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Supplemental Information

miR-1266 Contributes to Pancreatic Cancer Progression and Chemoresistance by the STAT3 and NF-κB Signaling Pathways

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Supporting Information

Figure S1. miR-1266 is upregulated in pancreatic cancer and correlated with poor prognosis. (a) miR-1266 expression levels was markedly elevated in pancreatic cancer tissues compared with normal pancreatic tissues as assessed by analyzing the TCGA pancreatic cancer miRNA sequencing dataset (Normal, n = 4; Pancreatic cancer, n = 178). $P < 0.001$. **(b)** miR-1266 expression levels was elevated in pancreatic cancer tissues compared with normal pancreatic tissues as assessed by analyzing the pancreatic cancer miRNA expression profiling from E-GEOD-32678 dataset (Normal, n = 7; Pancreatic cancer, n = 25). $P < 0.05$. **(c and d)** Kaplan–Meier analysis of overall and progression-free survival curves of patients with pancreatic cancer with high miR-1266 expression versus low miR-1266 expression in the TCGA pancreatic cancer dataset. The best cutoff point was chosen by using X-tile software with log-rank test.

Figure S2. The correlation of chemotherapeutic response with overall and progression-free survival in pancreactic cancer patients. (a and b) Kaplan–Meier analysis of overall **(a)** and progression-free **(b)** survival curves of patients with pancreatic cancer with PD/SD versus CR/PR. $P < 0.001$, **(c and d)** Kaplan–Meier analysis of overall **(c)** and progression-free **(d)** survival curves of patients with pancreatic cancer with PD/SD versus CR/PR in the TCGA dataset. $P < 0.001$.

Figure S3. miR-1266 promotes chemoresistance in pancreatic cancer cells in vitro. (a) Real-time PCR analysis of miR-1266 expression in pancreatic cancer cells transduced with miR-1266 or transfected with anta-1266 compared to controls. Transcript levels were normalized by *U6* expression. Error bars represent the mean ± s.d. of three independent experiments. $*P < 0.05$. **(b)** Inhibition of miR-1266 increased the apoptotic ratio in the absence of GEM. Error bars represent the mean ± s.d. of three independent experiments. *P

< 0.05. **(c)** Annexin V-FITC/PI staining of the indicated cells treated with gemcitabine (10 μM) for 36 h. Error bars represent the mean ± S.D. of three independent experiments. *P < 0.05. **(d)** The JC-1 staining showed that silencing miR-1266 decreased the mitochondrial potential in a dose-dependent manner in pancreatic cancer cells. Error bars represent the mean ± S.D. of three independent experiments. *P < 0.05. **(e and f)** Analysis of the activities of caspase-9 **(e)** and caspase-3 **(f)** were detected by the cleaved forms of these two proteins. Error bars represent the mean ± S.D. of three independent experiments. *P < 0.05. **(g)** Western blotting analysis of Bcl-2, Bcl-xL, Mcl-1 and Survivin in the indicated cells. **(h)** The effect of miR-1266 on proliferation of pancreatic cancer cells was assessed by MTT assay.

Figure S4. Inhibition of miR-1266 sensitizes pancreatic cancer cells to gemcitabine in vivo. (a) Xenograft model in nude mice. Representative images of tumor-bearing mice on day 10 and day 45 in AsPC-1 cells. Mice were euthanized, and tumors from each experimental group were excised. **(b)** After 10 days of inoculating AsPC-1 cells, mice were intraperitoneal injected with 50 μg/g gemcitabine (GEM) two times each week for 4 weeks. Tumor volumes in the low-dose anta-1266, high-dose anta-1266 and scramble groups were measured from the fifth day at five days interval. Data presented are the mean ± s.d. **(c)** Tumor weights of each group. **(d)** The overall survival of mice in the indicated group. **(e)** After 10 days of inoculating PANC-1 cells, mice were intraperitoneal injected with 50 μg/g gemcitabine (GEM) two times each week for 4 weeks. Tumor volumes in the high-dose anta-1266 and control groups were measured from the fifth day at five days interval. Data presented are the mean ± s.d. **(f)** Tumor weights of each group. **(g)** The Caspase-3 activity in the tumor tissues formed by the low-dose anta-1266, high-dose anta-1266 and scramble groups in AsPC-1 and PANC-1 cells respectively. **(h)** After 10 days of inoculating BxPC-3 cells, mice were intraperitoneal injected with 50 μg/g gemcitabine (GEM) two times each week for 4 weeks. Tumor volumes in the miR-1266-overexpressing and control groups were

measured from the fifth day at five days interval. Data presented are the mean ± s.d. **(i)** Tumor weights of each group. **(j)** Western blotting of SOCS3, PTPN11, ITCH and TNIP1 expression in scramble and anta-1266 (H.D.) tumor groups. α-Tubulin served as the loading control.

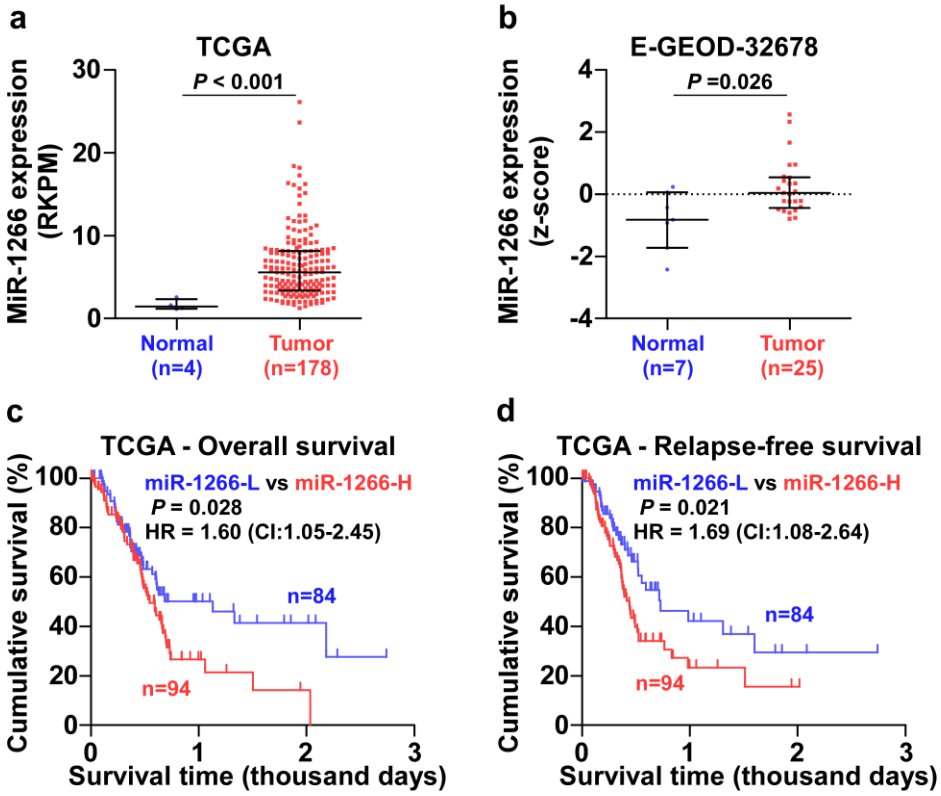
Figure S5. The inhibitors of STAT3 signaling Stattic and S3I-201 and the inhibitors of NF-κB signaling LY2409881 and JSH-23 repress STAT3 and NF-κB activity in a dose-dependent manner in pancreatic cancer cells. (a-d) STAT3 inhibitors Stattic and S3I-201, or NF-κB inhibitors LY2409881 and JSH-23 showed potent inhibition of the STAT3 and NF-κB reporter activities in pancreatic cancer cells. Error bars represent the mean ± s.d. of three independent experiments. *$P < 0.05$.

Figure S6. Wild-type sequence and mutant sequences of 3′UTRs in SOCS3, PTPN11, ITCH and TNIP1. (a) Predicted miR-1266 targeting sequence and mutant sequences in 3′ UTRs of SOCS3, PTPN11, ITCH and TNIP1. **(b and c)** Individual silencing of these targets rescued the STAT3 (E) and NF-κB (F) activity repression in miR-1266-silencing cells.

Figure S7. Recurrent gains and hypomethylation contribute to miR-1266 overexpression in pancreatic cancer tissues. (a) The percentage of deletion, diploid and gain in the pancreatic cancer samples from TCGA. **(b)** The average expression level of miR-1266 in pancreatic cancer patients with gains was higher than those without gains in the pancreatic cancer dataset TCGA. Each bar represents the median values ± quartile values. **(c)** The percentage of deletion, diploid and gain in our pancreatic cancer samples, ANT and benign pancreatic lesions. **(d)** The average expression level of miR-1266 in pancreatic cancer tissues with gains was higher than those without gains. Each bar represents the median values ± quartile values. **(e)** Methylation level of miR-1266 promoter in the pancreatic cancer

dataset from TCGA. **(f)** Methylation level of miR-1266 promoter using cg06706204 in our pancreatic cancer tissues, ANT and benign pancreatic lesions. Methylation ratio: methylation percentage in each tissue. **(g)** Real-time PCR analysis of miR-1266 expression levels in pancreatic cancer tissues with different methylation ratio. Transcript levels were normalized to *U6* expression.

Supplement figure 1



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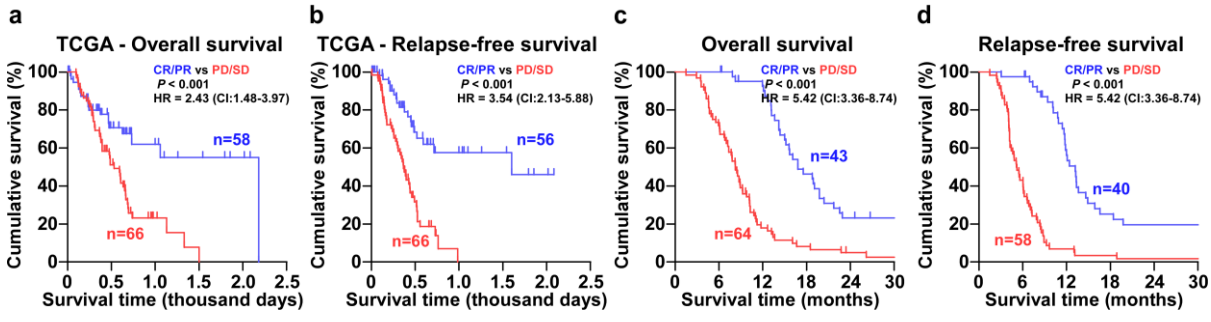

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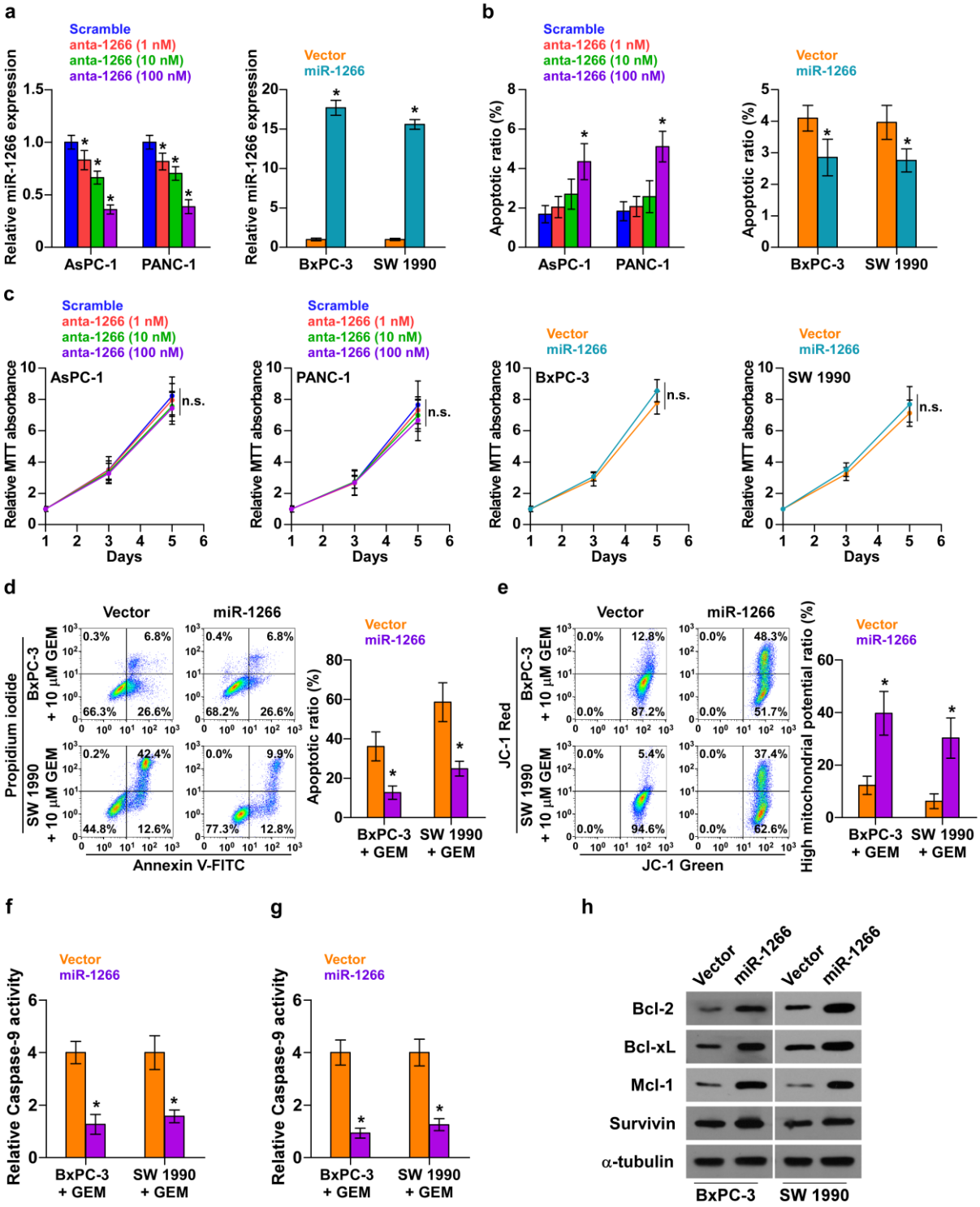

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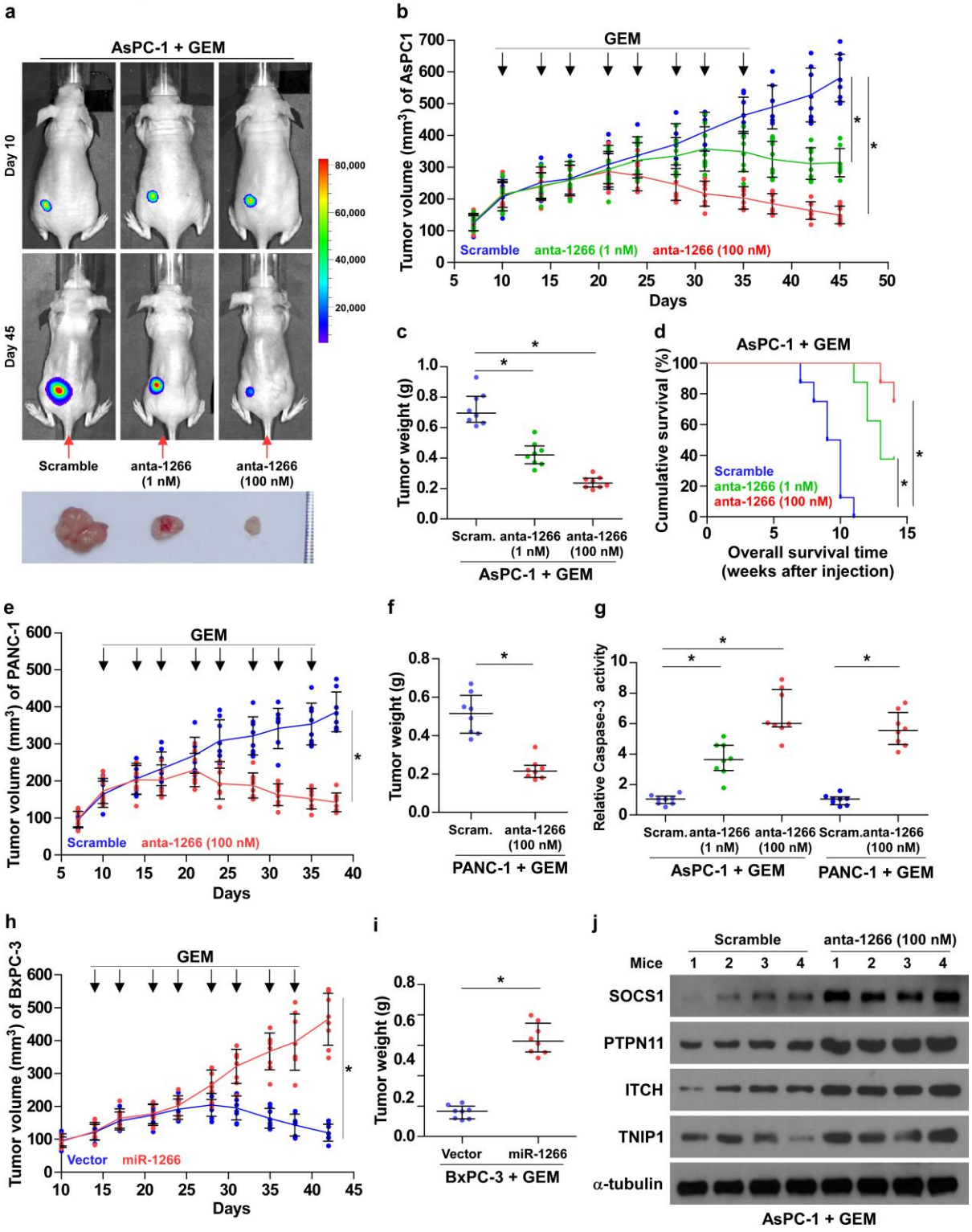

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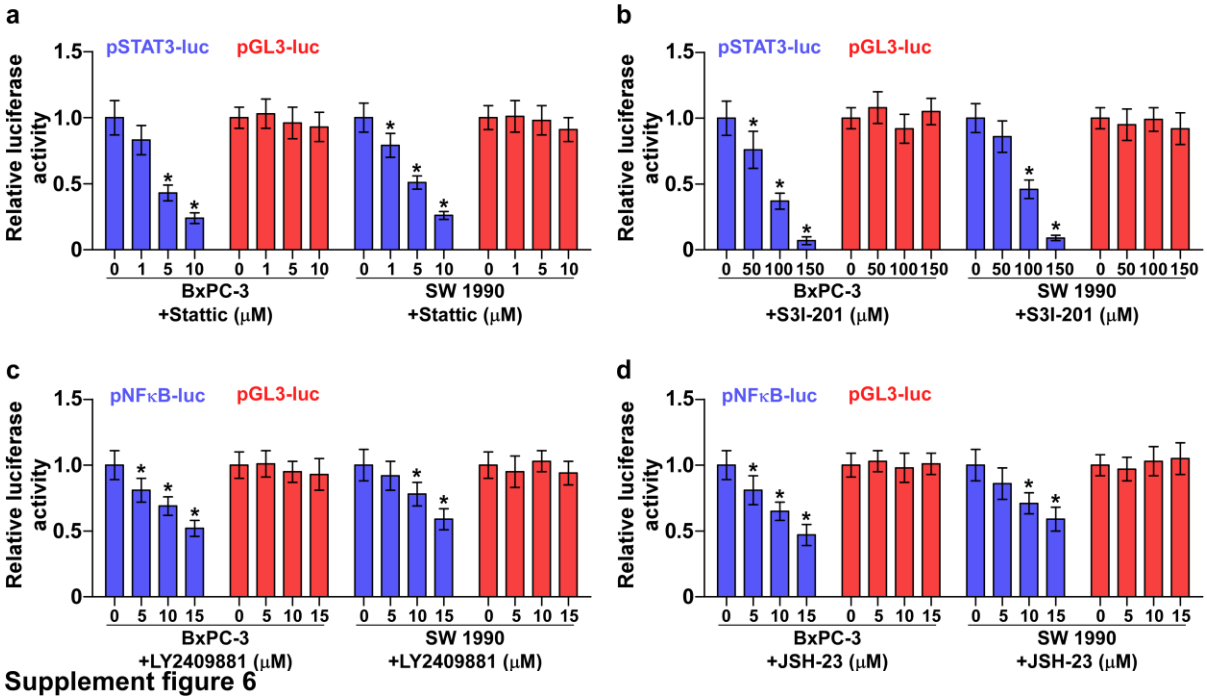

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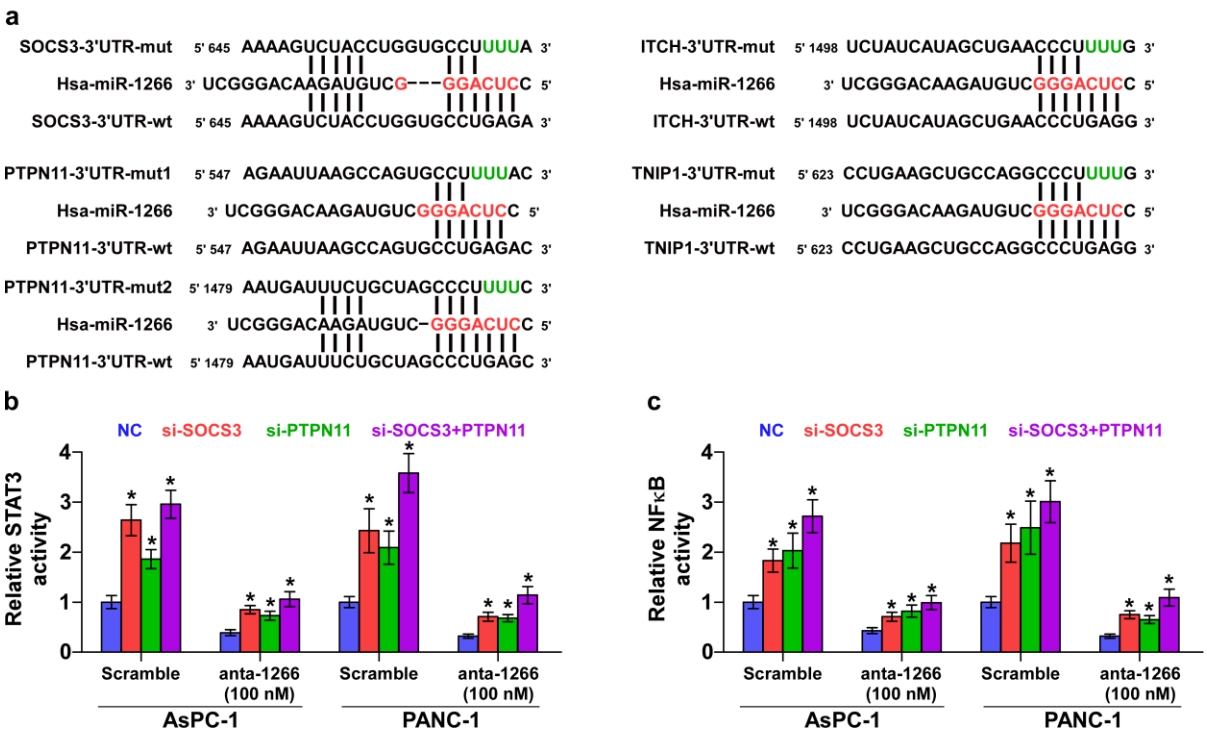

Supplement figure 7