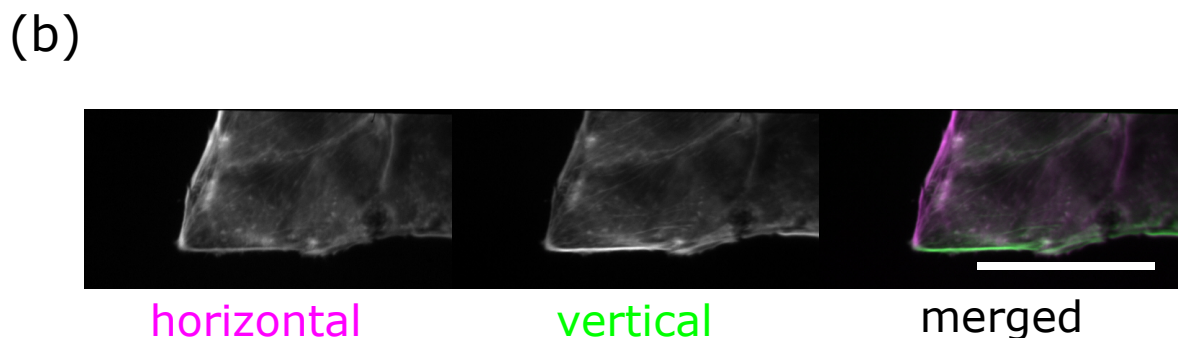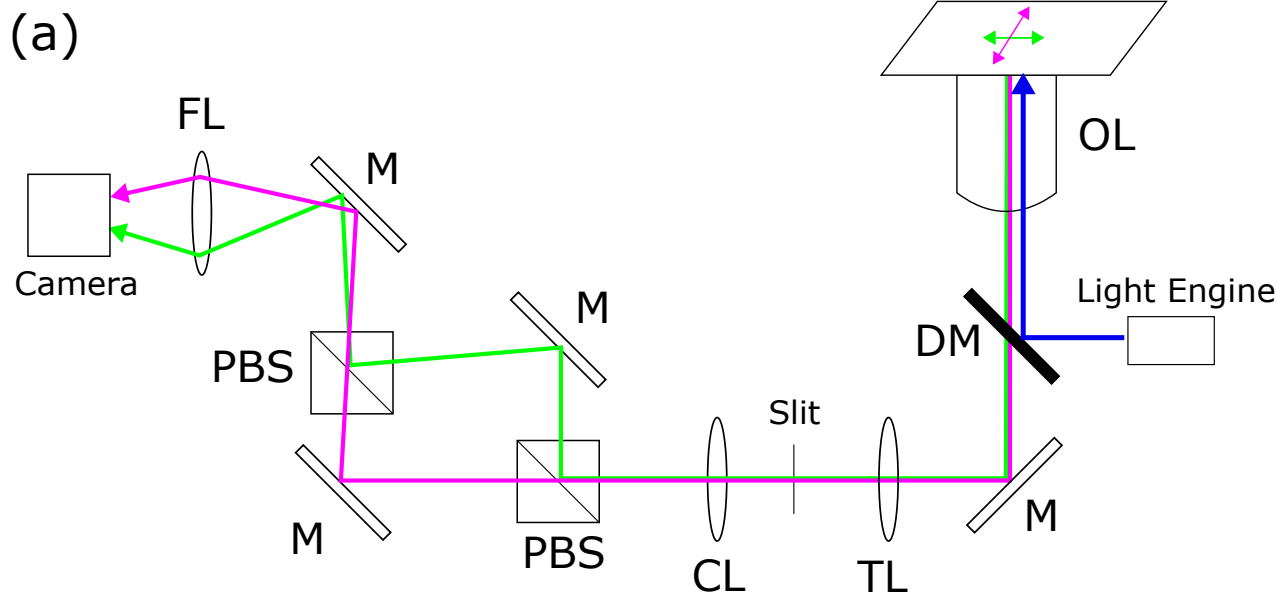# Supplementary Figure 1

(a)

(b)

horizontal   vertical   merged

Fluorescence polarization microscope (FPM) setup. (a) Schematic drawing of our FPM. OL, objective lens; DM, dichroic mirror, M, mirror; TL, tube lens; CL, collimating lens; PBS, polarization beam splitter; FL, focusing lens. (b) Fluorescence image of fixed LLC-PK1 stained with Atto565-phalloidin. Polarized fluorescence along 0° (left, horizontal polarization) and the polarized fluorescence along 90° (middle, vertical polarization) were shown in pseudocolors, magenta and green, respectively before merged (right). (Scale bar: 50 μm)

Supplementary Data

Sequence information

cpGFP$^{CT3/10}$

MGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYP
DHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKL
EYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTV
LSKDPNEKRDHMVLHEYVNAAGITLGMDELYKGGGSSKGEELFT<u>EAAAKAGS</u>*

(The underlined amino acids are added to the cpGFP.)

cpGFP-Lifeact (L18)

MGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYP
DHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKL
EYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTV
LSKDPNEKRDHMVLHEYVNAAGITLGMDELYKGGGSSKGEELFT<u>EAAAKEA</u>VADLIKKFESISKEE
GDATVDTNSS*

(The underlined amino acids are the helical linker.)

cpGFPΔC-UtrCHΔN (GU14)

MGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYP
DHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKL
EYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTV
LSKDPNEKRDHMVLHEYVNAAGITLGMDELYKGGGSSKGEELF<u>EAAAKAA</u>VQKKTFTKWINARFS
KSGKPPINDMFTDLKDGRKLLDLLEGLTGTSLPKERGSTRVHALNNVNRVLQVLHQNNVELVNIGG
TDIVDGNHKLTLGLLWSIILHWQVKDVMKDVMSDLQQTNSEKILLSWVRQTTRPYSQVNVLNFTT
SWTDGLAFNAVLHRHKPDLFSWDKVVKMSPIERLEHAFSKAQTYLGIEKLLDPEDVAVRLPDKKSII
MYLTSLFEVLPQQVTID*

(The underlined amino acids are the helical linker.)

UtrCHΔC-mEGFPΔN (UG7)

MAKYGEHEASPDNGQNEFSDIIKSRSDEHNDVQKKTFTKWINARFSKSGKPPINDMFTDLKDGRKL
LDLLEGLTGTSLPKERGSTRVHALNNVNRVLQVLHQNNVELVNIGGTDIVDGNHKLTLGLLWSIILH
WQVKDVMKDVMSDLQQTNSEKILLSWVRQTTRPYSQVNVLNFTTSWTDGLAFNAVLHRHKPDLF
SWDKVVKMSPIERLEHAFSKAQTYLGIEKLLDPEDVAVRLPDKKSIIMYLF<u>EAAAKA</u>ELFTGVVPIL
VELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQH
DFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNS
HNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNE
KRDHMVLLEFVTAAGITLGMDELYK*

(The underlined amino acids are the helical linker.)