

## Supplementary materials

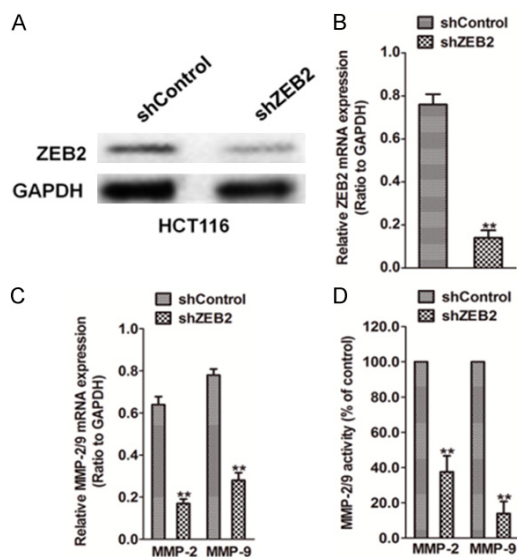
### MMP-2/9 activity assay

The activity of MMP-2/9 was determined by QuickZyme Mouse MMP-2/9 activity assay (QuickZyme BioSciences, Netherlands) according to the manufacturer's instructions. Briefly, after transfection for 24 h, HCT116 cells were washed with fresh medium and replaced with serum-free medium. After additional 24 h, the medium was collected and centrifuged at 10000 g for 10 min. Respective supernatant was added to the 96-well strip coated with MMP-2/9 antibody and incubated at 4°C overnight. After washing with wash buffer for 4 times, 50 µl assay buffer was added into the well, followed by adding 50 µl detection reagent. After incubation at 37°C for 1 h, OD405 was measured with Tecan (Männedorf, Switzerland).

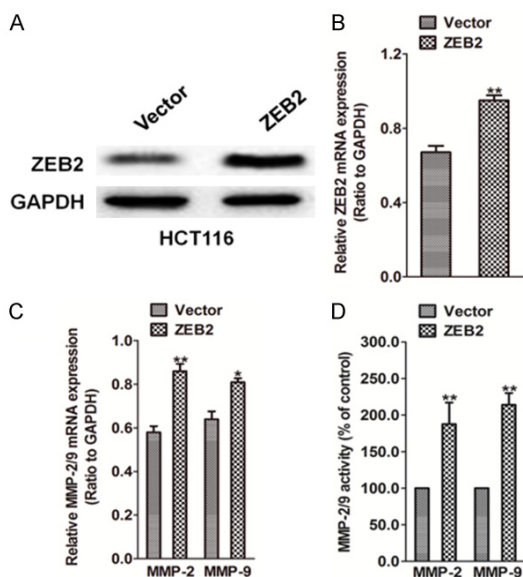
**Supplementary Table 1.** Association of ZEB2 expression with clinic-pathological factors from colon cancer patients

Clinicopathological variables	N	ZEB2 expression score (mean ± SD)	P-value
Age (years)			
< 60	57	5.63 ± 3.53	0.512
≥ 60	69	4.12 ± 3.15	
Tumor differentiation			
Well	52	4.19 ± 2.86	0.304
Moderate	49	4.78 ± 1.95	
Poor	25	5.02 ± 2.03	
Infiltration depth			
T <sub>1</sub> + T <sub>2</sub>	54	4.89 ± 1.14	0.003
T <sub>3</sub> + T <sub>4</sub>	72	4.92 ± 2.65	
Lymph node metastasis			
N <sub>0</sub>	66	3.96 ± 2.47	< 0.001
N <sub>1-3</sub>	60	5.47 ± 2.12	
Distant metastasis			
M <sub>0</sub>	84	4.69 ± 2.18	0.002
M <sub>1</sub>	42	5.54 ± 2.4	
TNM stage			
I	11	2.23 ± 2.52	< 0.001
II	37	6.01 ± 3.33	
III	55	5.18 ± 2.96	
IV	23	6.47 ± 3.40	

## ZEB2 promotes colorectal cancer metastasis



**Supplementary Figure 1.** A. RT-PCR analysis of the ZEB2 RNA level in HCT116 cells that were stably transfected with ZEB2 shRNA or the vector control. B. HCT116 cells were stably transfected with ZEB2 shRNA or the vector control. Western blot analysis of ZEB2 expression in the indicated cells was performed and  $\alpha$ -Tubulin was used as an internal control. C. Real-time PCR was carried out to evaluate the mRNA expression level of MMP-2/9 in ZEB2 down-expression and control cells. D. Activity of MMP-2/9 in control and ZEB2 knock-down cells was determined by MMPs activity assay.



**Supplementary Figure 2.** A. qRT-PCR analysis of HOXD9 levels in the established cell lines. B. Western blot analysis of HOXD9 levels in the established cell lines. C. Real-time PCR was carried out to evaluate the mRNA expression level of MMP-2/9 in ZEB2 over-expression and control cells. D. Activity of MMP-2/9 in control and ZEB2 knock-in cells was determined by MMPs activity assay.