

Supplementary Information:

Changes in cell morphology and actin organization in embryonic stem cells cultured under different conditions

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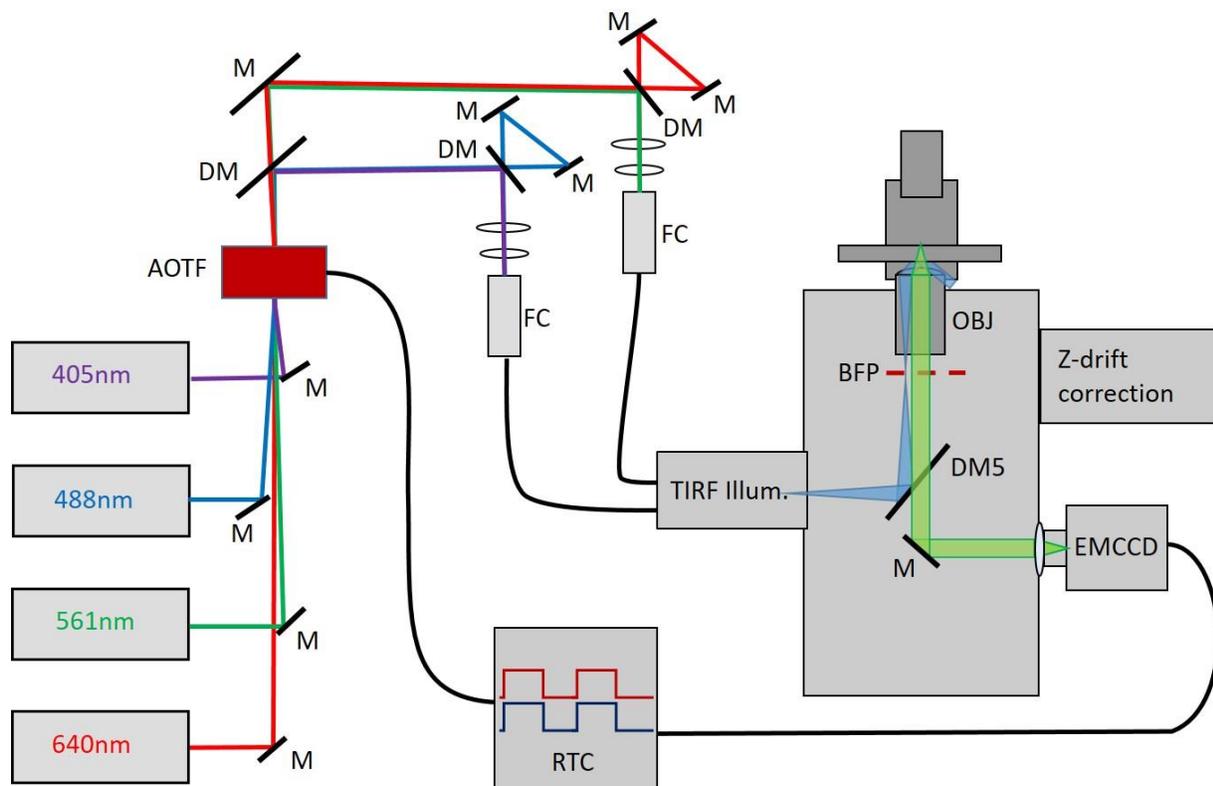


Fig. S1: Schematic of the super-resolution setup. Lasers of wavelengths 405 nm (40 mW, Olympus), 488 nm (200 mW, Cobolt), 561 nm (200 mW, Cobolt), and 640 nm (200 mW, Toptica) are directed by mirrors through the aperture of an acousto-optic tunable filter (AOTF) (AA Opto Electronic). The beams are split by dichroic mirrors (DM) (AHF Analysentechnik), and the 405 and 488 nm beams are coupled into one single-mode optical fiber (FC) (Qioptic); likewise, the 561 and 640 nm beams are coupled into a second optical fiber. The TIRF illuminator allows motorized control of the focus position of the excitation light on the back

focal plane (BFP) of the objective (OBJ) (1.45 NA, UAPON OTIRF, Olympus). Drift in the axial direction is continuously corrected by the Z-drift correction module (IX3-ZDC, Olympus). Images are captured on an EMCCD camera (ImagEM X2, Hamamatsu), which is coupled to the real-time controller (RTC, Olympus), that synchronizes the camera shutter with the AOTF in order to minimize sample illumination and bleaching.

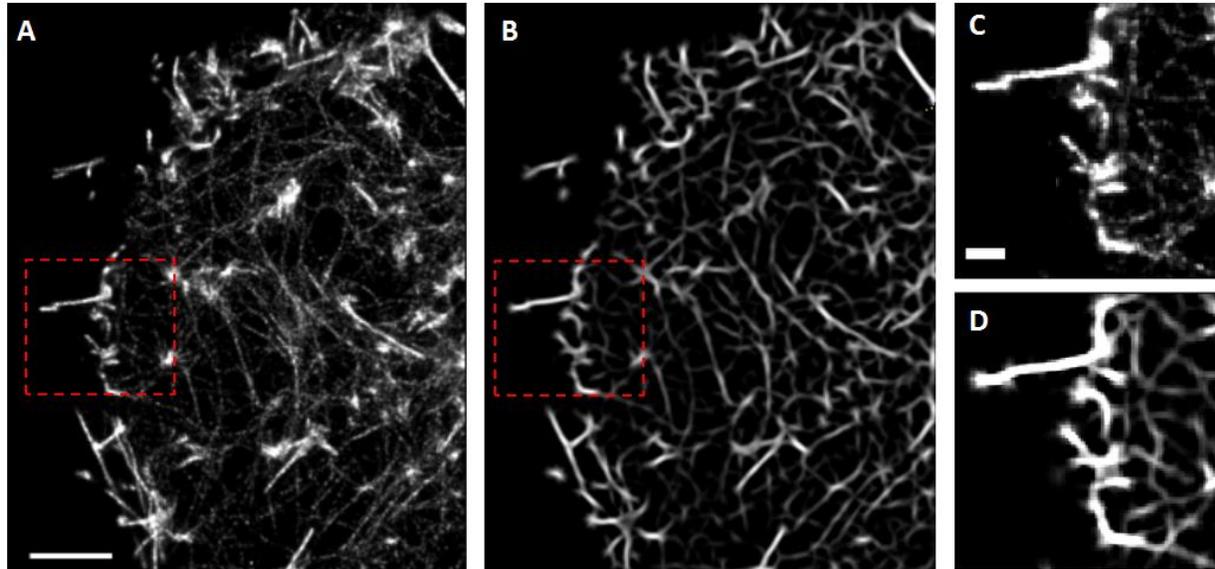


Fig. S2: Spatial resolution enhancement. **(A)** Reconstructed STORM image of the actin cytoskeleton in a stem cell in 2i media. **(B)** Image corresponding to (A) after 2D Gabor filtering. **(C-D)** Magnified views of the red boxed regions in images (A) and (B), respectively. Scale bar shown in (A) is also valid in (B) and is 2 μm . Scale bar shown in (C) is also valid in (D) and is 0.5 μm .