

Supplemental Table 1: Primers and plasmids used to generate the expression constructs for these studies

Primer Type	Insert plasmid	Destination plasmid	Modification to Destination plasmid	Final Plasmid	Primer Sequence
Megaprimer	myc-BioID2-MCS (Addgene #74223)	pLKO.1-puro-CMV-TagRFP (Sigma #SHC012)	Replacement of tRFP with myc-BioID2	URCF1 pLKO.1 myc-BioID2	AGATCCGCTAGCGACGCCACCATGGAACAAAACTCATCTCAGAAGAGG ATGGGGCCCCCGTTCTGCAGTCAGCGGTTAAACTTAAGCTTGGTACCGA
Megaprimer	GFP-CytoB5RR (Borgese)	URCF1 pLKO.1 myc-BioID2	Addition of linker between myc and BioID2 SGLRSRAQASNSDLEGGGSGGGSGGGGS	URChb4 pLKO.1 myc-linker-BioID2	ATGGAACAAAACTCATCTCAGAAGAGGATCTCTCCGGACTCAGATCTCGAGCTC CTTCAGCCAGATCAGGTTCTTGAAGTCGGATCCGCCACCTCCAGATC
Megaprimer	GFP-MYO19 ⁸²⁴⁻⁹⁷⁰	URChb4 pLKO.1 myc-linker-BioID2	Insertion of MYO19 ⁸²⁴⁻⁹⁷⁰ between linker and BioID2	URCL5 pLKO.1 myc-linker-MYO19 ⁸²⁴⁻⁹⁷⁰ -BioID2	TGGAACAAAACTCATCTCAGAAGAGGATCTCATGGCCTGCCTTGTCTGCTAAAGA CTTGAGCTCGAGATCTGAGTCCGGACCCAGCCAGTGAAGGC
Deletion	n/a	URO13 MYO19 ⁸⁶⁶⁻⁹⁷⁰ -EGFP	Removal of MYO19 amino acids 866-897	URDS10 MYO19 ⁸⁹⁸⁻⁹⁷⁰ -EGFP	CTCGAGCTCAAGCTTATGAGTAGCTACACTGTCCAG CTGGACAGTGTAGCTACTCATAAGCTTGAGCTCGAG
Quickchange	n/a	URDS10 MYO19 ⁸⁹⁸⁻⁹⁷⁰ -EGFP	Mutation of well-conserved basic residues in the MYO19 Miro-binding sequence	UREM4 MYO19 ⁸⁹⁸⁻⁹⁷⁰ -EGFP K923A RK927-28AA	GCTGCCTCAGGGATCGATAGCGTTTCACTGCGCAGCGTCTCCACTGCGGTATGC GCATACCGCAGTGGAGACGCTGCGCAGTGAACGCTATCGATCCCTGAGGCAGC
Megaprimer	mCherry-Lamina-C-18 (Addgene #55068)	pRK5-myc-Miro2 (Addgene #47891)	Addition of mcherry	URDT12 mchr-Miro2	ATCTCCGAGGAGGACCTGGGATCTATGGTGAAGGCGGAGGAG GATGCGCACGTCCCGCCTCATGCTTCCGCTTCCGCCGG
Megaprimer	mCherry-Lamina-C-18 (Addgene #55068)	pRK5-myc-Miro2 T18N (Addgene #47897)	Addition of mcherry	URDU4 mchr-Miro2 T18N	ATCTCCGAGGAGGACCTGGGATCTATGGTGAAGGCGGAGGAG GATGCGCACGTCCCGCCTCATGCTTCCGCTTCCGCCGG
Megaprimer	mCherry-Lamina-C-18 (Addgene #55068)	pRK5-myc-Miro2 ΔTM(Addgene #47901)	Addition of mcherry	URDV3 mchr-Miro2 ΔTM	ATCTCCGAGGAGGACCTGGGATCTATGGTGAAGGCGGAGGAG GATGCGCACGTCCCGCCTCATGCTTCCGCTTCCGCCGG
Quickchange	n/a	URDT12 mchr-Miro2	Mutation of amino acid A13 to V for GTP-bound state	URDW1 mchr-Miro2 A13V	CTTCCCACCTGGACCTCGCCAGTAA TTACTGGCGGAGGTCCAGGTGGGGAAG
Megaprimer	EGFP-Syne2 (Luxton lab)	URDT12 mchr-Miro2	Swap of mitochondrial insertion sequence with KASH domain to localize to nuclear membrane	UREL7 mchr-Miro2-KASH	CCCTTCTCCTTCTGGCTCCGGATGCCACCTCGACAGCC CAAGTTGGGCCATGGCGGCTATCTAGACTAGGTGGGAGGTGG
Megaprimer	pcDNA3.1-MYO10-HMM-Nanotrap (Addgene #87255)	UREL7 mchr-Miro2-KASH	Swap of Miro2 for an anti-GFP nanobody	URFB5 mchr-Nanotrap ^{green} -KASH	CATCCGGCGGAAGCGGAAGCAGATATCTGATGGCCAGGTTCAACT GGCATCCGGAGCCAGAAGGAAGAACTTCCACCTTTAGAGCTCACCGTCACCTGAGT
Quickchange	n/a	URDT12 mchr-Miro2	Mutations to acidic residues in switch I of Miro2 N-terminal GTPase	URFF9 mchr-Miro2 switch I mut.	GGGAGGGACCGCCGCGGGAAACGCGCGCCACCAG CTGGTGGCGCGGCGTTCCCGCGGCGGTCCCTCCC
Quickchange	n/a	URFF9 mchr-Miro2 switch I mut.	Mutations to acidic residues in switch II of Miro2 N-terminal GTPase	URFJ75 mchr-Miro2 switch I & switch II mut.	GACTACTCAGCAGCCGCGCAGACGGCCGCGGCTGCGGGAG CTCCCGCAGCGCCGCGGCGTCTGCGCGGCTGCTGAGTAGTC
Megaprimer	pGEX6P1-mCherry-Nanobody (Addgene #70696)	pEGFP-C1 (Clontech)	Insertion of an anti-mcherry nanobody to the C-terminus of GFP	URET3 GFP-Nanotrap ^{red}	CTGCAGTCGACGGTACCAGCATGGCACAGGTTTCACTGTTG GTTATCTAGATCCGGTGGATCCCGGTTATGTAACGGGCTGCTAACGGTAAC
Megaprimer	pcDNA3.1-MYO10-HMM-Nanotrap (Addgene #87255)	pGEX6P1-mCherry-Nanobody (Addgene #70696)	Swap of anti-GFP nanobody for an anti-mcherry nanobody (LaM4)	UREK2 MYO10-HMM-Nanotrap ^{red}	GAATCTGCAGATATCTGATGGCCAGATGGCACAGGTTTCACTGTTG CTCTAGACTCGAGCGGCCGCTCATTTATGTAACGGGCTGCTAACGGTAAC