Legends for Supplemental Videos

Video S1. FRAP reveals that IIB-SK1·2 is less dynamic than IIB-WT. MRC-5 SV1 TG1 cells were cotransfected with pEGFP-IIB-IIB-SK1·2 (shown on the right) and pmCherry-IIB-WT (shown on the left), and photobleaching was performed. Images were captured using a LSM 510 META microscope at 1 frame/10 sec. The playback speed is 5 frames/sec. Still images are displayed in Fig. 4A.

Video S2. Photoconversion reveals that IIB-SK1·2 is less dynamic than IIB-WT. MRC-5 SV1 TG1 cells were transfected with phmKikGR-IIB-WT (left) or phmKikGR-IIB-SK1·2 (right) and photoconversion was performed. Images were captured using an inverted microscope (Ti-E) and confocal laser microscope system (A1R) at 1 frame/3 min. The playback speed is 5 frames/sec. Bar, 5 μm.

Video S3. Photoconversion reveals that IIB-SK1·2 is less dynamic than IIB-WT. Enlarged movie of **Video S2**. Bar, 2 μm. Still images are displayed in Fig. 4D.

Video S4. IIB-SK1·2 accumulates in the posterior region of migrating cells. MEF/3T3 Tet-Off cells were cotransfected with pEGFP-IIB-SK1·2 and pmCherry-IIB-WT. Images were captured using an inverted microscope (HS All-in-One Fluorescence Microscope BZ-9000; KEYENCE) equipped with an objective lens (CFI Plan Apo λ 20×/0.75 NA; KEYENCE). The cells were maintained in DMEM/F12 (1:1) (GIBCO) supplemented with 10% FBS, and were warmed at 37°C in a Stage Top Incubator (INU-KI-F1; Tokai Hit) during observation. The images were captured at 1 frame/3 min and analyzed using Multipoint Time-lapse BZ-H2TL software (KEYENCE). The playback speed is 10 frames/sec. Still images are displayed in Fig. S2.

Video S5. IIB-SK1·2 remains in the posterior of the daughter cells during postmitotic spreading. MEF/3T3 Tet-Off cells were cotransfected with pEGFP-IIB-SK1·2 and pmCherry-IIB-WT. Images were captured using an inverted microscope (Ti-E; Nikon), equipped with a cooled CCD camera (ORCA-R2; Hamamatsu Photonics) and an objective lens (Plan Flour 40×/0.75 NA; Nikon). Cells were maintained in DMEM/F12 (1:1) (GIBCO) supplemented with 10% FBS, and warmed at 37°C in a chamber (INUBG2TF-WSKM; Tokai Hit) during observation. The images were captured at 1 frame/5 min and analyzed using MetaMorph software (Molecular Devices). The playback speed is 10 frames/sec. Bar, 20 μm. Still images are displayed in Fig. S3.

Supplemental Table S1

Table S1. Primers used for mutagenic PCR.

Use	Primers*
NMHC-IIB-SK1	
Forward	5'-TTTTGAGAAGCTTCAAGTTCAGCATGAGTTTCTTCACACTTCTGCTTCTCTTCTGCTAACAGCTGG-3'
Reverse	5'-GGAATCCCGCTCACTCAGCACAGAACTATTTAAGATTAAGAATGCCCTCGAGGAAGCCCTGGAGG-3'
NMHC-IIB-SK2	
Forward	5'-GCAGGATCTTTCCCTCTTCATGTTCAAGAGATGCCTCTGCCTCCTCCAGCTGGGTCCTCATTTCC-3'
Reverse	5'-GCATCCAGCTTGAGTTGAACCAAGTCAAGTCTGAGGTTGATAGAGACCTGCAAACCAGGGATGAGC-3'
NMHC-IIB-SK3	
Forward	5'-TCTGCATGGACTCCACGATTCTAATGTGGTTTCTCTTCATCTGGTCATTCTGCTCATCCCTGGTTTGC-3'
Reverse	5'-GCACACTGGATGCTGAGATCAGGAGCAGGAATGATGCCATTAGGTCAAAGAAAAAGATGGAGATAGACC-3'
NMHC-IIA-SK1	
Forward	5'-TTTTGAGAAGCTTCAAGTTCAGCATGAGTTTCTTCACACTTCTGCTTCTCCTCCGCCAGGAGCTGG-3'
Reverse	5'-GGAATCCCGCTCACTCAGCACAGAACTATTTAAGATTAAGAATGCCCTGGAGGAAGCCATGGAGC-3'
NMHC-IIA-SK2	
Forward	5'-GCAGGATCTTTCCCTCTTCATGTTCAAGAGATGCCTCTGCCTCTTCCAGCTGCGTCTTCATCTCC-3'
Reverse	5'-GCATCCAGCTTGAGTTGAACCAAGTCAAGTCTGAGGTTGATCGGGACCTGCAGGGCCGGGACGAGC-3'
nmRLC	
Forward	5'-CTCGAGCCACCATGTCGAGCAAAAAGGCA-3'
Reverse	5'-GGTACCTCAGTCATCTTTGTCTTTGG-3'
HA tagged nmELC	
Forward	5'-GAATTCCCACCATGTGTGACTTCACCGAAG-3'
Reverse	5'-AAGCTTTCATGCATAGTCCGGGACGTCATACGGATAGCCATTCAGCACCATGCGGACGAGC-3'
L21 insertion	
Forward	5'-GGATCTCGGTCCGAAACCAACTCCTAAAAAACCGCCACCATGTCGTACTACCATCAC-3'
Reverse	5'-GTGATGGTAGTACGACATGGTGGCGGTTTTTTAGGAGTTGGTTTCGGACCGAGATCC-3'
mCherry	
Forward	5'-GCTACCGGTCGCCACCATGG-3'
Reverse	5'-TGAGTACTTGTACAGCTCGTCCATGC-3'

*Underscores indicate primer sequences annealing to the template.

Supplemental Figure S1

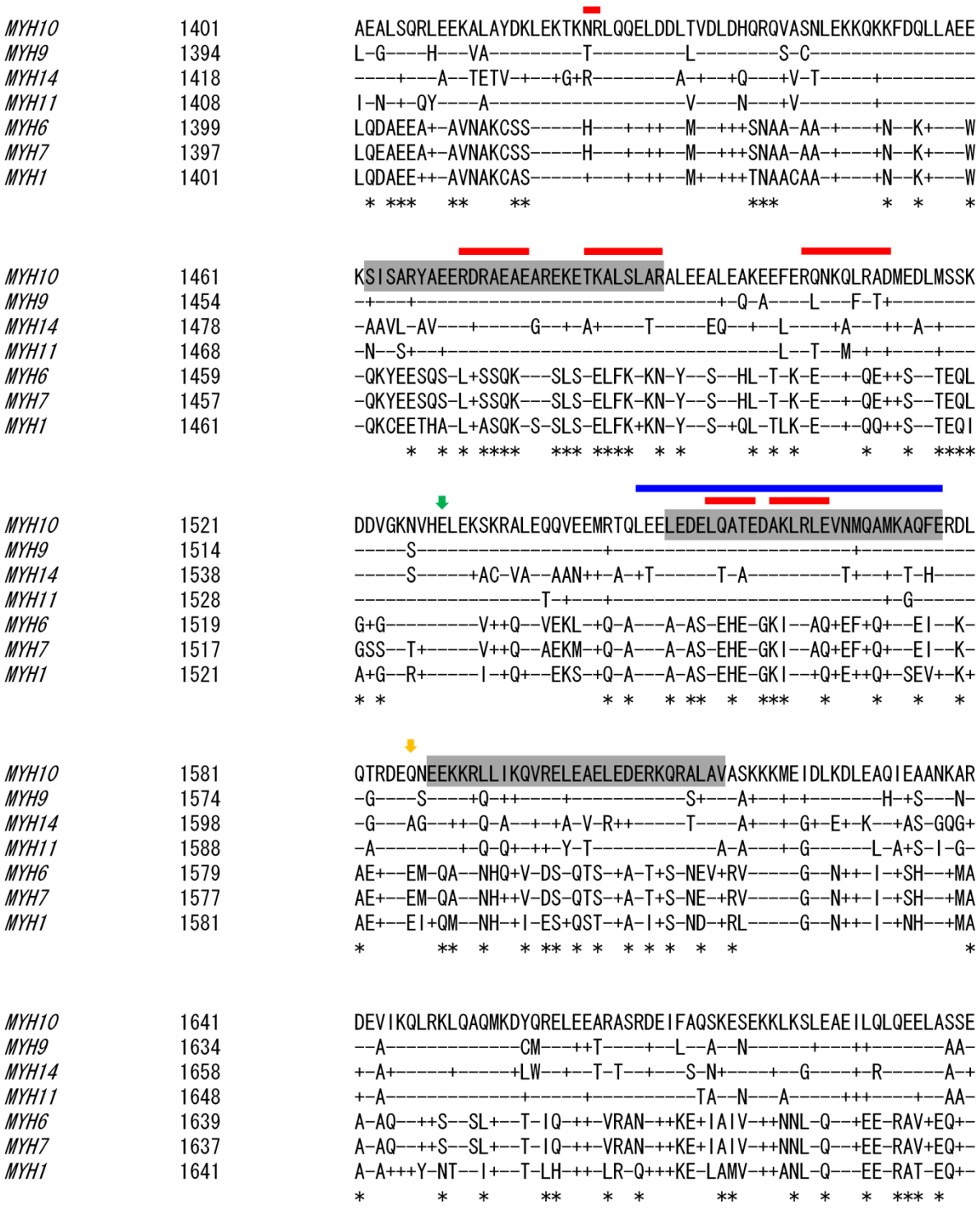

Figure S1. Sequence alignment of human class II myosin heavy chains in regions corresponding to *MYH10* (amino acids 1401–1700). Sequence data were obtained from GenBank. Identical and similar amino acid residues to *MYH10* are indicated by dash and plus sign, respectively. Asterisks indicate the amino acid residues conserved among nonsarcomeric type (*MYH9*, *MYH10*, *MYH11* and *MYH14*) but not conserved to sarcomeric type (*MYH1*, *MYH6* and *MYH7*). Gray shades indicate the region 1 (S1462-R1490), 2 (L1551-E1577) and 3 (E1588-A1617), from the N-terminus, respectively. Sequence data were obtained from

GenBank: *MYH10* (NMHC-IIB; NP_005955); *MYH9* (NMHC-IIA; NP_002464); *MYH14* (NMHC-IIC; NP_079005); *MYH11* (smooth muscle MHC; NP_001035202); *MYH1* (fast skeletal muscle MHC; NP_005954); *MYH6* (cardiac muscle MHC α; NP_002462); *MYH7* (cardiac muscle MHC β; NP_000248). A blue bar and red bars indicate the RLC-interacting region proposed by the in vitro crosslink experiments of Olney et al. (Olney et al. 1996) and Salzameda et al. (Salzameda et al. 2006), respectively. An orange arrow indicates the second skip residue from heptad repeat in the myosin tail, which is a putative second bending position. A green arrow also indicates the putative second bending position predicted by the single molecule negative stain electron microscopy with an image processing technique (Burgess et al., 2007).

Supplemental Figure S2

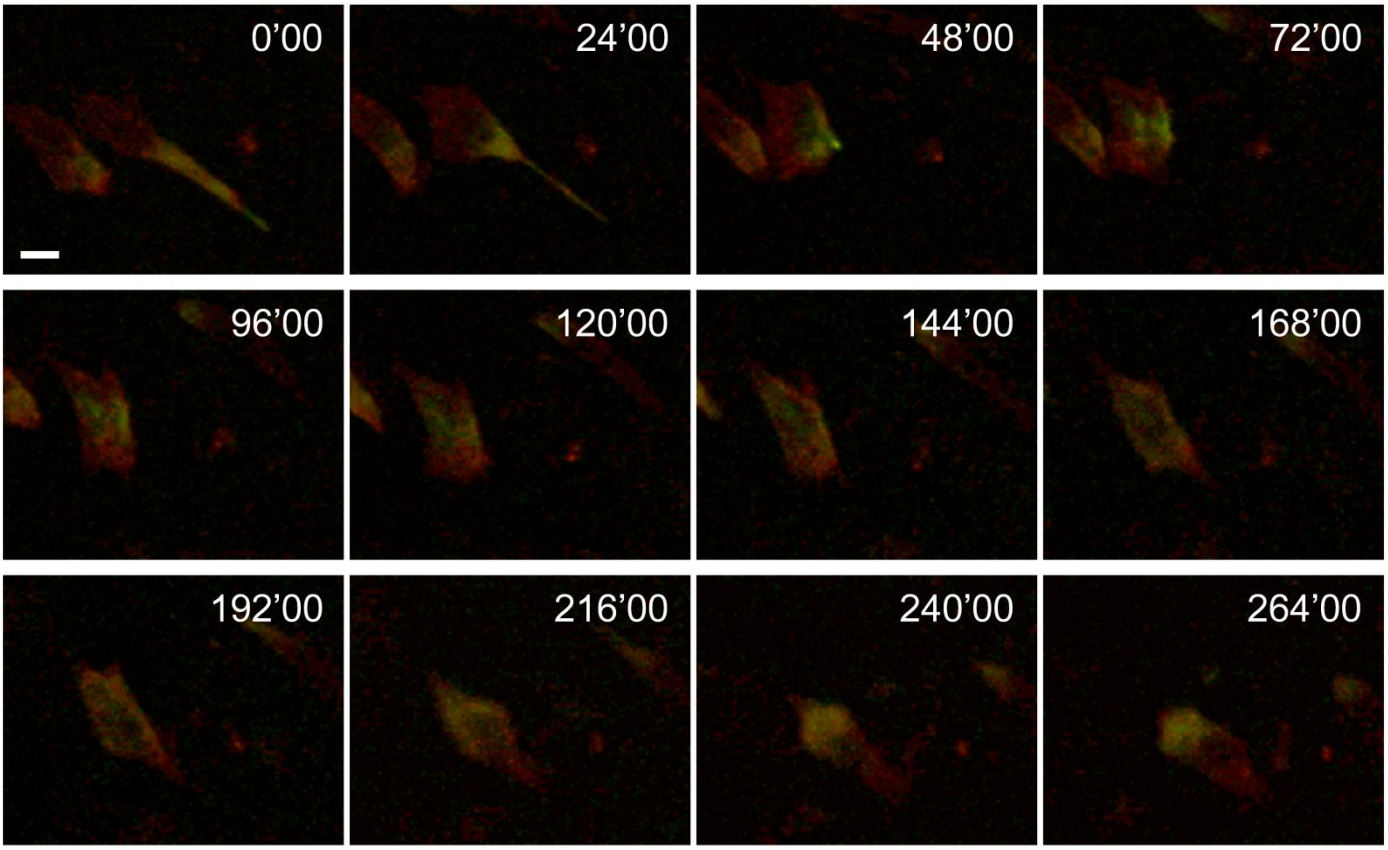

Figure S2. Still images from Video S4 focused on a direction change of migrating MEF/3T3 Tet-Off cells coexpressing EGFP-IIB-SK1·2 and mCherry-IIB-WT. Bar, 20 μm.

Supplemental Figure S3

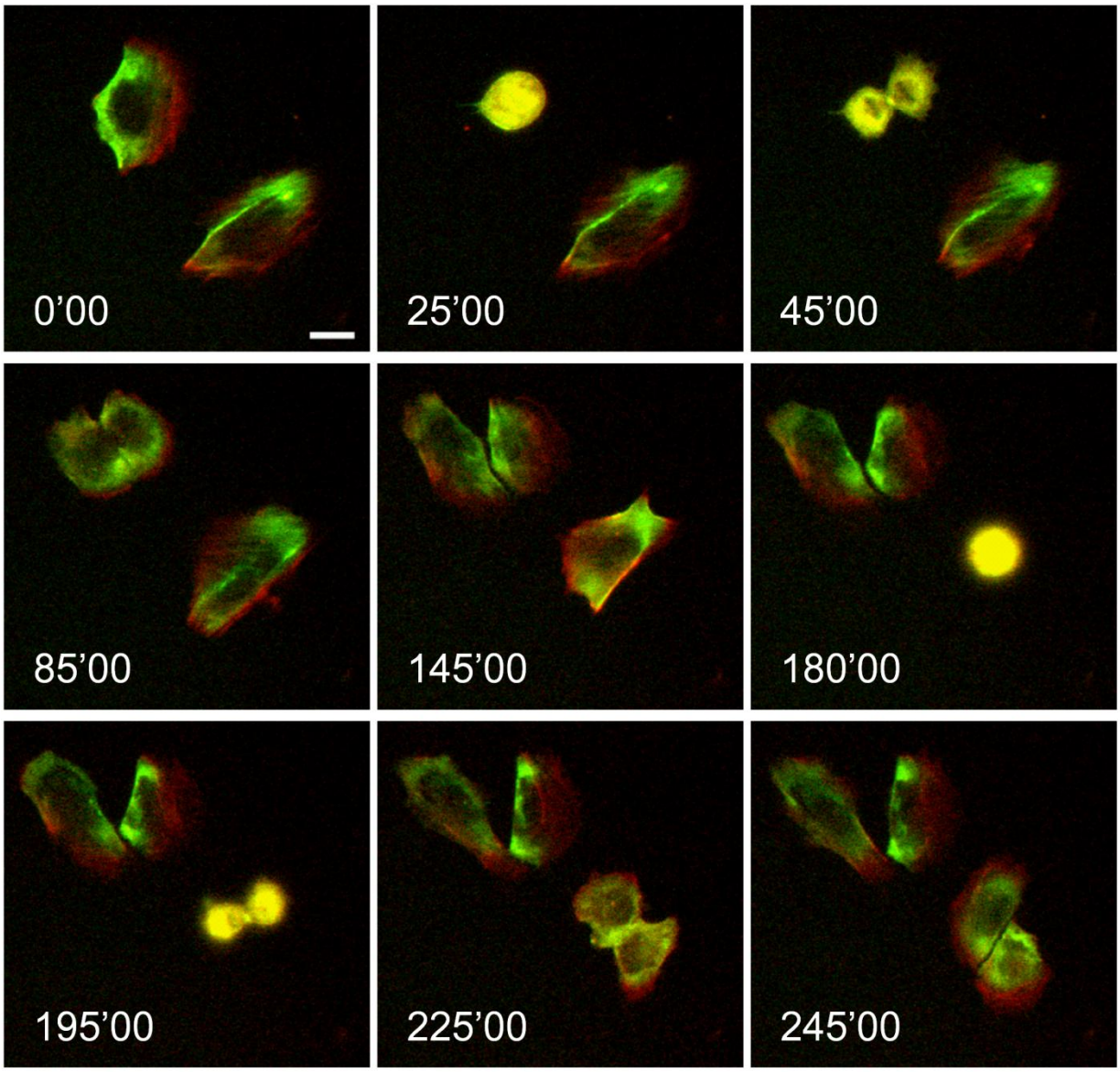

Figure S3. Still images from Video S5 focused on cytokinesis and postmitotic spreading of MEF/3T3 Tet-Off cells coexpressing EGFP-IIB-SK1·2 and mCherry-IIB-WT. Bar, 20 μm.

Supplemental Figure S4

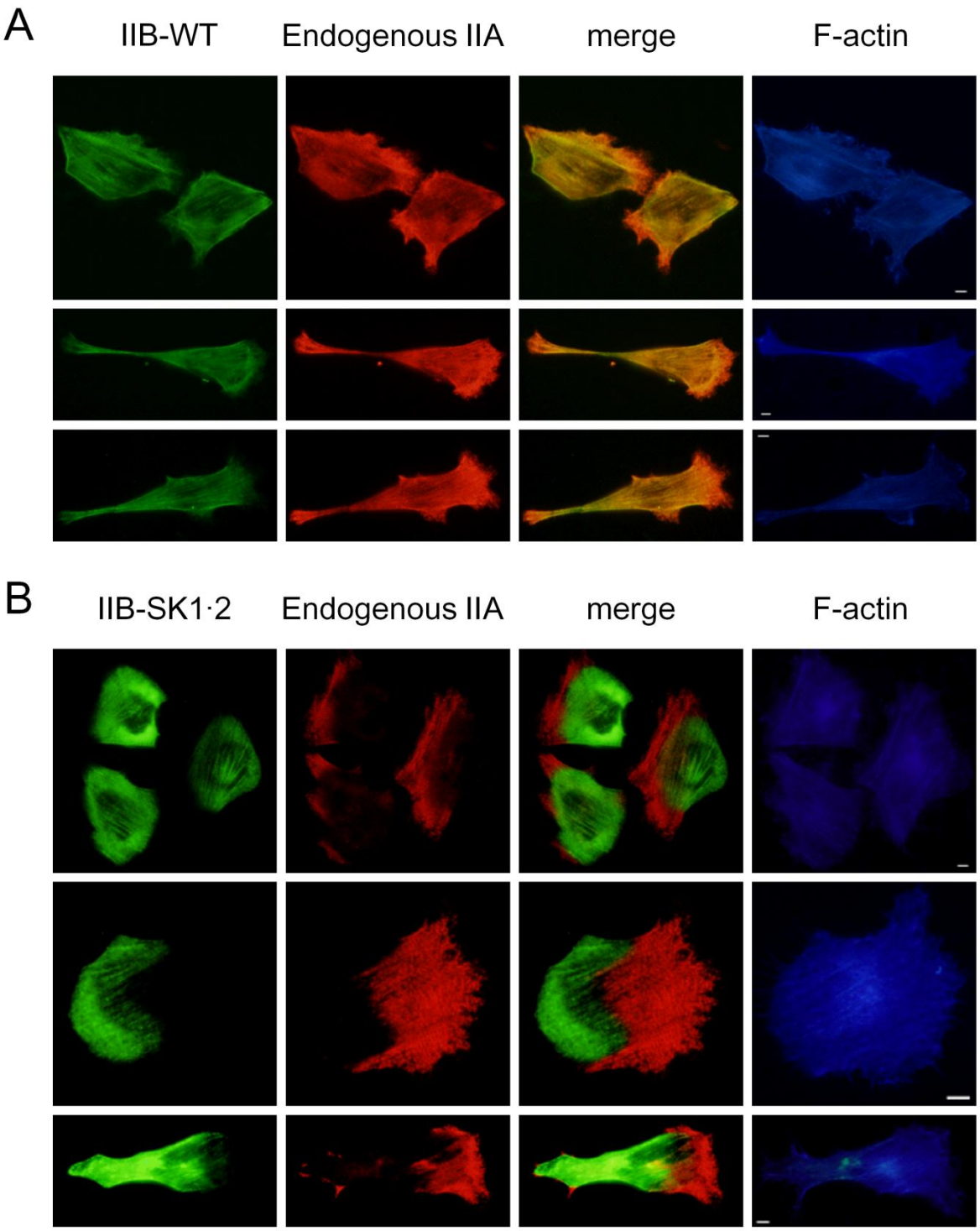

Figure S4. Localization of endogenous myosin IIA in HeLa Tet-Off cells expressing EGFP-IIB-WT (A), or EGFP-IIB-SK1·2 (B). Second column is images of endogenous myosin IIA staining. Third column is merged images of EGFP-IIBs and endogenous myosin IIA. Fourth column is images of F-actin stained by coumarin-phalloidin. The other representative images corresponding to Fig. 8D were shown. Bar, 5 μm.