V-ATPASE V_0 SECTOR SUBUNIT a1 IN NEURONS IS A TARGET OF CALMODULIN*

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Running head: V-ATPase V_0 sector subunit a1 interacts tightly with calmodulin

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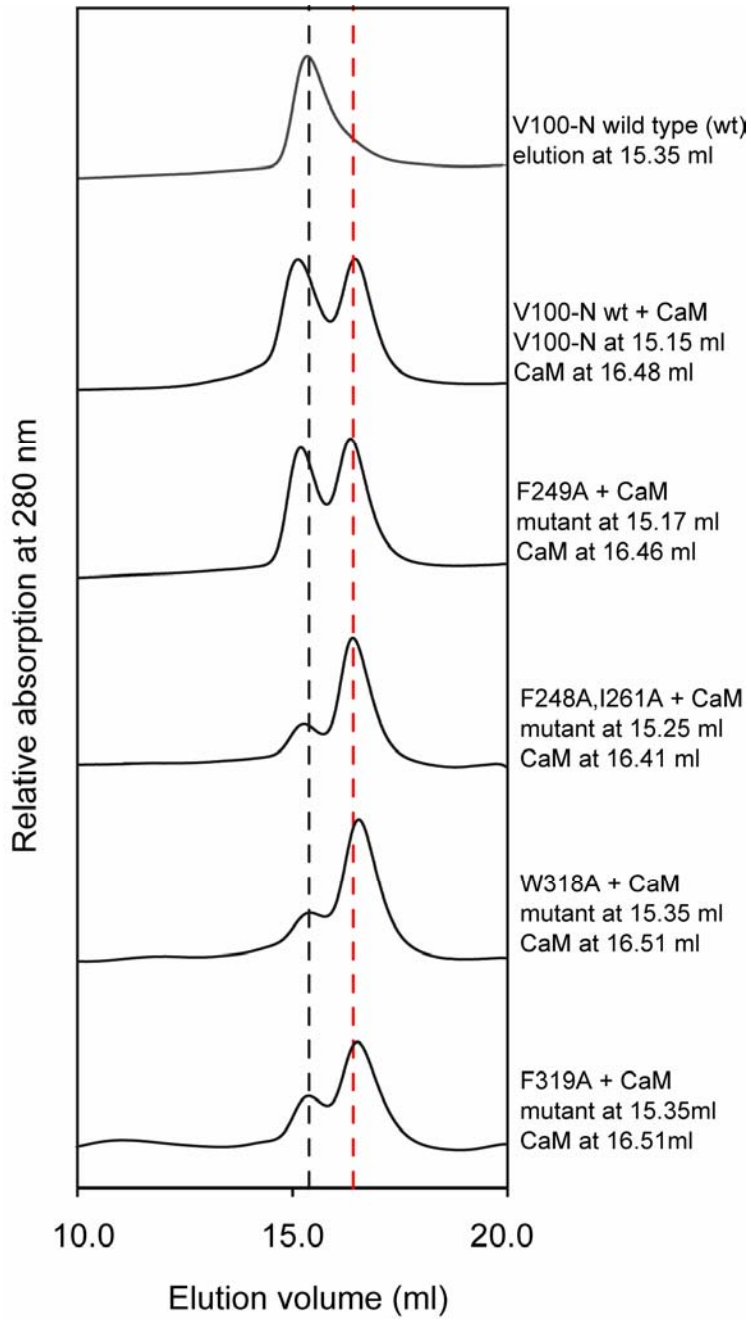
SUPPLEMENTARY DATA

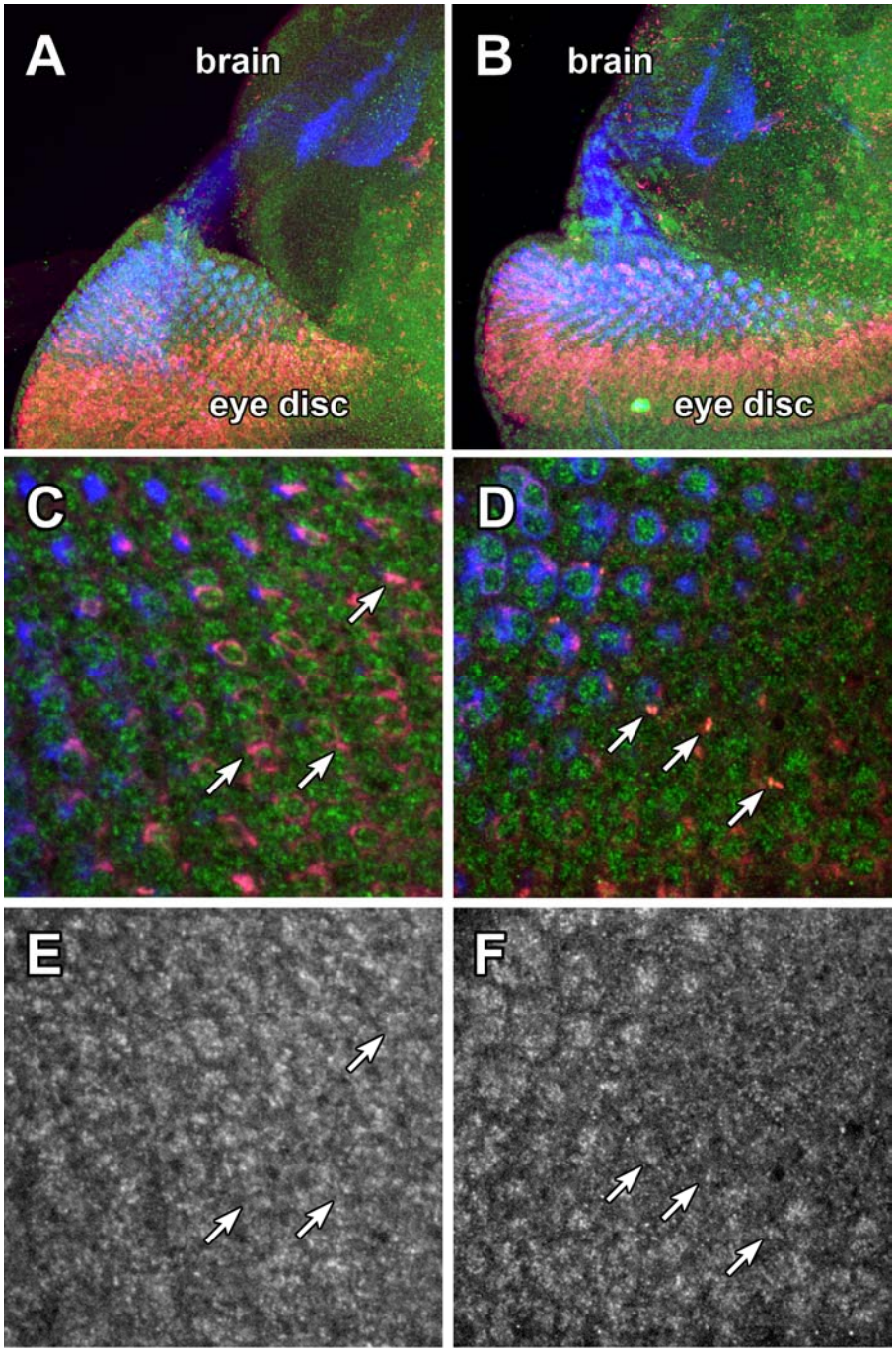
Supplementary Table 1. Assessment of the effects of mutations of residues in two putative CaM-binding domains in V100-N on binding of Ca^{2+}·CaM by pull down and gel filtration assays.

Location	Mutation	Ca^{2+}·CaM binding to V100-N	
		Pull down	Gel filtration
	None, wild-type	Yes	Yes (see Figure 1)
Segment 1	F248A	Yes	Yes
	F249A	Yes	Yes
	F248A+I261A	Yes	Yes
Segment 2	W318A	No	No
	F319A	No	No (see Figure 1)
	F319A+I328A[a]	n.d.	n.d.
	F319A+L332A[a]	n.d.	n.d.

[a] Ca^{2+}·CaM binding could not be determined (n.d.) or assayed because the double mutations resulted in insoluble V100-N.



Supplementary Fig. 1. Binding of CaM (Ca^{2+} loaded) to native V100-N and different mutant forms from replacement of residues on two putative CBD segments (segments 1 and 2; see main paper) assessed by analytical gel filtration. Segment 1 has the sequence 245FII**F**FQGDQLKTRVKK**I**CEGFRATLYP271, and segment 2 has the sequence 314NLKNW**F**VKVRKIKA**I**YHT**L**NLFNLD338.



Supplementary Fig. 2. Loss of CaM-binding causes no obvious defects in V100 or CaM localization during photoreceptor development. Shown are larval eye discs and photoreceptor projection in the larval brain. Photoreceptors are rendered mutant using the ey35FLP system and replaced with wild type (*A*, *F*, *E*) or mutant (*B*, *D*, *F*) v100. Blue: Chaoptin (24B10, a photoreceptor-specific marker), red: anti-V100, green: anti-CaM. V100 as well as CaM are present in cell bodies as well as developing synapses and exhibit an indistinguishable, punctuate and partly overlapping localization pattern.