Supplementary Data

Figure S5

A

PANC-1

Relative gene expression (normalized to GAPDH)

B

PANC-1

Cell Number (x 10^6/3 days)

C

PANC-1

Relative mRNA level (normalized to GAPDH)

D

CyPA

Dose (nM)

E

PANC-1/

100µg/ml CHX

Time (h)

F

MIA PaCa-2

Cell number x 10^6

PANC-1

Cell number x 10^6
Figure S5. CyPA promotes HAb18G/CD147-CD44s-pSTAT3 signaling and correlated pancreatic cell growth.

(A) Effect of exogenous human recombinant CyPA on mRNA levels of MMP1, MMP2, MMP9 in PANC-1 cells. Serum-starved PANC-1 cells were treated with CyPA for 24 hours at the concentrations of 0, 1, 10 and 100 nmol/L. Treatment with phosphate-buffered saline for 24 hours was used as a negative control.

(B) Effect of exogenous human recombinant CyPA on the cell growth of PANC-1 cells. Serum-starved PANC-1 cells were treated with 10 nmol/L CyPA for 72 hours. Treatment with phosphate-buffered saline for 72 hours was used as a negative control.

(C) Effect of exogenous human recombinant CyPA on mRNA levels of HAb18G/CD147 and CD44s (left panel), STAT3, cyclinD1 and survivin (right panel) in PANC-1 cells. CyPA treatment was as indicated in A).

(D) HAb18G/CD147, CD44s, pSTAT3, tSTAT3, cyclinD1 and survivin protein levels in serum-starved PANC-1 cells treated with CyPA for different time points (right) at different concentrations (left). Treatment with phosphate-buffered saline was used as a control. α-tubulin was included as a loading control.

(E) CD147 and EGFR protein expression in PANC-1 cells under 100 µg/ml CHX or/and 100 nmol/L CyPA treatment at different time point. Left: The determination of CD147 protein half-life (2 hour) with 100µg/ml CHX incubation for 0-8 hours. EGFR was included as control protein. Right: CHX treatment abolished the CyPA promoted-CD147 protein level enhancement.

(F) Cell growth assay in MIA PaCa-2 and PANC-1 cells treated with or without STAT3 inhibitor WP1066 (5µmol/L) and/or CyPA (100 nmol/L).
Figure S6

A

Relative gene expression (normalized to GAPDH)

PANC-1  MIA PaCa-2  HEK293

CD147  CD44

NTC  A6

CD147  CD44

NTC  A6

CD147  CD44

NTC  A6

NTC  A6

B

CyPA

HAb18G/CD147

CD44s

β-actin

PANC-1  MIA PaCa-2  HEK293

A6  A6

A6  A6

CD14  EGFP

C

HAb18G/CD147  CD44s

DAPI

Merge

D

HAb18G/CD147-CD44s correlation

Spearman $r = 0.4961$

$P < 0.001$

95% CI: 0.368-0.606

E

HAb18G/CD147  CD44s

a)

b)
Figure S6. HAb18G/CD147 and CD44s are co-expressed and associated in pancreatic cancer.

(A) CD44s mRNA expression in PANC-1, MIApCa-2 and HEK293 cells before and after knock-down or knock-in of HAb18G/CD147. Serum-starved cells were treated with or without 100 nmol/L CyPA for 30 min.

(B) CD44s protein levels in PANC-1, MIApCa-2 and HEK293 cells with or without CyPA stimulation.

(C) Co-immunolabeling of HAb18G/CD147 (green) and CD44s (red) in PNAC-1 cells. Magnification: 400X. Arrows indicated co-localization and co-expression of HAb18G/CD147 and CD44s in the cell membranes.

(D) Correlation between HAb18G/CD147 and CD44s expression in pancreatic cancer patients.

(E) Representative morphology of HAb18G/CD147 and CD44s immunostaining in pancreatic cancer tissues.