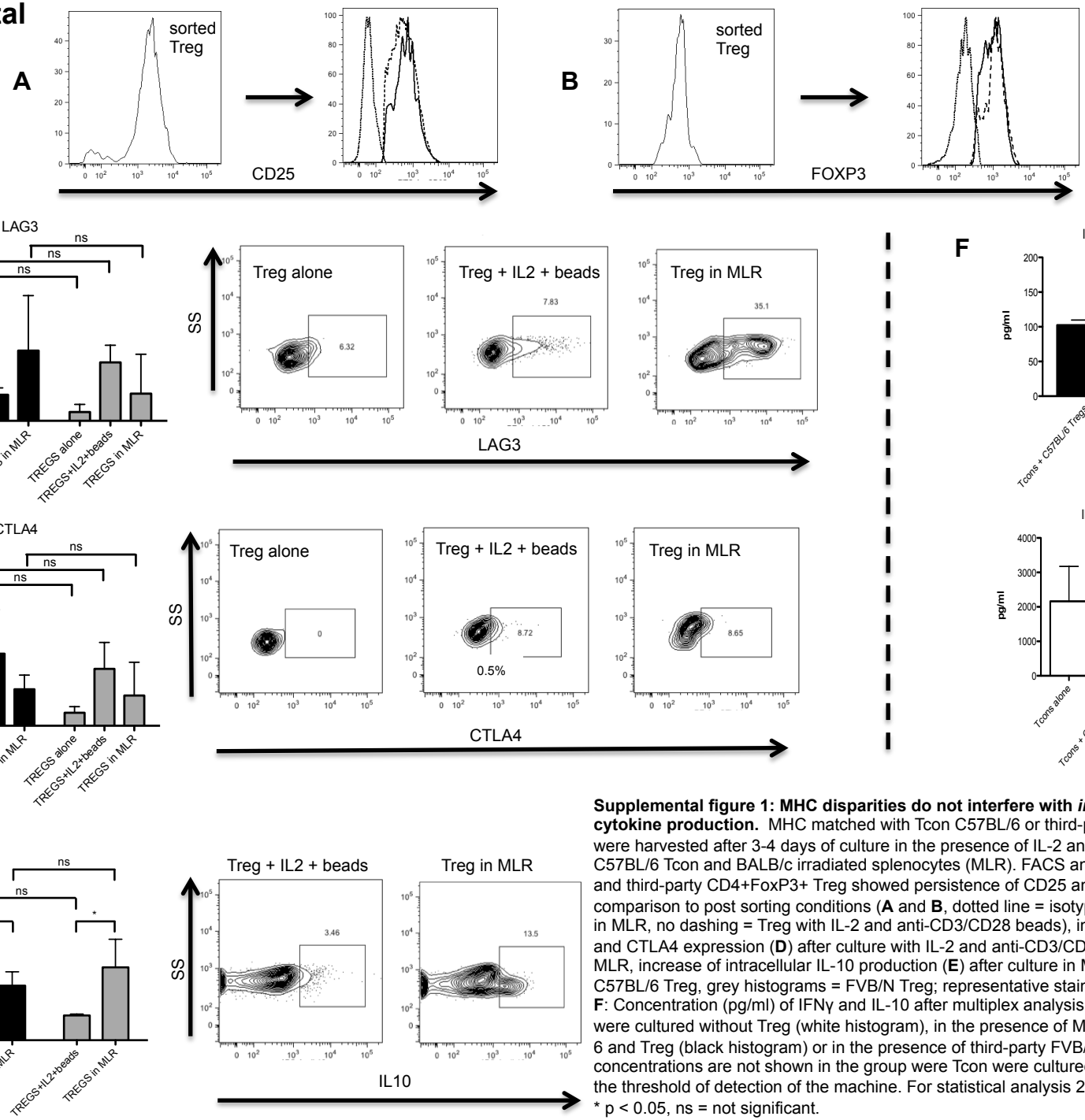
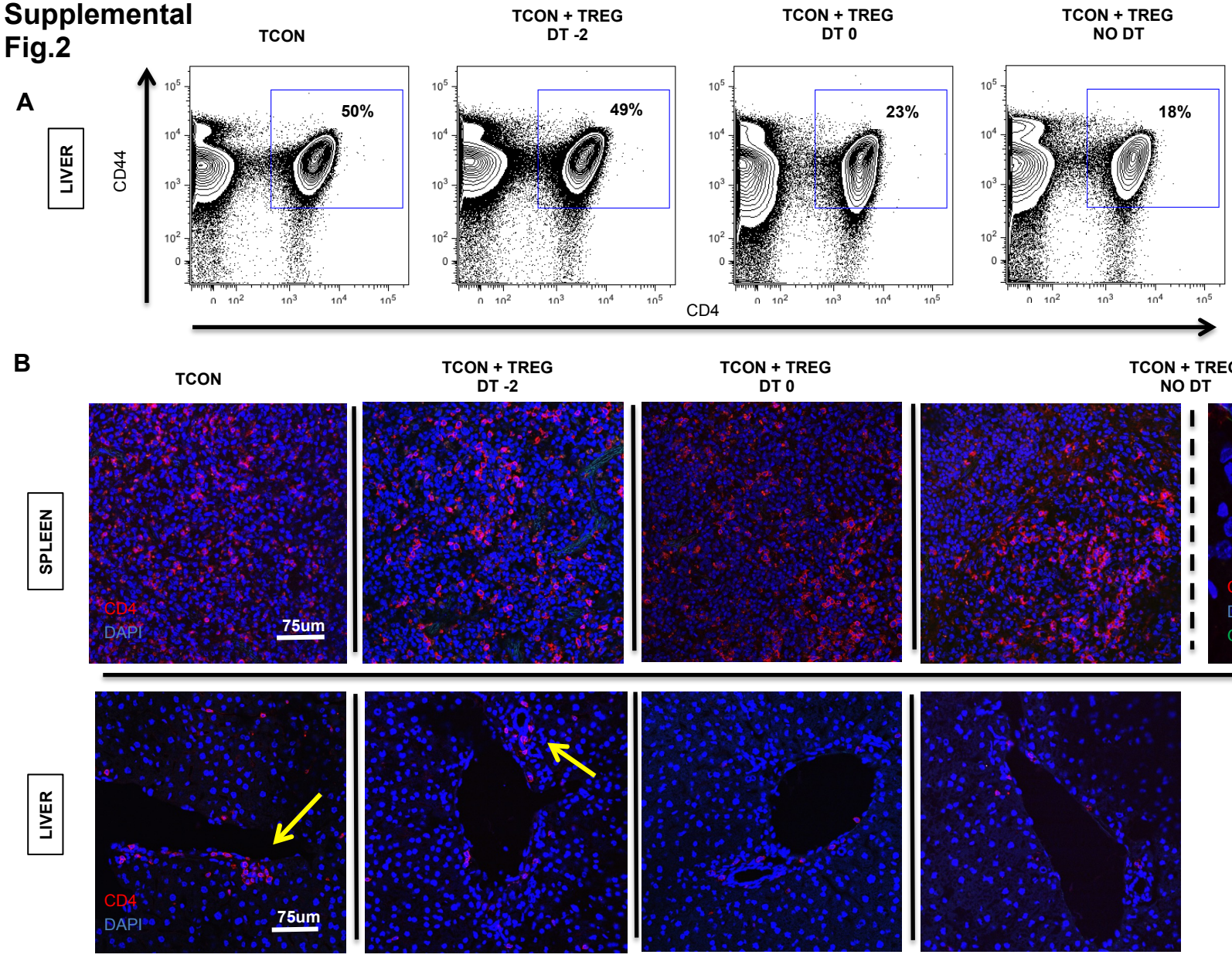
Supplemental Fig.1



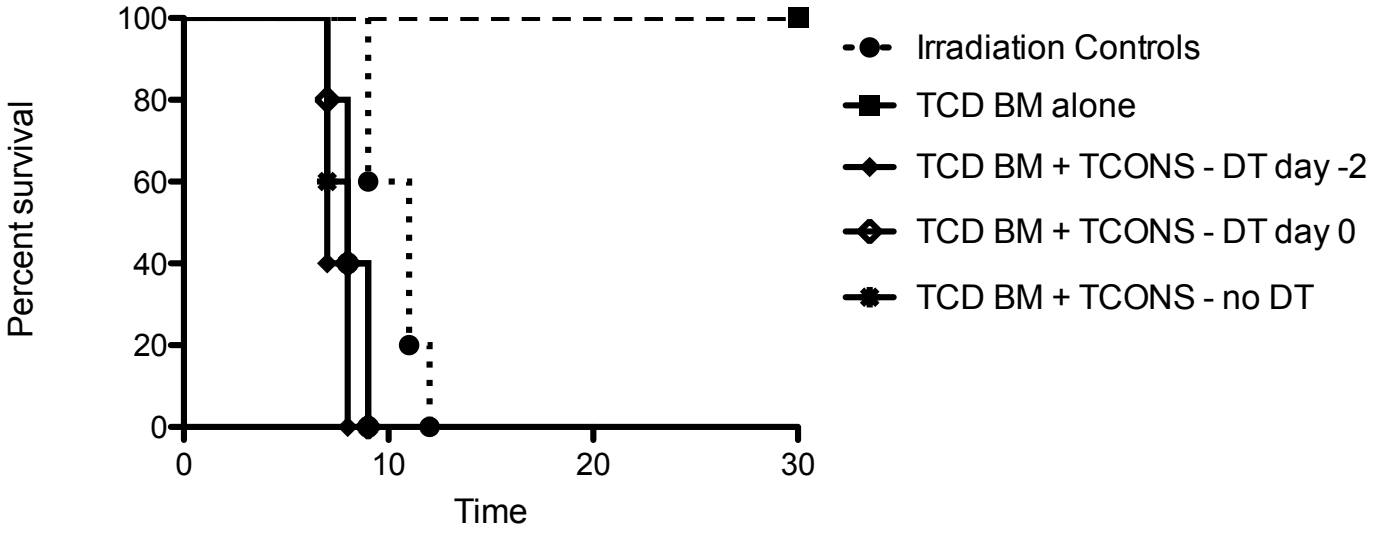
Supplemental figure 1: MHC disparities do not interfere with *in vitro* Treg activation and cytokine production. MHC matched with Tcon C57BL/6 or third-party FVB/N CD4+FoxP3+ Treg were harvested after 3-4 days of culture in the presence of IL-2 and anti-CD3/CD28 beads or with C57BL/6 Tcon and BALB/c irradiated splenocytes (MLR). FACS analysis of both MHC matched and third-party CD4+FoxP3+ Treg showed persistence of CD25 and FoxP3 expression in comparison to post sorting conditions (**A** and **B**, dotted line = isotype controls, dashed line = Treg in MLR, no dashing = Treg with IL-2 and anti-CD3/CD28 beads), increase of LAG3 expression (**C**) and CTLA4 expression (**D**) after culture with IL-2 and anti-CD3/CD28 beads or after culture in the MLR, increase of intracellular IL-10 production (**E**) after culture in MLR (black histograms = C57BL/6 Treg, grey histograms = FVB/N Treg; representative stained samples are also shown). **F**: Concentration (pg/ml) of IFNγ and IL-10 after multiplex analysis of supernatants when Tcon were cultured without Treg (white histogram), in the presence of MHC matched with Tcon C57BL/6 and Treg (black histogram) or in the presence of third-party FVB/N Treg (grey histogram). IL-10 concentrations are not shown in the group were Tcon were cultured alone as they were inferior to the threshold of detection of the machine. For statistical analysis 2-tailed student *t* test was used, * $p < 0.05$, ns = not significant.

Supplemental Fig.2



Supplemental figure 2: Treg reduce Tcon infilitration in the liver even if depleted two days after transplantation. Percentage of $CD4^+CD44^+$ donor cells in the liver of mice that received Tcon and did not receive Treg transfer (Tcon), Tcon + Treg + DT at day -2 and -1 (Tcon + Treg DT -2) or Tcon + Treg + DT at day 0 and +1 (Tcon + Treg DT 0) or only Tcon + Treg (Tcon + Treg no DT) is shown. Representative FACS samples are reported (**A**). Representative samples of spleen and liver harvested at day +6 after transplantation are shown after confocal microscopy analysis. Cryosections were stained with anti-CD4 PE and microscopic images are shown at different magnifications as reported in the figure. Less $CD4^+$ cells infiltrated portal spaces in the liver of mice that received Treg transfer and DT at day 0 or no DT in comparison to mice that received no Treg transfer or Treg transfer and DT at day -2 (red signals and yellow arrows). GFP^+ Treg were only detectable in the samples from mice that received Treg transfer and no DT (green signals and white arrows, **B**).

Supplemental Fig.3



Supplemental figure 3: DT does not increase GvHD lethality. Survival of transplanted BALB/c recipient mice after injection of C57BL/6 Tcon only (✱), Tcon + DT at day -2 and -1 (◆) and Tcon + DT at day 0 and +1 (◇) are shown. Mice that received TCD BM only (dashed line, ■) and mice that were lethally irradiated but not transplanted (dotted line, ●) were used as controls. For statistical analysis of mouse survival Kaplan-Meier test was used. Data is representative of one of two experiments (minimum 5 mice/group)