UNG2 Fluorescence Polarization Binding Assay

Ac-UNG2(1-25)-FITC	Ac-MIGQKTLYSFFSPSPARKRHAPSPE{Lys(FITC)}
UNG2(1-25)-FITC	MIGQKTLYSFFSPSPARKRHAPSPE{Lys(FITC)}
Ac-UNG2(1-13)-FITC	Ac-MIGQKTLYSFFSP{Lys(FITC)}
UNG2(1-13)-FITC	MIGQKTLYSFFSP{Lys(FITC)}

UNG2 Truncation Fluorescence Polarization Competition assay

Ac-UNG2(1-13)-WT-NH2	Ac-MIGQKTLYSFFSP-NH2
Ac-UNG2(1-13)-I2D-NH2	Ac-MDGQKTLYSFFSP-NH2
Ac-UNG2(1-10)-NH2	Ac-MIGQKTLYSF-NH2
Ac-UNG2(1-7)-NH2	Ac-MIGQKTL-NH2
Ac-UNG2(1-4)-NH2	Ac-MIGQ-NH2 *This is also the crystallographic construct
Ac-UNG2(1-3)-NH2	Ac-MIG-NH2
Ac-UNG2(1-2)-NH2	Ac-MI-NH2
Ac-UNG2(1)-NH2	Ac-M-NH2

Consensus Degron Screen

Ac-MIGQKTLYSFFSP{Lys(FITC)}
Ac-AIGQKTLYSFFSP{Lys(FITC)}
Ac-GIGQKTLYSFFSP{Lys(FITC)}
Ac-IIGQKTLYSFFSP{Lys(FITC)}
Ac-LIGQKTLYSFFSP{Lys(FITC)}
Ac-PIGQKTLYSFFSP{Lys(FITC)}
Ac-VIGQKTLYSFFSP{Lys(FITC)}
Ac-NIGQKTLYSFFSP{Lys(FITC)}
Ac-CIGQKTLYSFFSP{Lys(FITC)}
Ac-QIGQKTLYSFFSP{Lys(FITC)}
Ac-SIGQKTLYSFFSP{Lys(FITC)}
Ac-TIGQKTLYSFFSP{Lys(FITC)}
Ac-YIGQKTLYSFFSP{Lys(FITC)}
Ac-RIGQKTLYSFFSP{Lys(FITC)}
Ac-DIGQKTLYSFFSP{Lys(FITC)}
Ac-EIGQKTLYSFFSP{Lys(FITC)}
Ac-HIGQKTLYSFFSP{Lys(FITC)}
Ac-KIGQKTLYSFFSP{Lys(FITC)}
Ac-FIGQKTLYSFFSP{Lys(FITC)}
Ac-WIGQKTLYSFFSP{Lys(FITC)}
Ac-MAGQKTLYSFFSP{Lys(FITC)}
Ac-MGGQKTLYSFFSP{Lys(FITC)}
Ac-MLGQKTLYSFFSP{Lys(FITC)}
Ac-MMGQKTLYSFFSP{Lys(FITC)}
Ac-MPGQKTLYSFFSP{Lys(FITC)}
Ac-MVGQKTLYSFFSP{Lys(FITC)}

Ac-MNGQKTLYSFFSP{Lys(FITC)}
Ac-MCGQKTLYSFFSP{Lys(FITC)}
Ac-MQGQKTLYSFFSP{Lys(FITC)}
Ac-MSGQKTLYSFFSP{Lys(FITC)}
Ac-MTGQKTLYSFFSP{Lys(FITC)}
Ac-MYGQKTLYSFFSP{Lys(FITC)}
Ac-MRGQKTLYSFFSP{Lys(FITC)}
Ac-MDGQKTLYSFFSP{Lys(FITC)}
Ac-MEGQKTLYSFFSP{Lys(FITC)}
Ac-MHGQKTLYSFFSP{Lys(FITC)}
Ac-MKGQKTLYSFFSP{Lys(FITC)}
Ac-MFGQKTLYSFFSP{Lys(FITC)}
Ac-MWGQKTLYSFFSP{Lys(FITC)}
Ac-MIAQKTLYSFFSP{Lys(FITC)}
Ac-MIIQKTLYSFFSP{Lys(FITC)}
Ac-MILQKTLYSFFSP{Lys(FITC)}
Ac-MIMQKTLYSFFSP{Lys(FITC)}
Ac-MIPQKTLYSFFSP{Lys(FITC)}
Ac-MIVQKTLYSFFSP{Lys(FITC)}
Ac-MINQKTLYSFFSP{Lys(FITC)}
Ac-MICQKTLYSFFSP{Lys(FITC)}
Ac-MIQQKTLYSFFSP{Lys(FITC)}
Ac-MISQKTLYSFFSP{Lys(FITC)}
Ac-MITQKTLYSFFSP{Lys(FITC)}
Ac-MIYQKTLYSFFSP{Lys(FITC)}
Ac-MIRQKTLYSFFSP{Lys(FITC)}
Ac-MIDQKTLYSFFSP{Lys(FITC)}
Ac-MIEQKTLYSFFSP{Lys(FITC)}
Ac-MIHQKTLYSFFSP{Lys(FITC)}
Ac-MIKQKTLYSFFSP{Lys(FITC)}
Ac-MIFQKTLYSFFSP{Lys(FITC)}
Ac-MIWQKTLYSFFSP{Lys(FITC)}

Stability reporter plasmids	
PCS2-UNG(WT)-GFP-IRES-mCherry	
PCS2-UNG-MDD-GFP-IRES-mCherry	
PCS2-UNG-MID-GFP-IRES-mCherry	
PCS2-UNG-MLG-GFP-IRES-mCherry	
PCS2-UNG-MAG-GFP-IRES-mCherry	
PCS2-UNG-MAIG-GFP-IRES-mCherry	
PCS2-UNG-MCIG-GFP-IRES-mCherry	
PCS2-UNG-MDG-GFP-IRES-mCherry	
PCS2-UNG-MFG-GFP-IRES-mCherry	
PCS2-UNG-MTG-GFP-IRES-mCherry	
PCS2-UNG-MSG-GFP-IRES-mCherry	
PCS2-UNG-MWG-GFP-IRES-mCherry	
PCS2-UNG-MGG-GFP-IRES-mCherry	
PCS2-UNG-MCG-GFP-IRES-mCherry	
PCS2-QKI-GFP-IRES-mCherry	
PCS2-HPF1-GFP-IRES-mCherry	
PCS2-NUP62-GFP-IRES-mCherry	
PCS2-TMEM50B-GFP-IRES-mCherry	
PCS2-VPS37B-GFP-IRES-mCherry	
FAM72A mammalian expression vectors	
3xHA-FAM72A-WT	
3xHA-FAM72A-F8R	
3xHA-FAM72A-A32H	
3xHA-FAM72A-Y83A	
3xHA-FAM72A-Y83F	
3xHA-FAM72A-V85F	
3xHA-FAM72A-V85R	
3xHA-FAM72A-N97R	
3xHA-FAM72A-N98R	
UNG expression for co-IPs	
PCS2-UNG-WT-3xFLAG	
PCS2-UNG-MLG-3xFLAG	
PCS2-UNG-MAIG-3xFLAG	
PCS2-UNG-MCIG-3xFLAG	
PCS2-UNG-MDG-3xFLAG	
PCS2-UNG-MTG-3xFLAG	
PCS2-UNG-MSG-3xFLAG	
PCS2-UNG-MGG-3xFLAG	
PCS2-UNG-MCG-3xFLAG	
Fluorescence Polarization Assay Plasmids	
pLIB-6xHis-hFAM72A	
pET24a-6xHis-SUMO-zFAM72A-WT	*This is also the crystallographic construct
pET24a-6xHis-SUMO-zFAM72A-F6R	

pET24a-6xHis-SUMO-zFAM72A-A30H
pET24a-6xHis-SUMO-zFAM72A-Y81A
pET24a-6xHis-SUMO-zFAM72A-Y81F
pET24a-6xHis-SUMO-zFAM72A-V83F
pET24a-6xHis-SUMO-zFAM72A-V83R
pET24a-6xHis-SUMO-zFAM72A-N95R
pET24a-6xHis-SUMO-zFAM72A-N96R
Sf9 Pulldown Plasmids
pLIB-Strep-hFAM72A
pLIB-UNG2_1-25_WT_-TEV-GST-His
pLIB-UNG2_1-25_M0M1D_-TEV-GST-His
pLIB-UNG2_1-25_I2D_-TEV-GST-His
pLIB-UNG2_1-25_G3D_-TEV-GST-His
pLIB-UNG2_1-25_Q4G_-TEV-GST-His
pLIB-UNG2_1-25_K5D_-TEV-GST-His
pLIB-UNG2_1-25_T6R_-TEV-GST-His
pLIB-UNG2_1-25_L7D_-TEV-GST-His
pLIB-UNG2_1-25_Y8G_-TEV-GST-His
pLIB-UNG2_1-25_S9R_-TEV-GST-His
pLIB-UNG2_1-25_F10D_-TEV-GST-His
pLIB-UNG2_1-25_F11D_-TEV-GST-His
pLIB-UNG2_1-25_S12R_-TEV-GST-His
pLIB-UNG2_1-25_P13D_-TEV-GST-His

Retroviral expression vectors	**Source**
pMX-PIE (**P**uromycin-**I**RES-**E**GFP)	Gift (B.R. San-Martin)
pMX-CIG (m**C**herry-**I**RES-**G**418)	This study
pCL-Amphotropic	Gift (B.R. San-Martin)

CRISPR vectors	**Source**
px459 v2.0	Addgene #62988

Recombinant expression vectors	**Source**
pMX-CIG N' HA-(G4S)1-FAM72A	This study
pMX-CIG N' HA-(G4S)3-FAM72A	This study
pMX-PIE mUNG2 WT	Gift (Y. Feng)
pMX-PIE mUNG2 I2V, G3D	This study

Cloning and mutagenesis	Primer sequence (5' → 3')	Purpose
pMX-PIE-F-seq	TAGACGGCATCGCAGCTTG	Sequence verification of cDNA insert in pMX-PIE
pMX-PIE-R-seq	GCTTCGGCCAGTAACGTTAG	Sequence verification of cDNA insert in pMX-PIE
pMX-PIE-FAM72A-HA-G4S-3-F	GGTTCTGGCGGTGGCGGTTCTGGCGGTGGCGGTTCTTCTACCAACAACTGTACTT	Cloning of N' HA-(G4S)3 onto mFAM72A
pMX-PIE-FAM72A-HA-G4S-3-R	GCCACCGCCAGCGTAGTCTGGGACGTCGTATGGGTACATGGTGGCGGATCC	Cloning of N' HA-(G4S)3 onto mFAM72A
pMX-PIE-FAM72A-HA-G4S-1-F	TACGCTGGCGGTGGCGGTTCTTCTACCAACAACTGTACTTTC	Cloning of N' HA-(G4S)1 onto mFAM72A
pMX-PIE-FAM72A-HA-G4S-1-R	GTCTGGGACGTCGTATGGGTACATGGTGGCGGATCC	Cloning of N' HA-(G4S)1 onto mFAM72A
pMX-PIE-UNG2-I2V F	CGCCACCATGGTGGGCCAGAAGA	Site-directed mutagenesis of mUNG2 to generate degron mutants
pMX-PIE-UNG2-I2V R	AATTCGGATCCGGCAGTC	Site-directed mutagenesis of mUNG2 to generate degron mutants
pMX-PIE-UNG2-G3D F	CACCATGATCGACCAGAAGACCC	Site-directed mutagenesis of mUNG2 to generate degron mutants
pMX-PIE-UNG2-G3D R	GCGAATTCGGATCCG	Site-directed mutagenesis of mUNG2 to generate degron mutants
sgRNA for CRISPR/Cas9 mutagenesis (target exon #-guide #)	**Primer sequence (5' → 3')**	**Purpose**
hFAM72A (e1-1) F	CACCGAAACAAGTGCTCAGCTCTAG	Cloning into px459 (NGG PAM)
hFAM72A (e1-1) R	AAACCTAGAGCTGAGCACTTGTTT	Cloning into px459 (NGG PAM)
hFAM72A (e2-1) F	CACCGAATCTGCAAATGTAAACTGA	Cloning into px459 (NGG PAM)
hFAM72A (e2-1) R	AAACTCAGTTTACATTTGCAGATT	Cloning into px459 (NGG PAM)
hMAEA (e2-1) F	CACCGAGCTGGAGAAGACGTTGAG	Cloning into px459 (NGG PAM)
hMAEA (e2-1) R	AAACCTCAACGTCTTCTCCAGCT	Cloning into px459 (NGG PAM)
hMAEA (e2-2) F	CACCGACCAGCCACGTCACCATGG	Cloning into px459 (NGG PAM)
hMAEA (e2-2) R	AAACCCATGGTGACGTGGCTGGT	Cloning into px459 (NGG PAM)
Genotyping primers for amplifying sgRNA-targeted regions	**Primer sequence (5' → 3')**	**Purpose**
hFAM72A exon 1 F	TAGGTCCCGCAATTTGAATTTTAGC	464bp amplicon
hFAM72A exon 1 R	AGCATTACATCAGGACTGACCA	-
hFAM72A exon 2 F	TTCTACCCTTCTTTGTAGGGG	936bp amplicon
hFAM72A exon 2 R	CCCCCTAACCTACATTGCCTT	-
hMAEA exon 2 F	GTTCAGCAGGGCTTTTGTGG	580bp amplicon
hMAEA exon 2 R	CTGGACACTCCTGGAGCAAG	-