Supplementary Figure Legend

Supplementary Figure 1. EBNA1 activated the TGFβ1-SMAD3 axis. (A) SB431542 blocked the migration of Treg cells induced by EBNA1. (B) Upregulated gene sets of EBNA1-negative and -positive NPC cells in the hallmark gene set database according to GSEA. (C) TGFβ1 and SMAD3 mRNA expression in EBNA1-positive and -negative NPC cell lines. (D) The p-SMAD3 level was positively correlated with the expression of EBNA1 and TGFβ1, advanced clinical stage, higher risk of recurrence, metastasis and death in NPC patients.

Supplementary Figure 2. miR-200a is a downstream factor of the TGFβ1-SMAD3 axis. (A) TGFβ1 suppressed miR-200a expression in a time-dependent manner. (B) SB431542 rescued the suppressive effect of TGFβ1 on miR-200a and reversed a decrease in the expression levels of miR-200a induced by TGFβ1. (C) The miR-200a expression was elevated in a time-dependent manner after blockade of autocrine TGFβ signalling activity by SB431542. (D) miR-200a had little effect on the transcription of TGFβ1 and SMAD3.

Supplementary Figure 3. CXCL12 is a target gene of miR-200a. (A) Putative miR-200a-binding sites in CXCL12 3'UTR predicted by Targetscan. (B) The miR-200a has little effect on CXCL12 transcription by qRT-PCR. (C) Dual luciferase reporter vectors carrying the putative CXCL12 3'UTR wild type binding site elements and the corresponding mutated sequences. (D) overexpression of CXCL12 promotes migration of Treg cells, and miR-200a overexpression inhibited movement of Treg cells and significantly suppresses the stimulatory effect of CXCL12 on migration of Treg cells. (E) overexpression of CXCL12 decreased the suppressive effect of miR-200a on migration of Treg cells, and suppression of CXCL12 by specific siRNA reduced migration of Treg cells. (F) CXCL12 was positively correlated with EBNA1 expression, infiltration density of Treg cells, advanced clinical stage, high risk of recurrence, metastasis and death in NPC patients.

Supplementary Figure 4. c-JUN suppressed miR-200a transcription. (A) Six putative c-JUN-binding sites of miR-200a upstream of the 3 kbp promoter region. (B) Dual luciferase reporter vector with miR-200a upstream of 3 kbp wild-type promoter sequence (WT) and vectors with site1 deletion mutation (MUT1), site6 deletion mutation (MUT6) and both site1 and site6 deletion mutations (MUT1+6). (D) The c-JUN and p-c-JUN levels were positively correlated with advanced clinical stage, high risk of recurrence and metastasis in NPC patients.

Supplementary Figure 5. TGFβ1 and SB431542 had little effect on CXCL12 transcription. (A) The effect of TGFβ1 or SB431542 on CXCL12 mRNA level was not significant. (B) Various concentrations of miR-200a mimics reduced migration of Treg cells in a dose-dependent manner.

Supplementary Figure 6. CXCL12 mediates recruitment of Treg cell by upregulating CXCR4 receptor of Treg cells. (A) AMD3100 suppressed migration of Treg cells induced by CXCL12. (B) Fitted curves in HCCLM3 model were generated for the bodyweight of each group(*p<0.05; **p<0.01; ***p<0.001). (C) Fitted curves in Hepa1-6 model were generated for the bodyweight of each group(*p<0.05; **p<0.01; ***p<0.001).