SUPPLEMENTARY FIGURE LEGENDS

Figure 1S. Annexin A6 fusion proteins maintain the properties of native annexin A6.
(**A**) and (**B**) HEK293 cells expressing annexin A6YFP-tH and GFP-tH were mixed, and homogenized in the presence of 0.2 mM Ca^{2+}. Lysates were extracted with 0.5% Triton X-100 (TX-100) and fractionated on a discontinuous 5-30% flotation sucrose gradient in 0.2 mM Ca^{2+}, as shown. Fractions were collected from the top of the gradient and analyzed by SDS-PAGE and Western blotting with (A) anti-GFP MAbs or (B) anti-annexin A6 and anti-RhoA MAbs. Graphs show the distribution of annexin A6YFP-tH, GFP-tH, endogenous annexin A6 and RhoA in gradient fractions, expressed as % of the total protein.
(**C**) Gradient fractionation of HEK293 cell lysates containing annexin A6YFP-tK and GFP-tK was performed as described in (A and B) and analyzed by Western Blotting with anti-GFP MAbs. Low-density membrane fractions 3 and 4; soluble proteins in 40% sucrose fraction 8, pellet fraction 9P.

Figure 2S. PM-localized annexin A6 reduces EGF-stimulated Ca^{2+} entry into A431 cells.
A431 cells were transiently transfected with annexin A6YFP-tH or mCherry-tH constructs, co-cultured and stimulated with 100 ng EGF in the presence of 2 mM Ca^{2+}. (**A**) shows a representative recording of 5 cells each in the same field and (**B**) an average of 3 independent experiments (n = 9 coverslips), *$p<0.05$.

Figure 3S. Annexin A2 does not influence Ca^{2+} entry in annexin A2YFP-tH cells
(**A**) Annexin A2 was expressed as a fusion with YFP and the H-Ras anchor sequence. Live HEK293 cells producing A2YFP-tH were analyzed by confocal microscopy under resting conditions, and after stimulation with 2 mM Ca^{2+}- 5μM ionomycin. Annexin A2YFP-tH was targeted to the PM at rest, and at high Ca^{2+}. Bar = 5 μm.
(**B**) Annexin A2YFP-tH expressing cells were co-cultured with mCherry-tH control, loaded with Fluo-3/AM and stimulated with 10 μM carbachol (CCh), followed by SERCA inhibition with 1 μM thapsigargin (TG), and activation of SOCE with 2 mM and 5 mM $[Ca^{2+}]_{ex}$. In a representative experiment, responses in mCherry-tH- expressing cells (control, black line) were compared to annexin A2YFP-tH cells (grey line).
(**C**) The graph shows an average of 4 independent experiments (n=16 fields) ± SEM. There was no statistically significant difference between the responses in annexin A2YFP-tH cells compared to the mCherry-tH control.

Figure 4S. Re-loading with 3 mM Ca^{2+} restores the ER contents in annexin A6YFP-tH cells
(**A**) A typical recording of SOCE stimulation from co-cultured A6YFP-tH and mCherry-tH cells, incubated in Ca^{2+}-free buffer. ER $[Ca^{2+}]$ levels are reduced compared to the control.
(**B**) Same cells as in (A) pre-incubated in 3 mM $[Ca^{2+}]_{ex}$ for 2 h. Immediately before the experiment, cells were perfused with Ca^{2+}-free buffer, and TG-induced SOCE stimulated.
(**C**) Graph shows average of 4 independent experiments ± SEM (n=8 coverslips for control, n=11 coverslips for reloaded cells), *$p<0.05$.

Figure 5S. Annexin A6 binds purified actomyosin
Smooth muscle actomyosin and annexin A6 were purified as described (58). The absence of lipid contamination in both preparations was confirmed by thin-layer chromatography.
Purified annexin A6 (**Anx 6**; 2 μg of protein) was recombined with the indicated amounts of purified actomyosin (**AM**) in NCS buffer (120 mM KCl, 20 mM imidazol, pH 7.0) (final volume = 40 μl) containing 1 mM EGTA and 1.2 mM $CaCl_2$ **(+)** or 1mM EGTA alone **(-)**. The samples were incubated for 10 minutes at ambient temperature. Pellets obtained after centrifugation at 12 000 x g for 30 minutes were made up to a final volume of 40 μl with NCS buffer. Equal aliquots of the resulting pellets (**pel**) and supernatants (**sup**) were analyzed by SDS-PAGE.

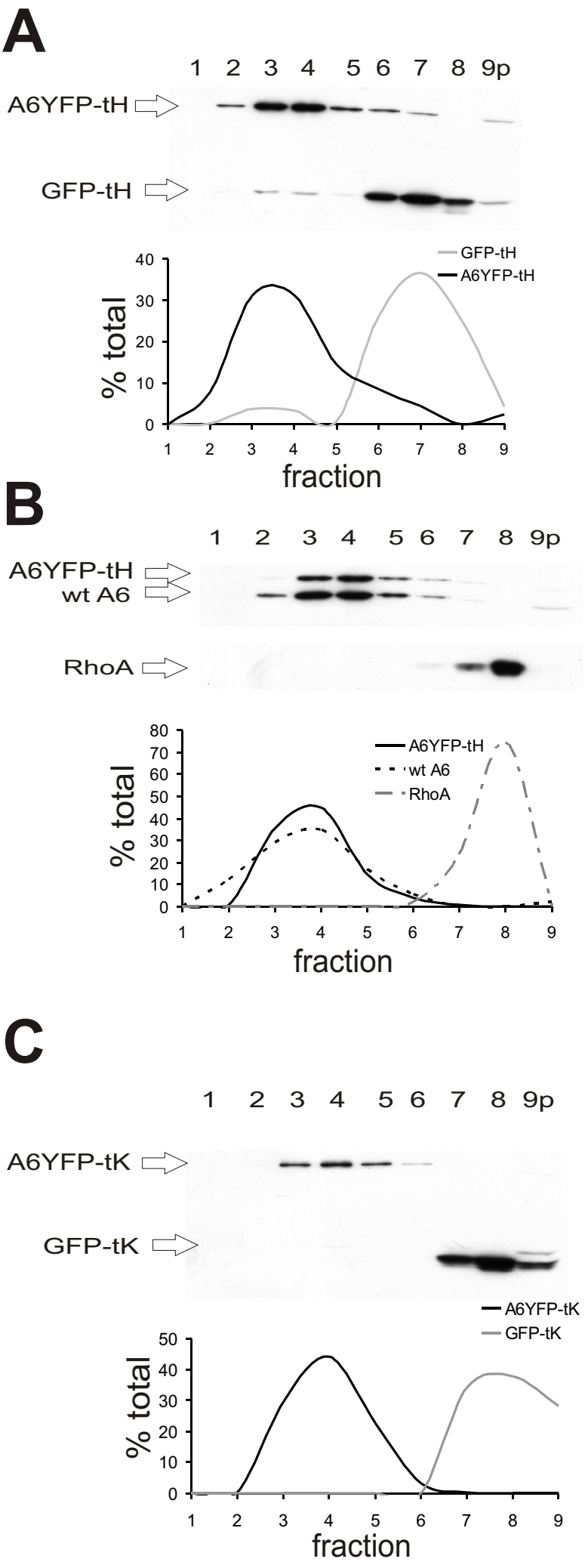
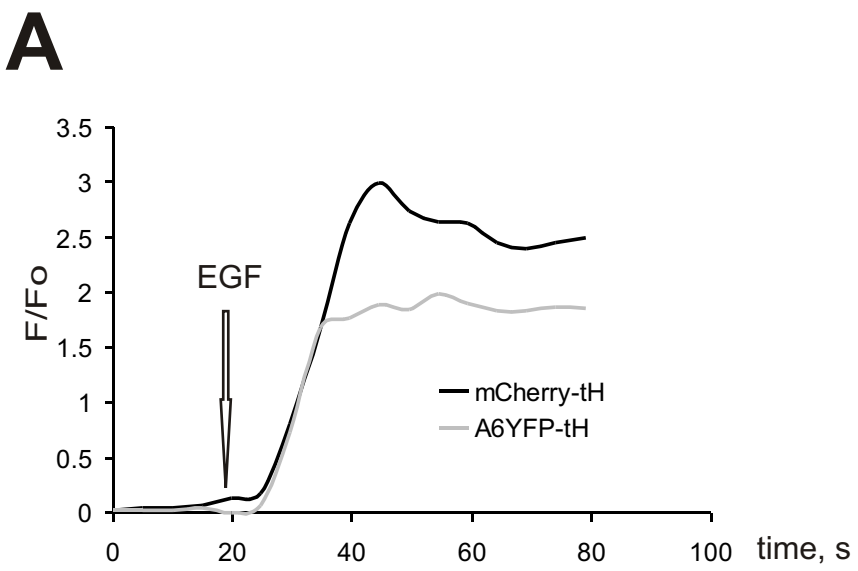

A
1 2 3 4 5 6 7 8 9p
A6YFP-tH
GFP-tH
GFP-tH
A6YFP-tH
% total
fraction
B
1 2 3 4 5 6 7 8 9p
A6YFP-tH
wt A6
RhoA
A6YFP-tH
wt A6
RhoA
% total
fraction
C
1 2 3 4 5 6 7 8 9p
A6YFP-tK
GFP-tK
A6YFP-tK
GFP-tK
% total
fraction

Fig. 1S


A
3.5
3
2.5
2
1.5
1
0.5
0
F/Fo
EGF
mCherry-tH
A6YFP-tH
0
20
40
60
80
100
time, s

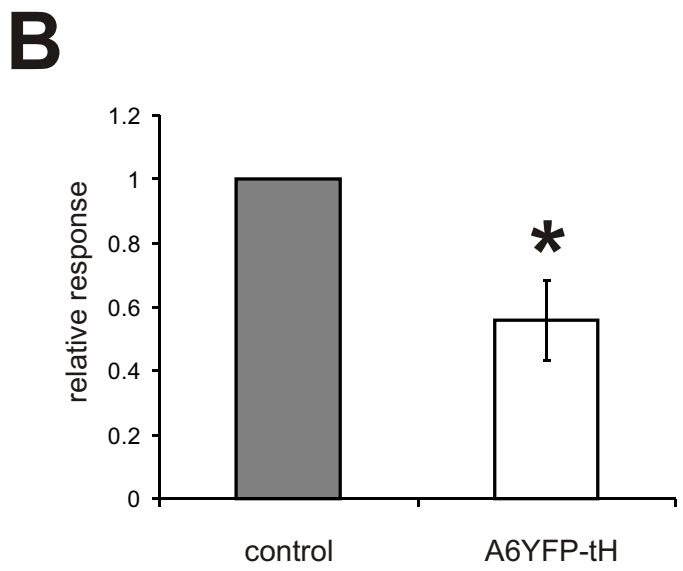
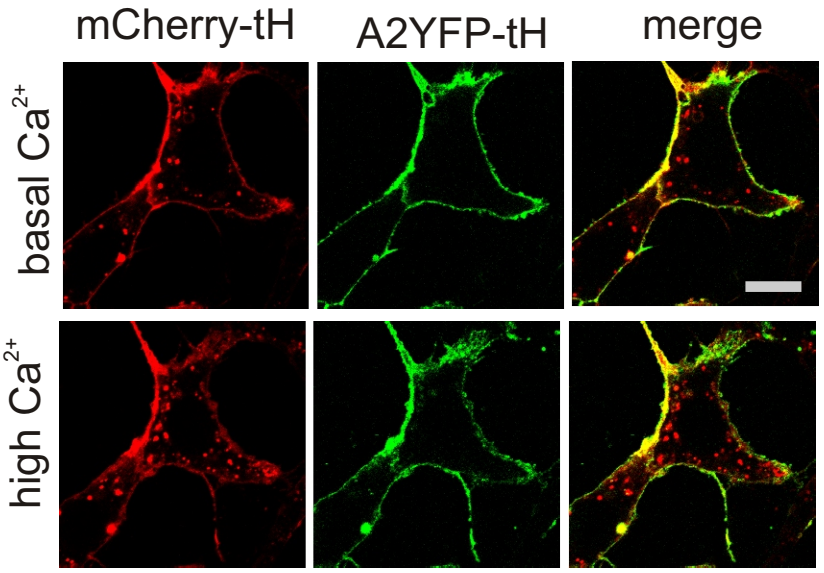
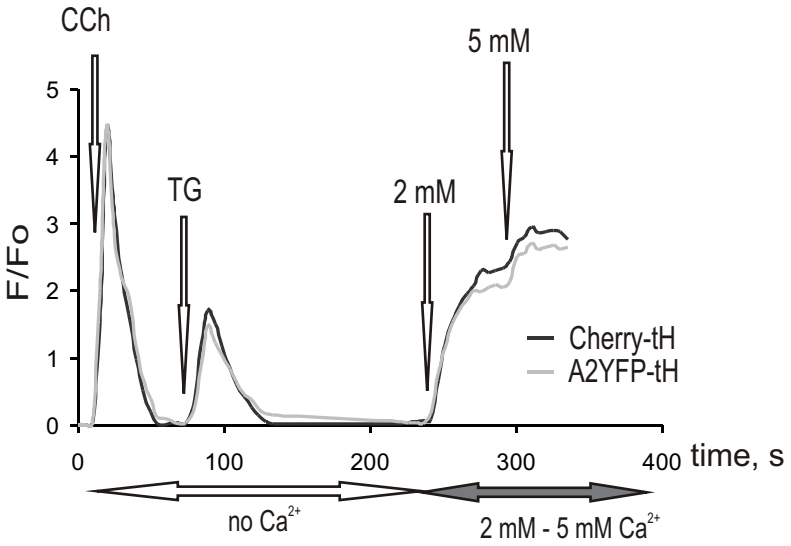

B
1.2
1
0.8
0.6
0.4
0.2
0
relative response
*
control
A6YFP-tH

Fig. 2S

A


mCherry-tH
A2YFP-tH
merge
basal Ca2+
high Ca2+

B


CCh
5 mM
TG
2 mM
F/Fo
0
1
2
3
4
5
0
100
200
300
400
time, s
Cherry-tH
A2YFP-tH
no Ca2+
2 mM - 5 mM Ca2+

C

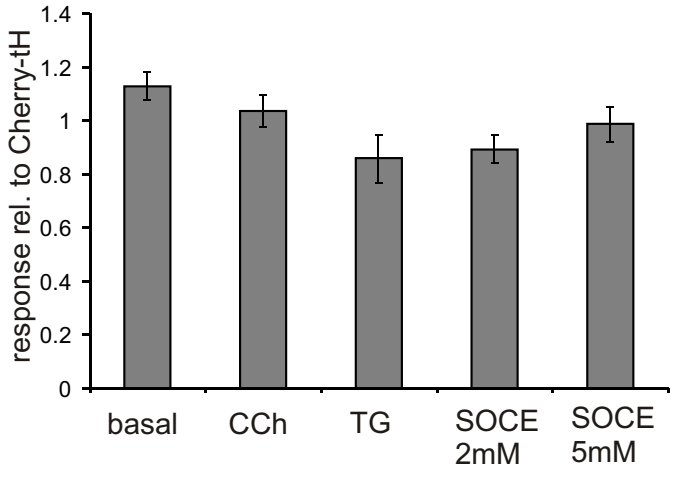

response rel. to Cherry-tH
0
0.2
0.4
0.6
0.8
1
1.2
1.4
basal
CCh
TG
SOCE 2mM
SOCE 5mM

Fig. 3S

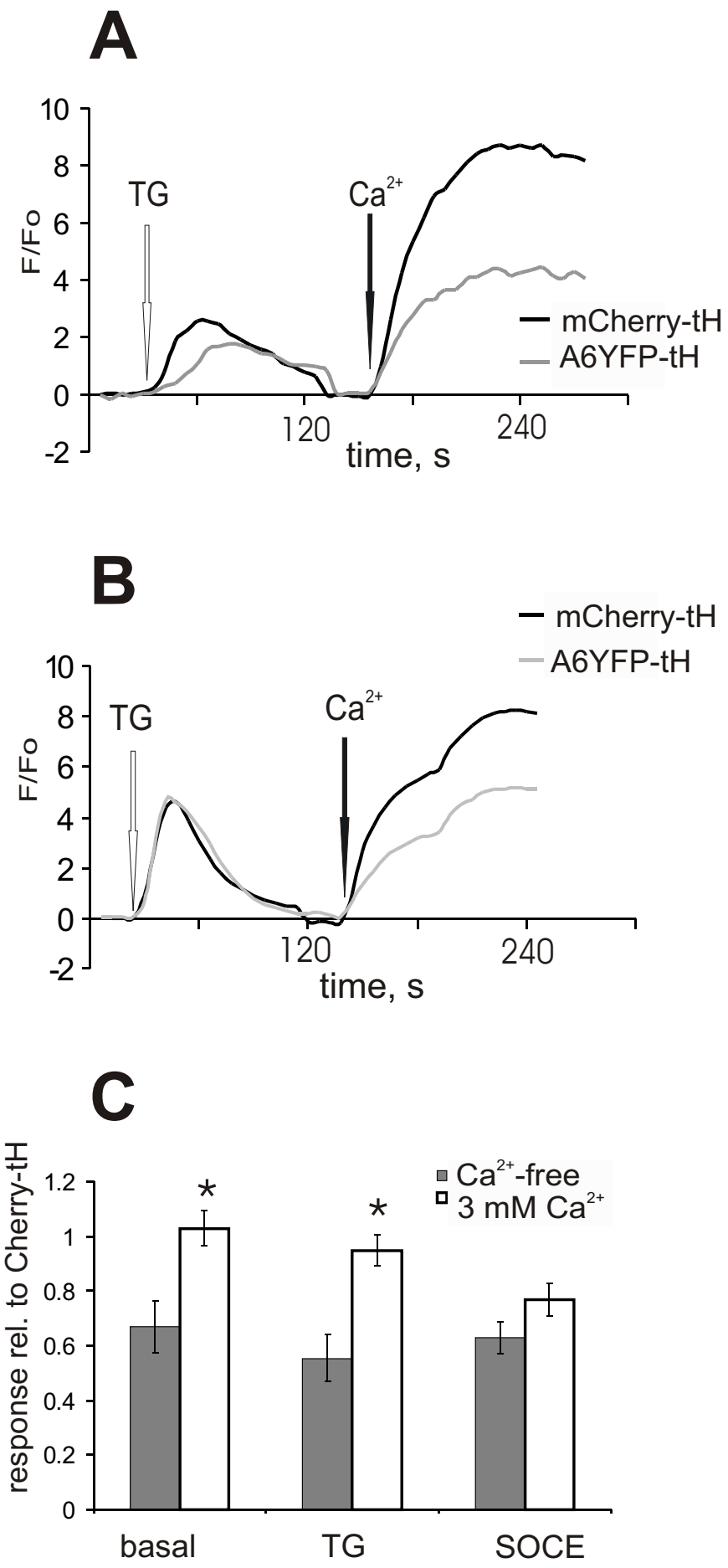

A
10
8
6
4
2
0
-2
F/Fo
TG
Ca2+
mCherry-tH
A6YFP-tH
120
240
time, s
B
mCherry-tH
A6YFP-tH
10
8
6
4
2
0
-2
F/Fo
TG
Ca2+
120
240
time, s
C
response rel. to Cherry-tH
1.2
1
0.8
0.6
0.4
0.2
0
Ca2+-free
3 mM Ca2+
*
*
basal
TG
SOCE

Fig. 4S

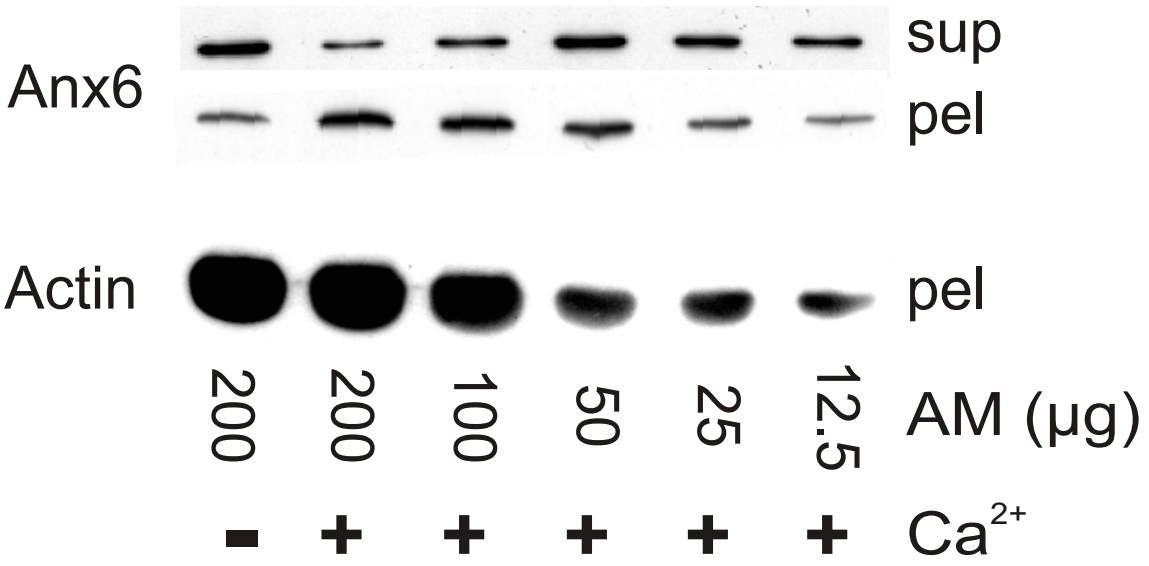


Fig. 5S