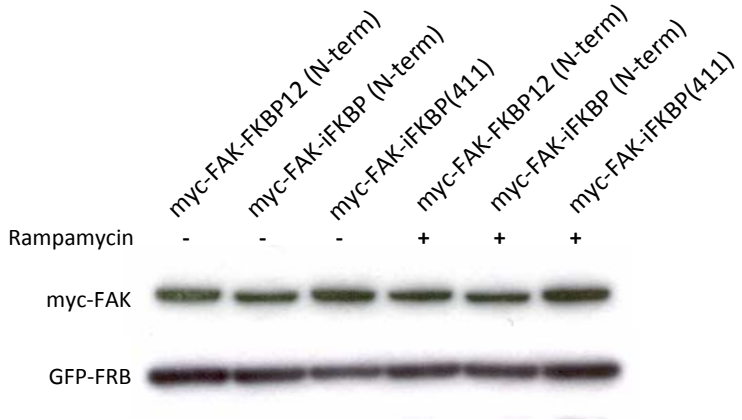
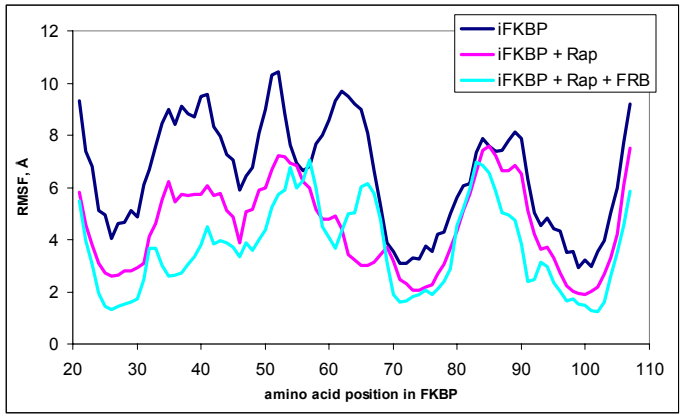
Supplementary Fig. S1



Supplementary Fig. S1. Input levels for myc-FAK constructs and GFP-FRB in cell lysates.

Nature Biotechnology: doi:10.1038/nbt.1639

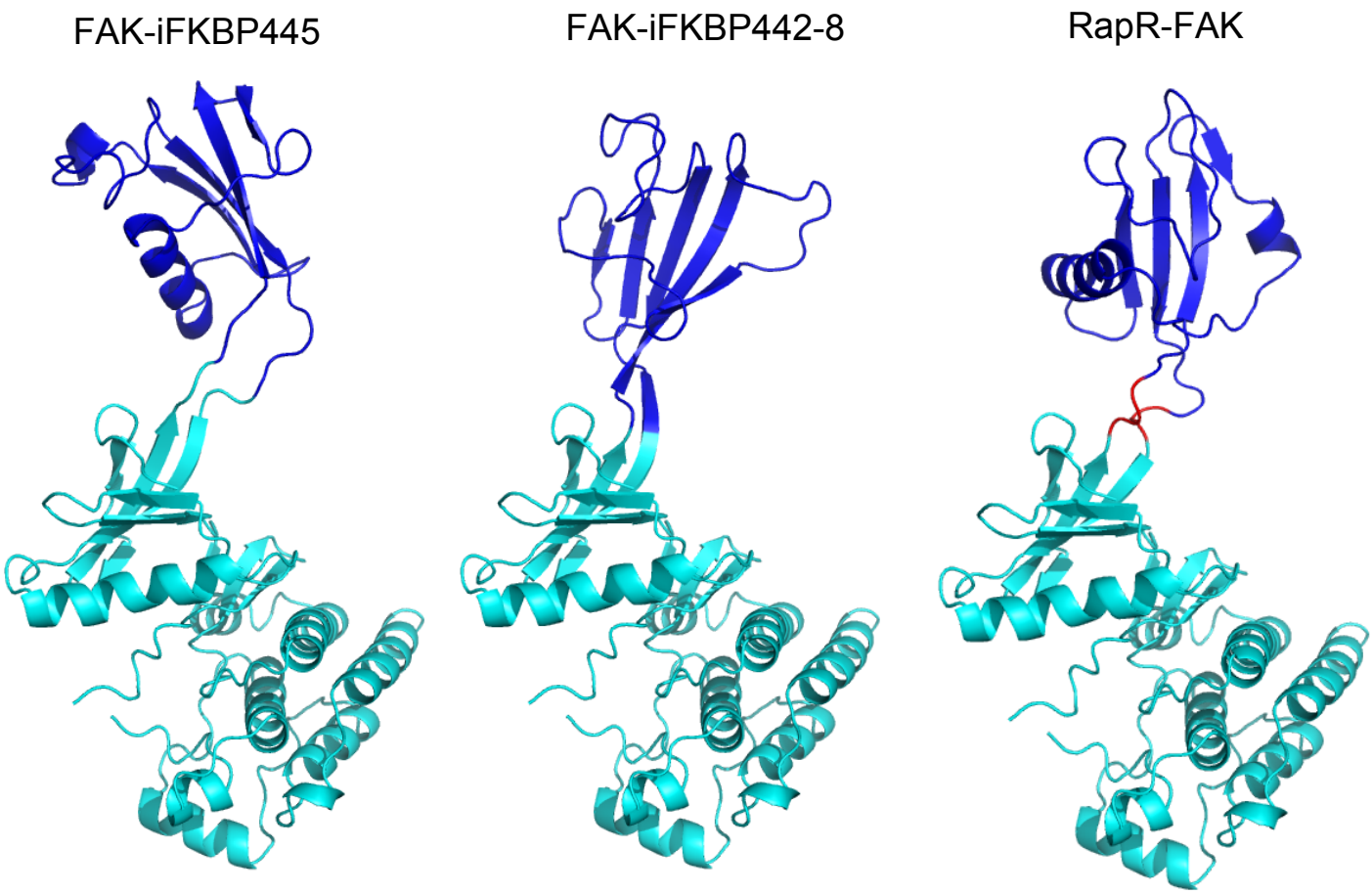
Supplementary Fig. S2



Supplementary Fig. S2. Root mean square fluctuation (RMSF) of each amino acid residue in iFKBP, with and without ligands.

Nature Biotechnology: doi:10.1038/nbt.1639

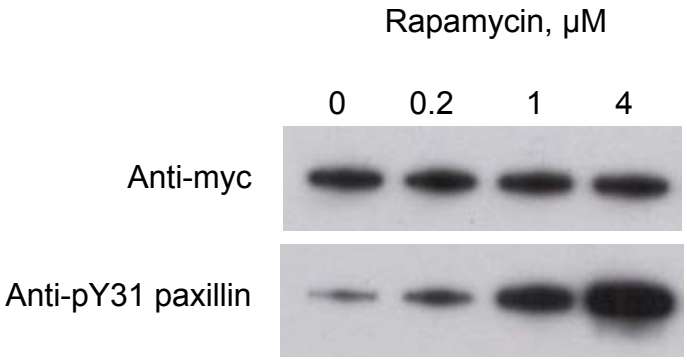
Supplementary Fig. S3



Supplementary Fig. S3. Predicted structures of FAK with different iFKBP insertions in the Ala442-Met448 loop of FAK (FAK = cyan, iFKBP insert = blue, linkers = red). In FAK-iFKBP445, the iFKBP insert replaces FAK amino acid Glu445. The FAK-iFKBP442-448 construct has an iFKBP insert replacing Ala442-Met448, without any linkers. In the RapR-FAK construct the iFKBP insert and Gly-Pro-Gly linkers replace Ala442-Met448.

Nature Biotechnology: doi:10.1038/nbt.1639

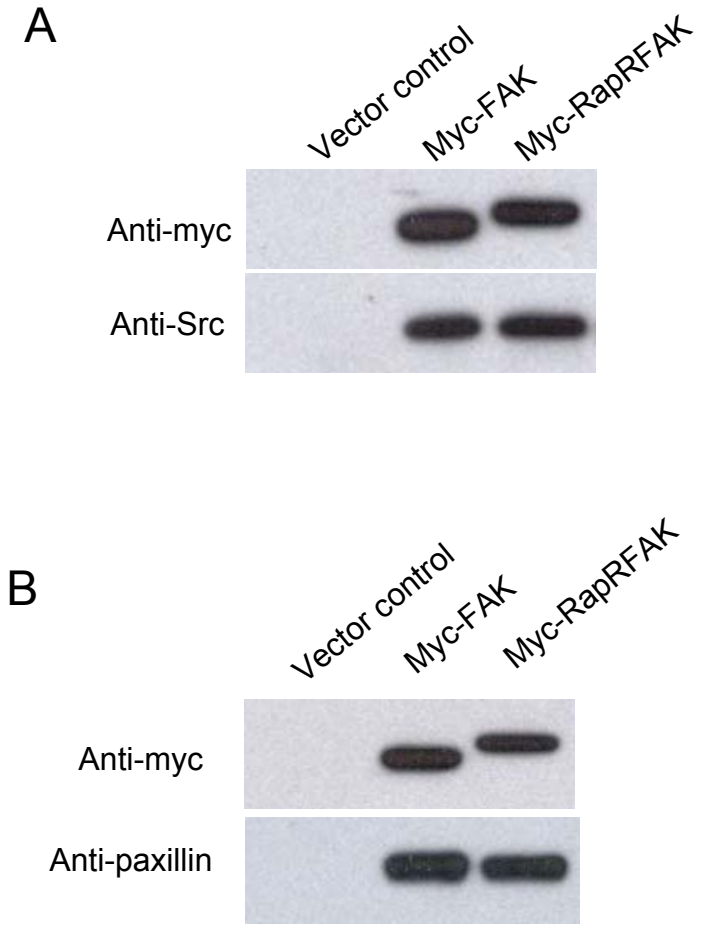
Supplementary Fig. S4



Supplementary Fig. S4. Regulation of RapR-FAK by rapamycin alone. 293T cells transfected with myc-RapR-FAK were treated with the indicated amount of rapamycin for 1 hour. Myc-RapR-FAK was immunoprecipitated and tested *in vitro* using N-terminal fragment of paxillin as a substrate.

Nature Biotechnology: doi:10.1038/nbt.1639

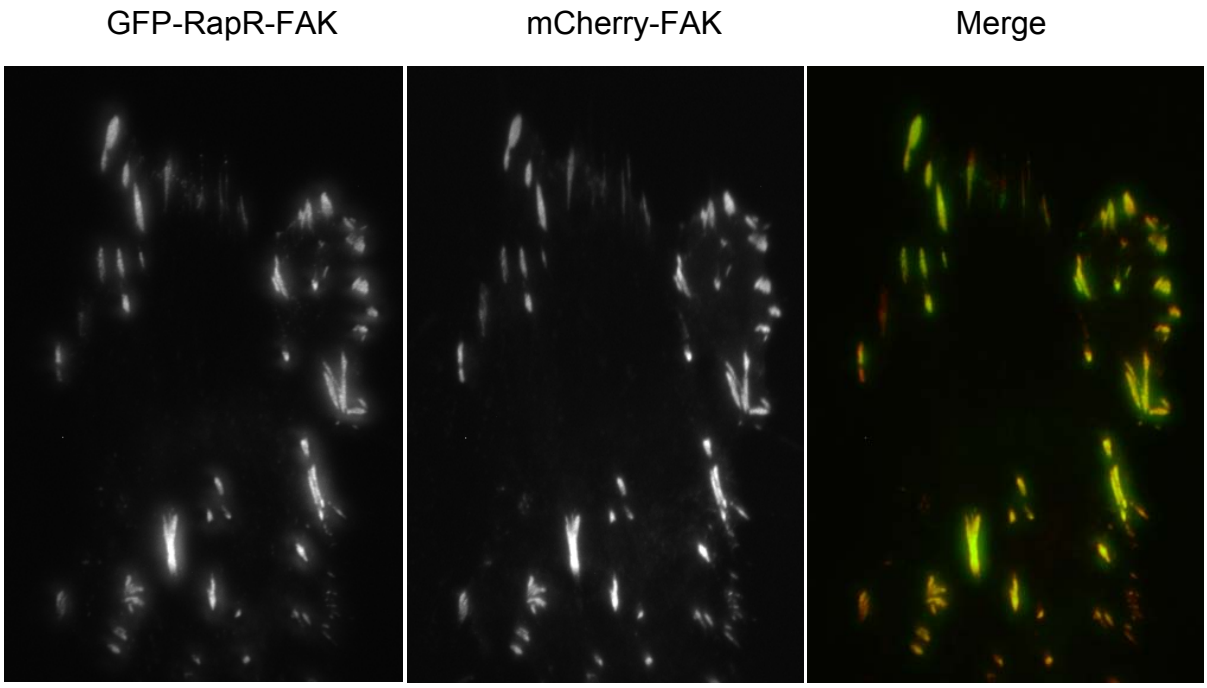
Supplementary Fig. S5



Supplementary Fig. S5. Interaction of RapR-FAK with binding partners. HEK293T cells were co-transfected with the indicated FAK construct and either Src **(A)** or paxillin **(B).** The FAK constructs were immunoprecipitated using anti-myc antibody. Co-immunoprecipitation of Src and paxillin was detected using anti-Src and anti-paxillin antibody.

Nature Biotechnology: doi:10.1038/nbt.1639

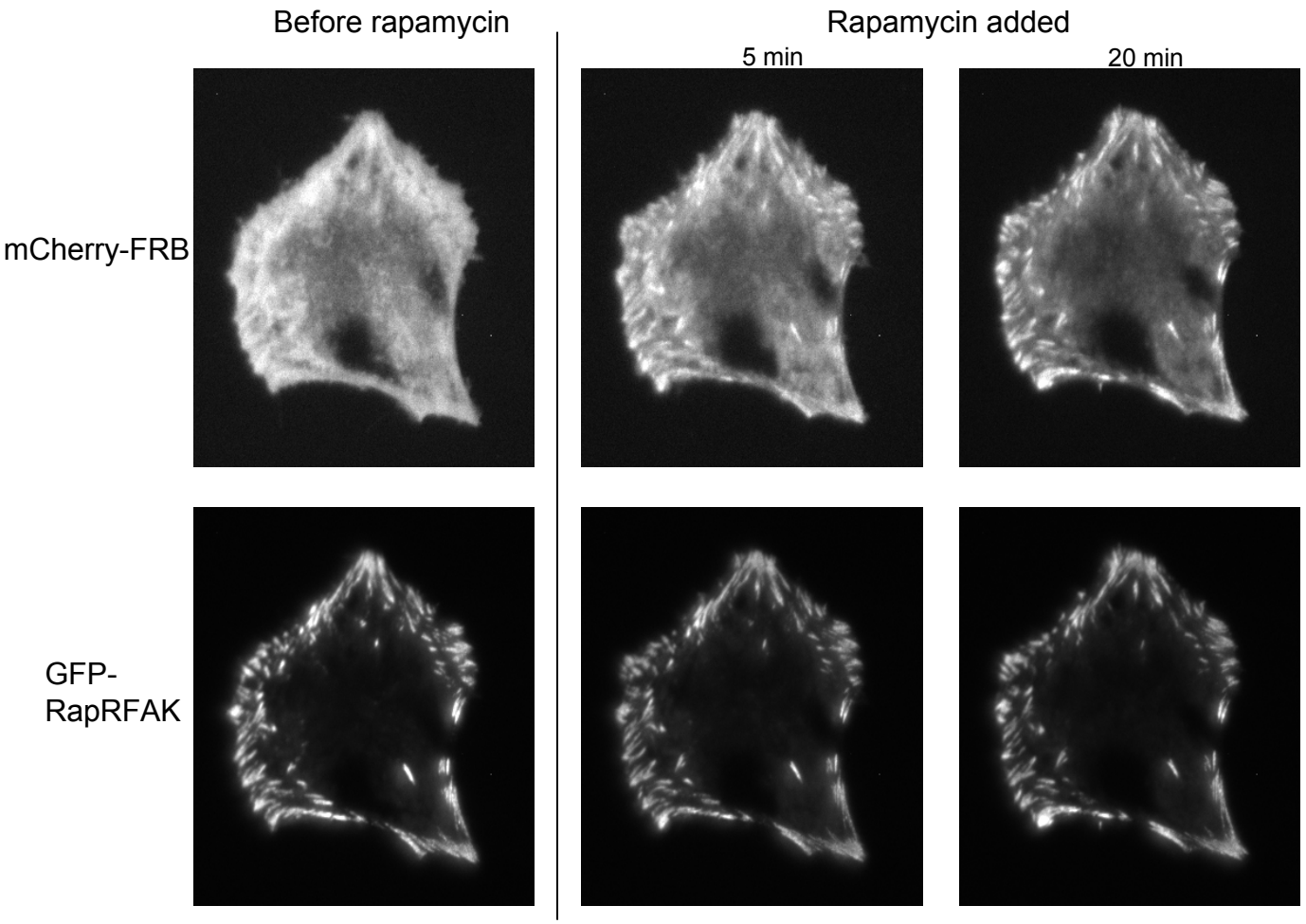
Supplementary Fig. S6



Supplementary Fig. S6. Co-localization of wild-type FAK and RapR-FAK in HeLa cells. GFP-RapR-FAK and mCherry-FAK were co-expressed in HeLa cells and imaged using total internal reflection fluorescence (TIRF) microscopy.

Nature Biotechnology: doi:10.1038/nbt.1639

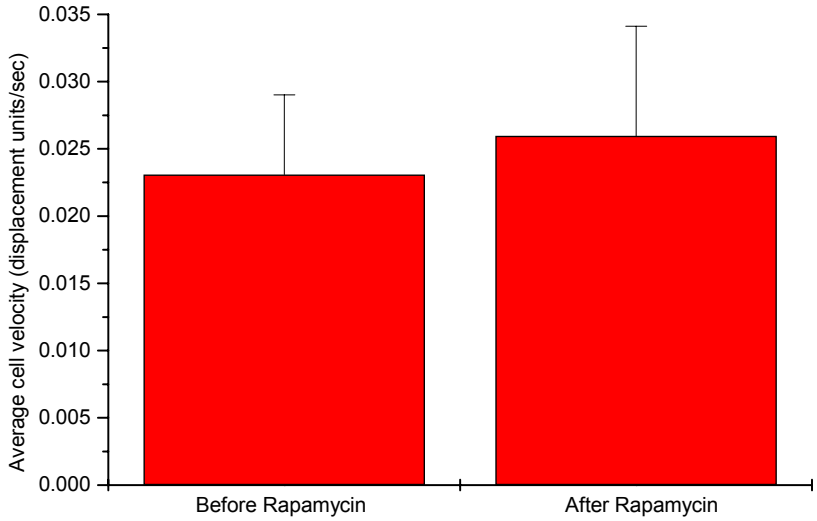
Supplementary Fig. S7



Supplementary Fig. S7. Colocalization of FRB with RapR-FAK upon treatment with rapamycin. HeLa cells co-expressing mCherry-FRB and GFP-RapR-FAK were imaged using TIRF microscopy before and after addition of rapamycin.

Nature Biotechnology: doi:10.1038/nbt.1639

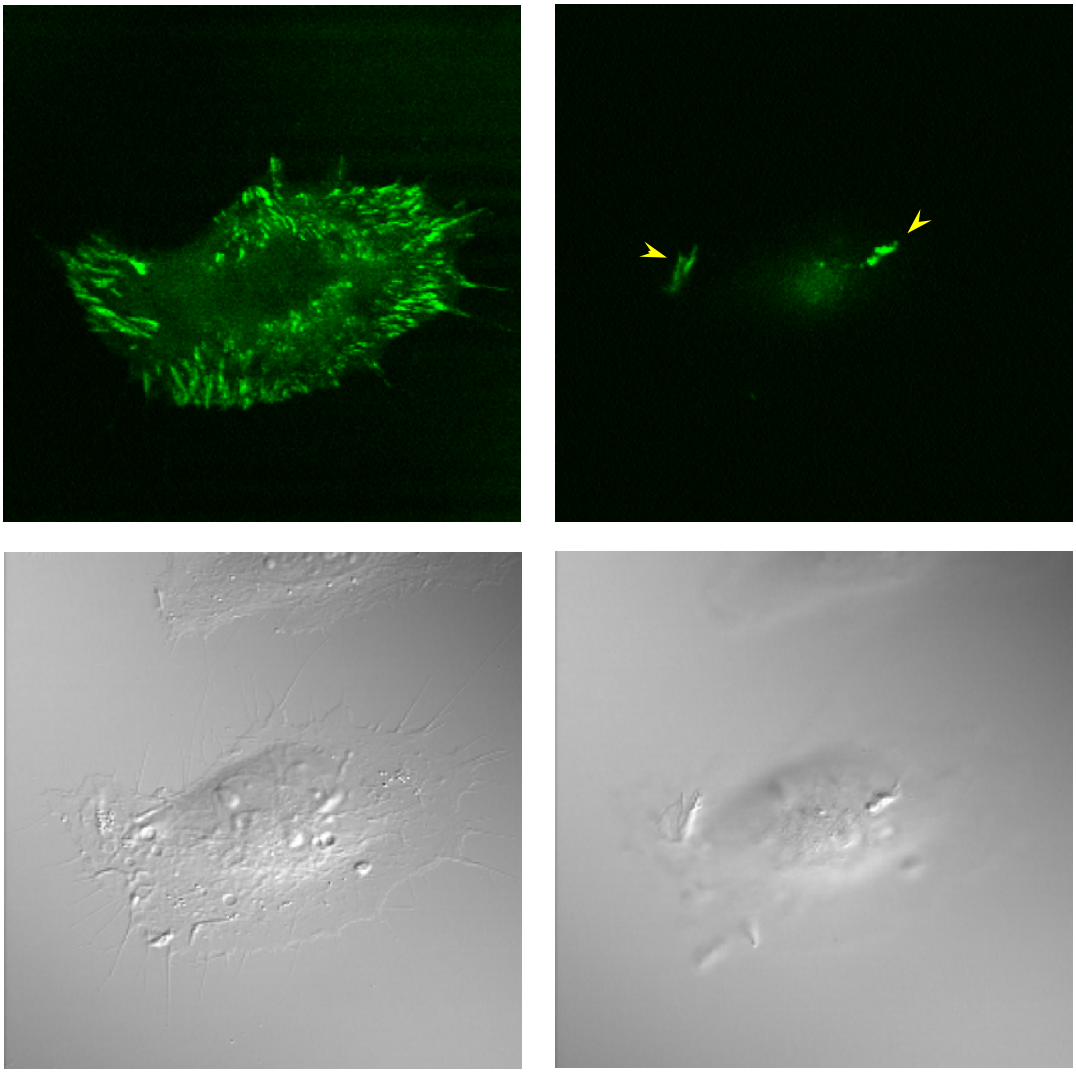
Supplementary Fig. S8



Supplementary Fig. S8. Effect of RapR-FAK activation on migration of HeLa cells. Cells expressing GFP-RapR-FAK and mCherry-FRB were imaged for 4 hours before and 10 hours after addition of rapamycin (18 cells total). Cells tracking and velocity calculation was done using Metamorph software (Molecular Devices).

Nature Biotechnology: doi:10.1038/nbt.1639

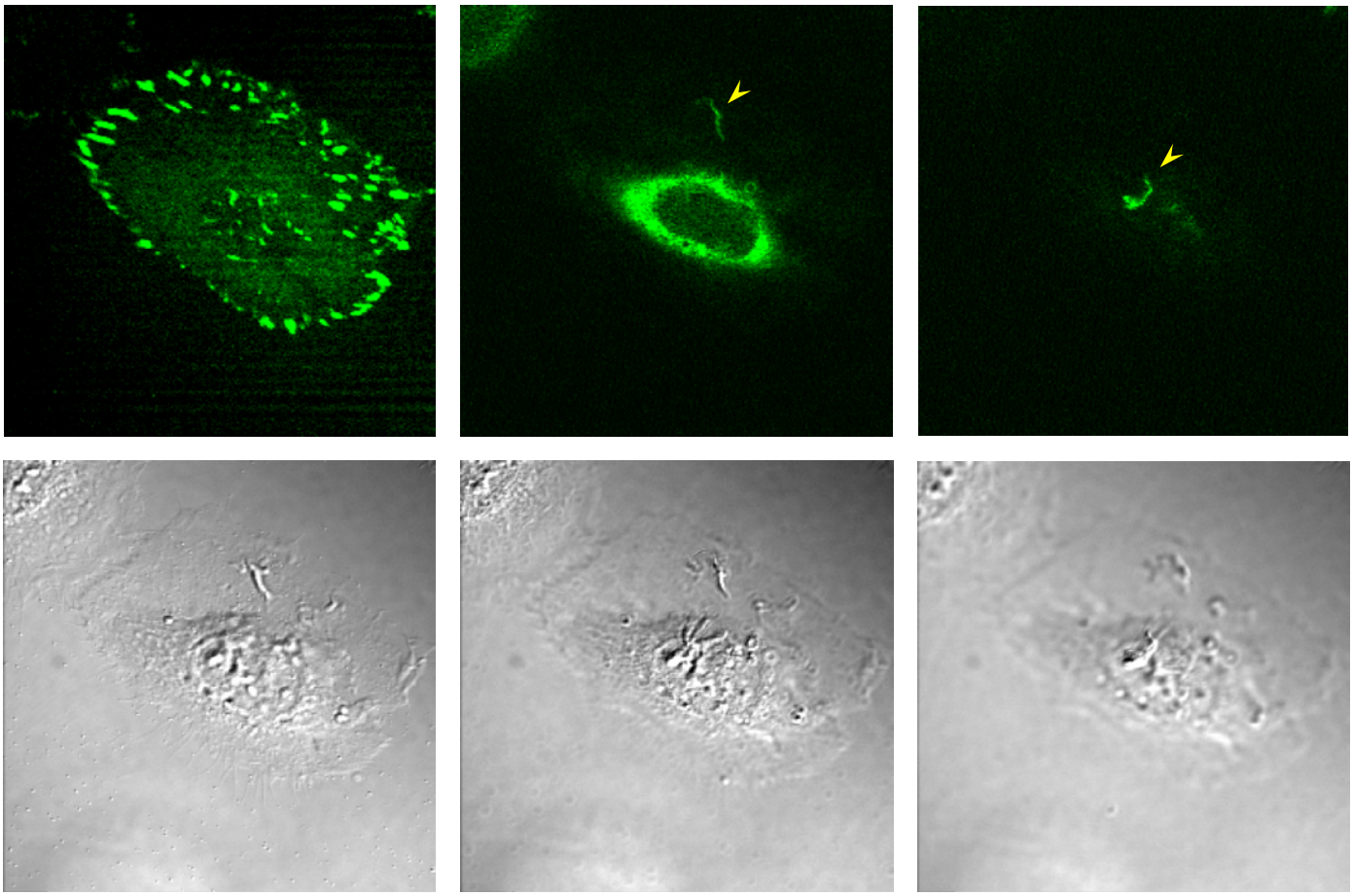
Supplementary Fig. S9



Supplementary Fig. S9. Localization of GFP-RapR-FAK-YM to dorsal ruffles. GFP-RapR-FAK-YM and mCherry-FRB were co-expressed in HeLa cells. Live cells were imaged on an Olympus Fluoview 1000 confocal microscope using a 60x objective 1 hour after addition of rapamycin. Fluorescence images (top row) show different confocal sections of the same cell. Corresponding DIC images are in the bottom row. Localization of GFP-RapR-FAK-YM to dorsal ruffles is indicated by arrowheads.

Nature Biotechnology: doi:10.1038/nbt.1639

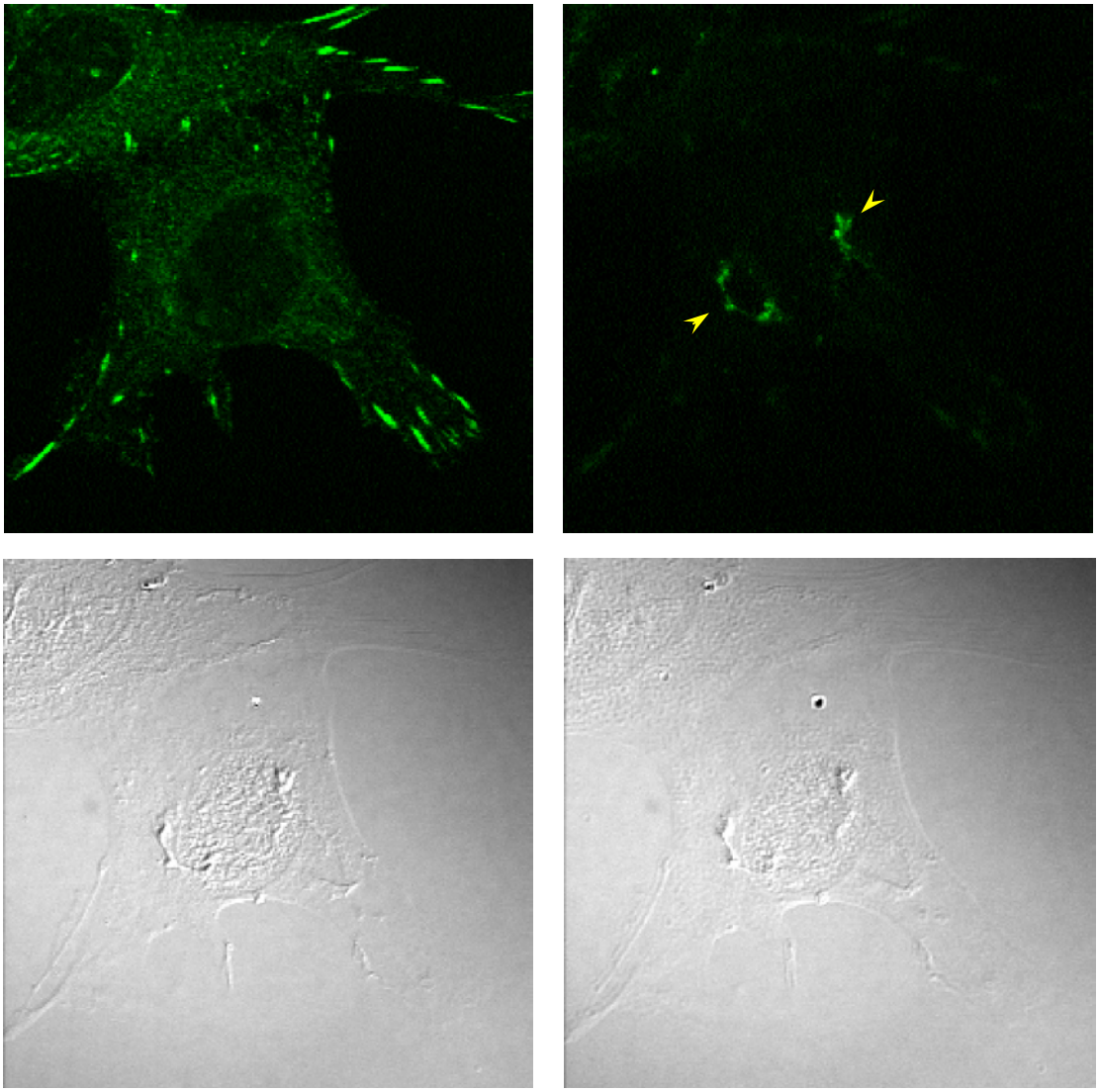
Supplementary Fig. S10



Supplementary Fig. S10. Localization of wild-type GFP-FAK to dorsal ruffles stimulated by myc-RapR-FAK-YM. GFP-FAK, myc-RapR-FAK-YM and mCherry-FRB were co-expressed in HeLa cells. Live cells were imaged on an Olympus Fluoview 1000 confocal microscope using a 60x objective 1 hour after addition of rapamycin. Fluorescence images (top row) show different confocal sections of the same cell. Corresponding DIC images are in the bottom row. Localization of GFP-FAK to dorsal ruffles is indicated by arrowheads.

Nature Biotechnology: doi:10.1038/nbt.1639

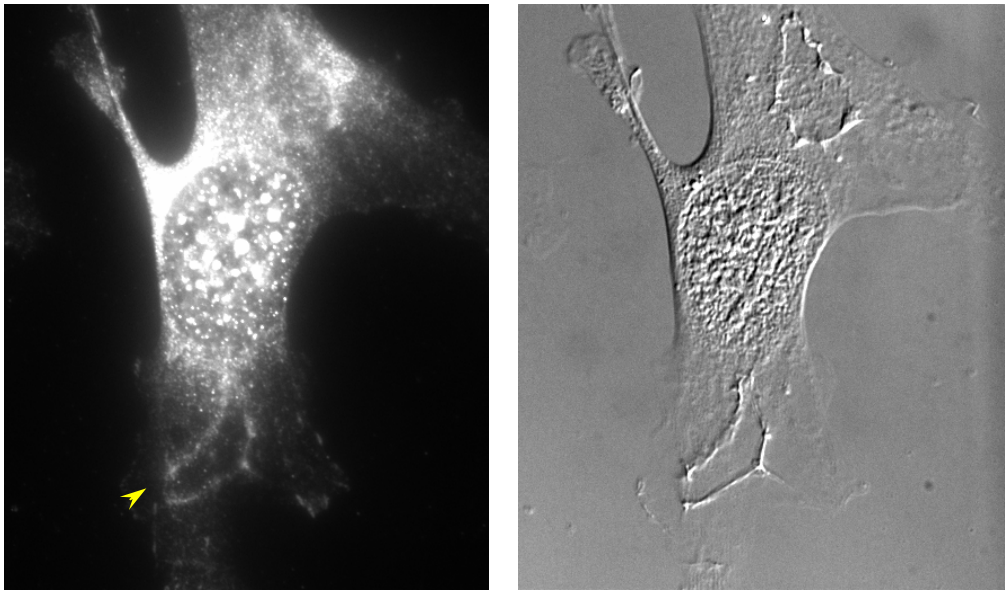
Supplementary Fig. S11



Supplementary Fig. S11. Localization of endogenous FAK to dorsal ruffles stimulated by PDGF. Mouse embryonic fibroblasts were treated with 30 ng/ml PDGF for 5 min. Cells were fixed and stained with anti-phospho-Tyr397-FAK antibody. Stained cells were imaged using an Olympus Fluoview 1000 confocal microscope with a 60x objective. Fluorescence images (top row) show different confocal sections of the same cell. Corresponding DIC images are in the bottom row. Localization of FAK to dorsal ruffles is indicated by arrowheads.

Nature Biotechnology: doi:10.1038/nbt.1639

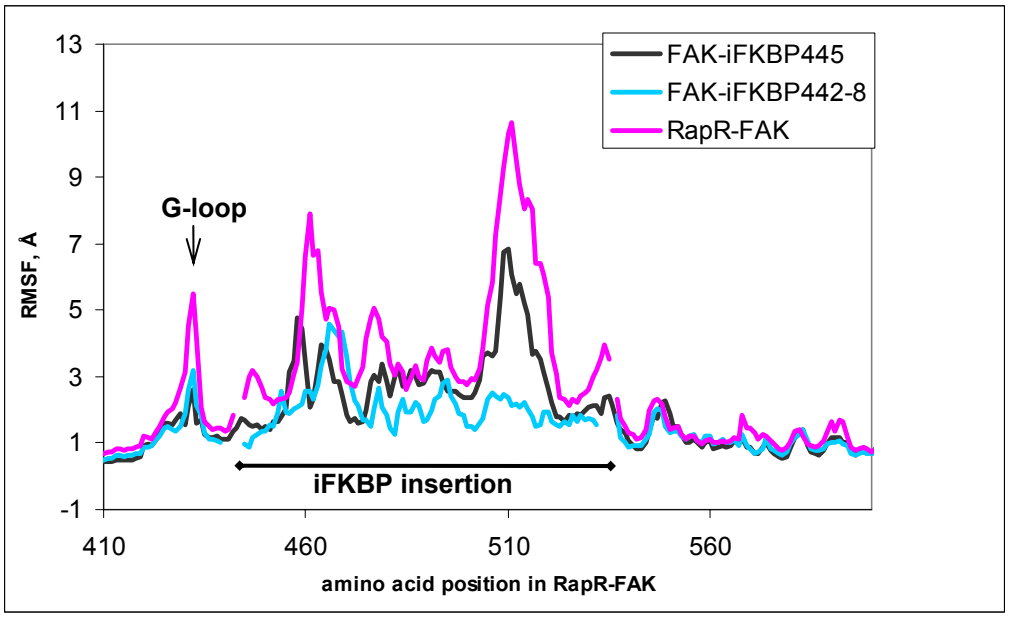
Supplementary Fig. S12



Supplementary Fig. S12. Localization of endogenous FAK to dorsal ruffles stimulated by PDGF. Mouse embryonic fibroblasts we treated with 30 ng/ml PDGF for 5 min. Cells were fixed and stained with anti-phospho-Tyr397-FAK antibody. Localization of FAK to dorsal ruffles is indicated by the arrowhead.

Nature Biotechnology: doi:10.1038/nbt.1639

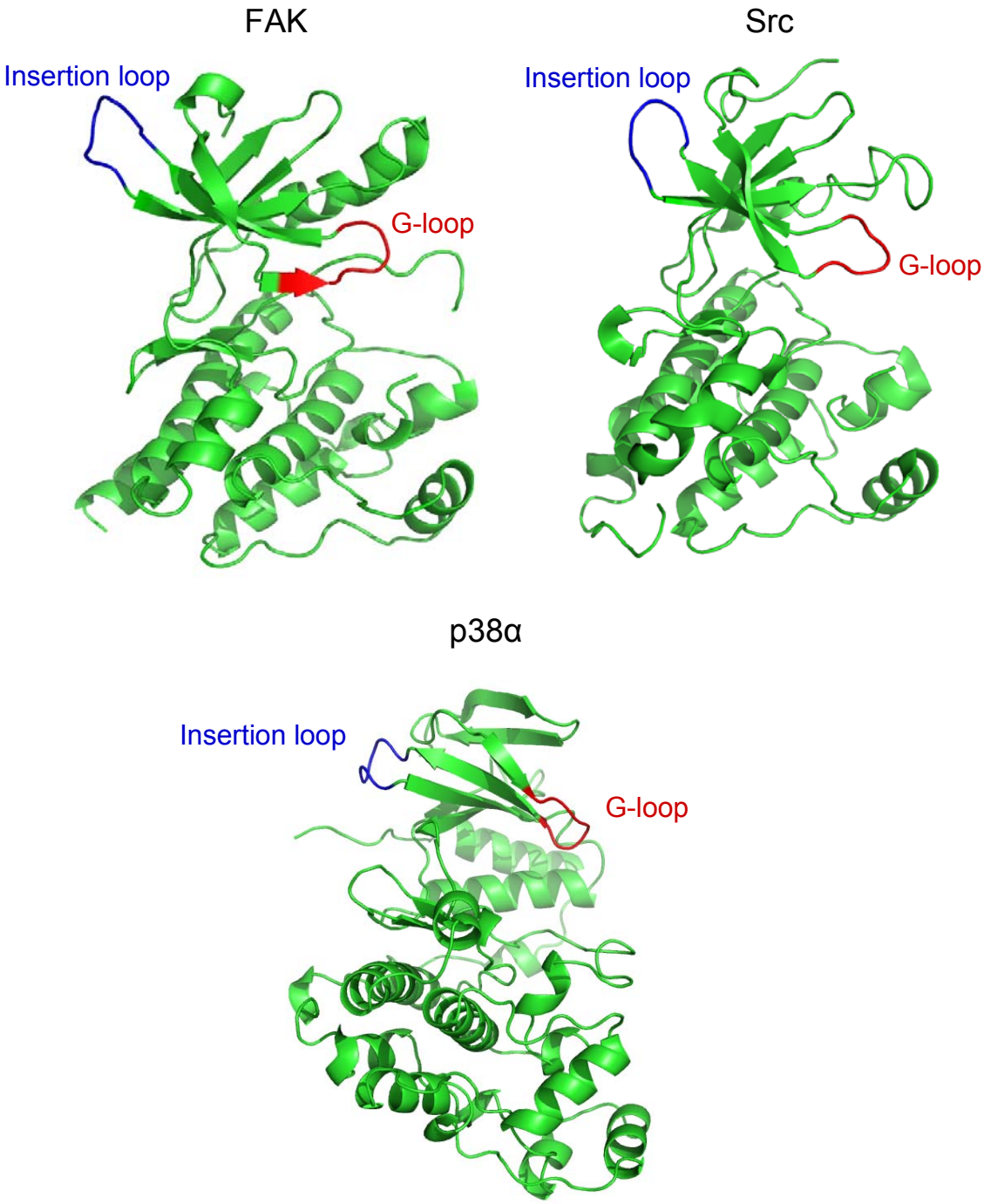
Supplementary Fig. S13



Supplementary Fig. S13. Root mean square fluctuation (RMSF) of each amino acid residue in the catalytic domain of FAK with different iFKBP insertions. Breaks in the graphs for RapR-FAK and FAK-iFKBP442-8 are created due to the lack of amino acids present in FAK-iFKBP445. All three constructs are depicted in Fig. 2B.

Nature Biotechnology: doi:10.1038/nbt.1639

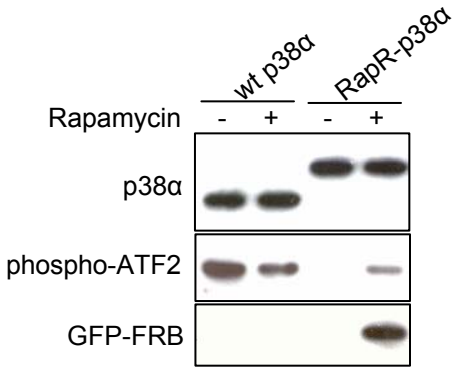
Supplementary Fig. S14



Supplementary Fig. S14. Comparison of catalytic domains structure for FAK (PDB ID: 2J0M), Src (PDB ID: 1YOJ) and p38 (PDB ID: 1p38). The loop where iFKBP is inserted (insertion loop) is depicted in blue. The G-loop is depicted in red.

Nature Biotechnology: doi:10.1038/nbt.1639

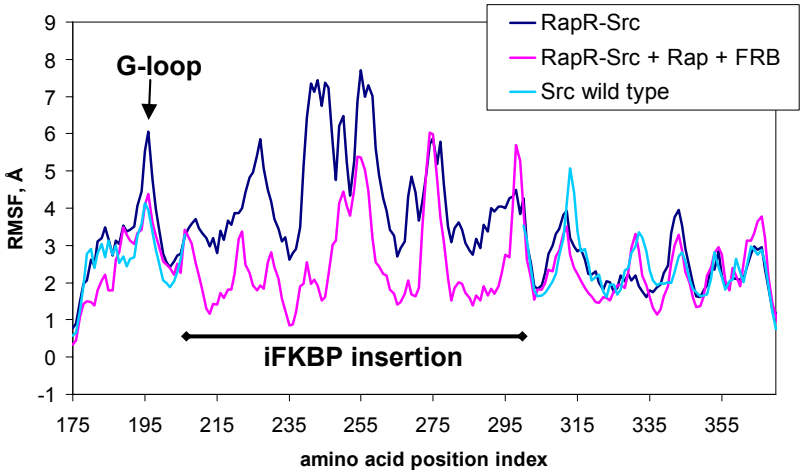
Supplementary Fig. S15



Supplementary Fig. S15. Regulation of p38α kinase by insertion of iFKBP. HEK293T cells co-expressing the indicated flag-tagged p38α construct and GFP-FRB were treated with either 200 nM rapamycin or ethanol solvent control. The kinase activity of p38α was tested *in vitro* using ATF2 as a substrate.

Nature Biotechnology: doi:10.1038/nbt.1639

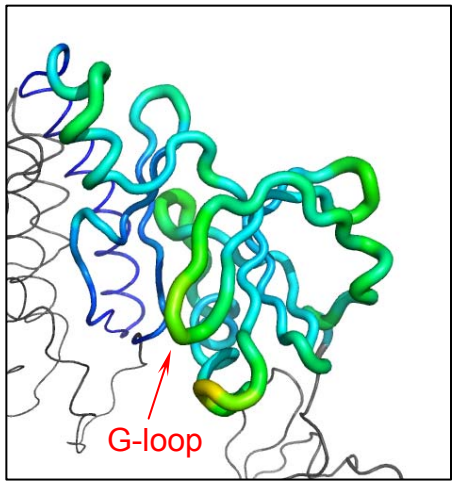
Supplementary Fig. S16



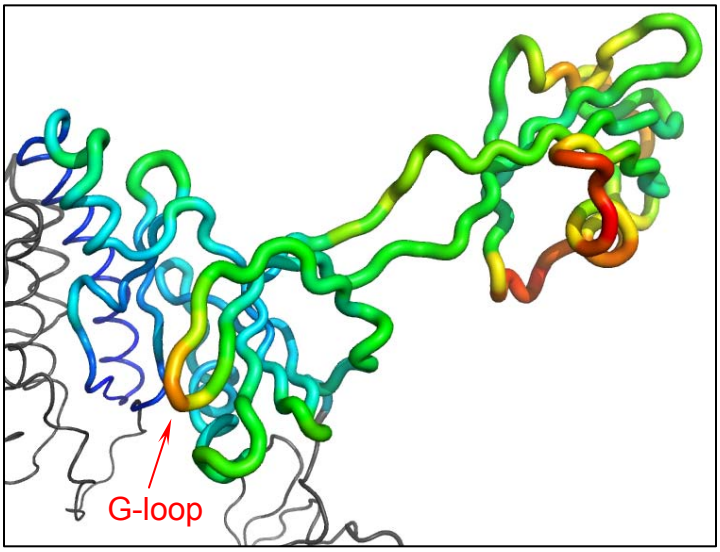
Supplementary Fig. S16. Root mean square fluctuation (RMSF) for each amino acid residue of the catalytic domains of wild-type Src and Src with iFKBP insertion (RapR-Src). The break in the graph for wild-type Src corresponds to the iFKBP insert. The arrow indicates the G-loop.

Nature Biotechnology: doi:10.1038/nbt.1639

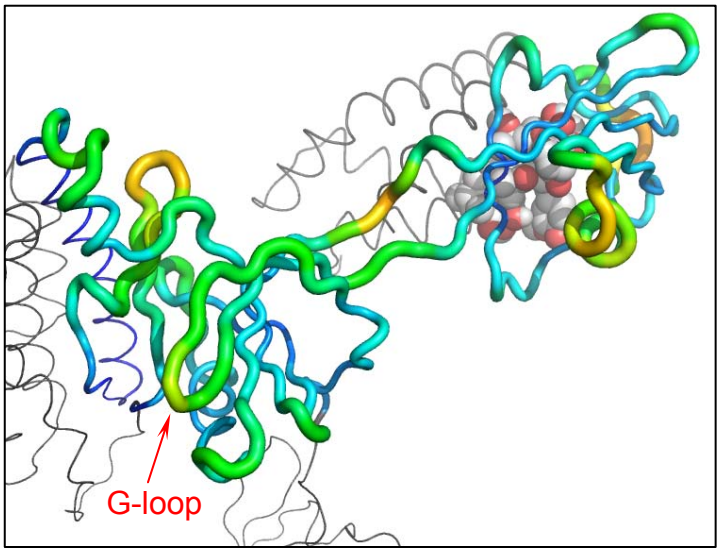
Supplementary Fig. S17



Wild-type Src



RapR-Src

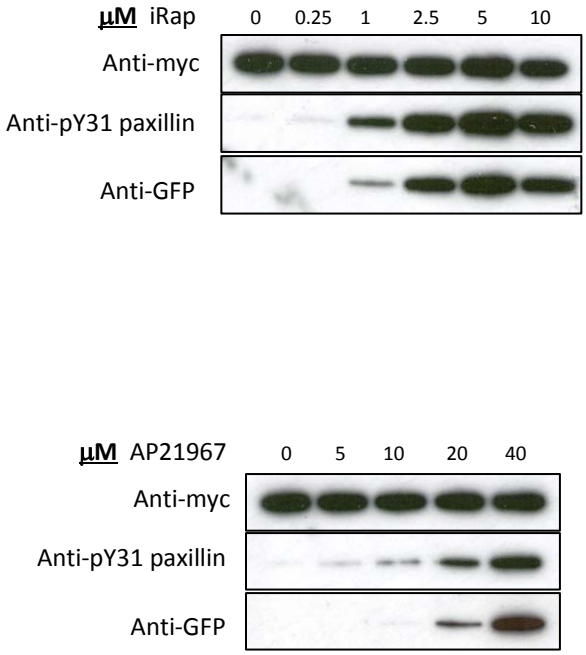


RapR-Src + Rap + FRB

Supplementary Fig. S17. Tube representations of the catalytic domain of wild–type Src and RapR-Src. Warmer colors and thicker backbone correspond to higher RMSF values, reflecting the degree of free movement within the structure. The red arrows point to the G-loop within the catalytic domain.

Nature Biotechnology: doi:10.1038/nbt.1639

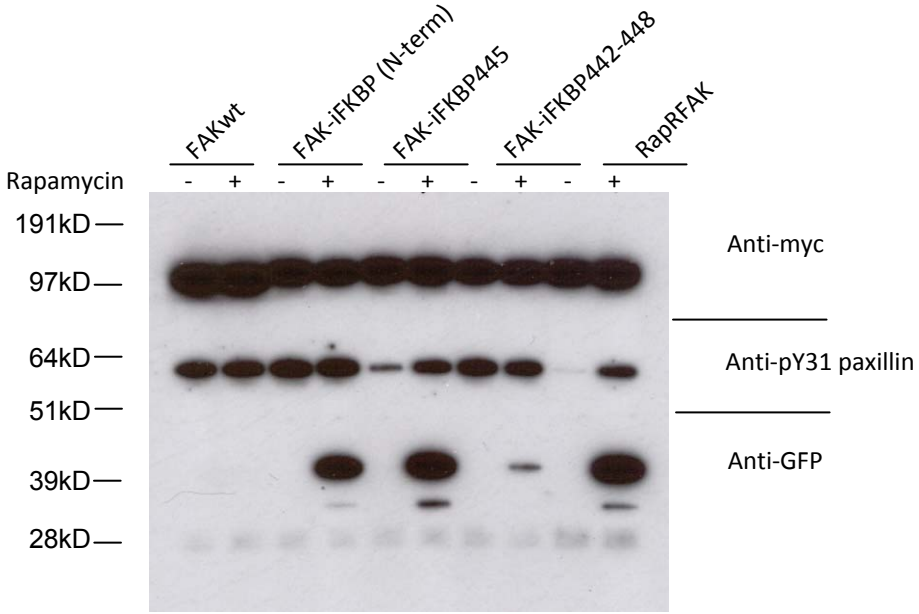
Supplementary Fig. S18



Supplementary Fig. S18. Regulation of RapR-FAK with non-immunosuppressive analogs of rapamycin. HEK293 cells co-transfected with myc-RapR-FAK-YM and GFP-FRB constructs were treated with indicated amount of iRap or AP21967 for 1 hour. RapR-FAK-YM kinase was immunoprecipitated and tested in an *in vitro* kinase assay using N-terminus of paxillin as a substrate.

Nature Biotechnology: doi:10.1038/nbt.1639

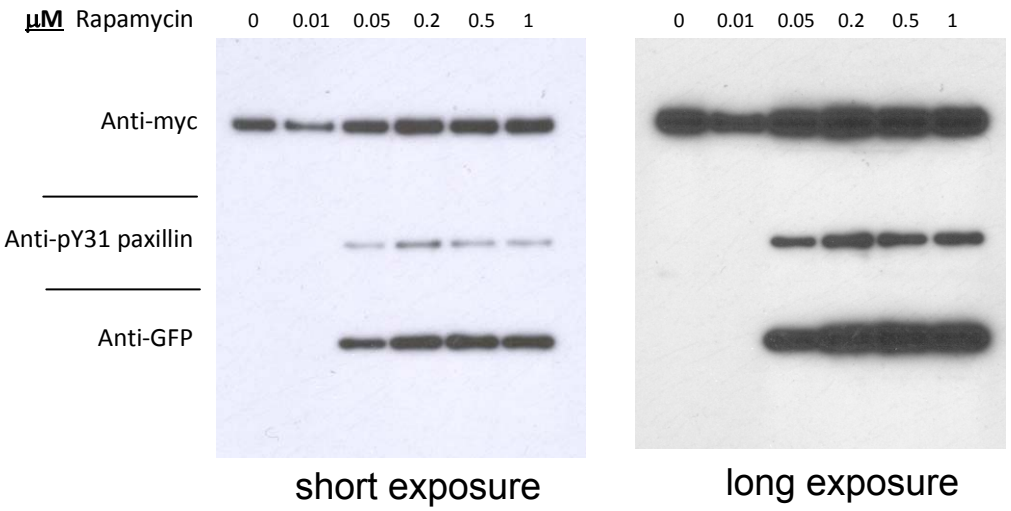
Supplementary Figure S19a



Supplementary Figure S19a. Supplementary figure for Figure 2a.

Nature Biotechnology: doi:10.1038/nbt.1639

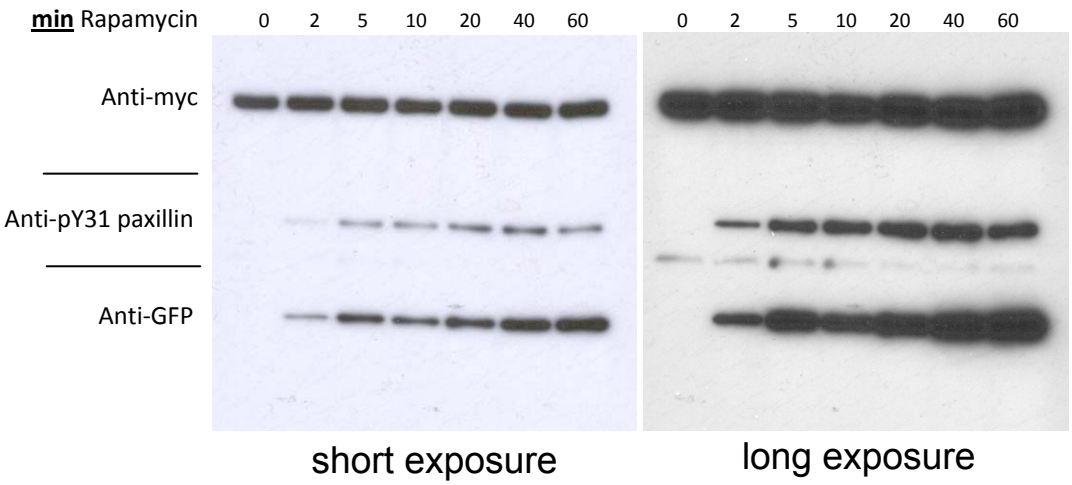
Supplementary Figure S19b



Supplementary Figure S19b. Supplementary figure for Figure 2c.

Nature Biotechnology: doi:10.1038/nbt.1639

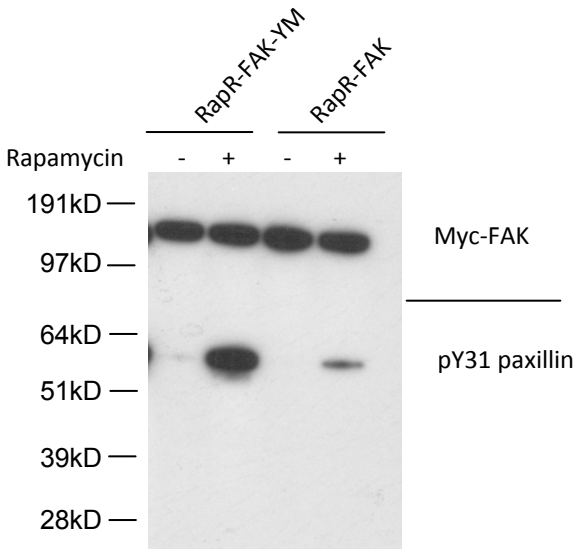
Supplementary Figure S19c



Supplementary Figure S19c. Supplementary figure for Figure 2d.

Nature Biotechnology: doi:10.1038/nbt.1639

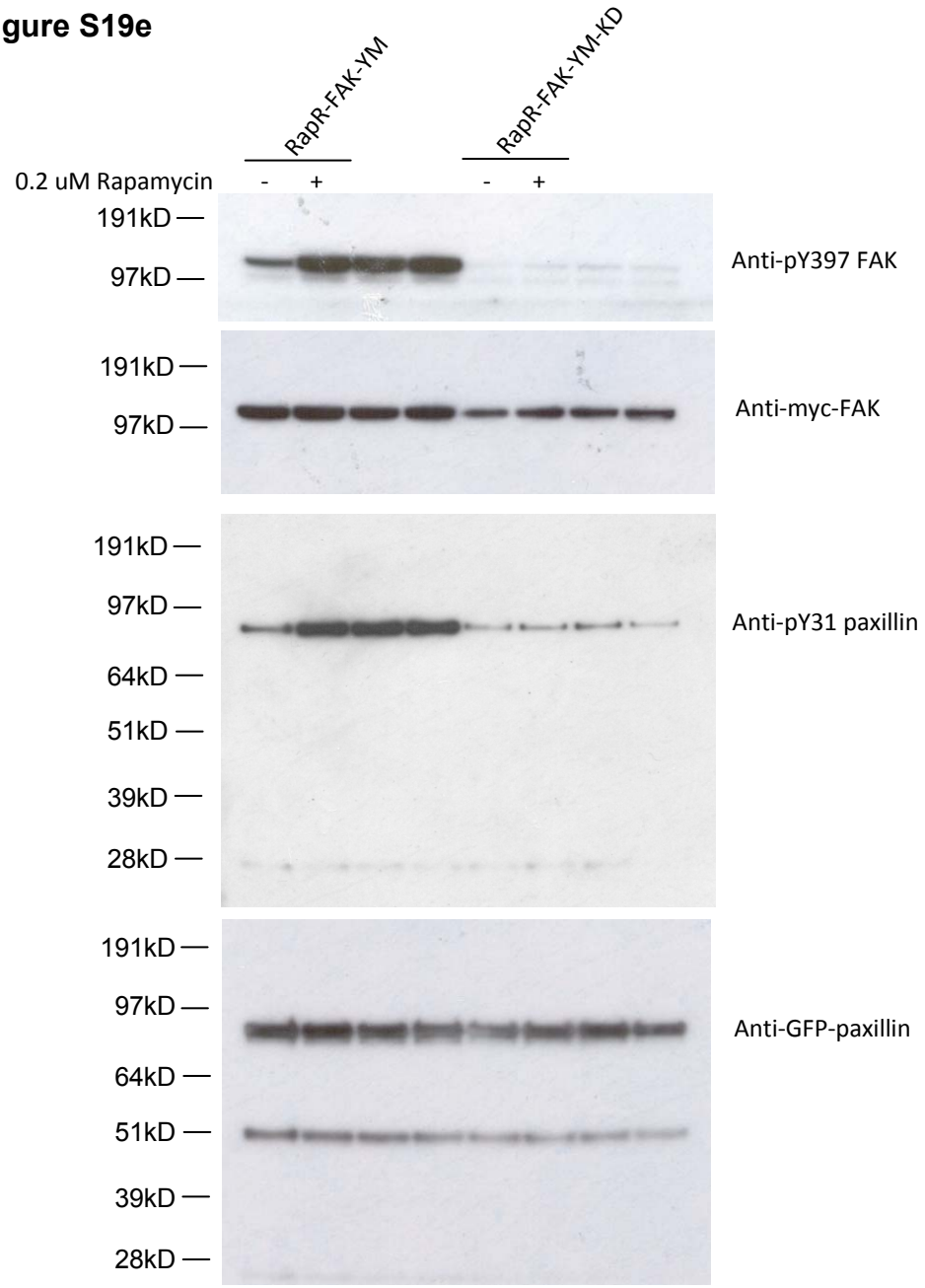
Supplementary Figure S19d



Supplementary Figure S19d. Supplementary figure for Figure 2e.

Nature Biotechnology: doi:10.1038/nbt.1639

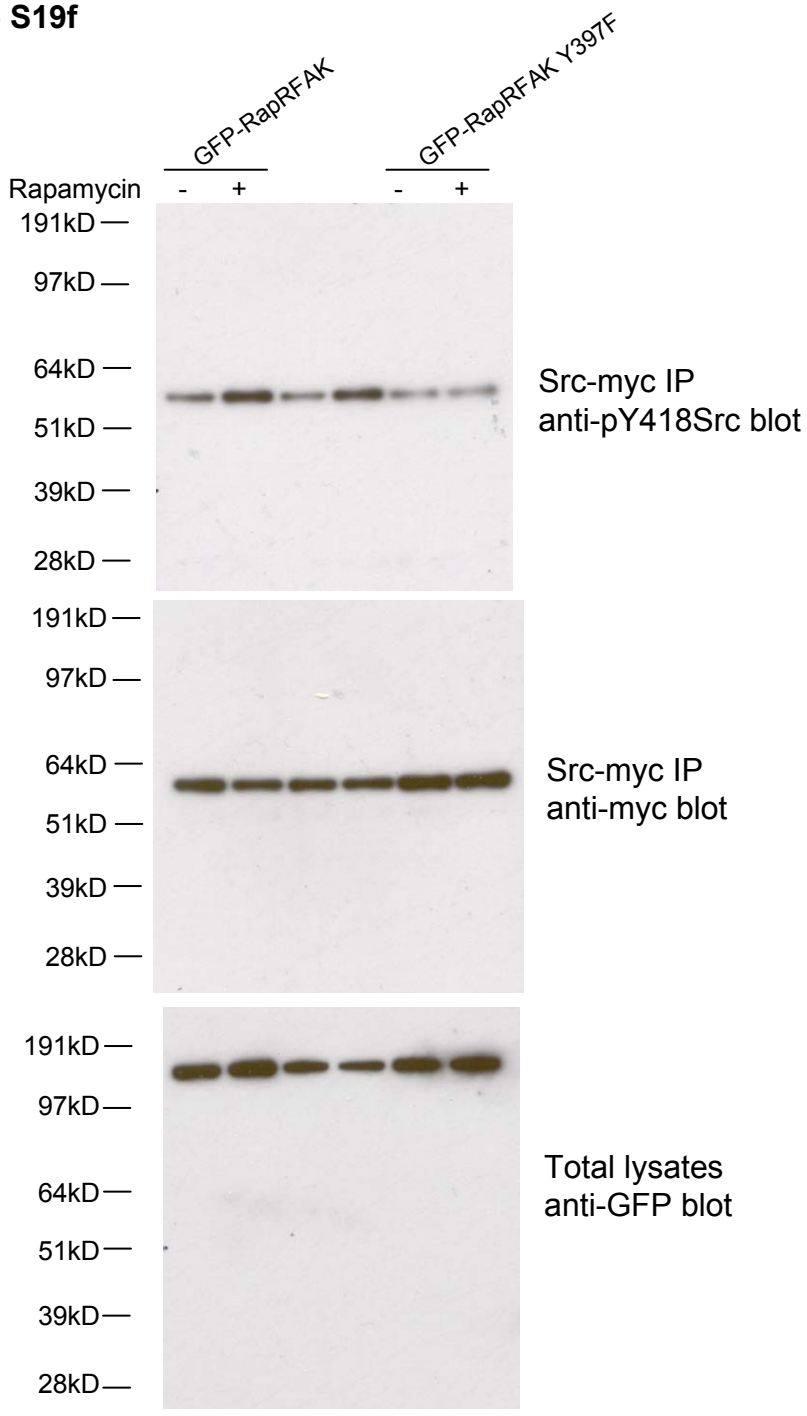
Supplementary Figure S19e



Supplementary Figure S19e. Supplementary figure for Figure 2f.

Nature Biotechnology: doi:10.1038/nbt.1639

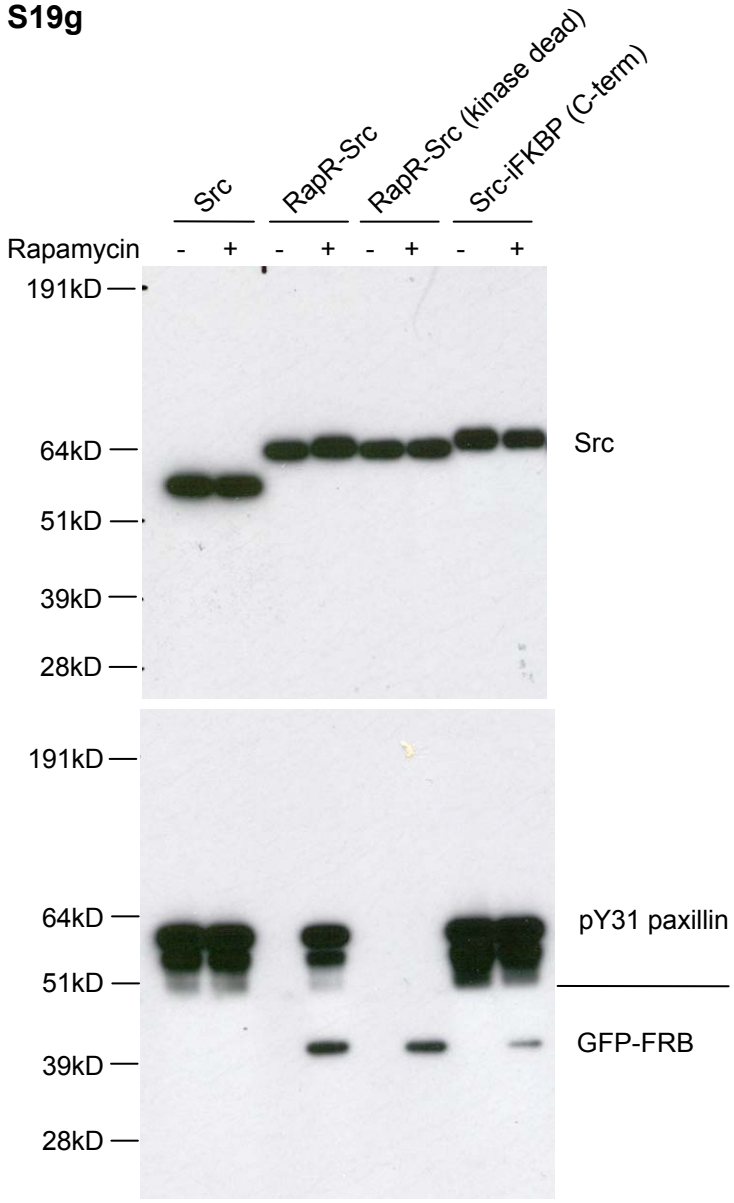
Supplementary Figure S19f



Supplementary Figure S19e. Supplementary figure for Figure 3d.

Nature Biotechnology: doi:10.1038/nbt.1639

Supplementary Figure S19g



Supplementary Figure S19e. Supplementary figure for Figure 4e.

Nature Biotechnology: doi:10.1038/nbt.1639

Supplementary Movie S1. HeLa cells co-transfected with GFP-RapR-FAK-YM and mCherry-FRB were filmed for 30 min before and 60 min after addition of rapamycin. Images were taken at one minute intervals.

Supplementary Movie S2. HeLa cells co-transfected with GFP-RapR-FAK-YM and mCherry-FRB were pretreated with rapamycin for 1 hour. Cells generating large dorsal protrusions were filmed for 30 min before and 30 min after addition of PP2 compound (5 μM final concentration). Images were taken at one minute intervals.

Supplementary Movie S3. Molecular dynamics simulations demonstrating free movement of the G-loop (red) and iFKBP insertion (magenta) within RapR-FAK catalytic domain without the binding ligands (top) and in complex with rapamycin and FRB (bottom). The simulations were performed as described in the Materials and Methods section.

Nature Biotechnology: doi:10.1038/nbt.1639