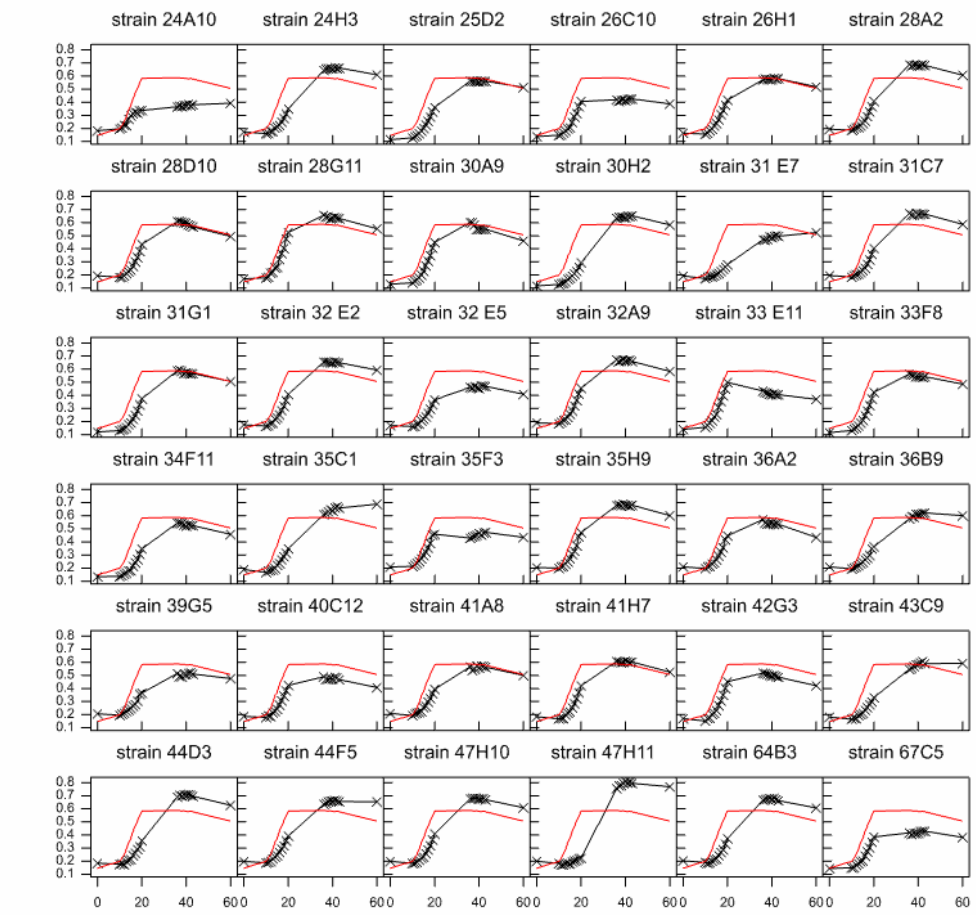
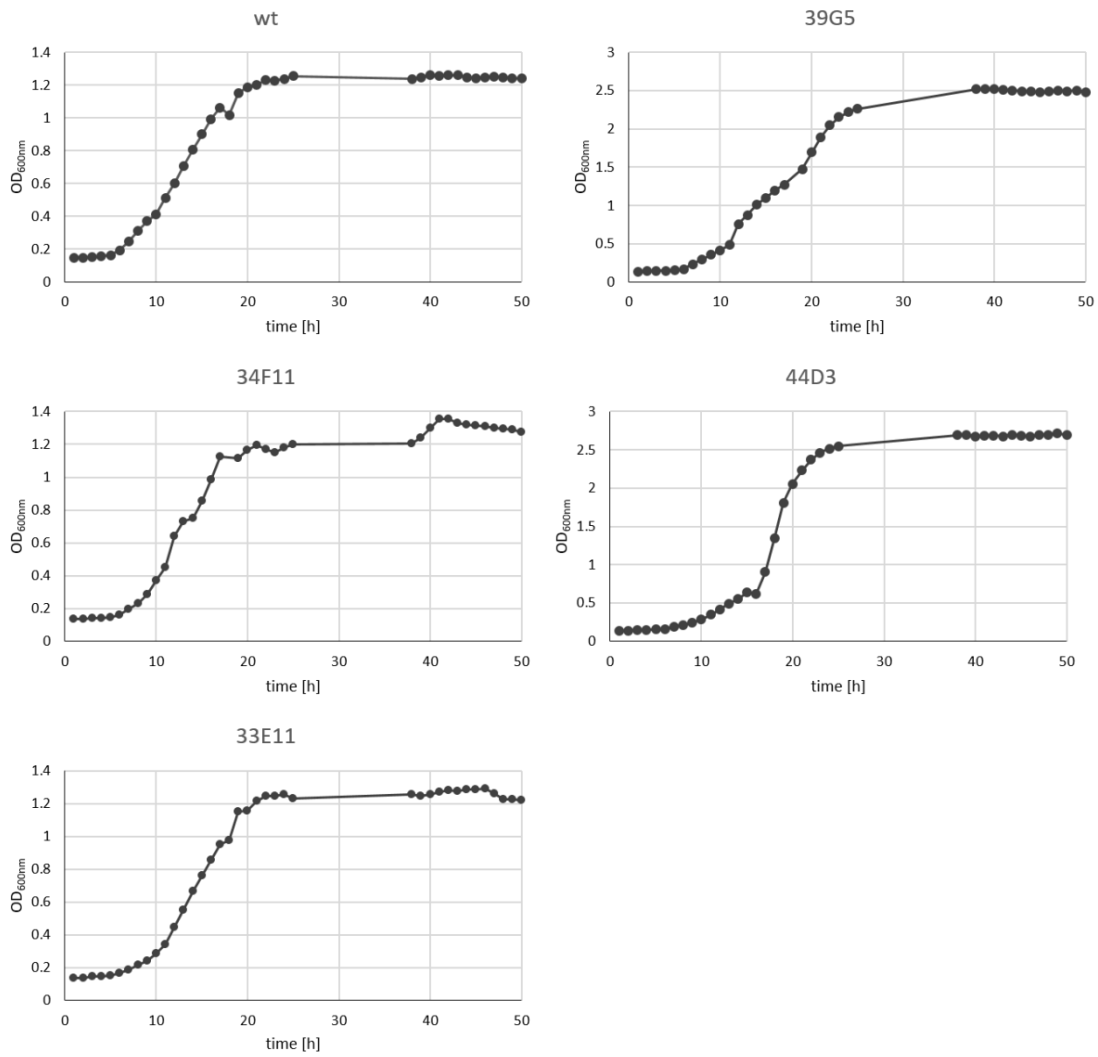
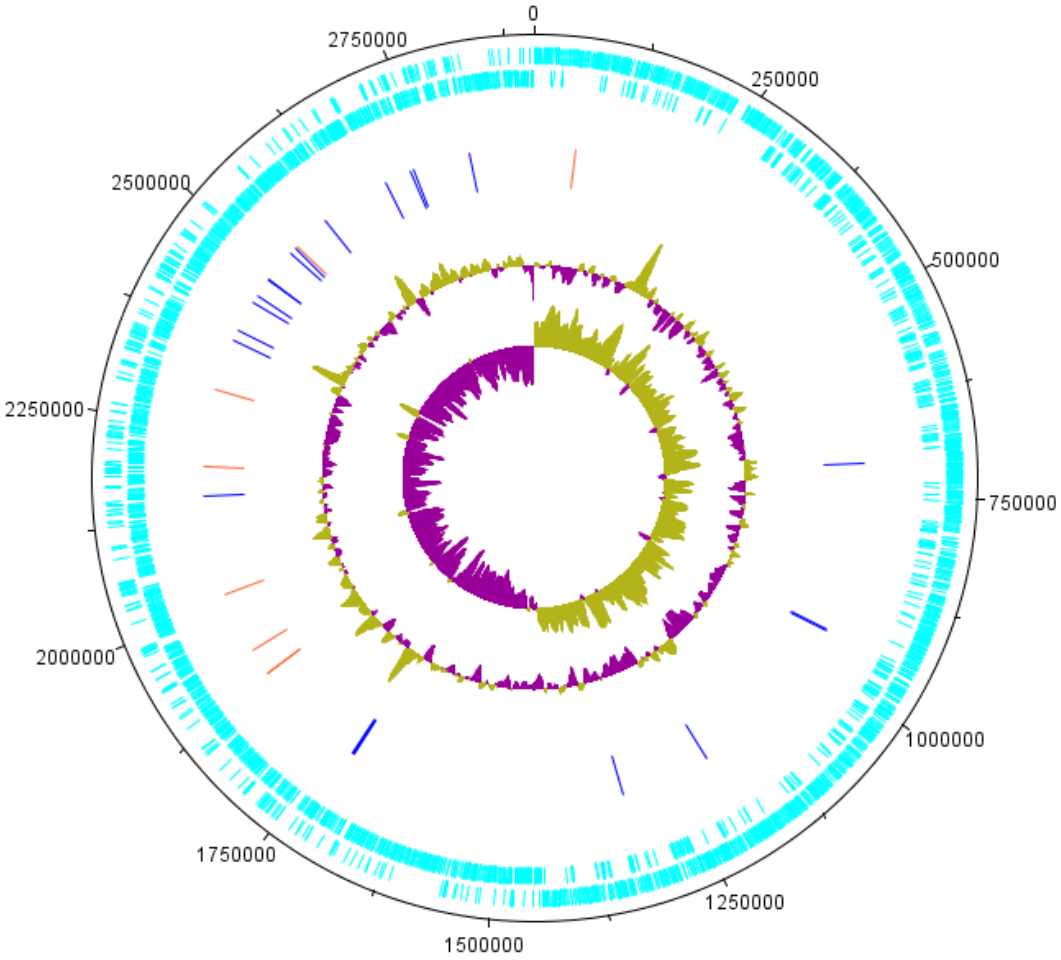
Supplementary information



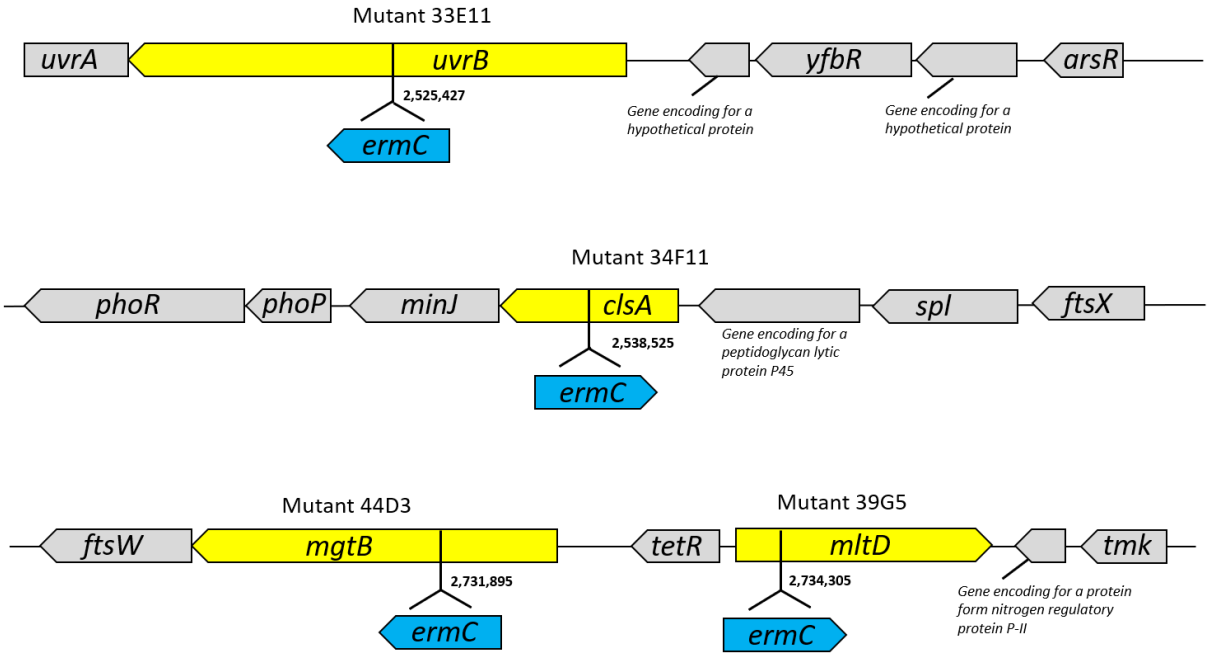
Supplementary Figure S1. Growth of 36 transposon mutants of the *Listeria monocytogenes* 15G01 strain in MWB at 30°C determined by optical density measurements at 595 nm. A 96-well plate with each well containing 200 µL of MWB was inoculated with an overnight culture of *L. monocytogenes* 15G01 and mutants grown in TSBYE at 37°C using a 96-well replicator. The turbidity of the wells was measured with a microplate reader at a wavelength of 595 nm at given time points. The readings were averaged and plotted against measured time points to produce a growth curve. OD_{595nm} values of the samples were corrected by subtracting the OD_{595nm} values for uninoculated media. The wild-type growth curve is shown in red.



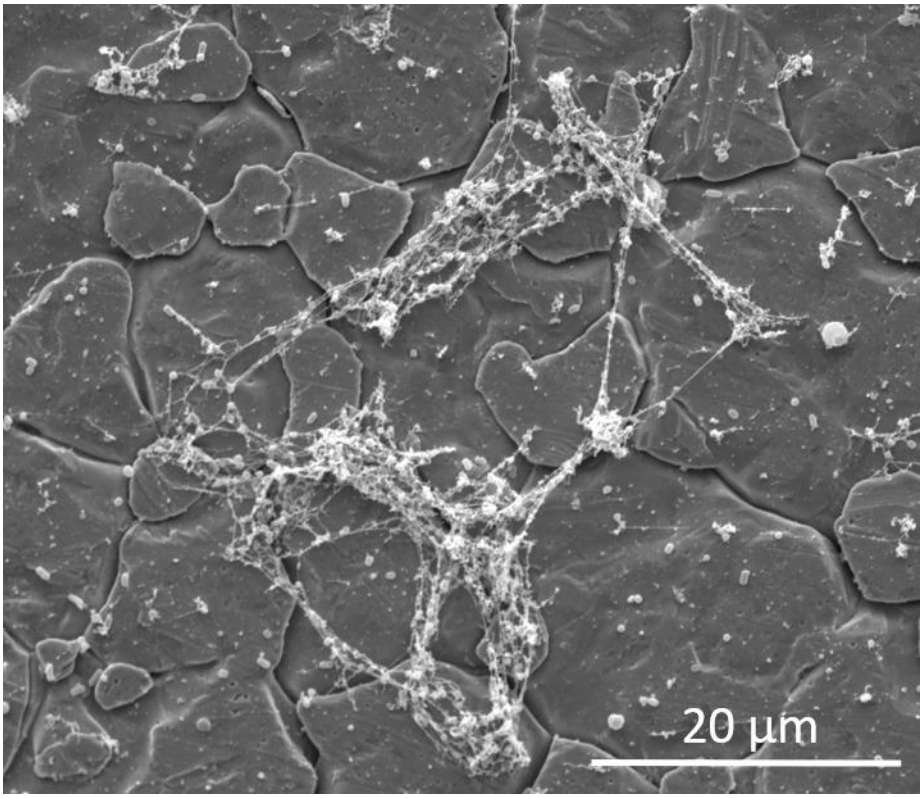
Supplementary Figure S2. Growth of the *Listeria monocytogenes* strain 15G01 (wt) and its four mutants in MWB at 30°C measured with an automated microplate reader at defined time points at 600 nm. The mutants have been grown without selective antibiotics to eliminate its effects on growth. The growth curves pictured are the means of two measurements.



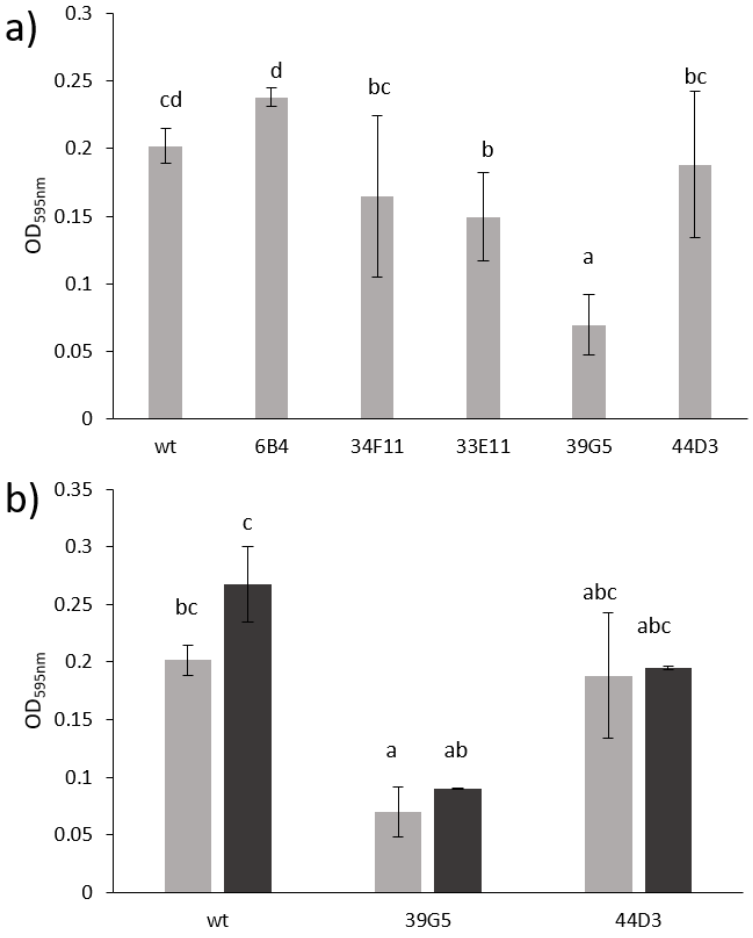
Supplementary Figure S3a. A representation of the genome of *Listeria monocytogenes* EGD showing the locations of transposon insertion sites associated with biofilm formation in *L. monocytogenes* 15G01. The outer ring represents the scale in bp, protein coding sequences are shown in turquoise, the middle ring highlights genes with a transposon insertion leading to greater biofilm formation (orange) and low biofilm formation (blue) in 15G01 and the inner ring (purple/green) shows the G+C % content plot with the GC skew. The image was generated using DNAPlotter (version 1.11) available from www.sanger.ac.uk.



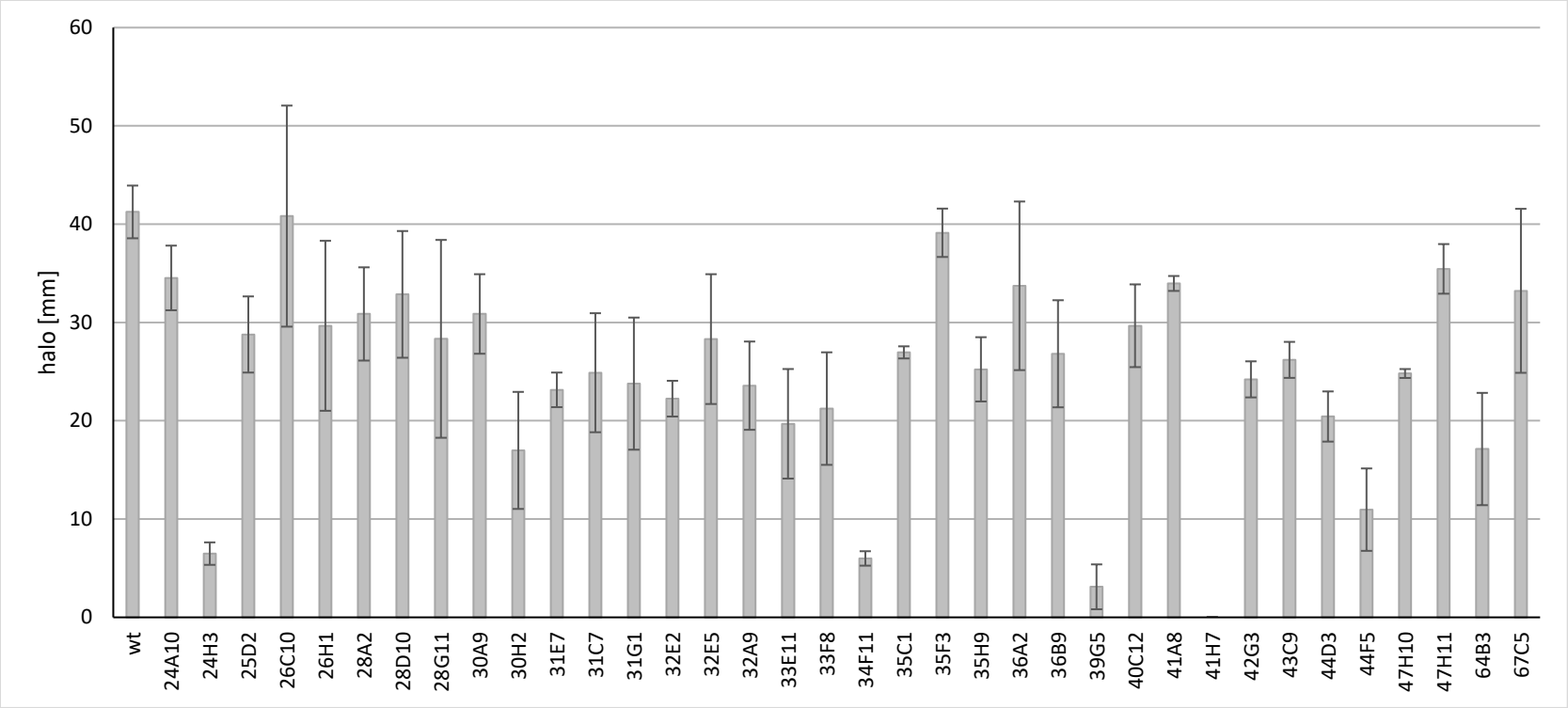
Supplementary Figure S3b. Co-ordinates of the transposon insertions of the four genes hit by the transposon in *L. monocytogenes* 15G01.



Supplementary Figure S4. Scanning electron microscopy image of a biofilm formed by the *flaA* mutant (41H7) after 7 d incubation at 30°C on stainless steel coupons coated with mussel juice at a 5000x magnification.



Supplementary Figure S5. Attachment of the *Listeria monocytogenes* 15G01 (wt) strain and the five mutant strains on polystyrene after 30 min at 30°C in MWB (a) and attachment of *L. monocytogenes* 15G01 (wt) and the mutants in MWB (light grey bars) and in MWB-5mM Mg^{2+} (dark grey bars) (b). Error bars represent the standard deviation of two experiments with n=12. Letters in common indicate no significant difference.



Supplementary Figure S6. Motility of the *Listeria monocytogenes* 15G01 (wt) strain and the 36 mutant strains after 48 h of incubation at 30°C measured as halo produced in TSA+0.25% agar. The error bars represent standard deviations of three independent experiments carried out with three replicates each.

Supplementary Table S1. Growth of 36 transposon mutants of the *Listeria monocytogenes* 15G01 strain in MWB at 30°C determined by optical density measurements at 595 nm.

To compare the mutant strains to the wild-type, logistic growth curves were fitted to each repetition of each strain (using the FITCURVE procedure in Genstat version 17, 2014). Because of the declines noted for some strains at 60 h, the 60 h data were excluded. The parameters of the curves were then compared to those for the wild-type replicates using t-tests.

All the strains were significantly different from the wild-type on at least one parameter. The most consistent difference was that all but two strains (24A10 and 33E11) had significantly higher m. The other notable feature was that most strains grew slower (lower b) than the wild-type, which is quite likely due to antibiotic presence.

The mean parameters (from the eight repetitions) are tabulated below, along with standard errors (s.e — based on the difference between the repetitions) and p values for the difference from the wild-type. P values are colour coded yellow (significant at p = 0.05 and value is higher than the wild-type) and turquoise (significant at p = 0.05 and value is lower than the wild-type).

	a (starting level)			Slope at time = m			c (how much line rises)			m (midpoint of increasing phase)		
Strain	Mean	s.e.	p	Mean	s.e.	p	Mean	s.e.	p	Mean	s.e.	p
Wild-type	0.15	0.004		0.053	0.001		0.44	0.012		14.6	0.55	
24A10	0.17	0.001	<.001	0.021	0.001	<.001	0.20	0.001	<.001	14.5	0.34	0.949
24H3	0.16	0.003	0.066	0.043	0.001	<.001	0.50	0.009	0.001	21.4	0.21	<.001
25D2	0.11	0.002	<.001	0.037	0.001	<.001	0.45	0.011	0.459	19.3	0.20	<.001
26C10	0.15	0.005	0.989	0.043	0.002	0.004	0.27	0.002	<.001	17.0	0.39	0.003
26H1	0.15	0.001	0.589	0.040	0.001	<.001	0.43	0.008	0.404	18.6	0.15	<.001
28A2	0.19	0.001	<.001	0.049	0.001	0.108	0.50	0.011	0.002	20.5	0.31	<.001
28D12	0.18	0.002	<.001	0.048	0.001	0.013	0.41	0.006	0.056	19.1	0.33	<.001
28G11	0.17	0.004	0.001	0.054	0.001	0.374	0.47	0.011	0.086	17.8	0.12	0.001
30A9	0.13	0.003	0.002	0.048	0.001	0.015	0.44	0.006	0.889	17.8	0.24	<.001
30H2	0.11	0.004	<.001	0.036	0.001	<.001	0.54	0.004	<.001	22.9	0.50	<.001
31 E7	0.17	0.003	<.001	0.022	0.002	<.001	0.32	0.011	<.001	23.1	0.60	<.001
31C7	0.19	0.002	<.001	0.050	0.001	0.123	0.48	0.006	0.009	20.5	0.32	<.001
31G1	0.12	0.002	<.001	0.046	0.001	0.001	0.45	0.004	0.211	19.5	0.53	<.001
32 E2	0.16	0.004	0.032	0.047	0.001	0.006	0.49	0.011	0.006	20.0	0.21	<.001
32 E5	0.16	0.003	0.019	0.034	0.001	<.001	0.30	0.005	<.001	18.5	0.28	<.001
32A9	0.18	0.001	<.001	0.049	0.001	0.071	0.48	0.007	0.005	19.3	0.18	<.001
33 E11	0.16	0.002	0.020	0.055	0.001	0.220	0.27	0.011	<.001	15.5	0.08	0.151
33F8	0.12	0.003	<.001	0.045	0.002	0.003	0.43	0.014	0.589	18.1	0.14	<.001
34F11	0.13	0.003	0.004	0.038	0.001	<.001	0.40	0.010	0.034	19.7	0.25	<.001
35C1	0.16	0.004	0.050	0.035	0.002	<.001	0.49	0.006	0.002	22.0	0.41	<.001
35F3	0.22	0.002	<.001	0.040	0.000	<.001	0.24	0.003	<.001	16.2	0.14	0.025
35H9	0.20	0.003	<.001	0.052	0.001	0.818	0.49	0.009	0.007	19.2	0.41	<.001
36A2	0.20	0.002	<.001	0.044	0.001	<.001	0.34	0.004	<.001	18.2	0.32	<.001

36B9	0.19	0.002	<.001	0.034	0.001	<.001	0.41	0.010	0.135	21.0	0.13	<.001
39G5	0.20	0.002	<.001	0.031	0.001	<.001	0.31	0.010	<.001	19.4	0.18	<.001
40C12	0.18	0.003	<.001	0.039	0.001	<.001	0.30	0.009	<.001	17.5	0.40	0.001
41A8	0.20	0.003	<.001	0.038	0.001	<.001	0.36	0.003	<.001	19.5	0.20	<.001
41H7	0.17	0.001	0.001	0.050	0.001	0.084	0.43	0.007	0.756	19.3	0.29	<.001
42G3	0.16	0.007	0.296	0.045	0.003	0.044	0.35	0.013	<.001	17.4	0.23	0.001
43C9	0.16	0.003	0.115	0.030	0.002	<.001	0.43	0.009	0.496	21.8	0.50	<.001
44D3	0.17	0.002	<.001	0.044	0.001	<.001	0.53	0.006	<.001	22.0	0.42	<.001
44F5	0.18	0.004	<.001	0.040	0.001	<.001	0.47	0.017	0.112	20.7	0.18	<.001
47H10	0.18	0.002	<.001	0.050	0.001	0.194	0.49	0.008	0.002	20.4	0.26	<.001
47H11	0.18	0.003	<.001	0.050	0.000	0.104	0.63	0.009	<.001	28.1	0.33	<.001
64B3	0.19	0.003	<.001	0.051	0.001	0.449	0.49	0.006	0.003	21.1	0.23	<.001
67C5	0.15	0.004	0.858	0.038	0.002	<.001	0.27	0.003	<.001	17.3	0.32	0.001