SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure 1. Activated CAF display increased expression of α-SMA and Tenascin C. hFB, LX-2, hCAF and human CCA cells were grown to subconfluency and mRNA was isolated. α-SMA (panel A) and tenascin c (panel B) mRNA expression was quantified by real-time PCR, normalized to 18S rRNA (mean ± SEM; n=3; ** p≤0.01).

Supplemental Figure 2. Expression of Bcl-2 proteins varies between CCA cell lines and corresponds to navitoclax induced Bax activation. Whole cell lysates were prepared from the untreated human CCA cell lines HuCCT-1, Mz-ChA-1, KMCH and KMBC. Cell lysates were subject to immunoblot analysis of Bcl-2 proteins. Full-length blots/gels are presented in Supplementary Figure 6.

HuCCT, KMCH and KMBC cells were grown to approximately 50% confluency on glass chamber slides and treated with navitoclax (1 μM) for 24 hrs. Cells were then analyzed by fluorescence microscopy using a conformation-specific antibody (6A7) against activated Bax. Bax positive cells are plotted as percentage of total cells (panel C; mean ± SEM; n=4; ** p≤0.01).

Supplemental Figure 3. Pretreatment of quiescent fibroblasts with TGF-β induces sensitivity to navitoclax. Human quiescent fibroblast were incubated with TGF-β (10 ng/ml; 24 hrs) or vehicle and subsequently treated with navitoclax (1 μM) or vehicle for 48 hrs. Apoptosis was then assessed by DAPI-staining and fluorescence microscopy (upper graph; mean ± SEM; n=3; * p≤0.05) or fluorescent analysis of caspase 3/7 activity displayed as fold change compared to vehicle control (lower graph; mean ± SEM; n=6; * p≤0.05).
**Supplemental Figure 4. Navitoclax induces apoptosis in rat CAF but not BDEnue cells.** Rat control fibroblasts (rFb) and rat cancer associated fibroblasts (rCAF) cells were grown to subconfluency and treated with the indicated doses of navitoclax or vehicle for 48 hrs. Cells were analyzed for apoptotic nuclear morphology by DAPI-staining and fluorescence microscopy (panel A; mean ± SEM; n=3; * p ≤ 0.05).

rCAF and BDEnue rat CCA cells were grown on multiwell plates and treated with navitoclax (1 μM) or vehicle for 48 hrs. Apoptosis was measured by DAPI-staining and fluorescence microscopy (panel B, upper graph; mean ± SEM; n=3; * p ≤ 0.05) or fluorometric analysis of caspase3/7 activity displayed as fold change compared to vehicle control (panel B, lower graph; mean ± SEM; n≥5; ** p ≤ 0.01).

**Supplemental Figure 5. Navitoclax induces Bax activation and Smac release predominantly in stromal cells in-vivo.**

Frozen sections of BDEnue tumors from animals treated with two doses of vehicle or navitoclax (5mg/kg) were fixed in 4% paraformaldehyde and permeabilized with 0.02% CHAPS at 37°C or fixed with 100% Acetone at -20°C respectively. Sections were then stained for activated Bax employing the conformation specific antibody 6A7 (green) and co-stained for α-smooth muscle actin (α-SMA; red) (Panel A). Areas of α-SMA negative tumor cells are outlined. In panel B, frozen sections were stained for the mitochondrial marker Smac (labeled in green) with a counterstaining for cytokeratin-7 (CK-7, red). CK-7 positive tumor cells are outlined. Note that the punctate mitochondrial Smac staining is lost in the stromal compartment after navitoclax treatment as Smac undergoes rapid proteosomal degradation after release from the mitochondria (Hu and Yang, JBC 2003). Nuclei were marked with DAPI (blue).