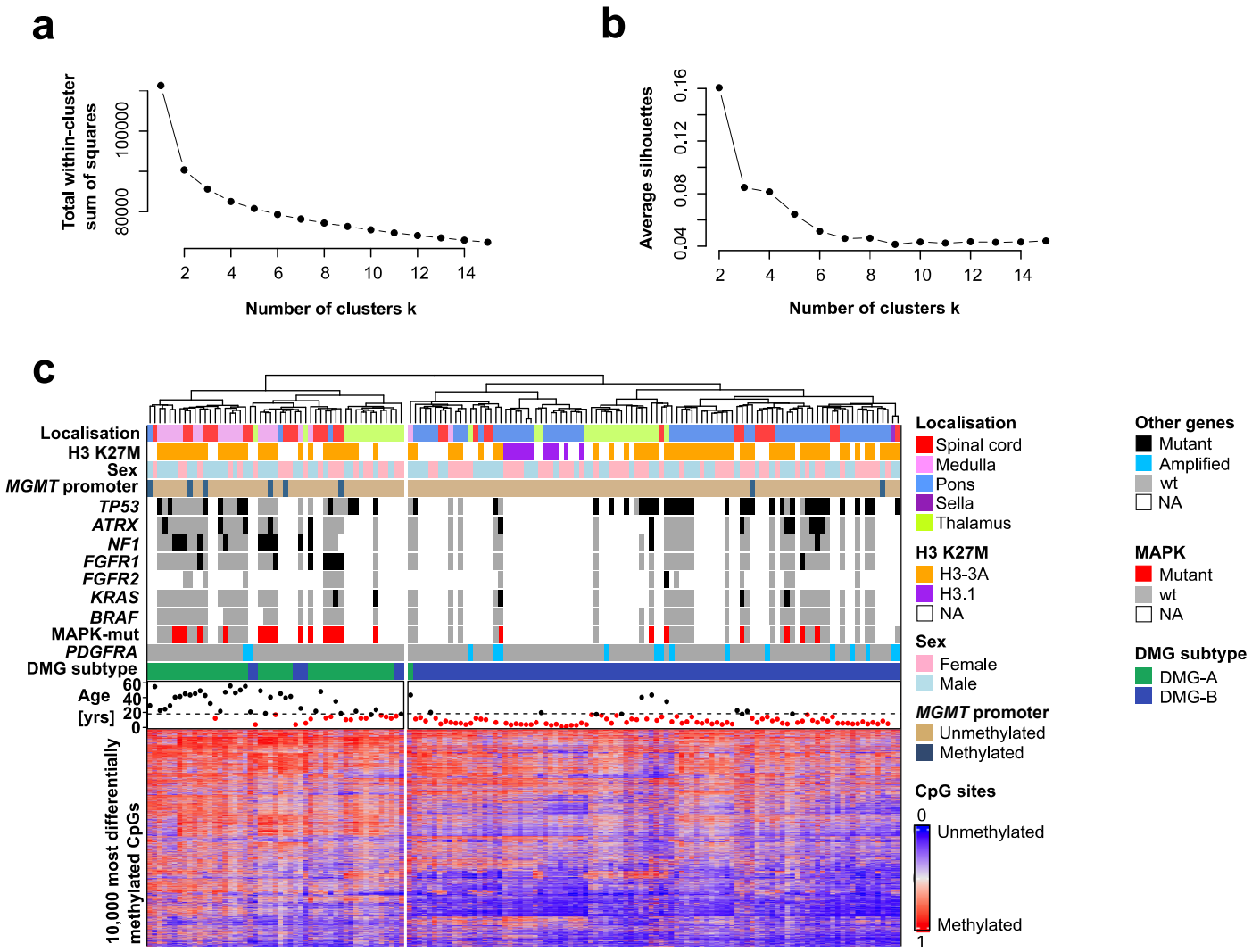

Online Resource 3: k-means clustering confirms the two DMG subtypes. Consensus clustering of 149 H3 K27M-mutant DMGs (**c**) confirms the presence of two different subtypes of DMGs, DMG-A and DMG-B. The optimal number of two clusters was determined using the elbow method (**a**) and the silhouette method (**b**), and proved the robustness of the number of two main clusters from Fig. 1a. Only minor differences were visible: eight cases switched the cluster compared to the unsupervised hierarchical cluster analysis from Fig. 1 (DMG-A green, DMG-B blue). Otherwise, results were comparable. Most *FGFR1*-mutant cases clustered together in one subcluster of DMG-A cases, while H3.1 cases clustered together in neighbouring subclusters of DMG-B.

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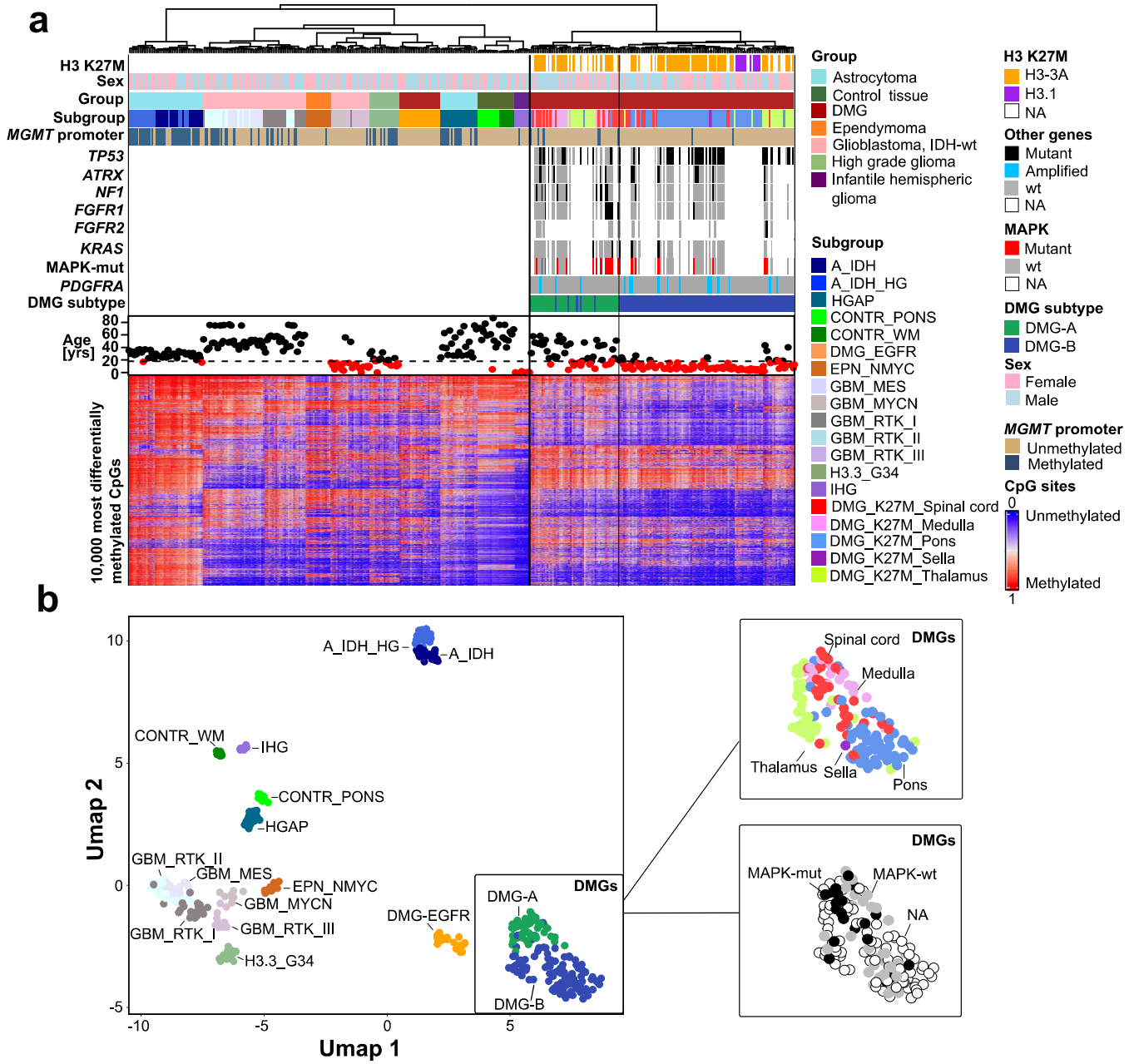

Online Resource 4: Unsupervised hierarchical clustering and Uniform Manifold Approximation and Projection (UMAP) for Dimension Reduction of DMGs with different reference classes prove the robustness of the results

Unsupervised hierarchical clustering (**a**) and UMAP **(b)** of the DMGs with a reference cohort of 227 cases from different glioma entities and normal CNS tissue confirm the two DMG-subtypes from Fig. 1a. In **a,** the cases from DMG-A in Fig. 1a are shown in green, those from DMG-B in blue. Only five cases switched the cluster. Again, the DMG-A cluster contained DMGs with more medullary DMGs (**a,** see also upper right panel in **b**) and more mutations in genes associated with the MAPK-signalling pathway (**a,** see also lower right panel in **b**). The DMG-B cluster contained more pontine DMGs (**a** and upper right panel in **b**) and *TP53*-mutant cases (**a**). Reference methylation classes: Astrocytoma, *IDH*-mutant (A_IDH); High grade astrocytoma *IDH*-mutant (A_IDH_HG); High-grade astrocytoma with piloid features (HGAP); Control tissue, pons (CONTR_PONS); Control tissue, white matter (CONTR_WM); Diffuse midline glioma, *EGFR*-altered (DMG_EGFR); Ependymoma, subclass NMYC (EPN_NMYC); Glioblastoma, *IDH*-wildtype, subclass mesenchymal (GBM_MES); Glioblastoma, *IDH*-wildtype, subclass *MYCN* (GBM_MYCN); Glioblastoma *IDH*-wildtype, subclass RTK I (GBM_RTK_ I), Glioblastoma *IDH*-wildtype, subclass RTK II (GBM_RTK_II), Glioblastoma *IDH*-wildtype, subclass RTK III (GBM_RTK_III); Diffuse hemispheric glioma, H3 G34-mutant (H3.3_G34); Infant-type hemispheric glioma (IHG)

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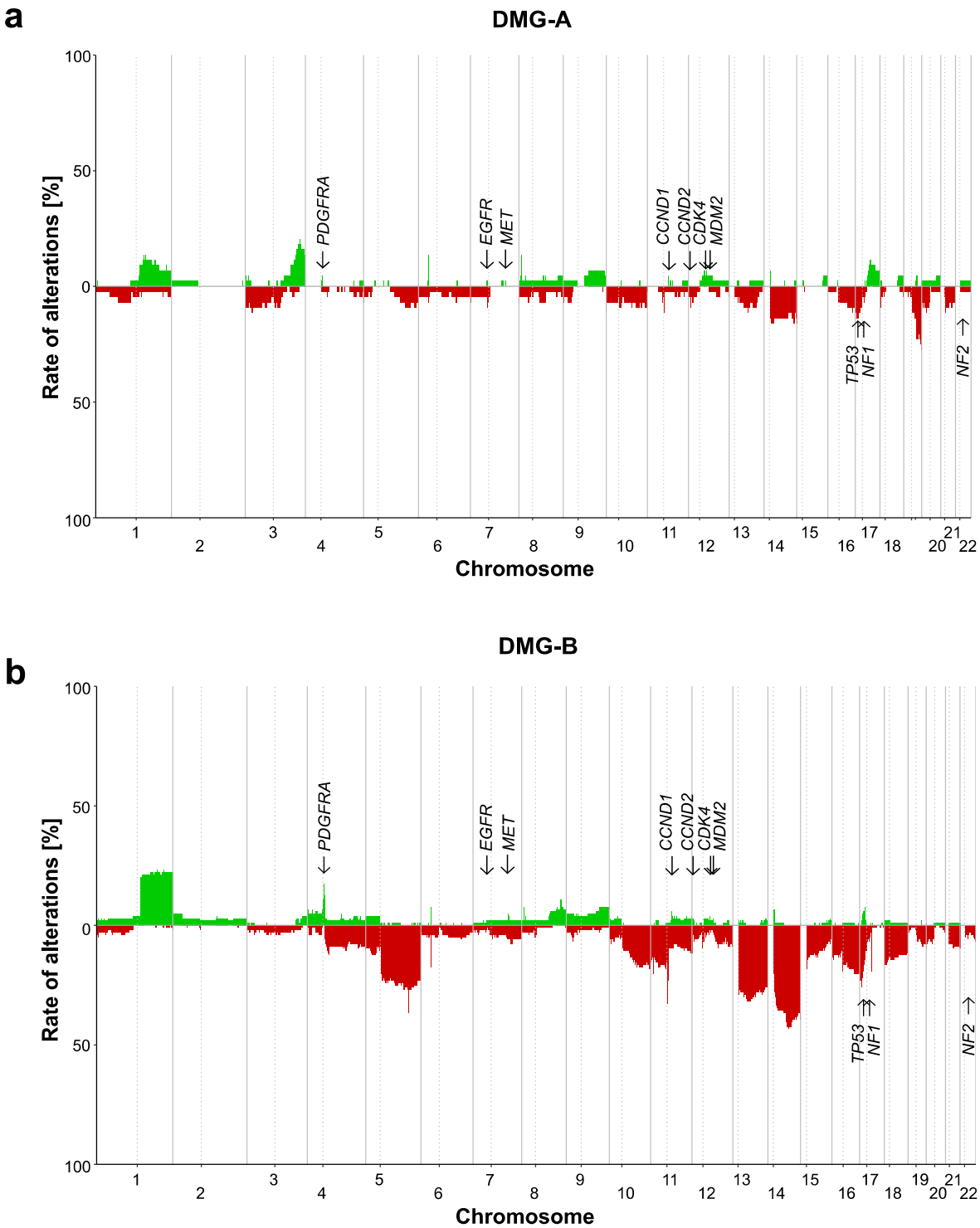

Online Resource 5: DMG-B have significantly more copy number alterations than DMG-A Cumulative copy number plots of cases from DMG-A (**a**) and DMG-B (**b**). Rate of gains in green, rate of losses in red. Arrows at the top mark the loci of selected proto-oncogenes that were amplified in some DMGs. Arrows at the bottom mark the loci of selected tumour suppressors that were deleted in some DMGs. Mean CNV load/Mb: DMG-A 183.5 ± 168.5, DMG-B 258.1 ± 127.0 ($p < 0.001$)

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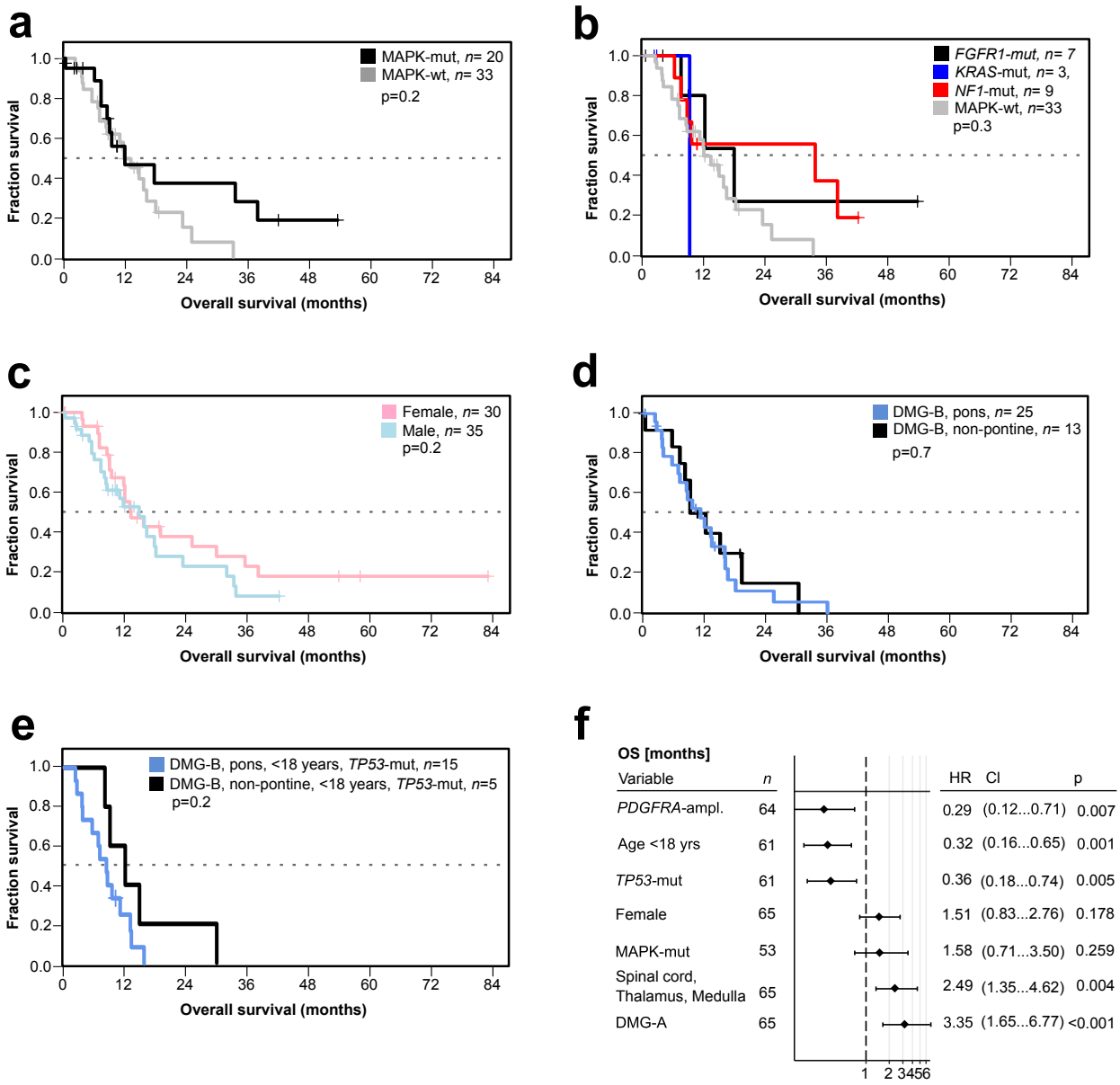

Online Resource 6: **MAPK-status and sex do not influence survival**. MAPK-status in general (**a**), as well as individual MAPK-mutations (**b**) and sex (**c**) did not influence survival (MAPK-mutant: median OS= 12.0 ± 15.3 months, MAPK-wild type: median OS= 11.8 ± 7.4 months, p= 0.2*; FGFR1*-mut: median OS= 17.9 ± 28.3 months, *NF1*-mut: median OS= 33.8 ± 25.0 months, *KRAS*-mut: median OS= 9.0 ± 13.9 months, p= 0.3; female: median OS= 13.0 ± 17.5 months, male: median OS= 14.8 ± 12.1 months, p= 0.2). (**d**) The survival of patients with DMG-B of non-pontine localisations was as poor as the survival of patients with pontine DMG-B (DMG-B, non-pontine: median OS= 10.5 ± 8.1 months, DMG-B, pons: median OS= 11.0 ± 7.8 months, p= 0.7). (**e**) The survival of pediatric patients with *TP53*-mutant, non-pontine DMG-B was as poor as the survival of pediatric patients with *TP53*-mutant, pontine DMG-B (DMG-B, non-pontine, <18 years, *TP53*-mutant: median OS= 12.0 ± 8.9 months, DMG-B, pons, <18 years, *TP53*-mutant: median OS= 8.3 ± 4.5 months, p= 0.2). (**f**) In a univariate cox regression, age (paediatric vs. adult), tumour localisation (non-pontine vs. pontine), *PDGFRA*-status (balanced vs. amplified) and *TP53*-status (mutant vs. wt) significantly influence survival. However, these factors were dependent on the subtype attribution that has the largest effect on survival

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