

Appendix material for: FKBP8 recruits LC3A to mediate Parkin-independent mitophagy

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Appendix Table S1. Plasmids and oligonucleotides used in this study

Gateway cloning vectors

<u>Plasmid</u>	<u>Description</u>	<u>Source</u>
pDest15	bacterial GST fusion expression vector, T7 promoter	Invitrogen
pDEST-Myc	mammalian Myc-tag fusion expression vector, CMV and T7 promoters	[1]
pDest-3xFlag	mammalian triple flag fusion expression vector, CMV	[2]
pDest-Cerulean-C1	mammalian Cerulean fusion expression vector, CMV	this study
pDest-EGFP-C1	mammalian EGFP fusion expression vector, CMV	[1]
pDest-EYFP-C1	mammalian EYFP fusion expression vector, CMV	this study
pDest-mCherry-C1	mammalian mCherry fusion expression vector, CMV	[3]
pDest-mCherry-EYFP-	mammalian mCherry-EYFP fusion expression vector,	this study
pDest-EGFP-Flp-In-	mammalian Flp-In expression vector, Tet inducible	[4]
pENTR1A, -2B, -3C	gateway entry vectors	Invitrogen

Gateway entry clones (made by traditional subcloning or site-directed mutagenesis)

<u>Plasmid</u>	<u>Description</u>	<u>Source</u>
pENTR-FKBP8	human FKBP8 in entry vector	Imogene's
pENTR-FKBP8 F24A/L26A	point mutant of pENTR-FKBP8	this study
pENTR-FKBP8 N412K	point mutant of pENTR-FKBP8	This study
pENTR-FKBP8 N412K/F24A/L26A	point mutations of pENTR-FKBP8	This study
pENTR-FKBP8 Δ TM	Deletion of Transmembrane domain of	this study
pENTR-FKBP8-Mito	TM domain swapped with mitochondrial targeting sequence in	this study
pENTR-FKBP8-ER	TM domain swapped with ER targeting sequence in pENTR-FKBP8	this study
pENTR-BNIP3	human BNIP3 in entry clone	this study
pENTR-NIX	human NIX in entry clone	[5]
pENTR-OMP25-TM (109-145)	Transmembrane domain of human OMP25 cloned in ENTRY vector	This study
pDONOR221-OMP25-TM (109-145)	Transmembrane domain of human OMP25 cloned in DONOR vector	this study
pENTR-LC3A	human LC3A in entry vector	[3]
pENTR-LC3A F52A	Point mutation of LC3A	this study
pENTR-LC3B	human LC3B in entry vector	[3]
pENTR-LC3B D19A/F52A	Point mutation of LC3B	this study
pENTR-LC3B F52A/L53A	Point mutation of LC3B	this study
pENTR-LC3B F52A/F108A	Point mutation of LC3B	this study
pENTR-LC3B F108A	Point mutation of LC3B	this study
pENTR-LC3C	human LC3C in entry vector	[3]
pENTR-GABARAP	human GABARAP in entry vector	[3]
pENTR-GABARAP-L1	human GABARAP-L1 in entry vector	[3]
pENTR-GABARAP-L2	human GABARAP-L2 in entry vector	[3]

Gateway expression clones (made by Gateway LR reactions)

<u>Plasmid</u>	<u>Description</u>	<u>Source</u>
pDest-Myc-FKBP8	Myc-tag expression of FKBP8	this study
pDest-Myc-FKBP8	Myc-tag expression of FKBP8 LIR mutant	this study
pDest-Cerulean-FKBP8	Cerulean fluorescent expression of FKBP8	this study
pDest-Cerulean-FKBP8 F24A/L27A	Cerulean fluorescent expression of FKBP8 LIR mut	this study
pDest-EGFP-FKBP8	EGFP fluorescent expression of FKBP8	this study
pDest-EGFP-FKBP8- Δ TM	EGFP fluorescent expression of FKBP8	this study
pDest-EGFP-FKBP8-Mito	EGFP fluorescent expression of FKBP8 on	this study
pDest-EGFP-FKBP8-ER	EGFP fluorescent expression of FKBP8 on ER	this study
pDest-mCherry- FKBP8	mCherry fluorescent expression of FKBP8	this study
pDest-mCherry-FKBP8F24A/L27A F24A/L27A	mCherry fluorescent expression of FKBP8 LIR mutant	this study
pDest-mCherry-EYFP-FKBP8	mCherry-EYFP fluorescent expression of	this study
pDest-Cerulean-FKBP8 N412K	Cerulean fluorescent expression of FKBP8 N412K	this study
pDest-Cerulean-FKBP8 N412K/F24A/L26A	Cerulean fluorescent expression of FKBP8 N412K/F24A/L26A	this study
pDest-3xFlag-FKBP8	FLAG-tag expression of FKBP8	this study
pDest-3xFlag-FKBP8	FLAG-tag expression of FKBP8 LIR mutant	this study
pDest-Myc-BNIP3	Myc-tag expression of BNIP3	this study
pDest-Cerulean-BNIP3	Cerulean fluorescent expression of BNIP3	this study
pDest-mCherry-BNIP3	mCherry-fluorescent expression of BNIP3	this study
pDest-Myc-NIX	Myc-tag expression of NIX	this study
pDest-Cerulean-NIX	Cerulean fluorescent expression of NIX	this study
pDest-mCherry-NIX	mCherry-fluorescent expression of NIX	this study
pDest-mCherry-EYFP-OMP25-TM	expression vector for mCherry-EYFP fusion with TM domain of OMP25	this study
pDest-mCherry-OMP25-TM	mCherry-fluorescent expression of OMP25-TM	this study
pDest15-LC3A	GST-LC3A fusion protein expression	[3]
pDest15-LC3B	GST-LC3B fusion protein expression	[3]
pDest15-LC3C	GST-LC3C fusion protein expression	[3]
pDest15-GABARAP	GST-GABARAP fusion protein expression	[3]
pDest15-GABARAP-L1	GST-GABARAP-L1 fusion protein	[3]
pDest15-GABARAP-L2	GST-GABARAP-L2 fusion protein	[3]
pDest-3XFlag-LC3A	FLAG-tag expression of LC3A	this study
pDest-3XFlag-LC3B	FLAG-tag expression of LC3B	this study
pDest-3XFlag-GABARAP-L1	FLAG-tag expression of GABARAP-L1	this study
pDest-EYFP-LC3A	EYFP fluorescent expression of LC3A	this study
pDest-EGFP-Flp-In-LC3A	For stable inducible EGFP fluorescent	this study

Other vectors

<u>Plasmid</u>	<u>Description</u>	<u>Source</u>
pGEX4T-3	bacterial GST fusion expression vector, tac	Amersham
dsRed2-Mito	mammalian expression vector for mitochondrial	Clontech

ssEGFP-KDEL	Mammalian EGFP fusion vector for an ER	[6]
mCherry-EGFP-OMP25-TM	expression vector for mCherry-EGFP fusion with mitochondrial	[7]
pSpCas9(BB)-2A-Puro	gRNA construct for CRISPR/Cas9 system	Addgene
pGEMT-easy	Cloning of PCR products from KO cells	Promega

Selected oligonucleotides used in this study

Oligonucleotide description	5'-3' oligonucleotide sequence
Mitochondria targeting of FKBP8	CTGATCCTGGCCATGCTGGCCATCGGCCTGTTTCAGCCTGGG CGCCTTCATCAAGATCATCCAGCTGAGGAAGAACAAC
ER targeting of FKBP8	ATCACCACCGTGGAGAGCAACAGCAGCTGGTGGACCAACT GGGTGATCCCCGCCATCAGCGCCCTGGTGGTGGCCCTGATG TACAGGCTGTACATGGCCGAGGAC
CRISPR guide RNA-Forward	CACCGGTTGTCCCATGTCCTCCAG
CRISPR guide RNA-Reverse	AAACCTGGAGGACATGGGACAACC
Amplification of genomic DNA exon 2 of FKBP8 for CRISPR KO verification -Forward	GTAAGTGGATGGGGTTGAGGAT
Amplification of genomic DNA exon 2 of FKBP8 for CRISPR KO verification -Reverse	CTTTGCTCCTTACCCAGAATGT

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