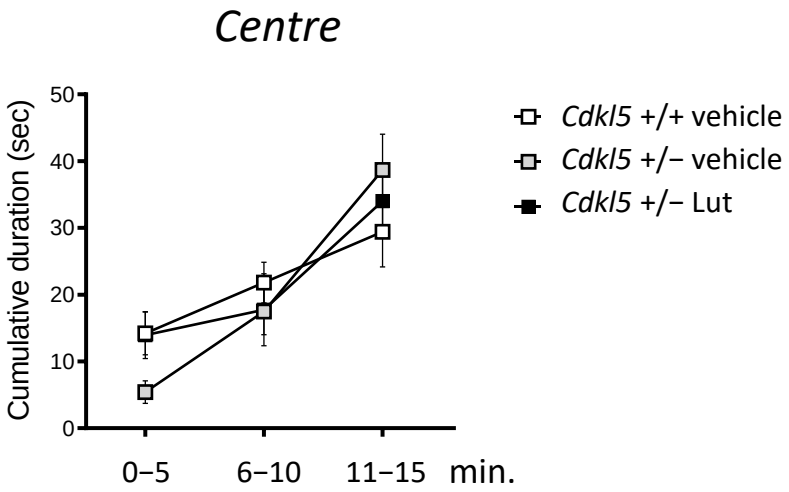


Figure S1. Anxiety-like behavior in *Cdkl*5 +/− mice and effect of luteolin treatment.

Time (cumulative duration) spent in the center of the open-field arena. Vehicle-treated *Cdkl*5 +/+ (n = 19), *Cdkl*5 +/− (n = 18) mice, and luteolin-treated *Cdkl*5 +/− (n = 17) mice spent a comparable time in the center of the arena, suggesting that hyperactivity in *Cdkl*5 +/− was not due to increased anxiety. Fisher's LSD test after two-way ANOVA.

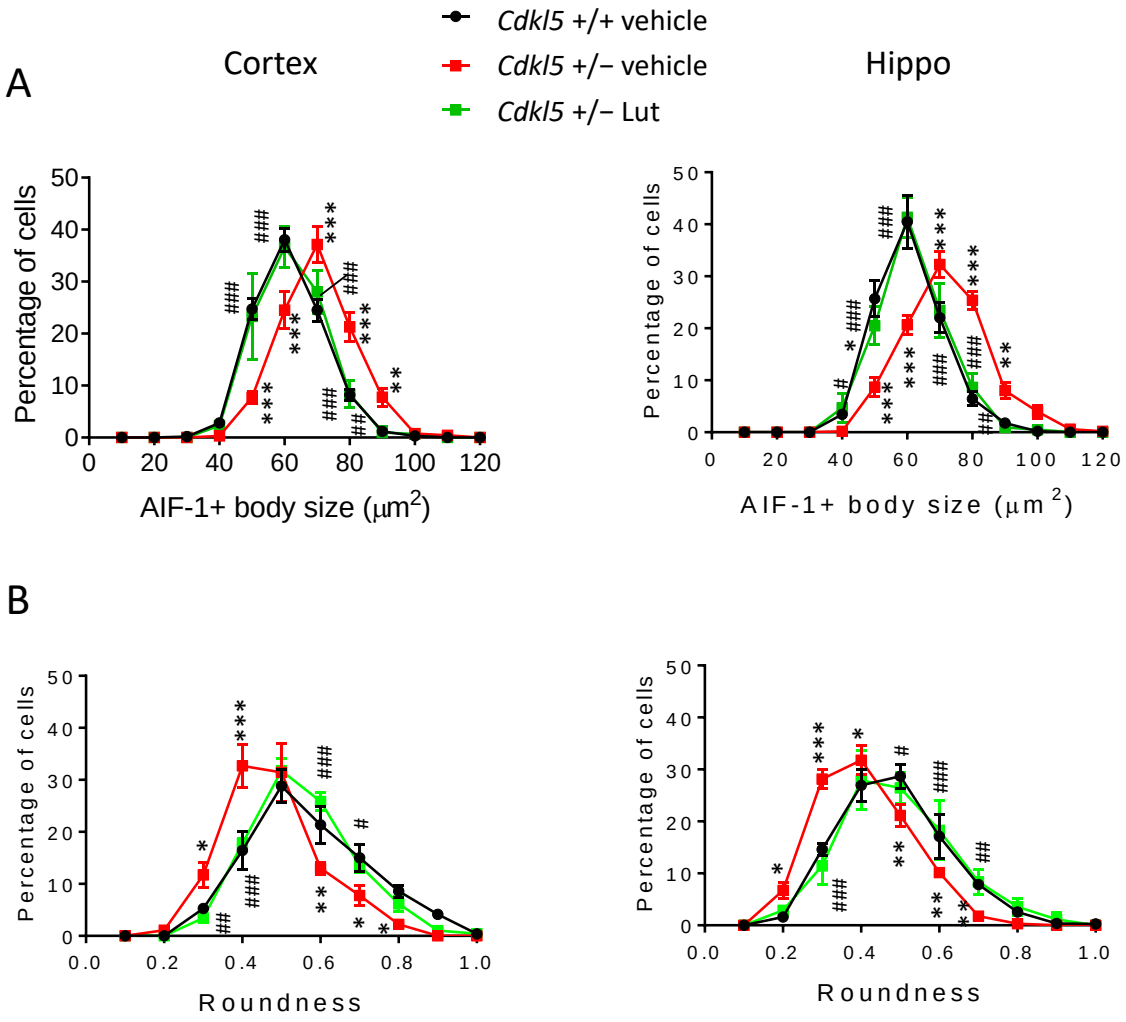


Figure S2. Luteolin treatment completely rescues microglia overactivation in the brain of *Cdkl*5 +/− mice. (A,B) Distribution analysis of microglial cell soma area (A) and microglial cell circularity (roundness; B) in the somatosensory cortex (on the left) and hippocampus (on the right) of vehicle-treated *Cdkl*5 +/+ (n = 5) and *Cdkl*5 +/− (n = 6) mice and luteolin-treated *Cdkl*5 +/− (n = 4) mice. The enlarged body size and irregular shape of microglia cells in the brain of *Cdkl*5 +/− mice was completely restored in 20-day luteolin treated *Cdkl*5 +/− mice. The results in A and B are presented as means ± SEM. *p<0.05, **p<0.01, ***p<0.001 as compared to the vehicle-treated *Cdkl5* +/+ mice; #p<0.01, ##p<0.01, ###p<0.001 as compared to the vehicle-treated *Cdkl5* +/− mice. Fisher's LSD test after two-way ANOVA.

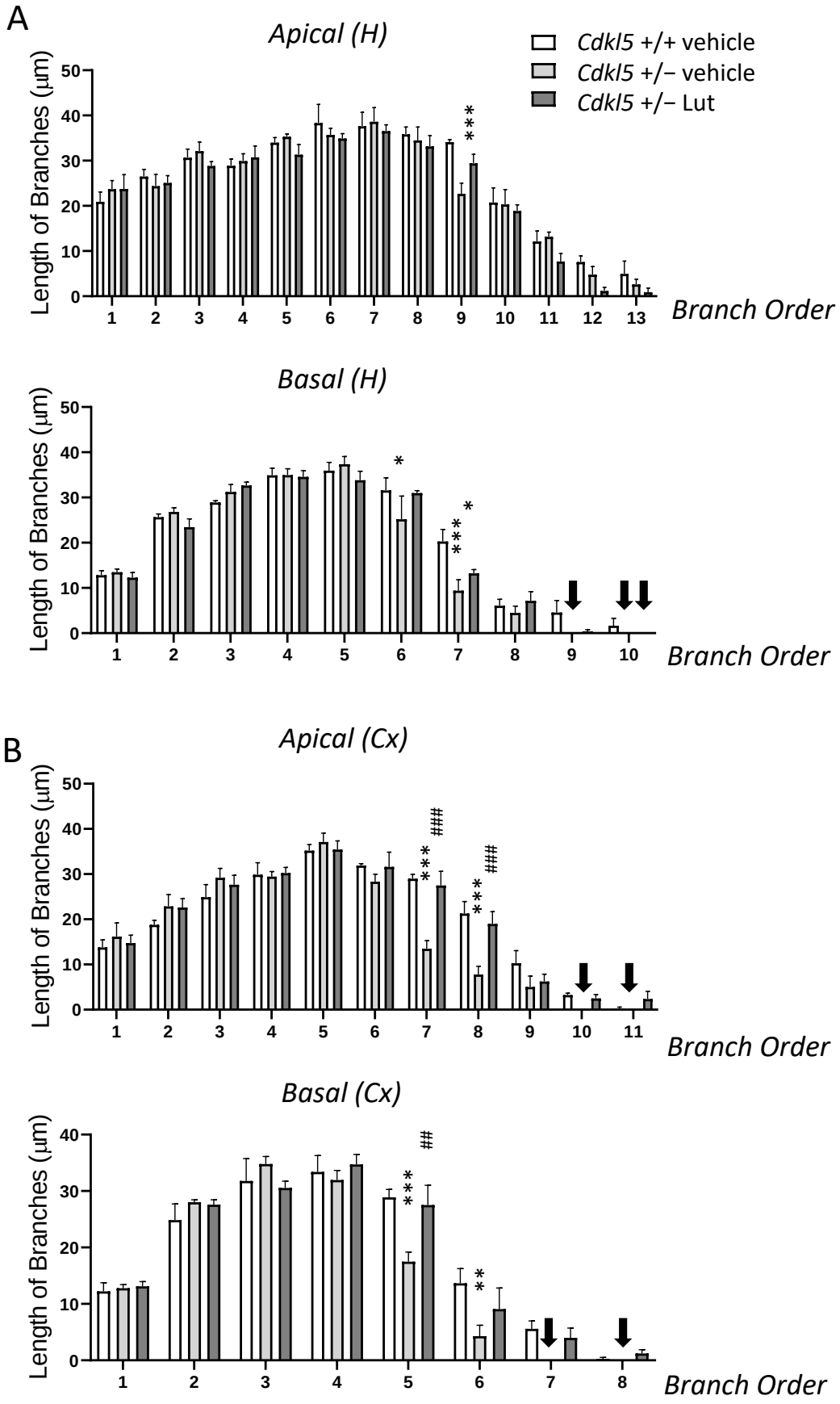

A
Apical (H)
Cdkl5 +/+ vehicle
Cdkl5 +/− vehicle
Cdkl5 +/− Lut
Length of Branches (µm)
Branch Order
Basal (H)
B
Apical (Cx)
Basal (Cx)

Figure S3. Effect of luteolin treatment on mean length of dendritic branches of hippocampal and cortical neurons in *Cdkl*5 +/− mice. (A,B) Quantification of the length of branches of different orders of apical dendrites (upper panel) and basal dendrites (lower panel) in Golgi-stained hippocampal (A) and cortical (B) pyramidal neurons of vehicle-treated *Cdkl5* +/+ (n = 4) and *Cdkl5* +/− (n = 4) mice and luteolin-treated *Cdkl5* +/− (n = 4) mice. Black arrows indicate the lack of branches of that order. H = hippocampus; CX = cortex. Values in A and B are represented as means ± SEM. *p<0.05, **p<0.01, ***p<0.001 as compared to the vehicle-treated *Cdkl*5 +/− condition; ##p<0.01, ###p<0.001 as compared to the vehicle-treated *Cdkl*5 +/− condition. Fisher's LSD test after two-way ANOVA.

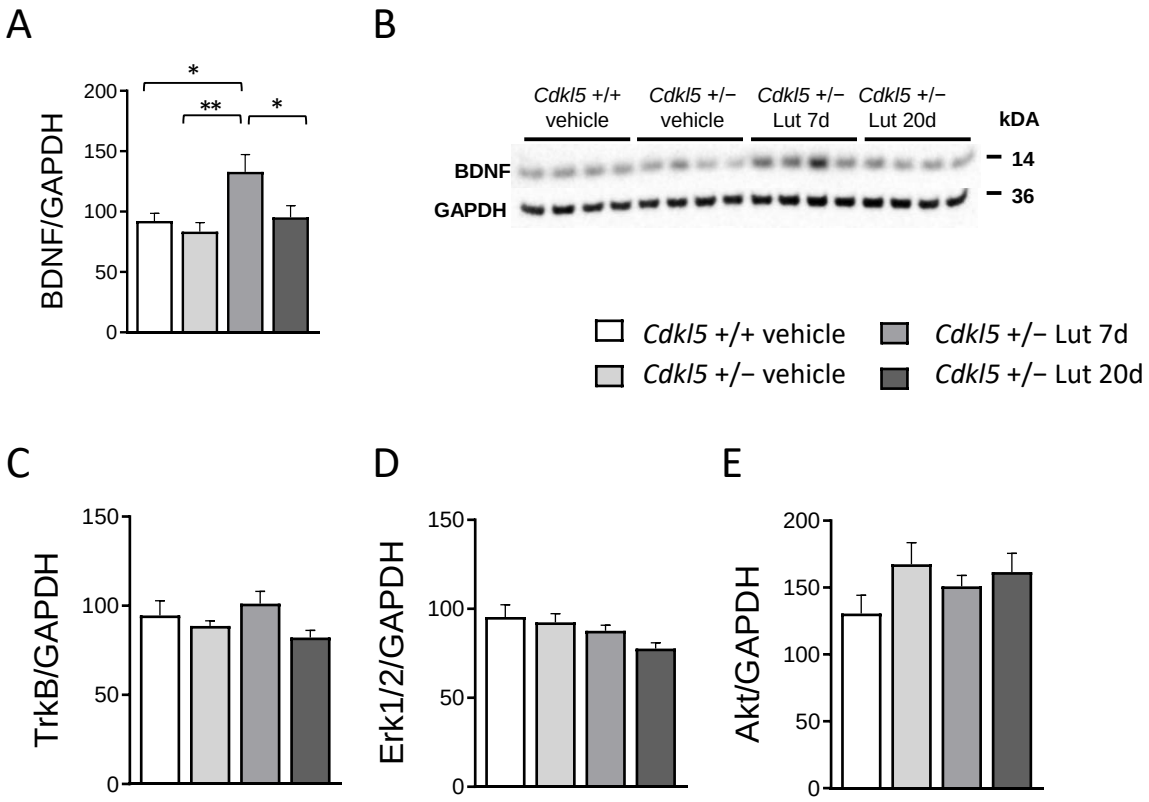


Figure S4. Effect of luteolin treatment on BDNF, TrkB, Erk 1/2 and Akt protein levels in the cortex of *Cdkl*5 +/− mice. (A,B) Western blot analysis of BDNF levels in somatosensory cortex homogenates from vehicle-treated *Cdkl5* +/+ (n = 4) and *Cdkl5* +/− (n = 4) mice, 7-day luteolin treated *Cdkl5* +/− (Lut 7d, n = 4) and 20-day luteolin treated *Cdkl5* +/− (Lut 20d, n = 4) mice. The histogram in A shows mature BDNF protein levels normalized to GAPDH protein levels. **(B)** Example of immunoblot for BDNF; part of the BDNF immunoblot was previously published in [50]. **(C-E)** Histograms show protein levels of TrkB (B), Erk 1/2 (C) and Akt (D) normalized to GAPDH levels in somatosensory cortex homogenates from vehicle-treated *Cdkl5* +/+ (n = 4) and *Cdkl5* +/− (n = 4) mice, 7-day luteolin treated *Cdkl5* +/− (Lut 7d, n = 4) and 20-day luteolin treated *Cdkl5* +/− (Lut 20d, n = 4) mice. Data are expressed as a percentage of vehicle-treated *Cdkl5* +/+ mice. Values represent mean ± SEM. *p<0.05, **p<0.01 (Fisher's LSD test after two-way ANOVA).

					Half emispheres		
Cohort	**N° of animals**	**Genotype**	**Treatment**	**Behavioral studies**	**IHC**	**Golgi staining**	**WB**
1	10	*Cdkl5* +/+	Vehicle	10	-	-	-
	10	*Cdkl5* +/−	Vehicle	10	-	-	-
	10	*Cdkl5* +/−	Lut 20	10	-	-	-
2	11	*Cdkl5* +/+	Vehicle	11	-	-	-
	8	*Cdkl5* +/−	Vehicle	8	-	-	-
	8	*Cdkl5* +/−	Lut 20	8	-	-	-
3	11	*Cdkl5* +/+	Vehicle	-	14	4	4
	14	*Cdkl5* +/−	Vehicle	-	20	4	4
	14	*Cdkl5* +/−	Lut 20	-	20	4	4
	4	*Cdkl5* +/−	Lut 7	-	-	-	4

Table S1. List of cohorts of treated mice and their use. WB: western blotting, IHC: immunohistochemistry.

	Target	Description	Dilution	Manufacturer
Primary Antibodies	AIF-1	Rabbit polyclonal	IHC 1:300	Thermo Fisher Scientific
	Ki67	Rabbit monoclonal	IHC 1:200	Thermo Fisher Scientific
	DCX	Goat polyclonal	IHC1:100	Santa Cruz Biotechnology
	DCX	Rabbit polyclonal	IHC1:300	Thermo Fisher Scientific
	BrdU	Rat monoclonal	IHC 1:200	Abcam
	PSD95	Rabbit polyclonal	IHC 1:200	Abcam
	GAPDH	Rabbit polyclonal	WB 1:5000	Sigma-Aldrich
	BDNF	Rabbit polyclonal	WB 1:500	Santa Cruz
	P-TrkB (Ser816)	Rabbit polyclonal	WB 1:500	Millipore
	TrkB	Rabbit polyclonal	WB 1:500	Santa Cruz
	P-Erk1/2 (Thr202/Tyr204)	Rabbit polyclonal	WB 1:1000	Cell signaling
	Erk1/2	Rabbit polyclonal	WB 1:1000	Cell signaling
	P-Akt (Ser473)	Rabbit polyclonal	WB 1:1000	Cell signaling
	Akt	Rabbit polyclonal	WB 1:1000	Cell signaling
Secondary Antibodies	Goat anti-Rabbit IgG HRP-conjugated		WB 1:5000	Jackson ImmunoResearch
	Biotinylated anti-goat IgG		IHC 1:200	Vector Bio-Labs
	Goat anti-mouse IgG Cy3-conjugated		IHC 1:200	Jackson ImmunoResearch
	Goat anti-rat IgG Cy3-conjugated		IHC 1:200	Jackson ImmunoResearch
	Goat anti-rabbit IgG Cy3-conjugated		IHC 1:200	Jackson ImmunoResearch

Table S2. List of primary and secondary antibodies. WB: western blotting, IHC: immunohistochemistry.

	Before treatment	End of the treatment	
	Vehicle	**Vehicle**	p
***Cdkl5* +/+**	20.37 ± 0.32	20.36 ± 0.30	n.s.
	Vehicle	**Vehicle**	
***Cdkl5* +/−**	20.72 ± 0.44	20.87 ± 0.37	n.s.
p	n.s.	n.s.	
	Luteolin	**Luteolin**	
***Cdkl5* +/−**	20.23 ± 0.62	20.82 ± 0.48	n.s.
p	n.s	n.s	

Table S3. Effect of treatment with luteolin on body weight. Body weight in grams of *Cdkl5* +/+ (vehicle n = 21) and *Cdkl5* +/− (vehicle n = 18 or luteolin n = 18) mice at the beginning and at the end of a 20 days of daily treatment with vehicle or luteolin (10 mg/kg). Values are represented as mean ± SEM, n.s. not significant (two-tailed Student's t-test).