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Supplemental information

Acute reorganization of postsynaptic $GABA_A$ receptors reveals the functional impact of molecular nanoarchitecture at inhibitory synapses

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Olah et al., Figure S1

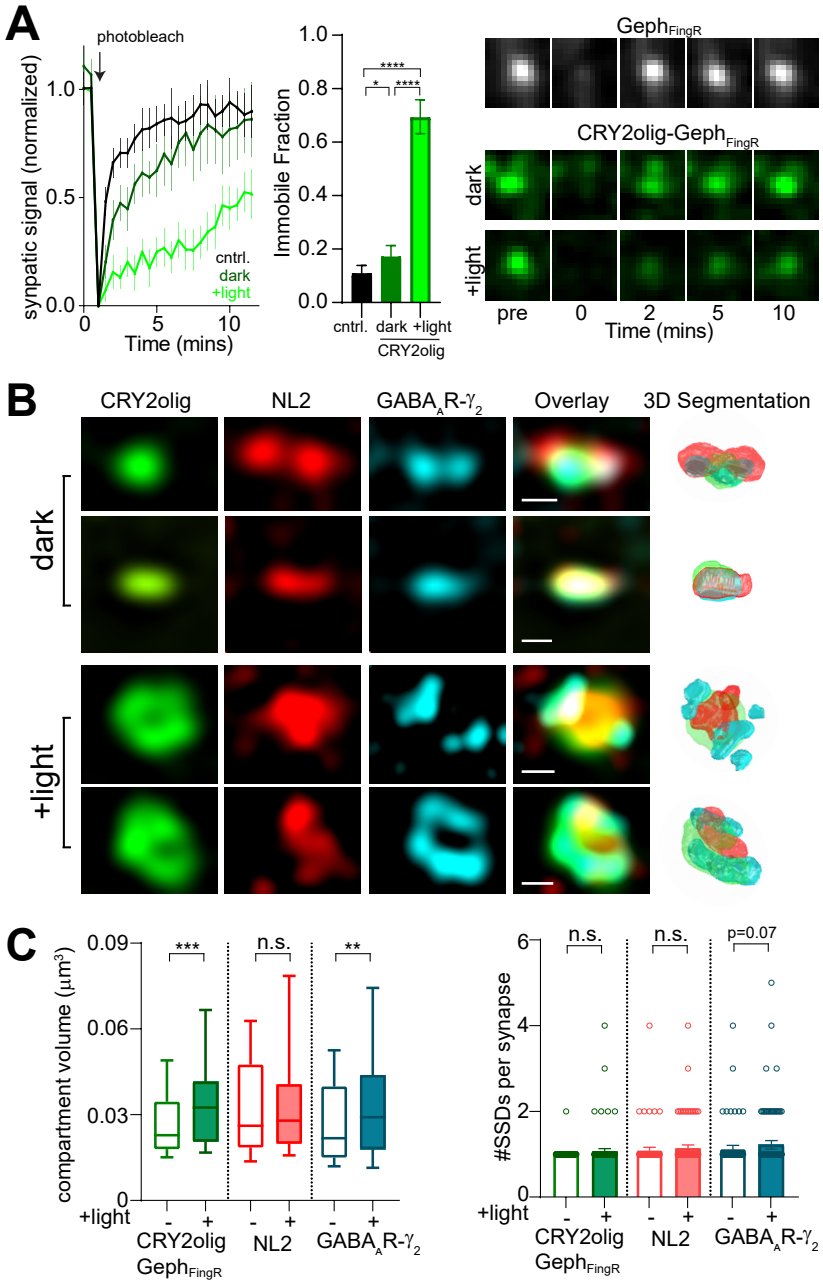

Figure S1. Geph$_{FingR}$ clustering stabilizes it at synapse and does not perturb NL2 nanostructure (Related to Figure 1).

A. The rate of fluorescence recovery after photobleaching is reduced for CRY2olig-Geph$_{FingR}$-mScarlet after exposure to blue light. Geph$_{FingR}$-mScarlet (black) at inhibitory synapses rapidly recovers with a $t_{1/2}$=41.6±8 sec, immobile fraction (IF) = 0.11±0.027 (11 synapses, 3 cells). Before exposure to blue light, CRY2olig-Geph$_{FingR}$-mScarlet (dark green) recovers at inhibitory synapses with slightly delayed kinetics with a $t_{1/2}$=93.9±16.8 sec, IF = 0.17±0.04 (10 synapses, 3 cells). After exposure to blue light (2 mins, 1 sec pulse every 30 sec), CRY2olig-Geph$_{FingR}$-mScarlet recovery is drastically reduced with a $t_{1/2}$=121.2±86.2 sec, IF = 0.7±0.06 (12 synapses, 3 cells). Error bars represent 95% CI. Immobile fractions for each condition are shown in the middle plot. $^{*}p < 0.05$; $^{****}p < 0.0001$; ordinary one-way ANOVA, multiple comparisons test with Tukey's correction. Right panel, example synapse fluorescence for Geph$_{FingR}$-mScarlet (gray, top), CRY2olig-Geph$_{FingR}$-mScarlet before light (green, middle), and CRY2olig-Geph$_{FingR}$ mScarlet after light exposure (green, bottom).
B. Representative SIM images and 3D reconstructions for CRY2olig-Geph$_{FingR}$-GFP (green), NL2 (red) and GABA$_A$Rγ2 (cyan) before and after exposure to 10min blue light. Scale bars = 0.2 μm.
C. Quantification of synaptic compartment volume (10-90th percentile) and mean number of subsynaptic domains (SSDs) for CRY2olig-Geph$_{FingR}$, NL2, and GABA$_A$Rγ2 at inhibitory synapses before and after 10 min blue light illumination. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$; Mann-Whitney test. N=2 cultures, n=94-132 synapses.

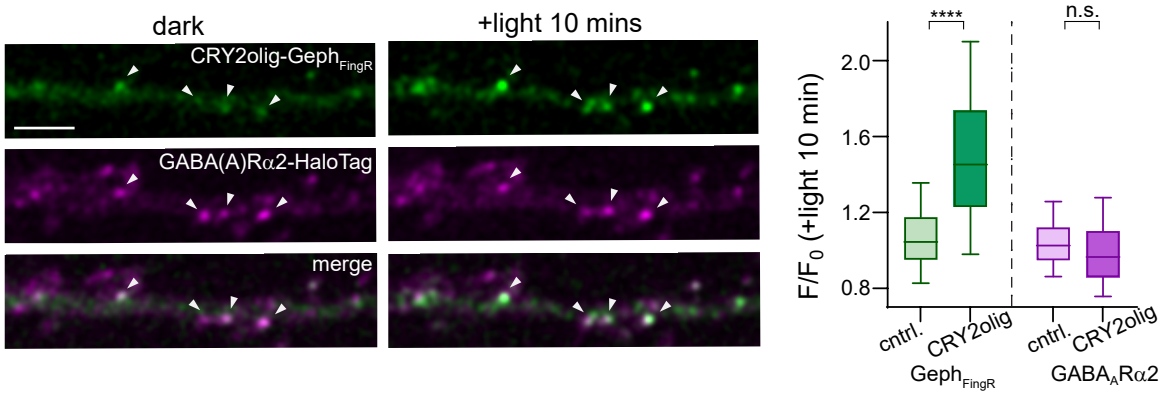

Figure S2. CRY2olig-Geph$_{FingR}$ clustering does not perturb expressed GABA$_A$Rα2-Halo levels at synapses (Related to Figure 2).

Representative confocal images of CRY2olig-Geph$_{FingR}$-GFP and GABA$_A$Rα2-HaloTag + JF635i before and 10 min following light exposure. Scale bar = 2 μm. Right, synaptic fluorescent intensity levels (normalized to dark levels) 10 min following light exposure for CRY2olig-Geph$_{FingR}$-GFP and GABA$_A$Rα2-HaloTag + JF635i. n.s. not significant; ****p < 0.0001; Mann-Whitney test. N=2 cultures, n=290-350 synapses.

Olah et al., Figure S3

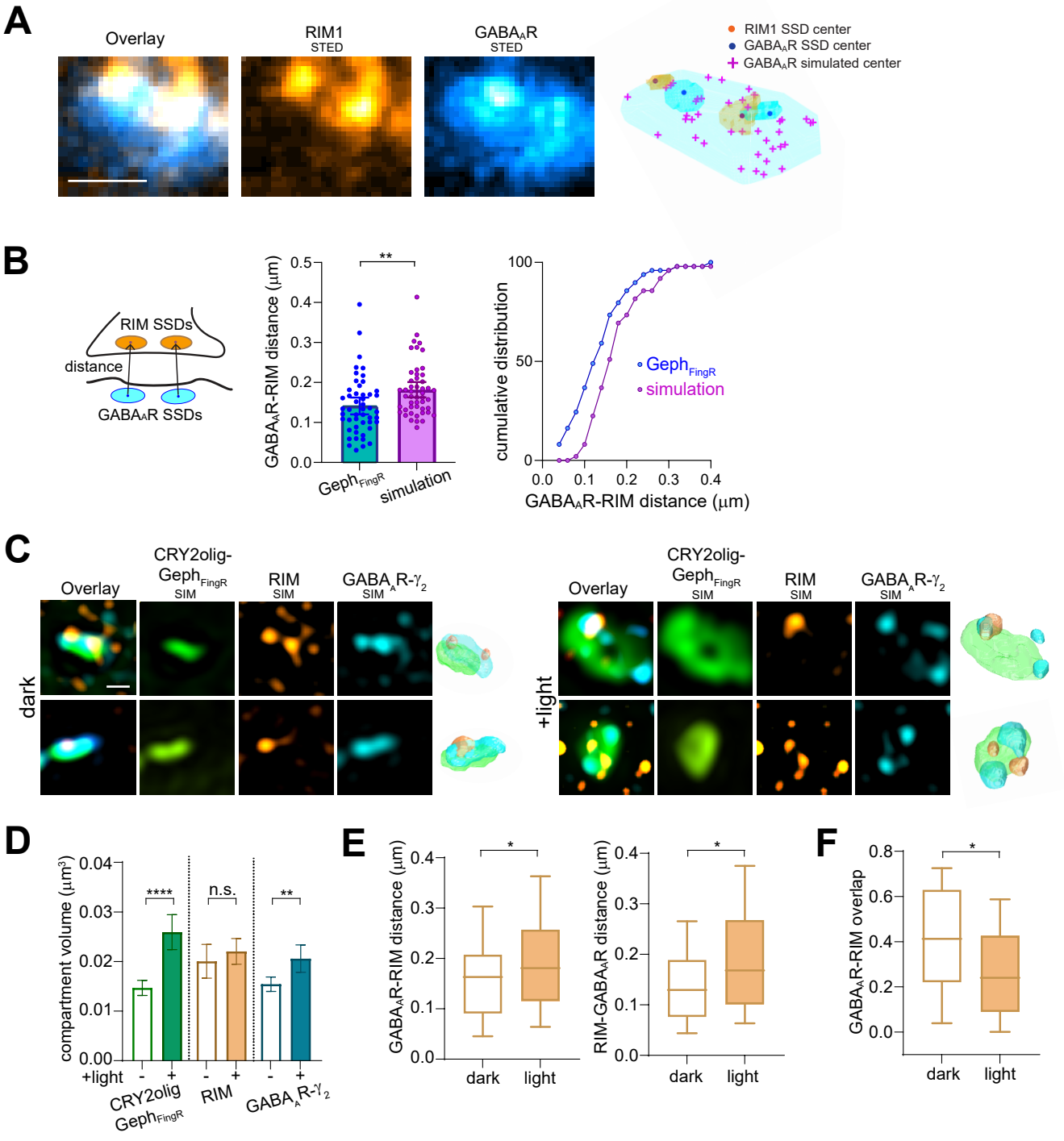

Figure S3. Nanoalignment between $GABA_AR$s and RIM1 is perturbed by CRY2olig-$Geph_{FingR}$ clustering (Related to Figure 3).

A. Left, representative STED image of an example synapse labeled for RIM1 (orange) and $GABA_AR\gamma2$ (cyan). Scale bar = 200 nm. Right, segmented SSDs for $GABA_AR\gamma2$ (dark cyan) and RIM1 (orange) along with the entire $GABA_AR\gamma2$ synaptic region (light cyan) and randomly simulated locations within the region (magenta crosses).

B. Left, schematic of 3D maximum intensity center to center distances. Middle, average $GABA_AR$-RIM1 distance per synapse in control cells expressing $Geph_{FingR}$-GFP compared with simulation values in which $GABA_AR$ locations were randomly placed within the same total synaptic volume. All data points are shown with bar at mean value. Error bars represent 95% CI. $^{**}p < 0.01$; Mann-Whitney test. Right, same data plotted as a cumulative distribution.

C. Representative SIM images and 3D reconstructions of synapses from cells expressing CRY2olig-$Geph_{FingR}$-GFP (green) labeled for RIM1 (orange) and $GABA_AR\gamma2$ (cyan) before and 10 min after exposure blue light. Scale bar = 0.2 µm.

D. Average compartment volume of CRY2olig-$Geph_{FingR}$-GFP, $GABA_AR\gamma2$ and RIM1 at synapses from cells expressing CRY2olig-$Geph_{FingR}$-GFP before (dark) and after light induced clustering. Error bars represent 95% CI. $^{*}p < 0.05$; Mann-Whitney test. N=3 cultures, n=81-203 synapses.

E. Left, average $GABA_AR\gamma2$ to RIM1 (left) and RIM1 to $GABA_AR\gamma2$ (right) distance per synapse in cells expressing CRY2olig-$Geph_{FingR}$-GFP before (dark) and after light induced clustering. 10-90th percentile of distribution plotted. $^{*}p < 0.05$; Mann-Whitney test. N=2 cultures, n=81-203 synapses.

F. Fractional volume overlap between $GABA_AR\gamma2$ SSDs and RIM1 SSDs per synapse. 10-90th percentile of distribution plotted. $^{*}p < 0.05$; Mann-Whitney test. N=2 cultures, n=81-203 synapses.

Olah et al., Figure S4

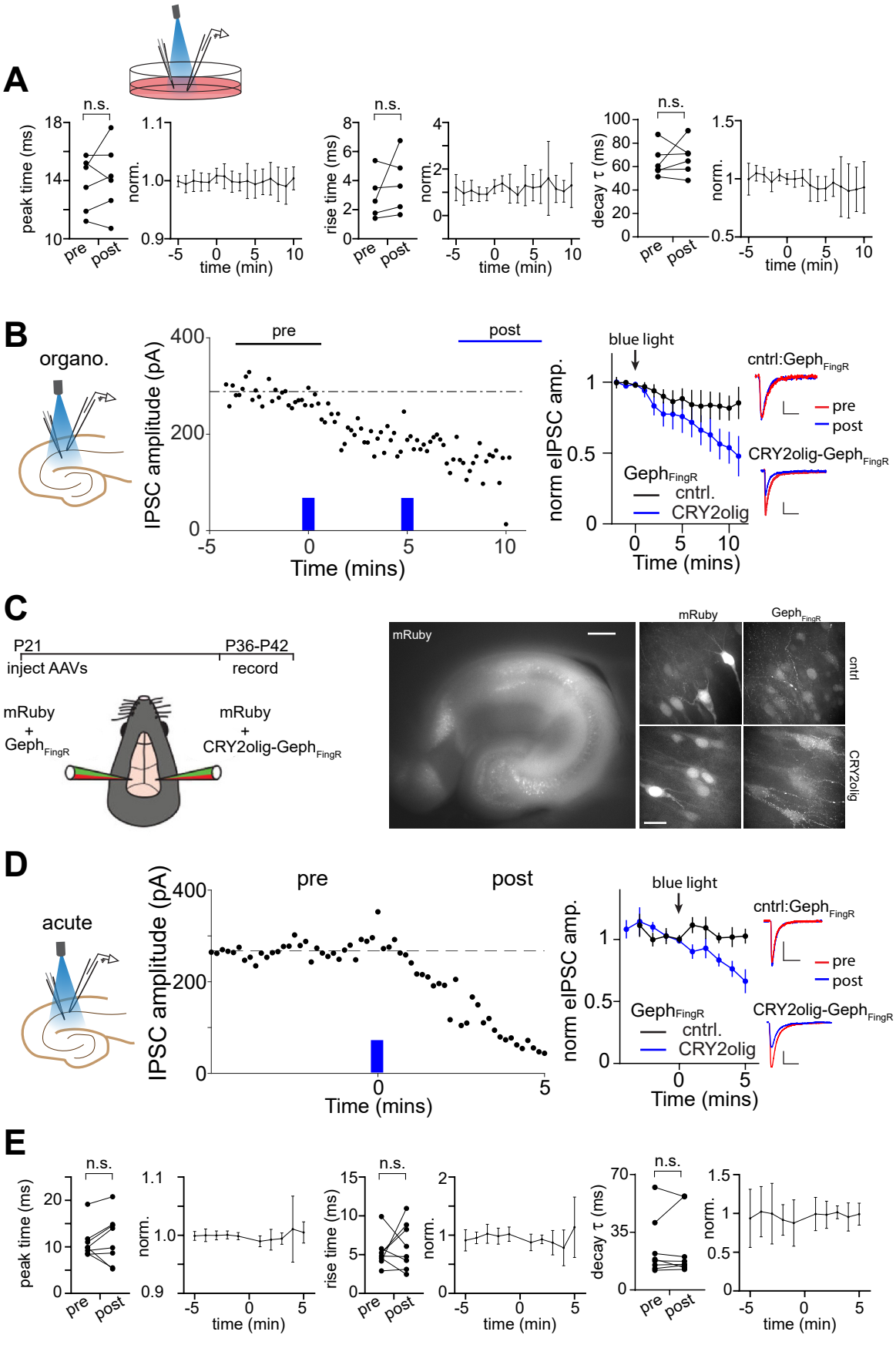

Figure S4. IPSC amplitudes and kinetics following CRY2olig-Geph$_{FingR}$ clustering in different neuronal preparations (Related to Figure 4).

A. IPSC kinetic parameters measured from dissociated hippocampal neurons (from main Fig. 4A-D). IPSC time to peak, rise time (10-90%) and decay lifetime (weighted 2-component exponential fit) plotted for neurons expressing CRY2olig-Geph$_{FingR}$ pre and 10 min post light exposure. n=6 cells from 5 different cultures. n.s. not significant, 2-way ANOVA.

B. IPSCs measured from organotypic hippocampal slices biolistically transfected with CRY2olig-Geph$_{FingR}$ or Geph$_{FingR}$ as a control. Results from a representative cell expressing CRY2olig-Geph$_{FingR}$ are shown with averaged time course shown to the right. Blue bars represent blue light illumination (1-2 sec each pulse). Representative traces recorded from control (Geph$_{FingR}$-GFP, top) or CRY2olig-Geph$_{FingR}$-GFP expressing cells (bottom) during baseline (pre-light exposure, red) and 8-11 min post light exposure (blue) are shown to the right. N=5 cultures, n=5 cells. Scale bar top, 30 pA, 15 msec, scale bar bottom, 80 pA, 15 ms.

C. Mice were stereotaxically injected with AAVs encoding mRuby, along with either CRY2olig-Geph$_{FingR}$-GFP or Geph$_{FingR}$-GFP. mRuby was included to identify the infection site without activating CRY2olig. Example of acute hippocampal slice from an infected animal, scale bar = 200 µm. The right panels show infected CA1 pyramidal cell bodies expressing mRuby and either CRY2olig-Geph$_{FingR}$-GFP (bottom panels) or Geph$_{FingR}$-GFP (top panels), scale bar = 25 µm.

D. Example IPSC amplitudes from an infected CA1 pyramidal neuron recorded from an acute hippocampal slice as in (C) with averaged IPSC amplitudes plotted to the right. Representative IPSC traces recorded from control (Geph$_{FingR}$-GFP, top) or CRY2olig-Geph$_{FingR}$-GFP expressing cells (bottom) during baseline (pre-light exposure, red) and 4-5 min post light exposure (blue) are shown to the right. Scale bar top, 30 pA, 33 msec, scale bar bottom, 200 pA, 40 ms. N = 3 animals, n=8 cells.

E. IPSC kinetic parameters measured from CA1 neurons from hippocampal slices. IPSC time to peak, rise time (10-90%) and decay lifetime (weighted 2-component exponential fit) plotted for neurons expressing CRY2olig-Geph$_{FingR}$ pre and 5 min post light exposure. n=8 cells from 3 animals. n.s. not significant, 2-way ANOVA.