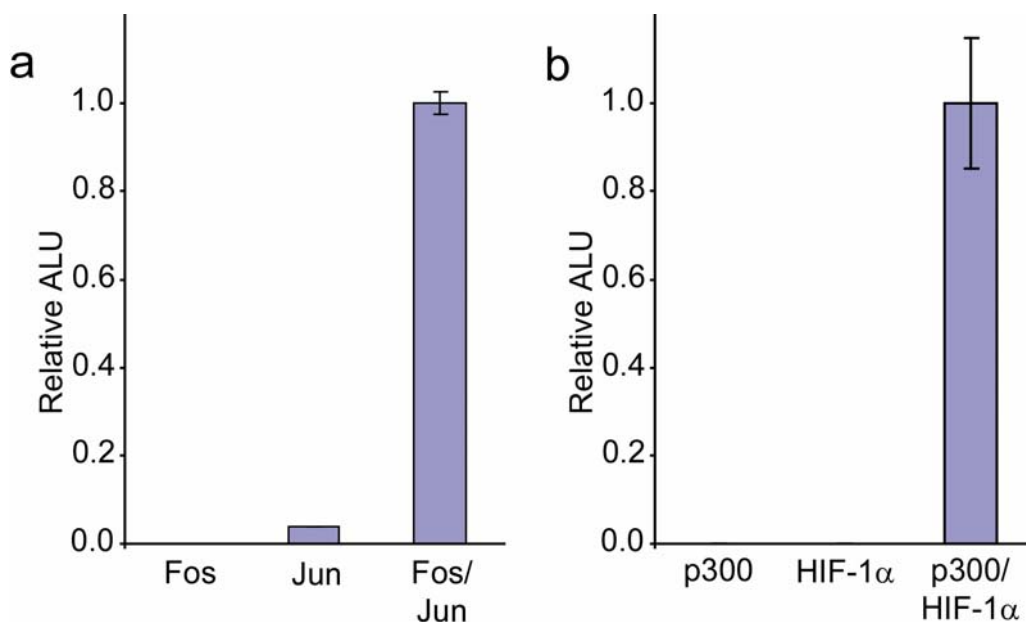


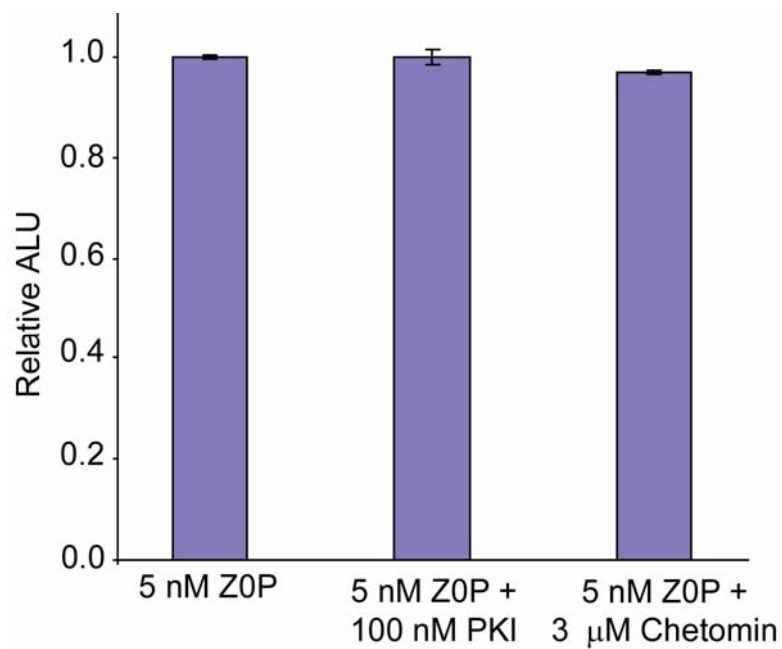
A General and Rapid Cell-Free Approach for the Interrogation of Protein-Protein, Protein-DNA, and Protein-RNA Interactions and their Antagonists Utilizing Split-Protein Reporters

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Supplementary Figure S1. Detection of Fos/Jun and p300/HIF-1 α interactions. (a) Association dependent firefly luciferase reassembly of Fos-NFluc and CFluc-Jun fusions utilizing *in vitro* transcribed mRNA in a rabbit reticulocyte lysate translation system. A significant signal increase is observed when Fos-NFluc and CFluc-Jun are co-expressed over either fusion protein alone. (b) Association dependent firefly luciferase reassembly of p300-NFluc and CFluc-HIF-1 α fusions utilizing *in vitro* transcribed mRNA in a rabbit reticulocyte lysate translation system. A significant signal increase is observed when p300-NFluc and CFluc-HIF-1 α are co-expressed as compared to either fusion protein alone.



Supplementary Figure S2. Interrogating off target effects of the PKI (peptide) and chetomin (small molecule) protein-protein interaction inhibitors on the DNA dependent firefly luciferase reassembly system (ternary complex: PBSII-NFluc, CFluc-Zif268, and 5nM target dsDNA oligonucleotide). No decrease in signal is observed upon addition of either 100 nM PKI or 3 μM chetomin to the ternary complex.

Reassembly Pairs	Split-Protein Fusion	Agonist	Antagonist
NVenus-Zif268 PBSII-CVenus	Venus (1-157) Venus (158-238)	Z0P dsDNA Target	
N β Lac-Zif268 PBSII-C β Lac	β Lactamase (26-196) β Lactamase (198-290)	Z0P dsDNA Target	
PBSII-NFluc CFluc-Zif268	Firefly Luciferase (2-416) Firefly Luciferase (398-550)	Z0P dsDNA Target	hpDNA-Zif268
NFluc-Zif268 PBSII-CFluc	Firefly Luciferase (2-398) Firefly Luciferase (394-550)	Z0P dsDNA Target	
NGluc-Zif268 PBSII-CGluc	Gaussia Luciferase (17-109) Gaussia Luciferase (110-185)	Z0P dsDNA Target	
Pum2-NFluc CFluc-Pum1	Firefly Luciferase (2-416) Firefly Luciferase (398-550)	ss 5'-CAUGGUGUAUAUAGUCUUUUGAUAUAGCGGC-3'	ss 5'-CCAGAAUUGUAUAUUCG-3'
MBD2-NFluc CFluc-Zif268	Firefly Luciferase (2-416) Firefly Luciferase (398-550)	ss 5'-GCGTA _m CGTACGCCACGCCACCG-3'	
PKI-NFluc CFluc-PKA	Firefly Luciferase (2-416) Firefly Luciferase (398-550)		PKI Peptide
FRB-NFluc CFluc-FKBP	Firefly Luciferase (2-416) Firefly Luciferase (398-550)	Rapamycin	
p300-NFluc CFluc-Hif1 α	Firefly Luciferase (2-416) Firefly Luciferase (398-550)		Chetomin
Fos-NFluc CFluc-Jun	Firefly Luciferase (2-416) Firefly Luciferase (398-550)		

Supplementary Table S1. Z0P dsDNA Target (5'-GCGTAGCGTGGGCGGTGTGGAAACACCG-3')Protein fusions, reporter fragments, agonists, and antagonists used.

Supplementary Methods

Fos/Jun and p300/HIF-1 α association dependent reassembly of firefly luciferase.

Duplicate 25 μ L translations were carried out in Flexi-Rabbit Reticulocyte Lysate (Promega) according to the manufacturer's protocol using 2 pmol of each mRNA encoding for either Fos-NFluc, CFluc-Jun, or both, and 0.5 μ L of RNasin Plus (Promega). Translations were incubated at 30 °C for 90 minutes and assayed by combining 20 μ L of translation solution with 80 μ L of Steady-Glo Luciferase System (Promega). Light emission was monitored using a Turner TD-20e luminometer with a 3 second delay and a 10 second integration time.

Duplicate 25 μ L translations were carried out in Flexi-Rabbit Reticulocyte Lysate (Promega) according to the manufacturer's protocol using 2 pmol of each mRNA encoding for either p300-NFluc, CFluc-HIF-1 α , or both, 10 μ M ZnCl₂ and 0.5 μ L of RNasin Plus (Promega). Translations were incubated at 30 °C for 90 minutes and assayed by combining 20 μ L of translation solution with 80 μ L of Steady-Glo Luciferase System (Promega). Light emission was monitored using a Turner TD-20e luminometer with a 3 second delay and a 10 second integration time.

Determination of off target effects of PKI and chetomin on signal generation.

Duplicate 25 μ L translations were carried out in Flexi-Rabbit Reticulocyte Lysate (Promega) according to the manufacturer's protocol using 2 pmol of each mRNA encoding for PBSII-NFluc (residues 2-416) and CFluc-Zif268 (residues 398-550), 10 μ M

ZnCl₂, 0.5 µL of RNasin Plus (Promega), and 25 nM Z0P target DNA (**Table S1**). Translations were incubated at 30 °C for 90 minutes followed by the addition of either 10 µM PKI, 300 µM chetomin, or DMSO and allowed to incubate at room temperature for 30 minutes. Light emission was assayed by adding 20 µL of translation to 80 µL of Steady-Glo Luciferase Assay System (Promega). Final concentrations in the assays were 5 nM DNA, 5 nM DNA plus 100 nM PKI, and 5 nM DNA and 3 µM chetomin. Light emission was monitored 1 minute after Steady-Glo addition using a Wallac 1420 VICTOR 3™ V luminometer with a 1 second integration time.

Complete Reference 48 Citation

Kung, A. L.; Zabludoff, S. D.; France, D. S.; Freedman, S. J.; Tanner, E. A.; Vieira, A.; Cornell-Kennon, S.; Lee, J.; Wang, B. Q.; Wang, J. M.; Memmert, K.; Naegeli, H. U.; Petersen, F.; Eck, M. J.; Bair, K. W.; Wood, A. W.; Livingston, D. M. *Cancer Cell* **2004**, 6, 33-43.