

#	Clonal Frequency And Fold Expansion of Detected pSTAT3-inhibited iTregs at day +21	Frequency		Fold
		Day 0	Day +21	Expansion
1	CTGTCGGCTGCTCCCTCCCAGACATCTGTGACTTCTGTGCCAGCAGTTACTCGTACAGTAGCAATCAGCCCCAGCATTTTGGTGAT	6.45E-06	1.91E-04	29.57
2	AAGATCCAGCCTGCAAAGCTTGAGGACTCGGCCGTGTATCTCTGTGCCAGCAGCTTAGACCTCAATCAGCCCCAGCATTTTGGTGAT	3.22E-06	5.45E-05	16.90
3	AGCACCTTGGAGCTGGGGGACTCGGCCCTTATCTTTGCGCCAGCAGCTTGAGCCCAACGCGAACACTGAAGCTTTCTTTGGACAA	3.22E-06	5.45E-05	16.90
4	GAGCTCTCTGGAGCTGGGGGACTCAGCTTTGTATTTCTGTGCCAGCAGCGTCCGCCGGCCCTCCAATGGAGCAGTACTTCGGGCCG	3.22E-06	2.72E-05	8.45
5	CAGCCCTCAGAACCAGGGACTCAGCTGTGACTTCTGTGCCAGCACTCGACCGGGACAGGGGAACACTGAAGCTTTCTTTGGACAA	3.22E-06	2.72E-05	8.45
6	GAGTTGGCTGCTCCCTCCCAGACATCTGTGACTTCTGTGCCAGCAGTTACTCGTACAGTAGCAATCAGCCCCAGCATTTTGGTGAT	3.22E-06	2.72E-05	8.45
7	GAGTCGGCTGCTCCCTCCCAGACATCTGTGACTTCTGTGCCAGCAGTTACTCGTACAGTAGCAATCAGCCCCAGCATTTTGGTGAT	3.22E-06	2.72E-05	8.45
8	AAGATCCAGCCTGCAGAGCTTGGGGACTCGGCCGTGTATCTCTGTGCCAGCAGCTTAGACCTCAATCAGCCCCAGCATTTTGGTGAT	3.22E-06	2.72E-05	8.45
9	CTCACTCTGGAGTCCGCTACCAGCTCCCAGACATCTGTGACTTCTGTGCCATCAGGGGATTAGACAATGAGCAGTTCTTCGGGCCA	3.22E-06	2.72E-05	8.45
10	CACATCAATCCCTGGAGCTTGGTGACTCTGCTGTGTATTTCTGTGCCAGCAGGGGCTCTGGGGCCAACGTCTGACTTTCGGGGCC	3.22E-06	2.72E-05	8.45
11	AATGTGAGCACCTTGGAGCTGGGGGACTCGGCCCTTATCTTTGCGCCAGCAGCAAGAGTACGTGCTGGAGCAGTACTTCGGGGCCG	3.22E-06	2.72E-05	8.45
12	AAGATCCAGCCTGCAGAGCTTGGGGACTCGGCCGTGTATCTCTGTGCCAGCAGCTTACACTACCGGGGAGACCCAGTACTTCGGGCCA	3.22E-06	2.72E-05	8.45
13	TCTAAGAAGCTCCTTCTCAGTGACTCTGGCTTCTATCTCTGTGCCTGTCAAAGGGGGGTTAGGGCTGAAGCTTTCTTTGGACAA	3.22E-06	2.72E-05	8.45
14	AGCACCTTGGAGCTGGGGGACTCGGCCCTTATCTTTGCGCCAGCAGAGGGGACAGGGATTACACCGGGGAGCTGTTTTTGGAGAA	9.67E-06	8.17E-05	8.45
15	CAGCCTGCAAAGCTTGAGGACTCGGCCGTGTATCTCTGTGCCAGCACCCGGGACGGCCGGTCTAGATATGGCTACACCTTCGGTTCG	6.45E-06	5.45E-05	8.45
16	TCCACGGAGTCAGGGGACACAGCACTGTATTTCTGTGCCAGCAGCAAAGGAACGGACTAGCGGTGCTACGAGCAGTACTTCGGGGCCG	8.06E-05	5.17E-04	6.42
17	TCCACGGAGTCAGGGGACACAGCACTGTATTTCTGTGCCAGCAGCAAAGGAACGGACTAGCGGTGCTACGAGCAGTACTTCGGGGCCA	3.22E-05	1.91E-04	5.91
18	ACGATCCAGCGCACACAGCAGGAGGACTCGGCCGTGTATCTCTGTGCCAGCAGCTTAGACCTCAATCAGCCCCAGCATTTTGGTGAT	6.45E-06	2.72E-05	4.22
19	CAGCGCACACAGCAGGAGGACTCGGCCGTGTATCTCTGTGCCAGCACCCGGGACGGCCGGTCTAGATATGGCTACACCTTCGGTTCG	3.55E-05	1.09E-04	3.07
20	AAGATCCAGCCCTCAGAACCAGGGACTCAGCTGTGACTTCTGTGCCAGCAGTCGGACGGGAATCAGCCCCAGCATTTTGGTGAT	9.67E-06	2.72E-05	2.82
21	CCTGTCCCTAGAGTCTGCCATCCCAACCAGACAGCTTTACTTCTGTGCCACCAGTGATTTGCCGATGGCTACACCTTCGGTTCG	9.67E-06	2.72E-05	2.82
22	CTGACGATCCAGCGCACACAGCAGGAGGACTCGGCCGTGTATCTCTGTGCCAGCAGCTTATTGAACACTGAAGCTTTCTTTGGACAA	9.67E-06	2.72E-05	2.82
23	TTGGAGCTGGACGACTCGGCCCTGTATCTCTGTGCCAGCAGTCCCCGGAGGGCAGGGGGGCCGCTACGAGCAGTACTTCGGGGCCG	9.67E-06	2.72E-05	2.82
24	GGCTGCTCCCTCCAAACATCTGTGACTTCTGTGCCAGCAGTTAGCTCAAACGTCCGGGGTAACTACGAGCAGTACTTCGGGGCCG	9.67E-06	2.72E-05	2.82
25	AACCTGAGCTCTCTGGAGCTGGGGGACTCAGCTTTGTATTTCTGTGCCAGCAGCGGGGAGGGTGAAACACCATATATTTTGGAGAG	2.26E-05	5.45E-05	2.41
26	GAGTCCGCCAGCACCAACCAGACATCTATGTACTTCTGTGCCAGCAGTTTTCTGGACAGAAAAAATCAGCCCCAGCATTTTGGTGAT	2.58E-05	2.72E-05	1.06

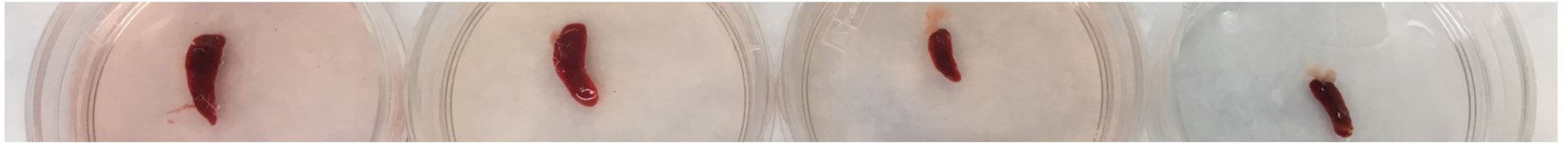
Supplemental Table 1: Human pSTAT3-inhibited iTregs expand and are detectable in vivo. NSG mice were transplanted with a 1cm² human skin graft, followed by 5x10⁶ PBMCs plus 1x10⁵ pSTAT3-inhibited iTregs as described. Genomic DNA from an aliquot of pSTAT3-inhibited iTregs (1x10⁶) at day 0, as well as harvested human T cells (1x10⁶) from a recipient spleen at day +21, were sequenced using Adaptive Biotechnologies multiplex kit to identify unique TCR Vβ clones. The data was analyzed and characterized per the international ImMunoGeneTics information system. The table shows the individual pSTAT3-inhibited iTreg clones detected at day +21 from a representative mouse.

PBMCs Only

**PBMCs +
DMSO-Tregs**

**PBMCs +
S3i-Tregs**

No PBMCs



Supplemental Figure 1: Recipient spleen-size is reduced after adoptive transfer of human pSTAT3-inhibited iTregs. NSG mice received a human skin graft, allogeneic PBMCs (5×10^6), and pSTAT3-inhibited (S3i) or control DMSO-treated iTregs (1×10^5). On day +21, the mice were humanely euthanized and the recipient spleens were harvested and processed for analysis by flow cytometry. Representative mouse spleens are shown from 1 or 3 experiments.

Supplemental Table 2: Key reagents

Antibodies				
Antigen:	Fluor:	Manufacturer:	Clone:	Catalog #
CD127	AF647	BD Biosciences	HIL-7R- M21	558598
CD4	FITC	ThermoFisher	OKT4	11-0048-42
CD25	PECy7	ThermoFisher	M-A251	557741
Foxp3	PE	BD Biosciences	259D/C7	560046
Ki67	PE	ThermoFisher	20Raj1	12-5699-42
CD4	PECy7	ThermoFisher	SK3	25-0047-42
CD45RO	FITC	BD Biosciences	UCHL1	555492
CD62L	PECy7	Biolegend	DREG-56	304822
IL-4	APC	ThermoFisher	8D4-8	17-7049-42
IFN-γ	FITC	BD Biosciences	B27	554700
IL-17	BV421	Biolegend	BL168	512322
CLA	FITC	Biolegend	HECA- 452	321306
Anti-STAT3 (total)	PE	BD Biosciences	M59-50	560391
CD3	FITC	ThermoFisher	OKT3	11-0037-42

CD4	PE	ThermoFisher	RPA-T4	12-0049-42
Chemicals, Peptides, and Recombinant Proteins				
Live Dead Fixable Yellow Stain		ThermoFisher		L34959
Live Dead Fixable Aqua Stain		ThermoFisher		L34965
S3I-201		Moffitt Cancer Center		
Human recombinant IL-2		R&D		202-IL/CF
Critical Commercial Assays				
CD25 Microbeads, Human		Miltenyi Biotec		130-092-983