Supporting Information

Wu et al. 10.1073/pnas.1209397109

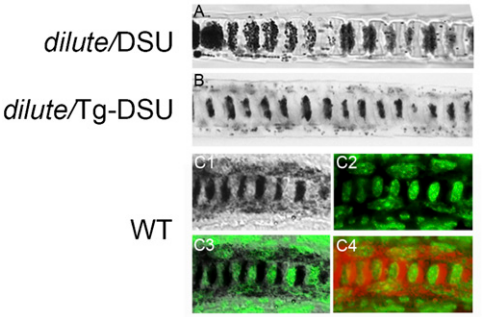


Fig. S1. The likely overexpression of melanoregulin appears to suppress further the already reduced level of melanosome transfer exhibited by *dilute* melanocytes. Shown are the distributions and densities of pigment within the central shaft of the hair from a *dilute* mouse that is WT at DSU (*dilute*/DSU) (*A*), and a *dilute* mouse that possesses four to eight integrated copies of a BAC transgene containing the WT DSU structural gene (again on a WT DSU background) (*dilute*/Tg-DSU) (*B*). The pigment-free spaces in hair are caused by the presence of the keratinocyte's nucleus, which is shown for WT hair stained with DAPI in *C* (*C* 1, pigment distribution; *C* 2, DAPI stain; *C* 3, overlay of *C* 1 and 2; *C* 4, overlay of *C* 1 and 2 where the black pigment in *C* 1 has been pseudocolored red).

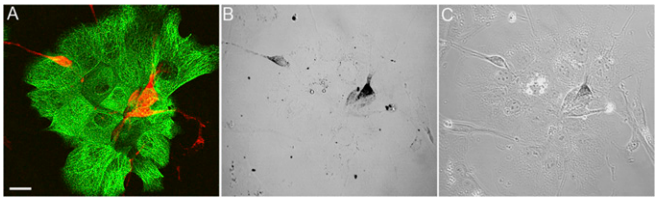


Fig. S2. Keratinocytes are present in early mouse primary skin cultures. Shown is a primary culture from a *dilute* mouse that has been stained with an antibody to the Kit receptor to visualize the shape of melanocytes (red signal in *A*) and with an antibody to keratin 14 to visualize the shape of keratinocytes (green signal in *A*). *B* and *C* are the corresponding bright-field and phase-contrast images, respectively. Note that this field also contains portions of two fibroblasts, which are negative for both markers and which are barely visible in the upper and lower left corners of the transmitted-light images. (Scale bar: 20 μm.)

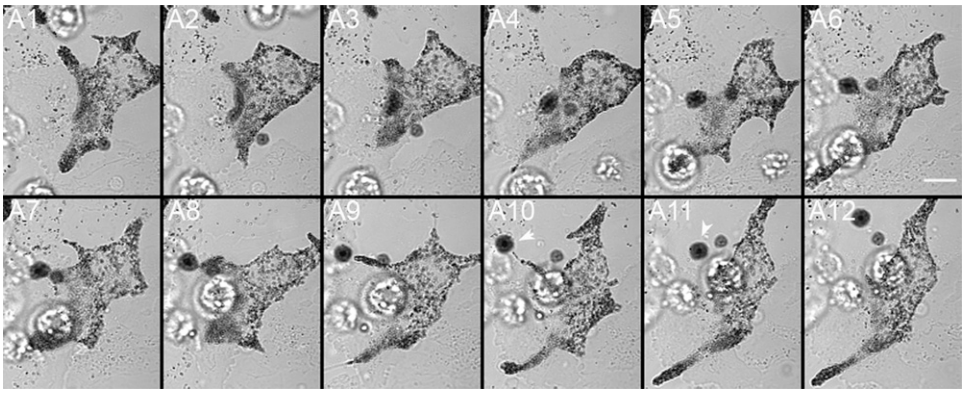


Fig. S3. The still images (and Movie S5, from which these still images were taken) show a shedding event that occurred from the center of a melanocyte. The white arrows mark the position of the forming package in the frame most likely immediately preceding (*A* 10) and the frame most likely immediately following (*A* 11) the release of the package. These still images are 20 min apart. (Scale bar: 11 μm.)

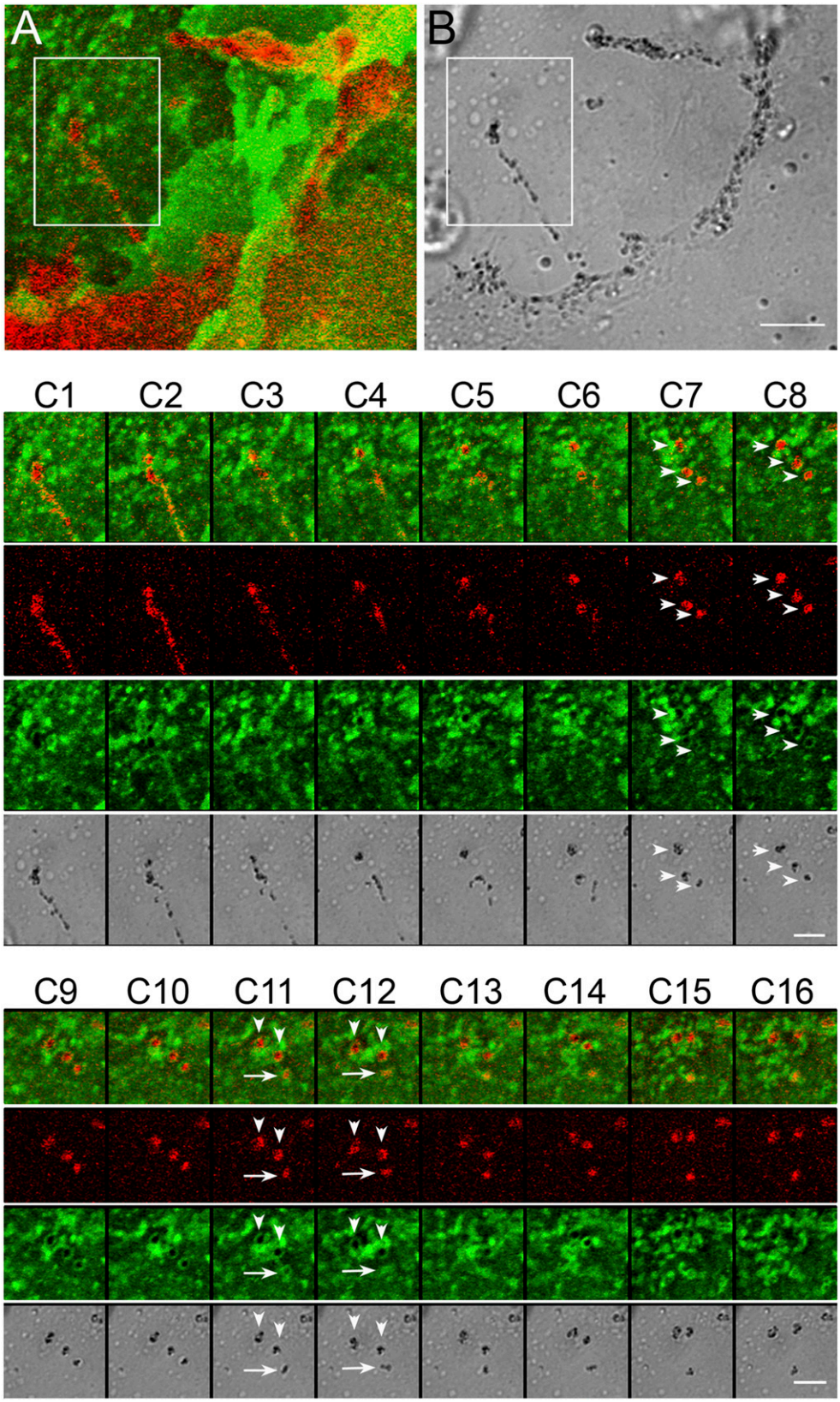


Fig. S4. Generation of three packages from the same melanocyte dendrite. *A* and *B* show a melanocyte/keratinocyte pair in which the long dendrite within the boxed region yields three melanosome packages in succession (see also Movie S8, from which these two images were taken). The still images in *C*, which also were taken from Movie S8, demonstrate the thinning of the dendrite and the abscission steps leading to the formation of the three packages (arrowheads in *C* 7 and 8) and the subsequent phagocytosis of one of these packages (the package indicated by arrows in *C* 11 and 12) by the keratinocyte. Still images C 1–16 were taken at 1, 78, 124, 156, 164, 184, 204, 260, 280, 294, 300, 306, 312, 318, 324, and 330 min, respectively. (Scale bars: 6.5 μm in *A* and *B*; 3 μm in *C*.)

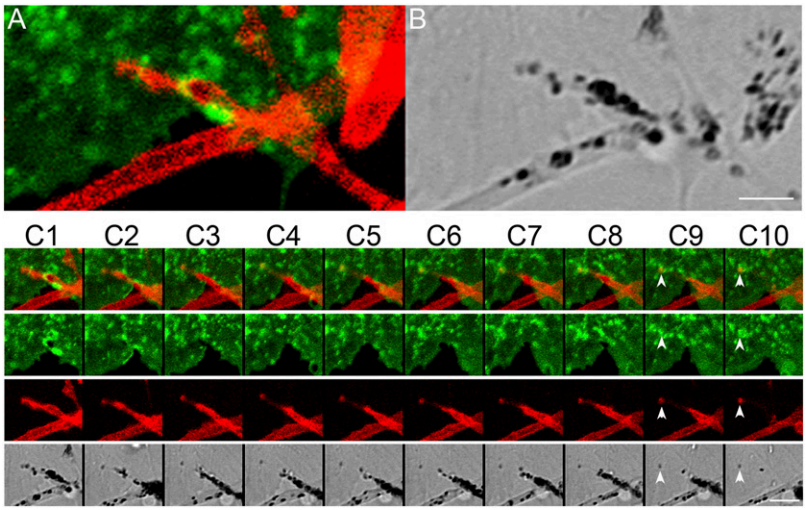


Fig. S5. In some cases, melanosome packages can contain only a single melanosome. *A* and *B* show a melanocyte/keratinocyte pair in which the dendrite yields a melanosome package containing what appears to be a single melanosome (see also Movie S9, from which these two images were taken). The still images in *C*, which also were taken from Movie S9, highlight this event. The white arrowheads in these still images mark the position of the forming single-melanosome package in the frame immediately preceding (*C* 9) and the frame immediately following (*C* 10) its release. The still images in *C* 1 and 2 are 30 min apart. The still images in *C* 3–10 are 6 min apart. (Scale bars: 3.5 μm in *A* and, *B*; 5.6 μm in *C*.)

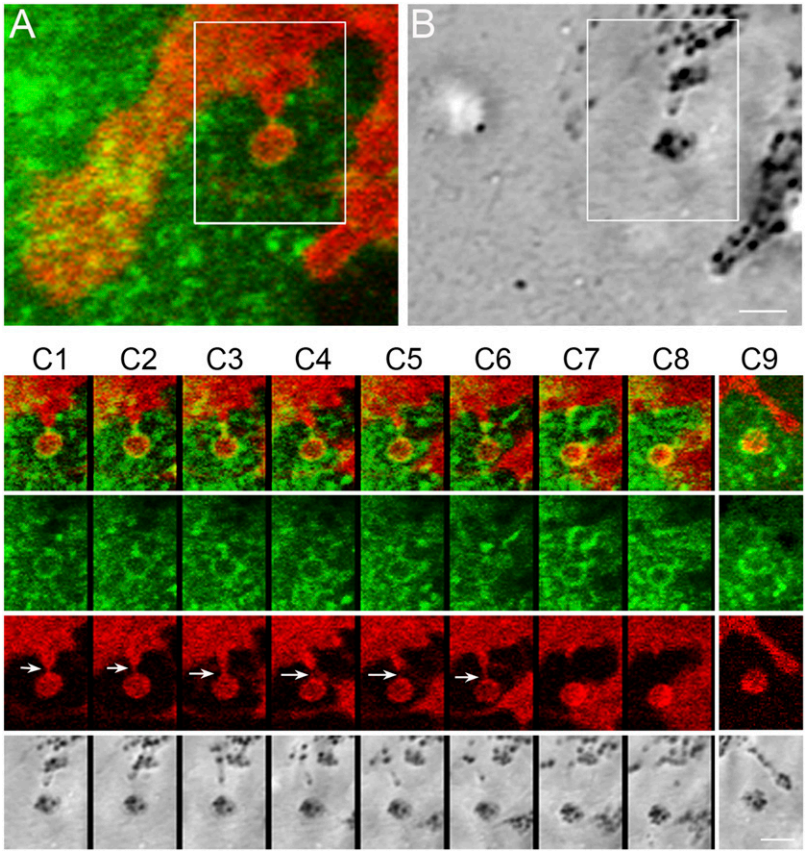
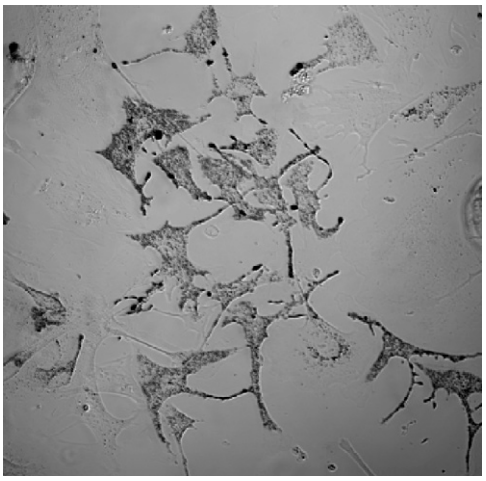
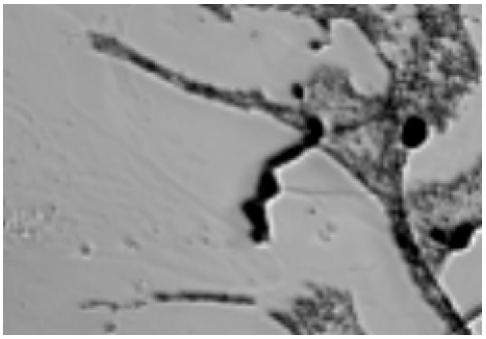


Fig. S6. Shedding of a melanosome package in the absence of any obvious stretching force. *A* and *B* show a melanocyte/keratinocyte pair in which the melanosome package within the boxed area forms without any obvious external stretching force (see also Movie S11, from which these two images were taken). The still images in *C*, which also were taken from Movie S11, highlight this shedding event, which looks very much like the formation and subsequent separation of a "hanging droplet." The white arrows in *C* 1–6 mark the thinning of the connection between this bud-like package and the melanocyte up to the point of abscission (*C* 7). The still images in *C* 1–8 are 2 min apart; the image in *C* 9 is 30 min later. (Scale bars: 3.8 μm.)



Movie S1. Time-lapse movie of a primary skin culture from a WT mouse. The time interval between images is 2 min. The movie plays at 3,600× real time.

Movie S1

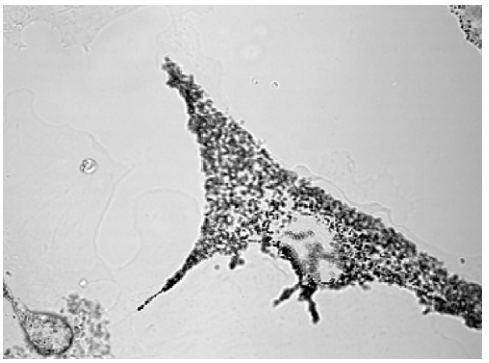


Movie S2. Time-lapse movie of a primary skin culture from a WT mouse. The time interval between images is 2 min. The movie plays at 1,800× real time.

Movie S2

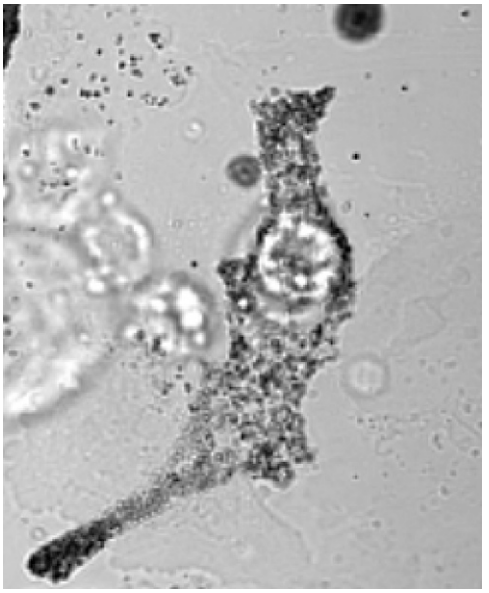
Movie S3. Time-lapse movie of a primary skin culture from a WT mouse. The time interval between images is 2 min. The movie plays at 3,600× real time.

Movie S3



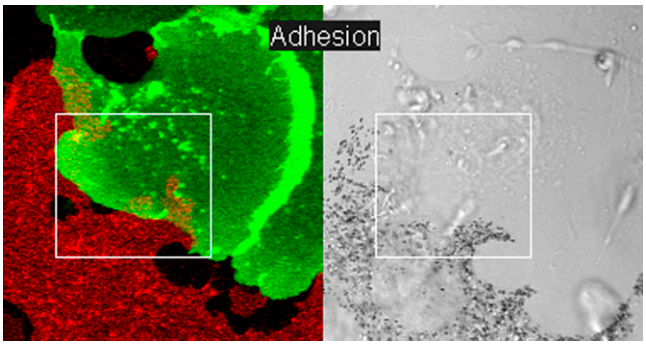
Movie S4. Time-lapse movie of a primary skin culture from a WT mouse. The time interval between images is 2 min. The movie plays at 3,600× real time.

Movie S4



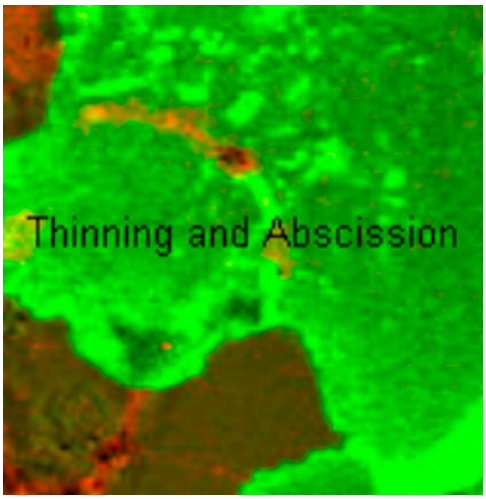
Movie S5. Time-lapse movie of a primary skin culture from a WT mouse. The time interval between images is 2 min. The movie plays at 3,600× real time.

Movie S5



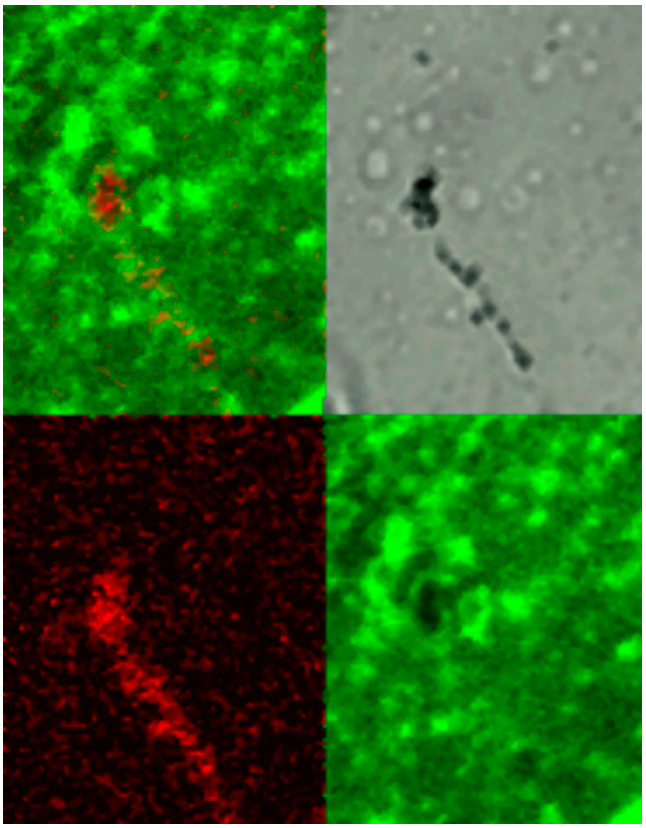
Movie S6. Time-lapse movie of a primary skin culture from a "Holly Skin" mouse. The time interval between images is 2 min. The movie plays at 1,800× real time.

Movie S6



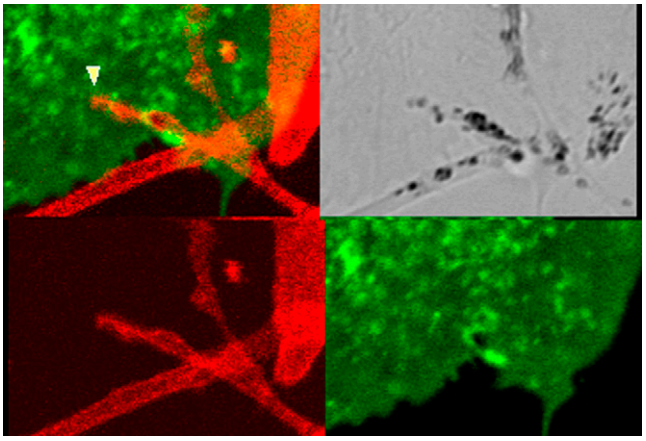
Movie S7. Time-lapse movie of a primary skin culture from a Holly Skin mouse. The time interval between images is 2 min. The movie plays at 1,800× real time.

Movie S7



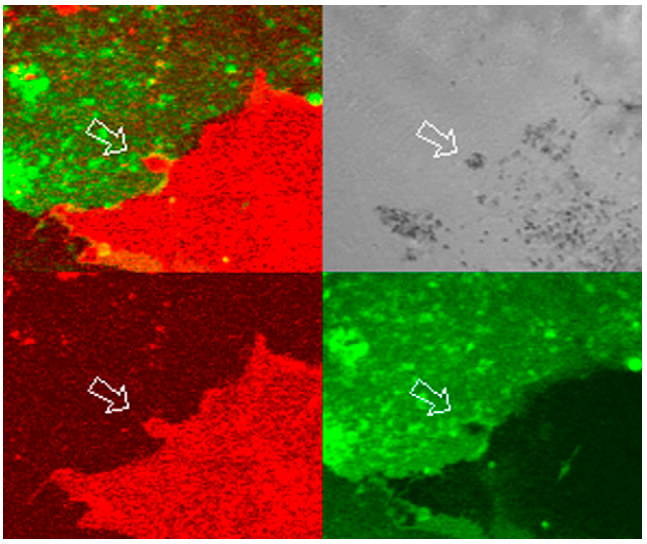
Movie S8. Time-lapse movie of a primary skin culture from a Holly Skin mouse. The time interval between images is 2 min. The movie plays at 3,600× real time.

Movie S8



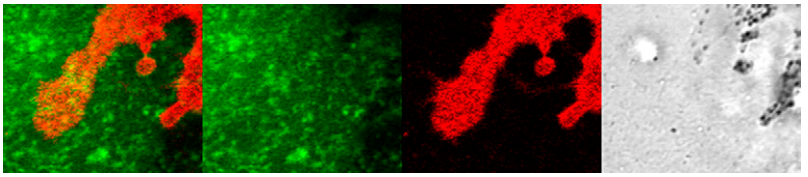
Movie S9. Time-lapse movie of a primary skin culture from a Holly Skin mouse. The time interval between images is 2 min. The movie plays at 1,800 × real time.

Movie S9



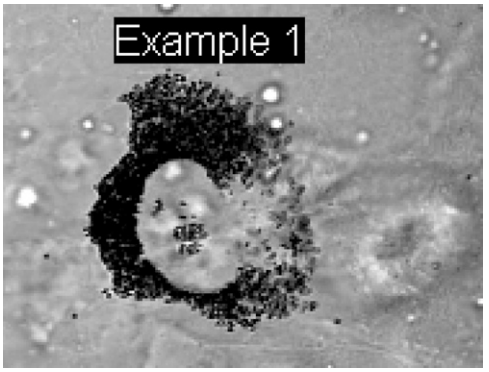
Movie S10. Time-lapse movie of a primary skin culture from a Holly Skin mouse. The time interval between images is 2 min. The movie plays at 1,800× real time.

Movie S10



Movie S11. Time-lapse movie of a primary skin culture from a Holly Skin mouse. The time interval between images is 2 min. The movie plays at 1,800× real time.

Movie S11



Movie S12. Time-lapse movie of a primary skin culture from a *dilute/dsu* mouse. The time interval between images is 2 min. The movie plays at 1,800× real time.

Movie S12