Extended data legends

Extended Data Figure 1 | Kinetics of diploidization in mouse haESCs. a, DNA content histogram of haESCs (day 12 after sorting), stained with Hoechst 33342 and analyzed by flow cytometry. DNA content is quantified relative to genome duplication (1n, 2n, and 4n). The corresponding cell-cycle phases of haploid and diploid cells are indicated. **b,** Comparison of cell cycle profiles and DNA content of haESCs stained with Hoechst 33342 and analyzed by flow cytometry at early and late passages following haploid enrichment by FACS. **c,** DNA content histograms of haESCs across passages after sorting. **d,** Quantification of cells based on their DNA content (1n, 2n, and 4n) from the cell cycle profiles shown in c. **e,** Stabilization of the haploid state following extended ESC culture and multiple rounds of 1n cell enrichment by FACS. The haploid state of haESCs maintained over different passages is compared. The passage from initial derivation (p) and the number of 1n enrichments by FACS (s = sorting) are indicated. DNA quantification was performed after 8 passages from the last sorting event. **f,** Morphology of haploid and diploid ESCs cultured on gelatin-coated wells. Top, brightfield images showing colony distribution (scale, 50μm). Bottom, magnified view of a single colony (scale, 20μm).

Extended Data Figure 2 | Supplementation of TCA cycle substrates does not promote stabilization of the haploid state. a, Differential expression analysis of genes involved in citrate, aconitate, and β-CG metabolism. Bar plots show average fold changes relative to the mean expression in diploid ESCs. Results were normalized to five internal reference genes (β-Actin, Eif4a, Dnmt1, Rrm2, and Tbp). P-values were calculated using Welch's modified unpaired t-test (ns > 0.05, * < 0.05, **< 0.01).**b,** Comparison of cell cycle profiles and DNA content of haESCs stained with Hoechst 33342 and analyzed by flow cytometry supplemented for 6 days with the indicated TCA compounds. **c,** Quantification of 1n fraction of cells supplemented with indicated TCA components for 6 and 12 days. Two biological replicates are shown (n = 2).

Extended Data Figure 3 | Comparative 13C Metabolic Flux Analysis Reveals Conserved TCA Cycle Dynamics in Haploid and Diploid ESCs. a, Schematic representation of glycolysis and the TCA cycle. **b**, Absolute quantification (detected ion intensity) of the indicated metabolites in haploid, diploid, and diploidized ESC lines

(n = 3). **c–l**, Bar plots showing the fractional labeling patterns (proportion of 13C-labeled metabolites) of isotopes in glycolysis and TCA cycle intermediates after 24-hour incubation in medium supplemented with 25 mM 13C-glucose. Metabolic profiling of haploid, diploid, and diploidized ESCs (n = 3) is compared to unlabeled controls

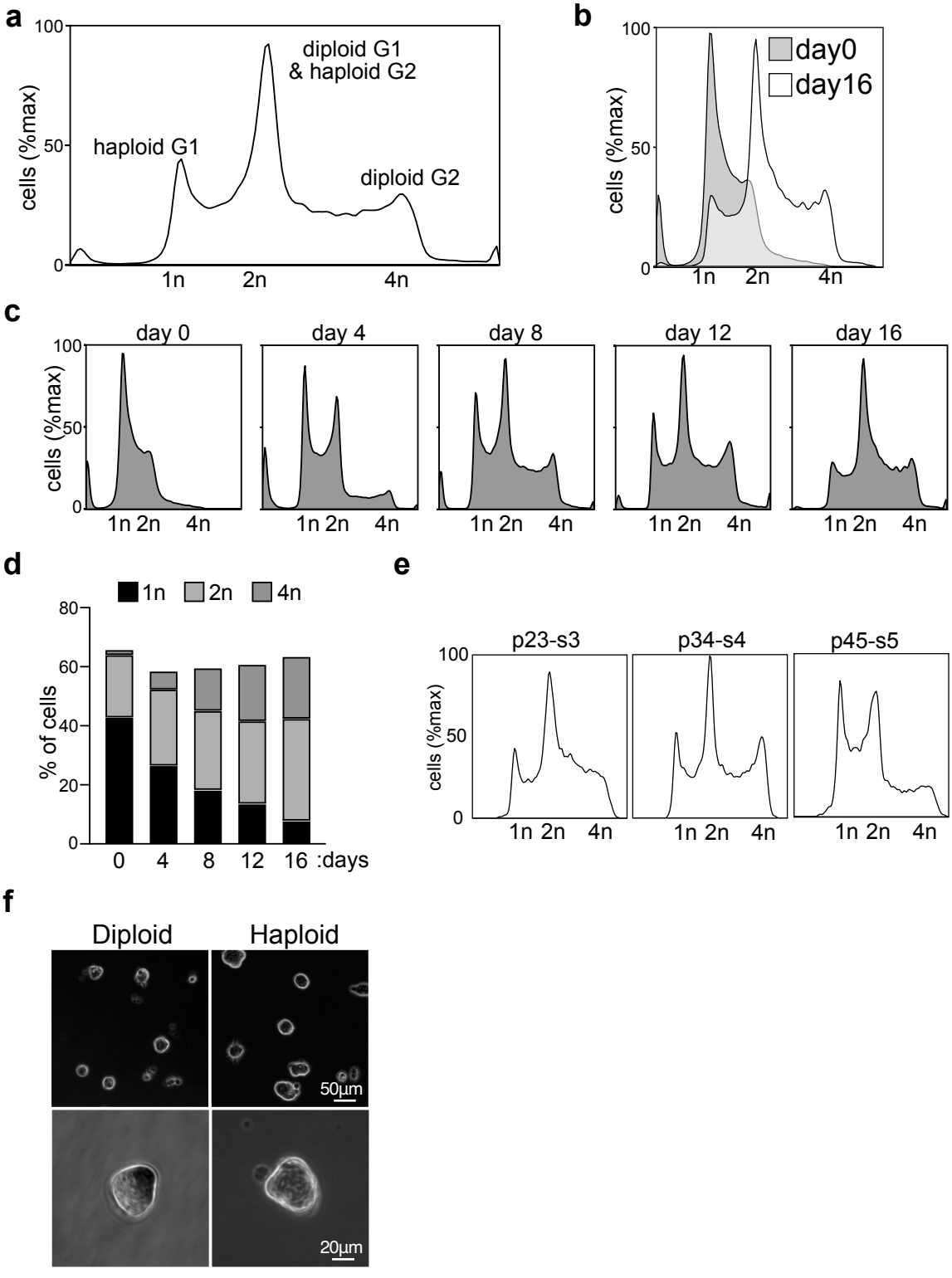
Extended Data Figure 4 | Haploid ESCs exhibit higher mitochondrial density and perturbed activity. a, Mitochondrial abundance in haploid and diploid ESCs, quantified by flow cytometry using MitoTracker Green FM staining. Data are presented as violin and box plots, showing the distribution of fluorescence intensity in haploid ESCs relative to diploid samples (n = 2). **b**, Mitochondrial membrane potential in haploid and diploid ESCs, assessed by flow cytometry using MitoTracker CMXRos. Data are presented as violin and box plots, showing the distribution of fluorescence intensity in haploid ESCs normalized to diploid samples (n = 2). CCCP-treated cells serve as a depolarization control. **c**, Mitochondrial proton leak kinetics measured using the Seahorse XF Mito Stress Test. Replicates and comparable time points are grouped for haploid and diploid ESCs.

Extended Data Figure 5 | Haploid ESCs show increased of NADPH/NADP+ ratio. **a**, Haploid and diploid ESCs show a similar proliferation rate. Bar plot showing proliferation rate of diploid, haploid, and diploidized ESCs plated and grown for 2 days. Results are normalized to the mean of diploid cells. **b**, Ceramide abundance in metabolomic analysis. Data are presented as relative abundance, normalized to all detected metabolites in the metabolomic analysis. P-values were calculated using Welch's modified unpaired t-test (ns > 0.05 and **< 0.01). **c**, Myriocin promotes haploid diploidization. Haploid ESCs were treated with Myriocin (10μM) for 4 days. DNA content histograms of Hoechst33342-stained cells analyzed by flow cytometry.

Extended Data Figure 6 | NADPH affects Aurora kinases phosphorylation during mitosis of Haploid ESCs. a, Differentiating haploid ESCs show decreased Aurora A/B phosphorylation during prometaphase. Quantification of Aurora A/B phosphorylation staining per prometaphase. Statistical significance was determined using Welch's unpaired t-test (ns > 0.05, **< 0.01). **b,** Aurora A/B phosphorylation staining during prometaphase in haploid cells with and without mitoSTH/NOX expression. Chromosomes were stained with DAPI.

Extended Data Figure 7 | Chemical decrease of mitochondrial oxidative stress improves haploid stability. a, Effects of FCCP, mitoTEMPO and resveratrol of haploid ESC stability. Cells were treated as indicated for 6 passages. Representative DNA content histograms, with the fraction of cells displaying 1n and 4n DNA content in the untreated sample indicated. DNA content was assessed using Hoechst 33342 staining and flow cytometry. **b,** haESCs treated with mitoTEMPO (mT) and resveratrol (R) show increased haploid fraction upon multiple passages. Cells were grown with or without mT+R combination for 6 passages. Representative DNA content histograms, with the fraction of cells displaying 1n and 4n DNA content in the untreated sample indicated. DNA content was assessed using Hoechst 33342 staining and flow cytometry.

Extended data 1



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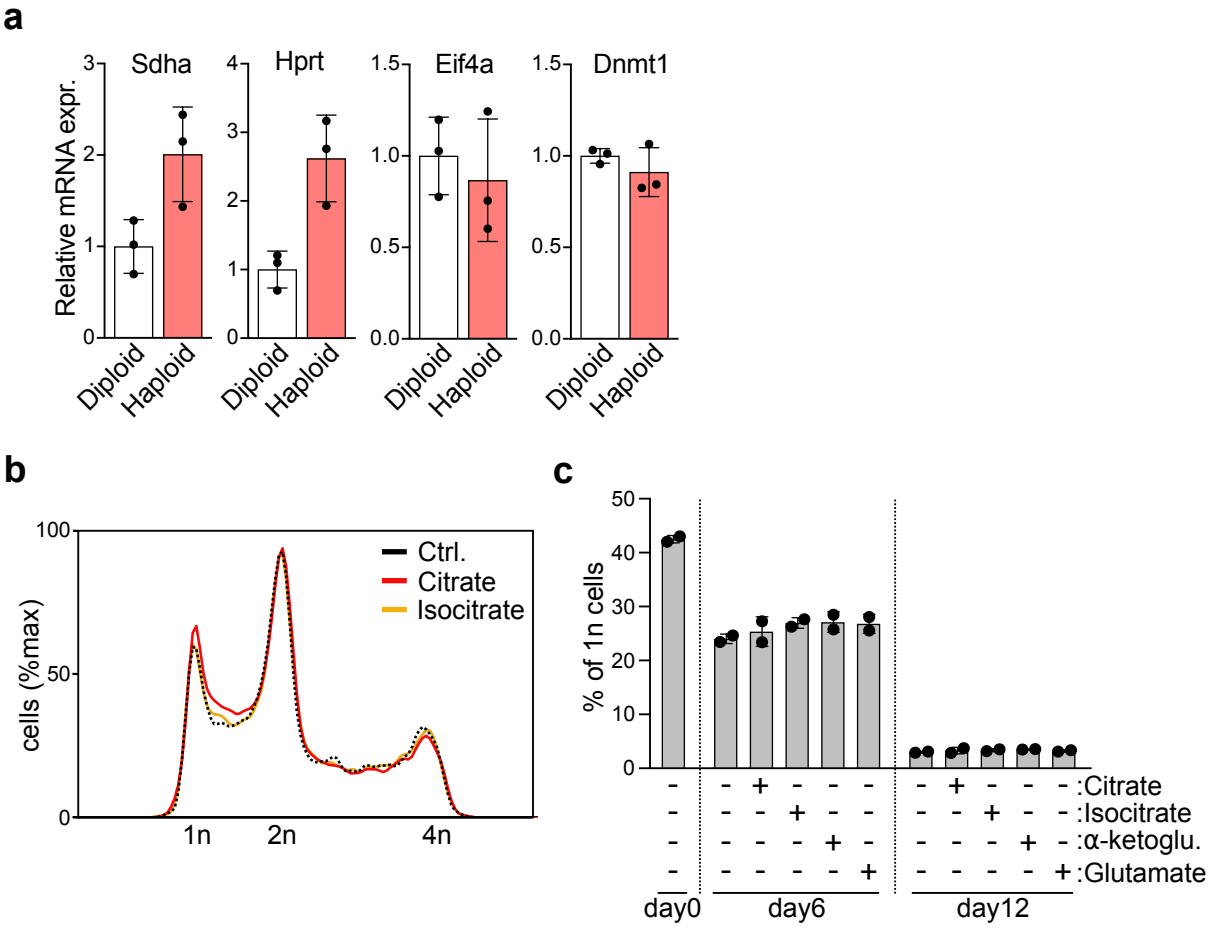

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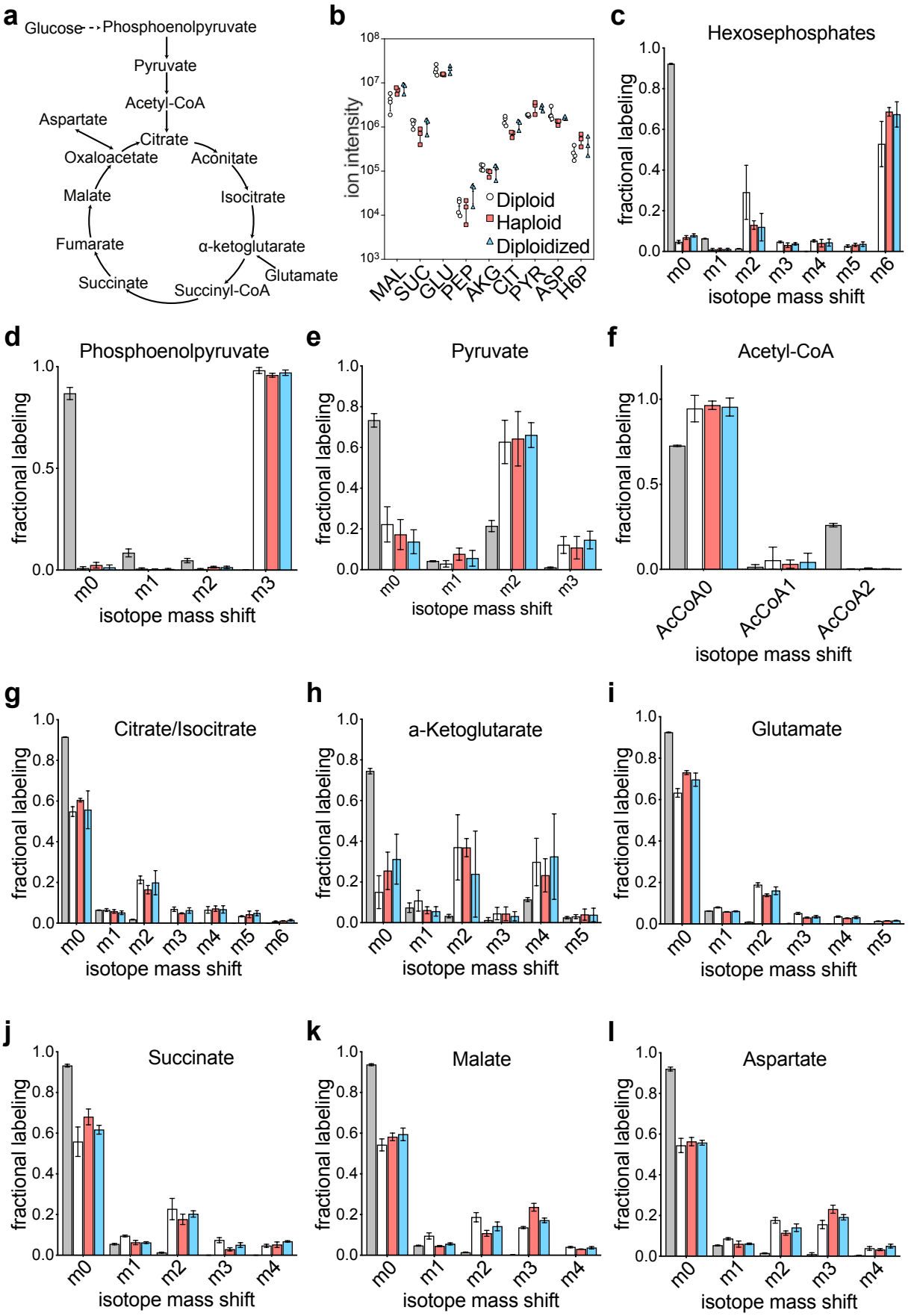

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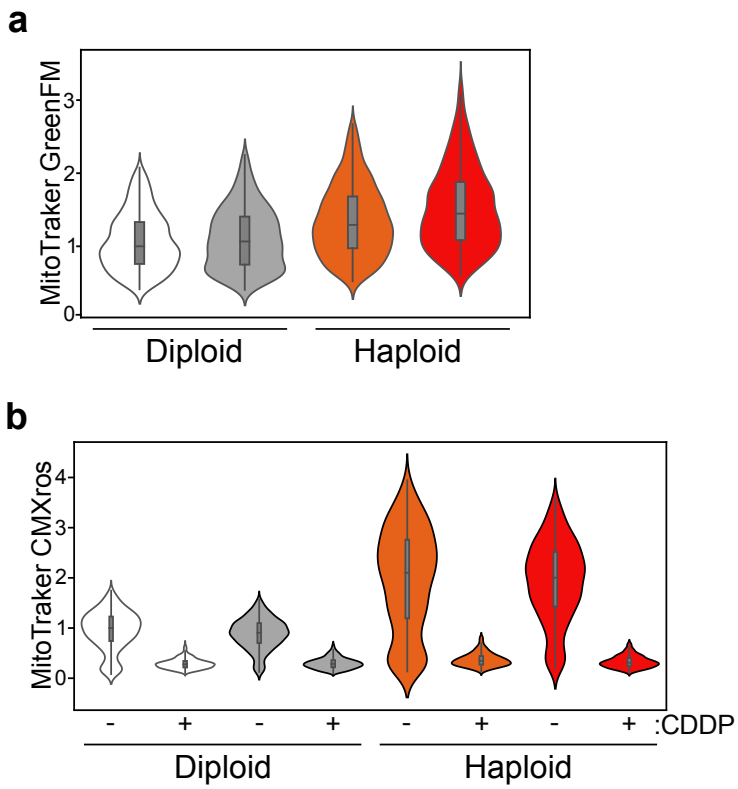

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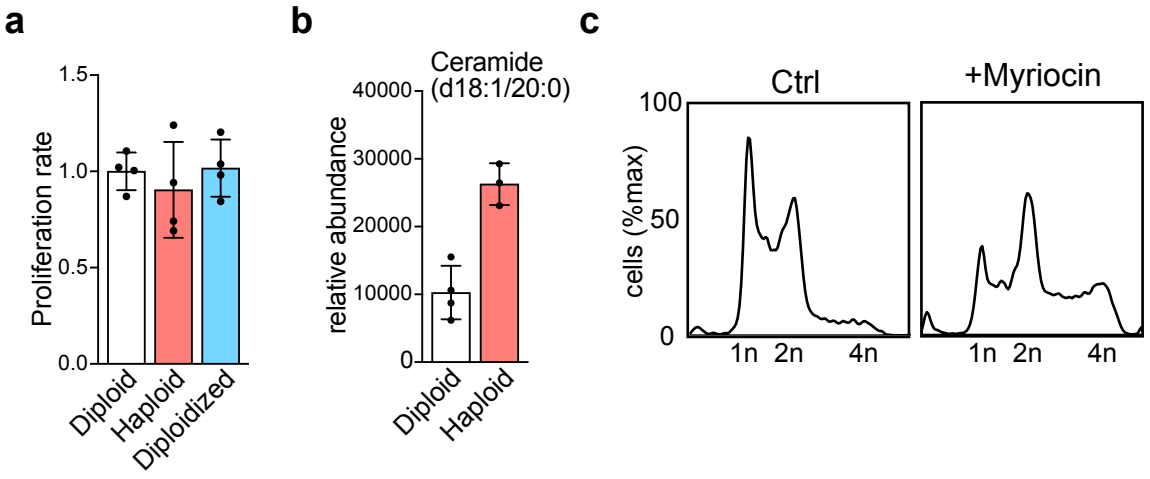

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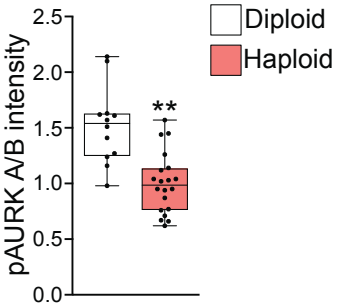

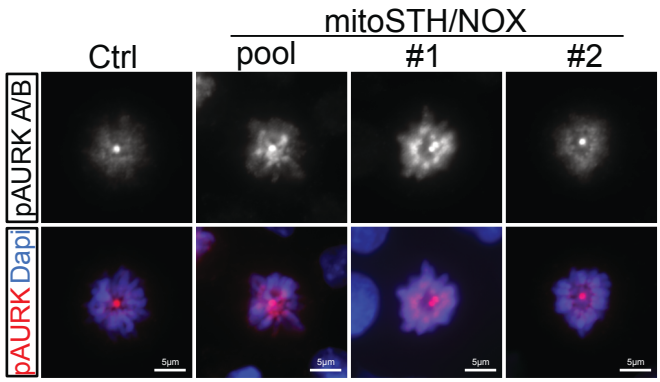

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