Wang et al., http://www.jcb.org/cgi/content/full/jcb.201312109/DC1

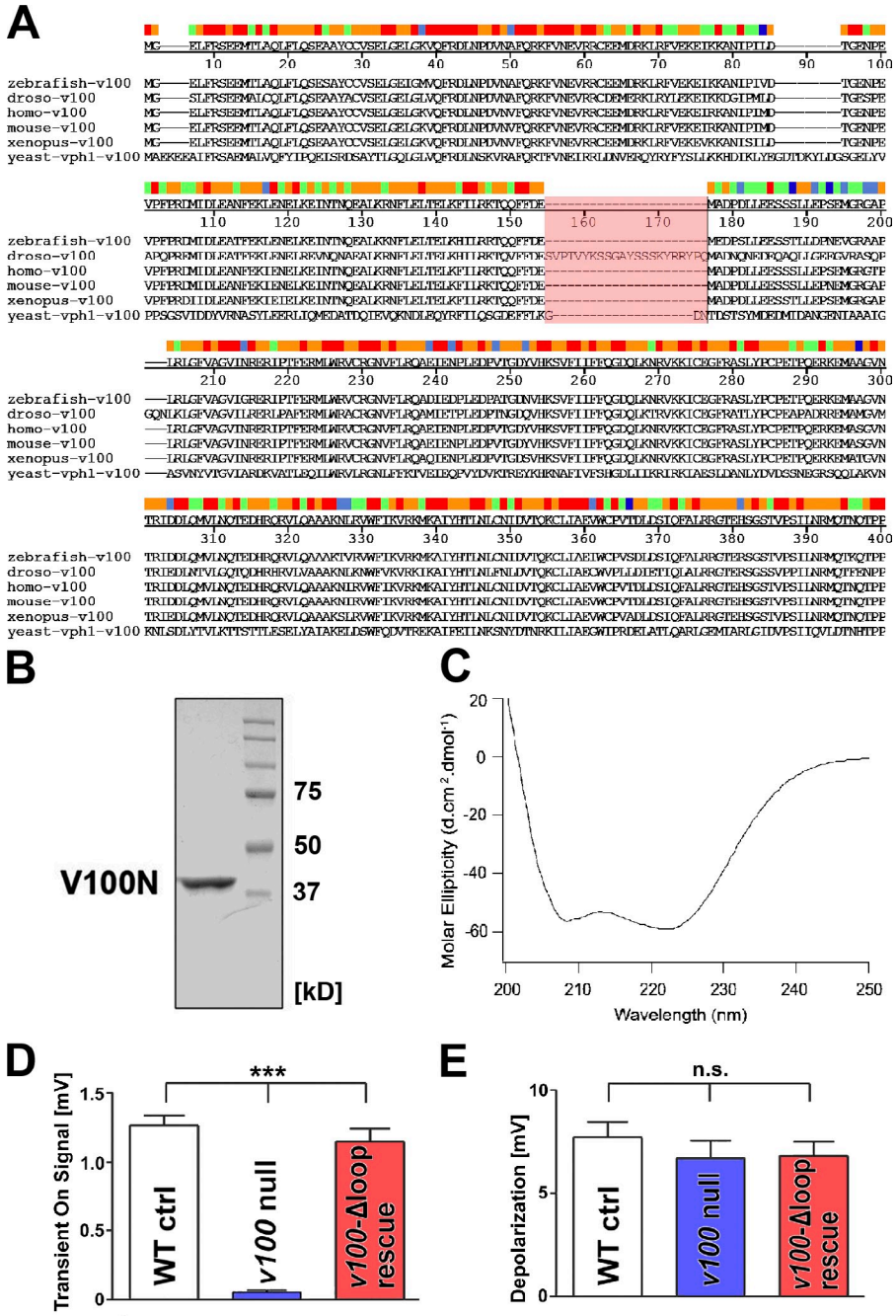

Figure S1. **Deletion of a partially hydrophobic 20–amino acid loop allows purification of a soluble 407–amino acid N-terminal protein and does not affect the in vivo function of full-length V100.** (A) Alignment of the N termini of V0a1 orthologues reveals a stretch of amino acids in the longest *Drosophila* isoform (shown at position 155–175 in the alignment and 142S-163Q in *Drosophila* [droso] V100) that is not present in yeast, *Xenopus*, zebrafish, mouse, or human. The red shaded sequence marks the *Drosophila*-specific amino acids deleted in the V100-Δloop. The color code marks the level of conservation across the shown sequences, from most to least conserved: red (100%), orange, green, light blue, and dark blue. (B) Coomassie gel of bacterial purified V100N-Δloop. (C) CD spectra reveal characteristic double minima at 208 and 222 nm, indicating significant α-helical character. (D) Expression of full-length V100-Δloop in null mutant photoreceptor neurons full rescues neurotransmission in ERG recordings. ctrl, control. ***, $P < 0.001$. (E) ERG depolarization is not affected by expression of V100-Δloop . WT, wild type. Error bars show SEMs.

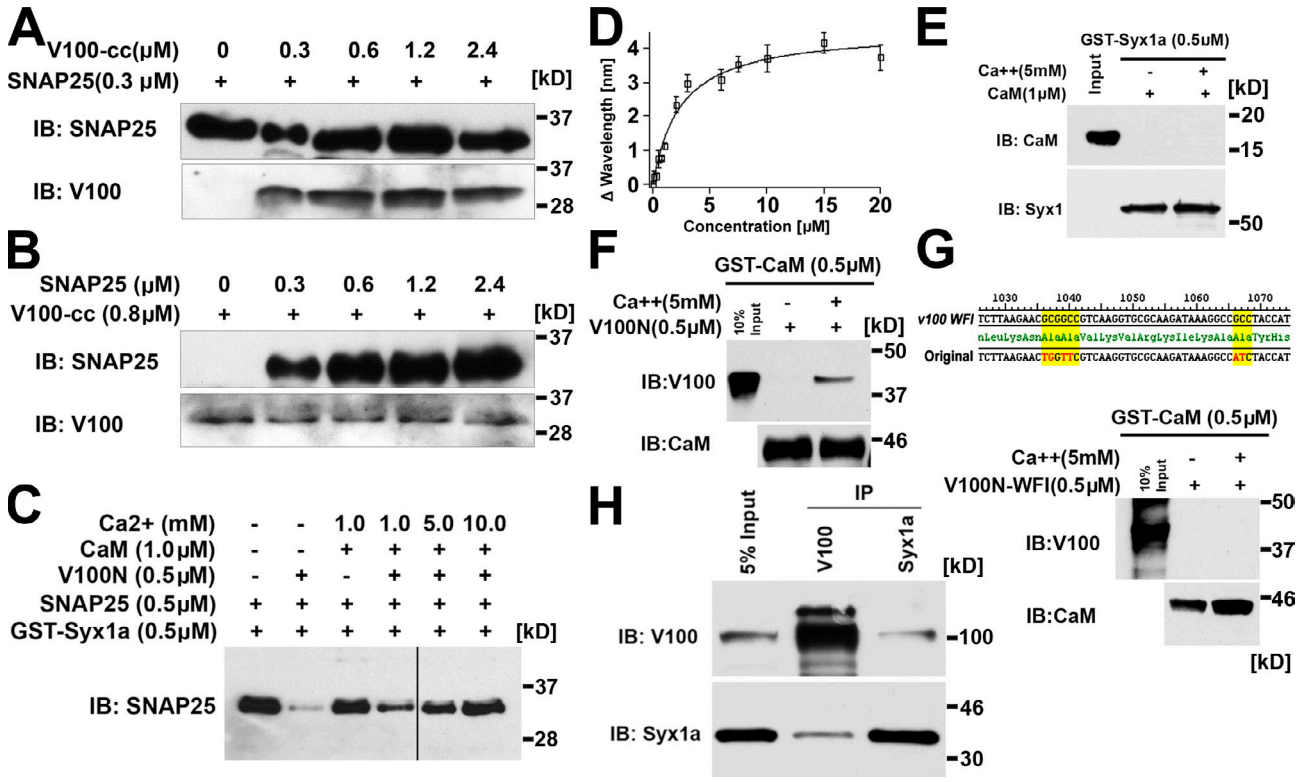

Figure S2. **Supplementary biochemical interaction experiments.** (A and B) A 180–amino acid N-terminal V100 fragment without the CaM binding site binds to Syx1A but does not compete with SNAP25 binding. (A) GST-Syx1A pull-down with increasing amounts of V100-short (amino acids 10–143). (B) GST-Syx1A pull-down with increasing amounts of SNAP25. Note that there is no binding competition in either experiment. (C) GST pull-downs using Ca^{2+} concentrations of 1 mM and higher exhibit an increasing efficiency in the release of the competitive binding of V100N to Syx1A as revealed by increased SNAP25 binding. The black line indicates the removal of an intervening lane for presentation purposes. (D–G) CaM directly interacts with V100N in a calcium-dependent manner. (D) Binding affinity curve for V100N with Ca^{2+}–CaM using BLI (quantification shown in Table S1). Error bars show SEMs. (E) GST-Syx1A pull-down shows no direct CaM binding under our experimental conditions. (F) GST-CaM pull-down with V100N in the presence and absence of Ca^{2+}. (G, top) Amino acid mutant to generate *v100*^{WFI}. Yellow shaded areas show the exact nucleotides and amino acids changes in the *v100*^{WFI} mutant. Red letters show the nucleotides in the wild-type sequence, which have been changed in the *v100*^{WFI} mutant. (bottom) Pull-down analogous to F with V100N^{WFI} reveals loss of CaM binding. (H) Co-IPs from adult fly brains reveal that only a small percentage of Syx1A exists in a complex with V100 in neurons at a given time point. Under our experimental conditions, most V100 protein in a protein extract can be immunoprecipitated with anti-V100 antibodies. In contrast, only ∼5% of the much more abundant Syx1A protein is immunoprecipitated with anti-Syx1A antibodies. In each case, the other protein is coimmunoprecipitated, indicating that ≤50% of V100 in homogenized fly brain tissue is in a complex with Syx1A, whereas only 1% or less of the more abundant Syx1A in the brain is complexed with V100. Note that some interactions may be lost as a result of dissociation in the detergent extract. IB, immunoblot.

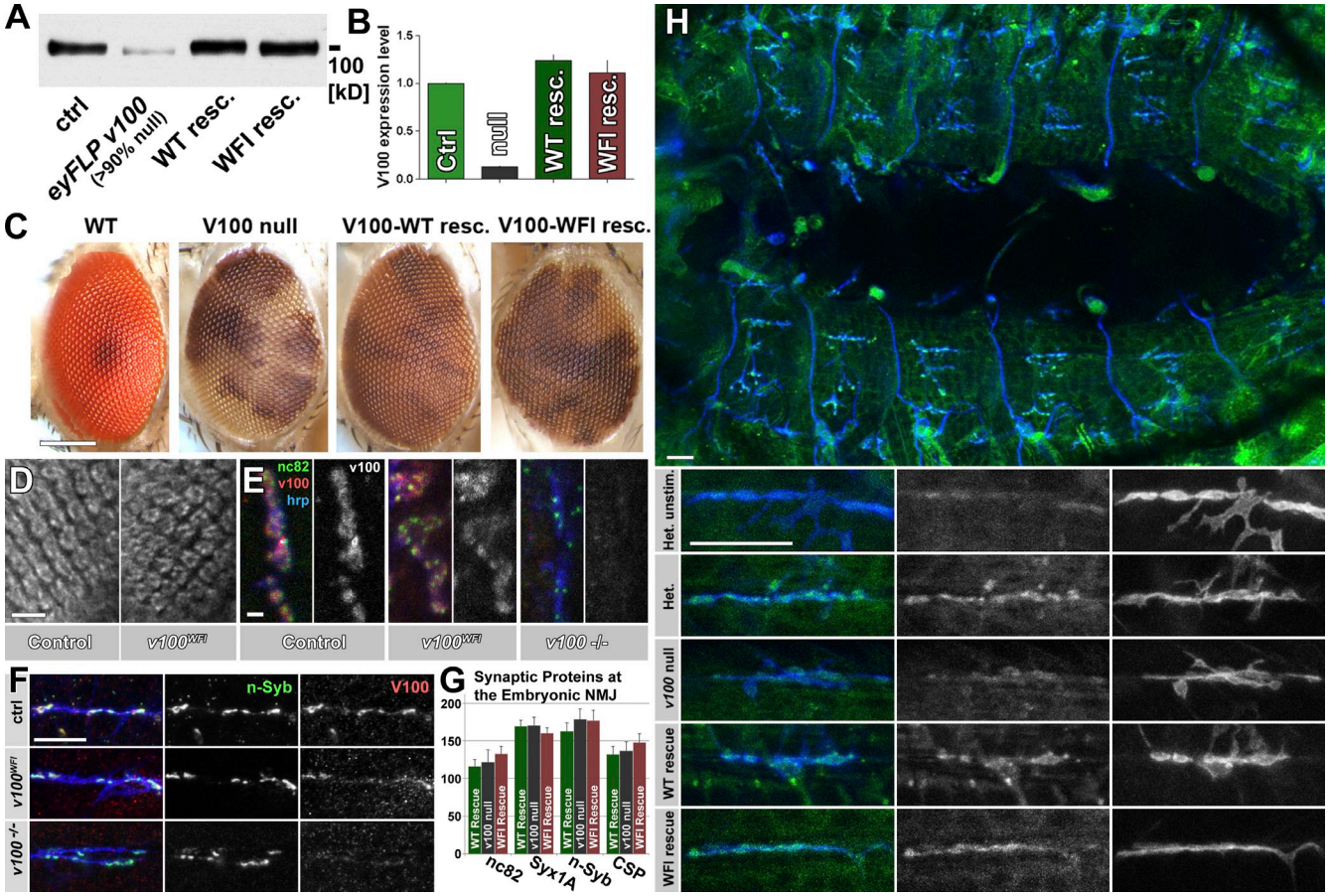

Figure S3. **Generation of CaM binding–deficient V100^{WFI} with low expression at endogenous levels, localizes to synapses, does not disrupt synaptic protein localization, and rescues FM1-43 uptake.** (A and B) Western blot from dissected eyes and probed with V100 antibody determines endogenous expression levels of V100^{WFI} and V100^{WT} in null mutant photoreceptors compared with wild-type (WT) photoreceptors. Note that loss of *v100* is embryonically lethal, and >90% mutant eyes were generated using the *eyeless-flippase* (*eyFLP*) mosaic method as described previously (Hiesinger et al., 2005). resc., rescue. (C) Photoreceptor-specific expression of *v100^{WT}* and *v100^{WFI}* in *v100*-null mutant eyes (glass multiple reporter–Gal4 at 18°C) causes no developmental defects or other notable toxicity (see ERG recordings in Fig. 3, A–C). (D) Adult photoreceptor synapses immunolabeled for V100. (E) Embryonic NMJs immunolabeled for active zones (nc82), V100, and neuronal membrane (HRP). Single V100 channels are shown in grayscale. (F) Embryonic NMJs immunolabeled for n-Syb and V100. (G) Quantification of immunolabels as in F. (H, top) Complete wild-type embryo fillet after FM1-43 uptake experiment, fixation, and counterlabeling of neuronal membranes with HRP (blue). (bottom) Representative images of individual NMJs for the indicated genotypes and stimulation conditions. Quantification in Fig. 4 C. ctrl, control. Error bars show SEMs. Bars: (C) 200 µm; (D, F, and H) 10 µm; (E) 1 µm.

Table S1. **Binding of V100N to Syx1A, SNAP25, and Ca^{2+}–CaM based on BLI**

Interaction	Parameters			
	K_d	k_{on}	k_{off}	R^2
	M	$M^{-1}s^{-1}$	s^{-1}	
V100N–Syx1A	2.25×10^{-6}	1.02×10^{3}	2.29×10^{-3}	0.98
V100N–SNAP25	4.50×10^{-6}	0.60×10^{3}	2.68×10^{-3}	0.90
V100N–Ca^{2+}–CaM	2.40×10^{-6}	0.50×10^{3}	1.20×10^{-3}	0.95
Syx1A–SNAP25	0.13×10^{-6}			

Syx1A–SNAP25 was measured by pull-down and Western immunoblotting as in Rickman et al. (2004).

References

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Rickman, C., F.A. Meunier, T. Binz, and B. Davletov. 2004. High affinity interaction of syntaxin and SNAP-25 on the plasma membrane is abolished by botulinum toxin E. *J. Biol. Chem.* 279:644–651. http://dx.doi.org/10.1074/jbc.M310879200