Supplemental information
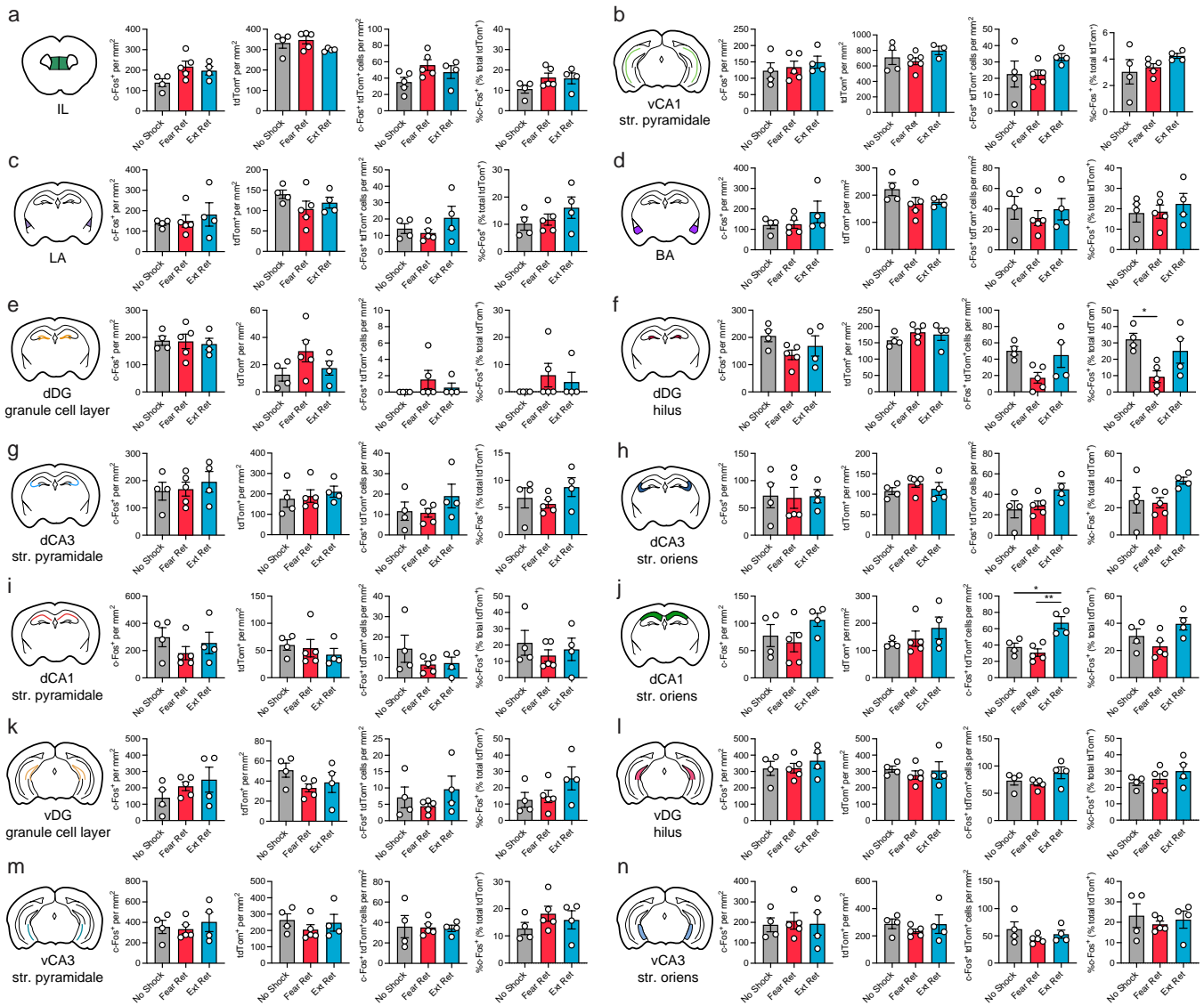
Ventral hippocampal interneurons govern extinction

# and relapse of contextual associations

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Supplementary Figure 1. Additional regions of interest for c-Fos-based analysis of general and SST-IN-specific following fear or extinction retrieval, related to Fig. 1. (a-n) Quantification of c-Fos+ cell density, tdTom+ cell density, c-Fos+ tdTom+ cell density, and c-Fos+ (% total tdTom+ cells) from **(a)** infralimbic medial prefrontal cortex (IL), **(b)** vCA1 str. pyramidale, **(c)** lateral amygdala (LA), **(d)** basal amygdala (BA), **(e)** dorsal dentate gyrus (dDG) granule cell layer, **(f)** dDG hilus, **(g)** dorsal CA3 str. pyramidale, (**h**) dorsal CA3 str. oriens, **(i)** dCA1 str. pyramidale, **(j)** dCA1 str. oriens, **(k)** vDG granule cell layer, **(l)** vDG hilus, **(m)** vCA3 str. pyramidale and **(n)** vCA3 str. oriens related to Fig. **1a-f** analyzed by one-way ANOVA. c-Fos+ (% total tdTom+ cells) from dDG hilus: $F_{(2, 10)} = 5.72$, $p < 0.05$. c-Fos+ tdTom+ cell density from dCA1 str. oriens: $F_{(2, 10)} = 12.0$, $p < 0.01$. No Shock: $n = 4$; Fear Ret: $n = 5$; Ext Ret: $n = 4$. * $p < 0.05$, ** - $p < 0.01$ Tukey's post-hoc test.
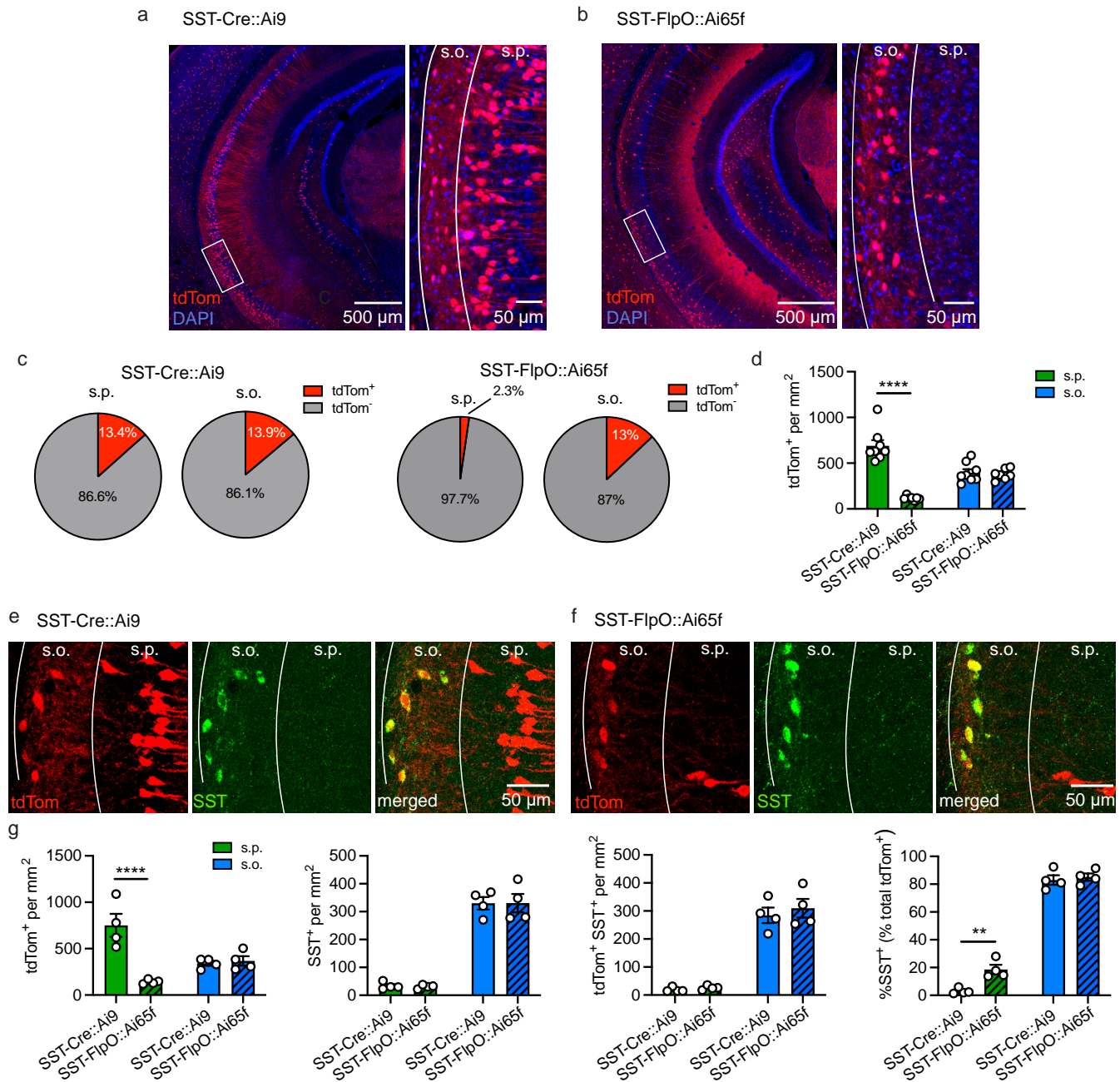
Supplementary Figure 2. Comparison of recombination selectivity in SST-Cre versus SST-FlpO mice, related to Fig. 1. (a-b): Representative image of vCA1 in **(a)** SST-Cre::Ai9 and **(b)** SST-FlpO::Ai65f mice. Solid white box indicates region of magnification in right panel. **(c)** tdTom+ and tdTom- cells as a proportion of DAPI+ cells in vCA1 s.p. and s.o. layers. **(d)** tdTom+ cell density in vCA1 s.p. and s.o. layers analyzed by two-way ANOVA. $F_{(1, 26)} = 45.2$, $p < 0.0001$. **(e-f)** Representative image of SST immunolabeling and tdTom expression in vCA1 of **(e)** SST-Cre::Ai9 and **(f)** SST-FlpO::Ai65f mice. **(g)** tdTom+, SST+, tdTom+ SST+ cell density, and SST+ cells (% of tdTom+ cells) in vCA1 s.p. and s.o., analyzed by two-way ANOVA. tdTom+ density: $F_{(1, 12)} = 21.4$, $p < 0.001$. SST+ (% total tdTom+): $F_{(1, 12)} = 5.12$, $p < 0.05$. **a-d:** SST-Cre::Ai9: $n = 8$; SST-FlpO::Ai65f: $n = 7$. **e-g**: SST-Cre::Ai9: $n = 4$; SST-FlpO::Ai65f: $n = 4$. ** - $p < 0.001$, **** - $p < 0.0001$ by Šidák's post hoc test.
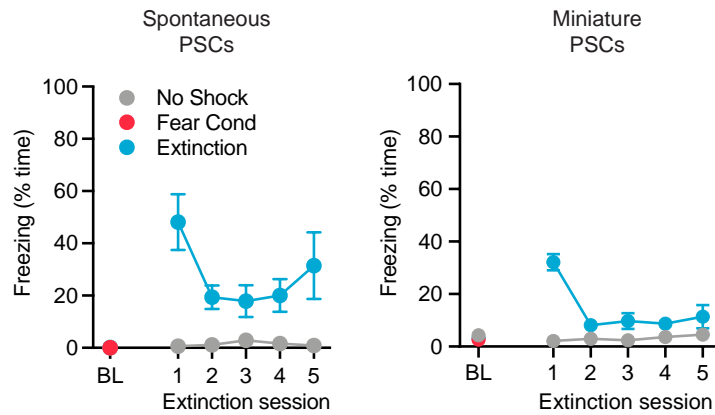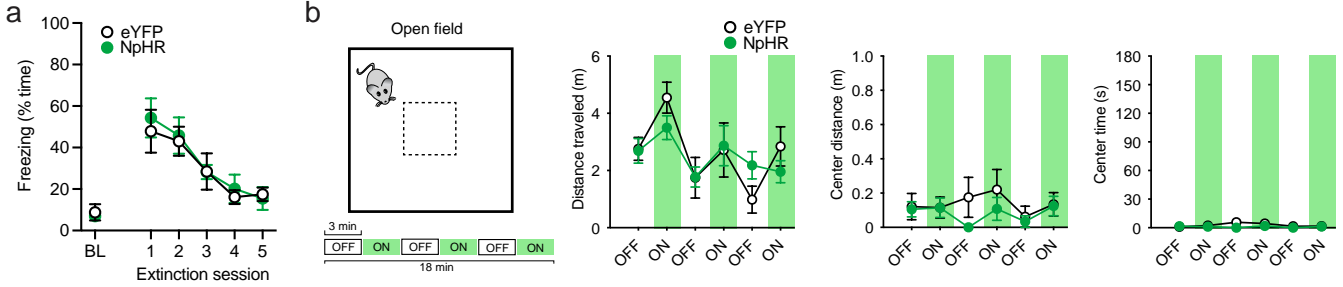
Supplementary Figure 3. Freezing levels for mice used in synaptic electrophysiology experiments, related to Fig. 2. Freezing was quantified during the pre-shock baseline period during contextual fear conditioning and during each 5 min extinction session. Spontaneous postsynaptic currents (PSCs); No Shock: n = 6 mice; Fear Cond: n = 4 mice; Extinction: n = 5 mice. Miniature PSCs; No Shock: n = 5 mice; Fear Cond: n = 5 mice; Extinction: n = 3 mice.
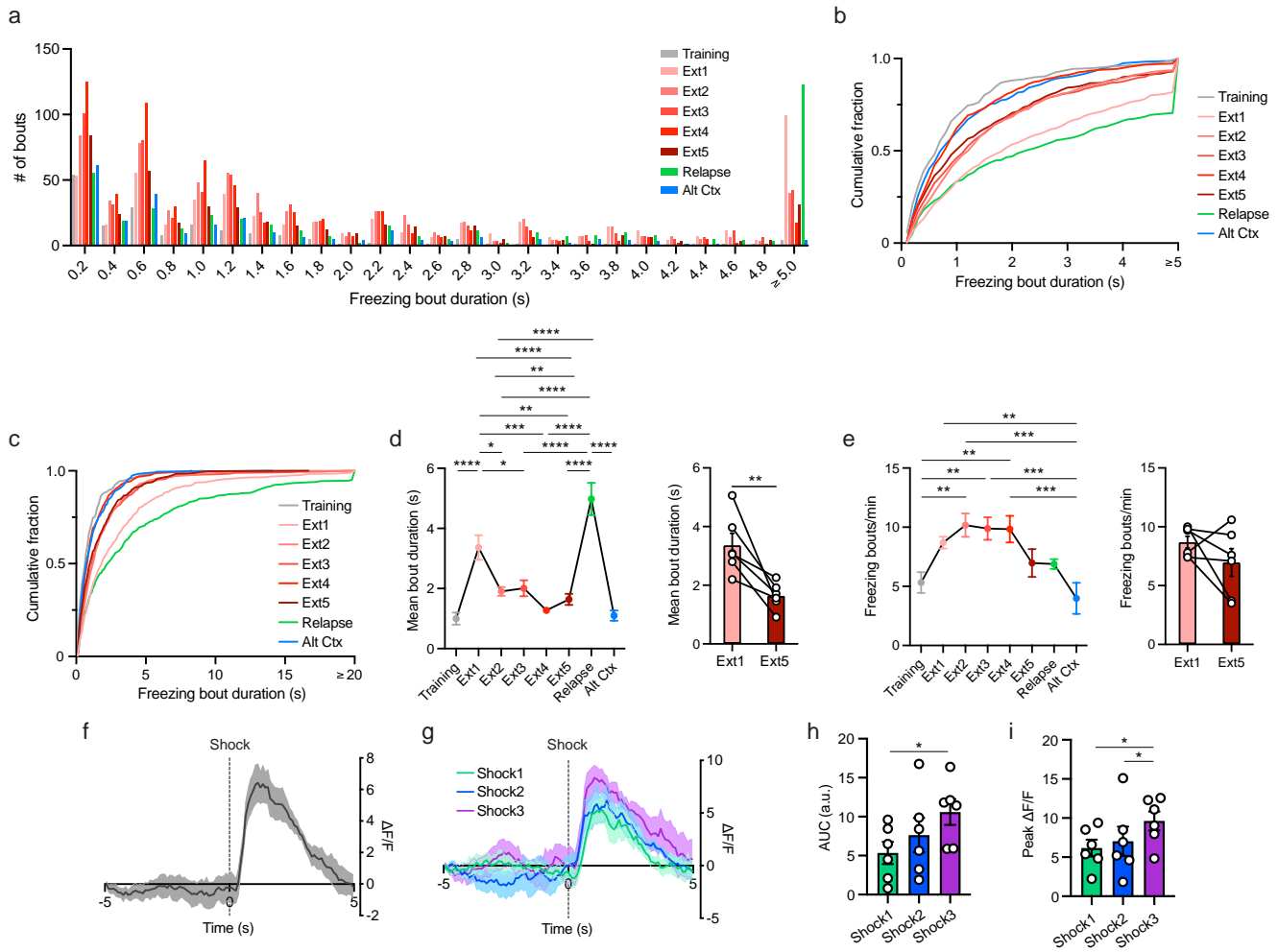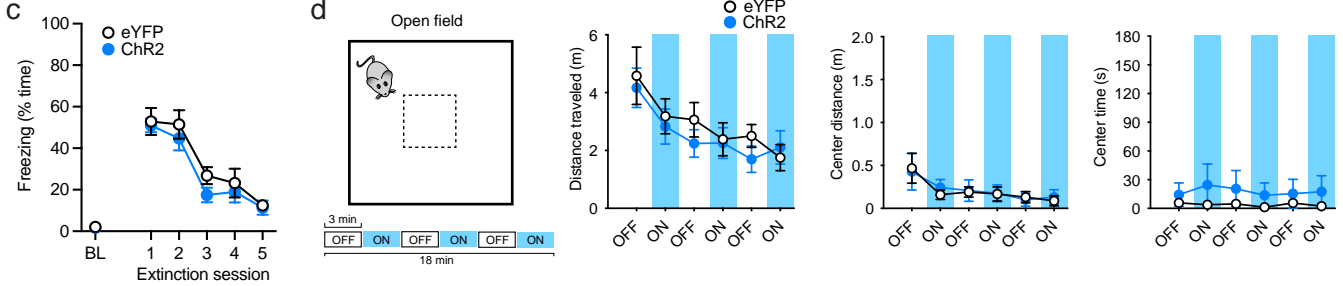
Supplementary Figure 4. Freezing bout analysis and vCA1 SST-IN shock-related Ca²⁺ responses, related to Fig. 3. (a-b) Histogram **(a)** and cumulative distribution **(b)** of freezing bout length across behavioral sessions in Fig. 3, with bouts ≥5 s as the maximum value. **(c)** Same as **(b)**, but with bouts ≥20 s as the maximum value. **(d)** Mean bout duration across behavioral sessions (left), with break-out for comparison of extinction sessions 1 and 5 for individual animals (right), analyzed by one-way repeated-measures ANOVA. $F_{(7, 35)}$ = 23.6, p < 0.0001. **(e)** Freezing bout frequency across behavioral session (left), with break-out for comparison of extinction sessions 1 and 5 for individual animals, analyzed by one-way repeated-measures ANOVA. $F_{(7,35)}$ = 8.22, p < 0.0001. **(f)** Mean ΔF/F from ±5 s of shock responses from during contextual fear conditioning. **(g)** ΔF/F from ±5 s of individual shocks. **(h-i)** Quantification of area under the curve (AUC) **(h)** and peak amplitude **(i)** of mean ΔF/F from of 0 to 2 s of each shock onset, as analyzed by one-way RM ANOVA. AUC: $F_{(2, 10)}$ = 4.95, p < 0.05. Peak: $F_{(2, 10)}$ = 7.26, p < 0.05. n = 6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Tukey's post-hoc test **(d-e, h-i)**.
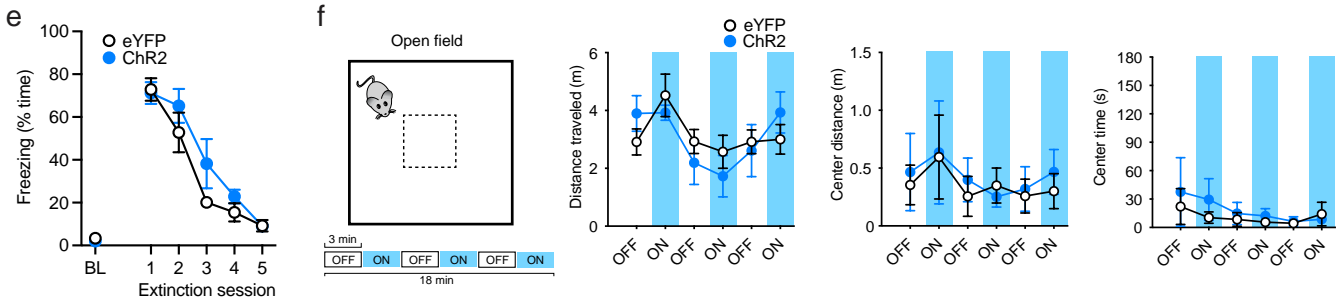
Supplementary Figure 5. Extinction-related freezing and open field metrics for optogenetic manipulations of SST-INs, extinction-related SST-INs and PV-INs, related to Figs. 4-6. (a-b) Additional analysis associated with photoinhibition of SST-INs in Fig. 4. of **(a)** freezing during the baseline period of the training session and first 3 min of each of the 5 extinction sessions, and **(b)** total distance traveled, center distance, and time in center from open field test. Photostimulation occurred in 3 min epochs beginning 3, 9, and 15 min into the 18 min test with light parameters identical to manipulations in fear conditioning contexts. **(c-d)** Analysis equivalent to that shown in **a-b** but associated with photoexcitation of SST-INs in Fig. 3. **(e-f)** Analysis equivalent to that shown in **a-b** but associated with photoexcitation of extinction-tagged SST-INs in Fig.5. **(g-h)** Analysis equivalent to that shown in **a-b** but associated with photoexcitation of PV-INs in Fig. 6. **a-b**: eYFP: n = 6; NpHR: n = 6. **c-d**: eYFP: n = 5; ChR2: n = 4. **e-f**: eYFP: n = 5; ChR2: n = 4. **g-h**: eYFP: n = 6; ChR2: n = 6.
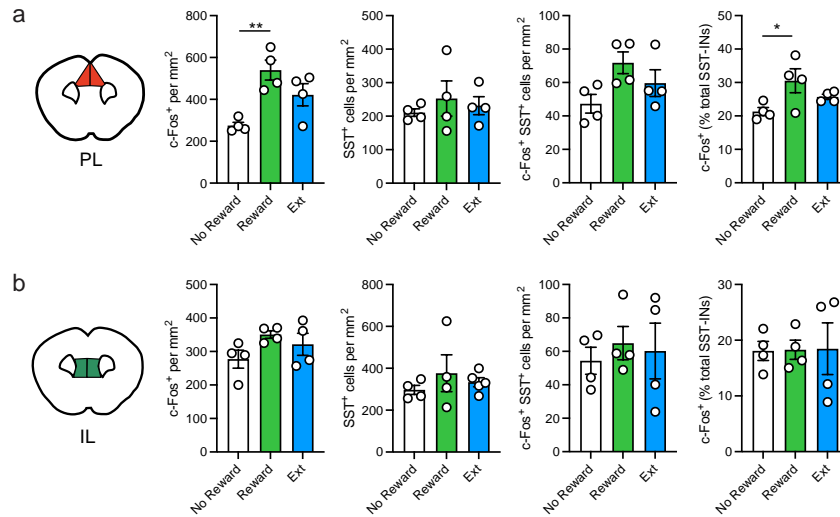
Supplementary Figure 6. Additional analysis of SST-IN activation during retrieval of reward extinction, related to Fig. 7. (a-b) c-Fos+ cell density, SST-IN density, c-Fos+ SST-IN density, and c-Fos+ SST-INs (% total SST-INs) from (**a**) prelimbic cortex (PL) and (**b**) infralimbic cortex (IL) during retrieval of an extinguished contextual reward memory analyzed by one-way ANOVA. c-Fos+ cells per mm^2: $F_{(2, 9)} = 10.0$, $p < 0.01$. c-Fos+ (% total SST-INs): $F_{(2, 9)} = 4.28$, $p < 0.05$. No Reward: $n = 4$; Reward: $n = 4$; Extinction: $n = 4$. * - $p < 0.05$; ** - $p < 0.01$ by Tukey's post-hoc test.