# H4K12ac is regulated by estrogen receptor-alpha and is associated with BRD4 function and inducible transcription

## Supplementary Material

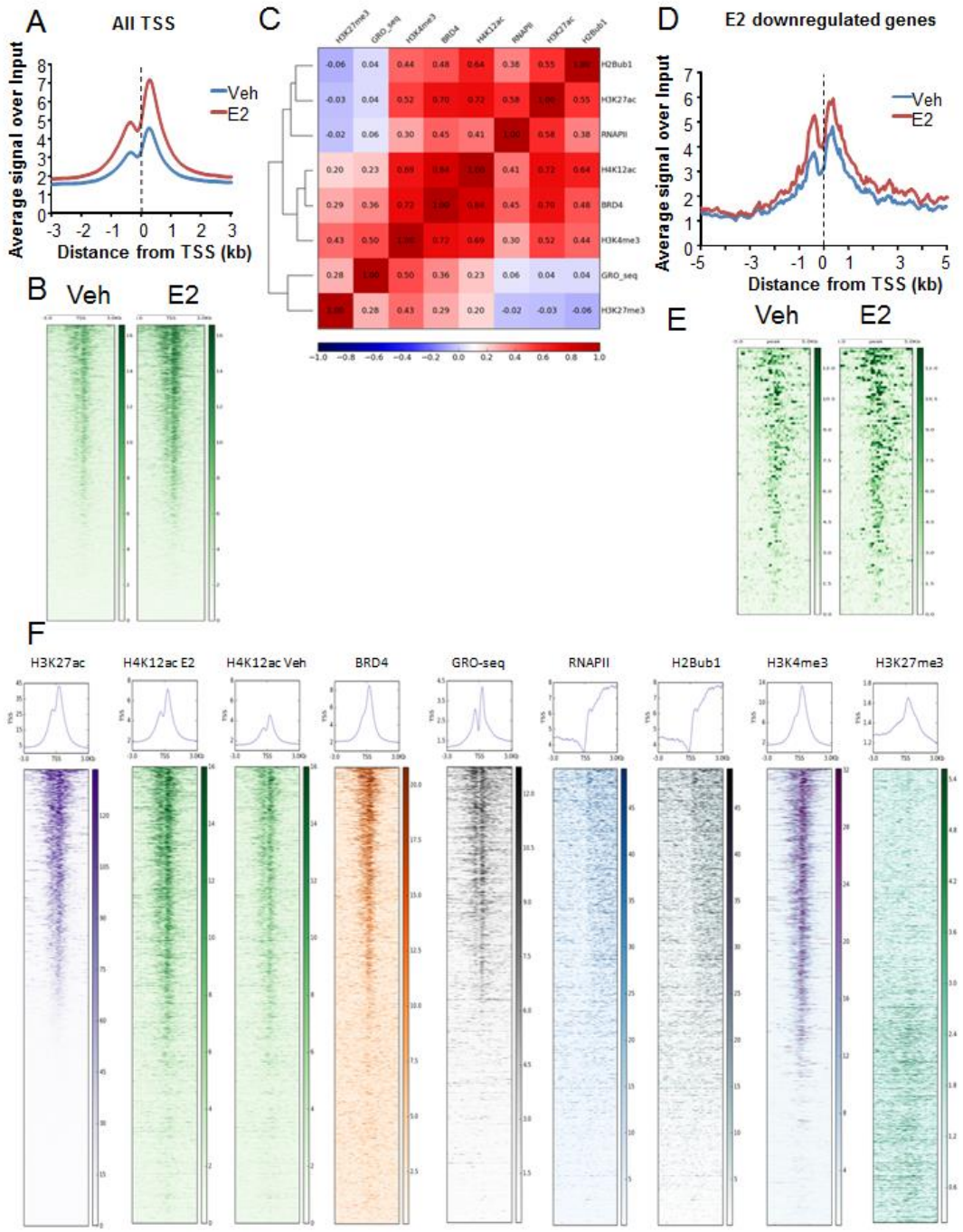
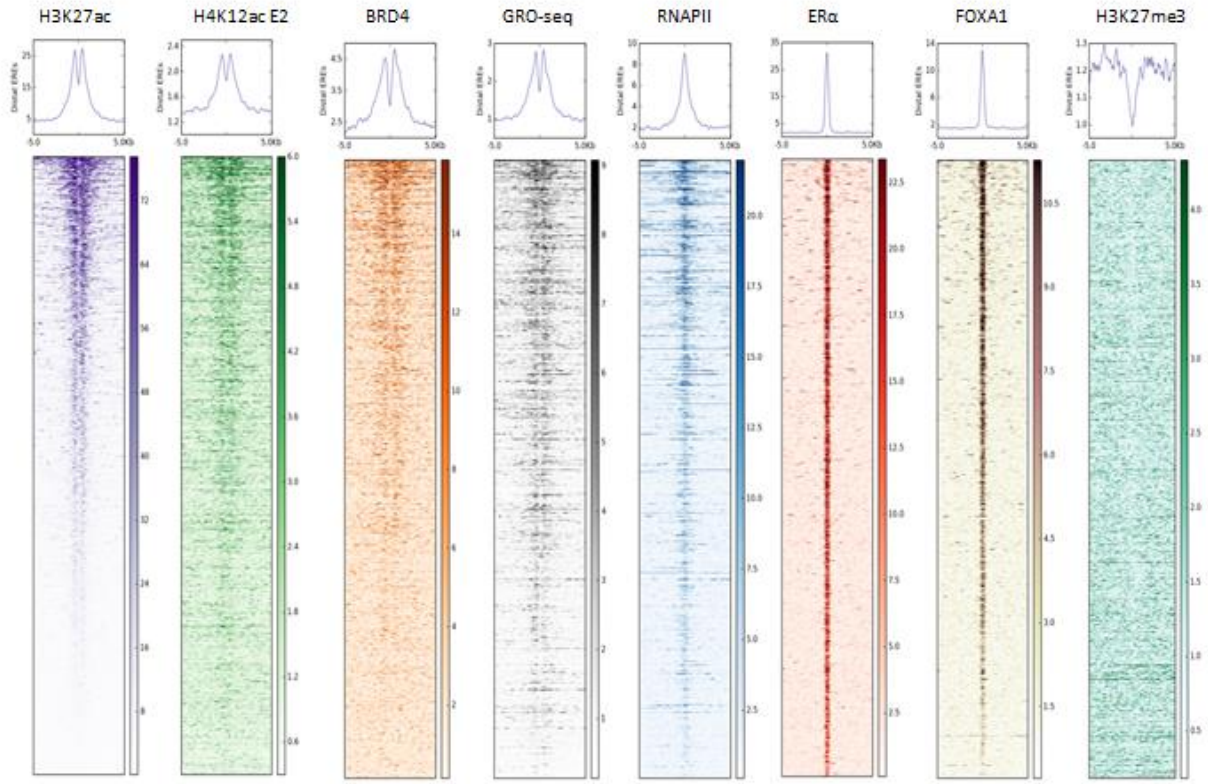
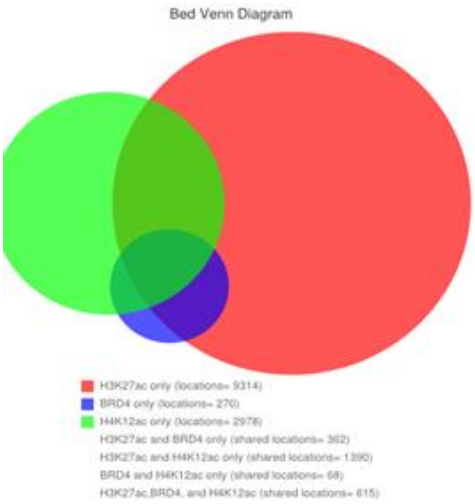


**Figure S1:** (A) Average genomic binding profiles of H4K12ac around TSS and 3 kb upstream and downstream of all the genes under vehicle (Veh) and estrogen-treated (E2) conditions. The X-axis shows the distance from the TSS of the genes in kilobase pairs. TSS is indicated by a black dotted line. (B) Heatmaps showing genomic binding profiles of H4K12ac around TSS and 3 kb upstream and downstream of all genes under vehicle (Veh) and estrogen-treated (E2) conditions. Center of the heatmap represents TSS. Color key of the heatmaps is shown on the side. (C) Correlation plot showing the heatmap with the Pearson's correlation coefficient values for H4K12ac, BRD4, H3K27ac, H3K4me3, H2Bub1, GRO-seq, RNAPII and H3K27me3 on TSS and 3 kb downstream region of all genes. Color key of the heatmap is shown at the bottom of the plot. (D) Average genomic binding profiles of H4K12ac around TSS and 3 kb upstream and downstream of estrogen-downregulated genes under vehicle (Veh) and estrogen-treated (E2) conditions. The X-axis shows the distance from the TSS of the genes in kilobase pairs. TSS is indicated by a black dotted line. (E) Heatmaps showing genomic binding profiles of H4K12ac around TSS and 3 kb upstream and downstream of all genes under vehicle (Veh) and estrogen-treated (E2) conditions. Center of the heatmap represents TSS. Color key of the heatmaps is shown on the side. (F) Heatmaps showing genomic binding profiles of H3K27ac, H4K12ac with estrogen treatment (H4K12ac E2) and without estrogen treatment (H4K12ac Veh), BRD4, nascent RNA transcription (GRO-seq), RNAPII, H2Bub1, H3K4me3 and H3K27me3 around TSS and 3 kb upstream and downstream of estrogen-induced genes. Density of the signals is arranged according to average H3K27ac signals from high to low. Center of the heatmap represents TSS. Color key of the heatmaps is shown at their side.

A




B




C

| MSigDB ID | -log10(Binomial p value) |
|---|---|
| GARY_CD5_TARGETS_UP | 13.52352575 |
| HALMOS_CEBPA_TARGETS_DN | 13.33967441 |
| ENK_UV_RESPONSE_KERATINOCYTE_UP | 12.62124881 |
| MITSIADES_RESPONSE_TO_APLIDIN_UP | 12.48514476 |
| UDAYAKUMAR_MED1_TARGETS_DN | 11.28121698 |
| SPIELMAN_LYMPHOBLAST_EUROPEAN_VS_ASIAN_UP | 10.86018449 |
| LOPEZ_MESOTHELIOMA_SURVIVAL_OVERALL_DN | 10.66214835 |
| LUI_THYROID_CANCER_CLUSTER_1 | 10.57082474 |
| LEONARD_HYPOXIA | 10.49309238 |
| AMIT_EGF_RESPONSE_40_HELA | 10.48841179 |
| MANALO_HYPOXIA_UP | 10.20579563 |
| SMID_BREAST_CANCER_RELAPSE_IN_LIVER_DN | 9.951153225 |
| BENPORATH_MYC_TARGETS_WITH_EBOX | 9.541881349 |
| KIM_WT1_TARGETS_DN | 9.410117725 |
| STEIN_ESR1_TARGETS | 9.347385294 |
| NUYTTEN_NIPP1_TARGETS_DN | 9.187657414 |
| CHARAFE_BREAST_CANCER_LUMINAL_VS_MESENCHYMAL_UP | 9.173701107 |
| LIU_CDX2_TARGETS_DN | 9.132821234 |
| STEIN_ESTROGEN_RESPONSE_NOT_VIA_ESRRA | 9.099766622 |
| MILI_PSEUDOPODIA_HAPTOTAXIS_DN | 9.000357398 |
| MOHANKUMAR_TLX1_TARGETS_UP | 8.956431432 |
| AMIT_DELAYED_EARLY_GENES | 8.784263588 |
| BLUM_RESPONSE_TO_SALIRASIB_UP | 8.718630696 |
| SMID_BREAST_CANCER_BASAL_DN | 8.598820282 |
| WANG_CISPLATIN_RESPONSE_AND_XPC_UP | 8.350592797 |
| DIRMEIER_LMP1_RESPONSE_EARLY | 8.195201105 |
| CREIGHTON_ENDOCRINE_THERAPY_RESISTANCE_1 | 8.186093246 |
| GOZGIT_ESR1_TARGETS_DN | 8.132177326 |
| DOANE_BREAST_CANCER_ESR1_UP | 7.887568282 |
| VICENT_METASTASIS_DN | 7.821348176 |
| WINTER_HYPOXIA_UP | 7.709285749 |
| TONKS_TARGETS_OF_RUNX1_RUNX1T1_FUSION_HSC_DN | 7.706635456 |
| ELVIDGE_HYPOXIA_UP | 7.674113621 |
| FRASOR_RESPONSE_TO_ESTRADIOL_UP | 7.645330415 |

**Figure S2:** (A) Heatmaps showing genomic binding profiles of H3K27ac, H4K12ac with estrogen treatment (H4K12ac E2) and without estrogen treatment (H4K12ac Veh), BRD4, nascent RNA transcription (GRO-seq), RNAPII, ERα, FOXA1 and H3K27me3 around distal EREs and 5 kb upstream and downstream with all estrogen-treated (E2) conditions. Density of the signals is arranged according to H3K27ac signals from high to low. Center of the heatmap represents center of the distal ERE region. Color key of the heatmaps is shown on the side. (B) Venn diagram showing the overlap of distal binding regions of H3K27ac (red), H4K12ac (green) and BRD4 (blue). (C) GREAT analyses on distal regions which oveelpas with H3K27ac, H4K12ac and BRD4 occupancy. The names of the Molecular signature database (MSigDB) pathways are denoted with their –log 10 binomial p-values. Estrogen-specific pathways are highlighted in pink and other breast cancer-related pathways in brown.

# E2 upregulated genes

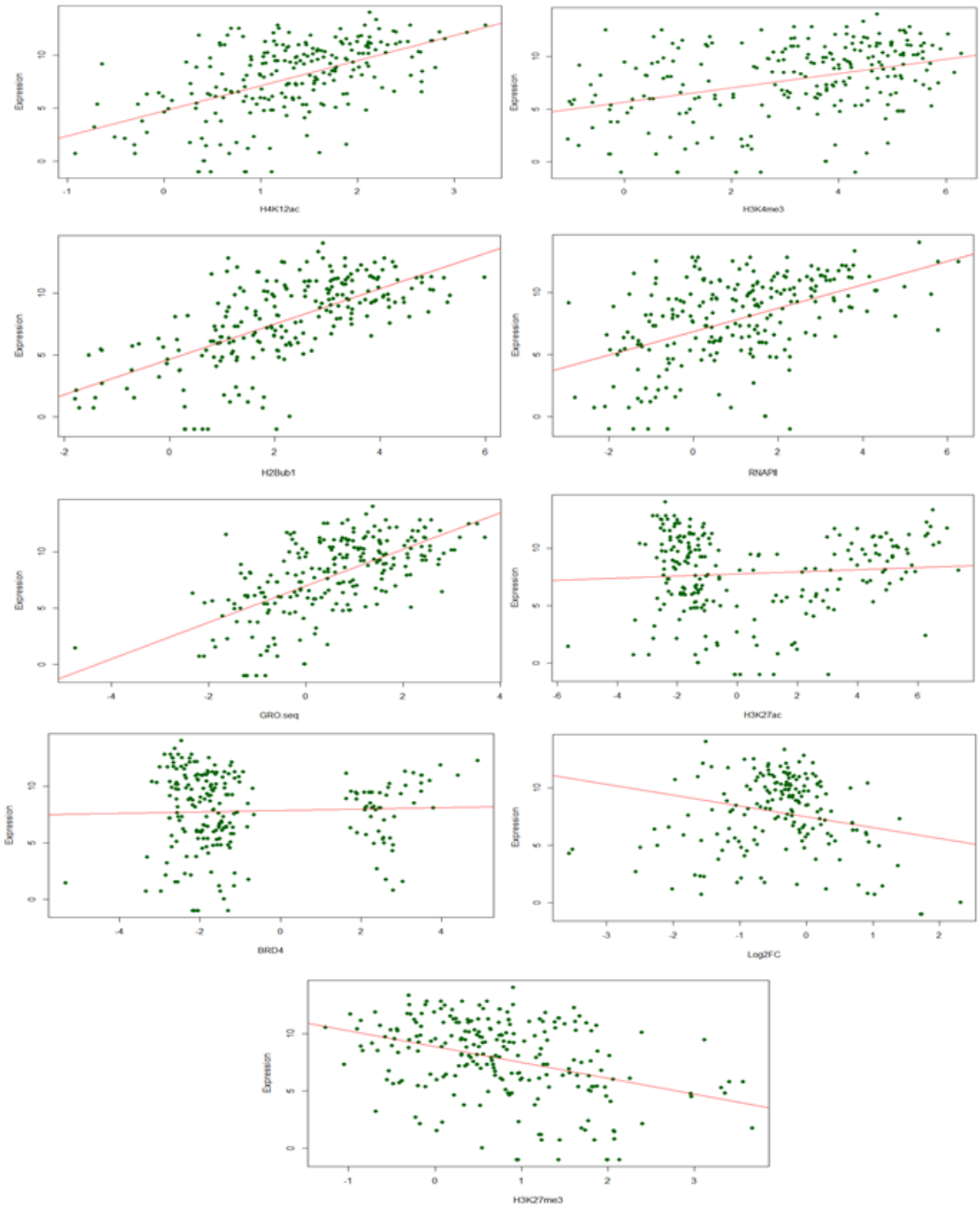


**Figure S3:** Scatterplots showing the relationship between various ChIP-seq signals (H4K12ac, H3K4me3, BRD4, H2Bub1, RNAPII, GRO-seq, H3K27ac, H3K27me3, logarithmic fold changes of siBRD4 compared to siCont (Log2FC)) and absolute gene expression in logarithmic values for estrogen upregulated genes. Each dot in the plot represents a single gene. Red line indicates the correlation.

## All genes

| | GRO.seq | H2Bub1 | H3K4me3 | RNAPII | H3K27me3 | BRD4 | H3K27ac | H4K12ac | Expression | Log2FC |
|---|---|---|---|---|---|---|---|---|---|---|
| GRO.seq | 1 | 0.737716 | 0.607588 | 0.740766 | -0.16243 | 0.109476 | 0.29921 | 0.59734 | 0.573008 | -0.07581 |
| H2Bub1 | 0.737716 | 1 | 0.604958 | 0.617574 | -0.02786 | 0.093484 | 0.262579 | 0.69864 | 0.600286 | -0.08857 |
| H3K4me3 | 0.607588 | 0.604958 | 1 | 0.623692 | 0.181553 | 0.085577 | 0.288095 | 0.749786 | 0.521682 | -0.12752 |
| RNAPII | 0.740766 | 0.617574 | 0.623692 | 1 | -0.165 | 0.018243 | 0.206677 | 0.635878 | 0.553889 | -0.09979 |
| H3K27me3 | -0.16243 | -0.02786 | 0.181553 | -0.165 | 1 | 0.074421 | -0.01146 | 0.069792 | -0.30611 | 0.010353 |
| BRD4 | 0.109476 | 0.093484 | 0.085577 | 0.018243 | 0.074421 | 1 | 0.675574 | 0.036245 | 0.011641 | -0.01329 |
| H3K27ac | 0.29921 | 0.262579 | 0.288095 | 0.206677 | -0.01146 | 0.675574 | 1 | 0.224662 | 0.183565 | -0.04418 |
| H4K12ac | 0.59734 | 0.69864 | 0.749786 | 0.635878 | 0.069792 | 0.036245 | 0.224662 | 1 | 0.497749 | -0.09188 |
| Expression | 0.573008 | 0.600286 | 0.521682 | 0.553889 | -0.30611 | 0.011641 | 0.183565 | 0.497749 | 1 | -0.21114 |
| Log2FC | -0.07581 | -0.08857 | -0.12752 | -0.09979 | 0.010353 | -0.01329 | -0.04418 | -0.09188 | -0.21114 | 1 |

## E2 regulated genes

| | GRO.seq | H2Bub1 | H3K4me3 | RNAPII | H3K27me3 | BRD4 | H3K27ac | H4K12ac | Expression | Log2FC |
|---|---|---|---|---|---|---|---|---|---|---|
| GRO.seq | 1 | 0.677545 | 0.536808 | 0.703225 | -0.22096 | 0.040218 | 0.112583 | 0.510785 | 0.629388 | -0.09888 |
| H2Bub1 | 0.677545 | 1 | 0.555719 | 0.496261 | -0.05379 | 0.136138 | 0.189556 | 0.686092 | 0.640592 | -0.02342 |
| H3K4me3 | 0.536808 | 0.555719 | 1 | 0.572254 | 0.242915 | 0.018732 | 0.233546 | 0.721774 | 0.375121 | 0.029958 |
| RNAPII | 0.703225 | 0.496261 | 0.572254 | 1 | -0.19608 | -0.0191 | 0.091506 | 0.540041 | 0.490185 | -0.0855 |
| H3K27me3 | -0.22096 | -0.05379 | 0.242915 | -0.19608 | 1 | 0.050236 | 0.040627 | 0.068655 | -0.36606 | 0.081474 |
| BRD4 | 0.040218 | 0.136138 | 0.018732 | -0.0191 | 0.050236 | 1 | 0.591603 | 0.008577 | 0.038346 | -0.21605 |
| H3K27ac | 0.112583 | 0.189556 | 0.233546 | 0.091506 | 0.040627 | 0.591603 | 1 | 0.161019 | 0.07871 | -0.04206 |
| H4K12ac | 0.510785 | 0.686092 | 0.721774 | 0.540041 | 0.068655 | 0.008577 | 0.161019 | 1 | 0.540699 | -0.10892 |
| Expression | 0.629388 | 0.640592 | 0.375121 | 0.490185 | -0.36606 | 0.038346 | 0.07871 | 0.540699 | 1 | -0.24406 |
| Log2FC | -0.09888 | -0.02342 | 0.029958 | -0.0855 | 0.081474 | -0.21605 | -0.04206 | -0.10892 | -0.24406 | 1 |

**Figure S4:** Pearson correlation coefficient (R) values were shown in tables representing the relationship between various ChIP-seq signals (H4K12ac, H3K4me3, BRD4, H2Bub1, RNAPII, GRO-seq, H3K27ac, H3K27me3, logarithmic fold changes of siBRD4 compared to siCont (Log2FC)) and absolute gene expression for all genes and estrogen-induced genes.