Challenge 12 : Unraveling Epigenetic Signatures associated with Prognostic Gene Expression profiles in Glioblastoma

Team members:

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Mentor:

Dr. Salma Abozeid

Introduction

Glioblastoma (GB) is a highly aggressive brain cancer with poor prognosis

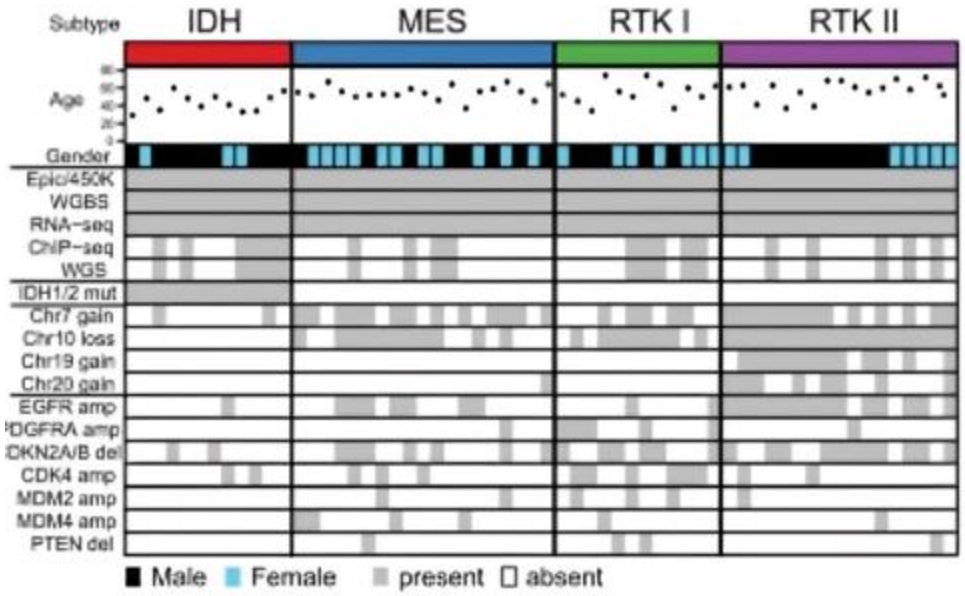
Epigenome studies reveal critical roles of DNA methylation and histone acetylation.“

Subtypes: IDH, RTK I, RTK II, MES.

Understanding epigenetic signatures can improve the Prognostic strategies

The Epigenome of GB Subtypes

- IDH subtype: global hypermethylation.
- RTK I & RTK II subtypes: hypomethylation In specific regions
- MES subtype: highly aggressive with poor prognosis



Wu, Y., Fletcher, M., Gu, Z. et al. Glioblastoma epigenome profiling identifies SOX10 as a master regulator of molecular tumour subtype. Nat Commun 11, 6434 (2020).

The problem:

1

- ✓ GB shows high heterogeneity
- ✓ No standard care of treatment
- ✓ 50% of patients are unresponsive to treatment by TMZ

2

- ✓ Epigenetic modifications (methylation and acetylation) are crucial

3

- ✓ Subtypes exhibit distinct epigenetic patterns affecting prognosis.

Challenge is to:

Identify	Profile	Uncover
Identify and characterize SE/E specific GB	SE associated genes and prognostic markers in GB.	potential epigenetic therapeutic targets for future studies.

Methods

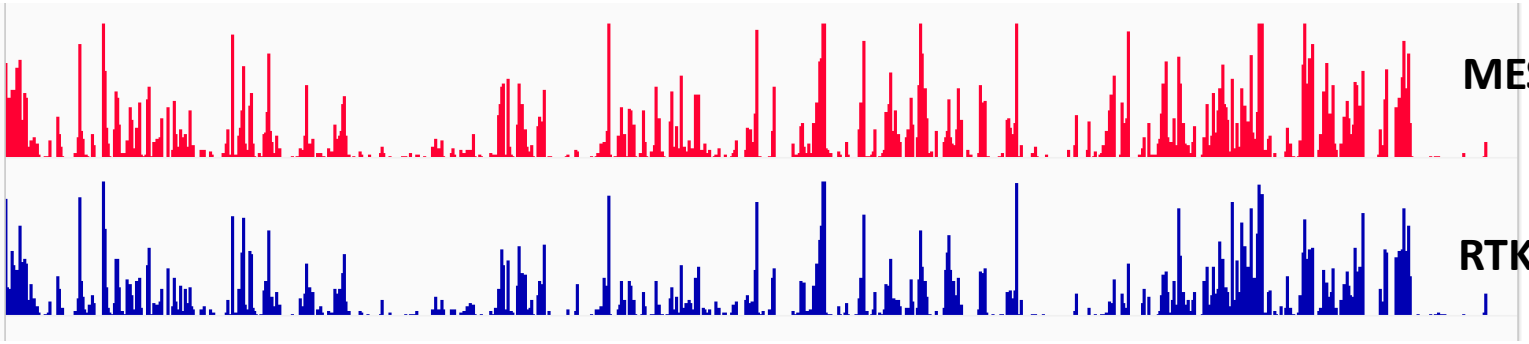
BedTools	ChIPSeeker	Reactome PW
For overlapping methylation and acetylation peaks	For obtaining the peak annotation using Granges	For obtaining functional annotations

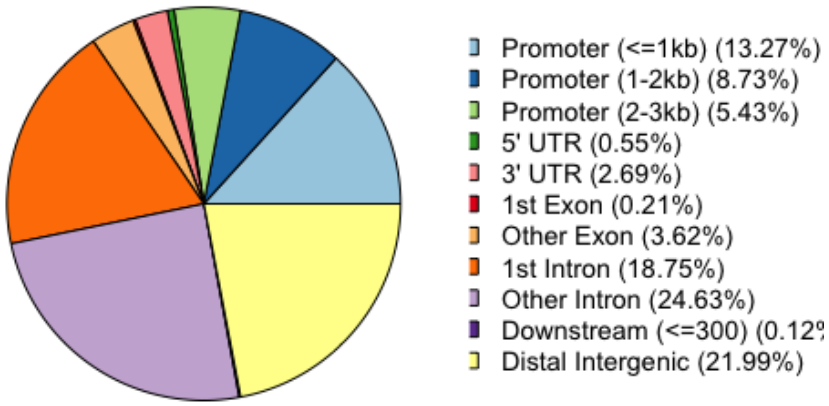
Methods

IGV	GREAT	MEMEsuite/ JASPER
For Visualization and extraction of genomic sequences	For identifying of prognostic markers associated with super enhancers	For Motif and TFs identifications

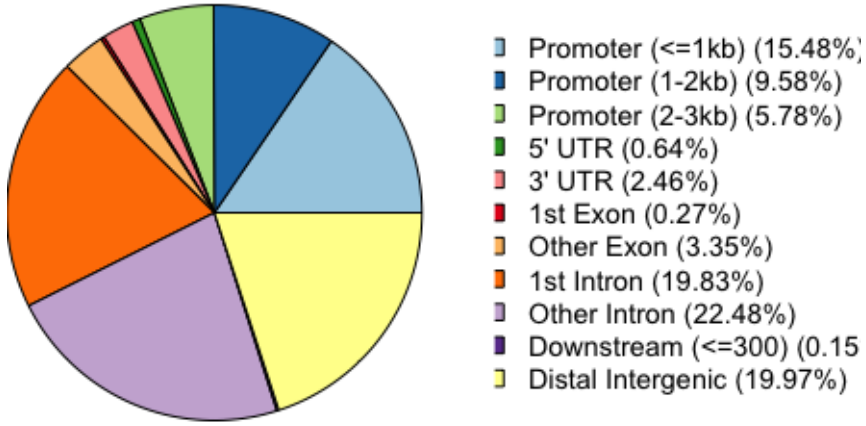
Results:

1. Enhancer peak and functional annotation in GB per subtype

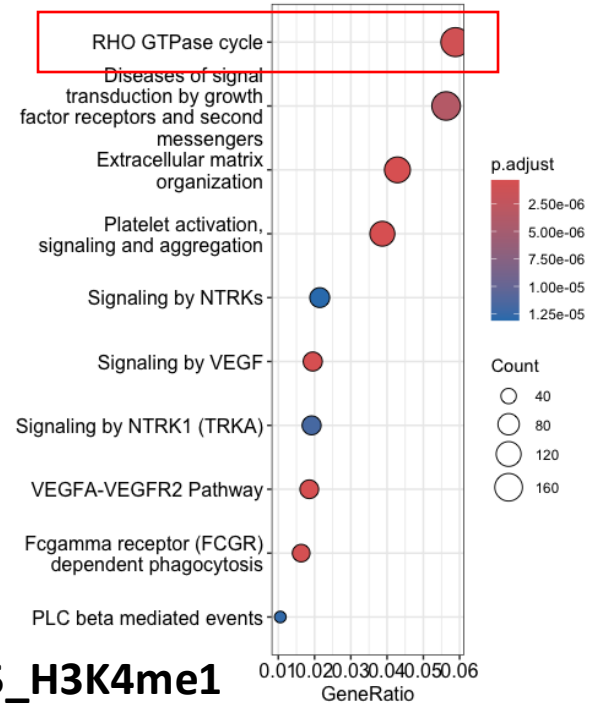




MES_H3K4me1



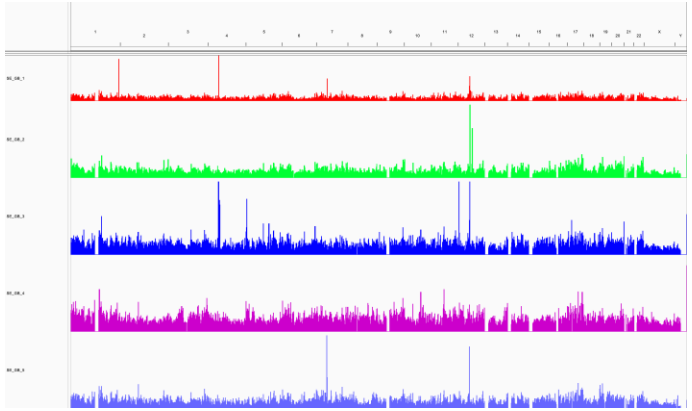
RTK_I_ H3K4me1



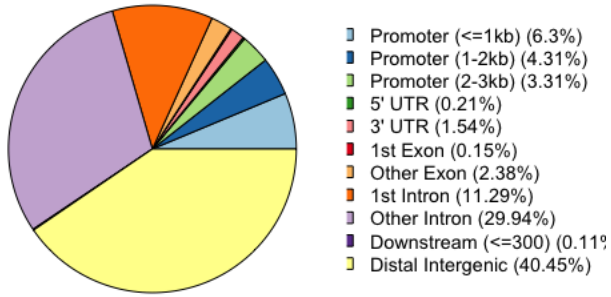
MES_H3K4me1

Results:

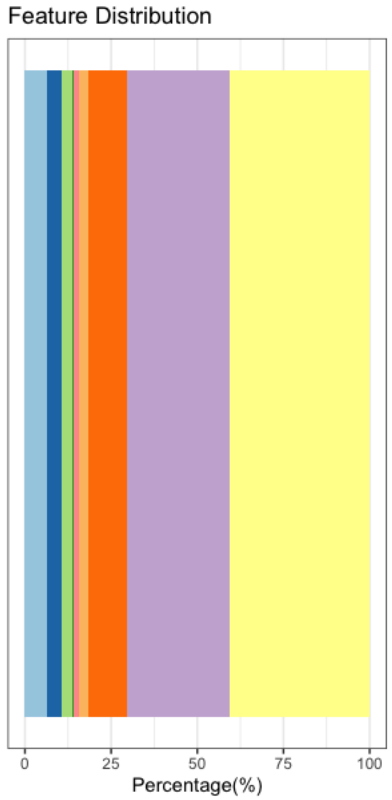
2. Analysis and Visualization of Super-Enhancers of GB

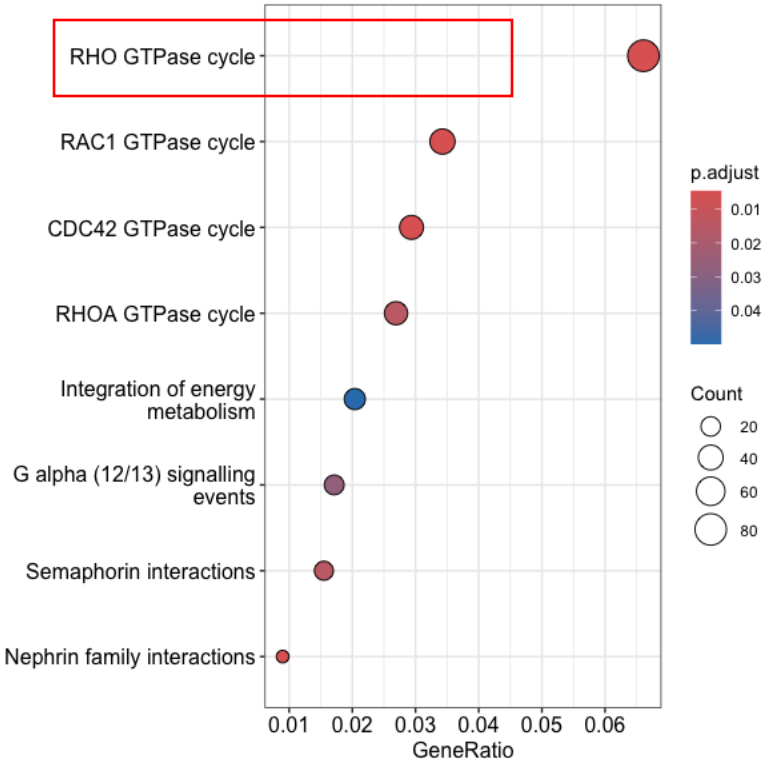


IGV screenshot of SE sequences in five GB patients



We identified 224 specfic SE in GB tissues



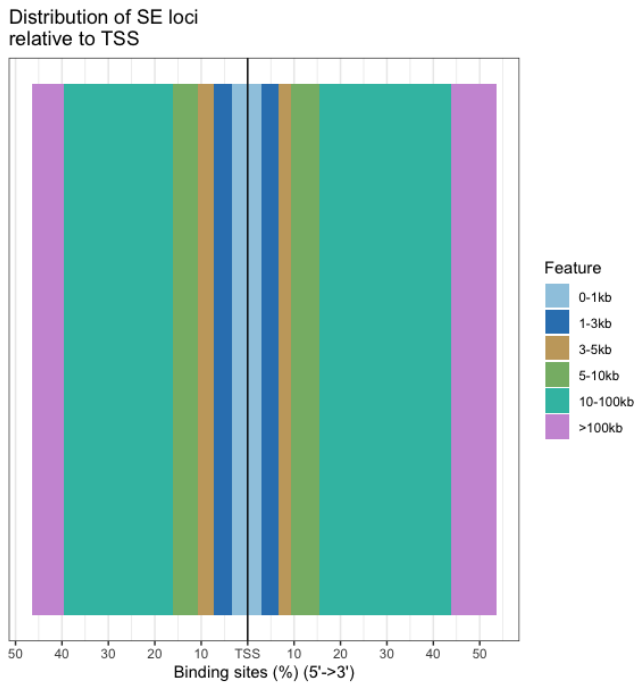


Peak anntation and Functional annotation of SE using ChIPSeeker and Reactomepw

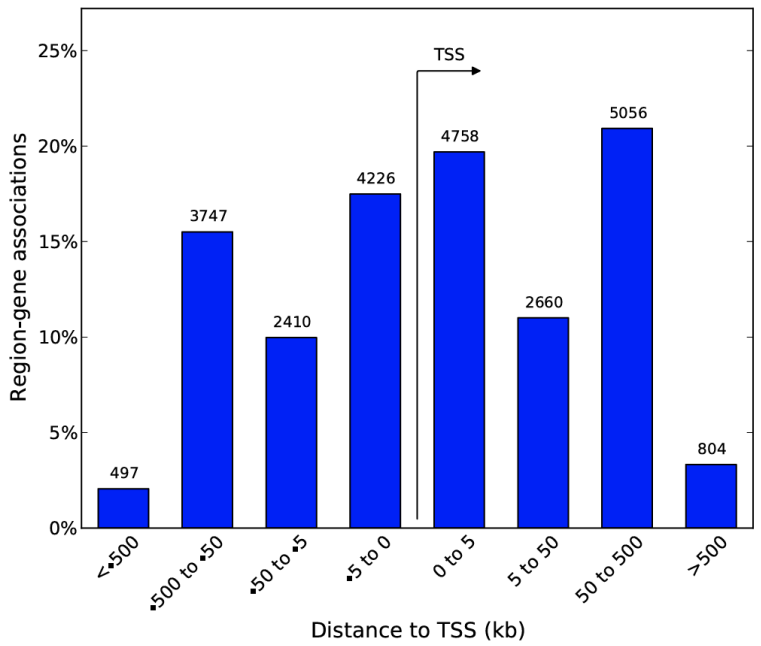
Results:

3. SE-gene association using GREAT tool

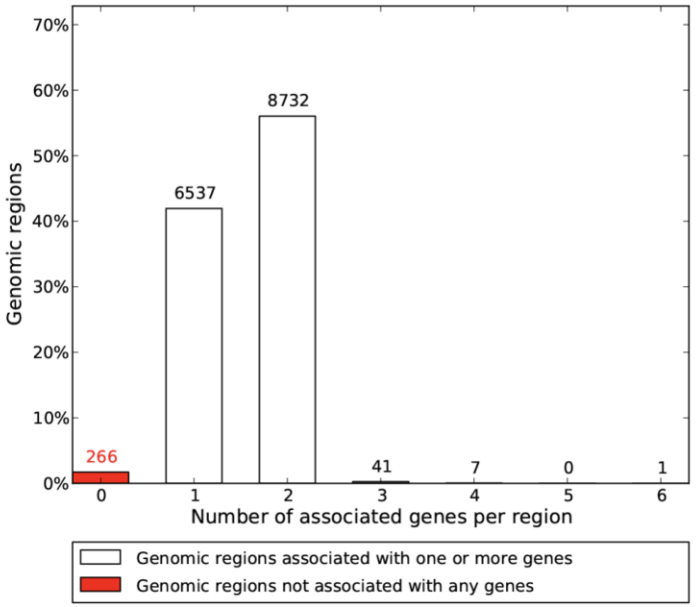
A



B



C



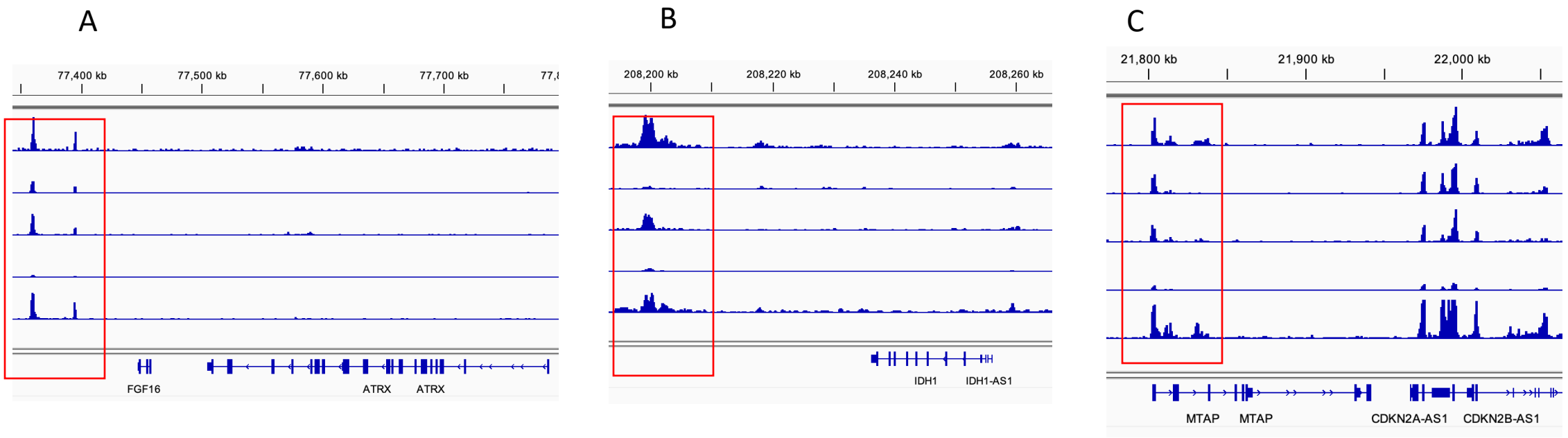
A) And B) Distribution of SE loci relative to TSS

C) Genes associated with SE regions in GB

Genes associated with SE regions in GB, among genes were IDH , CDKN2A and ATRX (known prognostic markers)

Results:

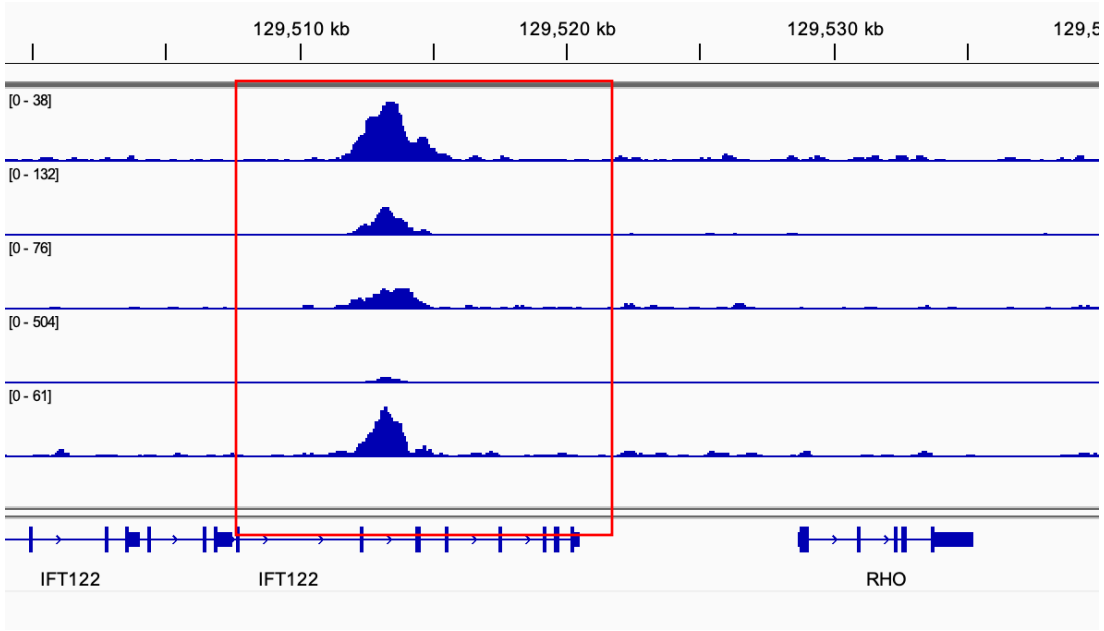
4. Super-enhancer associated with prognostic markers



Shown are CHIP-seq. (H3K27ac) tracks around the (a)ATRX, (b) IDH and, c) CDKN2A loci (GRCh38/hg38))

Results:

5. SE-associated to Rho is 20,000 bases upstream of Rho gene



Shown are ChIP-seq. (H3K27ac) tracks around the RHO locus (GRCh38/hg38))

The Role of Rho GTPases in Motility and Invasion of Glioblastoma Cells

Houssam Al-Koussa, Oula El Atat, Leila Jaafar, Hagop Tashjian, and Mirvat El-Sibai

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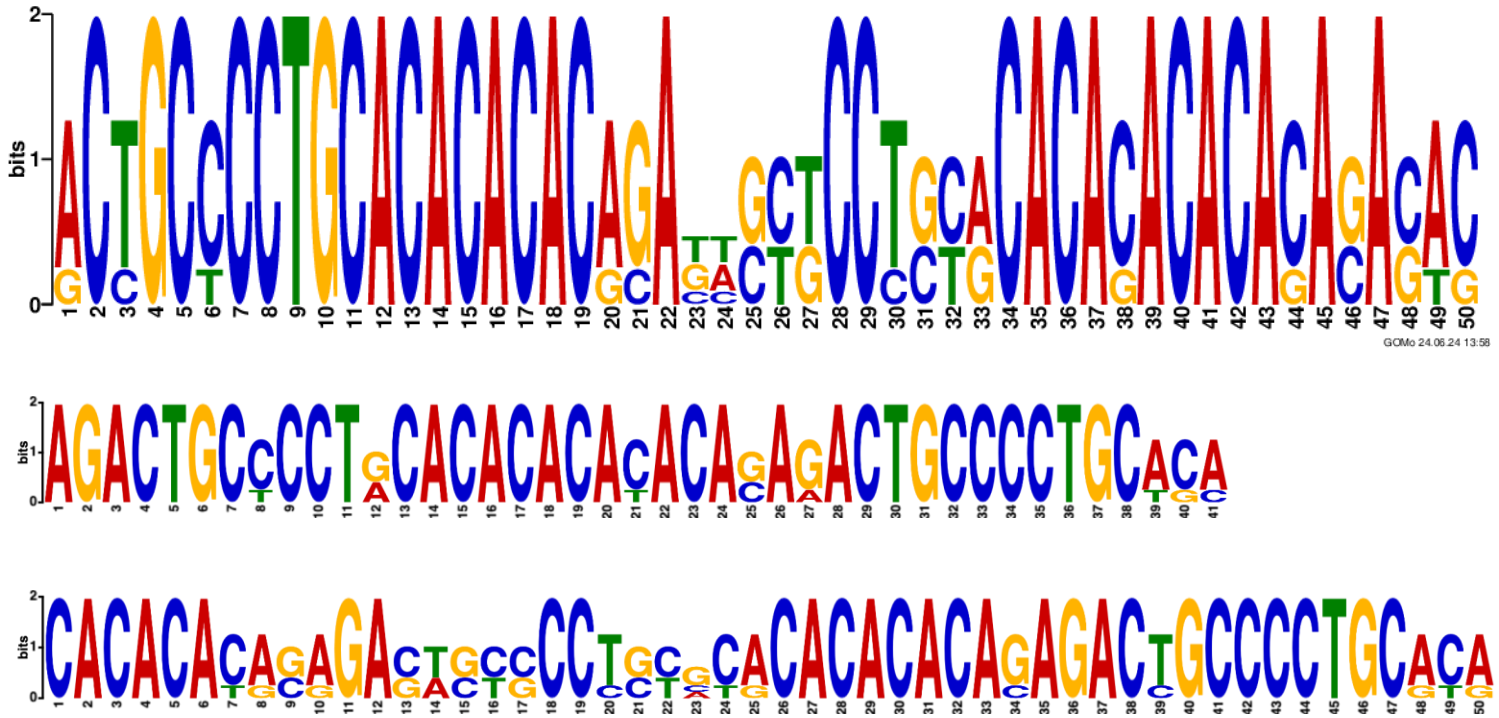
The Rho family of small GTPases, which includes Rac1, Cdc42, and RhoA, is an important family whose members are key regulators of the invasion and migration of glioblastoma cells.

Results:

6. Motif identification of SE-associated with Rho and functional annotation using GOMO



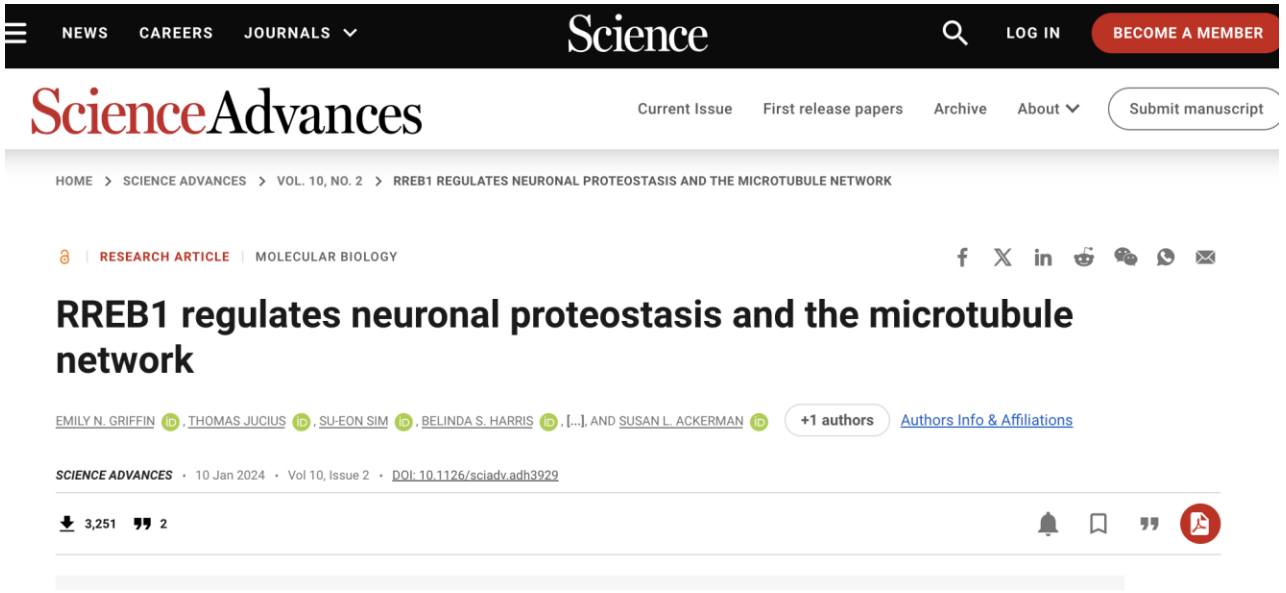
Top 5 specific predictions

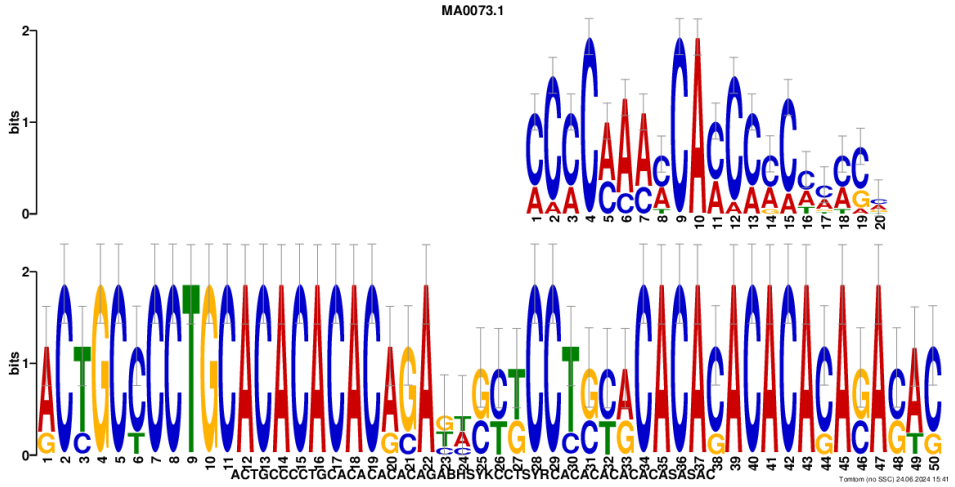
BP anterior/posterior pattern formation
MF growth factor binding
MF extracellular-glutamate-gated ion channel activity
BP epidermis development
BP negative regulation of transcription from RNA polymerase II promoter

Three motifs have five occurrences in SE loci near Rho gene

Results:

7. TFs targets of SE-associated with Rho



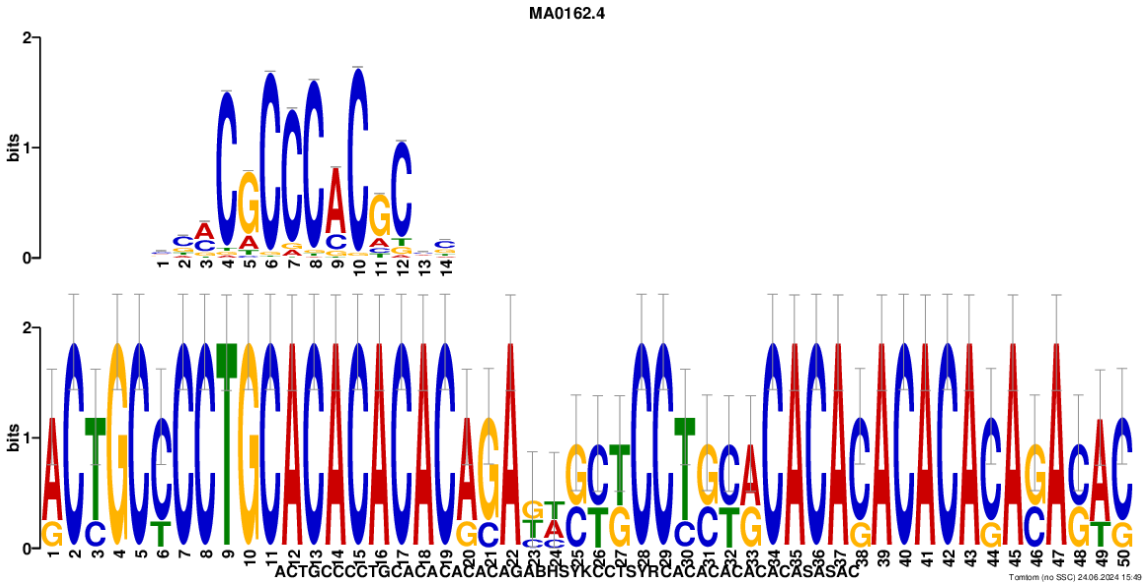


Name [MA0073.1 (RREB1)]

Using JASPAR2022_CORE_vertebrates_non-redundant_v2 database, the most significant motif could be targeted by 7 TFs: RREB1, EGR1, KLF9, ZSCAN4, MSANTD3 and NR5A1.

Results:

7. TFs targets of SE-associated with Rho



Name [MA0162.4 (EGR1)]

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Expression and prognostic value of the transcription factors EGR1 and EGR3 in gliomas

Arnon Møldrup Knudsen, Ida Eilertsen, Susanne Kielland, Mikkel Warming Pedersen, Mia Dahl Sørensen, Rikke Hedegaard Dahlrot, Henning Bünsow Boldt, Sune Munthe, Frantz Rom Poulsen & Bjarne Winther Kristensen

Scientific Reports **10**, Article number: 9285 (2020) | Cite this article

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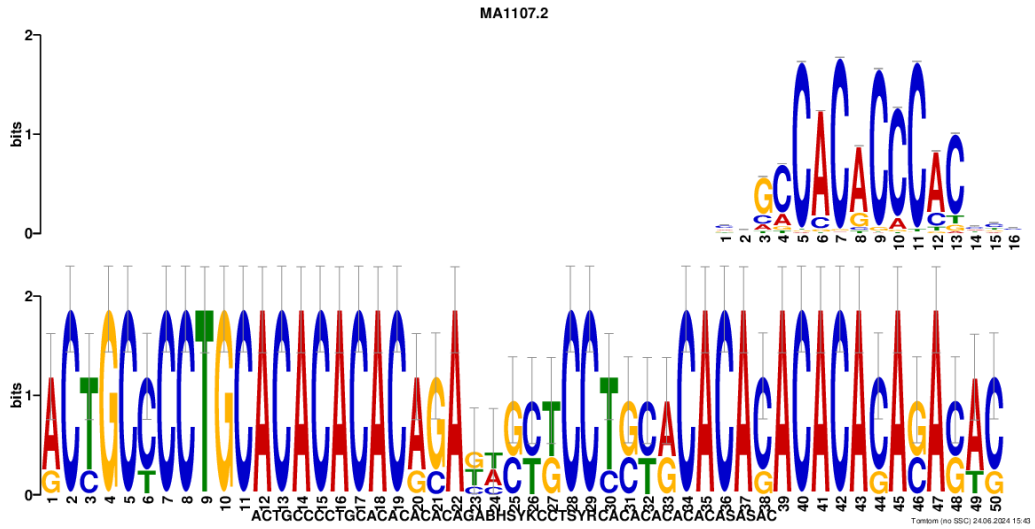

Name MA1107.2 (KLF9)

JBC JOURNAL OF BIOLOGICAL CHEMISTRY
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J Biol Chem. 2014 Nov 21; 289(47): 32742–32756. PMCID: PMC4239625
Published online 2014 Oct 6. doi: 10.1074/jbc.M114.588988 PMID: 25288800

Kruppel-like Factor-9 (KLF9) Inhibits Glioblastoma Stemness through Global Transcription Repression and Integrin α6 Inhibition[*] [S]

Mingyao Ying,[‡§] Jessica Tilghman,[‡¶,1] Yingying Wei,[‖,1] Hugo Guerrero-Cazares,[**] Alfredo Quinones-Hinojosa,[¶**‡‡] Hongkai Ji,[§§] and John Laterra[‡§¶‡‡,2]

Results:

7. TFs targets of SE-associated with Rho

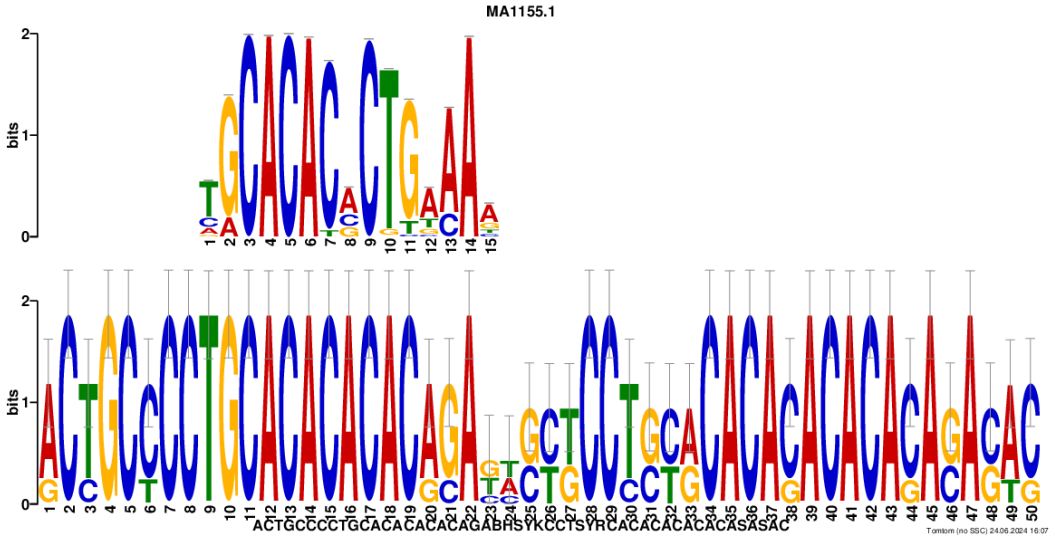
nature portfolio

Oncogene. 2020; 39(26): 4970–4982.
Published online 2020 Jun 7. doi: 10.1038/s41388-020-1333-1

PMCID: PMC7314663
NIHMSID: NIHMS1594445
PMID: 32507861

ZSCAN4 facilitates chromatin remodeling and promotes the cancer stem cell phenotype

Benjamin A. Portney,[#1] Michal Arad,[#1] Aditi Gupta,[1] Robert A. Brown,[1] Raju Khatri,[1] Phyo Nay Lin,[1] Andrea M. Hebert,[1,2] Kristen H. Angster,[1,2] Lorna E. Silipino,[2] W. Alex Meltzer,[1] Rodney J. Taylor,[2,3] and Michal Zalzman[1,2,3,4]



Name MA1155.1 (ZSCAN4)

They reported that ZSCAN4 leads to a functional histone 3 hyperacetylation at the promoters of OCT3/4 and NANOG, leading to an upregulation of CSC factors.
Its depletion leads to downregulation of CSC markers, severely affects tumor growth. They suggested that ZSCAN4 plays an important role in the maintenance of the CSC phenotype, indicating it is a potential therapeutic target in HNSCC.

Conclusion:

1. We found SE associated with GB prognostic markers such as IDH, ATRX and CDKN2A, further investigations are needed to the regulatory effect of these SE.
2. The functional annotation of both E and SE in GB tissues showed Rho GTPase is the most signficant in all GB patients
3. SE-associated to Rho (SE-Rho) could be linked to Glioblastoma progression.
4. RREB1, EGR1, KLF9, ZSCAN4 TFs bind to SE-Rho, this in fact support the importance to further investigate the SE-Rho regulatory pathway
5. Future studies using in-vivo animal models and cell lines is required to validate the effect of the inhibition SE-Rho
6. Inhibitors of SEs could be a potential therapeutic target in treatment of Glioblastoma

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10 PRINT "THANK YOU"
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