Supplemental Figure 1

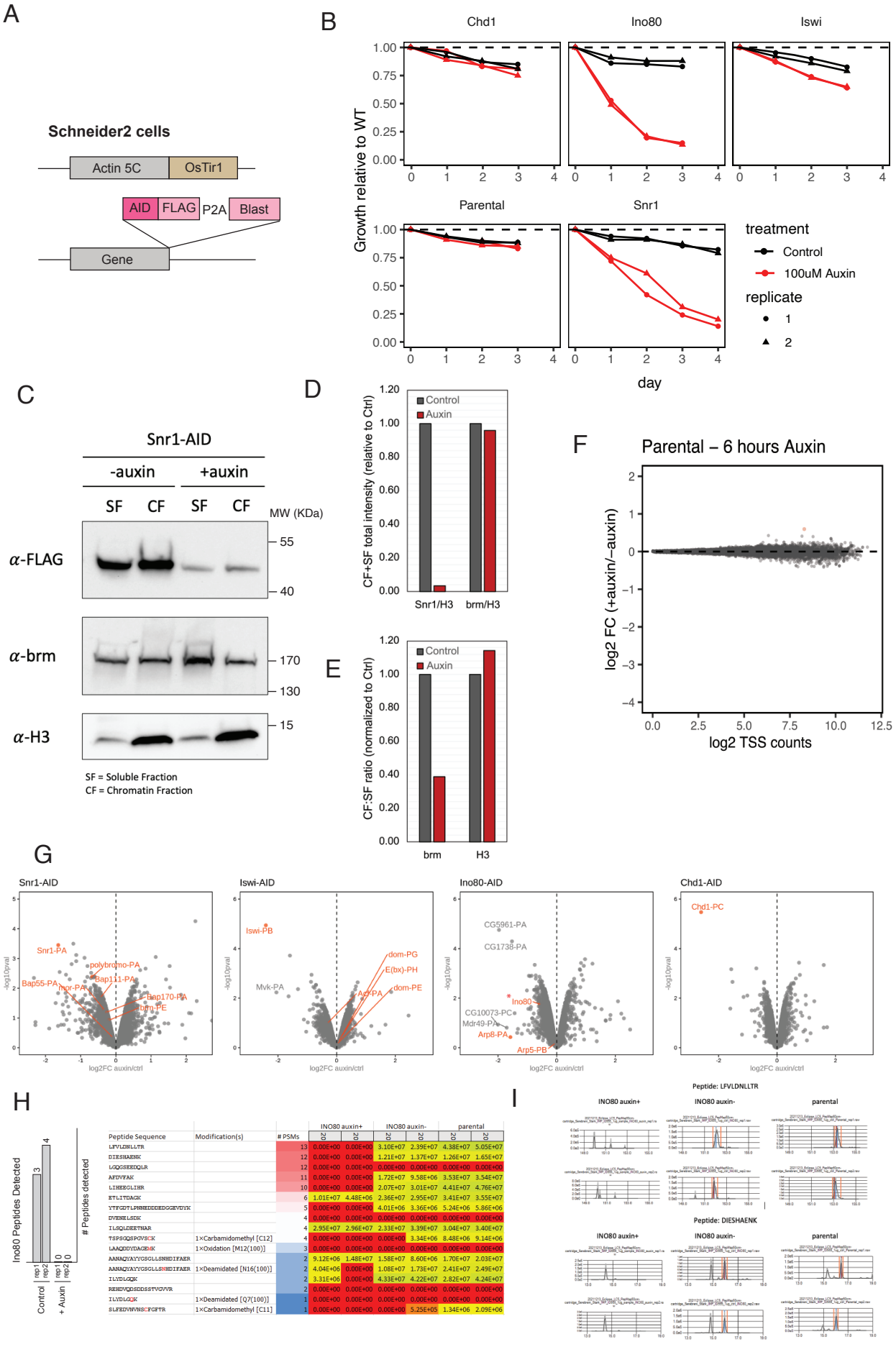

Figure S1 – Characterization of S2 AID cell lines, Related to Figure 1. A) Diagram of OsTir1 integration and AID tagging scheme in Drosophila S2 cells. B) Competition assays of tagged cell lines with growth relative to WT untagged cell lines. Two replicates shown, each in the absence (black lines) or presence (red lines) of auxin. C) Western blot against FLAG (Snr1), Brm, and H3. Nuclei were fractioned into soluble vs chromatin bound fractions, before and after Auxin treatment. D) Quantification of summed intensity from CF and SF per condition, normalized to H3 summed intensity E) Quantification of CF to SF ratio for Brm and H3, before and after Auxin treatment, normalized to Control ratio. F) MAplot of Parental cell line comparing 6h Auxin treatment to control, significantly affected genes (at FDR<0.05) in red G) Limma computed log2 Fold Changes and p-values from quantitative mass spectrometry before and after auxin treatment of AID cell lines. H) Detected peptides before and after Ino80 depletion and table showing In080 peptide sequences, total #peptide-spectrum matches in all samples, and peptide areas per sample. The top 5 most frequently detected Ino80 peptides were lowly abundant and could not be detected in the Auxin treatment sample, however manual inspection of peptide areas suggests at least 10 fold depletion after auxin treatment. More frequent Ino80 peptides (based off #PSMs, e.g. LFVLDNLLTR, DIESHAENK), were not quantified by the software in +auxin samples due to signals below the noise cutoff of 5E5 intensity counts per second. Less frequently identified peptides (e.g. ETLITDAGK, ILSQLDEETNAR, AANAQYAYYGSGLLSNHDIFAER, below-left) were likely erroneously quantified, probably due to coelution with other peptides. I) Manual evaluation of extracted ion chromatograms for the 5 most frequently identified peptides (e.g. LFVLDNLLTR, DIESHAENK, below-right) shows signals at least 10 times less intense in the auxin+ samples. From this we conclude that ino80 is likely at least 10-fold downregulated upon auxin treatment.

Supplemental Figure 2

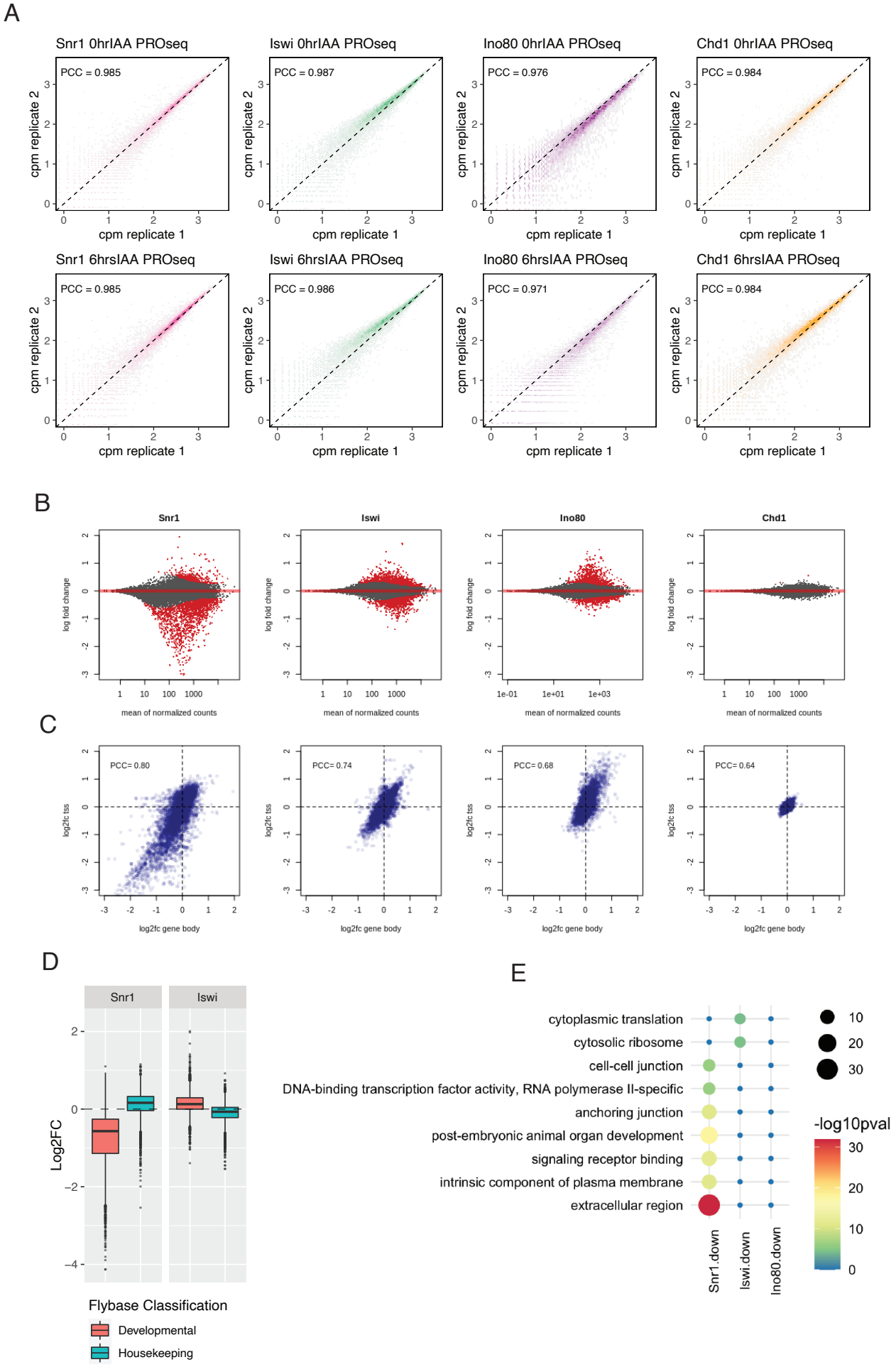

Figure S2 – PRO-seq of S2 cells upon degradation of chromatin remodeler core components, Related to Figure 1. A) Scatterplots of replicate PRO-seq cpms at TSSs. Pearson correlation coefficient indicated in top left corner of each plot. B) PRO-seq results using full gene analysis. Top row: MA plots with significantly affected genes in red (FDR<0.05). C) Comparison of log2fc calculated using TSS counts vs using gene counts, with PCC indicated. D) PRO-seq log2 Fold Changes calculated by DeSeq2 differential expression in Snr1-AID cell line and Iswi-AID cell lines. TSSs split by Developmental or Housekeeping classification based of expression entropy in Drosophila tissues from Flybase. E) GO term enrichment of significantly affected genes after Snr1, Iswi, or Ino80 depletion.

Supplemental Figure 3

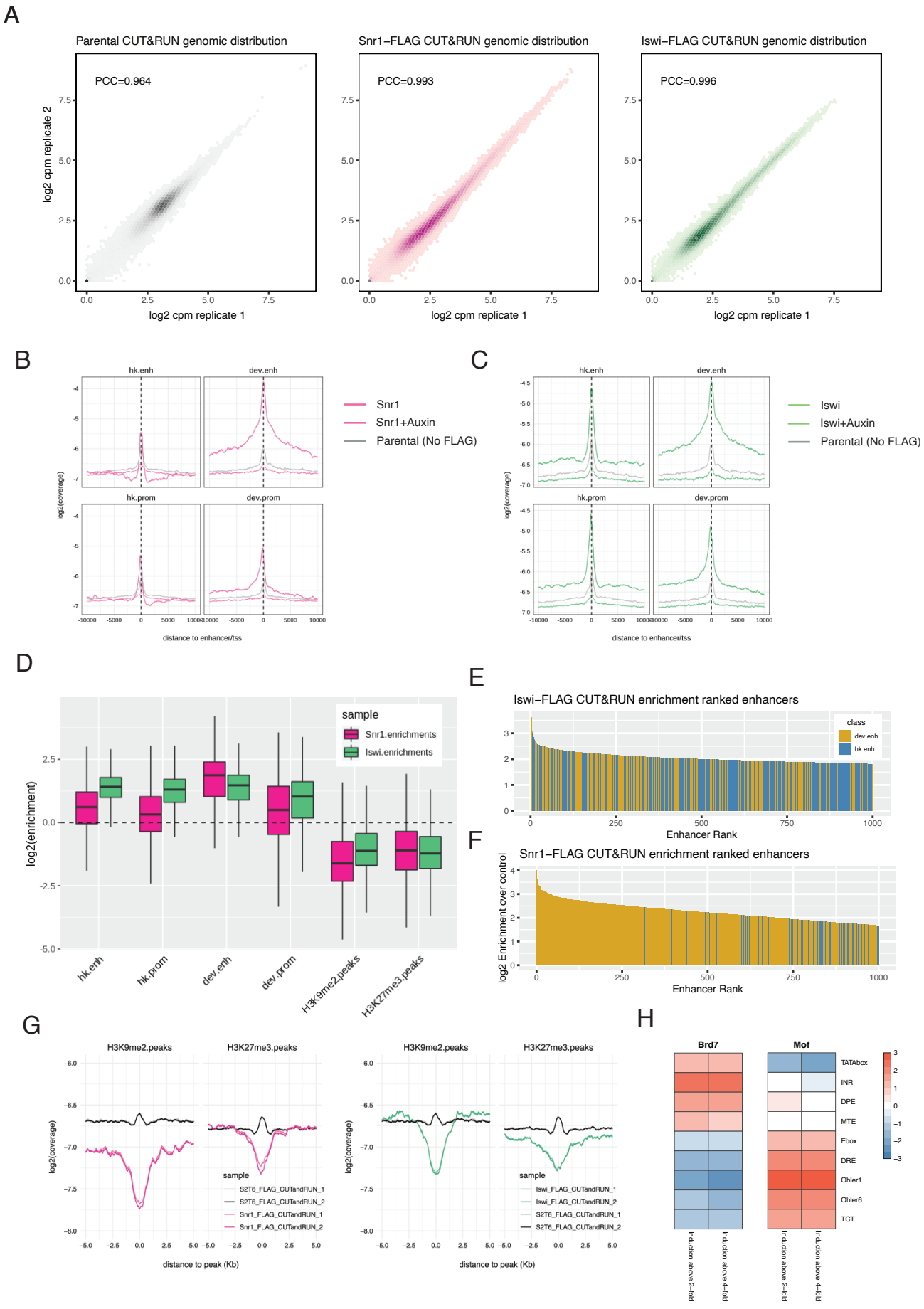

Figure S3. Chromatin remodeler occupancy in S2 cells assessed by CUT&RUN, Related to Figures 2-3. A) 2D Denisty plot of Parental, Snr1, and Iswi anti-FLAG CUT&RUN cpm calculated from genome wide bins of 1Kb, with PCC indicated. B) Anti-FLAG CUT&RUN coverage at developmental and housekeeping promoters/enhancers. Coverage for Snr1-AID cell line before (solid line) and after 6 hours of Auxin treatment (dashed line), compared against parental no-FLAG CUT&RUN (grey line). C) As in B but for Iswi-AID D) Anti-FLAG CUT&RUN enrichments at genomic regions, including housekeeping and developmental enhancers/promoters, as well as silenced regions such as H3k9me2 (Heterochromatin) and H3K27me3 (Polycomb) marked region. Boxplot show CUT&RUN enrichment distribution against no-Tag control at each element for Snr1 (pink) and Iswi (green) cells. E) Waterfall plots showing the top 1000 enriched enhancers for Iswi-AID CUT&RUN. Enhancers coloured by type, where housekeeping are blue and developmental are gold. F) As in D but for Snr1-AID. G) Anti-FLAG CUT&RUN coverage at H3K9me2 peaks and H3k27me3 peaks in Drosophila S2 cells. Left: Snr1 (pink) vs. Parental (grey) Right: Iswi (green) vs. Parental (grey). H) Motif enrichment of STAP-seq promoters activated >2 or >4 fold over GFP by Brd7 recruitment (left) or Mof recruitment (right).

Supplemental Figure 4

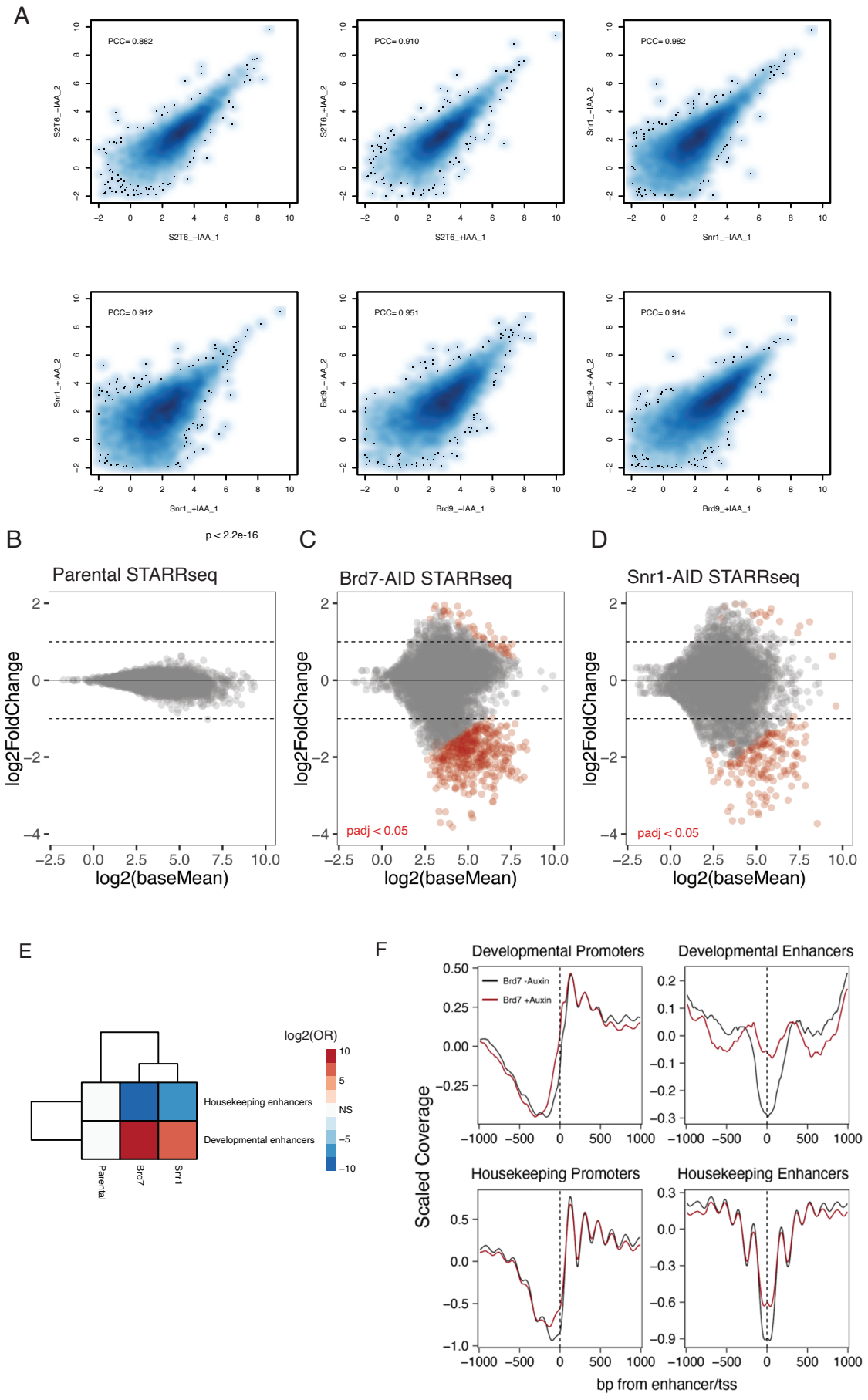

Figure S4. Enhancer activity changes after Snr1 and Brd7 depletion, Related to Figure 4. A) 2D Density plot of Parental, Snr1 and Brd7 genome wide mixed STARR-seq cpm at enhancer summits, for control and Auxin experiments. Pearson correlation coefficient indicated in each plot. B-D) MA plots of Parental, Brd7-AID, and Snr1-AID STARRseq screens plotting DESeq2 shrunken log2fc of enhancer activity auxin/control against DESeq2 basemean. Significantly affected enhancers with FDR<0.05 are colored in red. E) Enrichment of Housekeeping or developmental type enhancers in significantly downregulated enhancers in STARR-seq screens. Non-significant enrichments are colored with white. F) MNase scaled coverage of Brd7-AID cell line in Control (Grey line) and 12hrs Auxin treatment (Red line).

Supplemental Figure 5

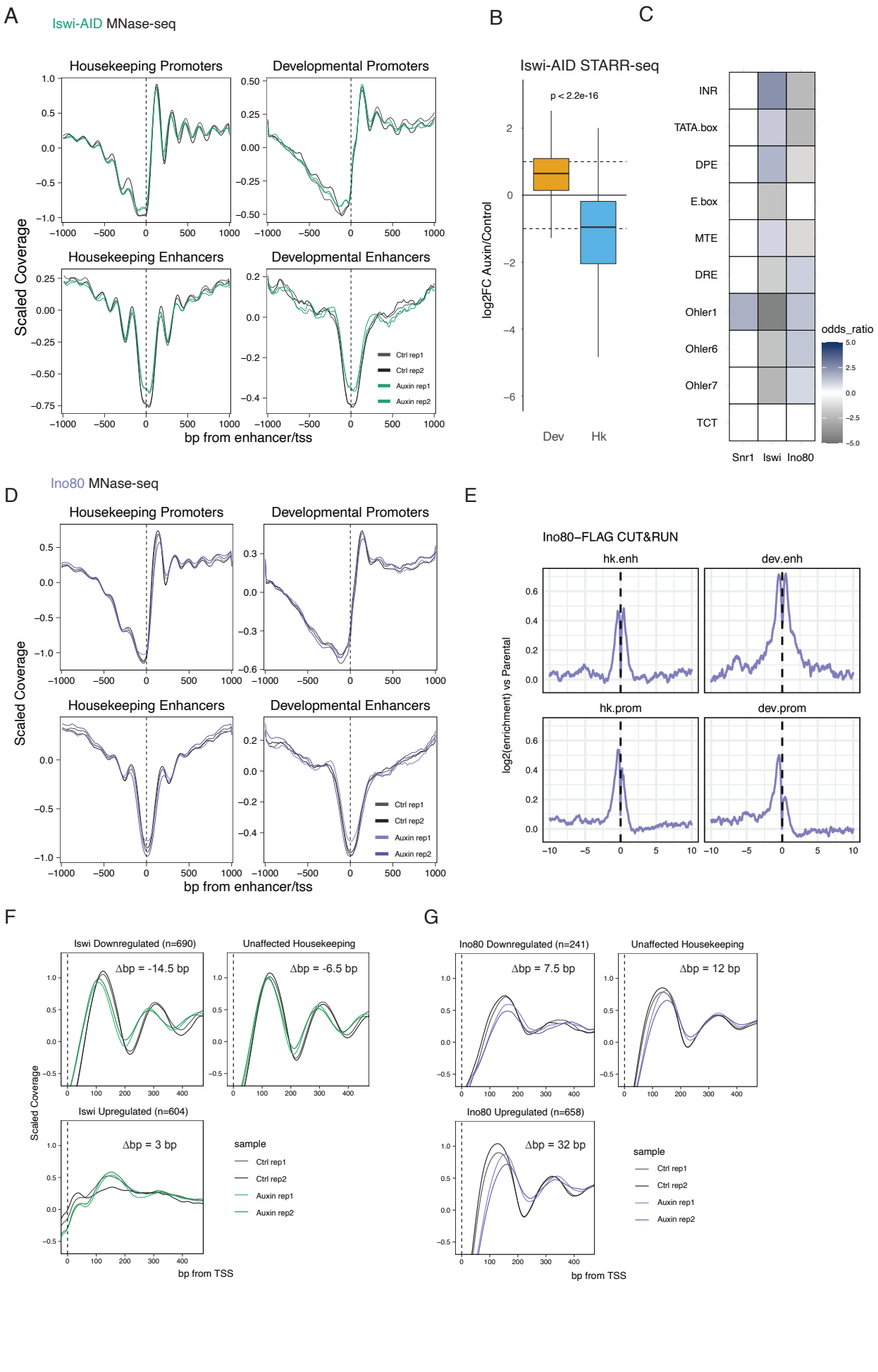

Figure S5. Iswi and Ino80 regulate nucleosome positioning at housekeeping promoters, Related to Figure 5. A) MNase-seq scaled coverage at Developmental/Housekeeping enhancers and promoters, before and after 12 hours auxin treatment in Iswi-AID cell lines. Two replicates shown. B) STARR-seq of Iswi-AID cell line after 24 hours Auxin treatment compared to control. Log2 Fold Change of enhancer activity for all developmental enhancers and all housekeeping enhanncers shown and p-value ofWilcoxon rank-sum test between developmental and housekeeping enhancer log2FCs C) Motif enrichment in significantly upregulated TSSs from PRO-seq differential expression. Non-significant enrichments coloured in left white. D) As in A, except for Ino80 AID. E) Anti-FLAG CUT&RUN enrichment coverages in Ino80 cell line. F) Scaled MNase-seq coverage of Iswi-AID (2 replicates) at different gene subsets defined by transcriptional change in PRO-seq: downregulated, unaffected housekeeping, and upregulated. Average change in +1 nucleosome position displayed for each plot. G) As in F but for Ino80

Supplemental Figure 6

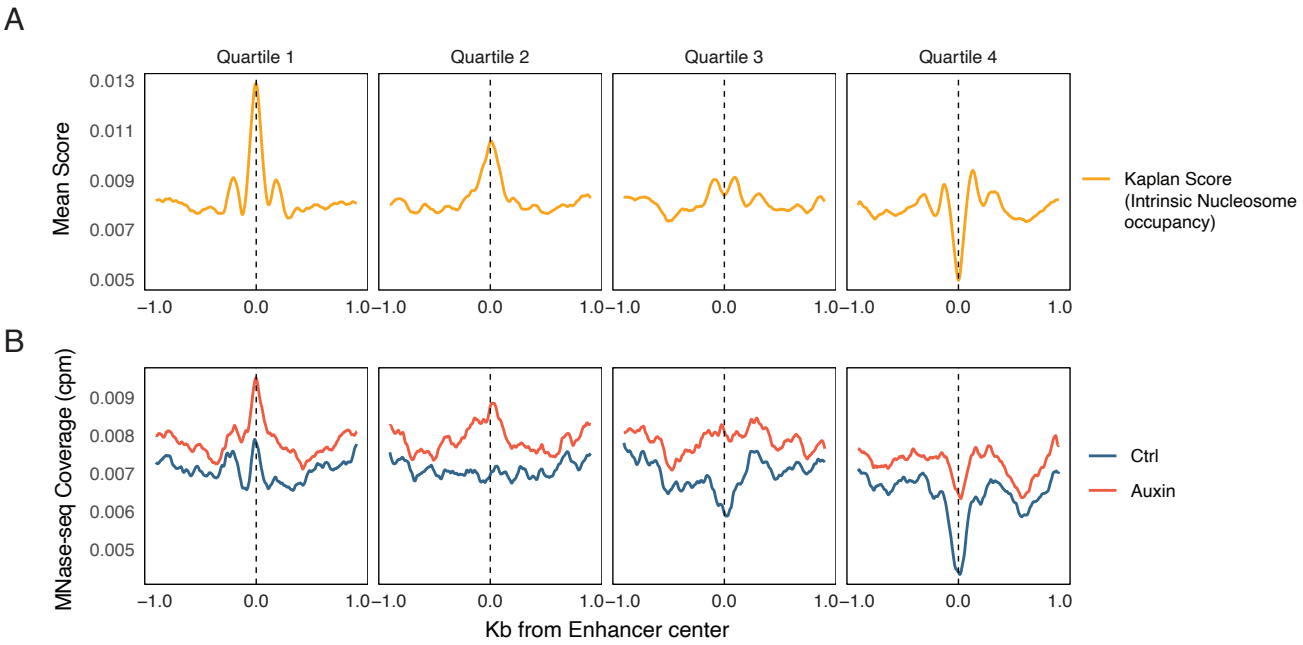

Figure S6 – Nucleosome occupancy changes at enhancers grouped by Kaplan score, Related to Figure 6. A) Nucleosome Occupancy Score coverages from Kaplan et al. 2009 for developmental enhancers (n=1010) split into quartiles based on ranked occupancy score at enhancer center. B) Coverage of MNase-seq on Snr1-AID cell line at developmental enhancer quartiles. Control coverage in blue and Auxin treated coverage in Red.