Cell Reports, Volume *26*

Supplemental Information

Nanoscale Subsynaptic Domains Underlie the Organization of the Inhibitory Synapse

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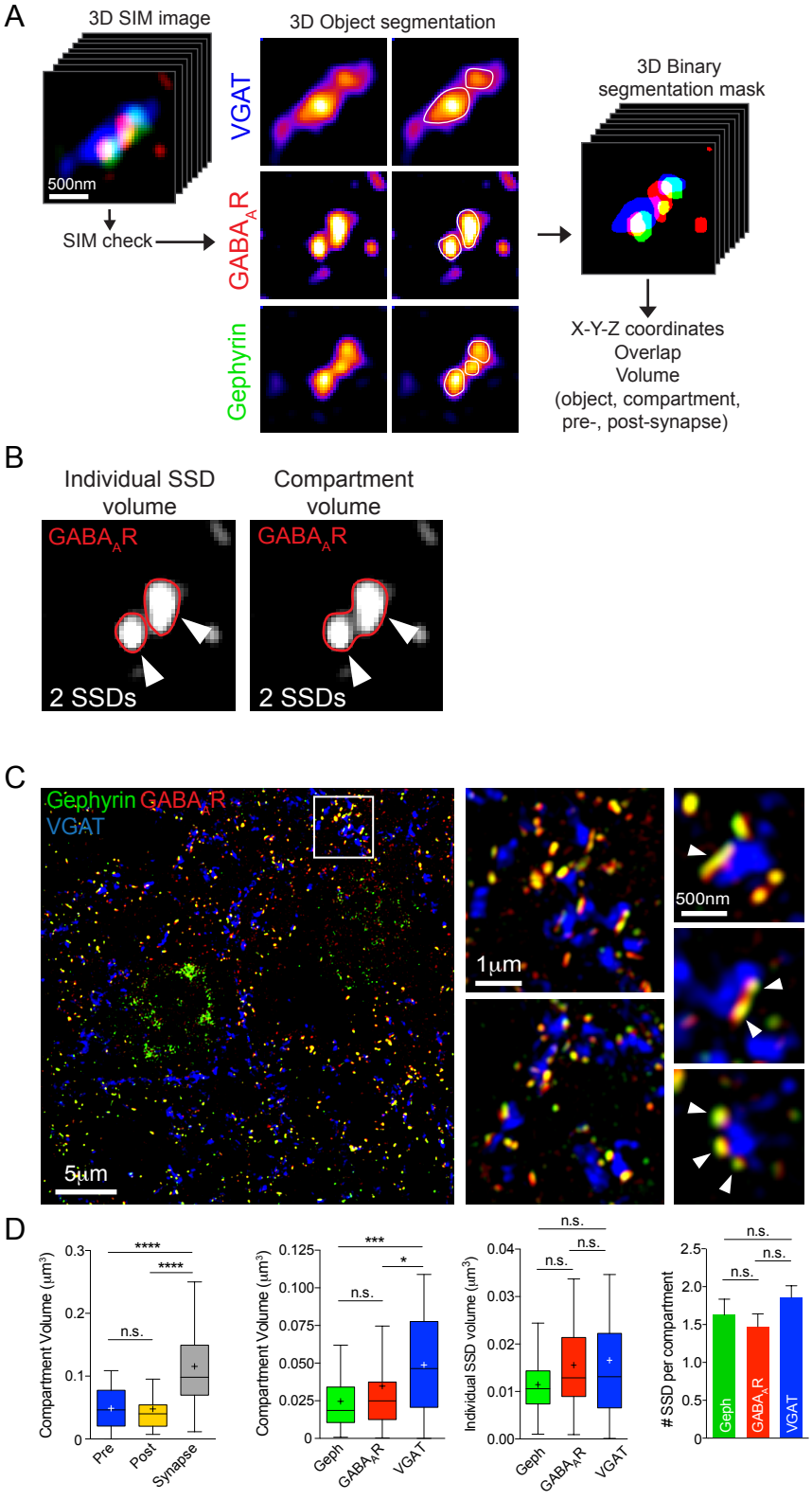

Figure S1 Related to Figure 2. 3D segmentation analysis of SIM images and 3D-SIM in acute brain slices. A. 3D segmentation protocol of 3D-SIM image, providing binary segmentation mask from which numerous parameters can be extracted. **B**. Representation of measurements of 'individual SSD volume' and 'compartment volume' for a GABA$_A$R compartment with 2 SSDs.
C. Maximum projection SIM image of cortical slice (layer 2/3) labeled with antibodies to gephyrin, GABA$_A$Rs and VGAT. White boxes highlight magnified regions. **D.** Quantification of compartment volume, individual SSD volume and number of SSDs per synapse, n=58 synapses, 10 neurons, Kruskal-Wallis tests. Cross denotes mean, horizontal line denotes median.

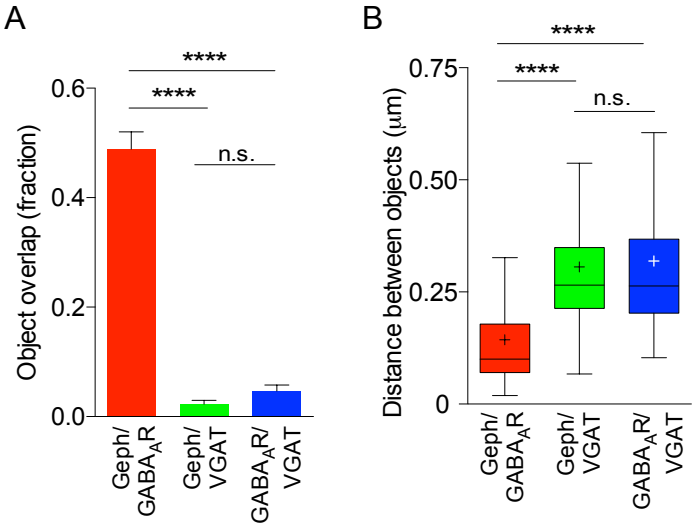

Figure S2 Related to Figure 3. Overlap and distance analysis of inhibitory synapses in acute brain slices.
A. Mean overlap fraction for gephyrin, $GABA_A$Rs and VGAT, p****<0.0001, Kruskal-Wallis, n= 58 synapses. Data are represented as mean ± SEM.
B. Box plot of center-to-center distances between neighboring SSDs, p****<0.0001, Kruskal-Wallis, n=83-89 SSDs. Cross denotes mean, horizontal line denotes median.

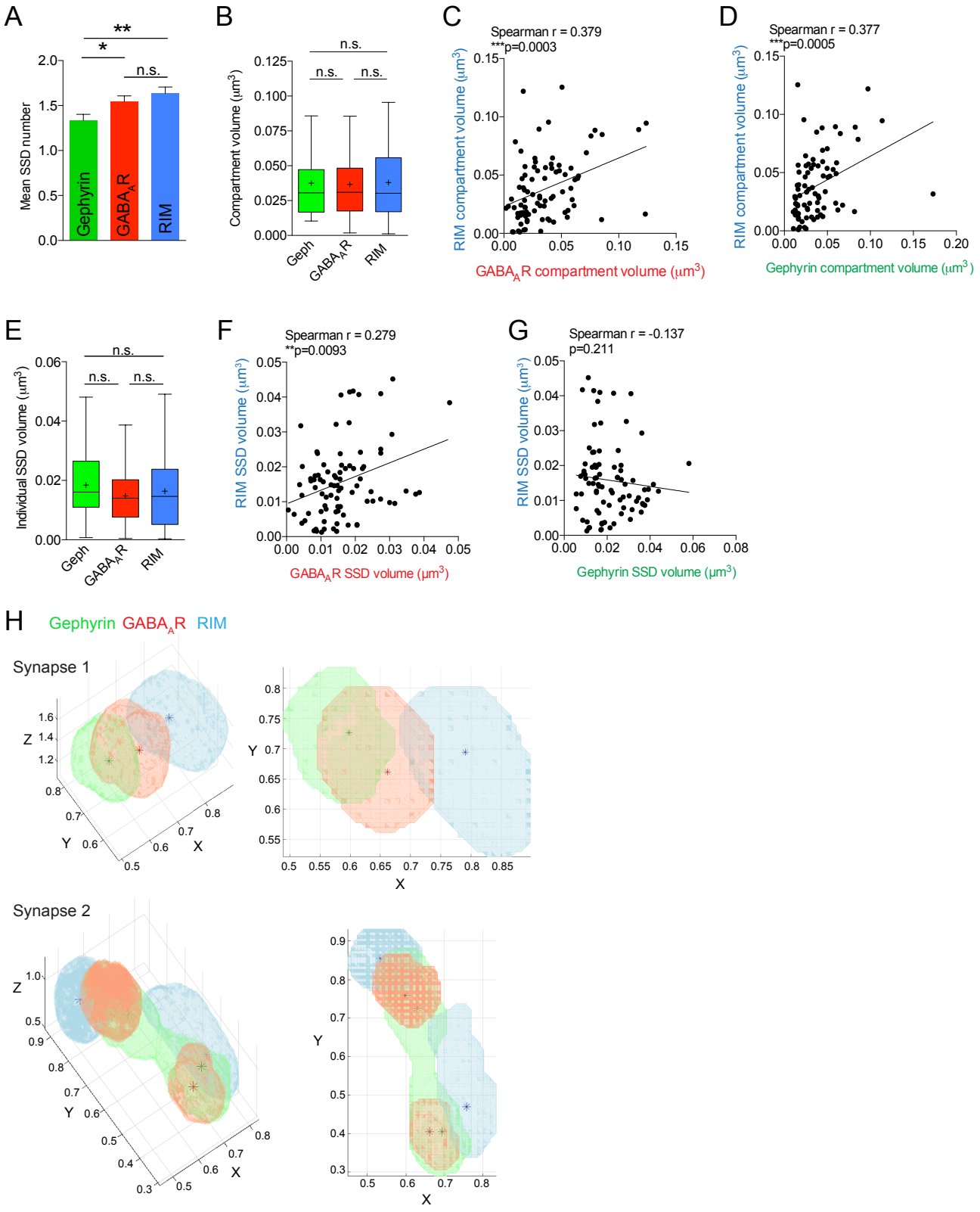

Figure S3 Related to Figure 5. Volume data for gephyrin, GABA$_A$Rs and RIM SSDs.

A. Mean number of SSDs per compartment for gephyrin, GABA$_A$R and RIM, p***<0.0010, Kruskal-Wallis, Dunn's post hoc, n=92 synapses. Data are represented as mean ± SEM.

B. Box plot of compartment volumes for gephyrin, GABA$_A$R and RIM, n.s., Kruskal-Wallis, Dunn's post hoc, n= 92 synapses. Cross denotes mean, horizontal line denotes median.

C. Correlation plot of RIM compartment volume and GABA$_A$R compartment volume per synapse, n= 92 synapses.

D. Correlation plot of RIM compartment volume and gephyrin compartment volume per synapse, n= 92 synapses.

E. Box plot of individual SSD volumes for gephyrin, GABA$_A$R and RIM, n.s, Kruskal-Wallis, Dunn's post hoc, n= 92 synapses. Cross denotes mean, horizontal line denotes median.

F. Correlation plot of RIM individual SSD volume and GABA$_A$R individual SSD volume per synapse, n= 92 synapses.

G. Correlation plot of RIM individual SSD volume and gephyrin individual SSD volume per synapse, n= 92 synapses.

H. Further examples of 3D and 2D rendering of inhibitory synapses. Stars denote maximun intensity points, units=µm.

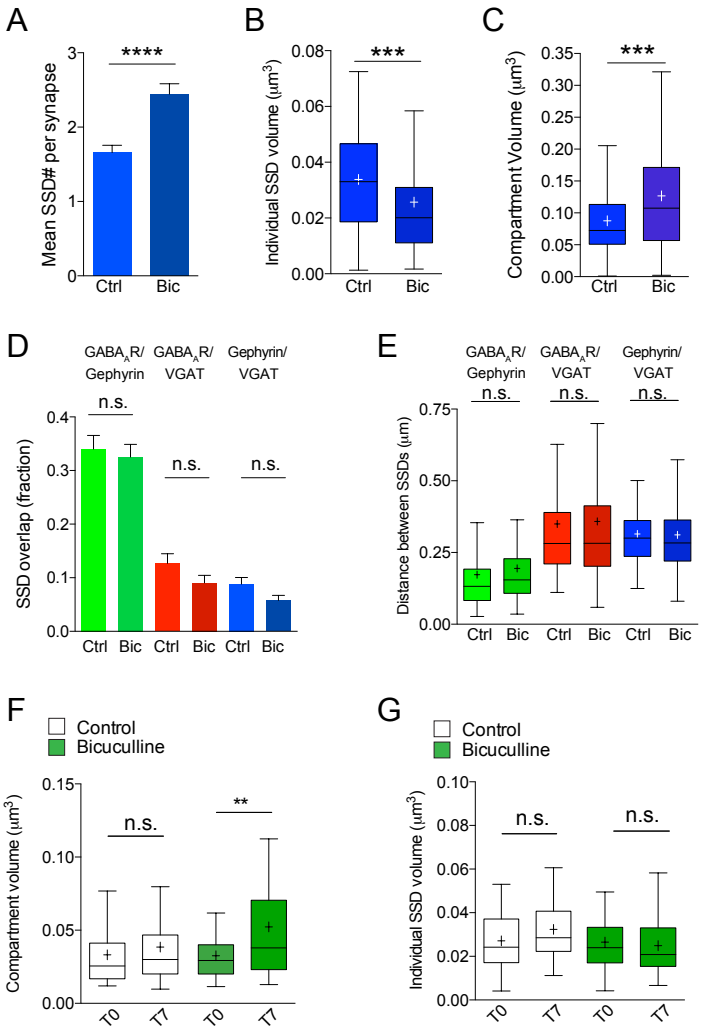

Figure S4 Related to Figure 6. Further measurements of inhibitory synapses.
A. Mean number of SSDs per VGAT compartment in control or bicuculline treated conditions.,****p<0.0001, n= 85/97 synapses, Mann-Whitney test. Data are represented as mean ± SEM.
B. Box plot of VGAT individual SSD volume in control and bicuculline conditions, ***p=0.0001, n= 85/97 synapses, Mann-Whitney test. Cross denotes mean, horizontal line denotes median.
C. Box plot of VGAT compartment volume in control and bicuculline conditions, p=0.002, n= 48/73 synapses, t-test. Cross denotes mean, horizontal line denotes median.
D. Mean overlap fraction for gephyrin, $GABA_A$Rs and VGAT in control or bicuculline (Bic) treated conditions. Data are represented as mean ± SEM.
E. Box plot of center-to-center distances between neighboring SSDs for gephyrin, $GABA_A$Rs and VGAT in control or bicuculline treated conditions. Cross denotes mean, horizontal line denotes median.
F. Box plot of gephyrin compartment volume in control and bicuculline conditions at time 0 and 7 hours, p=0.002, n= 48/73 synapses, t-test. Cross denotes mean, horizontal line denotes median.
G. Box plot of gephyrin individual SSD volume in control and bicuculline conditions at time 0 and 7 hours, p>0.05, n= 48/73 synapses, t-test. Cross denotes mean, horizontal line denotes median.