Extended Data Figure 8 | Validation of ependymoma subgroup-specific super enhancer genes. a, H3K27ac profiles at the ependymoma-specific super enhancer locus *IGF2BP1* in the Heidelberg cohort ($n = 24$ independent samples) with at least three biological replicates per subgroup, with the exception of ST-EPN-SE, which is shown as a biological duplicate. **b**, *IGF2BP1* gene expression derived from RNA-seq data for the Heidelberg cohort ($n = 24$ independent samples) with a horizontal bar for each subgroup indicating the mean. **c**, **d**, Normalized survival of PF-EPN-A (S15) primary cultures (**c**) and EP1-NS cell cultures (**d**) following shRNA knockdown of *IGF2BP1* with two independent non-overlapping shRNA constructs compared to shCONTROL.1. Experiments performed as six technical replicates and independently validated in three biological replicates. Horizontal bars indicates mean values. **e**, H3K27ac profiles at the ependymoma-specific super enhancer locus *CACNA1H* in the Heidelberg cohort with at least three biological replicates per subgroup, with the exception of ST-EPN-SE, which is shown as a biological duplicate. **f**, H3K27ac profiles surrounding the *CACNA1H* locus in a ST-EPN-RELA model (EP1-NS), a PF-EPN-A model (S15) and a normal neural stem cell control performed in biological duplicates. **g**, *CACNA1H* gene expression derived from RNA-seq data for the Heidelberg cohort ($n = 24$ independent samples) with a horizontal bar for each subgroup indicating indicating the mean. **h**, **i**, Normalized survival of PF-EPN-A (S15) primary cultures (**h**) and EP1-NS (**i**) cell cultures following shRNA knockdown of *CACNA1H* with two shRNA constructs compared to shCONTROL.1. Experiments performed as four technical replicates and independently validated in three biological replicates. Horizontal bars indicate mean values. **j**, Normalized cell survival of EP1-NS, S15, and NSC194 cells treated with increasing concentrations of mibefradil. Shown are technical triplicates, results replicated in biological triplicates. **k**, Overlay of ATAC-seq and H3K27ac-seq data centred upon ATAC-seq peak regions identified in the ST-EPN-RELA cell culture EP1-NS. **l**, CRISPR–dCAS9 targeting of *CACNA1H* active enhancers impairs *CACNA1H* expression. H3K27ac-seq (top) and ATAC-seq (bottom) surrounding the *CACNA1H* locus, indicating regions targeted by CRISPR–dCAS9 sgRNA complexes. Region 1 (R1) indicates a negative control region devoid of H3K27ac (green), while regions 2–4 (R2–R4) indicate experimental regions under evaluation. Experiments replicated in biological duplicates. **m**, Gene expression for various sgRNA constructs relative to a 'dummy' targeting control (D103), negative control (green), and uninfected control. All group comparisons were made using a two-sided Wilcoxon rank-sum test; error bars show s.d. and horizontal bars indicate mean value. Experiments were replicated in biological triplicates.

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