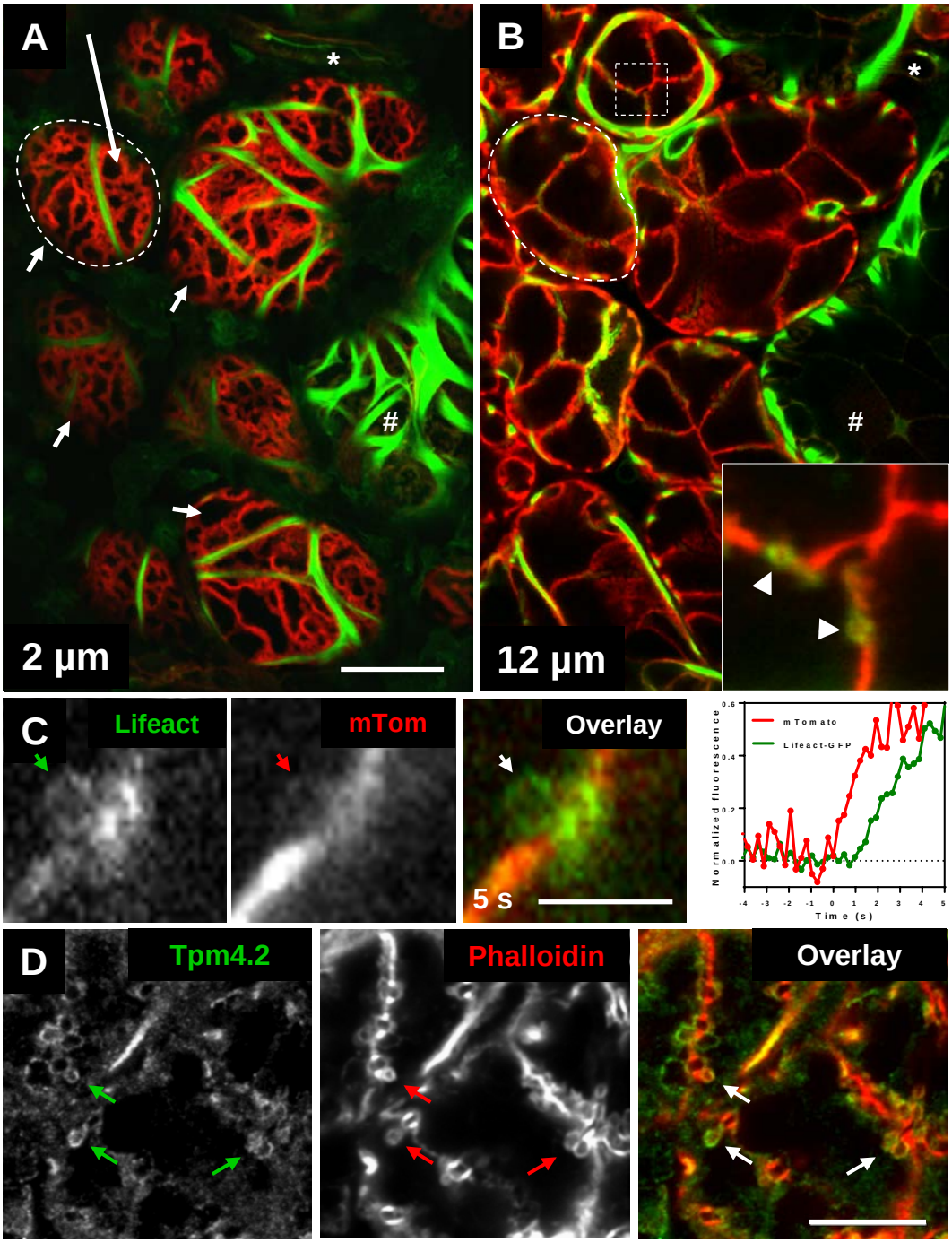


Figure S1. Actin is enriched on apical membranes in submandibular salivary glands. Tpm4.2 is present on the granules. Related to Figure 1. (A-B) Overview of submandibular salivary gland acinar cells in mTomato x Lifeact-GFP mice imaged with intravital confocal microscopy. Snapshots of salivary glands showing membrane marker mTomato (red) and F-actin marker Lifeact-GFP (green) taken at 2 or 12 µm depth [individual acini (arrows), blood vessels (*), ducts (#)]. Single acinus is outlined by dashed line. At the surface slice (A) mTomato delineates microridges of basolateral plasma membrane of acinar epithelial cells, while myoepithelial cells wrapping around the acini are seen as spindle-like structures enriched in green fluorescence. Inset in (B) shows a canaliculus cross-section showing APM enriched in F-actin (green, arrowheads). Scale bar is 20 µm and the inset width is 10.5 µm. (C) Example image and fluorescence trace from timelapse imaging of exocytosis in mTomato x Lifeact-GFP mice at 241 ms temporal resolution. Single frame at 5 s after the onset of actin polymerization is shown with fused granule visible in both channels (arrows). See also Fig1 C-D. Scale bar = 3 µm (D) Detection of Tpm4.2 and actin (TRITC-phalloidin) on fused granules (arrows) in fixed salivary gland section of wild-type mouse 10 min post isoproterenol injection. Scale bar = 10 µm.

	Granule overlap scores
Mouse1	181 / 183
Mouse2	244 / 244
Mouse3	200 / 203
Mouse4	185 / 185
Total	810 / 815

Table S1. Tpm3.1 and actin filaments co-localize on fused secretory granules in mouse submandibular salivary glands. Relates to Figure 1E. Isoproterenol-stimulated salivary glands from WT mice (n = 4) were collected, fixed and stained for F-actin and endogenous Tpm3.1. Quantification of phalloidin and Tpm3.1 overlap on fused granules is shown. Results are expressed as number of granules positive for both phalloidin and Tpm3.1 versus total granules scored. Five granules out of 815 were labeled with phalloidin and did not exhibit anti-Tpm3.1 antibody staining.

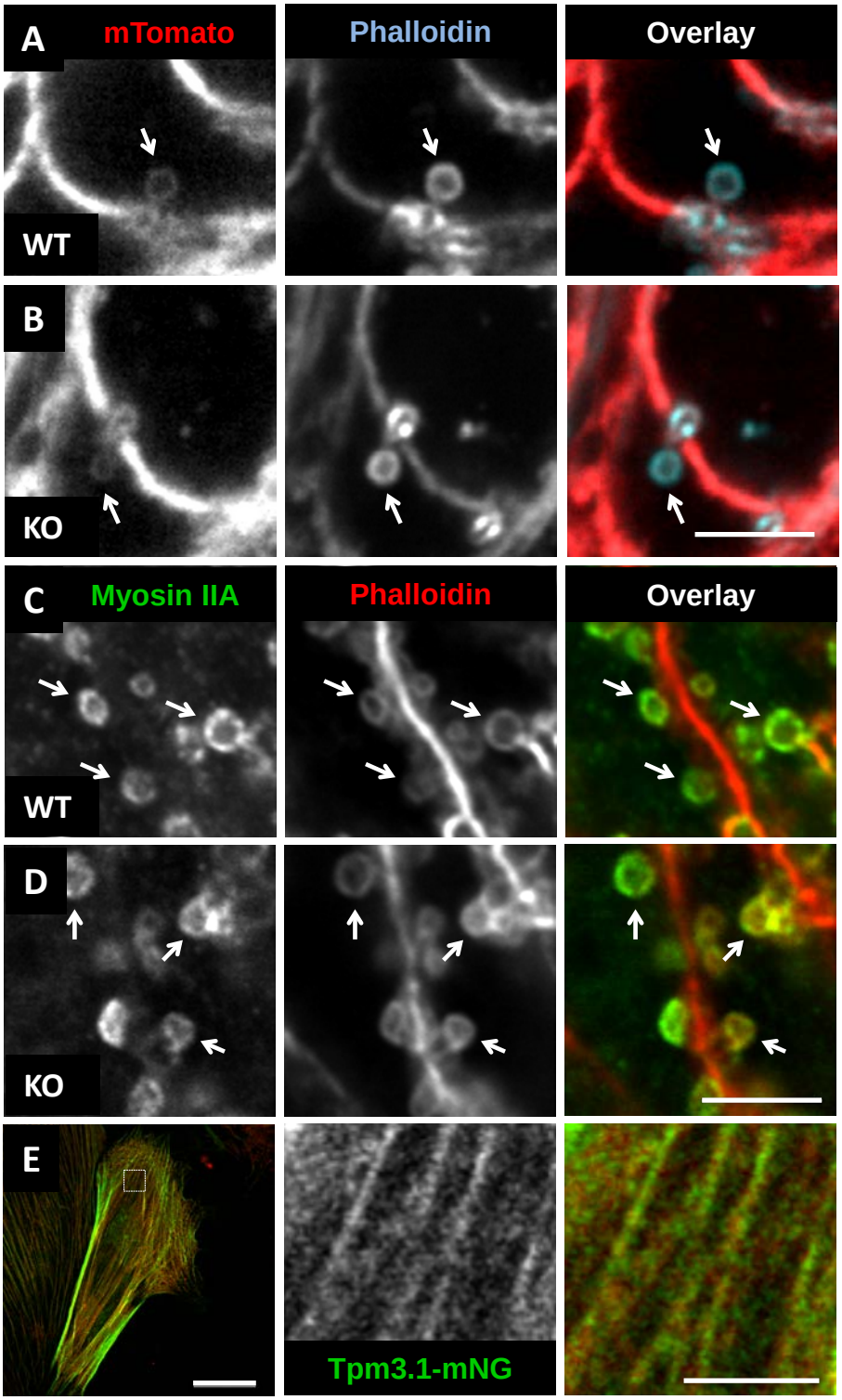


Figure S2. F-actin coat assembly is independent of Tpm3.1 Relates to Figure 2. (A-B) Representative sections of salivary glands from (A) mTomato (WT) and (B) Tpm3.1 KO x mTomato (KO) mice after 5 min isoproterenol stimulation were stained for F-actin (Alexa647-phalloidin, cyan). Fused secretory granules are shown (arrows). No abnormalities in actin coat assembly on the granules were observed indicating that actin recruitment is not affected by the ablation of Tpm3.1. (C-D) Sections of salivary glands from (C) wild-type (WT) and (D) Tpm3.1 KO (KO) mice after 10 min isoproterenol stimulation were stained with anti-myosin IIA antibody (green) and TRITC-phalloidin (red). Fused secretory granules are visible (arrows). No obvious differences in myosin IIA recruitment on the granules were observed. Scale bars for panels A-D are 5 µm. (E) MEF cell isolated from Tpm3.1-NG KI mouse embryo, relates to Fig. 3. Confocal image of Tpm3.1-NG (green) and endogenous Tpm3.1 (red) detected by anti-Tpm3.1 antibody are localized onto stress fibers (right panels). Scale bar for the main image is 30 µm and 5 µm for the zoomed view.

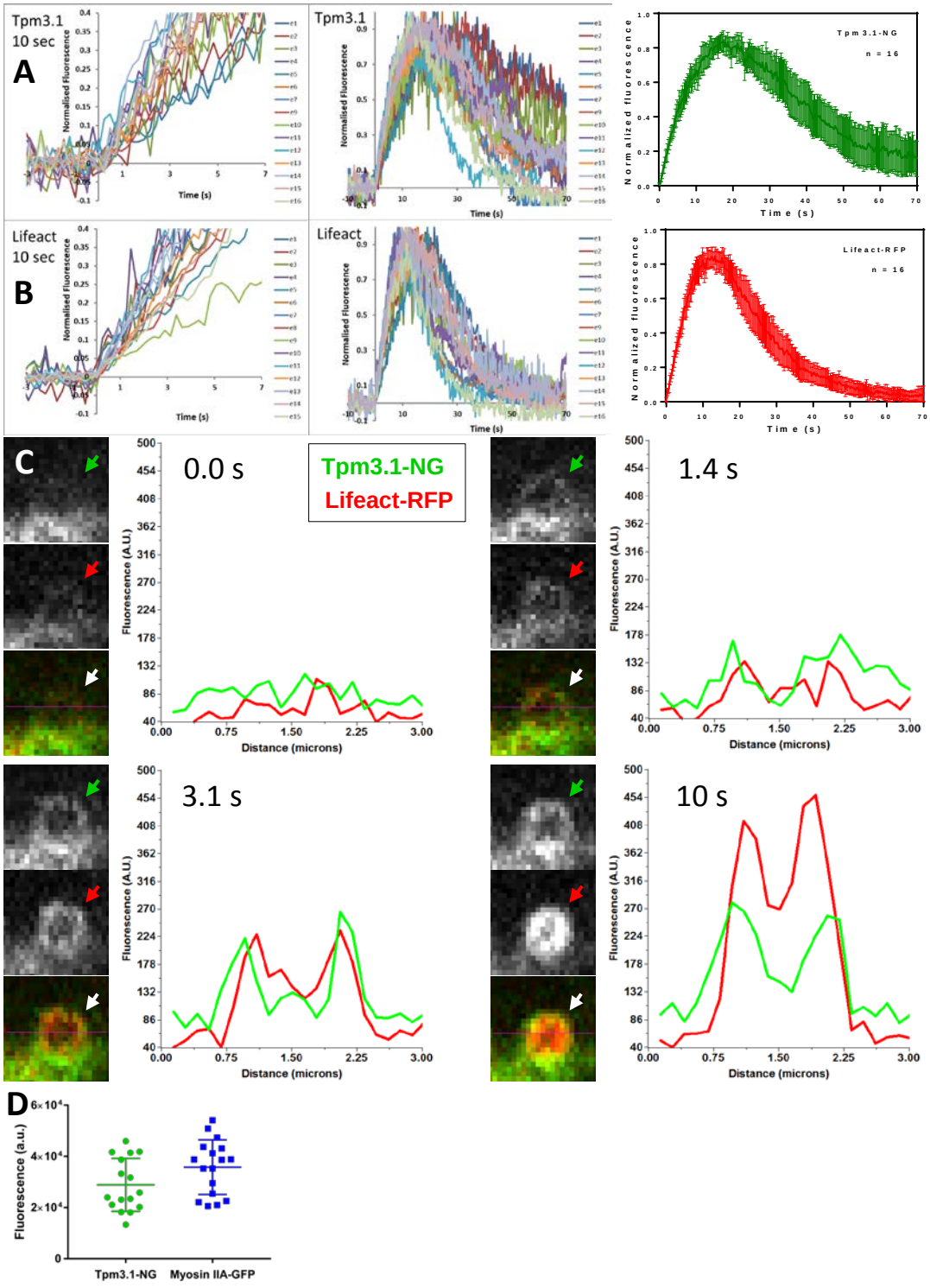

Tpm3.1
10 sec
A
Normalised Fluorescence
Time (s)
Tpm3.1
Normalised Fluorescence
Time (s)
Tpm 3.1-NG
n = 16
Normalized fluorescence
Time (s)
Lifeact
10 sec
B
Normalised Fluorescence
Time (s)
Lifeact
Normalised Fluorescence
Time (s)
Lifeact-RFP
n = 16
Normalized fluorescence
Time (s)
C
0.0 s
Tpm3.1-NG
Lifeact-RFP
Fluorescence (A.U.)
Distance (microns)
1.4 s
Fluorescence (A.U.)
Distance (microns)
3.1 s
Fluorescence (A.U.)
Distance (microns)
10 s
Fluorescence (A.U.)
Distance (microns)
D
Fluorescence (a.u.)
Tpm3.1-NG
Myosin IIA-GFP

Figure S3. Normalised fluorescence intensity traces of Tpm3.1NG and Lifeact-RFP recruitment kinetics from multiple secretory granule fusion events. Related to Figure 4. (A-B) Live intravital confocal was performed to capture *de novo* cytoskeleton assembly after isoproterenol-stimulated secretory granule fusion in Tpm3.1-NG KI x Lifeact-RFP mouse. Data were recorded in time-lapse mode at a rate of 241 ms per frame. Fluorescence intensity profiles were acquired by drawing a small region of interest around fusing granules. Normalised fluorescence traces of Tpm3.1-NG (A) and Lifeact-RFP (B) for 16 granule fusion events are shown. Fusion events were synchronised by setting one frame before Lifeact-RFP fluorescence increase above baseline as t = 0 s. Fluorescence traces spanning 10 s and 70 s are shown, with mean normalized intensities +/- 95% CI of all traces presented in rightmost panels. (C) Line scan (line shown in magenta in the overlay images) fluorescence intensity plots and corresponding images from a single secretory granule fusion in Tpm3.1-NG KI (green arrows) x Lifeact-RFP (red arrows) mouse. Four timepoints from the acquisition sequence are shown. Images were captured at 140 nm per pixel spatial and 241 ms per frame temporal resolution. Image width and line length are 3 µm. (D) Using identical imaging parameters, time-lapse images were acquired of MyoIIA-GFP KI x Lifeact-RFP mice. To compare the brightness of the Tpm3.1-NG and MyoIIA-GFP raw maximal fluorescence intensity values for each granule were plotted as mean +/- SD. The fluorescence reached maximum on the granules between 20-40 seconds after fusion. Student's t-test revealed no significant difference between the means ($p = 0.068$).

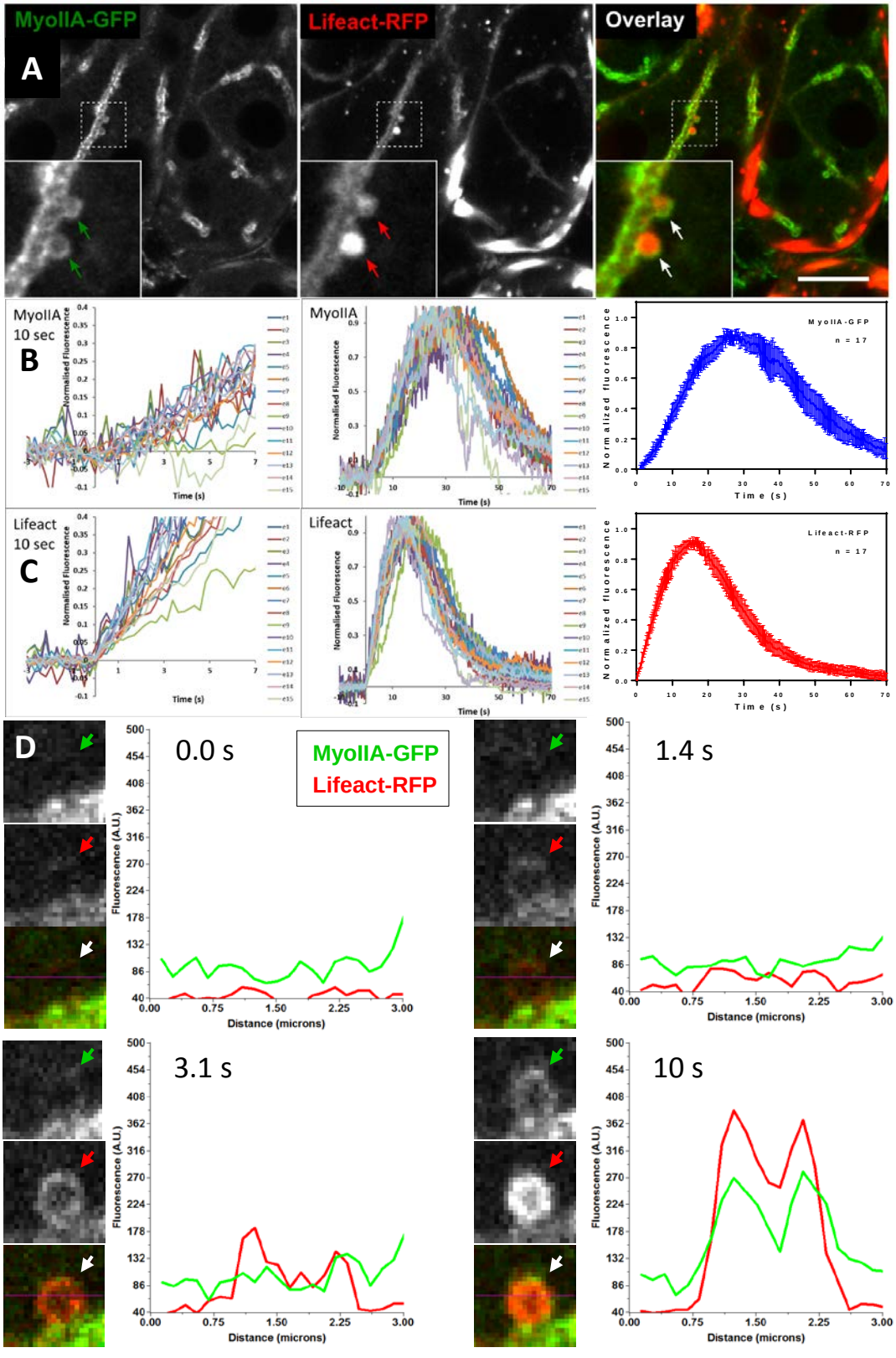
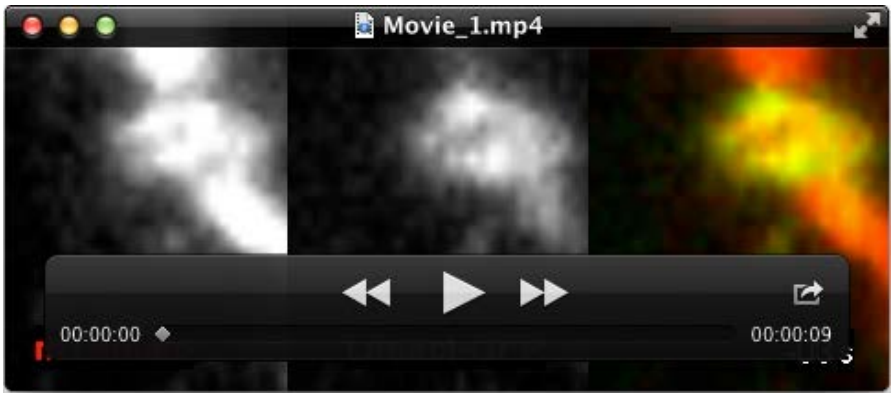


Figure S4. MyoIIA-GFP KI mouse cross with Lifeact-RFP images and normalised fluorescence intensity traces from multiple secretory granule fusion events. Related to Figure 4. (A) Snapshots of intravital confocal imaged salivary acini in situ in MyoIIA-GFP KI x Lifeact-RFP mice. Both F-actin and Myosin IIA are enriched at the canaliculi/ APM of acinar cells. Inset shows 2 fused granules (arrows) with recruitment of actin and Myosin IIA onto the granules after exocytosis is stimulated by isoproterenol. Scale bar is10 µm and the width of the insets is 6.5 µm. (B-C) Normalised fluorescence traces of MyoIIA-GFP (B) and Lifeact-RFP (C) for 17 granule fusion events are shown. Fluorescence traces spanning 10 s and 70 s are shown, with mean normalized intensities +/- 95% CI of all traces presented in rightmost panels. (D) Line scan (line shown in magenta) fluorescence intensity plots and corresponding images from a single secretory granule fusion in myosin IIA KI (green arrows) x Lifeact-RFP (red arrows) mouse. Four timepoints from acquisition sequence are shown. Images were captured at 140 nm per pixel spatial and 241 ms per frame temporal resolution. Image width and line length are 3 µm.



Movie 1. Actin filaments form a scaffold around secretory granules after fusion with the apical plasma membrane in mouse salivary acinar cells in vivo. Related to Figures 1A-D and S1C.

Intravital confocal imaging of actin filament assembly during isoproterenol-stimulated secretory granule exocytosis in submandibular salivary gland acinar cells in an mTomato x Lifeact-GFP mouse. A close-up view of a single exocytosis event is shown with granule membrane marked by mTomato (red) and actin filaments labeled by Lifeact-GFP (green). Time zero is set to one frame first appearance of Lifeact-GFP signal on the secretory granule. Contrast and brightness was adjusted in each channel for optimal visualization of the granule at early stage post-fusion. Time-lapse image frames were acquired at 944 ms intervals and playback was set to 7.5 frames per sec (approximately 7.8 x real time). Time is displayed in seconds. Scale bar = 3 μm.

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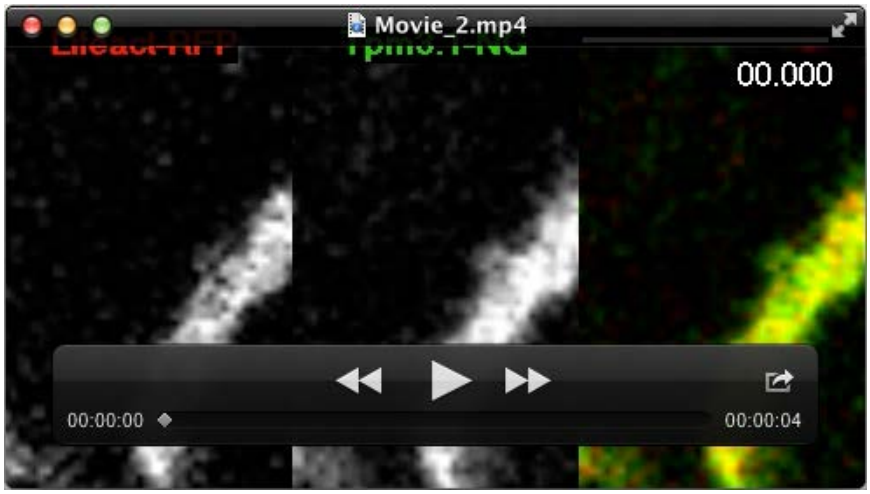
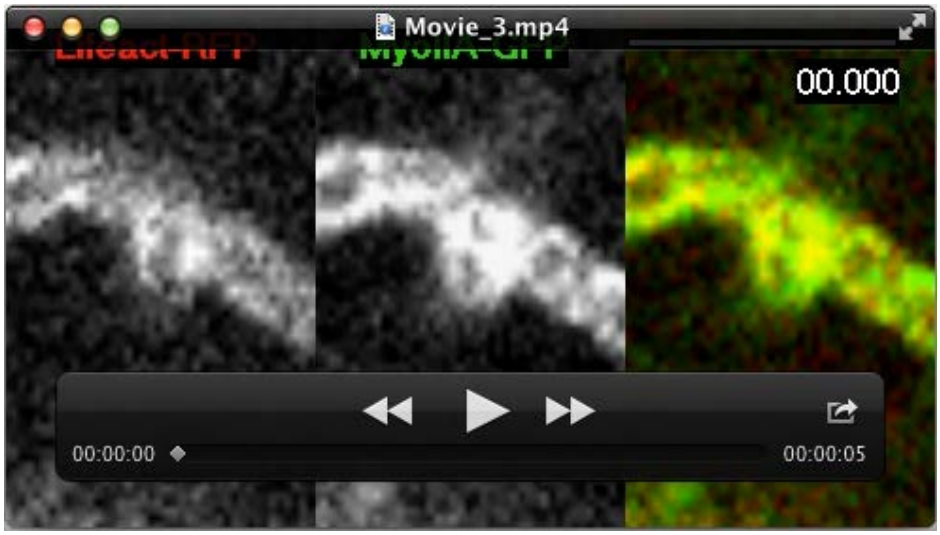


Movie 2. Initial actin and Tpm3.1 filaments simultaneously assemble around fused secretory granules in mouse acinar cells in vivo. Related to Figures 4A and S3.
Intravital confocal imaging of actin filament assembly during secretory granule exocytosis in submandibular salivary gland acinar cells in Tpm3.1-NG KI x Lifeact-RFP mouse. Single exocytosis event acquired at higher temporal resolution (241 ms image frame intervals) is shown. Tpm3.1-NG (green) and Lifeact-RFP (red) appear to be recruited simultaneously around the granule. Time zero is set to one frame prior to first appearance of Lifeact-RFP signal on the secretory granule. Playback was set to 60 frames per sec (approximately 16x real time). Time is displayed in seconds.milliseconds. Scale bar = 5 µm.



Movie 3. Myosin IIA-GFP recruitment follows Initial actin filament assembly around fused secretory granules in mouse acinar cells in vivo. Related to Figures 4B and S4.
Intravital confocal imaging of actin filament assembly during secretory granule exocytosis in submandibular salivary gland acinar cells in Myosin IIA-GFP KI x Lifeact-RFP mouse. Single exocytosis event acquired at higher temporal resolution (241 ms image frame intervals) is shown. The initial recruitment of Myosin IIA-GFP (green) around the granule seems to lag a few seconds behind the first appearance of Lifeact-RFP (red). Playback was set to 60 frames per sec (approximately 16x real time). Time is displayed in seconds.milliseconds. Scale bar = 5 µm.