Table SI

Oligonucleotides used for preparing expression constructs and sequencing.

Name	Sequence
Cloning NLSs into pEGFPN1 (Clontech) using the HindIII and PstI restriction sites.	
Cdc5lNLS1 HPF	AGCTTGCCACCATGAAGAAGGCCAAGAGGAAAGCAAGAGAGAAACTGCA
Cdc5lNLS1 HPR	GTTTCTCTCTTGCTTTCCTCTTGGCCTTCTTCATGGTGGCA
Cdc5lNLS2 HPF	AGCTTGCCACCATGAAAAGAAGAGAACTTCGACTGCA
Cdc5lNLS2 HPR	GTCGAAGTTCTCTTCTTTTCATGGTGGCA
Cdc5lNLS3 HPF	AGCTTGCCACCATGAAGAAAAGAAAAAGGAAGAGACTGCA
Cdc5lNLS3 HPR	GTCTCTTCCTTTTTCTTTTCTTCATGGTGGCA
Cdc5lNLS4 HPF	AGCTTGCCACCATGAGAAAAAAAGACAAACAGCATTTGAAAAGGAAAAAACTGCA
Cdc5lNLS4 HPR	GTTTTTTCCTTTTCAAATGCTGTTTGTCTTTTTTTCTCATGGTGGCA
SV40NLSHPF	AGCTTGCCACCATGCCTAAAAAGAAGCGTAAAGTCCTGCA
SV40NLSHPR	GGACTTTACGCTTCTTTTTAGGCATGGTGGCA
NucNLSHPF	AGCTTGCCACCATGAAGCGTCCGGCAGCCACTAAGAAGGCAGGTCAAGCGAAAAAGAAAAAGCTGCA
NucNLSHPR	GCTTTTTCTTTTTCGCTTGACCTGCCTTCTTAGTGGCTGCCGGACGCTTCATGGTGGCA

Name	Sequence
Cloning CDC5L and truncated mutants with N-terminal HA tags in pEGFPC3 (Clontech), with removal of the eGFP cDNA from the vector. The NheI and SacII restriction sites were used.	
NForNHeI	TTAGCTAGCGCCACCATGTACCCATACGACGTCCCAGACTACGCCATGCCTCGAATTATGATCAAG
CRevSacII	TAACCGCGGAACTGTACTTCAGAATTTTG
hdelFor	TTGGCTAATACTCAGGGAGAATCTGATTTACCATCAGCTA
idelFor	GAAAAACTCTTGCACTTGCAGATTTCAGATGCAGAACTC
jdelFor	CTCACCAATGTGGACACCGAGCTGGAAGAACGTGAAATA
kdelFor	ACTATTCTAAGACCCTTATTGGTGCAGGAGATGGAAGTG
ldelFor	GACAGAATTGAATCACTTAATGACTTATGGGACCA
arevSacII	TTTCCGCGGTCACTCGGCATTTTCTGGTAGAAC
brevSacII	TTTCCGCGGTCAAGATTCTTTTTTCCTTTTCAA
cRevSacII	TTTCCGCGGTCAAGCTTTATCCAGAAGAAATTCAT
hdelRev	TGATGGTAAATCAGATTCTCCCTGAGTATTAGCCAAGCG
idelRev	TTCTGCATCTGAAATCTGGTGCAAGAGTTTTTCCT
jdelRev	TTCACGTTCTTCCAGCTCGGTGTCCACATTGGTGAGGGCCAT

Cloning AID and C-terminally altered mutants in pEGFPN1 using the HindIII and PstI

restriction sites.

HindAIDF	AGAAGAAAGCTTGCCACCATGGACAGCCTCTTGATGAAC
PstAIDR	AGAAAGCTGCAGAAGTCCCAAAGTACGAAATGCG
PstAIDF193R	AGAAAGCTGCAGAAGTCCCAAAGTACG
PstAID188xR	AGAAGACTGCAGGTCATCAACCTCATACAGGGG

Cloning AID/ A3G NTD into pOPTG (kind gift of Dr. O. Perisic, Cambridge) using the NdeI and BamHI restriction sites.

AIDNdeF	CAAACATATGATGGACAGCCTCTTGATGAACCGG
AIDBamHR	CTTTGGATCCTCAAAGTCCCAAAGTACGAAATGC
A3GNdeF	CAAACATATGATGAAGCCTCACTTCAGAAAC
A3GBamHR	CTTTGGATCCCTACGAGTGTCTGAGAATCTCCCCC

Name	Sequence
Cloning NLSs in pGEX4T1 (GE Healthcare) using the BamHI and XhoI restriction sites.	
cdc5LNLS1BXF	GATCCATGAAGAAGGCCAAGAGGAAAGCAAGAGAGAAAC
cdc5LNLS1BXR	TCGAGTTTCTCTCTTGCTTTCCTCTTGGCCTTCTTCATG
cdc5LNLS2BXF	GATCCATGAAAAGAAGAGAACTTCGAC
cdc5LNLS2BXR	TCGAGTCGAAGTTCTCTTCTTTTCATG
cdc5LNLS3BXF	GATCCATGAAGAAAAGAAAAAGGAAGAGAc
cdc5LNLS3BXR	TCGAGTCTCTTCCTTTTTCTTTTCTTCATG
cdc5LNLS4BXF	GATCCATGAGAAAAAAAGACAAACAGCATTTGAAAAGGAAAAAAC
cdc5LNLS1BXR	TCGAGTTTTTTCCTTTTCAAATGCTGTTTGTCTTTTTTTCTCATG
SV40NLSBXF	GATCCATGCCTAAAAAGAAGCGTAAAGTCc
SV40NLSBXR	TCGAGGACTTTACGCTTCTTTTTAGGCATG
NucNLSBXF	GATCCATGAAGCGTCCGGCAGCCACTAAGAAGGCAGGTCAAGCGAAAAAGAAAAAGC
NucNLSBXR	TCGAGCTTTTTCTTTTTCGCTTGACCTGCCTTCTTAGTGGCTGCCGGACGCTTCATG
PRP19NLSBXF	GATCCATGCGCAAGAAGAGAGGGAAGC
PRP19NLSBXR	TCGAGCTTCCCTCTCTTCTTGCGCATG
PLRG1NBXF	GATCCATGCACAAACGAAAAC
PLRG1NBXR	TCGAGTTTTCGTTTGTGCATG
PLRG1CBXF	GATCCATGCCAGAAATTATCAAGAGAAAGAGAC
PLRG1BXR	TCGAGTCTCTTTCTCTTGATAATTTCTGGCATG
PRPF31BXF	GATCCATGCGGAAGAAGCGAGGCGGCCGCAGGTACCGCAAGATGAAGGAGC

	GGC
PRPF31BXR	TCGAGCCGCTCCTTCATCTTGCGGTACCTGCGGCCGCCTCGCTTCTTCCGCATG

Cloning β-galactosidase in pEGFPN1 to generate pBGalGFPN1 using the BamHI restriction site.

BGalBamHIF	AGAAGAGGATCCAGCCACCATGACCATGATTACGGAT
BGalBamHIR	AGAAGAGATCCCCTTTTTGACACCAGAC

Name	Sequence
Quikchange site-directed mutagenesis of pBGalFPN1/AID$_{[F193A]}$.	
K10A	CAGCCTCTTGATGAACCGGAGGGCGTTTCTTTACCAATTCAAAAATG
V18SR19V	CGGAGGAAGTTTCTTTACCAATTCAAAAATAGCGTCTGGGCTAAGGGTCGG
W20K	CAATTCAAAAATGTCCGCAAGGCTAAGGGTCGGCGTGA
G23S	CCGCTGGGCTAAGAGTCGGCGTGAGAC
R50G	CACTGGACTTTGGTTATCTTGGCAATAAGAACGGC
R112D	GAGGATCTTCACCGCGGACCTCTACTTCTGTGAG

Cloning CTNNBL1 and N-terminally truncated mutants in pOPTH using NdeI and BamHI restriction sites.

NAPNdeF	TATATCATATGATGGACGTGGGCGAACTTCTGAG
NAP77NdeF	GAGGATCTTCACCGCGGACCTCTACTTCTGTGAG
NAPBamR	TAATAGGATCCCTAGAAGTTCTCCAGC

PCR amplification of nuclear transport factor ORFs from a human spleen cDNA library (Invitrogen) and subcloning into pcdna3.1/3xHA (Ref. 1) and pGEX4T1 using the BamHI restriction site.

Kpna1F	GCATAGGGATCCGCCACCATGACCACCCCAGGAAAAGA
Kpna1R	ACAATGGGATCCTCAAAGCTGGAAACCTT
Kpna2F	AGAAGAGGATCCGCCACCATGTCCACCAACGAGAATGCT
Kpna2R	AGAAGAGGATCCCTAAAAGTTAAAGGTCCCAG
Kpnb1F	CCGAAGGGATCCGCCACCATGGAGCTGATCACCATTCT
Kpnb1R	CCGAAGGGATCCTCAAGCTTGGTTCTTCAGTTT
RanF	AGAAGAGGATCCGCCACCATGGCTGCGCAGGGAGA
RanR	AGAAAGGGATCCTCACAGGTCATCATCCTCAT
CRM1F	AGAAGAGGATCCGCCACCATGCCAGCAATTATGACAA
CRM1R	GTACCGGGATCCTTAATCACACATTTCTTCTG

Cloning CTNNBL1 and truncated mutants into pEGFPN1 using the HindIII and SacII restriction sites.

NAPHindF	TTTTAAGCTTGCCACCATGGACGTGGGCGAACTT
DeltaNLSHindF	TTTTAAGCTTGCCACCATGCAAACTGGTACTCGAGAACGC
NLSHindF	TTTTAAGCTTGCCACCATGAAACGTCCCCGGGATGATG
NAPSBR	TTTTGGATCCCCGCGGGAAGTTCTCCAGCAAGCCCAG

Name	Sequence

Cloning karyopherin αs into pOPTH (kind gift of Dr. O. Perisic, Cambridge) using the NdeI and BamHI restriction sites.

hKapa1F	CGCATAGACCACCCCAGGAAAAGAGAACTTTCG
hKapa1R	CGGGATCCTCAAAGCTGGAAACCTTCCATAGG
hKapa2F	CGCATATGTCCACCAACGAGAATGCTAATAC
hKapa2R	CGGGATCCCTAAAAGTTAAAGGTCCCAGGAG

Cloning CTNNBL1 N-terminal domain fragments into pOPTG using the NdeI and BamHI restriction sites.

26-33F	CGCATATGCAGAAGATGCGTCGGAACAAACTTAGGGATCCCG
26-33R	CGGGATCCCTAAGTTTGTTTCCGACGCATCTTCTGCATATGCG
13-33/44F	CGCATATGAGGGGCACAAAACGTCCCCGG
13-44R	CGGGATCCCTATTCTTCCCGATAGCGGCCG
13-33R	CGGGATCCCTAAGTTTGTTTCCGACGCATC
13-33/44R13A	CGCATATGGCGGGCACAAAACGTCCCCGGGATG
13-33/44K16A	CGCATATGAGGGGCACAGCGCGTCCCCGGGATG
13-33/44K16AR17A	CGCATATGAGGGGCACAAAAGCGCCCCGGGATG
13-33/44 R13AK16AR17A	CGCATATGGCGGGCACAGCGGCGCCCGCGGATG
1-77F	CGCATATGGACGTGGGCGAACTTCTGAGTACCAG
1-77R	CGGGATCCCTATGGCTCCTCCTCTTCCTCTTTCCTC

Sequencing CTNNBL1.

NAPF1	ATGGACGTGGGCGAACTTCTGAGC
NAPF2	CTTCTACAGTGGCTGTTGAAGAGG
NAPF3	GGGCAGCAGCGGACCCGGCTTC
NAPR1	CTAGAAGTTCTCCAGCAAGCCCAGG
NAPR2	GGCATGGTCCAGCACTTTCAGGGC
NAPR3	GGCATGGTCCAGCACTTTCAGGGC
pOPTHORFR	GGCAGCAGCCAACTCAGCTTCC

Sequencing pOPTG/pOPTH constructs.

T7F	TAATACGACTCACTATAGGG
T7R	CTAGTTATTGCTCAGCGG

Sequencing pGEX4T1 constructs.

pGEX5'	GGGCTGGCAAGCCACGTTTGGTG
pGEX3'	CCGGGAGCTGCATGTGTCAGAGG

Cloning AID into pMXs-ires-GFP

AIDF	GCGGATCCGCCACCATGGACAGCCTC
AID-HAR	GCGAATTCTCAAGCGTAATCTGGAACATCGTATGGGTATCCTCCT CCAAGTCCCAAAG
SV40R	GCGAATTCTCAGACTTTACGCTTCTTTTTAGGAGCGTAATCTGG
CDC5LNLS3R	GCGAATTCTCATCTCTTCCTTTTTCTTTTCTTAGCGTAATCTGG

Reference

1. Mund T, Pelham HR (2009) Control of the activity of WW-HECT domain E3 ubiquitin ligases by NDFIP proteins. *EMBO Rep* 10: 501-507.

Table SII

Buffers

Lysis Buffer	0.1% Triton X-100 20 mM Tris.HCl pH 8.0 125 mM NaCl 3 mM $MgCl_2$ 10% glycerol Complete protease inhibitor cocktail (Roche) 150 U ml^{-1} benzonase (Novagen) 400 μg ml^{-1} lysolecithin (Sigma)
Buffer A	20 mM Hepes pH 7.3 110 mM potassium acetate 1 mM EGTA 2 mM magnesium acetate 10% glycerol 0.2% Tween-20 0.2% BSA 5 mM 2-mercaptoethanol
Buffer B	50 mM Tris.HCl pH 7.5 200 mM NaCl 5mM 2-mercaptoethanol
Buffer C	20mM Tris.HCl pH 7.4 100 mM NaCl 0.1% Triton X-100 5 mM dithiothreitol
Buffer D (pH 8.0)	50 mM sodium phosphate 150 mM NaCl 20 mM imidazole 5 mM 2-mercaptoethanol