Fig. S1- TetradecylTriMethylAmmonium Bromide (MiTMAB^{TM}) completely abolished synaptic vesicle endocytosis after stimulation. FM1-43 images prior to the unloading of 7 DIV cerebellar granule cells labeled in the absence (Aa) or in the presence (Ab) of MiTMAB (5µM, 10min) at normal (1.3 mM $CaCl_2$) Ca^{2+} concentrations. Ac and Ad represent phase contrast images of cells in control and MiTMAB, respectively. B, shown are examples of individual unloading kinetics in control and in the presence of MiTMAB. Note the higher noise level in MiTMAB experiments due to the lower signal tonoise ratio. C, Mean initial fluorescence (a.u.) in control and MitMAB-treated synaptic boutons, ($p \leq 0.01$; ANOVA). D, Frequency histogram of FM1-43 initial fluorescence in control and MiTMAB-treated cells. (E) Cumulative probability of the initial FM1-43 fluorescence measured prior to unloading with 50 mM KCl in the presence or absence of MiTMAB (5 µM, 10 minutes) at normal (1.33 mM) $CaCl_2$ concentrations.

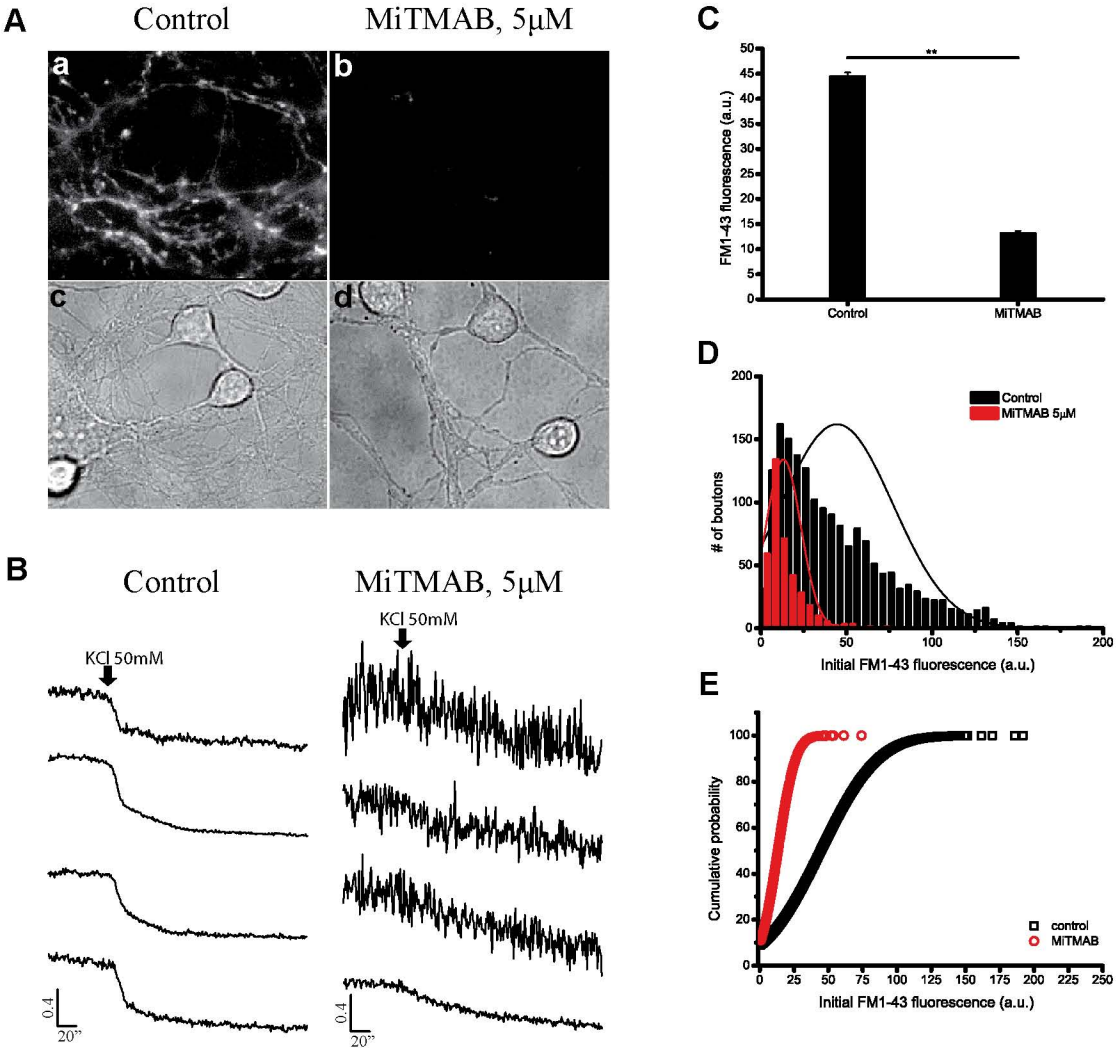

A
Control
MiTMAB, 5µM
a
b
c
d
B
Control
MiTMAB, 5µM
KCl 50mM
KCl 50mM
0.4
20"
0.4
20"
C
**
FM1-43 fluorescence (a.u.)
Control
MiTMAB
D
Control
MiTMAB 5µM
# of boutons
Initial FM1-43 fluorescence (a.u.)
E
Cumulative probability
control
MiTMAB
Initial FM1-43 fluorescence (a.u.)

Fig. S2. Neuronal maturation of cerebellar granule cells involves an increase in the expression of synaptic proteins. (A) Solubilized extracts of cell cultures at 3, 7 and 14 DIV were resolved by SDS-PAGE (7.5% acrylamide) and analyzed by a standard Western blotting procedure. Quantification of dynamin, syntaxin, synaptojanin and synapsin expression normalized to the GADPH or β-tubulin content in cerebellar granule cell cultures at 3, 7 and 14 DIV. (B) Histogram showing the mean (±SEM) protein content normalized to values at 3 DIV. n=3 experiments performed on different cultures. * $p \leq 0.05$, ** $p \leq 0.01$ when compared with values at 3DIV, t-test.

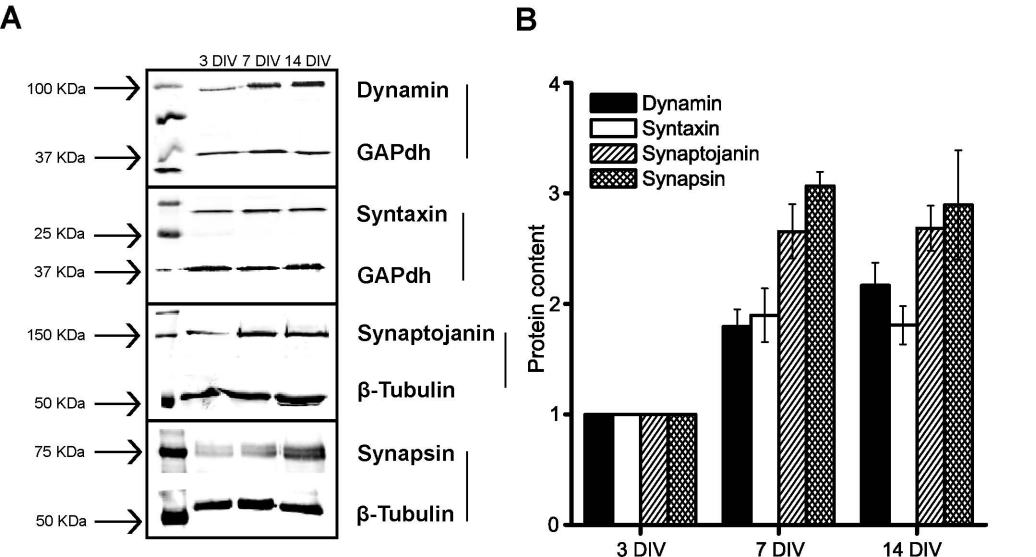
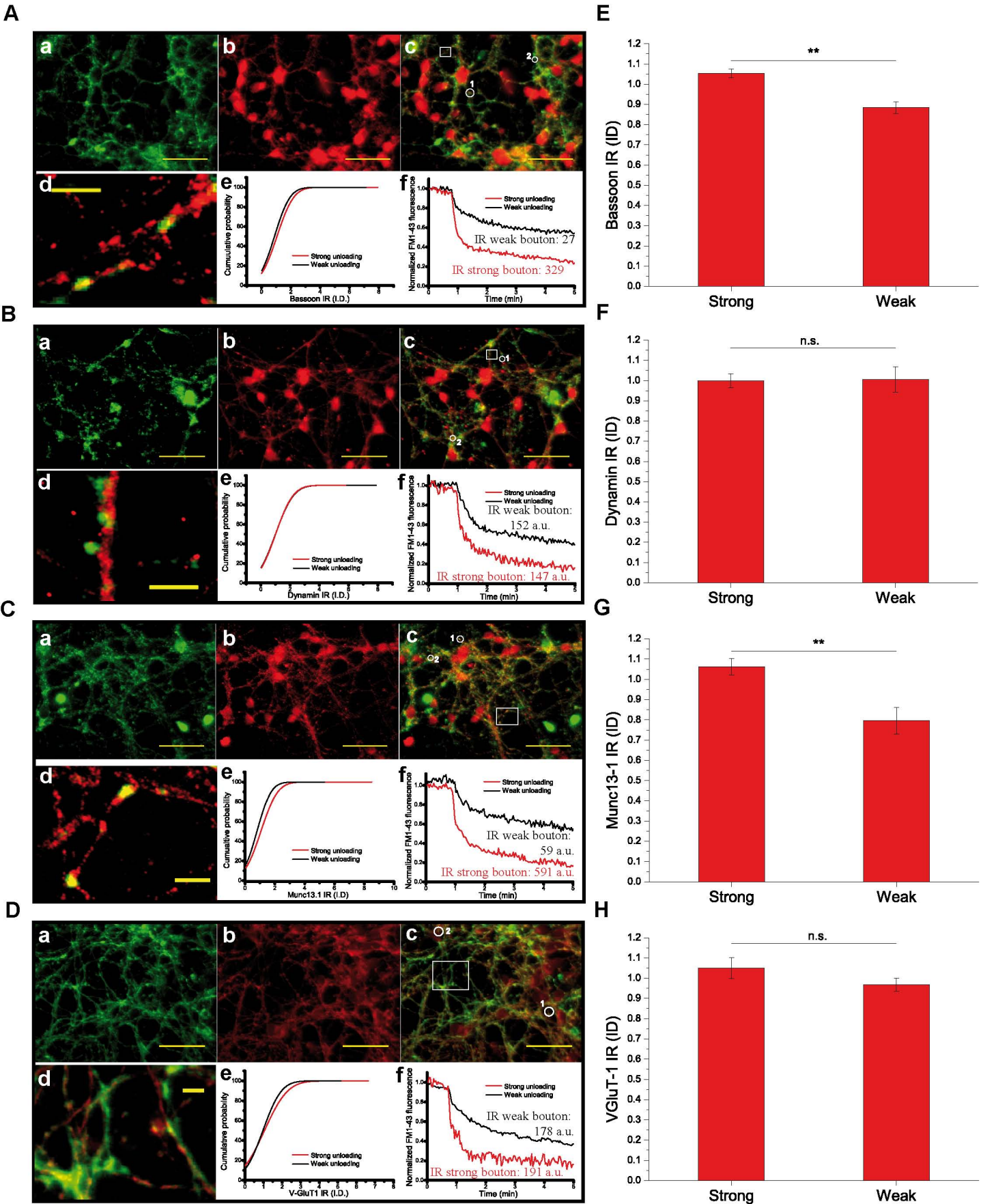

A
3 DIV 7 DIV 14 DIV
100 KDa
Dynamin
37 KDa
GAPdh
Syntaxin
25 KDa
37 KDa
GAPdh
150 KDa
Synaptojanin
β-Tubulin
50 KDa
75 KDa
Synapsin
50 KDa
β-Tubulin
B
Dynamin
Syntaxin
Synaptojanin
Synapsin
Protein content
0
1
2
3
4
3 DIV
7 DIV
14 DIV

Fig. S3. Synaptic boutons with strong FM-1-43 unloading contain more bassoon and munc13-1 than the nerve terminals with weak FM1-43 unloading. The expression of dynamin and vGlut1 is similar between the two groups of nerve terminals. (a) FM1-43 image, (b) immunofluorescence image, and merged FM-1-43 and immunofluorescence images at low (c) and at high magnifications (d), (e) cumulative immunofluorescence of bassoon (A), dynamin (B), Munc13-1 (C) and vGlut1 (D) in nerve terminals with strong and weak FM1-43 unloading profiles. (f) An example of nerve terminals with strong and weak unloading kinetics (shown as 1 and 2 in panel c, respectively). Differences in protein immunoreactivity (IR) expressed as the normalized integrated density (ID) of the two synaptic bouton populations for bassoon (E), dynamin (F), Munc13-1 (G) and vGlut1 (H). Integrated densities were normalized to the mean value of each experiment. The results shown in E, F, G and H represent the means ± s.e.m of at least three experiments. n.s. $p \geq 0.05$ and ** $p \leq 0.01$, when comparing nerve terminals with strong versus weak unloading profiles (t-test).


A
a
b
c
d
e
f
Cumulative probability
Strong unloading
Weak unloading
Bassoon IR (I.D.)
Normalized FM1-43 fluorescence
IR weak bouton: 27
IR strong bouton: 329
Time (min)
B
IR weak bouton: 152 a.u.
IR strong bouton: 147 a.u.
Dynamin IR (I.D.)
C
IR weak bouton: 59 a.u.
IR strong bouton: 591 a.u.
Munc13.1 IR (I.D)
D
IR weak bouton: 178 a.u.
IR strong bouton: 191 a.u.
V-GluT1 IR (I.D.)
E
Bassoon IR (ID)
**
Strong
Weak
F
Dynamin IR (ID)
n.s.
G
Munc13-1 IR (ID)
H
VGluT-1 IR (ID)