## Supplemental Figure Legends

### Supplemental Figure 1

**(A)** Comparison of gene dependency scores (Mean β-scores) of 8 significant genes between pediatric and adult HGG cell lines. Student t-test was performed to determine the significance of the adjusted p-value.

**(B)** Comparison of MCL1 RNA expression (z-score) with the mean IHC scores of pediatric HGG TMA. One-way Anova test was performed to determine the significance. Multiple comparison was corrected by using Bonferroni's test. p=ns.

### Supplemental Figure 2

**(A)** Bar plot represents Log10 $IC_{50}$ of AZD5991 and S63845 in a panel of pediatric and adult HGG cell lines.

**(B)** Scatterplot showing correlation of *MCL1* gene effect (Mean β-scores) of HGG cell lines ($n^{(Adult)} = 2$, $n^{(Pediatric)} = 11$) with S63845 drug response (AUC). Pearson's correlation test was used to determine r and significance p.

**(C)** Waterfall plot showing the negative and positive correlated fatty acid metabolism gene dependencies with *MCL1* gene dependency in pHGG. Pearson's correlation test was used to determine r and significance p.

**(D)** Scatterplots showing correlation between the eight-fatty acid metabolism genes with *MCL1* gene dependency in pHGG. Pearson's correlation test was used to determine r and significance p.

**Supplemental Figure 3**

**(A)** Waterfall plot showing the negative and positive correlated features with AZD5991 response in pHGG.

**(B)** Scatterplot showing correlation between methylation status of cluster 1 CpG sites against AZD5991 response in pediatric HGG cell lines. Pearson's correlation test was used to determine r and significance p.

**Supplemental Figure 4**

**(A)** Pyrograms of control, methylated and unmethylated cg00300298. "c" bisulphite control, "T-C" CpG site of interest.

**(B-C)** Box plot comparing the distribution of cg00300298 methylation status between pediatric and adult HGG subtypes in **(B)** cell lines and **(C)** patient samples. Student t-test, $^*p<0.05$, ns = not significant.

**(D)** Scatterplot showing correlation between methylation status of cg00300298 vs Mean β-score of *MCL1* dependency in pediatric HGGs. Pearson's correlation coefficient, $p < 0.05$. Pink highlighted points indicate the methylated samples which are non-dependent on MCL1.

**(E)** Scatterplot showing correlation between ABALON RNA levels vs methylation status of cg00300298 in pediatric HGGs. Pearson's correlation coefficient, p =ns.

**(F)** Scatterplot showing correlation between BCL-xL protein levels vs methylation status of cg00300298 in pediatric HGGs. Pearson's correlation test was used to determine r and significance p, $p < 0.05$.

## Supplemental Figure 5

**(A)** Table represents Fisher's exact test p values for methylation beta-score cut-offs ranging from 0.7-0.4 in cell lines. Student t-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns=not significant.

**(B)** Boxplots illustrates the methylation patterns of the individual CpG sites (cg00300298, cg08257293, cg12873919, cg13989999, and cg18787420 across and adult and pediatric non-malignant brain tissue, derived from an external dataset (36). Student t-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns=not significant.

## Supplemental Figure 6

**(A)** Scatterplot showing the correlation between replicates of BT12 and BT16 in vivo genomic screens.

**(B)** Gene dependency scores of *MCL1* and core-essential scores of BT12 and BT16 cell lines. Red – MCL1, Grey – Core -essentials.

## Supplemental Table Legends

### Supplemental Table S1

In vitro CRISPR control metrics summary of adult and pediatric HGG cell lines, related to Figure 1

### Supplemental Table S2

Layout and diagnosis of cores in adult and pediatric tissue microarrays (TMAs), related to Figure 1

### Supplemental Table S3

Random Forest (RF) features of AZD5991 response in HGG, related to Figure 3

### Supplemental Table S4

Beta-scores (Methylation scores) of the 44 BCL2L1 CpG sites, related to Figure 3

### Supplemental Table S5

Clinical data of patient samples, related to Figure 4

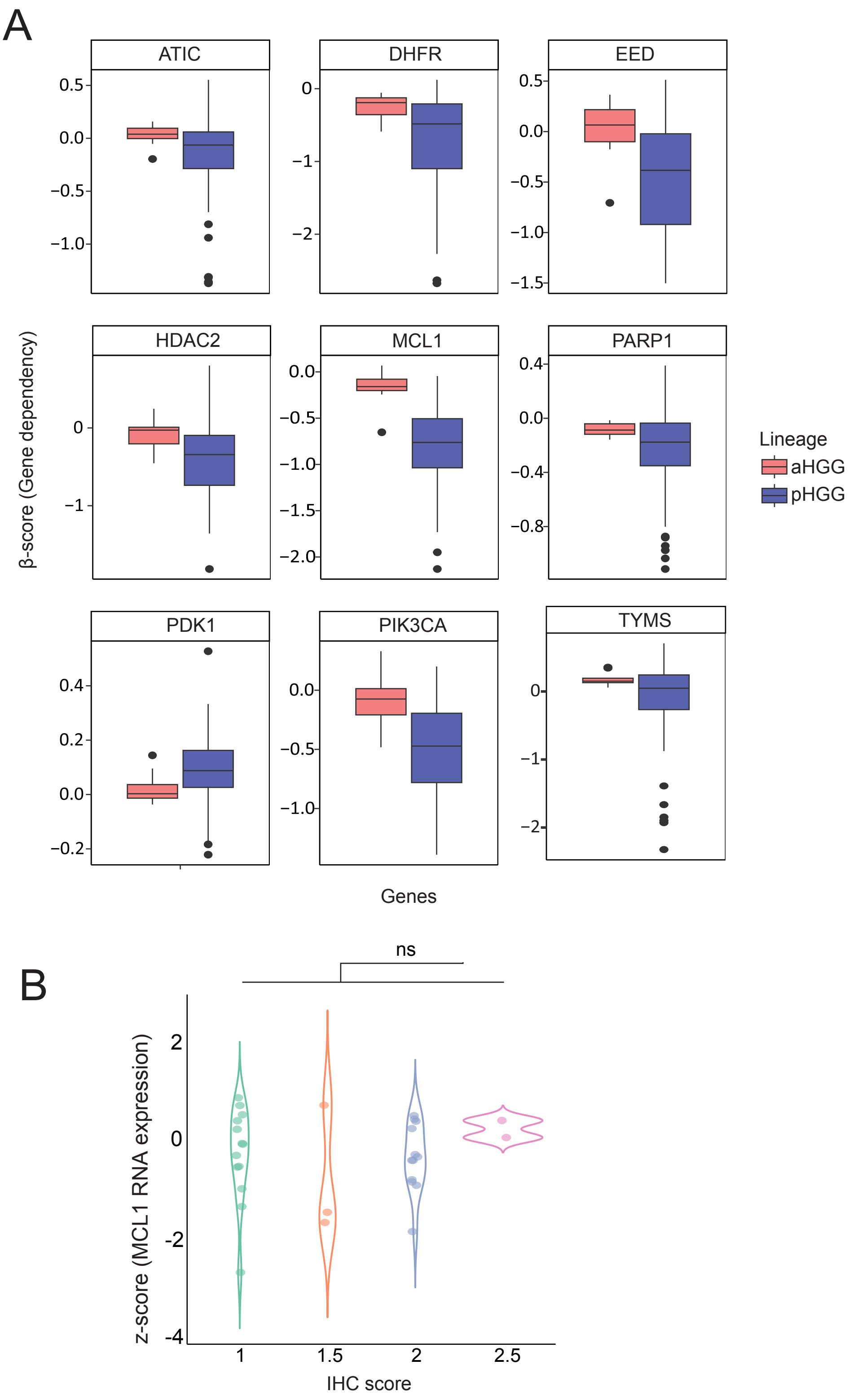
### Supplemental Table S6

Beta-scores (Methylation) of 5 overlapping cluster 1 CpG sites, related to figure 5

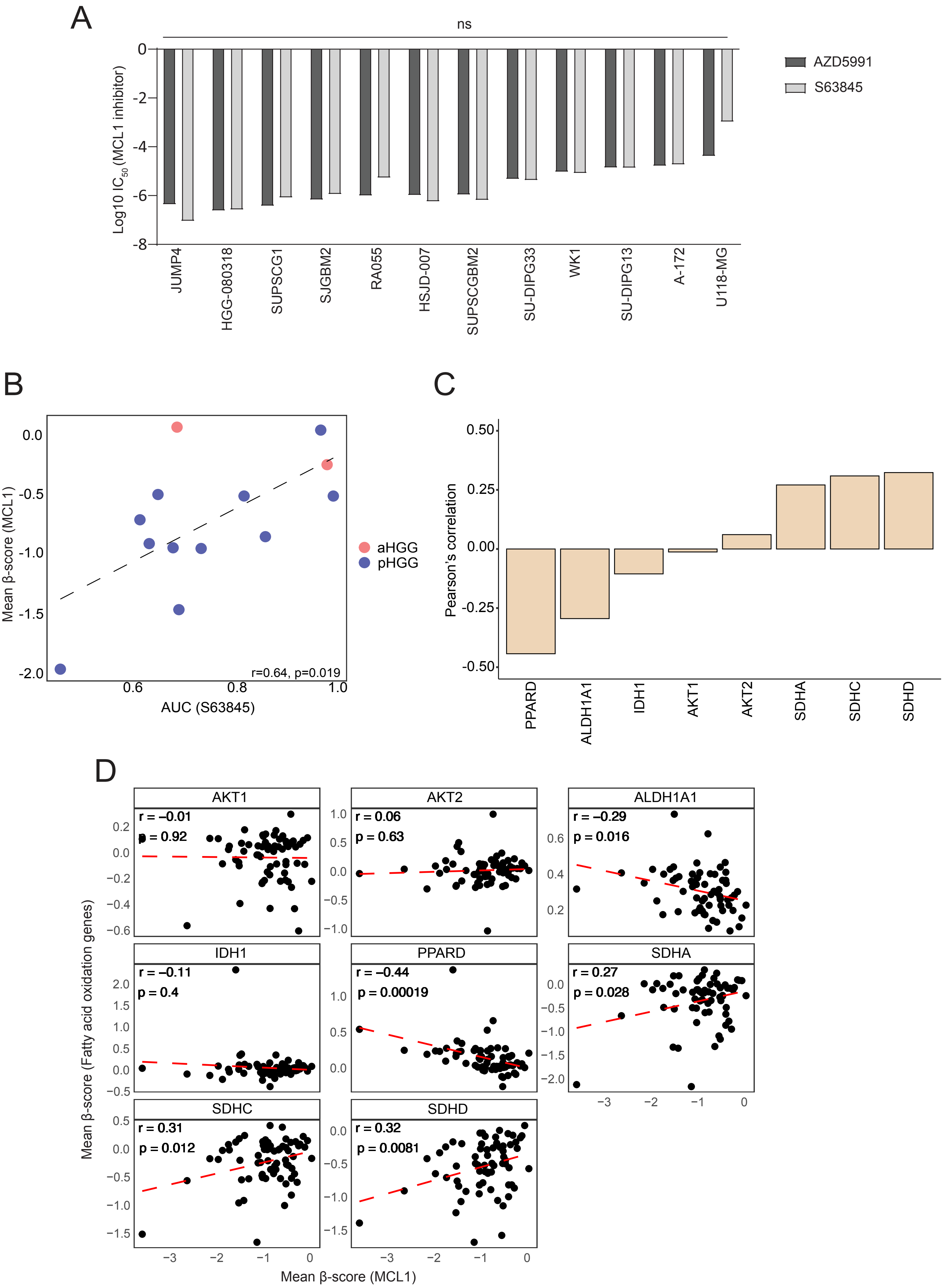
### Supplemental Table S7

In vivo CRISPR control metrics summary of BT12 and BT16 ATRT cell lines, related to Figure 6

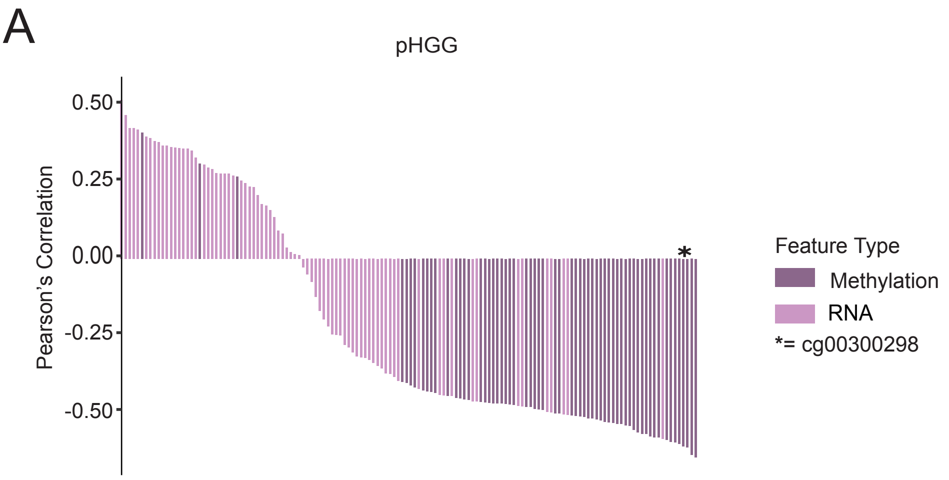
# Supplemental Figure 1

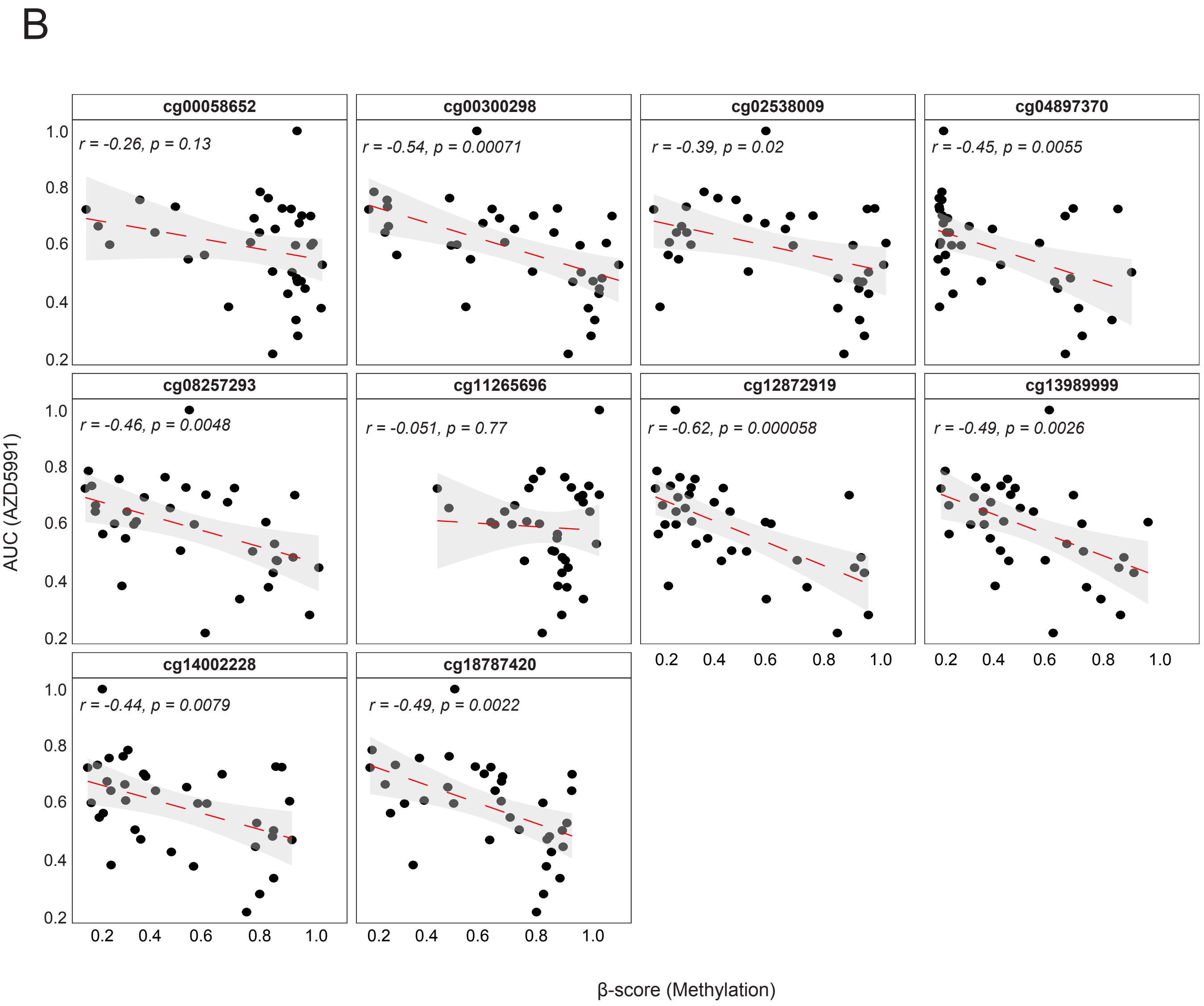


# Supplemental Figure 2

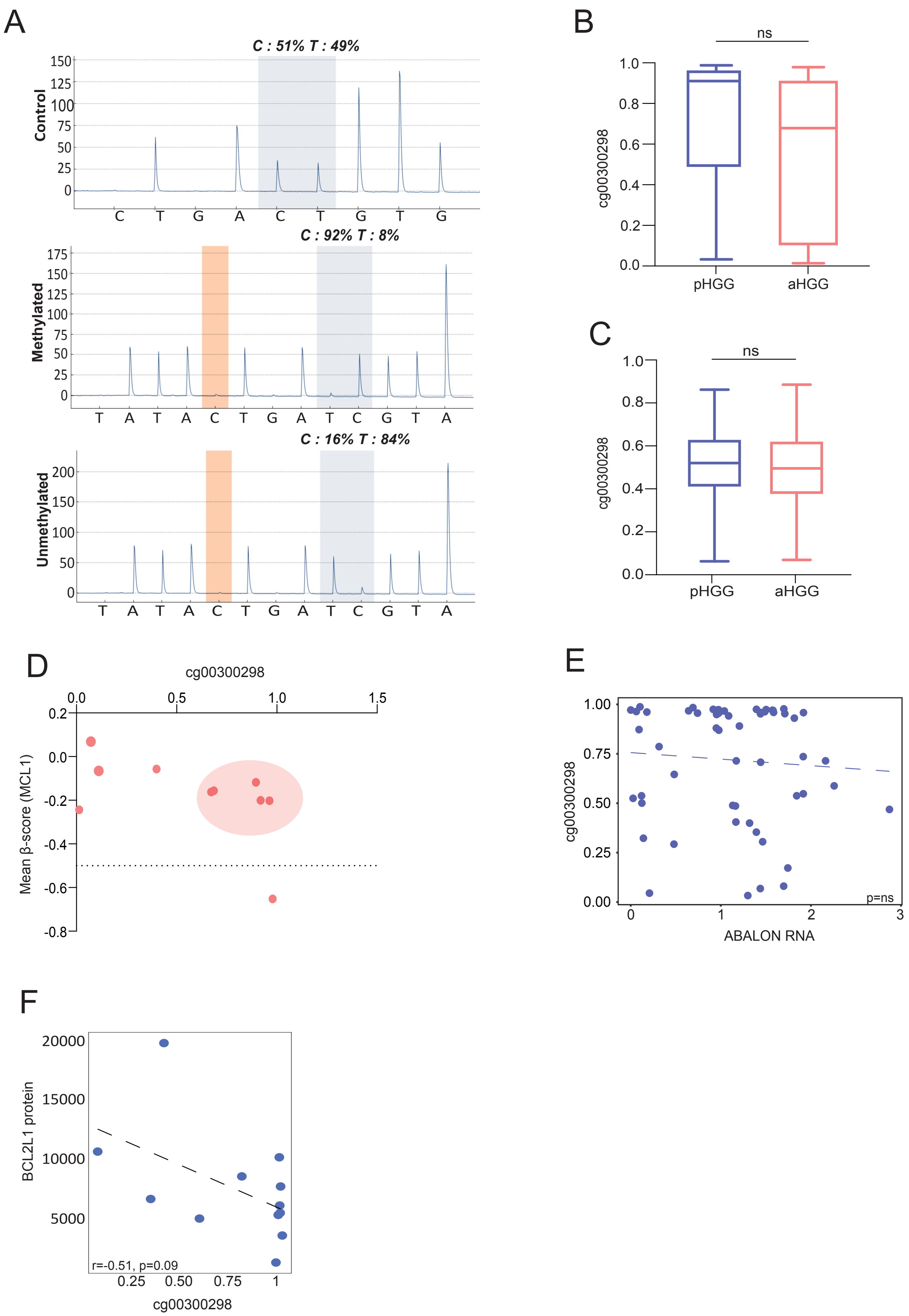


# Supplemental Figure 3





# Supplemental Figure 4

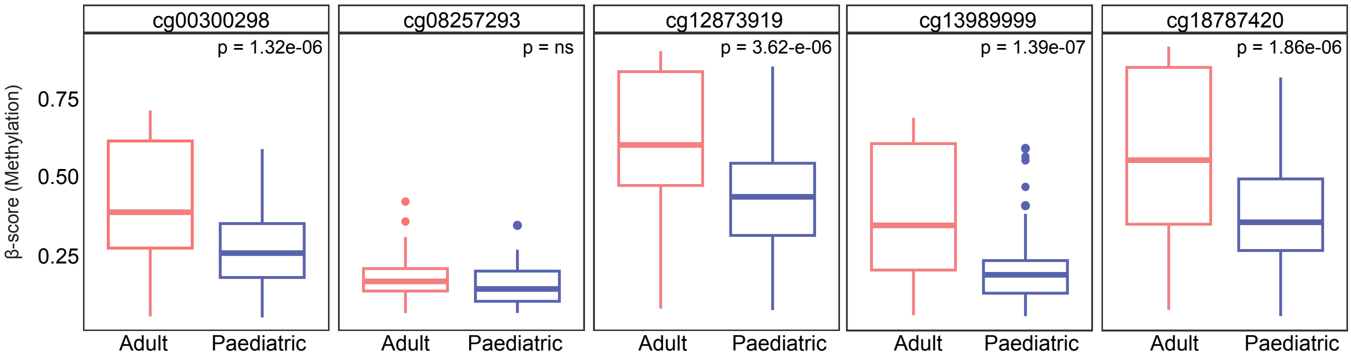


# Supplemental Figure 5

A

| Methylated Phenotype Threshhold (β-score) | Fisher’s Exact Test (p-value) |
|---|---|
| ≥0.7 | *** |
| ≥0.6 | ** |
| ≥0.5 | * |
| ≥0.4 | ns |

B



# Supplemental Figure 6