**Long noncoding RNA *SChLAP1* forms a growth promoting complex with HNRNPL in human glioblastoma through stabilization of ACTN4 and activation of NF-κB signaling**

**Supplementary Figure Legends**

**Supplementary Figure S1.** (**A**) RNA-ISH performed on non-neoplastic brain tissues derived from four regions of the brain, including parietal, temporal, and occipital lobes, and the basal ganglia. Scale bar = 100 µm (top) or 50 µm (bottom).

**Supplementary Figure S2. Silencing of *HNRNPL* inhibits proliferation of GBM cells**. (**A**) Summary of proteins forming protein-complexes with *SChLAP1* antisense and sense, respectively. (**B**) Western blot to examine knockdown efficiency of siRNAs for *GRSF1*, *ENO1*, *HNRNPL* and *HSP90AB1*. (**C**) Growth curves of U118MG and LN229 cells transfected with control and siRNAs targeting *GRSF1*, *ENO1*, *HNRNPL* and *HSP90AB1* as determined using cell counting. (n.s. = not significant, \*\**P* < 0.01)

**Supplementary Figure S3. *SChLAP1* promotes the growth of GBM cells *in vitro* and *in vivo****.* U118MG and BG7 cells were infected with empty or *SChLAP1*-, *SChLAP1*-Δexon2- and *SChLAP1*-AS-expressing lentiviral constructs. Ectopic expression efficiency was analysed with (**A**) qRT-PCR. GAPDH served as the internal control. Cell growth was examined with (**B**) cell counting. (**C, D**) *In vivo* bioluminescent images and quantification of U118MG and BG7-NC and -*SChLAP1*-OE, -*SChLAP1*-Δexon2-OE and *SChLAP1*-AS-OE derived xenografts at the indicated time points. (**E**) Kaplan–Meier survival analysis performed with survival data from mice implanted with U118MG and BG7-NC and -*SChLAP1*-OE, -*SChLAP1*-Δexon2-OE and *SChLAP1*-AS-OE cells. Log-rank test, *SChLAP1*-OE *vs* NC: *P* < 0.05; *SChLAP1*-Δexon2-OE or *SChLAP1*-AS-OE *vs* NC: *P* = n.s. (n.s. = not significant, \**P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001)

**Supplementary Figure S4.** Schematic representation ofthe folding and domains of HNRNPL in (**A, left panel**) and interpolated charges in (**A, right panel**). (**B**) Western blot analysis of ACTN4 and HNRNPL protein levels in *SChLAP1*-modified GBM cells. GAPDH was served as the internal control.

**Supplementary Figure S5. ACTN4 is highly expressed in GBM and is associated with poorer survival in glioma patients**. (**A**) Representative images of IHC staining with antibodies targeting ACTN4 in primary tissue microarrays and NBT. Representitave image of NBT was from frontal lobe, scale bar = 100 µm (top) or 50 µm (bottom). For glioma tissue microarrays, scale bar = 200 µm (top) or 50 µm (bottom). (**B**) Graphic representation of scoring performed on IHC staining for ACTN4 in primary tissue samples and NBT. Overall survival analysis of *ACTN4low* and *ACTN4high* groups in glioma patients from (**C**) Rembrandt and CGGA databases (Log-rank test, *P* < 0.001). (**D**) qRT-PCR to determine expression of *SChLAP1* and western blotting to detect corresponding protein levels of ACTN4 and HNRNPL in NHA and various GBM cell lines. For qRT-PCR, GAPDH served as the internal control for normalization, and expression of *SChLAP1* in GBM cell lines was assessed relative to expression of *SChLAP1* in NHA. (**E**) Correlation of *SChLAP1* and ACTN4 expression levels from primary tissue microarray data, two-tailed χ2 test, *P* < 0.001. (n.s. = not significant, \*\**P* < 0.01, \*\**P* < 0.001)

**Supplementary Figure S6.** (**A**) IHC performed on non-neoplastic brain tissues derived from four regions of the brain, including parietal, temporal, and occipital lobes, and the basal ganglia. Scale bar = 100 µm (top) or 50 µm (bottom).

**Supplementary Figure S7. *SChLAP1* activates NF-κB signaling through regulation of nuclear translocation of the p65 subunit**. (**A**) Heatmap showing the relationship between *ACTN4*-associated genes and representative clinical features. Correlation analysis was performed using CGGA mRNA-sequencing data to identify *ACTN4*-correlated genes. (**B**) Venn diagram illustrating common genes between *ACTN4*-correlated genes and Rel/ NF-κB target genes. Luciferase activity from NF-κB luciferase reporter constructs in control and *SChLAP1*-modified (**C**) LN229 and (**D**) U118MG cells. (**E**) Western blot to detect cytoplasmic and nuclear p65 protein in control and *SChLAP1*-modified LN229 and U118MG cells. (**F**) Immunofluorescence staining for p65 (Red) in control and *SChLAP1*-modified LN229 and U118MG cells showing cellular localization. Nuclei were labeled with DAPI (blue), and images were merged. Scale bar = 50 µm. (\**P* < 0.05, \*\**P* < 0.01)

**Supplementary Table S1.** Clinical data for individual patients.

**Supplementary Table S2.** Oligonucleotide sets used in this study

**Supplementary Table S3.** Plasmids used in this study

**Supplementary Table S4.** Primer sets used in this study

**Supplementary Table S5.** Association between *SChLAP1* expression and clinicopathological factors in glioma

**Supplementary Table S6.** Proteins binding with *SChLAP1* sense and antisense

**Supplementary Table S7.** Most significant decreasing protein partners of HNRNPL after *SChLAP1* knockdown

**Supplementary Table S8.** Association between ACTN4 expression and clinicopathological factors in glioma

**Supplementary Materials and Methods**

**Histologic evaluation system for RNA-ISH and IHC staining**

The intensity of staining for ACTN4-IHC and *SChLAP1*-ISH was scored using a scale of 0–3: 0 (negative), 1 (weakly positive, light yellow), 2 (moderately positive, yellowish brown), and 3 (strongly positive, brown). The percentages of positive staining cells were also scored based on a scale of 0–3: 0 (0%), 1 (1% to 33%), 2 (34% to 66%), and 3 (67% to 100%). The sum of the intensity and percentage scores was used as the final staining score. Low expression in tumors was defined as a total score of 0–3, and high expression was defined as a total score of 4–6.

**Antibodies used**

For western blotting, the following antibodies were used: HNRNPL (ab6106, Abcam; Cambridge, MA; 1:1000), Histone H3 (ab176842, Abcam; 1:1000), GRSF1 (ab205531, Abcam; 1:1000), ENO1 (ab155102, Abcam; 1:1000), HSP90AB1 (ab32568, Abcam; 1:1000), ACTN4 (ab108198, Abcam; 1:1000), GAPDH (ab181602, Abcam; 1:10000), p65 (#8242, Cell Signaling Technology; Beverly, MA; 1:1000), Flag-tag (#14793, Cell Signaling Technology; 1:1000), HA-tag (#3724, Cell Signaling Technology; 1:1000); Flag-tag (F1804, Sigma-Aldrich; St. Louis, MO; 1:1000); Ub (sc-8017, Santa Cruz Biotechnology; Dallas, TX; 1:200).

Anti-p65 (#8242, Cell Signaling Technology; 1:200) was used in immunofluorescence assays.

For immunohistochemistry, anti-ACTN4 (ab108198, Abcam), anti-ATRX (ab97508, Abcam) and anti-IDH1 (R132H) (Sigma) were used at a dilution of 1:200.

Co-IPs were performed with non-specific IgG or antibodies specific for HNRNPL (ab6106, Abcam; 4 µL), ACTN4 (ab108198, Abcam; 4 µL); HA-tag (#3724, Cell Signaling Technology; 4 µL), and Flag-tag (F1804, Sigma-Aldrich; 4 µL).