SUPPORTING INFORMATION

Isoform-Specific Disruption of AKAP-localized PKA Using Hydrocarbon Stapled Peptides

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 Parent sequence peptides did not gain intracellular access

a) Library of parent sequence stapled peptides lacking the addition of Lys residues or an N-terminal PEG_3 group. All peptides designed in the parent sequence libraries demonstrated limited water solubility. **b)** Hela cells were treated with 5 μM 5(6)-carboxyfluorescein labeled peptides of the original parent sequences in either a non-stapled or stapled format. Cells were pretreated with peptides for 6 hrs before washing, fixation and imaging by fluorescence microscopy. As expected, none of the compounds tested demonstrated notable cell permeability.

Supplementary Figure 2 Fluorescence polarization of 1K library of peptides

Fluorescence polarization of the 1K Lys-modified peptide library was measured using purified protein constructs of the D/D domains from either PKA-RI or PKA-RII. Peptides were plated at a final concentration of 10 nM and the D/D dimerization domains were tested over a concentration range of 0.1 nM to 100 μM. Data was collected in triplicates for each concentration measurement.

Supplementary Figure 3 Fluorescence polarization of 2K library of peptides

Fluorescence polarization of the 2K Lys-modified peptide library was measured using purified protein constructs of the D/D domains from either PKA-RI or PKA-RII. Peptides were plated at a final concentration of 10 nM and the D/D dimerization domains were

tested over a concentration range of 0.1 nM to 100 μM. Data was collected in triplicates for each concentration measurement.

Supplementary Figure 4 Fluorescence polarization of 3K library of peptides

Fluorescence polarization of the 3K Lys-modified peptide library was measured using purified protein constructs of the D/D domains from either PKA-RI or PKA-RII. Peptides were plated at a final concentration of 10 nM and the D/D dimerization domains were tested over a concentration range of 0.1 nM to 100 μM. Data was collected in triplicates for each concentration measurement.

Supplementary Figure 5 Cell permeability of STAD peptides in HeLa cells

Cell permeability of STAD-1, -2, and -3 are shown using HeLa cells at 40X magnification. Cells were treated with 5 μM of 5(6)-carboxyfluorescein-labeled peptides for 6 hrs before imaging.

Supplementary Figure 6 Cell permeability of STAD peptides in MDA-MB-231 cells

Cell permeability of STAD-1, -2, and -3 are shown using MDA-MB-231 cells at 40X magnification. Cells were treated with 5 μM of 5(6)-carboxyfluorescein-labeled peptides for 6 hrs before imaging.

Supplementary Figure 7 Cell permeability of STAD peptides in PC-3 cells

Cell permeability of STAD-1, -2, and -3 are shown using PC-3 cells at 40X magnification. Cells were treated with 5 μM of 5(6)-carboxyfluorescein-labeled peptides for 6 hrs

before imaging.

Supplementary Figure 8 Cell permeability of STAD scramble control peptides

Cell permeability of the scramble controls for STAD-1, -2, and -3 were tested in MDA-MB-231 cells. Cells were treated with 5 μM of 5(6)-carboxyfluorescein-labeled peptides for 6 hrs before imaging. All three peptides were found to gain intracellular access to the cytoplasm.

Supplementary Figure 9 PKA response in STAD-2-treated cells using nuclear-excluded AKAR4

a) The PKA response in HeLa cells pretreated with STAD-2 was measured using an AKAR4 probe that is excluded from the nucleus (by introduction of an NES sequence). This implies that the signal in diffusible AKAR4 (Fig.4e) was not due to nuclear contributions. b) The PKA response is shown in HeLa cells lacking peptide pretreatment using the pmAKAR4 probe. PKA activity is enhanced in response to Fsk/IBMX stimulation, and is inhibited by treatment with H89.

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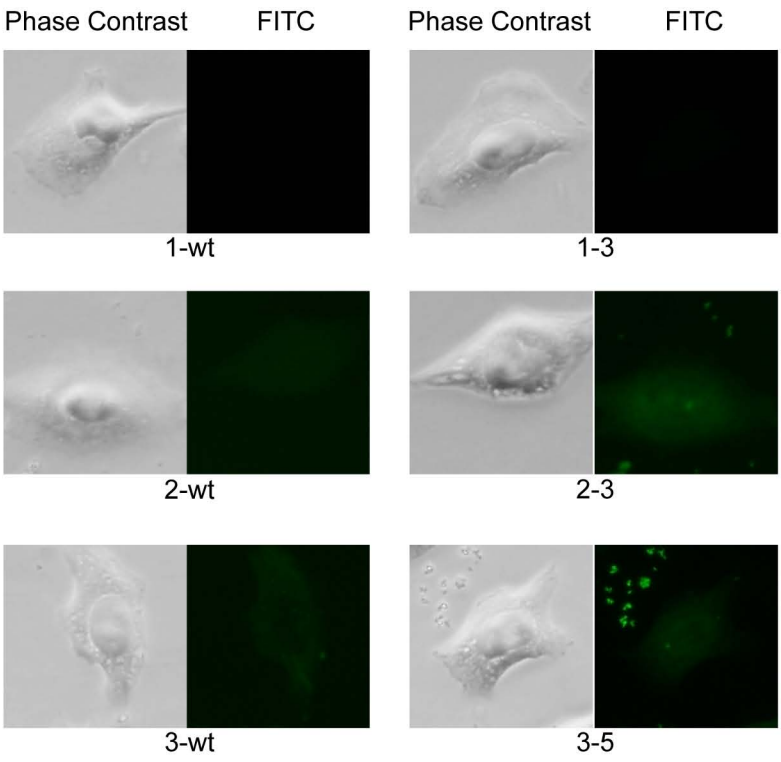
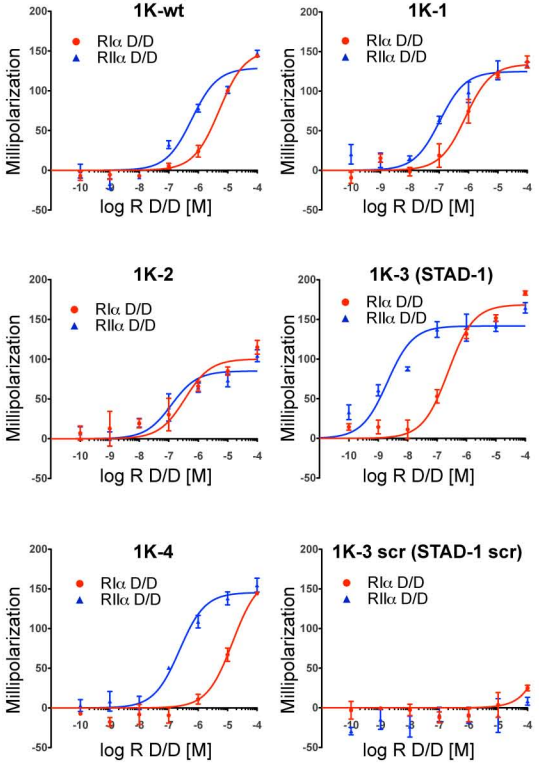
a

Template	Peptide	Parent Sequence Libraries
RIAD	1-wt	EQYANQLADQIIKEATE
	1-1	E*YAN*LADQIIKEATE
	1-2	EQYAN*LAD*IIKEATE
	1-3	EQYANQLAD*IIK*ATE
	1-4	EQYANQLADQII*EAT*
AKAP220	2-wt	SGLANFLVSEALSNALK
	2-1	S*LAN*LVSEALSNALK
	2-2	SGLAN*LVS*ALSNALK
	2-3	SGLANFLVS*ALS*ALK
smAKAP	3-wt	LYAQRLSEEIVRAVQQWA
	3-1	*YAQ*LSEEIVRAVQQWA
	3-2	LYAQ*LSE*IVRAVQQWA
	3-3	LYAQRLS*EIV*AVQQWA
	3-4	LYAQRLSEEIV*AVQ*WA
	3-5	LYAQRLSEEIVRAV*QWA*

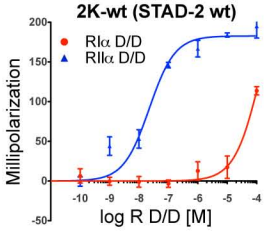
b

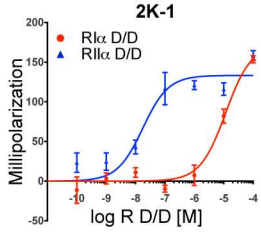


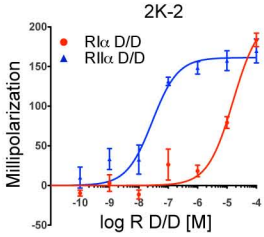
Wang, Y. et al, Supplementary Figure 1

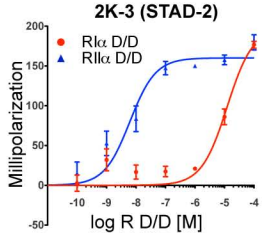


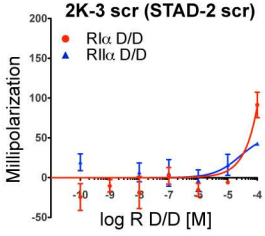
Wang, Y. et al, Supplementary Figure 2



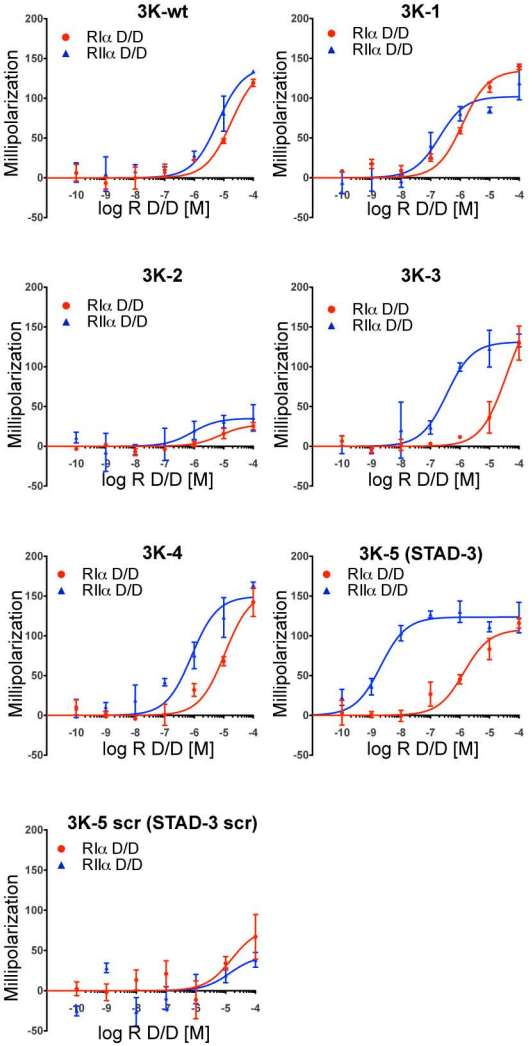






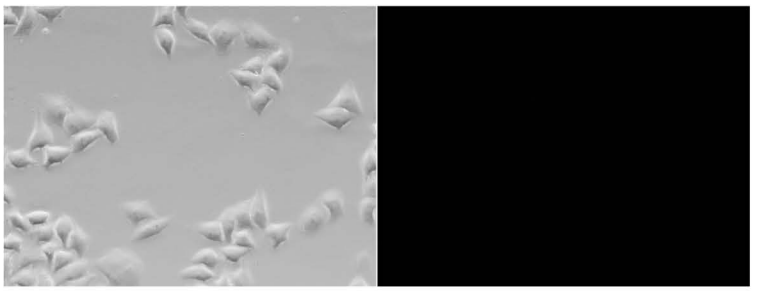


Wang, Y. et al, Supplementary Figure 3



Wang, Y. et al, Supplementary Figure 4

HeLa



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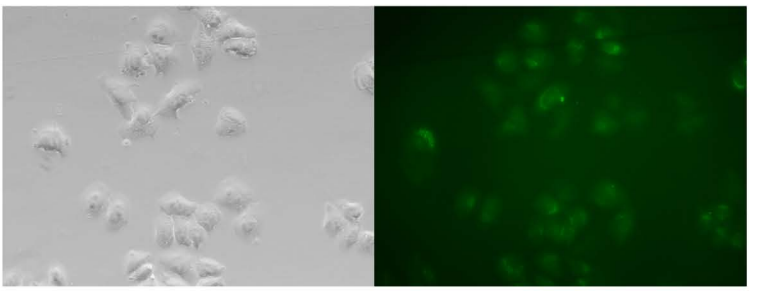

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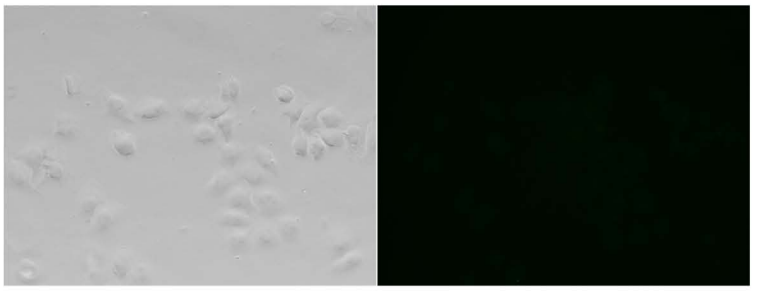

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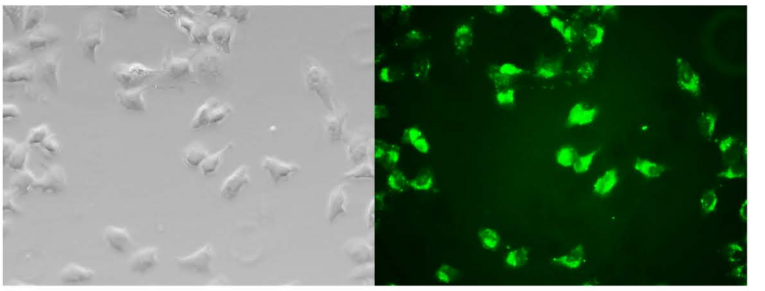

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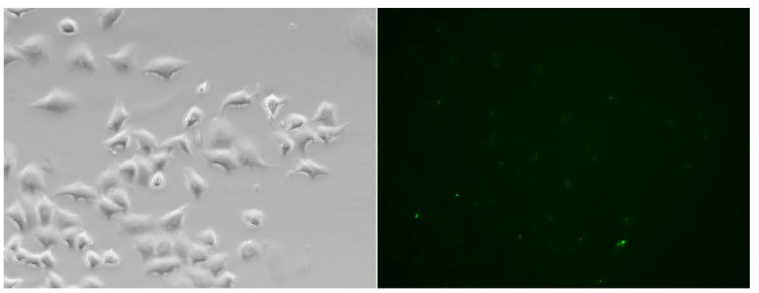

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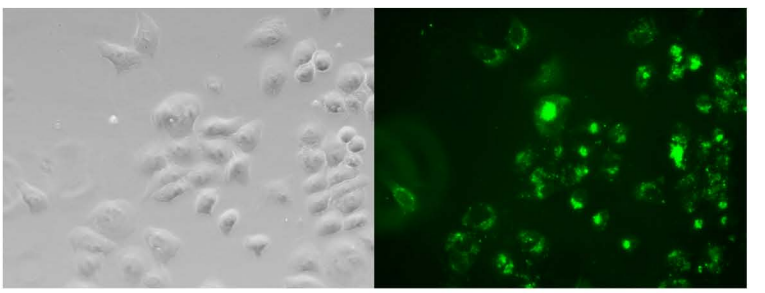

STAD-3

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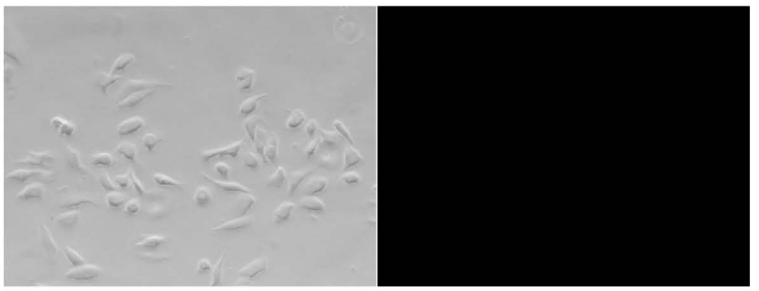
MDA-MB-231



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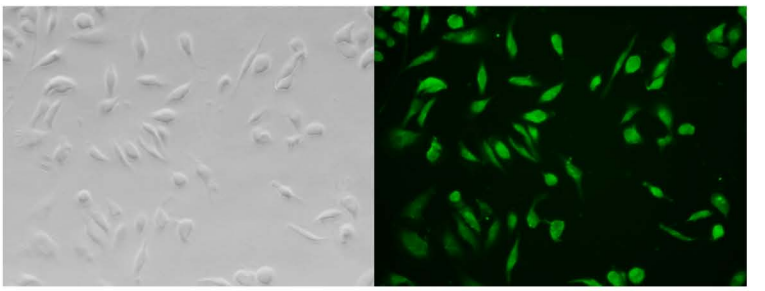

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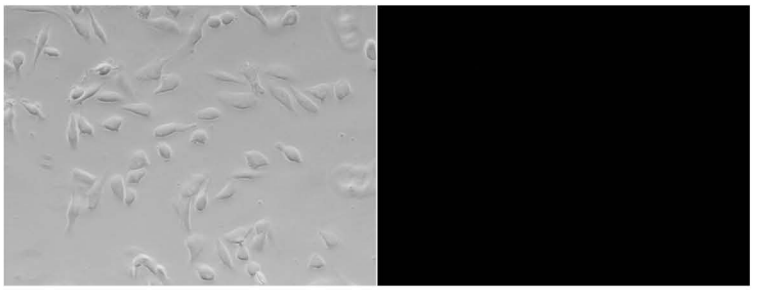

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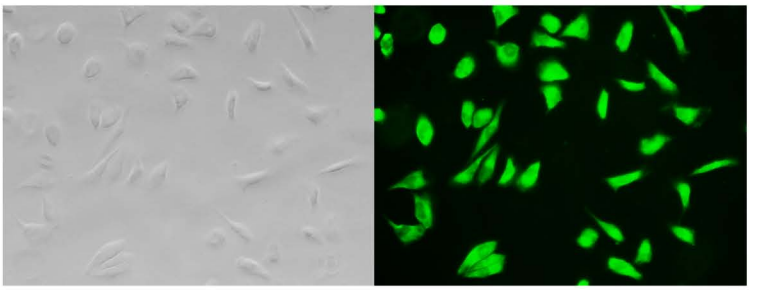

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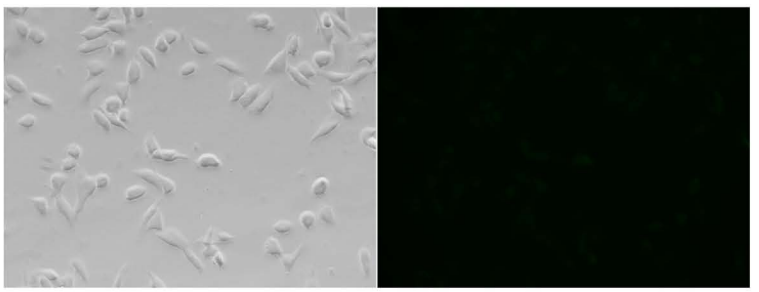

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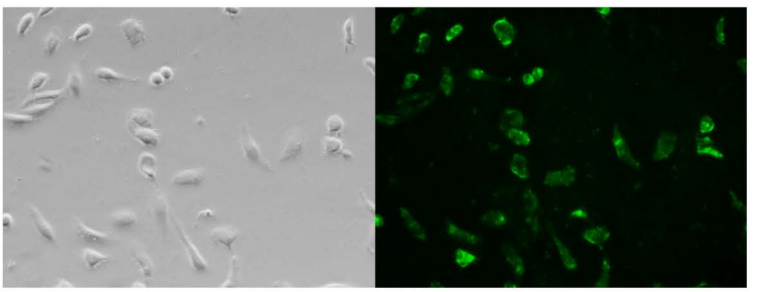

STAD-3

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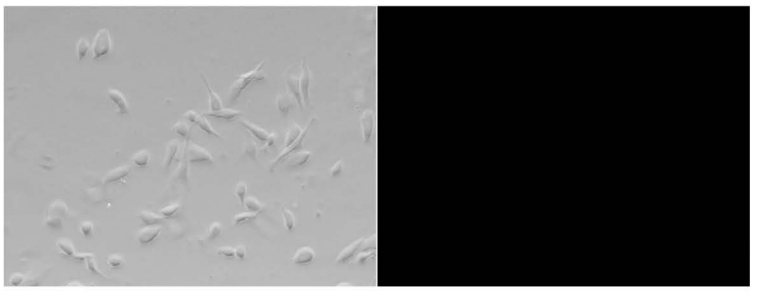
PC-3



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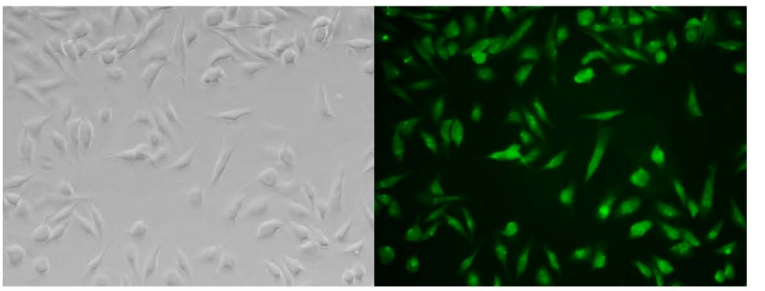

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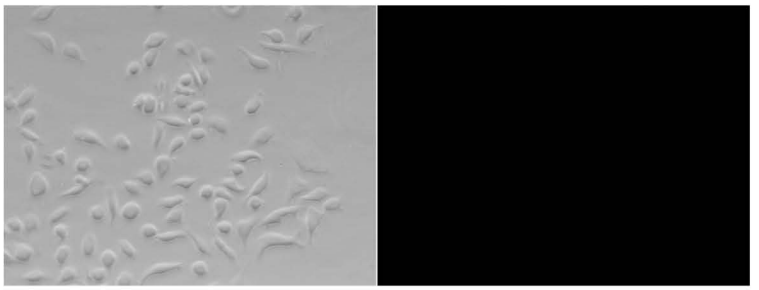

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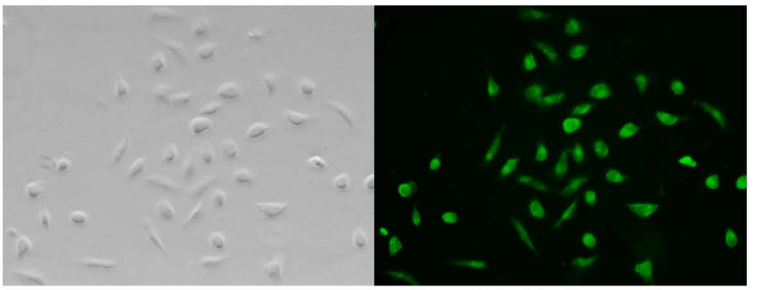

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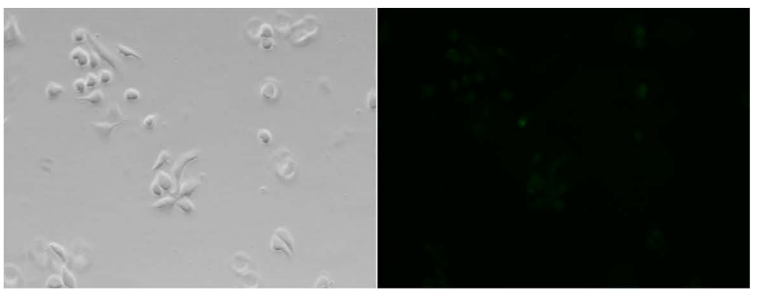

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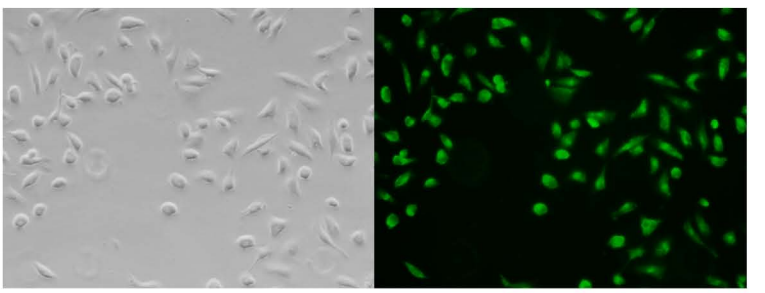

STAD-3

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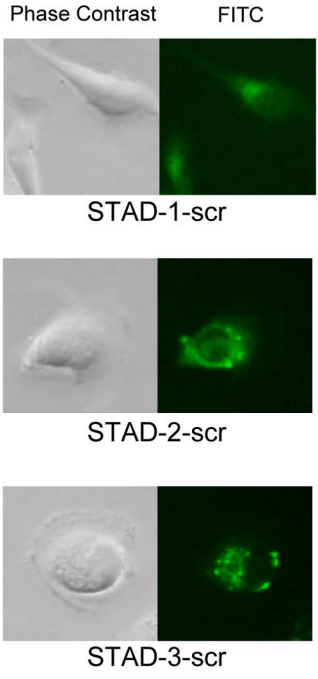
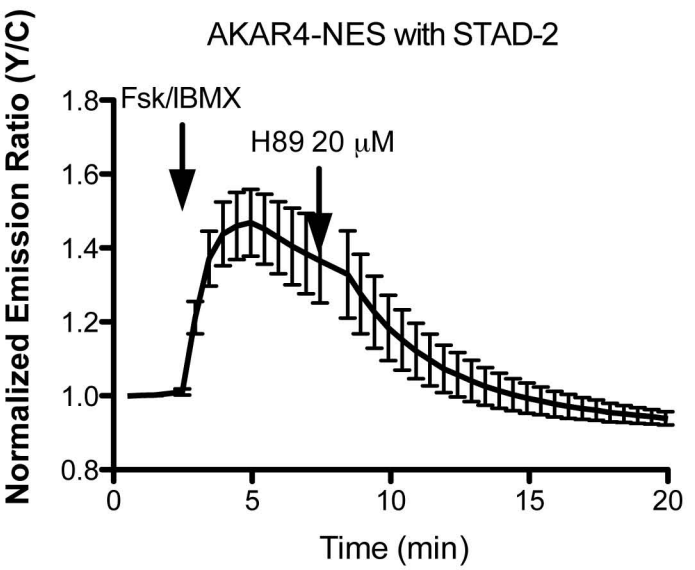


Wang, Y. et al., Supplementary Figure 8

a



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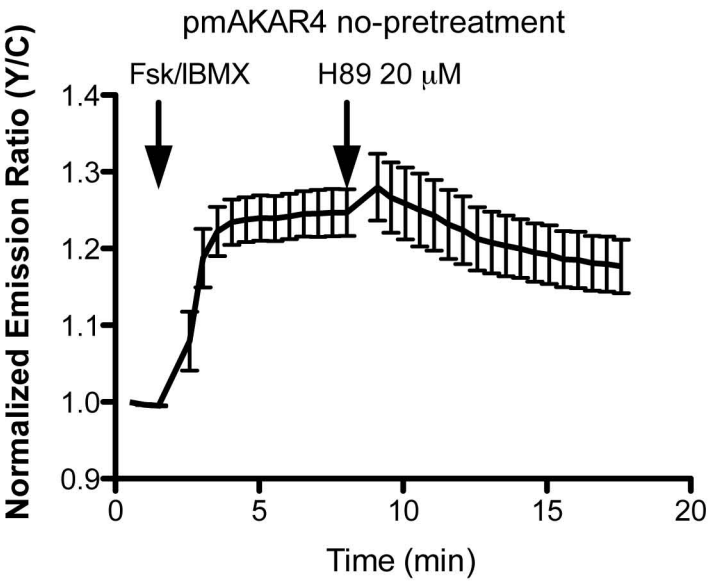

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