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Supporting Information

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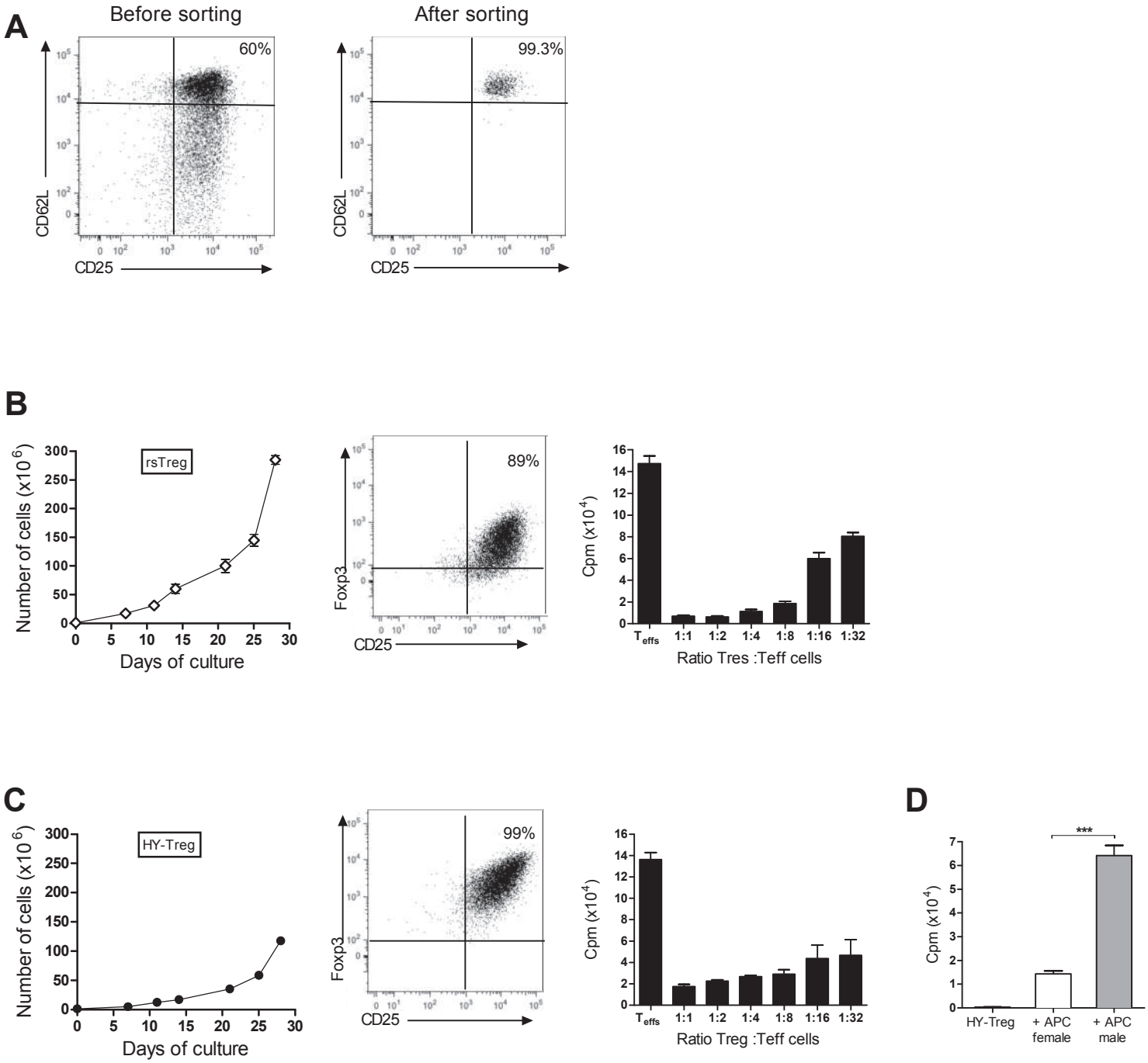
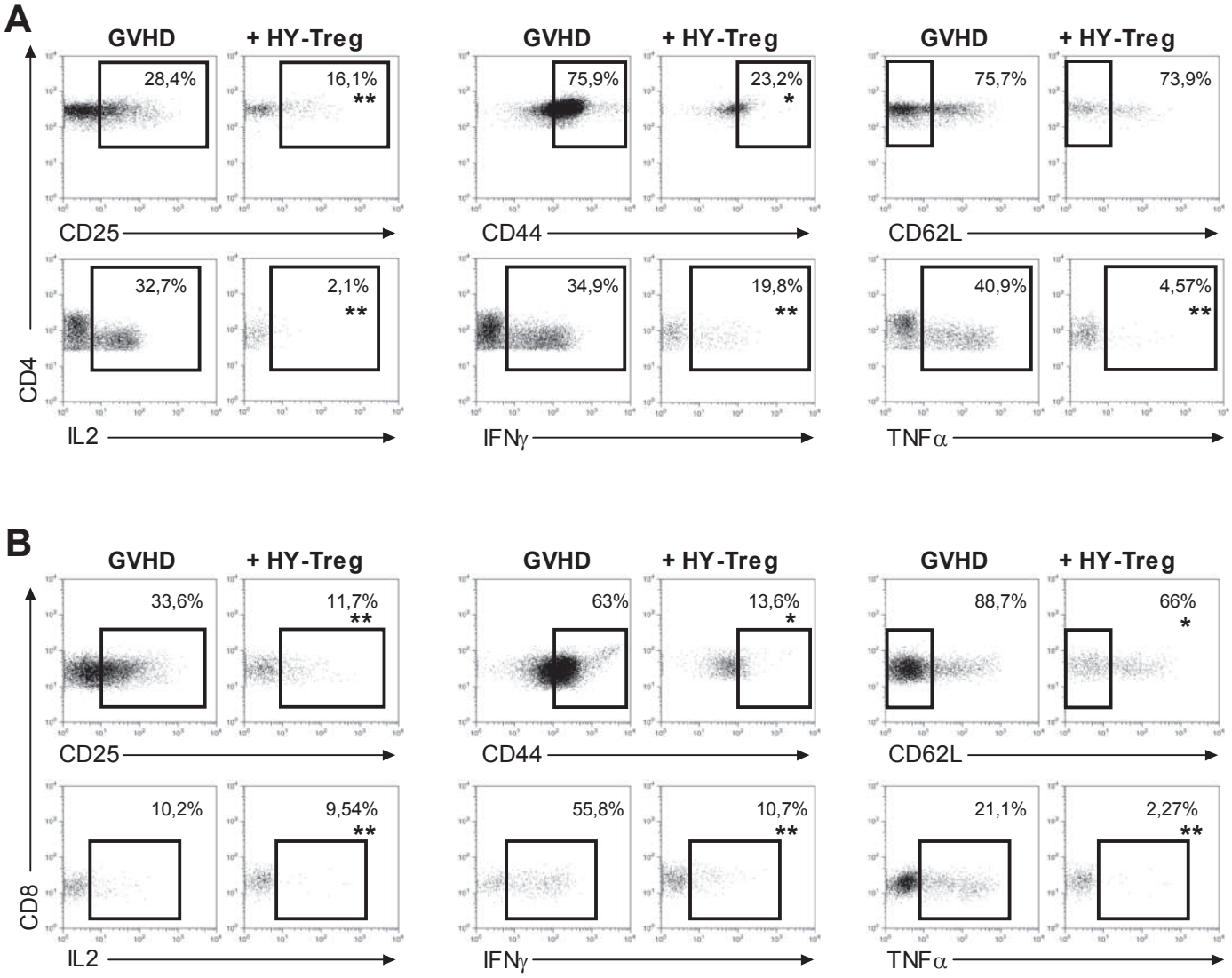
In vivo activation of transferred regulatory T cells specific for third-party exogenous antigen controls GVH disease in mice

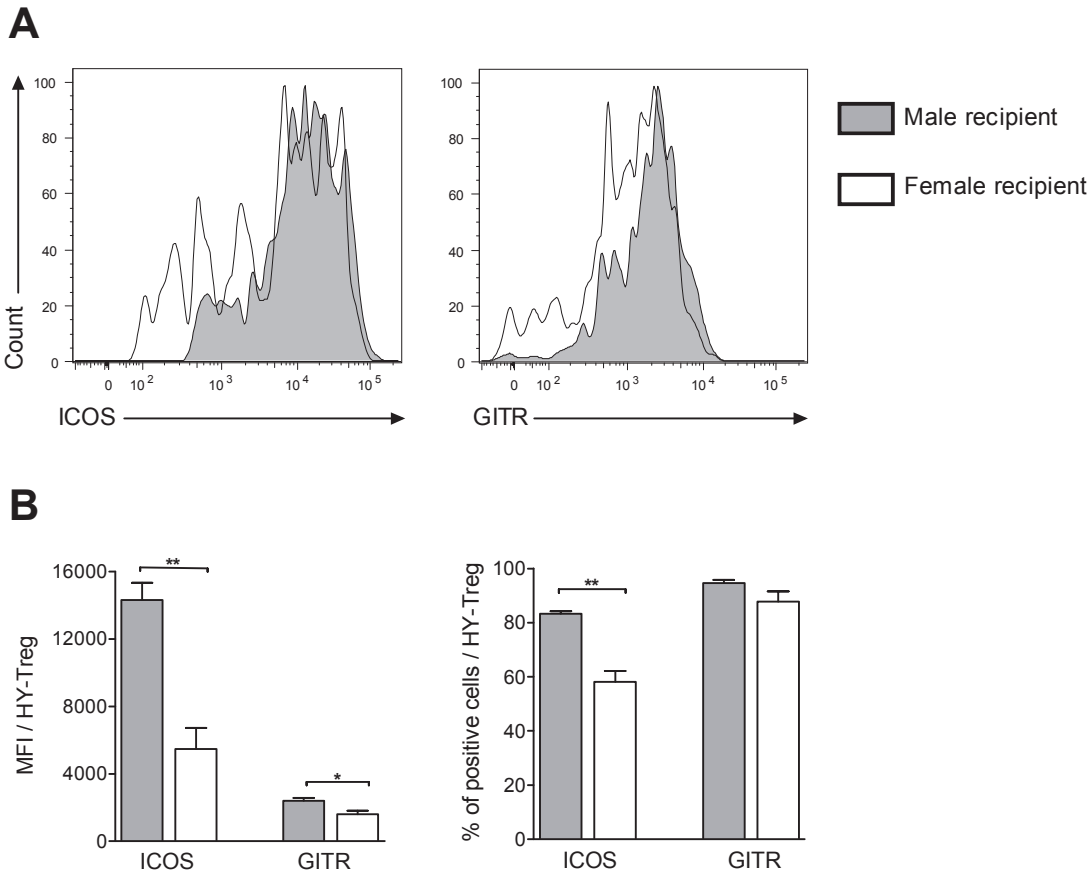
Supporting Information- Figure 1



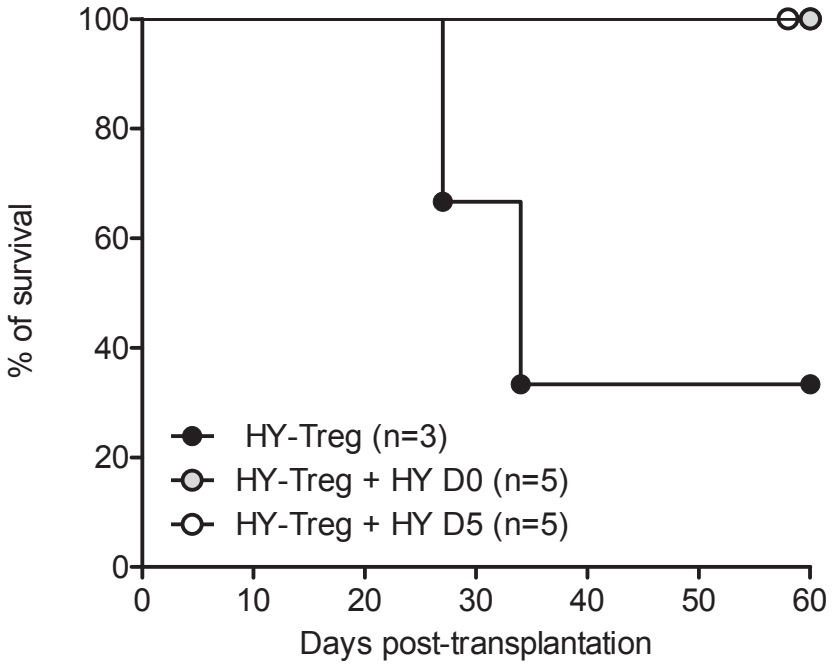
Supporting Information - Figure 2



Supporting Information - Figure 3



Supporting Information - Figure 4



Supporting informations - Figure legends

Figure 1. Expansion of antigen-specific Treg cells. (A) Isolation of highly purified Treg cells. Representative dot plot obtained before (left) and after Treg cells sorting (right). Highly purified Treg cells were cultured with C3H APC to select and expand rsTreg cells or with autologous B6 $CD8^+$ DCs loaded with HY peptide to expand HY specific Treg cells (HY-Treg). (B-C) Ex vivo expansion of rsTreg cells (B) and HY-Treg cells (C) during 30 days (left), phenotype of Treg cells by flow cytometry at the end of the culture and expression of $CD25^+Foxp3^+$ cells among $CD4^+$ cells is shown (middle). Suppressive activity of Treg cells was measured by in vitro proliferation of T cells cultured with DC and anti-CD3 and then determined by the incorporation of ^3H methyl-thymidine. Histograms represent mean ± SEM (right). (D) In vitro specific proliferation assay of HY-Treg cells in presence of APC obtained from B6 male or female was determined by 3H-methyl-thymidine incorporation. Data shown are pooled from 2 experiments performed. **p<0.01, two-tailed unpaired Student's *t* test.

Figure 2. HY-Treg cells prevent GVH disease by reducing activation and differentiation of donor Teff cells. Donor $CD4^+$ and $CD8^+$ Teff cells are analyzed at day 6 post-transplantation in the spleen of animals grafted as described in Fig.1A and identified using the $CD45.1^+$ congenic marker. Mean percentage ± SEM of $CD4^+$ (A) and $CD8^+$ (B) divided donor T cells ($CD45.1^+CFSE^{low}$) expressing membrane CD25, CD44, CD62L or intracellular IL-2, IFN-g and TNF-α with or without HY-Treg cells (n=4 in each group). Data shown are pooled from 2 experiments performed. *p<0.05, **p<0.01, two-tailed unpaired Student's *t* test.

Figure 3. Prevention of GVH disease is associated with high expression of activation markers on HY-Treg cells in the presence of their cognate antigen. HY-Treg cells are collected at day 6 post-transplantation in the spleen of female or male grafted animals, identified by their CD90.2+CD4+H2Kk- phenotype and analyzed for ICOS and GITR expression. (A) Representative histograms of ICOS (left) and GITR (right) expression by HY-Treg cells in male (grey) or in female (white) B6C3F1 recipients. (B) Mean MFI ± SEM (left) and percentage (right) of ICOS and GITR expressing HY-Treg cells in male (n=4, grey) or in female (n=4, white) B6C3F1 recipients. *p<0.05, **p<0.01, two-tailed unpaired Student's *t* test.

Figure 4. Treatment of GVH disease by delayed HY-Treg cells activation. Female B6C3F1 were lethally irradiated and grafted with BM cells, Teff cells and HY-Treg cells as described in Fig. 5A. Kaplan-Meier survival curves from control mice injected with Teff cells and HY-Treg cells (black circle), mice intravenously injected with 100µg HY peptide at D0, D2 and D5 (grey circle) or at D5, D7 and D9 (white circle) post-transplantation.