

Supplementary files

CAPS-1 requires its C2, PH, MHD1 and DCV domains for dense core vesicle exocytosis in mammalian CNS neurons

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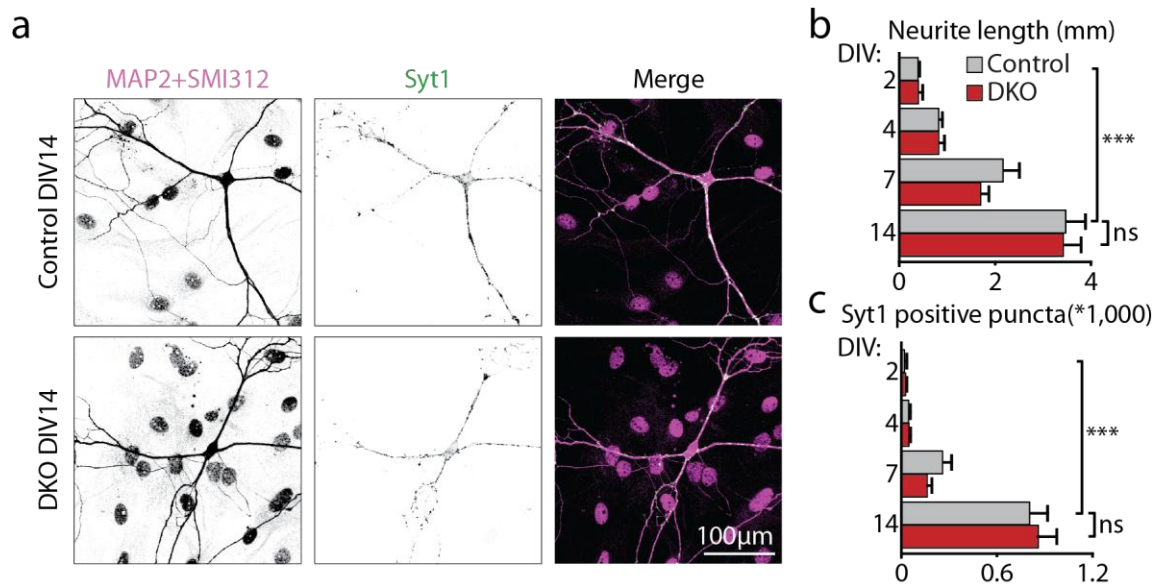


Figure S 1 – CAPS DKO neurons show no developmental defects

a: Representative images of CAPS-2 KO (control, top) and CAPS DKO (bottom) neurons at DIV 14, stained with dendrite marker (MAP2; magenta) and axonal marker (SMI312; magenta) and synaptotagmin 1 (syt1, green).

b: Total neurite length in mm of control and CAPS DKO neurons at DIV 2, 4, 7 and 14 in CAPS DKO and control neurons. 2-way ANOVA: genotype: $p = 0.53$ (not significant, ns), DIV: $p = 7.8 \times 10^{-25}$ (***), genotype*DIV: $p = 0.72$ (ns).

c: Number of syt1 positive puncta in control and CAPS DKO neurons at DIV 2, 4, 7 and 14. 2-way ANOVA: genotype: $p = 0.85$ (ns), DIV: $p = 9.0 \times 10^{-28}$ (***), genotype*DIV: $p = 0.71$ (ns).

Detailed information (average, SEM, n and detailed statistics) is shown in Table S1.

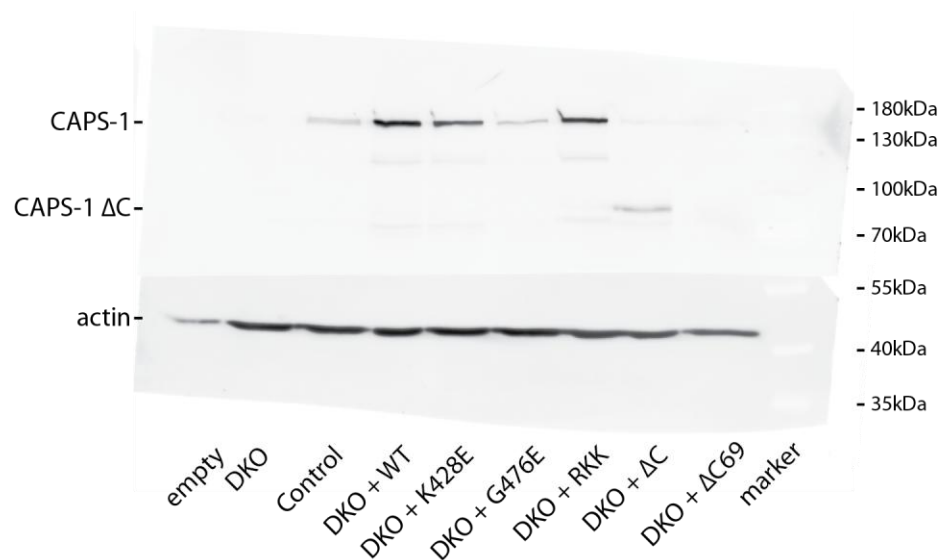


Figure S 2 – Western blot of CAPS DKO cortical neurons

Uncropped western blot of CAPS DKO cortical neurons infected with wild type or mutant CAPS-1 constructs and CAPS-2 KO control neurons (Figure 1b shows cropped blot). DKO + Δ69 are CAPS DKO cortical neurons infected with a mutant lacking the last 69 amino acids of the C-terminus¹, which was not further used in this study because of strongly reduced expression levels.

Supplementary References

1. Parsaud, L. et al. Calcium-dependent activator protein for secretion 1 (CAPS1) binds to syntaxin-1 in a distinct mode from Munc13-1. *J Biol Chem* 288, 23050–23063, doi:10.1074/jbc.M113.494088 (2013).

Table S 1 – Data and statistics

For each data figure, the measured variable, tested groups, average and SEM, n (independent cells) and N (independent weeks) are listed. Statistical tests were performed on all groups, unless specified otherwise. Statistical tests used $\alpha = 0.05$ and were two-tailed. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. N.A.: not applicable.

Figure	measured variable	group	average $\pm$ SEM	n (N)	statistics
1c	relative CAPS-1 level	CAPS DKO	0.05 ± 0.04	2	N.A.
		control	1.00 ± 0.74	2	
		DKO + WT	5.25 ± 2.32	2	
		DKO + K428E	3.62 ± 0.47	2	
		DKO + G476E	2.08 ± 0.79	2	
		DKO + RKK	7.60 ± 0.55	2	
		DKO + ΔC	2.91 ± 0.15	2	
1e	relative Manders' coefficient	control	0.48 ± 0.05	24 (4)	1-way ANOVA (CAPS conditions): $p = 0.067$ (ns)
		DKO + WT	0.61 ± 0.05	25 (4)	
		DKO + K428E	0.66 ± 0.07	9 (2)	
		DKO + G476E	0.48 ± 0.12	10 (2)	
		DKO + RKK	0.64 ± 0.08	14 (2)	
		DKO + ΔC	0.31 ± 0.05	5 (2)	
		VAMP	1.00 ± 0.03	26 (4)	
1f	Number CAPS-1 puncta	control	34.8 ± 7.7	5 (2)	N.A.
		DKO + ΔC	0.0 ± 0.0	5 (2)	
2b	number DCVs	control	$1.9 \pm 0.1 \cdot 10^3$	18 (4)	1-way ANOVA: $p = 0.42$ (ns)
		DKO	$2.3 \pm 0.2 \cdot 10^3$	17 (6)	
		DKO + WT	$2.4 \pm 0.2 \cdot 10^3$	38 (6)	
		DKO + K428E	$2.5 \pm 0.3 \cdot 10^3$	11 (3)	
		DKO + G476E	$2.3 \pm 0.3 \cdot 10^3$	16 (3)	
		DKO + RKK	$2.3 \pm 0.3 \cdot 10^3$	16 (3)	
		DKO + ΔC	$2.8 \pm 0.5 \cdot 10^3$	13 (3)	
2i	DCV exocytosis events	control	51 ± 9.7	18 (4)	1-way ANOVA: $p = 5.9 \cdot 10^{-9}$ (***) Post-hoc Dunnett's test (compared to control): DKO: $p = 2.0 \cdot 10^{-6}$ (***) DKO + WT: $p = 0.27$ (ns) DKO + K428E: $p = 8.5 \cdot 10^{-5}$ (***) DKO + G476E: $p = 2.7 \cdot 10^{-5}$ (***) DKO + RKK: $p = 7.4 \cdot 10^{-5}$ (***) DKO + ΔC : $p = 5.2 \cdot 10^{-5}$ (***) Post-hoc Dunnett's test (compared to DKO): DKO + K428E: $p = 1.0$ (ns) DKO + G476E: $p = 1.0$ (ns) DKO + RKK: $p = 0.96$ (ns) DKO + ΔC : $p = 1.0$ (ns)
		DKO	2.1 ± 0.8	17 (6)	
		DKO + WT	36.9 ± 6.3	38 (6)	
		DKO + K428E	4.3 ± 1.7	11 (3)	
		DKO + G476E	6.4 ± 1.9	16 (3)	
		DKO + RKK	8.8 ± 3.8	16 (3)	
		DKO + ΔC	5.4 ± 2.7	13 (3)	
2j	DCV release probability	control	$2.9 \pm 0.55 \%$	18 (4)	1-way ANOVA: $p = 2.2 \cdot 10^{-8}$ (***) Post-hoc Dunnett's test (compared to control): DKO: $p = 1.0 \cdot 10^{-6}$ (***) DKO + WT: $p = 0.045$ (*) DKO + K428E: $p = 3.2 \cdot 10^{-5}$ (***) DKO + G476E: $p = 8.0 \cdot 10^{-6}$ (***) DKO + RKK: $p = 2.5 \cdot 10^{-5}$ (***) DKO + ΔC : $p = 1.9 \cdot 10^{-4}$ (***) Post-hoc Dunnett's test (compared to DKO): DKO + K428E: $p = 1.0$ (ns) DKO + G476E: $p = 1.0$ (ns) DKO + RKK: $p = 0.98$ (ns) DKO + ΔC : $p = 0.98$ (ns)
		DKO	$0.11 \pm 0.05 \%$	17 (6)	
		DKO + WT	$1.8 \pm 0.33 \%$	38 (6)	
		DKO + K428E	$0.20 \pm 0.07 \%$	11 (3)	
		DKO + G476E	$0.31 \pm 0.10 \%$	16 (3)	
		DKO + RKK	$0.43 \pm 0.18 \%$	16 (3)	
		DKO + ΔC	$0.41 \pm 0.22 \%$	13 (3)	

Figure	measured variable	group	average ± SEM	n (N)	statistics		
3d	NH4 ⁺ _{max}	control	3.4 ± 0.45	8 (2)	Mann-Whitney U test p = 0.24 (ns)		
		CAPS DKO	2.9 ± 0.21	14 (2)			
3e	Fstim _{max}	control	0.43 ± 0.06	8 (2)	Mann-Whitney U test p = 0.042 (*)	t-test (comparing U-values of 3e (U = 26) and 3f (U = 7)) p = 4.9 *10 ⁻²⁰ (***)	
		CAPS DKO	0.27 ± 0.04	14 (2)			
3f	DCV exocytosis events	control	51 ± 9.7	18 (4)	Mann-Whitney U test p = 2.0 *10 ⁻⁸ (***)		
		CAPS DKO	2.1 ± 0.8	17 (6)			
S1b	neurite length (mm)	Control	DIV 2	0.38 ± 0.03	20 (2)	2-way ANOVA: genotype: p = 0.53 (ns) DIV: p = 7.8 *10 ⁻²⁵ (***) genotype*DIV: p = 0.72 (ns) Post-hoc Tukey test for DIV: DIV 2 vs 4: p = 0.33 (ns); DIV 2 vs 7: p = 2.2 *10 ⁻⁷ (***) DIV 2 vs 14: p = 4.4 *10 ⁻¹³ (***) ; DIV 4 vs 7: p = 1.4 *10 ⁻⁴ (***) DIV 4 vs 14: p = 4.4 *10 ⁻¹³ (***) ; DIV 7 vs 14: p = 4.2 *10 ⁻⁸ (***)	
		CAPS DKO	DIV 2	0.41 ± 0.06	13 (2)		
		Control	DIV 4	0.81 ± 0.08	20 (2)		
		CAPS DKO	DIV 4	0.84 ± 0.09	20 (2)		
		Control	DIV 7	2.14 ± 0.33	17 (2)		
		CAPS DKO	DIV 7	1.68 ± 0.17	19 (2)		
		Control	DIV 14	3.44 ± 0.41	20 (2)		
		CAPS DKO	DIV 14	3.38 ± 0.37	20 (2)		
S1c	syt1 positive puncta	Control	DIV 2	22 ± 4.6	20 (2)	2-way ANOVA: genotype: p = 0.85 (ns) DIV: p = 9.0 *10 ⁻²⁸ (***) genotype*DIV: p = 0.71 (ns) Post-hoc Tukey test for DIV: DIV 2 vs 4: p = 0.99 (ns); DIV 2 vs 7: p = 0.04 (*) DIV 2 vs 14: p = 4.4 *10 ⁻¹³ (***) ; DIV 4 vs 7: p = 0.07 (ns) DIV 4 vs 14: p = 4.4 *10 ⁻¹³ (***) ; DIV 7 vs 14: p = 4.4 *10 ⁻¹³ (**)	
		CAPS DKO	DIV 2	27 ± 6.5	13 (2)		
		Control	DIV 4	45 ± 10.5	20 (2)		
		CAPS DKO	DIV 4	48 ± 9.4	20 (2)		
		Control	DIV 7	259 ± 57	17 (2)		
		CAPS DKO	DIV 7	162 ± 31	19 (2)		
		Control	DIV 14	809 ± 112	20 (2)		
		CAPS DKO	DIV 14	862 ± 117	20 (2)		