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Supplemental Information

A Novel Mechanism of Doxorubicin Resistance and Tumorigenesis Mediated by MicroRNA-501-5p-Suppressed BLID

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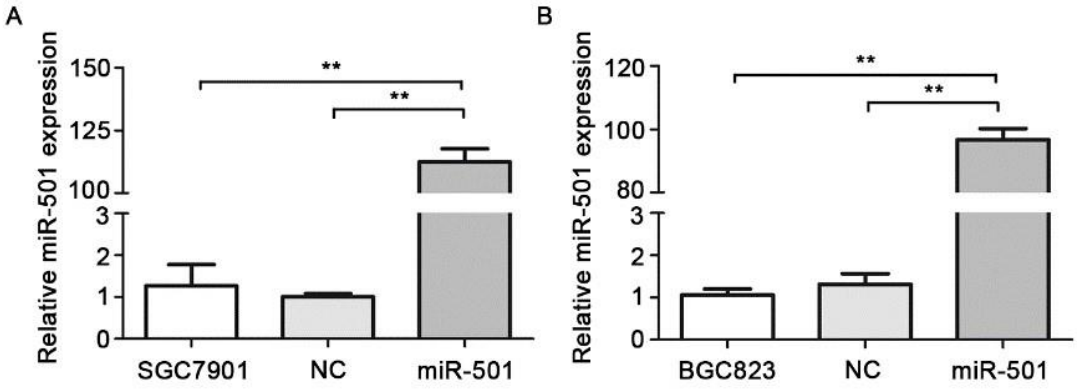

Figure S1 miR-501 is overexpressed in SGC7901 and BGC823 cells after transfected with miR-501 mimic. The level of miR-501 was detected by real-time qRT-PCR analysis. The negative miRNA (NC) and SGC7901 and BGC823 cells were the negative controls, respectively. The experiments were repeated for three times and the numbers are indicated as mean ± SD. $^{**}P < 0.01$.

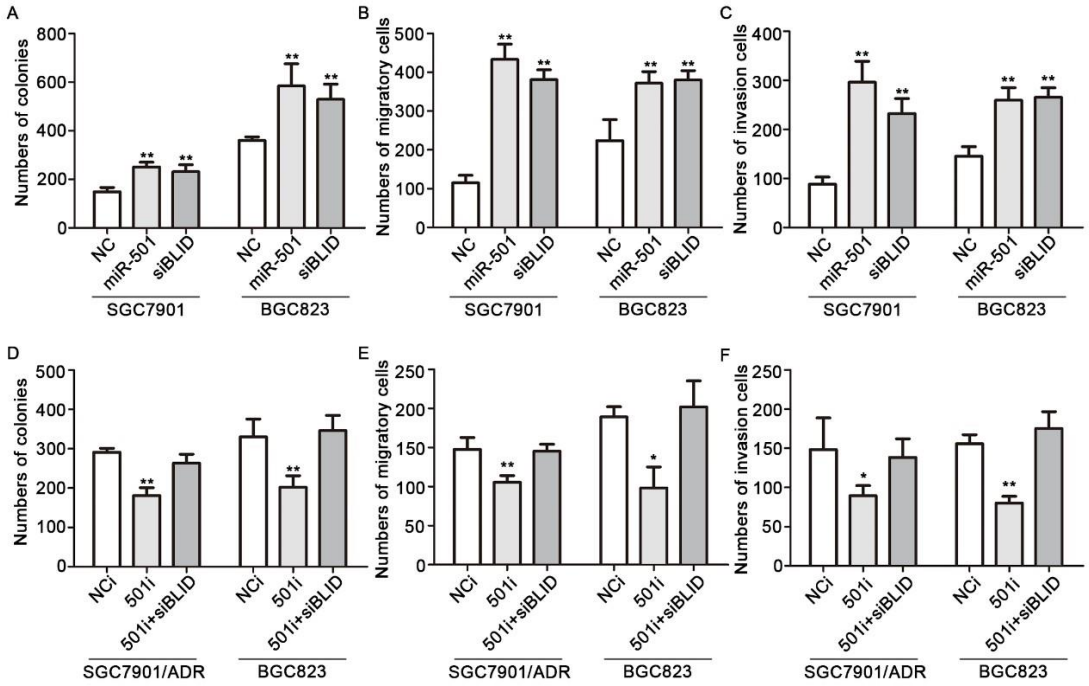

Figure S2 Graph data and statistical analysis of cell proliferation, migration and invasion. (A) SGC7901 and BGC823 cells were transfected with the miRNA mimics or siRNA described as above. The colony formation assay was performed to show the impact of miR-501 on cell growth. **(B)** Cell migratory potential was determined by the transwell migration assay without matrigel. **(C)** Cell invasive potential was determined by the transwell invasion assay with matrigel. **(D)** The endogenous miR-501 was knocked down by the inhibitor in SGC7901/ADR and BGC823 cells. The cell proliferative potential was detected by colony formation assay. Transwell assays with or without matrigel were performed to determine the potentials of migration **(E)** and invasion **(F)** in the miR-501 knockdown SGC7901 cells and BGC823 cells, respectively. All the experiments were repeated for three times and the numbers are indicated as mean ± SD. **P* < 0.05, ***P* < 0.01.

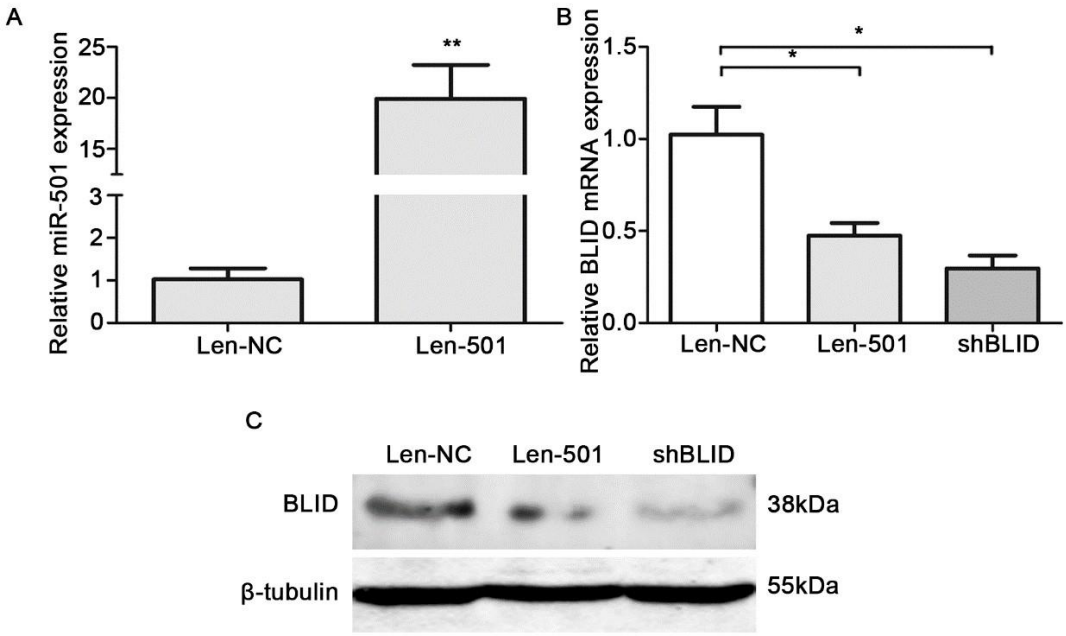

Figure S3 miR-501 is upregulated and BLID is downregulated in the miR-501 lentivirus infected SGC7901 cells. (A) SGC7901 cells were infected with lentiviral vectors of miR-501 (Len-501), negative control (Len-NC), or shRNA against BLID (shBLID). The expression of miR-501 was quantified by real-time qRT-PCR. **(B)** The expression of BLID mRNA was detected by real-time qRT-PCR. (C) The expression of BLID protein was analyzed by Western blot. The experiments were repeated for three times and the numbers are indicated as mean ± SD. *$P < 0.05$, **$P < 0.01$.

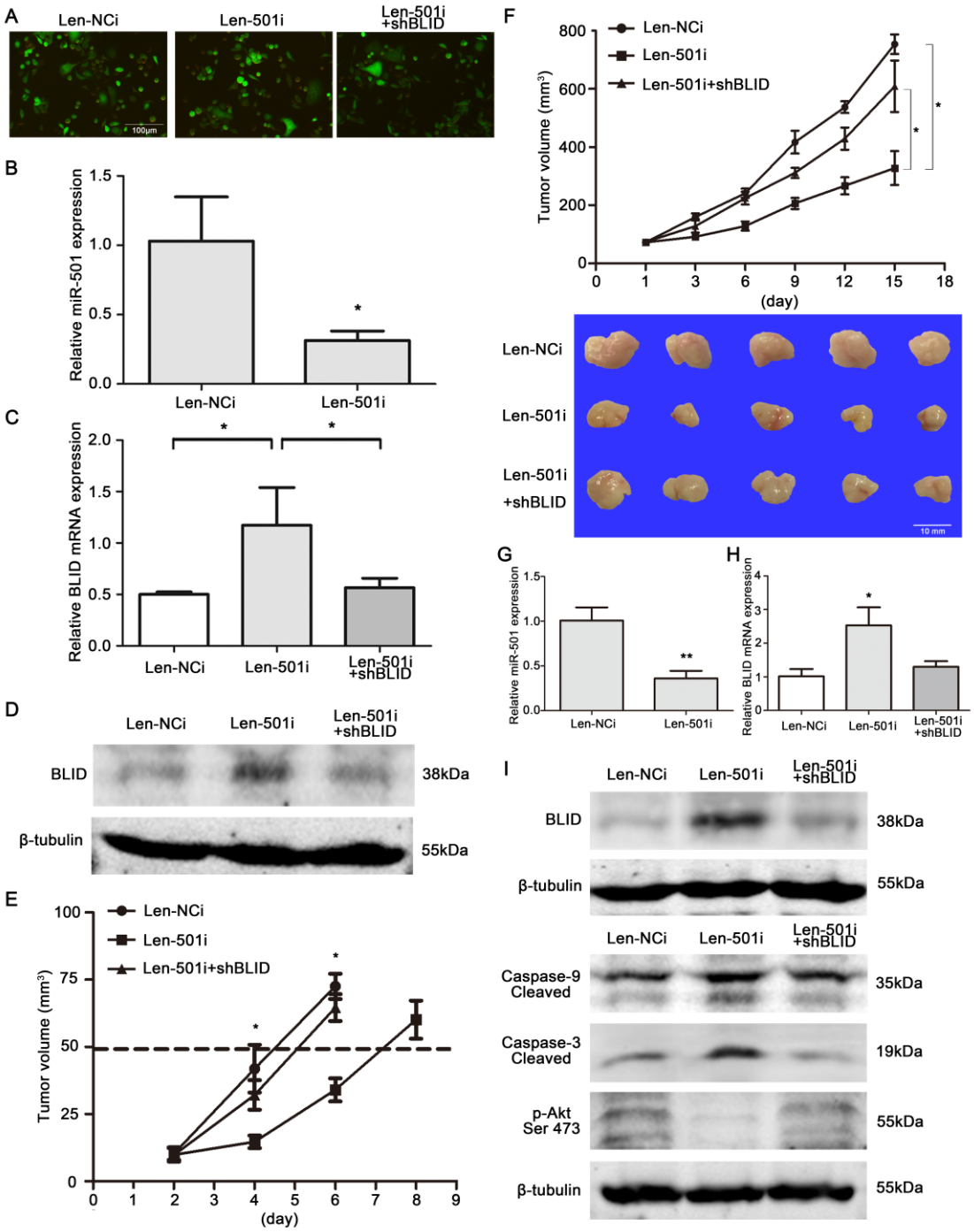

Figure S4 miR-501 knock-down reverses doxorubicin resistance and inhibits the growth of gastric cancer cell *in vivo*. (A) SGC7901 cells were infected with lentiviral vectors of miR-501 inhibitor (Len-501i), scrambled miRNA inhibitor (Len-NCi) or were co-infected with lentiviral vectors of miR-501 inhibitor and shRNA against BLID (Len-501i + shBLID). The cells with green fluorescence were observed under the fluorescence microscope and the representative pictures were shown. **(B)**

miR-501 expression was measured by real-time qRT-PCR in Len-NCi and Len-501i groups of cells. **(C)** BLID mRNA expression was detected by real-time qRT-PCR. **(D)** BLID protein expression was analyzed by Western blot. **(E)** The growing rate curve of the subcutaneous tumors in nude mice was shown. **(F)** The tumor volumes were measured and recorded at all the time points during ADR treatment. **(G)** The expression of miR-501 in tumor xenografts was quantified by real-time qRT-PCR. **(H)** The expression of BLID mRNA in the mice xenograft tumor tissues was detected using real-time qRT-PCR. **(I)** The protein expression of BLID, cleaved caspase-9/3 and p-Akt Ser 473 in the xenograft tumor tissues were analyzed by Western blot. Numbers are indicated as mean ± SD. *$P < 0.05$, **$P < 0.01$.

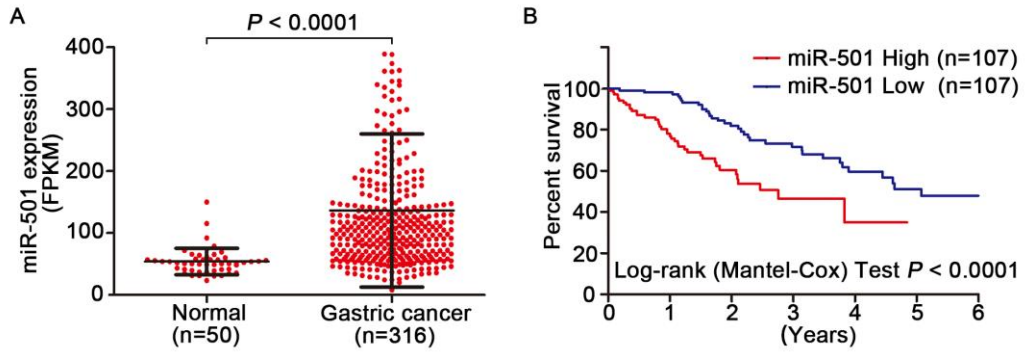

Figure S5 miR-501 is upregulated in the gastric cancer tissues and predicts the poor prognosis based on The Cancer Genome Atlas (TCGA) dataset analysis. (A) Comparison of miR-501 level in normal gastric tissues (n = 50) with gastric cancer tissues (n = 316). **(B)** Kaplan-Meier plots were used to estimate overall survival rate. Log rank test was performed to assess the differences in survival between the high (n = 107) and low of miR-501 expression group (n = 107). The experiments were repeated for three times and the numbers are indicated as mean ± SD.

Table S1 Primer sequences or lot numbers of real-time qRT-PCR

Name	Primer sequences or Lot numbers
BLID	Forward 5’ - CCTCTGCTGAGGCCCTGTAA - 3’
	Reverse 5’ - GCCTGAATAATGGGCGAAATC - 3’
GAPDH	Forward 5’ - CCACTCCTCCACCTTTGAC - 3’
	Reverse 5’ - ACCCTGTTGCTGTAGCCA - 3’
miR-501	Applied Biosystems Lot Number: P161011-002 H09 (Thermo Fisher Scientific, Waltham, MA)
RNU6B	Applied Biosystems Lot Number: P160808-004 E06 (Thermo Fisher Scientific, Waltham, MA)

Table S2 Sequences of synthesized oligonucleotides for miR-501 binding site(s) of BLID

3'UTR	Location	Sequences of oligonucleotides
BLID mRNA	87-242bp	Forward - wild type: 5'TCTAGATTCAATAAGACCCAATTCTTAACAGTCTT TTCTACCCACTTTTACCCATAACTTTTCCAAATTTG GTTCAAATTGTGCAGAGAAACAATAAAATTTTTAA AAAGGATAAACTGGCTAGTTAAAAGTAAATGGCAT TTAATTAAAACAAATCTTGCATCTAGA 3' Reverse - wild type: 5'TCTAGATGCAAGATTTGTTTTAATTAAATGCCATT TACTTTTAACTAGCCAGTTTATCCTTTTTAAAAATTT TATTGTTTCTCTGCATAATTTGAACCAAATTTGGAA AAGTTATGGGTAAAAGTGGGTAGAAAAGACTGTTA AGAATTGGTCTTATTGAATCTAGA 3' Forward - mutant: 5'TCTAGATTCAATAAGACCCAATTCTTAACAGTCTT TTCTACCCACTTTTACCCATAACTTTTCCAAATTTG GTTCAAATTGTGCAGAGAAACAATAAAATTTTTAA *CCCTTCG*AAACTGGCTAGTTAAAAGTAAATGGCAT TTAATTAAAACAAATCTTGCATCTAGA3' Reverse - mutant: 5'TCTAGATGCAAGATTTGTTTTAATTAAATGCCATT TACTTTTAACTAGCCAGTTTCGAAGGGTTAAAAAT TTTATTGTTTCTCTGCATAATTTGAACCAAATTTGG AAAAGTTATGGGTAAAAGTGGGTAGAAAAGACTG TTAAGAATTGGTCTTATTGAATCTAGA3'

Underlined bases are the predicted binding sites; italic base pairs are mutant.