

## Histone H3-wild type diffuse midline gliomas with H3K27me3 loss are a distinct entity with exclusive *EGFR* or *ACVR1* mutation and differential methylation of homeobox genes

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**Supplementary Table 1. CpG island probe positions of differentially methylated genes between H3-WT and H3K27M DMG tumours.** List of probes altered in the differential methylation analysis between the H3-WT and H3K27M DMG tumours including chromosome positions, Gencode gene name, log fold change (FC), P-value, adjusted P-value and B-value.

**Supplementary Fig. 1 MRI imaging and histological features of a 3 year-old male with H3-WT (*zcc183*) and EZHIP staining for *zcc339* with positive control (a-b)** Gadolinium-enhanced T1-weighted and T2-weighted axial images demonstrating the bilateral thalamic infiltrative H3-WT with scanty enhancement. (c) H&E and immunohistochemistry staining for *zcc183* including H&E (600x), H3K27M (600x), H3K27me3 (600x) and EZHIP (600x), as well as EZHIP positive staining control (400x). Immunohistochemical staining demonstrates negative nuclear staining for H3K27M mutant, loss of nuclear staining for H3K27me3 in tumour cells with preserved staining in inflammatory cells, and strong nuclear expression of EZHIP in tumor cells.

**Supplementary Fig. 2 Somatic mutation profile of 5 H3-WT tumours**

Circos plots showing the somatic genome-wide mutation profile of (a-b) *ACVR1* mutated H3-WT tumours, (c-d) *EGFR* mutated H3-WT tumours and (e) H3-WT tumour. The inner area shows the structural variants, the inner ring shows the minor allele ploidy, followed by the copy number variants (green are amplifications, and red are deletions), then somatic SNVs (C>A – blue, C>G – black, C>T – red, T>A – grey, T>C – light green, T>G – light pink).

**Supplementary Fig. 3 EZHIP expression is regulated by promoter methylation**

Heatmap representation of the 5 CpG islands in *EZH1P*. (a) The ZERO glioma tumour cohort with Mondal and Castel cohorts with histology highlighted (H3K27M DMG – red; ependymoma (EPD) other – pink; PFA-EPD – purple; posterior fossa B (PFB-EPD) – dark green; RELA fusion positive EPD (RELA-EPD) – light green; H3-WT – blue; H3-WT *zcc446* – brown; HGG – yellow; LGG – light blue) the TPM value (0 – white to 20 – dark green, grey – unknown TPM) for the corresponding sample (N=138) and (b) Mondal, Castel and ZERO H3-WT (purple, light blue and blue, respectively, and brown for *zcc446* H3-WT sample without *EZH1P* expression) and H3K27M DMG (H3.1 K27M – yellow; H3.3 K27M – red) (N=82).



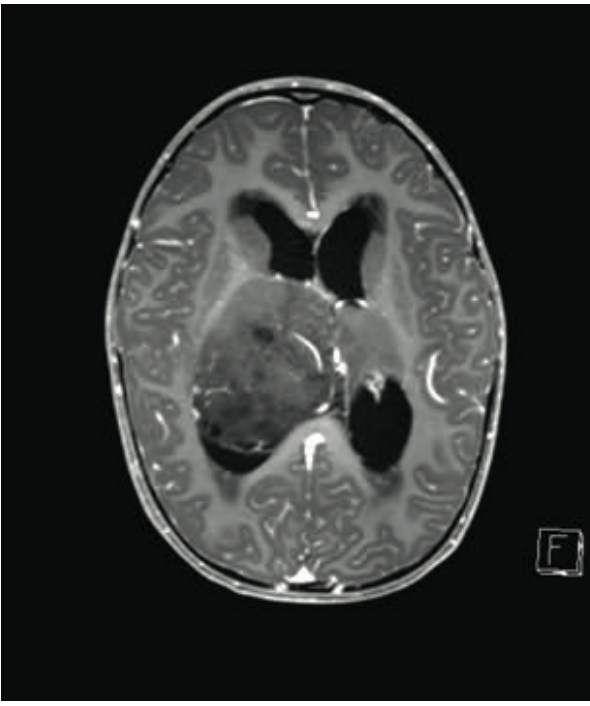




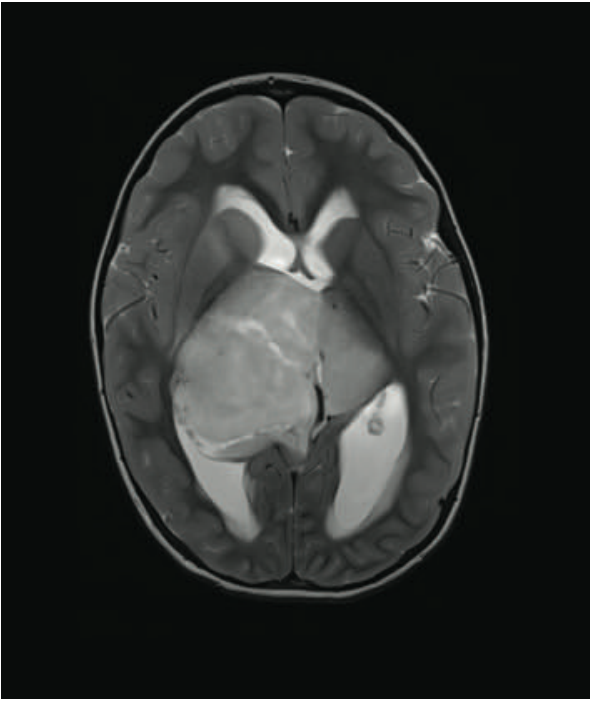


|            |       |           |                          |        |   |                                   |        |        |        |          |         |        |
|------------|-------|-----------|--------------------------|--------|---|-----------------------------------|--------|--------|--------|----------|---------|--------|
| cg23618344 | chr12 | 54423428  | chr12:54423427-54423712  | Island | HOXC4;HOXC5   | 5'UTR;5'UTR                       | 1.7558 | 3.6848 | 4.4407 | 9.66E-05 | 0.04738 | 1.3232 |
| cg12338417 | chr3  | 32860178  | chr3:32858194-32860506   | Island | TRIM71  | 1stExon                           | 4.9678 | 3.8600 | 4.4283 | 0.0001   | 0.04813 | 1.2919 |
| cg03206359 | chr5  | 172663793 | chr5:172663616-172664584 | Island | NKX2-5  | TSS1500                           | 2.8349 | 2.1806 | 4.4207 | 0.0001   | 0.04857 | 1.2725 |
| cg22132508 | chr3  | 138658878 | chr3:138656627-138659107 | Island | RP11-548O1.3  | 3'UTR                             | 2.2467 | 2.1449 | 4.4205 | 0.0001   | 0.04857 | 1.2721 |
| cg13441766 | chr5  | 134376442 | chr5:134374385-134376751 | Island | CTC-276P9.1;CTC-276P9.1;CTC-203F4.1;CTC-349C3.1;CTC-349C3.1 | TSS1500;TSS1500;5'UTR;5'UTR;5'UTR | 1.2663 | 4.4885 | 4.4202 | 0.0001   | 0.04858 | 1.2713 |
| cg07211474 | chr5  | 114515104 | chr5:114514716-114516220 | Island | TRIM36  | 5'UTR                             | 1.5131 | 0.4845 | 4.4198 | 0.0001   | 0.04859 | 1.2703 |

A



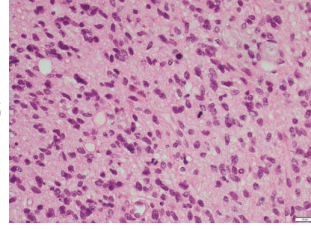
B



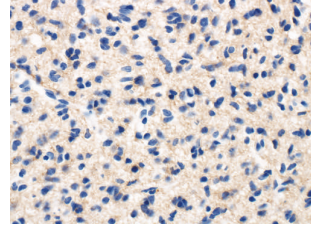
C

**zcc183**

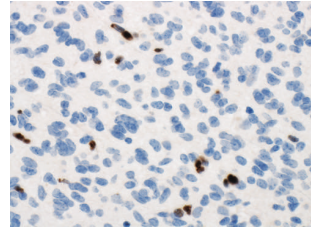
**H&E**



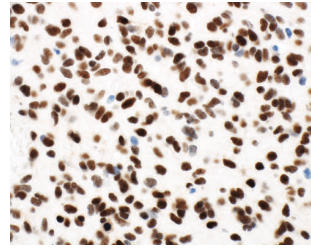
**H3K27M**



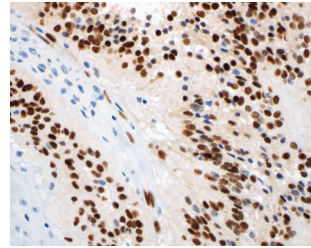
**H3K27me3**



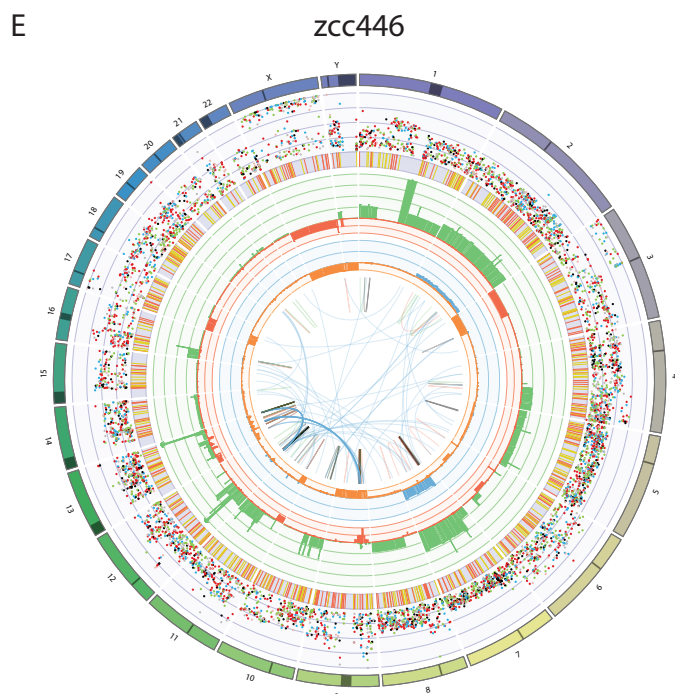
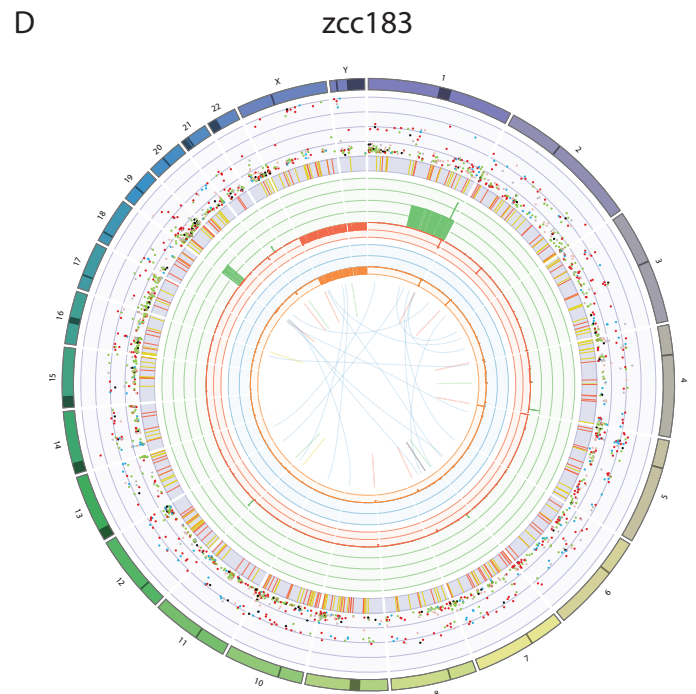
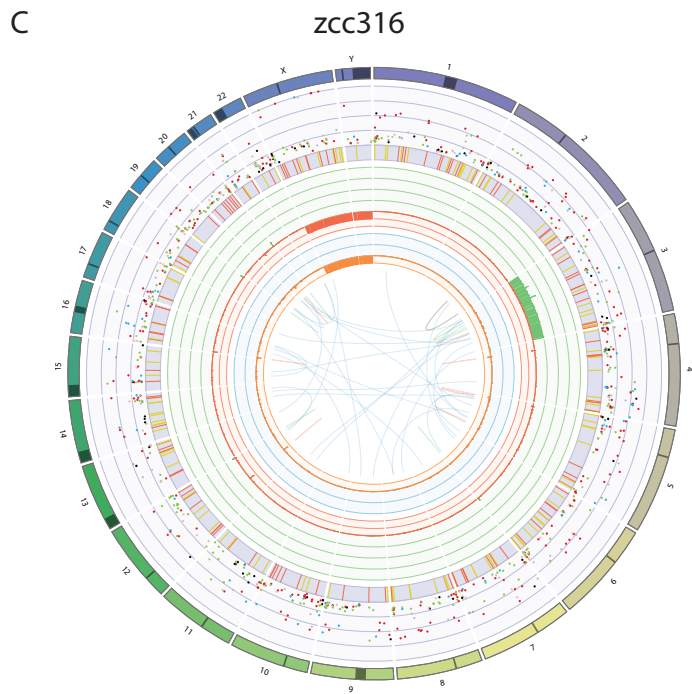
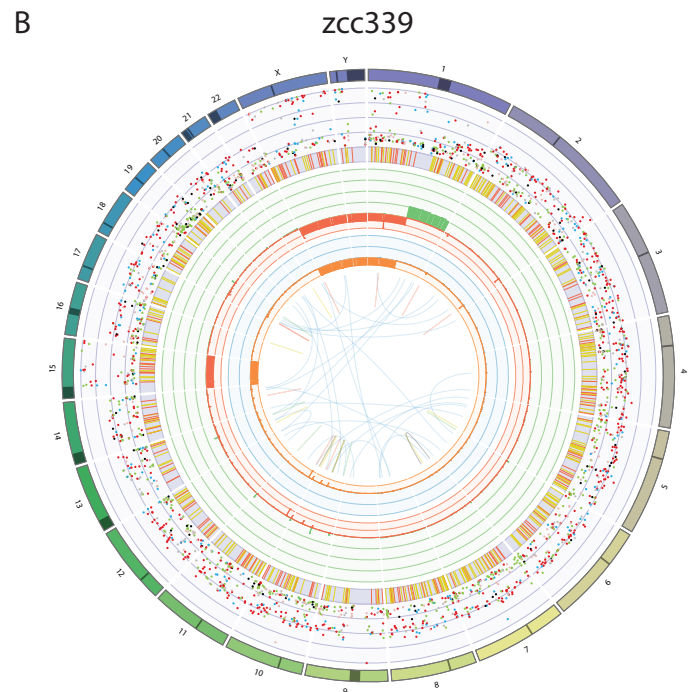
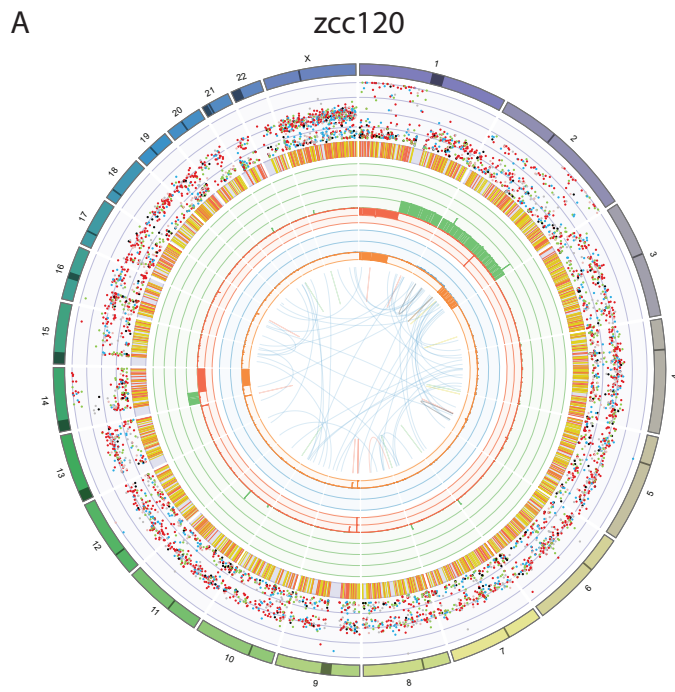
**EZHIP**



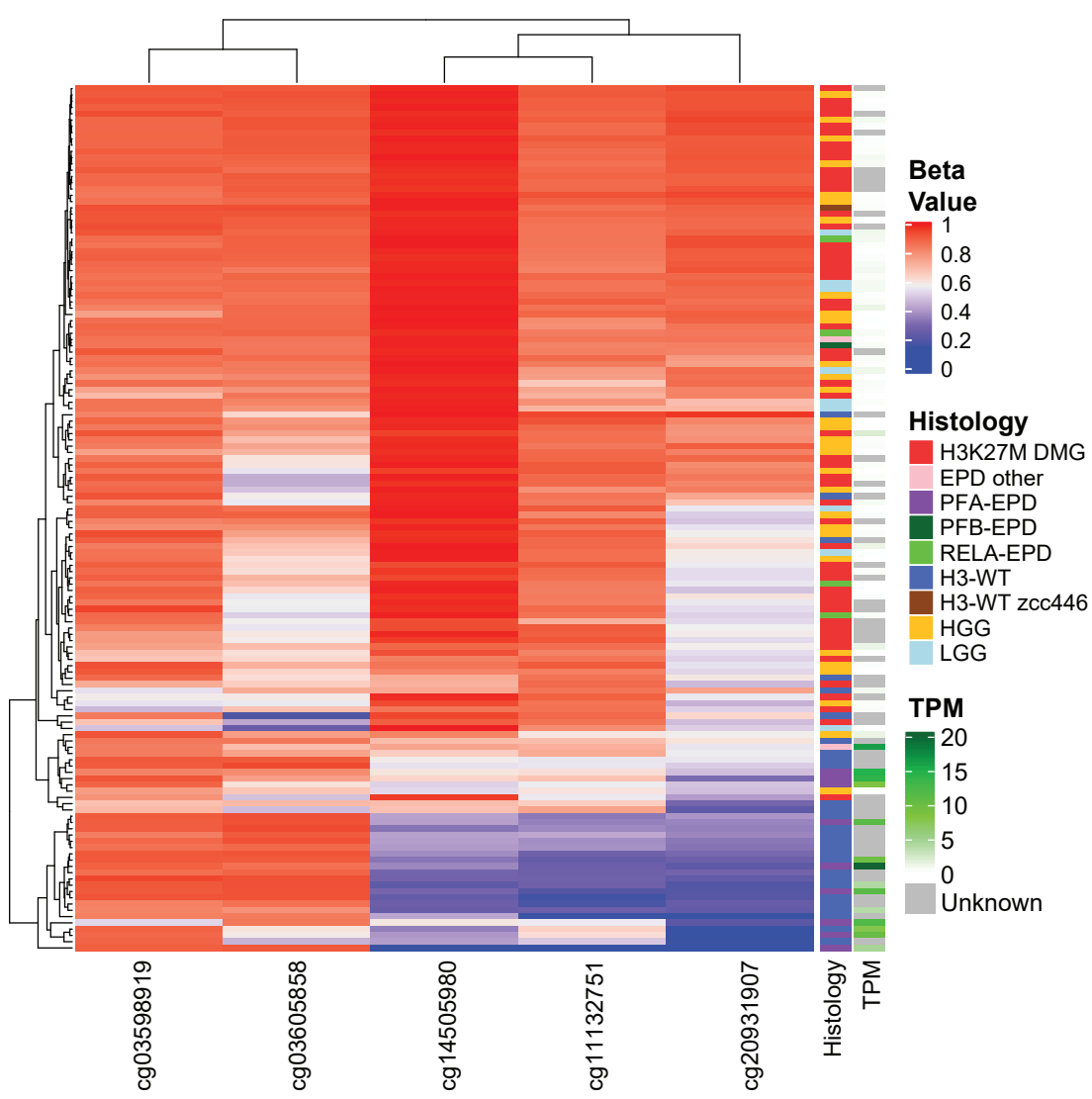
**EZHIP  
Positive  
Control**







A



B

