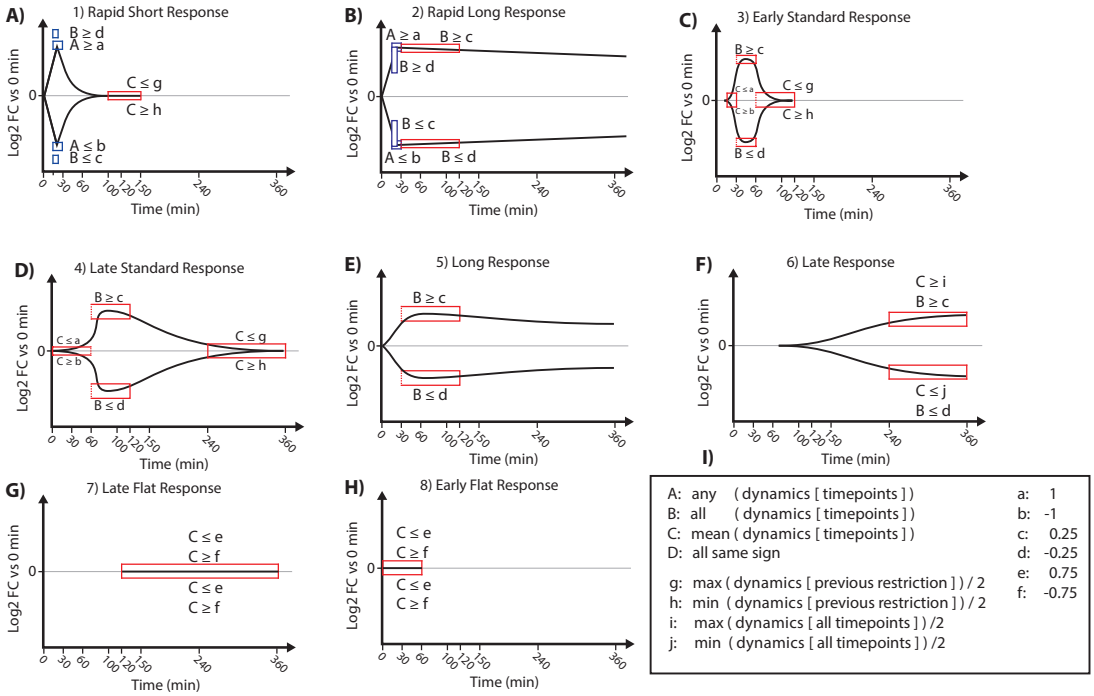
Fig. S1.

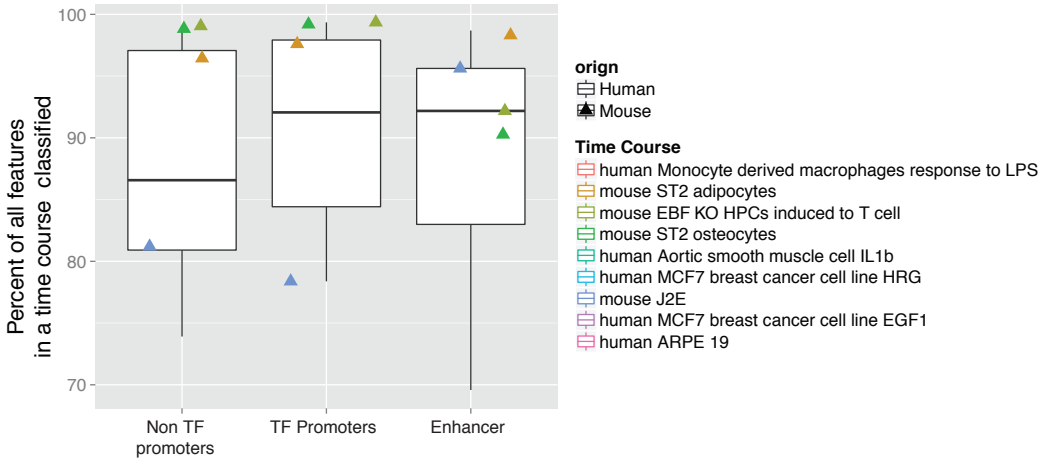


Rules describing the eight core responses patterns identified. For each core response (A-H) a schematic representation of both the up and downregulated response is shown. For each of these responses the rules created to classify the response are visualized as the combination of a colored box and at least one letter code consisting of a upper case and a lower case letter separated by ≤ or ≥. Text above the line apply to the upregulated response and vice versa. These rules can be interpreted as follows: The colored box gives the time range where the rule applies using solid lines for inclusion and dashed lines for exclusion of time points. Overlapping blue boxes are interpreted as "OR": at least one of them must be true while the red boxes describe rules that are required to be true. The upper case letter describes if and how the log2 fold change vs. t=0 values are summarized over the interval and the lower case letter describes the cutoff used.

For example: For the upregulated "long response" (panel E) these rules would be interpreted as follows. Since the red box convers the 30-120 min interval and is dashed in the left end and solid in the right end the rule is applied to all log2 fold change vs. t=0 values in the range > 30 up to ≤120. In the letter combination the uppercase letter is "B", meaning that we are evaluating the cutoff on all the log2 fold change values from the analyzed region (as seen from documentation in panel I). The lower case letter is "c" meaning a cutoff of 0.25. In summary: All log2 fold changes vs. t=0 values in the interval >30 up to 120 minutes must be larger than 0.25.

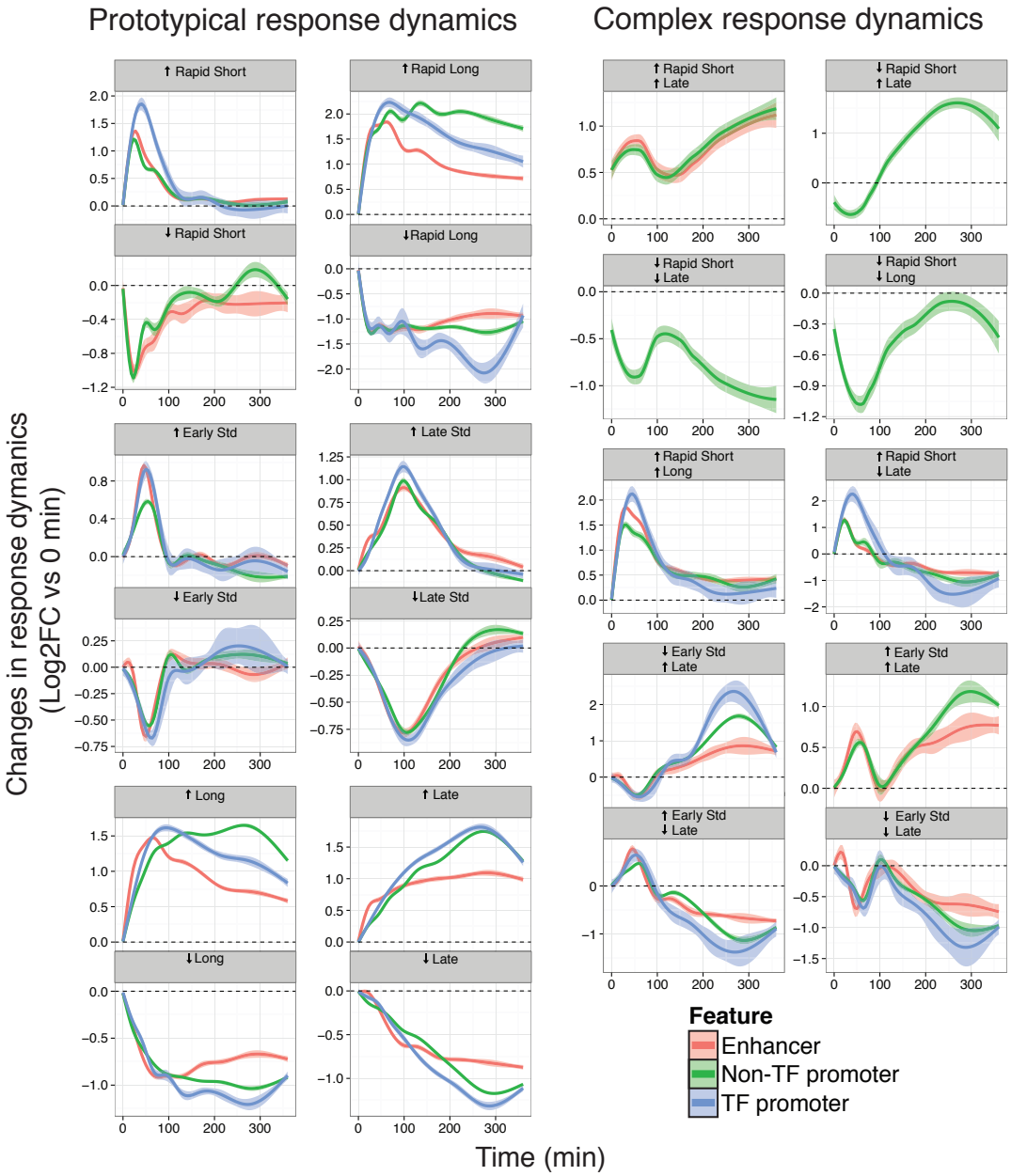
Note that 1) For complex response patterns only the following combinations was allowed: A+E, A+F, A+G, C+F and C+H. 2) If none of the rules applied, the feature was marked as 'unclassified'. 3) The "late response" we discuss in the article is comprised of H+F and the response we denote "early standard" is comprised of C + G. The R script where these rules are implemented can be found in Supplementary text.

Fig. S2



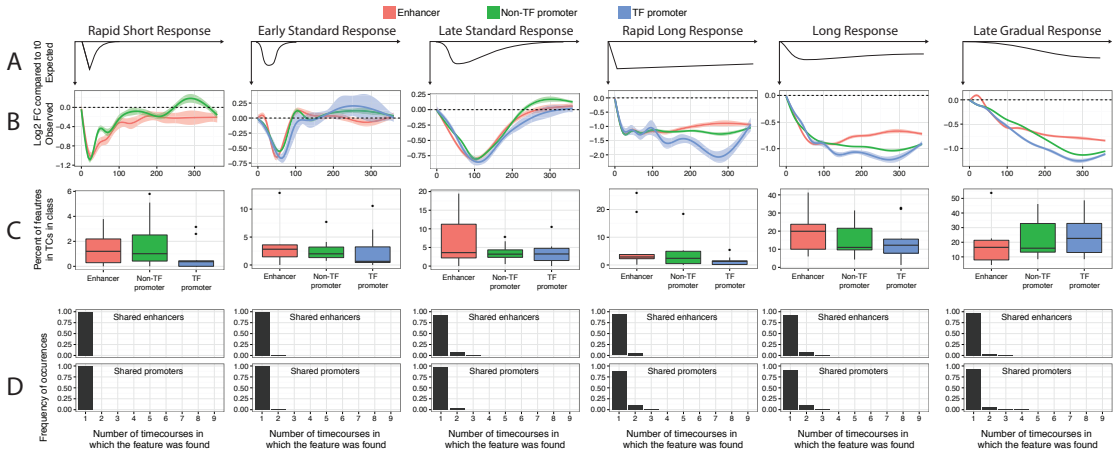
Percent of features classified. For each time course the percent of all significantly differentially expressed non-TF promoters, TF promoters and enhancers that were classified by our classification algorithm (see Fig. S2-S3) were calculated.

Fig. S3



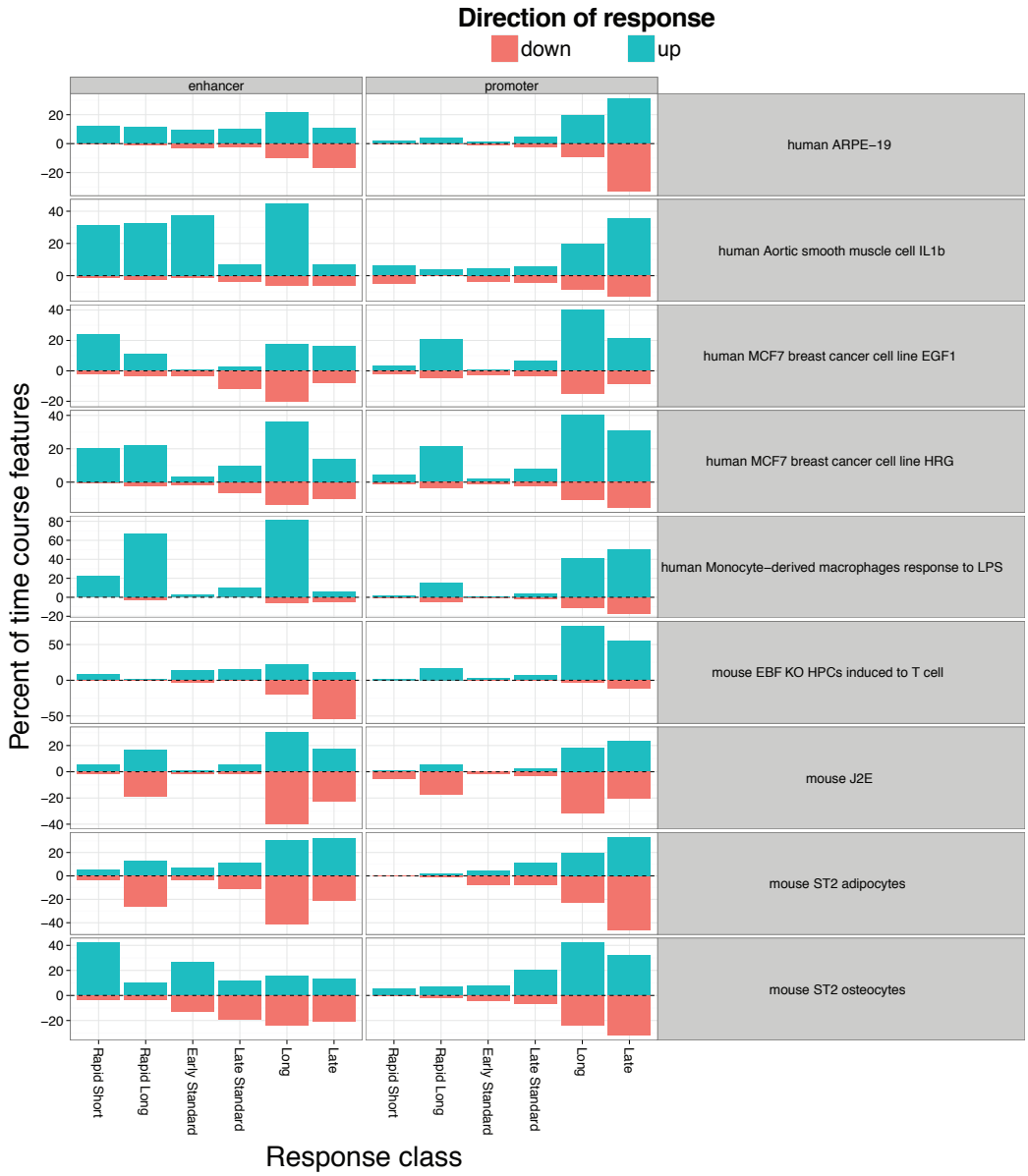
Empirical response pattern expression of all identified response classes (Extension of Figure 1C). The classification scheme identifying each of the response patterns described in Figure S1 was applied to significantly changing promoters (split up by TF promoters and remaining promoters) and enhancers across the 9 early response a time course (Table S1) (see main text for details). Y axis shows the mean log2 fold change of all promoters and enhancers classified into each response class over all time courses studied. The 95% confidence intervals of means are shown. X axis shows time in minutes. Note that all the six upregulated prototypical classes encompass enhancers and both types of promoters, and a response class was only plotted if it had 30 or more entries. Opposite (up and down) responses are combined.

Fig. S4



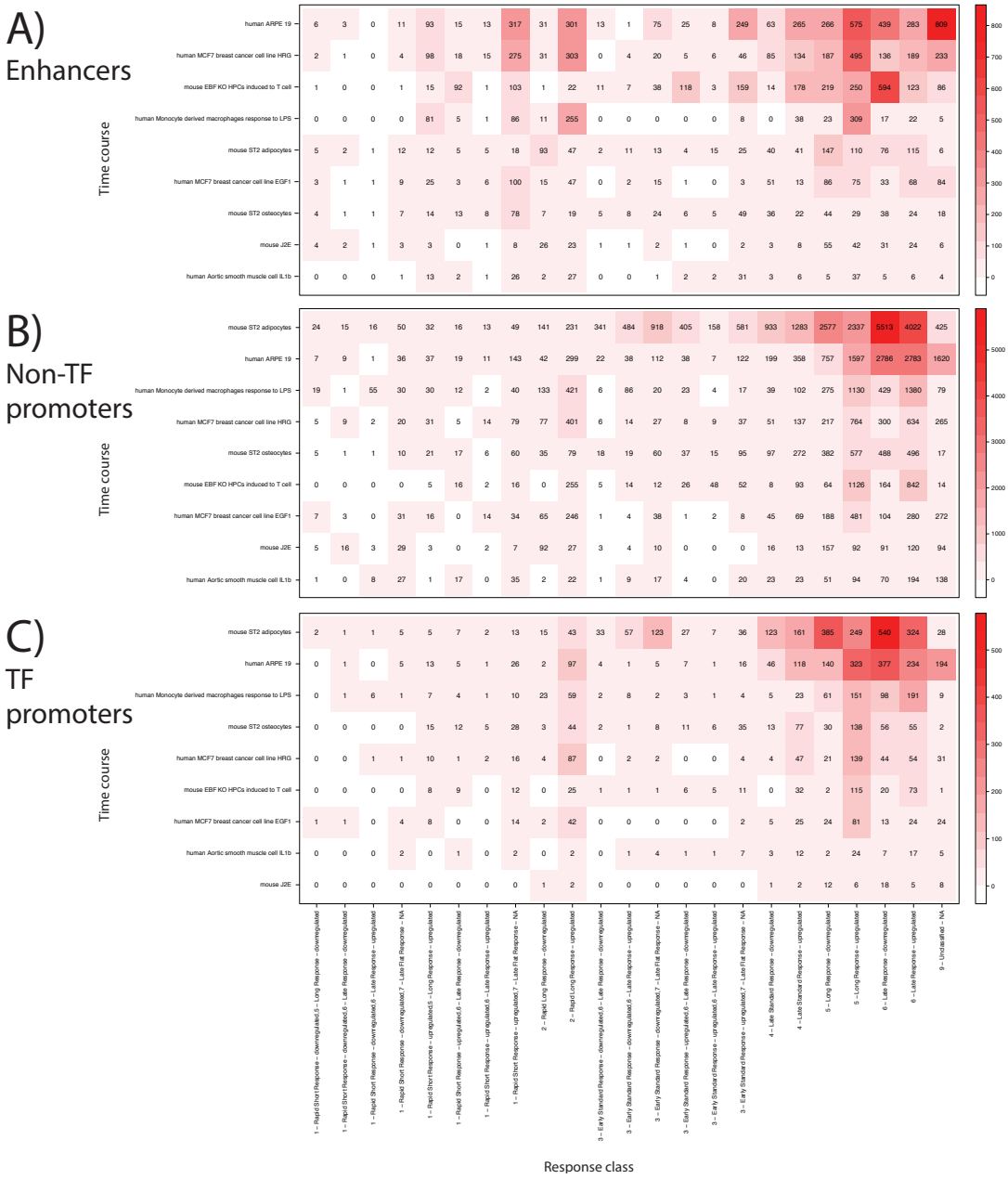
Rule-based clustering reveals general response patterns.
A. Stylistic representation of each of the major prototypical response patterns identified as described in the main text; Y axis shows log2 fold change vs time 0, X axis shows time in minutes. Only down-regulated response patterns are shown, but each class also has a corresponding up-regulated profile (Figure 1C).
B. The classification scheme identifying each of the response patterns described in A) was applied to signifi- cantly changing promoters (split up by TF promoters and remaining promoters) and enhancers across the 9 early response a time course (Table S1) (see main text for details). Y-axis shows the mean log2 fold change of all promoters and enhancers classified into each response class over all time courses studied. The 95% confidence intervals of means are shown. X-axis shows time in minutes.
C. Preference for enhancers, TF promoters and other promoters for respective classes, shown as boxplots summarizing the fractions of enhancers, TF promoters and other promoters from that class in all analyzed time courses. Early peaking response classes are enhancer-dominated, response classes describing a second expression wave are TF-promoter dominated while the response class describing a gradual increase over time is highly dominated by promoters of non-TF genes, suggesting transcriptional waves (enhancers, TFs and then their target genes).
D. Overlap between time courses in terms of enhancers and promoters in respective class. The figures show the frequency (Y axis) of the number of time courses (out of 9) sharing a specific feature (enhancer, TSS, etc.) (X axis).

Fig. S5



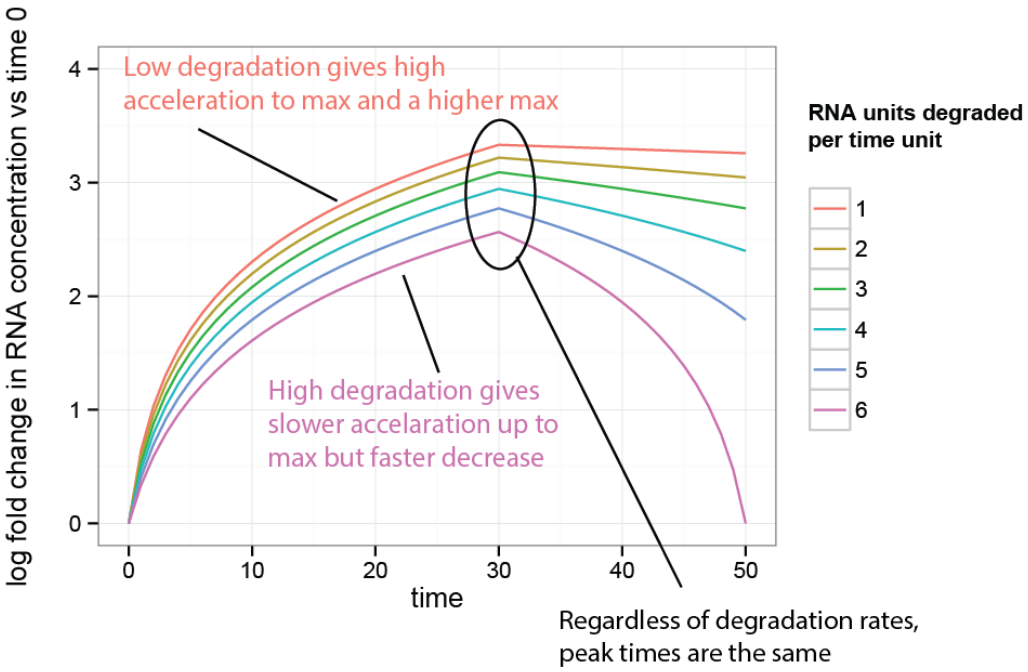
Size of up- and down-regulated responses. For each of the 6 prototypic response patterns (Figure 1C, S1-S4), in each time course, the percent of features (enhancer or promoter) classified as the upregulated response or downregulated response were extracted. The percentages were calculated separately for promoters and enhancers. Note that the y-axis used is positive on both side of zero which allows the up and downregulated responses to be plotted together

Fig. S6



Number of features classified into response classes. For each class (columns) the number of features (enhancers (panel A), Non-TF promoters (panel B) and TF-promoters (panel C)) classified into that class in each time course (rows) is annotated. Note that the time course (rows) are sorted after number of features individually in panels A, B and C while the columns remain the same. Note that the color scales in A, B and C are different.

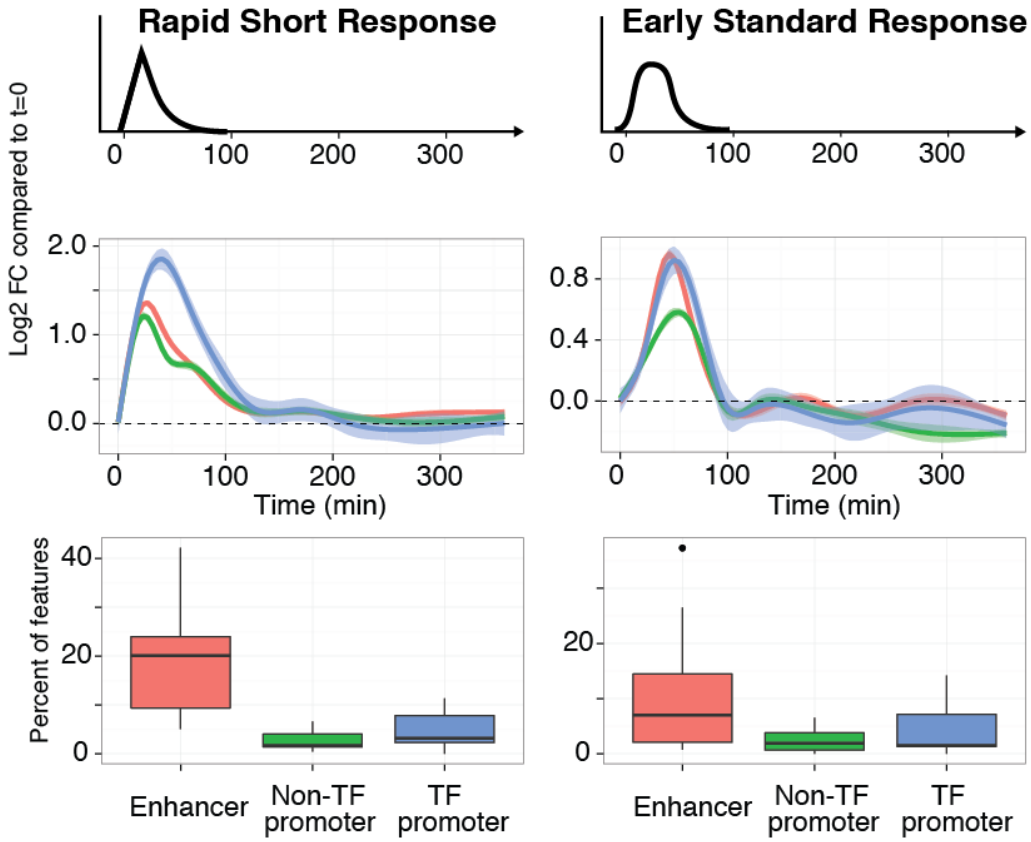
Fig. S7



Simulation of impact of degradation rate

In the simulation, RNAs are produced at 10 units per minute until 30 minutes, where RNAs are no longer produced (only decayed). The parameter that varies is the degradation rate, from 1 RNA unit per minute to 6 per minute. RNA degradation is always active in the simulation. Y axis shows RNA amount fold change vs time 0. X axis shows time in minutes

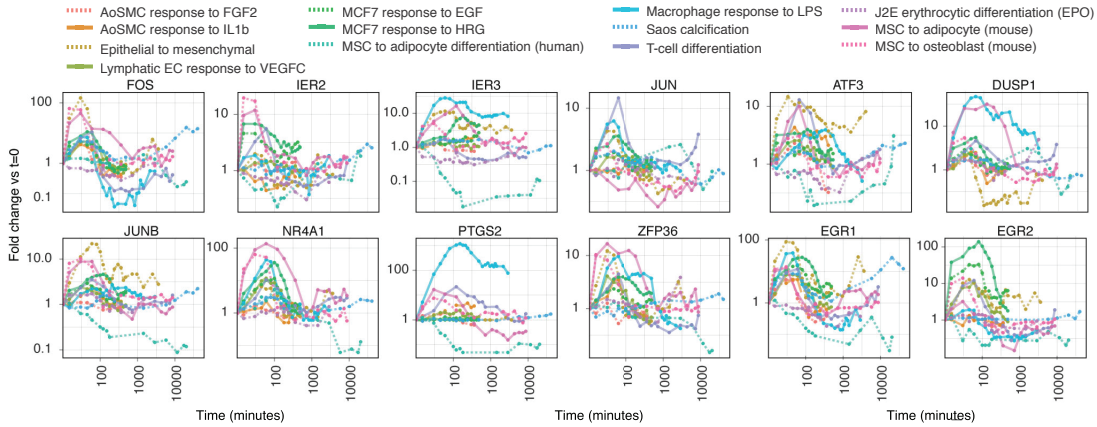
Fig. S8



Actual observations (part of Figure 1)

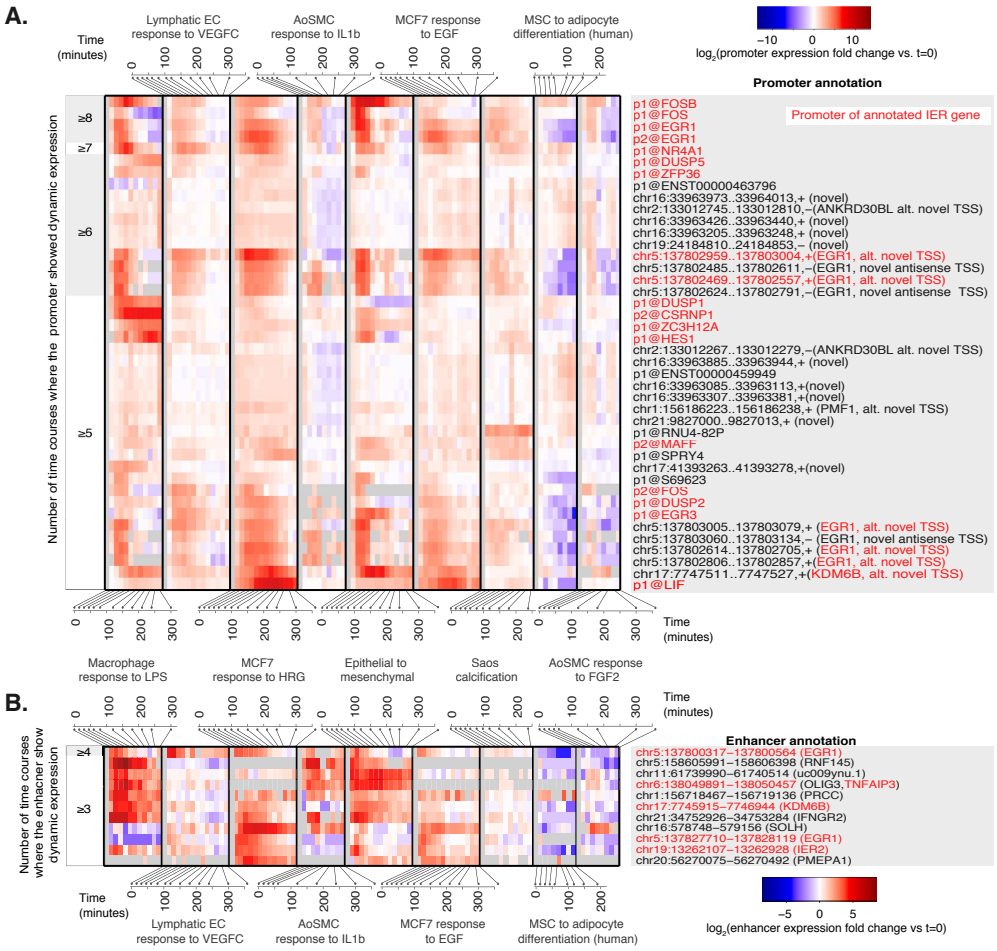
Only Rapid Short Response and Early Standard response are shown. Note that both genes and enhancers follow these responses (despite different degradation rates). Also note that enhancers reach their maximum very early (high acceleration). This is highly different from the response predicted by just varying degradation rates. Thus, they must be transcribed very early.

Fig. S9



Divergence of early gene response. Examples of highly variable expression over time of literature-derived immediate early response (IER) genes in time courses with high sampling in the first time points. Y-axis shows fold change vs. time 0 (log10-scaled), X-axis shows time in minutes (log10-scaled). Each box shows the expression of one IER gene for 13 time courses.

Fig. S10

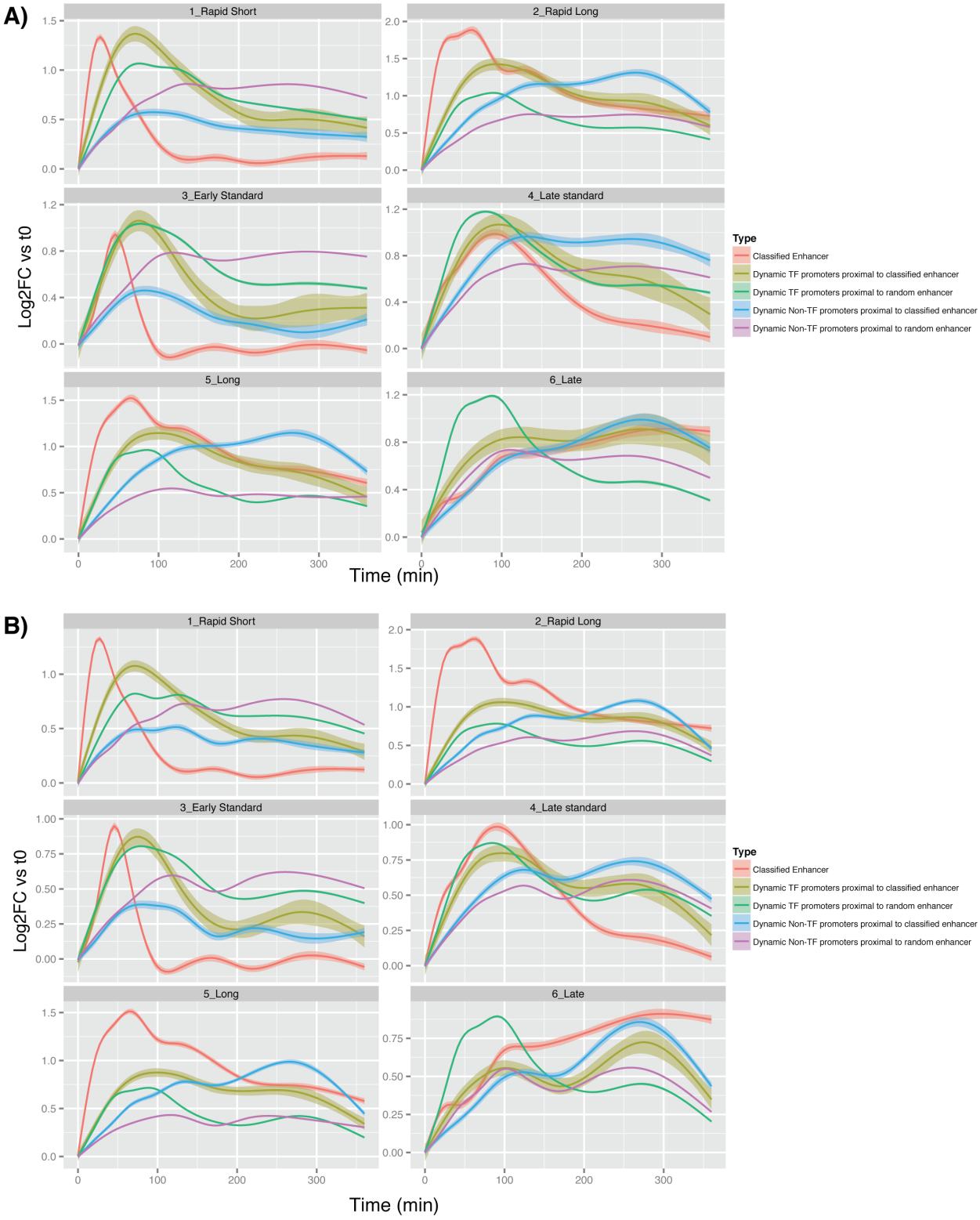


Early response genes and enhancers

A. Early response promoters in nine human time courses with high sampling density (Table S1). Rows show promoters with significant expression changes in the first 2 hours in at least five, six, or seven out of nine human time courses (see left-most column). Red color indicates up-regulation compared to time 0, blue indicates repression. Grey regions indicate no data. Each black box describes one of nine human time courses and their first measured time points. Promoter/gene annotation is shown on the right, following annotation convention of FANTOM5 (10). Novel promoters are indicated by hg19 coordinates; overlapping genes on the same strand are shown. Literature-annotated immediate early response genes are highlighted in red.

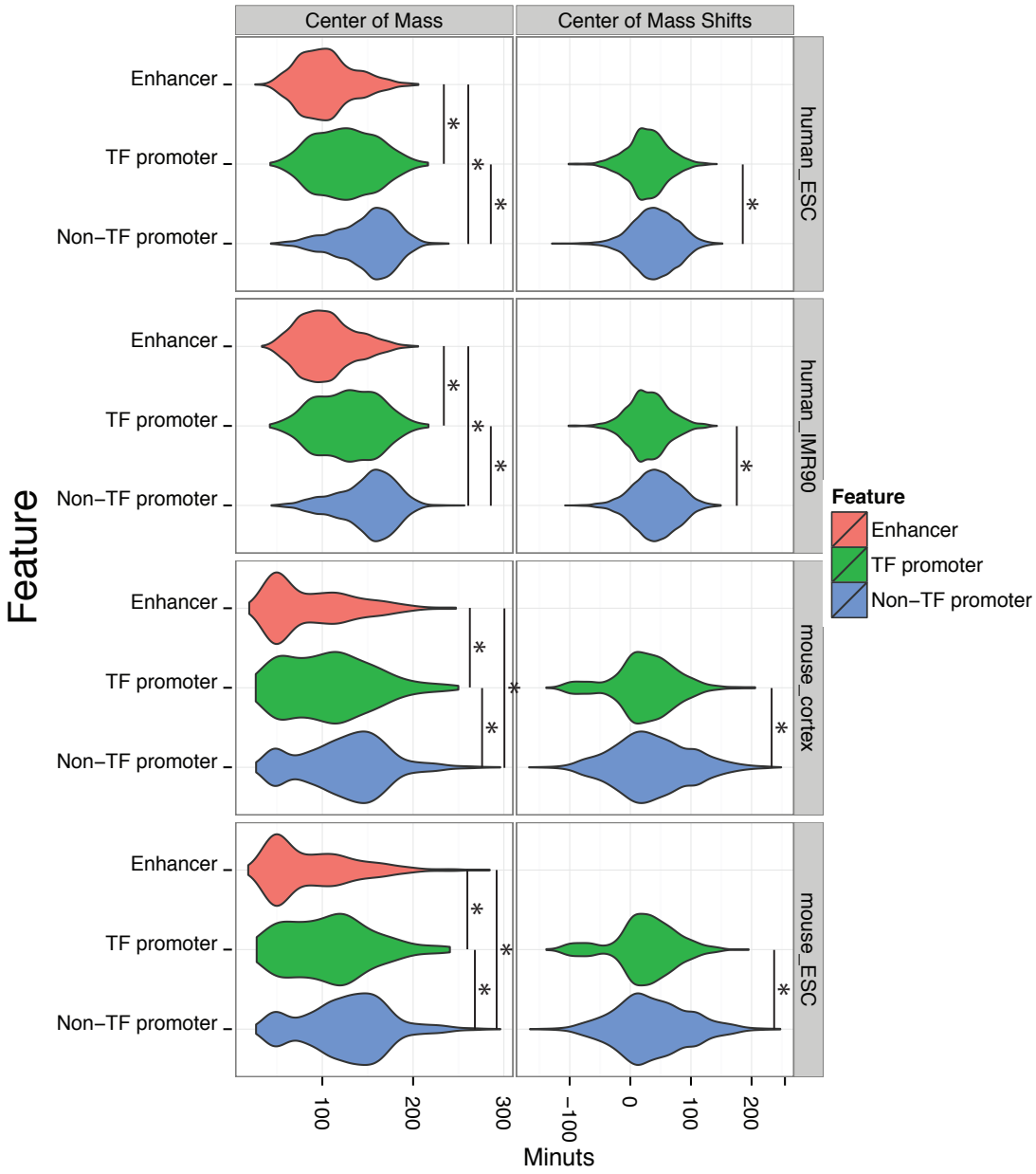
B. Discovery of early response enhancers as in panel A. Enhancer locations are shown on the right, together with the closest annotated genes (a single gene if the enhancer is intronic, the two closest genes on either strand if intergenic). Enhancers that are closest to known early response genes are highlighted in red.

Fig. S11



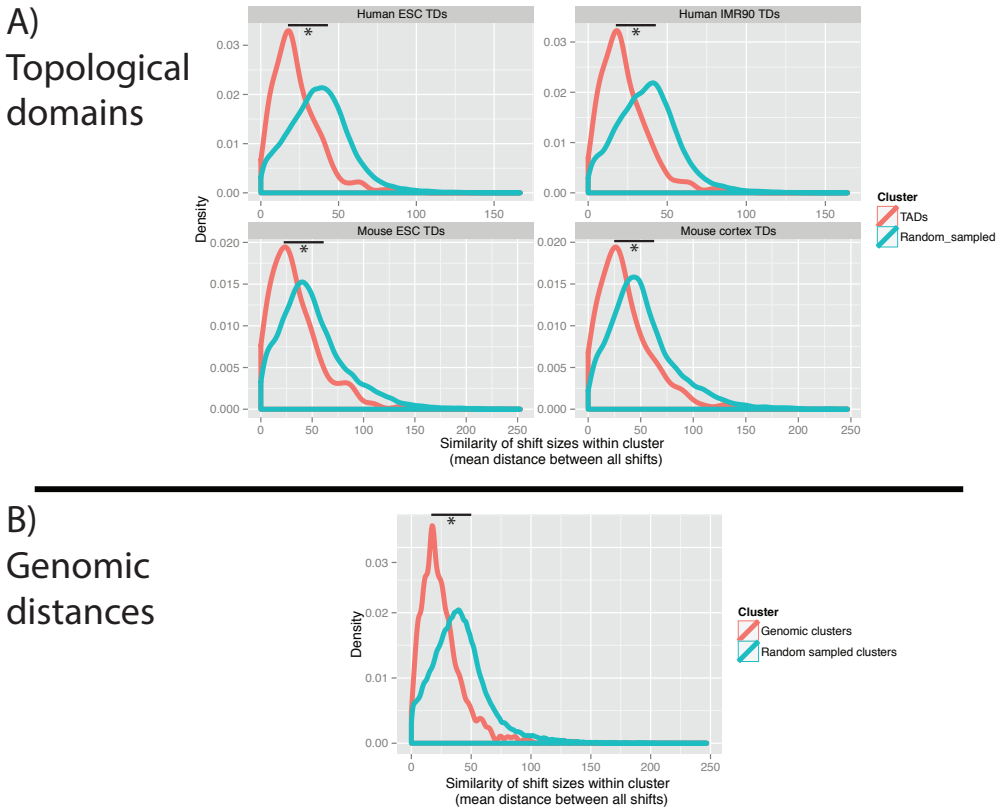
Expression of promoters in the vicinity of classified enhancers. Smoothed mean expression over time for all enhancers classified into the different response classes (header) and all proximal differentially expressed promoters either within 200kb (A) or within the same TAD (B), split by gene type. Controls for class specificity constitute promoters proximal to randomly sampled enhancers. Shaded areas indicate 95% confidence intervals.

Fig. S12



Center of Mass and Shift sizes for TAD defined enhancer promoter pairs. Left column: For each TAD dataset (rows) Distribution of Center of Mass (CM) of expression changes (the time point where 50% of expression changes have occurred over a time course) for enhancers, TF-promoters and promoters of other genes. Right column: difference in CM ("shift") between paired enhancers and promoters within the same TAD as a function of gene type. Asterisks indicate significance (all $P < 0.05$. Mann-Whitney U test) and black dots indicate 25, 50 (median) and 75 percentiles of distributions.

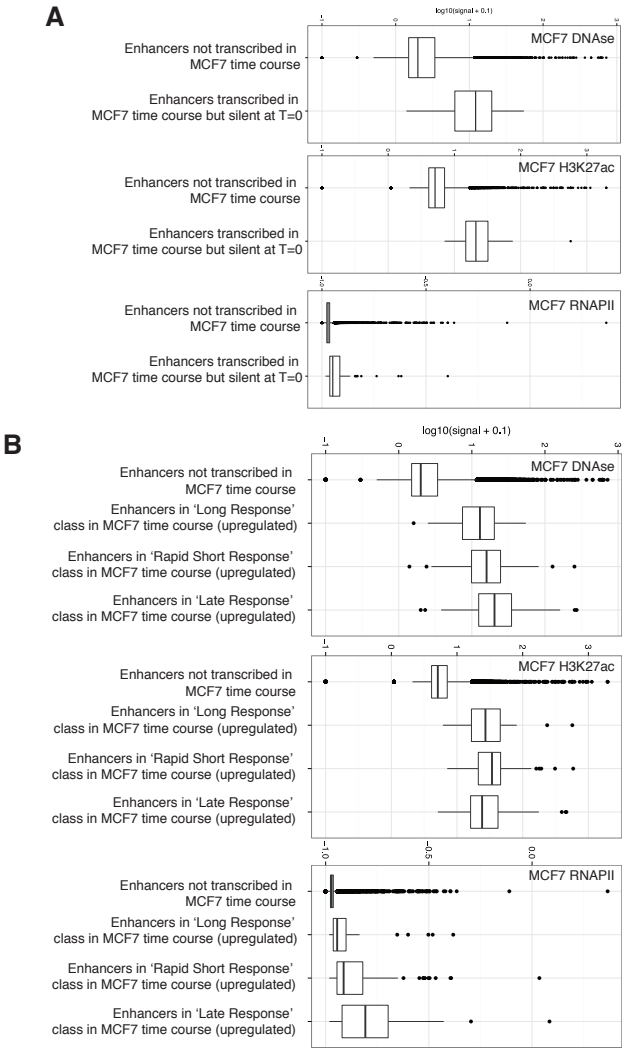
Fig. S13



Similarity of enhancer-promoter CM shifts within clusters

The similarity of all enhancer-promoter CM shifts within clusters of enhancers-promoter pairs either defined (as described in Supplementary methods) by the four sets of topological domains (TADs) (A) or genomic distances (B) was measure as the mean of the Euclidian distances between all comparisons of all CM shifts. The randomly sampled distribution was obtained as described in methods; briefly clusters of identical complexity as the empirical defined clusters were obtained. These consisted of an identical number of both promoters and enhancers. From these all possible enhancer-promoter CM shifts were calculated and compared as for the empirical clusters. Significant comparisons (all $P < 1.0\ e{-}14$. Mann-Whitney U test), are indicated with *.

Fig. S14



Comparison of initial chromatin stages as a function of enhancer transcription dynamics

A) ENCODE MCF7 ChIP-seq data measuring RNAPII and H3K27ac as well as DNase-seq was used to assess the chromatin state at time=0 in the MCF HRG time course. Enhancers were split as follows: those that did not have any CAGE signal in the MCF7 time course, and those that were dynamically expressed (upregulated). For the latter, we only took the subset that had no MCF7 CAGE tags at time=0, in order to focus on transcription going from a zero level and have no confounding baseline effects.

B) As in A, but splitting enhancers by their dynamics response classification in the MCF7 time course. Here, we have no requirement on the number of CAGE tags at time=0