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Tissue inducible Lifeact expression allows visualization of actin dynamics in vivo and ex vivo

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Abstract

We describe here the development and characterization of a conditionally inducible mouse model expressing Lifeact-GFP, a peptide that reports the dynamics of filamentous actin. We have used this model to study platelets, megakaryocytes and melanoblasts and we provide evidence that Lifeact-GFP is a useful reporter in these cell types ex vivo. In the case of platelets and megakaryocytes, these cells are not transfectable by traditional methods, so conditional activation of Lifeact allows the study of actin dynamics in these cells live. We studied melanoblasts in native skin explants from embryos, allowing the visualization of live actin dynamics during cytokinesis and migration. Our study revealed that melanoblasts lacking the small GTPase Rac1 show a delay in the formation of new pseudopodia following cytokinesis that accounts for the previously reported cytokinesis delay in these cells. Thus, through use of this mouse model, we were able to gain insights into the actin dynamics of cells that could only previously be studied using fixed specimens or following isolation from their native tissue environment.

Introduction

The actin cytoskeleton is essential for several important cell processes such as motility, cytokinesis, morphology as well as vesicle trafficking (Mitchison and Cramer, 1996; Pelham and Chang, 2002; Ridley, 2006). Visualization of the actin cytoskeleton in live and fixed cells is therefore essential for cell biologists. The most commonly used reagents to visualize the actin cytoskeleton in fixed cells is probably fluorescent phalloidin and less commonly anti-actin antibodies are used (Tang et al., 1989). However, imaging of live cell actin dynamics was first made possible through the use of microinjection of fluorescently labeled actin (Wang, 1987) and later by the transfection of cells with vectors encoding fluorescently tagged actin probes (Westphal et al., 1997). Although the expression of fluorescent protein fused to actin shows colocalization with native actin and does not grossly interfere with its

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polymerization (Ballestrem et al., 1998) it may not participate properly in formin-mediated actin assembly (Chen and Pollard, 2011).

An important recent development is the identification of a 17-amino-acid peptide (Lifeact) isolated from yeast that binds specifically to filamentous actin (F-actin) with a low affinity and appears to be a faithful reporter of actin dynamics without disrupting them (Riedl et al., 2008). Lifeact has now been successfully used in several studies of actin dynamics in cells (Era et al., 2009; Li et al., 2010; Renkawitz et al., 2009) and even in live mice (Riedl et al., 2010b) and appears to perturb actin dynamics less than other fusion protein based probes (Deibler et al., 2011). However, it has also been reported that Lifeact cannot visualize some forms of nuclear actin deposits (Munsie et al., 2009) but these rod structures do not represent native filaments (Nishida et al., 1987), so this is not perhaps surprising. Here we report the construction of a cre-inducible mouse for the expression of Lifeact in a tissue-specific context. This has helped us to overcome two specific obstacles- firstly, working with primary cells that are not amenable to transfection (e.g. platelets and megakaryocytes) and secondly studying cell behaviour in a native environment, such as melanoblasts migrating in embryo skin.

Megakaryocytes (Mks) are multi-nucleated bone marrow cells, which undergo endomitosis and greatly enlarge before producing platelets into the bloodstream. Platelets are anucleate cell fragments that are responsible for blood homeostasis. Because of the polyploidy and the endomitotic processes in Mks it is difficult to transfect these cells (Isakari et al., 2007) while platelets are completely refractory to DNA transfection as they have no nucleus. Actin dynamics have not been visualized before in live megakaryocytes to our knowledge, but they have been visualized in platelets using GFP-actin (Calaminus et al., 2008b) and with a mouse expressing constitutive GFP-Lifeact (Riedl et al., 2010a). We report here that the conditional Lifeact mouse allows the visualization of live actin dynamics in both cell types and would also permit *in vivo* studies, since platelet-factor-4 (Pf4Cre) can be used to induce Lifeact expression in megakaryocytes and platelets (Tiedt et al., 2007b).

Melanoblasts are neural crest derived cells that migrate out of the neural tube of the mouse at around embryo day E9.5 and continue to migrate in the dermis and eventually in the epidermis by E13.5. These cells have not been grown in culture and a system for understanding their live motility has only recently been developed (Li et al., 2011; Lindsay et al., 2011; Mort et al., 2010). We recently used Lifeact to visualize actin dynamics in melanoblasts migrating in embryonic skin explants (Li et al., 2011) and here we extend this analysis and determine how actin dynamics are altered in Rac1 depleted melanoblasts during cytokinesis. Therefore, we demonstrate that the Cre-inducible Lifeact-GFP mouse is an excellent system to investigate the actin cytoskeleton dynamics of cells both *in vivo* and *in vitro*.

Results

A transgenic mouse line was created expressing tissue specific Lifeact-GFP by specifically targeting to the *Hprt* locus of the mouse X chromosome, a locus known to be permissive for heterologous gene expression (Bronson et al., 1996; Cvetkovic et al., 2000; Guillot et al., 2000). To attain cell-type specific Lifeact-GFP expression we created a *Hprt*-targetable vector containing a strong heterologous CAAG promoter and a floxed neomycin resistance (PGK-Neo-R) STOP cassette upstream of a Lifeact-GFP fusion cDNA and polyadenylation signal. Site-specific recombination of this construct at the mutated *Hprt* locus of HM1 ES cells restores *hprt* activity, allowing very tight selection. Following confirmation that clones were correctly targeted, mouse lines were derived by injecting ESC into C57BL/6J

blastocysts according to standard protocols (Nagy et al., 2003). Cre-recombinase expressing cells remove the STOP cassette and allow expression of Lifeact-GFP (Fig. 1A).

To allow lineage-restricted gene expression, Lifeact-GFP f/f mice were crossed with mice expressing cre-recombinase, which is regulated by the megakaryocyte lineage specific platelet factor-4 promoter (Pf4Cre) (Tiedt et al., 2007a). The Pf4Cre Lifeact-GFP mice were viable and healthy. To determine Lifeact expression levels and the quality of the signal during *in vitro* cell spreading, we isolated Pf4Cre Lifeact-GFP Mks from murine bone marrow. With confocal microscopy we imaged spreading Mks on a basement membrane isolated from the mouse peritoneum. During real-time spreading a clear signal of Lifeact-GFP could be detected showing filamentous actin based structures, such as filopodia and lamellipodia. Furthermore, we were able to visualize the formation of actin-rich adhesions called podosomes (Fig. 1B and Supplementary Movie 1), Which co-localized with both Arp2/3 complex and Wasp (Fig. S1A, B). The average spreading area of Lifeact-GFP Mks were comparable to litter-matched controls, indicating normal actin dynamics (Fig. S1B).

To investigate the suitability of Lifeact-GFP in platelets, we isolated Pf4Cre Lifeact-GFP platelets from murine blood. We used total internal reflection (TIRF) microscopy, which images the first 100 nm of the bottom of the cells (Mattheyses et al., 2010) to examine real-time dynamics of F-actin. In this way we imaged spreading platelets forming filopodia (Fig. 1C, S3A and Supplementary Movie 2). The expression of Lifeact-GFP does not apparently compromise the spreading of platelets, as the Arp2/3 complex is located at the rim of the spread platelets in Lifeact positive platelets as well as in control platelets (Fig. S2A) and (Calaminus et al., 2008a). Furthermore, we analyzed the average surface area of Lifeact-GFP platelets and showed no difference compared to the control (Fig. S2B). Mks and platelets did not show any abnormalities in their morphology, during spreading or in functionality, confirming that the expression of Lifeact-GFP has no detectable impact on these cells under the tested experimental conditions.

To test the suitability of Lifeact-GFP expression in melanocyte lineage *in vivo*, we crossed Lifeact-GFP mice with mice expressing cre recombinase under control of the tyrosinase promoter (TyrCre) (Delmas et al., 2003). TyrCre Lifeact-GFP mice were born healthy at the expected Mendelian ratio, with no alteration in coat color (Data not shown). To characterize melanocyte Lifeact-GFP expression, melanocytes were isolated from 1-day old pups and counterstained with fluorescent phalloidin. Fluorescent phalloidin staining perfectly overlapped with Lifeact-GFP fluorescence (Fig. S3). Live imaging of melanocytes on 2D glass indicated that Lifeact-GFP labelled dynamic lamellipodial actin at the cell periphery, as well as stable stress fibers formed in the cell body (Fig. 1D and Supplementary Movie 3). In addition, we also studied localization of F-actin in melanoblasts *in vivo* by imaging E15.5 embryonic trunk skin as previously described (Li et al., 2011; Mort et al., 2010). Generally, Lifeact-GFP exhibited diffuse localization in the body of melanoblasts, but we could also detect strong Lifeact-GFP flashes at the tips of protrusions (Fig. 2A, yellow arrow and Supplementary Movie 4). We confirmed the specificity of Lifeact-GFP localization by comparing it with GFP localization in melanoblasts in embryo explants from TyrCre Z/EG embryos (Li et al., 2011; Novak et al., 2000). Z/EG is a double reporter transgene, which drives GFP-expression in the melanoblast lineage, when crossed to TyrCre background (Li et al., 2011). In contrast to Lifeact-GFP localization, GFP localized throughout the body and protrusions of melanoblasts (Fig. 2A and Supplementary Movie 4), thus confirming the specificity of Lifeact-GFP localization in melanoblasts *in vivo*. To test whether Lifeact-GFP compromised any cytoskeletal functions, we compared the melanoblast migration speed and persistence in TyrCre Lifeact-GFP and TyrCre Z/EG explants. The melanoblast migration speed, and persistence were indistinguishable between TyrCre Lifeact-GFP and TyrCre Z/

EG explants (Fig. 2B and C). Thus Lifeact-GFP had no effect on melanoblast migration within the epidermis.

We previously used TyrCre Z/EG Rac1 f/f mice to demonstrate that Rac1-depleted melanoblasts took 3-fold longer complete cytokinesis as compared to TyrCre Z/EG control mice (Li et al., 2011). To characterize this phenotype further, we crossed Lifeact-GFP mice onto the TyrCre Rac1 f/f background to visualize the actin dynamics during cytokinesis. Similar to melanoblasts in TyrCre Z/EG Rac1 f/f explants, melanoblasts in TyrCre Lifeact-GFP Rac1 f/f explants also showed a delay in cytokinesis (Fig. 3A and Supplementary Movie 5). Surprisingly, Rac1 knockout caused no delay during the early stages of cytokinesis, including the rounding up of the mother cell and furrow formation (Fig. 3B). About 10 minutes after the furrow formation, control daughter melanoblasts started forming F-actin rich protrusions (Fig. 3A, yellow arrow and Supplementary Movie 5), which led to daughter cell separation. However, Rac1 depleted daughter melanoblasts took about 60 minutes to initiate F-actin rich protrusions after the furrow formation (Fig. 3A and B; Supplementary Movie 5). Despite this huge delay in formation of protrusions, control and Rac1 depleted daughter melanoblasts took about the same time to separate once the F-actin rich protrusions were formed (Fig. 3B). Together, these data indicate that Rac1 is particularly required for formation of F-actin rich protrusions for daughter cell separation during cytokinesis. This defect does not occur in vitro in primary melanocytes grown on fibronectin coated 2D cell culture plate (Li et al., 2011) stressing the importance of studying cells in their native 3D environment.

Discussion

The Lifeact probe was developed in 2008 (Riedl et al., 2008) and mice constitutively expressing Lifeact were developed in 2010 (Riedl et al., 2010b) as a useful tool for investigation of actin dynamics in vivo. Since its development, many groups have found Lifeact to be extremely useful for cell biological experiments and certainly in our experience it can be brighter and less disruptive to cell dynamics than GFP-actin. We now have developed a conditional Lifeact-GFP mouse that allows selective Lifeact expression. This mouse will permit study of actin dynamics in tissues of interest with a normal nonfluorescent tissue background. We find that Lifeact expression works well when driven by different cre recombinases, such as TyrCre (Delmas et al., 2003) and PF4Cre (Tiedt et al., 2007a), so we expect this model to be widely useful for in vivo studies of actin dynamics.

As intravital imaging becomes increasingly popular, it is important to develop new tools to allow the study of molecular dynamics in live cells in mice. Lifeact-GFP is an excellent probe for actin dynamics in Mks and platelets and we could detect no morphologic or functional abnormalities of these cells expressing Lifeact-GFP. Mks form dynamic podosomes, which we image here for the first time in live cells. We also saw dynamic actin in platelet lamellipodia as platelets spread, indicating that Lifeact-GFP is expressed well in platelets. The expression of inducible Lifeact in these cell types makes it possible to study F-actin dynamics in real-time without interfering with other processes. However in order to fully understand mechanisms of the actin cytoskeleton in Mks and platelets further investigations will focus on in vivo imaging. It could thus be interesting in future studies to determine the involvement of actin in platelets during blood clot formation as well as the role of F-actin in Mks migrating from the osteoblastic to the vascular niche in vivo.

The role of Rac1 in cytokinesis is not well explored. It has previously been reported that Rac1 is inhibited at the cleavage furrow so that activation of RhoA can trigger contraction and ingression of the furrow (Kamijo et al., 2006). Our study using Lifeact-GFP has

revealed a previously unknown positive role for Rac1 in regulation of the separation of the two daughter cells. We initially found that melanoblasts lacking Rac1 showed delayed cytokinesis and that although cells rounded up with normal timing, they took longer to divide (Li et al., 2011). Use of Lifeact-GFP revealed that while melanoblasts concentrate most of their F-actin in the cleavage furrow, they do not separate from each other until they have formed F-actin based protrusions facing away from the furrow. Cytokinesis delay in Rac1 KO melanoblasts results from a large lag in the time taken to make the first F-actin based protrusions after the cytokinetic furrow ingresses. Thus, we reveal a positive role for Rac1 in melanoblast cytokinesis that would not have been possible to identify without live imaging of these cells in native embryo skin. When we cultured cells out into a fibronectin coated dish, they did not show a Rac1 dependent delay in cytokinesis (Li et al., 2011). It will be interesting to see whether Rac1 is required for cytokinesis with cells seeded on a surface with a defined stiffness that resembles the tissue stiffness in a living skin. Our study thus emphasized the importance of studying cells in vivo or ex vivo in the native tissue and environment.

Materials and Methods

Mouse Genetics

All experiments involving animals were performed according to UK home office regulations.

Generation of Lifeact-mEGFP Gene-Targeted Mice—Conditional Lifeact-mEGFP mice were generated by targeting a lox-stop-lox transgene under the control of a CAG promoter to the HPRT locus (Bronson et al., 1996; Samuel et al., 2009). The CAG lox-stop-lox transgene was constructed by firstly replacing the SA of plasmid pBigT (Srinivas et al., 2001) with the CAG promoter from pTurbo-Cre (gift of Prof. Tim Ley, The Genome Center at Washington University, St. Louis, US). The PGK promoter and the EM7 promoter sequence from pL452 were subsequently inserted into pBigT. CAAG by recombineering in *E. coli* (Liu and Bambara, 2003). Short 5' and 3' arms homologous to sequences within the Hprt targeting plasmid pSKB1 (Bronson et al., 1996) were then inserted up and downstream of the expression cassette to generate pHprt.CAAG.STOP. A cDNA encoding the LifeAct-mEGFP fusion protein was then cloned downstream of the lox-stop-lox. The transgene was then recombineered into pSKB1 (Bronson et al., 1996) to generate the final targeting vector.

The vector was linearised and electroporated into Hprt-deficient HM1 ES cells, cultured on a DR4 mouse embryonic fibroblast feeder layer (Tucker et al., 1997). Homologous recombinants were selected in medium containing HAT supplement (Sigma). Correct targeting of the vector to the Hprt locus on both the 5' and 3' sides was confirmed using PCR on genomic DNA prepared from HAT-resistant colonies. PCR genotyping was done using Expand Long Template (Roche) according to the manufacturer's recommendations. Primers used for genotyping targeted ES cells were 5' GTTGCTGAGGCAAAAATAGTGTAAT and CCATTACCGTAAGTTATGTAACGC and 3' CTACCTAGTGAGCCTGCAAACTG and ATGTAAGTGCTAGGAATTGAACCTG.

Following identification of correctly targeted clones, mouse lines were derived by C57BL/6J blastocysts according to standard protocols (Nagy et al., 2003). Germline transmission was identified by coat colour and correct transmission of the transgene was confirmed by PCR to the eGFP sequences using the primers AAGTTCATCTGCACCACCG and TCCTTGAAGAAGATGGTGCG.

Generation of Rac f/f, TyrCre and Pf4Cre mice—The Rac1 floxed mice in C57BL6/J background were previously described (Walmsley et al., 2003). Tyrosinase Cre A (TyrCre) mice in C57BL6/J background were previously described and were a kind gift of Lionel Larue (Delmas et al., 2003). There are no phenotypes associated with TyrCre^{+/-} Rac1 f/+ animals. Therefore, heterozygous genotype with genotypes: TyrCre^{+/-} Rac1 +/+, TyrCre^{0/0} Rac1 +/+, TyrCre^{0/0} Rac1 f/+ served as control for this study. LacZ/EGFP (Z/EG) mice in C57BL6/J background were previously described (Novak et al., 2000). The platelet-factor 4 Cre (Pf4Cre) mice were bred in C57BL6/J background as previously reported (Tiedt et al., 2007a). Genotyping of the transgenes was performed as previously described (Delmas et al., 2003; Novak et al., 2000; Tiedt et al., 2007; Walmsley et al., 2003) and by the company Transnetyx (Memphis, TN).

Cell culture

Primary mouse melanocytes were cultured as previously reported (Larue et al., 1992). Briefly, 1 day old TyrCre Lifeact-GFP mice were humanely sacrificed and briefly sterilized by rinsing twice in ice cold 70% ethanol for 5 seconds and twice in ice-cold PBS for 5 seconds. 1 cm² of skin, was removed and cut into small pieces and incubated in 2 ml of collagenase type 1 and 4 (Sigma) at 37°C, 5% CO₂ for 40 minutes. The epidermis and dermis were separated and transferred to 10 ml wash buffer, centrifuged at 1100 rpm at room temperature for 5 minutes and resuspended in 2 ml cell dissociation buffer (Gibco). After incubating at 37 °C and 5% CO₂ for 10 minutes, the tissue was repeatedly put through 18 g and then 20 g needles to further dissociate it and then resuspended and left to settle in 10 ml wash buffer for 10 minutes. The supernatant was centrifuged at 1100 rpm for 5 minutes and resuspended in 2 ml PBS and plated at a density of 1×10^6 cells per well of a 6-well tissue culture plate in melanocyte growth medium F-12 (Invitrogen) containing 200 nM TPA (Sigma) and 100 µg/ml primocin (InvivoGen). After 2 days in culture, 50 µg/ml Geneticin (Gibco) was added to the culture medium to select for melanocytes. Melanocytes were passaged at around 50% confluence. Mks and platelets were isolated from Pf4Cre Lifeact-GFP mice and litter matched controls as previously described (Mazharian et al., 2009; McCarty et al., 2005).

Immunofluorescence Staining

Melanocytes were plated on glass coverslip overnight, Mks were allowed to spread for 3 hours and platelets for 45 minutes at 37°C with 5% CO₂. The cells were fixed with 4% formaldehyde in phosphate-buffered saline (PBS) for 10 minutes at room temperature and permeabilized with 0.1 % Triton X-100 for 5 minutes. Primary antibody (Anti P34-Arc, Millipore; Anti-Wasp (B-9) Santa Cruz Biotechnology) was incubated for 30 minutes followed by secondary antibody (Invitrogen) and rhodamine phalloidin (Invitrogen) staining for 30 minutes. Coverslips were mounted with DAPI containing mounting media (Invitrogen). Pictures were taken with a Nikon A1R or Olympus FV 2000 confocal microscope using a 60× oil objective. For analysis of spread cells at least 40 platelets and 10 Mks were analysed per experiment using ImageJ 1.42q.

Live cell imaging

For 2D melanocyte imaging, cells were plated overnight in glass bottom dishes, which were coated with 10 µg/ml fibronectin (BD bioscience) for 1 hour at 37 °C, and then incubated with 1% heat inactivated bovine serum albumin for 20 minutes. Time-lapse video microscopy was performed using a Nikon TE2000 microscope in a 37°C chamber with 5% CO₂. Real-time spreading of platelets was done on glass-bottom dishes covered with 100µg/ml fibrinogen (Enzyme Research Laboratories) at 37°C, pictures were taken every 10 or 5 seconds for 30 minutes with a Nikon total internal reflection (TIRF) microscope using a

100× oil objective. Live cell imaging of Mks was done on a basement membrane isolated from the murine peritoneum (Hotary et al., 2006) and imaged with an Olympus FV2000 microscope using a 60× objective. Images were taken every minute.

Ex-vivo imaging of melanoblast migration

Experimental setup was as previously reported (Li et al., 2011)

Melanoblast migration speed in skin explants

Individual cells were tracked manually using ImageJ, Manual Tracking plugin, with distance/time measurements taken every 5 minutes for 3 hours. The persistence were calculated using Chemotaxis Tool plugin. More than 60 cells were examined from each of at least 3 different skin explants from different embryos.

Statistics

Mean values $\pm$ SEM and statistical analysis were determined using Graph Pad Prism 5.0 (Graphpad Software). Significance was calculated by using a two-tailed paired or unpaired students t-test. All experiments, if not stated otherwise, were done at least 3 times independently. A p-value smaller than 0.01 was considered to be significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Ballestrem C, Wehrle-Haller B, Imhof BA. Actin dynamics in living mammalian cells. *Journal of Cell Science*. 1998; 111:1649–1658. [PubMed: 9601095]
- Bronson SK, Plaehn EG, Kluckman KD, Hagaman JR, Maeda N, Smithies O. Single-copy transgenic mice with chosen-site integration. *Proceedings of the National Academy of Sciences*. 1996; 93:9067–9072.
- Calaminus SD, Thomas S, McCarty OJ, Machesky LM, Watson SP. Identification of a novel, actin-rich structure, the actin nodule, in the early stages of platelet spreading. *J Thromb Haemost*. 2008a; 6:1944–1952. [PubMed: 18761725]
- Calaminus SDJ, Thomas S, McCarty OJT, Machesky LM, Watson SP. Identification of a novel, actin-rich structure, the actin nodule, in the early stages of platelet spreading. *Journal of Thrombosis and Haemostasis*. 2008b; 6:1944–1952. [PubMed: 18761725]
- Chen Q, Pollard TD. Actin filament severing by cofilin is more important for assembly than constriction of the cytokinetic contractile ring. *The Journal of Cell Biology*. 2011
- Cvetkovic B, Yang B, Williamson RA, Sigmund CD. Appropriate Tissue- and Cell-specific Expression of a Single Copy Human Angiotensinogen Transgene Specifically Targeted Upstream of the HPRT Locus by Homologous Recombination. *Journal of Biological Chemistry*. 2000; 275:1073–1078. [PubMed: 10625648]
- Deibler M, Spatz JP, Kemkemer R. Actin Fusion Proteins Alter the Dynamics of Mechanically Induced Cytoskeleton Rearrangement. *PLoS ONE*. 2011; 6:e22941. [PubMed: 21850245]
- Delmas V, Martinozzi S, Bourgeois Y, Holzenberger M, Larue L. Cre-mediated recombination in the skin melanocyte lineage. *genesis*. 2003; 36:73–80. [PubMed: 12820167]
- Era A, Tominaga M, Ebine K, Awai C, Saito C, Ishizaki K, Yamato KT, Kohchi T, Nakano A, Ueda T. Application of Lifeact Reveals F-Actin Dynamics in *Arabidopsis thaliana* and the Liverwort, *Marchantia polymorpha*. *Plant and Cell Physiology*. 2009; 50:1041–1048. [PubMed: 19369273]
- Guillot PV, Liu L, Kuivenhoven JA, Guan J, Rosenberg RD, Aird WC. Targeting of human eNOS promoter to the HpRT locus of mice leads to tissue-restricted transgene expression. *Physiological Genomics*. 2000; 2:77–83. [PubMed: 11015585]

- Isakari Y, Harada Y, Ishikawa D, Matsumura-Takeda K, Sogo S, Ishida T, Taki T, Kiwada H. Efficient gene expression in megakaryocytic cell line using nucleofection. *Int J Pharm.* 2007; 338:157–164. [PubMed: 17331684]
- Kamijo K, Ohara N, Abe M, Uchimura T, Hosoya H, Lee J-S, Miki T. Dissecting the Role of Rho-mediated Signaling in Contractile Ring Formation. *Molecular Biology of the Cell.* 2006; 17:43–55. [PubMed: 16236794]
- Larue L, Dougherty N, Porter S, Mintz B. Spontaneous malignant transformation of melanocytes explanted from Wf/Wf mice with a Kit kinase domain mutation. *Proceedings of the National Academy of Sciences of the United States of America.* 1992; 89:7816–7820. [PubMed: 1380168]
- Li A, Dawson JC, Forero-Vargas M, Spence HJ, Yu X, Kwon I, Anderson K, Machesky LM. The Actin-Bundling Protein Fascin Stabilizes Actin in Invadopodia and Potentiates Protrusive Invasion. *Current Biology.* 2010; 20:339–345. [PubMed: 20137952]
- Li A, Ma Y, Yu X, Mort RL, Lindsay CR, Stevenson D, Strathdee D, Insall RH, Chernoff J, Snapper SB, et al. Rac1 Drives Melanoblast Organization during Mouse Development by Orchestrating Pseudopod-Driven Motility and Cell-Cycle Progression. *Developmental Cell.* 2011; 21:722–734. [PubMed: 21924960]
- Lindsay CR, Lawn S, Campbell AD, Faller WJ, Rambow F, Mort RL, Timpson P, Li A, Cammareri P, Ridgway RA, et al. P-Rex1 is required for efficient melanoblast migration and melanoma metastasis. *Nat Commun.* 2011; 2:555. [PubMed: 22109529]
- Liu Y, Bambara RA. Analysis of Human Flap Endonuclease 1 Mutants Reveals a Mechanism to Prevent Triplet Repeat Expansion. *Journal of Biological Chemistry.* 2003; 278:13728–13739. [PubMed: 12554738]
- Matheyses AL, Simon SM, Rappoport JZ. Imaging with total internal reflection fluorescence microscopy for the cell biologist. *Journal of Cell Science.* 2010; 123:3621–3628. [PubMed: 20971701]
- Mazharian A, Watson SP, Saverin S. Critical role for ERK1/2 in bone marrow and fetal liver, derived primary megakaryocyte differentiation, motility, and proplatelet formation. *Experimental Hematology.* 2009; 37:1238–1249.e1235. [PubMed: 19619605]
- McCarty OJT, Larson MK, Auger JM, Kalia N, Atkinson BT, Pearce AC, Ruf S, Henderson RB, Tybulewicz VLJ, Machesky LM, et al. Rac1 Is Essential for Platelet Lamellipodia Formation and Aggregate Stability under Flow. *Journal of Biological Chemistry.* 2005; 280:39474–39484. [PubMed: 16195235]
- Mitchison TJ, Cramer LP. Actin-based cell motility and cell locomotion. *Cell.* 1996; 84:371–379. [PubMed: 8608590]
- Mort RL, Hay L, Jackson IJ. Ex vivo live imaging of melanoblast migration in embryonic mouse skin. *Pigment Cell & Melanoma Research.* 2010; 23:299–301. [PubMed: 20067551]
- Munsie LN, Caron N, Desmond CR, Truant R. Lifeact cannot visualize some forms of stress-induced twisted f-actin. *Nat Meth.* 2009; 6:317–317.
- Nagy, A.; Gertsenstein, M.; Vintersten, K.; Behringer, R. *Manipulating the Mouse Embryo: A Laboratory Manual.* Third Edition. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 2003.
- Nishida E, Iida K, Yonezawa N, Koyasu S, Yahara I, Sakai H. Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells. *Proceedings of the National Academy of Sciences.* 1987; 84:5262–5266.
- Novak A, Guo C, Yang W, Nagy A, Lobe CG. Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon cre-mediated excision. *genesis.* 2000; 28:147–155. [PubMed: 11105057]
- Pelham RJ, Chang F. Actin dynamics in the contractile ring during cytokinesis in fission yeast. *Nature.* 2002; 419:82–86. [PubMed: 12214236]
- Renkawitz J, Schumann K, Weber M, Lammermann T, Pflücke H, Piel M, Polleux J, Spatz JP, Sixt M. Adaptive force transmission in amoeboid cell migration. *Nat Cell Biol.* 2009; 11:1438–1443. [PubMed: 19915557]
- Ridley AJ. Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. *Trends in cell biology.* 2006; 16:522–529. [PubMed: 16949823]

- Riedl J, Crevenna AH, Kessenbrock K, Yu JH, Neukirchen D, Bista M, Bradke F, Jenne D, Holak TA, Werb Z, et al. Lifeact: a versatile marker to visualize F-actin. *Nat Meth.* 2008; 5:605–607.
- Riedl J, Flynn KC, Raducanu A, Gartner F, Beck G, Bosl M, Bradke F, Massberg S, Aszodi A, Sixt M, et al. Lifeact mice for studying F-actin dynamics. *Nat Methods.* 2010a; 7:168–169. [PubMed: 20195247]
- Riedl J, Flynn KC, Raducanu A, Gartner F, Beck G, Bosl M, Bradke F, Massberg S, Aszodi A, Sixt M, et al. Lifeact mice for studying F-actin dynamics. *Nat Meth.* 2010b; 7:168–169.
- Samuel MS, Munro J, Bryson S, Forrow S, Stevenson D, Olson MF. Tissue selective expression of conditionally-regulated ROCK by gene targeting to a defined locus. *genesis.* 2009; 47:440–446. [PubMed: 19391117]
- Srinivas S, Watanabe T, Lin C-S, William C, Tanabe Y, Jessell T, Costantini F. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Developmental Biology.* 2001; 1:4. [PubMed: 11299042]
- Tang X, Lancelle SA, Hepler PK. Fluorescence microscopic localization of actin in pollen tubes: Comparison of actin antibody and phalloidin staining. *Cell Motility and the Cytoskeleton.* 1989; 12:216–224. [PubMed: 2655936]
- Tiedt R, Schomber T, Hao-Shen H, Skoda RC. Pf4-Cre transgenic mice allow the generation of lineage-restricted gene knockouts for studying megakaryocyte and platelet function in vivo. *Blood.* 2007a; 109:1503–1506. [PubMed: 17032923]
- Tiedt R, Schomber T, Hao-Shen H, Skoda RC. Pf4-Cre transgenic mice allow the generation of lineage-restricted gene knockouts for studying megakaryocyte and platelet function in vivo. *Blood.* 2007b; 109:1503–1506. [PubMed: 17032923]
- Tucker KL, Wang Y, Dausman J, Jaenisch R. A transgenic mouse strain expressing four drug-selectable marker genes. *Nucleic Acids Research.* 1997; 25:3745–3746. [PubMed: 9278500]
- Walmsley MJ, Ooi SKT, Reynolds LF, Smith SH, Ruf S, Mathiot A, Vanes L, Williams DA, Cancro MP, Tybulewicz VLJ. Critical Roles for Rac1 and Rac2 GTPases in B Cell Development and Signaling. *Science.* 2003; 302:459–462. [PubMed: 14564011]
- Wang YL. Mobility of filamentous actin in living cytoplasm. *The Journal of Cell Biology.* 1987; 105:2811–2816. [PubMed: 3693399]
- Westphal M, Jungbluth A, Heidecker M, Muhlbauer B, Heizer C, Schwartz J-M, Marriott G, Gerisch G.n. Microfilament dynamics during cell movement and chemotaxis monitored using a GFP, Δ actin fusion protein. *Current Biology.* 1997; 7:176–183. [PubMed: 9276758]

Figure 1. Murine Megakaryocytes, Platelets and Melanocytes expressing Lifeact-GFP

(A) Genetic background of the conditional Lifeact-GFP mouse. Construct for conditional EGFP-Lifeact expression was restored to the *hprt* locus. A CAG lox-stop-lox transgene was used to regulate the expression of Lifeact-GFP. pA: poly A tail, pink box: Human *hprt* promoter and exon 1 of the *hprt* gene, blue boxes: murine exons of the *hprt* gene. (B) Real-time spreading of Pf4Cre Lifeact-GFP Mk, Mk was preincubated for 1 hour on a basement membrane, images were taken every minute. Podosomes are highlighted in the inset. (C) Real-time TIRF movie of Pf4Cre Lifeact-GFP platelets spreading on fibrinogen for 7 min prior to addition of 1U/ml thrombin and images were taken every 5 seconds. (D) Live cell imaging of F-actin dynamics of TyrCre Lifeact-GFP melanocytes migrating on a fibronectin coated coverglass. Images captured every 5 min. Scale bars represent 10 μm.

Figure 2. Specific TyrCre Lifeact-GFP signal of melanoblasts in skin explants

(A) Up: Images of TyrCre Z/EG and TyrCre Lifeact-GFP melanoblasts in skin explants. Lifeact-GFP expression can be clearly seen in the cellular protrusions; the yellow arrow points to an example of an F-actin flash. Bottom: densitometric analysis of Lifeact-GFP signal with arrows at peak Lifeact-GFP intensities. (B) 2 hr tracks of individual melanoblast migration in skin explants. (C) Relative migration speed and persistence over 2 hours was plotted from 20 cells per explant from 3 explants. Error bars indicate mean $\pm$ SEM. Scale bar represents 10 μ m.

Figure 3. Melanoblasts of TyrCre Lifeact-GFP control and TyrCre Lifeact-GFP Rac1 f/f skin explants undergoing cytokinesis

(A) Representative stills from live-cell imaging of melanoblasts in TyrCre Lifeact-GFP control, TyrCre Lifeact-GFP Rac1 f/f skin explants undergoing cytokinesis with time interval as indicated. Red square represents initiation of furrowing and yellow square shows the formation of the first protrusion by daughter cell as indicated by yellow arrows. (B) Quantification of the duration between mother cell rounding up and furrow formation, furrow formation and protrusion formation and protrusion formation to separation of two daughter cells. Error bars indicate mean $\pm$ SEM from at least 15 divisions. **, $P < 0.01$. Scale bars represent 10 μ m.