Supplementary Information Guide:

a) **Supplementary Figure 1.** *1L* and *2L* rate analysis in two additional genetic backgrounds.

b) **Supplementary Figure 2.** Genomic analysis of S288c/YJM789 *1L* and *2L* clones.

c) **Supplementary Table 1.** Yeast strains used in this study.

d) **Supplementary Table 2.** Plasmids used in this study.

e) **Supplementary Table 3.** Sequencing and copy number analysis of rough Ura- CANR S288c/JAY291 clones.

f) **Supplementary Table S4.** Sequencing and copy number analysis of *1L* and *2L* S288c/JAY291 clones.

g) **Supplementary Table S5.** Sequencing and copy number analysis of *1L* and *2L* S288c/YJM789 clones.

h) **Supplementary Table S6.** Analysis of the frequency of sequenced clones possessing unselected CCNAs.

i) **Supplementary Table S7.** Proportion of unselected CCNAs affecting each chromosome.

j) **Supplementary Table S8.** Rates of *1L* and *2L* chromosome loss calculated using fluctuation analysis.

**Figure S1. *1L* and *2L* rate analysis in two diverged genetic backgrounds. (A)** Empirically derived rates of chromosome loss for each *1L*-selection (yellow) and *2L*-selection (yellow striped) in an isogenic S288c/S288c background. Black lines denote *theoretical 2L* rate predictions. Fold change between *theoretical 2L* rates and empirically derived *2L* rates (black lines vs. yellow striped) are noted. **(B)** Empirically derived rates of chromosome loss for each *1L*-selection (yellow) and *2L*-selection (yellow striped) in the hybrid S288c/YJM789 background. Black lines denote *theoretical 2L* rate predictions. Fold change between *theoretical 2L* rates and empirically derived *2L* rates (black lines vs. yellow striped) are noted.

**Figure S2. Genomic analysis of S288c/YJM789 *1L* and *2L* clones.**

**(A)** Percentage of *1L* and *2L* isolates with 0 (white), 1 (black), and ≥2 (yellow) unselected CCNAs. **(B)** Graph depicting the proportion of unselected CCNAs affecting each chromosome. Note that cells were trisomic for Chr12 (12+). **(C)** Karyotypes of the parent strain and all clones containing ≥1 unselected CCNA. For each chromosome, yellow bars denote the S288c homolog and black bars denote the YJM789 homolog. yChr12 is present at two copies. Colored boxes represent denoted karyotypic events.