Supplementary Figure 1: (**A**) Hind limb allograft survival showed a MST of 18.5 days in TBI alone (n=4), MST 24.5 days in TBI + CTLA4-Ig (n=4) , MST 80.5 days in MR1 alone (n=4) and MST 95.5 days in TBI + MR1 (n=3). Groups containing MR1 (MR1 alone and TBI+MR1) showed a significantly better VCA survival compared to TBI alone or TBI + CTLA4-Ig. (**B**) Donor-specific mixed chimerism was detected in animals treated with MR1 alone or TBI + MR1 alone in case animal survival was beyond 30 days after transplant. Percentage of K^d positive cells represented the proportion of cells expressiong H-2K^d among PBMC in the blood. Statistical differences were calculated by log-rank test.

Supplementary Figure 2: (**A**) Representative gating strategy for detection of donor haplotype MHC class I expression in $CD3^+$ T cells. A similar gating strategy was used for $B220^+$ B cells and $CD11b^+$ Macrophages. (**B**) At the time of sacrifice (POD70), mixed chimerims levels of central lymphoid organs (spleen) correlated with chimerism levels detected in peripheral blood. (Data ar representative of 4 mice per group from 2-3 independent expreriments, * $P<0.05$, ** $P<0.01$, NS = no significance, P values were calculated by 2-tailed *t* test)

Supplementary Figure 3: The production of IL-2, IL-10 or IL-17 in recipient $CD4^+$ T lymphocytes was measured to determine $CD4^+$ T cell reactivity. (**A**) IL-2 (**B**) IL-10 (**B**) IL-17 were measured at various time points im central lymphoid organs (LN). (Data ar representative of at least 3 mice per group from 2-3 independent expreriments, * $P<0.05$, ** $P<0.01$, NS = no significance, P values were calculated by 2-tailed *t* test)

Supplementary Figure 4: (**A**) Representative Treg-mediated suppression measured by CFSE dilution using flow cytometry. Conventional T cells ($CD4^+CD25^-$) were isolated from wild type C57BL/6 mice and labeled with CFSE. Cells were activated with anti-CD3 plus syngeneic bone marrow derived dendritic cells and cultured either alone or in the presence of various Tregs at different Treg-to-Responder ratios. After 72hr, proliferation was determined by CFSE dilution among viable $CD4^+$ T cells using flow cytometric analysis. (**B**) Tregs isolated from recipients treated with TBI + CoB showed stronger suppression compared to those of recipients treated with CoB alone. The latter showed a similar suppressive activity than natural Tregs isolated from wild type C57BL/6 animals.

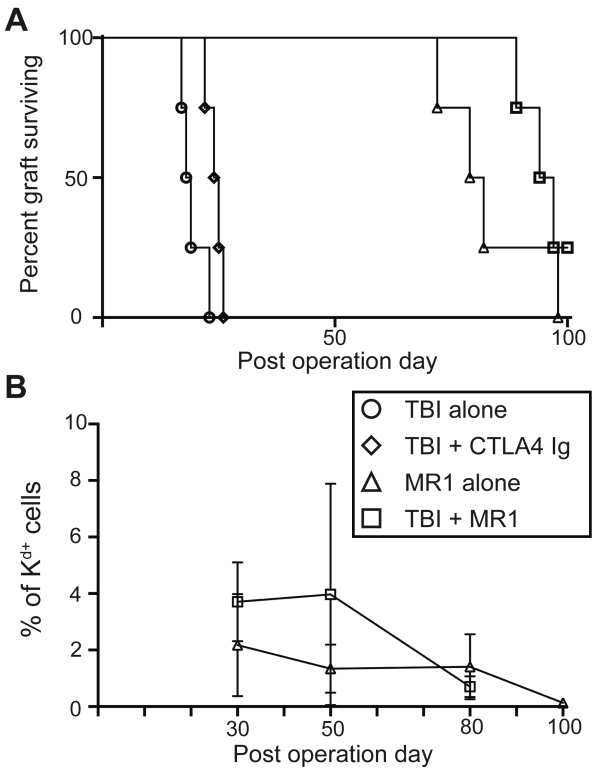
Supplementary Figure 5: (**A**) Representative gating strategy for detection of donor-derived $Foxp3^+$ T cells isolated from spleen. (**B**) While the overall percentage of $Foxp3^+$ $CD4^+$ T cells was comparable between both groups (left), the percentage of H-$2K^d$ expression on $Foxp3^+$ T cells was significally ($p>0.05$) higher in recipients treated with TBI + CoB compared to CoB at POD70. (Data ar representative of 4 mice per group from 2-3 independent expreriments, * $P<0.05$, ** $P<0.01$, NS = no significance, P values were calculated by 2-tailed *t* test)

Supplementary Figure 6: (**A**) Schematic illustration of modified treatment strategies. These included the combination of total body irradiation, CTLA4-Ig, and anti-CD154 mAb (MR1) in the presence of PC 61 either 7 or 30 days after transplant. Graft survival and mixed chimerism analysis were performed as outlined. (**B**) Depletion of Treg cells using PC61 did neither on POD 7 nor 30 have a deleterious effect on graft survival. In both groups, animals experienced survival up to 100

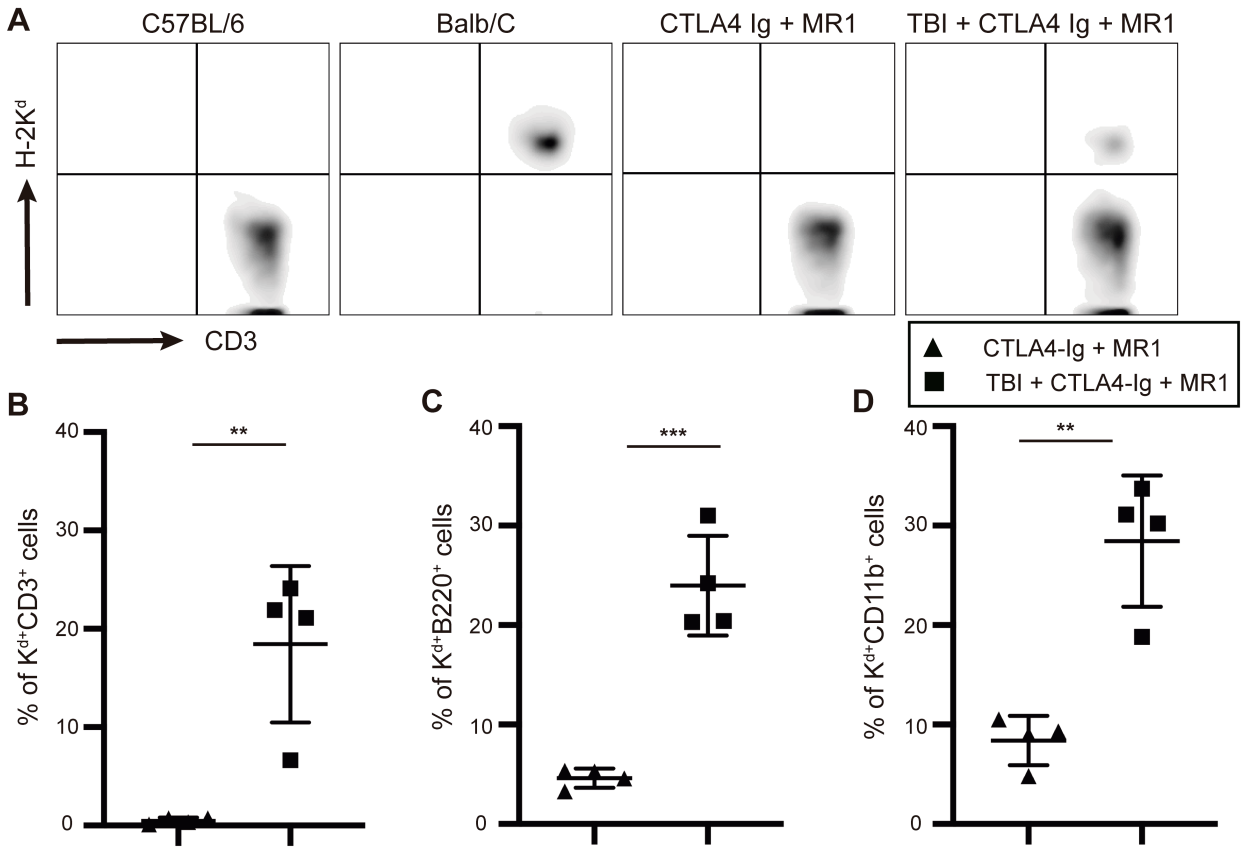
days (**C-E**) Furthermore, depletion of Treg cells using PC61 did not affect the establishment of multi-lineage stable mixed chimerisms. Statistical differences were calculated by log-rank test.

Supplementary Figure 7: (**A**) Representative gating strategy for detection of donor versus recipient derived APC in the recipient thymus. (**B**) The percentage of H-2K^{d} expression on CD11c^{+} dendritic cells in the thymus was significally ($p<0.01$) higher in recipients treated with TBI + CoB compared to CoB at POD70. (Data ar representative of 4 mice per group from 2-3 independent expreriments, * $P<0.05$, ** $P<0.01$, NS = no significance, P values were calculated by 2-tailed *t* test)

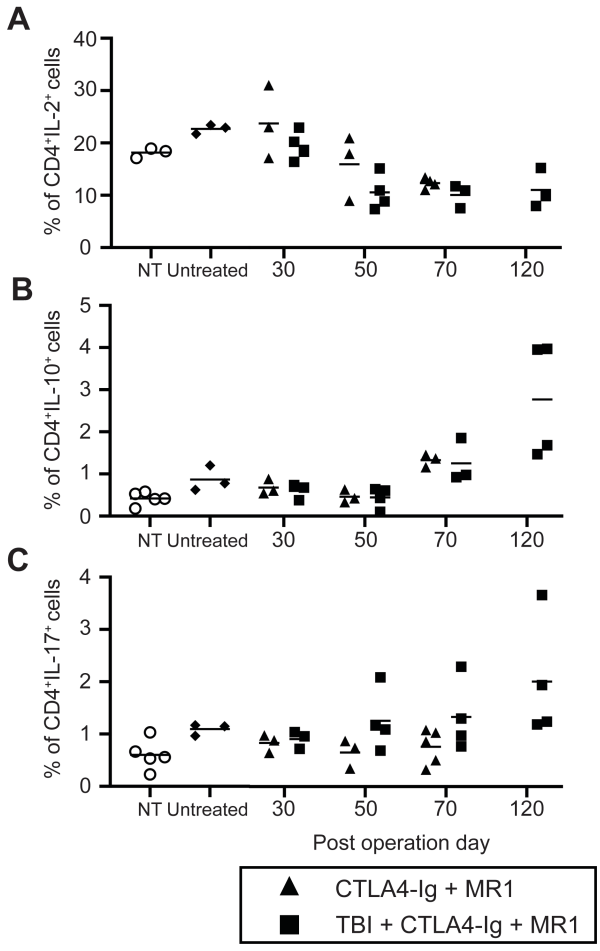
Supplementary Figure 1



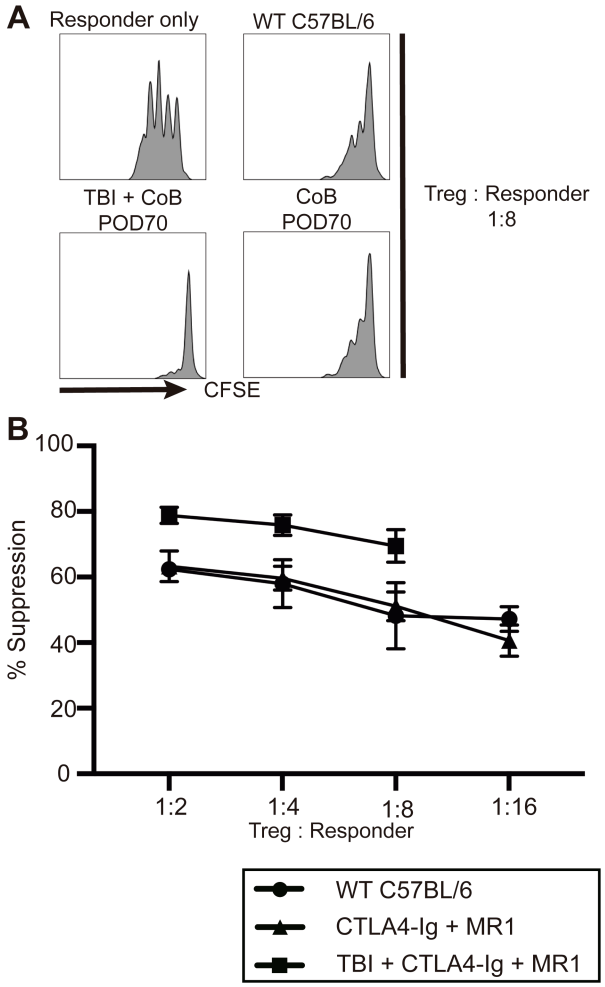
Supplementary Figure 2



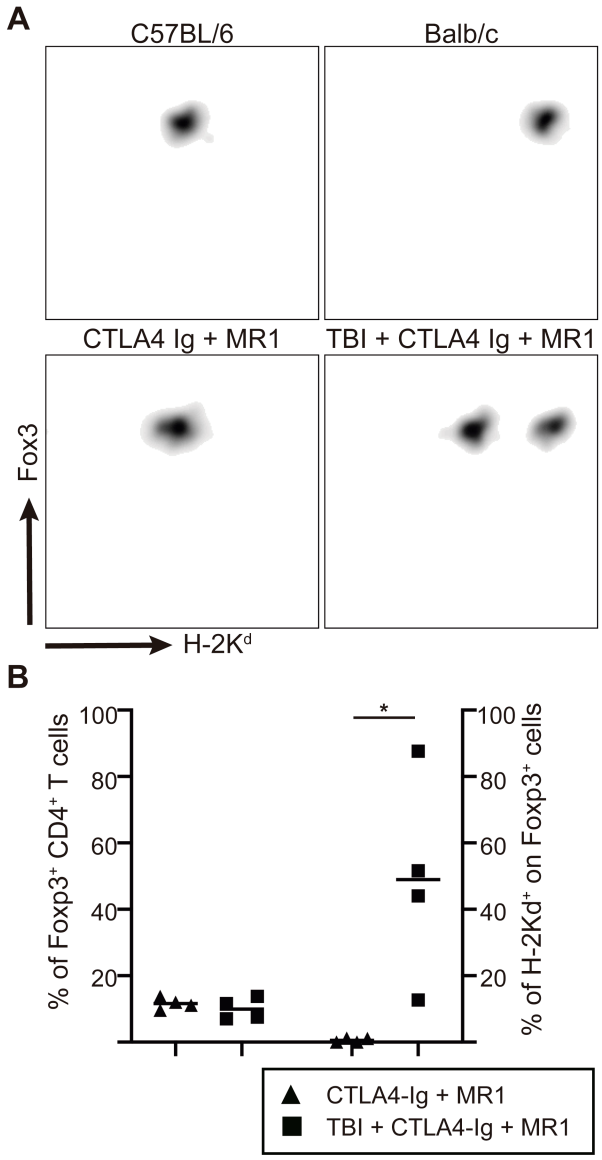
Supplementary Figure 3



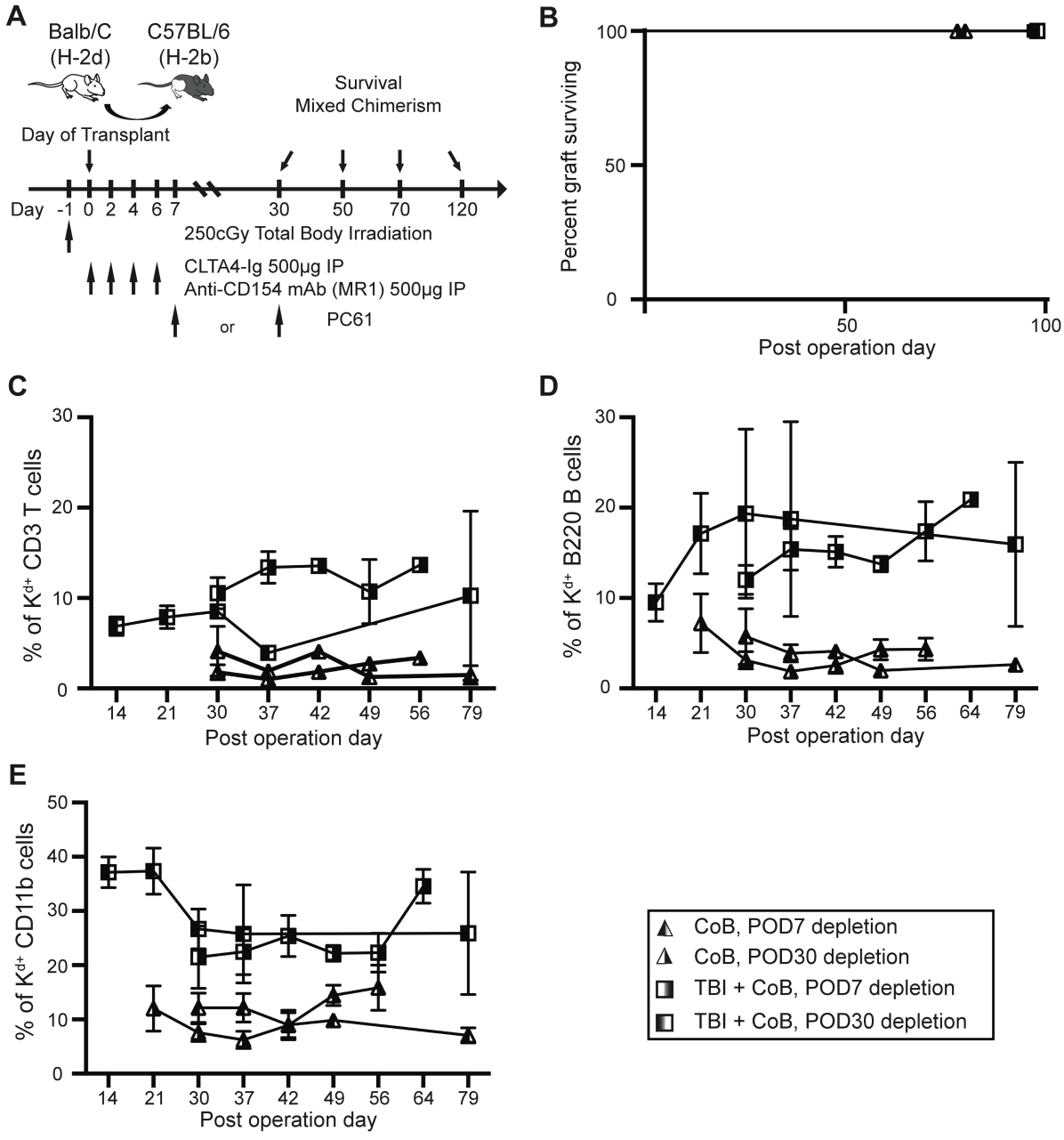
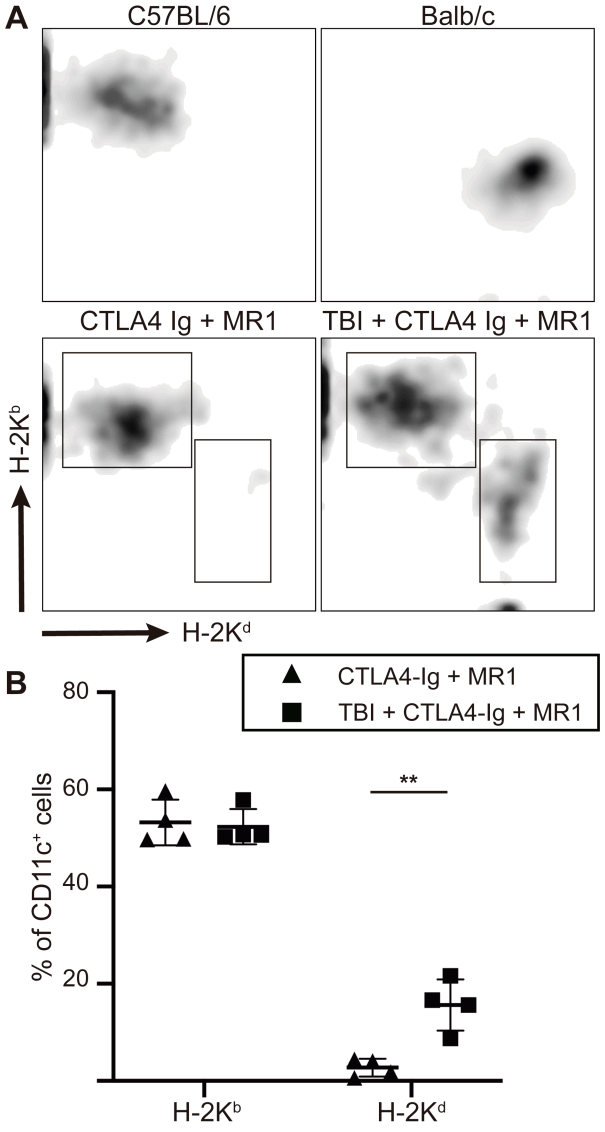
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7