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Supplemental information

miR-140 inhibits osteosarcoma progression by impairing USP22-mediated LSD1 stabilization and promoting p21 expression

Wei Liu, Dunwei Wang, Lidi Liu, Lin Wang, and Ming Yan

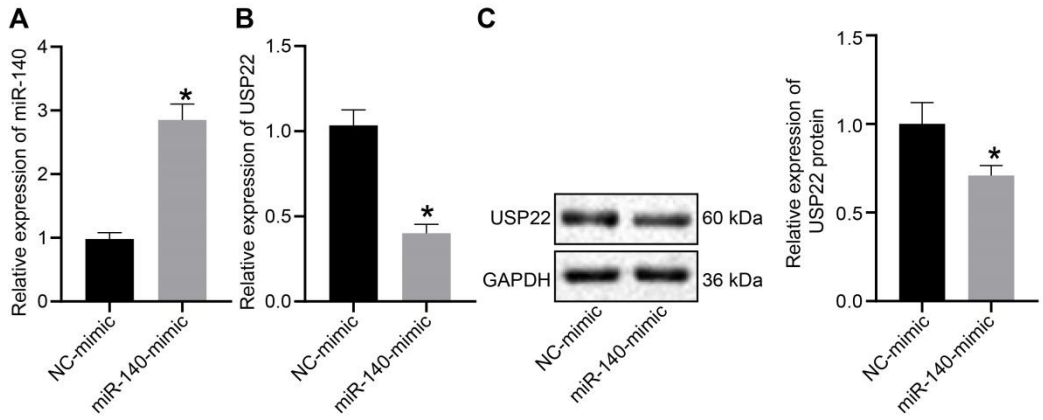

Figure S1. miR-140 inhibits the expression of USP22 in U2OS cells.

A, miR-140 expression in U2OS cells transfected with miR-140 mimic detected by RT-qPCR. B, USP22 expression in U2OS cells transfected with miR-140 mimic detected by RT-qPCR. C, USP22 protein expression in U2OS cells transfected with miR-140 mimic detected by Western blot. * $p < 0.05$ compared with U2OS cells transfected with NC mimic by unpaired *t*-test. Cell experiment was repeated three times.

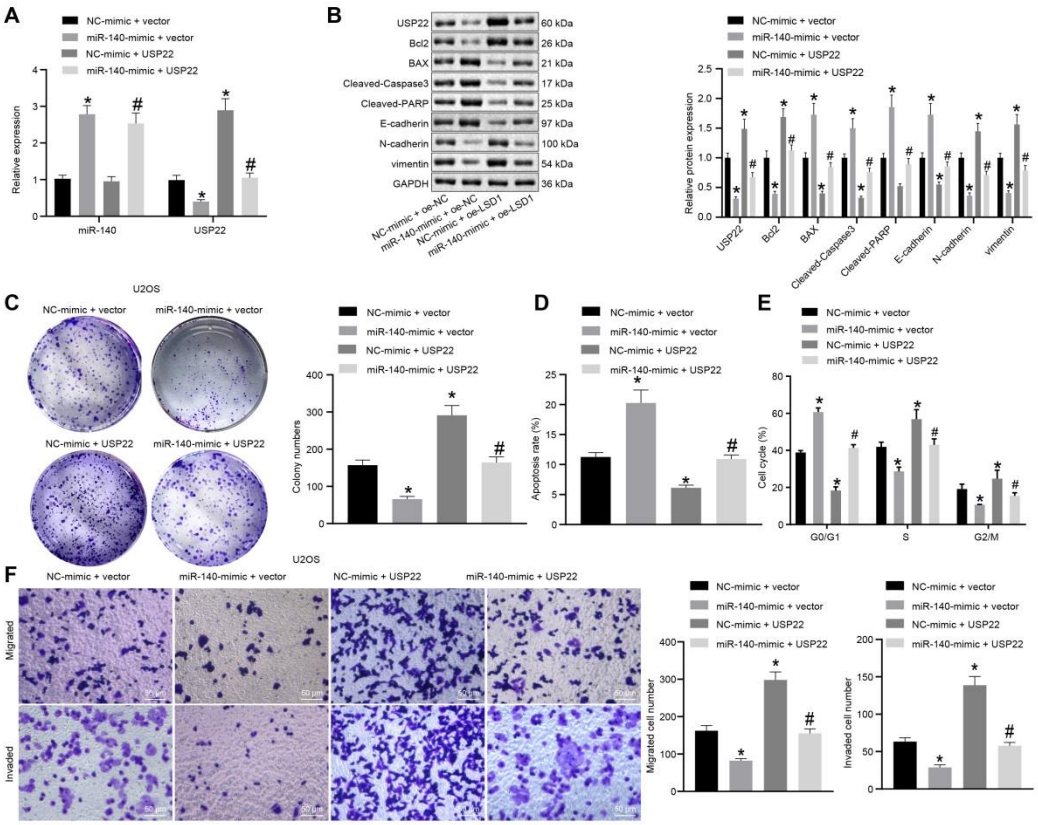

Figure S2. Overexpression of miR-140 inhibits cell migration, invasion and proliferation of U2OS cells.

A, Expression of miR-140 and USP22 in U2OS cells transfected with miR-140 mimic, USP22 or both examined using RT-qPCR. B, USP22, Bcl2, Bax, cleaved-caspase3, cleaved-PARP, E-cadherin, N-cadherin and vimentin expression in U2OS cells transfected with miR-140 mimic, USP22 or both examined using Western blot. C, D and E, Cell proliferation (C), apoptosis (D) and cycle (E) analysis in U2OS cells transfected with miR-140 mimic, USP22 or both evaluated using colony formation assay and flow cytometry, respectively. F, Invasion and migration of U2OS cells transfected with miR-140 mimic, USP22 or both assessed using Transwell assay (200 ×). * $p < 0.05$ compared with U2OS cells transfected with both NC mimic and empty vector. # $p < 0.05$ compared with U2OS cells transfected with both miR-140 mimic and empty vector. Comparison of data among multiple groups is analyzed by one-way ANOVA. Each experiment was repeated three times.

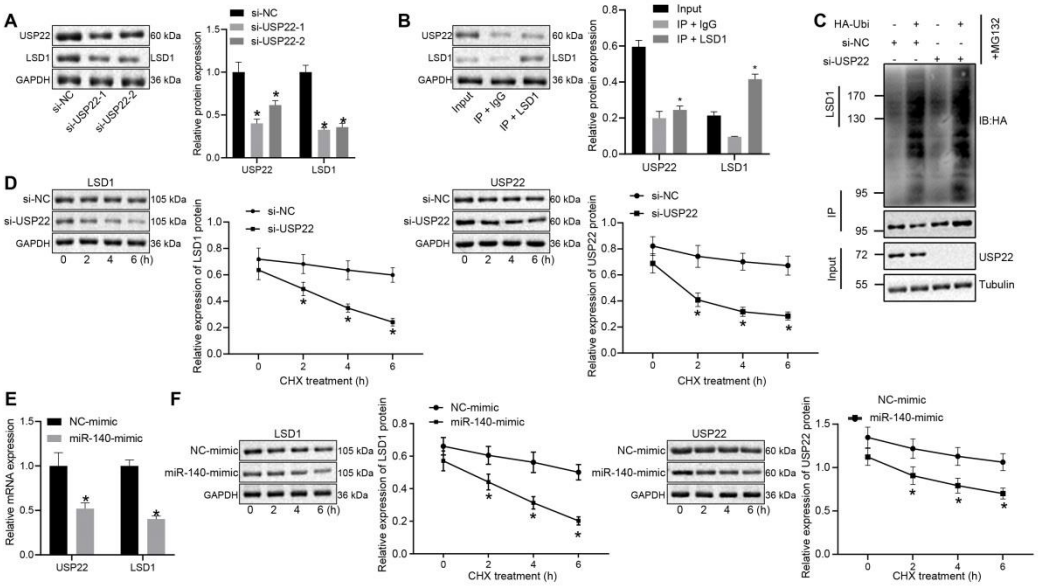

Figure S3. USP22 suppresses LSD1 ubiquitination and degradation in U2OS cells.

A, USP22 and LSD1 expression in U2OS cells transfected with si-USP22-1 or si-USP22-2 detected using Western blot. B, Interaction of USP22 with LSD1 in U2OS cells determined using Co-IP. C, Effects of USP22 knockdown on LSD1 ubiquitination in U2OS cells examined using Co-IP. D, Effects of USP22 knockdown on LSD1 protein stability in CHX-treated U2OS cells. E, Protein expression of USP22 and LSD1 in U2OS cells transfected with miR-140 mimic evaluated using Western blot. F, Effects of overexpressed miR-140 on LSD1 protein stability in CHX-treated U2OS cells. * $p < 0.05$ compared with U2OS cells treated with si-NC or NC mimic. Data between two groups were compared using unpaired *t*-test. Data among multiple groups were analyzed using one-way ANOVA, while data at different time points were compared using repeated measures ANOVA. Cell experiment was repeated for three times.

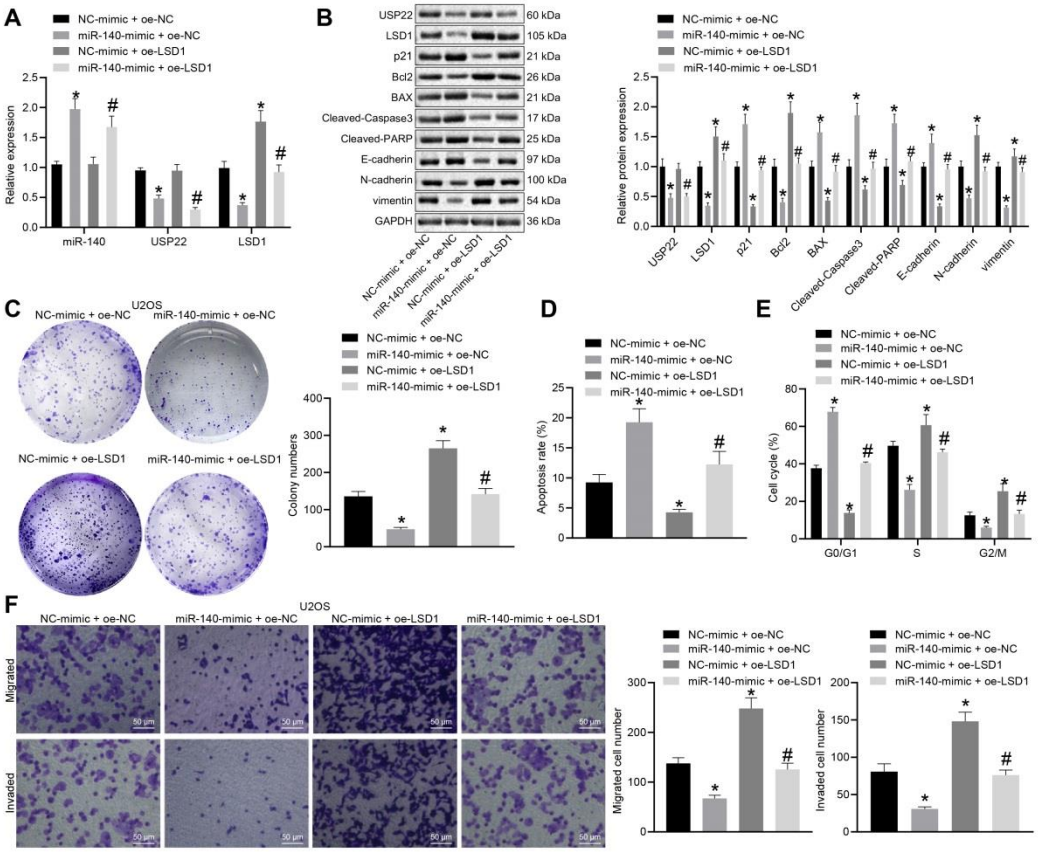

Figure S4. Poor p21 expression and amplified LSD1 expression in osteosarcoma tissues.

A, mRNA expression of p21 and LSD1 detected by RT-qPCR in osteosarcoma tissues (n = 65). B, Protein expression of p21 and LSD1 detected by Western blot in osteosarcoma tissues (n = 65). * $p < 0.05$ compared with adjacent normal tissues by paired *t*-test.

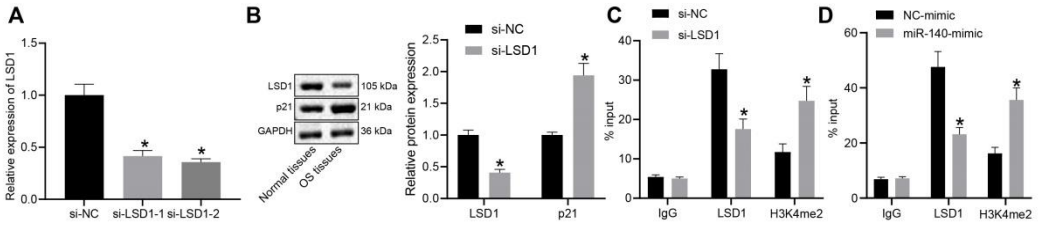

Figure S5. miR-140 suppresses U2OS cell proliferation, migration and invasion by downregulating LSD1 *via* USP22.

A, Expression of miR-140, USP22 and LSD1 in HOS cells transfected with miR-140-mimic, oe-LSD1 or both detected using RT-qPCR. B, Protein expression of Bcl2, BAX, cleaved-caspase3, cleaved-PARP, E-cadherin, N-cadherin and vimentin in U2OS cells transfected with miR-140-mimic, oe-LSD1 or both assessed using Western blot. C, Evaluation of U2OS cell proliferation using the colony formation assay upon transfection with miR-140-mimic, oe-LSD1 or both. D and E, U2OS cell apoptosis (D) and cell cycle (E) assessed using flow cytometry upon transfection with miR-140-mimic, oe-LSD1 or both. F, Transwell assay examining U2OS cell invasion and migration upon transfection with miR-140-mimic, oe-LSD1 or both (200 ×). * $p < 0.05$ compared with U2OS cells transfected with both NC-mimic and oe-NC. # $p < 0.05$ compared with U2OS cells transfected with both miR-140-mimic and oe-NC. Data comparison among multiple groups is analyzed by one-way ANOVA. Each experiment was repeated three times.

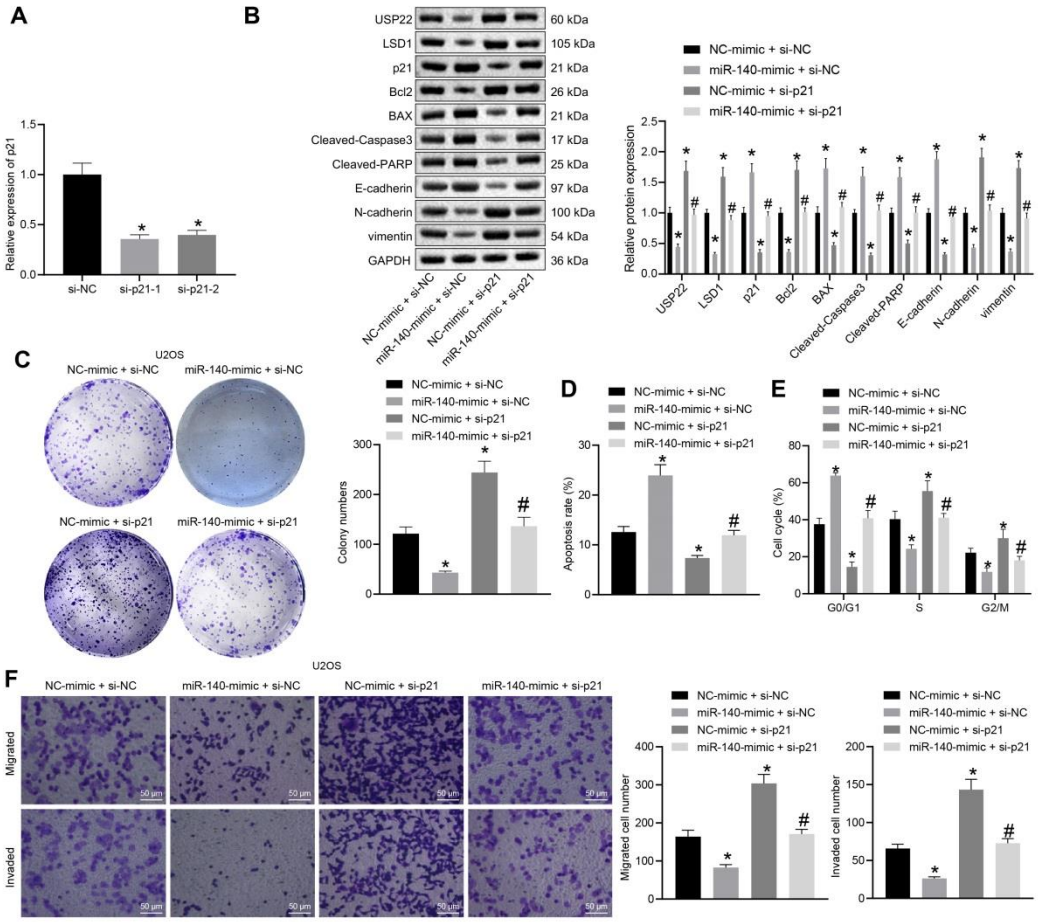

Figure S6. miR-140 enhances p21 H3K4me2 methylation through the USP22/LSD1 axis in U2OS cells.

A, RT-qPCR analysis of LSD1 expression in U2OS cells transfected with si-LSD1-1 or si-LSD1-2. B, Western blot for LSD1 and p21 expression in U2OS cells transfected with si-LSD1. C, ChIP detection of H3K4me2 enrichment and the presence of LSD1 on the p21 promoter region in U2OS cells transfected with si-LSD1. D, Effects of miR-140 overexpression on the enrichment of H3K4me2 and LSD1 on the p21 promoter region in U2OS cells. * $p < 0.05$ compared with U2OS cells transfected with si-NC or NC-mimic. The data comparison between two groups is analyzed by unpaired *t* test. The comparison among multiple groups is analyzed by one-way ANOVA. Each experiment was repeated three times.

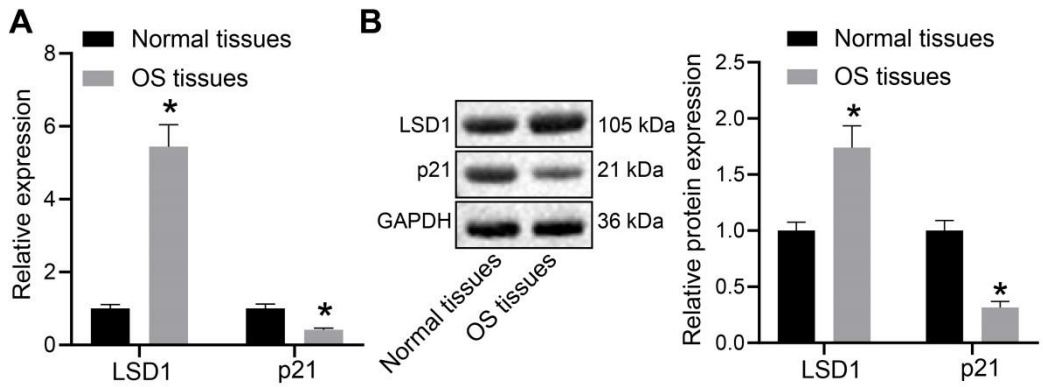

Figure S7. miR-140 hinders the malignant phenotypes of U2OS cells *via* p21 upregulation.

A, Silencing efficiency of siRNA targeting p21 detected by RT-qPCR in U2OS cells. B, Protein expression of p21, USP22, LSD1, Bcl2, BAX, cleaved-caspase3, cleaved-PARP, E-cadherin, N-cadherin and vimentin detected by Western blot in U2OS cells transfected with miR-140 mimic, si-p21 or both. C, D, and E, Analysis of proliferation (C), apoptosis (D) and cell cycle stage (E) of U2OS cells transfected with miR-140 mimic, si-p21 or both using the colony formation assay and flow cytometry. F, Transwell assay examining U2OS cell invasion and migration upon transfection with miR-140 mimic, si-p21 or both (200 ×). * $p < 0.05$ compared with U2OS cells transfected with si-NC or NC-mimic. # $p < 0.05$ compared with U2OS cells transfected with miR-140-mimic and si-NC. Comparison of data among multiple groups is analyzed by one-way ANOVA. Each experiment was repeated three times.

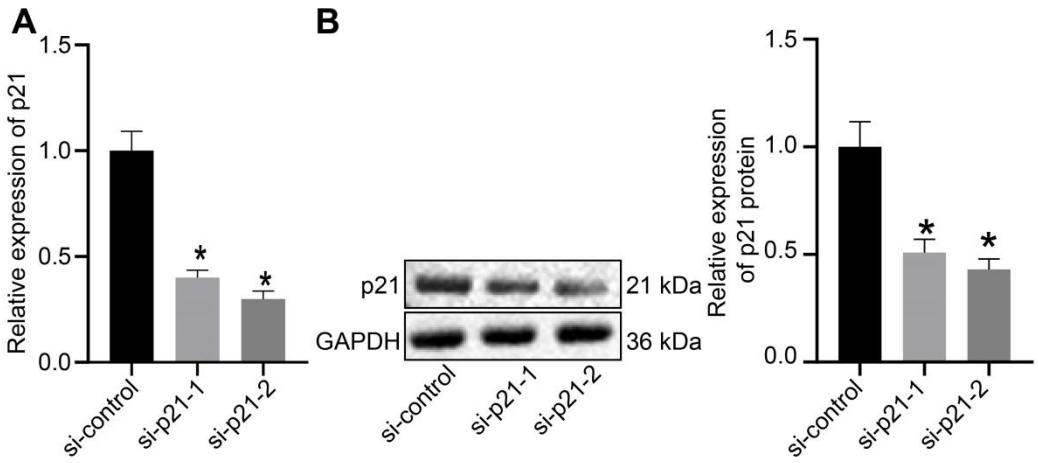

Figure S8. Transfection efficiency of shRNAs targeting p21.

A, p21 mRNA expression detected by RT-qPCR. B, p21 protein expression detected by Western blot. * $p < 0.05$ compared with sh-control by unpaired *t*-test.