



Supplementary Figure 8. Nix-depleted PDAC cells normalize mitochondrial mass and upregulate Bnip3 in hypoxic conditions.

A) Representative images of H&E staining and IHC staining for Nix, Ki67 and p-Erk in tumors from end-stage (survival study) KPC and KPCNix^{Δ/Δ} tumors. Black arrows: epithelial compartment, blue arrows: stromal compartment. Scale bar: 50 μ m. B) Flow cytometry analysis of tumor organoids derived from KPC and KPC Nix^{Δ/Δ} mice and grown in low glucose medium, stained with MitoTracker Green, DiIC15 and MitoSoxRed. Median fluorescent intensity (MFI) normalized to means for KPC organoids, n=3 biological replicates per genotype. C) Western blot for levels of Tomm20, Nix, and Hsp90 (loading control) for whole cell lysates from tumor organoids as in (B). D) Proliferation measured by iRFP intensity in siNT or siNIX FA6 cells grown in normoxia (20% O₂) or hypoxia (1% O₂). E) Immunoblot for Hif1a, Nix, Bnip3, total ERK (p42/p44, loading control) in siNT and siNIX FA6 cells grown in low glucose medium in hypoxic (1% O₂) and normoxic (21% O₂) conditions. F) Immunoblot for Bnip3 and Hsp90 (loading control) in age-matched (16-week old) KPC and KPCNix^{Δ/Δ} organoids grown in low glucose medium in hypoxic (0.5% O₂) and normoxic (21% O₂) conditions.