**Supplementary Figure 1. Prediction of TRIP-Br1 mitochondrial localization.** MitoProt software program, a computational method to predict mitochondrial localization of target protein, was used to predict the possibility of TRIP-Br1 mitochondrial localization using the full-length TRIP-Br1 amino acid sequence. The Mitoprot software is available at ftp://ftp.biologie.ens.fr/pub/molbio. Based on the protein localization prediction program (TargetP 1.1 Server), VDCA1, a well-known mitochondrial protein with the number of amino acid residues similar to TRIP-Br1, was used as a control. VDAC1 has a high probability of export into mitochondria. However, TRIP-Br1 does not carry the typical mitochondrial leader sequence. Therefore, it has a low possibility of mitochondrial translocation.

**Supplementary Figure 2. A,B** MCF7<sup>WT-TRIP-Br1</sup> and MCF7<sup>KD-TRIP-Br1</sup> cells were cultured in a confocal dish for 24 h, followed by staining with Mitotracker dye (100 nM) for 30 min. TRIP-Br1 localization in mitochondria was determined via two-color immunofluorescence microscopy using anti-TRIP-Br1 (yellow) and Mitotracker dyes (red). These cells were visualized under a confocal microscope. Representative fluorescent images are visualized with confocal microscopy (scale bar, 20 μm).

Supplementary Figure 3. Mice strains were genotyped via PCR as described previously [63].

**Supplementary Figure 4.** Potential phosphorylation sites amino acid sequences of TRIP-Br1 protein predicted with NetPhos 2.0 Server. Targeted potential phosphorylation sites were mutated by changing serine to alanine. Mitochondrial TRIP-Br1 levels are shown after STS treatment.

# **Supplementary Figure 1**

TRIP-Br1 Input sequence length : 233 aa

VALUES OF COMPUTED PARAMETERS				
Net charge of query sequence Analysed region Number of basic residues in targeting sequence Number of acidic residues in targeting sequence <b>Cleavage site</b> Cleaved sequence	 -28 10 5 0	:	not	predictable

		HYDROPHOBIC SCALE USED							
		GES KD		GVH1	ECS				
H17	:	1.176	1.353	0.185	0.551				
MesoH	:	-0.031	0.468	-0.183	0.295				
MuHd_075	:	16.929	13.174	4.465	4.026				
MuHd_095	:	16.679	11.844	5.013	4.232				
MuHd_100	:	14.339	11.578	4.121	3.290				
MuHd_105	:	18.576	12.091	5.196	3.839				
Hmax_075	:	-25.083	-7.233	-7.833	-2.053				
Hmax_095	:	-21.000	-4.987	-7.951	-1.015				
Hmax_100	:	-26.800	-7.600	-8.948	-1.800				
Hmax_105	:	-22.400	-4.783	-8.199	-1.237				

Probability of export to mitochondria: 0.0608

# **Supplementary Figure 2**



### **Supplementary Figure 3**

KO-2 Control KO-1 KO-3 Control KO-1 KO-2 KO-3

WT primer

KO primer

### **Supplementary Figure 4**

