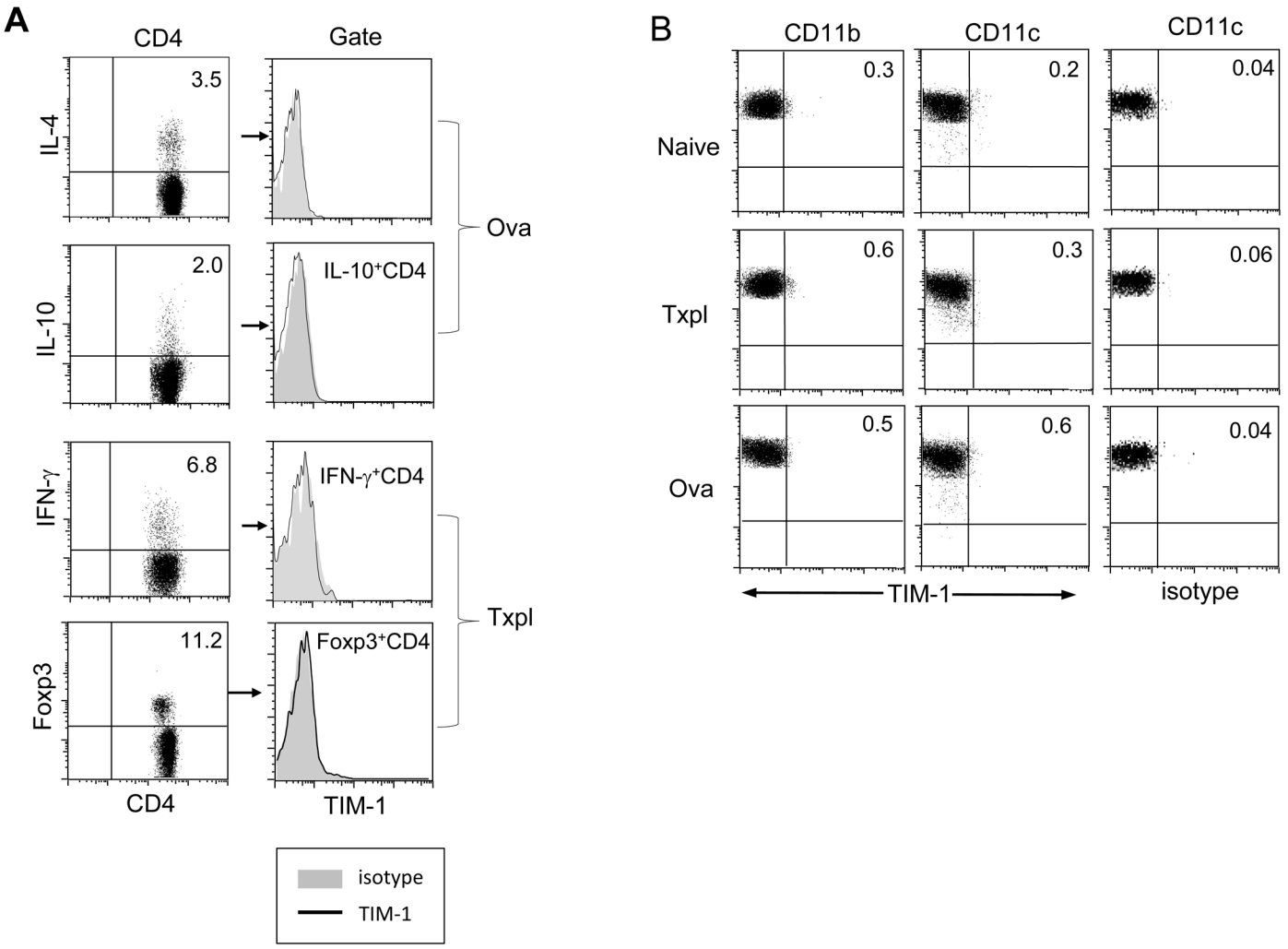
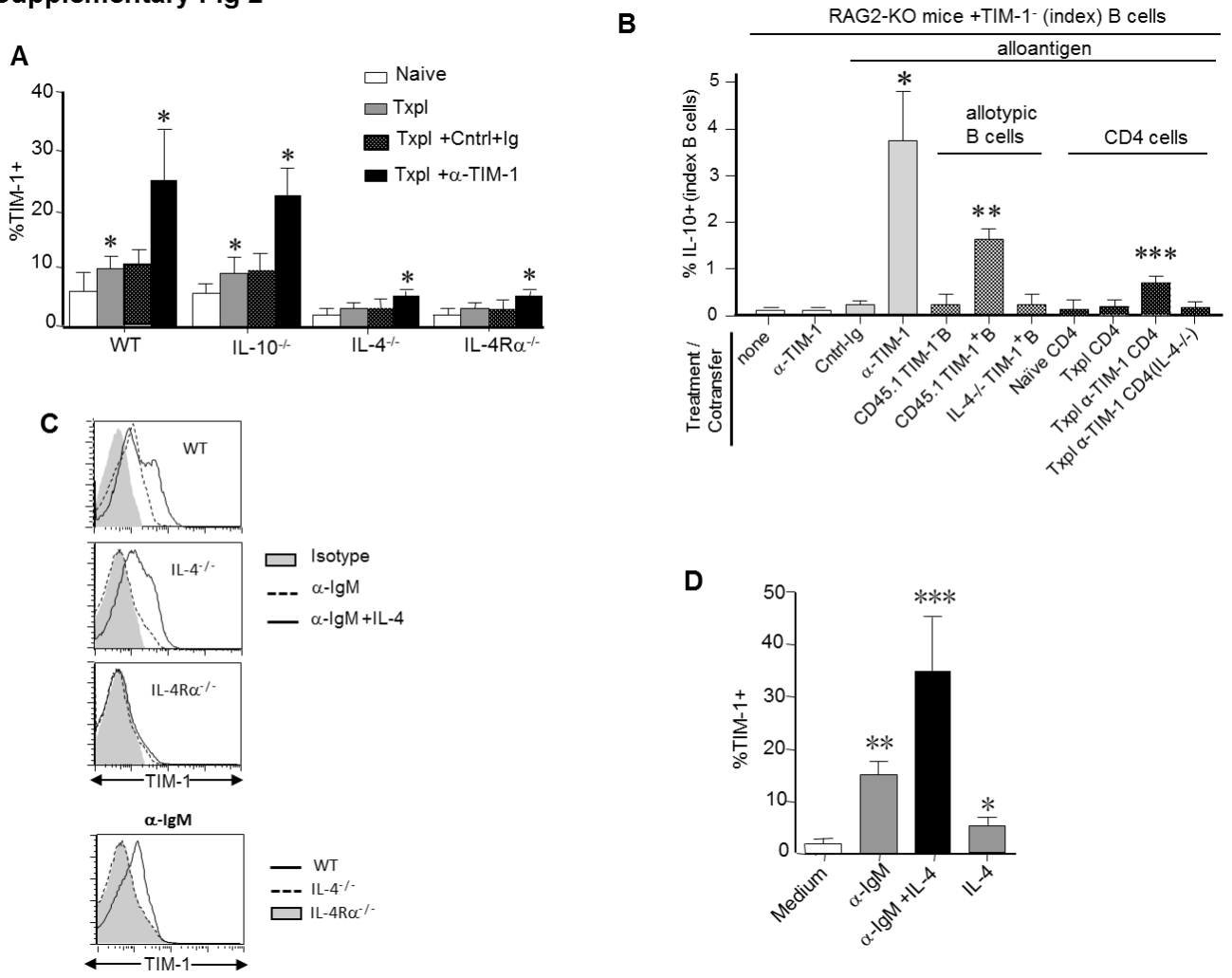
Supplementary Fig 1



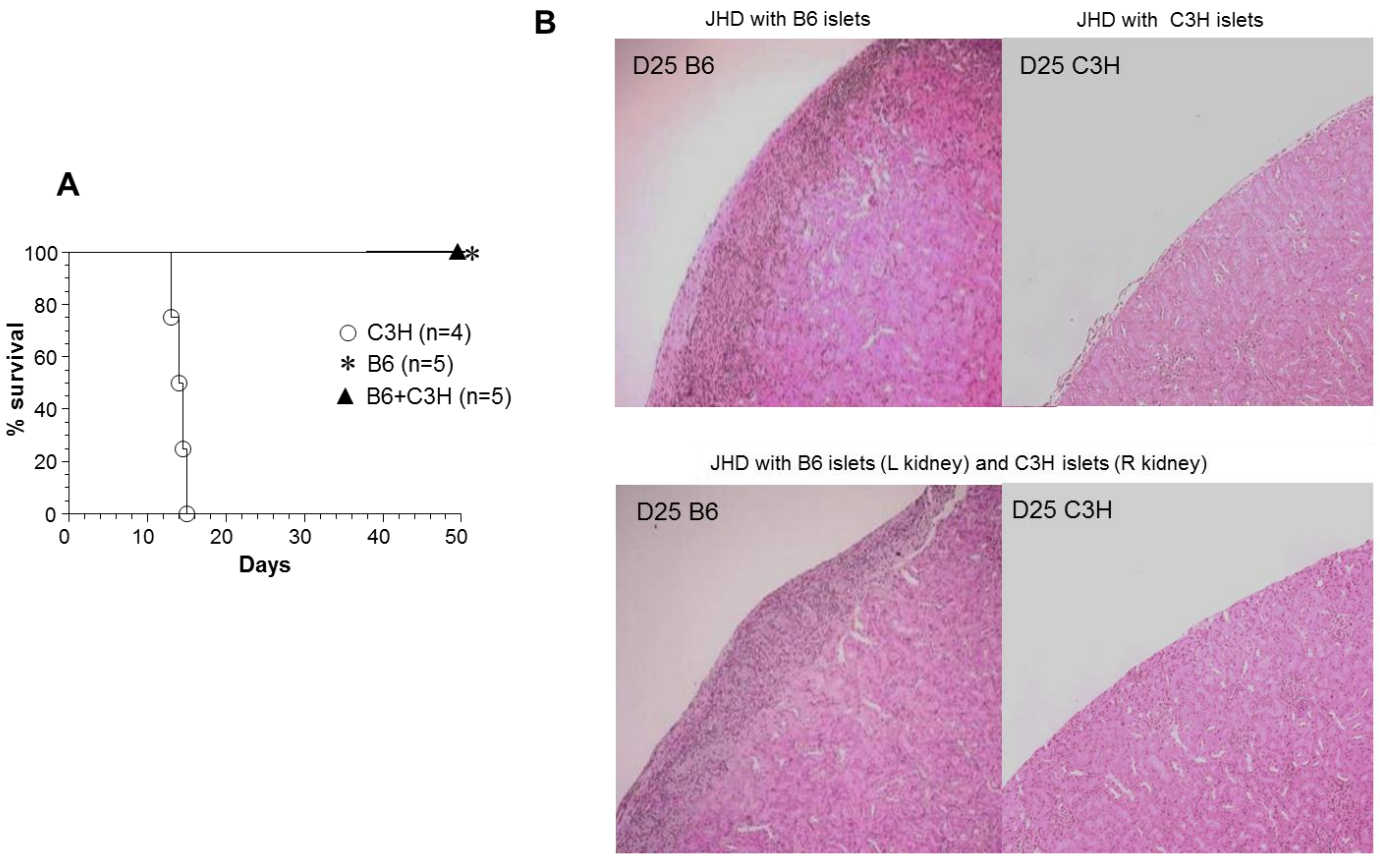
Supplementary Figure 1: TIM-1 expression on CD4 subsets and innate immune cells before and after in vivo activation. Representative expression of TIM-1 on splenocytes determined by flow cytometry in naïve BALB/c mice vs. BALB/c mice 14d after immunization with C57BL/6 islets (Txpl) or Ovalbumin (Ova). (**A**) TIM-1 expression on CD4 subsets: CD4 subsets were detected by intracellular cytokine or Foxp3 expression (left column). Shown are Th2 (IL-4 and IL-10) expression after Ova and Th1 (IFNγ) and Treg (Foxp3) expression after Txpl on CD4 cells. Numbers show the percent of cells expressing cytokines in each histogram. In the right column, CD4 cells expressing each cytokine were examined for TIM-1 expression (black line) compared to isotype control (solid gray). (**B**) TIM-1 expression gated on $CD11b^+$ and $CD11c^+$ cells from spleen of naïve or immunized (Ova or Txpl) BALB/c mice. Isotype controls for TIM-1 staining were used to set cursors (shown for $CD11c^+$ cells). n=5 mice/group.

Supplementary Fig 2



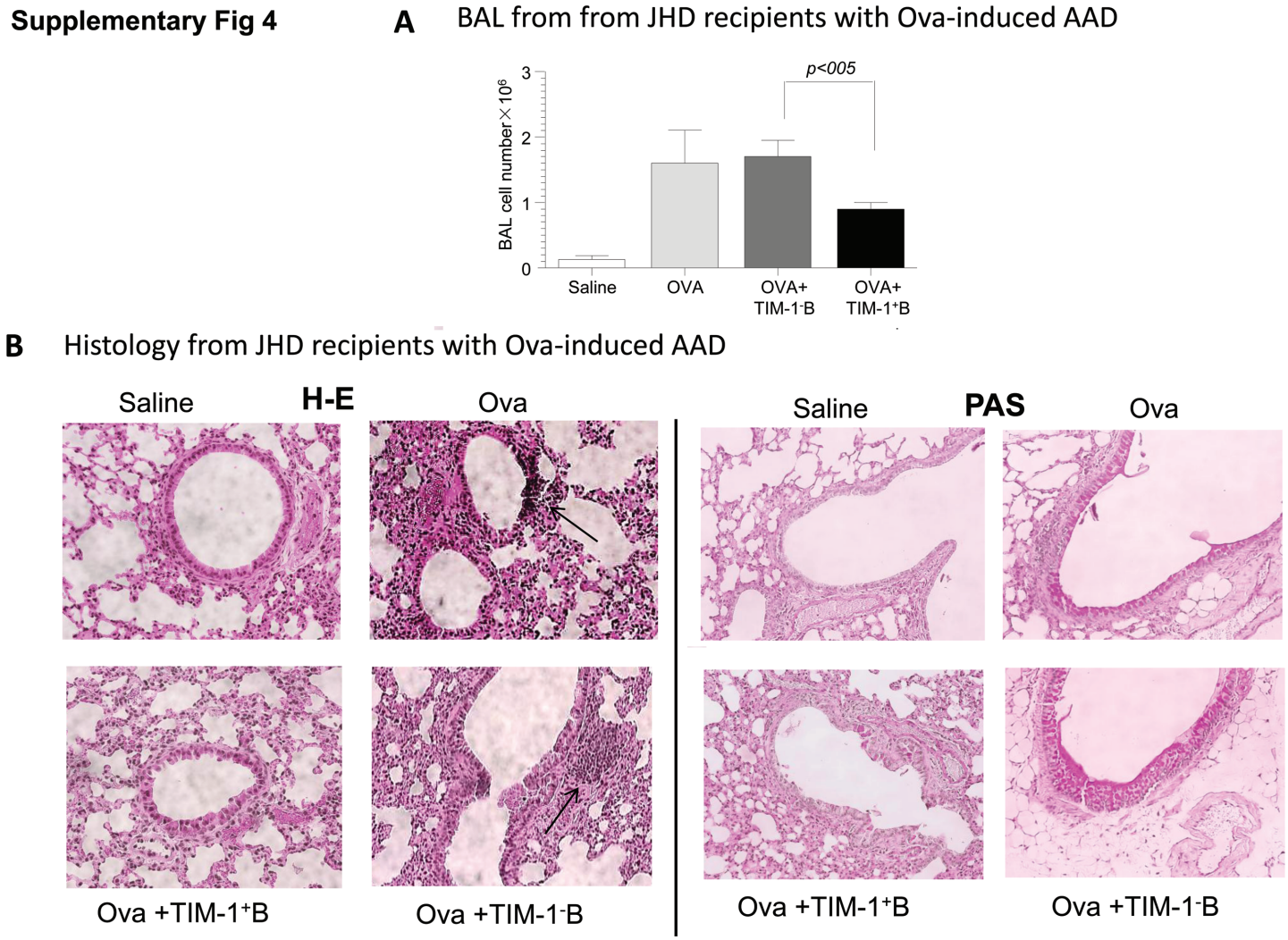
Supplementary Figure 2: TIM-1 expression in vivo and in vitro, is dependent on BCR ligation and IL-4 signaling. (**A**) Frequency (mean+SD) of TIM-1$^+$ cells amongst splenic CD19$^+$ B cells from WT, IL-10$^{-/-}$, IL-4$^{-/-}$, or IL-4R$\alpha^{-/-}$ naïve BALB/c mice or those that received C57BL/6 islet allografts with or without α-TIM-1 treatment (d14). n=5 mice/group. $*$ p<0.05 vs. WT. **(B)** 10^7 sorted TIM-1$^-$ ("index") B cells from naïve BALB/c mice were adoptively transferred into syngeneic RAG2-KO mice that were naïve or those that received C57BL/6 islets. Recipient mice were treated with control Ig (contrl-Ig) or α-TIM-1, or received 10^7 sort-purified allotypic TIM-1$^-$ or TIM-1$^+$ B cells from allo-stimulated wt or IL-4$^{-/-}$ BALB/c mice. Alternatively, mice received 10^7 sort-purified CD4 cells from wt or IL-4-/- BALB/c mice (naïve; allostimulated (Txpl); or allostimulated and treated with α-TIM-1 (Txpl +α-TIM-1)). Shown is frequency (mean+SD) of IL-10 expression on index B cells in spleen 14d after transfer (determined by flow cytometry). n=3 mice/group. *p<0.01 vs. Cntrl-Ig or naïve recipients. **p<0.05 TIM-1$^+$ B vs. cotransfer of other B cells. ***p<0.05 Txpl α-TIM-1 CD4 vs. other CD4 cell co-transfers. (**C**) Upper panel: Representative histograms showing TIM-1 expression by WT, IL-4$^{-/-}$ or IL-4R$\alpha^{-/-}$ TIM-1$^-$ B cells after stimulation with α-IgM (dashed line) vs. α-IgM plus IL-4 (solid line). Isotype control is shown (gray fill). n=3 independent experiments. Lower panel: Overlay of histograms from above comparing TIM-1 expression on α-IgM treated WT, IL-4$^{-/-}$, or IL-4R$\alpha^{-/-}$ TIM-1$^-$ B cells. **(D)** Frequency (mean+SD) of TIM-1 expression on purified CD19$^+$ TIM-1$^-$ B cells after in vitro culture (48 hours) in media alone or in the presence of α-IgM and/or rIL-4, as indicated. $*$, **, *** p<0.01 vs. each other group. n= 4 independent experiments.

Supplementary Fig 3



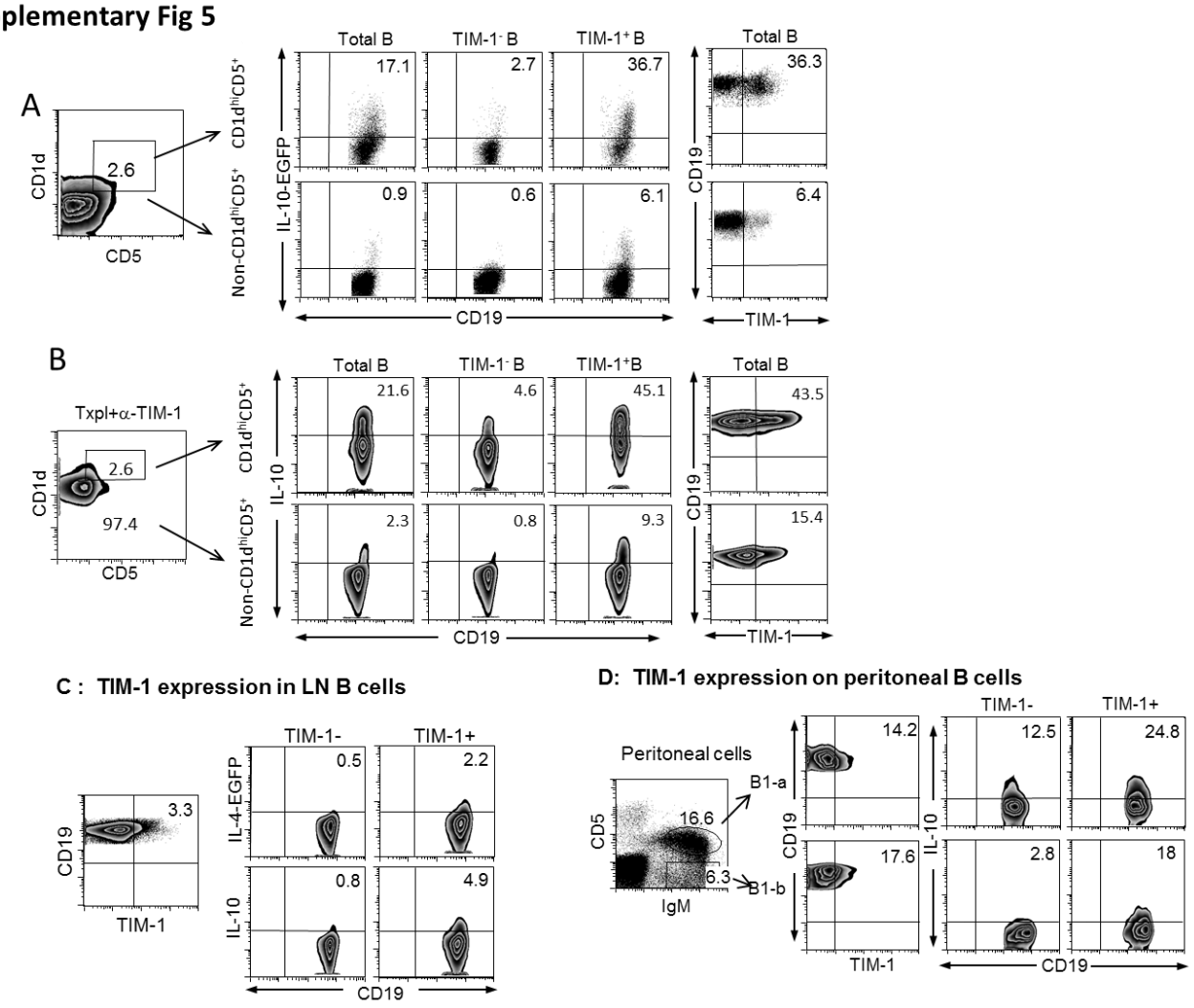
Supplementary Figure 3: TIM-1$^+$ B cells exhibit antigen-specific regulatory activity even when relevant and irrelevant antigens are present in the same host. 1X10^7 sort-purified TIM-1$^+$ splenic B cells from BALB/c mice allo-stimulated with C57BL/6 splenocytes (ip) (d14) were transferred into otherwise untreated JHD recipients of islet allografts from the same donor strain (C57BL/6), an unrelated strain (C3H), or both strains (with C57BL/6 islets under the left kidney capsule and C3H islets under the right kidney capsule). **(A)** Kaplan-Meir plots of graft survival. (**B**) Representative H+E staining of allografts taken from a cohort (3 mice/group) of recipients described above that were sacrificed on d25.

Supplementary Fig 4



Supplementary Figure 4: TIM-1+ B cells transferred from mice with local inhalational tolerance (LIT) reduce acute allergic airway disease (AAD). Mice were immunized with OVA + alum (as in Fig 1) on days 0 and 7. 5 days later, mice were exposed to 1% aerosolized OVA for 1 h/day for either 3 days (AAD) or 40 days (LIT), as previously described (31). TIM-1$^+$B or TIM-1$^+$B cells (10^7) from spleens of LIT mice were injected (iv) into JHD recipients that were then subjected to AAD. Positive control mice received no cell transfer (Ova), and negative control mice received aerosolized saline rather than Ova. Mice were sacrificed 24 h after the last aerosol treatment and the lungs were assessed for the development of AAD. n= 3 mice per group. **(A)** BAL cellularity (nucleated cell count). (**B**) Representative histological sections: H+E staining (left panel) demonstrating increased peribronchiolor infiltration by leukocytes as well as collections of infiltrating cells (arrows) in lungs from Ova and Ova +TIM-1$^-$ B recipients, compared to Saline or Ova + TIM-1$^+$ B cell recipients. PAS-staining (right panel) reveals goblet cell hyperplasia and mucous overproduction (eosinophillic material) in Ova and Ova +TIM-1$^-$ B recipients, compared to Saline or Ova + TIM-1$^+$ B cell recipients.

Supplementary Fig 5



Supplementary Fig 5: TIM-1$^+$ B cells are highly enriched for IL-10 expression in different mouse strains, after transplantation, and in LN and peritoneum in addition to spleen.

(**A and B**) Representative expression of CD5 vs. CD1d on CD19$^+$ B cells (left plot) with the CD1dHiCD5$^+$ population indicated by the rectangular gate. The right panel shows IL-10 and TIM-1 expression on CD19$^+$ (Total B), and on TIM-1$^+$ and TIM-1$^-$ B cells within the CD1dHiCD5$^+$ and non-CD1dHiCD5$^+$ B cell populations. Data are similar to Fig 7A (naïve BALB/c mice), except that here, **(A)** shows splenocytes from naïve C57BL/6 IL-10 reporter mice, and **(B)** shows splenocytes from α-TIM-1 treated BALB/c allograft recipients (d14). The numbers in each flow cytometry plot represent the percent of gated cells within the designated area. Data are representative of 3-6 mice/group. (**C**) Representative expression of TIM-1, IL-4 and IL-10 on B cells in peripheral LN from naïve BALB/c mice. CD19$^+$ B cells were assessed for TIM-1 expression (left plot) and cytokine expression was assessed on TIM-1$^+$ and TIM-1$^-$ B cell gates (right panel). The numbers in each flow cytometry plot represent the percent of gated cells within the designated area. Data are representative of three independent experiments. (**D**) Representative expression of TIM-1 and IL-10 on peritoneal B1 subsets. Peritoneal washout cells from naïve BALB/c mice were divided into B1a and B1b subsets based on CD5 expression (left plot). CD19$^+$ B cells were assessed for TIM-1 expression and IL-10 expression was assessed on TIM-1$^+$ and TIM-1$^-$ B cells. Data are representative of 5 independent experiments. The numbers in each flow cytometry plot represent the percent of gated cells within the designated area.