Poëa-Guyon et al., http://www.jcb.org/cgi/content/full/jcb.201303104/DC1

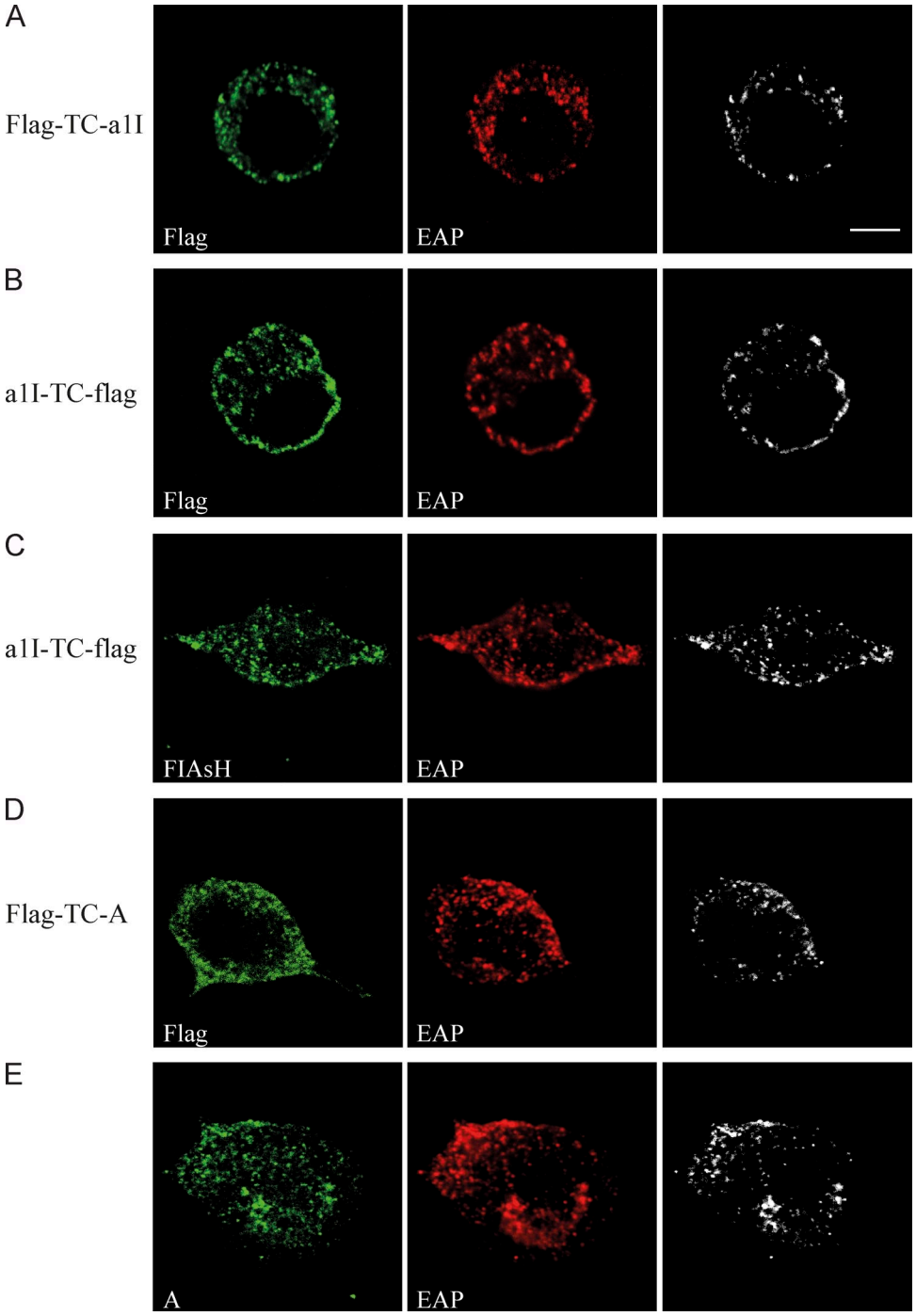


Figure S1. **The insertion of the Flag-TC tags does not affect targeting of a1-I or A subunits to secretory granules in PC12 cells.** (A–C) a1-I subunits carrying Flag and TC tags in the N-terminal (A) or C-terminal position (B and C) were detected using anti-Flag antibodies (Flag) or FlAsH-EDT_2 binding to the TC tag (FlAsH). (D and E) The recombinant Flag-TC-A (D) and endogenous (E) A subunits have similar subcellular distributions. Secretory granules that contain the reporter protein CgA-EAP were labeled with anti-EAP antibodies (EAP).The merge images depicting colocalization are shown on the right. Bar, 5 µm.

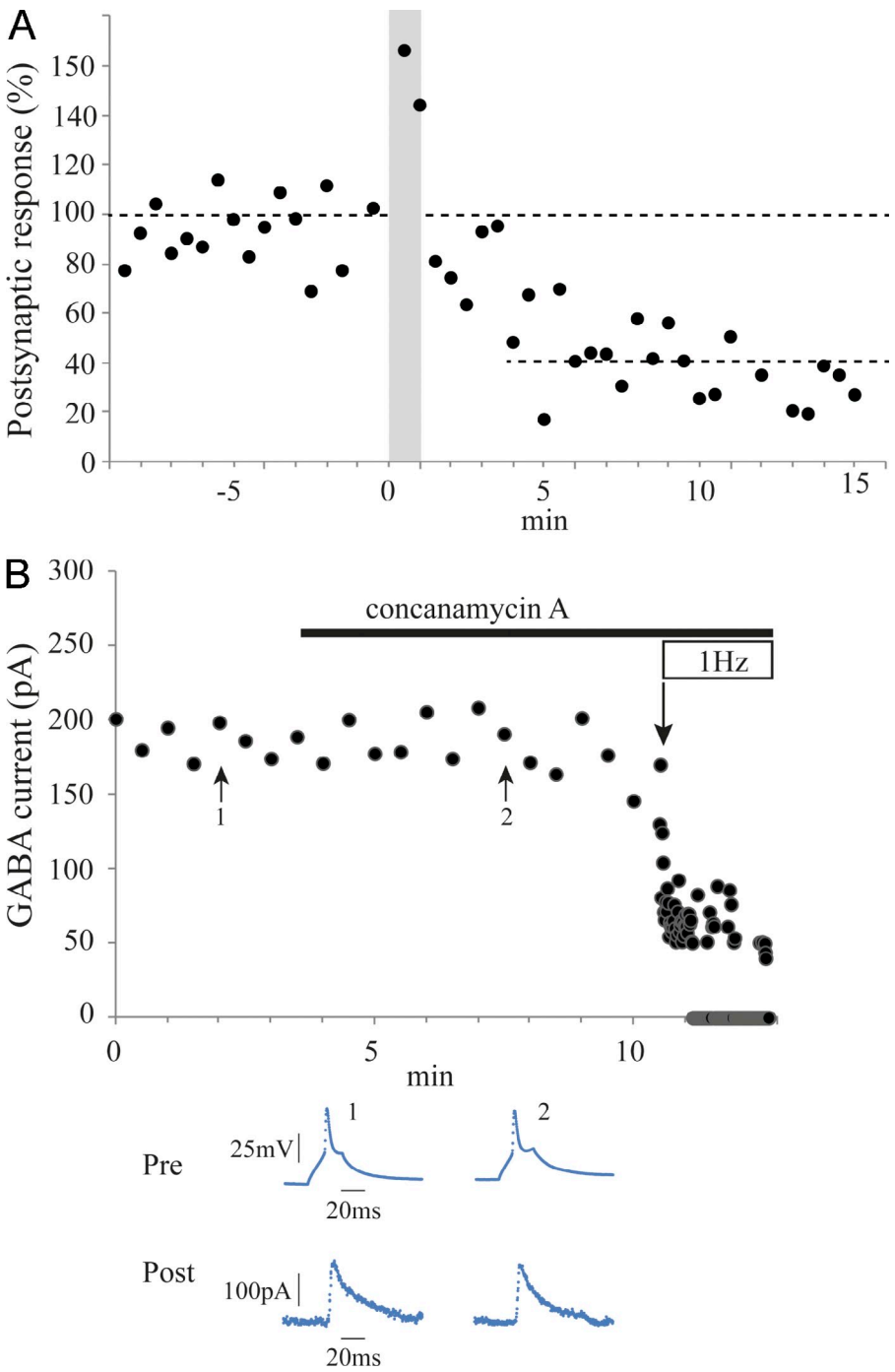


Figure S2. **Photoinactivation of the neuronal a1-I V0 subunit impairs neurotransmitter release independently from V-ATPase proton transport.** (A) Mouse cortical glutamatergic neurons expressing a V0 a1-I subunit with a C-terminal TC-Flag tag were treated as described in Fig. 1. Photoinactivation of a1-I-TC-Flag resulted in a rapid reduction of the postsynaptic response amplitude (in ~2 min after the flash), which remained stable at this lower amplitude. Data shown are from a single representative experiment that was repeated twice. Gray shading corresponds to the photoinactivating flash, and dotted lines correspond to the mean amplitude of the postsynaptic response (as in Fig. 1 C). (B) Inhibition of V-ATPase proton transport by 1 µM concanamycin A did not affect γ-aminobutyric acid (GABA) release triggered by low frequency stimulations for >10 min. Depression of release was only observed at higher frequency (1 Hz) of stimulation. Examples of presynaptic action potentials (Pre) and postsynaptic currents (Post) before (1) and after (2) introduction of concanamycin A. Graph shows a representative experiment that was repeated twice.

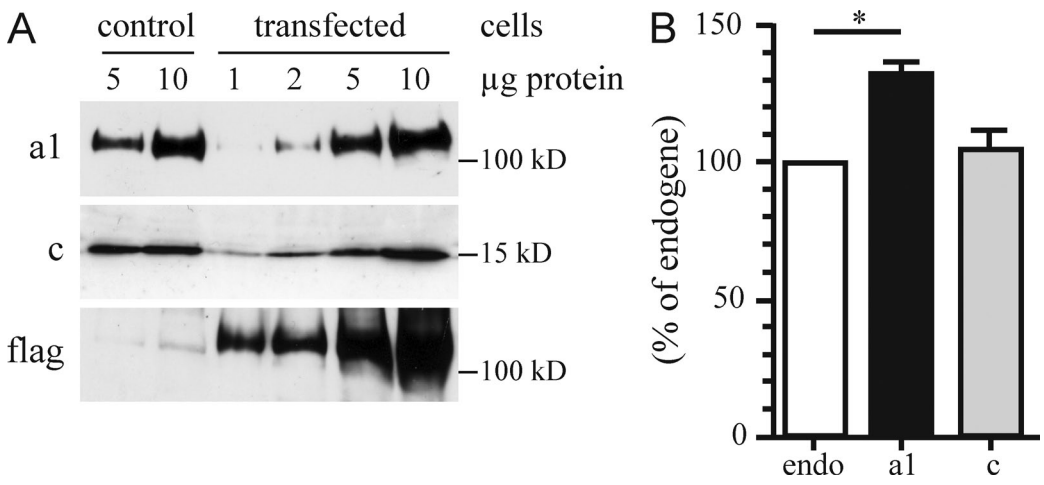
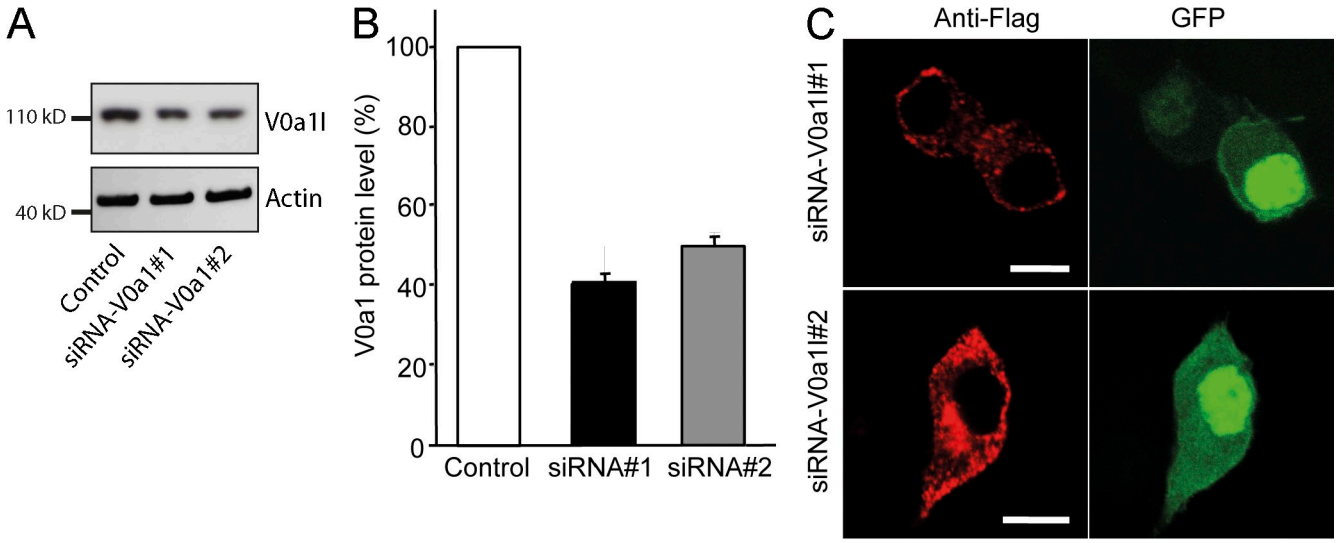


Figure S3. **Relative amounts of recombinant Flag-TC–tagged a1-I and endogenous a1 subunits in transfected PC12 cells.** (A and B) PC12 cells were transfected to coexpress Flag-TC-a1-I and GFP. The percentage of transfected cells (7%; mean of four transfections) was evaluated from the proportion of GFP-expressing cells. Amounts of a1, c, and Flag-tagged subunits in control and transfected cells were estimated on Western blots 48 h after transfection (A) and quantified in three independent experiments (B). Data are means ± SEM. *, $P < 0.05$.



D

	Control	siRNA#1	*p*	siRNA#1 + Rescue	*p*	siRNA#2	*p*	siRNA#2 + Rescue	*p*
Number of cells	36	25		25		25		25	
Number of spikes (total)	649	201		427		307		474	
Number of spike per cell	18 ± 4	8 ± 4	0.01	17 ± 5	ns	12 ± 3	0.05	19 ± 3	ns
Number of spikes (analyzed)	576	148		362		243		369	
I_{max} (pA)	60.5 ± 4.5	33.7 ± 3.6	0.01	60.7 ± 4.2	ns	37.8 ± 2.9	0.05	61.8 ± 2.3	ns
Half Width (ms)	28.4 ± 3.6	36.5 ± 2.9	0.05	28.8 ± 2.4	ns	32.8 ± 3.7	ns	30.9 ± 2.8	ns
Q (fC)	1890 ± 80	820 ± 40	0.01	1910 ± 90	ns	960 ± 90	0.01	1820 ± 50	ns
Slope (pA/ms)	6.3 ± 1.2	5.1 ±1.3	ns	6.1 ± 1.3	ns	5.4 ± 0.8	ns	6.5 ± 1.2	ns
Rise Time (ms)	10.1 ± 1.3	14.1± 1.2	0.05	10.8 ± 1.9	ns	13.5 ± 1.1	ns	11.5 ± 1.8	ns
Decay time (ms)	54.3 ± 3.3	65.8 ± 2.5	0.01	55.4 ± 3.7	ns	62.4 ± 3.9	0.05	54.1 ± 2.1	ns
spike with foot (%)	28	30		25		23		25	
Number of foot analyzed	161	44		90		55		92	
Number of foot per cell	4.5 ± 0.8	1.8 ± 1	0.05	3.6 ± 0.7	ns	2.2 ± 1.2	0.05	3.7 ± 0.8	ns
I foot (pA)	4.5 ± 0.6	3.2 ± 0.6	0.05	4.0 ± 0.9	ns	3.7 ± 0.7	ns	4.6 ± 0.8	ns
Q foot (fC)	23.4 ± 2.5	13.3 ± 3.2	0.01	22.1 ± 2.6	ns	18.2 ± 4.5	ns	23.1 ± 2.9	ns
t foot (ms)	15.1 ± 2.6	9.9 ± 2.1	0.05	14.4 ± 2.9	ns	12.9 ± 2.4	ns	13.2 ± 3.9	ns

Figure S4. **Overexpression of an siRNA-resistant TC-a1-l construct rescues catecholamine release defects after V0 a1 silencing.** (A) Two different siRNAs targeting bovine V0 a1 (Thermo Fisher Scientific) reduced V0 a1 expression level after 96 h. Actin served as a loading control. (B) Quantification of the silencing effect on V0 a1 expression ($n = 6$). Data are means ± SEM. (C) Chromaffin cells nucleoporated with the indicated V0 a1 siRNA and coexpressing a rat V0 a1-l bearing a Flag-TC tag and GFP were stained with an anti-Flag antibody revealing the granular distribution of the rat V0 a1-l-Flag protein. Bars, 5 µm. (D) 96 h after nucleofection, two siRNAs targeting V0 a1 significantly affected various amperometric signal parameters when catecholamine release was evoked by a 10-s application of 100 mM KCl. Coexpression of a siRNA-resistant V0 a1-l bearing a Flag-TC tag and GFP rescued all parameters to the control condition.

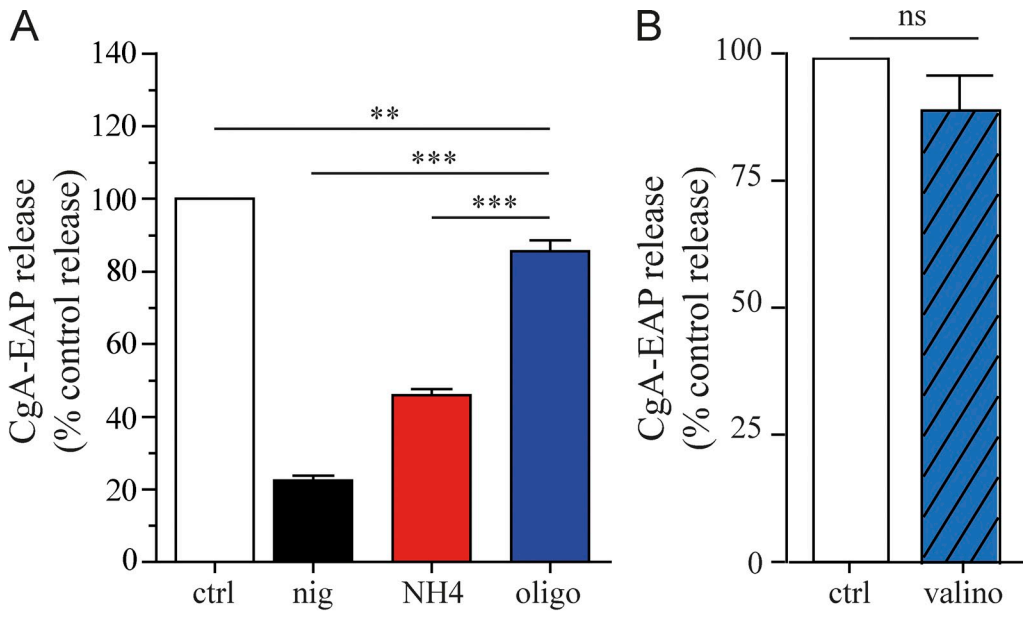


Figure S5. **CgA-EAP release is only slightly inhibited by the mitochondrial F1F0-ATPase inhibitor oligomycin A.** (A and B) PC12 cells expressing CgA-EAP were preincubated 15 min with 5 µM nigericin (nig), 20 mM NH_4Cl, or 5 µM oligomycin A (oligo; A) or 5 µM valinomycin (valino; B), and release was estimated. Data were obtained from eight (A) or five (B) different cell cultures. Data are means ± SEM. **, P < 0.01; ***, P < 0.001. ctrl, control.

Table S1. **Effect of CALI treatment and V-ATPase subunits overexpression on catecholamine release from chromaffin cells**

Parameters	Control	FlAsH 5–10 min	FlAsH 30 min	TC-a1-I	a1-I-TC	TC-A
Number of cells	94	44	46	35	29	48
Number of spikes (total)	1,695	751	798	667	526	869
Number of spike per cell	18 ± 3	17 ± 4	17 ± 3	19 ± 3	18 ± 3	18 ± 2
Number of spikes (analyzed)	1,403	648	702	583	469	399
I_{max} (pA)	59.8 ± 3.5	57.9 ± 3.9	60.2 ± 4.4	57.8 ± 2.5	60.8 ± 2.7	60.1 ± 4.3
Half width (ms)	27.8 ± 3.2	26.9 ± 2.4	28.3 ± 2.8	27.8 ± 3.2	30.3 ± 3.1	29.7 ± 3.4
Q (fC)	1,790 ± 90	1,740 ± 60	1,810 ± 80	1,760 ± 90	1,720 ± 80	1,760 ± 120
Slope (pA/ms)	6.0 ± 0.8	5.9 ±1.2	6.1 ± 1.1	6.4 ± 0.9	6.9 ± 1.2	6.2 ± 1.7
Rise time (ms)	10.7 ± 1.3	10.1± 1.2	10.8 ± 1.5	10.5 ± 1.1	11.5 ± 1.5	10.8 ± 1.2
Decay time (ms)	55.4 ± 3.1	54.8 ± 2.6	56.9 ± 3.9	53.4 ± 3.6	54.4 ± 2.7	55.5 ± 2.1
Spike with foot (%)	28	24	26	28	25	23
Number of foot analyzed	393	143	158	136	108	99
Number of foot per cell	4.5 ± 0.9	3.8 ± 0.8	4.5 ± 0.7	4.3 ± 0.9	4.5 ± 0.9	4.1 ± 0.9
I foot (pA)	4.1 ± 0.6	4.2 ± 0.5	4.0 ± 0,7	4.7 ± 0.7	4.1 ± 0.8	4.4 ± 0.6
Q foot (fC)	21.2 ± 2.3	23.3 ± 3.8	22.5 ± 2.9	22.2 ± 2.5	23.3 ± 2.2	20.8 ± 2.3
t foot (ms)	14.9 ± 2.1	13.9 ± 4.0	14.3 ± 2.8	15.9 ± 2.2	14.2 ± 3.0	14.8 ± 3.9

Chromaffin cells successively incubated with the FlAsH-EDT_2 probe and 1 mM BAL were illuminated for 1 min, and catecholamine release was evoked by a 10-s application of 100 mM KCl onto the recorded cell and measured by carbon fiber amperometry 5–10 min or 30 min after the flash (FlAsH). Catecholamine release from chromaffin cells coexpressing either the V0 a1-I or the V1 A subunits bearing a Flag-TC tag, and GFP was recorded in the absence of treatment and flash (TC-a1-I, a1-I-TC, and TC-A, respectively). Control release was measured from untransfected cells in the absence of any treatment (control). No statistical variations compared with control were observed in the recorded parameters. *I* foot, foot current amplitude; *t* foot, foot current duration.