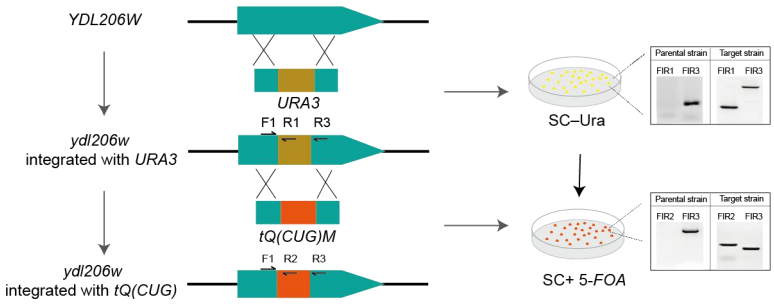
a

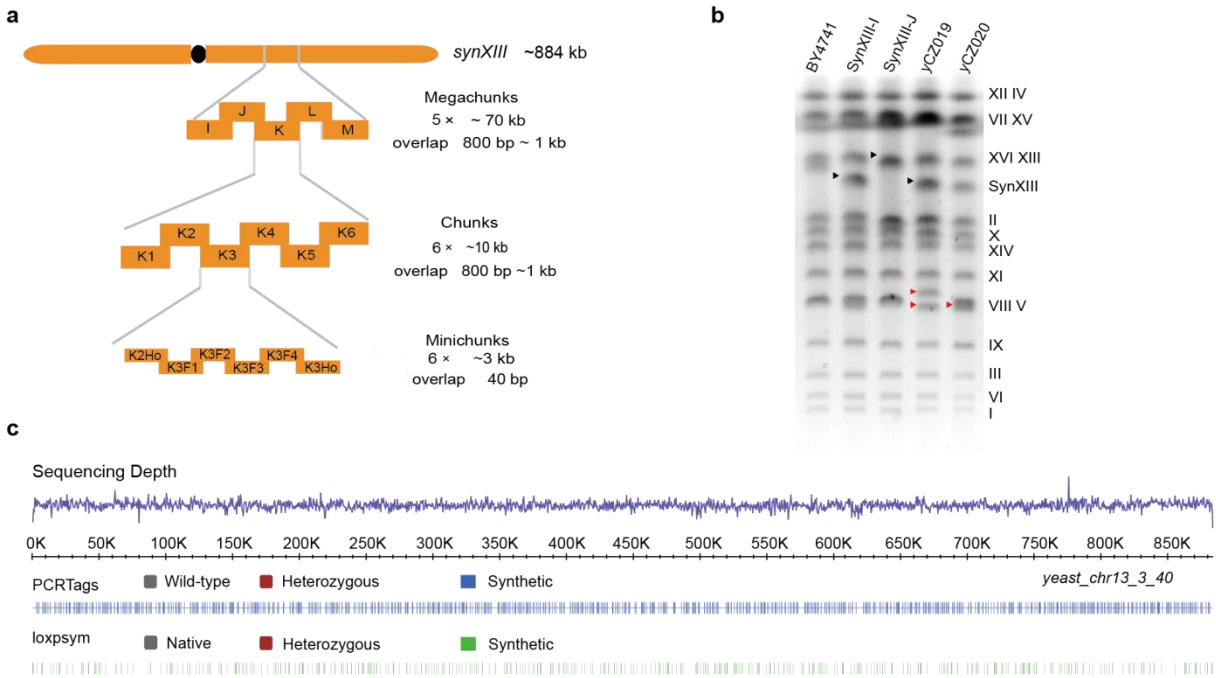
Information for *chrXIII* tRNAs

Gene	Number of tRNA on *chrXIII*	Number of tRNA on others
tY(GUA)	2	7
tQ(CUG)	1	0
tG(GCC)	1	15
tD(GUC)	1	16
tP(UGG)	1	10
tS(AGA)	1	10
tR(UCU)	2	10
tK(UCU)	1	13
tM(UCU)	1	11
tV(AAC)	3	11
tA(AGC)	2	9
tW(CCA)	1	5
tE(UUC)	1	14
tF(GAA)	1	10
tH(GUG)	1	7
tL(CAA)	1	9

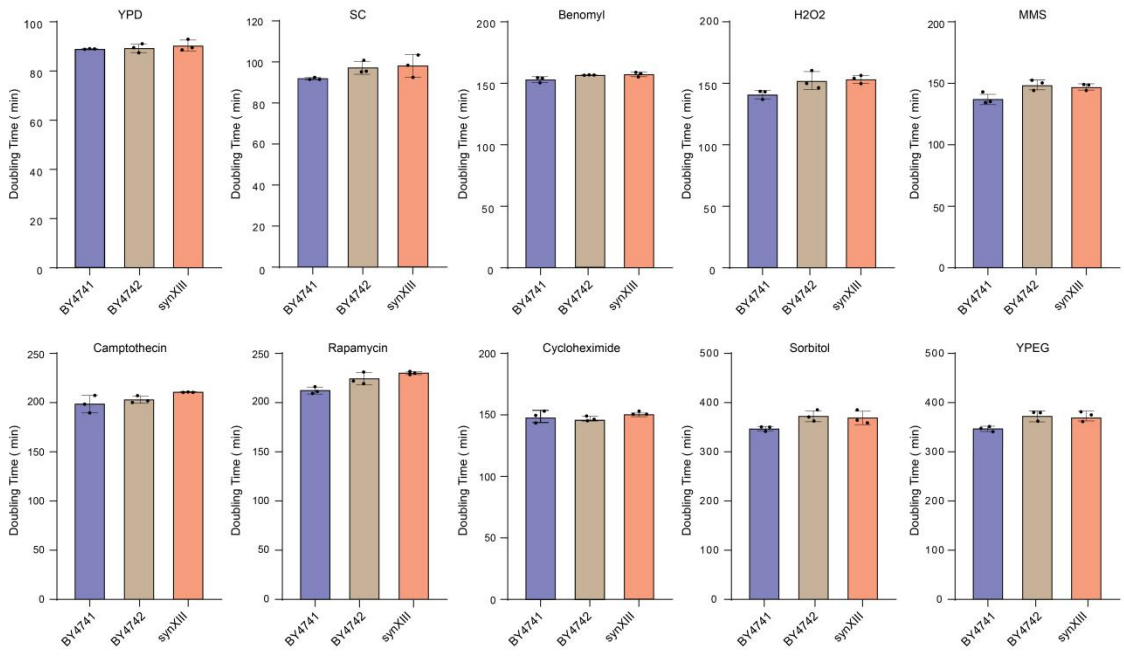
b



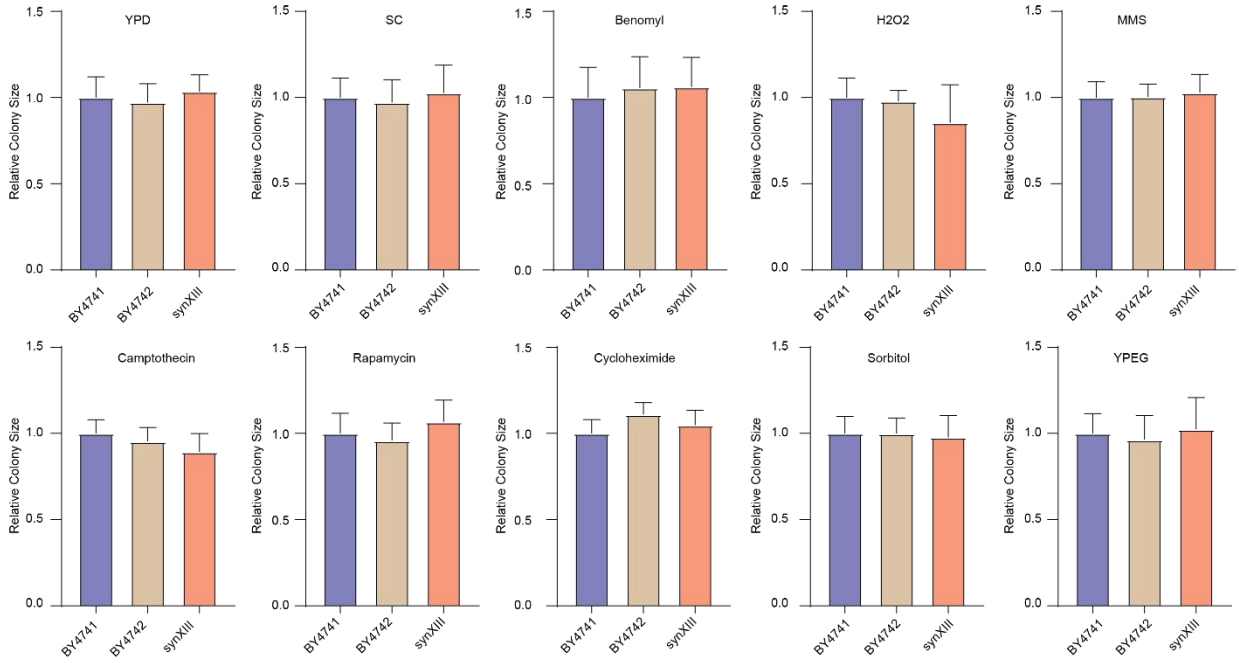
Supplementary Fig. 1 Summary of tRNAs in *chrXIII* **a** Summary of tRNA in *chrXIII* and compared with other 15 chromosomes. **b** The design of *tQ(CUG)* relocation into *YDL206W*.



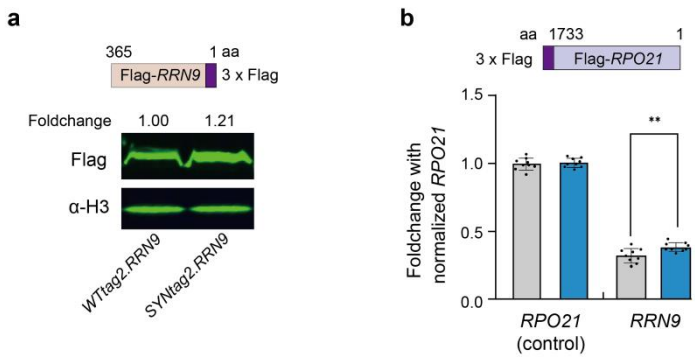
Supplementary Fig. 2 Synthetic chromosome XIII construction **a** The intact *synXIII* chromosome are segmented stepwise into ~50 kb megachunks (as K, L, M), further divided into ~10kb chunks (such as K1, K2, K3) and further divided into ~3 kb minichunks (such as K1FI, K1F2) according to the experimental design. More details are provided in Supplementary text. **b** Pulsed-field gel electrophoresis demonstrated the karyotype in synXIII strains compared with wildtype. The black arrow indicated the *synXIII* chromosome. The red indicated chrV and chrVIII. **c** Whole genome sequencing analysis of synthetic chromosome XIII. The sequencing depth indicates its average in 500-bp windows. The PCRtags and loxPsym site were identified by the sequencing reads containing its synthetic-specific sequence (supporting read ≥ 5).



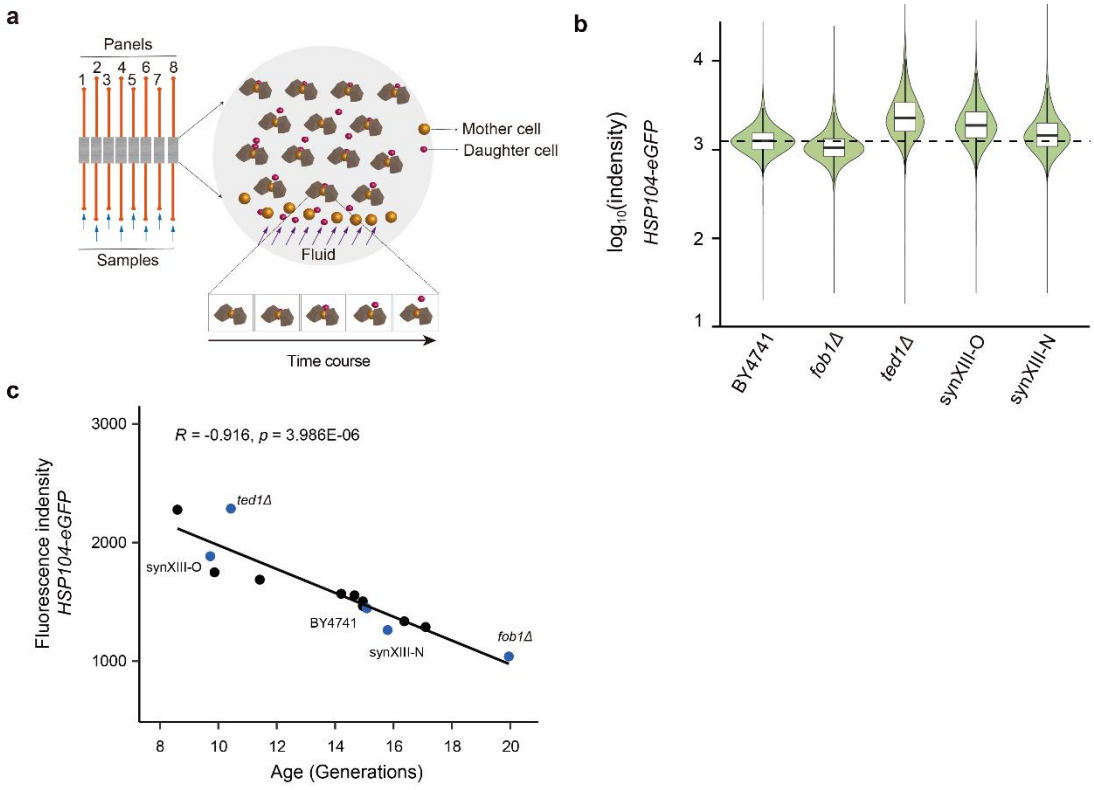
Supplementary Fig. 3. Doubling times of corresponding strains in different medium ($n = 3$). The data are presented as the mean and SD. Source data are provided as a Source Data file. Dilutions of overnight cultures of synXIII and wild-type (BY4741 and BY4742) strains to OD= 0.1, were used for growth curve for 24h. Conditions include: YPD; SC ;YPD + Benomyl; YPD + H_2O_2 (1 mM, 2 h pretreatment); YPD + methyl methane sulfone (MMS, 0.01% v/v); YPD + Camptothecin (0.1 μg/mL); YPD + Rapamycin (0.2 μg/mL); YPD + Cycloheximide (10 μg/mL, 2 h pretreatment); YPD + Sorbitol (1 M); YPD, yeast extract peptone dextrose; YPEG, yeast extract peptone glycerol ethanol; SC, synthetic complete. Source data are provided as a Source data file.



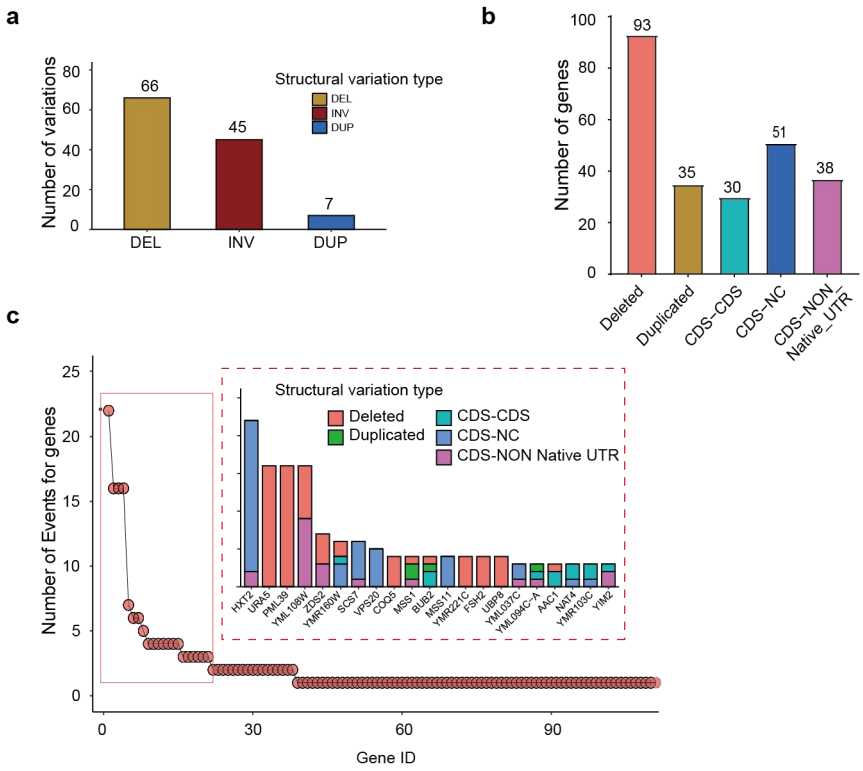
Supplementary Fig. 4. Colony size analysis of corresponding strains under different conditions. The average colony size of BY4741 was set to 1.0. The data are presented as the mean and SD. Source data are provided as a Source Data file. ~300 colonies of synXIII and wild-type (BY4741 and BY4742) strains were plated on different conditions. Conditions include: YPD; SC; YPD + Benomyl; YPD + H_2O_2 (1 mM, 2 h pretreatment); YPD + methyl methane sulfone (MMS, 0.01% v/v); YPD + Camptothecin (0.1 μg/mL); YPD + Rapamycin (0.2 μg/mL); YPD + Cycloheximide (10 μg/mL, 2 h pretreatment); YPD + Sorbitol (1 M); YPD, yeast extract peptone dextrose; YPEG, yeast extract peptone glycerol ethanol; SC, synthetic complete. Source data are provided as a Source data file.



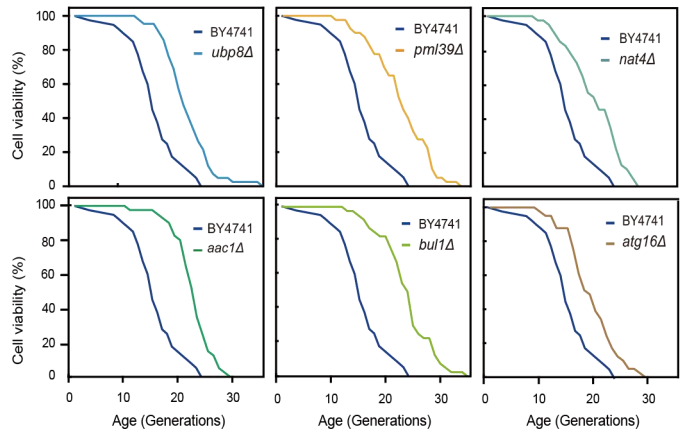
Supplementary Fig. 5. Quantitative analysis of RRN9 **a** The corresponding expression level of Rrn9 protein with wildtype and synthetic PCRTag2 by immunoblot. WTtag2 RRN9, expressed from the locus RRN9 in BY4741; SYNTag2 RRN9, expressed from the locus RRN9 with the synthetic PCRtag2 replacing the native PCRtag in BY4741; Protein level was quantified as a fold change. **b** RNAPII-ChIP analyses at *RRN9* by RT-PCR. The fold change of RNAPII ratios was normalized to levels of *RPO21* with 3 biological replicates and repeated 3 times. The statistical confidence is calculated by one-sided T-test, and error bars represent SD.Source data are provided as a Source data file.



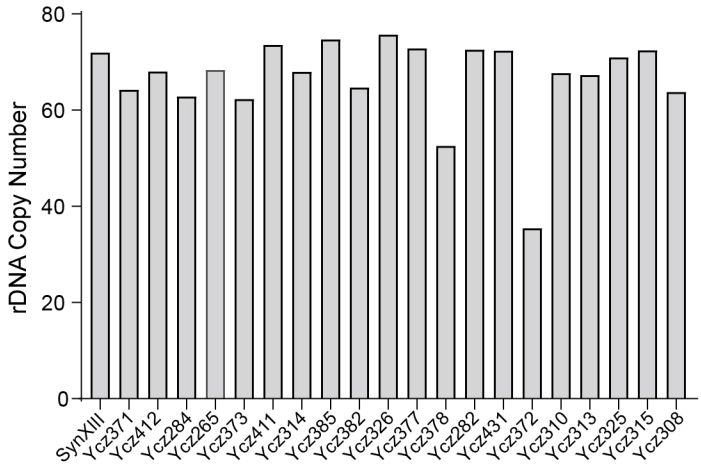
Supplementary Fig. 6 Lifespan measurement using *HSP104* as an aging biomarker. **a** Schematic for microfluidic system for automating lifespan measurement. Each chip with 8 micro-channels (left) is sealed on a thin glass slide (bottom). Geometries of the channels (red) and chambers (brown). Arrows show the directions of fluid flow, the mother cell with the bigger size stuck by chamber and the daughter cell washed away by fluid. The divisions of mother cell represent its replicative lifespan. **b** Comparison of *HSP104-eGFP* expression for BY4741, *ted1Δ*, *fob1Δ,* synXIII-O and synXIII-N. The *ted1Δ* and *fob1Δ* strains were constructed by using *URA3* to replace the ORF region of *TED1* and *FOB1*. The fluorescence intensity of *HSP104-eGFP* was measured by FACS (~100,000 cells for each sample).Source data are provided as a Source data file. c The correlation between HSP104-eGFP and the replicative lifespan across genetic variant mutants.Source data are provided as a Source data file.



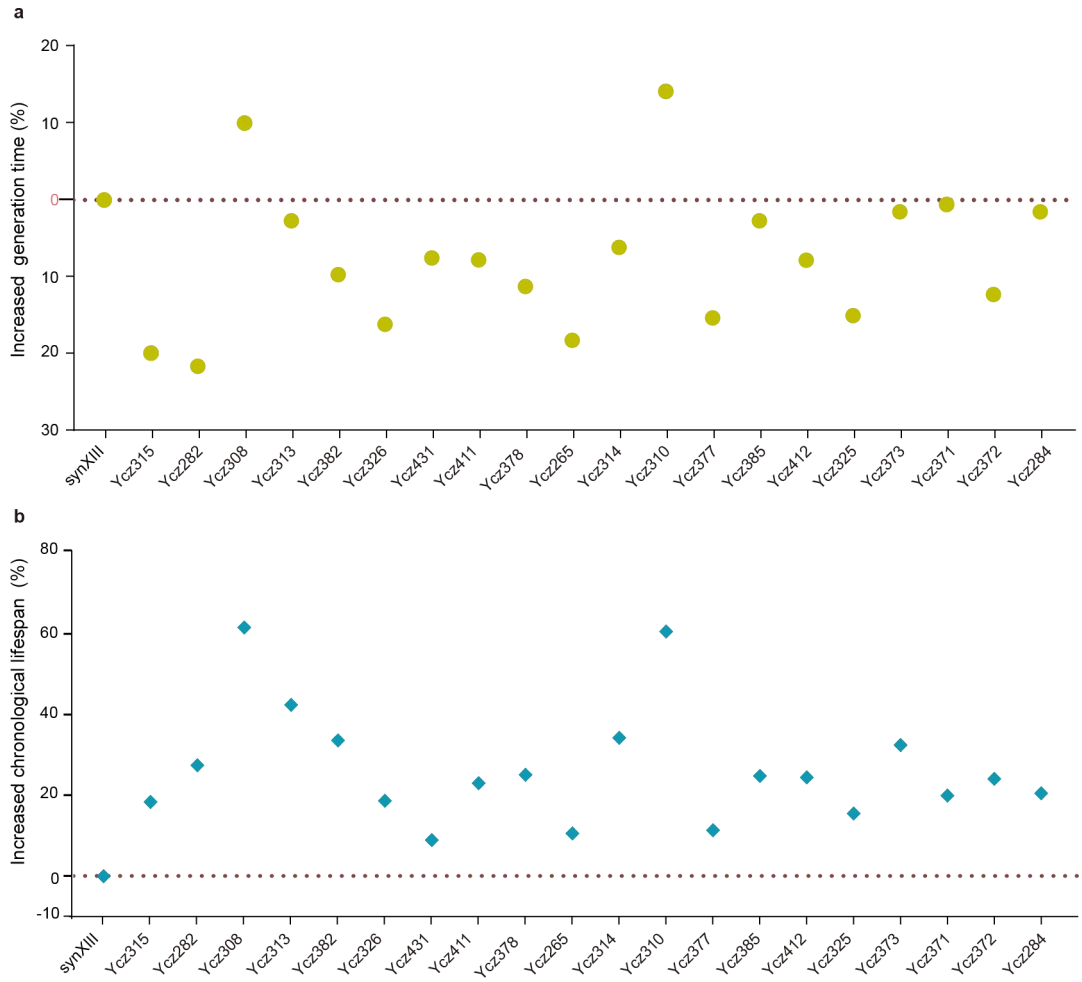
Supplementary Fig. 7 Screening and identification of potential aging regulators from the long-lived SCRaMbLEd *synXIII* strains. **a** Overview of SCRaMbLE structural variants including deletion (DEL), inversion (INV), and duplication (DUP) on *synXIII* chromosome of in total 20 screened strains. **b** Overview of SCRaMbLEd genes involved and the types of variations.Source data are provided as a Source data file. **c** The variant representation per gene (the number of times a variant is represented) in the collection of 20 long-lived SCRaMbLEants. The blue dots represent the selected genes for lifespan measurement in panel D (frequency ≥ 3). The heat map shows the distribution of 5 different variant types for each selected gene. The color indicates the frequency of different rearrangement event types for target genes in these SCRaMbLEd strains. CDS-CDS, CDS-NC, CDS-UTR represent the gene of 3'-UTR with a replacement by a CDS, non-coding (NC) sequence or non-native UTR respectively. Source data are provided as a Source data file.



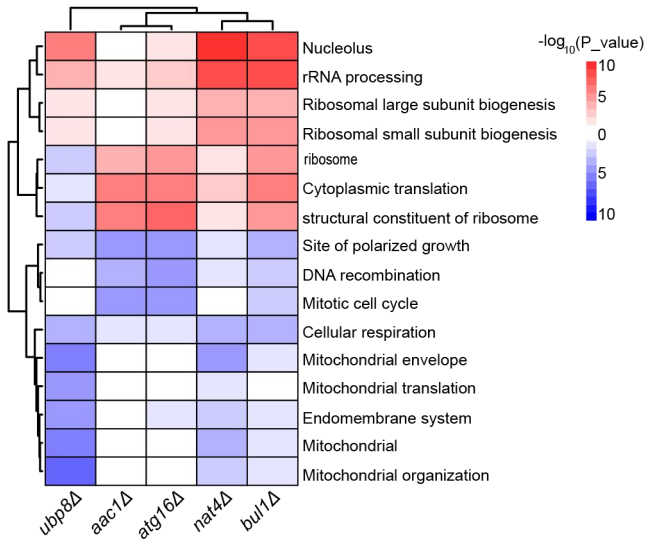
Supplementary Fig. 8 Identification of potential aging regulators from the mutants. Survival curves of BY4741 and each single null mutant strains with three replicates (n = 40 for each replicate).Source data are provided as a Source data file.



Supplementary Fig. 9 Estimation of the corresponding rDNA copy number of *synXIII* and 20 SCRaMbLEd derivatives by relative sequencing depth. Source data are provided as a Source data file.



Supplementary Fig. 10 Analysis of generation time and chronological lifespan of 20 long-lived SCRaMbLEd strain compared to *synXIII*. **a** Increased mean generation times compared to *synXIII*. For each strain, the generation time was calculated by the average of 40 single yeast cell. Source data are provided as a Source data file. **b** Increased mean chronological lifespan compared to *synXIII*. For each strain, the chronological lifespan was calculated by the average of 40 single yeast cell.Source data are provided as a Source data file.

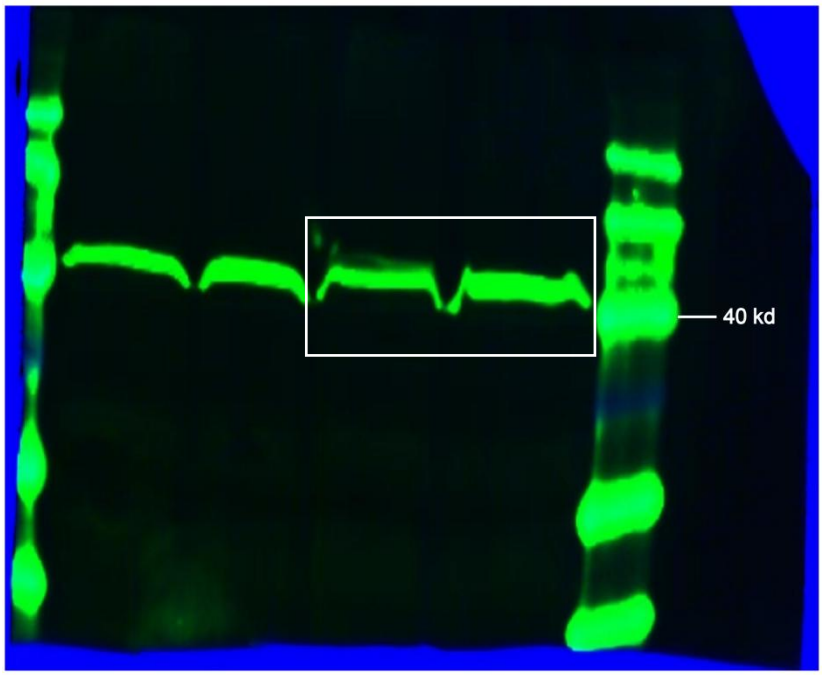


Supplementary Fig. 11 Transcriptome profiling of null mutant strains compared to BY4741. The heatmap represents the statistical significance of Gene Ontology (GO) enrichment of differentially expressed genes: red, up-regulated; blue, down-regulated.Source data are provided as a Source data file.

Supplementary table. Summary of *synXIII* design

Modification	Design feature	Number	Base alteration
Replacement	wild type telomere -> Universal Telomere Cap [a]	2	16880->1378
	629 pairs of WT PCR tags and 629 pairs of SYN PCRTags	1258	15879
	stop codon TAG -> TAA	100	100
Deletion	transposable element region[b]	36	28246
	gene	21	30187
	tRNA	21	1691
	snoRNA	2	175
	intron [c]	17	2707
Insertion	LoxPsym site	333	11322

a. Wild-type telomeres together with its adjacent subtelomeric regions are replaced with universal telomere caps. **b**. Transposable element region include these functional features of LTR retrotransposons, transposable element genes and long terminal repeats. **c**. Nine introns were retained as these introns reside in ribosomal subunit coding genes.



Source data of uncropped scans of immunoblot used in Supplementary Fig. 5a