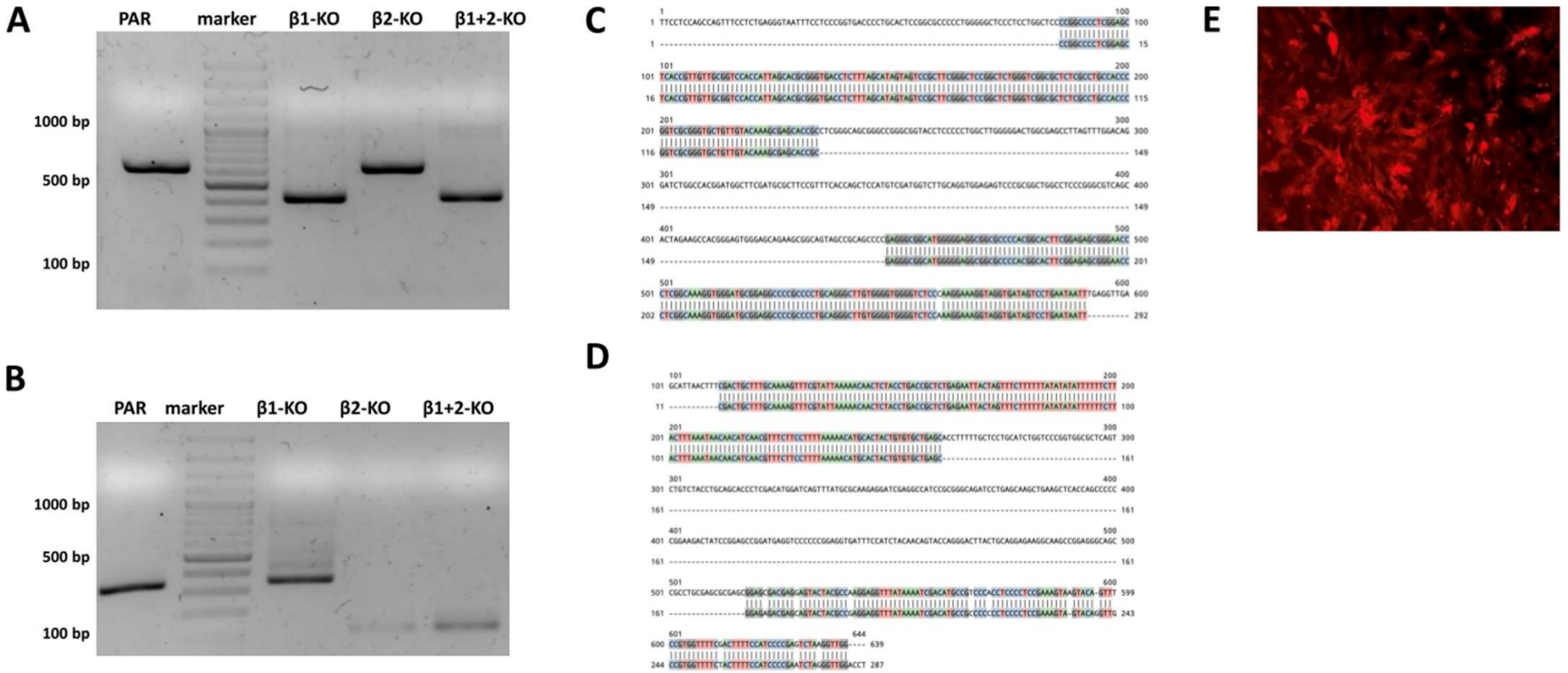
Supplementary material

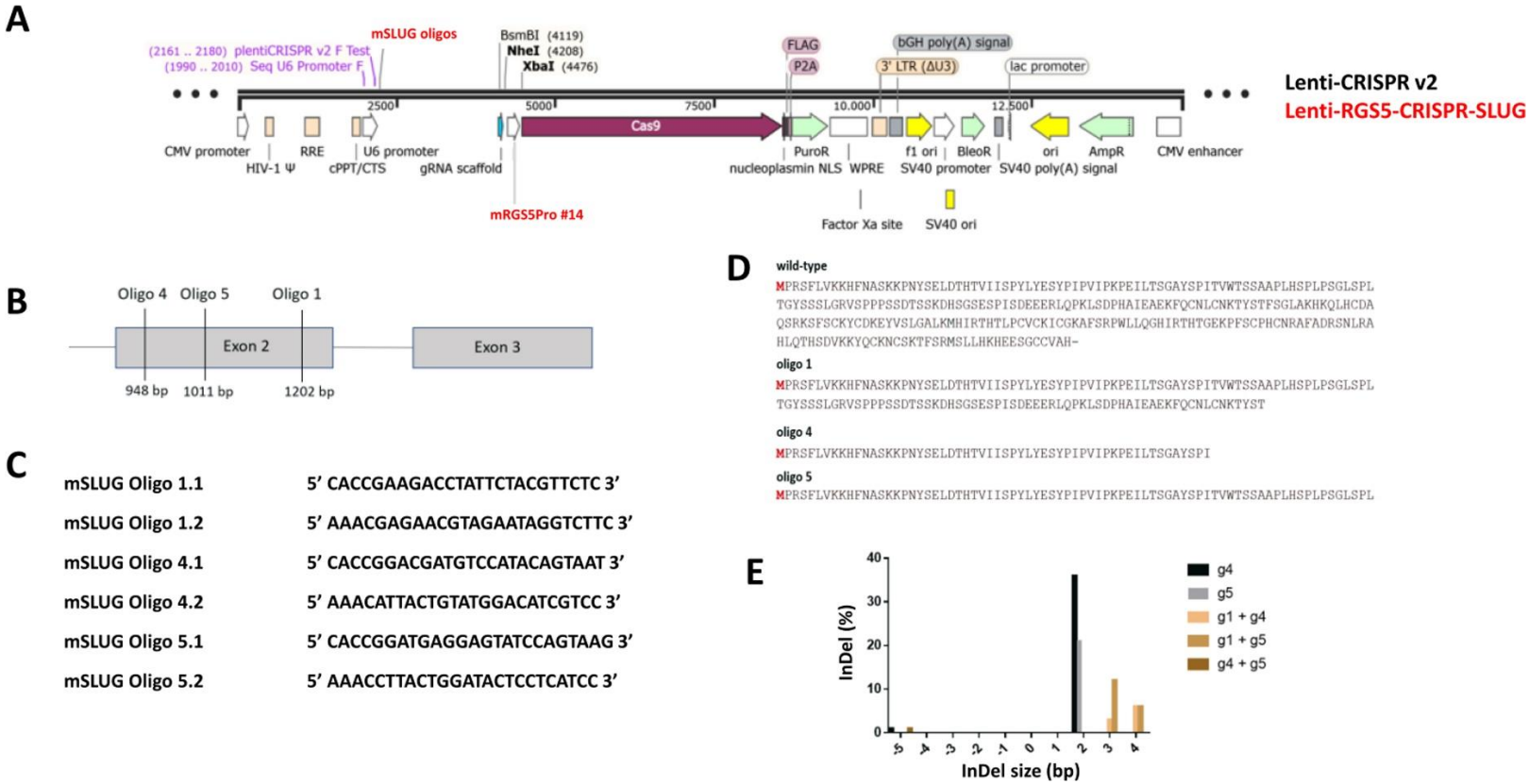
- **Supplementary Figures 01 – 11**
- **Supplementary Tables 1 - 2**

Supplementary Figure 01



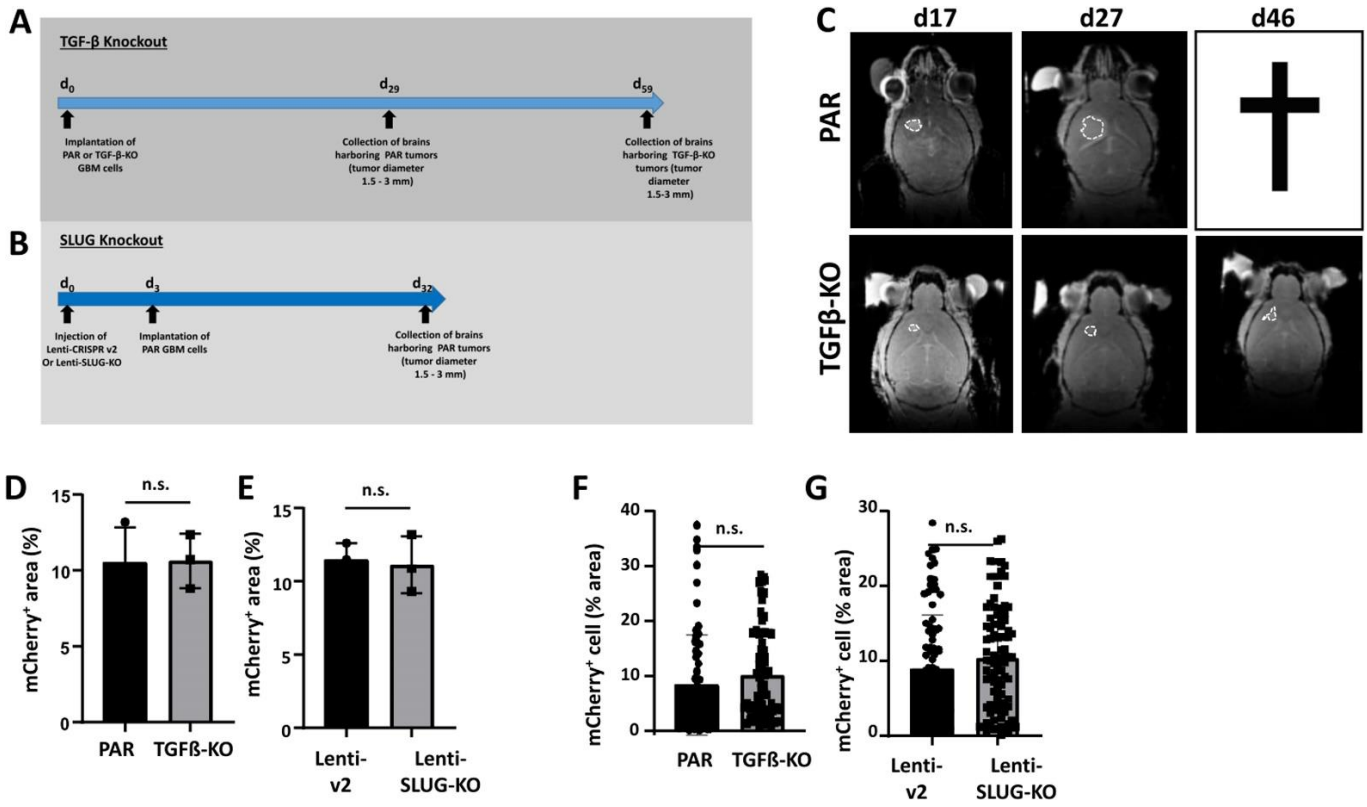
Supplementary Figure 1. Verification of GL261 TGF-β1 + β2 knockout cells. **A/B.** Agarose gel electrophoresis of PCR-fragments generated from genomic DNA of parental (PAR) and TGF-β knockout GL261 cells using **(A)** TGF-β1 and **(B)** TGF-β2 specific primer (n=2, one representative experiment is shown). **C/D**. Evaluation of the **(C)** TGF-β1 KO and **(D)** TGF-β2 KO by genomic DNA sequencing (upper lane: parental cells, lower lane: TGF-β-KO cells). E. GL261-KO mCherry expressing cells.

Supplementary Figure 02



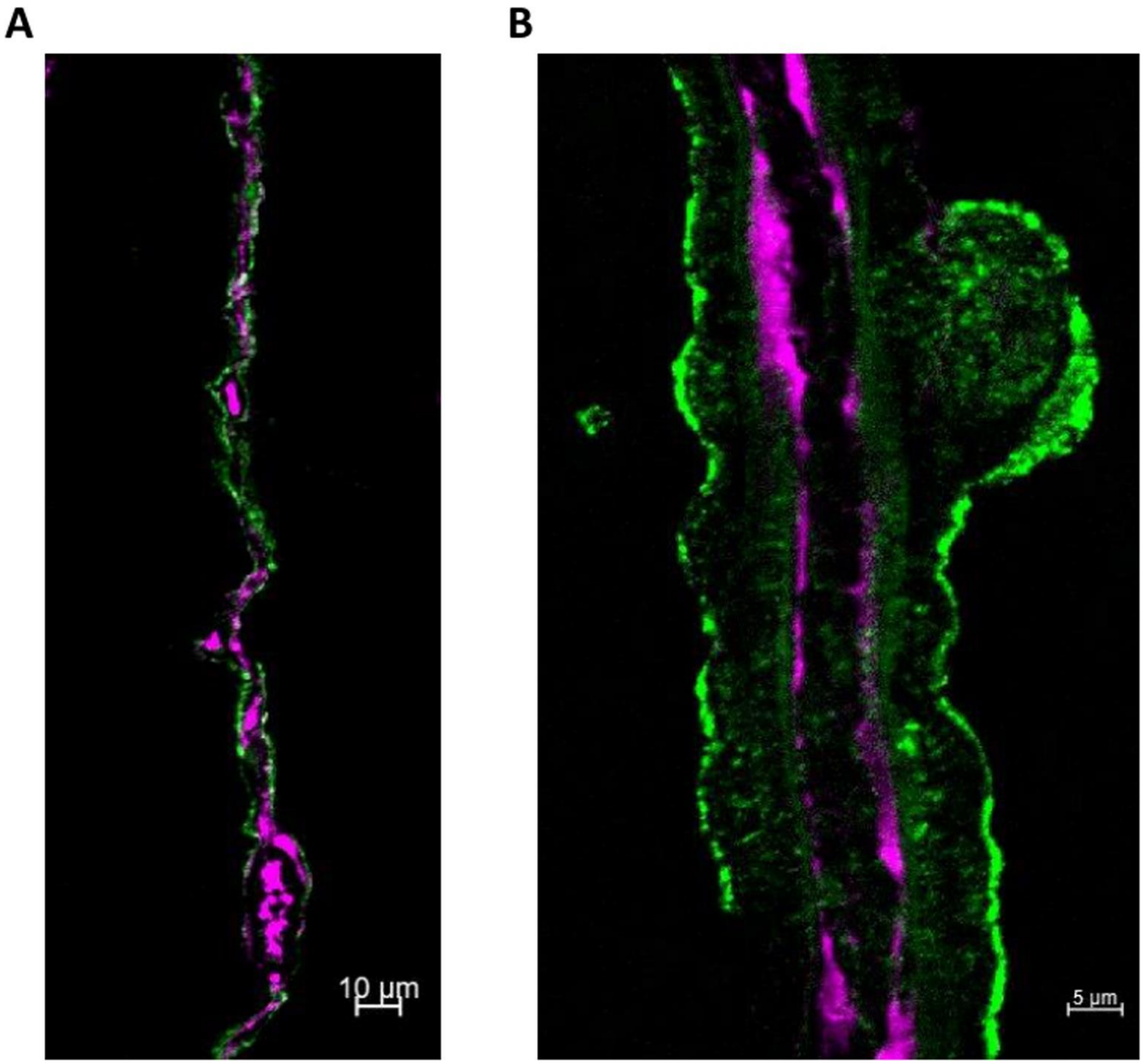
Supplementary Figure 2. Generation of mouse SLUG-specific pLentiCRISPR v2. **A.** Structure of Lenti-CRISPR v2 and Lenti-RGS5-CRISPR-SLUG. **B.** Targeting sides of sgSLUG Oligos in the murine SLUG gene. **C.** Sequence of the mSLUG guide oligos downstream of the U6 promoter of pLenti-CRISPR v2. **D.** Predicted deletion of the murine SLUG protein after crispering using the different Lenti-RGS5-CRISPR-SLUG constructs. **E.** Prediction of target efficacy by SLUG sgRNAs # 1, 4 and 5..

Supplementary Figure 03



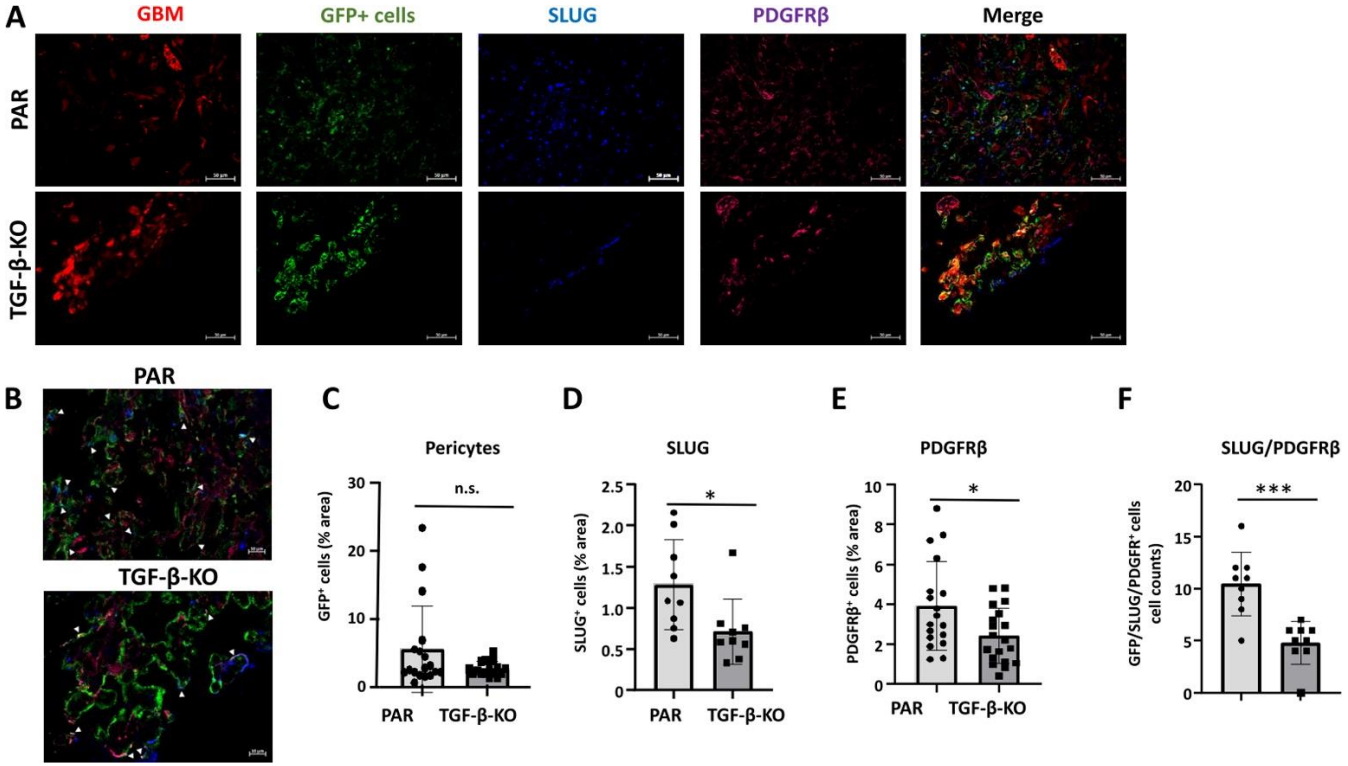
Supplementary Figure 3. Verification of tumor growth in the different mouse experimental groups. **A/B.** Time scales of treatment in TGF-β-KO mice **(A)** and mice injected with lentiviruses to knockout SLUG in RGS5+ VAMCs **(B)**. **C.** Preliminary experiment demonstrating the growth of PAR and TGFβ GBM bearing mice determined by MRI analyses (dottet lines indicate the tumor border). **D.** Retroactive analysis of tumor size in the analyzed brains of mice harboring PAR tumors (day 29 after GBM cell implantation) and from mice harboring TGF-β-KO tumors (day 59 after tumor cells implantation (n=3 mice per group, 10 x magnification, SD, t-test, n.s.: not significant). **E.** Retroactive analysis of tumor size in the analyzed brains from mice that were injected with either Lenti-V2 or Lenti-SLUG-KO (day 29 after GBM cell implantation; n=3 mice per group, 10 x magnification, SD, t-test, n.s.: not significant). **F.** Retroactive analysis of tumor size (% area of mCherry positive cells in analyzed tissue slices) in the brains harvested from mice harboring PAR tumors (day 29 after GBM cell implantation) and from mice harboring TGF-β-KO tumors (day 59 after tumor cells implantation (n=3 mice per group and 32 slices per mouse, 25 x magnification, SD, t-test, n.s.: not significant). **G.** Retroactive analysis of tumor size (% area of mCherry positive cells in analyzed tissue slices) in the brains harvested from mice that were injected with either Lenti-V2 or Lenti-SLUG-KO (day 29 after GBM cell implantation; n=3 mice per group and 32 slices per mouse, 25 x magnification, SD, t-test, n.s.: not significant).

Supplementary Figure 04



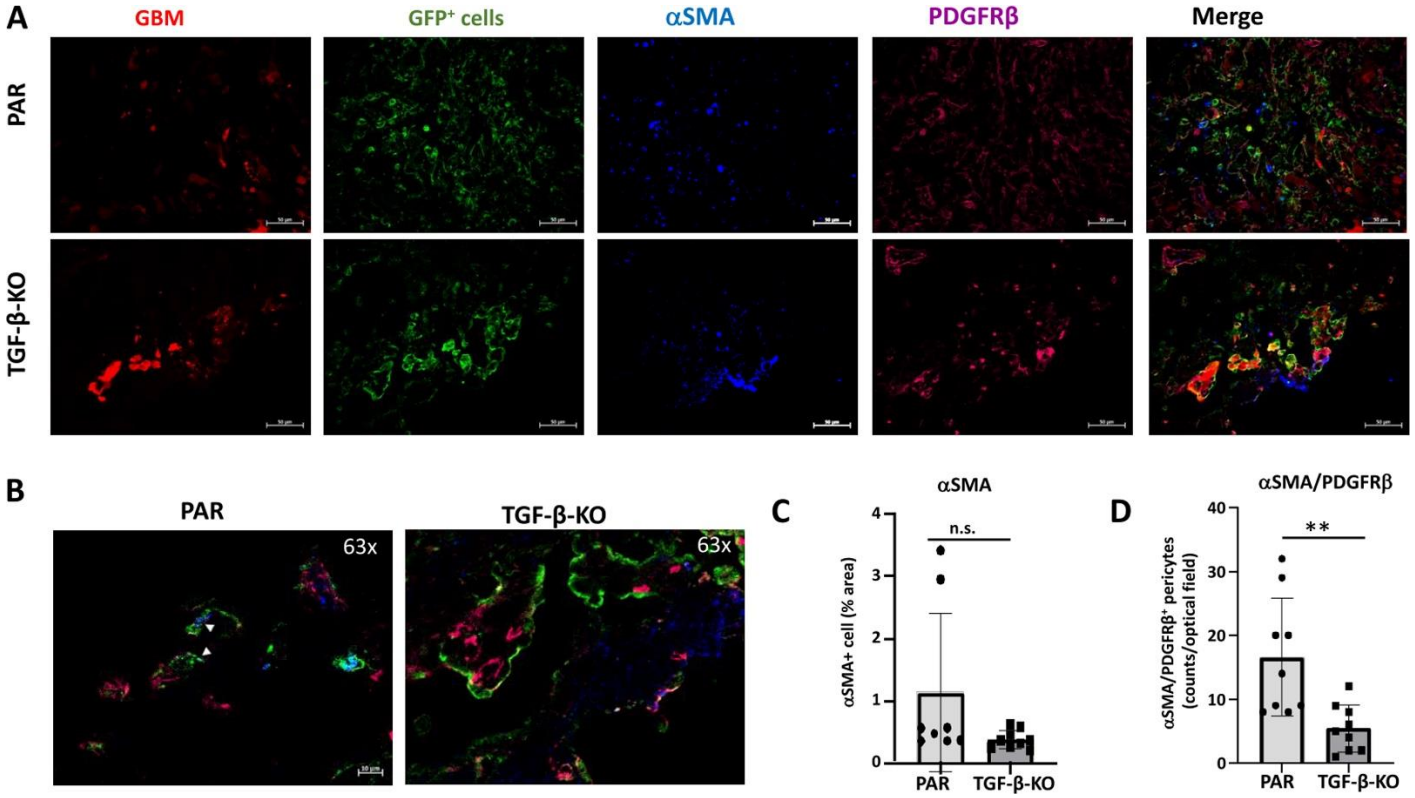
Supplementary Figure 4. Specificity of GFP staining in VAMCs of RGS5-GFP-reporter mice. Visualization of CD31 and GFP co-localization in the mouse brain. Figure shows representative areas of GFP and CD31 double staining in the contralateral, non-tumor bearing hemisphere of a RGS5-GFP-reporter mouse brain tissue harboring a PAR tumor. **A.** GFP signal adjacent to CD31 positive vessels (magenta) is shown (25 x magnification, scale bar: 10 µm). **B.** GFP positive VAMCs wrap around CD31-positive ECs (100 x magnification, scale bar: 5 µm).

Supplementary Figure 05



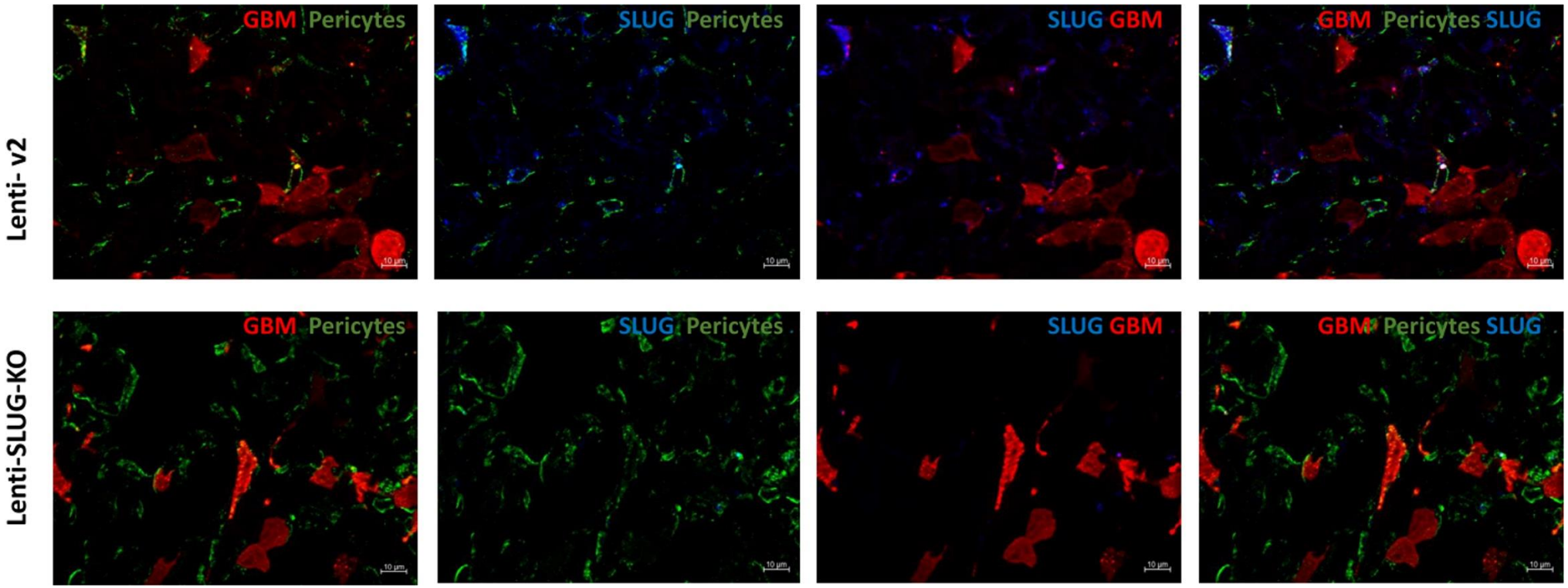
Supplementary Figure 5. Identification of SLUG and PDGFRβ in GBM associated VAMCs in the infiltration zone of PAR- and TGF-β-KO-GBM bearing RGS5-GFP reporter mice. A. Immuno-fluorescence of VAMCs (green), SLUG (blue), PDGFRβ (magenta) in the infiltration zone of GL261 tumors (red). One picture of each mouse is exemplarily shown. 25x magnification. **B.** White arrows indicate GFP/SLUG/PDGFRβ-triple positive cells, 63x magnification, bars: 50 µm. **C-E.** Quantification of pericytes **(C)**, SLUG- **(D)** and PDGFRβ-**(E)** positive cells (n=3 mice per group, 18 slices per group. **F.** Quantification of SLUG/PDGFRβ/GFP positive cells (n=3 mice, 3 sections/mouse). C-F: SEM, t-test, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Supplementary Figure 06



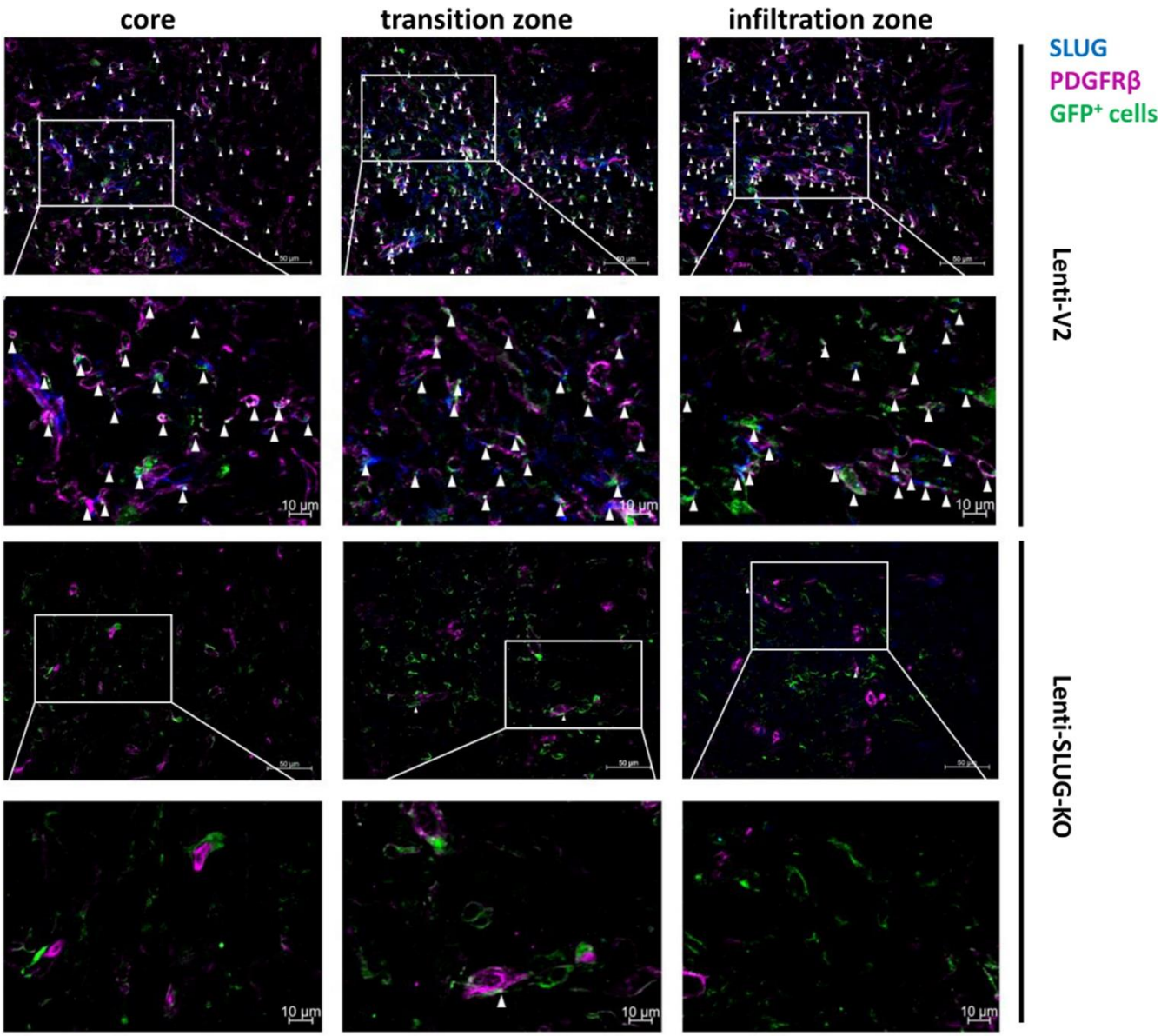
Supplementary Figure 6. Identification of αSMA and PDGFRβ in GBM-associated VAMCs in the infiltration zone of PAR- and TGF-β-KO-GBM bearing RGS5-GFP reporter mice. A. Immunofluorescence of VAMCs (green), αSMA (blue), PDGFRβ (magenta) in the infiltration zone (GL261: red). One picture is exemplarily shown, 25x magnification, bars: 50 µm. **B.** Merged photograph of GFP/αSMA/PDGFRβ triple positive cells indicated by white arrows, 64x magnification. **C.** Quantification of GFP positive and **D.** of αSMA/PDGFRβ/GFP positive cells (n=3 mice, 3-9 sections per mouse, SEM, t-test, ** p<0.01, **** p<0.0001).

Supplementary Figure 07



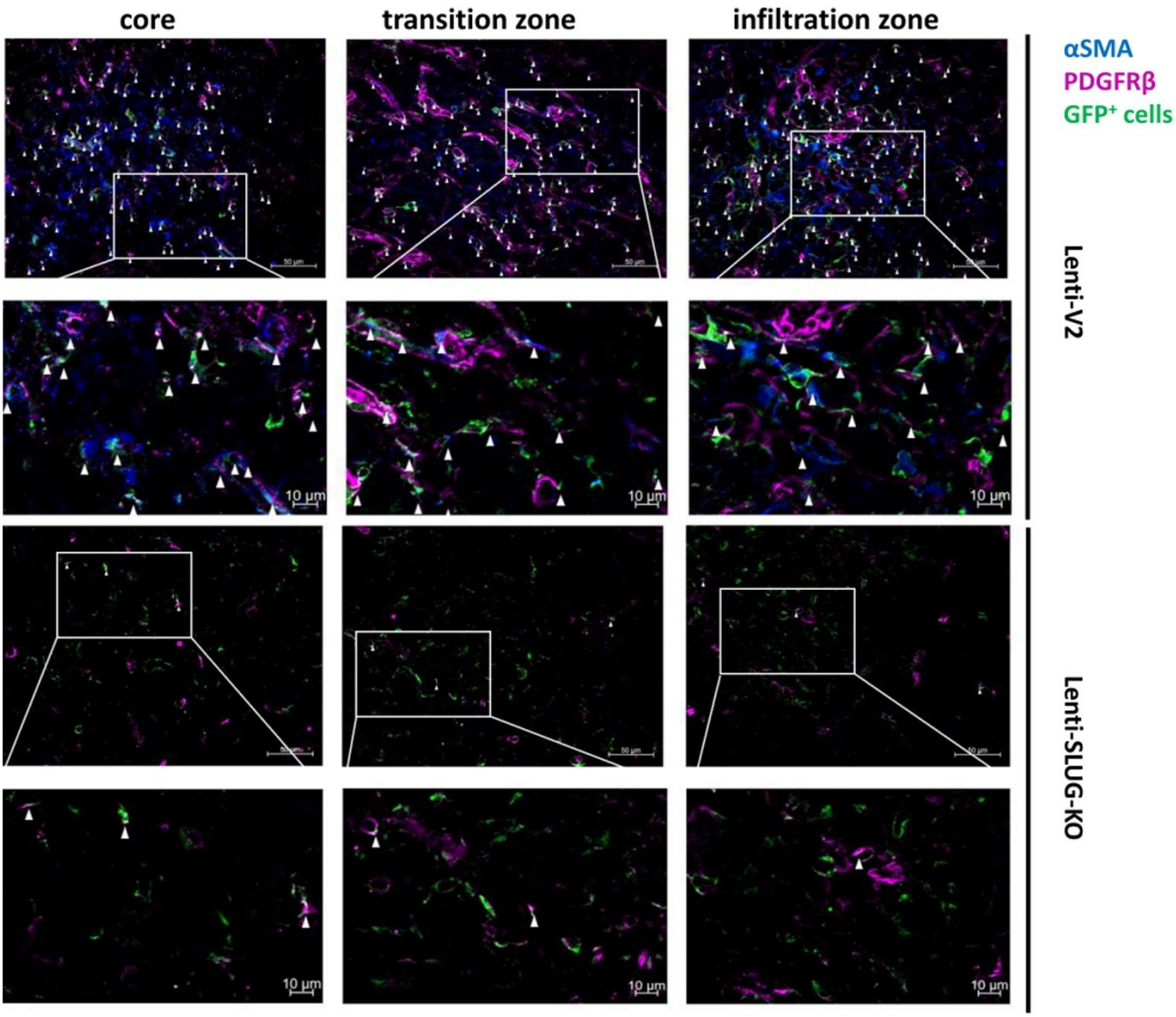
Supplementary Figure 7: Detection of SLUG expression in the tumor area of PAR-GBM harboring RGS5-GFP reporter mice that received an injection of Lenti-CRISPRv2 or Lenti-RGS5-CRISPR-SLUG (64x magnification, bars: 10 µm).

Supplementary Figure 08



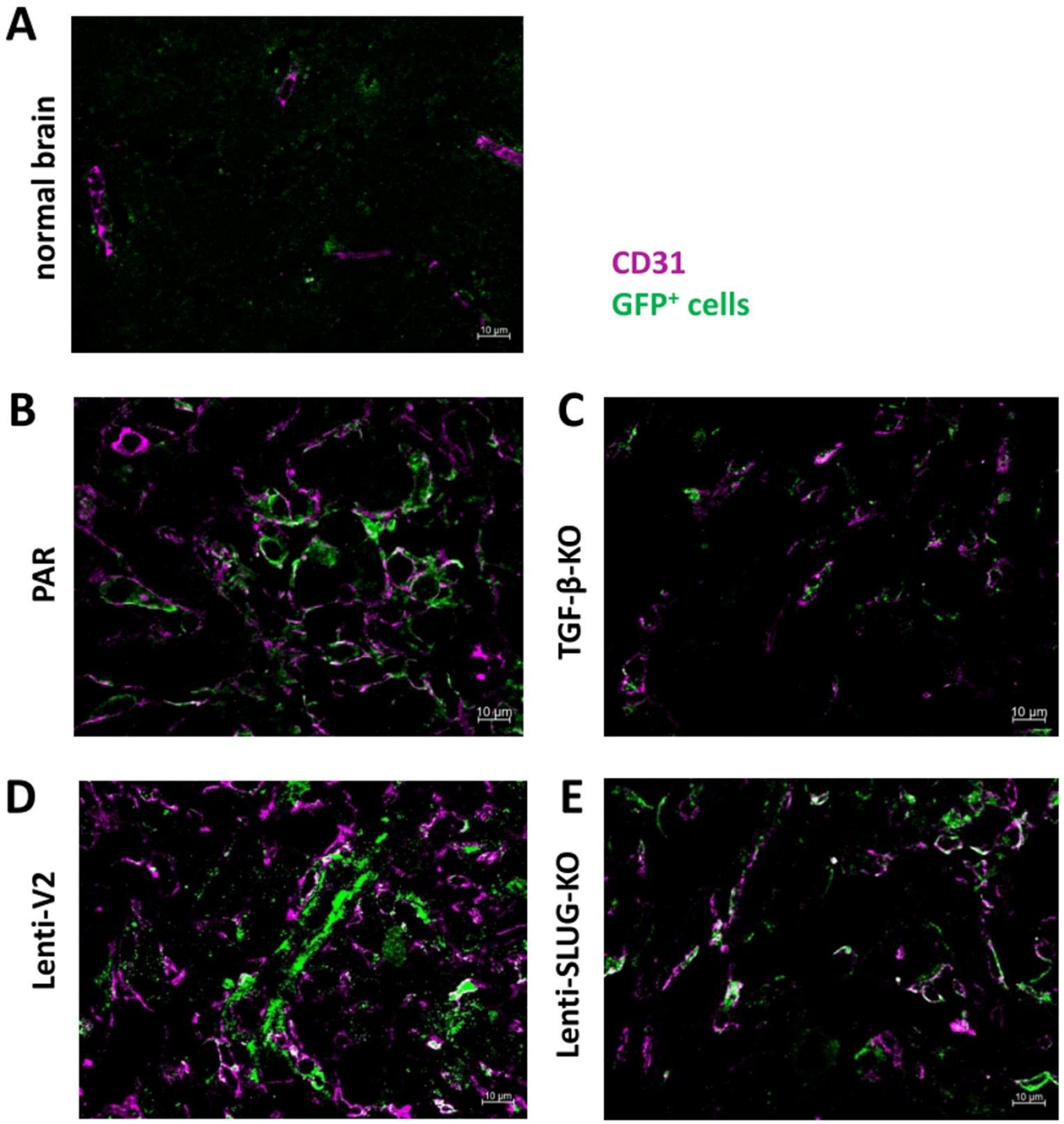
Supplementary Figure 8. Identification of SLUG/PDGFRβ double positive VAMCs in the tumor core, transition and infiltration zone of PAR-GBM bearing RGS5-GFP reporter mice that received an intrastriatal injection of either Lenti-v2 or Lenti-SLUG-KO. SLUG/PDGFRβ/GFP triple positive cells were counted manually (white arrows). One picture is exemplarily shown (n=2 mice, 24 slices/mouse; bars: upper panels: 50 µm, lower panels: 10 µm).

Supplementary Figure 09



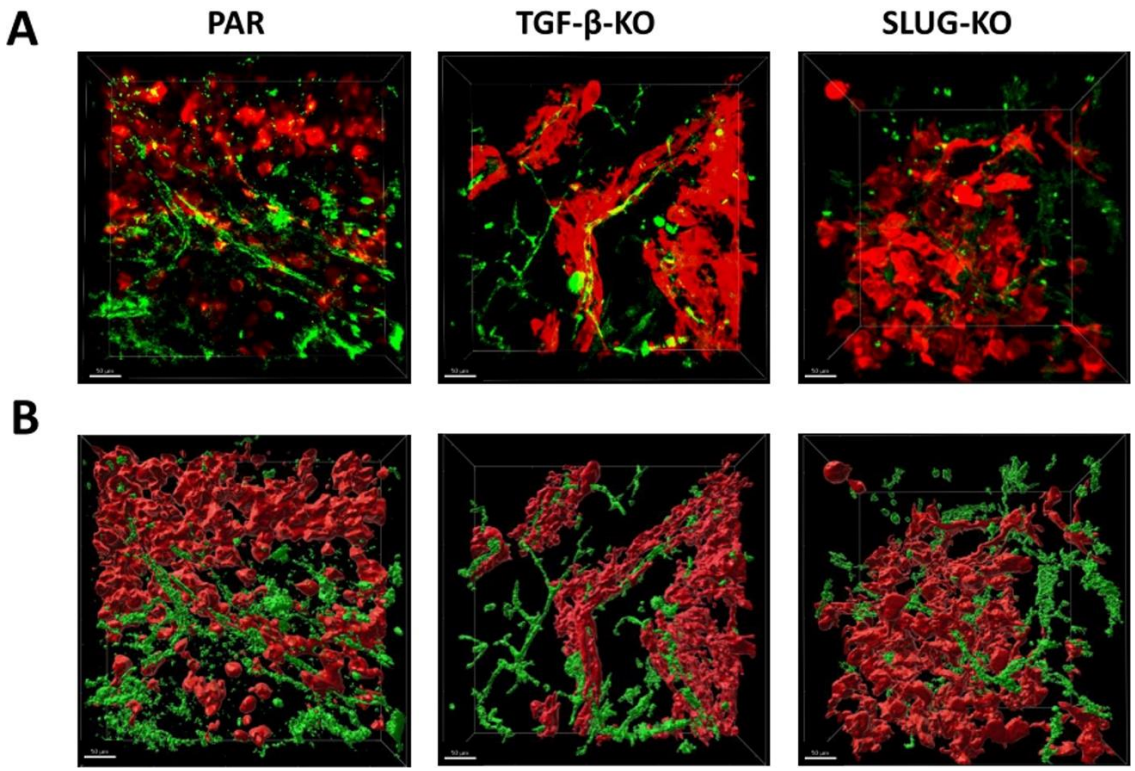
Supplementary Figure 9. Identification of αSMA/PDGFRβ double positive VAMCs in the tumor core, transition and infiltration zone of PAR-GBM bearing RGS5-GFP reporter mice that received an intrastriatal injection of either Lenti-v2 or Lenti-SLUG-KO. αSMA/PDGFRβ/GFP triple positive cells were counted manually (white arrows). One picture is exemplarily shown (n=2 mice, 24 slices/mouse; bars: upper panels: 50 µm, lower panels: 10 µm).

Supplementary Figure 10



Supplementary Figure 10. Visualization of vessel structure and VAMC coverage. The vascular structure consisting of endothelial cells (CD31, magenta) and adjacent VAMCs (GFP, green) is shown for the contralateral, no tumor containing hemisphere, in the normal brain **(A)**, in PAR **(B)**, in TGF-β-KO tumors **(C)** as well in mice harboring PAR tumors and injected with either Lenti-V2 **(D)** or Lenti-SLUG-KO **(E)**. Images were taken at 63x magnification (n=2 mice per group; Scale bars: 10 µm).

Supplementary Figure 11



Supplementary Figure 11. Visualization of intratumoral vessel morphology after knocking out TGF-β in GBM cells or specifically knocking out SLUG in VAMCs. Pictures exemplarily show CD31 (green) and mCherry fluorescence (GBM cells, red) in a RGS5-GFP mouse bearing either a PAR tumor without further treatment (PAR, left), in a mouse bearing a TGF-β-KO tumor (TGF-β-KO, middle), or in a mouse bearing a PAR tumor that received an intrastriatal injection of Lenti-SLUG-KO (SLUG-KO, right) prior **(A)** and post **(B)** 3D-reconstruction by using Imaris. All images were taken at 20x magnification (n=1 mouse per group; Scale bars 50 µm).

Supplementary Table 01

F1 5' AAA*GCTAGC*CACACCTGCATCATCAGAGCCTGGAC 3'

F4 5' AAA*GCTAGC*TGGCTGTTCTCTCCTTCATAGCTAC 3'

R1 5' AAA*TCTAGA*TGGAAAGTCCCAGCTTTAGTGAATAAA 3'

R2 5' AAA*TCTAGA*ATCAGTTTCATCTCAGCGGGACCCC 3'

R3 5' AAA*TCTAGA*GTGTCTCAGGGACTTTTTTTCCCCCAG 3'

R4 5' AAA*TCTAGA*TTTGGCTGGCGGCTTAGATCCTCAAC 3'

Supplementary Table 1. Primers used for the amplification of the murine RGS5 minimal promoter (NheI and XbaI cleavage sites are shown in italic letters).

Supplementary Table 02

mSLUG Oligo 1.1 5' CACCGAAGACCTATTCTACGTTCTC 3'

mSLUG Oligo 1.2 5' AAACGAGAACGTAGAATAGGTCTTC 3'

mSLUG Oligo 4.1 5' CACCGGACGATGTCCATACAGTAAT 3'

mSLUG Oligo 4.2 5' AAACATTACTGTATGGACATCGTCC 3'

mSLUG Oligo 5.1 5' CACCGGATGAGGAGTATCCAGTAAG 3'

mSLUG Oligo 5.2 5' AAACCTTACTGGATACTCCTCATCC 3'

Supplementary Table 2: Oligos serving as guide RNAs and used for cloning into pLentiCRISPR v2.