Supplementary Information S2
Molecular Analysis

DNA was extracted from paraffin embedded samples using the AllPrep FFPE DNA/RNA extraction kit (Qiagen) and from frozen samples using the QIAamp DNA mini kit (Qiagen). RNA from frozen samples was extracted using mirVana (ThermoFisher).

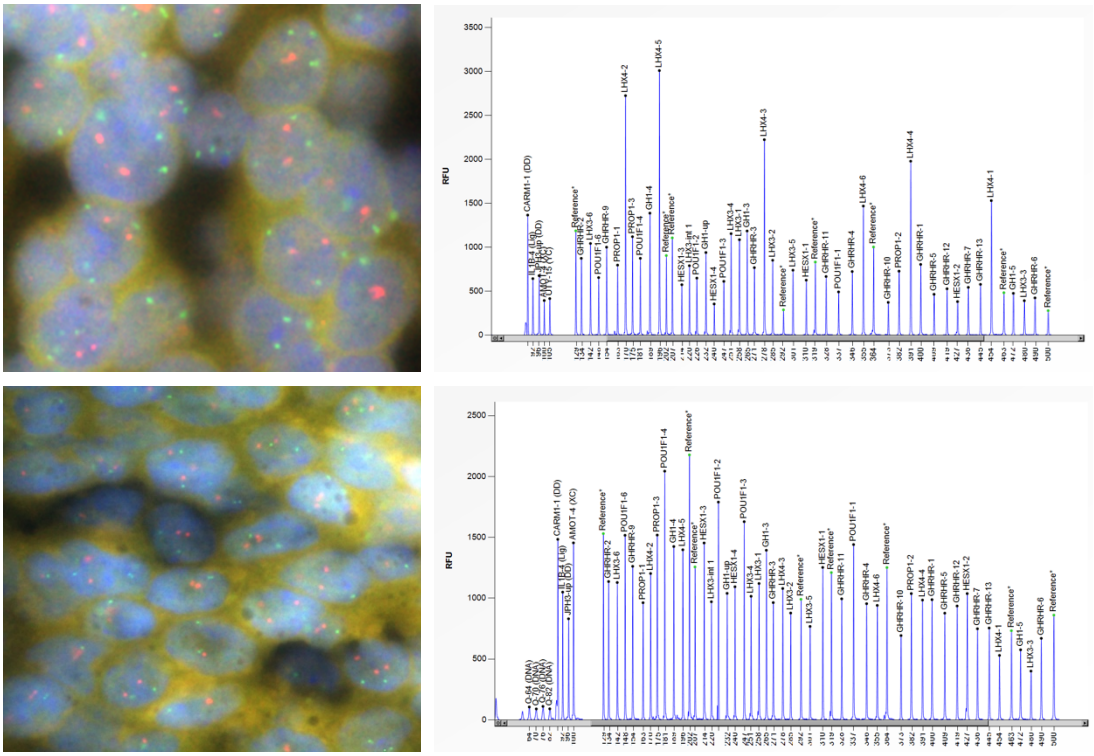
DNA methylation profiles were generated using Infinium HumanMethylation450 BeadChip arrays (Illumina) and deposited at GSE182707. Subgroups were assigned using Heidelberg classifiers v11b4 and v12.3 as previously described[1]. Calibrated scores >0.8 supported diagnosis, although 31/34 cases had scores >0.9.

1q status was evaluated by DNA methylation profiling, fluorescent in-situ hybridisation (FISH) and multiplex ligation-dependent probe amplification (MLPA) as previously described[1]. There was good correlation between methods. The Fleiss-Kappa statistic was 0.615 ($p<0.001$) and 0.708 ($p=0.008$) where two and three tests were performed respectively.

Quantitative real-time PCR determined *hTERT* expression as previously described[25].

Immunohistochemistry was performed in triplicate on four micrometre tissue microarrays for ReLA, TNC, H3K27Me3 and pAKT. Antigen retrieval was with sodium citrate buffer (pH6). Protein blocking was with normal goat or horse serum (Vector Labs) before five minute peroxidase blocking (DAKO). H3K27me3 (Tri-Methyl-Histone H3, Rabbit mAb, 1:500; Cell Signalling Technology), pAKT (Phospho-Akt, ser473, Rabbit mAb, 1:50; Cell Signalling Technology), and TNC (Tenascin-C, E-9, Mouse mAb, 1:50; Santa Cruz Biotec) primary antibodies were incubated for one hour at room temperature. Positive controls were human tonsil (H3K27me3, RelA), breast carcinoma (pAKT), and epidermoid carcinoma (TNC). Target antigens were detected using the DAKO Envision Detection Kit. For TNC, biotinylated universal antibody anti-rabbit/mouse (Vector, UK) diluted in horse serum was applied, followed with Vectastain avidin/biotin complex reagent (Vectastain-Elite ABC kit, Vector,

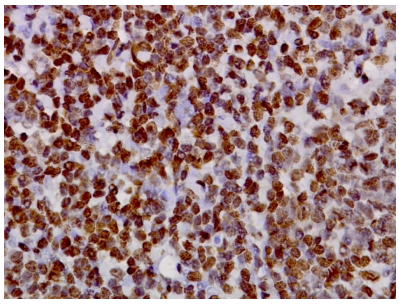
UK). For all target antigens, diaminobenzidine chromogen was applied for five minutes. H3K27me3, RelA, pAKT and TNC were scored as either negative or positive.



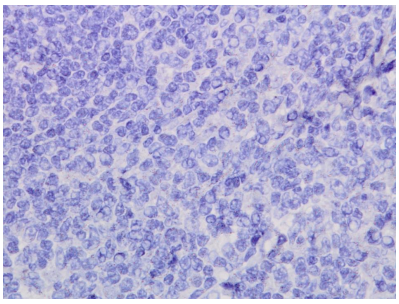
Example of 1q25 gain and no gain using both FISH and MLPA technique. Using Vysis FISH probes, spectrum green fluorescence donates 1q25 status. Using the SALSA MLPA probemix the LHX4 gene (6 exons) is located on chromosome 1q25.2, and alterations in electropherogram peaks represents changes in copy number. Coffalyser software (MRC Holland) was used for all MLPA analysis of raw data.

H3.3K27me3

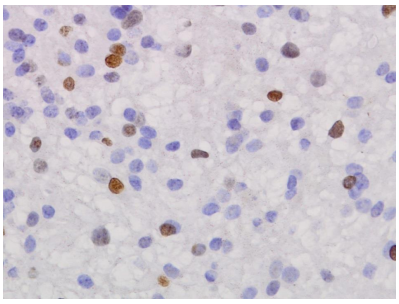
Tonsil control - POS



Tonsil control - NEG



Ependymoma - POS

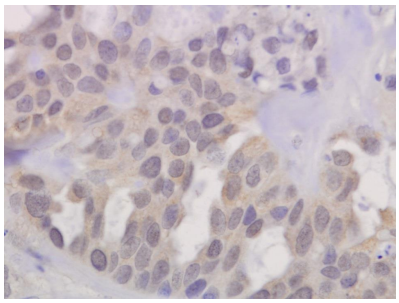


H3.3K27m3	CST #9733	Rabbit	Steamer, Sodium citrate buffer, pH 6	1:500	1hr @ RT	Tonsil

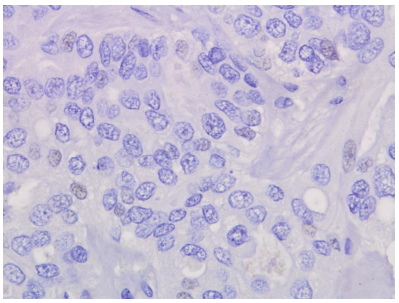
All x40 magnification

PI3K (PAKT)

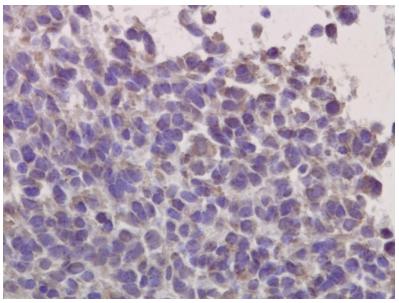
BC control - POS



BC control - NEG



Ependymoma - POS



PI3K (PAKT)	CST #3787	Rabbit	Steamer, Sodium citrate buffer, pH 6	1:50	1hr @ RT	Breast carcinoma

All x40 magnification