Supplemental Information

Interferon β production by the epidermis is mediated by an antimicrobial peptide and the MAVS signaling pathway

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This Supplementary file includes:

Supplemental Figures S1-S7 and Legends

Supplemental Table S1

Supplemental Experimental Procedures

Supplemental References

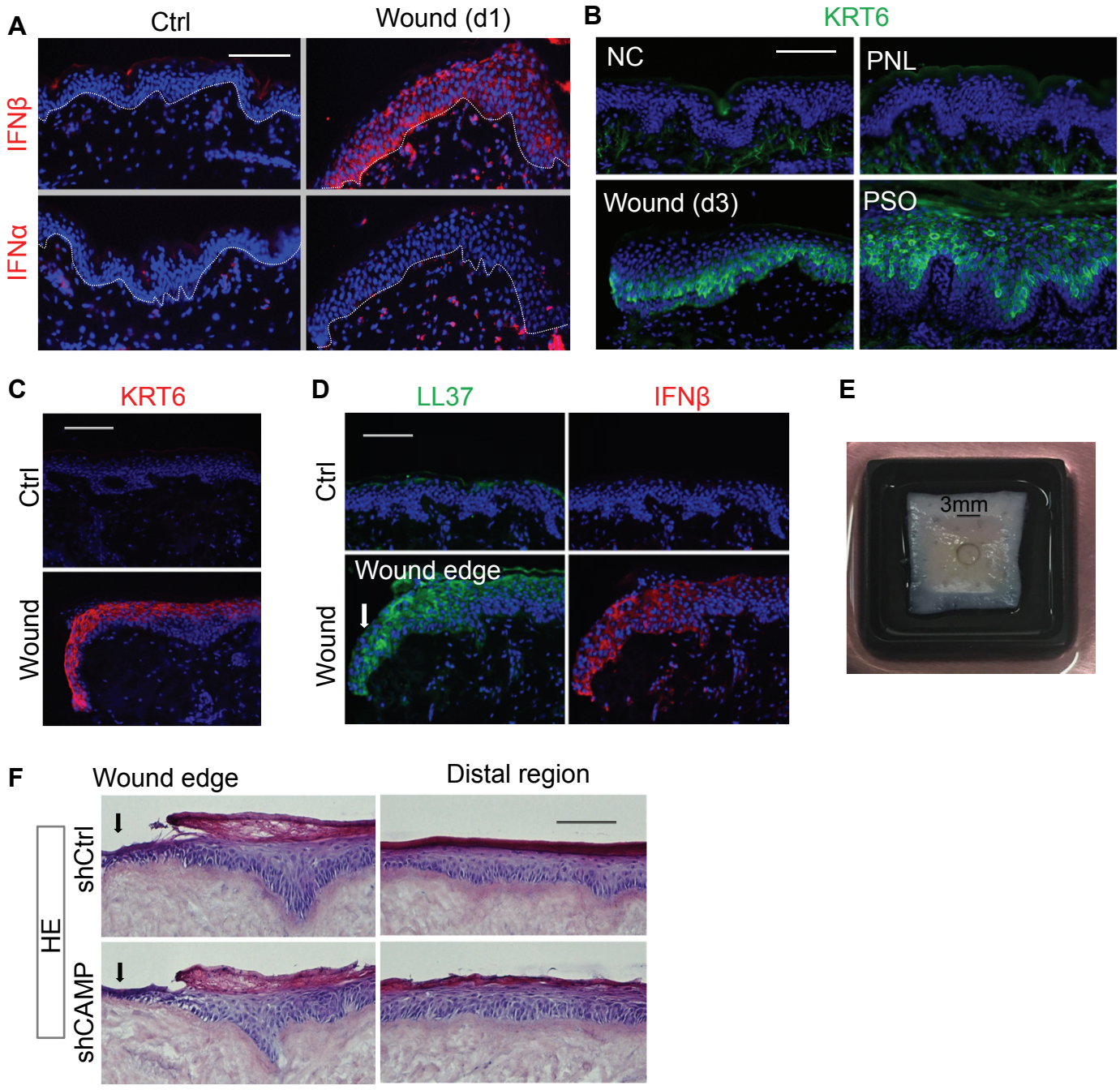
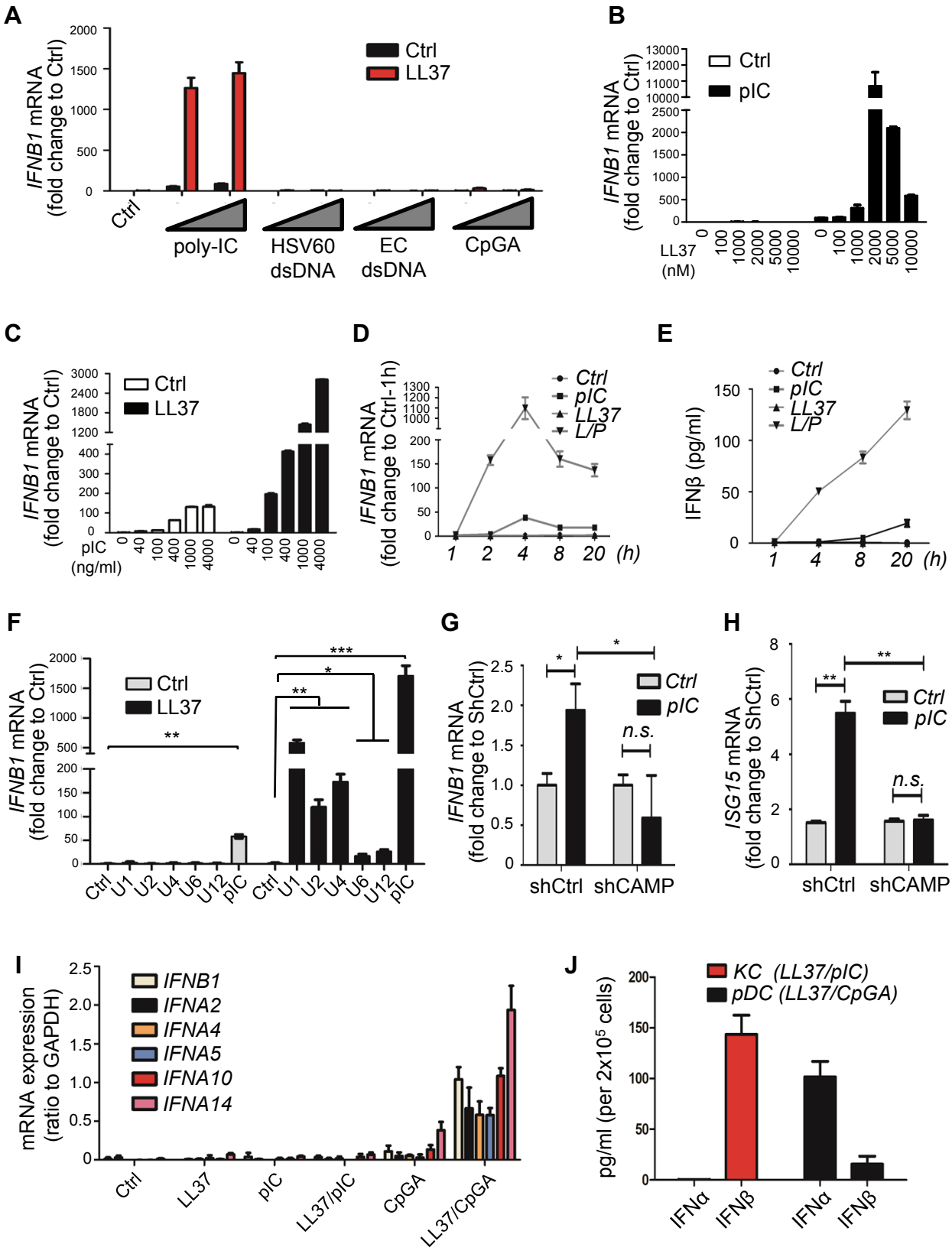


Figure S1 (related to Figure 1) **In vivo, ex vivo and in vitro human skin wound models** (**A**) Skin sections of control or wounded (day 1) skin were immunostained with IFNβ or IFNα antibodies as indicated. White dashed line indicates the epidermal/dermal junction. (**B**) Skin sections of normal human control (NC), normal wounded skin (day3), psoriasis non-lesional (PNL) or psoriasis lesional (PSO) were stained with KRT6 (green) antibody as indicated. Nuclei were counterstained with DAPI (blue). Scale bar, 100 µm. (**C**) Skin sections of normal human skin or ex vivo wounded (day 2) human skin were stained KRT6 (green) antibody. Nuclei were counterstained with DAPI (blue). Scale bar, 100 µm. (**D**) Skin sections of ex vivo wounded skin (day 2) immunostained with LL37 or IFNβ antibodies as indicated. Scale bar, 100 µm. (**E**) In vitro skin wounding: 3D human skin constructs were wounded using a 3 mm punch biopsy and skin was collected at day3 for analysis. (**F**) Hematoxylin and eosin (HE) staining of skin sections of wounded 3D skin. Wound edge and distal unwounded areas are shown. Scale bar, 100 µm.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IFNβ

Figure S2 (related to Figure 2) **LL37 enhances IFNβ production by dsRNA in keratinocytes.** (**A**) NHEKs were pretreated with 2 μM LL37 or vehicle control prior to treatment with increasing doses (0.2 ug/ml or 1 ug/ml) of pIC or various dsDNA as indicated for 4 hrs. IFNβ mRNA fold induction was analyzed by RTqPCR analyses (n=3). (**B**) NHEKs were pretreated with 2 μM LL37 then increasing doses of pIC. Relative IFNβ mRNA fold induction at 4 hrs was examined by RTqPCR analyses (n=3). (**C**) NHEKs were pretreated with increasing doses of LL37 as indicated, then treated with 0.4 ug/ml pIC. Relative *IFNB* mRNA fold induction at 4 hrs was examined by RTqPCR analyses. (**D-E**). Time course analyses of IFNβ mRNA (**E**) and IFNβ protein secretion (**F**). NHEKs were pretreated with LL37 or control before treating with various snRNA as indicated. Conditioned medium was collected at 18 hrs to measure IFNβ protein secretion (n=3). (**B**) NHEKs were pretreated with LL37 or control before treating with various snRNA as indicated. Relative *IFNB* mRNA fold induction at 4 hrs was examined by RTqPCR analyses. (**G-H**). 3D skin constructs made with control or CAMP knockdowned NHEKs were treated with vehicle control or pIC for 6 hrs prior to being subjected to RTqPCR analyses of IFNB1 (**G**) or ISG15 (**H**) mRNA expression (n=3). (**I**). Human pDCs were pretreated with 2 μM LL37 or vehicle control prior to treatment with pIC or CpGA for 6 hrs. The expression of various type1 IFNs was analyzed by RTqPCR (mRNA ratio to GAPDH is shown). (**J**). IFNα and IFNβ secretion from LL37/pIC activated NHEKs or LL37/CpGA activated human pDCs (6 hrs) by ELISA. All error bars indicate mean ± s.e.m. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (one way Anova).

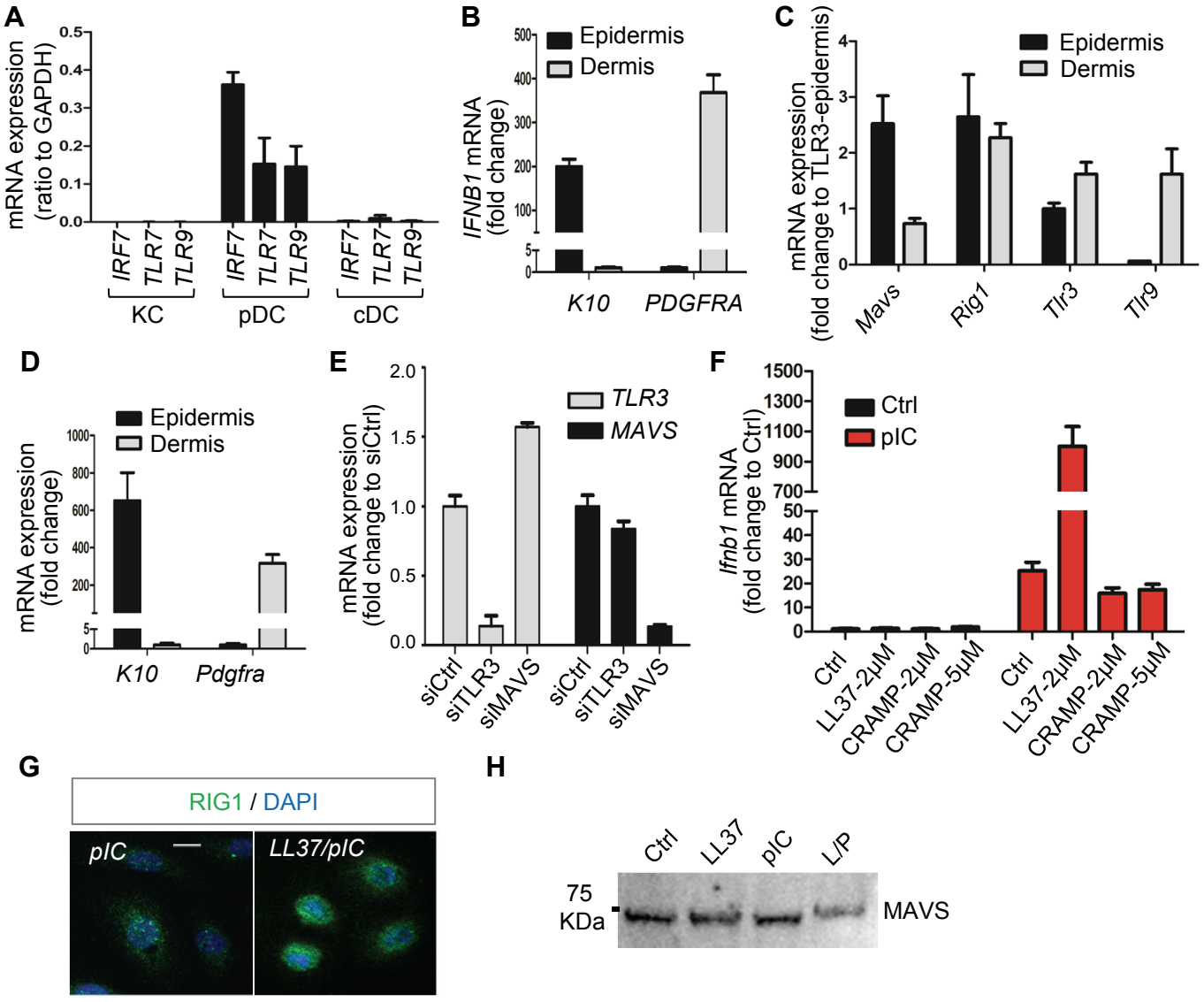

Figure S3 (related to Figure 3) **MAVS/RIG1/MDA5 pathway is abundantly expressed in epidermal keratinocytes and activated by dsRNA/LL37.** (**A**) RTqPCR analyses of relative mRNA levels of IRF7, TLR7 and TLR9 in NHEKs, human pDCs and cDCs (**A**) (n=3; ratios to GAPDH were shown). (**B**) RTqPCR analyses of relative mRNA levels of *K10* (a keratinocyte specific gene) and *PDGFRA* (a fibroblast specific gene) in separated human skin epidermis and skin dermis as indicated. Fold change of K10 mRNA is to dermis, and fold change of PDGFRA mRNA is to epiermis.(**C**) RTqPCR analyses of relative mRNA levels of *Mavs, Rig1, Tlr3* and *Tlr9* in separated epidermis and dermis from mouse skin. (**D**) RTqPCR analyses of relative mRNA levels of *K10* and *Pdgfra* in separated mouse skin epidermis and skin dermis as indicated. Fold change of K10 mRNA is to dermis, and fold change of Pdgfra mRNA is to epiermis. (**E**) RTqPCR analyses of *MAVS* and *TLR3* in NHEKs transfected with control siRNA, TLR3 siRNA or MAVS siRNA. (**F**) Mouse KCs were pretreated with vehicle control, LL37 (2 μM), or CRAMP (2 μM or 5 μM) then stimulated with pIC for 4 hrs. Relative mRNA levels of *Ifnb1* were examined by RTqPCR analyses (n=3). (**G**) NHEKs treated with LL37/pIC or vehicle control were immunostained with anti-RIG1 antibody (green). Nuclei were counterstained with DAPI (blue). Scale bar, 20 μm. (**H**). Western-blotting of cell extracts from NHEKs treated with various combination of pIC and LL37 using anti-MAVS antibody. All error bars indicate mean ± s.e.m. * P<0.05, ** P<0.01, *** P<0.001 (one way Anova).

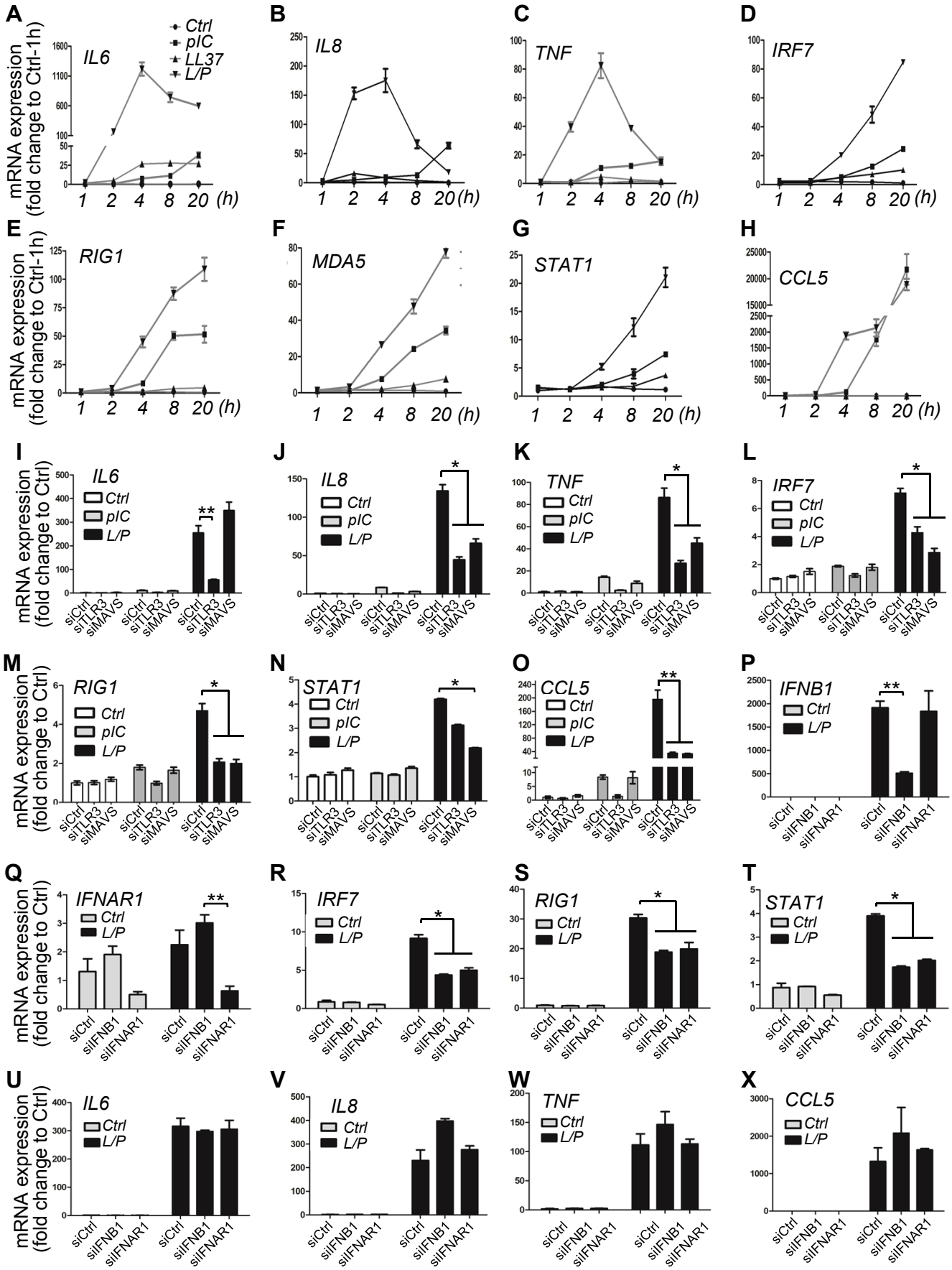
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Figure S4 (related to Figure 3) **Regulation of pro-inflammatory cytokines and IFN responsive genes by LL37 and dsRNA.** (**A-G**). Time course analyses of relative mRNA levels of *IL6* (**A**), *IL8* (**B**), *TNF* (**C**), *IRF7* (**D**), *RIG1* (**E**), *MDA5* (**F**), *STAT1* (**G**) and *CCL5* (**H**), in NHEKs cells treated with various combination of LL37 and pIC as indicated. (**H-M**). NHEKs transfected with control siRNA, TLR3 siRNA or MAVS siRNA were treated with vehicle control, or LL37/pIC as indicated. Relative fold induction of *IL6* (**I**), *IL8* (**J**), *TNF* (**K**), *IRF7* (**L**), *RIG1* (**M**), *STAT1* (**N**) and *CCL5* (**O**) mRNA is shown. (**P-Q**) RTqPCR analyses of *IFNB* and *IFNAR1* in NHEKs transfected with control siRNA, IFNAR1 siRNA or IFNB1 siRNA. (**R-X**) NHEKs transfected with control siRNA, IFNAR1 siRNA or IFNB1 siRNA were treated with vehicle control, or LL37/pIC. Relative fold induction of *IRF7* (**R**), *RIG1* (**S**), *STAT1* (**T**), *IL6* (**U**), *IL8* (**V**), *TNF* (**W**), and *CCL5* (**X**) mRNA is shown. All error bars indicate mean ± s.e.m. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (one way Anova).

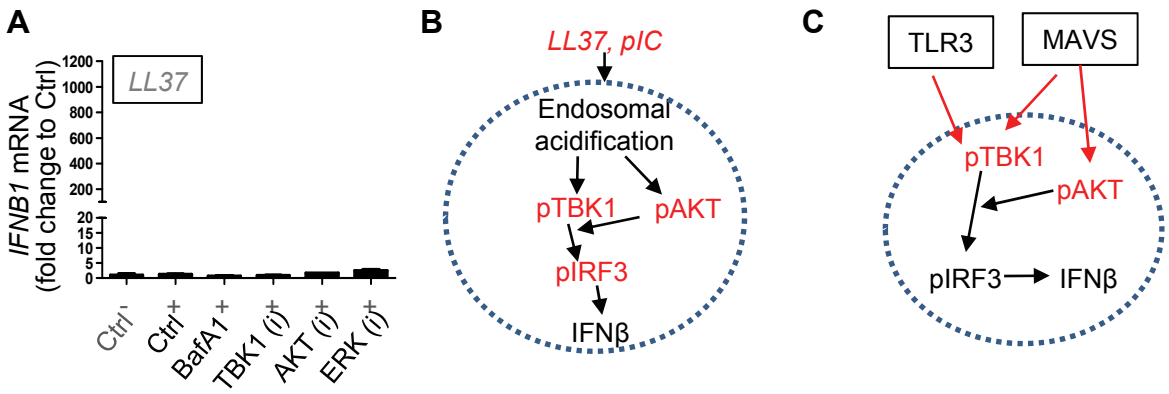

Figure S5 (related to Figure 4 and Figure 5) **IFNβ production in response to dsRNA and LL37 is dependent on TBK1/AKT and MAVS.** (**A**). NHEKs were pretreated with LL37 for 5 hrs prior to RTqPCR analyses of *IFNB* mRNA expression. Note that this graph is the LL37 with inhibitors control for Figure 5B. (**B**). Proposed model for how LL37/pIC regulates TBK1/AKT/IRF3 activation. Endosomal acidification plays a central role in regulating TBK1 and AKT phosphorylation in response to LL37/pIC. Both TBK1 and AKT are required for IRF3 phosphorylation and the subsequent induction of IFNβ in response to LL37/dsRNA. (**C**) Proposed model for how TLR3 and MAVS are involved in TBK1/AKT/IRF3 activation. Both MAVS and TLR3 are involved in TBK1 phosphorylation in response to LL37/pIC, whereas AKT phosphorylation by LL37 only depends on MAVS, but not TLR3.

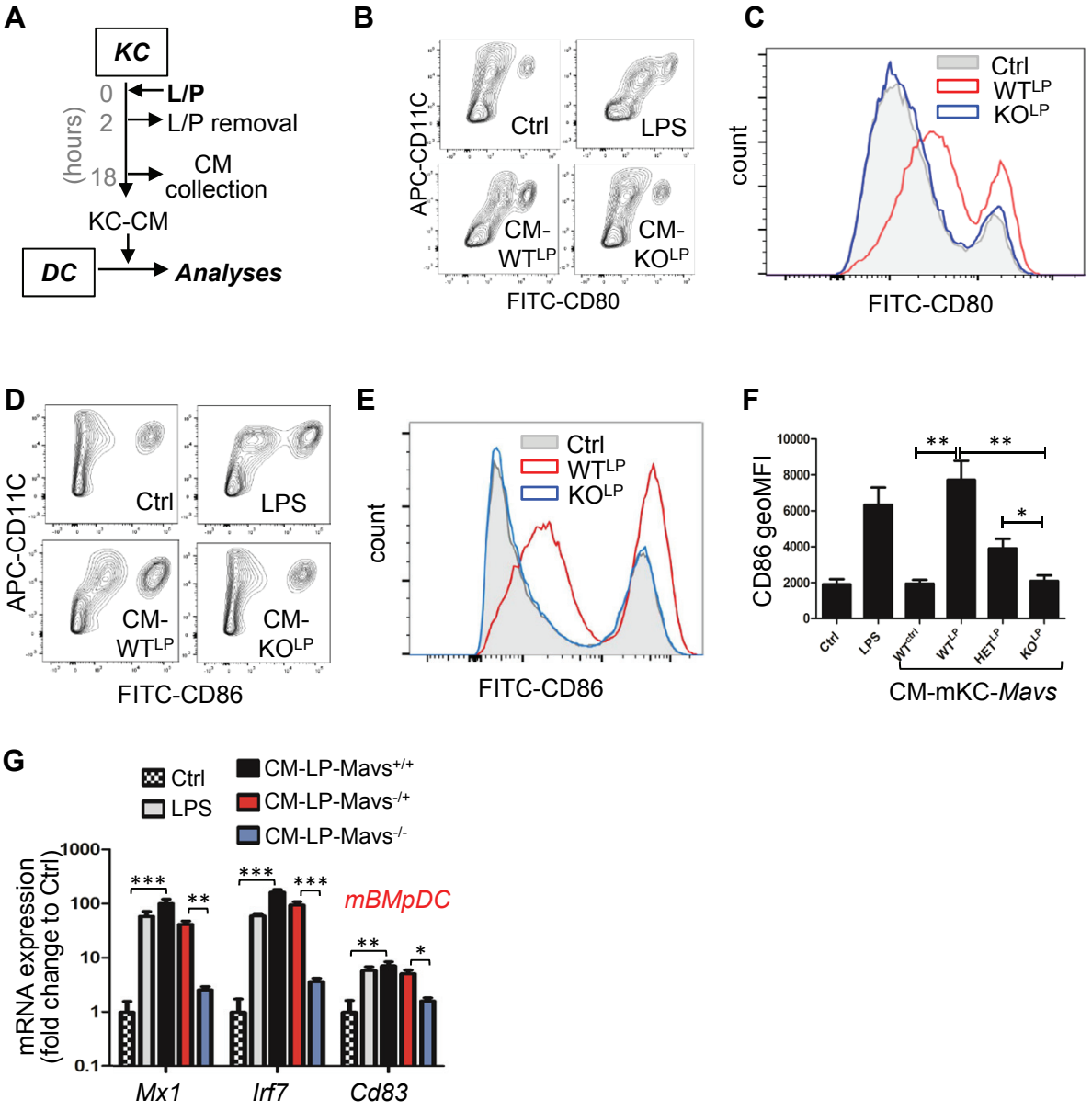

Figure S6 (related to Figure 6) **MAVS-mediated secretion of IFNβ from keratinocytes promotes activation/maturation of dendritic cells.** (**A**). Experiment scheme of collection of keratinocyte (KC) conditioned medium (CM) to stimulate dendritic cell in vitro. (**B**). FACS analyses of surface expression of FITC-CD80 and APC-CD11C in mouse BMDC stimulated with KC-CM as indicated for 48 hrs. (**C**). Histogram of surface expression of FITC-CD80 in mouse BMDC stimulated with KC-CM as indicated for 48 hrs. (**D**). FACS analyses of surface expression of FITC-CD86 and APC-CD11C in mouse BMDC stimulated with KC-CM as indicated for 48 hrs. (**E**). Histogram of surface expression of FITC-CD86 in mouse BMDC stimulated with KC-CM as indicated for 48 hrs. (**F**). Quantification of the geometric MFI of FITC-CD86 (n=3) of Figure S6D. (**G**) RTqPCR analyses of *Mx1, Irf7* and *Cd83* mRNA expression in mouse BMpDC treated with KC-CM (6 hrs) as indicated (n=3). All error bars indicate mean ± s.e.m. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (one way Anova).

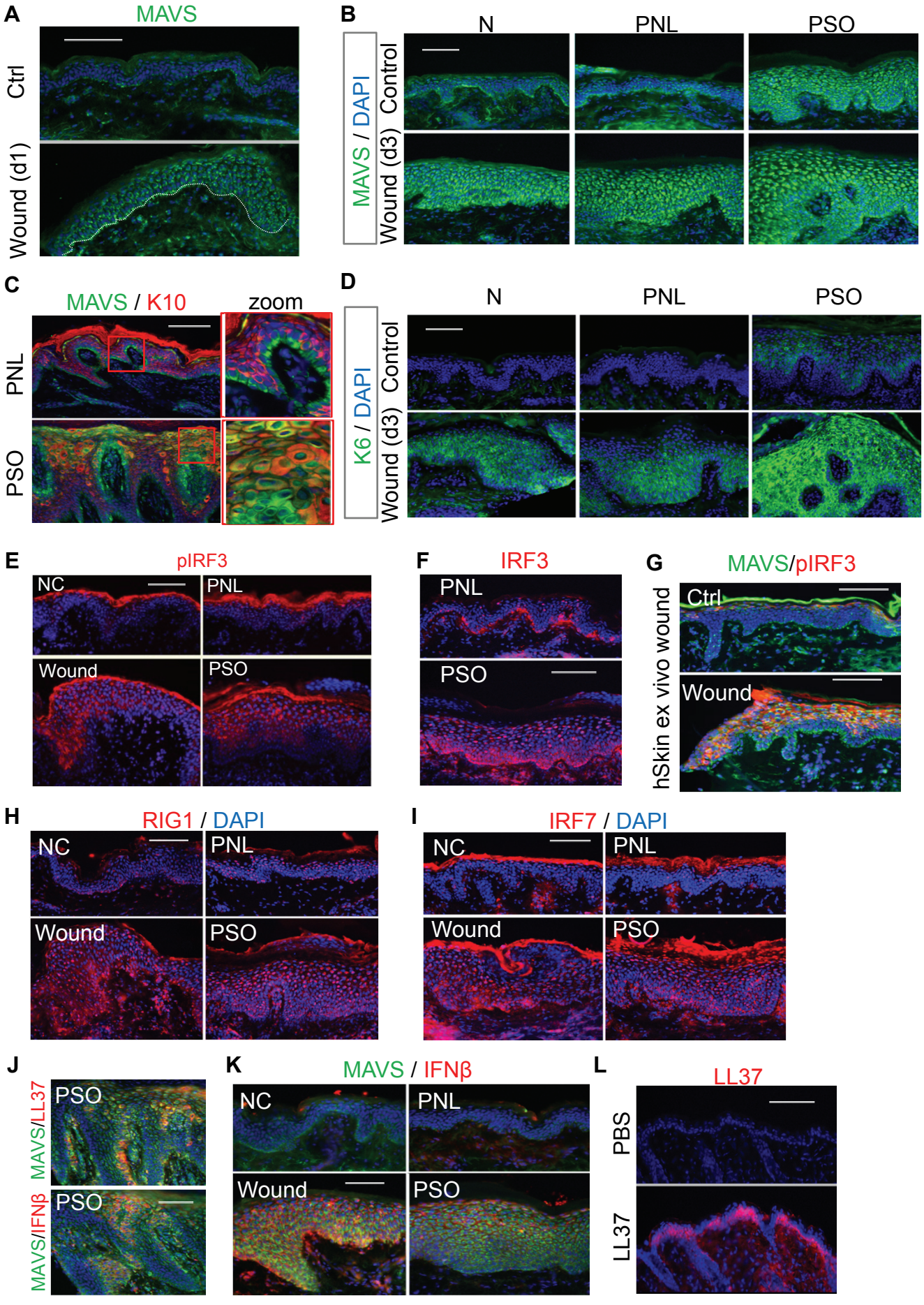
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Figure S7 (related to Figure 7) **MAVS and type1 IFN pathway are activated in wounded/psoriatic skin epidermis**. (**A**). Wounded skin (day 1) sections were immunostained with IFNβ or IFNα antibodies as indicated. White dashed line indicates the epidermal/dermal junction. (**B-D**). MAVS (**B**), MAVS and K10 (**C**), or KRT6 (**D**) immunostaining on skin sections from control or wounded (day3) normal human skin (N), psoriasis non-lesional (PNL) or psoriasis lesional (PSO) skin as indicated. Nuclei was counterstained with DAPI (blue). Note that K10 is a keratinocyte early differentiation marker expressed only in suprabasal cells. MAVS was express in basal KCs that do not express K10 in PNL epidermis, but MAVS was induced in K10 positive suprabasal KCs in PSO epidermis. (**E-F**). pIRF3 (**E**) or IRF3 (**F**) immunostaining on skin sections from normal control (NC), or wounded (day 3) human skin, psoriasis non-lesional (PNL) or psoriasis lesional (PSO) skin as indicated. Nuclei were counterstained with DAPI (blue). **(G)** Immunostaining of ex vivo skin wounds or unwounded control skin with MAVS (green) and pIRF3 (red) antibodies. (**H-K**) Immunostaining of RIG1 (**H**), IRF7 (**I**), MAVS (green) with LL37 or IFNβ (red) (**J**), or MAVS (green) with IFNβ (red) (**K**) of human skin sections as indicated. (**L**) Skin of *Mavs*$^{-/+}$ or *Mavs*$^{-/-}$ mice was injected intradermally with LL37. Representative image of LL37 immunostaining of PBS or LL37 injected skin. All images are representative image of n=3~5 human samples/group. All scale bars in this figure, 100 μm.

Table S1. List of primers used for RT-qPCR (related to experimental procedure "Reverse transcription-quantitative PCR analyses"). Gene symbols and forward and reverse primer sequences for each gene are shown in the table below. Note that predeveloped TaqMan gene expression assays detecting human *MAVS* or mouse *Mav*s gene expression in human psoriasis cDNA samples or in K5-IL-17C skin cDNA were purchased from Applied Biosystems.

Gene	Strand	Primer sequence
GAPDH	Forward	TGGGCTACACTGAGCACCAG
	Reverse	GGGTGTCGCTGTTGAAGTCA
IFNB1	Forward	GGGACTGGACAATTGCTTCAA
	Reverse	GCAGTACATTAGCCATCAGTCACTTAA
IFNA2	Forward	GCTTGGGATGAGACCCTCCTA
	Reverse	CCCACCCCCTGTATCACAC
IFNA4	Forward	TGCTGGTGCTCAGCTACAAA
	Reverse	TGGTGGCCATCAAACTCCTC
IFNA5	Forward	TGGTGCTCAACTGCAAGTCA
	Reverse	GGCTTGAGCCTTCTGGAACT
IFNA10	Forward	TCCCCCAGGAGGAGTTTGAT
	Reverse	TCCCAAGCAGCAGATGAGTC
IFNA14	Forward	ATGACCTGGAAGCCTGTGTG
	Reverse	TCTTCACAGCCAGGATGGAG
MAVS	Forward	TGGAGTCCTCCTCTGACCTG
	Reverse	GGCTGGAAGGAGACAGATGG
RIG1	Forward	TGCGAATCAGATCCCAGTGTA
	Reverse	TGCCTGTAACTCTATACCCATGT
MDA5	Forward	GTGCATGGAGGAGGAACTGT
	Reverse	AGAGCCTGTTAACTCTTGGACA
TLR3	Forward	AGCTGGAAAATCTCCAAGAGC
	Reverse	TTGCGTGAAAACACCCTGGA
TLR4	Forward	AAAATCCCCGACAACCTCCC
	Reverse	AGAGGTGGCTTAGGCTCTGA
TLR5	Forward	GCCGGTCCTGTGTTTGGAAT
	Reverse	GGTGAGGTTGCAGAAACGATAAA
TLR6	Forward	TTCTCCGACGGAAATGAATTTGC
	Reverse	CAGCGGTAGGTCTTTTGGAAC
TLR7	Forward	TCCTTGGGGCTAGATGGTTTC
	Reverse	TCCACGATCACATGGTTCTTTG
TLR8	Forward	ATGTTCCTTCAGTCGTCAATGC
	Reverse	TTGCTGCACTCTGCAATAACT
TLR9	Forward	CTGCCTTCCTACCCTGTGAG
	Reverse	GGATGCGGTTGGAGGACAA
IFNAR1	Forward	ATTTACACCATTTCGCAAAGCTC
	Reverse	TCCAAAGCCCACATAACACTATC
CCL5	Forward	CTGCTTTGCCTACATTGCCC
	Reverse	TCTTCTCTGGGTTGGCACAC
IL6	Forward	AATTCGGTACATCCTCGACGG
	Reverse	TTGGAAGGTTCAGGTTGTTTTCT
TNF	Forward	GCTGCACTTTGGAGTGATCG
	Reverse	GGGTTTGCTACAACATGGGC
IL8	Forward	TCCAAGCTGGCCGTGGCTCT
	Reverse	CTGTGTTGGCGCAGTGTGGTCC
ISG15	Forward	GCGCAGATCACCCAGAAGAT

	Reverse	TCCTCACCAGGATGCTCAGA
IRF7	Forward	CCCACGCTATACCATCTACCT
	Reverse	GATGTCGTCATAGAGGCTGTTG
STAT1	Forward	ATCAGGCTCAGTCGGGGAATA
	Reverse	TGGTCTCGTGTTCTCTGTTCT
K10	Forward	ATGGCAACTCACATCAGGGG
	Reverse	GCGCAGAGCTACCTCATTCT
PDGFRA	Forward	TTGAAGGCAGGCACATTTACA
	Reverse	GCGACAAGGTATAATGGCAGAAT
MX1	Forward	GTTTCCGAAGTGGACATCGCA
	Reverse	CCCACCCCCTGTATCACAC
CD83	Forward	GAGAAACCTAAGTGGCAAGGTG
	Reverse	AGGACAATCTCCGCTCTGTAT
Hprt	Forward	GTTAAGCAGTACAGCCCCAAA
	Reverse	AGGGCATATCCAACAACAAACTT
Mavs	Forward	CTGCCTCACAGCTAGTGACC
	Reverse	CCGGCGCTGGAGATTATTG
Tlr3	Forward	TCCCCGGGGTGTTTCCAGAC
	Reverse	GGTGGGGGTTCAGTTGGGCG
Tlr9	Forward	TCTCCCAACATGGTTCTCCGTCG
	Reverse	GCAGGGTACCCAGGGCCAGA
mK10	Forward	GTCCACTGGTGATGTGAATGT
	Reverse	CCAGACCCTGAACAGTACGTC
Pdgfra	Forward	ATGAGAGTGAGATCGAAGGCA
	Reverse	CGGCAAGGTATGATGGCAGAG
Ifnb1	Forward	AGCTCCAAGAAAGGACGAACA
	Reverse	GCCCTGTAGGTGAGGTTGAT
Ifna2	Forward	GCCATCCCTGTGCTGCGAGA
	Reverse	GCAGCAGATGAAGCCTTTGATGTGA
Ifna4	Forward	CCCTGCTGGCTGTGAGGACA
	Reverse	ACCTCCCAGGCACAGAGGCT
Isg15	Forward	GAGCTAGAGCCTGCAGCAAT
	Reverse	TCACGGACACCAGGAAATCG
Rig1	Forward	CAGATCCGAGACACTAAAGGGA
	Reverse	TCCTCATCAGCCTTGCTTTCA
Mx1	Forward	GACCATAGGGGTCTTGACCAA
	Reverse	AGACTTGCTCTTTCTGAAAAGCC
Cd83	Forward	CGCAGCTCTCCTATGCAGTG
	Reverse	GTGTTTTGGATCGTCAGGGAAT

Supplemental Experimental Procedures

Chemicals and antibodies

Synthetic LL37 and CRAMP peptides was synthesized and purchased from Genemed synthesis Inc (San Antonio, TX), All TLR ligands, including Pam3CSK, LTA, pIC, Malp2, Flagellin, R848, HSV60-dsDNA, EC-dsDNA and CpGA, were purchased from invivogen (Invivogen, San Diego, CA). snRNAs including U1, U2, U4, U6 and U12 were generated by in vitro transcription as described previously (Borkowski et al., 2015). Bafilomycin A1 (blocker for endosomal acidification) was purchased from Sigma (St Louis, MO). Cay10567 (TBK1inhibitor), Wortmannin (PI3K/AKT inhibitor), U0126 (ERK1/2 inhibitor) were all purchased from EMD Millipore (Billerica, MA). RNaseIII was purchased from ThermoFisher Scientific (Waltham, MA). Human recombinant GM-CSF, human recombinant IL4, human Flt3L were purchased from BioLegend (San Diego, CA), and mouse recombinant GM-CSF was purchased from R&D Systems (Minneapolis, MN).

Antibodies

Rabbit anti-LL-37 antibody was made from our lab as described previously (Dorschner et al., 2001). Rabbit anti-IFNβ1, rabbit anti-IFNα, sheep anti-CAMP, rabbit anti-phosphoIRF3 (Ser386; used for IHC), rabbit anti-IRF3 (used for IHC), rabbit anti-ISG15, rabbit anti-IRF7, goat anti-RIG1, mouse anti-Aconitase are from Abcam (Cambridge, MA). Rabbit anti-MAVS mouse anti-phosphoTyrosine, rabbit anti-phosphoTBK1, rabbit anti-TBK1, rabbit anti-phosphoAKT, mouse anti-AKT, rabbit anti-phosphoEKR1/2, mouse anti-ERK1/2 rabbit anti-phosphoIRF3 (Ser396; used for WB) and rabbit anti-IRF3 (used for WB), are from Cell Signaling (Danvers, MA). Mouse anti-MAVS are from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-GAPDH antibody is from Fitzgerald (Acton, MA). Mouse anti-KRT6 was purchased from Pierce-Thermo Scientific (Rockford, IL). Mitoview green (mitochondrial tracker) was purchased from Biotium, INC (Hayward, CA). The SYTO RNASelect Green Fluorescent cell Stain was purchased from ThermoFisher Scientific (Waltham, MA). APC anti-human CD11C, PE anti-human CD86, APC anti-mouse Cd11c, FITC anti-mouse CD80, and FITC anti-mouse CD86 antibodies for FACS analyses were purchased from eBioscience (San Diego, CA). Control IgG or IFNα or IFNβ neutralizing antibodies were purchased from BioLegend (San Diego, CA).

Model of ex vivo wounded human skin

Healthy skin was obtained from excess skin tissue removed from mole removal surgery. The skin biopsies were cut into 4x4 mm square pieces (ex vivo wounded skin) and incubated in culture for 2 days in DMEM supplemented with 10% FBS and antibiotics. Uncultured fresh tissue (day 0 control) or day2 cultured skin (wounded) samples were embedded in OCT for immunohistochemistry.

Mice

Mavs$^{-/-}$ mice (in B6/129 background) was originally purchased from Jackson Laboratory and was backcrossed to B6/129 wildtype to generate the heterozygous *Mavs*$^{-/+}$ mice for further breeding to generate *Mavs*$^{+/+}$, *Mavs*$^{-/+}$ or *Mavs*$^{-/-}$ littermate mice at the University Research Center at the University of California, San Diego (UCSD). All animal experiments were done on sex-matched 7~12 weeks' old mice and are approved by the UCSD Institutional Animal Care and Use Committee. Shaved dorsal skin of *Mavs*$^{-/+}$ or *Mavs*$^{-/-}$ mice was injected intradermally with 50ul 320uM LL37 (to the right side of the dorsal skin) or the vehicle control PBS (to the left side of the dorsal skin) every 12 hrs. 8 hours after the third injections, skin samples were collected for RTqPCR analyses.

Histology, immunohistochemistry (IHC) and immunocytochemistry (ICC)

Tissue biopsies were directly embedded in OCT compound, and frozen sections were fixed in 4% PFA for 10 mins prior to Hematoxylin/Eosin (H&E) staining or immunofluorescence staining as described before (Zhang et al., 2015). Note that for RNA staining, we used 4% PFA prefixed, sucrose cryoprotected skin tissues to maximally preserve RNA stability. For IHC, fixed and permeabilized tissue sections were treated with Image-iT FX reagent (invitrogen) before incubating with primary antibodies followed by appropriate 488- or Cy3-coupled secondary antibodies. Nuclei were counter-stained with DAPI. For ICC analyses, NHEKs were grown on 8-well chamber slides (Thermo Scientific Nunc) coated with attachment factor (Invitrogen), and after indicated treatments, cells were fixed in 4% PFA for 10 mins prior to immunostaining as described above for IHC analyses. All images were taken with an Olympus BX41 microscope (widefield) or Zeiss LSM510 confocal microscope as indicated. All images shown are representative image of n=3~5 human samples or cell treatments/group.

Reverse transcription-quantitative PCR (RTqPCR) analyses

Total cellular RNA was extracted using the PureLink RNA isolation kit with DNase1 digestion to remove genomic DNA contamination (Life Technologies) and 500 ng of RNA was reverse transcribed to cDNA using iScript cDNA synthesis kit (Bio-rad). Quantitative, real-time PCR was performed on the CFX96 real time system (Biorad) using 2X SYBR Green Mix from Biotool (Houston, TX). All of the primers except primers for type1 IFNs used were designed to span at least one exon to minimize the possibility of nonspecific amplification from the genomic DNA. Note that because type1 IFNs have only one exon without intron, it is critical to include the DNase1 digestion step during RNA prep as described above and RNA only controls are included for each qPCR to confirm that there was no non-specific amplification from genomic DNA using these primers. The expression of *GAPDH* gene was used as a house keeping gene to normalize data in human samples or cells. Mouse *Hprt* was used as a house keeping gene to normalize data in mouse samples or cells. Specific primer sequences are shown in supplementary Table S1. *MAVS* expression in psoriatic skin cDNA samples was performed using the pre-developed Taqman Gene Expression Assays (Applied Biosystems, Foster City, CA) and *RPLP0* was used as internal housekeeping gene control as described previously (Johnston et al., 2013). *Mavs* expression in K5-IL-17C skin cDNA samples was performed using the pre-developed Taqman Gene Expression Assays

(Applied Biosystems, Foster City, CA) and *Gapdh* was used as internal housekeeping gene control as described previously (Johnston et al., 2013).

Protein extraction and Immunoblotting analyses

NHEKs were lysed in a denaturing lysis buffer containing 20 mM HEPES pH 7.4, 250mM NaCl, 2 mM EDTA, and 1% SDS supplemented with completed proteinase inhibitor cocktail as well as 50 mM sodium floride, 5mM *N*-ethylmaleimide, 100μM hemin chloride to maximally preserve protein post-transational modifications as described previously (Zhang et al., 2012b). Lysates were boiled for 3 mins homogenized by sonication using digital sonifier (Branson) followed by centrifugation to remove DNA and cell debris. Protein concentrations were measured by BCA protein assay kit (Thermo Scientific Inc., Rockford, IL). For immunoblotting, 20ug of protein was separated on a 10% Tris-Glycine precast gel (Biorad), transferred to PVDF membrane (Biorad), followed by immunoblotting using indicated primary antibodies followed by fluorescent secondary antibodies (LICOR) and imaging using fluorescent Odyssey System (LICOR). All western blot images shown in the paper are representative of 3 independent experiments.

IFNα and IFNβ ELISA

Human IFNα and IFNβ Platinum ProcartaPlex Simplex ELISA Kits were purchased from eBioscience (San Diego, CA) and samples were analyzed by Luminex machine and IFNα and IFNβ concentration in NHEK or pDC conditioned medium was determined according to manufacturer's instruction.

Dendritic cell culture and stimulation

Fresh human PBMCs were purchased from ixcells Biotechnology (San Diego, CA) and cells were used within a few hours after collection. Mouse bone marrow (BM) cells were isolated from C57BL/6 wildtype mouse femur. Classical DCs were differentiated from human adherent PBMCs with human GMCSF (50 ng/ml) and human IL4 (25 ng/ml) or from mouse BM cells with mouse GM-CSF (25 ng/ml) in RPMI1640 supplemented with 10% FBS, 50 μM 2-ME and antibiotics for 6 days. Human pDCs were isolated from total PBMCs using pDC MACS isolation kit from Miltenyl Biotec (San Diego, CA). Mouse pDCs were differentiated from BM cells for 7 days with 100 ng/ml human Flt3L, and pDCs were purified with anti-B220 MACS beads as described before (Nakagawa et al., 2014). Purity of each cell type is greater than 80% as confirmed by FACS analyses. To stimulate DCs, LPS (0.5 μg/ml) or CpGA (1 μM), or LL37 (2 μM), poly IC (0.4 μg/ml), or 1:1 v/v of KC-CM was added to 0.2~0.5 x 10^6 DCs in 24 well dishes. Control IgG or IFNα or IFNβ neutralizing antibody was added to KC-CM at 10 μg/ml in KC-CM before adding the KC-CM to DC culture. RNA and supernatant was collected at 6 hrs after stimulation and FACS analyses for surface expression of CD11c, CD80, CD86 and CD83 were performed by flow cytometry 24~48 hrs after stimulation and analyzed by FlowJo V10 software.

T cell proliferation assay

Autologous naïve CD3 T cells were purified from fresh human PBMCs using CD3 MACS isolation kit from Miltenyl Biotec (San Diego, CA). The CD3 negative adherent monocytes was used to generate classical DCs as described in the "Dendritic cell culture and stimulation" section. Purified CD3 T cells and monocyte-derived cDCs from the same donor were used in T cell proliferation assay. Monocyte derived cDCs were treated for 24 hrs with KC-control-CM with IgG, or KC-LP-CM with IgG, or KC-LP-CM with IFNβ neutralizing antibody, cells were then washed to remove KC-CM before adding to T cells. Purified CD3+ T cells (3×10^5) were labeled with CFSE (2 μM) then co-cultured with cDC that had been pretreated with KC-CM at a ratio of 1:5 (DCs: T cells). After 5 days in culture, the CFSE fluorescence was determined by flow cytometry and FlowJo V10 software.

Supplemental References:

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