Figure S1

A

Primary fibroblasts utilized in this study (Coriell Institute Biobank)

Cat#	Nom	Sex	Age	Tissue
Controls				
GM08447	Con.1	F	<1	ear
GM05659	Con.2	M	1	chest
GM00969	Con.3	F	2	n.d.
GM02036	Con.4	F	11	n.d.
GM03377	Con.5	M	19	arm
GM03440	Con.6	M	20	leg
Trisomy 13				
GM00526	T13.1	M	n.d.	
GM02948	T13.2	M	n.d.	

Cat#	Nom	Sex	Age	Tissue
Trisomy 18				
GM00734	T18.1	F	18FW	
GM03538	T18.2	F	n.d.	
Trisomy 21				
GM04616	T21.1	F	<1	n.d.
GM04592	T21.2	F		
AG05397	T21.3	M	1	thorax
AG06922	T21.4	M	2	thorax
GM02767	T21.5	F	14	n.d.
AG08941	T21.6	F	19	arm
AG08942	T21.7	M	21	arm

B

Average gene expression per chromosome (log_2 ratios)

Chr	# genes	T13.1	T13.2	T18.1	T18.2	T21.1	T21.2	T21.3	T21.4	T21.5	T21.6	T21.7	Con.1	Con.2	Con.3	Con.4	Con.5	Con.6
1	1103	-0.02	0.00	0.00	0.00	0.00	-0.02	-0.01	0.00	0.01	-0.01	-0.01	0.01	0.00	0.00	0.00	0.00	0.00
2	712	0.01	-0.04	-0.01	-0.02	-0.04	-0.02	0.00	-0.02	-0.03	-0.02	0.02	-0.04	-0.03	0.01	0.01	0.02	0.00
3	608	-0.01	-0.05	0.01	0.00	-0.03	-0.03	0.00	-0.04	-0.02	0.03	0.03	0.00	0.01	0.01	0.00	0.00	-0.02
4	381	0.00	0.04	0.01	-0.01	0.02	0.03	0.01	-0.01	-0.01	0.01	0.02	-0.02	-0.04	-0.02	-0.03	0.05	0.00
5	488	0.00	0.04	0.03	-0.01	0.01	0.03	-0.01	-0.02	-0.05	-0.02	0.02	-0.03	0.02	0.00	-0.02	0.02	-0.03
6	549	-0.04	-0.03	0.00	-0.02	-0.03	-0.08	-0.04	-0.05	-0.05	-0.04	-0.02	-0.04	0.02	-0.02	-0.03	0.02	0.01
7	482	0.03	0.03	0.02	0.02	0.04	0.01	0.01	0.02	0.03	0.01	0.02	0.01	-0.01	-0.02	-0.02	0.00	0.00
8	379	-0.05	-0.08	-0.09	-0.02	-0.02	-0.02	0.00	0.00	0.01	0.00	0.00	0.01	-0.06	0.00	0.01	-0.02	0.01
9	438	-0.06	-0.05	-0.03	-0.05	0.00	0.04	0.01	-0.01	0.02	0.02	-0.01	0.05	-0.05	0.02	0.03	-0.04	0.00
10	421	-0.03	-0.07	-0.04	-0.04	-0.04	0.01	-0.02	-0.02	-0.03	-0.04	0.02	-0.04	0.04	-0.04	-0.03	0.04	-0.03
11	628	-0.03	0.01	-0.01	0.01	-0.01	0.00	-0.02	0.03	-0.01	-0.01	-0.04	0.02	-0.02	0.00	0.02	-0.02	-0.01
12	565	0.00	0.01	0.01	0.01	-0.01	-0.03	0.00	-0.03	-0.03	-0.07	-0.01	-0.02	0.01	0.00	-0.03	0.01	0.03
13	183	0.59	0.60	0.06	0.00	-0.01	0.00	0.01	-0.06	0.05	-0.03	0.06	-0.08	-0.02	0.00	-0.02	0.04	-0.01
14	364	0.00	-0.01	0.01	0.02	0.00	0.02	0.04	0.01	-0.01	0.01	0.04	0.00	0.02	0.02	0.00	-0.01	-0.01
15	343	-0.01	0.02	-0.01	-0.03	-0.03	0.02	0.01	0.00	-0.02	0.02	0.01	0.01	0.00	0.00	-0.03	0.03	-0.02
16	489	0.01	0.04	0.01	0.03	0.05	0.05	0.01	0.04	0.03	0.03	0.00	0.04	0.02	0.02	0.03	-0.03	0.03
17	643	0.03	0.01	-0.04	-0.02	0.03	0.02	0.00	0.02	0.03	0.01	-0.02	0.03	-0.02	0.01	0.02	-0.03	0.02
18	145	0.00	0.05	0.61	0.65	0.03	0.06	0.03	0.03	0.04	0.02	0.04	0.00	-0.03	0.03	-0.02	-0.04	0.04
19	790	-0.01	-0.02	-0.02	-0.03	-0.02	-0.03	-0.02	0.02	-0.02	-0.02	-0.07	0.01	0.03	0.01	0.02	-0.01	0.02
20	293	-0.02	-0.01	-0.02	0.01	-0.01	0.01	-0.01	0.00	-0.03	0.02	-0.01	0.06	-0.01	0.00	0.02	-0.03	0.00
21	92	0.02	0.00	0.09	0.05	0.58	0.51	0.58	0.58	0.63	0.52	0.52	-0.07	0.05	0.00	0.02	0.01	-0.01
22	256	-0.10	-0.09	-0.11	-0.05	-0.05	0.00	-0.03	0.02	-0.01	0.04	-0.02	0.04	0.01	-0.03	0.03	0.00	0.00
X	349	0.03	0.00	0.02	-0.01	0.01	-0.05	-0.06	-0.06	0.05	0.01	-0.03	-0.01	0.07	-0.01	0.02	0.00	-0.04

C

Outliers genes from normal distributions

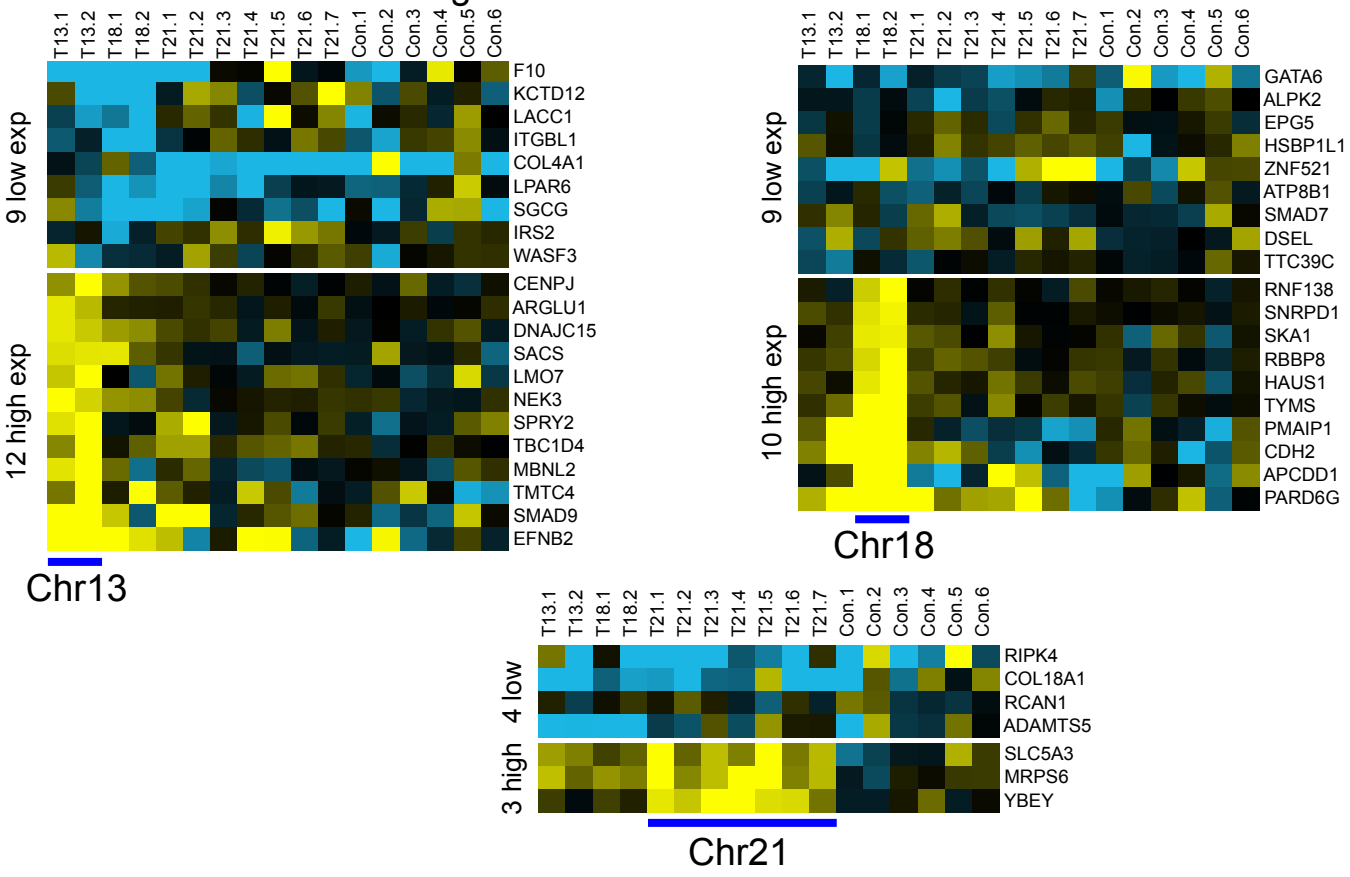


Figure S1. Transcript levels increase proportionally with gene copy number in trisomic primary fibroblasts

Cell lines utilized in this study. All cell lines were obtained from the Coriell Institute (https://www.coriell.org). Cat# = Catalogue number, Nom = nomenclature used in the manuscript. Sex, age, and tissue of the donor are listed. **B.** The values of the averaged gene expression per chromosome calculated for each cell line are shown. The triplicated chromosomes are highlighted in red. **C.** Gene expression of triplicated genes that are outliers in the fits of the normal distributions for each trisomic cell lines is shown. Outliers were identified by fitting the distributions to a Gaussian curve using least squares regression methods and defining the points above or below the curve.

Figure S2

A

Fibroblasts immortalized with hTERT
RNASeq data from Sullivan et al.

Cat#	Nomenclature	Sullivan
Controls		
GM08447	Con.1a-b	DS_F2DA_1-2
GM05659	Con.2a-b	DS_M1YR_1-2
GM00969	Con.3a-b	DS_F2YR_1-2
GM02036	Con.4a-b	DS_F11YR_1-2
GM03377	Con.5a-b	DS_M19YR_1-2
GM03440	Con.6a-b	DS_M20YR_1-2
Trisomy 21		
GM04616	T21.1a-b	TS_F3DA_1-2
AG05397	T21.2a-b	TS_M1YR_1-2
AG06922	T21.3a-b	TS_M2YR_1-2
GM02767	T21.4a-b	TS_F14YR_1-2
AG08941	T21.5a-b	TS_F19YR_1-2
AG08942	T21.6a-b	TS_M21YR_1-2

T cells were isolated from
10 individuals with T21 and
7 controls

Sullivan	Nom
D21_F17YR	Con.1
D21_F22YR	Con.2
D21_F29YR	Con.3
D21_F31YR	Con.4
D21_M31YR	Con.5
D21_M38YRA	Con.6
D21_M38YRB	Con.7
T21_F32YR	T21.1
T21_F45YR	T21.2
T21_F4YR	T21.3
T21_M12YR	T21.4
T21_M13YR	T21.5
T21_M13YRA	T21.6
T21_M13YRB	T21.7
T21_M14YR	T21.8
T21_M1YR	T21.9
T21_M33YR	T21.10

Monocytes were isolated from
10 individuals with T21 and
7 controls

Sullivan	Nom
D21_F17YR_5112016	Con.1
D21_F29YR_5112016	Con.2
D21_M31YR_5112016	Con.3
D21_M38YR_5112016	Con.4
D21_F22YR_5312016	Con.5
D21_F31YR_5312016	Con.6
D21_M38YR_5312016	Con.7
T21_F32YR_5112016	T21.1
T21_F45YR_5112016	T21.2
T21_M13YRA_5112016	T21.3
T21_M13YRB_5112016	T21.4
T21_F4YR_5312016	T21.5
T21_M1YR_5312016	T21.6
T21_M12YR_5312016	T21.7
T21_M13YR_5312016	T21.8
T21_M14YR_5312016	T21.9
T21_M33YR_5312016	T21.10

EBV immortalized lymphoblastoid
lines three T21 and three D21 come
from the Nexus Clinical Data Registry
and Biobank at the University of Colorado

Sullivan	Nom
D21_F7YR_1A	Con.1a
D21_F7YR_2A	Con.1b
D21_F14YR_1B	Con.2a
D21_F14YR_2B	Con.2b
D21_F18YR_1C	Con.3a
D21_F18YR_2C	Con.3b
T21_F9YR_1A	T21.1a
T21_F9YR_2A	T21.1b
T21_F13YR_1B	T21.2a
T21_F13YR_2B	T21.2b
T21_F20YR_1C	T21.3a
T21_F20YR_2C	T21.3b

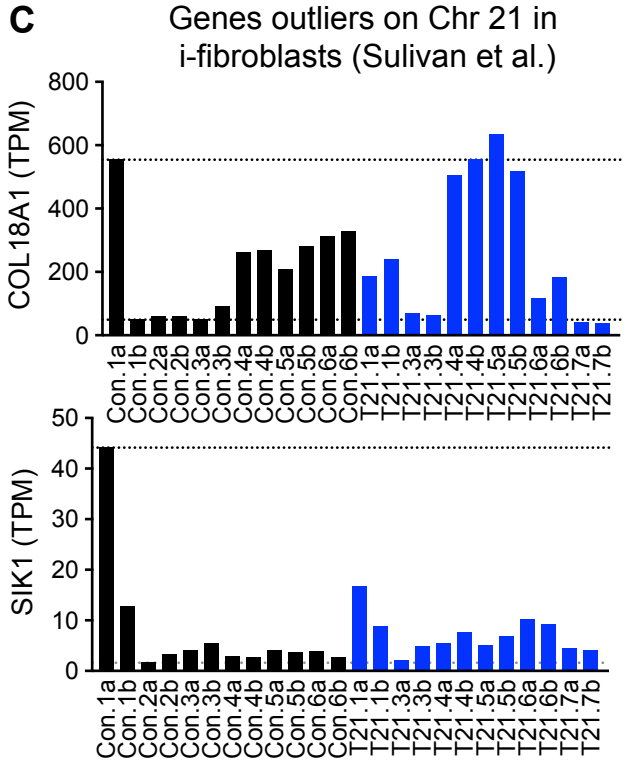
B

Letau.	Nom
rpkm_Ts1	T21.1
rpkm_Ts2	T21.2
rpkm_Ts3	T21.3
rpkm_Ts4	T21.4
rpkm_Ts5	T21.5
rpkm_Ts6	T21.6
rpkm_Ts7	T21.7
rpkm_Ts8	T21.8
rpkm_N1	Con.1
rpkm_N2	Con.2
rpkm_N3	Con.3
rpkm_N4	Con.4
rpkm_N5	Con.5
rpkm_N6	Con.6
rpkm_N7	Con.7
rpkm_N8	Con.8

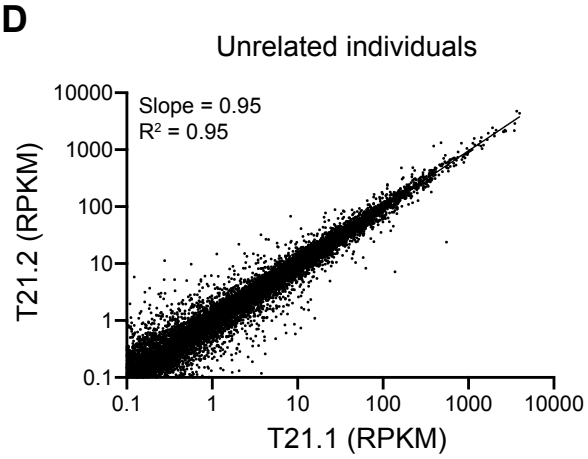
Replicates from Monozygotic twins

Letau.	Nom
rpkm_T2N_Rep0	MZ.1
rpkm_T2N_Rep1	MZ.2
rpkm_T2N_Rep2	MZ.3
rpkm_T2N_Rep3	MZ.4
rpkm_T1DS_Rep0	MZ.T21.1
rpkm_T1DS_Rep1	MZ.T21.2
rpkm_T1DS_Rep2	MZ.T21.3
rpkm_T1DS_Rep3	MZ.T21.4

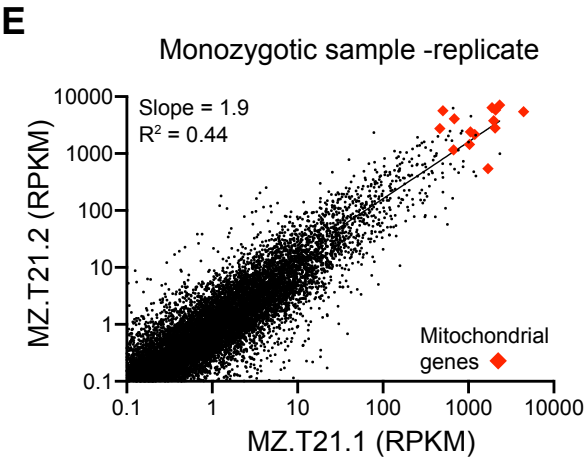
C



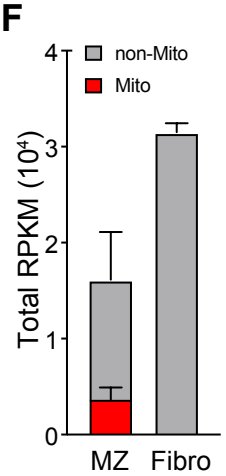
D



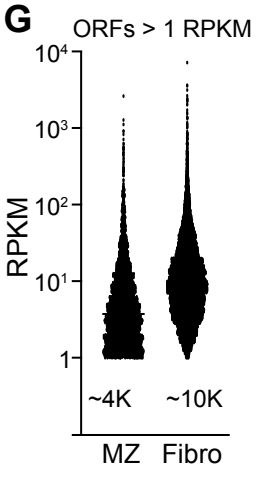
E



F



G



H

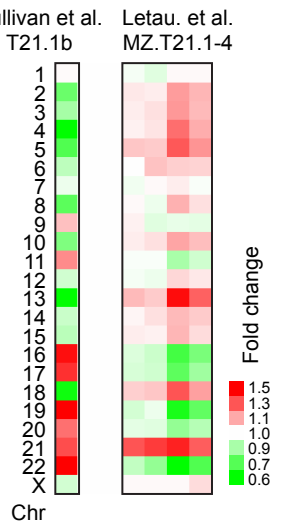


Figure S2. Transcript levels increase proportionally with gene copy number in distinct trisomic cell lines

A. Nomenclature of the cell lines analyzed by Sullivan et al. (20). **B.** Nomenclature of the cell lines analyzed by Letourneau et al. (16). **C.** Example of a couple of outliers of the fits of the distributions of triplicated genes in the data for the fibroblasts analyzed by Sullivan et al. **D.** Linear regression analysis of the RNA counts of the transcriptomes from two unrelated individuals. **E.** Linear regression analysis of the RNA counts of the biological replicates of the transcriptome from one monozygotic twin with trisomy 21. **F**. The average total reads of 8 transcriptomes from the monozygotic twins are almost half when compared to the average of the total reads from primary fibroblasts from unrelated individuals. In red, ~20% of the total reads come from mitochondrial DNA. **G**. Distribution of the transcript reads that show 1 RPKM of greater in both sets of data. **H.** Comparison of the average gene expression per chromosome of the outlier transcriptomes in the Sullivan et al. and Letourneau et al. studies.

Figure S3

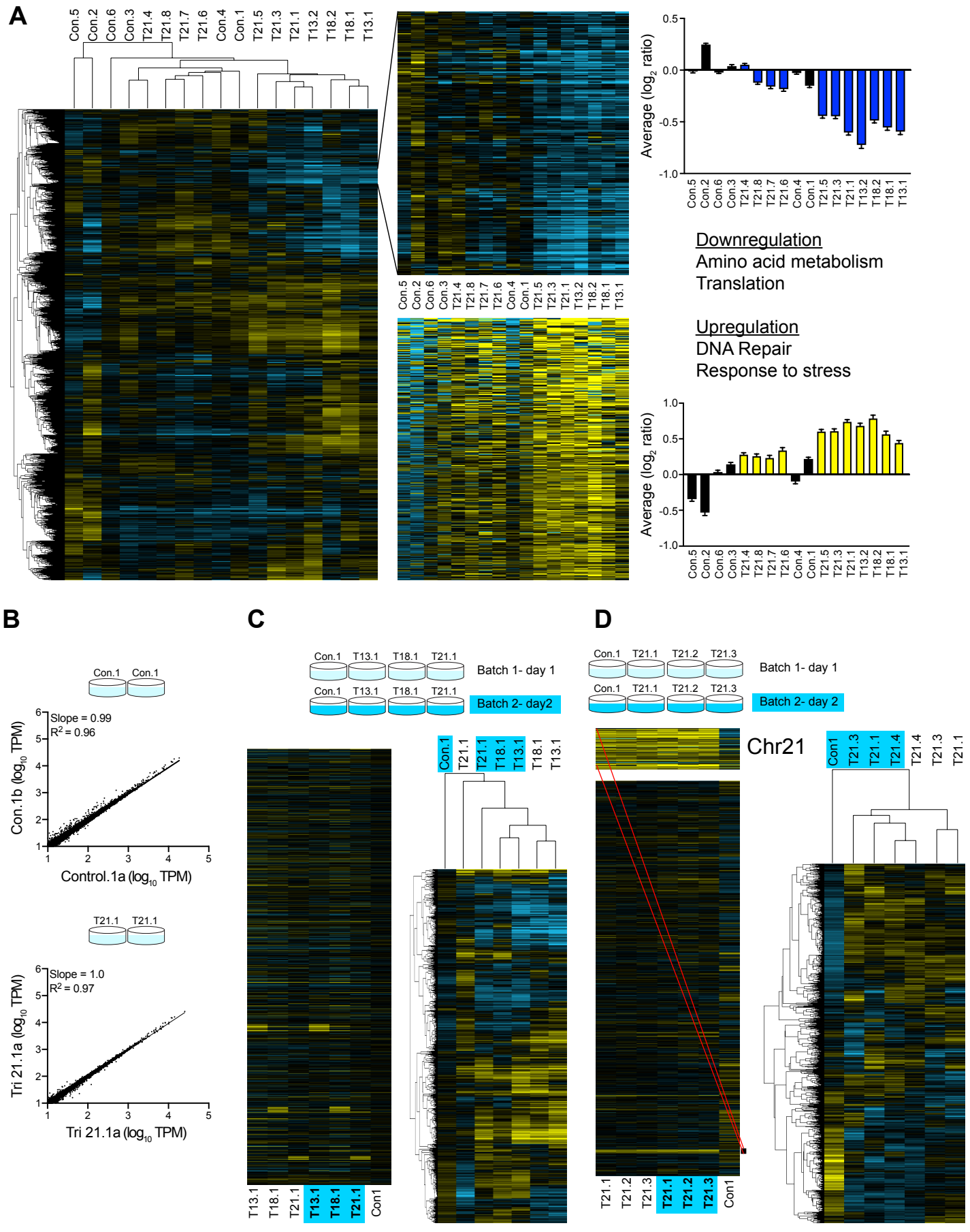

A
Con.5 Con.2 Con.6 Con.3 T21.4 T21.8 T21.7 T21.6 Con.4 Con.1 T21.5 T21.3 T21.1 T13.2 T18.2 T18.1 T13.1
Average (log2 ratio)
Downregulation
Amino acid metabolism
Translation
Upregulation
DNA Repair
Response to stress
B
Con.1 Con.1
Slope = 0.99
R2 = 0.96
Con.1b (log10 TPM)
Control.1a (log10 TPM)
T21.1 T21.1
Slope = 1.0
R2 = 0.97
Tri 21.1a (log10 TPM)
Tri 21.1a (log10 TPM)
C
Con.1 T13.1 T18.1 T21.1
Batch 1- day 1
Con.1 T13.1 T18.1 T21.1
Batch 2- day2
Con.1 T21.1 T21.1 T18.1 T13.1 T18.1 T13.1
T13.1 T18.1 T21.1 T13.1 T18.1 T21.1 Con1
D
Con.1 T21.1 T21.2 T21.3
Batch 1- day 1
Con.1 T21.1 T21.2 T21.3
Batch 2- day 2
Chr21
Con1 T21.3 T21.1 T21.4 T21.4 T21.3 T21.1
T21.1 T21.2 T21.3 T21.1 T21.2 T21.3 Con1

Figure S3. Gene expression patterns of human primary fibroblasts

A. Hierarchical clustering analyses of the expression patterns of primary fibroblasts analyzed in this study (left heatmap). Down (middle top) and upregulated (middle bottom) cluster of genes in primary trisomic fibroblasts. Bars represent the average change in gene expression of the down and unregulated clusters (right). **B.** Linear regression analysis of the transcriptome profiles of one control and one trisomy 21 cell lines grown in parallel show a high degree of reproducibility. **C-D**. Hierarchical clustering analyses of the expression patterns of primary fibroblasts grown months apart show that culture conditions significantly influence the patterns of gene expression. Cluster patterns are driven by the time of culture in each comparison (blue boxes).

Figure S4

A

Pearson r between RNA and protein profiles

	T13.1.prot	T13.2.prot	T18.1.prot	T18.2.prot
Con.1.rna	-0.30	-0.14	-0.34	-0.19
Con.2.rna	0.01	-0.04	-0.03	0.15
T13.1.rna	0.50	0.39	0.33	0.29
T13.2.rna	0.20	0.57	0.22	0.27
T18.1.rna	0.41	0.41	0.63	0.46
T18.2.rna	0.29	0.37	0.36	0.65
T21.1rna	0.13	0.30	0.10	0.27
T21.3.rna	-0.03	-0.07	-0.11	0.04
T21.4.rna	0.08	0.16	0.04	0.15

	Con.1a.prot	Con.1b.prot	Con.2.prot	Con.4.prot	Con.6.prot
Con.1.rna	0.41	0.42	-0.44	0.09	0.00
Con.2.rna	-0.16	-0.18	0.49	-0.01	-0.03
Con.4.rna	0.20	0.22	-0.23	0.55	0.17
Con.6.rna	0.07	0.09	-0.29	0.07	0.42

	T21.1.prot	T21.2prot	T21.5prot	T21.6prot	T21.7prot
T21.1.rna	0.40	0.31	0.20	0.16	0.18
T21.2.rna	0.11	0.57	0.20	0.27	0.26
T21.5.rna	0.16	0.31	0.58	0.32	0.26
T21.6.rna	0.03	0.27	0.24	0.50	0.27
T21.7.rna	-0.02	0.32	0.19	0.28	0.53

B

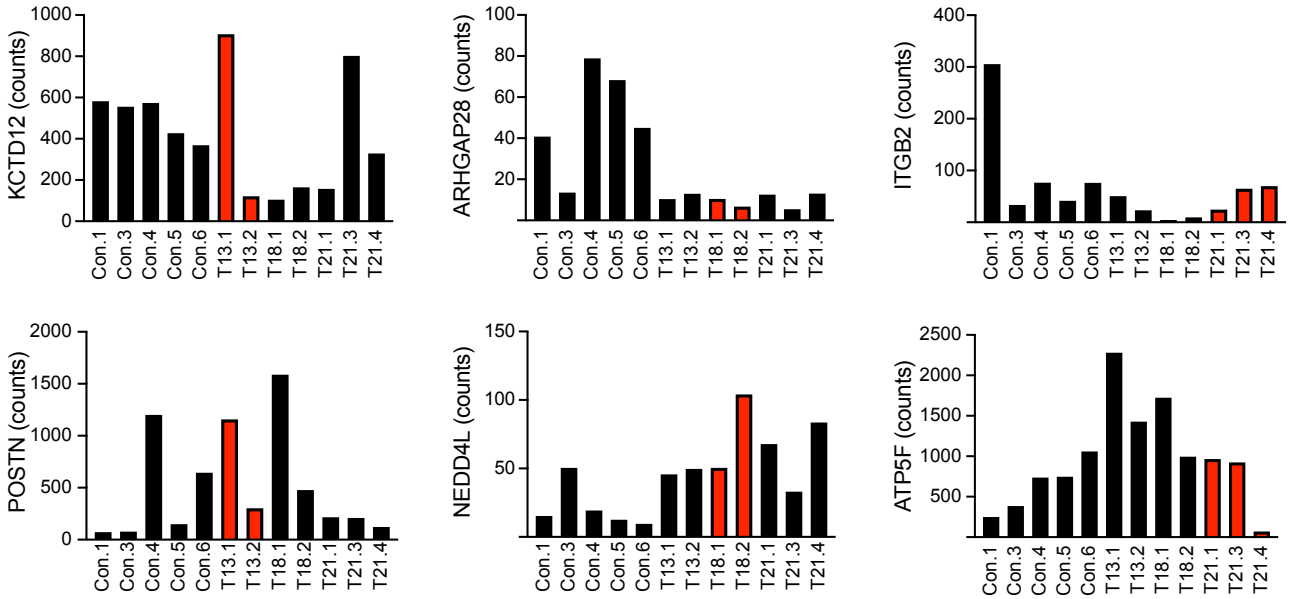


Figure S4. Protein levels proportionally increase with copy number in trisomic fibroblasts
A. Pearson correlation coefficients were calculated for the transcriptome and proteome profiles of the indicated cell lines. **B**. Examples of the peptide counts of a few triplicated genes that are outliers in the fits of the normal distributions. Red bars correspond to peptide counts of the triplicates genes.

Figure S5

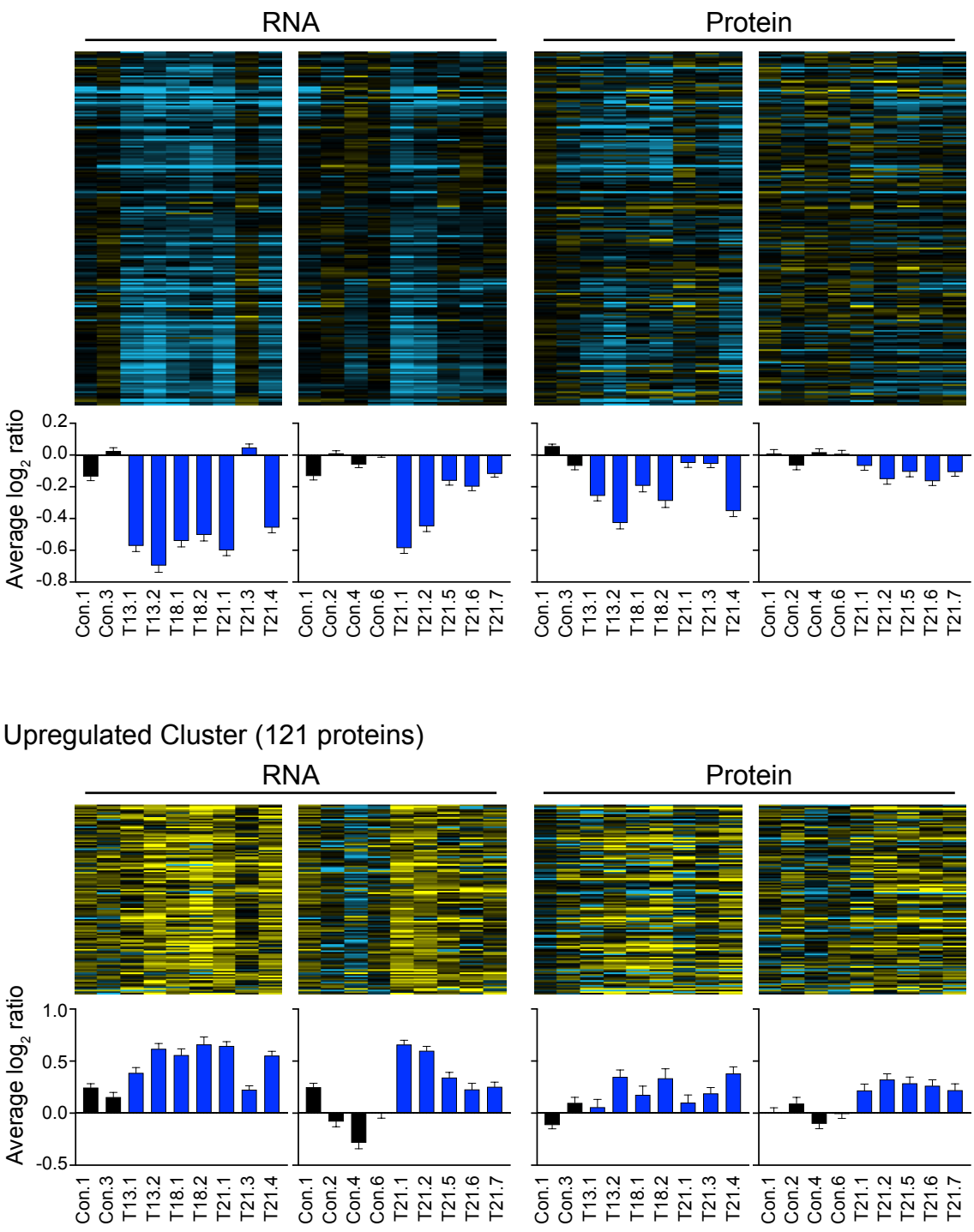


Figure S5. Cluster of down and upregulated genes in trisomic fibroblasts
Down and upregulated clusters of genes identified in Figure S3 lead to changes in protein levels.
Bars represent average change in transcript (left) and protein levels (left) in each of the cell lines analyzed.

Figure S6

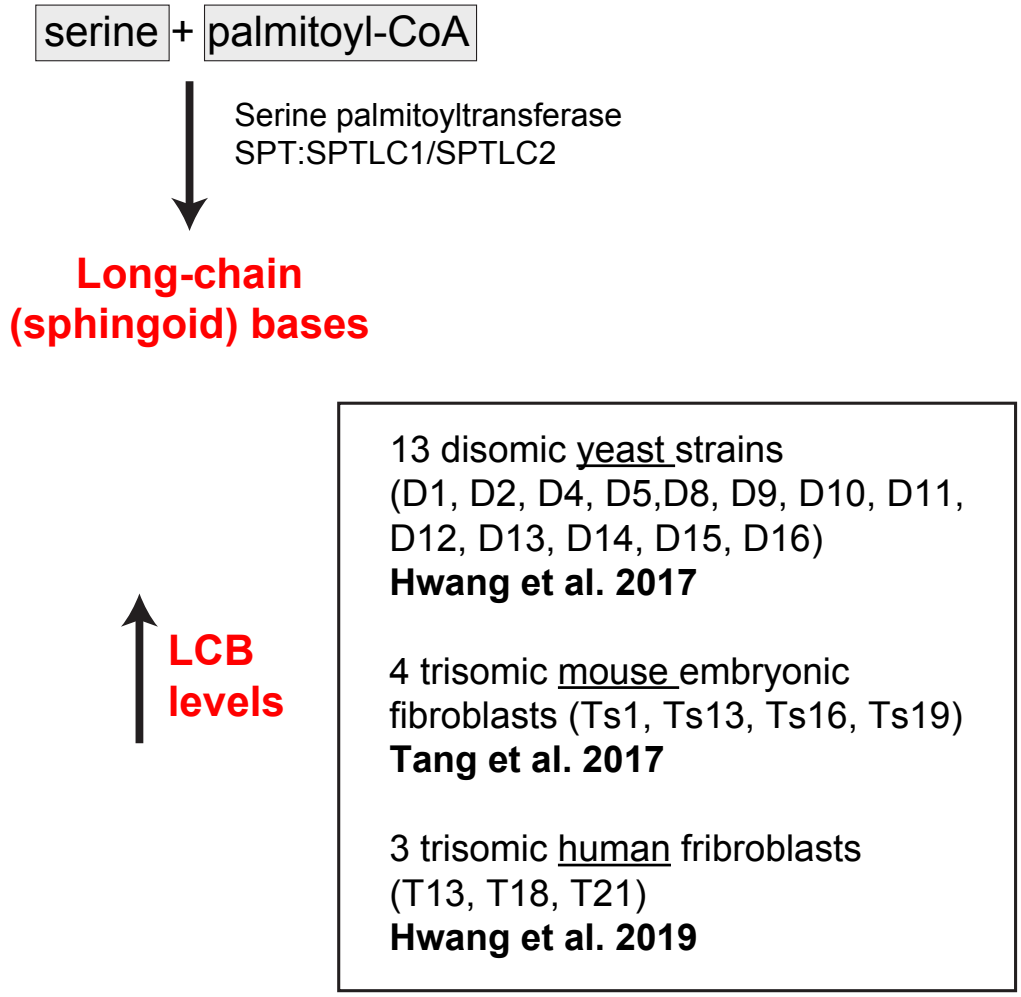


Figure S6. Sphingolipid synthesis is upregulated in aneuploid cells
Schematic of the biochemical pathway for the serine-dependent synthesis of sphingolipids is shown. LCB levels are elevated in aneuploid yeast, mouse, and human cells independent of the independent of the identity of the extra chromosome.

Figure S7

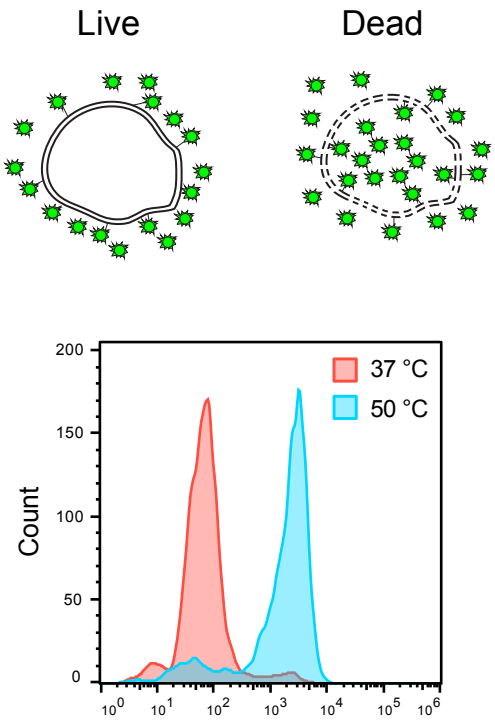


Figure S7. Assay for cell viability
The LIVE/DEAD™ Fixable Green Dead Cell Stain Kit uses a dye that reacts with free amines yielding intense fluorescent staining. The dye's reactivity is restricted to the cell surface in viable cells resulting in less intense fluorescence than non-viable cells. As a control experiment, cells were heat shocked for 30 minutes. Viable control cells are in red and heat treated non-viable cells are in blue.

Supplementary Experimental Procedures

Protein extraction and digestion

Frozen cell pellets were lysed with RIPA buffer (150 mM NaCl, 1% NP40, sodium deoxycholate 0.5%, SDS 0.1%, TRIS-HCl 25 mM pH 7.4, DTT 5 mM) and insoluble material was removed by pelleting at 20k x g for 10 min. The supernatant was methanol/chloroform precipitated and dried on the benchtop. Dry peptide pellets were resuspended in 8 M urea, 50 mM ammonium bicarbonate (ambic). Protein concentrations were measured using the DC protein assay kit (BioRad). Proteins were reduced by adding DTT to a final concentration of 5 mM and incubating for 30 min at RT, then alkylated with 15 mM iodoacetamide in the dark at RT for 30 min. An additional 5 mM DTT was added to quench the iodoacetamide. The urea concentration was diluted to 2 M by adding 3 volumes of 50 mM ambic. Proteins were digested overnight at room temperature with lysyl endopeptidase (lysC, Wako, Richmond, VA) at a ratio 1:125 enzyme:protein at RT. Samples were diluted to 1 M urea with 50 mM ambic and digested with Trypsin (Promega #V5111) for 10 hours at 1:125 enzyme:protein at 37°C. Digestion was stopped by the addition of formic acid (FA) to a final concentration of 2%. Acidified peptides were loaded onto pre-equilibrated Sep-Pak tC18 cartridges (Waters) and the columns were washed with 1% FA. Bound peptides were eluted with 70% acetonitrile (ACN), 1% FA, dried, and re-suspended in water. Protein concentration was measured using the Pierce colorimetric peptide assay (Thermo, #23275). 50 µg of peptides from each sample was aliquoted and dried down, then re-suspended in 100 ul of 0.5 M HEPES, pH = 8.5. Peptides were labeled by the addition of 0.25 mg TMT in anhydrous ACN for 60 min at RT. Labeling was quenched with 8 µl 5% hydroxylamine and acidified with 16 µl neat FA. A small aliquot (5 µl) of each labeled sample was removed and mixed in equal volumes from each of the 10 channels within each TMT set, desalted on C18 STAGE tips (55), and analyzed using a 85 min LC-MS/MS/MS method with synchronous precursor selection (SPS) enabled to generate TMT reporter ions from MS2 fragment ions. Reporter ions were scanned at 60,000 resolution in the orbitrap. The final sample mixes for each 10plex were made based on the relative intensity of total reporter ions from each in order to achieve equal levels.

Peptide Fractionation

Combined TMT-labeled peptides were de-salted, resuspended in 300 µl buffer A (5% ACN, 10 mM NH_4HCO_3, pH 8) and separated by high-pH reverse-phase HPLC (56) on a C18 column (Waters, #186003570, 4.6 mm x 250 mm, 3.5 µ ID) using a 50 min gradient from 18% to 38% buffer B (90% ACN, 10 mM NH_4HCO_3, pH 8) with a flow rate = 0.8 ml/min. Fractions were collected over 45 min at 28 sec intervals beginning 5 min after the start of gradient in a 96-well plate. These original fractions were pooled into 12 samples each containing four fractions (only 48 of 96 fractions were used) by sampling at equal intervals across the gradient, i.e. by combining fractions 1/25/49/73, 3/27/51/75, 5/29/53/77, ….13,37,61,95. This pooling strategy minimizes overlap between fractions. The pooled samples were dried down, resuspended in 50 µl of 5% FA, and desalted on STAGE tips.

LC-MS/MS Analysis

Each fraction was analyzed on a Thermo Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific) equipped with an Easy nLC-1000 UHPLC (Thermo Fisher Scientific). Peptides were separated with a gradient of 6–25% ACN in 0.1% FA over 115 min and introduced into the mass spectrometer by nano-electrospray as they eluted off a self-packed 40 cm, 75 µm (ID) reverse-phase column packed with 1.8 µm, C18 resin (Sepax Technologies, Newark, DE). They were detected using the real-time search (RTS) MS3 method (57) which employs a data-dependent Top10-MS2 method and a RTS triggered SPS-MS3 to collect reporter ions. For each cycle, one full MS scan was acquired in the Orbitrap at a resolution of 120,000 with automatic gain control (AGC) target of 5×10^5 and maximum ion accumulation time of 100 ms. Each full scan was followed by the individual selection of the most intense ions, using a 2 sec cycle time for collision-induced dissociation (CID) and MS2 analysis in the linear ion trap for peptide identification using an AGC target of 1.5×10^4 and a maximum ion accumulation time of 50 ms. Ions selected for MS2 analysis were excluded from reanalysis for 60 s. Ions with +1, >+5, or unassigned charge were excluded from selection. Collected MS2 spectra were searched in real time against a database of all uniprot reviewed human sequences (uniprot.org, downloaded May 1, 2019) using binomial score cutoff of 0.75. Passing spectra triggered synchronous precursor selection of up to 10 MS2 ions for HCD fragmentation and MS3 scanning in the orbitrap at resolution = 60,000 using a maximum ion time of 200 ms. The gene close-out feature was enabled and applied to each set of

12 fractions and used to limit the selection and collection of MS3 reporter ions to a maximum of 10 peptides per protein across all 12 runs in each 10plex set.

Peptide Searching and Filtering and Protein Quantification

MS/MS spectra were then searched to match peptide sequences using SEQUEST v.28 (58) and a composite database containing all reviewed human uniprot protein sequences (uniprot.org, downloaded May 1, 2019) and their reversed complement. Search parameters allowed for two missed cleavages, a mass tolerance of 20 ppm, a static modification of 57.02146 Da (carboxyamidomethylation) on cysteine, and dynamic modifications of 15.99491 Da (oxidation) on methionine and 229.16293 Da on peptide amino termini and lysines.

Peptide spectral matches were filtered to a 2% false-positive rate using the target-decoy strategy (59) combined with linear discriminant analysis (LDA)(60) using the SEQUEST Xcorr and ΔCn scores, mass error, charge, and the number of missed cleavages. Further filtering based on the quality of quantitative measurements (reporter ion sum signal-to-noise ≥ 200, isolation specificity >0.7) resulted in a final protein FDR < 1% for both 10plex experiments.

Total protein intensity values for each sample were derived from the summed reporter ion intensities from the corresponding channel from all peptides mapping to that protein. Values were normalized to account for small variations in sample mixing based on the sum intensity of values from all proteins in each channel within each 10plex. Relative abundances for each protein across samples within each 10plex were calculated as the fraction of the total intensity derived from each channel. The average $\log_2$ [Trisomy/WT] was calculated and used for all subsequent analysis. Proteome data is available: https://massive.ucsd.edu, ID#MSV000085706 and are available for download at ftp://massive.ucsd.edu/MSV000085706.