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Supplemental Information

Dysregulation of the Synaptic Cytoskeleton in the PFC Drives Neural Circuit Pathology, Leading to Social Dysfunction

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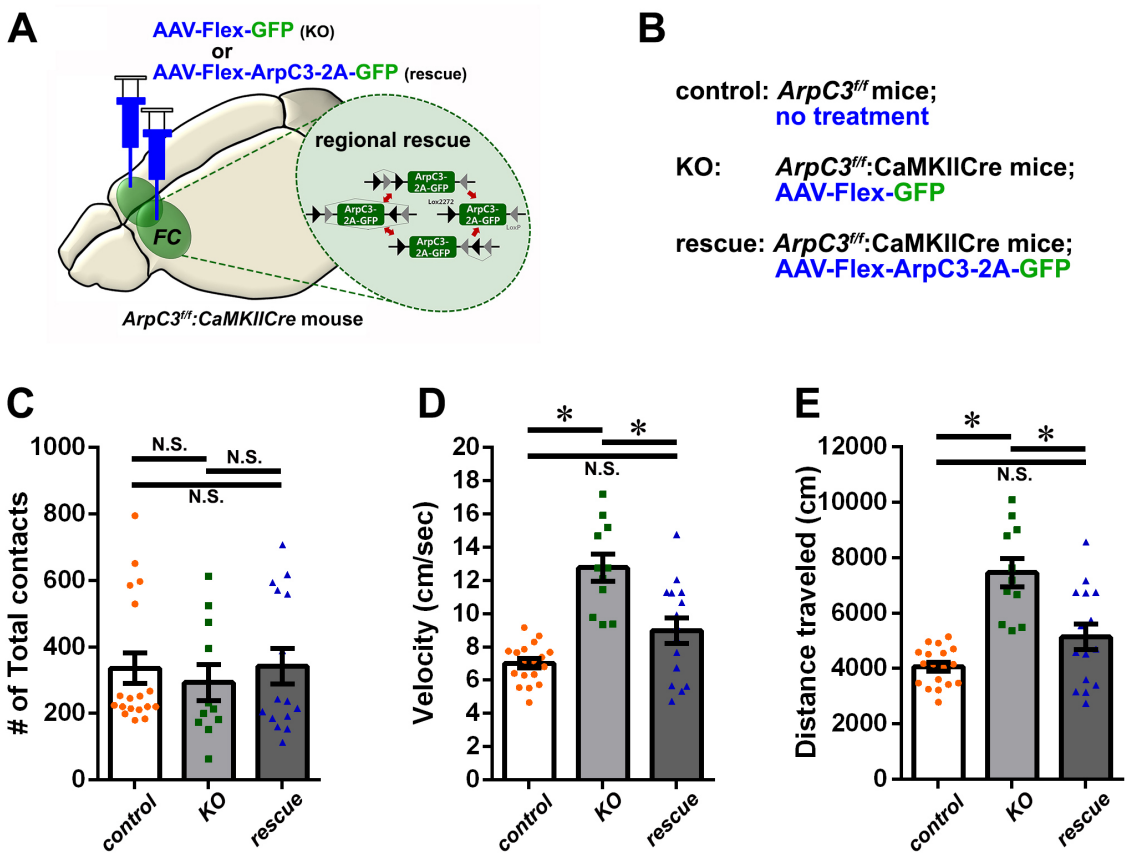

Figure S1. (Related to Figure 1). Regional rescue of ArpC3 within the frontal cortex does not affect the frequency of exploring each stimulus (social and nonsocial), but does normalize locomotor activity in *ArpC3$^{f/f}$:CaMKIICre* KO mice. (A) Schematic Illustration representing the selective re-expression of ArpC3 and GFP in *CaMKII:Cre* positive KO neurons in FC region. Inset shows the regional rescue strategy of Cre-dependent ArpC3 expression. (B) Descriptions of genotypes and treatments for each group of mice. (C) Total number of contacts with both social and non-social stimuli. (D) Mean velocities of each group. The velocity of KO mice is significantly higher than that of control (*p<0.0001), which is normalized by rescue in the PFC (*p=0.0005). (E) Total distances traveled of each group of mice. The distance moved is increased in KO mice (*p<0.0001), which is normalized by rescue in the PFC (*p=0.0005). *ArpC3$^{f/f}$* (control mice; n=18 male and female mice), *ArpC3$^{f/f}$:CaMKII:Cre*-GFP (KO; bilateral GFP virus; n=11 male and female mice), and *ArpC3$^{f/f}$:CaMKII:Cre*-ArpC3 (rescue; bilateral ArpC3 virus; n=15 male and female mice) mice. *p<0.05. Data are presented as mean ±SEM.

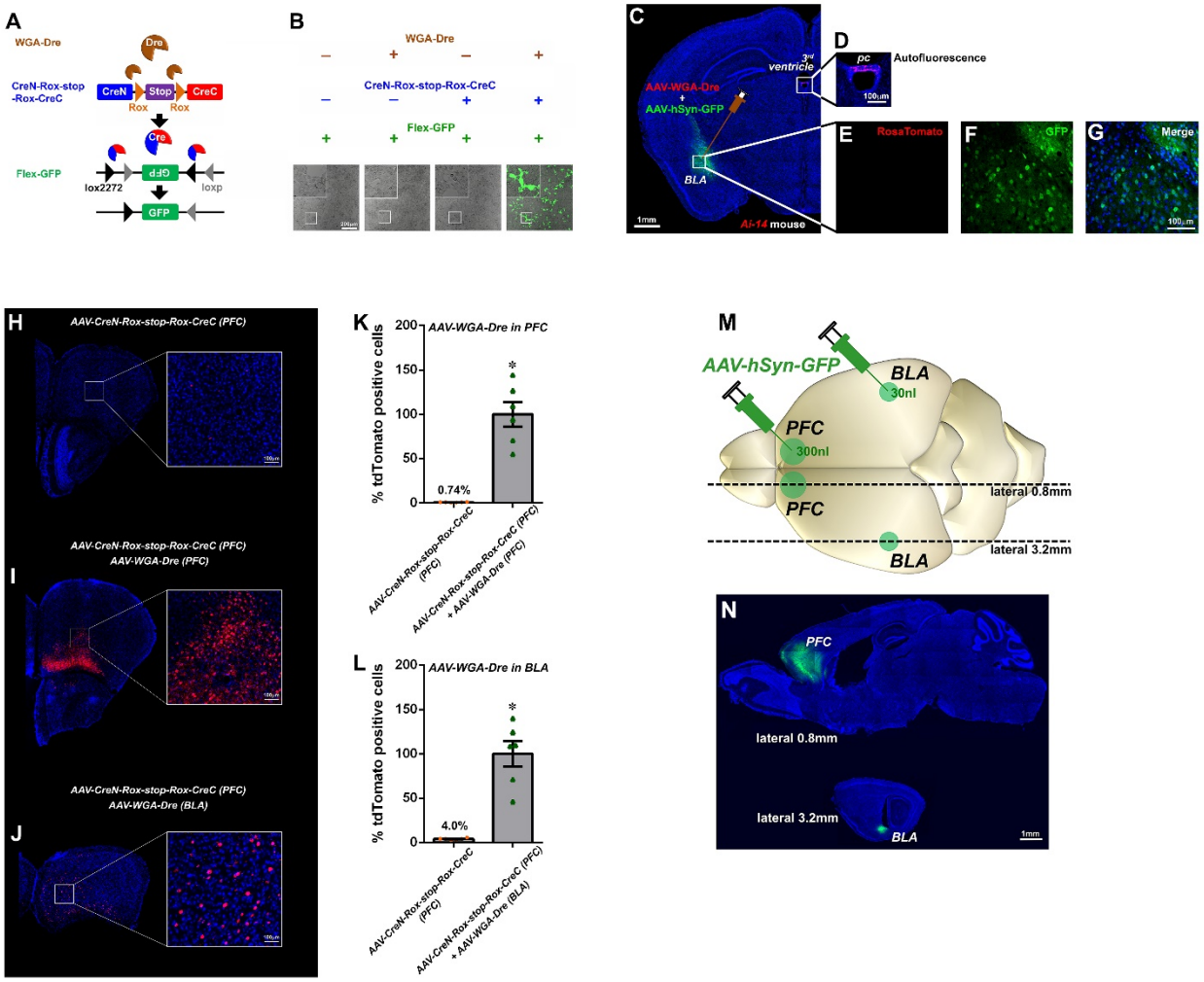

Figure S2. (Related to Figure 2). Validation for the specificity of the Dre-dependent Cre expression system. (A) Schematic illustration of the Dre-dependent Cre expression using a combined Dre and split-Cre system, which is visualized by the Cre-dependent GFP expression (Flex-GFP). (B) Combinational expression of WGA-Dre and CreN-Rox-stop-Rox-CreC with Flex-GFP in HEK293T cells to test the specificity of the system. (C-G) In vivo testing of the inability of Dre alone to mediate LoxP dependent recombination. (C) Expression of tdTomato and GFP in amygdala of *Ai-14* mouse two-weeks after the injection of AAV-*WGA-Dre* and the AAV-*hSyn-GFP* (positive control for infection). (D) Auto-fluorescence detected in the posterior commissure (pc) around 3rd ventricle, demonstrating imaging settings could detect faint signals in the tdTomato channel. (E) Under these imaging settings tdTomato fluorescence was not detected in the BLA region in which GFP was expressed (F), demonstrating that Dre recombinase does not non-specifically recombine LoxP sites *in vivo*. (G) Overlay of the three channels; tdTomato, GFP, and DAPI (blue). (H-L) In vivo test of AAV-*CreN-Rox-stop-Rox-CreC* using mouse brain. (H) tdTomato expression in the PFC of *Ai-14* mouse two-weeks after PFC injection of AAV-*CreN-Rox-stop-Rox-CreC*, (I) PFC injection of both AAV-*CreN-Rox-stop-Rox-CreC* and AAV-*WGA-Dre*, and (J) PFC injection of

AAV-*CreN-Rox-stop-Rox-CreC* with BLA injection of AAV-*WGA-Dre*. (K) Compared to the group of co-injection with AAV-*WGA-Dre* in PFC (I), AAV-*CreN-Rox-stop-Rox-CreC* injection without AAV-*WGA-Dre* (H) produced 0.74% of tdTomato-positive cells. (L) When compared to the circuit injection group (AAV-*CreN-Rox-stop-Rox-CreC* in PFC, AAV-*WGA-Dre* in BLA) (J), AAV-*CreN-Rox-stop-Rox-CreC* injection without AAV-*WGA-Dre* (H) produced 4% of tdTomato-positive cells in PFC. n=6 for all three groups. **p*<0.0001. (M) Schematic illustration of the AAV-*hSyn-GFP* injections into the PFC (300nl; $1X10^{13}$ GC/ml) and BLA (30nl; $1X10^{13}$ GC/ml) that are same site/titer/volumes used in all the experiments in this study. Dotted lines indicate the sagittal planes containing PFC (later 0.8mm) and BLA (lateral 3.2mm). (N) The GFP signals were specifically detected in the PFC and BLA regions that are not overlapped with each other, indicating that the AAV viruses were diffused to the restricted regions from injection sites. Data are presented as mean ±SEM.

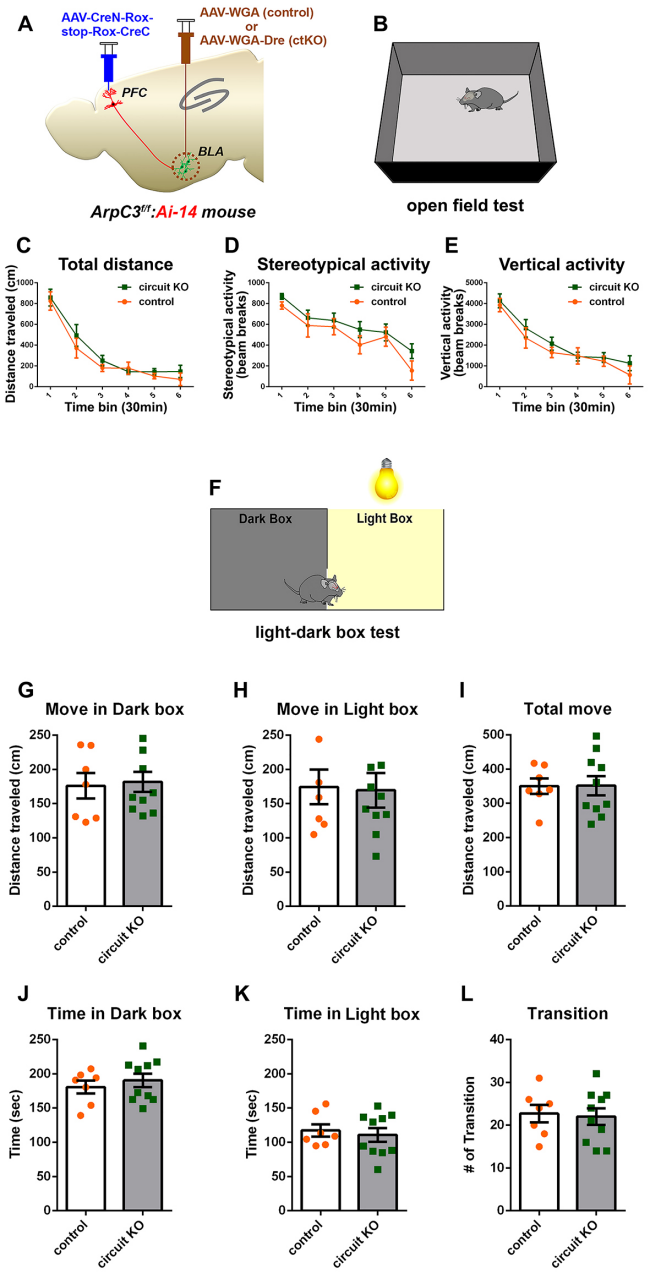

Figure S3. (Related to Figure 3). Open field and light-dark box tests of the ArpC3 ctKO mice. (A) Schematic illustration of the ctKO strategy using the circuit-selective expression of Cre in the *ArpC3$^{f/f}$:Ai-14* mice. (B) Schematic of open field test. Three hours of open filed test revealed that the total distance traveled (C), stereotypical activity (D), and vertical activity (E) of the ArpC3 ctKO mice (green; n=10) were not different from those of control mice (orange; n=7). (F) Schematic illustration of light and dark box test. The distance traveled in dark (G) and light (H) boxes, the total distance moved in both boxes (I), the time spent in dark (J) and light (K) box, and the transition number between both boxes (L) of ArpC3 ctKO mice (n=10) were not different from those of control mice (n=7). Data are presented as mean ±SEM.

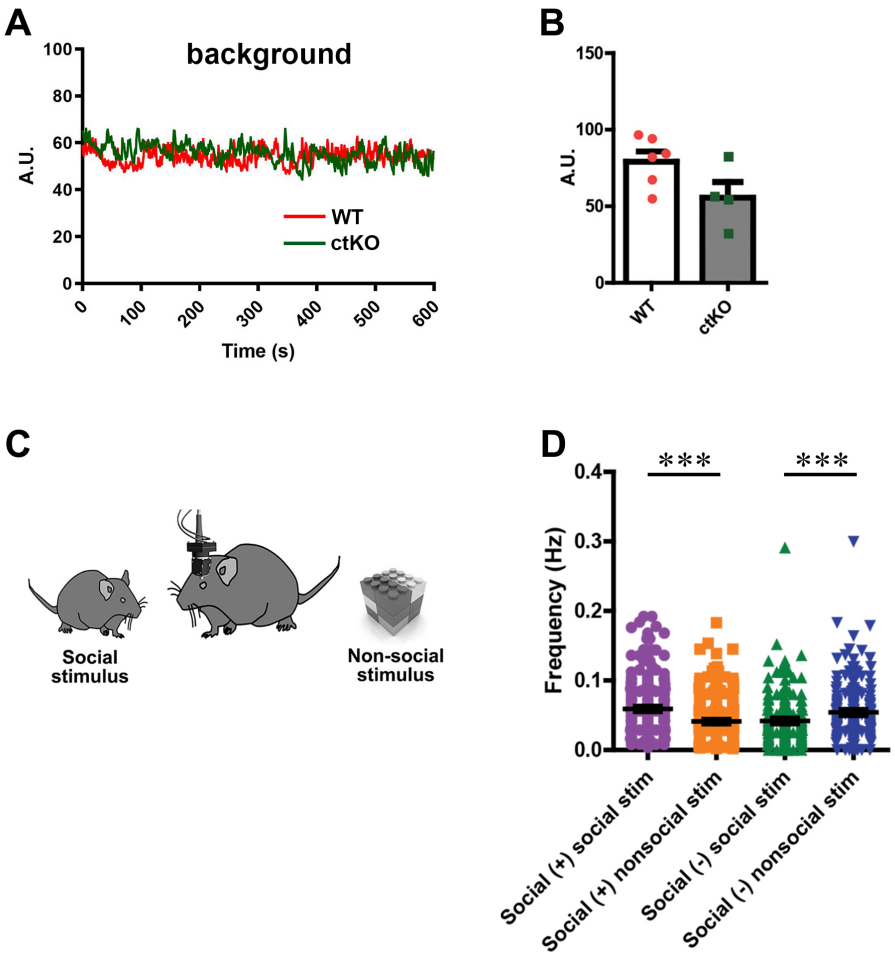

Figure S4. (Related to Figure 5). Social affiliation test and monitoring of the basal fluorescence during brain endoscopy. (A) The representative of basal fluorescence between WT and ctKO during calcium recording. (B) There is no difference in the basal fluorescence between the groups. n=6 for WT, n=4 for ctKO. (C) Schematic illustrating the brain endoscopic analysis during social affiliation test. (D) Social-categorized WT neurons preferentially respond to social stimulus rather than non-social object. Social (+) neurons are significantly more active when animals are in close state with social stimulus (purple dots) rather than with non-social stimulus (orange dots, ***p<0.0001). In contrast, social (-) neurons are significantly more active when the animals explore around non-social stimulus (blue dots) rather than social stimulus (green dots, ***p<0.0001). *** $p < 0.001$. All data are presented as mean ±SEM.

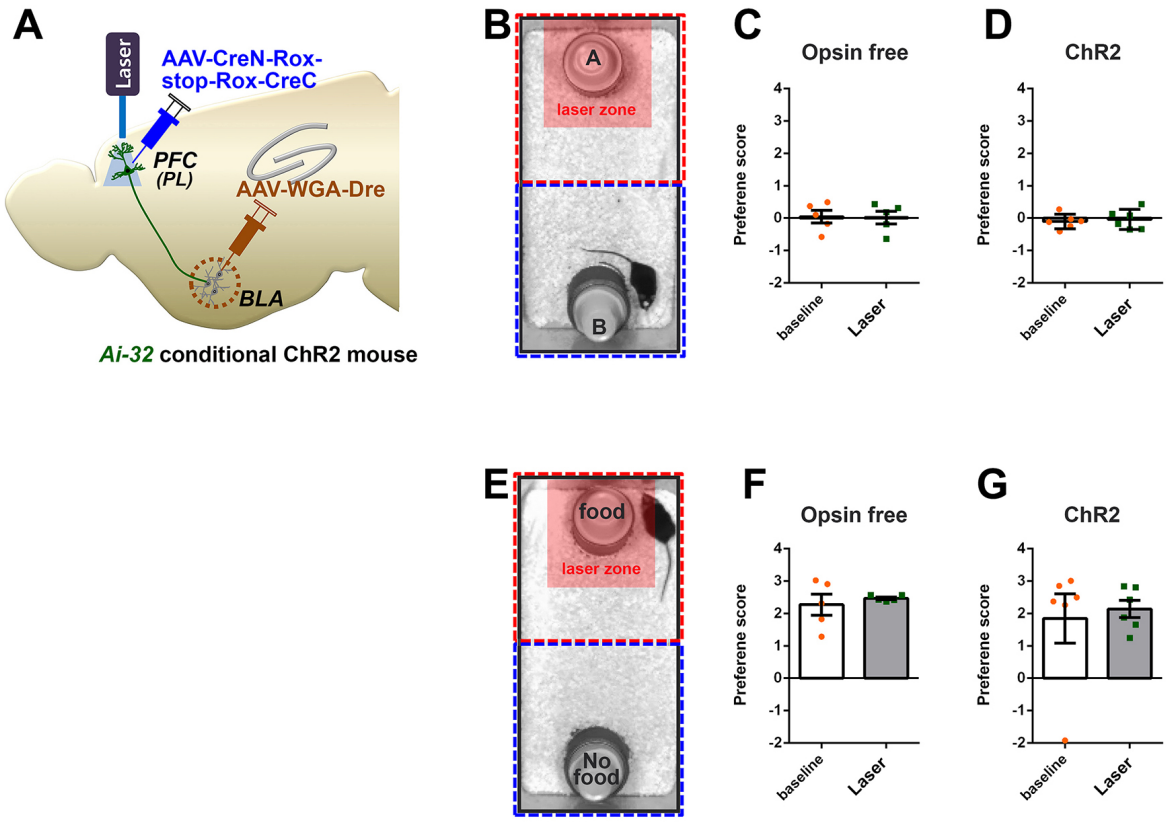

Figure S5. (Related to Figure 6). The effects of optogenetic stimulation on the aversiveness and motivation. (A-D) Circuit-selective optogenetic activation of the PL to BLA projection does not drive place preference and does not affect food-based motivation. (A) Schematic representation of the strategy for circuit selective expression of ChR2 and the optogenetic approach to activate the PL to BLA circuit. (B) Schematic of the testing field consisting of two identical non-social arenas (two identical objects in cup A and cup B), which has a virtual laser activation zone around the one of the cups. Opsin free control (C, n=5) and ctKI-ChR2 mice (D, n=6) do not show place preferences demonstrating the stimulus is not aversive. (E) Schematic of the testing field consisting of food and no-food (object) arenas, which have a virtual zone that triggers stimulation upon entering the laser zone around a cup containing food pellets. The food deprived (for 24 hours) opsin free control (F, n=5) and ctKI-ChR2 (G, n=6) mice similarly prefer the food zone under both baseline conditions (no laser) and with 5Hz stimulation of the PL-BLA circuit, demonstrating optogenetic activation does not affect appetite-mediated motivation. Data are presented as mean ±SEM.

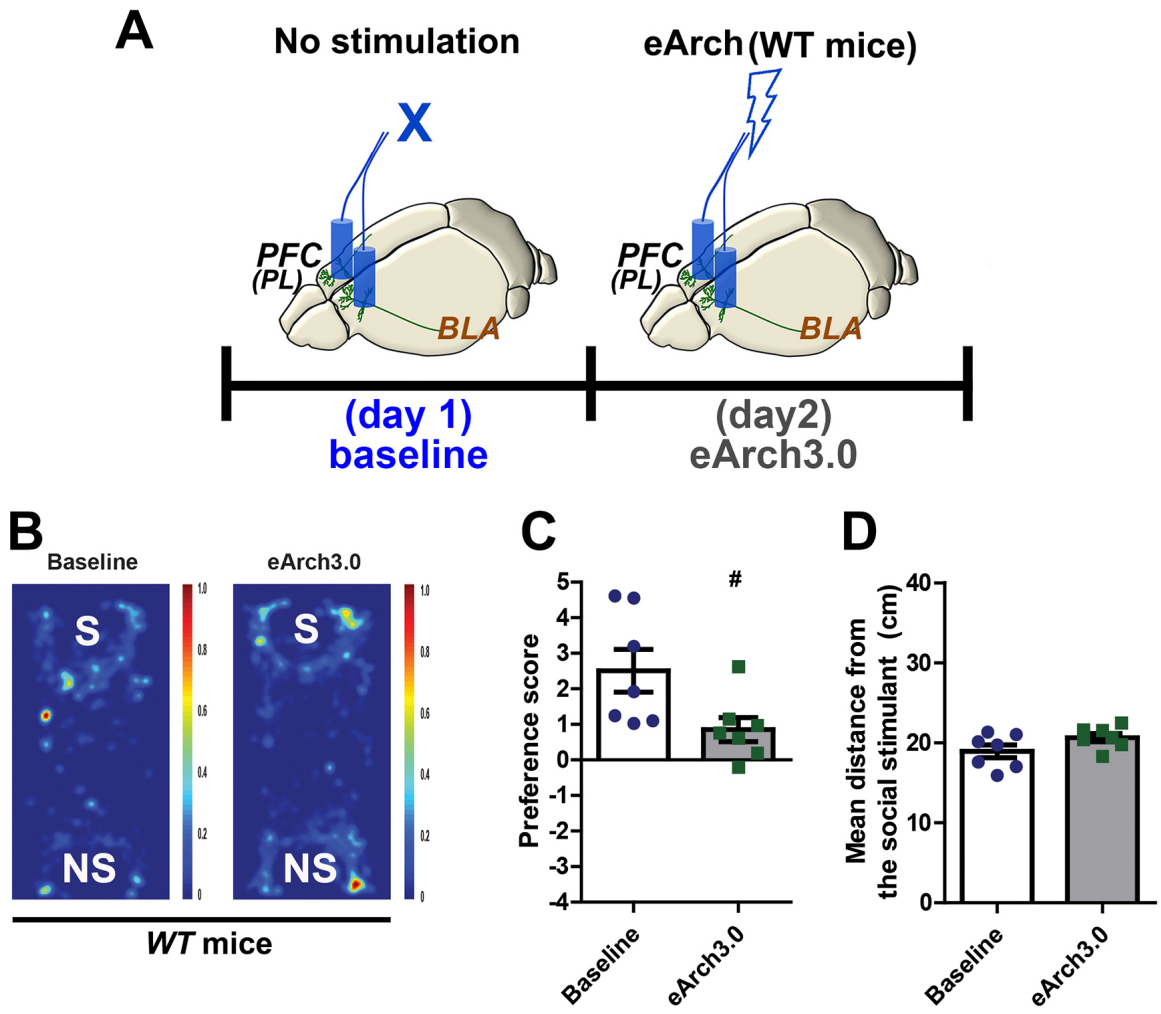

Figure S6. (Related to Figure 7). Conditional optogenetic inactivation of the PL-BLA circuit marginally influences social interaction of WT mice. (A) The time schedule for the real-time social preference tests with schematic illustrations of the circuit-selective optogenetic inactivation. (B) Representative heat maps of movement traces between social versus non-social stimuli without laser (baselines) or with eArch3.0-mediated optical inactivation within the social stimulus zone. S; social stimulus, NS; nonsocial stimulus. (C) Graph of preference score for social versus non-social stimulus (blue, baseline; green, eArch3.0 inactivation. n=7 male mice for each group). p=0.0735 for baseline versus eArch3.0. (D) Average distance between the experimental mouse and social stimulus. p=0.204 for baseline versus eArch3.0. $^{\#}p$<0.1. Data are presented as mean ±SEM.

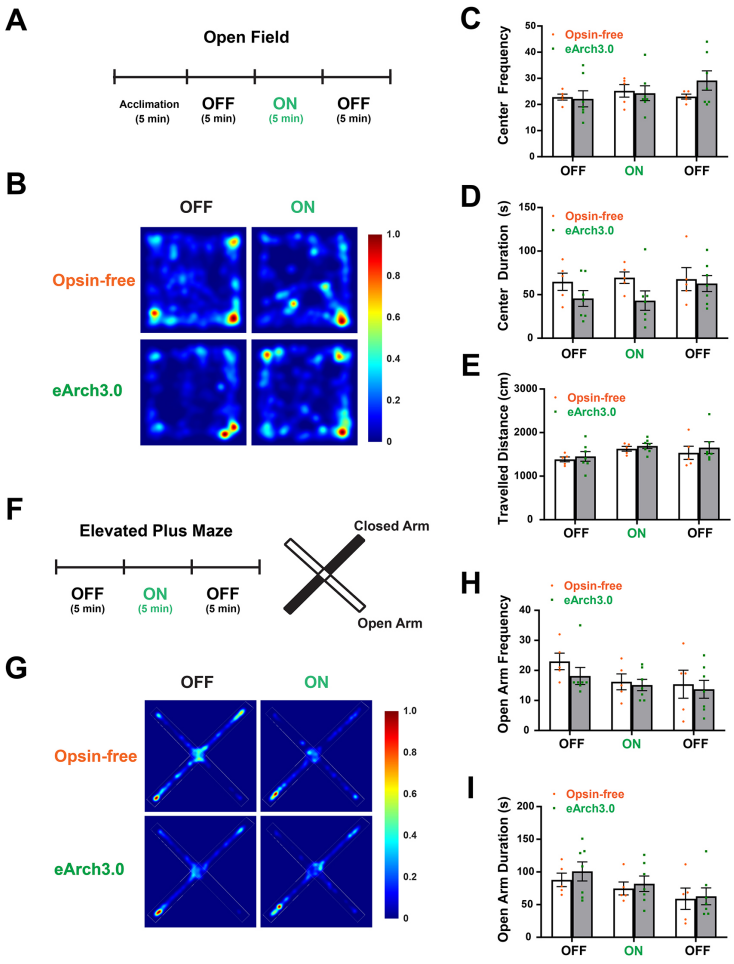

Figure S7. (Related to Figure 7). Optogenetic suppression of PL-BLA circuit does not affect general anxiety of the wild type mice. (A) Illustration of the open field testing procedure. Open field testing consisted of 5 min acclimation and consecutive 5 min epochs with alternating laser stimulation (OFF-ON-OFF). Continuous green laser stimulation was given during the Laser epoch. (B) Representative heat maps of movement traces during OFF and ON epochs of the open field test. (C) PL-BLA circuit inactivation does not alter the entry frequency to the center area. (E) PL-BLA circuit inactivation does not alter the duration in center area of open filed when compared to the opsin-free controls. (E) PL-BLA circuit inactivation does not change activity level in the open field. (F) Illustration of the elevated plus maze testing procedure and mappings indicating the locations of the open arms (white) and the closed arms (black). Elevated plus maze testing consisted of consecutive 5 min epochs with alternating laser stimulation (OFF-ON-OFF). Continuous green laser stimulation was given during the ON epoch. (G) Representative heat maps of movement traces during OFF and ON epochs of the elevated plus maze test. (H) PL-BLA circuit inactivation does not alter the frequency of open arm entry. (I) PL-BLA circuit inactivation does not change the duration in open arm. Data are presented as mean ±SEM.

Table S1. Statistical Results. Related to all Figures

Figure	Test type	n	Statistical significance	F/t value & effects
1D	Two-way ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 18, 11, 15	Posthoc tests. NS-S Vs. NS-NS control: p<0.0001 KO: p=0.5228 rescue: p=0.0002	There are effects of Trial ($F_{(1, 41)}$=45.24, p<0.0001), Genotype ($F_{(2, 41)}$=4.981, p=0.0116), and Trial*Genotype interaction ($F_{(2, 41)}$=4.425, p=0.0182)
2H	Independent *t*-test	In order 3, 3	p<0.0001	$t_{(4)}$=33.74
3D	Two-way ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 7, 10	Posthoc tests. control Vs. ctKO NS-NS: p>0.9999 NS-S: p=0.0062	There are effects of Genotype ($F_{(1, 30)}$=6.123, p=0.0192) and Trial*Genotype interaction ($F_{(1, 30)}$=4.425, p=0.0463)
3E	Independent *t*-test	In order 7, 10	p=0.2403	$t_{(15)}$=1.2223
3F	Independent *t*-test	In order 7, 10	p=0.8037	$t_{(15)}$=0.2530
3G	Independent *t*-test	In order 7, 10	p=0.8019	$t_{(15)}$=0.2554
4D	Independent *t*-test	In order 9, 15	p=0.1645	$t_{(22)}$=1.438
4E	Two-sample Kolmogorov-Smirnov test	In order 353, 1339	p<0.0001	$D_{(1692)}$ = 0.1564
4F	Independent *t*-test	In order 9, 15	p=0.0062	$t_{(22)}$=3.029
4G	Two-sample Kolmogorov-Smirnov test	In order 344, 1324	p<0.0001	$D_{(1668)}$ = 0.3414
4I	Independent *t*-test	In order 15, 18	p=0.2983	$t_{(31)}$=1.058
4J	Two-sample Kolmogorov-Smirnov test	In order 680, 1482	p=0.5489	$D_{(2162)}$=0.0367
4K	Independent *t*-test	In order 15, 18	p=0.0068	$t_{(31)}$=2.903
4L	Two-sample Kolmogorov-Smirnov test	In order 665, 1464	p<0.0001	$D_{(2129)}$=0.2378
4N	Independent *t*-test	In order 13, 14	p=0.0219	$t_{(25)}$=2.445
4O	Two-sample Kolmogorov-Smirnov test	In order 1299, 1401	p<0.0001	$D_{(2700)}$=0.1435
4P	Independent *t*-test	In order 13, 14	p=0.0508	$t_{(25)}$=2.052
4Q	Two-sample Kolmogorov-Smirnov test	In order 1286, 1387	p<0.0001	$D_{(2673)}$=0.1903
4S	Independent *t*-test	In order 13, 14	p=0.0219	$t_{(25)}$=2.445
4T	Two-sample Kolmogorov-Smirnov test	In order 1299, 1239	p=0.0280	$D_{(2538)}$=0.0577
4U	Independent *t*-test	In order 13, 14	p=0.0508	$t_{(25)}$=2.052
4V	Two-sample Kolmogorov-Smirnov test	In order 1286, 1226	p=0.2261	$D_{(2512)}$=0.0414
5E	Independent *t*-test	In order 184, 176	p<0.0001	$t_{(358)}$=8.811
5F	Independent *t*-test	In order 184, 176	p<0.0001	$t_{(358)}$=13.55
5G	Independent *t*-test	In order 184, 176	p<0.0001	$t_{(358)}$=6.257

5K	Two-way ANOVA	In order 87,97,95,81	Interaction p=0.0206 Social p<0.0001 Group p<0.0001	$F_{(1,356)}$ = 5.411 $F_{(1,356)}$ = 21.01 $F_{(1,356)}$ = 107.9 Bonferroni posthocs WT social p < 0.001
5L	Two-way ANOVA	In order 87,97,95,81	Interaction p=0.0857 Social p<0.0001 Group p<0.0001	$F_{(1,356)}$ = 2.936 $F_{(1,356)}$ = 38.49 $F_{(1,356)}$ = 90.87 Bonferroni posthocs WT social p < 0.001 ctKO social p < 0.01
6F	Paired *t*-test	In order 5	p=0.1966	$t_{(4)}$=1.548
6G	Paired *t*-test	In order 5	p=0.2703	$t_{(4)}$=1.278
6I	Paired *t*-test	In order 6	p=0.0038	$t_{(5)}$=5.076
6J	Paired *t*-test	In order 6	p=0.0028	$t_{(5)}$=5.451
7D	Paired *t*-test	Number of pairs 6	p=0.043	$t_{(5)}$=2.691
7E	Paired *t*-test	Number of pairs 6	p=0.039	$t_{(5)}$=2.779
S1C	One-way ANOVA followed by Bonferroni's multiple comparisons.	In order 18, 11, 15	Posthoc tests. control Vs. KO: p>0.9999 Control Vs. rescue: p>0.9999 KO Vs. rescue: p>0.9999	No effect was found
S1D	One-way ANOVA followed by Bonferroni's multiple comparisons.	In order 18, 11, 15	Posthoc tests. control Vs. KO: p<0.0001 Control Vs. rescue: p=0.1017 KO Vs. rescue: p=0.0005	There are effects of Treatment (virus) ($F_{(2, 41)}$=20.12, p<0.0001
S1E	One-way ANOVA followed by Bonferroni's multiple comparisons.	In order 18, 11, 15	Posthoc tests. control Vs. KO: p<0.0001 Control Vs. rescue: p=0.0587 KO Vs. rescue: p=0.0005	There are effects of Treatment (virus) ($F_{(2, 41)}$=21.09, p<0.0001
S2K	Independent *t*-test	In order 6, 6	P<0.0001	$t_{(10)}$=7.220
S2L	Independent *t*-test	In order 6, 6	P<0.0001	$t_{(10)}$=6.724
S3C	Two-way ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 6, 10	No statistical difference was found between control and ctKO groups in all 6 time points	There is time effect ($F_{(5, 70)}$=47.69, p<0.0001
S3D	Two-way ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 6, 10	No statistical difference was found between control and ctKO groups in all 6 time points	There is time effect ($F_{(5, 70)}$=14.41, p<0.0001
S3E	Two-way ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 6, 10	No statistical difference was found between control and ctKO groups in all 6 time points	There is time effect ($F_{(5, 70)}$=31.63, p<0.0001
S3G	Independent *t*-test	In order 7, 10	P=0.8083	$t_{(15)}$=0.2470
S3H	Independent *t*-test	In order 7, 10	P=0.8985	$t_{(15)}$=0.1297
S3I	Independent *t*-test	In order 7, 10	P=0.9795	$t_{(15)}$=0.0261
S3J	Independent *t*-test	In order 7, 10	P=0.5010	$t_{(15)}$=0.6895
S3K	Independent *t*-test	In order 7, 10	P=0.6534	$t_{(15)}$=0.4581
S3L	Independent *t*-test	In order 7, 10	P=0.8088	$t_{(15)}$=0.2463
S4B Social (+)	Paired *t*-test	In order 270	p<0.0001	$t_{(269)}$=17.18
S4B Social (-)	Paired *t*-test	In order 189	p < 0.0001	$t_{(188)}$=15.51
S4D	Independent *t*-test	In order 6, 4	p=0.0745	$t_{(8)}$=2.050

S5C	Paired *t*-test	In order 5	p=0.3810	$t_{(4)}$=0.9837
S5D	Paired *t*-test	In order 6	p=0.6311	$t_{(5)}$=0.5110
S5F	Paired *t*-test	In order 5	p=0.6022	$t_{(4)}$=0.5651
S5G	Paired *t*-test	In order 6	p=0.6463	$t_{(5)}$=0.4879
S6C	Paired *t*-test	In order 7	p=0.0735	$t_{(6)}$=2.166
S6D	Paired *t*-test	In order 7	p=0.7254	$t_{(6)}$=1.424
S7C 1st OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.867	No effect was found
S7C ON	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.821	No effect was found
S7C 2nd OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.202	No effect was found
S7D 1st OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.190	No effect was found
S7D ON	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.098	No effect was found
S7D 2nd OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.751	No effect was found
S7E 1st OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.639	There is time effect ($F_{(2, 20)}$=4.292, p<0.05
S7E ON	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.454	There is time effect ($F_{(2, 20)}$=4.292, p<0.05
S7E 2nd OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.575	There is time effect ($F_{(2, 20)}$=4.292, p<0.05
S7H 1st OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.264	No effect was found
S7H ON	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.743	No effect was found
S7H 2nd OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.755	No effect was found
S7I 1st OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.521	There is time effect ($F_{(2, 20)}$=4.423, p<0.05
S7I ON	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.667	There is time effect ($F_{(2, 20)}$=4.423, p<0.05
S7I 2nd OFF	Mixed ANOVA with repeated measure followed by Bonferroni's multiple comparisons.	In order 5, 7	p=0.861	There is time effect ($F_{(2, 20)}$=4.423, p<0.05