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Supplemental Information

XBP1-KLF9 Axis Acts as a Molecular Rheostat to Control the Transition from Adaptive to Cytotoxic Unfolded Protein Response

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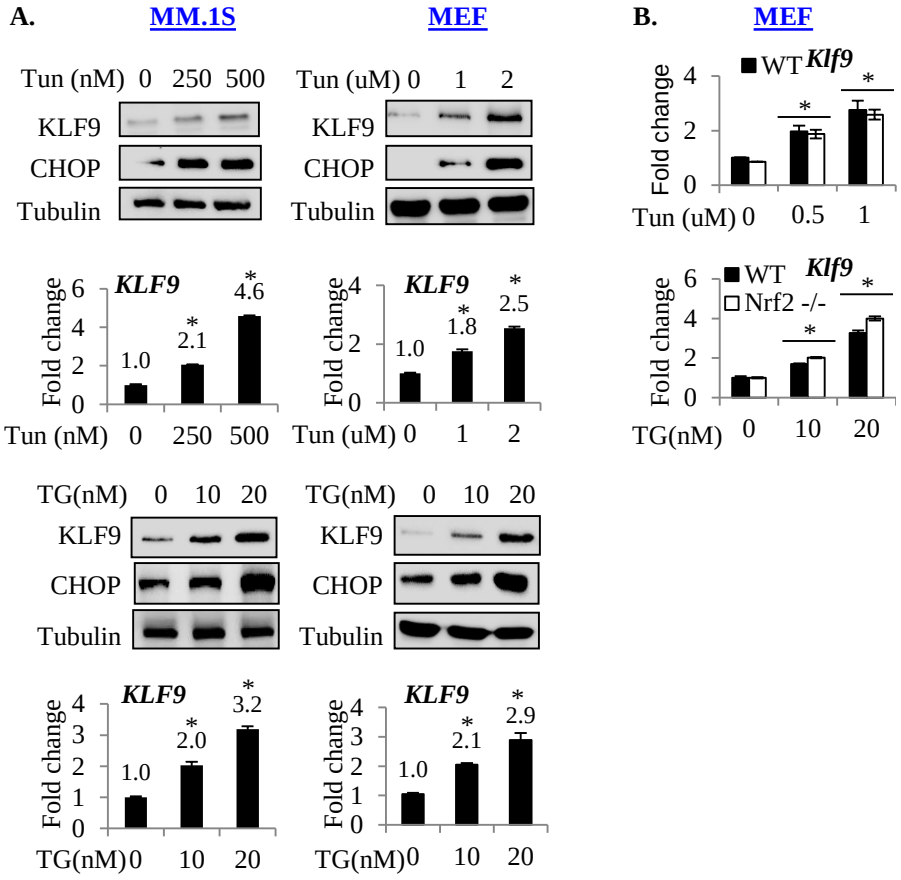


Figure S1. ER stress induces expression of KLF9, Related to Figure 1

A. Indicated cells were treated with indicated doses of tunicamycin (Tun) or thapsigargin (TG) for 24hrs and probed in Q-RT-PCR (upper panels, KLF9/β-actin signal ratios are shown) or immunoblotting with indicated antibodies. **B.** Wildtype (WT) or Nrf2$^{-/-}$ MEFs were treated with indicated doses of tunicamycin (tun) or thapsigargin (TG) for 24hrs and probed in Q-RT-PCR (upper panels, Klf9/β-actin signal ratios are shown). Representative images shown. All data represent mean ± SEM of 2 or more biological replicates. Statistical significance was analyzed using two-tailed Student t-Test. A $p < 0.05$ (∗) was considered significant.

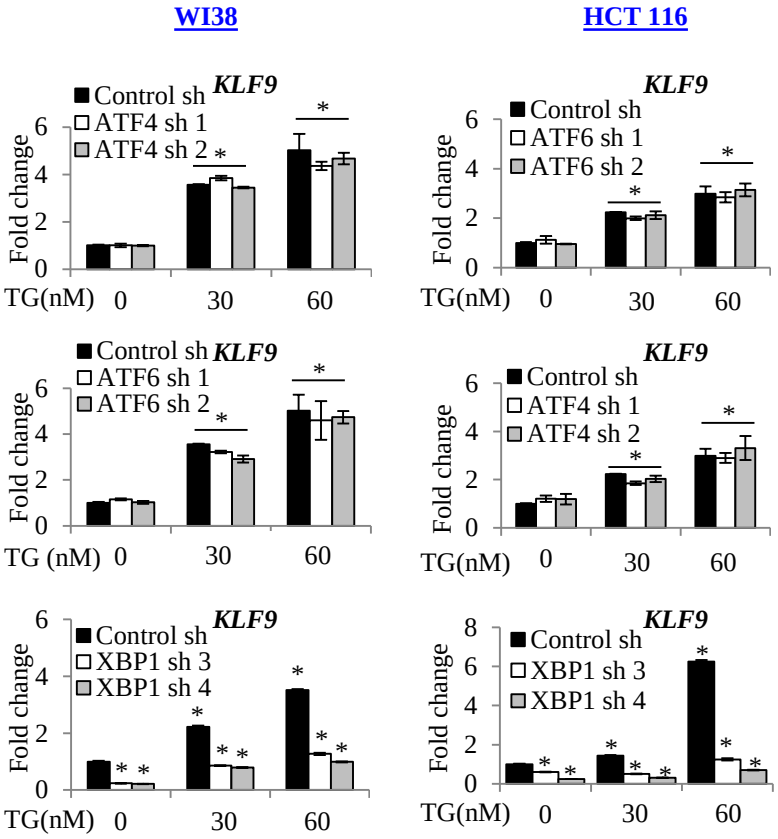


Figure S2. KLF9 induction of ER stress depends on ER stress, Related to Figure 2
Indicated cells transduced with control shRNA (Control sh) or shRNAs to indicated genes were treated with indicated doses of thapsigargin (TG) followed by Q-RT-PCR (KLF9/β-actin signal ratios are shown). All data represent mean ± SEM of 2 or more biological replicates. Statistical significance was analyzed using two-tailed Student t-Test. A $p < 0.05$ (∗) was considered significant.

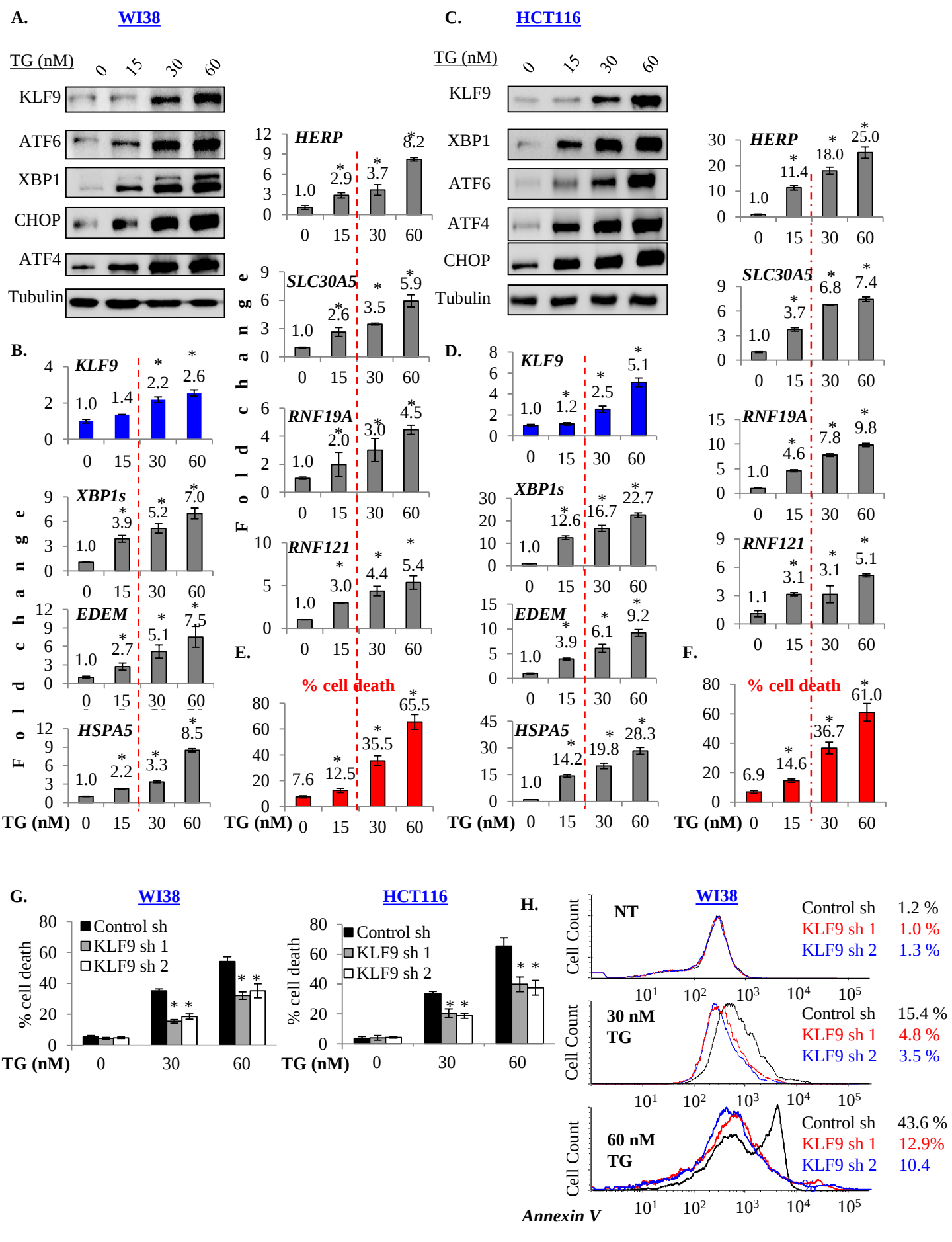
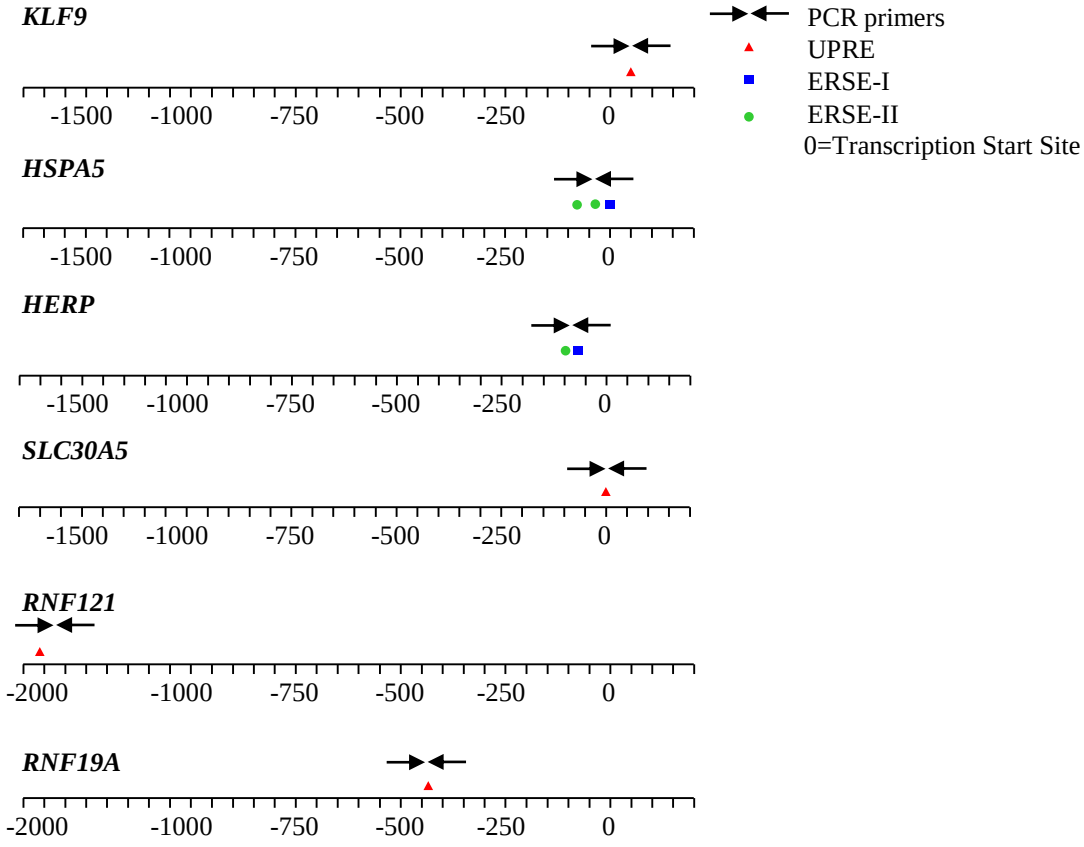

A.
WI38
TG (nM)
0
15
30
60
KLF9
ATF6
XBP1
CHOP
ATF4
Tubulin
C.
HCT116
TG (nM)
0
15
30
60
KLF9
XBP1
ATF6
ATF4
CHOP
Tubulin
B.
KLF9
1.0
1.4
2.2
2.6
XBP1s
1.0
3.9
5.2
7.0
EDEM
1.0
2.7
5.1
7.5
HSPA5
1.0
2.2
3.3
8.5
TG (nM) 0 15 30 60
Fold change
HERP
1.0
2.9
3.7
8.2
SLC30A5
1.0
2.6
3.5
5.9
RNF19A
1.0
2.0
3.0
4.5
RNF121
1.0
3.0
4.4
5.4
E.
% cell death
7.6
12.5
35.5
65.5
TG (nM) 0 15 30 60
D.
KLF9
1.0
1.2
2.5
5.1
XBP1s
1.0
12.6
16.7
22.7
EDEM
1.0
3.9
6.1
9.2
HSPA5
1.0
14.2
19.8
28.3
TG (nM) 0 15 30 60
HERP
1.0
11.4
18.0
25.0
SLC30A5
1.0
3.7
6.8
7.4
RNF19A
1.0
4.6
7.8
9.8
RNF121
1.1
3.1
3.1
5.1
F.
% cell death
6.9
14.6
36.7
61.0
TG (nM) 0 15 30 60
G.
WI38
HCT116
% cell death
Control sh
KLF9 sh 1
KLF9 sh 2
TG (nM) 0 30 60
H.
WI38
NT
30 nM TG
60 nM TG
Cell Count
Annexin V
Control sh 1.2 %
KLF9 sh 1 1.0 %
KLF9 sh 2 1.3 %
Control sh 15.4 %
KLF9 sh 1 4.8 %
KLF9 sh 2 3.5 %
Control sh 43.6 %
KLF9 sh 1 12.9%
KLF9 sh 2 10.4

Figure S3

Figure S3. KLF9 is upregulated by toxic doses of thapsigargin, Related to Figure 3
Indicated cells treated with the indicated doses of thapsigargin (TG) for 24hrs were probed in immunoblotting with the indicated antibodies (**A**, **C**) or in Q-RT-PCR (shown are ratios of signal for an indicated gene and β-actin) (**B**, **D**). **E, F.** The viability of cells treated as in A or D was assessed *via* trypan blue viability assay. **G, H.** Cells were transduced with control shRNA (Cl) or KLF9 shRNAs (sh1, sh2)), treated with thapsigargin (Tg) for 48hrs and probed in trypan blue viability assay (G) or Annexin V apoptosis assay (H). Representative images shown. All data represent mean ± SEM of 2 or more biological replicates. Statistical significance was analyzed using two-tailed Student t-Test. A $p < 0.05$ (∗) was considered significant.

A.



B.

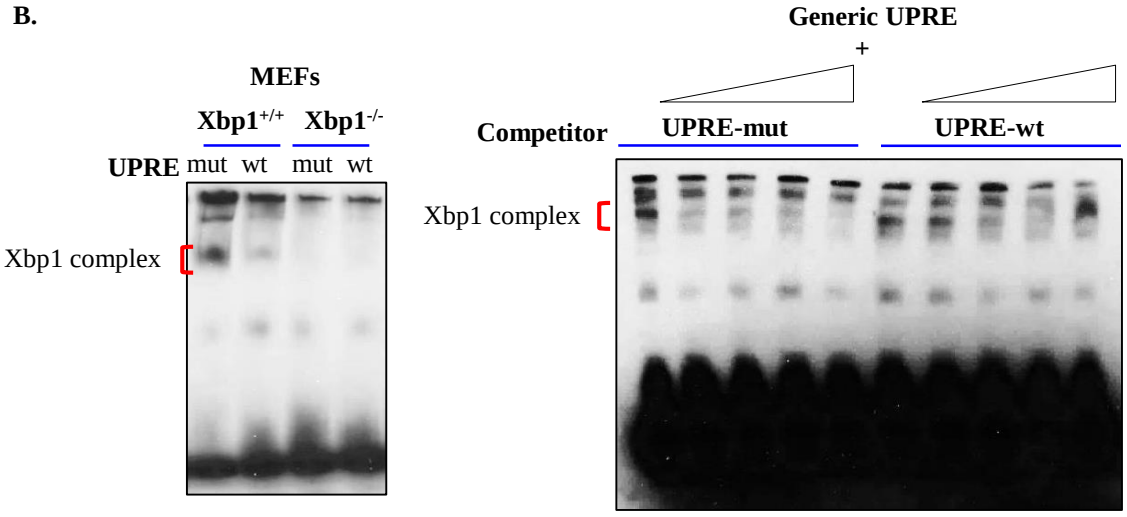


Figure S4. XBP1s binding to *KLF9* promoter, Related to Figure 4

A. Schematics representation of XBP1s binding sites in the promoter of studied genes. Arrows denote PCR primers used in ChIP assay. **B.** Full scale gel images corresponding to Figure 4E,F. Representative images shown.

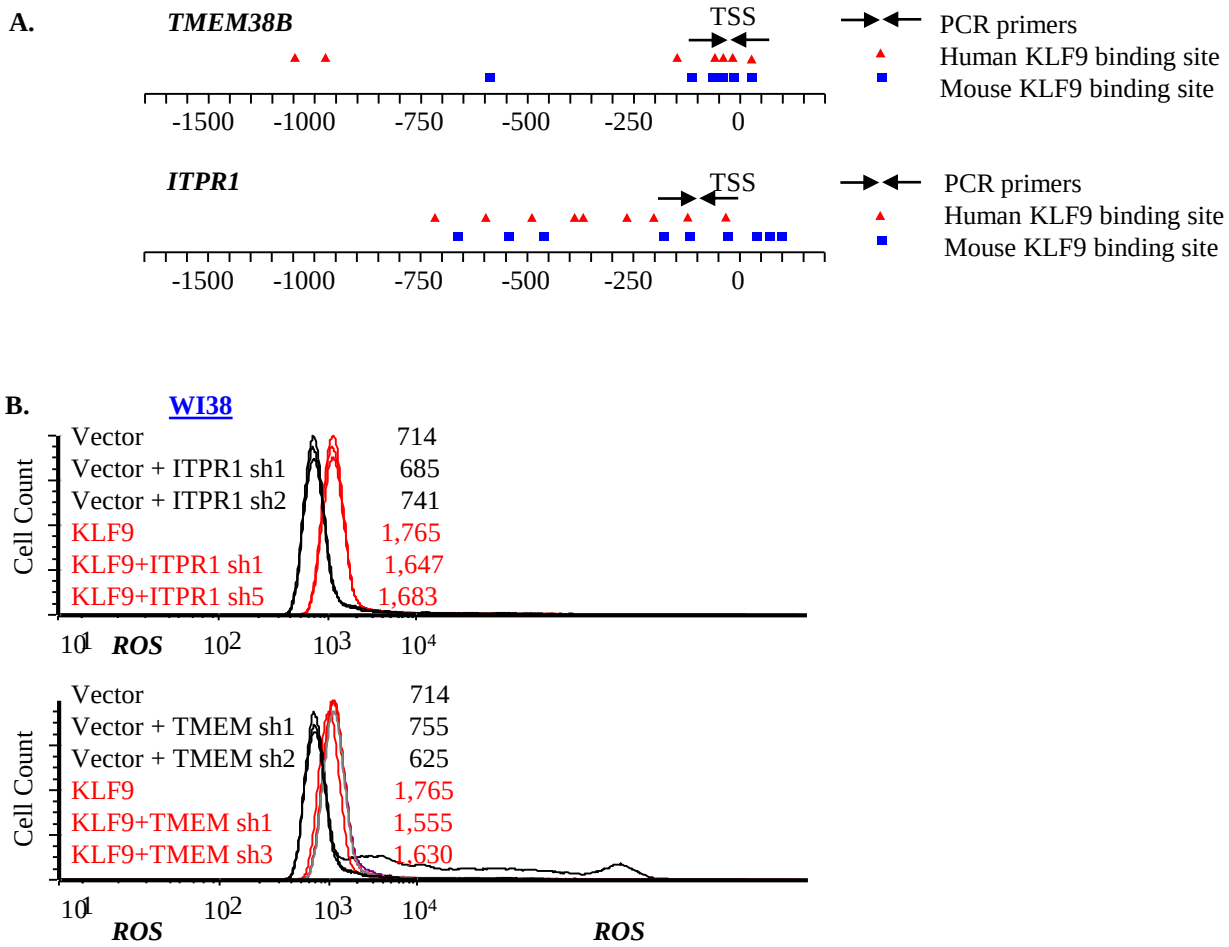


Figure S5. TMEM38B and ITPR1 are KLF9 targets, Related to Figure 5.
A. Schematics representation of KLF9 binding sites in the promoter of indicated genes. Arrows denote PCR primers used in Q-PCR on DNAs obtained in ChIP assay. **B.** WI38 cells expressing the indicated constructs were probed in ROS-FACS analysis. Representative images shown.

Table S1. KLF9 depletion decreases ER-induced cell death, Related to Figure 3

WI38	% Cell Death		
Tun. μM	0	1	2
Control sh	5.5	35.2	54.2
KLF9 sh1	4.6	15.4	32.1
KLF9 sh2	4.9	18.5	35.2

HCT116	% Cell Death		
Tun. μM	0	1	2
Control sh	3.6	22.6	47.6
KLF9 sh1	4	14.2	29.5
KLF9 sh2	4.3	13.5	28.4

Table S2. ITPR1 and TMEM38B are direct KLF9 target genes, Related to Figure 5

Genes oppositely regulated by KLF9 overexpression and depletion (RNA-Seq)	Genes involved in regulation of ER biogenesis	Genes whose expression changed ≥2 fold by KLF9 overexpression (Q-RT-PCR)	Genes interacting with KLF9 (ChIP assay)
AADAC	AMFR	ATP2A1	ITPR1
AMFR	ATP2A1	ITPR1	TMEM38B
ASPHD1	BSCL2	PPP1R15A	
ATP2A1	EIF2AK2	SDF2L1	
BSCL2	ERN1	TMEM38B	
CCND1	ITPR1	TRAM2	
CD48	JPH4		
CDS1	PPP1R15A		
COL4A3BP	SDF2L1		
CYP26A1	TMEM38B		
CYP39A1	TRAM2		
CYP4F2			
DEGS2			
DERL3			
DUOXA1			
EDA			
EGR1			
EIF2AK2			
ERN1			
ERP27			
EXTL1			
FAM129A			
FOS			
HLA-DOB			
HLA-DRA			
HSD11B1			
HSPA6			
HSPB2			
HSPB3			
ITPR1			
JPH1			
JPH4			
JSRP1			
LRMP			
MLANA			
NR4A2			
OLFM1			
P4HA3			
PPP1R15A			
PTGS2			
RYR1			
SCAMP5			
SDF2L1			
SOAT2			
TMEM38B			
TRAM2			

Table S3. TMEM38B depletion decreases ER-induced cell death, Related to Figure 5

	% Cell Death		
Tun. μM	0	1	2
Control sh	2.9	32.7	46
TM sh1	3.5	19.8	32
TMsh2	3.8	22.6	30.2

Table S4. PCR primers and probes, Related to STAR Methods

Q-RT-PCR primers	
Name	**Sequence 5'-3'**
Hs_CHOP L	AGAACCAGGAAACGGAAACAGA
Hs_CHOP R	TCTCCTTCATGCGCTGCTTT
Hs_EDEM L	CAAGTGTGGGTACGCCACG
Hs_EDEM R	AAAGAAGCTCTCCATCCGGTC
Hs_HSPA5 L	TGTTCAACCAATTATCAGCAAACTC
Hs_HSPA5 R	TTCTGCTGTATCCTCTTCACCAGT
Hs_HERP L	TTCTGGGAAGCTGTTGTTGG
Hs_HERP R	TCACATTGCACACCAGATGC
Hs_SLC30A5 L	ATCACACCAATGCTGCCAAG
Hs_SLC30A5 R	TGATCACAGCCATTCACACC
Hs_RNF19A L	ATGCCAGCACCAAAGCAATG
Hs_RNF19A R	TGTGCCTGAATTTGGATGGC
Hs_RNF121 L	TTCAGACAGTGTGTGTGCTG
Hs_RNF121 R	TTGCTTCTTTCCCACGATGC
Hs_DNAJB9 L	TCGGAGGGTGCAGGATATTAG
Hs_DNAJB9 R	CGCTCTGATGCCGATTTTGG
Hs_XBP1s L	CTGAGTCCGAATCAGGTGCAG
Hs_XBP1s R	ATCCATGGGGAGATGTTCTGG
Mm_Edem L	TTTTGAGTGGGTTGCCAGAC
Mm_Edem R	TCTGCTTTCCAACCCAATGG
Mm_Hspa5 L	TCGGGCCAAATTTGAAGAGC
Mm_Hspa5 R	CAACACTTTCTGGACAGGCTTC
Mm_Tmem38b L	TGCCATGCTCCATTGCTTTG
Mm_Tmem38b R	ACTAGGTCACGAGGGCAAAAG
Mm_Itpr1 L	AAACCACGTGCTTCATCTGC
Mm_Itpr1 R	TTTCACCAGCACGATGAAGC
TaqMan Probes	
Name	**Cat #**
Hs_KLF9	Hs00230918_m1
Hs_B-Actin	Hs99999903_m1
Hs_TMEM38B	Hs00216531_m1
Hs_ITPR1	Hs00181881_m1
Hs_XBP1s	Hs00231936_m1
Mm_Klf9	Mm00495172_m1
Mm_b-actin	Mm00607939_s1
ChIP primers	
Name	**Sequence 5'-3'**
Hs_KLF9 promoter L	AAACGGAACAGTCGGATTGG
Hs_KLF9 promoter R	TCACGTCACAAACCAAACCC
Hs_ITPR1 promoter L 1	GGTTTCTCGTGCAAACCCGA
Hs_ITPR1 promoter R 1	CCCCCTTCCCTGGAATCCA
Hs_ITPR1 promoter L 2	CGGCCCCAGTGACACCTG

Hs_ITPR1 promoter R 2	GGCTGGGCCCTAAATAGACG
Hs_TMEM38B promoter L 1	GTGACTTGCACTGTGGTTGC
Hs_TMEM38B promoter R 1	AGGAGTAGGAGAGGGCACAG
Hs_TMEM38B promoter L 2	AACTCTGAGCGTCTCCCCTT
Hs_TMEM38B promoter R 2	GTGAGGAGTAGGAGAGGGCA
Hs_HSPA5 promoter L	GGAGCAGTGACGTTTATTGCG
Hs_HSPA5 promoter R	TCGTTGGAGGCCGTTCATTG
Hs_HERP promoter L	CGATTGGGCCACGTTGGG
Hs_HERP promoter R	GCTTCGGGCGCCTTTTATAG
Hs_SLC30A5 promoter L	AGCTTCAATGCGACACAACG
Hs_SLC30A5 promoter R	TTGCCAAGCCACGTCATCAG
Hs_SDF2L1 promoter L	AGCGATCCAACAACCTCAAG
Hs_SDF2L1 promoter R	GCGTGAACAGTTGGTGAAGA
Hs_TRAM2 promoter L	GCGTCTTCACCAACTGTTCAC
Hs_TRAM2 promoter R	TTTGATTGGTCCACGCTTCC
Hs_PPP1R15A promoter L	TGAGGCAGGAGAATCGCTTG
Hs_PPP1R15A promoter R	TTTGAGGCGGAGTTTTGCTC
Hs_ATP2A1 promoter L	GCAACAACTTGTGGCTTTGC
Hs_ATP2A1 promoter R	AAAGGGAGCAGAGACACAGAG
Hs_RNF121 promoter L	AGCATTTTGGGAGGTTGACG
Hs_RNF121 promoter R	CAGGGTTTCACCATGTTGGC
Hs_RNF19a promoter L	AAGCTGGTTGTTTCCGAAGC
Hs_RNF19a promoter R	TGGCTCTTGTCTGGCATCAG
EMSA Probes	
Name	**Sequence 5'-3'**
UPRE L (Btn lable)	GGTCGAGACAGGTGCTGACGTGGCGATTCCCC
UPRE R (Btn lable)	GGGGAATCGCCACGTCAGCACCTGTCTCGACC
KLF9 WT L (Btn lable)	AGGGGGTTTGGTTTGTGACGTGATGGGATTCT
KLF9 WT R (Btn lable)	AGAATCCCATCACGTCACAAACCAAACCCCCT
KLF9 Mut L (Btn lable)	AGGGGGTTTGGTTTGTGACGTGGTGGGATTCT
KLF9 Mut R (Btn lable)	AGAATCCCACCACGTCACAAACCAAACCCCCT
UPRE L (unlabled)	GGTCGAGACAGGTGCTGACGTGGCGATTCCCC
UPRE R (unlabled)	GGGGAATCGCCACGTCAGCACCTGTCTCGACC
KLF9 WT L (unlabled)	AGGGGGTTTGGTTTGTGACGTGATGGGATTCT
KLF9 WT R (unlabled)	AGAATCCCATCACGTCACAAACCAAACCCCCT
KLF9 Mut L (unlabled)	AGGGGGTTTGGTTTGTGACGTGGTGGGATTCT
KLF9 Mut R (unlabled)	AGAATCCCACCACGTCACAAACCAAACCCCCT