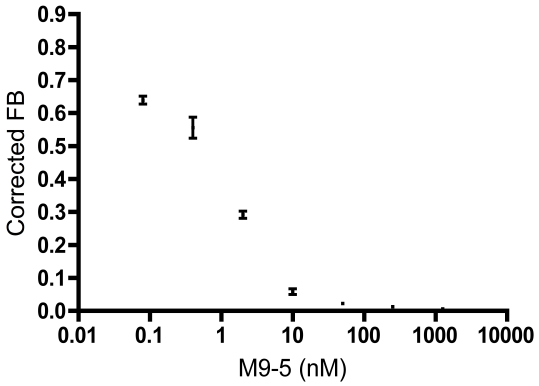
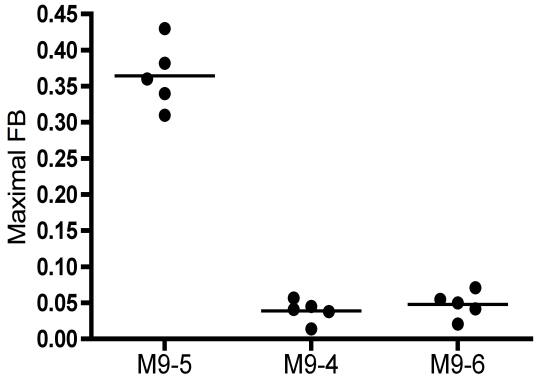
Supplementary Figure 1



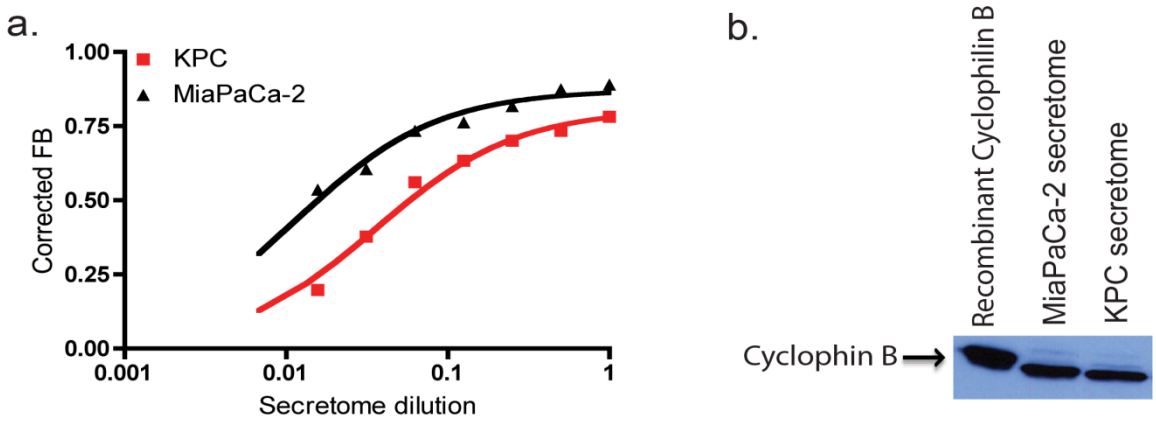
Supplementary Figure 1: Competition assay to determine K_d of the M9-5 aptamer. The MiaPaCa-2 secretome (30 µl; 3.5 ng/µl) was co-incubated with 5 µl of increasing concentrations of unlabelled M9-5 aptamer (X-axis) and 5 µl of ^{32}P-end-labeled M9-5 aptamer (5000 cpm/µl; containing trace amounts of radiolabeled-RNA). The mixtures were incubated at 37°C for 30 minutes to allow the aptamer and proteins to equilibrate, and radioactive filter-binding assays were performed as previously described. The mean and SD of two independent binding experiments are plotted.

Supplementary Figure 2



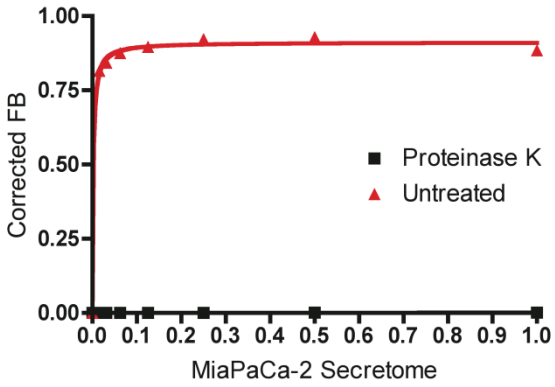
Supplementary Figure 2: M9-5 aptamer binds to the pancreatic cancer patient sera. The same set of pancreatic cancer patient sera (n=5) were analyzed for M9-4, M9-5 and M9-6 binding by using the radioactive filter-binding assay. M9-4 and M9-6 demonstrated no significant binding to the sera as compared to the M9-5 aptamer.

Supplementary Figure 3



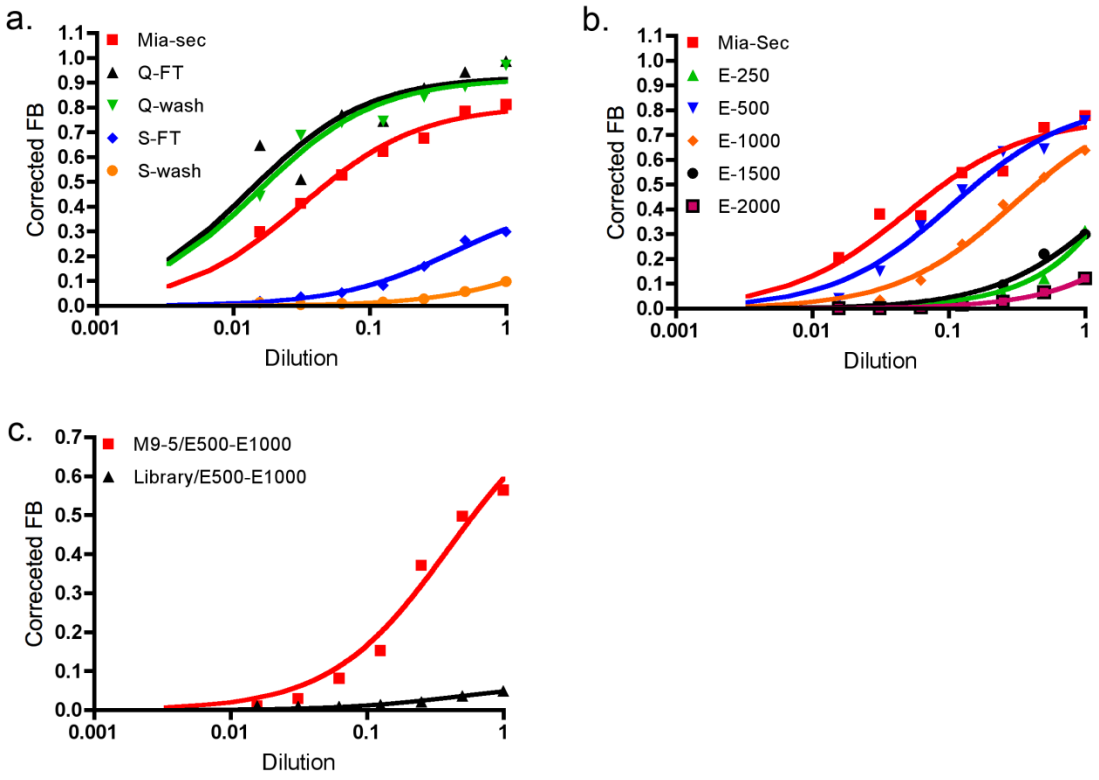
Supplementary Figure 3: M9-5 aptamer binds to KPC secretome. (a) Pancreatic tumor cells collected from the KPC mice were cultured *in vitro*(1) and the secretome was collected as described previously. M9-5 aptamer bound to the KPC secretome in a radioactive filter-binding assay with affinity comparable to the MiaPaCa-2 secretome. (b) Western blot analysis on KPC secretome could detect Cyclophilin B (CypB). MiaPaCa-2 secretome and recombinant CypB was used as positive controls.

Supplementary Figure 4



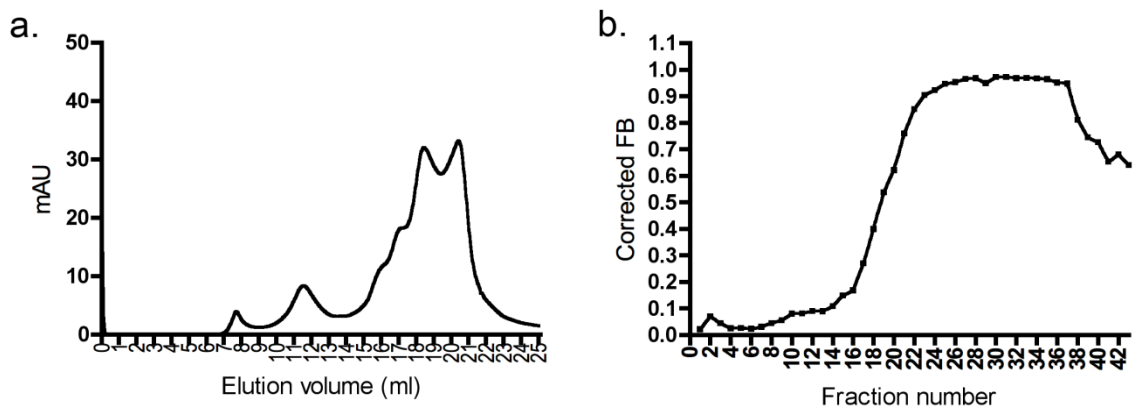
Supplementary Figure 4: The M9-5 target is protein. MiaPaCa-2 secretome (30 µl of 0.2 µg/µl) secretome was treated with 1 µl (20 µg/µl) of Proteinase-K and incubated at 37°C for 15 minutes. M9-5 radioactive filter binding assay was performed. M9-5 binding to the MiaPaCa-2 secretome was completely abolished by the Proteinase-K treatment.

Supplementary Figure 5



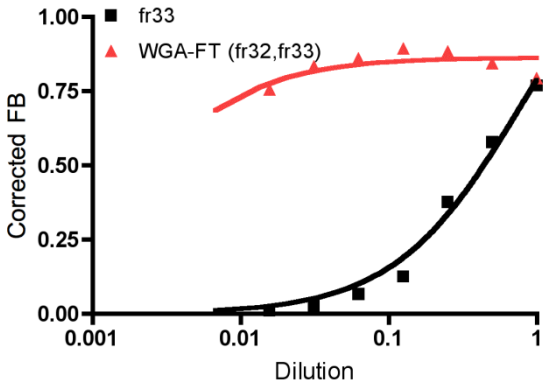
Supplementary Figure 5: The M9-5 target binds to a cation exchange column. (a) MiaPaCa-2 secretome was loaded onto either cation exchange (S) or anion exchange (Q) columns. The flow-through (FT) containing unbound protein fractions and the column washings were tested for M9-5 binding using radioactive filter binding assay. M9-5 binding activity is present in the unfractionated secretome (Mia-sec), anion exchange FT (Q-FT), and wash (Q-wash) but not the cation exchange FT (S-FT) and wash (S-wash), indicating that the M9-5 target binds to the cation exchange column. (b) The bound proteins were sequentially eluted from the cation exchange column (S) by using buffer "F" containing; 250 mM NaCl (E-250), 500 mM NaCl (E-500), 1000 mM NaCl (E-1000), 1500 mM NaCl (E-1500), and 2000 mM NaCl (E-2000). The eluted fractions were equilibrated against Buffer F and each fraction was tested for the M9-5 binding activity. Binding activity was identified in the E-500 and E-1000 fractions. (c) The E-500 and E-1000 fractions were pooled together, and binding activity was compared to the starting RNA library to confirm that binding activity was specific.

Supplementary Figure 6



Supplementary Figure 6: Fractionation of the cation exchange column eluate by molecular weight. (a) The eluate fractions with binding activity from the cation exchange column (S) (E-500 and E-1000) were pooled together and the high salt buffer was exchanged against Buffer F. The equilibrated protein fraction was loaded onto a Superose ™ 6 10/300 GL column (GE healthcare). Proteins were eluted in 24 ml of Buffer F and fractions were collected using ÄKTApurifier™ (GE healthcare) Fast protein liquid chromatography (FPLC). The elution was monitored by UV-absorption at 280 nM and is indicated as mAU (milli absorbance unit). Fraction collection was started from 6 ml of elution volume; 9 fractions (1 ml/fraction) were collected to 15 ml, and 34 fractions (0.25 ml/fraction) were collected from 15 to 24 ml. (b) Individual fractions were tested for M9-5 binding activity. High binding activity was detected in fractions 24 through 37, corresponding to an approximate molecular weight range of 15 to 30 kDa.

Supplementary Figure 7



Supplementary Figure 7: The M9-5 is target present in the WGA-agarose column flow-through. (a) Fractions 32 and 33 from the gel filtration column were pooled together and loaded onto the WGA-lectin column. High binding activity was present in the WGA lectin column flow-through, suggesting that the M9-5 target does not possess the N-acetyl glucosamine (GlcNAC) and terminal GlcNAC moieties that typically bind this lectin. The input (fraction 33) was used as the binding control.

Supplementary Table 1: Pancreatic cancer patient data.

Clinical characteristic		Mean M9-5 FB[2] ± SD
Age	< 65 (n=15)	0.27 ± 0.13
	≥ 65 (n=9)	0.26 ± 0.08
Gender	Male (n=10)	0.24 ± 0.09
	Female (n=14)	0.30 ± 0.12
Tumor location	Head (n=18)	0.28 ± 0.10
	Tail (n=6)	0.24 ± 0.14
Clinical stage[1]	IA-IIA (n=4)	0.30 ± 0.12
	IIB (n=12)	0.24 ± 0.11
	III (n=8)	0.29 ± 0.12
CA19-9	< 40 U/ml (n=10)	0.24 ± 0.07
	≥ 40 U/ml (n=14)	0.29 ± 0.13

[1]Stage determined by radiographic and pathologic data, when available. Not all patients underwent surgical exploration.
[2]FB = fraction bound of M9-5 to undiluted serum

Supplementary Table 2: Mass spectrometric analysis

Protein Name	Accession Number	Predicted MW[1]	Predicted Protein pI[2]	WGA 1D Gel Band	Unique Peptides Identified[3] Fr 27	Fr 30	Fr 34	Fr 37
Peptidyl-prolyl cis-trans isomerase B (Cyclophilin B)	**PPIB_Human**	**24 kDa**	**9.25**	**10**	**4**	**7**	**16**	**6**
Metalloproteinase inhibitor 2	TIMP2_Human	24 kDa	6.48	8	-	-	3	-
Metalloproteinase inhibitor 1	TIMP1_Human	23 kDa	8.47	4	7	8	5	-
Non-histone chromosomal protein HMG-14	HMGN1_Human	11 kDa	9.61	2	-	-	-	-
Heterogeneous nuclear ribonucleoprotein G	HNRPG_Human	42 kDa	10.06	2	-	2	-	-

[1] Predicted molecular weight of the non-processed protein within SwissProt database.
[2] Protein pI predicted from http://ca.expasy.org/tools/pi_tool.html
[3] Unique peptides identified mascot database searches of LC-MS/MS data at a calculated false positive rate of 0.0%

Raw data are available for download at www.proteomecommons.org (M9-5 aptamer)

Supplementary Methods

SELEX Protocol: The sequence of the DNA template oligonucleotide, 5′ primer and the 3′ primer are 5′-GGGAGGACGATGCGG-N_{40}-CAGACGACTCGCCCGA-3′ (N_{40} represents a 40-nucleotide random region with equimolar quantifies of A, T, C, and G), 5′-GGGGGAATTCTAATACGACTCACTATAGGGAGGACGATGCGG-3′ and 5′-TCGGGCGAGTCGTCTG-3′, respectively. DNA oligonucleotides were synthesized by Oligos, Etc. We used SELEX protocols identical to those described by Layzer et al.(2). Briefly, the double stranded DNA template was prepared by annealing the DNA template oligonucleotide to the 5′ primer then filling in the template with Exo-Klenow (NEB). The starting RNA library was generated by *in vitro* transcription using natural purines and 2′ -fluoro-modified pyrimidines (TriLink Biotechnologies) and a modified T7 RNA polymerase(3) then gel-purified. Following each round of selection, protein-RNA complexes were extracted from the nitrocellulose membranes. The RNA was reverse transcribed using the 3′ primer then amplified with the 5′ and 3′ primers using standard PCR conditions. The PCR product was then used as the template for *in vitro* transcription of the next round of RNA. At the end of the selection, the PCR products of the desired rounds were digested with *Eco*RI and *Bam*HI (NEB) and directionally cloned into linearized pUC19 for sequencing and generation of clonal RNA transcripts.

Mass spectrometric analysis of M9-5 target: 1D gel bands were subjected to in-gel tryptic digestion as previously described(4). Extracted peptides were brought to dryness using vacuum centrifugation and resuspended in 20 µl 2% acetonitrile, 0.1% formic acid (FA). In-solution FPLC fractions were desalted/buffer-exchanged into 50 mM ammonium bicarbonate, pH 8.0 using a Zeba Spin 7K MWCO gel-filtration column (Sigma) and were normalized to 0.1 ug/ul following a Bradford total protein concentration assay was performed (Bio-Rad). Solution samples were reduced for 20 min at 70°C with 5

mM dithiolthreitol and alkylated for 45 min at RT with 10 mM iodoacetamide. Sequencing grade modified trypsin (Promega) was then added at a 50:1 protein-to-enzyme ratio and allowed to proceed for 18 hr at 37C. 10 µl of each peptide mixture were separated on a Waters NanoAcquity UPLC equipped with a 1.7 um BEH130 75 um X 25 cm reversed-phase column using a linear gradient from 5% acetonitrile, 0.1% FA to 40% acetonitrile, 0.1% FA over 30 minutes at 400 nL/min. Eluting peptides were analyzed following electrospray ionization by a Waters Synapt HDMS G1 mass spectrometer (in-gel sample) or a Waters Synapt HDMS G2 mass spectrometer (in-solution sample) operating in a data-dependent mode of acquisition. Mass spectra were processed with Mascot Distiller (Matrix Science) and were then submitted to Mascot searches (Matrix Science) against a SwissProt_Human database with either 20 ppm precursor or 10 ppm precursor and 0.04 Da product ion tolerances for Synapt G1 or Synapt G2 data, respectively. Searched spectra were imported into Scaffold v3.0 (Proteome Software) and scoring thresholds were set to yield a minimum of 99% protein confidence (implemented by the PeptideProphet algorithm) based on decoy database searches(5). A minimum of two unique peptides from each protein was required for identification.

References:

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3. Knudsen, S.M., Robertson, M.P., and Ellington, A.D. 2002. In vitro selection using modified or unnatural nucleotides. *Curr Protoc Nucleic Acid Chem* Chapter 9:Unit 9 6.
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5. Searle, B.C. 2010. Scaffold: a bioinformatic tool for validating MS/MS-based proteomic studies. *Proteomics* 10:1265-1269.