Supplemental figure legends

Figure S1: Optical fiber placements for optogenetic experiments; photoexcitation effects on striatal eCBs, related to Figure 1.

(**A**) Optical fiber placements for mPFC$^{\rightarrow}$BLA photoexcitation experiment.

(**B**) mPFC$^{\rightarrow}$BLA photoexcitation during extinction training did not change eCB levels in dorsal striatum (paired t-test for AEA YFP versus ChR2: $t(24)=0.169$, $P=0.8675$, $\eta_p^2=0.01$, n=15/YFP, n=11/ChR2 and paired t-test for 2-AG YFP versus ChR2: $t(22)=0.912$, $P=0.3717$, $\eta_p^2=0.04$, n=11 mice/YFP and n=8 mice/ChR2). (**C**) Optical fiber placements for mPFC$^{\rightarrow}$BLA photostimulation + systemic SR141716A experiment.

(**D**) Optical fiber placements for mPFC$^{\rightarrow}$BLA photoexcitation + intra-BLA SR141716A experiment.

Data in B are mean±SEM.

Figure S2: **Pharmacological effects on GRAB$_{eCB2.0}$ biosensor-measured eCBs at mPFC$^{\rightarrow}$BLA neurons, related to Figure 2.**

(**A**) GRAB$_{eCB2.0}$ signal following intraperitoneal injection of vehicle (paired t-test for vehicle versus baseline: $t(3)=2.314$, $P=0.1036$, $g=0.57$, n=4) or injection of the CB1R antagonist, SR141716A (5 mg/kg), (paired t-test SR141716A versus baseline: $t(4)=1.316$, $P=0.2585$, $g=0.12$, n=5).

(**B**) GRAB$_{eCB2.0}$ signal following intraperitoneal injection of the CB1R agonist, WIN 55,212-22 (5 mg/kg), (paired t-test for WIN 55,212-22 versus baseline: $t(7)=2.685$, $P=0.0313$, $g=0.56$, n=8) or a combination of SR141716A (5 mg/kg) and WIN 55,212-22 (5 mg/kg) (paired t-test WIN 55,212-22 + SR141716A versus baseline: $t(3)=1.540$, $P=0.2211$, $g=0.42$, n=4).

(**C**) GRAB$_{eCB2.0}$ signal following intraperitoneal injection of the FAAH/MAGL dual inhibitor, JZL195 (20 mg/kg), (paired t-test for JZL195 versus baseline: $t(3)=4.402$, $P=0.0217$, $g=1.31$, n=4) or a combination of JZL195 (20 mg/kg) and SR141716A (5 mg/kg) (paired t-test for JZL195+ SR141716A versus baseline: $t(2)=0.424$, $P=0.7127$, $g=0.16$, n=3).

(**D**) $GRAB_{eCB2.0}$ signal following intraperitoneal injection of the FAAH inhibitor, URB597 (3 mg/kg), (paired t-test for URB597 versus baseline: $t(5)=3.241$, $P=0.0229$, $g=0.45$, n=6) or a combination of SR141716A (5 mg/kg) and URB597 (3 mg/kg) (paired t-test for URB597 + SR141716A versus baseline: $t(2)=0.120$, $P=0.9152$, $g=0.03$, n=3).

(**E-F**) Behavior (**E**) and *in vivo* fiber photometry (**F**) procedure for $GRAB_{eCB2.0}$ biosensor recordings following pre-extinction intraperitoneal injection of vehicle or SR141716A (5 mg/kg) (Veh n=9, SR n=9).

(**G**) Optical fiber placements for pre-extinction SR141716A experiment.

(**H**) $GRAB_{eCB2.0}$ signal at conditioning US presentation (ANOVA US-effect: $F(1,16)=2.911$, $P<0.1073$, $\eta_p^2=0.04$, drug group-effect: $F(1,16)=40.280$, $P<0.0001$, $\eta_p^2=0.53$, interaction: $F(1,16)=2.645$, $P=0.1234$, $\eta_p^2=0.03$, Holm-Šídák's tests base-Veh versus US-Veh: $P<0.0001$, base-SR versus US-SR: $P=0.0042$) and CS presentation (see Table S1 for CS presentation-related statistical results).

(**I**) $GRAB_{eCB2.0}$ signal during early extinction training CS on (see Table S1 for CS on-related statistical results) and CS off (ANOVA event-effect: $F(1,14)=1.353$, $P=0.2643$, $\eta_p^2=0.04$, drug group-effect: $F(1,14)=6.119$, $P=0.0268$, $\eta_p^2=0.17$, interaction: $F(1,14)=1.446$, $P=0.2491$, $\eta_p^2=0.04$, Holm-Šídák's tests base-Veh versus CS off-Veh: $P=0.0552$, base-SR versus CS off-SR: $P=0.3529$, base-Veh versus base-SR: $P=0.9771$, CS off-Veh versus CS off-SR: $P=0.1999$) periods.

Data are mean±SEM population average Z-scores normalized to 5-minute pre-injection baseline (A-D) and normalized to 5-second pre-event baseline (H and I). ***$P<0.001$, **$P<0.01$, *$P<0.05$.

Figure S3: Behavioral measures related to $GRAB_{eCB2.0}$ biosensor measurements, related to Figure 3.

(**A**) Freezing levels during $GRAB_{eCB2.0}$ recordings (n=16). Higher levels of CS-related freezing on conditioning trial 3 versus trial 1 (paired t-test for trial 3versus trial 1: $t(15)=2.531$, $P=0.0231$, $\eta_p^2=0.30$). Lower levels of CS-related freezing on extinction retrieval 2 versus Early (first 5-trial block) extinction training (paired t-test for retrieval 2 versus early extinction training: $t(15)=4.473$, $P=0.0004$, $\eta_p^2=0.57$). Higher levels of CS-related freezing on fear renewal versus Early (first 5-trial block) extinction training (paired t-test for renewal versus early extinction training: $t(14)=4.437$, $P=0.0006$, $\eta_p^2=0.58$).

(**B**) US-related $GRAB_{eCB2.0}$ signal in response to each of 3 conditioning US presentations (n=16). US1 (paired t-test for US1 versus baseline: $t(15)=5.322$, $P<0.0001$, $\eta_p^2=0.65$), US2 (paired t-test for US 2 versus baseline: $t(15)=4.039$, $P=0.0011$, $\eta_p^2=0.52$), US3 (paired t-test for US 3 versus baseline: $t(15)= 4.892$, $P=0.0002$, $\eta_p^2=0.61$).

(**C**) Correlation between population-average $GRAB_{eCB2.0}$ signal and average freezing during extinction training.

(**D**) Correlations between individual instances of CS on-related $GRAB_{eCB2.0}$ signal and CS on-related freezing during extinction training.

(**E**) Correlation between individual instances of CS off-related $GRAB_{eCB2.0}$ signal and CS off-related freezing during extinction training.

(**F**) Correlation between neutral cage movement velocity during instances of movement and $GRAB_{eCB2.0}$ signal during the corresponding period.

(**G**) Procedure for food consumption assay in fasted and sated state (n=8 mice).

(**H,I**) Time course (**H**) and average (ANOVA state-effect: $F(1,14)=3.525$, $P=0.0814$, $\eta_p^2=0.78$, food availability-effect: $F(1,14)=5.353$, $P=0.0364$, $\eta_p^2=0.13$, interaction: $F(1,14)=3.647$, $P=0.0769$, $\eta_p^2=0.09$, Holm-Šídák's tests fasted retrieve versus fasted baseline: $P=0.0195$, stated retrieve versus sated baseline: $P=0.7794$, fasted baseline versus sated baseline: $P=0.9590$, fasted retrieve versus sated retrieve: $P=0.0243$) (**I**) $GRAB_{eCB2.0}$ signal during food retrieval in sated and fasted state.

(**J**) Amount of food eaten (paired t-test for sated versus fasted: $t(7)=10.960$, $P<0.0001$, $\eta_p^2=0.94$) and number of consumption bouts (paired t-test for sated versus fasted: $t(7)=0.055$, $P=0.9576$, $\eta_p^2=0.01$) in the food consumption assay.

(**K-O**) Optical fiber placement for $GRAB_{eCB2.0}$ biosensor male mice (**K**), female mice (**L**), unpaired (**M**), CS-only (**N**), and US-only (**O**) experiments.

(**P**) Schematic of hypothetical model of stimulus-related eCB activity, and corresponding glutamatergic transmission, at $mPFC^{\rightarrow}BLA$ synapses. High eCB activity during CS off periods, particularly during early extinction when violation of the learned CS-shock association is most prominent, is hypothesized to result

in correspondingly low (CS on) suppression of mPFC→BLA synaptic glutamate release, whereas low eCB activity during CS on periods, especially during late extinction when the CS has been repeatedly experienced without shock, would result in disinhibition of CS-related glutamate release.

Figure S4: ***In vitro*** **neuronal recordings of eCB-mediated responses at vmPFC→BLA synapses, related to Figure 4.**

(**A**) Procedure for measuring effects of the synthetic cannabinoid, CP-55,940 (CP), on ChR2-mediated optically-evoked recordings at vmPFC→BLA synapses (n=4 Veh/n=4 CP).

(**B-D**) Effects of bath application of CP on feed-forward oIPSC PPR (unpaired t-test for CP versus vehicle (Veh): $t(38)=4.356$, $P<0.0001$, $\eta_p^2=0.33$, n=18-22 cells) (**B**), spontaneous EPSC (sEPSC) frequency (unpaired t-test for CP versus Veh: $t(37)=3.145$, $P=0.0033$, $\eta_p^2=0.21$, n=13-26 cells) and amplitude (unpaired t-test for CP versus Veh: $t(37)=1.416$, $P=0.1650$, $\eta_p^2=0.05$, n=13-26 cells) (**C**) and spontaneous IPSC (sIPSC) frequency (unpaired t-test for CP versus Veh: $t(31)=2.465$, $P=0.0194$, $\eta_p^2=0.16$, n=14-19 cells) and amplitude (unpaired t-test for CP versus Veh: $t(31)=4.035$, $P=0.0003$, $\eta_p^2=0.34$, n=14-19 cells) (**D**).

(**E**) Procedure for measuring effects of the MAGL inhibitor, JZL184 (JZL), on ChR2-mediated optically-evoked recordings at vmPFC→BLA synapses (n=3 Veh/n=3 JZL).

(**F-H**) Effects of bath application of JZL on feed-forward oIPSC PPR (unpaired t-test for JZL versus Veh: $t(22)=0.039$, $P=0.9689$, $\eta_p^2=0.01$, n=11-13 cells) (**F**), sEPSC frequency (unpaired t-test for JZL versus Veh: $t(31)=2.364$, $P=0.0245$, $\eta_p^2=0.15$, n=7-26 cells) and amplitude (unpaired t-test for JZL versus Veh: $t(39)=0.602$, $P=0.5504$, $\eta_p^2=0.01$, n=15-26) (**G**) and sIPSC frequency (unpaired t-test for JZL versus Veh: $t(33)=2.156$, $P=0.0385$, $\eta_p^2=0.12$, n=16-19 cells) and amplitude (unpaired t-test for JZL versus Veh: $t(32)=4.308$, $P=0.0001$, $\eta_p^2=0.37$, n=16-18 cells) (**H**).

(**I**) Procedure for measuring effects of the FAAH inhibitor, PF-3845 (PF), on ChR2-mediated optically-evoked recordings at vmPFC→BLA synapses (n=5 Veh/n=5 PF).

(**J-L**) Effects of bath application of PF on feed-forward oIPSC PPR (unpaired t-test for PF versus Veh: $t(31)=2.648$, $P=0.0126$, $\eta_p^2=0.18$, n=15-18 cells) (**J**), sEPSC frequency (unpaired t-test for PF versus Veh: $t(69)=5.426$, $P<0.0001$, $\eta_p^2=0.30$, n=32-39, n=33-39 cells) and amplitude (unpaired t-test for PF versus Veh: $t(70)=2.810$, $P=0.0064$, $\eta_p^2=0.10$, n=33-39 cells) (**K**) and sIPSC frequency (unpaired t-test for PF versus Veh: $t(60)=4.818$, $P<0.0001$, $\eta_p^2=0.28$, n=27-35 cells) and amplitude (unpaired t-test for PF versus Veh: $t(60)=5.247$, $P<0.0001$, $\eta_p^2=0.31$, n=27-35 cells) (**L**).

Data are mean±SEM. ****$P<0.0001$, ***$P<0.001$, **$P<0.01$, *$P<0.05$.

Figure S5: **Neural and behavioral analyses of CRISPR-Cas9 mutation, related to Figure 5.**

(**A**) Example low (upper left) and high (upper right) magnification images of mCherry (Cre virus) and GFP (Flex-KASH virus) expression in mPFC, and example low magnification image of mCherry expression in BLA (lower left) of a vmPFC$^{\rightarrow}$BLA^{Cnr1} intact mouse.

B) Example image depicting EGFP-L10a expression in a Rosa26^{fsTRAP} mouse expressing the CRISPR-Cas9 mutated virus construct.

(**C**) BLA *Cnr1* mRNA expression measured by rt-PCR in EGFP-tagged mRNA-enriched samples (unpaired t-test for intact versus mutated: $t(13)=0.919$, $P=0.3747$, $\eta_p^2=0.06$, n=7 intact/n=8 mutated).

(**D**) Percent BLA-projecting dmPFC and vmPFC neurons expressing *Cnr1* mRNA, as quantified by Basescope (n=4/dmPFC, n=3/vmPFC).

(**E**) Effects of vmPFC$^{\rightarrow}$BLA^{Cnr1} mutation (n=10 intact/n=11 mutated) on number of consumption bouts (ANOVA state-effect: $F(1,19)=125.400$, $P<0.0001$, $\eta_p^2=0.45$, group-effect: $F(1,19)=2.980$, $P=0.1005$, $\eta_p^2=0.06$, interaction: $F(1,19)=3.010$, $P=0.0990$, $\eta_p^2=0.01$, Holm-Šídák's tests intact sated versus fasted: $P<0.0001$, mutated sated versus fasted: $P<0.0001$, sated intact versus sated fasted: $P=0.3611$, fasted intact versus fasted mutated: $P=0.0582$), amount of food eaten (ANOVA state-effect: $F(1,18)=180.200$, $P<0.0001$, $\eta_p^2=0.60$, group-effect: $F(1,18)=1.3149$, $P=0.2667$, $\eta_p^2=0.02$, interaction: $F(1,18)=1.031$, $P=0.3235$, $\eta_p^2=0.01$, Holm-Šídák's tests intact sated versus fasted: $P<0.0001$, mutated sated versus fasted: $P<0.0001$, sated intact versus sated mutated: $P=0.5232$, fasted intact versus fasted mutated: $P=0.2842$) and

body weight (ANOVA state-effect: $F(1.442, 25.95)=22.110$, $P<0.0001$, $\eta_p^2=0.40$, group-effect: $F(1,18)=0.536$, $P=0.4736$, $\eta_p^2=0.01$, interaction: $F(2,36)=0.213$, $P=0.8096$, $\eta_p^2=0.01$, Holm-Šídák's tests intact versus mutated before surgery: $P=0.9905$, intact versus mutated sated: $P=0.8523$, intact versus mutated fasted: $P=0.8523$).

(**F-H**) Effects of vmPFC$^{\rightarrow}$BLA^{Cnr1} mutation (n=9 intact/n=11 mutated for all anxiety tests) on percent dark compartment duration (unpaired t-test for intact versus mutated: $t(18)=2.212$, $P=0.0401$, $\eta_p^2=0.21$) in the light-dark exploration test (LD) test (F), percent open arm duration (unpaired t-test for intact versus mutated: $t(18)=1.273$, $P=0.2190$, $\eta_p^2=0.08$), closed arm duration (unpaired t-test intact for intact versus mutated: $t(18)=1.023$, $P=0.3199$, $\eta_p^2=0.05$) and total distance moved (unpaired t-test for intact versus mutated: $t(18)=0.0574$, $P=0.9548$, $\eta_p^2=0.01$) in the elevated plus-maze (EPM) (**G**), and number of marbles buried (unpaired t-test for intact versus mutated: $t(18)=0.634$, $P=0.5341$, $\eta_p^2=0.02$) in the marble burying test (MB) (**H**).

(**I**) Effects of dmPFC$^{\rightarrow}$BLA^{Cnr1} mutation (n=8 intact/n=8 mutated) on number of consumption bouts (ANOVA state-effect: $F(1,14)=35.120$, $P<0.0001$, $\eta_p^2=0.30$, group-effect: $F(1,14)=2.287$, $P=0.1527$, $\eta_p^2=0.08$, interaction: $F(1,14)=0.002$, $P=0.9684$, $\eta_p^2=0.01$, Holm-Šídák's tests intact sated versus fasted: $P=0.3266$, mutated sated versus fasted: $P=0.3266$, sated intact versus sated fasted: $P=0.0017$, sated mutated versus fasted mutated: $P=0.0017$), amount of food eaten (ANOVA state-effect: $F(1,14)=125.400$, $P<0.0001$, $\eta_p^2=0.51$, group-effect: $F(1,14)=4.581$, $P=0.0504$, $\eta_p^2=0.10$, interaction: $F(1,14)=2.455$, $P=0.1394$, $\eta_p^2=0.01$, Holm-Šídák's tests intact sated versus fasted: $P<0.0001$, mutated sated versus fasted: $P<0.0001$, sated intact versus sated mutated: $P=0.1834$, fasted intact versus fasted mutated: $P=0.0305$) and body weight (ANOVA state-effect: $F(1.023, 14.32)=49.020$, $P<0.0001$, $\eta_p^2=0.37$, group-effect: $F(1,14)=0.383$, $P=0.5460$, $\eta_p^2=0.10$, interaction: $F(2,28)=0.460$, $P=0.6359$, $\eta_p^2=0.01$, Holm-Šídák's tests intact versus mutated before surgery: $P=0.8374$, intact versus mutated sated: $P=0.8374$, intact versus mutated fasted: $P=0.8374$).

(**J-L**) Effects of dmPFC$^{\rightarrow}$BLA^{Cnr1} mutation (n=8 intact/n=8 mutated mice across all anxiety tests) on percent dark compartment duration (unpaired t-test for intact versus mutated: $t(14)=0.011$, $P=0.9917$,

η_p^2=0.01) in the light-dark exploration test (LD) test (**F**), percent open arm duration (unpaired t-test for intact versus mutated: $t(14)$=0.313, P=0.7588, η_p^2=0.01), closed arm duration (unpaired t-test for intact versus mutated: $t(14)$=0.382, P=0.7084, η_p^2=0.01) and total distance moved (unpaired t-test for intact versus mutated: $t(14)$=0.434, P=0.6706, η_p^2=0.01) in the elevated plus-maze (EPM) (**G**), and number of marbles buried (unpaired t-test for intact versus mutated: $t(14)$=0.438, P=0.6681, η_p^2=0.01) in the marble burying test (MB) (**H**).

Data are mean±SEM. *P<0.05, #P<0.05 (versus sated).

Figure S1, Related to Figure 1, Optical fiber placements for optogenetic experiments; photoexcitation effects on striatal eCBs.

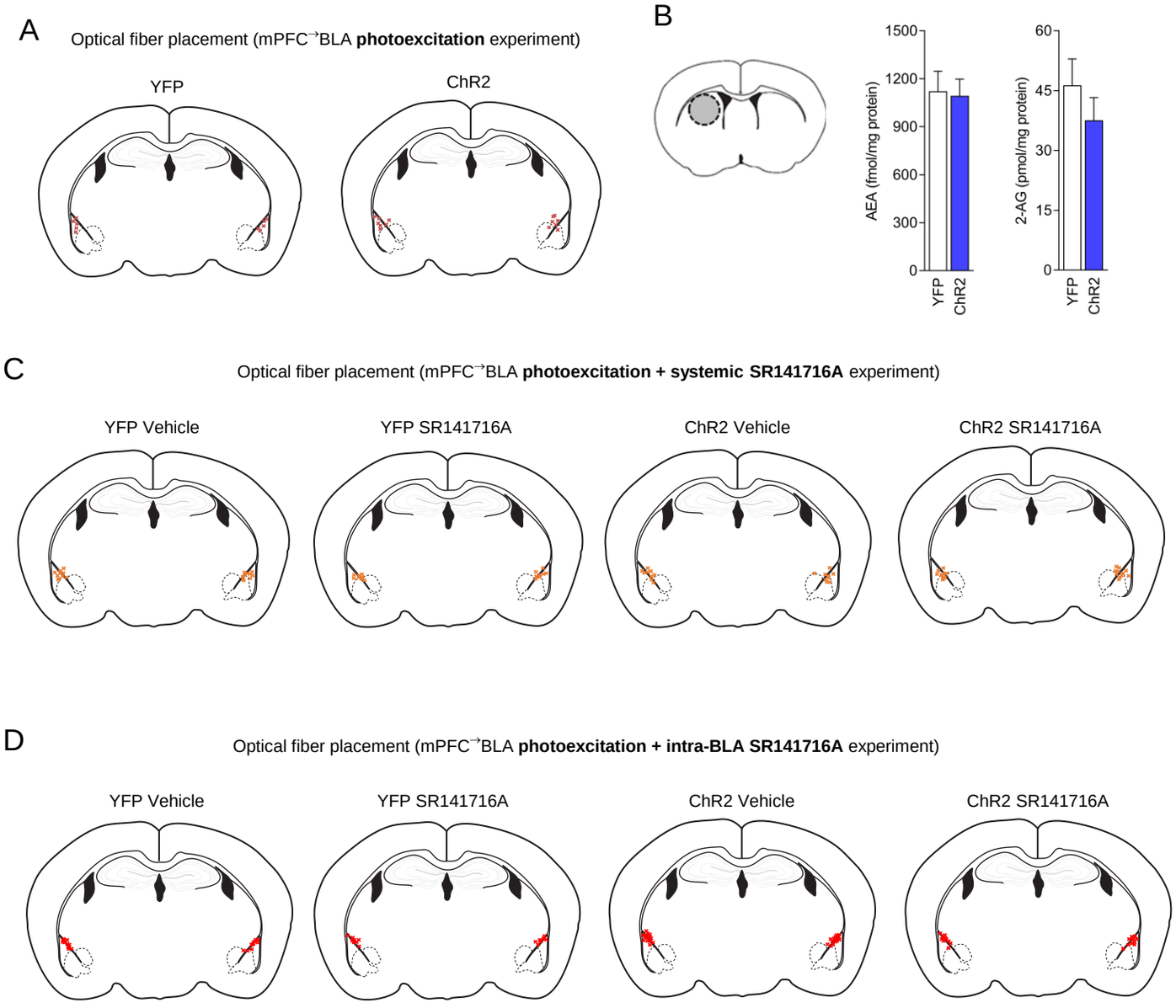

Figure S2, Related to Figure 2, Pharmacological effects on $GRAB_{eCB2.0}$ biosensor-measured eCBs at mPFC→BLA neurons.

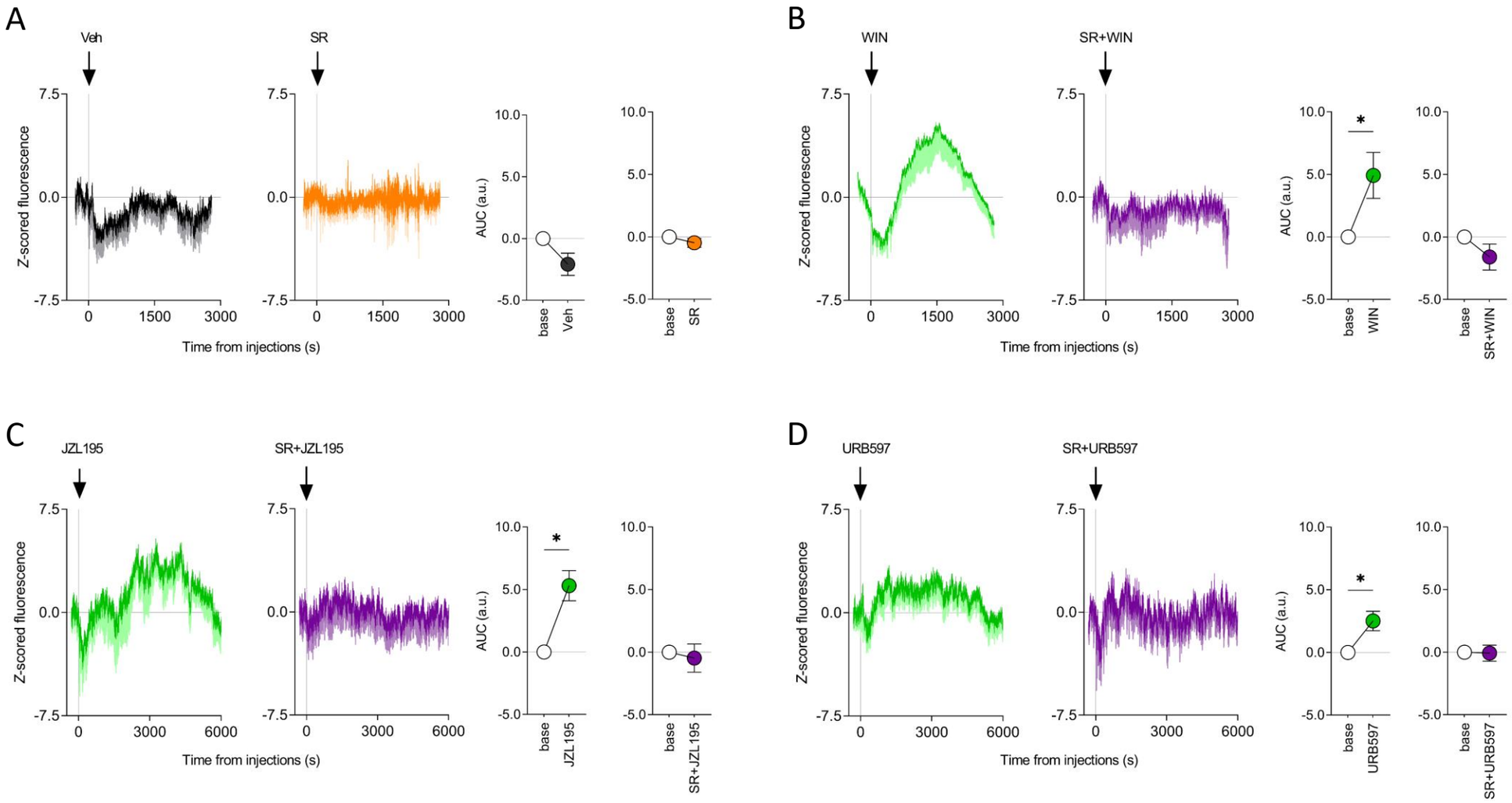

Figure S3, Related to Figure 3, Behavioral measures related to $GRAB_{eCB2.0}$ biosensor measurements

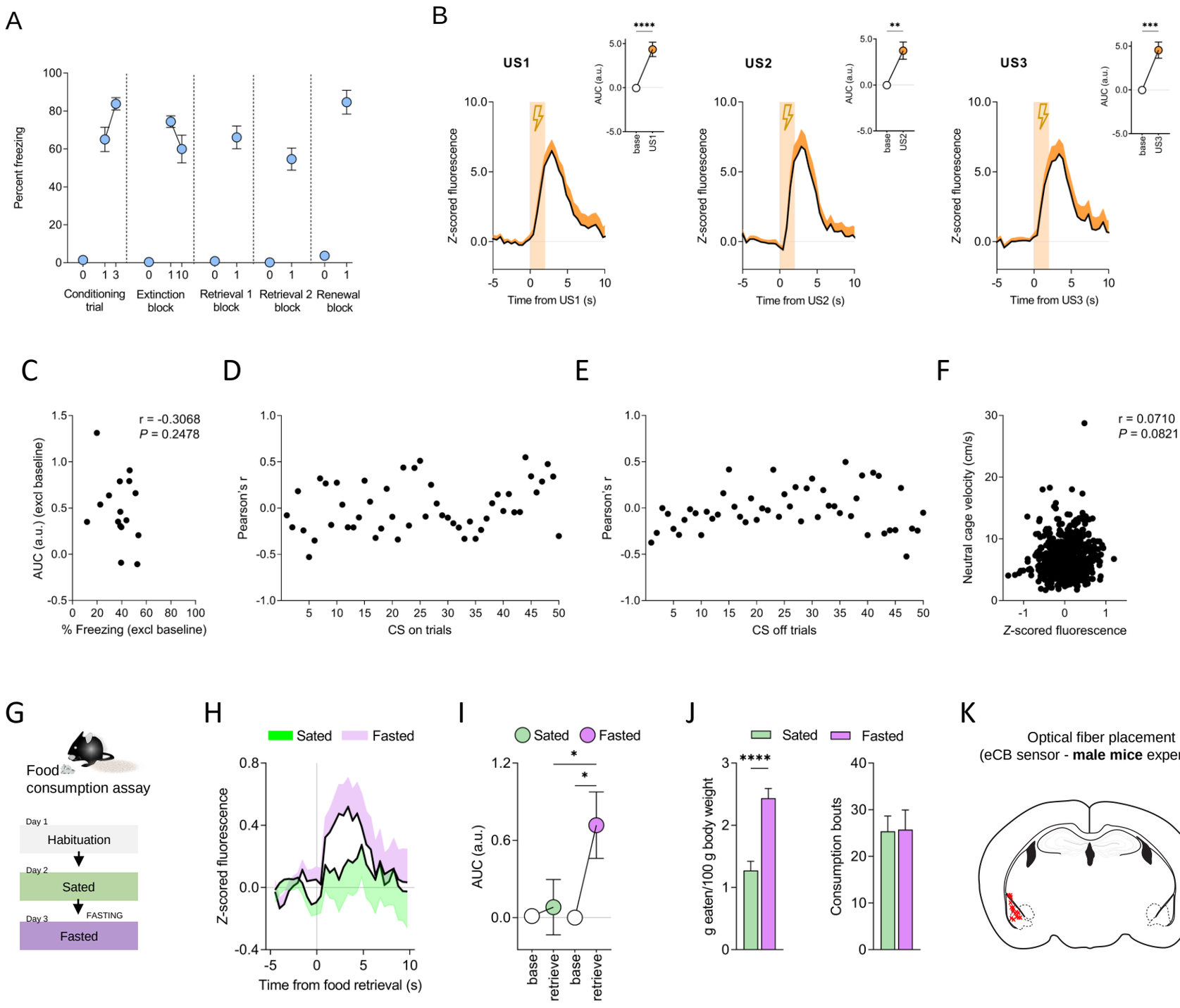

Figure S4, Related to Figure 4, *In vitro* neuronal recordings of eCB-mediated responses at vmPFC→BLA synapses

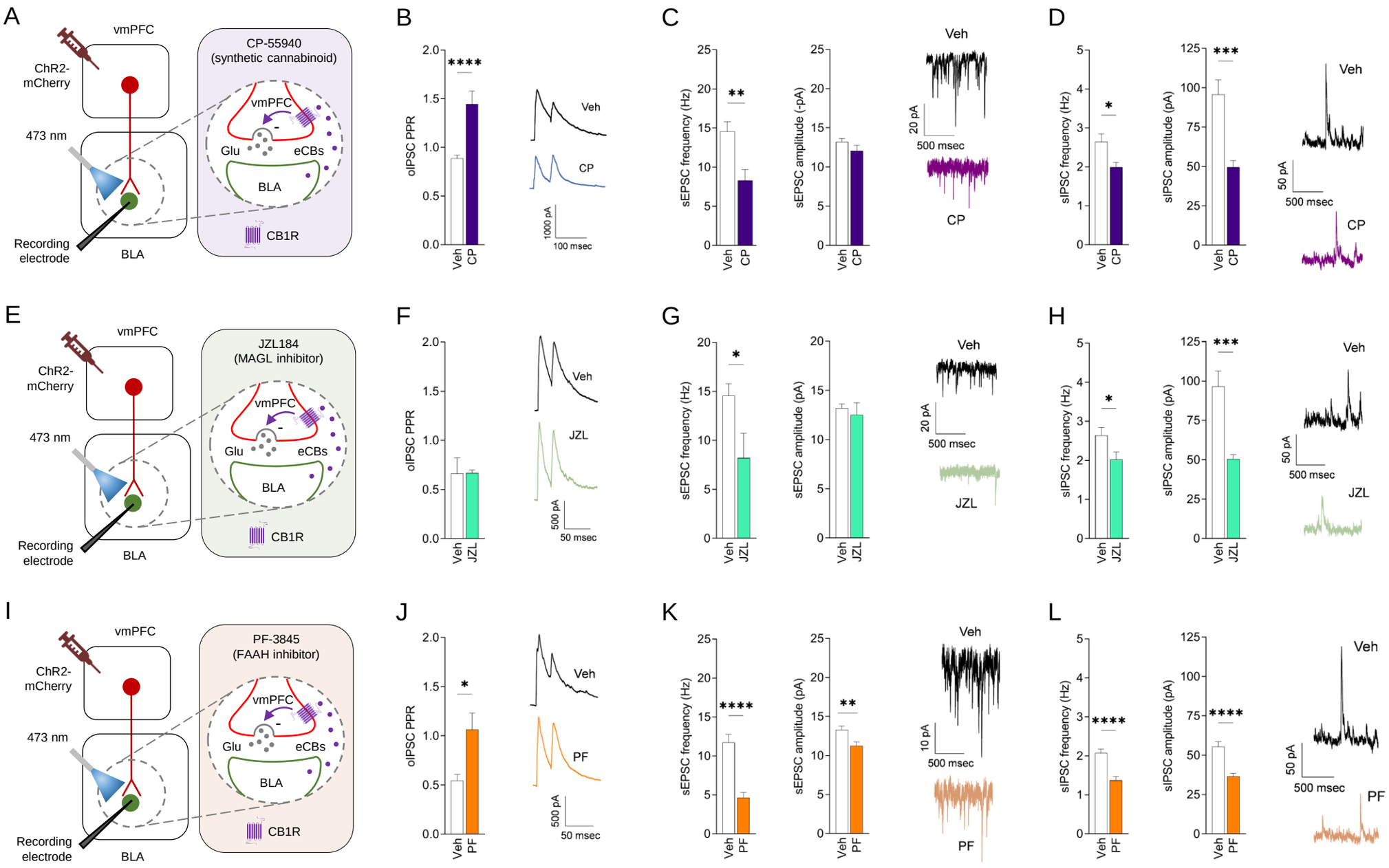

Figure S5, Related to Figure 5, Neural and behavioral analyses of CRISPR-Cas9 mutation