Supplemental Information for "Quantifying the interplay between rapid bacterial evolution within the mouse intestine and transmission between hosts"

Kimberly S. Vasquez[1], Lisa Willis[2], Nate J. Cira[2], Katharine M. Ng[2], Miguel F. Pedro[3], Andrés Aranda-Díaz[2], Manohary Ranjendram[2], Feiqiao Brian Yu[4], Steven K. Higginbottom[1], Norma Neff[4], Gavin Sherlock[5], Karina B. Xavier[3], Stephen R. Quake[2,4], Justin L. Sonnenburg[1,4], Benjamin H. Good[6,7,*], Kerwyn Casey Huang[1,2,4,*]

[1]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA

[2]Department of Bioengineering, Stanford University, Stanford, CA 94305, USA

[3]Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal

[4]Chan Zuckerberg Biohub, San Francisco, CA 94158, USA

[5]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA

[6]Department of Physics, University of California at Berkeley, Berkeley, CA 94720, USA

[7]Department of Applied Physics, Stanford University, Stanford, CA 94305, USA

[*]To whom correspondence should be addressed: bhgood@stanford.edu, kchuang@stanford.edu

Supplemental Figure Legends

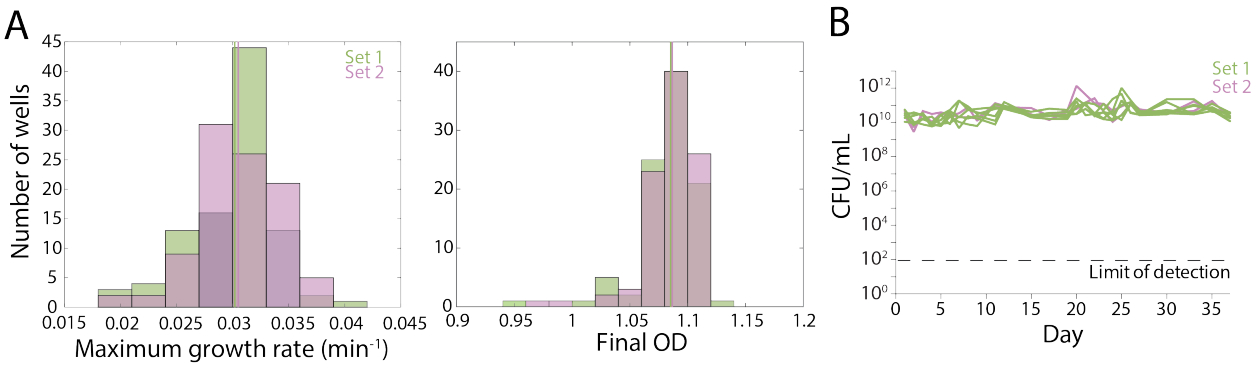

Figure S1: Growth dynamics of the barcoded *E. coli* libraries, related to Figure 1.

A) The individual barcoded *E. coli* from Set 1 (green) and Set 2 (pink) showed similar maximum growth rates and final OD_{600} values. Vertical lines represent the mean of each set.

B) Culturable densities of bacteria on LB agar plates from the feces of all mice were approximately constant.

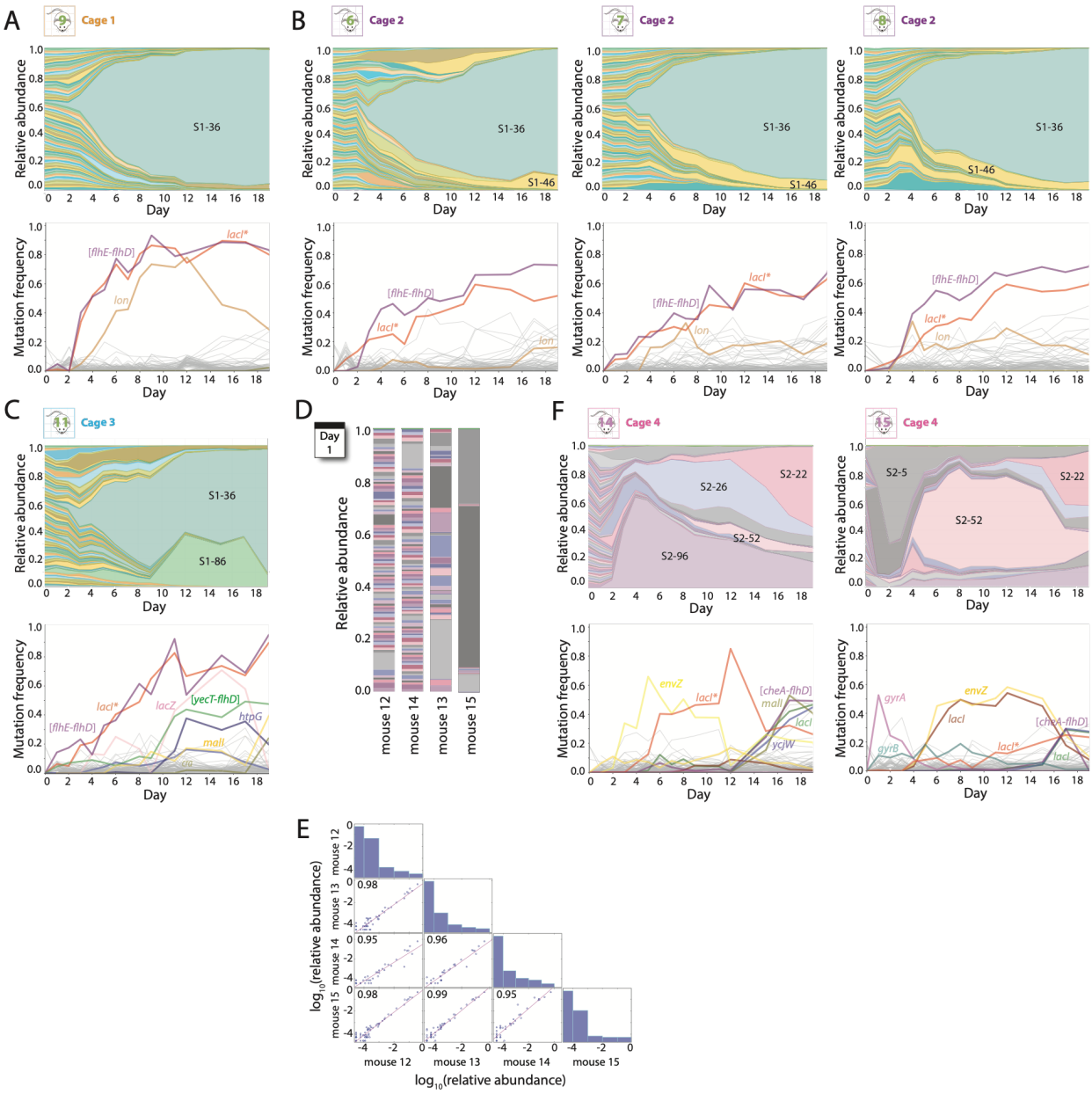

Figure S2: Additional barcode and metagenomic tracking of S1 and S2 mice, related to Figure 2.

A-C) Relative abundances of barcodes (top) and mutations (bottom) over the first 19 days in S1 mice not shown in Figure 2.

D) Relative abundances of all S2 mice on day 1 after colonization. An apparent bottleneck was observed in mice 13 and 15 that led to the dominance of a few barcodes, whereas mice 12 and 14 had relatively even abundances.

E) Pearson correlation coefficients of relative abundances between all pairs of S2 mice were very close to 1 on day 19, indicating similar compositions after 19 days of co-housing. Histograms along the diagonal show the distribution of barcode abundances in each mouse.

F) Relative abundances of barcodes (top) and mutations (bottom) over the first 19 days in S2 mice not shown in Figure 2.

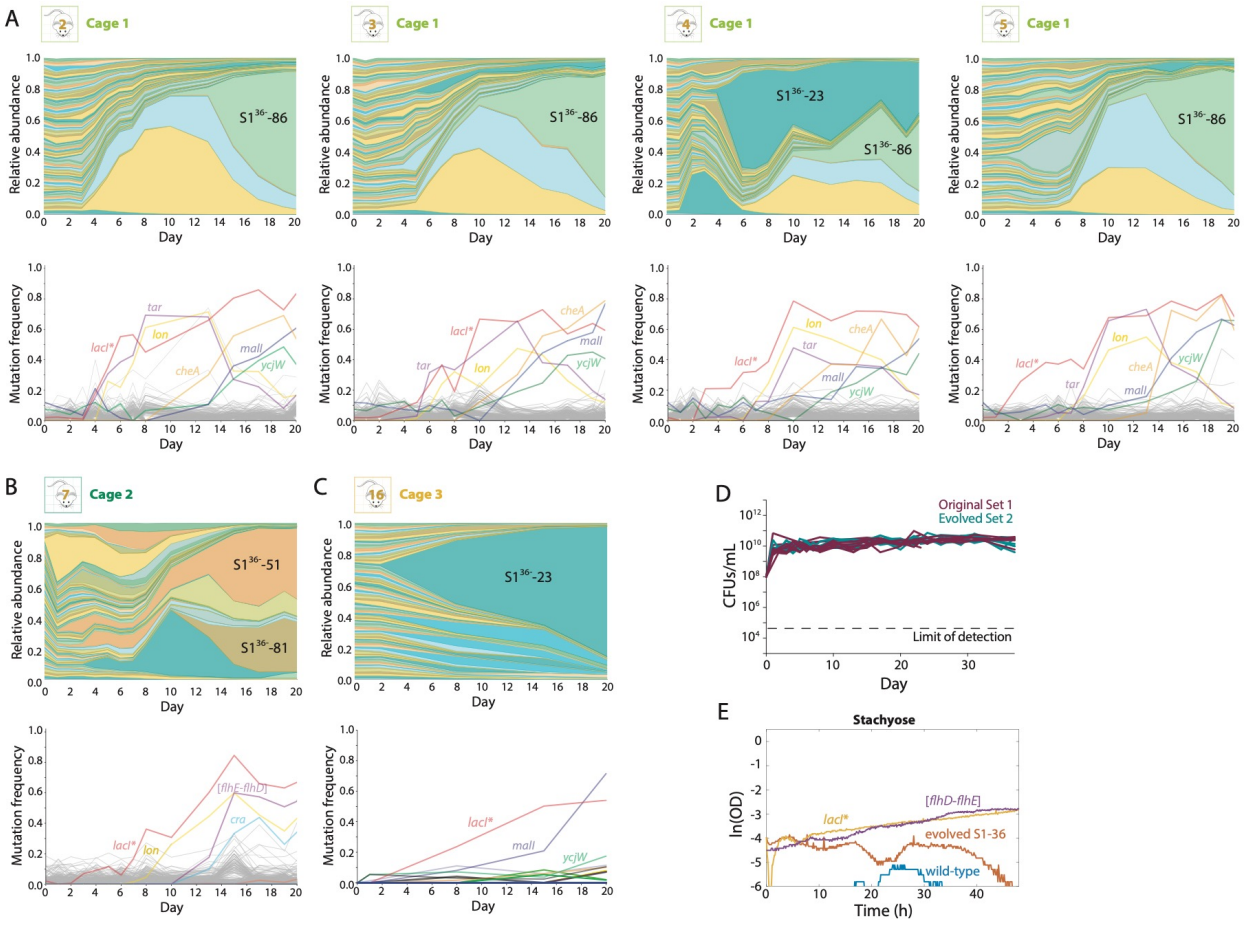

Figure S3: Additional barcode and metagenomic tracking of S1^{36-} and S2* mice, related to Figure 3.

A-C) Relative abundances of barcodes (top) and mutations (bottom) over the first 20 days in S1^{36-} mice not shown in Figure 3.

D) Culturable densities of bacteria on LB agar plates from the feces of all mice were approximately constant.

E) Growth curves of the wild-type parent, evolved S1-36, a *lacI** mutant, and an [*flhD-flhE*] mutant in M9 supplemented with stachyose in a Biolog plate.

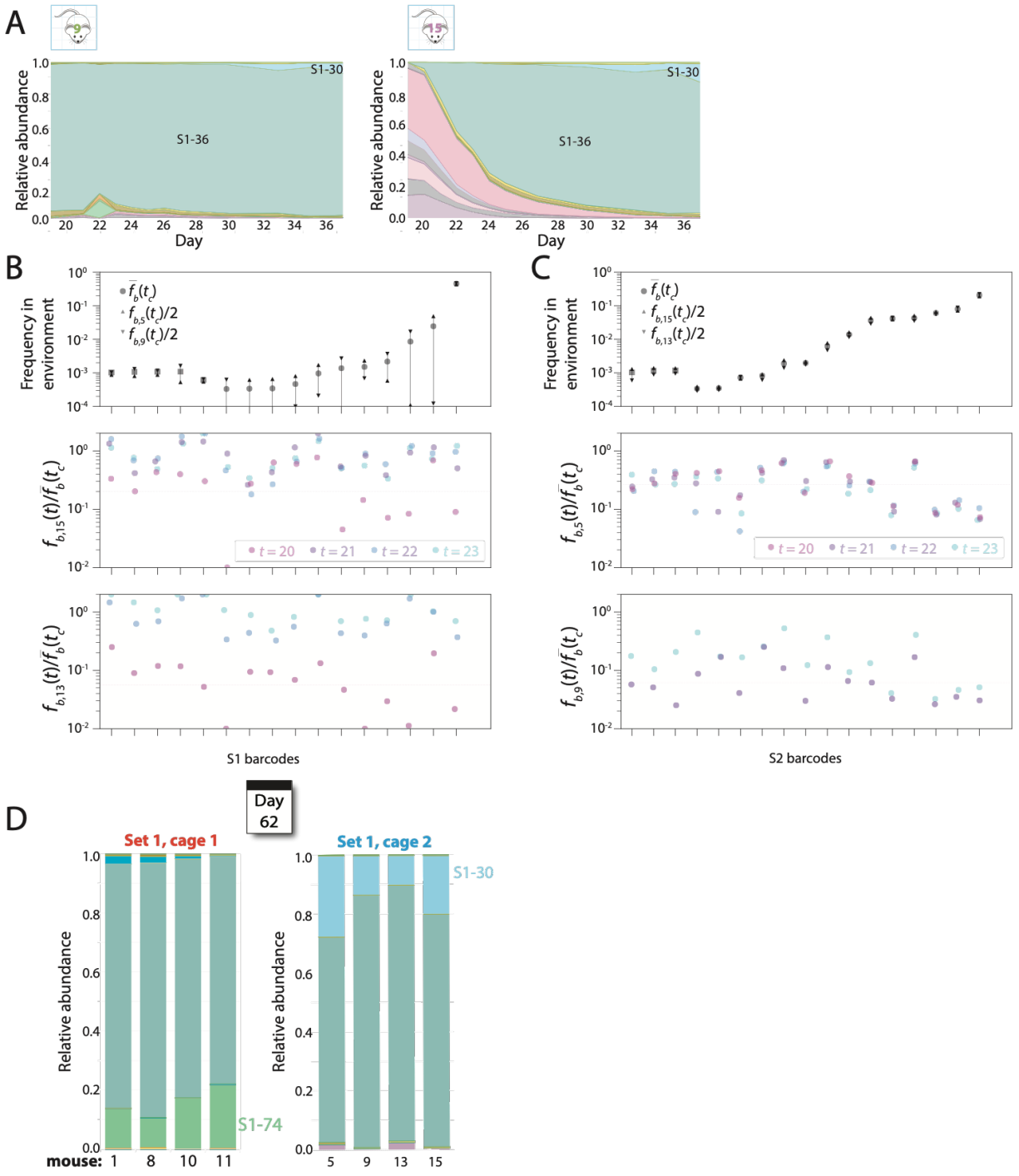

Figure S4: Cross-housed mouse data supports a migration rate of ~10%, related to Figure 4.

A) Relative abundances of S1 (shades of green) and S2 (shades of pink) barcodes in after 19 days in mice initially colonized with S1 (left) or S2 strains (right) that are not shown in Figure 4, related to Figure 4B.

B) Top: frequencies of the most abundant S1 barcodes present in S1 mice on day 19. Circles represent the mean of individual barcodes across mice 5 and 9, squares represent course-grained barcodes that represent collections of barcodes whose individual frequencies were $<10^{-3}$. Triangles are individual measurements in mice 5 and 9. Middle, bottom: the abundance of the S1 barcodes in S2 mice 15 (middle) and 13 (bottom) on days 20-23 (directly after cross-housing) normalized to the mean abundance in S1 mice at the time of cross-housing ($\bar{f}_b(t_c)$), which represents a time- and barcode-dependent migration rate. Pink dotted line is the mean migration rate on day 20, which is ~10%.

C) Top: frequencies of the most abundant S2 barcodes present in S2 mice on day 19. Circles represent the mean of individual barcodes across mice 15 and 13, squares represent course-grained barcodes that represent collections of barcodes whose individual frequencies were $<10^{-3}$. Triangles are individual measurements in mice 15 and 13. Middle, bottom: the abundance of the S2 barcodes in S1 mice 5 (middle) and 9 (bottom) on days 20-23 (directly after cross-housing) normalized to the mean abundance in S2 mice at the time of cross-housing ($\bar{f}_b(t_c)$). Pink (middle) and

purple (bottom) dotted lines are the mean migration rate on day 20 or day 21, respectively, which were ~10% similar to (A).

D) Barcode relative abundances after 62 days remained similar across S1 mice within each of cage 1 and cage 2, although S1-74 bloomed in cage 1 while S1-30 bloomed in cage 2.

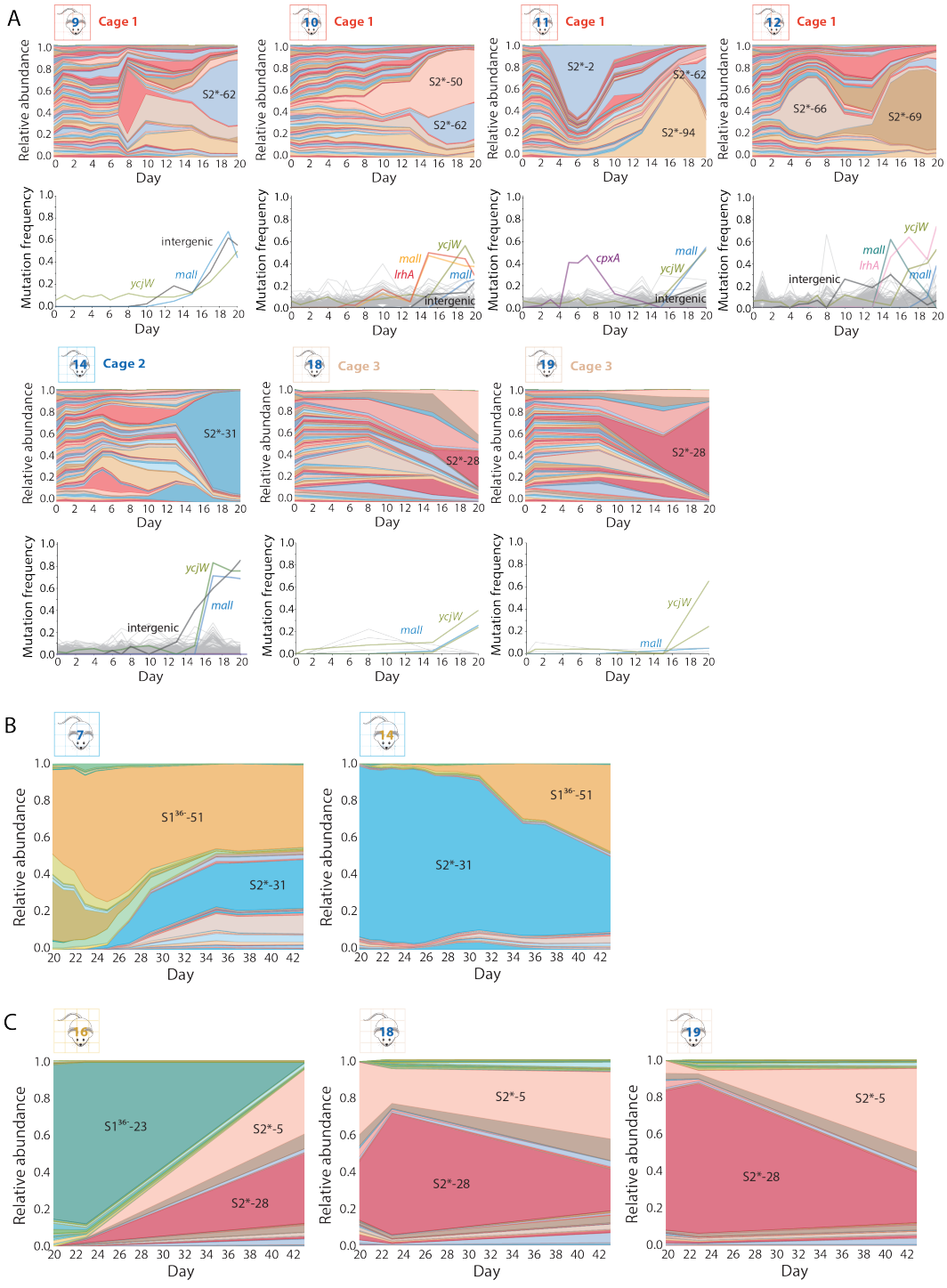

Figure S5: Additional barcode and metagenomic tracking of S1^{36-} and S2* mice, related to Figure 5.

A) Relative abundances of barcodes (top) and mutations (bottom) over the first 20 days in S2* mice not shown in Figure 5A.

B) Relative abundances of barcodes after day 20 in cross-housed $S1^{36-}$ and S2* mice not shown in Figure 5D.

C) Relative abundances of barcodes in cross-housed $S1^{36-}$ and S2* mice from cages 2 not shown in Figure 5F.

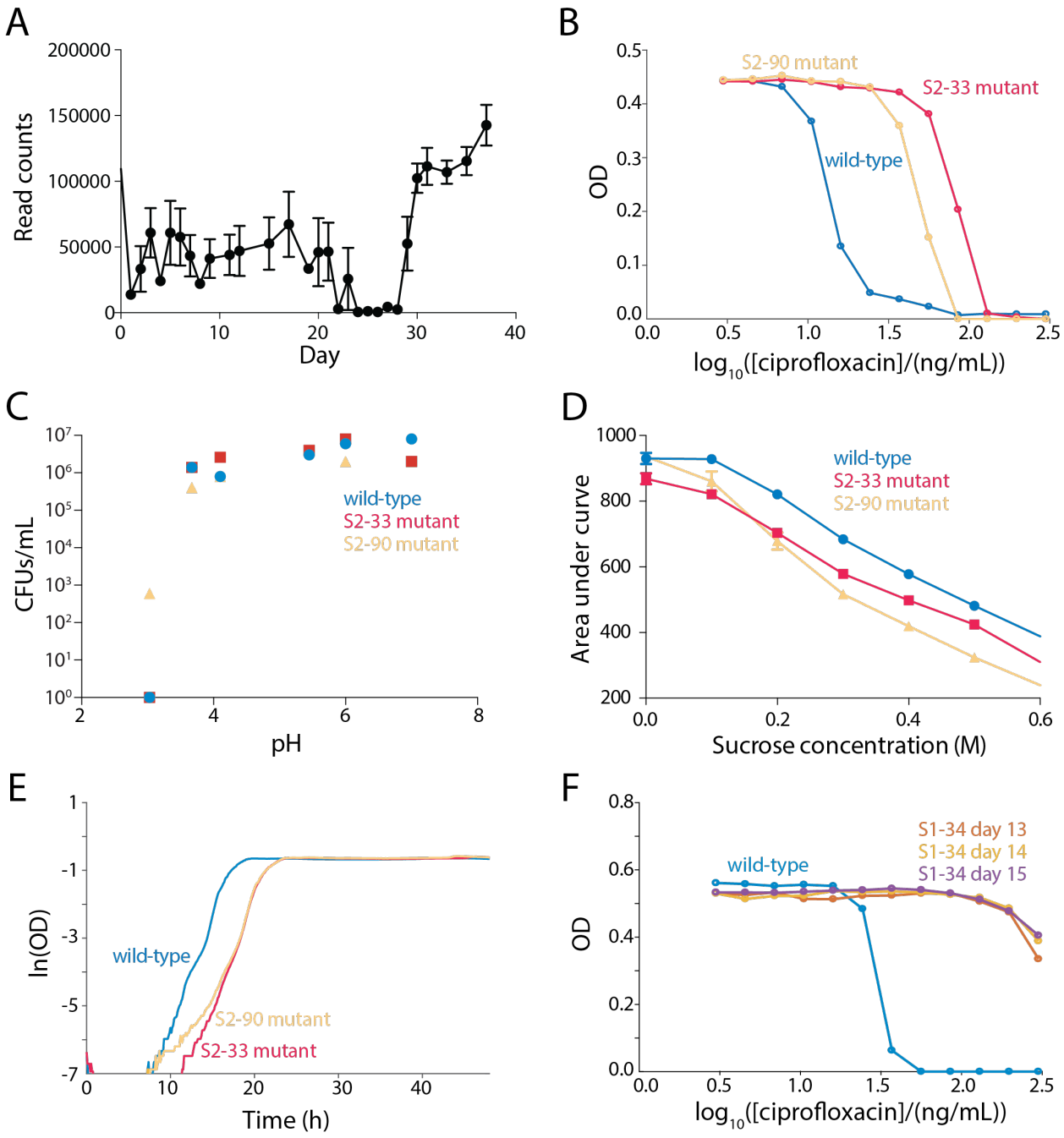

Figure S6: Growth phenotypes of ciprofloxacin-resistant clones, related to Figure 6.

A) Read counts from barcode sequencing of all ciprofloxacin-treated mice dropped during treatment (days 19-21) and remained low until day 29. Error bars represent 1 standard error of the mean.

B) The S2-33 *gyrA* mutant and S2-90 *gyrB* mutant exhibited higher IC_{50} to ciprofloxacin than the wild-type parent.

C) Culturable bacterial densities after 24 h in M9 salts at a range of pH values were similar for wild type, the S2-33 *gyrA* mutant, and the S2-90 *gyrB* mutant.

D) Area under the curve after 24 h of growth was slightly higher for wild type compared with the S2-33 *gyrA* and S2-90 *gyrB* mutants across a range of sucrose concentrations.

E) The S2-33 *gyrA* and S2-90 *gyrB* mutants exhibited a longer lag than wild type in unsupplemented LB.

F) Three S1-34 *gyrA* mutants isolated on different days displayed higher IC_{50} than the wildtype parent strain.