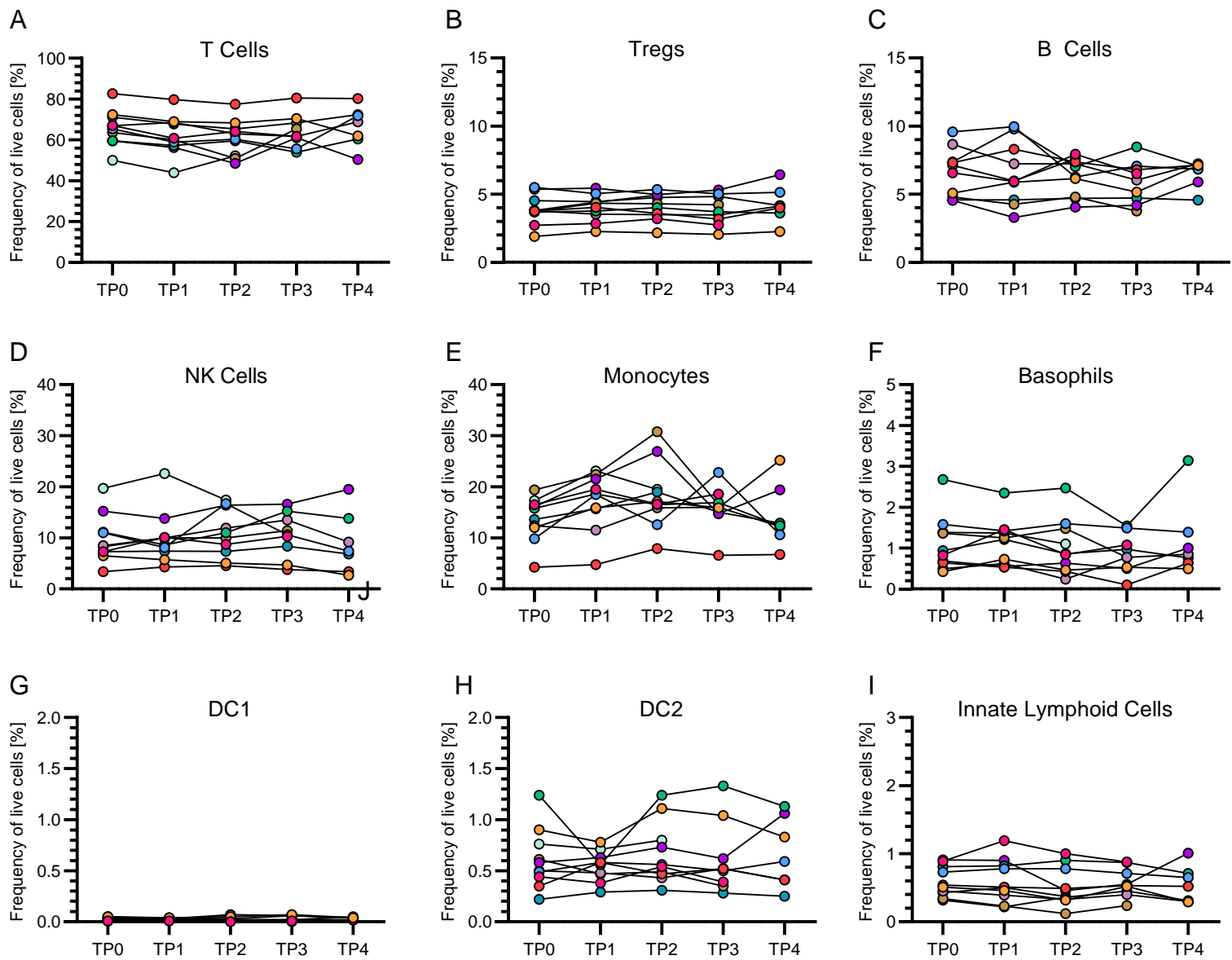


Supplemental information

Reduction of IFN-I responses by plasmacytoid dendritic cells in a longitudinal trans men cohort

Benjamin Grünhagel, Malte Borggrewe, Sven Hendrik Hagen, Susanne M. Ziegler, Florian Henseling, Laura Glau, Rebecca-Jo Thiele, Maria Pujantell, Varshi Sivayoganathan, Benedetta Padoan, Janna M. Claussen, Arne Düsedau, Jana Hennesen, Madeleine J. Bunders, Stefan Bonn, Eva Tolosa, Christian F. Krebs, Christoph Dorn, and Marcus Altfeld

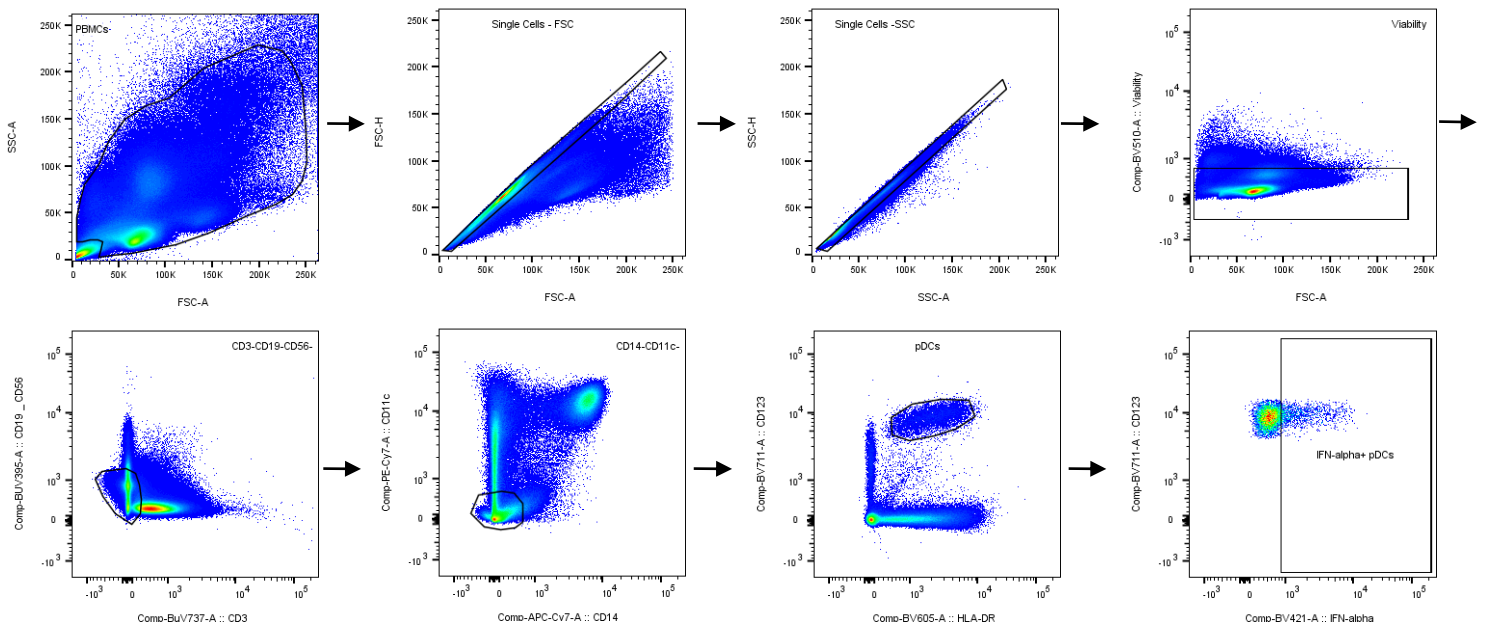
Supplemental Figure S1. Population Frequencies.



Supplemental Figure S1. Population Frequencies. *Related to Figure 2.*

Frequencies of immune cell populations over time under testosterone injections in $n=10$ trans men, determined by flow cytometry. Frequencies did not change significantly over time for (A) T Cells ($R^2=0.82$, $p=0.38$), (B) Regulatory T Cells ($R^2=0.93$, $p=0.23$), (C) B Cells ($R^2=0.74$, $p=0.3$), (D) Natural Killer Cells ($R^2=0.86$, $p=0.26$), (E) Monocytes ($R^2=0.63$, $p=0.3$), (F) Basophils ($R^2=0.85$, $p=0.75$), (G) DC1 Dendritic Cells ($R^2=0.69$, $p=0.42$), (H) DC2 Dendritic Cells ($R^2=0.8$, $p=0.29$), (I) Innate Lymphoid Cells ($R^2=0.85$, $p=0.18$), as assessed by extra sum-of-squares F test comparing a linear regression model with a shared slope and individual intercepts to a respective model with a hypothetical slope of 0.

Supplemental Figure S2. Gating Strategy for the Identification of pDCs.

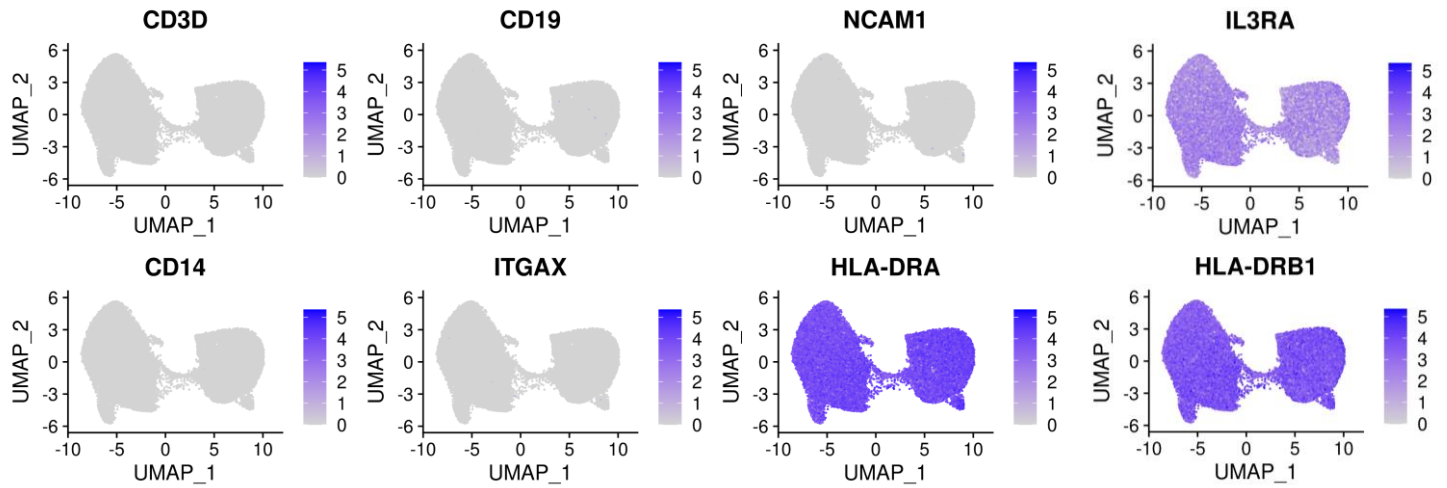


Supplemental Figure S2. Gating Strategy for the Identification of pDCs. *Related to Figure 3.*

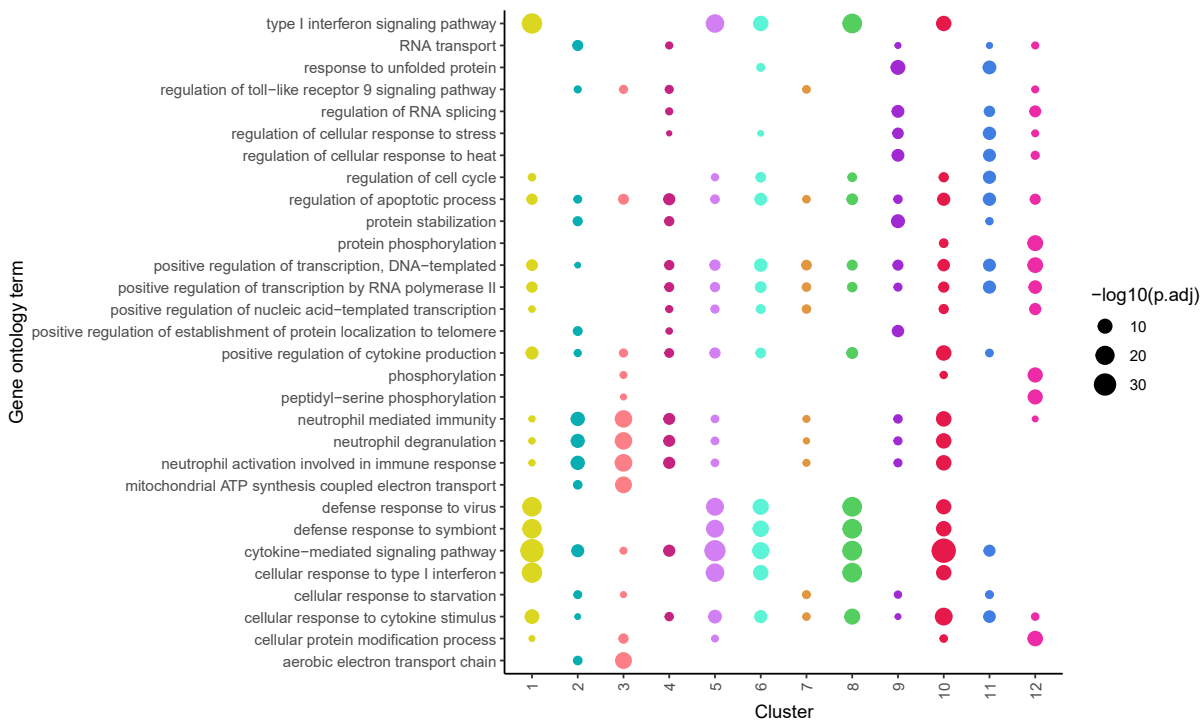
Gating strategy for identification of pDCs and cytokine producing pDCs, shown in one representative donor. After setting a broad PBMC gate and excluding doublets using FSC and SSC, viable cells were selected for further analysis. pDCs were defined as CD3-CD19-CD56-CD14-CD11c-HLA-DR+CD123+ cells. Equivalent gating strategies were used for FACS-isolation of pDCs and population frequency experiments.

Supplemental Figure S3. scRNA seq of pDCs.

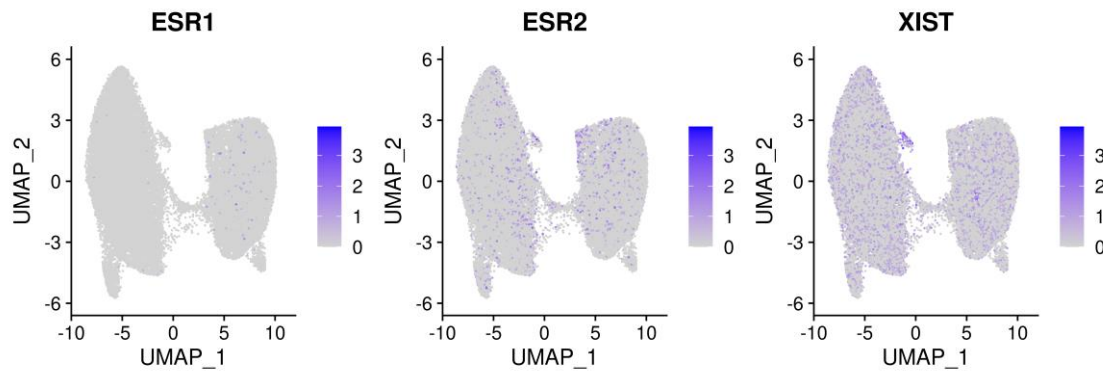
A



B



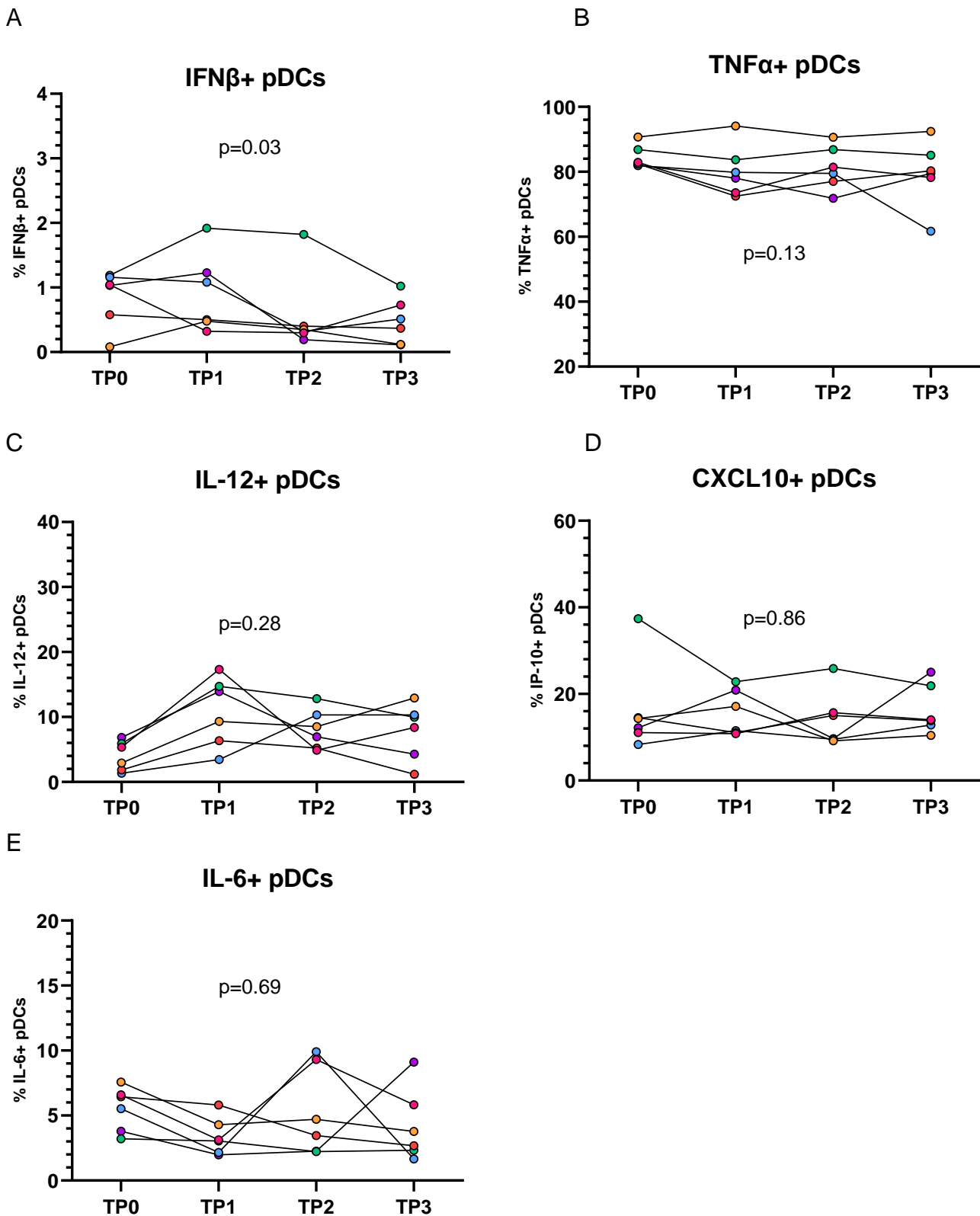
C



Supplemental Figure S3. scRNA seq of pDCs. Related to Figure 3.

(A) mRNA expression of indicated markers used for FACS-isolation of pDCs (CD3-CD19-NCAM1-CD14-ITGAX-HLA-DR+IL3RA+). (B) Top 5 GO terms associated with markers from each pDC cluster and enrichment of these GO terms across all cluster. (C) UMAP of ESR1, ESR2, XIST transcripts.

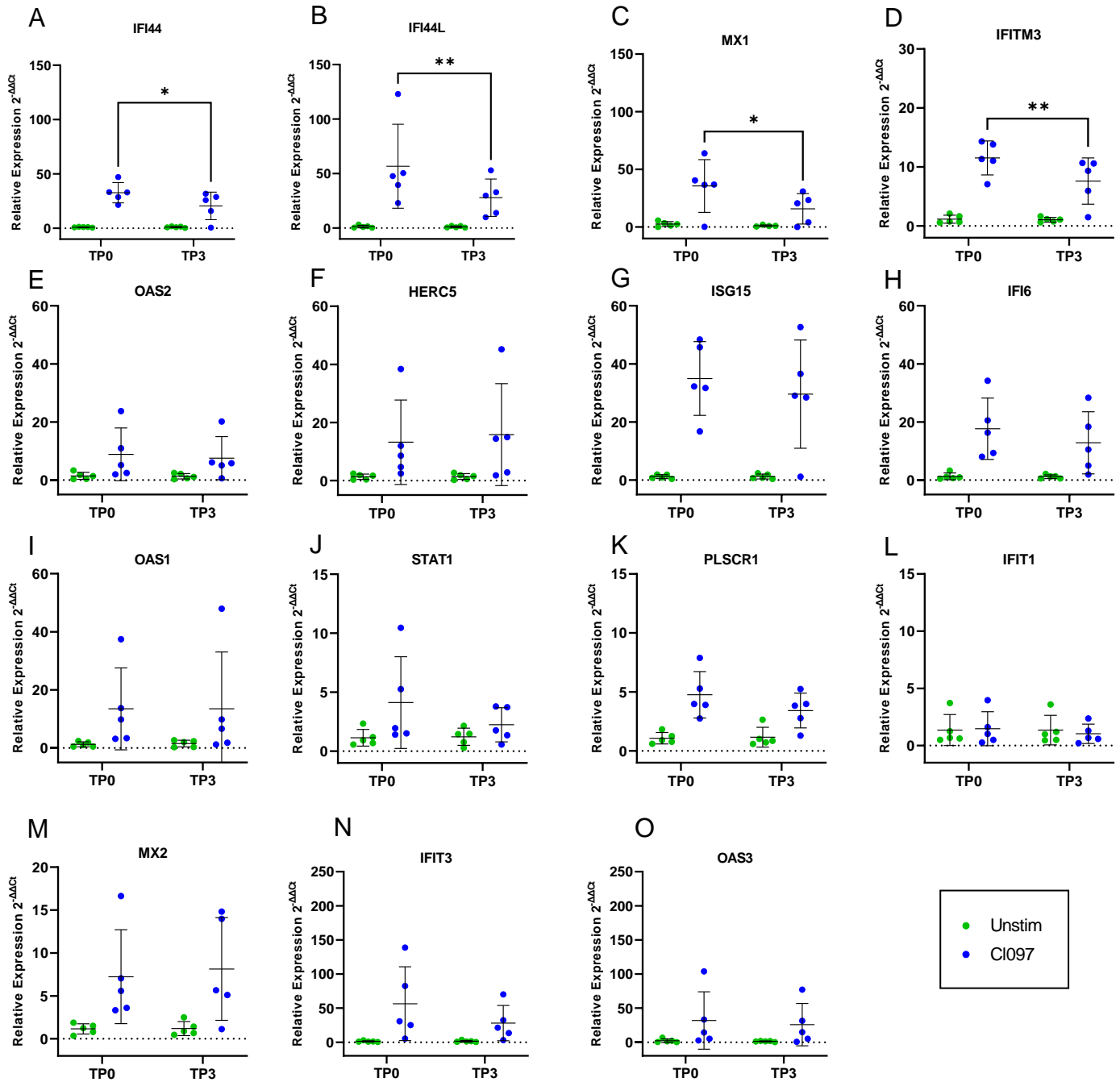
Supplemental Figure S4. Cytokine Production by pDCs.



Supplemental Figure S4. Cytokine Production by pDCs. Related to Figure 5.

Frequencies of cytokine positive pDCs following TLR7/8-stimulation of PBMCs over time under testosterone injections in six trans men, determined by intracellular cytokine staining for flow cytometry. (A) Frequencies of IFN β -producing pDCs in n=6 trans men decreased significantly over time under testosterone administration ($R^2=0.68$, $p=0.03$). Frequencies did not change significantly over time for (B) TNF α ($R^2=0.68$, $p=0.13$), (C) IL-12 ($R^2=0.32$, $p=0.28$), (D) CXCL10 ($R^2=0.65$, $p=0.86$), (E) IL-6 ($R^2=0.19$, $p=0.69$). Statistical significance was assessed by extra sum-of-squares F test comparing a linear regression model with a shared slope and individual intercepts to a respective model with a hypothetical slope of 0.

Supplemental Figure S5. Single Plots of Interferon-Stimulated Genes.



Supplemental Figure S5. Single Plots of Interferon-Stimulated Genes. *Related to Figure 6.*

Interferon-stimulated gene (ISG) expression of PBMCs for both TLR7/8-stimulation (blue) and without stimulation (green), displayed for each ISG. TLR7/8-mediated ISG expression was significantly reduced at TP3 compared to TP0 for (A) IFI44 ($p=0.02$), (B) IFI44L ($p=0.007$), (C) MX1 ($p=0.01$), (D) IFITM3 ($p=0.006$). For (E) OAS2, (F) HERC5, (G) ISG15, (H) IFI6, (I) OAS1, (J) STAT1, (K) PLSCR1, (L) IFIT1, (M) MX2, (N) IFIT3 and (O) OAS3, reduction was not significant. Statistical significance was assessed by two-way ANOVA followed by Fisher's least significant difference test (A-B, D-O) or mixed effect analysis (C). Dotted line represents 0. Mean with SD indicated as error bars.

* $p < 0.05$, ** $p < 0.01$.

Supplemental Table S5: Interferon-Stimulated Gene Primers

Gene	Sequence fw	Sequence rev
B2M	TGCTGTCTCCATGTTTGATGATCT	TCTCTGCTCCCCACCTCTAAGT
HERC5	TGCACTCTCAAAGGTGGTG	CCAGGCCTAGTTGTCCACAT
IFI44	AGCCTGTGAGGTCCAAGCTA	ATCTGCAGCCCATAGCATT
IFI44L	TATGTGTGTTGGCTGGGAGA	GGGCCTGCATACCTCATAGA
IFI6	AGGATGAGGAGTAGCCAGCA	TTGGGAGGTTGAGACAGGAG
IFIT1	GGCAAACCAACCGTCTCTA	TTTGAGATGGGGTCTCGTTC
IFIT3	GAACATGCTGACCAAGCAGA	CAGTTGTGTCCACCCTTCT
IFITM3	TCGCCTACTCCGTGAAGTCT	CATAGGCCTGGAAGATCAGC
ISG15	GAGAGGCAGCGAACTCATCT	CTTCAGCTCTGACACCGACA
MX1	GCCACAAGGCACCTAAGTC	ATTCTCCAAACCTGCGCTCT
MX2	AAGCAGTATCGAGGCAAGGA	TCGTGCTCTGAACAGTTTGG
OAS1	GAGCTCCTGACGGTCTATGC	GTTTCGTGAGCTGCCTTCTC
OAS2	GCTCCTATGGACGAAAACA	TGAACCCATCAAGGGACTTC
OAS3	GACCTAAGGGATGGCTGTGA	CAGGAACTGAAGGCTCAGG
PLSCR1	GGTTTACTTTGCAGCGGAAG	ACCAGGAGGAGCTTGGATTT
STAT1	CCGTTTTTCATGACCTCCTGT	TGAATATCCCCGACTGAGC