Supporting Information

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SI Materials and Methods

Generation of MTreg Clonal Populations and Assessment of Suppressive Functions. Clonal MTreg populations were generated by limiting dilution culture of ex vivo-sorted $CD45RA^{-}CD25^{+}CD127^{low}CD4^{+}$ T cells with irradiated feeder cells, phytohemagglutinin (PHA), and IL-2. Suppressive function was assessed by coculture of carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled $CD4^{+}$ responders (2×10^4 per well) with suppressors at a cell ratio of 1:1 in the presence of 2×10^4 per well irradiated allogeneic $CD14^{+}$ cells and PHA as described previously (1). Growth (assessed by CFSE dilution, 100 − % undivided cells) of wells with suppressor cells (experimental group) was compared with that of wells without suppressors (control). The percentage of suppression was determined as follows: 100 − (growth of experimental group/growth of control) × 100.

1. Valmori D, Merlo A, Souleimanian NE, Hesdorffer CS, Ayyoub M (2005) A peripheral circulating compartment of natural naive CD4 Tregs. *J Clin Invest* 115:1953–1962.

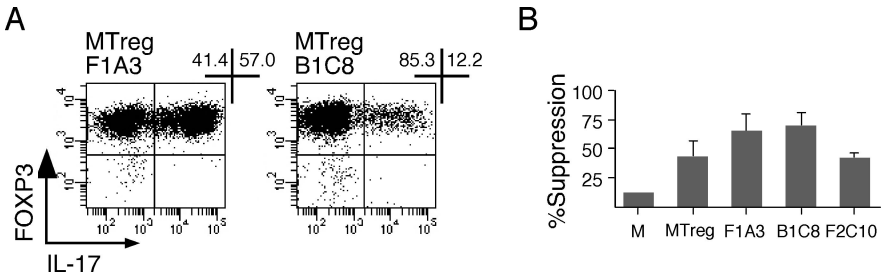

Fig. S1. Assessment of suppressive function of Treg-derived clonal populations. (*A*) Ex vivo-sorted MTregs were cloned under limiting dilution conditions. Obtained clonal populations were stimulated for 4 h with PMA/ionomycin, stained with anti-FOXP3 and anti-IL-17 antibodies, and analyzed by flow cytometry. Dot plots for 2 clones obtained from 2 donors are shown. (*B*) Suppressive function of MTreg clones F1A3 and B1C8, of a control Treg FOXP3^{+}IL-17^{-} clone (F2C10), and of ex vivo-sorted M and MTreg populations, used as internal controls, was assessed by coculture of CFSE-labeled responders with suppressors (cell ratio 1:1) in the presence of monocytes and PHA, as detailed in the *SI Materials and Methods*.