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Supplemental information

Overactive PKA signaling underlies the hyperalgesia in an ADHD mouse model

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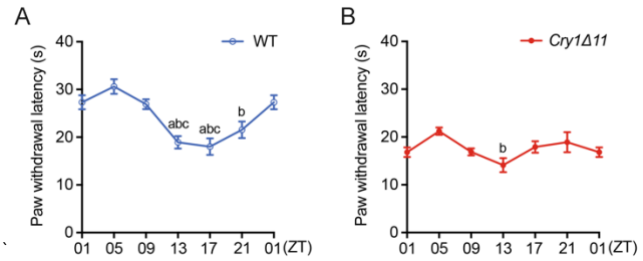

Figure S1. Sidak's multiple comparisons of mean paw withdrawal latencies at different time points for WT (A) and *Cry1Δ11* mice (B), Related to Figure 1. The letters a, b and c above the curves indicated the data at that time point was significantly different as compared to the data at ZT01, ZT05 and ZT09, respectively. Data are presented as mean ± SEM (n=5 mice/group/time point). One-way ANOVA with *post hoc* Sidak analysis.

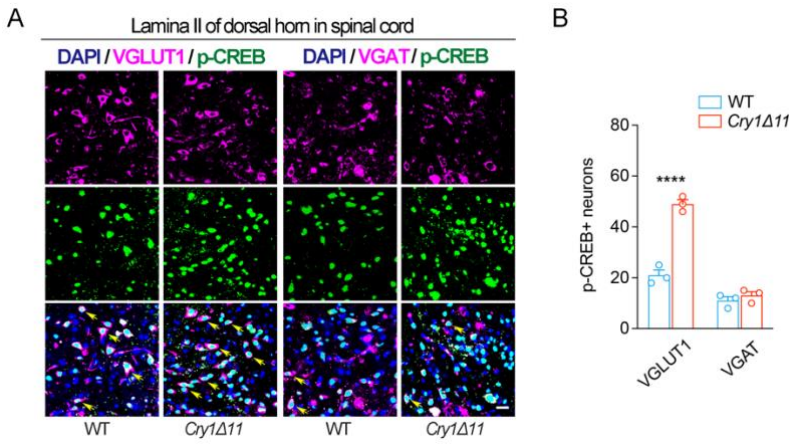

Figure S2. Localization of p-CREB in the excitatory and inhibitory neurons, Related to Figure 3. (A) Representative immunofluorescence images, showing p-CREB, VGLUT1, VGAT and DAPI in the spinal cords of WT and *Cry1Δ11* mice. Representative co-localized neurons are indicated with arrows. (B) Quantification of neurons co-expressing p-CREB/VGLUT1 or p-CREB/VGAT in the lamina II of the dorsal horn in the spinal cords of WT and *Cry1Δ11* mice. Data are presented as means ± SEM. n=3 mice/genotype; ****P< 0.0001, 2-way ANOVA followed by Bonferroni's *t*-test.

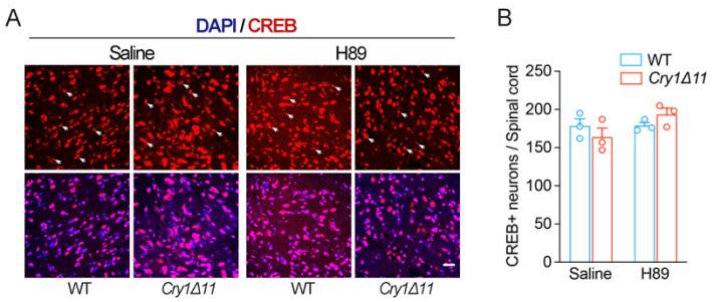

Figure S3. H89 treatment did not alter the number of CREB-positive neurons in the spinal cord, Related to Figure 5. (A) Representative immunofluorescence of CREB in the lamina II of the dorsal horn in the spinal cords. Representative positive neurons are indicated with arrows. (B) Quantification of CREB-positive neurons in the spinal cord. n=3 mice.

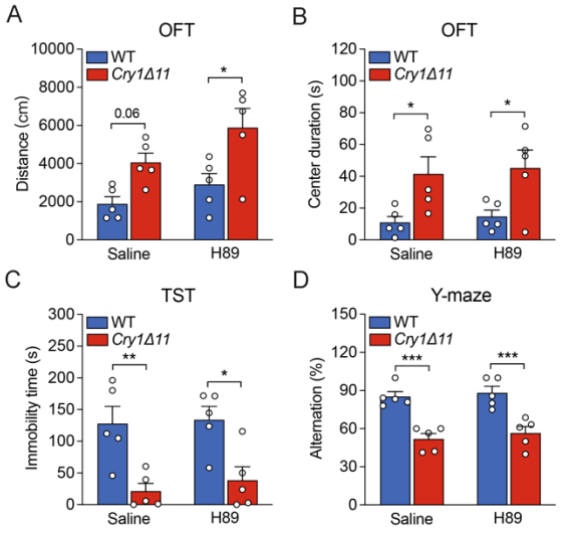

Figure S4. H89 treatment did not influence the ADHD-like behaviors in *Cry1Δ11* mice, Related to Figure 5. Saline or H89 (15 μg/kg) were injected i.t. during ZT01-05 and behaviors were measured at 60 minutes after injection. OFT, open field test; TST, tail suspension test. Data are presented as mean ± SEM; n= 5 mice/genotype/treatment, *P< 0.05, **P< 0.01, ***P< 0.001, 2-way ANOVA followed by Bonferroni's *t*-test.

Table S1. **The top 30 upregulated putative PKA phosphorylation sites identified in *Cry1Δ11* mice, Related to Figure 6.**

Gene name	Position	Modified sequence	P-value	Gene name	Position	Modified sequence	P-value
Prx	S1028	KVS*	<0.0001	Arpc1b	S310	KAS*	0.0171
Prx	S979	RDS*	0.0005	Spire1	S365	RHS*	0.0037
Plekha1	S375	RLS*	0.0009	Spire1	T370	RHS*	0.0037
Mylk	S1795	KSS*	0.0156	Tkt	S104	KIS*	0.026
Epb41	S703	RLS*	0.0311	Baiap3	S215	RSS*	0.0002
Tnik	S984	KIS*	0.0124	Mpz	S195	RLS*	0.0024
Rasgrp2	S147	KMS*	0.0152	Arhgap20	S669	KVS*	0.0049
Hcls1	S62	KVS*	0.0311	Sorbs2	S339	RKS*	0.0386
Rgl1	S520	RLS*	0.0264	Pdlim4	S119	RSS*	0.0052
Gprc5b	S368	KPS*	0.0242	Sh3rf3	S376	RHS*	0.0095
Plekhg3	S862	RES*	0.0013	Shroom3	S675	RQS*	0.0044
Layn	S299	KQS*	0.0129	Pllp	S9	KVS*	0.0018
Map4	S973	RVS*	0.0045	Dock2	S1704	RSS*	0.0309
Glcci1	S248	RQS*	0.0031	Plxnc1	S984	KQS*	0.0016
Lrch3	S415	RIS*	0.0184	Klc4	S566	RSS*	0.011

*, the phosphorylated Serine/Threonine.

Table S2. The top 30 downregulated putative PKA phosphorylation sites identified in *Cry1Δ11* mice, Related to Figure 6.

Gene name	Position	Modified sequence	P-value	Gene name	Position	Modified sequence	P-value
Slc4a4	S1029	KGS*	0.0488	Clip3	S399	KKS*	0.0025
Sgsm1	S229	RHS*	0.0438	Mepce	T188	KSS*	0.0006
Ryr1	S2844	KIS*	0.0006	Mepce	S192	KSS*	0.004
Hnrnpc	S306	RDS*	0.0377	Pitpnm1	S600	RGS*	0.0021
Ank2	S2422	KES*	0.0012	Nlgn2	S714	RLS*	0.0005
Rbm10	S89	RHS*	0.0038	Cul9	S1318	RPS*	0.0382
Pitpnc1	S119	KGS*	0.0078	Cwc22	S829	RDS*	0.0206
Pitpnc1	S122	KGS*	0.0078	Ube4b	S674	KDS*	0.0093
Chl1	S1148	KGS*	0.0149	Jup	S665	RVS*	0.0063
Top2b	S1387	KAS*	0.0039	Folh1	S10	RDS*	0.004
Ankrd34b	S496	RQS*	0.0053	Hepacam	S320	KDS*	0.0237
Pitpnm3	S343	KQS*	0.023	Srcin1	S588	KDS*	0.0035
G3bp1	S7	KPS*	0.0358	Rbm10	S723	RPS*	0.0257
Prkar2a	S96	RVS*	0.0377	Ifih1	S302	RVS*	0.0134
Prkaca	S339	RVS*	0.0055	Pclo	S1378	KVS*	0.0011

*, the phosphorylated Serine/Threonine.