Supplemental Figure Legends

Supplemental Figure 1: Effects of Metformin and Beclin-1 knock down on the accumulation of trisomic MEFs in culture.

(A) Wild-type (filled bars) and trisomic cells (open bars) were treated with metformin and cell number was determined 72 hours thereafter. Cell number is shown as the percentage of its untreated control.

(B) Cells were transfected with either empty vector or a Beclin1 knockdown shRNA construct. Cell number was determined 72 hours after AICAR treatment. The data presented are the mean ± standard deviation. **P*<0.05, ***P*<0.005, *t* test. Beclin1 knockdown efficiency was determined by immunoblotting using an anti Beclin1 antibody. Actin was used as a loading control in Western blots.

Supplemental Figure 2: Cell cycle duration, senescence and necroptosis are not increased in AICAR-treated trisomic MEFs.

(A) Cell cycle distribution of wild-type and trisomic cells as judged by DNA content analysis.

(B) Examples of β-galactosidase levels in trisomy 13 and littermate control cells in the presence or absence of AICAR. β-galactosidase staining is shown in blue.

(C) Wild-type (filled bars) and trisomic cells (open bars) were treated with nectrostatin-1 or with nectrostatin-1 and 0.2 mM AICAR (wild-type, grey bars; Ts, open bars). Cell number was determined after 3 days. The data presented are the mean ± standard deviation. **P*<0.05, ***P*<0.005, *t* test.

Supplemental Figure 3: Inhibition of the mTOR pathway does not affect the proliferative abilities of trisomic MEFs.

(A) Wild-type (filled bars) and trisomic cells (open bars) were treated with rapamycin or torin-1 and cell number was determined after 72 hours. **P*<0.05, ***P*<0.005, *t* test.

(B) Wild-type (grey bars) and trisomic cells (open bars) were treated with 0.2 mM AICAR and the indicated concentration of rapamycin or torin-1. Cell number was determined after 3 days.

(C) Cells were transfected with either empty vector (shown in Figure 3G because these experiments were performed at same time) or a mTOR knockdown shRNA construct. Note, that in contrast to Supplemental Figure 3B, wild-type cells were not dramatically affected by the simultaneous inhibition of mTOR and AMPK in this experiment. We speculate that the different methods employed to inactivate mTOR are responsible for this difference. Inactivation of mTOR by shRNA knock-down may not be as efficient as Rapamycin or Torin1-induced mTOR inhibition. Furthermore, in the shRNA experiment cells harboring low mTOR activity were cultured for two weeks before AICAR treatment. This may have allowed wild-type cells to adapt to low-level mTOR signaling, allowing them to survive the acute AICAR treatment.
(D) mTOR knockdown efficiency revealed by immunoblotting using an anti mTOR1 antibody.

Supplemental Figure 4: Effects of Chloroquine on aneuploid tumor cell lines.
Cells were treated with the indicated concentration of Chloroquine. Cell number was determined 3 days after the addition of compound and is shown as the percentage of the untreated control.
(A) Primary euploid cells (black symbol), MIN colon cancer cell lines (blue symbols) and aneuploid CIN colon cancer cells (red symbols).
(B) Cell number of euploid (black symbols) and aneuploid lung cancer cells (red symbols).
The data presented are the mean ± standard deviation. *P<0.05, **P<0.005, t test.

Supplemental Figure 5: AICAR induces Autophagy in human colon cancer cells.
(A) Quantitative RT-PCR analysis of mRNA abundance of the autophagy genes ATG1, ATG4, Beclin1, LC3, Bnip3 and GAPRAPL1. mRNA levels were quantified in two untreated MIN (HCT15 and LoVo) and CIN (HT29 and SW620) cells (filled bars) as well as AICAR treated (0.5 mM AICAR for 24 hours; open bars). RNA levels were normalized to those of the ribosomal RPL19 gene and normalized to untreated HCT15 cells. The data presented are the mean ± standard deviation. *P<0.05, **P<0.005, t test.
(B) MIN and CIN cells were treated with AICAR for 24 hours at the indicated doses and

the levels of inducible Hsp72 and non-lipidated LC3-I and lipidated LC3-II were determined by immunoblotting.

Supplemental Figure 6: Effects of 2-deoxyglucose on the accumulation of euploid and trisomic MEFs in culture.

(A) Wild-type (filled bars) and trisomic cells (open bars) were treated with 2-deoxyglucose and cell number was determined after 3 days.

(B, C) AMPK activity was analyzed by determining the extent of threonine172 phosphorylation on AMPK (B) or by *in vitro* kinase assays using the substrate peptide, IRS-1 S789 (C) in wild-type and trisomic cells after 24 hours of 2-deoxyglucose treatment. The data presented are the mean ± standard deviation. Quantifications of the ratio of phosphorylated AMPK/total AMPK protein normalized to untreated wild-type cells are shown underneath the P-AMPK blot.

Supplemental Figure 7: 17-AAG and Chloroquine induce p53-mediated apoptosis in trisomy 13 cells.

Cells were transfected with a p53 knockdown shRNA and treated with 17-AAG (A) or Chloroquine (B) at the indicated doses. Cell number was determined after 72 hours and is shown as the percentage of the untreated control. The data presented are the mean ± standard deviation. **P*<0.05, ***P*<0.005, *t* test.

Supplemental Tables

Table S1. Effects of selected compounds on euploid (WT) and trisomic (Ts) MEFs.

Table S2. shRNA sequences used in this study.

Table S3. Primers used for quantitative Real-Time PCR.

Experimental Procedures and Materials

Cellular effects of the chemicals used in this study

Compounds were selected that cause DNA damage, proteotoxic stress or metabolic stress (shown in Table S1). Aphidicolin (Sigma-Aldrich) is an inhibitor of DNA Polymerase α,

δ and ε. Camptothecin (Sigma-Aldrich) inhibits DNA topoisomersase1, and hydroxyurea (Sigma-Aldrich) reduces production of deoxyribonucleotides via inhibition of ribonucleotide reductase. Cisplatin (TOCRIS) and Doxorubicin (Sigma-Aldrich) interact directly with DNA and trigger DNA damage. 17-AAG (Invivogen) inhibits Hsp90. Cycloheximide (Sigma-Aldrich) and puromycin (Sigma-Aldrich) antagonize protein synthesis. Chloroquine (Sigma-Aldrich) inhibits Autophagy by disrupting the fusion of the autophagosome and lysosome. Lactacystin (Cayman) and MG132 (Sigma-Aldrich) are proteasome inhibitors. Tunicamycin (Sigma-Aldrich) disrupts early steps in glycoprotein synthesis. AICAR (Toronto Research Chemicals) and metformin (TOCRIS) induce energy stress and are agonists of AMPK, and Compound C (Calbiochem) inhibits the activity of AMPK and many other protein kinases. 2-Deoxyglucose (Sigma-Aldrich) blocks the function of glucose hexokinase, preventing glycolysis. Rapamycin (Sigma-Aldrich) and torin1 (a gift from Dr. D. Sabatini) inhibit mTOR.

Mouse Strains

All mouse strains were obtained from the Jackson Laboratory. Strains used to generate trisomic embryos are: Rb(1.2)18Lub/J and Rb(1.3)1Ei/J (for Ts1), Rb(11.13)4Bnr/J and Rb(13.16)1Mpl/J (for Ts13), Rb(6.16)24Lub and Rb(16.17)7Bnr (for Ts16), and Rb(5.19)1Wh/J and Rb(9.19)163H (for 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.

Human cell lines

Four primary human cell lines (CCD112 CoN, CCD841 CoN, WI38 and IMR90), five MIN lines (HCT-116, HCT-15, DLD-1, SW48 and LoVo), five CIN lines (Caco2, HT29, SW403, SW480 and SW620) and eight non-small cell lung cancer lines (A549, NCI-H520, NCI-H838, NCI-H1563, NCI-H1792, NCI-H2122, NCI-H2170 and NCI-H2347) were obtained from ATCC. The information on chromosome number and karyotypes was obtained from the NCI database and the COSMIC Dataset at the Sanger Institute. The p53 status of the cell lines used in Figure 6C and D was determined in (Berglind et al.,

2008; Hwang et al., 1999; Mukhopadhyay et al., 2005). All human cells were cultured in RPMI (Invitrogen) supplemented with 10 % FBS, 2 mM L-glutamine and 100 U/ml penicillin/streptomycin and maintained at 37°C with 5 % CO_2 in a humidified environment.

Primary MEF cell lines

Littermate-derived euploid and trisomic primary MEFs were described previously (Williams et al., 2008). All experiments were performed in at least three independent trisomic cell lines and analyzed together with euploid littermates that carried a single Robertsonian translocation. We used MEFs at early passages (≤p5) to ensure that karyotypic changes had not yet occurred. Two independent $Cdc20^{AAA}$ MEF cell lines were kindly provided by Dr. Pumin Zhang. $Bub1b^{(H/H)}$ mice were kindly provided by Dr. Jan van Deursen. MEFs from these mice were prepared as described in Williams et al. (2008). The MEF cells were cultured in DMEM (Invitrogen) supplemented with 10 % FBS, 2 mM L-glutamine and 100 U/ml penicillin/streptomycin. All cells were maintained at 37°C with 5 % CO_2 in a humidified environment.

Cell viability assay

In order to determine the effect of various compounds on wild-type and trisomic MEFs, we first tested the impact of each chemical using the alamarBlue viability assay (Invitrogen) in a 96-well format. Potential effects were confirmed using the trypan blue viability assay (Sigma-Aldrich) in a 6-well format.

To assess viability using the alamarBlue assay, cells were plated at a density of 2,000 cells per well in 96-well plates one day prior to drug treatments. 10 % alamarBlue (in 100 µl medium) was added after 3 days. After four hours, cell number was analyzed according to the manufacturer's instructions (Ahmed et al., 1994). For trypan blue staining, cells were plated at a density of $2x10^5$ cells per well in 6-well plates one day prior to drug treatment. At the indicated time points, cells were trypsinized and mixed with an equal volume of 0.4% trypan blue and cell number was counted by using the Cellometer AutoT4 (Nexcelom). Each analysis was performed three times. Standard deviations (SDs) are shown.

Immunoblotting and antibodies

Cultured cells were lysed in lysis buffer (50 mM Tris-HCl, pH7.4, 150 mM NaCl, 5 mM $MgCl_2$, 1 % Triton X-100, 0.5 % NP-40, 10 % glycerol, protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche)). Lysates were resolved on 4-15% SDS PAGE gels (Bio-Rad). PVDF blots were probed with the following primary antibodies: anti-AMPKα, anti-Phospho-AMPKα (Thr172), anti-p53, anti-Phospho-p53 (Ser15), anti-mTOR, anti-Hsp60, anti-Hsp90, anti-Beclin 1 and anti-Bax (all antibodies are from Cell Signaling Technology and used at a 1:000 dilution); anti-LC3 (Novus, 1:2,000); anti-Hsp72 (Stressgen, 1:1,000); anti-p21 (Santa Cruz, 1:1,000) and anti-actin (Sigma-Aldrich, 1:10,000).

Constructs and retroviral infection

A miR-30-based shRNA targeting construct for AMPKα1, Beclin 1, mTOR, p53 or Rb was subcloned into LTR-driven miR30 SV40-GFP (LMS) (MSCV-based vectors) (Dickins et al., 2005). Targeting sequences were selected based on RNAi Codex algorithms (Silva et al., 2005) or BIOPREDsi design (Huesken et al., 2005) (Table S2). The pBABEpuro GFP-LC3 was obtained from addgene (Kabeya et al., 2000). For production of high titer retrovirus, 293T cells were cotransfected with retroviral vector (20 μg) and helper DNA (10 μg) using *Trans*IT-LT1transfection reagent (Mirus). 24 hours after transfection, the culture supernatant was collected and filtered through a 0.45 μm filter and used to transduce wild-type and trisomic MEFs. A second infection was repeated after 24 hours. 24 hours after the second infection, target cells were placed into fresh medium and cultured for 3 days. GFP-positive cells were isolated by FACS.

AMPK kinase assays.

Wild-type and trisomic MEFs were treated with AICAR as indicated and cells were lysed in lysis buffer (20 mM Tris-HCl, pH7.5, 250 mM NaCl, 0.5 % NP-40, 10 % glycerol, 1 mM EDTA, 1 mM EGTA, protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche), 1mM DTT). 10 μg of lyaste were resuspended in 100 μl AMPK kinase assay buffer (Cyclex, CY-1182). After 30 minutes incubation at 30°C with a substrate

peptide, IRS-1 S789, the activity of AMPK was analyzed by measuring the phosphorylation level of IRS-1 S789, according to the manufacturer's instructions.

Fluorescence imaging

For GFP-LC3 fluorescence imaging, wild-type *GFP-LC3* and trisomic *GFP-LC3* MEFs were exposed to the indicated drug treatments in phenol red-free DMEM. All fluorescence images were acquired using a Nikon digital sight DS-Qi1 MC camera on a Nikon Eclipse T*i* inverted microscope. All single plane images were acquired and processed using the NIS-Element AR 3.0 software.

RT Quantitative PCR

Total RNA from MEFs was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. 1μg RNA was used to generate cDNAs using RETROscript kit (Ambion). qPCR was performed by mixing iQ SYBR Green Supermix (Bio-Rad) with the primers described in Table S3, and amplified using a LightCycler 480 II (Roche). The LightCycler software was used to determine gene expression levels and normalized to a ribosomal reference gene, RPL19.

Apoptosis assay

To assay apoptosis, $1x10^6$ cells were seeded on a 10 cm plate and exposed to the indicated treatments for 24 hours. 10^6 cells were resuspended in 1ml of Annexin V buffer and 10^5 cells were stained with propidium iodide and FITC-conjugated annexin V antibody (BD Pharmingen) for 15 minutes at room temperature before FACS analysis.

Cell Cycle Analysis

Exponentially growing cells (≤p5) were trypsinized and collected by centrifugation. Cells were fixed in 70 % ethanol and resuspended in propidium iodide and RNaseA in PBS. 20,000 events were counted on a FACScan Flowcytometer (Becton Dickinson) for each cell line and cell cycle distribution was determined using the FlowJo software (Tree Star Inc.).

Senescence-associated β-galactosidase activity assays

Senescent cells were identified using established protocols (Dimri et al., 1995). 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 (37 mM Citric acid, 126 mM Na_2HPO_4, 1 mg/ml X-Gal, 5 mM $K_4Fe(CN)_6$, 5 mM K3Fe$(CN)_6$, 150 mM NaCl, 2 mM $MgCl_2$). Cells were analyzed by light microscopy.

Mice xenografts.

Two MIN (HCT15 and LoVo) and two CIN (HT29 and SW620) cells were resuspended in PBS and inoculated s.c. into both flanks of 6-week old female nude mice. Nude mice were injected at the left flank with one MIN cell line and the right flank with one CIN cell line. Seven days after injection, animals were treated with daily i.p. injections of AICAR (500 mg/kg body weight), 17-AAG (80 mg/kg body weight), a combination of the two drugs, or an equal volume of vehicle (PBS). Tumor volume (mm^3) was measured every six days and estimated using caliper measurements using the formula $\pi /6 \times A \times B^2$ (A is the larger diameter, B is the smaller). All animal studies and procedures were approved by the MIT Institutional Animal Care and Use Committee.

Statistics

All data are shown as the mean ± standard deviation. Means were compared using the 2-tailed Student's *t* test. $P < 0.05$ was considered statistically significant in all calculations. All data analyses were performed using the Prism software package, version 4.

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