

Supplemental material

Klapproth et al., <https://doi.org/10.1083/jcb.201903109>

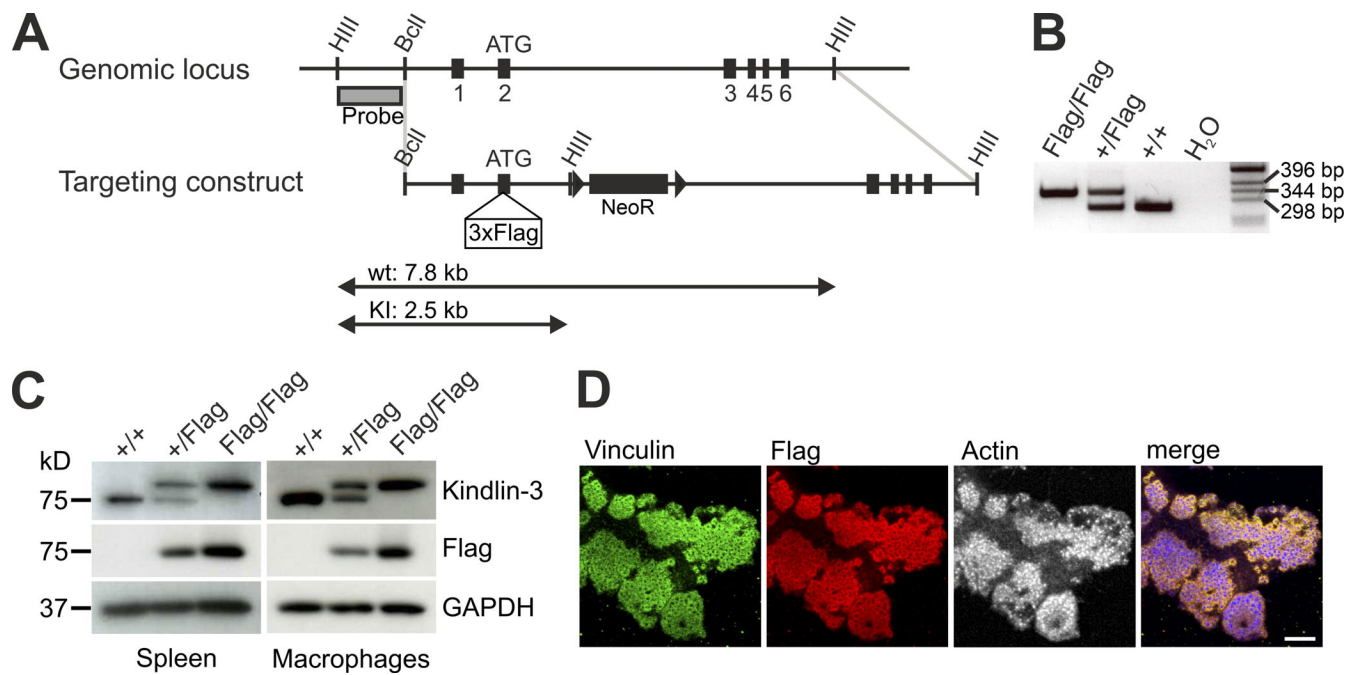


Figure S1. **Generation and characterization of Flag-tagged kindlin-3 knockin mice.** (A) Targeting strategy for the generation of mice expressing an N-terminally Flag-tagged version of kindlin-3, by targeting exon 2 of the endogenous kindlin-3 *klrsiv* gene locus. (B) Genomic PCR for genotyping WT (+/+), heterozygous (+/Flag) and homozygous (Flag/Flag) mutant animals. (C) Western blot analyses of lysates of spleen and bone marrow-derived macrophages from +/+, +/-Flag, and Flag/Flag mice. (D) IF staining for vinculin (green), flag peptide (red), and actin (white/blue in merge) of Flag/Flag preosteoclasts. Scale bar, 10 μ m.

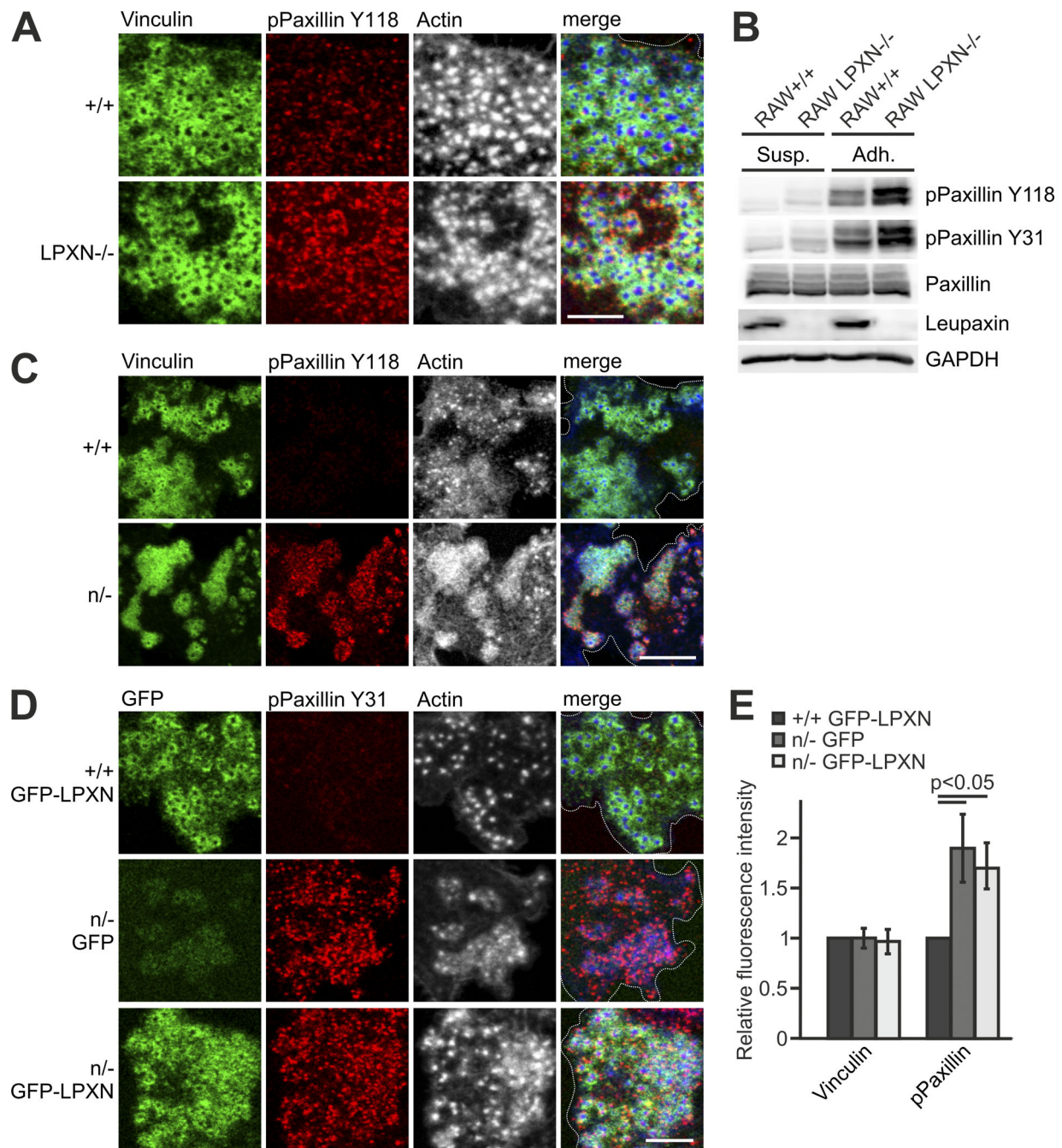


Figure S2. **Loss of leupaxin podosomal localization results in reduced paxillin phosphorylation.** (A) Confocal images of +/+ RAW cells and leupaxin^{-/-} RAW cells stained for vinculin (green), paxillin phosphorylated at Y118 (red), and actin (white/blue in merge). Scale bar, 5 μ m. (B) Analysis of paxillin phosphorylation in +/+ and leupaxin^{-/-} RAW cells kept either in suspension (Susp.) or adherent (Adh.) to fibronectin assessed by probing Western blots for paxillin phosphorylated at Y31 or Y118, total paxillin, and leupaxin. (C) Confocal images of vinculin (green), Y118-phosphorylated paxillin (red), and actin (white/blue in merge) IF stainings of preosteoclasts derived from WT and K3^{n/-} mice. Scale bar, 10 μ m. (D) Confocal images of +/+ preosteoclasts overexpressing GFP-tagged leupaxin and K3^{n/-} preosteoclasts transduced with either GFP alone or GFP-tagged leupaxin stained for phosphorylated paxillin at Y31 (red) and actin (white/blue in merge). Scale bar, 5 μ m. (E) Quantification of vinculin level and paxillin phosphorylation at Y31 in podosomal clusters of +/+ and n/- preosteoclasts transduced with GFP or GFP-tagged leupaxin by measuring MFI of confocal images. Values of WT cells expressing GFP-leupaxin were set to 1. n = 4. Dotted white lines mark cell borders.

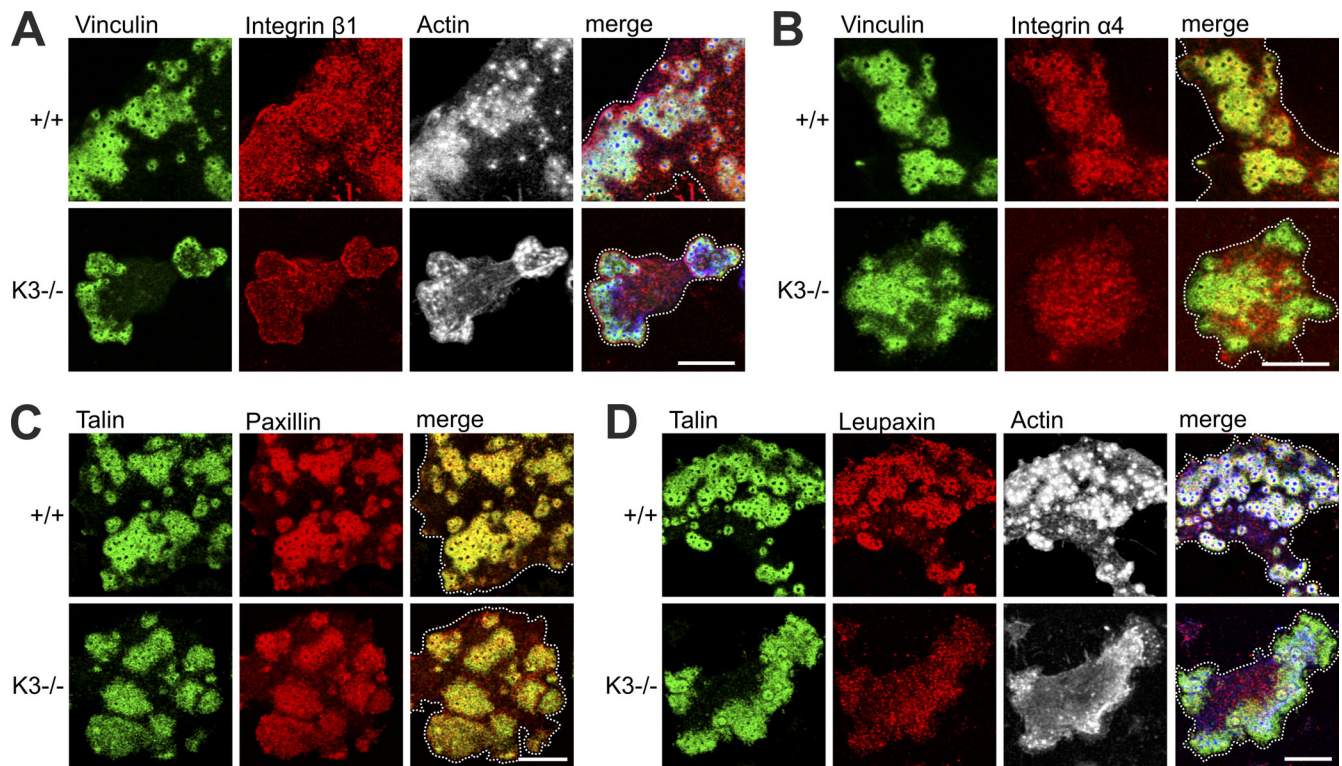


Figure S3. **In contrast to the plaque proteins vinculin, talin, and paxillin, $\beta 1$ and $\alpha 4$ integrins are not recruited to adhesion patches of kindlin-3-deficient preosteoclasts.** Confocal images of IF stainings for (A) vinculin (green), integrin $\beta 1$ (red), and actin (white/blue in merge); (B) vinculin (green) and integrin $\alpha 4$ (red); (C) talin (green) and paxillin (red); and (D) talin (green), leupaxin (red), and actin (white/blue in merge) in podosomes of K3^{+/+} and K3^{-/-} preosteoclasts. Scale bars, 10 μm . Dotted white lines mark cell borders.

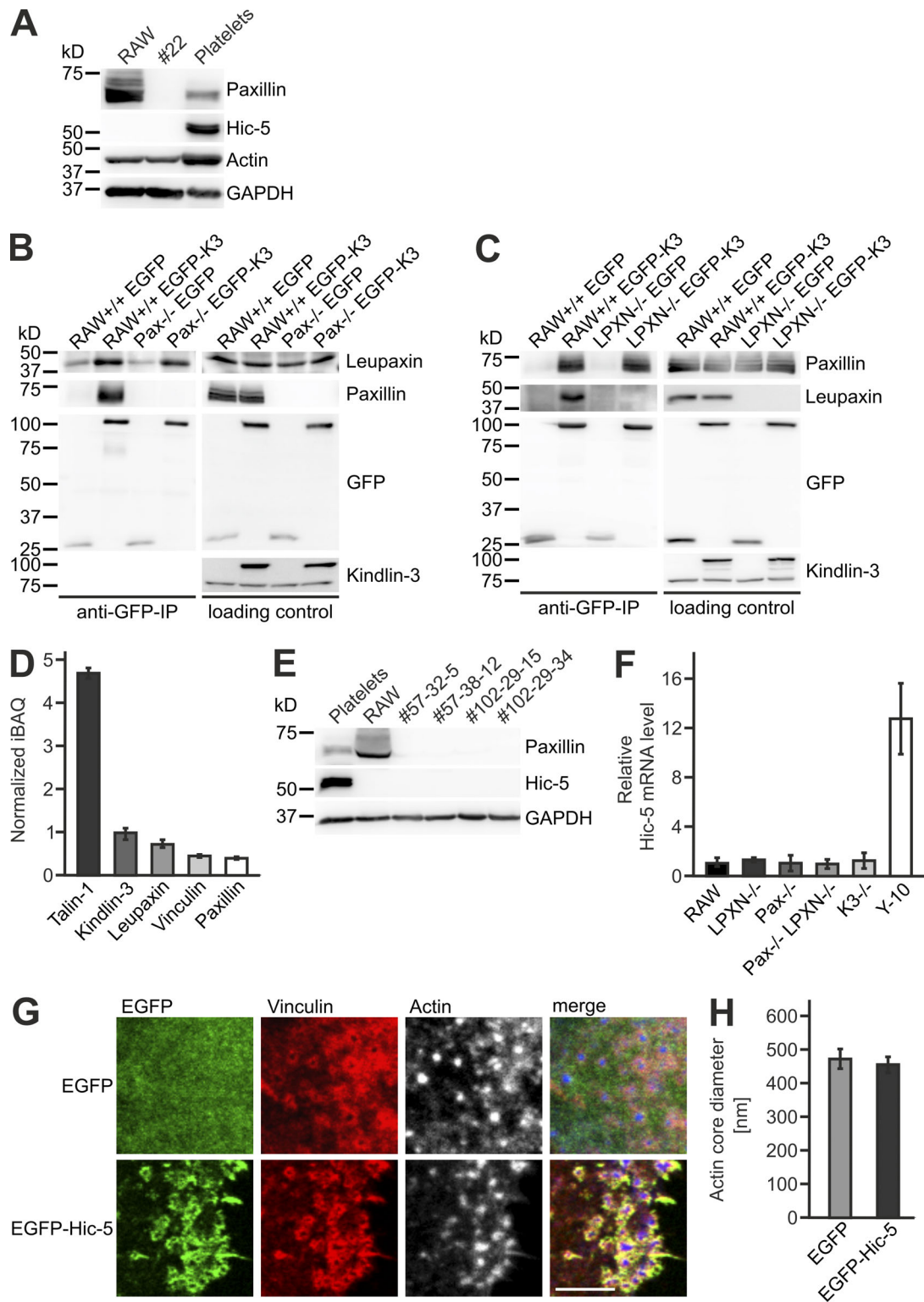


Figure S4. Paxillin and leupaxin do not compete for kindlin-3 binding in RAW cells. (A) Western blot analyses of +/+, paxillin^{-/-} (#22) RAW cells, and platelets for paxillin and Hic-5 expression. Actin and GAPDH served as loading controls. (B) GFP-IP from lysates of +/+ and paxillin^{-/-} RAW cells expressing either EGFP or EGFP-K3 analyzed for leupaxin and paxillin binding. (C) GFP-IP from lysates of WT and leupaxin-deficient RAW cells expressing either EGFP or EGFP-K3 analyzed for paxillin and leupaxin binding. (D) Quantification of the protein abundance of talin-1, kindlin-3, leupaxin, paxillin, and vinculin in RAW cells by whole proteome mass spectrometry analyses. All values are given in relation to the amount of kindlin-3. *n* = 4. (E) Western blot analyses of platelets and +/+ and different clones of paxillin/leupaxin dKO RAW cells for paxillin and Hic-5 expression. (F) Hic-5 mRNA level in +/+, leupaxin^{-/-}, paxillin^{-/-}, paxillin/leupaxin dKO, and K3^{-/-} RAW cells relative to GAPDH. WT controls were set to 1, and megakaryocyte precursor cell line Y-10 served as positive control. *n* = 4. (G) Confocal images of paxillin/leupaxin dKO RAW cells transfected with EGFP or EGFP-tagged Hic-5 stained for vinculin (red) and actin (white/blue in merge). Scale bar, 5 μ m. (H) Diameter of the podosomal actin cores in paxillin/leupaxin dKO RAW cells transfected with EGFP or EGFP-tagged Hic-5. *n* = 4.

Table S1. **Key resources**

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|---|--|---|---------------------------------------|---|
| Genetic reagent (<i>Mus musculus</i>) | K3 ^{-/-} | Moser et al., 2008 | RRID: MGI:2147790 | R. Fässler (Max Planck Institute of Biochemistry) |
| Genetic reagent (<i>M. musculus</i>) | K3 ^{n/-} | Klapproth et al., 2015 | RRID: MGI:3785479 | R. Fässler |
| Antibody | Rabbit anti-actin | Sigma-Aldrich | Cat. no. A2066; RRID: AB_476693 | WB (1:3,000) |
| Antibody | rabbit anti-cortactin | Cell Signaling Technology | Cat. no. 3502; RRID: AB_2115148 | WB (1:1,000) |
| Antibody | Rabbit anti-phospho-Cortactin Y421 | Cell Signaling Technology | Cat. no. 4569; RRID: AB_2276917 | WB (1:1,000) |
| Antibody | Rabbit anti-FAK | Cell Signaling Technology | Cat. no. 3285; RRID: AB_2269034 | WB (1:1,000) |
| Antibody | Rabbit anti-phospho-FAK Y397 | Cell Signaling Technology | Cat. no. 3283S; RRID: AB_2173659 | WB (1:1,000) |
| Antibody | Mouse anti-FLAG M2-Cy3 | Sigma-Aldrich | Cat. no. A9594; RRID: AB_439700 | IF (1:500) |
| Antibody | Mouse anti-GAPDH | Merck Millipore | Cat. no. CB1001; RRID: AB_2107426 | WB (1:20,000) |
| Antibody | Mouse anti-GFP | Homemade cell culture supernatant | | WB (1:3) |
| Antibody | Mouse anti-Hic-5 | BD Biosciences | Cat. no. 611164; RRID: AB_398702 | WB (1:500) |
| Antibody | Rabbit anti-His-tag | Cell Signaling Technology | Cat. no. 2365; RRID: AB_2115720 | WB (1:1,000) |
| Antibody | Alexa Fluor 488–conjugated anti-mouse IgG | Thermo Fisher Scientific | Cat. no. A-11001; RRID: AB_2534069 | IF (1:300) |
| Antibody | Alexa Fluor 546–conjugated anti-mouse IgG | Thermo Fisher Scientific | Cat. no. A-11003; RRID: AB_2534071 | IF (1:300) |
| Antibody | Alexa Fluor 488–conjugated anti-rabbit IgG | Thermo Fisher Scientific | Cat. no. A-11034; RRID: AB_2576217 | IF (1:300) |
| Antibody | Alexa Fluor 546–conjugated anti-rabbit IgG | Thermo Fisher Scientific | Cat. no. A-11010; RRID: AB_2534077 | IF (1:300) |
| Antibody | Alexa Fluor 546–conjugated anti-rat IgG | Thermo Fisher Scientific | Cat. no. A-11081; RRID: AB_2534125 | IF (1:300) |
| Antibody | IgG1 isotype control from murine myeloma | Sigma-Aldrich | Cat. no. M5284-.1MG; RRID: AB_1163685 | IP (5 µg) |
| Antibody | Anti-integrin α4-PE | BD Pharmingen | Cat. no. 553157; RRID: AB_394670 | IF (1:100) |
| Antibody | Anti-integrin αV-PE | BD Pharmingen | Cat. no. 551187; RRID: AB_394088 | IF (1:100) |
| Antibody | Anti-integrin β1 | R. Fässler (Max Planck Institute of Biochemistry) | | IF (1:5,000) |
| Antibody | Anti-integrin β3-PE | eBioscience | Cat. no. 12-0611-81; RRID: AB_465717 | IF (1:100) |
| Antibody | Rabbit anti-kindlin-3 | Markus Moser (Max Planck Institute of Biochemistry) | | IF (1:100), WB (1:3,000) |
| Antibody | Mouse anti-leupaxin | Abcam | Cat. no. ab181621 | IF (1:100), WB (1:1,000) |
| Antibody | Mouse anti-paxillin | Thermo Fisher Scientific | Cat. no. 610051; RRID: AB_397463 | WB (1:5,000), IP (5 µg) |
| Antibody | Rabbit anti-paxillin | Abcam | Cat. no. ab32084; RRID: AB_779033 | IF (1:300) |

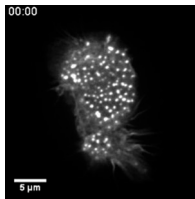
Table S1. **Key resources (Continued)**

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|---|---|----------------------------|--------------------------------------|-------------------------------|
| Antibody | Rabbit anti-paxillin Y31 | Thermo Fisher Scientific | Cat. no. 44-720G; RRID: AB_2533732 | IF (1:100), WB (1:1,000) |
| Antibody | Rabbit anti-paxillin Y118 | Thermo Fisher Scientific | Cat. no. 44-722G; RRID: AB_1501919 | IF (1:100), WB (1:1,000) |
| Antibody | Mouse anti-PTP-PEST | Thermo Fisher Scientific | Cat. no. MA1-12377; RRID: AB_2175233 | IF (1:100), WB (1:1,000) |
| Antibody | Rabbit anti-Pyk2 | Cell Signaling Technology | Cat. no. 3292; RRID: AB_2174097 | WB (1:1,000) |
| Antibody | Rabbit anti-phospho-Pyk2 Y402 | Cell Signaling Technology | Cat. no. 3291; RRID: AB_2300530 | WB (1:1,000) |
| Antibody | Anti-p-tyrosine (pY99) | Santa Cruz Biotechnology | Cat. no. sc-7020; RRID: AB_628123 | IF (1:50) |
| Antibody | Mouse anti-talin | Sigma-Aldrich | Cat. no. T3287; RRID: AB_477572 | WB (1:20,000) |
| Antibody | Rabbit anti-talin1 | Abcam | Cat. no. ab71333; RRID: AB_2204002 | IF (1:250) |
| Antibody | Mouse anti-vinculin | Sigma-Aldrich | Cat. no. V9131; RRID: AB_477629 | IF (1:500) |
| Peptide, recombinant protein | GeneArt Platinum Cas9 Nuclease (3 µg/µL) | Thermo Fisher Scientific | Cat. no. B25641 | |
| Peptide, recombinant protein | Fibronectin bovine plasma | Merck Millipore | Cat. no. 341631 | 5 µg/ml |
| Peptide, recombinant protein | Gelatin from pig skin, Oregon Green 488 Conjugate | Thermo Fisher Scientific | Cat. no. G13186 | 10 µg/ml |
| Peptide, recombinant protein | Recombinant mouse TNF-α | R&D Systems | Cat. no. 410-MT | 2 µg/ml |
| Commercial assay or kit | µMACS GFP Isolation Kit | Miltenyi | Cat. no. 130-091-125 | |
| Commercial assay or kit | µMACS DYKDDDDK Isolation Kit | Miltenyi | Cat. no. 130-101-591 | |
| Commercial assay or kit | Mouse Macrophage Nucleofactor Kit | Lonza | Cat. no. VAPA-1009 | |
| Commercial assay or kit | Glutathione Magnetic Agarose Beads | Jena Bioscience | Cat. no. AC-605-5 | |
| Commercial assay or kit | Pierce Protein A/G Magnetic beads | Thermo Fisher Scientific | Cat. no. 88802 | |
| Commercial assay or kit | PureCube 100 INDIGO Ni-Agarose beads | Cube Biotech | Cat. no. 75103 | |
| Commercial assay or kit | GeneArt Precision gRNA Synthesis Kit | Thermo Fisher Scientific | Cat. no. A29377 | |
| Commercial assay or kit | Neon Transfection System 10 µl Kit | Thermo Fisher Scientific | Cat. no. MPK1096 | |
| Commercial assay or kit | Lipofectamine LTX Reagent with PLUS Reagent | Thermo Fisher Scientific | Cat. no. A12621 | |
| Commercial assay or kit | Lipofectamine 3000 Transfection Reagent | Thermo Fisher Scientific | Cat. no. L3000015 | |
| Commercial assay or kit | RNeasy Mini Kit | Qiagen | Cat. no. 74104 | |
| Commercial assay or kit | iScript cDNA Synthesis Kit | Bio-Rad | Cat. no. 1708890 | |
| Commercial assay or kit | iQ SYBR Green Supermix | Bio-Rad | Cat. no. 1708880 | |
| Chemical compound, drug | M-CSF | Tebu-bio | Cat. no. 315-02-B | |
| Chemical compound, drug | Alexa Fluor 647 phalloidin | Thermo Fisher Scientific | Cat. no. A22287; RRID: AB_2620155 | IF (1:40) |
| Chemical compound, drug | RANKL | Tebu-bio | Cat. no. 315-11-B | |
| Software, algorithm | GraphPad Prism | GraphPad | RRID:SCR_002798 | |

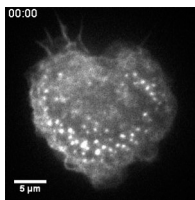
Table S1. **Key resources (Continued)**

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|------------------------------------|-------------------------------|-------------------------------|------------------------|------------------------|
| Software, algorithm | ImageJ | National Institutes of Health | RRID:SCR_003070 | |
| Software, algorithm | Photoshop | Adobe | RRID:SCR_014199 | |
| Other | Glass Bottom Microwell Dishes | MatTek Corporation | Cat. no. P35G-1.5-20-C | |

WB, Western blot; PMID, PubMed ID; RRID, Research Resource identifiers.



Video 1. **Time-lapse video of a WT preosteoclast expressing LifeAct-GFP acquired with a custom-made spinning disc confocal microscope.** Pictures were taken every 15 s for 5 min.



Video 2. **Time-lapse video of a K3^{n/-} preosteoclast expressing LifeAct-GFP acquired with a custom-made spinning disc confocal microscope.** Pictures were taken every 15 s for 5 min.

References

- Klapproth, S., F.A. Moretti, M. Zeiler, R. Ruppert, U. Breithaupt, S. Mueller, R. Haas, M. Mann, M. Sperandio, R. Fässler, and M. Moser. 2015. Minimal amounts of kindlin-3 suffice for basal platelet and leukocyte functions in mice. *Blood*. 126:2592–2600. <https://doi.org/10.1182/blood-2015-04-639310>
- Moser, M., B. Nieswandt, S. Ussar, M. Pozgajova, and R. Fässler. 2008. Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat. Med.* 14: 325–330. <https://doi.org/10.1038/nm1722>