

Figure S1. Peak score distribution.

Peaks were binned according to the number of tags per height. The number of peaks in each bin is plotted in a log₁₀ scale. In blue the E2 data is plotted, in red the minus ligand control. The minus ligand data shows that the majority of peaks are in the lower peak height bins, while the E2 data shows peak present through the entire range, indicating the presence of many high confidence binding sites that are E2 dependent.

Figure S2. Validation of ChIP-Seq data by qPCR.

Twenty ER α interaction sites were randomly selected and validated by ChIP-qPCR. Recovery at ER α binding sites was compared to background, i.e. the recovery at exon 2 of the myoglobin gene, a genomic region to which ER α does not bind. At a threshold of 2 fold over background all sites were validated as positive. The mean of 3 biological replicates is plotted and error bars represent the standard deviation.

Figure S3. ER α binding induced by different ligands.

Binding of ER α is affected but not abolished upon treatment with different ligands. Depicted is a representative example of residual binding of ER α in response to tamoxifen treatment, whereas binding is abolished in the presence of fulvestrant.

Figure S4. Evolutionary conservation of ER α binding sites.

For each binding site the mean PhastCons28 (28-way vertebrate conservation) score was calculated. The mean score for each group is plotted. As background the mean score of random genomic regions was calculated. All groups have significantly higher mean compared to background (p-value < 0.01).

Figure S5. Validation of RNAPII enrichment in the promoter.

RNAPII occupancy was measured for 6 genes by ChIP-qPCR at different positions in the promoter; 500 bp upstream of the TSS, at the TSS, 500 bp downstream and 1 kb downstream of the TSS. The mean of 3 independent replicates is plotted and error bars represent the standard deviation.

Figure S6. Phosphorylation status of RNAPII at promoters displaying PPEP.

ChIP-qPCR validation was performed with antibodies against RNAPII, N-terminal RNAPII and phospho-specific antibodies (serine 2, serine 5 and serine 7). The observation that Serine 5 phosphorylation and surprisingly serine 7 phosphorylation are present in the promoter region, while serine 2 phosphorylation is low or absent implies PPEP in the absence as well as presence of E2. The mean of three independent replicates is plotted.

Figure S7. Effect of antagonists on ER α and RNAPII occupancy in the *TFFI* locus. The left panel shows ER α and the right panel the RNAPII profile. In green the

minus ligand, in red the E2, in blue the tamoxifen and in purple the fulvestrant data is shown. The representative example shows a clear increase in ER α occupancy upon E2 and tamoxifen induction. Treatment with fulvestrant results in only limited ER α binding. RNAPII occupancy is increased only upon E2 treatment.

Figure S8. RNAPII validation.

For each cluster 7-8 genes were randomly selected and the RNAPII occupancy was measured by ChIP-qPCR. The mean of three independent replicates is plotted. Error bars represent standard deviation. Cluster 2 shows moderate downregulation of RNAPII occupancy upon E2 treatment while cluster 3 and 4 show a strong increase in RNAPII occupancy. Tamoxifen and fulvestrant result in diminishing levels on cluster 3 and 4 genes. Cluster 1 and 5 show a minor and less consistent alteration in RNAPII occupancy.

Figure S9. Primary transcript levels.

Changes in primary transcript was measured for 6 randomly chosen genes in the 5 clusters upon ligand treatment relative to minus ligand control (0 h). All primary transcript levels are normalized to that of ribosomal protein 19 (RSP19). The mean of 3 independent replicates is plotted. For 5/6 genes in cluster 2, primary transcript levels are decreased after 1 hour of E2 treatment and do not change significantly upon tamoxifen treatment. Fulvestrant treatment increases primary transcript levels. Genes in cluster 3 and 4 show strong upregulation of primary transcript levels already after 1 hour of E2 treatment; at later times the levels decrease while tamoxifen and

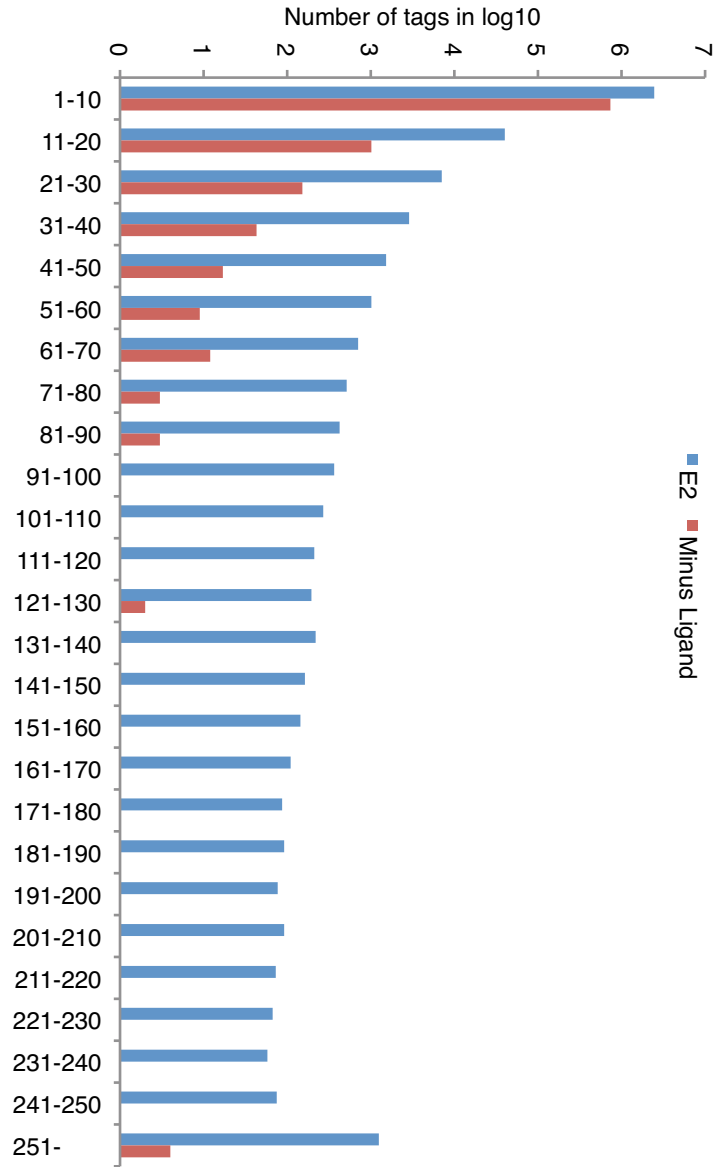
fulvestrant do elicit significant changes. Primary transcript levels of cluster 1 and 5 show only minor and not very consistent changes upon ligand treatment.

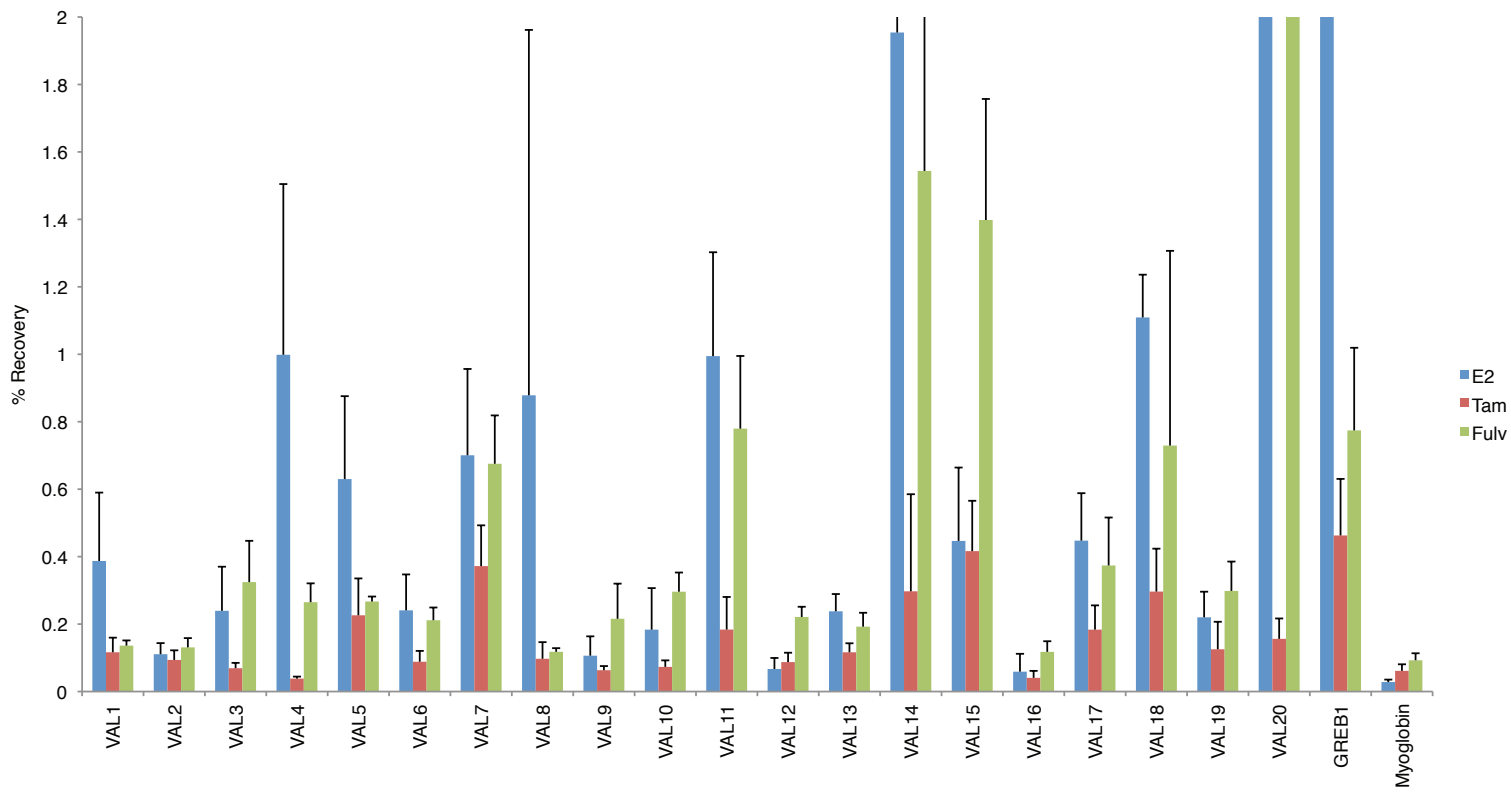
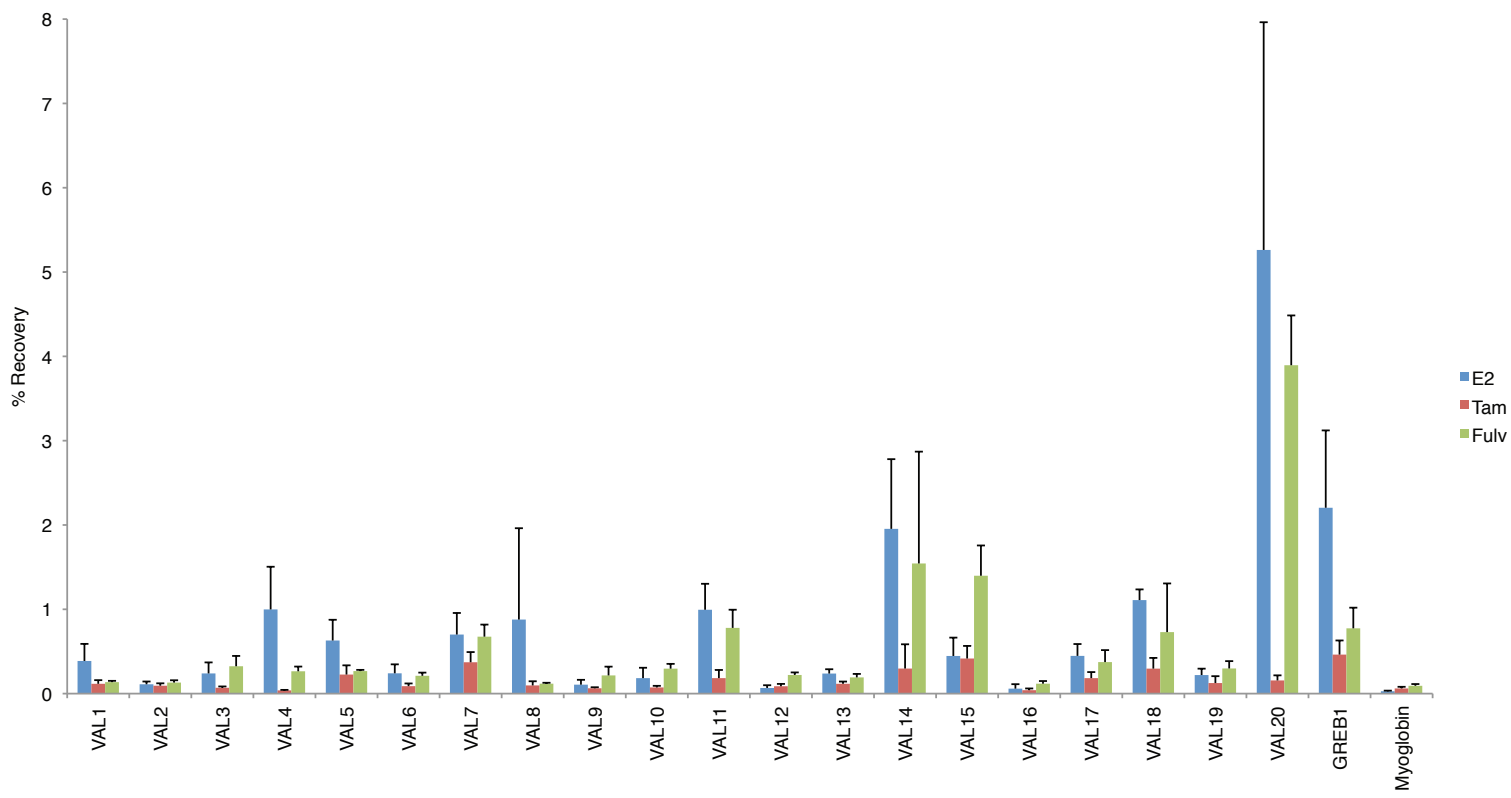
Figure S10. mRNA expression levels of the 5 clusters.

Changes in mRNA was analyzed as in Figure S9. mRNA levels were normalized to RSP19. The mean of 3 independent replicates is plotted. Cluster 2 shows downregulation of all genes following 8 hour of E2 as well as tamoxifen and fulvestrant treatment. In cluster 3, 2/6 genes show an increase in mRNA already after 1 h and at 3 h after E2 treatment 5/6 genes show elevated mRNA. Cluster 4 genes are delayed and reveal an increase in mRNA levels only after 3 h of E2 treatment. Tamoxifen and fulvestrant treatment diminished mRNA levels of cluster 3 and 4 genes. Cluster 1 genes show a decrease in mRNA after 8 h of tamoxifen treatment. Genes from cluster 5 show a rather inconsistent and temporary upregulation of mRNA levels.

Figure S11. RNAPII occupancy.

RNAPII occupancy over two genes. **A.** Myc gene. **B.** SIAH2 gene.





57 — ER α minus ligand

1 — ER α E2

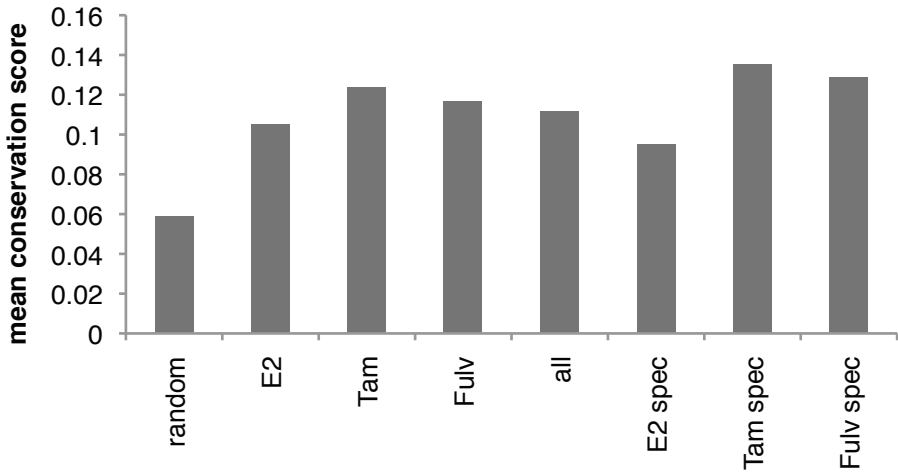
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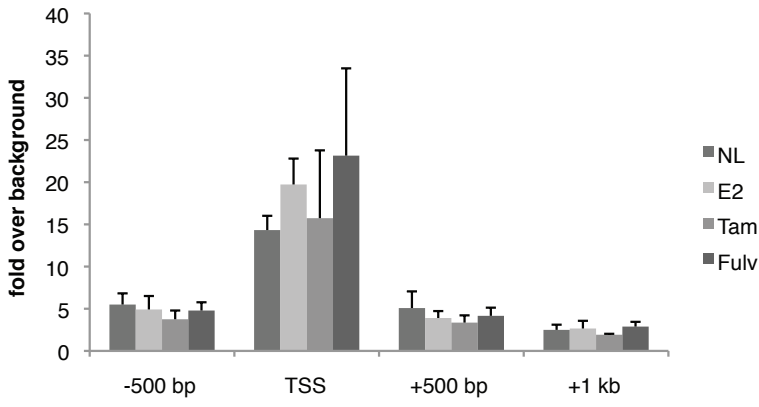
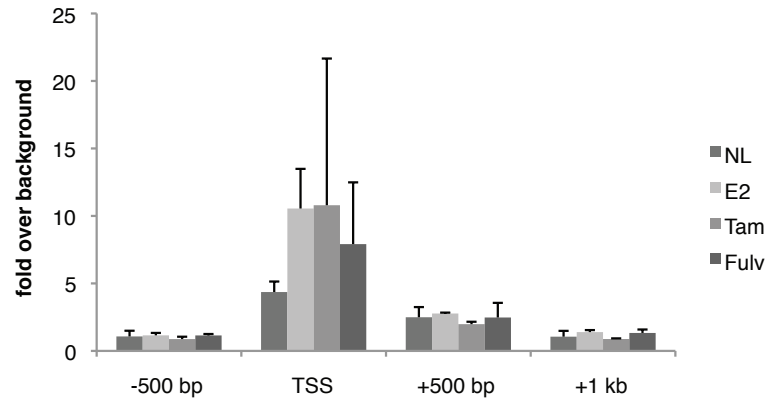
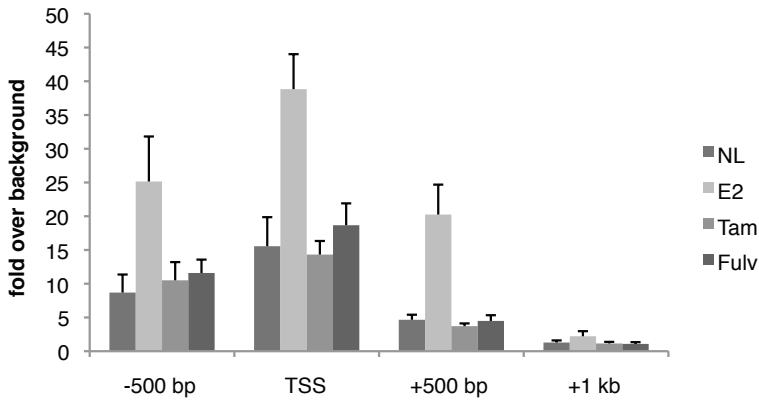
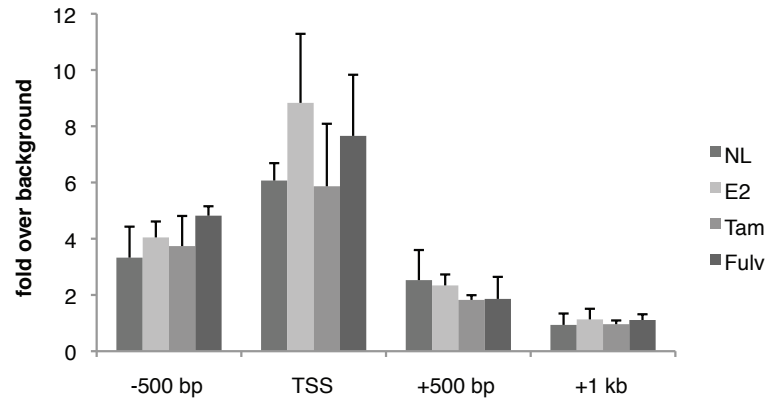
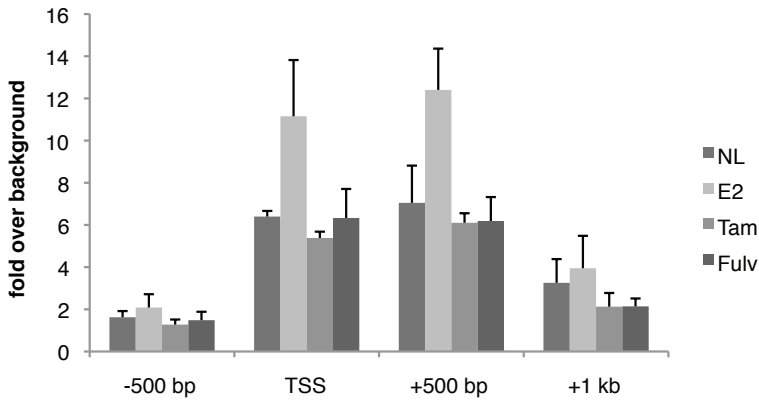
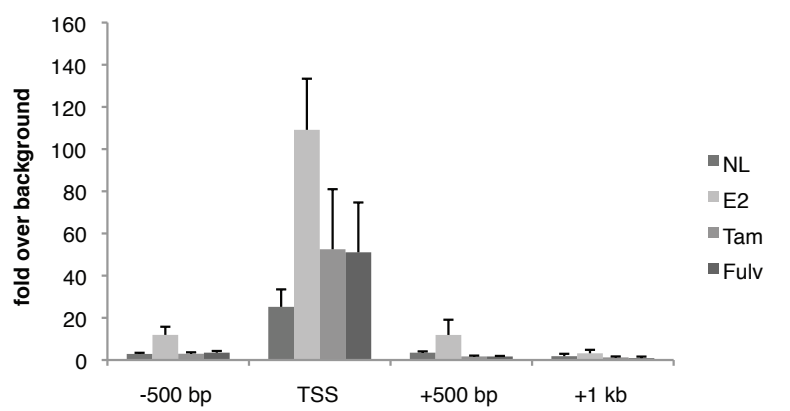
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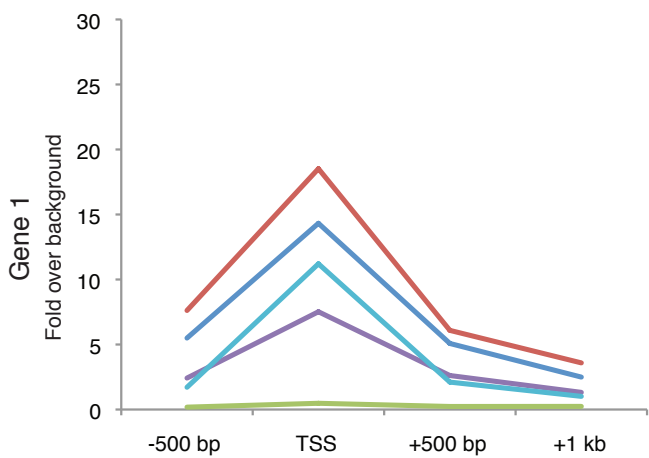
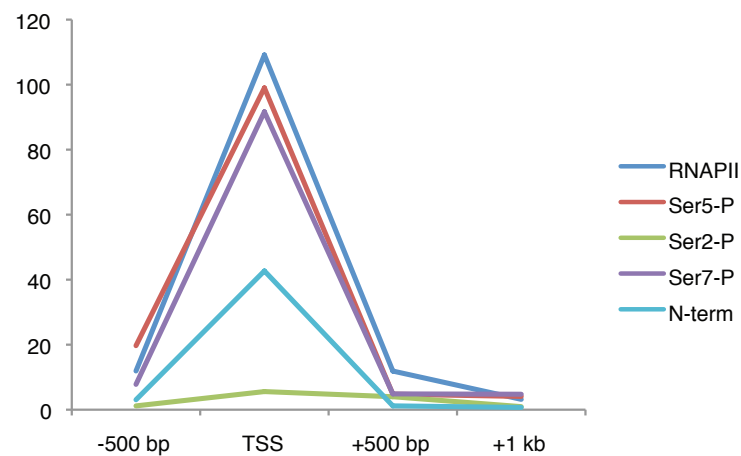
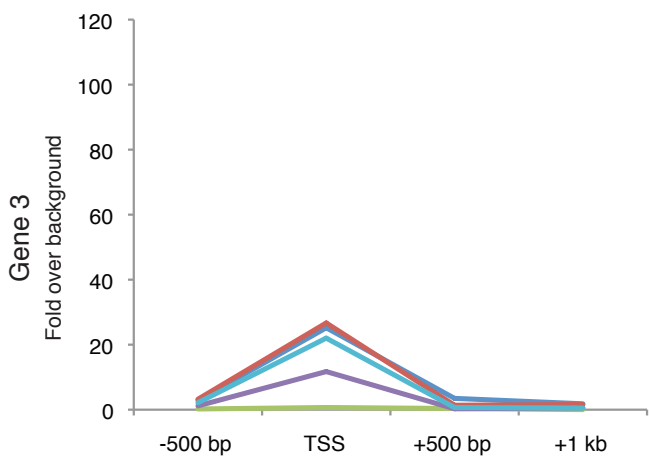
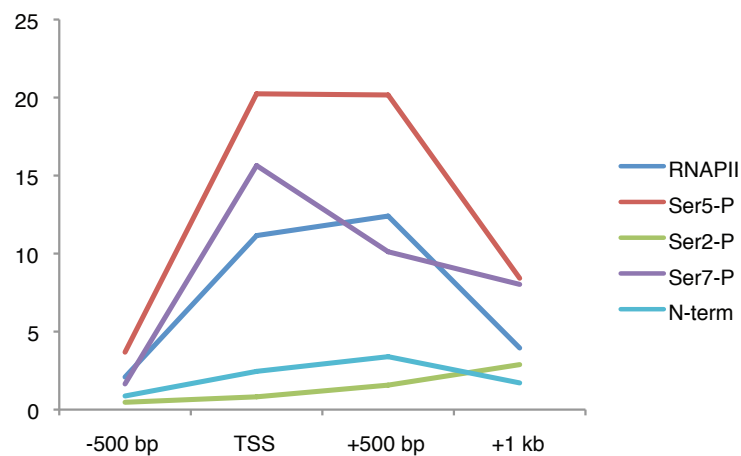
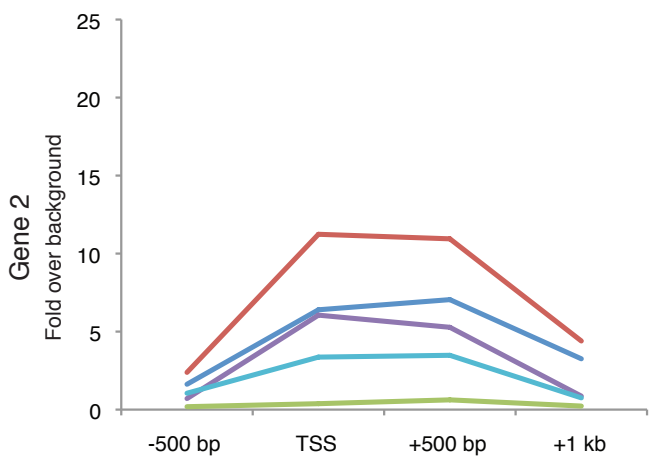
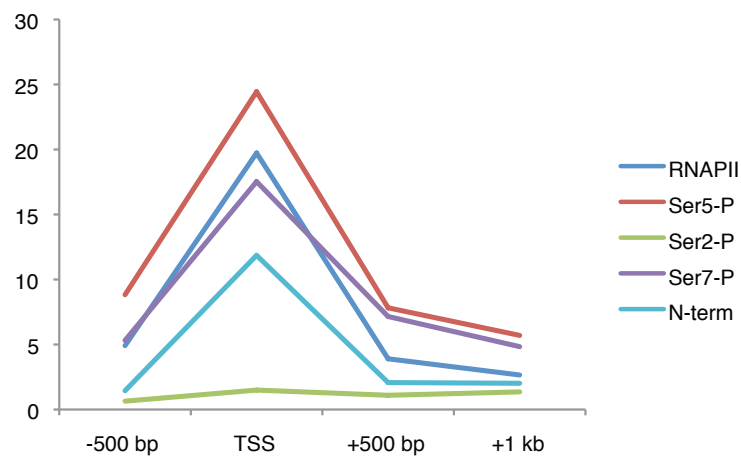
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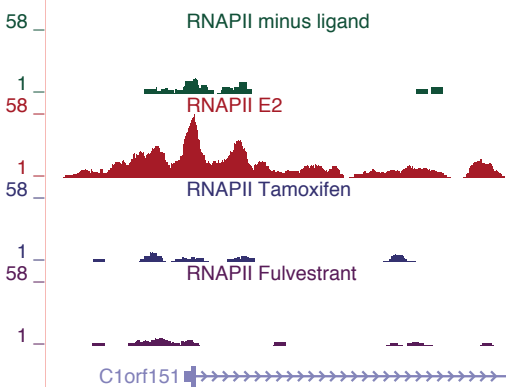
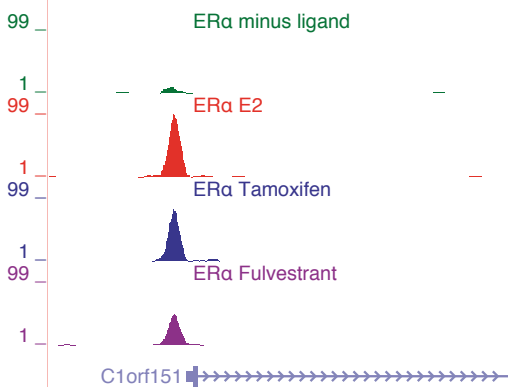


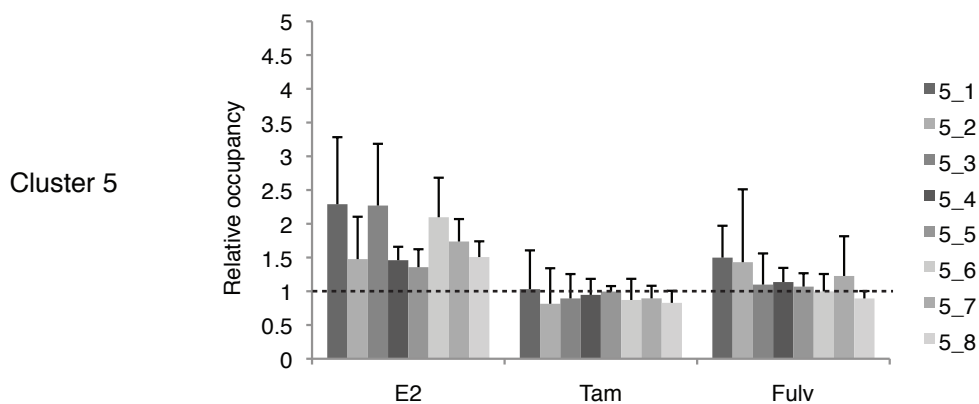
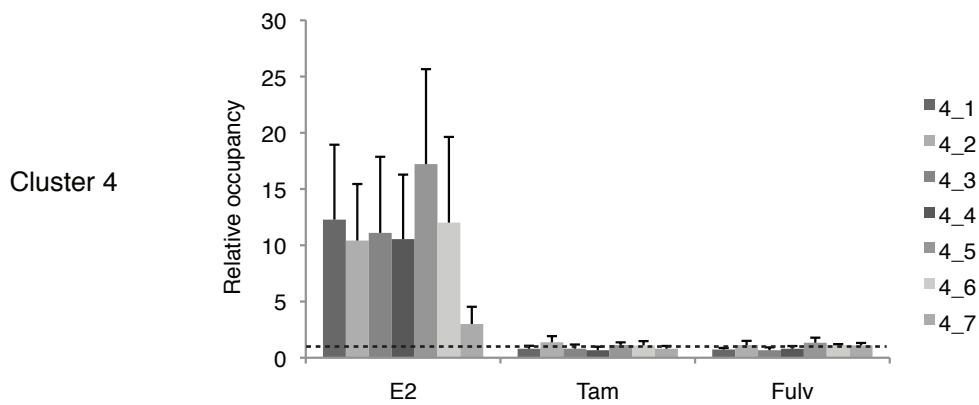
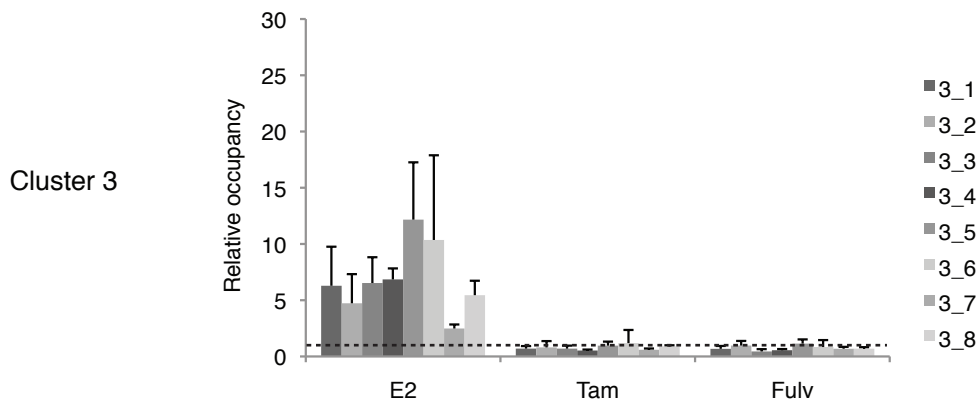
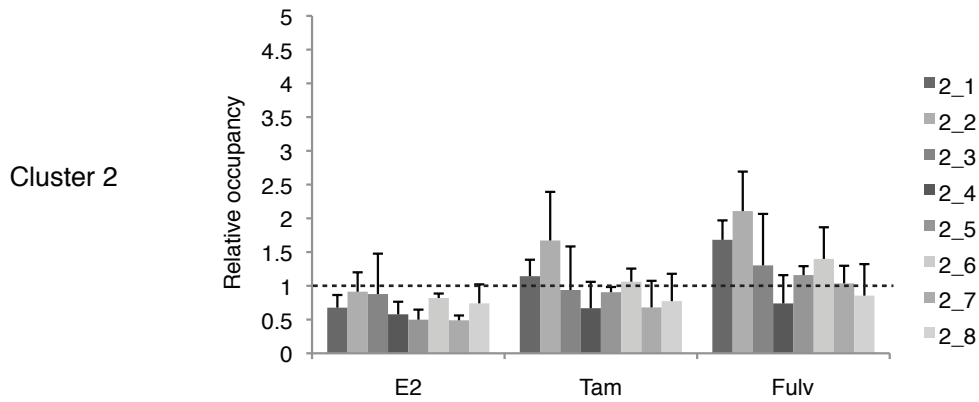
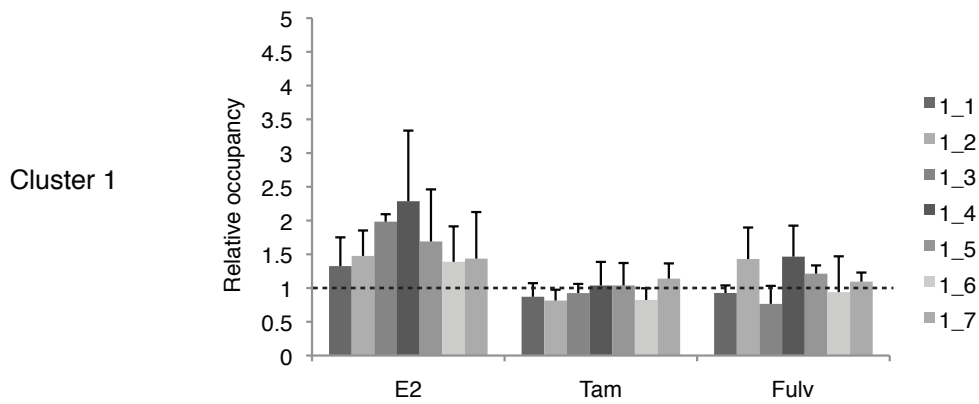
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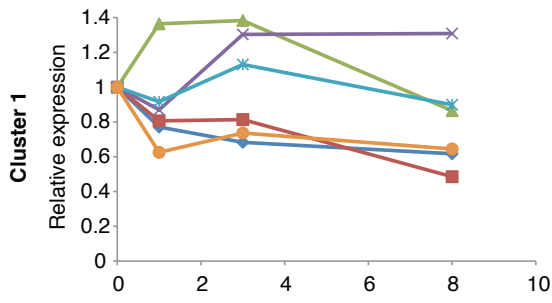
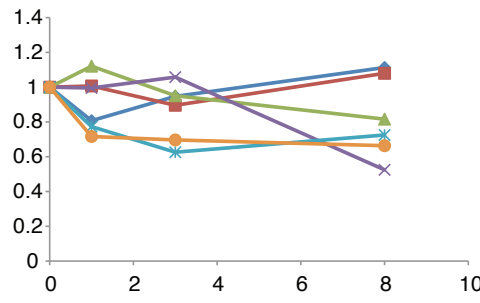
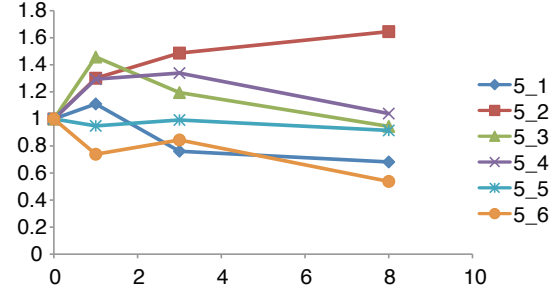
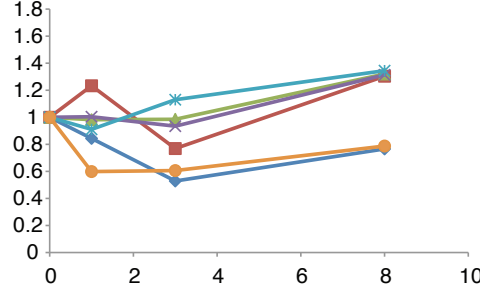
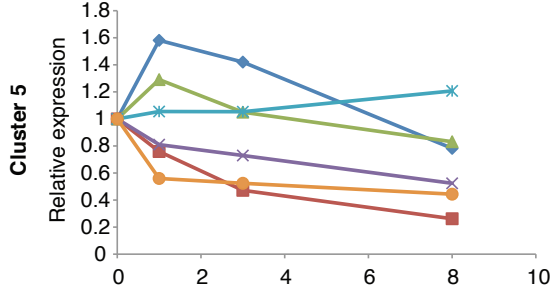
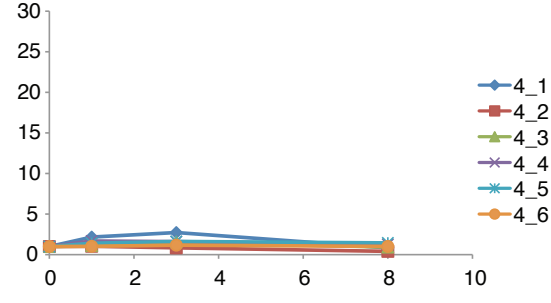
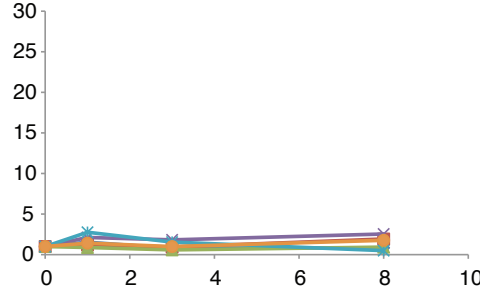
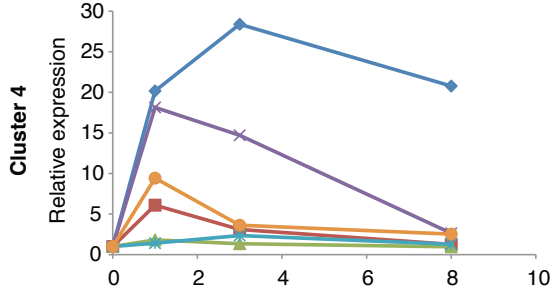
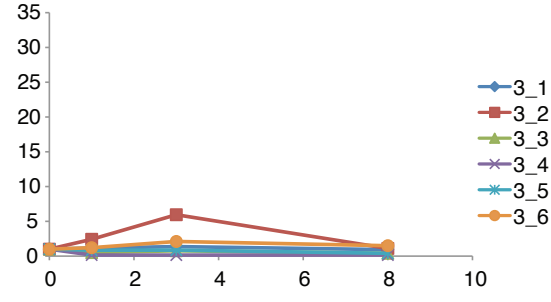
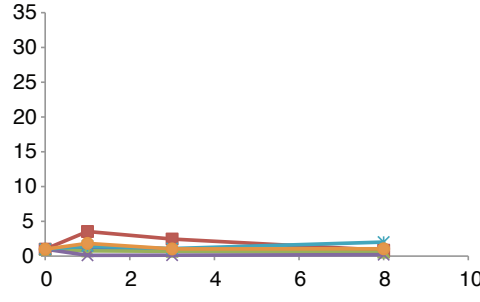
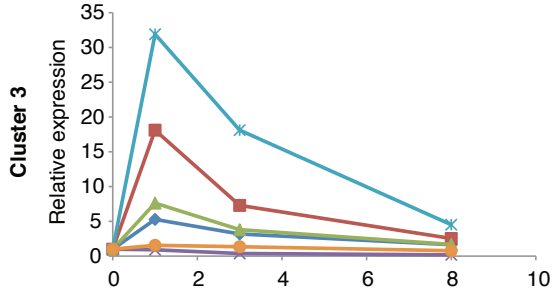
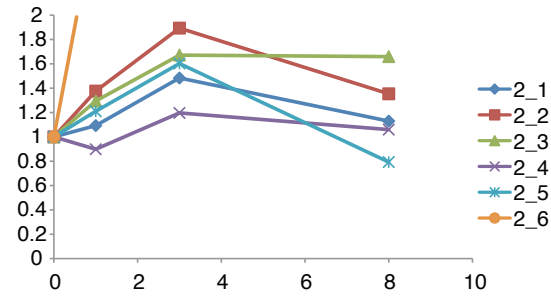
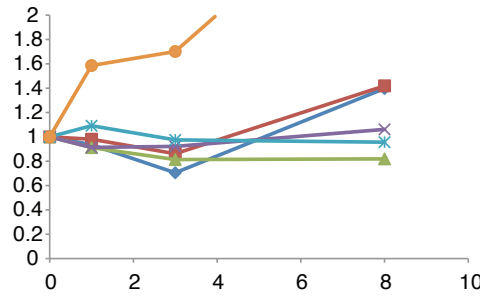
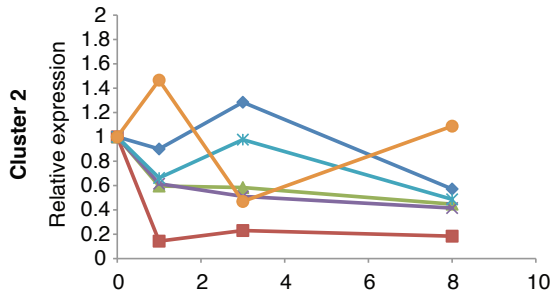
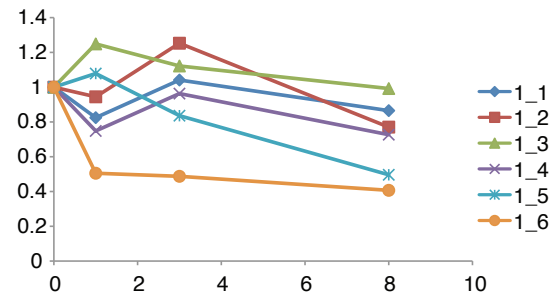


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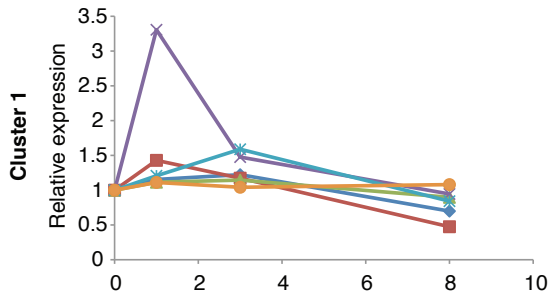
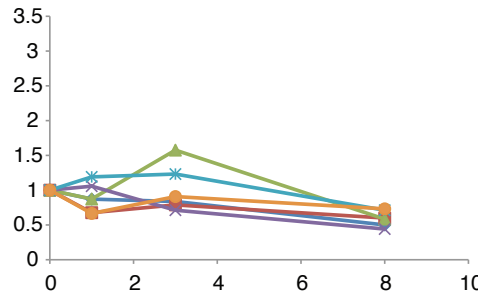
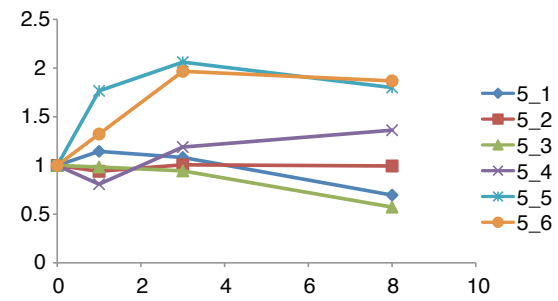
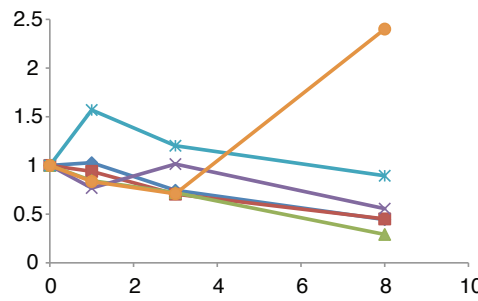
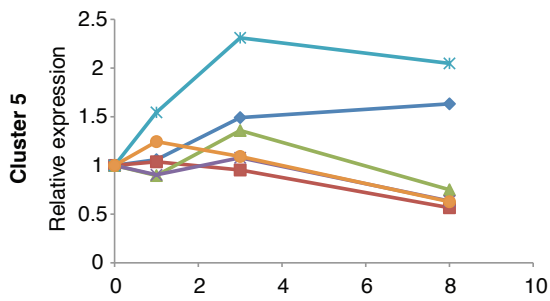
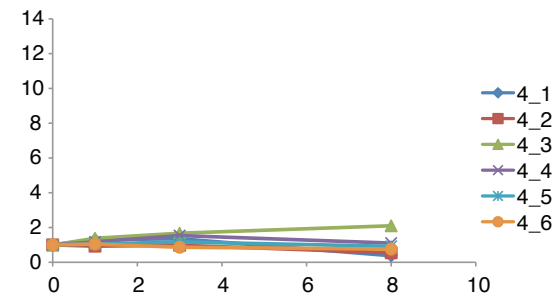
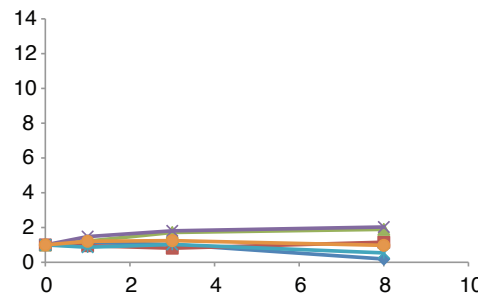
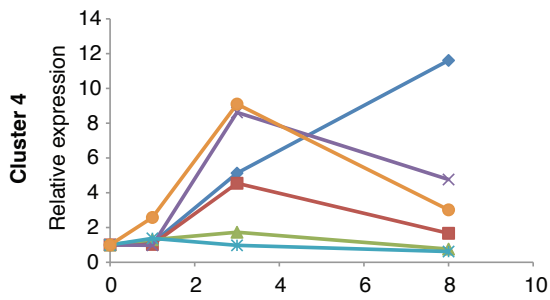
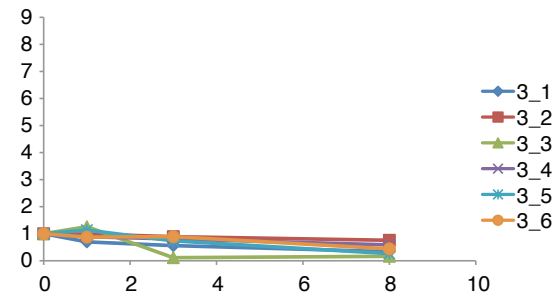
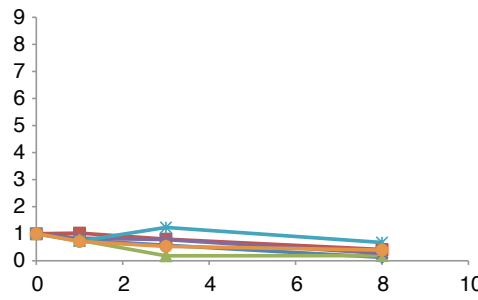
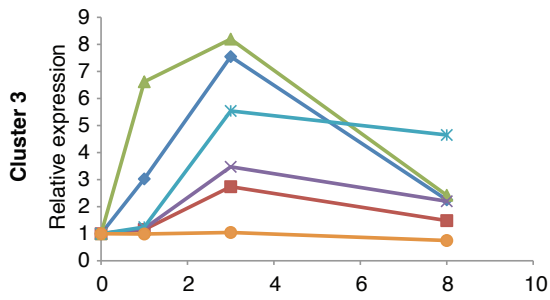
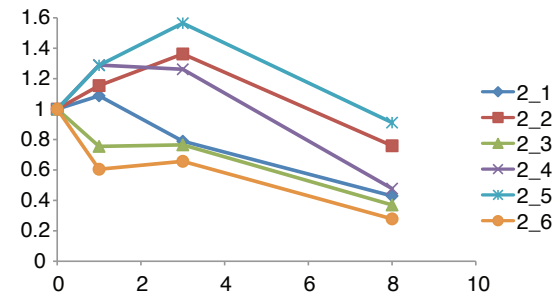
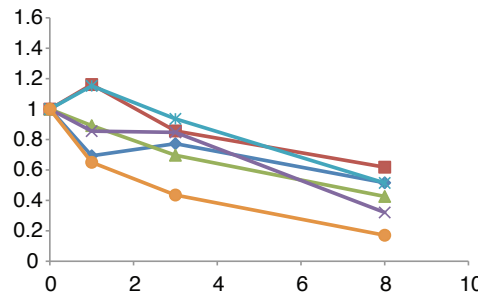
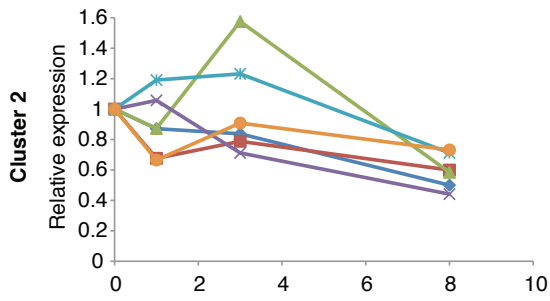
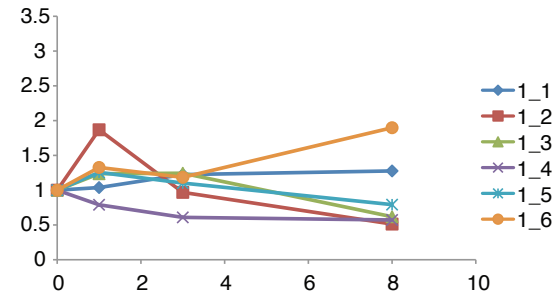


E2**Tamoxifen****Fulvestrant**

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