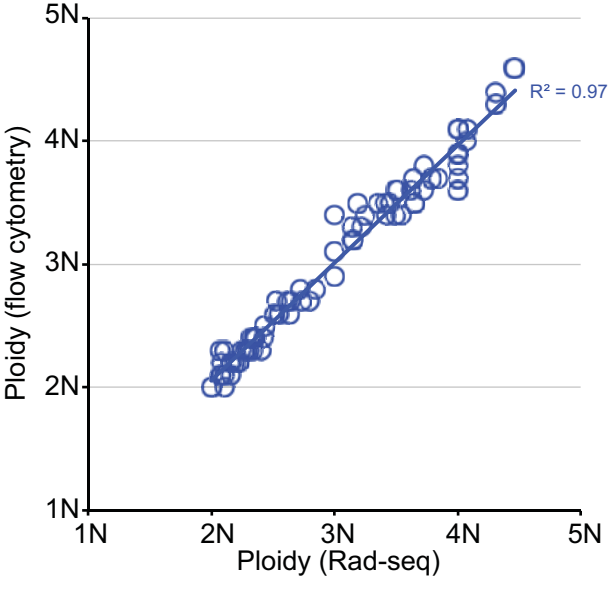

Figure S1. Correlation between ploidy estimates as determined by flow cytometry (y-axis) or ddRAD-seq analysis.

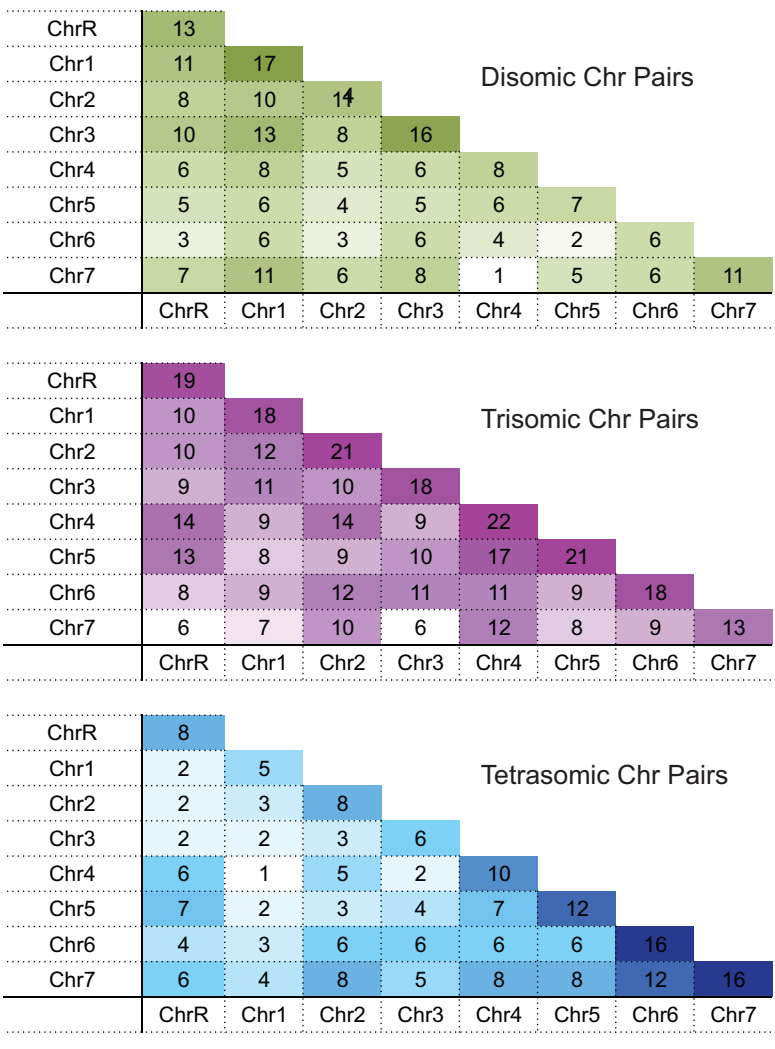

Figure S2. Frequency of balanced chromosomes in disomy, trisomy and tetrasomy. The number of isolates that have balanced chromsome pairs in either disomy (green), trisomy (purple) or tetrasomy (blue). The number indicated on the diagonal represents the total number of isolates of that copy number and chromosome. All other numbers represent the frequency in which that specific chromosome pair (the intersection of a particular row and column) is balanced, such that a single highly aneuploid strain can be represented multiple times.

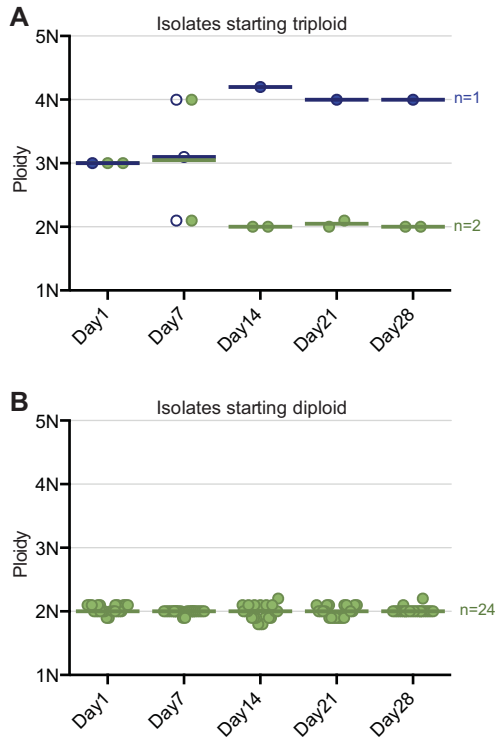

Figure S3. Ploidy dynamics of triploid and diploid isolates. Isolates that were initially **A)** triploid (tetraploid LOH-derived from YJB12712 (n=2) and YJB12779 (n=1)) or **B)** diploid (diploid LOH-derived from DSY919 (n=24)) were serially transferred daily in liquid YPD for 28 days. Flow cytometry for ploidy assessment was performed for each isolate on Days 7, 14, 21 and 28. Ploidy values for Day1 are the same data presented in Figures 1 and 2. Isolates are colored by ending ploidy: diploid (green) or tetraploid (blue). Isolates with only a single ploidy are displayed as filled circles and isolates with multiple ploidy subpopulations are displayed as open circles. The median for each class at every timepoint is indicated with a bar.

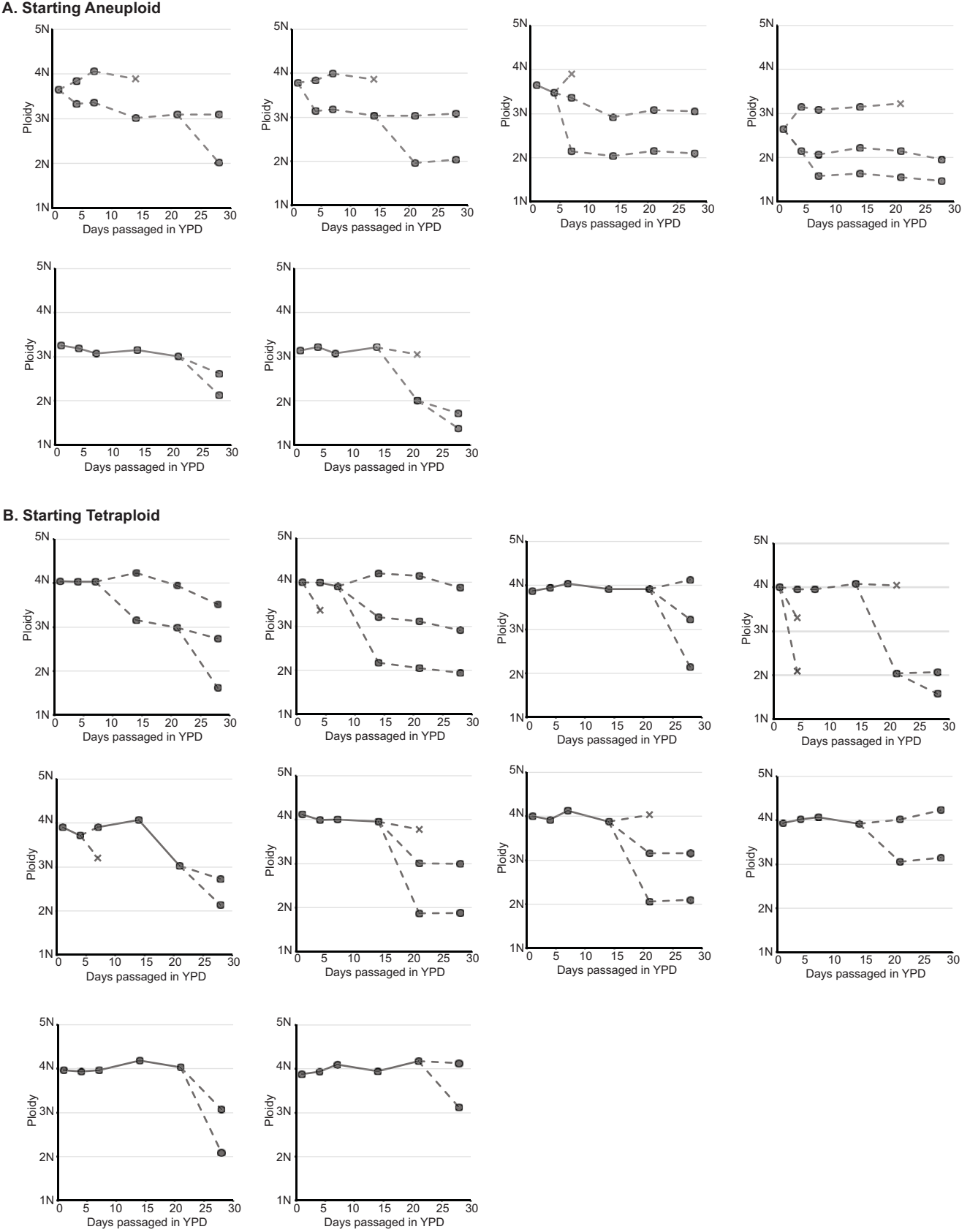

Figure S4: Ploidy trajectories of individual isolates that on Day28 had multiple ploidy populations. **A)** Isolates that were initially aneuploid (n=6). **B)** Isolates that were initially tetraploid (n=10). Ploidy was assessed after 4, 7, 14, 21 and 28 days of serial transfers in liquid YPD. Data for individual isolates across the passaging experiment are represented by circles and connected with lines. Dashed lines indicate timepoints with multiple ploidy subpopulations and X's represent ploidy subpopulations that became extinct (were not observed in subsequent passages of the same individual culture).

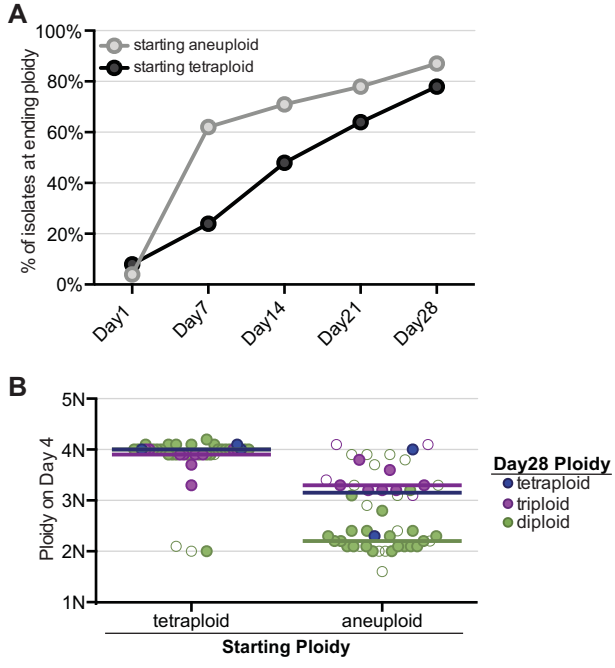

Figure S5. Timing of ploidy resolution in tetraploid and aneuploid isolates. A) The percentage of isolates that had reached their 'ending ploidy' (defined as Day28 ploidy), throughout the timecourse of the passaging experiment. Isolates that ended with multiple ploidy subpopulations (Figure S2) are included in this metric. B) Ploidy values of isolates on Day4. Isolates are colored by their ultimate ending ploidy: diploid (green), triploid (purple) or tetraploid (blue). Isolates with only a single ploidy are displayed as filled circles and isolates with multiple ploidy subpopulations are displayed as open circles. The median for each class is indicated with a bar of corresponding color.

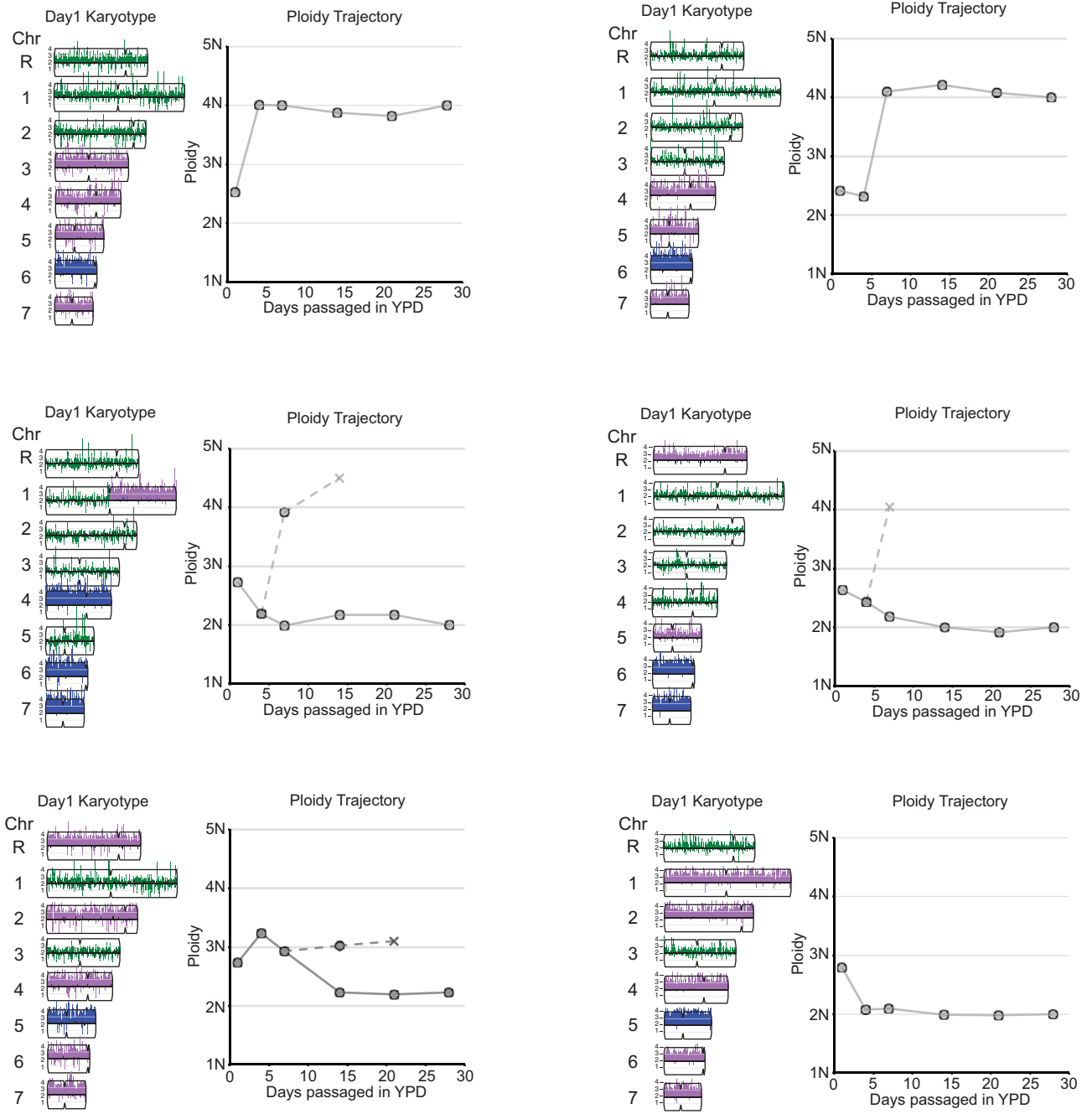

Figure S6. Long-term ploidy dynamics of complex aneuploid isolates. Six isolates displayed complex aneuploid karyotypes with di-, tri-, and tetrasomic chromosome combinations (left). Individual ploidy trajectories of the 28-day passaging experiment are shown on the right. Ploidy was assessed after 4, 7, 14, 21 and 28 days of serial transfers in liquid YPD. Data for individual isolates across the passaging experiment are represented by circles and connected with lines. Dashed lines indicate timepoints with multiple ploidy subpopulations and X's represent ploidy subpopulations that became extinct (were not observed in subsequent passages of the same individual culture).

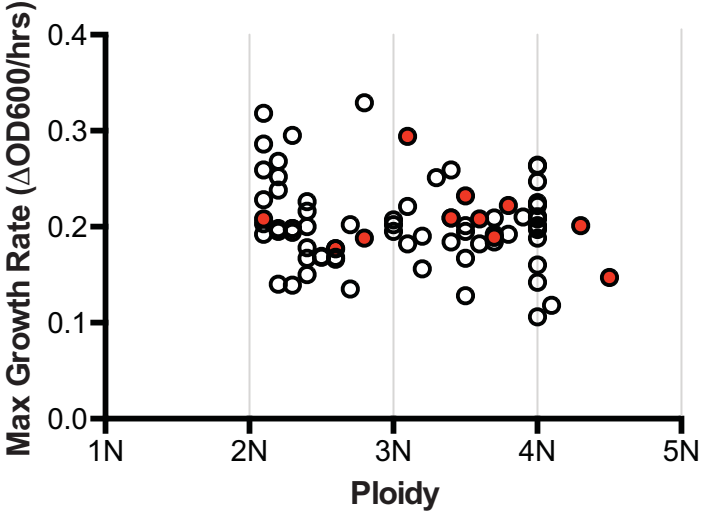

Figure S7: YPD growth rates on Day1. XY scatterplot of Day1 ploidy (x-axis) and corresponding growth rate in YPD (y-axis) for each isolate. Isolates that were subject to LOH selection (growth on 2-DOG) are open circles and isolates that were NOT selected for LOH (growth on YPD) are red filled circles.

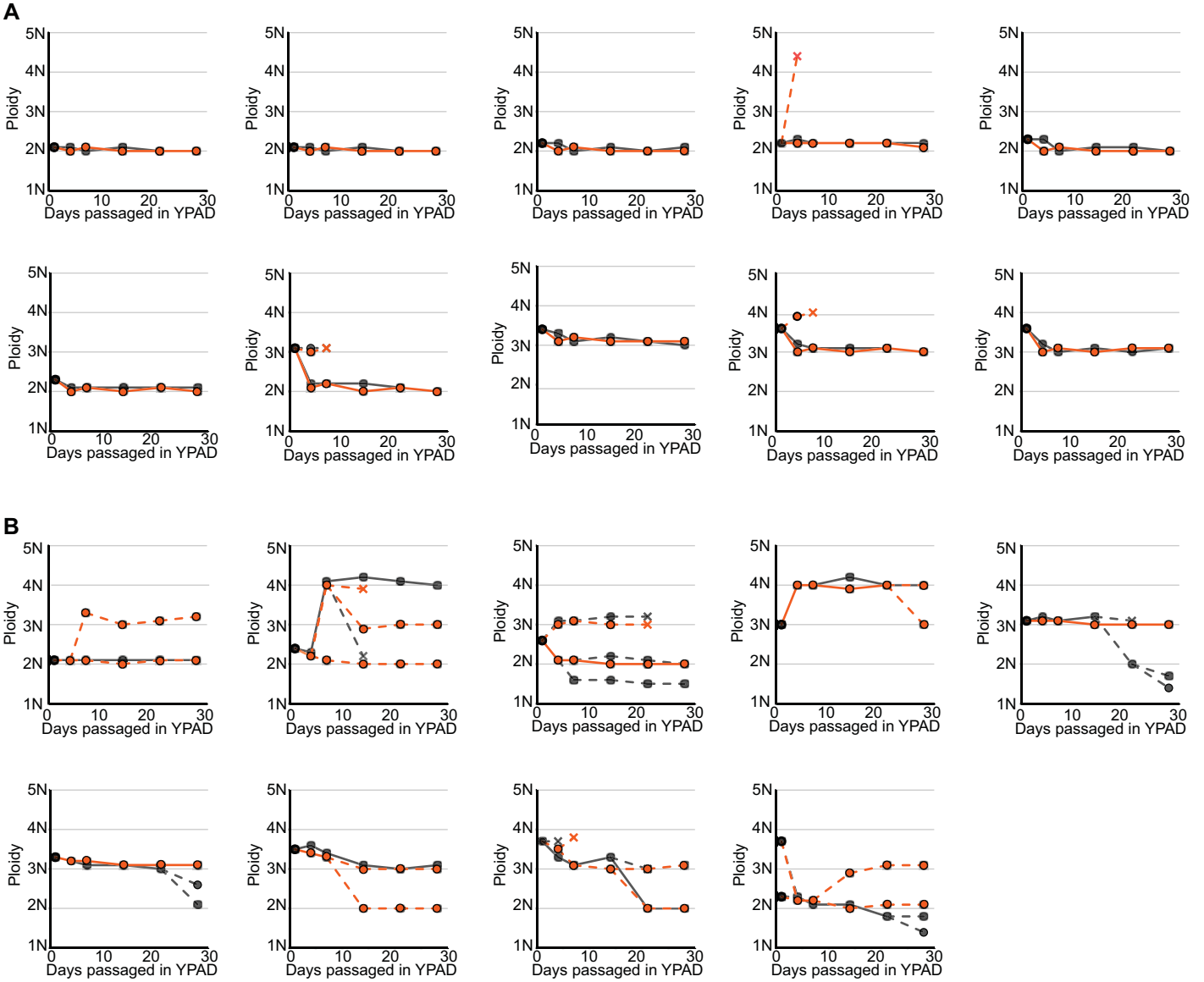

Figure S8. Ploidy potential of aneuploid isolates. Plots of ploidy trajectories of the same aneuploid YJB12779-derived isolates that were independently passaged twice. A) Isolates in which both passaging iterations followed similar ploidy trajectories and reached similar ploidy resolution (n=10). B) Isolates in which the two passaging iterations resulted in different ploidy trajectories and and ploidy resolutions (n=9). Data for a single isolate across the passaging experiment are represented by circles and connected with lines. Dashed lines indicated individual cultures with mixed ploidy subpopulations and X's represent discontinued ploidy subpopulations.
Black: Experiment 1; Orange: Experiment 2.

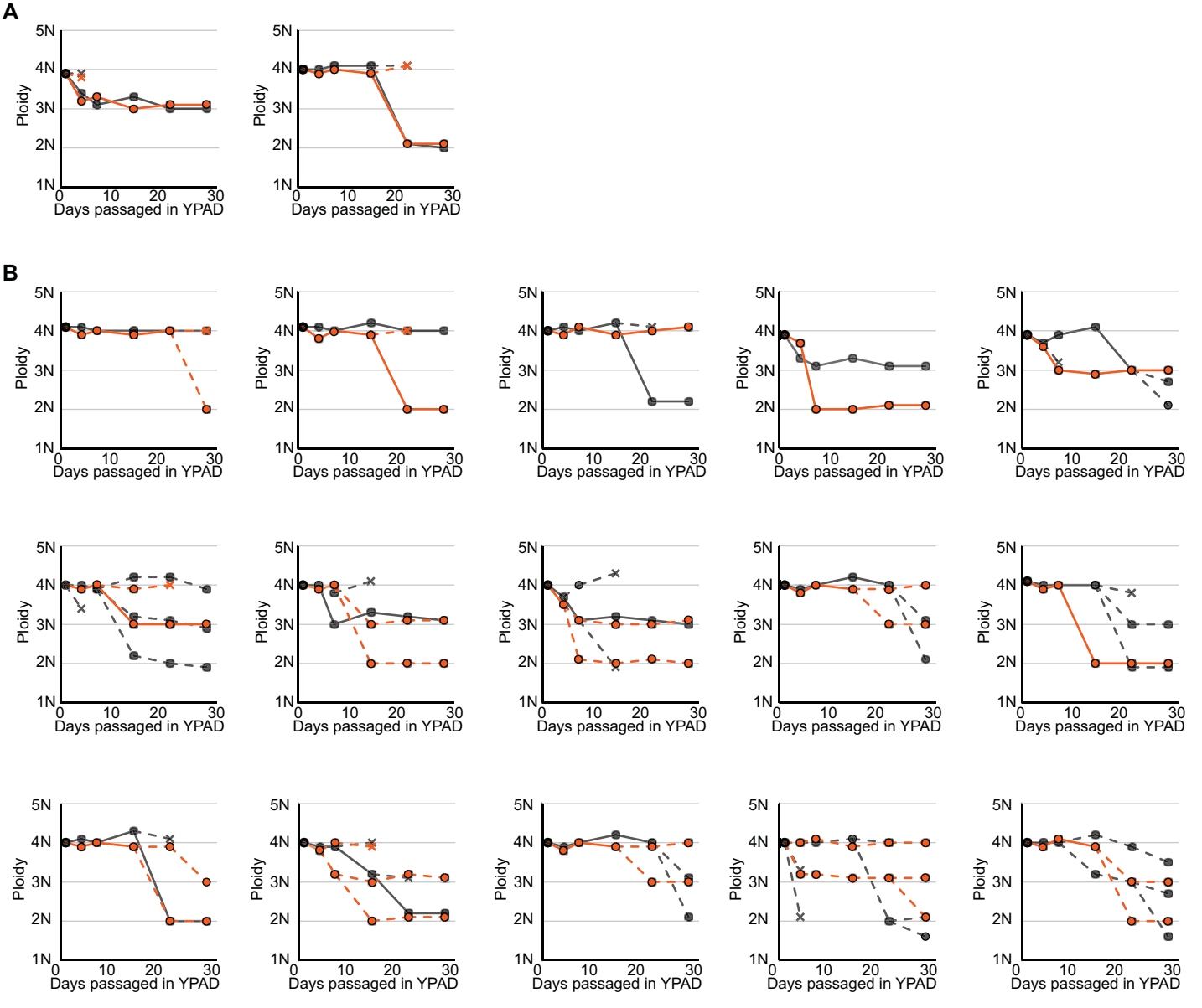

Figure S9. Ploidy potential of tetraploid isolates. Plots of ploidy trajectories of of the same tetraploid YJB12779-derived isolates that were independently passaged twice. A) Isolates in which both passaging iterations followed similar ploidy trajectories and reached similar ploidy resolution (n=2). B) Isolates in which the two passaging iterations resulted in different ploidy trajectories and and ploidy resolutions (n=15). Data for a single isolate across the passaging experiment are represented by circles and connected with lines. Dashed lines indicated individual cultures with multiple ploidy subpopulations and X's represent discontinued ploidy subpopulations. Black: Experiment 1; Orange: Experiment 2.

Table S1 Strains used in this study

Strain	Genotype
Tetraploids	
YJB12712	*MTLα/α/a/Δ; gal1Δ/gal1Δ/gal1Δ/GAL1; ura3Δ/ura3Δ/URA3/URA3 his- leu- ENO1-GFP:NAT/ENO1/ENO1/ENO1*
YJB12651	*MTLa/α/Δ/Δ; gal1Δ/gal1Δ/GAL1/GAL1; ura3Δ/ura3Δ/URA3/URA3; ade2Δ/ade2Δ/ADE2/ADE2; ENO1-GFP:NAT/ENO1-RFP:NAT/ENO1/ENO1*
YJB12779	*MTLa/a/α/Δ; gal1Δ/GAL1/GAL1/GAL1; ade2Δ/ade2Δ/ADE2/ADE2 his- leu-*
RBY18	*MTLα/a/Δ/Δ; gal1Δ/gal1Δ/GAL1/GAL1; ura3Δ/ura3Δ/URA3/URA3; ade2Δ/ade2Δ/ADE2/ADE2*
Diploids	
CHY477	*MTLα/Δ; ade2Δ/ade2Δ*
DSY917	*MTLa/a; gal1Δ/GAL1; his- leu-*
DSY919	*MTLα/α; gal1Δ/GAL1; his- leu-*
YJB12334	*MTLa/Δ; gal1Δ/gal1Δ/; ura3Δ/ura3Δ; ENO1-GFP:NAT/ENO1*
YJB12552	*MTLα/Δ; ade2Δ/ade2Δ ENO1-RFP:NAT/ENO1*