G3 Genes | Genomes | Genetics

The dynamics of diverse segmental amplifications in populations of *Saccharomyces cerevisiae* adapting to strong selection

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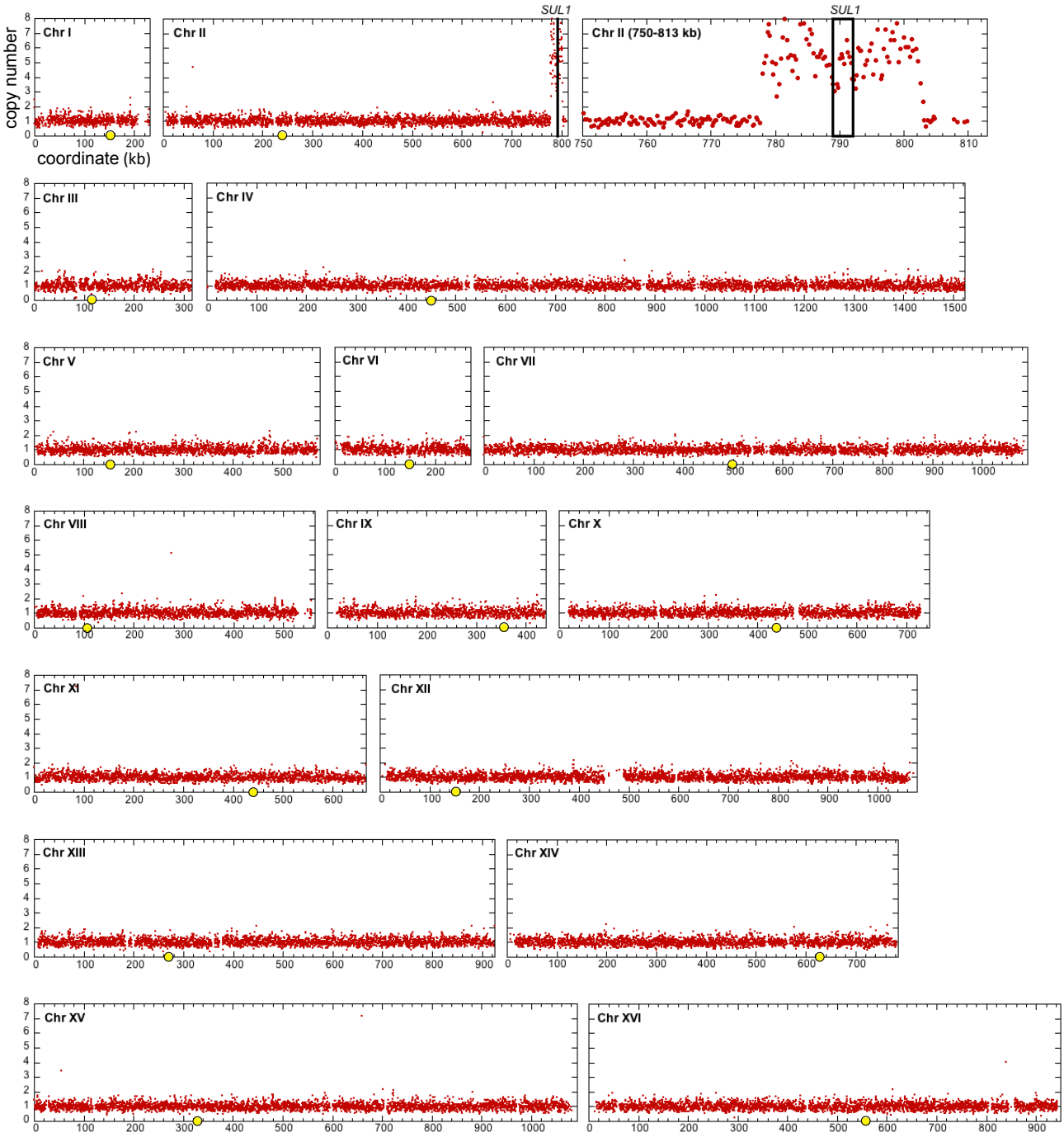
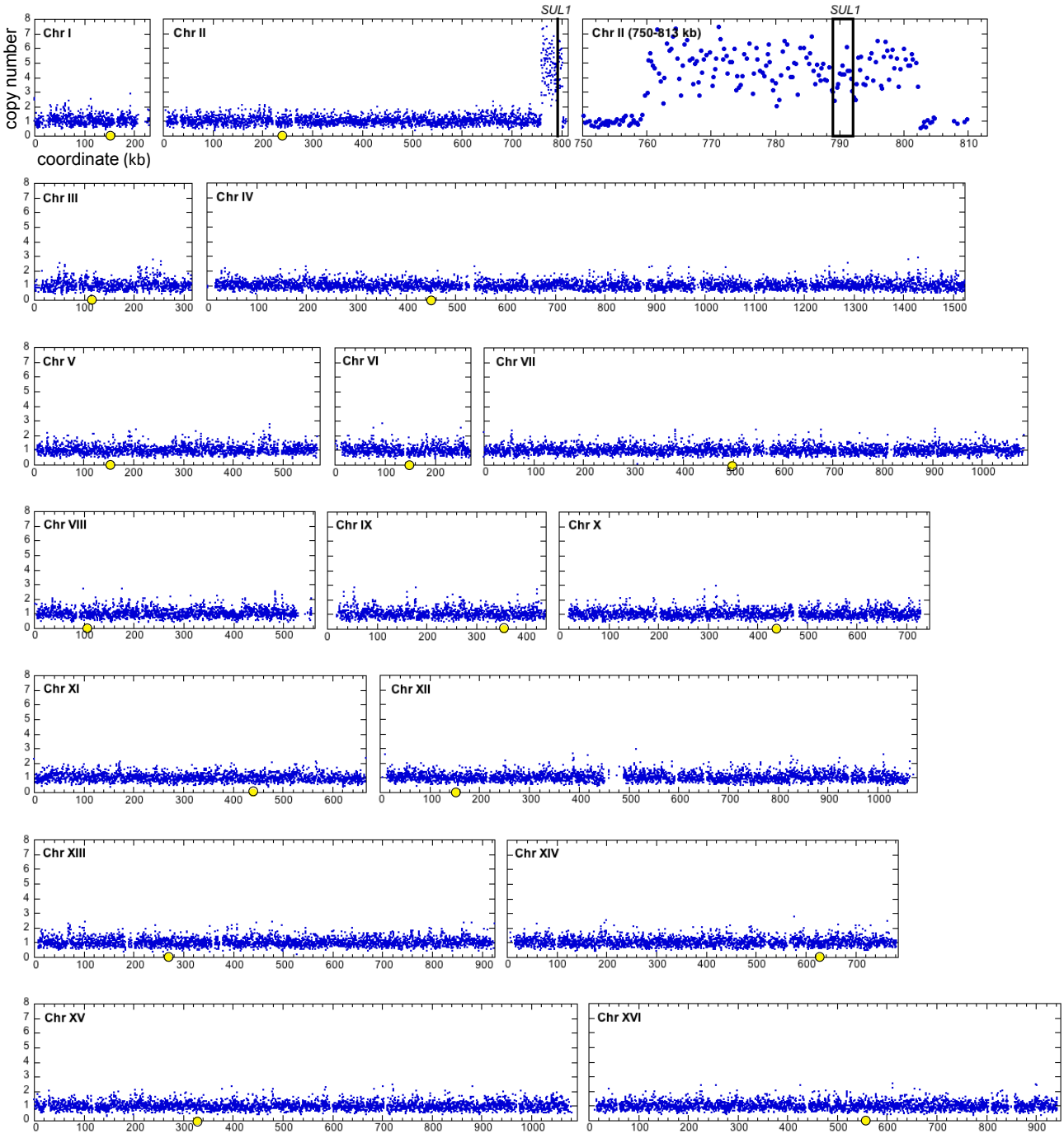
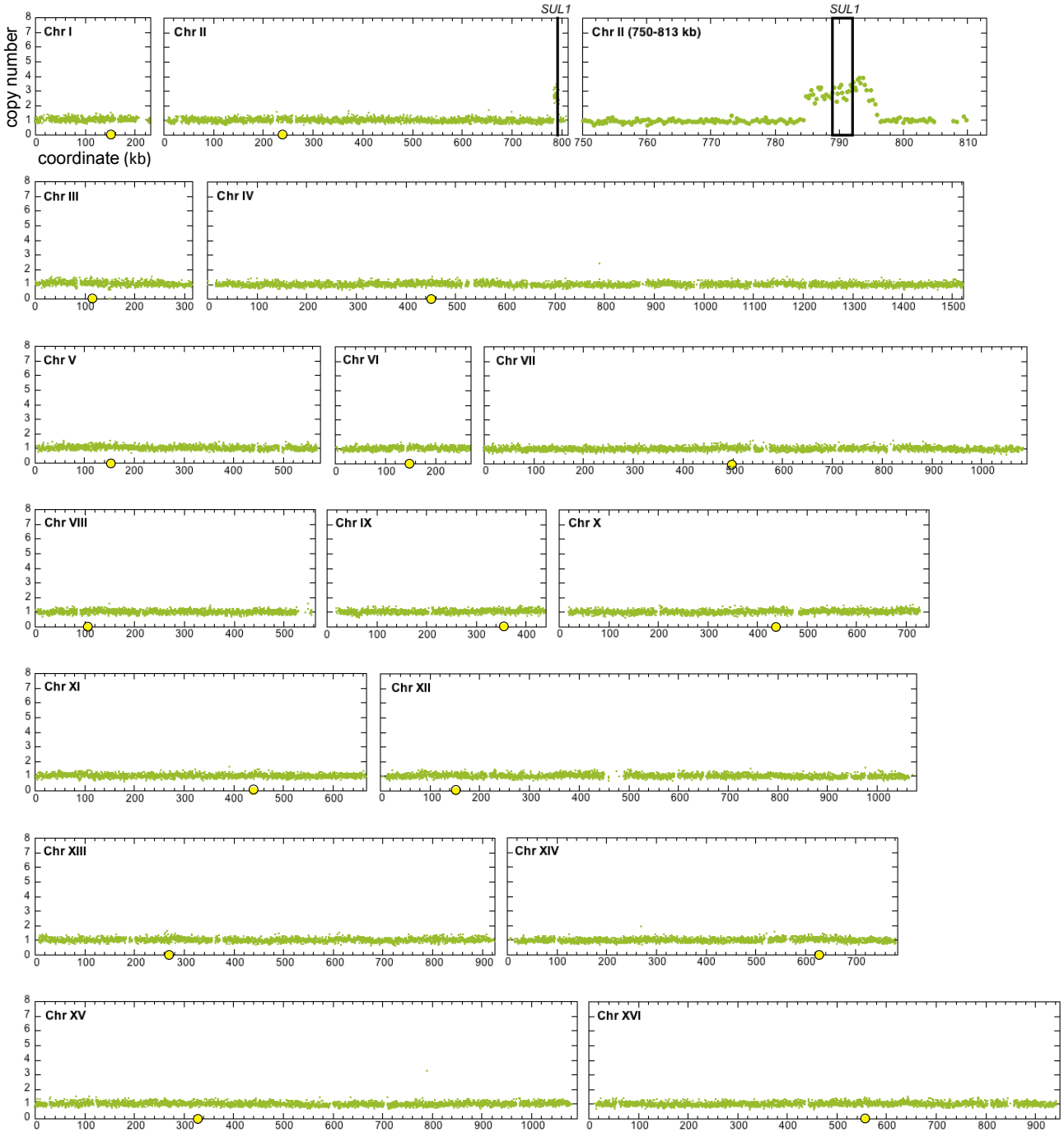
Supplementary Figure 1A: aCGH of Pop2 210 clone1



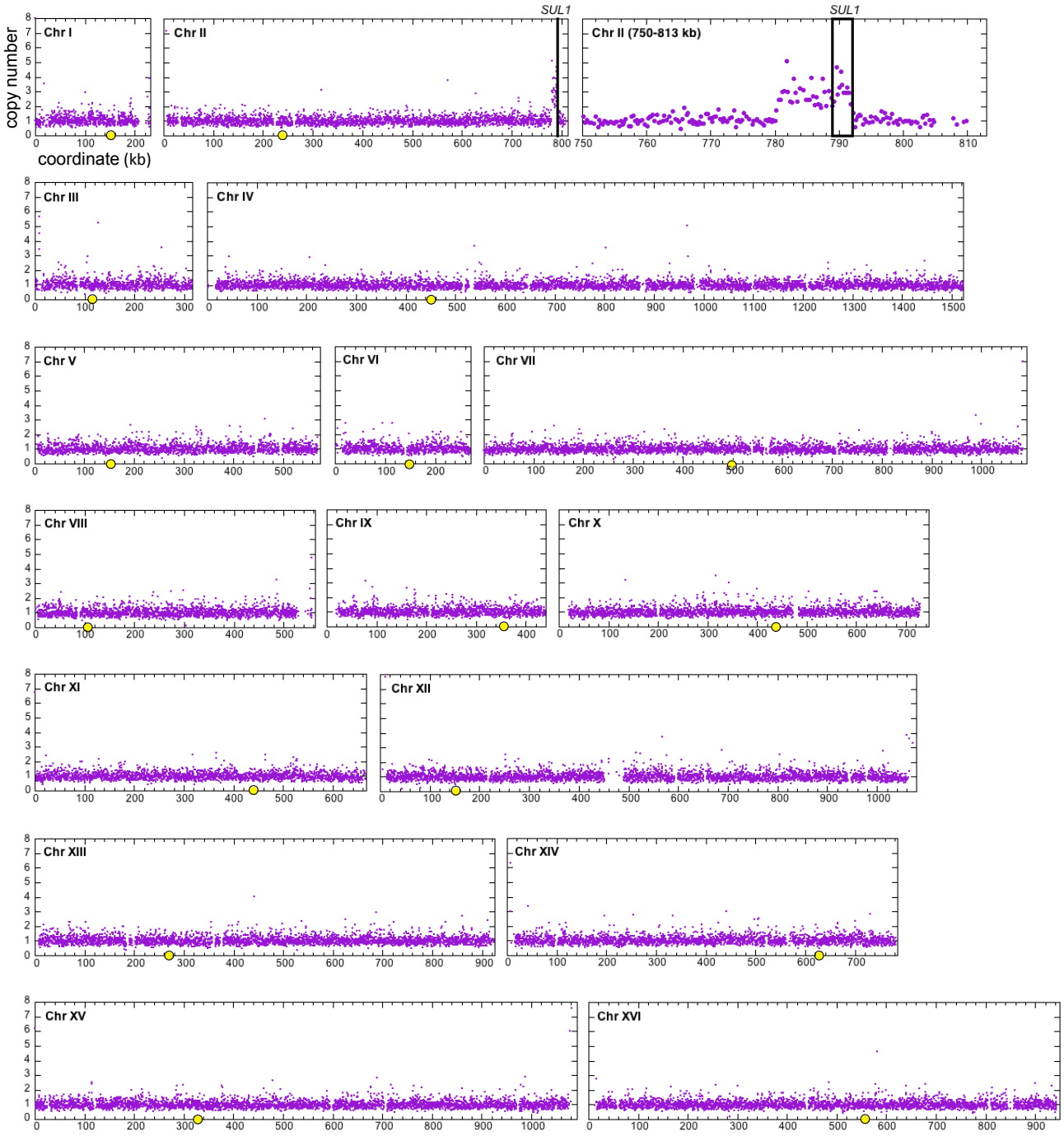
Supplementary Figure 1B: aCGH of Pop3 210 clone1



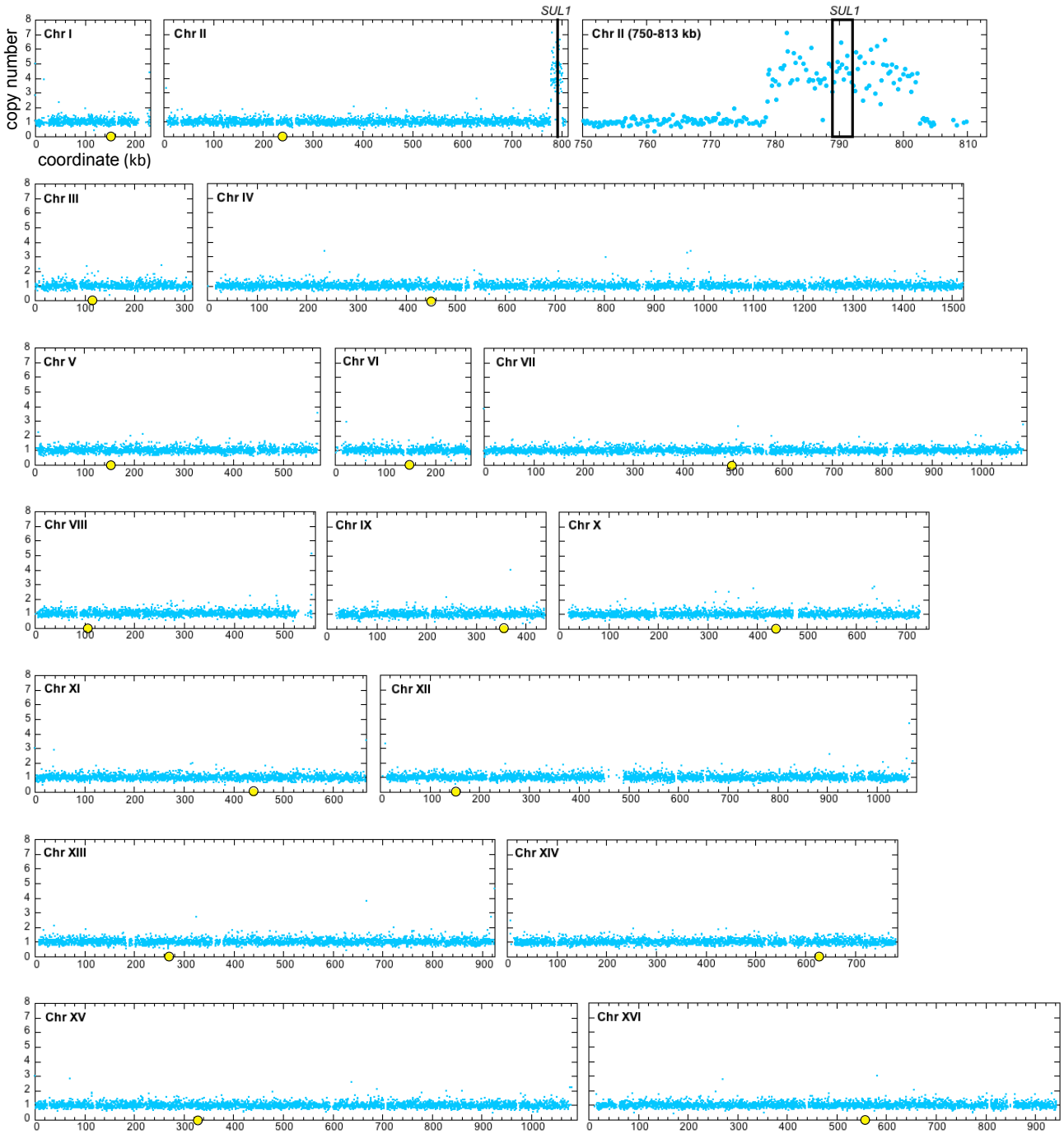
Supplementary Figure 1C: aCGH of Pop4 210 clone1



Supplementary Figure 1D: aCGH of Pop5 209 clone1



Supplementary Figure 1E: aCGH of Pop6 211 clone1



Supplementary Figure 1F: aCGH of Pop7 201 clone1

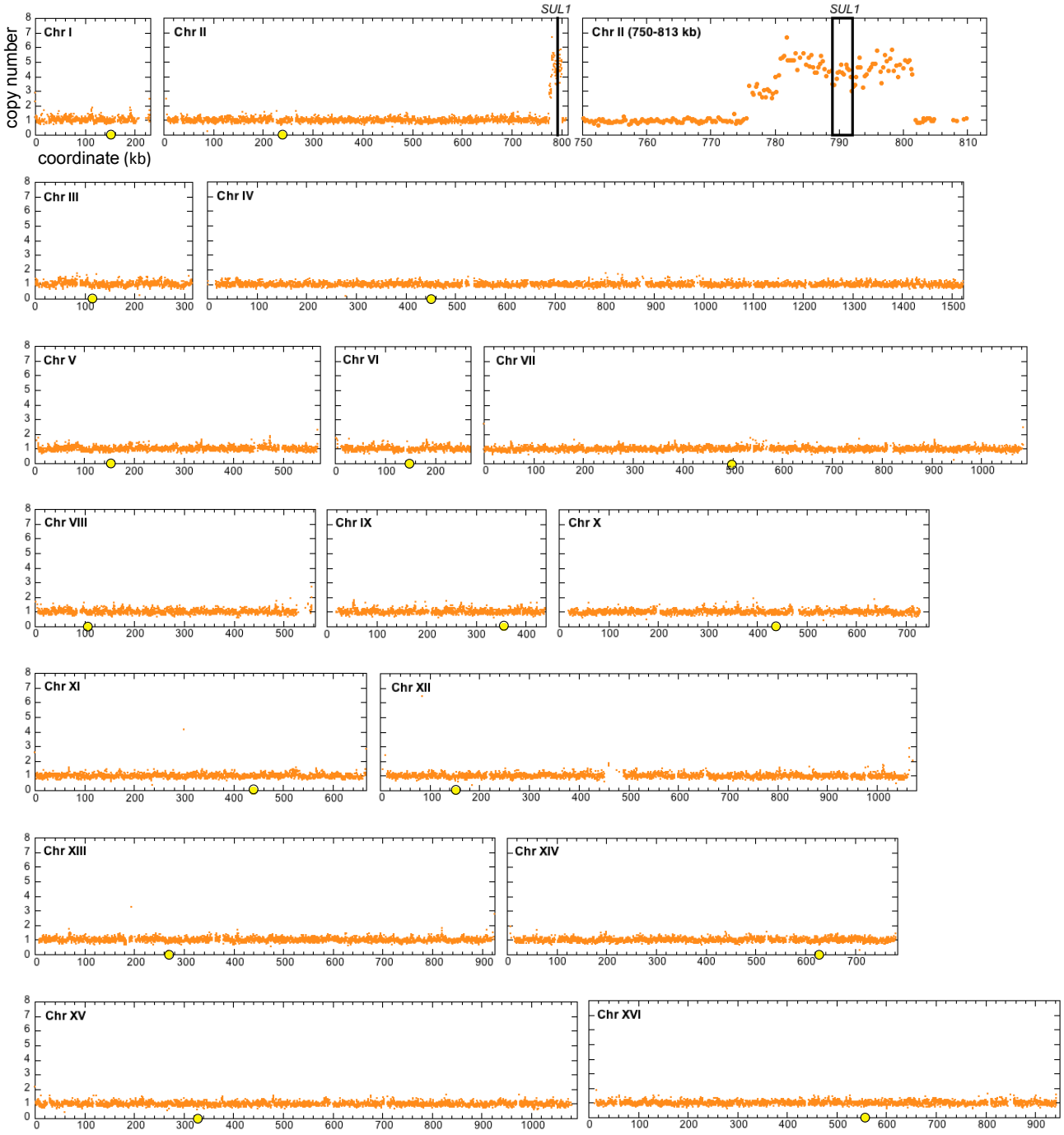


Figure S1 Whole genome copy number data based on aCGH of evolved clones. A to F) Copy number of each probe across the genome is shown for each chromosome in clones from populations 2 through 7. An expanded view of the last ~60 kb of chromosome II is shown to provide higher resolution for the genomic region that contains the *SUL1* locus.

Figure S2

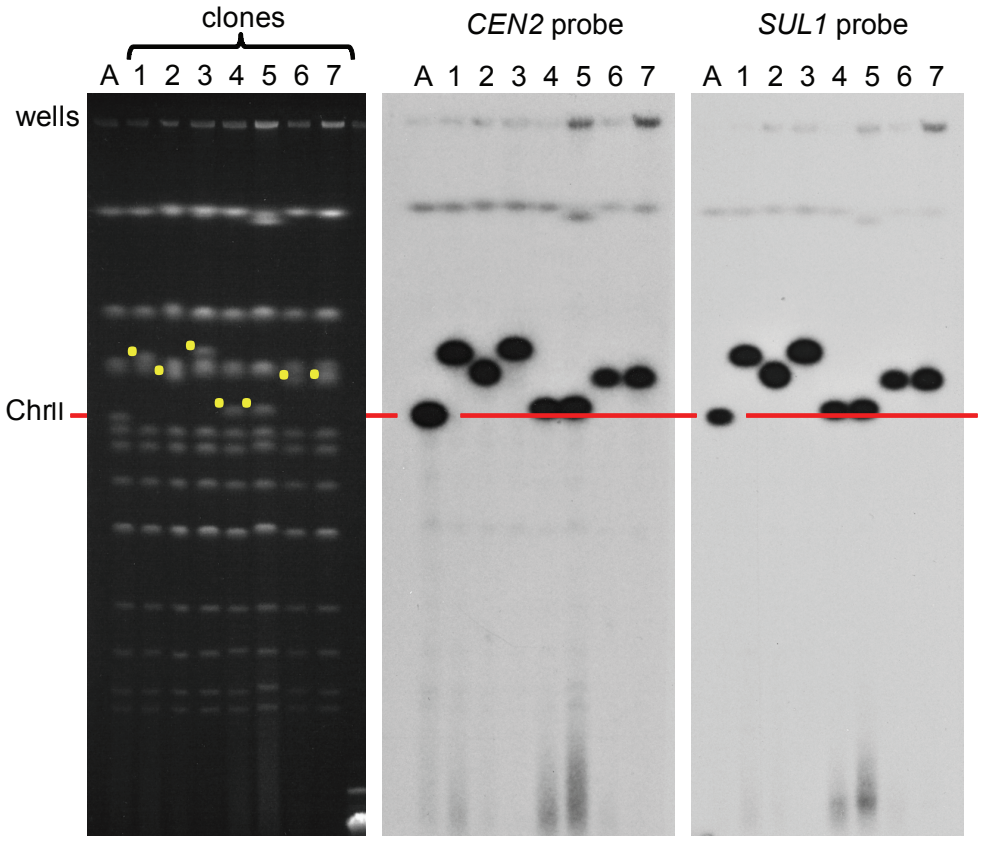


Figure S2 CHEF gel analysis of clones from the seven populations. LEFT: ethidium bromide stained CHEF gel of individual clones. The yellow dots to the left of specific bands indicate chromosomes unique to each evolved clone. RIGHT TWO PANELS: Autoradiograms of Southern blots hybridized with *CEN2* and *SUL1* probes show the identity of chromosome II and the location of the additional copies of *SUL1*.

Figure S3

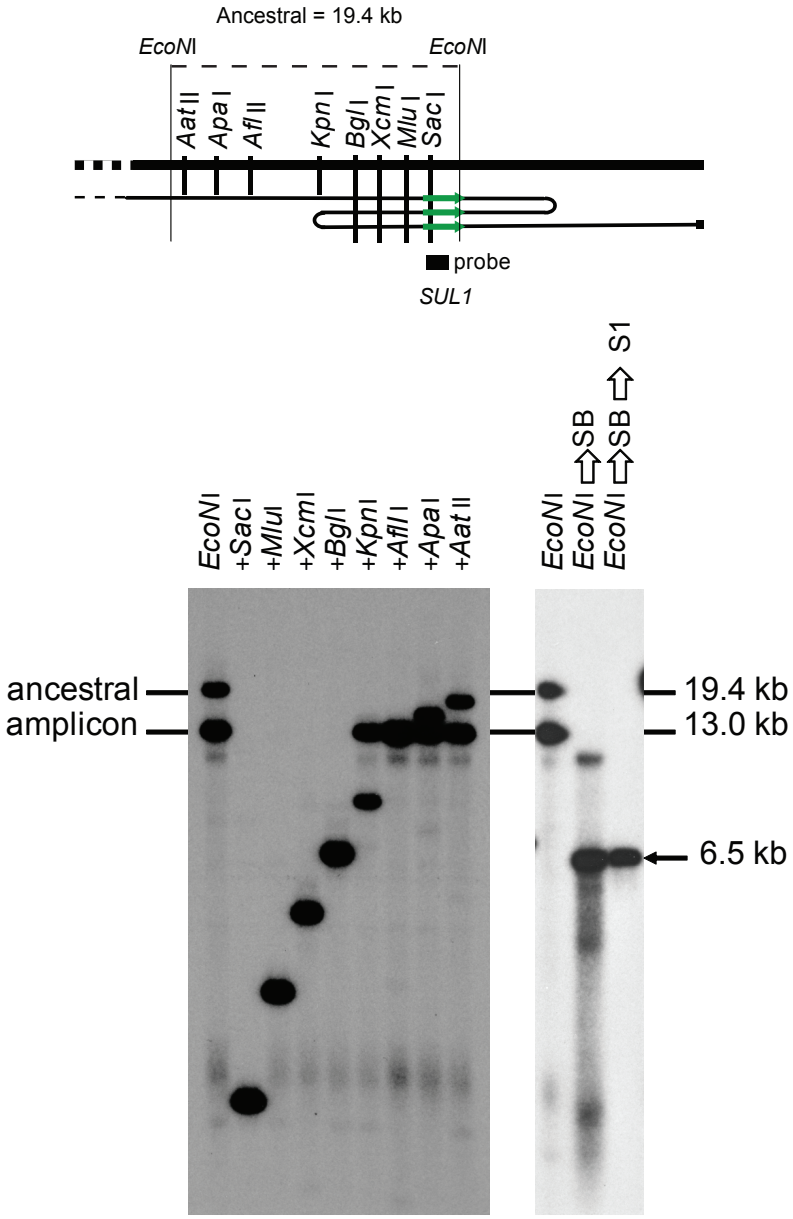
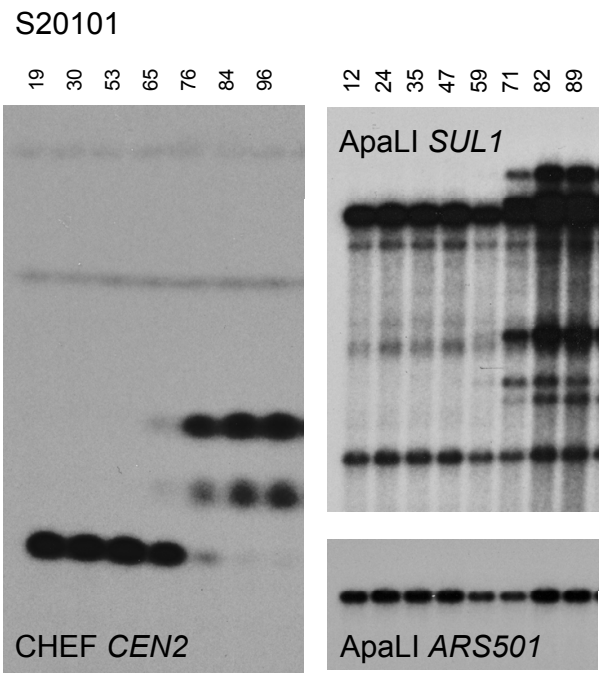
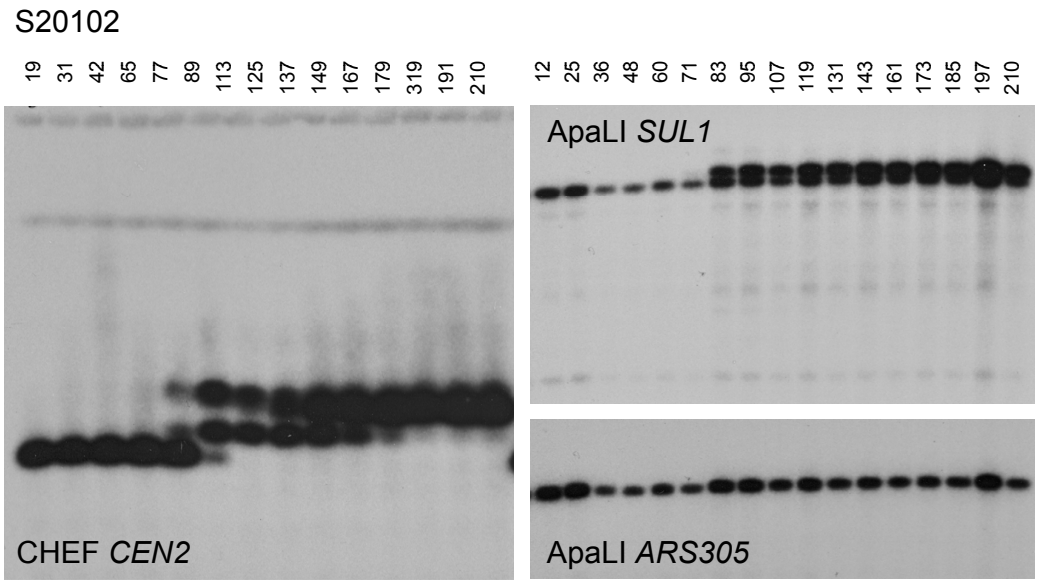


Figure S3. ***EcoN*I analysis of the centromere proximal junction of Pop4 201 clone1**. TOP: Map of the right telomeric region of chromosome II showing the position of *SUL1*, the relevant restriction enzyme sites, the deduced structure of Pop4 201 clone1 and the probe used for Southern blot analysis. BOTTOM LEFT: Indirect end-labeling of *EcoN*I double digests. The order of the lanes corresponds to the order in which the sites for the second restriction enzymes are found between the two *EcoN*I sites; the series of bands of increasing sizes in the Southern blot indicate that the ancestral *SUL1* fragment is intact. Fragments that contain the amplicon junction co-migrate with the expected fragments only up to the position of the junction. Second enzymes whose sites lie distal to the amplicon junction fail

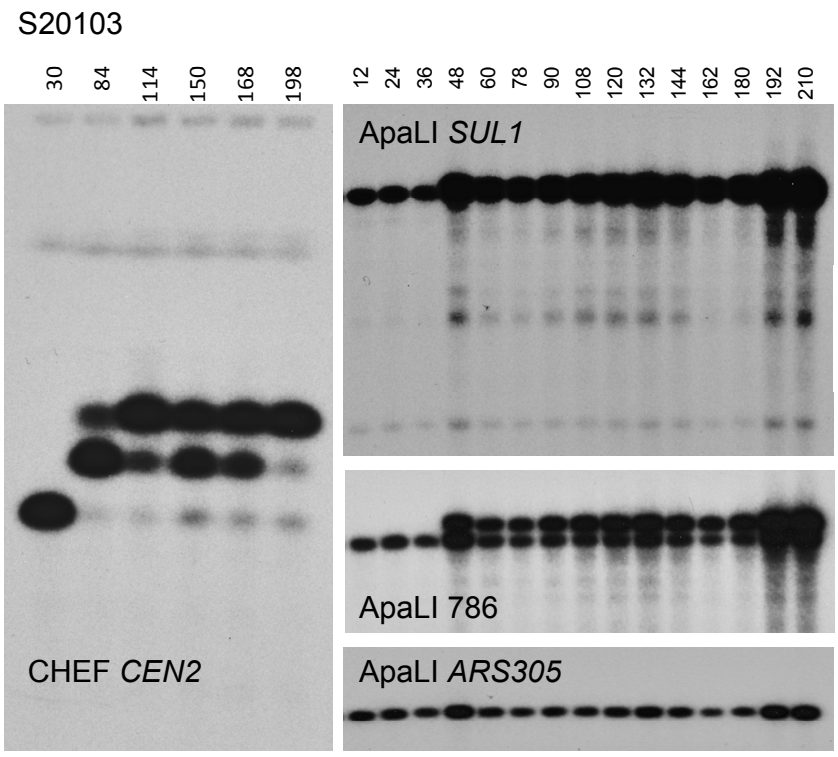
to make a second cleavage and produce the amplicon-specific *Eco*NI junction fragment. BOTTOM RIGHT: Southern analysis of the snap-back/S1-nuclease assay of Pop4 210 clone1 using a *SUL1* probe. The 13.0 kb *Eco*NI fragment generates an S1-resistant duplex molecule approximately half of its original size while the single strands of the ancestral fragment are degraded by S1.



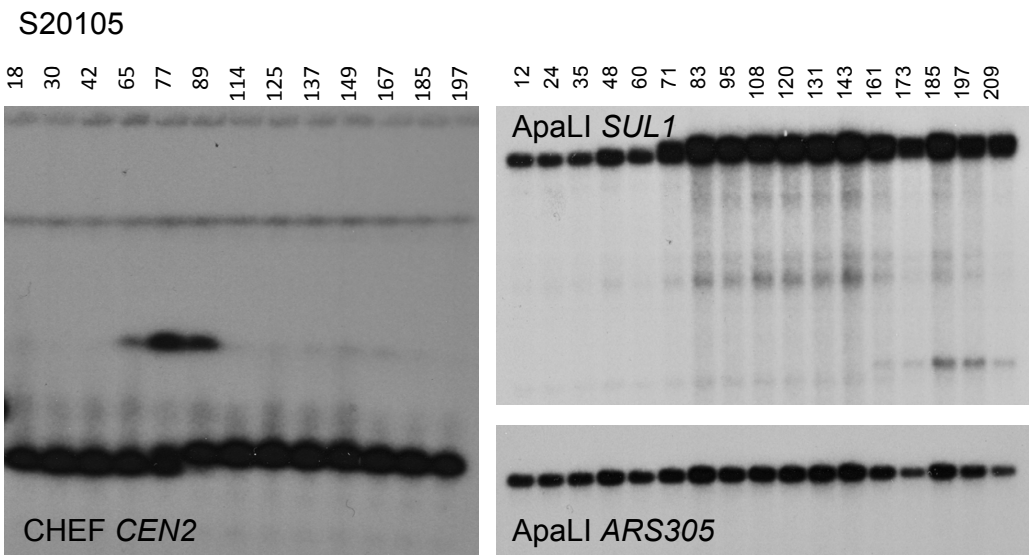
SuppFigure 4-1



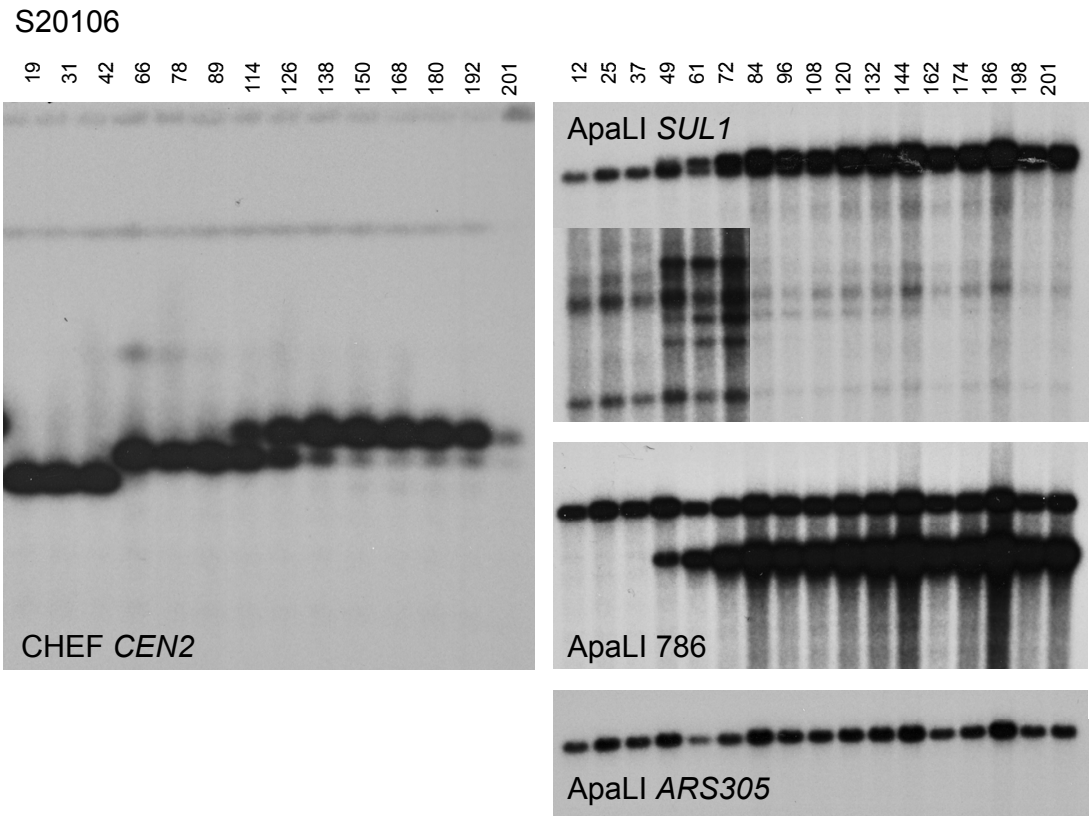
SuppFigure 4-2



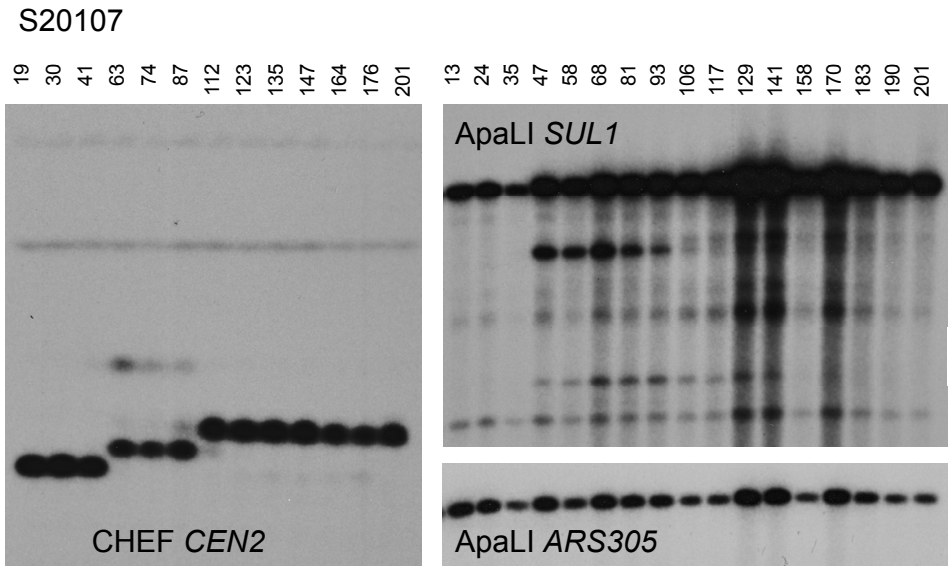
SuppFigure 4-3



SuppFigure 4-4



SuppFigure 4-5



SuppFigure 4-6

Figure S4 Gel analysis of evolving populations S20101-S20107. LEFT: CHEF gels of population samples at the indicated generations reveal the dynamics of chromosome II alterations in each of the seven evolution experiments. The hybridization probe is immediately adjacent to *CEN2*. Numbers across the top refer to generations of sulfate limited growth. RIGHT: *Apa*LI digestion and electrophoretic separation of population DNA samples from each of the seven evolution experiments. TOP: Hybridization of the blot with *SUL1* detects telomere proximal junction fragments in addition to the ancestral fragment. Hybridization with probe 786 detects centromere proximal junction fragments in addition to the ancestral fragment. For Pop6, an inset of a darker exposure reveals transient minor fragments that likely correspond to the transient low abundance form of chromosome II seen in the Pop6 CHEF gel. BOTTOM: Hybridization of the blot with *ARS305* serves as a loading control.

Supplementary Figure 5: aCGH of Pop3 201 clone10

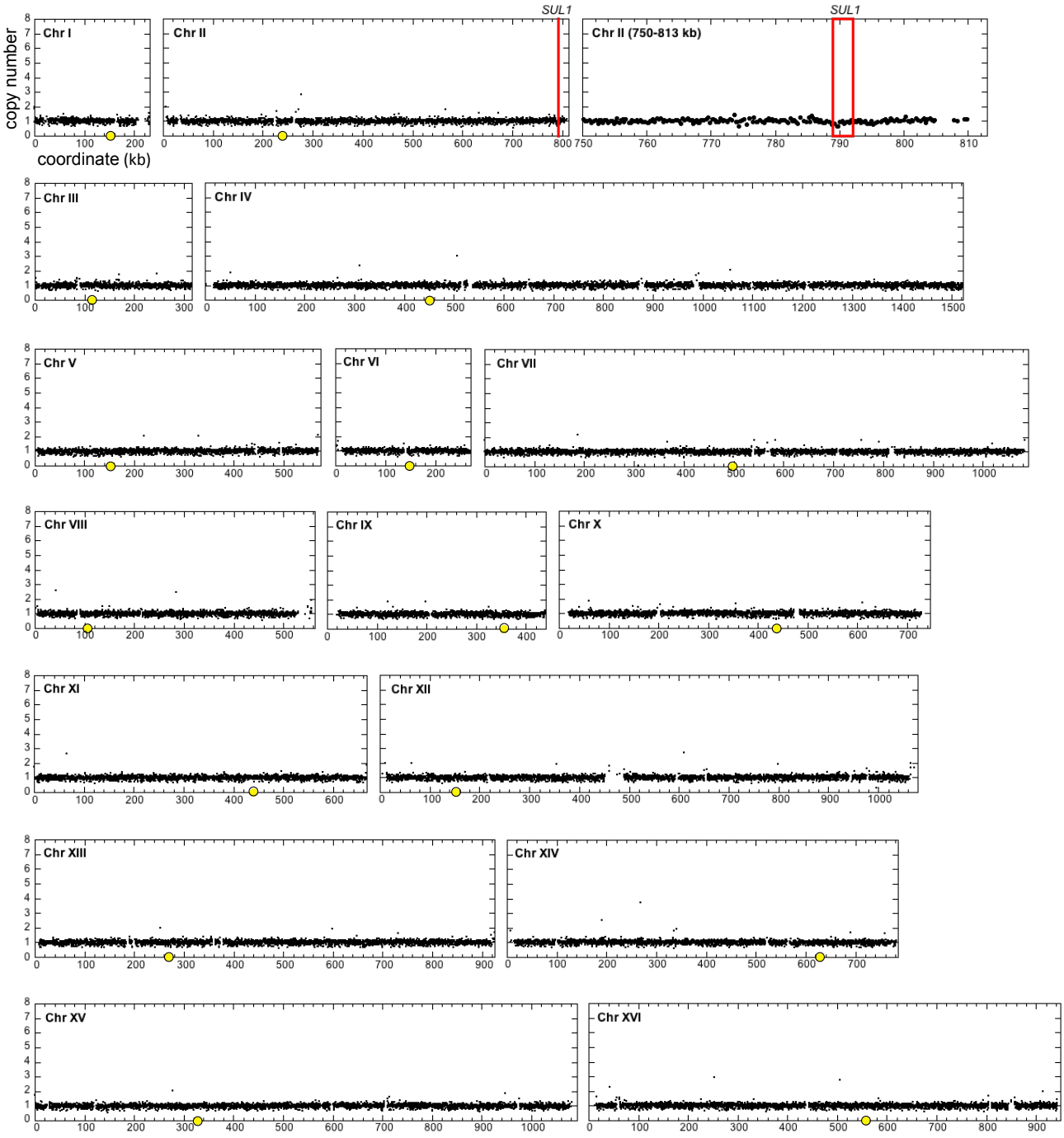
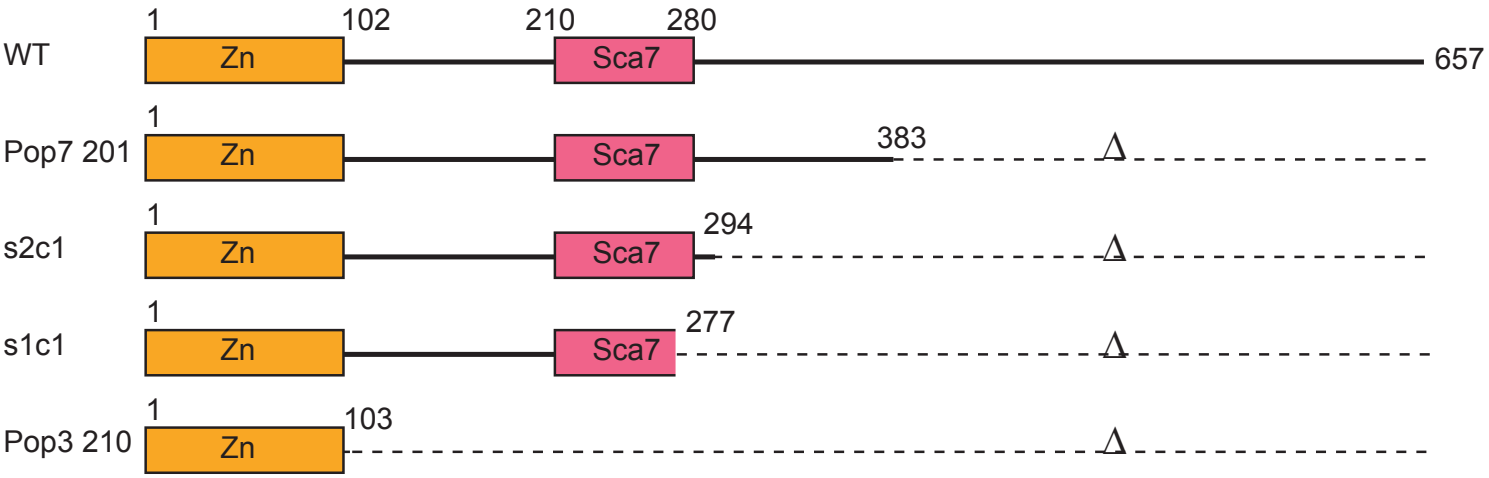


Figure S5 Whole genome copy number data from aCGH of Pop3 210 clone10. Copy number of each probe across the genome is shown for Pop3 210 clone10. An expanded view of the last ~60 kb of chromosome II is shown to provide higher resolution for the genomic region that contains the *SUL1* locus. No amplification at the *SUL1* locus or any other location of the genome was detected for this clone.

Figure S6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s2c1
s1c1
Pop3 210
Zn
Sca7
Δ
1
102
103
210
277
280
294
383
657

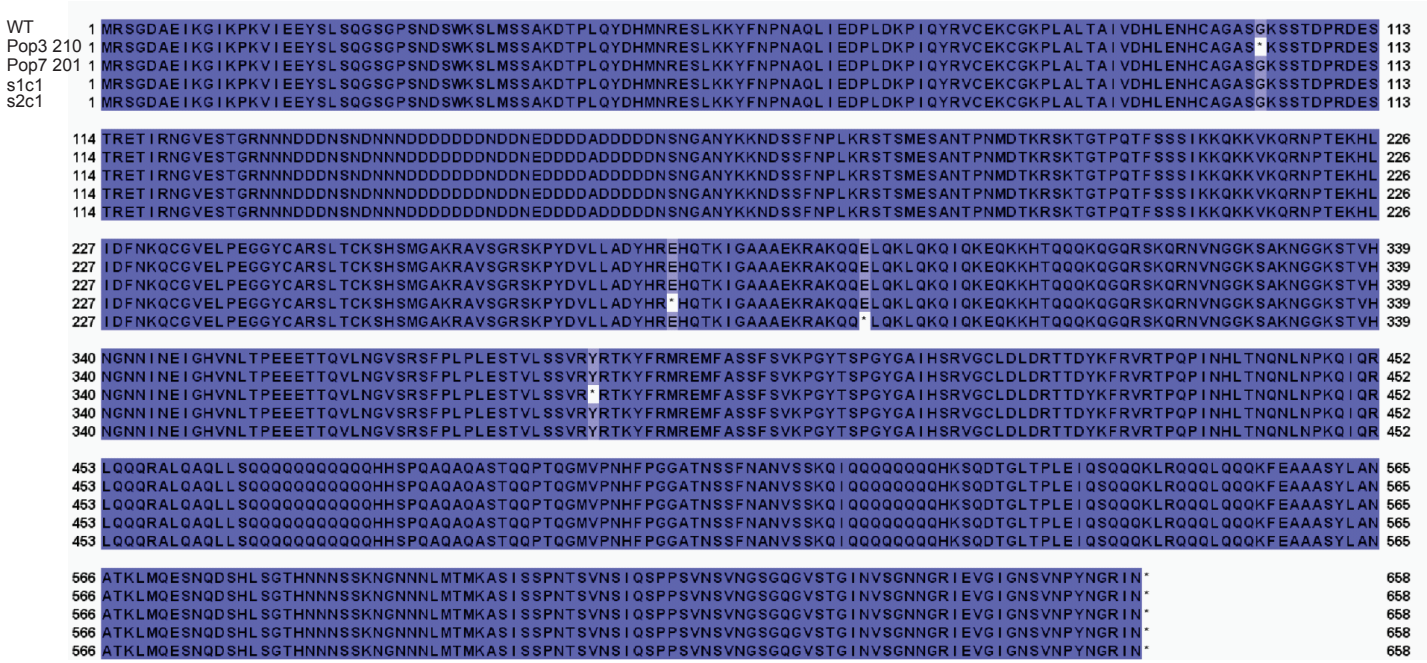
WT
Pop3 210
Pop7 201
s1c1
s2c1

Figure S6. *SGF73* mutations identified in four independent sulfate-limited evolutions. TOP: Diagrammatic representation of the Sgf73 protein. Boxes show protein domains. Residue numbering for the domains is shown above the protein. The extents of recovered truncations are shown by dashed lines with the last included amino acid indicated by the residue number. BOTTOM: ClustalW multiple alignment of Sgf73 sequences from a wildtype strain and four evolved strains carrying truncation mutations in *SGF73*.

File S1 Supporting Methods and Materials: NIB-and-Grab DNA isolation protocol

1. Pellet cells (~6 x 10^8), rinse in distilled water, and transfer to a 15 ml conical tube. (Cells may be frozen at - 20˚C at this point.). Add 0.2 ml NIB to the cell pellet and vortex briefly. (NIB = Nuclear Isolation Buffer: 17% glycerol, 50 mM MOPS buffer, 150 mM potassium acetate, 2 mM magnesium chloride, 0.5 mM spermidine, and 0.15 mM spermine; pH is adjusted to 7.2 after all ingredients are dissolved.) Add 0.9 ml acid-washed glass beads. (There will be no obvious liquid above the level of the beads.)

2. Vortex at the highest rate with a vigorous vortexer, 5 to 10 times for 30 seconds, returning the cells to ice for 30 seconds between bursts of vortexing. Check for cell lysis by microscopy. Continue vortexing until the desired percentage of broken cells is achieved (~90%). Add 0.6 ml NIB to the broken cells, vortex briefly and transfer the solution to a fresh microfuge tube. Repeat with another 0.6 ml of NIB. (There should now be ~1.3 ml transferred to the microfuge tube.) Spin down nuclei, cell walls and unbroken cells in a microfuge for 20-30 minutes at top speed at 4°C. Pipette off the supernatant. Keep the pellet on ice.

3. Resuspend the nuclei in 0.3 ml TEN (50 mM Tris, 50 mM EDTA, 100 mM NaCl) to which 1 ml of RNAse A (10 mg/ml) has been added. Keep on ice 5 minutes. Add 12 ml of 25% Sarkosyl. Invert tube gently a few times to mix. Add 1 ml proteinase K (20 mg/ml). Invert tube again a few times to mix and transfer to 37°C for 30 minutes.

4. Spin the lysed nuclei for 5 minutes at 4°C at top speed in a microfuge. Remove the supernatant with a pipette tip to a screw-cap microfuge tube. Add 0.3 ml phenol:chloroform:isoamyl alcohol (24:24:1). Invert a dozen times to mix the phases. Let it rest and repeat another dozen inversions. Spin the samples at top speed in a microfuge to separate the phases (the top aqueous phase should be clear).

5. Use a wide-bore tip or cut off the tip of a 200 µl pipette tip to increase the diameter of the opening and carefully remove the aqueous phase to a clean microfuge tube. It will take several attempts to recover all of the aqueous phase. Avoid taking any of the white interface. (There should be ~0.25 ml of the aqueous phase.)

6. Add 2 volumes (~0.5 ml) of absolute ethanol that contains 0.5 M potassium acetate. Slowly and gently mix the phases by inverting the tube. The DNA should spool. When all of the aqueous phase has been incorporated into the alcohol phase, the DNA spool should collapse and fall to the bottom of the tube. Pull off the liquid with a pipette tip and discard. To the DNA pellet, add 0.3 ml 70% ethanol. Spin 5 minutes at top speed. Aspirate the ethanol, invert the tube and let it air dry for 30 minutes.

7. Resuspend the DNA in 75 µl (or so) of TE (Tris pH 8.0; 1 mM EDTA) by gently flicking the tube a few times. Incubate the tube overnight (or longer) at 4˚C. It will probably take the DNA overnight or longer to completely go into

solution. Store the DNA at 4˚C. Be aware that there may be RNA nucleotides/fragments in the solution so an OD_{260} reading is likely to give an overestimate of the DNA concentration. Run a microliter on a gel to confirm the DNA concentration. The average size of the DNA is greater than 50 kb.

Supporting Tables S1-S3

Available for download at http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.113.009365/-/DC1 as Excel files.

Table S1. Copy number of *SUL1* locus in independent clones isolated from each experiment at generations ~50 and ~200 by qPCR.

Table S2. List of non-synonymous mutations detected in each clone.

Table S3. List of primers used in this study.