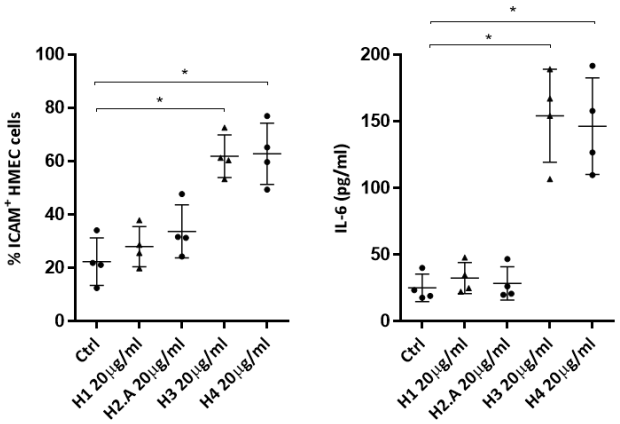
***Type of paper:*** *Brief reports*

**Title:** Endothelial cells activated by extracellular histones promote FoxP3$^{+}$ suppressive Treg cells *in vitro*

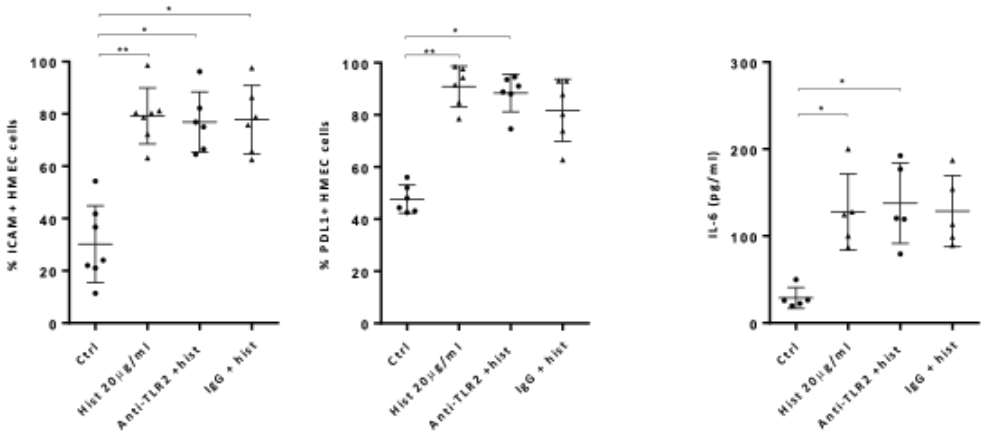
# Supplemental data

## Figure S1: Effect of histones H1, H2A, H3 and H4 on HMECs




ICAM-1 was assessed in HMEC cells activated with 20 µg/mL of purified individual histones (H1, H2.1, H3, H4). Percentage of positive cells were evaluated by cytometry. Levels of IL-6 in the supernatant were measured by ELISA. Kruskal-Wallis test,*$p$=0.006, n=4

## Figure S2: Anti-TLR2 effect on endothelial cells expression of ICAM-1, PDL-1 and secretion of IL-6

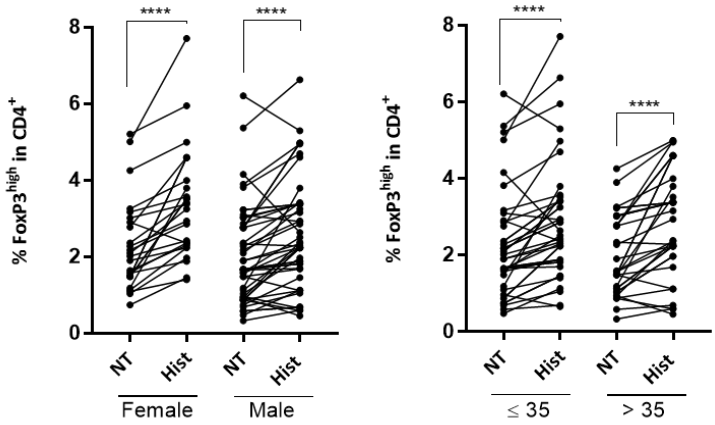



CD54 (ICAM-1) and CD106 (Vascular cell adhesion protein 1 (VCAM-1)) expression were measured on Human Microvascular Endothelial Cells (HMEC) after treatment with 20 µg/mL

histone, with or without IgG anti-TLR2 50 µg/mL or a control IgG antibody 50 µg/mL. Median Fluorescence Intensity (MFI) and percentage of positive cells were measured by cytometry. Kruskal-Wallis test,***$p$=0,002, n = 6

InterLeukin-6 was measured by ELISA in the supernatant. Kruskal-Wallis test,**$p$=0.013, n=5

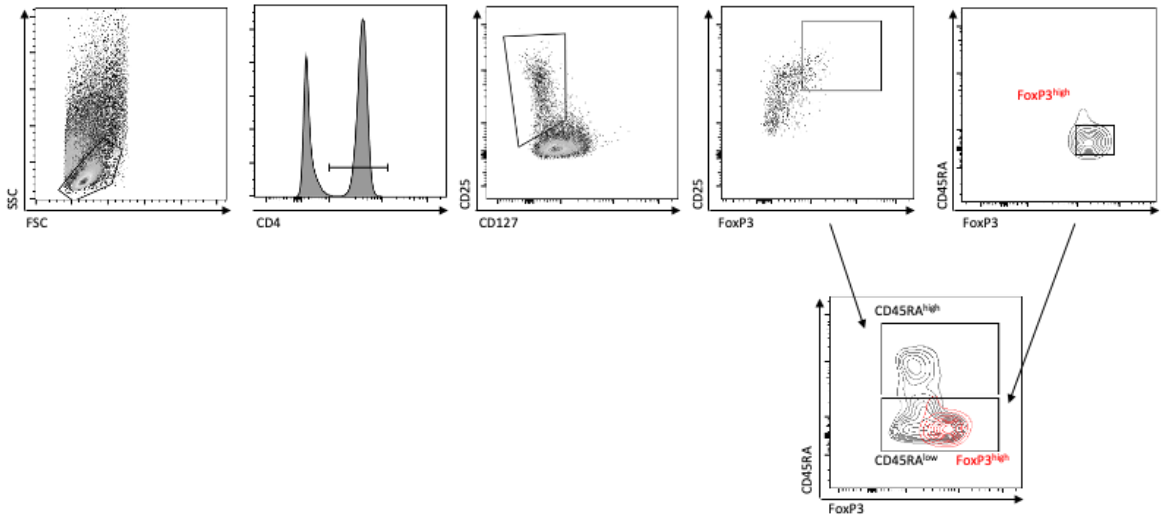
## Figure S3: Effect of sex and age of PBMCs donors on Treg expansion




Comparison of Treg expansion after 3-days of coculture, with or without histone pre-stimulation of HMECs. Left panel are results presented as percentage of $CD4^+$ $CD25^+$ $CD127^{low}$ $FoxP3^{high}$ among $CD4^+$ cells according to the sex of the PBMCs donors. Wilcoxon test, ****$p$<0,0001

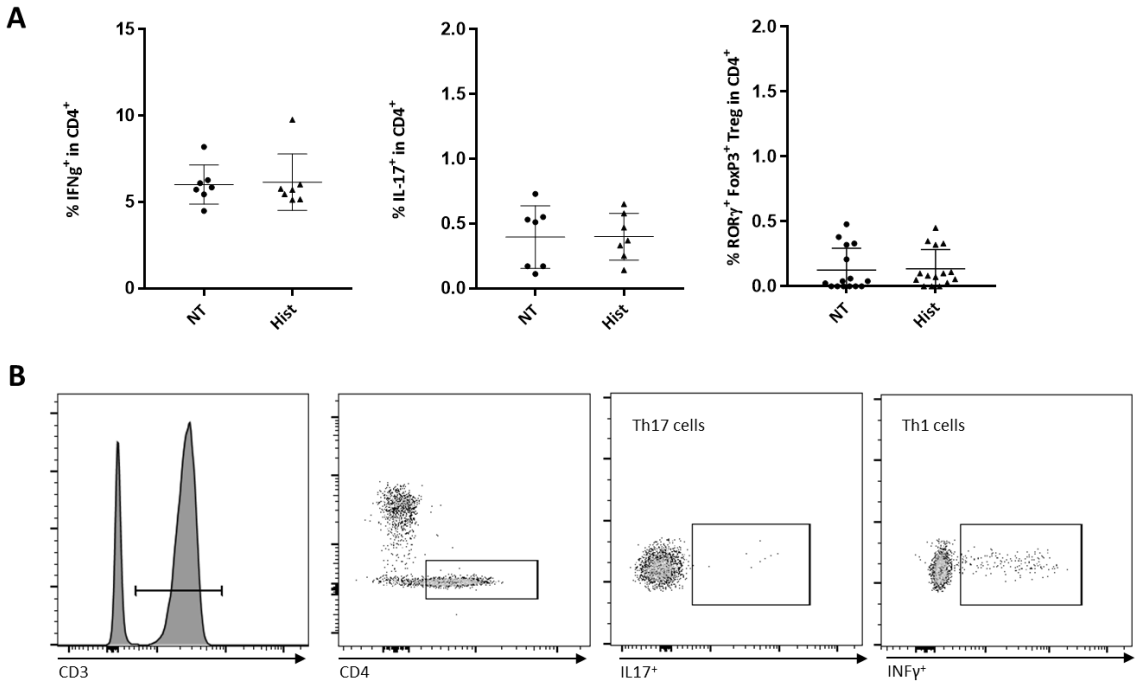
Right panel are results presented as percentage of $CD4^+$ $CD25^+$ $CD127^{low}$ $FoxP3^{high}$ among $CD4^+$ cells according to the age of the PBMCs donors. Age ≤ 35 years old, n=40; age > 35 years-old, n=33. 35 years-old corresponds to the median age of the PBMCs donors. Wilcoxon test, ****$p$<0,0001.

## Figure S4: Gating strategy for Treg cells identification and phenotype




Gating strategy to identify Treg cells after 3 days of coculture with HMEC stimulated with histone for 18h. Treg cells are $CD4^+$ $CD25^+$ $CD127^{low}$ $FoxP3^{high}$ T lymphocytes, memory Treg are $CD45^-$ and naïve Treg cells are $CD45RA^+$. Gating was performed using Fluorescence Minus One (FMO) control.

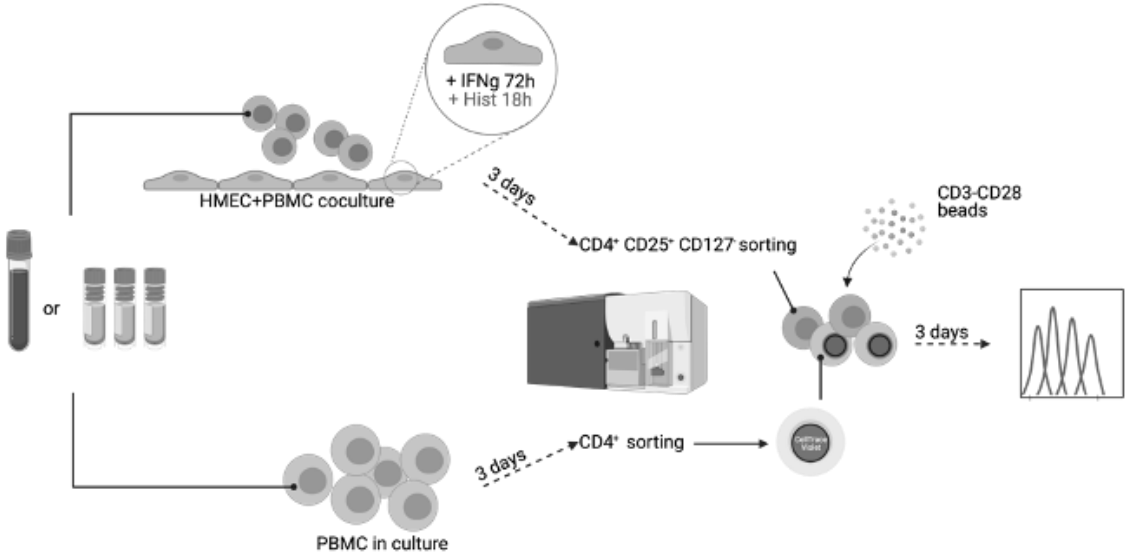
## Figure S5: Th17, Th1 and Treg RORγT$^+$ population at the end of 3-days cocultures



A/ Comparison of Th17, Th1 and RORγt-Treg$^{+}$ expansion after 3 days of coculture, with or without histone pre-stimulation of HMECs. Results are presented as percentages of respectively CD3$^{+}$ CD4$^{+}$ IL-17$^{+}$ and CD3$^{+}$ CD4$^{+}$ IFNγ$^{+}$ among CD4$^{+}$ cells. n=7 for Th1 and Th17 cells, n=30 for RORγt$^{+}$ Tregs cells. Paired t test (ns).

B/ Gating strategy for Th1 and Th17 cells. FoxP3 RORγt$^{+}$ are defined as CD4$^{+}$ CD25$^{+}$ CD127$^{low}$ FoxP3$^{high}$ RORγt$^{+}$.

## Figure S6: Suppression assay method




PBMCs were prepared from fresh donor blood or thawed, and cocultured as described above. We used methods previously described in literature [17], [52] for the following protocol. Treg cells are generated in our in vitro model, for 3 days in cocultures with histone-stimulated endothelial cells. Tregs were stained with CD4 FITC, CD25 PE, and CD127 PerCP-Cy5.5 and sorted on a BD FACS Aria II system. In parallel, autologous PBMCs were cultured in T25 flasks and CD4$^{+}$ T cells were sorted the same day as Tregs on BD FACS Aria II, with a CD4 FITC prior staining with CellTrace Violet proliferation kit (ThermoFisher). These cells are considered as responders T cell (Tresp). Sorted Tregs and stained Tresp were then cocultured together at different ratio for 3 days. We added Dynabeads T activator CD3/CD28 (ThermoFicher), 1/10 diluted. Proliferation of Tresp cells was analyzed by flow cytometry on BD FACS CantoII.

# Table S1: HMEC1 phenotype

| Percentage of HMEC positive cells | | NT | Hist | IFNg - | IFNg Hist |
|---|---|---|---|---|---|
| Adhesion molecules | CD31 | 55,3 | 55,5 | 61,45 | 63,15 |
| | CD54 | 3,07 | 21,7 | 49,9 | 59,5 |
| HLA molecules | HLA-DR | 0,00861 | 0,018 | 88,7 | 89,2 |
| | HLA-I | 95,8 | 96,6 | 81,5 | 75,3 |
| Co-stimulatory molecules | CD274 | 0,69 | 12,5 | 54,3 | 57,7 |

HMEC1 cells phenotype without any treatment (NT), with or without Interferon (IFN)γ at 30ng/ml for 3 days and with or without extracellular histones (hist) at 20µg/ml during 18h. Results are presented as percentage of positive cells for each marker. VCAM, 4-1 BBL, CD62P were not detectable by cytometry on HMEC1 cells.