



Is Comet or TUNEL a Better Indicator of Sperm Cell Abnormalities?

BYU Cell Biology & Physiology

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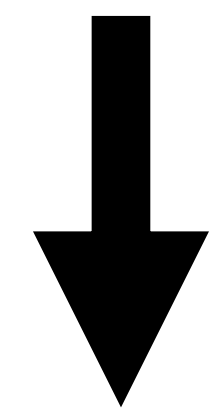
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Background

- **Sperm DNA methylation** and **DNA damage** correlate with fertility and sperm quality. High DNA damage should be associated with significant changes in DNA methylation
- **The Comet and TUNEL assay** are two common ways to quantify levels of sperm DNA damage
- **First, we assessed the relationship between sperm DNA damage and regional DNA methylation**
- **Second, we compared the outcomes of the two different DNA damage assays**
- Data taken from FAZST- the largest sperm DNA methylation dataset available

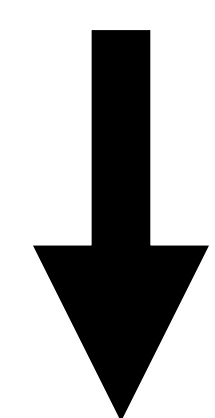
Methods

FAZST Data



Isolate scores from COMET and TUNEL assay...

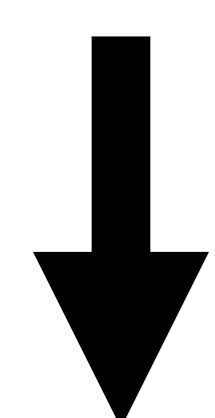
Take the top and low 10% of scores from each assay (high and low DNA damage group)



Sliding Window Analysis:

Analyze differentially methylated regions (DMR'S) between high and low damage groups

Pick most significant regions



GREAT Analysis:

Using GREAT program

Are those DMR's related to relevant gene ontology (GO) or cellular pathways?

Results

COMET vs. TUNEL

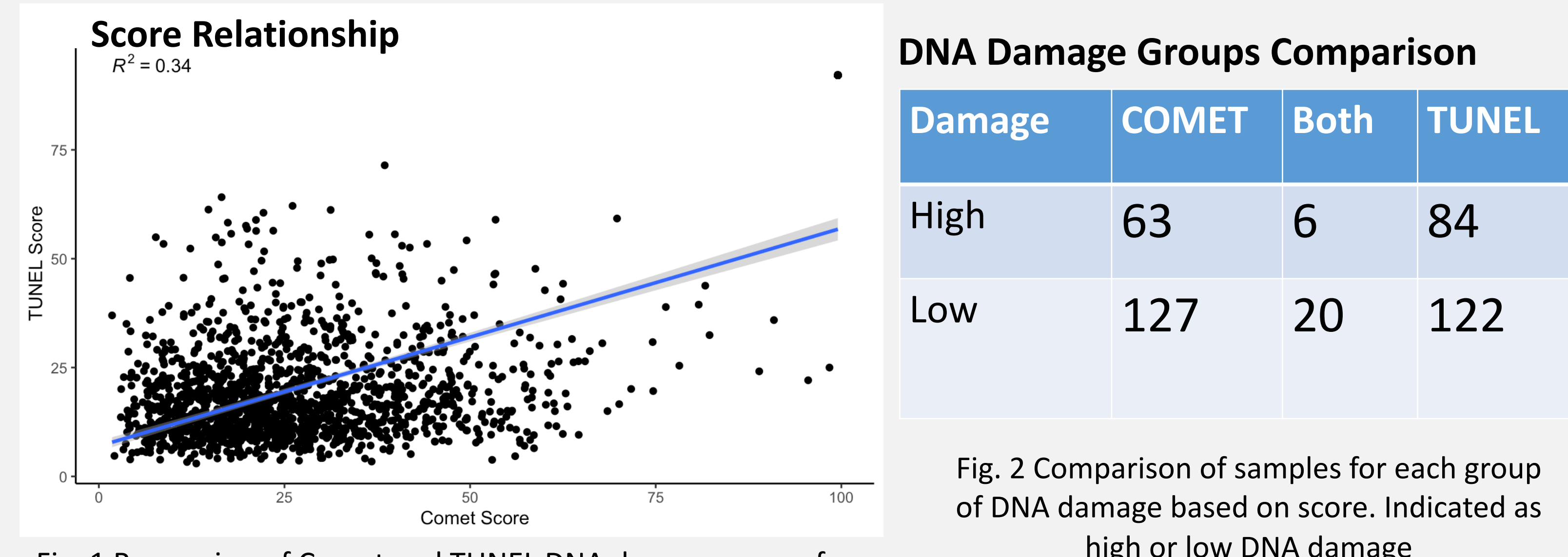


Fig. 1 Regression of Comet and TUNEL DNA damage scores from all patients (P<0.001)

Sliding Window Analysis

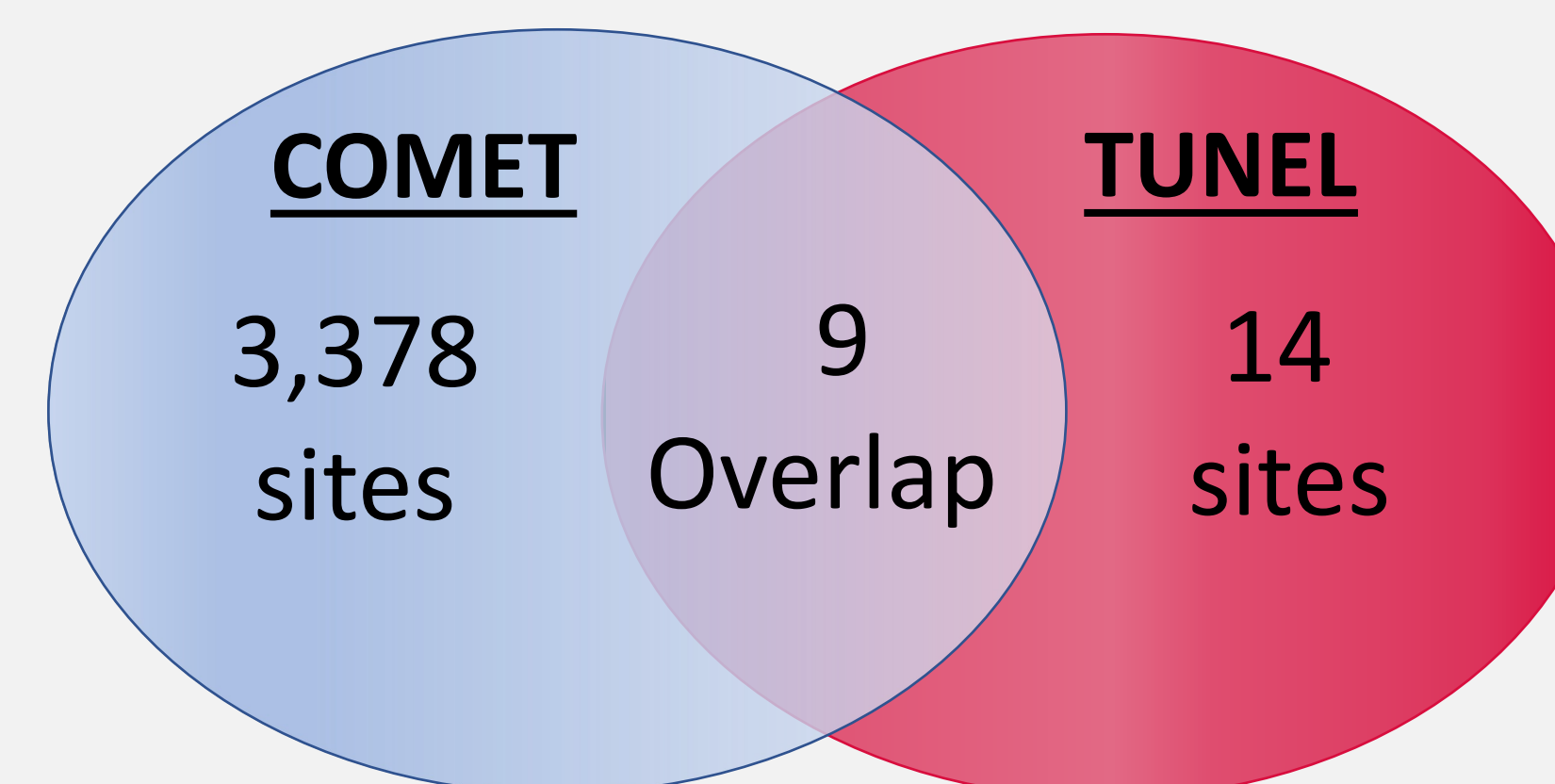


Fig. 3 Comparison of all differentially methylated regions in genome between high and low DFI and Comet Scores as found by the sliding window analysis

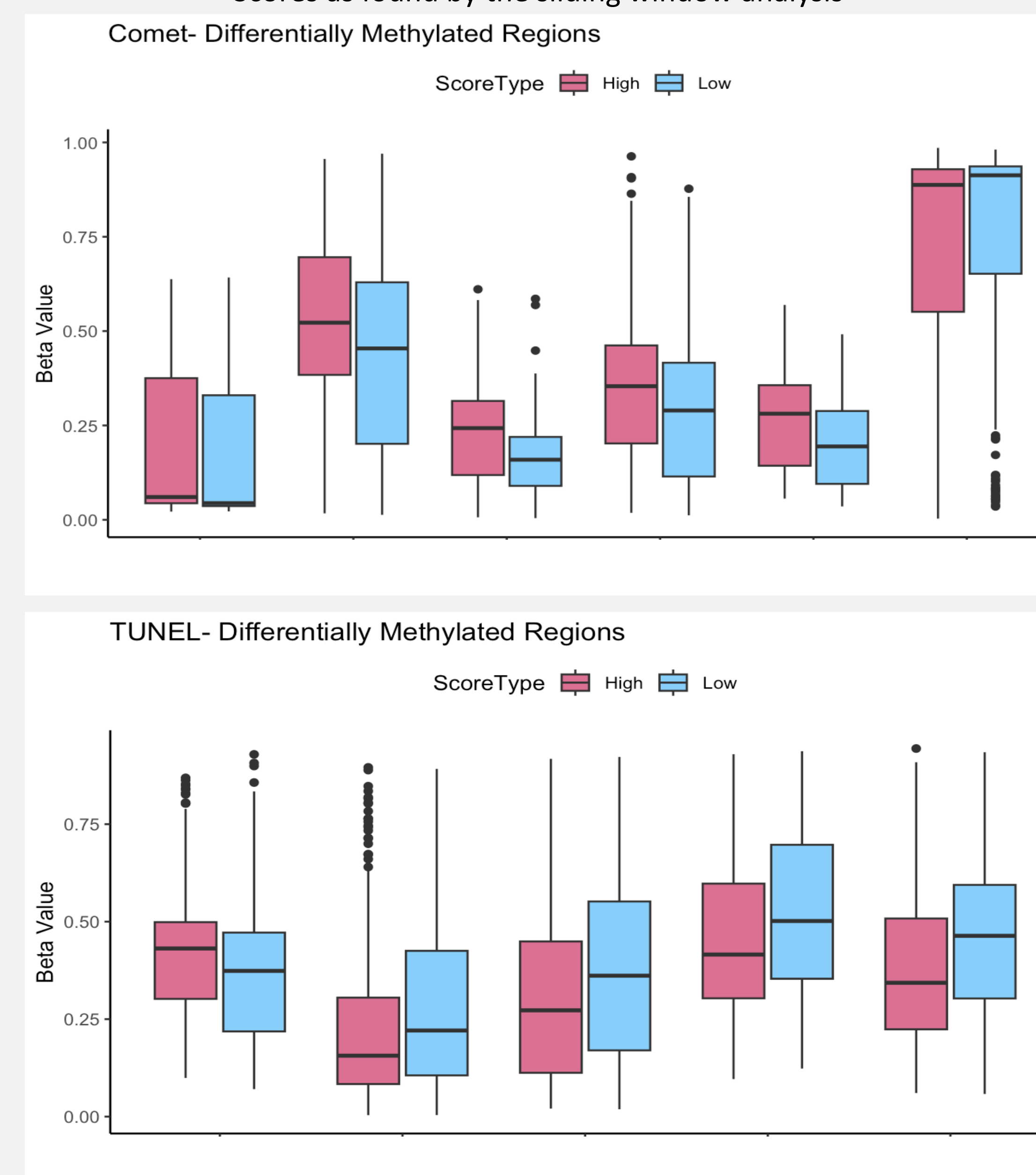


Fig. 4&5 Most significant differentially methylated regions (DMR's) of the genome. A difference > 0.07 in methylation signature was taken

GREAT Analysis

TUNEL Assay = NONE
COMET Assay= 2 GO pathways

COMET GO Pathways:

1. DNA Methylation involved in gamete generation
2. piRNA metabolic process

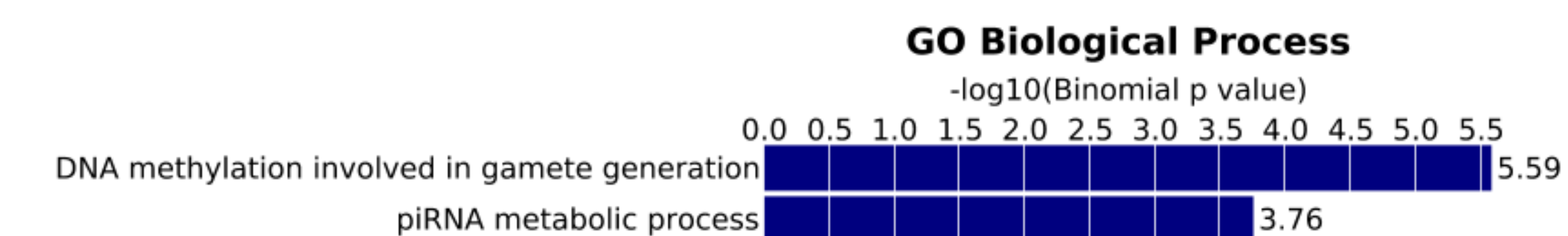


Fig. 6 Binomial p values relating COMET associated differentially methylated regions to gene-ontology processes

Genes Associated Per COMET Region

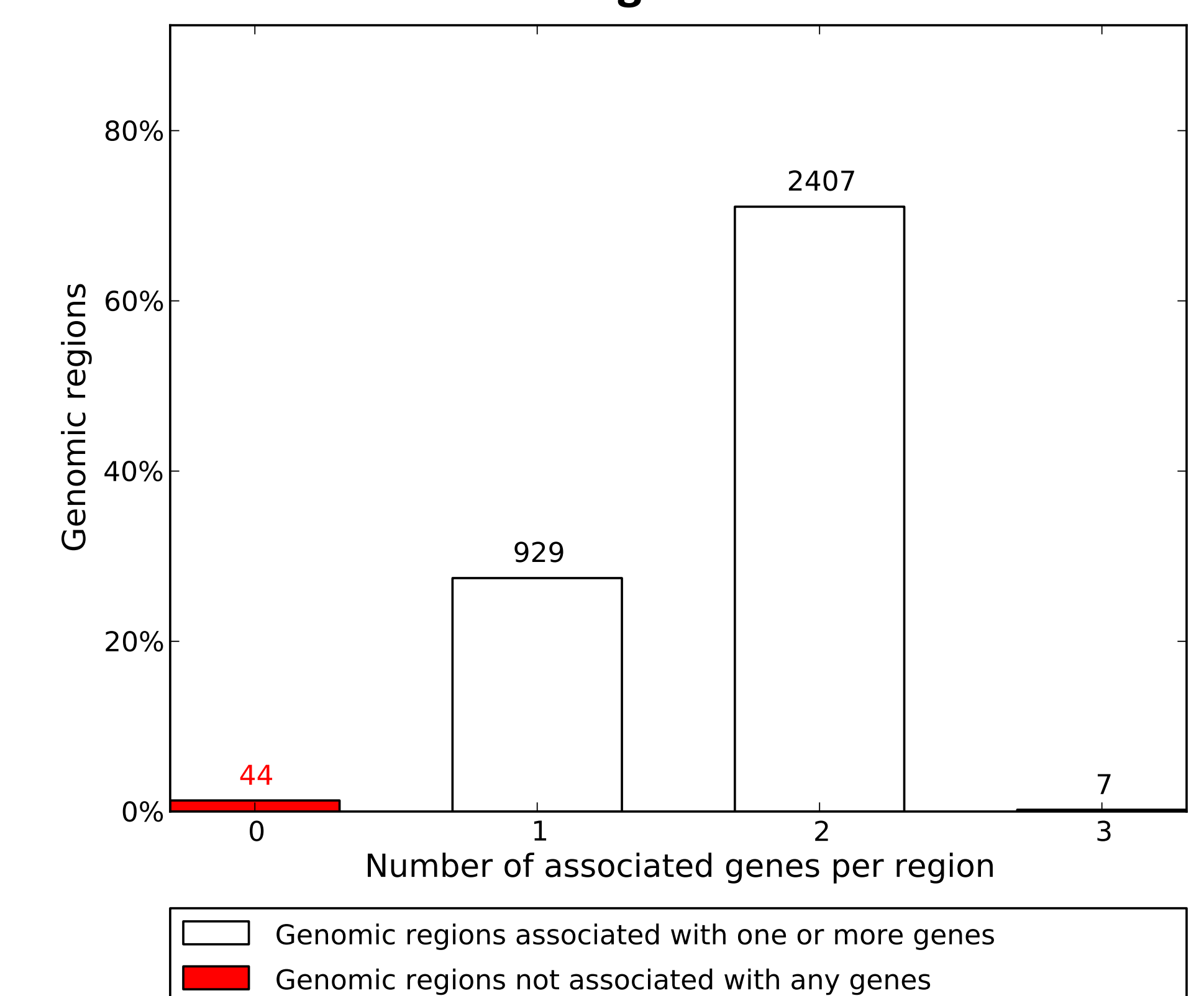


Fig. 7 Gene association graph of 3,387 differentially methylated regions between high and low COMET scores

Conclusion

- **The COMET assay seems to be a better candidate for measuring sperm cell health**
- Since COMET and TUNEL are used interchangeably to assess DNA damage, they should report similar deviations in DNA methylation, but they do not
- Although a regression of COMET and TUNEL show a positive relationship, **the COMET assay seems to have much higher association with methylation disruption**
- There are a tremendous number of COMET sites associated with germline development