Figure S1. Effects of overexpression of splicing factors on the alternative splicing of STAT3 exon 23. (A) Alternative splicing of STAT3 exon 23 were analyzed by RT-PCR. Diagrams on the right show the structures of STAT3 pre-mRNA and spliced products. Short lines above or below exons stand for primer positions. (B) The histogram summarized the effects of overexpression of splicing factors (including those in Figure 1D) on the relative ratio of STAT3 α vs β isoform. *: P<0.05; **: P<0.01
Figure S2. Overexpression of splicing factors was confirmed by western blot using anti-Flag (A), anti-GFP (B), anti-T7 (C), and anti-Myc antibody (D).
Figure S3. Overexpression of STAT3α rescued cell growth inhibition induced by PCBP1 overexpression in SCC-9 cells. SCC-9 cells were transfected with T7-PCBP1 expression lentivirus, STAT3α-GFP expression lentivirus, empty control lentivirus and/or GFP expression control lentivirus. Transfected cells were divided into four groups: T7-PCBP1 + GFP, Vector + GFP, T7-PCBP1 + STAT3, and Vector + STAT3.

(A) Cells were seeded into 12 well plates (1 × 10^5 cells per well). Cell number was counted at Day 2 and Day 4. Values represent means ± SE. (B) The histograms summarized the number of cells counted on Day 4. Data are the means ± SE, n = 3. LV: lentivirus. (C) Western blot displayed overexpression of exogenous T7 tagged PCBP1 and exogenous STAT3α-GFP fusion protein. GAPDH served as loading control. (D) Expression levels of the indicated STAT3 target genes (Bcl-xl and survivin) were analyzed by RT-PCR. GAPDH served as a loading control. (E, F) STAT3α overexpression rescues the inhibition of PCBP1 overexpression on the clonogenic ability of SCC-9 cells. One thousand cells were seeded into 6 cm plates and cultured for 10 days. Representative images are shown (E). (F) The histograms summarized the number of colonies. Data are the means ± SE, n = 3.
Figure S4. Knockdown of PCBP1 upregulates the ratio of STAT3 α/β and promotes the cell proliferation of OSCC cell. (A) PCBP1 was downregulated in CAL 27 or SCC-9 cells. The alternative splicing of exon 23 was detected by RT-PCR. (B) Knockdown efficiency of PCBP1, the expression of STAT3 and phosphorylated STAT3 were analyzed by western blot. GAPDH served as a loading control. (C, D) CAL 27 or SCC-9 cells were treated with anti-PCBP1 or non-specific (NS) siRNA on Day 0 and Day2. Cell numbers were counted on Day 2 and Day 4 (C). (D) The histograms show significant difference between PCBP1 knockdown and NS control groups on Day 4. Data are the means ± SE, n=3.
Figure S5. Mutant 4 abolished PCBP1's regulation of the alternative splicing of STAT3 exon 23. (A) RT-PCR analysis of alternative splicing of exon 23 in 293 cells transfected with minigene [wild-type (wt) or mutant (mt4)] in the presence or absence of T7-PCBP1 overexpression. Relative α/β represents the ratio of band intensities of isoform α vs β. GAPDH served as a loading control. Diagrams on the right show the structures of STAT3 minigene and spliced products. (B) Western blot confirmed the overexpression of T7 tagged PCBP1. GAPDH served as a loading control.