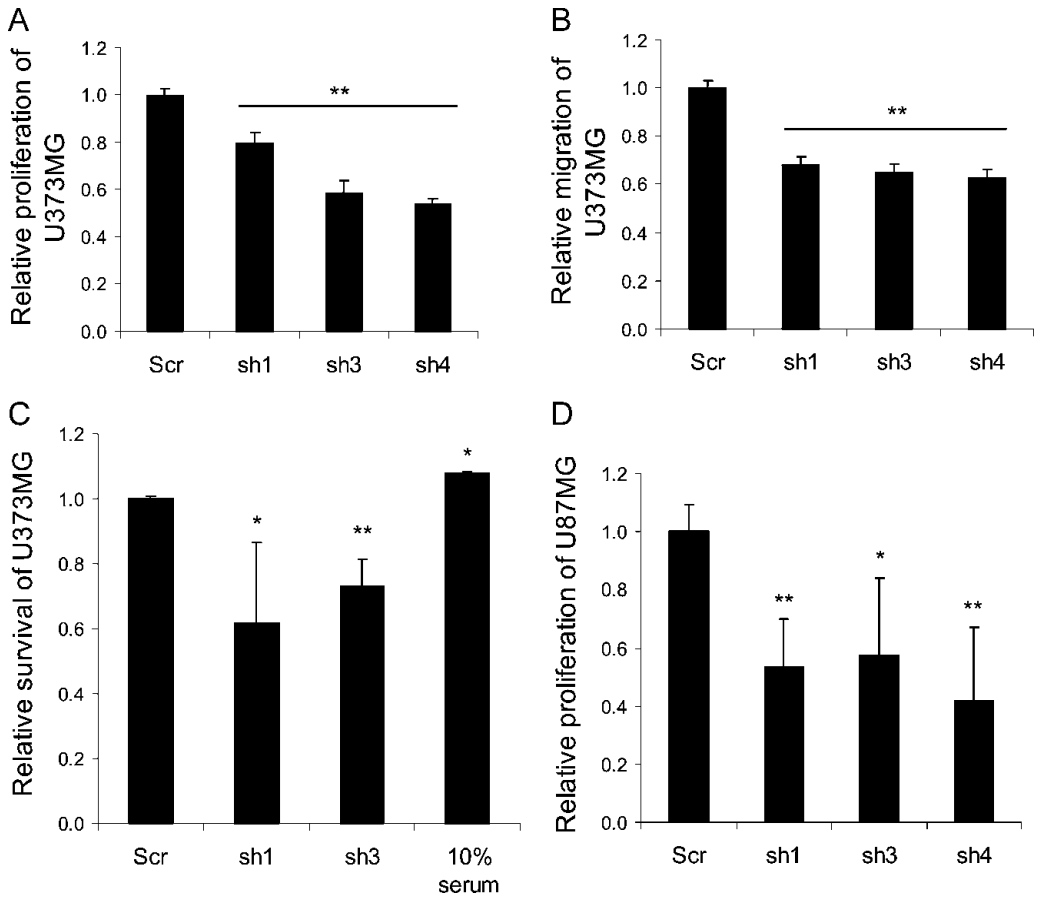


Figure W1. Suppression of NTN4 expression inhibit glioblastoma cell proliferation and motility. (A, B) Suppression of NTN4 gene expression in U373MG cells significantly reduced both cell mitogenic ability and motility compared with cells transfected with nontargeting scrambled control (Scr). (C) Suppression of NTN4 expression increased U373MG cell apoptosis caused by serum deprivation compared with the nontargeting control (Scr). Cells cultured in 10% serum were set as positive control. (D) Suppression of NTN4 gene expression also reduced U87MG cell mitogenicity. Mean ± SE, $n \geq 3$. $^{**}P < .01$. $^{*}P < .05$.

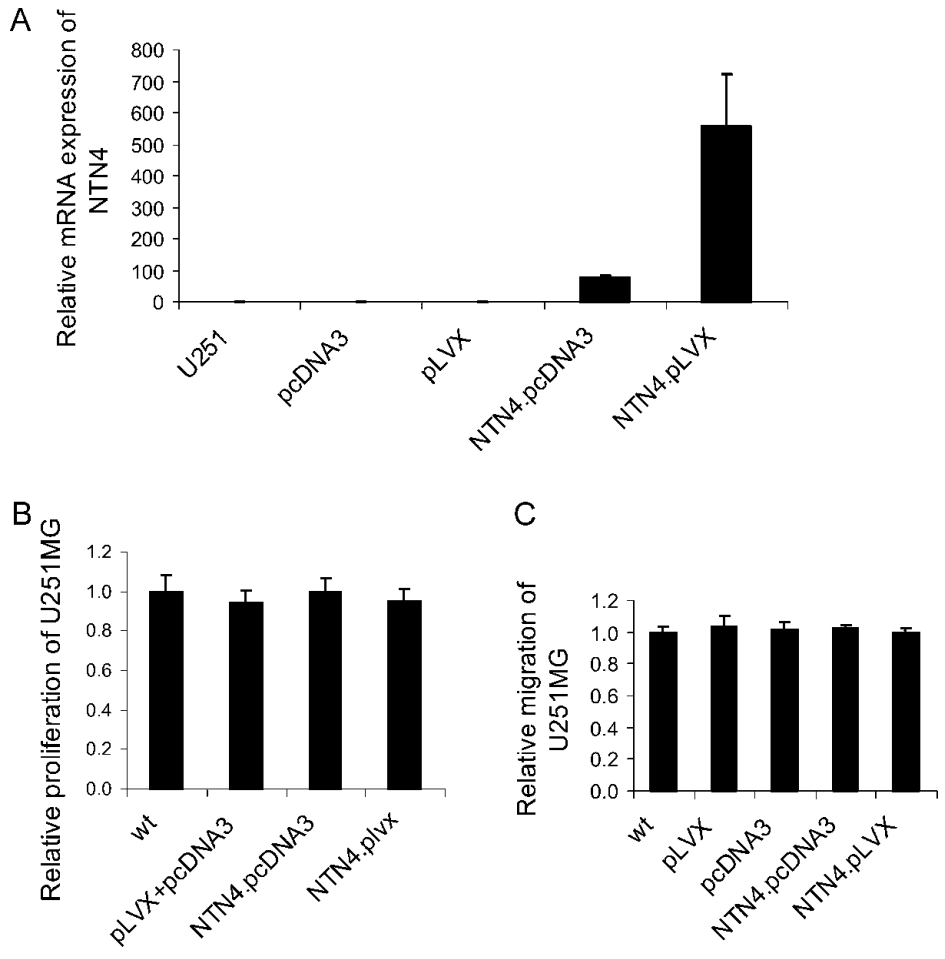


Figure W2. Stable overexpression of NTN4 has no significant effects on U251MG cell proliferation and migration. Q-RT-PCR was used to detect the expression of NTN4 at the mRNA level in NTN4-overexpressing U251MG cells. The NTN4 mRNA is increased ~50-fold in NTN4.pcDNA3-transfected U251MG cell and ~550-fold in NTN4.pLVX-transfected U251MG cell (A). The wild-type, empty vector control and NTN4-overexpressing U251MG cells proliferated (B) and migrated (C) at comparable rates.

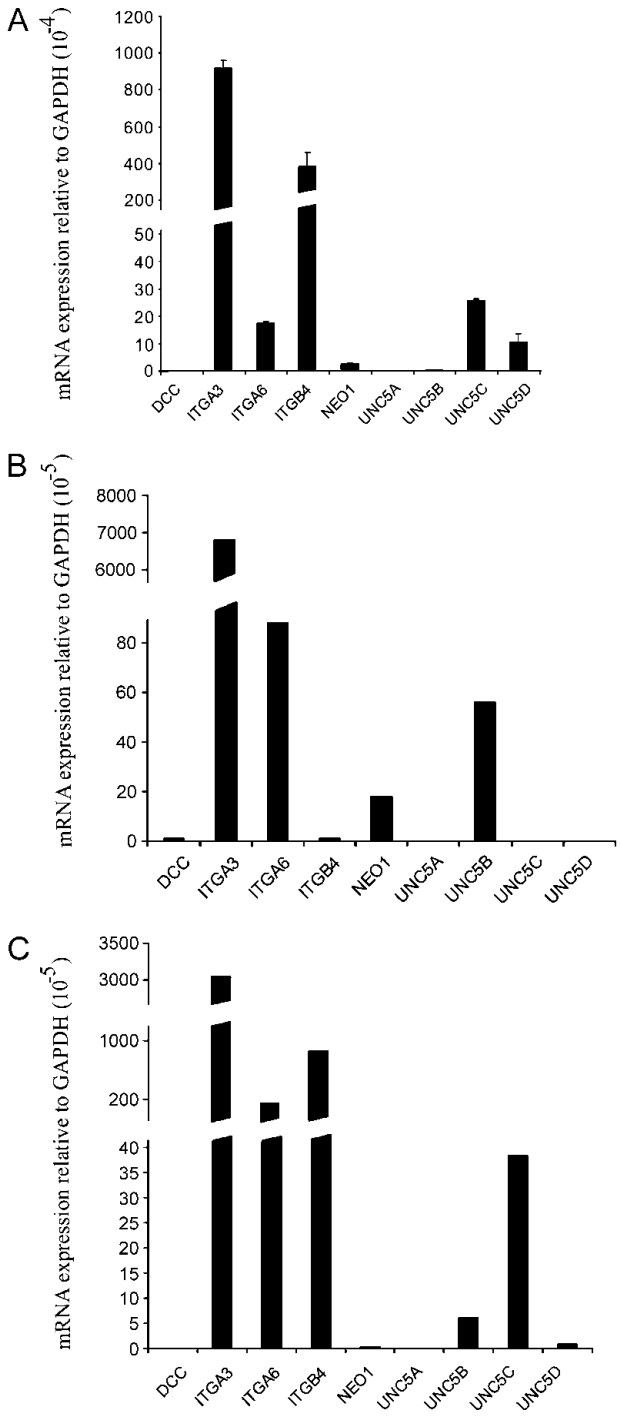
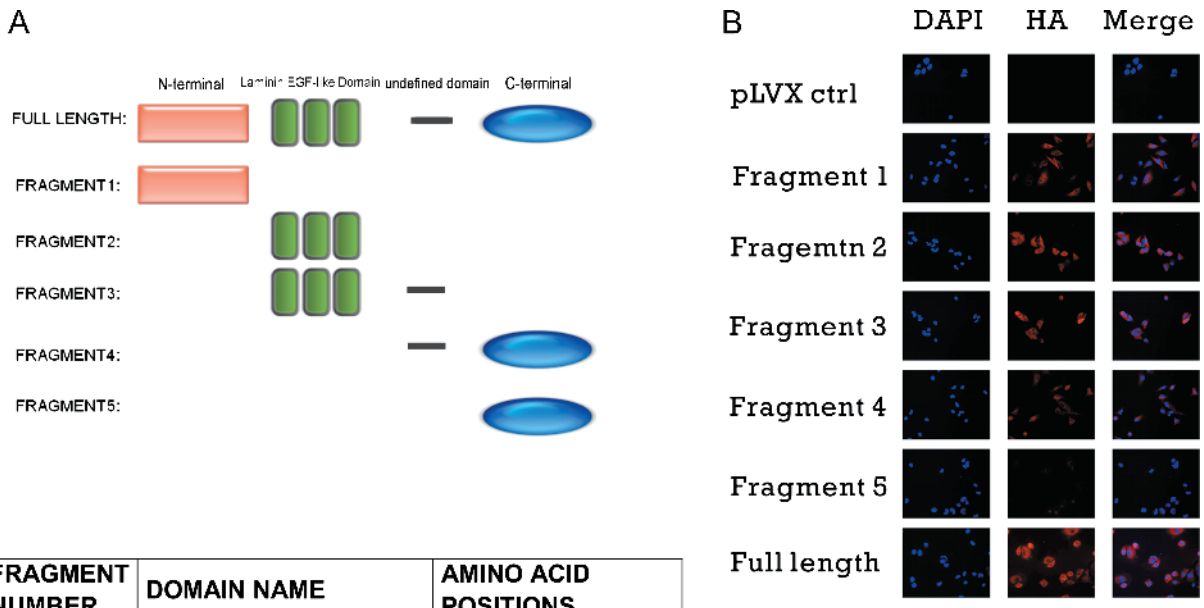


Figure W3. The expression of various receptors of NTN1 in glioblastoma cell lines. U251MG, U87MG, and U373MG cells were cultured to ~50% confluence and subsequently used for Q-RT-PCR. The expression levels of NTN1 receptors were compared with the expression of GAPDH. (A) U251MG cells express ITGA3, ITGA6, ITGB4, UNC5C, and UNC5D at decent levels; U251MG also express NEO1 and UNC5B at low levels. However, DCC and UNC5A were absent in U251MG. (B) U87MG cells express ITGA3, ITGA6, NEO1, and UNC5B at decent levels and express DCC and ITGB4 at low levels. However, UNC5A, UNC5C, and UNC5D are absent in U87MG. (C) U373MG cells express ITGA3, ITGA6, ITGB4, and UNC5C at decent levels and express NEO1, UNC5B, and UNC5D at low levels. However, DCC and UNC5A are absent in U373MG.



FRAGMENT NUMBER	DOMAIN NAME	AMINO ACID POSITIONS
Fragment 1	N-terminus	1-261
Fragment 2	Laminin EGF-like domain	261-445
Fragment 3	Laminin EGF-like domain and unidentified domain	261-516
Fragment 4	unidentified domain and C-terminus	445-628(without stop codon)
Fragment 5	C-terminus	516-628(without stop codon)
FL	FULL LENGTH	1-628(without stop codon)

Figure W4. TAP-TAG and mass spectrometry analysis. (A) We separated NTN4 full-length sequence to five fragments based on functional domains. (B, C) We successfully obtained U251MG cells expressing FLAG/HA–tagged peptides and full-length NTN4 cDNA. Immunofluorescence and immunoblot analysis with anti-HA antibody were performed to confirm target sequence expression.

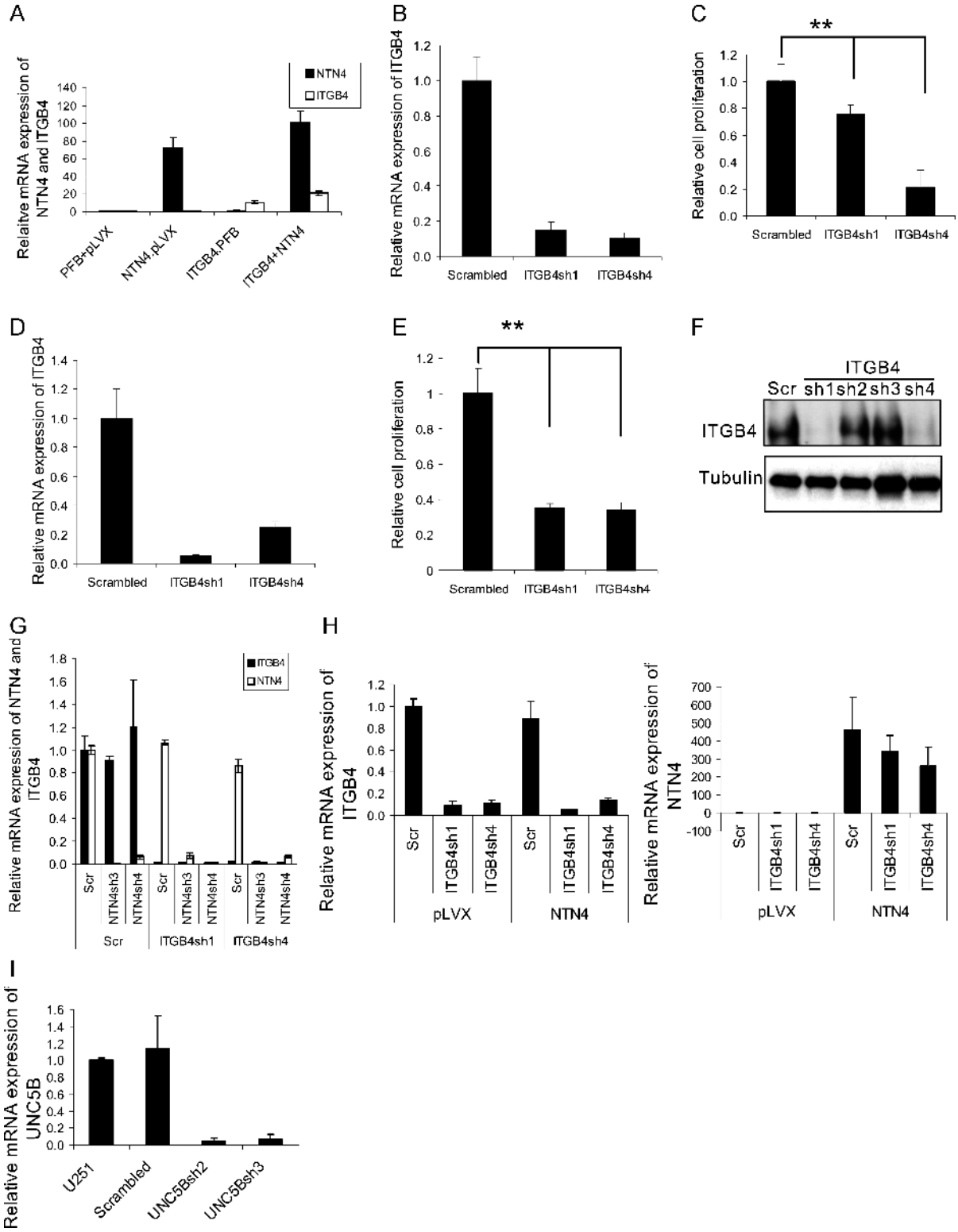


Figure W5. The expression level of NTN4 and ITGB4 in various cell lines and suppression of ITGB4 reduce U87MG and U373MG cell proliferation. (A) Q-RT-PCR was used to detect the expression of NTN4 and ITGB4 at mRNA level in NTN4/ITGB4 transfected cells. The expression of NTN4 increased ~70-fold more in NTN4.pLVX transfected U251MG cells and ~100-fold for NTN4/ITGB4 combined transfected U251MG cells. The expression of ITGB4 increased ~15-fold in ITGB4.PFB transfected U251MG cells and ~20-fold in NTN4/ITGB4 combined transfected U251MG cells. (B, C) Knocking down ITGB4 in U87MG reduced cell proliferation. (D, E) Suppression of ITGB4 in U373MG inhibited cell proliferation. (F) Two most efficient shRNAs of ITGB4 significantly inhibited ITGB4 expression in U251MG at protein level. (G) We successfully suppressed the expression of ITGB4 and NTN4 either alone or together in U251MG cells at the mRNA level. (H) We obtained U251MG cell lines with ITGB4 silencing and NTN4 overexpression. (I) Q-RT-PCR was performed to detect UNC5B silencing effects in U251MG cells. UNC5B shRNAs resulted in UNC5B mRNA reduction of approximately 96% for shRNA2 and 94% for shRNA3.

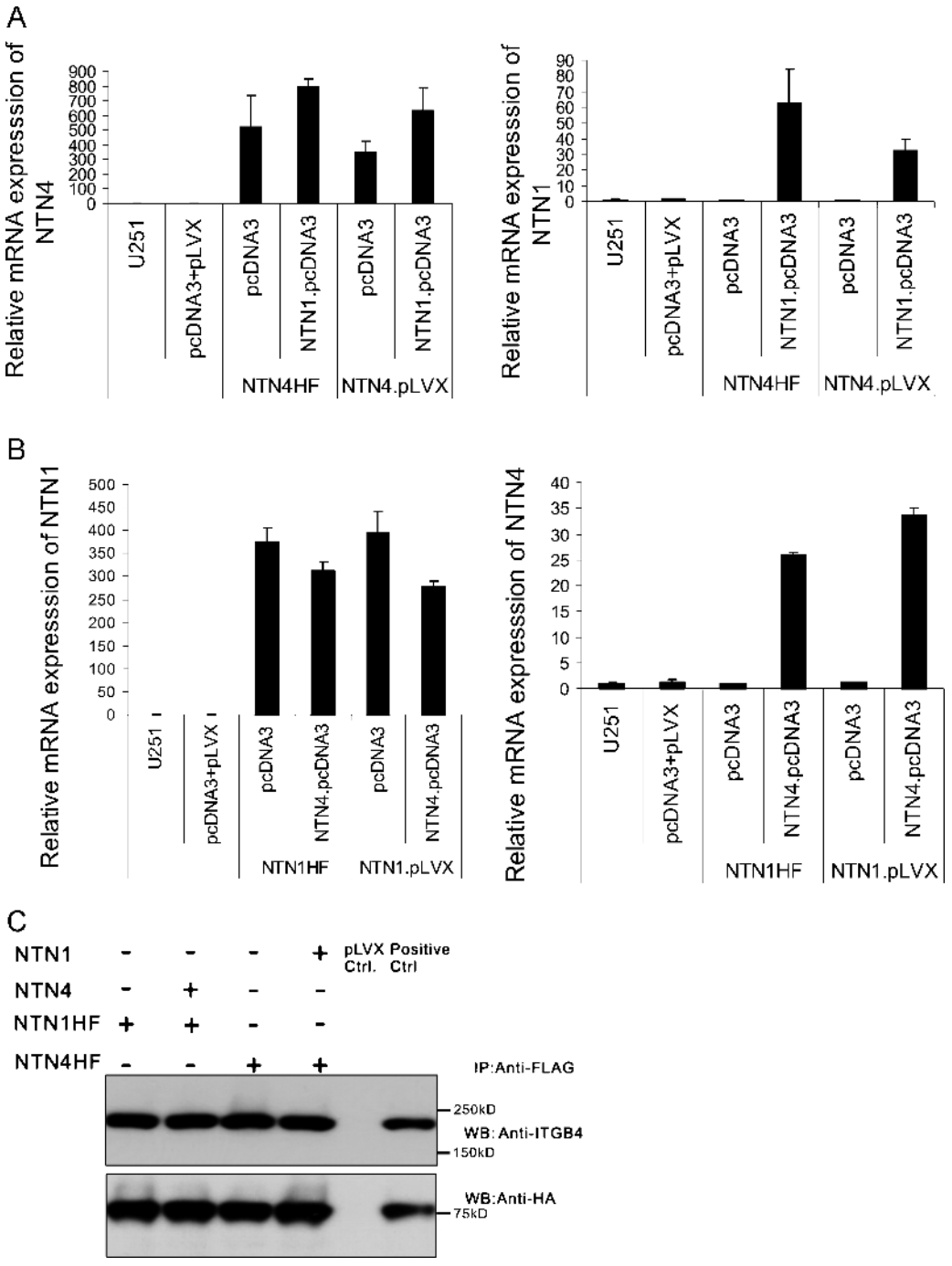


Figure W6. NTN4 binds to ITGB4 independently of NTN1/ITGB4 interaction. (A, B) We established two U251MG cell lines overexpressing NTNs, as either tagged or native proteins (NTN1FH/NTN4; NTN4FH/NTN1). Meanwhile, tagged NTN1-overexpressing U251 cell (NTN1FH) and tagged NTN4-overexpressing U251 cell (NTN4FH) were used as controls. We used Q-RT-PCR to determine the expression levels of NTN4 or NTN1 at the mRNA level in these cell lines. (C) The cells were lysed and subjected to immunoprecipitation with anti-FLAG agarose and immunoblot analysis with ITGB4 antibody. Overexpression of native NTN1 did not compete with NTN4 for binding to ITGB4. Conversely, overexpression of native NTN4 did not compete with NTN1 for binding to ITGB4. Immunoblot analysis with HA antibody was applied to confirm the equal expression levels.

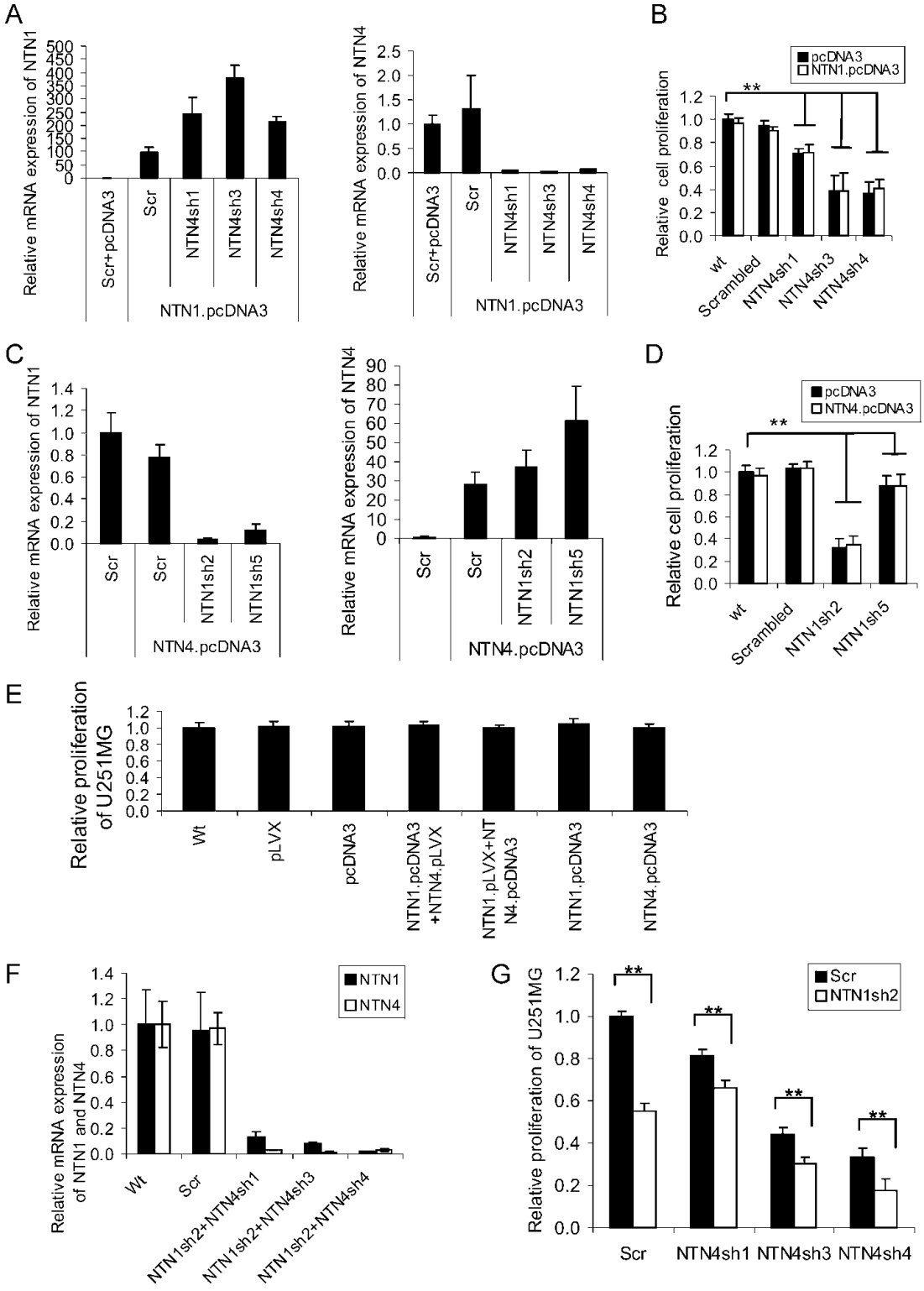


Figure W7. Functional relationships between NTN4 and NTN1 in the promotion of glioblastoma cell growth. (A) NTN1 was overexpressed in U251MG cells (NTN1.pcDNA3). In addition, NTN4 expression was silenced by the short hairpin system in NTN1-overexpressing U251 cells. (B) BrdU incorporation assay indicated that NTN1 overexpression did not alter cell growth. Furthermore, suppression of NTN4 in NTN1-overexpressing cells or control cells decreased cell proliferation to the same extent compared with nontransfected cells (wt) or to nontargeting control cells. (C) We knocked down NTN1 expression in U251MG cells. Q-RT-PCR was applied again to determine NTN1-silencing effects at the mRNA level. (D) Decreased NTN1 expression in control U251MG cells resulted in the inhibition of cell proliferation, and NTN4 overexpression did not neutralize this effect. (E) U251MG cells were stably transduced with the NTN4 gene, the NTN1 gene, or the empty vector control, respectively, or in combination. BrdU incorporation assay was performed. Single or combined overexpression of NTN4 or NTN1 in U251MG cells had no significant effects on cell proliferation compared with empty vector control. (F) U251MG cells were infected with scrambled control or NTN4/NTN1 shRNA separately or in combination. The expression levels were confirmed by Q-RT-PCR analysis at the mRNA level. (G) Suppression of NTN1 in NTN4 silenced U251MG cells was still able to reduce cell proliferation. Mean ± SE, $n \geq 3$. $^{**}P < .01$. $^{*}P < .05$.

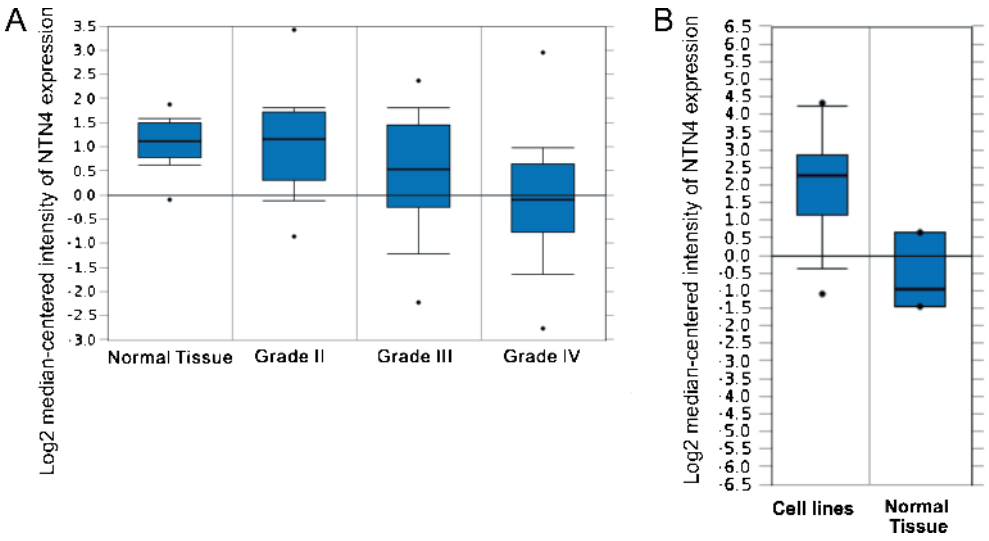


Figure W8. The expression of NTN4 during glioma progression and in glioblastoma cell lines. (A) Published microarray data from www.oncomine.org revealed that glioma cells tend to decrease NTN4 expression after glioma progression. The $\log_2$ median of centered intensities of NTN4 is 1.121 (normal tissue), 1.161 (grade 2 glioma), 0.535 (grade 3 glioma), −0.097 (grade 4 glioblastoma). (B) NTN4 expression in 20 glioblastoma cell lines *versus* normal brain tissue. The $\log_2$ median of centered intensities of NTN4 in 20 glioblastoma cell lines is 2.242, whereas the value is −0.983 in normal brain tissue. This suggests that most glioblastoma cell lines express NTN4.