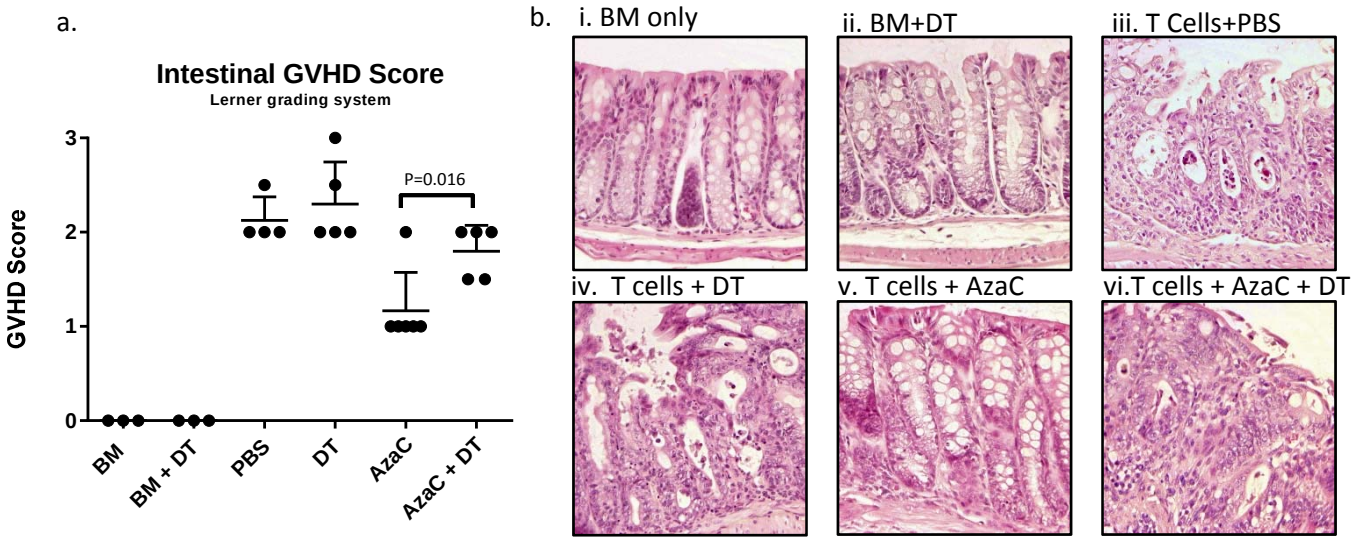
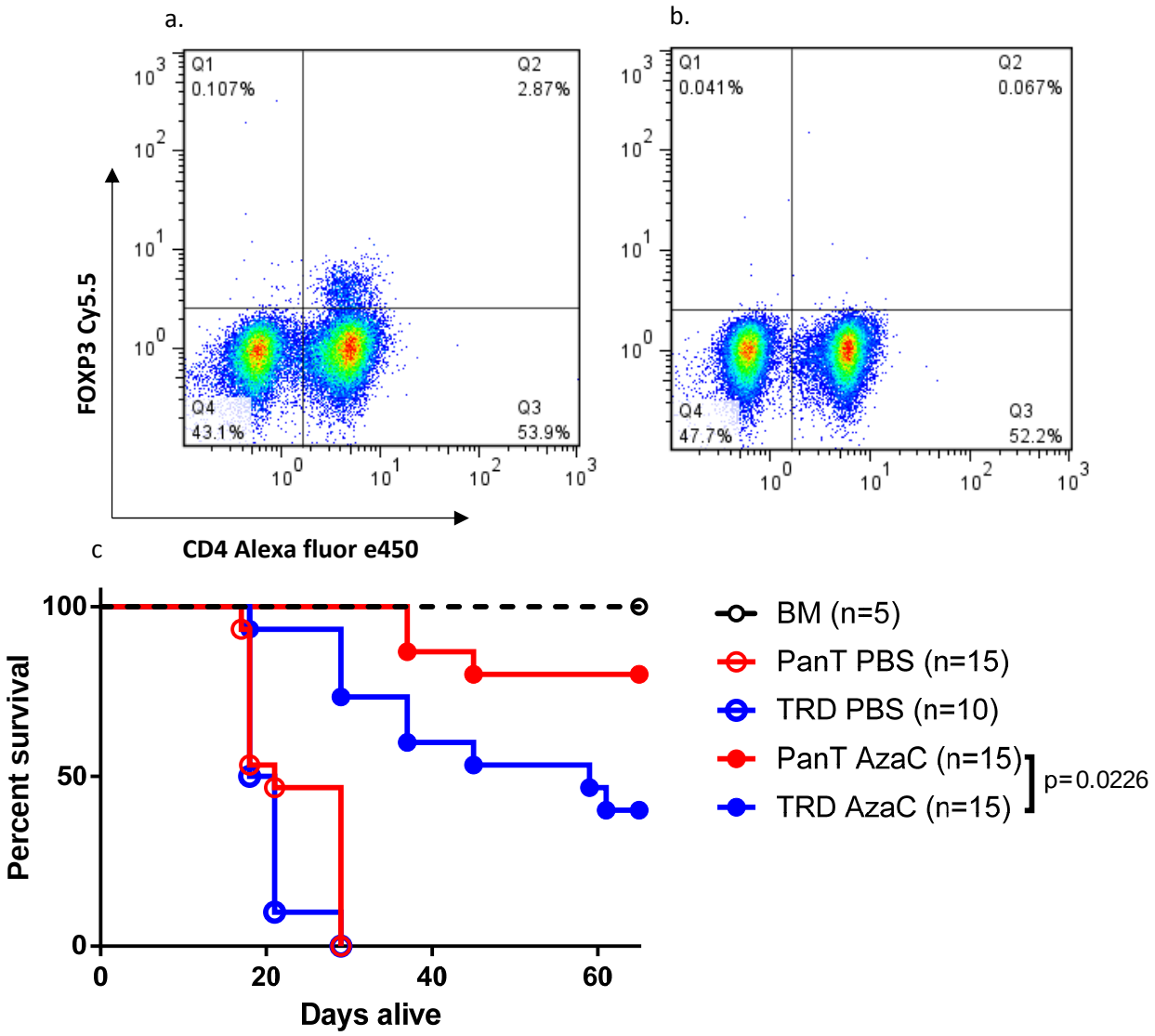
Supplemental Figure 1



Supplemental Figure 1. Gastrointestinal Histopathology. Balb/c mice were infused with $1x10^7$ B6.*Foxp3*$^{DTR/GFP}$ pan T cells 11 days after an initial T cell depleted (TCD) bone marrow (BM, CD45.1) transplant. They were subsequently treated with AzaC alone (AzaC+PBS), DT alone (PBS+DT), neither drug (PBS+PBS), or both drugs (AzaC+DT). Some mice received irradiation, but no cells (irr only), only TCD BM (BM only), or BM along with DT (BM +DT). Refer to the methods section for further technical regarding histopathology. Mice treated with AzaC alone had lower gastrointestinal GVHD, when graded blind by a veterinary pathologist (Lerner grading) (10) than mice treated with both AzaC and DT (Day 25 p=0.016). (a). Hematoxylin and eosin stained sections of the GI tract (b). BM only mice treated with PBS (i) or DT (ii). Mice receiving BM and T cells treated with PBS (iii), PBS and DT (iv), AzaC and PBS (v), and AzaC and DT (vi). n=3 to 5 mice per group.

Supplemental Figure 2



Supplemental Figure 2. The depletion of Tregs in MACS purified donor T cells (a) T cells isolated from B6.*Foxp3*$^{DTR/GFP}$ splenocytes using pan T isolation kit II (Miltenyi Biotec) (b) T cells isolated from B6.*Foxp3*$^{DTR/GFP}$ splenocytes using pan T isolation kit II supplemented with 1.5 µg of biotinylated anti-CD25 antibody (Clone 7D4 BD Pharminogen) per 1x10^7 splenocytes. A more detailed description of the protocol can be found in the methods section. (c) nTregs are required in the donor graft for optimal mitigation of GVHD by AzaC. Representative data from a single experiment. Lethally irradiated Balb/c were infused with either 1x10^7 nTreg depleted B6 pan T (TRD) or 1x10^7 nTreg replete pan T cells 11 days after initial BM transplant. Recipient mice were treated with PBS or AzaC on days +15, +17, +19 and +21. Mice receiving nTreg depleted pan T cells had significantly reduced survival when compared to mice receiving nTreg replete pan T following treatment with AzaC (Pan T AzaC Vs. TRD AzaC, p=0.0226).

Supplemental Table 1. PANTHER analysis. Pathways enriched in CD4 Teffs treated with AzaC.

Go Process	p=
regulation of cytokine production involved in immune response	1.90E-03
positive regulation of cell adhesion	9.49E-03
regulation of cell proliferation	1.05E-02
regulation of response to wounding	1.61E-02
regulation of cell adhesion	1.73E-02
negative regulation of cell proliferation	2.24E-02
regulation of production of molecular mediator of immune response	2.46E-02
regulation of inflammatory response	4.75E-02

Supplemental Table 2. PANTHER analysis. Pathways enriched in nTregs treated with AzaC.

Go Process	P=
immune system process	1.33E-07
positive regulation of biological process	1.38E-06
immune response	2.88E-06
regulation of immune system process	6.51E-06
leukocyte differentiation	5.73E-05
cell activation	9.46E-05
positive regulation of immune system process	1.03E-04
defense response	1.24E-04
T cell differentiation	1.84E-04
positive regulation of cellular process	2.17E-04
leukocyte cell-cell adhesion	5.52E-04
positive regulation of cell-cell adhesion	2.30E-03
positive regulation of T cell activation	2.49E-03
positive regulation of homotypic cell-cell adhesion	3.22E-03
positive regulation of leukocyte cell-cell adhesion	3.22E-03
positive regulation of cell adhesion	3.42E-03
immune system development	3.61E-03
lymphocyte differentiation	3.65E-03
single-organism process	4.51E-03
leukocyte activation	4.63E-03
biological regulation	5.49E-03
response to stress	5.89E-03
T cell aggregation	6.30E-03
T cell activation	6.30E-03
regulation of multicellular organismal process	6.73E-03
lymphocyte aggregation	6.88E-03
hematopoietic or lymphoid organ development	7.95E-03
positive regulation of lymphocyte activation	8.49E-03
positive regulation of macromolecule metabolic process	8.64E-03
leukocyte aggregation	8.90E-03
response to cytokine	9.21E-03
positive regulation of metabolic process	9.54E-03
regulation of homotypic cell-cell adhesion	1.01E-02
immune effector process	1.04E-02
single organism cell adhesion	1.13E-02
lymphocyte activation	1.23E-02
alpha-beta T cell differentiation involved in immune response	1.31E-02
regulation of cytokine production	1.49E-02
positive regulation of cytokine production	1.50E-02
alpha-beta T cell activation involved in immune response	1.61E-02
regulation of hemopoiesis	2.20E-02

alpha-beta T cell differentiation	2.23E-02
regulation of cellular process	2.25E-02
T cell differentiation involved in immune response	2.37E-02
regulation of cell-cell adhesion	2.52E-02
single organismal cell-cell adhesion	2.56E-02
regulation of response to external stimulus	2.59E-02
negative regulation of biological process	2.61E-02
regulation of lymphocyte activation	2.79E-02
cellular process	2.82E-02
positive regulation of leukocyte activation	2.85E-02
regulation of biological process	2.91E-02
cell adhesion	3.04E-02
regulation of leukocyte differentiation	3.10E-02
regulation of T cell activation	3.10E-02
response to other organism	3.23E-02
response to external biotic stimulus	3.23E-02
organic substance metabolic process	3.32E-02
regulation of cell adhesion	3.39E-02
biological adhesion	3.57E-02
hemopoiesis	3.99E-02
negative regulation of multicellular organismal process	4.11E-02
regulation of leukocyte cell-cell adhesion	4.28E-02
positive regulation of cell activation	4.45E-02
response to external stimulus	4.51E-02
metabolic process	4.52E-02
positive regulation of cellular metabolic process	4.89E-02