

Supplementary Material to:

**Tumor Antigen Specific iTreg Accumulate in the Tumor Microenvironment
and Suppress Therapeutic Vaccination**

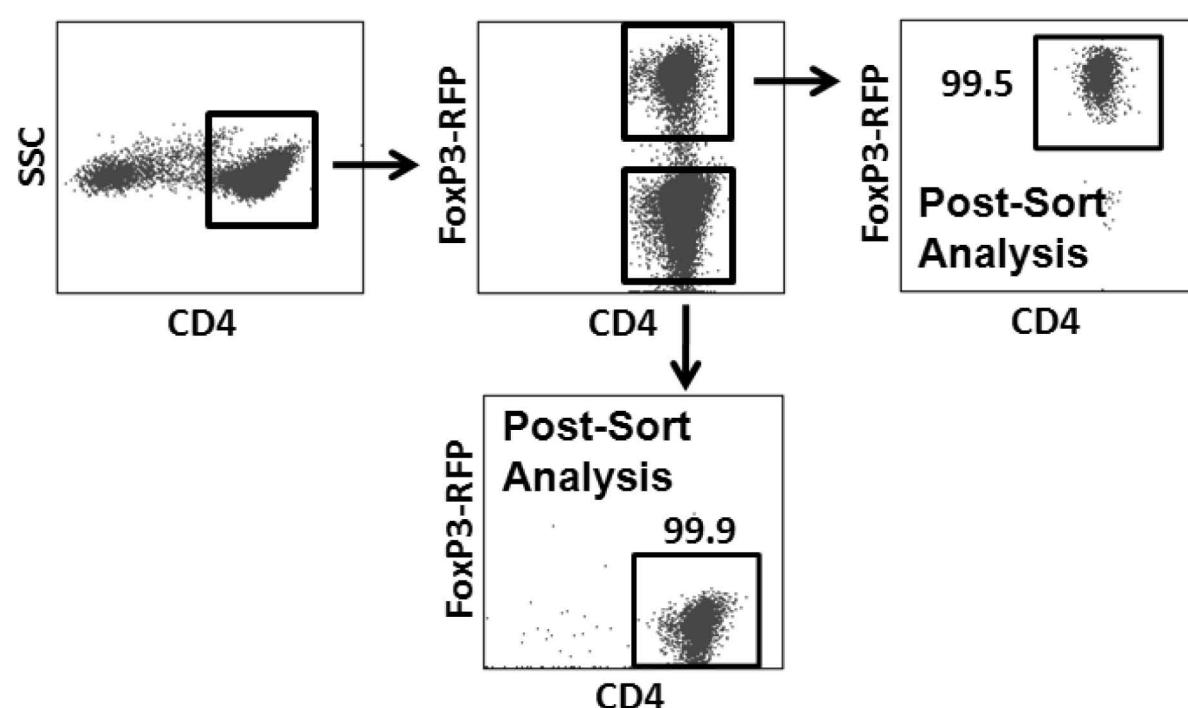
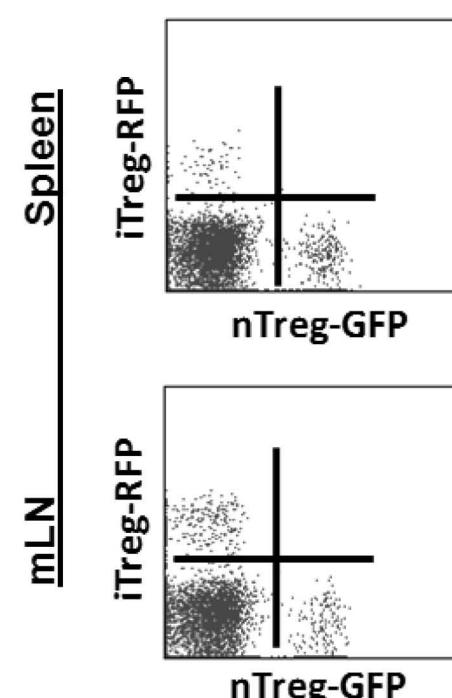
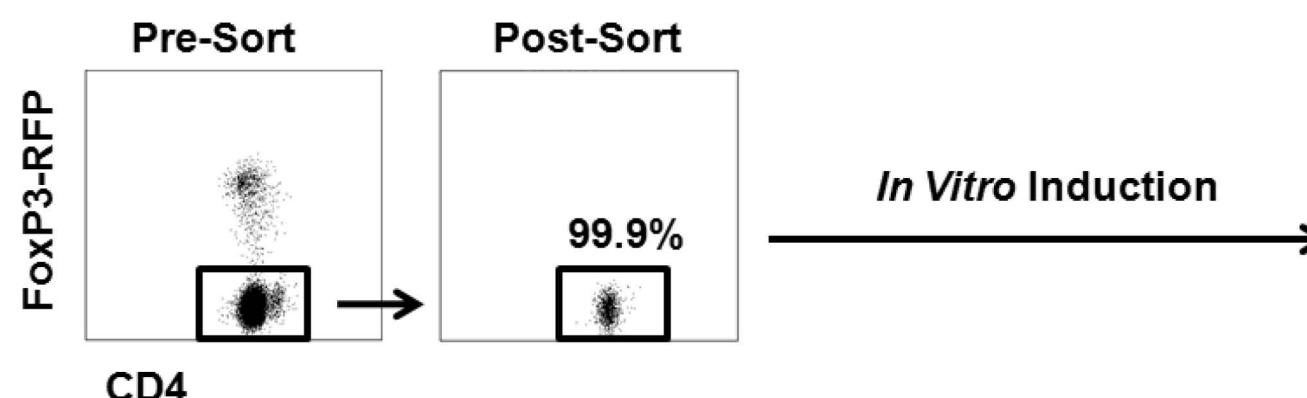
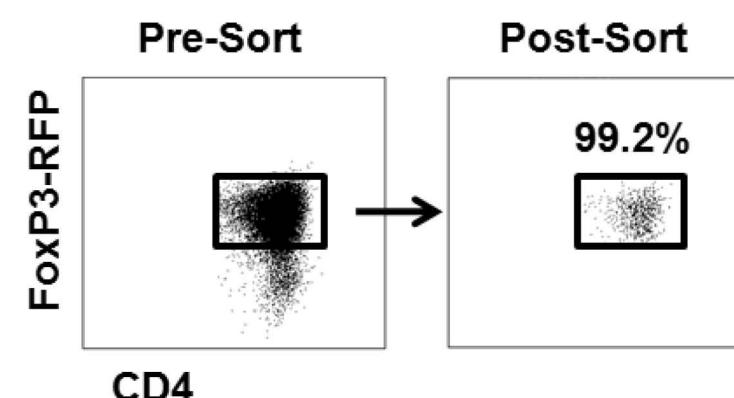
Taylor H. Schreiber, Dietlinde Wolf, Maria Bodero, Eckhard R. Podack

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Primer Segment	Sequence (5' → 3')
V β 4	GCCTCAAGTCGCTTCCAACCTC
V β 7	TACAGGGTCTCACGGAAAGAAGC
V β 8.2	CATTATTCATATGGTGCTGGC
V β 8.3	TGCTGGCAACCTTCGAATAGGA
V β 9	TCTCTCTACATTGGCTCTGCAGGC
V β 11	GCACTCAACTCTGAAGATCCAGAGC
V β 12	GATGGTGGGGCTTCAAGGATC
V β 14	ACGACCAATTCATCCTAAGCAC
C β	CTTGGGTGGAGTCACATTCT
J β 1.1	FAM-ACTGTGAGTCTGGTCCCTTACC

Supplemental Table 1: Primer sequences used for primary and secondary PCR reactions in TCR spectratyping analysis.

A.**nTreg-RFP and Tconv Sorting Strategy****B.****Polyclonal iTreg and nTreg Analysis Plots****C.****Pre-Gated on CD4+V α 2+V β 5+****D.****Pre-Gated on CD4+V α 2+V β 5+**

Supplemental Figure 1: Sorting and detection strategies for iTreg and nTreg populations. (A) Representative sorting strategy for purification of polyclonal Tconv (CD4+FoxP3-RFP negative) and nTreg (CD4+FoxP3-RFP positive) cells from FIR mice. (B) Representative analysis plots for detection of adoptively transferred polyclonal iTreg and nTreg in the mLN and spleen from CD4-/ mice as shown in Figure 1. (C) Example sorting profile for OT-II Tconv cells from OT-II/FIR mice. For some experiments the OT-II Tconv were used directly, and for some experiments these cells were subsequently used for *in vitro* induction to OT-II iTreg and re-sorted as shown in (D).