Expanded View Figures

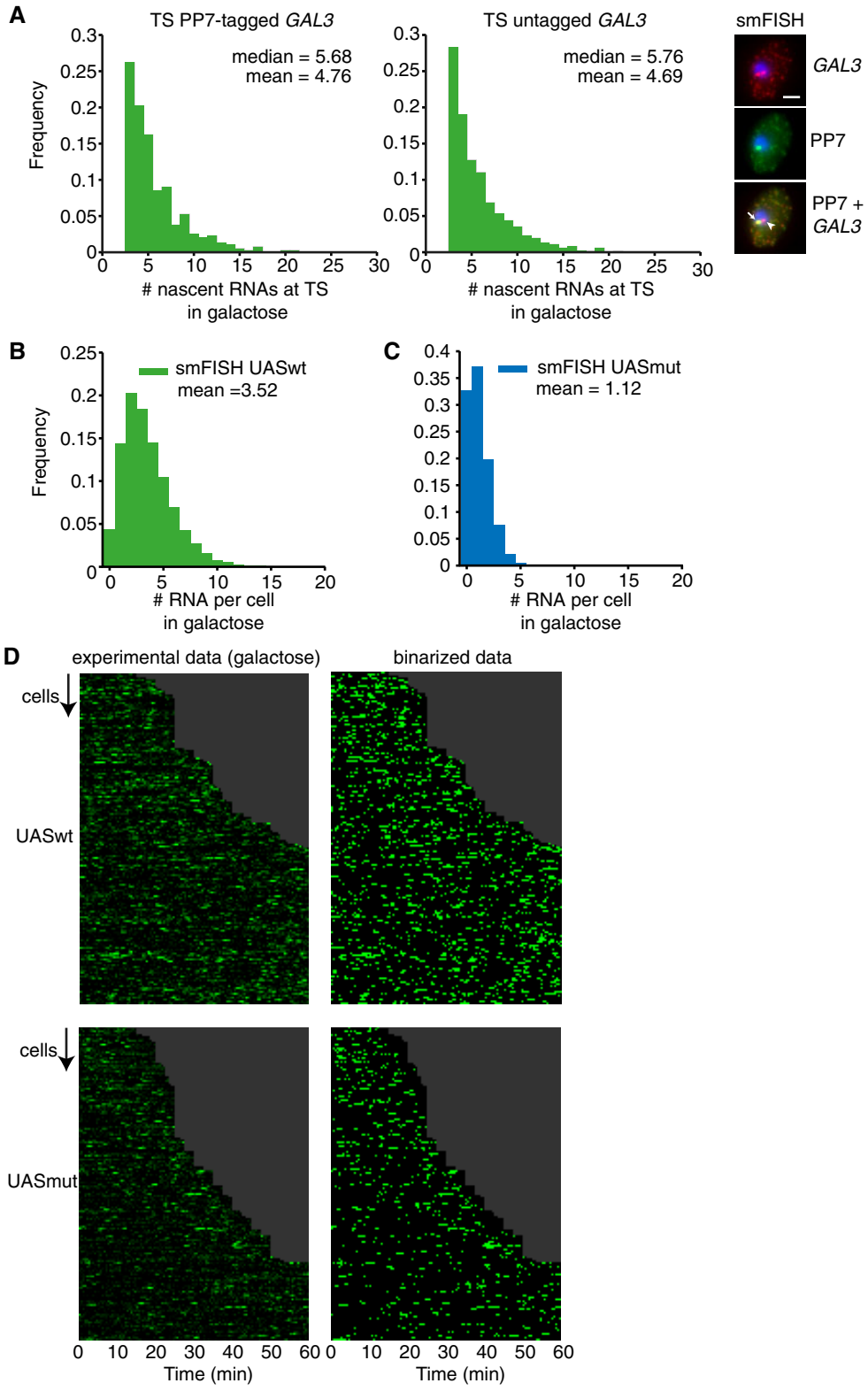

Figure EV1.

Figure EV1. Mutations in upstream activating sequence reduce burst size, but not burst frequency (related to Fig 1).

A *GAL3* transcription site intensity of heterozygous diploids with and untagged and PP7-tagged *GAL3* allele, as measured by colocalization with the PP7 TS with smFISH. An example cell is shown on the right, with the PP7-tagged *GAL3* allele indicated with an arrow and the untagged allele indicated with an arrowhead. The dynamic range and distribution of the tagged and untagged allele are similar, indicating that the PP7-tag does not affect transcription of *GAL3*. To prevent single RNAs from contributing to the TS distributions, TSs were defined as nuclear spots with 2.5-fold the median intensity of cytoplasmic RNAs. n = 2,716 cells. Scale bar: 2 μm.

B Distribution of the number of RNAs per cell determined by smFISH. A strain with PP7-tagged *GAL3* driven by the UASwt *GAL3* promoter was hybridized with PP7 probes. n = 11,839 cells.

C Same as (B) for a strain with UASmut *GAL3* promoter. n = 4,845 cells.

D Heatmap of transcription site intensity of individual cells (rows) in galactose of one of three independent experiments. Left plots show experimental data for UASwt and UASmut at *GAL3*, right plots show binarized data after thresholding. In all three experiments combined, 324 cells were analyzed for UASwt and 250 cells for UASmut.

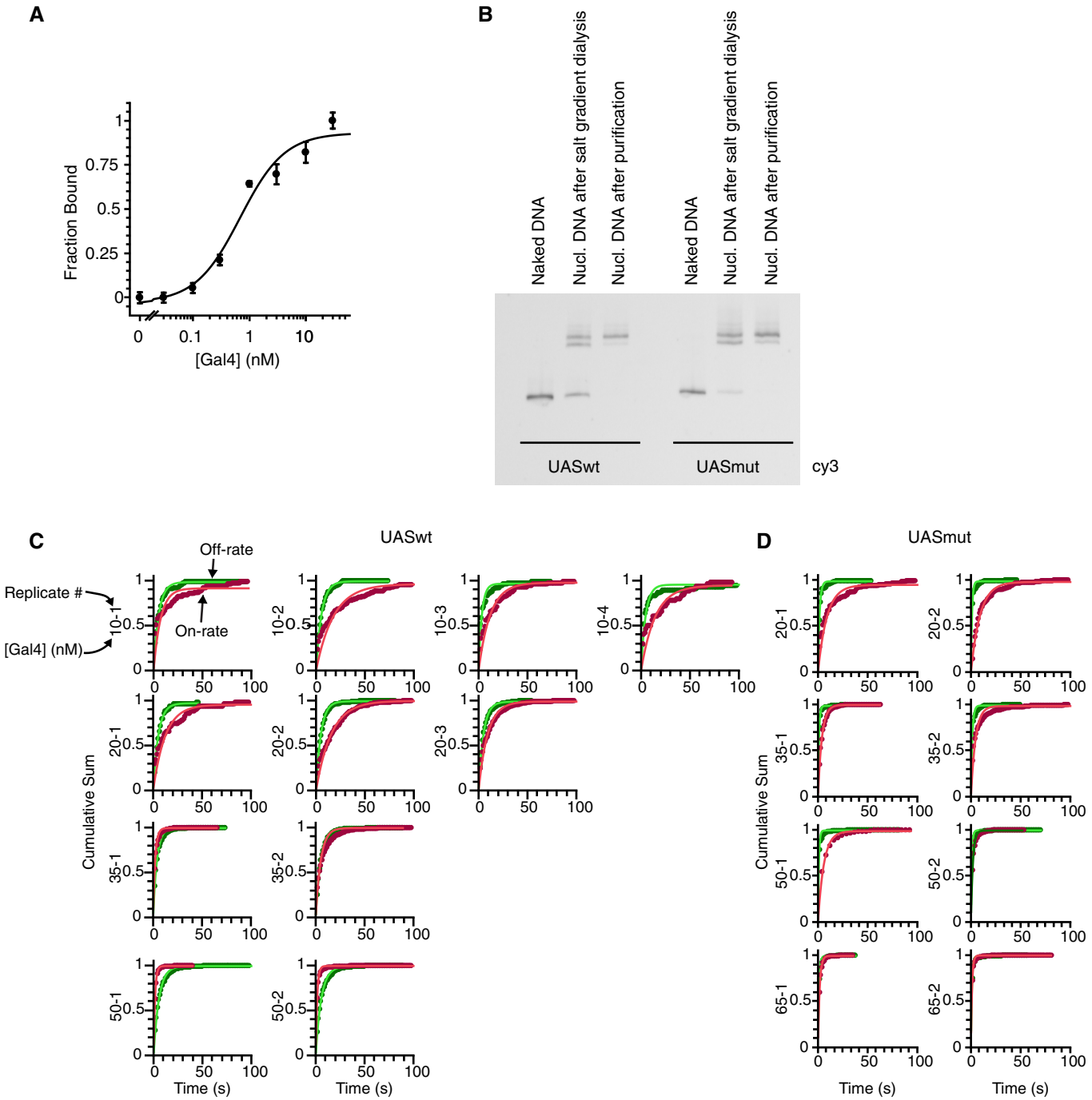

Figure EV2. Mutations in UAS reduce residence time of Gal4 on nucleosomal DNA *in vitro* (related to Fig 2).

A Titration of Gal4 binding to the UASwt sequence where binding is measured through PIFE. With 500 pM DNA, K_D = 680 ± 230 pM. Because the measured K_D is essentially equal to the concentration of DNA used in this experiment, binding is considered to be stoichiometric and we assume the actual K_D is much lower. In this experiment, ~80% of DNA molecules are bound at [Gal4] = 5 nM. For the competition experiments to measure relative affinities between the UASwt and UASmut seq, 5 nM Gal4 was used. n = 3. Errors indicate SD.

B Nucleosomes were reconstituted at a ratio of 1.25:1 of DNA:HO via salt gradient dialysis. Lanes 2 and 5 are nucleosome samples post salt gradient dialysis and before sucrose gradient purification. After dialysis, nucleosomes were loaded into 5–30% sucrose gradients and purified by centrifugation. Sucrose fractions containing nucleosomes were collected and concentrated. Lanes 3 and 6 are purified samples for UASwt and UASmut seq, respectively.

C Cumulative sum distribution for dwell times and unbound times for UASwt and UASmut single-molecule experiments. Numbers indicate Gal4 concentration and replicate number.

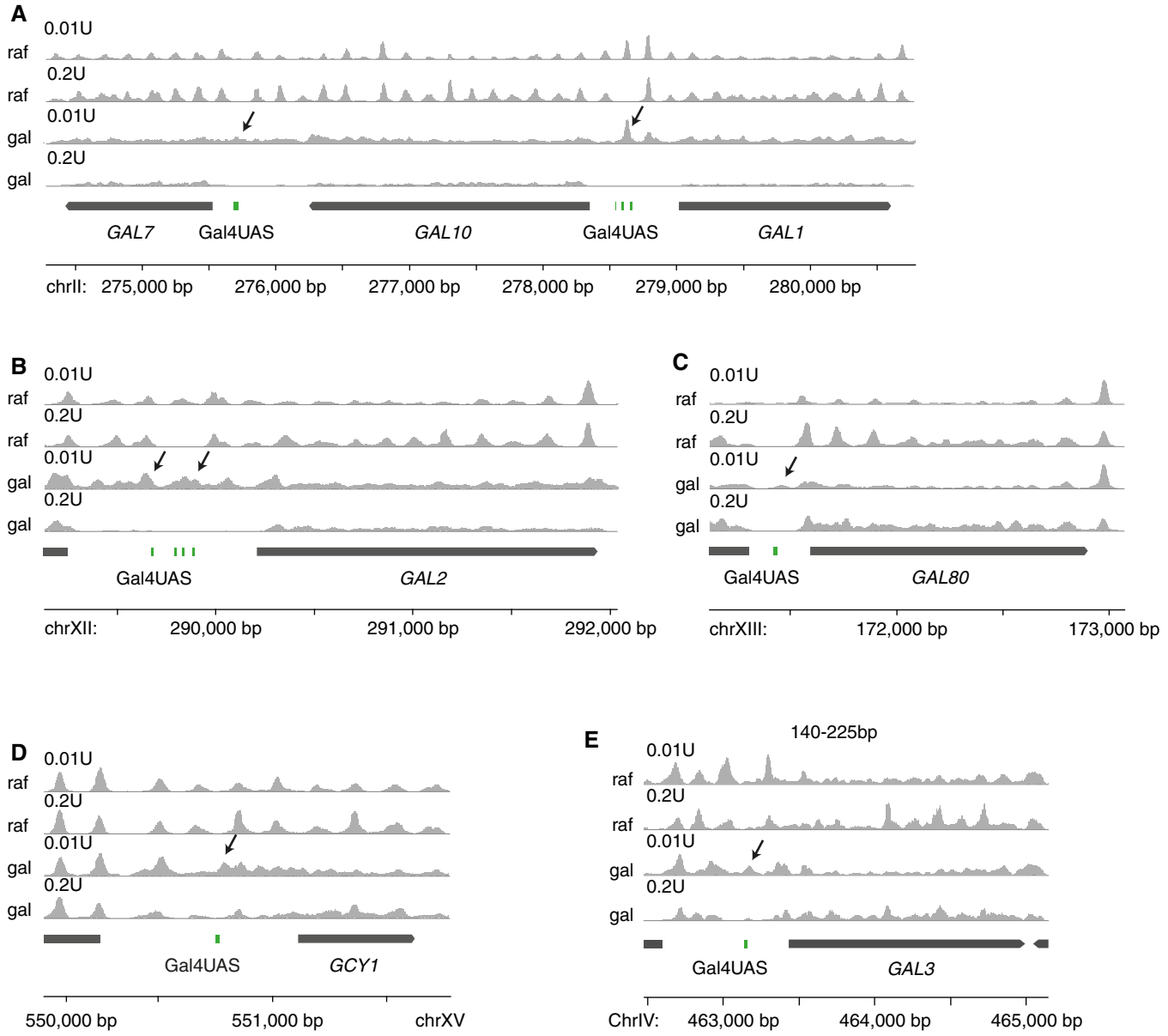

Figure EV3. Gal4 binds at the edge of a fragile nucleosome in galactose *in vivo* (related to Fig 3).

A–D Gal4 binds at the edge of a fragile nucleosome in galactose at many galactose response genes *in vivo*. Profiles of nucleosome midpoint positions (95–225 bp) by MNase-seq experiments at (A) *GAL1*, *GAL10*, *GAL7*, (B) *GAL2*, (C) *GAL80*, and (D) *GCY1*. Samples were digested with the indicated MNase concentrations in both raffinose (raf) and galactose (gal) containing media. Midpoints of nucleosomes are smoothed by 31 bp.

E Profile of nucleosome midpoint positions at *GAL3* using a higher threshold (140–225 bp), which gives the same result at Fig 3.

Data information: Arrows indicate the position of fragile nucleosomes.

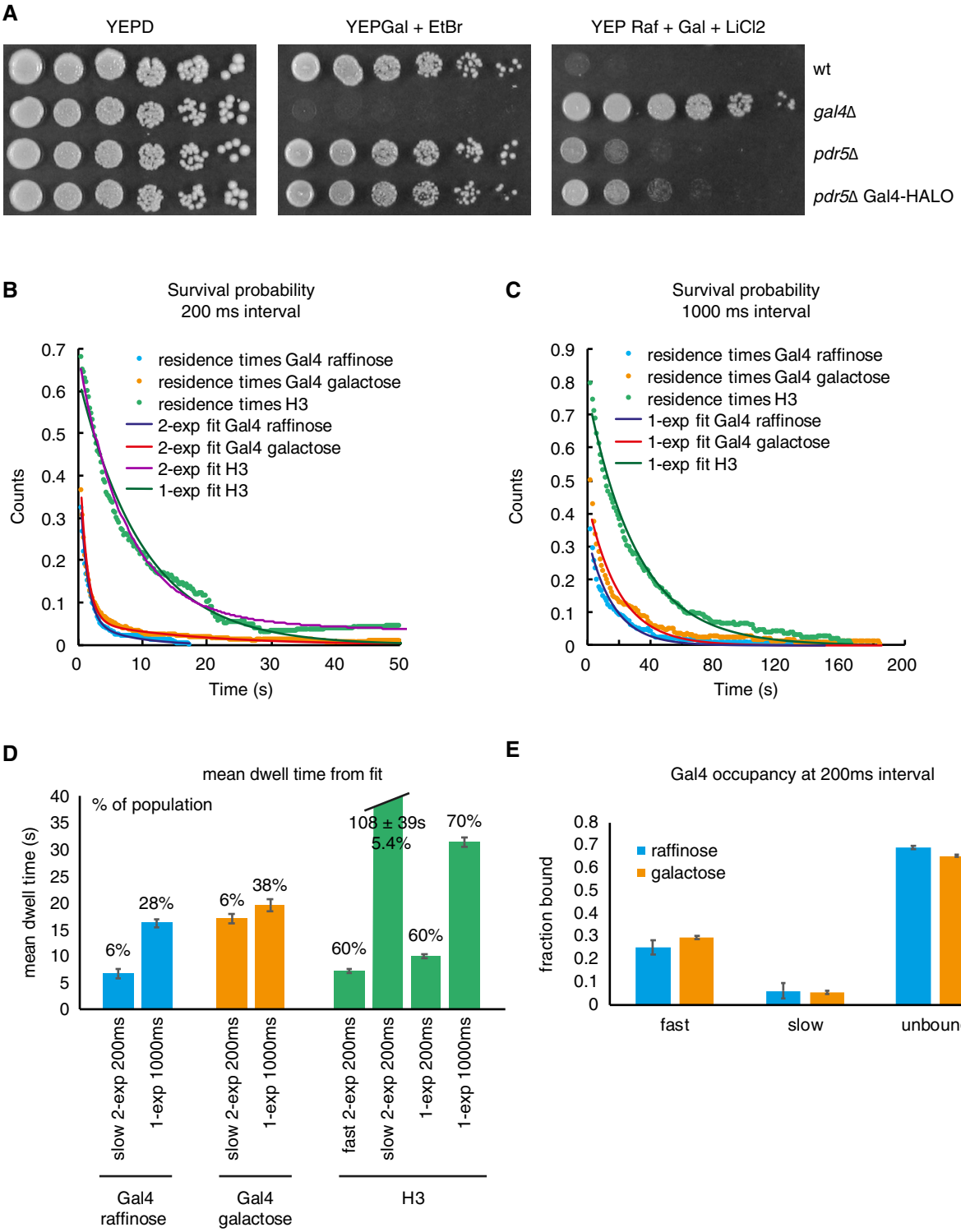

Figure EV4.

Figure EV4. Measurements of the Gal4 residence time *in vivo* shows two Gal4 populations, with long residence times colocalizing with target gene (related to Fig 4).

A Growth assay of wt, *gal4*Δ, *pdr5*Δ and *pdr5*Δ Gal4-HALO strains. Serial dilutions (fivefold) are shown after 3 days of growth on YEPD, YEPGal + EtBr, and YEP Raf + Gal + LiCl2.

B Survival probability of the duration of Gal4 and H3 tracks (after displacement thresholding) at 200 ms interval from cells grown in raffinose or galactose. H3 is fit by a single and bi-exponential, Gal4 by a bi-exponential distribution, indicating 2 Gal4 populations. Gal4 raffinose: n = 258 tracks in 30 cells, Gal4 galactose: n = 346 tracks in 25 cells, H3: n = 175 tracks in 34 cells.

C Same as for (A) at 1,000 ms interval. Both H3 and Gal4 are fit to a single-exponential fit. Gal4 raffinose: n = 153 tracks in 41 cells, Gal4 galactose: n = 238 tracks in 40 cells, H3: 213 tracks in 43 cells.

D Comparison of the mean residence times from data taken at 200 and 1,000 ms interval. The percentages above indicate the percentage of the population that the bar represents. H3 is a control, and is used to determine the longest measurable dwell time in our assay before we have bleaching effects. Although at 200 ms the 2-exp fit of H3 shows that 5% of the molecules have a long dwell time of 108 s, the majority of H3 (60%) show a residence time of 7 s that is similar to Gal4. As expected, the average H3 dwell time (from 1-exp fit) increases from 9.9 s at 200 ms to 31.2 s at 1,000 ms interval, indicating it is dominated by bleaching. Gal4 dwell time does not show the same increase between 200 and 1,000 ms interval, indicating that we are capturing the real dwell time. At 1,000 ms, we are well below the average dwell time of H3. Gal4 raffinose 200 ms: n = 258 tracks in 30 cells, Gal4 raffinose 1,000 ms: n = 153 tracks in 41 cells, Gal4 galactose 200 ms: n = 346 tracks in 25 cells, Gal4 galactose 1,000 ms: n = 238 tracks in 40 cells, H3 200 ms: n = 175 tracks in 34 cells, H3 1,000 ms: 213 tracks in 43 cells. Error bars indicate 95% CI.

E The fraction of fast- and slow-bound Gal4 molecules is similar in raffinose and galactose. Gal4 raffinose: n = 258 tracks in 30 cells, Gal4 galactose: n = 346 tracks in 25 cells. Error bars indicate SD.

Figure EV5. Galactose signaling regulates transcription levels by modulating burst frequency but not burst duration (related to Fig 6).

A Distribution of number of nascent RNAs at the *GAL10* transcription site determined by smFISH in wild-type cells grown in galactose.

B Same as (A) for *gal80*Δ cells.

C Average number of nascent RNA at the *GAL10* transcription site, determined by 8 smFISH experiments as in (A) for wt (n = 23,503 cells) and 4 experiments as in (B) for *gal80*Δ (n = 6,472 cells). Error bars indicate SEM.

D Distribution of number of nascent RNAs at the *GAL3* transcription site determined by smFISH in wild-type cells grown in galactose.

E Same as (D) for *gal80*Δ cells.

F Average number of nascent RNA at the *GAL3* transcription site, as determined by 3 smFISH experiments as in (D) for wt (n = 12,410 cells) and 4 experiments as in (E) for *gal80*Δ (n = 29,022 cells). Error bars indicate SEM.

G–J Autocorrelation of *GAL10* TS intensity traces from cells after induction with media containing (G) 2% galactose, n = 86 cells (H) 0.2% galactose, n = 79 cells (I) 0.02% galactose, n = 84 cells (J) 0.004% galactose, n = 53 cells, corrected for non-steady state effects (Lenstra *et al*, 2015). Dwell times and amplitudes are represented as bar graphs in Fig 6D and E. Shaded areas and errors indicate SEM.

K, L Autocorrelation of same-cell dose response of *GAL10* TS intensity traces from cells grown in (K) 0.004% galactose, and (L) after addition of 2% galactose (n = 13 cells), corrected for non-steady state effects (Lenstra *et al*, 2015). Dwell times and amplitudes are represented as bar graphs in Fig 6G and H. Shaded areas and errors indicate SEM.

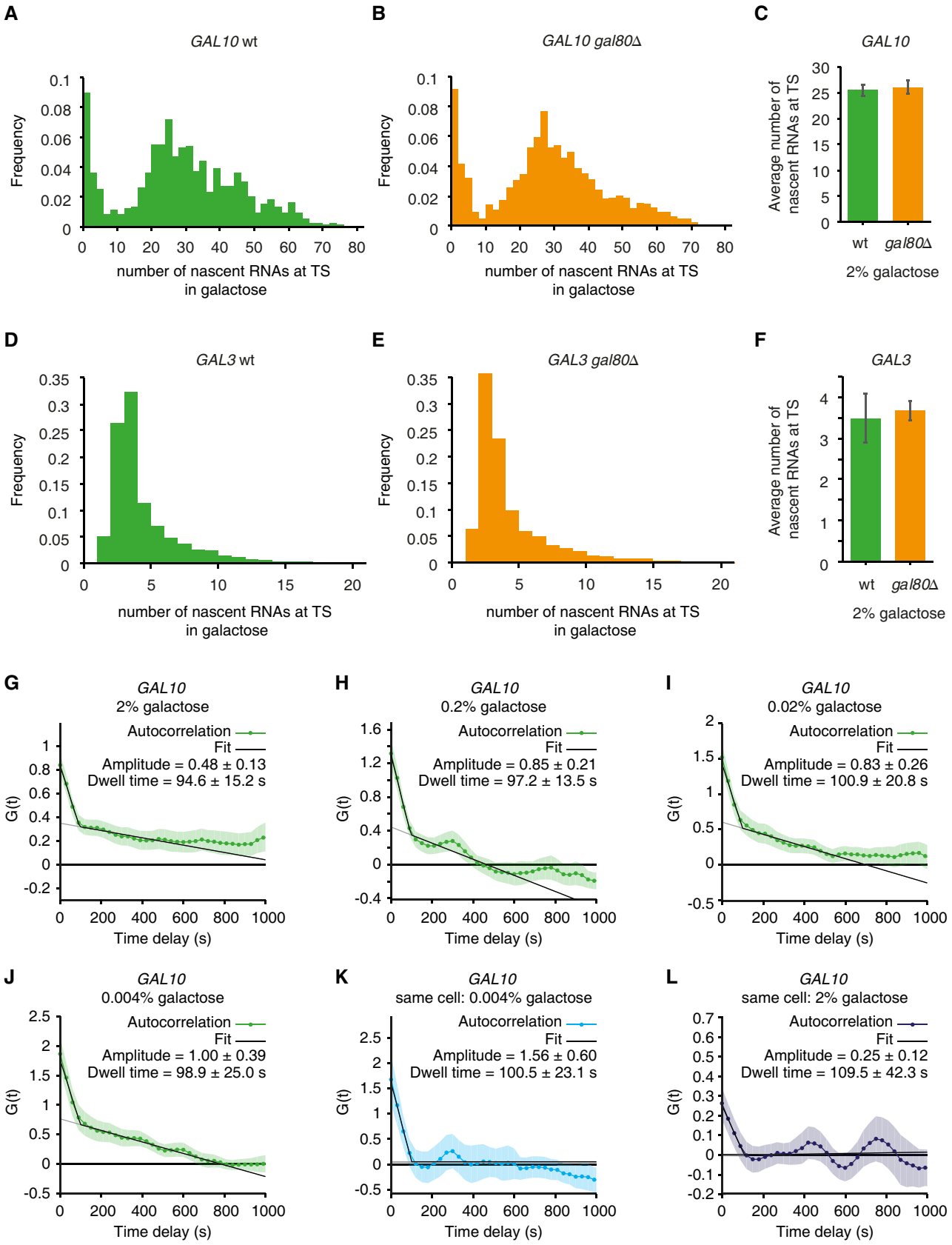
A
GAL10 wt
Frequency
number of nascent RNAs at TS in galactose
B
GAL10 gal80Δ
Frequency
number of nascent RNAs at TS in galactose
C
GAL10
Average number of nascent RNAs at TS
wt
gal80Δ
2% galactose
D
GAL3 wt
Frequency
number of nascent RNAs at TS in galactose
E
GAL3 gal80Δ
Frequency
number of nascent RNAs at TS in galactose
F
GAL3
Average number of nascent RNAs at TS
wt
gal80Δ
2% galactose
G
GAL10
2% galactose
Autocorrelation
Fit
Amplitude = 0.48 ± 0.13
Dwell time = 94.6 ± 15.2 s
G(t)
Time delay (s)
H
GAL10
0.2% galactose
Autocorrelation
Fit
Amplitude = 0.85 ± 0.21
Dwell time = 97.2 ± 13.5 s
G(t)
Time delay (s)
I
GAL10
0.02% galactose
Autocorrelation
Fit
Amplitude = 0.83 ± 0.26
Dwell time = 100.9 ± 20.8 s
G(t)
Time delay (s)
J
GAL10
0.004% galactose
Autocorrelation
Fit
Amplitude = 1.00 ± 0.39
Dwell time = 98.9 ± 25.0 s
G(t)
Time delay (s)
K
GAL10
same cell: 0.004% galactose
Autocorrelation
Fit
Amplitude = 1.56 ± 0.60
Dwell time = 100.5 ± 23.1 s
G(t)
Time delay (s)
L
GAL10
same cell: 2% galactose
Autocorrelation
Fit
Amplitude = 0.25 ± 0.12
Dwell time = 109.5 ± 42.3 s
G(t)
Time delay (s)

Figure EV5.