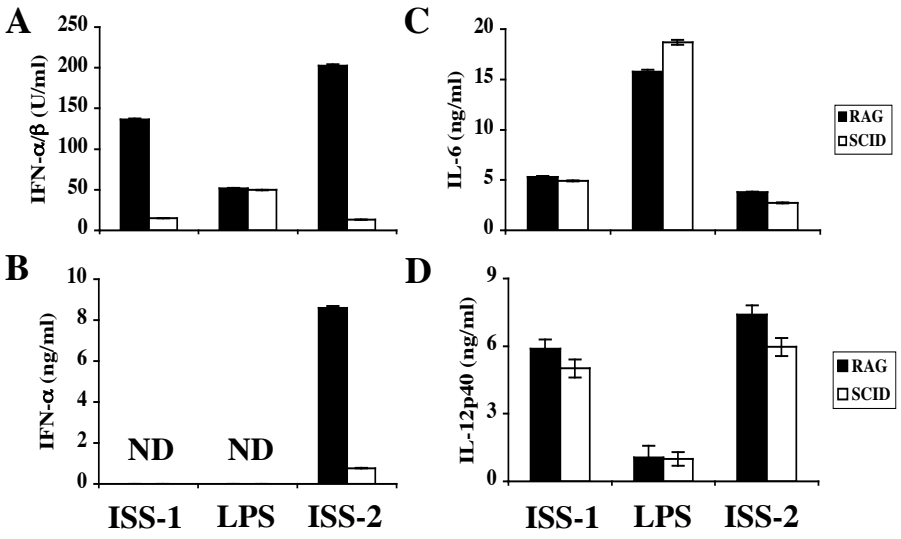
Supplemental Material

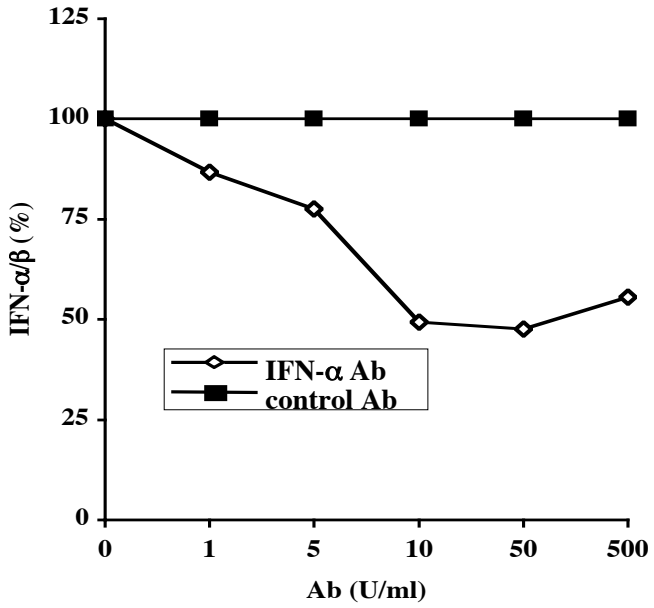
We evaluated the production of type 1 IFN by BM-PDC derived from *RAG1*$^{-/-}$ and SCID mice. BM-PDC were generated from bone marrow cells cultured with 100 ng/ml of hFlt3 ligand (PeproTech Inc. #300-19), and the levels of type 1 IFN generated upon stimulation with ISS-1 were determined by a bioassay and by an IFN-α ELISA kit (PBL Biomedical Laboratories). As shown in Supplemental Figure 1 below, we did not detect any IFN-α in the RAG- or SCID-derived BM-PDC supernatants after stimulation with ISS-1 and LPS (Supplemental Figure 2B) when tested by ELISA. In contrast, different levels of type 1 IFN in these supernatants were detected when tested by the bioassay. IFN-α levels were detectable by ELISA in the supernatants of BM-PDC upon stimulation with ISS-2, a class A CpG ODN that is known to elicit very high levels of this cytokine. As expected, the levels of IFN-α were by far higher in the supernatants of RAG-derived BM-PDC than in the supernatants of SCID-derived BM-PDC.



Supplemental Figure 1: **Comparison of assays for the measurement of type-1 IFN: Bioassay vs. ELISA.**
BM-PDC (10^6/ml) from 3 RAG1$^{-/-}$ and 3 SCID mice were pooled and stimulated with ISS-1 (10 μg/ml), LPS (50 ng/ml), and ISS-2 (10 μg/ml). Cytokine levels in the supernatants were measured 24 hrs later (triplicates). (A) Type 1 IFN (IFNα/β) levels (U/ml) by bioassay. (B) IFN-α levels (pg/ml) by ELISA (PBL). (C) IL-6 and (D) IL-12p40 levels (ELISA). ND - not detected.

The bioassay detects type 1 IFN activity, which consists of multiple IFN-α gene products and a single IFN-β gene product. In contrast, the ELISA kit (PBL) detects only a few species of IFN-α. To verify and to measure the relative IFN-α and IFN-β activities in the PDC supernatants, we neutralized IFN-α from the supernatants of ISS-stimulated BM-MDC. Thus, increasing concentrations of rabbit anti-mouse IFN-α antibody (PBL) and rabbit IgG (Jackson Immuno-Research Laboratories) were added to the supernatants and the resulting type 1 IFN was

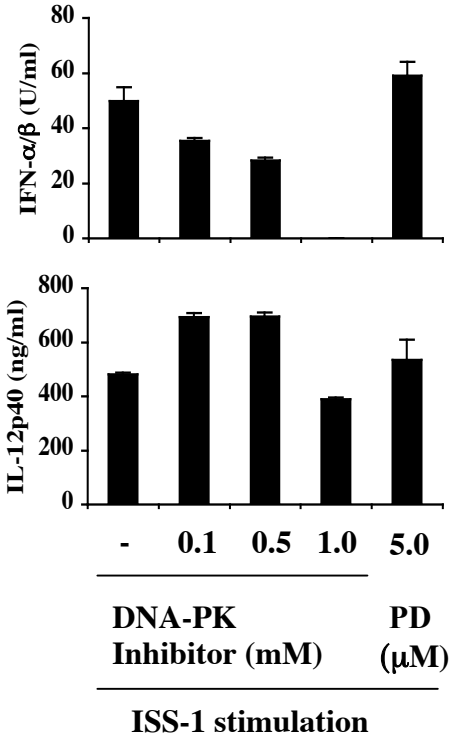
analyzed by bioassay. As shown below, IFN-α neutralization inhibited up to 50% of the total type-1 IFN activity in the supernatants.



Supplemental Figure 2: **IFN-α neutralization does not abolish type-1 IFN activity when tested by bioassay**

BM-MDC (10^6/ml) generated from 3 $RAG1^{-/-}$ mice were pooled and stimulated with ISS-1 (10 μg/ml). After 24 hrs the supernatants were collected and co-incubated with various concentrations of rabbit anti-mIFN-α IgG or control rabbit IgG overnight at 37^0C. Type 1 IFN levels were measured in the supernatants by bioassay. The level obtained for each control IgG concentration was considered as 100% of type 1 IFN (IFN-α/β) activity.

We performed a type 1 IFN bioassay using BM-MDC made from RAG deficient mice. These cells were stimulated with ISS-1 in the presence or absence of a novel DNA-PK inhibitor NU7026 (Calbiochem). As presented in the Figure below type 1 IFN production was inhibited by the DNA-PK inhibitor, while the IL-12 (p40) production was intact.



Supplemental Figure 3: **DNA-PK inhibitor suppresses type-1 IFN production from BMDC (MDC).**
BM-MDC (10^6/ml) from 2 *RAG1*$^{-/-}$ mice were pooled and co-incubated with or without various concentrations of DNA-PK or MEK inhibitor (control). Cells were stimulated with ISS-1 (10 μg/ml). Cytokine levels in the supernatants were measured 24 hrs later in triplicate. IL-12 (p40) levels were measured by ELISA (BD-Pharmingen) and type 1 IFN (IFN-α/β) levels (U/ml) were measured by bioassay.

We analyzed the response of BM-MDC and BM-PDC from RAG1 deficient and DNA-PK deficient mice (provided by Dr. Gloria Li, Memorial Sloan Kettering, NY). As shown in Supplemental Tables 1 and 2, after ISS or LPS stimulation the levels of IFN-α in the supernatants of these cells were below detection when measured by ELISA (PBL). In contrast, we detected type 1 IFN activity in these supernatants when tested by bioassay (Tables 1 and 2). The levels of type 1 IFN in the supernatants of BM-MDC and BM-PDC derived from RAG1 deficient animals were higher than those detected in BM-MDC and BM-PDC supernatants derived from DNA-PK deficient mice when tested by bioassay (Tables 1 and 2 below). These data combined with the SCID BM-MDC data presented in the main text indicate that DNA-PK is involved in type 1 IFN production by these two DC subsets upon TLR9 stimulation.

	IFN-α/β (U/ml)		IFN-α (pg/ml)		IL-6 (ng/ml)		IL-12p40 (ng/ml)	
	ISS-1	LPS	ISS-1	LPS	ISS-1	LPS	ISS-1	LPS
***RAG*$^{-/-}$**	190±10	40±5	ND	ND	13±2	28±2	284±21	96±8
***DNA-PK*$^{-/-}$**	35±5	35±5	ND	ND	12±1	26±2	272±17	98±10

Supplemental Table 1: **Reduced type-1 IFN production in BM-MDC from DNA-PK KO mice.**
BM-MDC (10^6/ml) from 3 RAG1 and 3 DNA-PK KO mice were pooled and stimulated with ISS-1 (10 μg/ml) or LPS (50 ng/ml). Cytokine levels in the supernatants were measured 24 hrs later in triplicate. Type 1 IFN levels (IFNα/β) were measured by bioassay (U/ml), IFN-α levels were measured by ELISA (pg/ml, PBL) and IL-6 and IL-12p40 levels were measured by ELISA (BD-Pharmingen). ND - not detected.

	IFN-α/β (U/ml)		IFN-α (pg/ml)		IL-6 (ng/ml)		IL-12p40 (ng/ml)	
	ISS-1	LPS	ISS-1	LPS	ISS-1	LPS	ISS-1	LPS
***RAG*$^{-/-}$**	527±10	15±0.5	ND	ND	25±2	13±2	227±14	44±15
***DNA-PK*$^{-/-}$**	89±5	22±2	ND	ND	22±1	10±2	200±20	29±9

Supplemental Table 2: **Reduced type-1 IFN production in BM-PDC from DNA-PK KO mice.**
BM-PDC (10^6/ml) from 3 RAG1 and 3 DNA-PK KO mice were pooled and stimulated with ISS-1 (10 μg/ml) or LPS (50 ng/ml). Cytokine levels were detected as described above for Table 1. ND - not detected.