Figure S1. TDO2 promotes liver cancer cell migration and invasion. (A and B) HepG2 and Huh7 cells were transfected with pTDO2 or pcDNA3.1, and cell metastasis was determined using (A) wound healing wound assays and (B) Transwell migration and Matrigel invasion assays. (C and D) Wound healing, and Transwell migration and invasion assays of liver cancer cells after siRNA-mediated TDO2 knockdown (siTDO2). Data are presented as the mean ± SEM. **P<0.01. TDO2, tryptophan 2,3-dioxygenase; pTDO2, TDO2 overexpression plasmid; siRNA, small interfering RNA.

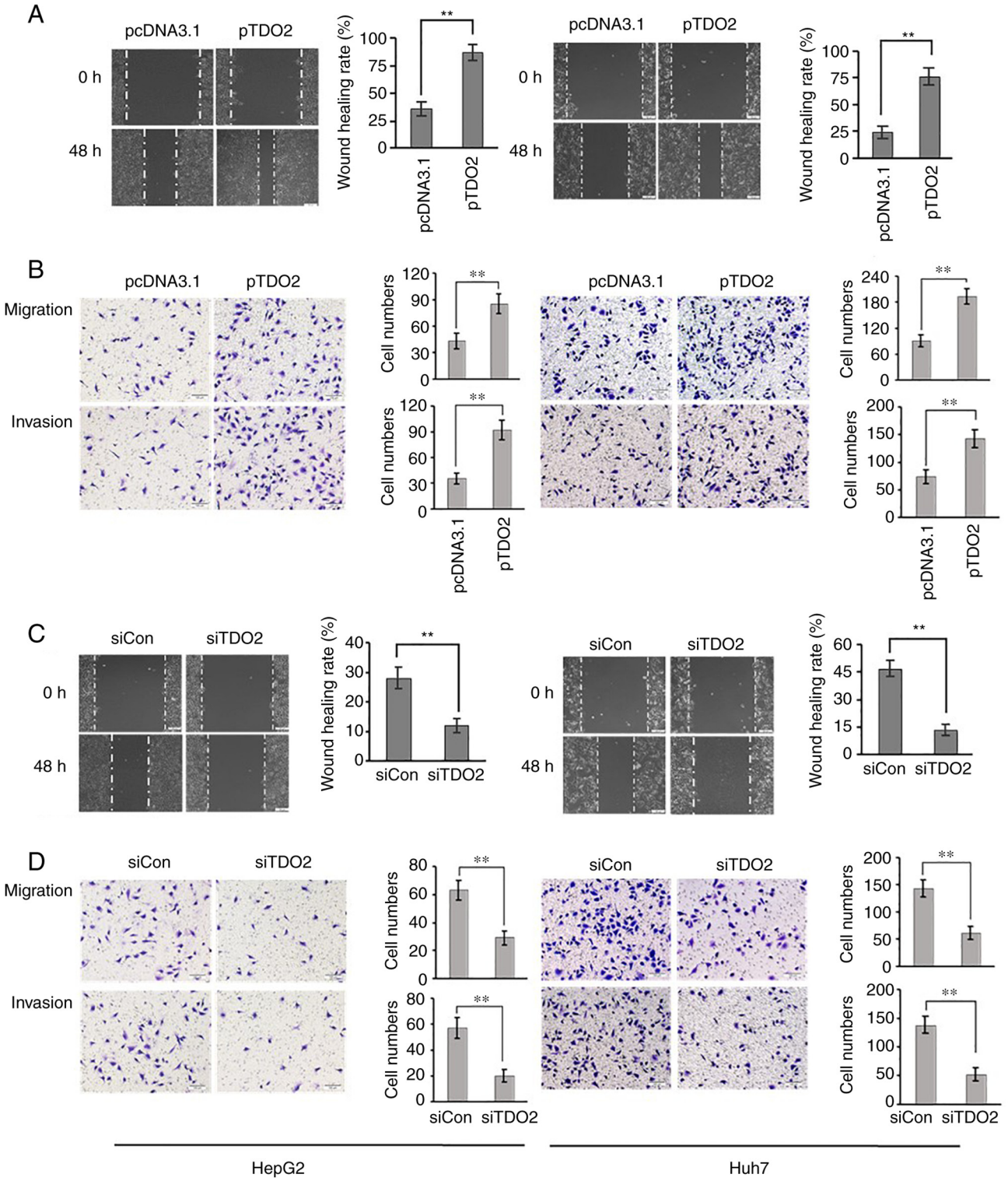

Figure S2. miR-140-5p inhibits migratory and invasive ability of liver cancer cells. (A) Wound healing assay indicated that miR-140-5p overexpression (miR-140-5p mimic group) inhibited wound closure in the HepG2 and Huh7 cells. (B) Overexpression of miR-140-5p reduced migration and invasion of HepG2 and Huh7 cells *in vitro*. (C and D) HepG2 and Huh7 cells were transfected with miR-140-5p inhibitor or negative control (NC), and cell migration and invasion were determined by wound healing, and Transwell migration and Matrigel invasion assays. Data are presented as the mean ± SEM. $^{**}P<0.01$. miR, microRNA.

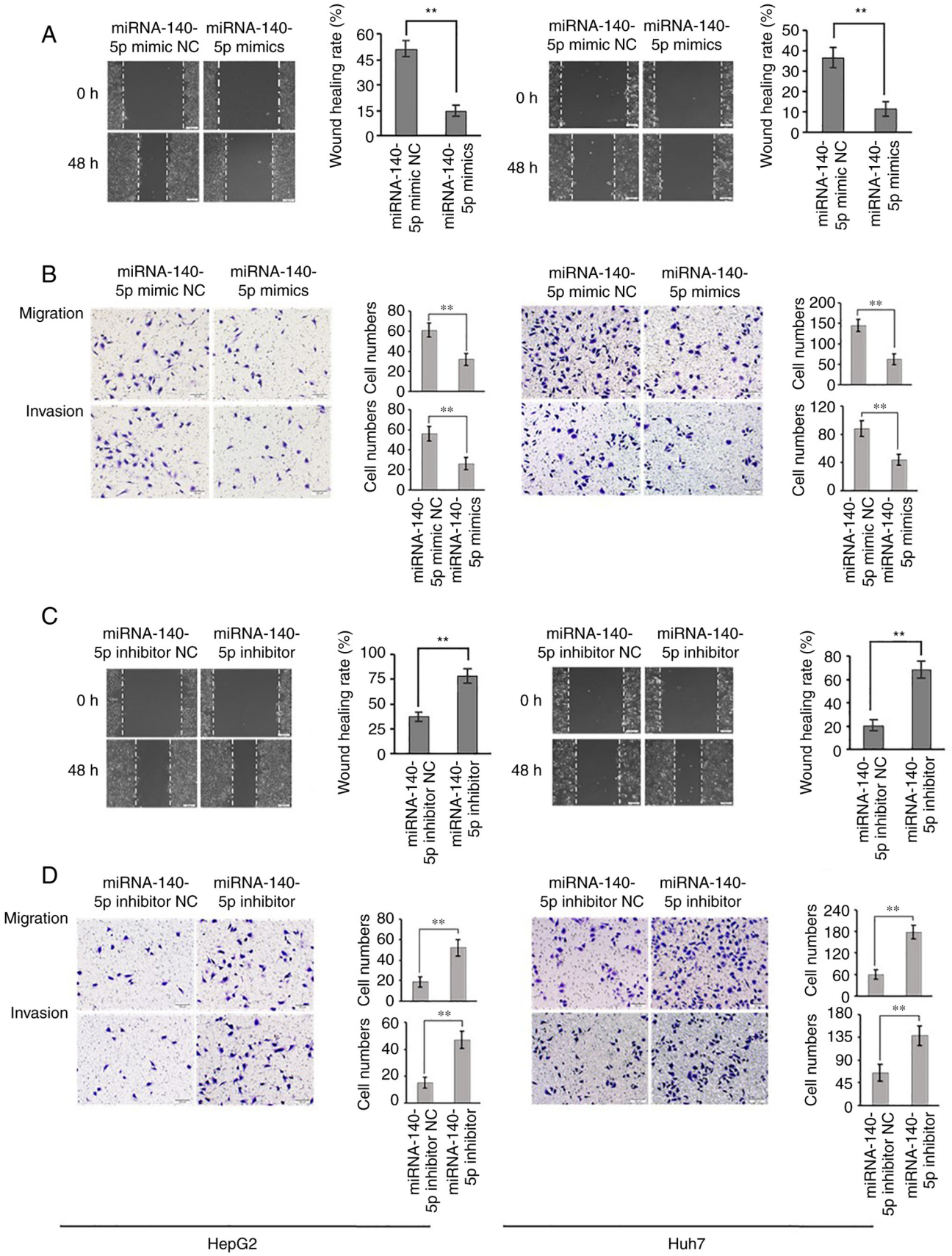

Figure S3. TDO2 does not affect the Wnt/β-catenin pathway. HepG2 and Huh7 cells were transfected with plasmids pTDO2 or pcDNA3.1. (A) Western blotting for the EMT markers E-cadherin, N-cadherin and fibronectin. (B) Western blot analysis of transfected HepG2 (left hand panels) and Huh7 (right hand panels) cell lines expressing either pCDNA3.1 or pTDO2 for c-Myc and β-catenin. Data are presented as the mean ± SEM. $^{**}P<0.01$; ns, not significant. EMT, epithelial-mesenchymal transition; TDO2, tryptophan 2,3-dioxygenase.

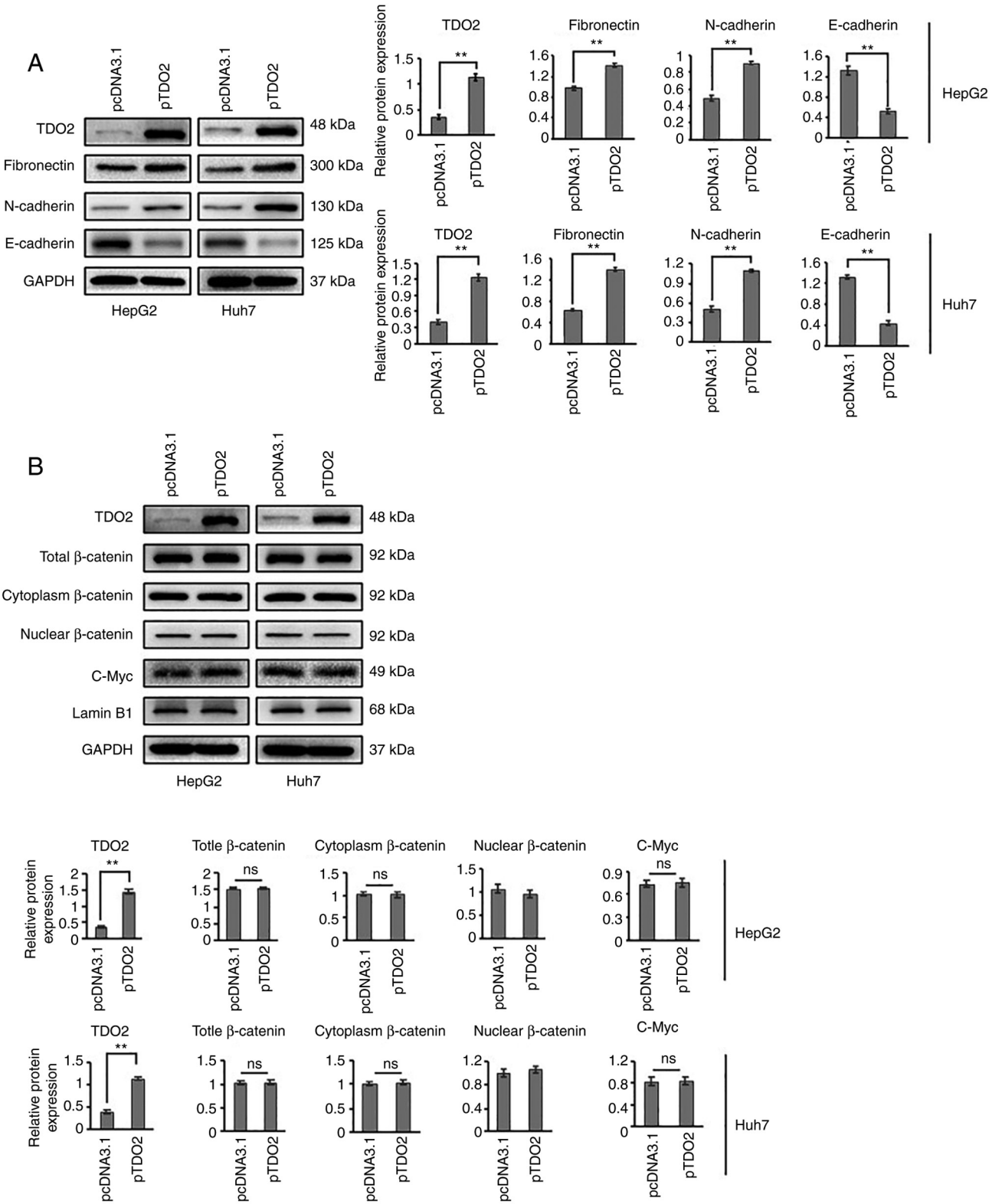

Figure S4. CD44 knockdown in TDO2-overexpressing cells attenuates TDO2-induced cell metastasis. (A-C) HepG2 and Huh7 cells were transfected with control pcDNA3.1, pTDO2, siRNA and CD44 siRNA as indicated. (A) CD44, MMP7 and TDO2 protein expression analysis. (B) Wound healing assays, and (C) Transwell migration and Matrigel invasion assays were performed 24 h after transfection. Data are presented as the mean ± SEM. $^{**}P<0.01$; ns. not significant. TDO2, tryptophan 2,3-dioxygenase; pTDO2, TDO2 overexpression plasmid; siRNA, small interfering RNA.

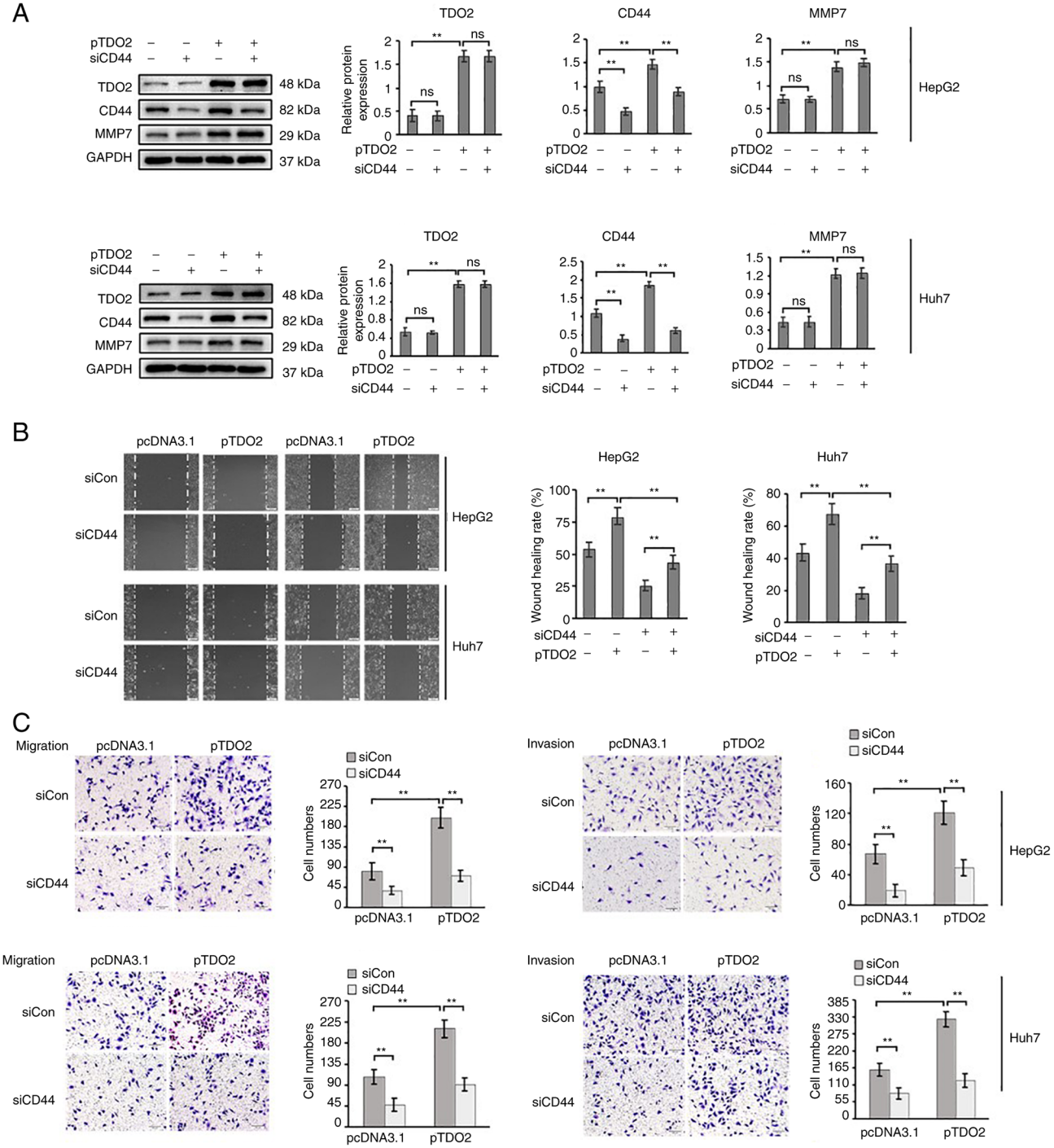

Figure S5. MMP7 knockdown in TDO2 overexpressing cells attenuates the TDO2-induced cell metastasis. (A-C) HepG2 and Huh7 cells were transfected with control pcDNA3.1, pTDO2, siCon and MMP7 siRNA (siMMP7) as indicated. (A) MMP7, TDO2 and CD44 protein expression analysis. (B) Wound healing, and (C) Transwell migration and Matrigel invasion assays were performed 24 h after transfection. Data are presented as the mean ± SEM. $^{**}P<0.01$; ns, not significant. TDO2, tryptophan 2,3-dioxygenase; pTDO2, TDO2 overexpression plasmid; siRNA, small interfering RNA; MMP, matrix metalloprotease.

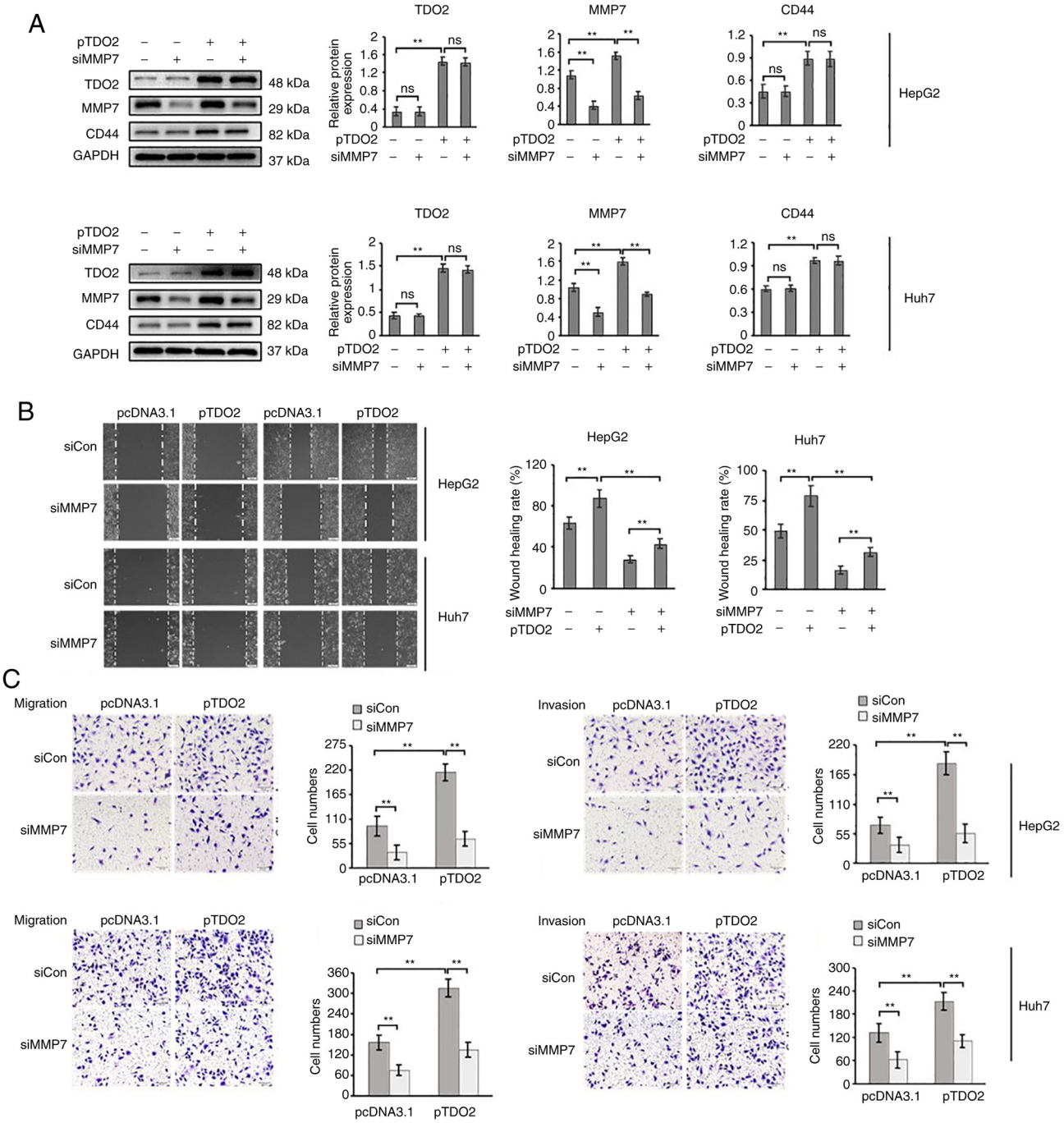

Figure S6. Effects of TDO2 on Wnt5a pathway activation. (A-C) HepG2 and Huh7 cells were transfected with control pcDNA3.1, pTDO2, siCon and Wnt5a siRNA (siwnt5a) as indicated. (A) Wnt5a, TDO2, P-PKC, CD44 and MMP7 protein expression analysis. (B) Wound healing assays, and (C) Transwell migration and Matrigel invasion assays were performed 24 h after transfection. Data are presented as the mean ± SEM. **P<0.01; ns, not significant. TDO2, tryptophan 2,3-dioxygenase; MMP, matrix metalloprotease; P-PKC, phospho-protein kinase C; pTDO2, TDO2 overexpression plasmid; siRNA, small interfering RNA.

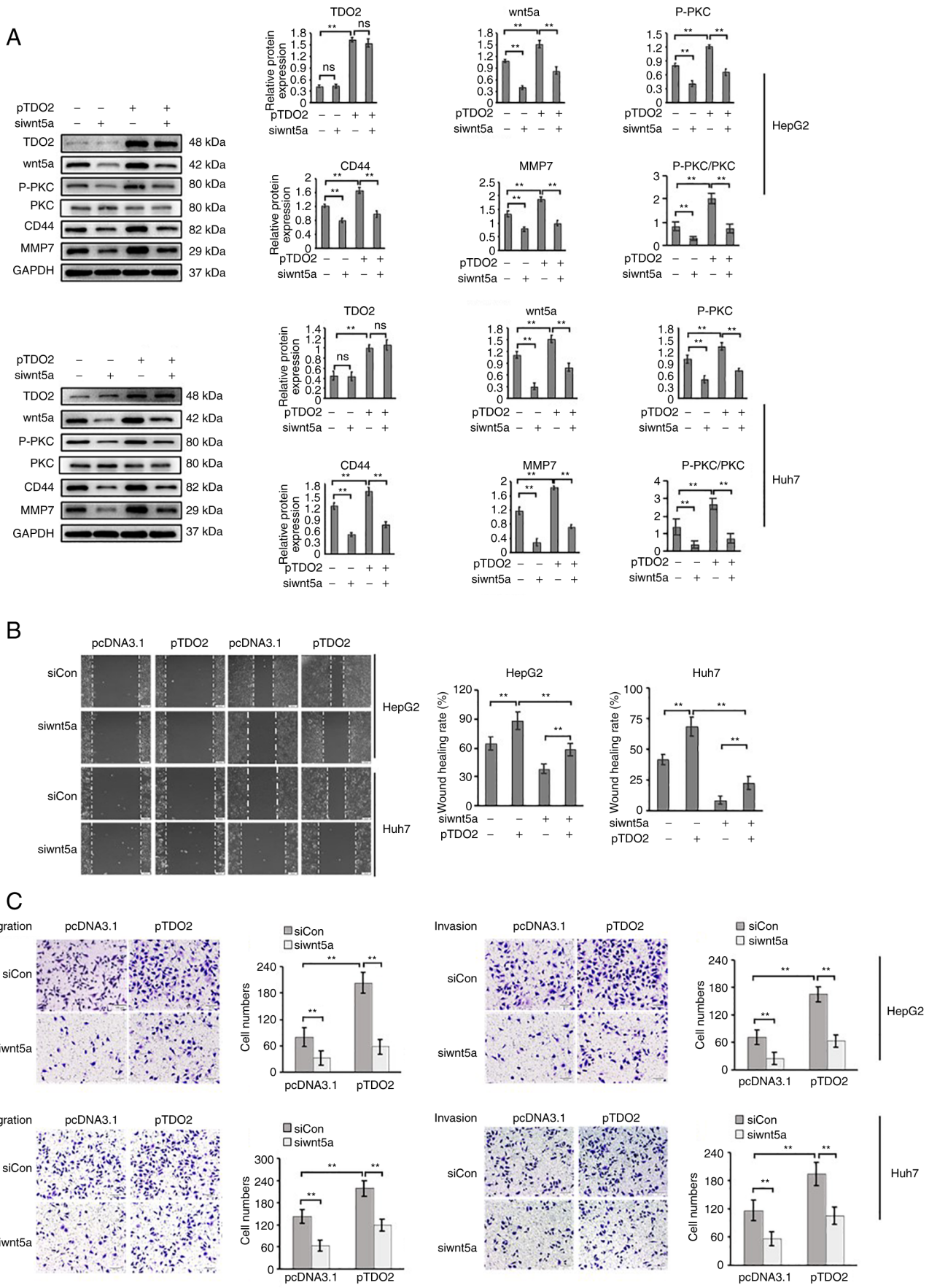

Figure S7. Effects of the PKC inhibitor, GÖ 6983, on cell migration and invasion. (A-C) HepG2 and Huh7 cells were transfected with control pcDNA3.1 or pTDO2, after which 1 μM GOE6983 was added to the culture medium 1 h post-transfection. (A) P-PKC, TDO2, Wnt5a, CD44 and MMP7 protein expression analysis. (B) Wound healing assays, and (C) Transwell migration and Matrigel invasion assays were performed 24 h after transfection. Data are presented as the mean ± SEM. **P<0.01; ns, not significant. TDO2, tryptophan 2,3-dioxygenase; pTDO2, TDO2 overexpression plasmid; P-PKC, phospho-protein kinase C.

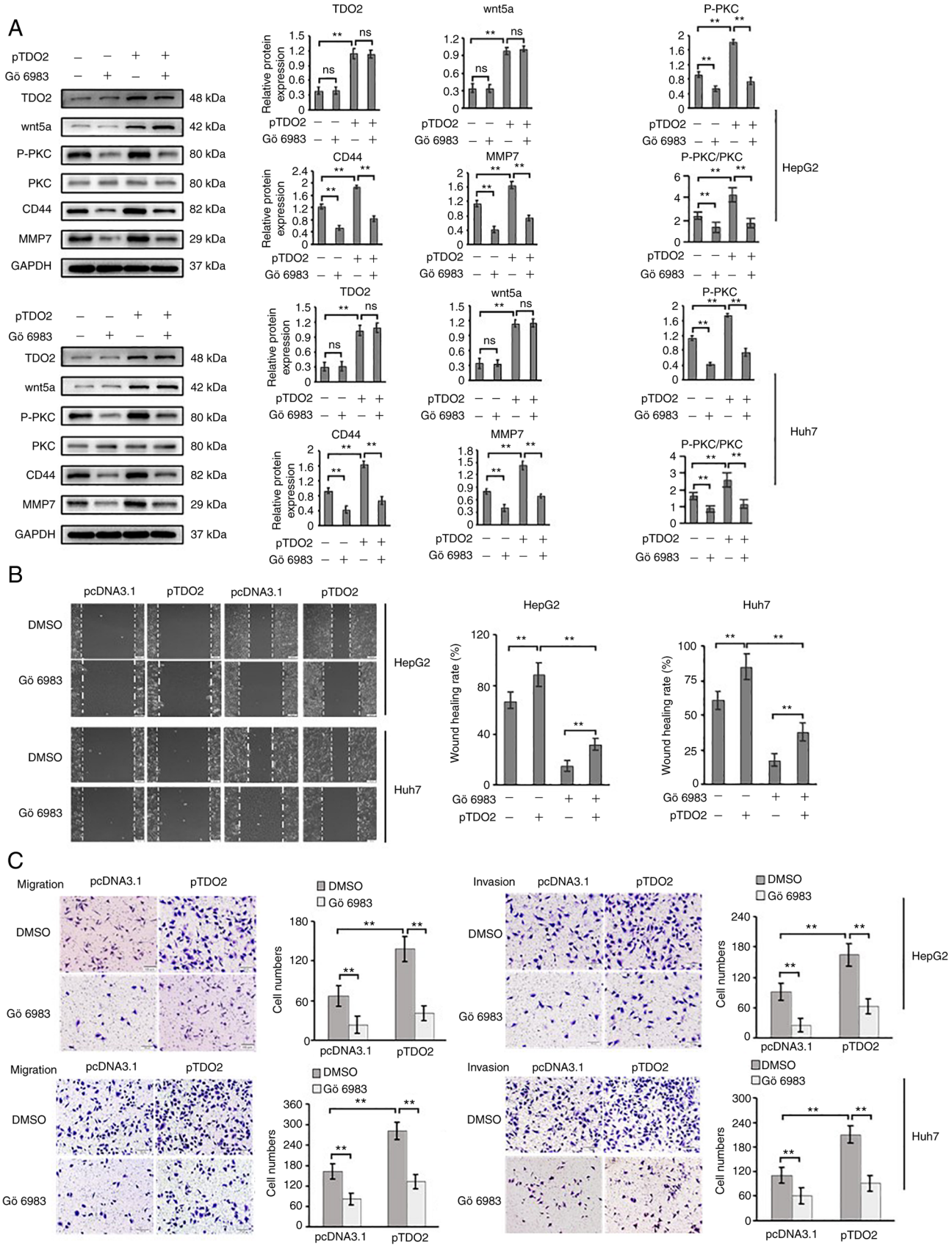

Figure S8. Effects of the ERK inhibitor, U0126, on cell migration and invasion. (A-C) HepG2 and Huh7 cells were transfected with control pcDNA3.1 or pTDO2, after which 10 μM U0126 GÖ 6983 was added to the culture medium 1 h post-transfection. (A) Phospho-PKC, wnt5a, TDO2 and P-ERK protein expression analysis. (B) Wound healing assays, and (C) Transwell migration and Matrigel invasion assays were performed 24 h after transfection. Data are presented as the mean ± SEM. **P<0.01; ns, not significant. TDO2, tryptophan 2,3-dioxygenase; pTDO2, TDO2 overexpression plasmid; P-PKC, phospho-protein kinase C.

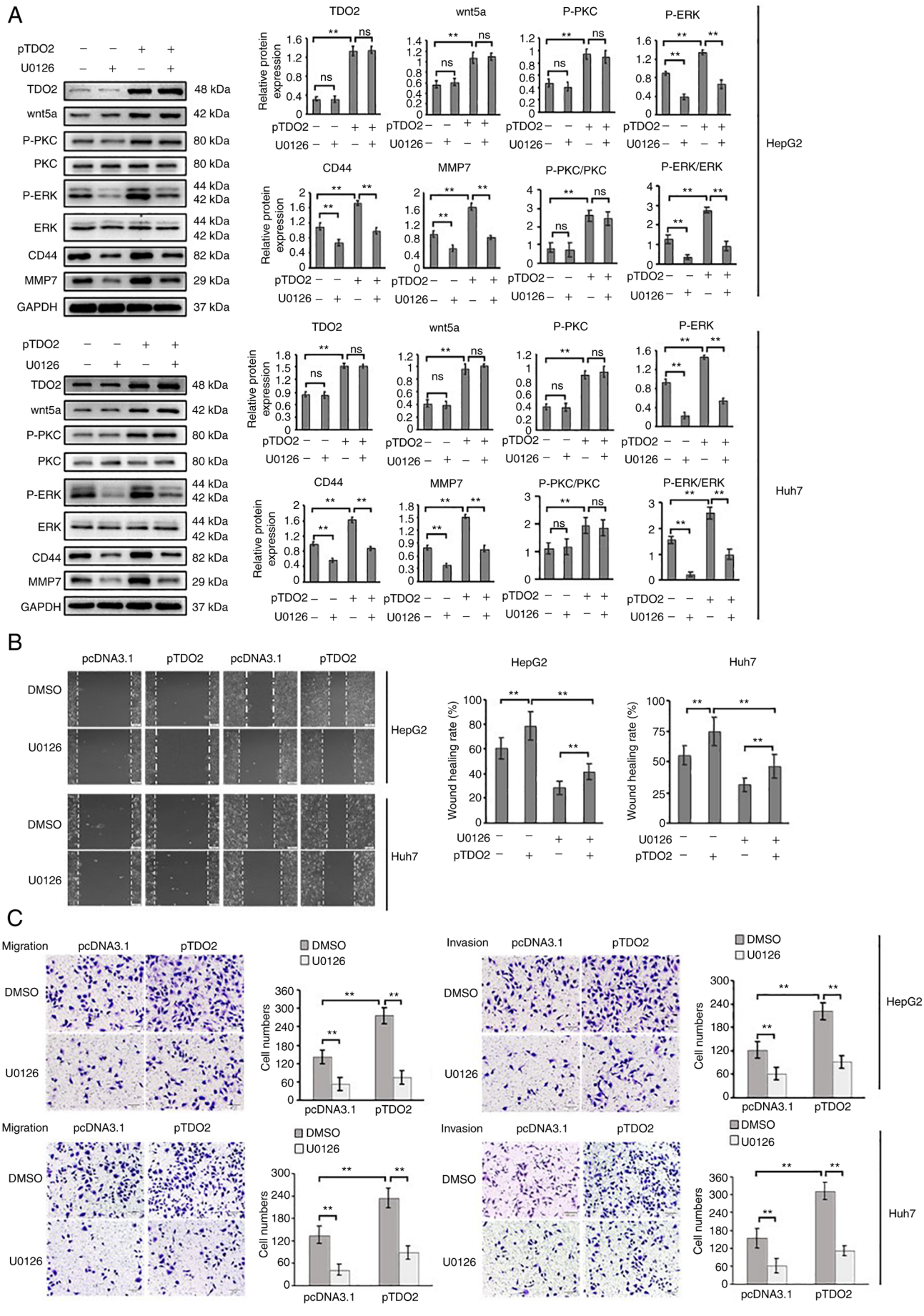

Figure S9. TDO2 promotes tumorigenesis in nude mice. HepG2 and Huh7 cells transduced with either control (Lv-Con) or TDO2 lentiviral (Lv-TDO2) constructs were subcutaneously implanted into nude mice. (A) Images of representative subcutaneous tumors excised from the flanks of the nude mice (n=3 per group). (B) Tumor volumes for HepG2 and HuH7 xenografts were measured weekly. (C) Xenograft tumors were analyzed for weight and volume after excision, alongside measurement of serum TDO2 levels after mice had been sacrificed. (D) Immunohistochemical images of representative mouse tumor sections stained for TDO2, Wnt5a, CD44 and MMP7. Scale bar, 50 mm. Data are presented as the mean ± SEM. **P<0.01. TDO2, tryptophan 2,3-dioxygenase; MMP, matrix metalloprotease.

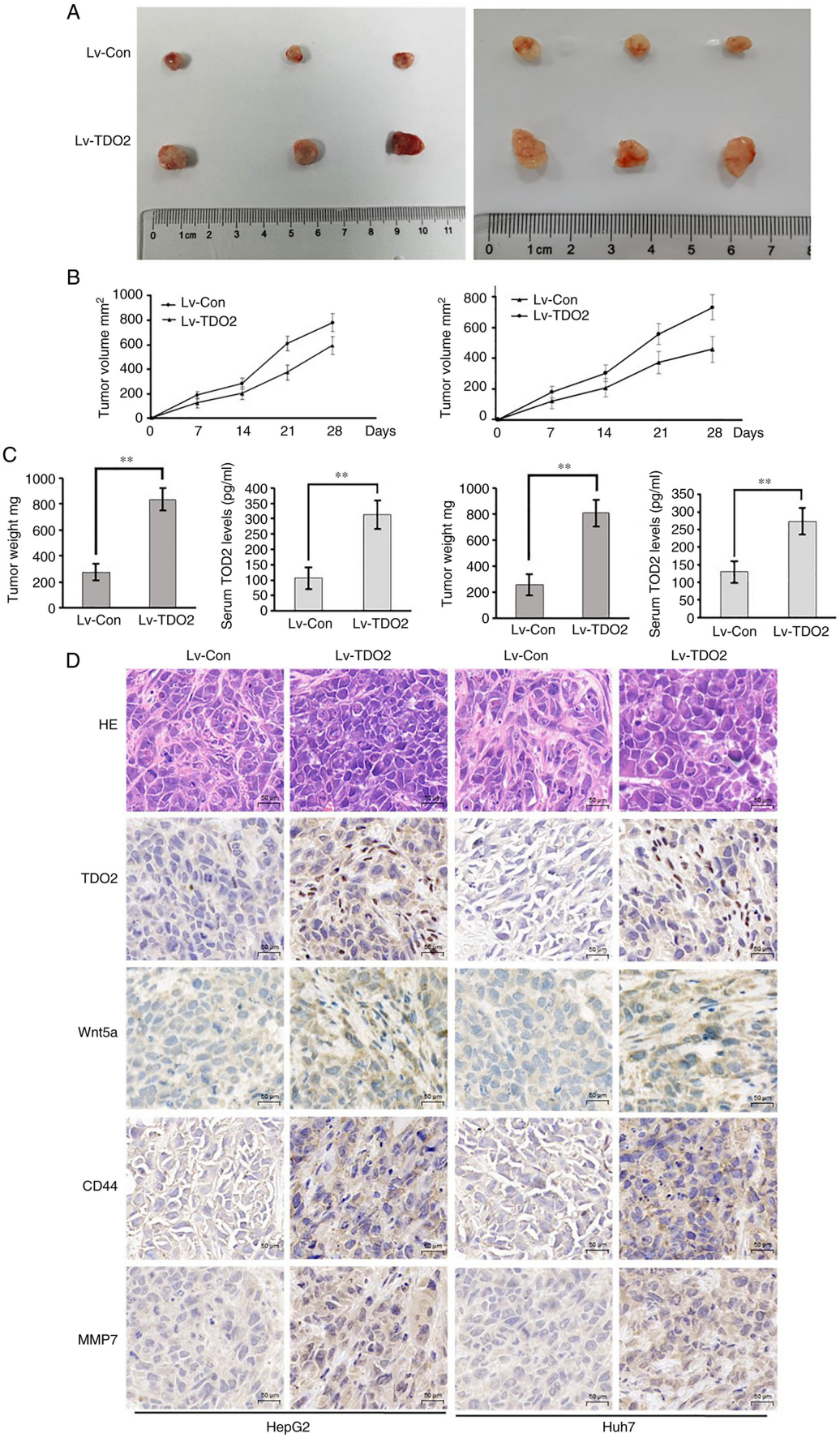

Figure S10. TDO2 overexpression partially abrogates the effects of azelnidipine on TDO2-induced cell metastasis. (A-C) HepG2 and Huh7 cells were transfected with control pcDNA3.1 or pTDO2, after which 15 nM azelnidipine was added to the culture medium 24 h post-transfection. (A) TDO2 protein expression analysis. (B) Wound healing assays, and (C) Transwell migration and Matrigel invasion assays were performed 24 h after transfection. Data are presented as the mean ± SEM. **P<0.01. TDO2, tryptophan 2,3-dioxygenase; pTDO2, TDO2 overexpression plasmid; P-PKC, phospho-protein kinase C; MMP, matrix metalloprotease.

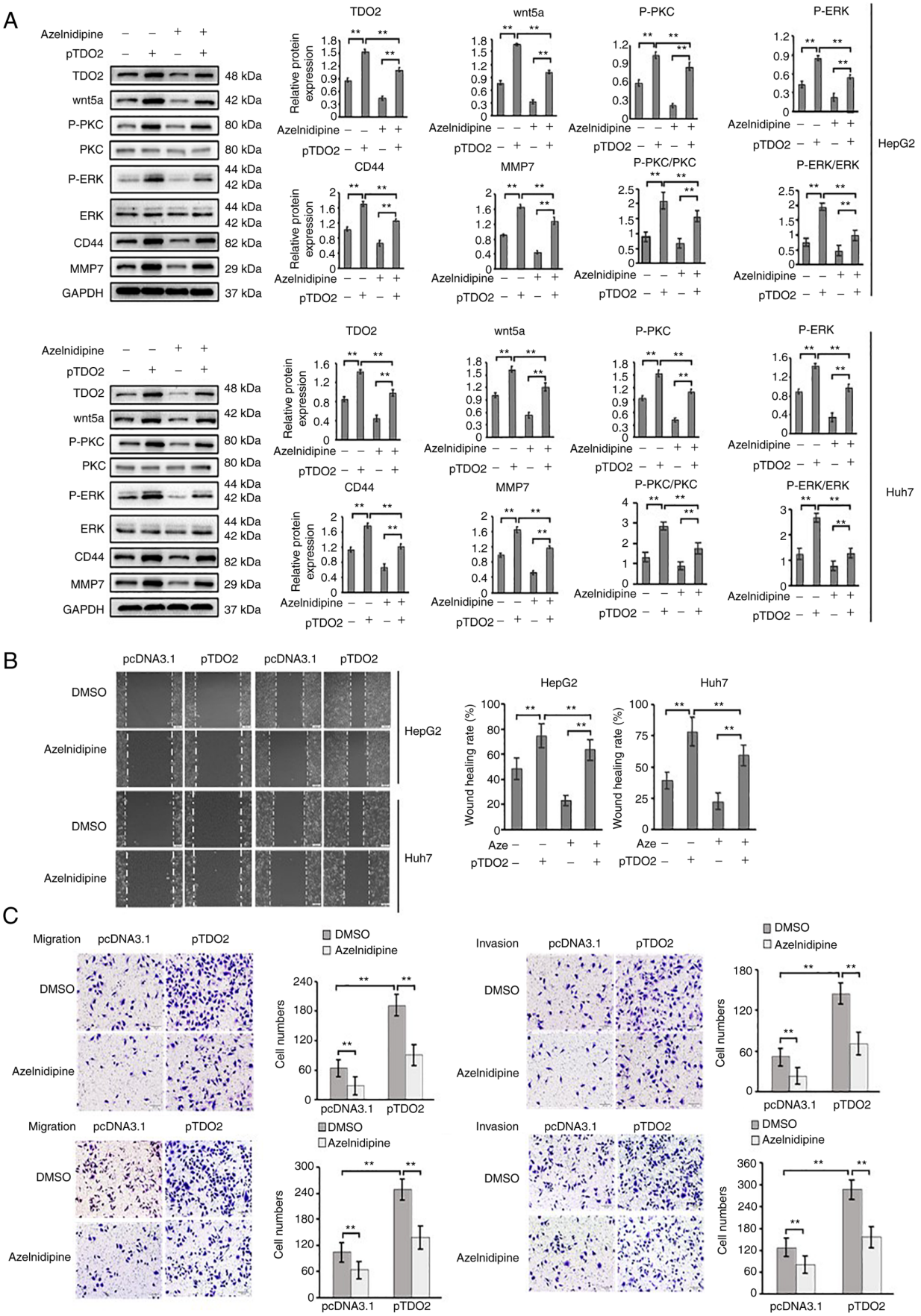

Figure S11. Inhibition of TDO2 expression by azelnidipine prevents tumorigenesis in nude mice. Oral administration of azelnidipine was followed by analysis of subcutaneous tumors following implantation of HepG2 and Huh7 cells into the nude mouse. Representative images of the tumor xenografts isolated from nude mice (n=3) for (A) HepG2 and (F) Huh7 cell lines. Estimated tumor volumes plotted over time under control or azelnidipine-treatment conditions for (B) HepG2 and (G) Huh7 cell lines. Weight of xenograft tumors under control vs. azelnidipine-treatment in (C) HepG2 and (H) Huh7 cell lines. Relative levels of serum TDO2 measured under control vs. azelnidipine-treatment in (D) HepG2 and (I) Huh7 cell lines. Immunohistochemical images of representative mouse tumor sections stained for TDO2, Wnt5a, CD44 and MMP7 from xenografts of (E) HepG2, and (J) Huh7 cell lines. Scale bar, 50 mm. Data are presented as the mean ± SEM. $^{**}P<0.01$. TDO2, tryptophan 2,3-dioxygenase; MMP, matrix metalloprotease.

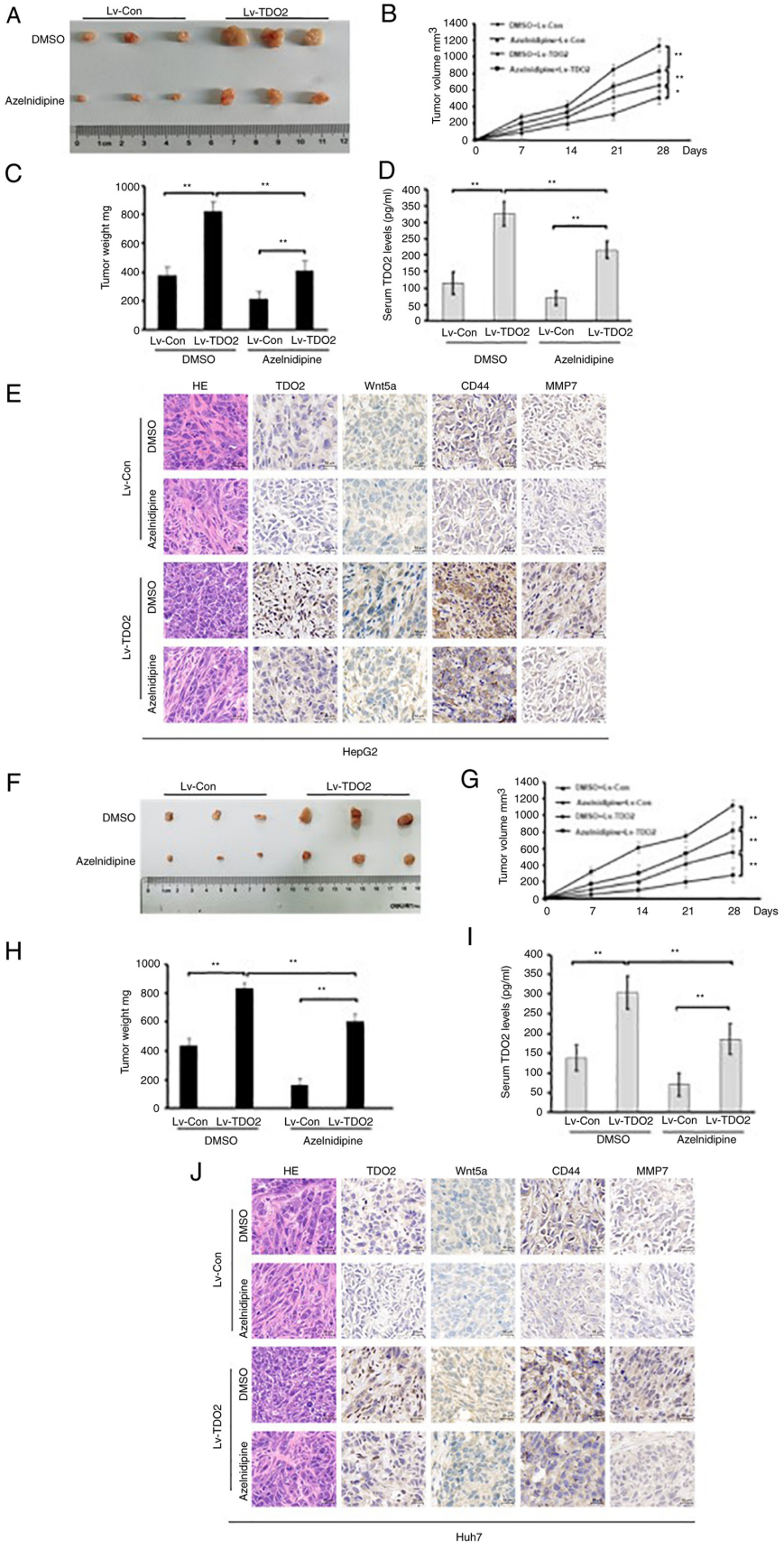

Figure S12. miR-140-5p inhibits the expression of CD44 and MMP7. (A) Overexpression of miR-140-5p (miR-140-5p mimics) reduced the expression of CD44 and MMP7. Data are presented as the mean ± SEM. **P<0.01. MMP, matrix metalloprotease.

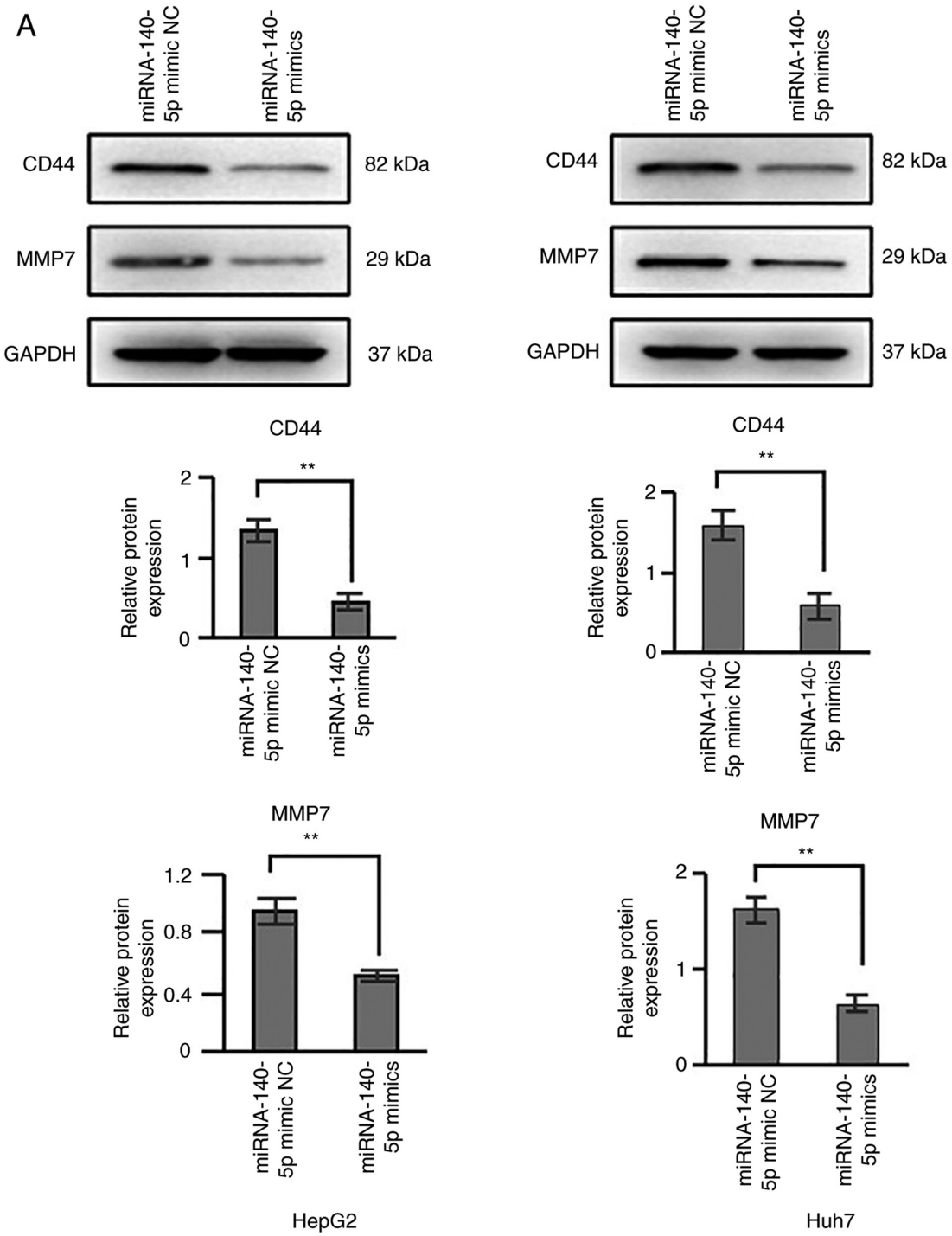

Figure S13. Nifedipine does not affect TDO2 expression. (A and B) Liver cancer cells were treated with 15 nM nifedipine, and TDO2 expression was determined by (A) western blotting and (B) reverse transcription-quantitative PCR 24 h after treatment. ns, not significant. TDO2, tryptophan 2,3-dioxygenase.