

The Roles and Prognostic Significance of ABI1-TSV-11 Expression in Patients with Left-sided Colorectal Cancer

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+Yu Zhang and Zhaohui Zhong contributed equally to this study

Supplementary material

Fig.S1

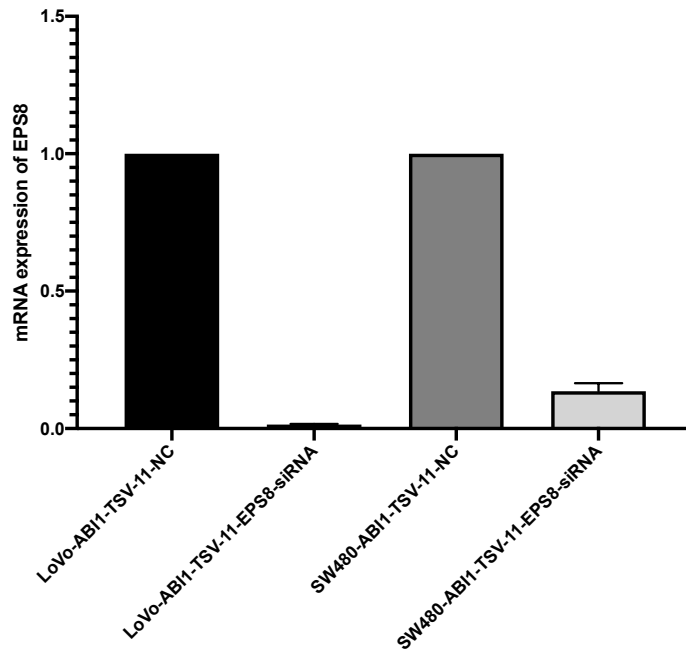


Fig. S1. Establishment of EPS8-knockdown cell lines by EPS8-siRNA in ABI1-TSV-11 overexpressing LoVo and SW480 cell lines.

qRT-PCR analysis using *EPS8* specific primers of in LoVo-ABI1-TSV-11 and SW480-ABI1-TSV-11 cell lines after EPS8 and NC siRNAs transfection.

Fig.S2

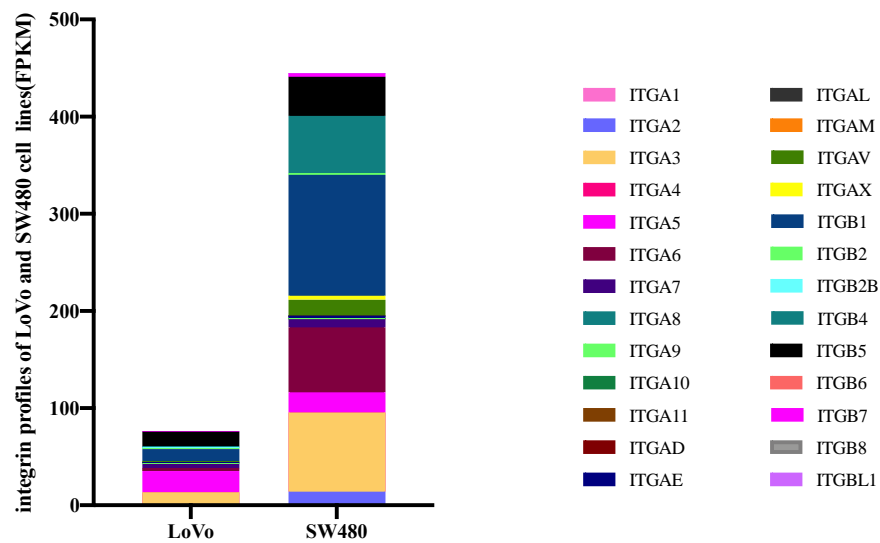


Fig. S2. integrins mRNA expression profiles of LoVo and SW480 cell lines using RNA sequencing data.

Fig. S3

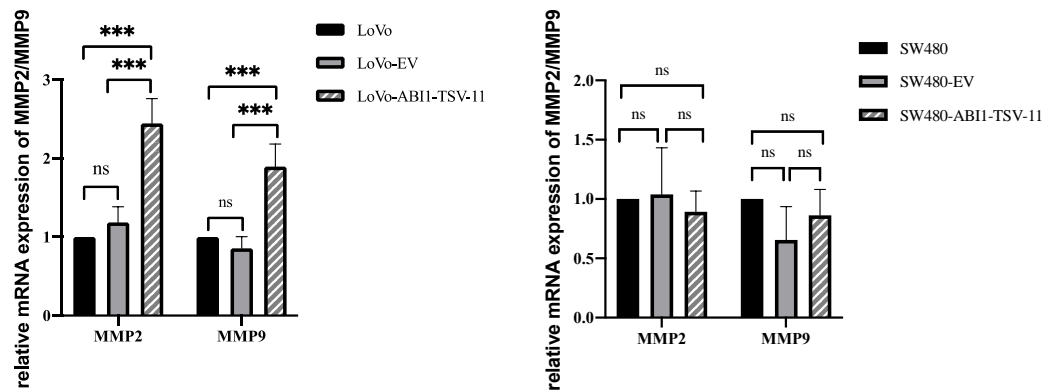


Fig. S3. mRNA expression of MMP2 and MMP9 of LoVo-ABI1-TSV-11, SW480-ABI1-TSV-11 and their corresponding controls

Fig.S4

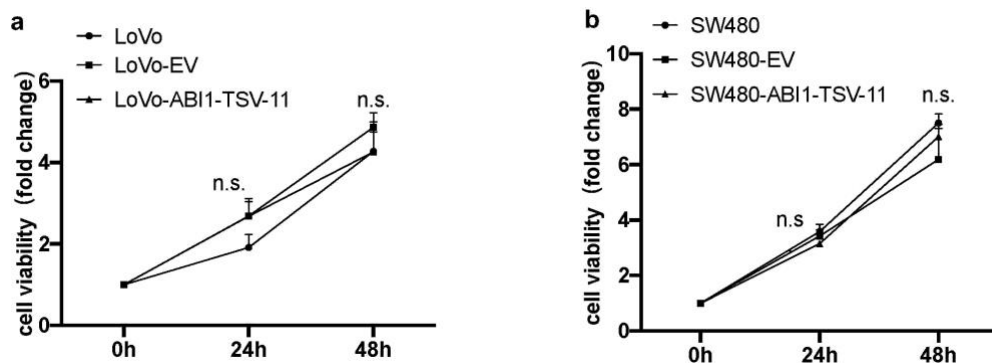
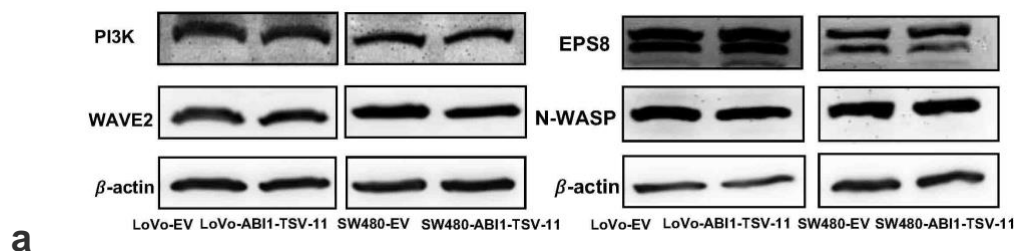
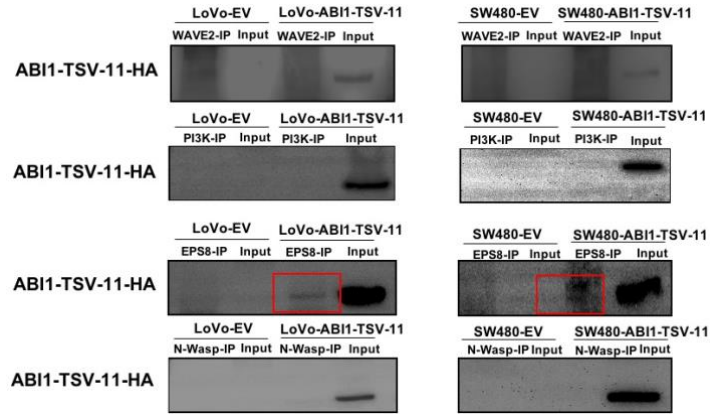


Fig. S4. ABI1-TSV-11 overexpression has no effects on proliferation and cell cycle in LoVo and SW480 cell lines *in vitro*.

a, Proliferation analysis of LoVo-ABI1-TSV-11 cell line and controls using Cell Counting Kit-8. b, Proliferation analysis of SW480-ABI1-TSV-11 cell line and controls using Cell Counting Kit-8. Data are presented as the mean \pm S.D. of three or four experiments (one-way ANOVA, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, not significant).

Fig. S5





b

Fig.S5. WAVE2, PI3K, EPS8, and N-WASP protein expression in LoVo-ABI1-TSV-11, LoVo-EV, SW480-ABI1-TSV-11, SW480-EV cells, and the interaction of ABI1-isoform-11 with EPS8.

a, Protein expression analysis of WAVE2, PI3K, EPS8, and N-WASP in LoVo-ABI1-TSV-11, SW480-ABI1-TSV-11, and their corresponding control cells. b, ABI1-isoform-11 interacts with EPS8 (shown in red boxes), not WAVE2, PI3K and N-WASP in LoVo-ABI1-TSV-11 and SW480-ABI1-TSV-11 cells.

Fig.S6

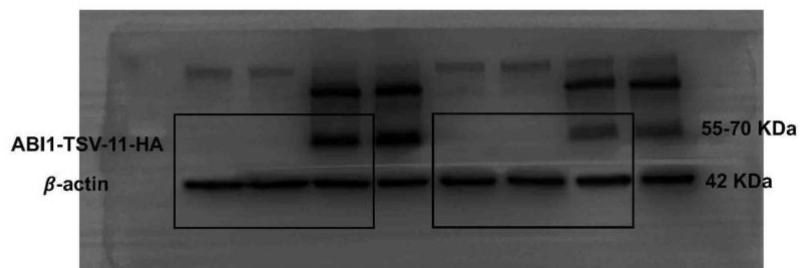
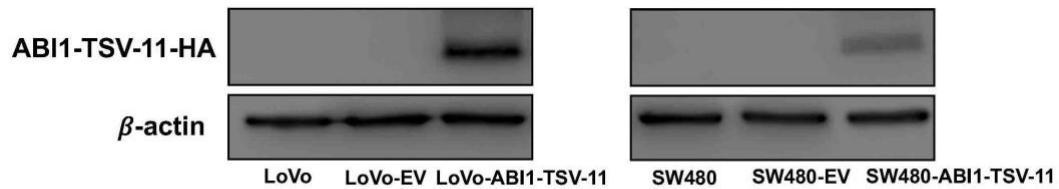


Fig.S6. Full length western blot of Fig.2d

Fig.S7

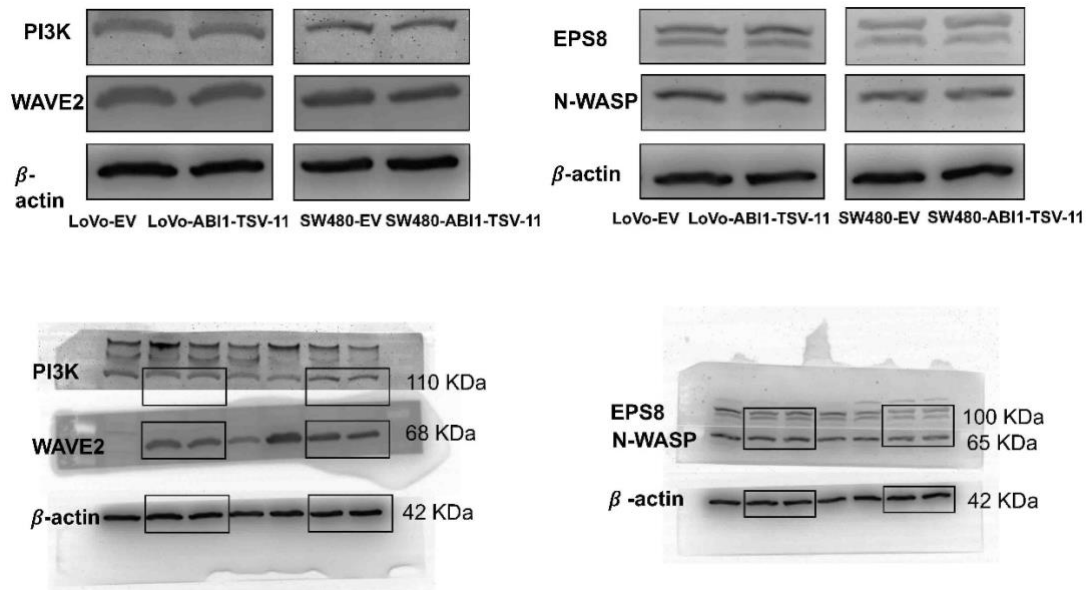


Fig.S7. Full length western blot of Fig.S5a

Fig.S7

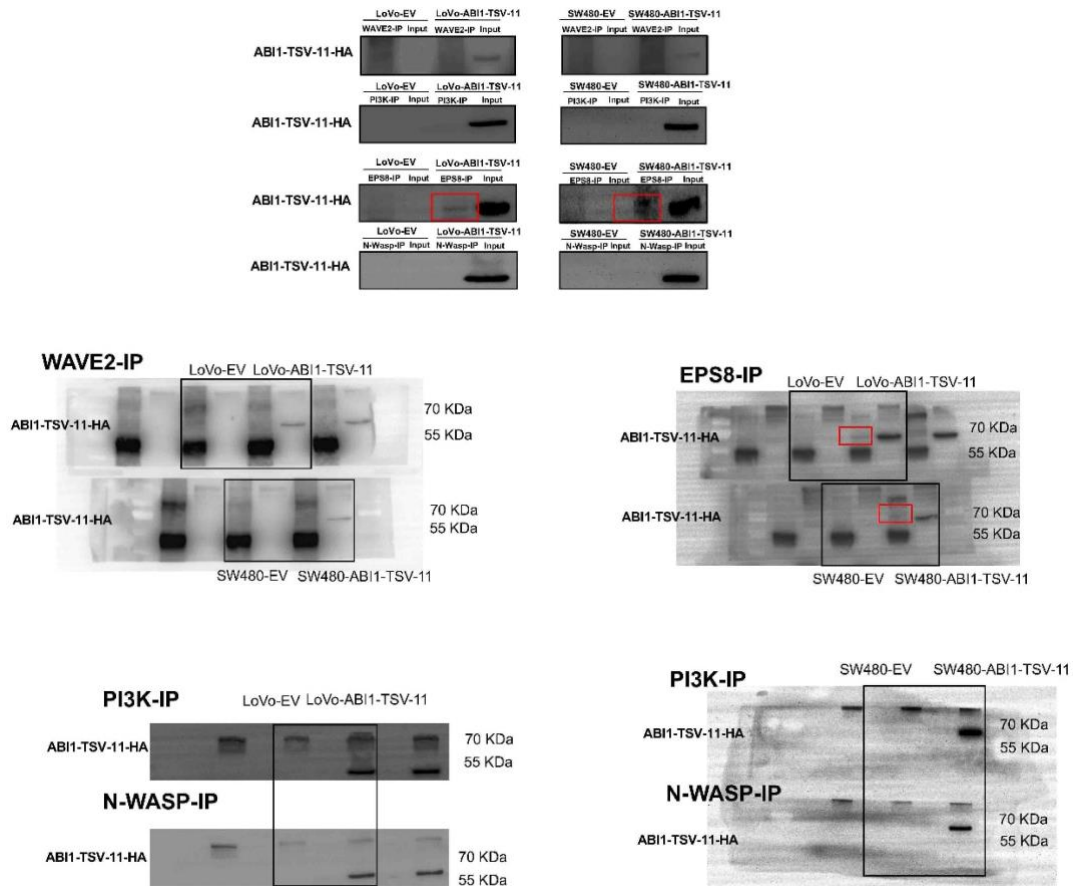


Fig.S8. Full length co-immunoprecipitation of Fig.S5b

Fig. S9

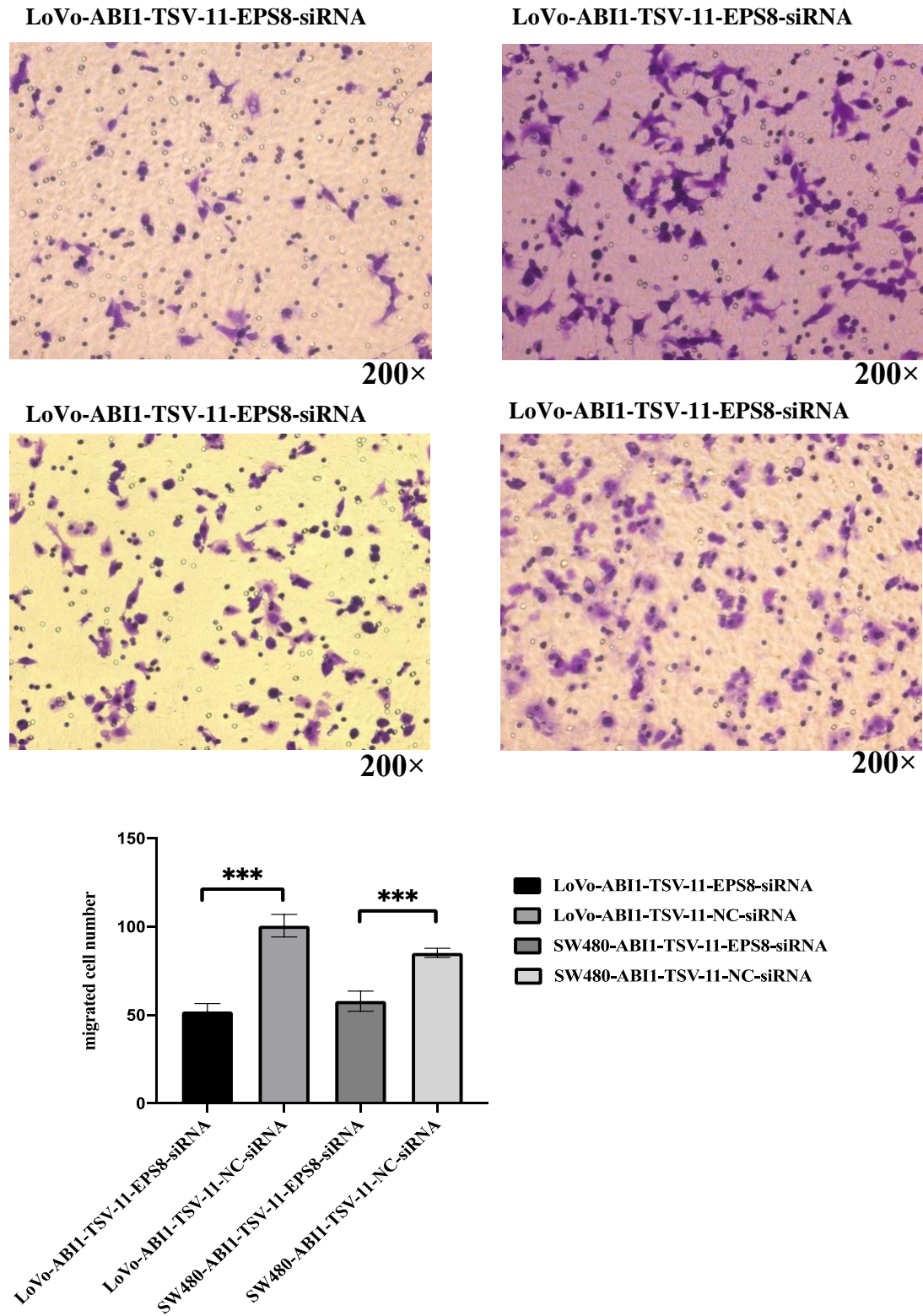


Fig.S9 EPS8 knockdown attenuates migration of LoVo-ABI1-TSV-11 and SW480-ABI1-TSV-11 cells in vitro (*P < 0.05, **P < 0.01, *P < 0.001, ns, not significant)**

Fig. S10

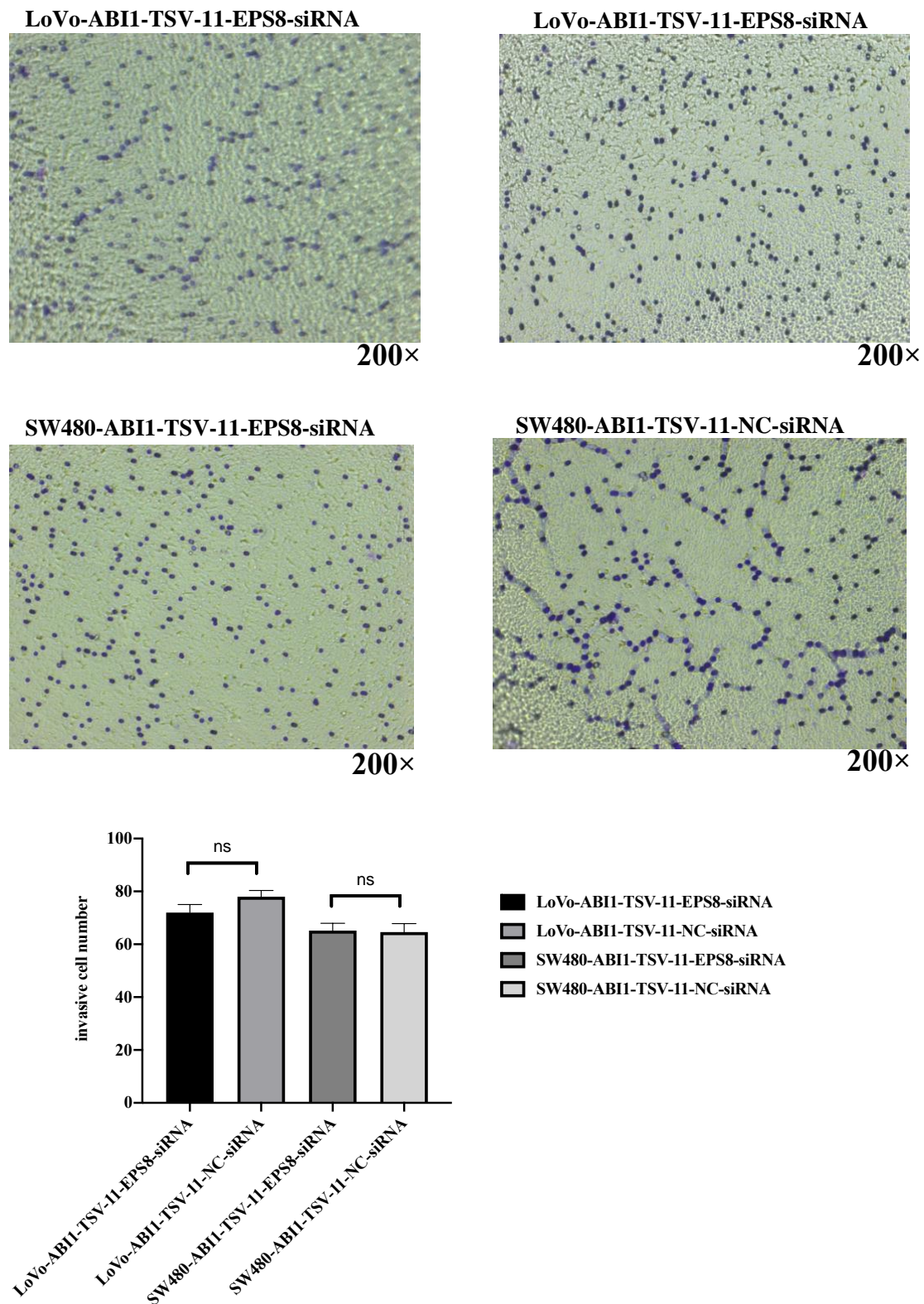


Fig. S10 EP8 Kncokdown does not change invasion of LoVo-ABI1-TSV-11 and SW480-ABI1-TSV-11 cell lines in vitro. (*P < 0.05, **P < 0.01, *P < 0.001, ns, not significant)**

Fig. S11

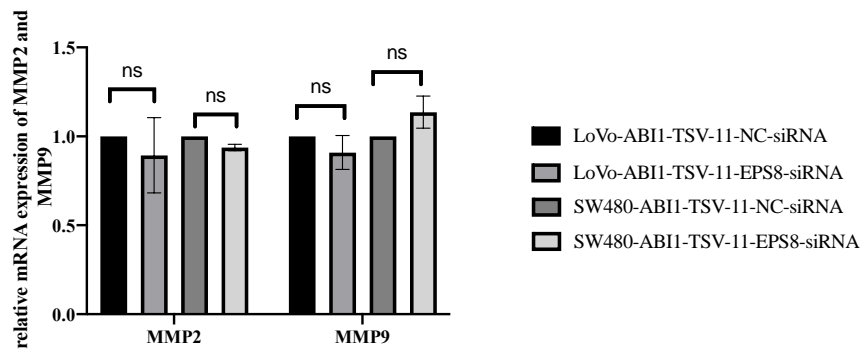
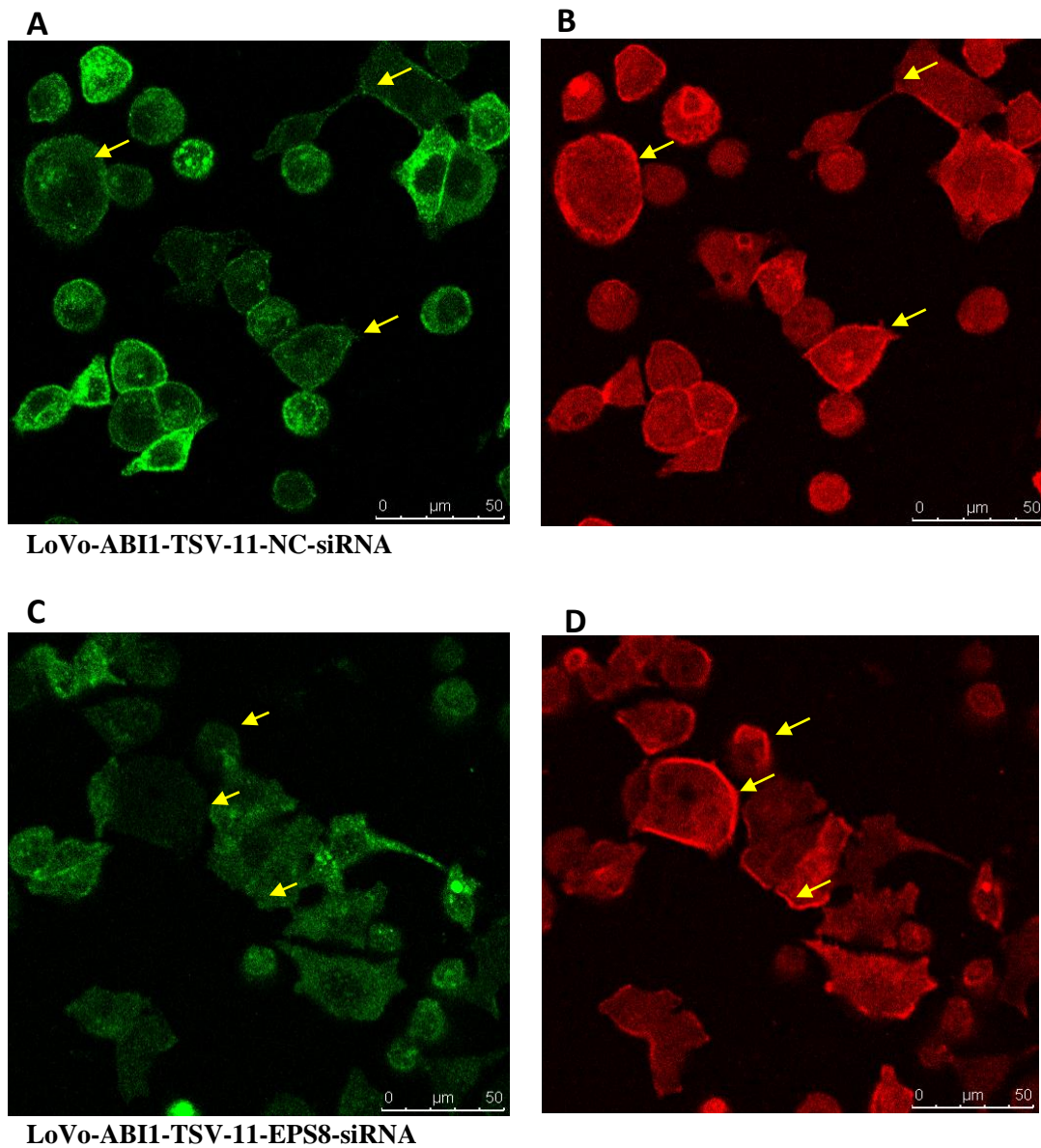
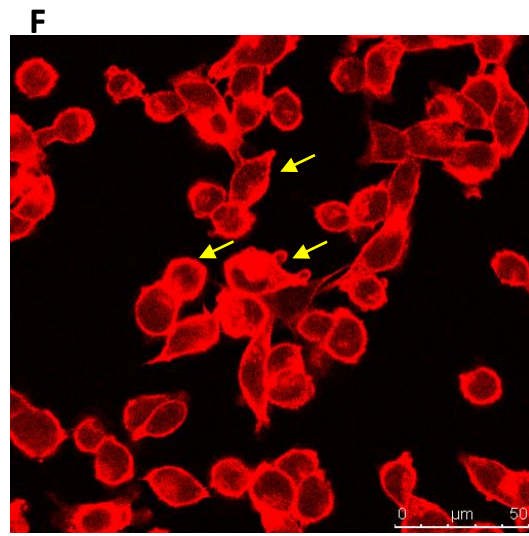
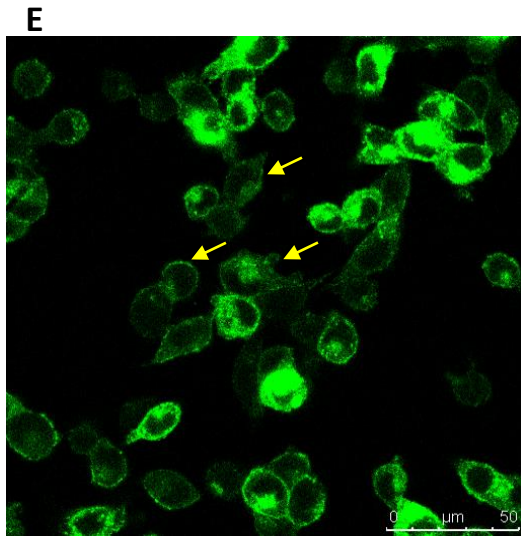


Fig. S11 mRNA expression of MMP2 and MMP9 of LoVo-ABI1-TSV-11-EPS8-siRNA, SW480-ABI1-TSV-11-EPS8-siRNA and their corresponding controls (*P < 0.05, **P < 0.01, *P < 0.001, ns, not significant)**

Fig. S12





SW480-ABI1-TSV-11-NC-siRNA

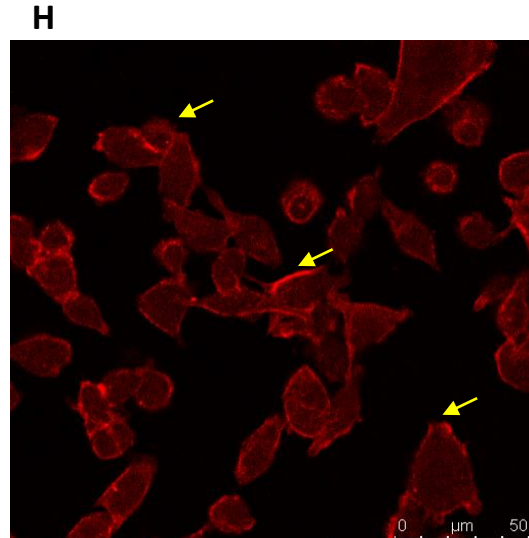
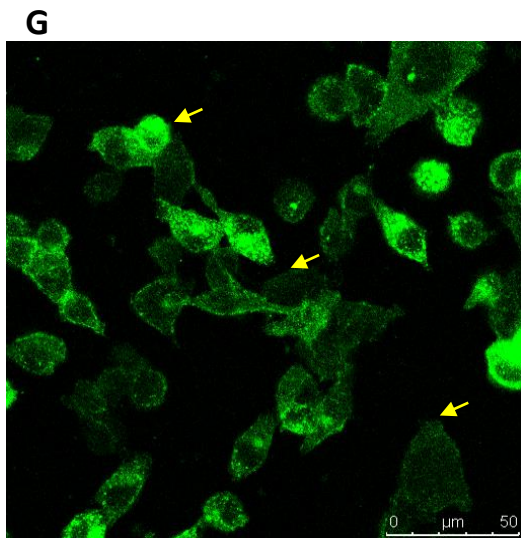


Fig.S12 knockdown EPS8 attenuates the colocalization of ABI-isoform-11 (Green) and F-actin (Red) in LoVo-ABI1-TSV-11 cell lines (A-D) and SW480-ABI1-TSV-11 cell lines (E-H) at the subcellular level.

Table S1 The primers for qRT-PCR assay.

| Primer Name | Primer Sequence (5'-3') |
|-------------------------------|--------------------------------|
| β -actin Forward primer | 5'-CATGTACGTTGCTATCCAGGC-3' |
| β -actin Reverse primer | 5'-CTCCTTAATGTACGCACGAT-3' |
| ABI1 Forward primer | 5'-ACACTGGGACGGAATACTCCTTAT-3' |
| ABI1 Reverse primer | 5'-ACACTGGGACGGAATACTCCTTAT-3' |
| MMP2 Forward primer | 5'-AGCGAGTGGATGCCGCCTTAA-3' |
| MMP2 Reverse primer | 5'-CATTCCAGGCATCTGCGATGAG-3' |
| MMP9 Forward primer | 5'-GCCACTACTGTGCCTTTGAGTC-3' |
| MMP9 Reverse primer | 5'-CCCTCAGAGAATCGCCAGTACT-3' |
| EPS8 Forward primer | 5'-GCCAACTTCTAATCGCCATA-3' |
| EPS8 Reverse primer | 5'-TCACTGTTGTTTCCTTGCTAC-3' |

Table S2 Antibodies or dyes used in this study.

| Name | Article number | Corporation name | Country | Dilution ratio |
|------------------------------------|----------------|---------------------------------------|---------|--|
| β -actin | TA-09 | Zhongshan Golden-bridge Biotechnology | China | 1:1000 (WB) |
| ABI1 | sc-30038 | Santa Cruz Biotechnology | USA | 1:200(WB) |
| HA-Tag | #3724 | Cell Signaling Technology | USA | 1:1000(WB) |
| WAVE2 | 07-410 | Millipore | USA | 1:1000 (WB) 1:50 (IP) |
| PI3K | #4228 | Cell Signaling Technology | USA | 1:1000 (WB) 1:50 (IP) |
| ESP8 | #43114 | Cell Signaling Technology | USA | 1:1000 (WB) 1:50 (IP) 1:100 (IF) |
| N-WASP | #4848 | Cell Signaling Technology | USA | 1:1000 (WB) 1:50 (IP) |
| TRIRC-labeled Goat Anti-Rabbit IgG | ZF-0316 | Zhongshan Golden-bridge Biotechnology | China | 1:50 (IF) |
| Phalloidin-iFluor-647 | ab176759 | Abcam | UK | 1X (IF) |
| DAPI | F01001-00 | GP Medical Technologies | China | 10ug/ml (IF) |



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May 18, 2020

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This letter confirms that the manuscript entitled *Elevated ABI1-TSV-11 expression is related to lymph node metastasis and poor prognosis of left-sided colorectal cancer, and promotes LoVo and SW480 cancer cell migration in vitro and lung metastasis in vivo by interacting with EPS8* by Dr. Yu et al has been copyedited by native English speakers with a related biomedical background in **BioMed Proofreading® LLC**.

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Fig.S13. Certificate for English copyediting service.