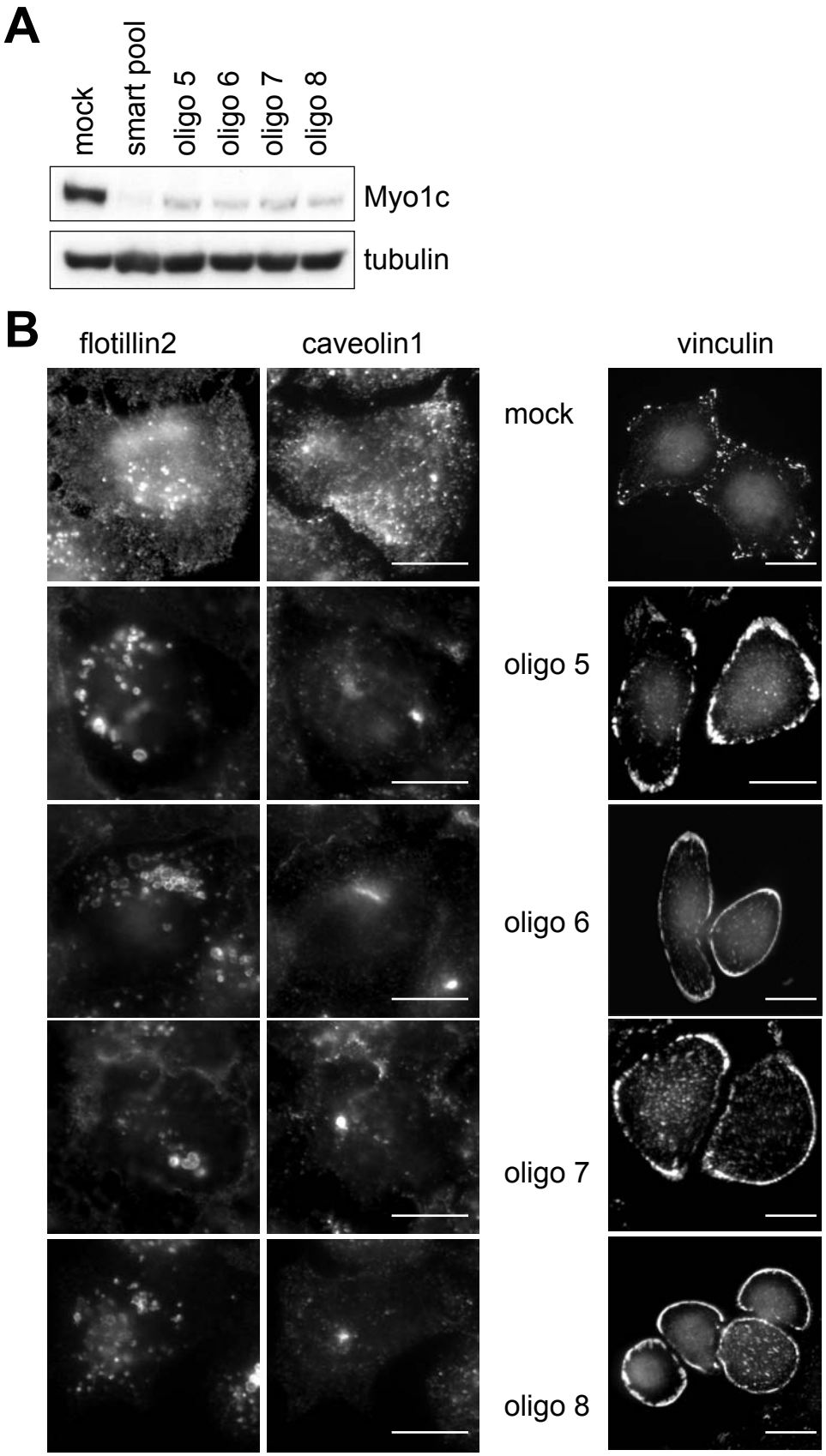

Fig. S1: Single siRNA oligos targeting Myo1c cause redistribution of lipid rafts and focal adhesions. (A) HeLa cells were either mock transfected, transfected with single siRNA oligos specific to Myo1c or with a siRNA smart pool, which combines all four oligos. Cell lysates were blotted and probed with antibodies to Myo1c and α-tubulin as a loading control to confirm the successful Myo1c depletion. **(B)** Mock and Myo1c depleted cells using single oligos were labeled with antibodies to flotillin2, caveolin1 or vinculin. Bars, 10μm

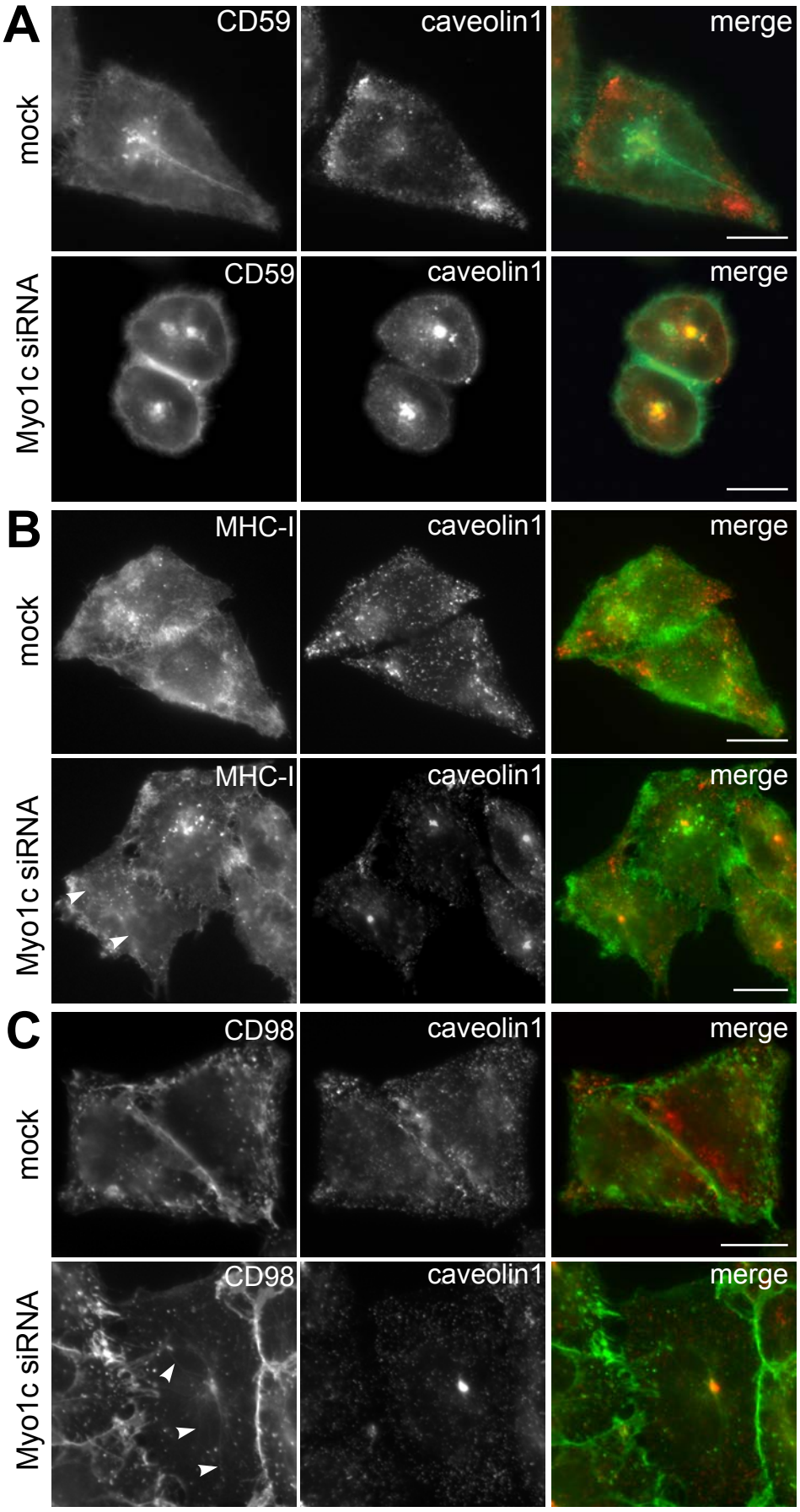

Fig. S2: Depletion of Myo1c redistributes CD59, but does not affect CD98 or MHC-class I localization. (A) HeLa cells were mock treated or treated with siRNA specific to Myo1c and stained with antibodies to caveolin1 and lipid raft marker CD59 for immunofluorescence microscopy. **(B, C)** Mock and Myo1c knockdown cells were incubated in the presence of antibodies to MHC-I **(B)** or CD98 **(C)** for 1 hour at 37°C, then fixed and labelled with anti-caveolin1 antibodies for immunofluorescence microscopy. Bars, 10μm

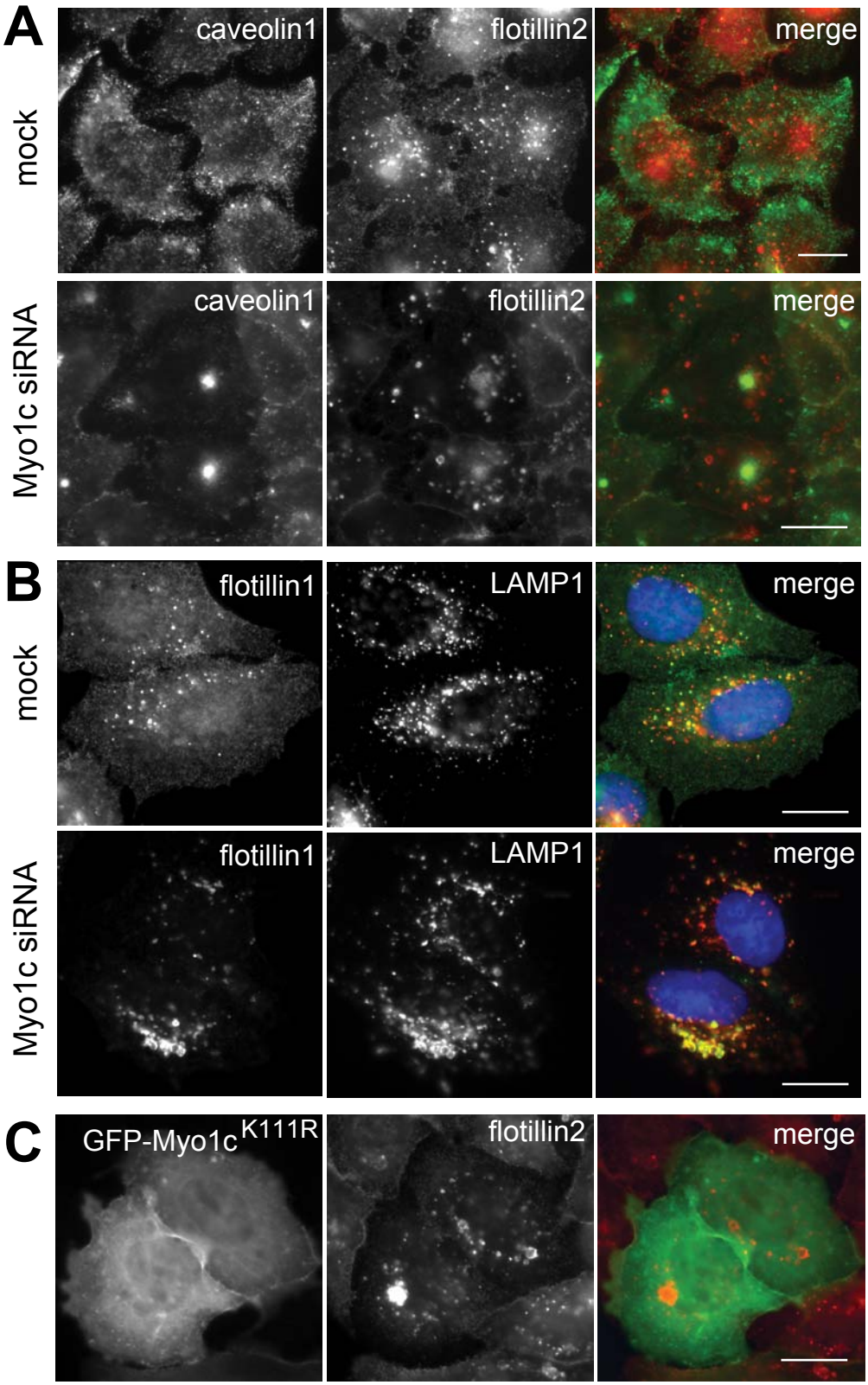

Fig. S3: Depletion of Myo1c redistributes lipid raft markers flotillin1 and 2. (A) HeLa cells were mock treated or treated with siRNA specific to Myo1c and stained with antibodies to caveolin1 and flotillin2 for immunofluorescence microscopy. **(B)** Mock or Myo1c depleted HeLa cells were labeled with antibodies to flotillin1 and the late endosomes/lysosome marker LAMP1 for confocal microscopy. Cell nuclei are shown in blue in the merged images. **(C)** HeLa cells transiently transfected with the dominant negative GFP-Myo1cK111R rigor mutant were labeled with antibodies to GFP and flotillin2. Bars, 10µm

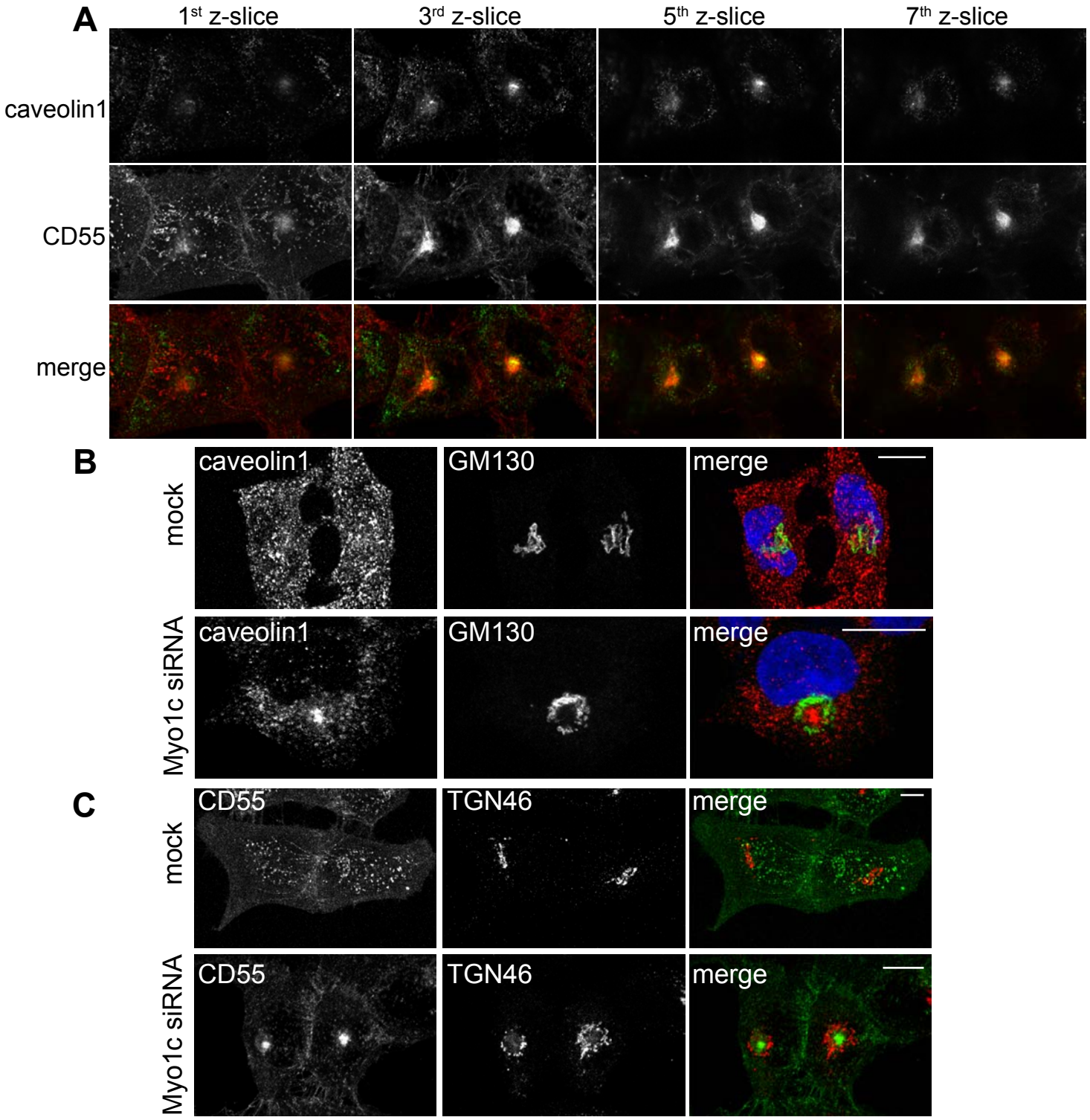

Fig. S4: Depletion of Myo1c causes accumulation of lipid rafts in the peri-nuclear position, which is distinct from the Golgi complex. (A) Myo1c depleted HeLa cells were labelled with anti-CD55 and anti-caveolin1 antibodies and z-stacks were captured using confocal microscopy. Images represent individual optical z-slices (0.33µm thickness). **(B, C)** Mock and Myo1c siRNA treated HeLa cells were stained with antibodies to caveolin1 and the Golgi marker GM130 **(B)** or CD55 and the trans Golgi protein TGN46 **(C)** for confocal microscopy. Images represent a confocal z projection of the whole cell. Bars, 10µm

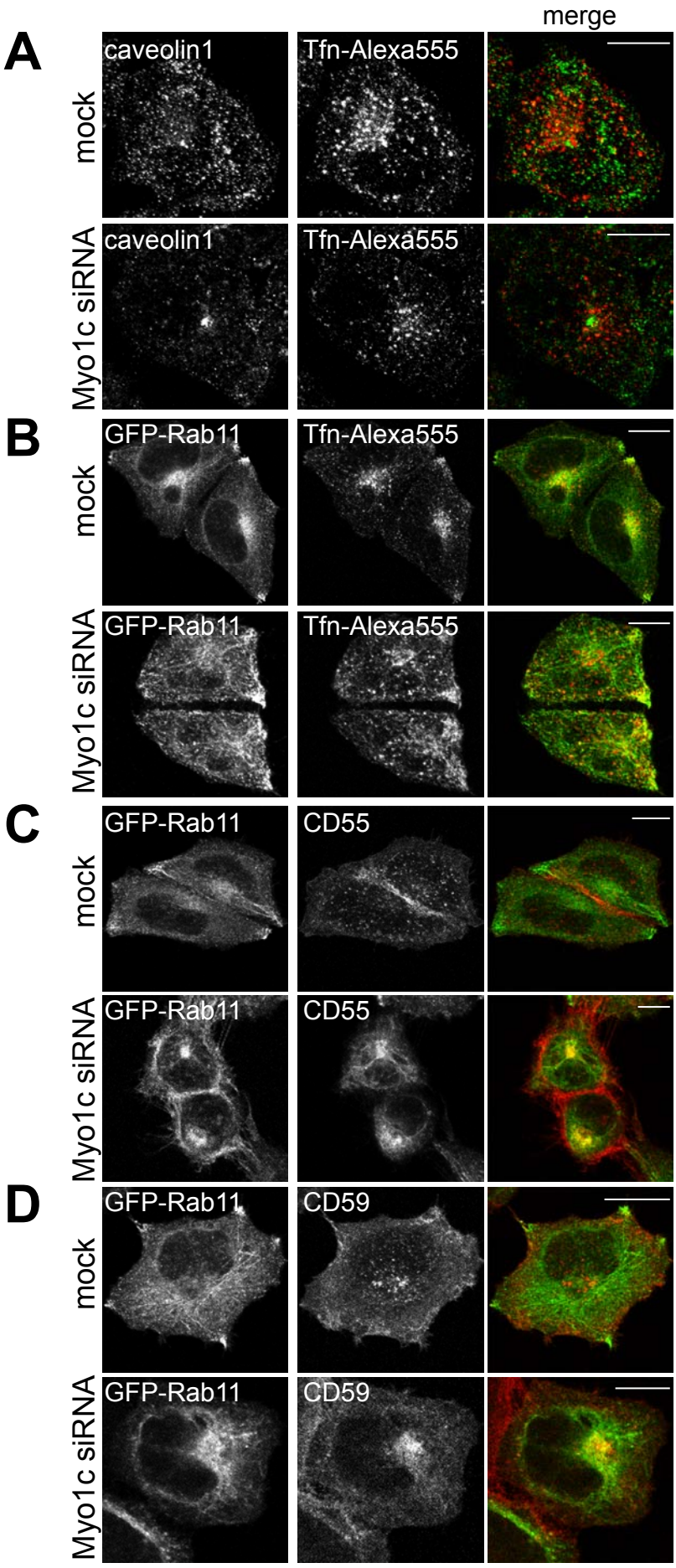

Fig. S5: Depletion of Myo1c does not change the distribution of the transferrin receptor or Rab11. (A) To detect the TfnR along the endocytic/recycling pathway mock or Myo1c depleted HeLa cells were incubated with Tfn-Alexa555 for 1 hour at 37°C, then fixed and labeled with caveolin1 antibodies for confocal microscopy. **(B)** Control and Myo1c knockdown HeLa cells stably expressing GFP-Rab11 were incubated with Tfn-Alexa555 for 1 hour at 37°C, fixed and imaged using a confocal microscope. **(C, D)** To visualize the distribution of lipid rafts in mock and Myo1c siRNA treated HeLa cells stably expressing GFP-Rab11, cells were incubated in the continuous presence of antibodies to CD55 **(C)** or CD59 **(D)** for 1 hour at 37°C, then processed for confocal microscopy. All images represent a confocal z projection of whole cells. Bars, 10μm