

FIGURE 1

A

AXL EXPRESSION	
NEGATIVE	POSITIVE
24% (10/42)	76% (32/42)
	WEAK MEDIUM/STRONG
	59% (19/32) 41% (13/32)
	INVASIVE CELLS
	47% (9/19) 92% (12/13)

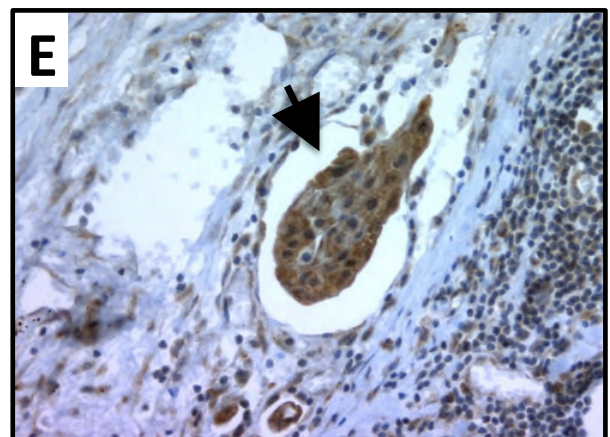
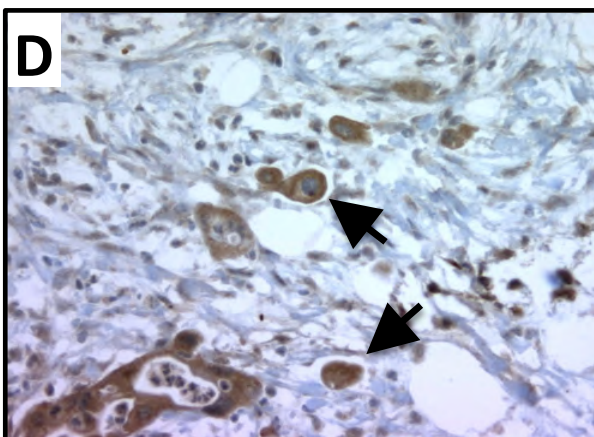
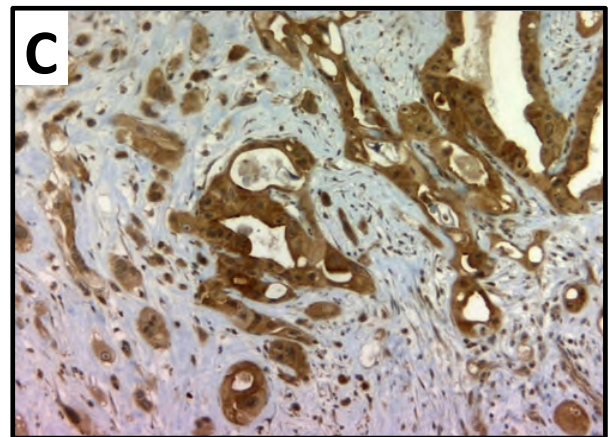
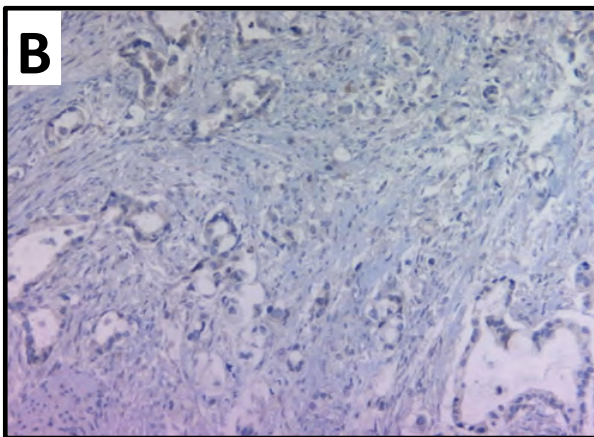


FIGURE 2

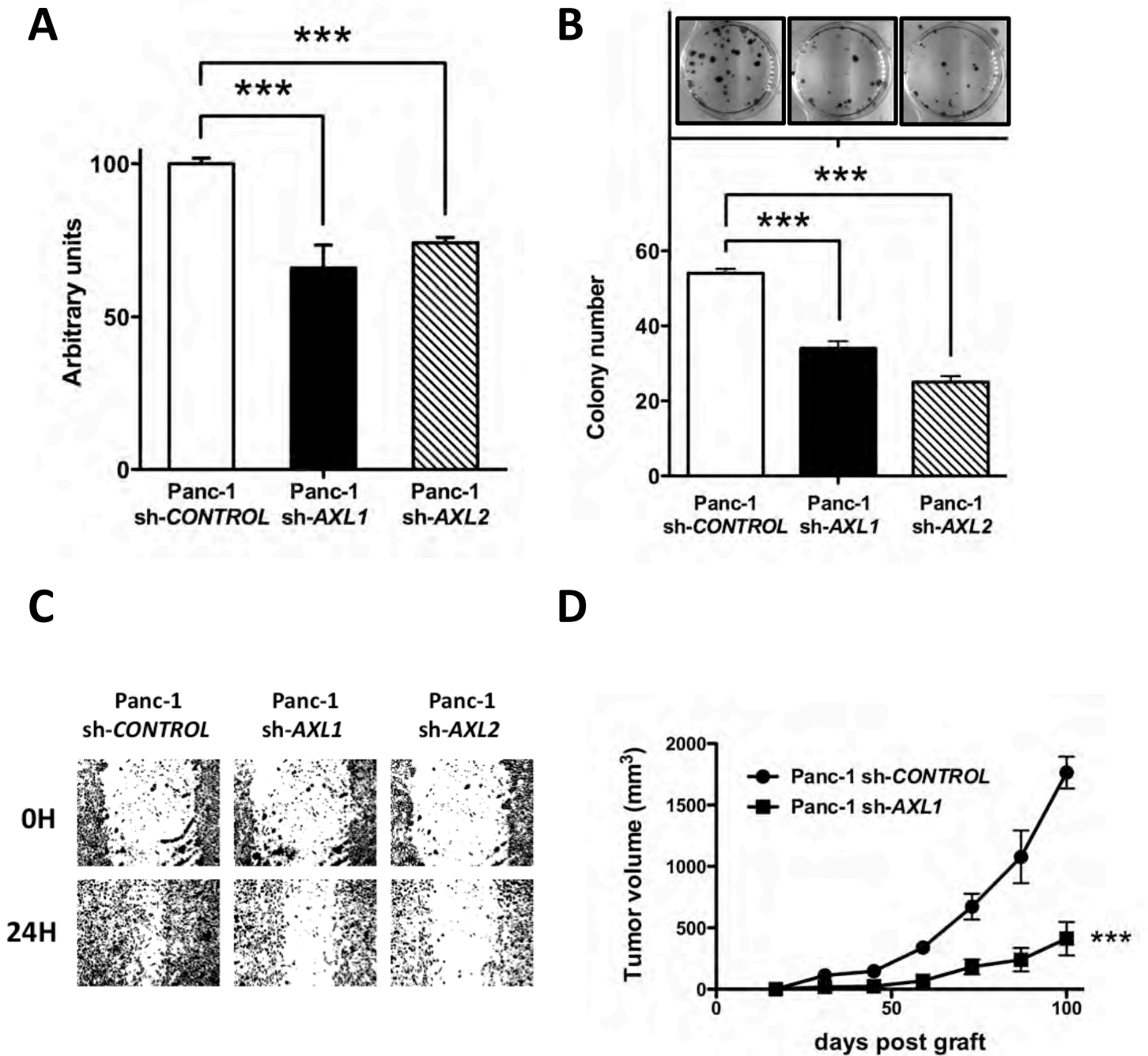
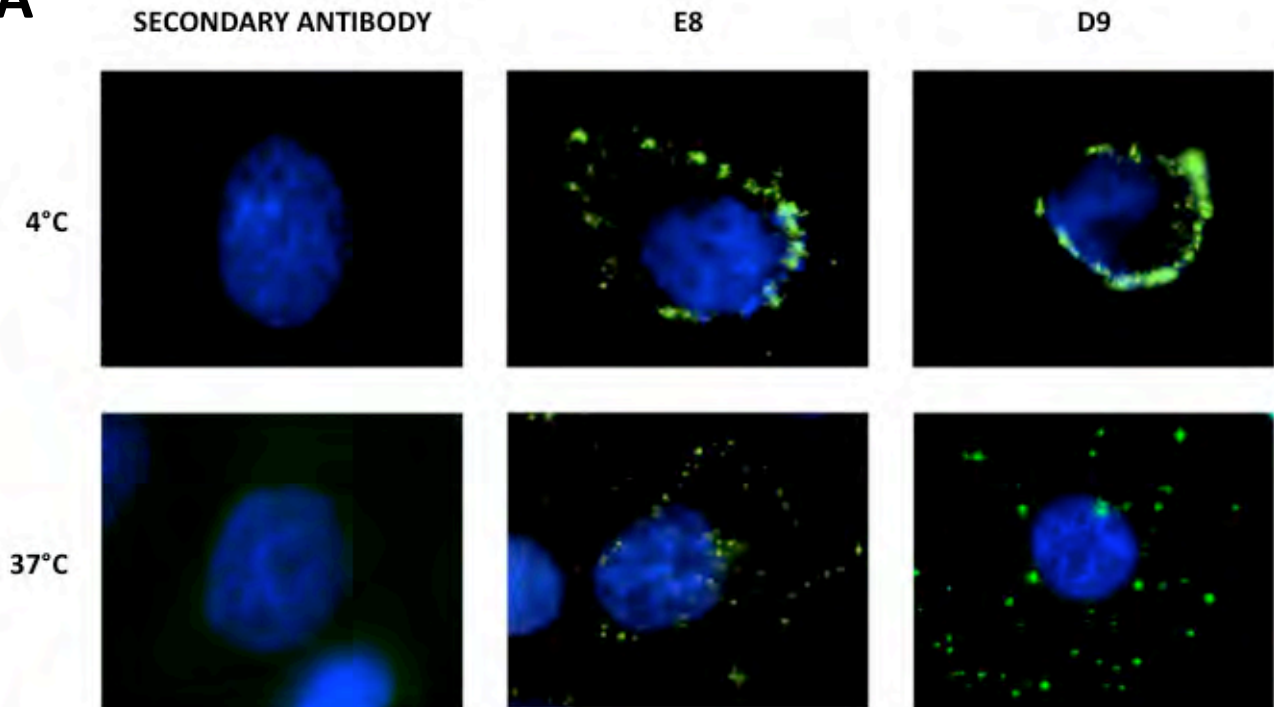
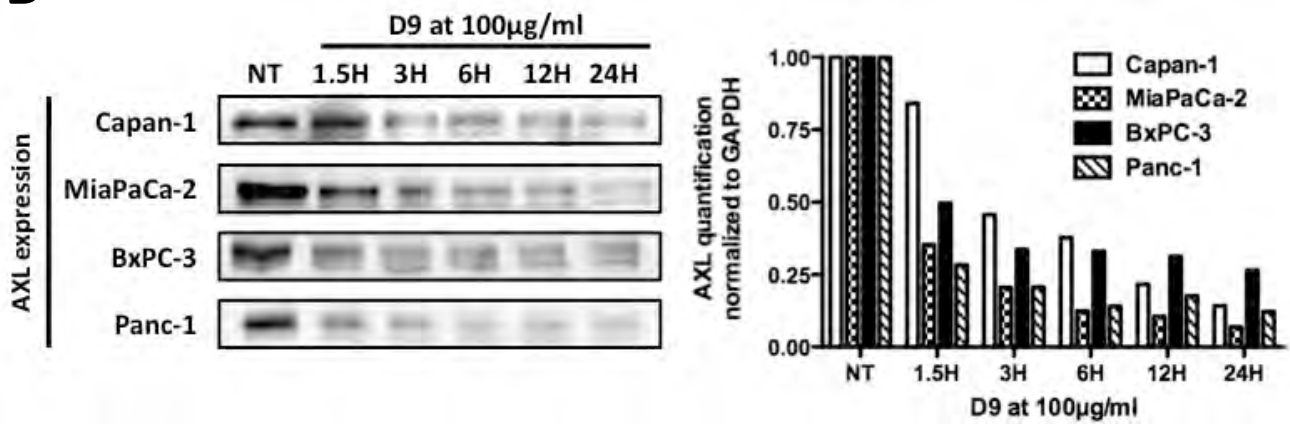


FIGURE 3

A



B



C

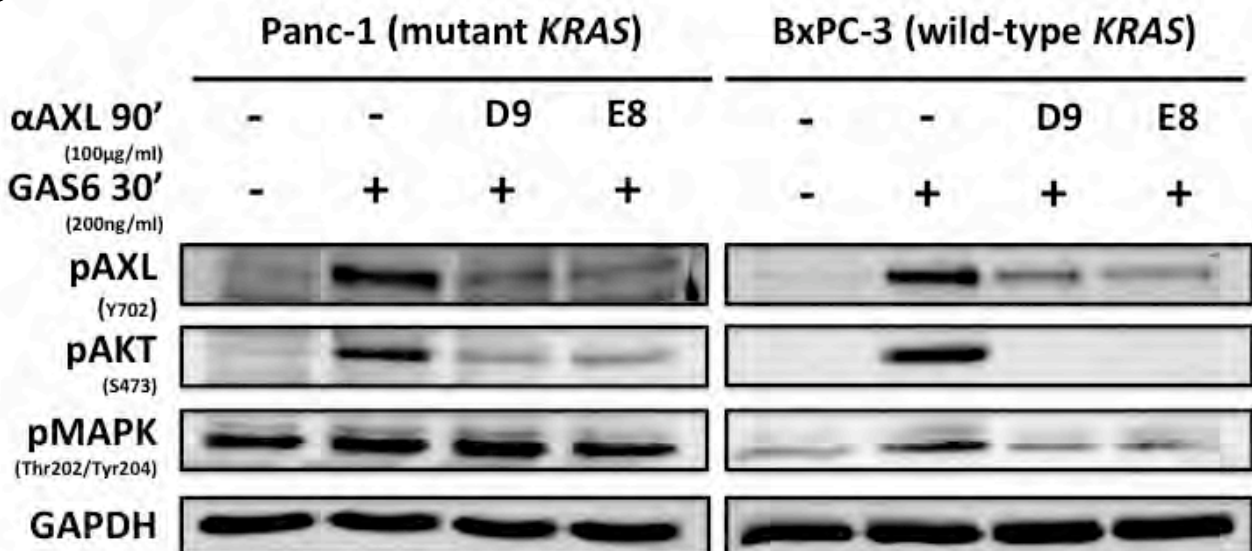
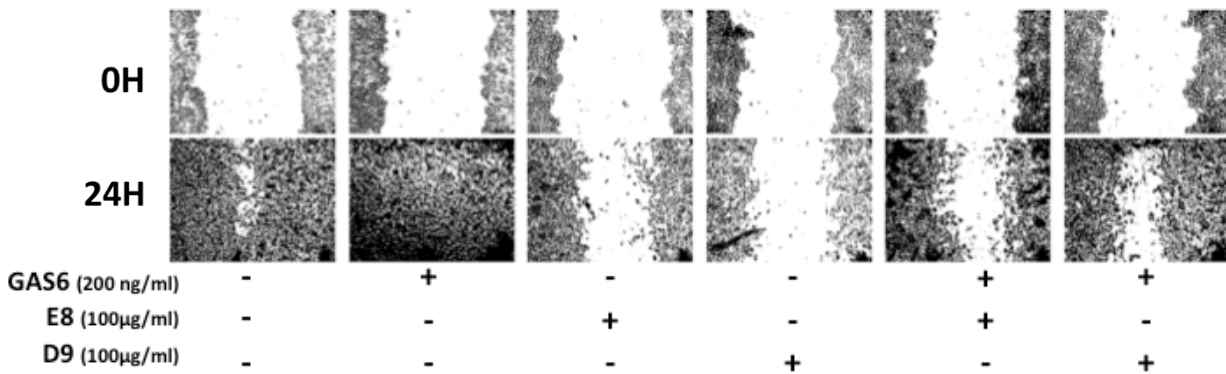
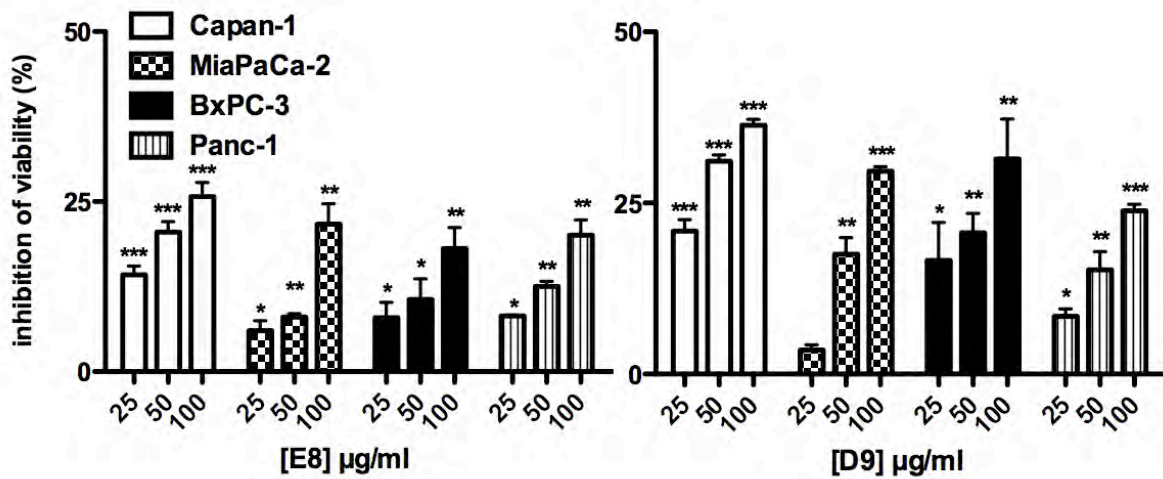


FIGURE 4

A



B



C

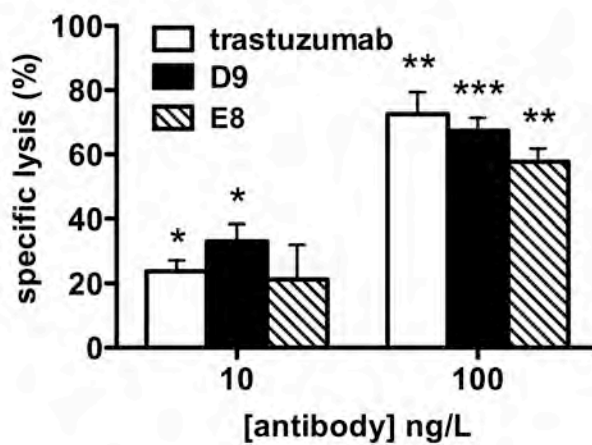
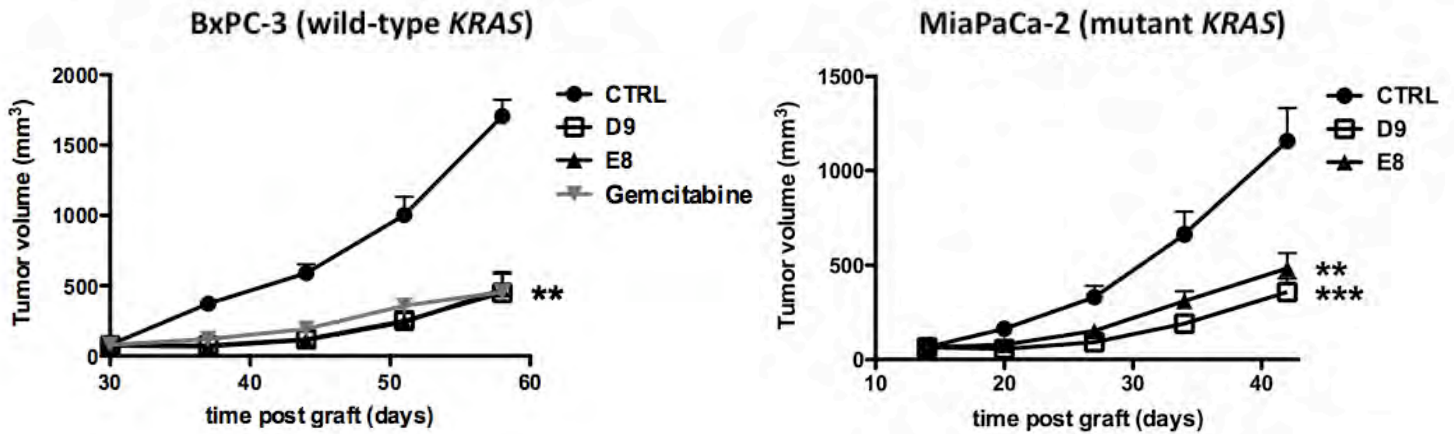
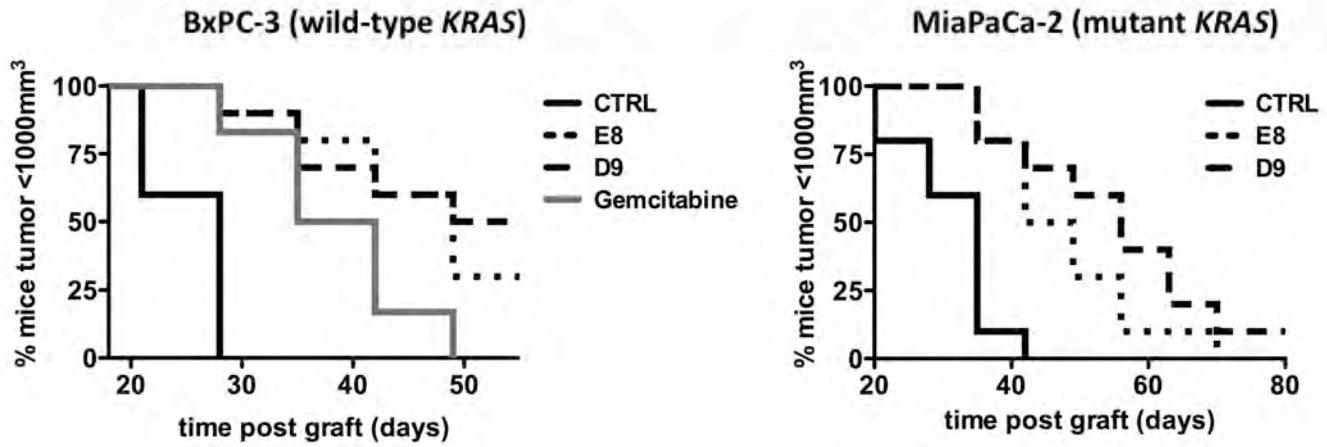


FIGURE 5

A



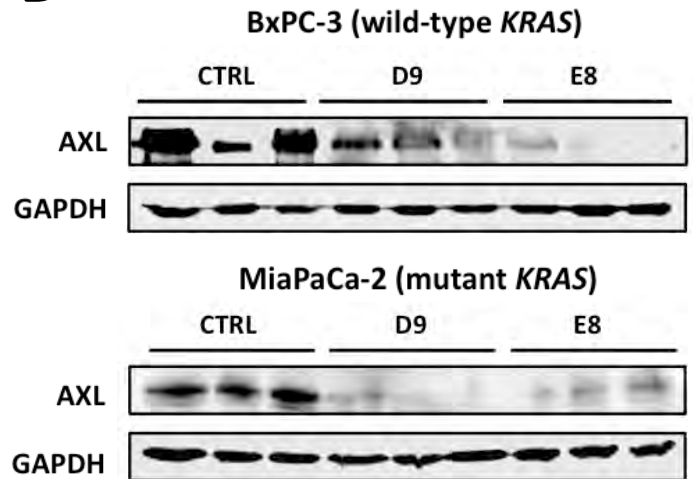
B



C

Xenograft	Treatment	Median (day)	Benefit (day)	Tumor free mice (%)
MiaPaCa-2	CTRL	35	0	0
	D9	56	+21	1/10 (10%)
	E8	42	+7	0
BxPc-3	CTRL	28	0	0
	D9	49	+21	3/10 (30%)
	E8	49	+21	3/10 (30%)
	Gemcitabine	35	+10	0

D



E

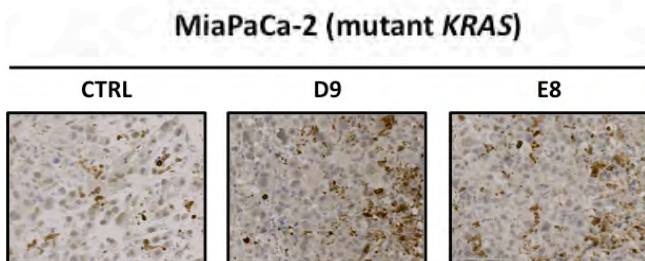
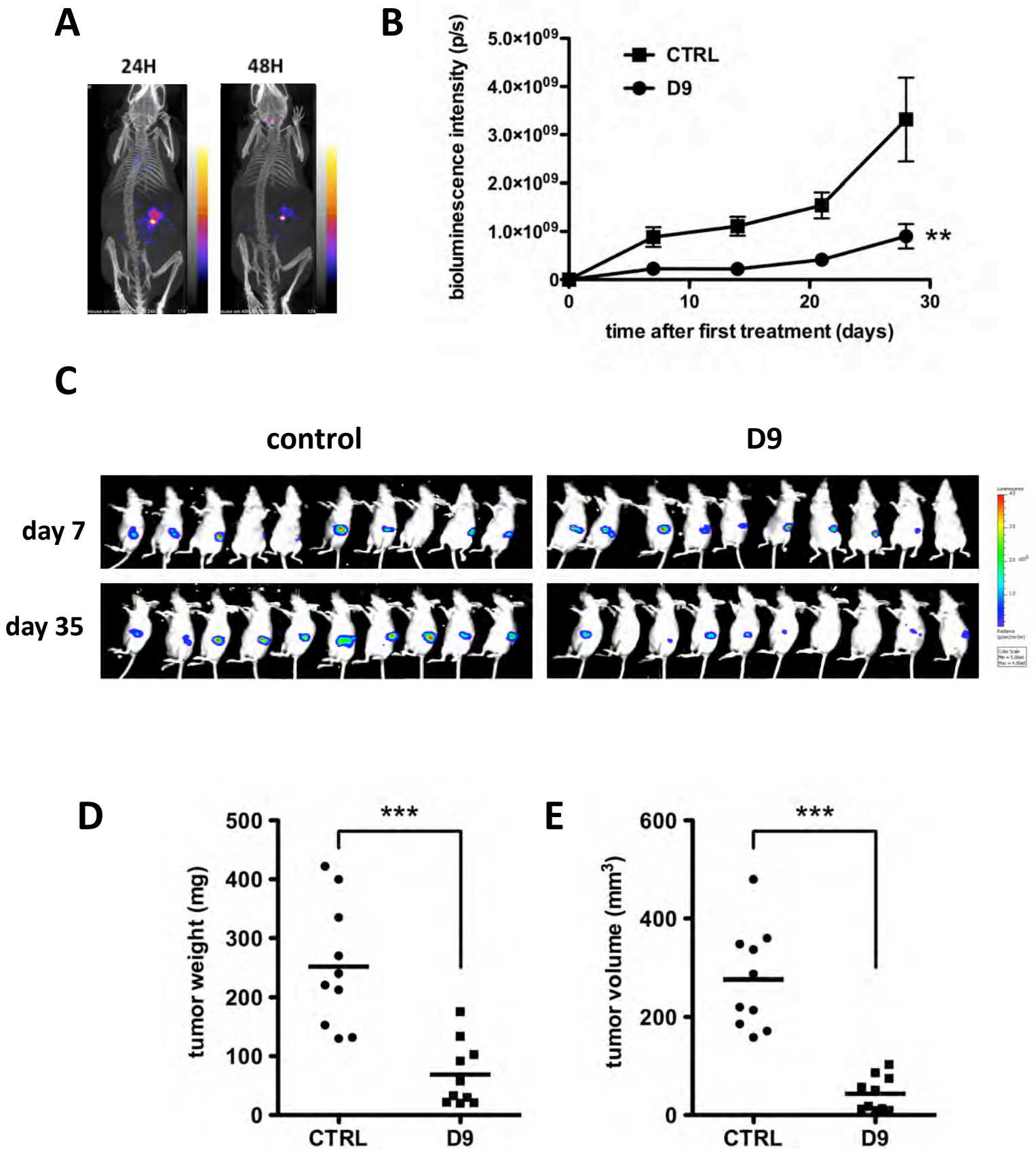
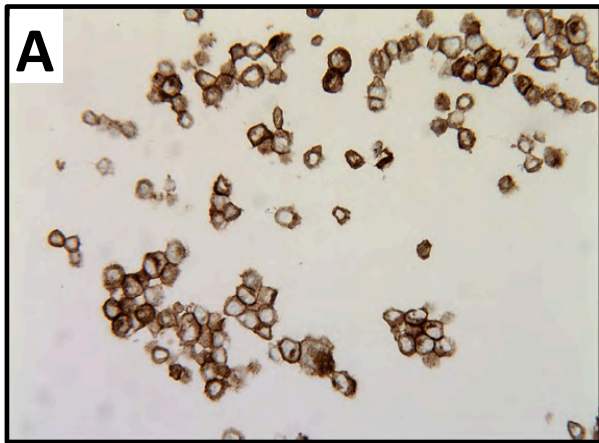


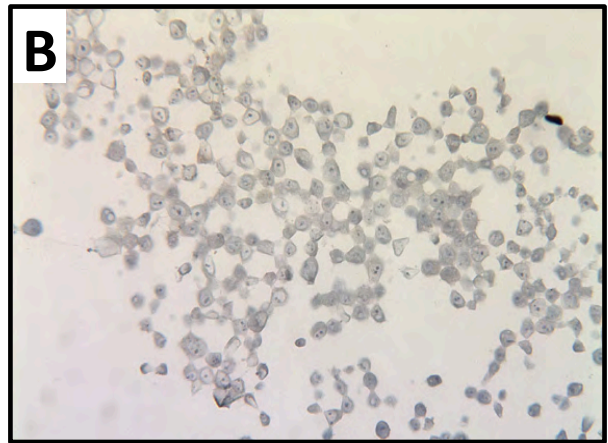
FIGURE 6



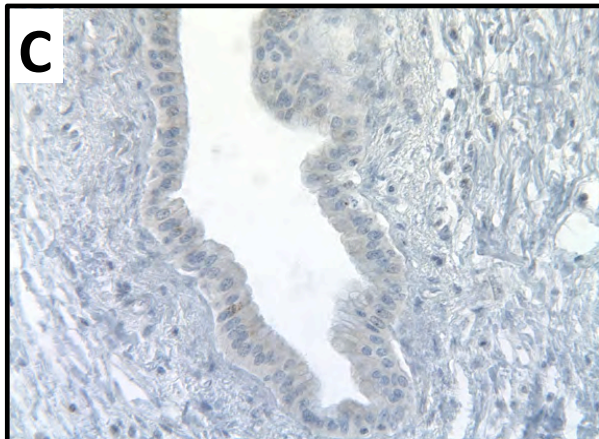
Panc-1 sh-*CONTROL*



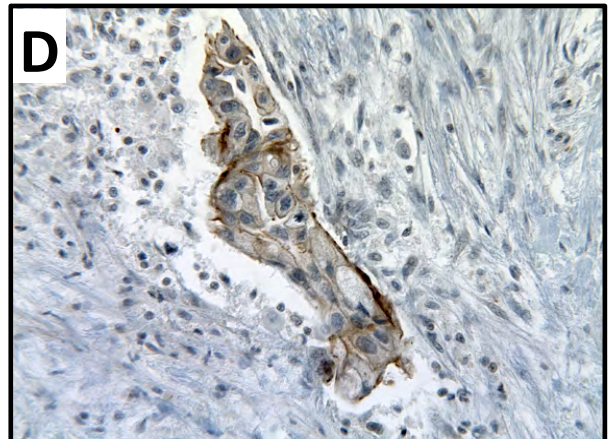
Panc-1 sh-*AXL1*



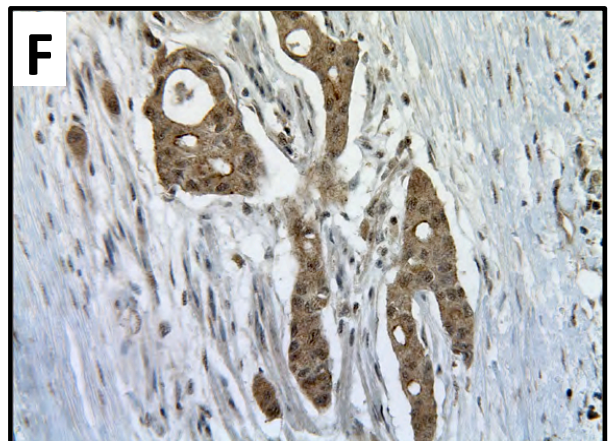
Normal Pancreatic Ductal Epithelium



Malignant Pancreatic Ductal Epithelium

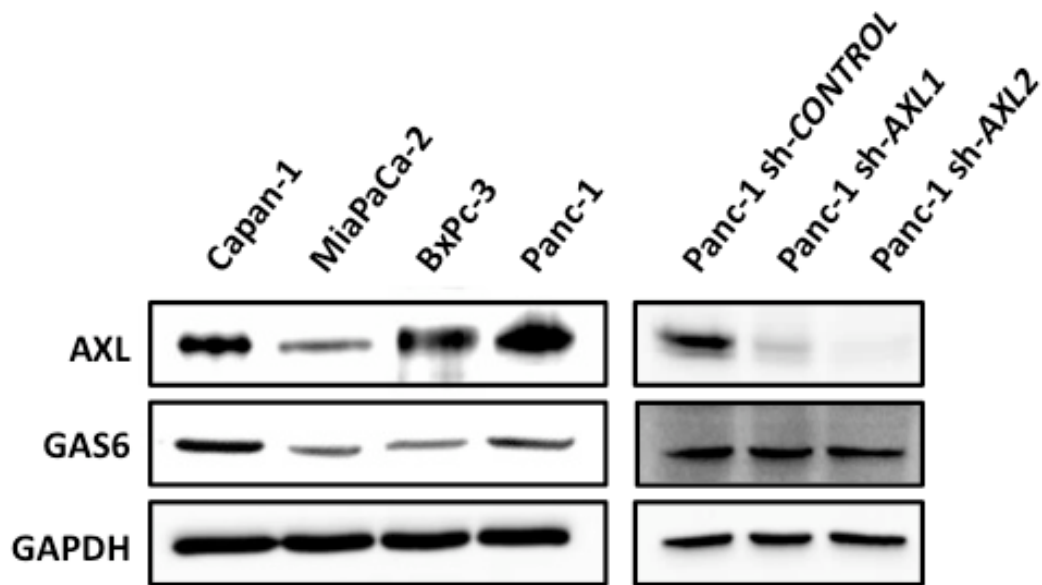
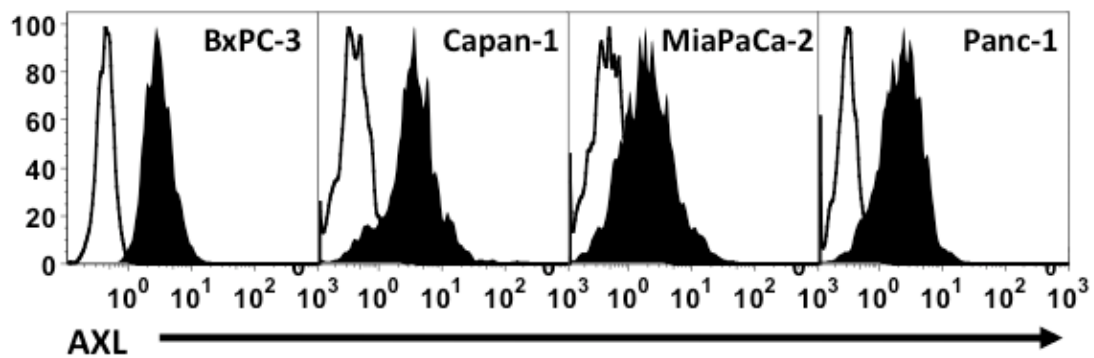


PATIENT 1

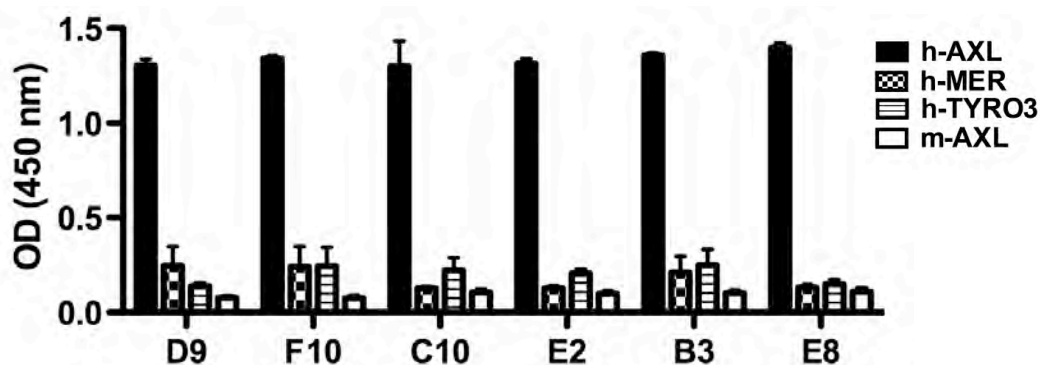
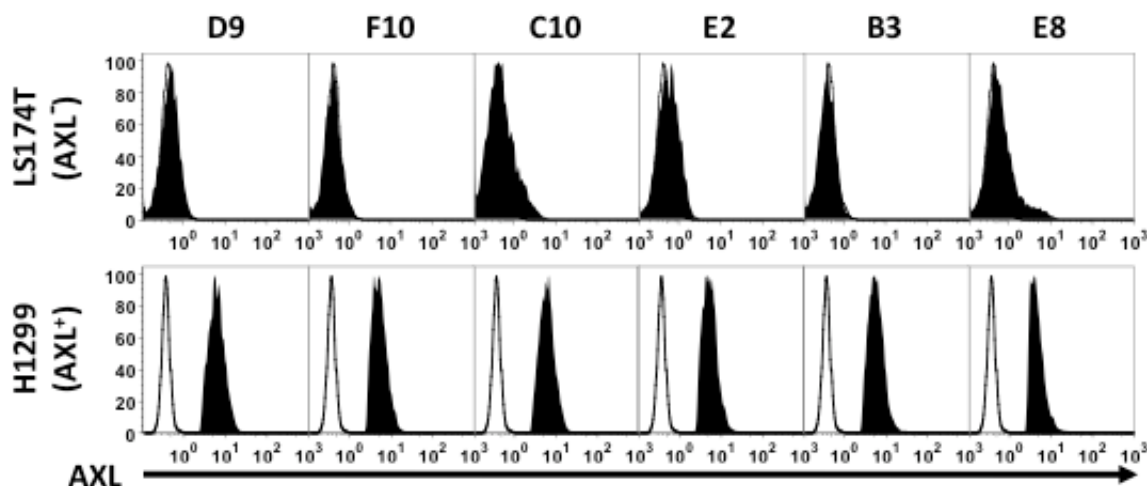
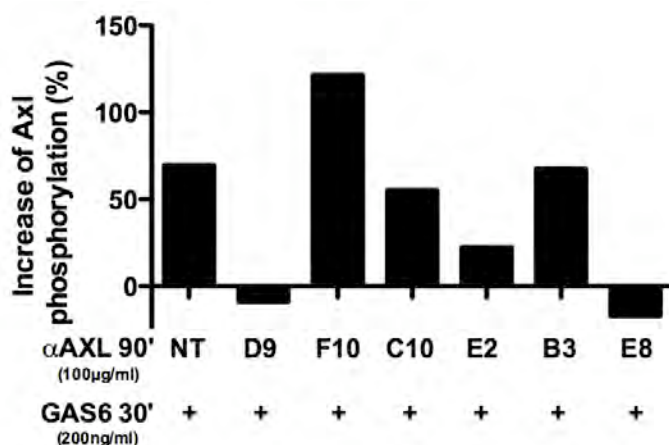
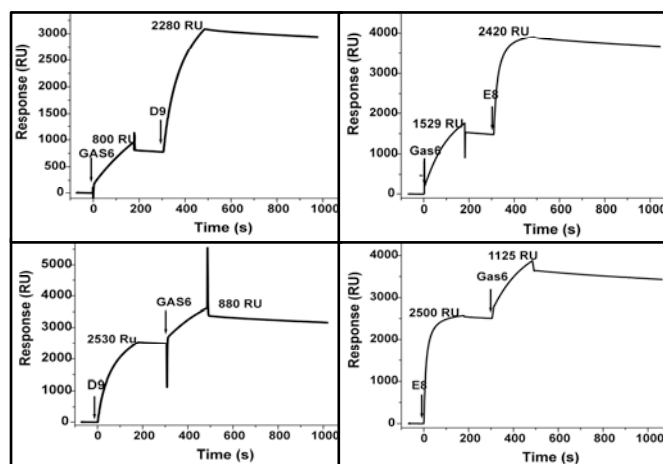


PATIENT 2

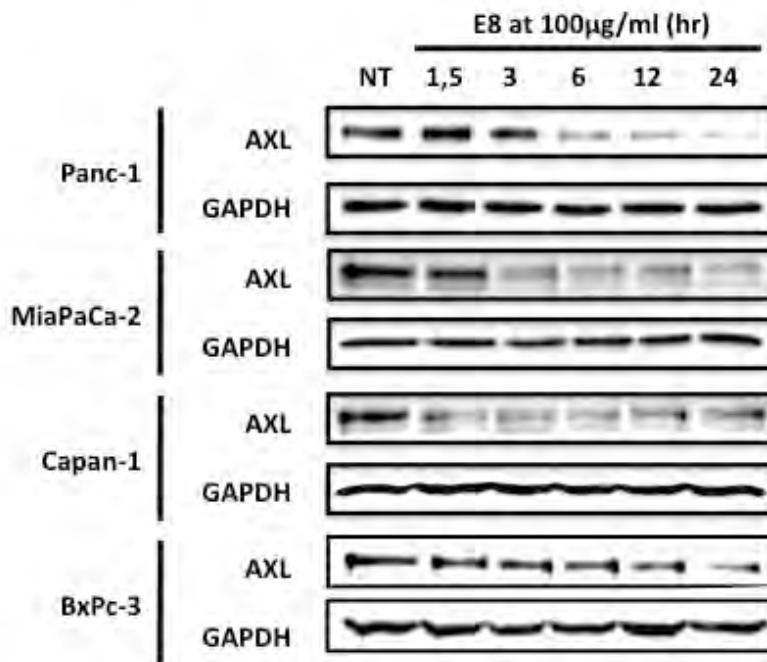
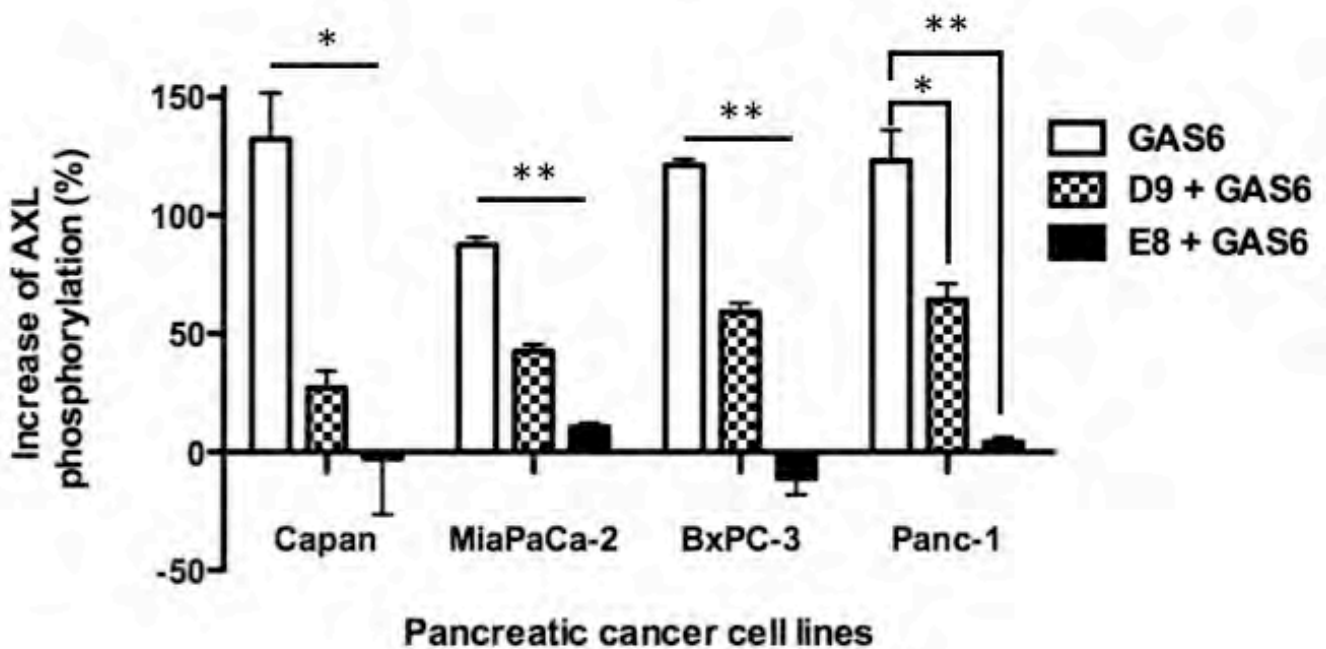
Supplementary Figure 1. AXL expression in Pancreatic Cancer. (A-B) Analysis of specificity of the rabbit polyclonal anti-AXL (ab72069, Abcam, Cambridge, UK) by IHC comparing staining between Panc-1 sh-*CONTROL* and Panc-1 sh-*AXL1*. (C-F) Expression of AXL in normal (C and E) and malignant (D and F) Pancreatic Ductal Epithelium by IHC.

A**B**

Supplementary Figure 2. AXL global and surface expression in pancreatic cancer cell lines. (A) Western blot analysis of AXL and GAS6 expression in four pancreatic cancer cell lines and in Panc-1 sh-AXL1, sh-AXL2 or sh-CONTROL cell line. (B) Cell surface AXL expression was observed by flow cytometry with an anti-AXL antibody.

A**B****C****D**

Supplementary Figure 3. Characterization and selection of anti-human AXL mAbs. (A) The specificity of the generated mAbs for human AXL was analyzed by ELISA against hAXL, hMER, hTYRO-3 and mAXL. (B) The ability of six selected mAbs to bind to AXL at the cell surface was analyzed by flow cytometry using AXL-positive H1299 cells. (C) Effect of the anti-AXL mAbs on AXL phosphorylation using the Phospho-Axl (PanTyr) Sandwich ELISA Kit (Cell Signaling Technology). H1299 cells were incubated with 200 ng/ml GAS6 after pre-incubation (1.5h) with 100 μg/ml of anti-AXL mAbs candidates. Results are presented as the percentage of phosphorylation relative to GAS6-treated control cells. (D) Analysis of the competition between D9 or E8 and GAS6 by Biacore assays. The anti-AXL mAbs D9 or E8 bound to a rhAXL-coated chip after saturation with GAS6 (upper panels), and GAS6 bound to a rhAXL-coated chip that was saturated with anti-AXL mAbs (lower panels).

A**B**

Supplementary Figure 4. D9 and E8 anti-AXL mAbs inhibit AXL activation a downregulation of the receptor. (A) AXL expression in Capan-1, MiaPaCa-2, BxPC-3 and Panc-1 cells was analyzed by western blotting at different time points after incubation with 100 µg/ml E8. (B) Cells were serum-starved overnight, pre-incubated or not with 100 µg/ml D9 or E8 for 1.5 hour and then stimulated with 200 ng/ml GAS6 for 30 minutes. AXL phosphorylation was measured with the Phospho-Axl (PanTyr) Sandwich ELISA Kit. The results are presented as the percentage of phosphorylation relative to the value in untreated, control cells.