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Supplemental Information

SOX2-Upregulated microRNA-30e Promotes the Progression of Esophageal Cancer via Regulation of the USP4/SMAD4/CK2 Axis

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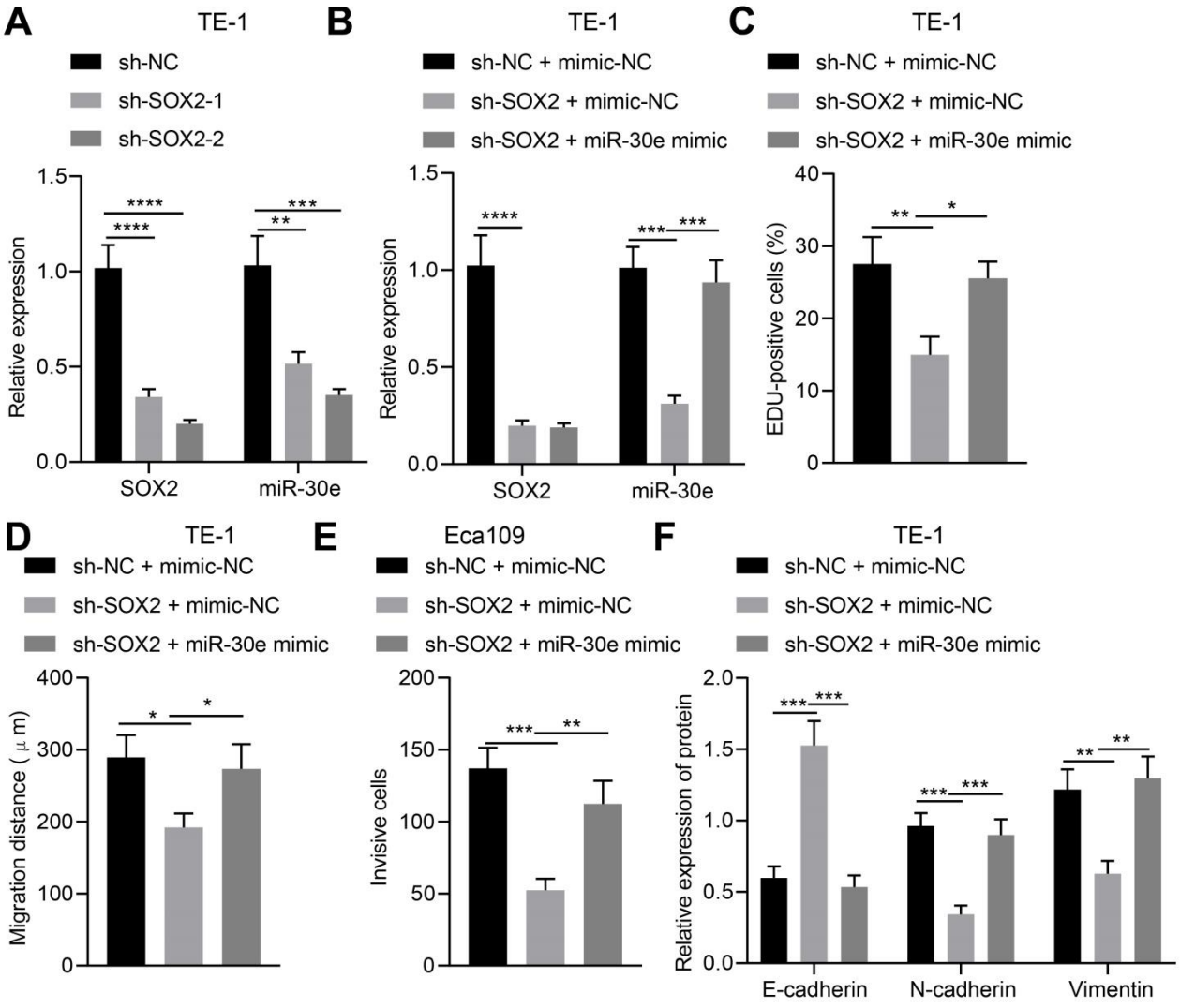
Supplementary Figure legends

Fig. S1. SOX2 promotes proliferation, migration, and invasion, as well as EMT of TE-1 cells *via* miR-30e upregulation *in vitro*. A, Expression of SOX2 and miR-30e in TE-1 cells transfected with sh-SOX2-1 or sh-SOX2-2 measured by RT-qPCR. B, Expression of SOX2 and miR-30e in TE-1 cells transfected with sh-SOX2 or in combination with miR-30e mimic measured by RT-qPCR. C, Proliferation of TE-1 cells detected by EdU assay (× 200). D, Migration of TE-1 cells detected by scratch test. E, Invasion of TE-1 cells detected by Transwell assay (× 200). F, Western blot analysis of EMT-related proteins (E-cadherin, N-cadherin, and Vimentin) in TE-1 cells. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$. Comparisons among multiple groups were performed using one-way ANOVA. Data are shown as mean ± standard deviation of three technical replicates.

Fig. S2. miR-30e promotes cell proliferation, migration, invasion, and EMT in TE-1 cells by targeting USP4 *in vitro*. A, Expression of USP4 and miR-30e determined by RT-qPCR in TE-1 cells transfected with miR-30e inhibitor or in combination with sh-USP4. B, Proliferation of TE-1 cells detected by EdU assay (× 200). C, Migration of TE-1 cells detected by scratch test. D, Invasion of TE-1 cells detected by Transwell assay (× 200). E, Western blot analysis of EMT-related proteins (E-cadherin, N-cadherin, and Vimentin) in TE-1 cells. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$. Comparisons among multiple groups were performed using one-way ANOVA. Data are shown as mean ± standard deviation of three technical replicates.

Fig. S3. SMAD4 decreased CK2 expression to suppress proliferation, migration, invasion, and EMT of TE-1 cells *in vitro*. A, mRNA expression of SMAD4 and CK2 in TE-1 cells treated with oe-SMAD4 detected by RT-qPCR. B, Western blot analysis of SMAD4 and CK2 proteins in TE-1 cells treated with oe-SMAD4. C, Enrichment of SMAD4 in CK2 promoter region determined by ChIP assay in oe-SMAD4-treated TE-1 cells. TE-1 cells were transfected with oe-NC, oe-SMAD4, and oe-SMAD4 + oe-CK2. D, Expression of SMAD4 and CK2 in TE-1 cells measured by RT-qPCR.

E, Proliferation of TE-1 cells detected by EdU assay (× 200). F, Migration of TE-1 cells detected by scratch test. G, Invasion of TE-1 cells detected by Transwell assay (× 200). H, Western blot analysis of EMT-related proteins (E-cadherin, N-cadherin, and Vimentin) in TE-1 cells. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$. Comparisons among multiple groups were performed using one-way ANOVA. Data are shown as mean ± standard deviation of three technical replicates.



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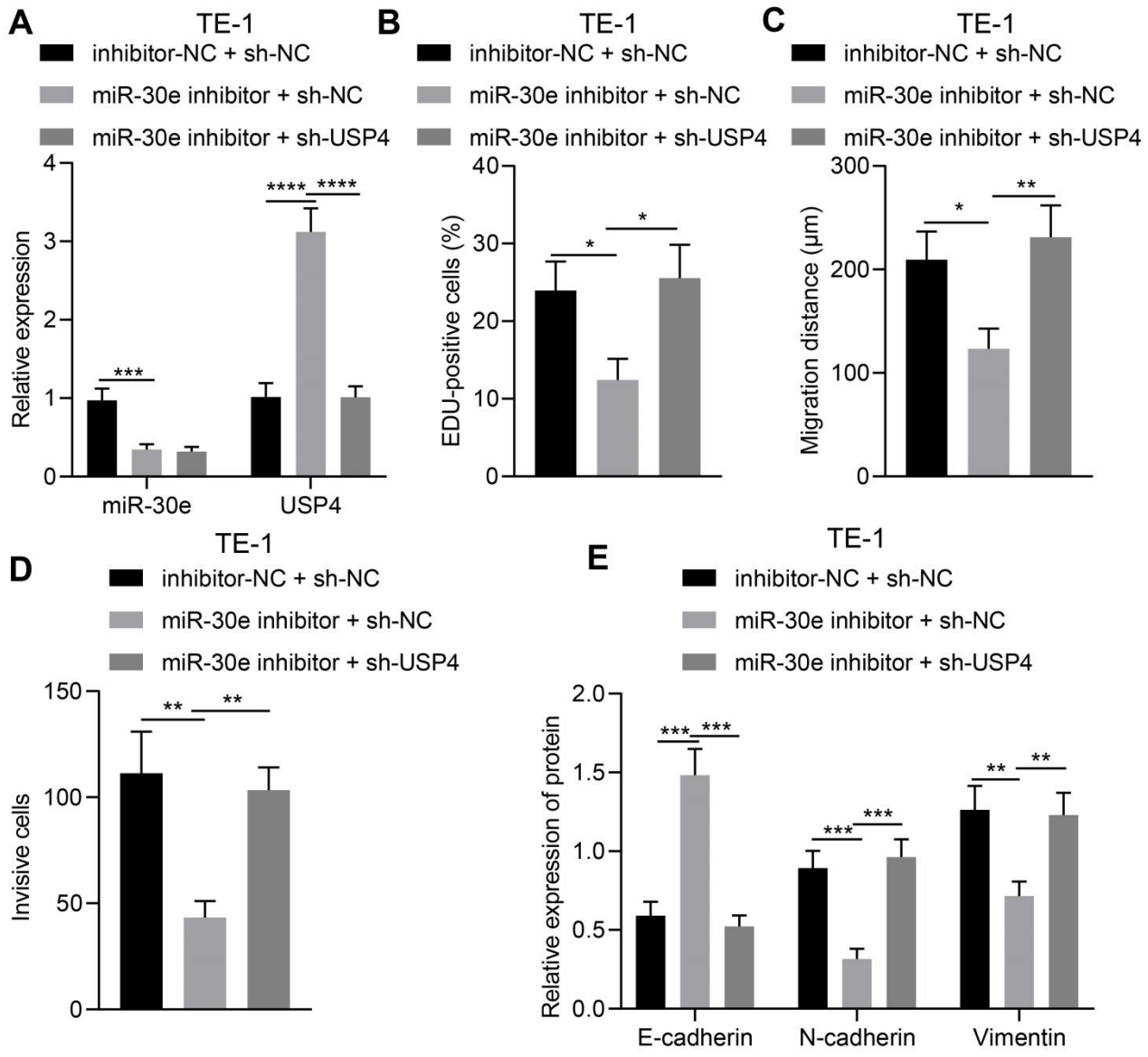

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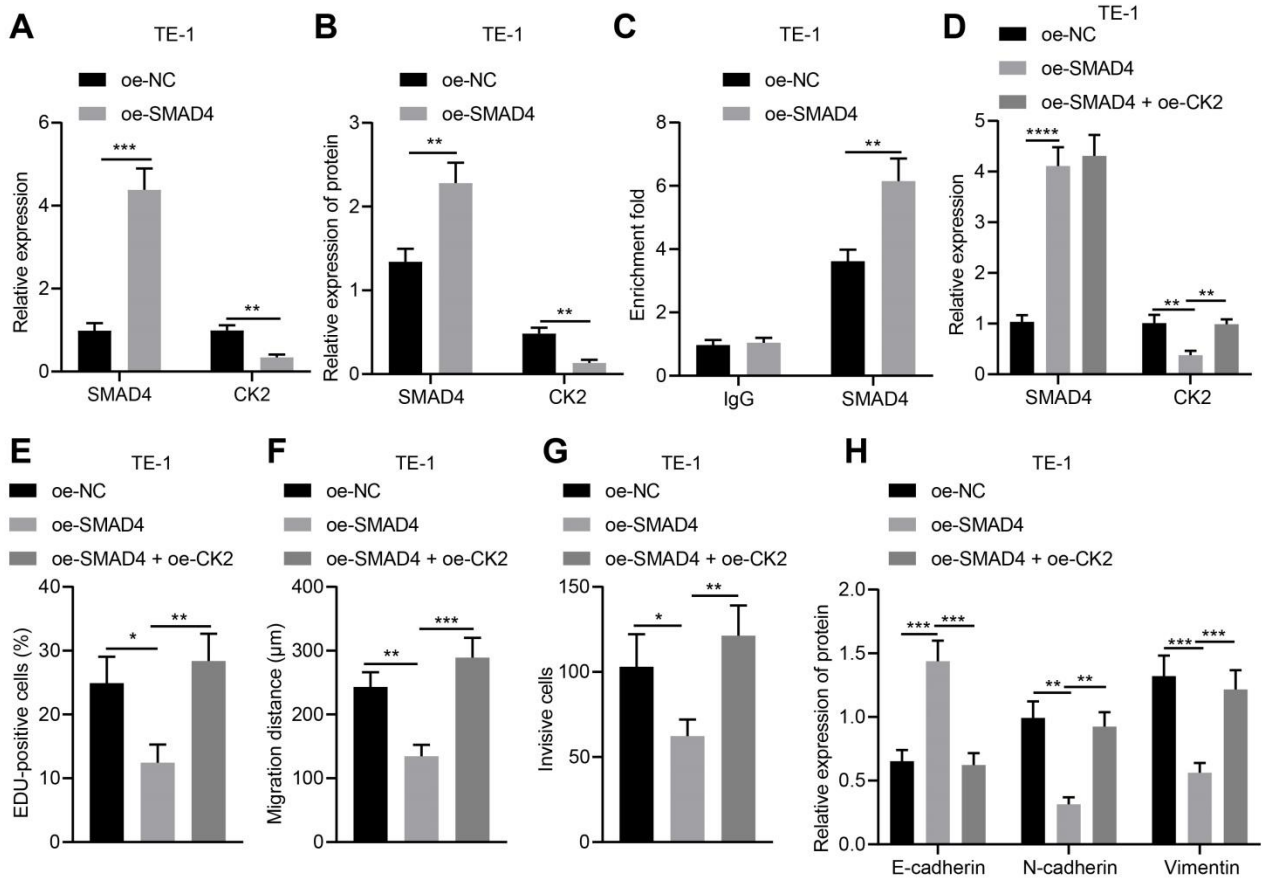

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