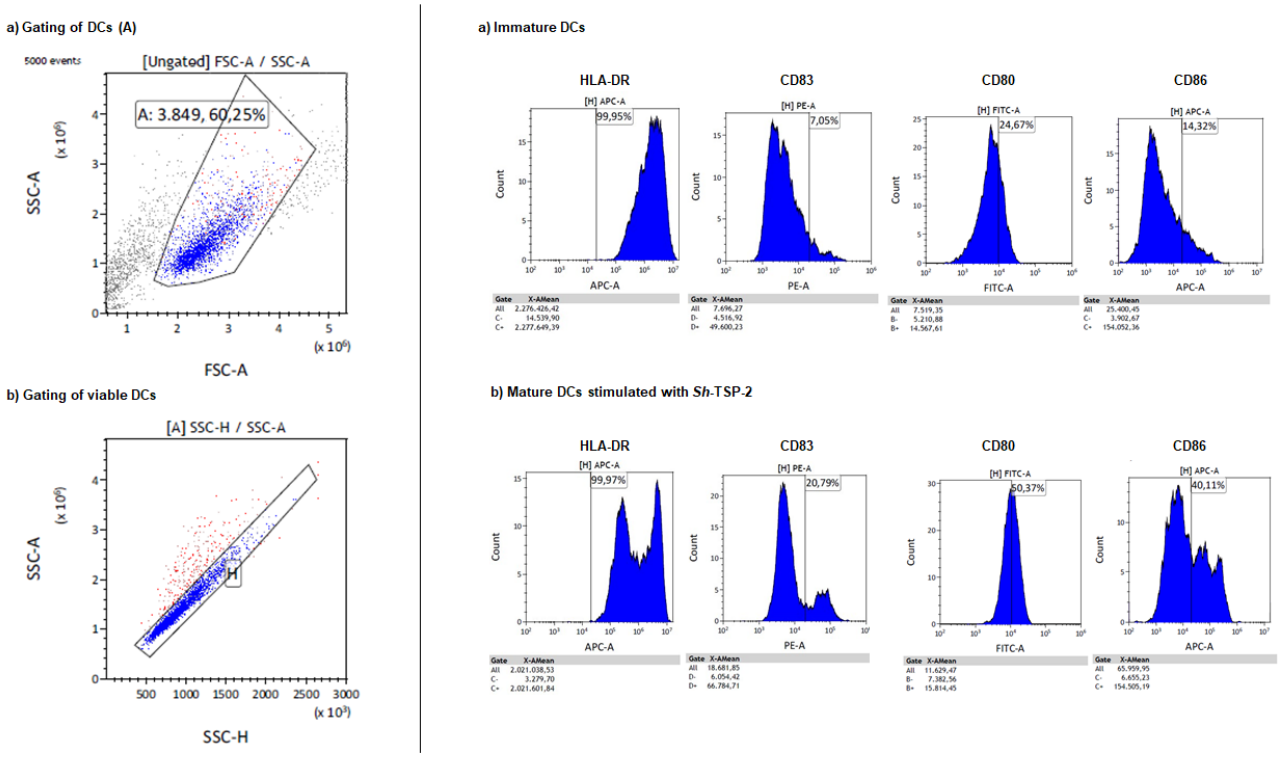
SUPPLEMENTARY INFORMATION

Supplementary methods. Flow cytometry.

Unstained cell samples (blanks) were used to set parameters of the flow cytometer, allowing for the exclusion of nonspecific fluorescent signals. A gate was created based on Forward Scatter-Area (FSC-A) and Side Scatter-Area (SSC-A) parameters to select cells by size and granularity, isolating the dendritic cell (DCs) population. Within this gated population, dead cells and debris were identified and excluded based on Side Scatter-Height (SSC-H) and SSC-A parameters, isolating only viable DCs cells for further analysis. The parameters established with the unstained controls were consistently applied during flow cytometric analysis across all samples, both those stained and unstained with the conjugated antibody. For each sample, the Mean Fluorescence Intensity (MFI) was recorded to quantify the expression levels of the markers (HLA-DR, CD80, CD83, CD86) on DCs. This approach is described in the figure below and ensures standardized, comparable measurements across all samples for accurate analysis.



The left panel shows an example of gating strategy. a) A gate was established based on FSC-A and SSC-A parameters to isolate the DCs population (Gate A). b) Within this gated population, a further gate was established based on SSC-H and SSC-A parameters to isolate viable DCs (Gate H). The right panel shows an example of Mean Fluorescence Intensity (MFI) values of DCs maturation markers. a) MFI of HLA-DR, CD80 CD83 and CD86 markers in immature DCs. b) MFI of HLA-DR, CD80, CD83 and CD86 markers in mature DCs stimulated for 16 hours with 10 µg/ml of *Sh*-TSP-2.

Supplementary Table 1. Primers used in RT quantitative PCR

Gene	Forward 5'-3'	Reverse 5'-3'
IFNγ	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
IL17	AGATTACTACAACCGATCCACCT	GGGGACAGAGTTCATGTGGTA
IL4	ACTTTGAACAGCCTCACAGAG	TTGGAGGCAGCAAAGATGTC
IL5	CCCACAAGTGCATTGGTGAA	CCTCAGAGTCTCATTGGCTATCAG
IL13	TGAGGAGCTGGTCAACATCA	CAGGTTGATGCTCCATACCAT
18S	ATTAAGGGTGTGGGCCGAAG	GGTGATCACACGTTCCACCT
IL10	TCAAGGCGCATGTGAACTCC	GATGTCAAACTCACTCATGGCT

The table shows the forward and reverse primers used in RT quantitative PCR assay to measure the level of expression of target cytokine genes.

Supplementary Table 2. Induction of DCs maturation markers by *S. haematobium* tetraspanins.

Marker	*Sh*-TSP-2				*Sh*-TSP-6				*Sh*-TSP-23			
	MFI	**Fold-change**	**95% CI**	**P**	**MFI**	**Fold-change**	**95% CI**	**P**	**MFI**	**Fold-change**	**95% CI**	**P**
HLA-DR	2743811	1.5	0.9-2.4	0.2307	3412539	1.5	1.4-2.2	0.0798	1752588	1.0	0.7-1.1	0.9265
CD80	10223	2.9	2.0-3.1	0.0100	7499	2.0	1.9-2.4	0.0730	6044	1.5	1.3-1.9	0.3824
CD83	29130	7.3	3.2-16.5	0.0188	13537	2.9	2.7-10.5	0.1070	3470	1.0	0.8-1.8	>0.9999
CD86	47683	1.6	0.9-1.9	0.2681	34435	1.1	1.0-1.6	0.4062	22128	0.7	0.5-1.8	0.9632

The table shows the median expression level (MFI) of maturation markers in DCs induced by *Sh*-TSP-2, *Sh*-TSP-6 and *Sh*-TSP-23, the median fold-change compared to unstimulated cells (Fold-change), together with its 95% confidence interval and the P-value of Kruskall-Wallis test of comparison with unstimulated cells.

Supplementary Table 3. Induction of DCs cytokine production by *S. haematobium* tetraspanins.

Cytokine	*Sh*-TSP-2				*Sh*-TSP-6				*Sh*-TSP-23			
	[pg/ml]	Fold-change	95% CI	P	[pg/ml]	Fold-change	95% CI	P	[pg/ml]	Fold-change	95% CI	P
IL1β	18.2	5.6	4.3-7.0	0.0097	15.6	3.1	2.5-5.1	0.0523	3.4	1.0	0.9-1.5	0.8292
IL6	11600.0	478.4	174.9-1231.0	0.0001	2581.1	121.2	26.4-160.7	0.0222	798.9	18.6	4.5-42.8	0.2439
TNF	8472.8	219.1	71.1-321.6	0.0015	388.8	7.3	7.0-25.0	0.0844	47.4	1.8	0.9-3.2	0.8292
IL12p70	14.0	7.6	4.3-8.5	0.0054	3.7	1.3	1.0-3.1	0.2587	2.8	1.0	0.7-1.9	0.8621
IL23	1560.0	5.0	2.1-9.2	0.0121	420.0	1.5	0.3-2.9	0.7292	230.0	0.9	0.5-1.6	0.681
IL4	1480.0	1.2	0.3-6.0	0.9312	1065.0	0.7	0.3-17.0	0.5603	2040.0	1.1	0.4-10.0	0.7138
IL13	9.1	1.6	1.5-7.0	0.0253	4.4	1.0	0.0-3.1	0.8963	3.2	0.6	0.3-1.0	0.2774
IL10	4998.5	342.0	199.7-486.2	0.0004	175.6	12.2	7.2-24.1	0.0521	24.3	2.4	0.7-3.2	0.5174

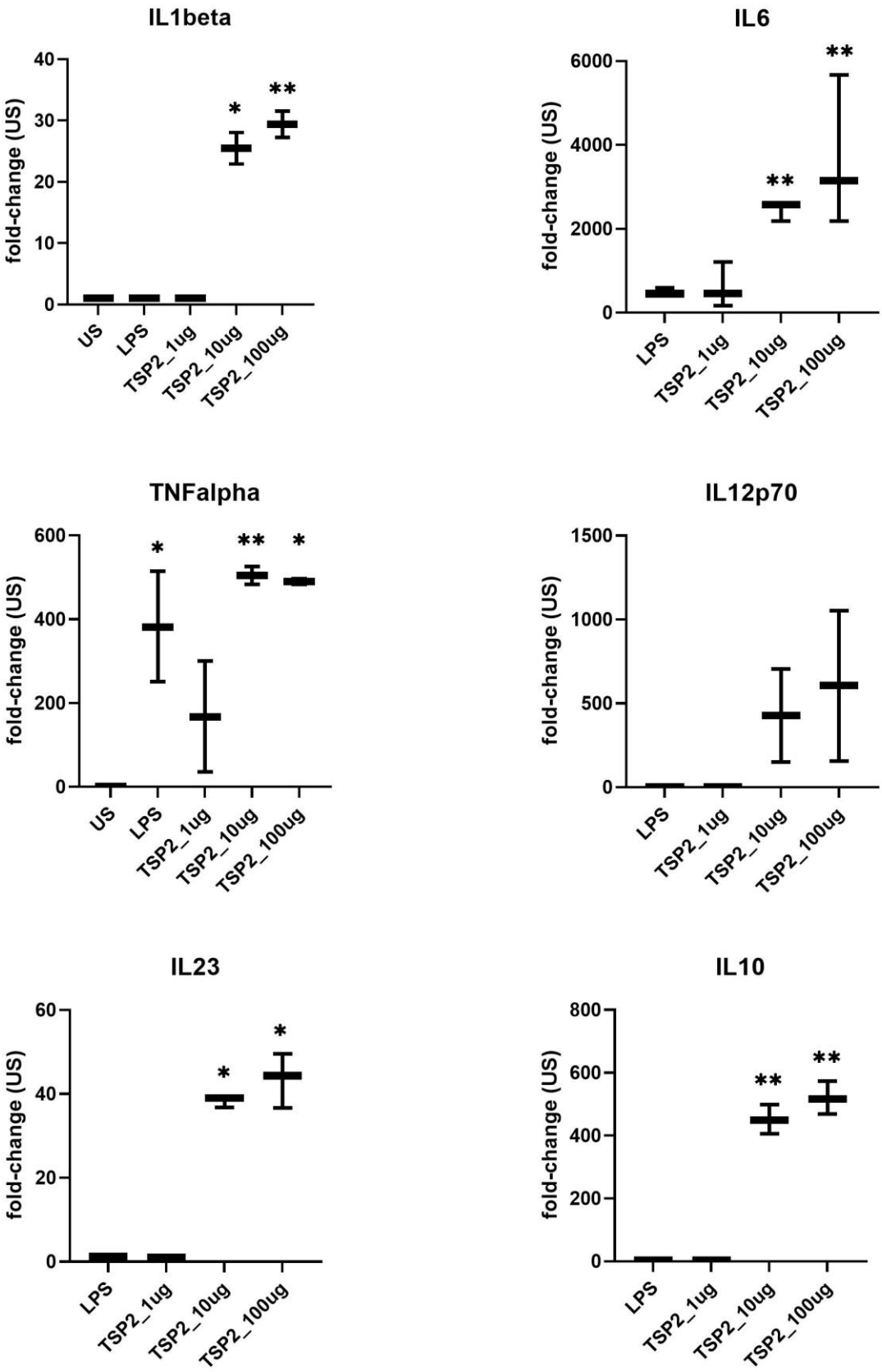
The table shows the median cytokine concentration [pg/ml] in supernatant of DCs induced by *Sh*-TSP-2, *Sh*-TSP-6 and *Sh*-TSP-23, the median fold-change compared to unstimulated cells, together with its 95% confidence interval and the P-value of Kruskall-Wallis test of comparison with unstimulated cells.

Supplementary Table 4. Induction of T CD4+ cells cytokine gene expression by *S. haematobium* tetraspanins.

Cytokine	*Sh*-TSP-2				*Sh*-TSP-6				*Sh*-TSP-23			
	$2^{-\Delta Ct}$	Fold-change	95% CI	P	$2^{-\Delta Ct}$	Fold-change	95% CI	P	$2^{-\Delta Ct}$	Fold-change	95% CI	P
IFNɣ	5.63	2.9	1.7-3.3	0.0303	1.39	0.8	0.2-2.1	>0.9999	4.39	1.6	1.2-2.5	0.2097
IL17	0.37	4.1	0.7-10.4	0.3916	0.37	4.1	0.2-14.7	0.4578	0.28	3.1	0.5-6.1	0.6477
IL4	0.01	1.4	1.4-1.9	0.3022	0.01	2.2	2.1-2.2	0.0391	0.02	3.1	0.7-3.1	0.0855
IL5	0.01	0.3	0.2-2.0	0.9092	0.02	1.8	1.1-3.8	0.1104	0.02	1.9	1.1-4.5	0.0872
IL13	0.03	0.9	0.7-1.4	0.7323	0.17	3.1	2.4-4.6	0.0226	0.07	1.8	1.4-2.7	0.1382
IL10	0.17	1.8	1.4-5.4	0.0401	0.07	1.0	0.5-1.3	0.8196	0.13	1.7	1.0-5.2	0.1104

The table shows the median cytokine gene expression level ($2^{-\Delta Ct}$ X100) in T CD4+ cells induced by *Sh*-TSP-2, *Sh*-TSP-6 and *Sh*-TSP-23, the median fold-change compared to unstimulated cells, together with its 95% confidence interval and the P-value of Kruskall-Wallis test of comparison with unstimulated cells.

Supplementary Figure 1. DCs cytokine production upon stimulation with *Sh*-TSP-2 at different concentrations and in presence of an LPS inhibitor.



The figure shows boxplots (median and 5-95% range) of the fold-change in cytokine concentration in supernatant of DCs stimulated with LPS and *Sh*-TSP-2 at different concentrations (1µg/ml, 10µg/ml, 100µg/ml) in presence of the LPS inhibitor colistin, compared to unstimulated DCs (US). The experiment was conducted with DCs from 3 donors.

The analysis was restricted to inflammatory (IL1β, IL6, TNF), Th1 (IL12p70), Th17 (IL23) and regulatory (IL10) cytokines for which a relevant (fold-change>2) and significant (p-value<0.05) increase in concentration was observed during the first experiment.

Asterisks indicated statistical significance of Kruskal Wallis rank test (* p-value≤0.05, ** p-value≤0.01, *** p-value≤0.001).

As shown in the figure, LPS did not induce an increase in cytokine production in presence of colistin. An exception to this observation was shown by TNF, probably as a result of experimental variation. On the contrary, *Sh*-TSP-2 at a 10µg/ml concentration did induce an increase in cytokine production even in presence of colistin, with no further increase at the higher 100µg/ml concentration, confirming previous results. Such increase was significant with the exception of IL12, likely because of the reduced number of donors and inter-donor variation.