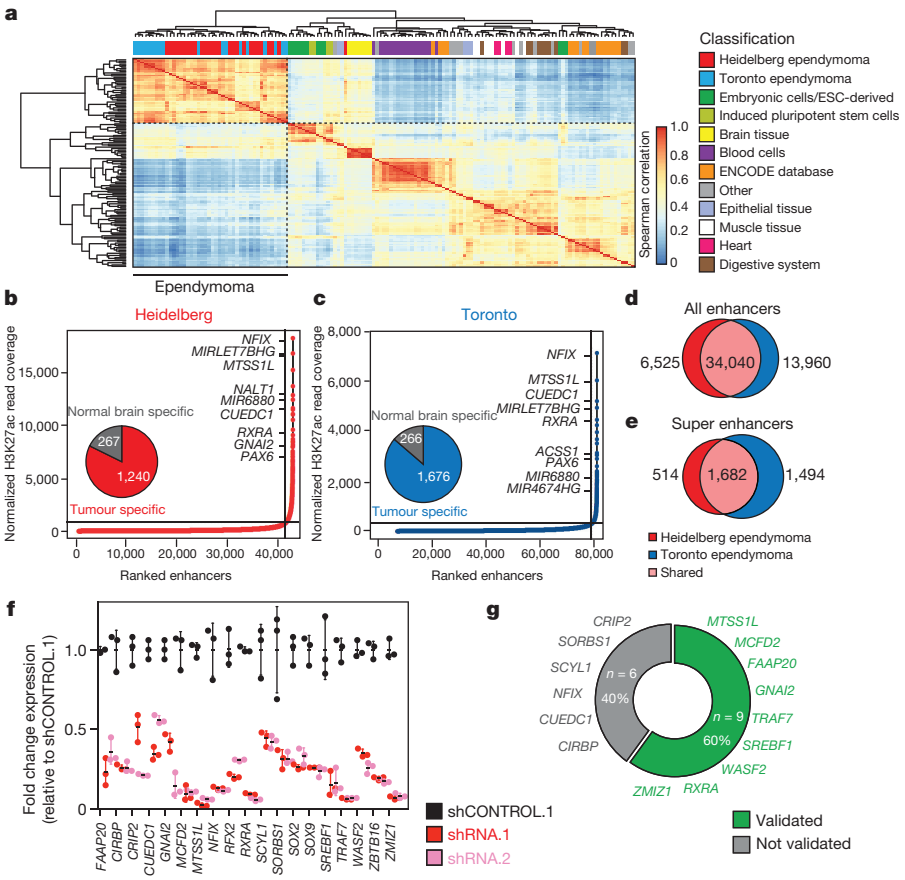


**Figure 1 | H3K27ac profiles define active regulatory elements of ependymoma. a**, Unsupervised hierarchical clustering of the top 10,000 variant enhancer loci detected in ependymomas compared to the Roadmap Epigenomics Consortium samples; $n = 143$ independent samples. **b**, **c**, Inflection plot indicating identified ependymoma super enhancers. **d**, **e**, Venn diagrams depicting the number of shared enhancers (**d**) and super enhancers (**e**) between the Heidelberg ($n = 24$) and Toronto ($n = 18$) independent ependymoma sample cohorts. **f**, Quantitative reverse transcription PCR showing knockdown efficiency of 15 ependymoma super-enhancer-associated genes ($n = 3$ technical replicates, error bars show s.d. Results were reproduced in independent biological duplicates). **g**, Percentage of top ependymoma super enhancer genes that demonstrate greater than 50% decrease in viability over seven days. Cell survival from knockdown of each gene was assayed and independently replicated as biological triplicates.

ChIP–seq), a histone mark of active chromatin, on two independent cohorts of fresh-frozen primary ependymoma specimens in two different facilities ('Heidelberg' and 'Toronto'), each with a different H3K27 acetylation-specific antibody. Our analysis focused on the intersection of shared enhancers between these two datasets, integrated with whole-exome sequencing (WES), whole-genome sequencing (WGS), RNA sequencing (RNA-seq), DNA copy-number analysis, and DNA methylation profiling (Extended Data Figs 1, 2; Supplementary Tables 1–7). 'Active' typical enhancers were defined as significant H3K27ac peaks more than 2.5 kb from the nearest transcriptional start site. To perform unsupervised hierarchical clustering, the top 10,000 variant enhancer loci from both cohorts were compared to the Roadmap Epigenomics and ENCODE databases[5] (Fig. 1a, Extended Data Figs 3, 4). Ependymoma enhancer profiles were distinct from those of other tissue types, marked by acquisition and loss of hundreds of enhancer loci (Extended Data Fig. 4). Consistent with prior literature, super enhancer domains were substantially associated with greater transcriptional load[6–9] (Extended Data Fig. 4). We identified 2,196 and 3,176 super enhancers in the Heidelberg and Toronto cohorts, respectively, and both cohorts shared a large proportion of super enhancer regions (Fig. 1b–e, Supplementary Tables 8–10, Extended Data Fig. 4). The vast majority of super enhancers were tumour-specific and enriched with cancer-associated genes reported in other solid cancers, including *PAX6*, *SKI*, *FGFRL1*, *FGFR1*, and *BOC* (Fig. 1b, c, Supplementary Table 10, Extended Data Fig. 4). Several of these genes, such as *EPHB2* and *CCND1*, have been previously validated as ependymoma oncogenes[10–12] (Extended Data Fig. 5).

To determine whether super enhancers reveal pathways and genes on which ependymoma cells depend, and which could be actionable by targeted therapy, the 15 top-ranking ependymoma super enhancer genes were validated in a series of 60 RNA interference short hairpin RNA (shRNA) knockdown time-course studies to demonstrate the feasibility of our approach to uncover novel cancer targets (Extended Data Fig. 6). Following transduction of ST-EPN-RELA patient-derived (EP1-NS) cells with shRNA constructs, the two most effective and specific shRNA constructs per gene were functionally validated (Fig. 1f). Globally, depletion of the top-ranking tumour-specific super enhancer genes impaired cell growth to varying degrees over seven days, compared to non-targeting shRNA controls (Extended Data Fig. 7). Using a stringent cut-off of shRNA-mediated growth inhibition by two independent shRNA constructs (shRNA.1 and shRNA.2) of at least 50% decrease in cell viability over seven days, a majority (60%) of ependymoma super enhancer genes were required for cellular maintenance, supporting super enhancer mapping as a viable approach for therapeutic target identification (Fig. 1g).

We next investigated whether the differences in enhancer landscapes between molecular subgroups of ependymoma reflect transcriptional differences. In both cohorts, unsupervised hierarchical clustering of all enhancers demonstrated an unbiased segregation of ependymoma molecular subgroups (Fig. 2a–d, Extended Data Fig. 5). Molecular differences between ependymoma subgroups were supported by robust segregation at the DNA methylation level (Fig. 2c). Subgroup-specific typical enhancers were enriched within large H3K27 acetylated domains (that is, super enhancers), and confirmed