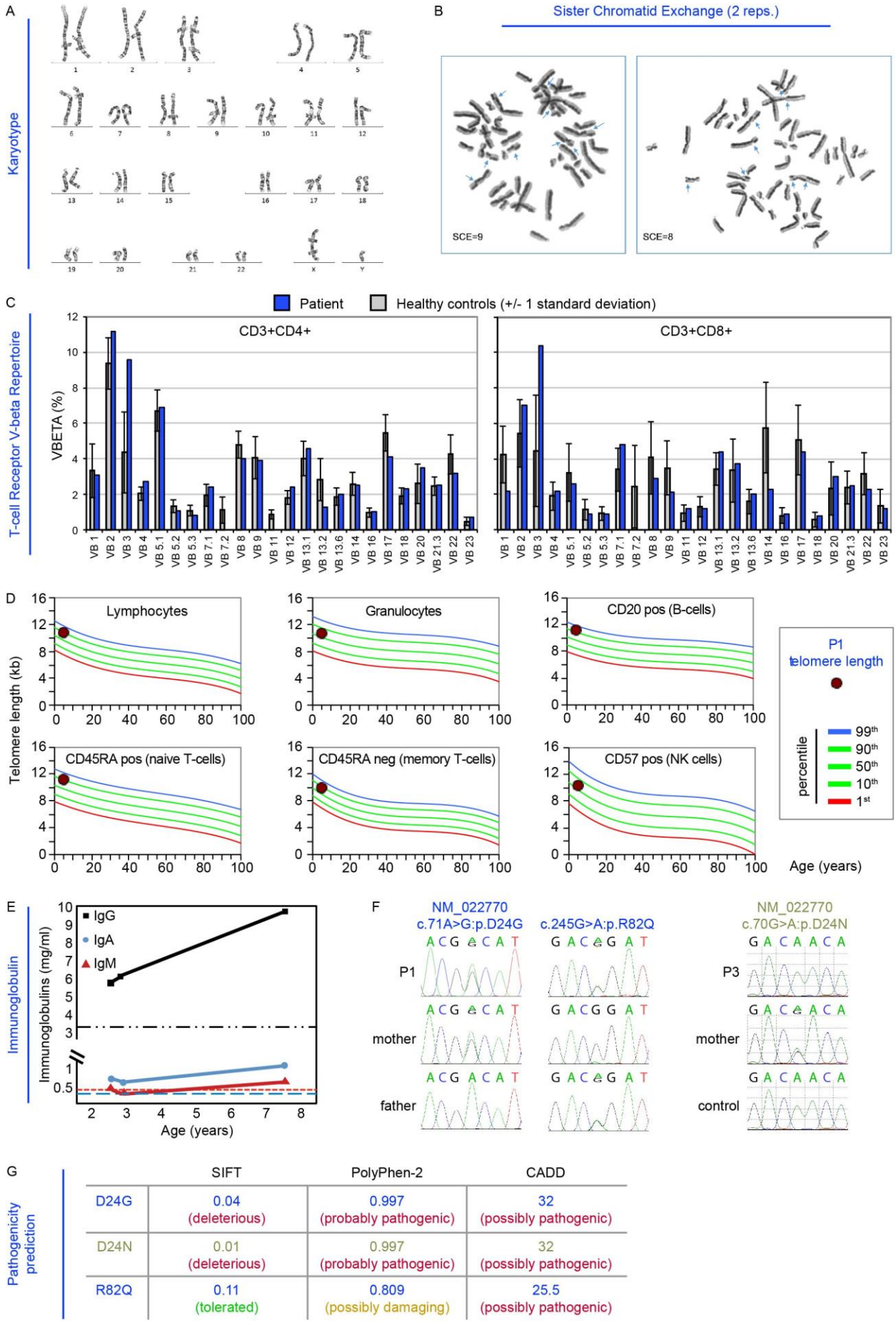
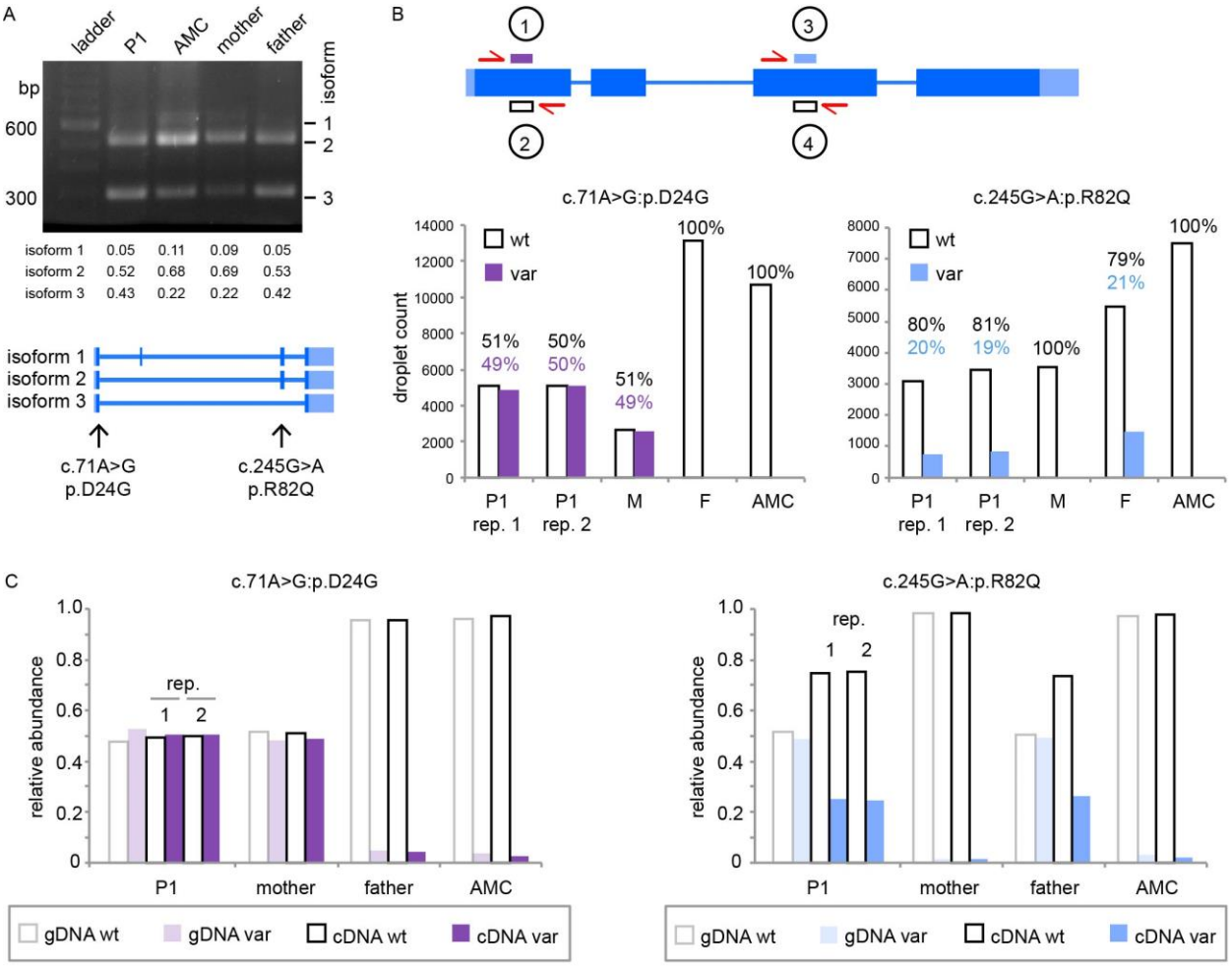
Supplementary material for

McQuaid et al. Hypomorphic GINS3 variants alter DNA replication and cause Meier-Gorlin Syndrome

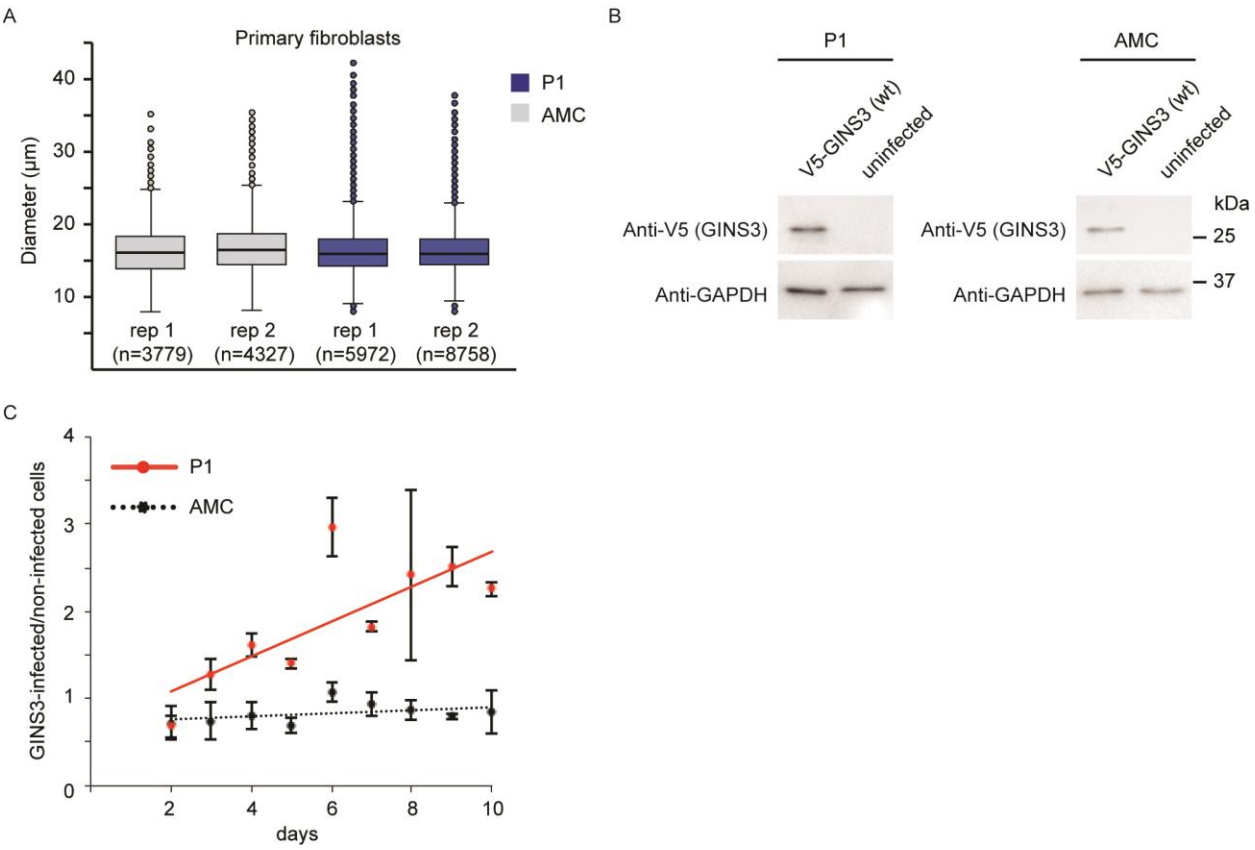


	SIFT	PolyPhen-2	CADD
D24G	0.04 (deleterious)	0.997 (probably pathogenic)	32 (possibly pathogenic)
D24N	0.01 (deleterious)	0.997 (probably pathogenic)	32 (possibly pathogenic)
R82Q	0.11 (tolerated)	0.809 (possibly damaging)	25.5 (possibly pathogenic)

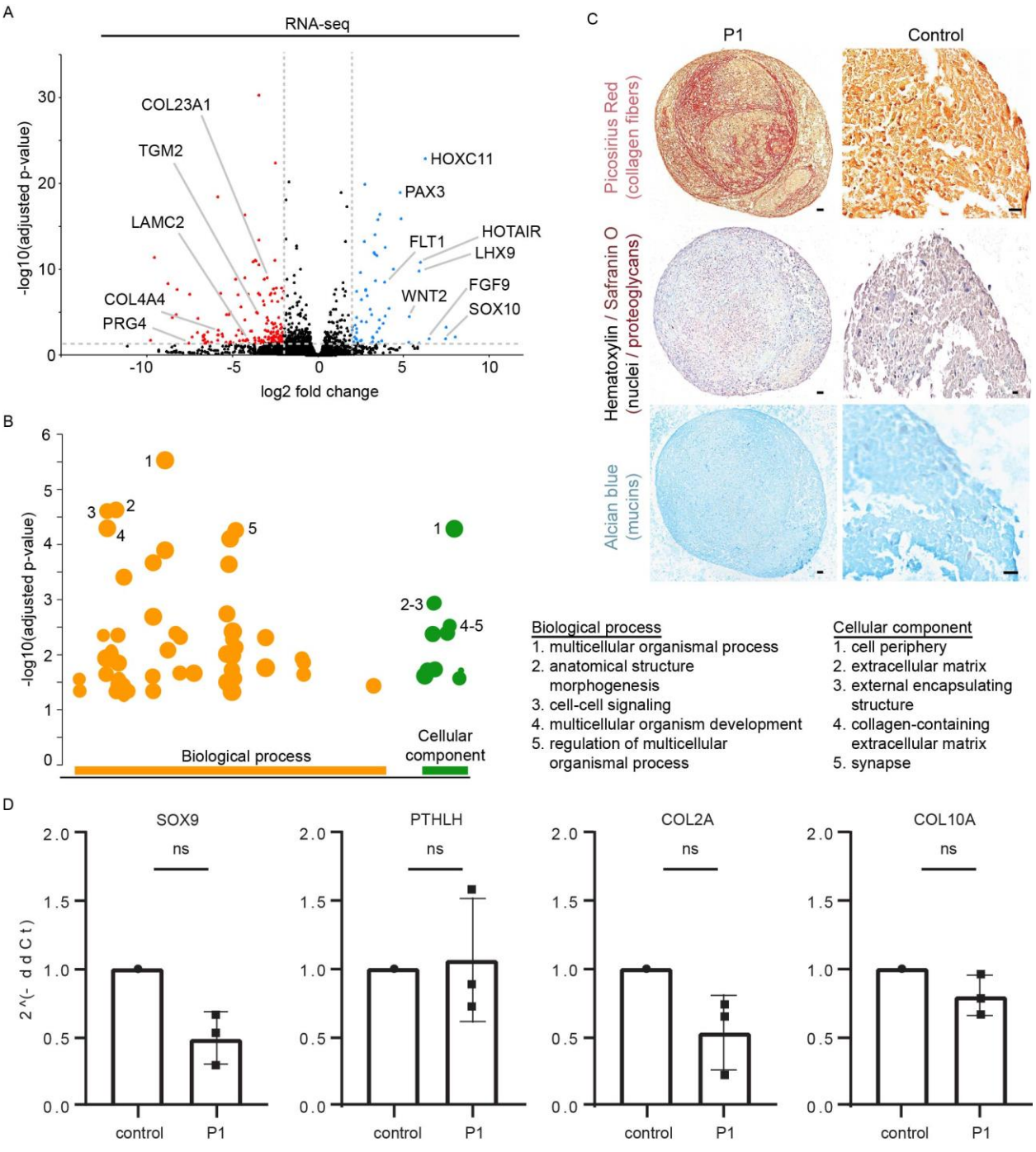
Supplementary Figure 1. Genetic characteristics of the patients. (A-B) Karyotype (A) and sister chromatid exchange (SCE) analyses (B) for P1. (C) Immunological profile for P1. (D) Telomere length analysis for blood cells in P1. Analysis was performed twice; representative results from one analysis are shown. (E) Quantification of blood immunoglobulins for P1. (F) Sanger sequencing showing *GINS3* heterozygosity in P1 and homozygozity in P3. (G) Pathogenicity prediction scores for the *GINS3* variants.



Supplementary Figure 2. Allelic balance in P1 fibroblasts (related to **Figure 3B**). (A) RT-PCR analysis of the 3 *GINS3* splicing isoforms in P1, age-matched control (AMC), or parent-derived primary fibroblasts. Relative abundance was determined using ImageJ. (B) Digital droplet PCR analysis of the c.71A>G (p. D24G) and c.245G>A (p.R82Q) *GINS3* alleles in P1 and parent-derived primary fibroblasts. TaqMan probes in purple and blue (1 and 3) annealed to the c.71A>G:p.D24G and c.245G>A:p.R82Q variant alleles respectively, while the probes in white (2 and 4) annealed to the WT alleles. (C) Genotyping of P1 fibroblasts using MassArray technology (1).

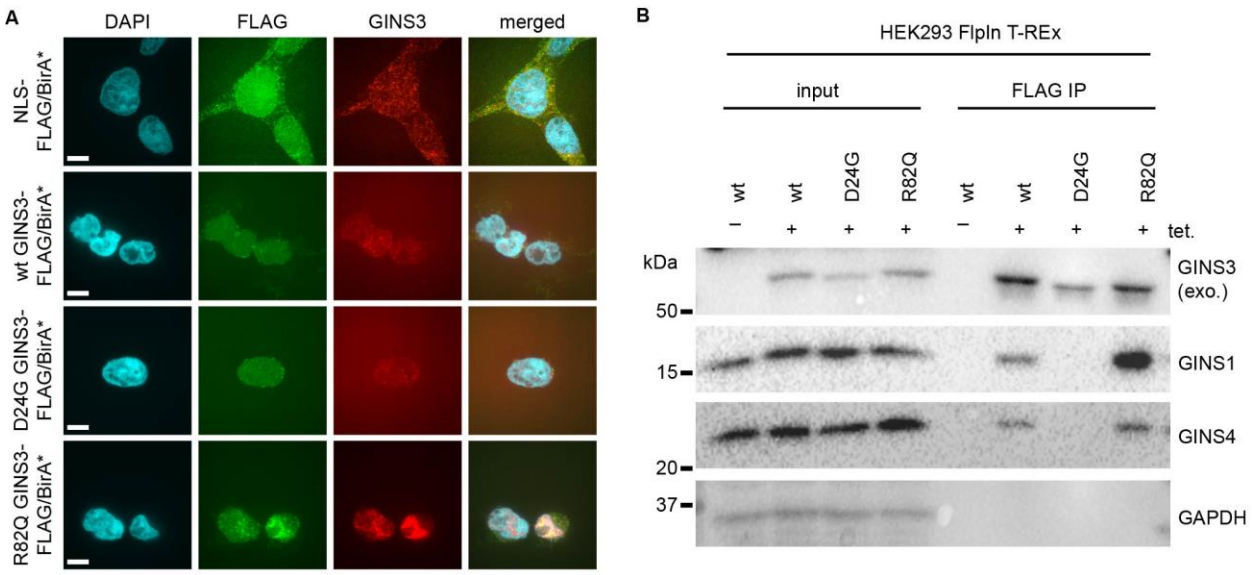


Supplementary Figure 3. Growth characteristics of P1 fibroblasts (related to **Figure 3**). (A) Comparison of cell size in P1 and age-matched control (AMC) fibroblasts. (B) Western analysis of primary fibroblasts infected with lentiviral particles expressing V5-tagged WT GINS3. (C) Proliferation of P1 and age-matched control (AMC) fibroblasts +/- lentiviral delivery of WT *GINS3*. Data was derived from 5 technical replicates and normalized to the first time point.

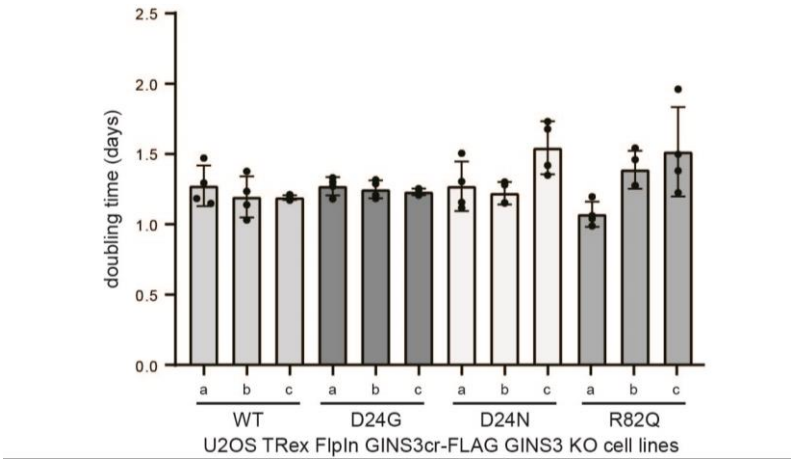


Supplementary Figure 4. Transcriptional changes in P1 do not prevent chondrogenic differentiation. (A) Volcano plot highlighting transcriptional changes observed by RNA-seq, when comparing P1 (n = 3) and control primary fibroblasts derived from both parents (n = 1 for each parental fibroblast line). (B) Gene ontology terms that are enriched in the differentially

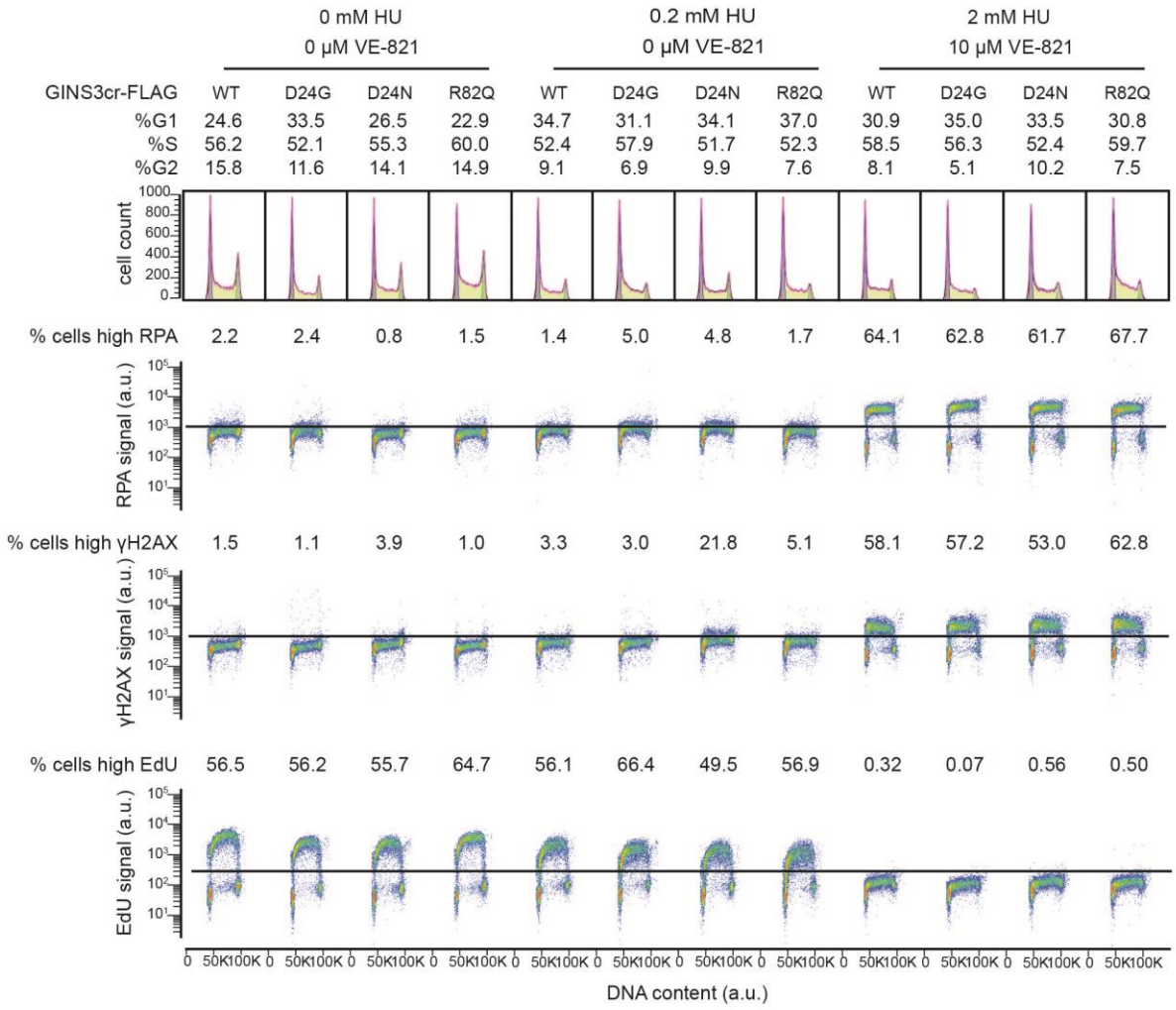
expressed genes. (C) P1 and age-matched control (AMC) fibroblasts were differentiated into chrondrocytes (see **Methods**). Fibroblast-derived chondrogenic pellets were fixed, embedded in paraffin, and stained with chondrogenic markers. Figures are representative of three replicates. Scale bars = 50 μm. (D) RT-qPCR analysis of early (*SOX9*, *PTHLH*) and late (*COL2A*, *COL10A*) chrodrogenic gene expression after chrondrogenic differentiation of primary fibroblasts. P1 expression levels were normalized to that of AMC fibroblasts.



Supplementary Figure 5. Co-immunoprecipitation of endogenous GINS1 with exogenous GINS3 is altered when exogenous GINS3 carries the D24G or R82Q mutation. (A) Immunolabeling of exogenous BirA*-FLAG-tagged and endogenous GINS3 in isogenic HEK293 Flp-In T-REx cells (induced with tetracycline for 24 hours), showing a nuclear distribution of the exogenous GINS3 proteins. Nuclear localization signal (NLS)-tagged BirA* is also shown. Scale bars = 10 µm. (B) Immunoprecipitation of the exogenous GINS3 proteins.

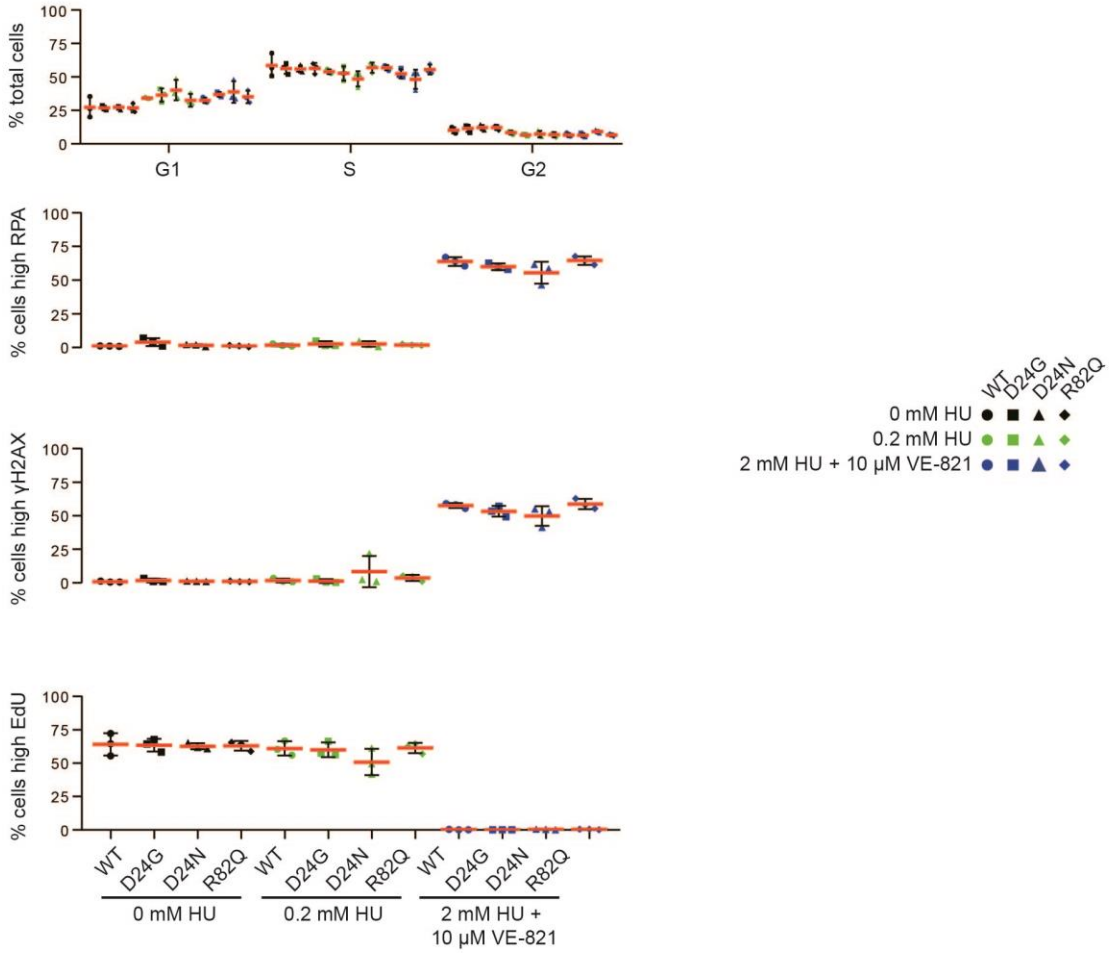


Supplementary Figure 6. Expression of exogenous GINS3 variants does not affect doubling time in U2OS cells. Doubling time of 3 independent clones (a, b, c) of each indicated genotype (KO = CRISPR-Cas9-mediated knockout). Height of the bars is mean of 4 independent measurements of doubling time; error bars indicate standard deviation.



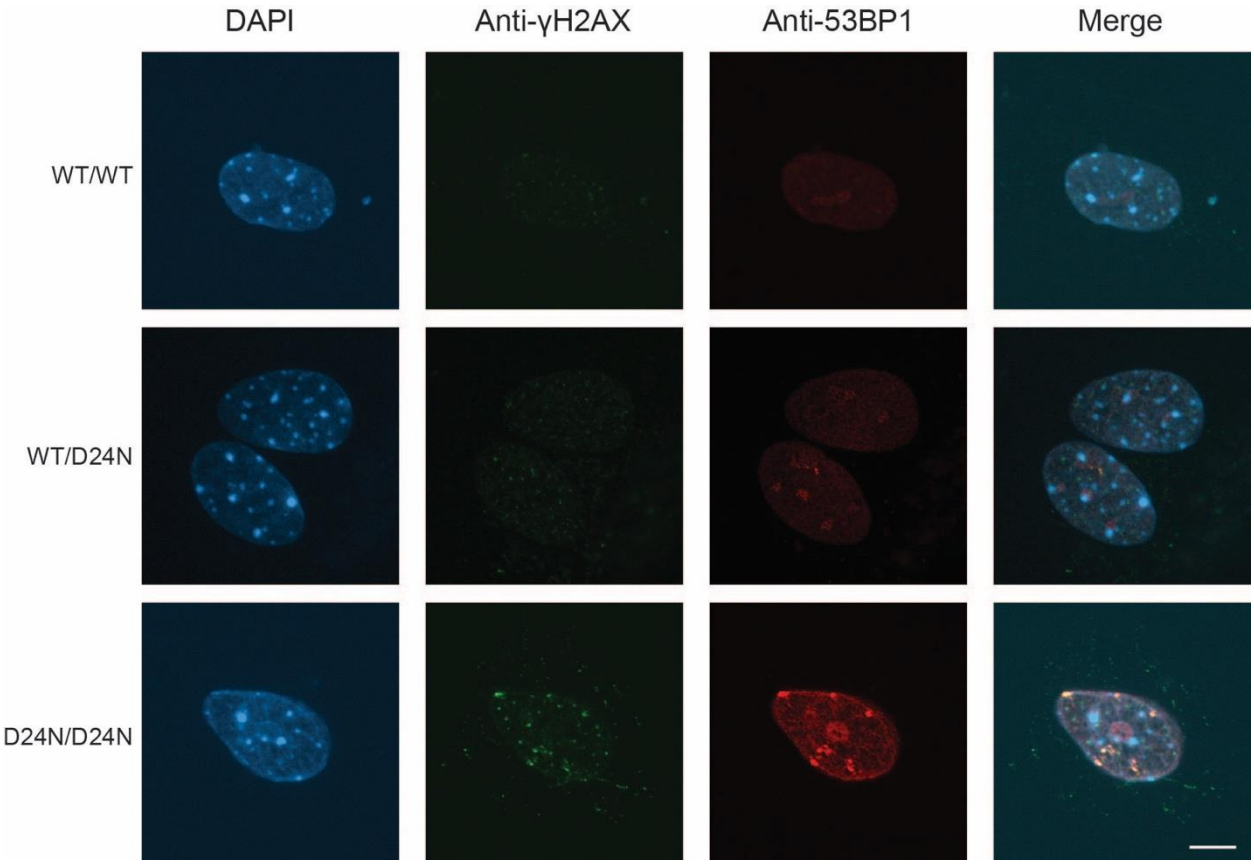
Supplementary Figure 7. Cell lines expressing GINS3 variants do not show significant changes in RPA or ɤ-H2AX accumulation on chromatin, in fraction of EdU positive cells, or in cell cycle distribution (related to **Figure 7A** and **Supplementary Figure 8**). Three independent cell lines were left untreated, treated with 0.2 mM HU for 6 hours or pre-treated with 10 µM VE-821 (an ATR inhibitor) for 30 minutes followed by 2 mM HU for 6 hours (as positive control). All cell lines were pulsed with 10 µM EdU 30 min prior to harvesting. Cells were treated with an extraction buffer containing 0.2 % Triton X-100 to remove non-chromatin bound material prior to fixation. Cells were stained with fluorescent antibodies against RPA and ɤ-H2AX, EdU was fluorescently labeled using click chemistry and DNA was stained with DAPI. Analysis was

performed by flow cytometry. Flow cytometry plots from a representative set of cell lines are shown to demonstrate gating strategy, while a summary of results from 3 independent cell lines for each genotype is shown in **Supplementary Figure 8**.



Supplementary Figure 8. Cell lines expressing GINS3 variants do not show significant changes in RPA or ɤ-H2AX accumulation on chromatin, in fraction of EdU positive cells, or in cell cycle distribution (related to **Figure 7A** and **Supplementary Figure 7**). Three independent cell lines were left untreated, treated with 0.2 mM HU for 6 hours or pre-treated with 10 μM VE-821 (an ATR inhibitor) for 30 minutes followed by 2 mM HU for 6 hours (as positive control). All cell lines were pulsed with 10 μM EdU 30 min prior to harvesting. Cells were treated with an extraction buffer containing 0.2 % Triton X-100 to remove non-chromatin bound material prior to fixation. Cells were stained with fluorescent antibodies against RPA and ɤ-H2AX, EdU was fluorescently labeled using click chemistry and DNA was stained with DAPI. Analysis was performed by flow cytometry. Results are a summary of results from 3 independent cell lines for

each genotype; flow cytometry plots from a representative set of cell lines are shown in **Supplementary Figure 7** to demonstrate gating strategy.



Supplementary Figure 9. Number and intensity of ɤ-H2AX and 53BP1 foci is increased in *Gins3*^{D24N}/*Gins3*^{D24N} MEFs. Figure shows representative images for analysis performed in **Figure 8C-D**. Scale bar = 10 μm.

Supplementary Table 1. Clinical Features of the seven patients with recessive *GINS3* mutations							
Phenotype	Patient 1 D24G/R82Q	Patient 2 D24N/D24N	Patient 3 D24N/D24N	Patient 4 D24N/D24N	Patient 5 D24N/D24N	Patient 6 D24N/D24N	Patient 7 D24N/D24N
Sex	M	F	M	F	M	F	F
Age at diagnosis	5 years	23 years	14 years	14 years	4 years	11 years	10 years
Age at recent examination	4.5 years	24 years	16 years	15 years	4 years	11 years	10 years
Prematurity	No (37 weeks)	NA	No	NA	No	NA	Yes
Antenatal Complications	IUGR	NA	IUGR	NA	IUGR	NA	No
Postnatal Complications	SGA	NA	SGA	NA	SGA	NA	SGA
Growth Parameters							
Birth weight (g)	2024(-2.2 SD)	NA	2000(-2.3 SD)	NA	2100(-2.3 SD)	NA	2100(-2.5 SD)
Birth length	NA	NA	NA	NA	NA	NA	NA
Birth head circumference (cm)	34.5 (0.8 SD)	NA	NA	NA	NA	NA	NA
Weight at examination (kg)	8.8 (-4.8 SD)	45.7 (- 1.7 SD)	37.7 (-2.7 SD)	46.9 (-0.76 SD)	10.5 (-3.5 SD)	31 (-1.1 SD)	13.2 (-4.6 SD)
Height at examination (cm)	82.3 (-5.2 SD)	149.2 (-2.2 SD)	155 (-2.1 SD)	148.5 (-2.1 SD)	92 (-2.4 SD)	136 (-1.11 SD)	108.2 (-4.5 SD)
Head circumference at examination (cm)	47.5 (-2.2 SD)	50.5 (-3.6 SD)	50 (-3.4 SD)	51.5 (-2.4 SD)	44 (-4.2 SD)	NA	44 (-6.4 SD)
Facial Characteristics							
Prominent forehead	Yes	No	No	No	Yes	NA	Yes
Prominent cranial vault	Yes	No	No	No	No	NA	No
Hypertelorism	No	No	No	No	No	NA	Yes
Down-slanted palpebral fissures	Yes	No	No	No	No	NA	No
Small posteriorly rotated ears	Yes	Yes	Yes	Yes	Yes	NA	Yes
Micro-/retrognathia	Yes	No	No	No	Yes	NA	Yes
Prominent nose	No	Yes	Yes	Yes	Yes	NA	Yes
Neurologic Features							
Motor delay	No	No	No	No	No	No	No
Speech delay	No	No	Yes	No	No	No	Yes

Cognitive disability	No	Mild	Mild	No	No	No	No
Structural brain abnormality	Yes	No	No	NA	Subcortical and basal ganglia calcification	No	No
Respiratory Features							
Recurrent infections	Yes	No	Yes	No	Yes	Yes	Yes
Reactive airway disease	Yes	No	No	No	Yes	Yes	No
Gastrointestinal Features							
Failure to thrive	Yes	No	Yes	No	Yes	Yes	Yes
Short stature with preserved HC	Yes	No	No	Yes	No	NA	No
Chronic diarrhea	Yes	No	No	No	No	No	NA
Sensitivities / Allergies	gluten	No	No	No	NA	NA	NA
Urogenital Anomalies							
Abnormal genitalia	Hypoplastic scrotum	No	No	No	Yes	No	No
Hypospadias	No	NA	No	NA	Yes	NA	NA
Cryptorchidism	Yes	NA	Yes	NA	Yes	NA	NA
Hypoplastic labia majora	NA	No	NA	No	NA	NA	NA
Renal anomalies/problems	No	NA	Yes (renal stones)	No	No	No	No
Skeletal Features							
Bilateral clinodactyly	Yes	No	No	No	Yes	NA	Yes
Phalangeal shortening	Yes	No	No	No	Yes	NA	Yes
Delayed skeletal maturation	Yes	No	No	No	NA	NA	NA
No patellae after 4 years of age	Yes	Yes	No	Yes	NA	NA	NA
Dermatological Features							
Café au lait macules	No	No	No	No	No	No	NA
Other Features							
Neutropenia	No	NA	Yes	NA	Yes	Yes	Yes
Hearing loss	No	No	Yes	No	NA	No	No
Abbreviations are as follows: M/F, Male/Female; IUGR, Intrauterine growth restriction; SGA, Small for gestational age; NA, Not available;							

HC, Head circumference.
No clinical data is available on patient 4 who has been lost to clinical follow-up

Supplementary Methods

Sequencing, rare variant identification and variant validation.

P1: Exonic DNA was selected using the Agilent SureSelect 50 Mb (V5) All Exon Kit, then sequenced on an Illumina HiSeq 2000. Read alignment, variant calling, and annotation were done as outlined for previous FORGE and Care4Rare Canada projects (2) with a pipeline based on Burrows-Wheeler Aligner (3), Picard (4), ANNOVAR (5), and custom annotation scripts. Variants were disregarded if they were present at >1 in the 1000 genome phase 1 dataset (April 2012 release), the 6500 exomes of the National Institute of Health Heart, Lung and Blood Institute, GO Exome Sequencing Project (Seattle, WA, USA, data downloaded March 10, 2012), and seen in more than 6 samples from our in-house database (～2000 exomes previously sequenced at the McGill University and Genome Quebec Innovation Centre). PCR amplification followed by bi-directional Sanger sequencing was used to validate and segregate the identified mutations.

P2-4: A family with three children affected by an MGS-like phenotype and homozygous c.70G>A (p.D24N) *GINS3* variants was identified through GeneMatcher. DNA of the three affected and eight unaffected siblings, as well as the mother, were genotyped using Axiom SNP Chip platform to determine the candidate autozygome as has been described previously (6). Exome sequencing was performed on the index using TruSeq Exome Enrichment kit (Illumina) following the manufacturer's protocol. The coding/splicing homozygous exome sequencing variants that are within the candidate autozygome were considered as likely candidates if present with frequency of <0.1% in publicly available variant databases (1000 Genomes, NHLBI Exome Sequencing Project, Exome Variant Server, and Genome Aggregation Database [gnomAD]) and a database of in-house ethnically matched exomes (Saudi Human Genome Program; totaling

2,379 exomes), and predicted to be pathogenic by the two in-silico prediction modules PolyPhen, SIFT and had a CADD score of >15.

P5-7: DNA libraries were constructed using Agilent SureSelect version 5 kits. Quality control for insert size and library representation were performed using an Agilent Tapestation and qPCR respectively. Sequencing (BGI Europe) was carried out using an Illumina HiSeq 4000 to an average depth of coverage of 150x with automated adapter trimming of the fastq sequences. DNA sequence quality metrics were carried out using FASTQC version: 0.11.7. Alignment, quality filtering and variant identification were undertaken using commercially available algorithms. Human reference assemblies were aligned against GRCh37.p13. QIAGEN Clinical Insight - Interpret software was used in sequence analysis and interpretation. The application was internally designed and developed by QIAGEN. All analyses were based on: QIAGEN Clinical Insight-Interpret (8.0.20210924), Ingenuity Knowledge Base (D-release).

Plasmids & site-directed mutagenesis

GINS3 cDNA (CCDS10796.1) was introduced into the pDONR223 (Invitrogen) and subjected to site-directed mutagenesis using the QuikChange methodology (Agilent) with the following primers: GINS3-D24G F, GINS3-D24G R, GINS3-D24N F, GINS3-D24N R, GINS3-R82Q F, and GISN3-R82Q R (all primer sequences are listed in Supplementary Table 2). Mutagenesis was verified by Sanger sequencing and constructs introduced into the pDEST-FLAG-BirA*. This plasmid and the control pDEST-BirA*-FLAG-GFP and pDEST- FLAG-NLS- BirA* were generously provided by Dr. Anne-Claude Gingras (Lunenfeld-Tanenbaum Research Institute).

A codon optimized version of *GINS3* cDNA with a C-terminal TEV site and 3xFLAG tag (GINS3cr-FLAG) was obtained from BioBasic Inc. The GINS3cr-FLAG ORF was amplified by PCR using primers attB1-GINS3 and attB2-GINS3 and transferred onto the plasmid pcDNA5/FRT/TO using the Gateway cloning system (Invitrogen) to obtain pcDNA5/FRT/TO-GINS3cr-FLAG. Site-directed mutagenesis was performed (using primers GINS3cr-D24G-F, GINS3cr-D24G-R, GINS3cr-D24N-F, GINS3cr-D24N-R, GINS3cr-R82Q-F and GINS3cr-R82Q-R) to insert point mutations in the GINS3cr ORF that would allow it to express D24G, D24N or R82Q GINS3 variant proteins.

Generation of GINS3-expressing human cell lines

HEK293 and U2OS Flp-In T-REx cell lines were cultured in complete medium (DMEM supplemented with 10% FBS (Wisent), 2 mM L-glutamine, 200 U/mL penicillin, 200 µg/mL streptomycin) supplemented with 5 µg/ml blasticidin and 75 µg/ml zeocin. To generate cell lines expressing wild-type or variant GINS3, HEK293 Flp-In T-REx cells (ThermoFisher Scientific) were transfected using Lipofectamine 3000 (ThermoFisher Scientific) as instructed by the manufacturer with a 1:9 ratio of the pcDNA5 or pDEST plasmid of interest, and the pOG44 plasmid (ThermoFisher Scientific) encoding the Flp recombinase. The transfected cells were subsequently selected in complete medium containing 5 µg/ml blasticidin and 200 µg/ml hygromycin; the concentration of hygromycin was reduced to 100 µg/ml the following day.

To generate cell lines expressing a CRISPR-resistant version of wild-type or variant GINS3 (GINS3cr-FLAG cell lines), the pcDNA5/FRT/TO-GINS3cr-FLAG plasmids, along with pOG44 (Invitrogen), were used to transfect a U2OS T-REx Flp-In cell line (Invitrogen) as described above for HEK293 T-REx Flp-In cell line, which allowed for the integration of the

Supplementary Table 2. Sequences of primers used in this study.

Primer name	**SEQUENCE (5' → 3')**
GINS3-D24G F	CTTTCTTTCTTTGGACGGCATCCTGATGTCCC ACG
GINS3-D24G R	CGTGGGACATCAGGATGCCGTCCAAAGAAAGAAAG
GINS3-D24N F	CTTTCTTTCTTTGGACAACATCCTGATGTCCCACG
GINS3-D24N R	CGTGGGACATCAGGATGTTGTCCAAAGAAAGAAAG
GINS3-R82Q F	CTTTTTGACAACAAGCGACAGATCCTTTCTGTGGAACTC
GINS3-R82Q R	GAGTTCCACAGAAAGGATCTGTCGCTTGTTGTCAAAAAG
GINS3C71-F	GGGCCTGAGGAGAACTTTCT
GINS3C71-R	CAGCTTCTCGTGGGACATC
GINS3C71-VIC	CTTTGGACGACATCC
GINS3C71-FAM	TTTGGACGGCATCC
GINS3C245-F	TGGCTGGCAAAAGGACTTT
GINS3C245-R	CCAACCCTCTTGGTAGATCTTG
GINS3C245-VIC	ACAAGCGACGGATC
GINS3C245-FAM	AACAAGCGACAGATC
C71_WT_F	ACGTTGGATGGCTTATTTCCGAGTGGAGTC
C71_W1_R	ACGTTGGATGCAGCTTCTCGTGGGACATC
C71_WT_E	AACTTTCTTTCTTTGGACG
C245_W1_F	ACGTTGGATGTTGAACTACCCTTGTGGCTG
C245_W1_R	ACGTTGGATGACCCTCTTGGTAGATCTTGG
C245_W1_E	TTTTGACAACAAGCGAC
PSF3-F1	GCCAACTTTGTTCTTGGTATTATATTTTTACAATAGAAGGGCA AGCATAGAAAGGCGGATCCCCGGGTTAATTAA
PSF3-INT-WT-R	TACAAGGAAACTCTGTCCCATCTGCTAGGACATCATCAATGT CATAGTAACCCATGCAGCGTACGGATATCAC
PSF3-INT-D8G-R	TACAAGGAAACTCTGTCCCATCTGCTAGGACACCATCAATGT CATAGTAACCCATGCAGCGTACGGATATCAC
PSF3-INT-D8N-R	TACAAGGAAACTCTGTCCCATCTGCTAGGACATTATCAATGT CATAGTAACCCATGCAGCGTACGGATATCAC
PSF3-PRS-F	AGGGCGAATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAA CTAGTGGATCCCCTGCCTGCAAGTGGTTTAAAG
PSF3-PRS-R	AGGTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGC CCGGGGGATCCACATAATGTTAAAGATGATTCAGATC
PSF3-F2	TGAATCTTATAAGGACACGAAAAGGTGGATGTTTAAAAAACG GATCCCCGGGTTAATTAA
PSF3-R1	TAGTTATTTTATATAGTGTATTTCTAAAGAAAAAGATGTGGA ATTCGAGCTCGTTTAAAC
PSF3-D8G-F	GTCCCATCTGCTAGGACACCATCAATGTCATAGTAAC
PSF3-D8G-R	GTTACTATGACATTGATGGTGTCCTAGCAGATGGGAC
PSF3-D8N-F	GTTACTATGACATTGATAATGTCCTAGCAGATGGG
ATTB1-GINS3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGAGCG AGGCCTACTTCAGAGTGG

Supplementary Table 2 continued.

Primer name	Sequence (5' → 3')
ATTB2-GINS3	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCACTTGTCGTC ATCGTCCTTG
GINS3CR-D24G-F	GGCTCATCAGGATGCCGTCCAGGCTCAGG
GINS3CR-D24G-R	CCTGAGCCTGGACGGCATCCTGATGAGCC
GINS3CR-D24N-F	GGCTCATCAGGATGTTGTCCAGGCTCAGGAA
GINS3CR-D24N-R	TTCCTGAGCCTGGACAACATCCTGATGAGCC
GINS3CR-R82Q-F	CAGCTCGACGCTCAGGATCTGCCGCTTGTTGTCGAACAG
GINS3CR-R82Q-R	CTGTTCGACAACAAGCGGCAGATCCTGAGCGTCGAGCTG
GINS3-GRNA1-F	CACCGTCGGAAATAAGCCTCTGACA
GINS3-GRNA1-R	AAACTGTCAGAGGCTTATTTCCGAC
GINS3-GRNA2-F	CACCGAGGAAGAAAGCGCCAAGGCG
GINS3-GRNA2-R	AAACCGCCTTGGCGCTTTCTTCCTC
MGINS3-GRNA	TCAGGATGTCGTCCAAAGAC
SSODN	CGCCCTCCTCATCCCTGACTGTGCTGCGAGCTGCCATGTCCGA GGCGTATTTCCCAGTGGAGTCGGGCGCTCTGGGGCCGGAGGA GAACTTTCTGTCTTTGGACAACATCCTCATGTCCCAGGAGAA GCTGCCGGTGCGGGTCGAGACGCCC
MGINS3-D24NSEQ-F	ATGTCCGAGGCGTATTTCCC
MGINS3-D24NSEQ-R	GCTGGAGCCAAAGCCAATGA

GINS3cr-FLAG ORFs into the genome at a consistent location; once integrated, the GINS3cr-FLAG ORFs were under the control of a tetracycline/doxycycline-inducible promoter.

To generate GINS3cr-FLAG cell lines in which the endogenous copy of the GINS3 gene was disrupted (GINS3 KO cell lines), oligonucleotides encoding guide RNAs targeting endogenous GINS3, but not codon-optimized GINS3cr, were synthesized (Invitrogen; GINS3-gRNA1-F, GINS3-gRNA1-R, GINS3-gRNA2-F, GINS3-gRNA2-R), annealed, phosphorylated and cloned into BsmBI/ClaI-digested pLentiCRISPRV2 (Addgene). Plasmids pLentiCRISPRV2-GINS3-1 and pLentiCRISPRV2-GINS3-2 were used to transfect GINS3cr-FLAG cell lines. Selection for plasmid was maintained for 3 days using 1 µg/mL puromycin and then removed; induction of GINS3cr-FLAG by addition of 1µg/mL doxycycline every 48 hours was maintained continuously after CRISPR transfection. After 5 weeks, individual cells were isolated by flow cytometry using a BD Aria III Sorter (BD) in order to obtain isogenic cell lines.

Allelic balance and gene isoforms

Droplet digital PCR (ddPCR) was performed using the QX200 Droplet Digital PCR system (Bio-Rad Laboratories). Custom TaqMan probes (part 4331349, Life Technologies) were designed to using Primer Express 3.0 software (Life Technologies) to target the variant alleles c71A>G (GINS3c71-F, GINS3c71-R, GINS3c71-VIC, GINS3c71-FAM) and c245G>A (GINS3c245-F, GINS3c245-R, GINS3c245-VIC, GINS3c245-FAM).

The reaction mix consisted of ddPCR SuperMix for Probes (Bio-Rad Laboratories) and the GINS3c71 or GINSc245 SNP genotyping oligos. Assays were validated by temperature gradient to ensure optimal separation of alternate and reference-allele-containing droplets. Cycling conditions for the reaction were 95°C for 10 min, followed by 45 cycles of 94°C for 30

sec and 60°C for 1 min, 98°C for 10 minutes and finally a 10°C hold on a Life Technologies Veriti thermal cycler. Data was analysed using QuantaSoft Analysis Pro software v1.0.596 (Bio-Rad Laboratories).

Samples were further genotyped for c.71 and c.245 positions using the MassARRAY Analyzer 4 System (Agena Biosciences, San Diego, CA, USA) using iPlex gold chemistry and analyzed using Typer 4.0 software. Briefly, each locus is amplified by PCR and a third primer that flanks the polymorphism site is extended by one base (E). Primers used were c71_WT_F, c71_W1_R, c71_W1_E, c245_W1_F, c245_W1_F and c245_W1_E. The extension reaction products were analyzed using MALDI-TOF mass spectrometry to determine the amount and type of molecules present in the sample.

RNA isolation and RT-qPCR

Total RNA was extracted from 4-week differentiated chondrogenic pellets, or from MEFs with TRIzol Reagent (Life Technologies). RNA was precipitated with isopropanol and GlycoBlue Coprecipitant (Life Technologies), and subsequently digested with DNaseI (Life Technologies). RNA was reverse transcribed with random hexamer primers and SuperScript III Reverse Transcriptase (Life Technologies). Target gene abundance was quantified by RT-qPCR on a QuantStudio 3 System (Applied Biosystems), using PowerUp SYBR Green Master Mix (Applied Biosystems) and gene-specific primers in triplicate. Gene expression measurements were normalized to RPL13A (for chondrogenic analysis) or to GAPDH and ACTB (for MEF analysis) as an internal control using the ΔΔCt method. Two-tailed unpaired t-tests were performed on control and patient ΔCt values using GraphPad Prism version 8. RT-qPCR primers are listed in Supplementary Table 3.

RNA-Seq

RNA-seq libraries were generated with NEBNext Ultra II Directional RNA library prep for Illumina with NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs) as per manufacturer's protocol. Briefly, 250 ng of total RNA spiked with SIRV Set 3 (Lexogen) with 12 PCR cycles was used to generate the libraries. Fragment sizes and concentrations of libraries were checked with a Bioanalyzer DNA High Sensitivity (Agilent) and NEBNext Library Quant Kit for Illumina (New England Biolabs). Libraries were sequenced on Illumina NextSeq500 with paired-end 150 bp read length. FASTQ sequences were aligned to hg19 and gene counts generated using STAR (v2.4.2a) (7). Differential gene expression analysis was performed using DESeq2 (v1.20.0) (8). Gene ontology term enrichment was determined using g:Profiler (9). RNAseq data have been deposited to the European Genome-Phenome (EGA): accession ID: EGAS00001006038.

BioID

HEK293 Flp-In T-REx cells were grown in 10 cm plates to 70% confluency and induced with 1 µg/mL doxycycline for 24 hours, with 50 µM biotin added during the last 8 hours. Cells were harvested by trypsinization, washed with PBS, pelleted, and at least ~0.1 g per sample snap frozen. To isolate biotinylated proteins, the cell pellets thawed and lysed on ice in 600 µl of freshly prepared lysis buffer containing 8M urea (Millipore Sigma), 50 mM HEPES pH 7.4, 1 mM PMSF, 1 mM DTT, 1% Triton X-100 (Millipore Sigma). Samples were sonicated on ice with a Sonic Dismembrator Model 120 (ThermoFisher Scientific) at 30% amplitude 2X for 10 sec interspaced by 10 sec pauses. Between the two sonications, 600 µl of

Supplementary Table 3. Sequences of primers used in RT-qPCR

Primer name	Sequence (5′→3′)	Isoform
RPL13A-F	GAGGTATGCTGCCCCACAAA	
RPL13A-R	GTGGGATGCCGTCAAACA	
SOX9-F	ACTCGCCACACTCCTCCTC	
SOX9-R	CCCTCTCGCTTCAGGTCA	
PTHLH-F	GAGGTGTCCCCTAACTCCAA	
PTHLH-R	TCTTTGTACGTCTCCACCTTGTT	
COL2A1-F	GGCAATAGCAGGTTCACGTA	
COL2A1-R	CTCGATAACAGTCTTGCCCC	
COL10A-F	GCTAAGGGTGAAAGGGGTTC	
COL10A-R	CTCCAGGATCACCTTTTGGA	
GINS3-UTR1-F	ACAGCTTCCAACCTCGTTCA	
GINS3-UTR1-R	ACGGAGGGATACGTCTGTGA	
GINS3-ISO-A-F	TGATGTCCCACGAGAAGCTG	Variant 1
GINS3-ISO-A-R	CAACAGAGCAAAACCCTGTGG	Variant 1
GINS3-ISO-B-F	TGATGTCCCACGAGAAGCTG	Variant 2
GINS3-ISO-B-R	AAGCTTGGAACCCTGTGGG	Variant 2
GINS3-ISO-C-F	GCGGTCCCACAGACTTTTATC	Variant 3
GINS3-ISO-C-R	TAAGCCCCTCTCCATCTCGT	Variant 3
MP21-F	GCAGCCGAGAGGTGTGAGCC	
MP21-R	GGACATGGTGCCTGTGGCTCTG	
MMCM6-F	TCGTCTCACACACTACGATCAC	
MMCM6-R	GCTAAGGGTAGGACAGCACAG	
MPCNA-F	TCCTTGGTACAGCTTACTCTGC	
MPCNA-R	AATTTTGGACATGCTGGTGAGG	
MGAPDH-F	CACCACCAACTGCTTAGCC	
MGAPDH-R	GTCTTCTGGGTGGCAGTGAT	

lysis buffer was added. The samples were then centrifuged at 16 500 x *g* for 10 minutes at 4°C. Fifty microliters of pre-washed Streptavidin Sepharose High Performance beads (Millipore Sigma) was added to each sample, after which samples were left to rotate overnight at 4°C. Beads were recovered by centrifugation at 1000 x *g* for 5 minutes at 4°C and washed by resuspending in 1 ml of wash buffer (8M Urea, 50 mM HEPES, pH 7.4) with 8 minutes of rotation at room temperature. Beads were again recovered through a 2 min centrifugation at 1000 x *g*, and the washing steps repeated 4 times, after which the beads were transferred to fresh low-binding tubes.

Protein digestion & LC-MS/MS analysis

Buffers were prepared using MS-grade water (ThermoFisher Scientific). Beads were washed 4 times with 1 ml of 20 mM ammonium bicarbonate, and once more with the same buffer containing 1 mM biotin. Protein reduction was carried out by incubating the beads in 50 µl of 20 mM ammonium bicarbonate buffer containing 10 mM DTT (ThermoFisher Scientific) for 30 minutes at 60°C with stirring (1250 rpm). Samples were cooled for 5 min at room temperature, and alkylation followed by adding 50 µl of 50 mM ammonium bicarbonate buffer containing 15 mM chloroacetamide (Millipore Sigma), for 1 hour at room temperature with stirring (1250 rpm) in the dark. Chloroacetamide was neutralized by adding 15 mM DTT (final concentration) and stirring for 3 minutes at room temperature. Proteins were then digested by adding 1 µg Pierce MS-grade trypsin (ThermoFisher Scientific) and incubating overnight at 37°C with stirring (1250 rpm).

Digestion was stopped by adding formic acid (FA, ThermoFisher Scientific) to a final concentration of 1%, followed by stirring for 5 minutes at room temperature. The beads were

spun at 2000 x *g* for 3 minutes and the supernatant transferred to a fresh low-binding tube. Beads were resuspended in 100 µl of buffer containing 60% acetonitrile (ACN, Millipose Sigma) and 0.1% FA, and stirred at 1250 rpm for 5 minutes at room temperature. The supernatant was collected and combined with the one obtained previously. Samples were then concentrated in a centrifugal evaporator at 60°C until completely dry (approximately 2 hours) and resuspended in 30 µl of 0.1% trifluoroacetic acid (TFA) buffer (Millipore Sigma). Peptides were purified using ZipTip 10-µl micropipette tips containing a C18 column (EMD Millipore). The ZipTip was first moistened by suctioning 10 µl of 100% ACN solution three times, then equilibrated by suctioning 10 µl of 0.1% TFA buffer three times. Ten microliters of each peptide sample was passed on the equilibrated ZipTip through 10 successive up-and-down pipetting cycles. This step was performed three times in order to pass the entire sample on the column. The ZipTips were then washed with 10 µl of 0.1% TFA buffer three times. Peptides were eluted into fresh low-binding microtubes, with 10 µl of a 50% ACN and 1% FA buffer. This step was carried out three times to obtain a final volume of 30 µl. The peptides were then concentrated in a centrifugal evaporator at 60°C until completely dry (approximately 30 minutes) and resuspended in 30 µl 1% FA buffer. Peptide concentration was determined using a NanoDrop spectrophotometer (ThermoFisher Scientific), after which samples were transferred to a glass vial, and stored at -20°C until ready for mass spectrometry.

For LC-MS/MS, 250 ng of each sample was injected into an HPLC (nanoElute, Bruker Daltonics) and loaded onto a trap column with a constant flow of 4 µl/min (Acclaim PepMap100 C18 column, 0.3 mm id x 5 mm, Dionex Corporation) then eluted onto an analytical C18 Column (1.9 µm beads size, 75 µm x 25 cm, PepSep). Peptides were eluted over a 2-hour gradient of acetonitrile (5-37%) in 0.1% FA at 500 nL/min while being injected into

a TimsTOF Pro ion mobility mass spectrometer equipped with a Captive Spray nano electrospray source (Bruker Daltonics). Data was acquired using data-dependent auto-MS/MS with a 100-1700 m/z mass range, with PASEF enabled with a number of PASEF scans set at 10 (1.27 seconds duty cycle) and a dynamic exclusion of 0.4 minute, m/z dependent isolation window and collision energy of 42.0 eV. The target intensity was set to 20,000, with an intensity threshold of 2,500.

The raw files were analyzed using MaxQuant 1.6.17.0 (10), and the Uniprot human proteome database (21/03/2020, 75,776 entries). The settings used for the MaxQuant analysis (with TIMS-DDA type in group-specific parameters) were: 2 miscleavages were allowed; fixed modification was carbamidomethylation on cysteine; enzymes were Trypsin (K/R not before P); variable modifications included in the analysis were methionine oxidation, protein N-terminal acetylation and protein carbamylation (K, N-terminal). A mass tolerance of 10 ppm was used for precursor ions and a tolerance of 20 ppm was used for fragment ions. Identification values "PSM FDR", "Protein FDR" and "Site decoy fraction" were set to 0.05. Minimum peptide count was set to 1. Label-Free-Quantification (LFQ) was also selected with a LFQ minimal ratio count of 2. Both the "Second peptides" and "Match between runs" options were also allowed.

Results were sorted using Prostar (Proteomics statistical analysis with R). Proteins positive for at least one of the "Reverse", "Only.identified.by.site" or "Potential.contaminant" categories were eliminated, as well as proteins identified from a single peptide. An SLSA (Structured Least Square Adaptative) and DetQuantile imputation were performed for POV (Partially Observed Value) and MEC (Missing in the Entire Condition) missing values, respectively. After a mean centering within each condition, the results were sorted in order to

keep proteins that were present in at least 2 of the 3 biological replicates for each condition. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [1] partner repository with the dataset identifier PXD028079.

Immunoprecipitation and immunolabeling

For immunoprecipitations, HEK293 Flp-In T-REx cells were seeded for 70% confluency and induced with 1 µg/mL tetracycline for 24 hours to express exogenous GINS3-BirA* fusion proteins. Cells were lysed in lysis buffer (50 mM Tris pH 7.7, 100 mM KCl, 2 mM EDTA, 0.1% NP-40, and 10% glycerol) supplemented with protease inhibitors, and briefly sonicated. DNA was digested with benzonase (EMD Millipore 71205-03) to a final concentration of 0.5 units/µL and left to gently rotate at 4°C for 1 hour. A total of 1 mg of protein was incubated with FLAG M2 beads (Millipore Sigma) for 3 hours at 4°C with gentle rotation. After incubation, beads were washed 3 times with lysis buffer and eluted by boiling in loading buffer.

For immunolabeling of HEK293 Flp-In T-Rex lines, cells were seeded on sterilized cover slips at 40% confluency. On the following day, HEK293 Flp-In T-REx cells were induced with 1 µg/mL tetracycline for 24 hours to express exogenous GINS3-BirA* fusion proteins. Cells were fixed with 2% paraformaldehyde with 0.2% Triton X-100 for 10 min and washed three times with PBS. Cells were further permeabilized with 0.5% Tergitol diluted in PBS for 10 minutes and then blocked with a 3% BSA, 1% Normal Goat Serum PBS solution for 1 hour at room temperature. Coverslips were transferred to a humidity chamber and incubated with primary antibody (1:1000 FLAG M2 Millipore Sigma F1804, or 1:1000 GINS3 Abcam AB177515) for 1 hour. After incubation cells were washed with PBS and incubated with secondary antibody (ATTO488-conjugated anti-mouse, Rockland 610-152-121S, or Alexa Fluor 594-conjugated

anti-rabbit, Jackson ImmunoResearch 111-585-008) diluted at 1:250 in blocking buffer for 30 minutes. Samples were then washed in PBS and incubated in DAPI diluted to 1:1000 in PBS. Coverslips were washed and mounted on slides with ProLong Gold Antifade mounting media (Invitrogen).

For immunolabeling of MEF lines, cells were seeded on sterile coverslips 24 hours prior to labeling procedure. After 24 hours, coverslips were washed twice with cold PBS, and then incubated 3 min with 0.5% Triton X-100 in PBS on ice. Coverslips were washed once with cold PBS, and then incubated 15 min on ice with 4% formaldehyde in PBS. Coverslips were washed once with cold PBS and incubated 10 min on ice with 0.5% Triton X-100 in PBS. Coverslips were washed with PBS followed by 0.05% Tween-20 in PBS (PBS-T). Coverslips were incubated overnight at 4˚C in blocking buffer (3% BSA in PBS-T) with 1 mM NaN_3, and then washed with blocking buffer. Coverslips were incubated with primary antibodies 1:500 anti-gH2AX (Millipore Sigma, 05-636) and 1:200 anti-53BP1 (Novus Biologicals, NB100-305) in blocking buffer for 1 hour at 37˚C, and then washed 3 times for 10 min in PBS-T. Coverslips were incubated with primary antibodies 1:500 Alexa Fluor 488 goat anti-mouse IgG (H+L) (Life Technologies, A11029) and 1:500 Alexa Fluor 594 goat anti-rabbit IgG (H+L) (Life Technologies, A11012) in blocking buffer with 20 µg/mL DAPI (Invitrogen) for 1 hour at 37˚C, and then washed 3 times for 10 min in PBS-T. Coverslips were mounted onto glass slides with Immuno-Fluore Mounting Medium (MP Biomedicals) and images were captured at 630 X magnification using a Zeiss Axio Imager Z2 microscope (Zeiss). To analyze MEF images in an unbiased manner, an algorithm created with the MATLAB platform (version R2019a; Mathworks, MA) was used. Briefly, an Otsu threshold was applied to DAPI images to generate a binary mask that could then be applied to all channels; small objects were removed, holes were

filled, and adjacent nuclei were separated using a watershed algorithm. All masks were examined manually and any incorrectly identified nuclei were discarded. Masked images were smoothed using a bandpass algorithm, and foci were identified as local maxima. To measure intensity within foci, local maxima were expanded to a radius of 3 pixels and used to generate a binary mask for each nucleus; average focal intensity per nucleus was obtained by taking the average of all non-zero pixels. A minimum of 50 cells were analyzed for each cell line.

Yeast strain generation

To generate point mutations in the *PSF3* gene, the *kanMX6* gene cassette flanked by *loxP* sites was amplified from plasmid pOM10 (11) using primer pairs PSF3-F1 and PSF3-int-WT-R, PSF3-F1 and PSF3-int-D8G-R and PSF3-F1 and PSF3-int-D8N-R (see table of yeast primers below for sequences); these primers include 5' overhangs to allow recombination of PCR products with the upstream and 5' region of the *PSF3* gene and contain no point mutations (to give Psf3 WT), c.23A>G (to give Psf3 D8G) and c.22G>A (to give Psf3 D8N), respectively. PCR products were transformed into BY4743 and positive transformants were selected on medium containing G418. Isolated transformants were subsequently transformed with pSH47 (12), a plasmid expressing Cre recombinase under the control of a galactose-inducible promoter. Positive transformants were selected on SC-ura medium, and isolates were cultured on SC-ura medium containing galactose as a carbon source to induce the expression of Cre and catalyze the removal of the *kanMX6* cassette from the 5' end of the *PSF3* gene. After confirmation of *kanMX6* cassette removal by PCR, isolates were transferred to minimal medium for sporulation. Tetrad spores were dissected and presence of *PSF3* point mutations was assessed by Sanger sequencing (IRIC Genomics Platform).

Yeast strains used in cycloheximide chase experiment were generated by a different method. The *PSF3* gene, plus 500 bp of upstream and downstream sequence, encompassing the *PSF3* promoter and terminator, was amplified using primers PSF3-pRS-F and PSF3-pRS-R and cloned into pRS316 (ATCC 77145) by gap repair to generate pRS-PSF3. A *3HA::natMX6* cassette was amplified from plasmid pFA6a-3HA-natNX6 (13) using primers PSF3-F2 and PSF3-R1 and cloned into pRS-PSF3 by gap repair to generate pRS-PSF3-3HA. Mutations were introduced into *PSF3* by site-directed mutagenesis with primer pairs PSF3-D8G-F and PSF3-D8G-R (D8G), and PSF3-D8N-F and PSF3-D8N-R (D8N) and verified by Sanger sequencing (IRIC Genomics Platform). Wild-type and mutant pRS-PSF3-3HA plasmids were used to transform BN23 (BY4347 *PSF3/psf3*Δ*::kanMX6*). Yeast strains were sporulated to obtain mat a haploid products carrying the plasmid and the *psf3*Δ*::kanMX6* deletion.

Yeast doubling time & S phase progression

Yeast cultures were inoculated at 0.001 OD in YPAD in a 96-well plate, and culture density was measured at 630 OD every 30 minutes for 24 hours. Doubling time was calculated based on the rate of growth during exponential growth phase for 8 separate cultures.

To synchronize yeast cells in G1 phase, actively growing cultures were diluted to 0.18 OD and incubated 3 hours in YPAD with 5 µg/mL alpha factor at 30˚C with shaking; an additional 5 µg/mL alpha factor was added after 1.5 hours. Synchronization was confirmed by observing schmoo morphology in upwards of 50% of cells. To release from G1 arrest, cells were washed once with YPAD, then cultured in fresh YPAD medium with 50 µg/mL pronase at 0.75 OD and 30˚C with shaking; culture samples were collected at indicated time points.

To fix samples, ~0.2 OD of culture was mixed with ethanol (final concentration = 70%) and incubated at room temperature for a minimum of 20 minutes before being stored at 4˚C. To prepare samples for analysis by flow cytometry, samples were incubated 3 hours at 42˚C with 400 µg/mL RNAse, followed by 30 minutes at 50˚C with 1 mg/mL Proteinase K. Samples were sonicated 10 seconds at 30% cycle duty, then incubated with Sytox Green (Invitrogen) for a minimum of 10 minutes prior to flow cytometry using a FACSCalibur (BD Biosciences). Analysis was performed using FlowJo software (BD Biosciences) with the Watson (Pragmatic) model for cell cycle analysis.

DNA combing analysis

One million cells were seeded in 100 mm dishes 24 hours prior to nucleotide analog labeling. To label, cells were washed once with PBS, then incubated 20 minutes with 8 mL medium containing 30 µM CldU (Sigma Aldrich) at 37˚C. CldU medium was removed and cells were incubated 20 minutes with 8 mL of medium containing 250 µM IdU (Sigma Aldrich) at 37˚C. Cells were collected by trypsinization and resuspended in PBS, and cell number was determined by hemocytometer counts. Preparation of DNA plugs and DNA combing was adapted from references (14, 15). 1.2 million cells from each treatment were pelleted, washed once in TNE50 (10 mM Tris-HCl pH 7.5, 20 mM NaCl, 50 mM EDTA), and then resuspended in TNE50 for a total volume of 200 µL. An equal volume of 1% low melting point agarose (Life Technologies) was added to each tube, and 4 plugs were prepared for each condition. Plugs were incubated 3 days in Proteinase K buffer (1 mg/mL proteinase K (Bio Basic), 1% N-laurelsarcosine (BioShop) in TNE50) at 50˚C, with buffer being changed once each day. Plugs were washed 5 times for 10 minutes with TNE50, then 1 plug from each condition was incubated

30 minutes in 1 µL YOYO-1 Iodide (ThermoFisher Scientific) in 150 µL TNE50. Plugs were then washed 3 times 5 minutes with 10 mL TE pH7.5, followed by 1 time in 2 mL MES-E buffer (50 mM MES pH 5.7, 1 mM EDTA). Plugs were resuspended in 2 mL fresh MES-E buffer and melted 30 minutes in a 70˚C water bath to create a DNA solution. DNA solutions were cooled to 42˚C prior to overnight digestion at 42˚C with 3 units beta-agarase. DNA solutions were combed onto silanized coverslips (prepared according to reference (15) using a Fibre Comb Molecular Combing System (Genomic Vision). Coverslips were heated 90 minutes at 60˚C, then mounted onto slides using cyanoacrylate glue. Coverslips were dehydrated by sequential 5-minute incubations in 70%, 90% and 100% ethanol. After drying 5 min at room temperature, coverslips were denatured by a 25-minute incubation in 1 M NaOH. Coverslips were washed 5 times 1 minute in PBS, followed by 1 time 5 minutes in PBS-T (0.05% Tween 20 in PBS). Coverslips were then blocked 30 minutes in a humid chamber at 37˚C with 50 µL blocking buffer (10% BSA in PBS-T). To label analogs CldU and IdU, coverslips were incubated in a humid chamber at 37˚C 60 minutes with 50 µL primary antibodies anti-BrdU (Abcam, rat monoclonal [BU1/75 (ICR1)], ab6326, 1:400; cross-reacts with CldU) and anti-BrdU (BD Biosciences, mouse monoclonal (Clone B44), 347580, 1:25; cross-reacts with IdU), followed by 60 minutes with 50 µL secondary antibodies Alexa Fluor 488 goat anti-mouse IgG (H+L) (Life Technologies, A11029, 1:100) and Alexa Fluor 594 goat anti-rat IgG (H+L) (Life Technologies, A11007, 1:100). To label single-stranded DNA, coverslips were incubated in a humid chamber at 37˚C 60 minutes with 50 µL anti-DNA antibody, single-stranded (EMD Millipore, mouse monoclonal (clone 16-19), MAB3034, 1:100), followed by 60 minutes with 50 µL Alexa Fluor 649 goat anti-mouse IgG (H+L) (Life Technologies, A21235, 1:100). Cover slips were mounted using Immuno-Fluore Mounting Medium (MP Biomedicals). Images were captured at 400 X

magnification using a Zeiss Axio Imager Z2 microscope (Zeiss), and analysis was performed using Image J software (NIH).

Senescence-associated beta-galactosidase (SA-B-gal) assay

Dishes of MEF cells were rinsed twice with PBS and fixed in 0.5% glutaraldehyde (Grade II, Sigma) in PBS for 15 minutes at room temperature. Dishes of fixed cells were washed twice with PBS + 1 mM $MgCl_2$, then stored in PBS + 1 mM $MgCl_2$ at 4˚C for 24 hours to 7 days. To assay for the presence of SA-B-gal enzyme activity, cells were covered in X-gal solution (1 mg/mL X-gal (BioShop), 5 mM $K_3Fe(CN)_6$ (Sigma), 5 mM $K_4Fe(CN)_6*3H_2O$ (Sigma) in PBS) and incubated at 37˚C until blue colour developed in approximately 10% of control cells, at which point cells were imaged at 10X with an Olympus IX73 microscope with SC30 colour camera.

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