Supplementary Figure 1: Identification of SVZ+/− methylome signature in HD training cohort. For identification of most differentially methylated probes of the 450k methylation array, dmpFinder (minfi package) was utilized and per CpG differences between MRI based SVZ+/− assignments was calculated (no p-value adjustment). To identify the most relevant CpGs and to adjust for misclassifications, the first up to the nth CpG of the 225 best ranked (p<0.001) CpGs were selected to obtain two main clusters by hierarchical cluster analysis (Euclidean distance, complete linkage). Each cluster derived classification was tested against the original MRI based SVZ+/− based assignment and a Chi-Squared test was calculated. The minimum p-value occurred at least twice (CpG 188, orange red vertical line) was used to obtain a reference clustering on which machine learning random forest classifier was trained.
Supplementary Figure 2: Cluster analysis with reference tumors from Heidelberg Neuropathology Department and their corresponding Pan-Glioma DNA-Methylation subtype assignment (RTKI: n=10, RTKII: n=11, H3.3 G34 mut: n=15, H3.3 K27 mut: n=19, IDHmut: n=11 and mesenchymal: n=16) together with the SVZ-HD cohort (n=54) and corresponding MRI SVZ+/- assignments (A). SVZMRI+/- tumors cluster together with GBM-IDH-wildtype subtypes (RTKI/II and MES). There was no apparent further subclustering /enrichment found, unless for RTKI within this main branch. Heatmap shows unsupervised clustering, Euclidean distance and Wards linkage of 30,000 differentially regulated CpGs selected by median absolute deviation (MAD). B: DNA methylation based subtype classification into RTKI/II/MES adapted from [1] shows the relative proximity of these subclasses compared to other glioma or CNS tumor entities. C: Heidelberg DNA-Methylome Classifier (https://www.molecularneuropathology.org/mnp) Version v11b4 assignment of all SVZM positive and negative tumors identified in this study (combined HD and TCGA cohort) shows presence of RTKI/II and MES GBM subtypes independent of SVZM assignment. ComBat was used to merge 450k data from both cohorts and k-means clustering for subtype assignment of TCGA cohort.

[1] Capper, D., et al. DNA methylation-based classification of central nervous system tumours. Nature 555, 469-47 (2018).
Supplementary Figure 3: Association between degree of resection and SVZ assignment. A: Survival analysis with SVZM/MRI assignment and degree of surgery (reference: gross total resection). Cox models. B: Mosaic plot and measurements of association between both variables.
### Supplementary Figure 4: Prognostic value of SVZM vs. MRI based SVZ+/− classification in multivariable survival analyses of additional clinical \{KPS, Age\} (A) molecular/treatment \{MGMT, Radio-Chemotherapy\} (B) variables (Cox-PH model; SVZ+ vs SVZ−; female vs male; KPS \(\geq 80\) vs < 80; unmethylated vs methylated). As MGMT methylation unsure classified samples were excluded from analysis.
Supplementary Figure 5: Differential methylation of SVZ tumors. A: Differentially regulated CpGs (SAM, 500 permutations, FDR < 0.05). B: Intersect between 15CpG RF signature and 439 differential CpGs. C: Most frequently identified genes by CpG annotation of the 439 intersect CpG set. D: Detailed visualization of the most prominently altered genomic locus on chromosome 4 which is highly abundant in the SVZM signature.
Supplementary Figure 6: CNV alterations on segment (A, all) and gene level, p < 0.05 (B, consensus). Fisher test.
**Supplementary Figure 7:** Mutations. A: Total numbers of non-silent mutations. B: Selected genes, with Wilson 95% confidence intervals. C: Glioblastoma driver mutations, IntOGen, access date: 2022-04-14. Font size corresponds to number of samples with mutations. Numbers of mutated samples / gene are shown below. Star marks DCAF12L2, identified as being differentially mutated.
Supplementary Figure 8

Nine ZNF177 CpGs were identified in consensus CpGs, ZNF599-ZNF177 alterations were detected on expression level and copy number level (loss). Our finding is in line with previous literature suggesting an important role ZNF599-ZNF177. It is among the top 10 oncoscore genes (07/2021) and differentially methylated/regulated e.g. in lung, head and neck and colorectal cancer [1-4].

1. Diaz-Lagares, A., et al., A Novel Epigenetic Signature for Early Diagnosis in Lung Cancer. Clin Cancer Res, 2016. 22(13): p. 3361-71.
2. Lleras, R.A., et al., Hypermethylation of a cluster of Kruppel-type zinc finger protein genes on chromosome 19q13 in oropharyngeal squamous cell carcinoma. Am J Pathol, 2011. 178(5): p1965-74.
3. Chung, H.H., et al., A Novel Prognostic DNA Methylation Panel for Colorectal Cancer. Int J Mol Sci, 2019. 20(19).
4. Piazza, R. et al., OncoScore: a novel, Internet-based tool to assess the oncogenic potential of genes. Sci Rep, 2017. 7.: 46290.
Supplementary Figure 9: (A) Analysis of single cell RNAseq data (Xierakis et al [2]) confined expression of LRBA mRNA (red/brown coloring of positive cells, right side) to the microglia compartment (dark blue cluster, left side) in healthy mouse brains.

B) In analogy, cellular distribution of key biomarker for identification of GBM subtypes as reported by Neftel et al. [1] was also allocated in this scRNAseq data, i.e. Vimentin (Vim) expression for the mesenchymal subtype (MES-like) was confined to endothelial cell (EC), vascular smooth muscle cells (VSMC) and olfactory ensheathing glia (OEG). Sox11 (NPC-like) expression was most frequently detected in neuronal restricted precursor cells (NRP) and immature neurons, to a lesser degree in oligodendrocyte precursor cells (OPC) and mature neurons. Plp1 (OPC-like) was abundantly expressed in Oligodendrocytes, OEG and a fraction of OPC, Gfap (AC-like) was expressed in a subset of Astrocytes, neural stem cells (NSC) and astrocyte-restricted precursors (ARP).

[1] Neftel, C., Laffy, J., Filbin, M.G., Hara, T., Shore, M.E., Rahme, G.J., et al. (2019). An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. Cell 178(4), 835-849 e821. doi: 10.1016/j.cell.2019.06.024.

[2] Xierakis, M., Liprnick, S.L., Innes, B.T., Simmons, S.K., Adiconis, X., Dionne, D., et al. (2019). Single-cell transcriptomic profiling of the aging mouse brain. Nat Neurosci 22(10), 1696-1708. doi: 10.1038/s41593-019-0491-3.
Supplementary Figure 10: Distribution of LRBA expression in mouse and human single cell glioblastoma data from the Brain Immune Atlas (https://www.brainimmuneatlas.org/). Mouse datasets show high Lrba expression in Microglia, human samples mostly in T-cells. Data accessed on 2022-04-28.

[1] Hannah Van Hove, Liesbet Martens, Isabelle Scheyltjens, Karen De Vlaminck, Ana Rita Pombo Antunes, Sofie De Prijck, Niels Vandamme, Sebastiaan De Schepper, Gert Van Isterdael, Charlotte L. Scott, Jeroen Aerts, Geert Berx, Guy E. Boeckxstaens, Roosmarijn E. Vandenbroucke, Lars Vereecke, Diederik Moechars, Martin Guilliams, Jo A. Van Ginderachter, Yvan Saeys and Kiavash Movahedi. A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. Nature Neuroscience 2019; 22:1021-35.
**Supplementary Figure 11:**
UMAP representation of methylation data with SVZM+ assignment and group centroids (median), showing a separation of SVZM+/− tumors.

Left column: HD data, right: TCGA data.
Upper row: 15 CpG set, middle row: HD 225 CpGs (see Suppl-Fig. 1), 214 CpGs were present in TCGA, bottom row: HD 321584 CpGs, TCGA: 305573 CpGs.
HD: Filtered for X,Y chr, repetitive sequences and SNPs.
TCGA: Intersect with probes present in HD data.
M-values.
### Supplementary Figure 12: Multivariate survival analysis using a parametric model with loglogistic distribution. RCHT: Radio-Chemotherapy.
**Supplementary Figure 13**: Cases with discordant MRI / methylome SVZ classification, see also Figure 2. Tumor extension on FLAIR sequence is marked in yellow, on T1 in red. Relevant ventricular borders are highlighted in green. A careful look at all dimensions (axial ax., coronal cor.) and sequences (T2 FLAIR and T1 contrast enhanced, T1ce) identified ventricle zone proximity in SVZM+ classified tumors. Tumor association with ventricular zone is also visible in MRI-SVZ+ but SVZM- tumor classification underscores the challenge to discriminate primary association vs. secondary invasion to this zone solely by imaging principles.

Clinical implications of a well-established classifier could be, besides prognosis prediction, a potential use in target volume delineation and inclusion of fibertracks along the SVZ. Furthermore, intentionally inclusion of parts of the SVZ in the target volume could be discussed. Treatment escalations in systemic therapies, RT and potentially tumro treating fields for SVZM+ pacientes are meritedbased on the inferior prognosis. A future perspective could be to train machine learning algorithms to predict SVZM allocation.
**Supplementary Figure 14:** Concordance of T2FLAIR and T1ce tumor localization assignment for tumors with equal T1ce MRI and SVZM classification for which T2 FLAIR sequences were available (n=39).

By FLAIR sequence as SVZ- classified tumors were in all but one case also T1ce SVZ- (left). FLAIR SVZ+ classified tumors were nearly equally classified as SVZ+-/- on T1ce sequence (right).
| Feature       | n  | %   |
|--------------|----|-----|
| All          | 47 | 100 |
| Sex          |    |     |
| female       | 21 | 44.680 |
| male         | 25 | 53.190 |
| Age          | 64.55 | 97.870 |
| [23.38;85.66] |    |     |
| RT           |    |     |
| yes          | 12 | 25.530 |
| TotalDose    | 60 | 19.150 |
| [32.7;160]   |    |     |
| TMZ          |    |     |
| yes          | 11 | 23.400 |
| TMZadjuvant  |    |     |
| yes          | 9  | 19.150 |

| Feature       | n  | %   |
|--------------|----|-----|
| All          | 132| 100 |
| Sex          |    |     |
| female       | 55 | 41.670 |
| male         | 75 | 56.820 |
| Age          | 61.94 | 98.480 |
| [21.8;85.66] |    |     |
| RT           |    |     |
| yes          | 39 | 29.550 |
| TotalDose    | 60 | 24.240 |
| [8;160]      |    |     |
| TMZ          |    |     |
| yes          | 36 | 27.270 |
| TMZadjuvant  |    |     |
| yes          | 32 | 24.240 |

| Feature       | n  | %   |
|--------------|----|-----|
| All          | 39 | 100 |
| Sex          |    |     |
| female       | 17 | 43.590 |
| male         | 22 | 56.410 |
| Age          | 61.35 | 100 |
| [44.18;85.66] |    |     |
| RT           |    |     |
| yes          | 31 | 79.490 |
| TotalDose    | 60 | 64.100 |
| [8;160]      |    |     |
| TMZ          |    |     |
| yes          | 30 | 76.920 |
| TMZadjuvant  |    |     |
| yes          | 27 | 69.230 |

**Supplementary Table 1:** Patient characteristics of the TCGA-GBM data. Median and ranges in brackets for age and RT total dose [Gy].