Supplementary Data 7

Primers used in this study.

Primer name	Sequence (5'-3')	Usage
Cell line knockout verification		
NAA30-KO gDNA F	CAACGGATTGATTAGCCCCGAA	HAP1 *NAA30*-KO, quality control, PCR, sequencing
NAA30-KO gDNA R	GACATATCGTATCGTCCGATCCTC	HAP1 *NAA30*-KO, quality control, PCR
NAA35-KO gDNA F	CGACAATGATTCCCATAGACACTTG	HAP1 *NAA35*-KO, quality control, PCR, sequencing
NAA35-KO gDNA R	ATCTTACCTCTTCAAAAACAGCAGC	HAP1 *NAA35*-KO, quality control, PCR
NAA38-KO gDNA F	GCATCCCAGCTACACACAGA	HAP1 *NAA38*-KO, quality control, PCR, sequencing
NAA38-KO gDNA R	GTACGCTTCAGTGAGCCACA	HAP1 *NAA38*-KO, quality control, PCR, sequencing
CRISPR/Cas9-mediated *NAA30* gene disruption in human MDA-MB-231 cell line		
NAA30 sgRNA F	CACC**g**AGCGGCGGCGAGCGATCCCG	Underlined: flanks matching the BsbI restriction site. Bold: inserted nucleotide to increase RNA Pol III initiation. Capital: align with *NAA30* or non-targeting sequence
NAA30 sgRNA R	AAACCGGGATCGCTCGCCGCCGCT**c**	
Non-targeting sgRNA F	CACC**g**TATTACTGATATTGGTGGG	
Non-targeting sgRNA F	AAACCCCACCAATATCAGTAATA**c**	
hU6 sequencing F	CGATACAAGGCTGTTAGAGAG	Human U6 forward sequencing primer.
Illumina sequencing		
PCR1 F	GAGGGCCTATTTCCCATGATTC	CRISPR library prep, PCR1
PCR1 R	GTTGCGAAAAAGAACGTTCACGG	CRISPR library prep, PCR1
PCR2 pLCV2 U6 amplicon F	AATGATACGGCGACCACCGAGATCTAC ACnnnnnnnnACACTCTTTCCCTACACGAC GCTCTTCCGATCTTTGTGGAAAGGACGA AACACCG	CRISPR library prep, PCR2 pLVC2: lentiCRISPR v2 nnnnnnnn: denotes i5 or i7 index underlined: denotes annealing sequence
PCR2 pLCV2 U6 amplicon R	CAAGCAGAAGACGGCATACGAGATnnnn nnnnGTGACTGGAGTTCAGACGTGTGCTC TTCCGATCTACTTGCTATTTCTAGCTCTA AAAC	
PCR2 pLCV2 tracr amplicon F	AATGATACGGCGACCACCGAGATCTAC ACnnnnnnnnACACTCTTTCCCTACACGAC GCTCTTCCGATCTACTTGCTATTTCTAGC TCTAAAAC	
PCR2 pLCV2 tracr amplicon R	CAAGCAGAAGACGGCATACGAGATnnnn nnnnGTGACTGGAGTTCAGACGTGTGCTC TTCCGATCTTTGTGGAAAGGACGAAAC ACCG	
Plasmid cloning, mutagenesis, and sequencing		
pCMV6-UBE2M I2G F	GATCGCCATGggCAAGCTGTTCTC	UBE2M mutagenesis
pCMV6-UBE2M I2A F	GATCGCCATGgcCAAGCTGTTCTC	UBE2M mutagenesis
pCMV6-UBE2M I2D F	GATCGCCATGgaCAAGCTGTTCTCGCTG	UBE2M mutagenesis
pCMV6-UBE2M I2E F	GATCGCCATGgagAAGCTGTTCTC	UBE2M mutagenesis
pCMV6-UBE2M I2L F	GATCGCCATGcTCAAGCTGTTCTCGCTG	UBE2M mutagenesis
pCMV6-UBE2M I2F F	GATCGCCATGtTCAAGCTGTTCTCGCTG	UBE2M mutagenesis
pCMV6-UBE2M I2Y F	GATCGCCATGtaCAAGCTGTTCTC	UBE2M mutagenesis
pCMV6-UBE2M I2P F	GATCGCCATGccCAAGCTGTTCTCGCTG	UBE2M mutagenesis
pCMV6-UBE2M Mut R	GCGGCGGCAGATCTCCTC	UBE2M mutagenesis

pCMV6-UBE2M del F	ACGCGTACGCGGCCGCTC	UBE2M deletion mutagenesis
pCMV6-UBE2M del R	GGCGATCGCGGCGGCAGATC	UBE2M deletion mutagenesis
NAA30 E321A F	GGTTGTTTTGGcAACCGAAATAAC	NAA30 E321A mutagenesis
NAA30 E321A R	TCATCACAGTCTCCCTCAAC	NAA30 E321A mutagenesis
T7 promoter F	TAATACGACTCACTATAGGG	Plasmid sequencing
CMV promoter F	GAGGTCTATATAAGCAGAGC	Plasmid sequencing
BGH R	TAGAAGGCACAGTCGAGG	Plasmid sequencing
M13 R	CAGGAAACAGCTATGAC	Plasmid sequencing
ADH1 promoter F	CCTCGTCATTGTTCTCGTTCC	Plasmid sequencing (yeast)
***Drosophila* knockout confirmation**		
CG11412/Naa30A F	CAAGGAAAGTGGAGGAAGTGC	Confirming *Naa30A* deletion
CG11412/Naa30A R	GGTATGTATCCCTCGCCAATG	Confirming *Naa30A* deletion

Peptides used in this study.

Peptide name	Sequence	Derived from	UniProt KB	Supplier	Usage
MIKLFSL (UBE2M)	MIKLFSL RWGRPVGRRRRPVRVYP	UBE2M	P61081	Innovagen AB	Acetylation assay
MYTLLSG (ARFRP1)	MYTLLSG RWGRPVGRRRRPVRVYP	ARFRP1	Q13795		
MLPSTSV (UNC50)	MLPSTSV RWGRPVGRRRRPVRVYP	UNC50	Q53HI1		
DDDIAAL (β-actin)	DDDIAAL RWGRPVGRRRRPVRVYP	β-actin	P60709	Biogenes	
Ac-MI-UBE2M-biotin	Ac-MIKLFSLKQQKK-biotin	UBE2M	P61081	Innovagen AB	Peptide pulldown
MI-UBE2M-biotin	MIKLFSLKQQKK-biotin	UBE2M	P61081		
ML-UBE2M-biotin	MLKLFSLKQQKK-biotin	UBE2M	P61081		
MY-UBE2M-biotin	MYKLFSLKQQKK-biotin	UBE2M	P61081		
MF-UBE2M-biotin	MFKLFSLKQQKK-biotin	UBE2M	P61081		
A-UBE2M-biotin	AIKLFSLKQQKK-biotin	UBE2M	P61081		
ME-UBE2M-biotin	MEKLFSLKQQKK-biotin	UBE2M	P61081		
R-UBE2M-biotin	RIKLFSLKQQKK-biotin	UBE2M	P61081		
F-UBE2M-biotin	FIKLFSLKQQKK-biotin	UBE2M	P61081		
R-nsP4-biotin	RIFSTIEGRTYK-biotin	Sindbis virus nsP4	-		
F-nsP4-biotin	FIFSTIEGRTYK-biotin	Sindbis virus nsP4	-		
G-nsP4-biotin	GIFSTIEGRTYK-biotin	Sindbis virus nsP4	-		