

CD90⁺ Human Dermal Stromal Cells Are Potent Inducers of FoxP3⁺ Regulatory T Cells

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Mesenchymal stromal cells (MSC)

- plastic-adherent, self-renewing, multipotent cells
- express a set of stem cell markers (e.g., CD90, CD105, CD73), but lack hematopoietic markers
- localized in virtually every prenatal and adult tissue, including human skin
- are immunomodulatory & suppress a great variety of lymphocytes and differentiate and expand Tregs
- immunomodulation is facilitated by cell–cell contact and the release of soluble factors (e.g., TGF- β , IL-10, HLA-G5)
- are hypoimmunogenic as they lack expression of HLA class II or costimulatory molecules

Regulatory T cells (Tregs)

- control the activation and expansion of aberrant, over- or self-reactive lymphocytes thereby preventing overwhelming pathophysiological immune response
- express CD4, CD25, and the transcription factor forkhead box P3 (FoxP3) and are mostly negative for CD127
- are generated in the thymus through presentation of self-peptides by thymus-resident stromal cells \Rightarrow naturally occurring (n)Tregs
- can be generated from naive CD4⁺CD45RA⁺ T cells *in vitro* and *in vivo*
- Thymic dendritic cells and/or stromal cells regulate the positive selection of self-reactive thymocytes and generate FoxP3⁺ Tregs via provision of costimulatory molecules (CD80, CD86) through ligation of CD28

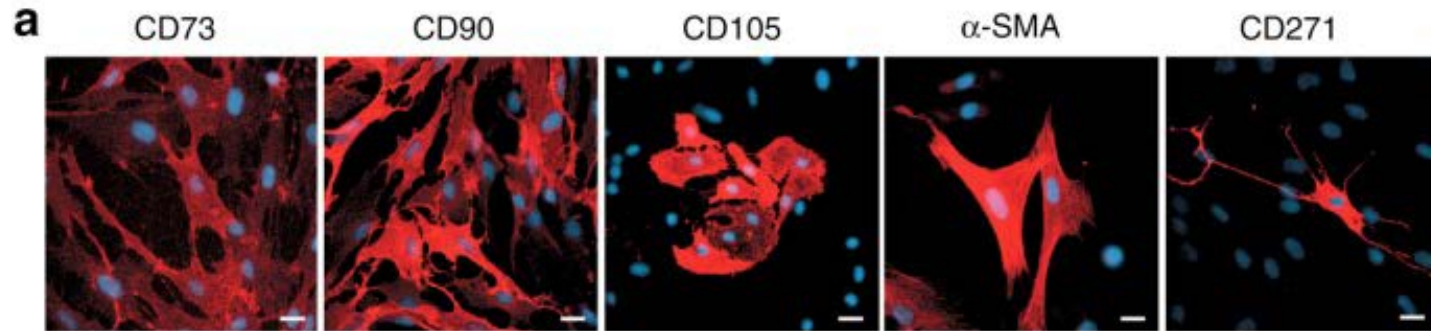
Aim of the study

- to determine whether dermal MSC subsets have immunosuppressive capacity
- to investigate whether the dermal MSC can induce the generation of Tregs
- to explore the differentiation potential of dermal MSC toward the endothelial lineage

Experimental design

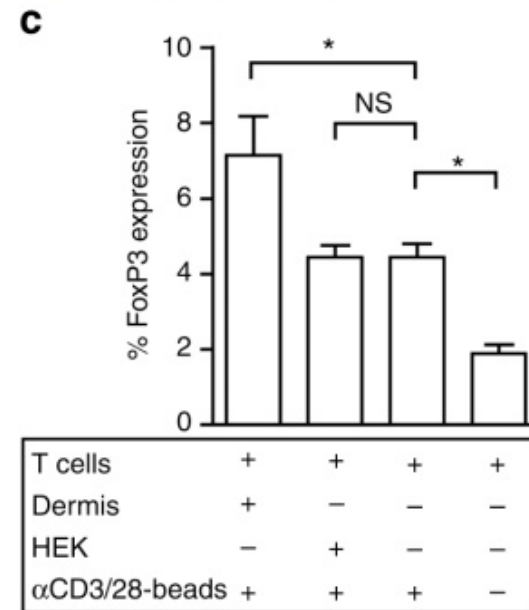
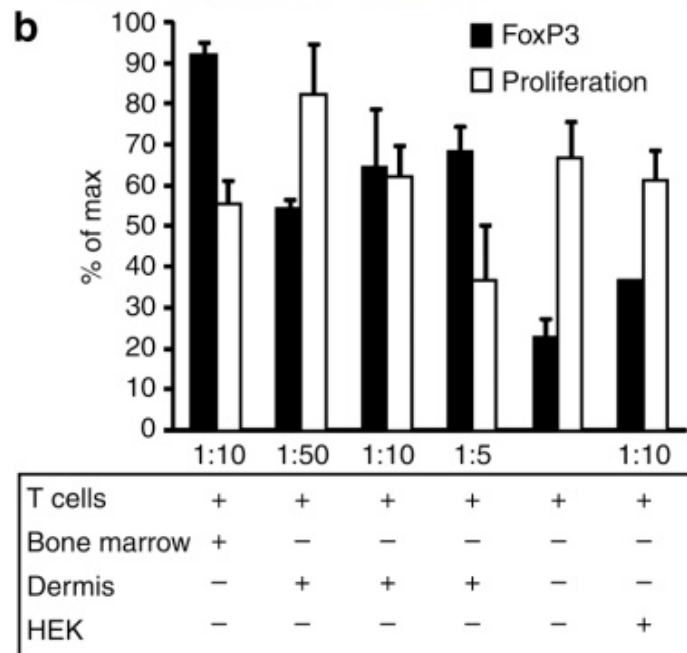
Carboxyfluoresceine succinimidyl ester (CFSE)-based division tracing coculture system with plastic-adherent dermal cells and CFSE-labelled T-cells stimulated via α CD3/CD28 beads

CFSE labeling is used to monitor distinct generations of proliferating cells by dye dilution. Live cells are covalently labeled with a very bright, stable dye. Every generation of cells appears as a different peak on a flow cytometry histogram.

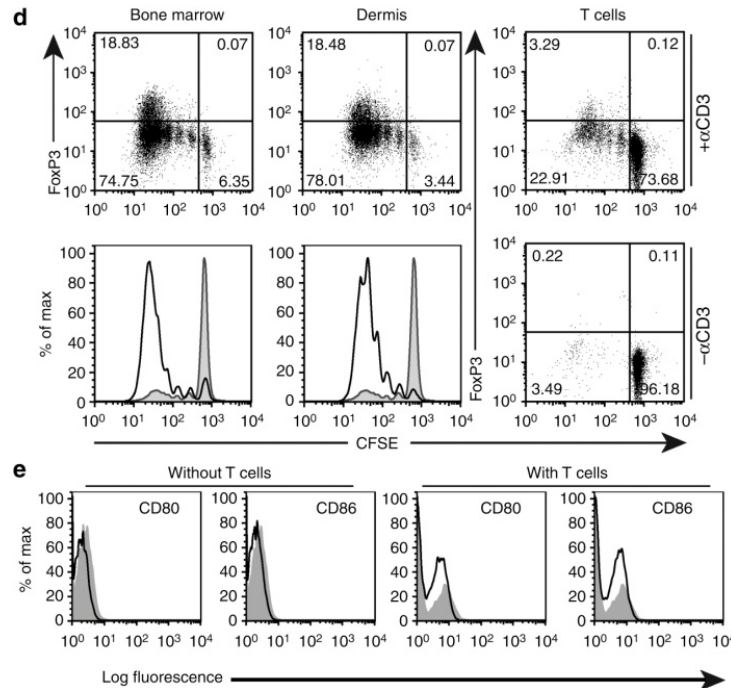


Phenotype of dermal cells – characterization by CLSM

Suppressive and FoxP3-inducing potential of plastic-adherent dermal cells



Dermal cells induce FoxP3 expression in CD25⁻CD4⁺CD45RA⁺ T cells irrespective of CD28 costimulation

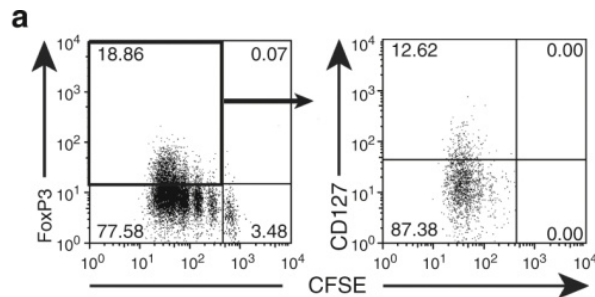


Generation of FoxP3⁺ T cells in the presence of dermal and BM cells

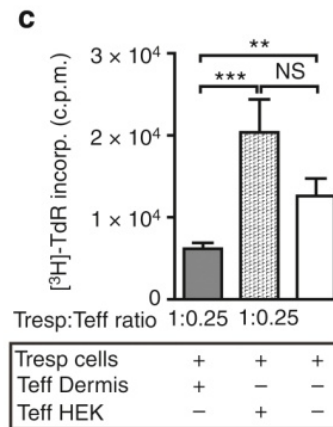
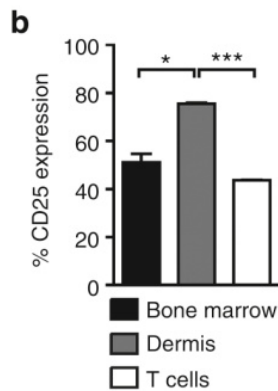
Proliferation of CD25⁻CD4⁺CD45RA⁺ T cells induced by dermal and BM cells

Dermal cells do not express CD80 or CD86 before and after co-culture with T cells

Dermal cells induce functional forkhead box P3⁺(FoxP3⁺) Tregs

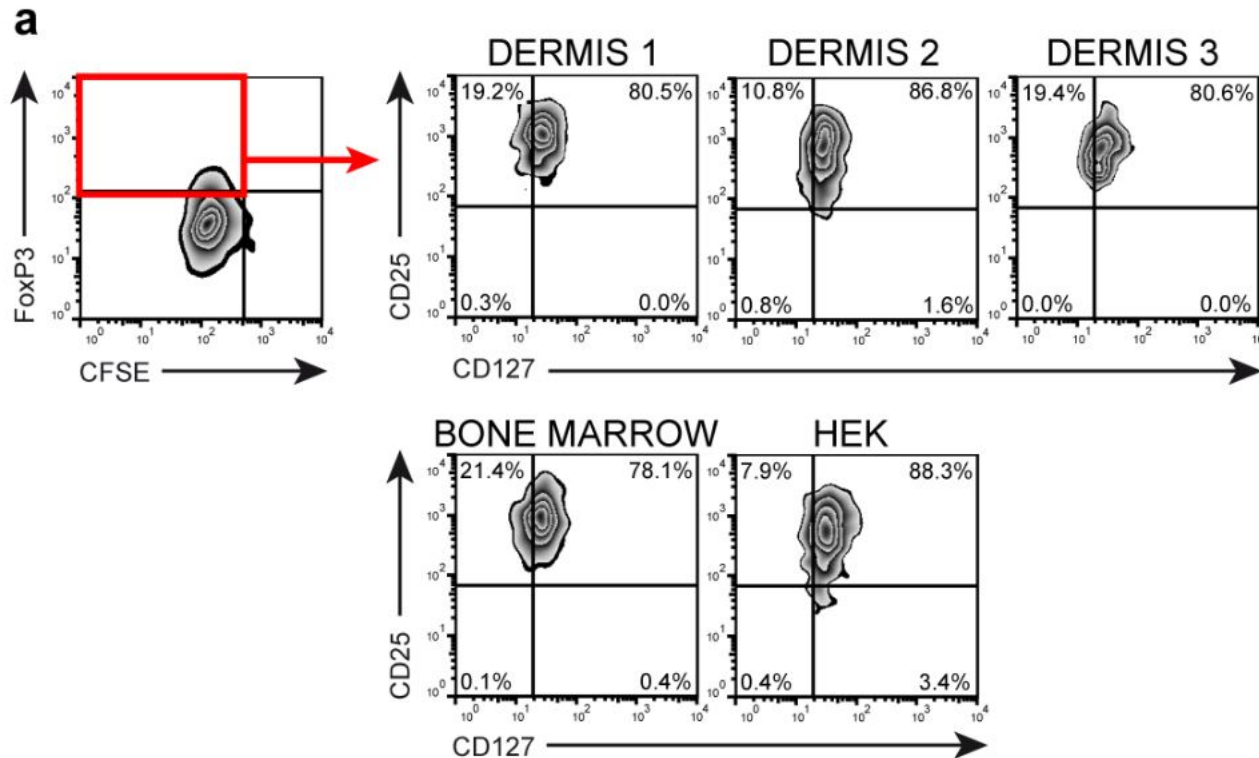


CD127 is downregulated in dermal cell-induced FoxP3⁺CD4⁺ T cells



Dermal cell-induced FoxP3⁺CD4⁺ T cells are functional as they significantly suppress the proliferation of CD25-depleted, αCD3/CD28-activated T cells

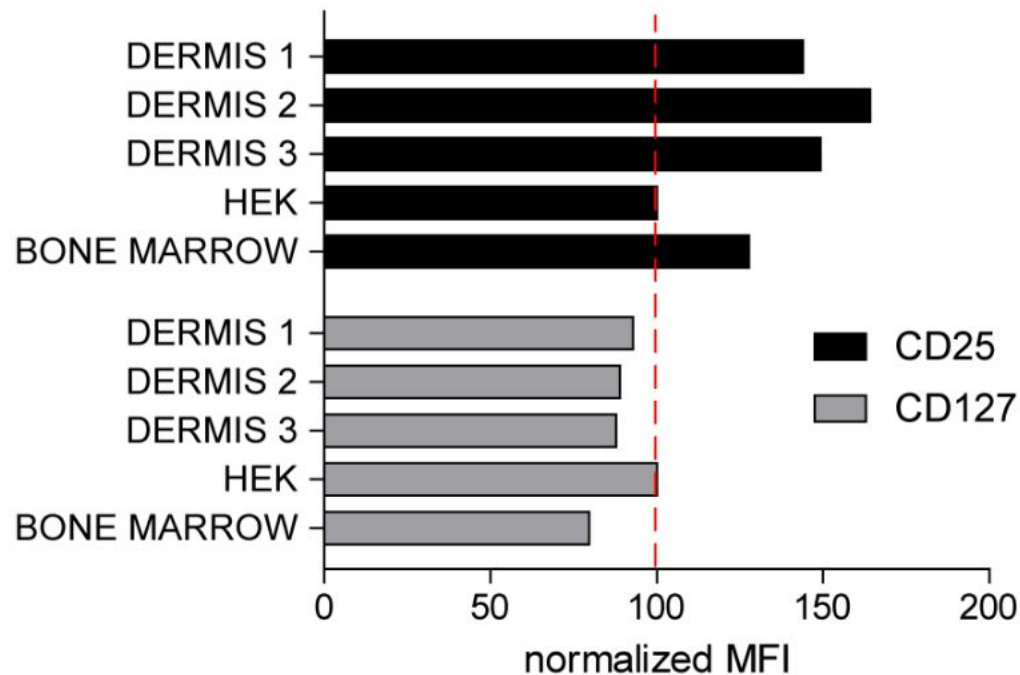
Co-expression pattern of CD25, CD127, and FoxP3 in α CD3-stimulated CFSE-labeled CD25⁻CD4⁺CD45RA⁺ T cells co-cultured with dermal cells, BM cells and HEK cells



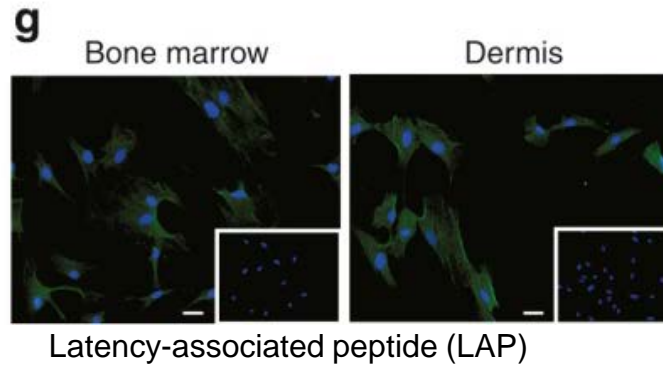
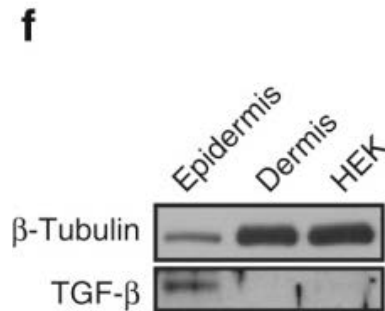
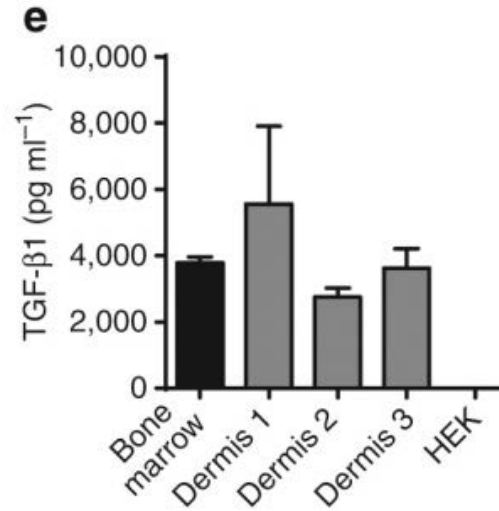
↑ CD25 ↓ CD127 in dermal and BM stromal cells

Expression level of CD25 and CD127

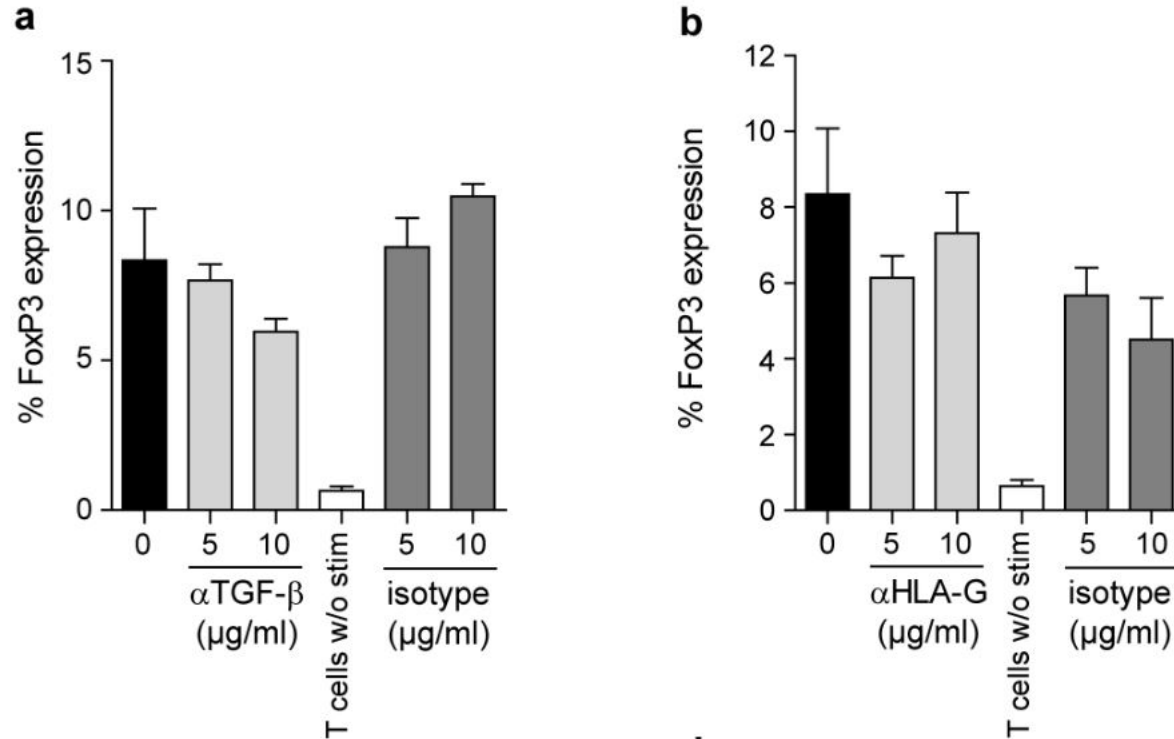
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TGF- β and FoxP3 induction

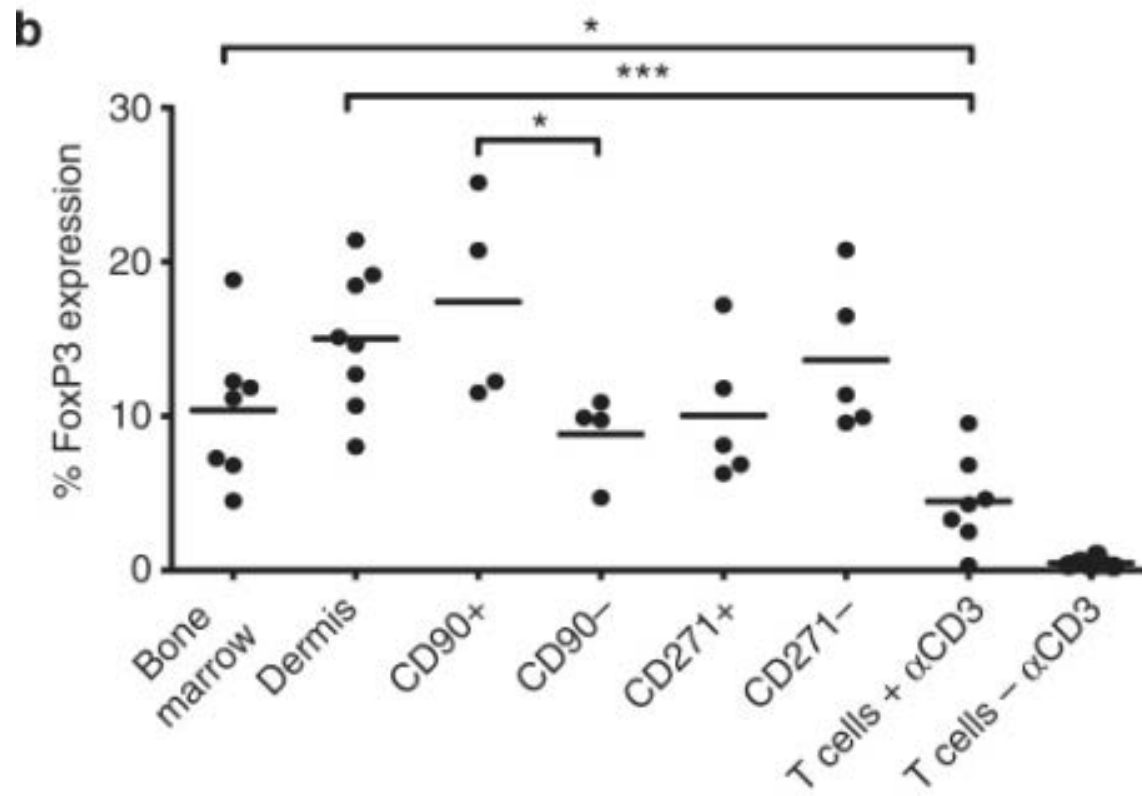


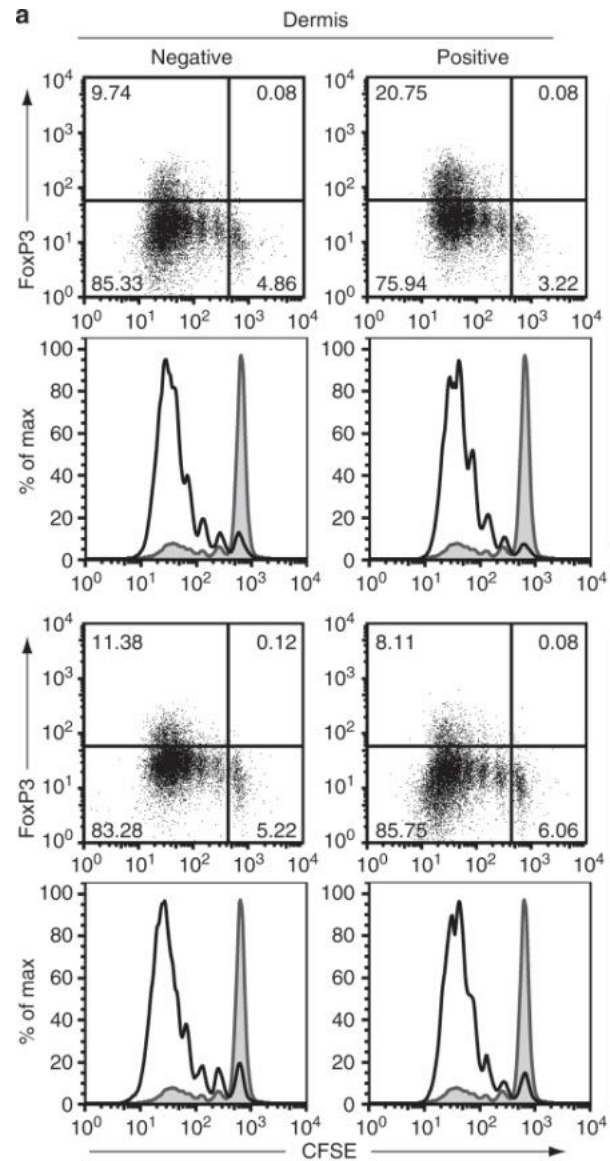
TGF- β is involved in dermis-induced FoxP3 expression



α TGF- β -blocking mAb reduces the number of FoxP3-expressing T cells co-cultured with dermal cells, reduction is concentration-dependent

Ability of different stromal cell subsets to induce FoxP3 in naive CD25⁻CD4⁺CD45RA⁺ T cells w/o provision of costimulatory molecules

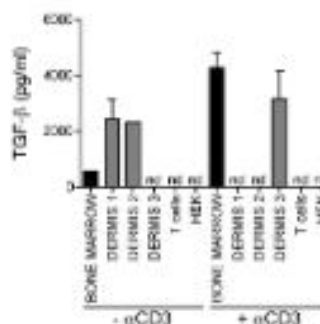
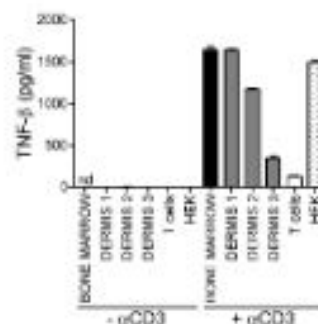
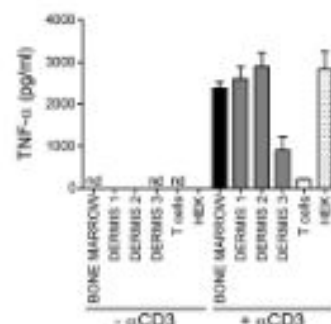
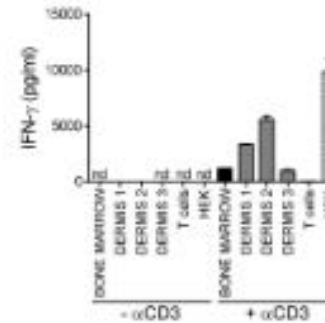
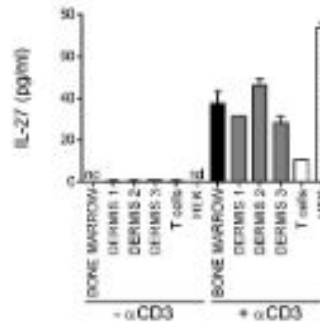
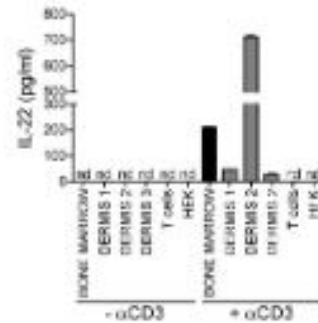
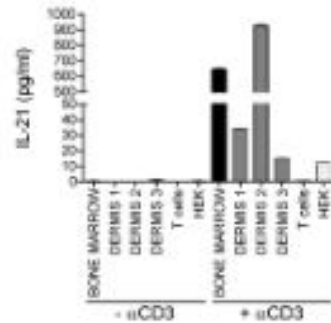
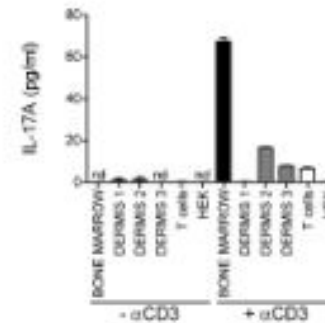
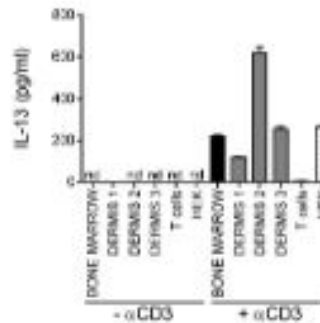
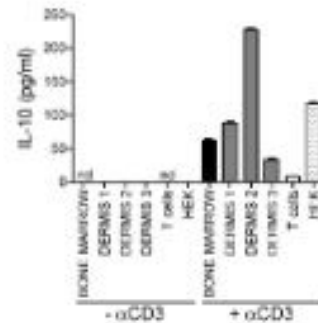
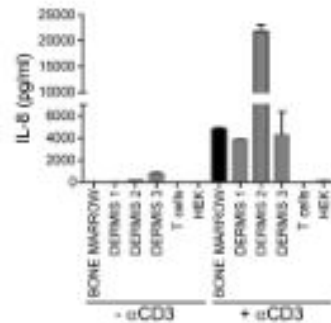
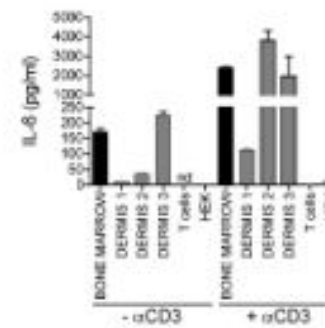
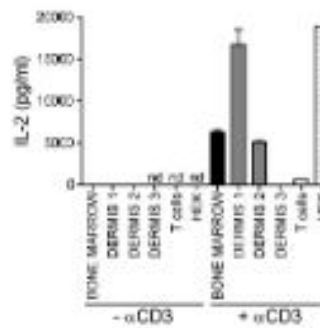
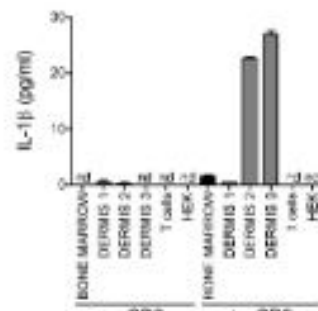
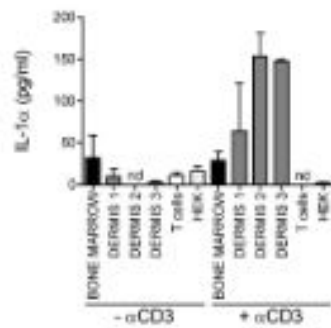




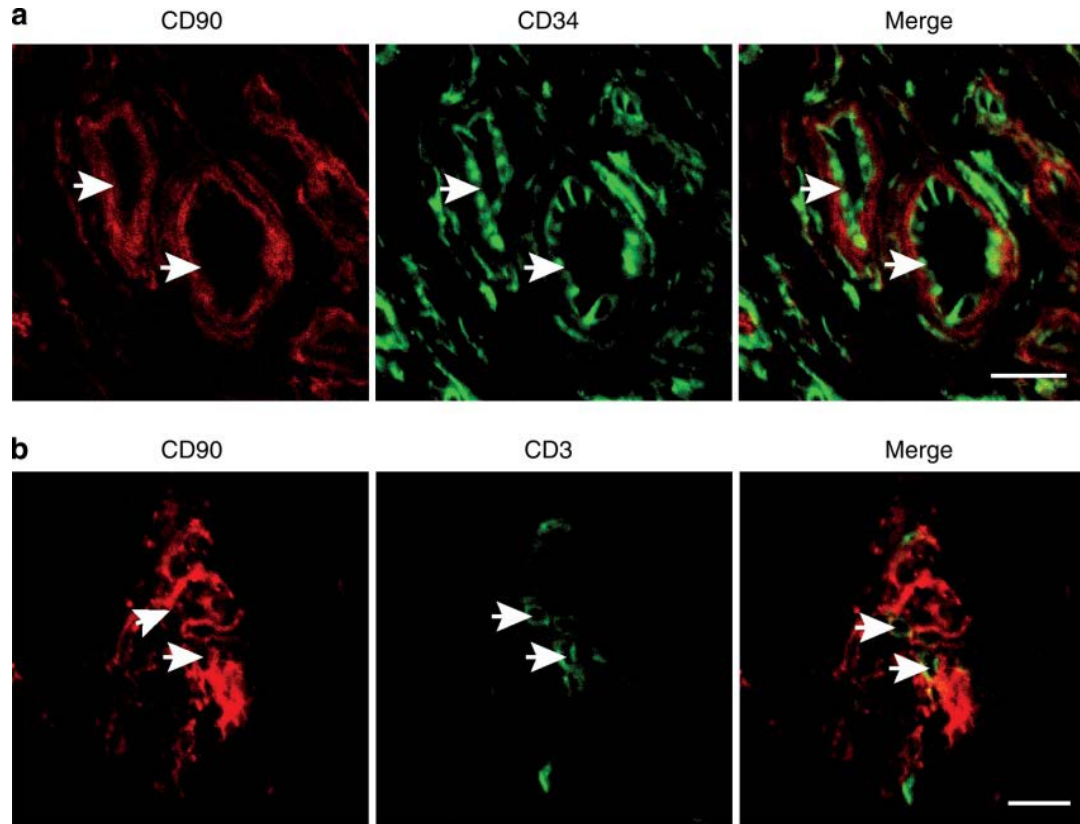
CD90⁺ dermal cells induce more FoxP3 than CD90⁻ cells

CD271⁻ dermal cells show a tendency to induce more FoxP3 compared to CD271⁺ dermal cells

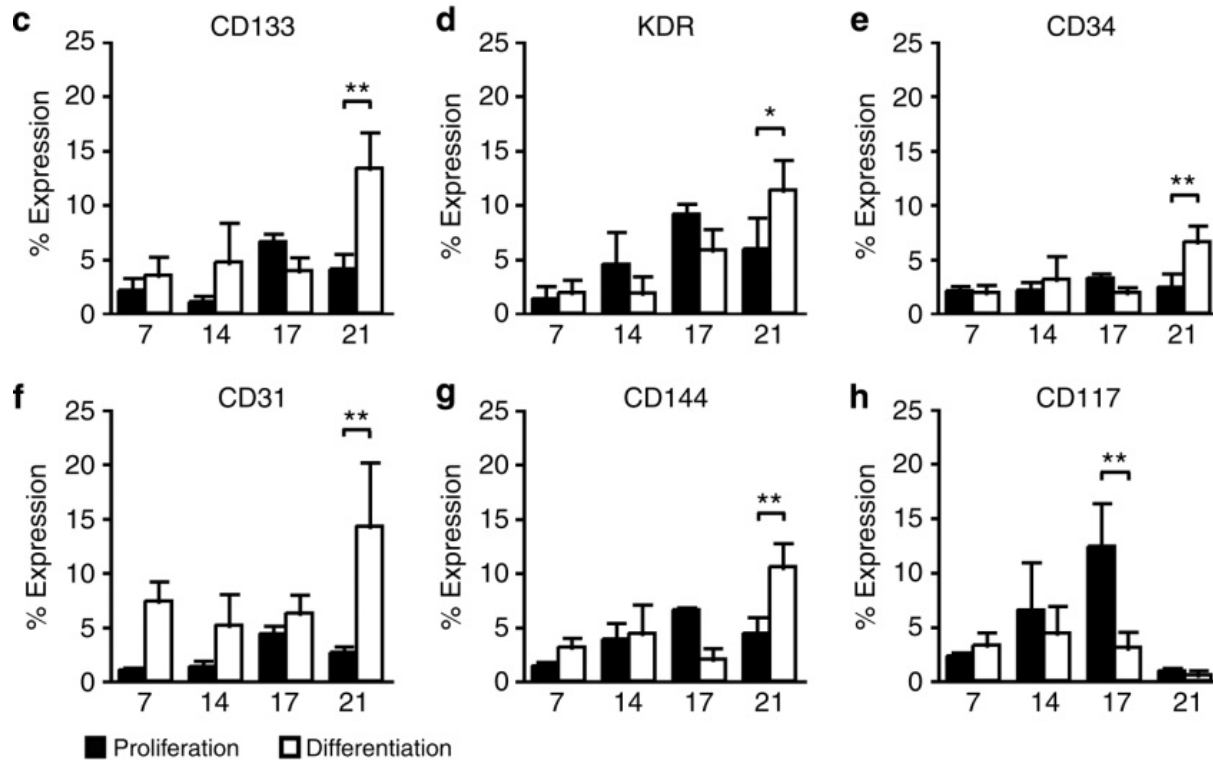
Cytokine profile upon co-culture of dermal cells, BM cells (+), HEK cells (-) and CD25⁻CD4⁺CD45RA⁺ T cells with and w/o α CD3-stimulation



CD90⁺ dermal cells are predominantly localized perivascularly



CD3⁺ T cells are in close proximity to CD90⁺ cells



CD90⁺ dermal cells in EC differentiation medium - ↑ expression of endothelial (progenitor) cell markers (CD133, VEGFR2, CD34) & mature endothelial cell markers (CD31, CD144)

Summary

- Plastic-adherent dermal cells suppress T-cell proliferation stimulated via α CD3/CD28 beads in a cell-density dependent manner
- Induction and maintenance of suppressive Tregs within healthy human skin is CD28-independent
- Dermal cells are able to expand natural Tregs and increase the percentage of activation-induced Tregs
- CD90⁺ dermal cells induce significantly higher percentages of FoxP3⁺ T cells compared with CD90⁻ cells
- plastic-adherent dermal cells are able to differentiate toward the endothelial lineage suggesting that CD90⁺ cells provide a local pool of vessel precursors



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