Figure S1. Apelin production by brain endothelial cells. (A) RT-PCR was used to check for the presence of the mRNA corresponding to the hits identified in the MS screen. Cytokines: 1. ADM, 2. APLN, 3. CTGF, 4. FSTL1, 5. PTX3, 6. IGFBP7, 7. MIF, 8. TGFβ2, 9. LGALS1; Proteases: 1. CST3, 2. SERPINE1, 3. TIMP1, 4. PRSS23, 5. CTSS, 6. SRGN; Extracellular matrix: 1. LGALS3BP, 2. FN1, 3. THBS1, 4. EFEMP1, 5. HSPG2, 6. LAMA5, 7. EDIL3. (B) Peptide sequences corresponding to apelin found in MS are indicated. Mass: observed mass; ppm: parts per million; Expectation: number of matches with equal or better scores that are expected to occur by chance alone. (C) Peptide View. MS/MS fragmentation of SLMLPDPGDGNLEDGNVRH sequence. (D) APLN mRNA expression in non-tumour and glioblastoma tissue using the TCGA, Rembrandt and Gravendeel databases. Data were analysed via the GlioVis platform (https://gliovis.shinyapps.io/GlioVis/).
Figure S2. In vivo characterisation of patient-derived glioblastoma cells with stem properties (GSC). (A) $5 \times 10^5$ patient-derived Glioblastoma cells with stem properties (GSCs #1, #2, #4 and #9) were implanted in flanks of nude mice and number of non-regressing palpable tumours was scored over time. n=4. (B) Different amounts of GSC#9 were implanted in flanks of nude mice and number of non-regressing palpable tumours was scored at week5. n=6. (C) $5 \times 10^5$ GSC#9 and GSC#1 cultured either as sphere (sph.) or adherent and differentiated (adh.) were implanted in flanks of nude mice and number of non-regressing palpable tumours was scored over time. n=8. (D) GSCs#9 were infected with control shRNA (shc), and shRNA targeting APLNR (sequence #1 and sequence #2) and 10^5 were implanted in female nude mice. Animals were sacrificed at week 5 post-surgery and the presence of tumours was determined by HE staining (absence, red; presence, green on the graph). n=5 mice/group. All panels are representative of n=3, unless specified.
Figure S3. Pre-tolerance studies of MM54 in healthy mice. (A) C57Bl/6 mice were injected twice a week with MM54 and monitored for toxicity. (B) weight loss, (C) cardiac, (D) kidney and (E) liver function. n=4 mice/group, means±SEM.
Figure S4. Efficacy of the APLNR antagonist MM193 in reducing xenograft progression. (A) Efficacy of the APLNR antagonist MM193 in reducing GSC#9 TS formation in response to increasing doses of the MM54 brother compound MM193 in endothelial cell conditioned medium (EC-CM) and apelin-supplemented mitogen-free media. n=3 means±SEM. **p<0.01; ***p<0.001. (B) Nude mice were inoculated with GSC#9 and monitored for tumour growth following treatment with MM193. n=6 mice/group, means±SEM. **p<0.01. (C) Analysis of cardiac frequency, blood pressure and glycaemic index in response to MM193 treatment in healthy C57Bl/6 mice. n=4 mice/group, means±SEM.