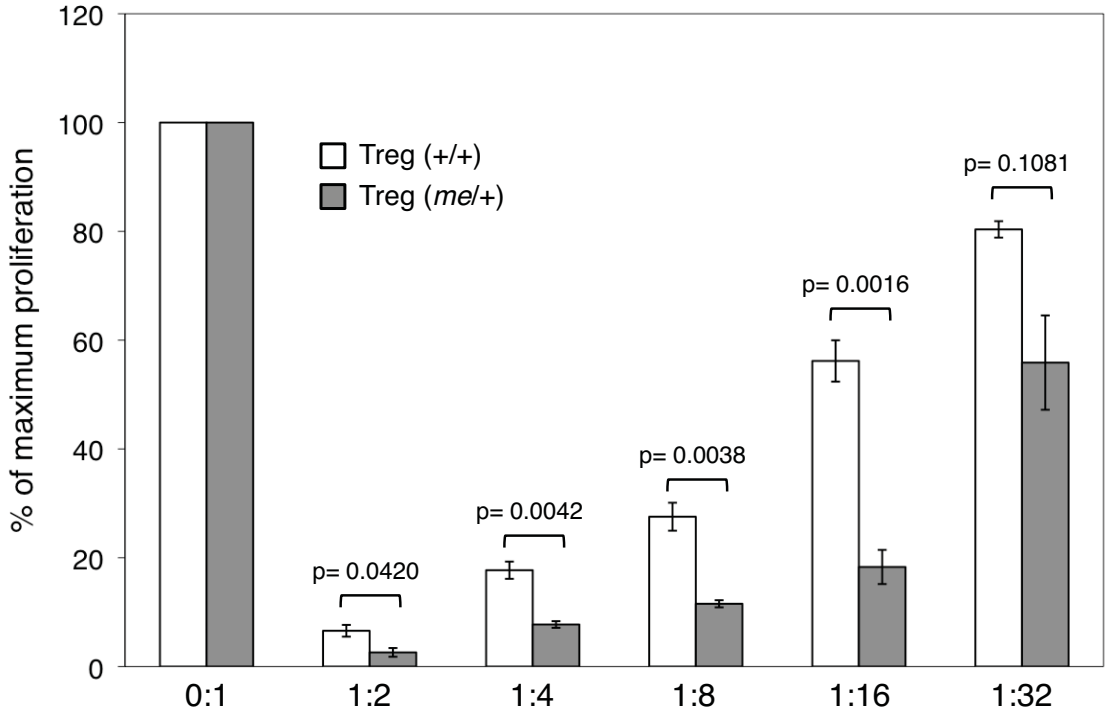
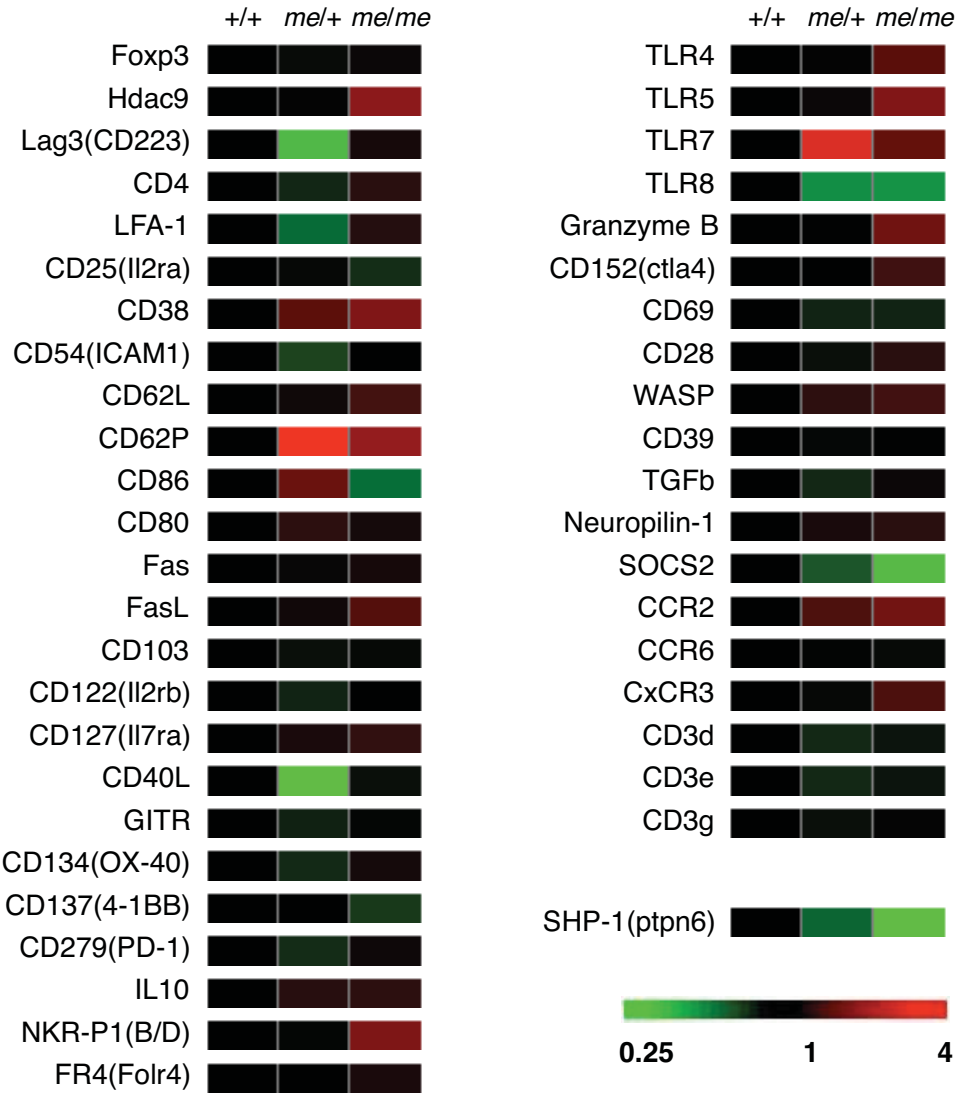
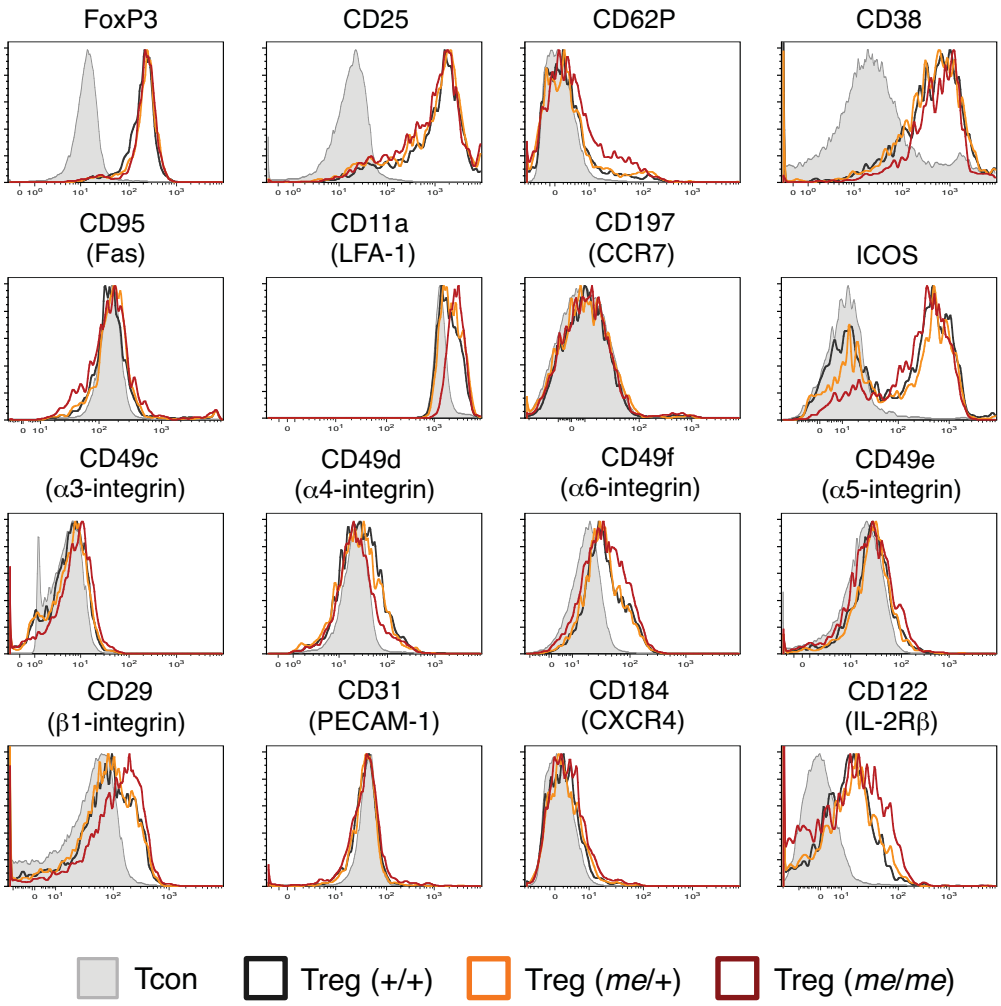


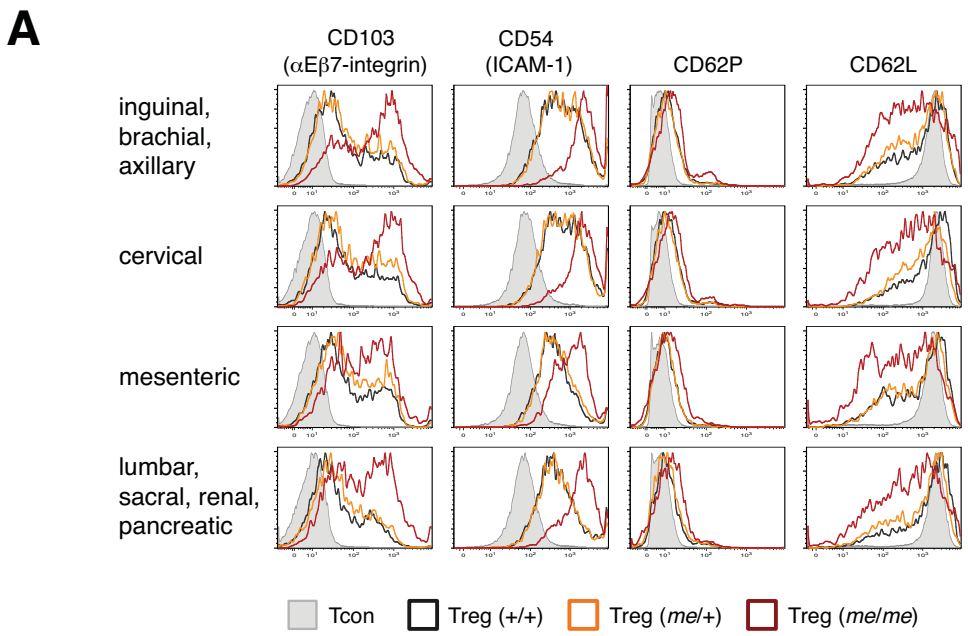
Supplementary Figure 1: Treg cells from adult SHP-1-deficient mice maintain the increased suppressive potential compared to wild type Treg cells. CD4+CD25+ T cells were purified from lymph nodes of 8 wks old +/+ and *me*/+ DO11.10 TCR-Tg mice and added to $2.5x10^4$ CD4+CD25- T cells from +/+ DO11.10 mice at the indicated ratios. Proliferation was measured by ^{3}H-thymidine incorporation in response to 125 ng/ml of OVA peptide. Proliferation of CD4+CD25- cells in the absence of Treg cells is set as 100%. Results shown are an average of three independent experiments with 1-3 mice each. Error bars indicate ±SEM.

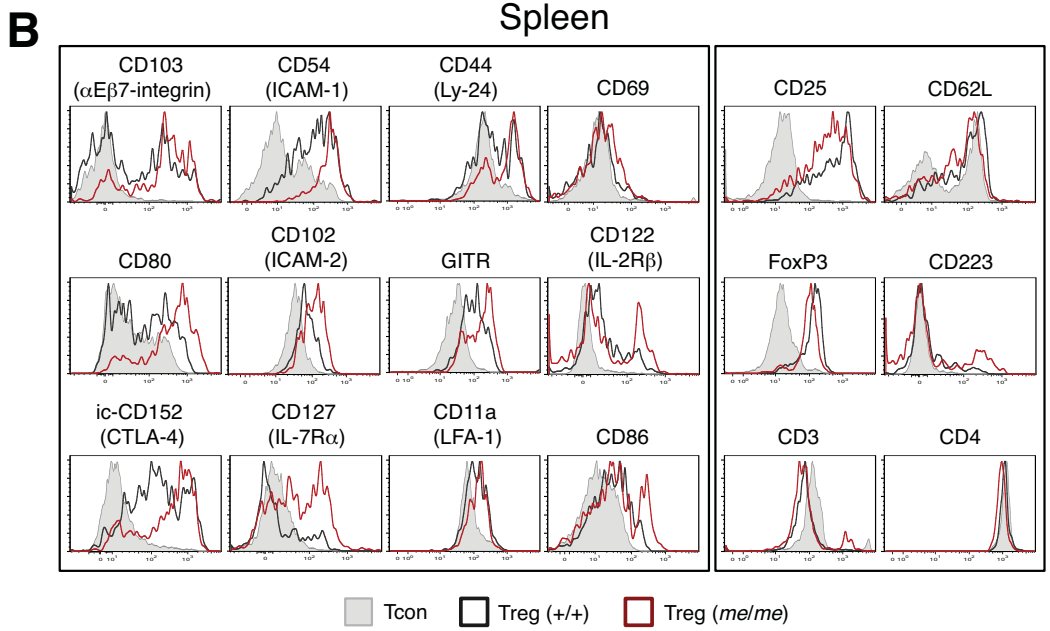


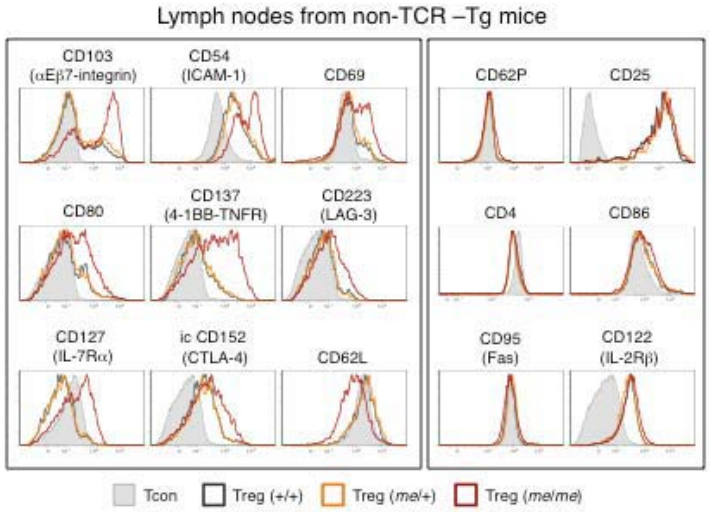
Supplementary Figure 2: +/+, *me*/+ and *me/me* Treg cells do not show significant differences in their gene expression profiles. Total RNA was isolated from freshly isolated CD4+CD25+ regulatory T cells from +/+, *me*/+ and *me/me* mice using Picopure RNA isolation kit (Molecular Devices, Sunnyvale, CA) and amplified with RiboAmp amplification kit (Molecular Devices, Sunnyvale, CA). For each group, Treg cells from three individual mice were processed separately. The Microarray was performed using Affymetrix GeneChip Mouse expression set 430 2.0 arrays at the Biomolecular research facility at the University of Virginia. The data were analyzed using the GeneSifter software for genetic analysis (Geospiza, Seattle, WA).
The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar, R., Domrachev, M., and Lash, A.E. 2002. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 30:207-210) and are accessible through GEO Series accession number GSE23600 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23600).



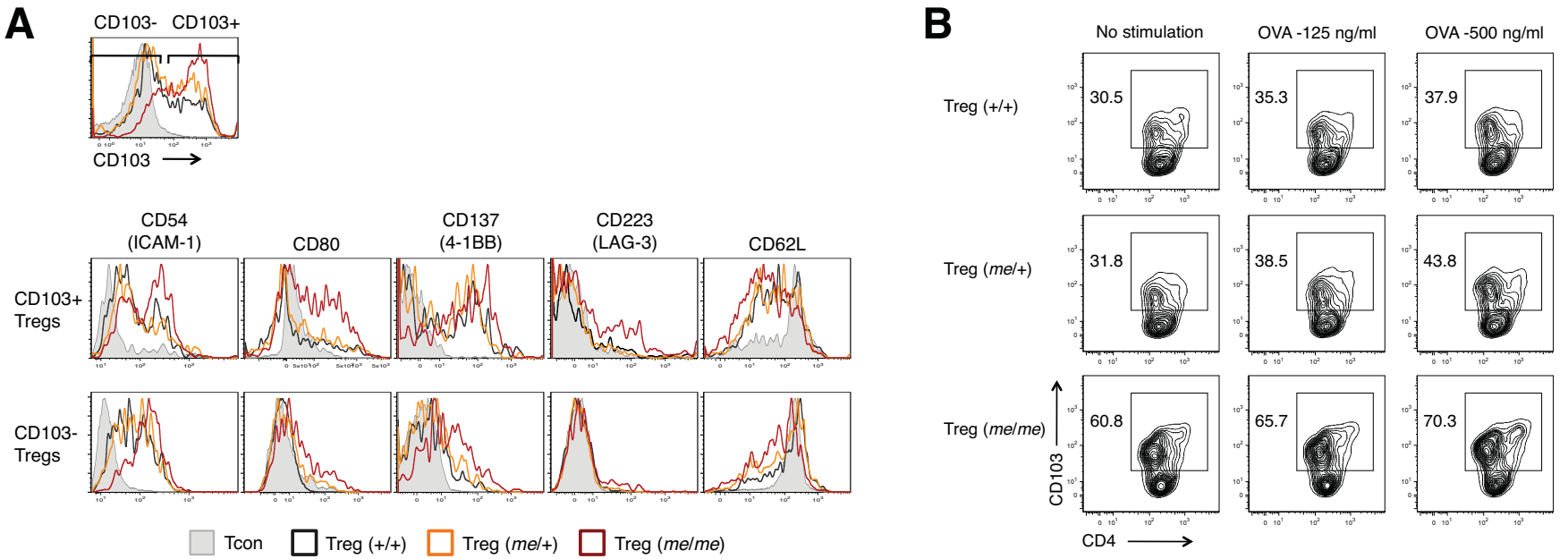
Supplementary Figure 3: Comparison of surface marker expression profiles.
$CD4^+$ T cells were isolated from lymph nodes of +/+, *me*/+ and *me*/*me* mice (DO11.10 TCR-Tg) and prepared for flow cytometric analyses. Stained cells were collected on a FACS caliber instrument and analyzed using FlowJo software. Gated populations of live $CD4^+CD25^-$ and $CD4^+CD25^+$ cells were analyzed for the expression of surface or intracellular proteins. Shaded histograms represent $CD4^+CD25^-$ T cells (Tcon) derived from +/+ mice.







Supplementary Figure 4: SHP-1 deficiency causes a more activated phenotype of Treg cells independent of the origin of Treg cells. (**A-C**) CD4+ T cells were isolated from indicated lymph nodes (**A**), spleen (**B**) or combined lymph nodes (**C**) of +/+, *me*/+ and *me*/*me* mice (**A** and **B** DO11.10 TCR-Tg; **C** non-TCR Tg) and prepared for flow cytometric analyses. Stained cells were collected on a FACS caliber instrument and analyzed using FlowJo software. Gated populations of live $CD4^+CD25^-$ and $CD4^+CD25^+$ cells were analyzed for the expression of surface or intracellular proteins. Shaded histograms represent $CD4^+CD25^-$ T cells (Tcon) derived from +/+ mice.



Supplementary Figure 5: *me/me* Treg cell population is enriched for CD103+ subpopulation. (**A**) Expression of surface markers in CD103+ and CD103- Treg cell subpopulations. CD4+ T cells were isolated from lymph nodes of +/+, *me*/+ and *me*/*me* mice and prepared for flow cytometric analyses. Stained cells were collected on a FACS Calibur instrument and analyzed using FlowJo software. Live $CD4^{+}CD25^{+}$ cells were gated based on the expression of CD103 followed by an analysis of the surface expression of indicated proteins. Shaded histograms represent $CD4^{+}CD25^{-}$ T cells (Tcon) derived from +/+ mice
(**B**) TCR stimulation of Treg cells increases surface expression of CD103. To assess whether the differences in CD103 expression between +/+, *me*/+, and *me*/*me* Treg cell populations are due to heightened sensitivity to TCR stimulation in the absence of SHP-1, Treg cells ($2x10^{5}$) isolated from +/+, *me*/+ and *me*/*me* mice were incubated with APCs (T-cell depleted splenocytes) and indicated amounts of OVA peptide for 20 h followed by staining for CD103, CD4 and CD25 and flow cytometric analyses. Percentages of CD103+ Treg cells are indicated. All Treg cells responded to peptide stimulation with a comparable increase in the percentage of CD103+ Treg cells. Moreover, the fraction of CD103+ Treg cells within the *me*/*me* Treg population was consistently higher than the percentage of CD103+ Treg cells within the +/+ Treg population indicating that increased sensitivity to TCR stimulation is not the cause of the enriched CD103+ Treg cell population observed in *me*/*me* mice. Control cells were cultured in the absence of OVA peptide for 20 h. Recombinant mIL-2 was added to all wells at a concentration of 30 U/ml.

	BMDC-Treg conjugates					
	[%]	Conjugate composition	Adjusted [%]	[%]	Conjugate composition	Adjusted [%]
	0 ng OVA peptide			100 ng OVA peptide		
Treg (+/+)	12.9	1 – 55.3 2 – 44.7 ≥3 – 0	18	26.8	1 – 49.4 2 – 42.3 ≥3 – 7.1	36
Treg (me/+)	22.8	1 – 59.5 2 – 36.5 ≥3 – 4.0	30.0	32.8	1 – 64.3 2 – 23.8 ≥3 – 10.7	42
Treg (me/me)	23.9	1 – 53.3 2 – 38.0 ≥3 – 7.6	33	35.3	1 – 49.2 2 – 39.5 ≥3 – 12.1	47
	0 ng OVA peptide			500 ng OVA peptide		
Treg (+/+)	18.4	1 – 54.1 2 – 37.6 ≥3 – 6.4	25	33.8	1 – 52.8 2 – 33.3 ≥3 – 13.4	45
Treg (me/+)	21.4	1 – 58.0 2 – 33.1 ≥3 – 10.2	30	44.1	1 – 50.2 2 – 36.0 ≥3 – 15.1	57
Treg (me/me)	20.6	1 – 48.2 2 – 39.7 ≥3 – 12.1	30	47.6	1 – 47.4 2 – 36.0 ≥3 – 17.8	61

Supplementary Figure 6: SHP-1 inhibits the conjugate formation between Treg cells and BMDCs. Data were obtained using the ImageStream[100] approach followed by data analysis with IDEAS™ analytical software, as presented in Figures 6 B and C. Concentrations of OVA peptide added in individual experiments are indicated. [%] indicates percentage of Treg cells found conjugated with BMDCs. "Conjugate composition" provides details of the number of Treg cells (1, 2, or ≥ 3) found conjugated per individual BMDC. This information was used to calculate the "adjusted [%]" using the following algorithm:

$$y = \frac{1 \times \%^a + 2 \times \%^b + 3 \times \%^c}{\%^a + \%^b + \%^c} \times \frac{\text{total \%}}{100}$$

$$\text{adjusted \%} = \frac{y}{100\text{-[total \%]} + y}$$

with $\%^a$,$\%^b$, and $\%^c$ being the respective percentages of 1, 2, ≥3 conjugates obtained from the analysis, as shown in the table under "conjugate composition", and total % being the sum of $\%^a + \%^b + \%^c$,

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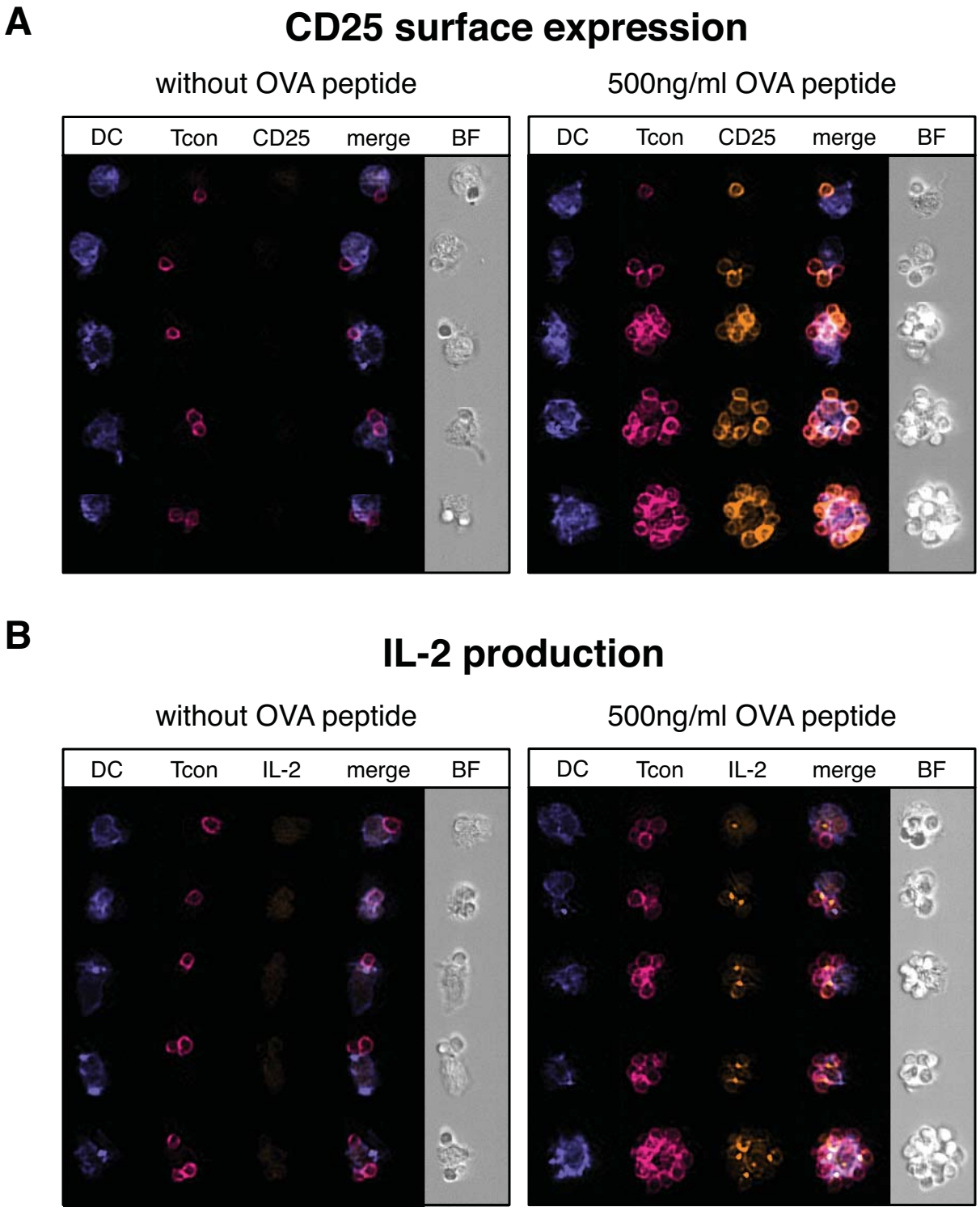

Supplementary Figure 7: Tcon cells conjugated to APCs express CD25 and IL-2 in an antigen-dependent manner.

(**A**) CD25 surface expression on Tcon cells conjugated to BMDCs without peptide (left) and with OVA peptide (right) (**B**) IL-2 expression in Tcon cells conjugated to BMDCs without peptide (left) and with 500 ng/ml OVA peptide (right).

	Tcon-BMDCs conjugates			Within Tcon-BMDCs conjugates						Tcon conjugated with BMDC w/o Treg [%]
	[%]	Conjugate composition	Adjusted [%]	Treg containing [%]	Conjugate composition	Adjusted [%]	no Treg [%]	Conjugate composition	Adjusted [%]	
100 ng OVA peptide										
Tcon *alone*	**16.9**	1 – 61.5 2 – 21.5 ≥3 – 13.1	**23.3**	**----**	**----**	**----**	**100**		**100**	**23.3**
Tcon plus **Treg (+/+)**	**9.15**	1 – 72.1 2 – 16.4 ≥3 – 11.5	**12.3**	**29.5**	1 – 55.6 2 – 26.7 ≥3 – 17.8	**33.5**	**70.5**	1 – 79.1 2 – 7.0 ≥3 – 14.0	**66.5**	**8.2**
Tcon plus **Treg (me/+)**	**7.9**	1 – 70.0 2 – 12.0 ≥3 – 16.0	**11.1**	**40.0**	1 – 70.0 2 – 20.0 ≥3 – 10.0	**42**	**58.0**	1 – 75.9 2 – 13.8 ≥3 – 10.3	**58**	**6.4**
Tcon plus **Treg (me/me)**	**8.0**	1 – 73.3 2 – 15.0 ≥3 – 8.3	**10.3**	**51.7**	1 – 64.5 2 – 22.6 ≥3 – 9.7	**55**	**48.3**	1 – 82.8 2 – 6.9 ≥3 – 6.9	**45**	**4.6**
500 ng OVA Peptide										
Tcon alone	**66.3**	1 – 71.4 2 – 18.4 ≥3 – 9.2	**73**	**----**	----	**----**	**100**		**100**	**73**
Tcon plus **Treg (+/+)**	**63.3**	1 – 64.9 2 – 17.5 ≥3 – 14.5	**72**	**47.2**	1 – 60.9 2 – 17.3 ≥3 – 16.4	**49.5**	**51.5**	1 – 68.3 2 – 17.5 ≥3 – 12.5	**50.5**	**36**
Tcon plus **Treg (me/+)**	**56.8**	1 – 68.8 2 – 14.4 ≥3 – 10.1	**64**	**53.6**	1 – 58.8 2 – 17.6 ≥3 – 14.3	**58.7**	**45.9**	1 – 79.4 2 – 11.8 ≥3 – 5.88	**41.3**	**26**
Tcon plus **Treg (me/me)**	**55.4**	1 – 61.3 2 – 20.4 ≥3 – 14.7	**65**	**67.2**	1 – 58.9 2 – 22.0 ≥3 – 16.1	**72**	**30.8**	1 – 71.4 2 – 15.6 ≥3 – 7.8	**28**	**18**

Supplementary Figure 8: SHP-1-deficient Treg cells are more efficient in preventing the formation of exclusive Tcon/BMDC conjugates. Data were obtained using the ImageStream^{100} approach followed by data analysis with IDEAS™ analytical software, as presented in Figure 8. Concentrations of OVA peptide added in individual experiments are indicated.

Left panel of table: Tcon-BMDC conjugates - [%] indicates percentage of Tcon cells found conjugated with BMDCs. "Conjugate composition" provides details of the number of Tcon cells (1, 2, or ≥ 3) found conjugated per individual BMDC. This information was used to calculate the "adjusted [%]" using the same algorithm as in Supplementary Figure 6.

Middle panel of table: Tcon-BMDC conjugates were further separated based on the presence of Treg cells in the conjugate. [%] indicates percentage of Tcon-BMDC conjugates that also contain Treg cells. "Conjugate composition" provides details of the number of Tcon cells (1, 2, or ≥ 3) found conjugated per individual conjugate. This information was used to calculate the "adjusted [%]" using a modification of the algorithm used in Supplementary Figure 6. : $\mathbf{y_{with\ Treg} = [(1x\%^a + 2x\%^b + 3x\%^c) / (\%^a + \%^b + \%^c)] \times [total\ \%] / 100}$ and $\mathbf{adjusted\ [\%] = y_{with\ Treg} / (y_{with\ Treg} + y_{without\ Treg})}$ with $y_{without\ Treg}$ being the value obtained from calculating the number of Tcon cells found in conjugates without Treg cells (see below).

Right panel of table: [%] indicates percentage of Tcon-BMDC conjugates that do not contain Treg cells. "Conjugate composition" provides details of the number of Tcon cells (1, 2, or ≥ 3) found conjugated per individual conjugate. This information was used to calculate the "adjusted [%]" using a modification of the algorithm used in Supplementary Figure 6. : $\mathbf{y_{without\ Treg} = [(1x\%^a + 2x\%^b + 3x\%^c) / (\%^a + \%^b + \%^c)] \times [\%] / 100}$ and $\mathbf{adjusted\ [\%] = y_{without\ Treg} / (y_{with\ Treg} + y_{without\ Treg})}$.

Right column of table: This "adjusted [%]" for 'Tcon-BMDC conjugates without Treg cells' was then multiplied with the original "adjusted [%]" in the left panel, which represents the % of input Tcon cells conjugated with BMDCs, to calculate the % of input Tcon cells that are conjugated to BMDCs without Treg cells in the conjugates. This represents the percentage of input Tcon cells that are potentially able to respond to antigen stimulation via up-regulation of CD25 and IL-2 production.