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Supplemental information

miR-519d-3p suppresses tumorigenicity and metastasis by inhibiting Bcl-w and HIF-1α in NSCLC

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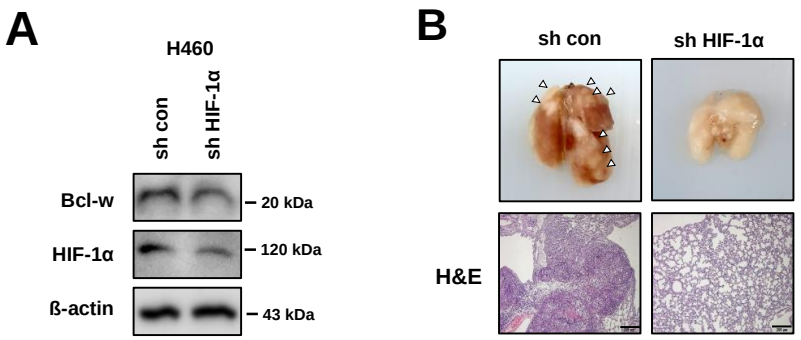

Figure S1. Expression of Bcl-w or HIF-1α is confirmed in cells used in animal experiments. (**A**) After transfection of H460 cells with sh HIF-1α, Bcl-w and HIF-1α expression levels were determined using Western blot analysis. β-actin was used as a loading control. (**B**) shHIF-1α cells were injected to tail vein in BALB/c nude mice (n=5, $1x10^6$ cells/mice). After 8 weeks, lung was harvested and subjected to H&E staining (scale bar 200μm). The data are presented as the mean ± S.D. ****P*<0.001. Student`s t-test.

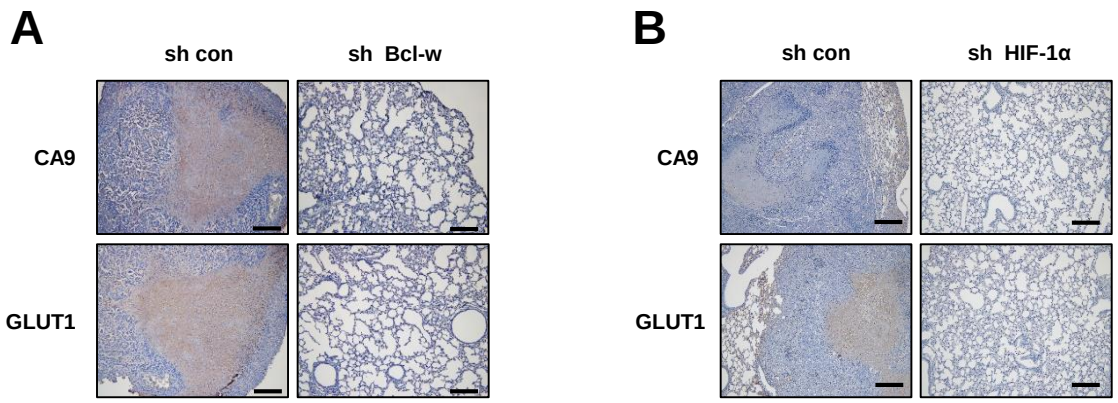

Figure S2. The expression of hypoxia markers decreases in pulmonary tumor tissues of Bcl-w or HIF-1α-knockdown mice. The expression of hypoxia markers CA9 and GLUT1 was detected by immunohistochemistry (IHC) staining. (**A**) The hypoxic regions in the pulmonary tumor formed after sh control or sh Bcl-w-transfected cells were injected into the tail vein of mice were indicated by staining with CA9 and GLUT1. (**B**) IHC images for hypoxic area within pulmonary tumor formed after sh con and sh HIF-1α Bcl-w-transfected cells were injected in mice were showed. scale bar 200μm.

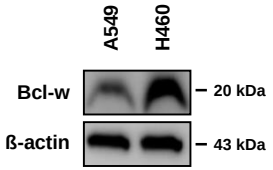

Figure S3. Basal level of Bcl-w expression is higher in H460 than in A549 cells. The expression of Bcl-w in A549 and H460 cells was determined by Western blot analysis.

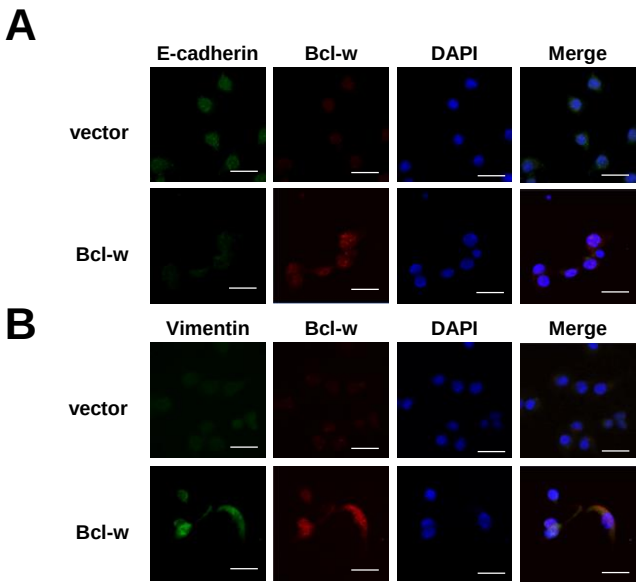

Figure S4. Overexpression of Bcl-w enhances mesenchymal properties. Immunofluorescence (IF) staining of E-cadherin (green) (**A**) or Vimentin (green) (**B**), Bcl-w (red), and DAPI (blue) in A549 cells: scale bar 50μm.

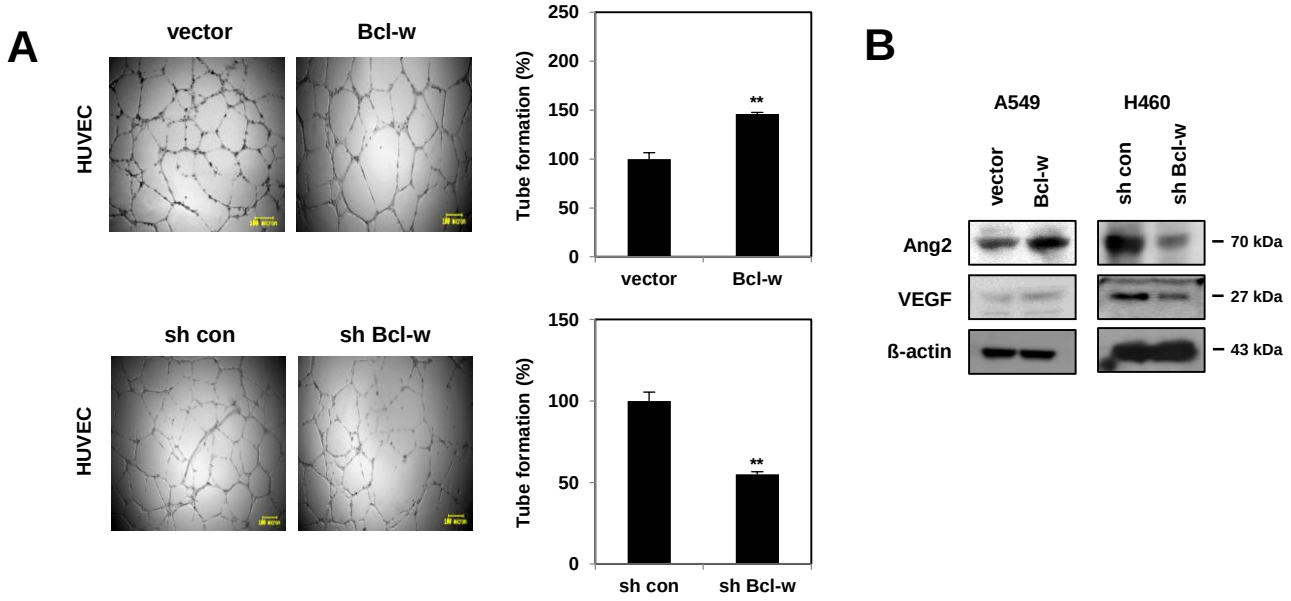

Figure S5. Bcl-w promotes tube formation ability in HUVECs. (**A**) After HUVEC cells were transfected with Bcl-w vector or Bcl-w shRNA, the effect of Bcl-w on angiogenesis was tube formation assay in matrigel. Scale bar 100um. (**B**) The expression level of angiogenesis-related factors, Ang2 and VEGF, were confirmed by Western blot analysis in H460 and A549 cells. The data are presented as the mean ± S.D. ***P*<0.01. Student`s t-test.

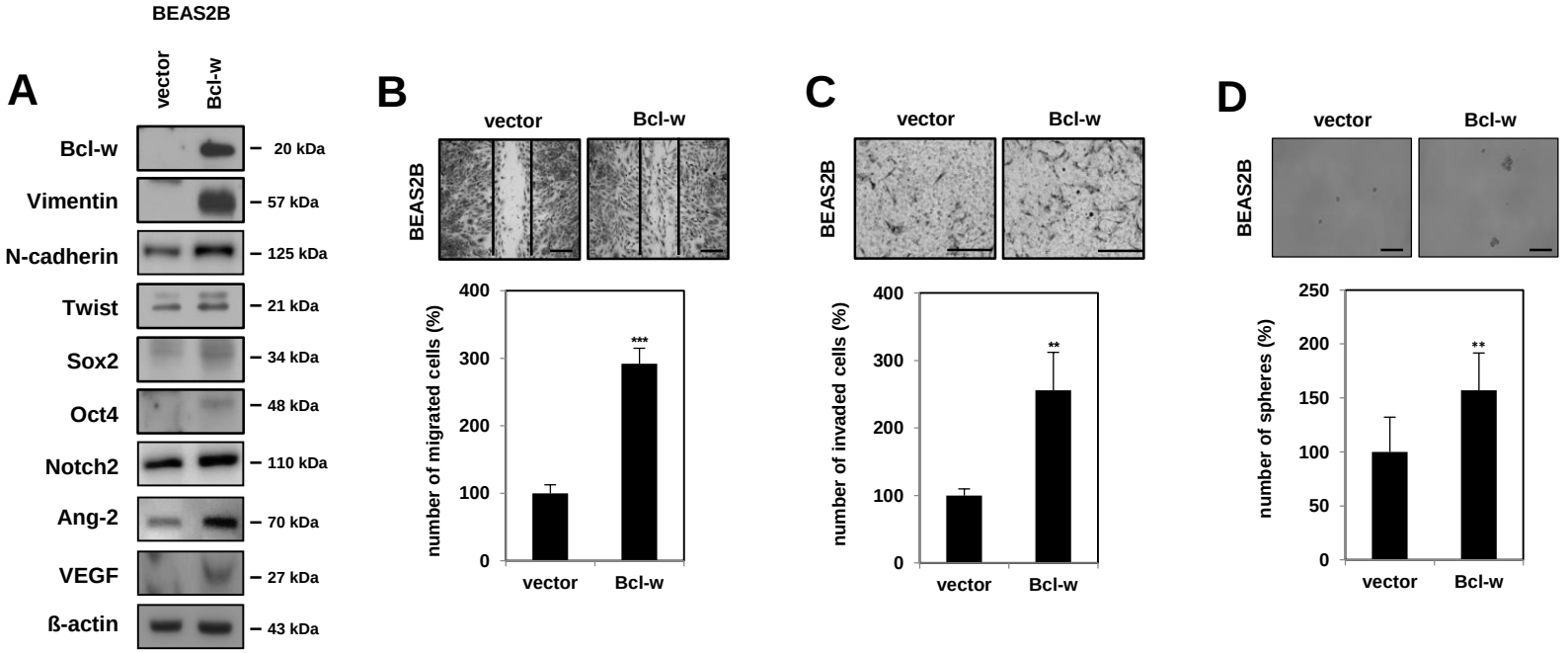

Figure S6. Bcl-w increases migratory and invasive abilities and stemness maintenance in normal lung cells, BEAS2B. After BEAS2B cells were transfected with Bcl-w overexpressing vector, indicated cells were subjected for (**A**) Western blot analysis with EMT and cancer stem-like cell markers, (**B**) wound healing assay, (**C**) invasion assay with matrigel-coated transwell, and (**D**) sphere forming assay. Scale bar 100um. The data are presented as the mean ± S.D. **P*<0.05; ***P*<0.01; ****P*<0.001. Student`s t-test.

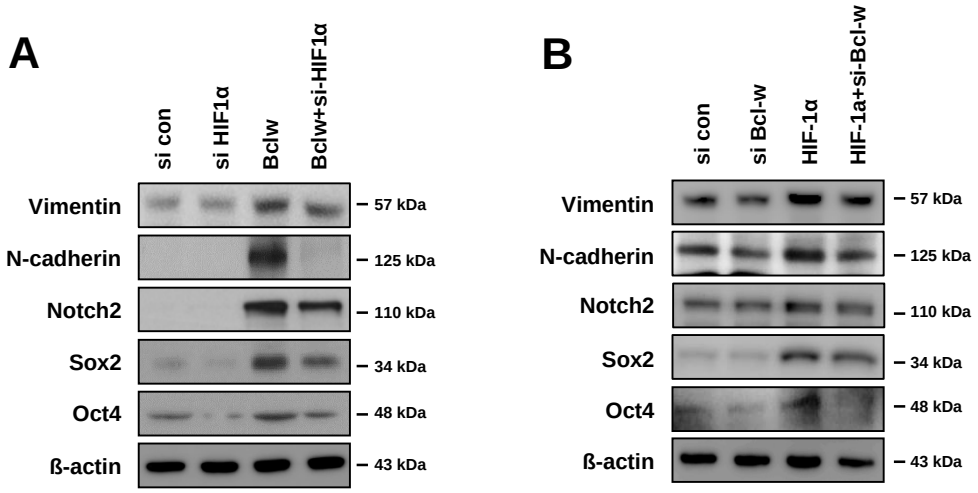

Figure S7. Bcl-w and HIF-1α positively correlate the expression of tumorigenic factors in lung cancer. (**A, B**) Western blot analysis was used to detect Vimentin, N-cadherin, Notch2, Sox2 and Oct4 expression in the indicated H460 cells. β-actin was used as a loading control.

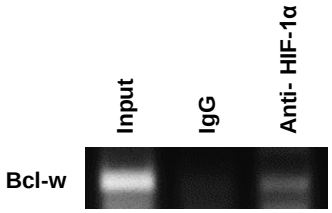

Figure S8. HIF-1α regulates the transcription of Bcl-w by directly binding to the promoter of Bcl-w. For chromatin immunoprecipitation (ChIP) analysis, H460 cells were treated with 100uM CoCl2 to induce hypoxia, and then the Bcl-w promoter fragment was pulled with HIF-1α or a control antibody.

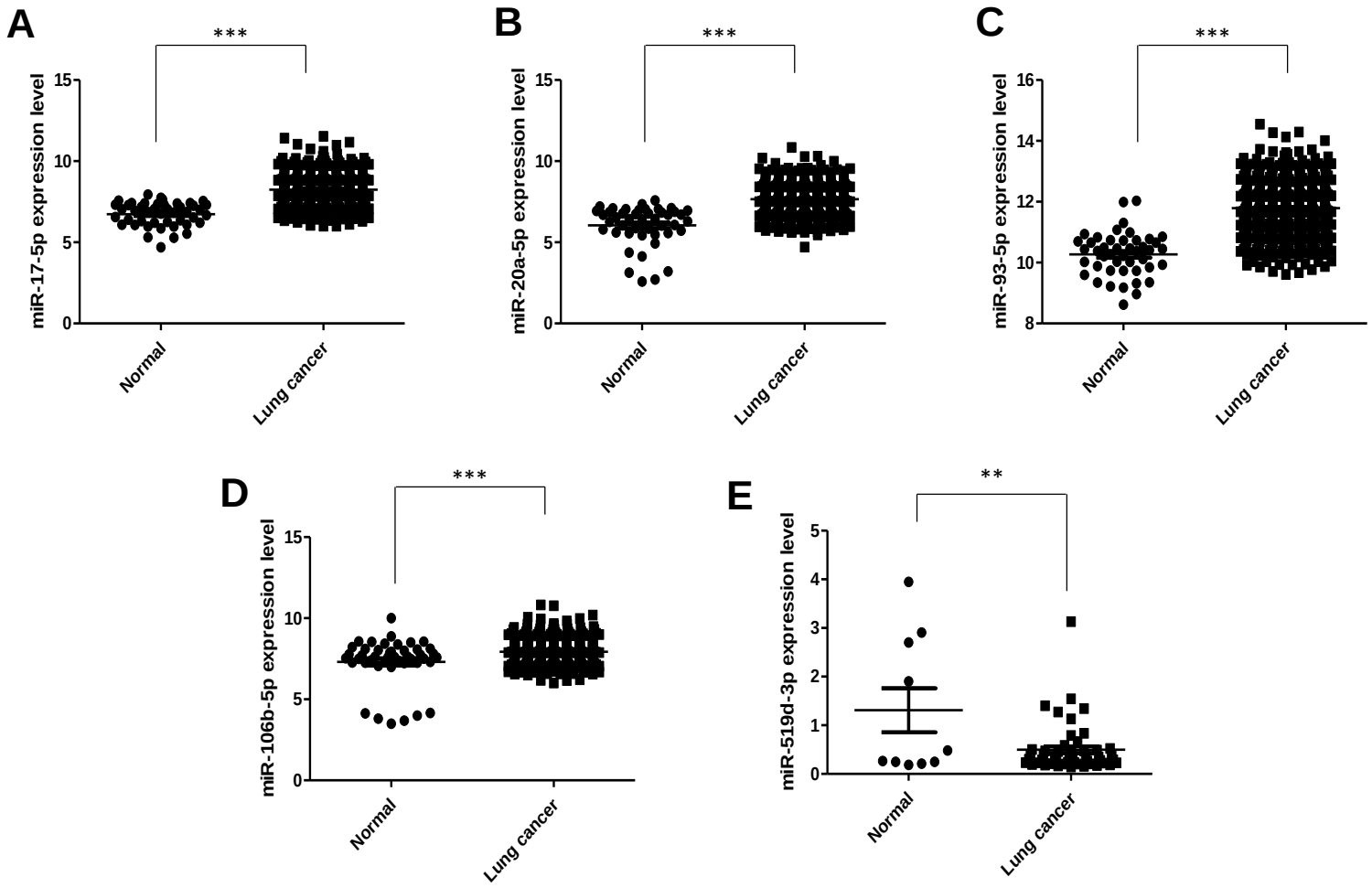

Figure S9. Expression analysis of 5 miRNA candidates in lung cancer patients using TCGA database. Expression of miRNA candidates (miR-17-5p (**A**), miR-20a-5p (**B**), miR-93-5p (**C**), miR-106b-5p (**D**) and miR-519d-3p (**E**)) was analyzed in lung cancer patients compared to the normal group. The indicated miRNA expression levels were plotted according to normal versus lung cancer tissues.The data are presented as the mean ± S.D. **P<0.01; ****P*<0.001. Student`s t-test.

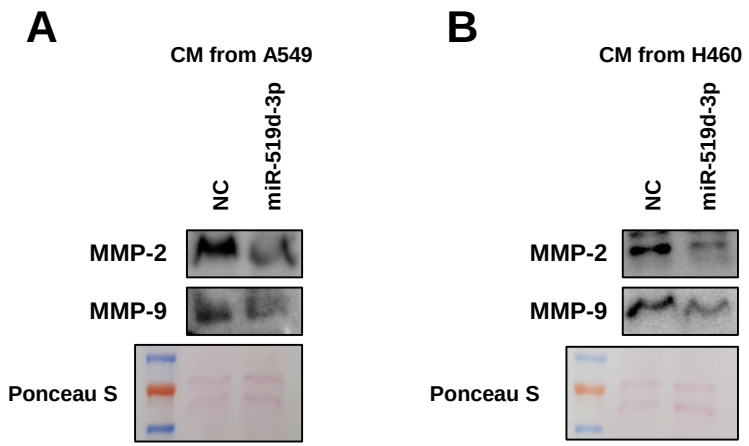

Figure S10. Overexpression of miR-519d-3p inhibits the secretion of MMP-2 and MMP-9. A549 (**A**) and H460 (**B**) cells were transfected with either negative control (NC) or miR-519d-3p mimic. Protein expression of MMP-2 and MMP-9 was confirmed in conditioned media (CM) collected from NC and miR-519d-3p-trasnsfected cells. The data are presented as the mean ± S.D**.**

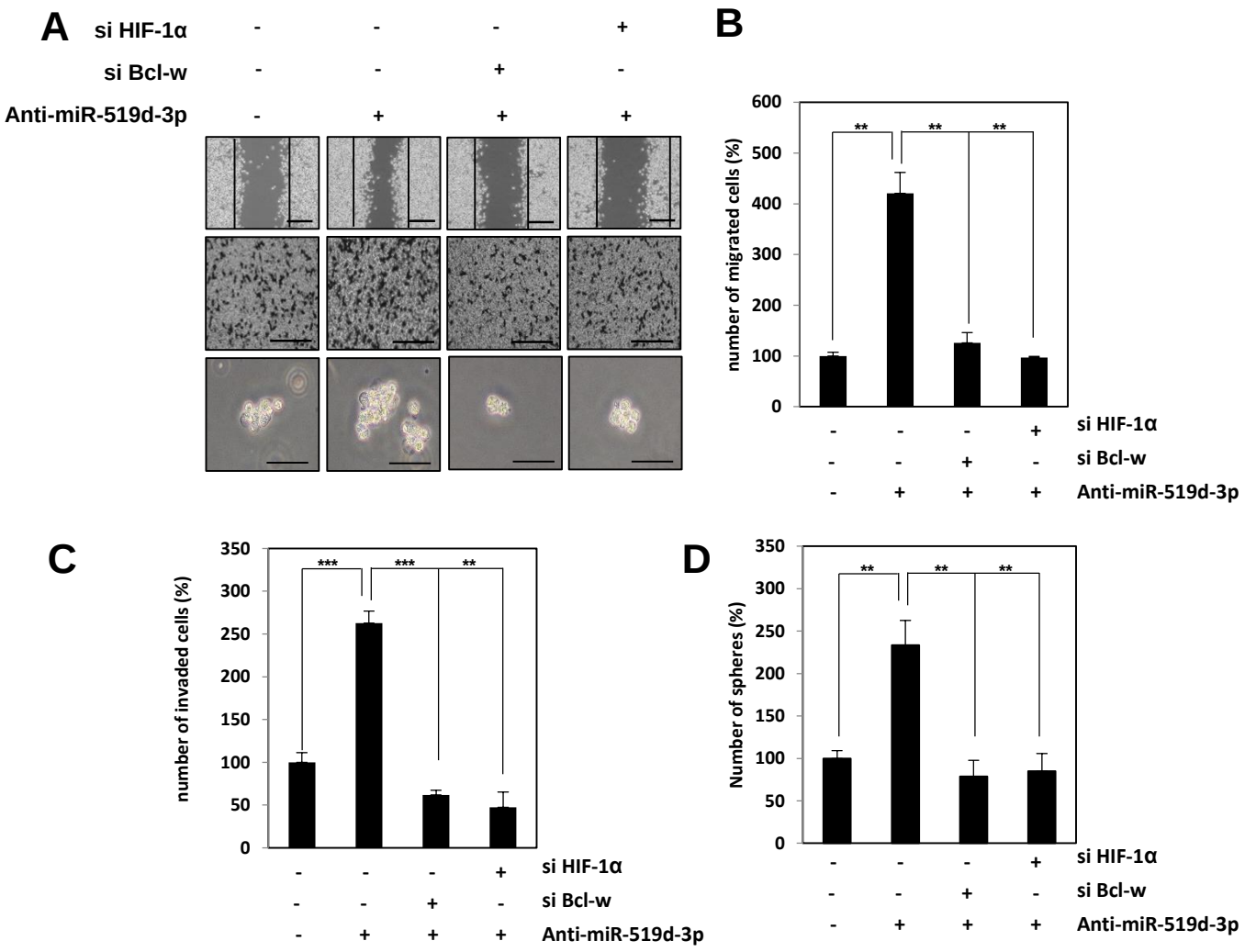

Figure S11. miR-519d-3p inhibitor increases cancer cell mobility, invasiveness and maintenance of stemness. (**A**) H460 cells were co-transfected with Bcl-w or HIF-1α siRNA and miR-519d-3p inhibitors and then assessed by wound healing assays (**B**), invasion assay with matrigel-coated transwell (**C**) , and sphere formation assay (**D**). Scale bar 100um. The data are presented as the mean ± S.D. ***P*<0.01; ****P*<0.001. Student`s t-test**.**