**Interleukin-6 secretion is limited by self-signaling in endosomes**

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**Supplementary Materials and methods**

*Cell culture*

MoDCs were derived from peripheral blood monocytes isolated from buffy coats from healthy volunteers (informed consent obtained and according to institutional and national ethics guidelines) by 6 days culturing with 300 U/ml IL-4 and 450 U/ml GM-CSF, as described [de Vries IJM, Eggert AAO, Scharenborg NM, et al (2002) J. Immunother. *25*, 429–438; Baranov M, Ter Beest M, Reinieren-Beeren I, et al (2014) J. Cell Sci. *127*, 1052–1064]. Monocyte-derived macrophages were derived from CD14+ monocytes isolated from peripheral blood monocytes with anti-CD14 conjugated magnetic beads (Miltenyi Biotec) by 6 days culturing with 20 ng/ml M-CSF (ProteinPrep). Plasmacytoid dendritic cells were isolated by positive selection with anti-BDCA4 conjugated magnetic beads and CD1c+ dendritic cells were isolated with anti-CD1c conjugated magnetic beads after B cell depletion (Miltenyi Biotec). All cells were cultured in RPMI-1640 medium (Gibco) supplemented with 2 mM ultraglutamine (Westburg), 10% fetal bovine serum (FBS) and 1% antibiotic-antimyotic (AA; PAA), except for HeLa cells which were cultured in high glucose DMEM with Glutamax and sodium pyruvate (Gibco), supplemented with 10% FBS and 1% AA. HeLa cell dissociation was performed with 2 mM EDTA for 5 mins at 37 ℃ and 5% $CO_2$ and cells were routinely passaged when 85-90% confluent. Where indicated, cells were treated with a log series up to 15 ng/ml of IL-6 (Cell Genix) for 20 minutes, 1 µg/ml LPS (Invivogen; from *E. coli* O111:B4; UltraPure; tlrl-3pelps) for 4, 6, 8, 18 or 24 hours, a combination of 2 µg/ml Poly(I:C) (Enzo Life Sciences) and 4 µg/ml R848 for 22 hours (Enzo Life Sciences), 10 µg/ml Brefeldin A (Sigma Aldrich), or 25 µM MG132 (Sigma). For dynamin inhibition, cells were incubated with 20 µM hydroxy-dynasore (Sigma-Aldrich) for 40 minutes. ELISA was used to measure soluble IL-6RA (DR600; R&D Systems), soluble gp130 (DGP00; R&D Systems) and IL-6 (88-7066-88; E Bioscience). Flow experiments were performed with 375,000 cells in a Sykes-Moore culture chamber (Bellco Glass) at 3 ml/hour flow rate. For blocking extracellular IL-6, we used 1 µg/ml of neutralizing mouse IgG1 (mabg-hil6-3; InvivoGen) and azide free mouse IgG1 as negative isotype control (MA1-10407; ThermoFisher).

BMDCs were generated from the femurs and tibias of 6–18 weeks old female wild-type mice (C57BL/6J). After isolation, bone marrow cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 1% L-glutamine (Lonza), 50 µM β-mercaptoethanol (Sigma-Aldrich) and 0.5% AA. For differentiation, medium was supplemented with either 200 ng/ml of FLT3L (Miltenyi) on the day of culture start (Day 0), or with 20 ng/ml of GM-CSF (Prepotech) on Day 0, with replenishment of medium on Day 3 and Day 6 (at a final concentration of 8.75 ng/ml GM-CSF each day), as described [Dingjan I, Verboogen DR, Paardekooper LM, et al (2016) Sci. Rep. *6*, 22064]. In both cases, the cells were harvested on Day 8. BMDCs were either not activated (0h) or activated with 1 µg/ml of LPS for 6h, 24h and 48 h.

*PCR*

mRNA was isolated from homogenized moDCs with the Quick-RNA MiniPrep kit (Zymo Research). RNA was reverse transcribed into cDNA with Moloney murine leukemia virus reverse transcriptase and random hexamers (Invitrogen). IL-6RA mRNA was amplified by PCR with the forward primer: CCCCTCAGCAATGTTGTTTGT and three reverse primers: GGCGTCGTGGATGACACAG, GGCAGTGGTACTGAAGAAGAA and CAGAACAATGGCAATGCAGA. To determine the occurrence of the short isoform of IL-6RA, the forward primer 6R5-1 (ACGCCTTGGACAGAATCCAG) and the reverse primer 6R3-2 (TGGCTCGAGGTATTGTCAGA) were used, as described [Horiuchi S, Koyanagi Y, Zhou Y, et al (1994) Eur. J. Immunol. *24*, 1945–1948]. The following primers were used for qPCR of IL-6: forward: AGTGAGGAACAAGCCAGAGC, reverse: AGCTGCGCAGAATGAGATGA; GUSB: forward: GACACGCTAGAGCATGAGGG, reverse: GGGTGAGTGTGTTGTTGATGG; and SOCS3: forward: CCTGCGCCTCAAGACCTTC, reverse: GTCACTGCGCTCCAGTAGAA.

*Transfection*

MoDCs were transfected with plasmid DNA or siRNA against IL-6RA (hIL-6R Stealth; HSS105342; Thermo Scientific) or VAMP3 (HSS113848, HSS113849, HSS113850; Thermo Scientific [Verboogen DRJ, Gonz âlez Mancha N, Ter Beest M & van den Bogaart G (2017) Elife *6*, 1–17] using a Neon Transfection system (Thermo Scientific) as described [Baranov M, Ter Beest M, Reinieren-Beeren I, et al (2014) J. Cell Sci. *127*, 1052–1064; Dingjan I, Verboogen DR, Paardekooper LM, et al (2016) Sci. Rep. *6*, 22064]. Genes coding for human IL-6 and human STAT3 were ordered as synthetic genes from GenScript (sequences below) and subcloned into the XhoI/BamHI and HindIII/AgeI sites of pEGFP-N1 (Clontech), respectively. The IL-6RA gene (HGl0398-M-N; Sino Biological) was subcloned into the EcoRI/SalI sites of pmCherry-N1 (Clontech). Epsin2-pmCherryC1 was a gift from Christien Merrifield (Addgene plasmid #27673) [Taylor MJ, Perrais D & Merrifield CJ (2011) PLoS Biol. *9*, e1000604].

*Construct for human IL-6*

Sequence of synthetic gene coding for human IL-6 flanked by XhoI and BamHI restriction sites:

CTCGA GATGA ACAGC TTCAG CACCA GCGCC TTCGG CCCCG TGGCC

TTCAG CCTGG GCCTG CTGCT GGTGC TGCCC GCCGC CTTCC CCGCC

CCCGT GCCCC CCGGC GAGGA CAGCA AGGAC GTGGC CGCCC CCCAC

CGCCA GCCCC TGACC AGCAG CGAGC GCATC GACAA GCAGA TCCGC

TACAT CCTGG ACGGC ATCAG CGCCC TGCGC AAGGA GACCT GCAAC

AAGAG CAACA TGTGC GAGAG CAGCA AGGAG GCCCT GGCCG AGAAC

AACCT GAACC TGCCC AAGAT GGCCG AGAAG GACGG CTGCT TCCAG

AGCGG CTTCA ACGAG GAGAC CTGCC TGGTG AAGAT CATCA CCGGC

CTGCT GGAGT TCGAG GTGTA CCTGG AGTAC CTGCA GAACC GCTTC

GAGAG CAGCG AGGAG CAGGC CCGCG CCGTG CAGAT GAGCA CCAAG

GTGCT GATCC AGTTC CTGCA GAAGA AGGCC AAGAA CCTGG ACGCC
ATCAC CACCC CCGAC CCCAC CACCA ACGCC AGCCT GCTGA CCAAG
CTGCA GGCCC AGAAC CAGTG GCTGC AGGAC ATGAC CACCC ACCTG
ATCCT GCGCA GCTTC AAGGA GTTCC TGCAG AGCAG CCTGC GCGCC
CTGCG CCAGA TGGAT CC

*Construct for human STAT3*

Sequence of synthetic gene coding for human STAT3 flanked by HindIII and AgeI restriction sites:

AAGCT TGATT TTAGC AGGAT GGCCC AATGG AATCA GCTAC AGCAG CTTGA
CACAC GGTAC CTGGA GCAGC TCCAT CAGCT CTACA GTGAC AGCTT CCCAA
TGGAG CTGCG GCAGT TTCTG GCCCC TTGGA TTGAG AGTCA AGATT GGGCA
TATGC GGCCA GCAAA GAATC ACATG CCACT TTGGT GTTTC ATAAT CTCCT
GGGAG AGATT GACCA GCAGT ATAGC CGCTT CCTGC AAGAG TCGAA TGTTC
TCTAT CAGCA CAATC TACGA AGAAT CAAGC AGTTT CTTCA GAGCA GGTAT
CTTGA GAAGC CAATG GAGAT TGCCC GGATT GTGGC CCGGT GCCTG TGGGA
AGAAT CACGC CTTCT ACAGA CTGCA GCCAC TGCGG CCCAG CAAGG GGGCC
AGGCC AACCA CCCCA CAGCA GCCGT GGTGA CGGAG AAGCA GCAGA TGCTG
GAGCA GCACC TTCAG GATGT CCGGA AGAGA GTGCA GGATC TAGAA CAGAA
AATGA AAGTG GTAGA GAATC TCCAG GATGA CTTTG ATTTC AACTA TAAAA
CCCTC AAGAG TCAAG GAGAC ATGCA AGATC TGAAT GGAAA CAACC AGTCA
GTGAC CAGGC AGAAG ATGCA GCAGC TGGAA CAGAT GCTCA CTGCG CTGGA
CCAGA TGCGG AGAAG CATCG TGAGT GAGCT GGCGG GGCTT TTGTC AGCGA
TGGAG TACGT GCAGA AAACT CTCAC GGACG AGGAG CTGGC TGACT GGAAG
AGGCG GCAAC AGATT GCCTG CATTG GAGGC CCGCC CAACA TCTGC CTAGA
TCGGC TAGAA AACTG GATAA CGTCA TTAGC AGAAT CTCAA CTTCA GACCC
GTCAA CAAAT TAAGA AACTG GAGGA GTTGC AGCAA AAAGT TTCCT ACAAA
GGGGA CCCCA TTGTA CAGCA CCGGC CGATG CTGGA GGAGA GAATC GTGGA
GCTGT TTAGA AACTT AATGA AAAGT GCCTT TGTGG TGGAG CGGCA GCCCT
GCATG CCCAT GCATC CTGAC CGGCC CCTCG TCATC AAGAC CGGCG TCCAG
TTCAC TACTA AAGTC AGGTT GCTGG TCAAA TTCCC TGAGT TGAAT TATCA
GCTTA AAATT AAAGT GTGCA TTGAC AAAGA CTCTG GGGAC GTTGC AGCTC
TCAGA GGATC CCGGA AATTT AACAT TCTGG GCACA AACAC AAAAG TGATG
AACAT GGAAG AATCC AACAA CGGCA GCCTC TCTGC AGAAT TCAAA CACTT
GACCC TGAGG GAGCA GAGAT GTGGG AATGG GGGCC GAGCC AATTG TGATG

CTTCC CTGAT TGTGA CTGAG GAGCT GCACC TGATC ACCTT TGAGA CCGAG
GTGTA TCACC AAGGC CTCAA GATTG ACCTA GAGAC CCACT CCTTG CCAGT
TGTGG TGATC TCCAA CATCT GTCAG ATGCC AAATG CCTGG GCGTC CATCC
TGTGG TACAA CATGC TGACC AACAA TCCCA AGAAT GTAAA CTTTT TTACC
AAGCC CCCAA TTGGA ACCTG GGATC AAGTG GCCGA GGTCC TGAGC TGGCA
GTTCT CCTCC ACCAC CAAGC GAGGA CTGAG CATCG AGCAG CTGAC TACAC
TGGCA GAGAA ACTCT TGGGA CCTGG TGTGA ATTAT TCAGG GTGTC AGATC
ACATG GGCTA AATTT TGCAA AGAAA ACATG GCTGG CAAGG GCTTC TCCTT
CTGGG TCTGG CTGGA CAATA TCATT GACCT TGTGA AAAAG TACAT CCTGG
CCCTT TGGAA CGAAG GGTAC ATCAT GGGCT TTATC AGTAA GGAGC GGGAG
CGGGC CATCT TGAGC ACTAA GCCTC CAGGC ACCTT CCTGC TAAGA TTCAG
TGAAA GCAGC AAAGA AGGAG GCGTC ACTTT CACTT GGGTG GAGAA GGACA
TCAGC GGTAA GACCC AGATC CAGTC CGTGG AACCA TACAC AAAGC AGCAG
CTGAA CAACA TGTCA TTTGC TGAAA TCATC ATGGG CTATA AGATC ATGGA
TGCTA CCAAT ATCCT GGTGT CTCCA CTGGT CTATC TCTAT CCTGA CATTC
CCAAG GAGGA GGCAT TCGGA AAGTA TTGTC GGCCA GAGAG CCAGG AGCAT
CCTGA AGCTG ACCCA GGTAG CGCTG CCCCA TACCT GAAGA CCAAG TTTAT
CTGTG TGACA CCAAC GACCT GCAGC AATAC CATTA CCTGC CGATG TCCCC
CCGCA CTTTA GATTC ATTGA TGCAG TTTGG AAATA ATGGT GAAGG TGCTG
AACCC TCAGC AGGAG GCAG TTTGA GTCCC TCACC TTTGA CATGG AGTTG
ACCTC GGAGT GCGCT ACCTC CCCCA TGCCA CCGGT

*Western blot*

For Western blot, moDCs were lysed in 1% SDS and 10 mM Tris-HCl, pH 6.8 and blotted to PVDF. Antibodies used were a polyclonal rabbit against IL-6RA (sc-661; Santa Cruz Biotechnology), a polyclonal rabbit against mouse IL-6RA (23457-1-AP; Proteintech), a rat IgG2a against α-Tubulin (YOL1/34; Novus biological), a rabbit monoclonal IgG against GAPDH (clone 14C10; Cell Signaling Technology), a rabbit polyclonal against VAMP3 (ab5789; Abcam), a rabbit monoclonal IgG against total STAT3 (clone 79D7; Cell Signaling), a rabbit monoclonal IgG against tyrosine phospho-STAT3 (Tyr705; clone D3A7; Cell Signaling), a rabbit monoclonal IgG against serine phospho-STAT3 (Ser727; #9134; Cell Signaling [Moravcová S, Červená K, Pačesová D & Bendová Z (2016) J. Neurosci. Res. *94*, 99–108]), and a goat polyclonal against SOCS3 (M-20; Santa Cruz Biotechnology). Secondary antibodies were conjugated to IRDye680 or IRDye800 (Li-Cor).

*Flow cytometry*

MoDCs were stained with mouse IgG1 against human IL-6RA conjugated to PE or APC (clone UV-4; Biolegend), a polyclonal rabbit against human IL-6RA (sc-661; Santa Cruz

Biotechnology), mouse IgG2a against human gp130 conjugated to PE (clone 2E1B02; Biolegend), rat IgG1 against human IL-6 conjugated to PE (clone MQ2-13A5; Biolegend) and mouse ascites IgG1 against human TfR (clone b3/25; Santa Cruz Biotechnology) combined with a secondary antibody labeled with Alexa fluor 488 (Life Technologies) under 0.1% saponin (Sigma) permeabilizing and non-permeabilizing conditions. A FACS Calibur (BD biosciences) was used and data was analyzed using FlowJo software.

*Immunofluorescence*

For immunofluorescence staining, 75,000 moDCs were cultured on 12 mm diameter glass coverslips. The cells were fixed with 4% paraformaldehyde (PFA), permeabilized with 0.1% saponin and blocked in PBS containing 20 mM glycine, 3% BSA and 0.1% saponin (CLSM-buffer). Cells were stained with the following primary antibodies in CLSM-buffer at a 1:100 dilution: polyclonal rabbit IgG against IL-6RA (sc-661; Santa Cruz Biotechnology), mouse IgG2a against IL-6 (sc-28343; Santa Cruz Biotechnology), mouse IgG1 against GM130 (610822; BD), mouse ascites IgG1 against TfR (clone b3/25; Santa Cruz Biotechnology), polyclonal rabbit against VAMP3 (ab5789; Abcam), polyclonal rabbit against VAMP8 (104 302; SySy), polyclonal goat against TGN38 (Clone C-15; Santa Cruz Biotechnology), mouse IgG1 against LAMP1 (328601; Biolegend), and mouse IgG1 against EEA1 (610456; BD). Secondary antibodies were labeled with Alexa fluor 488 or 647 (Life Technologies) and used at a 1:400 dilution. Cells were mounted in 100 mM Na-phosphate buffer at pH 7.4 with 68% glycerol and 4',6-diamidino-2-phenylindole (DAPI).

*Optical microscopy*

Fluorescence live cell imaging was performed with a Leica DMI6000 epi-fluorescence microscope fitted with a 63× 1.4 NA oil immersion objective. Focus was kept stable with the adaptive focus control (Leica). Confocal images were recorded with a Leica SP8 confocal laser scanning microscope with a 63× 1.20 NA water immersion objective. Overlap was quantified with an unbiased macro in FIJI that automatically selected imaged cell areas based on fluorescence intensity and then calculated Pearson correlation coefficients using the JACoP plugin. TIRF was performed on an Olympus IX71 inverted microscope equipped with a 150× 1.45 NA oil immersion objective and a Cell^TIRF illuminator and appropriate lasers for excitation (Olympus). Fluorescence emission was separated from the excitation light with a CMR-U-M4TIR-SBX dichroic mirror (Olympus) and FF01-530/43-25 emission band-pass filter (Semrock). Fluorescence from GFP and mCherry was separated with a T565lpxr (Chroma) dichroic mirror and FF01-530/43-25 (GFP; Semrock) and FF01-612/69-25 (mCherry; Semrock) emission filters. Images were recorded with an EM-CCD camera (C9100-13, Hamamatsu). The sample was kept at 37$^{o}$C by a sample heater (Pecon) and an objective heater (Tokai-Hit).

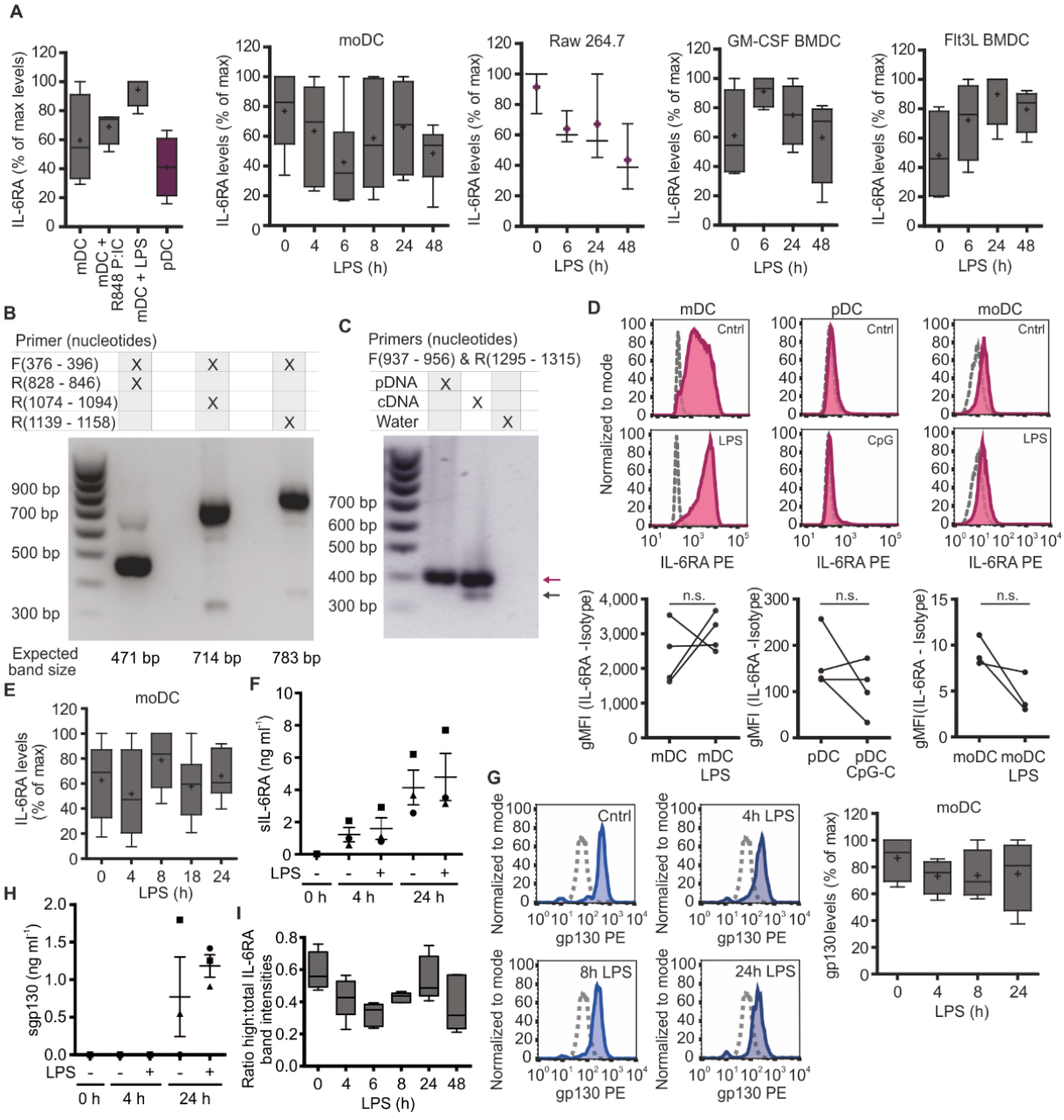
*Electron microscopy*

Primary rabbit IgG against IL-6RA at 100x dilution (Clone 23457-1-AP; Proteintech) or matching isotype control was pre-incubated with 10 nm diameter gold-labeled protein A (binds rabbit IgG) for 20 minutes. Subsequently, antibody mixes were incubated with the cells for 1 hour at 37$^{o}$C for their uptake. Cells were washed twice with PBS and fixed in 2%

glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 (PB). Post-fixation of cells was done with 1% (w/v) $OsO_4$ and 1% (w/v) potassium ferrocyanide in 0.1 M PB for 1 hour. Following dehydration with a graded ethanol series (30–100%) the cells were incubated in a 1:1 mixture of Epon resin and ethanol for 3.5 hour. After ethanol evaporation, the Epon resin was replaced and polymerized for 24 hour at 60$^{o}$C. 90 nm thin sections were cut with a microtome and specimens were imaged with a JEOL 1010 transmission electron microscope.

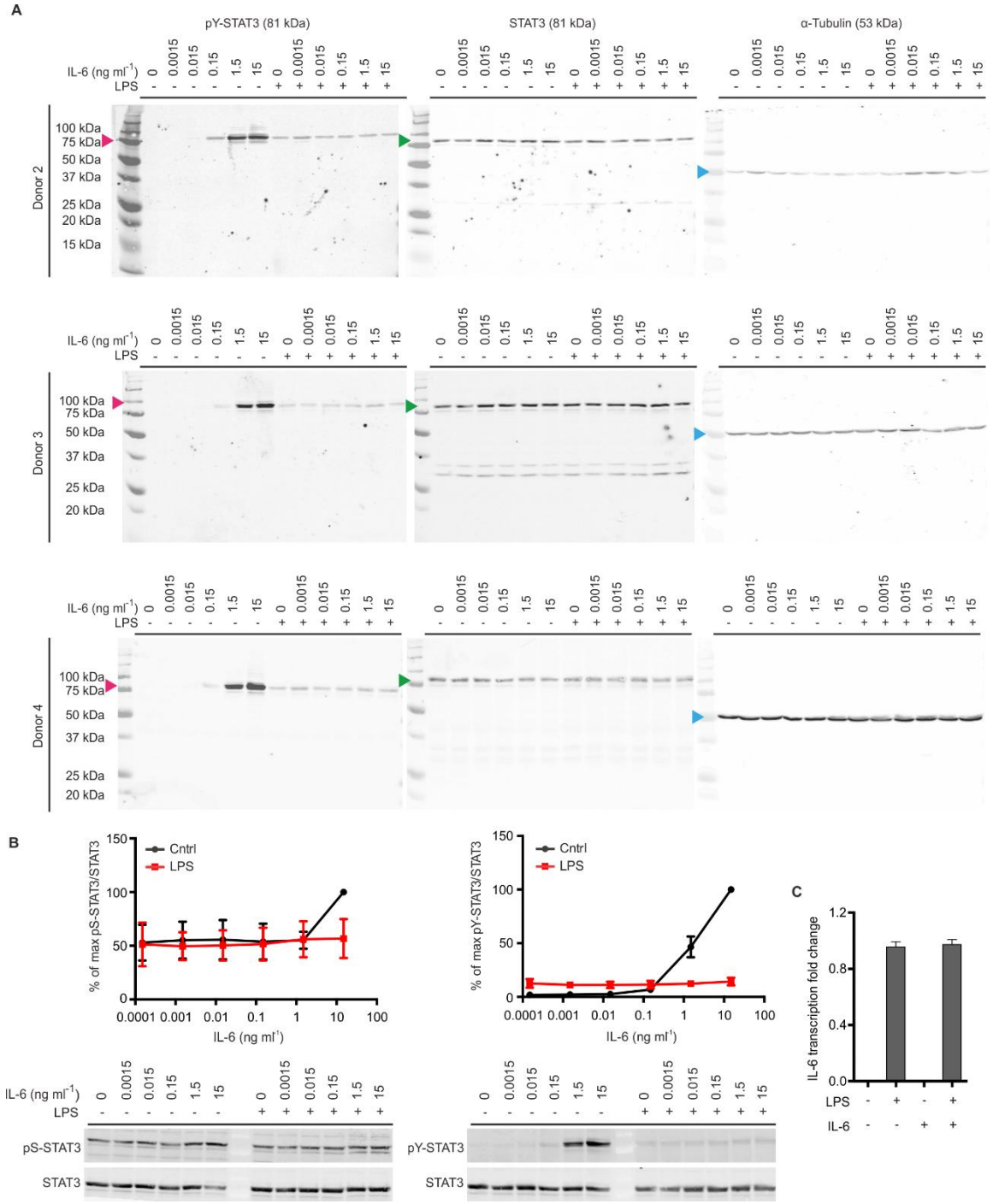
*Statistical analysis*

All data were analyzed using paired two-sided Student's t-tests (for datasets with two conditions), and ANOVA with post-hoc Bonferroni multiple comparison test (for >2 conditions). A value of $p < 0.05$ was considered statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

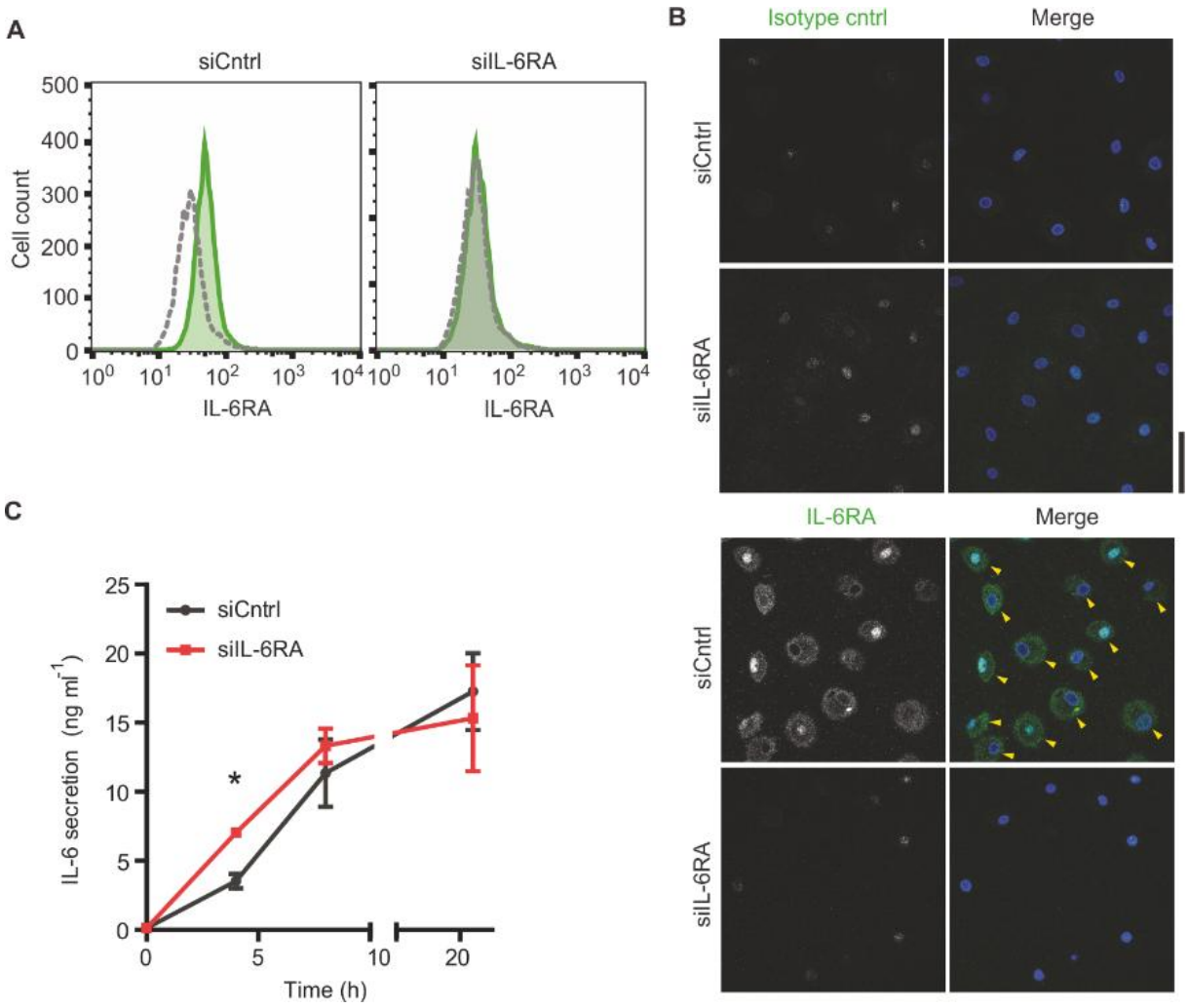



**Supplementary Figure S1. Levels of IL-6RA and gp130 in dendritic cells upon LPS stimulation.** (**A**) Quantification of IL-6RA protein levels by Western blot for CD1c+ myeloid dendritic cells (mDC) with or without overnight stimulation with LPS or a combination of Poly(I:C) and R848, plasmacytoid dendritic cells (pDC) and for moDCs, RAW264.7, and murine GM-CSF and FLT3L-differentiated bone marrow derived dendritic cells (BMDCs) stimulated with LPS for the times indicated (mean ± SEM from at least 4 donors or mice). The highest band intensity of IL-6RA per donor was used to calculate % of max (see main Figure 1A for representative blots). (**B**) mRNA expression of IL-6RA determined in moDCs by PCR. The position of the forward and reverse primers and the expected band sizes are indicated. (**C**) mRNA expression of IL-6RA isoforms determined by PCR. Plasmid DNA (pDNA) codes only for the long isoform 1 of IL-6RA (pink arrow); cDNA is derived from unstimulated moDCs. Grey arrow: short soluble isoform of IL-6RA. The forward primer 6R5-1 and the reverse primer 6R3-2 were used, as described [Horiuchi S, Koyanagi Y, Zhou Y, et

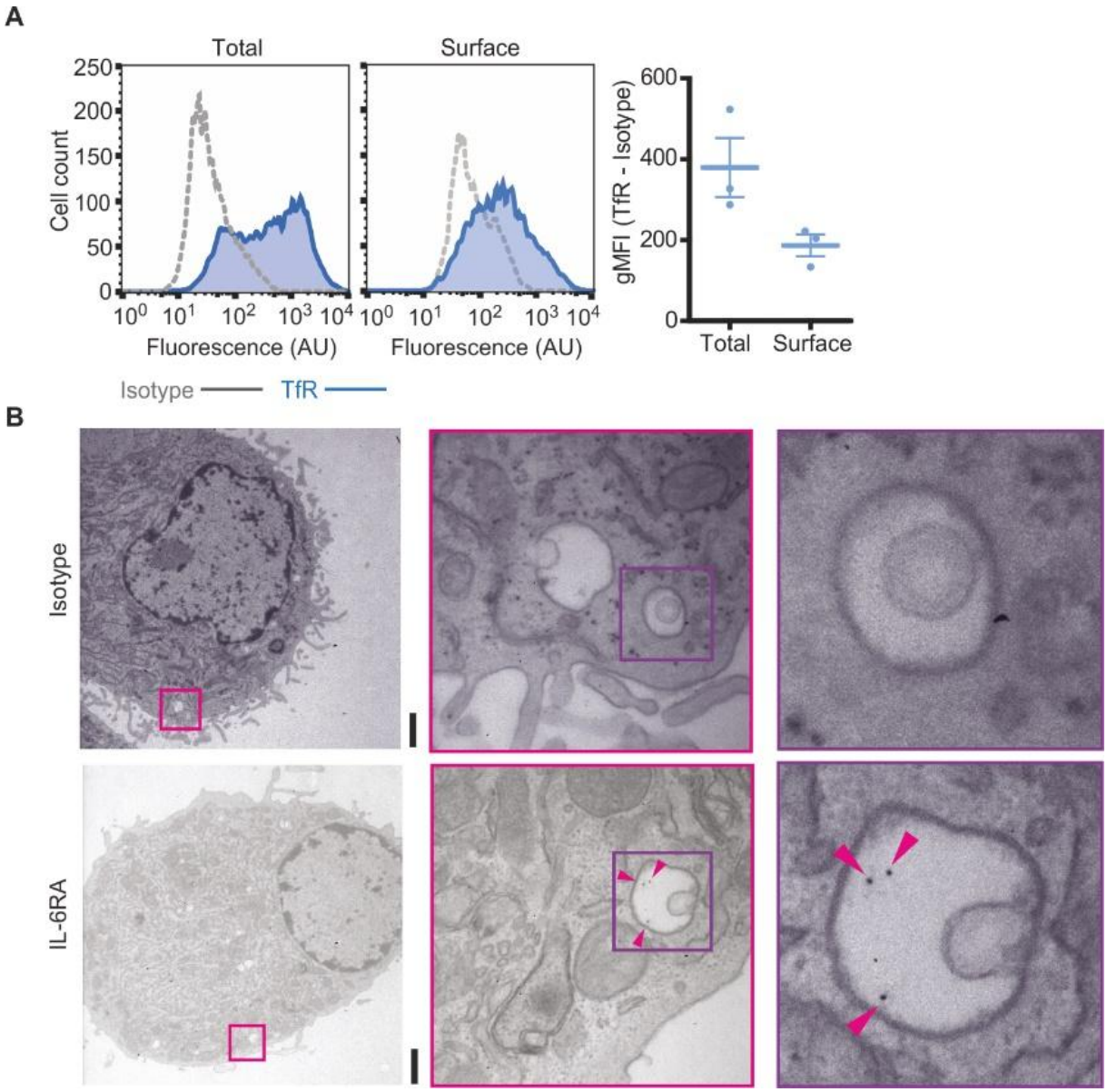
al (1994) Eur. J. Immunol. *24*, 1945–194]. (**D**) Quantification of IL-6RA protein levels by flow cytometry in mDCs treated overnight (16 h) with LPS, plasmacytoid DCs treated with CpG-C and moDCs with or without LPS for individual donors. Representative histograms from 1 donor are shown. Note the large variability among donors. (**E**) Quantification of IL-6RA protein levels by flow cytometry of moDCs upon LPS stimulation for 4, 8, 18 or 24 hours (mean ± SEM from 6 donors). The highest intensity IL-6RA per donor was used to calculate % of max. (**F**) Soluble IL-6RA (sIL-6RA) released by moDCs incubated 4 or 24 h in absence or presence of LPS by ELISA. (**G**) Quantification of gp130 expression by flow cytometry of moDCs upon LPS stimulation for 4, 8 or 24 hours (mean ± SEM from 4 donors). The highest intensity gp130 per donor was used to calculate % of max. Representative histograms from 1 donor are shown. (**H**) Soluble gp130 (sgp130) released by moDCs incubated 4 or 24 h in absence or presence of LPS by ELISA. (I) Ratios of high (fully glycosylated) over total band intensities of the moDC IL-6RA blots from main Figure 1A.



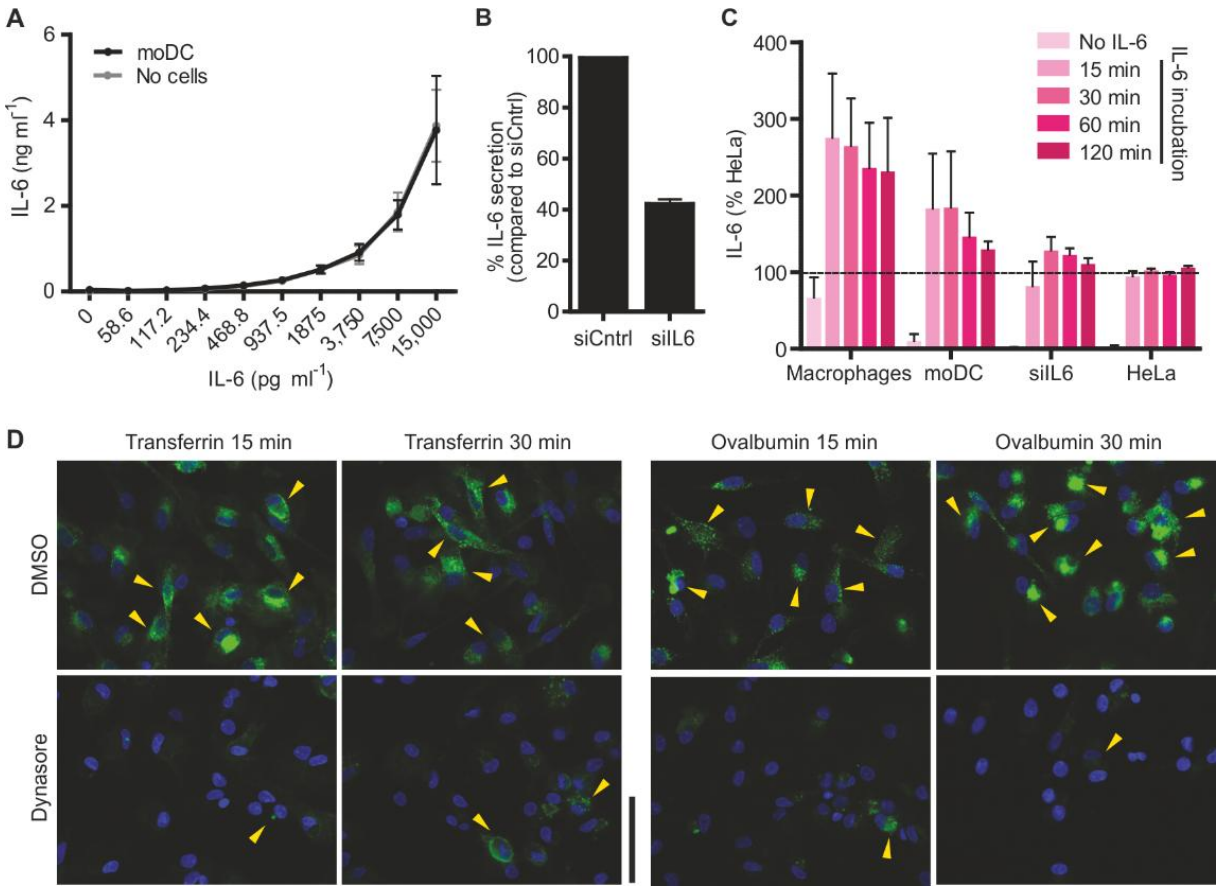

**Supplementary Figure S2. STAT3 tyrosine and serine phosphorylation upon IL-6 and LPS stimulation.** (**A**) Complete gels from main Figure 1B. STAT3 phosphorylation of tyrosine 705 (pY-STAT3) by Western blot. MoDCs were incubated for 20 min with the IL-6 concentrations indicated and with or without 4 hours LPS pre-incubation. α-Tubulin: loading control. Three donors are shown (4$^{th}$ donor in main Figure 1B). Arrow heads indicate the location of the quantified bands. (**B**) Same as panel A, but now for phosphorylation at serine 727 (left; pS-STAT3) and tyrosine 705 (right; pY-STAT3) for 3 donors. Graphs show quantification normalized to the maximum band intensities per donor. (**C**) IL-6 expression by qPCR for 4 h incubation in presence or absence of exogenous IL-6 and/or LPS (average ± SEM from 3 donors).



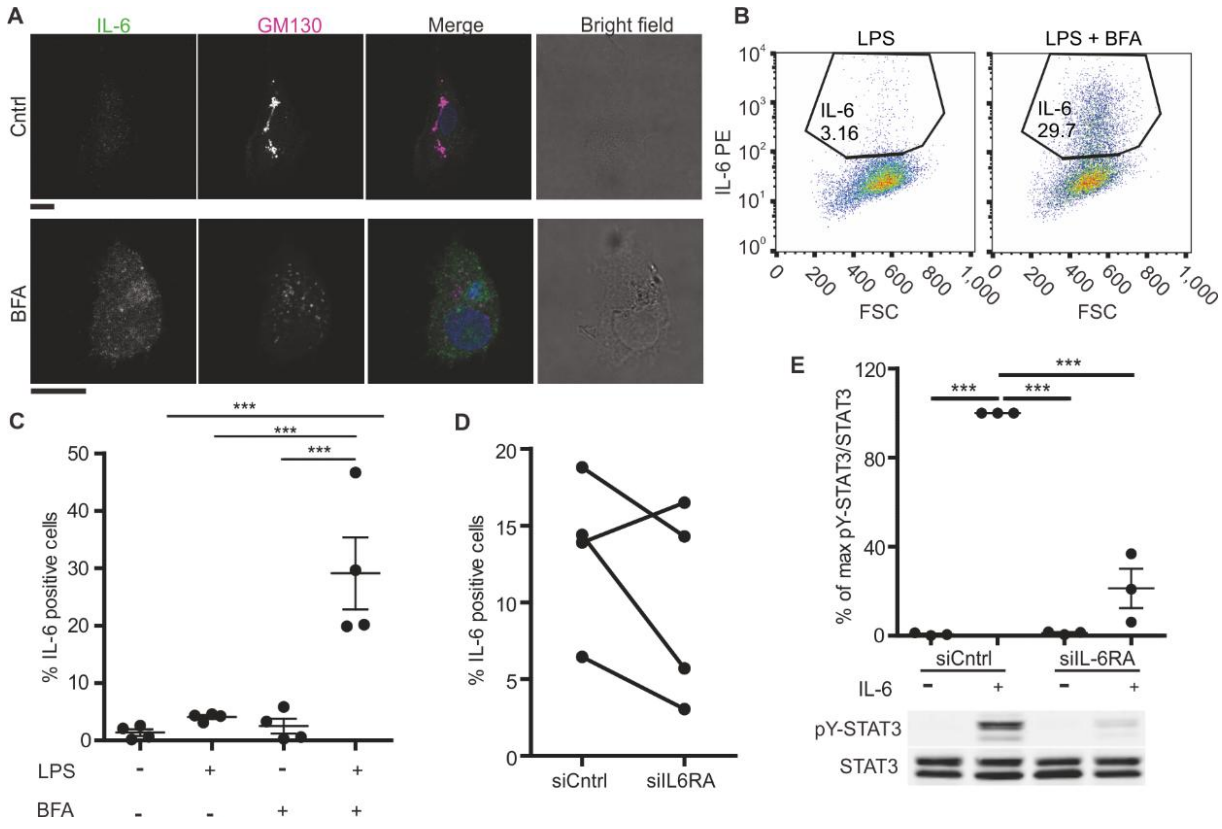

**Supplementary Figure S3. Control experiments for siIL-6RA.** (**A**) Validation of IL-6RA antibody for flow cytometry by siRNA knockdown in moDCs of IL6-RA (siIL-6RA). SiCntrl: non-targeting siRNA control. Representative histograms are shown for IL-6RA (green) and isotype control (dashed grey). (**B**) Same as panel A, but now with confocal microscopy with immunostaining for IL-6RA (green in merge) and DAPI (blue). Note that the antibody for IL-6RA shows some a-specific nuclear staining. Yellow arrowheads: IL-6RA-positive cells. Scale bar, 50 μm. (**C**) IL-6 secretion from LPS-stimulated moDCs over time with or without siIL-6RA (mean ± SEM from 3 donors).




**Supplementary Figure S4. Control experiments for localization of IL-6RA in dendritic cells.** (**A**) Flow cytometry of moDCs immunostained for the transferring receptor (TfR) with (total) or without permeabilization (surface). Left: representative histograms. Right: geometric mean fluorescence intensities (gMFI) for individual donors (isotype subtracted). (**B**) Transmission electron microscopy image of moDC that endocytosed antibody against IL-6RA or isotype control followed by immunogold staining. Pink arrowheads indicate immunogold particles. Scale bars, 200 nm.




**Supplementary Figure S5. Control experiments for uptake and breakdown of exogenous IL-6.** (**A**) Titration experiment of exogenous IL-6 in presence or absence of moDCs (mean ± SEM from 3 donors). IL-6 was measured by ELISA. (**B**) IL-6 secretion after 4 hours LPS stimulation upon siRNA silencing of IL-6 (siIL6; mean ± SEM from 4 donors). siCntrl: non-targeting siRNA control. (**C**) Breakdown of exogenous IL-6 by monocyte-derived macrophages, HeLa cells and moDCs with and without siRNA knockdown of IL-6 and after 15, 30, 60 or 120 minutes incubation determined by ELISA (mean ± SEM from at least 3 donors). (**D**) Confocal micrographs of moDCs treated with or without hydroxy-dynasore. Hydroxy-dynasore blocked uptake of fluorescently-labeled transferrin (left; green) and ovalbumin (right) at 15 and 30 minutes incubation. Blue: DAPI. Yellow arrowheads: transferrin and ovalbumin-positive cells. Scale bar, 50 µm.




**Supplementary Figure S6. Control experiments for the secretion of IL-6.** (**A**) Representative confocal images of LPS-treated moDCs with or without Brefeldin A (BFA). Cells were immunostained for IL-6 (green in merge) and GM130 (magenta). DAPI is in blue in merge. (**B**) Gating strategy of flow cytometry experiment for intracellular IL-6 accumulation in moDCs treated with LPS and/or BFA. IL-6PE: immunolabeling for IL-6 with a primary antibody labeled with phycoerythrin (PE). FSC: forward scatter. (**C**) Quantification of IL-6-positive cells from panel B for 4 individual donors (mean ± SEM). (**D**) Positive controls of main Figure 4B. MoDCs with non-targeting siRNA control (siCntrl) or siRNA knockdown of IL-6RA (siIL-6RA) were treated with LPS and BFA for 5 hours. The ratios between siCntrl and siIL-6RA were used to normalize % IL-6 positive cells in sIL-6RA samples. Individual donors are shown. (**E**) STAT3 phosphorylation (pY-STAT3; Tyr705) of moDCs treated with siIL-6RA in absence or presence of IL-6 for 20 minutes. Graph: quantification for 3 different donors. The highest pY-STAT3/STAT3 ratio per donor was used to calculate % of max pY-STAT3/STAT3. Blot from a representative donor is shown.

**Supplementary Movie S1.** Representative time lapse movie of moDC expressing human STAT3 conjugated to eGFP (STAT3-GFP; green) and empty mCherry (magenta) stimulated with 15 ng/ml IL-6 for 20 minutes.

**Supplementary Movie S2.** Representative time lapse movie of moDC expressing human STAT3 conjugated to eGFP (STAT3-GFP; green) and epsin 2 conjugated to mCherry (epsin2-mCherry; magenta) stimulated with 15 ng/ml IL-6 for 20 minutes. Note that the lower cell does not express epsin2-mCherry and shows retainment of STAT3-GFP in the nucleus.

**Supplementary Movie S3.** Representative time lapse movie of moDC expressing human IL-6 conjugated to GFP (IL-6-GFP; green in merge) and IL-6RA conjugated to mCherry (IL-6RA-mCherry; magenta). BF: bright field.