A simple fluorescent assay for the discovery of protein-protein interaction inhibitors

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SUPPORTING INFORMATION

Figure S1. Details of EGFP and EGFP-RAD52 expression plasmids and proteins

EGFP (MW = 29.1 kDa)

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MGSSHHHHHH SSGLVPRGSH MVSKGEELFT GVVPILVELD GDVNGHKFSV 50
SGEGEGDATY GKLTLKFICT TGKLPVPWPT LVTTLTYGVQ CFSRYPDHMK 100
QHDFFKSAMP EGYVQERTIF FKDDGNYKTR AEVKFEGDTL VNRIELKGID 150
FKEDGNILGH KLEYNYNSHN VYIMADKQKN GIKVNFKIRH NIEDGSVQLA 200
DHYQQNTPIG DGPVLLPDNH YLSTQSALSK DPNEKRDHMV LLEFVTAAGI 250
TLGMDELYK  259
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Note, the F64L\S65T EGFP mutations are highlighted in green and the thrombin cleavage site is underlined.

EGFP-RAD52 (MW = 76.2 kDa)

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MGSSHHHHHH SSGLVPRGSH MVSKGEELFT GVVPILVELD GDVNGHKFSV 50
SGEGEGDATY GKLTLKFICT TGKLPVPWPT LVTTLTYGVQ CFSRYPDHMK 100
QHDFFKSAMP EGYVQERTIF FKDDGNYKTR AEVKFEGDTL VNRIELKGID 150
FKEDGNILGH KLEYNYNSHN VYIMADKQKN GIKVNFKIRH NIEDGSVQLA 200
DHYQQNTPIG DGPVLLPDNH YLSTQSALSK DPNEKRDHMV LLEFVTAAGI 250
TLGMDELYKG GSGGSGGSGG SGGMSGTEEA ILGGRDSHPA AGGGSVLCFG 300
QCQYTAEEYQ AIQKALRQRL GPEYISSRMA GGGQKVCYIE GHRVINLANE 350
MFGYNGWAHS ITQQNVDFVD LNNGKFYVGV CAFVRVQLKD GSYHEDVGYG 400
VSEGLKSKAL SLEKARKEAV TDGLKRALRS FGNALGNCIL DKDYLRSLNK 450
LPRQLPLEVD LTKAKRQDLE PSVEEARYNS CRPNMALGHP QLQQVTSPSR 500
PSHAVIPADQ DCSSRSLSSS AVESEATHQR KLRQKQLQQQ FRERMEKQQV 550
RVSTPSAEKS EAAPPAPPVT HSTPVTVSEP LLEKDFLAGV TQELIKTLED 600
NSEKWAVTPD AGDGVVKPSS RADPAQTSDT LALNNQMVTQ NRTPHSVCHQ 650
KPQAKSGSWD LQTYSADQRT TGNWESHRKS QDMKKRKYDP S  691
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Figure S1. Details of EGFP and EGFP-RAD52 expression plasmids and proteins. All expression plasmids were made by Genscript®. Codons were optimized for expression in *Escherichia coli*. Enhanced green fluorescent protein (EGFP) was cloned into the pET28a plasmid using the NdeI site so that a thrombin cleavable, 6X His tag was placed at the N-terminus. The NT-EGFP-RAD52 was cloned into pET28a in a similar manner. For the later construct a disordered soluble linker was inserted between EGFP and RAD52. Note, the amino acid sequences with a dotted underline are disordered in the EGFP crystal structure (PDB ID 2Y0G). The soluble disordered linker has a double underline and the human RAD52 sequence is highlighted in yellow.