

Supporting Information

Identification of a Fragmented Small GTPase Capable of Conditional Effector Binding

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1. Stock Solutions

TBS buffer: 30 mM Tris-HCl (pH = 7.5), 250 mM NaCl, 5 mM MgCl₂, and 10% glycerol. Prior to use, 2 mM 2-mercaptoethanol was added.

Lysis buffer: 20 mM HEPES (pH = 7.5), 120 mM NaCl, 10% glycerol, and 2 mM EDTA.

Wash buffer: 50 mM Tris-HCl (pH = 7.6), 150 mM NaCl, 1% Triton X-100, and 20 mM MgCl₂.

2. Expression of Cdc42 Constructs in Bacterial Cells

BL21-CodonPlus (DE3)-RIPL E. coli cells were transformed with a vector expressing wild-type Cdc42 or the L61Cdc42 mutant. Protein expression was induced according to a previously described protocol.^{1,2}

3. Cdc42 Nucleotide Exchange in Bacterial Cell Lysates

Cells expressing wild-type Cdc42 were normalized to an OD₆₀₀ = 2.0 in 1 mL and were lysed using the B-PER™ Bacterial Protein Extraction Reagent (Life Technologies, 32 µL) following vendor provided protocols. Lysates containing 150 µg total protein were incubated with 25 mM EDTA and either 2 mM GDP or 1 mM GTP-γS for 45 min at 30 °C in order to facilitate nucleotide exchange. The reactions were then placed on ice, and quenched with 35 mM MgCl₂.

4. Preparation of PBD Beads

The Cdc42 binding domain (PBD) protein was amplified from the pET23-PBD (65-150)-N-Cys vector (Addgene, #13722),³ and cloned into a pGex-2T vector. The resulting GST-PBD protein construct was expressed in BL21 (DE3) cells by inducing with 0.3 mM IPTG for 3 hours at 37 °C.⁴ Cells were harvested by centrifugation at 3,220g for 30 min at 4 °C, washed with DI water, and frozen at -80 °C. For cell lysis, pellets were thawed and resuspended in 40 mL of 1x lysis buffer containing 1 mg/ml lysozyme and incubated for 20 min at 4 °C. The cells were then sonicated at 40% amplitude using 1 sec on and 1 sec off cycles for a total of 45 seconds. Lysates were clarified by centrifugation at 18,000g for 30 min and glutathione resin (Glutathione Sepharose 4 Fast Flow, GE Healthcare, 17-5132-01, 600 µL per 200 mL of cell culture) was added along with NP-40 (0.5%). The resulting mixture was incubated for 1 hr with rotation at 4 °C. Beads were then collected and washed in lysis buffer with 0.5% NP-40 (5 times), and another 3 times in lysis buffer with no NP-40.⁴ The resulting GST-PBD beads can be stored at 4 °C for up to one week.

5. Pull-Down of Cdc42 from Bacterial Cell Lysates

Total protein concentrations were adjusted to 3 µg/µL with wash buffer (excluding 1% Triton X-100). GST-PBD beads (30 µg immobilized protein) were added to 300 µL of cell lysate and the mixture was incubated for 30 min at 4 °C with rotation. GST-PBD beads were then collected via centrifugation at 800g for 5 min and washed four times with wash buffer. Beads were resuspended in 60 µL of SDS-PAGE loading dye, followed by denaturing at 95 °C for 10 minutes. The samples were centrifuged at 17,000g for 5 min and cooled to room temperature before loading on a 15% SDS-PAGE gel. Samples were transferred from the SDS-PAGE gel to

a PVDF membrane using standard protocols. The membrane was blocked with 5% milk in 1x TBST for 1 hr at room temperature. The membrane was washed three times with 1x TBST (15 minutes per wash), before incubating with a 1:10,000 dilution of anti-6X His-tag antibody conjugated to HRP (Abcam, ab1187) in 1x TBST containing 5% milk overnight at 4 °C with rotation. The next morning, the membrane was washed three times with 1x TBST (1 hr for the first wash, and 15 min for the second and third wash). Bands were visualized using SuperSignal West Dura Extended Duration Substrate (Life Technologies, 34075) on a Gel Doc XR+ system.

6. Cloning and Expression of the Cdc42 Fragment Library in Rabbit Reticulocyte Lysates

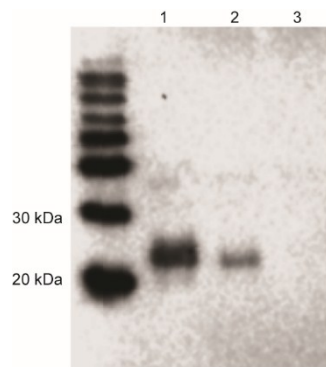
FRB and FKBP were amplified and cloned into pET-45b (Novagen, 71327-3) and pRSF-1b (Novagen, 71330-3) plasmids, respectively. L61Cdc42 N-terminal fragments were fused to the C-terminus of FRB in pET-45b, yielding a FRB-N-terminal Cdc42 fragment construct. L61Cdc42 C-terminal fragments were fused to the N-terminus of FKBP in pRSF-1b, yielding a Cdc42 C-terminal fragment-FKBP construct. These resulting constructs were amplified by PCR and a T7 RNA polymerase promoter and Kozak sequence were added according to previously published procedures.⁵ mRNA was transcribed from the resulting template DNA using the T7 RiboMAX Express RNA production system (Promega, P1320) and analyzed by agarose gel electrophoresis. The resulting mRNAs for potentially complementary fragments were translated in rabbit reticulocyte lysates (Promega, L4960) at a final reaction volume of 50 μ L. These reactions contained 33 μ L of rabbit reticulocyte lysate, 1 μ L amino acid mixture minus methionine, 40 μ M ³⁵S-methionine (PerkinElmer, NEG709A001MC, 1200Ci/mmol), 10 mM Mg(CH₃COO)₂, 70 mM KCl, 1 μ L RNasin Ribonuclease inhibitor (Promega, N2111), 2.5 μ L N-terminal Cdc42 fragment mRNA (2 μ g), 2.5 μ L C-terminal Cdc42 fragment mRNA (2 μ g), and 1 μ M rapamycin where indicated. The reactions were adjusted to 50 μ L with nuclease-free water. Constitutively active L61Cdc42 and dominant negative N17Cdc42⁶ were used as positive and negative controls. Reaction mixtures were incubated at 30 °C for 90 min.

7. Pull-Down of Reassembled Cdc42 from In Vitro Translation Reactions

The entire translation reaction mixture was incubated with 10 μ L of glutathione sepharose beads containing immobilized GST-PBD (2.95 μ g/ μ L) for 30 min at 4 °C with rotation. Reassembled Cdc42 fragments that bound to immobilized GST-PBD were purified by centrifugation at 800g for 5 min at 4 °C. Beads were then washed 4 times with wash buffer. Recovered proteins were analyzed by SDS-PAGE and visualized by autoradiography.

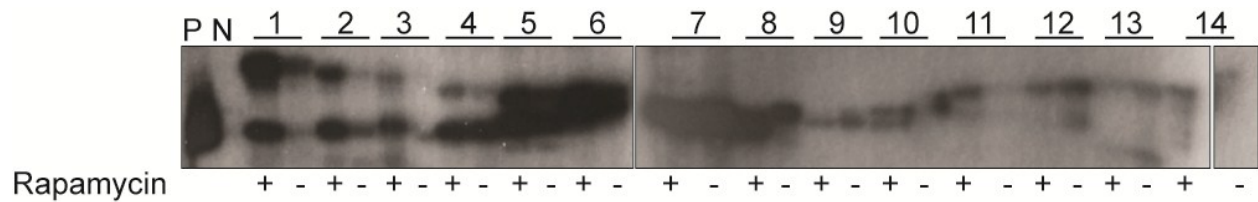
8. Supplementary Figures

Figure S1



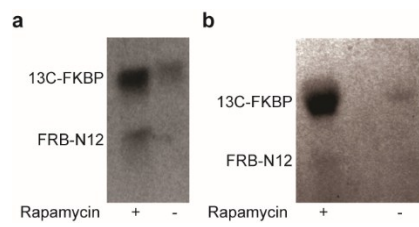
Wild-type Cdc42 cell lysate was exchanged with GDP or GTP- γ S respectively and incubated with immobilized PBD. The L61Cdc42 mutant was incubated with immobilized PBD under the same conditions. Purified Cdc42 was detected using an anti-His tag antibody (Abcam, ab1187). Lane 1: GTP- γ S bound Cdc42; Lane 2: L61Cdc42 mutant; Lane 3: GDP bound Cdc42. The expected molecular weight of Cdc42 is 22 kDa.

Figure S2



Cotranslation of FRB-N-terminal Cdc42 fragment and Cdc42 C-terminal fragment-FKBP constructs in rabbit reticulocyte lysates in the presence (+) or in the absence (-) of rapamycin. Reassembled Cdc42 was purified using immobilized PBD and visualized by autoradiography. Constitutively active L61Cdc42 is used as positive control (P) and the dominant negative N17Cdc42 mutant (N) is used as negative control. Refer to Tables S1 and S2 for protein sequences. Pair numbering is indicated above lanes, C-terminal fragments have larger molecular weights than N-terminal fragments for pairs 1-7. Due to the large number of samples, multiple gels are shown.

Figure S3



Independent replicates of cotranslations of FRB-N12 and 13C-FKBP constructs in rabbit reticulocyte lysates in the presence (+) or in the absence (-) of rapamycin where exposed to immobilized PBD and analyzed by SDS-PAGE. The N12 and 13C fragments consistently show increased effector binding in the presence of rapamycin. Translations were performed with different batches of rabbit reticulocyte lysate.

9. Supplementary Tables

Table S1. Full-Length Cdc42 Sequences

Protein	Amino Acid Sequence
Wild-Type Cdc42	QTIKCVVVG DGAVGKTCLLISYTTNKFPSEYVPTVFDNYAVTVMIGGEPYT LGLFDTAGQEDYDRLRPLSYPQTDVFLVCFSVSPSSFENVKEKWVPEIT HHCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPITPETA EKLARDLKAVKY VECSALTQRGLKNVFDEAILAALEPPETQPKRKCCIFHHHHHHH
L61Cdc42	QTIKCVVVG DGAVGKTCLLISYTTNKFPSEYVPTVFDNYAVTVMIGGEPYT LGLFDTAG L EDYDRLRPLSYPQTDVFLVCFSVSPSSFENVKEKWVPEIT HHCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPITPETA EKLARDLKAVKY VECSALTQRGLKNVFDEAILAALEPPETQPKRKCCIFHHHHHHH
N17Cdc42	QTIKCVVVG DGAVGK N CLLISYTTNKFPSEYVPTVFDNYAVTVMIGGEPYT LGLFDTAGLEDYDRLRPLSYPQTDVFLVCFSVSPSSFENVKEKWVPEIT HHCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPITPETA EKLARDLKAVKY VECSALTQRGLKNVFDEAILAALEPPETQPKRKCCIFHHHHHHH

Mutated amino acids are highlighted in red.

Table S2. Sequences of Cdc42 Fragments

Pair	Fragment	Amino Acid Sequence
Pair 1	FRB-N12	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGA
	13C-FKBP	MGVGKTCLLISYTTNKFSEYVPTVFDNYAVTVMIGGEPYTLGLFD TAGLEDYDRLRPLSYPQTDVFLVCFSSVSPSSFENVKEKWVPEITH HCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPITPETAELKARDLK AVKYVECSALTQRGLKNVFDEAILAALEPPETQPKRKCCIFGDPNW ELVYTARLQGGGSSGGGQISYASRGGVQVETISPGDGRTFPKRG QTCVVHYTG MLEDGKKFDSSRD RNKPFK FMLGKQEVIRGWEEGV AQMSVGQRAKL TISPDYAYGATGHPGIIPPHATLVFDVELLKLEHH HHHH
Pair 2	FRB-N29	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGA VGKTCLLISYTTNKFPS
	30C-FKBP	MGEYVPTVFDNYAVTVMIGGEPYTLGLFD TAGLEDYDRLRPLSYP QTDVFLVCFSSVSPSSFENVKEKWVPEITHHCPKTPFLLVGTQIDL RDDPSTIEKLAKNKQKPITPETAELKARDLKAVKYVECSALTQRGLK NVFDEAILAALEPPETQPKRKCCIFGDPNWE LVTARLQGGGSSG GGQISYASRGGVQVETISPGDGRTFPKRGQTCVVHYTG MLEDGK KFDSSRD RNKPFK FMLGKQEVIRGWEEGVAQMSVGQRAKL TISP DYAYGATGHPGIIPPHATLVFDVELLKLEHHHHHH
Pair 3	FRB-N35	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGA VGKTCLLISYTTNKFSEYVPTV
	36C-FKBP	MGFDNYAVTVMIGGEPYTLGLFD TAGLEDYDRLRPLSYPQTDVFL VCFSSVSPSSFENVKEKWVPEITHHCPKTPFLLVGTQIDLRDDPSTI EKLAKNKQKPITPETAELKARDLKAVKYVECSALTQRGLKNVFDEAI LAALEPPETQPKRKCCIFGDPNWE LVTARLQGGGSSGGGQISYA SRGGVQVETISPGDGRTFPKRGQTCVVHYTG MLEDGKKFDSSRD RNKPFK FMLGKQEVIRGWEEGVAQMSVGQRAKL TISPDYAYGAT GHPGIIPPHATLVFDVELLKLEHHHHHH
Pair 4	FRB-N47	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGA VGKTCLLISYTTNKFSEYVPTVFDNYAVTVMIG G
	48C-FKBP	MGEPYTLGLFD TAGLEDYDRLRPLSYPQTDVFLVCFSSVSPSSF ENVKEKWVPEITHHCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPIT

		PETAEK LARDLKAVKYVECSALTQRGLKNVFD EAILAALEPPETQP KRKCCIFGDPNWELVYTARLQGGGSSGGGQISYASRGVQVETIS PGDGRTFPKRGQTCVVHYTG MLEDGKKFDSSRD RNKPFK FMLGK QEVIRGWEEGVAQMSVGQRAKLTISP DYAYGATGHPGIIPPHATLV FDVELLKLEHHHHHH
Pair 5	FRB-N65	MAHHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTMIG GEPYTLGLFDTAGLE
	66C-FKBP	MGDYDRLRPLSYPQTDVFLVCF SVVSPSSFENVKEKWVPEITHHC PKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPITPETAEK LARDLKAVK YVECSALTQRGLKNVFD EAILAALEPPETQPKRKCCIFLQGGGSSG GGQISYASRGVQVETISPGDGRTFPKRGQTCVVHYTG MLEDGK KFDSSRD RNKPFK FMLGKQEVIRGWEEGVAQMSVGQRAKLTISP DYAYGATGHPGIIPPHATLVFDVELLKLEHHHHHH
Pair 6	FRB-N74	MAHHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTMIG GEPYTLGLFDTAGLE
	75C-FKBP	MGTDVFLVCF SVVSPSSFENVKEKWVPEITHHCPKTPFLLVGTQID LRDDPSTIEKLAKNKQKPITPETAEK LARDLKAVKYVECSALTQRGL KNVFD EAILAALEPPETQPKRKCCIFLQGGGSSGGGQISYASRGV QVETISPGDGRTFPKRGQTCVVHYTG MLEDGKKFDSSRD RNKPF K FMLGKQEVIRGWEEGVAQMSVGQRAKLTISP DYAYGATGHPGII PPHATLVFDVELLKLEHHHHHH
Pair 7	FRB-N82	MAHHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTMIG GEPYTLGLFDTAGLE
	83C-FKBP	MGSVVSPSSFENVKEKWVPEITHHCPKTPFLLVGTQIDLRDDPSTI EKLAKNKQKPITPETAEK LARDLKAVKYVECSALTQRGLKNVFD EAI LAALEPPETQPKRKCCIFLQGGGSSGGGQISYASRGVQVETISP GDGRTFPKRGQTCVVHYTG MLEDGKKFDSSRD RNKPFK FMLGKQ EVIRGWEEGVAQMSVGQRAKLTISP DYAYGATGHPGIIPPHATLVF DVELLKLEHHHHHH
Pair 8	FRB-N107	MAHHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTMIG GEPYTLGLFDTAGLE

		KEKWVPEITHHCPKT
	108C-FKBP	MGPFLLVGTQIDLRDDPSTIEKLAKNKQKPITPETAEKLARDLKAVK YVECSALTQRGLKNVFDEAILAALEPPETQPKRKCCIFLQGGGSSG GGQISYASRGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGK KFDSSRDNRNPKPFKMLGKQEVIRGWEEGVAQMSVGQRAKLTISP DYAYGATGHPGIIPPHATLVFDVELLKLEHHHHHH
Pair 9	FRB-N115	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTVMIG GEPYTLGLFDTAGLEDYDRLRPLSYPQTDVFLVCF SVSPSSFENV KEKWVPEITHHCPKTPFLLVGTQ
	116C-FKBP	MGIDLRDDPSTIEKLAKNKQKPITPETAEKLARDLKAVKYVECSALT QRGLKNVFDEAILAALEPPETQPKRKCCIFLQGGGSSGGGQISYAS RGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDR NPKPFKMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATG HPGIIPPHATLVFDVELLKLEHHHHHH
Pair 10	FRB-N120	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTVMIG GEPYTLGLFDTAGLEDYDRLRPLSYPQTDVFLVCF SVSPSSFENV KEKWVPEITHHCPKTPFLLVGTQIDLRD
	121C-FKBP	MGDPSTIEKLAKNKQKPITPETAEKLARDLKAVKYVECSALTQRGL KNVFDEAILAALEPPETQPKRKCCIFLQGGGSSGGGQISYASRGV QVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDNRNPKF KMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGII PPHATLVFDVELLKLEHHHHHH
Pair 11	FRB-N131	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTVMIG GEPYTLGLFDTAGLEDYDRLRPLSYPQTDVFLVCF SVSPSSFENV KEKWVPEITHHCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPI
	132C-FKBP	MGKQKPITPETAEKLARDLKAVKYVECSALTQRGLKNVFDEAILAA LEPPETQPKRKCCIFLQGGGSSGGGQISYASRGVQVETISPGDG RTFPKRGQTCVVHYTGMLEDGKKFDSSRDNRNPKPFKMLGKQEV RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDV ELLKLEHHHHHH
Pair 12	FRB-N137	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRRISKQQISYASRGGGSSGGGE LQTIKCVVVG DGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTVMIG GEPYTLGLFDTAGLEDYDRLRPLSYPQTDVFLVCF SVSPSSFENV

		KEKWVPEITHHCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPI
	138C-FKBP	MGTPETAEKLARDLKAVKYVECSALTQRGLKNVFDEAILAALEPPE TQPKRKCCIFLQGGGSSGGGQISYASRGGVQVETISPGDGRTFPK RGQTCVVHYTGMLLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEE GVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE HHHHHH
Pair 13	FRB-N151	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRISKQQISYASRGGGSSGGGE LQTIKCVVGDGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTMIG GEPYTLGLFDTAGLEDYDRLRPLSYPQTDVFLVCFSVSPSSFENV KEKWVPEITHHCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPIPE TAEKLARDLKA
	152C-FKBP	MGVKYVECSALTQRGLKNVFDEAILAALEPPETQPKRKCCIFLQGG GSSGGGQISYASRGGVQVETISPGDGRTFPKRGQTCVVHYTGML EDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKL TISPDYAYGATGHPGIIPPHATLVFDVELLKLEHHHHHH
Pair 14	FRB-N160	MAHHHHHHVGTGSNDDDDKSPDPMEMWHEGLEEASRLYFGERN VKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCR KYMKSGNVKDLTQAWDLYYHVFRISKQQISYASRGGGSSGGGE LQTIKCVVGDGAVGKTCLLISYTTNKFSEYVPTVFDNYAVTMIG GEPYTLGLFDTAGLEDYDRLRPLSYPQTDVFLVCFSVSPSSFENV KEKWVPEITHHCPKTPFLLVGTQIDLRDDPSTIEKLAKNKQKPIPE TAEKLARDLKAVKYVECSAL
	161C-FKBP	MGTQRGLKNVFDEAILAALEPPETQPKRKCCIFLQGGGSSGGGQI SYASRGGVQVETISPGDGRTFPKRGQTCVVHYTGMLLEDGKKFDS SRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAY GATGHPGIIPPHATLVFDVELLKLEHHHHHH

FRB is highlighted in blue, FKBP is shown in red, and the Cdc42 fragment is indicated in tan.
Multiple cloning sites and linkers are left black.

10. References

- 1 B. N. Goguen, G. S. Loving and B. Imperiali, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5058-5061.
- 2 P. Nalbant, L. Hodgson, V. Kraynov, A. Toutchkine and K. M. Hahn, *Science*, 2004, **305**, 1615-1619.
- 3 V. S. Kraynov, C. Chamberlain, G. M. Bokoch, M. A. Schwartz, S. Slabaugh and K. M. Hahn, *Science*, 2000, **290**, 333-337.
- 4 V. Benard and G. M. Bokoch, *Methods Enzymol.*, 2002, **345**, 349-359.
- 5 J. R. Porter, C. I. Stains, B. W. Jester and I. Ghosh, *J. Am. Chem. Soc.*, 2008, **130**, 6488-6497.
- 6 C. D. Nobes and A. Hall, *J. Cell Biol.*, 1999, **144**, 1235-1244.