

Mapping Immune Activity and Tissue Changes in Early Pancreatic Lesions

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Introduction and Methodology

Background and Objectives

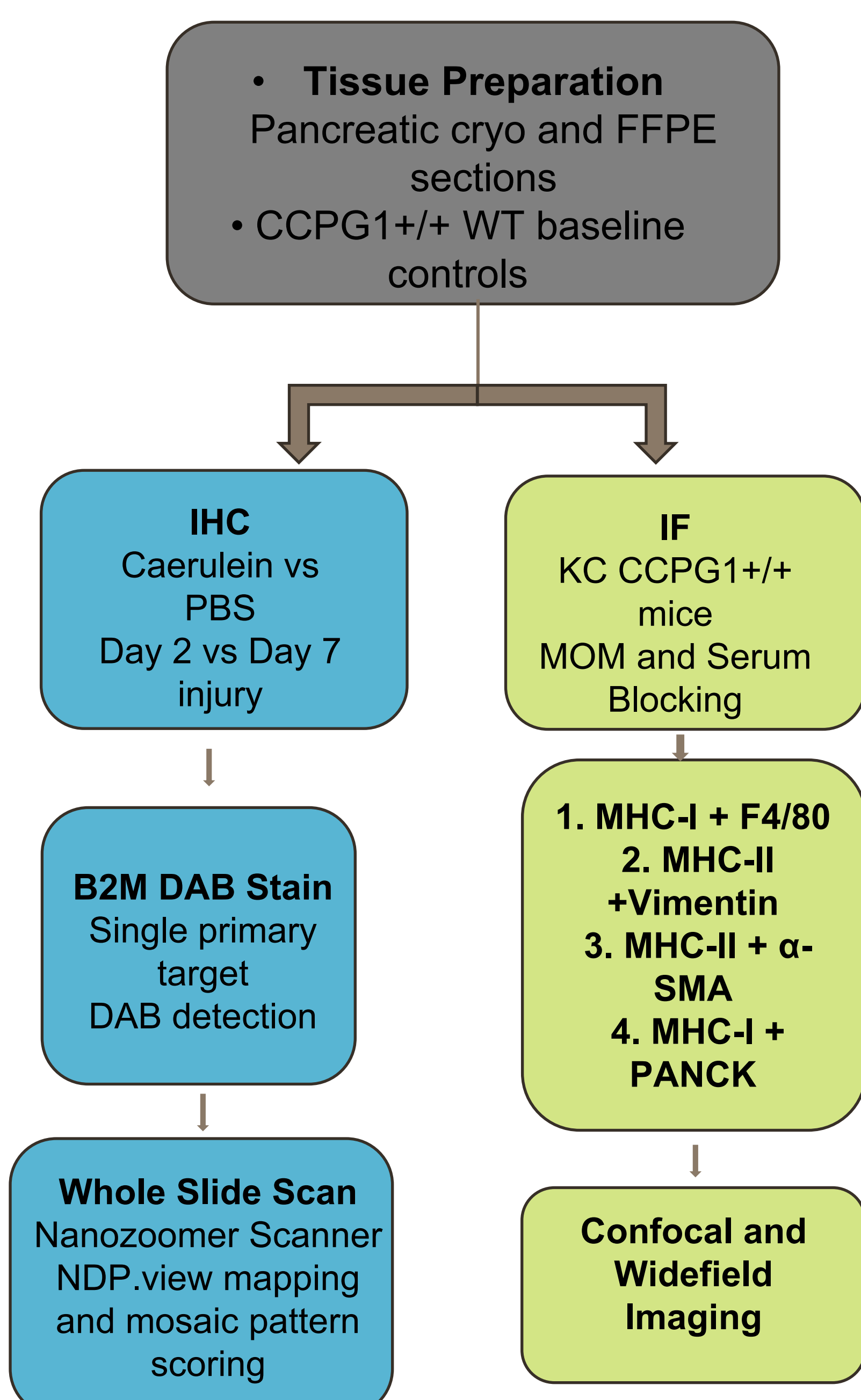
Mutant Kras and cerulein-induced pancreatitis drive acinar-to-ductal metaplasia (ADM) and precancerous lesion formation.

Objective: To map immune alarm signalling (MHC-I/B2M, MHC-II) during tissue injury and early lesion development, and identify which specific cell types (PANCK ducts/ADMs, F4/80+ macrophages, Vimentin + fibroblasts, and α -SMA for smooth muscle cells) drive this response.

Methods

IHC Protocol: FFPE pancreatic sections (CCPG1+/+) controls vs day 2 and day 7 cerulein-induced pancreatitis, KC mice) were stained for B2M (MHC -1 subunit) via DAB chromogen.

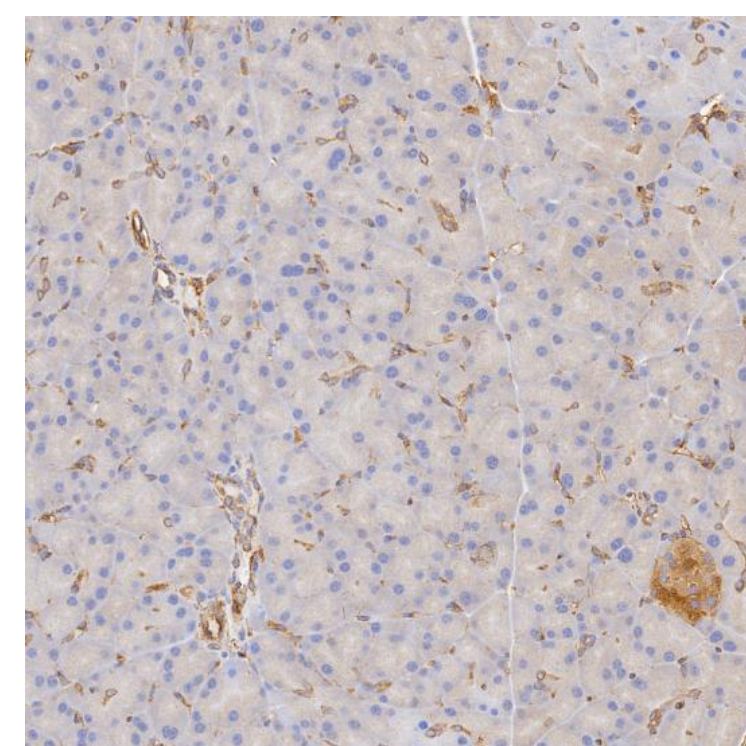
IF Protocol: Frozen cryosections (KC mutant/CCPG1 WT) blocked with Mouse-on-Mouse or protein buffer. Immunostained for primary targets (Amylase, MHC-I, F4/80, MHC-II, Vimentin, α -SMA, PANCK) + DAPI. Imaging done through widefield and confocal microscopy.



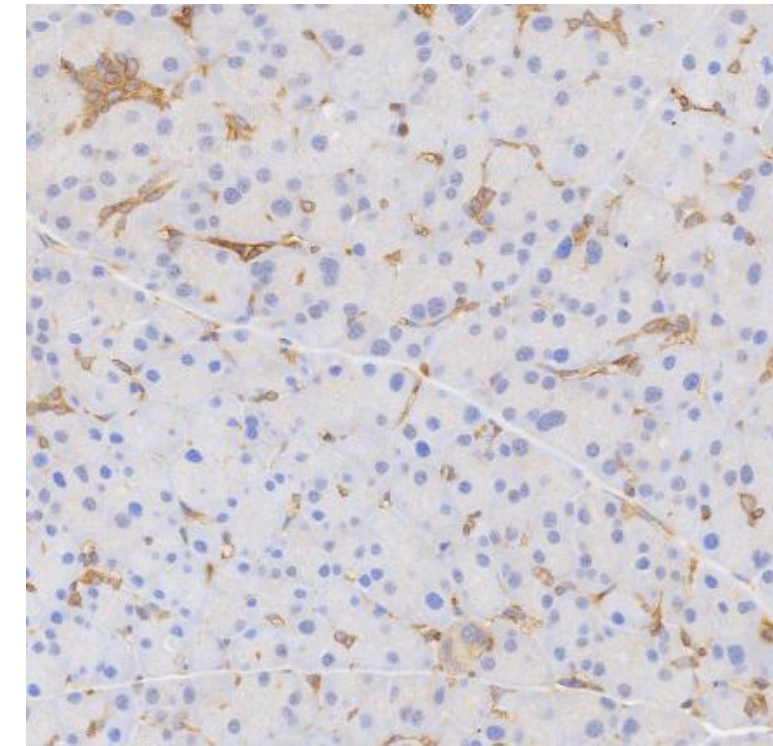
Acute Injury & Recovery (IHC 1)

Cerulein-induced pancreatitis causes B2M (immune protein component of MHC 1) upregulation as a result of inflammation. CCPG1(+/+) mice were used.

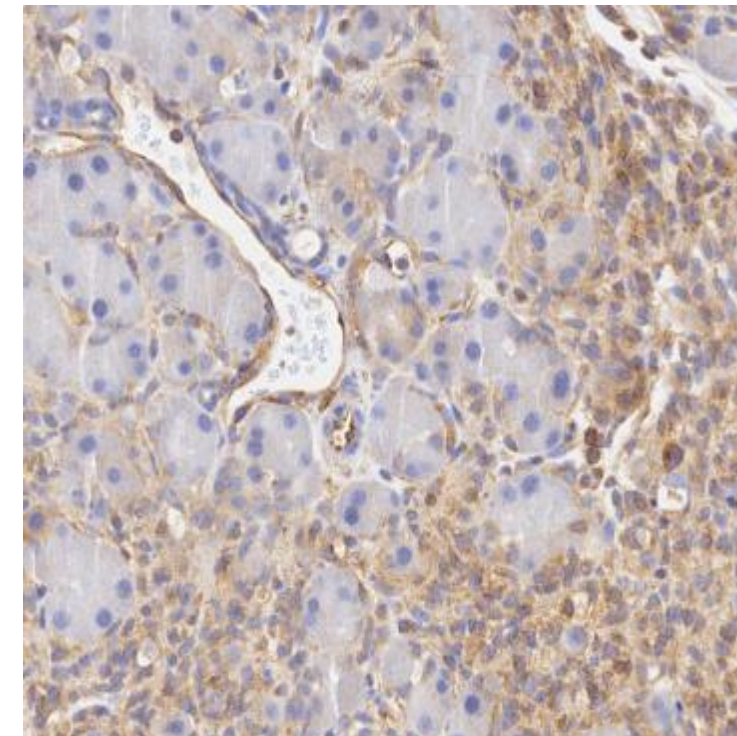
- PBS Controls:** Healthy acinar cells showed low, uniform expression of B2M.
- Injury (Day 2):** Cerulein triggered mosaic brown B2M staining, reflecting immune cell infiltration and stress.
- Tissue Recovery (Day 7):** Staining eventually improved to a uniform pattern, highlighting tissue repair.



PBS control



Day 7 Cerulein

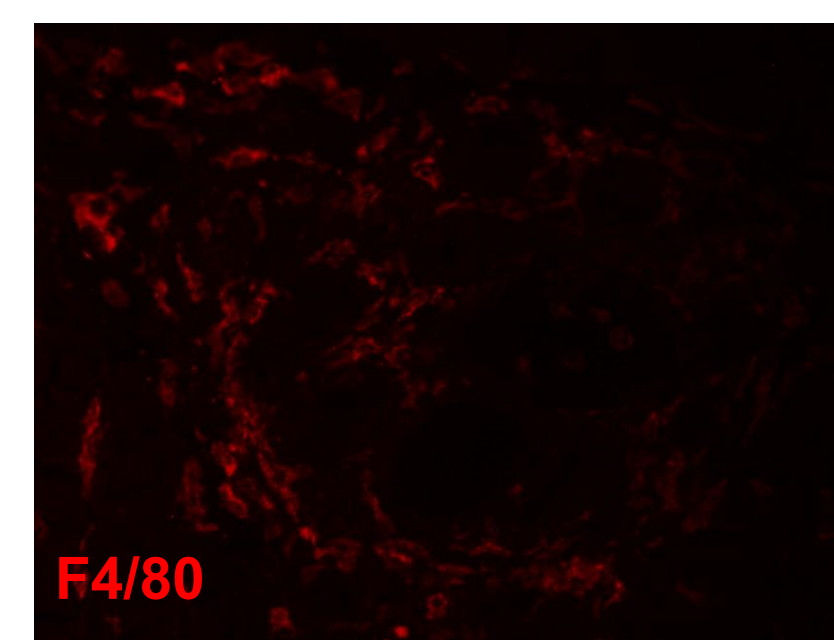
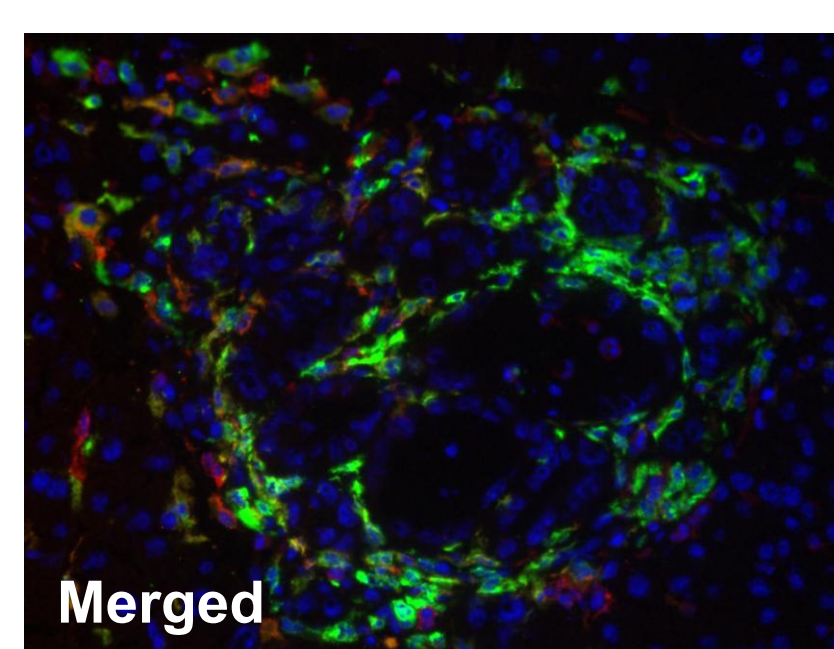
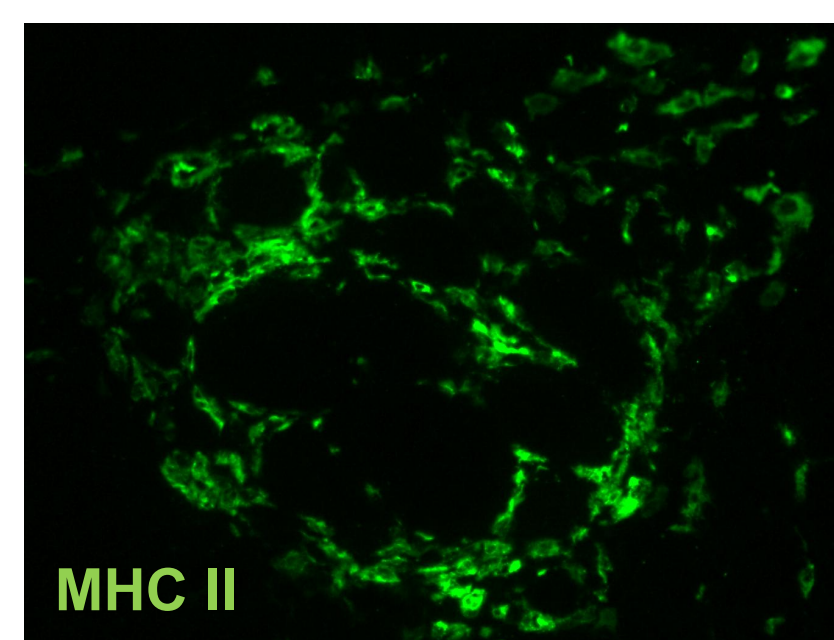


Day 2 Cerulein

Phenotyping Lesions (IF 1 +2)

Macrophages trigger immune warning signals in KC Lesions

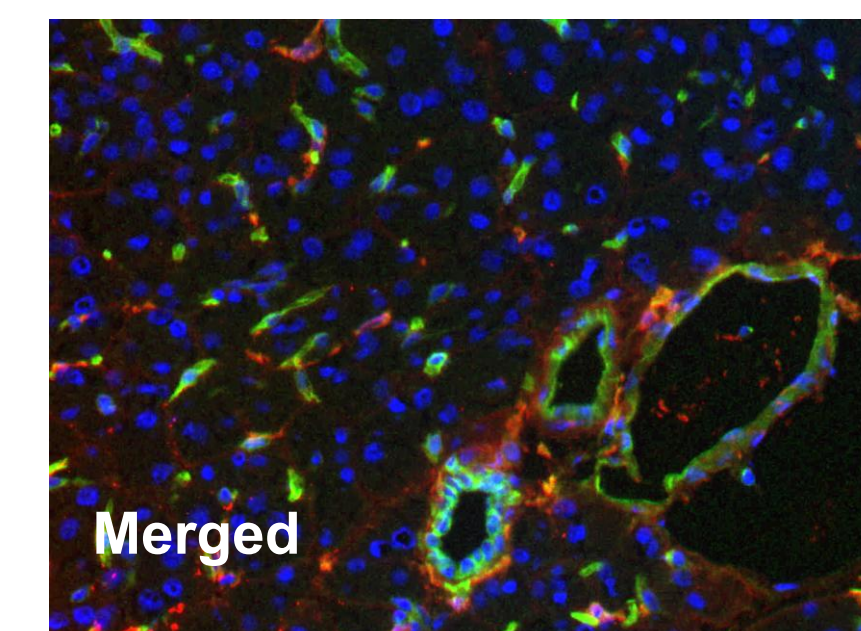
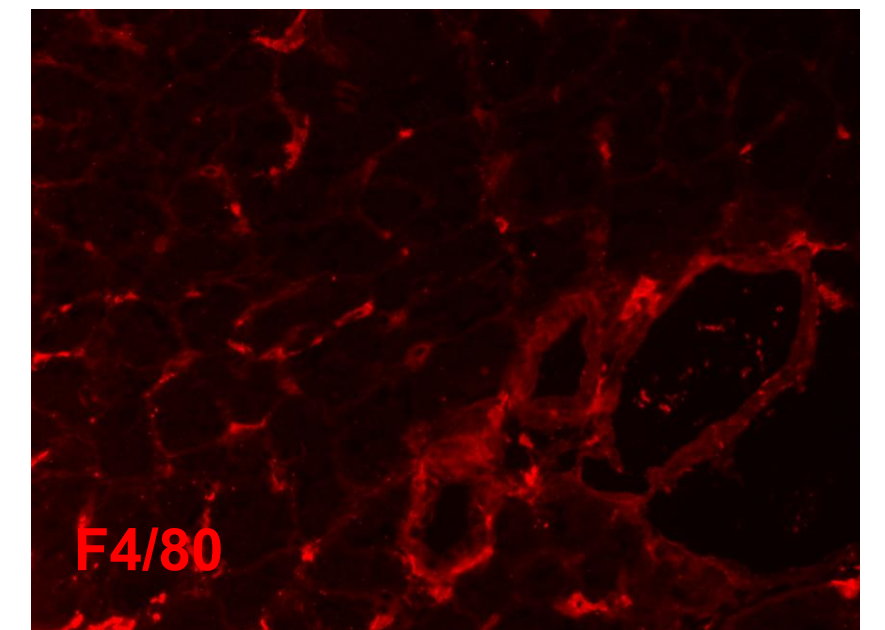
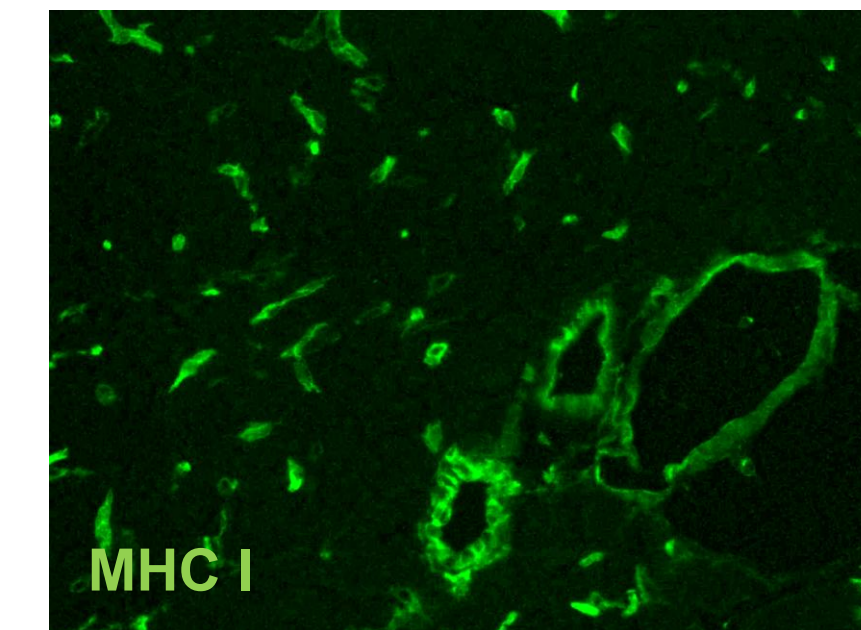
•**Macrophage Activation:** Strong colocalisation of F4/80 (Red) and MHC-II (Green) was seen. Shows infiltrating macrophages upregulate antigen presentation.



•**Cell Stress:** MHC-I was concentrated in *Kras*-mutant lesions rather than healthy acinar cells.

•**Cellular Identification:** Costaining confirmed that MHC-I and MHC-II populations around ADMs were mainly macrophages.

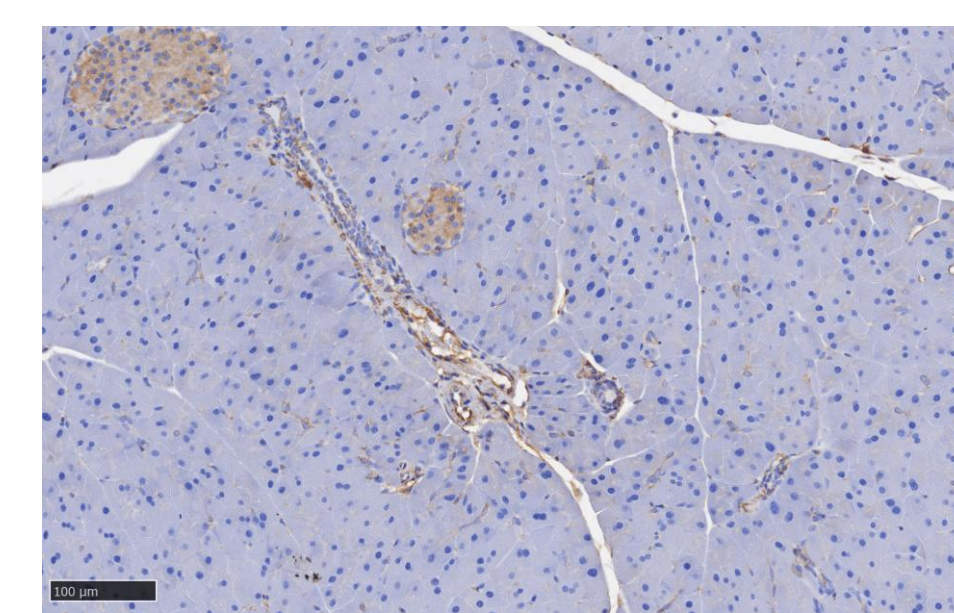
Macrophages trigger immune warning signals in KC Lesions



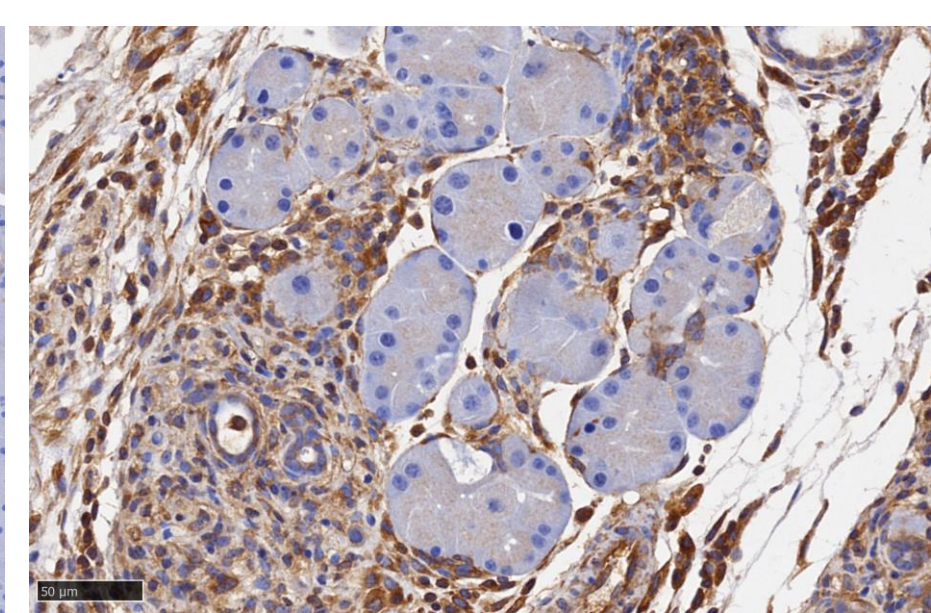
(Above) - Macrophages form a perimeter around ADMs. Confocal imaging shows F4/80 macrophages (Red) colocalising with MHC-I ductal membranes (Green).

B2M Expression in KC ADMs

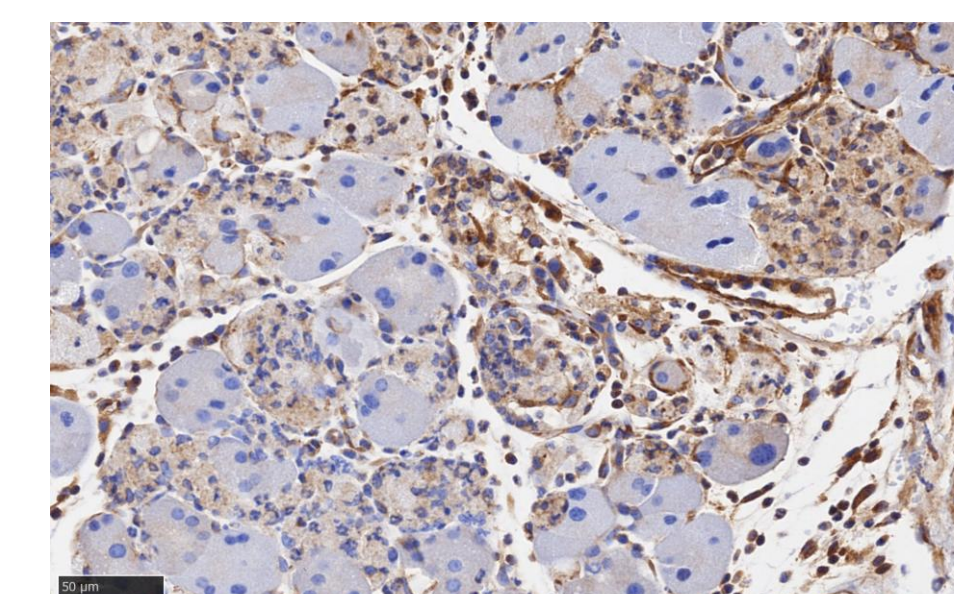
IHC 2 - Performed using B2M antibody on pancreatic tissue from KC mice and cerulein-treated mice (Day 2 and Day 7). Tissue slides were imaged to screen for healthy regions (with or without mosaic staining) and areas of ADMs.



PBS Control Day 7



Cerulein Day 7 With ADMs



Cerulein Day 2 with ADMs

Key Takeaways

- Cerulein inflammation induces short term B2M/MHC-I upregulation that recovers by Day 7, but *Kras* mutation drives heavy B2M display around ADMs.
- Macrophages are primary APCs : Infiltrating MHC1 and 2 populations co-localize mainly with F4/80 macrophages in lesion borders