Supplemental online materials:

Materials and Methods:

Mouse Strains

All mouse strains were obtained from the Jackson Laboratory. Strains used to generate trisomic embryos: Rb(1.2)18Lub/J and Rb(1.3)1Ei/J (Ts1), Rb(11.13)4Bnr/J and Rb(13.16)1Mpl/J (Ts13), Rb(6.16)24Lub and Rb(16.17)7Bnr (Ts16), and Rb(5.19)1Wh/J and Rb(9.19)163H (Ts19). All male compound Robertsonian heterozygous mice were mated with C57BL/6J females and embryos were collected at specific stages of embryogenesis by timed matings. All animal studies and procedures were approved by the MIT Institutional Animal Care and Use Committee.

Mouse Embryonic Fibroblast Derivation and Culture

Except for trisomy 1 lines, MEF lines were established as in (*S1*). Ts1 cell lines were created from 10.5 or 11.5 day postcoitum embryos and plated after overnight incubation in trypsin at 4°C followed by incubation for 30min at 37°C. Cells were plated on 1 to 3 6cm plates depending on the size of the embryo isolated. All cells were cultured in DMEM supplemented with 10% FBS, glutamine, penicillin, and streptomycin at 37°C. Cells were cultured at 37°C with 5% CO_2 in a humidified environment. In all experiments, cells were counted using a hemocytometer or Cellometer Auto T4 automated hemacytometer (Nexcelom).

Metaphase Spreads

Metaphase spreads were generated from early passage cell lines (≤p3). Exponentially growing cells were treated with colchicine (Sigma) at 50μg/ml for 4-6 hours. Cells were collected by trypsinization followed by centrifugation. Collected cells were incubated in 0.075mM NaCl hypotonic solution for 30 mins at 37°C. Following swelling in hypotonic solution, cells were washed in 3:1 methanol:acetic acid fix solution 3 times and resuspended in 50-200μl of fix solution and stored at -20°C. Slides were generated by

adding 7μl of fixed metaphase cells to a clean glass slide and spread by tilting the slide. The slide was subsequently held cells-side down over a boiling water bath for 5 seconds and then transferred to a heating block set at 90°C for 2-5 min. Slides were allowed to dry at room temperature overnight. Dried slides were stained with Geimsa (Sigma) or transferred to -20°C for storage prior to spectral karyotyping.

Spectral Karyotyping

Metaphase spreads were prepared and hybridized with SKYPaint Probe Mixture for Mouse Chromosomes (Applied Spectral Imaging) according to manufacturer instructions. After hybridization, slides were visualized with an Olympus BX61 microscope equipped with an ASI Spinning Disk Attachment (Applied Spectral Imaging). Images were then analyzed with SkyView 2.1.1 software (Applied Spectral Imaging)

Transcript Array

RNA was isolated from early passage (≤p3) cell lines by Trizol (Invitrogen). 5μg of total RNA was then reverse transcribed and labeled with GeneChip One-Cycle Target Labeling and Control Reagents as recommended by the manufacture (Affymetix) and hybridized to Affy Mouse 430A 2.0 Arrays (Affymetrix). Chips were analyzed and data was extracted for examination by GeneChip Operating Software (Affymetrix).

Expression Analysis

Affymetrix data analysis was performed using statistical tools provided by the r/Bioconductor projects (http://cran.r-project.org/; http://www.bioconductor.org/). Data import and quality control assessment was done using the Affy package (*S2*) and the AffylmGUI package (*S3*). Data was summarized and normalized using gcRMA (*S4*). Genes expressed at significantly different levels in the trisomies vs. wild-type, trisomy 13 vs wild-type and trisomy 16 vs wild-type comparisons were identified using the local pooled error test (LPE) with the BH correction to control for multiple hypothesis (*S5*). The commands for these differential expression tests and the related data file are available here (lpe.tar.gz). The summarized expression data (log2 scale) and differential expression results included in Table S1.

The boxplots summarizing fold change data by chromosomes were created in r. Two different sets of plots were created. The "expressed" set includes only expressed probes with average expression > 3 and variance across all samples < 0.2. "All" plots include all of the data. In all cases, fold change data were obtained by subtracting the average wild-type value from the average trisomy value (or the value itself if multiple data points were not available). Probes unmapped to specific chromosomes and those mapping to the Y chromosome were excluded. The commands and data required to create these plots are available here (boxplots.tar.gz).

Gene Set Enrichment Analysis (GSEA)

GSEA (http://www.broad.mit.edu/gsea/) was used to assess the significance of the increase in gene expression observed on the trisomic chromosomes. In comparisons between trisomic samples and wild-type samples, the gene set consisting of genes encoded by the trisomic chromosome has the highest normalized enrichment score and confidence values that approach 0. The files required for these comparisons and the results are available here (gsea.tar.gz).

Analysis of Functional Annotations

The GO Tree Machine (http://bioinfo.vanderbilt.edu/gotm/frame.php) was used to compare the functional annotations attached to differentially expressed genes in the Ts13 vs. wild-type comparison with those annotations attached to all gene products interrogated by the MOE_430A_2 chip.

Accumulation assays.

Exponentially growing early passage MEFs were plated at a density of $1x10^5$ cells on individual wells of multiple 6-well plates. All cells were plated in a final volume of 3 mls of medium. Each day wells were trypsinized and counted in triplicate. For "fed" cultures, the medium was aspirated and replaced with fresh medium every other day throughout the course of the experiment. Statistical analysis was performed using a 2-way nested ANOVA with all data points from days 3, 5, and 7. The data of all

accumulation assays employed for these statistical analyses are shown in Supplemental Table 4.

Metabolic Analysis

Different components of 1.5 mls of tissue culture medium were analyzed with an MBS7100 (YSI) according to the manufacturers specifications. To prepare samples for analysis, the medium was collected from the cells and cleared by centrifugation. The supernatant was transferred to a fresh tube and samples were kept at -20°C until analyzed. To determine the amount of analyte produced or used in the medium, all samples were compared to identical medium that was not exposed to cells. Statistical analysis was performed using a 2-way nested ANOVA with all data points obtained from days 3, 5, and 7 of the accumulation assays. The data of all accumulation assays employed for these statistical analyses are shown in Supplemental Table 4.

Cell volume Determination.

Cellular volume was determined using a Multisizer 3 Coulter Counter (Becton Dickinson) counting 5000 events according to the manufacturer instructions.

Cell Cycle Analysis

Exponentially growing cells ≤p5 were collected by trypsinization followed by centrifugation. Cells were fixed in 70% ethanol and stored at -20°C until analyzed. Cells were then resuspended in propidium iodide and RNAseA in PBS. For each cell line at least 20,000 events were collected on a FACScan Flowcytometer (Becton Dickinson). Cell cycle analysis was determined using FloJo software (Tree Star Inc.).

Proliferation Assays.

To determine the proliferative capacity and spontaneous immortalization of cell lines we used a modified serial passaging protocol as in Todaro and Green (*S6*). Briefly, passage 3 MEFs were plated on two 6-well plates (12 wells total) at a density of $5x10^5$ cells per well. Cells were allowed to grow, and on day 3 cells were trypsinized and counted for

the number of cells per well. Cells were then pooled and replated at $5x10^5$ cells per well. This process was repeated until there were either not enough cells to plate, or until immortalization (determined by an increase in cell proliferation) had taken place. The passage when immortalization had taken place was determined by modeling the doublings per passage and fitting them using the following equation using Prism software (Graphpad Software).

Y1=slope1*x + intercept1
intercept2 = slope1*x0 + intercept1
Y2=slope2*(x-x0) + intercept2
Y=IF((x<x0), y1, y2)

Senescence-associated β-galactosidase assays

Identification of senescent cells was performed using established protocols (*S7*). Briefly, cells on tissue culture plates were washed three times with PBS and fixed with 3% formaldehyde in PBS for 5 minutes. The cells were then washed three times with PBS, and incubated overnight at 37°C with freshly prepared staining solution (37mM Citric acid, 126mM Na2HPO4, 1mg/ml X-Gal, 5mM K4Fe(CN)6, 5mM K3Fe(CN)6, 150mM Nacl, 2mM MgCl2). Cells were visualized by light microscopy.

Fig. S1

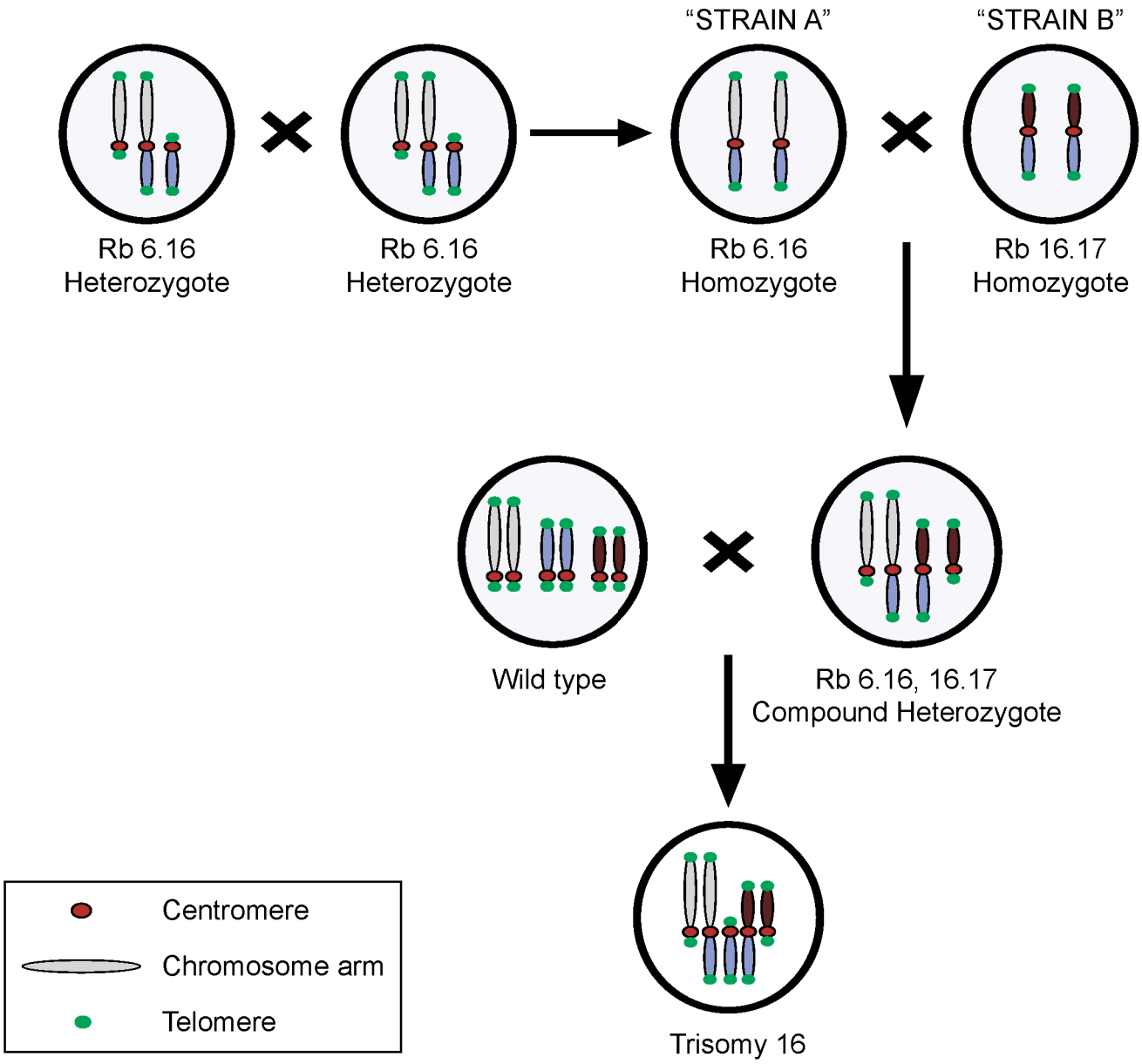

"STRAIN A"
"STRAIN B"
Rb 6.16
Heterozygote
Rb 6.16
Heterozygote
Rb 6.16
Homozygote
Rb 16.17
Homozygote
Wild type
Rb 6.16, 16.17
Compound Heterozygote
Trisomy 16
Centromere
Chromosome arm
Telomere

Fig. S2

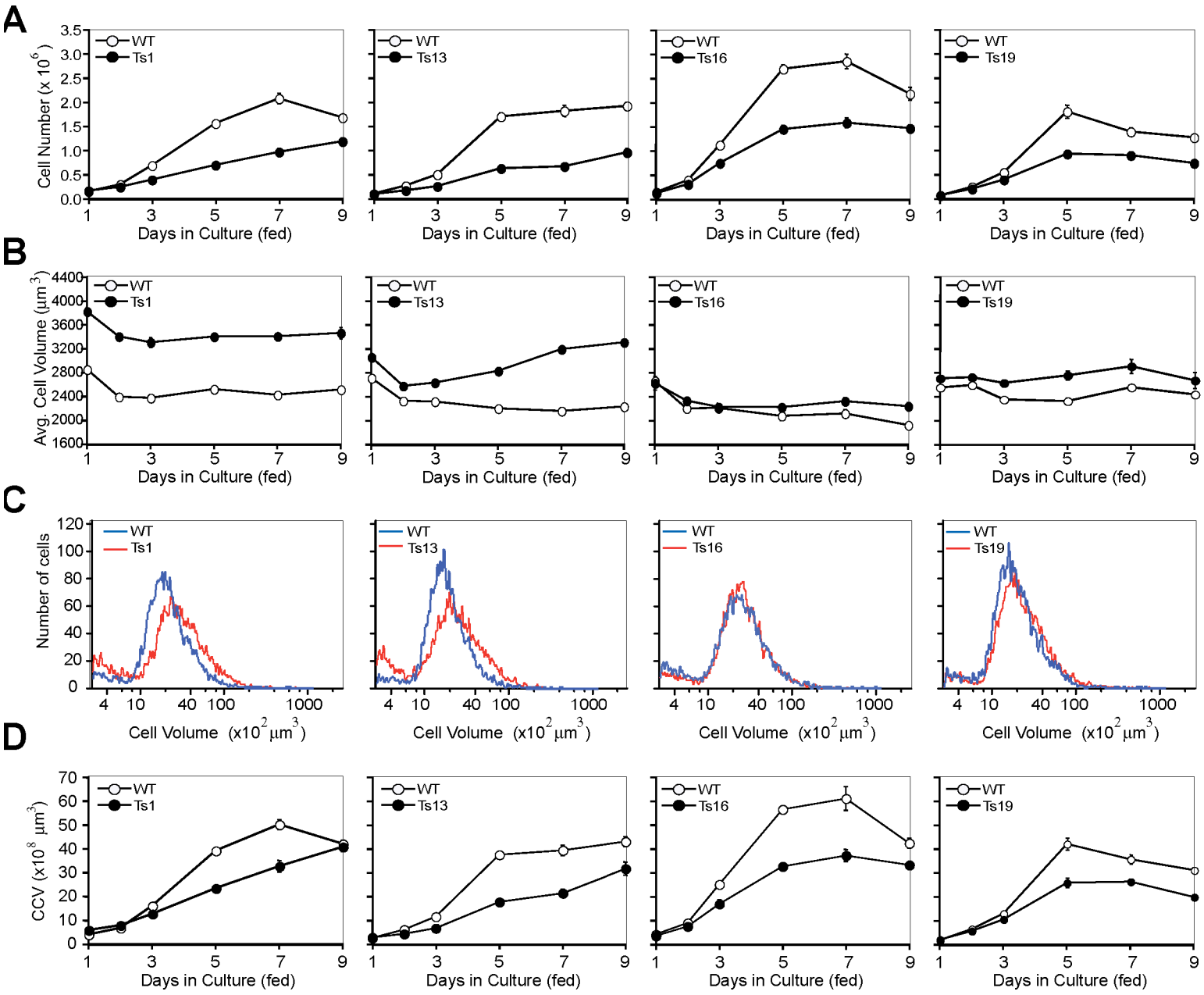

A
Cell Number (x 10^6)
WT
Ts1
Ts13
Ts16
Ts19
Days in Culture (fed)
B
Avg. Cell Volume (µm^3)
C
Number of cells
Cell Volume (x10^2 µm^3)
D
CCV (x10^8 µm^3)

Fig. S3

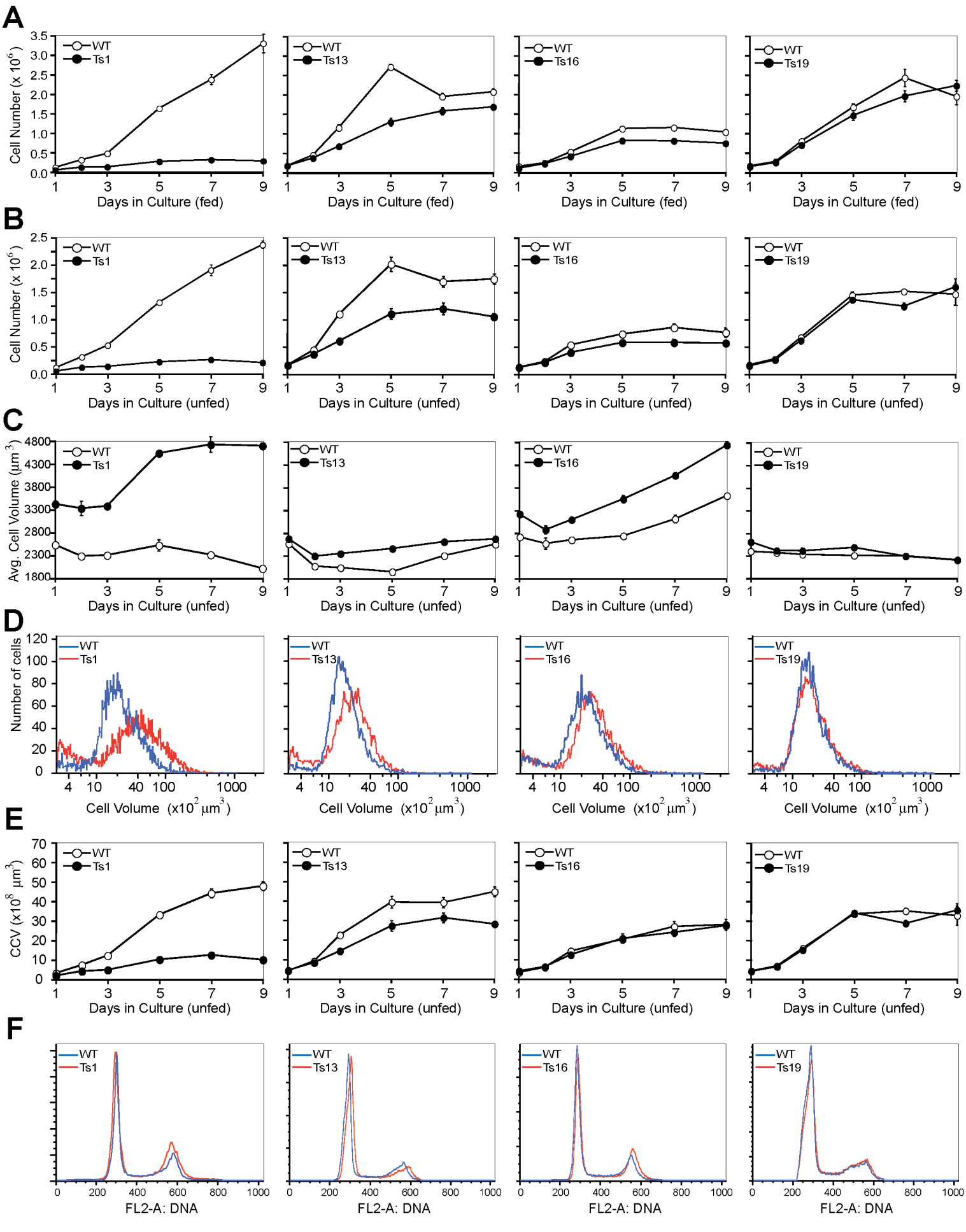
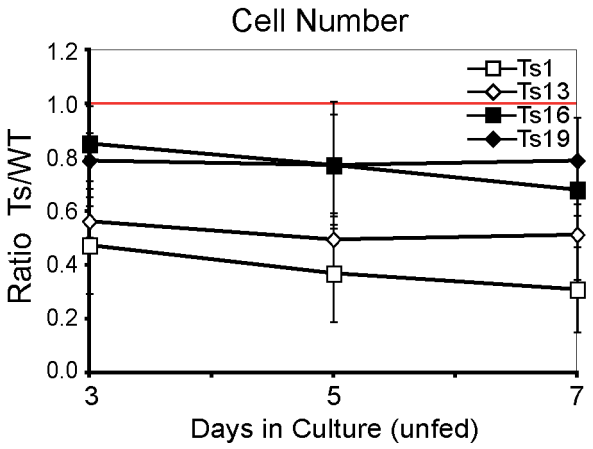
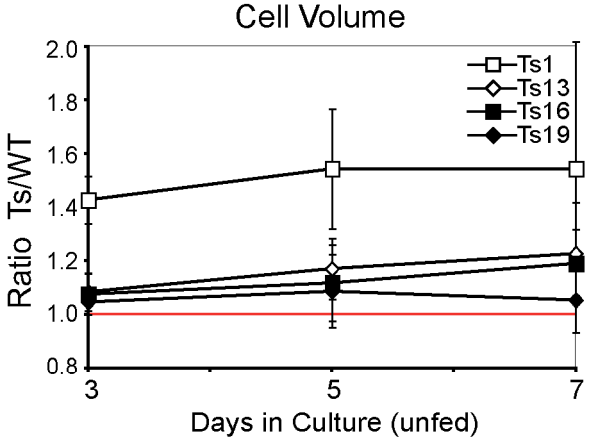

A
Cell Number (x 10^6)
WT
Ts1
Ts13
Ts16
Ts19
Days in Culture (fed)
B
Days in Culture (unfed)
C
Avg. Cell Volume (µm^3)
D
Number of cells
Cell Volume (x10^2 µm^3)
E
CCV (x10^8 µm^3)
F
FL2-A: DNA

Fig. S4

A


Cell Number
Ts1
Ts13
Ts16
Ts19
Ratio Ts/WT
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3
5
7
Days in Culture (unfed)

B


Cell Volume
Ts1
Ts13
Ts16
Ts19
Ratio Ts/WT
2.0
1.8
1.6
1.4
1.2
1.0
0.8
3
5
7
Days in Culture (unfed)

C

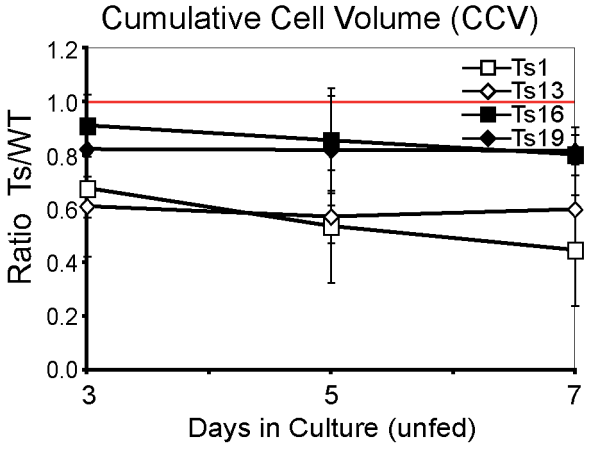

Cumulative Cell Volume (CCV)
Ts1
Ts13
Ts16
Ts19
Ratio Ts/WT
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3
5
7
Days in Culture (unfed)

Fig. S5

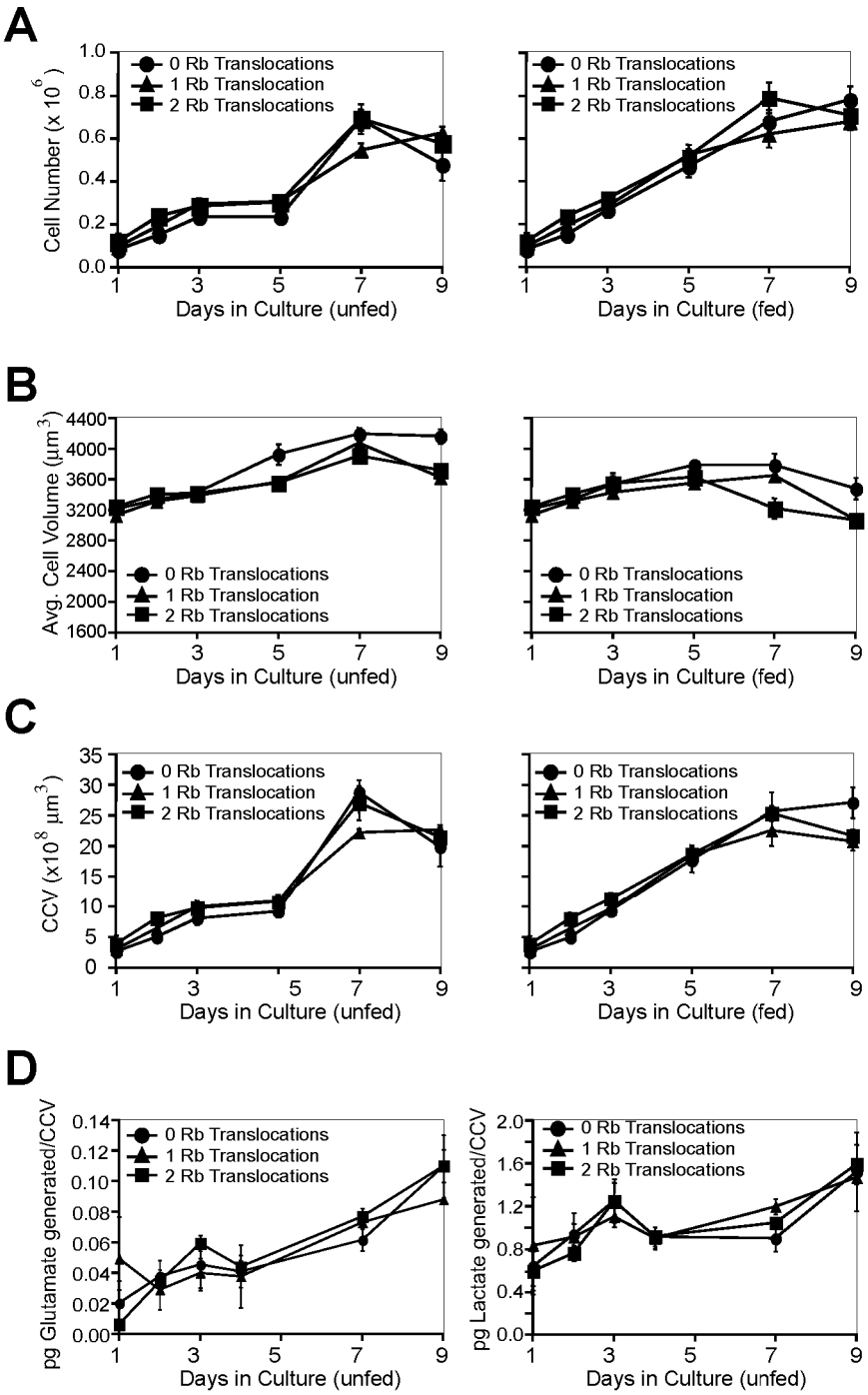

A
Cell Number (x 10^6)
0 Rb Translocations
1 Rb Translocation
2 Rb Translocations
Days in Culture (unfed)
Days in Culture (fed)
B
Avg. Cell Volume (μm^3)
C
CCV (x10^8 μm^3)
D
pg Glutamate generated/CCV
pg Lactate generated/CCV

Fig. S6

A

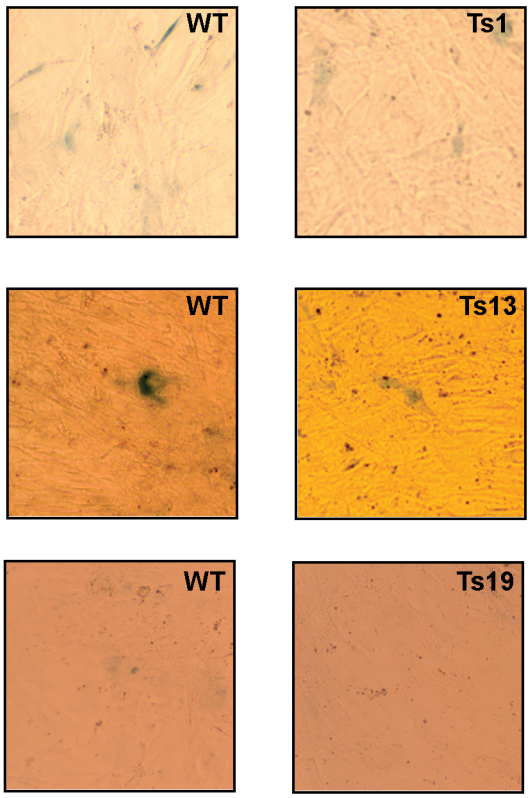


Fig. S7

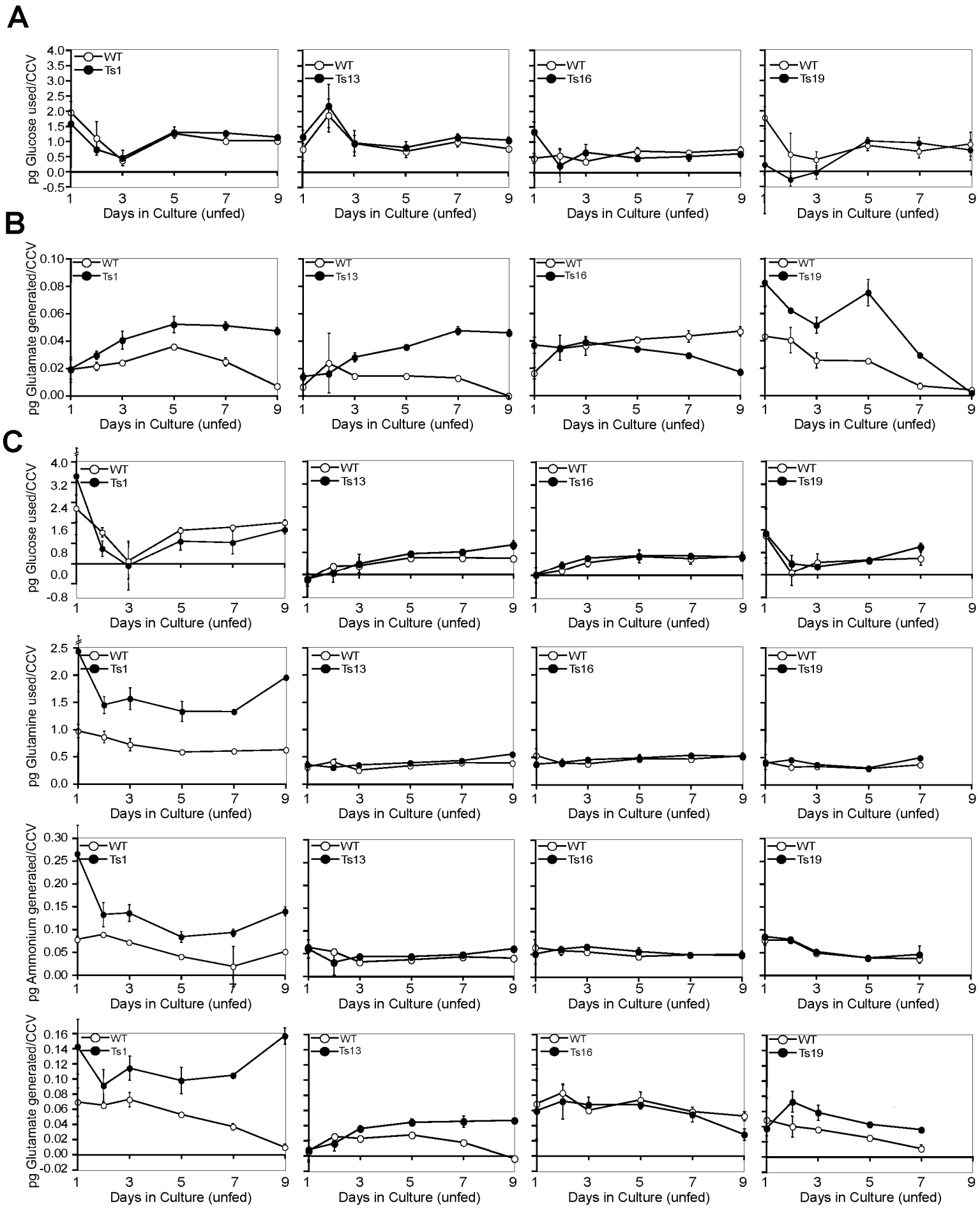

A
B
C
WT
Ts1
Ts13
Ts16
Ts19
pg Glucose used/CCV
pg Glutamate generated/CCV
pg Glutamine used/CCV
pg Ammonium generated/CCV
pg Glutamate generated/CCV
Days in Culture (unfed)

Fig. S8

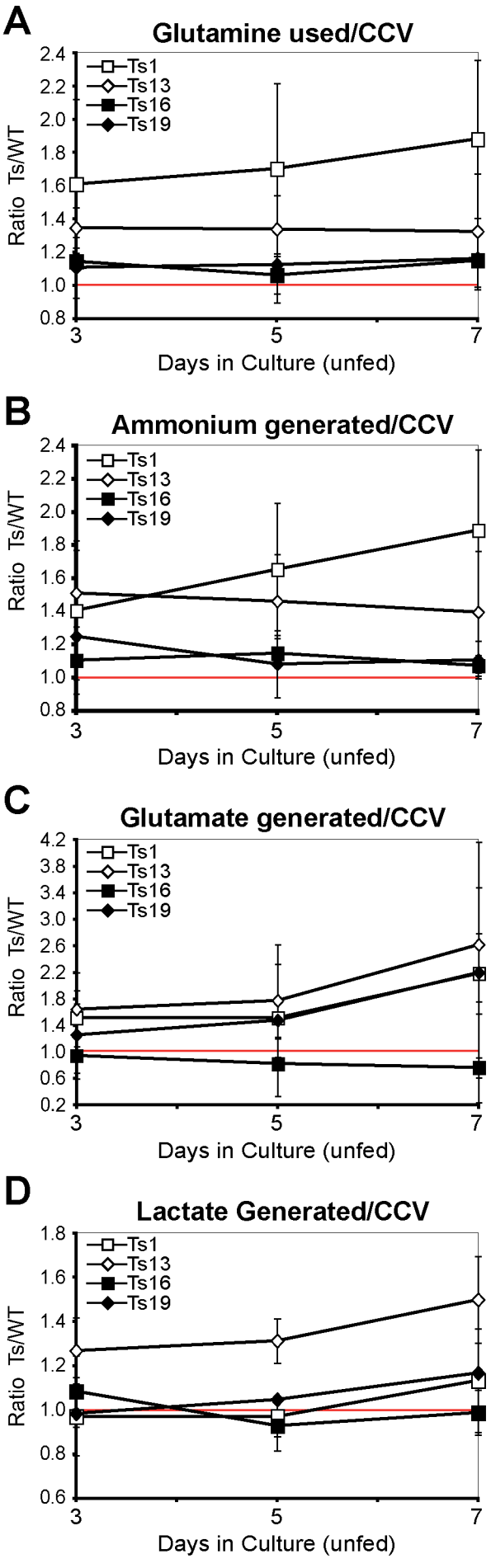
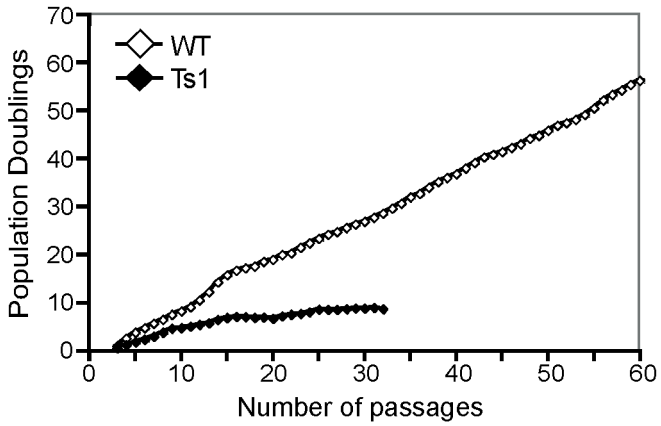

A
Glutamine used/CCV
Ts1
Ts13
Ts16
Ts19
Ratio Ts/WT
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
3
5
7
Days in Culture (unfed)
B
Ammonium generated/CCV
Ts1
Ts13
Ts16
Ts19
Ratio Ts/WT
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
3
5
7
Days in Culture (unfed)
C
Glutamate generated/CCV
Ts1
Ts13
Ts16
Ts19
Ratio Ts/WT
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
1.0
0.6
0.2
3
5
7
Days in Culture (unfed)
D
Lactate Generated/CCV
Ts1
Ts13
Ts16
Ts19
Ratio Ts/WT
1.8
1.6
1.4
1.2
1.0
0.8
0.6
3
5
7
Days in Culture (unfed)

Fig. S9

A


70
60
50
40
30
20
10
0
Population Doublings
WT
Ts1
0
10
20
30
40
50
60
Number of passages

Supplemental Figures:

Supplemental Figure 1: The breeding scheme used to generate trisomic embryos using trisomy 16 as an example.

Homozygous mouse strains carrying a Robertsonian translocations (Rb) between chromosome 16 and 6 [Rb(6.16); Strain A] were mated with Rb(16.17) homozygous animals (Strain B) to obtain Rb(6.16)/Rb(16.17) compound heterozygous mice. These compound heterozygous mice were then mated to wild-type mice. A meiotic non-disjunction event occurring in the male germ line of the compound Rb(6.16)/Rb(16.17) heterozygote will result in offspring that are trisomic for the chromosome common to the Rb fusion chromosomes. Similar breeding schemes were used to generate the other trisomic embryos and cell lines in this study.

Supplemental Figure 2: Effects of aneuploidy on cell proliferation when the medium is changed every two days.

Wild-type and trisomic cell lines were plated and grown with the medium changed every other day (*S8*), Wild-type (open circles) and trisomic cells (closed circles) were analyzed daily to determine cell number (A), cell volume (B), the distribution of cell volumes in the culture at day 5 (C) and cumulative cell volume (CCV), i.e. number of cells x average cell volume (D). Note that the absence of small size particles in (D) indicates that cells are not undergoing lysis. The data for each column come from the same cell line.

Supplemental Figure 3: Proliferation defects in independent trisomic MEFs.

Wild type (open circles) and Ts cells (closed circles) that originated from embryos of independent crosses were analyzed as described in Fig. 2. The data for each column come from the same cell line. The examples shown for trisomy 16 and 19 represent cases where the effects of the extra chromosome on cell proliferation and CCV were subtle or not detectable.

(A; B) Growth of early passage (p3) trisomic cells under "fed" (A; medium was changed every two days) or "unfed" (B; medium was not changed) conditions.

(C, D) C shows the average cell volume of cells under growth conditions where the medium was not changed (unfed) and D the distribution of cell volumes in the culture at day 5 of the accumulation assay. Note that the low amounts of small size particles in (D) indicates that cells are not undergoing lysis.
(E) Analysis of the cumulative cell volume during the accumulation assay.
(F) DNA content analysis of wild-type and trisomic cells passage 2.

Supplemental Figure 4: Summary of the proliferation characteristics in trisomic cells shown as the ratio between Ts and wild-type cells.

The ratio of cell number (A), cell volume (B) and cumulative cell volume (CCV) (C) between trisomic and wild-type cells was determined for each experiment (Ts1 N=3, Ts13 N=4, Ts16 N=4, Ts19 N=4). The graph shows the mean of these ratios. Error bars are +/- SD. A value of 1 indicates no difference between trisomic and wild-type cells, a value below 1 indicates a decrease in the trisomic cells, a value above 1 an increase.

Supplemental Figure 5: Robertsonian chromosomes do not affect cell proliferation and metabolism.

Wild-type cells (0 Robertsonian translocations; closed circles), cells carrying one Robertsonian chromosome (1 Robertsonian translocation; closed triangles) and cells carrying two Robertsonian chromosomes (2 Robertsonian translocations; closed squares) were grown and analyzed as described in Figure 2 and either medium was not changed (unfed) or changed every two days (fed). Samples were taken at the indicated times to determine cell number (A), cell volume (B), cumulative cell volume (C) and glutamate and lactate production (D).

Supplemental Figure 6: Senescence-associated β-galactosidase staining is not altered in cultures of trisomic cells.

Examples of β-galactosidase staining of Ts1 (passage 3; A), Ts13 (passage 4; B) and Ts19 (passage 5; B) and littermate controls are shown. β-galactosidase staining is shown in blue.

Supplemental Figure 7: Additional examples of the metabolic characteristics of Ts cell lines in unfed accumulation assays.

(A, B) The amount of glucose uptake (A) and glutamate generated (B) per CCV in the tissue culture supernatants of cells analyzed in Figure 3A - C was examined at the indicated times. (C) Examples of trisomic cells in which the alterations in metabolic activity were subtle or not detectable. Tissue culture supernatants of the experiments shown in Fig. S3 were subjected to metabolic analyses (*S8*) and the amount of glucose (top panels) and glutamine (second panels) used and ammonium (third panels) and glutamate (bottom panels) generated per CCV was determined at the indicated times.

Supplemental Figure 8: Summary of the metabolic analyses in trisomic cells shown as the ratio between Ts and wild-type cells.

The ratio of glutamine use (A), and ammonium (B), glutamate (C) and lactate (D) production between trisomic and wild-type cells was determined per CCV for each experiment (Ts1 N=3, Ts13 N=4, Ts16 N=3, and Ts19 N=4). The graph shows the mean of these ratios. Error bars are +/- SD. A value of 1 indicates no difference between trisomic and wild-type cells, a value below 1 indicates a decrease in the trisomic cells, a value above 1 an increase.

Supplemental Figure 9: Rate of spontaneous immortalization of primary Ts1 MEFs.

Cells were serially passed in a 3T3 immortalization assay (*S8*) and the number of population doublings are shown as a function of number of passages.

Supplemental Table 2

Analysis of Functional Annotations

The GO Tree Machine (http://bioinfo.vanderbilt.edu/gotm/frame.php) was used to compare the functional annotations attached to differentially expressed genes in the Ts13 vs. wild-type comparison with those annotations attached to all gene products interrogated by the MOE_430A_2 chip

Ontology	Term	Observed	Expected	Ratio	Pval
In cellular component	cytoplasm	95	63.83	1.49	2.52766E-06
In cellular component	cytoplasmic part	76	47.65	1.59	4.31844E-06
In cellular component	extracellular matrix (sensu Metazoa)	16	4.65	3.44	1.59183E-05
In molecular function	structural molecule activity	25	9.88	2.53	1.69482E-05
In cellular component	extracellular matrix	16	4.74	3.38	2.01878E-05
In biologicial process	biosynthesis	39	20.42	1.91	4.6283E-05
In cellular component	microfibril	3	0.11	27.27	0.000106393
In molecular function	translation factor activity\, nucleic acid binding	9	1.91	4.71	0.00011786
In molecular function	translation regulator activity	9	2	4.5	0.000165627
In molecular function	metalloendopeptidase inhibitor activity	3	0.13	23.08	0.000186417
In biologicial process	cellular biosynthesis	34	17.99	1.89	0.000192036
In cellular component	fibril	3	0.13	23.08	0.000209281
In cellular component	intracellular non-membrane-bound organelle	41	23.44	1.75	0.00022016
In cellular component	non-membrane-bound organelle	41	23.44	1.75	0.00022016
In molecular function	translation initiation factor activity	7	1.25	5.6	0.000226768
In cellular component	extracellular matrix part	8	1.72	4.65	0.000303912
In cellular component	cytosol	17	6.62	2.57	0.000331688
In biologicial process	protein metabolism	68	47.53	1.43	0.000534428
In biologicial process	protein biosynthesis	22	10.28	2.14	0.000584449
In biologicial process	macromolecule metabolism	86	64.46	1.33	0.000676011
In cellular component	ribonucleoprotein complex	17	7.11	2.39	0.000752646
In cellular component	secretory granule membrane	3	0.2	15	0.000836289

Ontology	Term	Observed	Expected	Ratio	Pval
In biologicial process	macromolecule biosynthesis	23	11.43	2.01	0.001015629
In biologicial process	actin cytoskeleton organization and biogenesis	9	2.66	3.38	0.001361624
In biologicial process	positive regulation of nitric oxide biosynthesis	2	0.06	33.33	0.001379166
In biologicial process	cortical cytoskeleton organization and biogenesis	3	0.24	12.5	0.001450138
In biologicial process	cellular protein metabolism	63	44.86	1.4	0.001591974
In biologicial process	translation	9	2.75	3.27	0.00170316
In biologicial process	organelle organization and biogenesis	29	16.46	1.76	0.001805824
In biologicial process	branching morphogenesis of a tube	5	0.91	5.49	0.002004004
In molecular function	growth factor activity	9	2.81	3.2	0.002020676
In cellular component	actin cytoskeleton	10	3.37	2.97	0.002023397
In biologicial process	cellular macromolecule metabolism	63	45.47	1.39	0.002252247
In biologicial process	morphogenesis of a branching structure	5	0.95	5.26	0.002470752
In biologicial process	actin filament-based process	9	2.92	3.08	0.002591493
In molecular function	actin binding	11	4.08	2.7	0.002625215
In biologicial process	regulation of cAMP metabolism	2	0.09	22.22	0.00271894
In biologicial process	neuromuscular physiological process	2	0.09	22.22	0.00271894
In molecular function	polysaccharide binding	6	1.55	3.87	0.004401433
In molecular function	CoA desaturase activity	2	0.11	18.18	0.004402233
In molecular function	stearoyl-CoA 9-desaturase activity	2	0.11	18.18	0.004402233
In biologicial process	regulation of cyclic nucleotide metabolism	2	0.11	18.18	0.004467048
In biologicial process	synaptic vesicle transport	3	0.35	8.57	0.004544293
In biologicial process	response to heat	3	0.35	8.57	0.004544293
In cellular component	zymogen granule membrane	2	0.11	18.18	0.004753858
In biologicial process	actin filament polymerization	3	0.37	8.11	0.005431038
In molecular function	structural constituent of ribosome	9	3.27	2.75	0.005453345
In molecular function	pattern binding	6	1.63	3.68	0.005739269
In biologicial process	regulation of biosynthesis	8	2.75	2.91	0.006232064
In biologicial process	cell proliferation	16	8.01	2	0.006425686
In cellular component	cytosolic part	6	1.68	3.57	0.006477502
In biologicial process	regulation of actin polymerization and/or depolymerization	4	0.76	5.26	0.006597145
In biologicial process	regulation of actin filament length	4	0.76	5.26	0.006597145
In biologicial process	regulation of nitric oxide biosynthesis	2	0.13	15.38	0.00660536

Ontology	Term	Observed	Expected	Ratio	Pval
In molecular function	cytoskeletal protein binding	13	5.95	2.18	0.006753222
In molecular function	extracellular matrix structural constituent	5	1.2	4.17	0.006855044
In cellular component	intracellular part	139	121.86	1.14	0.006963264
In cellular component	zymogen granule	2	0.13	15.38	0.00702603
In biologicial process	cell organization and biogenesis	44	30.7	1.43	0.007082138
In biologicial process	translational initiation	4	0.8	5	0.00804722
In cellular component	contractile fiber	5	1.25	4	0.008047324
In cellular component	cytoskeleton	23	13.57	1.69	0.008755605
In biologicial process	protein polyubiquitination	2	0.15	13.33	0.009116342
In biologicial process	regulation of nucleotide metabolism	2	0.15	13.33	0.009116342
In biologicial process	eating behavior	2	0.15	13.33	0.009116342
In cellular component	leading edge	5	1.3	3.85	0.009314195
In molecular function	calcium ion binding	22	12.85	1.71	0.009329813
In molecular function	insulin-like growth factor binding	3	0.45	6.67	0.009773724
In cellular component	lamellipodium	4	0.85	4.71	0.009867445
In biologicial process	protein folding	9	3.59	2.51	0.0099209

Supplemental Table 3

Cell Line	Passage	Cells Counted	Ave. Chromosome Arms	Std. Dev.	Wilcoxon Rank p (two-sided)
WT-1	44	81	127.8	23.3	3.09×10^{-10}
Ts13-1	44	17	79.8	4.4	
WT-2	40	28	80.0	8.0	4.19×10^{-5}
Ts13-2	40	64	74.4	24.0	
WT-3	41	58	66.8	22.8	0.44
Ts13-3	41	23	71.0	31.4	
WT-1	40	53	76.1	22.1	0.30
Ts16-1	40	71	77.0	23.2	
WT-2	39	23	76.1	22.3	1.82×10^{-05}
Ts16-2	39	53	109.1	33.0	
WT-3	46	39	106.2	23.5	5.68×10^{-11}
Ts16-3	46	30	63.3	14.3	
WT-1	35	32	76.3	13.8	0.03
Ts19-1	35	62	79.7	11.8	

Analysis of the chromosome number in spontaneously immortalized MEF cultures.
Each set represents independent, littermate matched trisomic and wild type cell line pairs, carried through a 3T3 protocol until immortalization. Metaphase spreads were prepared from post-immortalization cultures. The number of chromosome arms was determined by counting all arms (one Robertsonian translocation chromosome yields 2 chromosome arms). Note: while the differences might be significant between the immortalized wild-type and trisomic MEF cultures within a given experiment, the differences are not consistent across all immortalized cell lines.

Supplemental References

S1. B. R. Williams *et al.*, *Curr Biol* **12**, 648 (Apr 16, 2002).

S2. B. M. Bolstad, Collin, F., Brettschneider, J., Simpson, L., Cope, L. Irizarry, R. A. and Speed, T. P., in *Bioinformatics and computational biology solutions using R and Bioconductor* V. C. R. Gentleman, S. Dudoit, R. Irizarry, W. Huber, Ed. (Springer Science+Business Media, New York, 2005) pp. 33-47.

S3. G. K. Smyth, in *Bioinformatics and computational biology solutions using R and Bioconductor* V. C. R. Gentleman, S. Dudoit, R. Irizarry, W. Huber, Ed. (Springer Science+Business Media, New York, 2005) pp. 397-420.

S4. B. M. Bolstad, Irizarry, R. A., Gautier, L. and Wu, Z, in *Bioinformatics and computational biology solutions using R and Bioconductor* V. C. R. Gentleman, S. Dudoit, R. Irizarry, W. Huber, Ed. (Springer Science+Business Media, New York, 2005) pp. 13-32.

S5. N. Jain *et al.*, *Bioinformatics* **19**, 1945 (Oct 12, 2003).

S6. G. J. Todaro, H. Green, *J Cell Biol* **17**, 299 (May, 1963).

S7. G. P. Dimri *et al.*, *Proc Natl Acad Sci U S A* **92**, 9363 (Sep 26, 1995).

S8. See Supporting Online Material.