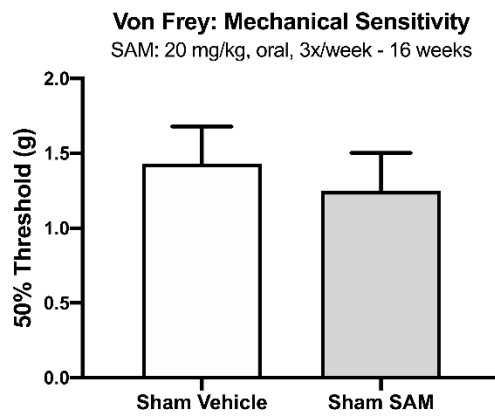
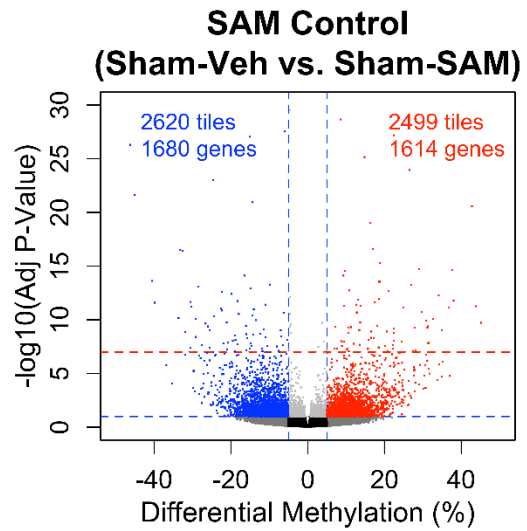


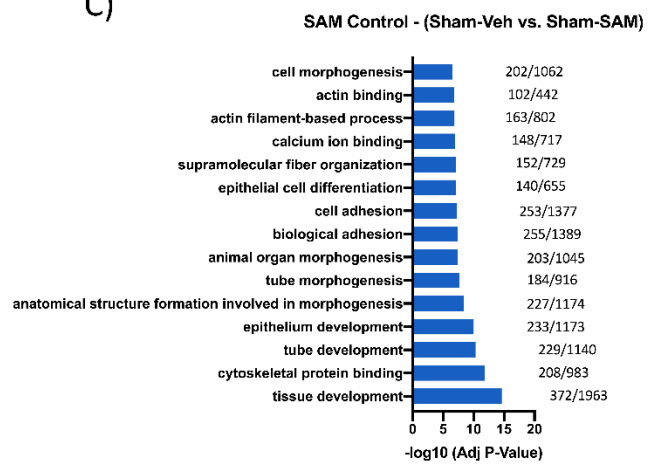
A)



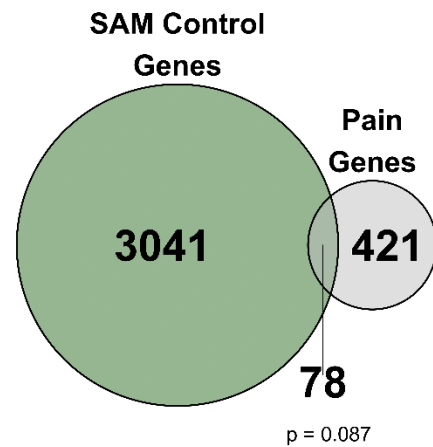
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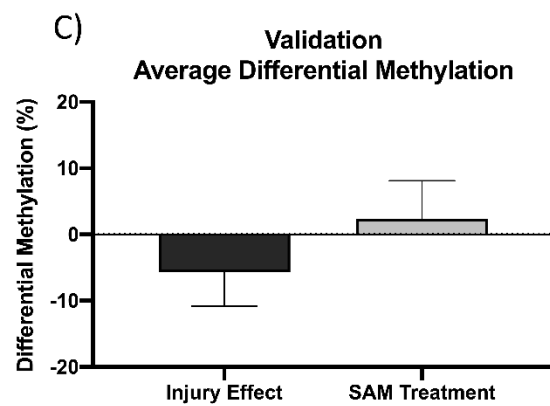
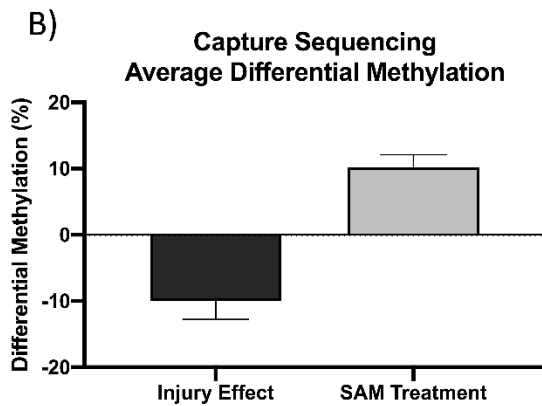
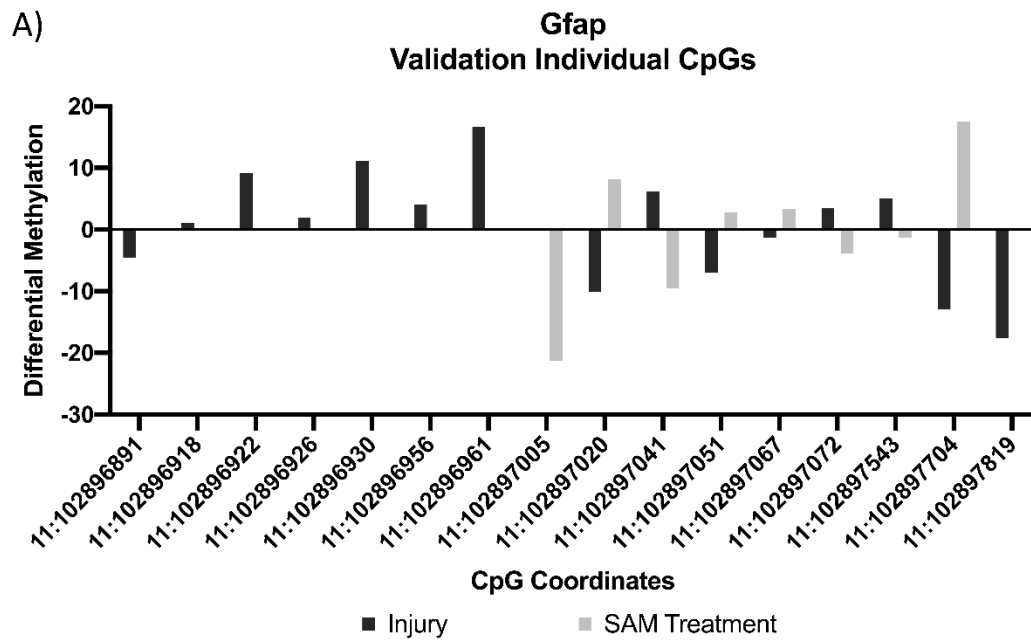
C)



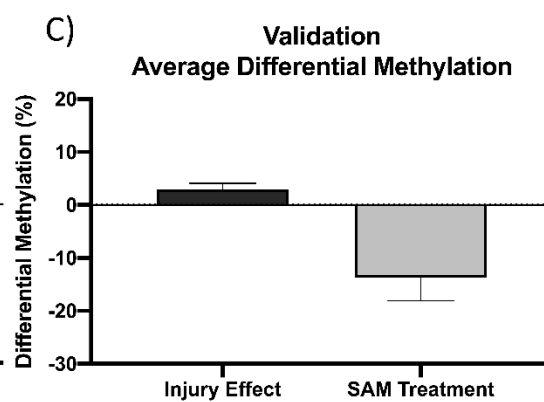
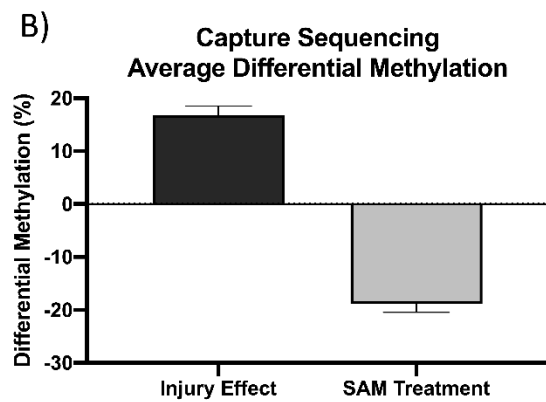
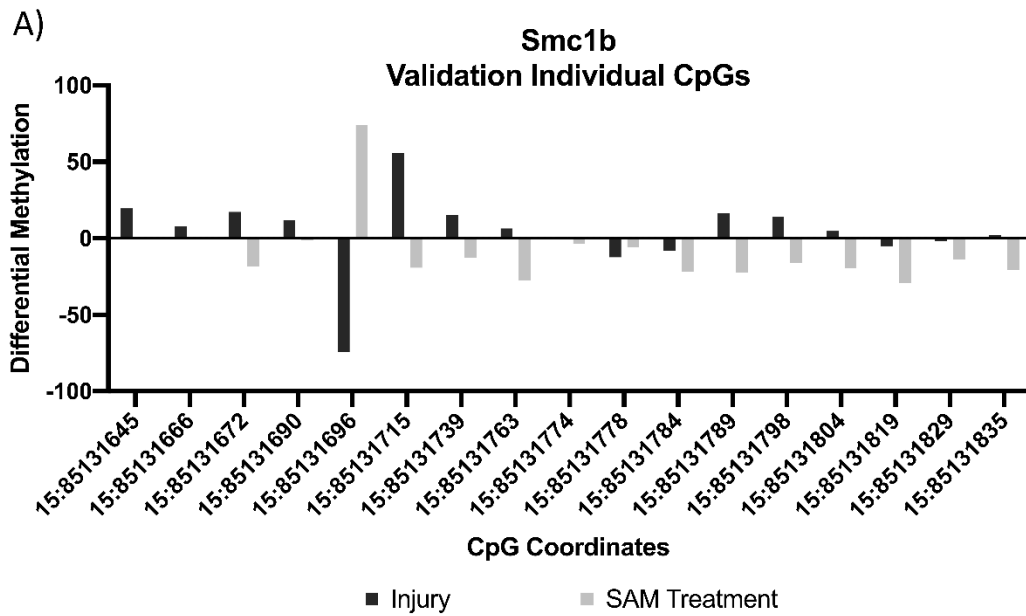
D)



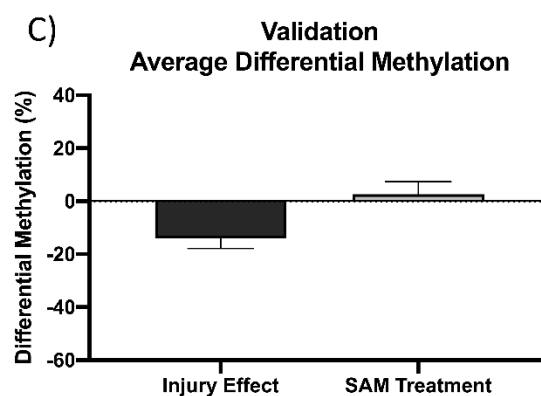
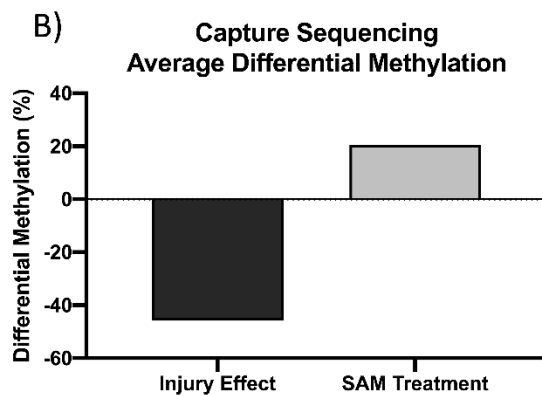
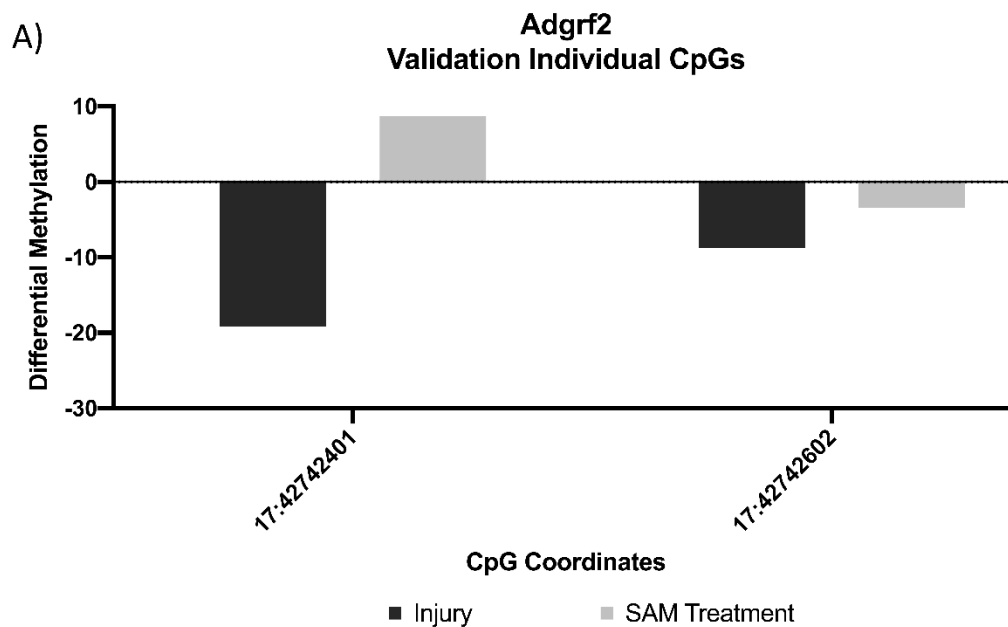
*Supplemental Figure 1: Effect of SAM in Sham control mice.* A) Repeated administration of SAM over a 4 month period does not affect mechanical hypersensitivity in Sham animals compared to vehicle-treated animals. Sham-Vehicle: n=6, Sham-SAM: n=6. Unpaired t-test. Adapted from Gregoire et al. 2016 [27] B) Volcano plot depicts the magnitude and statistical significance of promoter region tile differential methylation during SAM control comparison. Tiles are displayed in terms of positive or negative methylation with the control group as the reference point (x-axis), against  $-\log_{10}(\text{adjusted p-value})$ ; y-axis). Blue horizontal dashed line indicates an adjusted p-value threshold of 0.1, red horizontal dashed line indicates an adjusted p-value threshold of  $1 \times 10^{-7}$ . The blue vertical dashed line indicates methylation difference thresholds of 5% and -5%. Red: hypermethylated; blue: hypomethylated; dark grey: non-significant but with methylation differences of  $> 5\%$ ; light grey: significant but with methylation differences of  $< 5\%$ , black = not different. Sham-Vehicle: n=3, Sham-SAM: n=3. C) The top 15 gene ontologies enriched for differentially methylated genes by SAM in uninjured animals (3119 differentially methylated genes identifying 106 enriched ontologies). Displayed ontologies are filtered for a maximum size of 2000 genes and are from the Biological Processes or Molecular Function domains. Listed are the number of differentially methylated genes / total number of genes in each ontology. D) Displayed is the number of differentially methylated genes (green), the number of pain genes detected (grey), and the number of differentially methylated pain genes (overlap) for the Sham-SAM vs. Sham-Vehicle comparison. Hypergeometric test. SNI: spared nerve injury; SAM: s-adenosyl methionine.



*Supplemental Figure 2 – Gfap DNA Methylation validation by targeted bisulfite sequencing.* A) Differential methylation between Injury and SAM Treatment at each of the individual CpGs (genomic position on x-axis) within the tiling regions of interest identified as differentially methylated by capture sequencing. B) Average differential methylation identified by capture sequencing. Error bars represent the standard error of differential methylation when multiple tiling regions are annotated to the gene of interest. C) Average differential methylation identified by targeted bisulfite sequencing. Error bars represent standard error across animals (n=6-9).

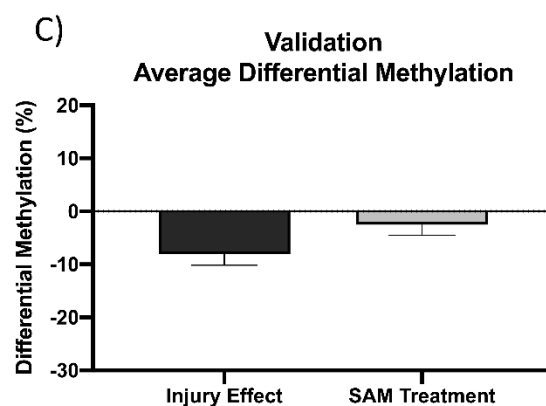
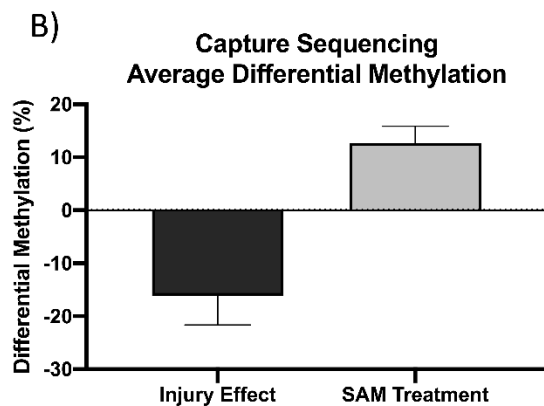
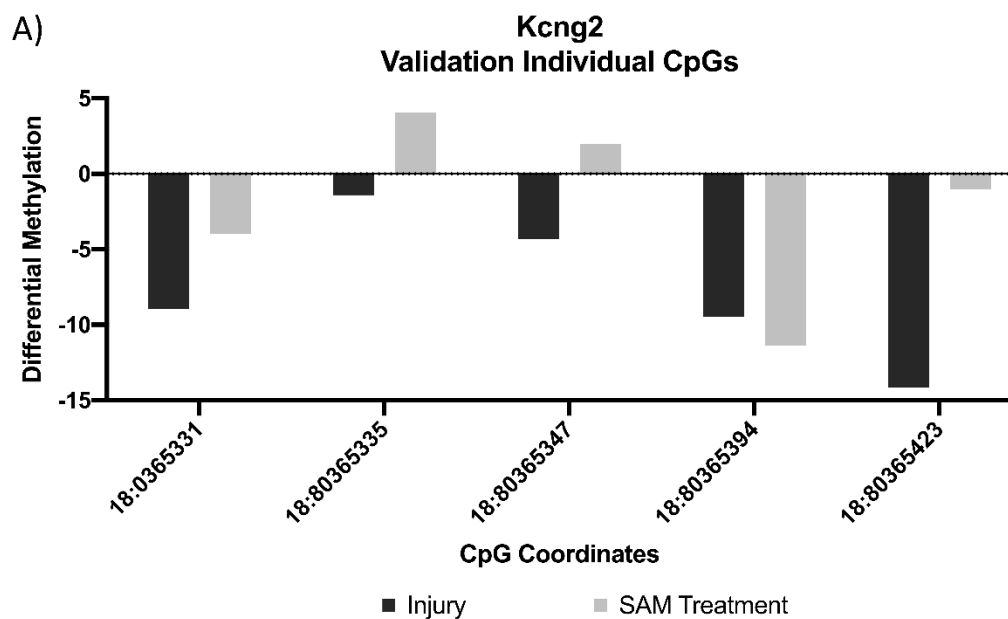


*Supplemental Figure 3 – Smc1b DNA Methylation validation by targeted bisulfite sequencing.* A) Differential methylation between Injury and SAM Treatment at each of the individual CpGs (genomic position on x-axis) within the tiling regions of interest identified as differentially methylated by capture sequencing. B) Average differential methylation identified by capture sequencing. Error bars represent the standard error of differential methylation when multiple tiling regions are annotated to the gene of interest. C) Average differential methylation identified by targeted bisulfite sequencing. Error bars represent standard error across animals (n=6-9).



*Supplemental Figure 4 – Adgrf2 DNA Methylation validation by targeted bisulfite sequencing.* A) Differential methylation between Injury and SAM Treatment at each of the individual CpGs (genomic position on x-axis) within the tiling regions of interest identified as differentially methylated by capture sequencing. B) Average differential methylation identified by capture sequencing. Error bars represent the standard error of differential methylation when multiple tiling regions are annotated to the gene of interest. C) Average differential methylation identified by targeted bisulfite sequencing. Error bars represent standard error across animals (n=6-9).





*Supplemental Figure 5 – Kcng2 DNA Methylation validation by targeted bisulfite sequencing.* A) Differential methylation between Injury and SAM Treatment at each of the individual CpGs (genomic position on x-axis) within the tiling regions of interest identified as differentially methylated by capture sequencing. B) Average differential methylation identified by capture sequencing. C) Average differential methylation identified by the validation targeted bisulfite sequencing. B) Average differential methylation identified by capture sequencing. Error bars represent the standard error of differential methylation when multiple tiling regions are annotated to the gene of interest. C) Average differential methylation identified by targeted bisulfite sequencing. Error bars represent standard error across animals (n=6-9).

### **Supplemental Table Legends**

**Supplemental Table 1 – Probe Bed File.** Genomic coordinates for the bisulfite capture-sequencing probes

**Supplemental Table 2 – Blacklist Bed File.** Genomic coordinates for regions known to have anomalous, unstructured, and high signal/read counts in next gen sequencing

**Supplemental Table 3 – Differentially methylated tiles identified in Injury.**

**Supplemental Table 4 – Differentially methylated tiles identified in SAM.**

**Supplemental Table 5 – Differentially methylated tiles that undergo a reversal after SAM Treatment of SNI animals.**

**Supplemental Table 6 – Gene ontologies enriched for differentially methylated genes identified in Injury.**

**Supplemental Table 7 – Gene ontologies enriched for differentially methylated genes identified in SAM.**

**Supplemental Table 8 – Gene ontologies enriched for differentially methylated genes that undergo a reversal after SAM Treatment of SNI animals.**

**Supplemental Table 9 – Differentially methylated pain-related genes in both Injury and SAM conditions**