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**Supplementary Fig. 1:** Construction, characterization and growth of the trisomic and tetrasomic cell lines.

(A) Schematic overview of the chromosome transfer by micronuclei fusion.

(B) aCGH analysis of the used cell lines.

(C) mFISH karyograms of HCT116 and HCT116 5/4. The image shows one representative karyogram out of 10.

(D) Time in interphase as measured by time lapse fluorescence imaging reveals extended interphase in HCT116 H2B-GFP 5/4 in comparison to the original disomic cell line.

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**Supplementary Fig. 2:** Correlations and alignments of quantified proteome changes.

(A) Comparison of technical (left), biological (middle) and label swapped (right) replicas of SILAC experiments. Pearson coefficients are stated (r) in the plots. Yellow dots represent the proteins coded on chromosome 5. A small population of proteins that do not follow the same trend can be identified when comparing forward and reverse labeling experiments (right panel). These proteins are not significantly enriched for any pathway (Fisher exact test, Benjamini-Hochberg FDR < 2%), suggesting that the effect of label swap is random and does not affect the pattern of pathway down- or upregulations.

(B) DNA and protein ratios of the clones HCT116 H2B-GFP 5/4, HCT116 H2B-GFP 5/3, HCT116 3/3, RPE-1 5/3 12/3 and RPE-1 21/3 aligned in respect to their chromosome position (for details see Supplementary Information).
Supplementary Fig. 3: Density distribution of genome, transcriptome and proteome.

(A) Density distribution of DNA changes in comparison to protein changes in HCT116 derived aneuploid cell lines. The upper plots (green lines) represent the density distribution for disomes; the lower plots (red lines) represent the density distribution for supernumerary chromosomes.

(B) Density distribution of mRNA changes in comparison to protein changes in RPE-1 derived aneuploid cell lines. The upper plots (green lines) represent the density distribution for disomes; the lower plots (red lines) represent the density distribution for supernumerary chromosomes. Note that whereas the medians are nearly identical for the disomes, the levels of proteins coded on the supernumerary chromosomes are significantly shifted towards 0. The numbers in parenthesis represent total number of quantified genes and proteins, respectively.
Supplementary Fig. 4: Analysis of macromolecular complexes.

(A) Density distributions of proteins coded on the extra chromosomes assigned to the category “protein complexes” (CORUM) in comparison to the remaining proteins from the aneuploid population. Thick lines represent density distributions of proteins that belong to a category as defined in CORUM. Thin lines represent all proteins except proteins analyzed within the given category. Median values are labeled by dotted lines. “Category” and “not Category” classes that are significantly different (Wilcoxon rank sum test, p<0.05) are marked in the graphs with an asterisk.

(B) DNA, RNA and protein abundance of subunits of protein complexes that contain at least one subunit coded on the extra chromosome 5 in the HCT116 5/4 cell line. The proteins from the tetrasomic chromosome are marked by an arrow. Medians with the interquartile range are shown in the plots.

Supplementary Figure 4, Stingele, Stoehr et al.
**Supplementary Fig. 5:** Significantly altered pathway regulations in aneuploid cell lines.

A The pathway alterations are almost identical in all aneuploid clones regardless the type of the extra chromosome (trisomy of chr. 3 versus tetrasomy of chr. 5) and the origin of the aneuploidy (derived from RPE-1). Proteome data were used for the analysis.

B Similar patterns of changes in pathway regulation can be observed when comparing data obtained by proteome analysis with data from transcriptome analysis.

C Pattern of changes in pathway regulation based completely on mRNA data.

D The observed pathway regulations are not an artifact of the copy number changes of genes coded on the extra chromosomes, since excluding the proteins coded on chr. 5 from the analysis did not affect the identified pathways. Proteome data were used for the analysis.
Supplementary Fig. 6: The autophagic flux is not impaired in trisomic and tetrasomic cell lines. Representative images of cells after transfection with the double tagged mRFP-GFP-LC3. Yellow foci represent phagosomes (both GFP and mRFP signals visible), red foci represent lysosomes (only mRFP signal is insensitive to the acidic pH). Bar 10 μm. Bafilomycin inhibits autophagic flux in all tested cell lines.
Supplementary Fig. 7: Aneuploid cells show increased levels of p62 and ubiquitin foci.

(A) Aneuploid HCT116 derivatives show elevated levels of p62 and ubiquitin. This is further increased after autophagy inhibition using bafilomycin A1. Plots represent the signal intensity and colocalization along the indicated gray line. Red line-p62, green line – ubiquitine.

(B) Increased p62 and ubiquitin levels were also observed in RPE-1 5/3 12/3.
Supplemental Experimental Procedures

Array comparative genomic hybridization (aCGH)

Genomic DNA for aCGH analysis was extracted using the Qiagen Gentra Puregene Kit according to manufacturer’s instructions. The aCGH analysis was performed by IMGM laboratories, Martinsried, Germany. Commercially available human genomic DNA (Promega) was used as a reference sample for all 4x44K array based analyses (HCT116, HCT116 3/3, HCT116 5/4, HCT116 H2B-GFP 5/3, HCT116 H2B-GFP 5/4, RPE-1 and RPE-1 5/3 12/3). RPE-1 H2B-GFP 21/3 was analyzed by SurePrint 4x180K G3 Human CGH Microarray. gDNA extracted from HCT116 was used as a reference for the high density CGH analysis of HCT116 5/4 by the 2x400K array. gDNA concentration and DNA absorbance ratio (260nm/280nm) were measured by NanoDrop ND-1000 UV-VIS Spectrophotometer (PeqLab). 1 μg of gDNA was used for each reaction. gDNA integrity was tested on an 1.0 % agarose gel stained with ethidium bromide (EtBr). 1.0 μg gDNA of each sample was subjected to restriction digestion with a combination of Alu I and Rsa I restriction enzymes. The digested gDNA samples were directly labeled with exo-Klenow fragments and random primers by incorporation of Cy-5 dUTP (dUTP = 2'-deoxyuridine 5'-triphosphate) for the experimental samples and Cy-3 dUTP for the reference samples (Genomic DNA Enzymatic Labeling Kit, Agilent Technologies). After purification, each experimental sample was combined with its respective reference sample and hybridized to respective arrays. All microarrays have been washed with increasing stringency using Oligo aCGH Wash Buffers (Agilent Technologies) followed by drying with acetonitrile (Sigma-Aldrich). Fluorescent signal intensities for both dyes were
detected with Scan Control 8.4.1 Software (Agilent Technologies) on the Agilent DNA Microarray Scanner and extracted from the images using Feature Extraction 10.5.1.1 Software (Agilent Technologies). The software Feature Extraction 10.5.1.1 as well as the software Genomic Workbench 5.0.14 was used for quality control, statistical data analysis and visualization. Raw microarray data were normalized. ADM-2 aberration algorithm was applied together with centralization algorithm. Aberrations for all samples were filtered from the whole genome data and analyzed based on a threshold of log2 ≥ 0.39 for amplifications and log2 ≤ -0.30 for deletions with at least five consecutive aberrant probes.

Chromosome spreads

Cells were grown to 70-80 % confluency, treated with 50 ng/ml colchicine for 3-5 h, collected by trypsinization and centrifuged at 1000 rpm for 10 min. Pellets were resuspended in 75 mM KCl and incubated for 10-15 min at 37° C. After centrifugation at 800 rpm for 10 min, cell pellets were resuspended in 3:1 methanol/acetic acid to fix the cells. Cell pellets were washed several times in 3:1 methanol/acetic acid, spread on a wet glass slide and air dried at 42° C for 5 min.

Paints

Chromosome spreads were prepared as described above. Each sample was labeled with probes for two different chromosomes: a transferred chromosome and a control chromosome. Probes (Chrombios GmbH, Raubling, Germany) for chromosome 2, 3, 5 and 21 were tagged with TAMRA, FITC, Cy-5 and TAMRA, respectively. The chromosomes were labeled according to the manufacturer’s instructions and
counterstained with DAPI. Images were obtained by a fully automated Zeiss inverted microscope.

**Multicolor FISH (mFISH)**

Cells were prepared as described for chromosome spreads, the mFISH was performed by the Chrombios GmbH, Raubling, Germany as follows: Approximately 30 μl of cell suspension were dropped on a glass slide. To remove excess of cytoplasm, slides were treated with pepsin (0.5 mg/ml in 0.01 M HCl, pH 2.0) at 37°C for 40 min. Slides where then washed 10 min in 2x SSC at room temperature followed by ethanol washes (70, 90, and 100 %) and air dried. Human chromosome painting probes were labeled with different haptens (Biotin, Digoxigenin) and fluorochrome coupled dUTPs (TAMRA, TexasRedTM, DEAC), respectively, by standard DOP-PCR protocols. For better distinction of chromosomes, each individual chromosome was labeled with at least two different haptens/fluorochromes. Chromosomes were denatured for 1 min at 70°C in 2x SSC, 70 % formamide. Slides were then dehydrated in 70 %, 90 % and absolute ethanol for 5 min each and air dried. The probe pool was ethanol precipitated and re-suspended in 5 μl hybridization buffer (50 % formamide, 2x SSC, 10 % dextran sulfate). The DNA was denatured at 85°C for 5 min, pre-annealed at 37°C for about 60 min, and applied to the denatured chromosomes. The site of hybridization was covered with an 18x18 mm cover slip and mounted with rubber cement. Slides were incubated for overnight at 37°C. After hybridization the cover slip was removed and the slides were washed in 2x SSC for about 8 min at room temperature and in 0.4 x SSC/0.1 % Tween for 1 min at 70°C. Signal detection for
indirect labels was performed with an anti-digoxigenin antibody labeled with FITC (Roche Diagnostics) diluted 1:200 in 4 x SSC/5 % BSA and avidin-Cy™-5 (Amersham) diluted 1:400. Incubation was for 30 min. The slide was then washed twice in 4 x SSC/0.1 % Tween, 10 min each time. For microscopy, the slide was mounted in antifade solution containing DAPI. In situ hybridization signals were analyzed on a Zeiss Axioplan II microscope equipped with narrow band pass emission filters (ChromaTechnology) for each of the six fluorochromes including the DAPI counter stain. Each image plain was recorded separately with a cooled CCD camera (SenSys, Photometrics). Chromosomes were then displayed in false colors and merged on the computer. Camera control, image capture and merging were done with SmartCapture VP software (DigitalScientific, Cambridge, UK). For the identification of chromosomes and possible chromosome rearrangements, each image plain was analyzed separately. Karyotyping of mFISH images was done using the image processing software SmartType (DigitalScientific, Cambridge, UK).

**mRNA array**

mRNA was purified using the Qiagen mRNeasy mini kit. mRNA array analysis was conducted by IMGM laboratories as follows: An aliquot of the total RNA samples was used to determine RNA concentration and purity on the NanoDrop ND-1000 spectral photometer (PeqLab). RNA samples were analyzed by the 2100 Bioanalyzer (Agilent Technologies) capillary electrophoresis. 500 ng total RNA was introduced into an RT-IVT reaction. Prior to RT-IVT, the total RNA samples were spiked with *in-vitro* synthesized polyadenylated transcripts (One-Color RNA Spike-In Mix, Agilent Technologies) which serve as an internal labeling control for linearity, sensitivity and accuracy. The spiked total RNA was reverse transcribed into cDNA
and then converted into labeled cRNA by in-vitro transcription (Quick-Amp Labeling Kit One-Color, Agilent Technologies) incorporating Cy-3-CTP. For the analysis the Agilent Whole Human Genome Oligo Microarrays (4x44K format) were used in combination with a One-Color based hybridization protocol. Signals on the microarrays were detected with the Agilent DNA Microarray Scanner. Raw microarray data were quantile-normalized.

**Growth curves**

300,000 cells were seeded in each well of a six well dish and subsequently time points were taken every 24 h for 5 days. Cells were quantified either by counting four fields in a haemocytometer or by a Beckman Coulter Particle Count and Size Analyzer Z2. The mean values were used to generate the growth curves.

**Cell death analysis**

Cells were grown in a 10 cm dish to 70-80 % confluency and collected by trypsinization. After resuspending the cells in DMEM + 10 % FCS, propidium iodide was quickly added to the cells to a final concentration of 69 µM. Until FACS analysis, samples were kept on ice. Samples were measured by the FACScalibur (Becton Dickinson) using the Cell Quest Pro 5.2.1 (BD Bio Sciences) software in the FL-3 channel. FACS profiles were analyzed by FlowJo 7.6.1 software (https://rsb.info.nih.gov.ij/).

**β-galactosidase assay**

Control HCT116 and HCT116 5/4 samples were treated for 7 days with 0.2 µM doxorubicin to obtain β-galactosidase positive (i.e. senescent) cells. Control cells as well as untreated cells were grown in a 6 well dish to 70-90 % confluency. Cells
were then washed three times with PBS and fixed at room temperature for 5 min in 3% formaldehyde/1x PBS. Cells were washed again twice in 1x PBS and treated with staining solution (5 mM K$_3$Fe(CN)$_6$, 2 mM MgCl$_2$, 150 mM NaCl, 30 mM citric acid/phosphate buffer, 5 mM K$_4$Fe(CN)$_6$, 1 mg/ml X-gal) for 2 h at 37° C. Bright field images were taken by a fully automated Zeiss inverted microscope (AxioObserver Z1), using a 20x air objective.

**Thymididine release**

1,000,000 cells were seeded on a 6 cm dish and thymidine was added at 2 mM final concentration for 30 h. To release the cells from the thymidine block, cells were washed three times with PBS. Fresh DMEM + 10 % FCS including penicillin and streptomycin was added and cells were further incubated at 37° C. Samples for the time course were taken every hour. The samples were used either for DNA content measurement by FACScalibur or for immunoblotting.

**Immunofluorescence evaluation**

For LC3 immunofluorescence intensity analysis, the background of images was subtracted and levels were adjusted equally for all analyzed samples. For p62 immunofluorescence intensity analysis, the cell cytoplasm was additionally stained by HCS cell mask red dye (Invitrogen) before fixation to allow automated cell segmentation; mean intensity of p62 signal/cell was extracted from the Slidebook software. For analysis of the p62/ubiquitin co-localization, cells were additionally stained with anti-ubiquitin P4D1.

For all image analyses, the background was subtracted and fluorescence intensity levels were equally adjusted. Line intensities to visualize co-localization were
generated by Slidebook software (3i) using the line intensity tool. Ubiquitin-FITC signal was in general low. To allow direct comparison of the ubiquitin signal with the p62 signal, the ubiquitin signal intensity was multiplied by the factor 40 in all images.

**Autophagy flux assay**

HCT116, HCT116 3/3 and HCT116 5/4 were seeded one day before transfection, so that the cells were at 40 % confluency at the day of transfection. The ptfLC3 plasmid (mRFP-GFP-LC3, Addgene) was transfected using Lipofectamin LTX™ and PLUS™ reagent. Two days after transfection, signals were imaged with a 100x oil objective by using a 561 nm (mRFP) and 473 nm (GFP) laser. Since transfection levels were different in every cell, the fluorescence levels were adjusted individually for each cell. To evaluate autophagy activity and autophagic flux, we quantified the number of GFP-positive and mRFP-positive LC3 foci within a fixed area (2500 voxels). Only foci with median fluorescence intensity higher than the median of the entire area were counted.

**SILAC labeling**

All used cell lines were cultured as adherent cells in DMEM with high glucose. DMEM devoid of arginine and lysine was supplemented with 10 % FBS dialyzed with a cutoff of 10 kDa (Invitrogen) and 1x penicillin/streptomycin. For SILAC labeling, arginine and lysine were added in either light (Arg0; Lys0) or heavy (Arg10; Lys8) form to a final concentration of 33.6 µg/ml for arginine and 73 µg/ml for lysine. L-arginine (Arg0), L-lysine (Lys0, L-^{13}C_{6}^{15}N_{4}-arginine (Arg10) and L-^{13}C_{6}^{15}N_{2}-lysine (Lys8) were purchased from Sigma-Aldrich; sequencing grade-modified trypsin was purchased from Promega. RPE-1 and HCT116 cells were grown in heavy (Arg10; Lys8) SILAC medium for 8 to 10 doubling times and tested for full incorporation. Tri-
and tetrasomic cells were grown for 3-4 doublings in light (Arg0; Lys0) medium. HCT116 5/4 and all RPE-1 measurments were performed as double labeling experiments in three biological replicates. The HCT116 5/4 measurements contains in addition technical replicates and a label switch. HCT116 3/3, HCT116 H2B-GFP 5/3 and 5/4 were measured once.

**Cell lysis and protein digestion**

Labeled cells were washed twice with ice-cold PBS and the pellets were stored at -80°C. Proteins were extracted and digested following the recently described filter-aided sample preparation (FASP) protocol. In brief, cells were lysed with 4% sodium dodecyl sulfate (SDS), 0.1 M dithiothreitol (DTT) in 100 mM Tris/HCl pH 7.6 followed by an incubation for 5 min at 95°C and subsequent sonication for 15 min. Samples were centrifuged with high speed and the supernatants were transferred into new tubes. Protein concentrations of the samples were detected using a tryptophan-fluorescence assay and Bradford measurements, respectively. Proteins from the samples to be compared were mixed 1:1 according to the protein amount and 200-300 µg of the mixture was transferred on Microcon YM-30 filter tubes (Millipore). SDS was replaced by urea and finally 50 mM ammonium bicarbonate (ABC) buffer. Proteins were alkylated and digested with trypsin (1:50 w/w, modified sequencing grade) on the filter over night at 37°C. The arising peptides were eluted from the membrane by 0.5 M NaCl and ddH2O for OFFGEL fractionation and anion exchange fractionation, respectively.

**Peptide separation using an OFFGEL fractionator**
Peptides were desalted using 3M Empore HP Extraction disk cartridges (C18-SD; 7 mm/3 ml; Varian). The eluate was concentrated to remove the majority of acetonitrile and the peptide concentration was measured using a NanoDrop instrument (Thermo Fisher Scientific). A total of 100-150 µg peptides per experiment were separated based on their isoelectric point (pl) using the Agilent 3100 OFFGEL fractionator (Agilent) in combination with commercially available IPG DryStripes, 13 cm, pH 3-10 (GE Healthcare) diluted 1:50 in 5 % glycerol. Peptides were focused for 20 kVh at maximum current of 50 µA and maximal power of 200 mW. The 12 peptide fractions were transferred in 96-well plates and acidified by adding 10 µl of an acidic solution (30 % ACN, 5 % AcOH, 10 % TFA) prior to desalting with StageTips (Empore disk, C\textsubscript{18} Reversed phase)\textsuperscript{2} and LC-MS/MS analysis.

**Anion Exchange fractionation**

Peptide concentrations were measured by using a NanoDrop instrument. About 30 µg of peptides were fractionated into six fractions by using a recently described anion exchange method\textsuperscript{3}. In short, peptides were loaded at pH 11 on an anion exchange material plugged into a pipette tip and peptides were eluted from the material by using six different pH buffer solutions (pH11, 8, 6, 5, 4 and 3). All six fractions were desalted with StageTips (Empore disk, C18 Reversed phase) prior to LC-MS/MS analysis.

**Liquid-Chromatography Mass Spectrometry (LC-MS)**

Fractions of peptide mixtures were analyzed using nanoflow liquid chromatography (LC-MS/MS) on an EASY-nLC\textsuperscript{TM} system (Proxeon Biosystems, Odense, Denmark) online connected to the LTQ Orbitrap XL or LTQ Orbitrap Velos instrument (Thermo
Fisher Scientific, Bremen, Germany) through a Proxeon nanoelectrospray ion source

5 µl of the peptide samples were directly autosampled onto a 15 cm column (75
µl inner diameter; Proxeon Biosystems) packed in-house with 3 µm reversed phase
beads (ReproSil-Pur C18-AQ, Dr. Maisch). Peptides were separated and directly
electro-sprayed into the mass spectrometer using a 240 min method including a
linear gradient from 2 % to 30 % ACN in 0.5 % AcOH over 170 min at a constant
flow of 250 nl/min. The LTQ Orbitrap XL and LTQ Orbitrap Velos were operated in
data-dependent mode switching automatically between full scan MS and MS/MS
acquisition. Instrument control was through Tune 2.6.0. and Xcalibur 2.1.0. Full scan
MS spectra (m/z 300 – 1650) were acquired in the Orbitrap analyzer after
accumulation to a target value of 10^6 in the linear ion trap. Spectra were acquired
with a resolution of 60,000 at 400 m/z. For LTQ Orbitrap Velos data, the 10 to 15
most intense ions with charge states ≥ +2 were sequentially isolated with a target
value of 5,000 and fragmented using collision-induced dissociation (CID) in the
linear ion trap with normalized collision energy of 30-35 %. On the LTQ Orbitrap
Top7 peaks were fragmented using the same range for normalized collision energy
as on the LTQ Orbitrap Velos. HCD fragmentation on the LTQ Orbitrap Velos has
been performed for Top10 with 4-5E4 ions with subsequent analysis in the Orbitrap
cell with 7500 resolution. Normalized collision energy of 35 % has been used. For
CID, the activation q was set to 0.25 and the activation time was set to 30 ms and 10
ms for LTQ Orbitrap and LTQ Orbitrap Velos, respectively. The ion selection
threshold was set to 500 counts for CID-MS/MS and 5000 counts for HCD-MS/MS.
Maximum ion accumulation times of 1000 ms and 500 ms for full scans and 150 ms
and 25 ms for CID-MS/MS scans were set for the LTQ Orbitrap XL and the LTQ Orbitrap Velos, respectively. Maximum filling times for HCD-MS/MS were set to 150 ms. Standard mass spectrometry parameters were set for all experiments as follows: 2.2 kV spray voltage; no sheath and auxiliary gas; 200° C heated capillary temperature; predicted and normal automatic gain control (AGC) enabled for Velos analyses, for Orbitrap data normal AGC was enabled; 110 V Tube lense voltage (LTQ Orbitrap) and 50-60 % S-lense RF level (LTQ Orbitrap Velos), respectively; if used, a lock mass of m/z 445.120024 has been applied ; for LTQ Orbitrap Velos measurements, the lock mass abundance was set to 0 %. 

Sample processing

HCT116 derived samples were prepared using OFFGEL fractionation followed by LC-MS/MS performed in CID mode. RPE-1 samples were separated using strong anion exchange fractionation and consecutive LC-MS/MS with CID fractionation. SuperSILAC samples were fractionated with strong anion exchange fractionation and in the following analyzed using HCD fractionation.

Data analysis

*.RAW files of all double labeling HCT116 experiments were analyzed together using the in-house developed software MaxQuant ( ; version 1.0.14.10), which performs peak list generation, SILAC- and extracted ion current-based quantitation, calculated posterior error probability and false discovery rate based on MASCOT (Matrix Science, London, UK; version 2.2.04) search engine results, peptide to protein group assembly, and data filtration and presentation. All created MS files were analyzed together, but separate ratios were provided for the different
experiments. Data were searched against the human International Protein Index protein sequence database (ipi.HUMAN.v3.62.dec, 168418 forward and reversed protein sequences from EBI Database) supplemented with frequently observed contaminants such as human keratins, bovine serum proteins and proteases and concatenated with reversed copies of all sequences (target-decoy database, ⁸). Scoring was performed in MaxQuant as described previously ⁹. Parent masses and fragment ions were searched with a mass tolerance of 7 ppm and 0.5 Da, respectively. We required strict trypsin specificity, however, allowing up to two missed cleavage sites. Fixed modification of cysteine carbamidomethylation (Cys 57.021464 Da) and variable modifications for N-acetylation of proteins (N-term 42.010565) and oxidation of methionine (Met 15.994915 Da) have been set. The minimum peptide length was restricted to at least 6 amino acids. The created peak lists were uploaded to Mascot and the generated files (*.dat) together with the underlying data were further processed in MaxQuant. False discovery rates on peptide and protein level were fixed to 1 %, including automatic filtering on peptide length, mass error precision estimates, and peptide scores of all forward and reversed peptide identifications. Re-Quantification and match between runs functions were applied. Reported protein groups had to be identified by at least one unique peptide to be accepted. Quantitation was based on unique and razor peptides only and a minimum of 2 ratio counts was required. Complete protein and peptide lists as well as the underlying *.RAW files are available on the TRANCHE database (https://proteomecommons.org/tranche/).
*.RAW files from RPE-1 and the SuperSILAC HCT116 experiments were processed with MaxQuant using version 1.2.0.25 and 1.2.0.29, respectively. In contrast to the previous version, an own search engine – Andromeda – is implemented into the workflow that replaces the Mascot Search. Data were searched against the human International Protein Index protein sequence database ipi.HUMAN.v3.68.dec. The parameters are defined as in the HCT116 search described in detail above.

**Analysis of CGH data**

For all samples, the raw values ("r BGSubSignal") were divided by the corresponding control background signal ("g BGSubSignal") producing background subtracted ratios. Subsequently, the ratios of ratios of aneuploid to diploid samples were calculated. The values were converted into log2 ratios. Since the median slightly differed from 0, the median of the whole population was subtracted leading to a normalized population centered at 0. High resolution CGH ratios depicted in log10 were transformed into log2 ratios to have comparable values on all levels. All CGH data are available on the TRANCHE database.

**Analysis of the mRNA data**

Three biological replicas have been performed for all analyzed samples. In a first step, all ratios were transformed in log2 values and the ratios of the biological replicas were averaged by using the median. Subsequently, the control value was subtracted from the aneuploid sample to form the ratio aneuploid/control. mRNA data are uploaded to the TRANCHE database.
Analysis of the protein data

The protein list output file (proteinGroups.txt) was first filtered to remove contaminants and reverse entries and the ratios were transformed in log2 values. Median ratios were calculated for technical replicates of the same biological replicates. For the biological replicas E1, E2 and E3 the median ratio was calculated (HCT116 5/4). Only median ratios calculated from at least two underlying biological replicates were taken into account for further analyses. The combined median ratio was used for the comparison of CGH, mRNA and protein data HCT116 5/4. For the other aneuploid HCT116 cell lines, normalized protein ratios were used. RPE-1 data were processed similarly in a separate dataset.

Combination of the pre-processed DNA, mRNA and protein results

All generated CGH and mRNA log2 entries were matched to the corresponding protein entries and merged into a table. Supplementary Table 1 contains all information that has been generated within this study, and combines CGH, mRNA and protein results for all different clones. CGH data were combined with the protein data using chromosome position information from Ensembl (http://www.ensembl.org/index.html) included in Uniprot. mRNA data were combined with the protein entries by using the specific “Probe set” names and the corresponding Uniprot entries. Subsequently, CGH data were filtered for entries on chromosome 5 and 3, respectively, that were not present in trisomic or tetrasomic state. The thresholds for filtering were set at 0.65 and 0.6 for HCT116 5/4 and HCT116 H2B-GFP 5/4, respectively, for trisomic entries at 0.3. For each ratio column, one additional column has been created in which all entries below these
thresholds have been deleted (named with the additive term “filtered”). RPE-1 data were not filtered, since no deletions occurred on CGH level. Only filtered populations have been used for further analyses. Total chromosome positions were calculated for each entry by adding up the single chromosome position values of all chromosomes starting from chromosome 1 to chromosome X. For downstream analyses, required columns have been extracted from the basic dataset and only entries with ratios determined in all required sub-columns were used.

**Two-dimensional annotation enrichment analysis**

Significant deregulations of categorical annotations within one population in comparison to a second population was analyzed using a two-dimensional annotation distribution analysis\(^{10}\). As categories, Gene Ontology (GO) biological processes (GOBP), molecular functions (GOMF) and cellular components (GOCC), KEGG pathways, proteins within complexes (CORUM) and the underlying chromosomes have been taken into account. In brief, we calculate whether the expression of proteins of a given category is significantly different from the remaining population by applying a non-parametric two-sided (two-sample) Wilcoxon-Mann-Whitney test. Multiple hypothesis testing is controlled by using a Benjamini-Hochberg false discovery rate threshold of 2 %. A relative regulation value between -1 and 1 depending on the average rank of the underlying proteins are calculated for each significant category. Values close to 1 indicate protein categories strongly concentrated at the high numerical distribution, whereas values close to -1 relate to a population at the low end of the distribution. Data used for the 2D annotation
distribution analysis are listed in the Supplementary Table 2. Data were plotted in R, highlighting specific categories as indicated in Supplementary Table 2.

**Density histograms for different populations**

Density histograms have been generated using the R software. Different populations were extracted from the main population and plotted together in one figure via using the `density` function. Moreover, additional information such as median ratios of the subpopulation as well as the underlying numbers for the different populations were extracted and depicted within the plots. All ratios were used as log2 values. Proteins associated to a complex were extracted using the CORUM database (http://mips.helmholtz-muenchen.de/genre/proj/corum/). The *Kinase Activity* subpopulation was extracted from the GOMF terms searching for the term “kinase activity”. Single entries from subpopulations (CORUM, Chr5 or GOMF Kinase activity) are depicted in the figures below the graph with black vertical lines to clarify the amount of underlying hits.

**Detailed Analysis of kinases and complexes**

Complex proteins as well as proteins with kinase activity from the HCT116 5/4 data were extracted via the CORUM and GOMF term “kinase activity” as mentioned above.

Multimolecular Complex Analysis: Complexes that contain at least one protein and corresponding mRNA and DNA entries coded on the tetrasomic chromosome 5 and at least 5 entries in total were analyzed. Only proteins with existent mRNA and DNA information were used for this analysis.
Kinase Analysis: DNA, mRNA and proteins from the tetrasomic chromosome 5 classified as coding for kinases were plotted by using the GraphPad Prism software.

**Boxplot analysis**

For better comparison of protein subpopulations, specific categories were extracted from the complete dataset (e.g. GOBP “autophagy”). Ratios from different experiments were compared by using the `geom_boxplot` function from the R package ggplot2, plotting the different experiments next to each other. Specific proteins were highlighted within each population with colored spots according to the legend. Horizontal lines within the box represent the median of the population. Lower and upper hinge represent the 25% and 75% quantile, respectively. Lower whisker and upper whisker represent the ‘lower hinge – 1.5 * IQR’ and ‘upper hinge + 1.5 * IQR’, respectively. Significances were calculated using a non-parametric two-sample Mann-Whitney t-test.
Details of mass spectrometry runs

HCT116 cell lines

A total of 7548 proteins were identified by analyzing all 196 *.RAW files with MaxQuant. The numbers of quantified proteins/entries are listed for all individual measurements.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Name in Suppl. Table 1</th>
<th>Folder name on TRANCHE</th>
<th># of files</th>
<th># of unfiltered quantified entries (filtered)</th>
<th>Remarks</th>
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<td>HCT116 H2B-GFP 5/4</td>
<td>Protein HCT116 H2B-GFP 5/4</td>
<td>Aneuploidy_HCT116_RAW_C4</td>
<td>13</td>
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<td>Tetrasome for Chr.5 vs control</td>
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<td>HCT116 H2B-GFP 5/3</td>
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<td>Protein HCT116 3/3</td>
<td>Aneuploidy_HCT116_RAW_Chr3_1</td>
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<td>Aneuploidy_HCT116_RAW_Chr3_2</td>
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</table>
RPE-1 cell lines

A total of 5678 protein have been identified by 36 *.RAW files with MaxQuant. An overall number of 5200 proteins has been quantified.

<table>
<thead>
<tr>
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<td>RPE1 5/3 12/3</td>
<td>Prot 5/3 12/3</td>
<td>RPE1 Aneuploidy RAW Chr512 Chr21</td>
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Hash Codes for TRANCHE database

Experiment E1_1 ("Aneuploidy_HCT116_RAW_OG1"):  
Passphrase: StorchovAneu10  
GGURAkJD1nVntlod0cT6DADFSHpxcR9cVALh5CZX7TY/ccRUiqgt8MvC2dR42M1r7XB0xhd6qWqijJRr2UbBjbNAK6MAAAAAAVbA==

Experiment E1_2 ("Aneuploidy_HCT116_RAW_OG3"):  
Passphrase: StorchovAneu10  
CDWD7iS/zyBA6aBXZ7hGsTdNw1WRswyQZNeRN+/vVKezG CJvfOPhc/Hs/TypKgTodEMH+yiS+xvWz/OkWP7JyCTG7sAAAAAAAOKg==

Experiment E1_3 ("Aneuploidy_HCT116_RAW_OG4"):  
Passphrase: StorchovAneu10  
OrKi7CIVTvK7QLDT+QgWll5i8ZIsyIk5i/3as53tknbWZ2NYonLgwqnzNfUrFGyDWXUF8UE1wowJbDYR1HeHU9BekQAAAAAAAALg==

Experiment E2_Cell, mixed on cell level ("Aneuploidy_HCT116_RAW_M2WT"):  
Passphrase: StorchovAneu10  
jk6F0iNSQYooYoTvoJcoZmzuGZzRgblNydX+YyJ7gGsZEjjsEvgmQcUp3zOCis9W/FqFazftXZgkcPsukkQlqQHU8oAAAAAAAAPzw==

Experiment E2_Prot, mixed on protein level ("Aneuploidy_HCT116_RAW_MS2WT"):  
Passphrase: StorchovAneu10
Experiment E3 ("Aneuploidy_HCT116_RAW_MSChr5"):
Passphrase: StorchovAneu10
Eaur+4UmZySJ1rlMRxI mttqPva61BBxPg8gL/ImQ3AN8CHmaKzrvjvT8/2GJsMiY6onwz+3F3SPhbs+62z1pjxg3YAAAAAAAARjQ==

Experiment HCT116 3/3, first half ("Aneuploidy_HCT116_RAW_Chr3_1"):
Passphrase: StorchovAneu10
EPNepMq6/Ys3s+ZSAwcWdKjEIaItF5+clBNDBpn7DzGr/b/Mu8tKG5p54Ld6kl/lzIbFsVylWwfrhjGUpVR0RJSoAAAAAAAQAQ4Q==

Experiment HCT116 3/3, second half ("Aneuploidy_HCT116_RAW_Chr3_2"):
Passphrase: StorchovAneu10
Q0mWyrER5FjastQZdNO7/2rESTCmrJSBROV7zd6lLpBKSov0TZNd9FqEcDzvJMSThYo+T1SPxefN0XT//ZdfpqmvQAAAAAAAAMfA==

Experiment HCT116 H2B-GFP 5/3 ("Aneuploidy_HCT116_RAW_Clone6"):
Passphrase: StorchovAneu10
uhGfa5UaUoCq/QyorfeFr4YCMzIFVtJLbychfy4i+dgEE2SdKOu456dWdNlmL5h6dN4e3e03Lii0y8xQxT/05+iAAAAAAAAMfA==

Experiment HCT116 H2B-GFP 5/4 ("Aneuploidy_HCT116_RAW_Clone4"):
Passphrase: StorchovAneu10
7+A7jFe4pYRwmE/Rmqad4c0nDTDWzOxwTyUtU1RBOob6LhHEOvJ/CCTC8Qg5hu2HcXXvMMf3QiziXIS4/dHCamZlcMAAAAAANKw==

MaxQuant Output Files HCT116:
Passphrase: Aneuploidy
Y7wT6/0Y1WADTs8ztTyL+dXWNkS7VmAxyiwHO3WaWsuAlr+Pwus6KOfimOjizzB8KCTbjk8l5M1Y/j3Lk77iixcmvMAAAAAAL5A==

Washes ("Aneuploidy_HCT116_RAW_Washes"):
Passphrase: StorchovAneu10
EgN1+l4ENsJUc7J+4fexirq7KMy/c48r+Cau6aJcg+BtpD56ESP/loYMjWxytC1jfCCw x0NNB2bD5fSWWZI+Gbxj5hkAAAAAZiA==

CGH Data HCT116 ("HCT116 CGH data"):
Passphrase: Aneuploidy
CsbJTGGYGMWChedvMEF/Hsu0Cqs4TkoJ5U0cU70DK+2cOjSRsNVMEUgazZyfH QXm0pZwuXXQ5INCgs85tNBvr+wrEAAAAAAAGAA==
mRNA Data HCT116 ("HCT116 mRNA data"):
Passphrase: Aneuploidy
DMkLRPtJ6HI8PNdMAV8AD1Jx4p4Lo2HMMY+HYzGYfhoZkne9msHHleaQcMtJNjqa
O1cv/MsK7FC7l/0okAI9ajB+EYDDDDDDDAG2Q==

CGH Data RPE-1 ("RPE1 CGH data"):
Passphrase: Aneuploidy
1BL412GkH54q/NiAKKXcXgb90EzmqFey49V3HfArcUULPMFLJfbOT947xmiOnizq77H
g642TcBvLOsIHzx5SrLqosjQDDDDDDDDDDDDDDQ==

MaxQuant Output Files RPE-1 ("RPE1 MaxQuant Output files"):
Passphrase: Aneuploidy
KDYSI6P5U7v8Z35QspCGL/CAczMg3JcWGksM+lxXH6I6QYKTEXNswiFgLWs3SW4
ilzvT7olsukXqQoxj6swzZSPfEDDDDDDDDDDDDDDQ==

Experiment RPE-1 Chr5Chr12 and Chr21 ("RPE1 Aneuploidy RAW Chr512
Chr21"):
Passphrase: Aneuploidy
pBFT+7uXDVFlaWS/A0yfz/EB1eedk7dGFj9okbT7HbqUT4TNvIE5unYGsOFr0tW2Sy2
WuHioulES2hJqaW2w1f/GgDDDDDDDDDDDDDDw/g==
Supplemental References


