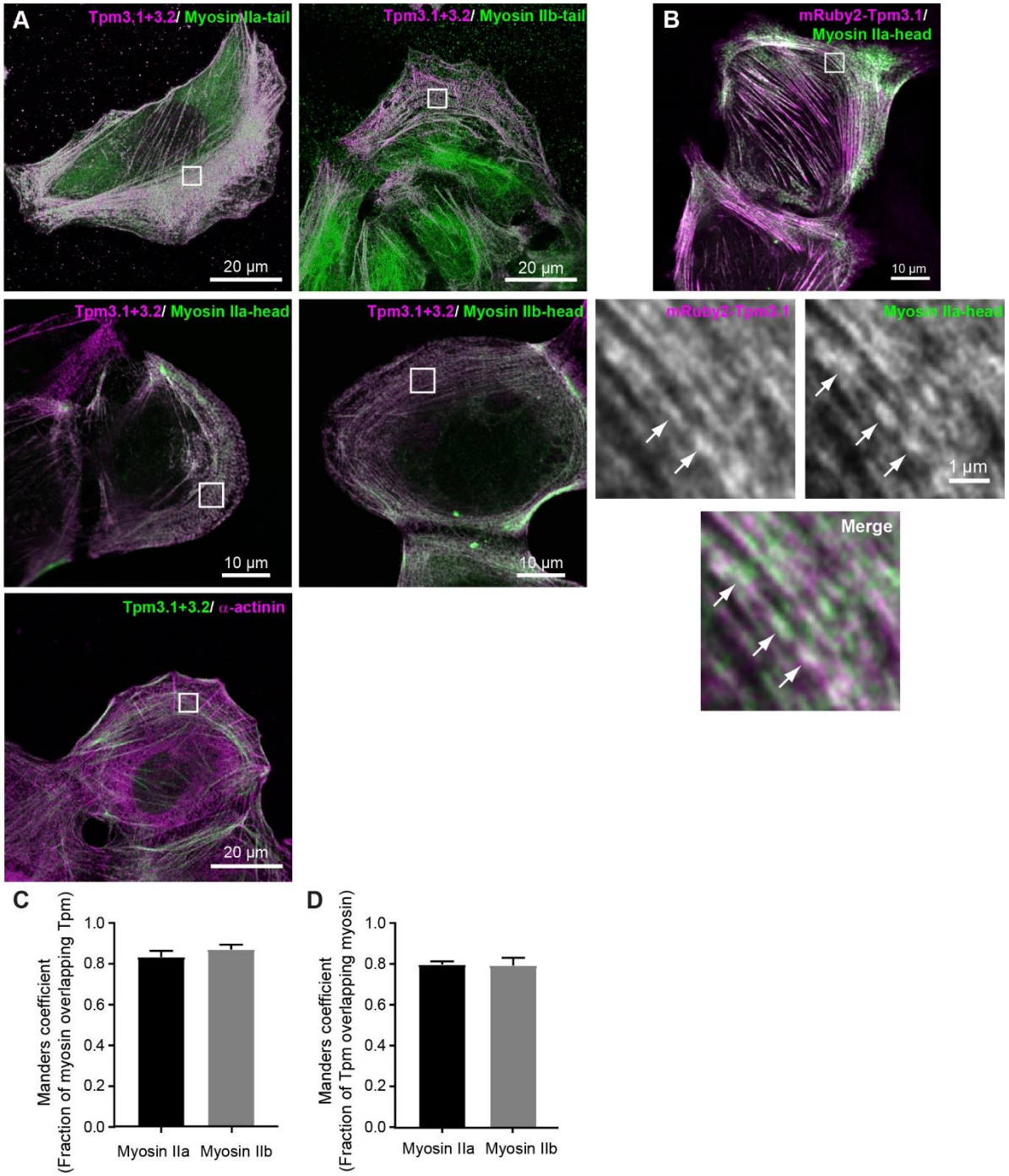
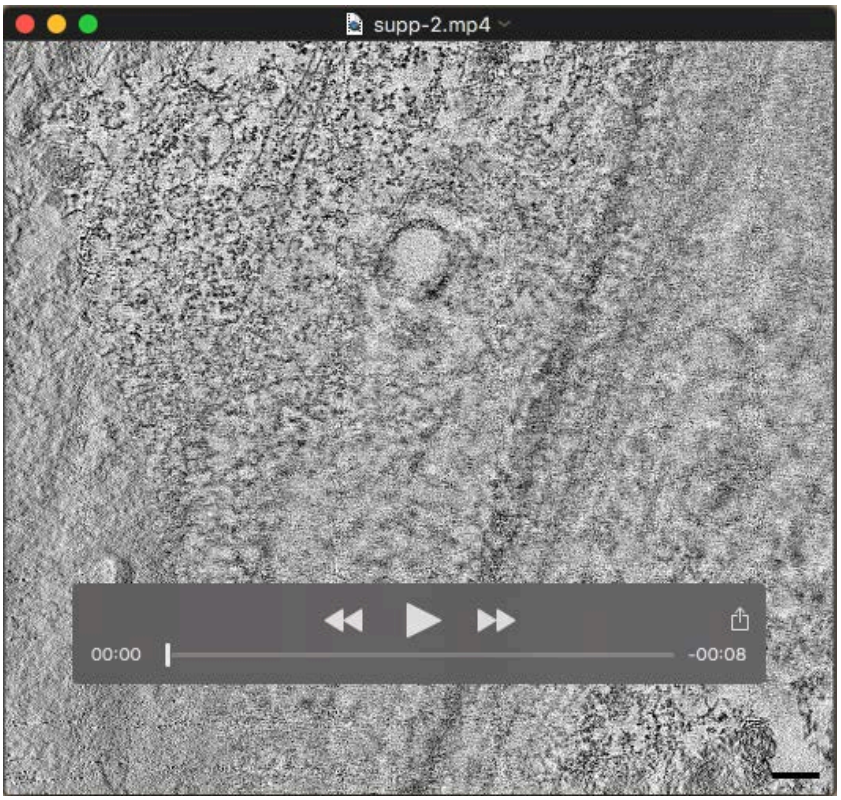
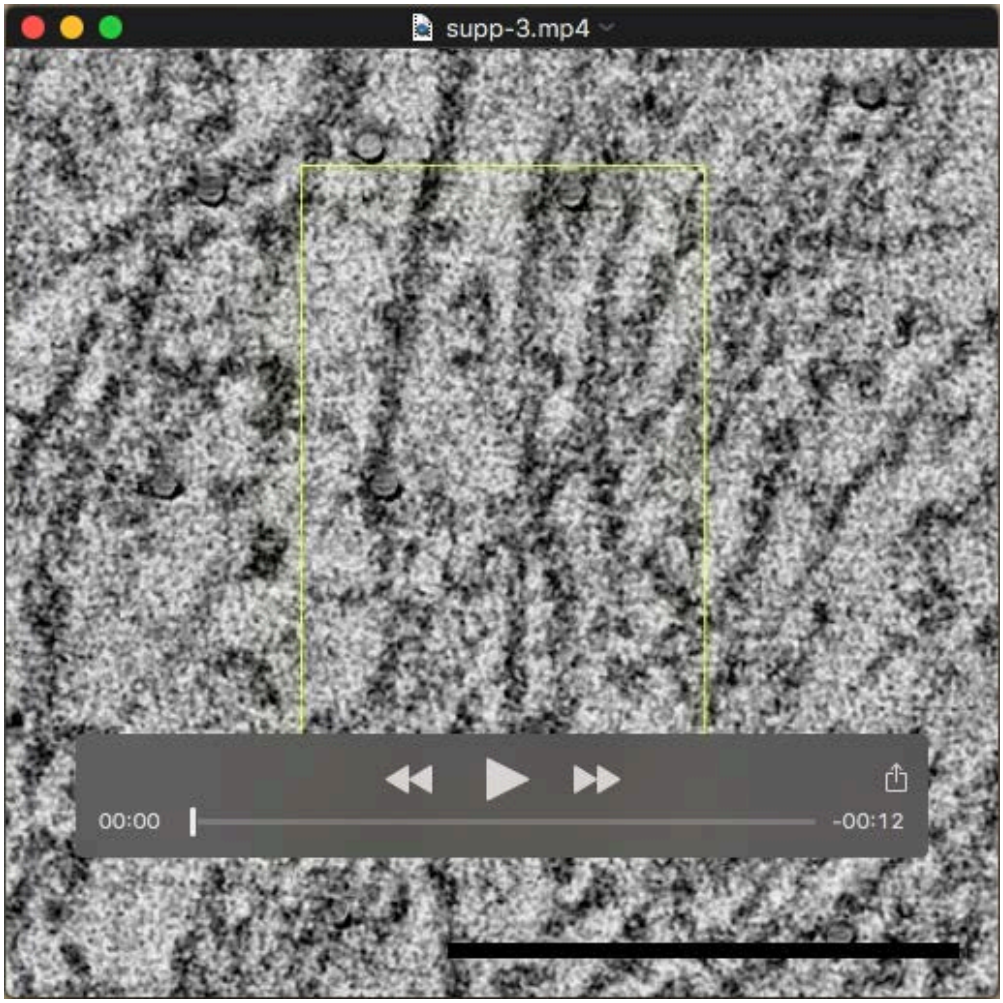


Figure S1. Tpm3.1/3.2 and Tpm4.2 co-localise with non-muscle II heads, but not non-muscle myosin II tails or α-actinin. (A) Confocal images of U2OS cells that were imaged in higher resolution in Figure 2. White boxes indicate the region imaged by STED or airyscan in Figure 2. (B) Live cell airyscan image of U2OS cells co-transfected with Ruby2-N-Tpm3.1 and myosin IIa-mEmerald demonstrate that the two constructs co-localise together at repetitive striations (arrows) along stress fibres. (C, D) Airyscan images from 3 independent experiments with 6-10 images per group per experiment were analysed for co-localisation between Tpm3.1/3.2 and Tpm4.2 with myosin IIa-mEmerald and myosin IIb-mEmerald. Graphs show mean ± SD of (C) fraction of myosin overlapping tropomyosin and (D) fraction of tropomyosin overlapping myosin.



Movie 1. Tpm3.1-APEX2 decorated actin filaments show a striated organization of Tpm3.1/3.2 in stress fibres. Tomogram reconstruction showing the 3D organization of Tpm3.1/3.2 coated actin filaments within a cultured homozygous Tpm3.1-APEX2 +/+ primary MEF. Dark enrichments correspond with Tpm3.1-APEX2 enriched regions (tomogram depth = 200 nm). Scale bar = 200 nm.



Movie 2. Tpm3.1/3.2 coated actin filaments are intact in sections processed for electron microscopy. A movie of showing a tomogram of Tpm3.1/3.2 coated actin filaments within a cultured homozygous Tpm3.1-APEX2 +/+ primary MEF. Dark enrichments correspond with APEX2-Tpm3.1 enriched regions (tomogram depth = 200 nm). Zoom-in isosurface rendering of the selected volume (magenta) highlights the intactness of the actin filaments. Scale bar = 200 nm.