Supplemental data for:

Integrin $\alpha_{IIb}\beta_3$ in a membrane environment remains the same height after Mn^{2+} activation when observed by cryo-electron tomography

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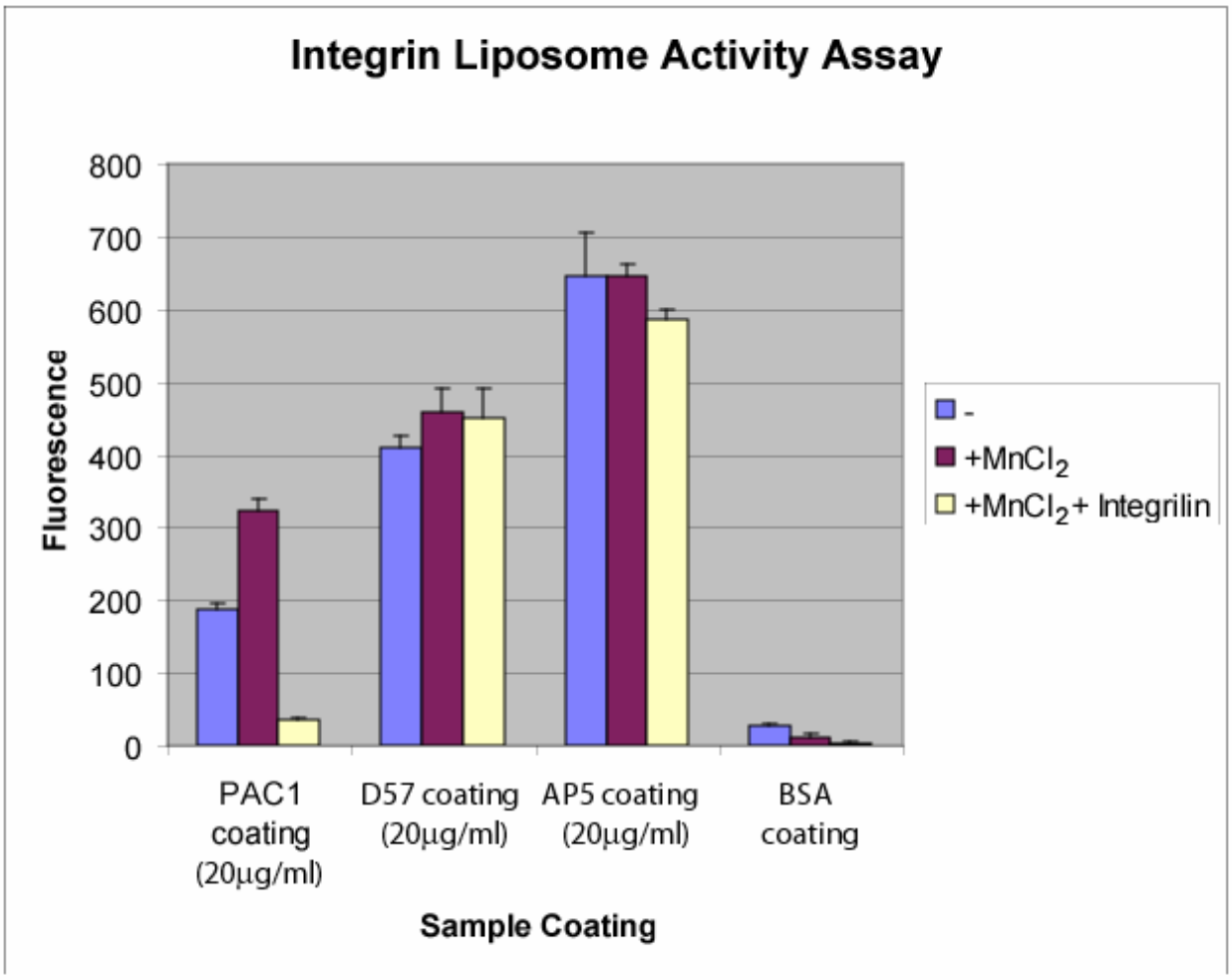

Figure S1. Results of integrin activation assay using antibody PAC-1, antibody D57 and antibody AP3. Each measurement is the average of 3 repeats and the error bars in the figure show the range of data. Note that these data and the purified integrins were

obtained at U. C. San Diego and were done subsequent to the experiments described in the main article.

Coatings:

D57 antibody: constitutively bind to β3. Not conformation or activation specific.
AP3 antibody: constitutively binds β3. Not conformation or activation sensitive.
PAC1: ligand-mimicking, activation-specific antibody.
Integrilin: competitive ligand inhibitor. Functions with a mechanism similar to an RGD peptide.

Procedures:

Liposomes are reconstituted with Fluorescein labeled lipids (PECF) with the ratio of (DMPC:DMPG:PECF = 25:25:1 molar ratio). The plates were coated with different antibody overnight at 4 degree and then blocked with 100mg/ml BSA. The plates were washed for 4 times with TBS buffer and liposome added to each well with conditions indicated in the graph. Integrilin as a specific inhibitor was added as a negative control to show that the Mn2+ induced binding is integrin specific. The binding mixture were incubated at 37 degree for 4 hours and subsequently washed 4 times. Then 100 µl of TBS with 1% Triton-X100 was added before taking the reading at 485/535. The assay were done simultaneously with empty liposomes (liposomes with no integrin but otherwise the same) as control reading of the background non-specific interaction of lipid and antibody. The empty liposome fluorescence were subtracted from the integrin liposome fluorescence to correct the background. The data was presented above.

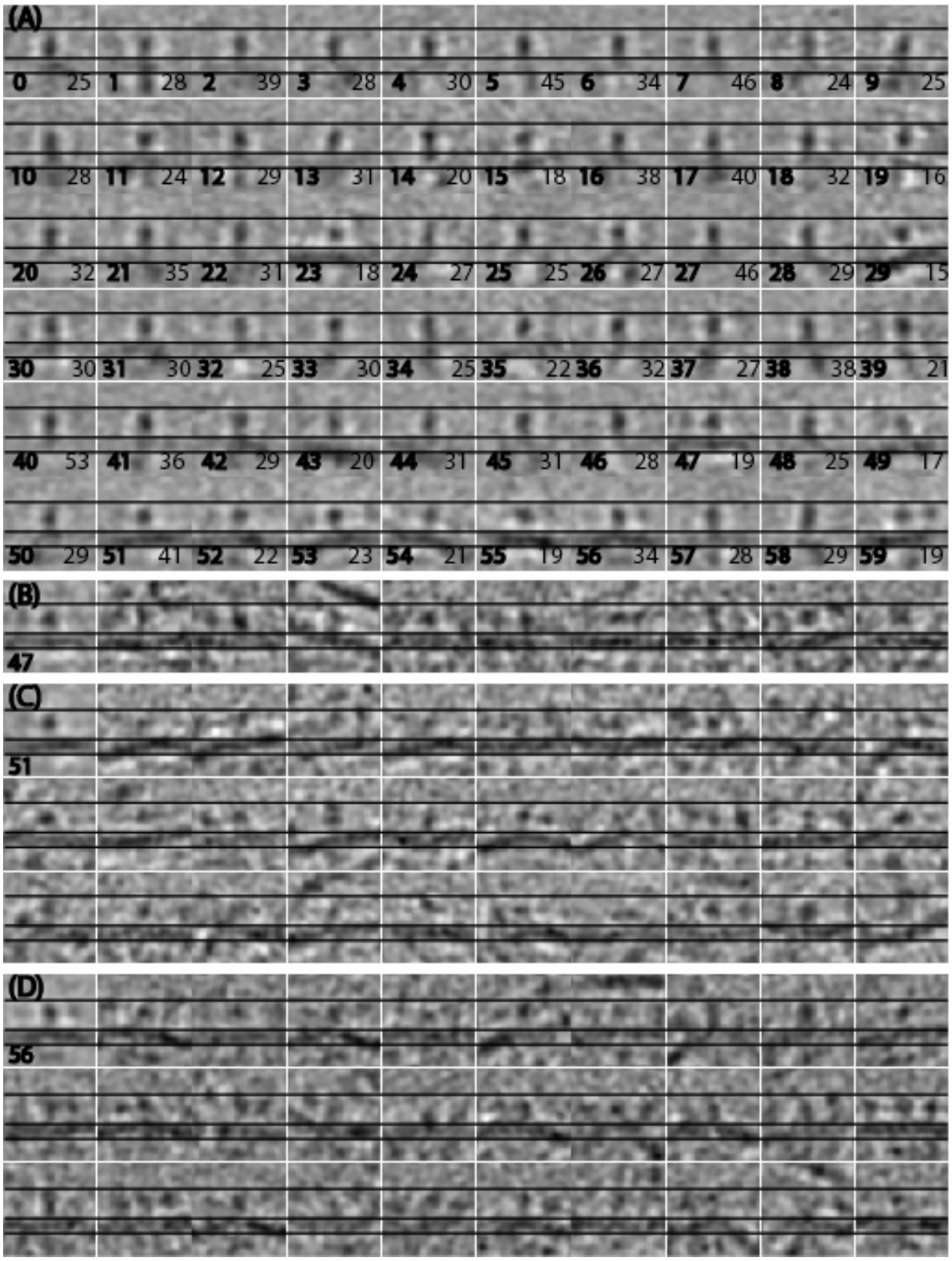
(A)
0 25 1 28 2 39 3 28 4 30 5 45 6 34 7 46 8 24 9 25
10 28 11 24 12 29 13 31 14 20 15 18 16 38 17 40 18 32 19 16
20 32 21 35 22 31 23 18 24 27 25 25 26 27 27 46 28 29 29 15
30 30 31 30 32 25 33 30 34 25 35 22 36 32 37 27 38 38 39 21
40 53 41 36 42 29 43 20 44 31 45 31 46 28 47 19 48 25 49 17
50 29 51 41 52 22 53 23 54 21 55 19 56 34 57 28 58 29 59 19
(B)
47
(C)
51
(D)
56

Figure S2. Projections of the inactive integrin molecules. Each panel is 360 Å × 360 Å (64 pixels x 64 pixels). The projections are computed from 11 sections (61 Å) through the center of the subvolume containing the integrin density. (A) 60 classes of inactive integrins. Integrin subvolumes include predominately molecules extending out the top and bottom of the tomogram, in which position, the membrane density is not visible [1]. Thus, most of the classes do not display the membrane density or show it weakly despite the fact that the averaging takes account of the missing wedge, which helps prevent the membrane density from being washed out in the averages. None the less, subvolumes tended to separate between those that contained the membrane and those that did not during the multivariate statistical analysis. Class number is indicated in the lower left hand corner of the individual panels in bold font and the number of class members in the lower right hand corner in normal font. The three horizontal lines, which are positioned identically in all panels, indicate the top of the integrin, the top of the membrane and the bottom of the membrane and are separated by 110 Å and 60 Å from the central line. (B-D) Class members from classes 47, 51 & 56 which show the bilayer with clarity. The class average is shown in the first panel (upper left hand corner) and identified by the class number and the number of class members. For space reasons, some classes do not show all members. Note that the density that the subvolumes are aligned to is in the center of each panel approximately in the center of the 110 Å wide space.

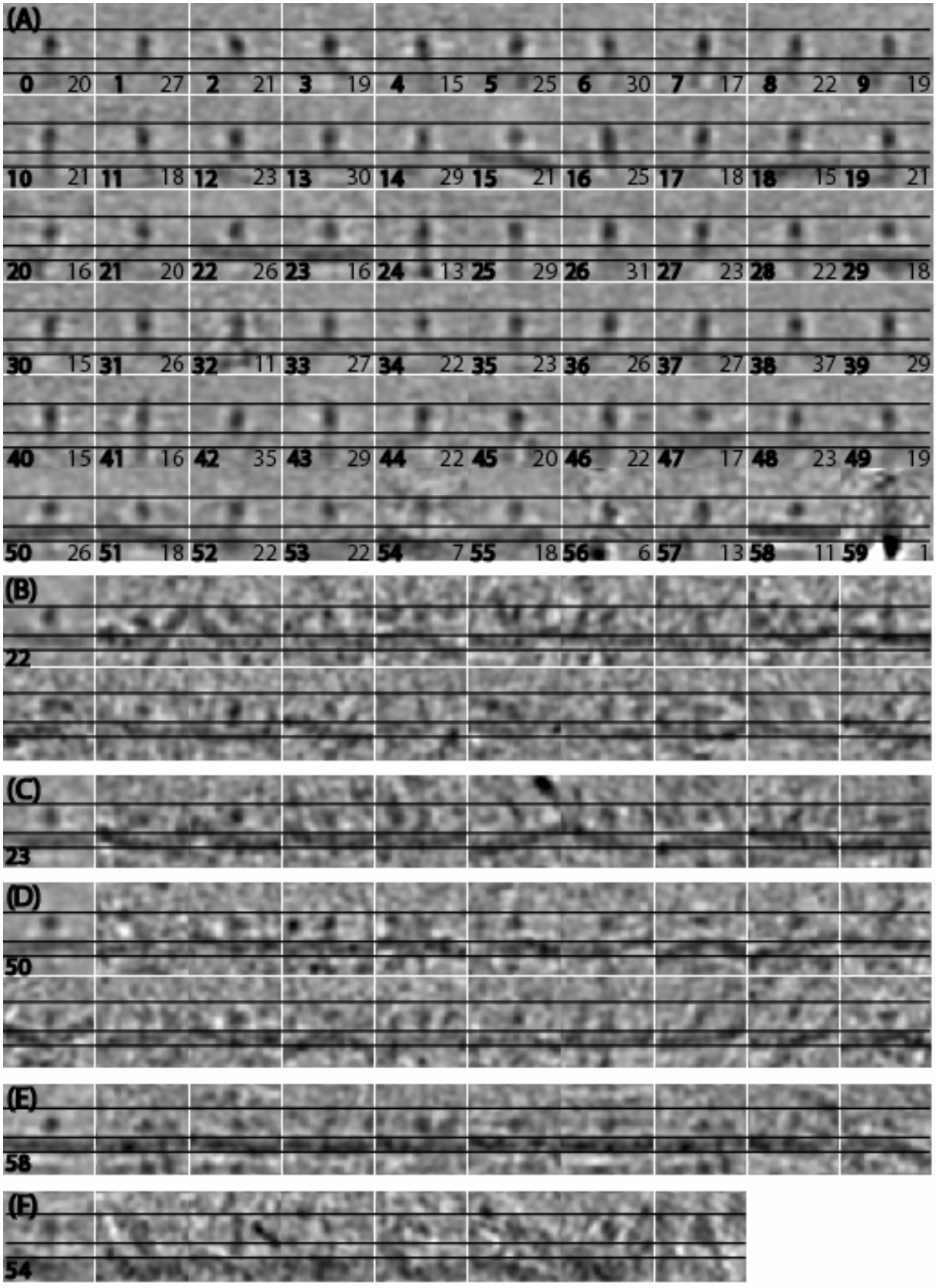
(A)
0 20 1 27 2 21 3 19 4 15 5 25 6 30 7 17 8 22 9 19
10 21 11 18 12 23 13 30 14 29 15 21 16 25 17 18 18 15 19 21
20 16 21 20 22 26 23 16 24 13 25 29 26 31 27 23 28 22 29 18
30 15 31 26 32 11 33 27 34 22 35 23 36 26 37 27 38 37 39 29
40 15 41 16 42 35 43 29 44 22 45 20 46 22 47 17 48 23 49 19
50 26 51 18 52 22 53 22 54 7 55 18 56 6 57 13 58 11 59 1
(B)
22
(C)
23
(D)
50
(E)
58
(F)
54

Figure S3. Projections of active integrin molecules. The layout and description of each panel is the same as for Figure 2S. (A). 60 classes of active integrins. (B-F) Class members from classes 22, 23, 50, 58 and 54 which show the bilayer clearly. The class average is shown in the first panel (upper left hand corner) and identified by the class number and the number of class members. For space reasons, some classes do not show all members.

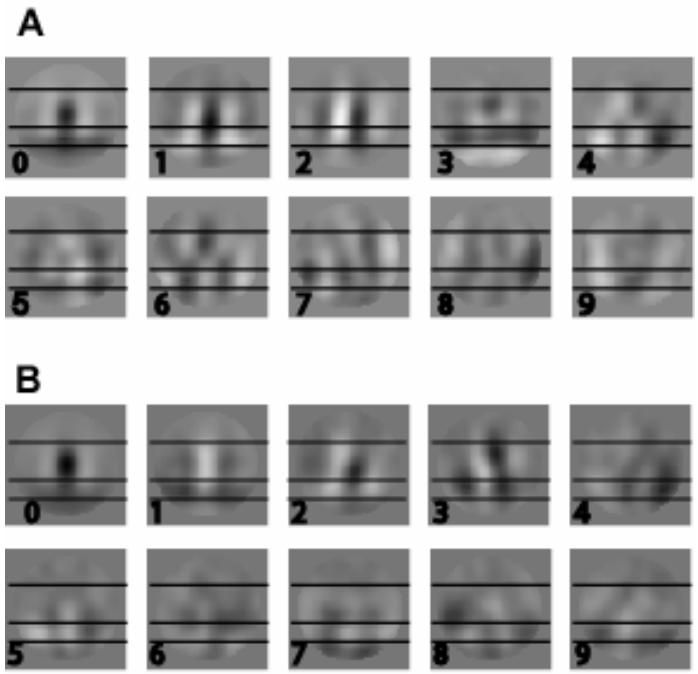

Figure S4. Eigenimages (factors) from the classification of active and inactive integrins obtained from the final classification step. The numbers denote the factor with the sorting done according to eigenvalue from highest (0) to lowest (9). The gray scaling is done from highest to lowest density for the individual panels unweighted by eigenvalue. (A) Inactive integrin eigenimages. (B) Active integrin eigenimages. The mask for both (A) and (B) consisted of a sphere of 55 pixels diameter (309 Å) centered within the

subvolume. The mask includes the membrane density which explains why top and bottom views separated from side views during the classification.

References

1. Dierksen, K., Typke, D., Hegerl, R., Walz, J., Sackmann, E. & Baumeister, W. (1995). Three-dimensional structure of lipid vesicles embedded in vitreous ice and investigated by automated electron tomography. *Biophys. J.* 68, 1416-1422.