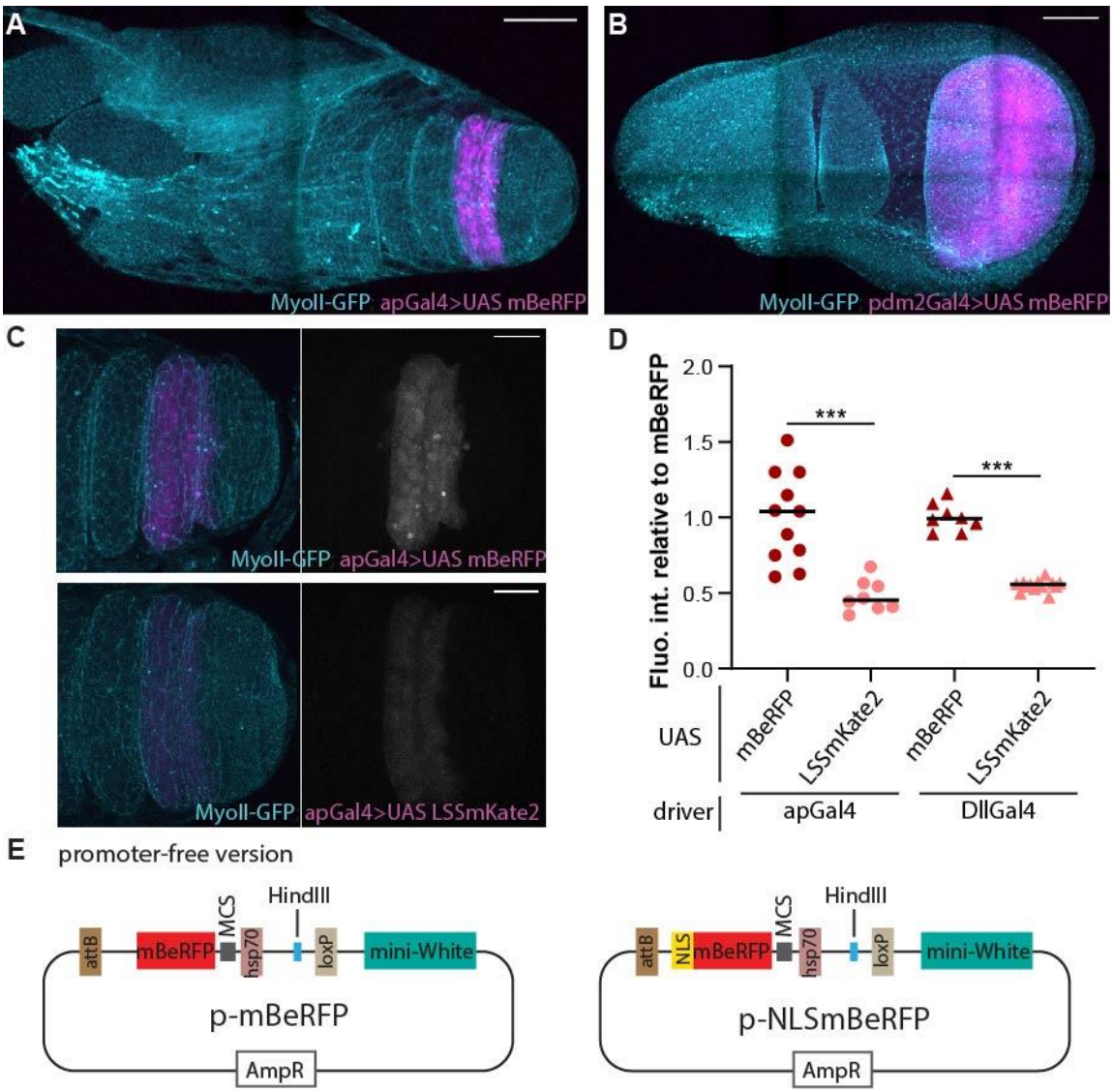


Fig. S1. A) Z-projection of an imaginal leg disc expressing endogenous MyoII-GFP (cyan) and cytoplasmic mBeRFP (magenta) in the apterous domain of expression. **B)** Z-projection of an imaginal wing disc expressing endogenous MyoII-GFP (cyan) and cytoplasmic mBeRFP (magenta) in the wing pouch (pdm2 expression domain). **C)** Z-projection of an imaginal leg disc expressing endogenous MyoII-GFP (cyan) and either cytoplasmic mBeRFP or LSSmKate2 (magenta) in the apterous domain of expression. **D)** Dot plot representing the fluorescence intensity of mBeRFP and LSSmKate2 relative to the mean fluorescence of all mBeRFP-expressing legs using two distinct drivers (*apterous*-Gal4 or *distalless*-Gal4). n=11, 8, 8 and 13 respectively for apGal4>UASmBeRFP, apGal4>UASLSSmKate2, DllGal4>UASmBeRFP and DllGal4>UASLSSmKate2. Black lines indicate the median. Statistical significance has been calculated using Student *t*-test. ***p>0.001. **E)** promoter-free versions of mBeRFP vectors, allowing versatile applications such as the expression of cytoplasmic, nuclear or fused version of mBeRFP after integration of a promoter of interest at the HindIII restriction site. Scale bars represent 50 µm and 20 µm respectively in A and B.

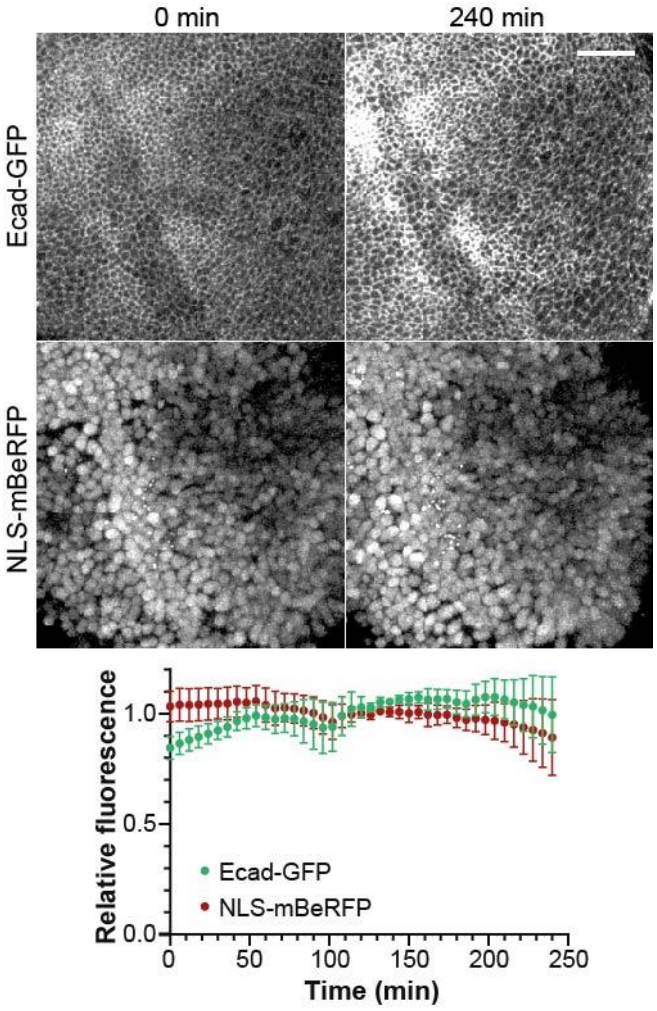


Fig. S2. Live imaging of an imaginal wing disc to compare the photobleaching of mBeRFP and GFP. (Top) Time lapse of a wing disc expressing endogenous Ecad-GFP and nuclear mBeRFP in the pdm2 domain of expression. Acquisitions of Z-stacks (41 planes) were done every 6 minutes during 4 hours. Scale bar represents 20 µm. (Bottom) Fluorescence decay of mBeRFP and GFP measured from live experiments on wing discs, upon 458 nm laser source excitation. Data represent mean ± s.e.m.

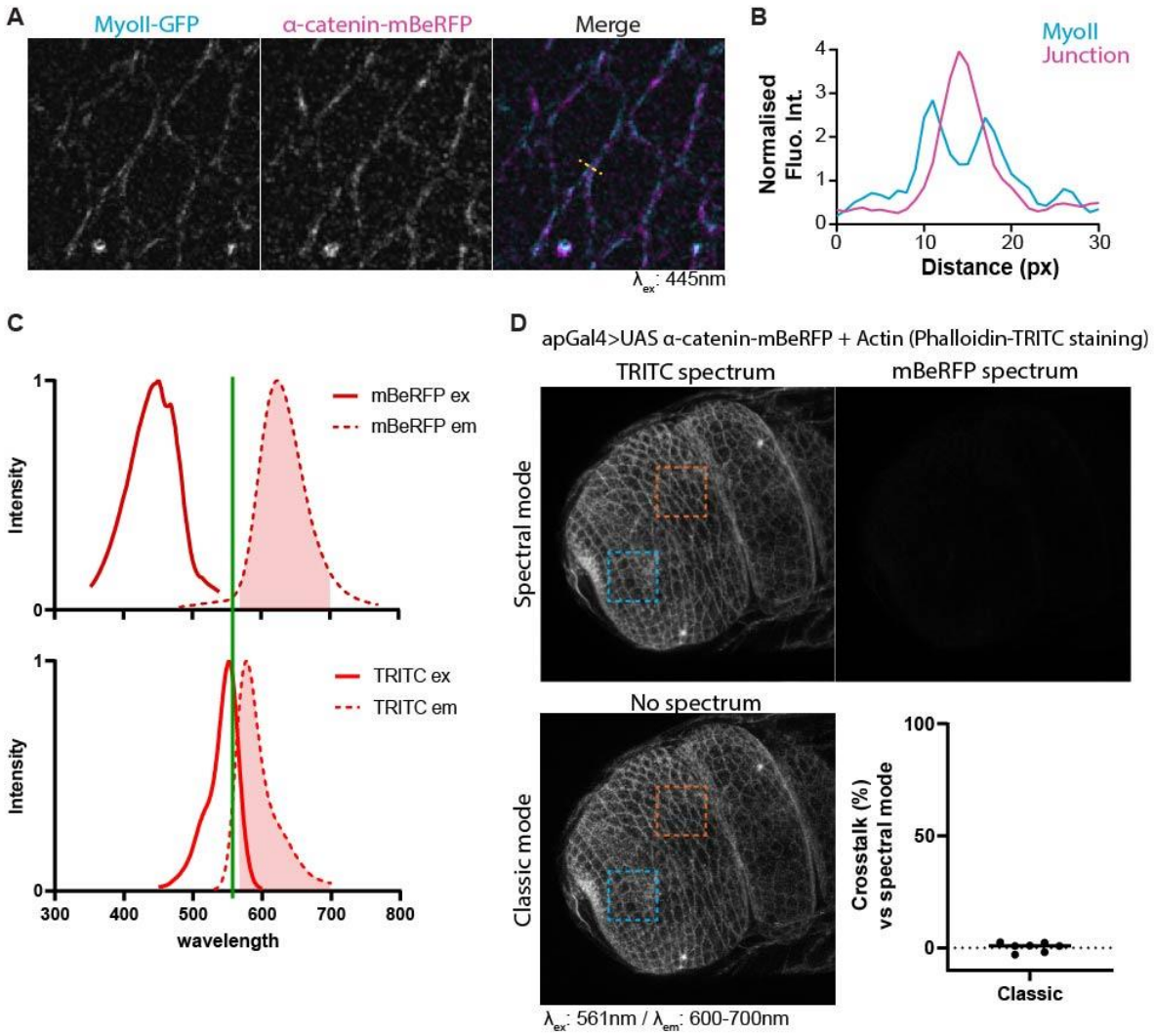
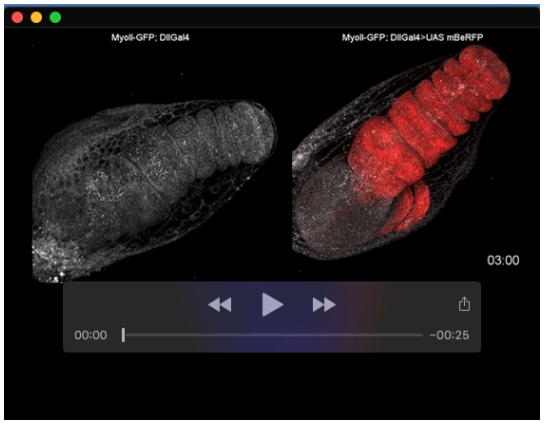
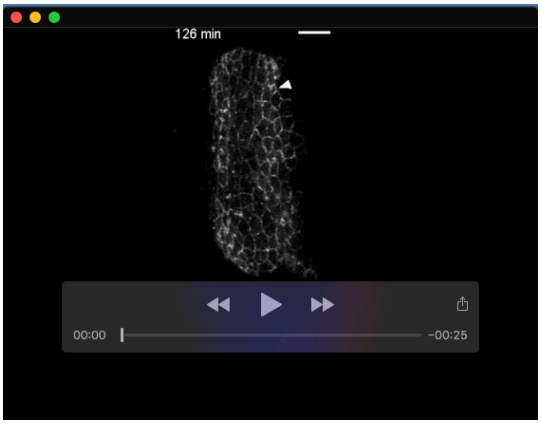


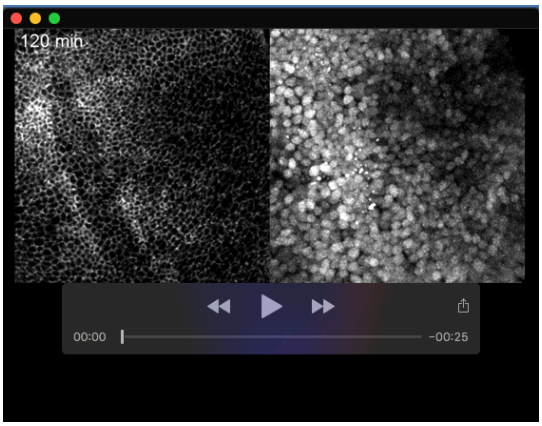
Fig. S3. A) RIM experiment. Z-projection showing MyoII-GFP (cyan) and alpha-catenin-mBeRFP (magenta) at the level of adherens junction in *MyoII-GFP; ap-Gal4, UAS-alpha-catenin-mBeRFP* leg disc. **B)** Graph represents the intensity profile of MyoII-GFP and alpha-catenin-mBeRFP at the level of the yellow dashed line. **C)** mBeRFP and TRITC excitation and emission spectra showing the emission windows (570 to 700 nm) as well as the excitation wavelength used (green line - 561 nm) . **D)** Test of the potential crosstalk between TRITC dye and mBeRFP. Z-projection of the distal part of a leg disc showing the actin stained with phalloidin-TRITC and UAS alpha-catenin-mBeRFP imaged using spectral (top) or classical confocal mode (bottom). Orange and blue dashed squares point out respectively inside and outside areas used to measure the crosstalk. (see graph). n=7



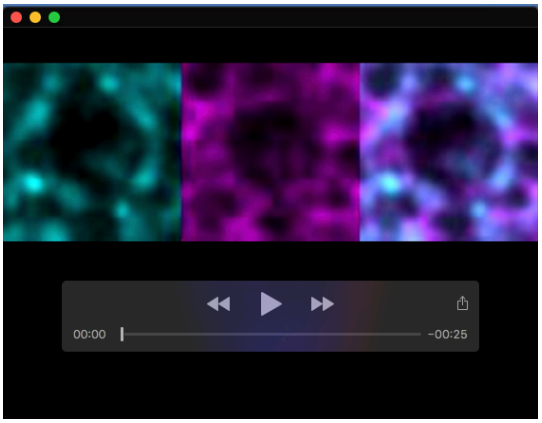
Movie 1. Development of leg discs expressing *MyoII-GFP* alone as a control or with *Dll-Gal4, UAS mBeRFP*.



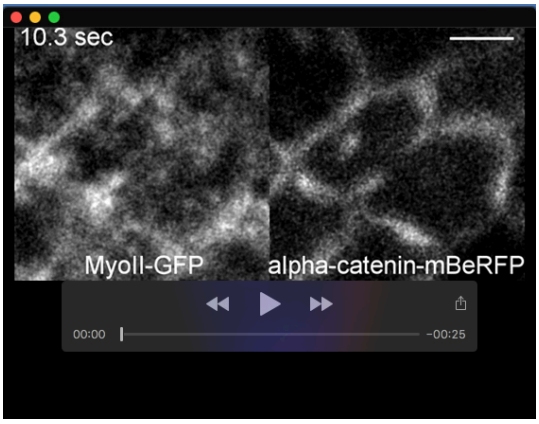
Movie 2. Time-lapse of distal part of a leg disc expressing *ap-Gal4, UAS-alpha-catenin-mBeRFP*. Arrows indicate apical cell extrusion.



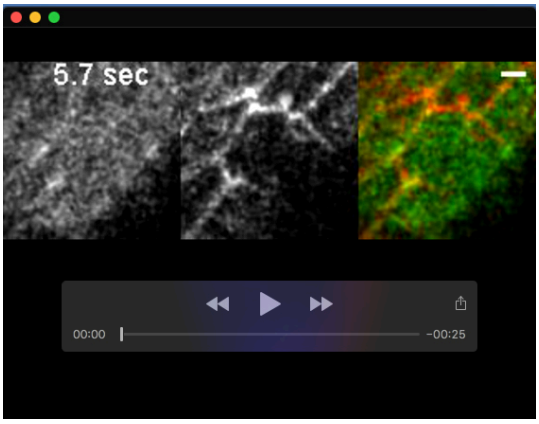
Movie 3. Time-lapse of Drosophila wing disc expressing *E-cadherin-GFP; pdm2-Gal4, UAS-NLS-mBeRFP*.



Movie 4. Cell division observed in a wing disc expressing *MyoII-GFP; pdm2-Gal4, UAS-alpha-catenin-mBeRFP.*



Movie 5. FRAP experiment in a leg disc expressing *MyoII-GFP; ap-Gal4, UAS-alpha-catenin-mBeRFP*



Movie 6. Laser ablation experiment in a leg disc expressing *MyoII-GFP; ap-Gal4, UAS-alpha-catenin-mBeRFP*