

Article

Deletion of NEMO Inhibits EMT and Reduces Metastasis in KPC Mice

Miltiadis Tsemmelis ¹, Kanishka Tiwary ², Katja Steiger ³, Nadine Sperb ¹, Melanie Gerstenlauer ¹, Uta Manfras ¹, Harald J. Maier ^{1,4}, Patrick C. Hermann ², Lap Kwan Chan ^{1,5,6,*} and Thomas Wirth ^{1,*}

Supplementary Materials

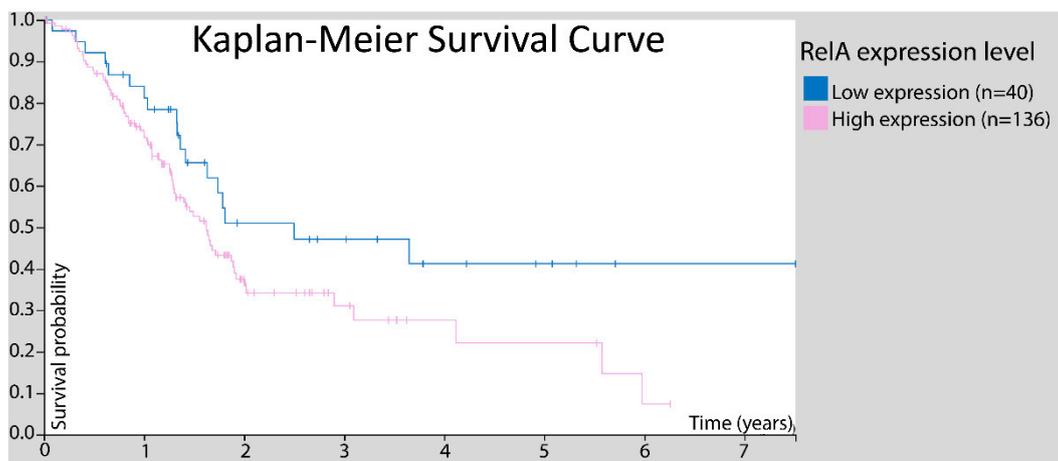
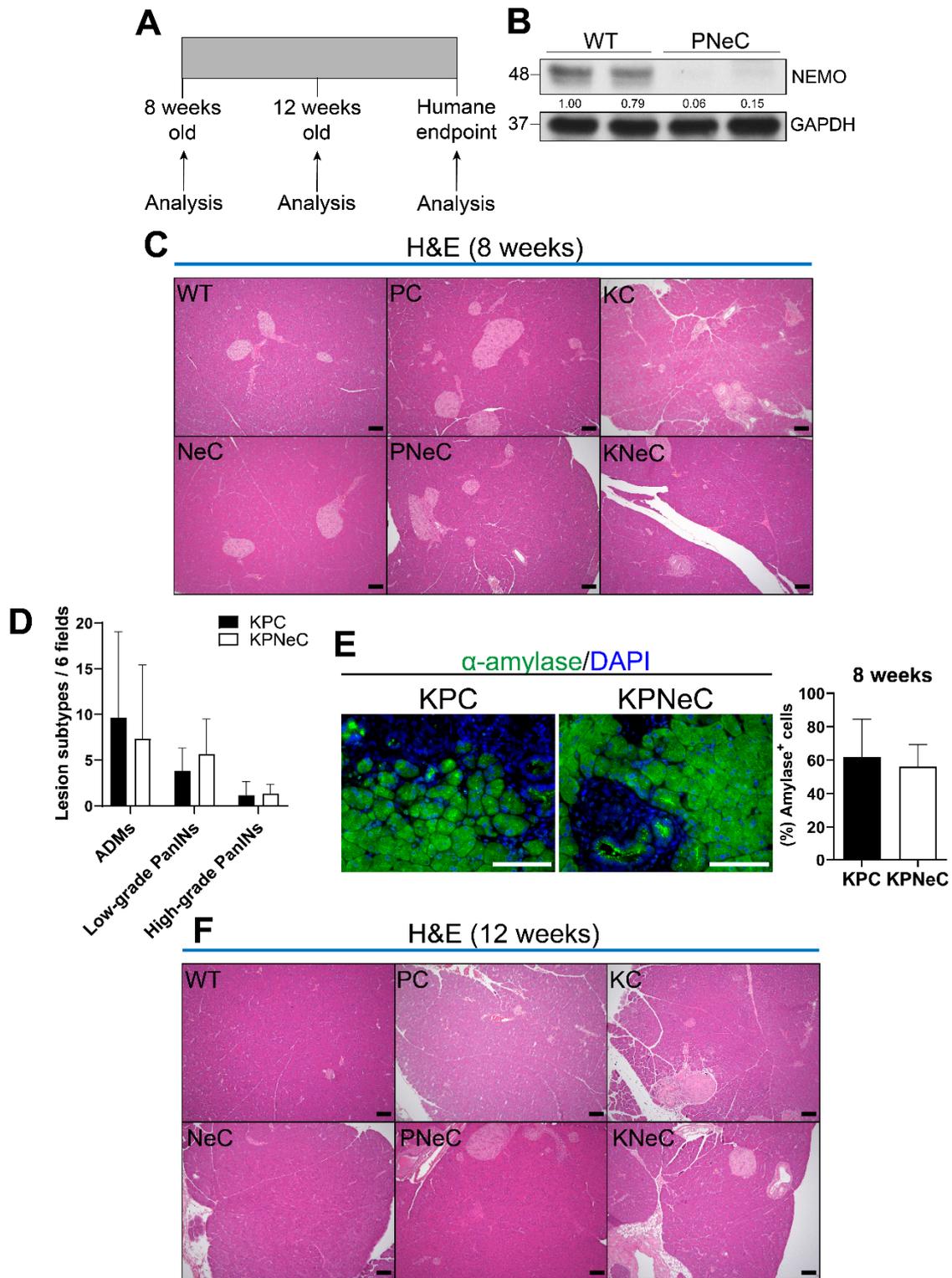


Figure S1. Kaplan-Meier survival analysis for low expression (blue line) and high expression (pink line) of RelA (p65) in PDAC patients. The figure is derived and modified from the human protein atlas (HPA) website (v20.proteinatlas.org/ENSG00000173039-RELA/pathology/pancreatic+cancer).



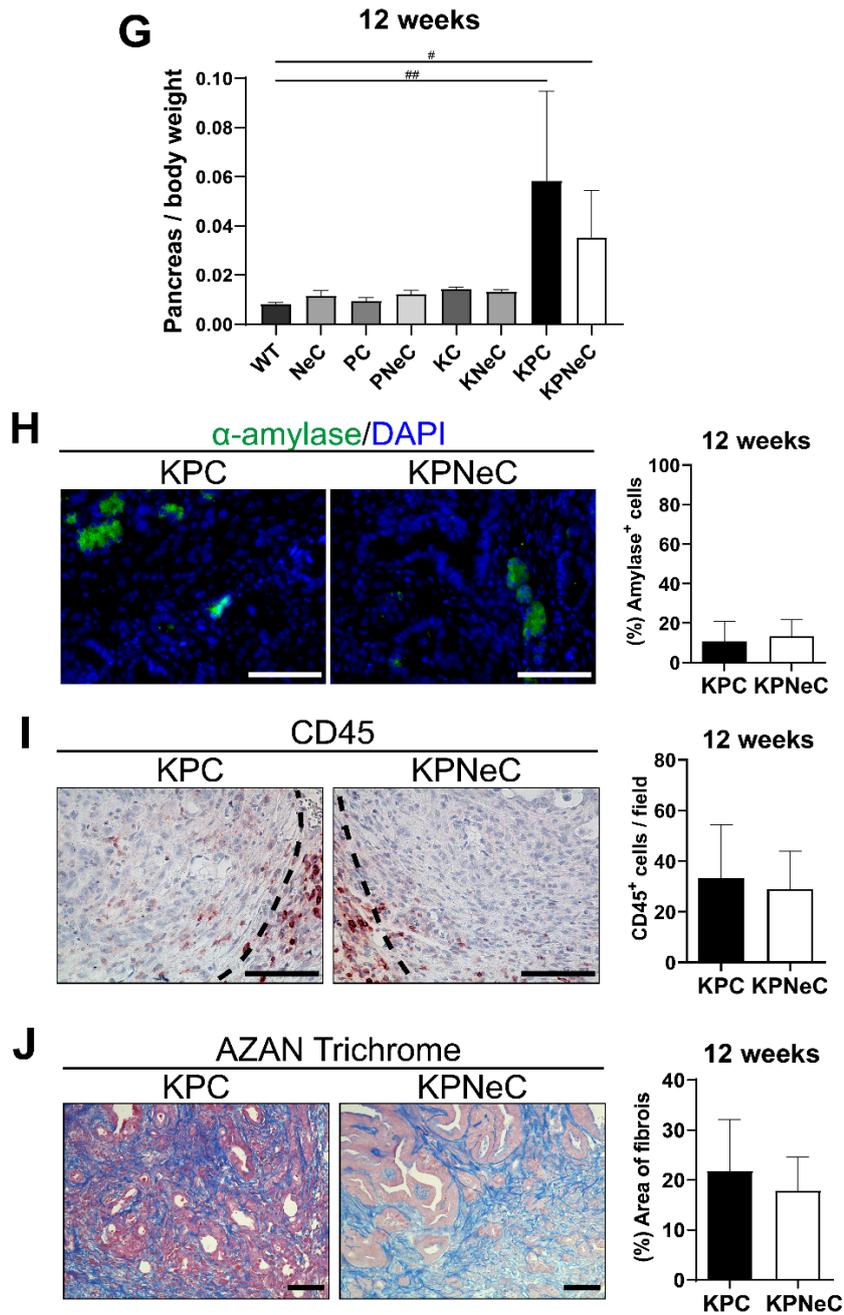


Figure S2. Analysis of pancreata of 8-week-old and 12-week-old mice. (A) Schedule of analysis of the different mouse models at the time point of 8 weeks, 12 weeks or at their humane endpoint (HEP). (B) Western blot analysis of pancreatic protein extracts from wild-type (WT) and Pdx1-Cre;p53^{fl/fl};NEMO^{fl/fl} (PNeC) mice. GAPDH was used as a loading control. (C) H&E staining on pancreatic sections of 8-week-old WT, Pdx1-Cre;NEMO^{fl/fl} (NeC), Pdx1-Cre;p53^{fl/fl} (PC), PNeC, Pdx1-Cre;KRAS^{G12D} (KC) and Pdx1-Cre;KRAS^{G12D};NEMO^{fl/fl} (KNeC) mice. (D) Quantification of acinar-ductal metaplasias (ADMs), low-grade and high-grade PanINs on pancreatic sections of 8-week-old KPC and KPNeC mice (N=6 mice/group). (E) Left: Visualization of α -amylase⁺ cells on pancreatic sections of 8-week-old KPC and KPNeC mice. Nuclear staining with DAPI, scale bar: 100 μ m. Right: Percentage of field covered by α -amylase⁺ cells to the field covered by the total number of cells (N=6 mice/group). (F) H&E staining on pancreatic sections of 12-week-old WT, NeC, PC, PNeC, KC and KNeC mice. (G) Quantification of pancreatic weight to body weight ratio for the indicated groups (KPC and KPNeC groups: N=8 mice/group, rest of the groups: N \geq 3 mice/group; one-way analysis of variance with Dunn's multiple comparison test: #p<0.05, ##p<0.01). (H) Left: Visualization of α -amylase⁺ cells on pancreatic sections of 12-week-old KPC and KPNeC mice. Nuclear staining with DAPI, scale bar: 100 μ m. Right: Percentage of field covered by α -amylase⁺ cells to the field covered by the total number of cells (N=6 mice/group). (I) Left: Visualization of CD45⁺ cells on pancreatic sections of 12-week-old KPC and KPNeC mice. Scale bar: 100 μ m. Dashed line: Separation of immune cells from rest of the tumor, scale bar: 100 μ m. Right: Quantification of CD45⁺ cells on pancreatic sections (N=6 mice/group). (J) Left: Visualization of fibrosis by Heidenhain's azocarmine aniline blue stain (AZAN) staining on pancreatic sections of KPC and KPNeC mice, scale bar: 100 μ m. Right: Quantification of fibrotic area (N \geq 4 mice/group).

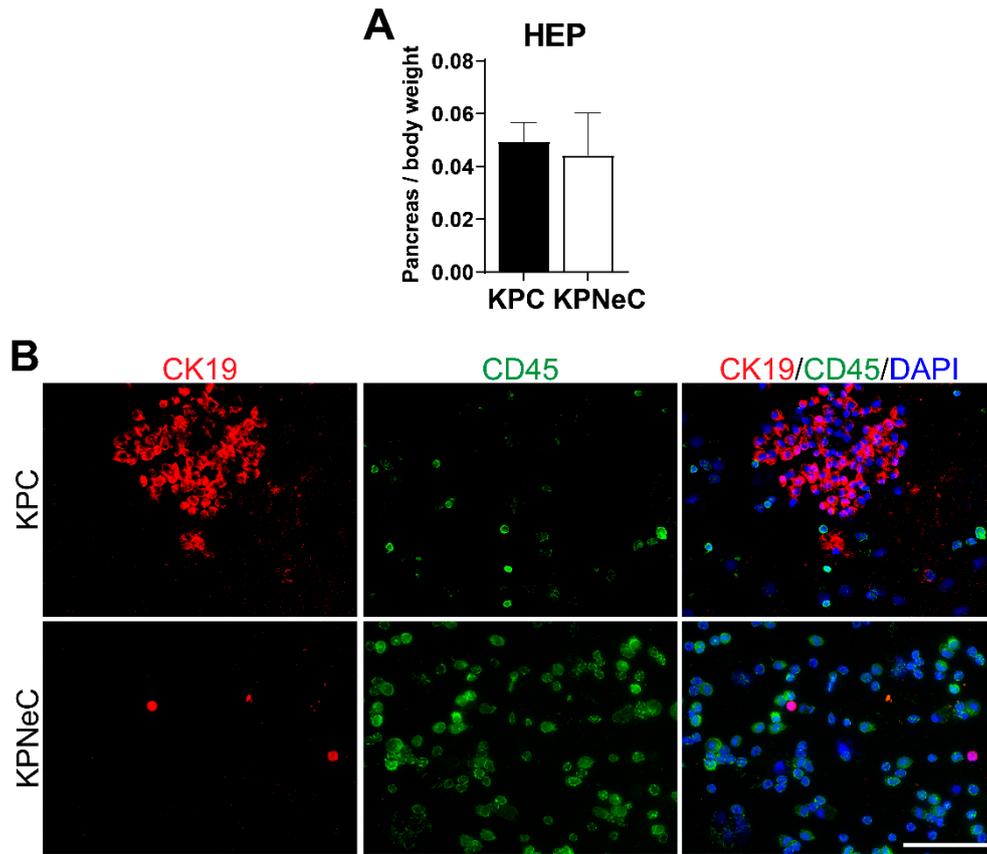


Figure S3. Analysis of KPC and KPNeC mice at their HEP. (A) Quantification of pancreatic weight to body weight ratio of KPC and KPNeC mice at their HEP. (N=9 mice/group). (B) Visualization of CK19⁺ and CD45⁺ ascitic cells isolated from HEP-analyzed KPC and KPNeC mice. Nuclear staining with DAPI, scale bar: 100 μ m.

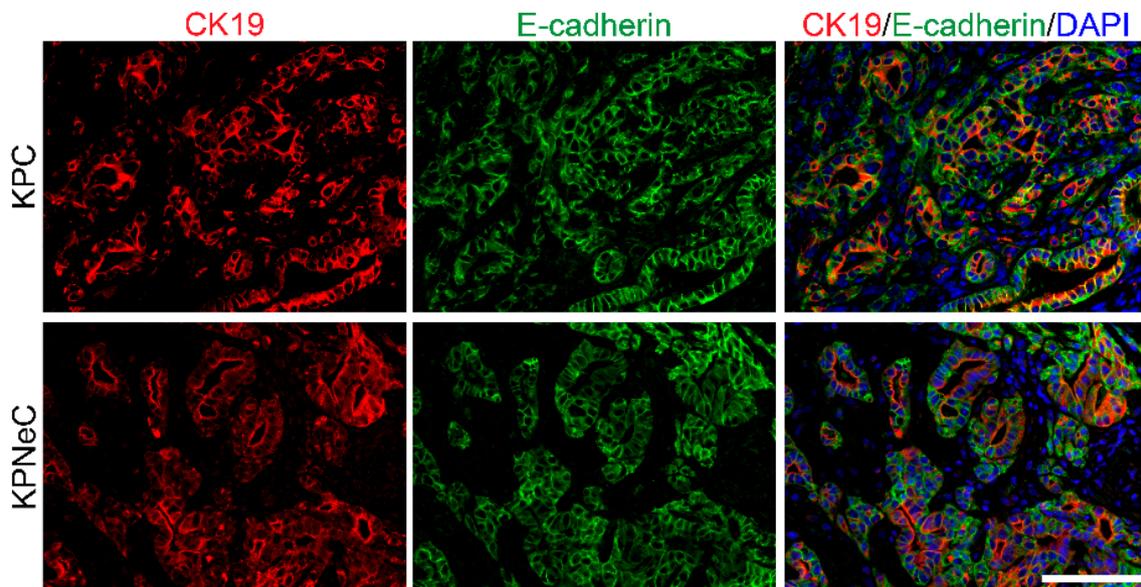


Figure S4. Visualization of CK19⁺ and E-cadherin⁺ cells on pancreatic sections of 12-week-old KPC and KPNeC mice. Nuclear staining with DAPI, scale bar: 100 μ m.

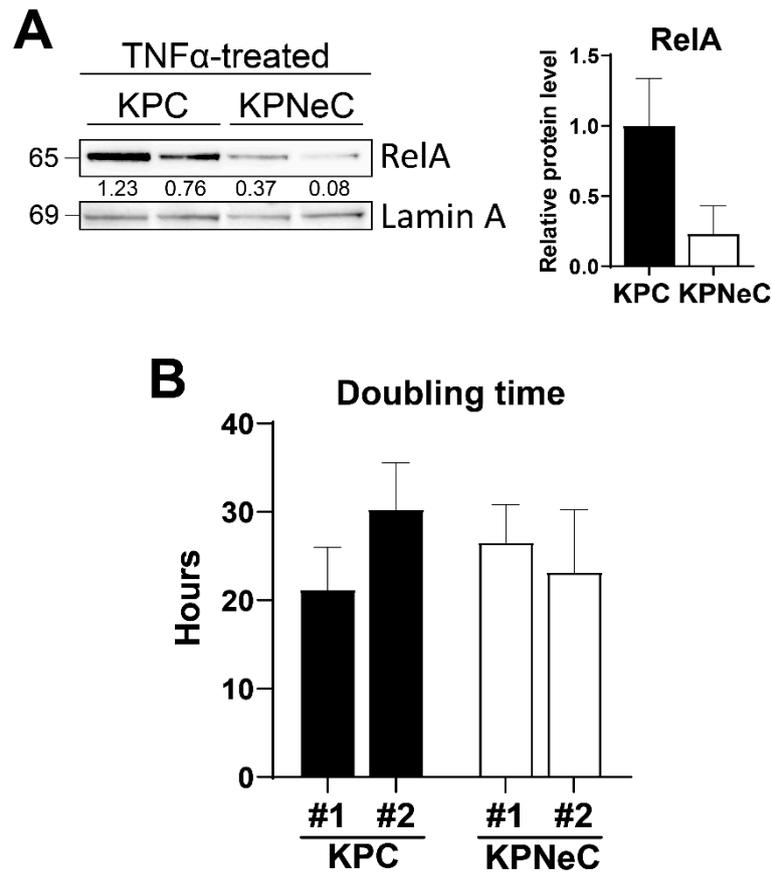


Figure S5. NEMO deletion inhibits NF- κ B signaling in KPC primary cancer cells. (A) Left: Western blot analysis of nuclear protein extracts isolated from TNF α -treated KPC and KPNeC primary cancer cells. Lamin A was used as a loading control. Right: Quantification of the western blot analysis. The diagram shows the quantification of the RelA/Lamin A given relative to KPC cells, which were set to 1 (N=2/group). (B) Average time required for the doubling/proliferation of cancer cells derived from 2 different KPC and 2 different KPNeC mice.

Table S1. Primers and primary antibodies used in the study.

Gene	Forward primer sequence	Reverse primer sequence
Cdh1	agtgtttgctcggcgtct	gcaaagccatgaggagacc
Cdh2	aaccatctcaggacagc	ccactgcatgtgctctcaag
Snai1	cttgtgtctgcacgacctgt	aggagaatggcttctacca
Snai2	cattgccttgctgcaag	cagtgagggcaagagaaagg
Timp1	gcaaagagcttctcaagacc	agggatagataaacagggaaacact
Twist1	gtcagctacgccttctcc	tccttctctggaacaatgaca
Vim	gtaccggagacaggtgcagt	ttcttccatctcacgcatc
Zeb1	aggtgatccagcacaacg	ggtggcgtggagtcagag
Zeb2	gacagtgcgtggaggag	gatgcagggggctgattat

Western Blot: Primary antibodies				
Antibody	Company	Catalog number	Dilution	Host Species
E-cadherin	Cell Signaling	3195S	1:1000	rabbit
GAPDH	Santa Cruz	sc-25778	1:1000	rabbit
Lamin A/C	Santa Cruz	sc-6215	1:1000	goat
NEMO/IKK γ	BD Biosciences	611306	1:1000	mouse
N-cadherin	Abcam	ab76011	1:1000	rabbit
RelA (p65)	Santa Cruz	sc-372	1:1000	rabbit
Slug	Cell Signaling	9585S	1:1000	rabbit

ZEB1	Santa Cruz	sc-25338	1:1000	rabbit
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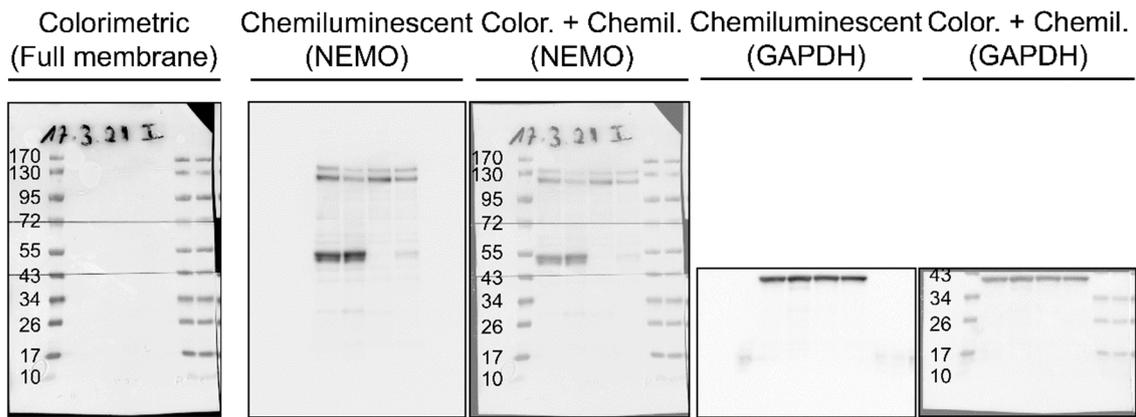
Immunofluorescence: Primary antibodies

Antibody	Company	Catalog number	Dilution	Host Species
α -amylase	MilliporeSigma	3195S	1:400	rabbit
CD45	BD Biosciences	550539	1:100	rat
CK19	MilliporeSigma	sc-25778	1:150	rabbit
CK19	Santa Cruz	sc-33111	1:100	goat
Ki67	ThermoFischer	611306	1:100	mouse
Vimentin	Abcam	ab7783	1:100	rabbit

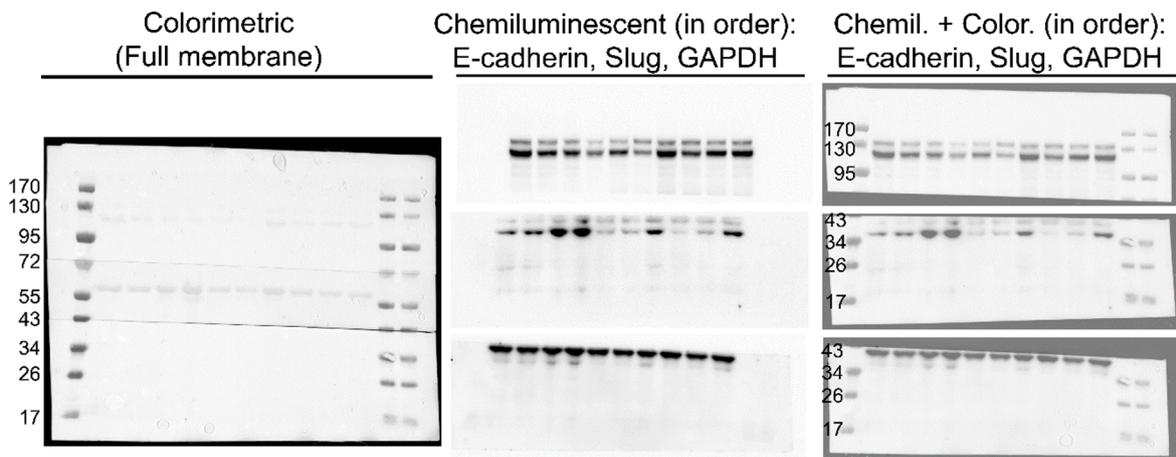
Immunohistochemistry: Primary antibody

Antibody	Company	Catalog number	Dilution	Host Species
CD45	BD Biosciences	550539	1:100	rat
Snail+Slug	Abcam	ab85936	1:100	rabbit

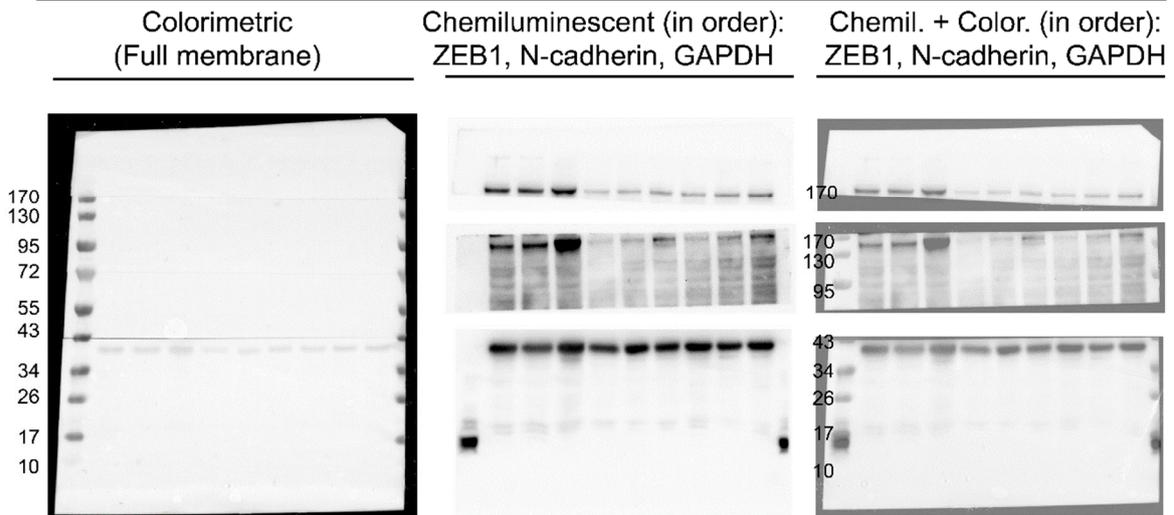
Membrane for NEMO



Membrane for E-cadherin, Slug

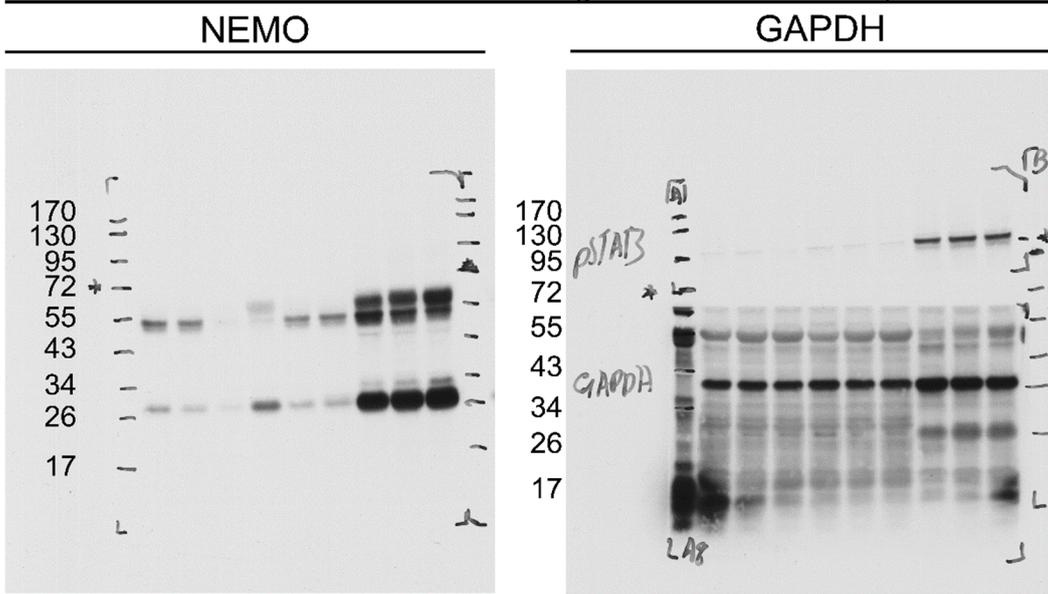


Membrane for ZEB1, N-cadherin



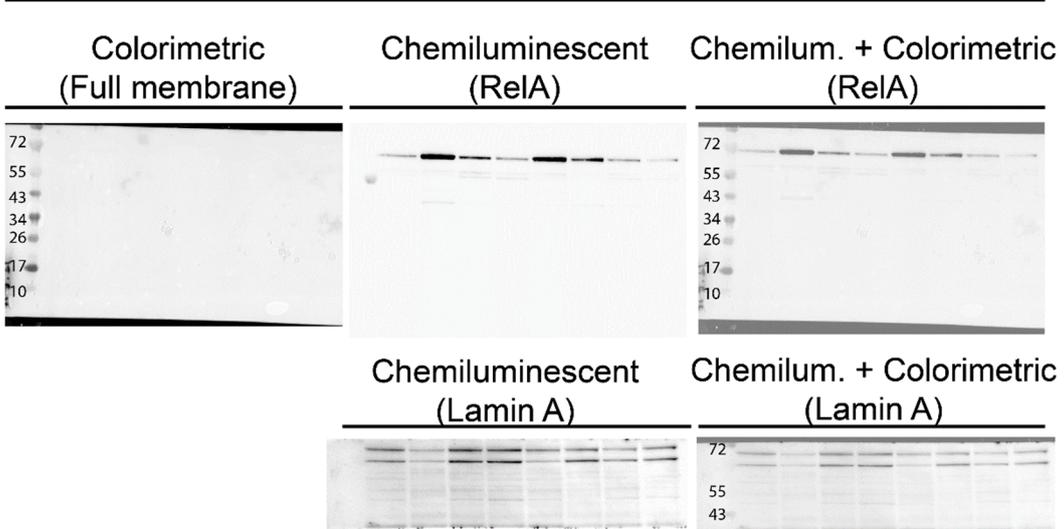
Membranes for western blot analysis depicted on Figure 4

Membrane for NEMO (pancreatic tissue)



Membrane for western blot analysis depicted on Figure S2

Membrane for RelA



Membrane for western blot analysis depicted on Figure S5