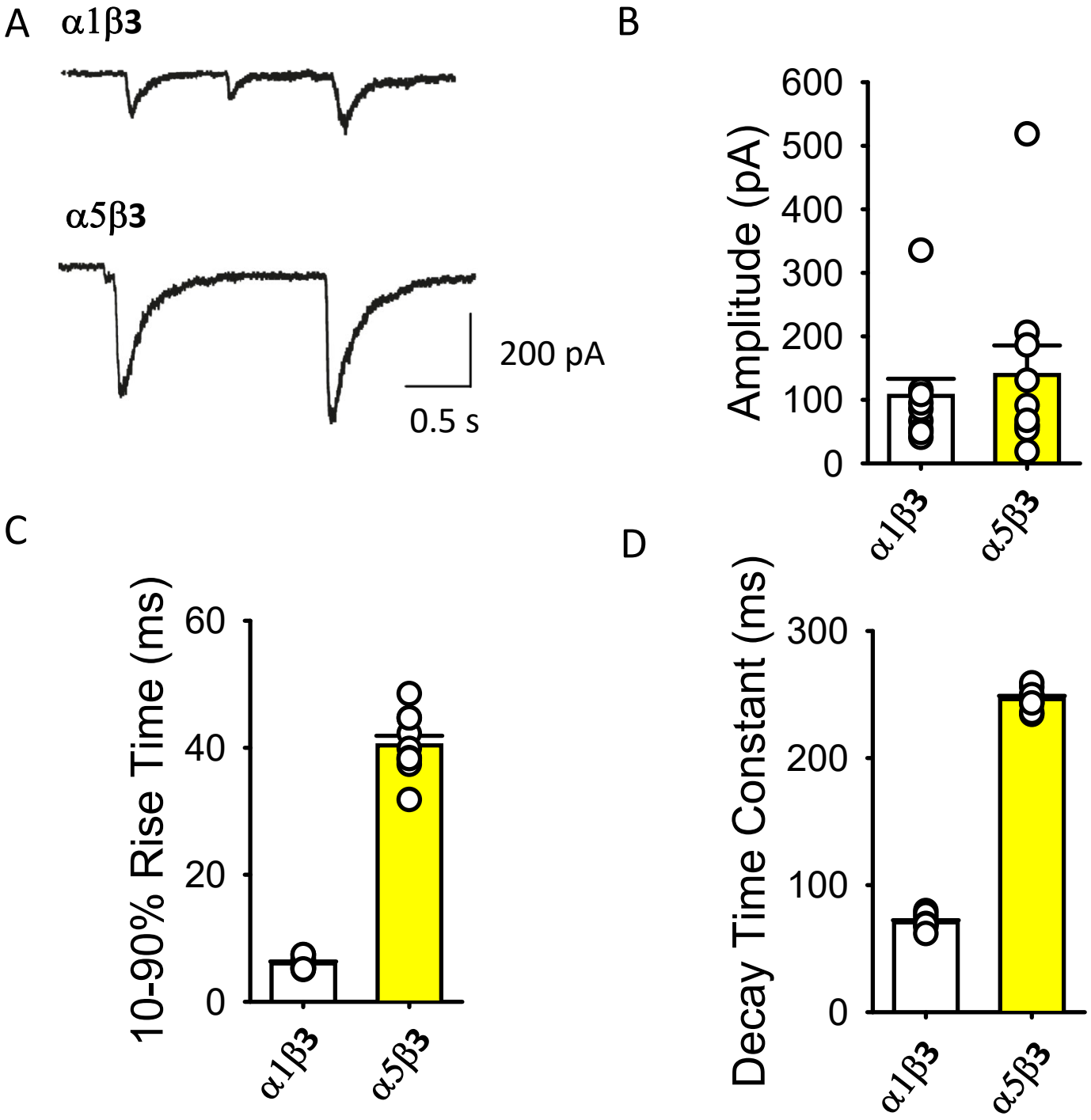


Figure S1. IPSC properties of α1β3 and α5β3 receptors lacking the γ2L subunit. (A) Representative inhibitory postsynaptic currents (iPSCs) recorded from artificial synapses formed between cortical neurons and HEK293 cells expressing α1β3 or α5β3 GABAARs in the absence of the γ2L subunit. Averaged currents from individual cells are shown below each set of representative traces.(B) Quantification of iPSC peak amplitude for α1β3 and α5β3 receptors. Receptors lacking γ2L generated smaller synaptic currents than their corresponding γ2L-containing receptors shown in Figure 2.(C) Quantification of iPSC 10-90% rise time. Removal of γ2L significantly slowed current activation kinetics in both α1β3 and α5β3 receptors. (D) Quantification of iPSC decay time constants. Receptors lacking γ2L exhibited substantially slower decay kinetics than γ2L-containing receptors, consistent with altered receptor gating and synaptic function. These data were used to assess γ2L incorporation into mutant receptors by comparison with the kinetic properties of β3G32R-containing receptors shown in Fig. 2.

Data are presented as mean ± SEM. Individual symbols represent single recorded cells. Statistical comparisons were performed using one-way ANOVA. followed by appropriate multiple-comparisons tests. N=10 cells from 3 different preparations.

Receptor	Amplitude (pA)	Rise Time (ms)	Decay Time (ms)
α1β3γ2L	260+/-30	2.4+/-0.4	40+/-1
α1β3G32Rγ2L	80+/-10	2.3+/-0.1	30+/-2
α1β3E180Gγ2L	N/A	N/A	N/A
α1β3	110+/-30	6.1+/-0.3	71+/-2
α5β3γ2L	230+/-20	3.3+/-0.1	150+/-3
α5β3G32Rγ2L	110+/-20	3.5+/-0.1	66+/-2
α5β3E180Gγ2L	N/A	N/A	N/A
α5β3	140+/-50	41+/-2	246+/-3

Table S1. Synaptic current properties of wild-type and mutant $GABA_ARs$ measured in artificial synapses.

Mean peak amplitude, 10-90% rise time, and decay time constant of inhibitory postsynaptic currents (iPSCs) recorded from artificial synapses formed between cortical neurons and HEK293 cells expressing the indicated $GABA_AR$ subunit combinations. Data are shown for receptors containing wild-type β3, β3G32R, or β3E180G subunits in the presence of γ2L (Figure 2) and for α1β3 and α5β3 receptors lacking γ2L (Figure S1). Values are presented as mean ± SEM.

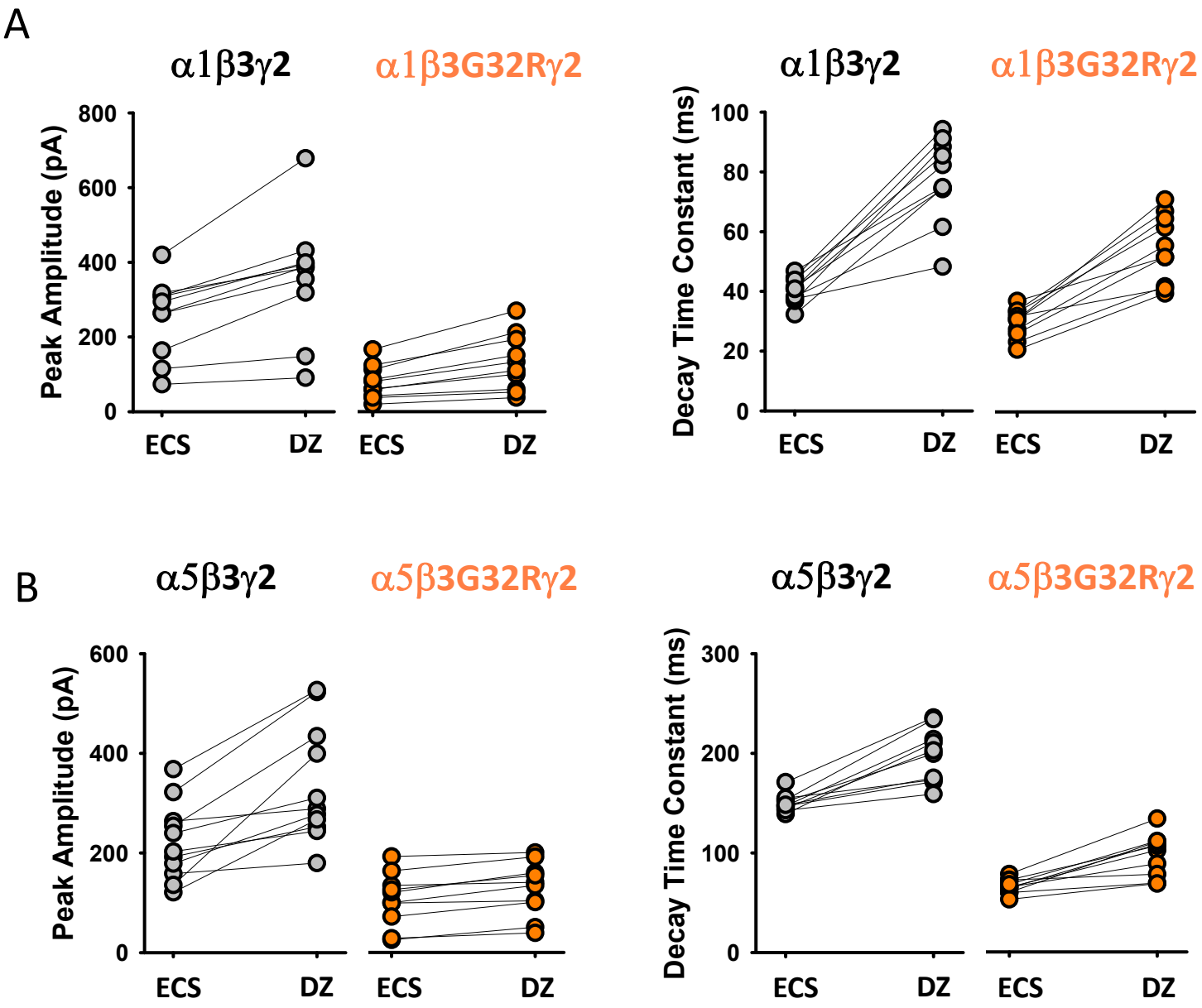


Figure S2. Diazepam potentiates synaptic currents mediated by wild-type and β3G32R-containing $GABA_A$Rs.

(A and B) Paired-cell analysis of DZ (1 μM) potentiation of IPSC peak amplitude (left) and decay time constant (right) in cells expressing α1β3γ2L, α1β3G32Rγ2L, α5β3γ2L, or α5β3G32Rγ2L receptors. Each pair of connected points represents measurements obtained from the same cell before and during DZ application. DZ increased both IPSC amplitude and decay time in all receptor subtypes and all cells examined. IPSCs were recorded for 10 min under baseline conditions and subsequently during 5 min of local diazepam (1 μM) application to the recorded cell.

Figure S3

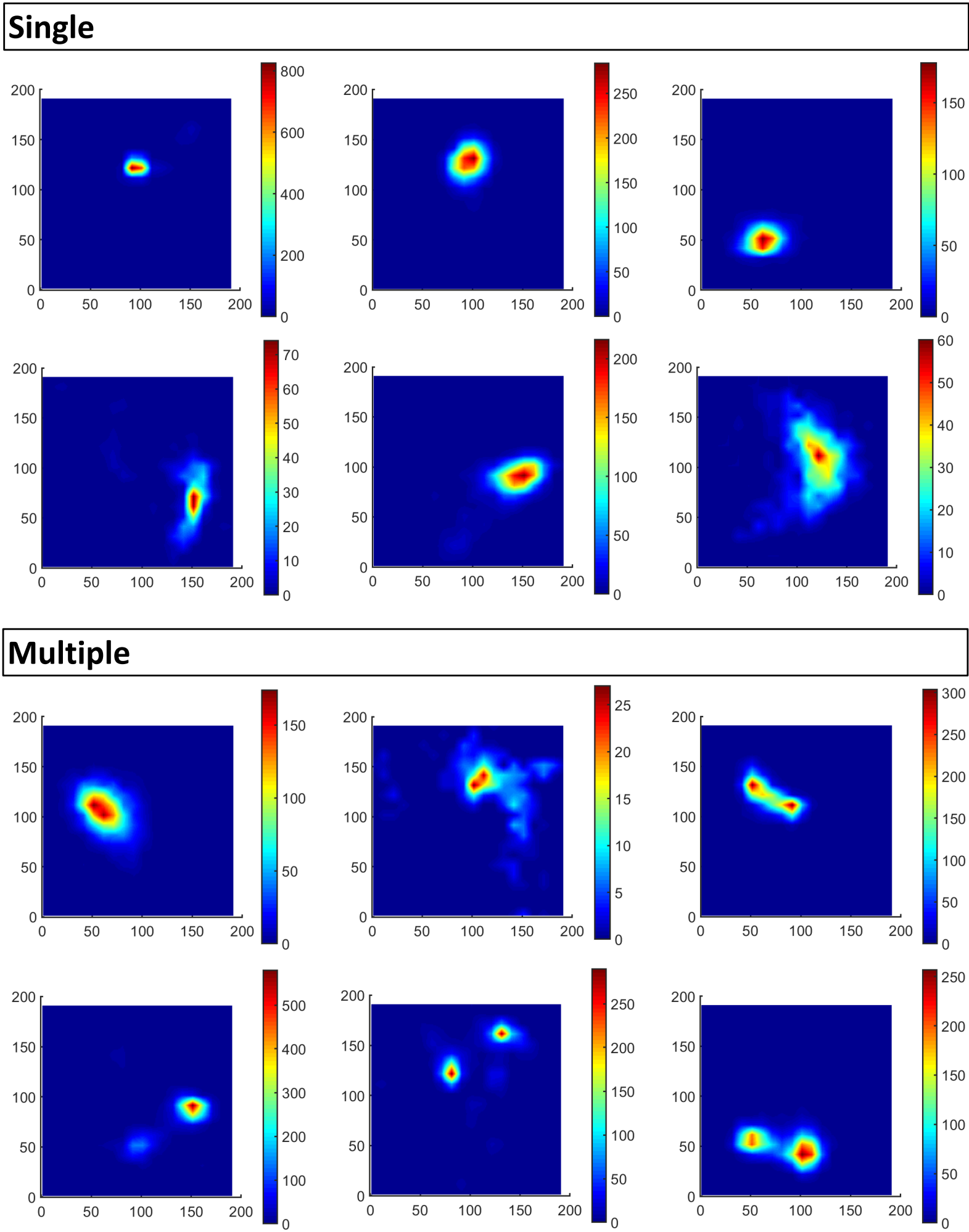


Figure S3

Multiple

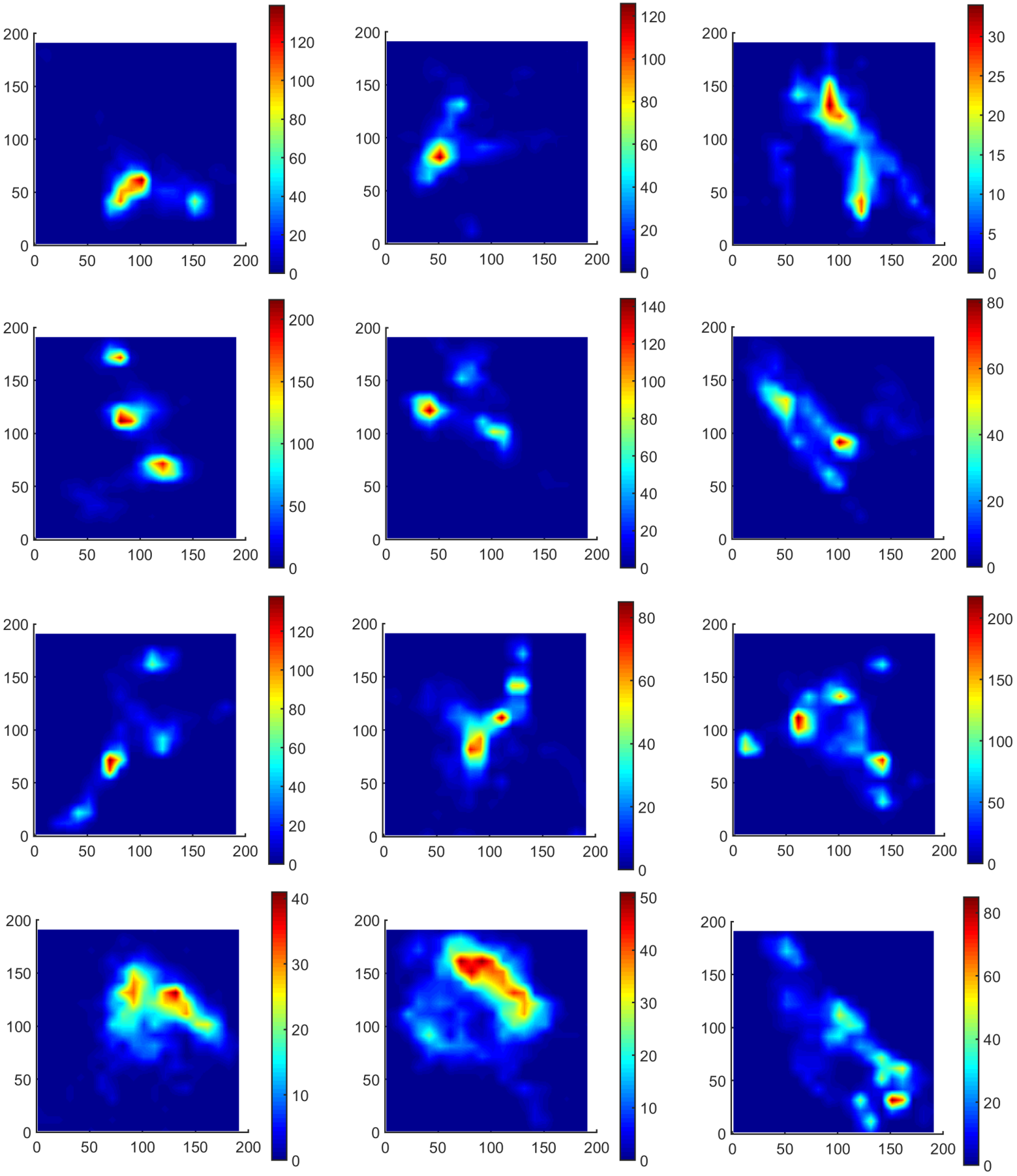


Figure S3. Examples of synaptic $GABA_AR$ nanodomain organization identified by localization density analysis.

Representative examples of β3-containing $GABA_AR$ distributions at inhibitory synapses reconstructed from single-molecule localizations and analyzed by localization density mapping. Synapses containing a single receptor nanodomain as well as multiple receptor nanodomains are shown. Receptor nanodomains were identified from local increases in localization density within individual synaptic receptor assemblies. Examples were pooled from neurons expressing wild-type β3, β3G32R, and β3E180G subunits. Examples are provided to illustrate the diversity of single- and multi-nanodomain synapses identified by the analysis and are not intended to represent genotype-specific differences.