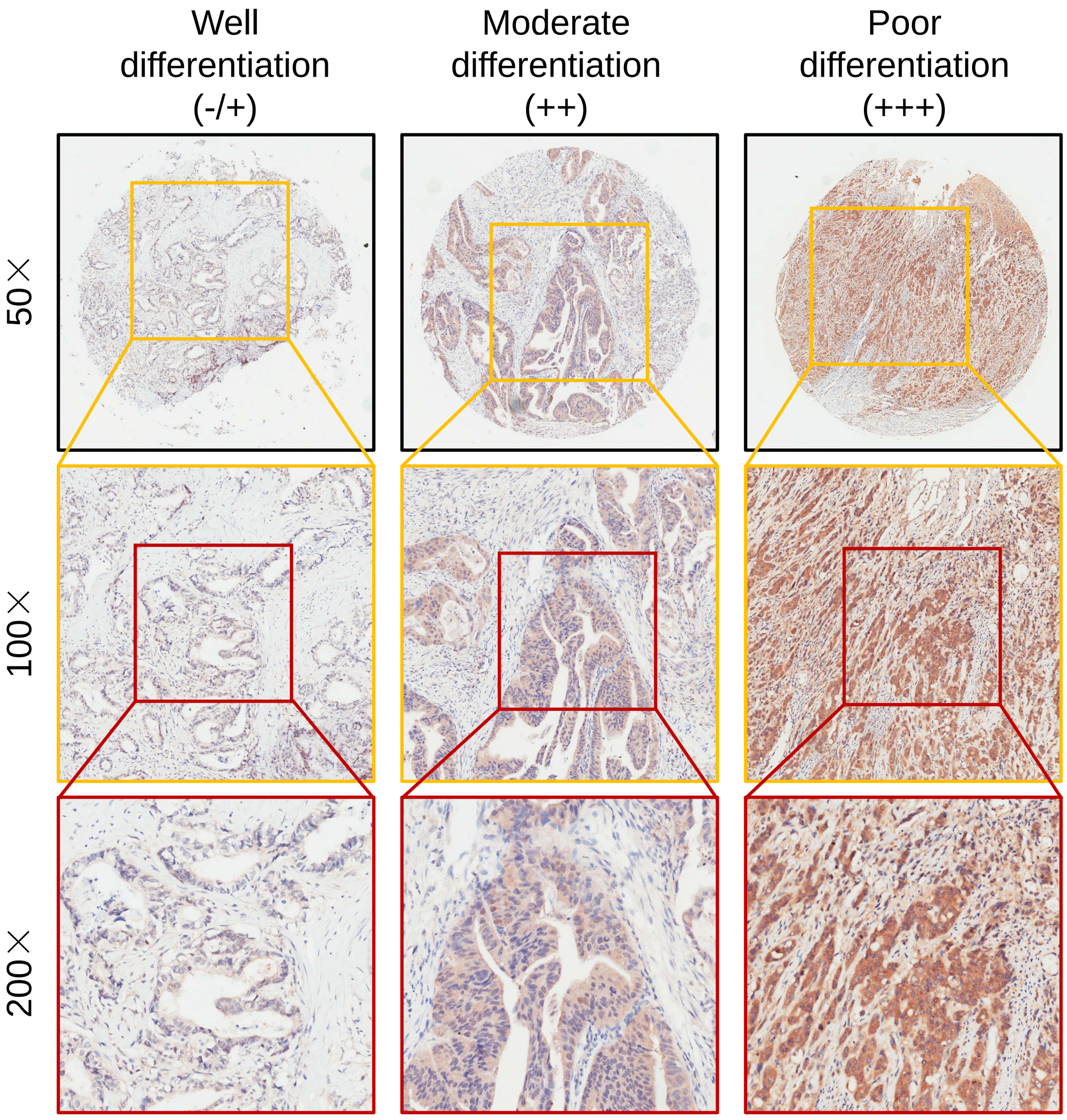
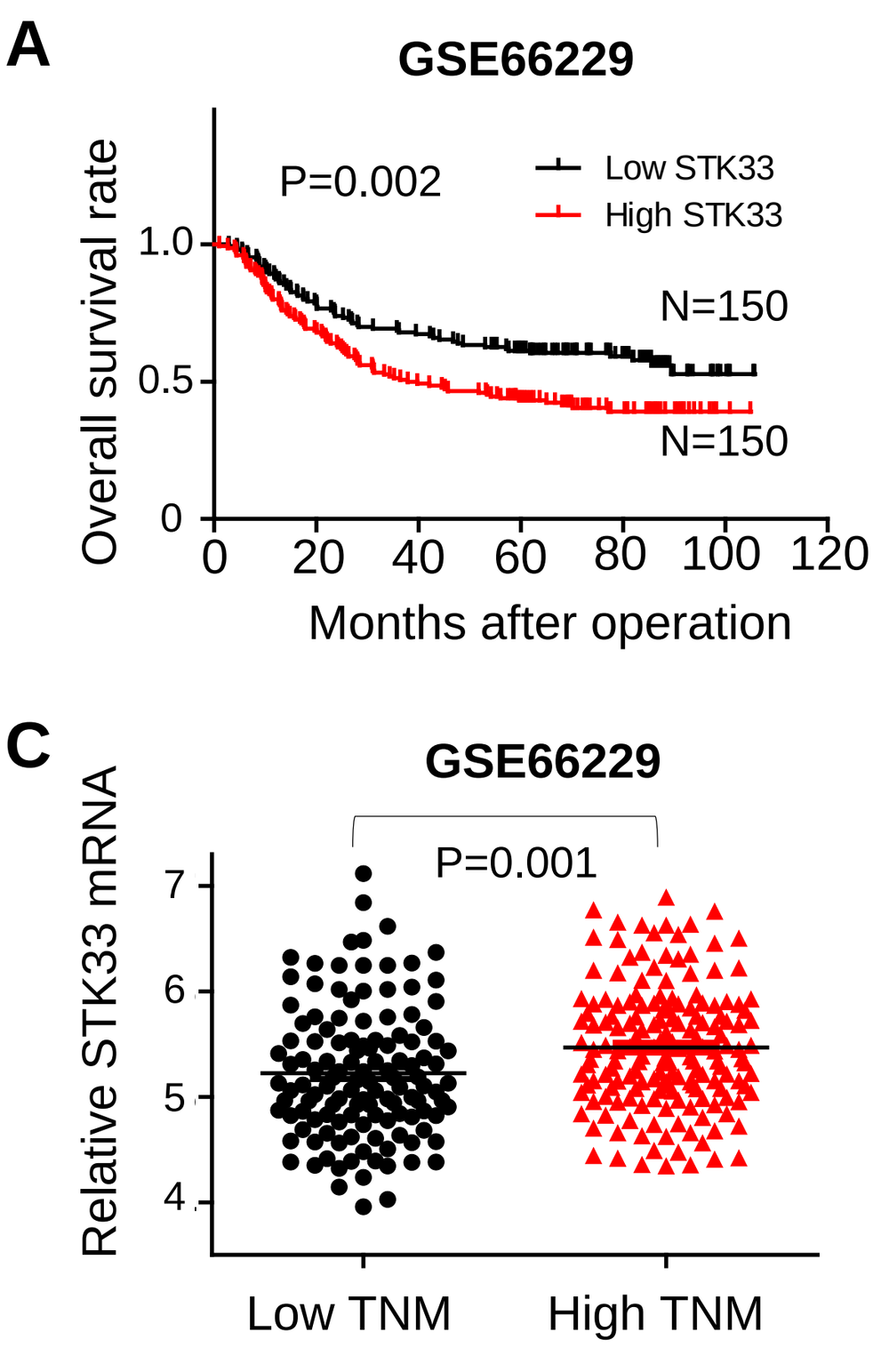
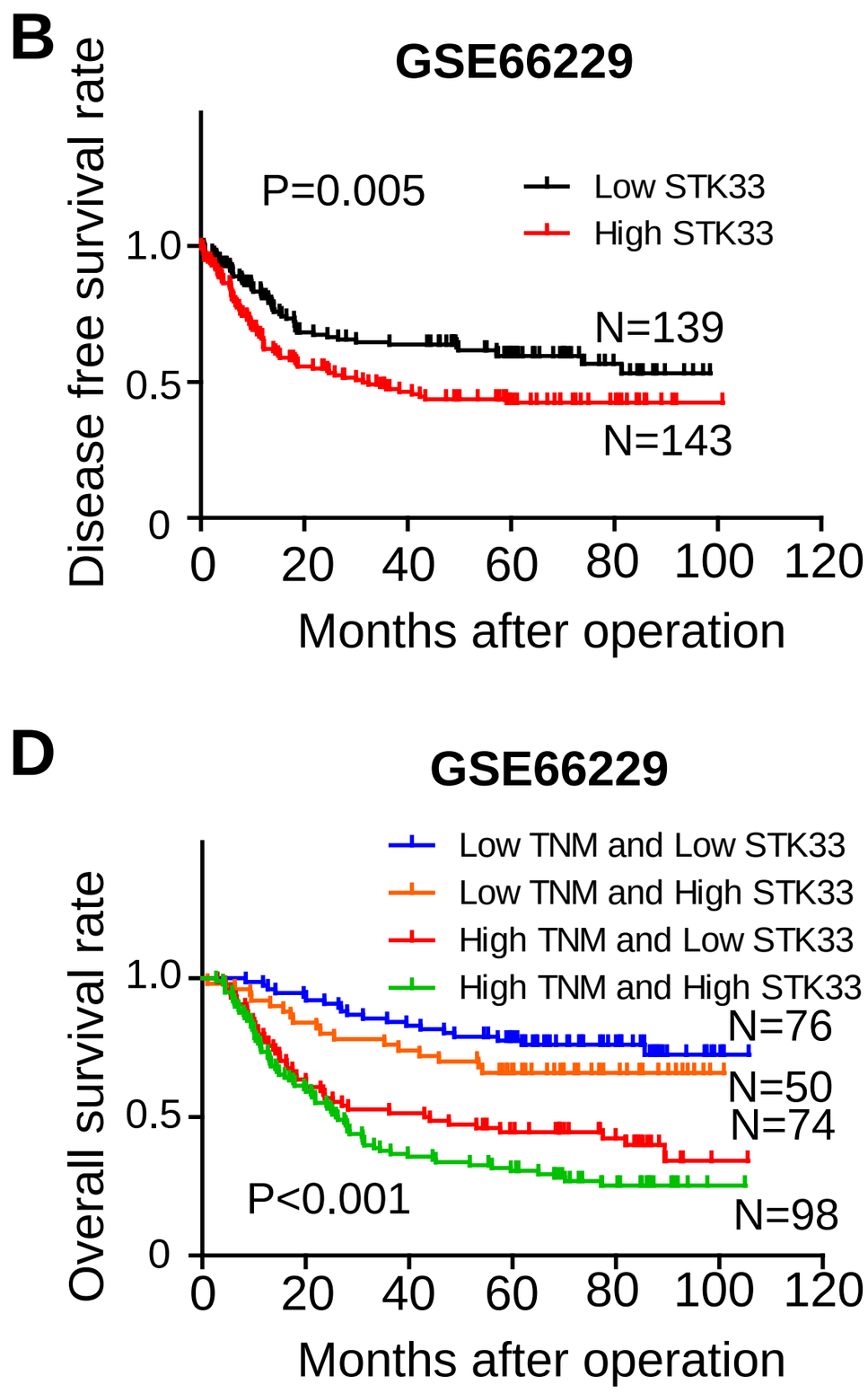
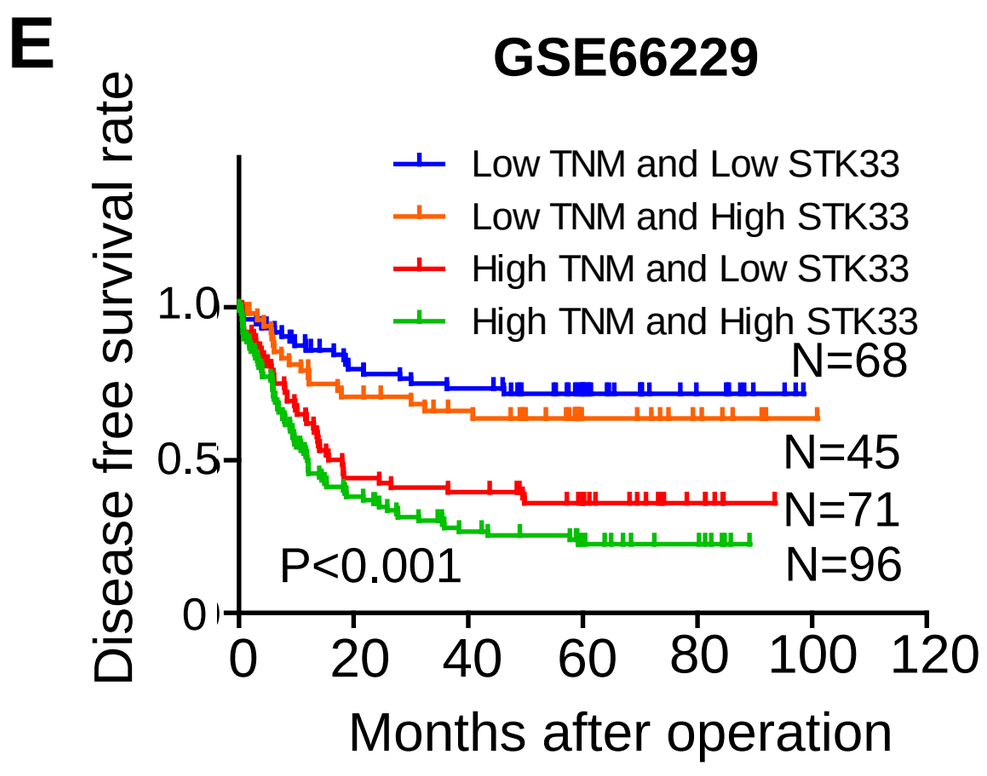


Supplementary Figure S1.

Immunohitochemical analysis of STK33 in TMAs of gastric cancer tissue specimens with different differentiation grade. Gastric specimens were immunostained with a specific anti-STK33 antibody. Expression of STK33 was estimated based on the percentage and intensity of the stained tumor cells. Representative images of STK33 expression in gastric cancer specimens with different differentiation grades are shown. All sections were individually scored by 2 investigators under an Olympus CX31 microscope (Olympus, Center Valley, PA).

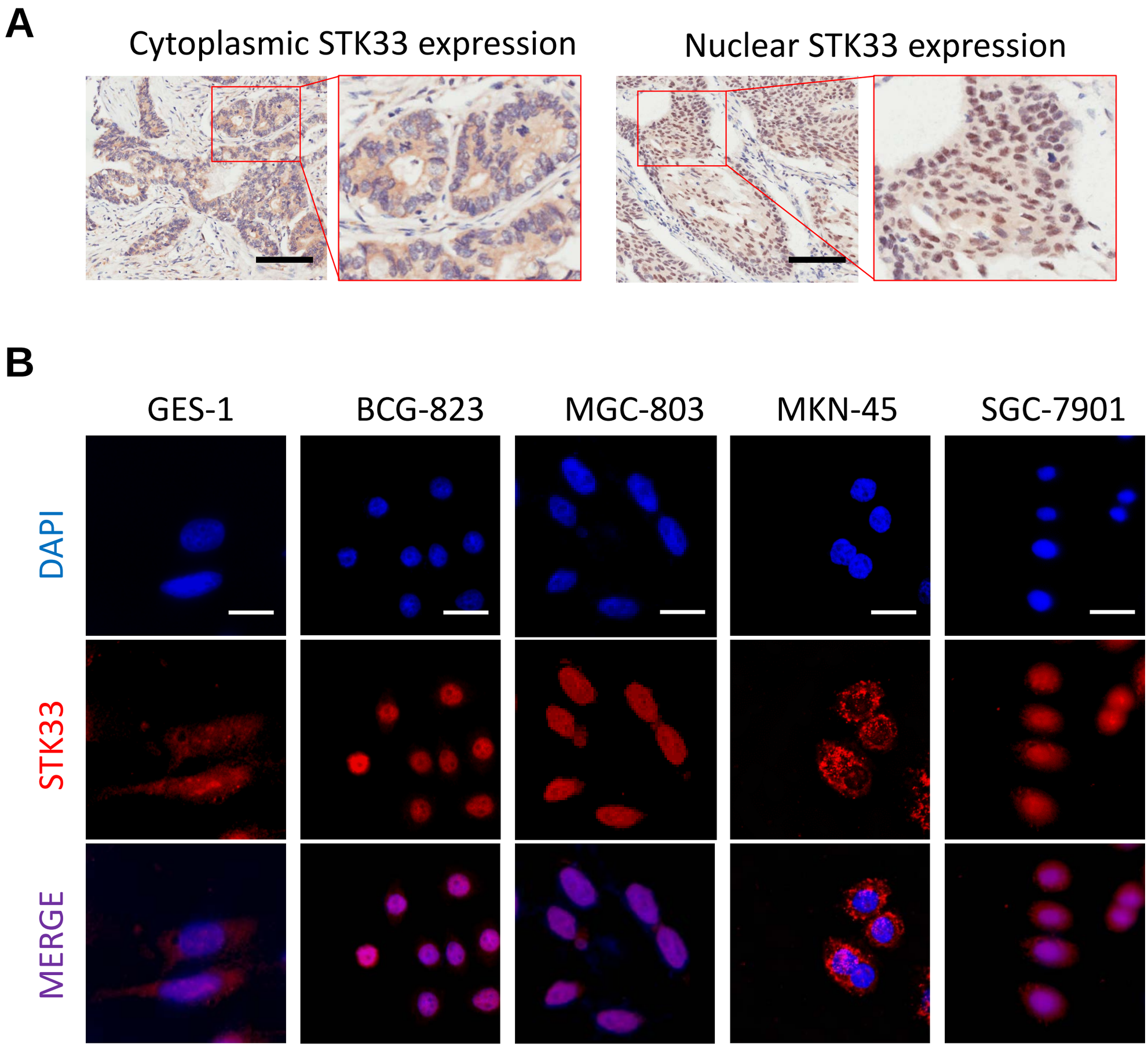






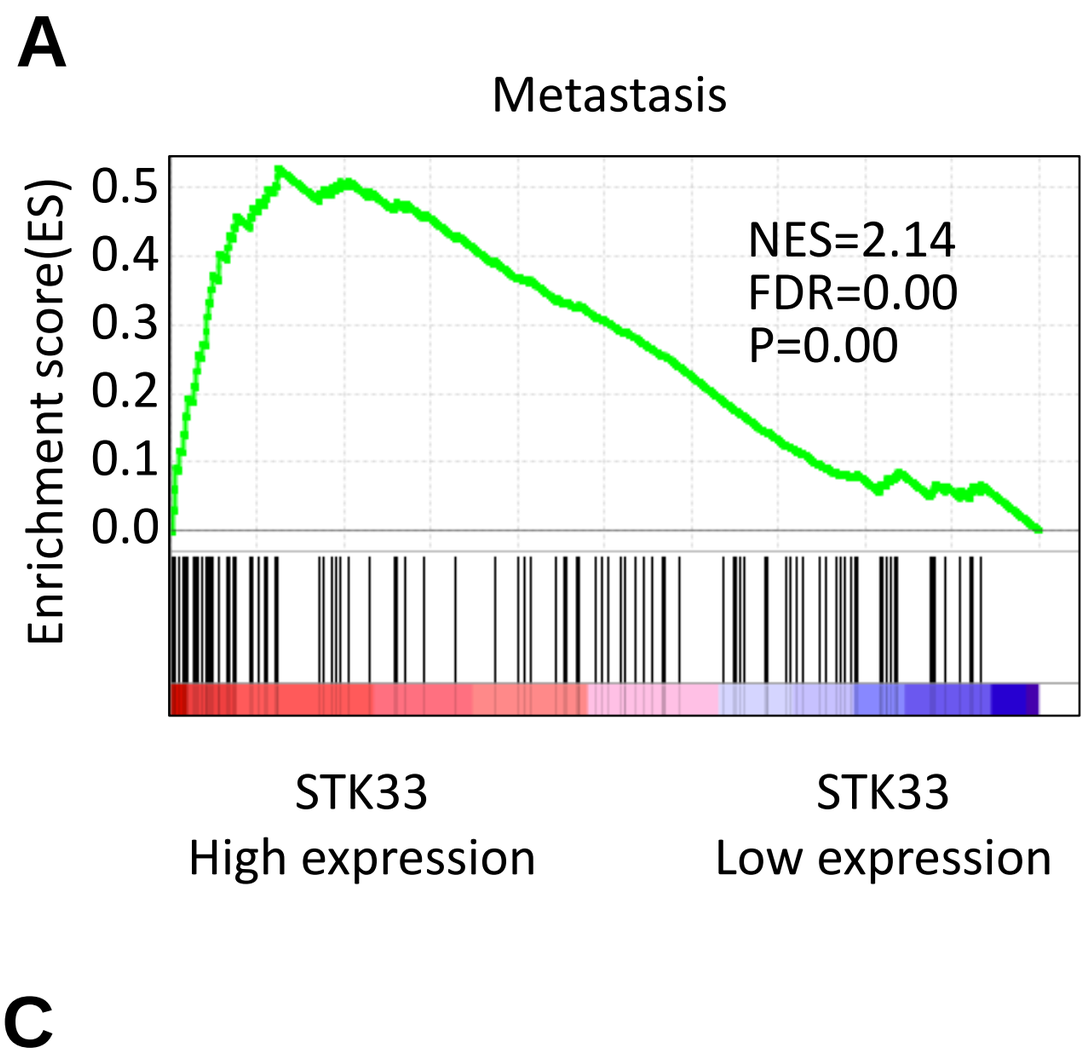
Supplementary Figure S2.

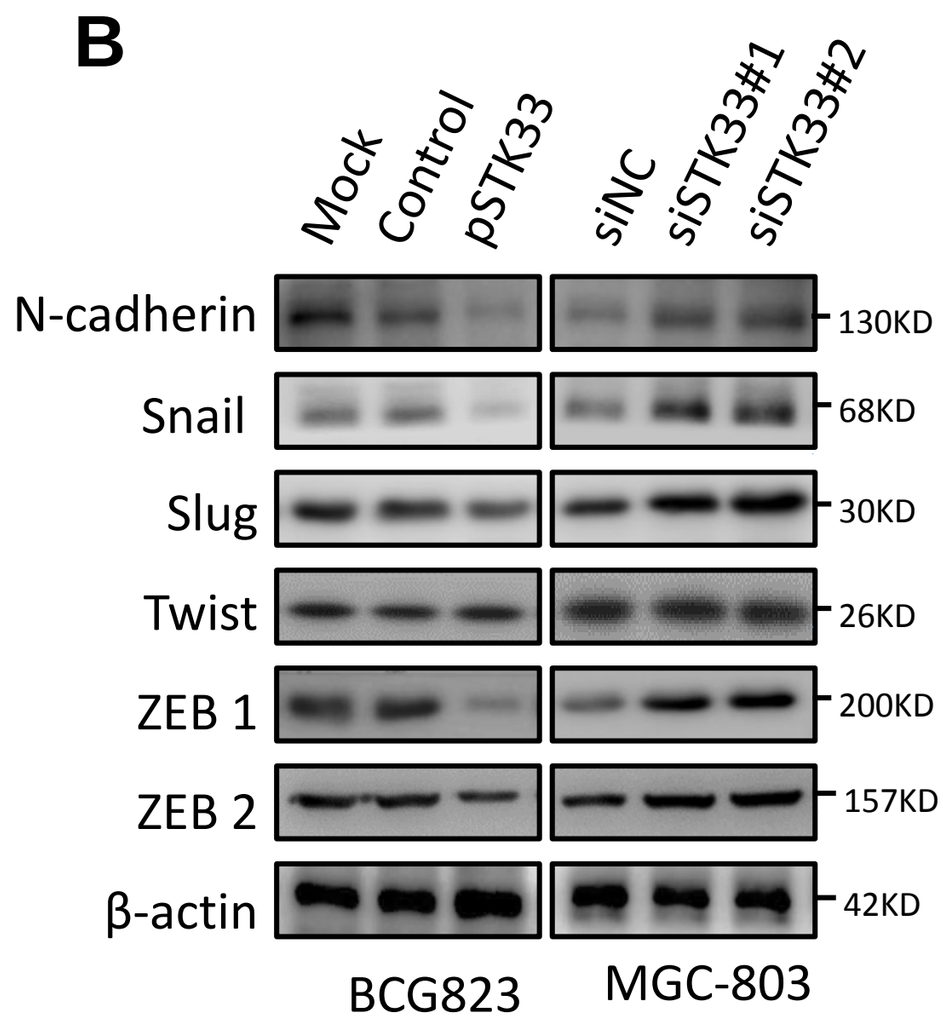
Estimates of OS and DFS of gastric cancer patients from GSE66229 (N=300). A, data from GSE66229 showed that elevated STK33 expression was associated with reduced OS rate in patients with GC. The patients were stratified into two groups (Low STK33 and High STK33) based on the median STK33 expression value. **B**, data from GSE66229 showed elevated STK33 expression was associated with reduced DFS rate in patients with GC. **C**, data from GSE66229 showed STK33 expression was increased in GC specimens with advanced TNM stage. Low TNM, stage I and II; High TNM, stage III and IV. **D**, data from GSE66229 showed STK33 predicted poorer OS of GC patients adjusted by TNM stages. **E**, data from GSE66229 showed STK33 predicted poorer DFS of GC patients adjusted by TNM stages.

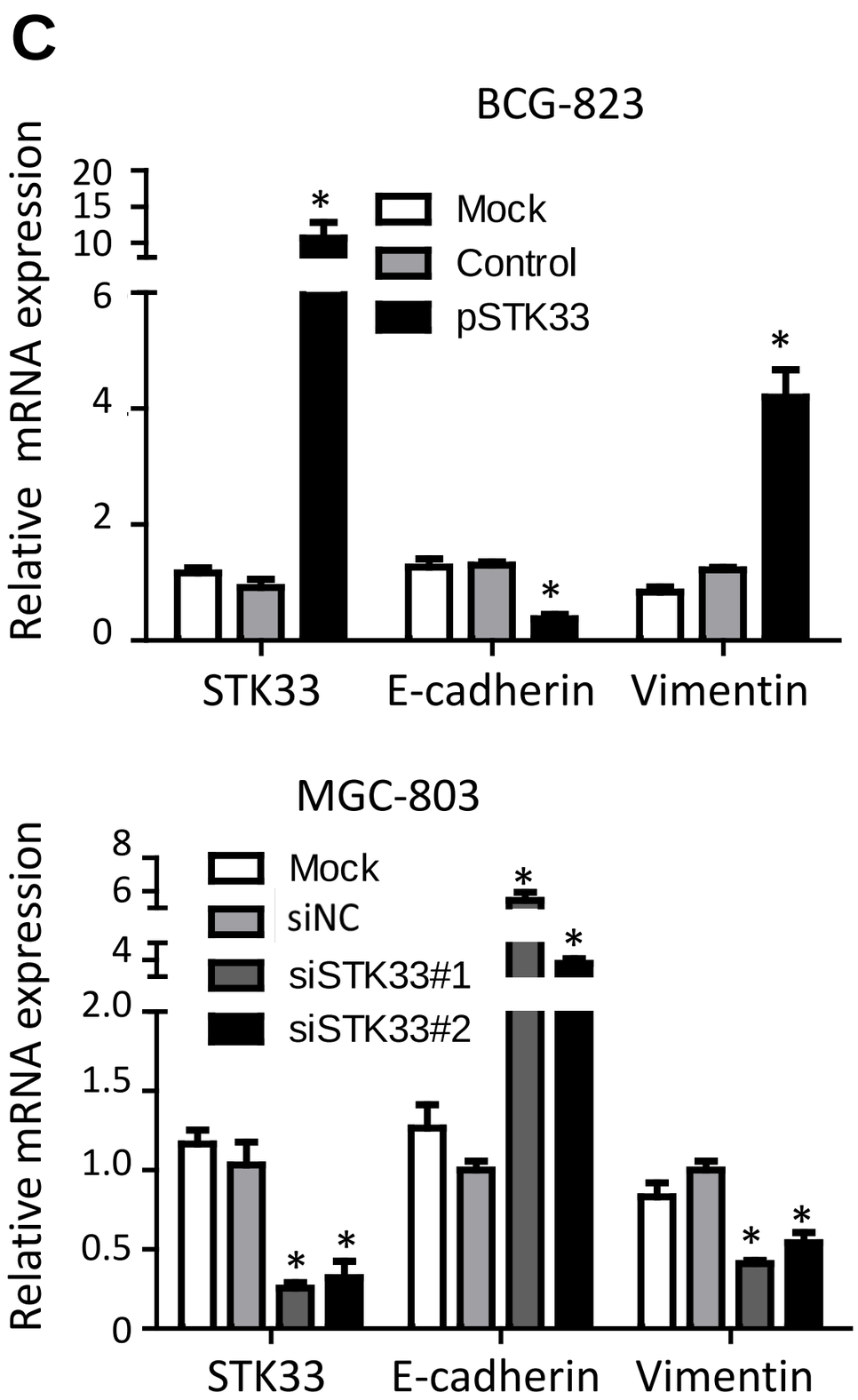


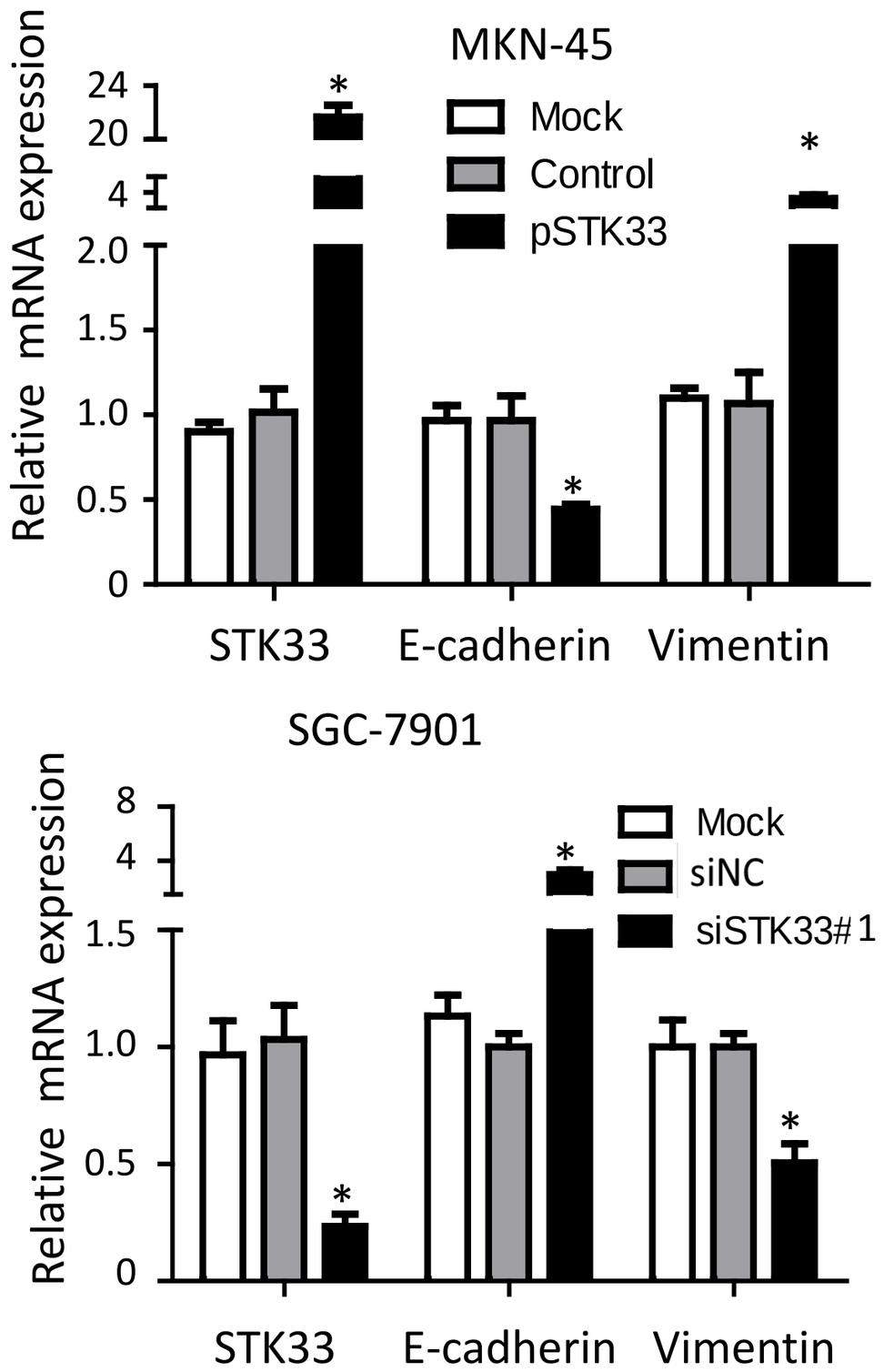
Supplementary Figure S3.

STK33 expression patterns in human gastric cancer tissues and cell lines. A, representative images of nuclear and cytoplasmic staining for STK33 in gastric cancer tissue specimens are shown. Scale bars: 50 μm. **B**, STK33 expression patterns in non-malignant (GES-1) and malignant (BCG-823, MGC-803, MKN-45, SGC-7901) gastric cells lines. All cell lines were cultured *in vitro* and immunofluorescently stained for STK33 (red) and nuclei (DAPI, blue). Localization of STK33 expression was shown in the merged images. Scale bars: 20 μm.



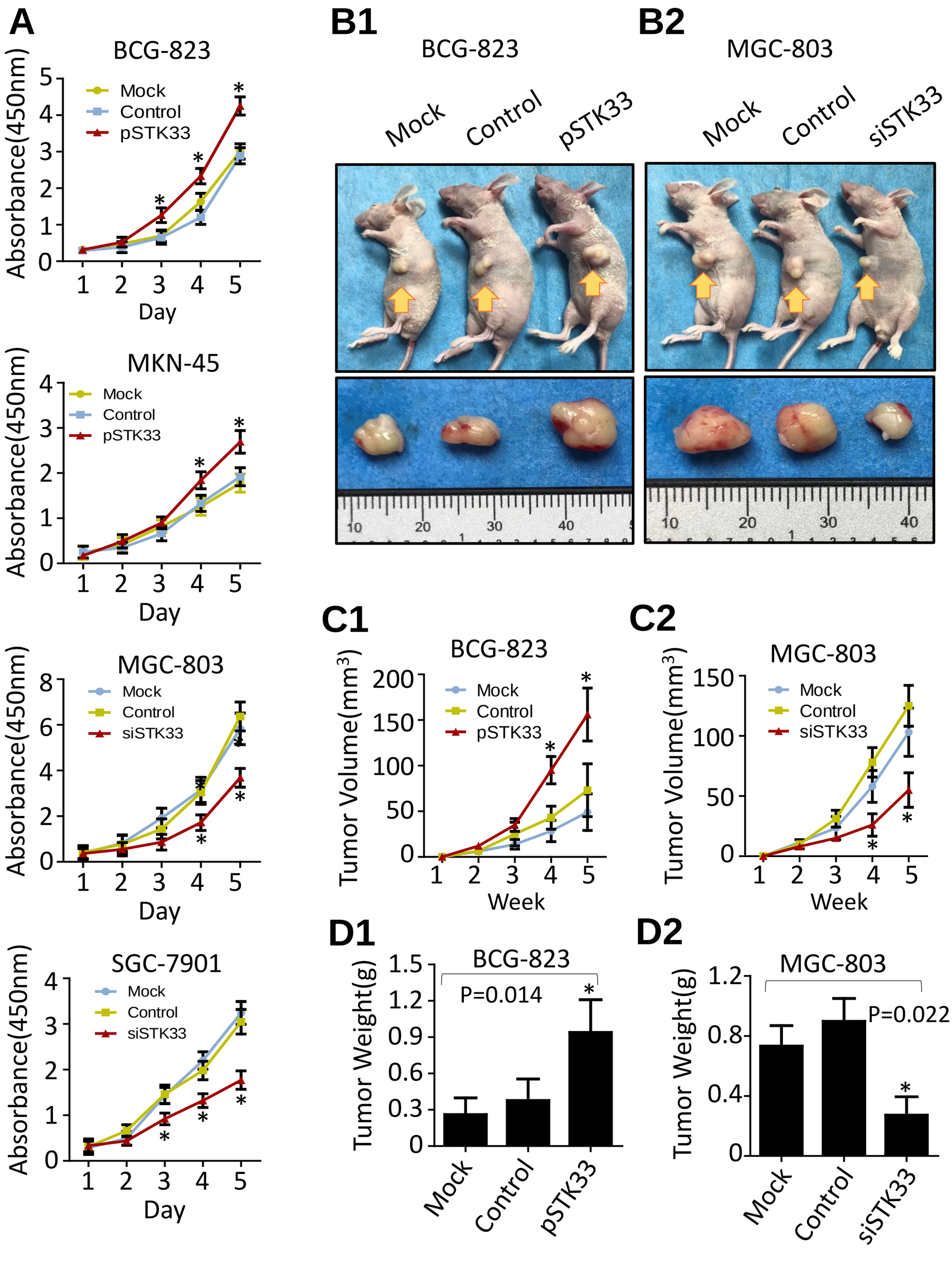






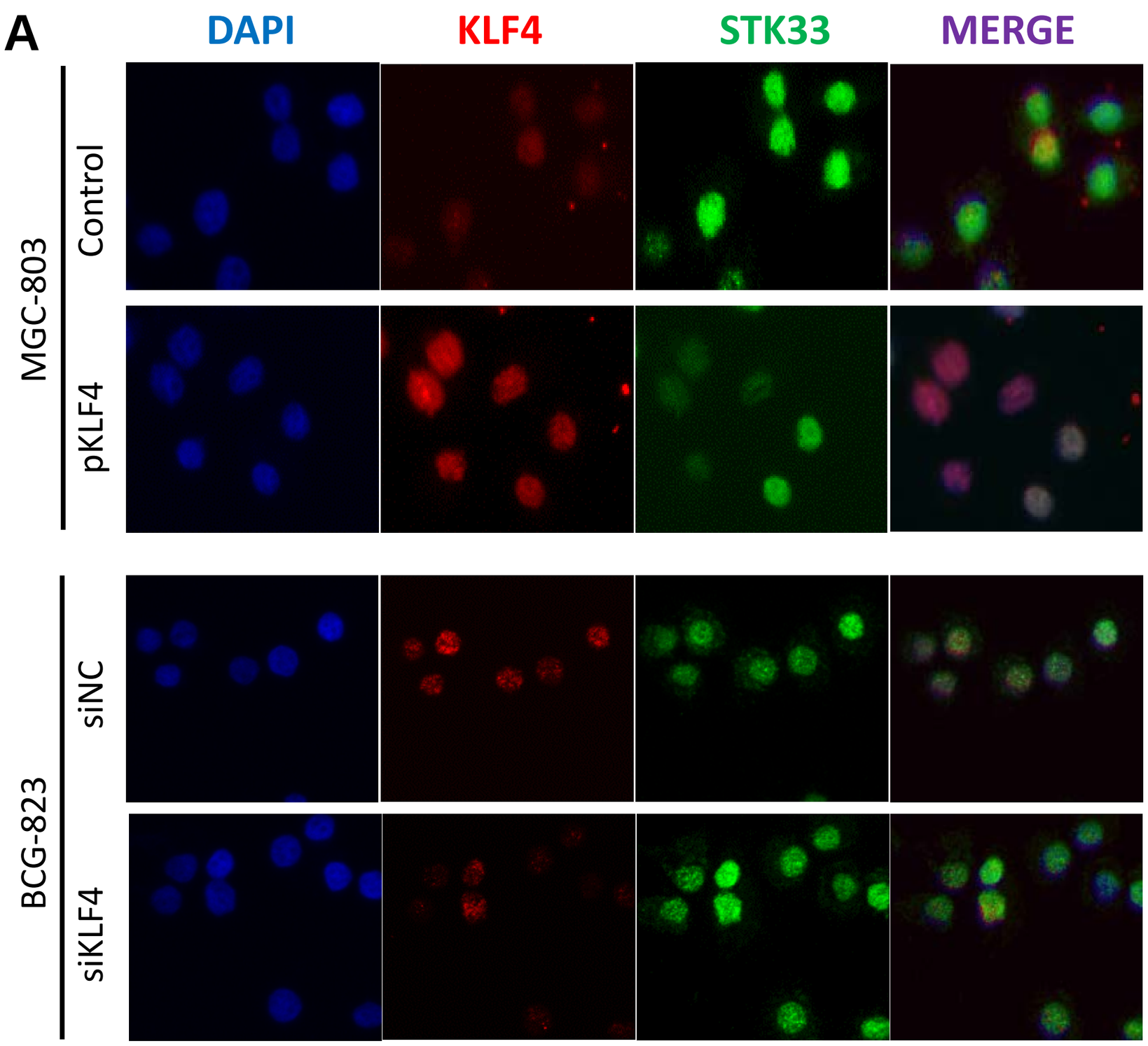
Supplementary Figure S4.

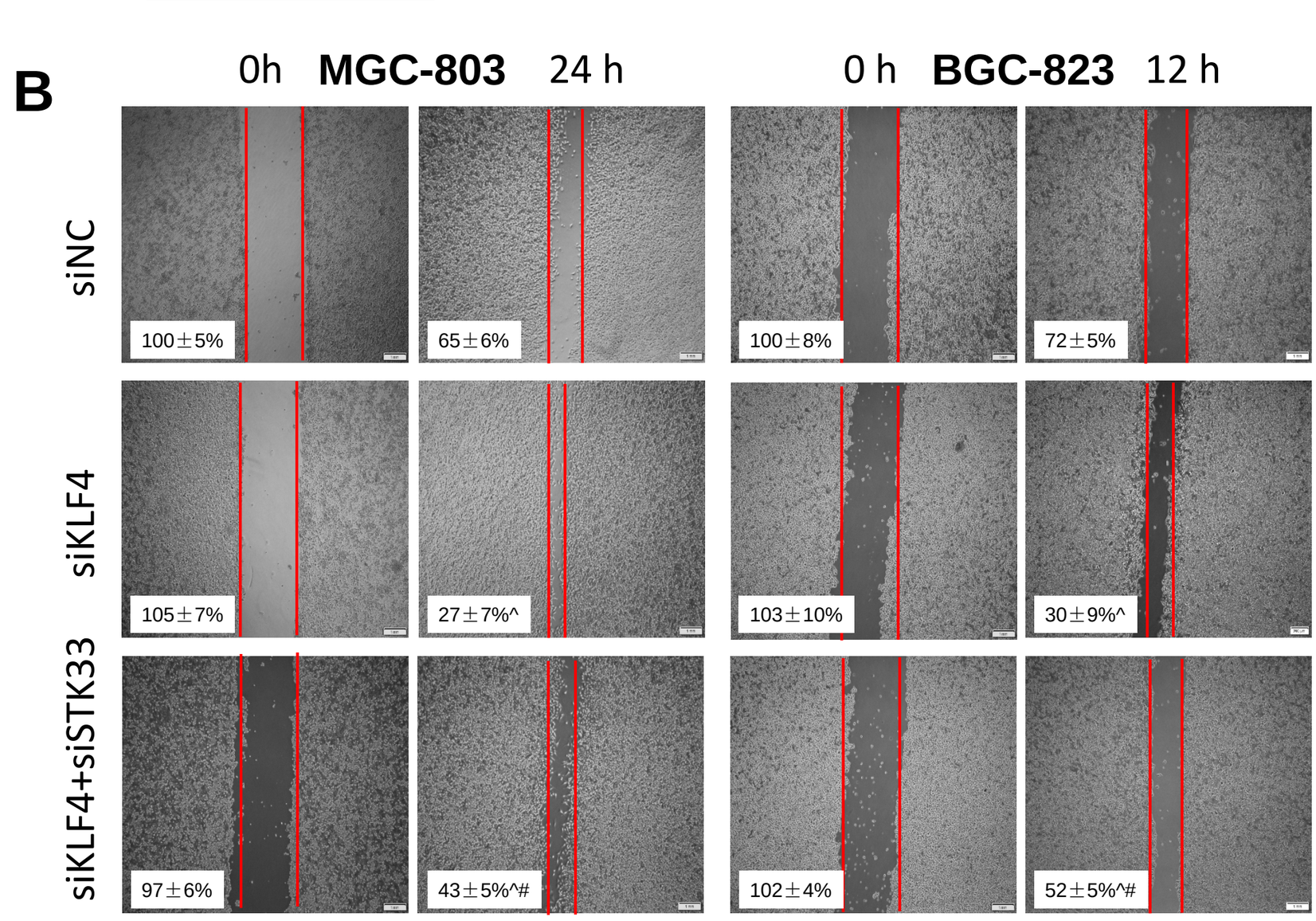
Effect of altered STK33 expression on gastric cancer metastasis and EMT. A, GSEA on the TCGA dataset. Enrichment plots of gene expression signatures for metastasis according to STK33 expression level. NES, normalized enrichment score. **B**, expression of EMT markers determined by Western blot in gastric cancer cell lines with altered STK33. **C**, relative mRNA expression of E-cadherin and Vimentin induced by dysregulated STK33 expression in gastric cancer cells. BCG-823 and MKN-45 cells were transfected with pSTK33, whereas MGC-803 and SGC-7901 cells were transfected with siSTK33 for 48 hours. Change of mRNA expression of E-cadherin and Vimentin were examined by quantitative PCR with specific primers.*P<0.05.



Supplementary Figure S5.

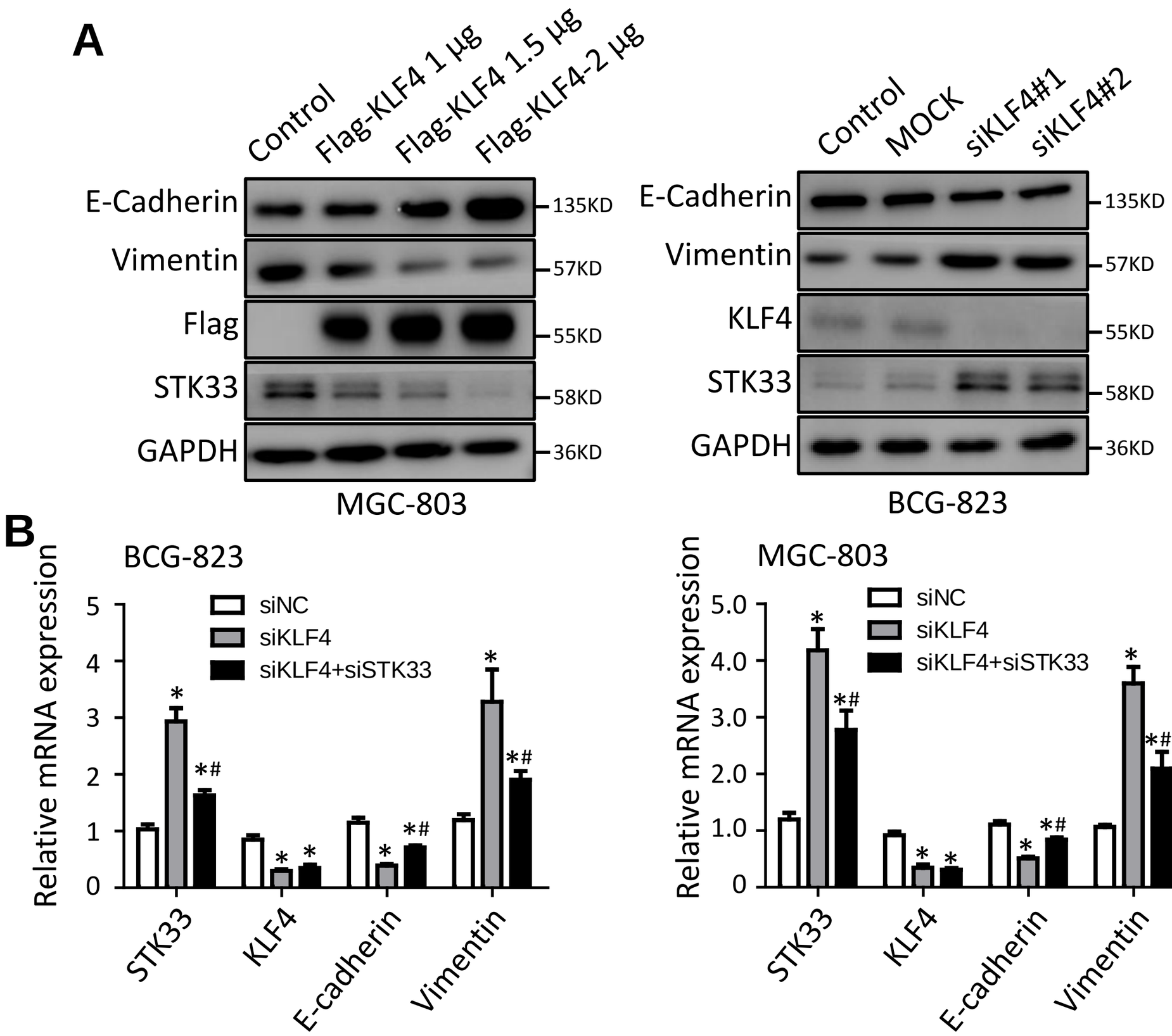
Effect of altered STK33 expression on gastric cancer growth *in vitro* and *in vivo*. A, assessment of GC cell growth *in vitro* by cell counting kit-8 (CCK-8) at the indicated time points. **B-D,** BCG-823 cells transfected with pSTK33 and MGC-803 cells transfected with siSTK33 were injected subcutaneously on the left thigh of nude mice (1×10^6 cells per mouse, five mice per group). Gross tumors (**B1** and **B2**), tumor weights (**C1** and **C2**), and tumor growth curves (**D1** and **D2**) are shown. The formula to calculate the tumor volume is $ab^2/2$, a represents the longest diameter of the tumor, and b represents the diameter perpendicular to the longest. *$P < 0.05$.





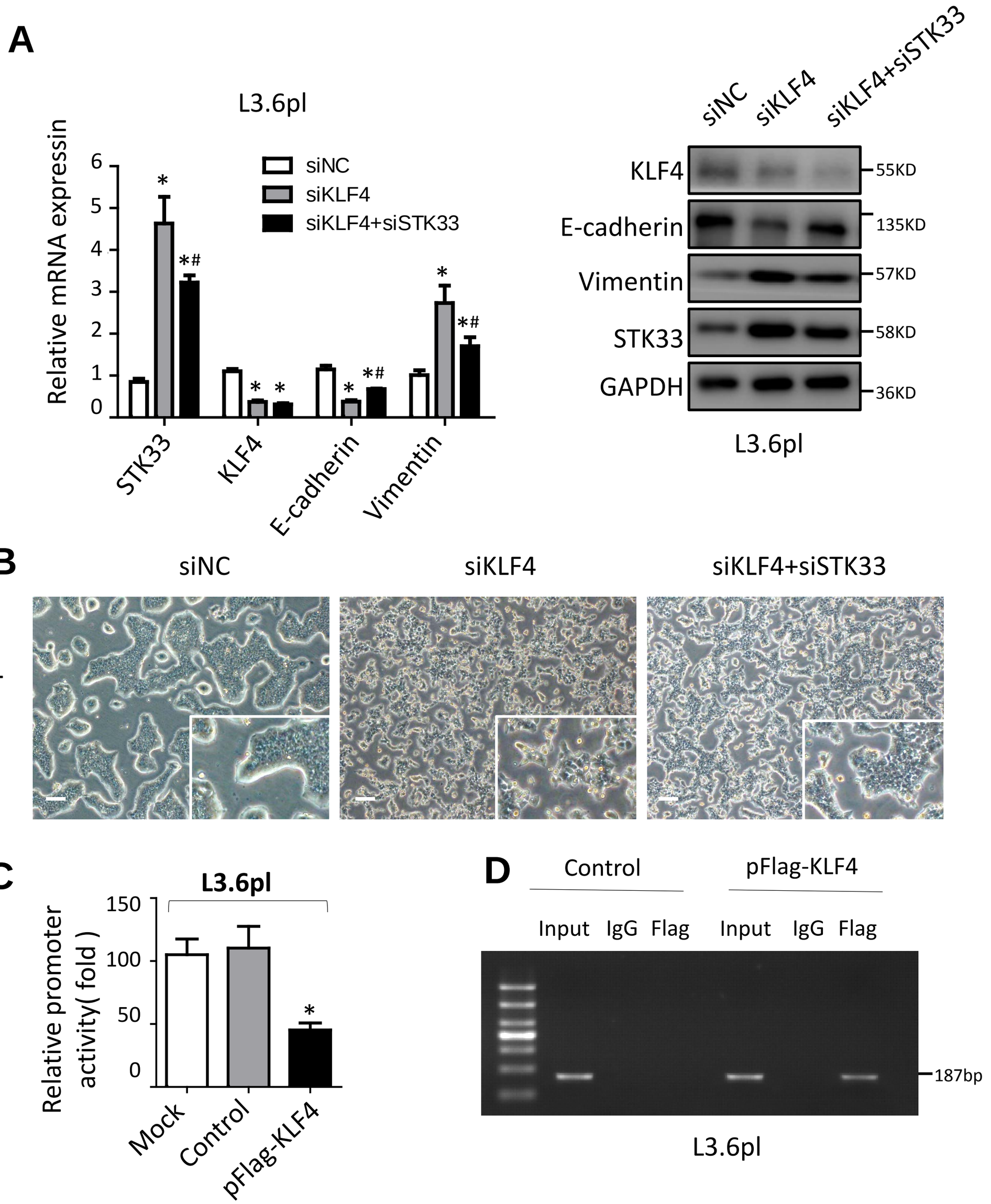
Supplementary Figure S6.

Identification of STK33 as a downstream target gene of KLF4. A, BCG-823 transfected with pFlag-KLF4 and MGC-803 transfected with siKLF4 were cultured *in vitro* for 48h and immunofluorescently stained for KLF4 (red), STK33 (green) and nuclei (DAPI, blue). Images were pseudocoloured using an Olympus microscope. **B**, Wound healing assay of effect of alerted expression of STK33 on KLF4-mediated migration in gastric cancer cells. ^Statistically significant when compared with siNC group ($P < 0.05$); #statistically significant when compared with siKLF4 group ($P < 0.05$)



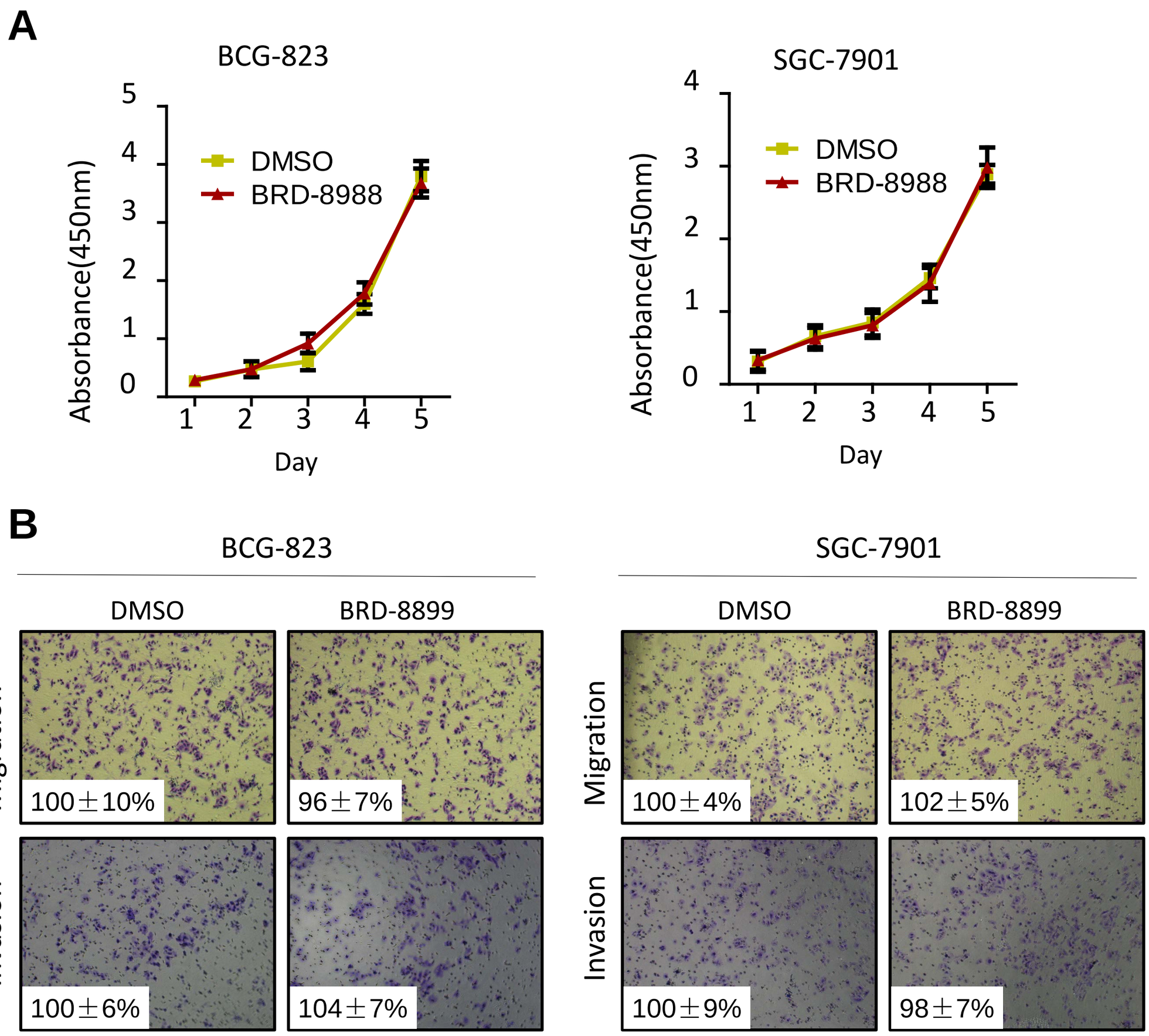
Supplementary Figure S7.

KLF4 negatively regulated STK33-induced invasion and EMT. A, expression of E-cadherin, Vimentin, KLF4 and STK33 determined by Western blot in siKLF4-transfected (BCG-823) and pFlag-KLF4-transfected (MGC-803) GC cell lines. 1μg, 1.5μg and 2μg KLF4-overexpressing plasmid vector and two specific siRNA for KLF4 were respectively used in the indicated group. **B**, relative mRNA expression of E-cadherin, Vimentin, STK33 and KLF4 in siKLF4-transfected GC cells with or without knockdown of STK33. **P*<0.05 compared with siNC group; #*P*<0.05 compared with siKLF4 group. **C**, Phase-contrast photomicrographs showing that downregulation of STK33 partially reverted the morphologic changes typical of EMT after knockdown of KLF4. Scale bars: 100 μm.



Supplementary Figure S8

Identification of transcriptional inhibition on STK33 expression by KLF4 in pancreatic cancer cells. A, negative regulation of STK33 mRNA and protein expression by KLF4 in pancreatic cancer cell line (L3.6pl). **P*<0.05 compared with siNC group ; #*P*<0.05 compared with siKLF4 group. **B,** phase-contrast photomicrographs demonstrating that knockdown of STK33 partially inhibited EMT induced by downregulation of KLF4 in L3.6pl cells. Scale bars: 100 μm. **C**, regulatory effect of KLF4 on STK33 promoter activity determined by dual luciferase assay. **D**, identification of KBS2 as binding sits of KLF4 on STK33 promoter by a ChIP assay. **P<0.05.*



Supplementary Figure S9

STK33 kinase inhibitor BRD-8899 failed to inhibit STK33-induced cell proliferation, migration and invasion in GC. BCG-823 and SGC-7901 cells were transfected with a pSTK33 for 48 hours. Cells were plated in 96-well plates or 24-well plates and then treated with BRD8988 or DMSO for 72 hours. **A**, CCK-8 assay showed that BRD-8899 did not inhibit proliferation of BCG-823 and SGC-7901 cells with overexpressed STK33 expression. **B**, Transwell assays illustrated that BRD-8899 did not inhibit migration and invasion of STK33-tansfected gastric cancer cells. **P<0.05.*