Supplementary Information

Title:

Doc2b Ca^{2+} binding site mutants enhance synaptic release at rest at the expense of sustained synaptic strength

Authors:

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Supplementary Table 1



Supplementary Figure S1: Liposome binding by GST fusion proteins depends on PS and Ca²⁺ and is not attributable to GST, thrombin or glutathione. (**A**) SDS-PAGE analysis of recombinant GST-C2AB or GST alone expressed in E.coli. F indicates full-length fusion protein, eluted from glutathione-Sepharose beads with 5 mM soluble glutathione; T indicates a protein fragment eluted from the beads by proteolytic cleavage with thrombin; R indicates the residual GST fragment remaining on the beads after thrombin cleavage, subsequently eluted with 5 mM glutathione. Predicted molecular weights are indicated at the right. (**B**) Kinetic traces of phospholipid binding, quantitated as absorption at 350 nm in presence of 834 nM [Ca²⁺]_{free} and liposomes containing DOPC/DOPS (80/20) unless indicated otherwise. After 60 s (arrow) the indicated sample was added: C2AB (50 µl of fraction 'T' shown in panel A), GST (50 µl of fraction 'F'), thrombin (50 µl containing 2.5U, equal amount as in fractions 'T'), glutathione (50 µl of 5 mM glutathione, equal as in fractions 'F'). For the 'no PS' control, 100% DOPC liposomes were used. For the 'no Ca^{2+'} control, a buffer containing 10 mM EGTA was used. (**C**) As a measure for total phospholipid binding, the A350 from the last 20 s was averaged. Graph represents mean ± sem of 2-4 assays (C2AB: n=4; others: n=2).



Supplementary Figure S2. Doc2b expression levels in wildtype and Doc2a,b double knockout (DKO) neurons. Western blots from (**A**) DKO and (**B**) WT high density neuron cultures overexpressing wildtype or mutant Doc2b confirming the overexpression of Doc2b^{WT} and mutants by lentiviral particles. Each lane contains the lysate from 100K cortical cells transduced at 1 DIV and harvested at 17 DIV. (**C**) Endogenous Doc2b could not be detected in standard conditions but became detectable by loading larger sample volumes of cortex or total brain lysate (µg indicates total wet weight of brain tissue). Doc2b immunoreactivity in overexpressing neurons was higher compared to endogenous levels in non-transfected wildtype neurons. For cropped blots, full-length blots are available in the replication dataset as detailed in the Data Availability Statement.



Supplementary Figure S3. Doc2b^{DN} and Doc2b^{6A} mutations enhance the frequency of spontaneous release in wildtype hippocampal neurons. (**A**) Representative mEPSC recordings in wildtype neurons overexpressing eGFP (ctrl), Doc2b^{WT} or mutants (Doc2b^{DN}, Doc2b^{6A}), cultured in networks in presence of 1 μ M TTX and 20 μ M gabazine. (**B**) Quantification of spontaneous neurotransmitter release frequency and (**C-F**) amplitude, charge, rise and decay time. (**G**) Representative recordings in isolated neurons cultured on microglial islands (autapses). (**H-L**) Quantification of the frequency and postsynaptic parameters. Data are represented as mean ±SEM;. Kruskall Wallis ANOVA, Pairwise Post-hoc tests (***, p<0.005).). Red stars signs indicate outliers. The number of cells (n) and the number of independent experiments (N) are indicated as "n/N".



Supplementary Figure S4. Doc2b mutants affect short term plasticity in WT autaptic neurons. (A) Averaged trace of a single EPSC from wildtype neurons overexpressing Doc2b^{WT} and mutants. (B) Quantification of 1st EPSC amplitude and charge (C). (D) Paired-pulse ratio. (E) Single EPSC guantal content corresponding to the ratio of the EPSC charge and mEPSC charge. Doc2b^{DN} and Doc2b^{6A} affect vesicle release probability and thus short-term plasticity. (F-L) Rundown during repetitive stimulation for 100 AP at 5 Hz. (F) Absolute (G), normalized total charge (H) and normalized synchronous release. (I) Cumulated absolute and (J) normalized total charge from the entire train. (K) Proportion of the synchronous and asynchronous component. (L) Recovery pulse 2s after 5 Hz stimulation. (M-S) Rundown during repetitive stimulation for 100 AP at 40 Hz. (M) Absolute and (N) Normalized total charge. (O) Normalized asynchronous release. (P) Cumulated absolute and (Q) normalized total charge during the entire train. (R) Proportion of the synchronous and asynchronous component. (S) Recovery, measured after a 40 Hz train. Data are represented as mean ±SEM; Kruskall Wallis ANOVA and one way repeated ANOVA, Pairwise Post-hoc tests (*, p<0.05, **, p<0.01, ***, p<0.005). Red stars signs indicate outliers. The number of recordings (n) and the number of independent experiments (N) are indicated as "n/N".



Supplementary Figure S5. Overexpression of wildtype Doc2b does not affect calcimycininduced neurotransmitter release. Calcimycin was perfused to directly induce Ca^{2+} influx and bypass voltage-dependent Ca^{2+} channels. (**A**) Typical example of single EPSC in naïve wildtype autaptic neurons; (**B**) the EPSC induced by puff application of calcimycin for 100 s. (**C**) Quantification of EPSC amplitude. (**D**) Calcimycin-induced response peak amplitude and (**E**) total charge transfer. Data are represented as mean ±SEM. Red stars signs indicate outliers. The number of recordings (n) and the number of independent experiments (N) are indicated as "n/N".

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ing site mutations cause constitutive phospholipid binding of C2A and C2AB fragments of Doc2b	Figure references	Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 36 nM [Ca2+]free = 36 nM [Ca2+]free = 655 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 10941 nM Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 36 nM	Itest (between g (2) Kruskal-W: Statistical result H(2)=11,580 H(2)=10,593 H(2)=10,593 H(2)=10,593 H(2)=6,228 H(2)=11,580 H(2)=11,580 H(2)=12,80 H(2)=1,580 H(2)=1,580 H(2)=1,580 H(2)=2,8,346 H(2)=2,8,346 H(2)=8,346 H(2)=8,346 H(2)=8,346 H(2)=8,346	proups com allis Test p-value p=0,003 p=0,006 p=0,006 p=0,007 p=0,003 p=0,015 p=0,015 p=0,015 p=0,015 p=0,015	Statistical test (2)	Effect size (r) 2,990 2,484 2,656 2,735 n.a. 3,700 3,063 3,000 3,000 3,000 3,000 3,000 3,000 2,409 2,676 2,409 2,676 2,409	Accepted α-sign 0,05 Descriptive and groups Accepted α-sign 0,05 Descriptive and groups	pairwise compar comparisons ificance pairwise compar comparisons	Sample n =15, N = 5 independent experiment ison Corrected p- value N = 12, N = 4 independent ison Corrected p- value
inding site mutations cause constitutive phospholipid binding of C2A and C2AB fragments of Doc2b	Figure references	Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 36 nM [Ca2+]free = 163 nM [Ca2+]free = 509 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 10941 nM Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 36 nM [Ca2+]free = 36 nM [Ca2+]free = 163 nM [Ca2+]free = 163 nM	Itest (between g (2) Kruskal-Wi Statistical result Fill H(2)=11,580 H(2)=9,620 H(2)=10,593 H(2)=10,593 H(2)=10,288 H(2)=10,288 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=10,200 H(2)=11,580 H(2)=10,200 H(2)=10,200 H(2)=10,200 H(2)=8,346 H(2)=8,346 H(2)=8,346 H(2)=6,500 H(2)=0,200 H(2)=0,200	proups com allis Test p-value p=0,003 p=0,006 p=0,005 p=0,001 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,010 p=0,015 p=0,010 p=0,015 p=0,015 p=0,015 p=0,015 p=0,015	Statistical test (2)	Effect size (r) 2,990 2,484 2,656 2,735 n.a. 3,700 3,063 3,000 3,000 3,000 3,000 3,000 3,000 2,676 2,409 2,676 2,409 1,876 2,675	Accepted α-sign 0,05 Descriptive and groups Accepted α-sign 0,05 Descriptive and groups	ficance pairwise compar comparisons ficance pairwise compar comparisons	Sample n =15, N = 5 independent experiment ison Corrected p- value N = 4 independent ison Corrected p- value
+ binding site mutations cause constitutive phospholipid binding of C2A and C2AB fragments of Doc2b	Pigure references	Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 63 nM [Ca2+]free = 163 nM [Ca2+]free = 655 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 10941 nM Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 36 nM [Ca2+]free = 163 nM [Ca2+]free = 163 nM [Ca2+]free = 163 nM	Itest (between g (2) Kruskal-Wi Statistical result Fesuit H(2)=11,580 H(2)=9,620 H(2)=10,593 H(2)=10,593 H(2)=10,593 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=1,580 H(2)=1,580 H(2)=1,580 H(2)=2,8,346 H(2)=9,846 H(2)=9,846 H(2)=9,846	proups com allis Test p-value p=0,003 p=0,006 p=0,005 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 groups com allis Test p=0,015 p=0,015 p=0,015 p=0,015 p=0,023	Statistical test (2)	Effect size (r) 2,990 2,484 2,656 2,735 n.a. 3,700 3,063 3,000 3,000 3,000 3,000 3,000 2,676 2,409 2,676 2,409 1,876 2,842	Accepted α-sign 0,05 Descriptive and groups Accepted α-sign 0,05 Descriptive and groups	ficance pairwise compar comparisons ificance pairwise compar comparisons	Sample n =15, N = 5 independent experiment ison Corrected p- value N = 12, N = 4 independent ison Corrected p- value
Ca2+ binding site mutations cause constitutive phospholipid binding of C2A and C2AB fragments of Doc2b	Eigure references	Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 76 nM [Ca2+]free = 36 nM [Ca2+]free = 655 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 10941 nM [Ca2+]free = 10941 nM [Ca2+]free = 10941 nM [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 7 nM [Ca2+]free = 65 nM	Itest (between g (2) Kruskal-Wi Statistical result Fesult H(2)=11,580 H(2)=9,620 H(2)=10,288 H(2)=10,593 H(2)=10,288 H(2)=10,593 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=1,580 H(2)=2,583 H(2)=8,346 H(2)=8,346 H(2)=8,346 H(2)=6,500 H(2)=7,423	proups com allis Test p-value p=0,003 p=0,008 p=0,008 p=0,008 p=0,001 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 groups com allis Test p=0,015 p=0,015 p=0,015 p=0,015 p=0,010 p=0,015 p=0,010 p=0,010 p=0,010 p=0,007 p=0,024	Statistical test (2)	Effect size (r) 2,990 2,484 2,656 2,735 n.a. 3,700 3,063 3,000 3,000 3,000 3,000 3,000 3,000 3,000 2,409 2,676 2,409 2,676 2,409 1,876 2,842 2,143	Accepted α-sign 0,05 Descriptive and groups Accepted α-sign 0,05 Descriptive and groups	ficance pairwise compar comparisons ificance pairwise compar comparisons	Sample n =15, N = 5 independent experiment ison Corrected p- value N = 12, N = 4 independent ison Corrected p- value
3. Ca2+ binding site mutations cause constitutive phospholipid binding of C2A and C2AB fragments of Doc2b	P. Figure references	Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 36 nM [Ca2+]free = 655 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 10941 nM Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 66 nM [Ca2+]free = 66 nM [Ca2+]free = 80 nM	Itest (between g (2) Kruskal-W: Statistical result Fesult H(2)=11,580 H(2)=10,288 H(2)=10,288 H(2)=10,283 H(2)=10,283 H(2)=14,332 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=14,580 H(2)=14,580 H(2)=8,346 H(2)=8,346 H(2)=8,346 H(2)=8,346 H(2)=7,423 H(2)=7,423 H(2)=7,423 H(2)=8,769 H(2)=8,769	proups com p-value p=0,003 p=0,006 p=0,005 p=0,005 p=0,003 p=0,015 p=0,015 p=0,015 p=0,015 p=0,015 p=0,015 p=0,015 p=0,015 p=0,016 p=0,017 p=0,024 p=0,012	Statistical test (2) (2) (2) (2) (2) (2) (2) (2) (2) (2) (2) (2) (2) (2) (2) parison)	Effect size (r) 2,990 2,484 2,656 2,735 n.a. 3,700 3,063 3,000 3,000 3,000 3,000 3,000 3,000 2,676 2,409 2,676 2,409 1,876 2,842 2,143 2,531	Accepted α-sign 0,05 Descriptive and groups Accepted α-sign 0,05 Descriptive and groups	ificance pairwise compar comparisons ificance pairwise compar comparisons	Sample n =15, N = 5 independent experiment ison Corrected p- value N =12, N = 4 ison Corrected p- value Corrected p- value
rre 3. Ca2+ binding site mutations cause constitutive phospholipid binding of C2A and C2AB F	- Figure reference	Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 36 nM [Ca2+]free = 65 nM [Ca2+]free = 655 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 10941 nM Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 6,5 nM [Ca2+]free = 103 nM [Ca2+]free = 163 nM [Ca2+]free = 6,5 nM [Ca2+]free = 8,3 nM [Ca2+]free = 8,3 nM [Ca2+]free = 1075 nM	Itest (between g (2) Kruskal-W: Statistical result	proups com allis Test p-value p=0,003 p=0,006 p=0,005 p=0,005 p=0,003 p=0,015 p=0,015 p=0,015 p=0,015 p=0,015 p=0,024 p=0,037 p=0,024 p=0,024 p=0,024 p=0,024 p=0,024 p=0,024 p=0,024	Statistical test (2)	Effect size (r) 2,990 2,484 2,656 2,735 n.a. 3,700 3,063 3,000 3,000 3,000 3,000 3,000 3,000 2,409 2,676 2,409 2,676 2,409 1,876 2,409 2,676 2,409 2,676 2,409 1,876 2,409 2,676	Accepted α-sign 0,05 Descriptive and groups Accepted α-sign 0,05 Descriptive and groups	ficance pairwise compar comparisons ificance pairwise compar comparisons	Sample n =15, N = 5 independent experiment ison Corrected p- value N = 12, N = 4 independent ison Corrected p- value
igure 3. Ca2+ binding site mutations cause constitutive phospholipid binding of C2A and C2AB fragments of Doc2b	T Figure references	Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 36 nM [Ca2+]free = 655 nM [Ca2+]free = 655 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 10941 nM Statistica Parameter [Ca2+]free = 2,2 nM [Ca2+]free = 7 nM [Ca2+]free = 36 nM [Ca2+]free = 36 nM [Ca2+]free = 509 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 655 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 1075 nM [Ca2+]free = 2,27 nM	Itest (between g (2) Kruskal-Wi Statistical result F H(2)=11,580 H(2)=9,620 H(2)=10,593 H(2)=10,593 H(2)=10,593 H(2)=10,593 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=11,580 H(2)=1,580 H(2)=1,580 H(2)=1,580 H(2)=1,580 H(2)=8,346 H(2)=8,346 H(2)=9,269 H(2)=8,346 H(2)=7,423 H(2)=7,538 H(2)=7,538 H(2)=4,154 H(2)=4,154	proups com allis Test p-onol p=0,003 p=0,008 p=0,006 p=0,005 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,003 p=0,010 p=0,015 p=0,015 p=0,015 p=0,015 p=0,012 p=0,024 p=0,022 p=0,023 p=0,125	Statistical test (2)	Effect size (r) 2,990 2,484 2,656 2,735 n.a. 3,700 3,063 3,000 3,000 3,000 3,000 3,000 3,000 2,409 2,676 2,409 2,676 2,409 1,876 2,842 2,143 2,531 2,176 n.a.	Accepted α-sign 0,05 Descriptive and groups Accepted α-sign 0,05 Descriptive and groups	ficance pairwise compar comparisons ificance pairwise compar comparisons	Sample n =15, N = 5 independent experiment ison Corrected p- value N = 12, N = 4 independent ison Corrected p- value

Supplementary Table1. Statistical analysis

		Statistica	l test (between)	groups com	parison)		Accepted α-signi	ficance	Sample
e	88		(1) Kruskal-W	allis Test			0.01		n - 107 N - 5
ient ient	len	Statistic	al test (within g	roups comp	arison)				independent
lefic	refe		Pairwise con	parison	-		0.05		experiment
s ent	Brue		Statistical		Statistical	Effect size	Descriptive and	pairwise compar	ison
ants 1 Do	Ē	Parameter	result	p-value	test	(r)	groups	comparisons	Corrected p-
mut se ir ron:	<u> </u>						eGFP*: 10.01 ± 1.424	eGFP vs WT	p=1
site elea: neu							WT*: 13,27 ± 1,568	eGFP vs DN	p=0,000
ng-∢ is re pal	в	mEPSC frequency	H(3)=64,111	p=0,000	(1)	4,568	DN*: 28,08 ± 3,226	eGFP vs 6A	p=0,000
indi eou							0A . 33,23 ± 2,100	WT vs 6A	p=0,002
2*-b Itan								DN vs 6A	p=0,095
por por	C C	mEPSC amplitude	H(3)=1,465	p=0,690	(1)	n.a.	00ED*: 00.25 + 0.204		n=1
c2b of s							WT*: 85.44 ± 8.168	eGFP vs W1	p=1
. Do	D	mEPSC charge	H(3)=11.767	p=0.008	(1)	0.838	DN*: 86,10 ± 9,856	eGFP vs 6A	p=0,591
re 4 que				• <i>`</i>			6A*: 123,65 ± 10,402	WT vs DN WT vs 64	p=1
fre.								DN vs 6A	p=0,010 p=0,027
	E	mEPSC rise	H(3)=3,599	p=0,308	(1)	n.a.			
	F	mEPSC decay	H(3)=6,849	p=0,077	(1)	n.a.			
		Statistica	l test (between i	aroups com	narison)		Accepted a-signi	ficance	Sample
	8		⁽¹⁾ Kruskal-W	allis Test	partoony		0.025	illinoo	Jacobi
	Buo	Statistic	al test (within a	rouns comn	arison)				n = 139, N = 5 independent
	efer	Julion	Painvise com	narison	anoony		0.05		experiment
	5		T all wise con	ipanson			Descriptive and	pairwise compar	ison
	ligu	Parameter	Statistical	p-value	Statistical	Effect size	Descriptive und	puil Wise compar	Corrected p-
	-		result		test	(r)	groups	comparisons	value
	В	1st EPSC amplitude	H(3)=6,481	p=0,090	(1)	n.a.			
ş	c	1st EPSC charge	H(3)=6,279	p=0,099	(1)	n.a.	Accented a signi	ficanco	Comple
uror	8	Statistica	⁽¹⁾ KruekaLW	allie Toet	parison		Accepted u-sign	licalice	Sample
nei	and a	Statistic	al toet (within a		aricon)		0,023	n = 102, N = 5	
ient	sfere	510115110	Doinviso com	noricon	ansonj		0.05	experiment	
efic	99		Pairwise con	ipanson			Descriptive and	nairwise compar	ison
2-d	lgi	Parameter	Statistical	p-value	Statistical	Effect size	Descriptive and	pairwise compar	Corrected p.
å			result		test	(r)	groups	comparisons	value
Ë	D	1st EPSC quantal content	H(3)=3,476	p=0,324	(1)	n.a.		6	
ssion in	D	1st EPSC quantal content Statistica	H(3)=3,476 I test (between s	p=0,324 groups com	(1) parison)	n.a.	Accepted α-sign	ficance	Sample
pression in	D secue	1st EPSC quantal content Statistica (1) On	H(3)=3,476 I test (between e-way repeated n ⁽²⁾ Kruskal-W	p=0,324 groups com neasures AN allis Test	(1) parison) IOVA	n.a.	Accepted α-signi 0,008	ficance	Sample n = 139, N = 5
n depression in	eferences D	1st EPSC quantal content Statistica (1) On Statistic	H(3)=3,476 I test (between e-way repeated n ⁽²⁾ Kruskal-Wa ral test (within g	p=0,324 groups com neasures AN allis Test roups comp	(1) parison) IOVA arison)	n.a.	Accepted α-signi 0,008 0.05	ficance	Sample n = 139, N = 5 independent experiment
term depression in	Ire references	1st EPSC quantal content Statistica (1) On Statistic	H(3)=3,476 I test (between e-way repeated n ⁽²⁾ Kruskal-W al test (within g Pairwise con	p=0,324 groups com neasures AN allis Test roups comp parison	(1) parison) IOVA arison)	n.a.	Accepted α-signi 0,008 0.05 Descriptive and	ficance pairwise compar	Sample n = 139, N = 5 independent experiment ison
hort-term depression in	Figure references C	1st EPSC quantal content Statistica (*) On Statistic Parameter	H(3)=3,476 I test (between e-way repeated n ⁽²⁾ Kruskal-W. al test (within g Pairwise com Statistical result	p=0,324 groups com neasures AN allis Test roups comp iparison p-value	(1) parison) IOVA arison) Statistical test	n.a. Effect size (r)	Accepted a-signi 0,008 0.05 Descriptive and groups	ficance pairwise compar comparisons	Sample n = 139, N = 5 independent experiment ison Corrected p-
e short-term depression in	Figure references	1st EPSC quantal content Statistica (¹⁾ On Statistic Parameter	H(3)=3,476 I test (between e-way repeated rn ⁽²⁾ Kruskal-W. al test (within g Pairwise com Statistical result	p=0,324 groups com neasures AN allis Test roups comp pparison p-value	(1) parison) IOVA arison) Statistical test	n.a. Effect size (r)	Accepted α-signi 0,008 0.05 Descriptive and groups eCEP*-0.00 + 0.063	ficance pairwise compar comparisons	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1
ance short-term depression in	Figure references	1st EPSC quantal content Statistica (¹⁾ On Statistic Parameter	H(3)=3,476 I test (between e-way repeated rn ⁽²⁾ Kruskal-W. al test (within g Pairwise com Statistical result	p=0,324 groups com measures AN allis Test roups comp parison p-value	(1) parison) (OVA arison) Statistical test	n.a. Effect size (r)	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN	Sample n = 139, N = 5 independent experiment ison Corrected p-value p=1 p=0,001
enhance short-term depression in	E Figure references	1st EPSC quantal content Statistica (1) On Statistic Parameter PP ratio 1	H(3)=3,476 I test (between e-way repeated r ⁽²⁾ Kruskal-W. al test (within g Pairwise con Statistical result H(3)=19,818	p=0,324 groups com measures AN allis Test roups comp parison p-value p=0,000	(1) parison) (OVA arison) Statistical test	n.a. Effect size (r) 1,681	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,002
o6A enhance short-term depression in	E Figure references	1st EPSC quantal content Statistica (¹) On Statistic Parameter PP ratio 1	H(3)=3,476 I test (between e-way repeated r ⁽²⁾ Kruskal-W. al test (within g Pairwise corr Statistical result H(3)=19,818	p=0,324 groups com neasures AN allis Test rooups comp parison p-value p=0,000	(1) parison) OVA arison) Statistical test	n.a. Effect size (r) 1,681	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,68 ± 0,025	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A WT vs 6A	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,002 p=0,057 n=1 46
oc2b6A enhance short-term depression in	D Eigure references	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1	H(3)=3,476 I test (between e-way repeated rn ⁽²⁾ Kruskal-W. al test (within g Pairwise com Statistical result H(3)=19,818	p=0,324 groups com neasures AN allis Test roups comp parison p-value p=0,000	(1) parison) IOVA arison) Statistical test	n.a. Effect size (r) 1,681	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,68 ± 0,025	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DA wT vs DA WT vs 6A DN vs 6A	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,002 p=0,014 p=1
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DN and Doc2b6A enhance short-term depression in	E Figure references	1st EPSC quantal content Statistica (1) On Statistic Parameter PP ratio 1 PP ratio 2	H(3)=3,476 I test (between e-way repeated r ⁽²⁾ Kruskal-W al test (within g Pairwise con Statistical result H(3)=19,818 H(3)=23,150	p=0,324 groups com neasures AN allis Test rooups comp pparison p-value p=0,000	(1) parison) (OVA arison) Statistical test (2) (2)	n.a. Effect size (r) 1,681	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,68 ± 0,036 WT*: 0,84 ± 0,034 WT*: 0,84 ± 0,036 WT*: 0,84 ± 0,036 WT*: 0,74 ± 0,029 6A*: 0,75 ± 0,028	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A WT vs 6A DN vs 6A eGFP vs 6A eGFP vs 0N eGFP vs 0A WT vs DN	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,002 p=0,146 p=1 p=0,0002 p=0,0002 p=0,0002 p=0,001
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ure 5. Ca^{2k} binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	D securalization of the securation of the secura	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 3 PP ratio 4	H(3)=3,476 I test (between e-way repeated r ⁽²⁾ Kruskal-W. al test (within g Pairwise com Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454	p=0,324 groups com neasures AN allis Test roups comp pparison p-value p=0,000 p=0,000 p=0,000	(1) parison) IOVA arison) Statistical test (2) (2) (1) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,68 ± 0,025 eGFP*: 0,92 ± 0,036 WT*: 0,84 ± 0,034 DN*: 0,75 ± 0,028 eGFP*: 0,93 ± 0,038 WT*: 0,86 ± 0,035 DN*: 0,75 ± 0,026 6A*: 0,74 ± 0,022 eGFP*: 0,89 ± 0,040 WT*: 0,84 ± 0,031 DN*: 0,75 ± 0,026 6A*: 0,77 ± 0,017	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A CGFP vs DN eGFP vs DN	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,002 p=0,146 p=1 p=0,002 p=0,014 p=0,002 p=0,014 p=0,002 p=0,013 p=0,456 p=0,013 p=0,465 p=0,013 p=0,023 p=0,002 p=0,003 p=0,002 p=0,002 p=0,003 p=0,023 p=0,042 p=1
Figure 5. Ca^{2k} binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	D securalization of the secural securation secural secural securation secural securation secural securation se	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 3 PP ratio 4	H(3)=3,476 I test (between e-way repeated r ⁽²⁾ Kruskal-W al test (within g Pairwise con Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454	p=0,324 groups com neasures AN alis Test roups comp parison p-value p=0,000 p=0,000 p=0,000	(1) parison) (OVA arison) Statistical test (2) (2) (1) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,68 ± 0,025 eGFP*: 0,92 ± 0,036 WT*: 0,84 ± 0,034 DN*: 0,74 ± 0,029 6A*: 0,75 ± 0,028 eGFP*: 0,93 ± 0,038 WT*: 0,84 ± 0,035 DN*: 0,75 ± 0,026 6A*: 0,75 ± 0,026 6A*: 0,75 ± 0,026 6A*: 0,77 ± 0,017 eGFP*: 0,89 ± 0,040 WT*: 0,84 ± 0,031 DN*: 0,75 ± 0,026 6A*: 0,77 ± 0,017	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN WT vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs MT eGFP vs DN eGFP vs CA DN vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs CA WT vs DN	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,002 p=0,146 p=1 p=0,002 p=0,014 p=0,002 p=0,014 p=0,002 p=0,013 p=0,013 p=0,013 p=0,013 p=0,003 p=0,003 p=0,003 p=0,002 p=0,003 p=0,0042 p=1 p=1 p=0,042 p=1
Figure 5. Ca^{2k} binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	F F	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 3 PP ratio 4	H(3)=3,476 I test (between e-way repeated r (2) Kruskal-W al test (within g Pairwise con Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454	p=0,324 groups com neasures AN alis Test roups comp parison p-value p=0,000 p=0,000 p=0,000	(1) parison) (OVA arison) Statistical test (2) (2) (1) (2) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735	Accepted α-sign 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,66 ± 0,030 6A*: 0,68 ± 0,025 eGFP*: 0,92 ± 0,036 WT*: 0,84 ± 0,034 DN*: 0,75 ± 0,028 eGFP*: 0,89 ± 0,040 WT*: 0,84 ± 0,031 DN*: 0,75 ± 0,026 6A*: 0,77 ± 0,017 eGFP*: 0,89 ± 0,026 WT*: 0,87 ± 0,026 N*: 0,77 ± 0.027	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN WT vs 6A DN vs 6A WT vs DN WT vs 6A DN vs 6A WT vs DN WT vs 6A DN vs 6A eGFP vs WT eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs A WT vs DN WT vs 6A DN vs 6A DN vs 6A eGFP vs DN eGFP vs DN	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,012 p=0,016 p=1 p=0,016 p=1 p=0,014 p=0,002 p=0,014 p=0,002 p=0,013 p=0,013 p=0,013 p=0,013 p=0,013 p=0,013 p=0,002 p=0,003 p=0,002 p=0,003 p=0,023 p=0,042 p=1 p=0,010 p=0,010
Figure 5. Ca^{24} binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	F F F	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 2 PP ratio 3 PP ratio 4 PP ratio 5	H(3)=3,476 I test (between e-way repeated r (2) Kruskal-W al test (within g Pairwise con Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454 H(3)=22,178	p=0,324 groups com neasures AN allis Test roups comp pparison p=0,000 p=0,000 p=0,000 p=0,000 p=0,000	(1) parison) (OVA arison) Statistical test (2) (2) (1) (2) (2) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735 1,881	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,66 ± 0,030 6A*: 0,68 ± 0,025 eGFP*: 0,92 ± 0,036 WT*: 0,84 ± 0,034 DN*: 0,75 ± 0,028 eGFP*: 0,93 ± 0,038 WT*: 0,84 ± 0,035 DN*: 0,75 ± 0,026 6A*: 0,74 ± 0,022 eGFP*: 0,89 ± 0,040 WT*: 0,89 ± 0,040 WT*: 0,89 ± 0,026 6A*: 0,77 ± 0,017 eGFP*: 0,89 ± 0,026 MT*: 0,87 ± 0,026 6A*: 0,77 ± 0,017	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN WT vs CA DN vs CA DN vs CA EGFP vs WT eGFP vs WT eGFP vs DN eGFP vs DN wT vs CA DN vs CA DN vs CA EGFP vs DN eGFP vs DN wT vs CA DN vs CA eGFP vs DN	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,017 p=0,146 p=1 p=0,002 p=0,014 p=0,002 p=0,014 p=0,002 p=0,013 p=0,0456 p=0,013 p=0,0475 p=0,0475 p=0,003 p=0,002 p=1 p=1 p=0,003 p=0,042 p=1 p=0,042 p=1 p=0,042 p=1 p=0,042 p=1 p=0,010
Figure 5. Ca^{24} binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	F F F	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 3 PP ratio 4 PP ratio 5	H(3)=3,476 I test (between e-way repeated r (2) Kruskal-W al test (within g Pairwise con Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454 H(3)=22,178	p=0,324 groups com neasures AN allis Test roups comp pparison p=0,000 p=0,000 p=0,000 p=0,000 p=0,000	(1) parison) (OVA arison) Statistical test (2) (2) (1) (2) (2) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735 1,881	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,66 ± 0,030 6A*: 0,66 ± 0,030 eGFP*: 0,92 ± 0,036 WT*: 0,84 ± 0,034 DN*: 0,75 ± 0,028 eGFP*: 0,93 ± 0,038 WT*: 0,86 ± 0,035 DN*: 0,75 ± 0,026 6A*: 0,74 ± 0,032 eGFP*: 0,89 ± 0,040 WT*: 0,84 ± 0,031 DN*: 0,75 ± 0,026 6A*: 0,77 ± 0,027 eGFP*: 0,89 ± 0,040 WT*: 0,87 ± 0,026 6A*: 0,77 ± 0,027 eGFP*: 0,89 ± 0,026 WT*: 0,87 ± 0,026 GA*: 0,78 ± 0,015	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A WT vs DN WT vs 6A DN vs 6A eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs A WT vs DN WT vs 6A DN vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs A WT vs DN WT vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs CA WT vs DN WT vs 6A DN vs 6A eGFP vs DN eGFP vs CA WT vs DN WT vs 6A DN vs 6A eGFP vs DN eGFP vs CA WT vs DN WT vs 6A DN vs 6A eGFP vs DN eGFP vs DN	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,001 p=0,002 p=0,057 p=0,146 p=1 p=1 p=0,000 p=0,002 p=0,014 p=0,003 p=0,475 p=0,0475 p=0,0475 p=0,084 p=1 p=1 p=0,003
Figure 5. Ca^{24} binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	D securate and the second seco	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 3 PP ratio 4 PP ratio 5	H(3)=3,476 I test (between ⁽²⁾ Kruskal-W al test (within g Pairwise con Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454 H(3)=22,178	p=0,324 groups com neasures AN allis Test roups comp parison p-value p=0,000 p=0,000 p=0,000 p=0,000	(1) parison) (OVA arison) Statistical test (2) (2) (1) (2) (2) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735 1,881	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,68 ± 0,025 eGFP*: 0,92 ± 0,036 WT*: 0,84 ± 0,034 DN*: 0,74 ± 0,029 6A*: 0,75 ± 0,028 WT*: 0,86 ± 0,035 DN*: 0,75 ± 0,028 eGFP*: 0,89 ± 0,026 6A*: 0,77 ± 0,017 eGFP*: 0,89 ± 0,026 WT*: 0,87 ± 0,026 MT*: 0,77 ± 0,026 GA*: 0,77 ± 0,026 MT*: 0,77 ± 0,026 MT*: 0,77 ± 0,026 MT*: 0,77 ± 0,026 MT*: 0,78 ± 0,026 WT*: 0,87 ± 0,026 WT*: 0,87 ± 0,026 WT*: 0,78 ± 0,015 eGFP*: 0,89 ± 0,026 MT*: 0,78 ± 0	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A mT vs DN wT vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs A mT vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs A mT vs CA DN vs 6A eGFP vs DN wT vs 6A DN vs 6A mT vs DN mT vs 6A DN vs 6A eGFP vs DN eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A eGFP vs DN eGFP vs VT eGFP vs DN eGFP vs VT eGFP vs DN eGFP vs VT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs VT eGFP vs DN eGFP vs VT eGFP vs DN eGFP vs VT eGFP vs DN eGFP vs VT eGFP vs VT e	Sample n = 139, N = 5 independent experiment corrected p- value p=1 p=0,001 p=0,016 p=0,017 p=0,018 p=1 p=0,002 p=0,014 p=0,013 p=1,456 p=0,475 p=0,013 p=0,002 p=0,013 p=0,003 p=0,023 p=0,002 p=1 p=0,002 p=1 p=0,002 p=1,002 p=0,003 p=0,0042 p=1 p=0,003 p=0,002 p=1 p=0,003 p=0,002 p=1 p=0,002 p=1 p=0,002 p=1 p=0,002 p=1 p=0,002 p=1 p=0,002 p=1 <tr< td=""></tr<>
Figure 5. Ca 24 binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	D secure age of the second sec	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 3 PP ratio 4 PP ratio 5	H(3)=3,476 I test (between ⁽²⁾ Kruskal-W, al test (within g Pairwise con Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454 H(3)=22,178	p=0,324 groups com neasures AN allis Test roups comp parison p-value p=0,000 p=0,000 p=0,000 p=0,000	(1) parison) (OVA arison) Statistical test (2) (2) (1) (2) (2) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735 1,881	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,68 ± 0,025 eGFP*: 0,92 ± 0,036 WT*: 0,84 ± 0,034 DN*: 0,74 ± 0,029 6A*: 0,75 ± 0,028 WT*: 0,86 ± 0,035 DN*: 0,75 ± 0,028 6A*: 0,75 ± 0,026 6A*: 0,74 ± 0,031 DN*: 0,75 ± 0,026 6A*: 0,77 ± 0,017 eGFP*: 0,89 ± 0,026 WT*: 0,87 ± 0,026 MT*: 0,78 ± 0,026 MT*: 0,78 ± 0,026 MT*: 0,78 ± 0,015 6A*: 0,77 ± 0,015 6A*: 0,78 ± 0,026 MT*: 0,89 ± 0,026 WT*: 0,89 ± 0,026 WT*: 0,89 ± 0,026 WT*: 0,89 ± 0,025 A*: 0,78 ± 0,025 eGFP*: 0,89 ± 0,029 WT: 0,91 ± 0,025	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs A wT vs DA WT vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A eGFP vs WT eGFP vs DN wT vs 6A DN vs 6A eGFP vs DN wT vs 6A DN vs 6A eGFP vs DN wT vs 6A DN vs 6A eGFP vs DN eGFP vs DN	Sample n = 139, N = 5 independent experiment corrected p- value p=1 p=0,001 p=0,017 p=0,016 p=1 p=0,002 p=0,014 p=0,013 p=0,014 p=0,013 p=0,013 p=0,013 p=0,013 p=0,010 p=0,023 p=0,024 p=1 p=0,003 p=0,002 p=1 p=0,002 p=1 p=0,002 p=1 p=0,002 p=1 p=0,002 p=1
Figure 5. Ca ²⁺ binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	F F F	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 2 PP ratio 3 PP ratio 4 PP ratio 5 PP ratio 6	H(3)=3,476 I test (between e-way repeated r ⁽²⁾ Kruskal-W. al test (within g Pairwise corr Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454 H(3)=22,178 H(3)=32,730	p=0,324 groups com neasures AN neasures AN pp=0,000 p=0,000 p=0,000 p=0,000 p=0,000 p=0,000 p=0,000	(1) parison) (OVA arison) Statistical test (2) (2) (2) (2) (2) (2) (2) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735 1,881 2,776	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,66 ± 0,030 6A*: 0,68 ± 0,025 eGFP*: 0,92 ± 0,036 WT*: 0,84 ± 0,034 DN*: 0,74 ± 0,029 6A*: 0,75 ± 0,028 eGFP*: 0,83 ± 0,038 WT*: 0,86 ± 0,035 DN*: 0,75 ± 0,026 6A*: 0,75 ± 0,026 6A*: 0,75 ± 0,026 6A*: 0,77 ± 0,027 eGFP*: 0,89 ± 0,026 WT*: 0,87 ± 0,026 DN*: 0,77 ± 0,026 CDN*: 0,78 ± 0,025 DN*: 0,78 ± 0,025 DN*: 0,78 ± 0,029 WT*: 0,89 ± 0,029 WT*: 0,89 ± 0,021 0,75 ± 0,025 DN*: 0,78 ± 0,025 DN*: 0,80 ± 0,021	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A DN vs 6A DN vs 6A CFP vs DN eGFP	Sample n = 139, N = 5 independent experiment ison Corrected p- value p=1 p=0,002 p=0,014 p=1 p=0,002 p=0,014 p=1 p=0,002 p=0,013 p=1 p=0,456 p=0,475 p=0,002 p=0,013 p=0,002 p=0,003 p=1 p=0,002 p=0,003 p=1 p=0,002 p=0,003 p=1 p=0,002 p=0,003 p=1 p=0,003 p=1 p=0,003 p=1 p=0,003 p=1 p=0,003 p=1 p=0,003 p=1 p=0,0458 p=0,015
Figure 5. Ca ²⁺ binding site mutants Doc2bDN and Doc2b6A enhance short-term depression in	D secuences F F F F	1st EPSC quantal content Statistica (†) On Statistic Parameter PP ratio 1 PP ratio 2 PP ratio 2 PP ratio 3 PP ratio 4 PP ratio 5 PP ratio 6	H(3)=3,476 I test (between e-way repeated r ⁽²⁾ Kruskal-W. al test (within g Pairwise corr Statistical result H(3)=19,818 H(3)=23,150 F(3,75)=8,465 H(3)=20,454 H(3)=22,178 H(3)=32,730	p=0,324 groups com neasures AN neasures AN p-value p=0,000 p=0,000 p=0,000 p=0,000 p=0,000 p=0,000 p=0,000	(1) parison) (OVA arison) Statistical test (2) (2) (1) (2) (2) (2) (2) (2)	n.a. Effect size (r) 1,681 1,964 0,859 1,735 1,881 2,776	Accepted α-signi 0,008 0.05 Descriptive and groups eGFP*: 0,90 ± 0,063 WT*: 0,78 ± 0,039 DN*: 0,78 ± 0,039 DN*: 0,78 ± 0,039 DN*: 0,78 ± 0,036 wT*: 0,84 ± 0,034 DN*: 0,75 ± 0,028 eGFP*: 0,93 ± 0,038 wT*: 0,84 ± 0,035 DN*: 0,75 ± 0,028 eGFP*: 0,93 ± 0,028 wT*: 0,86 ± 0,029 6A*: 0,75 ± 0,026 GA*: 0,74 ± 0,022 eGFP*: 0,89 ± 0,040 wT*: 0,84 ± 0,031 DN*: 0,75 ± 0,026 GA*: 0,77 ± 0,022 6A*: 0,77 ± 0,026 wT*: 0,89 ± 0,026 wT*: 0,89 ± 0,026 wT*: 0,89 ± 0,027 6A*: 0,78 ± 0,015 eGFP*: 0,89 ± 0,022 oA*: 0,78 ± 0,025 DN*: 0,78 ± 0,025 DN*: 0,80 ± 0,021 6A*: 0,80 ± 0,021	ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs ON eGFP vs ON eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs CA WT vs CA WT vs CA DN vs CA eGFP vs WT eGFP vs DN eGFP vs CA WT vs DN WT vs CA DN vs CA CGFP vs CA WT vs DN WT vs CA DN vs CA EGFP vs DN eGFP vs D	Sample n = 139, N = 5 independent experiment experiment ison Corrected p- value p=1 p=0,002 p=0,146 p=1 p=0,002 p=0,014 p=0,002 p=0,014 p=0,013 p=1 p=0,014 p=0,015 p=0,002 p=0,013 p=0,002 p=1 p=0,002 p=0,003 p=0,003 p=0,003 p=0,003 p=0,005 p=0,015 p=0,015

		Statistica	l test (between g	groups com	parison)		Accepted α-signi	ficance	Sample
	s		⁽¹⁾ Kruskal-W	allis Test			0,0167		
	ence	Statistic	al test (within a	rouns comp	arison)				n = 107, N = 5 independent
	refer	Statistic					0.05		experiment
	nre		Pairwise com	iparison					
	Б.		Statistical		Statistical	Effect size	Descriptive and	pairwise compar	ison
		Parameter	result	p-value	test	(r)	groups	comparisons	Corrected p- value
	E	Cumulative total charge	H(3)=2,380	p=0,497	(1)	n.a.			
							eGFP*: 59,94 ± 5,61	eGFP vs WT	p=0,606
		Cumulative					WT*: 70,91 ± 5,07	eGFP vs DN	p=0,338
	F	normalized	H(3)=27 255	p=0.000	(1)	2 664	DN*: 44,72 ± 2,25	eGFP vs 6A	p=0,015
us		charge		p 0,000		2,001	6A*:39,30 ± 1,85	WT vs DN	p=0,002
nro								WT vs 6A	p=0,000
nei							0GED*: 0.02 ± 0.012 ve	DN VS 6A	p=1
рко							0,17 ± 0,013 0,17 ± 0,013	eGFP vs WT	p=0,013
ns in		Synchronous &					± 0,014 v3 0,24 ± 0,014 DN*: 0.79 ± 0.022 vs 0.21	eGFP vs DN	p=0,391
Ilatio	G	Asynchronous charge	H(3)=10,386	p=0,016	(1)	1,005	± 0,022 6A*: 0.81 + 0.016 vs 0.19 +	eGFP vs 6A	p=1
stimu		proportion					0,016	WT vs DN	p=1
itive								WT vs 6A	p=0,263
repet							oCEP*: 0.52 ± 0.050	DN vs 6A	p=1
<u>Bu</u>							WT* 0.60 + 0.049	AGEP vs DN	p=0,217
nri				p=0,013	(1)		DN*: 0.48 + 0.031	eGFP vs 6A	p=1
pu	н	Recovery 5Hz	H(3)=10,714			1,036	6A* 0 42 + 0 024	WT vs DN	p=0.221
Mo								WT vs 6A	p=0,013
P								DN UN 6A	n-1
5								DN VS 6A	p=1
h ru		Statistica	l test (between g	groups com	parison)		Accepted a-signi	ficance	Sample
ength ru	ses	Statistica	I test (between s	g <mark>roups com</mark> allis Test	parison)		Accepted α-signi 0,0167	ficance	Sample
tic strength ru	iferences	Statistica Statistic	⁽¹⁾ Kruskal-Wa al test (within g	groups com allis Test roups comp	parison) arison)		Accepted α-signi 0,0167 0.05	ficance	Sample n = 101, N = 5 independent experiment
ynaptic strength ru	ure references	Statistica Statistic	l test (between s ⁽¹⁾ Kruskal-Wa al test (within g Pairwise com	groups com allis Test roups comp Iparison	parison) arison)		Accepted α-signi 0,0167 0.05		Sample n = 101, N = 5 independent experiment
ct synaptic strength ru	Figure references	Statistica Statistic	I test (between ((1) Kruskal-W- al test (within g Pairwise com Statistical	groups com allis Test roups comp nparison	parison) arison) Statistical	Effect size	Accepted α-signi 0,0167 0.05 Descriptive and	pairwise compar	Sample n = 101, N = 5 independent experiment
iffect synaptic strength ru	Figure references	Statistica Statistic Parameter	l test (between (⁽¹⁾ Kruskal-Wa al test (within g Pairwise com Statistical result	groups com allis Test roups comp parison p-value	parison) arison) Statistical test	Effect size (r)	Accepted a-signi 0,0167 0.05 Descriptive and groups	pairwise compar comparisons	sample n = 101, N = 5 independent experiment ison Corrected p- value
ts affect synaptic strength ru	Figure references	Statistica Statistic Parameter	l test (between (⁽¹⁾ Kruskal-Wi al test (within g Pairwise com Statistical result H(3)=1.022	groups com allis Test roups comp parison p-value	arison) arison) Statistical test	Effect size (r)	Accepted α-signi 0,0167 0.05 Descriptive and groups	pairwise compar comparisons	sample n = 101, N = 5 independent experiment ison Corrected p- value
tants affect synaptic strength ru	Figure references	Statistica Statistic Parameter Cumulative total charge	I test (between (⁽¹⁾ Kruskal-W- al test (within g Pairwise com Statistical result H(3)=1,082	proups com allis Test roups comp aparison p-value p=0,781	arison) atison) Statistical test	Effect size (r) n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eCEP* 43 34 + 3 69	pairwise compar comparisons	n = 101, N = 5 independent experiment ison Corrected p- value
nutants affect synaptic strength ru	E Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative	I test (between (⁽¹⁾ Kruskal-Wa al test (within g Pairwise com Statistical result H(3)=1,082	groups com allis Test roups comp aparison p-value p=0,781	arison) arison) Statistical test	Effect size (r) n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45.85 ± 3.40	pairwise compar comparisons eGFP vs WT eGFP vs DN	sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0.114
2b mutants affect synaptic strength ru	E Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative normalized	I test (between (⁽¹⁾ Kruskal-Wa al test (within g Pairwise com Statistical result H(3)=1,082	groups com allis Test roups comp aparison p-value p=0,781	arison) arison) Statistical test (1)	Effect size (r) n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75	pairwise compar comparisons eGFP vs WT eGFP vs 6A	p=-1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024
oc2b mutants affect synaptic strength ru	Z Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative normalized charge	i test (between (⁽¹⁾ Kruskal-W; al test (within g) Pairwise com Statistical result H(3)=1,082 H(3)=19,425	proups com allis Test roups comp aparison p-value p=0,781 p=0,000	arison) arison) Statistical test (1) (1)	Effect size (r) n.a. 1,933	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09	DIVVS 6A ficance comparisons eGFP vs WT eGFP vs DN eGFP vs DN	p=-1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,014 p=0,024 p=0,010
i. Doc2b mutants affect synaptic strength ru	Z Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative normalized charge	i test (between (⁽¹⁾ Kruskal-W: al test (within g Pairwise com Statistical result H(3)=1,082 H(3)=19,425	proups com allis Test roups comp parison p-value p=0,781 p=0,000	arison) arison) Statistical test (1) (1)	Effect size (r) n.a. 1,933	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09	DIVVS 6A	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,010 p=0,010 p=0,011
e 6. Doc2b mutants affect synaptic strength ru	Z Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative normalized charge	I test (between (⁽¹⁾ Kruskal-W: al test (within g Pairwise com Statistical result H(3)=19,425	proups com allis Test roups comp parison p-value p=0,781 p=0,000	arison) arison) Statistical test (1) (1)	Effect size (r) n.a. 1,933	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09	DIVVS 6A ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A WT vs 6A DN vs 6A	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,014 p=0,001 p=1
Figure 6. Doc2b mutants affect synaptic strength ru	Z Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous charge proportion	i test (between (⁽¹⁾ Kruskal-W: al test (within g Pairwise com Statistical result H(3)=1,082 H(3)=19,425 H(3)=7,705	proups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,053	arison) arison) Statistical test (1) (1)	Effect size (r) n.a. 1,933 n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09	pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs DA WT vs 6A DN vs 6A	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p-value p=1 p=0,114 p=0,010 p=0,011 p=1
Figure 6. Doc2b mutants affect synaptic strength ru	Z Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous charge proportion	I test (between ((1) Kruskal-W: al test (within g Pairwise com Statistical result H(3)=19,425 H(3)=7,705	proups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,053	arison) arison) Statistical test (1) (1) (1)	Effect size (r) n.a. 1,933 n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064	DIVVS 6A ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A WT vs 6A DN vs 6A eGFP vs WT	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,010 p=0,010 p=0,011 p=0,011 p=0,011 p=0,011
Figure 6. Doc2b mutants affect synaptic strength ru	A Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous charge proportion	I test (between ((1) Kruskal-W: al test (within g Pairwise com Statistical result H(3)=19,425 H(3)=7,705	proups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,053	arison) arison) Statistical test (1) (1) (1)	Effect size (r) n.a. 1,933 n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT*: 0,99 ± 0,076	DIVVS 6A ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A WT vs 6A DN vs 6A eGFP vs WT eGFP vs WT	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024 p=0,001 p=1 p=0,011 p=1
Figure 6. Doc2b mutants affect synaptic strength ru	Elgure references	Statistica Statistic Parameter Cumulative total charge Cumulative charge Synchronous & Asynchronous charge proportion Recovery 40Hz	i test (between ((1) Kruskal-W/ al test (within g) Pairwise corr Statistical result H(3)=1,082 H(3)=7,705 H(3)=16,682	groups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,053	arison) arison) Statistical test (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT*: 0,99 ± 0,076 DN*: 0,076 ± 0,061	DIVVS GA ficance pairwise compar comparisons eGFP vs DN eGFP vs DN WT vs GA DN vs GA DN vs GA eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024 p=0,001 p=1 p=0,011 p=0,011 p=0,012 p=0,013 p=0,738 p=0,738
Figure 6. Doc2b mutants affect synaptic strength ru	Eigure references	Statistica Statistic Parameter Cumulative total charge Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous & Asynchronous charge proportion Recovery 40Hz	i test (between ((1) Kruskal-W; ial test (within g) Pairwise corr Statistical result H(3)=1,082 H(3)=19,425 H(3)=7,705 H(3)=16,682	groups com allis Test roups comp parison p-value p=0,781 p=0,000 p=0,053	arison) arison) Statistical test (1) (1) (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT*: 0,99 ± 0,076 DN*: 0,99 ± 0,076	DIVVS 6A ficance comparisons eGFP vs DN eGFP vs DN WT vs 6A DN vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs A	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024 p=0,010 p=1,014 p=0,011 p=1,014 p=0,013 p=1,0181 p=1,0738 p=0,025
Figure 6. Doc2b mutants affect synaptic strength ru	P. Figure references	Statistica Statistic Parameter Cumulative total charge Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous & Asynchronous charge proportion Recovery 40Hz	i test (between ((1) Kruskal-W: ial test (within g Pairwise com Statistical result H(3)=1,082 H(3)=19,425 H(3)=7,705 H(3)=16,682	groups com allis Test roups comp parison p-value p=0,781 p=0,000 p=0,053	arison) arison) Statistical test (1) (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT*: 0,99 ± 0,076 DN*: 0,076 ± 0,061 6A*: 0,68 ± 0,030	DIVVS 6A ficance pairwise compar comparisons eGFP vs WT eGFP vs 6A WT vs 6A DN vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs DN eGFP vs DN wT vs 6A DN vs 6A DN vs 6A	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024 p=0,010 p=0,011 p=1 p=0,011 p=0,012 p=0,013 p=0,014 p=0,015 p=0,025 p=0,021 n=1
Figure 6. Doc2b mutants affect synaptic strength ru	P Elgure references	Statistica Statistic Parameter Cumulative total charge Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous & Asynchronous charge proportion Recovery 40Hz	I test (between ((1) Kruskal-W: al test (within g Pairwise com Statistical result H(3)=19,425 H(3)=7,705 H(3)=16,682 H(3)=1073	proups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,0053 p=0,001	arison) arison) Statistical test (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT*: 0,99 ± 0,076 DN*: 0,076 ± 0,061 6A*: 0,68 ± 0,030	DIVVS 6A ficance pairwise compar comparisons eGFP vs WT eGFP vs 6A WT vs DN WT vs 6A DN vs 6A eGFP vs WT eGFP vs DN eGFP vs DN wT vs DN wT vs 6A DN vs 6A DN vs 6A	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,010 p=0,010 p=0,011 p=0,012 p=1 p=0,738 p=0,025 p=0,001 p=1
Figure 6. Doc2b mutants affect synaptic strength ru	P Eigure references	Statistica Statistic Parameter Cumulative total charge Cumulative charge Synchronous & Asynchronous & Asynchronous charge proportion Recovery 40Hz RRP size (pC)	i test (between ((1) Kruskal-W/ al test (within g Pairwise corr Statistical result H(3)=1,082 H(3)=19,425 H(3)=7,705 H(3)=16,682 H(3)=1,073	proups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,001 p=0,784	arison) arison) Statistical test (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660 n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT*: 0,99 ± 0,076 DN*: 0,976 ± 0,061 6A*: 0,68 ± 0,030 eGFP*: 0,349 ± 0.032	DIVVS 6A ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A DN vs 6A DN vs 6A WT vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs A WT vs DA WT vs CA WT vs CA WT vs CA	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024 p=0,001 p=1 p=0,181 p=1,0,738 p=0,025 p=0,011 p=1
Figure 6. Doc2b mutants affect synaptic strength ru	Eigure references	Statistica Statistic Parameter Cumulative total charge Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous charge proportion Recovery 40Hz RRP size (pC)	i test (between ((1) Kruskal-W: al test (within g) Pairwise corr Statistical result H(3)=1,082 H(3)=19,425 H(3)=7,705 H(3)=16,682 H(3)=1,073	groups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,001 p=0,001 p=0,784	arison) arison) Statistical test (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660 n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT: 0,99 ± 0,076 DN*: 0,076 ± 0,061 6A*: 0,68 ± 0,030 eGFP*: 0,349 ± 0,032 WT: 0,430 ± 0,050	DIVVS 6A ficance pairwise compar comparisons eGFP vs DN eGFP vs DN WT vs 6A DN vs 6A DN vs 6A WT vs DN wT vs 6A eGFP vs WT eGFP vs WT eGFP vs WT eGFP vs WT eGFP vs WT	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024 p=0,010 p=1,011 p=0,011 p=0,011 p=0,011 p=1 p=0,011 p=1 p=0,011 p=1 p=0,025 p=0,001 p=1 p=0,0348
Figure 6. Doc2b mutants affect synaptic strength ru	P Eigure references	Statistica Statistic Parameter Cumulative total charge Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous & Asynchronous charge proportion Recovery 40Hz RRP size (pC)	i test (between ((1) Kruskal-W: ial test (within g) Pairwise corr Statistical result H(3)=1,082 H(3)=19,425 H(3)=7,705 H(3)=16,682 H(3)=10,73 H(3)=10,73	groups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,001 p=0,784	parison) arison) Statistical test (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660 n.a.	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,31 ± 0,064 WT*: 0,99 ± 0,076 DN*: 0,076 ± 0,061 6A*: 0,68 ± 0,030 eGFP*: 0,349 ± 0,032 WT*: 0,430 ± 0,050 DN*: 0,472 ± 0,051	DIN VS 6A ficance pairwise compar comparisons eGFP vs DN eGFP vs DN eGFP vs 6A DN vs 6A DN vs 6A eGFP vs DN eGFP vs 6A WT vs DN WT vs 6A DN vs 6A DN vs 6A	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024 p=0,010 p=1,014 p=0,011 p=0,011 p=0,012 p=1 p=0,013 p=1 p=0,025 p=0,025 p=1,0348 p=0,348 p=0,010
Figure 6. Doc2b mutants affect synaptic strength ru	R Sigure references	Statistica Statistic Parameter Cumulative total charge Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous & Asynchronous charge proportion Recovery 40Hz RRP size (pC) Release probability	I test (between ((1) Kruskal-W. al test (within g Pairwise com Statistical result H(3)=1,082 H(3)=19,425 H(3)=7,705 H(3)=16,682 H(3)=16,682 H(3)=11,073 H(3)=11,453	proups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,053 p=0,001 p=0,784 p=0,010	arison) arison) Statistical test (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660 n.a. 1,150	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT*: 0,99 ± 0,076 DN*: 0,076 ± 0,061 6A*: 0,68 ± 0,030 eGFP*: 0,349 ± 0,032 WT*: 0,430 ± 0,050 DN*: 0,072 ± 0,051 6A*: 0,560 ± 0,051	DIVVS 6A ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A WT vs DN WT vs 6A DN vs 6A DN vs 6A WT vs DN WT vs DN WT vs A DN vs 6A DN vs 6A WT vs DN WT vs A DN vs 6A WT vs DN WT vs A DN vs 6A WT vs DN WT vs A DN vs 6A DN vs 6A WT vs DN WT vs A DN vs 6A DN vs 6A WT vs DN eGFP vs DN eGFP vs DN eGFP vs DN eGFP vs DN	p=1 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,024 p=0,010 p=0,011 p=1 p=0,012 p=1 p=0,025 p=0,025 p=0,025 p=0,348 p=0,010 p=1
Figure 6. Doc2b mutants affect synaptic strength ru	P R S	Statistica Statistic Parameter Cumulative total charge Cumulative total charge Cumulative normalized charge Synchronous & Asynchronous & Asynchronous charge proportion Recovery 40Hz RRP size (pC) Release probability	I test (between ((1) Kruskal-W: al test (within g Pairwise com Statistical result H(3)=1,082 H(3)=19,425 H(3)=7,705 H(3)=7,705 H(3)=16,682 H(3)=10,73 H(3)=11,453	proups comp allis Test roups comp parison p-value p=0,781 p=0,000 p=0,053 p=0,001 p=0,784 p=0,010	arison) arison) Statistical test (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Effect size (r) n.a. 1,933 n.a. 1,660 n.a. 1,150	Accepted α-signi 0,0167 0.05 Descriptive and groups eGFP*: 43,34 ± 3,69 WT*: 45,85 ± 3,40 DN*: 33,88 ± 4,75 6A*: 29,20 ± 3,09 eGFP*: 0,81 ± 0,064 WT*: 0,99 ± 0,076 DN*: 0,076 ± 0,061 6A*: 0,68 ± 0,030 eGFP*: 0,349 ± 0,032 WT*: 0,430 ± 0,050 DN*: 0,472 ± 0,051	DIVVS 6A ficance pairwise compar comparisons eGFP vs WT eGFP vs DN eGFP vs 6A WT vs DN WT vs 6A DN vs 6A eGFP vs WT eGFP vs DN WT vs 6A DN vs 6A WT vs DN WT vs 6A DN vs 6A eGFP vs WT eGFP vs WT eGFP vs DN WT vs 6A DN vs 6A DN vs 6A eGFP vs DN WT vs 6A DN vs 6A DN vs 6A DN vs 6A EGFP vs DN WT vs 6A DN vs 6A DN vs 6A EGFP vs DN WT vs 6A EGFP vs DN WT vs 6A EGFP vs DN WT vs 6A DN vs 6A EGFP vs DN WT vs 6A EGFP vs DN EGFP vs CA EGFP vs DN EGFP vs CA EGFP vs CA EGF	p=11 Sample n = 101, N = 5 independent experiment ison Corrected p- value p=1 p=0,114 p=0,010 p=0,010 p=0,011 p=1 p=0,011 p=1,001 p=1 p=0,181 p=1 p=0,001 p=1 p=0,025 p=0,001 p=1 p=0,348 p=0,010 p=1 p=0,010 p=1

r, ct ype		Statistica	il test (between g	groups com	Accepted α-significance		Sample		
c 2b W ot affe n wildt	Ges		⁽²⁾ Kruskal-Wa	allis Test	0,01		- 460 N - 2		
of Dou bes no	eferen	Statistic	al test (within gr	roups comp	arison)		0.05		n = 168, N = 3 independent
sion 6A do 8 num ns	gure r		Pairwise com	parison	expe		experiment		
res 2b pse	Ĕ	ц.	Statistical		Statistical	Effect size	Descriptive and	pairwise compar	ison
erexpi nd Doc r synaj nei		Parameter	result	p-value	test	(r)	groups	comparisons	Corrected p- value
<u>o</u> a O	В	Synapse number	H(3)=3,452	p=0,327	(2)	n.a.			
.7 ° DN (go)	В	Dendritic length	F(3,117)=0,465	p=0,707	(1)	n.a.			
hol 2 h	В	Synapses/µm	H(3)=5,247	p=0,155	(2)	n.a.			
Po Do	В	Synapse area	H(3)=4,883	p=0,181	(2)	n.a.			
Ĕ	В	Soma area	H(3)=0,476	p=0,924	(2)	n.a.			

Note Note <th< th=""><th></th><th></th><th>Statistica</th><th>il test (between g</th><th>groups com</th><th colspan="2">Accepted α-significance</th><th>Sample</th></th<>			Statistica	il test (between g	groups com	Accepted α-significance		Sample		
Non- Statistical test (within groups comparison) 0.05 Independent experiment comparison B Parameter Statistical result p-value p-value Statistical test Effect size (r) Descriptive and pairwise comparison Corrected value B mEPSC frequency H(3)=73,308 p=0,000 (1) 5,863 GFP*: 4,33 ± 0,544 eGFP vs MT p=1 C mEPSC frequency H(3)=73,308 p=0,000 (1) 5,863 GFP*: 4,33 ± 0,544 eGFP vs MT p=1 D mEPSC frequency H(3)=73,308 p=0,000 (1) n.a. GFP*: 4,33 ± 0,544 eGFP vs MT p=1 D mEPSC aregolitude H(3)=73,308 p=0,000 (1) n.a. MT vs NA p=0,000 D mEPSC aregolitude H(3)=2,639 p=0,040 (1) n.a. n=218, N = 6 Statistical test (within groups comparison) 0.05 Corrected p- value 0.05 corrected p- value Parameter Statistical resuit p-value Statis	pal	se		⁽¹⁾ Kruskal-W	allis Test			0.01		n - 183 N - 4
Verticity Pairwise comparison 0.05 experiment Parameter Statistical result p-value Statistical test Effect size Descriptive and pairwise comparison Corrected p- value B mEPSC frequency H(3)=73,308 p=0,000 (1) 5,863 Descriptive and pairwise comparison Corrected p- value	am	enc	Statistic	al test (within g	roups comp				independent	
And by the second of	bod	efer		Pairwise com	parison		0.05	experiment		
Best Parameter Statistical result p-value Statistical test Effect size (r) Groups comparisons Corrected p- value B mEPSC frequency H(3)=73,308 p=0,000 (°) 5,863 eGFP*:4,33±0,544 eGFP*:8 AT p=1. C mEPSC frequency H(3)=73,308 p=0,000 (°) n.a. eGFP*:4,33±0,544 eGFP*:8 AA p=0,000 C mEPSC frequency H(3)=73,308 p=0,000 (°) n.a. eGFP*:4,33±0,544 eGFP*:8 AA p=0,000 C mEPSC frequency H(3)=73,308 p=0,000 (°) n.a. eGFP*:4,032 wT vs 6A p=0,000 C mEPSC frequency H(3)=73,908 p=0,840 (°) n.a. eGFP*:0,62±1,033 eGFP*:0,62±1,033 eGFP*:0,62±1,033 eGFP*:0,62±1,033 eGFP* vs DN p=0,400 G Groups fististical test (within groups comparison) 0.05 independent experiment Groups Fainteer Statistical test (within groups comparison) 0.05 Groups Groups Gro	hip	Figure r					Descriptive and	pairwise compar	ison	
B mEPSC frequency H(3)=73,308 p=0,000 (1) 5,863 eGFP*x33±0.544 eGFP vs BA p=0,000 C mEPSC area H(3)=73,308 p=0,000 (1) 5,863 eGFP vs CA p=0,000 C mEPSC area H(3)=1.851 p=0.604 (1) n.a. - - D mEPSC frequency H(3)=1.937 p=0.604 (1) n.a. -	n wildtype		Parameter	Statistical result	p-value	Statistical test	Effect size (r)	groups	comparisons	Corrected p- value
B mEPSC frequency H(3)=73,308 p=0,000 (1) 5,863 WT: 4,64 ± 0.628 eGFP vs DA p=0,000 DN*: 15,67 ± 1,830 eGFP vs CA p=0,000 DN*: 15,67 ± 1,830 eGFP vs CA p=0,000 C mEPSC frequency H(3)=1,851 p=0,604 (1) n.a. DN vs 6A p=0,000 E mEPSC frequency H(3)=2,678 p=0,644 (1) n.a. DN vs 6A p=1 F mEPSC frequency H(3)=0,839 p=0,840 (1) n.a. DN vs 6A p=1 F mEPSC frequency H(3)=0,839 p=0,840 (1) n.a. DN vs 6A p=1 F mEPSC frequency H(3)=0,839 p=0,840 (1) n.a. D.05 n=218, N = 6 Independent est (within groups comparison) 0.05 Descriptive and pairwise comparison User frequency n=218, N = 6 independent experiment B M mEPSC frequency H(3)=104,468 p=0,000 (1) 7,075 GGFP vs DN p=1 <	ein							eGFP*: 4,33 ± 0,544	eGFP vs WT	p=1
B mEPSC frequency H(3)=73,308 p=0,000 (1) 5,863 DN*: 15,67 ± 1,830 eGFP vs 6A p=-0,000 C mEPSC amplitude H(3)=73,308 p=0,000 (1) n.a. mEV WT vs DN p=0,000 D mEPSC amplitude H(3)=7,873 p=0,604 (1) n.a. mEV wT vs 6A p=0,000 D mEPSC decay H(3)=7,937 p=0,604 (1) n.a. mEV mEV <th>as</th> <td></td> <td></td> <td></td> <td></td> <td></td> <td> </td> <td>WT*: 4,64 ± 0,628</td> <td>eGFP vs DN</td> <td>p=0,000</td>	as							WT*: 4,64 ± 0,628	eGFP vs DN	p=0,000
Bit Incl Sc inequency In(c)=13,300 p=0,000 Incl Sc inequency p=0,000 WT vs 6A p=0,000 C mEPSC area H(3)=1,851 p=0,604 (0) n.a. p=1 D mEPSC area H(3)=2,678 p=0,604 (0) n.a. p=1 D mEPSC area H(3)=2,678 p=0,644 (0) n.a. p=1 D mEPSC area H(3)=1,937 p=0,644 (0) n.a. n=218, N = 6 n=218, N = 6 n=218, N = 6 independent experiment No 5A p=1 No 5A p=1 No 5A p=1 No 4A p=1 No 4A p=1 No 4A p=1 No 5A p=0,000 No 5A p=1 No 4A p=1 No 4A p=1 <td< td=""><th>ele</th><td>в</td><td>mEPSC frequency</td><td>H(3)-73 309</td><td>n=0.000</td><td>(1)</td><td>5.963</td><td>DN*: 15,67 ± 1,830</td><td>eGFP vs 6A</td><td>p=0,000</td></td<>	ele	в	mEPSC frequency	H(3)-73 309	n=0.000	(1)	5.963	DN*: 15,67 ± 1,830	eGFP vs 6A	p=0,000
Perform WT vs 6A p=0,000 D mEPSC amplitude H(3)=1,851 p=0,604 (*) n.a. D mEPSC free H(3)=1,851 p=0,604 (*) n.a. F mEPSC free H(3)=1,937 p=0,586 (*) n.a. F mEPSC free H(3)=0,839 p=0,840 (*) n.a. F mEPSC decay H(3)=0,839 p=0,840 (*) n.a. F mEPSC decay H(3)=0,839 p=0,840 (*) n.a. F mEPSC decay H(3)=0,839 p=0,840 (*) n.a. F mEPSC frequency H(3)=104,468 p-value Statistical test (r) n.a. Parameter Statistical result p-value Statistical test (r) Bescriptive and pairwise comparison value H mEPSC frequency H(3)=104,468 p=0,000 (*) 7,075 Descriptive and pairwise comparison Value I mEPSC frequency H(3)=104,468 p=0,020	IS I	Ŭ	mer so nequency	11(3)=73,300	p=0,000		3,005	6A*: 16,51 ± 1,842	WT vs DN	p=0,000
Verticity Mathematical mEPSC area (1) M(3)=1,851 (2) p=0,604 (3)=1,837 (2) (1) (1) n.a. Dives 6A p=1 C mEPSC area (1) mEPSC area (1) H(3)=1,851 (2) p=0,604 (1) (1) n.a.	106								WT vs 6A	p=0,000
C mEPSC amplitude D H(3)=2,678 mEPSC rise H(3)=2,678 F p=0,604 p=0,404 (i) n.a. n.a. E mEPSC rise mEPSC decay H(3)=0,839 F p=0,586 (i) n.a. n.a. n.a. F mEPSC decay H(3)=0,839 F p=0,640 (ii) n.a. n.a. n.a. F mEPSC decay H(3)=0,839 F p=0,840 (ii) n.a. n.a. n.a. F mEPSC decay H(3)=0,839 F p=0,840 (ii) n.a. n.a. n.a. Statistical test (within groups comparison) 0.01 n.a. n.a. n.a. Parameter Statistical result p-value Statistical test Effect size (r) Descriptive and pairwise comparison Corrected p- value H mEPSC frequency H H(3)=104,468 p=0,000 (ii) 7,075 Descriptive and pairwise comparison DN*: 31,90 ± 1,736 Corrected p- value I mEPSC area H(3)=4,737 p=0,492 (ii) n.a. NT*: 9,71 ± 1,171 eGFP vs DN p=0,000 I mEPSC area H(3)=4,373 p=0,224 iii n.a. NT vs 6A p=0,	ano								DN vs 6A	p=1
O mEPSC area mEPSC frequency H(3)=104,78 H(3)=0,803 p=0,444 (i) n.a. F mEPSC decay H(3)=0,803 p=0,840 (ii) n.a. F mEPSC decay H(3)=0,803 p=0,840 (ii) n.a. F mEPSC decay H(3)=0,803 p=0,840 (iii) n.a. F mEPSC decay H(3)=0,833 p=0,840 (iii) n.a. F mEPSC decay H(3)=0,833 p=0,840 (iii) n.a. F Statistical test (within groups comparison) 0.01 n = 218, N = 6 I Pairwise comparison 0.05 Corrected p-value Pairwise comparison Descriptive and pairwise comparison Corrected p-value P Pairwise comparison I Descriptive and pairwise comparison Corrected p-value P Pairwise comparison I I P=0,000 (i) T,075 Effect size Groups Comparisons Corrected p-value P MEPSC frequency H(3)=104,468 p=0,000	but	С	mEPSC amplitude	H(3)=1,851	p=0,604	(1)	n.a.			
E mEPSC rise H(3)=1,937 p=0,586 (i) n.a. F mEPSC decay H(3)=0,839 p=0,840 (ii) n.a. Statistical test (between groups comparison) Accepted α-significance Sample Image: Statistical test (within groups comparison) 0.01 n = 218, N = 6 Image: Statistical test (within groups comparison) 0.05 n = 218, N = 6 Image: Statistical test (within groups comparison) 0.05 Corrected p-value Parameter Statistical result p-value Statistical test Effect size Parameter Statistical result p-value Statistical test p-value Statistical test Provide (r) Descriptive and pairwise comparison H mEPSC frequency H(3)=104,468 p=0,000 (i) 7,075 GeGPP': 10,62 ± 1,093 eGFP vs GA p=0,000 Image: Statistical test p=0,000 (ii) 7,075 GeGPP vs GA p=0,000 Image: Statistical test p=0,000 (iii) 7,075 GeGPP vs GA p=0,000 Image: Statistical test	sbc	D	mEPSC area	H(3)=2,678	p=0,444	(1)	n.a.			
Note F mEPSC decay H(3)=0,839 p=0,840 (*) n.a. Statistical test (between groups comparison) Accepted α-significance Sample (*) n.a. 0.01 n = 218, N = 6 independent experiment Big Statistical test (within groups comparison) 0.05 n = 218, N = 6 independent experiment Parameter Statistical result p-value Statistical test Effect size (r) Descriptive and pairwise comparison Corrected p- value H mEPSC frequency H(3)=104,468 p=0,000 (*) 7,075 GeGFP*: 10,62 ± 1,093 eGFP vs MT p=1 WT*: 9,71 ± 1,171 eGFP vs DN p=0,000 (*) 7,075 MT*: 9,71 ± 1,171 eGFP vs ON p=0,000 I mEPSC frequency H(3)=104,468 p=0,000 (*) n.a. MT*: 9,71 ± 1,736 eGFP vs ON p=0,000 I mEPSC area H(3)=2,407 p=0,492 (*) n.a. MT*: 5,62 ± 0,137 eGFP vs NT p=0,238 L mEPSC decay Time H(3)=18,609 p=0,000 (of	E	mEPSC rise	H(3)=1,937	p=0,586	(1)	n.a.			
Statistical test (between groups comparison) Accepted α-significance Sample 1 REPSC frequency H(3)=104,468 p=0,000 (1) n=218, N=6 independent experiment 1 mEPSC area H(3)=2,407 p=0.492 (1) n.a. NT vs 6A p=0,000 1 mEPSC rise time H(3)=9,060 p=0,029 (1) n.a. NT vs 6A p=0,000 1 mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 MT vs 6A p=0,327 0.61 mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 MT vs 6A p=0,000	cy	F	mEPSC decay	H(3)=0,839	p=0,840	(1)	n.a.			
Open parameter Statistical result p-value Statistical test effect size (r) Descriptive and pairwise comparison n = 218, N = 6 independent experiment Parameter Statistical result p-value Statistical test Effect size (r) Descriptive and pairwise comparison Corrected p- value H mEPSC frequency H(3)=104,468 p=0,000 (1) 7,075 Effect size (r) eGFP*: 10,62 ± 1,093 eGFP vs DN p=0,000 DN*: 31,90 ± 1,736 eGFP vs DN p=0,000 (1) 7,075 Effect size (r) WT*: 9,71 ± 1,171 eGFP vs DN p=0,000 DN*: 31,90 ± 1,736 eGFP vs DN p=0,000 (1) n.a. WT*: 9,71 ± 1,171 eGFP vs A p=0,000 DN*: 31,90 ± 1,736 eGFP vs A p=0,000 (1) n.a. WT*: 9,71 ± 1,171 eGFP vs A p=0,000 DN*: 31,90 ± 1,736 eGFP vs A p=0,000 (1) n.a. WT vs 6A p=0,000 I mEPSC areaa H(3)=2,407 p=0,492 (1) n.a. UT vs 6A p=0,000 L <	nen Is	rences	Statistica	il test (between g	groups com	Accepted α-significance		Sample		
Be objective and pairwise comparison) independent experiment Pairwise comparison) independent experiment Pairwise comparison) Descriptive and pairwise comparison) Pairwise comparison) Pairwise comparison) Pairwise comparison) Pairwise comparison Pairwise comparison Pairwise comparison Parameter Statistical test (within groups comparison) Pairwise comparison Pairwise comparison Parameter Statistical test (within groups comparison) Pairwise comparison Pairwise comparison Pairwise comparison Parameter Statistical test (within groups comparison) Pairwise comparison	freq			⁽¹⁾ Kruskal-Wa	allis Test	0.01		n = 218. N = 6		
Pairwise comparison 0.05 experiment Parameter Statistical result p-value Statistical test Effect size (r) Descriptive and pairwise comparison Corrected p- value H mEPSC frequency H(3)=104,468 p=0,000 (1) 7,075 GGPP*: 10,62 ± 1,093 eGFP vs WT p=1 WT*: 9,71 ± 1,171 eGFP vs DN p=0,000 (1) 7,075 DN*: 31,90 ± 1,736 eGFP vs GA p=0,000 I mEPSC amplitude H(3)=2,407 p=0,492 (1) n.a. Interpendent	he		Statistic	al test (within g	roups comp	0.05	independent			
Parameter Statistical result p-value Statistical test Effect size (r) Descriptive and pairwise comparison Corrected p- value H mEPSC frequency H(3)=104,468 p=0,000 (1) 7,075 Effect size (r) eGFP*: 10,62 ± 1.093 eGFP vs WT p=1 WT*: 9,71 ± 1,171 eGFP vs DN p=0,000 (1) 7,075 DN*: 31,90 ± 1,736 eGFP vs AA p=0,000 I mEPSC amplitude H(3)=2,407 p=0,492 (1) n.a. DN vs 6A p=1 J mEPSC rise time H(3)=2,407 p=0,492 (1) n.a. Effect size Effect size <t< td=""><th>cet</th><td>refe</td><td></td><td>Pairwise com</td><td>parison</td><td>U.U5</td><td>experiment</td></t<>	cet	refe		Pairwise com	parison	U.U5	experiment			
B D Parameter Statistical result p-value Statistical test Effect size (r) groups comparisons Corrected p-value 4000 H mEPSC frequency H(3)=104,468 p=0,000 (1) 7,075 eGFP*: 10,62 ± 1,093 eGFP vs WT p=1 WT*: 9,71 ± 1,171 eGFP vs DN p=0,000 (1) 7,075 DN*: 31,90 ± 1,736 eGFP vs AA p=0,000 WT vs 3,00 ± 2,300 WT vs DN p=0,000 (1) n.a. DN vs 6A p=1 J mEPSC area H(3)=2,407 p=0,492 (1) n.a. UVT vs 6A p=0,000 K mEPSC rise time H(3)=9,060 p=0,224 (1) n.a. L mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 eGFP*: 6,07 ± 0,127 eGFP vs 6A p=0,237 GC A*: 6,64 ± 0,222 WT vs DN p=0,012 WT vs 6A p=0,000 (1) 1,260 GGFP vs 6A p=0,000 GGFP vs 6A p=0,000 (1) 1,2	han	e,					Descriptive and	ison		
H mEPSC frequency H(3)=104,468 p=0,000 (1) 7,075 eGFP*: 10,62 ± 1,093 eGFP vs DN p=1 WT*: 9,71 ± 1,171 eGFP vs DN p=0,000 (1) 7,075 EGFP*: 10,62 ± 1,093 eGFP vs DN p=0,000 DN*: 31,90 ± 1,736 eGFP vs DA p=0,000 DN*: 31,90 ± 1,736 eGFP vs 6A p=0,000 DN*: 31,90 ± 1,736 eGFP vs 6A p=0,000 ON vs 6A p=0,000 J mEPSC area H(3)=4,373 p=0,224 (1) n.a. K mEPSC rise time H(3)=9,060 p=0,029 (1) 0,614 K mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 EGFP*: 6,07 ± 0,127 eGFP vs 6A p=0,237 0N*: 6,36 ± 0,185 eGFP vs 6A p=0,000 (1) 1,260 EGFP*: 6,64 ± 0,222 WT vs DA p=0,0012	tions en	Figu	Parameter	Statistical result	p-value	Statistical test	Effect size (r)	groups	comparisons	Corrected p- value
H mEPSC frequency H(3)=104,468 p=0,000 (1) 7,075 WT*: 9,71 ± 1,171 eGFP vs DN p=0,000 DN*: 31,90 ± 1,736 eGFP vs 6A p=0,000 DN*: 31,90 ± 1,736 eGFP vs 6A p=0,000 6A*: 30,06 ± 2,300 WT vs DN p=0,000 MT vs 6A p=0,000 1 mEPSC amplitude H(3)=2,407 p=0,492 (1) n.a. J mEPSC rise time H(3)=9,060 p=0,029 (1) n.a. K mEPSC rise time H(3)=9,060 p=0,029 (1) 0,614 L mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 WT*: 5,62 ± 0,137 eGFP vs 6A p=0,237 ON*: 6,36 ± 0,185 eGFP vs 6A p=0,021 DN*: 6,36 ± 0,122 WT vs DN p=0,022	nte							eGFP*: 10,62 ± 1,093	eGFP vs WT	p=1
H mEPSC frequency H(3)=104,468 p=0,000 (1) 7,075 DN*: 31,90 ± 1,736 eGFP vs 6A p=0,000 0	E						[WT*: 9,71 ± 1,171	eGFP vs DN	p=0,000
Image: Solution Image: Sol	p.	н	mEPSC frequency	H(3)=104 468	n=0.000	(1)	7 075	DN*: 31,90 ± 1,736	eGFP vs 6A	p=0,000
Matrix Matrix<	002		mer do nequency	11(3)=104,400	p 0,000		1,010	6A*: 30,06 ± 2,300	WT vs DN	p=0,000
Beg Image: metric constraints metric constrai	ă								WT vs 6A	p=0,000
I mEPSC amplitude H(3)=2,407 p=0,492 (1) n.a. J mEPSC area H(3)=4,373 p=0,224 (1) n.a. K mEPSC rise time H(3)=9,060 p=0,029 (1) 0,614 L mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 eGFP*: 6,07 ± 0,127 eGFP vs DN p=1 DN*: 6,36 ± 0,185 eGFP vs GA p=0,027 (1) 1,260 WT*: 5,62 ± 0,137 eGFP vs GA p=0,227 GA*: 6,64 ± 0,222 WT vs DN p=0,000 (1) 1,260 WT vs GA p=0,000	pu								DN vs 6A	p=1
J mEPSC area H(3)=4,373 p=0,224 (1) n.a. K mEPSC rise time H(3)=9,060 p=0,029 (1) 0,614 L mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 eGFP*: 6,07 ± 0,127 eGFP vs MT p=0,238 MT*: 5,62 ± 0,137 eGFP vs 6A p=-0,327 EGFP vs 6A p=-0,327 6A*: 6,64 ± 0,222 WT vs DN p=0,012	z,		mEPSC amplitude	H(3)=2,407	p=0,492	(1)	n.a.			
K mEPSC rise time H(3)=9,060 p=0,029 (1) 0,614 ci L mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 eGFP*: 6,07 ± 0,127 eGFP vs WT p=0,238 WT*: 5,62 ± 0,137 eGFP vs DN p=1 DN*: 6,36 ± 0,185 eGFP vs 6A p=0,327 6A*: 6,64 ± 0,222 WT vs DN p=0,012	2P	J	mEPSC area	H(3)=4,373	p=0,224	(1)	n.a.			
ci eGFP*:5,07±0,127 eGFP*0s W1 p=0,238 WT*:5,62±0,137 eGFP vs DN p=1 DN*:6,36±0,185 eGFP vs 6A p=0,237 6A*:6,64±0,222 WT vs 6A p=0,000	0	К	mEPSC rise time	H(3)=9,060	p=0,029	(1)	0,614	-050+0.07 -0.407		- 0.000
L mEPSC decay Time H(3)=18,609 p=0,000 (1) 1,260 (1) 01/2 0,222 WT vs DN p=0,002 (1) 01/2 0,222 WT vs DN p=0,012 (1) 01/2 0,222 WT vs DN p=0,000 (1) 01/2 0,222 WT vs DN p=0,000 (1) 01/2 0,222 WT vs DN p=0,012 (1) 01/2 0,222 WT vs DN p=0,000 (1) 01/2 0,22								eGFP*: 6,07 ± 0,127	eGFP VS W1	p=0,238
B L mEPSC decay Time H(3)=18,609 p=0,000 (¹) 1,260 LIN [™] : 0,30 ± 0,185 eGFP Vs 6A p=0,327 6A*: 6,64 ± 0,222 WT vs DN p=0,012 WT vs 6A p=0,000 WT vs 6A p=0,000	S2							WIT: 5,62 ± 0,137	eGFP vs DN	p=1
©A ⁻ : 0,04 ± 0,222 WT VS DN P=0,012 WT vs 6A P=0,000	an	L	mEPSC decay Time	H(3)=18,609	p=0,000	(1)	1,260	DIN". 0,30 ± 0,185	EGFP VS 6A	p=0,327
• WIVS 6A p=0,000	-igu							0A": 0,04 ± 0,222	WIVS DN	p=0,012
	_									p=0,000

		Statistica	l test (between ç	groups com	Accepted α-significance		Sample		
	Sec	⁽¹⁾ On	e-way repeated m	neasures AN		0,025		n = 157. N = 6	
	Le Le	Statiatia	(2) Kruskal-Wa	allis Test	aricon		-,		independent
	ele	Statistic	Pairwise com	oups comp parison		0.05		experiment	
	Ē						Descriptive and pairwise compa		ison
	Figu	Parameter	Statistical result	p-value	Statistical test	Effect size (r)	groups	comparisons	Corrected p- value
	В	1st EPSC amplitude	F(3,111)= 1,427	p=0,239	(1)	n.a.			
							eGFP*: 137,96 ± 12,691	eGFP vs WT	p=0,211
						[WT*: 98,096 ± 10,515	eGFP vs DN	p=1
	с	1st EPSC charge	H(3)=11,041	p=0,012	(2)	0,881	DN*: 163,43 ± 17,967	eGFP vs 6A	p=1
		-					6A*: 174,28 ± 19,616	WT vs DN	p=0,043
								DN VS 6A	p=0,013
		Statistica	l test (between g	roups com	parison)		Accepted α-signi	ficance	Sample
	s	⁽¹⁾ On	e-way repeated m	neasures AN	IOVA				
			(2) Kruskal-Wa	allis Test			0,008		n = 165, N = 6
	fere	Statistic	al test (within gr	oups comp	arison)		0.05		experiment
	e e		Pairwise com	parison			C.U.D		experiment
	anu		Statistical		Statistical	Effoct eizo	Descriptive and	pairwise compar	ison
s	Ē	Parameter	result	p-value	test	(r)	groups	comparisons	Corrected p- value
ILOI							eGFP*: 0,86 ± 0,037	eGFP vs WT	p=1
ner							WT*: 0,85 ± 0,031	eGFP vs DN	p=0,013
tic	D	PP ratio 1	H(3)=21,561	p=0,000	(2)	1,680	DN*: 0,70 ± 0,029	eGFP vs 6A	p=0,005
itap							6A". 0,70 ± 0,020	WT vs 6A	p=0,007
โลเ								DN vs 6A	D=1
۲.							eGFP*: 1,01 ± 0,034	eGFP vs WT	p=1
/ in							WT*: 0,96 ± 0,026	eGFP vs DN	p=0,002
icity	D	PP ratio 2	F(3,108)=12,620	p=0,000	(1)	0.899	DN*: 0,81 ± 0,025	eGFP vs 6A	p=0,000
asti				• •			6A*: 0,78 ± 0,024	WT vs DN	p=0,006
ld r								DN VS 6A	p=0,000
tern							eGFP*: 0.96 ± 0.020	eGFP vs WT	p=1
ort					(1)		WT*: 0,94 ± 0,022	eGFP vs DN	p=0,000
sh	р	PP ratio 3	F(3.108)=15.793	000.0=q		0,917	DN*: 0,82 ± 0,017	eGFP vs 6A	p=0,000
ect	-		. (-,,,	F -,			6A*: 0,78 ± 0,022	WT vs DN	p=0,004
aff								WT vs 6A	p=0,000
ints			<u> </u>				eGEP*: 0.94 + 0.017	eGEP vs WT	p=1
Inte							WT*: 0,91 ± 0,015	eGFP vs DN	p=0,000
u q		PP ratio 4	H(3)=48 157	p=0.000),000 ⁽²⁾	3,749	DN*: 0,82 ± 0,012	eGFP vs 6A	p=0,000
0C 2		11 1000 4	11(0)=10,101	p 0,000			6A*: 0,79 ± 0,019	WT vs DN	p=0,000
0.D								WT vs 6A	p=0,000
ŝ							eGEP*: 0.89 + 0.017	PGEP vs WT	p=1
ung							WT*: 0.91 ± 0.014	eGFP vs DN	p=0.003
Ē		PP ratio 5	H(3)-40 339	n=0.000	(2)	3 1/10	DN*: 0,82 ± 0,011	eGFP vs 6A	p=0,000
		11 1440 5	11(0)-10,000	p 0,000		0,140	6A*: 0,80 ± 0,013	WT vs DN	p=0,000
								WT vs 6A	p=0,000
	┝──┤						eGEP*: 0.80 + 0.000		p=1 n=0.159
							WT*: 0.94 + 0.011	eGFP vs DN	p=0,100
		PP ratio 6	LI(2)-52 625	n-0.000	(2)	4 007	DN*: 0,84 ± 0,010	eGFP vs 6A	p=0,000
		FFIAUUU	H(3)=52,025	p=0,000		4,097	6A*: 0,81 ± 0,014	WT vs DN	p=0,000
								WT vs 6A	p=0,000
		Statistica	l tost (botwoon (TOUDE COM	narison)		Accented a signi	DN vs 6A	p=1
	8	Statistica	⁽¹⁾ KniekaLW/	allis Teet	pansonj		0.05	licance	n = 122 N = 6
	enc	Statistic	al test (within or	inis rest	arison)		0,00		independent
	efer	Stations	Pairwise com	parison	unoony		0.05		experiment
	9			puncen			Descriptive and	pairwise compar	ison
	igu	Parameter	Statistical	p-value	Statistical	Effect size	droupo	comparison	Corrected p-
	<u> </u>		result		iest	(1)	groups	comparisons	value
							eGFP*: 0,89 ± 0,009	eGFP vs WT	p=0,933
							WT*: 0,94 ± 0,011	eGFP vs DN	p=0,175
	E	1 st EPSC quantal content	H(3)=9,292	p=0,026	(1)	0,838	6A* 0.81 + 0.014	WT vs DN	p=0,009
							0/1.0,0120,014	WT vs 6A	p=0.012
								DN vs 6A	p=0,177

		Statistica	l test (between g	groups com	parison)		Accepted α-signi	ficance	Sample
	so a		(1) Kruskal-W	allis Test			0,0167		
	ence	Statistic	al test (within a	roups comp	arison)				n = 157, N = 6 independent
	refer		Painvise con	narison	,		0.05		experiment
	en e			ipanion			Description and		
	Ē	Deservator	Statistical		Statistical	Effect size	Descriptive and	pairwise compai	ison
		Parameter	result	p-value	test	(r)	groups	comparisons	value
	1	Cumulative total charge	H(3)=4,21	p=0,240	(1)	n.a.			
							eGFP*: 61,51 ± 3,277	eGFP vs WT	p=0,292
		Cumulative					WT*: 72,13 ± 3,391	eGFP vs DN	p=0,000
	L I	normalized	H(3)=59 983	p=0.000	(1)	4 787	DN*: 44,23 ± 2,352	eGFP vs 6A	p=0,000
		charge		P 0,000		.,	6A*: 41,57 ± 2,121	WT vs DN	p=0,000
								WT vs 6A	p=0,000
	L	0						DN vs 6A	p=1
	к	Asynchronous & Asynchronous charge proportion	H(3)=1,977	p=0,577	(1)	n.a.			
suc							eGFP*: 0,52 ± 0,025	eGFP vs WT	p=0,012
ŭ							WT*: 0,67 ± 0,035	eGFP vs DN	p=1
ne		Recovery 5Hz	H(3)-24 758	n=0.000	(1)	1 076	DN*: 0,50 ± 0,019	eGFP vs 6A	p=0,654
tic	Ľ	Necovery 5112	11(3)=24,730	p=0,000		1,570	6A*: 0,48 ± 0,018	WT vs DN	p=0,000
tap								WT vs 6A	p=0,000
au								DN vs 6A	p=1
EN EN		Statistica	l test (between g	groups com	parison)		Accepted α-signi	ficance	Sample
<u>,</u>	8		⁽¹⁾ Kruskal-W	allis Test			0,0167		
ity	e e	Statiatia	al toot (within a		aria on)				n = 144, N = 6
Istic	stere	Statistic	ai test (within g	roups comp	ansonj		0.05		experiment
n pla	9.9		Pairwise con	nparison					
ern	igu		Statistical		Statistical	Effect eize	Descriptive and	pairwise compar	ison
ort t	۳.	Parameter	result	p-value	test	(r)	aroups	comparisons	Corrected p-
shc					(1)		. .	-	value
ct	<u>Р</u>	Cumulative total charge	H(3)=4,868	p=0,182	(1)	n.a.	- OFD*: 20.02 · 2.205		- 1
affe		Cumulativa					eGFP*: 39,83 ± 2,265	eGFP VS W I	p=1
ts		cumulauve					WI ⁻ . 40,07 ± 3,034 DN*: 24.86 ± 1.401	eGFP VS DN	p=0,000
tan	Q	charge	H(3)=42,608	p=0,000	(1)	3,551	6A* 26 08 + 1 821	WT vs DN	p=0,000
Ĩ		charge					0/1 : 20,00 ± 1,021	WT vs 6A	p=0.000
5b i								DN vs 6A	p=1
00							eGFP*: 0,14 ± 0,008 vs	AGED VS WT	n=0.994
							0,86 ± 0,008	2011 13 111	p=0,034
ŝ		Synchronous &					WT*: 0,15 ± 0,007 vs 0,85	eGFP vs DN	p=0.027
ann							± 0,007		• -,
Fig		Asynchronous					+ 0.012	eGFP vs 6A	p=0,893
	R		H(3)=8,082	p=0,044	(1)	0,674	6A*: 0.17 ±0.013 vs 0.83 ±		
		charge proportion					0,013	W I VS DN	p=1
								WT vs 6A	p=1
								DN vs 6A	p=0,991
							eGFP*: 0,76 ± 0,035	eGFP vs WT	p=0,000
							WT*: 1,15 ± 0,063	eGFP vs DN	p=1
	s	Recovery 40Hz	H(3)=52.978	p=0.000	(1)	4,415	DN*: 0,70 ± 0,029	eGFP vs 6A	p=0,038
	-			F -,		.,	6A*: 0,62 ± 0,028	WT vs DN	p=0,000
								WT vs 6A	p=0,000
	Η _T	PPP size (nC)	LI(2)=2.490	n=0.477	(1)			DIN VS 6A	p=0,816
	⊢'−	KKP size (pC)	H(3)=2,489	p=0,477		n.a.	0CED*: 0.240 + 0.022	ACED VALUE	n=1
							eGFF". 0, 319 ± 0,023	eGFF VS W I	p=1
							W 1*: 0,280 ± 0,021	eGFP VS UN	p=0,054
	U	Release probability	H(3)=23,666	p=0,000	(1)	1,980	DIN*: 0,421 ± 0,031	EGFP VS 6A	p=0,005
	1						6A*: 0,430 ± 0,025	WTVS DN	p=0,004
								10/1 VO CA	n_0 000
								WTVS 0A	p=0,000
								DN vs 6A	p=0,000 p=1
		Statistics	I tost (botworn		narieon)		Accented a cient	DN vs 6A	p=0,000 p=1
t		Statistica	l test (between j	groups com	parison)		Accepted α-signi	DN vs 6A	p=0,000 p=1 Sample

		Juliolicu	i teat (between §	ji oups com	Accepted a-significance		Jampie		
a not	8	⁽¹⁾ One	e-way repeated n	neasures AN	IOVA		0,05		n - 54 N - 3
does	re referenc	Statistic	al test (within gr	oups comp		0.05		independent	
oc 2b charç			Pairwise com	parison			0.05		experiment
۵ŭ	lgu		Ctatistical		Chatiatian	Effect size	Descriptive and	pairwise compar	ison
ildtype ed EPS	Ē	Parameter	result	p-value	test	(r)	groups	groups comparisons	Corrected p- value
fwi	С	1st EPSC amplitude	F(1,27)=0,035	p=0,854	(1)	n.a.			
opu		Statistica	l test (between g	groups com	Accepted α-signi	Sample			
ssio	8		⁽²⁾ Mann-Whit	ney test		0,025	n = 49 N = 3		
xpre	erend	Statistic	al test (within gr	oups comp		0.05		independent	
vere t cal	erefe		Pairwise com	parison		0.05		experiment	
fec O	l n		Statistical		Statistical		Descriptive and	pairwise compar	ison
ure S4 af	Ē	Parameter Statistical p-v	p-value	test	(r)	groups	comparisons	Corrected p- value	
Fig	D	Calcimycin peak amplitude	U=281	p=0,718	(2)	n.a.			
	E	Calcimycin total charge(nC)	U=269	p=0,548	(2)	n.a.			