

## ZEB1 interacts with HDGF and promotes development of endometrial cancer

**Supplementary Table 1.** The sequences of shRNA were used in this study

|        |   |           |   |
|--------|---|-----------|---|
| shHDGF | 1 | Sence     | 5'CcgggtgCCGTGAAATCAACAGCCAACCTCGAGTTGGCTGTTGATTTCACGGCATTTTTg 3' |
|        |   | Antisence | 5'aattcaaaaatgCCGTGAAATCAACAGCCAACCTCGAGTTGGCTGTTGATTTCACGGCA 3'  |
|        | 2 | Sence     | 5'CcgggaACGAGAAAGGAGCGTTGAACTCGAGTTCAACGCTCCTTTCTCGTTCTTTTTg 3'   |
|        |   | Antisence | 5'aattcaaaaagaACGAGAAAGGAGCGTTGAACTCGAGTTCAACGCTCCTTTCTCGTTC 3'   |
|        | 3 | Sence     | 5'CcggcgAGAACAACCTACTGTCAACTCGAGTTGACAGTAGGGTTGTTCTCGTTTTg 3'     |
|        |   | Antisence | 5'aattcaaaaacgAGAACAACCTACTGTCAACTCGAGTTGACAGTAGGGTTGTTCTCG 3'    |
| shZEB1 | 1 | Sence     | 5'CcggccTCTCTGAAAGAACACATTACTCGAGTAATGTGTTCTTTCAGAGAGTTTTT 3'     |
|        |   | Antisence | 5'aataaaaacctCTCTGAAAGAACACATTACTCGAGT AATGTGTTCTTTCAGAGAGG 3'    |
|        | 2 | Sence     | 5'ccgggcTGTTGTTCTGCCAACAGTTCTCGAGAACTGTTGGCAGAAACAGCTTTTT 3'      |
|        |   | Antisence | 5'aataaaaagcTGTTGTTCTGCCAACAGTTCTCGAGA ACTGTTGGCTGAACA ACAGC 3'   |
|        | 3 | Sence     | 5'ccggccTACCACTGGATGTAGTAAACTCGAGTTTACTACATCCAGTGGTAGGTTTTTg 3'   |
|        |   | Antisence | 5'aattcaaaaaccTACCACTGGATGTAGTAAACTCGAGTTTACTACATCCAGTGGTAGG 3'   |

**Supplementary Table 2.** The sequences of siRNA were used in this study

| Name    | Sequence                                |
|---------|---|
| si-ZEB1 | 1 Sence 5'GGCAAGUGUUGGAGAAUA dTdT 3'    |
|         | Antisence 3'dTdT CCGUUCACAACCUUUAUU5'   |
|         | 2 Sence 5'CCAGAAUACACAGGGUUA dTdT 3'    |
|         | Antisence 3'dTdT GGUCUUUAUGUGUCCAAU 5'  |
|         | 3 Sence 5'GGACAGCACAGUAAUUA dTdT 3'     |
|         | Antisence 3'dTdT CCUGUCGUGUCAUUUAGAU5'  |
| si-HDGF | 1 Sence 5'GAAACGAGAUUGAAUGCAC dTdT 3'   |
|         | Antisence 3'dTdT CUUUGCUCUAGCUUACGUG 5' |
|         | 2 Sence 5'CUAAGCGUUUCCUCCUUA dTdT 3'    |
|         | Antisence 3'dTdT GAGUUCGCAAAGGAGGAU 5'  |
|         | 3 Sence 5'CCAUACGAUUGACGAGAUG dTdT 3'   |
|         | Antisence 3'dTdT GGUAUGCUAACUGCUCUAC 5' |

**Supplementary Table 3.** The primers and probes used in this study

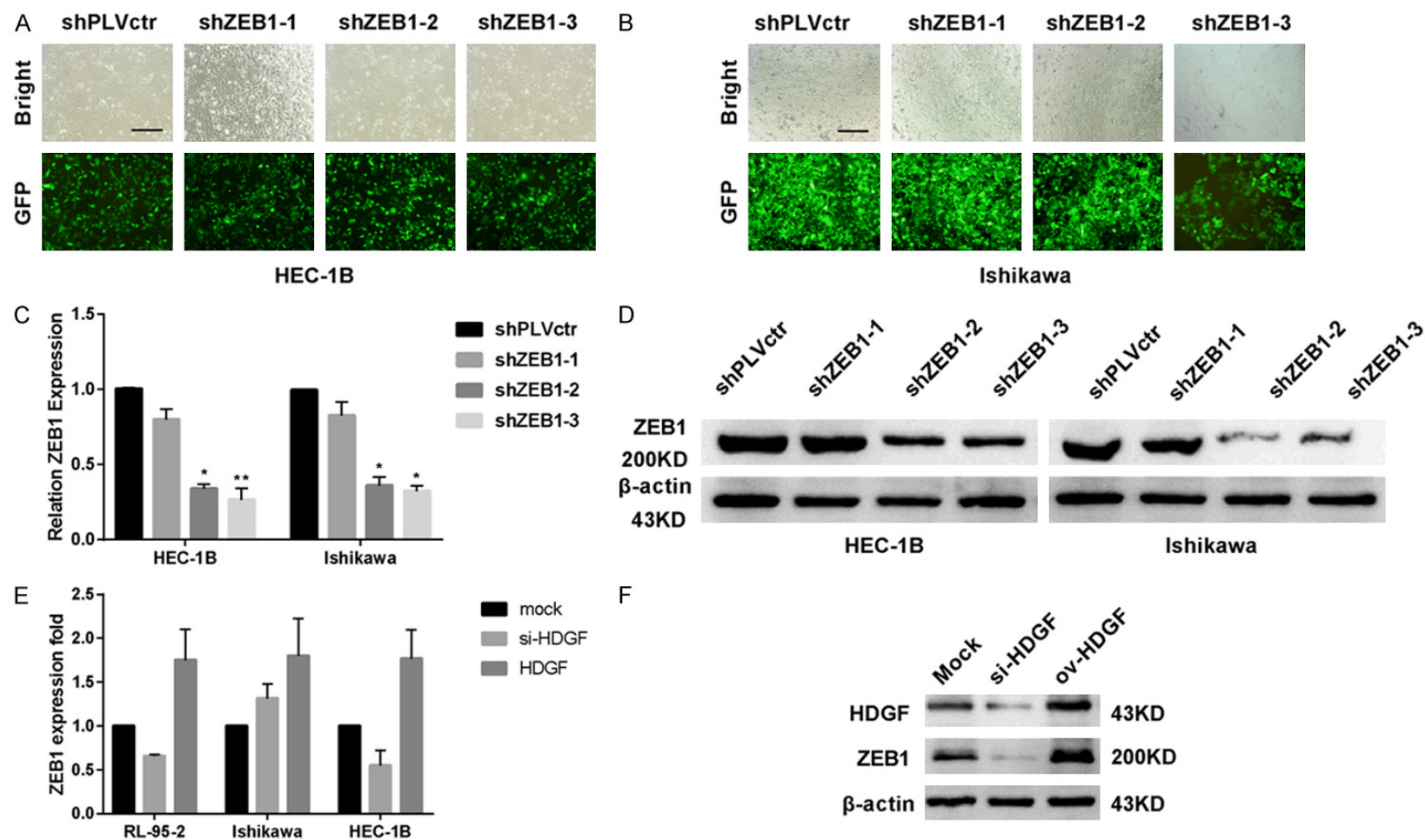
| Primers name                     | Sequence   |
|----------------------------------|--|
| ZEB1                             | Forward AGCAGTGAAGAGAAGGGAATGC                                   |
|                                  | Reverse GGTCTCTCAGGTGCCTCAG                                      |
| HDGF                             | Forward CGTGTACAGACGTCCACT                                       |
|                                  | Reverse CTCCTTGGCTGGCTCATCA                                      |
| ARF                              | Forward ATCTGTTTACAGTCTGGGACG                                    |
|                                  | Reverse CCTGCTTGTGGCAAATACC                                      |
| CHIP-ZEB1-Site1                  | Forward CTGTAATCCAGTACTCAGG                                      |
|                                  | Reverse AGAATCTCGCTCTGTTGCC                                      |
| CHIP-ZEB1-Site2                  | Forward GGACTATTACACTGACTGCAT                                    |
|                                  | Reverse GCAAGAAAACAGCCAAGCA                                      |
| CHIP-ZEB1-Site3                  | Forward TGCCAGTTCAGTCATACC                                       |
|                                  | Reverse GAATGCCAATAAACCAACCC                                     |
| EMSA-ZEB1-WT probe               | TGGCACACACCTGTAAAGAATGCCCCCTGTGCCACCCACCTCCGAAAAGAGGTAGCACCTGG   |
| EMSA-ZEB1-WT competition         | TGGCACACACCTGTAAAGAATGCCCCCTGTGCCACCCACCTCCGAAAAGAGGTAGCACCTGG   |
| EMSA-ZEB1-mut- site1 probe       | TGGTCTCTACTTAAAGAATGCCCCCTGTGCCACCCACCTCCGAAAAGAGGTAGCACCTGG     |
| EMSA-ZEB1-mut-site2 probe        | TGGCACACACCTGTAAAGAATTAATATCTTGCCACCCACCTCCGAAAAGAGGTAGCACCTGG   |
| EMSA-ZEB1-mut-site3 probe        | TGGCACACACCTGTAAAGAATGCCCCCTGTGCCAATATCTACTATCGGCTATTCTGTTGATCTA |
| EMSA-ZEB1-mut-3-site competition | TGGTCTCTACTTAAAGAATTAATATCTTGCCAATATCTACTATCGGCTATTCTGTTGATCTA   |

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**Supplementary Table 4.** A list of antibodies used for Western blot, IHC staining, IF, Co-IP, CHIP

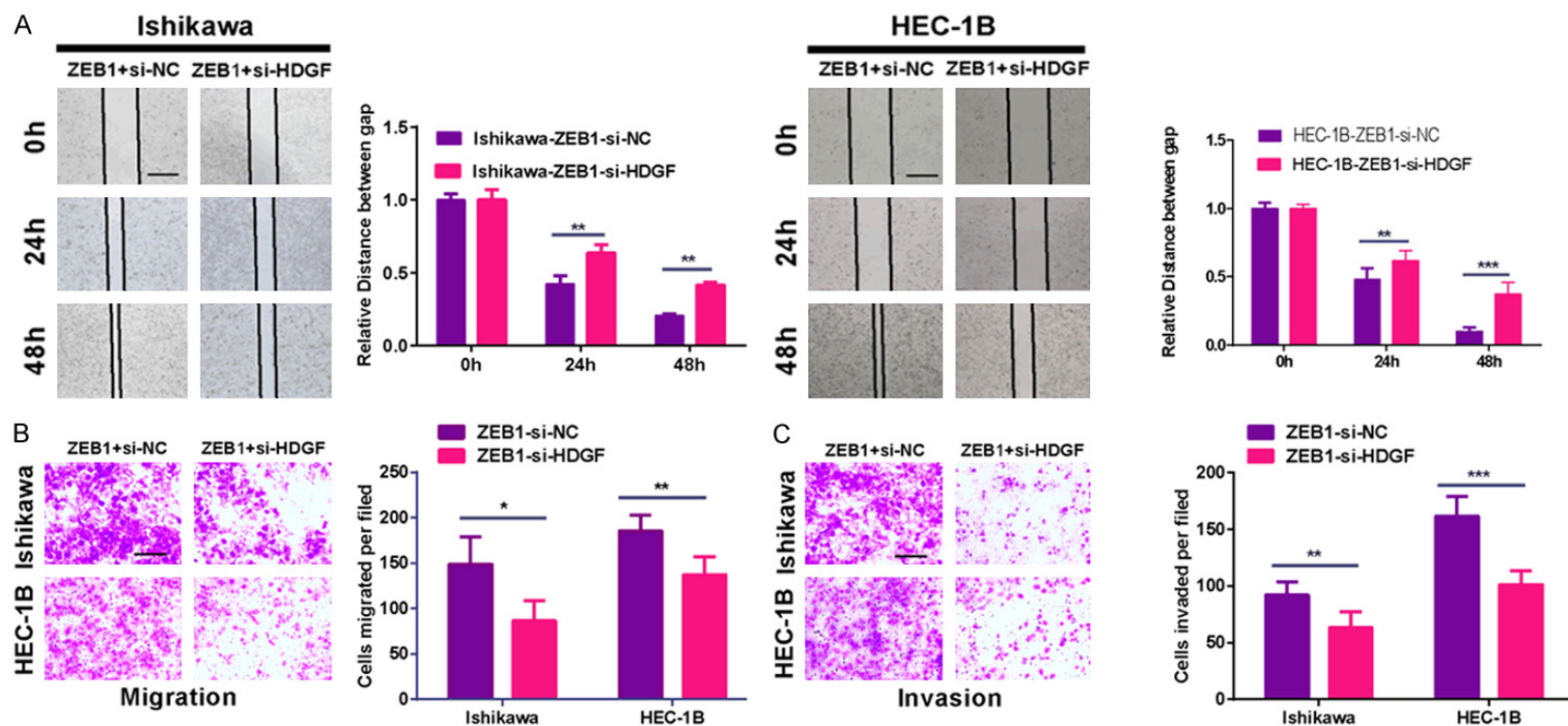
| Name of antibody | Cat. No    | Company     | Mol weight | Dilution (WB/IHC/CHIP/EMSA)                    |
|------------------|------------|-------------|------------|--|
| ZEB1             | 3396S      | CST         | 200 kDa    | 1:1000 (WB); 1:200 (IP); 1:50 (EMSA)           |
| ZEB1             | Ab203829   | abcam       | 200 kDa    | 1:250 (IHC); 1:100 (IF)                        |
| E-cadherin       | 3195S      | CST         | 135 kDa    | 1:1000 (WB); 1:200 (IF)                        |
| N-cadherin       | 13116S     | CST         | 140 kDa    | 1:1000 (WB)                                    |
| $\beta$ -catenin | 8480S      | CST         | 92 kDa     | 1:1000 (WB); 1:50 (IP)                         |
| Snail            | 3879S      | CST         | 29 kDa     | 1:1000 (WB)                                    |
| lamin B1         | 13435S     | CST         | 68.45 kDa  | 1:1000 (WB)                                    |
| Vimentin         | 5741S      | CST         | 57 kDa     | 1:1000 (WB); 1:100 (IF)                        |
| HDGF             | 60064-1-Ig | Proteintech | 40 kDa     | 1:1000 (WB); 1:50 (IHC); 1:100 (IF)            |
| HDGF             | 11344-1-AP | Proteintech | 40 kDa     | 1:200 (IP)                                     |
| Flag-Tag         | F1804      | Sigma       | -          | 1:100 (Co-IP); 1:1000 (WB)                     |
| His-Tag          | 12698s     | CST         | -          | 1:100 (Co-IP); 1:3000 (Pull down); 1:1000 (WB) |
| $\beta$ -actin   | sc-1616    | Santa       | 43 kDa     | 1:1000 (WB)                                    |
| PCNA             | 10205-2-AP | Proteintech | 36-38 kDa  | 1:30 (IHC)                                     |
| Ki67             | Ab16667    | abcam       | -          | 1:100 (IHC)                                    |
| TCF4             | 22337-1-AP | Proteintech | 72 kDa     | 1:1000 (WB); 0.5-4.0 $\mu$ g (Co-IP)           |
| Normal IgG       | 2729       | CST         | -          | 1:10 (IP)                                      |

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**Supplementary Figure 1.** A and B. HEC-1B and Ishikawa cells were transfected by lentiviruses containing shPLVctr or shZEB1. Scale bar: 25  $\mu$ m. C. ZEB1 expression was detected after transfection with lentiviruses. D. ShRNA against ZEB1 as indicated in EC cells by Western blot. E. ZEB1 expression was detected after transfection with si-RNA (HDGF) or HDGF plasmid. F. ZEB1 expression was detected by Western blot. ARF served as a loading control in QPCR assay.  $\beta$ -actin was used as a loading control in Western blot assay. Data were shown as the mean  $\pm$  SD, \*P < 0.05; \*\*P < 0.01.

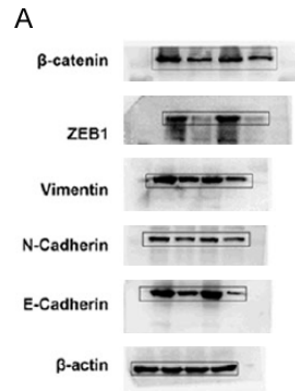
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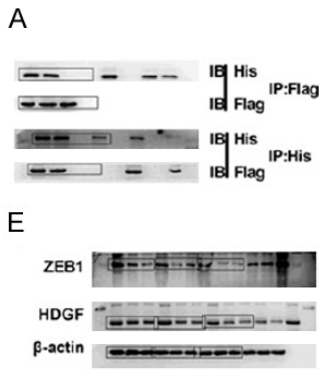
**Supplementary Figure 2.** HDGF mediates ZEB1 to promote EC migration and invasion by modulating  $\beta$ -catenin-induced EMT signal. A. Scratch migration assay indicated that suppression of HDGF in ZEB1-overexpressed EC cells reversed the ability of migration. Scale bar: 200  $\mu$ m. B and C. Cell migration and invasion were assessed via transwell and boyden assays. Scale bar: 25  $\mu$ m. Data were shown as the mean  $\pm$  SD, \* $P$  < 0.05; \*\* $P$  < 0.01; \*\*\* $P$  < 0.001.

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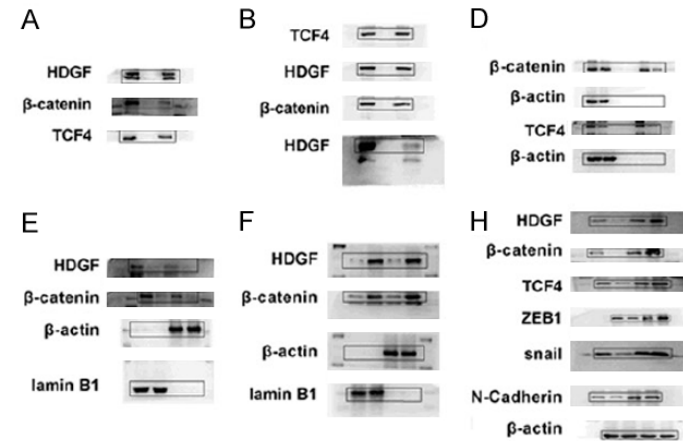
**Figure 2**



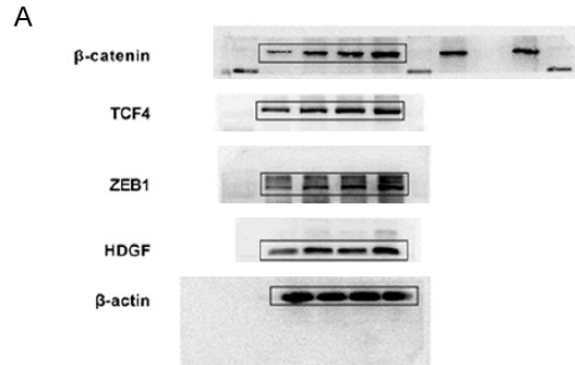
**Figure 3**



**Figure 5**



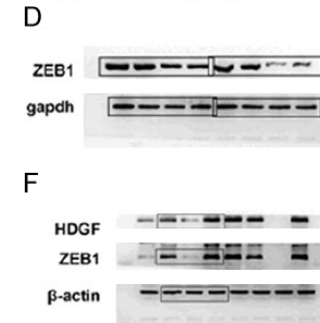
**Figure 6**



**Figure 7**



**Supplementary figure 1**



Supplementary Figure 3. The uncropped gels/blots.