SUPPLEMENTAL INFORMATION

Supplemental Figures

Figure S1, related to Figure 1. Mutations to ISW2 complex subunits extend lifespan.

Yeast replicative lifespan experiments were carried out for strains bearing deletions for *ITC1* (A), *DLS1* (B), *DPB4* (C), or a catalytically inactive allele *isw2-K215R* (D). See replicative lifespan statistics in Table S1.

Figure S2, related to Figure 3. The DNA damage response and stress response pathways, but not the Ty1 retrotransposition, is involved in aging and calorie restriction.

(A) Quantification of Ty1 genome copy number by real-time PCR for genomic DNA samples extracted from young and old cells prepared in non-restricted (NR, 2% glucose) and calorie restricted (CR, 0.05% glucose) media. (B) Quantification of Ty1 expression levels by real-time PCR for the cDNA derived from total RNA extracted from young and old cells prepared in non-restricted (NR, 2% glucose) and calorie restricted (CR, 0.05% glucose) media. Three pairs of real-time PCR primers targeting different regions of the Ty1 transcript were used in this experiment. (C) Ty1 *de novo* integration patterns were compared between cells cultured under NR or CR (0.5% and 0.05% glucose) conditions. The plasmid pVIT41 bearing a uniquely marked copy of Ty1 under the control of a GAL promoter and the control plasmid pNB19 bearing a copy of Ty1 of the same design but with a mutant integrase (IN) were transformed into WT and *isw2*Δ cells. Transformed cells were grown in selective media containing either dextrose (Dex) as a control or galactose (Gal) for Ty1 induction for 2 days. Genomic DNA was then extracted and de novo Ty1 integration was assessed by PCR. (D) Yeast replicative lifespan assay was carried out for wild-type Bdp1, or various Bdp1 truncation mutants. See lifespan statistics in

Table S1. (E) Histogram for RNA-seq analysis showing changes in anti-sense transcription in old cells compared to young cells. (F) Statistically significant overlap between genes derepressed more than 1.5 folds in *isw2*Δ (Fazzio et al., 2001) and genes showing Isw2 localization at promoters or ORF regions (Whitehouse et al., 2007). (G) Gene ontology (GO) analysis with GO clustering was performed for the list of genes up-regulated by 1.5 folds in strains deleted for *isw2*Δ comparing to wild-type (Fazzio et al., 2001). The bar graph shows the most significantly enriched GO clusters and their enrichment scores. See also Tables S2. (H) GO clustering analysis for genes showing Isw2 localization at promoters or ORF regions. Only GO categories with a p-value <0.01 were included in calculation of enrichment score.

Figure S3, related to Figure 3. Deletion of *ISW2* or calorie restriction increases cellular resistance to DNA damaging agents, methyl methanesulfonate (MMS) and camptothecin (CPT), an effect dependent on *RAD51*.

(A) Expression of *RAD51* and control genes in wild-type (WT) and *isw2*Δ cells cultured by real-time PCR analysis of cDNA derived from extracted RNA. Expression levels were normalized to *ACT1* gene. Error bars, standard error of the mean (SEM). Statistical *p*-value by student t-test, n=7. (B) Chromatin immunoprecipitation assay with anti-FLAG antibody (Sigma, F3165) for the FLAG-tagged wild-type (WT) and the FLAG-tagged catalytic mutant *isw2-K215R* cells at Isw2 regulated genomic loci as well as control loci. Data are expressed as percentage input. Error bars, SEM. (C) Yeast phenotype assay for independent isolates of various *isw2*Δ alleles on non-restricted (NR, 2% glucose) and calorie restricted (CR, 0.05% glucose) synthetic complete (SC) media with or without 0.04% MMS. Equal amount of cells were serial-diluted by 5 folds and spotted on media as shown. Plates were incubated at 30 °C for 2-4 days

before pictures were taken. (D) Yeast phenotype assay to test resistance to CPT. Equal amount of cells were serial-diluted by 5 folds and spotted on non-restricted (NR, 2% glucose) and calorie restricted (CR, 0.05% glucose) SC media with or without 250 µg/ml CPT as shown. Plates were incubated at 30 °C for 2-4 days before pictures were taken.

Figure S4, related to Figure 4. RNA-seq reveals gene expression change under CR conditions.

Histogram showing distribution of gene expression changes for cells grown in CR compared to NR conditions.

Figure S5, related to Figure 5. Examples nucleosome-seq tracks showing similar nucleosome positioning changes

Examples nucleosome-seq tracks showing similar nucleosome positioning changes in WT CR and *isw2*Δ NR, at stress response genes up-regulated in both CR and *isw2*Δ cells (A), Isw2-regulated genes (B), and genes showing Isw2-dependent chromatin remodeling (C). Arrows and shaded areas highlight altered nucleosomes.

Figure S6, related to Figure 6. Test of conservation for Isw2 regulated aging pathway.

(A-B) Deletion of *ISW2* delays replicative senescence in yeast *tlc1*Δ cells. Two representative pairs of individual haploid spore products from *TLC1/tlc1Δ ISW2/isw2Δ* diploid cells, which illustrate the dramatic change in cell density upon senescence and the significant delay for senescence in *isw2*Δ *tlc1*Δ compared to *tlc1*Δ cells. (C) Multiple alignment for worm ATHP-2 and Itc1/ACF1 orthologs showing a strong similarity. (D) Relative mRNA expression in human fetal lung fibroblast IMR90 for the 10 tested human homologs of yeast stress response

genes up-regulated in both *isw2*Δ and CR. See also Tables S4. (E) Relative mRNA expression in human fetal lung fibroblast IMR90 for the 13 tested genes, whose promoters are bound by SMARCA5. See also Tables S6. * $p<0.05$.

Supplemental Tables

Table S1, related to Figures 1, 2, 3, S1, and S2. Statistical data for yeast replicative lifespan experiment in this study.

See supplemental Excel file: Table S1.xlsx

Table S2, related to Figures 3 and S2. Isw2 repressed genes directly involved in stress response pathways based on functional annotation from SGD.

Stress response pathways	**Genes up-regulated by at least 1.5 fold in isw2Δ cells**
Autophagy	*ATG15, ATG2, LAP4, ATG1*
DNA damage response	*RAD51, DDI1, DUN1, HSM3, IMP2', RNR3, DDR48, CRT10, PIN4, HIM1,*
Heat shock response	*HSP30, HSP104, SSE2, HSP42, GRE3*
Osmotic stress response	*FMP43, YML131W, HOR2, HLR1, MSN1, YBR016W, GRE3*
Oxidative stress response	*RCK1, HOR2, PRX1, OYE3, GRE3, MAE1, CSR1, POS5*
Proteosome and ubquitination	*UBX3, SDS24, CDC26, ASI3, UBX4*
Protein degradation in vacuole	*LAP4, PRB1*

Table S3, related to Figure 4. Upper and lower 1-SD cutoffs used to generate the list of genes for GO analysis for the CR and NR RNA-seq dataset.

	log2(CR/NR)	**CR/NR**
Mean	0.0411	1.0289
SD	1.1298	2.1883
Lower 1SD Cutoff	-1.0887	0.4702
Upper 1SD Cufoff	1.1709	2.2516

Table S4, related to Figures 4, 6, and S6. List of common genes in up-regulated stress response GO clusters in *isw2*Δ cells grown under normal conditions and wild-type cells grown under CR conditions. *Human homologs selected to test expression upon BAZ1A knockdown.

ORF	Gene	Description (retrieved from SDG)	Human homolog
YBL078C	ATG8	Component of autophagosomes and Cvt vesicles; undergoes conjugation to phosphatidylethanolamine (PE); Atg8p-PE is anchored to membranes, is involved in phagophore expansion, and may mediate membrane fusion during autophagosome formation	GABARAPL1* GABARAPL2
YBR016W		Tail-anchored plasma membrane protein containing a conserved CYSTM module; predicted to be palmitoylated; has similarity to hydrophilins, which are involved in the adaptive response to hyperosmotic conditions	CYSTM1*
YBR132C	AGP2	High affinity polyamine permease, preferentially uses spermidine over putrescine; expression is down-regulated by osmotic stress; plasma membrane carnitine transporter, also functions as a low-affinity amino acid permease	SLC7A13 SLC12A1
YDL091C	UBX3	UBX (ubiquitin regulatory X) domain-containing protein that interacts with Cdc48p, green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm in a punctate pattern	FAF1* FAF2
YDR096W	GIS1	JmjC domain-containing histone demethylase and transcription factor; involved in expression of genes during nutrient limitation; negatively regulates DPP1 and PHR1; activity is modulated by limited proteasome-mediated proteolysis; has a JmjC and a JmjN domain in the N-terminal region that interact, promoting Gis1p stability and proper transcriptional activity; contains transactivating domains TAD1 and TAD2 downstream of the Jmj domains and a C-terminal DNA binding domain	KDM4B* KDM4A KDM4DL
YER150W	SPI1	GPI-anchored cell wall protein involved in weak acid resistance; basal expression requires Msn2p/Msn4p; expression is induced under conditions of stress and during the diauxic shift; similar to Sed1p	WWP1*
YFR053C	HXK1	Hexokinase isoenzyme 1, a cytosolic protein that catalyzes phosphorylation of glucose during glucose metabolism; expression is highest during growth on non-glucose carbon sources; glucose-induced repression involves the hexokinase Hxk2p	HKDC1 HK3 GCK
YJR096W		Putative xylose and arabinose reductase; member of the aldo-keto reductase (AKR) family; GFP-fusion protein is induced in response to the DNA-damaging agent MMS	AKR1C3*
YLR178C	TFS1	Protein that interacts with and inhibits carboxypeptidase Y and Ira2p; phosphatidylethanolamine-binding protein (PEBP) family member; targets to vacuolar membranes during stationary phase; acetylated by NatB N-terminal acetyltransferase	PEBP1*
YLR206W	ENT2	Epsin-like protein required for endocytosis and actin patch assembly and functionally redundant with Ent1p; contains clathrin-binding motif at C-terminus	EPN3 EPN2

YLR327C	TMA10	Protein of unknown function that associates with ribosomes; putative homolog of the F1F0-ATPase synthase regulator Stf2p	
YML131W		Putative protein of unknown function with similarity to medium chain dehydrogenase/reductases; expression induced by stresses including osmotic shock, DNA damaging agents, and other chemicals; GFP-fusion protein localizes to the cytoplasm	PTGR1*
YMR191W	SPG5	Protein required for survival at high temperature during stationary phase; not required for growth on nonfermentable carbon sources	HSP90B1*
YOR134W	BAG7	Rho GTPase activating protein (RhoGAP), stimulates the intrinsic GTPase activity of Rho1p, which plays a role in actin cytoskeleton organization and control of cell wall synthesis; structurally and functionally related to Sac7p	GMIP*

Table S5, related to Figures 6. Statistical data for worm lifespan experiment in this study.

Figure	*athp-2* KD Lifespan	*athp-2* KD # of Worm	Control KD Lifespan	Control KD # of Worm	*p*-value
6B	27.8	406	24.1	394	1.97E-14
6C	25.7	105	22.9	113	1.40E-03

Table S6, related to Figures 6 and S6. List of SMARCA5-bound genes forming the stress response GO cluster (54). *Gene selected to test expression upon BAZ1A knockdown.

SMARCA5-bound genes forming the stress response GO cluster
APOA4, BARD1, BBC3, C17ORF70, CCND1, CEP63, CLPB*, CRY2, DDB2*, EIF2AK3*, EIF2B4, ERCC1*, ERN1*, ERO1L, ESCO1, FADS1, FBXO18, FBXO31, GNL1, H2AFX*, HIPK1, HSPA5*, JUN*, MAP4K3*, MAP4K5, MAPK14, MAPK8IP3, MRPS35, MYO6, NBN, NSMCE2, NUAK2, OGG1, PAPD7, PHLDA3, POLG2, POLH*, PPP1R15B, PRDX1*, PRMT6, RAD9A*, RB1CC1, RBM14, RBM38, RECQL4, RRM2B, SLC11A2, TERF2IP*, TRPV4, TYMS, VAPB, WRNIP1, XPA, XPC

Table S7, related to experimental procedures. Yeast strains used in this study.

See supplemental Excel file: Table S7.xlsx

Table S8, related to experimental procedures. Oligonucleotides used in this study.

See supplemental Excel file: Table S8.xlsx

Supplemental Experimental Procedures

Quantification of Relative Ty1 Genome Copy Number

Yeast genomic DNA was extracted and purified as the main text. All genomic DNA samples were diluted 10 folds before quantitative real-time PCR. Results were shown as the average levels detected by the three Ty1 primer pairs (Ty1-1, Ty1-2, and Ty1-3) normalized to the average levels detected by control primer pairs (*ACT1* and *IntV*). Primer sequences used in this study are listed in Table S8.

PCR Assay for *de novo* Ty1-targeted Integration

Ty1-targeted integration detected by PCR was performed exactly as described previously (Bachman et al., 2005). Primer sequences used in this study are listed in Table S8.

Chromatin Immunoprecipitation (ChIP)

Chromatin Immunoprecipitation assay was performed as described previously (Dang et al., 2009) with the following modifications. Protein G Dynabeads (Invitrogen) was used instead of Protein G agarose beads. Prior to immunoprecipitation, 2 μg of FLAG antibody (Sigma, F3165) was attached to the beads by incubating with beads from a 20 μl suspension in 20 μl blocking solution (0.5% w/v BSA in 1× PBS). Primer sequences used in this study are listed in Table S8.

Supplemental References

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