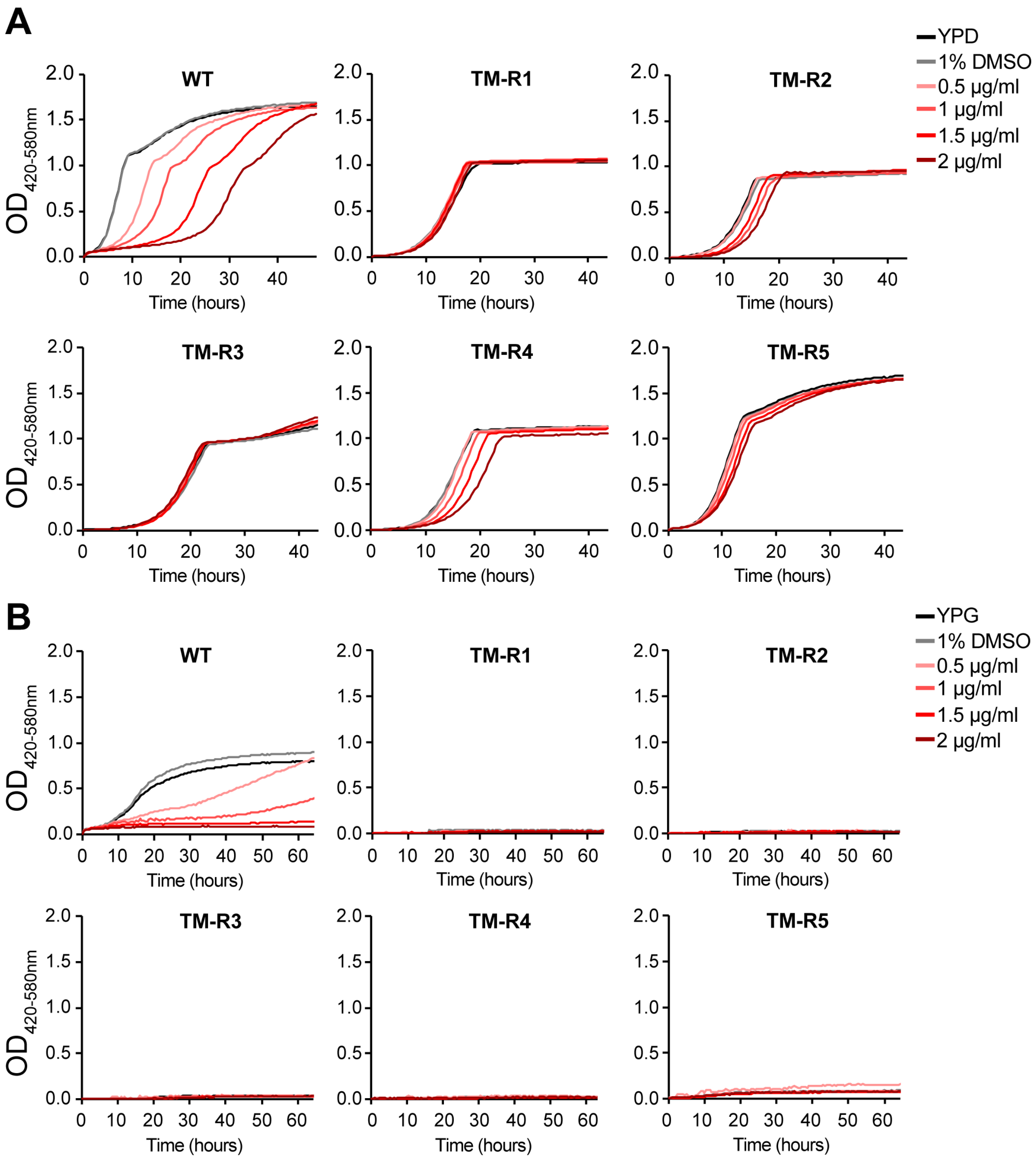

Fig. S1. Representative growth curves of the tunicamycin-resistant mutants. (A) Tunicamycin dose response of the tunicamycin-resistant (TM-R) mutants grown in YPD media. (B) TM-R mutants are unable to grow in media containing 3% glycerol (YPG) as a carbon source. Growth rates were analyzed in the presence of indicated concentration of tunicamycin or 1% DMSO as a control.

www.pnas.org/cgi/doi/10.1073/pnas.1804264115

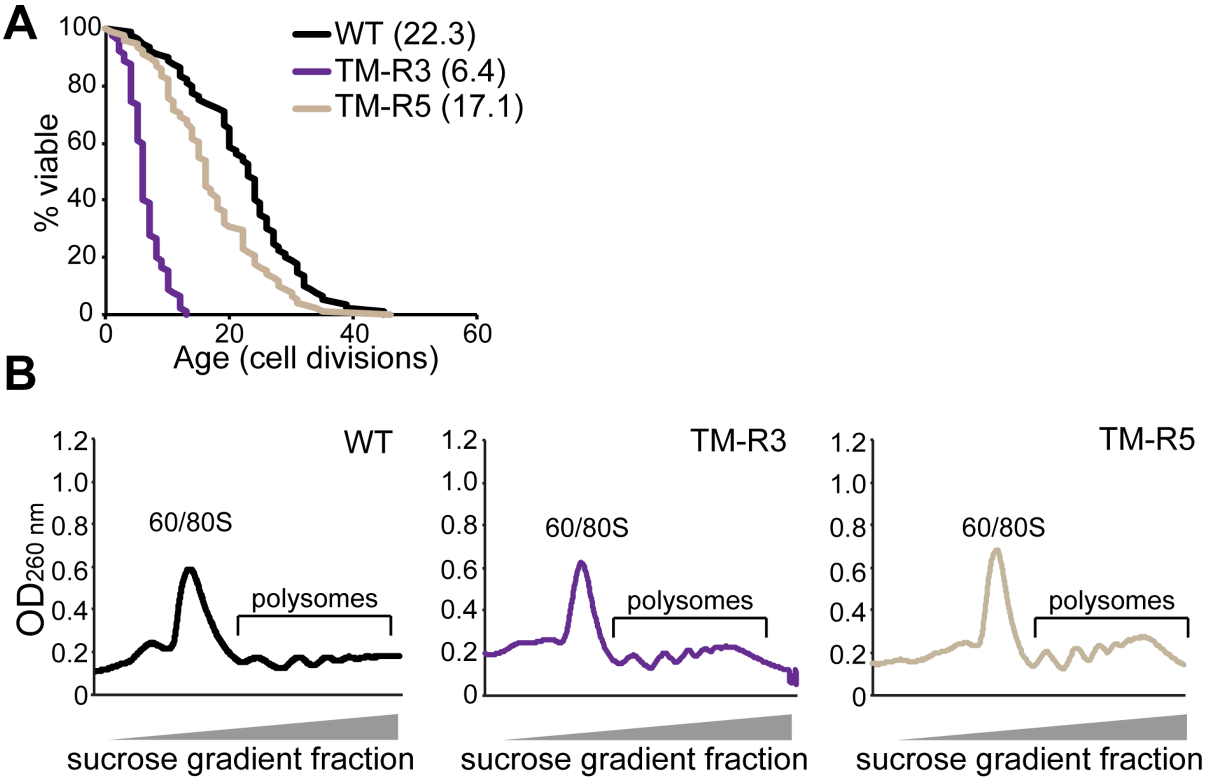

Fig. S2. Aneuploidy shortens replicative lifespan without reducing overall mRNA translation. (A) Survival curves for tunicamycin-resistant (TM-R) mutants and corresponding experiment-matched wild-type cells. Mean lifespans are shown in parentheses. (B) Polysome profiles of wild-type cells and tunicamycin-resistant (TM-R) mutants. Aliquots of cell extracts containing 50 OD_{260} units were loaded on top of sucrose gradients (10-50% wt/wt) and sedimented at 35,000 rpm at 4°C in a SW41 Ti rotor (Beckman) for 3 hrs. Fractions were collected using the Brandel gradient fractionation system and profiles were monitored at 254 nm.

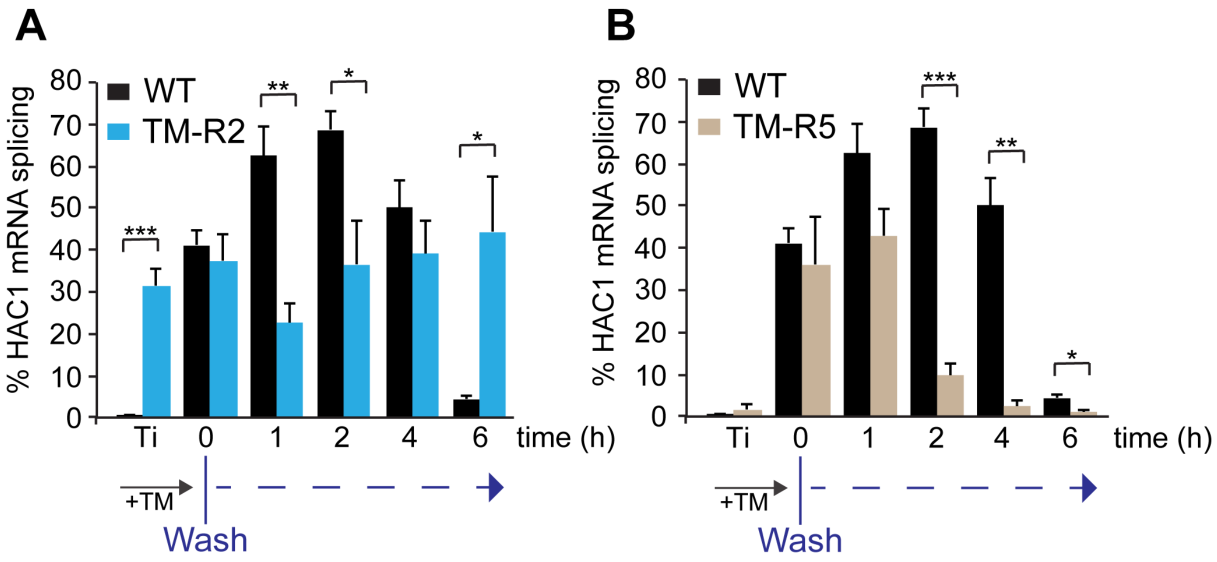

Fig. S3. Quantification of spliced *HAC1* mRNA after TM washout in the TM-R2 (A) and TM-R5 (B) mutants. Logarithmically growing cells were treated with 1 µg/ml TM for 30 min, cells were washed and incubated in fresh media for indicated time. Levels of spliced (spl) and un-spliced (us) *HAC1* mRNA were detected by RT-PCR. Error bars represent SEM (n=3). *p<0.05, **p<0.01, ***p<0.001, Student's *t* test.

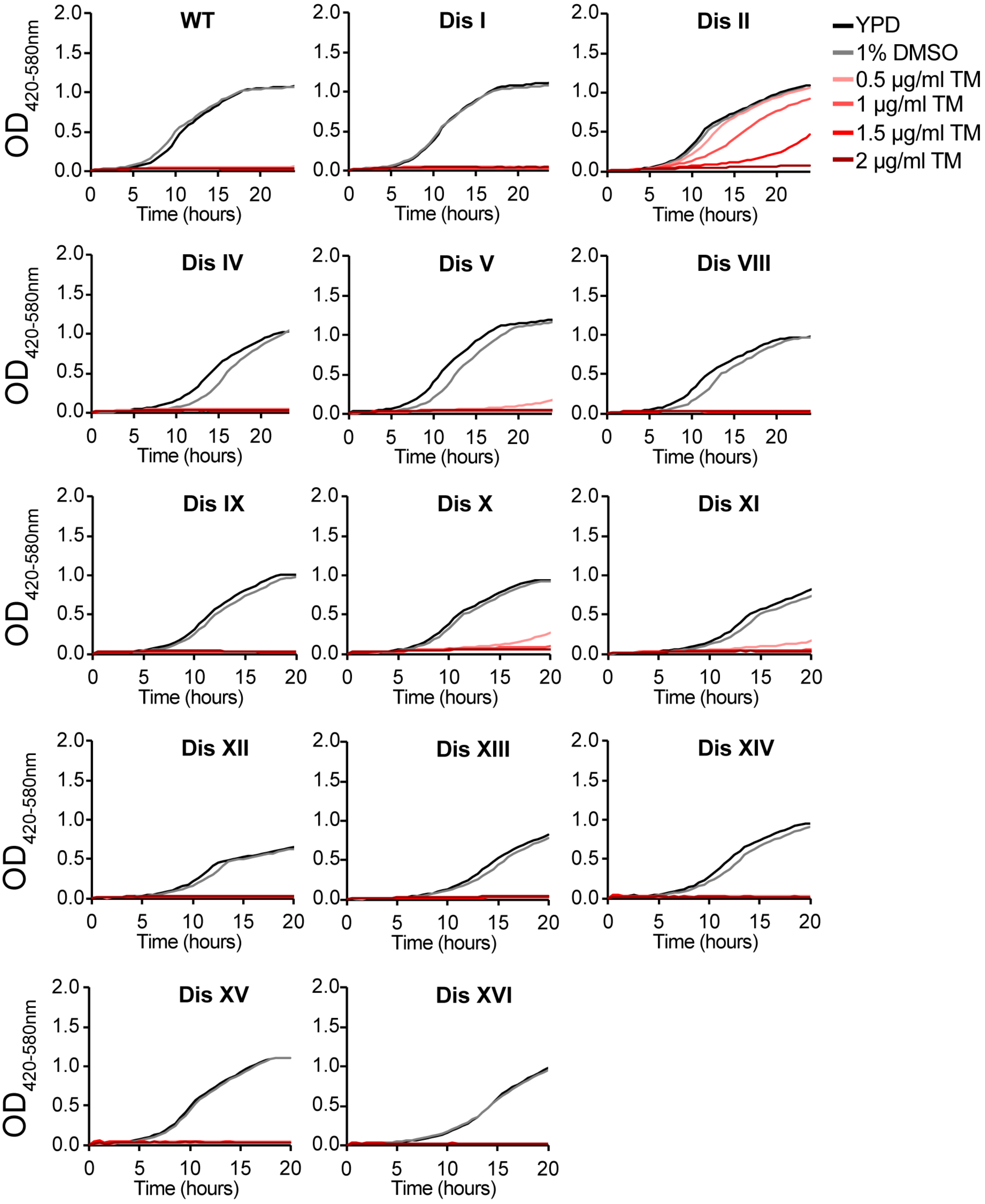

Fig. S4. Chr II duplication is sufficient to confer ER stress resistance to cells. Growth rates of wild-type (W303) cells and disomic (Dis) strains were analyzed in the presence of indicated concentration of tunicamycin or 1% DMSO as a control.

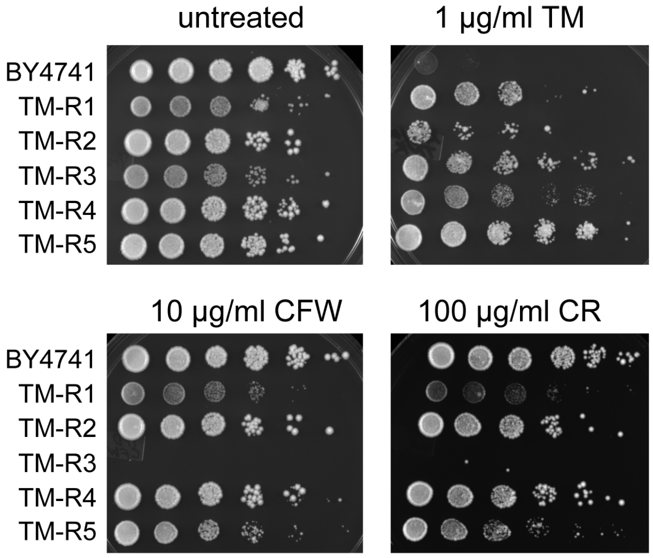

Fig. S5. Sensitivity of the TM-R mutants to cell-wall stress. Wild-type (BY4741) cells and TM-R strains were grown in YPD to mid-log phase. 10× serial dilutions of logarithmically growing cells were spotted on YPD plates containing the indicated concentrations of tunicamycin (TM), calcofluor white (CFW) or congo red (CR) and incubated for 48 h at 30°C.

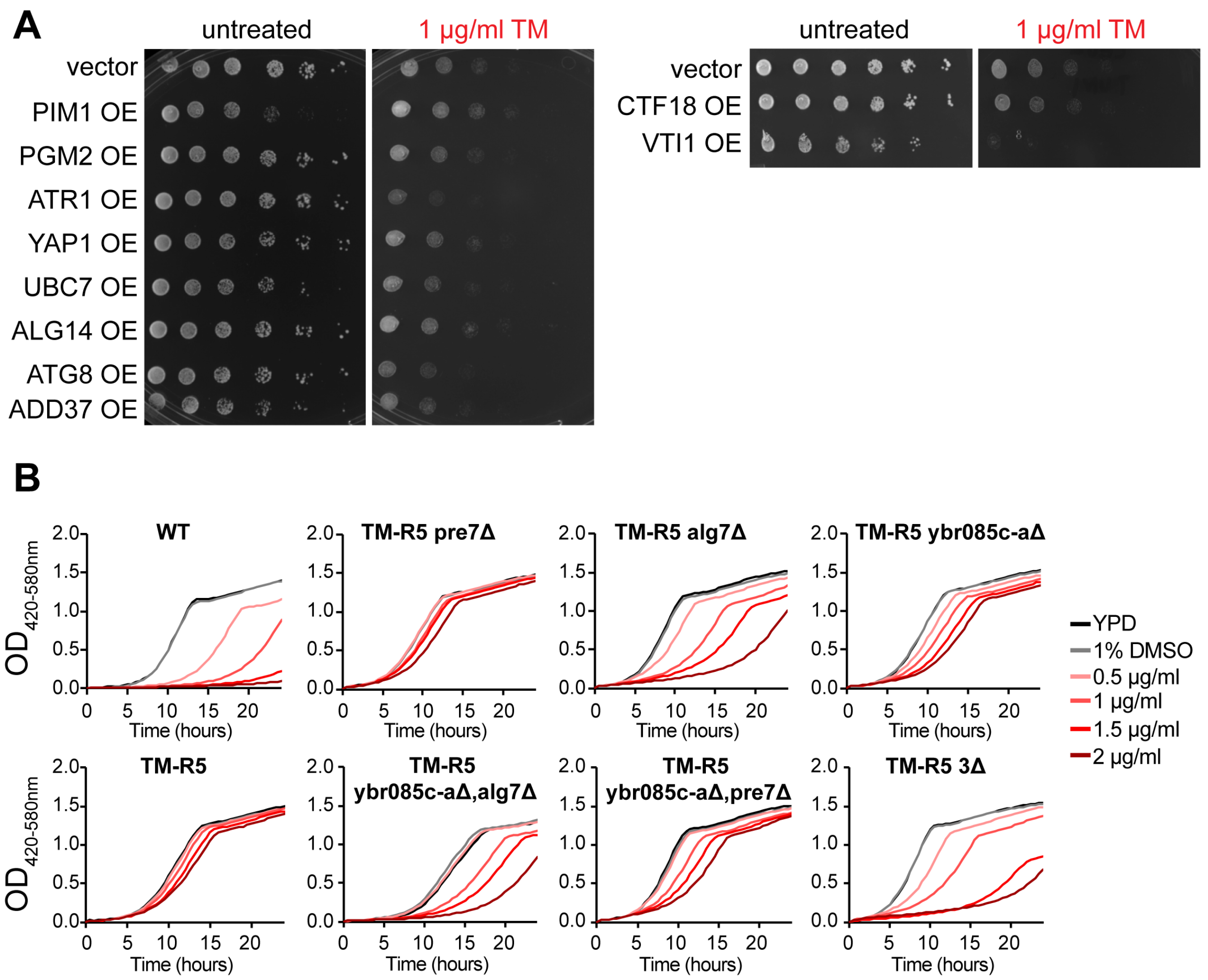

Fig. S6. Overexpression of *YBR085C-A*, *ALG7*, and *PRE7* in wild-type cells induces resistance to TM. (A) Resistance of strains to ER stress was determined using spot assays on SD agar plates lacking leucine in the presence of 1 µg/ml TM. (B) Simultaneous deletion of extra copies of *PRE7*, *ALG7*, and *YBR085C-A* (3Δ) prevents tunicamycin resistance in TM-R5 cells. Representative growth curves of the TM-R5 derived deletion mutants grown in YPD media in the presence of indicated concentration of tunicamycin or 1% DMSO as a control.

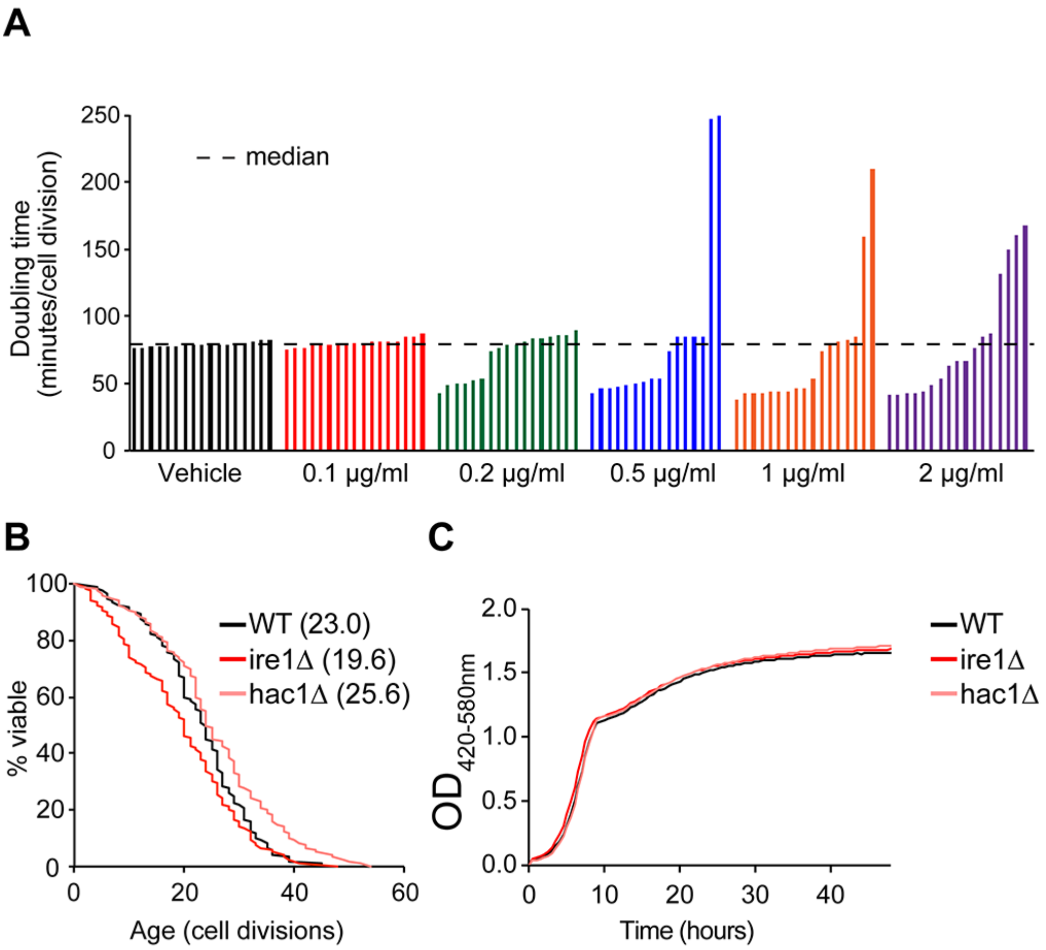

Fig. S7. (A) Chromosome duplications are not gained in response to low dose ER stress. Wild-type BY4741 cells were plated on YPD plates containing indicated concentrations of TM and grown at 30°C for 2-4 days. For each condition, 17 single colonies were then transferred into fresh media, and their growth rates were analyzed using standard YPD media in the absence of TM. Doubling time was calculated using the Yeast Outgrowth Data Analyzer (YODA) software. (B) Deletion of *IRE1* and *HAC1* genes does not significantly affect replicative lifespan. Mean lifespans are shown in parentheses. (C) Representative growth curves of the *ire1Δ* and *hac1Δ* mutants in YPD media.

Table S1. Yeast strains used in this study.

Designation	**Strain**	**Genotype**
LD001	BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
LD008	TM-R1	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
LD009	TM-R2	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
LD010	TM-R3	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
LD011	TM-R4	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
LD012	TM-R5	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
LD013	*ire1Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ire1Δ::KanMX6*
LD014	*hac1Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hac1Δ::KanMX6*
YCM256	W303	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, ade1::HIS3, lys2::KanMX6*
YCM257	W303, chr1 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, ade1::HIS3, ade1::KanMX6*
YCM258	W303, chr2 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, lys2::HIS3, lys2::KanMX6*
YCM259	W303, chr4 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, trp1::HIS3, trp1::KanMX6*
YCM260	W303, chr5 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, can1::HIS3, intergenic region betwn 187520-187620::KanMX6*
YCM261	W303, chr8 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, YHR006W to 007C::HIS3, YHR006W to 007C::KanMX6*
YCM262	W303, chr9 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, YIL009W to 008W::HIS3, YIL009W to 008W::KanMX6*
YCM263	W303, chr10 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, ura2::HIS3, ura2::KanMX6*
YCM264	W303, chr11 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, YKL006C-S to 006W::HIS3, YKL006C-S to 006W::KanMX6*
YCM265	W303, chr12 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, ade16::HIS3, ade16::KanMX6*
YCM266	W303, chr13 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, ura5::HIS3, ura5::KanMX6*
YCM267	W303, chr14 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, YNL005C to 004W::HIS3, YNL005C to 004W::KanMX6*
YCM268	W303, chr15 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, leu9::HIS3, leu9::KanMX6*
YCM269	W303, chr16 disome	*MAT***a**, *ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, met12::HIS3, met12::KanMX6*
CB027	TM-R5 Chr XIII marker strain	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ymr132cΔ::KanMX6*
CB028	TM-R5 Chr II marker strain	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr242wΔ::KanMX6*

CB029	TM-R5 *pre7Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pre7Δ::KanMX6*
CB059	TM-R5 *alg7Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 alg7Δ::KanMX6*
CB060	TM-R5 *ybr085c-aΔ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr085c-aΔ::KanMX6*
CB064	TM-R5 *ybr085c-aΔ, alg7Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr085c-aΔ::KanMX6 alg7Δ::URA3*
CB065	TM-R5 *ybr085c-aΔ, pre7Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr085c-aΔ::KanMX6 pre7Δ::LEU2*
CB066	TM-R5 *3Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr085c-aΔ::KanMX6 pre7Δ::LEU2 alg7Δ::URA3*

Table S2. Oligonucleotides used in this study.

Name	**Sequence**
TSL1 F	GTGTCGACATGGCTCTCATCGTGGCATC
TSL1 R	GTGCGGCCGCATTGGTCGTTAGAGAGAAG
YBR085C-A F	GTGTCGACATGTCATCTGCTCTATAC
YBR085C-A R	GTGCGGCCGCCACTCGTTCAAGCCTGGC
PGM2 F	GTGTCGACATGTCATTTCAAATTGAAAC
PGM2 R	GTGCGGCCGCTTAAGTACGAACCGTTG
TMA10 F	GTGTCGACATGACCAGAACTAGCAAATG
TMA10 R	GTGCGGCCGCTTAGATGTGGTATTGTTGC
PRX1 F	GTCCTAGGATGTTTAGTAGAATTTGTAGCG
PRX1 R	GTGGCCGGCCTTATTTCGACTTGGTGAATC
HSP26 F	GTCCTAGGATGTCATTTAACAGTCC
HSP26 R	GTGGCCGGCCGTCACTTTAGTTACC
PLB1 F	GTCCTAGGATGAAGTTGCAGAGTTTG
PLB1 R	GTGGCCGGCCCTAAATTAGACCGAAGAC
ACH1 F	GTCCTAGGATGACAATTTCTAATTTGTTAAAGC
ACH1 R	GTGGCCGGCCCTAGTCAACTGGTTCC
TPS1 F	GTCCTAGGATGACTACGGATAACGCTAAG
TPS1 R	GTGGCCGGCCTCAGTTTTTGGTGGCAGAG
ATR1 F	GTGTCGACATGGGCAATCAGTCATTAG
ATR1 R	GTGCGGCCGCCTAAGCCACAGTGCAATC
CSG2 F	GTGTCGACATGTCTACCACACTACTTTG
CSG2 R	GTGCGGCCGCCTAATGGTGGTATTTACC
PIM1 F	GTCCTAGGATGCTAAGAACAAGAACCAC
PIM1 R	GTGGCCGGCCTTAGTCCTTTTCCTTTTTAG
ADD37 F	GTCCTAGGATGGCTATTAAACCAACCAAAAG
ADD37 R	GTGGCCGGCCTTAATTAGGCATCGCA
YAP1 F	GTCCTAGGATGAGTGTGTCTACCGCCAAG
YAP1 R	GTGGCCGGCCTTAGTTCATATGCTTATTC
VTI1 F	GTGTCGACATGAGTTCCCTATTAATATC
VTI1 R	GTGCGGCCGCTTATTTAAACTTTGAGAAC
CTF18 F	GTCCTAGGATGGTTGATACCGCACCATAC
CTF18 R	GAGGCCGGCCTTATTCCCACAGGTTATTCC

RER2 F	GTCCTAGGATGGAAACGGATAGTGGTATAC
RER2 R	GTGGCCGGCCCAGACCTTTCTTAATTCAAC
ALG7 F	GTCCTAGGATGTTGCGACTTTTTCACTGGC
ALG7 R	GTGGCCGGCCTCAACGTACTGTCCATAG
NTH1 F	GTGTCGACATGAGTCAAGTTAATACAAG
NTH1 R	GTGCGGCCGCTCTCAACTATAGTCCATAG
UBC7 F	GTGTCGACATGTCGAAAACCGCTCAGAAAC
UBC7 R	GTGCGGCCGCTCAGAATCCTAATGATTTC
ATG8 F	GTGTCGACATGAAGTCTACATTTAAGTCTG
ATG8 R	GTGCGGCCGCCTACCTGCCAAATGTATTTTC
PRE8 F	GTCCTAGGATGACCGACAGATATTC
PRE8 R	GTGGCCGGCCTCATAATGCTTCTAATC
TFC1 F	GTGTCGACATGCCAGTGGAGGAGCCTC
TFC1 R	GTGCGGCCGCTTACAGATCGACTTCATC
PRE7 F	GTGTCGACATGGCCACTATTGCATCAG
PRE7 R	GTGCGGCCGCTTAATCTCTTTTTAGCTC
ALG14 F	GTGTCGACATGAAAACGGCCTACTTGG
ALG14 R	GTGCGGCCGCTTAAACAAGGATGCC
PRE5 F	GTGTCGACATGTTCAGGAACAATTAC
PRE5 R	GTGCGGCCGCCTAGATGTATTTAGCAAC
UMP1 F	GTGTCGACATGAATATCGTCCCACAAG
UMP1 R	GTGCGGCCGCTTAAATGCCTAATTG
SEC18 F	GTCCTAGGATGTTCAAGATACCTGG
SEC18 R	GTGGCCGGCCTTATGCGGATTGGGTCATC
YMR132C mut F	GAATTATAAGGCTCAATTGAAATTCAACTTTTCAATAACACTGTTTAGCTTGCCTCGTC
YMR132C mut R	TGGTACTCTATTTTGAAGCGTCTACGTTATGTAGATGATACGTTAGTATCGAATCGACAG
YBR242W mut F	ATAAAAAACAATTGTCAAAGAAAGCCTACATAAAAAAAATCTGTTTAGCTTGCCTCGTC
YBR242W mut R	CTTTGTACTTTTATGACGCAAAAGTTGGTATTTATGATAACGTTAGTATCGAATCGACAG
PRE7 mut F	AGAGTAGCCAAGACTATTGAACTATAAAGTTAAACAAAATCTGTTTAGCTTGCCTCGTC
PRE7 mut R	GATATTGGTCTATTTCTTTTTATACTATGATATGTATGCACGTTAGTATCGAATCGACAG

ALG7 mut F	AGTAGAGCAAGGCGGAGAACGGTAACAAAAAGTAGACTATCTGTTTAGCTTGCCTCGTC
ALGL7 mut R	TGCGTCATAAAAGTACAAAGTAACTACCAATACATAATCTCGTTAGTATCGAATCGACAG
YBR085C-A mut F	CTCAAGTTACCAACTACAATAAATTACATTATACTATAACCTGTTTAGCTTGCCTCGTC
YBR085C-A mut R	CATGAAGTGACTCGTATCGAACAAATATTTAGCCACTCGTCGTTAGTATCGAATCGACAG
ALG7 mut F2	GTAGAGCAAGGCGGAGAACGGTAACAAAAAGTAGACTATGATTGTACTGAGAGTGCACCACGCTTTTCAA
ALG7 mut R2	GCGTCATAAAAGTACAAAGTAACTACCAATACATAATCTTTAGTTTTGCTGGCCGCATCTTCTCAAATATG
PRE7 mut F2	GAGTAGCCAAGACTATTGAACTATAAAGTTAAACAAAATCTCGAGGAGAACTTCTAGTATATCTACATAC
PRE7 mut R2	GATATTGGTCTATTTCTTTTTATACTATGATATGTATGCATTAAGCAAGGATTTTCTTAACTTCTTCGGC
HAC1 RT-PCR F	CCGTAGACAACAACAATTTG
HAC1 RT-PCR R	CATGAAGTGATGAAGAAATC