SUPPLEMENTAL DATA

SUPPLEMENTAL TABLE S1.

Supplemental Table S1: Transfection efficiency of siLentFect in pancreatic cancer cell lines.

	SLF 2:1		SLF 4:1		SLF 6:1		SLF 8:1		SLF 10:1	
	NS	Lethal	NS	Lethal	NS	Lethal	NS	Lethal	NS	Lethal
BxPC3	84.3	3.7	82.2	8.0	76.0	1.0	66.5	3.8	54.6	0.6
MiaPaCa-2	91.5	17.3	90.0	8.8	91.9	7.2	100.6	6.6	97.9	6.1
Panc-1	83.7	6.2	85.6	5.9	69.1	1.7	78.1	2.2	71.2	1.5
AsPC-1	94.1	5.8	94.7	6.1	80.9	1.9	84.3	2.2	75.0	2.0
Su.86.86	92.9	36.5	96.0	19.8	91.9	9.8	90.2	4.0	79.1	1.7

SUPPLEMENTAL TABLE S1. Transfection efficiency of siLentFect in pancreatic cancer cell lines. In order to determine transfection efficiency, cells were reverse transfected with non-silencing (NS) or lethal control siRNA and incubated for 96 hours. Values shown are percent viability of buffer-treated wells. Higher amounts of transfection reagent often decreases cellular viability, so we chose the ratio used for each cell line (highlighted in red) based on the value that demonstrated a minimum decrease in viability with NS siRNA and a greater than 90% decrease in viability with lethal siRNA.

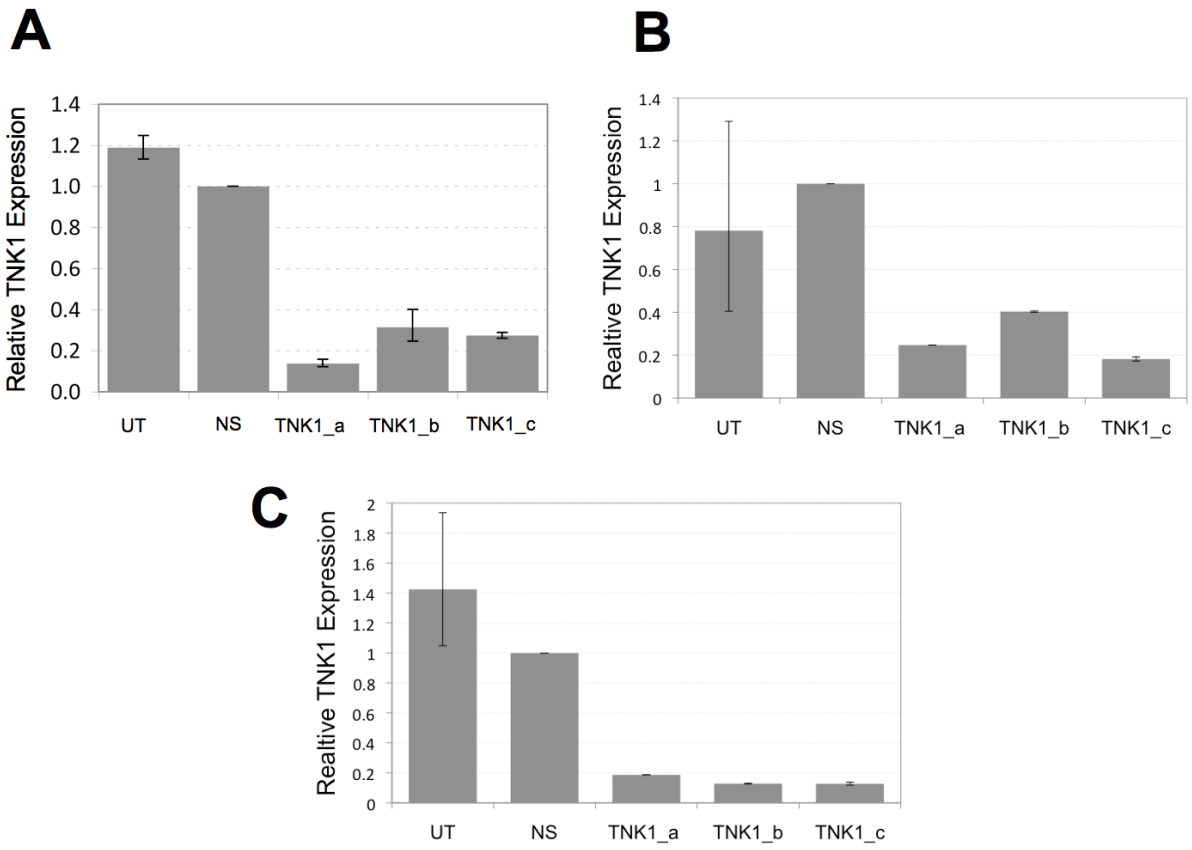
SUPPLEMENTAL TABLE S2:

Supplemental Table 2. Z-score values of hits from siRNA screening of kinases

siRNA	MiaPaCa2_1	MiaPaCa2_2	BxPC3_1	BxPC3_2	Norm_Fibro_1	Norm_Fibro_2
PLK1_A	-4.939	-5.008	-5.058	-7.319	-2.583	-2.871
CALM1_B	-3.640	-8.022	-5.700	-4.278	0.626	0.214
PRKCL1_B	-4.990	-6.798	-5.176	-4.225	-1.113	-1.515
TNK1_B	-4.379	-6.003	-1.009	-1.652	-1.366	-0.148
GRK4_B	-3.036	-4.001	-3.861	-3.920	-1.010	-0.916
MAPK11_A	-3.048	-5.367	-3.119	-3.385	-1.581	-0.581
PLK1_B	-3.195	-5.557	-4.264	-3.582	-0.694	-1.421
PTK9_B	-2.577	-6.457	-3.791	-1.780	0.213	-0.496
AKAP3_B	-3.182	-4.601	-3.228	-1.490	-1.082	-1.125
TNK2_B	-3.121	-5.639	-3.236	-1.176	-1.305	-1.365
CAMK1D_A	-2.391	-1.362	-3.493	-5.626	-1.757	-2.257
CIB3_B	-2.983	-3.325	-3.620	-1.656	-2.486	-1.917
EPHA5_B	-2.123	-1.403	-2.245	-2.374	-3.441	-3.351
MAPK12_A	-1.743	-1.668	-2.753	-1.596	-3.581	-3.191
UCK1_B	-2.072	-0.923	-2.891	-3.193	-2.178	-2.065
FLJ11149_A	-1.715	-2.327	-2.264	-2.038	-1.415	-1.701
MAP4K1_A	-2.254	-2.165	-1.672	-1.806	-2.653	-2.670
DAPK2_A	-2.306	-1.220	-2.113	-1.692	-2.179	-2.698
MAP4K2_A	-2.456	-1.564	-2.295	-2.212	-1.796	-1.894
CDK5R2_A	-3.316	-3.873	-1.976	-2.440	-0.061	-0.271
FLT4_B	-3.393	-3.006	-2.514	-2.632	1.298	1.223
PRKCL1_A	-1.824	-1.685	-1.905	-1.037	0.227	0.907
EPHA4_B	-2.461	-1.790	-1.816	-0.553	-0.823	-0.150
PTK9L_B	-1.811	-2.891	-2.133	0.177	-0.712	-0.707
MAP2K1IP1_B	-2.747	-1.811	-4.138	-2.666	-0.879	-1.187
PLK3_A	-1.974	-0.904	-4.716	-3.587	-1.064	-0.742
ROR2_A	-2.147	-0.722	-4.209	-2.896	-0.250	-0.704
PRKY_B	-1.834	-1.002	-1.882	-2.620	-0.910	-0.794
AKAP9_A	-1.943	-2.830	-2.486	-3.327	-0.427	-0.558
STK10_A	-0.586	-2.075	-3.102	-2.255	-0.376	-0.458
TAOK3_B	-2.242	-2.927	-3.418	-1.653	-0.881	-0.819
NEK3_A	-2.132	-2.315	-3.323	-1.942	-0.068	-0.438

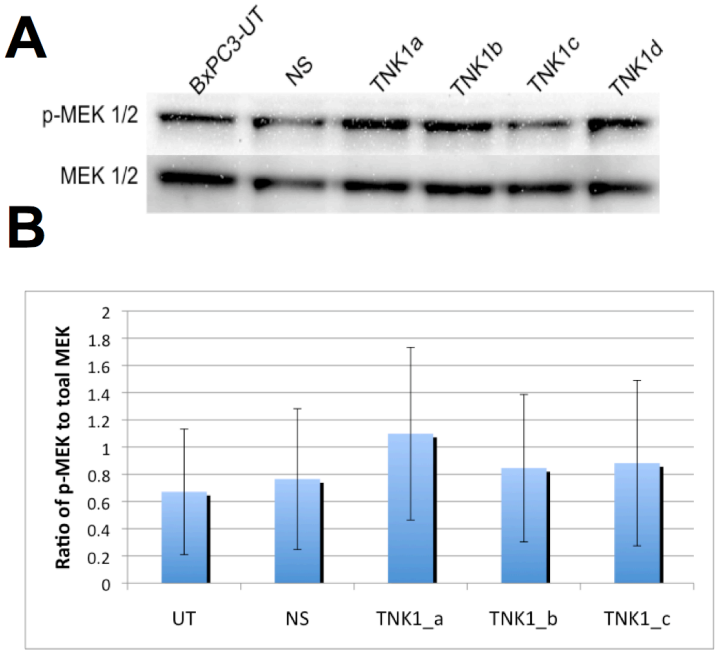
SUPPLEMENTAL TABLE S2. Z-score values of hits from siRNA screening of kinase. Cell viability data from each screen was normalized by z-score analysis for comparison. Hits had a cutoff of -1.65 for at least three of the four screens of MiaPaCa-2 and BxPC-3 cells. Z-score data for a normal fibroblast cell line is shown for comparison.

SUPPLEMENTAL FIGURE S1:



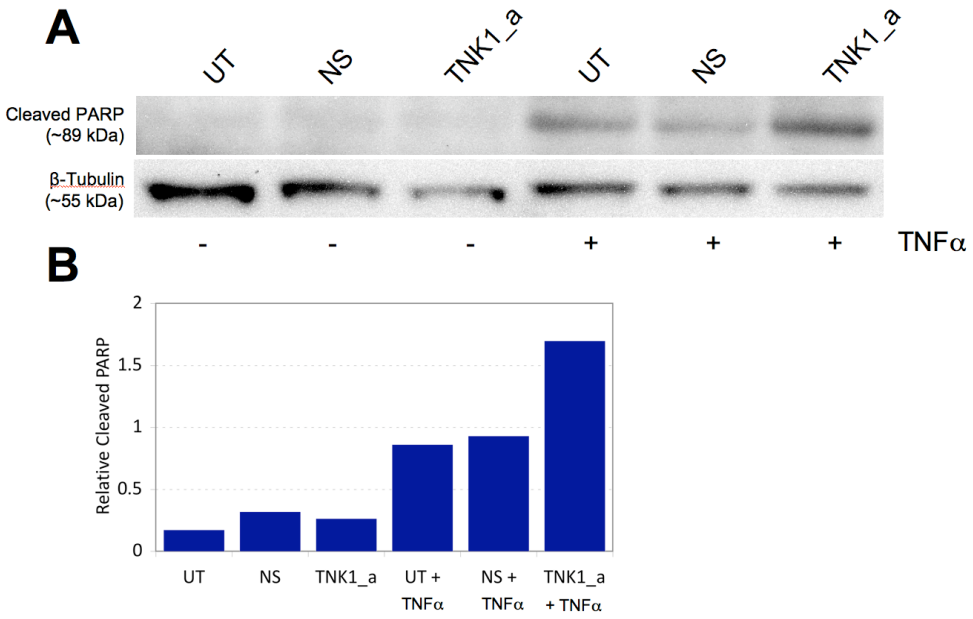
SUPPLEMENTAL FIGURE S1. Confirmation of TNK1 knockdown in pancreatic cancer cell lines. Pancreatic cancer cell lines (A) MiaPaCa-2, (B) AsPC-1 and (C) Su.86.86 were incubated with siRNA sequences against TNK1, negative control non-silencing siRNA (NS) or left untreated (UT) and allowed to grow for 48 hours. RNA was isolated and expression of TNK1 was determined by qRT-PCR analysis. All samples were normalized against GAPDH.

SUPPLEMENTAL FIGURE S2:



SUPPLEMENTAL FIGURE S2. TNK1 knockdown does not affect phosphorylation of MEK1/2. A, BxPC3 cells were treated with non-silencing or TNK1 siRNA for a total of 96 hours. Whole cell lysates were prepared and p-MEK1/2 (Ser217/221) expression was analyzed by western blotting along with levels of total MEK1/2. B, Densitometry analysis was completed to determine the ratio of p-MEK 1/2 (Ser217/221) to total MEK 1/2.

SUPPLEMENTAL FIGURE S3:



SUPPLEMENTAL FIGURE S3. PARP cleavage in TNK1 siRNA- and TNFα-treated cells. A, BxPC3 cells were treated with non-silencing or TNK1 siRNA for a total of 96 hours. Samples were incubated with 20 ng/mL TNFα for 12 hours and whole cell lysates were analyzed for cleavage of PARP by western blotting. Protein expression of β-tubulin was completed to ensure appropriate loading of all lanes. B, Densitometry analysis graphically illustrates the increase in cleaved PARP expression. Units of measurement are expressed as the ratio of cleaved PARP to β-tubulin. UT: untreated, NS: non-silencing siRNA treated.