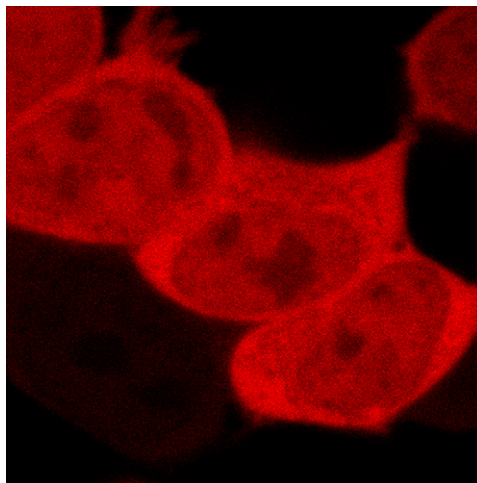


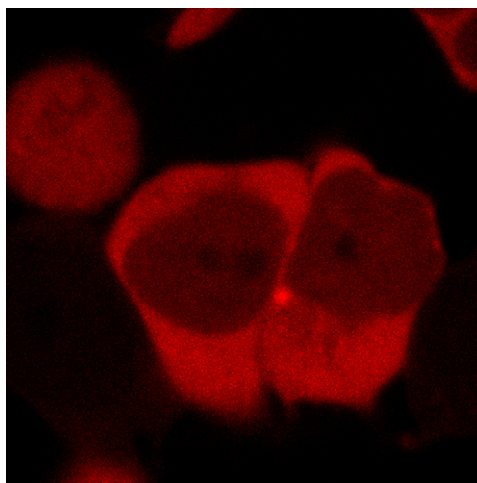
# Supporting Information

Skwarczynska et al. 10.1073/pnas.1212990110



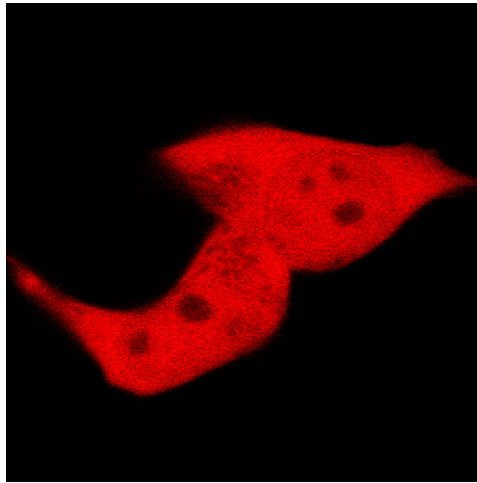
**Movie S1.** (nuclear exclusion\_5 $\mu$ M FC) mCherry-CT52M1 is cotransfected with T14-3-3 $\Delta$ C-M1-GFP. T14-3-3 $\Delta$ C-M1-GFP is localized in the cytoplasm, and mCherry-CT52M1 shuttles between the cytoplasm and nucleus. After the addition of 5  $\mu$ M fusicoccin (FC) to the HEK293T cells, mCherry-CT52M1 forms a complex with T14-3-3 $\Delta$ C-M1-GFP, thereby trafficking into the cytoplasm. Time frame: 60 min.

[Movie S1](#)



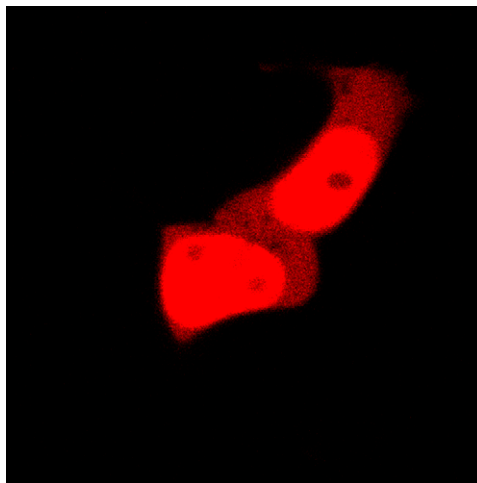
**Movie S2.** (nuclear exclusion\_revers) The reversibility of the nuclear-excluded mCherry-CT52M1 is demonstrated by rinsing the cells with FC-free medium. The ternary complex of mCherry-CT52M1, T14-3-3 $\Delta$ C-M1-GFP and FC dissociates, so that part of the cytoplasmic population of mCherry-CT52M1 trafficks back into the nucleus. Time frame: 60 min.

[Movie S2](#)



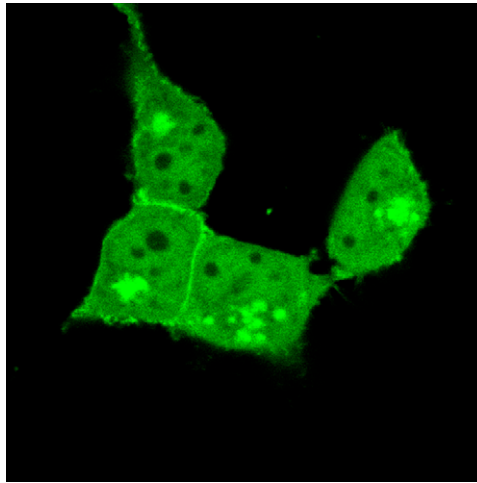
**Movie S3.** (nuclear accumulation\_5μM FC) mCherry-CT52M1 is cotransfected with T14-3-3cΔC-M2-NLS-GFP. T14-3-3cΔC-M2-NLS-GFP is localized in the nucleus, and mCherry-CT52M1 shuttles between the cytoplasm and nucleus. After the addition of 5 μM FC to the HEK293T cells, mCherry-CT52M1 forms a complex with T14-3-3cΔC-M2-NLS-GFP and thereby is translocated into the nucleus. Time frame: 60 min.

[Movie S3](#)



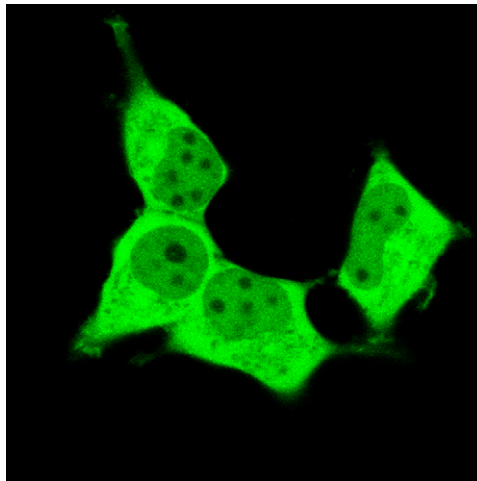
**Movie S4.** (nuclear accumulation\_revers) The reversibility of the nuclear-accumulated mCherry-CT52M1 is demonstrated by rinsing the cells with FC-free medium. The ternary complex of mCherry-CT52M1, T14-3-3cΔC-M2-NLS-GFP, and FC dissociates, so that part of the nuclear population of mCherry-CT52M1 trafficks back into the cytoplasm. Time frame: 60 min.

[Movie S4](#)



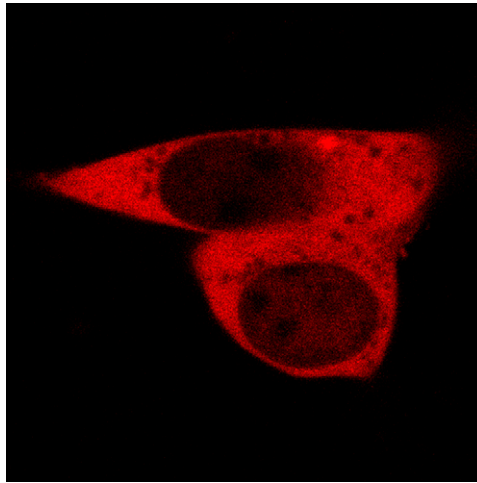
**Movie S5.** (pm recruitment\_5 $\mu$ M FC) *N*-Myr-mCherry-CT52M1 is cotransfected with T14-3-3c $\Delta$ C-M2-GFP. T14-3-3c $\Delta$ C-M2-GFP shuttles between nucleus and cytoplasm, and *N*-Myr-mCherry-CT52M1 is recruited to the plasma membrane. After the addition of 5  $\mu$ M FC to the HEK293T cells, T14-3-3c $\Delta$ C-M2-GFP forms a complex with *N*-Myr-mCherry-CT52M1 and thereby is recruited within few seconds to the plasma membrane. Time frame: 30 min.

[Movie S5](#)



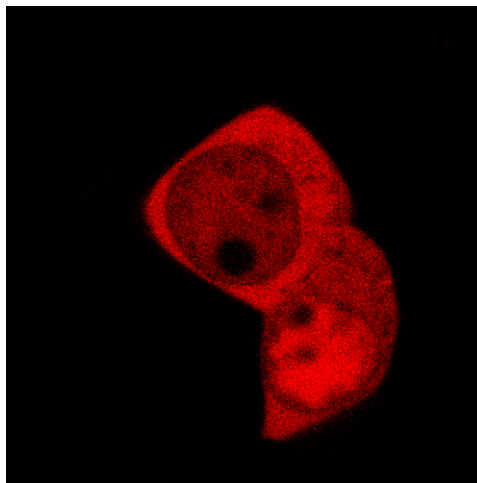
**Movie S6.** (pm recruitment\_revers) The reversibility of the plasma membrane-recruited T14-3-3c $\Delta$ C-M2-GFP is demonstrated by rinsing the cells with FC-free medium. The ternary complex of *N*-Myr-mCherry-CT52M1, T14-3-3c $\Delta$ C-M2-GFP, and FC dissociates, allowing translocation of T14-3-3c $\Delta$ C-M2-GFP back into the cytoplasm and nucleus. Time frame: 30 min.

[Movie S6](#)



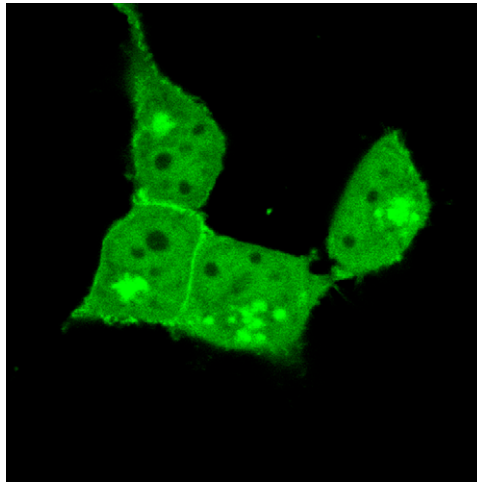
**Movie S7.** (p65 nuclear import\_5 $\mu$ M FC) mCherry-p65-CT52M1 is cotransfected with T14-3-3 $\Delta$ C-M2-NLS-GFP. T14-3-3 $\Delta$ C-M2-NLS-GFP is localized in the nucleus, and mCherry-p65-CT52M1 is localized in the cytoplasm. After the addition of 5  $\mu$ M FC to the HEK293T cells, mCherry-p65-CT52M1 forms a complex with T14-3-3 $\Delta$ C-M2-NLS-GFP and thereby is translocated into the nucleus. Time frame: 90 min.

[Movie S7](#)



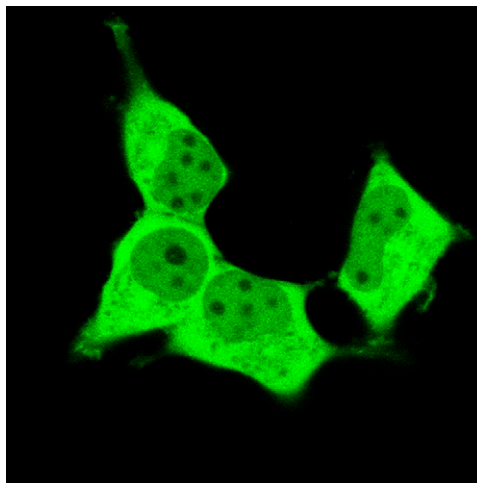
**Movie S8.** (p65 nuclear import\_revers FC.avi) The reversibility of the nuclear-accumulated mCherry-p65-CT52M1 is demonstrated by rinsing the cells with FC-free medium. The ternary complex of mCherry-p65-CT52M1, T14-3-3 $\Delta$ C-M2-NLS-GFP, and FC dissociates, so that part of the nuclear population of mCherry-p65-CT52M1 trafficks back into the cytoplasm. Time frame: 80 min.

[Movie S8](#)



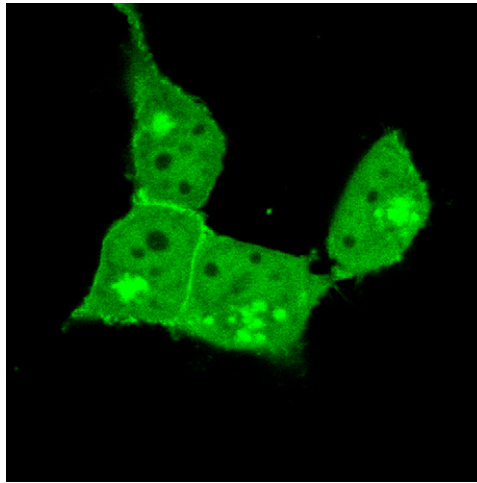
**Movie S9.** PM\_1 (pm recruitment\_5 $\mu$ M FC) *N*-Myr-mCherry-CT52M1 is cotransfected with T14-3-3c $\Delta$ C-M2-GFP. T14-3-3c $\Delta$ C-M2-GFP shuttles between nucleus and cytoplasm, and *N*-Myr-mCherry-CT52M1 is recruited to the plasma membrane. After the addition of 5  $\mu$ M FC to the HEK293T cells, T14-3-3c $\Delta$ C-M2-GFP forms a complex with *N*-Myr-mCherry-CT52M1 and thereby is recruited within few seconds to the plasma membrane. Time frame: 30 min.

[Movie S9](#)



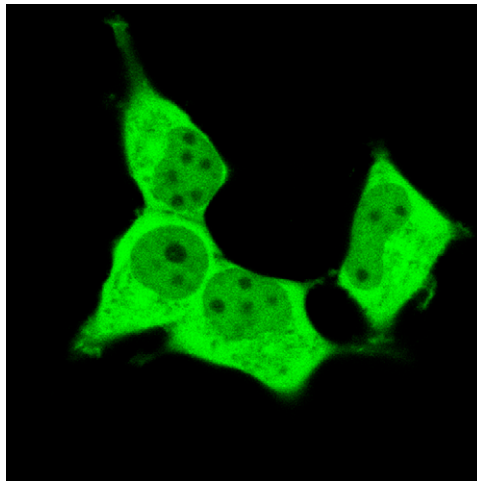
**Movie S10.** PM\_2 (pm recruitment\_revers) The reversibility of the plasma membrane-recruited T14-3-3c $\Delta$ C-M2-GFP is demonstrated by rinsing the cells with FC-free medium. The ternary complex of *N*-Myr-mCherry-CT52M1, T14-3-3c $\Delta$ C-M2-GFP, and FC dissociates, allowing translocation of T14-3-3c $\Delta$ C-M2-GFP back into the cytoplasm and nucleus. Time frame: 30 min.

[Movie S10](#)



**Movie S11.** PM\_3 (second pm recruitment\_5μM FC) *N-Myr-mCherry-CT52M1* is cotransfected with *T14-3-3cΔC-M2-GFP*. *T14-3-3cΔC-M2-GFP* shuttles between nucleus and cytoplasm, and *N-Myr-mCherry-CT52M1* is recruited to the plasma membrane. After the addition of 5 μM FC to the HEK293T cells, *T14-3-3cΔC-M2-GFP* forms a complex with *N-Myr-mCherry-CT52M1* and thereby is recruited within few seconds to the plasma membrane. Time frame: 30 min.

[Movie S11](#)



**Movie S12.** PM\_4 (second pm recruitment\_revers) The reversibility of the plasma membrane-recruited *T14-3-3cΔC-M2-GFP* is demonstrated by rinsing the cells with FC-free medium. The ternary complex of *N-Myr-mCherry-CT52M1*, *T14-3-3cΔC-M2-GFP*, and FC dissociates, allowing translocation of *T14-3-3cΔC-M2-GFP* back into the cytoplasm and nucleus. Time frame: 30 min.

[Movie S12](#)

## Other Supporting Information Files

[SI Appendix \(PDF\)](#)