BRG1 HSA domain interactions with BCL7 proteins are critical for remodeling and gene expression

Dietrich et al. 2023

Group 3:

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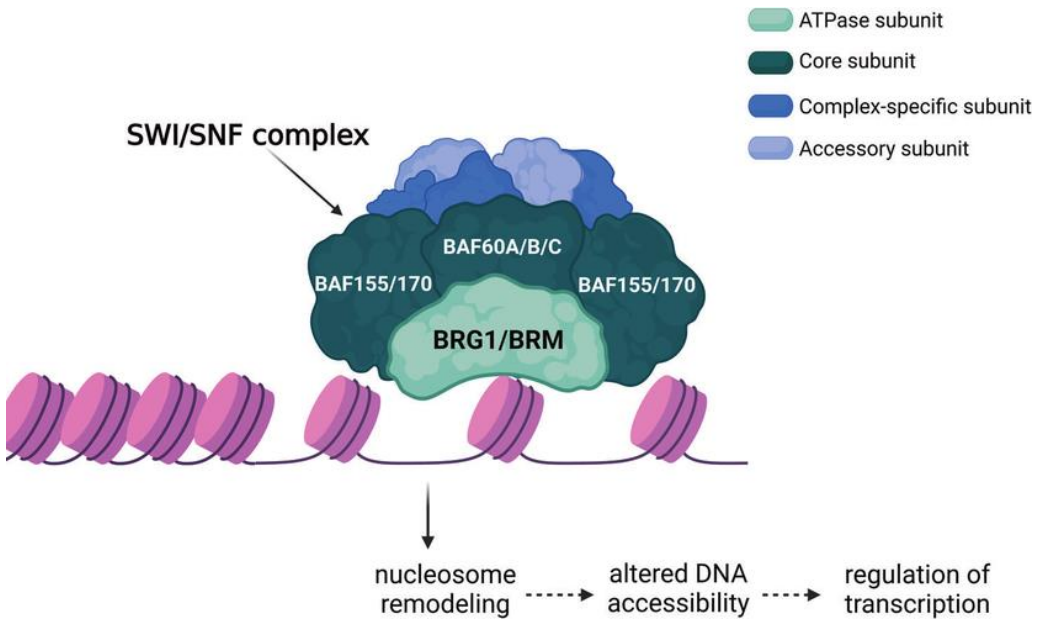
Background

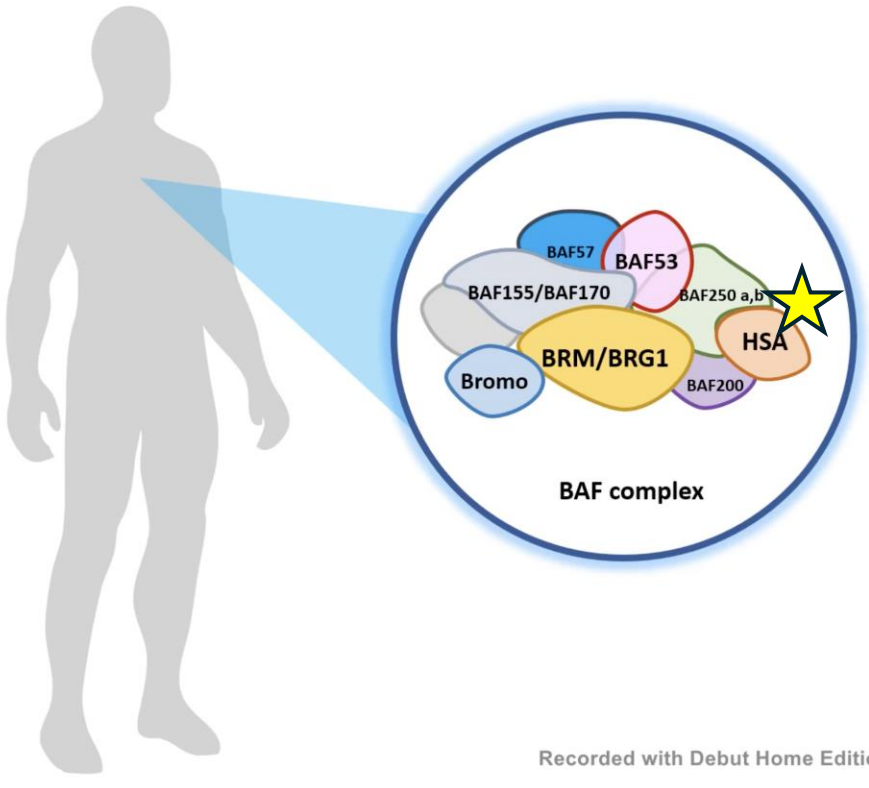
- SWI/SNF (SWItch/Sucrose Non-Fermentable) is a chromatin remodeling complex via critical subunits: **BRG1** and BRM enzymes/binding proteins
- Chromatin remodeling alters nucleosome structure which drives gene expression among many other biological processes including organismal development, DNA repair and transcriptional regulation
- Irregularities in chromatin remodeling can result in the development of cancer so further characterization of BRG1 was of interest



Source: Navickas et al. 2023

Background (continued)

- The HSA domain of BRG1 protein has been demonstrated to play a significant role in the function of BRG1
- Serves as a binding partner for other SWI/SNF proteins
- Contains many cancer associated mutations
- B-cell CLL Lymphoma 7 protein family (BCL7) are SWI/SNF complex members that bind to the HSA domain and are also associated with cancer
- Little research has been done to investigate role of BCL7 protein interactions with SWI/SNF members



Source: https://www.youtube.com/watch?app=desktop&v=G1KPBQBlKWE

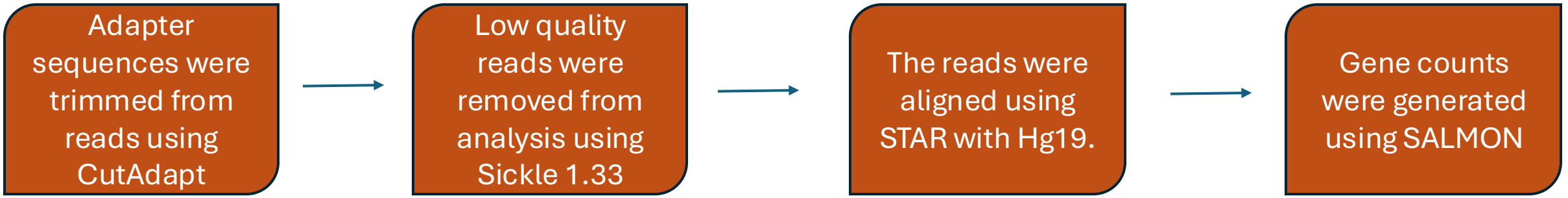
Methodology

- SW-13 adrenal carcinoma cells engineered to express either wild-type BRG1 (iBRG1) or HSA-deleted BRG1 (iΔHSA).
- Cells were induced with doxycycline for enhancement of BRG1 activity in the 24 hr samples.
- Samples prepared with the engineered cell lines can be grouped into a super series of four which further describes the NGS pipeline.
- Sample series:
- 1. The B-cell CLL/lymphoma 7 (BCL7) protein family members drive gene expression changes via interactions with the Brahma Related Gene 1 (BRG1) Helicase-Sant Associated domain (HSA) domain [24 hours]
- 2. The B-cell CLL/lymphoma 7 (BCL7) protein family members drive gene expression changes via interactions with the Brahma Related Gene 1 (BRG1) Helicase-Sant Associated domain (HSA) domain [LongTerm]
- 3. The B-cell CLL/lymphoma 7 (BCL7) protein family members drive gene expression changes via interactions with the Brahma Related Gene 1 (BRG1) Helicase-Sant Associated domain (HSA) domain [BCL7 knockdown]
- 4. The B-cell CLL/lymphoma 7 (BCL7) protein family members drive gene expression changes via interactions with the Brahma Related Gene 1 (BRG1) Helicase-Sant Associated domain (HSA) domain [CUT&RUN](Cleavage Under Targets & Release Using Nuclease)

Methodology

Data Preprocessing

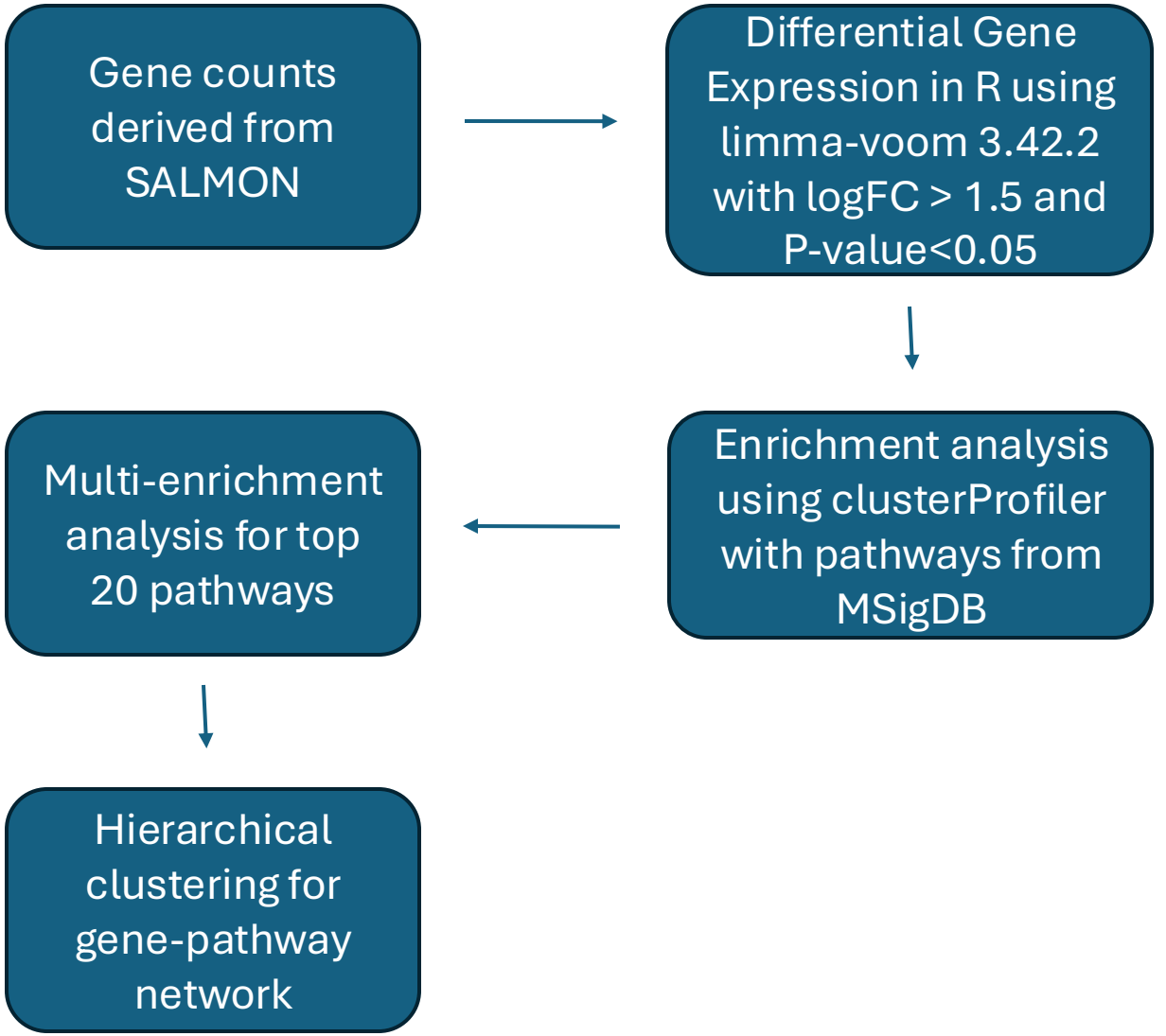
- **RNA Sequencing analysis**



Methodology

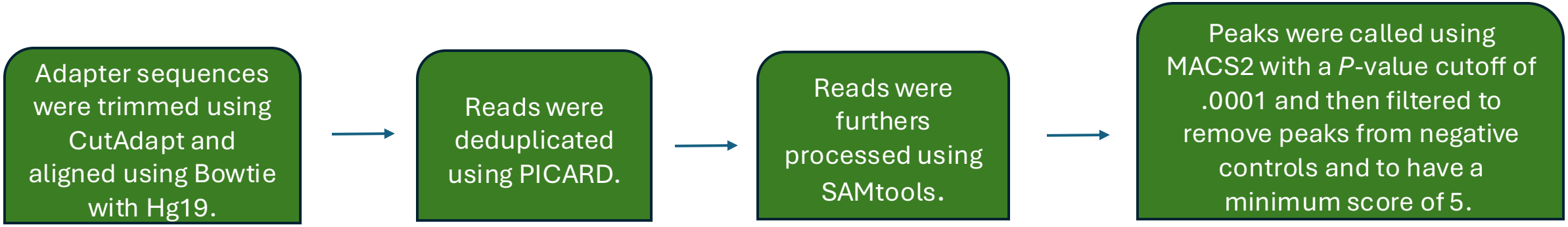
Downstream processing: RNA Sequencing analysis

- The gene counts were further processed for Different Gene Expression analysis using limma-voom 3.42.2.
- The differentially expressed genes were defined by fold change greater than 1.5 and pvalue of less than 0.05.
- DEGs were tested for enrichment in specific pathways using clusterProfiler with pathways from MSigDB in NetworkAnalyst.
- Top 20 pathways with P-value above 0.1 and two differentially expressed genes were further selected for multi-enrichment analysis.
- This gene-pathway matrix was subjected to hierarchical clustering to form a gene-pathway network cluster.



Methodology

CUT and RUN: Data Preprocessing



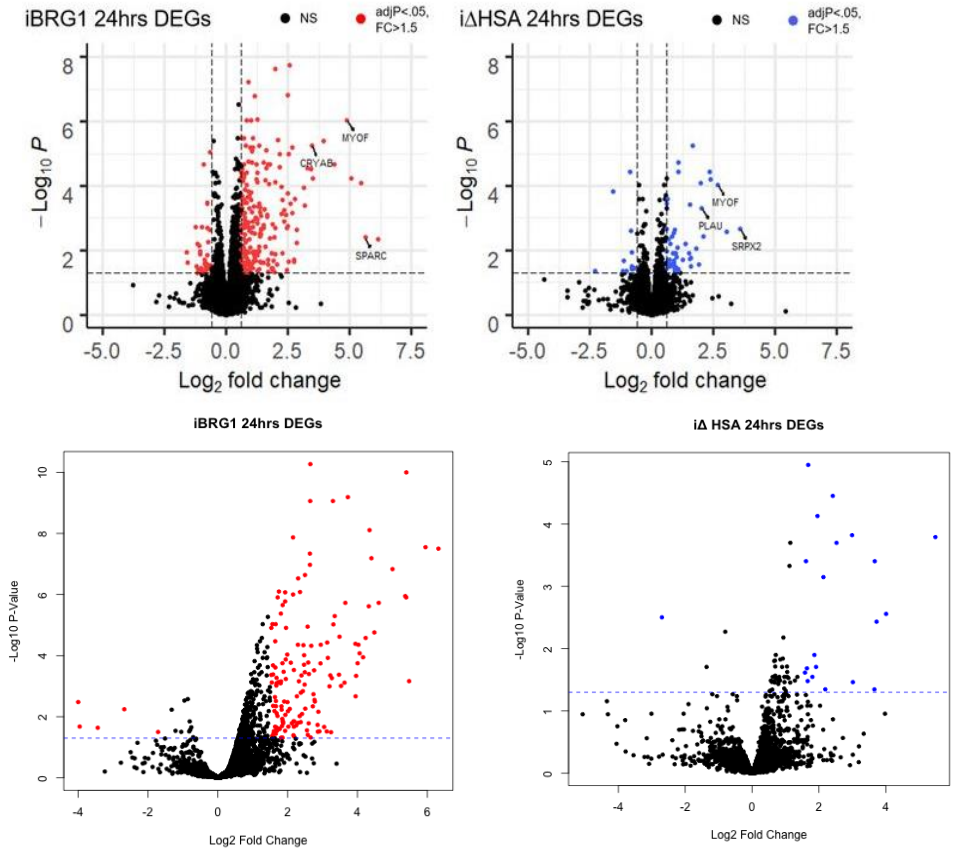
CUT and RUN: Downstream Processing

- The coverage profiles and heatmaps were developed using deeptools.

Results

Figure 1: HSA domain of BRG1 is necessary to drive a cancer- and senescence-associated gene expression profile.

- Dietrich et. Al identified 256 differentially expressed genes (DEGs) when iBRG1 was expressed compared with control cells, but only 68 DEGs when iΔHSA was expressed compared with control cells
- We identified 157 DEGs when iBRG1 was expressed and 21 DEGs for iΔHSA
- Differentially expressed genes defined as those with a fold change greater than 1.5 and an adjusted p-value of less than 0.05
- Possible reasons for difference in number of DEGs: normalization method (unmentioned), filtering, and newer versions of software were used
- Results supported prediction that HSA mutant (iΔHSA) had reduced ability to drive the expression of BRG1 target genes

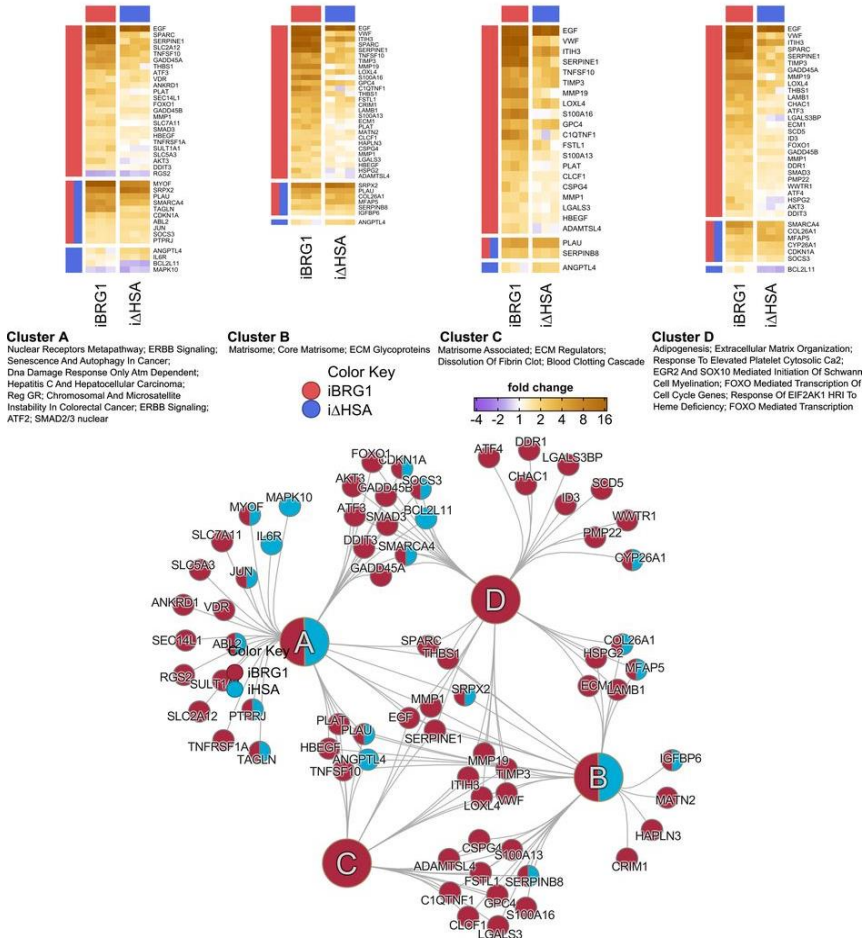


Description : volcano plots displays the differentially expressed genes identified in iBRG1 (left, red) or iHSA (right, blue) cells treated for 24 h of doxycycline compared with vehicle-treated cells.

Figure S1: Pathway analysis comparison between Dietrich et al. iBRG1 and iΔHSA 24hr DEGs

- Dietrich et al. observed significant enrichment of matrisome and extracellular matrix–associated genes in the iBRG1 which suggests that BRG1 expresses genes set that are highly implicated in cancer (Dietrich et al. 2023)

- Results support a model in which "re-expression of BRG1 in cells that do not express either SWI/SNF ATPase protein drives the expression of genes that would affect the development and progression of cancer, even via pathways that would normally undermine tumorigenesis" (Dietrich et al., 2023)

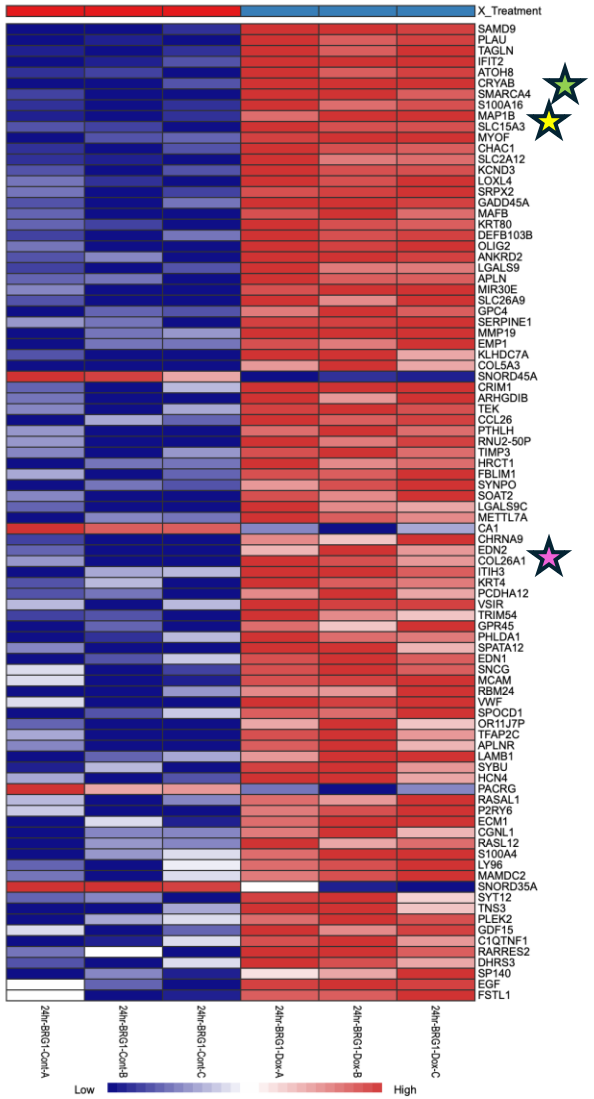


Description : Cluster plots displays the genes that make up each pathway with iBRG1 DEGs in red and iΔHSA DEGs in blue, and co-regulated genes displaying shared red and blue circles and Heatmaps of each pathway where red bars represent DEGs from iBRG1 samples and blue bars represent DEGs from iΔHSA samples.

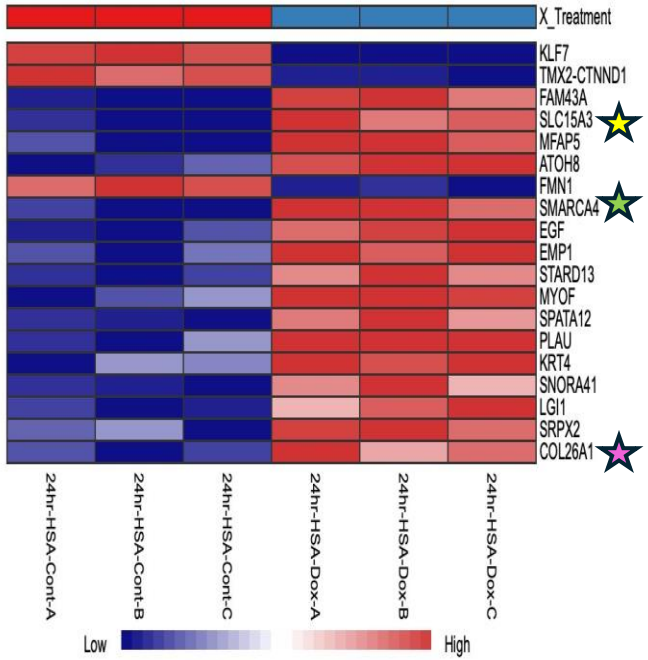
Figure S1: Pathway analysis for our iBRG1and iΔHSA 24hr DEGs

- Clear upregulation trend in the treatment groups (right side of each map)
- Significantly more DEGs in iBRG1 compared to iΔHSA suggests that the HSA domain plays an important role in regulating many genes
- SMARCA4 shows consistent change across both samples suggesting it is not affected by HSA deletion
- SLC15A3 and COL26A1 show more distinct variation between samples indicating a potential dependency on the HSA domain

iBRG1



iΔHSA

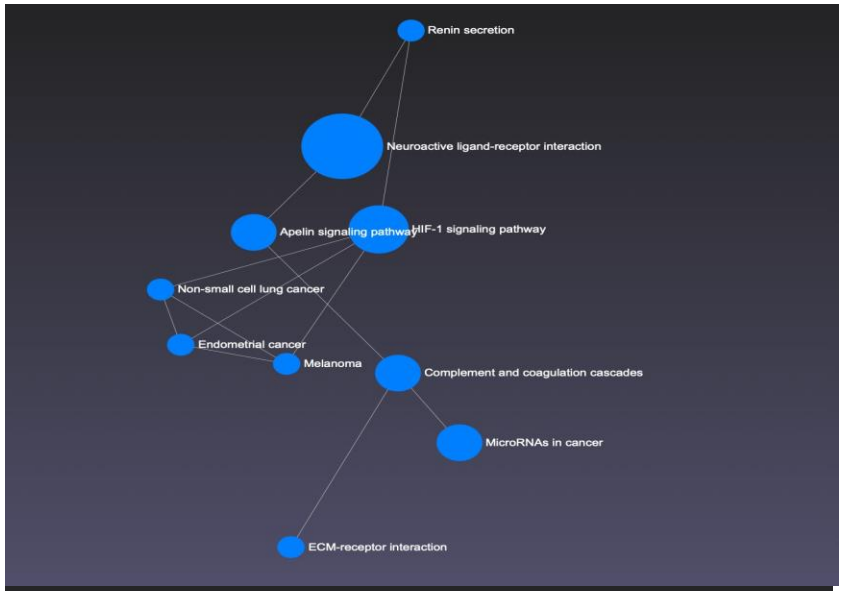


Description : Heatmaps for pathway analysis of all DEGs identified by RNA-seq in iBRG1 (left) or iΔHSA (right) cells after 24 h of doxycycline treatment.

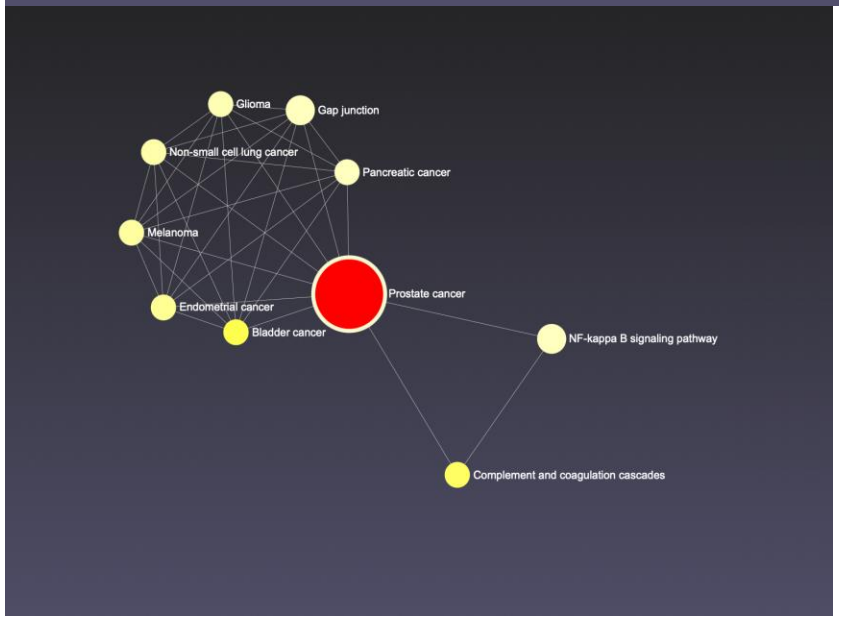
Figure S1: Pathway analysis for our iBRG1and iΔHSA 24hr DEGs

- Both cluster networks demonstrate pathways involved in cancer
- Shared pathways include non-small cell lung cancer, endometrial cancer, complement and coagulation cascades
- Even more pathways in iΔHSA involve cancer including pancreatic cancer, bladder cancer, melanoma and prostate cancer demonstrating the significant negative impacts that could arise from deletion of the HSA domain

iBRG1



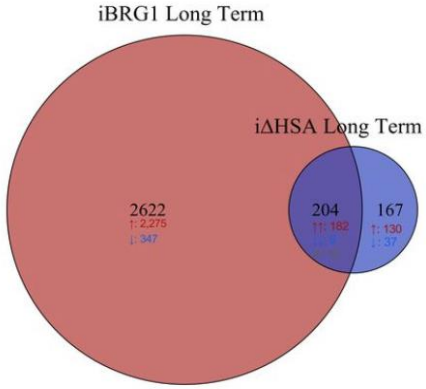
iΔHSA

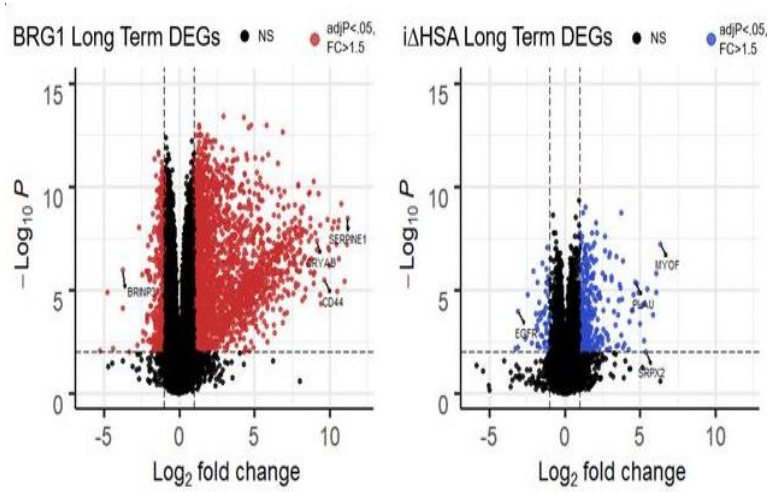


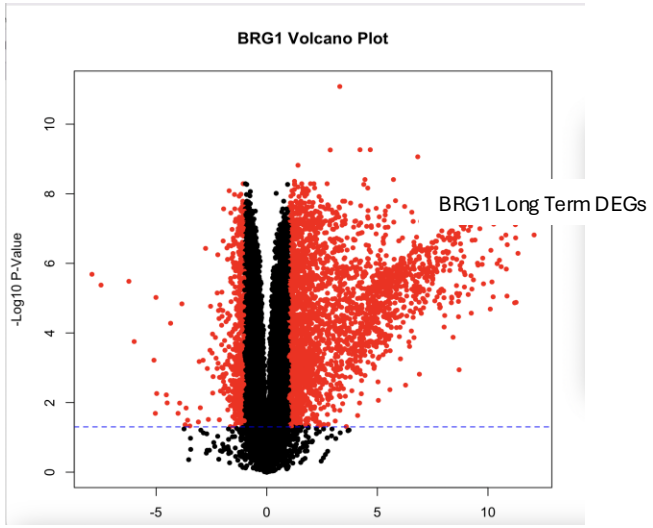
Description : Cluster networks display the genes that make up each pathway with iBRG1 (top) and iHSA (bottom) DEGs

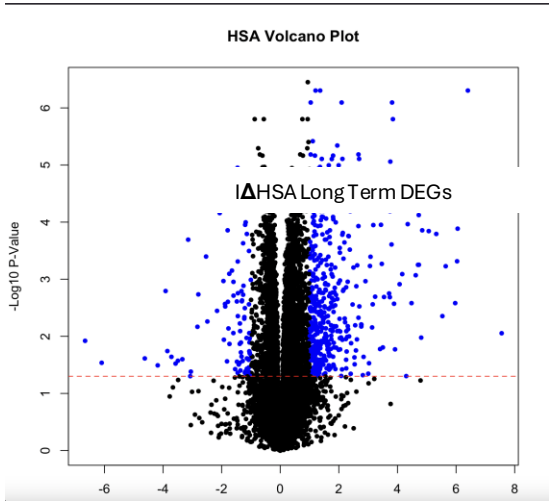
long-term BRG1 expression on cell proliferation, senescence, and gene expression profiles

- To explore the molecular mechanisms underlying these phenotypes, RNA-seq was used to analyze gene expression profiles.
- In the paper, a total of 2,622 differentially expressed genes (DEGs) were identified, while iΔHSA cells exhibited only 214 DEGs.
- In our analysis we have a total of 2,603 differentially expressed genes (DEGs) and 219 DEGs iΔHSA cells identified. Both of them suggest a significantly weaker transcription response in the absence of the HSA domain.
- Among the DEGs in iBRG1 cells, the majority (2,785 genes) were upregulated, while only 412 genes were downregulated. This indicates that BRG1 primarily acts as a transcriptional activator in SW-13 cells.
- The stark difference in the number of DEGs between iBRG1 and iΔHSA cells demonstrates that the HSA domain is critical for BRG1's ability to regulate transcription.





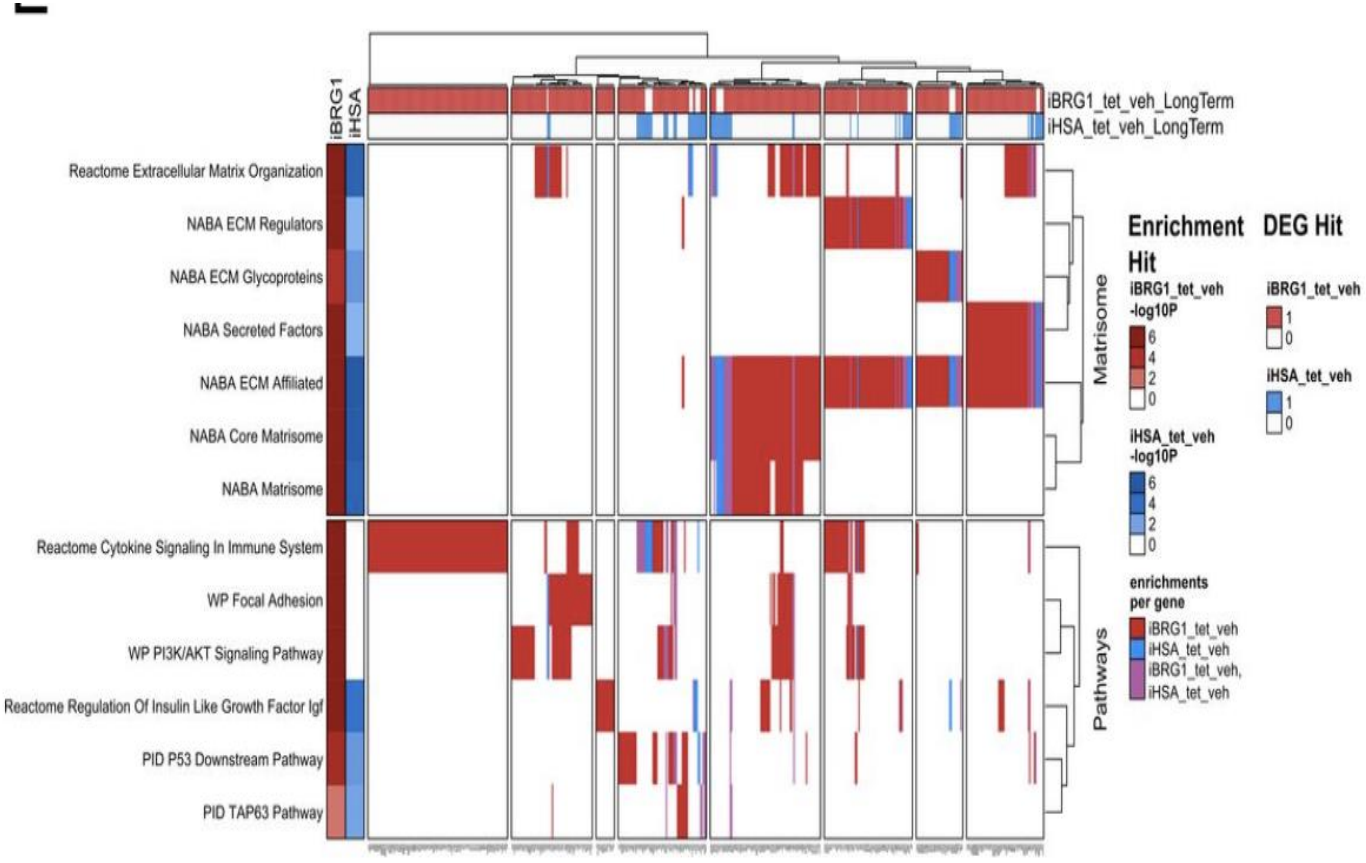




Description : Volcano plots displays the differentially expressed genes identified in iBRG1 (left, red) or iΔHSA (right, blue) treated for 14 d of continuous doxycycline compared with vehicle-treated cells and the Venn diagram of the overlaps between DEGs identified after 14 d of continuous doxycycline treatment of iBRG1 (red) or iΔHSA (blue) cells.

Pathway Enrichment Analysis: BRG1 Drives Key Biological Pathways

- Many genes altered by iBRG1 expression were significantly enriched in senescence and cancer-associated pathways
- Smaller number of pathways were also enriched in genes altered by iΔHSA expression reflecting the reduced transcriptional activity in the absence of the HSA domain
- This emphasizes the critical role of the HSA domain in enabling BRG1 to remodel chromatin and drive biological processes.

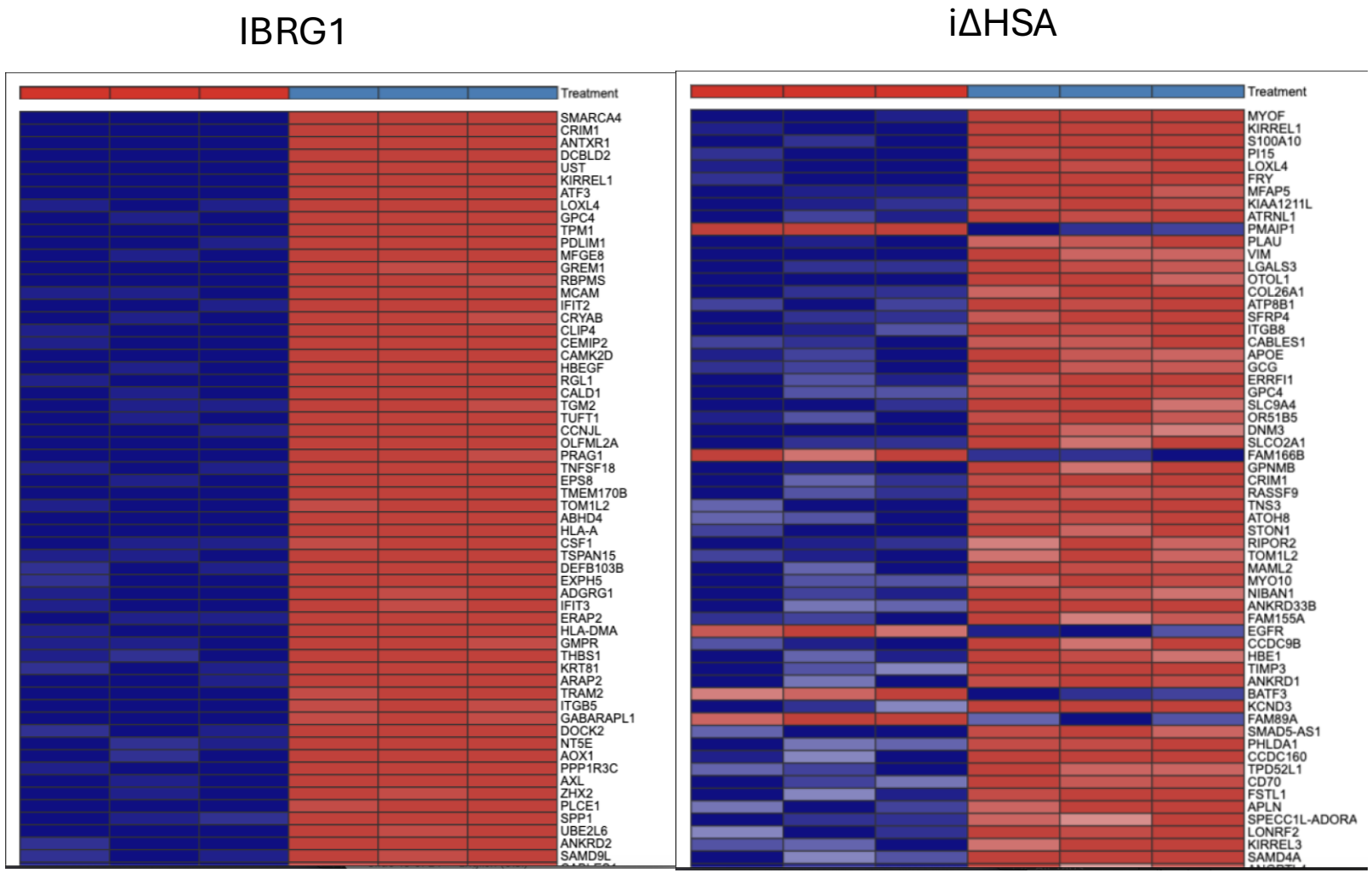


Description : Pathway analysis of all DEGs identified by RNA-seq in iBRG1 or iΔHSA cells after 14 d of continuous doxycycline treatment compared with vehicle treatment.

Long Term: Differentially expressed genes

- The iBRG1 heatmap (left) shows a significant number of genes that are strongly upregulated (red) or strongly downregulated (blue)
- In contrast, the iΔHSA heatmap (right) displays less pronounced gene expression changes

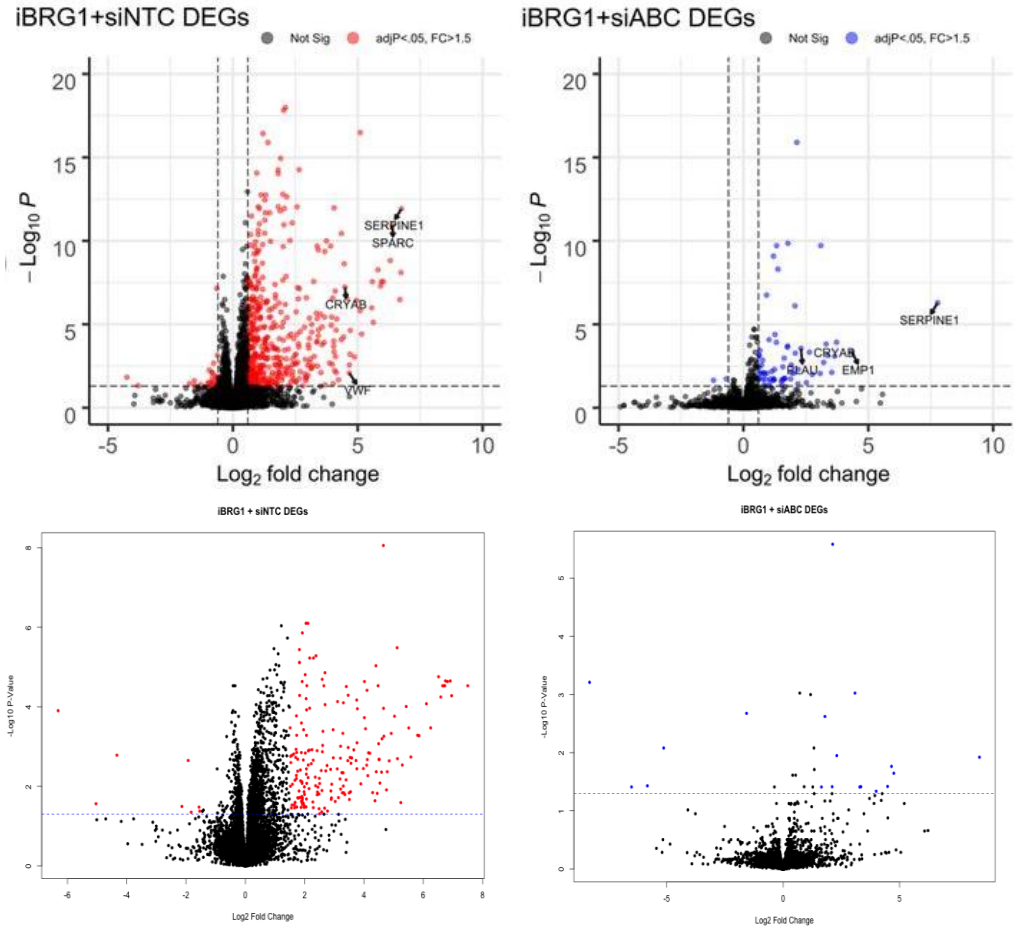
- The heatmaps clearly demonstrate that BRG1 with an intact HSA domain drives extensive gene expression changes. These changes are severely impaired in HSA-deleted BRG1 (iΔHSA) cells, confirming that the HSA domain is essential for BRG1's functionality.



Description : Heatmaps for pathway analysis of all DEGs identified by RNA-seq in iBRG1 (left) or iΔHSA (right) cells after long term doxycycline treatment.

Figure 5: BCL7 proteins are necessary for gene expression changes driven by BRG1.

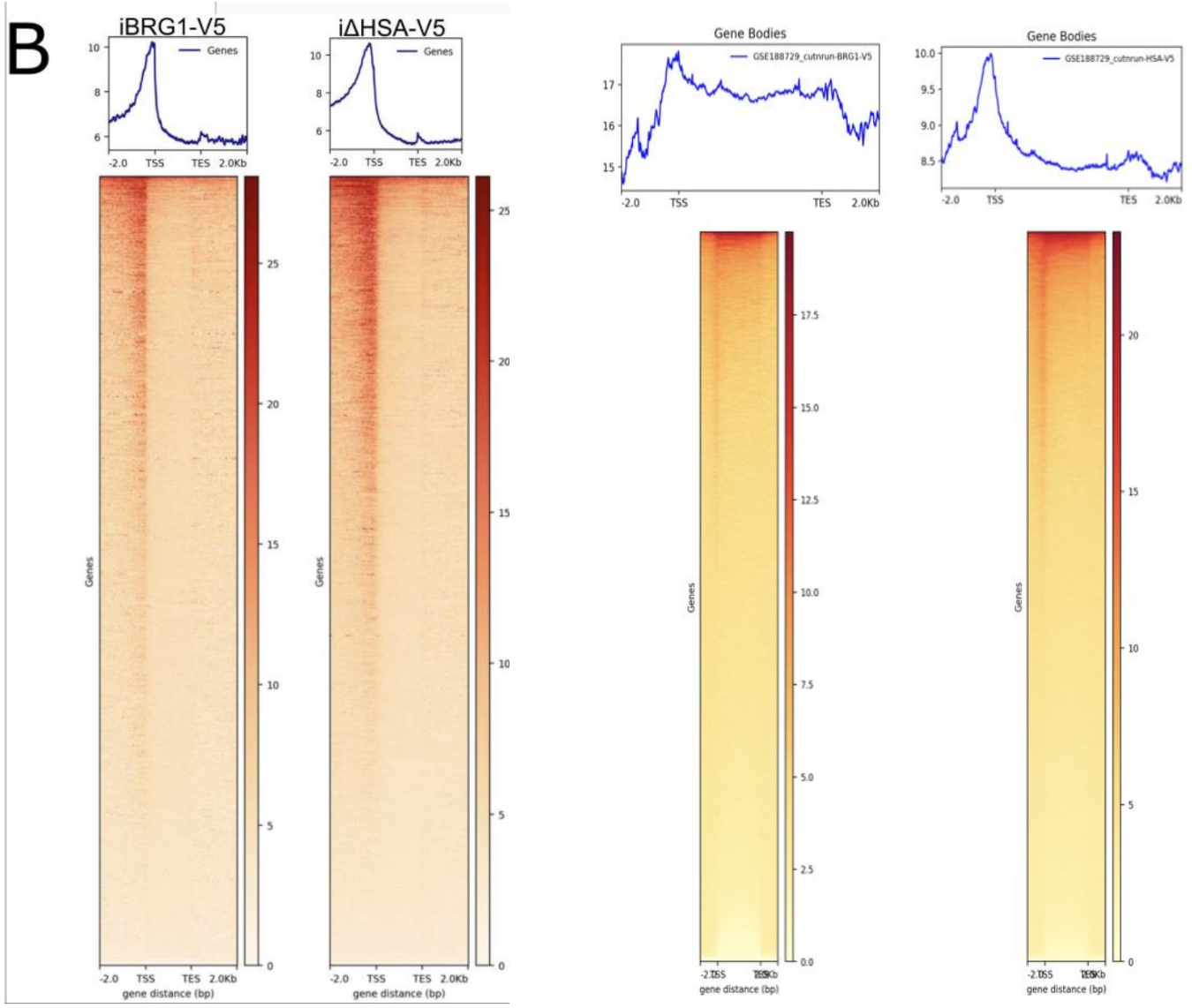
- Dietrich et. al predicted that the interaction with the BCL7 proteins is critical for the function of iBRG1 in SW-13 cells, and the loss of the interaction between iBRG1 and BCL7 proteins drives the phenotypes observed when iΔHSA is expressed.
- Knockdown of BCL7A, BCL7B, and BCL7C reduced the changes in gene expression driven by BRG1
- 61 BRG1-dependent DEGs were identified in siABC cells by Dietrich et. Al
- We observed 13 BRG1-dependent DEGs in siABC cells
- More upregulated DEGs confirms hypothesis that BRG1 mostly serves to upregulate genes and the HSA domain and its interactions with BCL7 are significant



Description : Volcano plots of DEGs identified by RNA-seq in iBRG1 cells after knockdown of a non-template control (left, red) or of BCL7A + BCL7B + BCL7C (right, blue).

Figure 2: Loss of the HSA domain does not alter binding of BRG1 to transcriptional start sites.

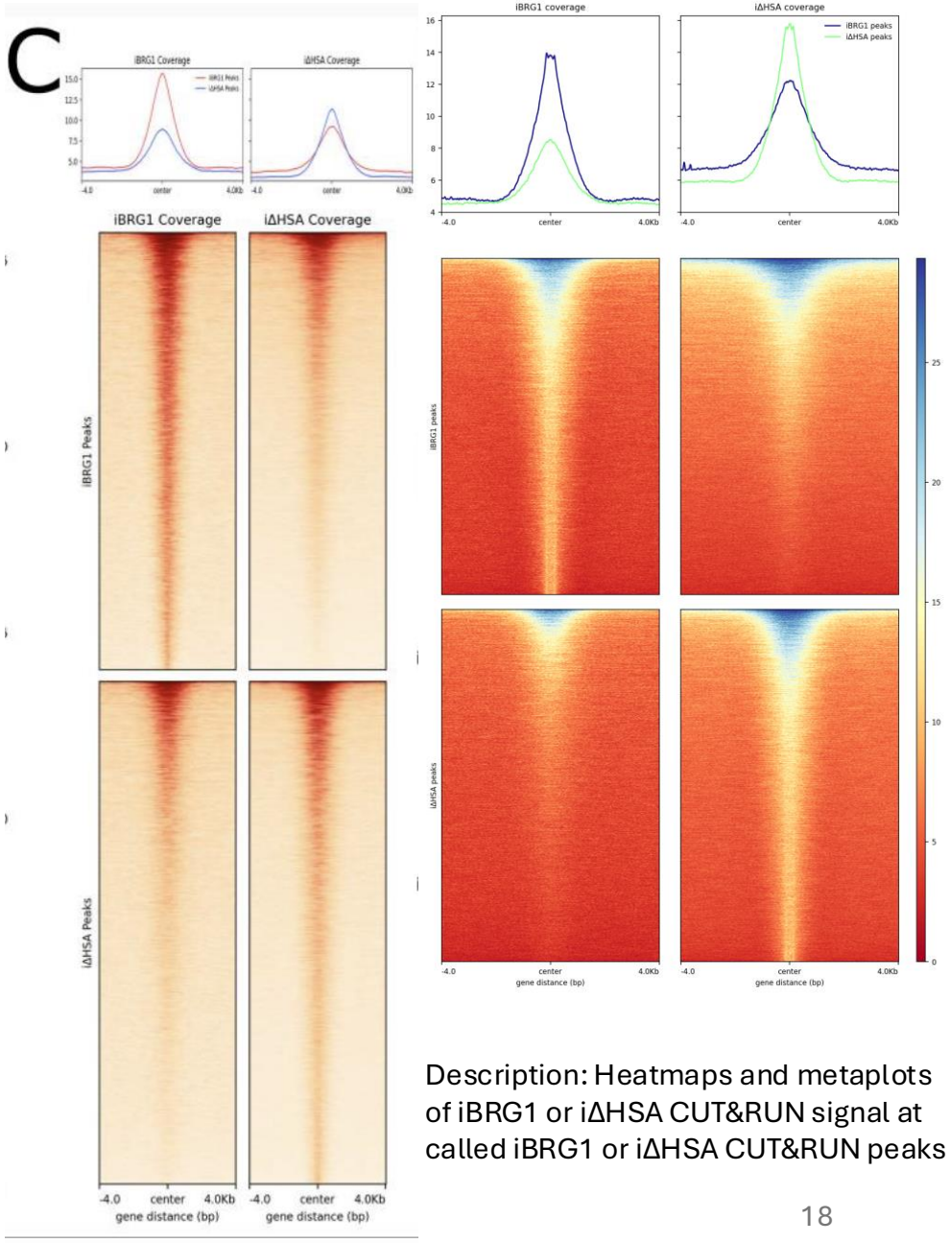
- The figures depict coverage plots of iBRG1 and iΔHSA over the transcriptional start site and the transcriptional end site scaled across all gene bodies.
- The comparative analysis between the two images suggests discrepancies in the iBRG1 plot.
- Variations in BRG1 TES peaks could result from differences in normalization, smoothing, matrix computation parameters in the TES region scaling, bin size or reference annotation files (e.g., GTF versions).



Description: Heatmaps and metaplots of iBRG1 or iΔHSA CUT&RUN signal in a 6-kb window around all transcriptional start sites, and they are scaled to represent all gene bodies.

Figure 2: Loss of the HSA domain does not alter binding of BRG1 to transcriptional start sites.

- Peaks represent binding activity between the protein of interest and area of a genomic sequence (eg. Higher peak corresponds to increased binding activity at that site)
- While there is a difference between the binding activity for iΔHSA (blue curve on left image, green curve on right image) at both identified peaks, it was determined that "the overlap of the peaks was similar and both peaks demonstrated a similar enrichment at genomic sites" (Dietrich et Al., 2023)
- "The HSA domain's role is not for DNA site binding specification" (Dietrich et Al., 2023)



Description: Heatmaps and metaplots of iBRG1 or iΔHSA CUT&RUN signal at called iBRG1 or iΔHSA CUT&RUN peaks

Discussion/Insight

- A key limitation in earlier research on BRG1-specific functions is the residual ATPase activity of BRM, which may compensate for BRG1 functional loss.
- This study tackles these limitations by employing a cell system deficient in both BRG1 and BRM, offering insights into the immediate and extended effects of reintroducing a single chromatin remodeler into a cancer cell model.
- Cancer-related pathways are significantly activated within 24 hours of treatment, with distinct differences between iBRG1 and iΔHSA gene expression, emphasizing the critical role of the HSA domain in BRG1 function.
- Loss of the HSA domain and reduced BCL7 expression produce comparable phenotypes, indicating that BCL7 proteins are critical for SWI/SNF complex function
- Long-term BRG1 expression induces significant proliferation suppression, senescence, and extensive transcriptional changes, predominantly enriching ECM-related and cancer-associated pathways, with essential for these effects.
- HSA domain is not necessary for localization to chromatin meaning another binding partner is most likely responsible for this difference

Source: Dietrich et al., 2023

Retrospective Analysis

- Tried to select a paper that had a clear protocol for data analysis such as stating all software/packages used as well as criteria for data filtering and statistical tests
- This paper did not have any available pipelines/code to directly compare so we had to do our analysis from scratch which was challenging as we believe it caused us to miss certain details that may have allowed us to produce better data and visuals or at least produce it with more ease (ex. multi-enrichment analysis was employed but no specific details or packages used were described so we had to recreate it to the best of our ability)
- The paper also analyzed ATAC-seq data which would have strengthened our analysis but they did not provide the ATAC-seq data so we were unable to include that in our recreation.
- While it provided a healthy challenge, in the future we think we would be more satisfied with our results had we chosen a paper that had a proper data analysis pipeline available .

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