SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Expression Constructs

The following previously published constructs were used for electroporation: pCAG-mGFP (Addgene plasmid 14757; (Matsuda and Cepko, 2007)), pEGFP-C1-fascin (Nemethova et al., 2008), pEGFP-N1-cofilin (DesMarais et al., 2004), pCAGG-GFP-GPI (Mukai et al., 2009), pCAGG-dnRac1-IRES-GFP (Shoval and Kalcheim, 2012), pCLGFPA (Scaal et al., 2004), pCAG-DsRed (Addgene plasmid 11151; (Matsuda and Cepko, 2004)) and PBX-pmKate2 (Sanders et al., 2013). pCAG-tubulin-GFP, pCAG-Aconitase-GFP and pCAG-frizzled7-GFP constructs were designed by us as follows: The vector pCIG (pCAGGS-IRES2-nucEGFP) containing CMV enhancer/chicken b-actin promoter and rabbit b-globin polyA was double digested using ClaI and NotI to eliminate the IRES-nucGFP sequence. The vector without the IRES-nucGFP sequence, named pCAG, was used for subsequent 2-fragment clonings.

Fragment 1 was eGFP, isolated by double-digestion (XhoI and NotI) from pEGFP-N3 (Clontech). Fragment 2 was tubulin, aconitase, or frizzled7, which were raised by PCR taking a self-constructed chicken cDNA library as a template and using the following primer pairs: For tubulin fwd: atcatcgaggtcggccgctgcctcatcctc, for aconitase fwd: atcatcgatcggcgctgcctcatcctc, for frizzled7 fwd: ccatcgaggtcggccgctgcctcatcctc, and rev: gagctcagagtccgctgcctcatcctc. PCR was performed using high fidelity KAPA polymerase ready to use kit (Peqlab). PCR products were purified by gel extraction (Qiagen) and digested with ClaI and XhoI. ClaI/XhoI digested PCR-based fragments were then ligated together with fragment 1 (eGFP digested with XhoI/NotI) into the pCAG vector digested with ClaI/NotI. To verify the identity and correctness of the cloned plasmids, restriction and sequence analyses (Eurofins GmbH) were performed.

Embryonic Manipulation and in ovo Electroporation

Fertilized chicken eggs (Gallus gallus domesticus, White Leghorn) were purchased from a local breeder (Produits agricoles Haas, Kaltenhouse, France). The eggs were incubated at 38˚C and staged according to Hamburger and Hamilton (HH) (Hamburger and Hamilton, 1992). Somite staging was done according to Christ and Ordahl (Christ and Ordahl, 1995), with the newly formed caudal most somite named I and the cranially adjacent somites staged by rising roman numbers. Embryos at HH16 were electroporated as described previously (Scaal et al., 2004). Briefly, eggs were fenestrated and vitelline membrane over somites I-IV was carefully removed. DNA constructs at the final concentration of 5-7 µg/µl were mixed with fast green (Sigma-Aldrich, 1% final concentration) to facilitate the visualization of the otherwise transparent DNA solution and injected into the somitocoele of somites I-IV. To achieve mosaic and distinct single cell pattern of the electroporated cells wherever required, we reduced the concentration of expression constructs to 0.5-1 µg/µl. Self-made platinum electrodes were placed in dorsal (+) to ventral (-) position (as shown in Fig. 1A) and 5 pulses of 7V for 10msec each with 100msec inter-pulse space were applied using a square wave pulse electroporator (Intracel, UK). Embryos were sealed with medical tape and reincubated until processed further for sectioning, staining or live imaging.

Surgical Removal of the Surface Ectoderm

Somites of HH16 embryos were electroporated with PBX-pmKate2 and incubated until stage HH19. The surface ectoderm overlying the electroporated somites was removed as described previously (Yang and Niswander, 1995). Briefly, 5 µl of 1.5% Nile blue A (Sigma-Aldrich, N5632) solution in water was poured over the electroporated somites resulting in the blistering of the surface ectoderm after a few minutes. Thereafter, the ectoderm was gently peeled away using a tungsten needle. The embryos were re-incubated for three hours after ectoderm removal and afterwards processed for slice preparation and for live imaging.
Embryo Processing, Embedding and Sectioning
After desired incubation periods, embryos were dissected from the eggs and immediately processed for overnight fixation in 4% PFA solution made in phosphate buffered saline (PBS) containing 0.1% Triton X-100. For vibratome sectioning, embryos were washed in PBS and equilibrated in 0.4% gelatine (Sigma-Aldrich)/27% BSA (AppliChem)/18% sucrose (Carl Roth, Germany) solution (GBS mix) until sunk down. The embryos were embedded overnight at room temperature in GBS mix which was supplemented by 5.5% formaldehyde (Sigma-Aldrich) solution. Embryos were cut out in blocks, mounted on a vibratome and 50µm free floating sections were collected in PBS for further staining procedures. For cryosectioning, fixed embryos were equilibrated overnight in 20% sucrose at 4˚C. Afterwards, they were put in OCT compound (Leica) at room temperature for 2 hours and embedded using the OCT compound on dry ice. 20 µm thick sections were cut using a cryostat (Leica), dried at 42˚C for 2 hours and kept at -20˚C until further processed for staining procedures.

Immunohistochemistry and Other Labeling Techniques
Immunohistochemistry was performed on 20µm thick cryosections. Briefly, the sections were washed three times in PBS containing 0.1% Triton X-100 (PBT) and incubated overnight at 4°C with desired primary antibodies (final concentration: 5µg/ml), which were diluted in 0.1% PBT solution containing 10% serum (same as the host for secondary antibody). Afterwards the sections were rinsed,washed frequently for one hour in 0.1% PBT and incubated with respective biotinylated secondary antibodies (1:500, 10% serum/0.1% PBT) overnight at 4˚C. The sections were again washed frequently for one hour in 0.1% PBT, incubated with streptavidin conjugated with cy3 (1:500, 0.1% PBT) overnight at 4˚C and mounted with DAPI Fluoromount-G (Southern Biotechnology) after frequent washing for one hour in 0.1% PBT. Following primary antibodies were used for immunohistochemical analysis: Monoclonal anti-dynein (intermediate chain), mouse ascites fluid (Sigma-Aldrich, D5167); Monoclonal anti-alpha tubulin produced in mouse (Sigma-Aldrich, T6199); Anti-kinesin heavy chain antibody [H2] (Abcam, ab23664). Biotin conjugated Donkey Anti-Mouse IgG (H+L) (715-065-151); Cy2-Streptavidin (016-220-084) and Cy3-Streptavidin (016-160-084) were purchased from Jackson ImmunoResearch.

Primary Culture of Dermomyotomal Cells
The electroporated somites were dissected and minced with a surgical scalpel to slurry. The minced tissue was collected in a tube containing DMEM/F-12 GlutMax, centrifuged at 1000 rpm for 10 min and trypsinized using 0.05% trypsin for 12min at 37˚C. Trypsinization was blocked with the same amount of FBS. The solution was pipetted up and down until homogenized. This homogeneous solution was centrifuged and cells were dissolved in DMEM/F-12 GlutaMAX containing 10% FBS and penicillin-streptomycin and cultured on laminin-coated coverslips. All reagents were purchased from Life Technologies. Cells were cultured for 24 hours, fixed with 4%PFA for 10 min at room temperature and processed for phalloidin staining and imaging.

Quantification and Statistical Analyses
Kymographic analysis was used to measure the extension and retraction velocities of the dermomyotomal FilIPs as well as the instantaneous and average velocities with which mGFP and frizzled7 puncta are transported along them. ImageJ (National Institutes of Health, Bethesda, MD) was used to make kymographs utilizing ‘multiple kymograph’ plug-in and the velocities were quantified using the macro ‘read velocities from tsp’. Thickness of the dermomyotomal cell protrusions was calculated manually. Mean fluorescence intensities in the subectodermal space were calculated using ImageJ. Statistical analyses were performed using Prism software (Graphpad). Error bars represent standard error of the mean (SEM).
SUPPLEMENTARY REFERENCES


SUPPLEMENTARY MOVIE LEGENDS

Movie 1. Stable Dermomyotomal FiLiPs. A representative mGFP electroporated dermomyotomal cell (HH18, Somite VII) exhibiting stable FiLiPs. Scale bar, 2 µm. Refer Fig. 1E.
Movie 2. Dynamic nature of FiLiPs.
A representative mGFP electroporated dermomyotom cell (HH18, Somite VII) exhibiting dynamic FiLiPs. Scale bar, 2 μm. Refer Fig. 1F.
Movie 3. FiLiPs are actin-based.
A 3D reconstruction of the z-stack images from a fixed transverse section of somite V of a HH16 embryo stained using Alexa Fluor 488 phalloidin. Green represents F-actin and blue represents nuclei, stained by DAPI.
Movie 4. FiLiPs are microtubule-based. A time lapse movie of a representative dermomyotomal cell (HH18, somite VIII) electroporated with tubulin-GFP and pmKate2 shows that all FiLiPs are microtubule-based. Scale bar, 5 µm. Refer Fig. 2F.
Movie 5. FiLiPs do not require the overlying surface ectoderm for their formation.
A representative time lapse movie of a pmKate2 electroporated dermomyotomal cell (HH17, somite VII) exhibiting FiLiPs after the surgical removal of the overlying surface ectoderm. Scale bar, 5 µm. Refer Fig. 3G.
Movie 6. Retrograde transport of mGFP puncta along a stable FiLiP.
Movie shows the retrograde transport of membrane puncta along a representative FiLiP formed by a mGFP electroporated dermomyotomal cell (HH18, Somite VII). Scale bar, 2 µm. Refer Fig. 4A.
**Movie 7. Retrograde transport of frizzled7-GFP puncta along a stable FiLiP.**
Movie shows the retrograde transport of frizzled7-GFP puncta along a FiLiP formed by a frizzled7-GFP electroporated dermomyotomal cell (HH18, Somite VII). Scale bar, 2 µm. Refer Fig. 4H.
### Table S1

Additional information about the supplementary movies such as resolution, frame per second used for making videos, objective and zoom factor used to acquire images is provided in the table below.

<table>
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<th>Movie Number (Resolution)</th>
<th>Frames per second (fps)</th>
<th>Time interval between each frame (sec)</th>
<th>Objective used (Mag/NA Imm.)</th>
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