#### Supplementary Figure 1. Cell processing schema.

Following a two day apheresis collection, cells undergo CD34 bead selection, followed by CD25 bead selection of the CD34 negative flow-through fraction. After CD25 enrichment, cells were stained with mAb against CD4 and CD127 and then further purified by flow cytometric sorting of CD4+CD127(dim) cells. At the time of apheresis, a fraction of apheresis product is removed and cryopreserved to provide the CD3 fraction.

#### Supplementary Figure 2. Dose escalation schema and DLT definition.

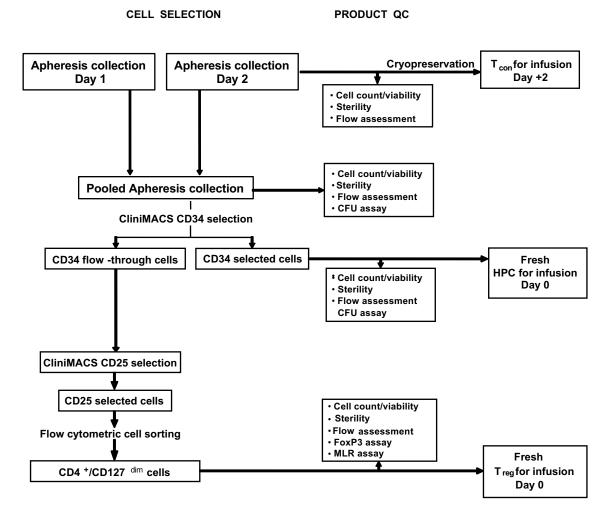
#### Supplementary Figure 3. Example mixed lymphocyte reaction suppression assay.

Proliferation of responder T cells were assessed by CFSE staining and flow cytometry (upper panels and lower left panel) and thymidine incorporation assay (lower right panel). Ratio of responders T cells (R) to target antigen presenting cells (S) and Treg (T) are provided. All assays repeated in triplicate, with significance of <0.01 (\*\*) by two-tailed student T test.

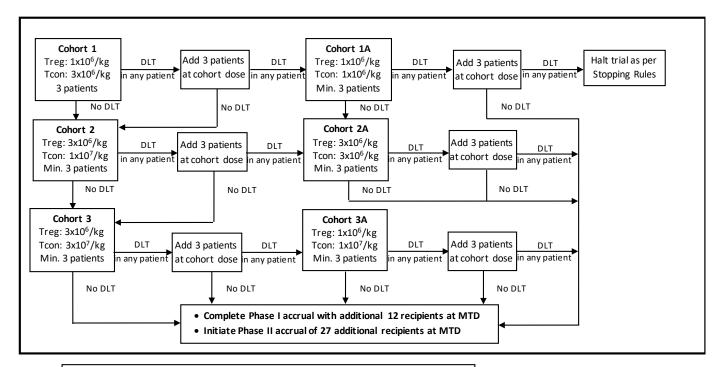
Supplementary Figure 4. Day of neutrophil and platelet engraftment after hematopoietic stem cell transplantation.

**Supplementary Figure 5. Treg phenotype by treatment group.** Peripheral blood Treg cell expression of surface and intracellular assessed by CyTOF on day +30 after HSCT. with Significance indicated as <0.05(\*) and <0.01 (\*\*) by two-tailed student T test.

### **Supplementary Figure 1.**

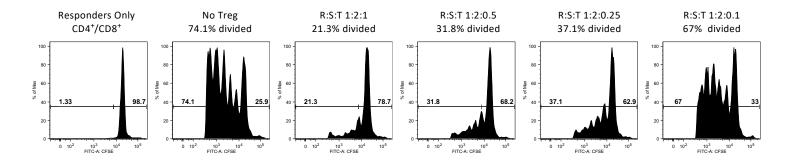


### **Supplementary Figure 2.**



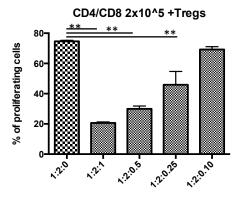
Dose Limiting Toxicity (DLT):

- Acute GvHD > grade 2
- Grade 4 neutropenia lasting to 28 days after HCT
- Grade 3 to 5 cytokine/release syndrome/acute infusion reaction

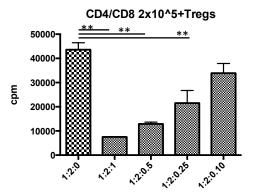


## Supplementary Figure 3.



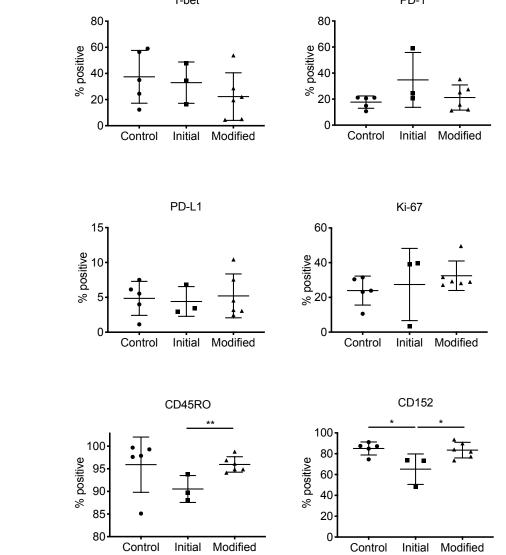


Thymidine



# Supplementary Figure 4.

	Subject	Neutrophil engraftment (Day+)	Platelet engraftment (Day+)
	5126	11	17
Initial protocol:	5201	10	14
Frozen T <sub>reg</sub>	5534	19	25
	5902	11	16
	6157	11	15
	6666	14	16
	6708	11	14
Modified protocol:	6784	12	10
Fresh T <sub>reg</sub>	6820	12	10
	6857	11	9
	6948	14	10
	7011	15	22
	Range	9 (10 – 19)	16 (9 – 25)
	Median	11	16



# Supplementary Figure 5

T-bet

PD-1