# Supplemental Data

# "groHMM: A Computational Tool for Identifying Unannotated and Cell Type-Specific Transcription Units from Global Run-On Sequencing Data"

**Chae *et al.* (2015)**

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## 1) Supplemental Tables

### Table S1. Public human GRO-seq data sets mined using groHMM.

| Cell Line | Treatments | n | Total Reads | HMM Parameters | | Number of Transcripts *(After Correcting for Errors)* |
|---|---|---|---|---|---|---|
| | | | | -LtProbB ($T$) | UTS ($\sigma^2$) | |
| **MCF-7** | E2 for 0,10, 40, 160 min. | 2 | 63,473,424 | 350 | 30 | 31,159 |
| **LNCaP** | DHT for 0, 60 min. | 1 | 17,567,377 | 150 | 40 | 28,864 |
| **AC16** | TNFα for 0, 10, 30, 120 min. | 2 | 108,646,713 | 250 | 10 | 31,676 |
| **IMR90** | N/A | 2 | 10,672,805 | 150 | 50 | 25,154 |

### Table S2. List of HMM and non-HMM based broad peak callers and their applicability to the analysis of GRO-seq data.

| Method | HMM-based? | Input Required? | Strand Specific Output? | Usable for GRO-seq data? | Reference |
|---|---|---|---|---|---|
| **groHMM** | Yes | No | Yes | Yes | 28 |
| **SICER** | No | Optional | No | Yes | 29 |
| **HOMER** | No | No | Yes | Yes | 30 |
| **RSEG** | Yes | Optional | No | Yes | 31 |
| **CCAT** | No | Yes | No | No | 53 |
| **ZINBA** | No | Yes | No | No | 54 |
| **BroadPeak** | No | Yes | No | No | 55 |
| **MACS2** (broad peak run option) | No | Optional | No | No | 56 |

**Table S3. Performance of each transcript-calling algorithm tested using GRO-seq data from MCF-7 cells with default parameter values.**

| Method | Number of Transcripts | Median Transcript Length (bp) | Error | | | TUA (Transcription Unit Accuracy) |
|---|---|---|---|---|---|---|
| | | | Merged Annotation | Dissociated Annotation | Total | |
| **groHMM** | 22,686 | 8,959 | 1,956 | 745 | 2,674 | 0.896 |
| **SICER** | 119,393 | 2,600 | 1,602 | 2,099 | 9,551 | 0.533 |
| **HOMER** | 129,061 | 1,573 | 731 | 1,029 | 9,241 | 0.323 |
| **RSEG** | 57,355 | 4,051 | 1,617 | 5,699 | 5,699 | 0.801 |

**Table S4. Comparison of transcription units called by groHMM using optimal parameters versus the average of all 50 explored parameter sets for *D. melanogaster* GRO-seq data.**

| Transcription Units | Number of Transcripts | Median Transcript Length (bp) | Error | | | Parameters | Value |
|---|---|---|---|---|---|---|---|
| | | | Merged Annotation | Dissociated Annotation | Rate | | |
| **Optimal** | 15,149 | 1,650 | 1,317 | 601 | 0.09 | -LtProbB ($T$)<br>UTS ($\sigma^2$) | 50<br>50 |
| **Average of 50 transcript sets** | 21,393 | 1,339 | 1,231 | 890 | 0.10 | -LtProbB ($T$)<br>UTS ($\sigma^2$) | 10..50<br>5..50 |
| **Consensus Annotation** | 10,542 | 2,661 | N/A | N/A | N/A | N/A | N/A |

**Table S5. Comparison of transcription units called by groHMM using optimal parameters versus the average of all 50 explored parameter sets for *C. elegans* GRO-seq data.**

| Transcription Units | Number of Transcripts | Median Transcript Length (bp) | Error | | | Parameters | Value |
|---|---|---|---|---|---|---|---|
| | | | Merged Annotation | Dissociated Annotation | Rate | | |
| **Optimal** | 25,180 | 1,800 | 2,989 | 581 | 0.10 | -LtProbB ($T$)<br>UTS ($\sigma^2$) | 20<br>50 |
| **Average of 50 transcript sets** | 22,009 | 4,078 | 2,854 | 472 | 0.11 | -LtProbB ($T$)<br>UTS ($\sigma^2$) | 10..50<br>5..50 |
| **Consensus Annotation** | 15,297 | 2,207 | N/A | N/A | N/A | N/A | N/A |

**Table S6. Top ten GO terms for the cell type-specific enhancer clusters.**

| *A. Cluster 1 (n =144)* | | | |
|---|---|---|---|
| **GO Terms** | **ID** | **-log10 p-value** | **Dispensability** |
| antigen processing and presentation of exogenous peptide antigen | GO:0002478 | 3 | 0 |
| proteolysis | GO:0006508 | 4 | 0 |
| response to oxidative stress | GO:0006979 | 4 | 0 |
| regulation of cell migration | GO:0030334 | 4 | 0 |
| response to abiotic stimulus | GO:0009628 | 4 | 0.092 |
| response to radiation | GO:0009314 | 4 | 0.094 |
| response to biotic stimulus | GO:0009607 | 3 | 0.101 |
| vasculature development | GO:0001944 | 4 | 0.108 |
| cytokinesis | GO:0000910 | 3 | 0.110 |
| cell activation | GO:0001775 | 4 | 0.136 |

| *B. Cluster 2 (n =86)* | | | |
|---|---|---|---|
| **GO Terms** | **ID** | **-log10 p-value** | **Dispensability** |
| protein localization to organelle | GO:0033365 | 4 | 0 |
| regulation of innate immune response | GO:0045088 | 4 | 0 |
| viral reproduction | GO:0016032 | 4 | 0.037 |
| cellular macromolecule catabolic process | GO:0044265 | 4 | 0.039 |
| chromosome segregation | GO:0007059 | 4 | 0.115 |
| interphase | GO:0051325 | 4 | 0.121 |
| regulation of ligase activity | GO:0051340 | 4 | 0.133 |
| microtubule-based process | GO:0007017 | 4 | 0.138 |
| induction of apoptosis | GO:0006917 | 4 | 0.147 |
| cytoskeleton organization | GO:0007010 | 4 | 0.151 |

| *C. Cluster 3 (n =36)* | | | |
|---|---|---|---|
| **GO Terms** | **ID** | **-log10 p-value** | **Dispensability** |
| RNA splicing | GO:0008380 | 4 | 0 |
| cellular component biogenesis | GO:0044085 | 4 | 0 |
| establishment of protein localization to organelle | GO:0072594 | 3 | 0.031 |
| respiratory electron transport chain | GO:0022904 | 3 | 0.117 |
| viral transcription | GO:0019083 | 3 | 0.296 |
| RNA catabolic process | GO:0006401 | 3 | 0.312 |
| translation | GO:0006412 | 4 | 0.341 |
| ncRNA metabolic process | GO:0034660 | 4 | 0.347 |
| translational initiation | GO:0006413 | 4 | 0.384 |
| ribonucleoprotein complex biogenesis | GO:0022613 | 4 | 0.458 |

| *D. Cluster 4 (n =57)* | | | |
|---|---|---|---|
| **GO Terms** | **ID** | **-log10 p-value** | **Dispensability** |
| behavior | GO:0007610 | 4 | 0 |
| positive regulation of leukocyte chemotaxis | GO:0002690 | 4 | 0.073 |
| C21-steroid hormone biosynthetic process | GO:0006700 | 4 | 0.100 |
| synaptic transmission | GO:0007268 | 4 | 0.105 |
| cell fate specification | GO:0001708 | 4 | 0.113 |

| | | | |
|---|---|---|---|
| calcium ion transport | GO:0006816 | 4 | 0.249 |
| multicellular organismal signaling | GO:0035637 | 4 | 0.391 |
| regulation of exocytosis | GO:0017157 | 4 | 0.476 |
| steroid metabolic process | GO:0008202 | 3 | 0.506 |
| endocrine process | GO:0050886 | 3 | 0.523 |

***E. Cluster 5 (n =112)***

| **GO Terms** | **ID** | **-log10 p-value** | **Dispensability** |
|---|---|---|---|
| biological adhesion | GO:0022610 | 3 | 0 |
| response to lipid | GO:0033993 | 4 | 0 |
| cardiocyte differentiation | GO:0035051 | 4 | 0 |
| ribosome biogenesis | GO:0042254 | 4 | 0 |
| RNA polyadenylation | GO:0043631 | 4 | 0.025 |
| cellular alkene metabolic process | GO:0043449 | 3 | 0.056 |
| olefin metabolic process | GO:1900673 | 3 | 0.058 |
| positive regulation of calcium ion transport | GO:0051928 | 4 | 0.076 |
| sulfur compound metabolic process | GO:0006790 | 3 | 0.080 |
| regulation of androgen receptor signaling pathway | GO:0060765 | 4 | 0.105 |

***F. Cluster 6 (n =255)***

| **GO Terms** | **ID** | **-log10 p-value** | **Dispensability** |
|---|---|---|---|
| circadian rhythm | GO:0007623 | 3 | 0 |
| RNA 3'-end processing | GO:0031123 | 4 | 0 |
| rhythmic process | GO:0048511 | 3 | 0 |
| DNA damage response, signal transduction by p53 | GO:0030330 | 4 | 0.026 |
| viral reproductive process | GO:0022415 | 4 | 0.030 |
| response to ionizing radiation | GO:0010212 | 4 | 0.067 |
| bone mineralization | GO:0030282 | 3 | 0.078 |
| Golgi vesicle transport | GO:0048193 | 4 | 0.100 |
| cell cycle checkpoint | GO:0000075 | 4 | 0.108 |
| generation of precursor metabolites and energy | GO:0006091 | 3 | 0.108 |

***G. Cluster 7 (n =18)***

| **GO Terms** | **ID** | **-log10 p-value** | **Dispensability** |
|---|---|---|---|
| regulation of leukocyte migration | GO:0002685 | 4 | 0 |
| extracellular matrix organization | GO:0030198 | 4 | 0.101 |
| regulation of behavior | GO:0050795 | 4 | 0.130 |
| regulation of angiogenesis | GO:0045765 | 3 | 0.138 |
| cellular response to interferon-gamma | GO:0071346 | 4 | 0.148 |
| extracellular structure organization | GO:0043062 | 4 | 0.374 |

**Table S7. Top ten GO terms for the non-cell type-specific enhancer cluster.**

| *Cluster 1 (n =136)* | | | |
|---|---|---|---|
| **GO Terms** | **ID** | **-log10 p-value** | **Dispensability** |
| proteolysis | GO:0006508 | 4 | 0 |
| microtubule-based process | GO:0007017 | 4 | 0 |
| response to abiotic stimulus | GO:0009314 | 4 | 0 |
| establishment of protein localization | GO:0048002 | 4 | 0 |
| regulation of ligase activity | GO:0016032 | 4 | 0.039 |
| response to radiation | GO:0051340 | 4 | 0.063 |
| mitotic prometaphase | GO:0000910 | 3 | 0.117 |
| chromosome segregation | GO:0007059 | 4 | 0.119 |
| induction of apoptosis | GO:0006917 | 4 | 0.130 |
| cell division | GO:0006913 | 4 | 0.132 |

## 1) Supplemental Figures

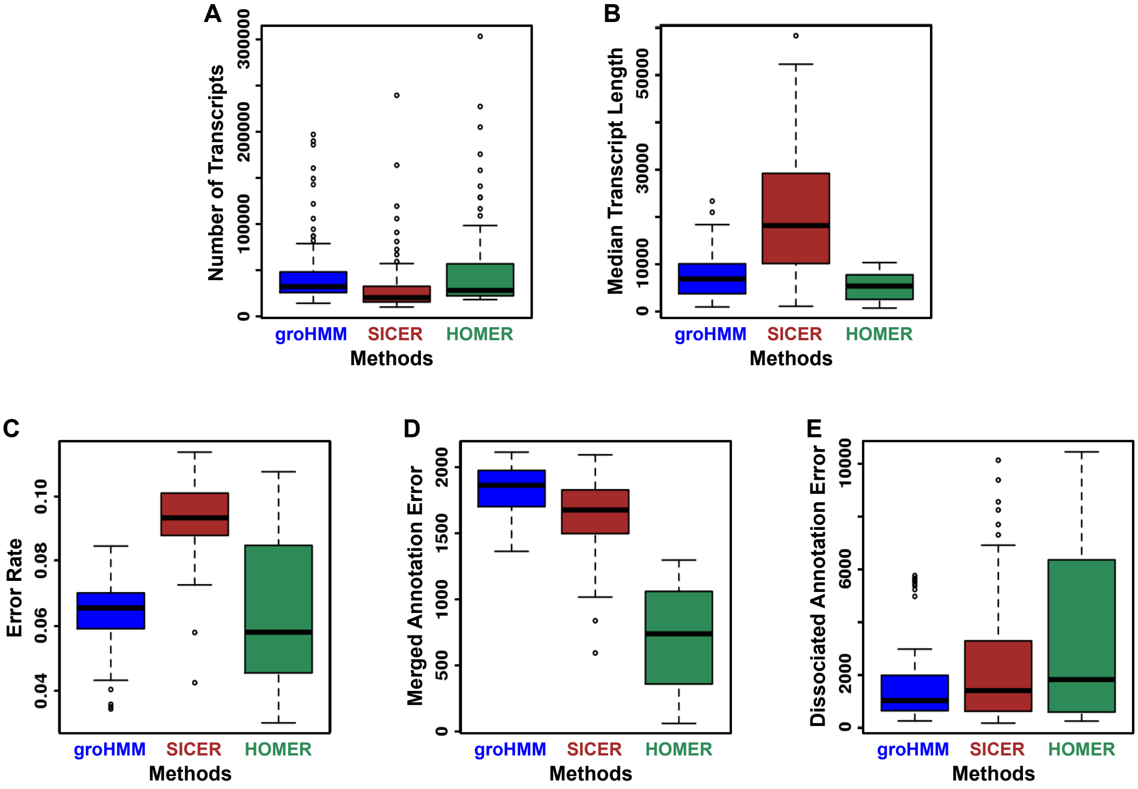


**Figure S1. Parametric space for explored 100 models comparing three transcript callers: groHMM, SICER, and HOMER.**
**(A)** Number of transcripts called.
**(B)** Median transcript length.
**(C)** Total error rate for two types of error (‘merged annotation error’ and ‘dissociated annotation error’).
**(D)** Number of occurrences of ‘merged annotation error.’
**(E)** Number of occurrences of ‘dissociated annotation error.’

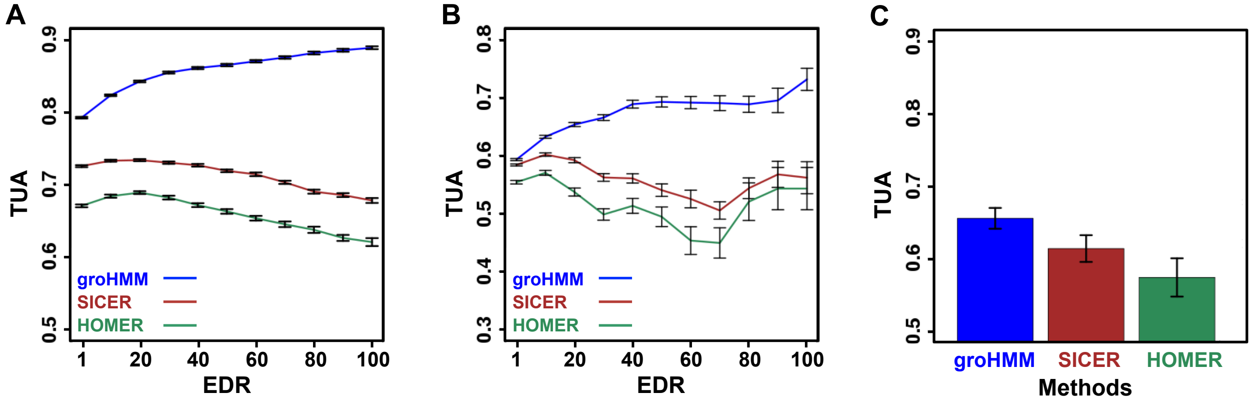


**Figure S2. Variations in TUA with gene expression patterns.**

**(A)** Evaluation of TUA when varying EDR (i.e., the smoothness of expression patterns) for mRNA genes.

**(B)** Evaluation of TUA when varying EDR (i.e., the smoothness of expression patterns) for lncRNA genes.

**(C)** TUA of called transcripts for well-expressed lncRNA annotations (n = 2,403). Ten percent of the annotations were bootstrapped with replacement (n = 100).

**Figure S3. Functional analysis of cell type-specific enhancers in three cell types.** *[See following page for the figure]*

In order to infer the function of the cell type-specific enhancers that we identified above, we used Gene Set Enrichment Analysis (GSEA) [39].

**(A)** Association matrix for the cell type-specific enhancers with functional gene sets. We determined the correlation of the transcription of each protein-coding gene with the transcription of each of the 1,052 cell type-specific enhancers. We then ranked the protein-coding genes based on the strength of their correlations and used these rankings to assign enrichment scores for all GSEA categories (i.e., gene ontology, or GO, terms) for each enhancer. Next, we performed hierarchical clustering analysis, displaying the normalized GSEA enrichment scores for each enhancer. Each row is a GO term with its associated normalized GSEA enrichment scores and each column represents an enhancer. This analysis identified seven clusters (**Table S5**). The GO terms in the clusters represent the characteristics of the cell type in which the enhancers are active. Red = positive association; green = negative association. The clusters were identified by using the cuttree function of R.

**(B)** Summary heatmap for the clusters shown in (A). The median values of each cluster were used for a more simple visual representation.

**(C)** Association matrix of non-cell type-specific enhancers with functional gene sets, as in (A). Analysis of 837 non-cell type-specific enhancers yielded fewer clusters and failed to group the enhancers from each cell type (**Table S6**).

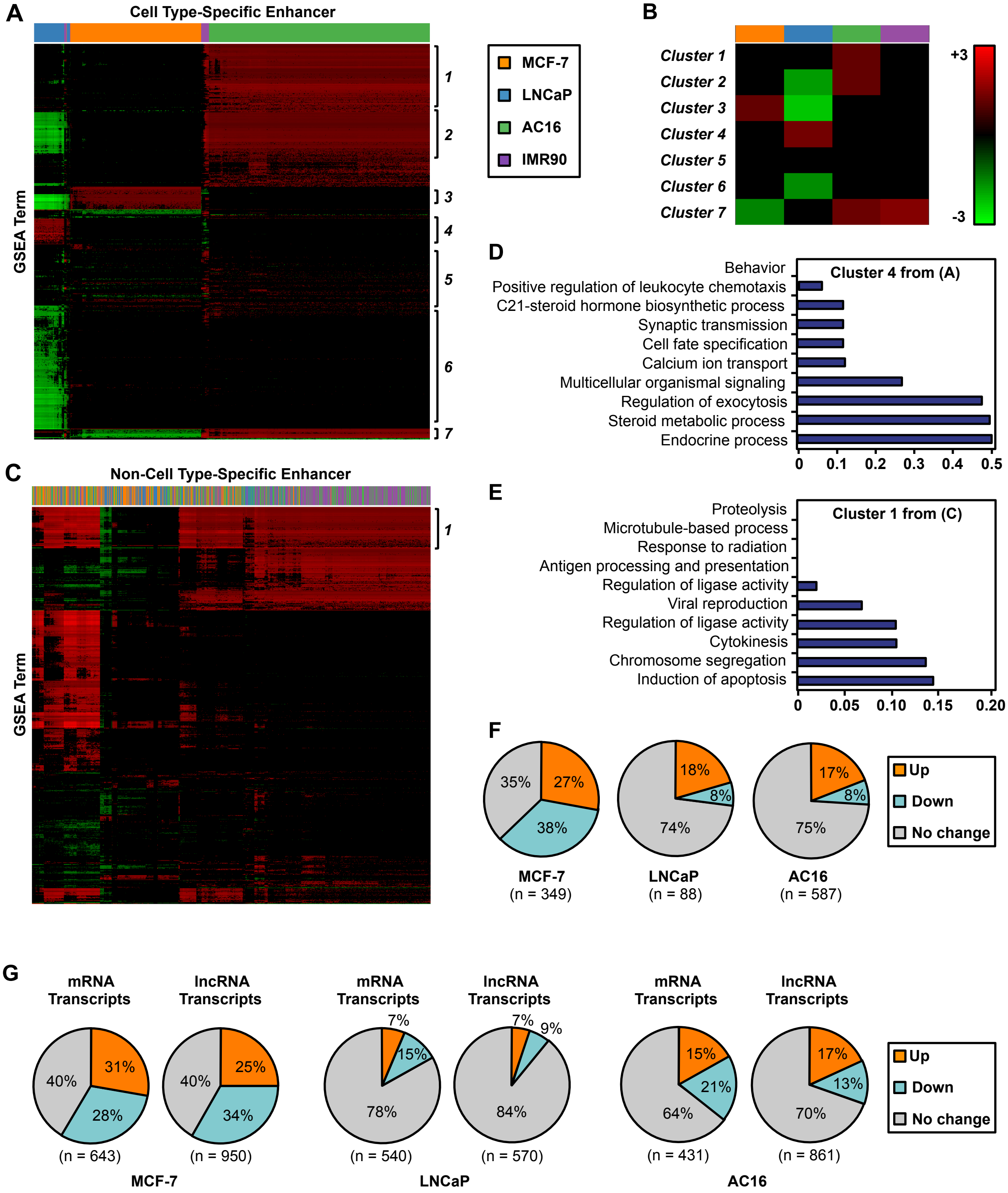
**(D)** The top ten GO terms for cluster 4 (n = 57) summarized by REVIGO. The p-values for all terms were < 0.0001 and ordered by 'dispensability,' which represents the non-redundancy of the term in the group [57]. The cluster 4 enhancers (panel A), which are active in LNCaP cells treated with dihydrotestosterone, are associated with GO terms related to steroid signaling, endocrine processes, and cellular signaling.

**(E)** Top ten GO terms for cluster 1 (n = 124) in (D) summarized by REVIGO (p-values < 0.001 and ordered by dispensability). The cluster 1 enhancers from the non-cell type-specific enhancer analysis (panel C), which are active in multiple cell types, are associated with GO terms related to a broader array of cellular processes.

**(F)** Pie charts showing regulation of enhancer transcription by treatment in each cell type (MCF-7, estradiol; LNCaP, dihydrotestosterone; AC16, tumor necrosis factor alpha). Regulation was called using FDR < 1% for MCF-7 and AC16, and p-value < 0.001 for LNCaP with edgeR. The data from the MCF-7, LNCaP, and AC16 cells gave us a unique opportunity to address this questions, given the availability of GRO-seq data sets from hormone-treated cells (MCF-7, estradiol; LNCaP, dihydrotestosterone; AC16, tumor necrosis factor alpha). When compared to the basal (untreated) condition, the treatments affected (either upregulated or downregulated) the transcription of between 25% and 65% of the cell type-specific enhancers within a given cell type, with the effects of the estradiol treatment in MCF-7 cells being most pronounced.

**(G)** Effects of treatments on mRNA and lncRNA transcript expression in three cell types. Pie charts showing the percent of protein-coding transcripts *(left)* and lncRNA transcripts *(right)* regulated by treatment in each cell type (MCF-7, estradiol; LNCaP, dihydrotestosterone; AC16, tumor necrosis factor alpha). Regulation was called using FDR < 1% for MCF-7 and AC16, and p-value < 0.001 for LNCaP using edgeR. The proportions of regulated protein-coding (mRNA) or lncRNA transcripts were similar to the proportions of regulated enhancer transcripts for each cell types.

## Figure S4.


A
Cell Type-Specific Enhancer
GSEA Term
1
2
3
4
5
6
7
MCF-7
LNCaP
AC16
IMR90
B
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
+3
-3
C
Non-Cell Type-Specific Enhancer
GSEA Term
1
D
Cluster 4 from (A)
Behavior
Positive regulation of leukocyte chemotaxis
C21-steroid hormone biosynthetic process
Synaptic transmission
Cell fate specification
Calcium ion transport
Multicellular organismal signaling
Regulation of exocytosis
Steroid metabolic process
Endocrine process
0
0.1
0.2
0.3
0.4
0.5
E
Cluster 1 from (C)
Proteolysis
Microtubule-based process
Response to radiation
Antigen processing and presentation
Regulation of ligase activity
Viral reproduction
Regulation of ligase activity
Cytokinesis
Chromosome segregation
Induction of apoptosis
0
0.05
0.10
0.15
0.20
F
35%
27%
38%
MCF-7
(n = 349)
18%
8%
74%
LNCaP
(n = 88)
17%
8%
75%
AC16
(n = 587)
Up
Down
No change
G
mRNA Transcripts
lncRNA Transcripts
mRNA Transcripts
lncRNA Transcripts
mRNA Transcripts
lncRNA Transcripts
40%
31%
28%
(n = 643)
40%
25%
34%
(n = 950)
MCF-7
7%
15%
78%
(n = 540)
7%
9%
84%
(n = 570)
LNCaP
15%
21%
64%
(n = 431)
17%
13%
70%
(n = 861)
AC16
Up
Down
No change