# Human Umbilical Vein Endothelial Cells foster conversion of $CD4^+CD25^-$ $Foxp3^-$ T cells into $CD4^+Foxp3^+$ Regulatory T Cells via Transforming Growth Factor-β

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## Supplementary Figure Legends

***Supplementary Figure S1. Female HUVECs induced Treg cells from $CD4^+$ T cells***

$CD4^+CD25^-Foxp3^-$ T cells were co-cultured with primary female HUVECs at cell-to-cell ratios of 1:1; 2:1 and 10:1 (T cells:HUVECs). After 48 and 72 hours, the number of $CD4^+Foxp3^+$ T cells was analyzed. At a cell-to-cell ratio of 1:1 female HUVECs significantly induced Treg cell generation at both time points. Results are presented as percentages (c). Representative flow cytometry plots are displayed for 48 hours (a) and 72 hours (b). Samples from 16 normal pregnant women were analyzed in duplicates. Data are presented as means ± S.E.M.. Statistical analysis among groups was performed using Two-way-ANOVA followed by Bonferroni correction for multiple comparisons. *=$p<0.05$; **=$p<0.01$.

***Supplementary Figure S2. Male HUVECs induced Treg cells from $CD4^+$ T cells***

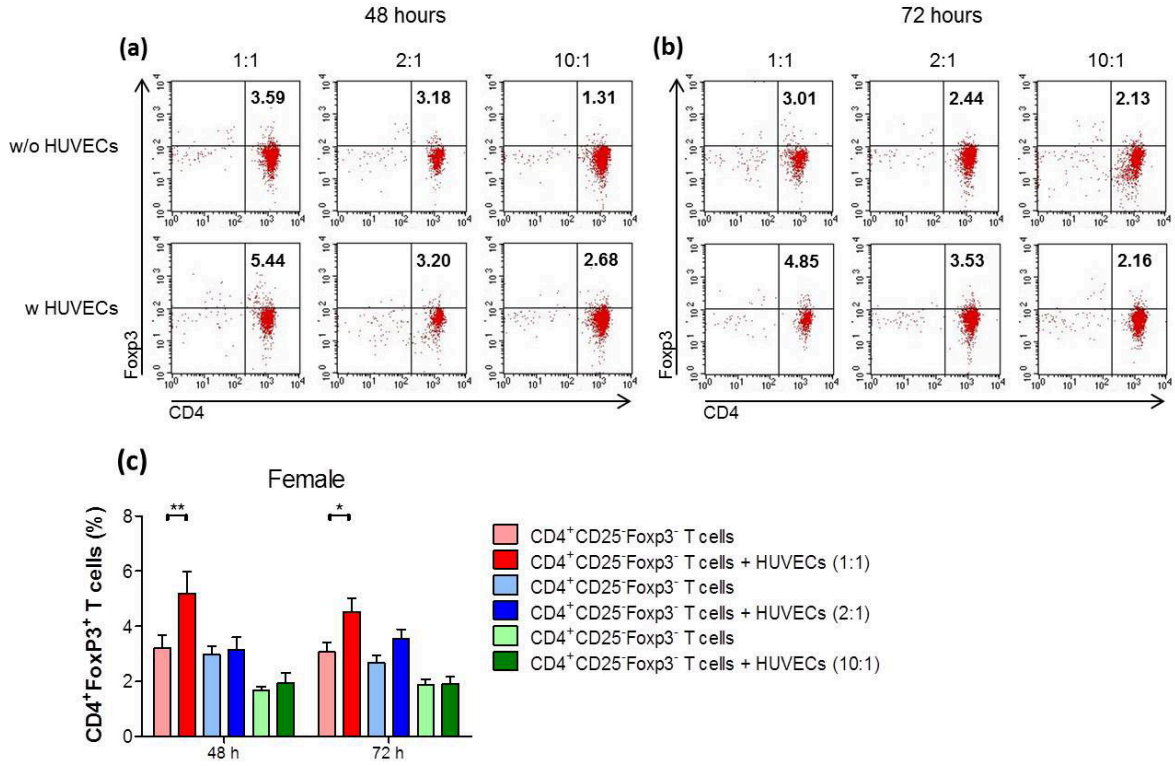
$CD4^+CD25^-Foxp3^-$ T cells were co-cultured with primary male HUVECs at cell-to-cell ratios of 1:1; 2:1 and 10:1 (T cells:HUVECs). After 48 and 72 hours, the number of $CD4^+Foxp3^+$ T cells was analyzed. Male HUVECs induced only slightly and non-significantly the generation of Treg cells. Results are presented as percentages (c). Representative flow cytometry plots are displayed for 48 hours (a) and 72 hours (b). Samples from 16 normal pregnant women were analyzed in duplicates. Data are presented as means ± S.E.M.. Statistical analysis among groups was performed using Two-way-ANOVA followed by Bonferroni correction for multiple comparisons.

***Supplementary Figure S3. HUVECs did not induce migration of $CD4^+$ T cells***

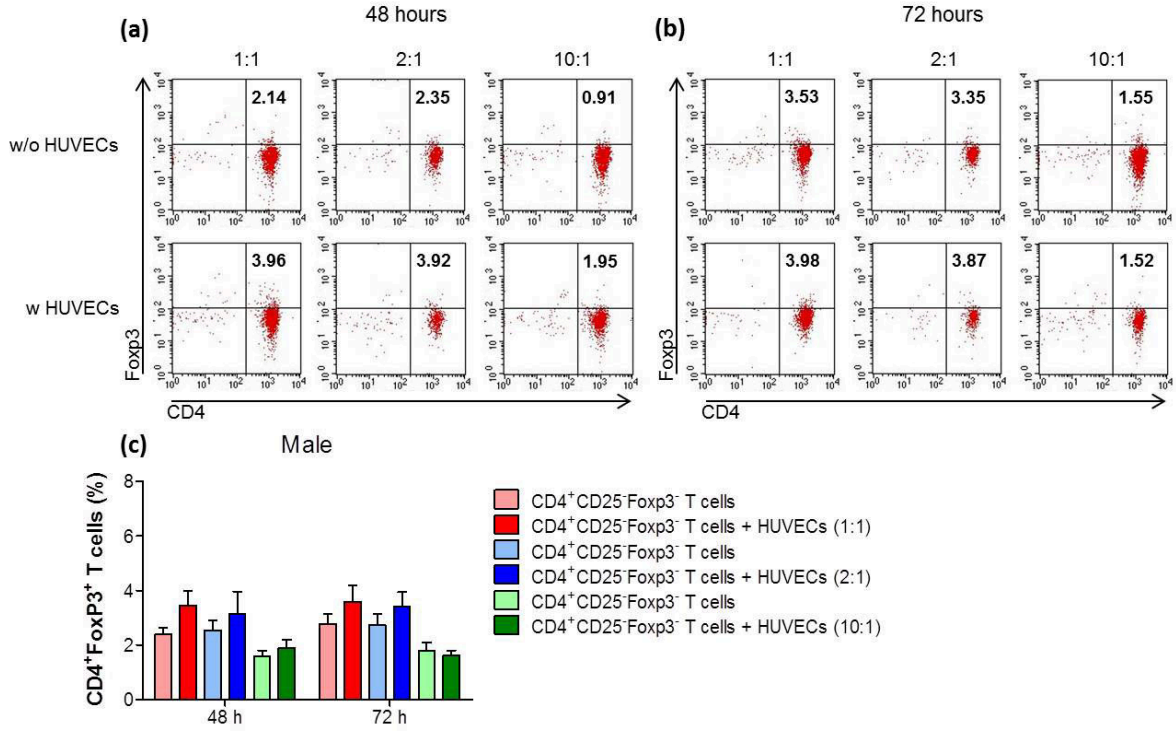
Using a two-chamber trans-well system $CD4^+CD25^-Foxp3^-$ T cells (upper chamber) were separated from female or male HUVECs (lower chamber). After 4, 8, 24 and 48 hours, the number of migrated T cells to the lower chamber was determined. Neither female (a) nor male

(b) HUVECs induced migration of T cells. T cells cultured alone (controls) revealed some spontaneous T cell migration. Samples from two normal pregnant women were analyzed in duplicates. Data are presented as means ± S.E.M.. Statistical analysis among groups was performed using Two-way-ANOVA followed by Bonferroni correction for multiple comparisons.

## Supplementary Figure S1




## Supplementary Figure S2



**Supplementary Figure S3**