Supplementary material:

Electrophysiology

Slice preparation: Adult male mice were anesthetized then decapitated; the brain was rapidly removed and placed in ice-cold oxygenated (95% O_2/5% CO_2) artificial cerebral spinal fluid (aCSF) containing (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 26 $NaHCO_3$, 2 $CaCl_2$ and 10 glucose. Coronal, 300 µm thick, acute brain slices containing the CEm (2-4 sections per mouse) were cut using a Vibratome VT1200 (Leica Biosystems). Slices were allowed to recover for at least 1 hour in aCSF before being transferred to a recording chamber constantly perfused with aCSF and gradually warmed to the recording temperature of 32-34^{o}C.

Electrophysiological recordings: Recording pipettes with a final tip resistance of 3-6 MΩ were pulled on a Sutter P-1000 and filled with solution containing (in mM) 130 K-gluconate, 10 KOH-HEPES, 10 KCl, 2 $MgCl_2$, 2 Mg-ATP, 0.3 Na_3-GTP and 10 QX-314. Evoked postsynaptic potentials were measured in current clamp configuration at a holding potential of 0 mV based on pilot experiments demonstrating that this holding potential maximized inhibitory postsynaptic potentials (IPSPs) and minimized excitatory postsynaptic potentials (EPSPs). Electrical stimulations were delivered using a concentric, bipolar platinum/iridium electrode with a 2-3 µm tip diameter (MicroProbes, USA) connected to a constant current stimulator (Digitimer, UK). The stimulating electrode was placed in the identical position each day, specifically in the middle of the BLA, centered on the lateromedial extent of the central amygdaloid nuclei, similar to as previously described (25). CEm was visually identified based on the location of anatomical structures including the BLA and lateral subdivision of the central amygdala. Cells were visualized using an upright microscope (BX51, Olympus) equipped with a 40x water immersion objective, infrared light with differential interference contrast and a digital camera. Cells exhibiting >20% changes in access resistance or holding current were excluded from analysis. Resting membrane potential was estimated immediately upon obtaining the whole-cell recording

configuration using current clamp recordings. Input resistance was calculated from the average of 5-10 10 mV test-pulses. The average series resistance for all groups was 13.7 ± 0.3 MΩ and did not differ between groups. Data were filtered at 2.9 kHz and sampled at 10 kHz with an EPC10 patch-clamp amplifier and analysed using PatchMaster and FitMaster software (HEKA Elektronic, Germany). At the end of an experiment, GABA receptors were blocked with 100 µM picrotoxin (Tocris Bioscience) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors were blocked using 10 µM 6,7-dinitroquinoxaline-2,3-dione (DNQX, Abcam).

Behavior

Analysis of freezing behavior: Behavior was recorded by a video camera and scored offline by a pixel-based analysis software (http://topowatch.sourceforge.net/, TopoWatch v0.3) that was validated by comparison to manual analysis by two independent observers. A detailed validation procedure of the analysis software has been published previously (Verma *et al.*, 2012).

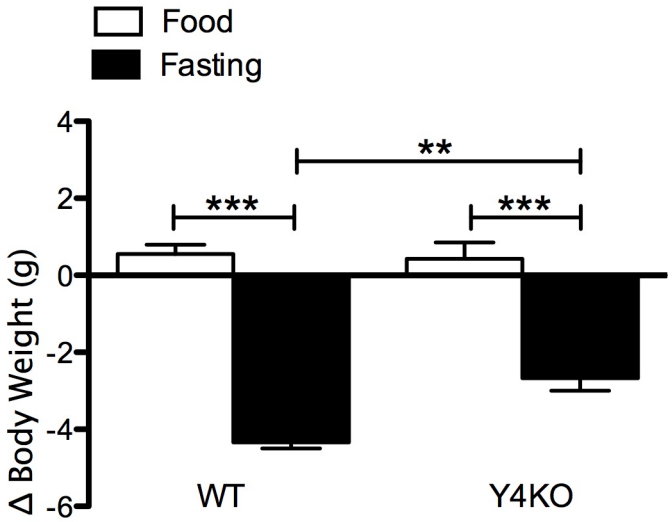
Determination of sensitivity threshold to the US: Naïve wildtype and Y4KO mice were placed individually into the conditioning box. After a 3 min habituation period, a series of electric foot shocks of increasing current intensity was applied (0.1-0.9 mA, 2 s, increase in 0.05 mA steps every 30 s). The sensitivity threshold was defined as the current at which the mice displayed each sign of the US sensitivity response (flinching, running, jumping and vocalization).

Histochemical Analysis

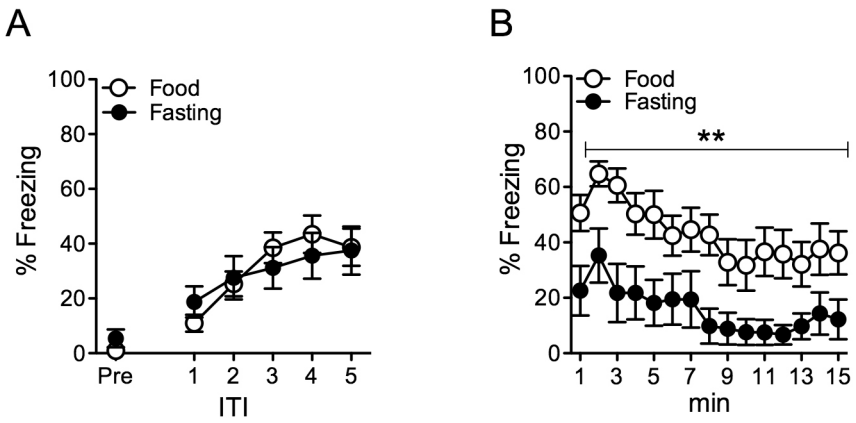
Immunohistochemistry: Immunohistochemical analysis was performed on free-floating, PFA-fixed, 40 µm thick coronal sections using indirect peroxidase labeling, as described previously [18]. The following antisera were used: polyclonal rabbit anti-cFos (1:20,000 PC38, Calbiochem), polyclonal rabbit anti-GABA (1:2,000 A2052, Sigma), polyclonal rabbit anti-FoxP2

(1:1,000, ab16046, Abcam), monoclonal mouse anti-PKCδ (1:500; BD Biosciences, #610397, Lot 23228). In brief, coronal sections were incubated free floating in 10% normal goat serum (Biomedica, Vienna, Austria) in Tris-HCl buffered saline (TBS; 50 mM, pH 7.2) for 90 min, followed by incubation with primary antiserum. The resulting complex was visualized by incubation with horseradish peroxidase-coupled secondary antibody (1:250 P0448; Dako, Vienna, Austria) at room temperature for 150 min, followed by tyramide amplification solution (1:100, TSA AMCA) for 6 min. After staining, sections were exposed to 0.01M HCL for 20 min at room temperature to denature HRP and first primary antibodies and incubation with the second primary antibody was performed as described for cFos except that TSA Fluorescein was used for staining. Sections were mounted on slides and covered using Vectashield mounting medium (Vector laboratories, Inc., Burlingame, USA). For visualizing PKCδ labeling, a biotinylated secondary horse anti-mouse antibody (1:200, Vectorlabs, Austria) was used followed by incubation with Alexa Fluor 546 labeled Streptavidin (1:100, Molecular Probes, Austria).

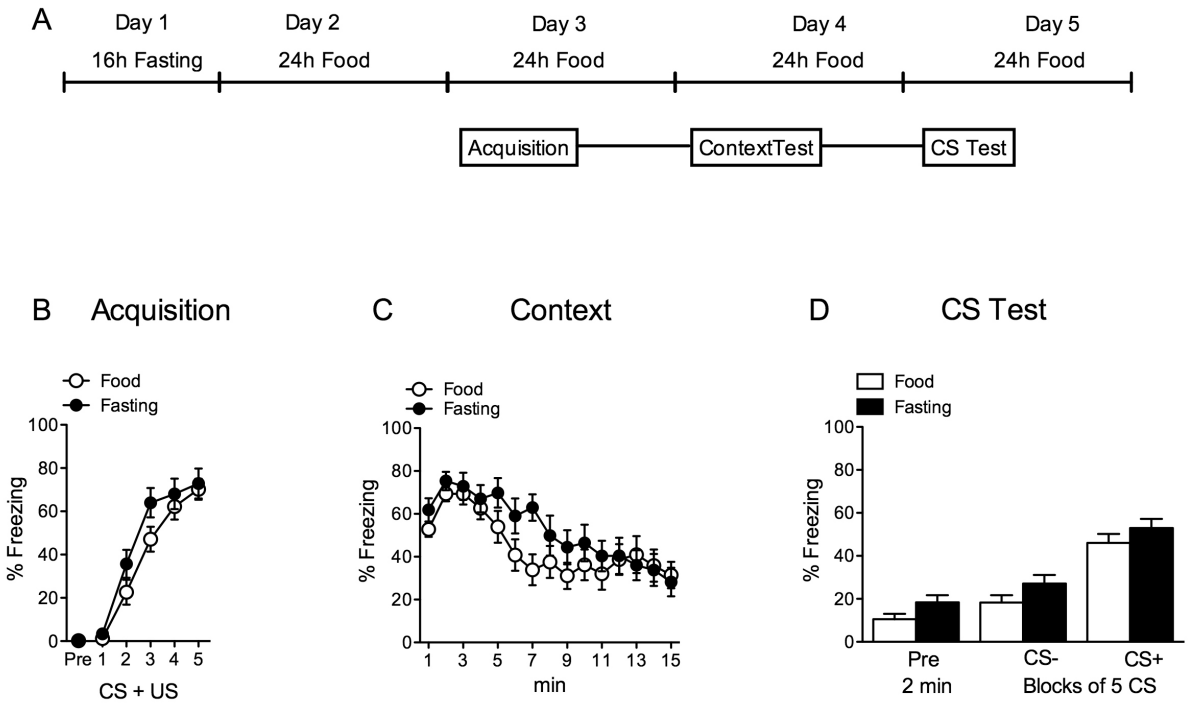
Number of cFos positive cells was obtained bilaterally from 6 matched sections per animal in the amygdala, hippocampus and hypothalamus at a magnification of 400 times in multiple separate fields and mean values were calculated for each mouse. Results are presented as number of immunoreactivity positive cells/section or per area and expressed as mean ± SEM. Analysis of dual labeling immunofluorescence was done as described elsewhere [18,66]. In brief, for each brain area depicting a region of interest, 4 matched sections per animal were processed for either cFos/GABA, cFos/FoxP2 or cFos/PKCδ for dual localization. Identification of dual labeled cells was performed at 400 times magnification within the respective brain area in each section.



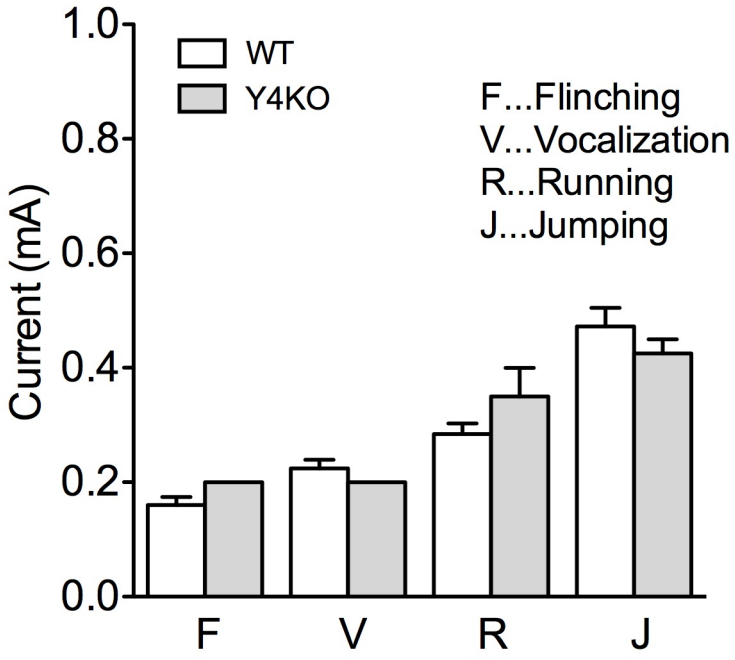
Supplemental fig. 1. Reduction of body weight in fasted wildtype and Y4KO mice. (A) The weight of wildtype and Y4KO mice was determined before and after a 16h fasting period and compared to respective non-fasted control groups. There was a mean reduction of 4.33g (17.4%) and 2.67g (13.36%) of bodyweight in fasted wildtype and Y4KO mice, respectively (one-way ANOVA: $F_{(3,30)}$=75.29, P<0.001; with *Bonferroni post-hoc* test: WT food vs. fasting: t=12.97, *P*<0.001, Y4KO food vs. fasting: t=6.96, *P*<0.001 and WT fasting vs. Y4KO fasting: t=3.95, *P*<0.01; ** *P*<0.01, *** *P*<0.001; WT: n=9, Y4KO: n=8).



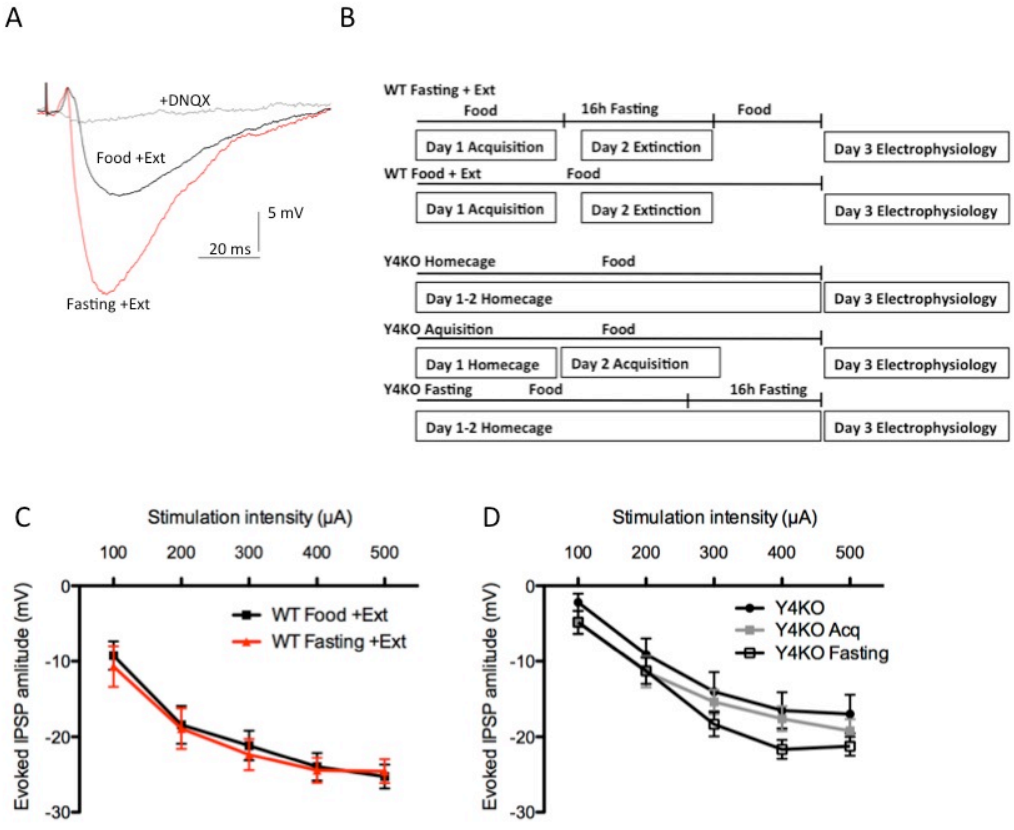
Supplemental fig. 2. Fasting before fear acquisition does not alter context fear acquisition but promotes context extinction by reducing context fear memory expression. (A) Wildtype mice were fasted for 16h before and during fear acquisition, followed by context extinction 24h later under fed conditions. No change was observed during context fear learning; however, (B) extinction of context fear was significantly faster in previously fasted mice compared to non-fasted controls. The apparent difference was predominantly due to reduced context fear expression as evidenced by reduced freezing already during the first minute. (two-way ANOVA for repeated measurement, $^{**}P<0.01$; Food: n= 11, Fasting: n=11).



Supplemental fig. 3. Acute short-term fasting (16h) followed by re-feeding (24h) did not change consecutive fear conditioning. (A) Fasting was performed for 16h (day1) followed by a period of re-feeding (24h on day 2). Fear conditioning and testing for context fear and cued fear was performed on day3-5. All mice had access to food *ad libitum* at least 24h before and during fear acquisition and fear testing. (B) Both, fasted and fed mice exhibited similar baseline freezing and acquisition of conditioned fear. (C) No changes in context freezing of mice that were fasted and re-fed before fear acquisition. (D) Mice that were fasted and re-fed before fear acquisition displayed unchanged CS-induced freezing 48h after fear conditioning. There was however, a trend towards a facilitation of fear acquisition (B, *P*=0.09) and increased fear expression in fasted and re-fed mice (D, *P*=0.07 for preCS and *P*=0.1 for CS-). (repeated two-way ANOVA for acquisition and context fear testing, Student's t-test CS testing; Food: n= 13, Fasting: n=13).

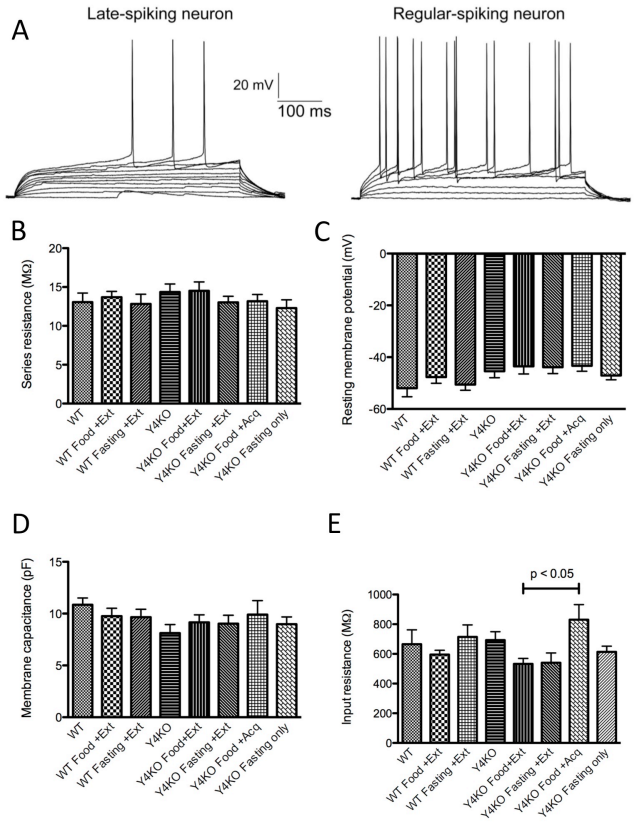


Supplemental fig. 4. Equal sensitivity threshold of wildtype and Y4 receptor KO mice. (A) In the fear conditioning box wildtype and Y4KO mice were exposed to increasing intensities of electric food shocks and the behavioral reaction was monitored (WT: n=10, Y4KO: n=6, Mann Whitney test).

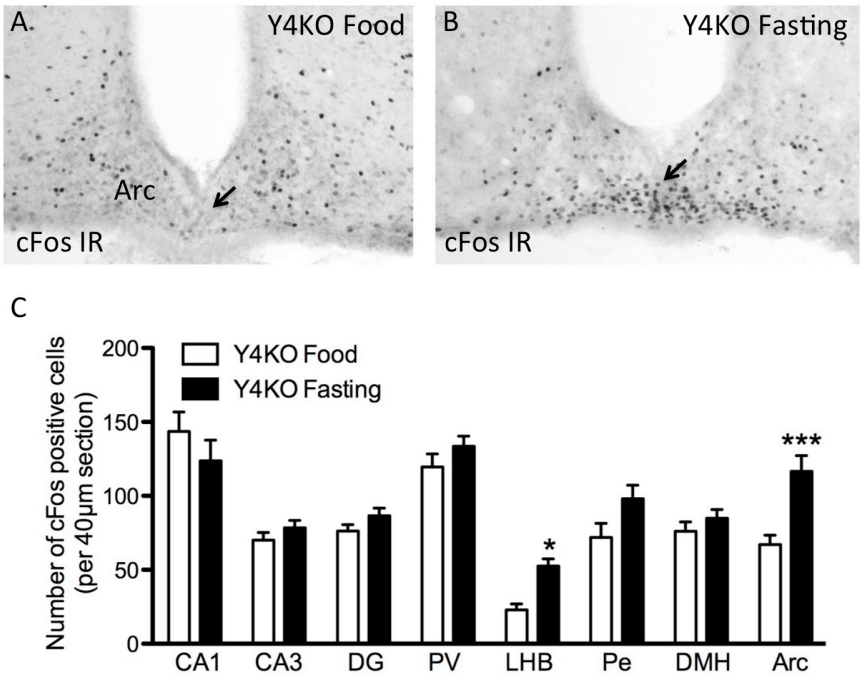


Supplemental fig. 5. Feed-forward inhibition in Y4KO mice is unchanged after acquisition or fasting alone. (A) Example traces depicting increased amplitude of an evoked IPSP in a CEm neuron of a Y4KO mouse upon fasting + fear extinction (red trace) compared to Food + extinction (black trace) and blockade by application of an AMPA receptor antagonist (+DNQX, gray trace), (B) experimental setup for wildtype mice with 16h fasting before and during extinction learning and Y4KO mice with homcage only, after fear acquisition and fasting alone. (C) Note that fasting + extinction did not increase feed-forward inhibition in wildtype mice compared to extinction alone, (D) no difference in BLA-mITC-CEm feed-forward-inhibition in Y4KO mice between homecage, fear acquisition and Y4KO mice that were only fasted but not fear conditioned (n = WT Food+ext 4 mice, 15 cells; WT Fasting+ext 3 mice, 12 cells; Y4KO 5

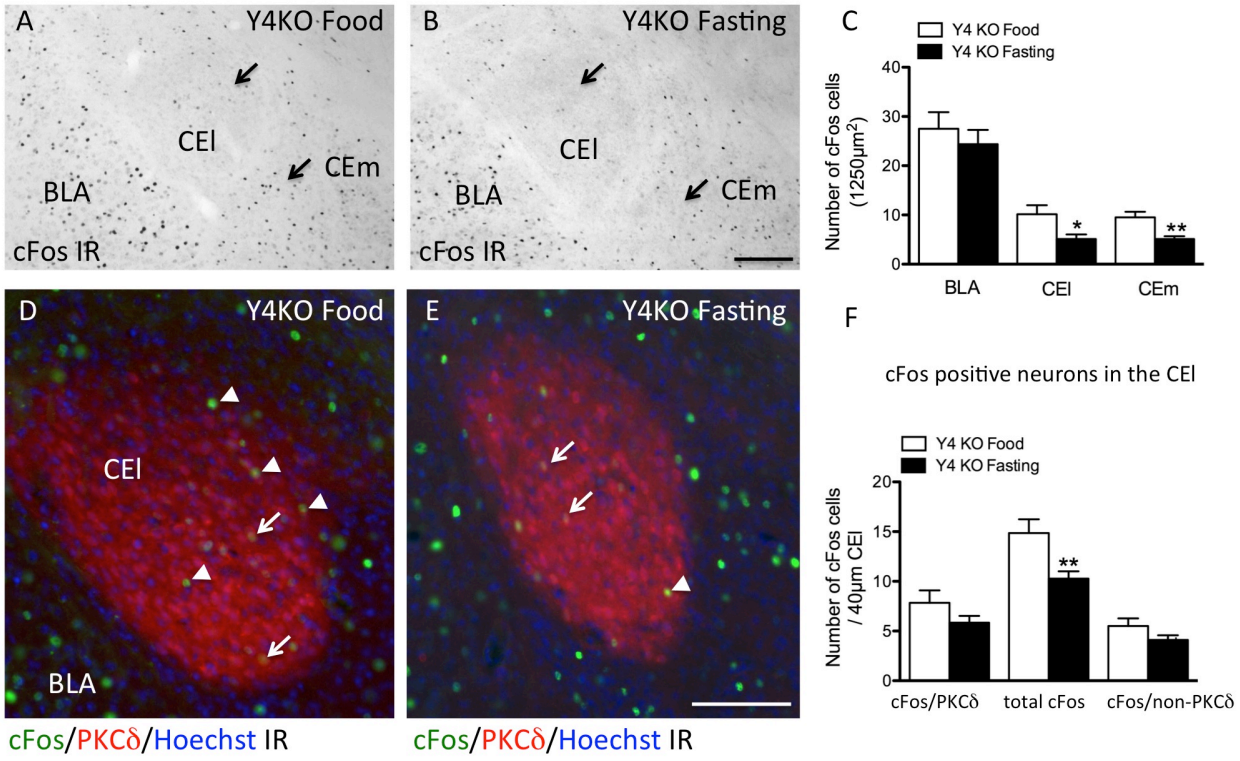
mice, 13 cells; Y4KO Acq 3 mice, 13 cells: Y4KO Fasting 3 mice, 14 cells, two-way ANOVA for repeated measurements).



Supplemental fig. 6. Membrane properties of CEm neurons. Two types of neurons were observed in the CEm, (A) example trace of a late-spiking neuron and of a regular spiking neuron. (B) Series resistance, (C) resting membrane potential, (D) membrane capacitance and (E) input resistance are shown. Note the increase in input resistance in Y4KO mice after fear acquisition. (n = WT 4 mice, 12 cells; WT Food+Ext 4 mice, 15 cells; WT Fasting+Ext 3 mice, 12 cells; Y4KO 5 mice, 13 cells; Y4KO Food+Ext 4 mice, 14 cells; Y4KO Fasting+Ext 4 mice, 12 cells; Y4KO Food+Acq 3 mice, 13 cells; Y4KO Fasting 3 mice, 14 cells, *P<0.05, one way ANOVA with *Bonferroni post hoc* test).



Supplemental fig. 7. Expression pattern of immediate early gene cFos after 16h of fasting or food available *ad libitum* followed by extinction training in Y4KO mice. (B) Increased expression of cFos in a fasted Y4KO mice in the arcuate nucleus of the hypothalamus compared to (A) a control Y4KO mouse with food available *ad libitum*, (C) histograms depicting quantification of cFos positive cells demonstrate increased cFos in the lateral habenula and in the arcuate nucleus of Y4KO mice after 16h of fasting or food available *ad libitum* followed by extinction training (Y4KO Food: n=8, Y4KO Fasting: n=8; **P*<0.05, ****P*<0.001, one way ANOVA with Bonferroni post hoc test).



Supplemental fig. 8. Expression of the immediate early gene cFos in the amygdala after 16h of fasting or food available *ad libitum* followed by extinction training in Y4KO mice. (A) Expression of cFos immunopositive cells in the amygdala of control Y4KO mice with food available *ad libitum* and (B) fasted Y4KO mice (arrows: note the reduced number of cFos positive cells in the CEl and CEm of fasted Y4KO mice in B, compared to fed Y4KO mice in A). (C) Histograms depicting quantification of cFos positive cells demonstrating a reduction in the CEl and CEm but not in the BLA of Y4KO mice after 16h of fasting or food available *ad libitum,* followed by extinction training. (D) Dual immunohistochemistry of PKCδ and cFos in a fed and (E) fasted Y4KO mice after fear extinction (arrows: example of PKCδ/cFos dual labeled neurons, arrowheads: example of cFos single labeled neurons) and (F) quantification depicting a significant reduction in cFos labeling in the CEl, however, non-significant reductions were seen in subpopulations of PKCδ and non-PKCδ neurons, suggesting that in the CEl, fasting does not

exclusively inhibit activation of PKCδ expressing neurons f; scale bar 100μm. (Y4KO Food: n=8, Y4KO Fasting: n=8; *P<0.05, **P<0.01, one way ANOVA with *Bonferroni post hoc* test).