5859	F (1,56) = 4.922	0.0306
S4B	F (1,55) = 9.860	0.0027	F (1,55) = 2.154	0.1479	F (1,55) = 4.977	0.0298
S4C	F (1,94) = 26.43	<0.0001	F (1,94) = 2.031	0.1574	F (1,94) = 1.354	0.2475
S4D	F (1,85) = 11.31	0.0012	F (1,85) = 0.574	0.4506	F (1,85) = 2.486	0.1186
S4E	F (1,84) = 8.308	0.0050	F (1,84) = 1.750	0.1894	F (1,84) = 0.430	0.5140

Supplementary Table 1