

Oncogenic Foxl2 is a chromatin-remodeling pioneer transcription factor in adult-type ovarian granulosa cell tumors

Veena K. Vuttaradhi^a, Eleonora Khlebus^a, Thomas Welte^a, Namrata Khurana^a, R. Tyler Hillman^{a,b,c}

^aDepartment of Genomic Medicine; ^bDepartment of Gynecologic Oncology and Reproductive Medicine; ^cCPRIT

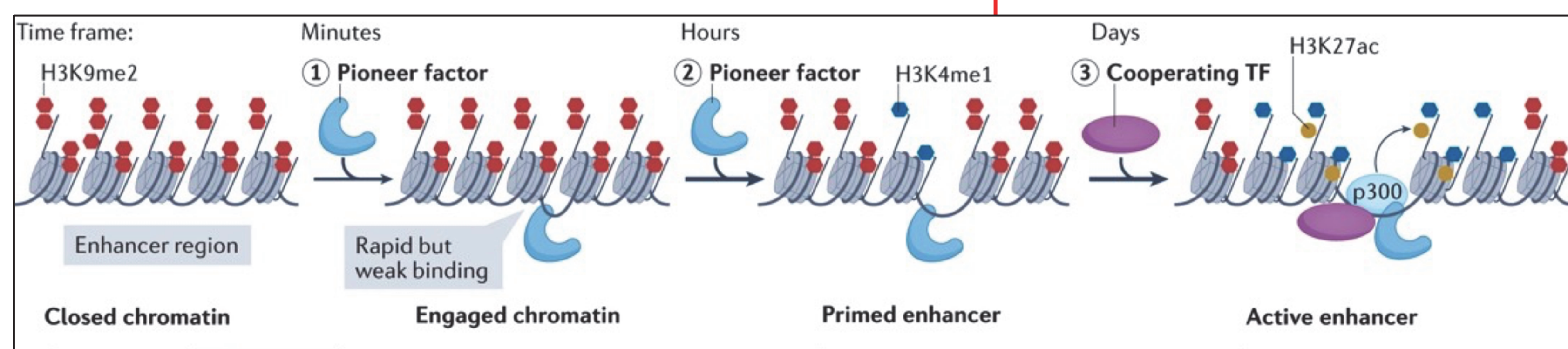
Scholar in Cancer Research-The University of Texas MD Anderson Cancer Center, Houston, TX, 77030, USA

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Background

- Adult-type granulosa cell tumors (aGCTs) are rare sex-cord stromal tumors that account for 5% of total ovarian cancers¹.
- A unique missense point mutation in the Forkhead domain-containing *FOXL2* (Foxl2 p.C134W) transcription factor is pathognomonic for aGCTs^{2,3}, but the oncogenic mechanism of this mutation is not known.
- Other Forkhead family transcription factors have well-described “pioneer” activity, binding to compacted, nucleosome-bound DNA and increasing accessibility for other regulatory proteins⁴ (Fig.1).



From: Balsalobre & Drouin, Nat Rev Molec Cell Biol, 2022.

Fig. 1 Priming of the enhancer region through binding of pioneer transcription factor to the closed chromatin

Objectives

- To develop novel cell culture model systems and
- To determine whether oncogenic Foxl2-C134W has pioneering activity in aGCTs.

Methods

- We used CRISPR/Cas9 editing to generate isogenic aGCT cells lacking either the *FOXL2* wild-type allele (single knock-out; SKO) or both the mutant and wild-type *FOXL2* alleles (double knock-out; DKO) (Fig. 2).
- ATAC-Seq and endogenous Foxl2 ChIP-Seq were performed on these isogenic lines to determine the differential chromatin accessibility at Foxl2-bound regulatory regions across genotypes.
- ENCODE pipelines and data standards were used for analysis and the irreproducible discovery rate was used to identify high-reliability ATAC-seq and ChIP-seq peaks.
- De novo* motifs were identified with the STREME algorithm.

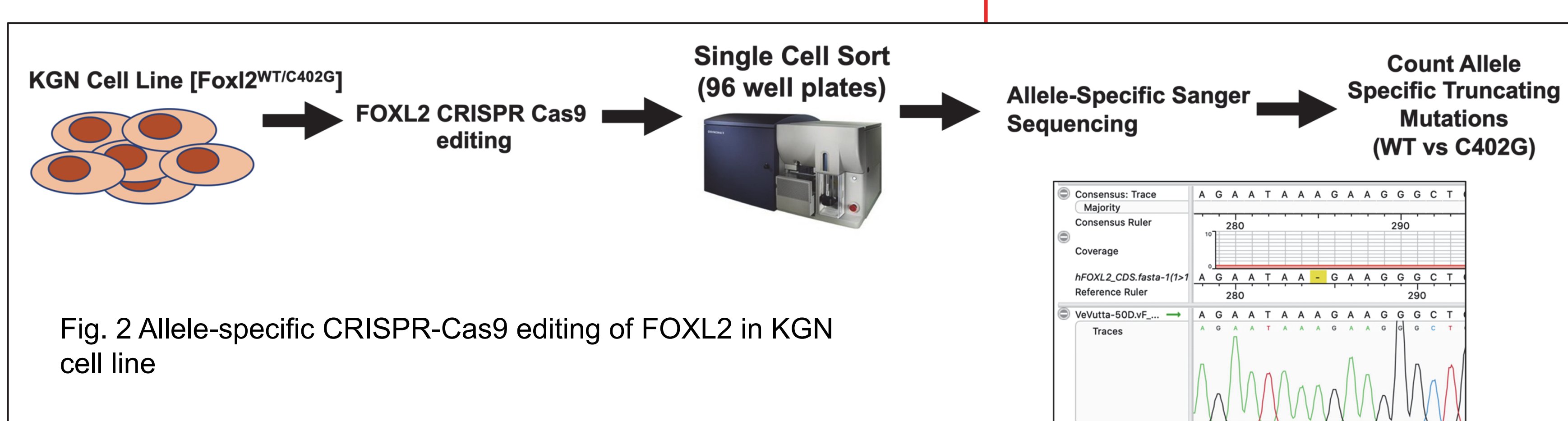


Fig. 2 Allele-specific CRISPR-Cas9 editing of FOXL2 in KGN cell line

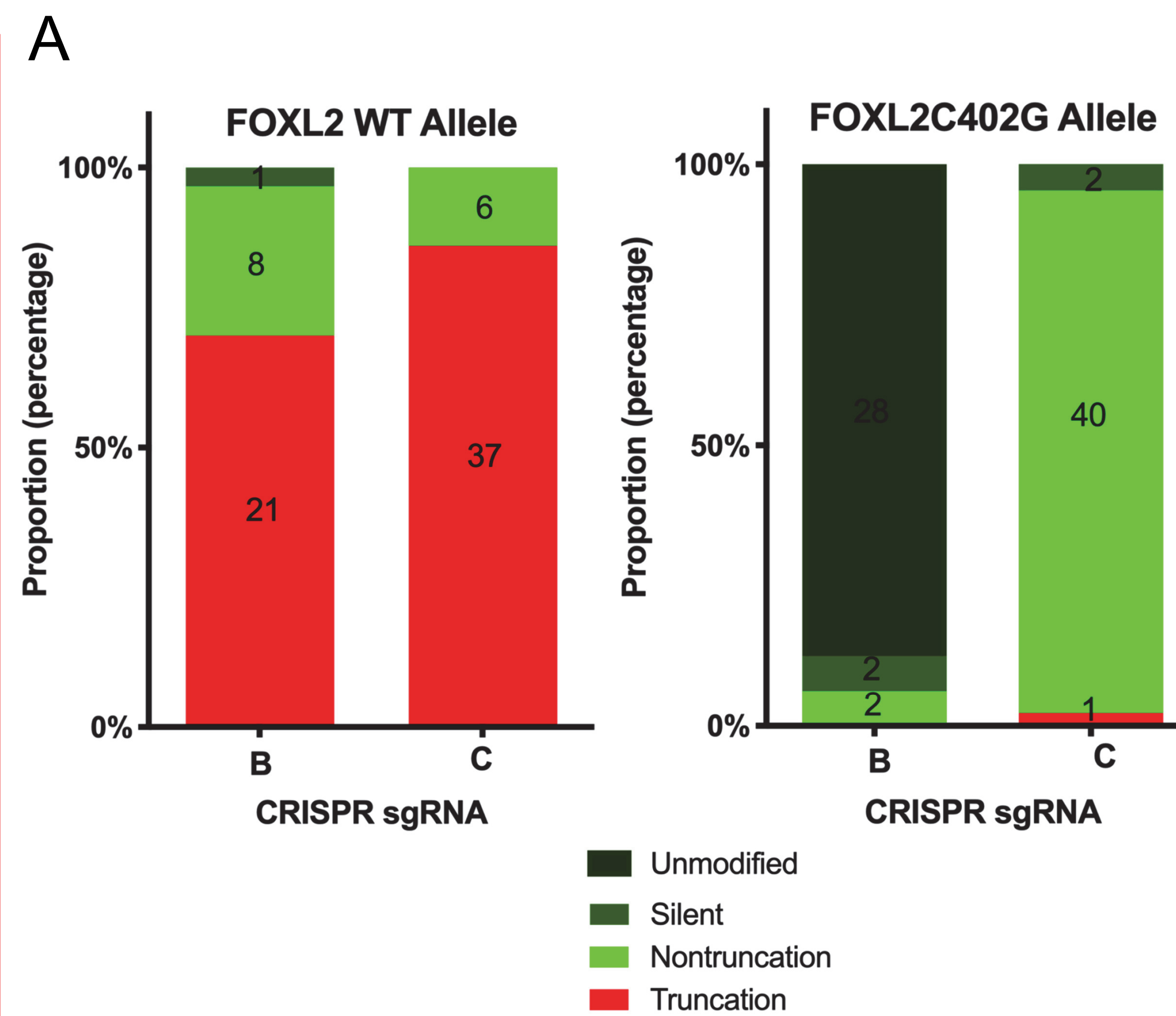
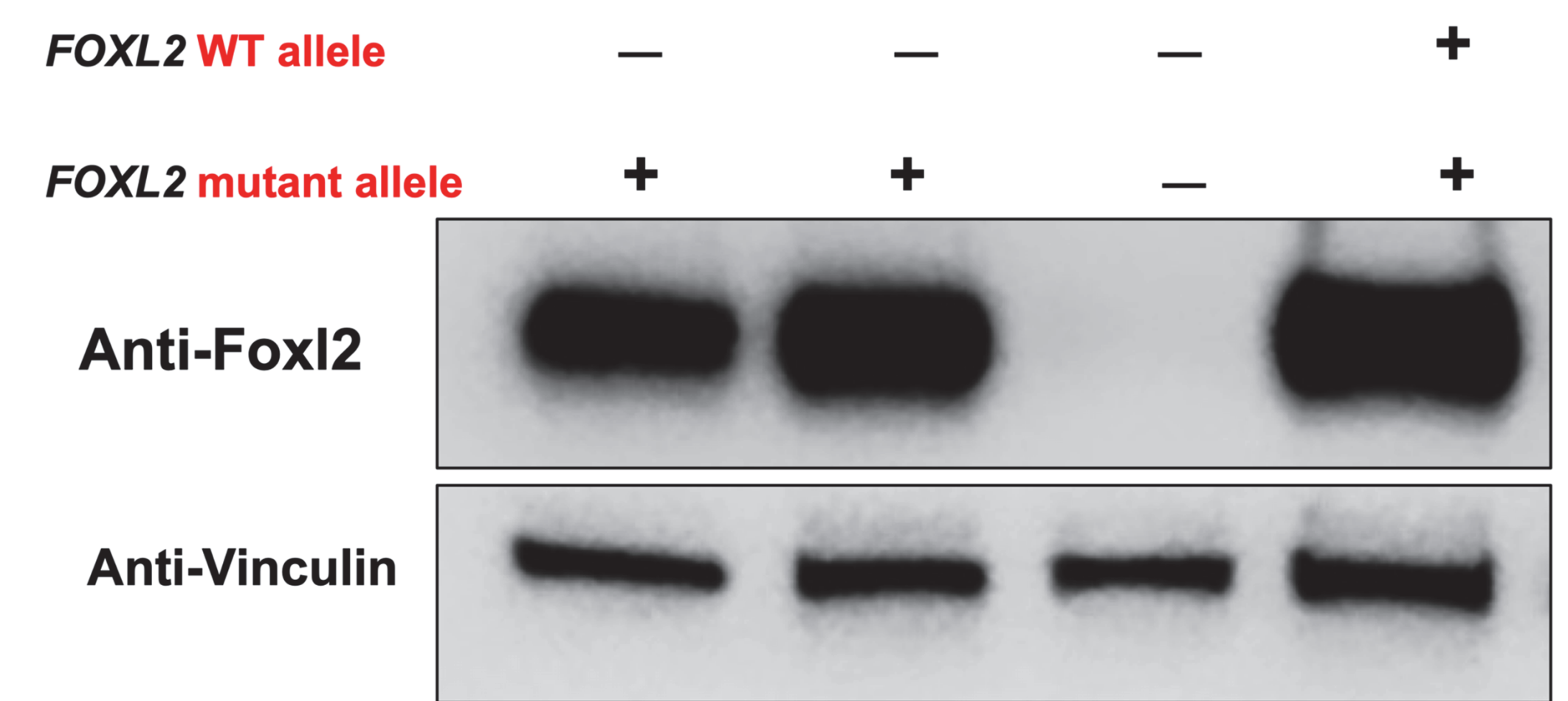


Fig. 3A Allele-specific Sanger sequencing data of FOXL2- edited KGN- lines; B Foxl2 expression across FOXL2-edited KGN- lines

B



Results

- Using the allele-specific CRISPR-Cas9 method, two specific, novel KGN-*FOXL2*^{-/-} and KGN-*FOXL2*^{-/C134W} isogenic cell lines were isolated (Fig. 3 A & B).
- Intense negative selection against the inactivation of FOXL2c.C402G was observed only in 1% of KGN-*FOXL2* edited lines.
- Endogenous ChIP-seq of Foxl2-C134W from the SKO cell line identified 1147 high-confidence peaks. *De novo* motif discovery performed on Foxl2-C134W ChIP-seq peaks identified the canonical Foxl2 binding motif ($P = 1.4 \times 10^{-7}$) in 44.9% of peaks but also identified a novel variant motif ($P = 6.7 \times 10^{-15}$) in 68.5% of peaks (Fig 4 A).
- Median chromatin accessibility at Foxl2-C134W peak regions, as measured by ATAC-seq, was significantly decreased in the DKO cells compared to either SKO or parental cell lines ($P < 2 \times 10^{-16}$ for both comparisons) (Fig 4 B).
- No difference was observed between SKO and parental cell lines ($P = 0.19$).
- Decreased chromatin accessibility at Foxl2-C134W ChIP-seq peaks in the DKO cells was driven by peaks containing the novel variant Foxl2 binding motif.

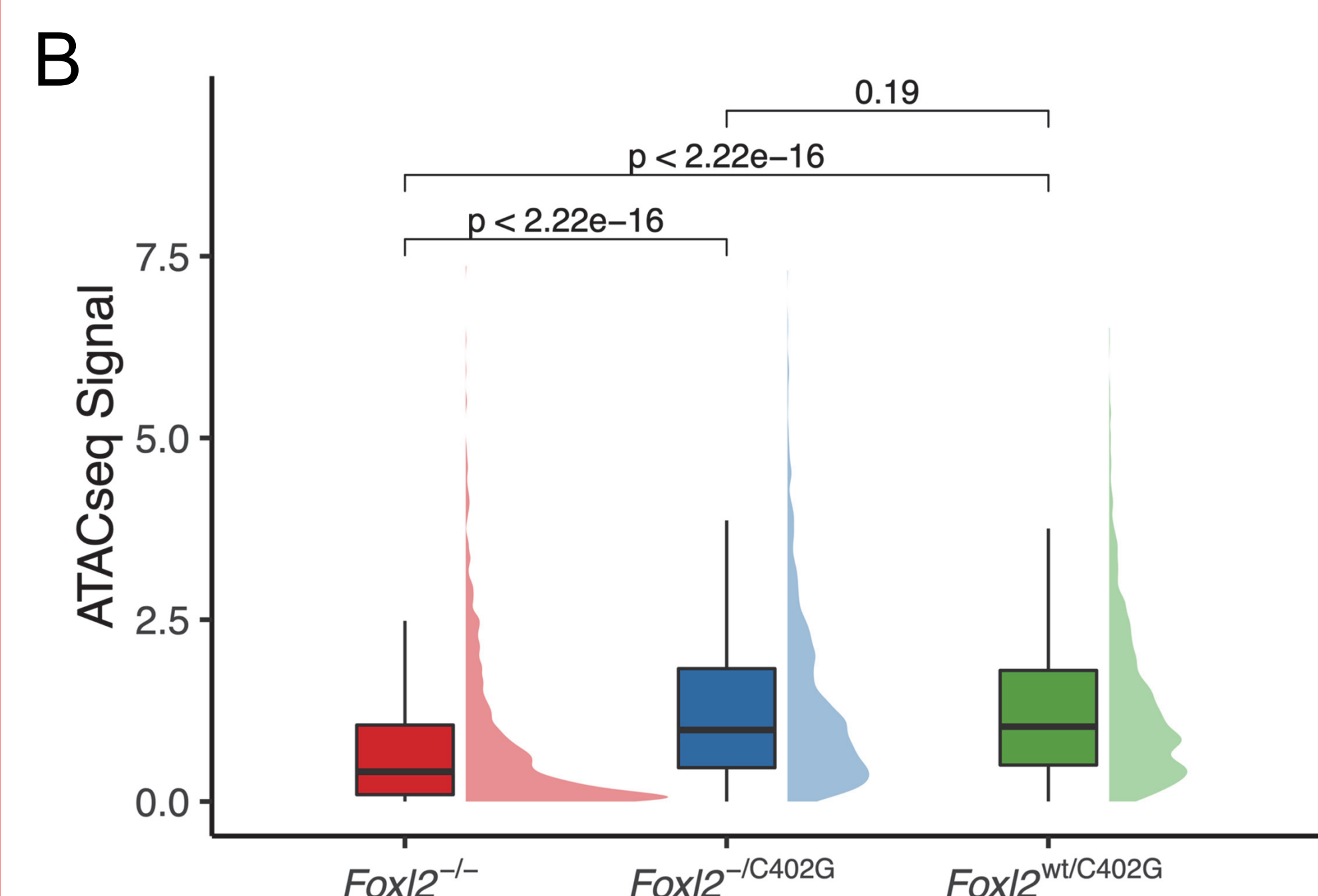
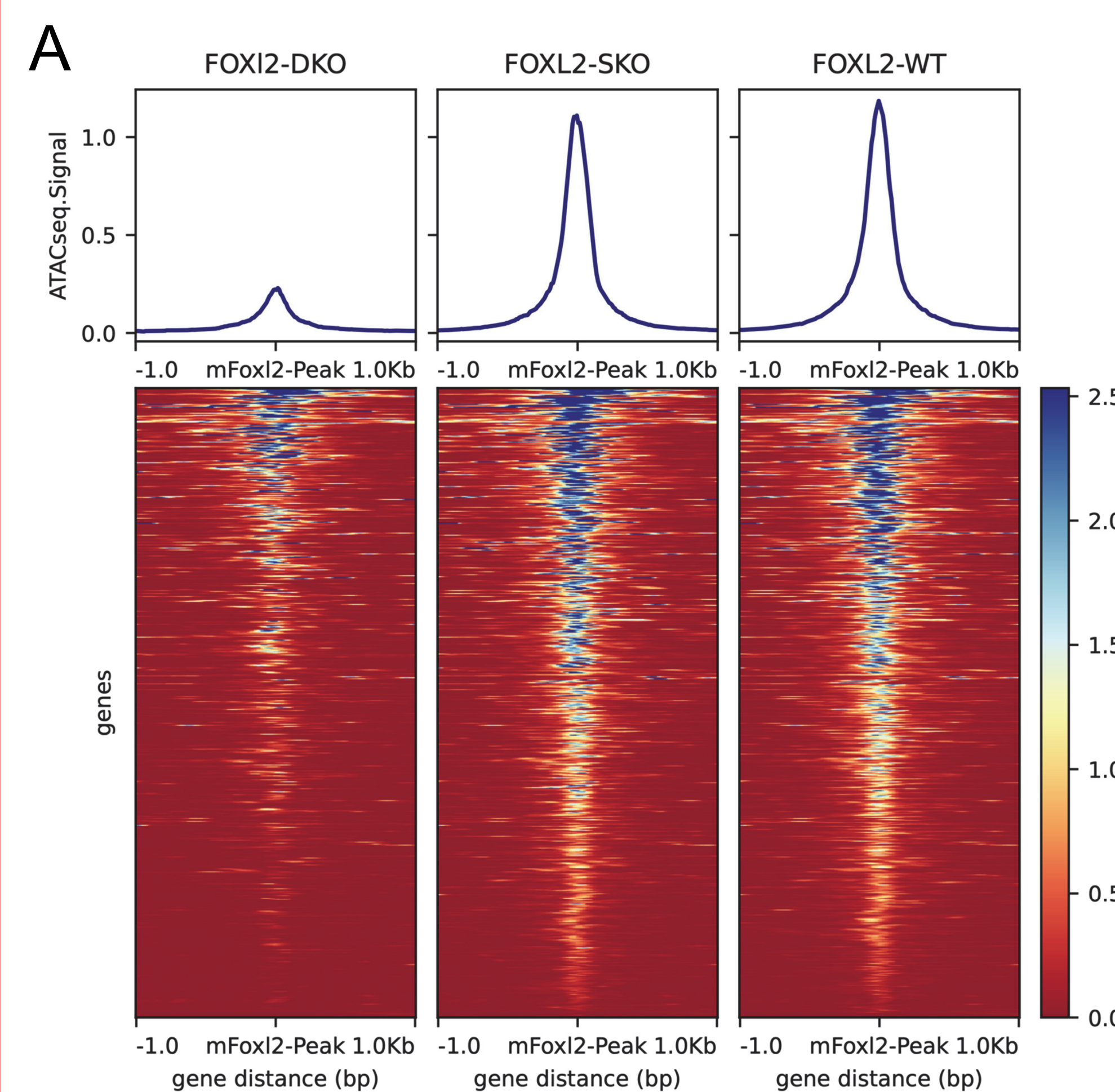


Fig. 4A & B Chromatin accessibility in Foxl2 ChIP-Seq peaks by genotypes

Conclusions

- Foxl2-C134W exhibits “pioneering” activity, increasing chromatin accessibility at key gene regulatory elements.
- The oncogenic mechanism of Foxl2-C134W in granulosa cell tumors may involve changes in DNA binding specificity, redirecting this pioneering function to sites containing a novel variant Foxl2 binding motif.

References

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