A small molecule compound targeting STAT3 DNA-binding domain inhibits cancer cell proliferation, migration, and invasion

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Supplemental Figures

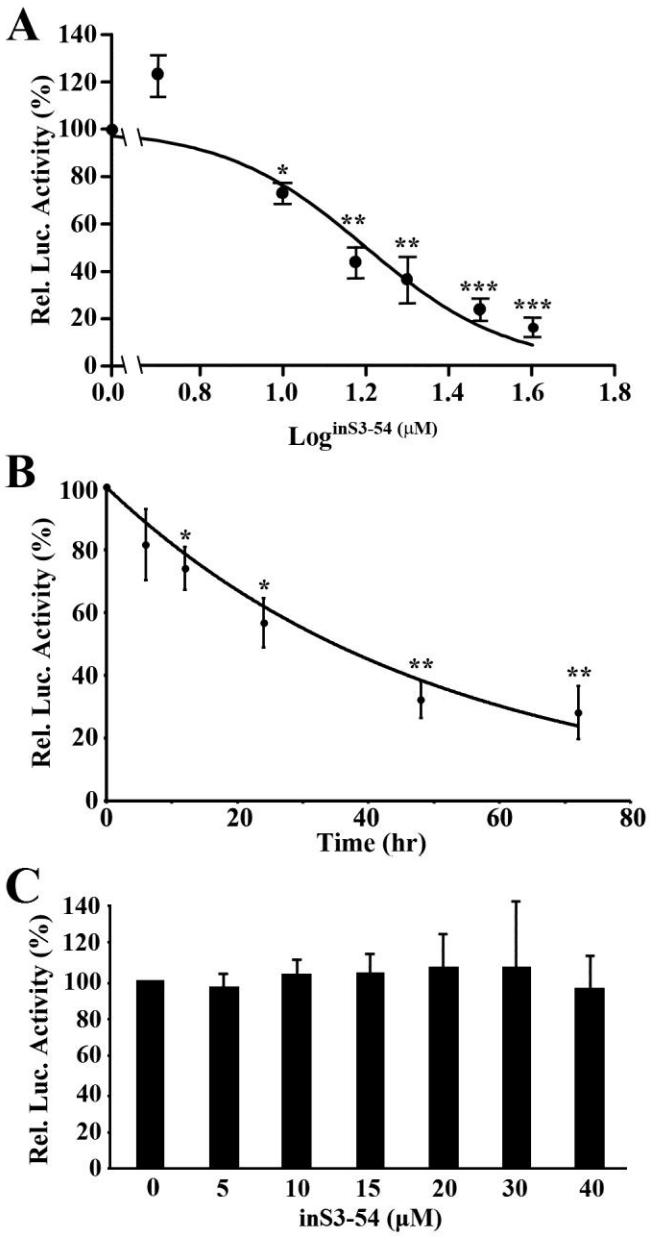

Figure S1. Effect of inS3-54 on STAT3-dependent and independent luciferase reporter expression. (A and B). Effect on STAT3-dependent luciferase reporter expression. MDA-MB-231 cells harboring a STAT3-dependent luciferase reporter construct were treated with increasing concentration of inS3-54 for 72 hrs (A) or with 20 μM inS3-54 for various times (B) followed by luciferase reporter assay. (C). Effect on STAT3-independent luciferase expression. H1299 cells were transiently transfected with a luciferase reporter construct driven by a p27 promoter lacking STAT3-binding sequence followed by treatment with different concentrations of inS3-54 for 48 hrs. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

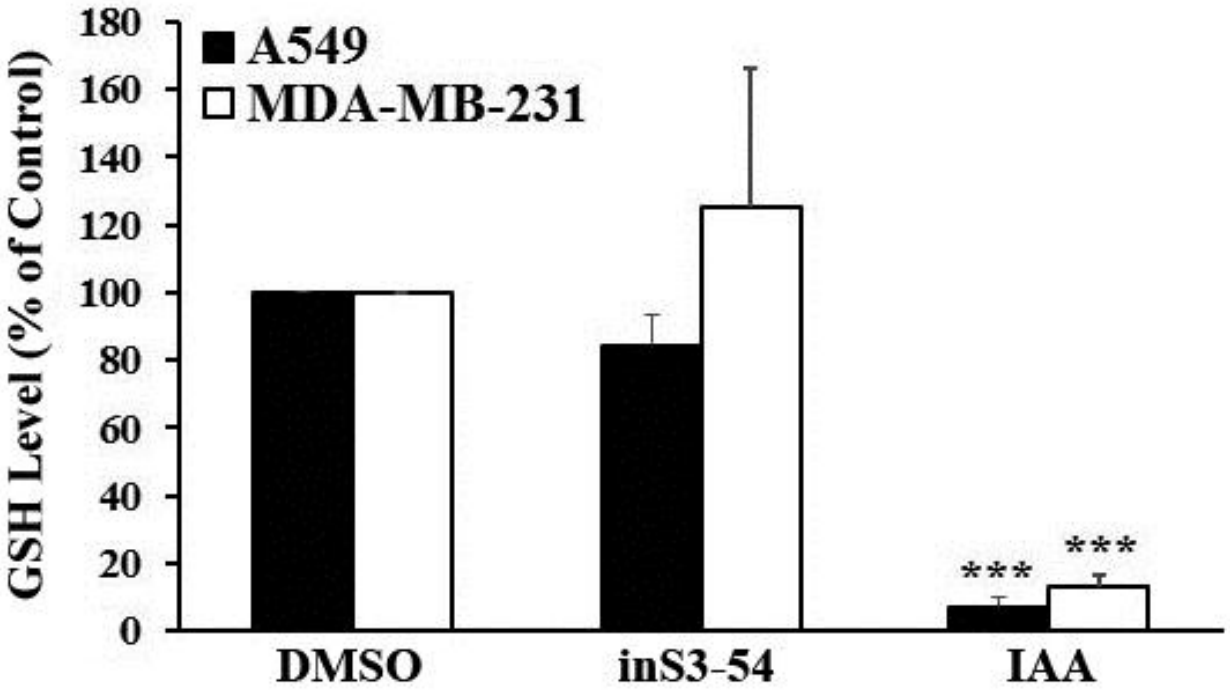

Figure S2. Effect of inS3-54 glutathione level. A549 and MDA-MB-231 cells were first treated with DMSO or 20 µM inS3-54 for 48 hours or with 15 mM iodoacetamide (IAA) for 30 minutes followed by determination of glutathione level using the GSH-Glo™ glutathione assay kit (Promega, Madison, WI, USA) per manufacturer's instructions. (*** $p<0.001$).

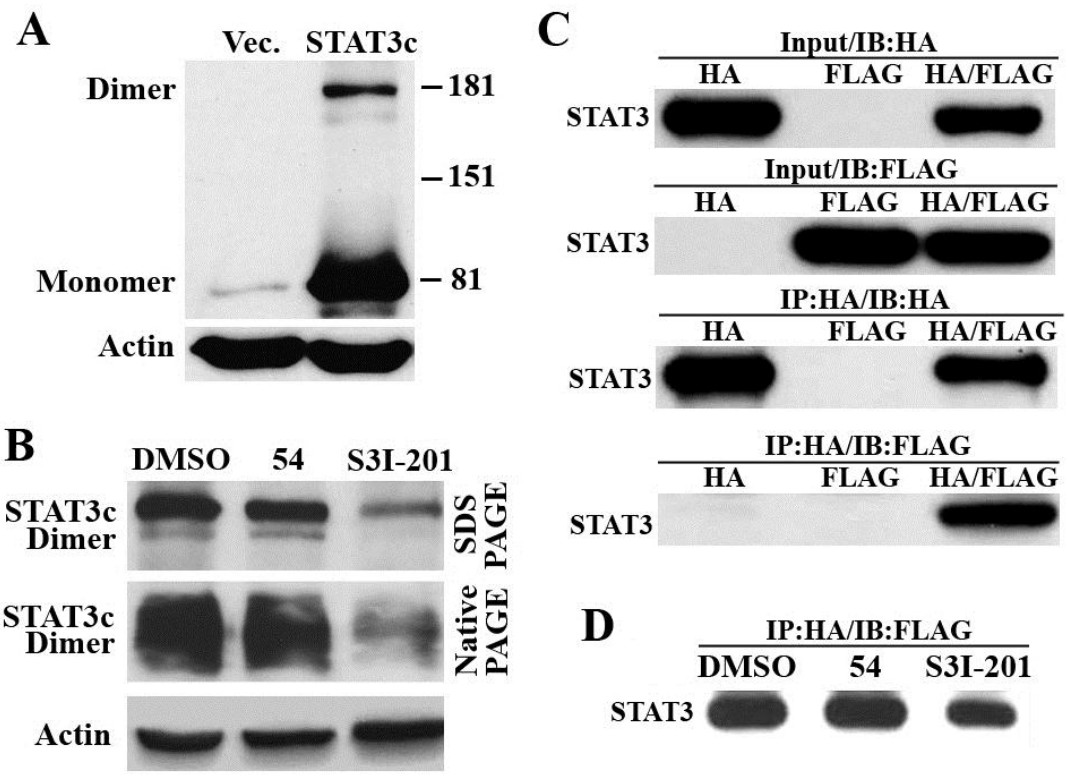

Figure S3. InS3-54 does not affect STAT3 dimerization. (A) Western blot analysis of STAT3c expression. H1299 cells were transiently transfected with vector control or STAT3c cDNA followed by lysate preparation, separation by non-reducing SDS-PAGE, and Western blot analysis using STAT3 antibody or actin antibody for a loading control. (B) Western blot analysis of STAT3 dimerization. H1299 cells-expressing STAT3c were treated with DMSO vehicle control, 20 μM inS3-54 or S3I-201 for 24 hrs followed by lysate preparation, separation by non-reducing SDS-PAGE or non-denaturing PAGE, and Western blot analysis of STAT3 dimerization status. Actin was used as a loading control. (C) Co-expression and immunoprecipitation of HA- and FLAG-tagged STAT3. Lysates from H1299 cells transfected with HA-tagged, FLAG-tagged STAT3, or both were subjected to co-immunoprecipitation using HA antibody and Western blot analyses with HA or FLAG antibodies as we previously described (*J Biol Chem 279*, 19781-19789). (D) Effect of inS3-54 on co-immunoprecipitation. H1299 cells co-transfected with HA- and FLAG-tagged STAT3 were treated with DMSO control, 20 μM inS3-54 or S3I-201 followed by co-immunoprecipitation with HA antibody and Western blot analysis with FLAG antibody.

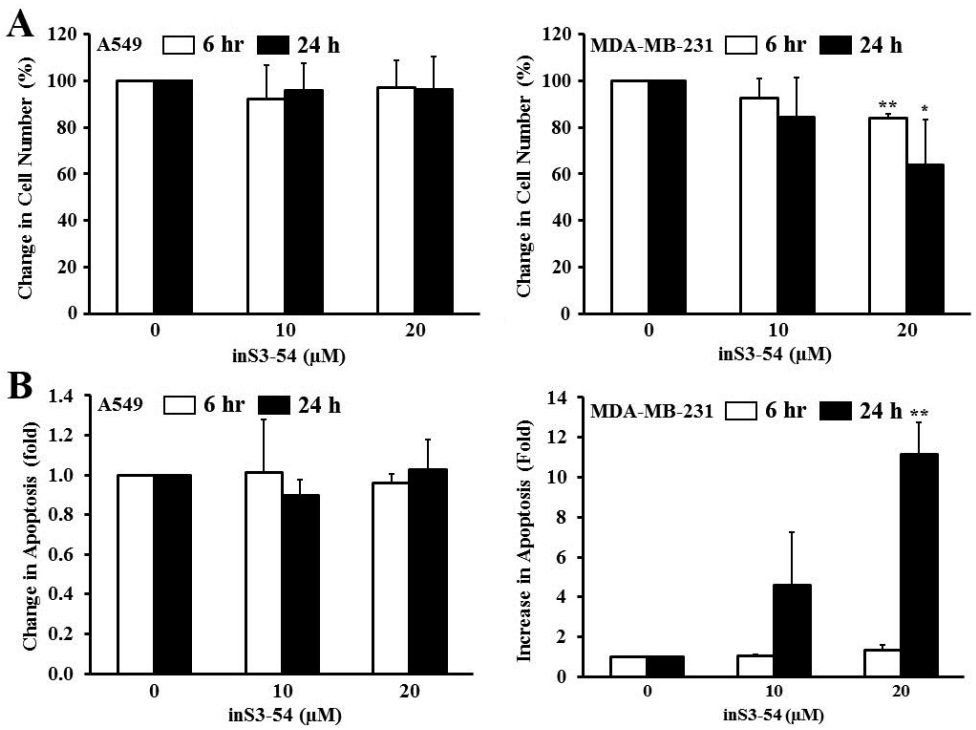

Figure S4. Effect of inS3-54 on cell growth and apoptosis of confluent cells. 100% confluent A549 and MDA-MB-231 cells were treated with 0 (DMSO vehicle control), 10 or 20 μM inS3-54 for 6 or 24 hrs followed by determination of change in cell number for proliferation (A) or ELISA for apoptosis (B). (** $p < 0.01$; *$p<0.05$).

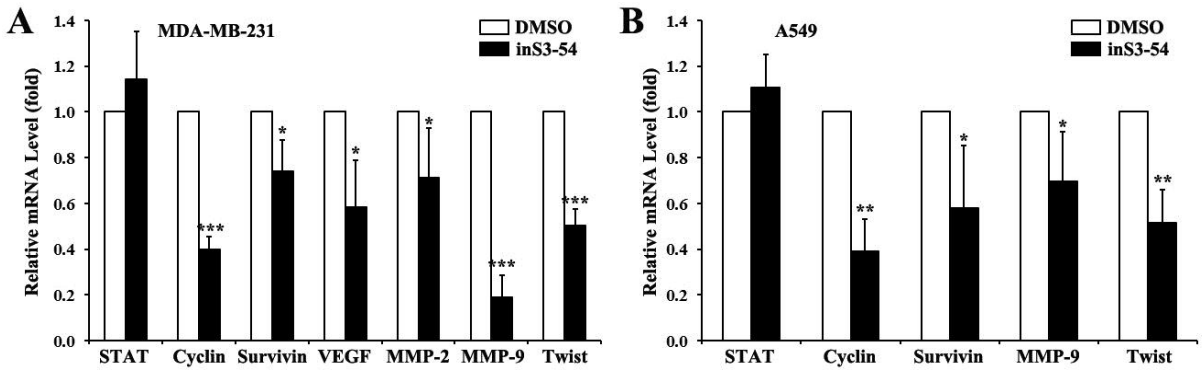

Figure S5. Effect of inS3-54 on mRNA level of STAT3 downstream target genes. MDA-MB-231 (A) and A549 (B) cells were treated with DMSO control or 20 μM inS3-54 followed by extracting total RNA and real-time RT-PCR analysis. InS3-54 inhibited mRNA level of STAT3 downstream targets in both cells lines. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Supplemental Table

Table S1. Primers for real-time PCR.

STAT3	F: GGCCCCTCGTCATCAAGA R: TTTGACCAGCAACCTGACTTTAGT
CyclinD1	F: CTTCCTCTCCAAAATGCCAG R: AGAGATGGAAGGGGGAAAGA
Survivin	F: TGCCTGGCAGCCCTTTC R: CCTCCAAGAAGGGCCAGTTC
VEGF	F: TACCTCCACCATGCCAAGTG R: GATGATTCTGCCCTCCTCCTT
MMP-1	F: AGCTAGCTCAGGATGACATTGATG R: GCCGATGGGCTGGACAG
MMP-2	F: TAGCATGTCCCTACCGAGTCT R: ATTGGATGGCAGTAGCTGC
MMP-9	F: TGACAGCGACAAGAAGTG R: CAGTGAAGCGGTACATAGG
MMP-10	F: ATCCAAGAGGCATCCATACC R: TCAACCTTAGGCTCAACTCC
Twist	F: CGGGAGTCCGCAGTCTTA R: TGAATCTTGCTCAGCTTGTC
GAPDH	F: AAGGACTCATGACCACAGTCCAT R: CCATCACGCCACAGTTTCC