Supporting Information

Engineering Modular Protein Interaction Switches by Sequence Overlap

Nathan A. Sallee, Brian J. Yeh and Wendell A. Lim

Table S1. Domain – Ligand Pairs Used to Construct Overlap Switches

Domain	Ligand(s)	K_d	PDB Code	Reference (Affinity, PDB)
Human C-Raf1 Ras-binding domain (RBD)	Human H-Ras(GTP)	18 nM	1C1Y	S1, S2
Human WASP p21-binding domain (PBD)	Human Cdc42(GTP)	164 nM	1CEE	measured, S3
Human WASP inhibitory segment (IS)	Human WASP cofilin helix	2 μM	1EJ5	S4, S4
Human FK506-Binding Protein-12 (FKBP)	FK506 small molecule	0.4 nM	1FKJ	S5, S6
E. coli dihydrofolate reductase (DHFR)	Methotrexate small molecule	0.6 nM	1RX3	S7, S8
Human 14-3-3ζ domain	Designed PKA-phosphorylatable peptide: RRYHpSLPFI[a]	low nM	1QJA	S9, S10
Mouse α-Syntrophin PDZ domain	Voltage-gated sodium channel peptide: VKESLV or Rat nNOS PDZ domain	7 μM 1 μM	2PDZ 1QAV	measured, S11 S12, S13
Human NHERF (EBP50) PDZ domain #1	Human β2 adrenergic receptor peptide: RNCSTNDSLL	18 nM	1GQ4	S14, S15
Human phosphatase hPTP1E PDZ domain #2	Human Fas receptor peptide: NFRNETQSLV	~30 μM	3PDZ	S16, S17
Mouse C-Crk SH3 domain #1	Mutant C3G peptide: YPPPALPPKRRR	20 nM	1CKA	S18, S19
Mouse Grb2 SH3 domain #1	Mouse SOS-1 peptide: PPPVPPRRR	5 μM	1GBQ	S18, S20
Human Hck SH3 domain	Mouse SOS-1 peptide: PPPVPPRRR	5.6 μM	4HCK	S18, S21
S. cerevisiae Abp1 SH3 domain	S. cerevisae Ark1 peptide: KKTKPTPPPKPSHLK	20 nM	1JO8	S22, S23
S. cerevisiae Sho1 SH3 domain	S. cerevisae Pbs2 peptide: NKPLPPLPVAGSSKV	1.3 μM	-	S22, NA

[a] "pS" denotes phosphoserine

Table S2. Details of 25 Engineered Switches

Domain-Domain Overlaps:

#	N-terminal module	C-terminal module	Overlap sequence[a]	Behavior
1	Human C-Raf1 RBD	Mouse C-Crk SH3 domain #1	…LQVDFL EYVRAL… …LQVDAL…	Only binds to Ras, not to SH3 ligand
2	Human NHERF PDZ domain #1	Human C-Raf1 RBD	…NAVRLLVVDPS NTIRVFLPNKQ… …NAVRVLLVNKQ…	Switch protein is insoluble
3	Human WASP PBD	Rat nNOS PDZ domain	…DPDLRSLFSRA NVISVRLFKRK… …DPDLVRLFKRK…	Switch protein is insoluble
4	Human FKBP-12	Human hPTP1E PDZ #2	…FDVELLKLE FEVELAKND… …FDVELLKLD…	Only binds to FK506, not to PDZ ligand
5	Rat nNOS PDZ domain	Human WASP PBD	…LGPPTKAV IGAPSGFK… …IGPPSKFK…	Only binds to Syn PDZ, not Cdc42
6	Human C-Raf1 RBD	Human WASP IS	…IG-EELQVDFL VGWDPQNGFDV… …VGWDELNVFDV…	Only binds to Ras, not to the cofilin helix
7	*E. coli* DHFR	Human hPTP1E PDZ #2	…YCFEILERR DIFEVELAK… …YCFEVLLIK…	Only binds to methotrexate, not to PDZ ligand
8	*E. coli* DHFR	Mouse Grb2 SH3 domain #1	…YCFEILERR GSMEAIAKY… …YCFEALAKY…	Binds both ligands, not mutually exclusive
9	Mouse α-Syntrophin PDZ domain	Human WASP PBD	…EVKYMK KKKISK… …EVKYMKKISK…	**Functional interaction switch** (Switch **1** in main text)
10	Mouse α-Syntrophin PDZ domain	Human WASP PBD	…LEVKYMK KKKISKA… …LEVKKKISKA…	**Functional interaction switch**
11	Mouse α-Syntrophin PDZ domain	Human WASP PBD	…LEVKYMK KKKISKAD… …LEVKYISKAD…	Only binds to PDZ ligand, not Cdc42
12	Human NHERF PDZ domain #1	Human WASP PBD	…VVDPE KKKIS… …VVDPKKKIS…	**Functional interaction switch**
13	Mouse C-Crk SH3 domain #1	Human WASP PBD	…IPVPYVEKY IGAPSGFKH… …IPAPYVFKH…	Only binds to SH3 ligand, not Cdc42
14	Mouse C-Crk SH3 domain #1	Human WASP PBD	…PYVEK KKKIS… …PYVEKKKIS…	Binds both ligands, not mutually exclusive
15	Mouse C-Crk SH3 domain #1	Human WASP PBD	…VPYVEK KKKISK… …KKYVSK…	Only binds to Cdc42, not to SH3 ligand
16	Human Hck SH3 domain	Human WASP PBD	…NYVAR KKKISK… …NYVAKKISK…	Only binds to SH3 ligand, not to Cdc42

Table S2. (continued)

Peptide-Domain Overlaps:

#	N-terminal module	C-terminal module	Overlap sequence[a]	Behavior
17	Crk SH3-binding peptide: YPPPALPPKRRR	Human WASP PBD	…LPPKRRR KKKISKAD… …LPPKKRISKAD…	**Functional interaction switch** (Switch **2** in main text)
18	Abp1 SH3-binding peptide: KKTKPTPPPKPSHLK	Human WASP PBD	…PSHLK KKKIS… …PSHLKKKIS…	Binds both ligands, not mutually exclusive
19	Human WASP PBD	Crk SH3-binding peptide: YPPPALPPKRRR	…SRAGIS YPPPALP… …SRAGPPPALP…	Binds both ligands, not mutually exclusive
20	Human WASP PBD	Abp1 SH3-binding peptide: KKTKPTPPPKPSHLK	…SRAGIS KKTKPT… …SRAGKKTKPT…	Binds both ligands, not mutually exclusive

Peptide-Peptide Overlaps:

21	Grb2/Hck SH3-binding peptide: PPPVPPRRR	14-3-3ζ-binding peptide: RRYHSLPFI	…VPPRRR RRYHSL… …VPPRRRYHSL…	**Functional interaction switch**
22	Crk SH3-binding peptide: YPPPALPPKRRR	14-3-3ζ-binding peptide: RRYHSLPFI	…PPKRRR RRYHSL… …PPRRRRYHSL…	**Functional interaction switch** (Switch **3** in main text)
23	14-3-3ζ-binding peptide: RRYHSLPFI	Grb2/Hck SH3-binding peptide: PPPVPPRRR	…YHSLPFI PPPVPPR… …YHSLPPPVPPR…	Binds both ligands, not mutually exclusive
24	14-3-3ζ-binding peptide: RRYHSLPFI	Crk SH3-binding peptide: YPPPALPPKRRR	…YHSLPFI YPPPALPPK… …YHSLPPALPPK…	Binds both ligands, not mutually exclusive
25	14-3-3ζ-binding peptide: RRYHSLPFI	Sho1 SH3-binding peptide: NKPLPPLPVAGSSKV	…YHSLPFI NKPLPPL… …YRSLPPL…	**Functional interaction switch**

[a] In the overlap sequence column, the top sequence is the N-terminal module, the middle is the C-terminal module, and the bottom is the sequence used in the overlapped, chimeric protein.

Timescale of Switching

In order to address whether the binding competition of our switches is occurring on a physiologically-relevant timescale, we used mathematical modeling and experimental approaches. Because we have measured the K_d values of switch **1** for its two ligands (Figure S1), we can model the kinetics of switching between its bound states, making a small number of assumptions. We found that the affinities of the Syntrophin PDZ domain and WASP PBD that constitute switch **1** are roughly the same in the switch as the affinities of the individual domains. The K_d's of Syn PDZ and switch **1** for VKESLV peptide are identical at 7 μM. Therefore, it is fair to assume that the affinity of switch **1** for nNOS PDZ (which binds to the same site as the peptide ligand) is 1 μM (the same as the Syn PDZ-nNOS PDZ affinity). Kinetic studies of PDZ-ligand interactions have shown that they have similar on rates (k_{on}) that fall in the range of 4-10 $\mu M^{-1}\ s^{-1}$, but that their off rates (k_{off}) vary more widely, leading to differences in affinity.[S24] Assuming that the on rate of the Switch **1**-nNOS interaction falls in the middle of this range (7 $\mu M^{-1}\ s^{-1}$), we get an off rate of 7 s^{-1} ($K_d \cdot k_{on}$). Similarly, kinetic analyses of the interactions of multiple fragments of WASP with Cdc42(GTP) have shown that the k_{on} values are very similar for the different fragments (0.19 $\mu M^{-1}\ s^{-1}$) and that differences in affinity are due to changes in k_{off}.[S25] Because our measured K_d of 247 nM for the switch **1**-Cdc42(GTP) interaction is very similar to the affinity of the wild-type WASP PBD, we assume that the value of k_{on} is similarly 0.19 $\mu M^{-1}\ s^{-1}$, giving us a k_{off} value of 0.047 s^{-1}.

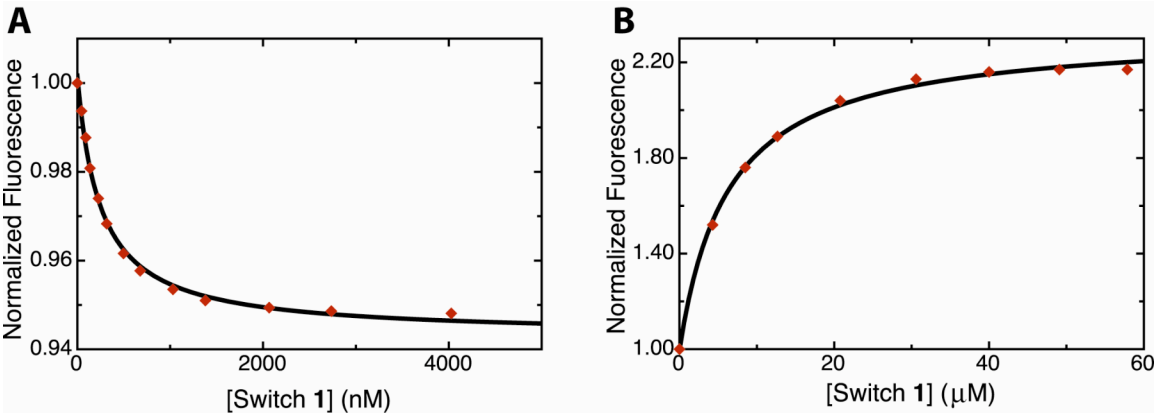

Figure S1. Switch **1** fluorescence perturbation affinity measurements. (A) Binding of switch **1** to Cdc42 G12V loaded with the fluorescent nucleotide analog Mant-GMPPNP gave a K_d of 247 nM. (B) Binding of switch **1** to dansylated VKESLV peptide gave a K_d of 7 μM.

Conversion between the bound states of switch **1** can be modeled as:

$$\text{nNOS} + \text{Switch } \mathbf{1} \underset{k_{off}(\text{NOS})}{\overset{k_{on}(\text{NOS})}{\rightleftharpoons}} \text{nNOS-Switch } \mathbf{1}$$

$$\text{Cdc42} + \text{Switch } \mathbf{1} \underset{k_{off}(\text{Cdc})}{\overset{k_{on}(\text{Cdc})}{\rightleftharpoons}} \text{Cdc42-Switch } \mathbf{1}$$

Using these equations, we made a simple deterministic model[S26] of switch binding equilibria using MATLAB (MathWorks). Using the estimated values of the kinetic parameters above, we calculated the timescale in which switch **1** goes from the nNOS-bound state to the Cdc42-bound state and vice versa. In our pulldown experiments in the body of the paper, we saw switch binding to 1 μM GST-nNOS was almost totally disrupted by 80 μM Cdc42 G12V. We therefore simulated this experiment by starting with 1 μM nNOS-bound Switch **1** and adding 100 μM Cdc42(GTP) at time zero. Under these conditions, we found that the switch is 99% in the Cdc42-bound state in 1.15 seconds (Figure S2A). The same experiment in the reverse direction (from Cdc42-bound to 90% nNOS-bound), starting with 1μM Cdc42-bound Switch **1** and 100 μM nNOS, was estimated to take 58 seconds (Figure S2B). This fits with our experimental data that show the Cdc42-switch **1** interaction can be disrupted by nNOS, but not as efficiently as the reverse competition (not shown).

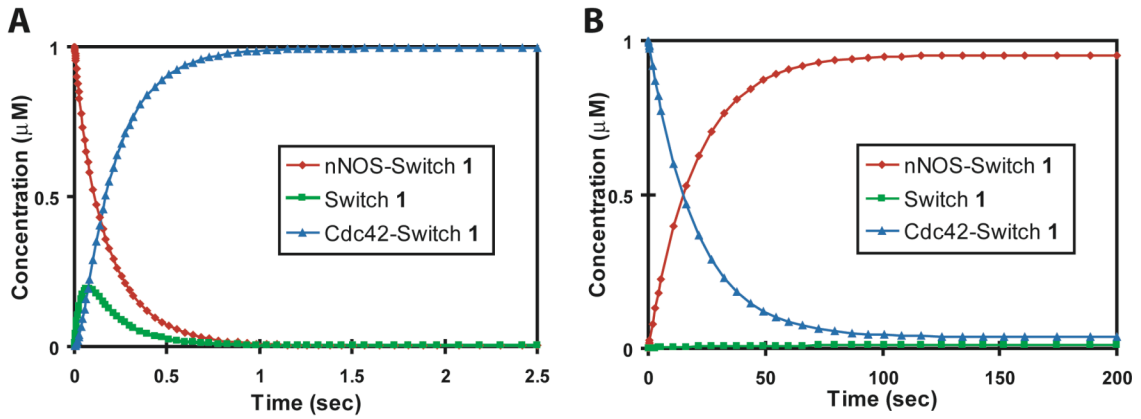

Figure S2. Simulation of competition between switch **1** bound states over time: unbound (green), nNOS-bound (red) and Cdc42-bound (blue). (A) Starting with 1 μM switch 100% bound to nNOS, addition of 100 μM Cdc42 drives switch **1** to 99% Cdc42-bound in just over 1 second. (B) Starting with 1 μM switch 100% bound to Cdc42, addition of 100 μM nNOS drives switch **1** to 90% nNOS-bound in 58 seconds.

To test this modeling against experimental data, we modified our GST pulldown assay to test switch competition over a brief time course (Figure S3). In the first step, the switch was bound to GST-tagged bait ligand and washed as described in the experimental section. Then, solutions of 100 μM of the competing ligand (Cdc42 G12V or 14-3-3ζ) were prepared and sufficient resin was added to bring the concentration of bait protein (with bound switch) to 1 μM. These were incubated for periods of 15, 30, 60 and 120 seconds with regular vortexing before the resin was quickly spun down and washed with PBS and 0.1% Triton X-100, then with PBS alone. As a control, the same incubations were carried out with buffer in place of the competing ligand. For switches **1**-**3**, the switch-bait interaction was mostly disrupted before the 120 second time point (Figure S3). Switch **1** in particular was almost completely unbound from nNOS within 15 seconds of Cdc42 addition (Figure S3A), in agreement with the above simulations. In each case the interaction was unaffected by incubation with buffer. As an additional control, we show that non-phosphorylated switch **3** bound to Crk SH3 was unperturbed by addition of 14-3-3ζ (Figure S3C).

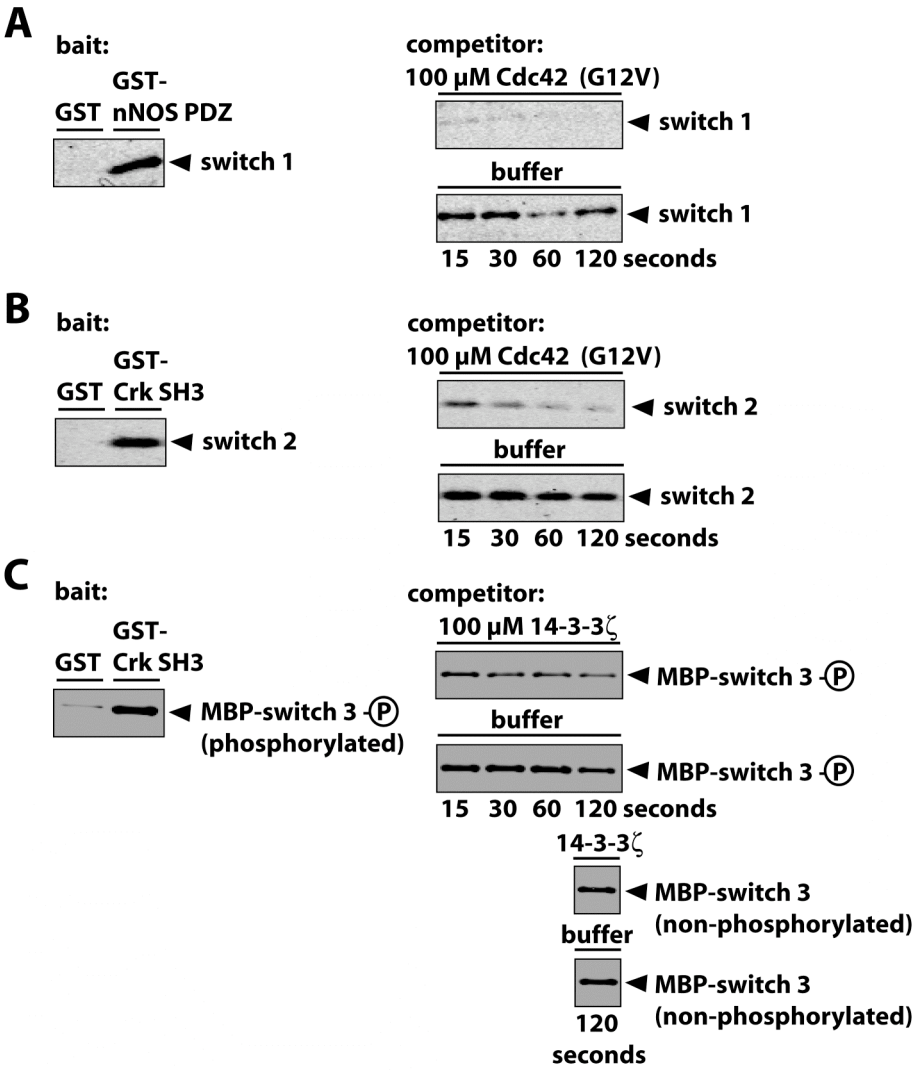

Figure S3. Competition pulldown time courses. (A-C) Switches **1**-**3** were first bound to GST-tagged bait protein (left side). Resin with bound switch was then added to solution containing 100 μM of competing ligand and incubated for the times shown. As a control, resin with bound switch was added to a solution of buffer alone. In panel C, switch **3** was tagged with maltose-binding protein (MBP) and 14-3-3ζ was shown to only compete with Crk SH3 binding when switch **3** was phosphorylated.

Supplemental references:

(S1) Herrmann, C.; Martin, G. A.; Wittinghofer, A. *J Biol Chem* **1995**, 270, 2901-2905.
(S2) Nassar, N.; Horn, G.; Herrmann, C.; Scherer, A.; McCormick, F.; Wittinghofer, A. *Nature* **1995**, 375, 554-563.
(S3) Abdul-Manan, N.; Aghazadeh, B.; Liu, G. A.; Majumdar, A.; Ouerfelli, O.; Siminovitch, K. A.; Rosen, M. K. *Nature* **1999**, 399, 379-383.
(S4) Kim, A. S.; Kakalis, L. T.; Abdul-Manan, N.; Liu, G. A.; Rosen, M. K. *Nature* **2000**, 404, 151-158.
(S5) Bierer, B. E.; Mattila, P. S.; Standaert, R. F.; Herzenberg, L. A.; Burakoff, S. J.; Crabtree, G.; Schreiber, S. L. *Proc Natl Acad Sci USA* **1990**, 87, 9231-9235.
(S6) Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *J Mol Biol* **1993**, 229, 105-124.
(S7) Appleman, J. R.; Howell, E. E.; Kraut, J.; Kuhl, M.; Blakley, R. L. *J Biol Chem* **1988**, 263, 9187-9198.
(S8) Sawaya, M. R.; Kraut, J. *Biochemistry* **1997**, 36, 586-603.
(S9) Yaffe M. B.; Rittinger K.; Volinia S.; Caron P. R.; Aitken A.; Leffers H.; Gamblin S. J.; Smerdon S. J.; Cantley L. C. *Cell* **1997**, 91, 961-971.
(S10) Rittinger, K.; Budman, J.; Xu, J.; Volinia, S.; Cantley, L. C.; Smerdon, S. J.; Gamblin, S. J.; Yaffe, M. B. *Mol Cell* **1999**, 4, 153-166.
(S11) Schultz J.; Hoffmuller U.; Krause G.; Ashurst J.; Macias M. J.; Schmieder P.; Schneider-Mergener J.; Oschkinat H. *Nat Struct Biol* **1998**, 5, 19-24.
(S12) Harris, B. Z.; Hillier, B. J.; Lim, W. A. *Biochemistry* **2001**, 40**,** 5921-5930.
(S13) Hillier, B. J.; Christopherson, K. S.; Prehoda, K. E.; Bredt, D. S.; Lim, W. A. *Science* **1999**, 284, 812-815.
(S14) Hall, R. A.; Ostedgaard, L. S.; Premont, R. T.; Blitzer, J. T.; Rahman, N.; Welsh, M. J.; Lefkowitz, R. J. *Proc Natl Acad Sci USA* **1998**, 95, 8496-8501.
(S15) Karthikeyan, S.; Leung, T.; Ladias, J. A. A. *J Biol Chem* **2002**, 277, 18973-18978.
(S16) Ekiel, I.; Banville, D.; Shen, S. H.; Gehring, K. *Biochem Cell Biol* **1998**, 76, 334-340.
(S17) Koslov, G.; Gehring, K.; Ekiel, I. *Biochemistry* **2000**, 39, 2572-2580.
(S18) Nguyen J. T.; Porter M.; Amoui M.; Miller W. T.; Zuckermann R. N.; Lim W. A. *Chem Biol* **2000**, 7, 463-473.
(S19) Wu, X.; Knudsen, B.; Feller, S. M.; Zheng, J.; Sali, A.; Cowburn, D.; Hanafusa, H.; Kuriyan, J. *Structure* **1995**, 3, 215-226.
(S20) Wittekind, M.; Mapelli, C.; Lee, V.; Goldfarb, V.; Friedrichs, M. S.; Meyers, C. A.; Mueller, L. *J Mol Biol* **1997**, 267, 933-952.
(S21) Horita, J. A.; Baldisseri, D. M.; Zhang, W.; Altieri, A. S.; Smithgall, T. E.; Gmeiner, W. H.; Byrd, R. A. *J Mol Biol* **1998**, 278, 253-265.
(S22) Zarrinpar, A.; Park, S. H.; Lim, W. A. *Nature* **2003**, 426, 676-680.
(S23) Fazi, B., Cope, M. J. T. V.; Douangamath, A.; Ferracuti, S.; Schirwitz, K.; Zucconi, A.; Drubin, D. G.; Wilmanns, M.; Cesareni, G.; Castagnoli, L. *J Biol Chem* **2002**, 277, 5290-5298.
(S24) Gianni, S.; Engstrom, A.; Larsson, M.; Calosci, N.; Malatesta, F.; Eklund, L.; Ngang, C. C.; Travaglini-Allocatelli, C.; Jemth, P. *J Biol Chem* **2005**, 280, 34805-34812.
(S25) Rudolph, M. G.; Bayer, P.; Abo, A.; Kuhlmann, J.; Vetter, I. R.; Wittighofer, A. *J Biol Chem* **1998**, 273, 18067-18076.
(S26) Ullah, M.; Schmidt, H.; Cho, K.-H.; Wolkenhauer, O. *IEE Proc-Syst Biol* **2006**, 153, 53-60.

Complete ref 17b:

Posern, G.; Zheng, J.; Knudsen, B. S.; Kardinal, C.; Müller, K. B.; Voss, J.; Shishido, T.; Cowburn, D.; Cheng, G.; Wang, B.; Kruh, G. D.; Burrell, S. K.; Jacobson, C. A.; Lenz, D. M.; Zamborelli, T. J.; Adermann, K.; Hanafusa, H.; Feller, S. M. *Oncogene* **1998**, *16*, 1903-1912.