

Description of Additional Supplementary Files

Supplementary Movie S1. Representative movie of spheroid stage intestinal organoid expressing *H2B-miRFP670*, *FUCCI-Cdt1-mCherry*, and *FUCCI-Geminin-Venus*. Top row shows a middle slice through the organoid, while the bottom row shows a maximum intensity projection of the 3D signal.

Supplementary Movie S2. Representative movie of mature stage intestinal organoid crypt regions expressing *Lgr5-GFP* and *FUCCI-G1-mKO2*. Top row shows a middle-slice through the organoid, while the bottom row shows a maximum intensity projection of the whole 3D signal.

Supplementary Movie S3-4. Representative movies of ear epidermis tissues bearing *Rbl1*^{-/-} single mutation (S3) or *Rb1*^{-/-}; *Rbl1*^{-/-} double mutation (S4) imaged every 12h for 8 days. The movie shows the tissue after the application of ethanol at 0h. A z-slice corresponding to the basal layer is shown at each time point. *K14-H2B-Cerulean* is shown in gray and *Cdt1-mCherry* FUCCI-G1 reporter is shown in red. Single cells are tracked from when they are born until either they exit G1 phase or until they divide into two daughter cells.

Supplementary Movie S5-6. Representative movies of a small patch of ear epidermal tissues bearing *Rbl1*^{-/-} single mutation (S5) or *Rb1*^{-/-}; *Rbl1*^{-/-} double mutation (S4). *K14-H2B-Cerulean* is shown in gray and *Cdt1-mCherry* FUCCI-G1 reporter is shown in red. Single cell tracking are shown in colored outlines. Z-slices corresponding to the middle of the basal layer are shown top left. More basal z-slices corresponding to the dermal collagen signal, which was used for registration and tracking, are shown top right. The bottom rows show a 3D rendering of the tissue patch without (left) or with (right) the corresponding 3D nuclear segmentations. For ease of viewing, only the center few segmented cells are shown in these views.

Supplementary Movie S7. An example 3D segmentation of all basal layer cells within a ~100µm x 100µm x 100µm in the mouse epidermis. *K14-H2B-Cerulean* is shown in blue and *K14-Actin-GFP* is shown in gray. The cortical and nuclear segmentations for each basal layer are shown in overlay as uniquely colored outlines.