Supplemental Data

Supplemental Tables

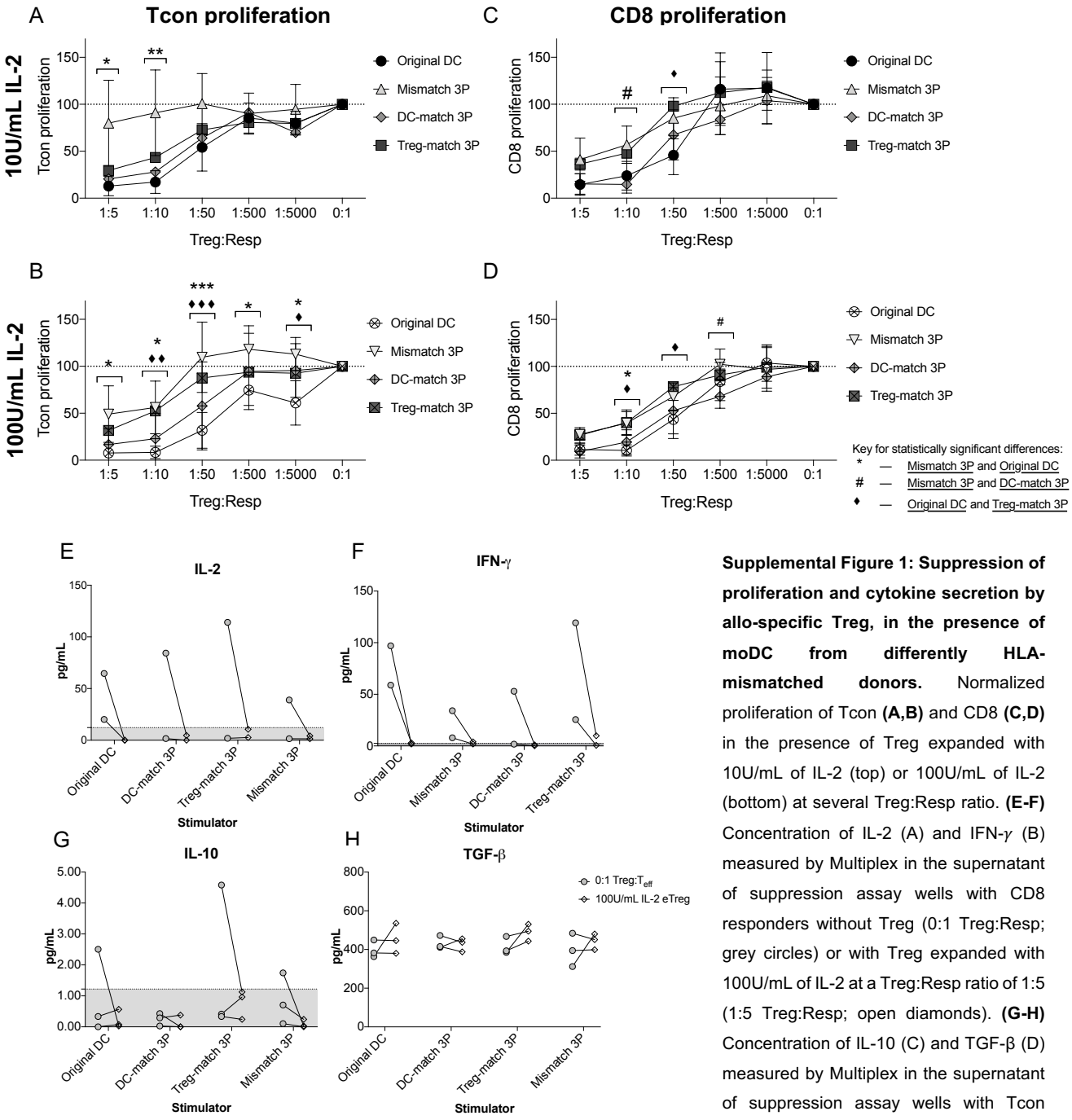
Table S1*. High-resolution HLA sequencing of donors used for experiments described in Figure 1, 2, 3, 4 and 6.*

EXP.1	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQA1	HLA-DQB1
Treg donor	A*02:01 A*23:01	B*18:01 B*49:01	C*07:01 C*07:01	DRB1*04:03 DRB1*04:05	DQA1*03:01 DQA1*03:03	DQB1*03:02 DQB1*03:02
Original DC donor	A*23:01 A*25:01	B*18:01 B*44:03	C*04:01 C*12:03	DRB1*04:01 DRB1*07:01	DQA1*02:01 DQA1*03:01	DQB1*02:02 DQB1*03:02
Mismatch 3P donor	A*32:01 A*33:01	B*14:02 B*14:02	C*08:02 C*15:05	DRB1*13:01 DRB1*16:01	DQA1*01:02 DQA1*01:03	DQB1*05:02 DQB1*06:03
EXP.2	**HLA-A**	**HLA-B**	**HLA-C**	**HLA-DRB1**	**HLA-DQA1**	**HLA-DQB1**
Treg donor	A*03:01 A*29:01	B*15:01 B*44:03	C*04:01 C*16:01	DRB1*07:01 DRB1*08:01	DQA1*02:01 DQA1*04:01	DQB1*04:02 DQB1*04:02
Original DC donor	A*23:01 A*25:01	B*18:01 B*44:03	C*04:01 C*12:03	DRB1*04:01 DRB1*07:01	DQA1*02:01 DQA1*03:01	DQB1*02:02 DQB1*03:02
Mismatch 3P donor	A*02:01 A*68:01	B*37:01 B*52:01	C*06:02 C*12:02	DRB1*13:03 DRB1*15:02	DQA1*01:03 DQA1*05:05	DQB1*06:01 DQB1*06:01
DC-match 3P donor	A*02:01 A*23:01	B*18:01 B*49:01	C*07:01 C*07:01	DRB1*04:03 DRB1*04:05	DQA1*03:01 DQA1*03:03	DQB1*03:02 DQB1*03:02
Treg-match 3P donor	A*02:01 A*02:01	B*07:02 B*07:02	C*07:02 C*07:02	DRB1*08:01 DRB1*15:01	DQA1*01:02 DQA1*04:01	DQB1*04:02 DQB1*04:02
EXP.3	**HLA-A**	**HLA-B**	**HLA-C**	**HLA-DRB1**	**HLA-DQA1**	**HLA-DQB1**
Treg donor	A*02:01 A*68:01	B*37:01 B*52:01	C*06:02 C*12:02	DRB1*13:03 DRB1*15:02	DQA1*01:03 DQA1*05:05	DQB1*06:01 DQB1*06:01
Original DC donor	A*02:05 A*30:02	B*18:01 B*50:01	C*05:01 C*06:02	DRB1*07:01 DRB1*13:01	DQA1*01:03 DQA1*02:01	DQB1*02:01 DQB1*06:03
Mismatch 3P donor	A*01:01 A*33:01	B*08:01 B*14:02	C*07:01 C*08:02	DRB1*01:02 DRB1*03:01	DQA1*01:01 DQA1*05:01	DQB1*05:01 DQB1*05:01
DC-match 3P donor	A*23:01 A*25:01	B*18:01 B*44:03	C*04:01 C*12:03	DRB1*04:01 DRB1*07:01	DQA1*02:01 DQA1*03:01	DQB1*02:02 DQB1*03:02
Treg-match 3P donor	A*29:01 A*29:01	B*51:01 B*51:01	C*02:02 C*14:02	DRB1*01:01 DRB1*11:03	DQA1*01:01 DQA1*05:05	DQB1*05:01 DQB1*06:01
EXP.4	**HLA-A**	**HLA-B**	**HLA-C**	**HLA-DRB1**	**HLA-DQA1**	**HLA-DQB1**
Treg donor	A*11:01 A*29:01	B*44:03 B*52:01	C*12:02 C*16:01	DRB1*07:01 DRB1*15:02	DQA1*01:03 DQA1*02:01	DQB1*02:02 DQB1*06:01
Original DC donor	A*29:01 A*29:01	B*51:01 B*51:01	C*02:02 C*14:02	DRB1*01:01 DRB1*11:03	DQA1*01:01 DQA1*05:05	DQB1*05:01 DQB1*06:01
Mismatch 3P donor	A*02:01 A*02:01	B*07:02 B*07:02	C*07:02 C*07:02	DRB1*08:01 DRB1*15:01	DQA1*01:02 DQA1*04:01	DQB1*04:02 DQB1*04:02
DC-match 3P donor	A*01:01 A*33:01	B*08:01 B*14:02	C*07:01 C*08:02	DRB1*01:02 DRB1*03:01	DQA1*01:01 DQA1*05:01	DQB1*05:01 DQB1*05:01
Treg-match 3P donor	A*02:05 A*30:02	B*18:01 B*50:01	C*05:01 C*06:02	DRB1*07:01 DRB1*13:01	DQA1*01:03 DQA1*02:01	DQB1*02:01 DQB1*06:03

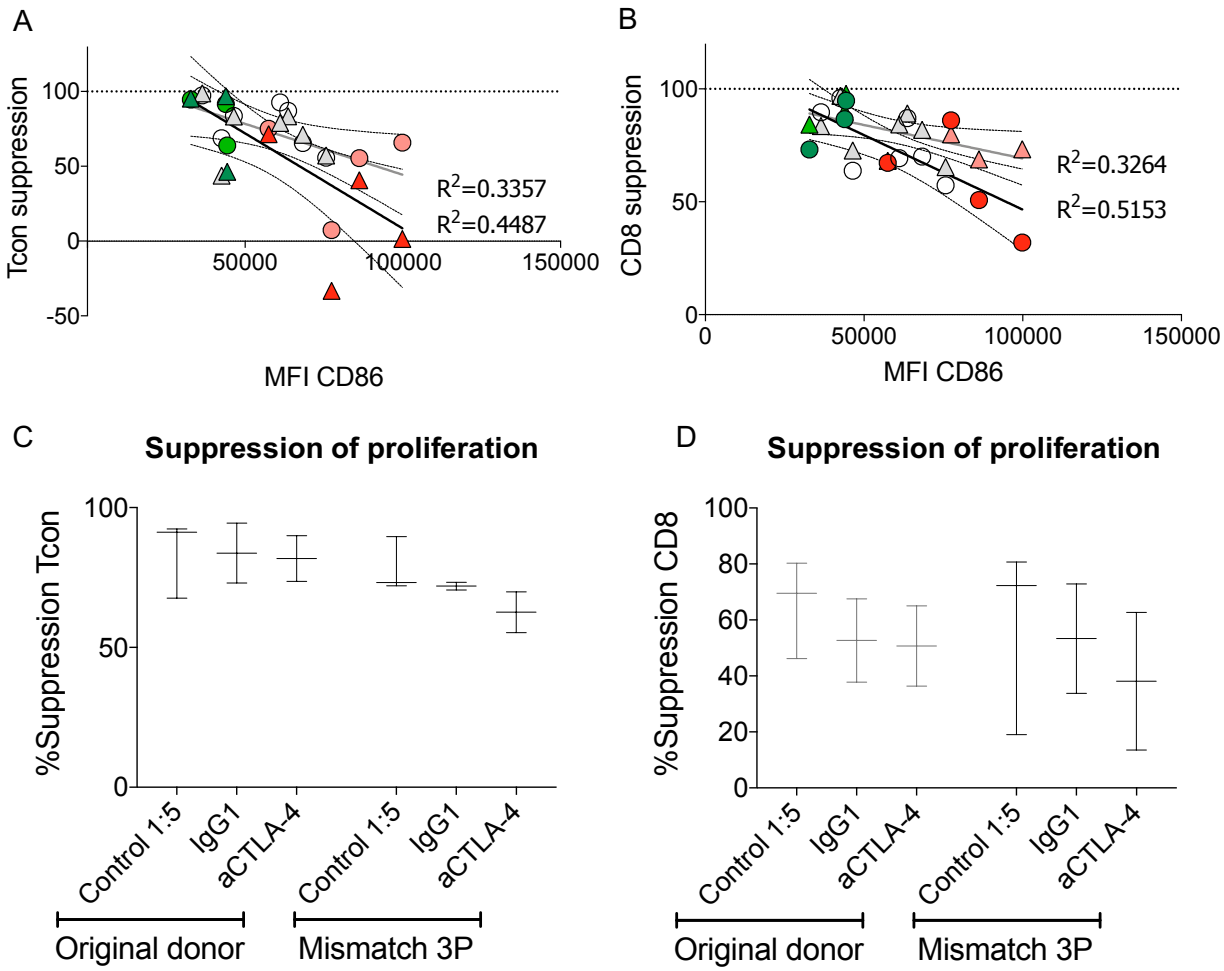
Table S2. High-resolution HLA sequencing of donors used for experiments described in Figure 5.

EXP.1	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQA1	HLA-DQB1
Treg donor	A*01:01 A*23:01	B*08:01 B*43:01	C*04:01 C*07:01	DRB1*03:01 DRB1*07:01	DQA1*02:01 DQA1*05:01	DQB1*02:01 DQB1*02:02
Original DC donor	A*30:01 A*30:02	B*42:01 B*58:01	C*07:01 C*17:03	DRB1*07:01 DRB1*09:01	DQA1*02:01 DQA1*03:02	DQB1*02:02 DQB1*02:02
Mismatch 3P donor	A*02:01 A*32:01	B*40:02 B*44:02	C*02:02 C*02:02	DRB1*13:01 DRB1*16:01	DQA1*01:02 DQA1*01:03	DQB1*05:02 DQB1*06:03
EXP.2	**HLA-A**	**HLA-B**	**HLA-C**	**HLA-DRB1**	**HLA-DQA1**	**HLA-DQB1**
Treg donor	A*30:01 A*30:02	B*42:01 B*58:01	C*07:01 C*17:03	DRB1*07:01 DRB1*09:01	DQA1*02:01 DQA1*03:02	DQB1*02:02 DQB1*02:02
Original DC donor	A*01:01 A*23:01	B*08:01 B*43:01	C*04:01 C*07:01	DRB1*03:01 DRB1*07:01	DQA1*02:01 DQA1*05:01	DQB1*02:01 DQB1*02:02
Mismatch 3P donor	A*11:01 A*24:02	B*15:01 B*37:01	C*03:03 C*06:02	DRB1*11:03 DRB1*13:01	DQA1*01:03 DQA1*05:05	DQB1*03:01 DQB1*06:03
EXP.3	**HLA-A**	**HLA-B**	**HLA-C**	**HLA-DRB1**	**HLA-DQA1**	**HLA-DQB1**
Treg donor	A*02:01 A*03:01	B*35:01 B*44:02	C*04:01 C*07:04	DRB1*01:01 DRB1*11:01	DQA1*01:01 DQA1*05:05	DQB1*03:01 DQB1*05:01
Original DC donor	A*11:01 A*24:02	B*15:01 B*37:01	C*03:03 C*06:02	DRB1*11:03 DRB1*13:01	DQA1*01:03 DQA1*05:05	DQB1*03:01 DQB1*06:03
Mismatch 3P donor	A*30:01 A*30:02	B*42:01 B*58:01	C*07:01 C*17:03	DRB1*07:01 DRB1*09:01	DQA1*02:01 DQA1*03:02	DQB1*02:02 DQB1*02:02
EXP.4	**HLA-A**	**HLA-B**	**HLA-C**	**HLA-DRB1**	**HLA-DQA1**	**HLA-DQB1**
Treg donor	A*11:01 A*24:02	B*15:01 B*37:01	C*03:03 C*06:02	DRB1*11:03 DRB1*13:01	DQA1*01:03 DQA1*05:05	DQB1*03:01 DQB1*06:03
Original DC donor	A*02:01 A*03:01	B*35:01 B*44:02	C*04:01 C*07:04	DRB1*01:01 DRB1*11:01	DQA1*01:01 DQA1*05:05	DQB1*03:01 DQB1*05:01
Mismatch 3P donor	A*01:01 A*02:01	B*51:01 B*73:01	C*15:02 C*15:02	DRB1*04:02 DRB1*04:05	DQA1*03:01 DQA1*03:01	DQB1*02:02 DQB1*02:02

Supplemental Figures



Supplemental Figure 1: Suppression of proliferation and cytokine secretion by allo-specific Treg, in the presence of moDC from differently HLA-mismatched donors. Normalized proliferation of Tcon **(A,B)** and CD8 **(C,D)** in the presence of Treg expanded with 10U/mL of IL-2 (top) or 100U/mL of IL-2 (bottom) at several Treg:Resp ratio. **(E-F)** Concentration of IL-2 (A) and IFN-γ (B) measured by Multiplex in the supernatant of suppression assay wells with CD8 responders without Treg (0:1 Treg:Resp; grey circles) or with Treg expanded with 100U/mL of IL-2 at a Treg:Resp ratio of 1:5 (1:5 Treg:Resp; open diamonds). **(G-H)** Concentration of IL-10 (C) and TGF-β (D) measured by Multiplex in the supernatant of suppression assay wells with Tcon responders without Treg (0:1 Treg:Resp; grey circles) or with Treg expanded with 100U/mL of IL-2 at a Treg:Resp ratio of 1:5 (1:5 Treg:Resp; open diamonds). As stimulators, we used moDC from the donor used in Treg expansion (Original DC donor; black circles), moDC from a 3rd party donor with partial HLA-matches to the Original DC donor (DC-match 3P; grey diamonds), moDC from a 3rd party donor with partial HLA-matches to the Treg donor (Treg-match 3P; dark grey squares) or moDC from a 3rd party donor completely mismatched to the Treg and to the Original DC donors (Mismatch 3P; grey triangles). Responder proliferation was measured by CTV dilution and normalized to control wells without Treg (0:1 Treg:Resp). *Samples were acquired on BD LSRFortessa™ X-20 and analysed using FlowJo. Data was obtained from 2-4 independent experiments. Statistical analysis of differences in responder proliferation with different stimulators were evaluated with multiple t-tests, by the Holm-Sidak method. Statistical significance was assumed when p<0.05.*



Supplemental Figure 2: Relation between CD86 expression on DC (A-B) and CTLA-4 (C-D) on the potency of suppression by Treg. (A-B) Correlation between CD86 MFI on DC stimulators and suppression of Tcon (A) and CD8 responders (B). The x axis shows the range of MFI, and y axis the **n**ormalized suppression detected after suppression assay with each DC. Triangles represent suppression by Treg expanded with 10U/mL of IL-2 and circles represent suppression by Treg expanded with 100U/mL of IL-2. Lines show linear regression if these data, with error bar and associated R^2. **(C-D) Effect of CTLA-4 blockade on Treg suppression.** Donor-specific Treg expanded with 100U/mL were cultured with Tcon (C) or CD8 (D) responders at a Treg:Resp ratio of 1:5 in the presence of aCTLA-4 or its isotype control (IgG1). As stimulators, we used moDC from the donor used in Treg expansion (Original DC donor), or moDC from a 3rd party donor completely mismatched to the Treg and to the Original DC donors (Mismatch 3P). Proliferation of responders was measured by CTV dilution, normalized to the proliferation of responders in controls wells without Treg, and suppression was calculated as the inverse of proliferation. *Samples were acquired on BD LSRFortessa™ X-20 and analysed using FlowJo. Data was obtained from 2 independent experiments. Statistical analysis of differences in responder proliferation or with different stimulators were evaluated with multiple t-tests, by the Holm-Sidak method. Statistical significance was assumed when p<0.05.*