Supporting Information

Endoplasmic reticulum stress-activated transcription factor ATF6α requires the disulfide isomerase PDIA5 to modulate chemoresistance

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Table S1. List of siRNAs used for screening and knock down experiments

	Sense	Antisense
AGR2	CUGAUUAGGUUAUGGUUUAUU	UAAACCAUAACCUAAUCAGUU
ATF6	GGCAGGACUACGAAGUGAUGA	AUCACUUCGUAGUCCUGCCCA
EP58	GGUCCUUCAUAGUAAGAUACA	UAUCUUACUAUGAAGGACCCA
ER29	CACUGGGGCAGUUAAGGUUGG	AACCUUAACUGCCCCAGUGUA
ERdj5-(I)	CUUCAACGAACUAGUUACACA	UGUAACUAGUUCGUUGAAGGU
ERdj5-(II)	GCAUAUGAAGUACUCAAAGUU	CUUUGAGUACUUCAUAUGCUC
ERO1Lβ	CAAGCCUCGAUCUGUUUAUCG	AUAAACAGAUCGAGGCUUGAA
Ero1p	CGAGCGCCCAGAUUUUCAACU	UUGAAAAUCUGGGCGCUCGAA
ERp18	CUCCUCGCACUUAAGUAUUUG	AAUACUUAAGUGCGAGGAGUA
ERp44-(I)	GAAGUAGCUCGGCAAUUAAUA	UUAAUUGCCGAGCUACUUCAU
ERp44-(II)	CUCUAUUGAGGGAUCGAGAUG	UCUCGAUCCCUCAAUAGAGUA
PDIA1-(I)	GGACGGUCAUUGAUUACAAUU	UUGUAAUCAAUGACCGUCCUG
PDIA1-(II)	CGCUGGAUGGUUUUAAGAAUU	UUCUUAAAACCAUCCAGCCUG
PDIA2	CGCUUGGUCAACCUUGAAACC	UUUCAAGGUUGACCAAGCGCA
PDIA3-(I)	GGAAUAGUCCCAUUAGCAAUU	UUGCUAAUGGGACUAUUCCUU
PDIA3-(II)	CCCUGAAGAUAUUUAGAGAUU	UCUCUAAAUAUCUUCAGGGUU
PDIA4-(I)	GCGAGUUUGUCACUGCUUUUU	AAAGCAGUGACAAACUCGCGG
PDIA4-(II)	CUAACAACCUGAGAGAAGAUU	UCUUCUCUCAGGUUGUUAGCG
PDIA5-(I)	CCUCGCUCAUUGAGAGAAUCU	AUUCUCUCAAUGAGCGAGGAG
PDIA5-(II)	GGAUGAUGCCGCAUUUCCAUU	UGGAAAUGCGGCAUCAUCCUC
PDIA6	GCGAGUCUCCUGUGGAUUAUG	UAAUCCACAGGAGACUCGCCU
TMX	GAGUGGACGGUUUAUCAU AAA	UAUGAUAAACCGUCCACUCAG

Table S2. List of primers used for q-PCR

	Forward primer (5'-3')	Reverse primer (5'-3')
BiP	GCTTATGGCCTGGATAAGAGG	CCACAACTTCGAAGACACCAT
Chop	ACCAAGGGAGAACCAGGAAACG	TCACCATTCGGTCAATCAGAGC
Ero1Lβ	GGTTTAGGAACTGCCCTGAAG	CAACTATTTCCTGTCGGGTGA
Gadd34	CCTCTACTTCTGCCTTGTCTCCAG	TTTTCCTCCTTCTCCTCGGACG
Grp94	CTATTCCGCCTTCCTTGTAGC	CCTCTTGGGTCAGCAATTACA
Herpud1	TCCTCCTCCTGACGTTGTAAA	TGCTCGCCATCTAGTACATCC
Orp150	GAAGATGCAGAGCCCATTTC	TCTGCTCCAGGACCTCCTAA
Pdia5	ACTGCTCAGAACCCGGAATA	GATGGTCCCTTGTCCTTTCA
Xbp1	GGAACAGCAAGTGGTAGA	CTGGAGGGGTGACAAC
Gapdh	AAGGTGAAGGTCGGAGTCAA	CATGGGTGGAATCATATTGG

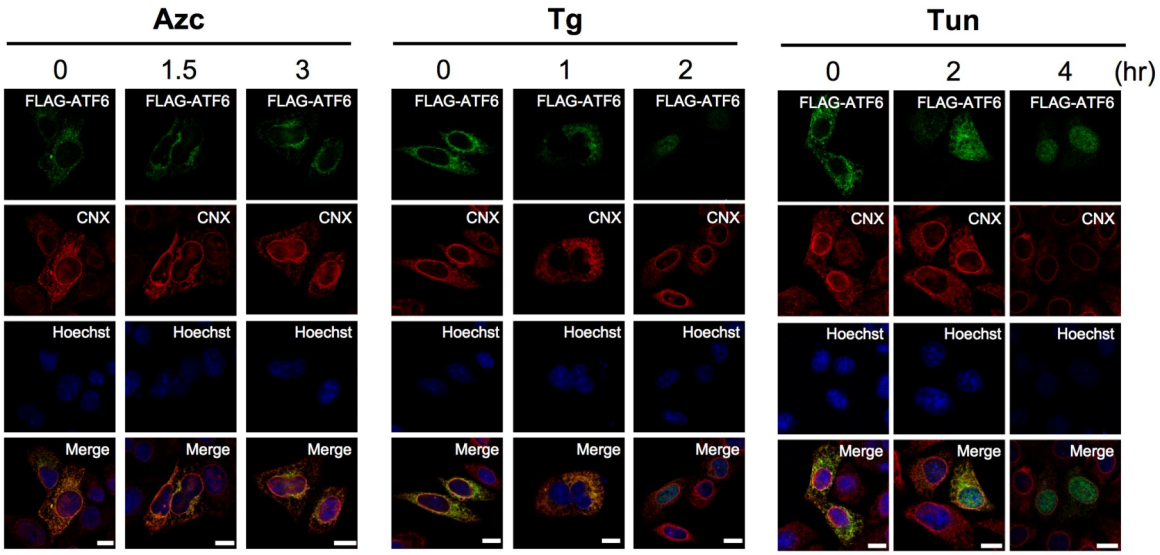

Fig. S1. ATF6α activation assay. HeLa cells transfected with FLAG-ATF6α plasmid for 24 h and treated with Azetidin-2-carboxylic acid (Azc, 10 mM), Thapsigargin (Tg, 500 nM) or Tunicamycin (Tun, 5 μg/ml) for the indicated time. Cells were then immunostained with anti-FLAG and anti-CNX (for ER) or anti-Giantin (for Golgi) antibodies. Cells were analyzed by confocal microscope. Bars, 10 μm.

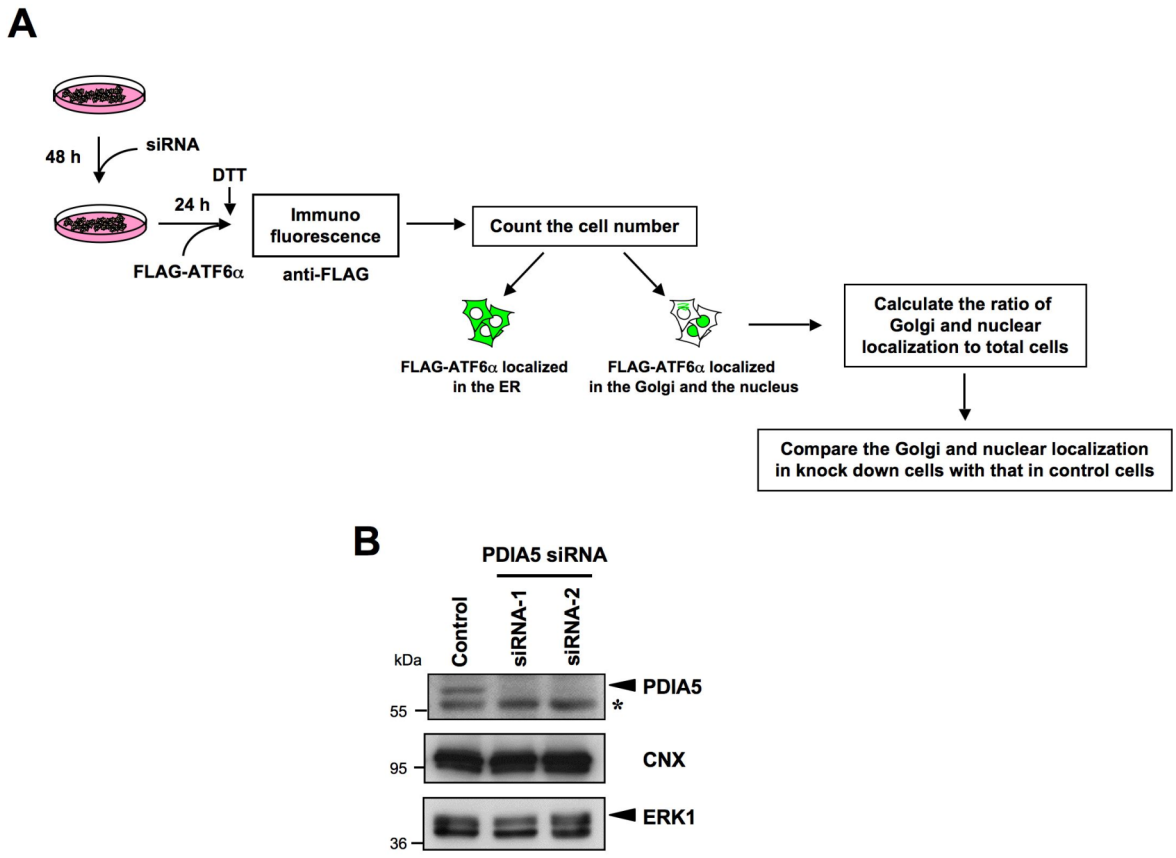

Fig. S2. Cell-based siRNA assay. (*A*) Schematic representation of the quantification method utilized for FLAG-ATF6**α** localization in control- or siRNA-transfected cells. HeLa cells were transfected with 25 nM siRNAs. Forty-eight hours after cells were then transfected with FLAG-tagged ATF6α (FLAG-ATF6α) for 24 h. Transfected cells were treated with 1 mM DTT for 2 h and co-stained with anti-FLAG and anti-CNX or anti-Giantin antibodies. All transfected cells were counted for the presence of FLAG-ATF6**α** in the ER, Golgi apparatus and nucleus. The ratio of cells localized in the Golgi apparatus and the nucleus to all FLAG-ATF6α-expressed and siRNA-transfected cells was calculated and compared with that in control cells. (*B*) The expression of PDIA5 proteins was silenced in HeLa cells by 25 nM siRNAs. Forty-eight hours after siRNA transfection, cell lysates were prepared using RIPA buffer and analysed by immunoblotting using antibodies against PDIA5 (1:500). Anti-CNX and anti-ERK1 antibodies were used as loading controls. The asterisk shows a nonspecific protein recognized by anti-PDIA5 antibodies.

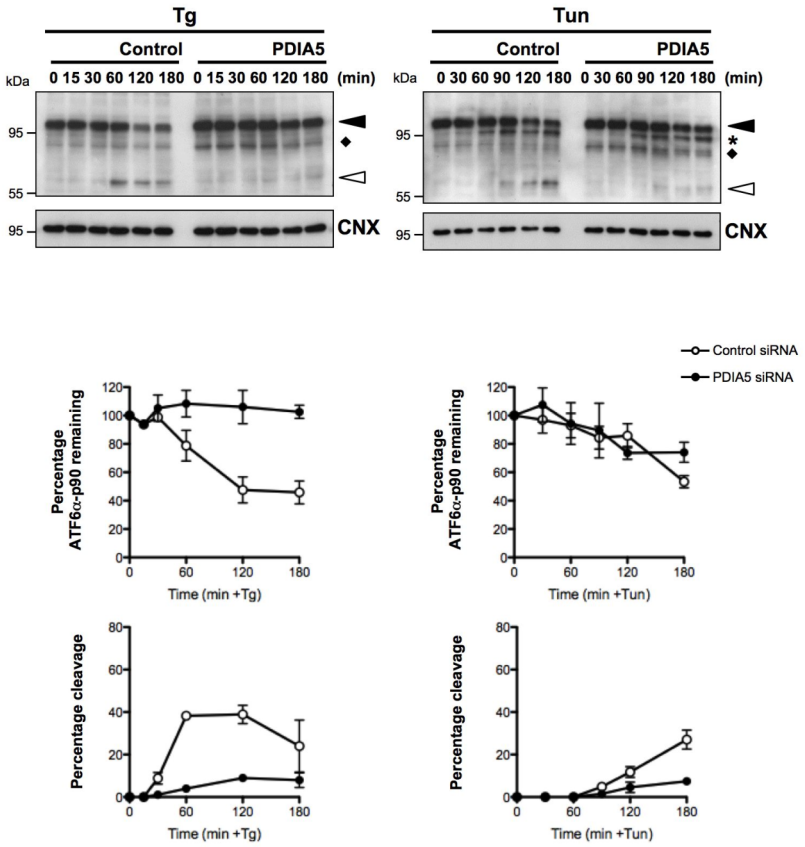

Fig. S3. Impact of PDIA5 silencing on ATF6α activation. Cleavage of endogenous ATF6α in HeLa cells exposed to Tg (500 nM) or Tun (5 µg/ml) as analyzed by immunoblot using anti-ATF6α antibodies. Full length and cleaved form of ATF6α indicates as black and white arrowheads, respectively. The asterisk shows the non-glycosylated form of ATF6α (right top panel). The black diamond represent a nonspecific protein recognized by mouse monoclonal anti-ATF6α antibodies. Time course quantification of full length (ATF6α-p90) and cytosolic portion (ATF6α-p50) of ATF6α upon treatment with the indicated ER stressor (bottom graphs).

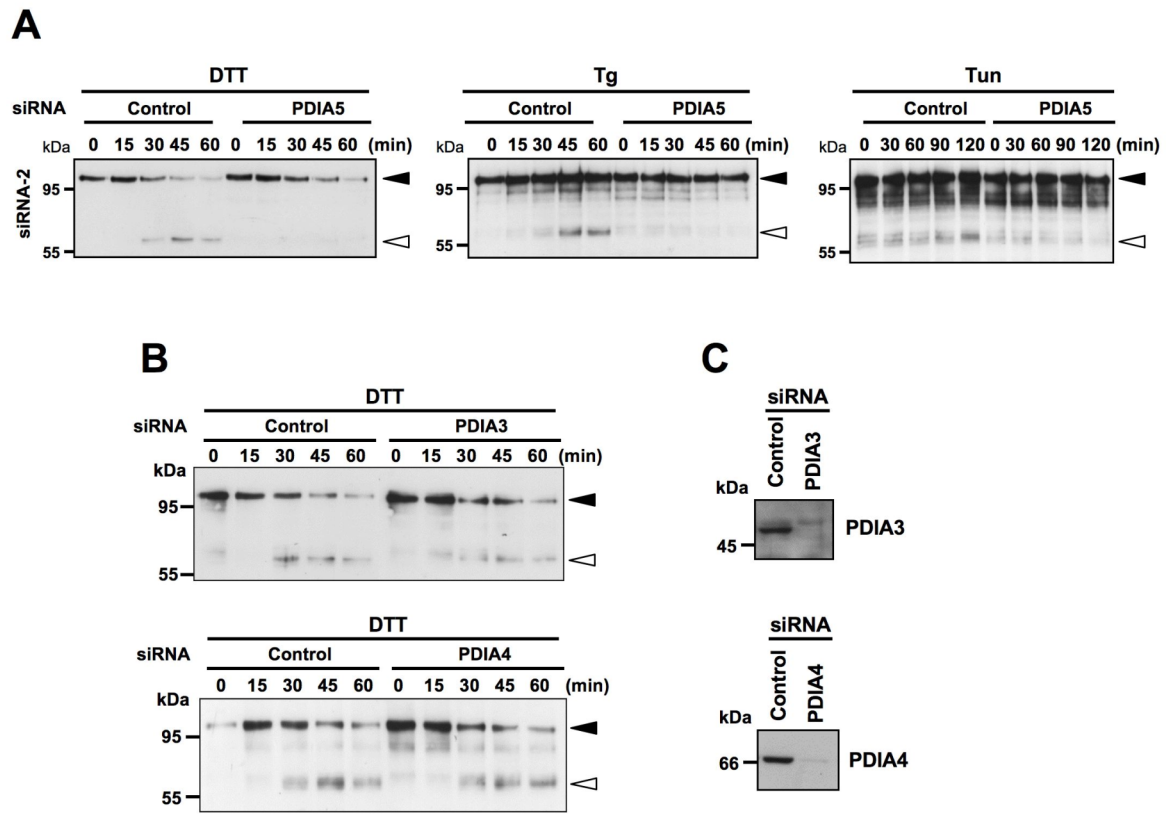

Fig. S4. Impact of PDIA3, PDIA4 and PDIA5 silencing on ATF6α activation. (*A*) siRNA against PDIA5 used in validation screen was transfected into HeLa cells stably expressing FLAG-ATF6α (HeLa-ATF6α). Seventy-two hours after siRNA transfection, cells were treated with DTT (1 mM), Tg (500 nM) or Tun (5 μg/ml) for the indicated time. Cell lysates were analyzed by immunoblotting using anti-ATF6**α** antibodies. ATF6α-p90 and ATF6α-p50 indicate as black and white arrowheads, respectively. (*B*) siRNAs against PDIA3 or PDIA4 were transfected into HeLa-ATF6**α** cells for 72 h. Cells were then treated with 1 mM DTT for the indicated period of time as in (*A*) and cell lysates were analyzed by immunoblotting using anti-ATF6α antibodies. (*C*) Expression of PDIA3 or PDIA4 was silenced in HeLa cells by 25 nM of siRNAs for 48 h. Cell lysates prepared by using RIPA buffer were analyzed by immunoblotting with antibodies against PDIA3 (1:1,000) or PDIA4 (1:4,000).

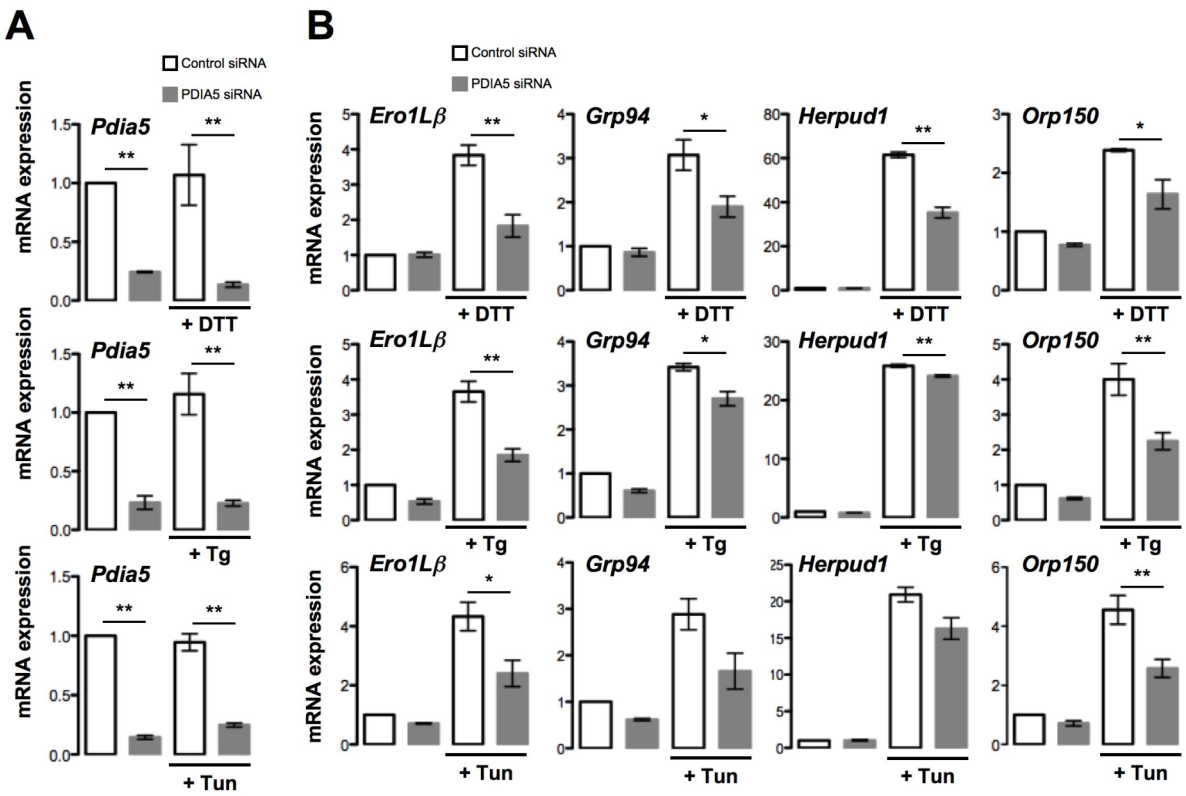

Fig. S5. Effects of PDIA5 silencing on ATF6α target genes and UPR signalling. (*A*) Expression of *Pdia5* mRNA upon silencing of PDIA5 using siRNA (25 nM) in HeLa cells. Forty eight hours after, cells were treated with indicated ER stressor for 2 h. Total RNA was extracted and analysed by q-PCR using primer pair for *Pdia5*. *(B)* Forty-eight hours after transfection cells were treated with with DTT, Tg or Tun for 2 h. Total RNA was isolated and analyzed by q-PCR using specific primers for ATF6α target genes (*Ero1Lβ*, *Grp94*, *Herpud1* and *Orp150*). Each mRNA expression was normalized to *Gapdh* mRNA. Data is shown as the average of three independent experiments ±SD (*$p < 0.05$ and **$p < 0.01$, as compared with control upon DTT treatment).

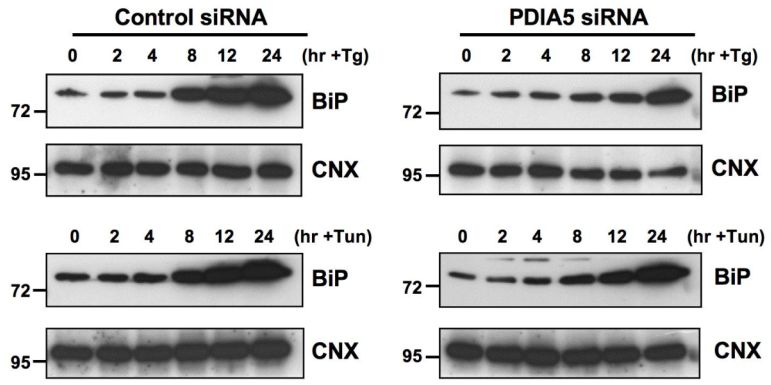

Fig. S6. Effects of PDIA5 silencing on ATF6α target genes and UPR signalling. BiP protein expression in control cells or PDIA5-silenced cells treated or not with Tg (500 nM) or Tun (5 μg/ml) was analyzed by immunoblotting using anti-KDEL antibody (upper panels). Anti-CNX antibodies was used as a loading control.

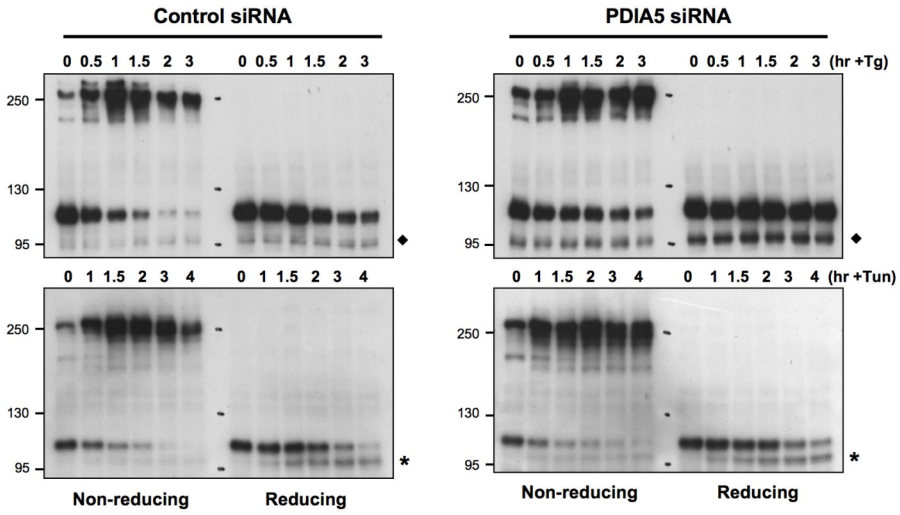

Fig. S7. Effects of PDIA5 silencing on ATF6α redox states upon Tg or Tun-mediated ER stress. Analysis of ATF6α redox state under conditions of PDIA5 silencing and Tg or Tun-induced ER stress. The asterisk (bottom panels) and black diamond (upper panels) show the non-glycosylated form of ATF6α and a nonspecific protein recognized anti-ATF6α antibodies, respectively.

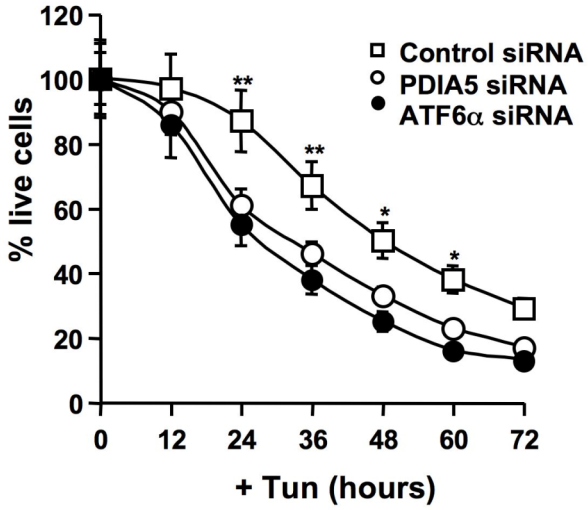

Fig. S8. Effects of PDIA5 or ATF6α silencing on ER stress resistance. HeLa cells were silenced for either PDIA5 or ATF6α for 48 h and treated with 5 µg/ml Tun for the indicated periods of time. Living cells were counted using Sulforhodamine B. Results are presented as the mean of 3 independent experiments ± SD.