



Figure S8 – Simple RNA polymerase pausing computational model simulation. Simulated expression profiles of TSSaRNAs differentially expressed during a growth curve relative to the control condition. Tiling microarray output simulation for a 35bp TSSaRNA in a 2kb cognate gene. Varying the only two model parameters it is possible to generate situations in which the cognate gene expression level remains constant over time and TSSaRNA levels can vary almost arbitrarily. Panels A to D are build mimicking our experimental setup displayed in manuscript's Figures 4 and Figure S7 exploring the parameter space. Vertical-axis – log2 ratios between simulated quantity of transcripts in each time point  $(I_t)$  and the amount simulated at reference time-point  $(I_{ref})$ . Black solid line – TSSaRNA expression profile. Black dashed line - cognate gene expression profile (constant over time and arbitrarily set to the same value of reference condition). Panel E shows all kinds of log2 rations that can be obtained for a TSSaRNA probe and its cognate gene when scanning the parameter space:  $\Delta \tau$  and  $\Delta t$ , "intrinsic transcription initiation interval" and "time spent stalled", respectively. This example scans  $\Delta \tau$ from 2 to 700 time units,  $\Delta t$  from 3 to 700 time units and simulates a 2kb gene with a 35bp TSSaRNA associated. Highlighted points in Panel E are examples of relatively constant TSSaRNA levels with an appropriately 3-fold difference in cognate gene level (light blue and purple circles, corresponding to  $\Delta t$ = 46 and  $\Delta \tau$  = 12 time units and  $\Delta t$  = 14 and  $\Delta \tau$  = 8 time units, respectively), and a 32-fold difference in TSSaRNA levels with relatively constant cognate gene levels (red and green circles, corresponding to  $\Delta t = 250$  and  $\Delta \tau = 4$  time units and  $\Delta t = 250$  and  $\Delta \tau = 84$  time units, respectively). Qualitatively, almost any complex dynamical behavior can be obtained if the pausing rhythm and the RNA polymerase arriving rate are jointly regulated by environmental clues.