Supplementary information for:

Nuclear Localized FAM21 Participates in NF-κB-Dependent Gene Regulation in Pancreatic Cancer Cells

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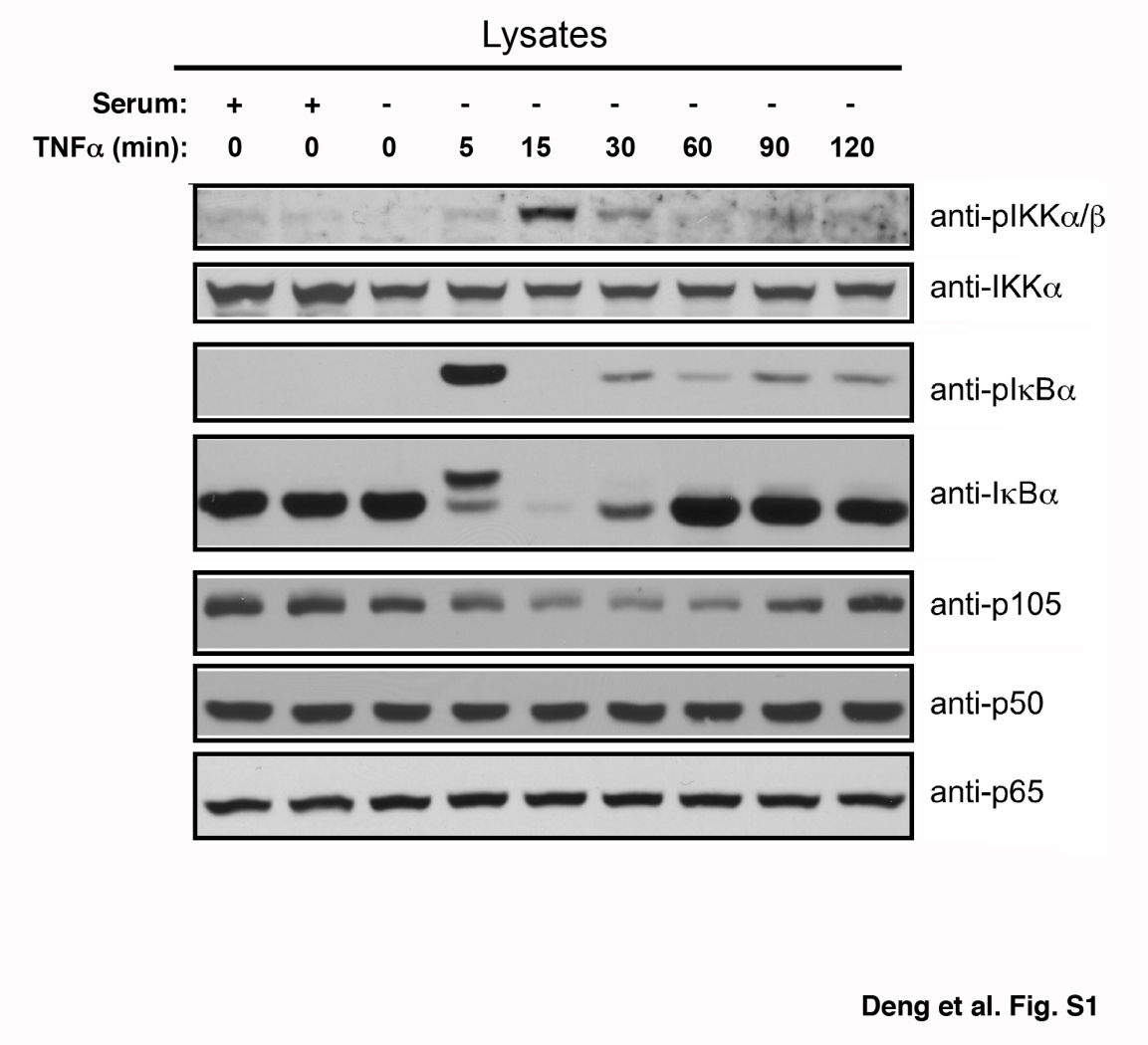


Fig.S1. Time kinetics of IKKα/β and IκBα phosphorylation and/or degradation following TNFα treatment.

Whole-cell extracts were from HeLa treated with TNFα for the indicated time points were prepared and subjected to Western blot analysis with indicated antibodies.

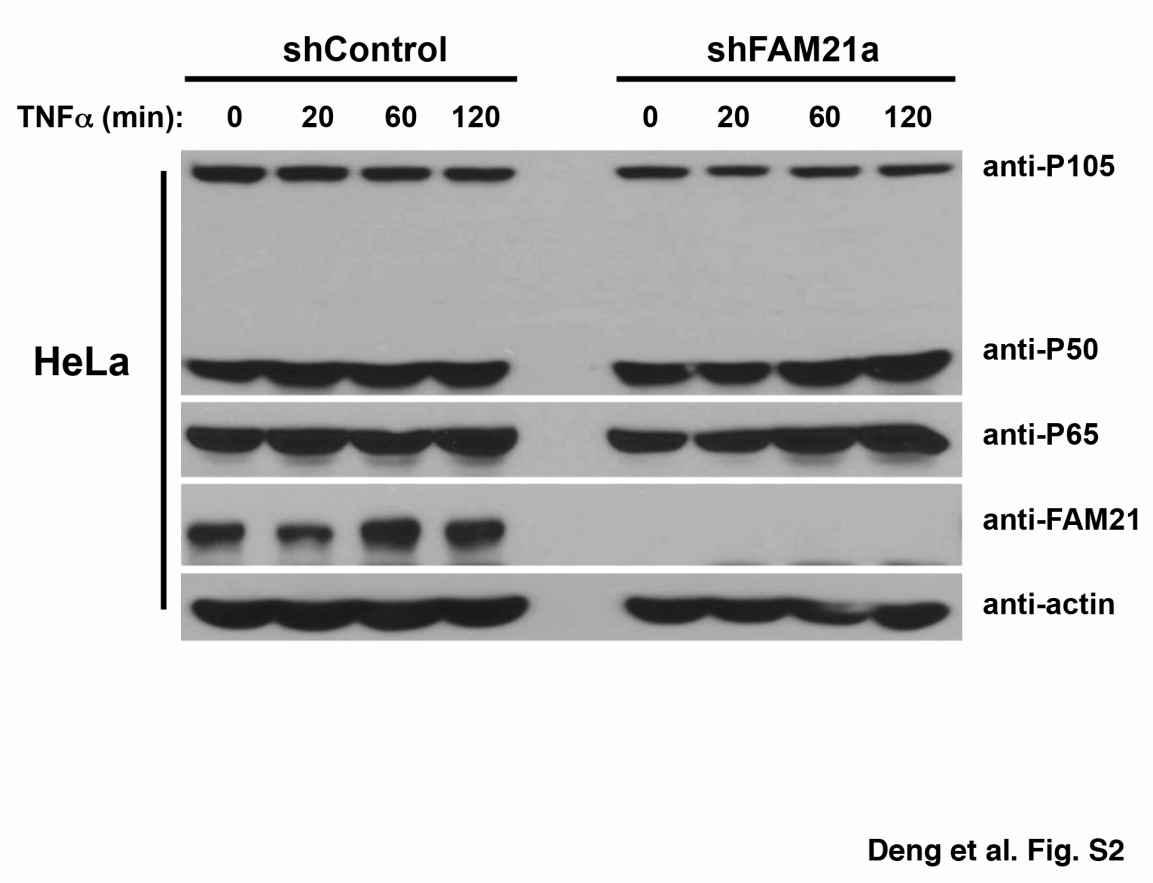
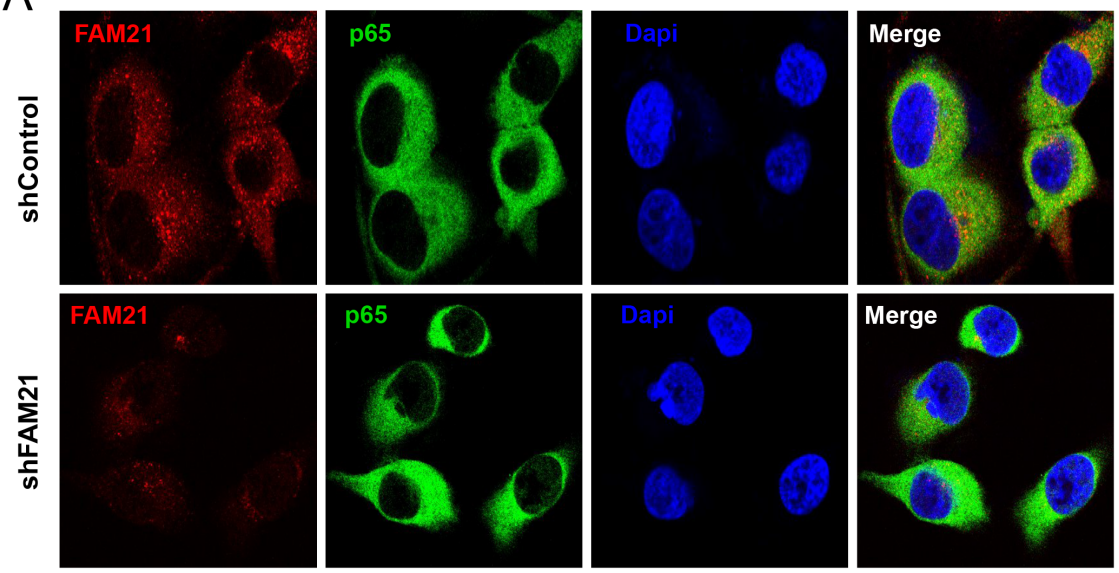


Fig. S2. Analysis of cytosolic p65, p50 protein levels in FAM21 knockdown HeLa cells.

Cytosolic extracts of control and shFAM21 stable HeLa cells treated with TNFα for indicated time points were examined by immunoblot analysis.

A



B

	Predicted bipartite NLS		
	Pos.	Sequence	Score
NLS2	507	DENKARAEKKVTLSSSKNLKPSSETKTQKG	5.7
NLS3	586	TAAKKQTLCLQAQREEKAKASELSKKKASA	6.2
NLS4	657	EAKAVKKTSLFEEDEEDDLFAIAKDSQKKTQRVS	6.5
NLS5	888	FSSAKSQPLVQEKKRVVKKDHSVDSFKNQKHP	6.5
NLS6	1191	KPAKKTNPFPLLEDEDDLFTDQKVKKNETKS	5.6
NLS7	1279	EKSKKKVEAKSIFDDDMDDIFSSGIQAKTTKPK	6.8

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Fig. S3. FAM21 and p65 Immunofluorescence and bipartite NLS. A. Immunofluoresent imaging for p65 in scramble control and shFAM21 Panc1 stable cells. B. Additional bipartite NLS sequences in the FAM21 tail domain as predicted by "cNLS Mapper".

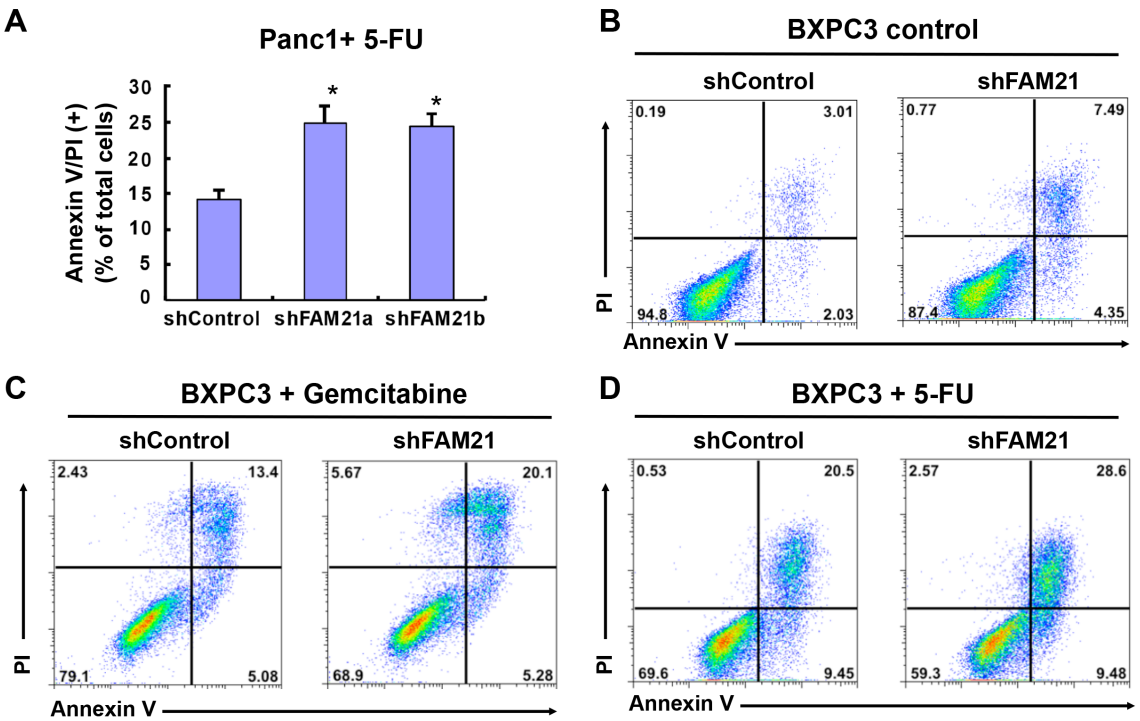

Fig.S4. Increased apoptosis in pancreatic cancer cells with FAM21 knockdown in response to drug treatment.

(A) Panc1 scramble control or shFAM21 stable cells were treated with indicated concentrations of 5-FU for 48 h. Cell apoptosis was determined by annexin V/PI staining and flow cytometry analysis. Results were quantified and shown as mean ± SD. * P-value< 0.05 compared to shControl. (B-D) BXPC3 scramble control and shFAM21 stable cells treated with solvent (B), or 0.25 μM gemcitabine (C) or 0.25 μg/ml 5-FU (D) for 48 h. Cell apoptosis was determined by flow cytometry following Annexin V/PI staining.

Table S1. Primers used for qRT-PCR

Primers	Sequence (5’ – 3’)
GAPDH-F	ACATCGCTCAGACACCATG
GAPDH-R	TGTAGTTGAGGTCAATGAAGGG
A20 -F	ATCATCCACAAAGCCCTCAT
A20- R	CCTTCCTCAGTACCAAGTCT
CCL2 -F	CCTCCAGCATGAAAGTCTCTG
CCL2 -R	TCTGCACTGAGATCTTCCTATTG
IL-1 -F	TGTATGTGACTGCCCAAGATG
IL-1 -R	TTAGTGCCGTGAGTTTCCC
IL-6 -F	CCACTCACCTCTTCAGAACG
IL-6 -R	CATCTTTGGAAGGTTCAGGTTG
SELE -F	TCCCTCTAGTTCCCCAGATG
SELE -R	AAGCCTTGAATCAGACGGAA

Table S2. Primers used for ChIP-PCR assay

Primers	Sequence (5' – 3')
SELE -F	AGGCATGGACAAAGGTGA
SELE -R	GTAAAGAGGAAATCCCCAAT
IL-1a promoter -F	GGCCTCAAGTGATTGTCCTG
IL-1a promoter -R	CAGTTGGAGTTTAAGCCATG
IL-1a intron1 -F	ATGCTGAATGTGGACTAAG
IL-1a intron1 -R	AGAACACCAGCCACCATC
IL-6 -F	AGGTTTCCAATCAGCCCC
IL-6 -R	AGCCTCAGACATCTCCAGT