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Supplemental information

Different priming states of synaptic vesicles underlie distinct release probabilities at hippocampal excitatory synapses

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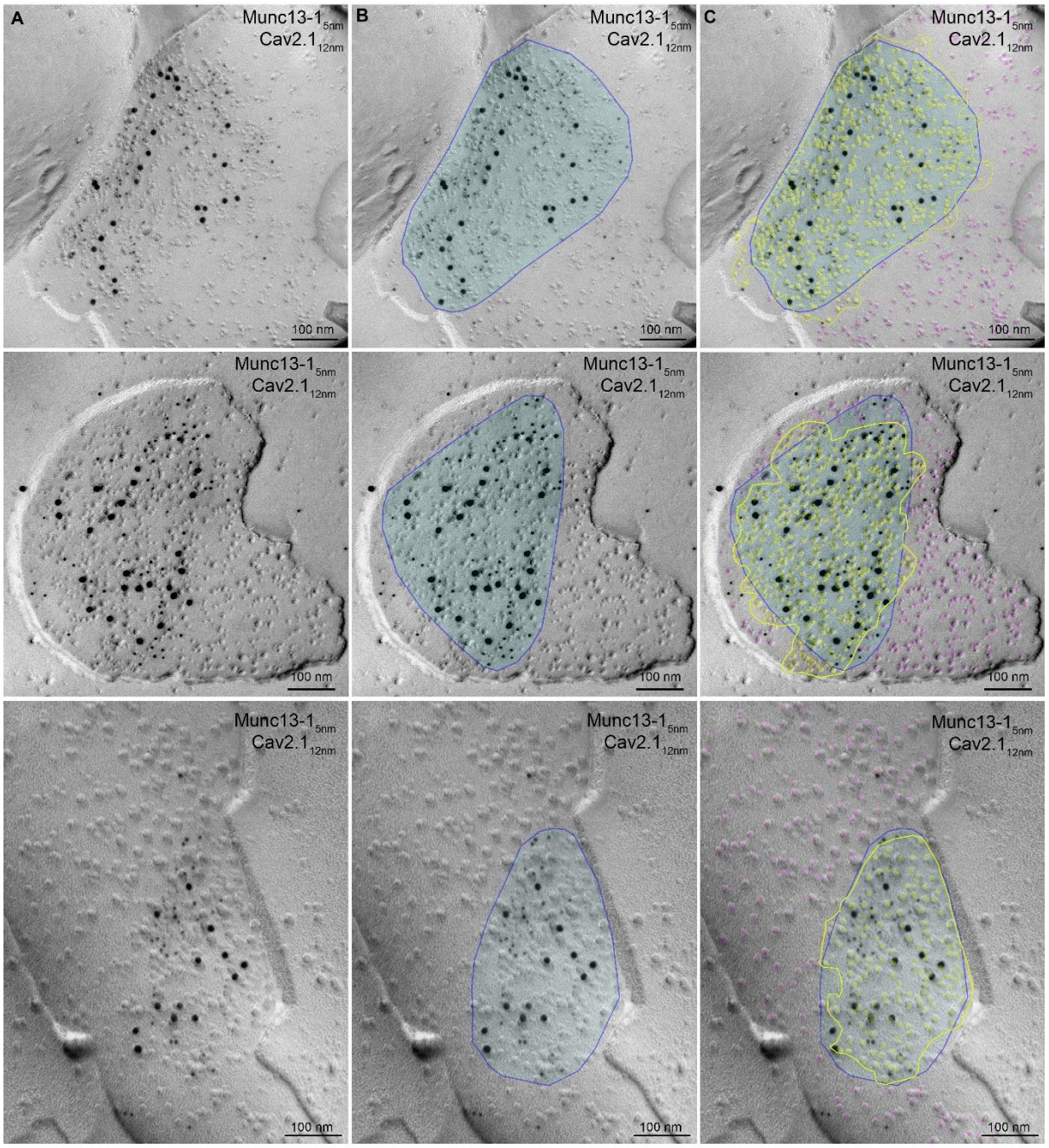
A
B
C
Munc13-1 5nm
Cav2.1 12nm
100 nm
D
AZ area (μm²)
0.4
0.3
0.2
0.1
0
Manual DBSCAN
E
Area overlap
(% of DBSCAN area)
100
75
50
25
0
F
IMP density (1/μm²)
3000
2500
2000
1500
1000
500
0
intra cluster
extra cluster

Figure S1: Comparison of active zone (AZ) areas determined by visual inspection or DBSCAN based outlining, Related to figure 2.

(A) Raw images of three axon terminal P-faces labeled for Munc13-1 and Cav2.1 with gold particles.

(B) AZ areas (blue) are outlined manually based on visual inspection of loose clusters of IMPs.

(C) AZ areas (yellow) are outlined based on DBSCAN clustering of all IMPs on the axonal P-face. Yellow dots label IMPs within the cluster, purple dots label IMPs outside the cluster.

(D) AZ areas determined by manual (blue) or DBSCAN based (yellow) outlining in 10 axon terminals.

(E) Manually outlined AZ areas largely overlap (mean ± SD = 92 ± 4%, CV = 0.04, n = 10) with the AZ areas determined by DBSCAN clustering of IMPs.

(F) Density of IMPs within DBSCAN determined clusters (2203.6 ± 489 IMP / μm^2) is on average 2.5 ± 1 times higher than outside the cluster (= 948.9 ± 244 IMP / μm^2).

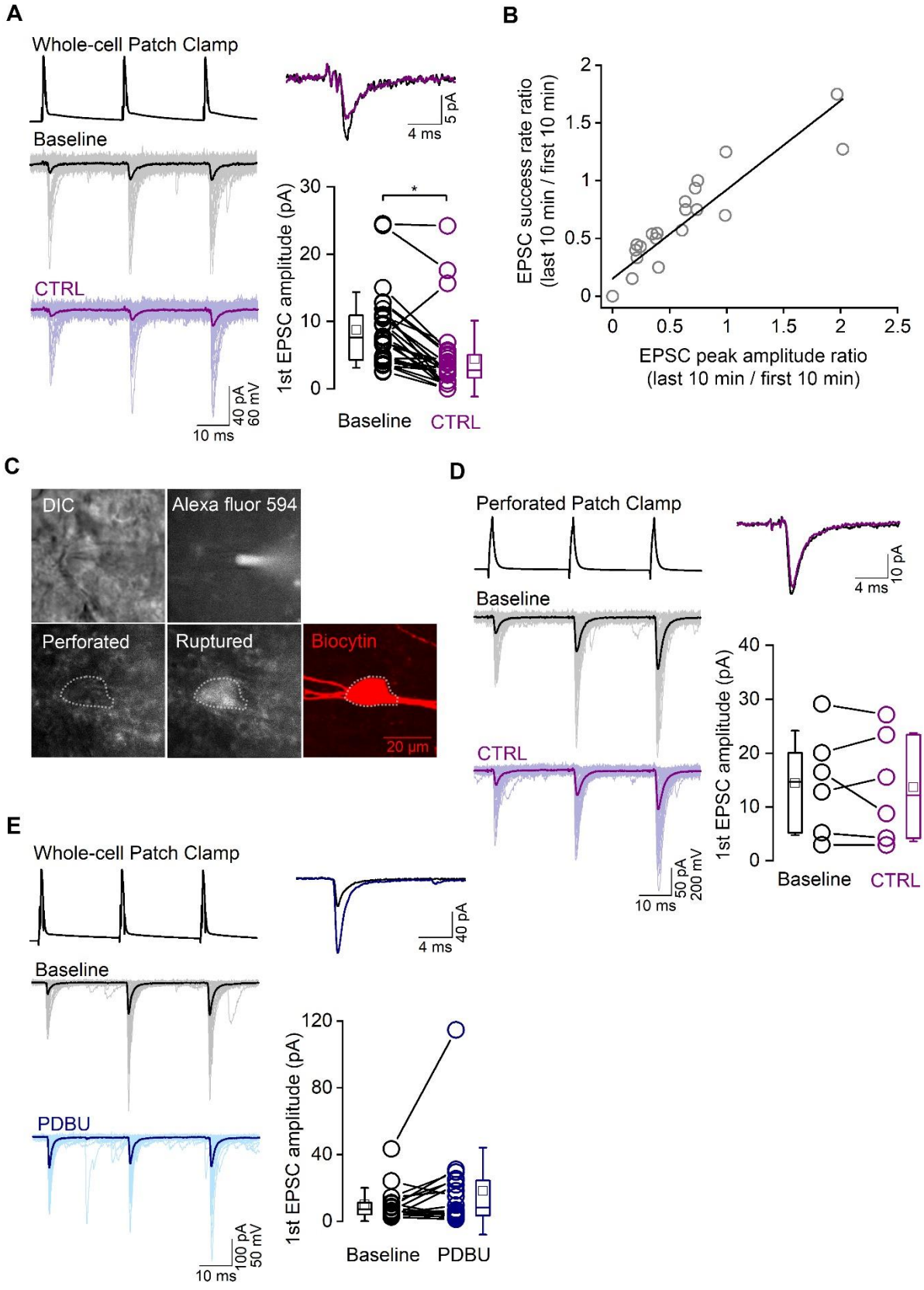
A
Whole-cell Patch Clamp
Baseline
CTRL
40 pA
60 mV
10 ms
5 pA
4 ms
1st EPSC amplitude (pA)
30
20
10
0
*
Baseline
CTRL
B
EPSC success rate ratio
(last 10 min / first 10 min)
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
EPSC peak amplitude ratio
(last 10 min / first 10 min)
C
DIC
Alexa fluor 594
Perforated
Ruptured
Biocytin
20 µm
D
Perforated Patch Clamp
Baseline
CTRL
50 pA
200 mV
10 ms
10 pA
4 ms
1st EPSC amplitude (pA)
40
30
20
10
0
Baseline
CTRL
E
Whole-cell Patch Clamp
Baseline
PDBU
100 pA
50 mV
10 ms
40 pA
4 ms
1st EPSC amplitude (pA)
120
80
40
0
Baseline
PDBU

Figure S2: Robust rundown of uEPSCs at CA1 PC – O-LM connections during 30 minutes of paired whole-cell recordings, Related to figure 7.

(A) Reduction of the amplitude of uEPSCs recorded in O-LM cells when the presynaptic PCs are recorded in whole-cell configuration. Individual (thin traces) and averaged (thick traces) uEPSCs evoked by three action potential at 40 Hz are shown from the beginning (black) and end (purple) of the recording period from the same pair (left). Superimposed averaged traces of the 1st uEPSCs at the beginning (black) and end (purple) of the recording (top right). The amplitude of the 1st uEPSCs is significantly reduced ($n = 28$, $p = 0.0001$, Wilcoxon signed-rank test) from the beginning (0 – 10 minutes) to the end (20 – 30 minutes) of a 30-minute-long recording period without any drug application (bottom right).

(B) Scatter plot between the uEPSCs peak amplitude ratios (last 10 min / first 10 min) and the success rate ratios (last 10 min / first 10 min) demonstrates that a larger rundown is accompanied by a larger increase in failure rate.

(C) Perforated patch-clamp recording of a PC. Differential interference contrast (DIC) image of a PC with an attached gramicidin-containing pipette after sealing (top, left). An epifluorescent image of the Alexa Fluor 594 filled pipette (top, right). After perforation of the membrane, the fluorophore in the pipette does not diffuse into the PC (bottom, left). When the plasma membrane ruptures, the Alexa 594 fluorophore diffuses into the PC (bottom, center). Maximum intensity projection image of the same PC after developing the biocytin with Cy3-coupled streptavidin.

(D) uEPSCs does not show a significant rundown ($n = 6$, $p = 0.83$, Wilcoxon signed-rank test) when the presynaptic PC is recorded in the perforated patch-clamp configuration. Panels are the same as in A.

(E) The effect of 1 μM PDBU on the uEPSCs when the presynaptic PCs were recorded in the whole-cell patch-clamp configuration. Panels are the same as in A. PDBU does not have a significant effect when not-corrected for the rundown ($n = 18$, $p = 0.117$, Wilcoxon signed-rank test). Box plots represent interquartile range, squares and whiskers indicate mean and SD in all panels.

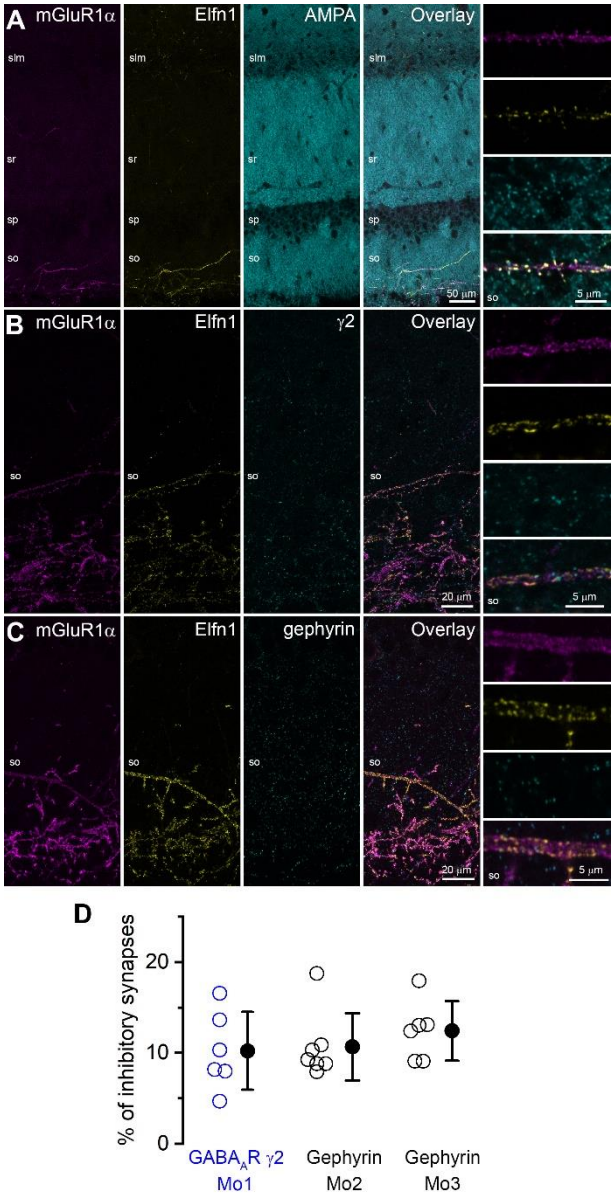

Figure S3: Distribution of inhibitory synapses on mGluR1α+ INs in CA1 stratum oriens, Related to STAR Methods.

(A) Immunofluorescent labeling for Elfn1, AMPA receptors and mGluR1α+ in the hippocampal CA1 area. (A right) High magnification view of a representative mGluR1α+ dendrite in stratum oriens (so) shows co-localization of Elfn1 and AMPA receptors.

(B) Immunofluorescent labeling for Elfn1, $GABA_AR$ γ2 subunit and mGluR1α+ in CA1 so. (B right) High magnification view of a representative mGluR1α+ dendrite.

(C) Immunofluorescent labeling for Elfn1, gephyrin and mGluR1α+ in CA1 so. (C right) High magnification view a representative mGluR1α+ dendrite.

(D) Percentage of inhibitory synapses on mGluR1α+ dendrites in so (n = 3 mice; Mo1, 10.2 ± 4.3%; Mo2, 10.7 ± 3.7%, Mo3, 12.4 ± 3.3 %). The proportion was calculated as the number of γ2 / gephyrin positive puncta divided by the sum of Elfn1 and γ2 /gephyrin puncta. Open circles indicate individual dendrites, filled circles represent mean ± SD.