Supplementary Materials: Cold Atmospheric Plasma-Treated PBS Eliminates Immunosuppressive Pancreatic Stellate Cells and Induces Immunogenic Cell Death of Pancreatic Cancer Cells

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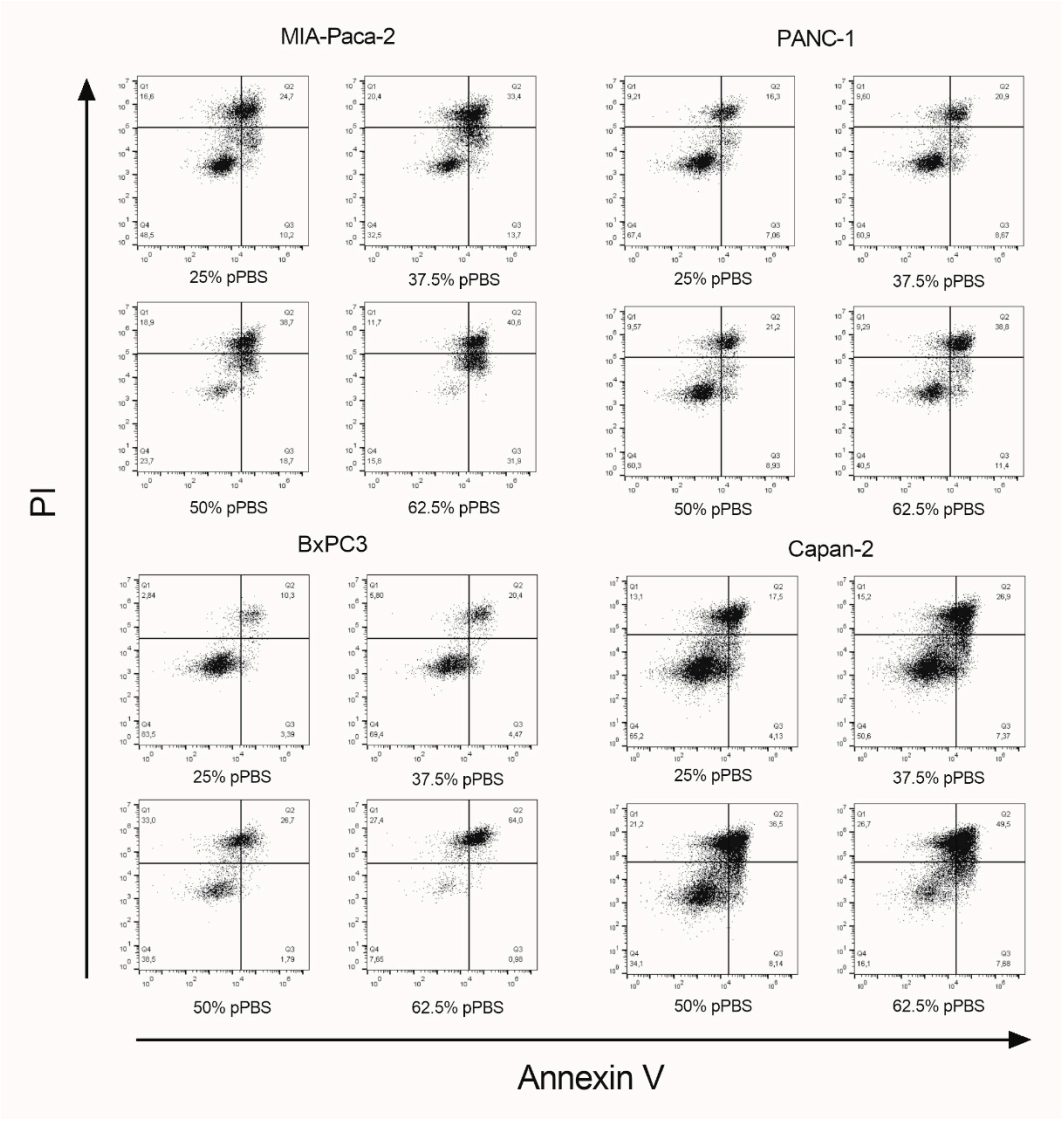

Figure S1. Dot plots showing the flow cytometric analysis of Annexin V and PI staining after 25%, 37.5%, 50% and 62.5% pPBS treatment in all PCC lines. Q1 = AnnV-/PI+; Q2 = AnnV+/PI+; Q3 = AnnV-/PI-; Q4 = AnnV+/PI-.

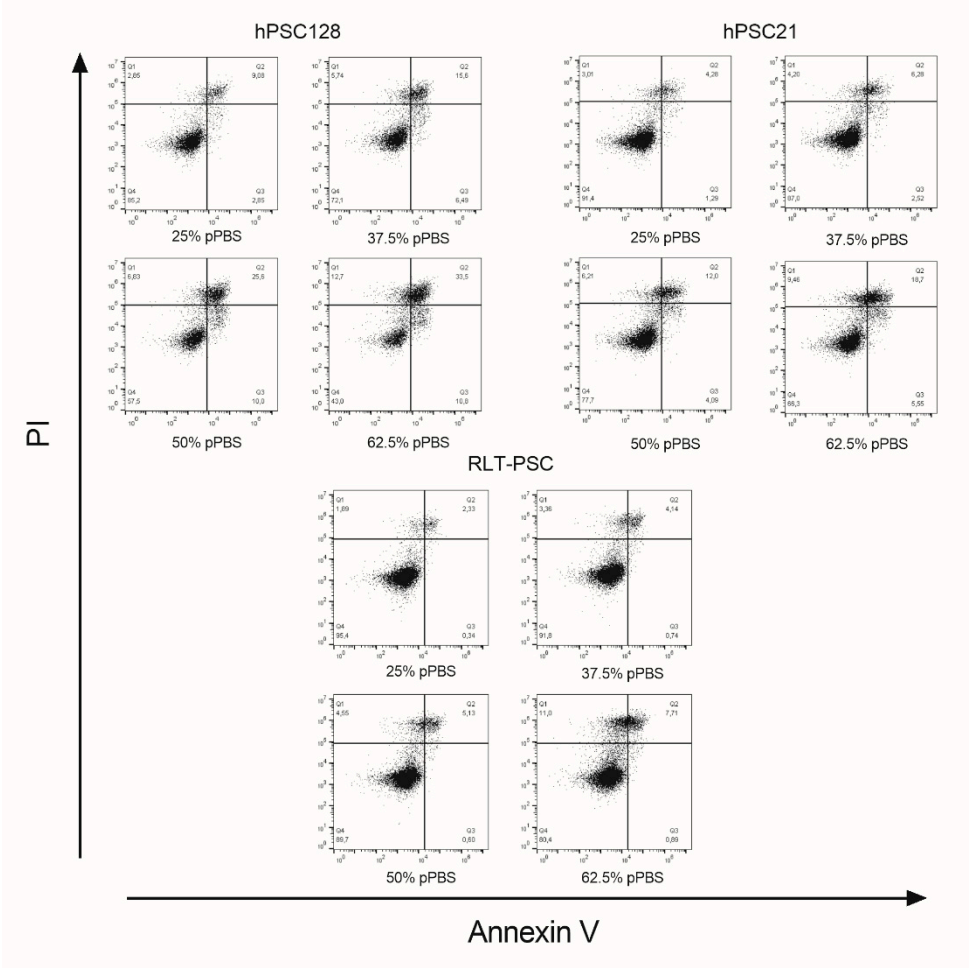

Figure S2. Dot plots showing the flow cytometric analysis of Annexin V and PI staining after 25%, 37.5%, 50% and 62.5% pPBS treatment in all PSC lines. Q1 = AnnV-/PI+; Q2 = AnnV+/PI+; Q3 = AnnV-/PI-; Q4 = AnnV+/PI-.

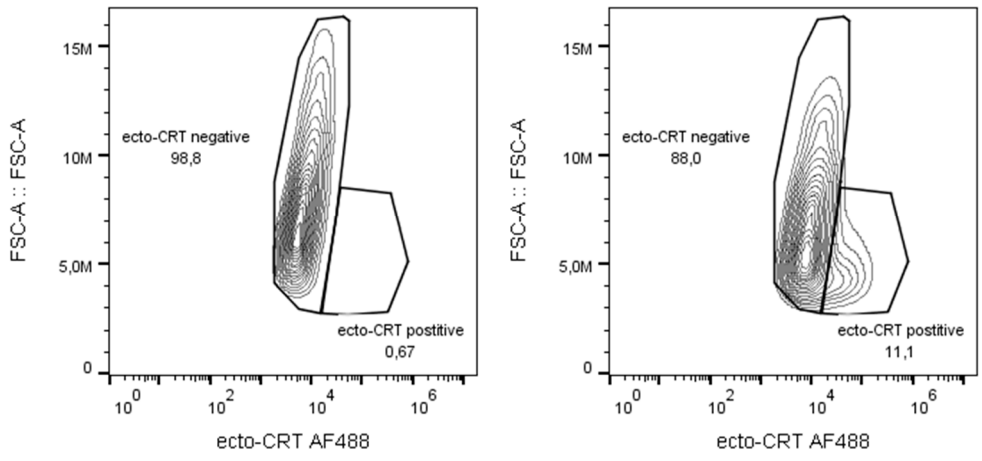

Figure S3. Gating strategy of surface exposure of ecto-CRT. Contour plots showing the flow cytometric analysis of ecto-CRT staining after 48h of 50% pPBS treatment (left) compared to untreated condition (right) in the BxPC3 cell line (PI- cells) showing the differences in Δ MFI (MFI treated vs MFI untreated).

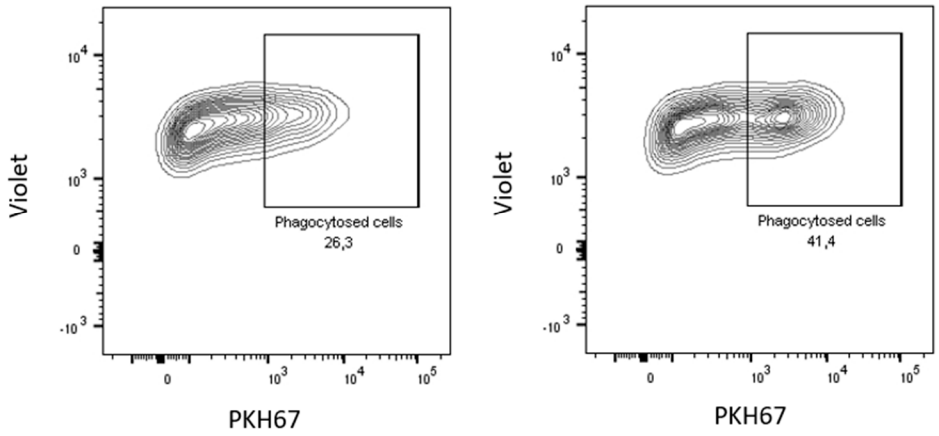

Figure S4. Gating strategy of phagocytosis. Contour plots showing flow cytometric analysis of percentage phagocytosis (left: MIA-Paca-2, untreated; right: MIA-Paca-2, 50% pPBS treatment). Target cells labelled with PKH67 dye and DC labeled with CellTracker Violet BMQC dye are cocultured for 48h (E:T ratio, 1:1). Phagocytosis of the PKH67+ target cells by violet labeled DC is expressed as the %violet+PKH67+ cells within the violet+ DC population.

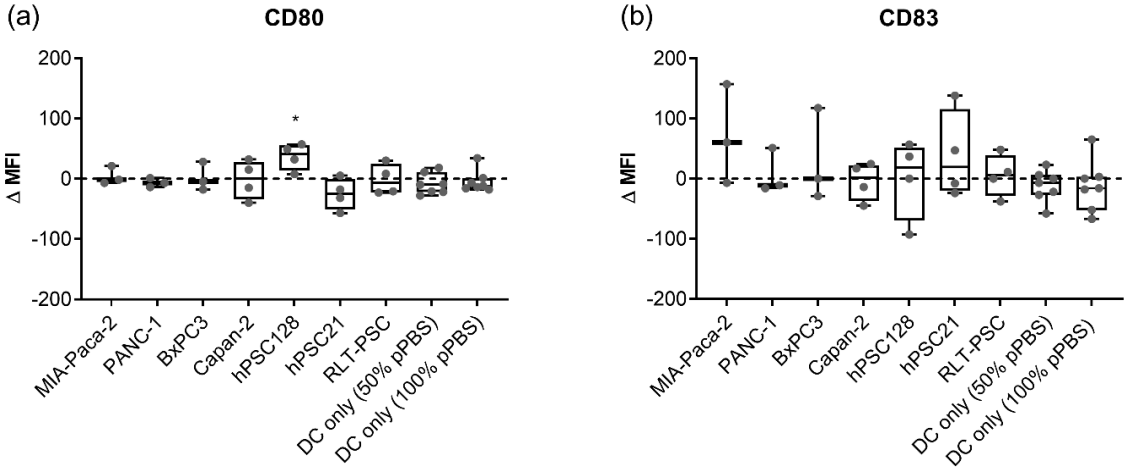

Figure S5. CD80 and CD83 expression on DC after coculture with pPBS-treated PSC and PCC. (**a**) Boxplot from minimum to maximum value of Δ MFI of the maturation marker CD80. (**b**) Boxplot from minimum to maximum value of Δ MFI of the maturation marker CD83. CD80 and CD83 expression is examined on immature DC after 48h of coculture of pPBS-treated PCC and PSC (E:T ratio, 1:1) and after pPBS treatment on immature DC without coculture using flow cytometry. Δ MFI represents [(MFI staining treated – MFI isotype treated) – (MFI staining untreated – MFI isotype untreated)]. Treatment of 50% pPBS is used for MIA-Paca-2 and Capan-2, treatment of 100% pPBS is used for PANC-1, BxPC3, hPSC128, hPSC21 and RLT-PSC. Every dot represents a different healthy donor with ≥3 donors used per cell line. $p<0.05$ significant differences compared to untreated control (*).