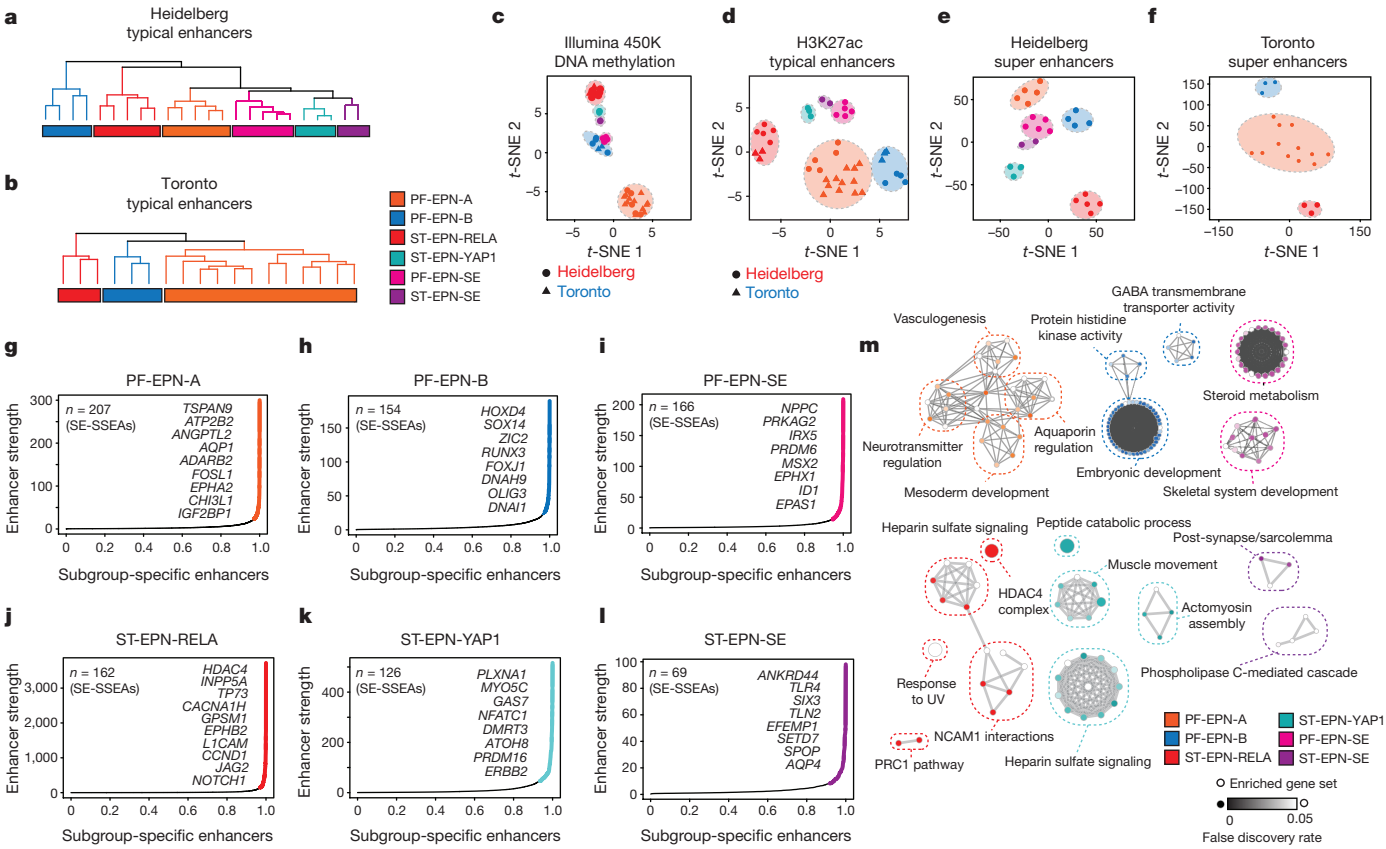

Figure 2 | Active enhancers delineate subgroups of ependymoma. **a**, **b**, Unsupervised hierarchical clustering of all H3K27ac enhancer loci in Heidelberg ($n = 24$) and Toronto ($n = 18$) independent sample cohorts. **c**, Combined *t*-distributed stochastic neighbour embedding (*t*-SNE) analysis of the top 10,000 variably methylated Illumina 450K CpG probes. **d**, Combined *t*-SNE analysis of all enhancer loci. $n = 43$ independent samples. **e**, **f**, *t*-SNE analysis of the H3K27ac marked super enhancer regions in ependymoma. $n = 42$ independent samples. **g**, **l**, Inflection plot indicating super enhancers with subgroup-specific enhancer activity (SE-SSEA) in ependymomas. $n = 24$ independent samples. **m**, G-Profiler pathway analysis of ependymoma subgroup super-enhancer-associated genes with significant enrichment indicated as the false discovery rate (FDR)-corrected *P* value. $n = 24$ independent samples.

by unsupervised segregation of ependymoma subgroups using super enhancer regions (Fig. 2e, f, Extended Data Fig. 5). We termed this distinct class of super enhancers with subgroup-specific enhancer activity SE-SSEAs, and similarly typical enhancers with subgroup-specific activity TE-SSEAs. Over 86% of SE-SSEAs observed in the Heidelberg cohort were confirmed by the Toronto cohort as active super enhancers in the respective subgroup (Extended Data Fig. 5), thus uncovering a distinct subset of super enhancers that were most common in the PF-EPN-A, PF-EPN-B and ST-EPN-RELA subgroups of ependymoma (Fig. 2g–l, Extended Data Fig. 5, Supplementary Tables 11–16). Owing to the low prevalence of ST-EPN-YAP1, ST-EPN-SE, and PF-EPN-SE tumours, these tumours were not represented in the Toronto cohort, and further downstream analysis was based on the Heidelberg cohort alone (Fig. 2g–l, Extended Data Fig. 5, Supplementary Tables 11–16). SE-SSEA genes were associated with subgroup-specific gene expression, further supporting the role of super enhancers as important contributors to transcriptional output (Extended Data Fig. 5, Supplementary Tables 11–16). SE-SSEA genes also converged on a subset of signalling pathways that distinguished the molecular subgroups of ependymoma, such as the polycomb repressive complex 1 (PRC1) and histone deacetylase (HDAC4) pathways in ST-EPN-RELA tumours, both of which can be inhibited by small molecules (Fig. 2m, Extended Data Fig. 5, Supplementary Table 17).

To translate identified SE-SSEA genes in subgroups of ependymoma into novel therapeutic leads, we first focused on ST-EPN-RELA tumours, where we observed an SE-SSEA proximal to *CACNA1H* and associated with its subgroup-restricted gene expression (Extended Data Fig. 8). CRISPR–dCas9-KRAB mediated repression of active constituent enhancers within the *CACNA1H* super enhancer resulted in downregulation of *CACNA1H* gene expression (Extended Data Fig. 8). Compared to a PF-EPN-A primary culture (S15-NS), cell proliferation of an ST-EPN-RELA patient-derived primary culture model (EP1-NS) was specifically impaired by shRNA-mediated knockdown of *CACNA1H* or pharmacologic blockade of its activity using the calcium channel inhibitor mibefradil (Extended Data Fig. 8). In a similar fashion, we found the super-enhancer-regulated gene *IGF2BP1* preferentially in a subset of PF-EPN-A tumours. shRNA-mediated targeting of *IGF2BP1* in PF-EPN-A ependymoma cultures, but not ST-EPN-RELA primary cultures, impaired cell proliferation, implicating *IGF2BP1* as a potential cancer dependency gene in PF-EPN-A ependymomas (Extended Data Fig. 8). Our findings thus identify candidate oncogenes that are associated with super enhancers as well as novel pathways specific to subgroups of ependymoma.

The regulation of cell-type-specific gene expression is often dominated by only a small number of core transcription factors out of the hundreds expressed within a given cell type[13]. As many important transcription factor motifs, such as FOSL1, FOSL2, SOX9, RFX2, and SOX2, were enriched across shared enhancers of ependymoma (Fig. 3a, Supplementary Table 18), we sought to identify the principal transcription factors of ependymoma that govern ependymoma cell identity across subgroups using core regulatory circuitry analysis[8,14] (Fig. 3b, Extended Data Fig. 9, Supplementary Table 19). A small set of highly active transcription factors was identified, including SOX9, RFX2, SOX2, ZBTB16, HES1, NFIA, and NFIB, which were highly expressed in ependymoma compared to a large collection of normal brain tissues (Fig. 3b, Extended Data Fig. 9). By contrast, transcription