Supplementary figure 1

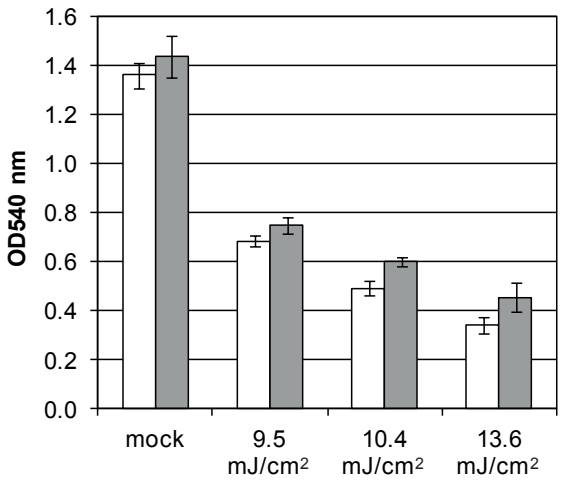

Figure S1. Cell viability declines with the dose of UVR and is not significantly different between WT and CD1d -/- MEKs. Cell Viability of (Figure 5A) performed by neutral red staining, quantify by OD_{540} (n=3, mean ± SD); representative experiment performed three times independently.

Supplementary figure 2

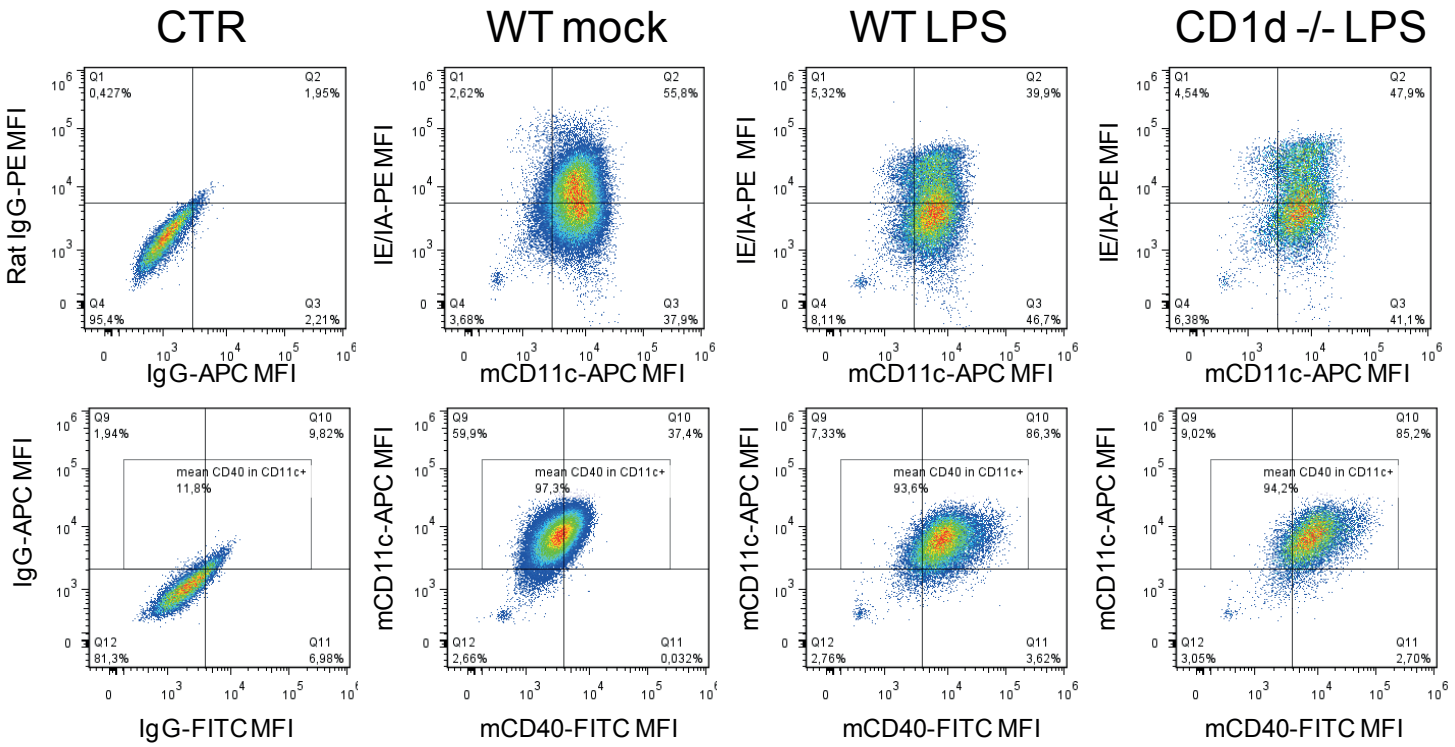

Figure S2. Similar expension and maturation of WT and CD1d -/- mouse bone marrow-derived dentritic cells (BMDCs) reveal by labeling of cell surface markers of dendritic cells. Both strains of BMDCs were cultivated for 10 days and stimulated with 250 ng/ml LPS for 24h. Isolated cells were then labeled with different fluoro-conjugated antibodies for mMHCII (IE/IA), mCD11c and mCD40 and analyze by flow cytometry. CTR: labeling of WT cells using fluoro-conjugated isotype control antibodies (LPS). MFI: Mean Fluorescence Intensity.

Supplementary figure 3

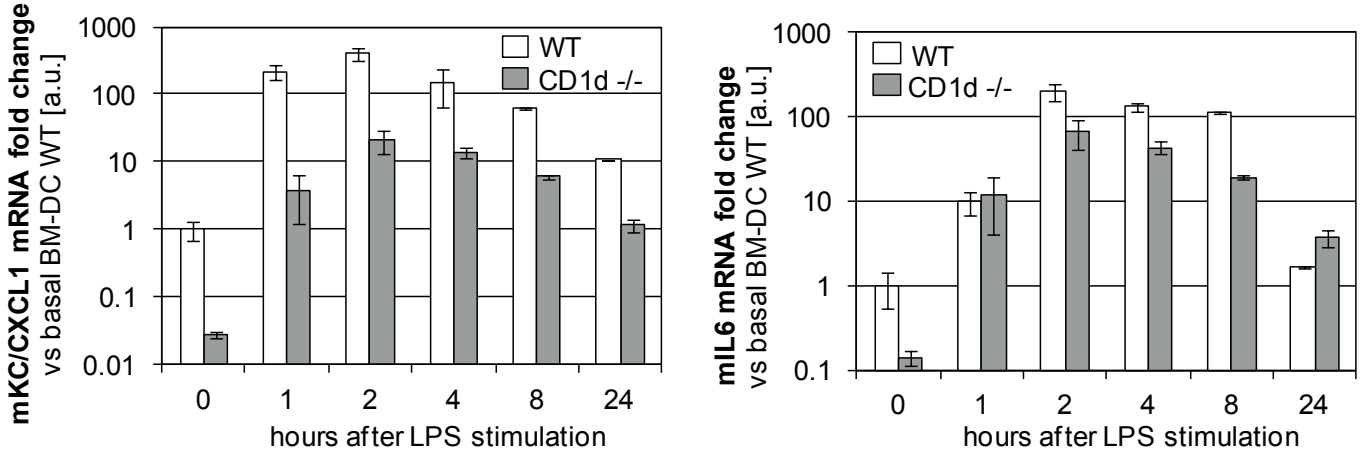

Figure S3. Kinetic of mKC/CXCL1 and mIL-6 mRNA expression in WT and CD1d -/- BMDC stimulated with 100ng/ml of LPS over 24h. Sample from Figure 5D and E (triplicates; mean ± SD), representative experiment performed three times independently. At the peak of mRNA expression (LPS 2h), the level of mKC/CXCL1 and mIL-6 mRNAs were lower in CD1d -/- compared to WT BMDCs.

Supplementary figure 4

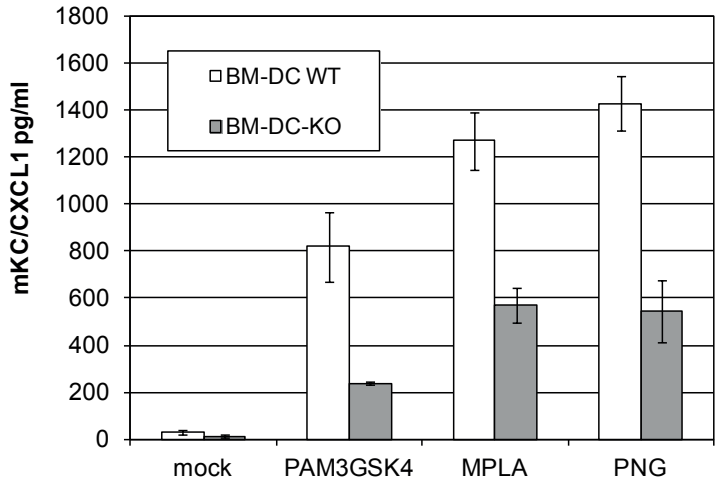

Figure S4: CD1d -/- dendritic cells induced less chemokine mKC/CXCL1 compare to wt cells after stimulation with different TLR agonists. ELISA of mKC/CXCL1 released in the medium of WT and CD1d -/- BMDC stimulated 24h with either with TLR2 or TLR4 agonists, respectively with the lipopetide PAM3 GSK4 (5mg/ml), monophosphoryl lipid A (MPLA, 1mg/ml) and with a non-specific TLRs agonist petidoglycan (PNG, 10mg/ml) (ELISA triplicates; mean ± SD).

Supplemental materials

Flow cytometry

Flow cytometry analysis was performed as described by (Lutz et al. 1999). BM-DC (1–5x105) were stained directly with phycoerythrin (PE)-conjugated monoclonal antibody (mAb) directed against major histocompatibility complex (MHC) class II (I-A/I-E PE Clone 2G9) or fluorescein isothiocyanate (FITC)-conjugated CD40 (3/23), or the appropriate fluorochome-conjugated isotype control mAb (all from BD PharMingen). Allophycocyanin (APC) anti-mouse CD11c and appropriate APC-conjugated isotype control were purchased from eBioscience. BMDC were incubated at 4–8 µg/ml of monoclonal antibody (mAb) in phosphate-buffered saline containing 0.1% sodium azide and 2% fetal calf serum for 30 min on ice in the dark. Samples were washed once in PBS and further incubated with LIVE⁄DEADR Fixable Violet Dead Cell Stain Kit (1:2000 in PBS) for further 30min on ice. Samples were washed once in PBS and were measured and analyzed with a Gallios™ (Beckman Coulter) flow cytometer; data represented with FlowJo 6.2.

UVB light sources.

A. In vivo

A bank of five Philips TL-40W/12 sunlamps (Philips, The Netherlands) provided one source of broadband UVB (280-320 nm). The irradiance of the TL-40W/12 sunlamps, measured using a PMA2200 radiometer and a PMA2101 UVB detector (SOLAR Light Co., PA), averaged 10 W/m^2. A BioSpectra System (Vilber Lourmat, Marne-La Vallee, France) comprising an irradiation chamber equipped with 312-nm illumination lamps, a sensor/dosimeter, calibrator and software provided another source of broadband UVB. The irradiance of the BioSpectra System averaged 27 W/m^2.

B. In vitro

To irradiate keratinocytes in culture, a single medisun® HF-54 (Schulze & Böhm GmbH, Germany) sunlight lamp was used. These lamps emit a continuous UVB spectrum from 280 to 315 nm. The intensity and spectral output of the radiation sources were measured using a PMA2200 radiometer and a PMA2101 UVB detector (SOLAR Light Co., PA).

UVB-irradiation of mice. The dorsal hair of the mice was removed with electric clippers and the shaved surface exposed to UVB. Mice exposed to UVB by Philips TL-40W/12 sunlamps were placed into a Plexiglass box separated into individual compartments by Plexiglass dividers and covered with a wire top that decreased the incident dose by 14 %. Each time a new group of mice were irradiated, the box was placed in the same position under the lamps to compensate for any uneven distribution of energy along the length of the bulbs. Mice were anaesthetized with pentobarbital (30 mg/kg administered i.p., Sanofi, Libourne, France) prior to UVB irradiation by the BioSpectra System or at different time points after irradiation by TL-40W/12 sunlamps. When applied to human skin, a dose of 430 mJ/cm^2 UVB would result in severe sunburn.

Cell viability with Neutral Red

After removing the supernatant (medium) cell were stained with 50µg/ml of Neutral Red (Sigma-Aldrich, MO, USA) for 2h in a new culture medium without serum. The adherent cells were after washed three time with 0.9% of NaCl, dry for 1 day, and lysed in a 1:1 solution of ethanol and acetic acid 2%. This solution was measured by OD 540nm to quantify the % of neutral incorporation. UVB untreated cells were considered as 100% survival cells.