Supplementary Information

Figure S1. Generation of FASTKD1 and FASTKD4 knockout cells.

A) left panel: Schematic of the PCR and restriction digest screen showing expected results for amplification of clones with: wild type sequences (WT), heterozygous deletions (-/+), homozygous deletions (-/-), and uncut sequences (-BstNI). Panel 2nd from the Right: example results obtained for an anti-FASTKD4 CRISPR/Cas9 construct treated clone (D4 CRISPR) compared to an untreated clone (WT). Right panel: example results obtained from bulk cells treated with an anti-FASTKD1 CRISPR/Cas9 construct (D1 CRISPR) or bulk wild type cells (WT). Lower panels: example screening of clones for candidate FASTKD1 or FASTKD4 knockout cell lines. Lanes corresponding to candidate cell lines are marked with an asterisk. The high cutting rate in the D1 screen is because it is a re-screen of candidates from a previous screen.

B) Confirmation of FASTKD4 knockouts by western blot with anti-FASTKD4 antibody. Left panel: Western blot of a HEK knockout cell line (HEK D4KO), compared to untreated HEK cells (HEK WT) with a marker lane in between. Top: anti-FASTKD4 signal. Bottom: anti-TOM20 signal on the same membrane as a loading control, with weak FASTKD4 signal still present. Right panel: a western blot as in the left panel using 143B cells. Multiple knockout cell lines (143B D4-KO), compared to untreated 143B cells (143B-WT).

C) Example sequencing results of a FASTKD4 KO cell. In this specific case the cell screened as heterozygous for a deletion in the FASTKD4 that was amplified. An allele with a 1 base pair insertion was detected. Genomic DNA was amplified by PCR, and then cloned into a vector and transformed into bacteria. Individual colonies were selected and the vector insert was sequenced to determine the sequence change. 8 colonies were selected for each clone to ensure every allele was sequenced. As expected, the mutations were always detected 3 base pairs upstream of the PAM motif where CRISPR/Cas9 is known to cut. The restriction site used in the PCR and restriction digest screen is indicated; note that the screen is leaky and only detects deletion mutations and not insertions.

Figure S2. A) Chimeric FASTK proteins do not rescue FASTK loss. Northern blot with ^{32}P labelled antisense ND6 and 7SL RNA probes. Equal amounts of total RNA from the indicated cells were loaded for analysis.
B) Immunofluorescence of the D0-D4^{RAP} chimeras. Top right panel: anti-FASTKD2 in green, Lower right panel anti-HA in red. HA tagged D0-D4^{RAP} still localizes to RNA granules.
C) Northern blot analysis with anti-ND5 or anti-CYB probes labeling ND5/CYB and precursor ND5-CYB. Anti-7SL probes were used for loading controls. Stability was assessed by transcriptional blockage with EtBr for the indicated times.

Supplementary Table 1. Plasmids, Primers, and antibodies.

Riboprobes

Name	Forward primer	Species
12S	ACTCAAAGGACCTGGCGGTGC	Human
16S	CCGTGAAGAGGCGGGCATAACAC	Human
MTND1	CTACGCCCTGATCGGCGCAC	Human
MTND2	CACCCCTCTGACATCCGGCCT	Human
MTND3	CCACCCCTTACGAGTGCGGC	Human
MTND4/4L	AAGCCCCCATCGCTGGGTCA	Human
MTND5	ACGCCCGAGCAGATGCCAAC	Human
MTND6 full-length	CGGTAATACGACTCACTATAGGGAGAcaccctaacaggtcaacctcCGGTAATACGACTCACTATAGGGAGAcaccctaacaggtcaacctc	Human
MTND6 CDS	CGG TAA TAC GAC TCA CTA TAG GGA GAC GAA TCA ACC CTG ACC CCT C	Human
MTND6 UTR	CGGTAATACGACTCACTATAGGGAGA GCTACCTCCCTGACAAGCGCCCGGTAATACG ACTCACTATAGGGAGA GCTACCTCCCTGACAAGCGCC	Human
MTCYB	CTCACTCCTTGGCGCCTGCC	Human
MTCO1	TGGAGGCCGGAGCAGGAACA	Human
MTCO2	CGCCCTCCCATCCCTACGCA	Human
MTCO3	GGCCCCCAACAGGCATCACC	Human
MTATP8/6	TGGCCCACCATAATTACCCCCA	Human
tRNA(Phe)	GTTTATGTAGCTTACCTCC	Human
tRNA(Glu)	GTT CTT GTA GTT GAA ATA C	Human
tRNA(LeuUUA/G)	GTTAAGATGGCAGAGCCCGG	Human
tRNA(Pro)	CGGTAATACGACTCACTATAGGGAGATCAGA GAAAAAGTC	Human
Tubulin	CGCGAAGCAGCAACCATGCG	Human
7SL	gccgggcgcggtggcgcgtg	Human

Riboprobes

Name	Reverse primer	Species
12S	CGGTAATACGACTCACTATAGGGAGA GGTGACGGGCGGTGTGTACG	Human
16S	CGGTAATACGACTCACTATAGGGAGAATCCA ACATCGAGGTCGTAAACCCT	Human
MTND1	CGGTAATACGACTCACTATAGGGAGA GGTCGTAGCGGAATCGGGGG	Human
MTND2	CGGTAATACGACTCACTATAGGGAGA GGCCTCCTAGGGAGAGGAGGGT	Human
MTND3	CGGTAATACGACTCACTATAGGGAGA AGGCCAGACTTAGGGCTAGGATGA	Human
MTND4/4L	CGGTAATACGACTCACTATAGGGAGA GGATGTAAGCCCGTGGGCGA	Human
MTND5	CGGTAATACGACTCACTATAGGGAGA GGCGCAGACTGCTGCGAACA	Human
MTND6 full-length	ATG ATG TAT GCT TTG TTT CTG	Human
MTND6 CDS	GAT TGT TAG CGG TGT GGT CG	Human
MTND6 UTR	AGGGGCAGGTTTTGGCTCGT	Human
MTCYB	CGGTAATACGACTCACTATAGGGAGAGCCTC ACGGGAGGACATAGCC	Human
MTCO1	CGGTAATACGACTCACTATAGGGAGACGGCG GGGTCGAAGAAGGTG	Human
MTCO2	CGGTAATACGACTCACTATAGGGAGA TACCCCCGGTCGTGTAGCGG	Human
MTCO3	CGGTAATACGACTCACTATAGGGAGA ATGCCAGTATCAGGCGGCGG	Human
MTATP8/6	CGGTAATACGACTCACTATAGGGAGA GGGGGCAATGAATGAAGCGAACAG	Human
tRNA(Phe)	CGGTAATACGACTCACTATAGGGAGATGT TTA TGG GGT GAT GTG AG	Human
tRNA(Glu)	CGGTAATACGACTCACTATAGGGAGATAT TCT CGC ACG GAC TAC	Human
tRNA(LeuUUA/G)	CGGTAATACGACTCACTATAGGGAGATGT TAA GAA GAG GAA TTG AAC	Human
tRNA(Pro)	CAG AGA ATA GTT TAA ATT AG	Human
Tubulin	CGGTAATACGACTCACTATAGGGAGATGGTC GGCCAGCTTGCGAAT	Human
7SL	CGGTAATACGACTCACTATAGGGAGA AGA GAC GGG GTC TCG CTA TG	Human

shRNAs

Name	Reference	Source
pLKO.1 shFASTK	Jourdain et. al. 2015 (9)	Sigma

Antibodies

Name	Reference	Source
FASTKD2	17464-1AP	Proteintech
FASTKD4	sc-373752	Santa Cruz Biotechnology
FLAG (monoclonal)	F3165	Sigma
FLAG (polyclonal)	F7425	Sigma
HA (monoclonal)	CO-MMS-101P-500	Covence
HA (polyclonal)	Ab9110	Abcam
Tom20	FL-145	Santa Cruz Biotechnology

Plasmids and primers

Name	Forward primer/Plasmid Description	cDNA source/Use
GFP	pWPT with GFP insert	P. Salmon
FASTK isoform 1	ATC GAT CGA CGC GTA TGA GGA GGC CGC GGG GGG AAC CCG G	Biocat
FASTK isoform 4	ATC GAT CGA CGC GTA TGA GGA GGC CGC GGG GGG AAC CCG G	Biocat
mitoFASTK	ATCGATCG ACGCGT atg ct**g** ag**g** gtc ctg ctc tc	Biocat
mitoFASTK-dRAP	ATCGATCG ACGCGT atg ct**g** ag**g** gtc ctg ctc tc	Biocat/For chimeras
FASTKD2	ATC GAT CGA CGC GTA TGT TGA CAA CTT TGA AGC C	Biocat/MRG Colocalization studies
FASTKD1-YFP	FASTKD1-YFP Fusion	Simarro et al., 2010
FASTKD4-YFP	FASTKD4-YFP Fusion	Simarro et al., 2010
px330 CRISPR /CAS9 plasmid	Plasmid for generating KO cell lines	addGene
D1 guide RNA	CAC CGA TTA ATG AAT GAC GAT ACC C	Clone into px330
D4 Guide RNA	CAC CGT TGG CCG ACT GAG ACT TGC C	Clone into px330
FASTKD4 D->A	GCT GAG GTG CTG CTG GCC AGT GAC GGC GAG TTT C	Mutagenesis of FASTKD4
D1ΔRAP	CACAGGTGTCGTGACGCGATGGAGGTCAGC GGTGGTGC	Gibson Assembly, Amplify without RAP domain (for Chimeras)
D4ΔRAP	CACAGGTGTCGTGACGCGATGGAGGTCAGC GGTGGTG	Gibson Assembly, Amplify without RAP domain (for Chimeras)
D1Rap Fw	TTGGAATTTTTGGATTCAAAAGCACTTTG	Amplify RAP domain (for Gibson generation of Chimeras)
D4 Rap Fw	ttcttgcggtgggagttcccc	Amplify RAP domain (for Gibson generation of Chimeras)
FASTKRap	atcgatcgTCTAGAcgctggcatttctgccggga	Amplify RAP domain (XbaI cut site)
KMU193	CCAAACGCGTGCCACCATGAAAAAAACACCT GTTTTCCTAG	Fixing of FASTKD1
KMU201	ATGCTTCTGTAACTAGTGCTTCAACTAGCGGG TCATGGGCCTCACCAGCAAACTGTTGTGTGA CGTAT	Fixing of FASTKD1

Name	Reverse primer	cDNA source/Use
FASTK isoform 1	CGA TCG ATT CTA GAG CCC CCT TCA GGC CCC CAG CGC AGG CCC	Biocat
FASTK isoform 4	CGA TCG ATT CTA GAG CCC CCT TCA GGC CCC CAG CGC AGG CCC	Biocat
mitoFASTK	CGA TCG ATT CTA GAG CCC CCT TCA GGC CCC CAG CGC AGG CCC	Biocat
mitoFASTK-dRAP	cga tcg att cta gac acc ctc tgg gca ggg tct	Biocat/For chimeras
FASTKD2	CGA TCG ATT CTA GAT TGT GTG CTT TGC ACA TTT AC	Biocat/MRG Colocalization studies
D1 guide RNA	AAA CGG GTA TCG TCA TTC ATT AAT C	Clone into px330
D4 Guide RNA	AAA CGG CAA GTC TCA GTC GGC CAA C	Clone into px330
FASTKD4 D->A	GAA ACT CGC CGT CAC TGG CCA GCA GCA CCT CAG C	Mutagenesis of FASTKD4
FASTKΔRAP	cgatcgatTCTAGAttcccgcaacaccagcacca	Amplify without RAP domain, XbaI cut site (for generation of Chimeras)
D1ΔRAP	ggggaactcccaccgcaagaaAGCAATCCTTTCAGCC CCTGGT	Amplify without RAP domain (for Gibson generation of Chimeras)
D4ΔRAP	CAA AGT GCT TTT GAA TCC AAA AAT TCC AAC GCT AGC CTC TTA GAC CCT GG	Amplify without RAP domain (for Gibson generation of Chimeras)
D1 Rap Rv	CAGGTCGACACGCGTGGACAAACATGACTTG ACTTCTCCAAATATACATTC	Amplify RAP domain (for generation of Chimeras)
D4 Rap Rv	CAAAGTGCTTTTGAATCCAAAAATTCCAACGC TAGCCTCTTAGACCCTGG	Amplify RAP domain (for Gibson generation of
KMU194	ATGCTTCTGTAACTAGTGCTTCAACTAGCGGG TCATGGGCCTCACCAGCAAACTGTTGTGTGA CGTAT	Fixing of FASTKD1
KMU202	GAAGCACTAGTTACAGAAGCATGGAGAAGGC TAGAAAGGTTTGATATTAAACTGCTCTCAGA	Fixing of FASTKD1