Figure S1, related to Figure 1

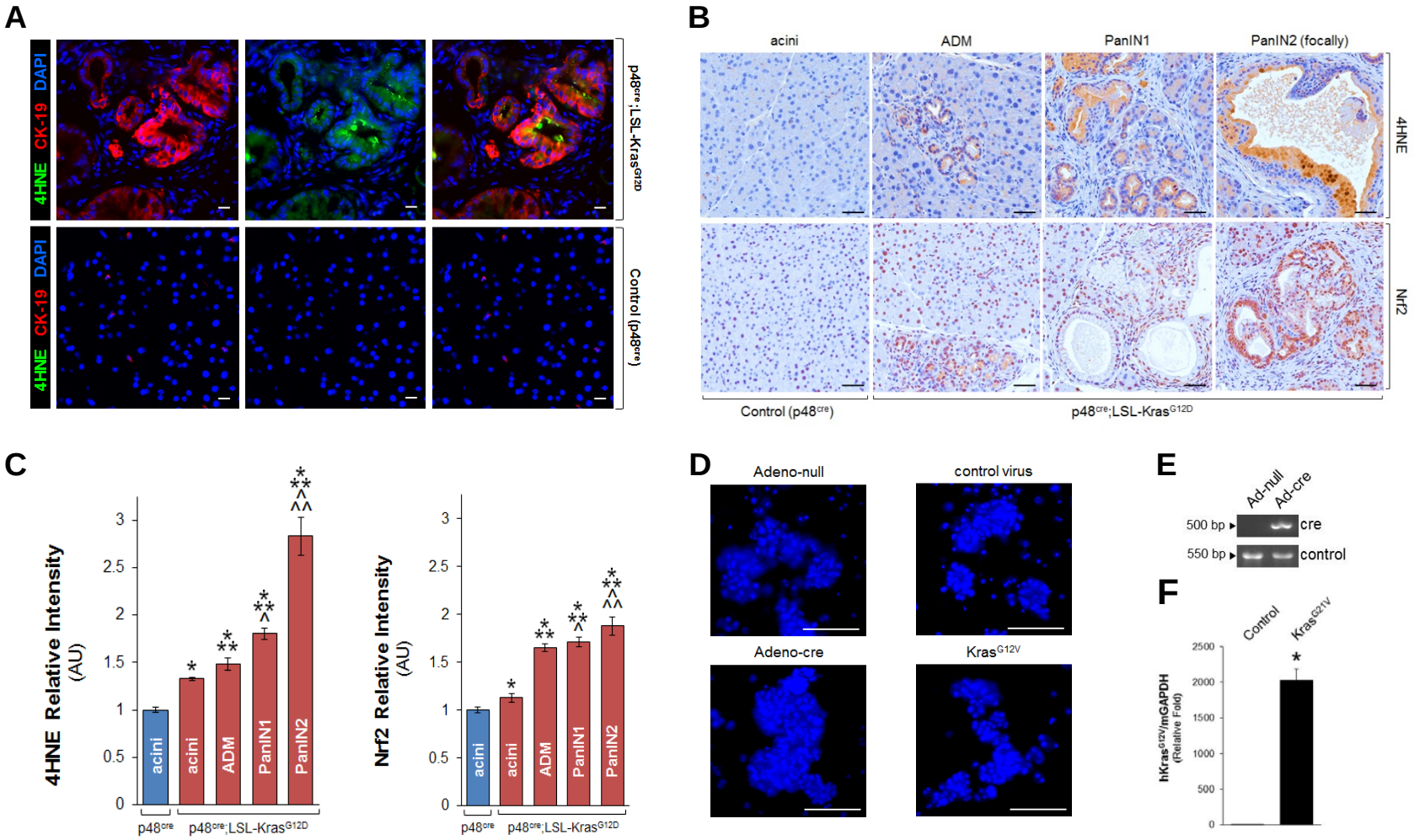

Figure S1, related to Figure 1: **A, B: Oxidative stress occurs in regions of acinar cell metaplasia and PanIN. A:** Pancreata of p48^{cre};LSL-Kras^{G12D} or control (shown is p48^{cre}) mice at an age of 6 months were analyzed for expression of CK-19 (ductal cell marker, indicates ADM) or presence of 4HNE (oxidative stress marker) using immunofluorescence labeling. DAPI marks the nuclei. The first two rows show single channels and right side shows a composite. The bar indicates 25 µm. **B: Occurrence of oxidative stress correlates with increased nuclear expression of Nrf2.** Pancreata of p48^{cre};LSL-Kras^{G12D} or control (shown is p48^{cre}) mice at an age of 6 months were analyzed by IHC for presence of 4HNE and Nrf2. The bar indicates 50 µm. **C: Relative increase in 4-HNE and Nrf2 expression in abnormal pancreatic structures.** Quantification of 4HNE and Nrf2 expression form S1B. IHC staining intensity was quantified using image J software and then normalized to the staining intensity in normal acinar cells of a control mouse (p48^{rce}). * indicates p<0.05 as compared to acini (control mouse), ** indicates p<0.05 as compared to acini (KC mouse), ^ indicates p<0.05 as compared to ADM, ^^ indicates p<0.05 as compared to PanIN1 (includes PanIN1A/PanIN1B). **D: Viability controls after infection with adenovirus or lentivirus.** In order to detect cell damage due to viral infection, viability of acinar cells was determined 48 hours post infection using the live cell dye Hoechst 33342. Panels of the left show controls for Fig. 1D and panels on the right controls for Fig.1E. The bars indicate 100 µm. **E, F: Expression controls for genes introduced by viral infection.** E shows controls for Fig. 1D in which gDNA was isolated 48 hours after viral infection and analyzed for cre expression using 5'-TCGCGATTATCTTCTATATCTTCAG-3' and 5'-GCTCGACCAGTTTAGTTACCC-3 as PCR primers. F shows controls for Fig.1E in which expression of human Kras^{G12D} 48 hours after infection was determined by qPCR using a primer set from Applied Biosystems (Kras Hs00270666_g1).

Figure S2, related to Figure 2

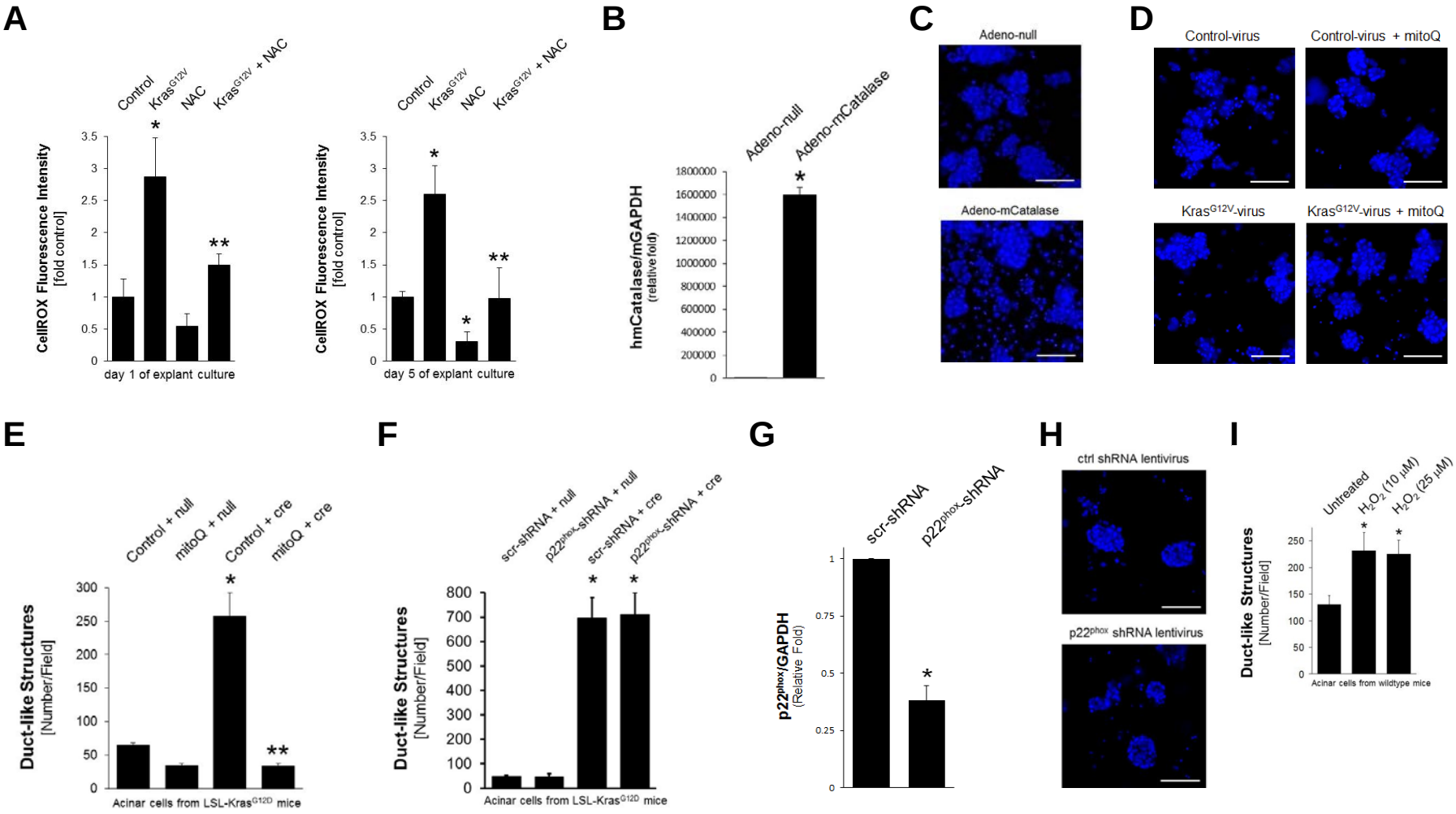

Figure S2, related to Figure 2: **ADM in explant culture is mediated by mitochondrial oxidative stress. A:** Control for Fig.2A. Detection and quantitation of reactive oxygen species (ROS) in live cells. At day 1 or day 5 of explant culture ROS were determined using CellROX® deep red as described in the Supplemental Experimental Procedures. * indicates statistical significance ($p<0.05$) as compared to control-treated cells; ** indicates statistical significance ($p<0.05$) as compared to $Kras^{G12V}$. **B, G:** 48 hours after infection, expression of mCatalase (B) or $p22^{phox}$ (G) was determined by qPCR using a primer sets from Applied Biosystems (p22phox Mm00514478_m1; Catalase Hs00156308_m1). * indicates statistical significance ($p<0.05$) as compared to adeno-null control. **C, D, H:** Viability controls after infection with adenovirus or lentivirus. In order to detect cell damage due to viral infection, viability of acinar cells was determined 48 hours post infection using the live cell dye Hoechst 33342. The bars indicate 100 μm. **E:** Primary mouse pancreatic acinar cells were isolated from LSL-$Kras^{G12D}$ mice, infected with adeno-null or adeno-cre, and then seeded in presence of mitoQ or control in 3D collagen explant culture. At day 5, ADM events (number of ducts per field) were counted. * indicates statistical significance ($p<0.05$) as compared to control + null; ** as compared to control + cre. **F:** Primary mouse pancreatic acinar cells were isolated from LSL-$Kras^{G12D}$ mice, double-infected (as indicated) with adeno-null or adeno-cre combined with lentivirus harboring control (scr-shRNA) or $p22^{phox}$-shRNA, and then seeded in 3D collagen explant culture. At day 5, ADM events (number of ducts per field) were counted. * indicates statistical significance ($p<0.05$) as compared to scr-shRNA + null. **I:** Primary mouse pancreatic acinar cells were isolated and seeded in 3D collagen explant culture in presence of indicated doses hydrogen peroxide. At day 9, ADM events (number of ducts per field) were counted. * indicates statistical significance ($p<0.05$) as compared to untreated cells.

Figure S3, related to Figure 3

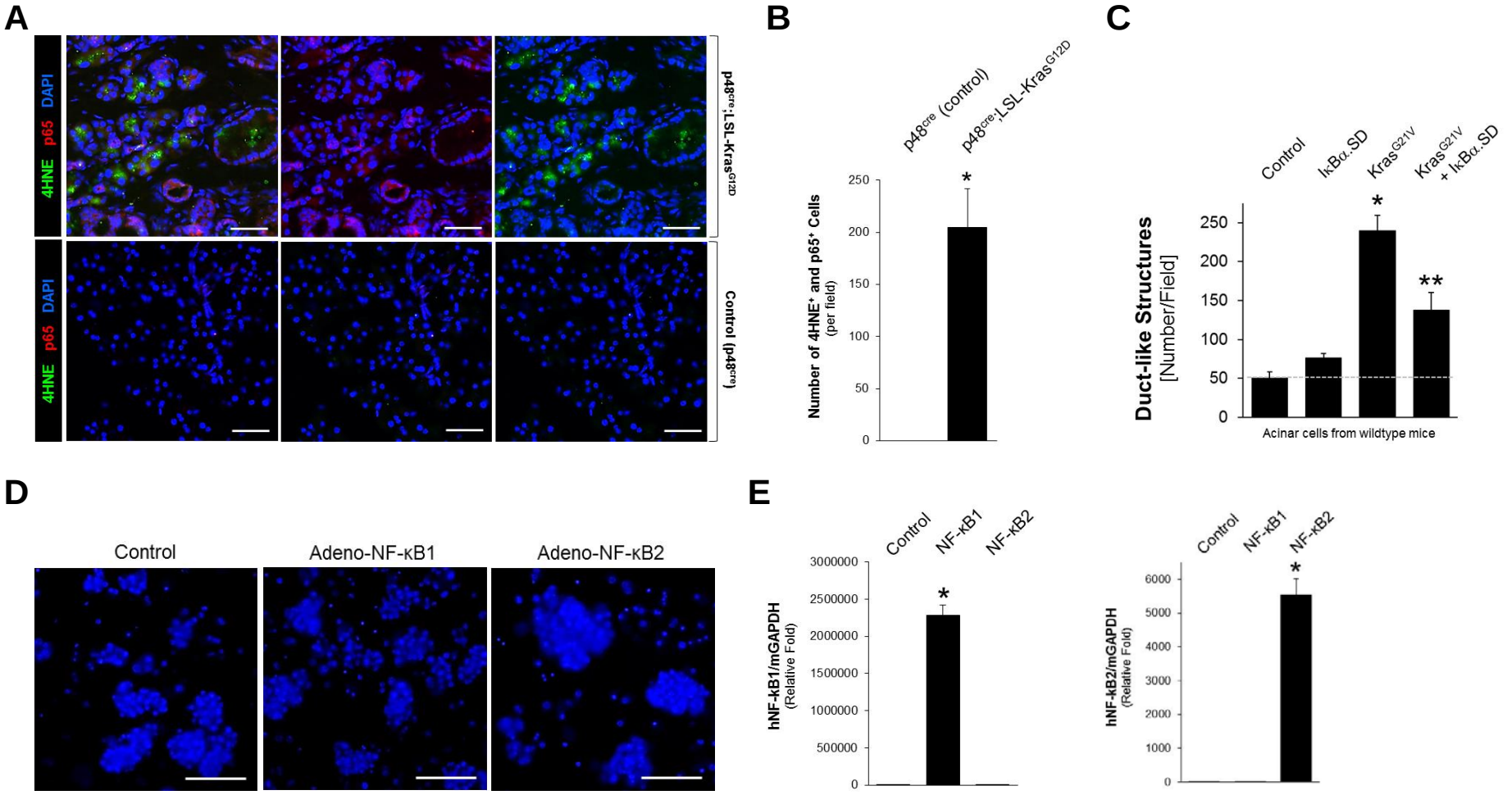

Figure S3, related to Figure 3: **Oncogenic Kras can drive ADM through the canonical NF-κB pathway. A:** Pancreatic sections from control mice or mice expressing the LSL-Kras^{G12D} allele under the p48 promoter (p48^{cre};LSL-Kras^{G12D}) were analyzed by immunofluorescence for expression of p65 (RelA) and 4HNE. Nuclei were stained with DAPI. Shown is a characteristic area of the pancreas. The bar indicates 25 µm. **B:** Quantification of 4HNE and p65 positive cells in A. N = 20 fields (covering the whole tissue sample) were analyzed using ImageJ. The asterisk indicates statistical significance ($p<0.05$) as compared to control. **C:** Primary mouse pancreatic acinar cells were isolated and adenovirally-infected with adeno-null (control, empty virus) or adeno-IκBα.SD (super-dominant IκBα), and lentivirus harboring control (null) or Kras^{G12V}, as indicated. Acinar cells then were seeded in 3D collagen explant culture. At day 5, ADM events (number of duct-like structures per field) were counted. * indicates statistical significance (p,0.05) as compared to control; ** indicates statistical significance ($p<0.05$) as compared to Kras^{G12V}. **D:** Viability controls for Fig. 3D. In order to detect cell damage due to viral infection, viability of acinar cells was determined 48 hours post infection using the live cell dye Hoechst 33342. The bars indicate 100 µm. **E:** Expression controls. 48 hours after infection, expression of NF-κB1 or NF-κB2 was determined by qPCR using a primer sets from Applied Biosystems (NF-κB1 Hs00765730_m1; NF-κB2 Hs01028901_g1). * indicates statistical significance ($p<0.05$) as compared to control.

Figure S4, related to Figure 4

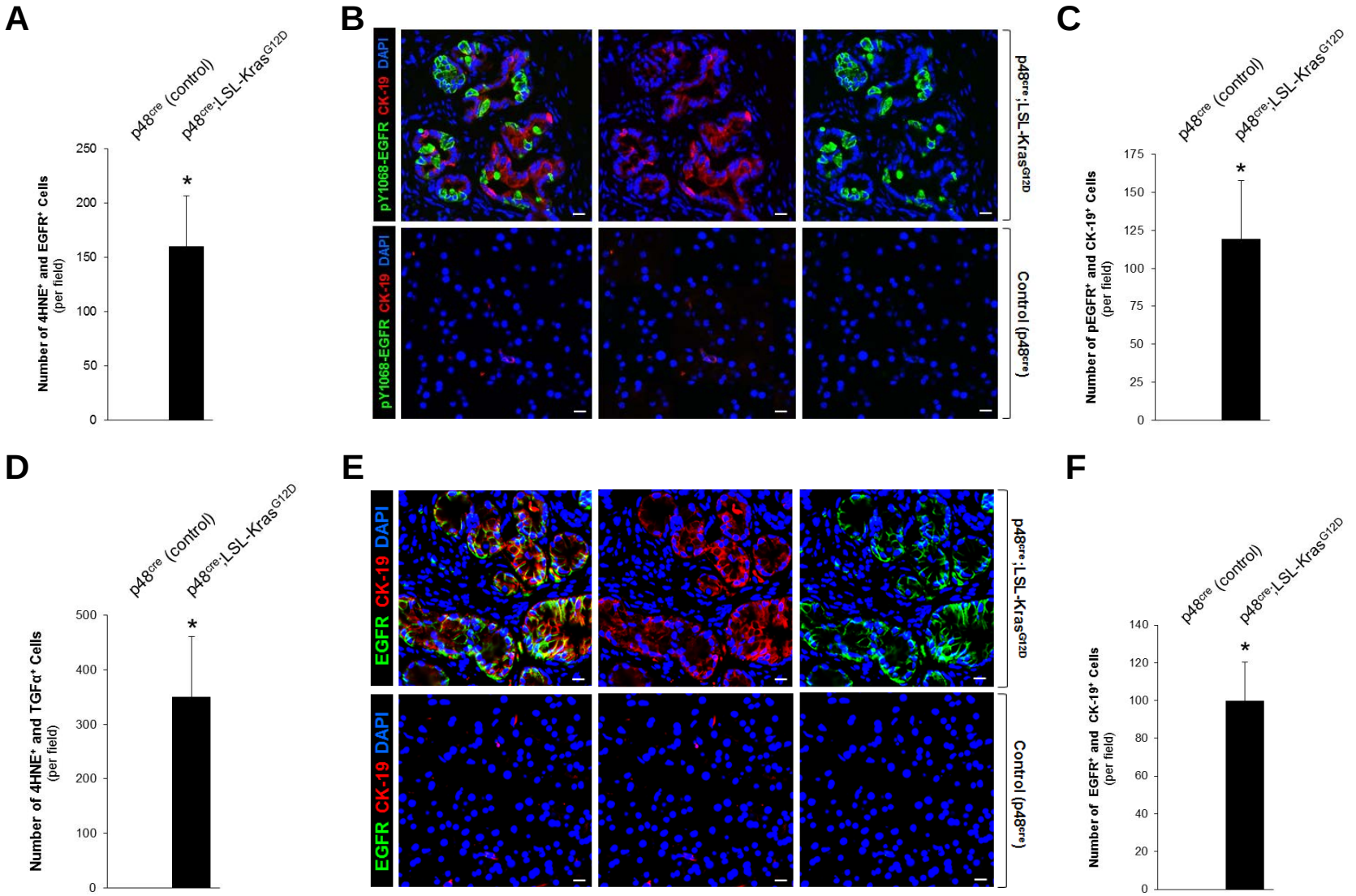

Figure S4, related to Figure 4: **A, C, D, F:** Quantification of cells positive for indicated markers. Suppl. Fig. S4A corresponds to Fig. 4B, Suppl. Fig. S4C to Suppl. Fig. S4B, Suppl. Fig. S4D corresponds to Fig. 4C and Suppl. Fig. S4F to Suppl. Fig. S4E. "fields" defines an area in the pancreas with n=20 fields covering the whole section. Analysis was performed using ImageJ. The asterisk indicates statistical significance ($p<0.05$) as compared to control. **B, E: Expression of pY1068-EGFR and EGFR in regions of pancreatic ADM and PanIN formation.** Pancreatic sections from control mice ($p48^{cre}$) or mice expressing the LSL-$Kras^{G12D}$ allele under the p48 promoter ($p48^{cre}$;LSL-$Kras^{G12D}$) were analyzed by immunofluorescence for expression of pY1068-EGFR (**B**) or EGFR (**E**) and the ductal marker CK-19. Nuclei were stained with DAPI. Shown is a characteristic area of the pancreas. The bar indicates 25 µm.

Figure S5, related to Figure 5

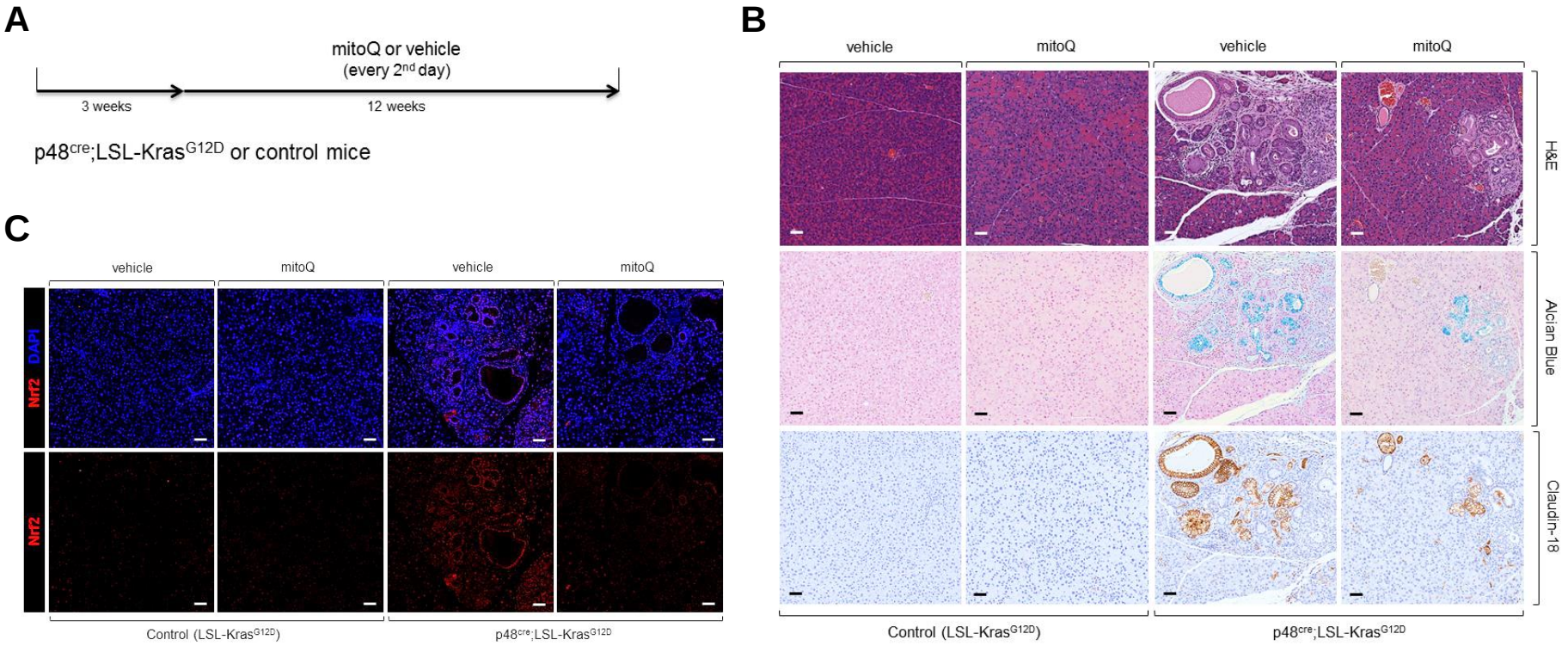

Figure S5, related to Figure 5: **Depletion of mitochondrial oxidative stress decreases abnormal pancreatic structures. A:** Treatment schedule for Figure 5. **B:** Tissue samples from Figure 5A additionally were stained with alcian blue or analyzed by IHC for Claudin-18 expression (marker for PanIN lesions). A representative area of the pancreas tissue under each condition is shown. Scale bar is 50 µm. **C:** Samples from Figure 5A additionally were analyzed by immunofluorescence for expression of Nrf2. Nuclei were stained with DAPI. Shown is a characteristic area of the pancreas. The bar indicates 50 µm.

Figure S6, related to Figure 6

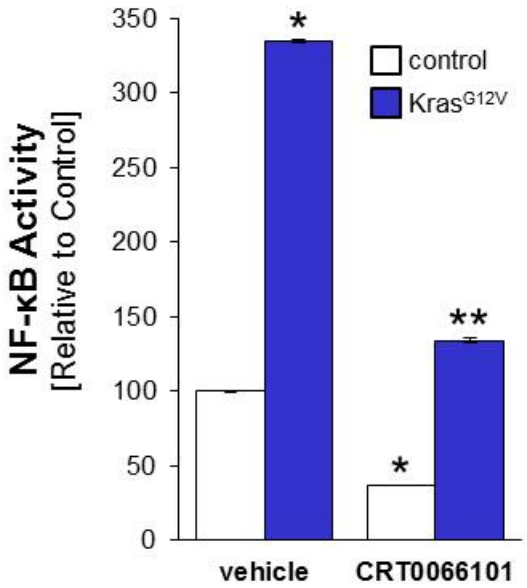

Figure S6, related to Figure 6: **Oncogenic Kras activates nuclear factor-κB through PKD.** Primary mouse pancreatic acinar cells were isolated, co-infected with lentivirus harboring control or $Kras^{G12V}$ virus and adenovirus harboring a NF-κB-luciferase reporter gene. Cells were seeded in presence of the PKD inhibitor CRT0066101 (2.5 μM), as indicated. 48 hours after virus infection, cells were isolated and a luciferase assay performed to measure NF-κB activity. * indicates statistical significance ($p<0.05$) as compared to control; ** indicates statistical significance ($p<0.05$) as compared to $Kras^{G12V}$.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Measurement of ROS Generation for Figure S2A

Primary pancreatic acinar cells in 3D explant culture at indicated times were loaded with 5 μM CellROX Deep Red (Life Technologies, Carlsbad, CA) and 2.5 μg/ml Hoechst 33342 for 30 minutes at 37 °C in complete Waymouth's media, washed 3 times with Live Cell Imaging Solution and emission was measured at 677 nm (excitation at 660 nm) for the CellROX Deep Red and at 460 nm (excitation at 355 nm) for Hoechst 33342 using a SpectraMax M5 fluorescent microplate reader (Molecular Device, Sunnyvale, CA). Signals obtained with CellROX Deep Red were normalized to Hoechst 33342.