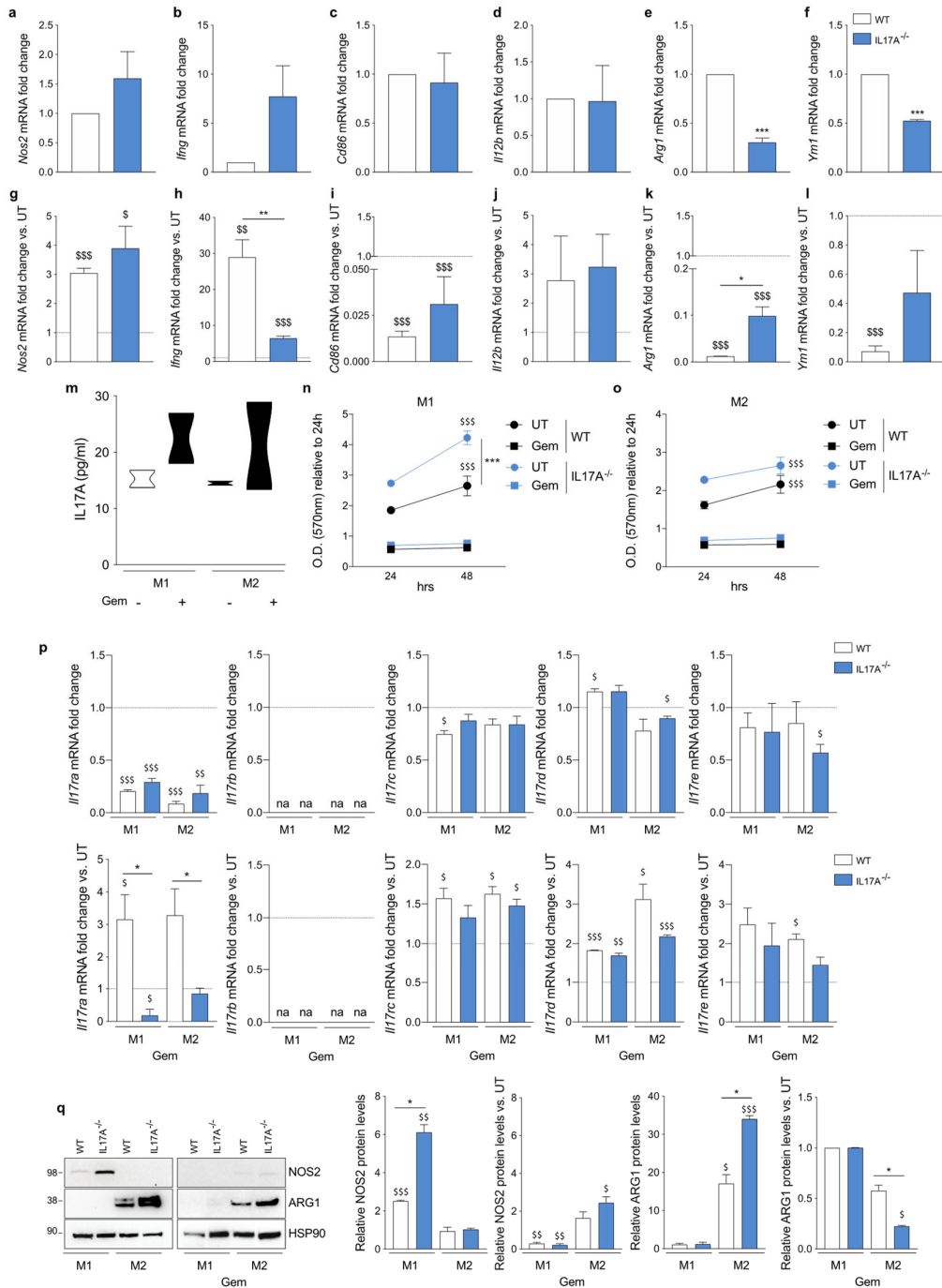


Supplementary Materials:

Supplementary Figure 1



Supplementary Figure S1. IL17A affects macrophage polarization, viability and receptor expression. *Nos2* (a), *Ifng* (b), *Cd86* (c), *Il12b* (d), *Arg1* (e) and *Ym1* (f) mRNA levels in WT (white bars) or IL17A^{-/-} (blue bars) M0 untreated bone-marrow-derived macrophages (BMDMs). ***P ≤ 0.001 values from IL17A^{-/-} macrophages were significantly different from those of WT cells. *Nos2* (g), *Ifng* (h), *Cd86* (i), *Il12b* (j), *Arg1* (k) and *Ym1* (l) mRNA levels in WT (white bars) or IL17A^{-/-} (blue bars) M0 treated with gemcitabine bone-marrow-derived macrophages (BMDMs). *P ≤ 0.05, **P ≤ 0.01 values from IL17A^{-/-} macrophages were significantly different from those of WT cells. *Nos2* (g), *Ifng* (h), *Cd86* (i), *Il12b* (j), *Arg1* (k) and *Ym1* (l) mRNA levels in WT (white bars) or IL17A^{-/-} (blue bars) M0 treated with gemcitabine bone-marrow-derived macrophages (BMDMs). IL17A levels

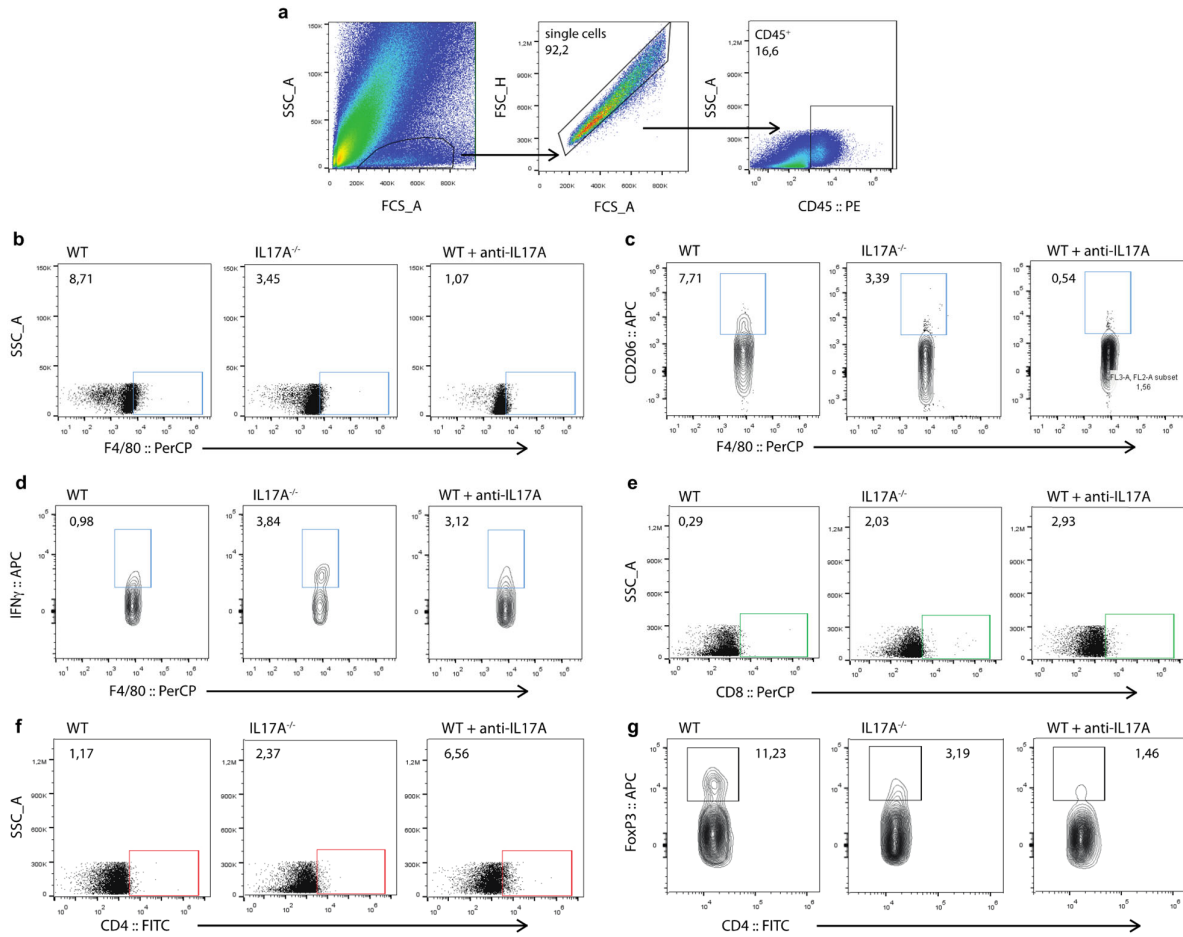
were quantified by ELISA in supernatants from M1- or M2-like WT BMDMs, untreated or treated with gemcitabine for 24 h (**m**). 24-48 h MTT assay performed with M1(**n**) or M2 (**o**) polarized WT (black symbols) or IL17A^{-/-} (blue symbols) BMDMs. ***P ≤ 0.001 values from IL17A^{-/-} macrophages were significantly different from those of WT cells. \$\$\$P ≤ 0.001 values from untreated macrophages were significantly different from those of gemcitabine-treated cells. *Il17ra*, *Il17rb*, *Il17rc*, *Il17rd* and *Il17re* mRNA levels in WT (white bars) or IL17A^{-/-} (blue bars) BMDMs (**p**). Graphs represent the fold-change versus relative untreated M0 cells (upper panels) or M1-like or M2-like cells (lower panels). Data are expressed as means ± SEM of biological replicates. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 values from IL17A^{-/-} were significantly different from those in WT macrophages. *P ≤ 0.05, **P ≤ 0.01, \$\$\$P ≤ 0.001 values from gemcitabine-treated macrophages were significantly different from those of M1-like or M2-like untreated cells. (**q**) Representative Western Blot images for NOS2 and ARG1 with proteins extracted from untreated M1- and M2-like macrophages or those with gemcitabine for 24 h. HSP90 was used as a loading control. Graphs represent the quantification of NOS2 and ARG1 bands from two independent immunoblotting experiments. *P ≤ 0.05 values from IL17A^{-/-} BMDMs were significantly different from those in WT macrophages. *P ≤ 0.05, **P ≤ 0.01, \$\$\$P ≤ 0.001 values from gemcitabine-treated macrophages were significantly different from those of M1-like or M2-like untreated cells.

Supplementary Table S1. Summary of changes induced by gemcitabine treatment in macrophages in the presence or absence of IL17A.

Markers and metabolic pathways		WT M1		IL17A ^{-/-} M1		WT M2		IL17A ^{-/-} M2	
		-	+	-	+	-	+	-	+
<i>Nos2</i> (mRNA)	Gem								
NOS2 (protein)			nd ^a		nd	nd		nd	
<i>Arg1</i> (mRNA)									
ARG1 (protein)		nd	nd	nd	nd				
<i>Ifng</i>									
<i>Cd86</i>									
<i>Il12b</i>									
<i>Ym1</i>									
PPP flux									
Lactate									
FAO									
TCA flux									
ETC									
ATP									
Glutaminase									

^a Not detected by Western Blotting.

Red boxes represent an increase, green boxes a reduction and black boxes unchanged levels compared with control samples: M0 untreated macrophages for all the conditions in the absence of gemcitabine, and M1- or M2-like untreated macrophages when gemcitabine is present.



Supplementary Figure S2. Gating strategy for immune cells infiltrating pancreatic cancer. Representative flow cytometry plots for intra-tumor cell populations, showing the gating strategy. Data are representative of $n = 5$ mice/group. Total CD45⁺ cells were first gated on single cells deriving from a Forward Scattered (FSC_A)/Side Scattered (SSC_A) plot (a). CD45⁺ cells were further gated for the subsets of interest, namely F4/80⁺ cells (b), CD206⁺ (c) and IFN γ ⁺ (d) gated on the F4/80⁺ cells, CD8⁺ (e), CD4⁺ (f) and FoxP3⁺ gated on the CD4⁺ cells (g).