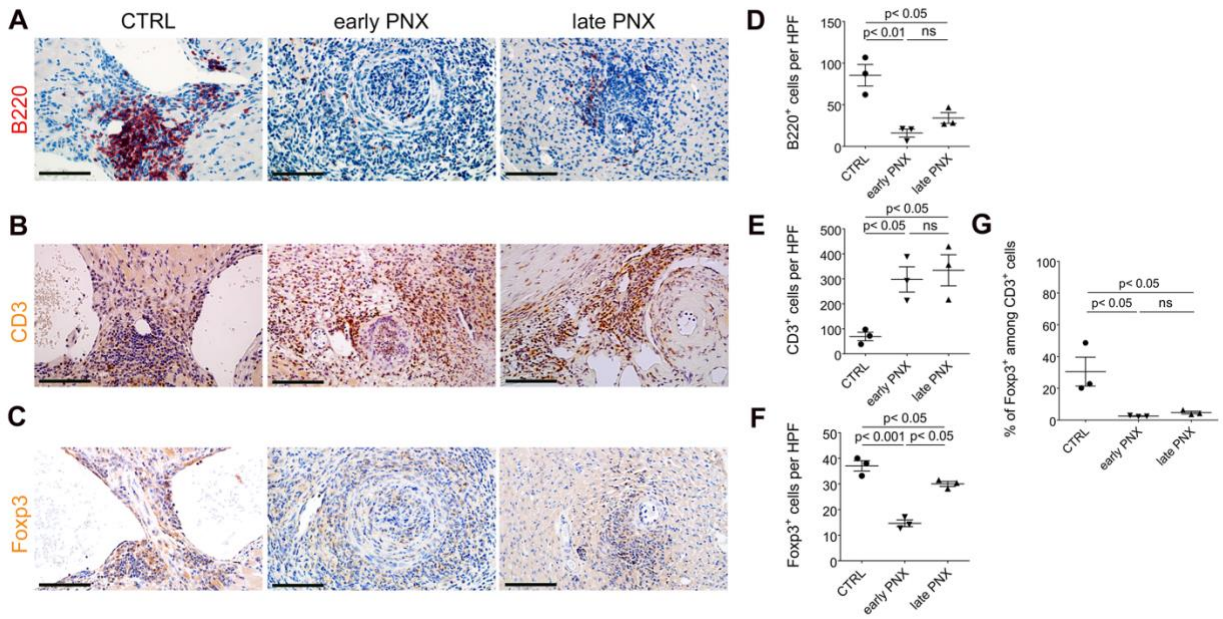

Figure S1. Foxp3+ and B cells are enriched in tolerant heart allografts. Representative immunostaining for (**A**) B cells (B220) (red), (**B**) T cells (CD3) (brown) and (**C**) Foxp3+ cells (brown) in Balb/c hearts that were transplanted into B6 mice that had received Balb/c lungs under peri-operative co-stimulatory blockade at least 30 days prior without (control (CTRL), left column), with removal of the tolerant pulmonary allograft 24 hours prior to cardiac transplantation (early pneumonectomy (PNX), middle column) and with removal of the tolerant pulmonary allograft 30 days after cardiac transplantation (late pneumonectomy (PNX), right column). Graphic representation of (**D**) B cells, (**E**) T cells, (**F**) Foxp3 cells per high power field (HPF) and (**G**) Foxp3 / CD3 ratios in heart allografts in control, early pneumonectomy and late pneumonectomy groups (6 HPFs analyzed per sample, n=3 mice per group). Data are expressed as mean ± S.E.M. One-way ANOVA was used to compare the means. ns= not significant. Scale bar 100μm.

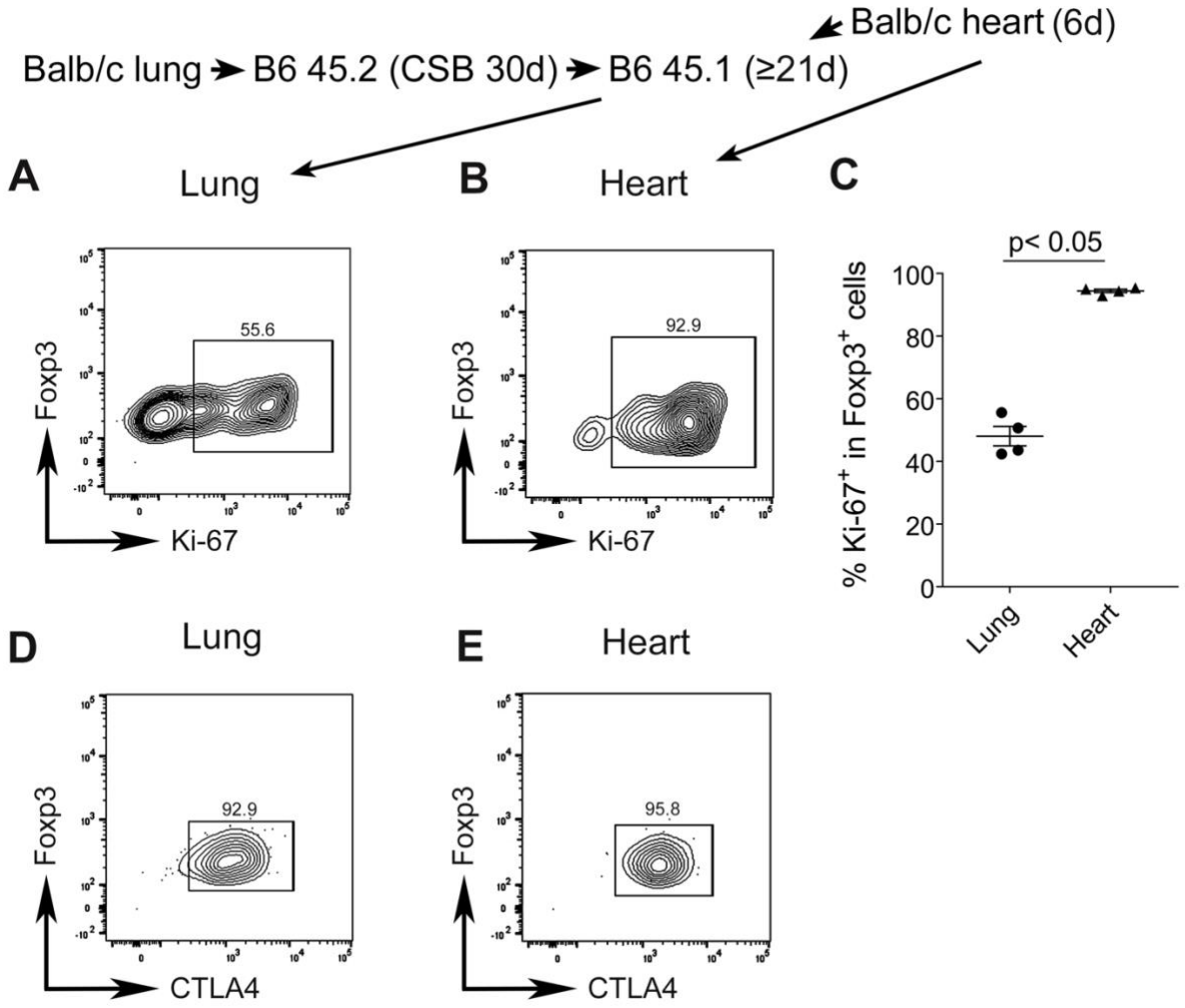

Figure S2. CD4+Foxp3+ T cells proliferate in lung and heart allografts. Representative contour plots depicting Ki-67 expression in CD90.2+CD45.2+CD45.1-CD4+CD8-Foxp3+ cells in (**A**) lung and (**B**) heart allografts 6 days after transplantation of Balb/c hearts into non-immunosuppressed B6 CD45.1 mice that had received tolerant Balb/c lung allografts at least 21 days prior. The Balb/c lung had originally been transplanted into a B6 CD45.2 host that was treated with peri-operative co-stimulatory blockade and re-transplanted 30 days later. (**C**) Graphic representation of percentage of

Ki-67 expressing CD90.2^{+}CD45.2^{+}CD45.1^{-}CD4^{+}CD8^{-}Foxp3^{+} cells in lung and heart allografts (n=4 per compartment). Data are expressed as mean ± S.E.M. Mann-Whitney U test was used to compare the means. Representative contour plots depicting intracellular CTLA4 expression in CD90.2^{+}CD45.2^{+}CD45.1^{-}CD4^{+}CD8^{-}Foxp3^{+} cells in (**D**) lung and (**E**) heart allografts 6 days after transplantation of Balb/c hearts into non-immunosuppressed B6 CD45.1 mice that had received tolerant Balb/c lung allografts at least 21 days prior. The Balb/c lung had originally been transplanted into a B6 CD45.2 host that was treated with peri-operative co-stimulatory blockade and re-transplanted 30 days later (n=2 per compartment).

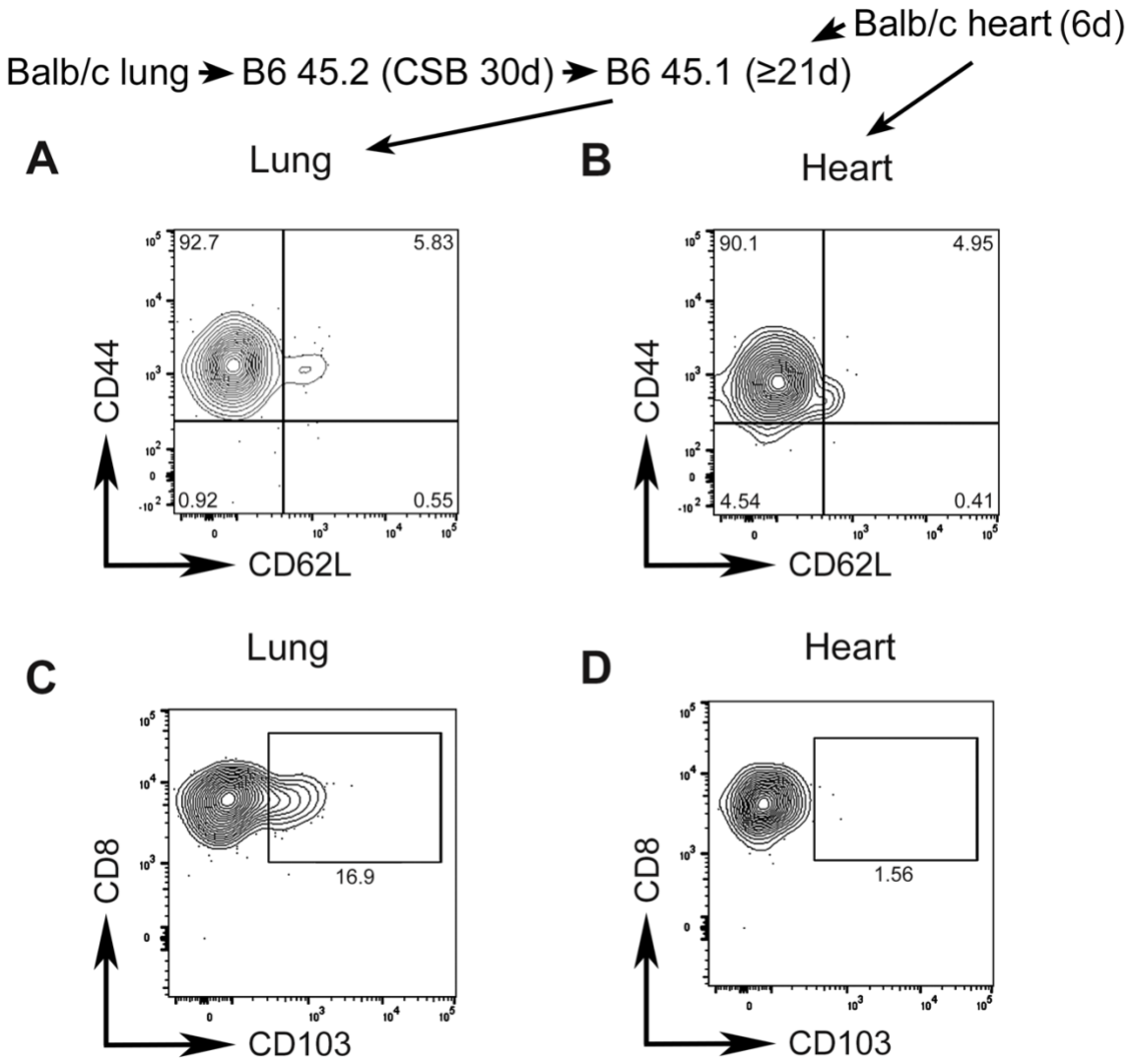

Figure S3. CD8+ T cells migrate from tolerant lung allografts to the periphery. Representative contour plots depicting CD44 and CD62L expression on CD90.2+CD45.2+CD45.1-CD4-CD8+ T cells in (**A**) lung and (**B**) heart allografts 6 days after transplantation of Balb/c hearts into non-immunosuppressed B6 CD45.1 mice that had received tolerant Balb/c lung allografts at least 21 days prior. The Balb/c lung had originally been transplanted into a B6 CD45.2 host that was treated with peri-operative

co-stimulatory blockade and re-transplanted 30 days later (n=4 per compartment). Representative contour plots depicting CD103 expression in $CD90.2^{+}CD45.2^{+}CD45.1^{-}CD4^{-}CD8^{+}$ T cells in (**C**) lung and (**D**) heart allografts 6 days after transplantation of Balb/c hearts into non-immunosuppressed B6 CD45.1 mice that had received tolerant Balb/c lung allografts at least 21 days prior. The Balb/c lung had originally been transplanted into a B6 CD45.2 host that was treated with peri-operative co-stimulatory blockade and re-transplanted 30 days later (n=2 per compartment).

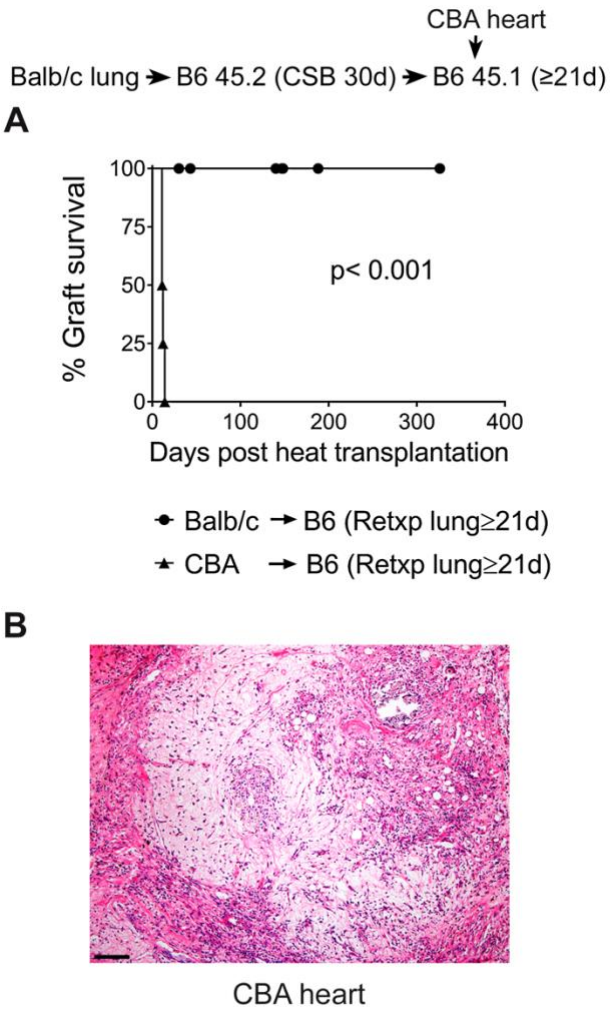

Figure S4. Peripheral tolerance is donor antigen-specific. (**A**) Kaplan-Meier survival curves of Balb/c (●) (n=7) or CBA (▲) (n=4) hearts that were transplanted into non-immunosuppressed B6 mice that received tolerant Balb/c pulmonary allografts at least 21 days before cardiac transplantation. The Balb/c lungs had been originally engrafted into B6 mice that received peri-operative co-stimulatory blockade and then re-transplanted at least 30 days later. (**B**) Histological appearance (H&E) of CBA hearts after transplantation into B6 mice into which a tolerant Balb/c lung allograft was re-transplanted at least 21 days prior. Scale bar 100μm.

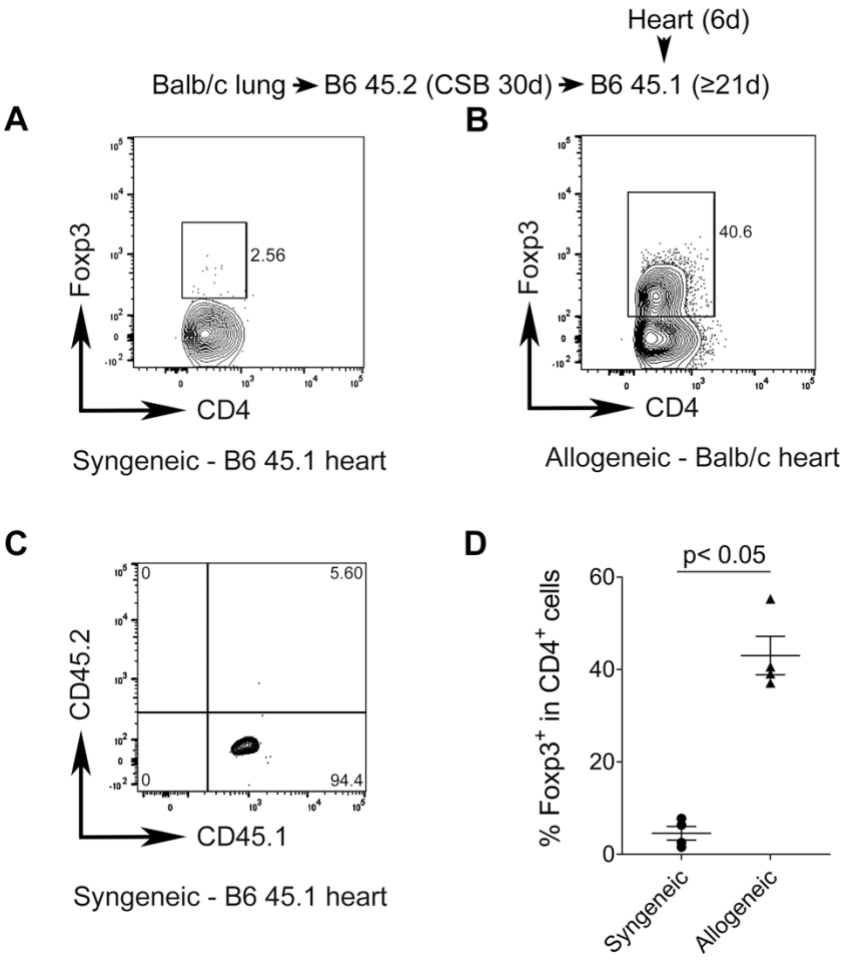

Figure S5. Few CD4+Foxp3+ T cells infiltrate syngeneic heart grafts. Representative contour plots depicting Foxp3-expressing CD4+ T cells in (**A**) syngeneic B6 or (**B**) allogeneic Balb/c heart grafts that were transplanted into non-immunosuppressed B6 CD45.1 mice that had received tolerant Balb/c lung allografts at least 21 days prior. The Balb/c lung had originally been transplanted into a B6 CD45.2 host that was treated with peri-operative co-stimulatory blockade and re-transplanted 30 days later. Plots are gated on live CD90.2+CD4+CD8- cells. (**C**) Majority of Foxp3-expressing CD4+ T cells in syngeneic grafts are derived from second recipient (CD45.1). Contour plot is gated on live Foxp3+ cells in (**A**). (**D**) Comparison of percentage of Foxp3-expressing CD4+ T cells

in syngeneic and allogeneic heart grafts. Data are expressed as mean ± S.E.M. Mann-Whitney U test was used to compare the means. n=4 per group.

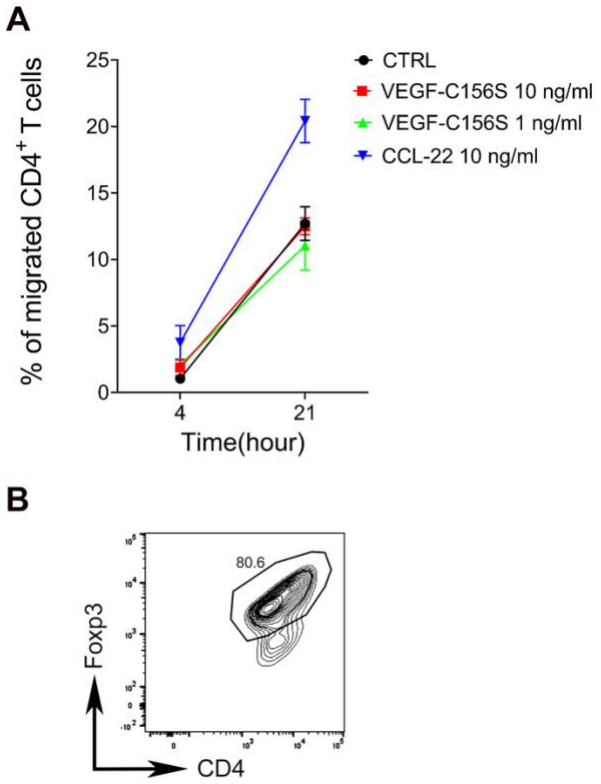

Figure S6. VEGF-C156S does not induce migration of CD4+Foxp3+ T cells. (**A**) Percentage of CD4+ T cells that migrated in response to CCL22, a chemoattractant for regulatory T cells, VEGF-C156S and control media (CTRL) 4 and 21 hours after initiation of transwell assay. Data from one representative experiment of two independent experiments are shown as mean ± S.E.M of three technical replicates. (**B**) Representative contour plot depicting Foxp3 expression on transmigrated CD4+ T cells 21 hours after initiation of transwell assay. Plot is gated on live CD90.2+CD4+ cells.

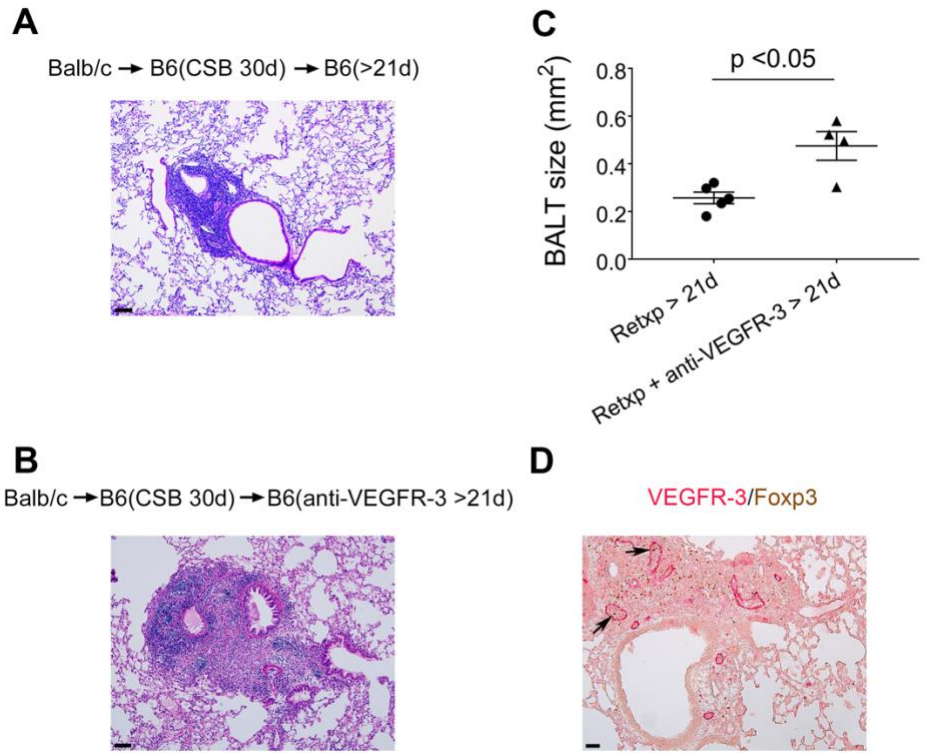

Figure S7. Cells accumulate in lymphatic vessels within bronchus-associated lymphoid tissue of lung allografts after treatment with anti-VEGFR-3. Histological appearance (H&E) of bronchus-associated lymphoid tissue (BALT) in Balb/c lungs that were initially transplanted into B6 recipients that received peri-operative co-stimulatory blockade (CSB) and then re-transplanted into (**A**) non-immunosuppressed untreated or (**B**) anti-VEGFR-3-treated B6 mice. Images depict grafts between 30 and 34 days after re-transplantation. Scale bars 100 μm. (**C**) Quantification of BALT size in conditions depicted in (**A**) (n=5) and (**B**) (n=4). Data are expressed as mean ± S.E.M. Mann-Whitney U test was used to compare the means. (**D**) Immunostaining of VEGFR-3 and Foxp3 in tolerant Bal/c lung allografts 31 days following re-transplantation into non-

immunosuppressed anti-VEGFR-3-treated B6 mouse. Arrows point to VEGFR-3+ lymphatic vessels within the BALT of the lung allografts. Scale bar 100 μm.

Video S1. Foxp3+ cells infiltrate heart allografts. Time-lapse intravital two-photon imaging of Foxp3+ cells (green) in Balb/c heart grafts 6 days after transplantation into B6 Foxp3 IRES-GFP recipient that was treated with peri-operative costimulatory blockade. Vessels appear red after intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 30 μm. Relative time is displayed in hrs:min:sec.

Video S2. Foxp3+ cells originating from tolerant lung allografts infiltrate heart allografts. Time-lapse intravital two-photon imaging of Foxp3+ cells (green) in Balb/c heart grafts 6 days after transplantation into B6 recipient that had received re-transplantation of tolerant Balb/c lung allograft 21 days prior. Balb/c lung had been initially transplanted into B6 Foxp3 IRES-GFP recipient that was treated with peri-operative costimulatory blockade and re-transplanted 30 days later. Vessels appear red after intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 30 μm. Relative time is displayed in hrs:min:sec.