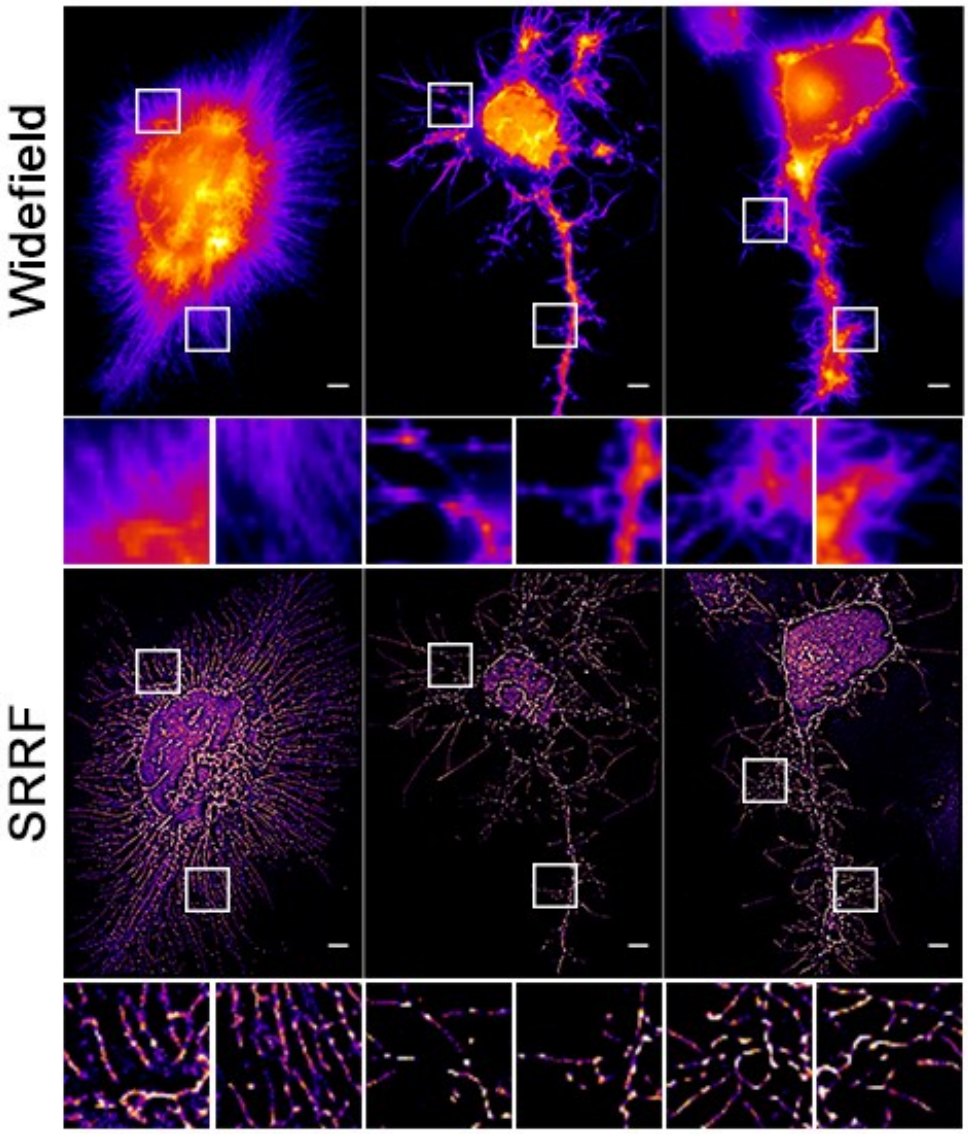
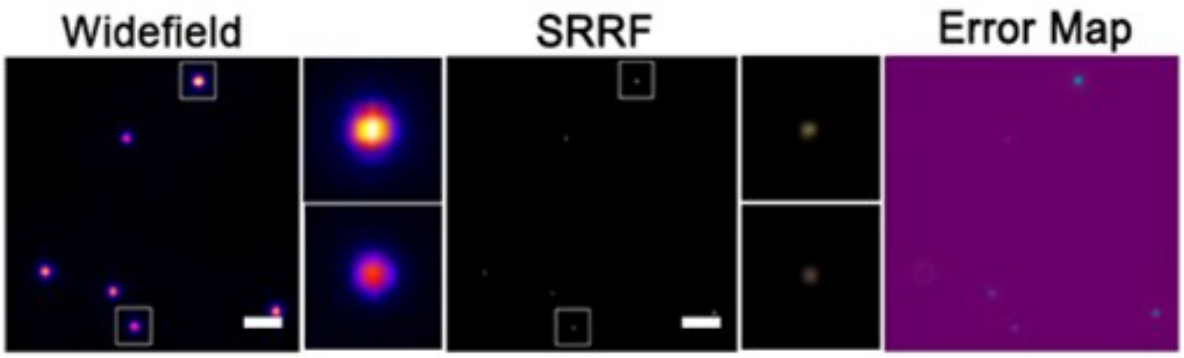
Electronic Supplementary Material (ESI) for Nanoscale.

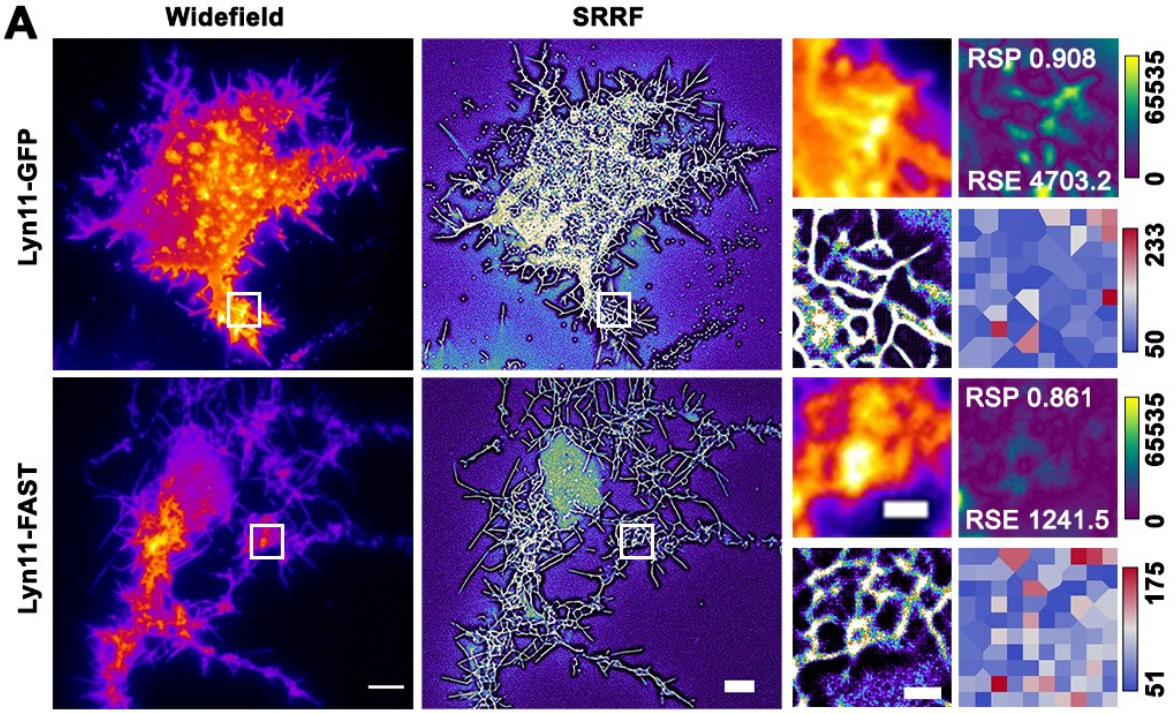
SUPPLEMENTARY INFORMATION:



Supplementary figure i: Visualization of retraction fibres at high resolution. Wide field images (top) and SRRF images (bottom) of live Neuro-2a cells expressing Lyn11-FAST in the presence of fluorogen. White regions highlight the retraction fibres at higher resolution, as shown in the widefield and SRRF panel. Scale bar: 2 µm.



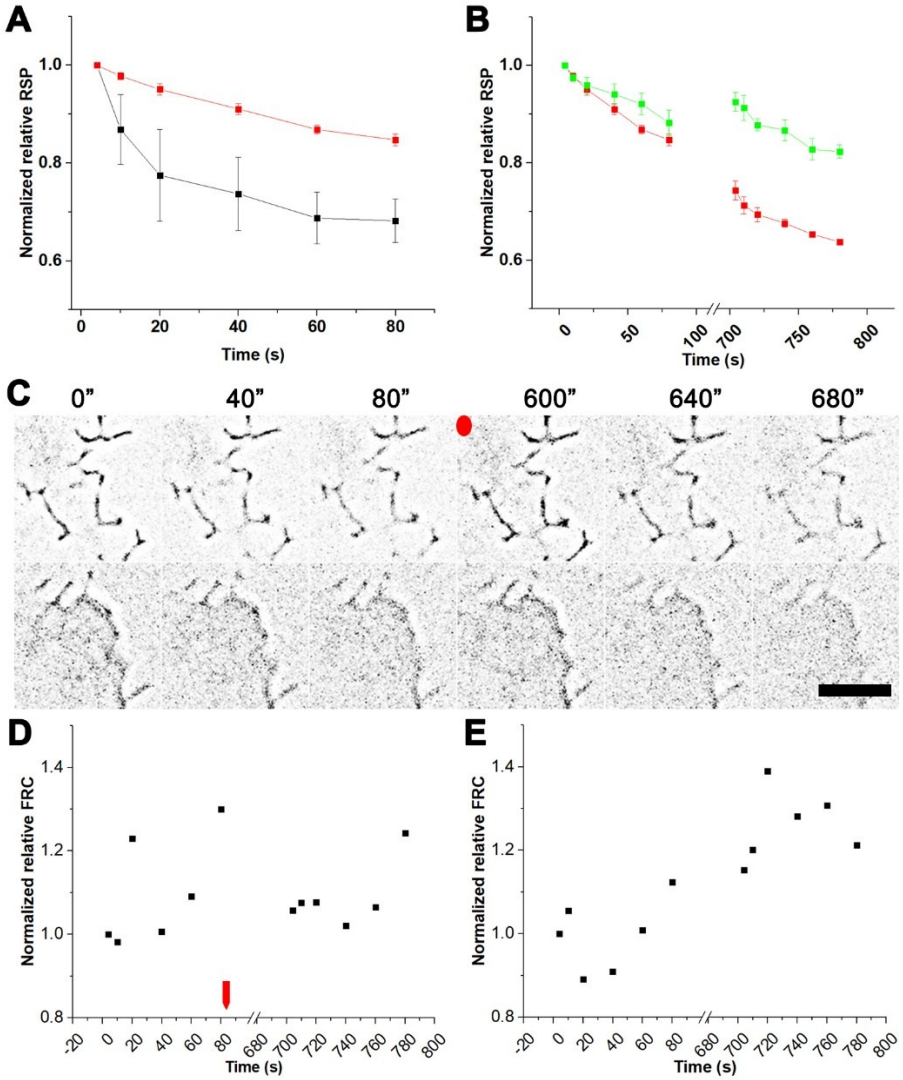
Supplementary Figure ii: Assessment of SRRF image reconstruction. Widefield and SRRF image of 100nm beads. White regions show the beads magnified on the right of the images. Resolution scaled Pearson coefficient was measured by comparing the diffraction limited image with SRRF image, thereby generating an error map using NanoJ-SQUIRREL. Scale bar: 2 µm.



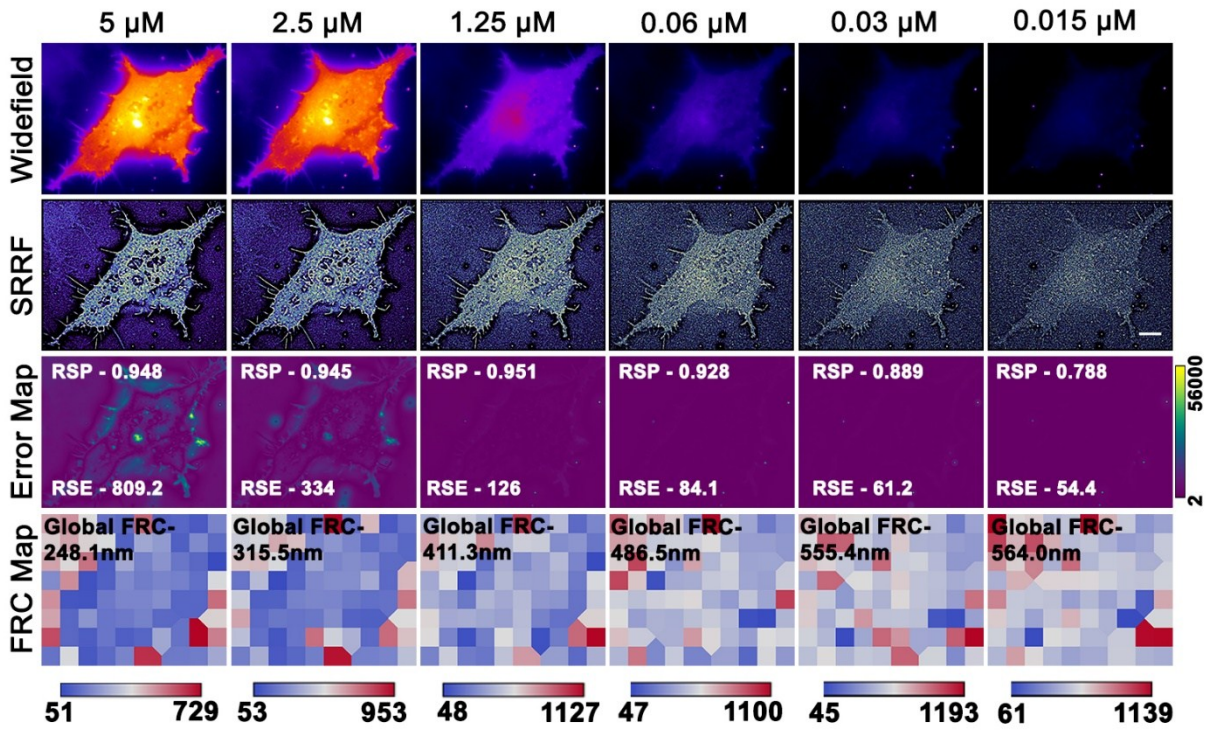
B

Construct	Spatial Resolution	Min FRC	Max FRC
Lyn11-GFP	109.13nm	50.16nm	261.56nm
Lyn11-FAST	104.10nm	49.083nm	245.46nm

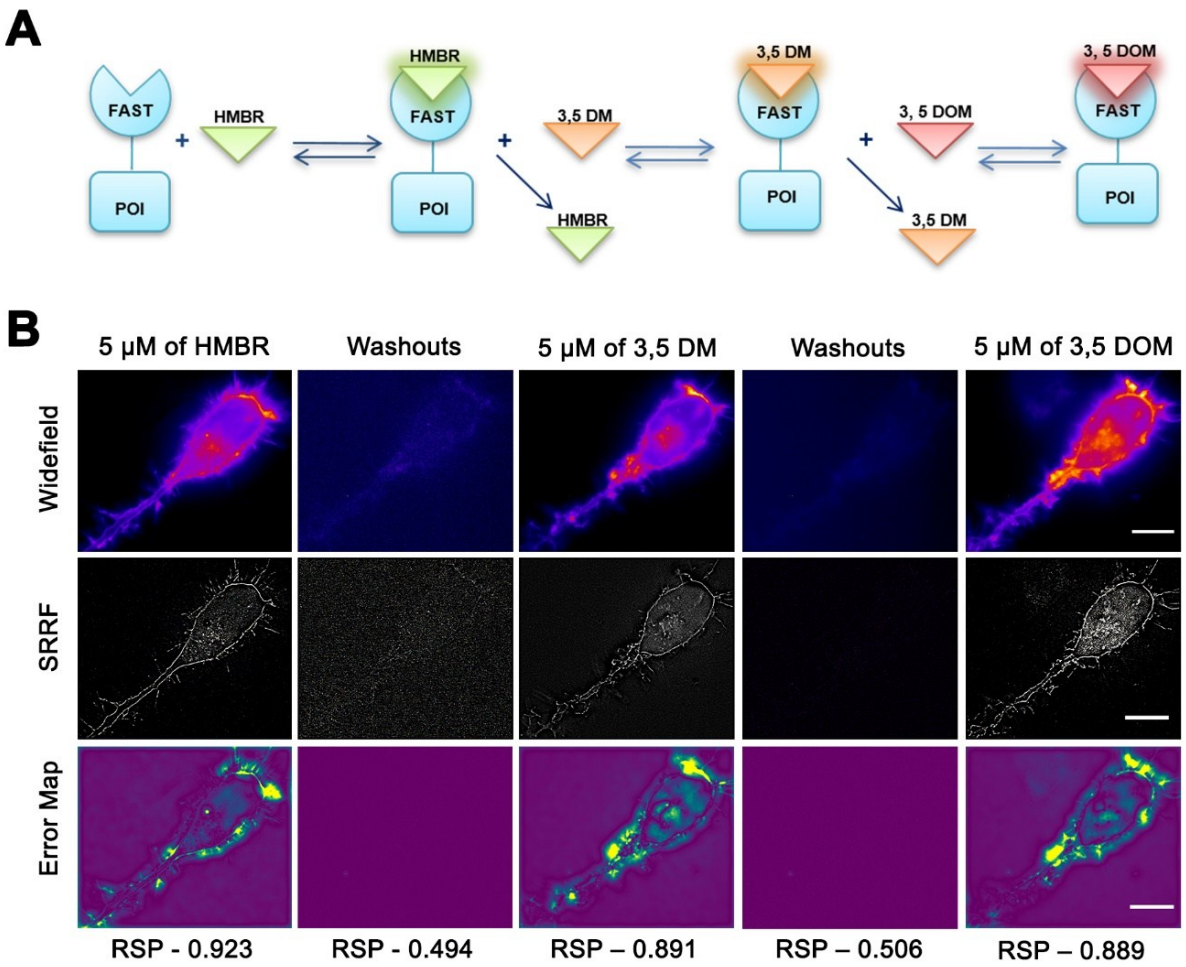
Supplementary figure iii: Comparison of FRC resolution between Lyn11-FAST and Lyn11-EGFP. A. Neuro-2a cells were transfected with Lyn11-EGFP and Lyn11-FAST. 3,5DM was used as a fluorogen for Lyn11-FAST. Corresponding maximum intensity projections of widefield images(left) and SRRF images (centre) are displayed with the FRC maps for the inset. B. Mean Spatial resolution and FRC range achieved for Lyn11-GFP and Lyn11-FAST (n = 10 cells). Scale bar: 1µm.



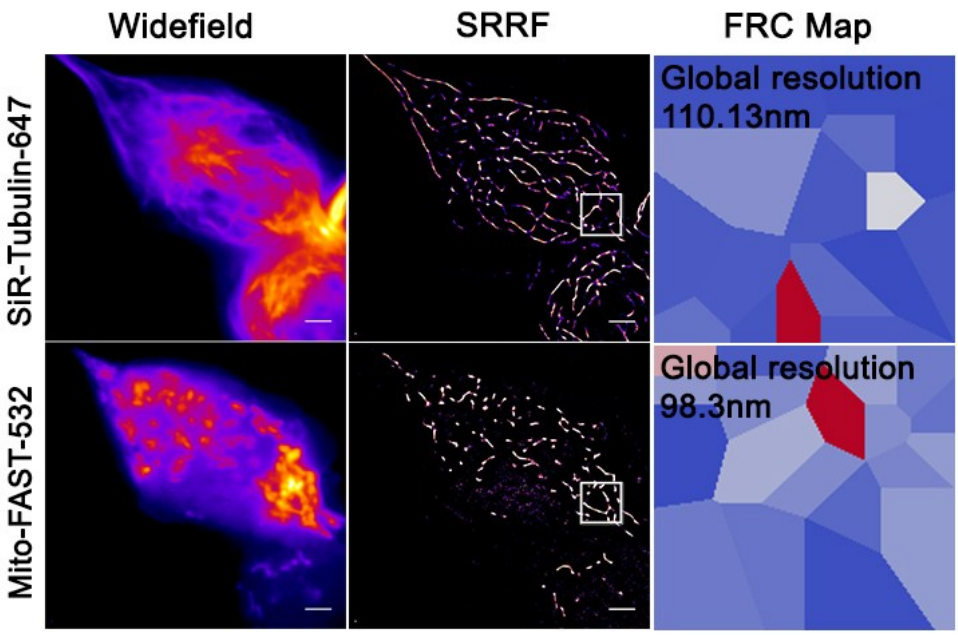
Supplementary figure iv: Comparison of FAST performance for time lapse SRRF imaging. A. The quality of the SRRF images for Lyn11-EGFP (black) declined sharply when compared to Lyn11-FAST (red) (n = 3 cells). This is represented by reduction in relative RSP values (mean ± S.E.M.) mimicking the intensity decay. B. Replenishment of FAST with fluorogen (green) allowed recovery of RSP while RSP of the control (red, n = 3 cells) decreased along time (Mean ± S.E.M.). The RSP value obtained in the first frame was used as reference to normalise the curves. C. A representative cell expressing Lyn11-FAST and undergoing replenishment of fluorogen (top panel), compared to control without replenishment (bottom panel). The red marker indicates the frame before which the replenishment was performed. Scale bar: 2.7 µm. D. The relative FRC changes over time for the representative cell depicted in top panel of C, where the red arrow indicates the replenishment. F. The plot indicates the relative FRC changes over time for the representative control cell in bottom panel C.



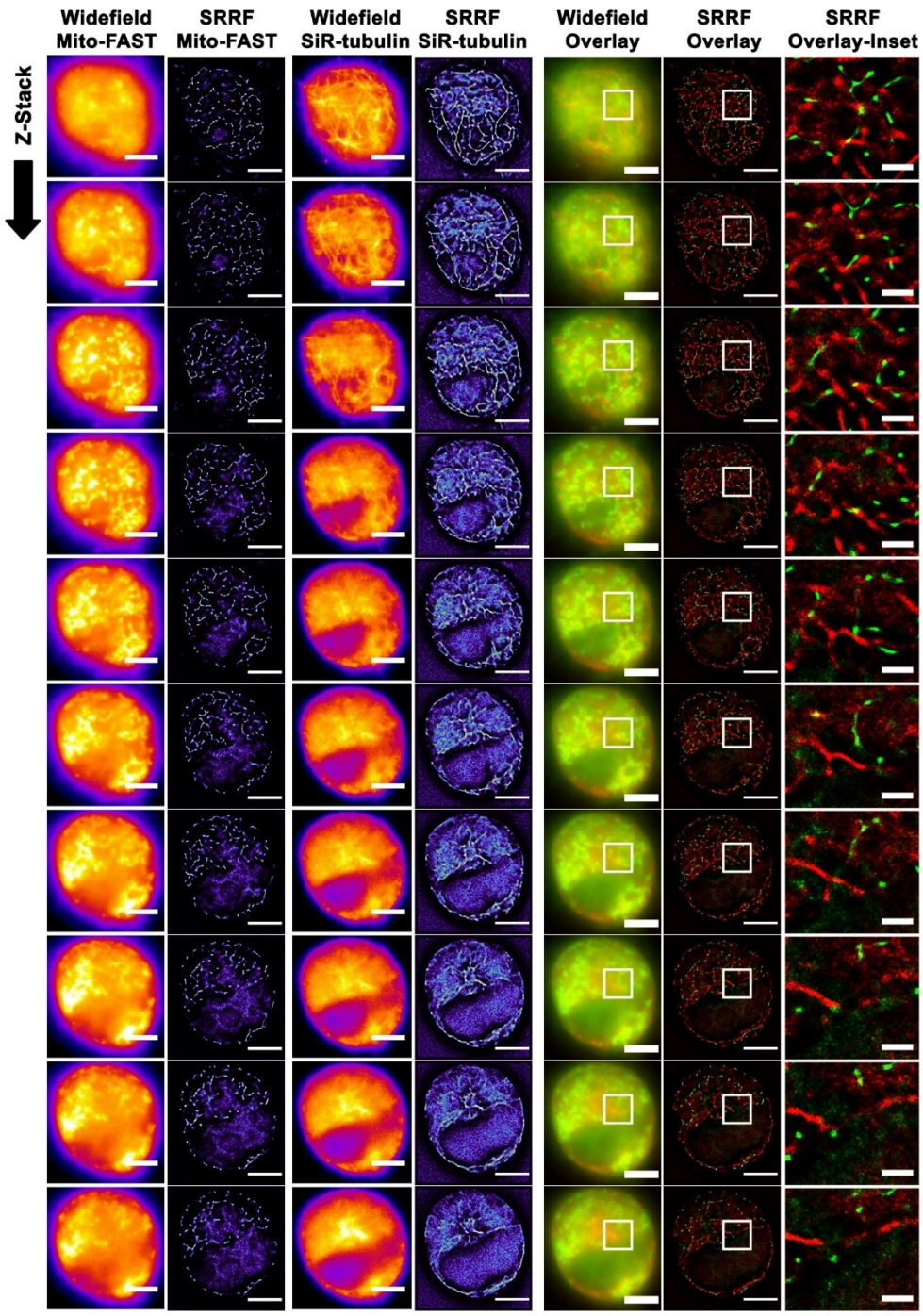
Supplementary figure v: Spatial resolution is dependent on fluorogen concentration. Lyn11-FAST was expressed in fixed Neuroblastoma cells in the presence of 3,5 DM. Concentration of 3,5 DM was sequentially reduced from 5 µM to 0.015 µM. Corresponding diffraction limited images as obtained by maximum intensity projection and their corresponding SRRF images are displayed for each concentration, with the Error and FRC Map. Scale bar: 1 µm. Error Map was calculated for each concentration with respect to the maximum intensity projection of the widefield images. Pseudocolour scale bar of Error Maps are indicated towards the right of the panel Error Map.



Supplementary figure vi: Sequential labelling of plasma membrane by stoke shifted FAST variants. A. A simplified scheme representing the fluorogen washouts, highlighting the reversibility of the fluorogen binding tag. B. Live Neuro-2a cells expressing Lyn11-FAST fusion proteins labelled with 5 µM HMBR, 3,5 DOM and 3,5 DM in sequential order with washouts. Error maps were generated correspondingly. Scale bar: 5 µm.



Supplementary figure vii: Simultaneous visualization of microtubules and mitochondria by SRRF. Neuroblastoma cells expressing Mito-FAST were treated with 5 µM of 3,5 DM and 100 nM SiR-tubulin. The FRC map is displayed for the marked region (white box). Scale bar: 1µm.



Supplementary Figure viii: 3D-stack of Mitochondria and Microtubules. 3D- stack of Neuro-2a cells expressing Mito-FAST with 5 µM of 3,5DM and 100 nM of SiR tubulin. Cells were imaged at 200 nm step size in Z plane for both the channels simultaneously. Widefield images were processed to obtain SRRF image stacks. Scale bar: 10 µm. Inset marked by white box is magnified to highlight the structural details of the dynamics within the Z-stack. Scale bar: 1 µm.