Supplemental Figure Legends

Figure S1 Related to Figure 1

(A) smFISH of the TFF1 intron in the *TFF1-MS2* cell line show multiple TFF1 transcription sites. A very small fraction of cells have 5 transcription sites suggesting ~5 alleles in this cell line. (B) 5 transcription sites can be observed in the TFF1 intron channel of Figure 1B. Transcription sites demonstrated by yellow triangles. (C) DNA FISH was used to validate the number of TFF1 alleles (yellow) in the *TFF1-MS2* clone. Two sets of BACs were used as controls with the green BACs on either side of the BAC1. (D) A two-state Hidden Markov model is used to identify the bursts. (E) Two complete single gene traces are shown in red and green. Active periods are segmented and pasted together to produce a continuous trajectory which reflects nascent RNA dynamics during active periods. (F) Concatenated trace from 100 individual gene trajectories. The shaded area corresponds to the region used for autocorrelation. (G) Autocorrelation analysis (red) and the fit of a deterministic model of RNA dwell time (black).

Figure S2 Related to Figure 2

(A) The TFF1 mRNA/cell distributions are broad and overlap distributions of other E2 concentrations. (B) Distributions for replicate 2 are shown. (C) The median nascent RNA at TFF1 transcription site is 3.29 and 1.60 for the pooled samples across all E2 concentrations for replicates 1 and 2 using smFISH. TFF1 exon spots which colocalized with TFF1 intron spots were designated as transcription sites. These transcription sites were then normalized by the median TFF1 exon intensity of the spots found in the cytoplasm of that specific cell. This yielded the number of nascent RNA that are at the transcription site. (D) Replicate 1 has considerably less cells and transcription sites, therefore we pooled all datasets and observe a median nascent RNA of 1.67 per transcription site (SE = 0.02). (E) The median nascent RNA is reproducible among the higher E2 doses for Replicate 2. The median is the 1.67 RNA/TS for the replicate 2 E2 doses. (F) Dose response for the *TFF1-MS2* cell line at 0, 0.05,0.5nM E2. One of the replicates is plotted. (G) Fraction of cells with at least 1 transcription site show similar activity. The *TFF1-MS2* single cell clone is slightly more active than the parental MCF7 population. Data is from smFISH TFF1 intron signal.

Figure S3 Related to Figure 4

Pairwise comparison of allele output and generation of the transcription cross-correlation. (A) Allele signal sum is computed as the area under the raw fluorescence trajectory (i.e. Fig. 2A black curve) after background subtraction for the entire time series. (B) Allele signal sum is computed as the area under the HMM curve (Fig. 2A red curve) for the entire time series. No background subtraction is necessary because the HMM curve is either 0 or 1. (C) Raw data for two alleles in the same cell, shown in red and green for presentation, even though both alleles are labeled with MS2-GFP. (D-E) Cross-correlations are computed in both directions: red to green,

and green to red. Gab(t) denotes the cross-correlation between allele ‘a’ and ‘b’. (F) The cross-correlations are averaged together. (G) Background correction is applied. Note the diminished error bars and the change in y-axis scaling.

Figure S4 Related to Figure 4

(A) Schematic of how correlation functions are computed from square pulse transcriptional behavior. The raw ‘burst’ is shown in red. Mathematical parameters are indicated. The time-dependent fluorescence is denoted as F(t). The squared burst is shown in red. The square of the time-dependent fluorescence is denoted as F(t)2. Drawn to scale. (B-E) Different scenarios of cross-correlation between transcriptional pulses from two alleles, denoted as red and green for clarity. N1 is the number of bursts from the red allele; N2 is the number of bursts from the green allele. M is the number of co-occurring bursts. (F) Random expectation for co-occurring bursts, according to STAR Methods Eqn. 18. The x-axis is the number of bursts of allele 1. The y-axis is the random expected value for the fraction of bursts of allele 2 which co-occur with a burst from allele 1. Burst duration (D)= 16 min. Total observation time (T) = 854 min.

Figure S5 Related to Figure 4

Cross-correlation and fractional overlap between bursts on a pairwise basis. (A) Gab(0) is plotted vs. separation between alleles. (B) Random control for alleles in different cells. (C) Same as panel (B) but with a greater number of pairs. (D) Single time point showing alleles separated by ~ 9.6 mm. (E) RMS distance as a function of time between two alleles where the separation is always less than a specific distance (6.4 mm). (F) RMS distance as a function of time between two alleles where the separation spans both the 6.4 mm threshold. (G-H) Fractional overlap of bursts per cell, comparisons in two directions. (I) Fractional overlap for random allele control.

Figure S6 Related to Figure 5

The coupled model fits both the live cell and FISH data. Active cumulative plots and model fits are shown in red and black respectively for (A) active, (B) inactive and (C) FISH data. The pooled histograms for the FISH data were used for the plots. (D) Flow chart describing data that was fit and models tested. (E) Example of signal segmentation of TFF1 staining in small intestine (right). Magnified region on right. Image credit to Human Protein Atlas.

Figure S7 Related to Figure 7

Entropy decreases in response to E2 dose across chromosome 11. (A) Three doses are plotted. Entropy is plotted in log base 2. (B) Two doses are plotted, 0 and Sat. E2. Entropy was calculated on 100kb bin contact map.

Movie Legends

Movie S1. Related to Figure 1. Uninduced *TFF1-MS2* cell shows no transcription sites. Cell was imaged for 105 minutes.

Movie S2. Related to Figure 1. Induced *TFF1-MS2* cell show multiple transcription sites active for short periods of time. Cell from Movie S1 was imaged for 14 hours immediately after addition of 100nM E2.

Movie S3. Related to Figure 1. *TFF1-MS2* cell line imaged on confocal microscope illustrates diffusing RNA in nucleoplasm. One transcription site is also observed in the middle of the nucleus. Cell was imaged for 40 seconds at saturating E2.

Movie S4. Related to Figure 1. TFF1 exhibits short periods of activity in cells at saturating E2. *TFF1-MS2* cells were imaged for over 14hrs.

Supplemental Table Legends

S1. Model comparisons, steady state occupancy and parameter values. Related to Figure 5.

S2. Ranked list of top 100 variable genes in MCF7 cells. Related to Figure 6.

S3. Oligos and smFISH probe sequences. Related to Figure 1.

S4. smFISH cell counts. Related to Figure 6.