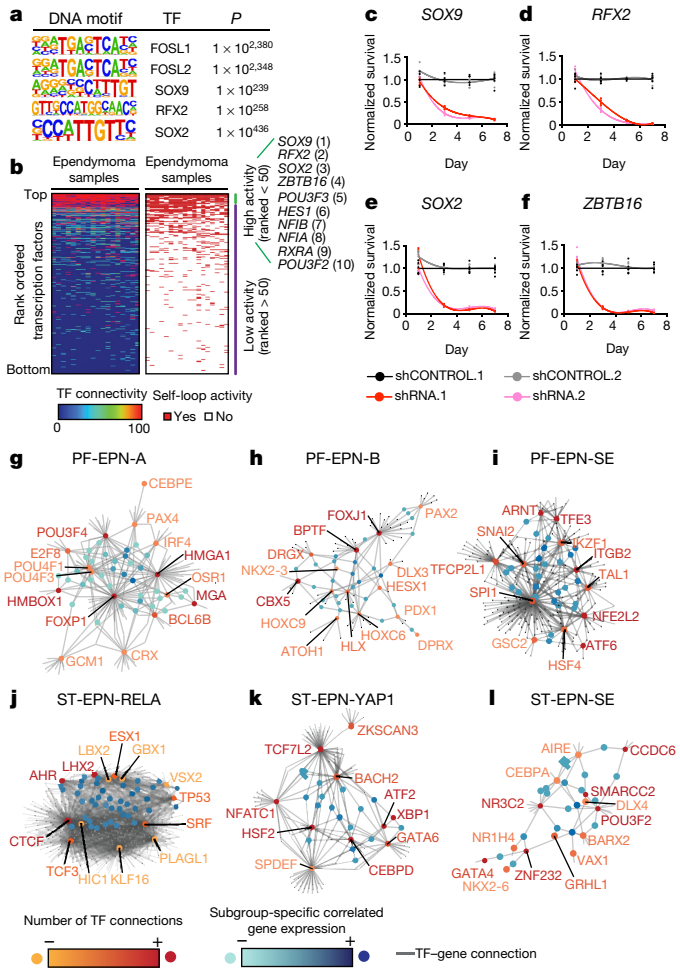

Figure 3 | Transcription factor circuitries of ependymoma. a, DNA motifs enriched within shared ependymoma-typical enhancers that overlay with ATAC-seq peaks derived from the EP1-NS cell culture model as determined by HOMER motif analysis (see Methods and Supplementary Table 18). TF, transcription factor. **b**, Heatmap of transcription factors ranked by predicted activity using core circuitry analysis (left) and presence or absence of self-loop activity (right). $n = 18$ independent samples from Toronto cohort. **c**–**f**, shRNA constructs targeting super-enhancer-associated genes ordered by normalized cell survival. Highlighted in red are shRNAs targeting super-enhancer-associated core transcription factors. Each gene assayed with six technical replicates and replicated in three independent biological experiments. **g**–**l**, Connections between subgroup-specific transcription factors integrated with gene expression in subgroups of ependymoma. $n = 24$ independent samples.

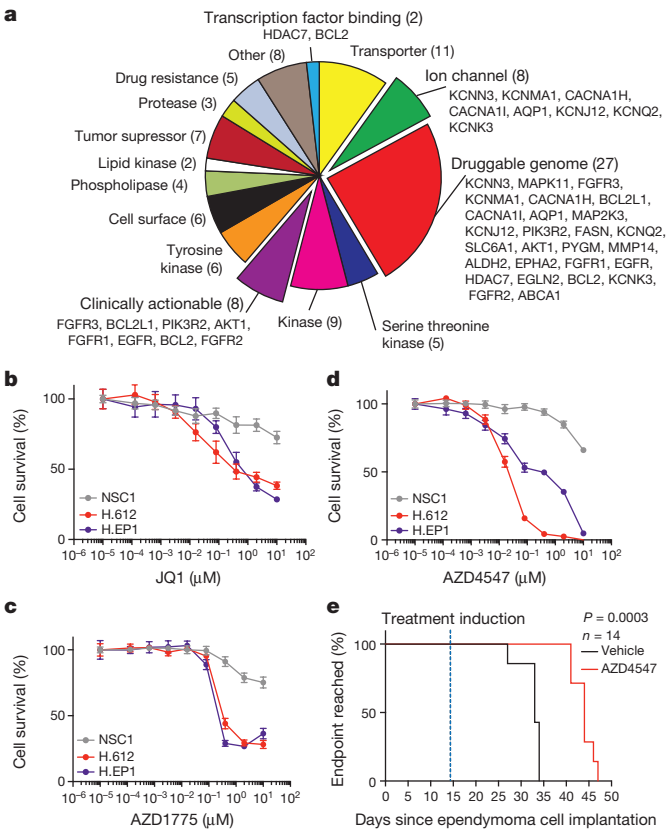

Figure 4 | Active regulatory maps identify candidate drugs against ependymoma. a, Pie chart of candidate drug compounds detected by integrating shared super enhancers with the Washington University Drug Gene Interaction Database. **b**–**d**, Ependymoma cells and neural stem cell line 1 (NSC1) controls treated with JQ1 (**b**), AZD1775 (**c**) or AZD4547 (**d**) for 72 h and assessed using an Alamar Blue stain. Error bars show s.d. Experiment performed as six technical replicates and replicated in biological triplicates. **e**, Kaplan–Meier curve for immunodeficient mice bearing H.612 ependymomas, treated with vehicle or AZD4547 (25 mg kg^{-1} d^{-1}). Significance of endpoint difference was assessed using a log-rank test. Median survival ratio of treatment (AZD4547):control (vehicle) is 44 days:33 days, and reported as a ratio of 1.333 with a 95% confidence interval of 0.4677–3.801.

factors that exhibited lower relative activity showed no significant difference in gene expression compared to normal brain (Extended Data Fig. 9). RNA interference (RNAi) was used to functionally demonstrate that the ependymoma core transcription factors *SOX9*, *RFX2*, *SOX2* and *ZBTB16* were essential for ependymoma cell maintenance (Fig. 3c–f, Extended Data Fig. 7). We hypothesized that this core model would be further specified by additional transcription factors that delineate the transcriptional differences between molecular subgroups of ependymoma. An integrative analysis was performed to assess subgroup-specific enhancers, the expression of their target genes within local topological associated domains[15], and the enrichment of subgroup-specific transcription factor-binding motifs at these subgroup-specific enhancer loci. Using this approach, we modelled regulatory circuitry maps of each molecular subgroup of ependymoma, as defined by distinct sets of transcription factors, which might be used to establish and/or maintain ependymoma subgroup identity (Fig. 3g–l, Supplementary Table 20).

We leveraged subgroup-specific super-enhancer-regulated transcription factors to provide further insight into the lineage programs of ependymoma (Extended Data Fig. 10). The rationale for these experiments stemmed from our observation that in zebrafish embryos, several subgroup-specific super enhancers were active in specific regions within the developing central nervous system (Extended Data Fig. 9). We identified a FOXJ1 transcription factor network that was enriched in PF-EPN-B ependymoma (Extended Data Fig. 10). FOXJ1 is expressed during mouse embryonic development at E13.5 (during the expansion of radial glial cells (RGCs), which are candidate cells-of-origin of ependymoma) and its expression is restricted in the regions surrounding the choroid plexus in the mouse forebrain and hindbrain (Extended Data Fig. 10). Compared to other brain tumour types, *FOXJ1* expression was increased in ependymomas, with the highest levels in PF-EPN-B tumours[16] (Extended Data Fig. 10). Furthermore, the ependymal differentiation program in RGC-derived *FOXJ1*-expressing cells versus *FOXJ1*-knockout cells was significantly and specifically enriched in PF-EPN-B ependymomas (Extended Data Fig. 10). From these data, we hypothesized that the transcriptional program of PF-EPN-B tumours closely resembles a more differentiated cell type along the ependymal lineage compared to ependymomas previously shown to match more primitive RGC precursor populations[11].