Supplemental information

LncRNA PVT1 promotes tumorigenesis of glioblastoma by recruiting COPS5 to deubiquitinate and stabilize TRIM24

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Supplementary Figures and Tables

**Fig. S1** Kaplan–Meier analyse association between *PVT1* level and prognosis of glioma patients in publicly available dataset GSE108476 and TCGA.

![Graphs showing Kaplan–Meier survival analysis](image)

**Fig. S2** (A,B) The RT-qPCR results show the efficiencies of *PVT1* knockdown in U251 and U373 cells and *PVT1* overexpression in LN229 cells. (C) Knockdown of *PVT1* supressed cell proliferation in U373 cells via CCK-8 assays. (D,E) Knockdown of *PVT1* inhibited colony formation ability in U373 cells. Scale bars, 10 mm. Data are presented as mean ± SEM. *p < 0.05. ** p < 0.01. *** p < 0.001.
Fig. S3 (A) WB analysis the expression of p-STAT3 after intervention of *PVT1* expression. (B) Re-expression of TRIM24 after *PVT1* deletion in U373 cells restores cell proliferation. (C,D) Re-expression of TRIM24 after *PVT1* deletion in U373 cells restores colony formation ability. (Scale bars, 10 mm). (E) WB analysis the expression of p-STAT3 after transfected with *PVT1*sh or TRIM24OE in U373 cells. Data are presented as mean ± SEM. *p < 0.05. **p < 0.01. ***p < 0.001.
Fig. S4 (A) WB analysis the expression of TRIM24 protein after overexpression of 30 human DUBs in 293T cells. (B) Quantification of TRIM24 protein levels in Fig.5C. Data are presented as mean ± SEM. ** $p < 0.01$. *** $p < 0.001$.

Fig. S5 Immunoblotting for detection of MYC-tagged USP4 and COPS6 pull down by PVT1 in 293T cells.
**Fig. S6** (A, B) RT-qPCR analysis effects of intervention of COPS5 expression on TRIM24 mRNA expression in glioma cells. (C) Knockdown of COPS5 decreases TRIM24 protein expression in U251 cells, while overexpression of COPS5 elevates TRIM24 protein expression. (D) Representative images of co-localization of TRIM24 with COP55 in U373 cells. Scale bar, 40 μm. (E) Quantification of TRIM24 protein levels in Fig. 6D. Data are presented as mean ± SEM. *p < 0.05. ** p < 0.01. *** p < 0.001.
Table S1. Sequence of shRNAs for *PVT1* and sgRNA for TRIM24

| RNAs       | shRNAs (5′-3′)                                                                 |
|------------|--------------------------------------------------------------------------------|
| *PVT1*-shRNA-1 | F:GATCCCCCAGCCCATCATGATGGTACTTTTCAAGAGAAGTAC        |
|            | CATCATGATGGCTGTTTTT                                                             |
|            | R:CTAGAAAAACAGCCATCATGATGGTACTTTCTCTTGAAAAGT                                    |
|            | ACCATCATGATGGCTGGGG                                                             |
| *PVT1*-shRNA-2 | F:GATCCCCGCACATTTCAAGGATACTATTTCAAGAGAAGTAT                                  |
|            | CCTGAAATGTGCCTTTTT                                                             |
|            | R:CTAGAAAAAGGCACATTTCAAGGATACTATCTCTTTGATAGT                                   |
|            | ATCCTGAAATGTGCGGG                                                             |
| TRIM24-sgRNA | F:CACCGACACGGCGCAAAGTGTCCAAC                                                  |
|            | R:AAACGTTGGACACTTGCGCGCGTGC                                                    |

Table S2. Primers for qRT-PCR assays

| Primer pairs | Sequence                      |
|--------------|-------------------------------|
| *PVT1*       | F:GCCCTTCTATGGGAATCCTA        |
|              | R:GGGGCAGAGATGAAATCGTAAT      |
| TRIM24       | F:TGTGAAGGACACTACTTTAAGAGA    |
|              | R:GCTCTGATACACGTCTTGGAGG      |
| β-actin (Human) | F:CATGTACGTTGCTATCCAGGC | |
|              | R:CTCCTTAATGTCACGCACGAT       |
| GAPDH (Human) | F:AGGGCTGTATTTAACTCTTGGT     |
|              | R:CCCCACTTGATTTTGAGGGA        |
| U6 (Human)   | F:TGGCACCAGCAACATGAA          |
|              | R:CTAAGTCATAGTCCCGCTAGAAGCA  |
Table S3. Correlation between *PVT1* expression and clinical features in GBM

| Characteristics                   | Total | Relative Expression of *PVT1* |   |   |
|-----------------------------------|-------|-------------------------------|---|---|
|                                   |       | Low  | High* | *P* Value† |
| Sex                               |       |      |       |            |
| Male                              | 23    | 10   | 13    | 0.352      |
| Female                            | 19    | 11   | 8     |            |
| Age                               |       |      |       |            |
| ≤65                               | 29    | 14   | 15    | 0.739      |
| >65                               | 13    | 7    | 6     |            |
| KPS score (preoperative)          |       |      |       |            |
| ≤70                               | 12    | 4    | 8     | 0.306      |
| >70                               | 30    | 17   | 13    |            |
| No. of lesions                    |       |      |       |            |
| Single                            | 32    | 19   | 13    | 0.07       |
| Multiple                          | 10    | 2    | 8     |            |
| Tumor size (cm³)                  |       |      |       |            |
| ≤10                               | 15    | 9    | 6     | 34         |
| >10                               | 27    | 12   | 15    |            |
| Necrosis Change                   |       |      |       |            |
| With                              | 19    | 6    | 13    | 0.03*      |
| Without                           | 23    | 15   | 8     |            |
| Edema                             |       |      |       |            |
| With                              | 31    | 14   | 17    | 0.483      |
| Without                           | 11    | 7    | 4     |            |
| Across The Center Line            |       |      |       |            |
| Yes                               | 11    | 3    | 8     | 0.16       |
| No                                | 31    | 18   | 13    |            |
| Recurrence                        |       |      |       |            |
| Yes                               | 31    | 12   | 19    | 0.035*     |
| No                                | 11    | 9    | 2     |            |
| Survival                          |       |      |       |            |
| Yes                               | 9     | 8    | 1     | 0.024*     |
| No                                | 33    | 13   | 20    |            |

* The median expression level of *PVT1* was used as the cutoff.

† Pearson’s chi-square tests were used to analyse the correlation between *PVT1* expression and clinical features, results were considered statistically significant at P <0.05.
Table S4. Mass spectrometry analysis of the proteins pulled down by PVT1 in U251 cells

| Number | Pro_acc | Pro_acc_name   | Gene name | Pro_cover (%) |
|--------|---------|----------------|-----------|---------------|
| 1      | O15164  | TIF1A_HUMAN    | TRIM24    | 38.3          |
| 2      | Q68E01  | INT3_HUMAN     | INTS3     | 27.1          |
| 3      | Q15029  | U5S1_HUMAN     | EFTUD2    | 24.8          |
| 4      | O00291  | HIP1_HUMAN     | HIP1      | 24.6          |
| 5      | P53396  | ACLY_HUMAN     | ACLY      | 19.8          |
| 6      | Q16531  | DDB1_HUMAN     | DDB1      | 17.6          |
| 7      | O14974  | MYPT1_HUMAN    | MYPT1     | 12.1          |
| 8      | Q13002  | GRIK2_HUMAN    | GRIK2     | 8.7           |
| 9      | P25092  | GUC2C_HUMAN    | GUCY2C    | 3.4           |