# Supplementary Materials

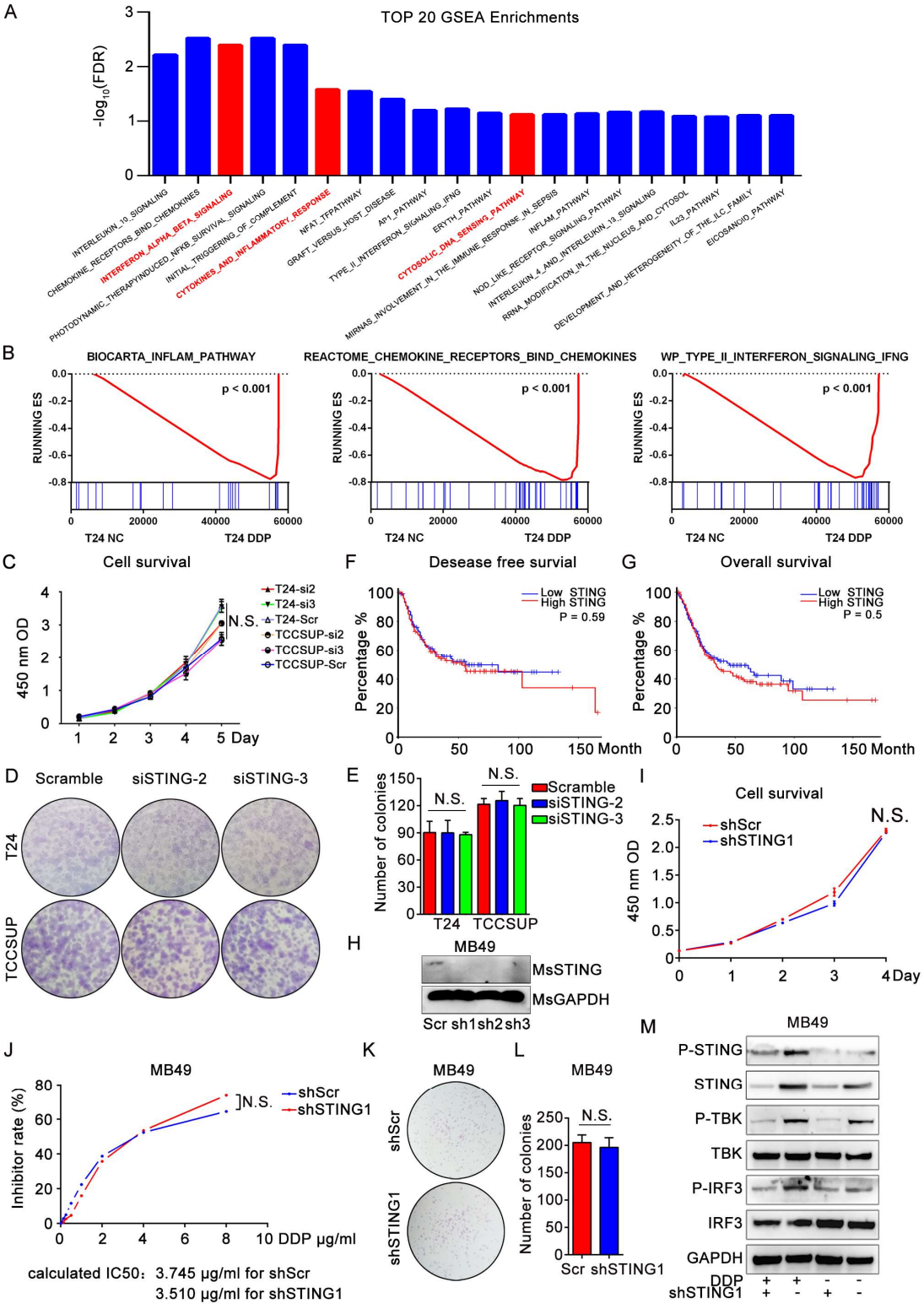


**Figure S1. Expression of STING did not affect proliferation of T24, TCCSUP and MB49 cell lines in vitro.** (A) Top 20 GSEA enrichment pathways in T24 cell lines with cisplatin treatment, 2 μg/ml for 24 h. (B) Inflam- and immune- relative enrichment pathway in T24 cell lines with cisplatin treatment, 2 μg/ml for 24 h. (C) T24 and TCCSUP cell lines were treated with non-targeting (Scramble, Scr) or two STING-targeting siRNA (si2 and si3) for 48 h before adding 1640 medium containing

10% CCK-8 regent. The 450 mm OD of each groups were measure at day 1, 2, 3, 4, 5. (D, E) Clonal formation assay of T24 and TCCSUP cell lines treated with non-targeting (Scramble, Scr) or two STING-targeting siRNA (si2 and si3) for 48 h. (F, G) Kaplan-Meier analysis of (F) overall survival rates and (G) disease-free survival rates stratified by low STING expression (n = 201) and high STING expression (n = 201) from The Cancer Genome Atlas (TCGA) database. The median was used as the dividing line, and STING upregulation showed none significantly associated with overall and disease-free survival rates. DDP, cisplatin. (H) Knockdown efficiency validation of shSTING lentivirus in MB49 cell lines. GAPDH was used as control protein. (I) MB49 cell lines were treated with non-targeting (Scramble, Scr) lentivirus or STING-targeting lentivirus for 48 h before adding 1640 medium containing 10% CCK-8 regent. The 450 mm OD of each groups were measure at day 0, 1, 2, 3, 4. (J) Calculated cisplatin IC50 of MB49 cell lines treated with non-targeting (Scramble, Scr) lentivirus or STING-targeting lentivirus for 48 h. (K, L) Clonal formation assay of MB49 cell lines treated with non-targeting (Scramble, Scr) lentivirus or STING-targeting lentivirus for 48 h. (M) Western blotting results of key proteins involved in cGAS-STING pathway in MB49 cell lines treated with 2 μg/ml cisplatin or PBS for 24 h combined with or without STING-targeting treatment. GAPDH was used as the control protein. DDP, cisplatin. N.S., P > 0.05.

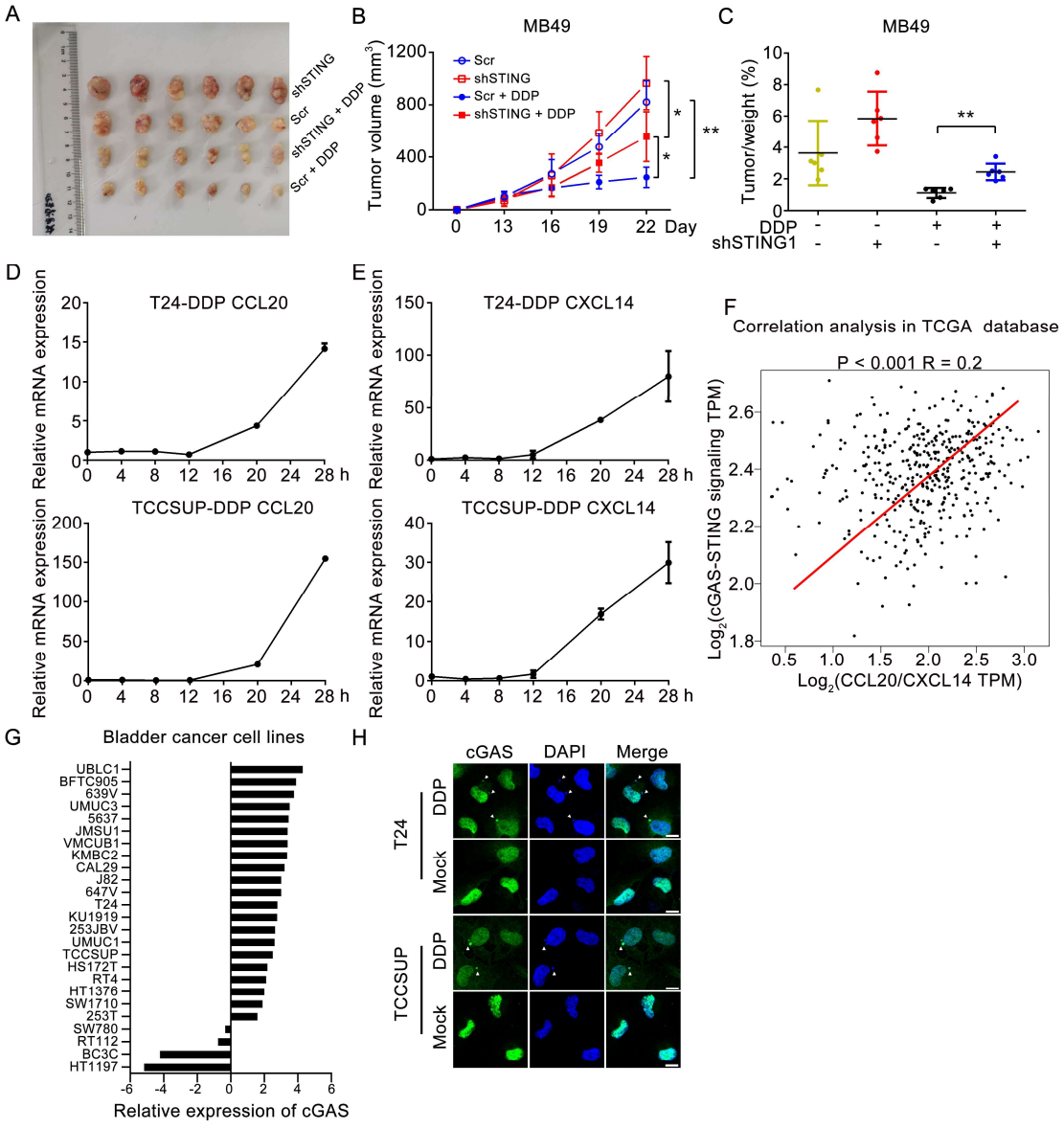


**Figure S2. Expression of STING did not affect proliferation and cisplatin sensitivity of MB49 cell lines.** (A) Different groups of tumor volumes in day 23. (B) Different groups of tumor proliferation curves. (C) Relative tumor weights of different groups. *P < 0.05, **P < 0.01. (D, E) T24 and TCCSUP cell lines were treated with 2 μg/ml cisplatin for 0 h, 4 h, 8 h, 12 h, 20 h, 28 h, respectively. CCL20

(D) and CXCL14 (E) mRNA were then analyzed by qRT-PCR. (F) Correlation analysis between cGAS-STING signaling and CCL20/CXCL14 expression in TCGA database. $P < 0.001$, $R = 0.2$. (G) Basic transcription levels of cGAS in bladder cancer cell line on CCLE database. (H) Cisplatin (2 μg/ml cisplatin for 24 h) induced micronuclei in T24 and TCCSUP cell lines. Scale bar represent 5 μm.

**Table S1.** The sequences of msSTING and siSTING and specific primers.

| shRNA | Sequences |
|---|---|
| MsSTING-1-Forward | CCGGAGAGGTCACCGCTCCAAATATCTCGAGATATTTGGAGCGGTGACCTCTTTTTTG |
| MsSTING-1-Reverse | AATTCAAAAAAGAGGTCACCGCTCCAAATATCTCGAGATATTTGGAGCGGTGACCTCT |
| MsSTING-2-Forward | CCGGATGATTCTACTATCGTCTTATCTCGAGATAAGACGATAGTAGAATCATTTTTTG |
| MsSTING-2-Reverse | AATTCAAAAAATGATTCTACTATCGTCTTATCTCGAGATAAGACGATAGTAGAATCAT |
| MsSTING-3-Forward | CCGGCAACATTCGATTCCGAGATATCTCGAGATATCTCGGAATCGAATGTTGTTTTTG |
| MsSTING-3-Reverse | AATTCAAAAACAACATTCGATTCCGAGATATCTCGAGATATCTCGGAATCGAATGTTG |
| siRNA | Sequences |
| siSTING-1 | CATGGTCATATTACATCGGAT |
| siSTING-2 | GCTGGCATGGTCATATTACAT |
| siSTING-3 | GTCCAGGACTTGACATCTTAA |
| PCR Primer | Sequences |
| CXCL1-Forward | ACAGTGTGTGGTCAACATTTCT |
| CXCL1-Reverse | AGCCCCTTTGTTCTAAGCCA |
| CXCL2-Forward | CAGTGTGTGGTCAACATTTCTCA |
| CXCL2-Reverse | TGCTCTAACACAGAGGGAAACA |
| CXCL3-Forward | CGCCCAAACCGAAGTCATAG |
| CXCL3-Reverse | TCTGGTAAGGGCAGGGACC |
| CXCL4-Forward | CCACACTTAACGGAGAGCCT |
| CXCL4-Reverse | AGGTGGTCTTCACACACAGG |
| CXCL5-Forward | TGCTGAGCTTTTTAGATGCCT |
| CXCL5-Reverse | AGACTATGAACCAATGAGACACA |
| CXCL6-Forward | ACGCTGAGAGTAAACCCCAA |
| CXCL6-Reverse | CCAGACAAACTTGCTTCCCG |
| CXCL7-Forward | GTGATCGGGAAAGGAACCCA |
| CXCL7-Reverse | AGCAGATTCATCACCTGCCA |
| CXCL8-Forward | TGGACCCCAAGGAAAACTGG |
| CXCL8-Reverse | AAGTTTCACTGGCATCTTCACTG |
| CXCL14-Forward | ATGAAGCCAAAGTACCCGCA |
| CXCL14-Reverse | CTTCTCGTTCCAGGCGTTGT |
| CXCL16-Forward | GGCACCTGACTCTAATACCTGA |
| CXCL16-Reverse | GCAGTGGCTGGTTAGTCCTAT |
| CCL5-Forward | CAGTCGTCCACAGGTCAAGG |
| CCL5-Reverse | TTCTCTGGGTTGGCACACAC |
| CCL19-Forward | CCATCCCTGGGTACATCGTG |
| CCL19-Reverse | GCAGTCTCTGGATGATGCGT |
| CCL20-Forward | AACCATGTGCTGTACCAAGAGT |
| CCL20-Reverse | AAGTTGCTTGCTTCTGATTCGC |
| CCL21-Forward | TGCAGCATCTGGACAAGACA |
| CCL21-Reverse | CCTTTAGGGGTCTGTGACCG |
| IL-6-Forward | CAGCCCTGAGAAAGGAGACAT |
| IL-6-Reverse | GGTTCAGGTTGTTTTCTGCCA |
| TNF-α-Forward | CCCGAGTGACAAGCCTGTAG |
| TNF-α-Reverse | TGAGGTACAGGCCCTCTGAT |
| INF-β-Forward | CAGCATCTGCTGGTTGAAGA |
| INF-β-Reverse | CATTACCTGAAGGCCAAGGA |

**Table S2. The sequencing coverage and quality statistics for each sample.**

| Sample ID | Total number of sequenced reads | Total number of uniquely mapped non-duplicate reads[a] | RNA integrity number (RIN) | Ratio of all reads aligned to rRNA regions to total uniquely mapped reads (rRNA rate) | Ratio of exon-mapped reads to total uniquely mapped reads (Expression Profile Efficiency) | Total number of detected transcripts with reads ≥ 1[b] |
|---|---|---|---|---|---|---|
| T24DDP2 | 43688566 | 41149832(94.19%) | >7 | unknown | 5765554923(91.2064%) | 24988 |
| T24DDP1 | 43426958 | 40877691(94.13%) | >7 | unknown | 5757569876(91.6351%) | 24731 |
| T24DDP3 | 41373478 | 38932654(94.1%) | >7 | unknown | 5384577085(90.0616%) | 25180 |
| T24NC2 | 46041354 | 43248261(93.93%) | >7 | unknown | 6152975627(92.4967%) | 23686 |
| T24NC3 | 43789786 | 41206904(94.1%) | >7 | unknown | 5889299096(92.9955%) | 23167 |
| T24NC1 | 46520052 | 43510283(93.53%) | >7 | unknown | 6229786100(93.1316%) | 23425 |