Hypo-osmotic–like stress underlies general cellular defects of aneuploidy

Hung-Ji Tsai1, Anjali R. Nelliat1,2, Mohammad Ikbal Choudhury3, Andrei Kucharavy1, William D. Bradford4, Malcolm E. Cook4, Jisoo Kim1, Devin B. Mair3, Sean X. Sun2,3, Michael C. Schatz6 & Rong Li1,2

Aneuploidy, which refers to unbalanced chromosome numbers, represents a class of genetic variation that is associated with cancer, birth defects and eukaryotic micro-organisms1,2–4. Whereas it is known that each aneuploid chromosome stoichiometry can give rise to a distinct pattern of gene expression and phenotypic profile4,5, it remains a fundamental question as to whether there are common cellular defects that are associated with aneuploidy. Here we show the existence in budding yeast of a common aneuploidy gene-expression signature that is suggestive of hypo-osmotic stress, using a strategy that enables the observation of common transcriptome changes of aneuploidy by averaging out karyotype-specific dosage effects in aneuploid yeast-cell populations with random and diverse chromosome stoichiometry. Consistently, aneuploid yeast exhibited increased plasma-membrane stress that led to impaired endocytosis, and this defect was also observed in aneuploid human cells. Thermodynamic modelling showed that hypo-osmotic–like stress is a general outcome of the proteome imbalance that is caused by aneuploidy, and also predicted a relationship between ploidy and cell size that was observed in yeast and aneuploid cancer cells. A genome-wide screen uncovered a general dependency of aneuploid cells on a pathway of ubiquitin-mediated endocytic recycling of nutrient transporters. Loss of this pathway, coupled with the endocytic defect inherent to aneuploidy, leads to a marked alteration of intracellular nutrient homeostasis.

Fig. 1 | Karyotype-independent transcriptomic response in heterogeneous aneuploid populations. a, Relative copy numbers of chromosomes (aneuploids/haploid) in different populations are represented by a colour gradient, in the heat map. Populations (pop. no.) 1 and 2 are heterogeneous populations that were generated from tetrad dissections; populations 3, 4 and 5 are heterogeneous populations that were generated using the MATa-selection method. Three previously published aneuploid strains with stable gain of specific chromosomes are shown for comparison. b, Correlation of differential gene-expression patterns between CAGE genes and those in cells exposed to the specified stress for 15 min (y axis). Grey dots, comparisons of all genes in both transcriptome analyses. Black circles, CAGE genes. Correlations were determined by Spearman’s rank correlation coefficient. Correlation scores and exact P values (for b–d) are shown in Supplementary Table 2; sample sizes in b–d are given in Source Data.

1Center for Cell Dynamics, Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, USA. 2Department of Chemical and Biomolecular Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, MD, USA. 3Department of Mechanical Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, MD, USA. 4Stowers Institute for Medical Research, Kansas City, MO, USA. 5Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA. 6Department of Computer Science, Johns Hopkins University, Baltimore, MD, USA. E-mail: rong@jhu.edu
Aneuploidy causes chromosome dosage-dependent changes in the expression of many genes, which results in phenotypic diversity. Whereas most aneuploid cells exhibit reduced fitness, karyotypically diverse populations exhibit high evolutionary adaptability. Extensive previous studies have revealed stress responses and genetic pathways in specific aneuploid strains or cell lines, but the unique transcriptomic patterns and phenotypic profiles that are associated with individual karyotypes make it difficult to discern the general consequence of aneuploidy. We therefore designed a scheme to analyse aneuploid populations that contain random karyotypes that are diverse enough to cancel out the dosage effects from specific karyotypes within the population. RNA sequencing analysis was performed on five of these aneuploid populations, in comparison with a reference haploid population. Despite having euploid-like chromosome stoichiometry, the heterogeneous aneuploid populations exhibited transcriptomic patterns that were different from that of the haploid population. The expression changes of several CAGE genes in individual aneuploid clones were consistent with those in aneuploid populations. Moreover, the average expression changes of CAGE genes of FM4-64 intensity in the cytoplasm versus at the plasma membrane (FM) from movies in d (left) or immediately after addition of 0.4 M sorbitol (right) over time. Ribbons denote 95% CI. Haploid cells, n = 205 and 206; aneuploid cells, n = 200 and 205 in the left and right subpanels, respectively. Fluorescence-activated cell sorting (FACS) profiles for transferrin uptake, showing less-efficient endocytosis in aneuploid NALM6 cells treated with MPS1 inhibitor (red), compared to the untreated diploid population (blue). Inset bar plot represents the percentages of cells that possess positive Alexa-Fluor-488-labelled transferrin signal within gated populations (green dotted line) (n = 3). Cell-surface stiffness was measured with atomic force microscopy in haploid and aneuploid cells (representative of three biological repeats). Scale bar, 5 μm. Average ratios (centre) and confidence intervals (CI) (endocytic patches in haploid and aneuploid lines outline the plasma membrane. Right, average travel distance away from plasma membrane (y axis) over time, with ribbons denoting 95% confidence intervals (CI) (endocytic patches in haploid and aneuploid cells, n = 158 and 255, respectively). Images from time-lapse movies of FM4-64 internalization in haploid and aneuploid cells (representative of three biological repeats). Scale bar, 5 μm. e, Average ratios (centre) and confidence intervals (CI) (endocytic patches in haploid and aneuploid lines outline the plasma membrane. Right, average travel distance away from plasma membrane (y axis) over time, with ribbons denoting 95% confidence intervals (CI) (endocytic patches in haploid and aneuploid cells, n = 158 and 255, respectively). Images from time-lapse movies of FM4-64 internalization in haploid and aneuploid cells (representative of three biological repeats). Scale bar, 5 μm. f, Average ratios (centre) and confidence intervals (CI) (endocytic patches in haploid and aneuploid lines outline the plasma membrane. Right, average travel distance away from plasma membrane (y axis) over time, with ribbons denoting 95% confidence intervals (CI) (endocytic patches in haploid and aneuploid cells, n = 158 and 255, respectively). Images from time-lapse movies of FM4-64 internalization in haploid and aneuploid cells (representative of three biological repeats). Scale bar, 5 μm. f, Average ratios (centre) and confidence intervals (CI) (endocytic patches in haploid and aneuploid lines outline the plasma membrane. 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environmental osmolarity) diminished the differential expression of genes that were shared between CAGE and the hypo-osmotic stress signature, as compared to haploid cells (Extended Data Fig. 2e). Faster nuclear export of Hog1 in aneuploid cells than in haploid cells after hyper-osmotic shock was also consistent with a higher intracellular turgor, as indicated by the slopes of force-displacement curves at high indentations that are grossly reduced by increasing medium osmolarity or cell membrane permeabilization (Fig. 2a, Extended Data Fig. 3a, b). Consistent with an increased turgor, cell lysis occurred faster in aneuploid populations than in haploid populations after cell wall weakening by treatment with zymolyase (Fig. 2b). This was not due to a thinner or faster removal of cell wall, and could be alleviated by the addition of sorbitol; the faster lysis was also observed in individual aneuploid clones (Extended Data Figs. 4a–d, 5). Moreover, aneuploid cells excreted more glycerol within a unit length of time than haploid cells, and exhibited hyper-phosphorylation of Slt2 (also known as Mpk1), which suggests a continuous hypo-osmotic stress response in aneuploid cells (Extended Data Fig. 4e, f).

Turgor pressure is expected to counter membrane invagination during endocytosis. Unlike the fast inward movement of endocytic foci marked by Abp1–GFP in haploid cells, Abp1–GFP-marked foci in aneuploid cells mostly moved along the cortex (Fig. 2c). The duration at the cortex of both Abp1 and Slal2, another endocytic patch protein, were significantly longer in aneuploid cells than in haploid cells, and no defective recruitment of these proteins was detected (Extended Data Fig. 6a–d). The loss of inward movement, and the longer duration at the cortex, of Abp1 in aneuploid cells were both rescued upon acute increase in environmental osmolarity (Extended Data Fig. 7a). The bulk plasma-membrane turnover, monitored through the internalization of the FM4-64 lipophilic dye, was also significantly slowed in aneuploid cells, as compared to haploid cells (Fig. 2d, e, Extended Data Fig. 6e). Overall, the phenotypes that are associated with aneuploidy are qualitatively consistent with those that are observed when haploid cells experience hypo-osmotic shock, and could be alleviated by increasing the external osmolarity (Fig. 2e, Extended Data Fig. 7).

To investigate whether human aneuploid cells also experience hypo-osmotic stress, we induced aneuploidy with random chromosome gains or losses from the mostly diploid NALM6 leukaemia cell line by inhibiting the MPS1 spindle-assembly checkpoint kinase, followed by a 40-h recovery. A transferrin uptake assay showed impaired endocytosis in the induced aneuploid population relative to the control population, and atomic force microscopy measurements showed higher average cell stiffness in the induced aneuploid population than in the control population (Fig. 2f, g, Extended Data Fig. 8). Furthermore, in NCI-60 cancer cell lines 'plasma membrane' was among the top significantly enriched gene ontology (GO) terms of those genes that are upregulated with increasing levels of chromosome instability, as defined by chromosomal numerical heterogeneity; the same gene ontology term (that is, plasma membrane) was significantly enriched among yeast CAGE genes (Supplementary Table 3).

Chromosome gain or loss leads to scaled changes in average levels of proteins encoded by aneuploid chromosomes. The disrupted proteome balance increases the amounts of free proteins that normally participate in protein complex formation, which would result in an increased intracellular solute concentration and lead to high cytoplasmic osmolarity in aneuploid cells (Fig. 3a). We modelled the effect of proteome imbalance on the basis of the principles of thermodynamics (Supplementary Methods). Simulations showed that the osmotic pressure increase due to proteome imbalance leads to cell swelling, the degree of which is a nonlinear function of the DNA content and is beyond that predicted by linear scaling with the genome size—a trend that is consistent with experimental measurements (Fig. 3b, Supplementary Methods). The cell swelling phenotype could not be attributed to misregulation of the cell cycle or cell polarity (Extended Data Fig. 6f, g), although these defects could affect cell size in specific aneuploid cells. Notably, this nonlinear cell-genome size scaling was also observed in the NCI-60 panel of cancer cells (Fig. 3c). Simulation of the turgor pressure also showed higher values in aneuploid cells than in haploid cells across a reasonable range of parameters (Extended Data Fig. 3c, d). Additionally, our theoretical model predicts an increased diffusion of cytosolic proteins and a decreased cell density for aneuploid cells compared to euploid cells; these predictions were also validated experimentally (Fig. 3d, e).
Fig. 4 | Dependency of aneuploid cells on the Art–Rsp5 pathway for fitness and nutrient homeostasis. a. Genome-wide deletion screen in heterogeneous aneuploid populations. b. Survival rates of aneuploid cells that contain specific mutation(s) (Supplementary Table 5). c. Microscopic colony growth of the three validated mutants. Grey dots represent the ratio of growth rate of a single aneuploid microcolony to average growth rate of haploid microcolonies that carry the same mutation. d. Schematic of subcellular locations of Art1, Vps51 and Yps5 in endocytic pathway and the function of Art1 and Rsp5 in ubiquitylating plasma-membrane-bound endocytic cargo. e. Hxt4 and Can1 turnover in response to glucose depletion and canavanine addition, respectively, in haploid and aneuploid populations. Grey dots represent the ratio of GFP intensity (plasma membrane:total). Relative GFP intensity at PM (PM:total) = 0.25***, 0.50*** and 0.75***. f. Heat maps showing fold changes in the relative abundance (to wild-type (WT) haploid cells) of intracellular free amino acids in wild-type and art1Δ haploid and aneuploid cells. g. Modelling of combinatorial effects of increased turgor pressure (ΔP externally increased) and reduced rate constant (ki) for transporter downregulation on the kinetics of substrate uptake. h. Uptake of the glucose analogue 2-NBDG in haploid or aneuploid wild-type or art1Δ cells over time (mean ± s.d.). i. Relationship of chromosome instability (CIN) level and the net influx of extracellular metabolites in the NCI-60 cancer cells (n = 54). Consumption of glucose and glutamine (coloured line with 95% CI ribbon) was significantly correlated with the level of chromosome instability. Thirty metabolites with positive net influx were plotted (other coloured lines). Spearman’s rank correlation scores and exact P values are consolidated in Supplementary Table 7. Box plots and violin plots (c and e) are defined as in Fig. 2 and Supplementary Methods. See Source Data for sample sizes (c, e and b) and exact P values (c and e), one tailed Mann–Whitney U test. ***P < 0.0001.

We next performed a genome-wide screen to identify non-essential gene deletions (open reading frame deletions) that are more detrimental to aneuploid populations that comprise diverse karyotypes than they are to haploid populations (Fig. 4a, Extended Data Fig. 9, Supplementary Methods, Supplementary Table 4). The primary screen revealed an enriched gene ontology term, ‘response to osmotic stress’, in gene deletions, which caused a low growth capacity in aneuploid populations; this included genes related to the cell wall integrity signalling pathway (such as wsc1 (also known as slg1)) and the aquaglyceroporin yps522 (Supplementary Table 3). Further validation experiments narrowed the candidates down to three mutants (art1Δ also known as ldb19, vps51Δ and yps52Δ) that generally reduced the viability and growth rate of aneuploid populations with broad karyotype diversity (as compared to haploid populations); by contrast, the growth defects of the other primary hits could be overcome, probably through karyotype selection (Fig. 4b–d, Supplementary Methods, Supplementary Table 5). art1Δ exhibited the lowest relative growth rates across nearly all cells of heterogeneous aneuploid populations (Fig. 4c). Art1 is an arrestin-related trafficking adaptor that targets the E3 ubiquitin ligase Rsp5 to promote endocytosis of plasma-membrane amino acid transporter art126,27. Heterogeneous art1Δ aneuploid, but not haploid, cells that carry a second deletion of other members of this gene family showed further reductions in viability (Fig. 4b, Supplementary Table 5). Furthermore, aneuploid cells that bear the rsp5-1 mutation also exhibited markedly reduced viability as compared to rsp5-1 haploid cells, at both permissive and semi-permissive temperatures (Fig. 4b, Supplementary Table 5).

The above findings implicate a possible general defect of aneuploid cells in the regulation of plasma-membrane nutrient transporters. In support of this notion, the glucose transporters Hxt3 and Hxt4 were not efficiently internalized in response to glucose depletion in a heterogeneous aneuploid population—unlike in haploid cells—even though the glucose-sensing pathway remained functional (Fig. 4e, Extended Data Fig. 10). Similarly, the turnover of arginine permease (Can1), after treatment with the toxic arginine analogue canavanine, was also reduced in aneuploid cells (Fig. 4e). The relative concentrations of free amino acids (in particular, glutamine) were altered in aneuploid cells, as compared to wild-type haploid cells, and art1Δ further exacerbated this imbalance (Fig. 4f, Supplementary Table 6). We constructed a flux-based model to understanding the general effect of aneuploidy-associated membrane stress on nutrient homeostasis (Supplementary Methods). Simulations using this model revealed that changes in the
turgor-associated parameter ($\Delta P^*$) and in the rate constant ($k_0$) for nutrient-regulated transporter downregulation would result in the most marked changes in nutrient homeostasis (Fig. 4g, Supplementary Methods), which was validated by glucose-uptake kinetics in wild-type and art1.∆ haploid and aneuploid cells (Fig. 4h). The influx of glucose and glutamine as compared to other carbohydrates and amino acids, respectively, are the most significantly and positively correlated with levels of chromosome instability among NCI-60 cancer cells ($^*$) (Fig. 4i, Supplementary Table 7), which suggests that metabolic remodelling may be a consequence of hypo-osmotic stress in aneuploid cancer cells with ongoing chromosome instability.

The data presented above uncover an aneuploidy-associated general stress state that may be explained by proteome imbalance. This hypo-osmotic-like stress state in aneuploid cells is chronic, unlike the transient osmotic shock responses of euploid cells. A downstream general endocytic defect underlies a metabolic dysregulation that can be observed in aneuploid yeast, and may also be evident in aneuploid cancer cells. This inherent defect in aneuploid cells also explains their survival and growth dependence on the Art→Rsp5 pathway. Rsp5 is a homologue of mammalian NEDD4, a member of the HECT family of E3 ubiquitin ligases. In mammalian cells, multiple E3 ligases—such as NEDD4 and MDM2—work together with arrestin-family adaptors to modulate the homeostasis of the plasma-membrane proteome. Thus, the deleterious effects of mutations that affect Art proteins and the Rsp5 E3 ubiquitin ligase might provide a proof-of-concept for how a common deficiency of aneuploidy may be targeted.

**Reporting summary**

Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1187-2.

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**Author contributions** H.-J.T. and R.L. designed the experiments. H.-J.T., A.R.N., M.I.C., W.D.B. and J.K. performed the experiments. Data were analysed by H.-J.T., A.R.N., M.I.C., M.E.C. and D.B.M. M.C.S. supervised the genomic and transcriptome analyses. A.R.N. and A.K. implemented the mathematical and biophysical models (written descriptions of model details can be found in the Supplementary materials). H.-J.T., A.R.N. and R.L. conceived and supervised the project.

**Competing interests** The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Generation of heterogeneous aneuploid populations without chromosome copy number bias for transcriptomic analyses. a, Generation of aneuploid cells through random meiotic segregation from a homozygous triploid (3N) yeast strain that bears a centromeric plasmid that carries the selection marker spHIS5 under the MATa-specific promoter STE2pr. Coloured rods represent chromosomes. b, Left, DNA content of a cohort of random aneuploid spore colonies (each represented by a grey dot, n = 75; see Source Data) produced through triploid meiosis and analysed by FACS; box plot and violin plot (defined as in Fig. 2 and Supplementary Methods) show the distribution of ploidy levels. Right, 192 random aneuploid colonies produced by triploid meiosis were pooled, and the resulting population subjected to quantitative PCR-based karyotyping (representative results from two biological repeats). Y axis represents the relative copy number (mean of the two arms) of each chromosome (dots) to the reference haploid yeast. There was no significant difference in copy number between each chromosome (one-way analysis of variance, P value = 0.998). c, Principal component analysis of RNA sequencing (RNA-seq) results in different populations. PC1 (x axis) shows the apparent difference between haploid (n = 2) and aneuploid (n = 5) populations. PC2 (y axis) reflects a small difference in the two methods of generation of aneuploid populations. Each dot represents one population. d, Gene expression in heterogeneous aneuploid populations (n = 5) relative to haploid populations (n = 2) in MA plot. x axis represents basal mean expression in log10 scale; y axis represents differential expression changes between aneuploid and euploid populations in log2 scale. P values calculated on the Wald statistic were corrected for multiple comparisons (Benjamini–Hochberg), and the resulting false discovery rate was further corrected for variance underestimation using an empirical null model. CAGE genes were identified as genes with final false discovery rate < 0.05 (see details in Supplementary Methods; exact P values in Supplementary Table 1). Each dot represents 1 gene, and 222 significantly differentially expressed (CAGE) genes common to all 5 aneuploid cell populations are labelled in dark red. e, Quantitative PCR with reverse transcription (RT–qPCR) validations of four significantly differentially expressed genes from the RNA-seq analysis in a heterogeneous aneuploid population (n = 1), and randomly picked individual aneuploid clones (n = 12). Black circles, individual aneuploid populations; red dot: a heterogeneous aneuploid population; green dot, aneuploid populations in RNA-seq (d). For individual aneuploid colonies, the expression change of each gene was normalized by the gene copy number (determined by qPCR), and then normalized to that of a non-CAGE gene (ACT1). Average gene-expression changes in the population and in the individual aneuploid clones followed similar trends (up- or downregulated) to those of heterogeneous populations in RNA-seq. Box plot representation as in Fig. 2 for measurements from the 12 aneuploid clones. f, Correlation analysis of expression changes of the genes implicated in the CAGE signature, showing positive correlation between aneuploid populations and stable aneuploid strains5 (n = 5) (Spearman’s rank correlation). Green circles represent mean expression changes of genes within the CAGE signature (213 in common in the transcriptomic data between aneuploid populations and the individual strains). Error bars, s.e.m. Only eight genes across all five strains appear to show opposite expression trend to CAGE. The trend line was fitted by linear regression (red dashed line) with grey ribbon (95% CI). Significant positive correlation was observed between the average gene-expression changes in the five aneuploid strains, and CAGE observed with the heterogeneous aneuploid population subjected to RNA-seq (P value = 1.609 × 10−7).
Extended Data Fig. 2 | See next page for caption.
**Extended Data Fig. 2 | Comparisons of gene-expression patterns between CAGE and several stress responses.**

**a.** Top, heat map showing negative correlation between CAGE and gene-expression changes of cells exposed to hyper-osmotic shock for different time periods. Colour gradient as in Fig. 1b. Scatter plot, genome-wide and CAGE correlation of gene-expression patterns between aneuploid versus haploid population (x axis) and cells exposed to hyper-osmotic stress for 15 min (y axis). Colour scheme as in Fig. 1c. Correlation scores from the Spearman’s rank correlation test and exact P value are in Supplementary Table 2; sample size (n) is documented in the Source Data for Fig. 1. Bottom, as for the top panel but for the correlation between CAGE and long-term DTT treatment (120 min).  

**b.** Heat map showing positive correlation of gene-expression changes in cells experiencing temperature down-shift (37 °C to 25 °C) with changes in cells experiencing hypo-osmotic shock (left), short-term DTT treatment (middle) or with CAGE (right). Correlation test and scores as in a; sample size (n) is documented in Source Data.  

**c.** The same MA plot as shown in Extended Data Fig. 1d with labels of environmental-stress response (ESR) genes (n = 857). Red and blue dots indicate ESR genes that were upregulated or downregulated, respectively, when cells were exposed to stress.  

**d.** Venn diagrams showing common genes between CAGE and ESR (left). No overlapping genes between upregulated genes in CAGE and ESR were found, and the same is true for downregulated genes in CAGE and ESR. By contrast, there are some overlaps between oppositely regulated CAGE and ESR genes.  

**e.** RT–qPCR monitoring expression changes of two CAGE genes that are also significantly differentially expressed under hypo- and hyper-osmotic shock conditions in a heterogeneous aneuploid population exposed to hyper-osmotic shock (0 to 1 M sorbitol). Multiple primer sets that target each gene were used. y axis indicates the ratio of gene expression of two CAGE genes between aneuploid and haploid populations over time, immediately after cells were shifted to medium with 1 M sorbitol (x axis). Note that one of the genes was upregulated (blue) in CAGE, and the other was downregulated (red). Plots show that these changes were alleviated by hyper-osmotic shock.  

**f.** Top, percentage of cells with nuclear-localized Hog1 as a function of time after different levels of acute hyper-osmotic exposure in haploid and aneuploid populations. y axis indicates the percentage of cells with nuclear-localized Hog1–GFP signal over time, immediately after cells were shifted to medium with 1 M sorbitol (x axis). Bottom, example images (two independent experiments) from two time points (t1 and t2, labelled with green lines in top panel) in haploid and aneuploid cells (n = 287 and 254; see Source Data for full information of sample size). Red arrows indicate diminished nuclear-localized Hog1. Note that Hog1 import occurred quickly, such that even at the first time point of measurement after osmotic shock, most cells displayed nuclear Hog1.
Turgor-pressure measurements of cells in a hyper-osmotic environment or experiencing cell permeabilization, and biophysical model predictions. 

a, Examples of force-displacement curves that show the difference of curve shapes from indented haploid and aneuploid cells in normal medium (YPD), or immediately after shift to medium containing 0.4 M sorbitol or pre-treated with 0.1% SDS (sample size (n) in Source Data). The pre-set force for this atomic force microscopy experiment was 1 nN. 

b, Box plots of slopes (nN nm$^{-1}$) computed at two parts of the curves in a: the first 10 nm of z-piezo displacement, which mostly reflects cell-wall stiffness, and the third quartile (Q3) of the curve, which is expected to be a contribution from cellular turgor. The results show that that increasing extracellular osmolarity (sorbitol, middle) or cell permeabilization (SDS, right) led to a marked reduction of intracellular turgor, and diminished the increased turgor in aneuploid cells to the level of that in haploid cells. One-tailed t-test; sample size (n) in Source Data. In YPD, at 0–10 nm, P value = 0.241; at Q3, P value = 0.029. In sorbitol, at 0–10 nm, P value = 0.329; at Q3, P value = 0.4765. In SDS, at 0–10 nm, P value = 0.9675; at Q3, P value = 0.5594. 

c, Model-simulated average turgor-pressure change (ratio between aneuploids and haploid cells) based on the combinations of two parameters—the abundance correlation of proteins that form large complexes (above 40 proteins per complex, such as ribosomes (x axis)) and the abundance correlation of proteins that form average complexes (base level (y axis)) across a reasonable range of values (0.7–0.95). Heat maps show the parameter scan without (left) and with (right) adjusting the abundance of large complexes to 20× the base concentration of complexes. 

d, Examples of box plots of simulated turgor pressure ratios (aneuploid and haploid populations, n = 18 and 2, respectively) using 0.85 abundance correlation of proteins that form base or large complexes, without (left) and with (right) the adjustment as in c. Each dot presents a simulated ratio of turgor based on experimental size measurements at each ploidy (see Fig. 3b). Box plots are as in Fig. 2.
Aneuploid cells display cell-wall stress phenotypes owing to hypo-osmotic imbalance. a, Representative electron micrographs of haploid (top) and aneuploid (bottom) cells, and box plots of measured cell-wall thickness showing no significant difference of cell-wall thickness between haploid (n = 15) and aneuploid (n = 15) cells (one-tailed t-test, P value = 0.152; measurements in Source Data). Scale bar, 1 μm. b, Mean intensity of fluorescently labelled concanavalin A on plasma membrane was measured in haploid and aneuploid cells at 0 and 20 min during zymolyase treatment (haploid cells, n = 50 and 31; aneuploids cells, n = 50 and 23, at 0 and 20 min, respectively). The fluorescence intensities were similar between haploid and aneuploid cells at these time points (one-tailed Mann–Whitney U test P value at 0 min = 0.1285 and at 20 min = 0.7286), which suggests rates of cell-wall removal by zymolyase were similar in the two populations. c, Haploid and aneuploid cells were treated with zymolyase to assess the tendency to burst by optical density measurement in normal or hyperosmotic (0.8 M sorbitol) environments. y axis represents the percentage of intact cells relative to time zero of treatment; x axis indicates the time immediately after addition of zymolyase with or without 0.8 M sorbitol. Each dot represents one measurement at each time point (n = 3). Plots show that the hyper-osmotic environment rescued the hypersensitivity of aneuploid cells to zymolyase, as compared to haploid cells. d, Nine individual aneuploid clones were tested for their zymolyase sensitivity, as described in Fig. 2b. Haploid and aneuploid cells are indicated by blue and red, respectively; each dot represents one measurement at each time point (n = 3). The plots show that the zymolyase hypersensitivity was associated with most aneuploid clones. e, Extracellular glycerol concentration was measured to assess the exportation of glycerol (glycerol in the supernatant of the cultures) during a two-hour timespan. Each dot represents one measurement in each culture (n = 3 for both haploid and aneuploid populations; black bar, mean concentration). f, Immunoblots for phosphorylated Slt2, total Slt2 and Pgk1 showed an increased phosphorylation of Slt2 in aneuploid cells, as compared to that in haploid cells. Fold changes of quantified phospho-Slt2 and Slt2 signal intensity between haploid and aneuploid cells were normalized by the Pgk1 signal of each respective population. Representative blot from two independent experiments (Supplementary Fig. 1, raw gel images). Box plots in a and b are as in Fig. 2.
Extended Data Fig. 5 | Electron micrographs of haploid and aneuploid cells used for the quantification of cell-wall thickness. Relates to cell-wall thickness measurements described in Extended Data Fig. 4a. Haploid and aneuploid cells were processed and imaged by using electron microscopy. Each electron microscopy panel was assembled as described in Supplementary Methods. Four measurements of cell-wall thickness were made from one cell in each image. Scale bars, 500 nm.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Aneuploid cells show impaired endocytosis but not general cell-cycle or polarity defects. a, b, Duration (left) and abundance (right, mean intensity of GFP signal) of Abp1 (a) and Sla2 (b) at endocytic patches were measured, comparing aneuploid and haploid cells. Duration and abundance of Abp1 signal are significantly greater in aneuploid cells, compared to haploid cells. Duration, endocytic patches in haploid and aneuploid cells, \( n = 146 \) and 255, respectively, \( P \) value < 2.94 \( \times 10^{-11} \). Abundance, endocytic patches in haploid and aneuploid cells, \( n = 144 \) and 237, respectively, \( P \) value < 2.2 \( \times 10^{-16} \) (one-tailed \( t \)-test). This is consistent with the delay in endocytic invagination observed in aneuploid cells, as the increase in abundance probably results from additional F-actin accumulation. Duration, but not abundance, of Sla2 signal was significantly greater in aneuploid cells. Duration, endocytic patches in haploid and aneuploid cells, \( n = 91 \) and 173, respectively, \( P \) value = 0.002758. Abundance, endocytic patches in haploid and aneuploid cells, \( n = 261 \) and 275, respectively, \( P \) value = 0.863 (one-tailed \( t \)-test). This is again consistent with the delay during endocytic invagination. These results also suggest that the endocytic defect of aneuploid cells was not due to a lack, or unstable localization, of these proteins to actin patches. c, Initial velocity of Abp1 during invagination was measured in single cells. Velocity of Abp1 inward movement in haploid cells is significantly faster than in aneuploid cells (haploid cells, \( n = 12 \); aneuploid cells, \( n = 20 \), \( P \) value = 2.237 \( \times 10^{-6} \) one-tailed \( t \)-test). Box plots in a–c are as in Fig. 2; full data are in Source Data. d, Average distance of Abp1 inward movement from cell cortex in individual aneuploid cells (\( n = 9 \); full data are in Source Data). The plots show average distance, with ribbons representing 95% CI. Abp1 in most aneuploid cells moves along the cortex. e, Net changes of FM4-64 signal intensity (arbitrary units) at plasma membrane and cytoplasm over time (related to Fig. 2e). Haploid cells, \( n = 205 \) and 206; aneuploid cells, \( n = 200 \) and 205 in left and right panels, respectively (full data are in Source Data for Fig. 2). Solid lines represent average fluorescent intensity; grey ribbons represent 95% CI. Bulk rate of FM4-64 internalization (mean ± s.d., per minute). Rate in haploid cells, 7.66 \( \times 10^{-4} \pm 1.49 \times 10^{-3} \); rate in aneuploid cells, 1.87 \( \times 10^{-4} \pm 4.42 \times 10^{-4} \); rate in haploid cells in sorbitol, 4.73 \( \times 10^{-4} \pm 9.35 \times 10^{-4} \); rate in aneuploid cells in sorbitol, 9.73 \( \times 10^{-4} \pm 1.16 \times 10^{-3} \). f, Correlation analysis between cell size (y axis) and cell-cycle profile. x axis, ratio of G1 and G2 peaks from FACS analysis (full data in Source Data) in aneuploid cells (red dots, \( n = 26 \)) (from experimental results in Fig. 3b, bottom). No correlation was found (Spearman’s rank correlation score = −0.037, \( P \) value = 0.856), which indicates that variation in cell-cycle distribution was not associated with the increased cell size in aneuploid populations. The trend line (dashed line) was fitted by linear regression (red dashed line) with grey ribbon (95% CI). g, Representative images of polarized cells with Abp1–GFP as a marker of actin patches, showing the preferential localization at the bud cortex (red arrowheads) indicative of polarized cells. We found that 97.6% of haploid and 93.2% of aneuploid small budded cells (\( n = 42 \) and 44, respectively; bud size roughly equal to or smaller than 1/2 of the mother cell, data collected from three independent experiments) exhibited a polarized actin-patch distribution. Scale bars, 5 \( \mu m \).
Extended Data Fig. 7 | Additional data showing that hypo-osmotic stress leads to increased cell size and endocytic slowdown in both haploid and aneuploid cells. 

a, Left, average distance that Abp1 travelled away from cell cortex under acute exposure to medium containing 0.4 M sorbitol (grey ribbon, 95% confidence intervals; haploid cells, n = 150; aneuploid cells, n = 136). No significant difference in the distance travelled inwardly by Abp1 patches was observed between haploid cells (top) and aneuploid cells (bottom) in this hyper-osmotic environment (Tukey contrasts on generalized linear model (GLM), multiple-testing-corrected P value = 0.0968). Right, duration (top) and abundance (mean intensity of GFP signal, bottom) of Abp1 signal at endocytic patches under acute treatment of 0.4 M sorbitol were measured, comparing aneuploid and haploid cells. The duration of the Abp1 signal at cortex between haploid and aneuploid cells was similar, whereas the abundance of Abp1 immediately before inward traverse remained different between the two groups. Duration, haploid cells, n = 144; aneuploid cells, n = 169; P value = 0.731. Abundance, haploid cells, n = 140; aneuploid cells, n = 121; P value = 3.106 × 10⁻¹⁰ (one-tailed t-test). b, Haploid cells experiencing hypo-osmotic stress (20 min after shifting from growth medium (SC) containing 1 M sorbitol to SC without sorbitol) displayed increased cell size compared to cells continuously growing in medium with 1 M sorbitol (n = 120 in both sets; one-tailed t-test, P value = 3.437 × 10⁻⁹). c, Cell surface stiffness (y axis) was measured with atomic force microscopy in haploid cells under hyper- (left) and hypo-osmotic (right) shock within 30 min of osmotic shift. Box plots show that cell stiffness was significantly different between cells growing in normal (SC) and hyper-osmotic (1 M sorbitol) environments (P value = 0.028, one-tailed t-test; sample size (n) and full data in Source Data), and trended downward or upward after a hyper- or hypo-osmotic shift, respectively. d, Average distance travelled and velocity (centre) of Abp1 from cell cortex (y-axis; ribbon, 95% CI) was monitored over time (x axis) after haploid cells were exposed to hypo-osmotic shock as in a. When cells were transferred from high (1 M sorbitol) to low (SC) osmolarity environment, the average distance travelled and their velocity became shorter (Tukey contrasts on GLM, 1 M sorbitol to SC, P value = 0.00783) and slower (Tukey contrasts on GLM, 1 M sorbitol to SC, P value = 0.0134), respectively (see Source Data for sample size (n)). e, Cell size decreases in haploid and aneuploid cells exposed to hyper-osmotic stress. Cells in haploid (left) and aneuploid populations (right) significantly reduced in size after being shifted from SC to medium with 1 M sorbitol, compared to populations grown continuously in SC medium (for haploid cells, n = 300 and 321; one-tailed t-test, P value < 2.2 × 10⁻¹⁶; for aneuploid cells, n = 151 and 237; one-tailed t-test, P value < 2.2 × 10⁻¹⁶). Full data and exact P values for a–e in Source Data. Box plots in a–c, e are as in Fig. 2.
Extended Data Fig. 8 | Hypo-osmotic stress associated with aneuploidy impairs efficient endocytic internalization in human cells.

**a**, Left, distribution of chromosome counts from metaphase spreads in two different groups: MPS1-inhibitor-treated (red, aneuploid cells) and untreated (blue, diploid cells). The treated population is more-aneuploid than the untreated population (two-sided Fisher exact test, \( P \) value = 0.002688, \( n = 30 \) in both populations) (full data are in Source Data). Right, three example images from the metaphase spreads.

**b**, FACS profiles in transferrin uptake assay (related to Fig. 2f). Three independent experiments were performed to assess uptake efficiency of transferrin in MPS1-inhibitor-treated (bottom, aneuploid) and untreated (top, diploid) cells. Histograms show the distribution of Alexa-Fluor-488-labelled transferrin signals (x axis) in the population (y axis). The same gate was applied to all three experiments to estimate the percentage of the population that was positive for Alexa Fluor 488.
Extended Data Fig. 9 | Genome-wide open reading frame deletion screen for mutations that reduce aneuploid cell fitness. Schematic of the construction of yeast triploid homozygous-deletion collection, and the production of karyotypically heterogeneous aneuploid ORF-deletion populations (see detailed description in Supplementary Methods).
Extended Data Fig. 10 | Aneuploidy prevents endocytic turnover of plasma-membrane transporters. a, Representative images of results quantified in Fig. 4e. Haploid and aneuploid populations with Hxt3–GFP (left) or Hxt4–GFP (middle) were grown with or without glucose. Hxt3–GFP and Hxt4–GFP remained on the plasma membrane after 20 h of glucose depletion in aneuploid, but not haploid cells. Haploid and aneuploid populations with Can1–GFP (right) were grown with or without canavanine. Can1–GFP in aneuploid, but not haploid, cells remained on the plasma membrane after four hours of treatment. Two independent experiments. Scale bars, 5 μm. b, Representative images show Mig1–GFP translocation out of the nucleus after glucose depletion, and the return of Mig1–GFP after glucose repletion, which demonstrates that glucose sensing is not impaired in aneuploid cells compared to euploid cells (three independent images of each condition from two independent experiments). Scale bar, 5 μm.
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☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

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Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Image acquisition softwares used were ImageJ, Nikon NIS Elements AR (v. 4.6), MetaMorph and Zeiss Zen.

Data analysis

Scripts for NGS downstream analysis used published tools bowtie2, HISAT2, samtools, HTseq and R packages DESeq2 and fdrtool. Custom R scripts were written for genetic screen data analysis. Nikon NIS Elements AR & Fiji plugin TrackMate were used for image analyses. FCS analysis was performed on Zen (Zeiss) image analysis software. Igor Pro software (Wavemetrics, USA) was used for analysis of AFM data. In the gene ontology analysis, redundant GO terms were determined using REVIGO. The SimBiology toolbox (Matlab R2016b) was used to perform simulations of the nutrient flux model. Software versions/methods are detailed in manuscript. All custom Unix shell & R scripts are deposited in a github repository and available upon request.

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Accession numbers are noted in the manuscript; codes for modeling are available in a public data server "https://github.com/RongLiLab/Tsai-et-al.-2019".

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Life sciences

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Sample size
Sample size should be large enough for each statistical analysis. Most experiments in this study included sample size (single cell measurement) more than 100. If less than 100, it is because of the nature of technical challenge in specific experiments. Otherwise, the criteria of sample size determination is noted in the manuscript.

Data exclusions
No data was excluded.

Replication
Reproducibility was confirmed

Randomization
This is not relevant to this study, which does not include any patient or clinical assessments.

Blinding
This is not relevant to this study, which does not include any patient or clinical assessments. Otherwise, for image quantifications, the analysis was performed blindly.

Reporting for specific materials, systems and methods

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Unique biological materials

Policy information about availability of materials

Obtaining unique materials

Describe any restrictions on the availability of unique materials OR confirm that all unique materials used are readily available from the authors or from standard commercial sources (and specify these sources).

Antibodies

Antibodies used

Phospho-p44/42 MAPK (Thr202/Tyr204) antibody (Cell Signaling Technology, #9101), Mpk1 antibody (Santa Cruz Biotechnology, E-9) and Pgk1 (Pgk1 antibody, ThermoFisher Scientific, 22C5DB)
### Eukaryotic cell lines

**Policy information about cell lines**
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- **Authentication**: No authentication in this study is required
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- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

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Methodology

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Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth
Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies
Describe the antibodies used for the ChiP-seq experiments, as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters
Specify the command line program and parameters used for read mapping and peak calling, including the ChiP, control and index files used.

Data quality
Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software
Describe the software used to collect and analyze the ChiP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

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Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Sample preparation is described in the manuscript

Instrument
Attune NxT

Software
FlowJo ver 10.3

Cell population abundance
Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy
No pre-gating was applied. The gating for population quantification is noted in the figure.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type
Indicate task or resting state; event-related or block design.

Design specifications
Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

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Acquisition

Imaging type(s)
Specify: functional, structural, diffusion, perfusion.

Field strength
Specify in Tesla

Sequence & imaging parameters
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State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

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Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

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Specify type of analysis:
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- ROI-based
- Both

**Statistic type for inference**
(See Eklund et al. 2016)
Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

**Correction**
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### Models & analysis

| n/a | Involved in the study |
|-----|-----------------------|
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| ☐   | Graph analysis |
| ☐   | Multivariate modeling or predictive analysis |

**Functional and/or effective connectivity**
Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

**Graph analysis**
Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

**Multivariate modeling and predictive analysis**
Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.