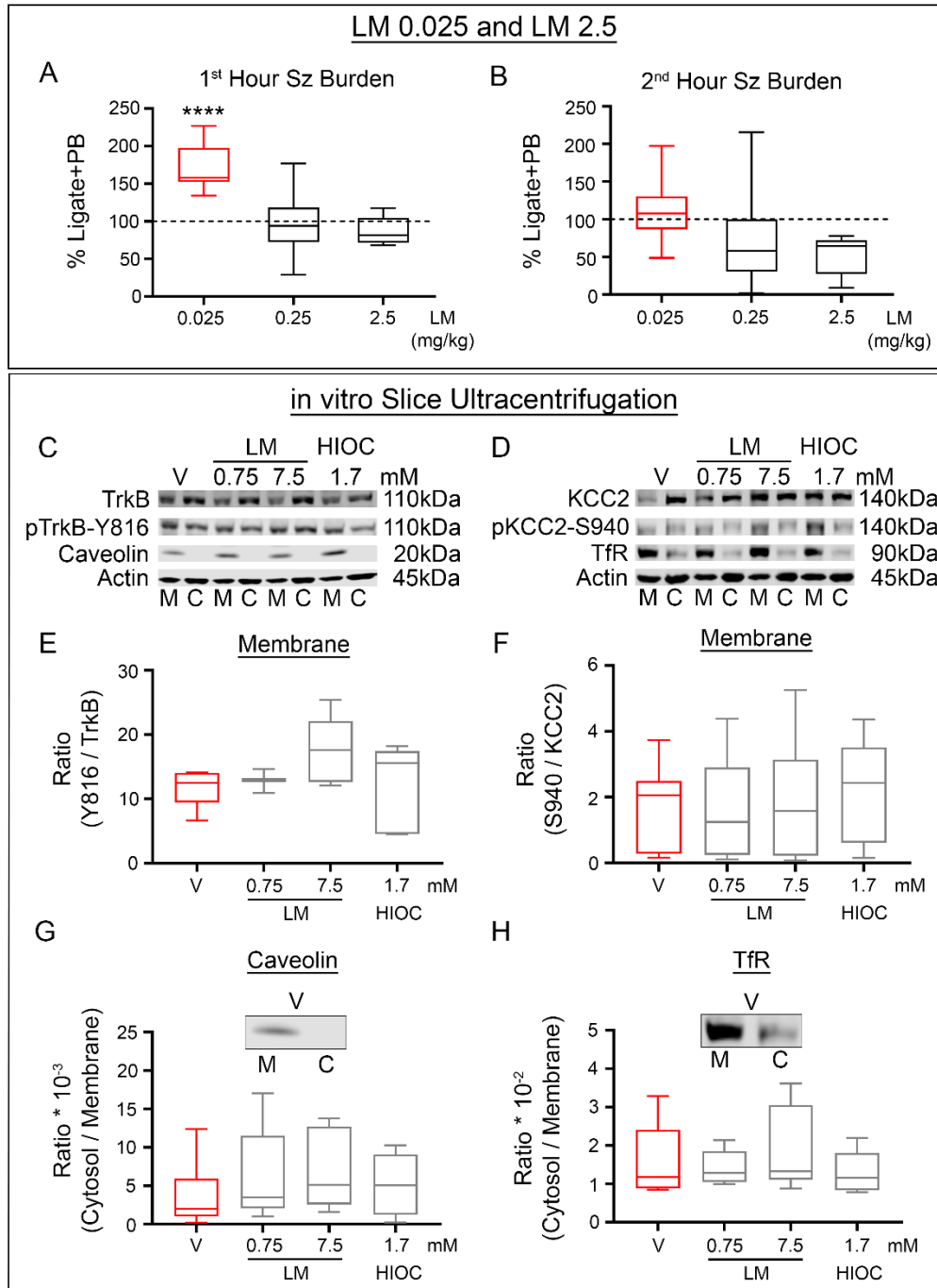1   **Supplementary Materials**

2   **Figure S1.**

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4   **Figure S1. Effect of LM graded doses on EEG seizure burdens as compared to effective**

5   **ASM dose and quantification of in vitro TrkB phosphorylation in naïve P7 brain slices. (A)**

1st hour EEG seizure burden as percent Ligate+PB for Post LM0.025, Post LM0.25, and Post LM2.5 treated P7 pups. **** $p < 0.001$ (Post LM0.025 vs. Ligate+PB) by one-way ANOVA. Number of mice (EEG): n = 28[16/12] (Ligate+PB [m/f]), 8[4/4] (Post LM0.025), 26[14/12] (Post LM), 8[4/4] (Post LM2.5). **(B)** 2nd hour EEG seizure burden as percent Ligate+PB for Post LM0.025, Post LM0.25, and Post LM2.5 treated P7 pups. **(C)** Representative Western blots showing membrane and cytosolic TrkB and pTrkB-Y816 expression after incubation with TrkB agonists. Plasma membrane fraction is denoted with an M, and proteins of interest were normalized to the membrane reference protein caveolin. Cytosolic fraction is denoted with a C, and proteins of interest were normalized to housekeeping protein β-actin. Number of mice and slices (WB): n = 2[4] (Vehicle [slices]), 2[4] (0.75mM LM), 2[4] (7.5mM LM), 2[4] (1.7mM HIOC). **(D)** Representative Western blots showing membrane and cytosolic KCC2 and pKCC2-S940 expression after incubation with TrkB agonists. **(E)** Ratio of pTrkB-Y816 to total TrkB at the plasma membrane. **(F)** Ratio of pKCC2-S940 to total KCC2 at the plasma membrane. **(G)** Ratio of caveolin-1 and **(H)** transferrin receptor fluorescence in the cytosolic fractions versus the membrane. All treatment groups showed similar expression levels, with membrane fraction fluorescence 2-3 orders of magnitude greater than the cytosolic fraction. Box-and-whisker plots show quartiles with median, and minima and maxima at the bottom and top whiskers respectively.
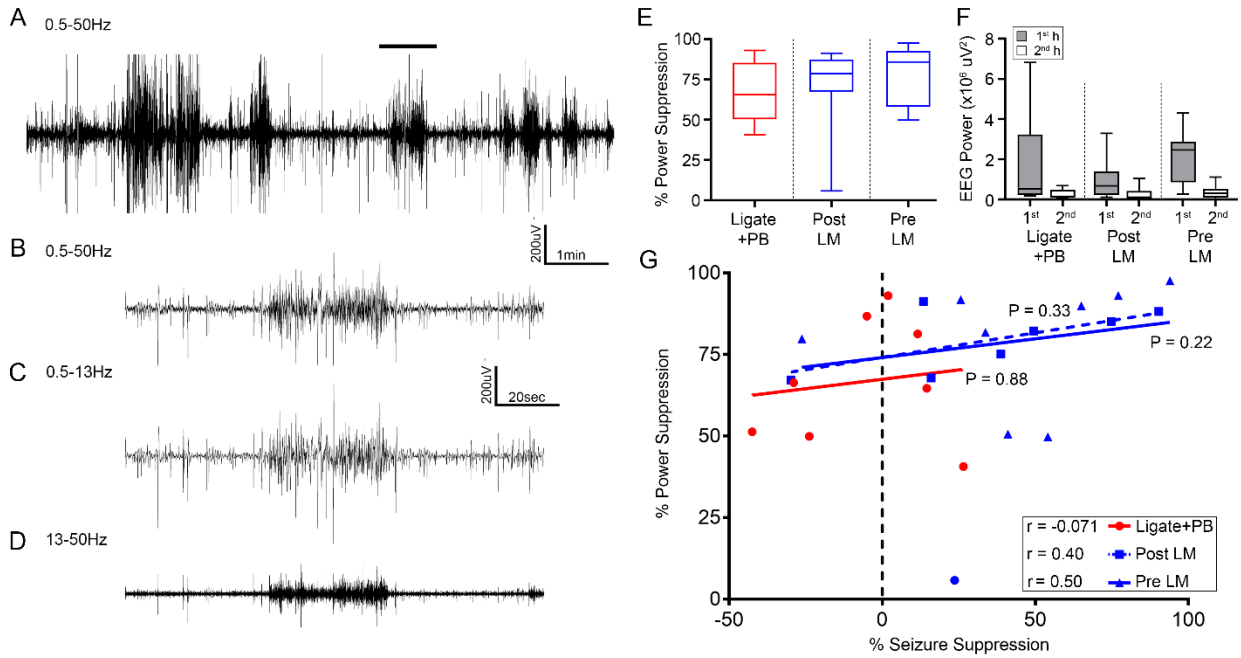
29   **Figure S2.**

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31   **Figure S2. EEG power alone failed to identify the LM-mediated rescue of PB-**

32   **refractoriness in P7 pups.** **(A)** Representative raw 10 min EEG trace from 0.5-50Hz of

33   refractory ischemic seizures from a neonatal P7 mouse pup. **(B)** A single ictal event from **A**

34   (solid bar – 2 min expanded timescale raw trace). **(C and D)** Filtered EEG trace of the same ictal

35   event in **B** filtered to show low frequency and high frequency components of the same ictal event

36   (0.5-13Hz and 13-50Hz). **(E)** EEG percent power suppression for Ligate+PB, Post LM, and Pre

37   LM treated P7 pups. Number of mice: n = 28 (Ligate+PB), 26 (Post LM), 27 (Pre LM). **(F)** EEG

38   1st and 2nd hour EEG powers for Ligate+PB, Post LM, and Pre LM treated P7 pups. **(G)** Percent

39   power suppression plotted as a function of percent seizure suppression. Post-hoc comparisons

40   were performed using Spearman's two-tailed nonparametric test. Box-and-whisker plots show

41   quartiles with median, and minima and maxima at the bottom and top whiskers respectively.

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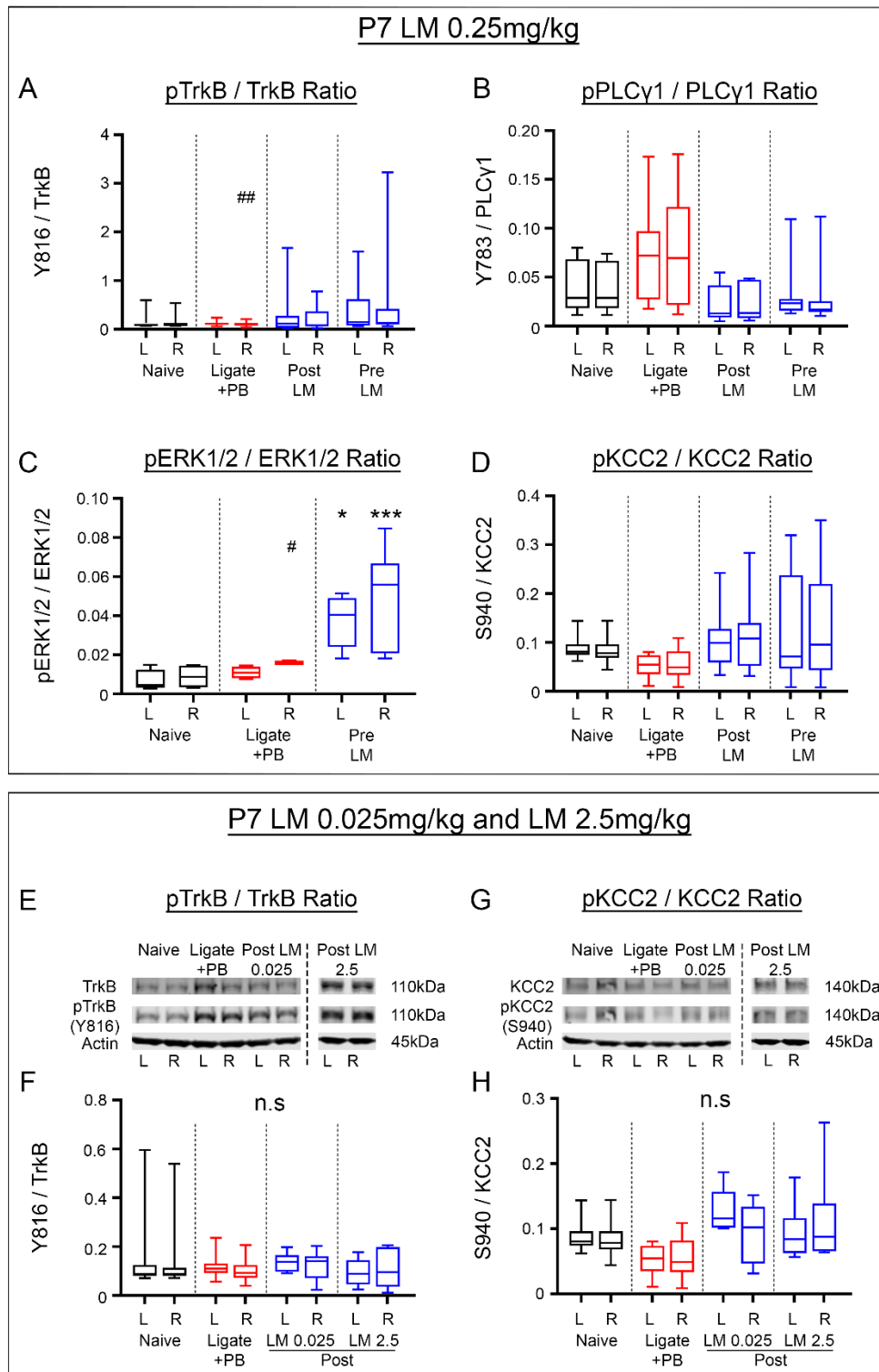
Figure S3.

Figure S3. Normalization of phospho-proteins to their total proteins in P7 ischemic pups.

(A) pTrkB-Y816 normalized to total TrkB for Naïve, Ligate+PB, Post LM, and Pre LM at P7. # signified hemispheric differences within groups, ## $p<0.01$ by two-tailed *t* test. Number of mice: n = 13 (Naïve), 19 (Ligate+PB), 13 (Post LM), 22 (Pre LM). **(B)** pPLCγ1-Y783 normalized to total PLCγ1 for Naïve, Ligate+PB, Post LM, and Pre LM at P7. **(C)** pERK1/2 normalized to total ERK1/2 for Naïve, Ligate+PB, and Pre LM at P7. * $p<0.05$, *** $p<0.001$ by one-way ANOVA. # $p<0.05$ by two-tailed *t* test. **(D)** pKCC2-S940 normalized to total KCC2 for Naïve, Ligate+PB, Post LM, and Pre LM at P7. **(E)** Representative Western blots showing contralateral (L) and ipsilateral (R) TrkB and pTrkB-Y816 expression in P7 pups treated with lower and higher graded doses of LM (LM0.025 and LM2.5 respectively). All proteins of interest were normalized to housekeeping protein β-actin. n = 13 (Naïve), 19 (Ligate+PB), 8 (Post LM 0.025), 13 (Post LM). **(F)** pTrkB-Y816 normalized to total TrkB for Naïve, Ligate+PB, Post LM0.025, and Post LM2.5. **(G)** Representative Western blots showing contralateral (L) and ipsilateral (R) KCC2 and pKCC2-S940 expression in P7 pups treated with graded doses of LM. All proteins of interest were normalized to housekeeping protein β-actin. **(H)** pKCC2-S940 normalized to total KCC2 for Naïve, Ligate+PB, Post LM0.025, and Post LM2.5. Box-and-whisker plots show quartiles with median, and minima and maxima at the bottom and top whiskers respectively.
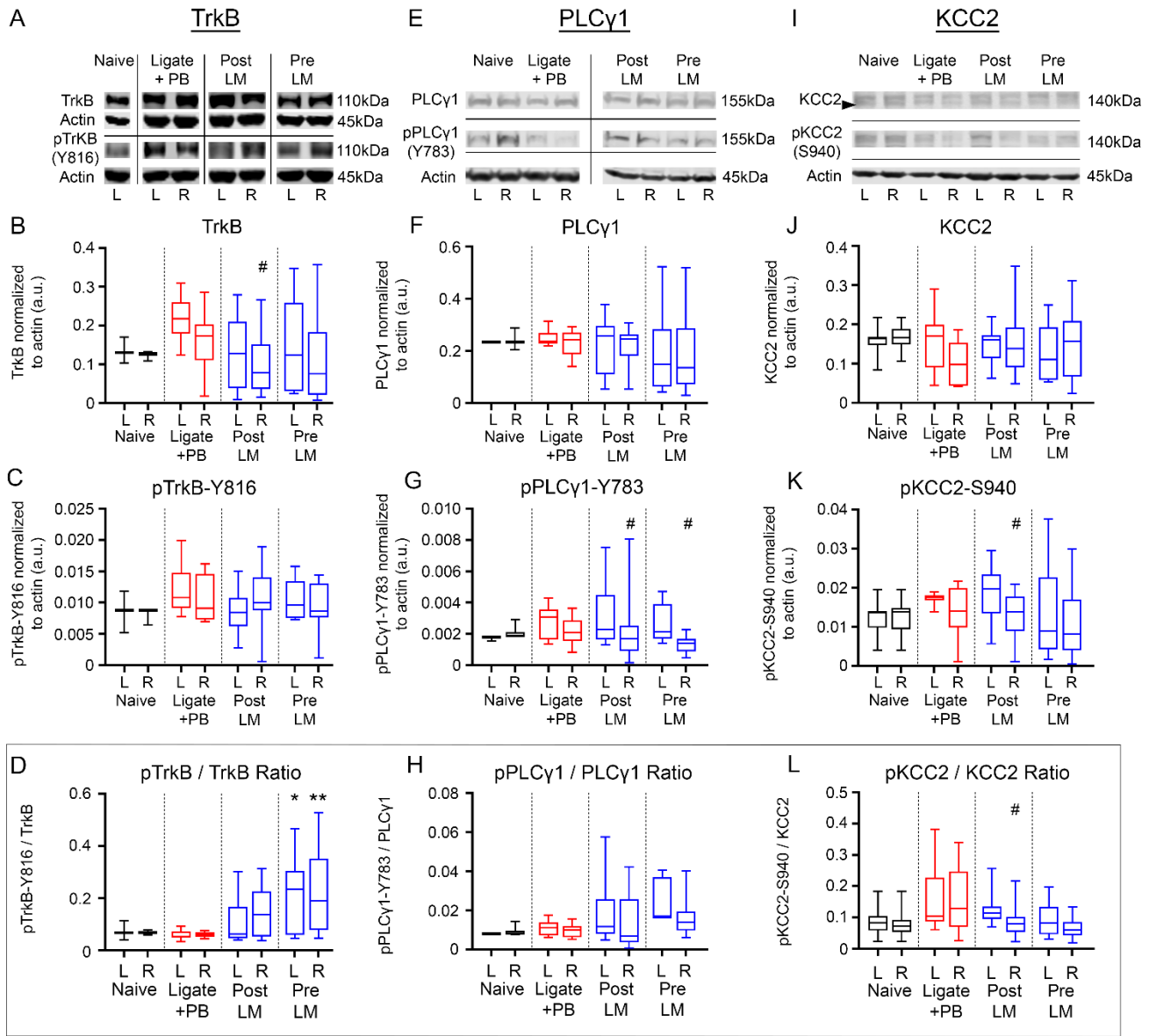
69 **Figure S4.**

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71 **Figure S4. TrkB-pathway activation was not significant in P10 ischemic pups.** All proteins

72 of interest were normalized to housekeeping protein β-actin. **(A)** Representative Western blots

73 showing TrkB and pTrkB-Y816 expression in P10 pups. Number of mice: n = 4 (Naïve), 9

74 (Ligate+PB), 12 (Post LM), 11 (Pre LM). **(B)** Contralateral (L) and ipsilateral (R) TrkB

75 expression 24h after ischemic insult for all treatment groups. # signified hemispheric differences

76 within groups. # p<0.05 by two-tailed *t* test. **(C)** Contralateral (L) and ipsilateral (R) pTrkB-

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Y816 expression 24h after ischemic insult for all treatment groups. **(D)** pTrkB-Y816 normalized to total TrkB for Naïve, Ligate+PB, Post LM, and Pre LM at P10. * $p<0.05$, ** $p<0.01$ by one-way ANOVA. **(E)** Representative Western blots showing PLCγ1 and pPLCγ1-Y783 expression. **(F)** Contralateral (L) and ipsilateral (R) PLCγ1 expression 24h after ischemic insult for all treatment groups. **(G)** Contralateral (L) and ipsilateral (R) pPLCγ1-Y783 expression 24h after ischemic insult for all treatment groups. # signified hemispheric differences within groups. # $p<0.05$ by two-tailed *t* test. **(H)** pPLCγ1-Y783 normalized to total PLCγ1 for Naïve, Ligate+PB, Post LM, and Pre LM at P10. **(I)** Representative Western blots showing KCC2 and pKCC2-S940 expression. **(J)** Contralateral (L) and ipsilateral (R) KCC2 expression 24h after ischemic insult for all treatment groups. **(K)** Contralateral (L) and ipsilateral (R) pKCC2-S940 expression 24h after ischemic insult for all treatment groups. # $p<0.05$ by two-tailed *t* test. **(L)** KCC2 normalized to total pKCC2-S940 for Naïve, Ligate+PB, Post LM, and Pre LM at P10. # signified hemispheric differences within groups. # $p<0.05$. Box-and-whisker plots show quartiles with median, and minima and maxima at the bottom and top whiskers respectively.
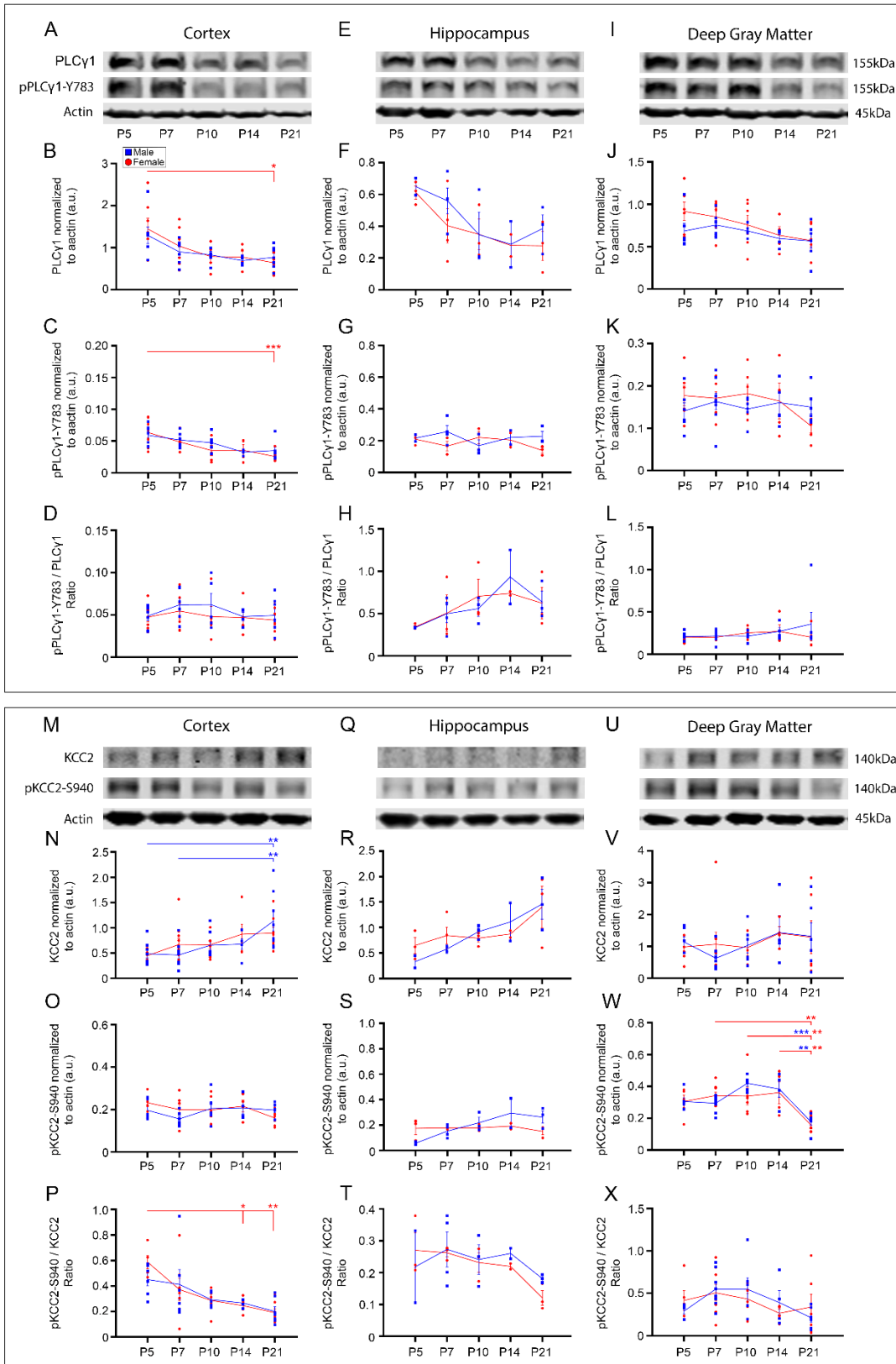
Figure S5.

Figure. S5. PLCγ1 and pPLCγ1-Y783 expression decreased significantly in female cortices, whereas KCC2 expression significantly increased in male cortices. All proteins were normalized to β-actin. **(A)** Representative Western blots showing PLCγ1 and pPLCγ1-Y783 expression in cortical tissue. **(B)** PLCγ1 and **(C)** pPLCγ1-Y783 expression in cortical tissue from P5 to P21. * $p<0.05$, *** $p<0.001$, **** $p<0.0001$ by two-way ANOVA. **(D)** pPLCγ1-Y783 normalized to total PLCγ1 in cortical tissue from P5 to P21. **(E)** Representative Western blots showing PLCγ1 and pPLCγ1-Y783 expression in hippocampal tissue. **(F)** PLCγ1 and **(G)** pPLCγ1-Y783 expression in hippocampal tissue from P5 to P21. **(H)** pPLCγ1-Y783 normalized to total PLCγ1 in hippocampal tissue from P5 to P21. **(I)** Representative Western blots showing PLCγ1 and pPLCγ1-Y783 expression in deep gray matter. **(J)** PLCγ1 and **(K)** pPLCγ1-Y783 expression in deep gray matter from P5 to P21. **(L)** pPLCγ1-Y783 normalized to total PLCγ1 in hippocampal tissue from P5 to P21. **(M)** Representative Western blots showing KCC2 and pKCC2-S940 expression in cortical tissue. **(N)** KCC2 and **(O)** pKCC2-S940 expression in cortical tissue from P5 to P21. ** $p<0.01$ by two-way ANOVA. **(P)** pKCC2-S940 normalized to total KCC2 in cortical tissue from P5 to P21. * $p<0.05$, ** $p<0.01$ by two-way ANOVA. **(Q)** Representative Western blots showing KCC2 and pKCC2-S940 expression in hippocampal tissue. **(R)** KCC2 and **(S)** pKCC2-S940 expression in cortical tissue from P5 to P21. **(T)** pKCC2-S940 normalized to total KCC2 in hippocampal tissue from P5 to P21. **(U)** Representative Western blots showing KCC2 and pKCC2-S940 expression in deep gray matter. **(V)** KCC2 and **(W)** pKCC2-S940 expression in deep gray matter from P5 to P21. ** $p<0.01$, *** $p<0.001$ by two-way ANOVA. **(X)** pKCC2-S940 normalized to total KCC2 in deep gray matter from P5 to P21. Number of mice per age group and region: n ≥4[2/2] (cortex [m/f]), ≥3[1/2] (hippocampus

124   [m/f]), ≥4[2/2] (deep gray matter [m/f]). Dot plots show all data and the mean ± 1 standard error

125   of the mean (SEM). Red (female) and blue (male) lines connect the means across age groups.

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147 **Table S1. Sample sizes for experimental paradigms**

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Sample size of pups in post-ligation EEG recordings			
Pups	**Ligate+PB**	**Post LM 0.25**	**Pre LM 0.25**
P7 total (M/F) [litters]	28 (16/12) [9]	26 (14/12) [6]	27 (14/13) [9]
P10 total (M/F) [litters]	11 (6/5) [3]	13 (6/7) [3]	11 (5/6) [3]
	Ligate+PB	**Post LM 2.5**	**Pre LM 2.5**
P7 total (M/F) [litters]	28 (16/12) [9]	8 (4/4) [2]	8 (4/4) [2]
	Ligate+PB	**Post LM 0.025**	
P7 total (M/F) [litters]	28 (16/12) [9]	8 (4/4) [1]	
	Ligate+PB	**Post HIOC**	**Pre HIOC**
P7 total (M/F) [litters]	28(16/12) [9]	6 (3/3) [2]	8 (4/4) [2]
	Ligate+PB	**Post DG**	**Pre DG**
P7 total (M/F) [litters]	28 (16/12) [9]	16 (8/8) [2]	15 (10/5) [2]

Sample size of pups that underwent Western Blot analysis after EEG recordings				
Pups	**Naïve**	**Ligate+PB**	**Post LM 0.25**	**Pre LM 0.25**
P7 total (M/F) [litters]	13 (6/7) [9]	19 (10/9) [7]	13 (5/8) [6]	22 (11/11) [9]
P10 total (M/F) [litters]	4 (2/2) [3]	9 (5/4) [3]	12 (6/6) [3]	11 (5/6) [3]
	Naïve	**Ligate+PB**	**Post LM2.5**	**Pre LM2.5**
P7 total (M/F) [litters]	13 (6/7) [9]	19 (10/9) [7]	8 (4/4) [2]	8 (4/4) [2]
	Naïve	**Ligate+PB**	**Post LM 0.025**	
P7 total (M/F) [litters]	13 (6/7) [9]	19 (10/9) [7]	8 (4/4) [1]	
	Naïve	**Ligate+PB**	**Post HIOC**	**Pre HIOC**
P7 total (M/F) [litters]	13 (6/7) [9]	19 (10/9) [7]	5 (3/2) [2]	6 (4/2) [2]
	Naïve	**Ligate+PB**	**Post DG**	**Pre DG**
P7 total (M/F) [litters]	13 (6/7) [9]	19 (10/9) [7]	6 (4/2) [2]	7 (4/3) [2]

Sample size of pups that underwent Western Blot analysis in Developmental Series			
Pups	**Cortex**	**Hippocampus**	**Deep Gray Matter**
P5 total (M/F) [litters]	4 (2/2) [3]	3 (1/2) [3]	4 (2/2) [3]
P7 total (M/F) [litters]	4 (2/2) [3]	6 (3/3) [3]	6 (3/3) [3]
P10 total (M/F) [litters]	4 (2/2) [3]	4 (2/2) [3]	4 (2/2) [3]
P14 total (M/F) [litters]	4 (2/2) [3]	4 (2/2) [3]	4 (2/2) [3]
P21 total(M/F) [litters]	4 (2/2) [3]	4 (2/2) [3]	4 (2/2) [3]

Sample size of pups that underwent in vitro drug incubation and Western Blot analysis			
Pups	**0.75mM LM**	**7.5mM LM**	**1.7mM HIOC**
P7 total (M/F) [litters]	2 (1/1) [1]	2 (1/1) [1]	2 (1/1) [1]

Sample size of pups from TrkB^{F616A} litters		
Pups	**WT$^{-/-}$ + 1NMPP1**	**F616A$^{+/+}$ + 1NMPP1**
P7 total (M/F) [litters]	18 (9/9) [5]	10 (6/4) [5]

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Table S2. Drugs, antibodies, and mice information

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Antibody	Dilution	Vendor	RRID
CD-1 Mouse	N/A	Charles River	022
C57 Black $TrkB^{F616A}$ Mouse	N/A	The Jackson Laboratory	022363
Phenobarbital (PB)	N/A	MilliporeSigma P5178-25G	57-30-7
LM22A-4	N/A	Tocris	4607
HIOC	N/A	Tocris	5961
Deoxygedunin (DG)	N/A	Gaia Chemical Company	L4250
1NMPP1	N/A	Apex Bio	B1299
mouse α KCC2	1:1000	Aviva Systems Biology OASE00240	AB_2721238
rabbit α pKCC2-S940	1:1000	Aviva Systems Biology OAPC00188	AB_2721198
mouse α TrkB	1:1000	BD Biosciences 610102	AB_397508
rabbit α pTrkB-Y816	1:500	Millipore ABN1381	AB_2721199
mouse α PLCγ1	1:1000	Thermo Fisher Scientific LF-MA0050	AB_2163544
rabbit α pPLCγ1-Y783	1:1000	Cell Signaling Technology 2821S	AB_330855
rabbit α Erk1/2	1:1000	Cell Signaling Technology 4695	AB_390779
rabbit α pErk1/2-Thr202/Tyr204	1:1000	Cell Signaling Technology 4377	AB_331775
mouse α actin	1:10000	LI-COR Biosciences 926-42213	AB_2637092
mouse α Transferrin Receptor	1:500	ThermoFisher Scientific	AB_2533029
rabbit α Caveolin-1	1:1000	Abcam	AB_444314
goat α mouse IgG, IRDye® 800CW Conjugated	1:5000	LI-COR Biosciences 926-32210	AB_621842
goat α rabbit IgG Antibody, IRDye® 680LT Conjugated	1:5000	LI-COR Biosciences 926-68021	AB_10706309

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