SUPPLEMENTAL FILE 1 (Creamer et al. 2016, doi: http://dx.doi.org/10.1101/063271)

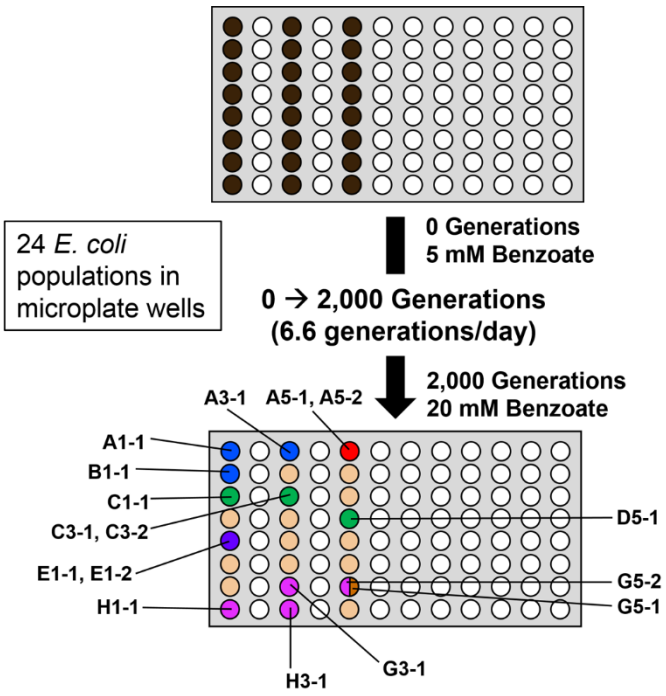

Figure S1. Design of evolution experiment. Twenty-four populations were founded from an overnight culture of *Escherichia coli* K-12 W3110. Each day, 2 µl of the previous culture was diluted into 200 µl fresh medium; the 1:100 dilution yields 6.6 generations per day. The medium consisted of LBK, 100 mM PIPES pH 6.5, supplemented with increasing concentrations of potassium benzoate: at 0 generations (gen), 5 mM benzoate; after 60 gen, 6 mM; 90 gen, 10 mM; 540 gen, 12 mM; 1,020 gen, 15 mM; 1,210 gen, 18 mM; 1580 gen, 20 mM. At 2,000 generations, eight clones were selected for genome sequencing, in pairs from each of four wells (clones A5-1, A5-2, C3-1, C3-2, E1-1, E1-2, G5-1, G5-2). A total of 24 clones were tested for chloramphenicol sensitivity in 5 mM benzoate medium, pH 6.5. Eight chloramphenicol-sensitive clones were selected for genomes sequencing (A1-1, A3-1, B1-1, C1-1, D5-1, G3-1, H1-1, H3-1). The color code for each well corresponds to the color of one of our 2000-generation isolates whose genome sequences we report (Fig. 1; Table S1).

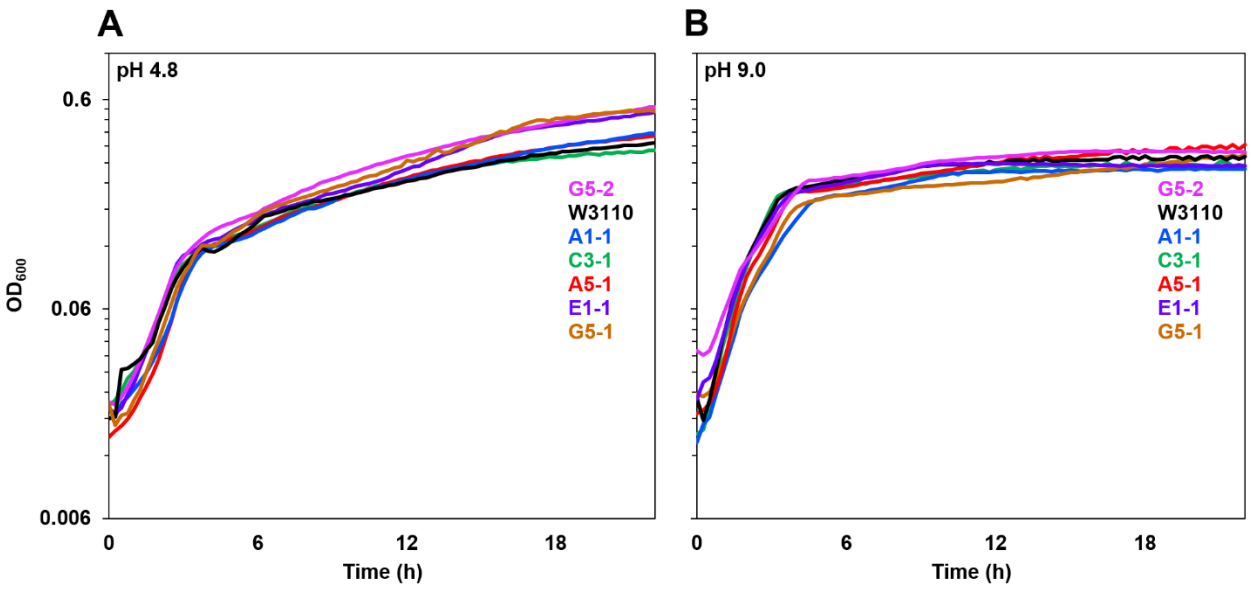

Figure S2. Benzoate-evolved strains show no growth difference at pH 4.8 and at pH 9.0 compared to ancestor strain. Benzoate-evolved strains and W3110 were cultured in LBK (**A**) 100 mM HOMOPIPES pH 4.8 and (**B**) 150 mM TAPS pH 9.0. For each strain, a curve with median cell density at 16 h is shown. Panels A and B show similar growth rate and 16-h cell density for the ancestral W3110 strain (black curve) compared to the benzoate-evolved strains (Friedman test; post-hoc Conover pairwise comparisons with Holm-Bonferroni adjusted p-values; N=8).

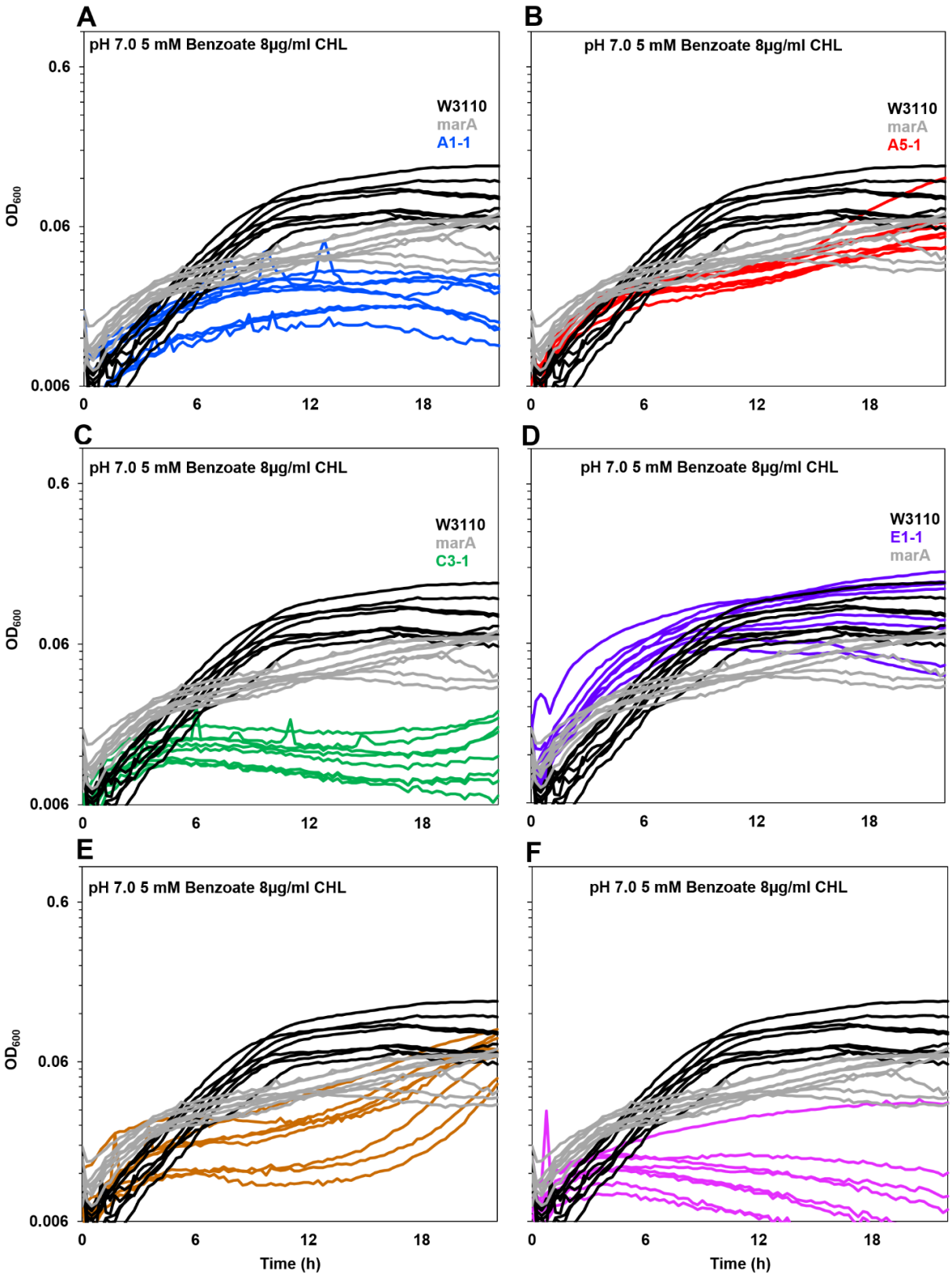

Figure S3. Chloramphenicol sensitivity of each strain cultured with 5 mM benzoate at pH 7.0. Each graph shows 8 replicates of a single benzoate-evolved strain compared individually with W3110 and with W3110 *marA*::*kanR*. The data set is the same as Figure 6C.

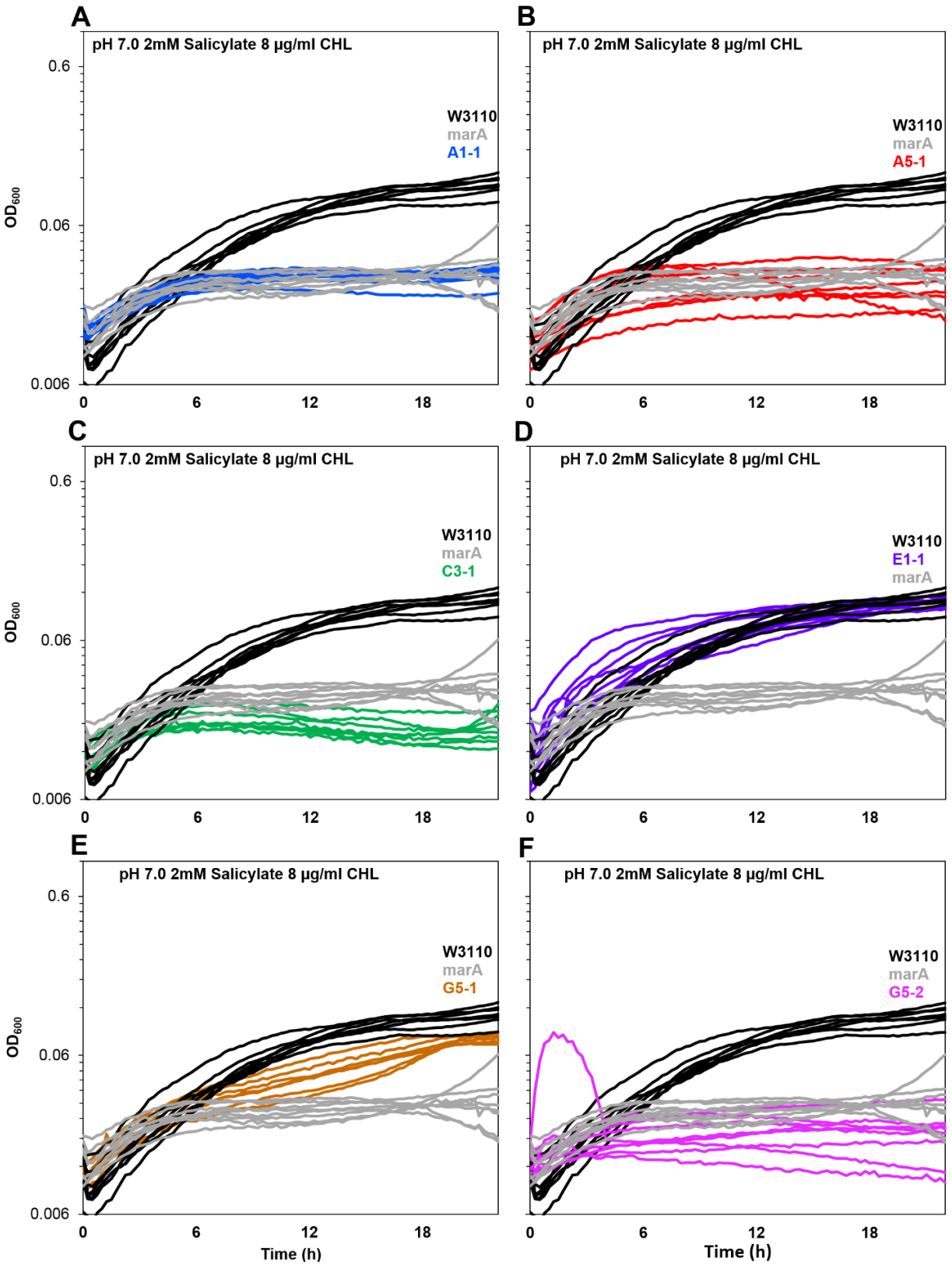

Figure S4. Chloramphenicol sensitivity of each strain cultured with 2 mM salicylate at pH 7.0. Each graph shows all 8 replicates of a single benzoate-evolved strain compared individually with W3110 and with W3110 *marA*::*kanR*. The data set is the same as Figure 6D.

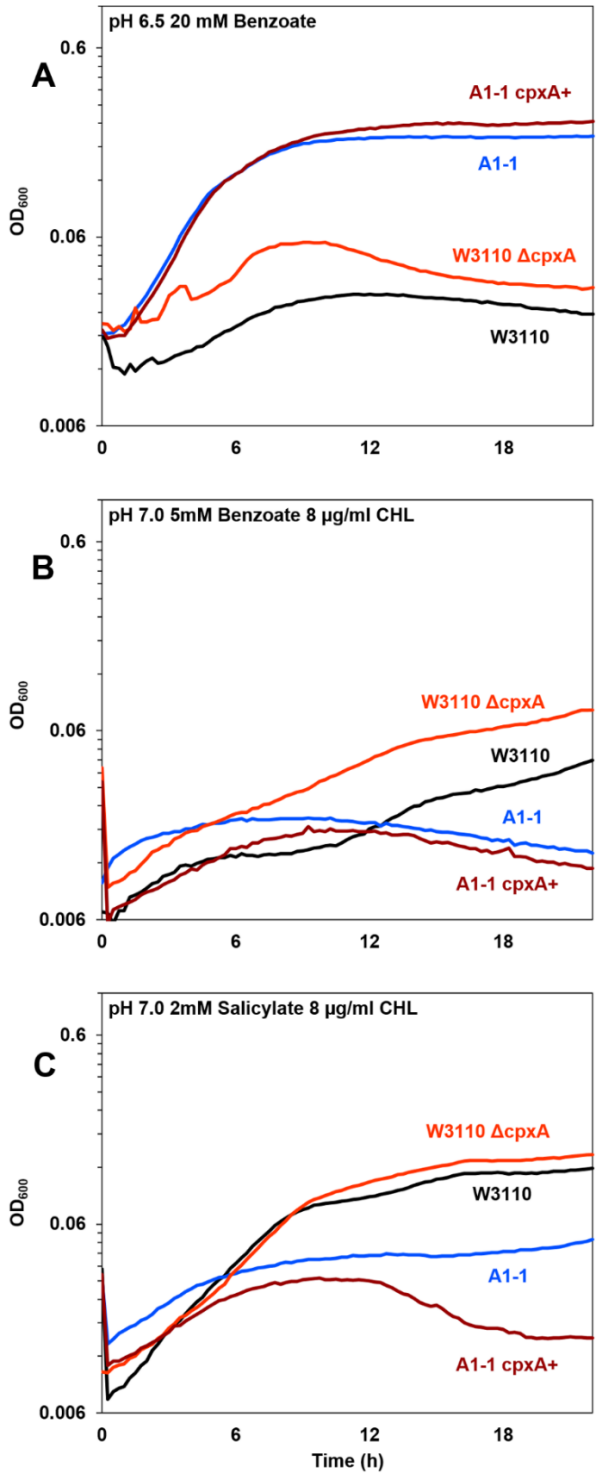

Figure S5. W3110 and A1-1 strains show no effect of *cpxA* alleles. Growth curves were conducted as for Figures 5 and 6. At 16 h, the cell density of strain W3110 *cpxA*::*kanR* showed no difference from that of W3110; and strain JLS1607 (A1-1 *fdhD75*8::*kanR cpxA*+) showed no difference from JLSK0001 (A1-1) (Friedman test; post-hoc Conover pairwise comparisons with Holm-Bonferroni adjusted p-values).