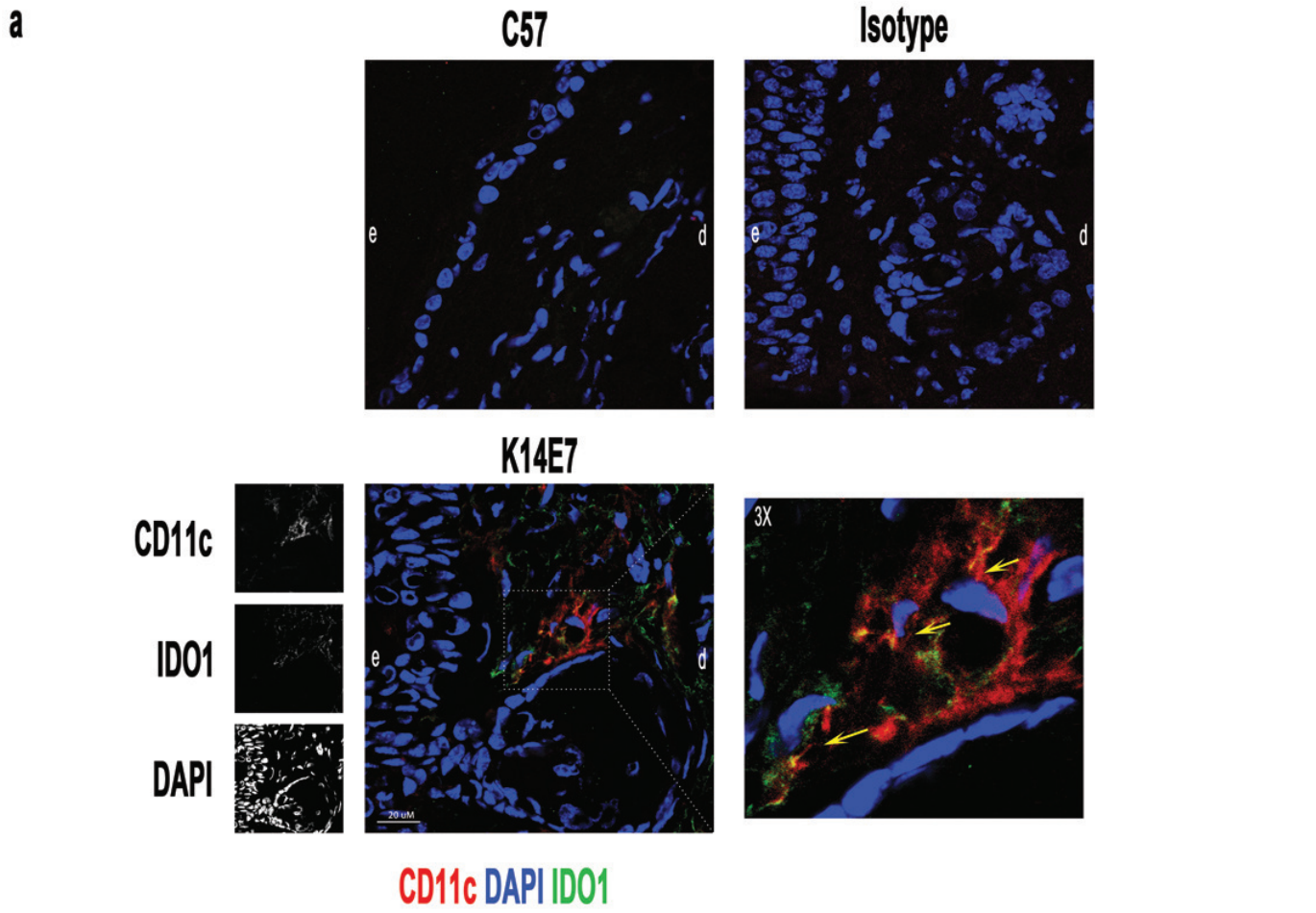


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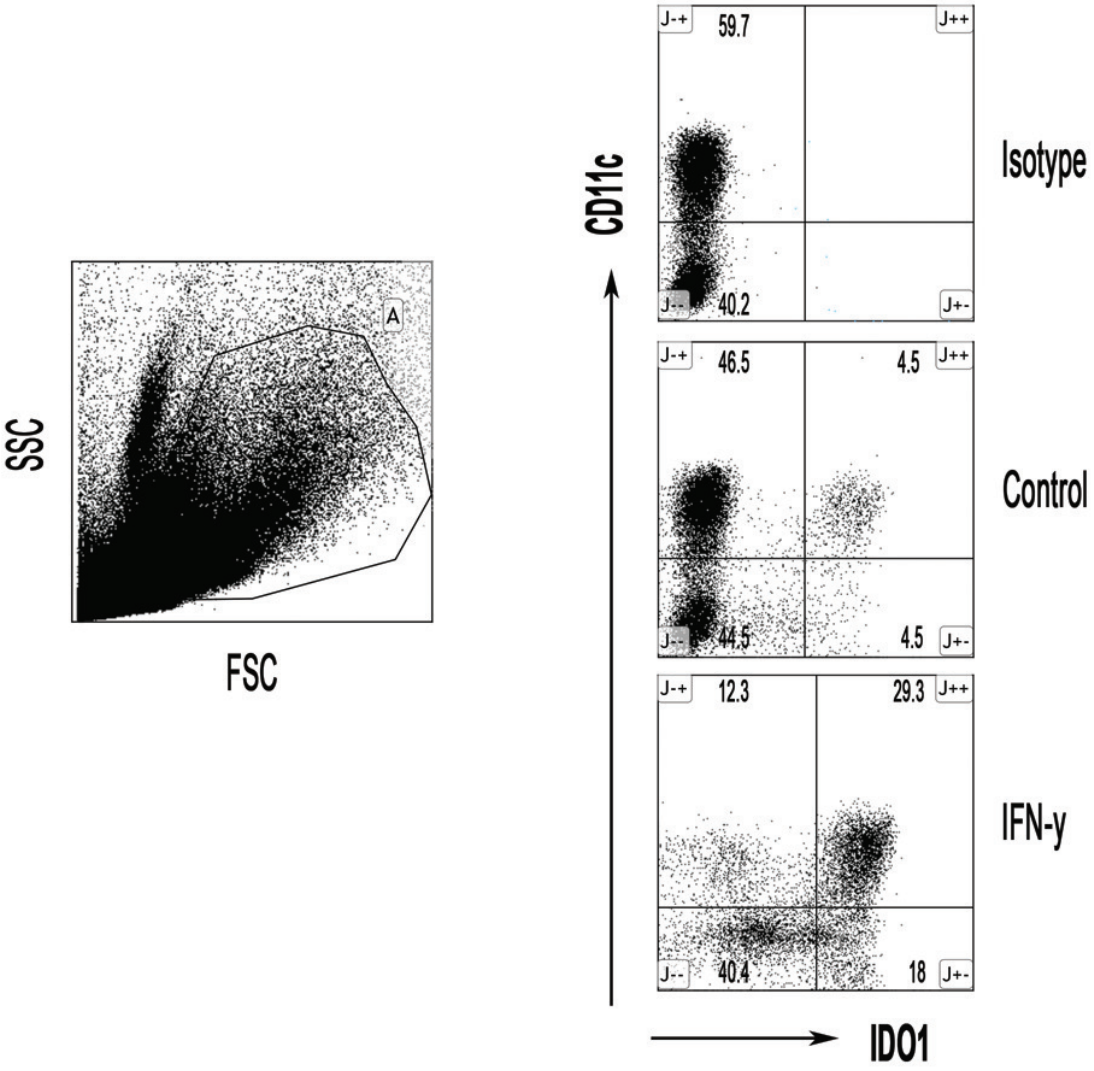


Mittal D et.al. Supplementary figure 2

## Supplementary methods

### Real-time PCR reaction

One microgram of total RNA was reverse transcribed using oligo dT, reverse transcriptase and RNAse inhibitor (AB Biosystems) in a 20µl total reaction volume. cDNA was diluted 1:5 and 2.5µl of cDNA/10µl reaction was used for SybrGreen real time PCR reactions. Each sample was run at least in duplicates and the results were analyzed using SDS software (AB Biosystems). The expression of different genes such as IDO1, IDO2 and TDO was normalized with ribosomal protein L32 (RPL32) and plotted as relative gene expression. For human samples, CIN-2/3 and surrounding normal cervical epithelial tissues (~10 $mm^3$) were collected and processed as above. The expression of human genes was normalized to human Hypoxanthine-guanine phosphoribosyltransferase -1 (HPRT-1). The list of primer pairs is listed in supplementary table 1.

### Flow cytometry

Anti-mouse monoclonal antibodies to CD3e (145-2C11), CD11b (553311), CD11c (HL3), CD45.2 (104), CD103 (557495), MHC-II (M5/114.15.2), langerin (DDX03628) and IFN-γR (MOB-47) and respective isotype control antibodies were purchased from BD Biosciences (San Jose, CA), Biolegend (San Diego, CA), Imgenex (San Diego, CA) and eBioscience (San Diego, CA). Ear skin was split into dorsal and ventral halves by forceps and was floated dermis side down in 1 mg/ml collagenase/dispase solution (Roche, Berlin, Germany) for 30 min at 37°C. After 30 min of incubation, skin was mechanically disrupted using forceps and passed through a

70μm cell strainer (BD Biosciences) to obtain single cell suspensions. Cells were preincubated with Fc block (Fcγ III/II receptor; BD Biosciences) for 10 min on ice before staining with predetermined optimal concentration of antibodies for 30 min at 4°C. For intracellular staining of IDO1, cells were fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) for 20 min on ice according to the manufacturer's instructions. Cells were subsequently stained using α-IDO1 (mIDO-48, BioLegend) for 30 min at 4°C and incubated with Alexa Fluor 488 conjugated secondary antibody. Flow-Count Fluorospheres™ (Beckman Coulter) were used for direct determination of absolute counts following the manufacturer's recommendations. Cells were acquired using a FACSCanto (BD Biosciences) or FACS Gallios (Beckman Coulter Inc) and analyzed with FlowJo (Tree Star Inc) or Kaluza (Beckman Coulter Inc) software.

For sorting DCs, inguinal and axillary lymph nodes were isolated from K14E7 mice and processed in collagenase/dispase. Resident (MHC-II$^{intermediate}$) and migratory (MHC-II$^{high}$) dendritic cells from the lymph nodes were sorted by flow cytometry (MoFlo, BD Biosciences) based on dual CD11c$^{+}$ and MHC-II$^{+}$ staining after the exclusion of dead cells by propidium iodide.

**Immunofluorescence staining**

Ear skin samples were kept in PLP buffer (37.5% 0.1M sodium periodate – 37.5% 0.2M poly L-lysine- 25% of 4% paraformaldehyde) for 24h at +4$^{o}$C. Samples were washed twice with PBS and dehydrated in 20% sucrose solution overnight at +4$^{o}$C. Samples were embedded in Tissue-Tek OCT and 7μM thick sections were blocked with 1% bovine serum albumin in 0.1M Tris-cl (pH 7.4) for 1 h before staining with anti-IDO (mIDO-48, BioLegend) and anti-CD11c (HL3,

BD Biosciences) primary antibodies overnight at +4$^0$C. Sections were incubated with appropriate Alexafluor conjugated secondary antibodies for 1 h at room temperature. Sections were then washed and stained with DAPI for 5 min. Images were acquired with Zeiss 510 Meta Confocal Microscope with Zen software (Zen software).

**Supplementary Table 1**: List of primers used for real-time PCR reaction

| **Gene** | **Forward (5'-3')** | **Reverse (5'-3')** |
|---|---|---|
| mIDO1 | CAATCAAAGCAATCCCCACTG | AAAACGTGTCTGGGTCCAC |
| mIDO2 | CCTAAAGAGTTACCGTGAGCAG | AACAAAAGGAATGGCAAGAGATC |
| mTDO | CTCAAGGTGATAGCTCGGATG | GAACTGTAGACTCTGGAAGCC |
| mRPL32 | AAGCGAAACTGGCGGAAAC | TAACCGATGTTGGGCATCAG |
| hIDO1 | TGGAGAAAGCCCTTCAAGTG | CCAGAACCCTTCATACACCAG |
| hIDO2 | AAGATAGCATTCGTGGCCTG | TGGAAATTGGGAACCTGGAG |
| hIFN-γ | GCATCGTTTTGGGTTCTCTTG | AGTTCCATTATCCGCTACATCTG |
| h IFN-γR1 | CGATTATGATCCCGAAACTACCTG | GGATACTGGAATCGCTAACTGG |
| hIFN-γR2 | CATTTTCGTTGCTGTCGGTG | CTCTAAGATGGGCTGAGTTGG |
| hHPRT | TCAGGCAGTATAATCCAAAGATGGT | AGTCTGGCTTATATCCAACACTTCG |

Prefix: h-human; m-mouse

**Supplementary figure 1.**

a) Dendritic cells in the dermis of K14E7 mice express IDO1

Immunofluorescence staining for IDO1 (green), CD11c (red) and DAPI (blue) in the skin of K14E7 mice and C57 mice. Images are representative from three skin samples and the experiment was repeated twice. Yellow arrow indicates $IDO1^+$ $CD11c^+$ cells. (d= dermis, e= epidermis; scale bar = 20μM).

b) IFN-γ induces IDO1 expression in the skin of IFN-$\gamma^{-/-}$E7 mice

Immunofluorescence staining for IDO1 (green), CD11c (red) and DAPI (blue) in the skin of IFN-$\gamma^{-/-}$E7 mice injected with IFN-γ (1ng/mouse) or PBS. Green arrow indicates $IDO1^+$ $CD11c^-$ cells and yellow arrow indicates $IDO1^+$ $CD11c^+$ DCs (d= dermis, e= epidermis; scale bar = 20μM).

**Supplementary figure 2.** IFN-γ induces IDO1 expression in splenic dendritic cells

Representative FACS plot showing MACS-purified (positive selection) splenic DCs 24 h after incubation with IFN-γ. CD11c and IDO1 staining along with isotype control staining for IDO1 with in MACS purified splenic DCs stimulated or not with IFN-γ (10ng/ml) for 24 h.