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# Atypical teratoid/rhabdoid tumors (ATRTs) with *SMARCA4* mutation are molecularly distinct from *SMARCB1* deficient cases

**Authors**

Dörthe Holdhof[1,2,#], Pascal D. Johann[3,4,5,#], Michael Spohn[2], Michael Bockmayr[1,2,6], Sepehr Safaei[1,2,7], Piyush Joshi[4,5], Julien Masliah-Planchon[8], Ben Ho[9], Mamy Andrianteranagna[8,10], Franck Bourdeaut[8,11], Annie Huang[8], Marcel Kool[4,5,12], Santhosh A. Upadhyaya[13], Anne E Bendel[14], Daniela Indenbirken[15], William D. Foulkes[16], Jonathan W. Bush[17,18], David Creytens[19], Uwe Kordes[1], Michael C. Frühwald[3], Martin Hasselblatt[20], and Ulrich Schüller[1,2,7]

[1]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Germany

[2]Research Institute Children's Cancer Center Hamburg, Germany

[3]Paediatric and Adolescent Medicine, Swabian Childrens' Cancer Center Augsburg, Germany

[4]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany.

[5]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ) and German Cancer Research Consortium (DKTK), Heidelberg, Germany.

[6]Institute of Pathology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany.

[7]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Germany

[8]INSERM U830, Laboratory of Translational Research in Pediatric Oncology, SIREDO Pediatric Oncology Center, Curie Institute, Paris, France.

[9]Division of Hematology & Oncology, Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Department of Paediatrics, University of Toronto, Toronto, ON, Canada

[10]INSERM U900, MINES ParisTech, PSL Research University, CBIO-Centre for Computational Biology, Curie Institute, Paris, France

[11]Departments of Genetics and of Oncopediatry and Young Adults, Curie Institute, Paris, France.

[12]Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands

[13]Department of Oncology, St Jude Children's Research Hospital, Department of Pediatrics, University of Tennessee Health Sciences Center, Memphis, Tennessee, USA

[14]Children's Hospitals and Clinics of Minnesota, Minneapolis, MN, USA

[15]Heinrich-Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany

[16]Department of Human Genetics, McGill University, Montreal, QC, Canada

[17]Division of Anatomical Pathology, British Columbia Children's Hospital and Women's Hospital and Health Center, Vancouver, British Columbia, Canada.

[18]University of British Columbia, Vancouver, British Columbia, Canada.

[19]Department of Pathology, Ghent University Hospital, Ghent, Belgium.

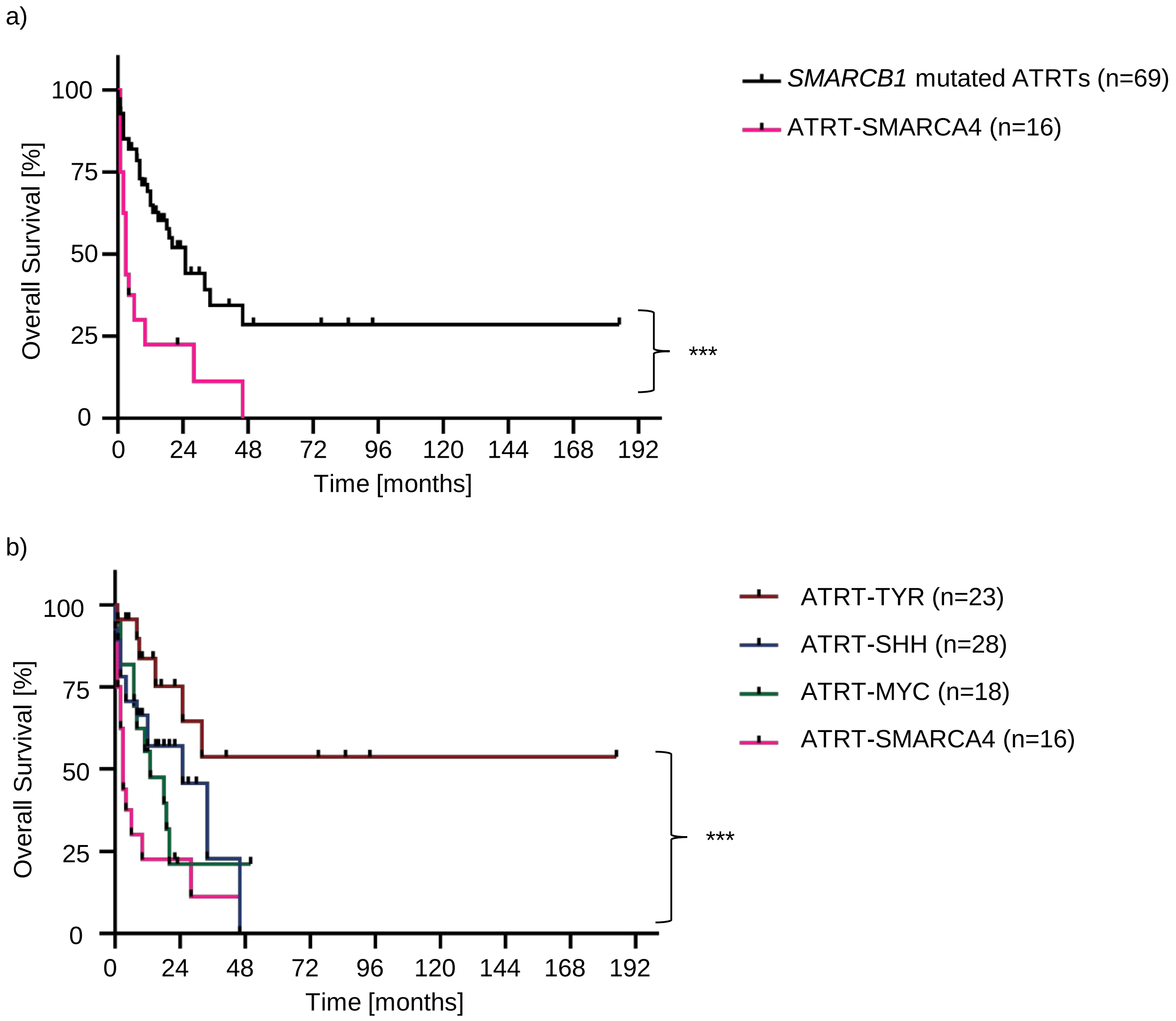
[20]Institute of Neuropathology, University Hospital Münster, Germany

[#]These authors contributed equally to this work.

**Corresponding author:**

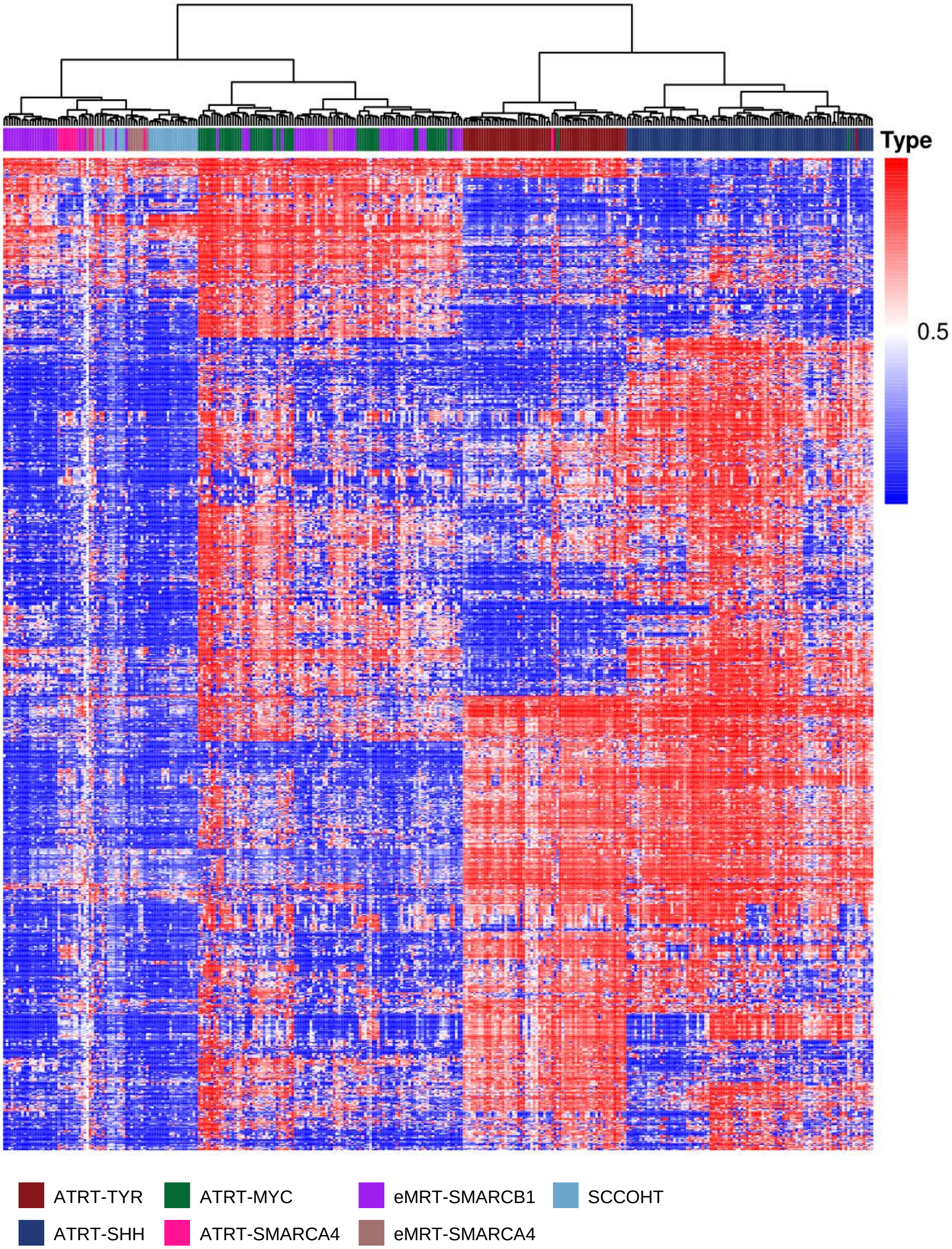
Ulrich Schüller, MD
Research Institute Children‘s Cancer Center Hamburg
Martinistrasse 52, N63 (HPI), D-20251 Hamburg, Germany
Phone: +49-40-426051240
Fax: +49-40-741040350
E-mail: u.schueller@uke.de

Supplementary Fig. 1

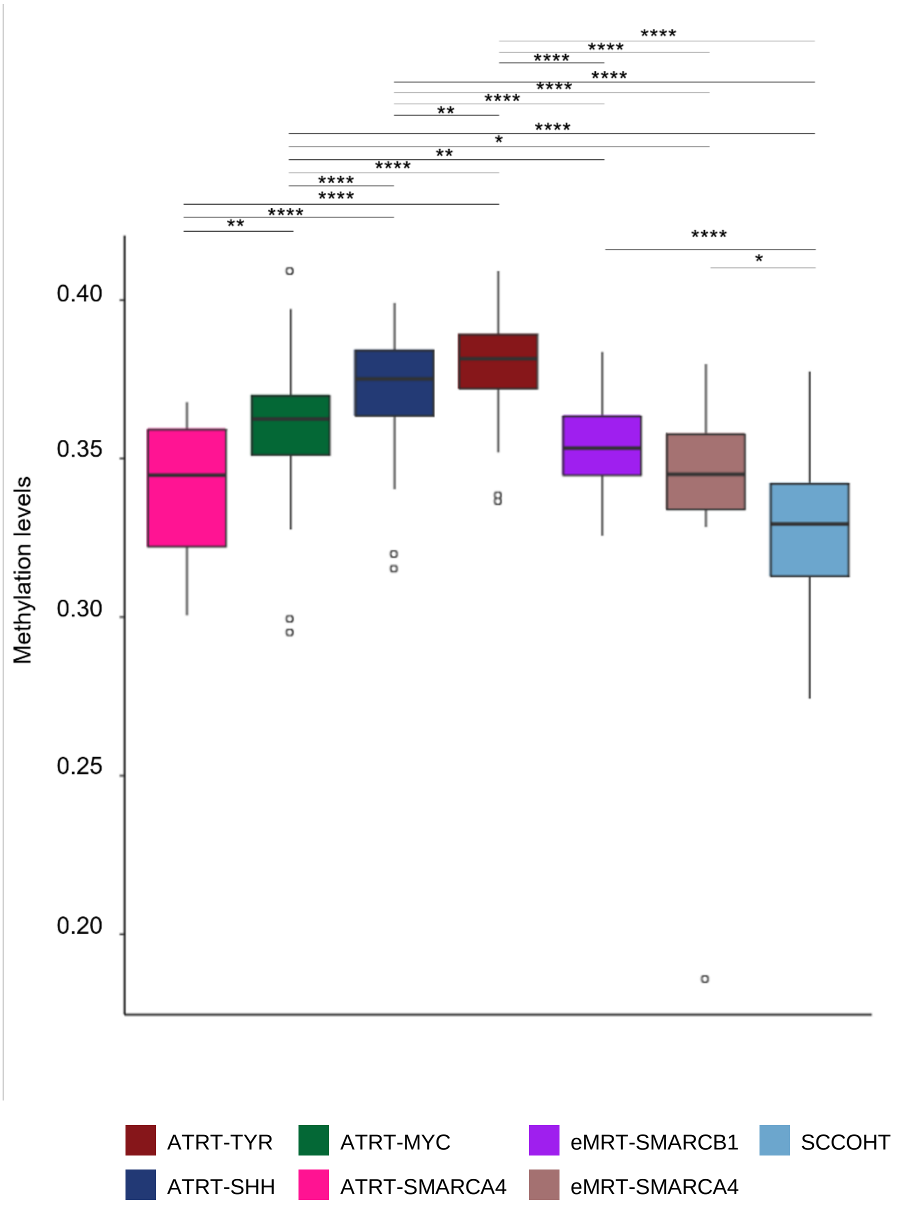


**Supplementary Fig. 1**

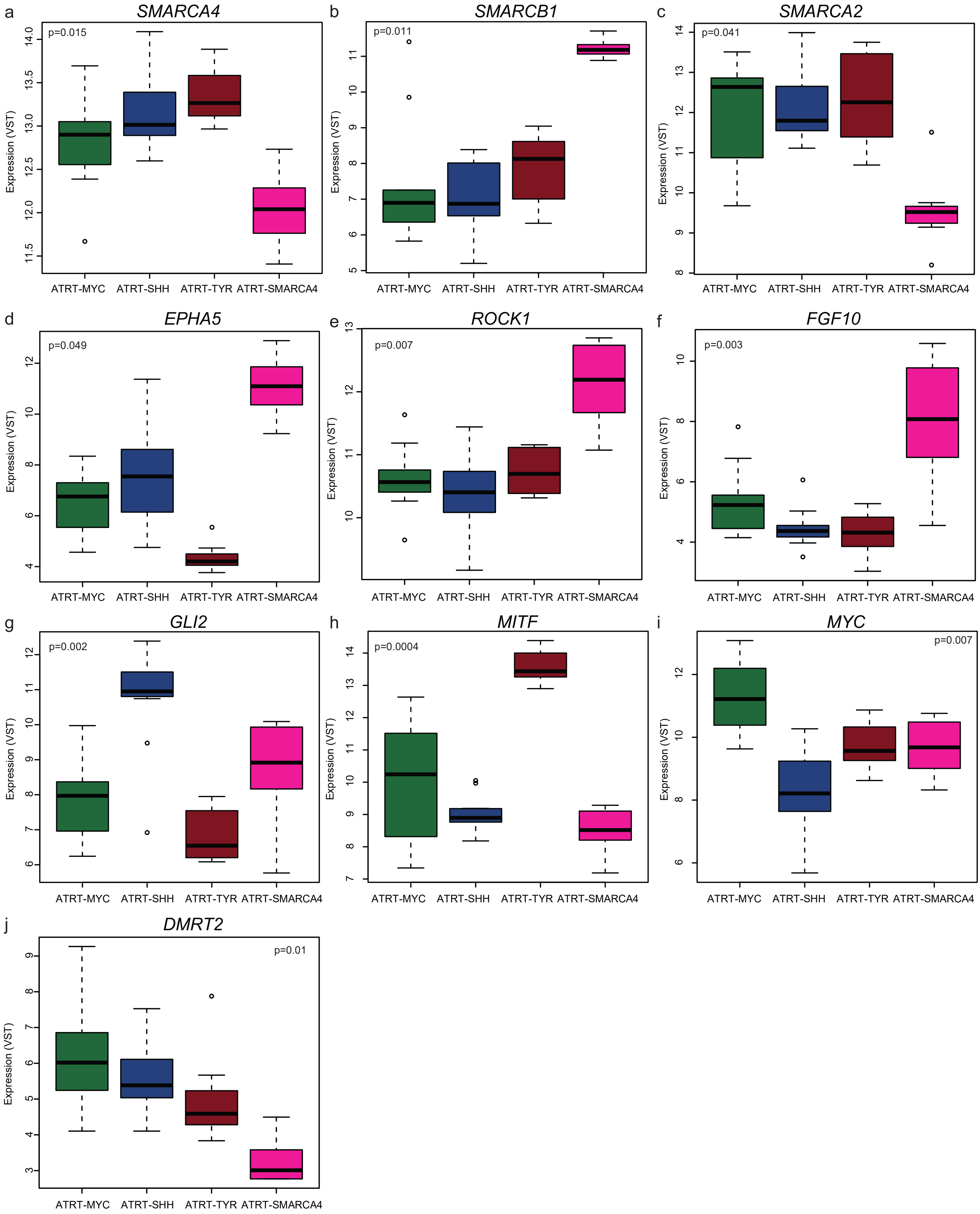
Kaplan Meier analysis for comparison of *SMARCB1* (n=69) and *SMARCA4* mutated ATRTs (n=16) shows significantly less survival in the latter (a). Kaplan Meier analysis for comparison of ATRT-TYR (n=23), ATRT-SHH (n=28), ATRT-MYC (n=18) and ATRT-SMARCA4 (n=16) depicts significant differences across all subgroups (b). Significance is based on a Log-Rank test. ***p<0.001. Note that this analysis included 5 published cases, for which tumor material was not available [5, 37].



**Supplementary Fig. 2**
Unsupervised hierarchical clustering of n=14 ATRT-SMARCA4 (pink), n=11 eMRT-SMARCA4 (brown), n=62 ATRT-TYR (dark red), n=93 ATRT-SHH (blue), n=53 ATRT-MYC (dark green), n=74 eMRT-SMARCB1 (purple), and n=28 SCCOHTs (light blue) using the 1,000 most variant probes is shown. The level of DNA methylation is indicated by a color scale representing beta values.

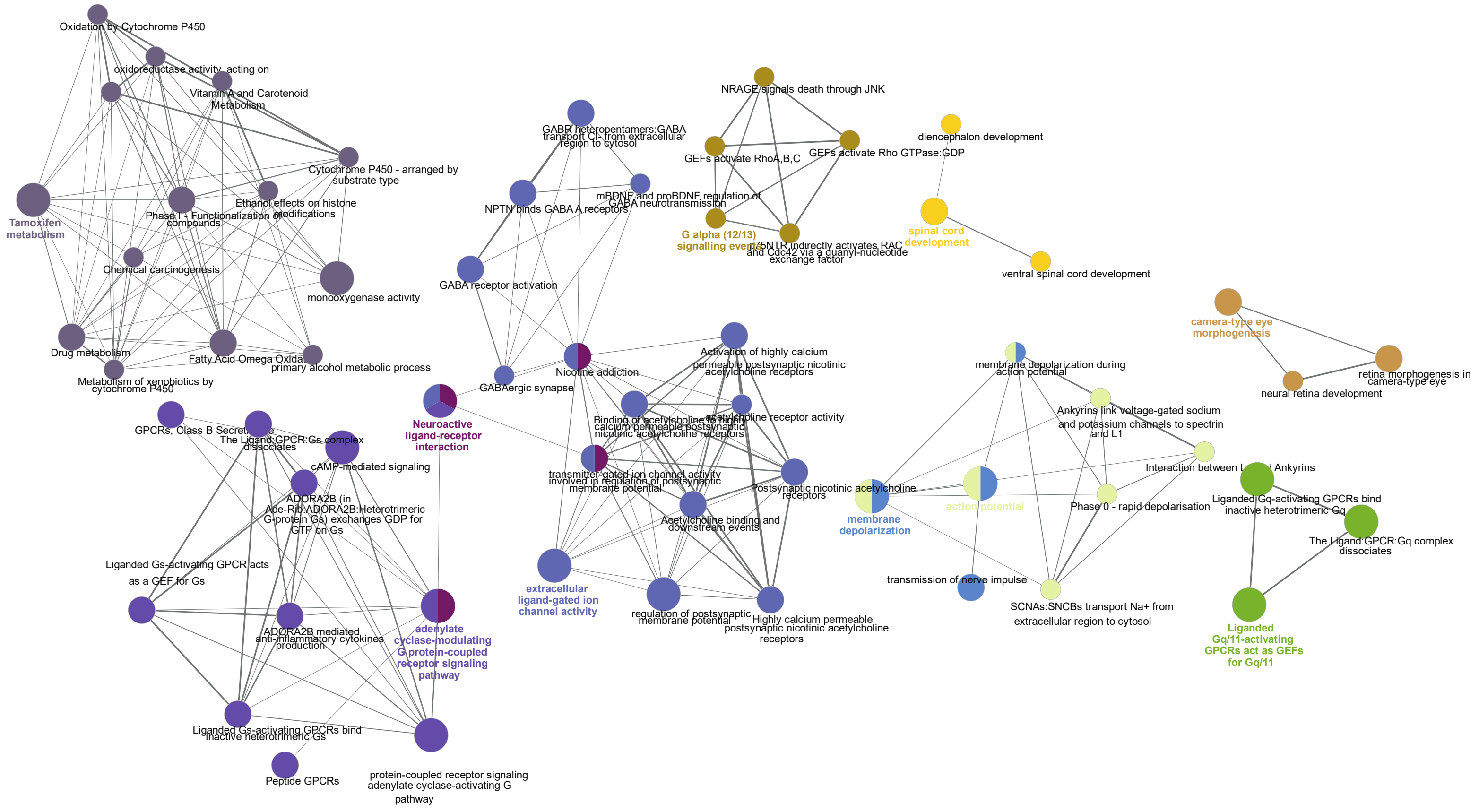


**Supplementary Fig. 3**
Genome-wide methylation levels of n=14 ATRT-SMARCA4 (pink), n=11 eMRT-SMARCA4 (brown), n=62 ATRT-TYR (dark red), n=93 ATRT-SHH (blue), n=53 ATRT-MYC (dark green), n=74 eMRT-SMARCB1 (purple), and n=28 SCCOHTs (light blue) are shown as boxplots where the y-axis indicates the β values derived from 450K/850K data. *p<0.05, **p>0.01, ***p>0.001, ***p>0.0001, not significant differences are not indicated.



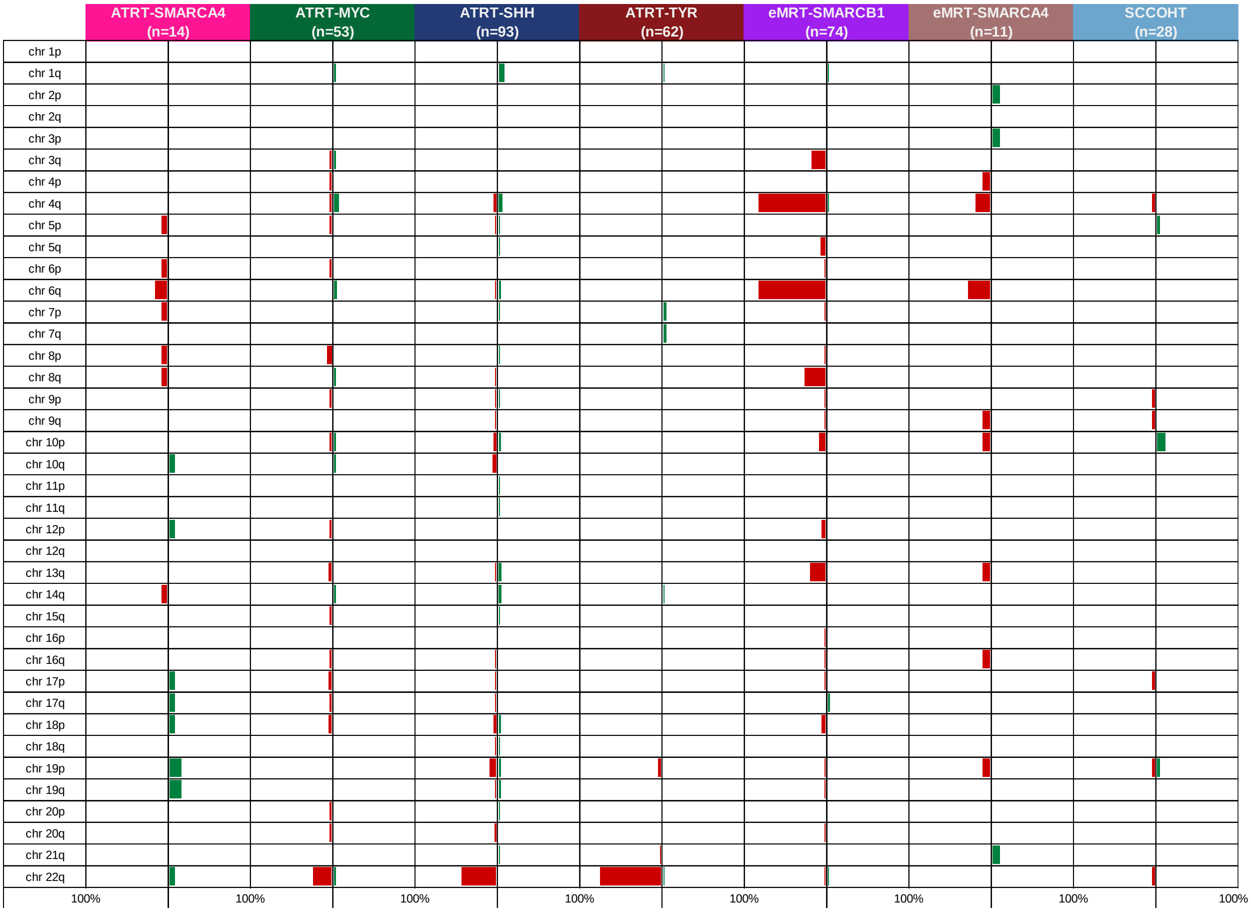
**Supplementary Fig. 4**
Relative expression levels of *SMARCA4* (a), *SMARCB1* (b), *SMARCA2* (c), *EPHA5* (d), *ROCK1* (e), *FGF10* (f), *GLI2* (g), *MITF* (h), *MYC* (i), and *DMRT2* (j) in the different ATRT subgroups are displayed as boxplots.

Supplementary Fig.5



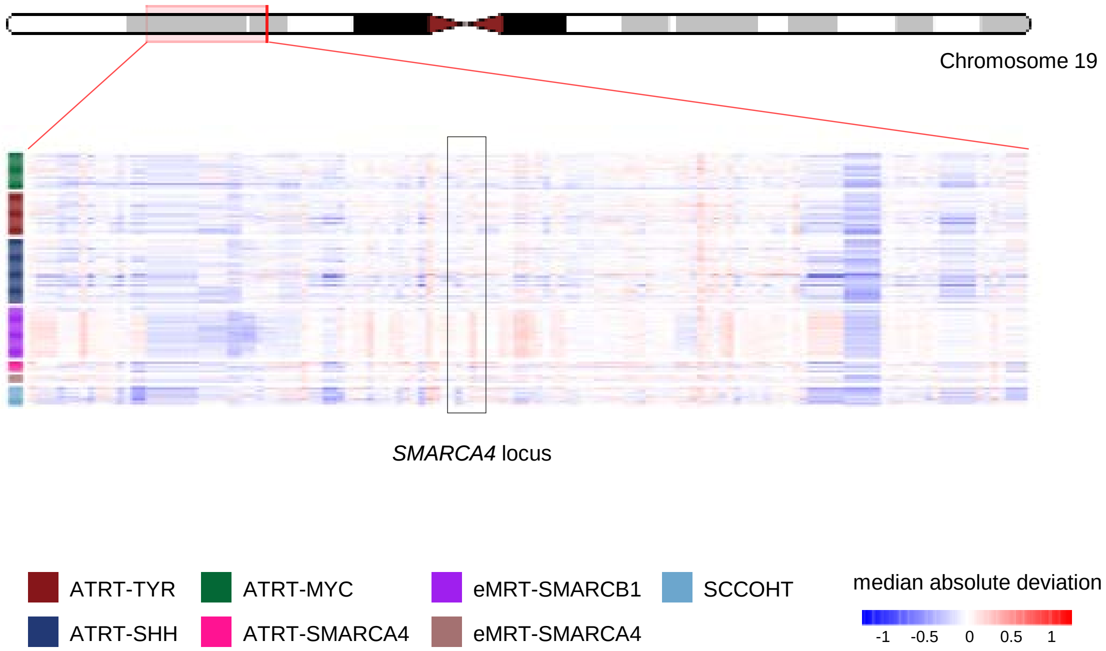
**Supplementary Fig. 5**

Network analysis of enriched terms in in *SMARCA4* mutated ATRTs compared to *SMARCB1* deficient ATRTs are depicted.



**Supplementary Fig. 6**
Overview of chromosomal gains and losses on q and p arms are shown as percentages of ATRT-SMARCA4 (n=14, pink), eMRT-SMARCA4 (n=11, brown), ATRT-TYR (n=62, dark red), ATRT-SHH (n=63, blue), ATRT-MYC (n=53, dark green), eMRT-SMARCB1 (n=74, purple), and SCCOHTs (n=28, light blue) calculated from 450K/850K DNA methylation data.

Supplementary Fig. 7



**Supplementary Fig. 7**
Chromosomal copy gains (red) and losses (blue) at the SMARCA4 locus inferred from 450K/850K DNA methylation data of ATRT-SMARCA4 (n=14, pink), eMRT-SMARCA4 (n=11, brown), ATRT-TYR (n=62, dark red), ATRT-SHH (n=63, blue), ATRT-MYC (n=53, dark green), eMRT-SMARCB1 (n=74, purple), and SCCOHTs (n=28, light blue) and summarized as a heatmap.

## Supplementary Table 1: Overview of tumor samples used in this study.

| **Type of Analysis** | ***SMARCA4* mutated ATRTs** | ***SMARCA4* mutated eMRTs** | ***SMARCA4* mutated MBs** | ***SMARCB1* mutated ATRT** | ***SMARCB1* mutated eMRT** | ***SMARCA4* wild type MB** | **SCCOHT** |
|---|---|---|---|---|---|---|---|
| Survival | n=11 present study<br>n=4 from [17]<br>n=1 from [2] | | | n=69 from [12] | | | |
| DNA methylation profiling | n=14 present study | n=10 present study<br>n=1 from [5] | n=2 present study<br>n=16 from [34] | n=12 present study<br>n=96 from [4,6,47] | n=74 from [6] | n=367 from [4] | n=1 present study<br>n=27 from [8] |
| RNA sequencing | n=8 present study | | | n=4 present study<br>n=24 from [22] | | | |
| Summary of all ATRT subgroups | n=14 present study<br>n=4 from [17]<br>n=1 from [2] | | | n≤321 from [19]<br>n=75 from [12] | | | |

**Supplementary Table 1: Overview of SMARCA4 mutated tumors without publicly available DNA methylation data prior to this study.** infra: infratentorial, n/a, data not available, supra: supratentorial

| **Case No.** | **Tumor location** | **Diagnosis** | **Sex** | **Age at diagnosis [years]** | **Overall survival [years]** | **dead /alive** | ***SMARCA4* mutation in the tumor** | **Predicted AA change** | **Coding impact** | **Clinical significance (ClinVar)** | **Clinical significance (ACMG)** | ***SMARCA4* germline mutation** | **SMARC4 protein (IHC)** | **References** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | Brain (infra) | MB (Group 3) | male | 8 | n/a | n/a | n/a | p.H1097Q | missense | n/a | Uncertain significance | n/a | retained | |
| 16 | Brain (infra) | MB (WNT) | female | 11 | n/a | n/a | n/a | p.E821K | missense | n/a | Uncertain significance | n/a | retained | |
| 17 | Ovary | SCCOHT | female | 14.4 | n/a | n/a | c.728dup | p.A245Gfs*42 | frameshift | n/a | Uncertain significance | n/a | n/a | |
| 18 | n/a | eMRT | n/a | n/a | n/a | dead | c.3168+1G>A | *Intron* | none | n/a | Pathogenic | yes | lost | |
| 19 | n/a | eMRT | male | n/a | n/a | n/a | c.4018G>A | p.E1340K | missense | n/a | Uncertain significance | n/a | lost | |
| 20 | Stomach | eMRT | female | 4.5 | n/a | alive | c.1943+1G>T | *Intron* | none | n/a | Pathogenic | yes | lost | |
| 21 | Kidney | eMRT | female | 0.6 | 1.5 | dead | c.3565C>T | p.R1189* | nonsense | Pathogenic | Pathogenic | yes | lost | Patient III3 [43] |
| 22 | Shoulder | eMRT | male | 0.3 | 5 | alive | n/a | n/a | n/a | n/a | n/a | n/a | lost | R907-18 [42] |
| 23 | Omentum | eMRT | male | 0.4 | <0.5 | dead | n/a | n/a | n/a | n/a | n/a | n/a | lost | R907-05 [42] |
| 24 | n/a | eMRT | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | |
| 25 | n/a | eMRT | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | |
| 26 | n/a | eMRT | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | |
| 27 | n/a | eMRT | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | |

**Supplementary Table 1: Overview of unpublished SMARCB1 mutated ATRTs included in this study.** infra: infratentorial, n/a, data not available, supra: supratentorial; √ analysis done; x analysis not done

| Case No. | Tumor location | Sex | Age at diagnosis [months] | Molecular subgroup | DNA methylation data | RNA sequencing data |
|---|---|---|---|---|---|---|
| 28 | infra | male | 8.4 | ATRT-MYC | √ | x |
| 29 | supra | male | 118.8 | ATRT-MYC | √ | √ |
| 30 | supra | male | 18 | ATRT-MYC | √ | x |
| 31 | supra | male | 2.4 | ATRT-MYC | √ | x |
| 32 | supra | male | 30 | ATRT-MYC | x | √ |
| 33 | supra | male | 38.4 | ATRT-SHH | √ | x |
| 34 | n/a | female | 15.6 | ATRT-SHH | √ | x |
| 35 | n/a | male | 13.2 | ATRT-SHH | √ | x |
| 36 | supra | male | 60 | ATRT-SHH | x | √ |
| 37 | infra | male | 12 | ATRT-TYR | √ | x |
| 38 | infra | female | 31.2 | ATRT-TYR | √ | x |
| 39 | supra | male | 19.2 | ATRT-TYR | x | √ |