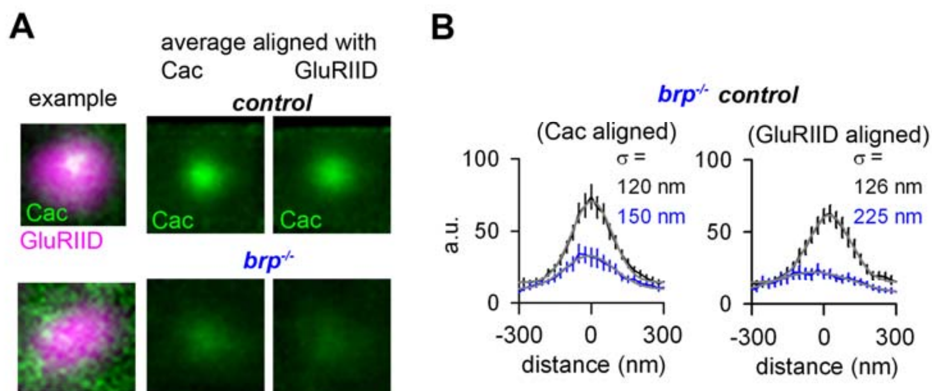
Supplemental Information for

Naked dense bodies provoke depression

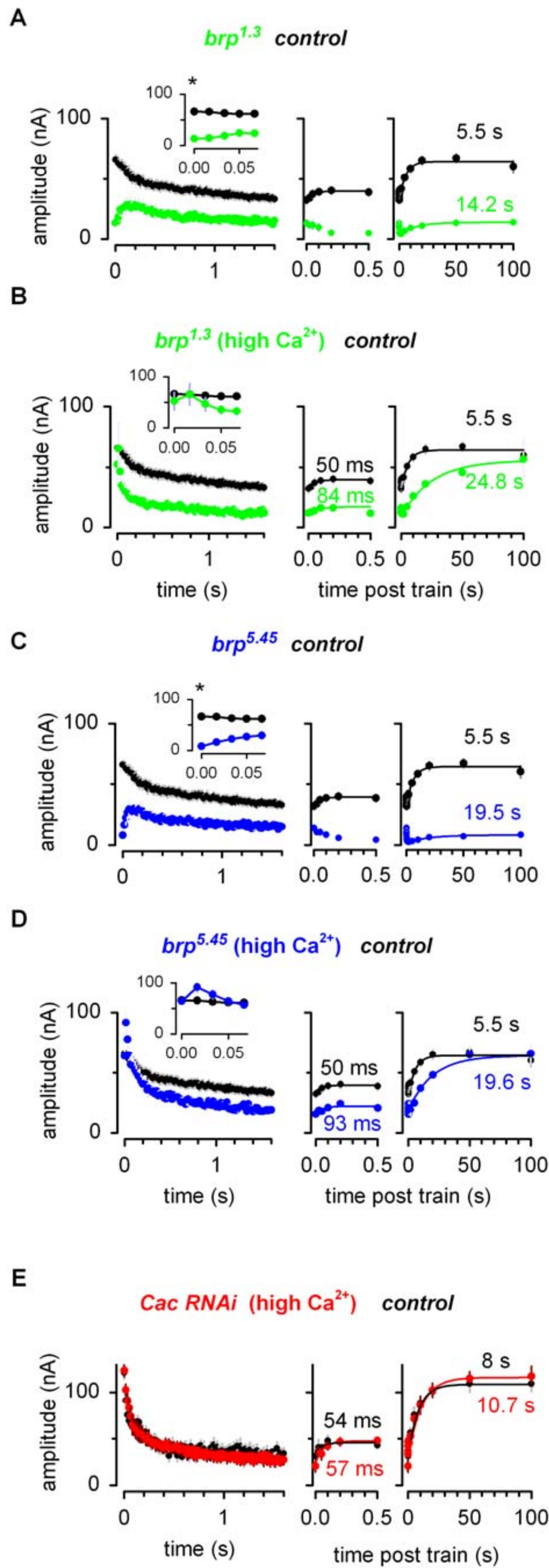
Stefan Hallermann, Robert J. Kittel, Carolin Wichmann, Annika Weyhersmüller, Wernher Fouquet, Sara Mertel, David Owald, Stefan Eimer, Harald Depner, Martin Schwärzel, Stephan J. Sigrist and Manfred Heckmann

SUPPLEMENTAL DATA



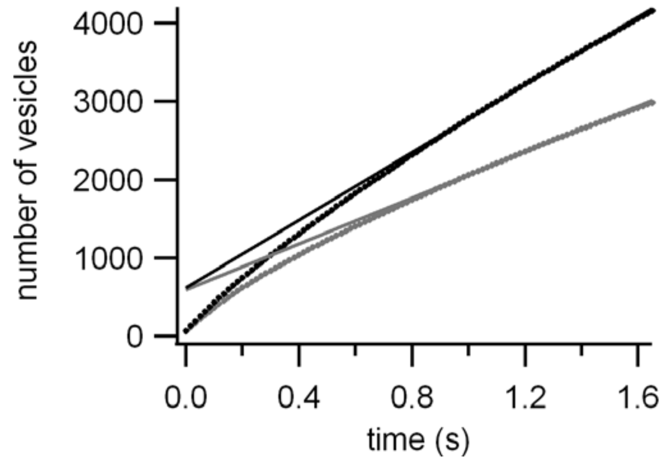
Supplementary Figure S1. Ca^{2+} channel clustering in *brp*$^{-/-}$ mutants

(A) GluRIID labels (magenta, confocal) were used to quantify the Ca^{2+} channel (Cac^{GFP}, green, STED) clusters in control animals (top row) and *brp*$^{-/-}$ mutants (*brp*^{69}; (Fouquet et al., 2009)). The Cac signals at synapses that appeared ideally planar (n = 15 each) were averaged after alignment with the postsynaptic GluRIID signal (right panels) or themselves (centre panels). While the example images were scaled up individually, all four averages were scaled up with the same factor. (B) Intensity profiles of the average Cac images in black for controls and in blue for *brp*$^{-/-}$ mutants with SEM error bars and the corresponding Gaussian fits (standard deviations given by σ) in black (control) and in blue (*brp*$^{-/-}$ mutants).



Supplementary Figure S2. Short term plasticity in *brp*$^{1.3}$ and *brp*$^{5.45}$ mutants and Ca^{2+} channel knockdowns

(A) Average EPSC amplitudes during trains of 100 stimuli at 60 Hz followed by stimuli with increasing intervals in controls (black, n = 21) and *brp*$^{1.3}$ mutants (green, n = 4). (B) The extracellular Ca^{2+} concentration for the experiments with *brp*$^{1.3}$ mutants was increased to 2.0 mM, to compensate for the impaired Ca^{2+} channel clustering and the correspondingly reduced initial EPSC amplitude (green, n = 4). (C) Average EPSC amplitudes during trains of 100 stimuli at 60 Hz followed by stimuli with increasing intervals in controls (black, n = 21) and *brp*$^{5.45}$ mutants (blue, n = 7). (D) The extracellular Ca^{2+} concentration for the experiments with *brp*$^{5.45}$ mutants was increased to 2.5 mM, to compensate for the impaired Ca^{2+} channel clustering and the correspondingly reduced initial EPSC amplitude (blue, n = 6). (E) To further validate this strategy, the density of Ca^{2+} channels was lowered independently of BRP by transgene-mediated RNA interference (RNAi) directed against the α1 subunit of the *Drosophila* Ca^{2+} channel (cacophony, Cac). The thereby reduced EPSC amplitude could be compensated by elevation of the extracellular Ca^{2+} channel concentration to 1.5 mM. In contrast to the results at *brp*$^{5.45}$ and *brp*$^{1.3}$ synapses, the average EPSC amplitudes during trains of 100 stimuli at 60 Hz and the subsequent recovery were normal in Cac RNAi animals (red, n = 5; control, black, n = 5).



Supplementary Figure S3. Back-extrapolation of released vesicles during 60 Hz trains in *brp*^{nude} mutants and controls

Peak EPSC amplitudes were related to number of vesicles assuming linear summation of miniature EPSCs (cf. Figure 2D). The data values from Fig 3C were integrated to give a plot of cumulatively released vesicles during 60 Hz trains. Data points in a range of 0.8-1.6 s were then fitted by linear regression, and back-extrapolated to time 0, to estimate the cumulative number of vesicles. For control (black) and *brp*^{nude} (grey), 610 and 590 readily releasable vesicles were obtained, respectively.