Supplemental material

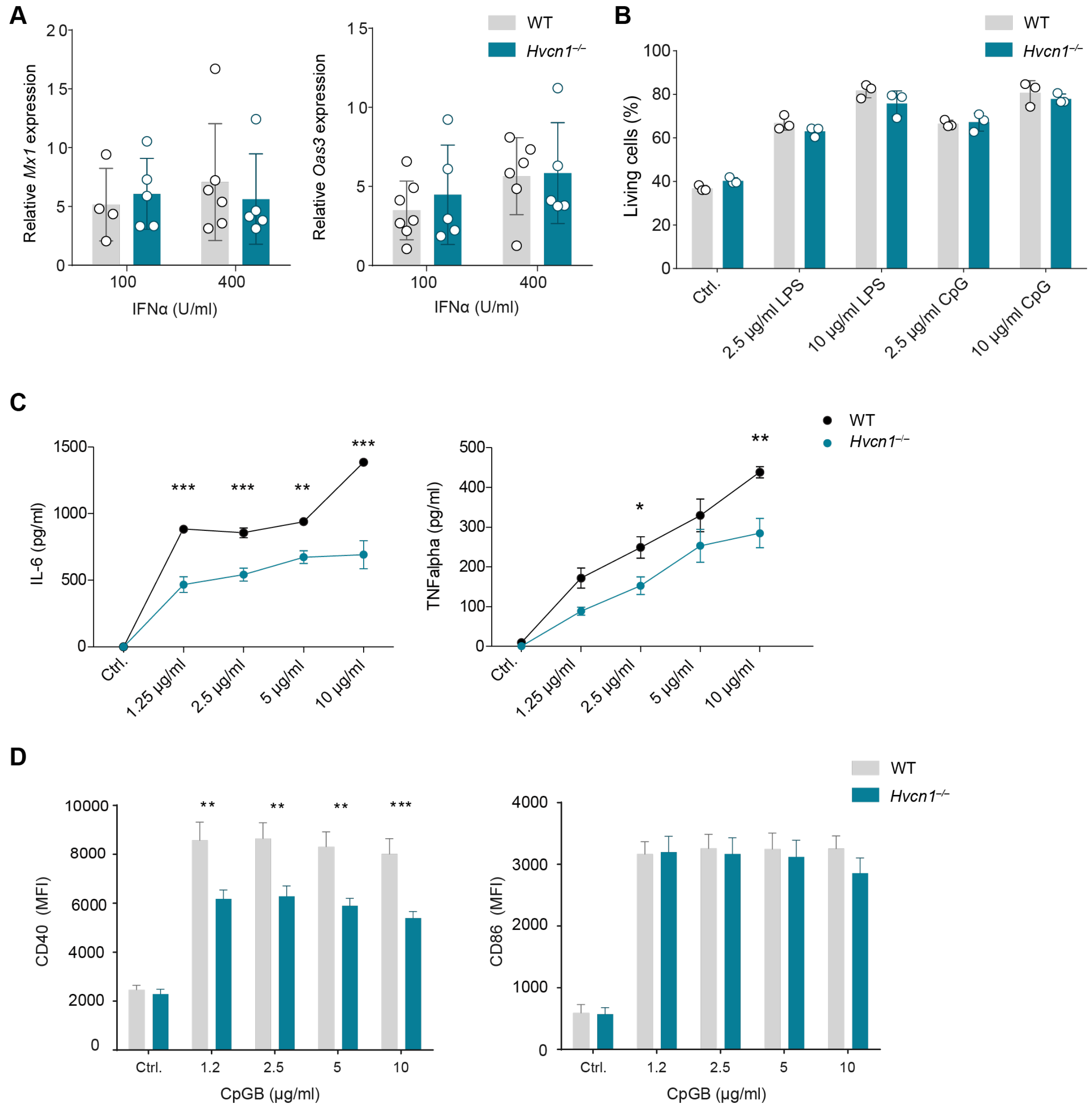

Figure S1. *Hv1*-deficiency does not affect IFNα sensitivity or survival of pDCs.
(A) Quantitative RT-PCR gene expression analysis of *Mx1* and *Oas3* in WT (*n* = 4–7) and *Hvcn1*–/– (*n* = 4–7) Flt3L-pDCs 18 hours after stimulation with IFNα. **(B)** Cell viability determined by flow cytometry in WT and *Hvcn1*–/– Flt3L-pDCs 18 hours after stimulation with LPS or CpGA. Percentage of living cells was determine using the Live/Dead near-IR fixable stain. Data presented as mean ± SEM and analyzed by Student´s t test. **(C)** IL-6 (left) and TNFα (right) concentrations measured by ELISA in the culture medium of WT (*n* = 3) and *Hvcn1*–/– (*n* = 3) Flt3L-pDCs 18 hours after stimulation with CpGB. **(D)** Surface expression of CD40 and CD86 determined by flow cytometry in WT (*n* = 3) and *Hvcn1*–/– (*n* = 3) Flt3L-pDCs 18 hours after stimulation with CpGA. Mean fluorescence intensity of each surface receptor was measured within the CD317+ cell population. Data presented as mean ± SEM and analyzed by Student´s t test (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

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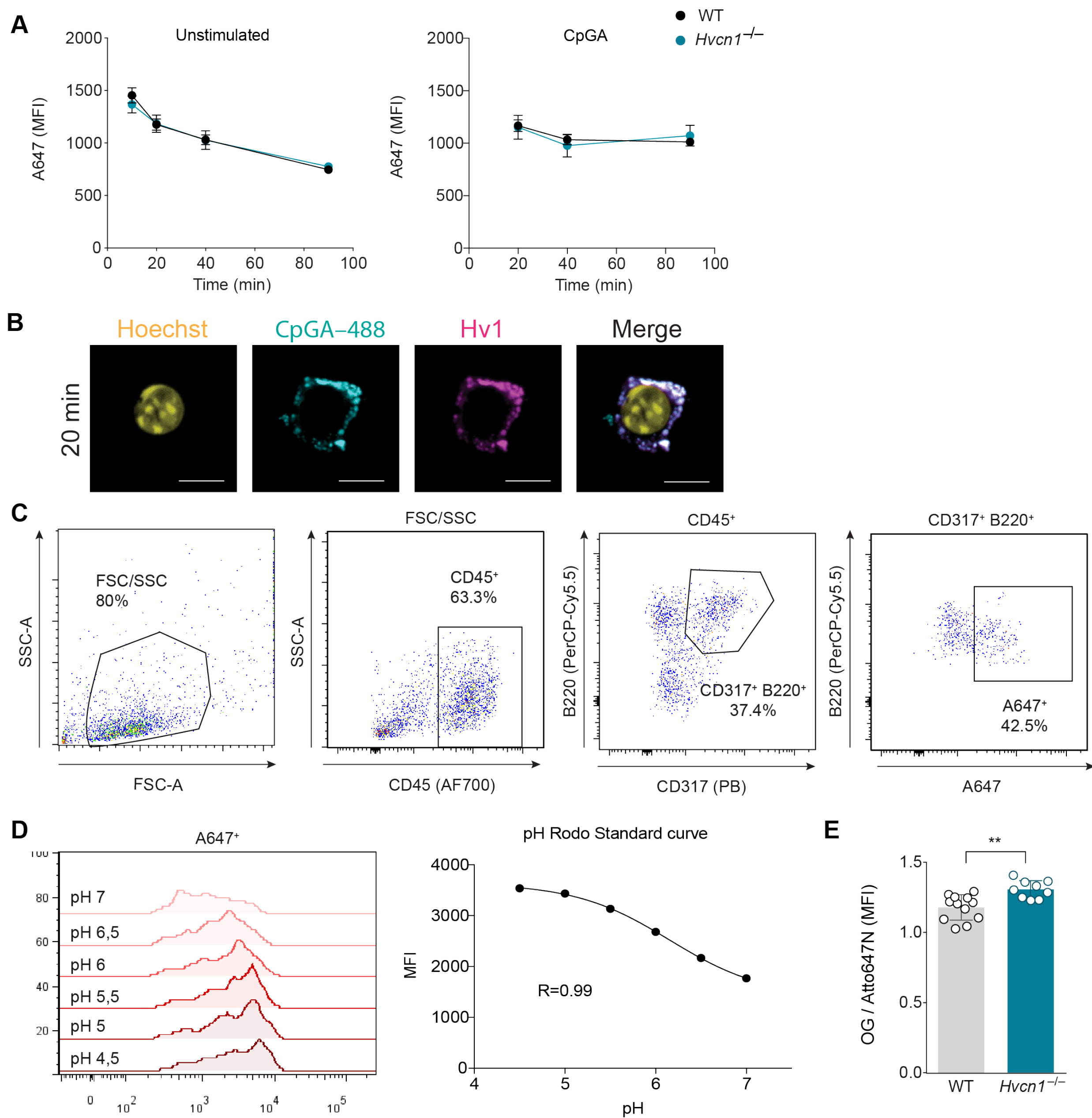

Figure S2. The pH-sensitive dye OGSE supports differential endosomal acidity in WT and *Hvcn1*$^{-/-}$ pDCs.
(**A**) Mean fluorescence intensity (MFI) of AF647 nanoparticles at different chase time points in unstimulated and CpGA-stimulated (10 µg/ml) Flt3L-pDCs obtained from bone marrow of WT (n = 3) and *Hvcn1*$^{-/-}$ mice (*n* = 3). Representative data from *n* = 2 experiments. (**B**) Representative immunolabeling of Hv1 (magenta) after stimulation for 20 minutes with 5 ng/µl Oregon green 488-labelled CpGA (cyan) of WT Flt3L-pDCs after transfection with HV1-HA. Scale bar: 5 µm. (**C**) Representative FACS plots and gating strategy for the identification of AF647+ Flt3L-pDCs. (**D**) Flt3L cells were pulsed with pHrodo®-conjugated nanoparticles and AF674-conjugated nanoparticles at a 1:1 proportion for 10 minutes. Cells were permeabilized with Triton x-100 and subsequently incubated for 10 minutes in saline buffers with different pH ranging from 4.5 to 7. Median fluorescence intensity (MFI) of pHrodo® was recorded for each pH standard within the AF647^{+} gate, and MFI values were plotted against the correspondent pH to create a standard curve (right). Representative MFI histograms of each pH standard are shown (left). **(E)** Flt3L-pDCs were treated with 10 µg/ml of a 1:1 mixture of Atto 647N-CpGA and Oregon green 488-CpGA for 90 minutes at 37ºC and analyzed by flow cytometry. MFI of Oregon Green 488 and Atto 647N was measured in living CD45^{+} CD317^{+} cells, and the ratio between the two different dyes was calculated. Data presented as mean ± SEM and analyzed by Student´s t test (***p* ≤ 0.01).

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