Figure S1

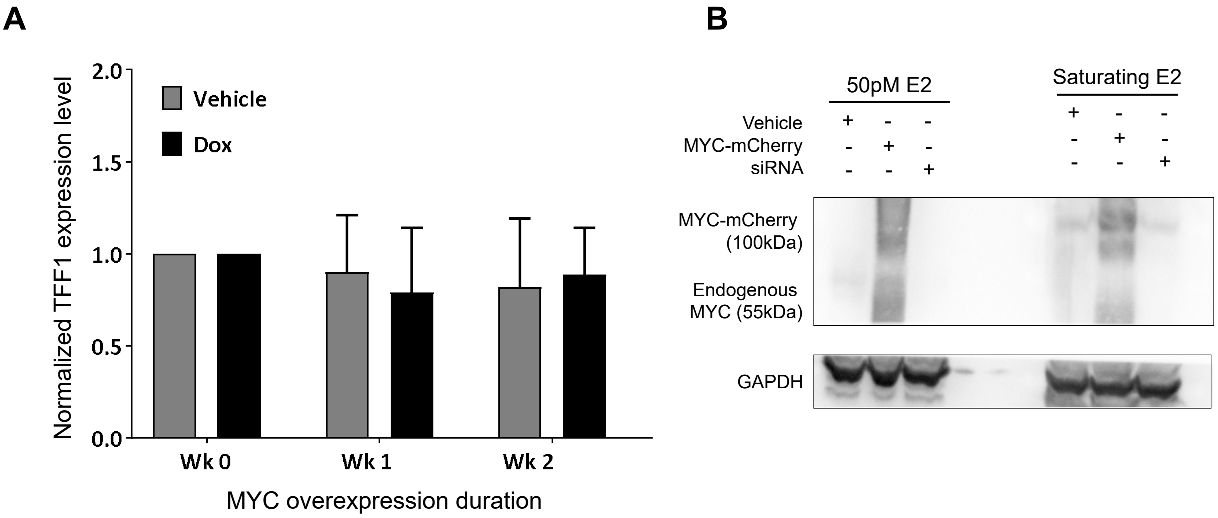


Figure S1. Related to Figure 1.

(A) RT-qPCR quantification of TFF1 expression level in MCF7 cells over a 2-week time course of MYC overexpression with a TetON MYC-EGFP inducible system. Bars represent mean+SD of three biological replicates.

(B) Western blot of MYC perturbed with vehicle, overexpression (MYC-mCherry plasmid) or knockdown (MYC siRNA) in MCF7 cells at 50pM and Saturating E2 levels.

Figure S2

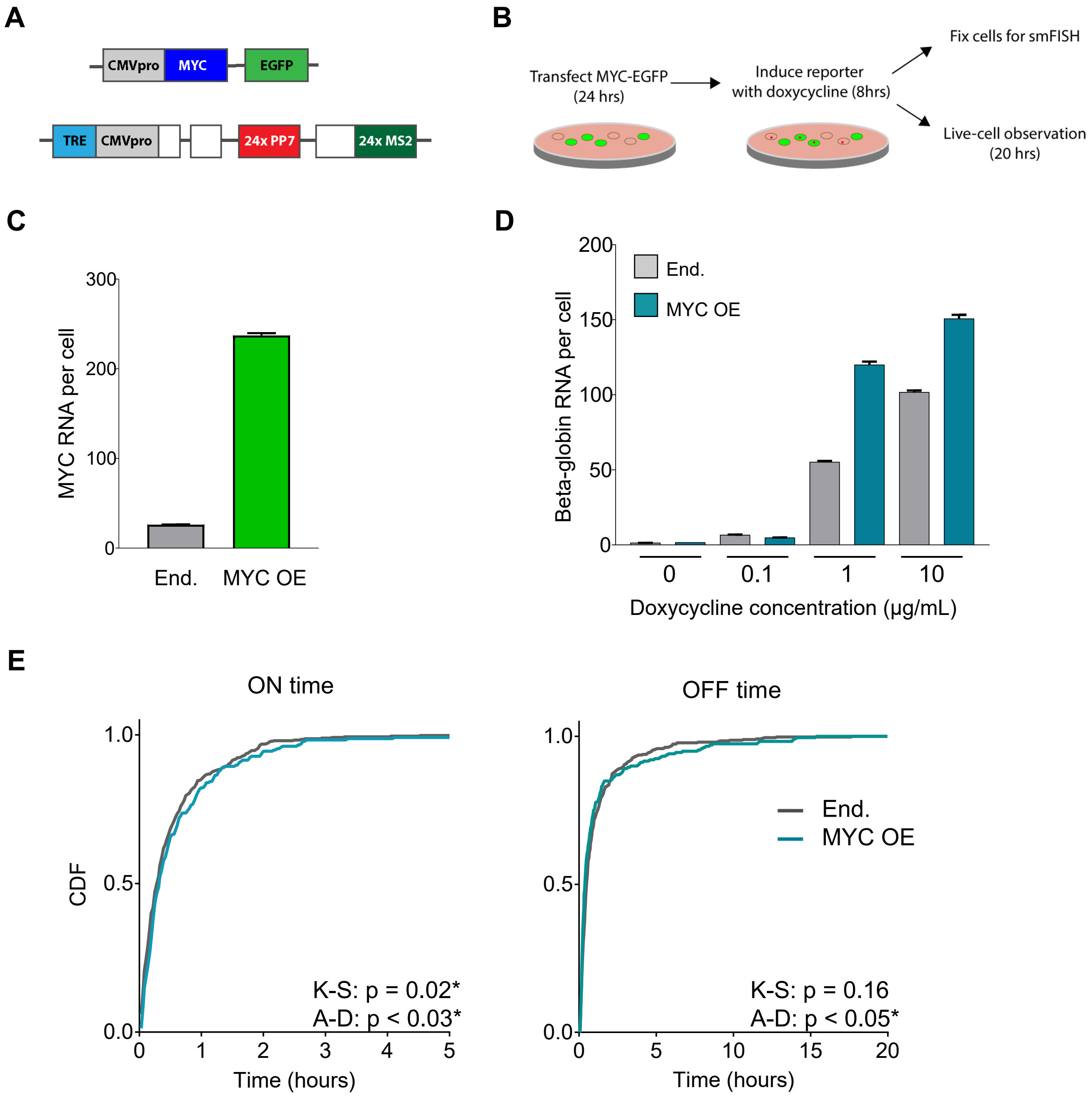


Figure S2. Related to Figure 2.

(A) Schematic of the TetON beta-globin reporter gene stably integrated in U2OS cells, and the MYC-EGFP transgene added as a plasmid transfection. For live-cell imaging, transcription activity was measured from the PP7 stem loop signal in intron 2 observed by the fluorescence of the interacting PP7-mCherry coat protein.

(B) Diagram of live-cell and smFISH experimental setup.

(C) smFISH quantification of MYC RNA per cell with endogenous levels of MYC (End.) and 24 hours MYC-EGFP overexpression (OE).

(D) smFISH quantification of induced beta-globin reporter expression with endogenous MYC levels or MYC overexpression.

(E) Normalized CDF of beta-globin reporter ON and OFF times with endogenous MYC levels or 24 hours MYC-EGFP transient overexpression. The beta-globin reporter was induced with 19.5µM doxycycline 6 hours prior to the start of the microscope acquisition period. Statistical significance from the Kolmogorov-Smirnov and Anderson-Darling tests are both reported.

Figure S3

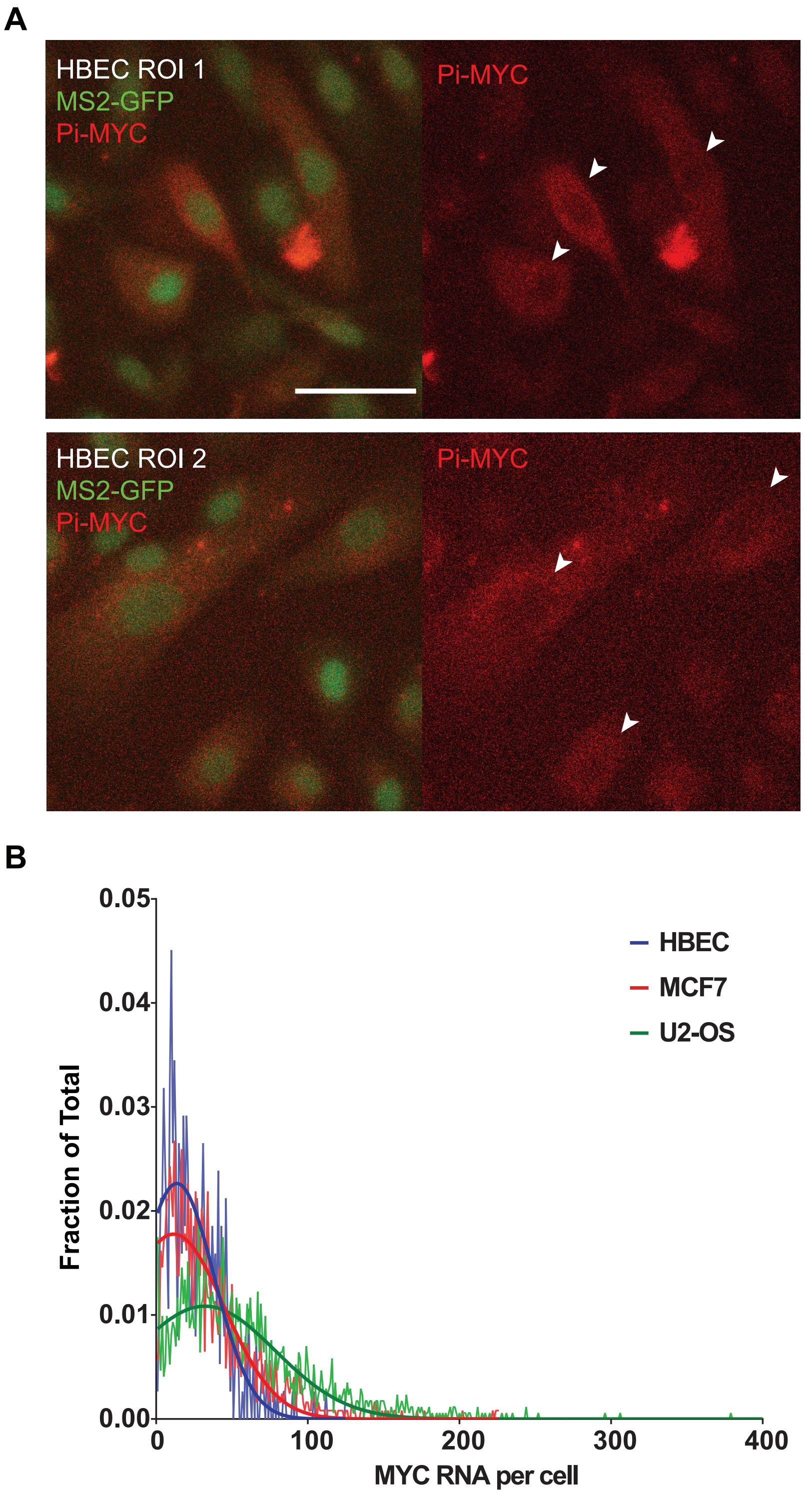
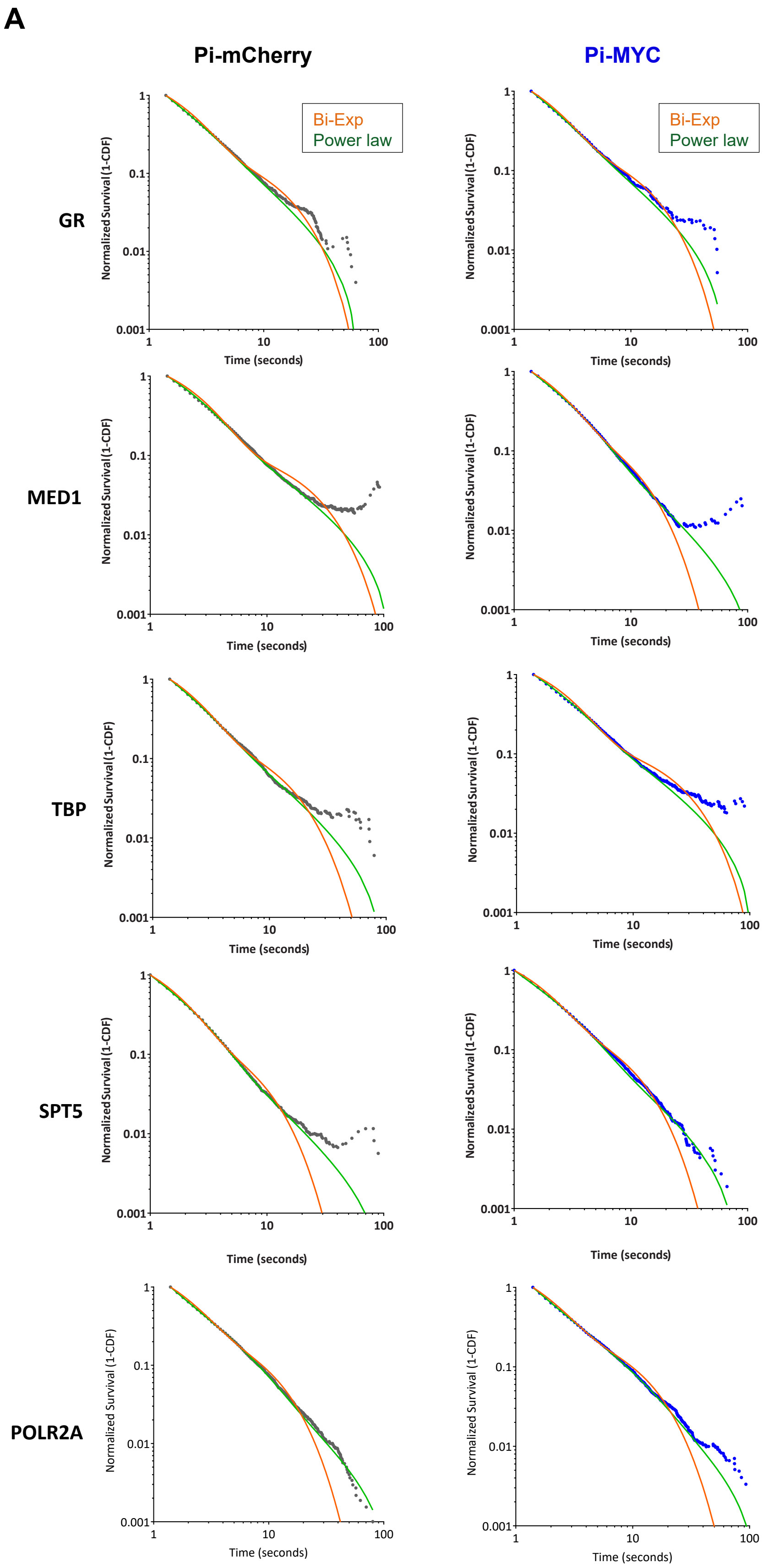


Figure S3. Related to Figure 7.

(A) HBEC cells stably expressing Pi-MYC. Nuclear exclusion in the absence of activating light is indicated by white arrows.

(B) smFISH quantification of endogenous MYC RNA per cell in HBEC (n=125 cells), MCF7 (n=226 cells), and U2OS (n=479 cells). Histograms are fitted to a Gaussian distribution.

Figure S4



B

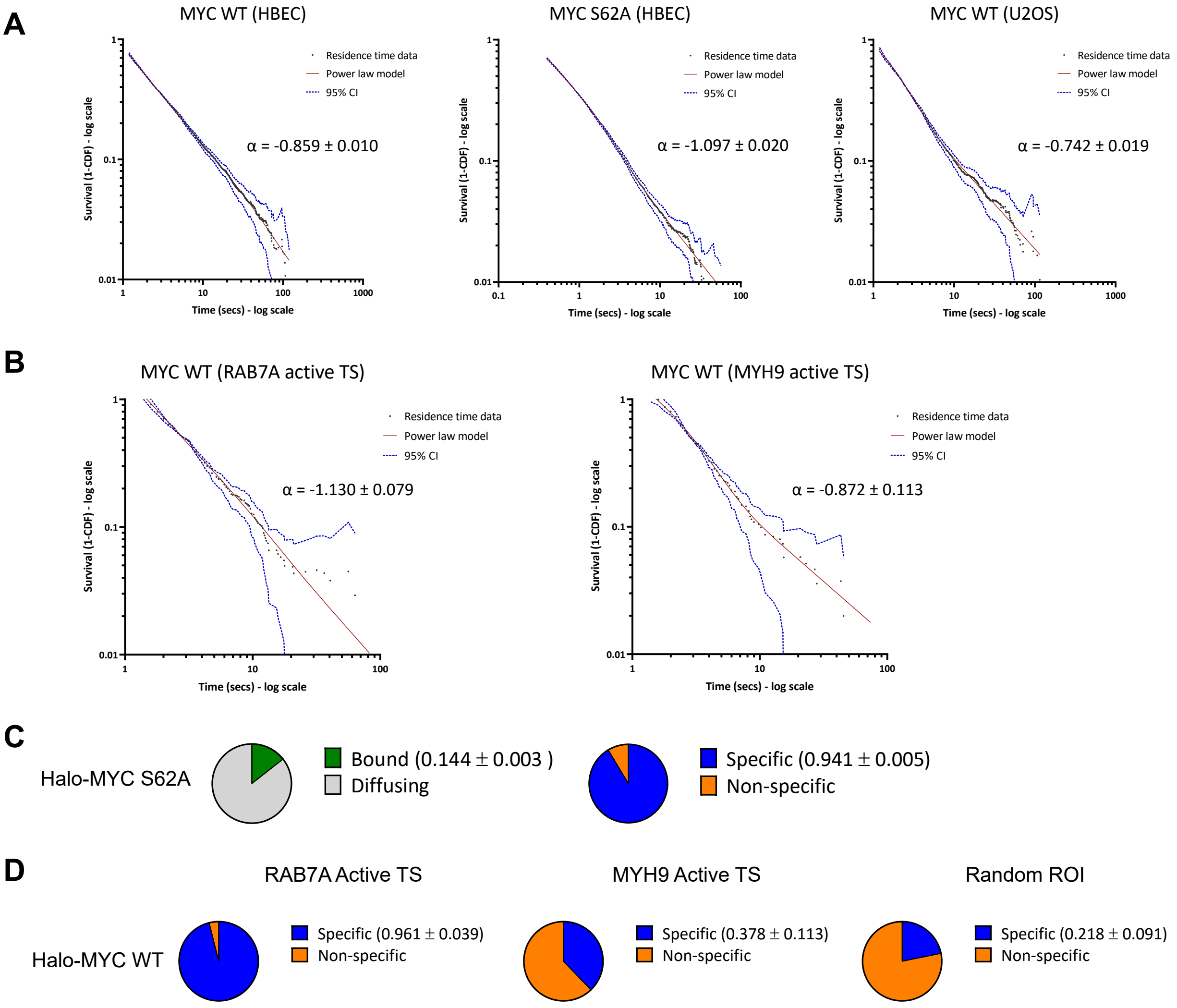
	BIC Values	
	Bi-exponential	Power Law
GR_Pi-mCherry	1.523E+06	1.099E+05
GR_Pi-MYC	1.239E+06	4.859E+04
MED1_Pi-mCherry	3.435E+07	6.032E+06
MED1_Pi-MYC	5.208E+07	1.897E+06
TBP_Pi-mCherry	1.409E+07	7.036E+05
TBP_Pi-MYC	5.200E+07	5.193E+05
SPT5_Pi-mCherry	6.679E+07	1.133E+06
SPT5_Pi-MYC	1.017E+07	5.595E+05
POLR2A_Pi-mCherry	1.985E+07	6.348E+05
POLR2A_Pi-MYC	3.664E+07	1.096E+05

Figure S4. Related to Figure 7.

(A) Raw dwell time data in HBEC Pi-mCherry or Pi-MYC expressing cells (gray and blue scatter plots) fit to a bi-exponential (orange) or power law (green) model.

(B) BIC values for each of the conditions fit to a bi-exponential or power law model.

Figure S5



E

	MYC (HBEC, global)	MYC S62A (HBEC, global)	MYC (U2OS, global)
K_1	0.503 ± 0.022	0.632 ± 0.027	0.559 ± 0.020
T_1 (sec)	1.99 ± 0.09	1.58 ± 0.07	1.79 ± 0.06
K_2	0.037 ± 0.002	0.036 ± 0.002	0.032 ± 0.003
T_2 (sec)	26.88 ± 1.46	28.09 ± 1.81	31.25 ± 2.65

Figure S5. Related to Figure 7.

(A) Survival plot of Halo-MYC and Halo-MYC^{S62A} dwell times fit to a power law distribution (red line) with 95% CI displayed (dashed blue line).

(B) As in (B), Survival plot of Halo-MYC dwell times at *RAB7A* and *MYH9* active transcription sites.

(C) Pie charts illustrating the Halo-MYC^{S62A} bound/diffusing fraction and specific bound/non-specific bound fraction in HBEC.

(D) Pie charts of the Halo-MYC specific bound/non-specific bound fraction in HBEC at the *RAB7A* active TS, *MYH9* active TS, and at random ROI.

(E) Rates and corresponding mean dwell time values resulting from fitting the data in (A) to a bi-exponential distribution.

Table S2

MYH9							
Function	Conventional non-muscle myosin; encoded protein is a myosin IIA heavy chain						
Transcription Sites							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	%Δ TS per cell
n Cells	8695	9866	7999	6531	9280.5	7265	
n TS	5798	6414	3884	2901	6106	3392.5	
TS per cell	0.667	0.650	0.486	0.444	**0.658**	**0.465**	**0.706**
Nascent RNA							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	Δ nascent RNA
Mean	8.3	8.1	7.4	7.4	**8.2**	**7.4**	**-0.8**
Median	7.3	7.2	6.6	6.7	7.2	6.6	
75% percentile	9.6	9.3	8.5	8.6	9.4	8.5	
SD	3.4	3.3	3.0	2.6	3.4	2.8	
Cellular RNA							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	Δ RNA per cell
Mean	149	132	148	135	**140.5**	**141.5**	**1.0**
Median	140	125	140	133	132.5	136.5	
75% percentile	184	171	180	181	177.5	180.5	
SD	73	76	69	79	74.5	74	

KPNB1							
Function	Member of importin beta family						
Transcription Sites							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	%Δ TS per cell
n Cells	4876	3965	3436	3511	4420.5	3473.5	
n TS	2398	2133	1457	1258	2265.5	1357.5	
TS per cell	0.492	0.538	0.424	0.358	**0.515**	**0.391**	**0.760**
Nascent RNA							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	Δ nascent RNA
Mean	6.0	5.9	5.6	6.1	**5.9**	**5.8**	**-0.1**
Median	5.3	5.1	5.0	5.4	5.2	5.2	
75% percentile	6.5	6.2	6.1	6.6	6.4	6.4	
SD	3.4	4.1	2.0	2.3	3.8	2.2	
Cellular RNA							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	Δ RNA per cell
Mean	101	95	72	61	**98**	**66.5**	**-31.5**
Median	93	89	65	51	91	58	
75% percentile	128	124	96	86	126	91	
SD	58	58	47	50	58	48.5	

RAB7A							
Function	RAB family member; regulates vesicular traffic in late endosomes and lysosomes						
Transcription Sites							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	%Δ TS per cell
n Cells	11562	9540	3546	4911	10551	4228.5	
n TS	4007	2318	1804	2897	3162.5	2350.5	
TS per cell	0.347	0.243	0.509	0.590	**0.295**	**0.549**	**1.864**
Nascent RNA							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	Δ nascent RNA
Mean	5.5	4.8	5.9	5.7	**5.2**	**5.8**	**0.6**
Median	4.2	3.9	4.2	4.3	4.0	4.3	
75% percentile	5.8	5.0	6.6	6.1	5.4	6.3	
SD	3.7	3.4	4.6	3.9	3.6	4.3	
Cellular RNA							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	Δ RNA per cell
Mean	49	45	53	59	**47**	**56**	**9.0**
Median	46	42	49	55	44	52	
75% percentile	61	59	66	72	60	69	
SD	29	28	33	35	28.5	34	

RPAP3							
Function	RNA polymerase II-associated protein						
Transcription Sites							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	%Δ TS per cell
n Cells	9459	6881	5852	5955	8170	5903.5	
n TS	2583	2110	2410	1941	2346.5	2175.5	
TS per cell	0.273	0.307	0.412	0.326	**0.290**	**0.369**	**1.273**
Nascent RNA							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	Δ nascent RNA
Mean	3.9	4.7	4.4	4.6	**4.3**	**4.5**	**0.2**
Median	3.5	3.7	3.9	4.0	3.6	3.9	
75% percentile	4.3	4.7	4.8	5.0	4.5	4.9	
SD	1.8	4.0	2.2	2.3	2.9	2.2	
Cellular RNA							
	veh1	veh2	dox1	dox2	veh (ave)	dox (ave)	Δ RNA per cell
Mean	8.1	8.8	13	11	**8.45**	**12**	**3.6**
Median	7	8	12	10	7.5	11	
75% percentile	11	13	17	15	12	16	
SD	5.9	7.4	7.4	7.8	6.65	7.6	

Table S2. Related to Figure 3.

Summary of smFISH RNA quantification measurements.