Table S1. Quantitative RT-PCR primer sequences

Primer	Forward Sequence	Reverse Sequence
α1 spectrin	AGAAATCCAACACCGAAGAGC	TCCAGGTCATCTGCGTCTCTC
β1 spectrin	CATCAGCGACCTCTACAAGGA	GAGCCCATGTTTTCCAGGTG
α2 spectrin	AGGGAGAACCTCCTAGAAGAGC	CTTCCCGGAACAACATGAACTT
β2 spectrin	ACACAGGAGACAAGTTCCGCTTCT	TCAACAGATGACACATCCCGTGGT
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

Figure S1. Histogram showing the distribution of pore sizes in proplatelet membrane skeletons (such as shown in Fig. 1)
Quantification of the lattice structure by morphometry shows its pores to have average areas of 6,033 + 2772 nm^2 (n=418 pores). Data was compiled from 10 different cytoskeletons of 0.5 μm^2 areas, with each area containing > 40 contiguous pores.

Figure S2. Immunoblot showing the presence of erythroid spectrin in megakaryocytes and platelet lysates from wild-type, but not platelets from mice deficient in erythroid alpha-spectrin (sph/sph)
Samples were probed with anti-α1 spectrin. Anti-α1 antibodies label a band in wild-type platelets and megakaryocytes. Accordingly, anti-α1 antibodies fail to recognize the erythroid spectrin isoform in lysates of sph/sph platelets. GAPDH was used as a loading control.

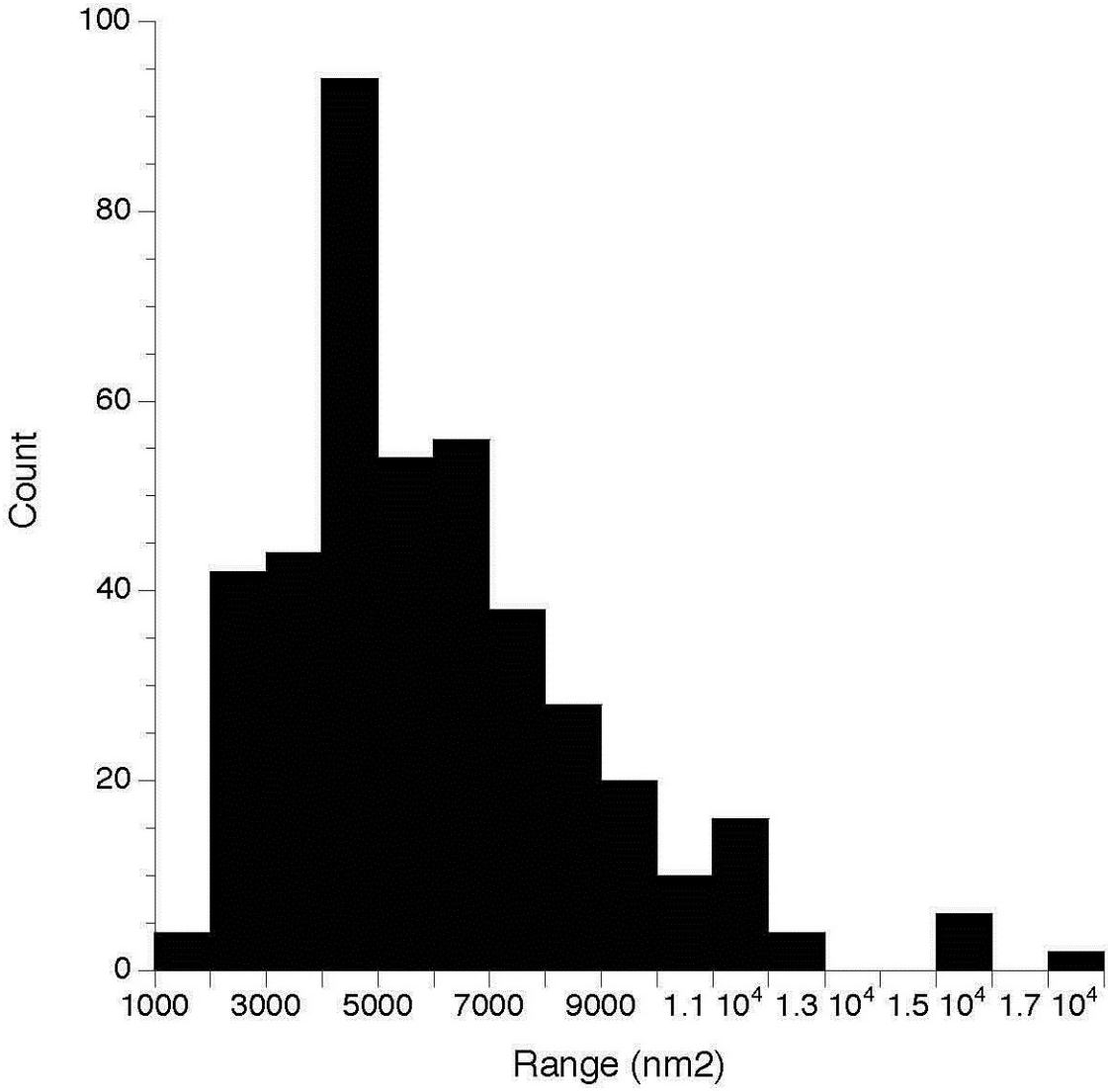
Figure S3. Immunofluorescence analysis of platelet spectrins
Localization of spectrin isoforms within resting human platelets. Left panels shows spectrin isoform staining, middle panels show filamentous actin localization (visualized by fluorescent phalloidin) and right panels show the merged images of spectrin and actin. Both α1 and β1 spectrins stain as punctate spots in the platelets. α2 and β2 spectrins also have a punctate or spotted distribution within human platelets. Scale bars represent 5μm.

Figure S4. Abnormal maturation of the invaginated membrane system in spα2N1-expressing megakaryocytes
(A) Thin-section electron microscopy images illustrating a typical mature control megakaryocyte (top panel) and an spα2N1-expressing megakaryocyte (bottom panel) containing an underdeveloped IMS. Corresponding high magnification views of the boxed, threshold areas are included. (B) Quantification of the IMS by measurement of the area per field of the threshold area. Data are expressed in % of megakaryocyte area occupied by the DMS and are the mean plus or minus one standard deviation for 10 control megakaryocytes and 10 spα2N1-expressing megakaryocytes. ** P<. 0.001 using Student unpaired t test. Experiments were repeated in triplicate.

Figure S5. Effect of OG permeabilization method on the integrity of the proplatelet plasma membrane
(A) Surface of untreated proplatelet. The plasma membrane is intact. (B) Proplatelet treated with 0.4% OG. Small holes are apparent in the proplatelet tips (arrows), ranging from 20 to 200 nm in diameter, although large regions of the membrane are left intact. The scale bar is 500 nm.

Figure S6. Quantification of western blots shown in Fig. 2
(A) Densitometric analysis of the immunoblots shown in Fig. 2A of three different experiments for quantitation of spectrin isoforms in starting cells (FLC), MKs at different days, platelets (Plt), ST3 cells and erythrocyte ghosts. (B) Quantitation of spectrin cytoskeleton distribution shown in Figure 2B from three different experiments in starting cells (FLC), MKs at different days and platelets. Western blots from three different experiments were quantified by densitometry (Adobe Photoshop CS3 software), and expressed as integrated density. For cytoskeletons data are presented as ratio of signals in pellet and supernatant with pellet.

Figure S1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60
40
20
0
Count
1000
3000
5000
7000
9000
1.1 10^4
1.3 10^4
1.5 10^4
1.7 10^4
Range (nm2)

Figure S2

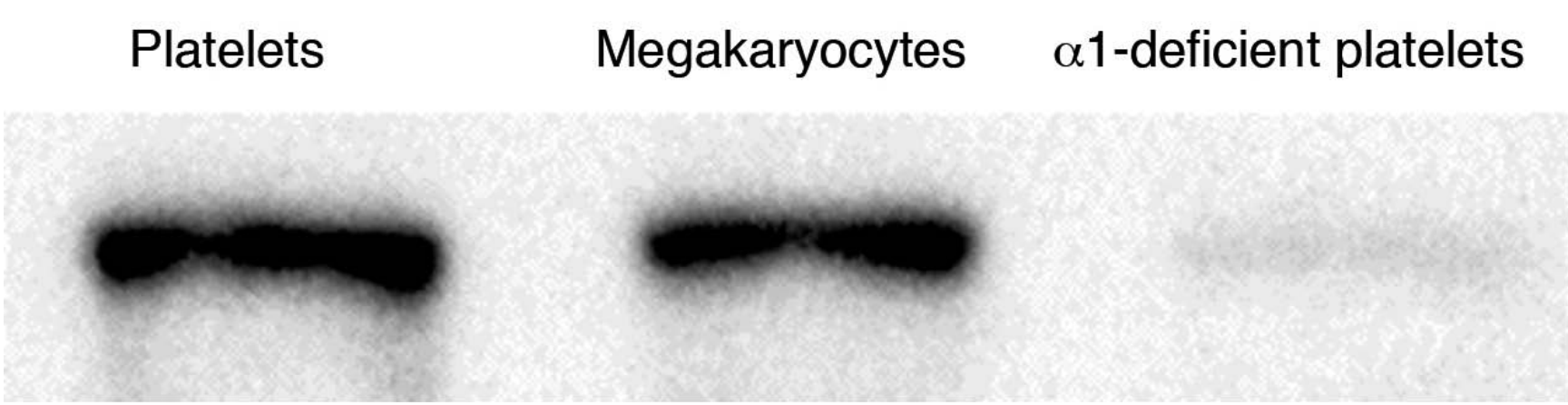


Figure S3

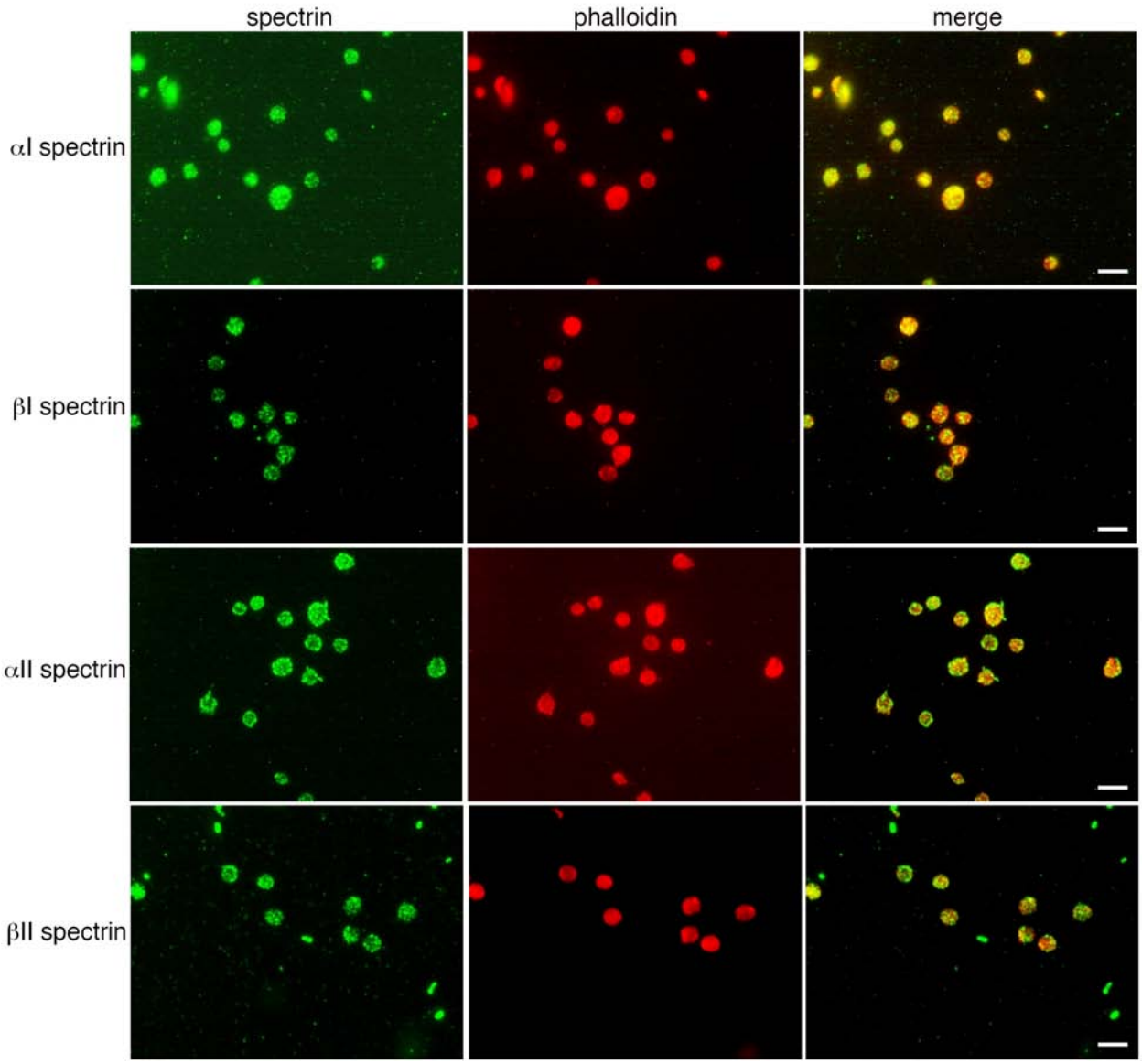
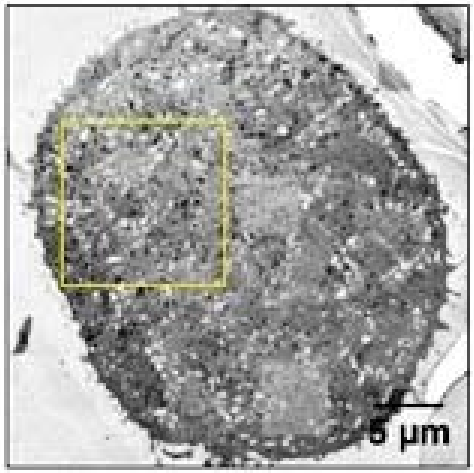
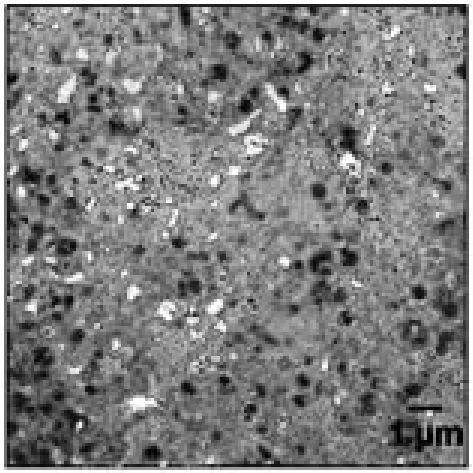

spectrin
phalloidin
merge
αI spectrin
βI spectrin
αII spectrin
βII spectrin

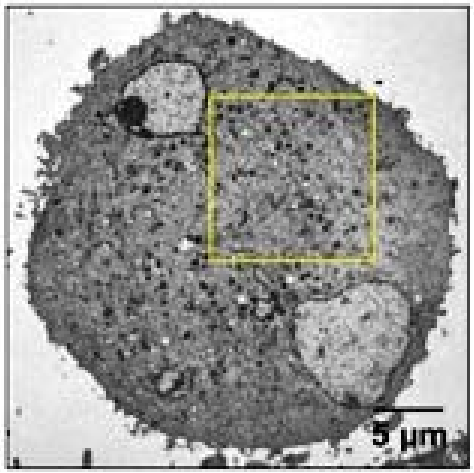
Figure S4

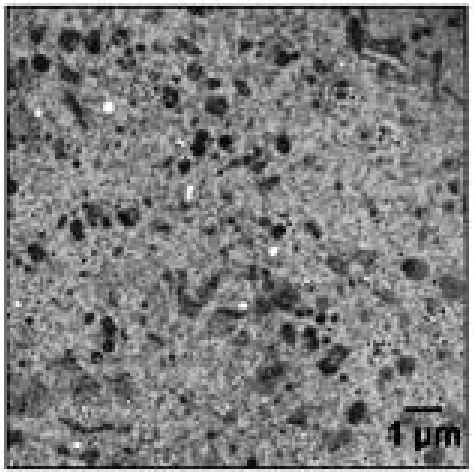
Control





spαIIN1





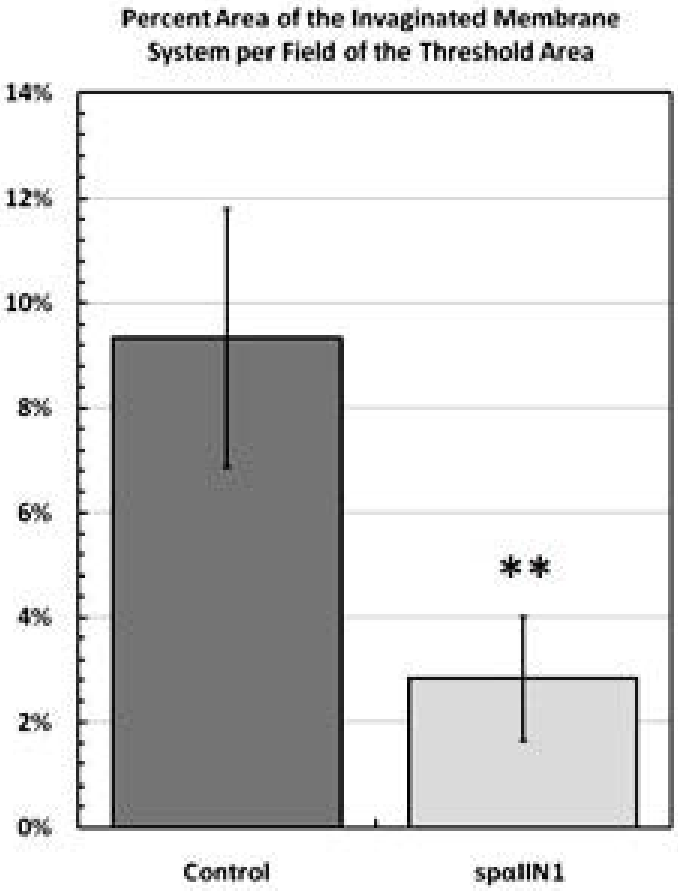
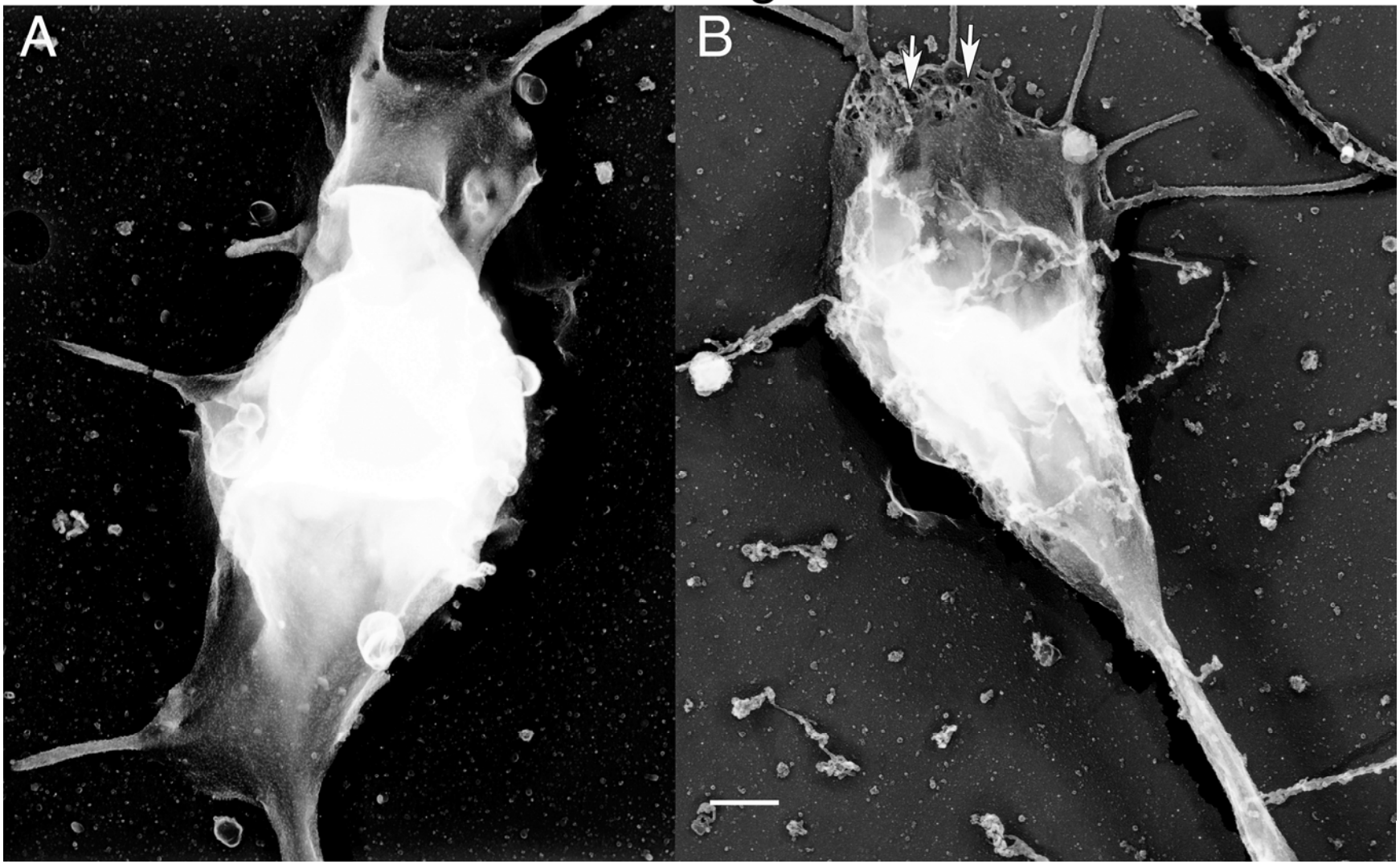
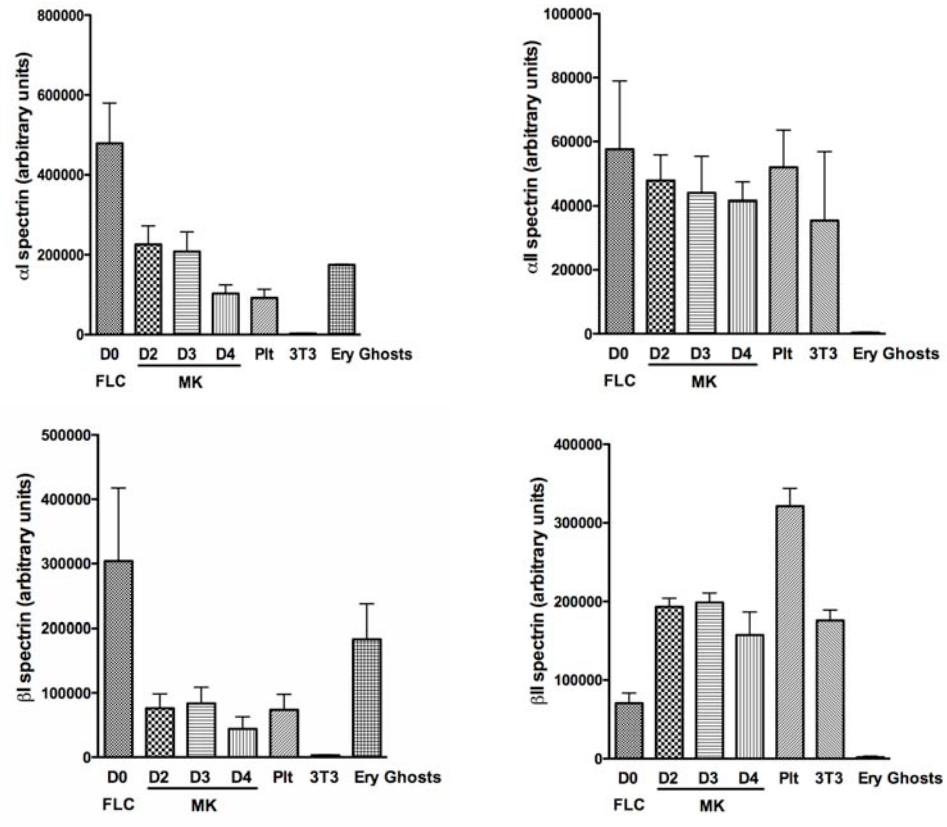


Figure S5



A


αI spectrin (arbitrary units)
αII spectrin (arbitrary units)
βI spectrin (arbitrary units)
βII spectrin (arbitrary units)
D0 D2 D3 D4 Plt 3T3 Ery Ghosts
FLC MK

B

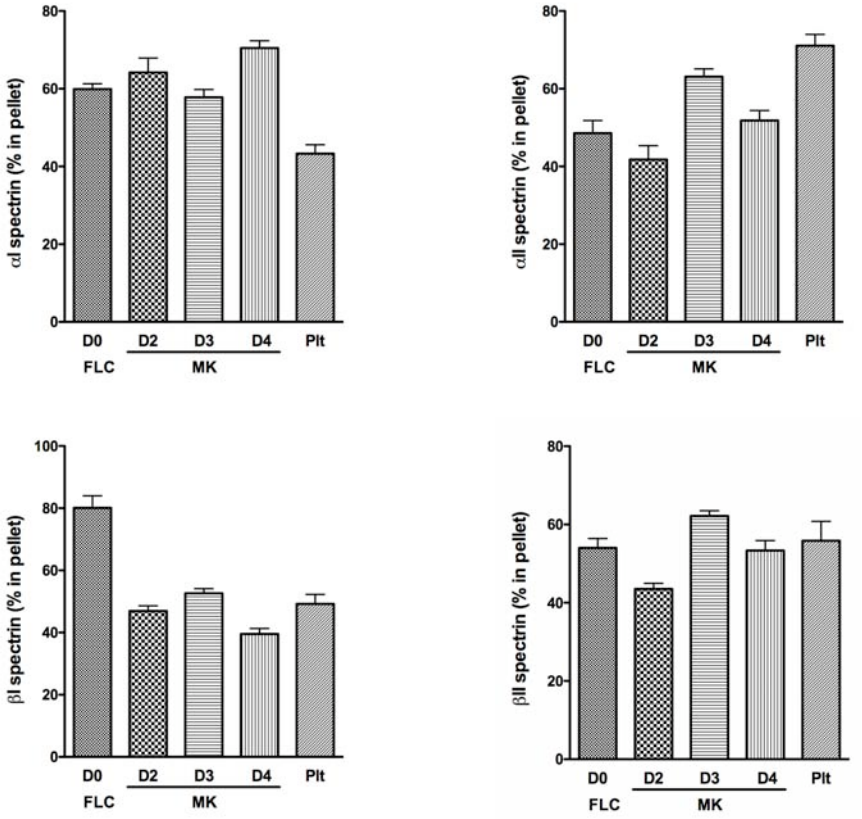

αI spectrin (% in pellet)
αII spectrin (% in pellet)
βI spectrin (% in pellet)
βII spectrin (% in pellet)
D0 D2 D3 D4 Plt
FLC MK

Figure S6