Supplementary Material for:

Combined targeting of TGF-beta, EGFR and HER2 suppresses lymphangiogenesis and metastasis in a pancreatic cancer model

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List of materials:

Supplementary Figure Legends

Supplementary Fig. 1. Lymphangiogenic genes are up-regulated in KRC and KIC tumors. **(A-B)** qPCR for the indicated mRNAs shows that LEC markers (A, *Lyve-1* and *Pdpn*) and lymphangiogenic genes (B, *Nrp1*, *Vegfc*, *Vegfd*, *Vegfr3*) are significantly increased in KRC (open bars) and KIC tumors (hatched bars) compared with control (C) pancreata (closed bars). Data are mean ± SEM from 5 mice per group. *, $P<0.05$; **, $P<0.01$.

Supplementary Fig. 2. TGF-β enhances lymphangiogenic gene expression. **(A)** qPCR shows that *Pdgfa*, *Vegfc* and *Vegfd* mRNA levels are significantly increased in KRC cells (open bars) compared with KC cells (closed bars), whereas none of these mRNAs are up-regulated in KSC cells (gray bars). **(B)** LYVE-1-positive LECs are not detectable in the stroma surrounding PanIN (left) or PCCs (middle) in KSC pancreata, and quantification (right) shows the marked decrease in LYVE-1 intensity compared with normal (C) pancreata. Scale bars, 50 μm. **(C)** qPCR shows that TGF-β1 (open bars, [0.5 nM]) does not up-regulate *Pdgfa*, *Vegfc* or *Vegfd* in KSC cells (left), whereas all three mRNAs are induced by TGF-β1 in KRC cells (right). Data in (A, C) are mean ± SEM of three independent experiments using two cell lines per GEMM. *, $P<0.05$; **, $P<0.01$.

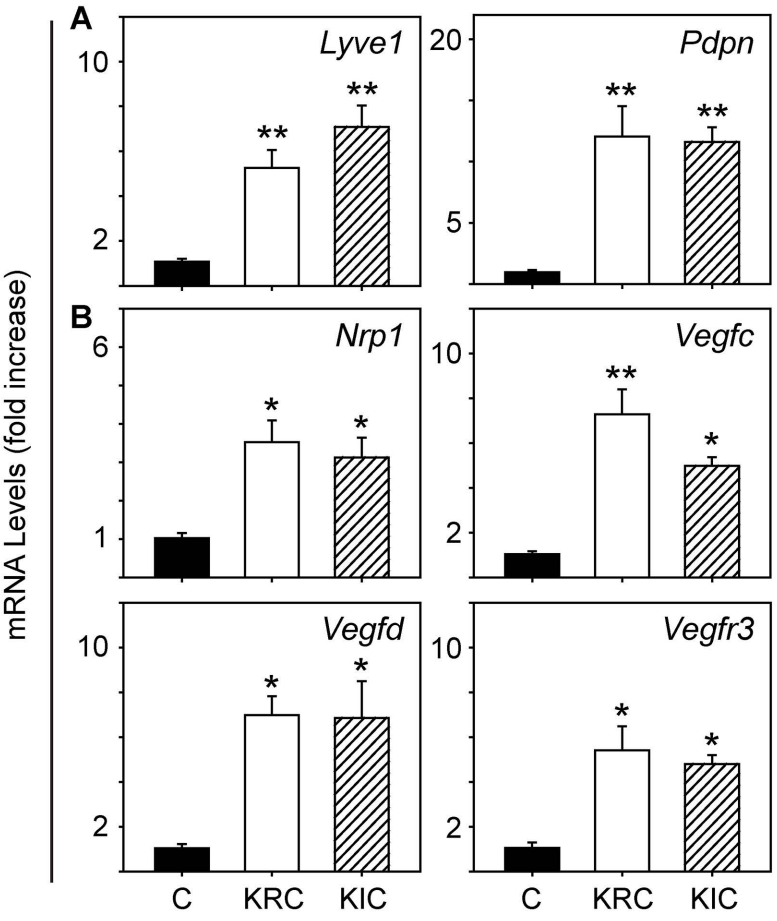
Supplementary Fig. 3. Lymphangiogenic factors are elevated in human pancreatic cancer cells. A heatmap shows the expression levels of the indicated mRNAs in *KRAS*-mutated human pancreatic cell lines. Normalized robust multi-array average (RMA) values from the Cancer Cell Line Encyclopedia were used to generate the heatmap. Red = upregulated; blue = down-regulated.

Supplementary Table 1: Lymphangiogenic factors that are up-regulated in KRC cells.

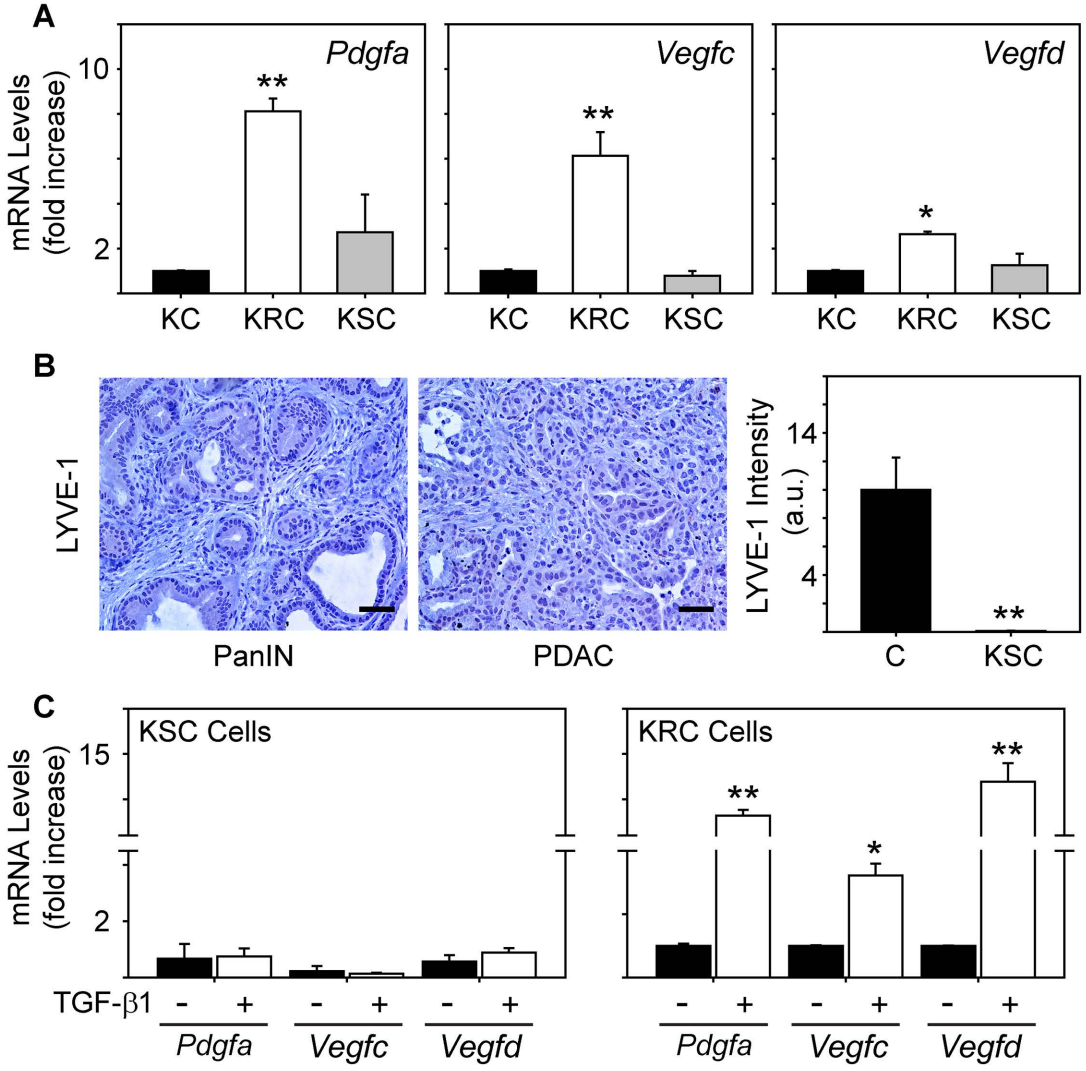
Number	Symbol	Fold Change	*P*-value	FDR
1	*Ccbe1*	30.36	7.67E-07	7.57E-05
2	*Cxcl12*	10.99	1.47E-07	4.61E-05
3	*Pdgfa*	4.64	2.80E-06	1.25E-04
4	*Vegfc*	4.54	1.58E-06	9.57E-05
5	*Pdgfb*	3.92	3.96E-05	5.22E-04
6	*Edn1*	3.08	1.34E-04	1.15E-03

Genes are ranked by fold change (FC), *P*-value and false discovery rate (FDR). A FC>1.5, *P*<0.01 and FDR<0.05 was considered statistically significant.

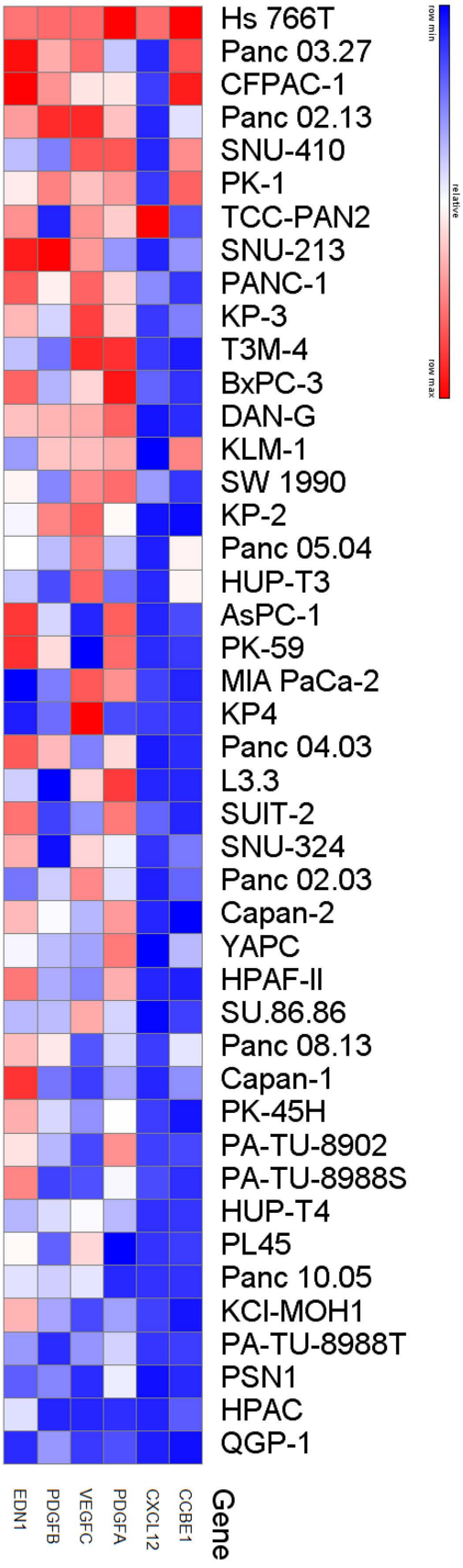
Supplementary Fig. 1



Supplementary Fig. 2



Supplementary Fig. 3



Supplementary Table 2: Lymph node involvement in angiogenic human PDACs.

	Angiogenic PDACs	**Non-angiogenic PDACs**
Number of patients	37	37
Lymph Node positivity	83.8% (31/37)	73.0% (27/37)
Lymph Node Ratio	*0.26*	0.19

Analysis of TCGA clinical data revealed that 37 patients in each angiogenic PDAC group had ≥12 lymph nodes examined for the presence of cancer cells. 31 patients in the angiogenic group had ≥1 positive node with an overall positive node ratio of 0.26, whereas only 27 patients in the non-angiogenic group had ≥1 positive and in this group, the node ratio was lower.