**The DNA endonuclease Mus81 regulates ZEB1 expression and serves as a target of BET4 inhibitors in gastric cancer**

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Supplementary Table S1. The primers for qRT-PCR

|  |  |  |
| --- | --- | --- |
| Gene | Forward primers | Reverse primers |
| GAPDH | AAGAAGGTGGTGAAGCAGG | GTCAAAGGTGGAGGAGTGG |
| Mus81 | AGCCCGAGTGATACTGCTG | TCCTCCTTGGTTAAGAAGTGGT |
| ZEB1 | TTACACCTTTGCATACAGAACCC | TTTACGATTACACCCAGACTGC |
| ZEB2 | GGAGACGAGTCCAGCTAGTGT | CCACTCCACCCTCCCTTATTTC |
| Twist1 | GTCCGCAGTCTTACGAGGAG | GCTTGAGGGTCTGAATCTTGCT |
| Snail1 | ACTGCAACAAGGAATACCTCAG | GCACTGGTACTTCTTGACATCTG |

Supplementary Table S2. The primers for CHIP

|  |  |  |
| --- | --- | --- |
|  | Forward primer | Reverse primers |
| ZEB1-1 | TATTCGAAGGAGGTGGGAAG | AGAAGCATCGGCTGACAGAT |
| ZEB1-2 | TAGGATCCCACGGTTCTACG | CACACGGTGCTTGTCTCACT |
| ZEB1-3 | TCCCCTCATCAAGGGAACTC | TGGCTGATTCTCCCTGTACC |
| Mus81-1 | TCCCCTCTACCTGTGTCTGG | GAGGCCTAGAGGAAGGAGGA |
| Mus81-2 | GAGGATCACCATTTCCTCCA | GAGGCTGGAGATGGAGACTG |

**Supplementary Figures and Legends**



**Supplementary Fig. S1 Mus81 expression changes in different gastric cancer tissues.** Mus81 expression was detected by western blotting.

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**Supplementary Fig. S2 Cell migration correlates with Mus81 levels in gastric cancer cells.** Transwell assays were performed to determine the migration of GES-1, AGS, SGC7901, MGC803, BGC823, HGC27 and MKN45 cells. Data are reported as the mean ± SD of three independent experiments.



**Supplementary Fig. S3 Efficiency of *Mus81* silencing and overexpression.** (A) *Mus81* expression was detected by qPCR in SGC7901 and BGC823 cells stably silenced for *Mus81* expression. (B) *Mus81* expression was detected by qPCR in GES-1 and MGC803 cells stably overexpressing *Mus81*.



**Supplementary Fig. S4 Mus81 had limited effect on cell proliferation of gastric cancer *in vitro* and *in vivo*.** (A) MTT assays to assess the role of Mus81 on the growth in SGC7901 and BGC823 cells. (B) Colony formation assays to determine the effect of Mus81 on the proliferation in SGC7901 and BGC823 cells. Values indicate the mean ± SD of three independent experiments (*t* test). (C) Xenograft studies to detect tumor growth after *Mus81* knockdown *in vivo* (n = 5, *t* test). *ns.* not significant.



**Supplementary Fig. S5 Mus81 has no effect on cell cycle in gastric cancer cells.** Cell cycle distribution was determined by flow cytometry analysis. Values indicate the mean ± SD of three independent experiments (*t* test)**.**

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**Supplementary Fig. S6 Knockdown *Mus81* has no effect on the expression of ZEB2, Twist1 and Snail.** (A, B and C) qPCR assays to detect the expression of ZEB2, Twist1 and Snail1 in *Mus81*- depleted gastric cancer cells. Values indicate the mean ± SD of three independent experiments (*t* test). (D) Western blotting to detect the expression of ZEB2 and Snail1 in *Mus81*-depleted gastric cancer cells. \* *P* < 0.05, *ns.* not significant.



**Supplementary Fig. S7** The chemical structures of small molecular inhibitors chemical structures.



**Supplementary Fig. S8 BRD4 does not directly regulate the expression of Mus81.** (A) Immunoprecipitation to investigate the potential interaction between BRD4 and Mus81. (B) ChIP assays to investigate the potential binding of BRD4 to *Mus81* promoter.



**Supplementary Fig. S9 Heatmap of the genes co-expressed with *Mus81* in 517 gastric cancer tissues and 36 gastric cancer cell lines.**



**Supplementary Fig. S10 AZD5153 decreases gastric cancer cells metastasis via Mus81 *in vivo*.** Mouse liver metastasis nodules in the control, *Mus81*-silenced, AZD5153 and *Mus81*-silenced plus AZD5153 groups (n = 5).



**Supplementary Fig. S11 Schematic model of BRD4-Sirt5-Mus81-ZEB1 axis was shown.** Mus81 promotes gastric cancer metastasis via regulating the transcription of ZEB1, BRD4 regulates Mus81expression through Sirt5, thereby AZD5153 inhibits gastric cancer metastasis by suppressing Sirt5/Mus81/ZEB1 axis.