Supplementary information

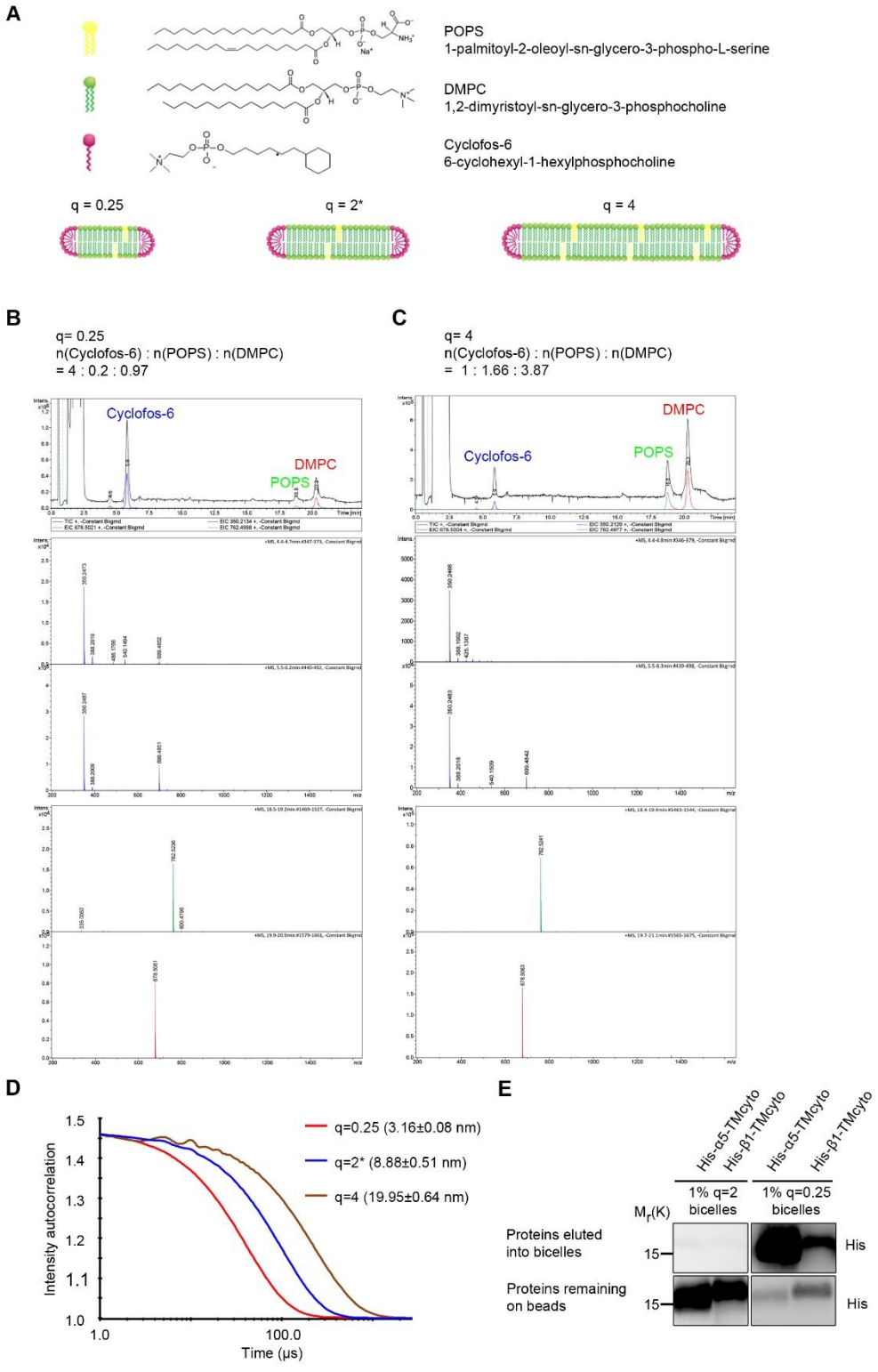

Figure S1. Lipid composition of bicelles. (A) Chemical structures of phospholipids used to generate the bicelles and cartoon illustration of architecture of and lipid distribution in bicelles. **(B, C)** The relative molar ratio of lipids and detergent in q=0.25 **(B)**, q=4 **(C)** bicelles was

determined by LC-MS. Cyclofos-6 (blue), POPS (green), DMPC (red). **(D)** Dynamic light scattering (DLS) curves of bicelles with different q-values to determine the hydrodynamic radii (nm) of the different bicelles (q=0.25: 3.16±0.08 nm; q=4: 19.95±0.64 nm; q=2*: 8.88±0.51nm, mean±s.e.m., n=3). **(E)** Western blot analysis for His-tagged α5 and β1 integrin TMcyto domains after incorporation into q=0.25 and q=2 bicelles. Proteins were successfully incorporated into q=0.25 bicelles but not into q=2 bicelles.

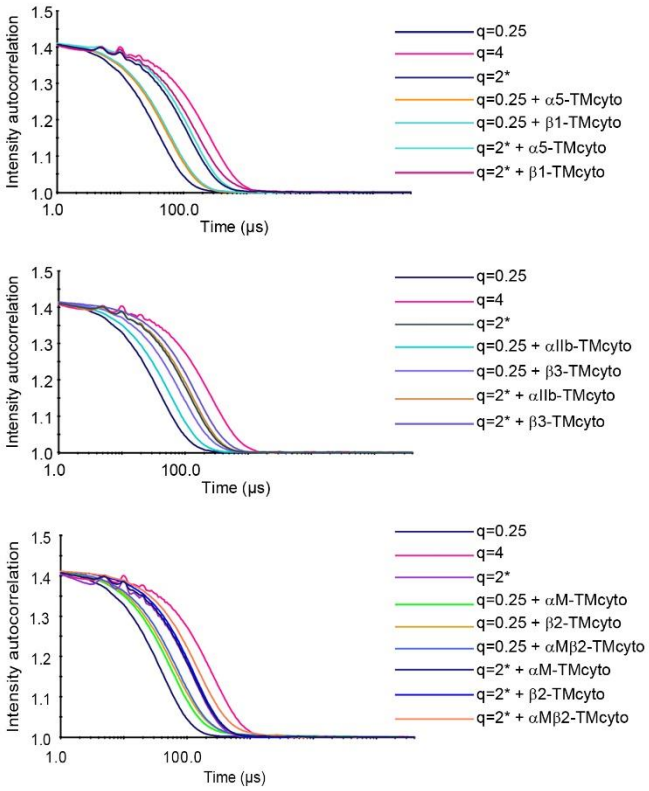

Figure S2. DLS measurement of bicelles before and after integrin TMcyto domain incorporation.

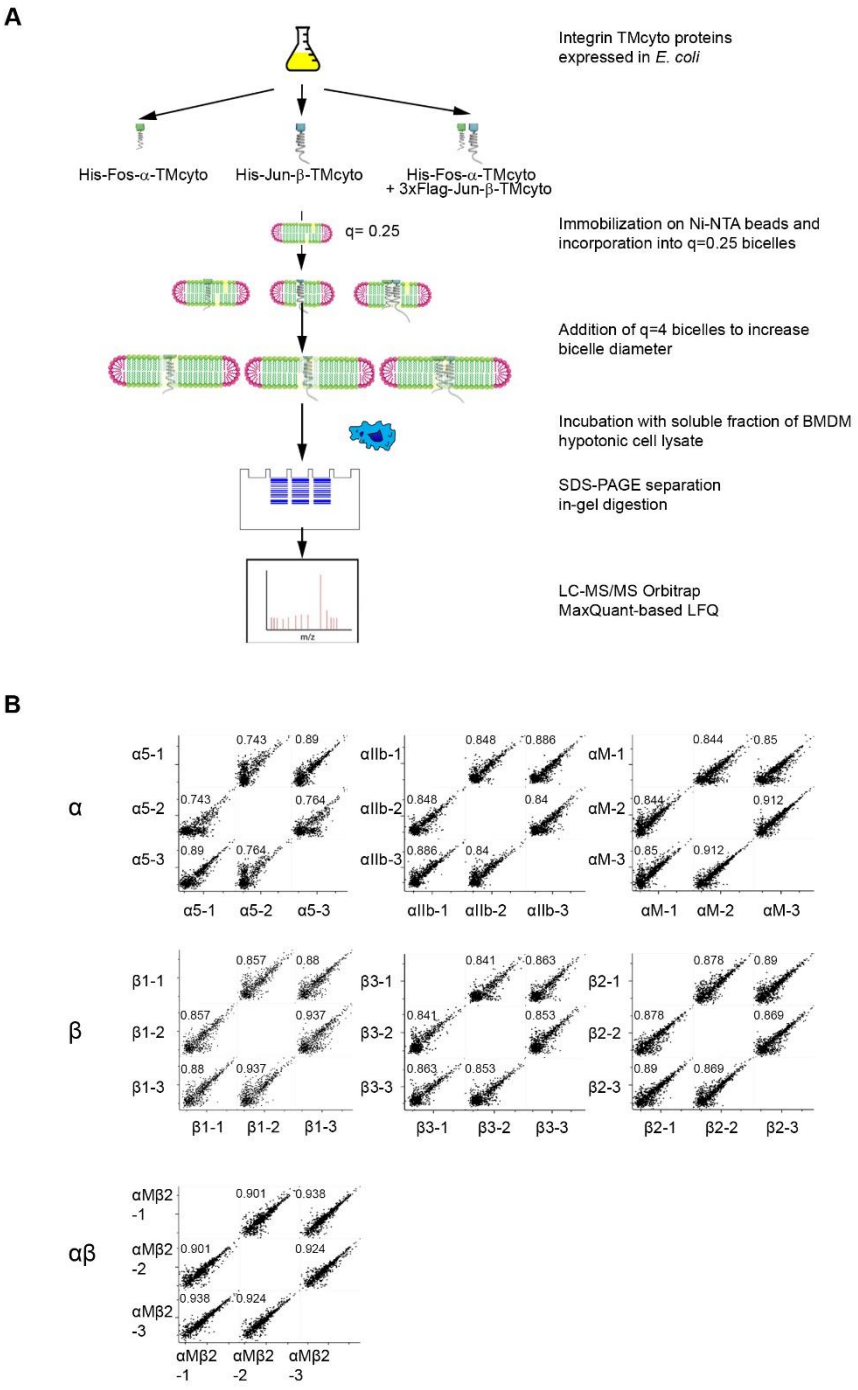

Figure S3. Workflow for incorporation of recombinant integrin TMcyto domains in bicelles, pull-downs and proteomic analysis. (A) Purified heterodimeric or monomeric integrin TMcyto domains were immobilized on Ni-NTA beads and incorporated into bicelle solutions (q=0.25). Bicelle size was increased by adding q=4 bicelles to obtain q=2* bicelles characterized by a hydrodynamic radius of ~10 nm, followed by the pull-down of proteins from hypotonic BMDM lysates. Interactors were identified by LC-MS/MS and analyzed by

MaxQuant LFQ intensity. **(B)** Multi-scatter plot of the protein LFQ intensities shows the reproducibility within the triplicate measurements of the different pull-downs. The indicated values correspond to Pearson correlation.

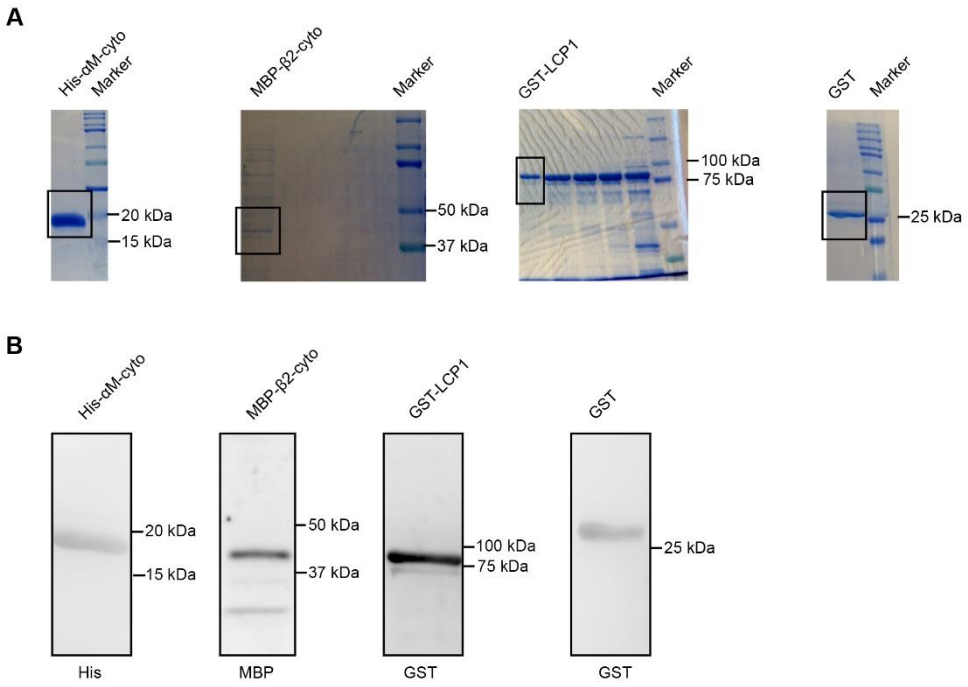

Figure S4. Expression of recombinant proteins used in pull-down experiments. (A, B) SDS-PAGE analysis of bacterially expressed and purified His-αM-cyto, MBP-β2-cyto, GST-LCP1, and GST stained either by Coomassie Blue **(A)** or by western blotting with antibodies against the indicated proteins **(B)**.

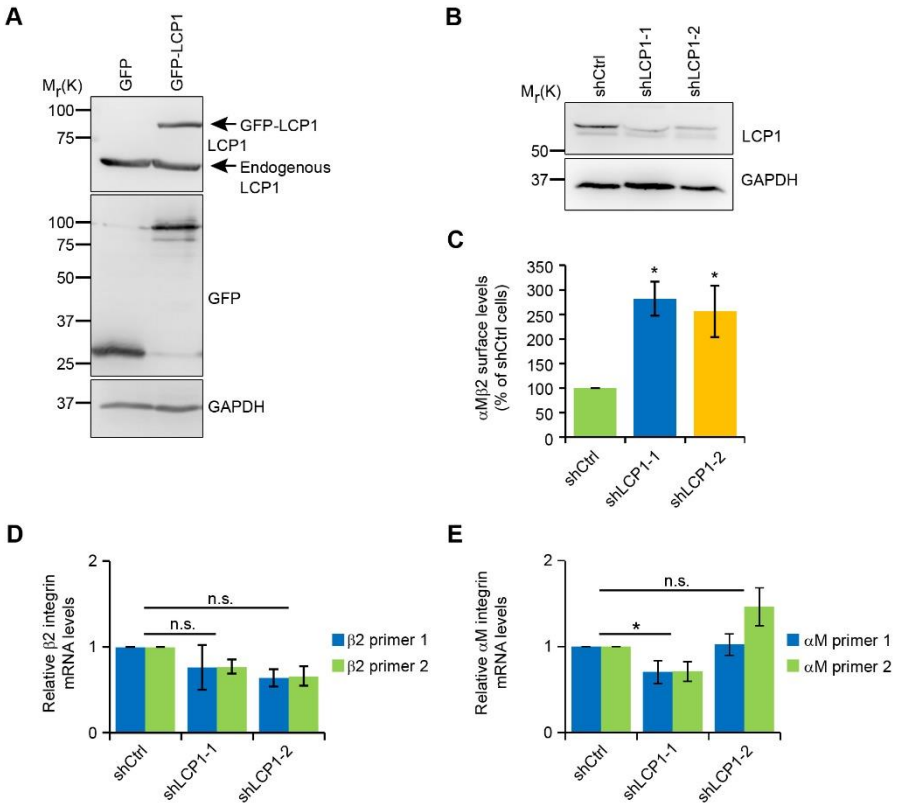

Figure S5. Regulation of αMβ2 integrin-surface levels by LCP1. (A) Western blot of GFP and GFP-LCP1 overexpressing PLB985 cells. **(B) Western blot analysis of** shRNA-mediated depletion of LCP1 in Raw264.7 cells. Cells were infected with shCtrl or two different LCP1 shRNA (shLCP1-1 and shLCP1-2). **(C)** FACS analysis of αMβ2 surface expression in LCP1-depleted Raw264.7 cells (values are normalized to shCtrl cells; mean±s.e.m., n=3, * $P < 0.05$). **(D, E)** Quantification of β2 **(D)** and αM integrin **(E)** mRNA levels in LCP1-depleted Raw264.7 cells determined by quantitative real-time PCR (values are normalized to shCtrl cells; mean±s.e.m., n=3, n.s. not significant, * $P < 0.05$).

Bicelles-integrin TMcyto	Hydrodynamic Radius (nm)
0.25-α5	4.54±0.22
0.25-β1	4.58±0.15
2*-α5	10.47±0.65
2*-β1	11.32±1.29
0.25-αIIb	12.65±1.67
0.25-β3	6.00±0.54
2*-αIIb	10.29±0.44
2*-β3	11.30±0.77
0.25-αM	4.41±0.20
0.25-β2	4.93±0.42
0.25-αMβ2	5.15±0.31
2*-αM	10.05±0.26
2*-β2	10.25±0.31
2*-αMβ2	11.17±0.49

Table S1: Hydrodynamic radii of bicelles after incorporation of indicated integrin α and β subunits determined by DLS.

Table S2: List of all proteins identified in pull-down experiments using bicelle-incorporated α or β TMcyto domains by mass-spectrometry (α5, β1, αIIb, β3, αM, and β2).

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Table S3: List of proteins with significant binding to lipid-incorporated α or β TMcyto domains. Overlap of proteins significantly binding to α or β TMcyto across the three different combinations (α5 vs β1; αIIb vs β3; αM vs β2).

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Table S4: List of proteins favoring binding to clasped αMβ2-TMcyto.

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