

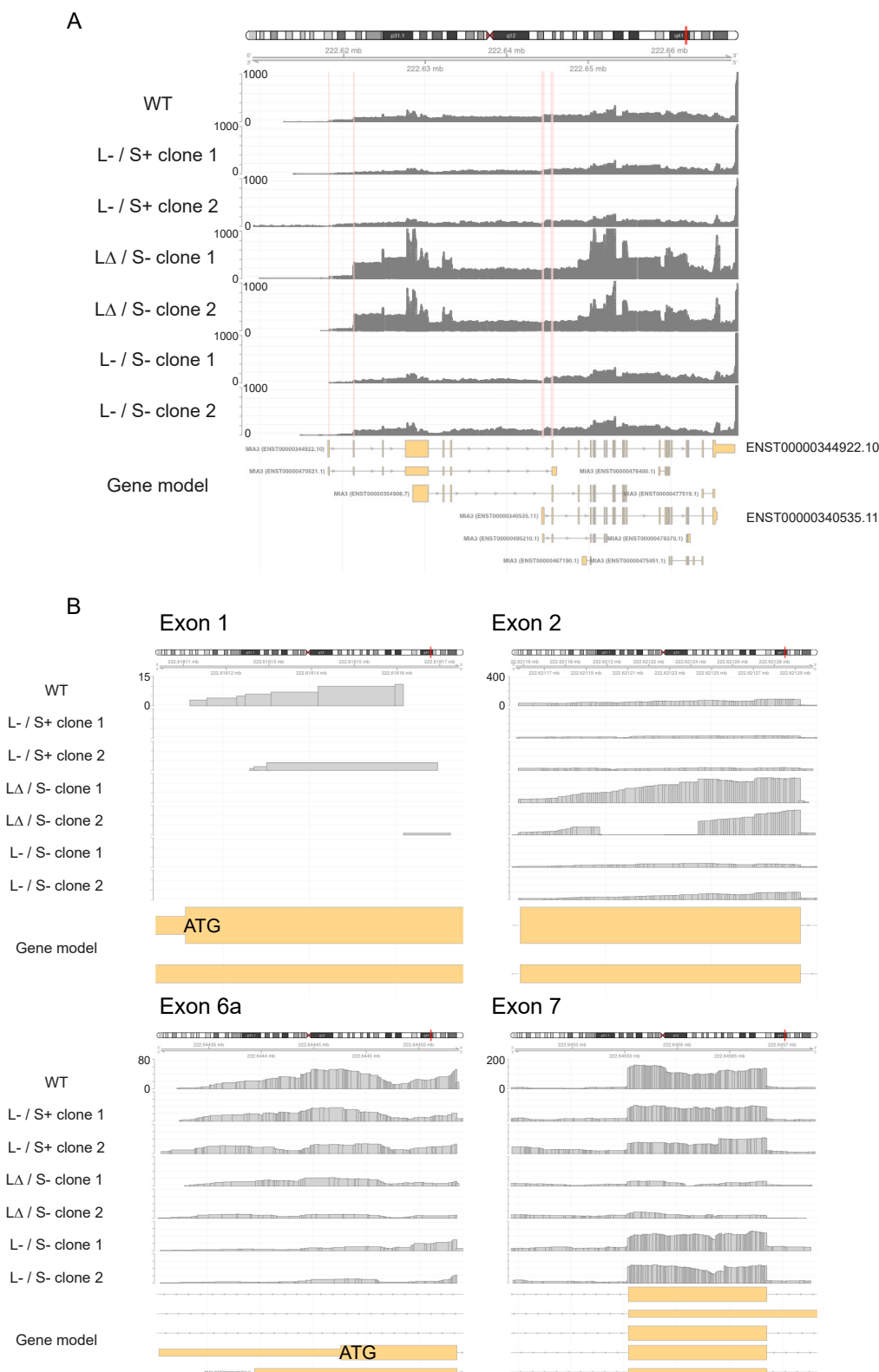


Fig. S1. RNAseq read mapping. A. Gene model for Mia3 gene (showing chr1:222,608,000 - 222,668,400) with read depth mapped for each cell line. B. Regions of particular interest (Exon 1, chr1:222,618,104 - 222,618,176; Exon 2, chr1:222,621,155 - 222,621,301; Exon 6a, chr1:222,644,301 - 222,644,593; Exon 7, chr1:222,645,442 - 222,645,734) are displayed as a chromosome ideogram, a genomic coordinate region track, and a genome annotation track showing putative gene models for MIA3. The gene annotation depicts predicted splice forms; ENST00000344922.10 denotes the major isoform TANGO1L, ENST00000340535.11 depicts TANGO1S. B. Exon 1 is significantly disrupted in all knockout cell lines with few if any reads around the core ATG site for TANGO1L. Exon 2 is also disrupted. Exon 6A shows the ATG start codon for TANGO1S. Exon 7 is the target of other gRNAs used. RNAseq data are derived from 3 independent RNA isolation and library preparations.

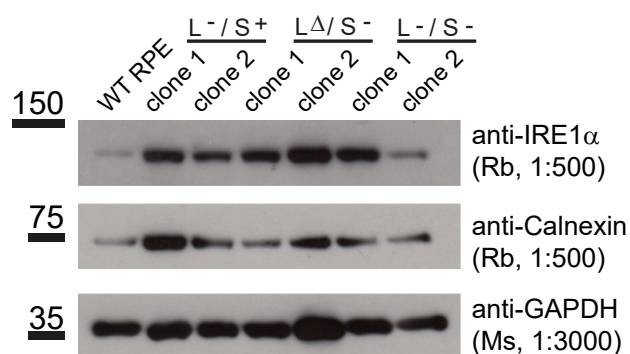


Fig. S2. Immunoblots show expression of IRE1a, calnexin, and GAPDH (as a loading control) in TANGO1 knockout cell lines. Uncropped blots at different exposures are included as Supplemental Data Set 8. Data are representative of n=3.

L-/S+	GO biological process complete	clone 1	clone 2	Average
	regulation of transcription by RNA polymerase II (GO:0006357)	1.5	1.6	1.6
	regulation of RNA metabolic process (GO:0051252)	1.37	1.45	1.4
	regulation of macromolecule biosynthetic process (GO:0010556)	1.37	1.4	1.4
	regulation of transcription, DNA-templated (GO:0006355)	1.36	1.45	1.4
	regulation of nucleic acid-templated transcription (GO:1903506)	1.36	1.45	1.4
	regulation of RNA biosynthetic process (GO:2001141)	1.36	1.45	1.4
	regulation of nucleobase-containing compound metabolic process (GO:0019219)	1.36	1.4	1.4
	regulation of cellular macromolecule biosynthetic process (GO:2000112)	1.36	1.41	1.4
	regulation of biosynthetic process (GO:0009889)	1.35	1.37	1.4
	regulation of cellular biosynthetic process (GO:0031326)	1.34	1.37	1.4
	regulation of gene expression (GO:0010468)	1.25	1.28	1.3
	regulation of nitrogen compound metabolic process (GO:0051171)	1.23	1.26	1.2
	regulation of primary metabolic process (GO:0080090)	1.23	1.26	1.2
	regulation of cellular metabolic process (GO:0031323)	1.22	1.24	1.2
L-/S-	GO biological process complete	clone 1	clone 2	Average
	COPII-coated vesicle cargo loading (GO:0090110)	8.06	10.95	9.5
	vesicle cargo loading (GO:0035459)	5.97	8.11	7.0
	intra-Golgi vesicle-mediated transport (GO:0006891)	5.53	5.63	5.6
	COPII vesicle coating (GO:0048208)	4.86	5.22	5.0
	vesicle targeting, rough ER to cis-Golgi (GO:0048207)	4.86	5.22	5.0
	COPII-coated vesicle budding (GO:0090114)	4.84	5.32	5.1
	vesicle targeting, to, from or within Golgi (GO:0048199)	4.84	5.01	4.9
	vesicle coating (GO:0006901)	4.81	4.91	4.9
	IRE1-mediated unfolded protein response (GO:0036498)	4.4	5.18	4.8
	retrograde vesicle-mediated transport, Golgi to endoplasmic reticulum (GO:0006891)	4.36	4.64	4.5
	vesicle budding from membrane (GO:0006900)	4.07	3.83	4.0
	vesicle targeting (GO:0006903)	4.03	3.81	3.9
	endoplasmic reticulum to Golgi vesicle-mediated transport (GO:0006888)	3.62	4.14	3.9
	Golgi organization (GO:0007030)	3.46	3.25	3.4
	endoplasmic reticulum unfolded protein response (GO:0030968)	3.07	3.76	3.4
L-/S-	GO biological process complete	clone 1	clone 2	
	intra-Golgi vesicle-mediated transport (GO:0006891)	6.52		
	retrograde vesicle-mediated transport, Golgi to endoplasmic reticulum (GO:0006891)	4.89		
	zinc ion transport (GO:0006829)	4.79		
	vesicle targeting, to, from or within Golgi (GO:0048199)	4.22		
	COPII vesicle coating (GO:0048208)	4.05		
	vesicle targeting, rough ER to cis-Golgi (GO:0048207)	4.05		
	vesicle coating (GO:0006901)	4.01		
	IRE1-mediated unfolded protein response (GO:0036498)	3.9		
	COPII-coated vesicle budding (GO:0090114)	3.83		
	vesicle targeting (GO:0006903)	3.65		
	vesicle budding from membrane (GO:0006900)	3.52		
	Golgi organization (GO:0007030)	3.48		
	endoplasmic reticulum to Golgi vesicle-mediated transport (GO:0006888)	3.42	2.66	
	Golgi vesicle transport (GO:0048193)	3.12		
	endoplasmic reticulum unfolded protein response (GO:0030968)	3.07		
	post-Golgi vesicle-mediated transport (GO:0006892)	2.7		
	establishment of vesicle localization (GO:0051650)	2.6		
	cellular response to unfolded protein (GO:0034620)	2.58		
	vesicle localization (GO:0051648)	2.54		
	vesicle organization (GO:0016050)	2.16	2.03	
	glycoprotein biosynthetic process (GO:0009101)	2.06		
	response to endoplasmic reticulum stress (GO:0034976)	2.03		

Fig. S3. Gene ontology analysis of pooled outcomes from MIA3 knockout cell lines. Tables show those terms enriched in each cell line. RNAseq data are derived from 3 independent RNA isolation and library preparations.

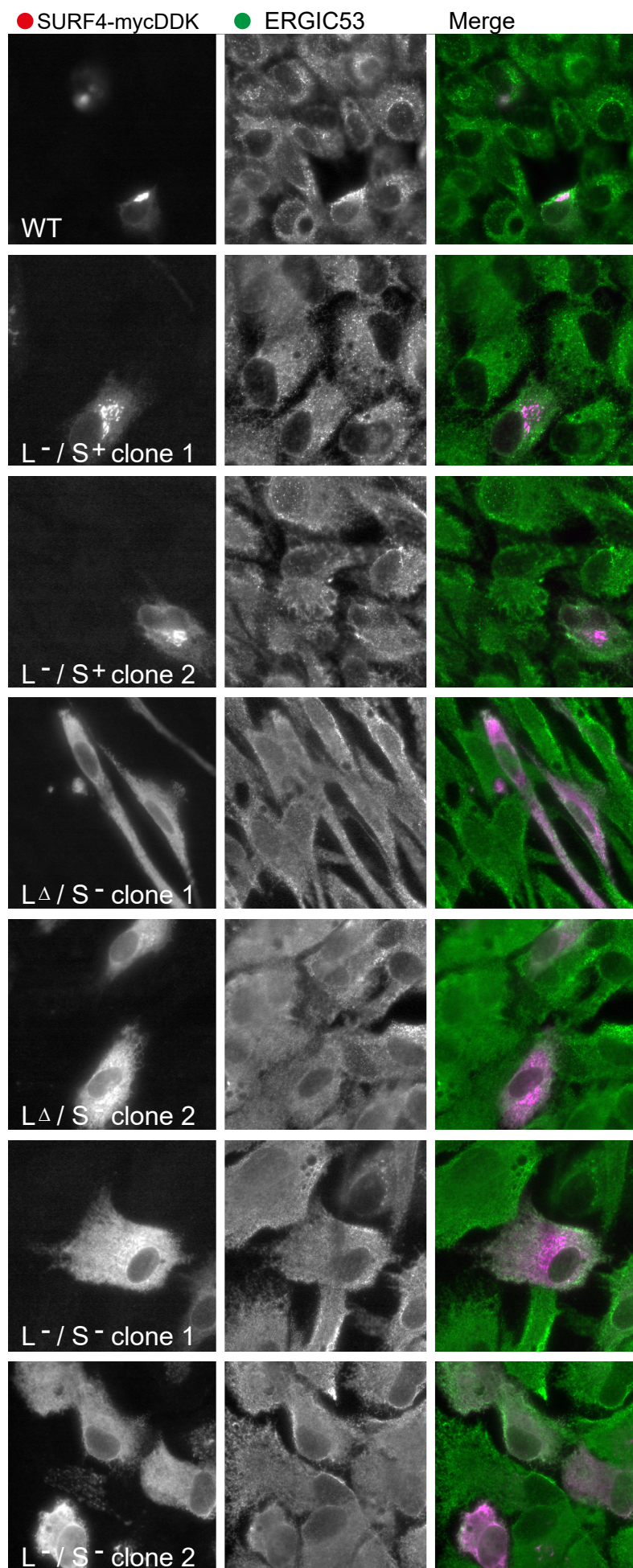


Fig. S4. Localization of SURF4-mycDDK expressed in each cell line. Cells were transfected with SURF4-mycDDK were then fixed and processed for immunofluorescence using to detect transfected cells and endogenous ERGIC53. >20 cells analysed from a single experiment.

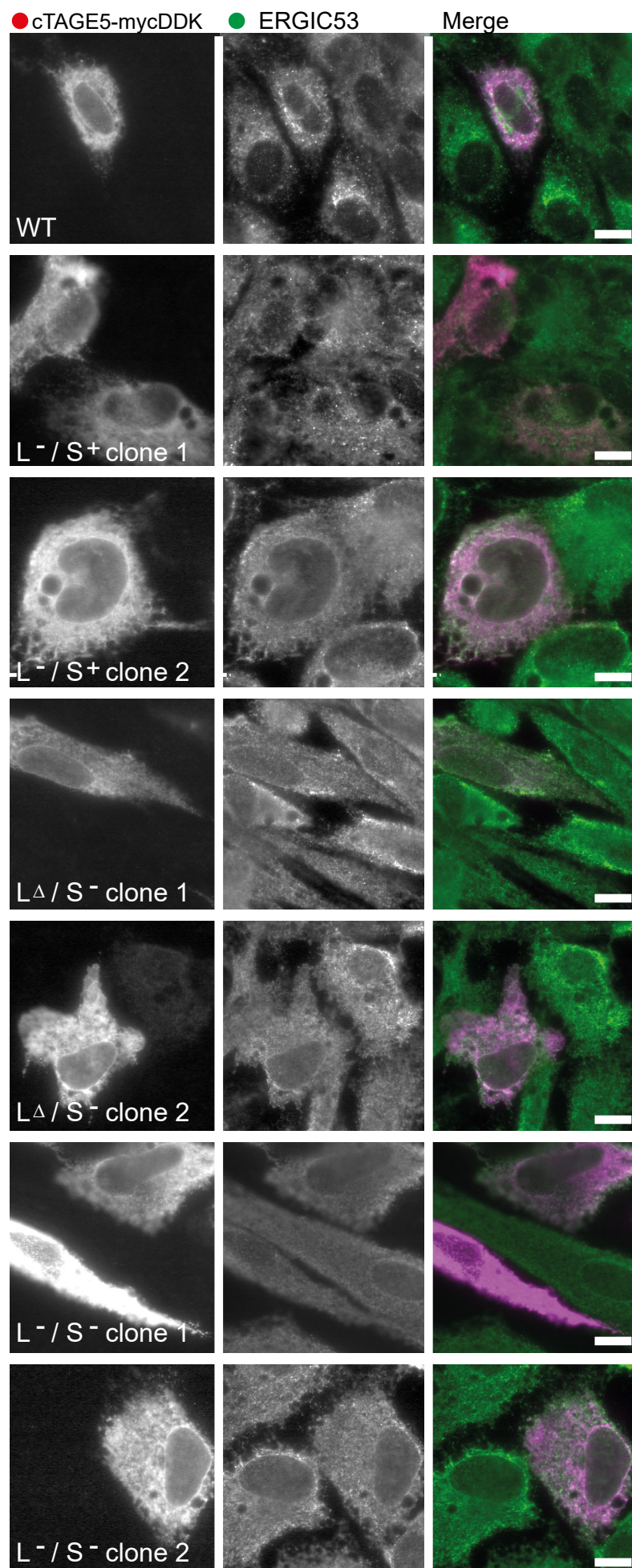


Fig. S5. Expression of cTAGE5-mycDDK cannot restore the typical localization of ERGIC53 to TANGO1 knockout cell lines. Transfected cells were detected using the FLAG epitope tag and co-labelled to detect endogenous ERGIC53. >20 cells analysed from a single experiment.