



Figure S2. The synaptic vesicle marker CSP and active zone marker Brp do not accumulate in NMJ terminal boutons in *vezl* mutants.

(A,B) Third-instar larval NMJs stained with anti-HRP (white) and anti-CSP (red). Terminal boutons are on the right (because of branching there are two terminal boutons in panel A). Scale bar is 10 μ m. Unlike HRP, the SV protein CSP is not enriched in terminal boutons in *vezl^{13.1}/Df(3R)Exel6180* mutants (the mean anti-CSP fluorescence intensity in the terminal bouton relative to non-terminal boutons is 1.01 (n = 7 animals, SEM = 0.11) in wild type and 1.06 (n = 7 animals, SEM = 0.04) in *vezl* mutants; p = 0.739 based on a two-tailed t-test).

(C,D) Structured illumination microscopy (SIM) images of terminal boutons stained with antibodies against HRP (blue), the major AZ protein Brp (green) and the post-synaptic glutamate receptor subunit GluRIIC (red). Scale bar is 10 μ m. In wild-type (C), Brp and GluRIIC are in apposition. In *vezl^{13.1}/Df(3R)Exel6180* mutants (D), Brp and GluRIIC remain in apposition, but are at decreased density. Barren areas appear to correlate with areas having increased anti-HRP staining (merge panel has reduced HRP intensity to show detail).

(E) Quantification of anti-Brp puncta density in terminal boutons. n = animals, indicated on each bar. Error bars indicate SEM. ***p < 0.001, based on a two-tailed t-test. Means, SEMs, and p values are available in Table S1.