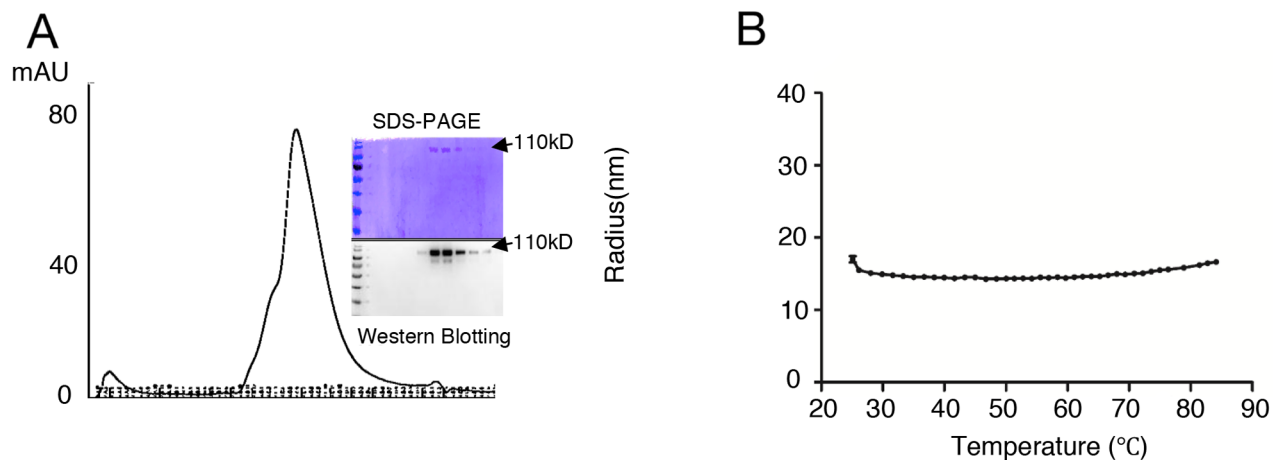
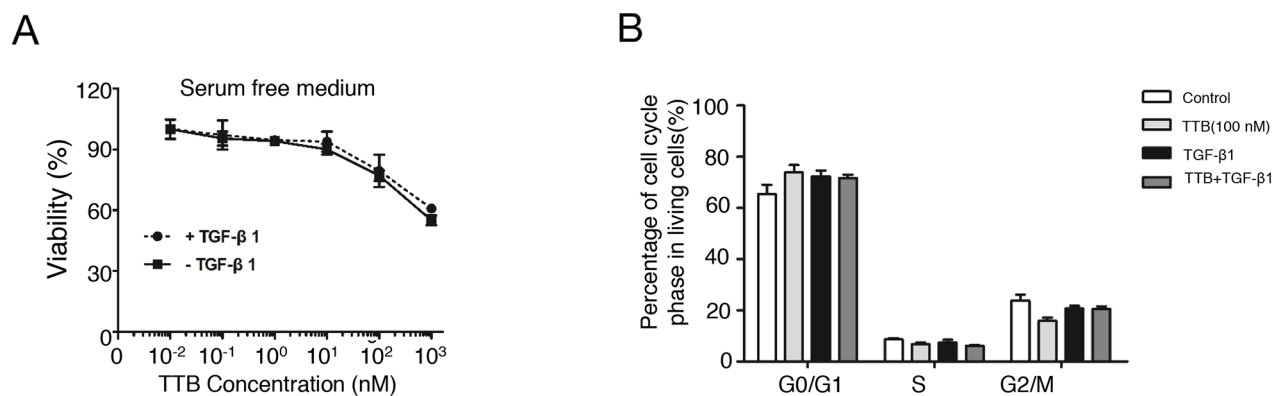


A targeted transforming growth factor-beta (TGF-β) blocker, TTB, inhibits tumor growth and metastasis

SUPPLEMENTARY MATERIALS

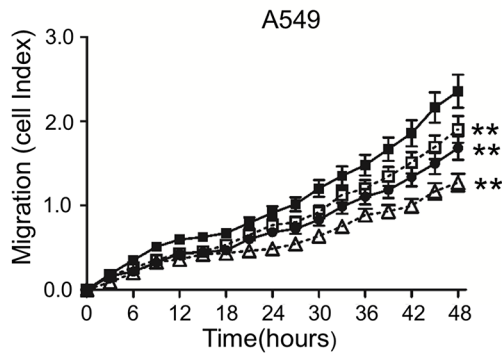


Supplementary Figure 1: Analysis of TTB protein. (A) Gel filtration analysis was performed to monitor the molecular weight. (B) Effects of the temperature on the protein particle sizes. Dynamic light scattering was performed to detect the thermo-stability.

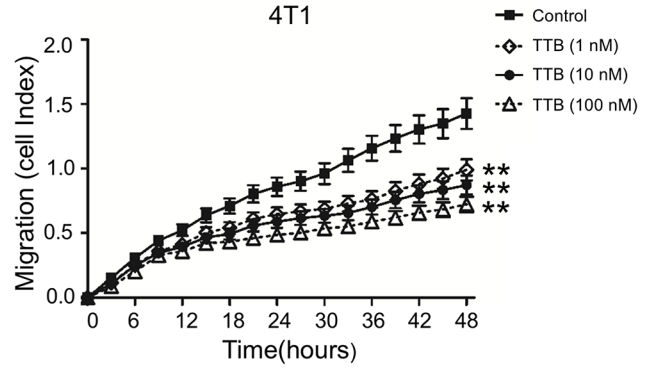


Supplementary Figure 2: (A) Cell viability using medium without serum, and **(B)** Cell cycle of A549 cultured in petri dish with indicated concentrations of TTB with or without TGF-β1 (20 pM). The bars represent the normalized values to control groups. Experiments have been repeated at least 3 times. The data are shown as the mean ± SEM.

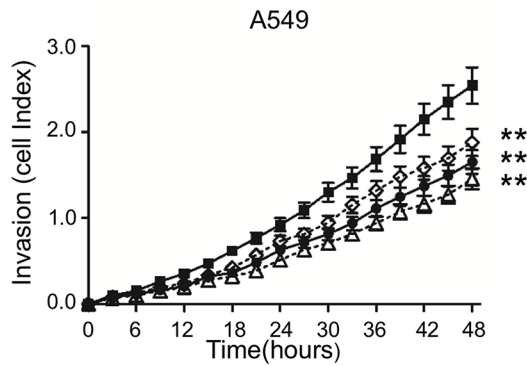
A



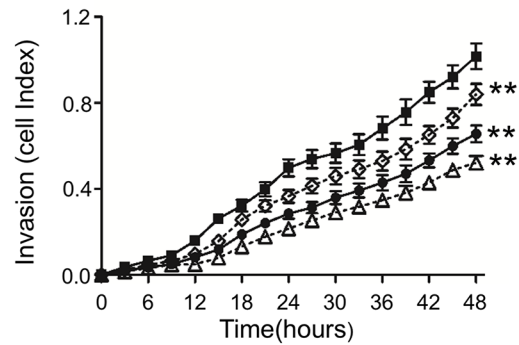
B



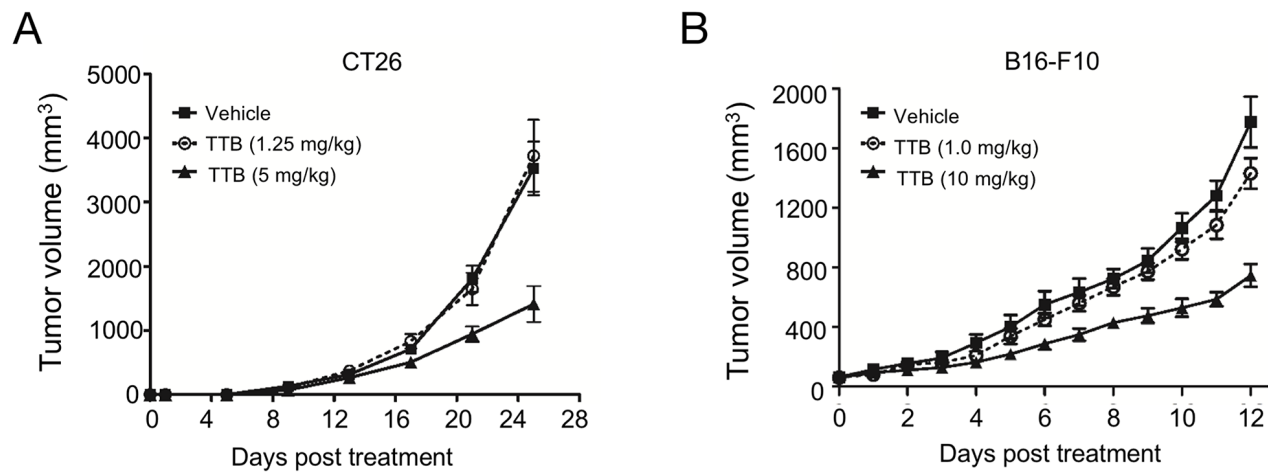
C



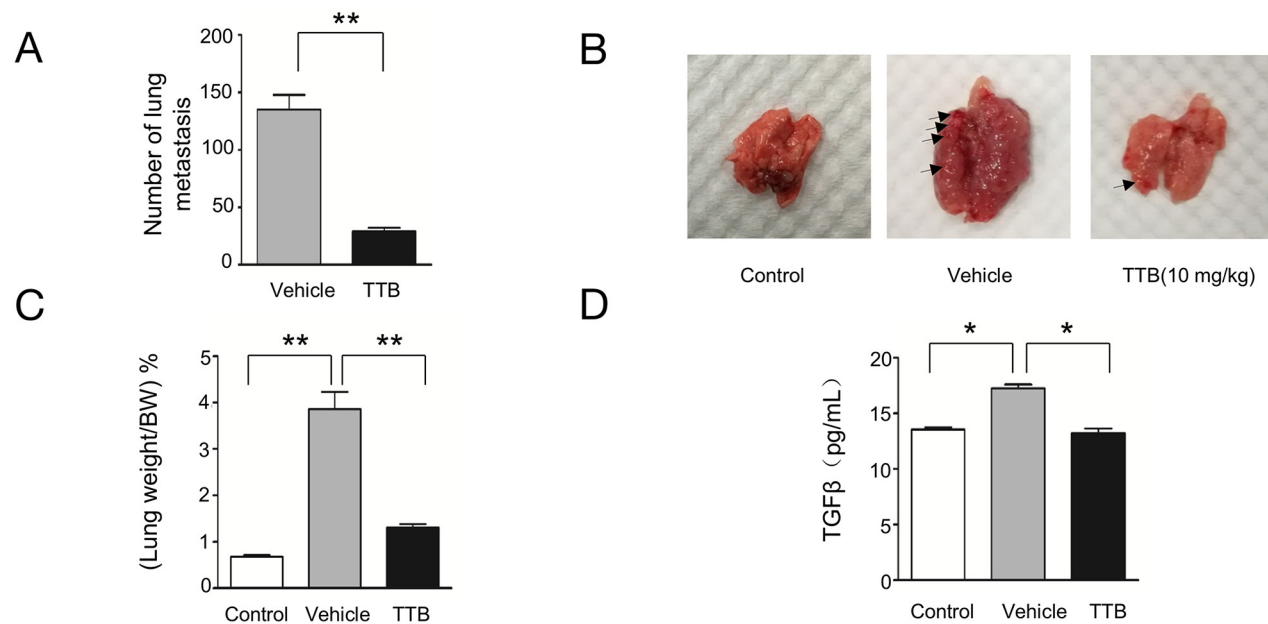
D



Supplementary Figure 3: Mobility of cancer cells detected using RTCA. A549 and 4T1 cells were seeded in E-plates and treated with TTB for 48 hours. Then cell index (CI) was measured every 30 min. (A) A549 and (B) 4T1 migration assays, (C) A549 and (D) 4T1 invasion assays were shown. (Control, solid line with square; TTB 1nM, dot line with diamond; TTB 10nM, solid line with filled circle; TTB 100nM, dot line with triangle, **P<0.01 vs. control). Experiments have been repeated at least 3 times. The data are shown as the mean \pm SEM.



Supplemental Figure 4: Tumor growth inhibition studies were performed as described in the Methods and Materials. (A) CT26 cells were s.c. injected into BALB/c mice (n = 8). Then, mice were intraperitoneally (i.p.) treated with vehicle or TTB every 3 days. The tumor volumes are shown as the mean \pm SEM (Vehicle, solid line with square; TTB 1.25mg/kg, dot line with circle; TTB 5mg/kg, solid line with filled circle). (B) B16-F10 cells were injected into C57BL/6 mice (n = 8). When tumor volume reached \sim 100 mm³, the mice were intraperitoneally (i.p.) treated with vehicle or TTB every 3 days. Growth of the tumors was determined. Experiments have been repeated at least 3 times. The data are shown as the mean \pm SEM.



Supplemental Figure 5: Tumor metastasis studies using 4T1 cells injected the left cardiac ventricle of female nude mice. The mice were treated with vehicle or TTB (10mg/kg) by i.p. injection every 3 days. On day 10, the mice were sacrificed. (A) The number of lung metastasis, (B) the representative lung images, (C) the percentage of lung weight/bodyweight, and (D) the serum levels of TGF- β , were determined accordingly. The data are shown as the mean \pm SEM (*P<0.05, **P<0.01).