Electronic Supplementary Material (ESI) for Integrative Biology.

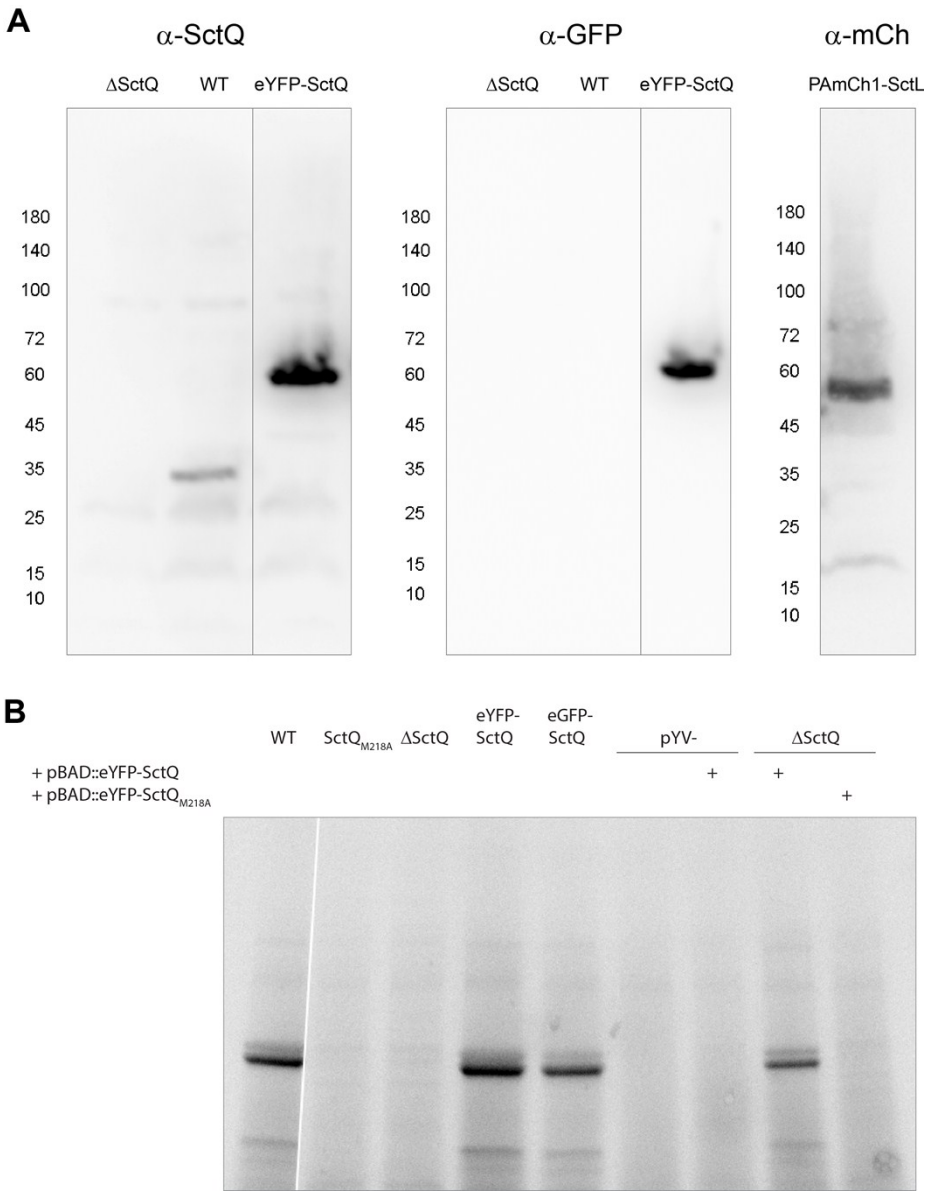

Fig. S1. The fusion proteins used in this study are stable and functional. (A) Immunoblot of total cellular proteins of *Y. enterocolitica* dHOPEMTasd strains expressing the denoted fusion proteins (as well as WT and *ΔsctQ* controls) probed against *Y. enterocolitica* SctQ (left), GFP (center), or mCherry (right) show expression of the full length fusion proteins (expected sizes of WT SctQ, 34.4 kDa; eYFP-SctQ, 62.8 kDa; PAmCherry1-SctL, 53.7 kDa) at higher expression levels compared to wild type, but with no detectable cleavage products. (B) Secretion profile of the indicated strains under secreting conditions. Cells were incubated at 37°C for three hours and the cell culture supernatant was TCA-precipitated. All samples were run and analyzed on the same gel after Coomassie staining; the white vertical line indicate omission of intermediate lanes. Gel loading was normalized by culture optical density at 600 nm, so that content from ~$3x10^9$ cells were used in each lane.

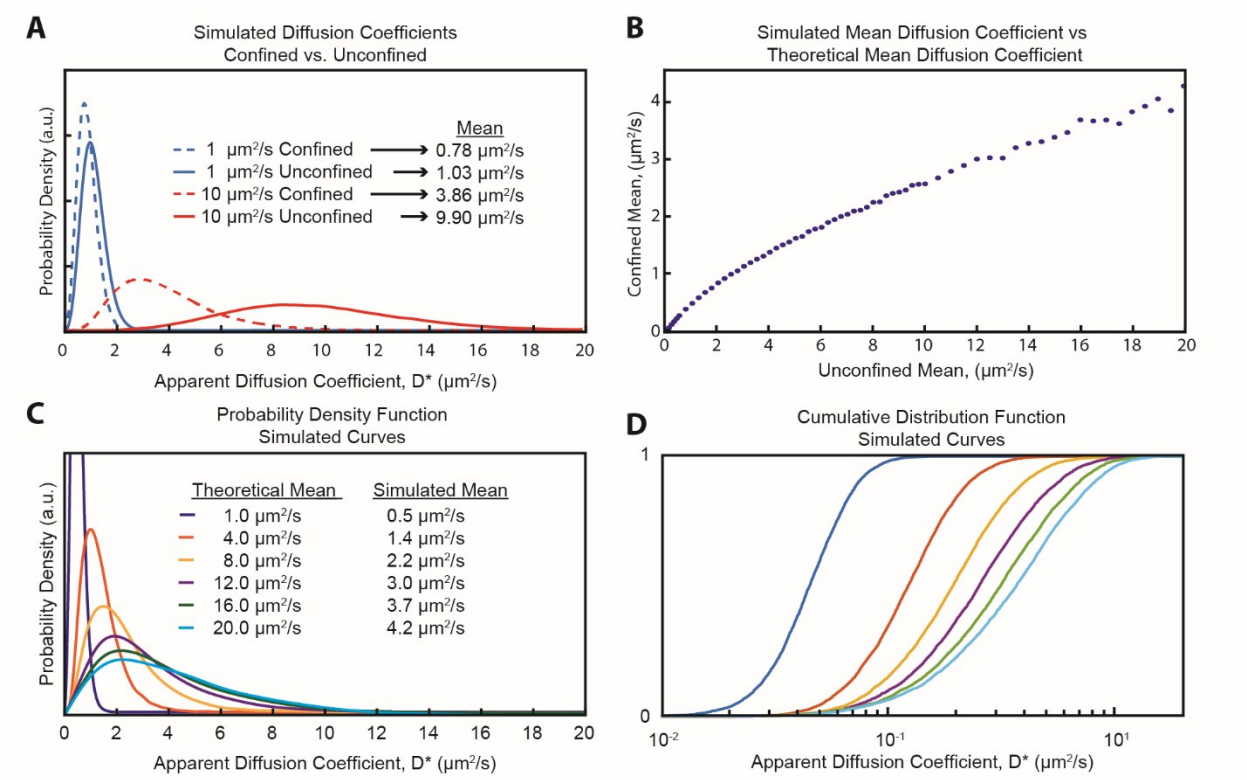

Fig. S2. Monte-Carlo simulation of apparent diffusion coefficient distributions. (A) Left-shift of apparent diffusion coefficient distributions due to cellular confinement for simulated trajectories. (B) Decrease in mean apparent diffusion coefficient for simulated trajectories when taking into account cellular confinement and motion blur. (C) Subset of simulated probability density functions when taking into account cellular confinement and motion blur. (D) Subset of simulated cumulative distribution functions when taking into account cellular confinement and motion blur. Colors are the same as in panel C.

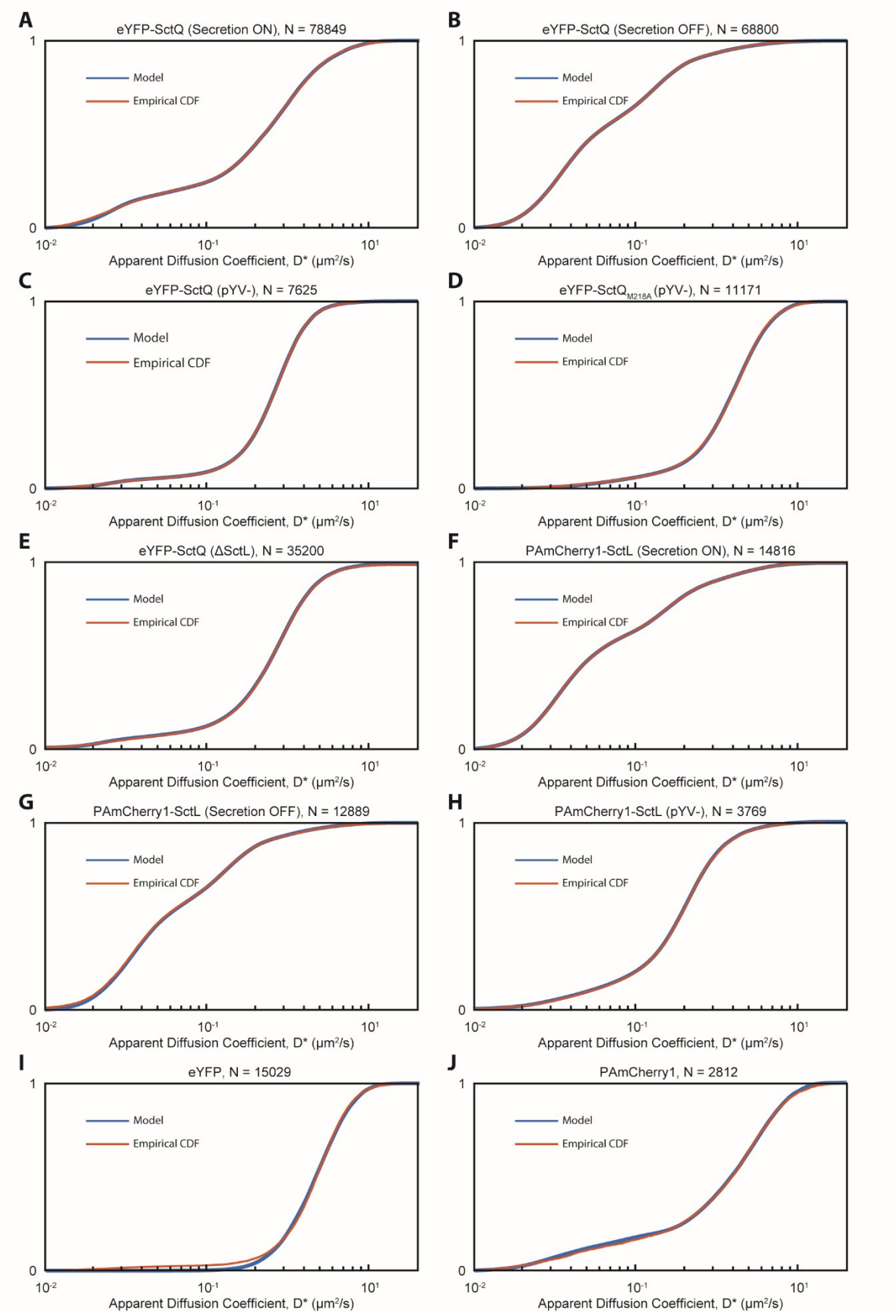
A
eYFP-SctQ (Secretion ON), N = 78849
B
eYFP-SctQ (Secretion OFF), N = 68800
C
eYFP-SctQ (pYV-), N = 7625
D
eYFP-SctQ$_{M218A}$ (pYV-), N = 11171
E
eYFP-SctQ (ΔSctL), N = 35200
F
PAmCherry1-SctL (Secretion ON), N = 14816
G
PAmCherry1-SctL (Secretion OFF), N = 12889
H
PAmCherry1-SctL (pYV-), N = 3769
I
eYFP, N = 15029
J
PAmCherry1, N = 2812
Model
Empirical CDF
1
0
10^{-2}
10^{-1}
10^{1}
Apparent Diffusion Coefficient, D* (μm²/s)

Fig. S3. Apparent diffusion coefficient distributions measured in live *Y. enterocolitica* cells and corresponding fits (shown as CDFs). (A) eYFP-SctQ Secretion ON, (B) eYFP-SctQ Secretion OFF, (C) eYFP-SctQ in pYV- background, (D) eYFP-$SctQ_{M218A}$ in pYV- background, (E) eYFP-SctQ, ΔSctL (F) PAmCherry1-SctL Secretion ON, (G) PAmCherry1-SctL Secretion OFF, (H) PAmCherry1-SctL in pYV- background, (I) eYFP, (J) PAmCherry1.

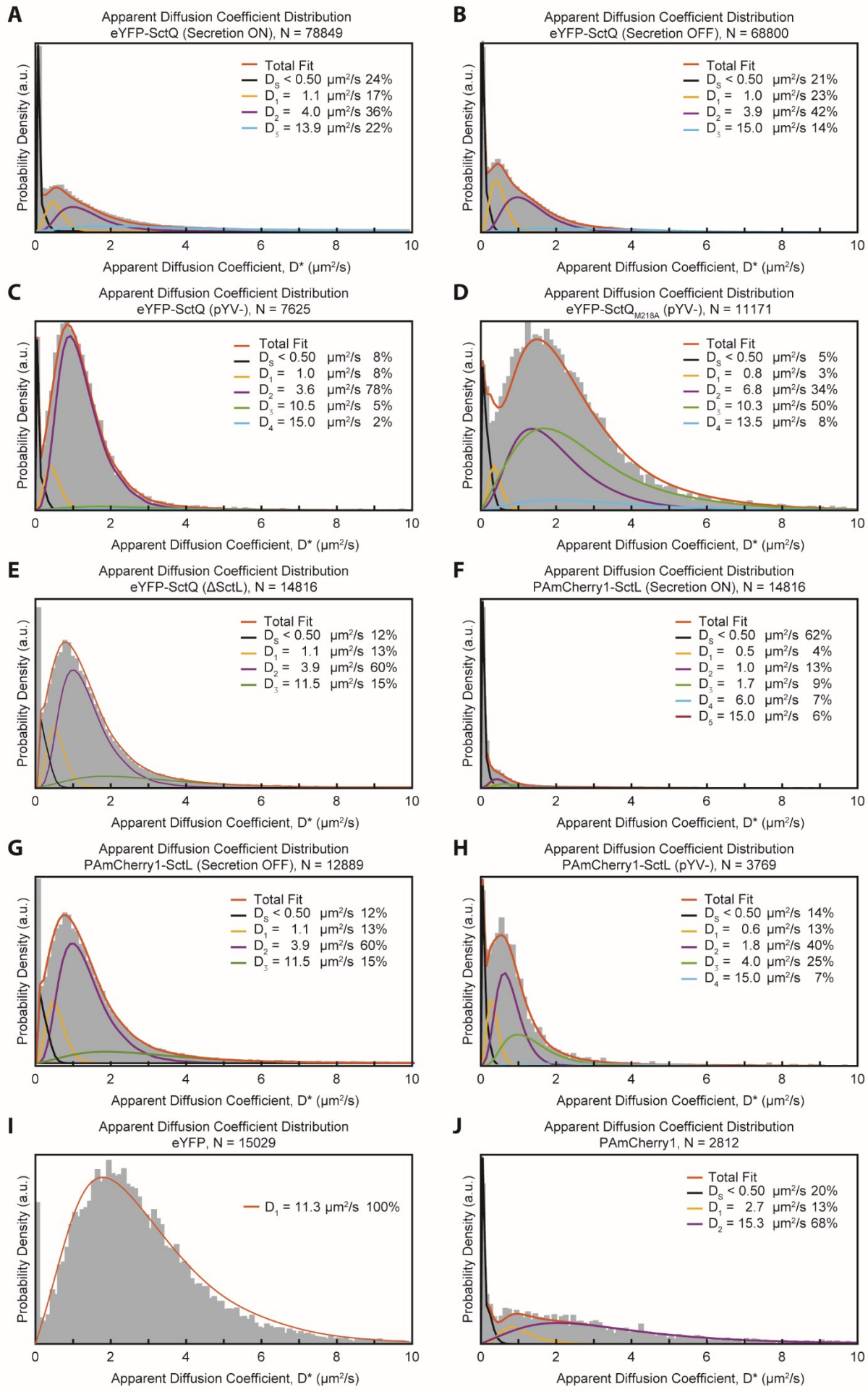
A
Apparent Diffusion Coefficient Distribution
eYFP-SctQ (Secretion ON), N = 78849
Total Fit
D_S < 0.50 µm²/s 24%
D_1 = 1.1 µm²/s 17%
D_2 = 4.0 µm²/s 36%
D_3 = 13.9 µm²/s 22%
B
Apparent Diffusion Coefficient Distribution
eYFP-SctQ (Secretion OFF), N = 68800
Total Fit
D_S < 0.50 µm²/s 21%
D_1 = 1.0 µm²/s 23%
D_2 = 3.9 µm²/s 42%
D_3 = 15.0 µm²/s 14%
C
Apparent Diffusion Coefficient Distribution
eYFP-SctQ (pYV-), N = 7625
Total Fit
D_S < 0.50 µm²/s 8%
D_1 = 1.0 µm²/s 8%
D_2 = 3.6 µm²/s 78%
D_3 = 10.5 µm²/s 5%
D_4 = 15.0 µm²/s 2%
D
Apparent Diffusion Coefficient Distribution
eYFP-SctQ$_{M218A}$ (pYV-), N = 11171
Total Fit
D_S < 0.50 µm²/s 5%
D_1 = 0.8 µm²/s 3%
D_2 = 6.8 µm²/s 34%
D_3 = 10.3 µm²/s 50%
D_4 = 13.5 µm²/s 8%
E
Apparent Diffusion Coefficient Distribution
eYFP-SctQ (ΔSctL), N = 14816
Total Fit
D_S < 0.50 µm²/s 12%
D_1 = 1.1 µm²/s 13%
D_2 = 3.9 µm²/s 60%
D_3 = 11.5 µm²/s 15%
F
Apparent Diffusion Coefficient Distribution
PAmCherry1-SctL (Secretion ON), N = 14816
Total Fit
D_S < 0.50 µm²/s 62%
D_1 = 0.5 µm²/s 4%
D_2 = 1.0 µm²/s 13%
D_3 = 1.7 µm²/s 9%
D_4 = 6.0 µm²/s 7%
D_5 = 15.0 µm²/s 6%
G
Apparent Diffusion Coefficient Distribution
PAmCherry1-SctL (Secretion OFF), N = 12889
Total Fit
D_S < 0.50 µm²/s 12%
D_1 = 1.1 µm²/s 13%
D_2 = 3.9 µm²/s 60%
D_3 = 11.5 µm²/s 15%
H
Apparent Diffusion Coefficient Distribution
PAmCherry1-SctL (pYV-), N = 3769
Total Fit
D_S < 0.50 µm²/s 14%
D_1 = 0.6 µm²/s 13%
D_2 = 1.8 µm²/s 40%
D_3 = 4.0 µm²/s 25%
D_4 = 15.0 µm²/s 7%
I
Apparent Diffusion Coefficient Distribution
eYFP, N = 15029
D_1 = 11.3 µm²/s 100%
J
Apparent Diffusion Coefficient Distribution
PAmCherry1, N = 2812
Total Fit
D_S < 0.50 µm²/s 20%
D_1 = 2.7 µm²/s 13%
D_2 = 15.3 µm²/s 68%
Probability Density (a.u.)
Apparent Diffusion Coefficient, D* (µm²/s)
0
2
4
6
8
10

Figure S4. Apparent diffusion coefficients distributions measured in live *Y. enterocolitica* cells and corresponding fits (shown as PDFs). (A) eYFP-SctQ Secretion ON, (B) eYFP-SctQ Secretion OFF, (C) eYFP-SctQ in pYV- background, (D) eYFP-$SctQ_{M218A}$ in pYV- background, (E) eYFP-SctQ, ΔSctL (F) PAmCherry1-SctL Secretion ON, (G) PAmCherry1-SctL Secretion OFF, (H) PAmCherry1-SctL in pYV- background, (I) eYFP, (J) PAmCherry1.

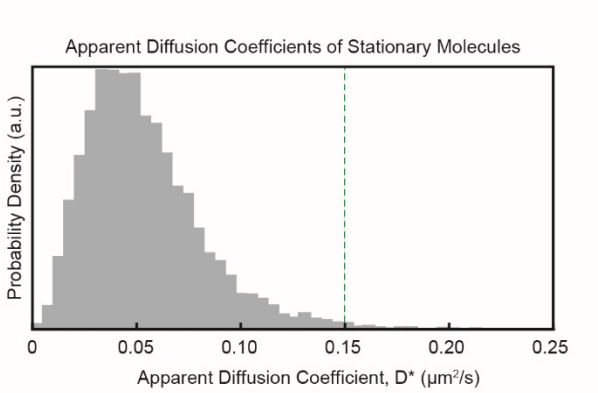

Fig S5. Diffusion coefficient distribution of simulated stationary emitters. Mean localization precisions for x, y, and z positions are 30 nm, 30 nm, and 50 nm, respectively. A threshold of 0.15 $\mu m^2/s$ (dashed green line) was chosen to score trajectories as originating from stationary emitters.

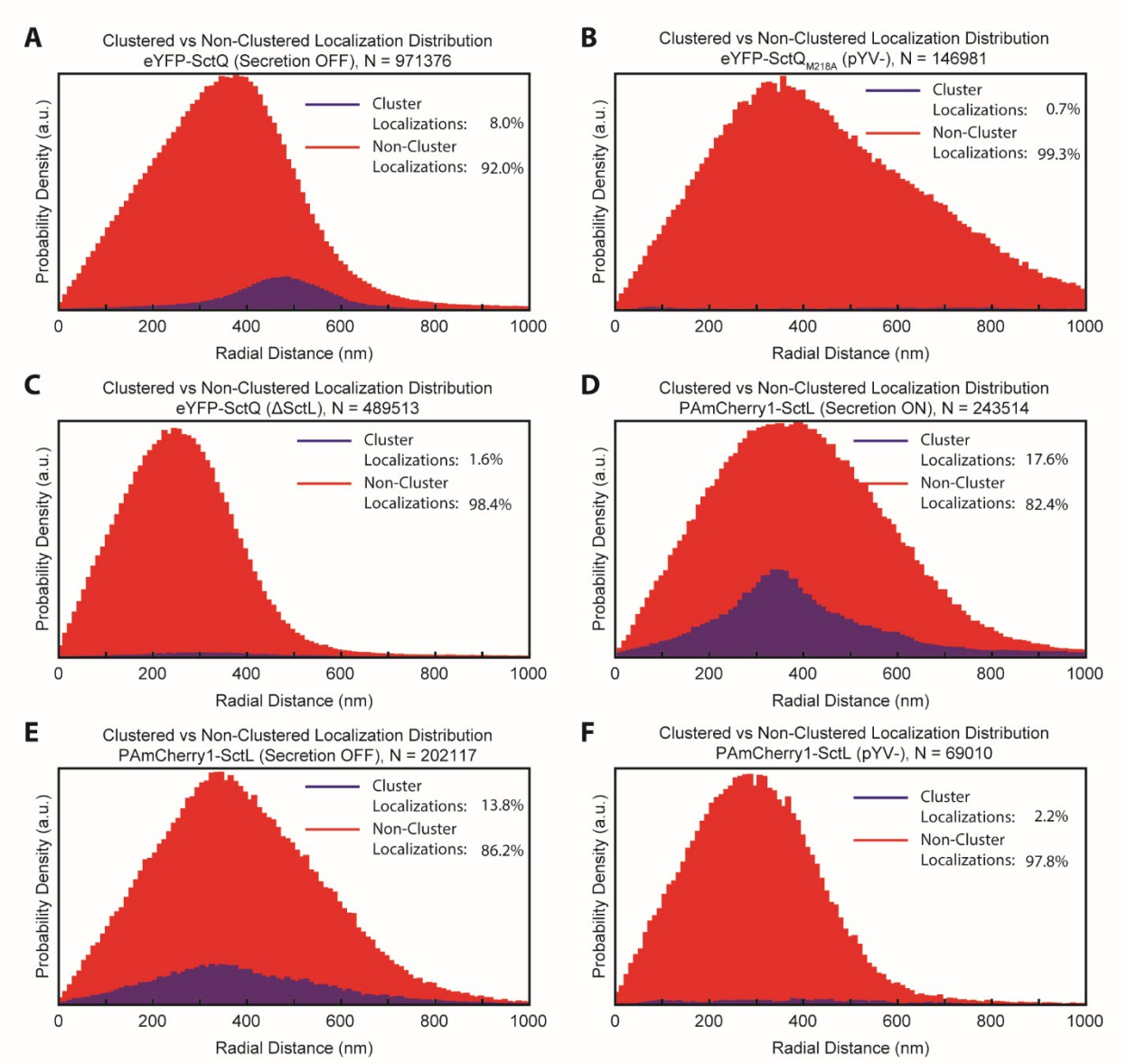

Fig. S6. Radial distribution functions of clustered vs. non-clustered single-molecule localizations. (A) eYFP-SctQ Secretion OFF, (B) eYFP-SctQ$_{M218A}$ in pYV- background, (C) eYFP-SctQ, ΔSctL (D) PAmCherry1-SctL Secretion ON, (E) PAmCherry1-SctL Secretion OFF, (F) PAmCherry1-SctL in pYV- background.

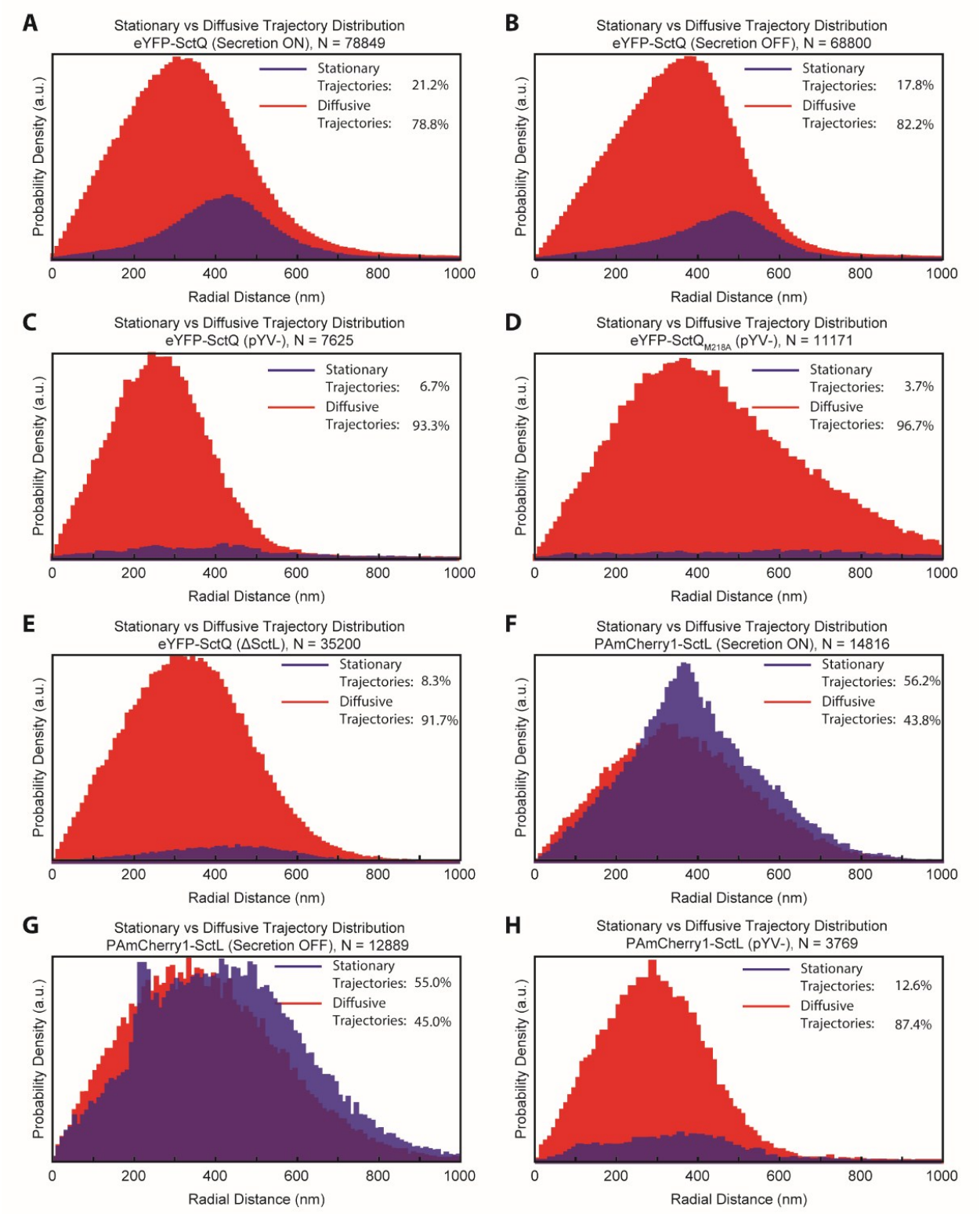
A
Stationary vs Diffusive Trajectory Distribution
eYFP-SctQ (Secretion ON), N = 78849
Stationary Trajectories: 21.2%
Diffusive Trajectories: 78.8%
B
Stationary vs Diffusive Trajectory Distribution
eYFP-SctQ (Secretion OFF), N = 68800
Stationary Trajectories: 17.8%
Diffusive Trajectories: 82.2%
C
Stationary vs Diffusive Trajectory Distribution
eYFP-SctQ (pYV-), N = 7625
Stationary Trajectories: 6.7%
Diffusive Trajectories: 93.3%
D
Stationary vs Diffusive Trajectory Distribution
eYFP-SctQM218A (pYV-), N = 11171
Stationary Trajectories: 3.7%
Diffusive Trajectories: 96.7%
E
Stationary vs Diffusive Trajectory Distribution
eYFP-SctQ (ΔSctL), N = 35200
Stationary Trajectories: 8.3%
Diffusive Trajectories: 91.7%
F
Stationary vs Diffusive Trajectory Distribution
PAmCherry1-SctL (Secretion ON), N = 14816
Stationary Trajectories: 56.2%
Diffusive Trajectories: 43.8%
G
Stationary vs Diffusive Trajectory Distribution
PAmCherry1-SctL (Secretion OFF), N = 12889
Stationary Trajectories: 55.0%
Diffusive Trajectories: 45.0%
H
Stationary vs Diffusive Trajectory Distribution
PAmCherry1-SctL (pYV-), N = 3769
Stationary Trajectories: 12.6%
Diffusive Trajectories: 87.4%
Probability Density (a.u.)
Radial Distance (nm)
0
200
400
600
800
1000

Fig. S7. Radial distribution functions of diffusive ($D > 0.15\ \mu m^2/s$) vs. stationary ($D < 0.15\ \mu m^2/s$) trajectories. (A) eYFP-SctQ Secretion ON, (B) eYFP-SctQ Secretion OFF, (C) eYFP-SctQ in pYV- background, (D) eYFP-$SctQ_{M218A}$ in pYV- background, (E) eYFP-SctQ, ΔSctL, (F) PAmCherry1-SctL Secretion ON, (G) PAmCherry1-SctL Secretion OFF, (H) PAmCherry1-SctL in pYV- background.

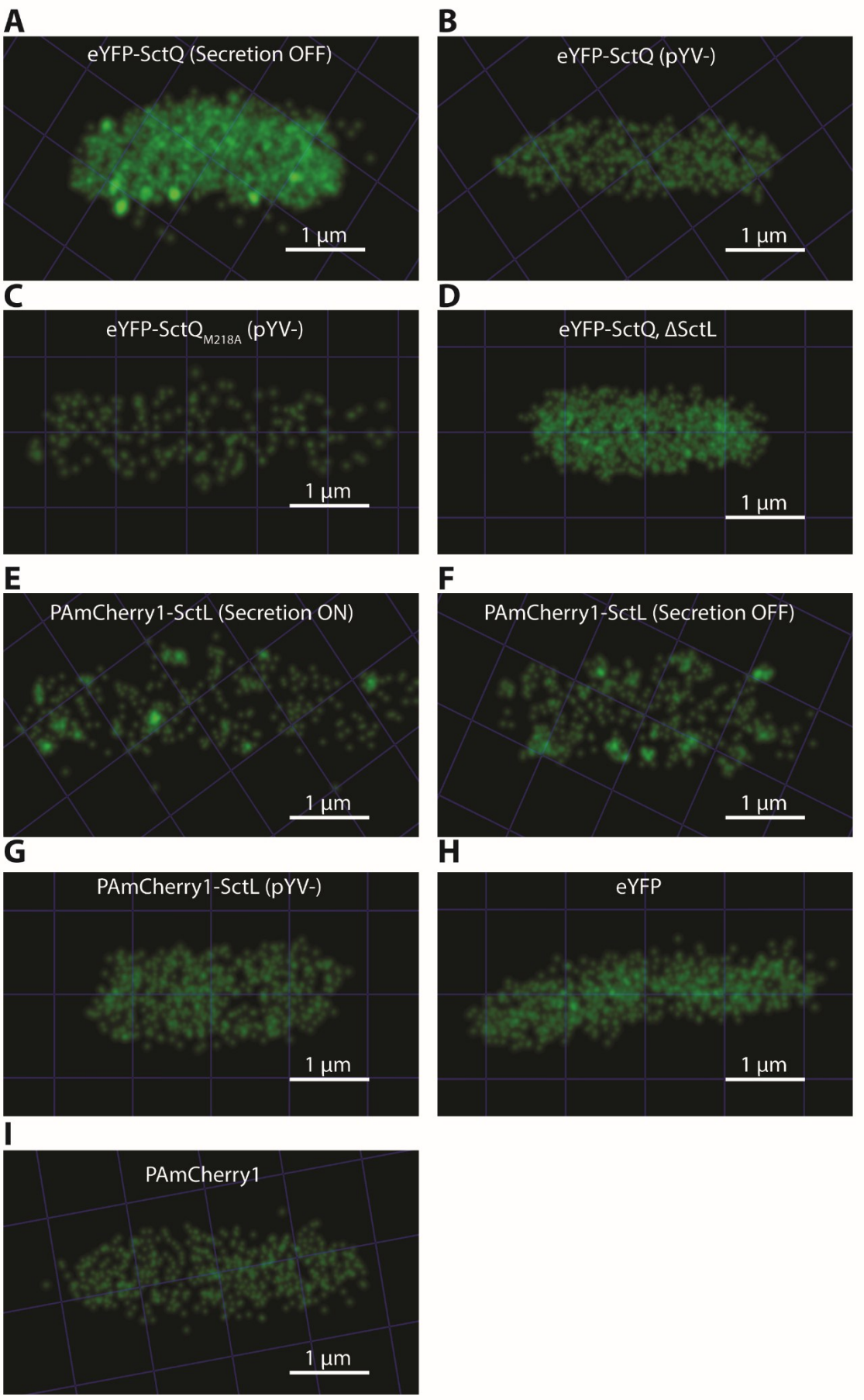
A
eYFP-SctQ (Secretion OFF)
1 µm
B
eYFP-SctQ (pYV-)
1 µm
C
eYFP-SctQ$_{M218A}$ (pYV-)
1 µm
D
eYFP-SctQ, ΔSctL
1 µm
E
PAmCherry1-SctL (Secretion ON)
1 µm
F
PAmCherry1-SctL (Secretion OFF)
1 µm
G
PAmCherry1-SctL (pYV-)
1 µm
H
eYFP
1 µm
I
PAmCherry1
1 µm

Fig. S8. Rendering of individual cells for each strain. (A) eYFP-SctQ Secretion OFF, (B) eYFP-SctQ in pYV- background, (C) eYFP-SctQ$_{M218A}$ in pYV- background, (D) eYFP-SctQ, ΔSctL (E) PAmCherry1-SctL Secretion ON, (F) PAmCherry1-SctL Secretion OFF, (G) PAmCherry1-SctL in pYV- background. (H) eYFP. (I) PAmCherry1

Table S1. List of strains and plasmids.

Strain Name	pYV Background	Characteristics	Ref
Wild-type	IML421	pYV40 *yopO*$_{\Delta 2\text{-}427}$ *yopE*$_{21}$ *yopH*$_{\Delta 1\text{-}352}$ *yopM*$_{23}$ *yopP*$_{23}$ *yopT*$_{135}$ Δ*asd*	[39]
pYV-	--	Lacking pYV40 plasmid	
AD4085	Wild-type	*egfp-yscQ*	[39]
AD4442	Wild-type	*eyfp-yscQ*	This work
AD4459	Wild-type	*pamcherry1-yscL*	This work
AD4601	Wild-type	*eyfp-yscQ,* ΔyscL	This work
AG0001	pYV-	pBAD-eYFP-YscQ	This work
AG0002	pYV-	pBAD-eYFP-YscQ$_{M218A}$	This work
AG0003	Wild-type	pAH12-eYFP	This work
AG0004	Wild-type	pAH12-PAmCherry1	This work
AG0005	pYV-	pBAD-PAmCherry1-YscL	This work
AD4419	Wild-type	*ΔyscQ*	[18]
	ΔyscQ	pBAD-eYFP-YscQ	This work
	ΔyscQ	pBAD-eYFP-YscQ$_{M218A}$	This work

Table S2: Confidence intervals obtained by bootstrapping.

eYFP-SctQ Secretion ON

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.16	0.17	0.19	1.06	1.09	1.14
State 2	0.35	0.36	0.37	3.84	3.92	4.21
State 3	0.20	0.23	0.24	13.43	13.62	15.00

eYFP-SctQ Secretion OFF

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.22	0.23	0.24	0.94	0.97	1.01
State 2	0.37	0.41	0.42	3.71	3.92	3.97
State 3	0.00	0.01	0.10	6.65	9.61	11.50
State 4	0.07	0.14	0.15	15.00	15.16	17.55

eYFP-SctQ pYV-

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.05	0.08	0.11	0.75	0.96	1.19
State 2	0.68	0.76	0.82	3.43	3.57	3.70
State 3	0.00	0.07	0.33	8.89	9.91	10.64
State 4	0.00	0.00	0.06	11.00	13.72	17.55

eYFP-SctQ$_{M218A}$ pYV-

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.03	0.03	0.04	0.71	0.80	1.03
State 2	0.15	0.35	0.51	6.53	6.84	7.70
State 3	0.24	0.50	0.95	10.06	10.31	11.7
State 4	0	0.07	0.19	11.97	13.55	15.1

PAmCherry1-SctL Secretion ON

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.01	0.04	0.09	0.41	0.55	0.65
State 2	0.05	0.13	0.18	0.82	1.01	1.09
State 3	0.05	0.08	0.12	1.33	1.71	1.97
State 4	0.05	0.07	0.09	5.09	5.88	6.00
State 5	0.05	0.06	0.07	13.68	14.97	17.88

PAmCherry1-SctL Secretion OFF

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.09	0.14	0.21	0.49	0.56	0.66
State 2	0.10	0.17	0.22	0.99	1.09	1.26
State 3	0.05	0.06	0.08	3.34	3.93	4.75
State 4	0.04	0.05	0.06	13.69	15.21	18.28

PAmCherry1-SctL pYV-

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.08	0.13	0.18	0.49	0.63	0.75
State 2	0.32	0.40	0.51	1.50	1.76	2.00
State 3	0.15	0.25	0.34	3.57	3.96	4.74
State 4	0.05	0.07	0.10	11.50	14.73	17.61

eYFP

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	1.00	1.00	1.00	11.12	11.26	11.38

PAmCherry1

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.08	0.13	0.19	2.10	2.84	3.90
State 2	0.62	0.67	0.72	14.64	15.34	15.89

eYFP-SctQ, ΔSctL

	Population Fraction			Diffusion Coefficient		
	min	**mean**	**max**	**min**	**mean**	**max**
State 1	0.12	0.13	0.16	1.04	1.12	1.21
State 2	0.58	0.60	0.61	3.81	3.89	4.07
State 3	0.14	0.15	0.16	11.36	11.57	11.81

Table S3: Mean apparent diffusion coefficients (D* > 0.15 μm²/s) for all data sets.

	Mean D* (μm²/s)
eYFP-SctQ Secretion ON	1.69
eYFP-SctQ Secretion OFF	1.43
eYFP-SctQ pYV-	1.30
eYFP-SctQ$_{M218A}$ pYV-	2.35
eYFP-SctQ, ΔSctL	1.33
PAmCherry1-SctL Secretion ON	1.10
PAmCherry1-SctL Secretion OFF	0.86
PAmCherry1-SctL pYV-	1.07
eYFP	2.87
PAmCherry1	2.93