Supplementary Figure legends

Supplementary Figure S1. PODXL1 and TRA-1-60 expression on patient PDAC tissues. (A) PODXL1-positive cells on the neoplastic glands detected by immunohistochemistry. Arrow indicated the strongly PODXL1-positive tumor cell at the neoplastic gland in PDAC tissues. (B) TRA-1-60 expression on PDAC tissues of the two patient cases detected by immunohistochemistry using anti-TRA-1-60 mAb (upper panel). Expression pattern on the patient tissues was similar to that of PODXL1. The budding tumor cells from the neoplastic gland was TRA-1-60-positive by immunofluorescence. (C) PODXL1 expression observed at the invasive front of advanced colon tubular adenocarcinomas and at the metastatic site of lymph node from patient tissues.

Supplementary Figure S2. Loss of the metastatic property in *PODXL1*-knockout clones derived from PDAC cells. (A) Genome sequencing analysis of the knockout clones (MiaPaca-2 clone#9, AsPC1 clone#2) generated by CRISPR/Cas9 system used in the study. (B) Loss of *PODXL1* significantly attenuated the lymph nodal metastasis (mesenteric lymph nodes) of PDAC cells in the liver metastatic mouse model *in vivo*. Number of the metastatic foci was summarized in the attached table. (C) *PODXL1*-knockout Panc-1 clone also showed loss of liver metastasis in comparison with the wild type *in vivo*.

Supplementary Figure S3. Histology of the metastatic tumors *in vivo*. (A) Liver metastatic tumors in mice with splenic MiaPaCa-2 injection (30 days post-injection). Multiple metastases by MiaPaCa-2 bearing *PODXL1* wild type (first column), which was positive for anti-human cytokeratin antibody (CAM5.2), in contrast to no intrahepatic micrometastasis in mice injected with *PODXL1*-KO MiaPaCa-2 (second column). Multiple metastatic foci to liver in mice with AsPC-1 bearing *PODXL1* wild type (third column), no intrahepatic micrometastasis in mice injected with *PODXL1*-KO AsPC1 (bottom column). (B) Histology of the metastatic focus of *PODXL1* wild type MiaPaCa-2 splenically injected mouse. (C) Histology of the metastatic focus of *PODXL1* wild type Panc-1 splenically injected mouse.

Supplementary Figure S4. Visualization of intracellular localization of chemokine receptor (C5aR, EP4, CX3CR1) in the presence of PODXL1 indicating the binding of these receptors to PODXL1 on co-transfected cells. 'Fluoppi' system revealed altered localization of C5aR, EP4-red and CX3CR1-red (membrane localization depicted with aggregated fluorescent dots) only in *PODXL1*-Ash co-transfected cells. (A) Fluoppi by cotransfection of *C5aR* with *PODXL1*. (B) Fluoppi by cotransfection of *EP4* with *PODXL1*, cotransfection of *CX3CR1* with *PODXL1*, respectively. (C) Binding assay to demonstrate direct coupling of PODXL1 with C5aR. Interaction between purified 3xHA-6xHis-tagged PODXL1 protein and 3xFlag-6xHis-tagged C5aR protein was examined by immunoprecipitaion using anti-HA beads. Left panel showed Coomassie brilliant blue staining of the sample with 3xHA-6xHis-tagged PODXL1 protein alone (left lane), the sample of the mixture of 3xHA-6xHis-tagged PODXL1 and 3xFlag-6xHis-tagged C5aR (middle lane), and the mixture of 3xHA-6xHis-tagged PODXL1 and heat-degraded 3xFlag-6xHis-tagged C5aR (right lane) by SDS-PAGE. Middle panel showed immunoblotting probed with anti-HA antibody (lane 1; 3xHA-6xHis-tagged PODXL1 alone, lane 2; 3xHA-6xHis-tagged PODXL1 + 3xFlag-6xHis-tagged C5aR, lane 3; 3xHA-6xHis-tagged PODXL1 and heat-inactivated 3xFlag-6xHis-tagged C5aR).

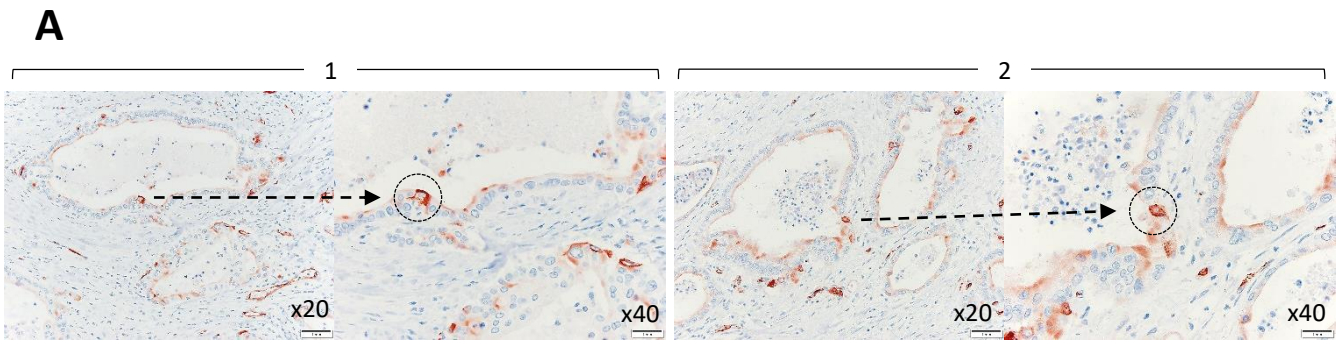
Supplementary Figure legends (continued)

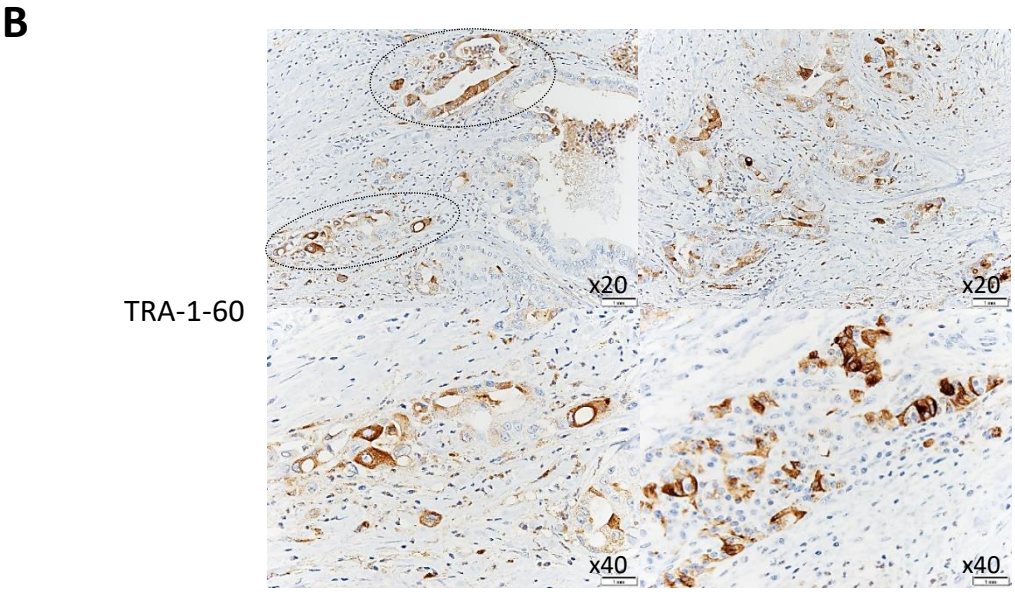
Right panel showed immunoblotting probed with anti-Flag antibody (lane 4; 3xHA-6xHis-tagged PODXL1 alone, lane 5; 3xHA-6xHis-tagged PODXL1 + 3xFlag-6xHis-tagged C5aR, lane 6; 3xHA-6xHis-tagged PODXL1 and heat-inactivated 3xFlag-6xHis-tagged C5aR).

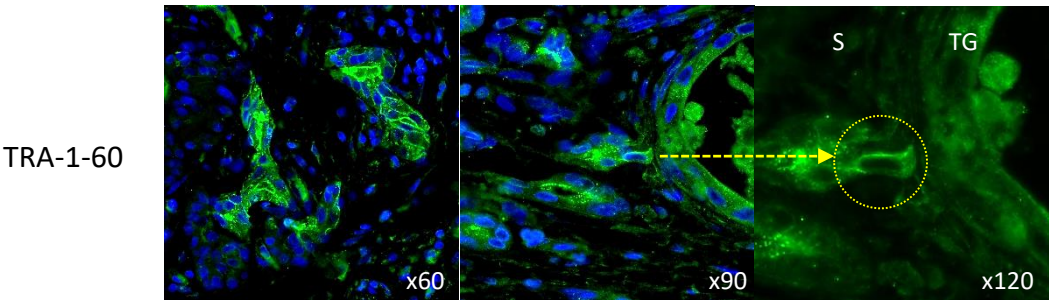
Supplementary Figure S5. Correlation of C5a/C5aR axis to PODXL1 for the cellular motility of PDAC cells. (A) Effect of C5AR on the cellular motility of MiaPaCa-2 cells. Effect of siRNA specific to C5aR demonstrated a decrease of motility of C5a-stimulated MiaPaca-2 cells with statistical significance (upper panel; graph). Crystal Violet staining of invaded MiaPaCa-2 cell with or without the siRNA treatment (lower panel; image). (C) Transwell chamber assay (invasion assay) demonstrated impacts of various chemokine ligands (CXCL8, CX3CL1, C5a, PEG2) on cellular motility of *PODXL1*-WT PDAC cells in comparison with *PODXL1*-KO cells (AsPC1, Panc-1).

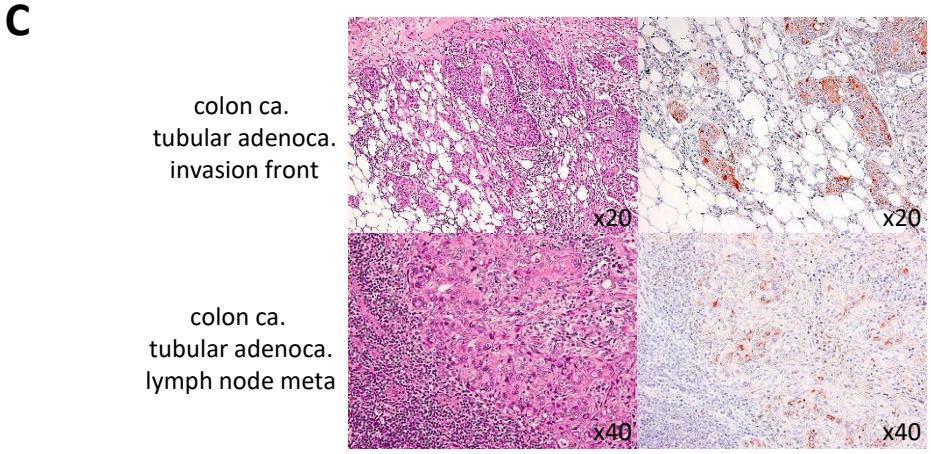
Supplementary Figure S6. Expression of C5aR was attenuated in *PODXL1*-KO PDAC cells. (A) Immunofluorescence using anti-C5aR mAb revealed strong membranous expression of C5aR in MiaPaCa-2, AsPC1, and Panc-1 bearing wild type of *PODXL1*, whereas its expression was prominently attenuated in their *PODXL1*-deficient (*PODXL1*-KO) clones. (B) Histology of liver metastasis developed in control IgG-treated mouse (HE and immunohistochemistry using anti-human cytokeratin, CAM5.2).

Supplementary Figure S7. C5aR expression was stably maintained between the primary site and metastatic site of the patient tissue with PDAC. C5aR was stably expressed on the metastatic cancer nests of the lymph node as well as PDAC nests at the primary site in the patient pancreas. Inset represented the hyperview in each tumor site.









Supplementary Figure S1

A

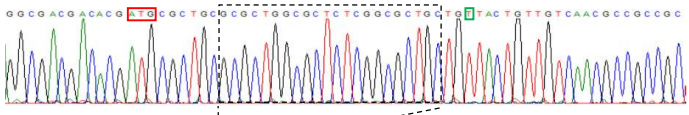
PODXL1 (NM_001018111) : ACGACACG**ATG**CGCTGCGCGCTGGCGCTCTCGGCGCTGCTG**C**TACTGTT

MiaPaCa-2 Pod1 WT : ACGACACG**ATG**CGCTGCGCGCTGGCGCTCTCGGCGCTGCTG**T**TACTGTT (c.C34>T: p.L12L in Exon 1)

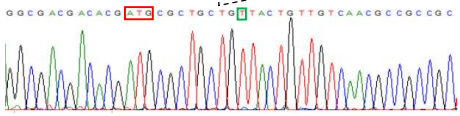
MiaPaCa-2 Pod1 KO #9: ACGACACG**ATG**CGCTGC----------TG**T**TACTGTT

deleted sequence

MiaPaCa-2 Pod1 WT



MiaPaCa-2 Pod1 KO #9 (homozygous KO)

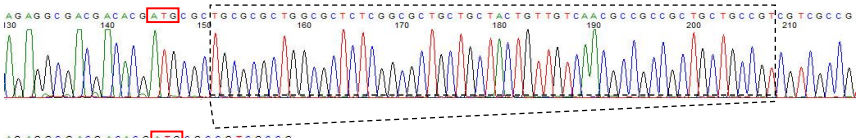


AsPC-1 Pod1 WT : ACGACACG**ATG**CGCTGCGCGCTGGCGCTCTCGGCGCTGCTGCTACTGTTGTCAACGCCGCCGCTGCTGCCGTCGTCGCCGTCGCCGTCGCCGT

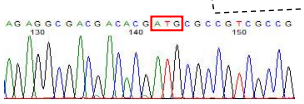
AsPC-1 Pod1 KO #2: ACGACACG**ATG**CGC----------CGTCGCCGTCGCCGT

deleted sequence

AsPC-1 Pod1 WT



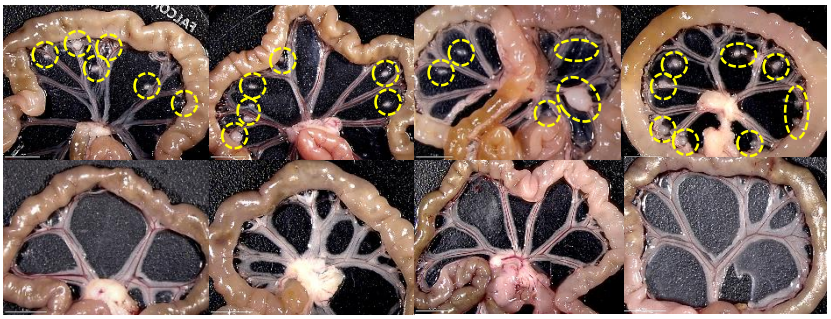
AsPC-1 Pod1 KO #2 (homozygous KO)



B

MiaPaCa-2 PODXL1 WT

MiaPaCa-2 PODXL1 KO #9



number of mesenteric LN metastasis

case / *PODXL1*	# 1	# 2	# 3	# 4
WT	6	6	5	8
KO	0	0	0	0

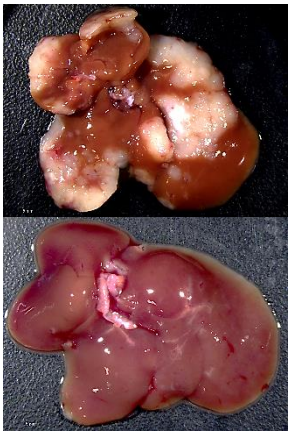
C

Panc-1

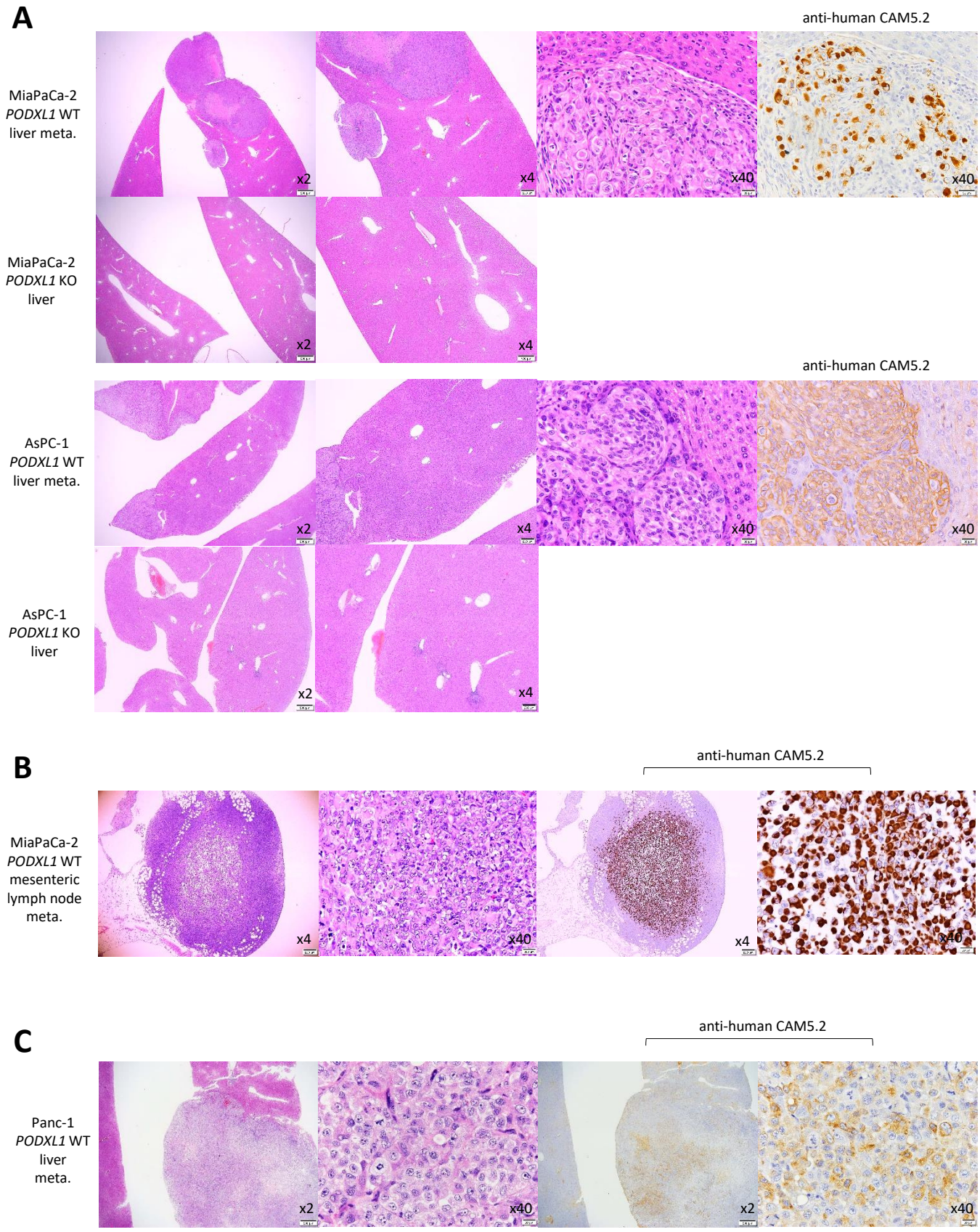
liver meta.

PODXL1 WT

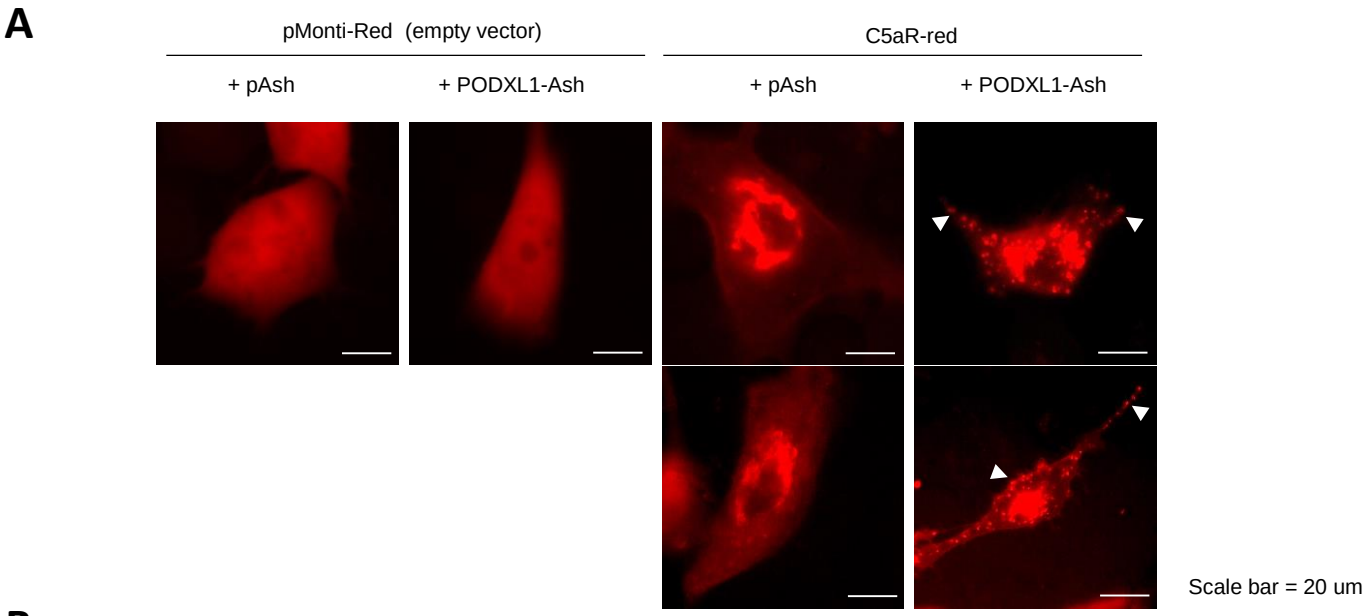
PODXL1 KO #13

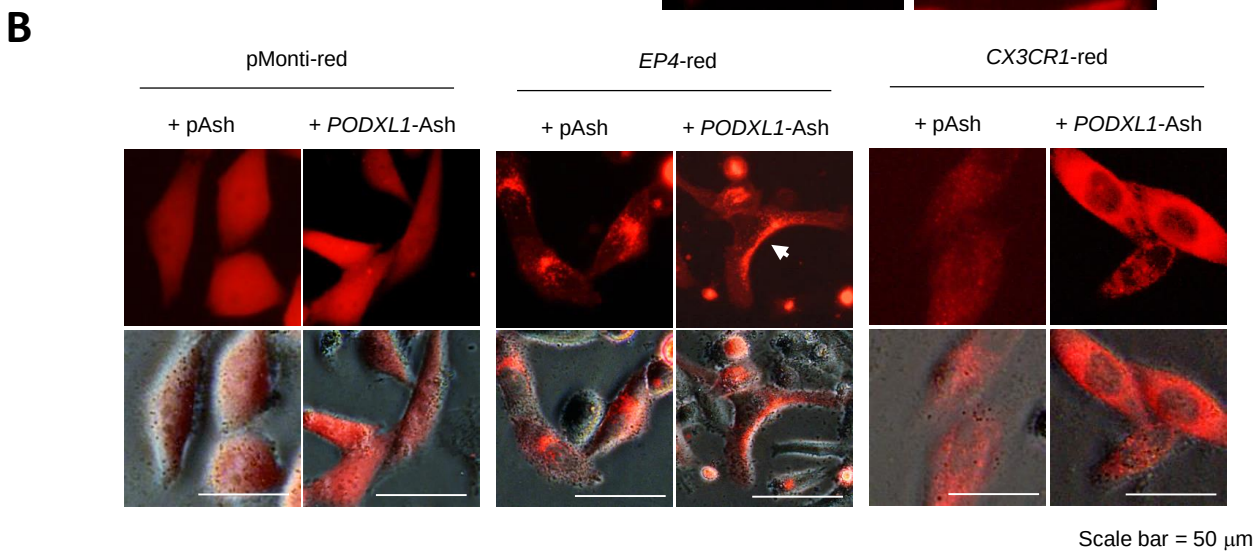


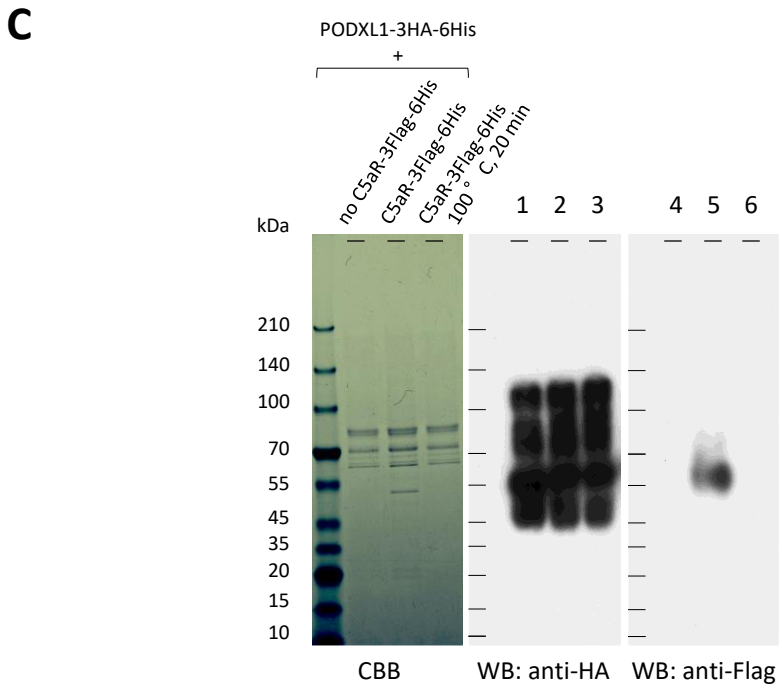
Supplementary Figure S2



Supplementary Figure S3

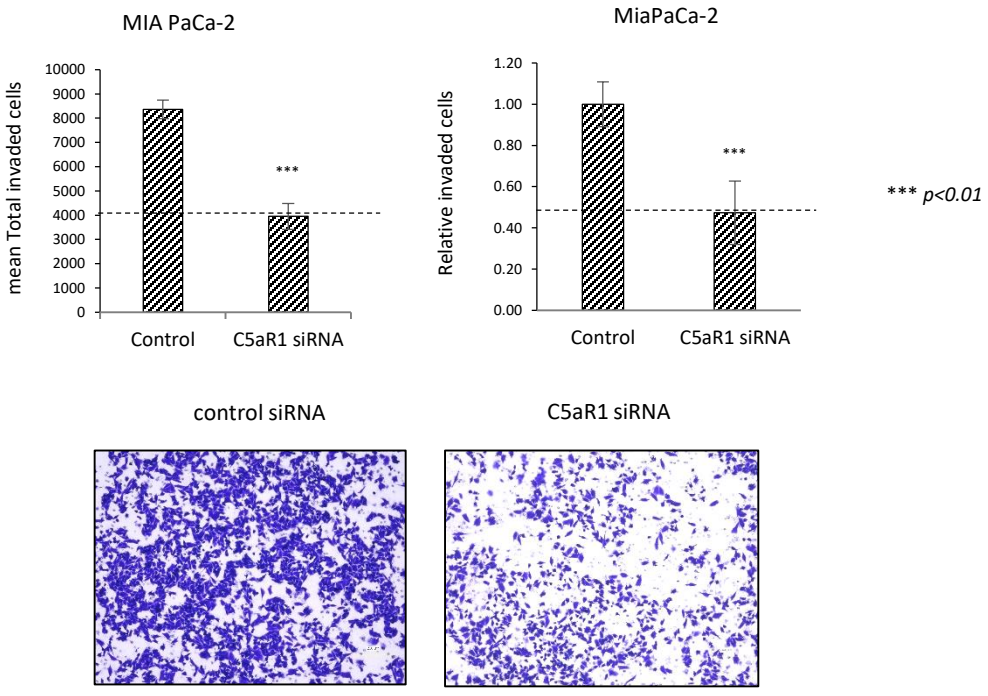






Supplementary Figure S4

A



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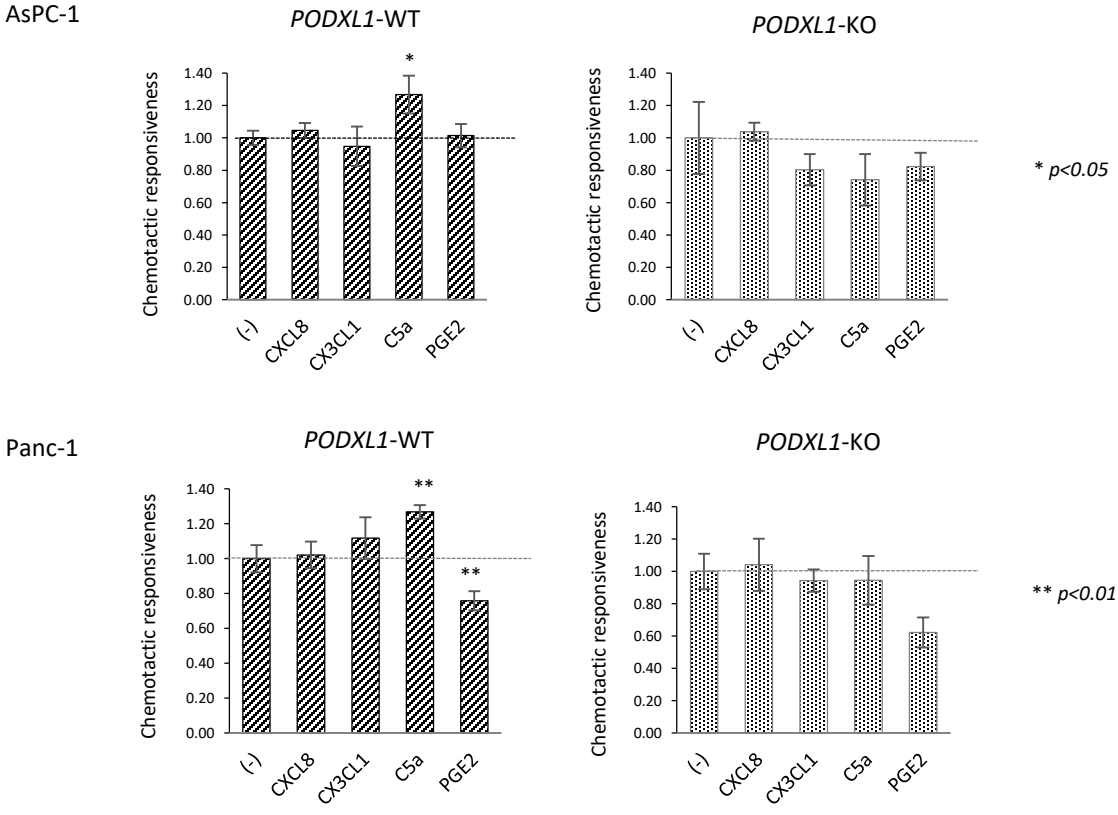
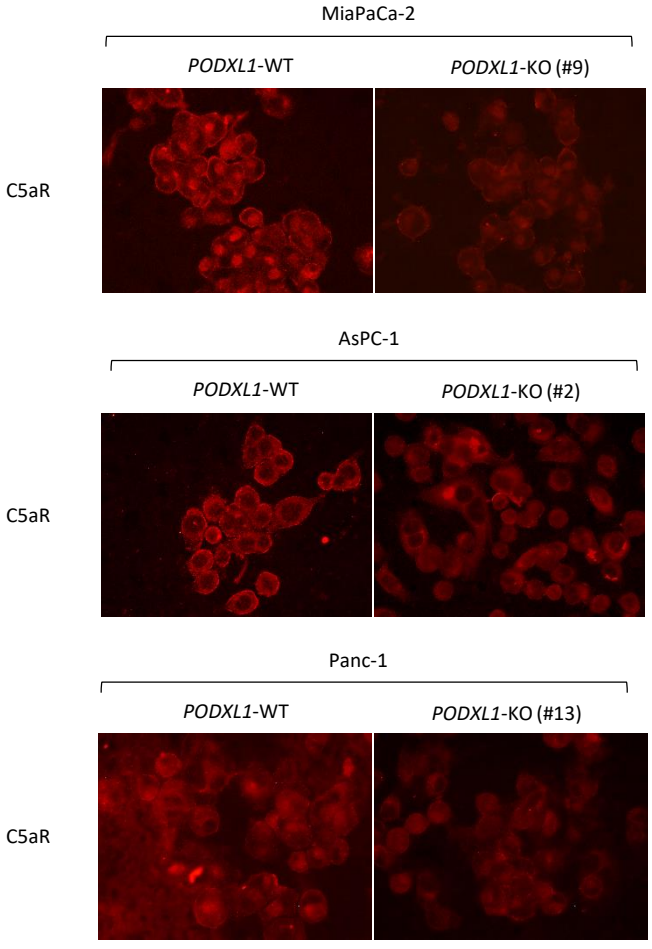


Supplementary Figure S5

A



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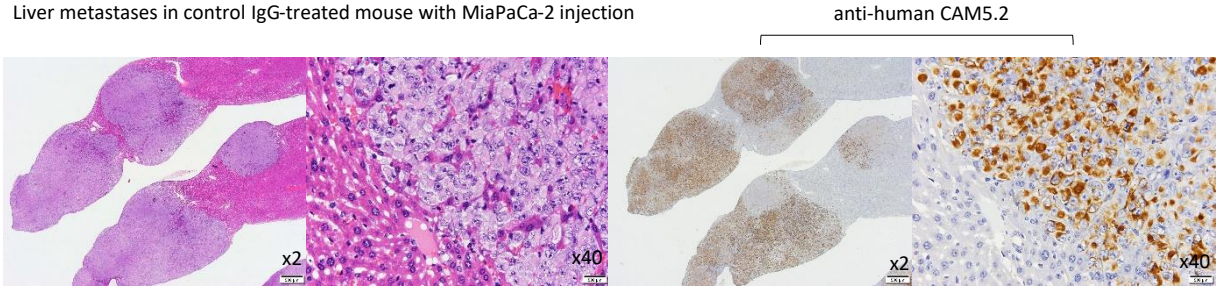
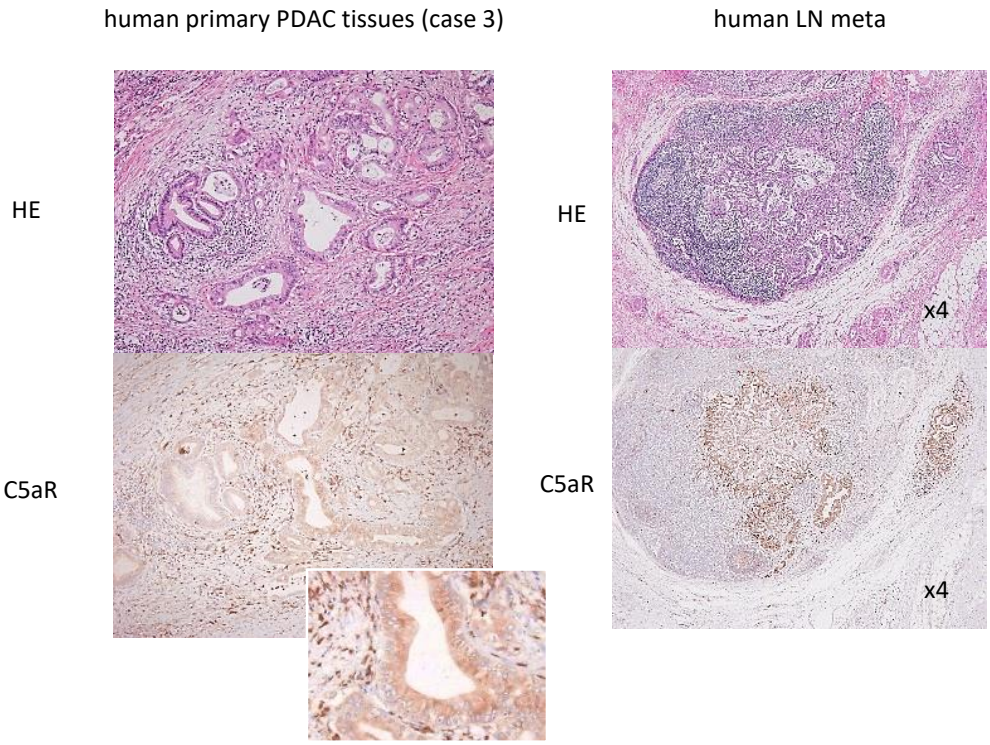


Supplementary Figure S6



Supplementary Figure S7