Supplementary information for

β2 integrins impose a mechanical checkpoint on macrophage phagocytosis

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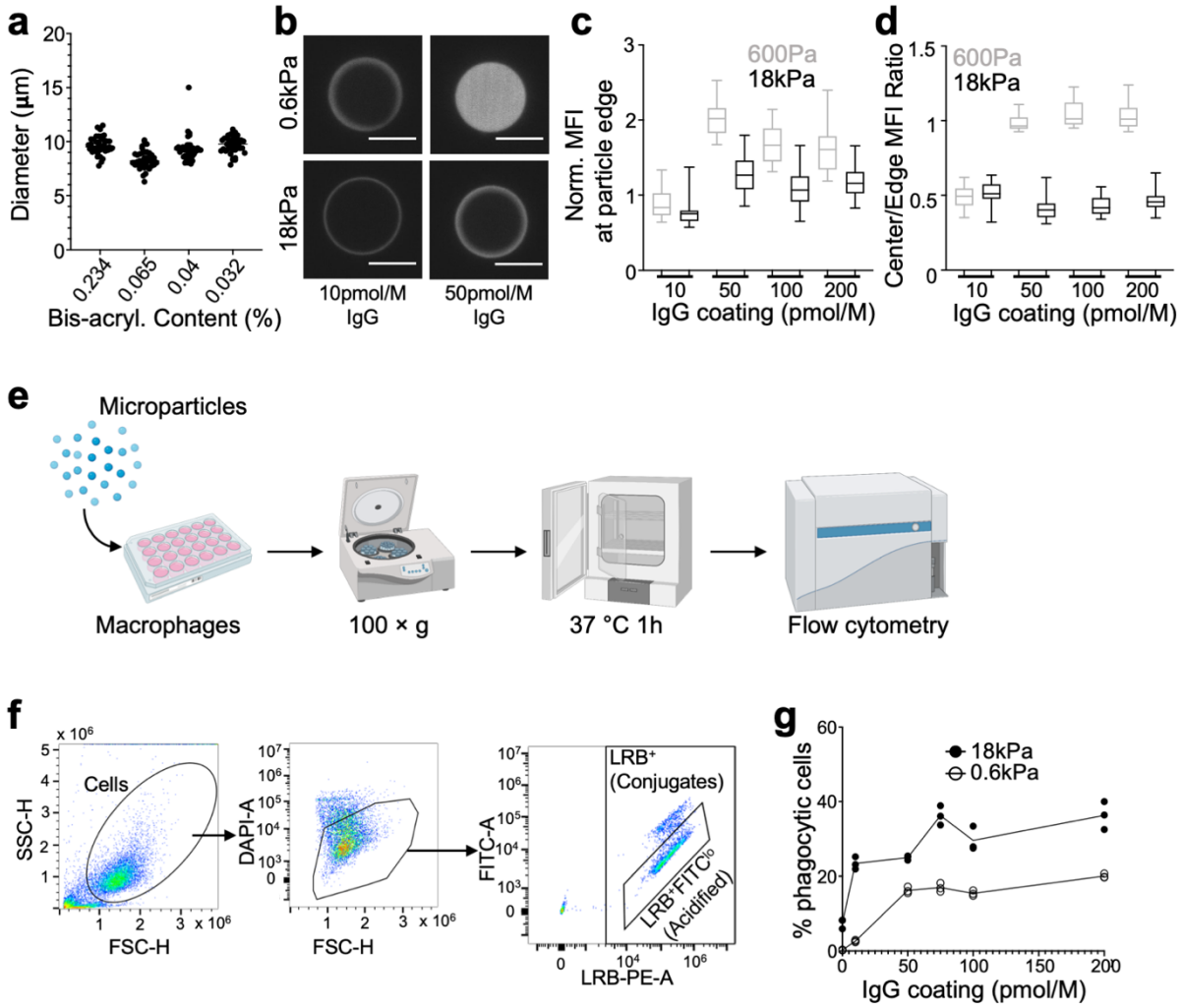
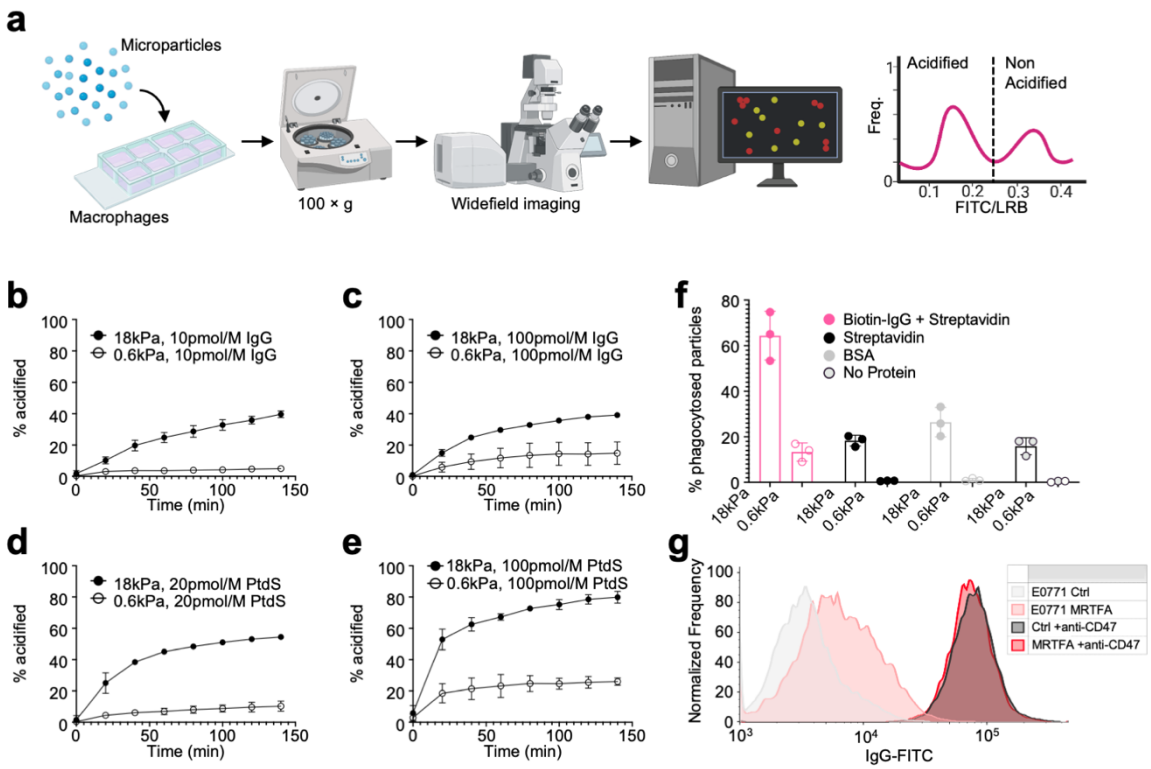
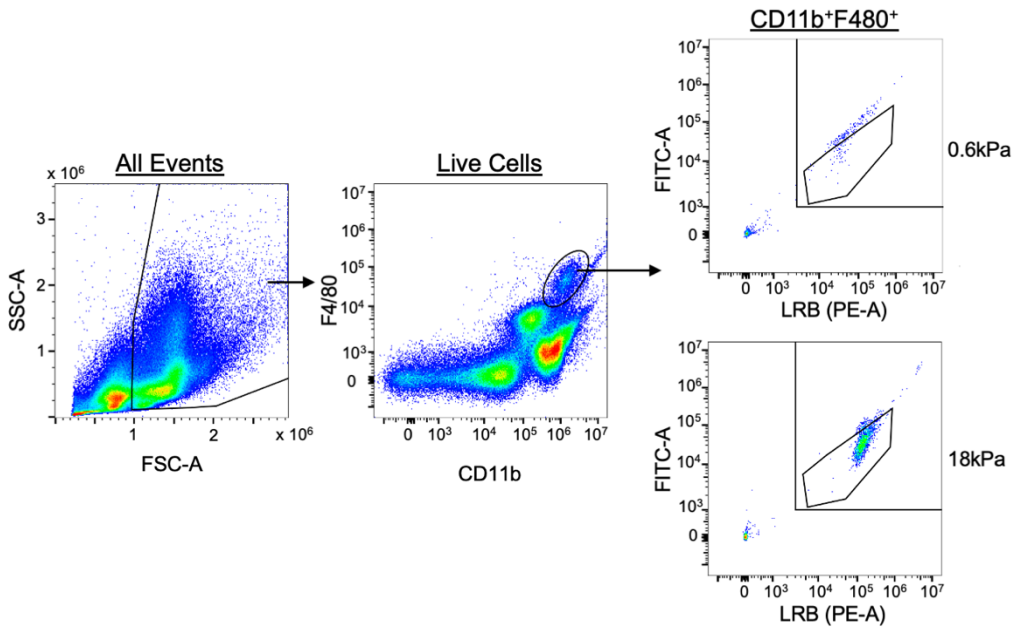


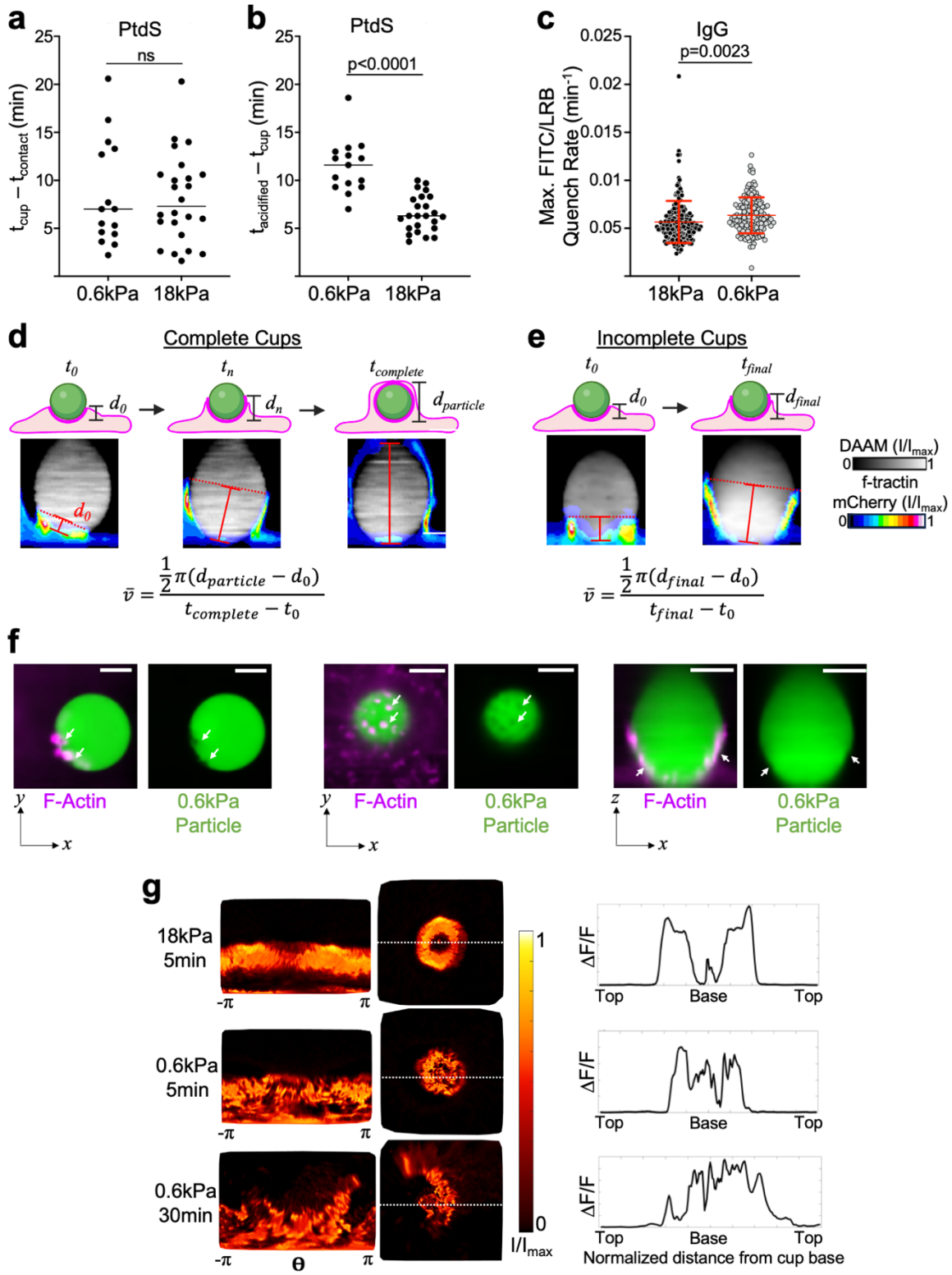
Supplementary Figure 1: A hydrogel microparticle system to interrogate phagocytic mechanosensing. a) Microparticle diameter, measured by confocal microscopy. n = 39 particles per condition. b) Confocal microscopy images of IgG-coated 18 kPa and 0.6 kPa particles counter-stained with anti-Mouse IgG. Scale bars = 10 µm. c-d) Quantification of effective IgG coating as a function of coating concentration. Maximum fluorescence intensity is normalized in each image to an AccuCheck normalization bead within the same field of view (not shown). Graphs show mean fluorescence intensity (MFI) at the particle edge (c) and the ratio of MFI at the center versus the edge of the particle (d). Boxes in c and d denote upper and lower quartiles, with error bars indicating maximum and minimum values. e) Schematic diagram for flow cytometric phagocytosis assay workflow. f) Representative gates for flow cytometric phagocytosis assay. Phagocytic efficiency is measured as $LRB^{+}FITC^{lo}$ events / all live cells. g) Phagocytic efficiency of stiff (18 kPa) and soft (0.6 kPa) DAAM particles, graphed as a function of IgG coating concentration. Schematic in e created with BioRender.com released under a Creative Commons



Supplementary Figure 2: Cargo rigidity promotes phagocytic uptake. a) Schematic diagram for wide-field microscopy-based phagocytosis assay and quantification. An automated analysis script was used to identify particles, measure FITC/LRB ratios, and categorize particles as acidified or non-acidified. b-e) BMDMs were challenged with 18 kPa or 0.6 kPa DAAM particles coated with IgG or PtdS, and phagocytic efficiency was measured over time by widefield live microscopy. n=3 technical replicates from values averaged across four fields of view per replicate. f) BMDMs were imaged together with 18 kPa or 0.6 kPa DAAM particles that had been coated as follows. Biotin-IgG + Streptavidin: standard coating protocol with 250 pmol/M Streptavidin and 10 pmol/M IgG + 50 μM FITC/LRB. Streptavidin: Particles conjugated with 250 pmol/M Streptavidin + 50 μM FITC/LRB. BSA: Particles conjugated with 250 pmol/M BSA + 50 μM FITC/LRB, No Protein: Particles conjugated with 50 μM FITC/LRB. Phagocytosis was quantified as the fraction of particles acidified per field of view after 2 h. Each point represents the mean of four fields of view in one technical replicate. Error bars denote SD, determined from 3 technical replicates. g) Representative flow cytometry plot of E0771 cells stained with anti-mouse IgG AF488, with or without pre-coating with mouse anti-CD47 antibody. Schematic in a created with BioRender.com



Supplementary Figure 3: Gating strategy for *in vivo* peritoneal macrophage phagocytosis assay. Macrophages were identified as $CD11b^{+}F4/80^{+}$ and phagocytic cells as $LRB^{+}FITC^{lo}$.



Supplementary Figure 4: Phagocytosis of soft targets is slow and prone to stalling. a-b) BMDMs were challenged with 18 kPa or 0.6 kPa PtdS-coated DAAM particles and imaged by widefield videomicroscopy. a) Recognition time, defined as the time difference between cup formation and

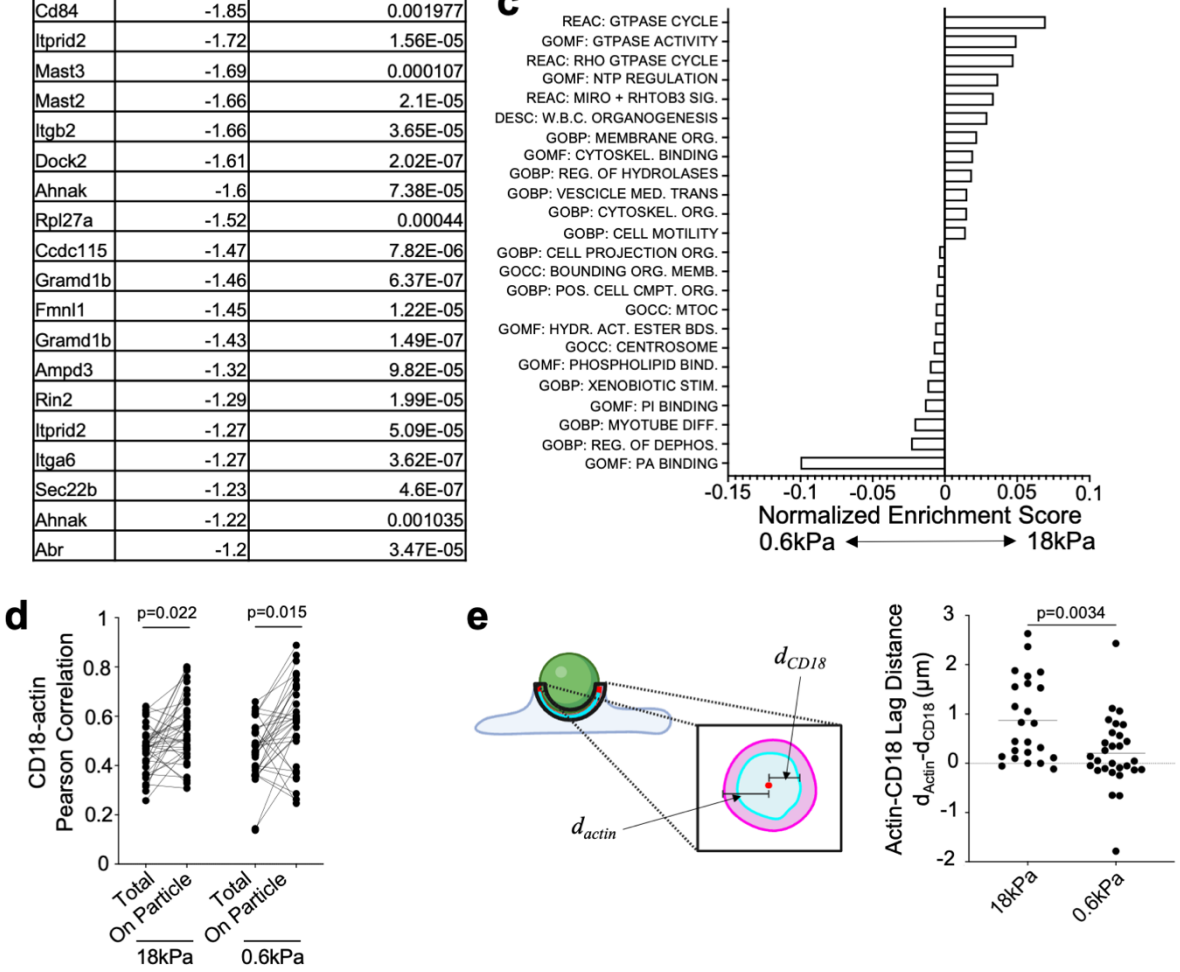
first contact. From left to right, n=15 and 24 cell-particle conjugates. b) Total acidification time, defined as the difference between particle acidification and cup formation. n=25 cells in each condition. In a-b, P-values determined by two-tailed Welch's t-test. ns: not significant. Mean values are indicated by horizontal lines in each column. c) BMDMs were challenged with 18 kPa or 0.6 kPa IgG-coated DAAM particles and imaged by widefield videomicroscopy. Graph shows maximum acidification rate, measured as the maximum rate of decrease in FITC/LRB ratio in each particle/cell interaction. From left to right, n=190 and 146 phagocytosis events, pooled from 3 technical replicates. Error bars indicate SD. P-value determined by two-tailed Welch's t-test. d-f) HoxB8-ER macrophages expressing F-tractin-mCherry were challenged with IgG-coated DAAM particles and imaged live by spinning disk confocal microscopy. d-e) Schematic diagram for quantification of cup advancement in live movies (see Methods), showing the protocol for cups that complete (d) and for cups that stall (e). f) Representative images of 0.6 kPa contacts highlighting interfacial F-actin protrusions. Left, z-slice through center of a particle with protrusion-induced distortions indicated by white arrows. Center, z-slice near the base of a particle, with upward protrusions and particle indentations indicated by white arrows. Right, Side-view of a particle with indentations in the particle base indicated by white arrows. Scale bars = 5μm. g) BMDMs were challenged with IgG-coated DAAM particles of different rigidities for various times, fixed and stained for F-actin, and then imaged by confocal microscopy. Center, stereographic views of representative phagocytic cups, formed on particles of the indicated stiffness at the indicated time. Views are centered on the base of the cup and colored by local F-actin intensity. Left, world map views of each cup, aligned such that the base of the phagocytic cup is downward. Right, linescan profiles of normalized F-actin across each cup, determined using the dotted lines in the central images. Schematics in d and e created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en.

a Phosphopeptides enriched on 18kPa

Gene	Log2FC	Adjusted p-value
Diaph2	-2.85	3.28E-05
Cfdp1	-2.39	1.86E-06
Hmox1	-2.23	1.53E-05
Itprid2	-2.19	9.2E-05
Cd84	-2.14	0.006444
Ahnak	-2.13	0.000169
Cd84	-1.85	0.001977
Itprid2	-1.72	1.56E-05
Mast3	-1.69	0.000107
Mast2	-1.66	2.1E-05
Itgb2	-1.66	3.65E-05
Dock2	-1.61	2.02E-07
Ahnak	-1.6	7.38E-05
Rpl27a	-1.52	0.00044
Ccdc115	-1.47	7.82E-06
Gramd1b	-1.46	6.37E-07
Fmnl1	-1.45	1.22E-05
Gramd1b	-1.43	1.49E-07
Ampd3	-1.32	9.82E-05
Rin2	-1.29	1.99E-05
Itprid2	-1.27	5.09E-05
Itga6	-1.27	3.62E-07
Sec22b	-1.23	4.6E-07
Ahnak	-1.22	0.001035
Abr	-1.2	3.47E-05

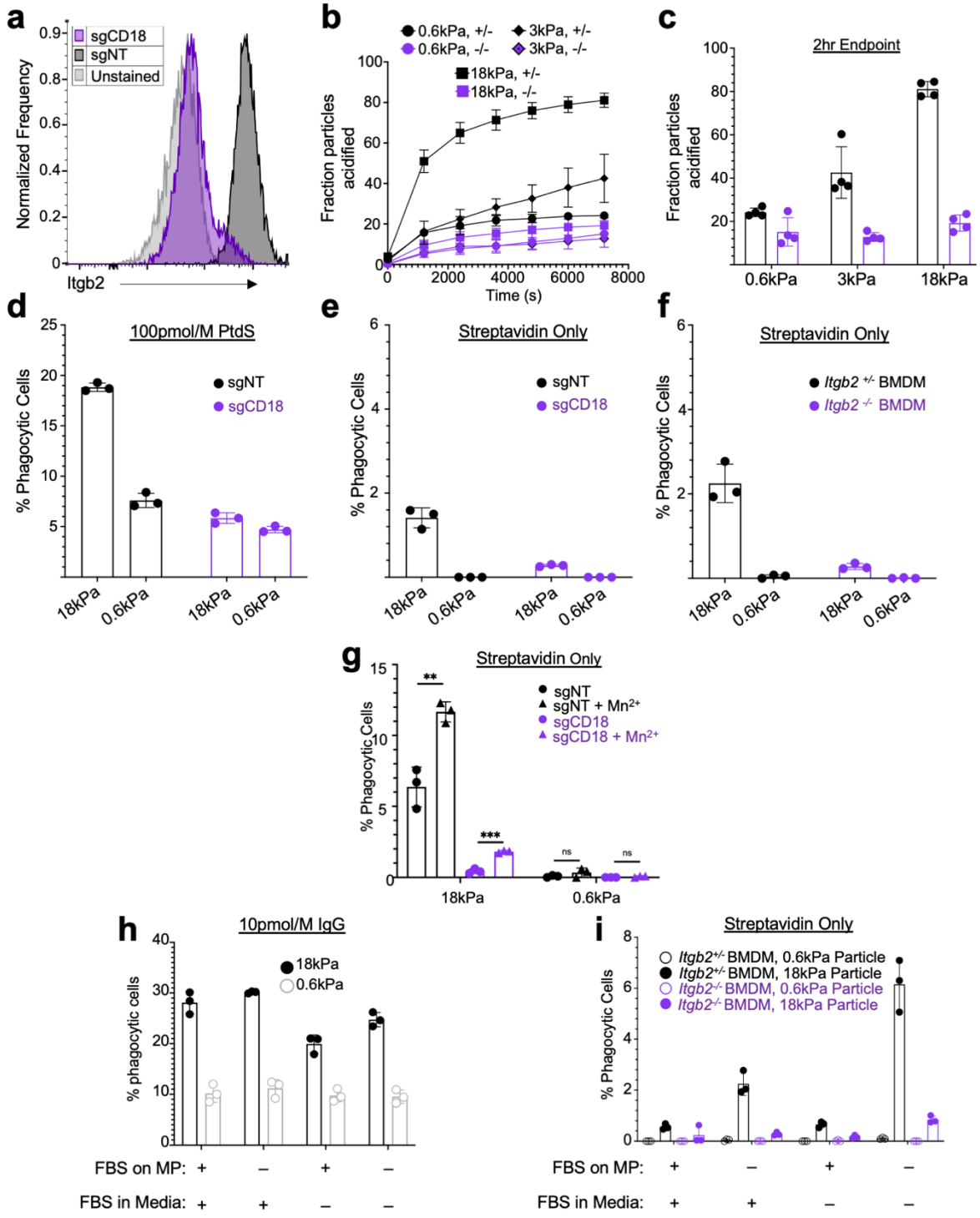
b Phosphopeptides enriched on 0.6kPa

Gene	Log2FC	Adjusted p-value
Nme1	1.04	2.32E-05
Usp15	1.06	6.59E-04
Ppp1r12a	1.12	7.71E-05
Gm20390	1.24	9.85E-06
Cip2a	1.24	2.99E-04
Ino80b	1.27	6.95E-04
Numa1	1.6	4.60E-04
Mtmr2	2.98	1.10E-05



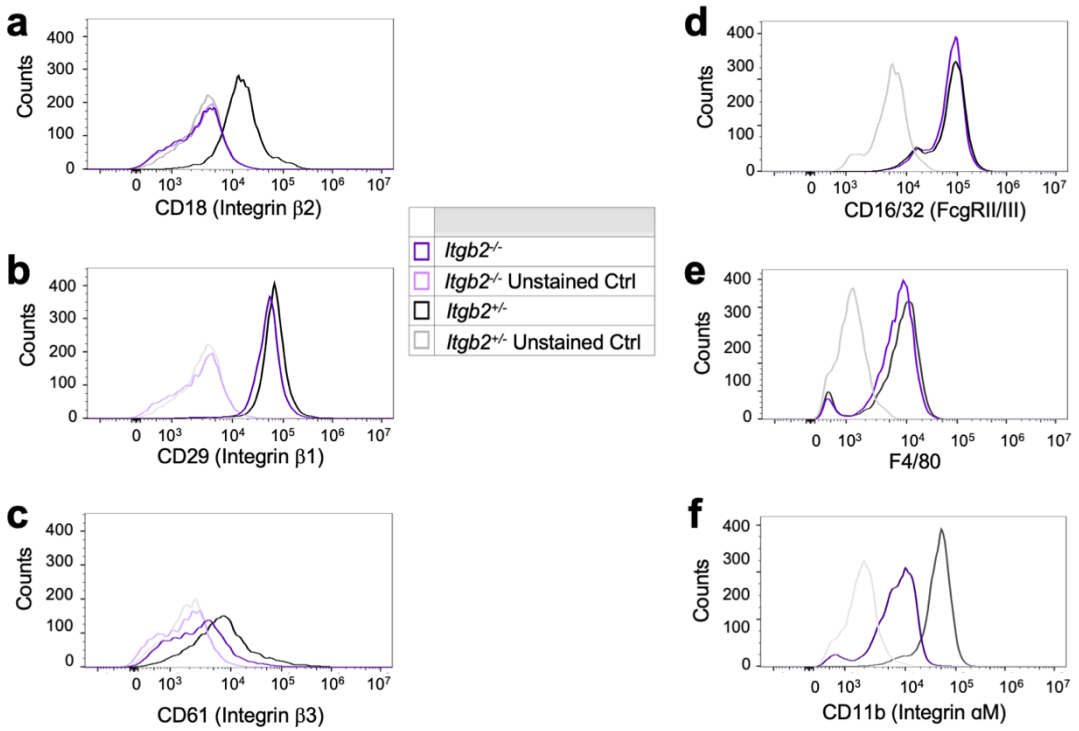
Supplementary Figure 5: β2 integrins respond differently to stiff and soft targets. a-c) BMDMs were challenged with stiff (18 kPa) and soft (0.6 kPa) IgG-coated DAAM particles and then subjected to phosphoproteomic analysis. Tables show genes with phosphopeptides enriched in the 18 kPa sample with Log2FC < -1.2 (a) or in the 0.6 kPa sample with Log2FC > 1 (b). Genes that appear in multiple rows contain multiple enriched peptides. See Methods for description of statistical analysis. c) Pathway enrichment analysis of the phosphopeptide data, showing all significantly enriched gene groups. Databases queried: REAC: Reactome Pathway Database, GOMF: Gene Ontology Molecular Function, GOBP: Gene Ontology Biological Process, GOCC: Gene Ontology Cellular Compartment, DESC: DESCARTES. d-e) BMDMs were challenged with

IgG-coated DAAM particles of different rigidities for various times, fixed and stained for F-actin and CD18, and then imaged by confocal microscopy. d) Colocalization between F-actin and CD18 (measured by Pearson's Correlation Coefficient) in the whole cell body (Total) and in the phagocytic cup (On Particle). From left to right, n=36 and 33 phagocytic cells. P-values determined by two-tailed paired t-test. e) CD18-actin lag distance, measuring the distance between the F-actin and CD18 signals at the leading edge of the phagocytic cup (see Methods), determined on 18 kPa and 0.6 kPa particles. Mean values are indicated by the horizontal line in each column. From left to right, n=24 and 28 phagocytic cups. P-values determined by two-tailed Welch's t-test. Schematic in e created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en.

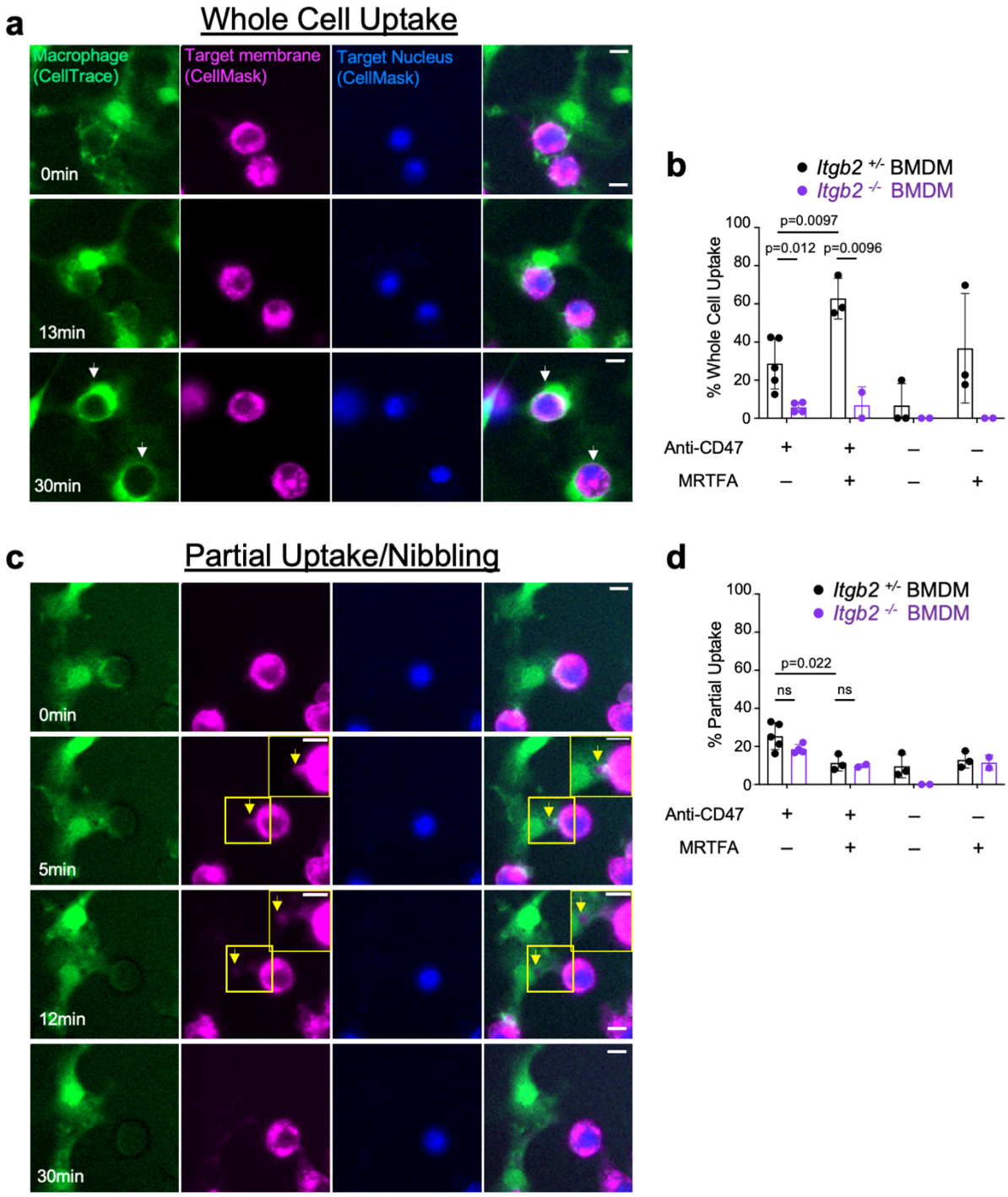


Supplementary Figure 6: β2 integrin signaling is necessary for phagocytic mechanosensing. a) Representative histogram from flow cytometric analysis of CD18 expression in Cas9^{+} HoxB8-ER derived macrophages transduced with either sgCD18 (purple) or sgNT (black) sgRNAs, compared to a unstained control. b-c) *Itgb2*$^{-/-}$ BMDMs and *Itgb2*$^{+/-}$ controls were imaged together with DAAM particles of differing rigidity coated with 10 pmol/M IgG. b) Kinetic analysis of

phagocytosis, using automated image analysis to identify acidified particles (see Methods). n = 3 technical replicates, each from 4 analyzed fields of view. c) Phagocytosis was quantified as the fraction of particles acidified per field of view after 2 h. n = 4 technical replicates, each from 4 analyzed fields of view. d-e) sgNT and sgCD18 HoxB8-ER macrophages were challenged with DAAM particles coated with PtdS (d) or streptavidin alone (e). Graph shows phagocytic efficiency measured by flow cytometry, n = 3 macrophage differentiations. f) *Itgb2*$^{-/-}$ BMDMs and *Itgb2*$^{+/-}$ controls were challenged with streptavidin-coated particles. Graph shows phagocytic efficiency measured by flow cytometry. g) Phagocytic efficiency of HoxB8-ER derived macrophages challenged with streptavidin-coated DAAM particles for 1 h in the presence or absence of 500 μM $MnCl_2$ added at the start of incubation. n=3 macrophage differentiations. P-values determined by two-tailed unpaired Student's T test. h) BMDMs were challenged in the presence or absence of serum (FBS in Media) with IgG-coated particles that were prepared either in the presence or the absence of serum (FBS on MP). Phagocytosis was assessed flow cytometrically after 1 h. n=3 technical replicates. i) *Itgb2*$^{-/-}$ BMDMs and *Itgb2*$^{+/-}$ controls were challenged in the presence or absence of serum (FBS in Media) with streptavidin-coated particles that were prepared either in the presence or the absence of serum (FBS on MP). Phagocytic efficiency was quantified by flow cytometry. n=3 technical replicates. All error bars denote SD.

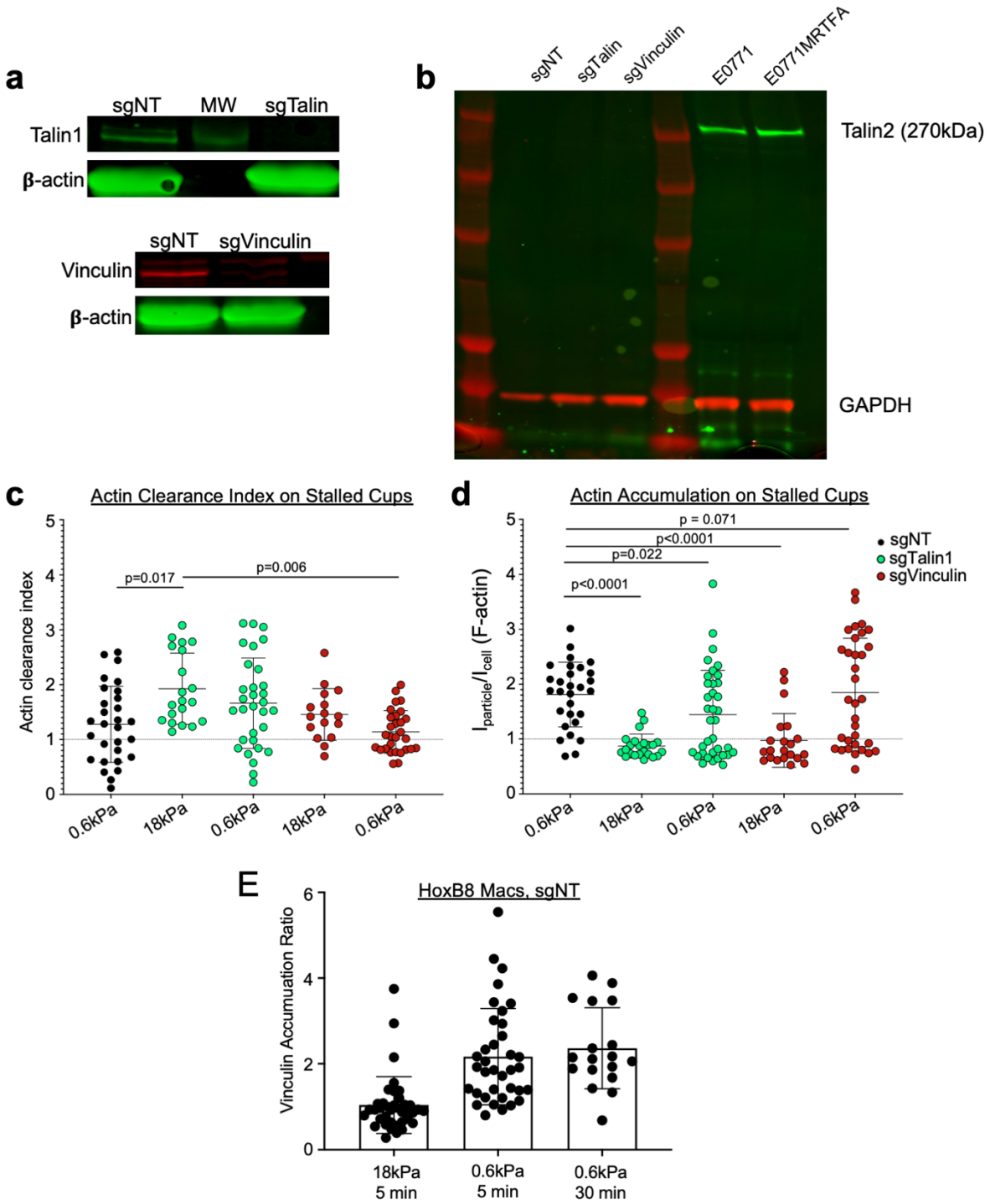


Supplementary Figure 7: *Itgb2*$^{-/-}$ macrophages develop normally and express equivalent levels of FcγRII/III. Representative flow cytometry plots of *Itgb2*$^{-/-}$ BMDMs and *Itgb2*$^{+/-}$ controls stained for various integrin and macrophage markers: a) CD18-APC-Cy7 (BioLegend 101414), b) CD29-APC-Cy7 (Biolegend 102225), c) CD61-APC (BD 564174), d) CD16/32-FITC (ThermoFisher Scientific 11-0161-82), e) F4/80-BV421 (BD 123137), f) CD11b-APC (Invitrogen 17-0112-82). All stains and measurements were performed independently.



Supplementary Figure 8: Macrophages attack cancer cells by gulping and nibbling. a) Representative time-lapse montages of macrophages engulfing whole live E0771 cancer cells incubated with anti-CD47 antibody. Whole-cell uptake (indicated by white arrows) was defined by internalization of target membrane (magenta) and nuclear stain (blue) within a corresponding cytoplasmic void in the macrophage (green). b) Quantification of whole cell uptake (See Methods)

of live E0771 target cells by *Itgb2*$^{-/-}$ BMDMs and *Itgb2*$^{+/-}$ controls. c) Representative time-lapse montage of a macrophage “nibbling” a small piece of membrane on a live target cell without nuclear uptake. Insets at higher contrast (yellow boxes) highlight the internalized membrane fragment (yellow arrows). d) Quantification of partial uptake (See Methods) of live E0771 target cells by *Itgb2*$^{-/-}$ BMDMs and *Itgb2*$^{+/-}$ controls. In b and d, for *Itgb2*$^{+/-}$ samples, from left to right, n=5, 3, 3, and 3 independent macrophage differentiations. For *Itgb2*$^{-/-}$ samples, from left to right, n=4, 2, 2, and 2 independent macrophage differentiations. P-values determined by two-tailed Student’s t-test. ns: not significant. All error bars denote SD. All scale bars = 10 μm.



Supplementary Figure 9: a) Western blot analysis of Cas9^{+} HoxB8-ER derived macrophages transduced with sgNT, sgTalin1, and sgVinculin. Talin1 expression is shown above (green) and Vinculin expression below (red). β-actin served as a loading control (green). MW = Molecular weight 250k band. See Methods for antibodies used. b) Western blot analysis of Talin2 expression in Cas9^{+} HoxB8-ER derived macrophages transduced with sgNT, sgTalin1, and sgVinculin.

E0771 cells were included as a positive control for Talin2 expression (green). GAPDH served as a loading control (red). Ladder = Precision Plus Kaleidoscope (Bio-Rad). c-d) $Cas9^+$ HoxB8-ER macrophages transduced with sgNT, sgTalin1, and sgVinculin were challenged for 30 min with 100 pmol/M IgG-coated DAAM particles. Graphs show F-actin clearance index (c) and F-actin accumulation ratio (d) for stalled phagocytic cups (see Methods). In c, from left to right, n=29, 23, 36, 21, and 33 cell-particle conjugates. In d, from left to right, n=29, 20, 31, 19, and 29 cell-particle conjugates. In c and d, P-values determined by Kolmogorov-Smirnov Test. e) Vinculin accumulation (see Methods) on phagocytic cups of sgNT HoxB8-ER macrophages. From left to right, n=38, 36, and 18 phagocytic cups. For all graphs, center bars denote mean and error bars denote SD.