

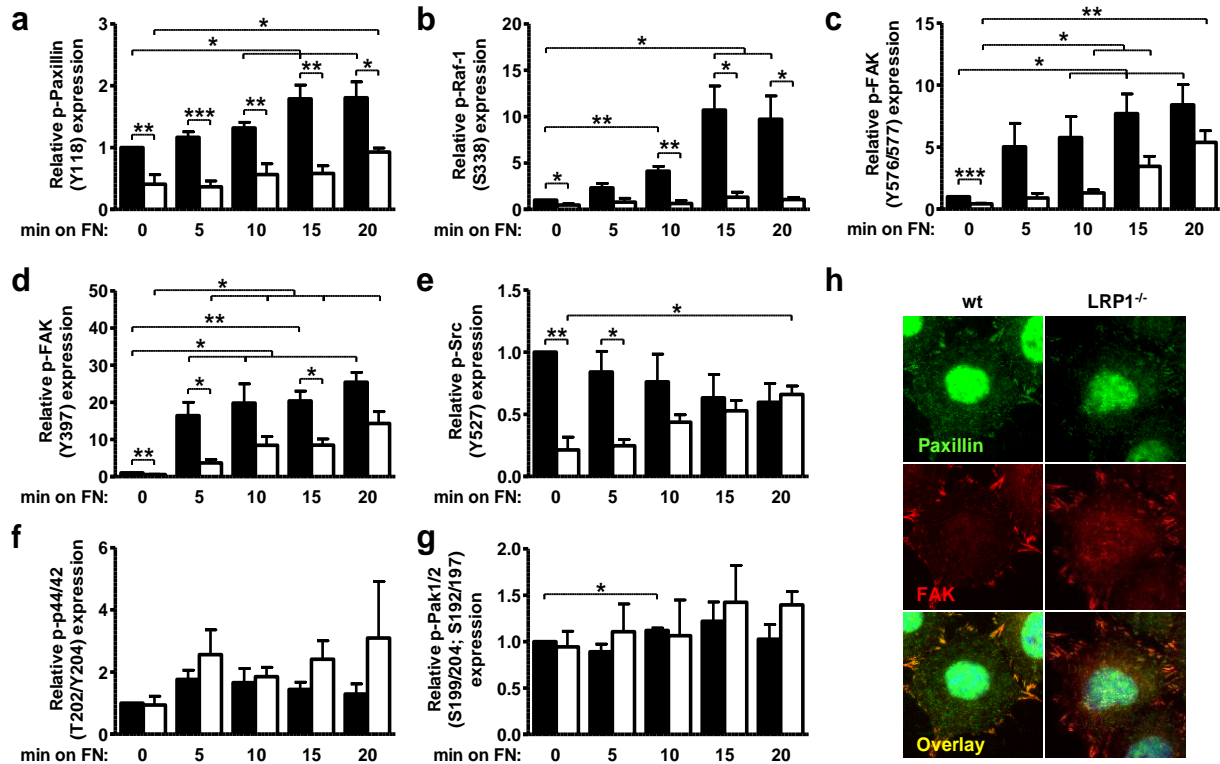
Supplementary Fig. S1 Effect of LRP1 depletion on cell adhesion, migration and cytoskeleton organization. **a** Representative images of crystal violet-stained wild-type or LRP1^{-/-} mouse embryonic fibroblasts (MEF) at 15 min after seeding onto fibronectin (FN) or vitronectin (VN)-coated plates (n = 4). **b** Representative images of primary human lung fibroblasts (pHLF) transfected with scrambled siRNA (si scr) or siRNA targeting LRP1 (si LRP1), plated onto fibronectin for 15 min and stained with crystal violet (n = 3). **c** Migration of wild-type and LRP1^{-/-} MEF on fibronectin as assessed by gap closure assay. Images were taken immediately after removing the silicone inserts (0 h) and after 16 h of culture. Representative images are shown (n = 5). **d** Phalloidin staining of wild-type and LRP1^{-/-} MEF. Nuclei were counterstained with DAPI, scale bar: 10 μ m (n = 3). **e** and **f** LRP1 knock-out in MEF **e** and knock-down in pHLF **f** were monitored by western blotting. β -actin served as a loading control

Title: Low density lipoprotein receptor-related protein 1 couples β 1 integrin activation to degradation

Journal: Cellular and Molecular Life Sciences

Lukasz Wujak, Ralph T Böttcher, Oleg Pak, Helena Frey, Elie El Agha, Ying Chen, Sigrid Schmitt, Saverio Bellusci, Liliana Schaefer, Norbert Weissmann, Reinhard Fässler, Malgorzata Wygrecka

Correspondence: Malgorzata Wygrecka; E-mail: malgorzata.wygrecka@innere.med.uni-giessen.de; Phone number: 0049 641 99 47482



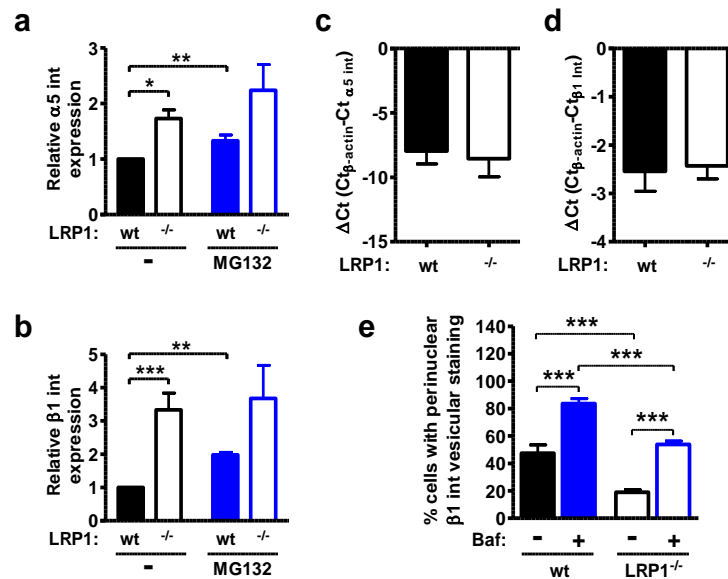
Supplementary Fig. S2 Effect of LPR1 depletion on phosphorylation and localization of focal adhesion molecules. **a** to **g** Densitometric analysis of the bands corresponding to phosphorylated and total forms of Paxillin **a**, Raf-1 **b**, FAK **c** and **d**, Src **e**, p44/42 **f** and Pak1/2 **g** detected in the protein lysates obtained from wild-type (black bars) and LRP1^{-/-} MEF (white bars) during adhesion to fibronectin. Results are expressed as a ratio of phosphorylated form to total protein band density. **h** Representative fluorescence microscopic images of wild-type and LRP1^{-/-} MEF at 40 min after seeding onto fibronectin stained with antibodies directed against Paxillin and FAK. Nuclei were counterstained with DAPI, scale bar: 10 μm (n = 3). Data in **a** to **g** are means ± SEM of three experiments; * $p \leq 0.05$, ** $p \leq 0.01$

Title: Low density lipoprotein receptor-related protein 1 couples $\beta 1$ integrin activation to degradation

Journal: Cellular and Molecular Life Sciences

Lukasz Wujak, Ralph T Böttcher, Oleg Pak, Helena Frey, Elie El Agha, Ying Chen, Sigrid Schmitt, Saverio Bellusci, Liliana Schaefer, Norbert Weissmann, Reinhard Fässler, Malgorzata Wygrecka

Correspondence: Malgorzata Wygrecka; E-mail: malgorzata.wygrecka@innere.med.uni-giessen.de; Phone number: 0049 641 99 47482



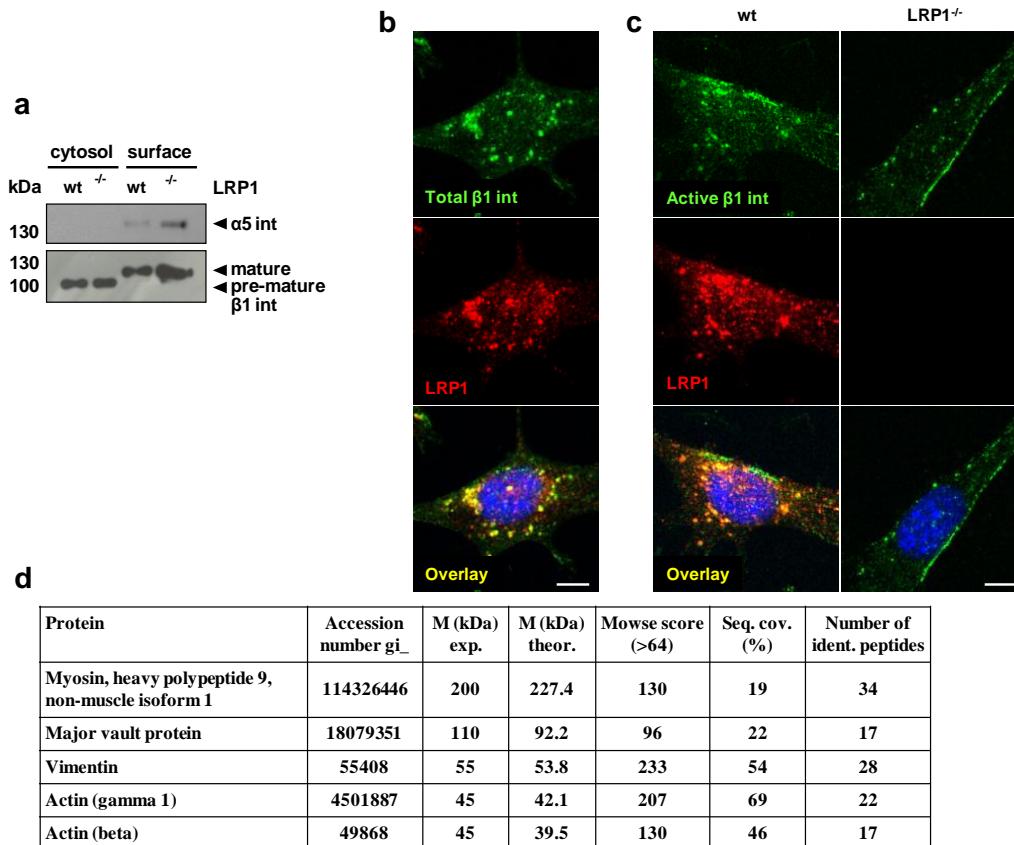
Supplementary Fig. S3 Protein and mRNA expression, and subcellular localization of integrin subunits in wild-type and LRP1^{-/-} MEF. **a** and **b** Densitometric analysis of the bands corresponding to $\alpha5$ **a** and $\beta1$ **b** integrin (int) detected in the protein lysates obtained from wild-type and LRP1^{-/-} MEF either untreated or treated with 10 μ M MG132 for 16 h. Data are expressed as a ratio of integrin to β -actin band density. **c** and **d** Real-time PCR analysis of $\alpha5$ **c** and $\beta1$ **d** integrin mRNA in wild-type and LRP1^{-/-} MEF. β -actin served as a reference gene. **e** Quantification of wild-type and LRP1^{-/-} MEF showing perinuclear vesicular staining for $\beta1$ integrin after treatment with 2.5 nM Bafilomycin A1 (Baf) for 16 h. For each cell type and condition, at least 50 cells were quantified from three independent experiments. Data are shown as a percentage of cells showing a perinuclear vesicular staining for $\beta1$ integrin. Data in **a** to **e** are means \pm SEM of three experiments; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Title: Low density lipoprotein receptor-related protein 1 couples $\beta1$ integrin activation to degradation

Journal: Cellular and Molecular Life Sciences

Lukasz Wujak, Ralph T Böttcher, Oleg Pak, Helena Frey, Elie El Agha, Ying Chen, Sigridd Schmitt, Saverio Bellusci, Liliana Schaefer, Norbert Weissmann, Reinhard Fässler, Malgorzata Wygrecka

Correspondence: Malgorzata Wygrecka; E-mail: malgorzata.wygrecka@innere.med.uni-giessen.de; Phone number: 0049 641 99 47482



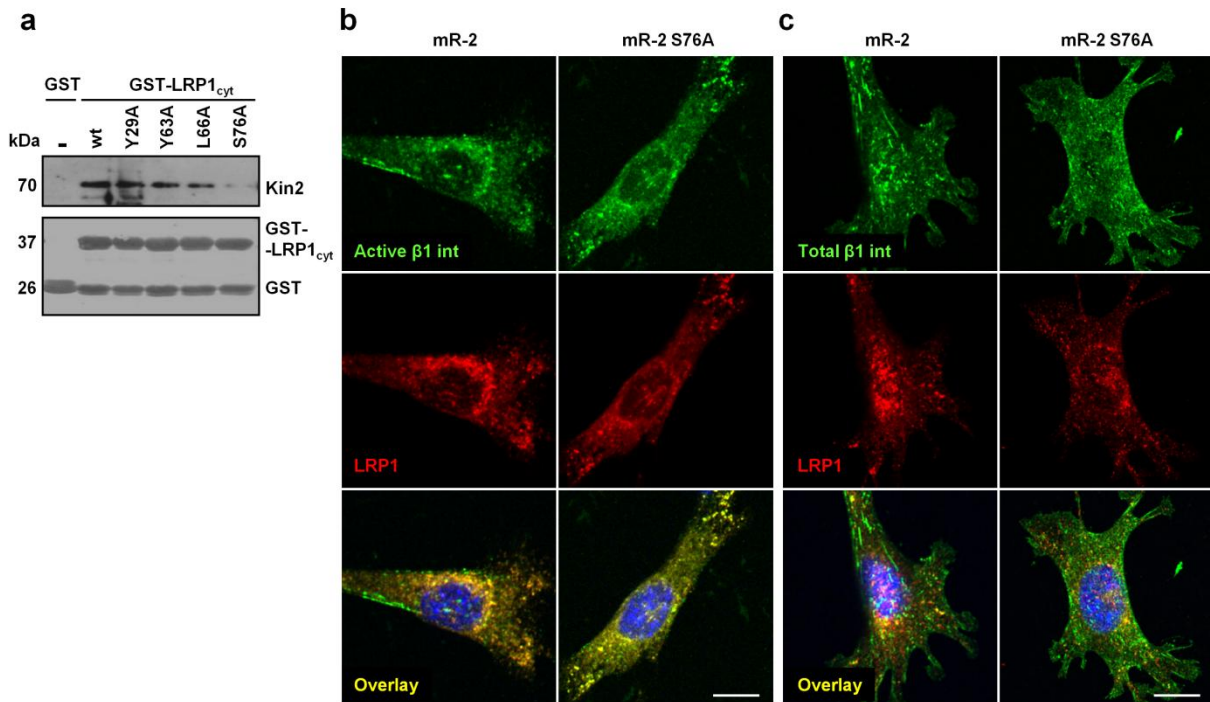
Supplementary Fig. S4 LRP1 regulates $\beta 1$ integrin intracellular trafficking. **a** Expression of $\alpha 5$ (upper panel) and $\beta 1$ (bottom panel) integrin (int) in cytosol and on the cell surface of wild-type or LRP1^{-/-} MEF was investigated by cell surface protein biotinylation. Western blots shown are representative of three experiments. **b** and **c** Subcellular distribution of LRP1 and endocytosed $\beta 1$ integrin in wild-type and LRP1^{-/-} MEF analyzed by confocal microscopy. Live cells were incubated with antibodies directed against total (clone MB1.2, **b**) or active (clone 9EG7, **c**) $\beta 1$ integrin for 1 h at 4 °C, shifted to 37 °C for 3 h 30 min, fixed and stained with anti-LRP1 antibody. Nuclei were counterstained with DAPI, scale bar: 5 μ m (n = 3). **d** Identification of proteins co-immunoprecipitated with anti-LRP1 antibody by MALDI-TOF mass spectrometry. Protein name, accession number (GenInfo Identifier, gi_), experimental (exp.) and theoretical (theor.) molecular mass in kDa, Mowse score, % of sequence coverage (seq. cov.) and number of identified peptides are listed. Mowse scores greater than 64 were considered a statistically significant match ($p < 0.05$)

Title: Low density lipoprotein receptor-related protein 1 couples $\beta 1$ integrin activation to degradation

Journal: Cellular and Molecular Life Sciences

Lukasz Wujak, Ralph T Böttcher, Oleg Pak, Helena Frey, Elie El Agha, Ying Chen, Sigrid Schmitt, Saverio Bellusci, Liliana Schaefer, Norbert Weissmann, Reinhard Fässler, Malgorzata Wygrecka

Correspondence: Malgorzata Wygrecka; E-mail: malgorzata.wygrecka@innere.med.uni-giessen.de; Phone number: 0049 641 99 47482



Supplementary Fig. S5 Effect of mutations in LRP1 cytoplasmic tail on kindlin2 binding and $\beta 1$ integrin subcellular distribution. **a** Determination of amino acid residues in the cytoplasmic tail of LRP1 critical for kindlin2 (Kin2) binding by GST pull-down. DNA sequence encoding GST-fused LRP1 cytoplasmic tail was mutated to create Y29A, Y63A, L66A or S76A variants or was left unaltered (wt). GST-fusion proteins were incubated with LRP1^{-/-} MEF lysates. Western blots shown are representative of four experiments. **b** and **c** LRP1^{-/-} MEF were transfected with vectors encoding unaltered LRP1 mini-receptor (mR)-2 or S76A variant. After seeding onto fibronectin, live cells were incubated with antibodies against active (clone 9EG7, **b**) or total (clone MB1.2, **c**) $\beta 1$ integrin (int) for 1 h at 4 °C, shifted to 37 °C for 3 h 30 min, fixed and incubated with anti-LRP1 antibody. Nuclei were counterstained with DAPI, scale bar: 5 μ m (n = 4)

Title: Low density lipoprotein receptor-related protein 1 couples $\beta 1$ integrin activation to degradation

Journal: Cellular and Molecular Life Sciences

Lukasz Wujak, Ralph T Böttcher, Oleg Pak, Helena Frey, Elie El Agha, Ying Chen, Sigrid Schmitt, Saverio Bellusci, Liliana Schaefer, Norbert Weissmann, Reinhard Fässler, Malgorzata Wygrecka

Correspondence: Malgorzata Wygrecka; E-mail: malgorzata.wygrecka@innere.med.uni-giessen.de; Phone number: 0049 641 99 47482