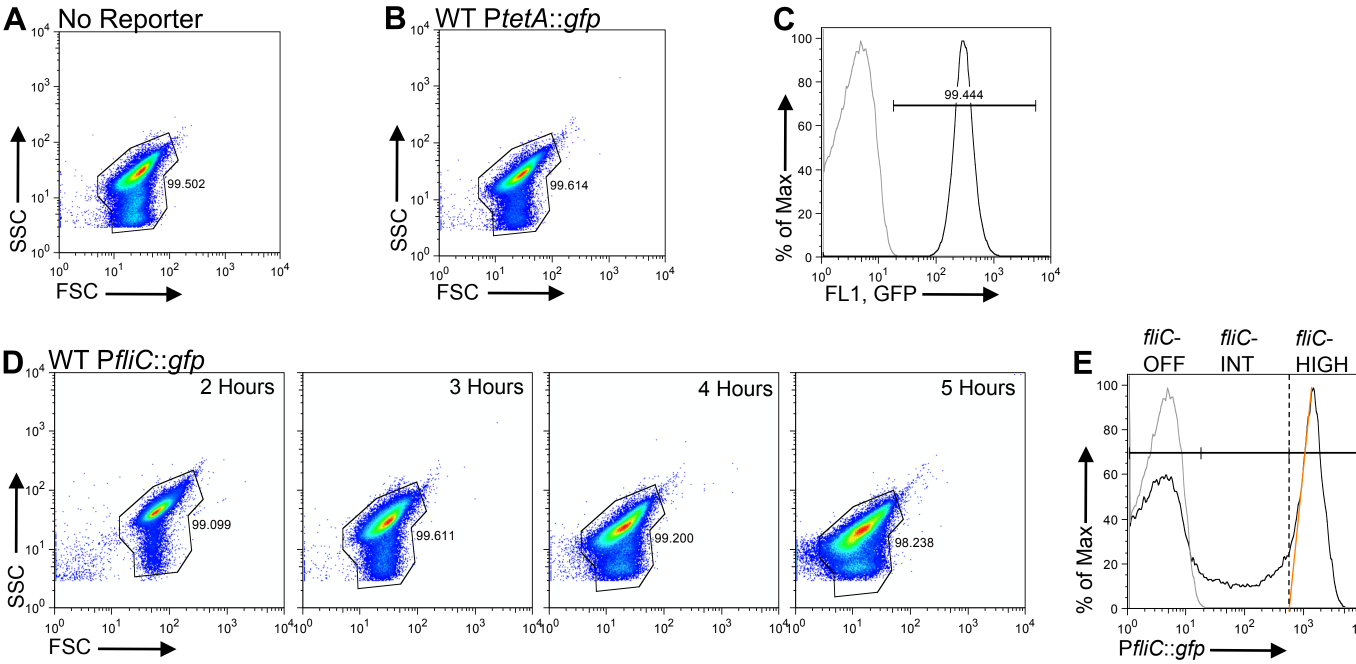

Figure S1: Gating of flow cytometry data for single cell analyses. A-D: Forward scatter (FSC, x-axis) by side scatter (SSC, y-axis) plots identify *Salmonella*-specific events. A two-lobed SSC distribution characterizes *Salmonella* and other rod-shaped organisms (Hewitt, C.J. et al. and Rychlik I. et al.)*.* Data were collected on a Becton Dickinson FACScan flow cytometer as indicated in the methods. A) FSCxSSC plot of total events for one sample of the GFP- WT strain (BC1662). 99.502% of events are within the *Salmonella*-specific gate. B) FSCxSSC plot of total events for one sample of the WT strain harboring a pBR322-based pP*tetA*::*gfp* constitutively expressed reporter (BC1967). 99.614% of events are within the *Salmonella*-specific gate. C) Overlay plot depicting GFP fluorescence of total events shown in (A) (GFP- sample, grey curve) and total events from (B) (constitutive GFP expression, black curve). 99.444% of total events from (B) are GFP+, indicating that they are *Salmonella* as opposed to particulate contaminants. D) FSCxSSC plots for representative WT 14028 pP*fliC*::*gfp* (BC2117) histograms from figure 2A. The *Salmonella*-specific gate was moved through the timecourse to accommodate the changing size, and therefore FSC, of the organisms. E) The *fliC*-OFF population was always defined as the range of fluorescence observed for the GFP- control (grey curve). For figures 2 and 4, the boundary between *fliC*-INT and *fliC*-HIGH was defined as the x-intercept of a line (orange) approximating the slope of the peak of highest expression for the 5 hour WT (BC2117) sample. For figure 5B, the *fliC*-HIGH gate was defined as the most intense peak of expression observed in those experiments (panel 16).

Hewitt, C.J, & Nebe-Von-Caron G. (2001) An Industrial Application of Multiparameter Flow Cytometry: Assessment of Cell Physiological State and its Application to the Study of Microbial Fermentations. *Cytometry* **44**: 179-187.

Rychlik, I., Cardova, L., Sevcik, M., & Barrow, P.A. (2000) Flow cytometry characterisation of *Salmonella typhimurium* mutants defective in proton translocating proteins and stationary-phase growth phenotype. *J Microbiol Methods* **42**: 255-263.

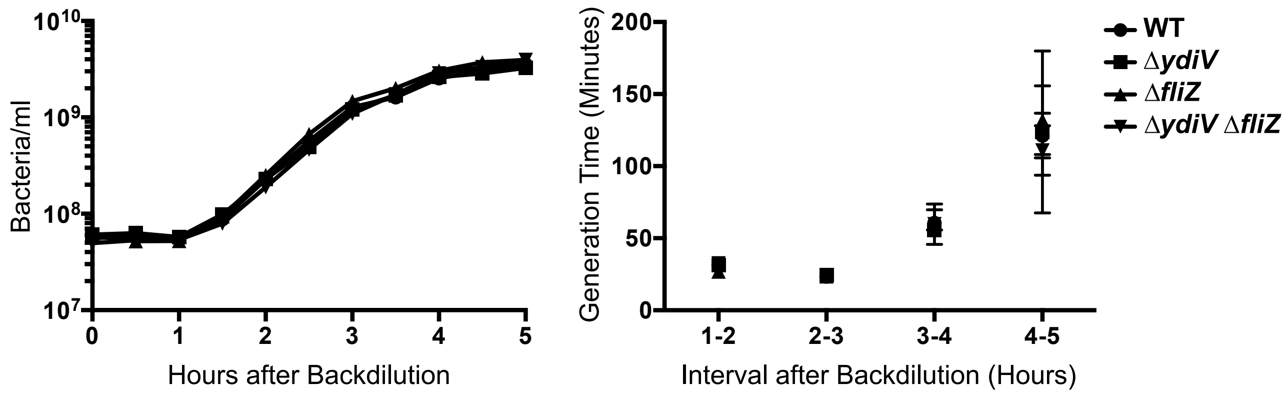

Figure S2: Growth curves for strains used in figures 2 and 4. (WT=BC2117, Δ*ydiV*=BC2118, Δ*fliZ*=BC2119 and Δ*ydiV* Δ*fliZ*=BC2675). Strains were grown as for flow cytometry experiments, in 2ml LB cultures with 100µg/ml carbenicillin, shaking at 225 rpm. Samples for direct counting on a Beckman Coulter Multisizer 4 were taken every 30 minutes. Left panel: Representative set of growth curves. Strains are in exponential growth phase from 1.5-3 hours after backdilution and post-exponential phase for the remainder of the timecourse. Right panel: Generation times for the four strains between 1-2, 2-3, 3-4 and 4-5 hours after backdilution (not calculated for lag phase (0-1 hour) since no appreciable growth occurred, as seen in left panel). Mean and standard deviation for each strain from three independent experiments is plotted for each hour-long interval. There were no significant differences between the strains in generation time within intervals, as determined by 2-way ANOVA.

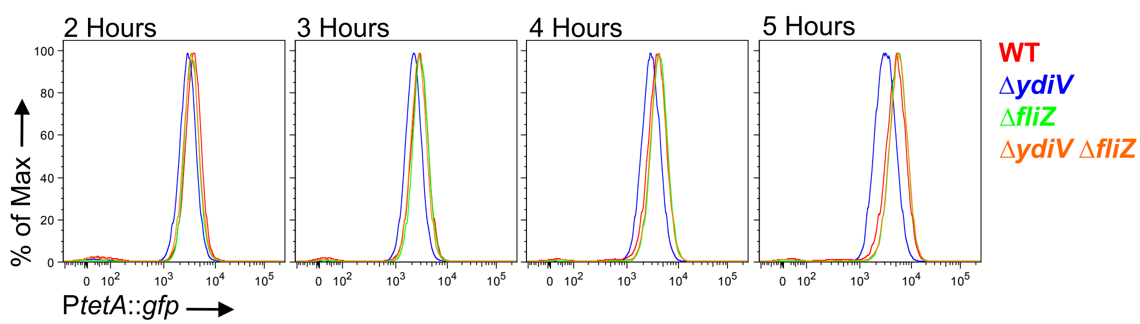

Figure S3: Monomodal expression of a constitutively expressed, plasmid-borne reporter fusion for WT and the three mutant strains across the timecourse. (WT: BC1967, Δ*ydiV*: BC1966, Δ*fliZ*: BC3703, Δ*ydiV* Δ*fliZ*: BC3704). Data were collected on a Becton Dickinson FACSCantoII flow cytometer using the following parameters: FSC-A 600, SSC-A 550, GFP 450, triggering on FSC-H 400 and SSC-H 200. Events shown fell within the *Salmonella*-specific gate, as shown in figure S1.

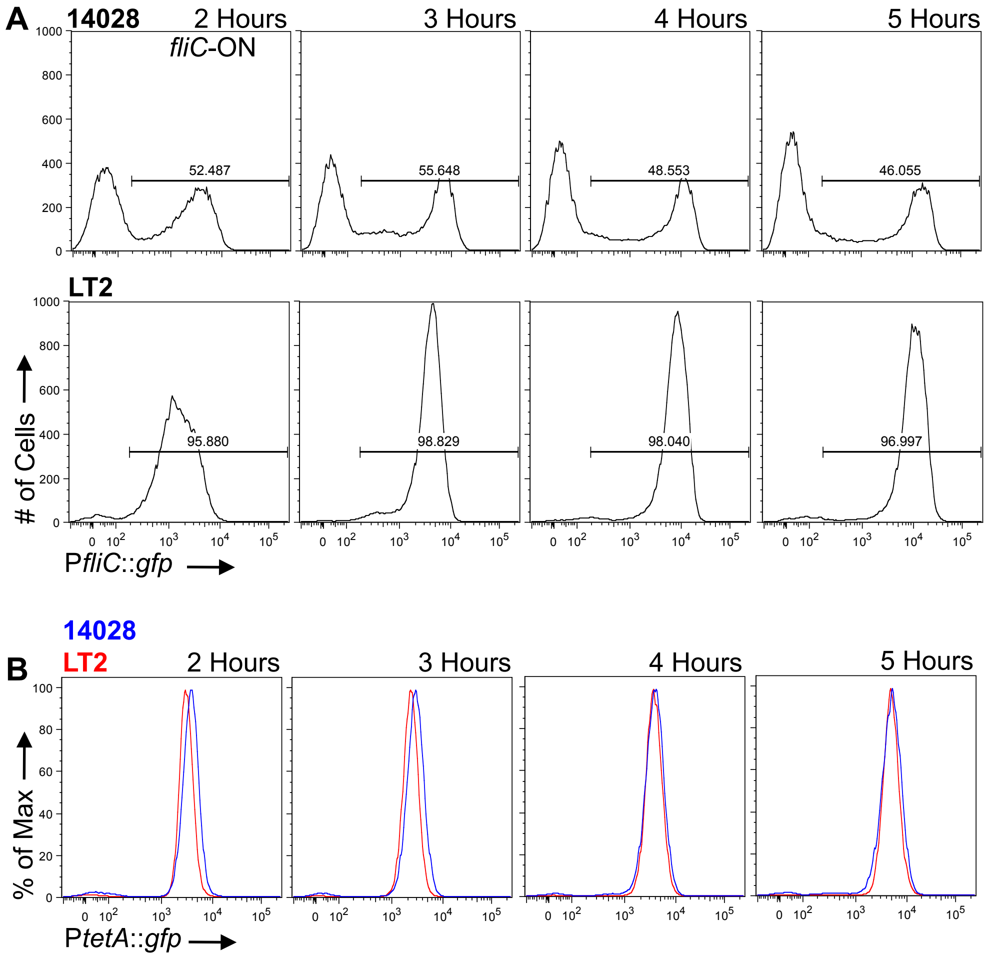

Figure S4: The census of *fliC* expression differs for *Salmonella* strains 14028 and LT2. All data were collected on a Becton Dickinson FACSCantoII flow cytometer using the following parameters: FSC-A 600, SSC-A 550, GFP 450, triggering on FSC-H 400 and SSC-H 200. Events shown fell within the *Salmonella*-specific gate, as shown in figure S1. A) *fliC* expression from 2 through 5 hours after backdilution for strains 14028 (BC2117, top panels) and LT2 (BC3616, bottom panels). 14028 maintains a *fliC*-OFF subpopulation throughout the timecourse, while most LT2 cells activate *fliC* expression by 2 hours. *fliC*-ON gate includes all fluorescence exceeding that of the GFP- control. B) The transcriptional pattern of a constitutively expressed promoter (pP*tetA*::*gfp*) fusion located on pBR322 is monomodal for both 14028 (BC1967) and LT2 (BC3705) throughout the timecourse.

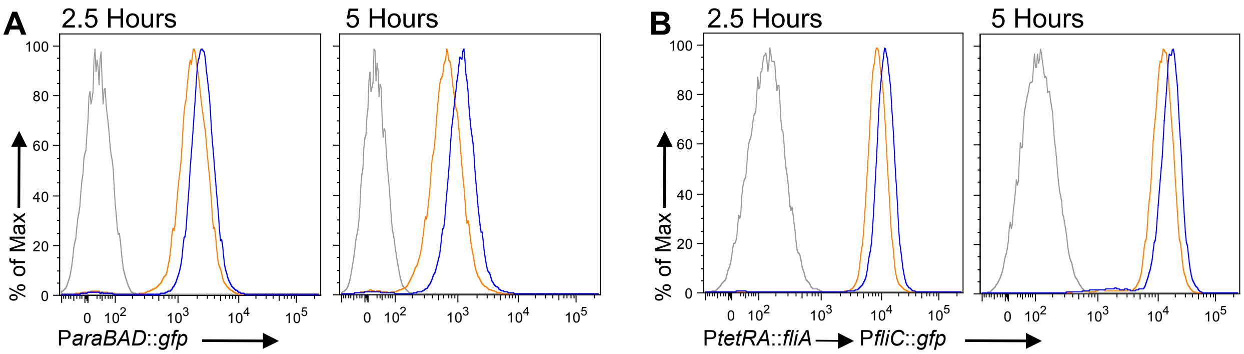

Figure S5: Unimodal expression from inducible promoters used in this study. Data were collected on a Becton Dickinson FACSCantoII flow cytometer using the following parameters: FSC-A 600, SSC-A 550, GFP 450, triggering on FSC-H 400 and SSC-H 200. Events shown fell within the *Salmonella*-specific gate, as shown in figure S1. For A and B, the left panel depicts GFP expression 2.5 hours after backdilution, and the right panel depicts GFP expression 5 hours after backdilution. A) Expression of GFP from P*araBAD* in the pJN105 vector (BC2414). Grey trace: no L-arabinose (L-ara), orange trace: 0.01% L-ara, blue trace: 0.1% L-ara. L-ara was added 2.5 hours before sampling for both timepoints, as for the experiments reported in figure 5B. B) *tetRA*-induced FliA drives unimodal P*fliC*::*gfp* expression (BC2804) Grey trace: 0μg ml^{-1} chlortetracycline (ct), orange trace: 1.6μg ml^{-1} ct, blue trace: 3.1μg ml^{-1} ct).

Table S1: Strains and plasmids used in this study, with construction intermediates

Strains		**Source**
BC156	*Salmonella* Typhimurium 14028	ATCC
BC1662	*Salmonella* Typhimurium 14028 *fljBA*::FRT	Stewart et al., 2011
BC1643	*Salmonella* Typhimurium 14028 *fljBA*::FRT *ydiV*::FRT	Stewart et al., 2011
BC1722	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT	This Study
BC2047	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT *ydiV*::FRT	This Study
BC2117	*Salmonella* Typhimurium 14028 *fljBA*::FRT pP*fliC*::*gfp,* carbR	This Study
BC2118	*Salmonella* Typhimurium 14028 *fljBA*::FRT *ydiV*::FRT pP*fliC*::*gfp*, carbR	This Study
BC2119	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT pP*fliC*::*gfp*, carbR	This Study
BC2675	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT *ydiV*::FRT pP*fliC*::*gfp*, carbR	This Study
BC2307	*Salmonella* Typhimurium 14028 *fljBA*::FRT *ydiV*::FKF::pKG136, kanR	This Study
BC3276	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT *ydiV*::FKF::pKG136 pJN105, kanR gentR	This Study
BC3277	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT *ydiV*::FKF::pKG136 p*fliZ,* kanR gentR	This Study
BC2264	*Salmonella* Typhimurium 14028 *fljBA*::FRT FlhC::3XFLAG, kanR	Stewart et al., 2011
BC2265	*Salmonella* Typhimurium 14028 *fljBA*::FRT *ydiV*::FRT FlhC::3XFLAG, kanR	Stewart et al., 2011
BC2266	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT FlhC::3XFLAG, kanR	This Study
BC2638	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT *ydiV*::FRT FlhC::3XFLAG, kanR	This Study
BC2298	*Salmonella* Typhimurium LT2 *ydiV*::FKF::pKG136, kanR (TH13742)	Wozniak et al., 2009
BC1697	*Salmonella* Typhimurium LT2 *ydiV*240::Tn10dTc(del-25) (*ydiV* T-POP, TH8757), tetR	Wozniak et al., 2009
BC3314	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT *ydiV*240::Tn10dTc(del-25) p*fliZ* pP*fliC*::*gfp*, tetR gentR carbR	This Study
BC2414	*Salmonella* Typhimurium 14028 *fljBA*::FRT p*gfp*, gentR	This Study
BC1091	*Salmonella* Typhimurium 14028 pKAS32::P*fliC*::*gfp*, carbR	Cummings et al., 2006
BC2804	*Salmonella* Typhimurium 14028 *fljBA*::FRT *flgM*::FRT pKAS32::P*fliC*::*gfp tetRA*::*fliA* tet-driven *fliA*, carbR tetR	This Study
BC1967	*Salmonella* Typhimurium 14028 *fljBA*::FRT pP*tetA*::*gfp* carbR	This Study
BC1966	*Salmonella* Typhimurium 14028 *fljBA*::FRT *ydiV*::FRT pP*tetA*::*gfp* carbR	This Study
BC3703	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT pP*tetA*::*gfp* carbR	This Study
BC3704	*Salmonella* Typhimurium 14028 *fljBA*::FRT *fliZ*::FRT *ydiV*::FRT pP*tetA*::*gfp* carbR	This Study
BC3616	*Salmonella* Typhimurium LT2 *fljBA*::FRT pP*fliC*::*gfp*, carbR	This Study
BC3705	*Salmonella* Typhimurium LT2 *fljBA*::FRT pP*tetA*::*gfp* carbR	This Study
Plasmids		
pP*fliC*::*gfp*	pSRB1, P*fliC*::*gfp* construct in pBR322 vector, carbR	Cummings et al., 2006
pJN105	Vector, gentR	Newman et al., 1999
p*fliZ*	*Salmonella* Typhimurium 14028 *fliZ* and native RBS cloned into pJN105, arabinose-induced expression, gentR	This Study
p*gfp*	*gfp* and RBS from pDW5 (Cummings et al., 2006) cloned into pJN105, arabinose-induced expression, gentR	This Study
pP*tetA*::*gfp*	pDW5, carbR	Cummings et al., 2006

Figure 2: Subpopulation Percentages			
Experiment 1			
Sample	Percent in fliC-HIGH Gate	Percent in fliC-INT Gate	Percent in fliC-OFF Gate
BC2117 2 Hr	4.91	51.2	43.9
BC2117 3 Hr	22.9	35.1	42.1
BC2117 4 Hr	31.1	18.8	50.1
BC2117 5 Hr	31.7	14.3	54
BC2119 2 Hr	5.25	45.7	49
BC2119 3 Hr	20.4	31.6	48
BC2119 4 Hr	21.1	28.4	50.5
BC2119 5 Hr	16.8	26.8	56.3
Experiment 2			
Sample	Percent in fliC-HIGH Gate	Percent in fliC-INT Gate	Percent in fliC-OFF Gate
BC2117 2 Hr	7.65	50.4	41.9
BC2117 3 Hr	25.6	33.5	40.8
BC2117 4 Hr	37	20.3	42.7
BC2117 5 Hr	38.8	16.2	45
BC2119 2 Hr	6.26	44.4	49.3
BC2119 3 Hr	26.6	28.4	45.1
BC2119 4 Hr	27.6	26.3	46.1
BC2119 5 Hr	21.5	27.8	50.8
Experiment 3	**(Representative Histograms)**		
Sample	Percent in fliC-HIGH Gate	Percent in fliC-INT Gate	Percent in fliC-OFF Gate
BC2117 2 Hr	7.23	42.7	50.1
BC2117 3 Hr	24.2	30.1	45.7
BC2117 4 Hr	35.3	17.4	47.2
BC2117 5 Hr	34.1	15	51
BC2119 2 Hr	6.29	45.3	48.5
BC2119 3 Hr	26.3	30.3	43.4
BC2119 4 Hr	26.2	26.6	47.2
BC2119 5 Hr	23	29.8	47.2
Averages			
Sample	Percent in fliC-HIGH Gate	Percent in fliC-INT Gate	Percent in fliC-OFF Gate
BC2117 2 Hr	6.6	48.1	45.3
BC2117 3 Hr	24.2	32.9	42.9
BC2117 4 Hr	34.5	18.8	46.7
BC2117 5 Hr	34.9	15.2	50.0
BC2119 2 Hr	5.9	45.1	48.9
BC2119 3 Hr	24.4	30.1	45.5
BC2119 4 Hr	25.0	27.1	47.9
BC2119 5 Hr	20.4	28.1	51.4

Figure 2: Mean Fluorescent Intensities			
Experiment 1			
Sample	fliC-HIGH,Mean,FL1-H	fliC-INT,Mean,FL1-H	fliC-OFF,Mean,FL1-H
BC2117 2 Hr	807	209	6.64
BC2117 3 Hr	908	257	5.05
BC2117 4 Hr	1351	208	4.78
BC2117 5 Hr	1641	191	4.66
BC2119 2 Hr	826	196	6.54
BC2119 3 Hr	926	273	4.77
BC2119 4 Hr	1253	245	4.44
BC2119 5 Hr	1438	223	4.4
Experiment 2			
Sample	fliC-HIGH,Mean,FL1-H	fliC-INT,Mean,FL1-H	fliC-OFF,Mean,FL1-H
BC2117 2 Hr	877	226	5.87
BC2117 3 Hr	936	250	5.14
BC2117 4 Hr	1367	207	4.81
BC2117 5 Hr	1528	215	4.38
BC2119 2 Hr	877	197	6.46
BC2119 3 Hr	996	272	4.93
BC2119 4 Hr	1300	253	4.55
BC2119 5 Hr	1422	242	4.35
Experiment 3	**(Representative Histograms)**		
Sample	fliC-HIGH,Mean,FL1-H	fliC-INT,Mean,FL1-H	fliC-OFF,Mean,FL1-H
BC2117 2 Hr	826	232	6.3
BC2117 3 Hr	971	239	4.86
BC2117 4 Hr	1421	196	4.52
BC2117 5 Hr	1596	193	4.16
BC2119 2 Hr	832	200	6.4
BC2119 3 Hr	973	270	4.49
BC2119 4 Hr	1253	255	4.13
BC2119 5 Hr	1386	241	3.88
Averages			
Sample	fliC-HIGH,Mean,FL1-H	fliC-INT,Mean,FL1-H	fliC-OFF,Mean,FL1-H
BC2117 2 Hr	836.7	222.3	6.3
BC2117 3 Hr	938.3	248.7	5.0
BC2117 4 Hr	1379.7	203.7	4.7
BC2117 5 Hr	1588.3	199.7	4.4
BC2119 2 Hr	845.0	197.7	6.5
BC2119 3 Hr	965.0	271.7	4.7
BC2119 4 Hr	1268.7	251.0	4.4
BC2119 5 Hr	1415.3	235.3	4.2

Figure 4: Subpopulation Percentages			
Experiment 1	**(Representative Histograms)**		
Sample	Percent in fliC-HIGH Gate	Percent in fliC-INT Gate	Percent in fliC-OFF Gate
BC2117 2 Hr	6.21	46.3	47.5
BC2117 3 Hr	30.2	32.7	37.1
BC2117 4 Hr	36.2	17.8	46
BC2117 5 Hr	37.2	15.2	47.6
BC2118 2 Hr	43.7	46	10.4
BC2118 3 Hr	34.2	53.7	12
BC2118 4 Hr	30.4	53.1	16.5
BC2118 5 Hr	31.8	48.6	19.7
BC2119 2 Hr	7.39	45.1	47.5
BC2119 3 Hr	28.4	25.2	46.3
BC2119 4 Hr	22.7	23.5	53.8
BC2119 5 Hr	24	27.3	48.7
BC2675 2 Hr	34.7	44.3	21
BC2675 3 Hr	22.4	58.9	18.7
BC2675 4 Hr	14.5	60	25.5
BC2675 5 Hr	14.1	54.6	31.2
Experiment 2			
Sample	Percent in fliC-HIGH Gate	Percent in fliC-INT Gate	Percent in fliC-OFF Gate
BC2117 2 Hr	8.98	46.7	44.3
BC2117 3 Hr	32.6	31.5	35.9
BC2117 4 Hr	33.1	18.8	48.1
BC2117 5 Hr	35.6	16.3	48.1
BC2118 2 Hr	44.6	44.4	10.9
BC2118 3 Hr	34.5	52.8	12.7
BC2118 4 Hr	29.2	52.9	17.9
BC2118 5 Hr	28.7	49.6	21.7
BC2119 2 Hr	10.4	46.3	43.3
BC2119 3 Hr	35	24	41
BC2119 4 Hr	29.1	23.8	47.1
BC2119 5 Hr	23.6	26.4	50
BC2675 2 Hr	43	42.6	14.4
BC2675 3 Hr	26.6	61.4	12
BC2675 4 Hr	16.1	65.3	18.6
BC2675 5 Hr	15.7	61.4	22.9

Figure 4: Subpopulation Percentages			
Experiment 3			
Sample	Percent in fliC-HIGH Gate	Percent in fliC-INT Gate	Percent in fliC-OFF Gate
BC2117 2 Hr	7.01	44.6	48.4
BC2117 3 Hr	26.1	29.8	44.1
BC2117 4 Hr	33.8	17	49.2
BC2117 5 Hr	34.6	14.2	51.2
BC2118 2 Hr	39.6	48.9	11.5
BC2118 3 Hr	34.7	54.4	10.9
BC2118 4 Hr	30.1	52.2	17.7
BC2118 5 Hr	30.3	48.7	21
BC2119 2 Hr	6.76	44	49.3
BC2119 3 Hr	31	23.7	45.3
BC2119 4 Hr	26.7	24.2	49.1
BC2119 5 Hr	22	27.9	50.2
BC2675 2 Hr	46.2	44.5	9.23
BC2675 3 Hr	32.8	58.5	8.67
BC2675 4 Hr	22.3	63.7	14
BC2675 5 Hr	19.7	61.7	18.7
Averages			
Sample	Percent in fliC-HIGH Gate	Percent in fliC-INT Gate	Percent in fliC-OFF Gate
BC2117 2 Hr	7.4	45.9	46.7
BC2117 3 Hr	29.6	31.3	39.0
BC2117 4 Hr	34.4	17.9	47.8
BC2117 5 Hr	35.8	15.2	49.0
BC2118 2 Hr	42.6	46.4	10.9
BC2118 3 Hr	34.5	53.6	11.9
BC2118 4 Hr	29.9	52.7	17.4
BC2118 5 Hr	30.3	49.0	20.8
BC2119 2 Hr	8.2	45.1	46.7
BC2119 3 Hr	31.5	24.3	44.2
BC2119 4 Hr	26.2	23.8	50.0
BC2119 5 Hr	23.2	27.2	49.6
BC2675 2 Hr	41.3	43.8	14.9
BC2675 3 Hr	27.3	59.6	13.1
BC2675 4 Hr	17.6	63.0	19.4
BC2675 5 Hr	16.5	59.2	24.3

Figure 4: Mean Fluorescent Intensities			
Experiment 1	**(Representative Histograms)**		
Sample	fliC-HIGH,Mean,FL1-H	fliC-INT,Mean,FL1-H	fliC-OFF,Mean,FL1-H
BC2117 2 Hr	794	226	5.89
BC2117 3 Hr	934	285	4.71
BC2117 4 Hr	1301	220	4.3
BC2117 5 Hr	1507	212	3.99
BC2118 2 Hr	1172	247	7.45
BC2118 3 Hr	1233	200	7.42
BC2118 4 Hr	1662	167	7.2
BC2118 5 Hr	1880	150	6.75
BC2119 2 Hr	834	209	6.17
BC2119 3 Hr	1035	270	4.65
BC2119 4 Hr	1301	241	4.19
BC2119 5 Hr	1465	244	3.94
BC2675 2 Hr	1287	229	6.82
BC2675 3 Hr	1444	192	6.19
BC2675 4 Hr	2195	149	6.26
BC2675 5 Hr	2452	128	6
Experiment 2			
Sample	fliC-HIGH,Mean,FL1-H	fliC-INT,Mean,FL1-H	fliC-OFF,Mean,FL1-H
BC2117 2 Hr	827	240	5.97
BC2117 3 Hr	959	277	4.87
BC2117 4 Hr	1276	214	4.47
BC2117 5 Hr	1441	209	4.16
BC2118 2 Hr	1172	249	7.12
BC2118 3 Hr	1248	199	7.38
BC2118 4 Hr	1641	162	7.2
BC2118 5 Hr	1877	139	6.65
BC2119 2 Hr	858	218	6.21
BC2119 3 Hr	1086	277	4.63
BC2119 4 Hr	1298	256	4.13
BC2119 5 Hr	1389	246	3.98
BC2675 2 Hr	1339	245	6.86
BC2675 3 Hr	1482	198	6.72
BC2675 4 Hr	2077	148	7.06
BC2675 5 Hr	2333	131	6.85

Figure 4: Mean Fluorescent Intensities			
Experiment 3			
Sample	fliC-HIGH,Mean,FL1-H	fliC-INT,Mean,FL1-H	fliC-OFF,Mean,FL1-H
BC2117 2 Hr	813	238	6.27
BC2117 3 Hr	967	255	4.89
BC2117 4 Hr	1381	204	4.48
BC2117 5 Hr	1575	200	4.12
BC2118 2 Hr	1129	244	7.77
BC2118 3 Hr	1236	207	7.42
BC2118 4 Hr	1671	170	7.28
BC2118 5 Hr	1783	145	6.73
BC2119 2 Hr	826	209	6.26
BC2119 3 Hr	1051	276	4.49
BC2119 4 Hr	1255	260	4.08
BC2119 5 Hr	1384	244	3.83
BC2675 2 Hr	1262	245	7.87
BC2675 3 Hr	1466	203	7.48
BC2675 4 Hr	2013	159	7.53
BC2675 5 Hr	2401	136	7.31
Averages			
Sample	fliC-HIGH,Mean,FL1-H	fliC-INT,Mean,FL1-H	fliC-OFF,Mean,FL1-H
BC2117 2 Hr	811.3	234.7	6.0
BC2117 3 Hr	953.3	272.3	4.8
BC2117 4 Hr	1319.3	212.7	4.4
BC2117 5 Hr	1507.7	207.0	4.1
BC2118 2 Hr	1157.7	246.7	7.4
BC2118 3 Hr	1239.0	202.0	7.4
BC2118 4 Hr	1658.0	166.3	7.2
BC2118 5 Hr	1846.7	144.7	6.7
BC2119 2 Hr	839.3	212.0	6.2
BC2119 3 Hr	1057.3	274.3	4.6
BC2119 4 Hr	1284.7	252.3	4.1
BC2119 5 Hr	1412.7	244.7	3.9
BC2675 2 Hr	1296.0	239.7	7.2
BC2675 3 Hr	1464.0	197.7	6.8
BC2675 4 Hr	2095.0	152.0	7.0
BC2675 5 Hr	2395.3	131.7	6.7