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Glucocorticoids promote intrinsic human Th17 differentiation

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20 **Capsule summary:**

21 Glucocorticoids (GCs) trigger human Th17 differentiation, a process in which IL-2 regulation
22 plays a mechanistic role. Th17 memory cells from healthy donors are similarly favored by
23 GCs, but this is not observed in psoriatic patients.

24

To the Editor:

Although Th17 cells are critical to host protection at epithelial barriers, they also play a pathogenic role in inflammatory and autoimmune diseases. Glucocorticoids (GCs) are frequently used to suppress undesired inflammation. However, human Th17 responses to GCs are not homogeneous, showing a clear influence of the patho-physiological context¹. In psoriasis, for example, Th17 cells are successfully suppressed by GCs, while in lupus erythematosus patients, Th17 cells are refractory to GC treatment. Furthermore, GC use has been repeatedly reported to be associated with increased IL-17 levels in so called refractory diseases, and one study on lupus erythematosus patients provided evidence that GC, but not other systemic therapies, are directly linked to increased Th17 responses². Consistently, GCs increased Th17 cells in murine T cell cultures, as well as in assays with CD8-depleted PBMCs from healthy donors^{3,4}. These findings cannot be explained by GC insensitivity, but rather indicate a stimulatory effect of GCs on Th17 responses, prompting us to investigate whether GCs are able to induce human Th17 cells.

We used an established protocol to induce the differentiation of naïve CD4 T cells from healthy individuals. T cells were differentiated in the presence or absence of dexamethasone (see online Fig. S1 and methods) for 12 days and gene expression of Th specifying transcription factors (TFs) were analyzed. Th17- and Treg-related TFs (RORC and FOXP3, respectively) were significantly increased by dexamethasone ($p \leq 0.05$), while those related to the Th1 (TBX21) and Th2 (GATA3) phenotypes were inhibited ($p \leq 0.001$). The Th22-related FOXO4 was unaffected (**Fig. 1A**). Furthermore, dexamethasone treatment significantly increased supernatant levels of IL-17 (**Fig. 1B**, $p \leq 0.05$, and online **Fig. S2**) as well as the frequency of IL-17⁺ T cells (**Fig. 1D**, $p \leq 0.05$, and online **Fig. S3**). Given that the amount of IL-17 in supernatants also depends on the final cell count in each culture condition, we investigated T cell numbers and proliferation at the end of our assays. Indeed, dexamethasone reduced the total number of cells (online **Fig. S4A**) and the percentage of proliferating cells (online **Fig. S4B**). Therefore, the level of IL-17 on a per-cell basis was approximately eight-times higher in dexamethasone-treated than in untreated cultures (**Fig.**

1C, $p \leq 0.05$). It is reasonable to speculate that GC could interfere on our cell count by favoring cell death, but T cell viability was not different in GC-treated and -untreated cultures, as determined by 7-AAD staining (online **Fig. S4C**). Remarkably, vitamins A (bexarotene and isotretinoin) and D (25D and 1,25D), which are, like GCs, hormones of the steroid superfamily, neither influenced the generation of IL-17⁺ T cells (online **Fig. S5A**) nor interfered with the stimulatory effect of dexamethasone on this cell type (online **Fig. S5B**).

We also found significantly higher levels of IL-10 in supernatants of dexamethasone-differentiated T cells (**Fig. 1E** and online **Fig. S2**), consistent with the reported upregulation of FOXP3. Further FACS analyses revealed that almost all dexamethasone-induced IL-17⁺ T cells co-expressed IL-10 (**Fig. 1F**), not only demonstrating that GCs induce an IL-10-secreting Th17 phenotype, but also highlighting often discussed compatibilities between Th17 and Treg differentiation programs^{S15-19}.

Reflecting the TF analyses, we found about 50% decline of Th1-related cytokines (IFN- γ and TNF- α) in the dexamethasone-treated T cell cultures (**Fig. 1G**, $p \leq 0.05$). The Th2-related IL-4, however, was not significantly different (**Fig. 1G**). Because more important than the level of single cytokines, it is the balance among cytokines that determines the final character of an immune response, we analyzed cytokine ratios. We verified a relative increase of IL-17 (**Fig. 1H** and online **Fig. S6A**), as well as IL-10 (online **Fig. S6B**), in comparison to Th1 and Th2 cytokines. Notably, IL-22 was not affected by dexamethasone, both in absolute (**Fig. 1G**) or relative levels (**Fig. S6C**). All in all, our results indicate that GCs favor a Th17/Treg environment in deprivation of Th1 and, to a lesser extent, of Th2 responses.

We observed increased RORC expression and IL-17 production by T cells in the absence of exogenous polarizing cytokines and antigen-presenting cells. This demonstrated that GCs are sufficient to activate an intrinsic Th17 differentiation program, suggesting a potential autocrine cytokine signaling mechanism behind this process. On this regard, we found that dexamethasone had no relevant effect on the secretion of IL-1 β , IL-6 and TGF- β

(online **Fig. S7A**), cytokines associated with Th17 development⁵, at day 3 and day 12 of the cultures. However, dexamethasone significantly reduced levels of IL-2 (**Fig. 1I**, $p \leq 0.01$ and online **Fig. S7B**), a recognized Th17 inhibitory factor^{6,7}. In fact, IL-2 lowering is a well-known suppressive effect of GCs with negative implications to the Th1 and Th2 fractions. As expected, IL-2 blocking alone enhances IL-17/IFN- γ ratios (**Fig. 1J**). The effects were comparable to those observed in dexamethasone-treated samples. Of note, the combination of dexamethasone and IL-2 blocking antibodies showed no additive effect (**Fig. 1J**). Conversely, additional exogenous IL-2 — at a dose corresponding to the differences observed in the dexamethasone-treated and untreated T cell cultures on day 3 — completely prevented dexamethasone-mediated increase in IL-17/IFN- γ and IL-17/TNF- α ratios (**Fig. 1K** and online **Fig. S8**). In sum, our data confirm a regulatory role for IL-2/IL-2R signaling in the Th17/Th1 balance and suggest IL-2 lowering as a mechanism by which GCs promote Th17 polarization.

One important function of Th17 and Th1 cells is the induction of epithelial immunity by triggering inflammation and antimicrobial responses. We assessed the impact of the cytokine balance generated by GC-differentiated T cells on the gene expression of antimicrobial peptides (AMPs) in normal human keratinocytes. While there were no significant changes in S100A7, RNase7, HBD3 and cathelicidin expression, supernatants from GC-differentiated T cells induced significantly higher expression of DEFB4 (**Fig. 2A**, $p \leq 0.05$) and CCL20 (**Fig. 2A**, $p \leq 0.001$). We also detected an enhanced expression of the pro-inflammatory cytokine pro-IL1 β , associated to a minimal increase of TNF- α (**Fig. 2A**, $p \leq 0.05$). IL-10 gene transcripts, in turn, were either not detected or very low in the keratinocyte cultures (not shown). Thus, supernatants from GC-differentiated T cells were equally efficient or even superior to those from GC-untreated cells at inducing AMPs and pro-inflammatory cytokines in epithelial cells, despite decreased IFN- γ (antimicrobial supportive) and increased IL-10 (antimicrobial restrictive). The unexpected immune-stimulatory character of these cells uncovers a GC-mediated potential benefit to host responses at barrier sites. Nevertheless,

GC-differentiated T cells still express IL-10, a common marker for non-pathogenic Th17 cells⁸. So known for their polyphenotypical character, a better understanding of the functional identity of Th17 cells triggered by GCs demands further disease-contextualized investigations.

Next, we wondered if GCs facilitate Th17 responses of skin-resident T cells. Memory T cells isolated from human skin, which predominantly display a CD4⁺ phenotype (online **Fig. S9**), were activated and cultured in the presence or absence of dexamethasone for supernatant analyses. Dexamethasone increased IL-17 and inhibited IFN- γ secretion (**Fig. 2B**), and thus, the IL-17/IFN- γ ratio (**Fig. 2C**). We then investigated if GCs regulate cytokine secretion of memory T cells in response to skin-relevant pathogens. Therefore, PBMCs were challenged with heat-killed *Candida albicans* or *Staphylococcus aureus*, and cultured in the presence or absence of dexamethasone for supernatant analyses. Dexamethasone significantly downregulated IFN- γ , yet sustained IL-17 secretion induced by *C. albicans* and *S. aureus* (**Fig. 2D**, $p \leq 0.05$), which therefore significantly raised IL-17/IFN- γ ratios (**Fig. 2E**, $p \leq 0.05$). This demonstrates that GCs not only promote Th17 polarization of naïve human T cells, but also modulate skin-resident and pathogen-specific memory T cells with a bias towards a Th17 phenotype, at the cost of Th1 responses. This inhibition of Th1 responses might be causally related to the observed risk for mucocutaneous infections, including candidiasis and cellulitis, in GC-treated individuals⁹. Sustained IL-17 production paralleling an inhibition of Th1 responses however might provide a mechanism that partially compensates for GC deleterious effects.

It is well known that GC responsiveness depends on the patho-physiological context. Thus, we compared PBMCs from healthy donors and individuals suffering from psoriasis, a prototypic Th17 disease, in which Th17 cells are suppressed by GCs. PBMCs were stimulated with heat-killed *C. albicans* and after 4 to 6 days, IFN- γ and IL-17 secretion was assessed. The data revealed a striking difference: in contrast to the sustained IL-17 secretion seen in healthy donors, IL-17 levels were significantly suppressed by GCs in supernatants of

psoriasis PBMCs (**Fig. 2F**). IFN- γ , on the other hand, was inhibited in both groups (**Fig. 2F**). These findings are in line with the currently accepted model of a context-dependent GC responsiveness.

Taken together, we identified GCs as promoters of human Th17 differentiation, which is regulated by IL-2. We also provide further evidence that the response of memory T cells to GC treatment is context-dependent. It is known that certain Th17 diseases are more GC-sensitive than others, but here, we show that in the absence of disease, Th17 cells are refractory to GC suppressive effects. The specific pathological aspects that alter the physiological response of Th17 to GC therapy remain to be clarified. Such insights are important to a better understanding of GCs desired and adverse effects. Moreover, the identification of IL-2 regulation as a mechanism for GC-induced Th17 differentiation defines IL-2 as a possible therapeutic agent ⁷ to counteract GC induction of Th17 cells, which should be addressed in future disease-specific studies.

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Conflict of interest disclosure

The authors have declared that no competing interests exist.

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Figure legends

Figure 1. GCs promote Th17 differentiation, a process that involves lowering of IL-2.

(A-I) Bead-activated naïve CD4 T cells were differentiated in the absence (media) or presence of 10^{-7} M dexamethasone (dexa) for 12 days. Bead-untreated cells (---) were also used as control. (A) Expression of TFs was assessed by qPCR. (B) IL-17 secretion was assessed by ELISA or CBA after PMA/ionomycin treatment, in absolute or (C) per cell levels. (D) IL-17⁺ cells were measured by ICS after PMA/ionomycin treatment. (E) IL-10 was assessed as in B. (F) IL-17⁺ and IL-10⁺ cells were co-measured by ICS, as in D. A representative plot of 4 experiments performed in triplicates is shown. (G) IFN- γ , TNF- α , IL-4 and IL-22 were assessed as in B. (H) Relative cytokine changes in ratios. (I) IL-2 levels in 3rd day supernatants were assessed as in B. (J) Naïve CD4 T cells were cultured as in A, combined or not (---) with anti-IL-2 or anti-IL-2R α +IL-2R β antibodies. IL-17 and IFN- γ secretion was measured as in B. (K) Naïve CD4 T cells were cultured as in A. 6250 pg/ml of IL-2 was added or not to dexamethasone-treated samples at the beginning of the T cell culture. IL-17, IFN- γ and TNF- α secretion was measured as in B and data displayed as cytokine ratios. Bar graphics display mean $\pm$ SEM of n assays, each one performed in triplicates. In A $n=6-10$, B $n=9$, C $n=9$, D $n=8$, E $n=7$, G $n=6-8$, H $n=6-8$, I $n=9$, J $n=2$ and K $n=5$. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Figure 2. Effect of GCs on T-cell-mediated cutaneous host defense. (A) Supernatants

from GC-differentiated T cells enhance AMP expression in keratinocytes. Human keratinocytes were cultured in growth media supplemented with day-12 supernatants from dexamethasone-differentiated or non-dexamethasone-differentiated CD4 T cells (1:5). Supernatants were generated as described for ELISA/CBA assays in Fig. 1. After 24 h of keratinocyte culture, expression of AMPs (black bars) and cytokines (white bars) were assessed by qPCR. (B-E) GCs upregulate IL-17/IFN- γ ratios in skin-resident memory T cells and pathogen-specific T cells. (B-C) Skin-resident T cells were activated with beads in the

presence or absence of 10^{-8} M dexamethasone (dexa). **(B)** IL-17 and IFN- γ were measured by ELISA in day-3 supernatants. **(C)** Cytokine data as ratios. **(D-E)** PBMCs were stimulated with heat-killed *Candida albicans* (CA) or *Staphylococcus aureus* (SA), in the presence or absence of 10^{-8} M dexamethasone. **(D)** IL-17 and IFN- γ were measured by ELISA in day-5 supernatants. **(E)** Cytokine data as ratios. **(F)** GCs suppress IL-17 production from psoriatic T cells. PBMCs from healthy donors and psoriatic patients were stimulated with CA with or without 10^{-8} M dexamethasone. IL-17 and IFN- γ were measured by ELISA in day-5 supernatants. Bar graphics in **A** represent mean $\pm$ SEM of 4-5 assays with supernatant from diverse donors. Bar graphics in **D**, **E**, and **F** represent mean $\pm$ SEM of n assays. In **B** $n=1$, **C** $n=1$, **D** $n=5$, **E** $n=5$ and in **F**, $n=9$ psoriatic patients and 8 healthy donors. * $p \leq 0.05$ and *** $p \leq 0.001$.

ONLINE REPOSITORY

Materials and methods**Ethics statement**

This study was conducted according to the principles expressed in the Declaration of Helsinki and approved by the Ethic Committee of the University of Cologne, Germany. All donors provided written informed consent for the collection of blood and subsequent analysis.

Cell isolation and culture

Peripheral blood mononuclear cell (PBMC) assays. PBMCs were extracted from blood or buffy coats from healthy donors or psoriatic patients using Ficoll-Paque (GE Healthcare, Freiburg, Germany). Psoriatic patients (n=9) were recruited from the Dermatology Department of the University of Cologne and required in-patient treatment. All patients had topical therapy, yet none of them was under systemic medication for psoriasis. The mean age of the patients and corresponding healthy controls was 49 years (range 25 - 79) and 45 years (range 28 - 65), respectively. In some assays, 2×10^5 cells/well were resuspended in enriched media (EM) with 10% human AB serum (PAN-Biotech, Aidenbach, Germany) and stimulated with heat-killed *Candida albicans* (0.5 cells/PBMC) or *Staphylococcus aureus* (5×10^6 cells/ml) (both InvivoGen, San Diego, CA) inside 96-well U-bottom plates. EM was composed of RPMI 1640 with L-glutamine, 1% sodium pyruvate, 0.5% essential amino acids, 0.5% nonessential amino acids, 1% penicillin/streptomycin, 10 mM Hepes and 55 μ M 2-mercaptoethanol (all Invitrogen, Darmstadt, Germany). After 1-2 h of antigen stimulation, cells were treated with dexamethasone (Sigma-Aldrich, Steinheim, Germany) at a concentration of 10^{-8} M (unless indicated otherwise) or not, and incubated at 37°C and 7% CO₂. On day 5, supernatants were harvested and stored at -20°C until cytokine analysis.

Naïve CD4 T cell assays. To better preserve the naïve-like quality of the cells, we worked here exclusively with fresh blood samples. Naïve CD4 T cells, in which IL-17-

producing T cells are virtually absent ¹, were isolated from PBMCs using MACS system (Miltenyi Biotec, Bergisch-Gladbach, Germany). We used an established protocol to induce the differentiation of naïve CD4 T cells ². Specifically, 5×10^4 cells/well were treated with anti-CD3/-CD28 coated beads (1 bead/cell) from the human T cell activation/expansion kit (Miltenyi Biotec) in 96-well U-bottom plates. Cells without beads were also used as non-activated controls. After 1-2 h, activated cells received 10^{-7} M dexamethasone or not, and were incubated at 37°C and 7% CO₂. In some cases, cells were alternatively stimulated with 10^{-7} M 25-hydroxyvitamin D (25D) (Biomol, Hamburg, Germany), 10^{-8} M 1,25-dihydroxyvitamin D (1,25D) (Biomol), 10^{-7} M isotretinoin (Sigma-Aldrich) or 10^{-7} M bexarotene (Sigma-Aldrich), combined or not with dexamethasone. In blocking experiments, anti-IL-2 and anti-IL-2R α +IL-2R β monoclonal antibodies (all R&D Systems, Minneapolis, MN) were added to the cultures. On day 5, plates were supplemented with 50 U/ml of rIL-2 (Miltenyi Biotec) and returned to incubator for additional 7 days. On day 12, cells were lysed for qPCR analyses. For the purpose of extracellular cytokine analyses, cells received a boosting cocktail (50ng/ml PMA + 0.5 μ g/ml ionomycin, both from Sigma-Aldrich) in fresh EM with 10% human serum. After 20-24 h, supernatants were harvested and stored at -20°C until analyses. In some experiments, cells were counted using Trypan blue exclusion at the end of the culture. If intended for intracellular cytokine staining, cells received the boosting cocktail for 5 h with additional Brefeldin A (BD Biosciences, Heidelberg, Germany) for the final 2.5 h.

Skin-resident T cell assays. We isolated T cells from normal human skin using a protocol that yields almost exclusively memory phenotypes ³. First, skin samples were freed from subcutaneous fat and minced into explants. 2-3 explants were placed over one skin grid (Cellsciences Pte Ltd, Melbourne, Australia) within a 24-well plate. Then, these wells received IMDM (Thermo Fischer Scientific, Germering, Germany) supplemented with 20% FCS (PAA Laboratories, Cölbe, Germany or PAN-Biotech, Aidenbach, Germany), 1% penicillin/streptomycin, 3.5 μ l/l β -mercaptoethanol and 100 U/ml IL-2 for further incubation at 37°C and 5% CO₂. Fresh media was given every 2 days. After 21 days, T cells were flushed

from grids and counted. 10^4 cells/well were resuspended in EM containing 10% human AB serum and stimulated with anti-CD3/-CD28 coated beads (3 beads/cell) in 96-well U-bottom plates. After 1.5 h, cells received 10^{-8} M dexamethasone or not and returned to incubator. On day 3, supernatants were harvested and stored at -20°C until cytokine analyses.

Keratinocyte assays. Normal human epidermal keratinocytes (NHEK) purchased from Promocell (Heidelberg, Germany) were recovered and expanded as suggested by the manufacturer. Cells were detached by trypsin/EDTA treatment and plated at 5×10^4 cells/well in keratinocyte growth media (Promocell). After a 4 h adherence period, media was supplemented with supernatants from GC- or non-GC-differentiated T cells (1:5) and returned to incubator (37°C and 5% CO_2). After additional 24 h, qPCR was performed.

Extracellular cytokine analyses

After a 20-24 h boosting step, cytokine analyses of T cell supernatants were performed by cytokine bead array (CBA) (BD Biosciences), according to the manufacturer's protocol. Targeted cytokines included IL-10, IL-17A, IFN- γ , TNF- α , free TGF- β , IL-6, IL-1 β and IL-2. Alternatively, cytokines were measured by sandwich ELISA with capture/detection mouse antibodies targeting human IL-22 (R&D), IL-17A, IL-4 (both eBioscience), IFN- γ and TNF- α (both Invitrogen, Frederick, MD). For IL-10 detection, we used an IL-10 ELISA kit (eBioscience).

Analyses of T cell phenotypes (surface FACS staining)

For analyses of T cell phenotypes, cells were stained with anti-CD25 PE, anti-CD4 APC (both BD Biosciences) and/or anti-CD8 FITC (Miltenyi Biotec) antibodies, as well as appropriate isotype controls. Fluorescence signals of cells were detected as background-corrected mean by FACS Calibur (BD Biosciences) and data analyzed with FlowJo software (Tree Star, Ashland, OR).

Intracellular cytokine staining (ICS)

After a 5 h boosting step, cells were fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences), as suggested by the manufacturer's protocol. Staining was performed using the following mouse monoclonal antibodies: anti-IL-17A Alexa-fluor-647, anti-IFN- γ PerCP-Cy5.5 (both BD Biosciences), anti-IL-10 FITC and anti-IL-22 PE (both eBioscience). Fluorescence signal of cells was detected as background-corrected mean by FACS Calibur and data analyzed with FlowJo software.

Analyses of T cell numbers, proliferation and viability

Naïve CD4 T cells were stained with CFSE (Sigma-Aldrich) ⁴ using a final concentration of 1.25 μ M per 10-100x10⁶ cells. Cells were recounted and cultured as previously described, at 10⁵/well. On day 12, supernatants were discarded and cells were resuspended in PBS containing 20% FCS and 0.1% sodium azide (FACS buffer) for FACS acquisition. Proliferation was assessed as the percentage of CFSE-negative cells. For assessing cell viability, washed cells were incubated with 5 μ l of 7-AAD (BD Biosciences) in FACS buffer for 5 min in the dark, just before FACS acquisition.

qPCR

mRNA was extracted from cells using RNeasy mini kit (Qiagen, Hilden, Germany), followed by cDNA transcription using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). mRNA expression was then assessed by qPCR with SYBR Green (Applied Biosystems, Darmstadt, Germany) or GoTaq Master Mix (Promega, Madison, WI), according to the manufacturers' protocols. Quantitect primer assays for hFOXP3, hTBX21, hGATA3, hpro-IL1 β and hIL-10 were from Qiagen. Primer sequences synthesized by Metabion (Munich, Germany) were previously reported for h36B4 ⁵, hFOXO4 ⁶, hCCL20 ⁷, hRNase-7 ⁸, hDEFB4 ⁹, hHBD3 ¹⁰, hCAMP ¹¹, hTNF- α ¹². hRORC primers were designed using Spidey ¹³ and Primer3 ¹⁴ programs: 5'-ATC TGG AGC TGG CCT TTC AT-3' sense, 5'-TGT AGA GTG GAG

GGA AAG CG-3' antisense. Gene expression was calculated using the comparative method for relative quantification ($2^{-\Delta Ct}$), with h36B4 as housekeeping gene. Final values were normalized by fold change to media control.

Statistical analyses

p -values were assessed by two-tailed Student's t -test. In some cases data were analyzed on log transformation of fold changes compared to media. For reference, statistical significance addressed * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. n refers to the number of independent assays performed with buffy coats or fresh blood samples from diverse donors.

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Supplementary Figure legends

Figure S1. T cell differentiation assay. (A) Graphical representation of our T cell differentiation protocol, as described in methods. Dexa: dexamethasone. (B) FACS analyses of magnetically isolated naïve CD4⁺ T cells used in A. Representative plots of one assay performed in triplicates are shown. *Left plots*, forward (FSC) and side scatter (SSC). *Right plots*, CD4/CD25 co-staining and corresponding isotype controls.

Figure S2. Kinetics of IL-17 and IL-10 production by GC-differentiated CD4 T cells. Bead-activated naïve CD4 T cells were differentiated in the absence (media) or presence of 10⁻⁷ M dexamethasone (dexa). Bead-untreated cells (---) were also used as control. Supernatants were harvested at different time points (specified in the graphic) and cytokines (IL-17 and IL-10) measured by ELISA. Data represents mean +/- SEM of 3 assays, each one performed in triplicates.

Figure S3. Dose response of dexamethasone on Th17 induction. Bead-activated naïve CD4 T cells were differentiated in the absence (media) or presence of different doses of dexamethasone (dexa) for 12 days. Bead-untreated cells (---) were also used as control. The frequency of IL-17⁺ cells was measured by ICS after PMA/ionomycin treatment. Bar graphic shows the number of cells as mean +/- SEM, for one representative assay performed in triplicates. * $p \leq 0.05$, ** $p \leq 0.01$.

Figure S4. GCs affect T cell number, proliferation and viability. (A) T cells cultured for the purpose of supernatant analyses (Fig. 1) were counted using Trypan blue exclusion. (B) Naïve CD4 T cells were CFSE-stained and cultured with activation beads in the absence or presence of 10⁻⁷ M dexamethasone (dexa). On day 12, proliferation was assessed as the percentage of CFSE-negative cells. (C) Non-CFSE-stained cells cultured as in B were incubated with 7-AAD on day 12 for viability analyses. Dead cells were assessed as the

percentage of 7-AAD-positive cells and dot plots show one representative assay. Bar graphics in **A**, **B** and **C** represent mean $\pm$ SEM of n assays. In **A** $n=9$, **B** $n=6$ and **C** $n=3$. * $p \leq 0.05$.

Figure S5. Vitamins D and A do not regulate Th17 differentiation. (**A-B**) Bead-activated naïve CD4 T cells were differentiated in the absence (media) or presence of 10^{-7} M dexamethasone (dexa) for 12 days. Alternatively, cells were stimulated with (**A**) 10^{-7} M 25-hydroxyvitamin D (25D), 10^{-8} M 1,25-dihydroxyvitamin D (1,25D), 10^{-7} M isotretinoin or 10^{-7} M bexarotene, separately or (**B**) combined with dexamethasone. Bead-untreated cells (---) were also used as control. The frequency of IL-17⁺ cells was measured by ICS after PMA/ionomycin treatment. Bar graphics in **A** and **B** display mean $\pm$ SEM of n assays, each one performed in triplicates. In **A**, the mean refers to the absolute number of cells ($n=8$). In **B**, the mean refers to the relative number of cells normalized to the media condition ($n=4$). * $p \leq 0.05$.

Figure S6. GCs increase relative levels of T cell-secreted IL-17 and IL-10, but do not affect IL-22 relative levels. (**A-C**) Bead-activated naïve CD4 T cells were differentiated in the absence (media) or presence of 10^{-7} M dexamethasone (dexa). Bead-untreated cells (---) were also used as control. On day 12, cytokine secretion was assessed by ELISA after PMA/ionomycin treatment. (**A**) Bar graphics display ratios representing relative IL-17 amounts in comparison to IL-4, as well as relative amounts of (**B**) IL-10 and (**C**) IL-22 in comparison to IFN- γ , TNF- α , and IL-4. Mean $\pm$ SEM refers to 6-8 assays. . ** $p \leq 0.01$ and *** $p \leq 0.001$.

Figure S7. GCs do not affect T cell secretion of Th17 polarizing cytokines (IL-1 β , IL-6 and TGF- β), but interfere with IL-2. (**A**) Bead-activated naïve CD4 T cells were differentiated in the absence (media) or presence of 10^{-7} M dexamethasone (dexa) for 3-12 days and supernatants were harvested after PMA/ionomycin treatment. IL-1 β , IL-6 and TGF-

β were analyzed by CBA. **(B)** 12-day supernatants were analyzed for their IL-2 content as described in **A**. Bar graphics display mean $\pm$ SEM of 4-10 assays, each one performed in triplicates. * $p \leq 0.05$.

Figure S8. IL-2 regulates Th17 induction by GCs. Bead-activated naïve CD4 T cells were differentiated in the absence (media) or presence of dexamethasone (dexa) for 12 days. IL-2 was added at different concentrations or not to dexamethasone-treated samples at the beginning of the T cell culture. After PMA/ionomycin treatment, supernatants were harvested for IL-17, IFN- γ and TNF- α assessment by ELISA or CBA. Bar graphics display ratios representing relative IL-17 amounts in comparison to IFN- γ and TNF- α . Mean $\pm$ SEM refers to 4 assays, each one performed in triplicates. * $p \leq 0.05$.

Figure S9. Characterization of isolated skin T cells. Human skin explants rested on metal grids under proper culture conditions (see methods). After 21 days, cells were flushed from the grids, counted and co-stained with CD4/CD8 antibodies or isotype-matched controls for FACS analyses.

Figure 1

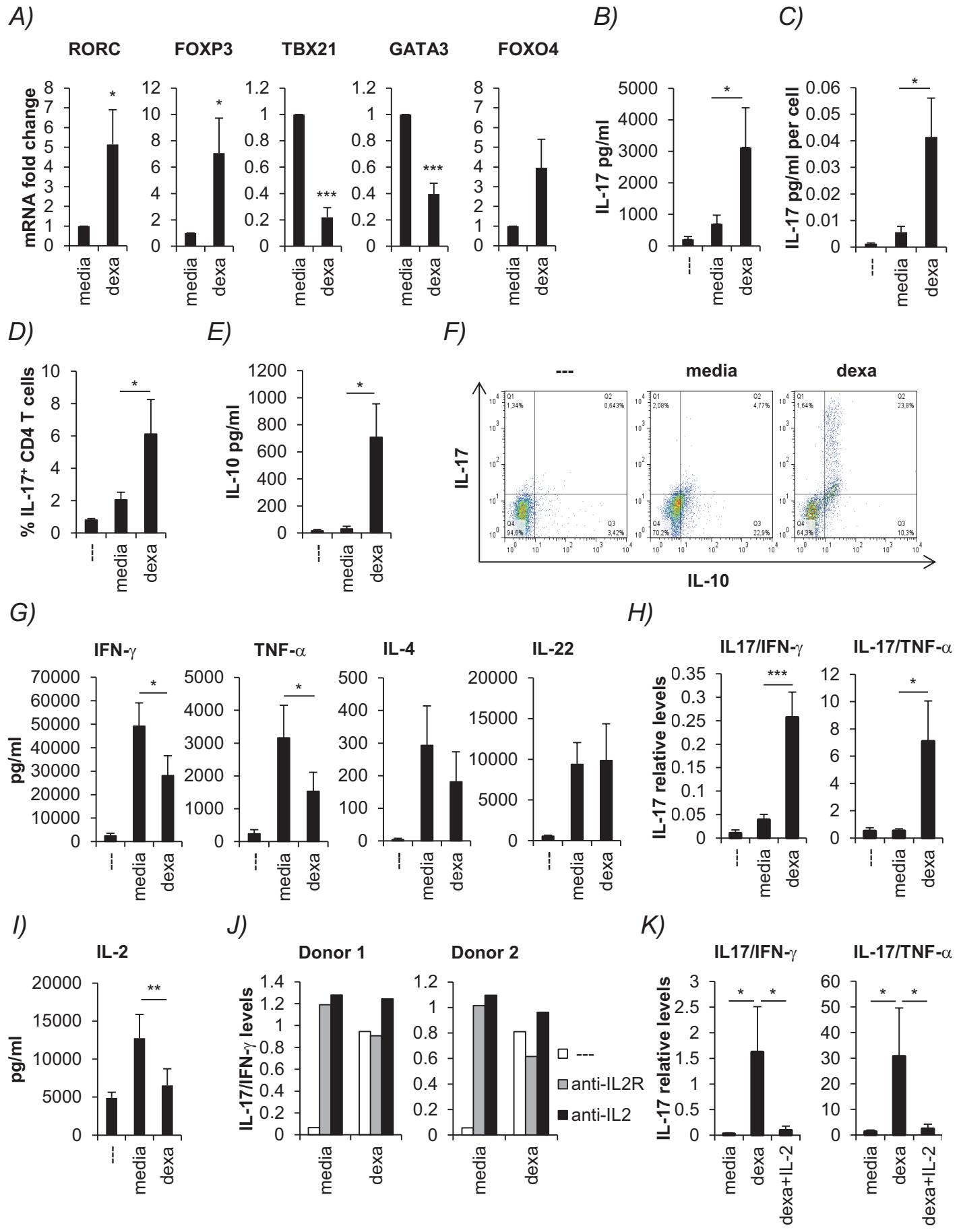
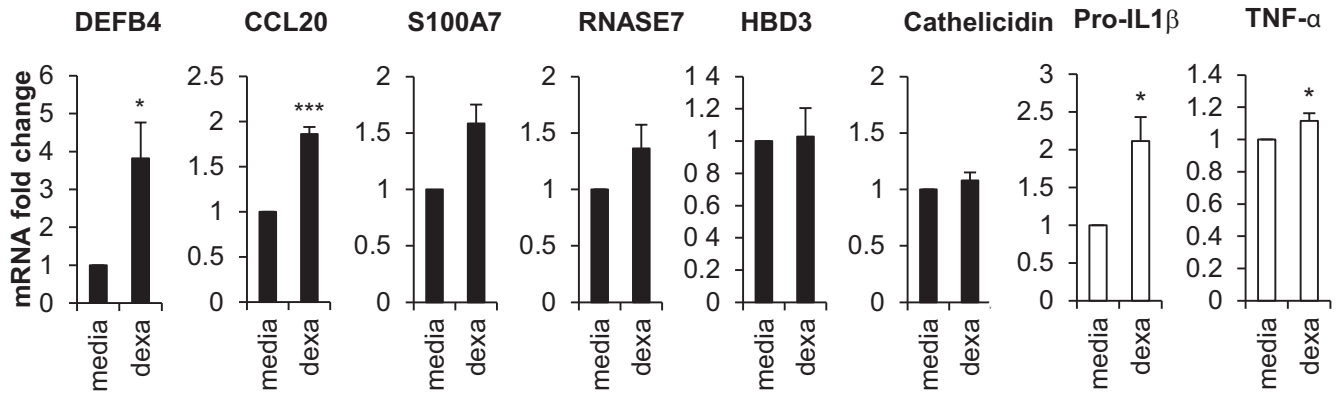
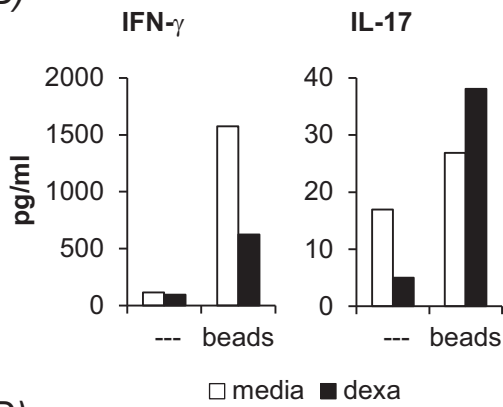


Figure 2

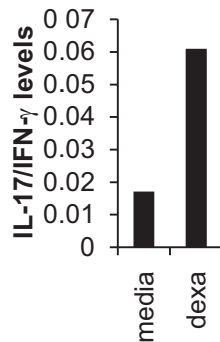
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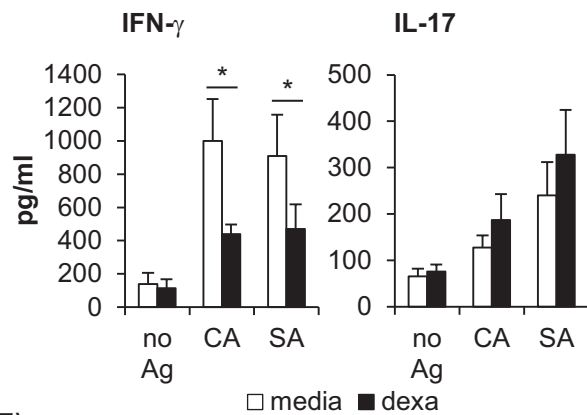
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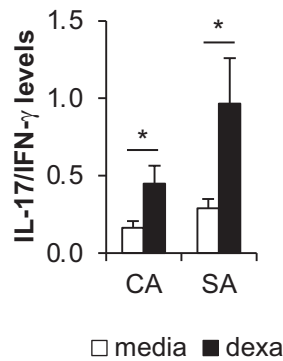
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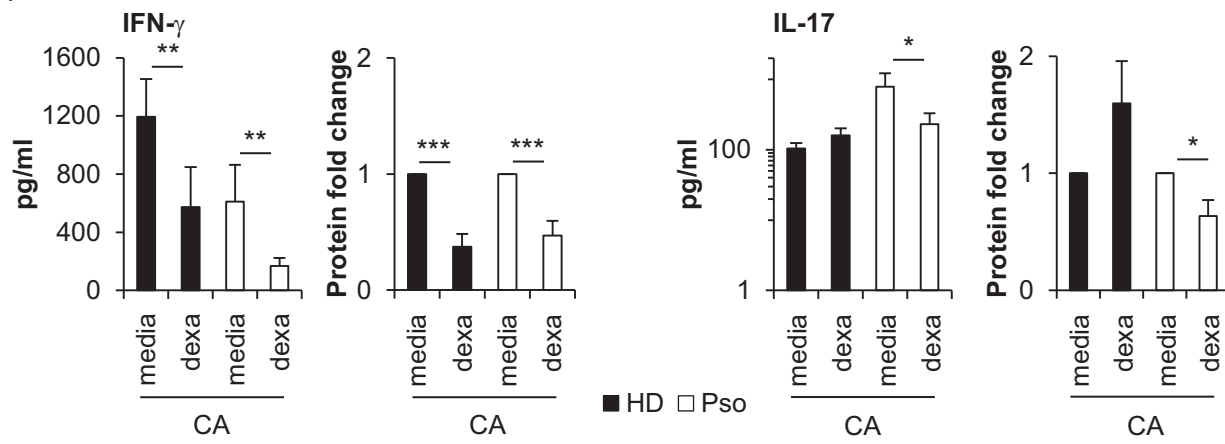
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E)



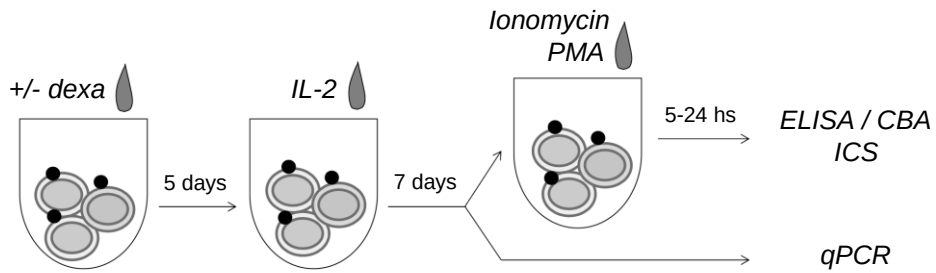
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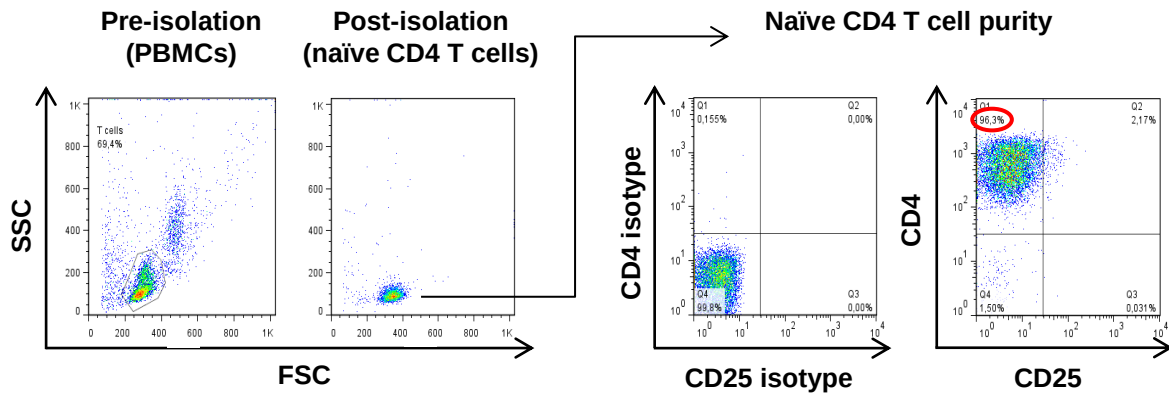
S1

A)

- Activation beads
- Naïve CD4 T cell

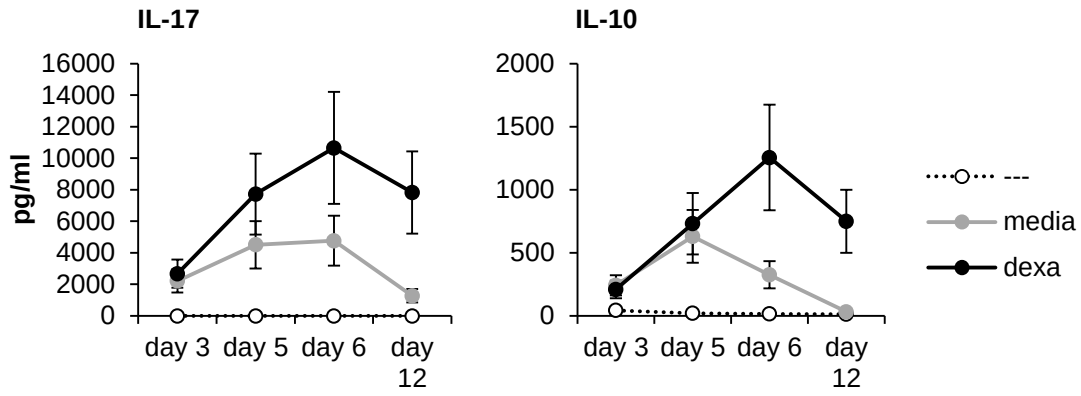


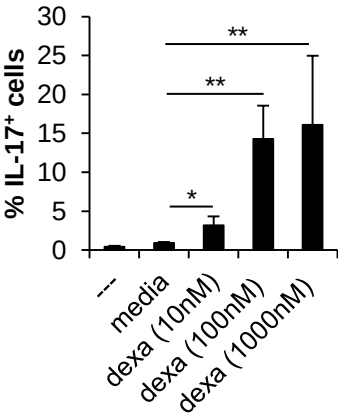
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S2

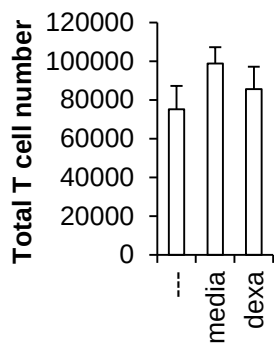
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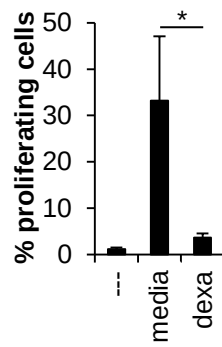


S4

A)



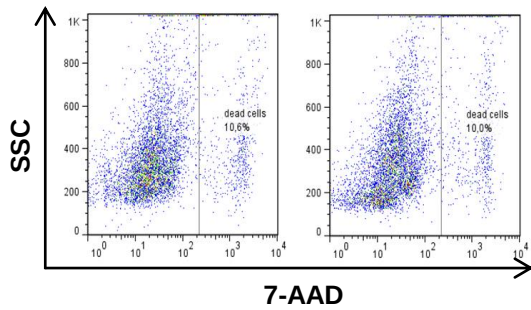
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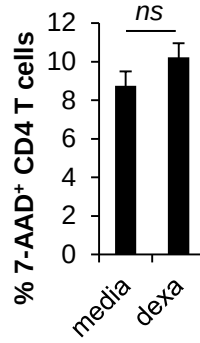
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Media

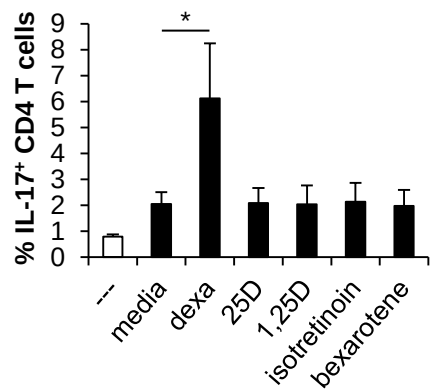
Dexa



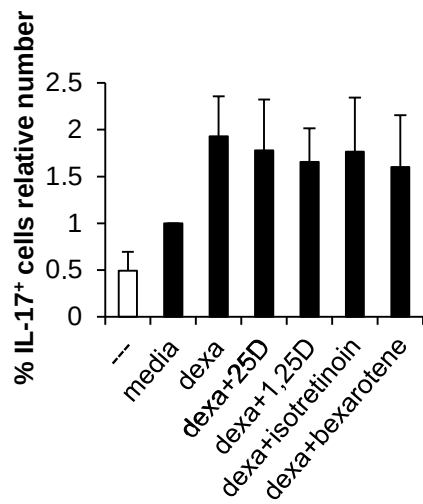
Dead CD4 T cell fraction



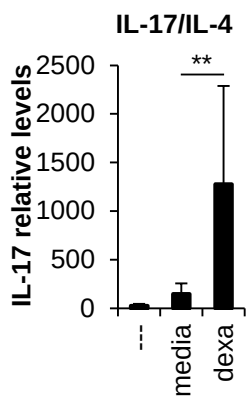
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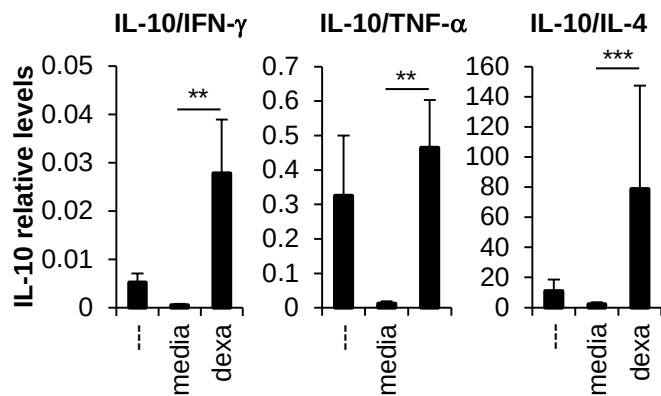
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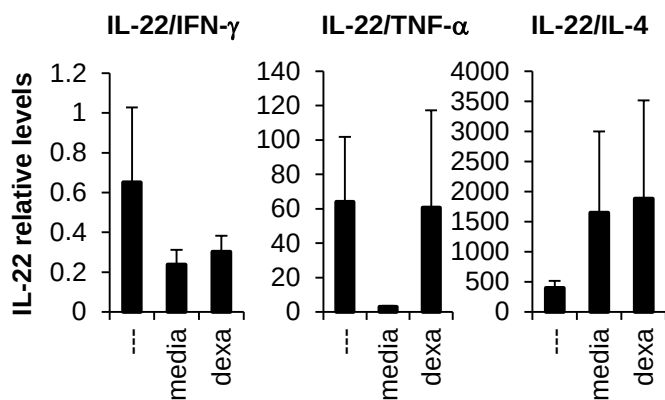
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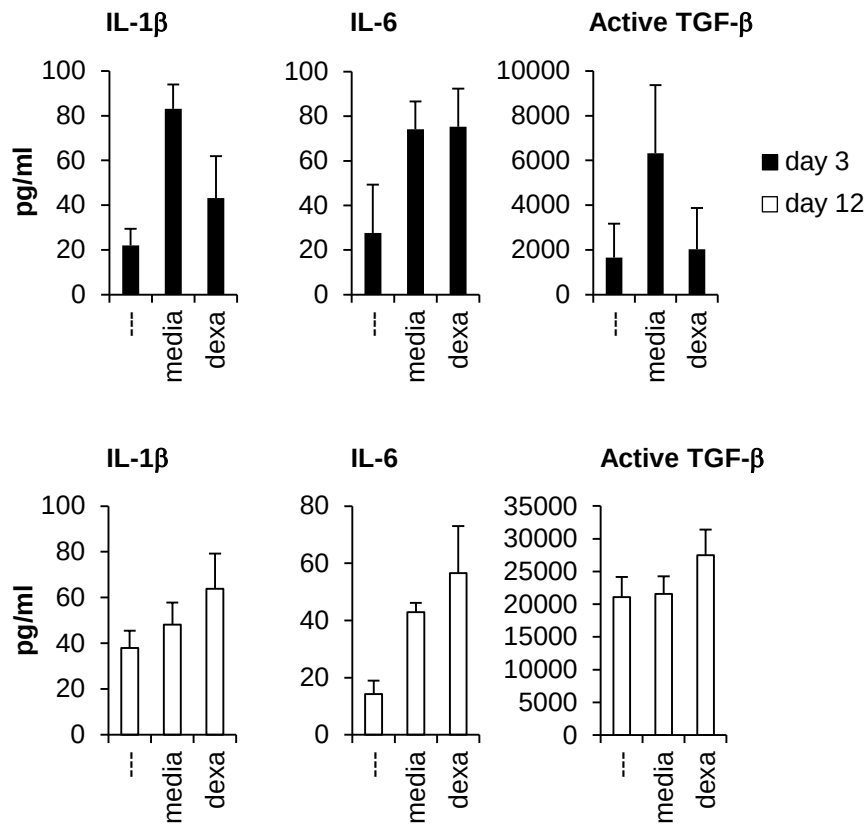


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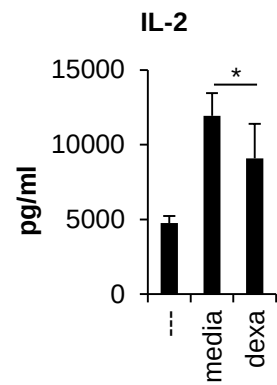


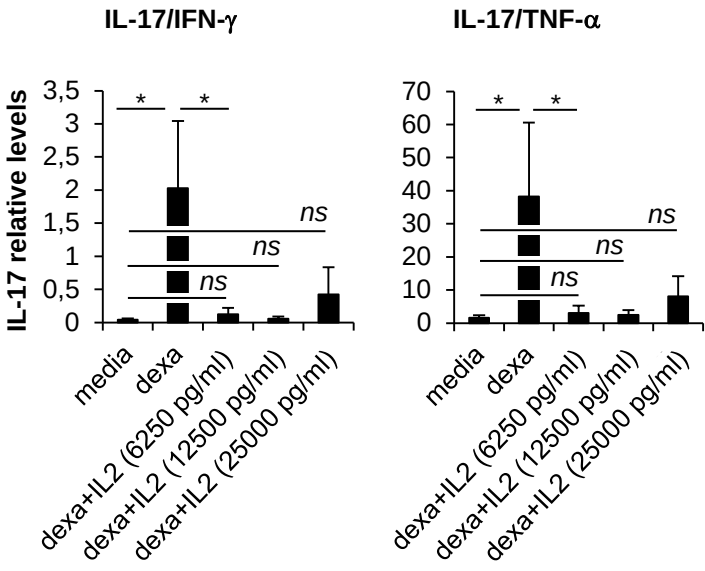
S7

A)



B)





Skin T cells

