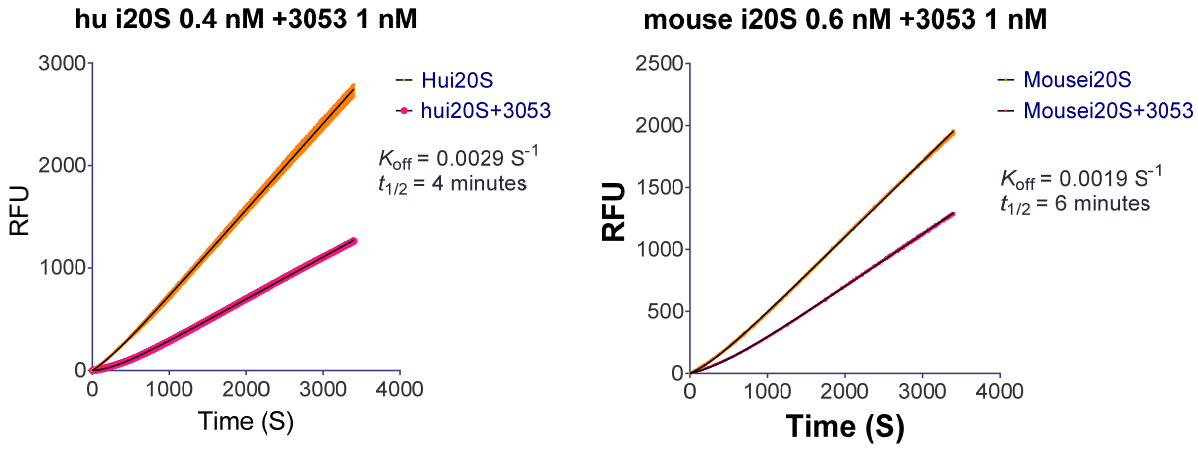

Figure S1. Time-dependent inhibition of β5i of hu i20S and mouse i20S by PKS3053, respectively.

The mixture of i20S (40 nM) and PKS3053 (100 nM) or DMSO at indicated concentrations were incubated for 30 minutes, prior to being diluted 100x into a solution of 25 μM of suc-LLVY-AMC in reaction buffer. The fluorescence of hydrolyzed AMC (Ex 360 nm / Em 460 nm) was immediately recorded for 1 hour. The curves were fit to non-linear least square equation that yielded k_{off} values 0.0029 s^{-1} for hu i20S and 0.0019 s^{-1} for mouse i20S.

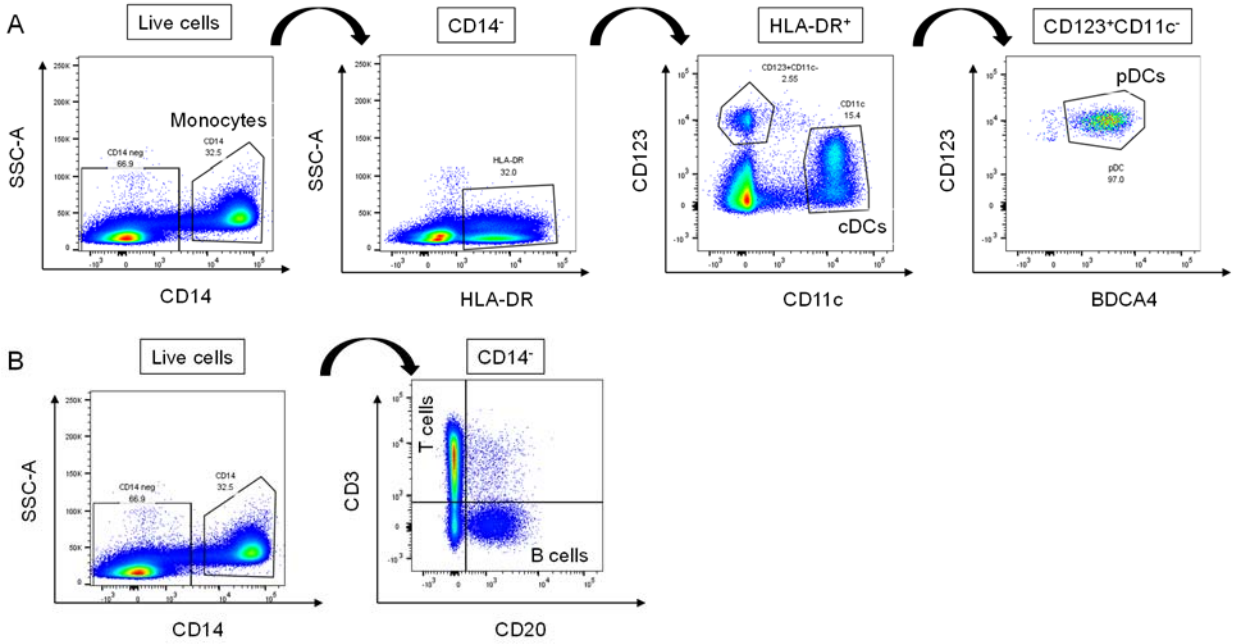

Figure S2. Gating strategy for the different immune cells within PBMCs.

PBMCs were prepared from HDs and cells were stained with CD14, HLA-DR, CD11c, CD123, BDCA4, CD3 and CD20 antibodies and analyzed by flow cytometry. (A,B) Representative dot plots of (A) Monocytes, identified as $CD14^+$ (left panel), pDCs, identified as $CD14^-HLA\text{-}DR^+CD11c^-CD123^+BDCA4^+$ (right panel), cDCs gated as $CD14^-HLA\text{-}DR^+$ $CD123^-BDCA4^-CD11c^+$ (middle right panel) and (B) B cells and T cells identified $CD14^-CD20^+$ and $CD14^-CD3^+$ respectively

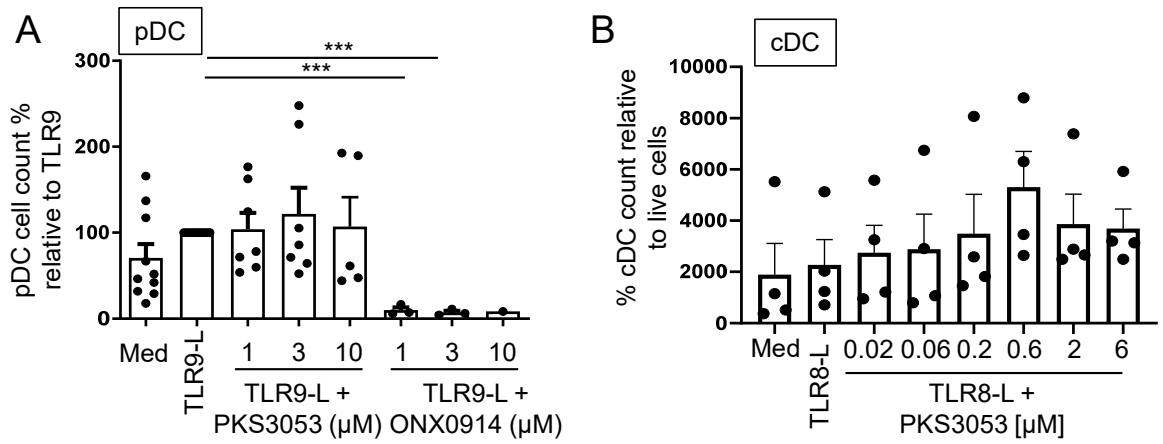

Figure S3. PKS3053, the β5i specific inhibitor is non-toxic on TLR-induced pDCs and cDCs

(A) Purified pDCs were cultured for 24h with media alone, with the TLR9 ligand CpG-C274 (0.1 μM) or with the TLR9-L and either PKS3053 or ONX0914 at the indicated concentration. The following day, the viability of pDCs was evaluated by flow cytometry (B) cDCs differentiated from monocytes were cultured for 24h with media alone, with the TLR8 agonist (ORN8-L) or with TLR8-L and PKS3053. After 24h, the viability of cDCs was evaluated by flow cytometry. All results are represented as a mean ± SEM and individual donors are shown. Statistical significance was evaluated using a Mann-Whitney U-test and *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

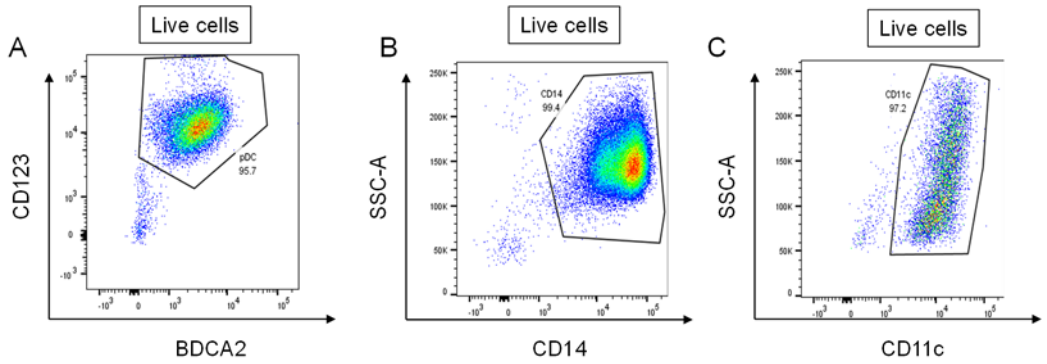

Figure S4. Gating strategy for purified pDCs, macrophages and cDCs.

(A) pDCs were purified by positive selection using miltenyi beads and cells were stained with CD123 and BDCA2 antibodies. Representative dot plot of 1 donor which shows that >95.5 % of purified cells are pDCs (B) Macrophages were differentiated from monocytes and cells were stained with CD14 antibody after 5 days. Representative dot plot of 1 donor which shows that >99 % of purified cells are $CD14^{+}$. pDCs (C) cDCs were differentiated from monocytes and cells were stained with CD11c antibody after 7 days. Representative dot plot of 1 donor which shows that >97 % of differentiated cells are cDCs.