

Figure S1

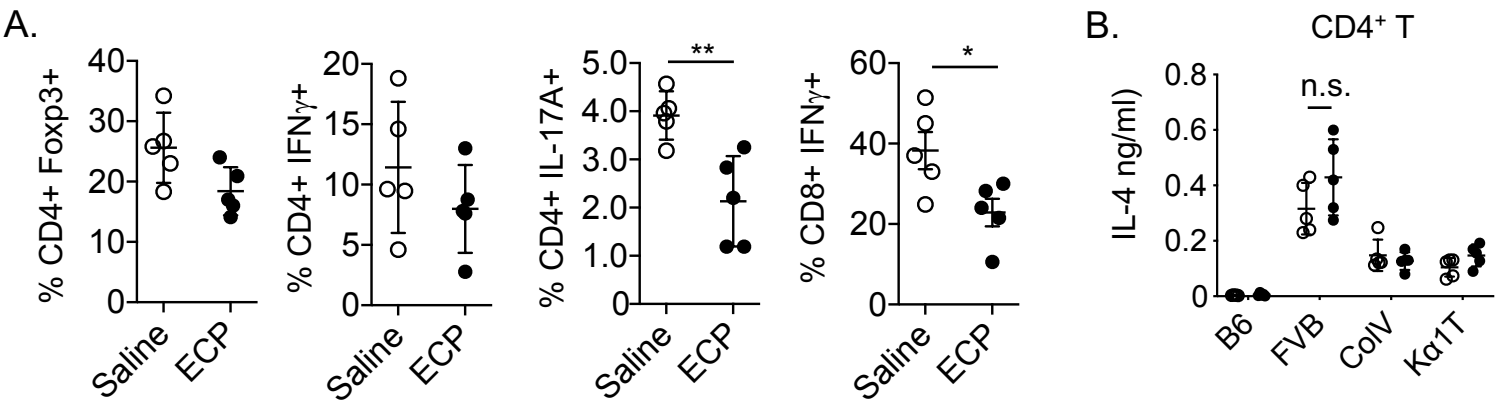


Fig. S1: ECP effects on T cell activation. (A) Intra-graft abundance for indicated effector T cells. Data shown is mean percent abundance \pm S.D.; 2-sided Mann Whitney U test; * $p < 0.05$, ** $p < 0.01$. (B) IL-4 expression following stimulation of saline (open circles) or ECP-treated (closed circles) intra-graft CD4⁺ T cells with B6 splenocytes (syngeneic antigens), FVB splenocytes (donor antigens) or B6 splenocytes pulsed with lung self-antigens Col V and K α 1T (N=5/group). Data shown are mean IL-4 culture supernatant concentration \pm S.D.; 2-sided Mann Whitney U test; where n.s. means non-significant.

Figure S2

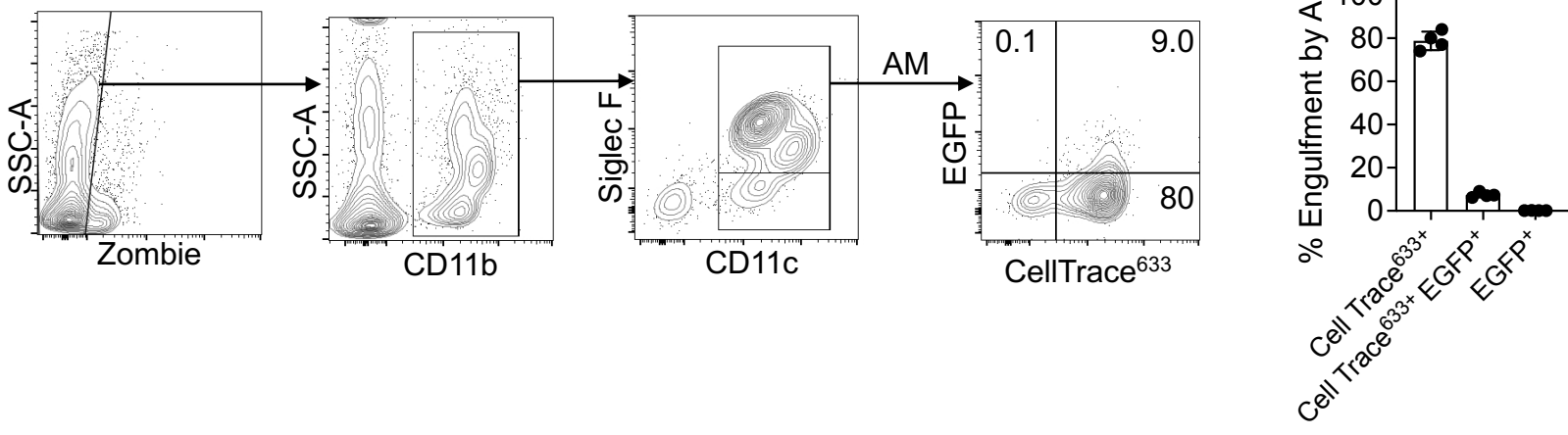


Fig. S2: ECP-treated leukocytes are rapidly engulfed by AM. Cell Trace⁶³³-labeled ECP-treated leukocytes and control EGFP⁺ non-ECP-treated leukocytes were co-transferred into 3T-FVB lung recipients and AM engulfment was analyzed two hours later. FACS plots are a representative result from 4 experiments. Histograms show pooled data of 4 experiments with mean AM percent engulfment \pm S.D.

Figure S3

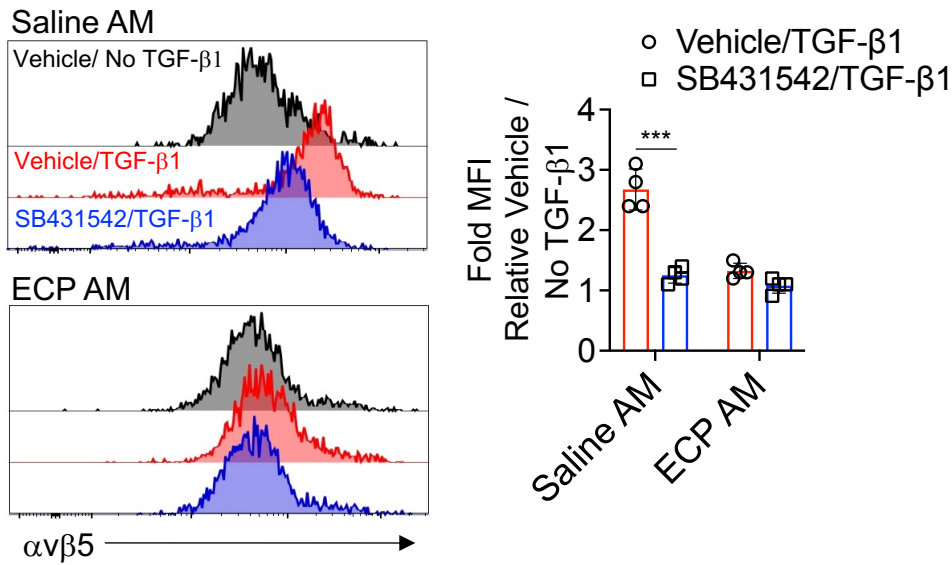


Fig. S3: ECP-treated leukocytes are engulfed by AM, which in turn induces resistance to TGF-β mediated αvβ5 upregulation. Saline and ECP-treated AM were pre-treated with 10 mM SB43152 or vehicle (DMSO) and then stimulated with or without TGF-β1. FACS plots shown are a representative result from 4 experiments. Fold MFI changes in αvβ5 expression is normalized to respective non-TGF-β-treated DMSO-pretreated controls. Data in bar graphs is pooled data from 4 experiments; Mean Fold MFI ± S.D.; 2-sided Mann Whitney U test; where ***p < 0.001 .

Figure S4

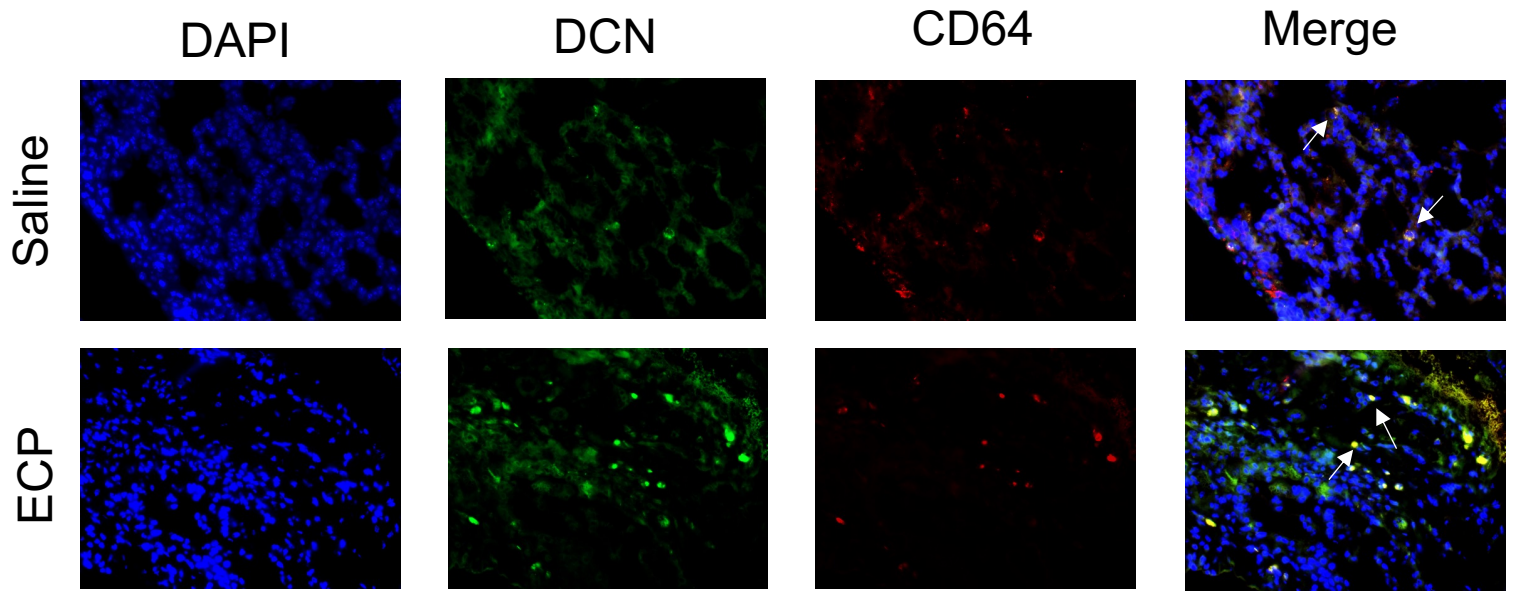


Fig. S4: ECP stimulates DCM expression in lung allograft CD64+ macrophages following induction of BOS pathogenesis. POD 16 3T-FVB allograft tissue treated with saline vehicle (N=3) or ECP (N=4) and analyzed for CD64 and DCN expression. Arrows show CD64⁺ DCN⁺ macrophages in alveolar spaces. Data shown are a representative result for each group.

Figure S5

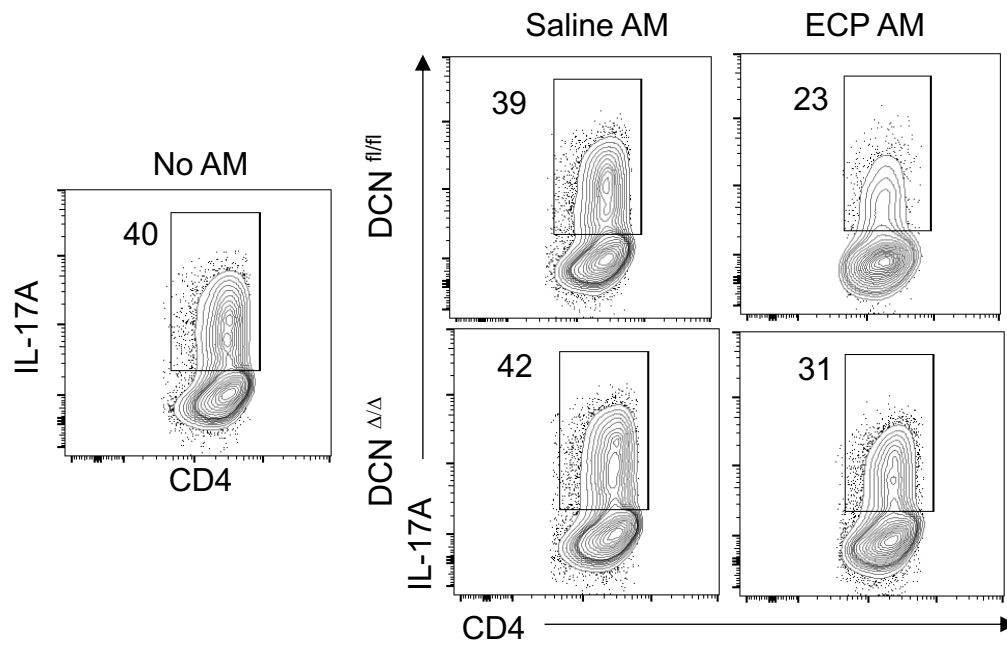


Fig. S5: ECP-treated AM inhibits T_h17 development in DCN expression-dependent manner. A representative FACS plot result from 5 experiments where plate bound CD3 ϵ and CD28 Abs were used to activate naïve B6 CD4⁺ T cells in the presence or absence of indicated AM conditioned supernatants added in a 1:1 v/v ratio to T_h17 polarization medium containing 10 ng/ml TGF- β 1. Intracellular IL-17A expression was assessed 4 days later.

Figure S6

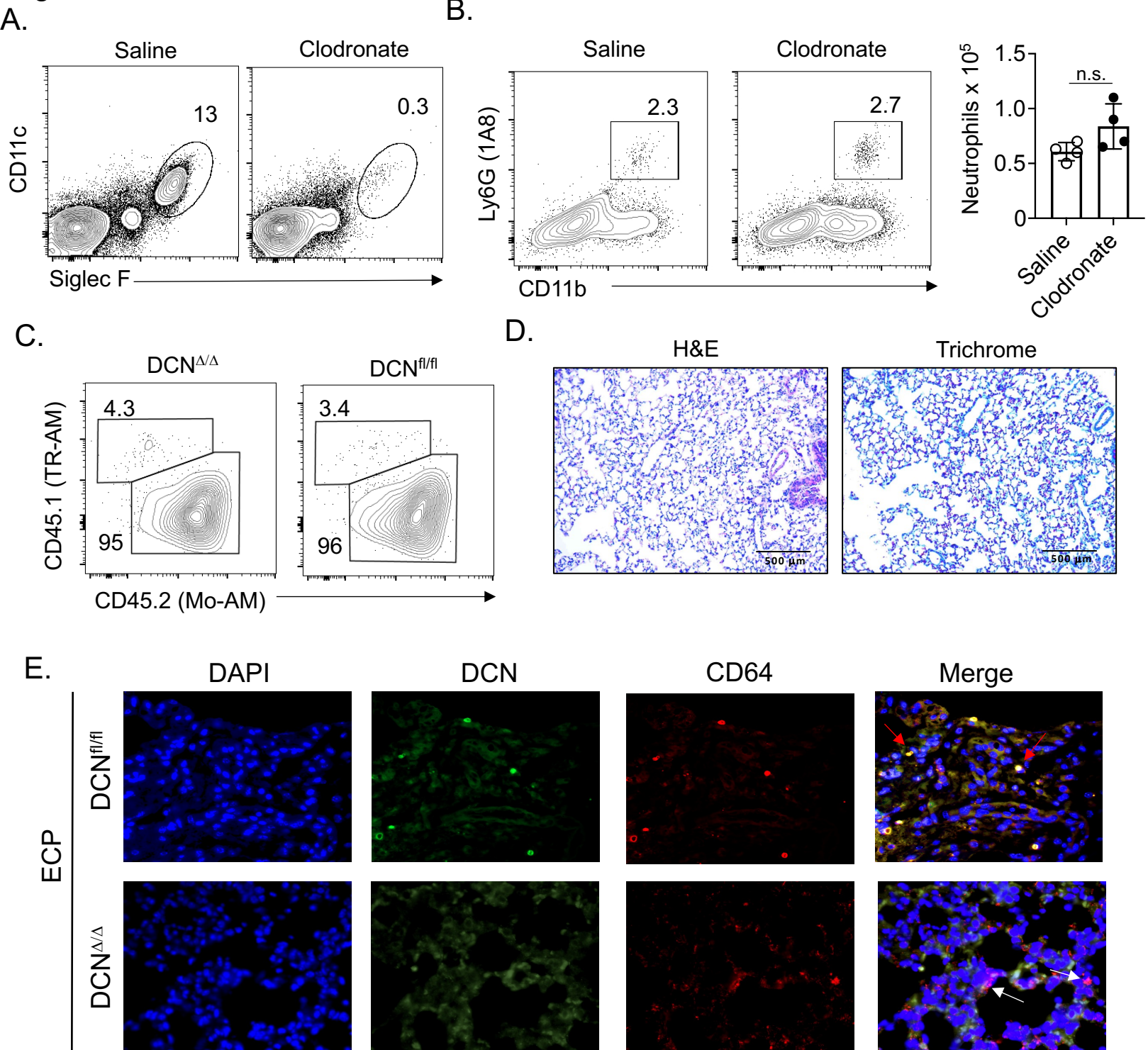
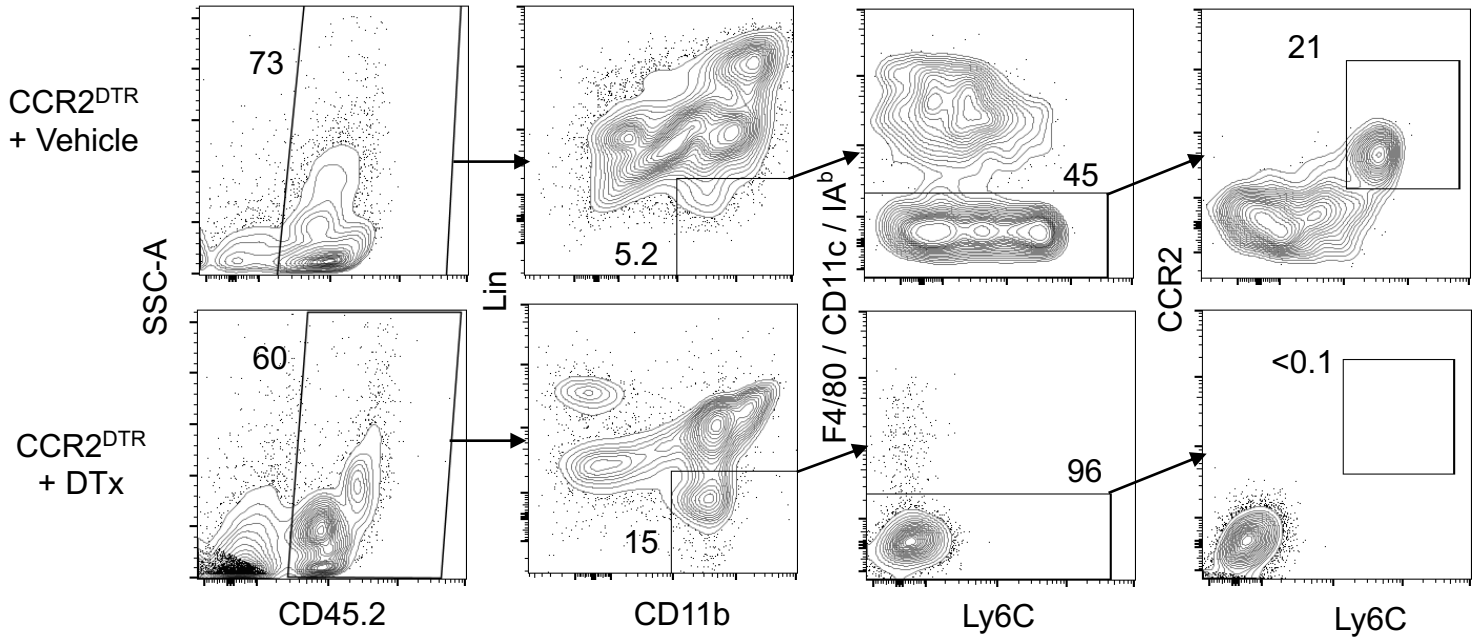


Fig. S6: Reconstitution with recipient-derived AM does not spontaneously induce OB lesions and validation of DCN antibody specificity. (A) AM depletion of donor lungs one day after clodronate depletion. Data shown is a representative FACS plot result from N=2/group (B) Intragraft neutrophilia in donor lungs one day after receiving clodronate liposomes. Data shown are representative FACS plots from N=4/group where histograms depict mean neutrophil numbers \pm S.D; 2-sided Mann Whitney U test; n.s. is non-significant. (C) Mo-AM generation in clodronate liposome-treated POD 7 DCN Δ/Δ and DCN $^{fl/fl}$ lung recipients of FVB lungs. Data shown are representative FACS plots from N=2/group. (D) POD 7 histology of a 3T-FVB allograft from a DCN Δ/Δ recipient that received clodronate liposome treatment. Histology shown is a representative result from 2 transplants. (E) POD 16 allograft tissue from ECP-treated DCN Δ/Δ and DCN $^{fl/fl}$ recipients of 3T FVB lungs evaluated for DCN expression within the CD64 compartment. Red arrows show CD64+ macrophages that express DCN. White arrows show CD64+ macrophages that lack DCN expression. Data shown are representative allograft stains from N=3/group.

A.



B.

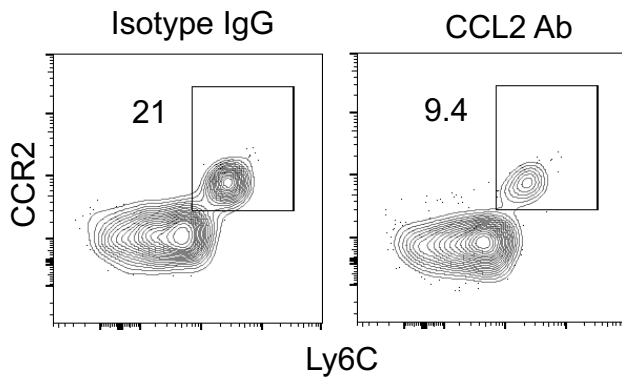


Fig. S7. Validation of monocyte depletion within lung recipients. (A) FACS plot gating strategy to measure allograft CCR2⁺ monocyte abundance of CCR2^{DTR} recipients of 3T FVB allografts 3 days after receiving PBS or 10 ng/g body weight of i.v. diphtheria toxin (DTx). Data shown is a representative result of 2 independent experiments where Lin represents a cocktail of CD90.2, B220, NK1.1 and Ly6G-specific Abs. **(B)** B6 recipients of 3T FVB allografts received 200 μ g i.v. of CCL2 neutralizing Abs on POD 6, 9 and 12 and assessed for blood peripheral CCR2⁺ accumulation on POD 16. FACS plots shown is representative result from 2 experiments.

Figure S8

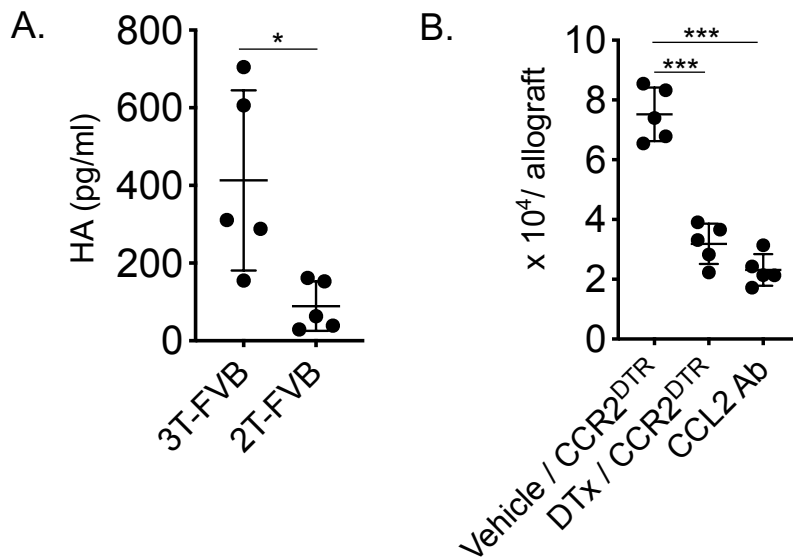


Fig. S8. HA accumulation in 3T-FVB allografts and CCR2 activity inhibition prevents intragraft Mo-AM accumulation. (A) HA concentration in the BAL fluid from POD 16 3T-FVB and 2T-FVB allograft. Data shown is a representative result from 2 determinations for N=5/group with a mean HA concentration \pm S.D.; 2-sided Mann Whitney U test; * $p < 0.05$. Intragraft 3T FVB Mo-AM numbers transplanted into POD16 CCR2^{DTR} recipients that received indicated treatments or transplanted into B6 recipients that received CCL2 Ab. Data shown is for N=5/group with mean Mo-AM numbers \pm S.D; 1-way ANOVA with Dunnett's multiple comparisons test; *** $p < 0.001$.

Figure S9

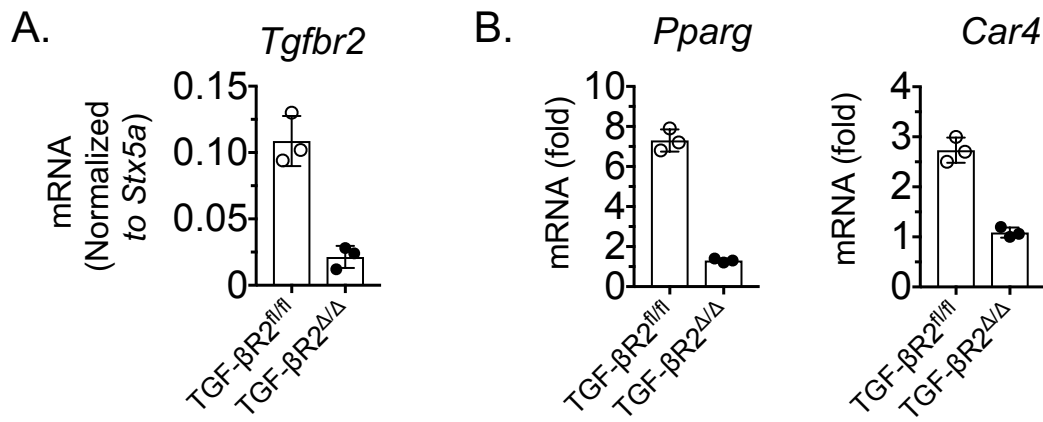


Fig. S9. Validation of TGF-βR2 deletion in CCR2⁺ monocytes. TGF-βR2^{fl/fl} and TGF-βR2^{Δ/Δ} mice were treated tamoxifen i.p. every other day for 10 days, rested for 5 days, FACS sorted for peripheral CCR2⁺ blood monocytes and assessed for (A) *Tgfbr2* transcript levels or (B) stimulated overnight with 5 ng/ml of TGF-β1 and measured for *Pparg* and *Car4* transcript levels. For (A) data shown is normalized to the macrophage housekeeping gene *Stx5a* and (B) shown normalized to TGF-βR2^{Δ/Δ} levels where bars represent means ± S.D. Data shown for A and B are representative results from 2 determinations.

Figure S10

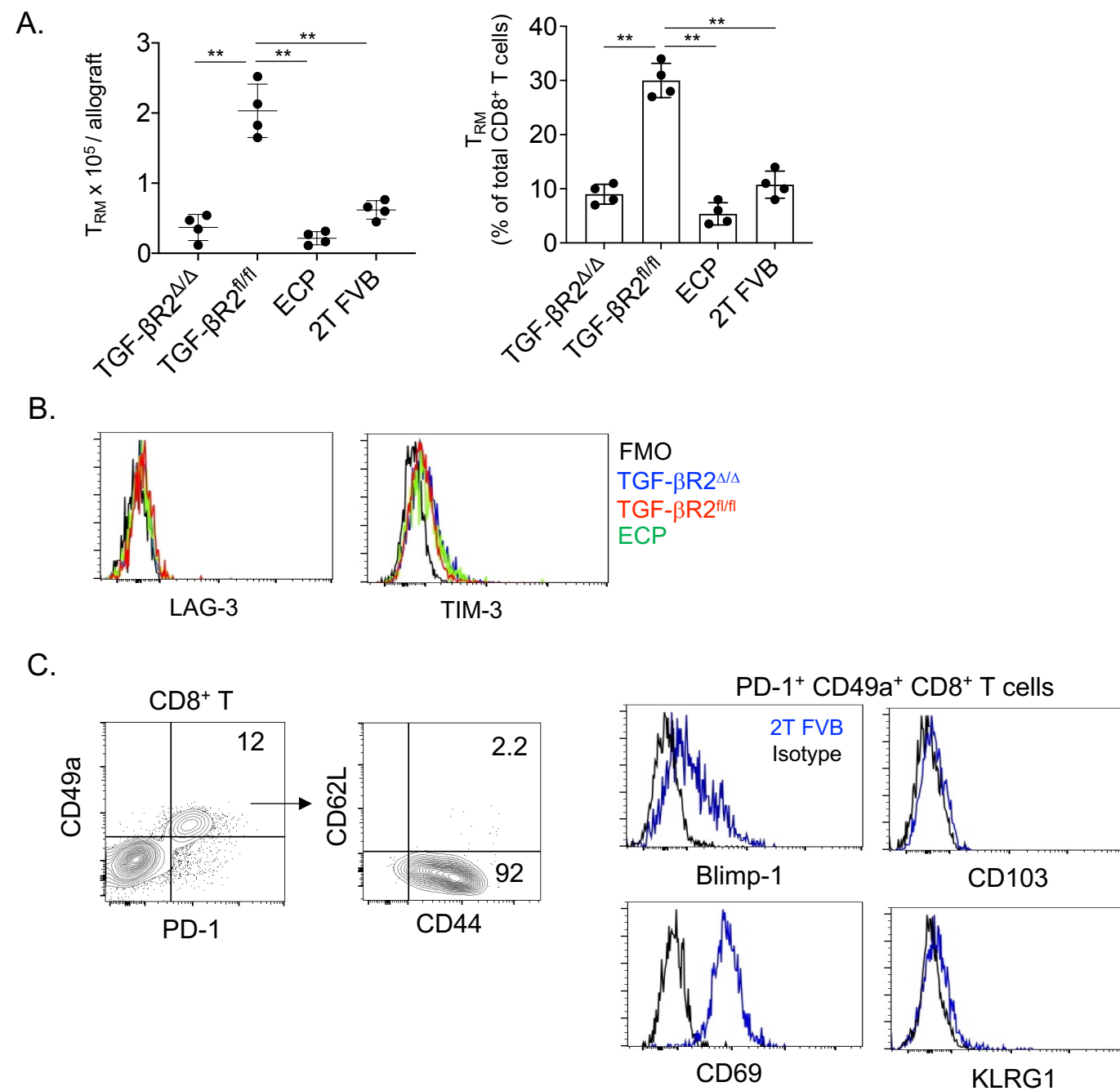


Fig. S10. T_{RM} phenotype and abundance in lung transplants. (A) Intra-graft numbers and percent abundance of T_{RM} for indicated transplant and treatment conditions. Bars represent mean levels \pm S.D.; N=4/group; 1-way ANOVA with Dunnett's multiple comparisons test $**p < 0.01$. (B) A representative FACS histogram result of T_{RM} LAG-3 and TIM-3 expression of N=4/group. (C) 2T-FVB allograft T_{RM} marker expression patterns. Data shown are a representative result from N=4/group.

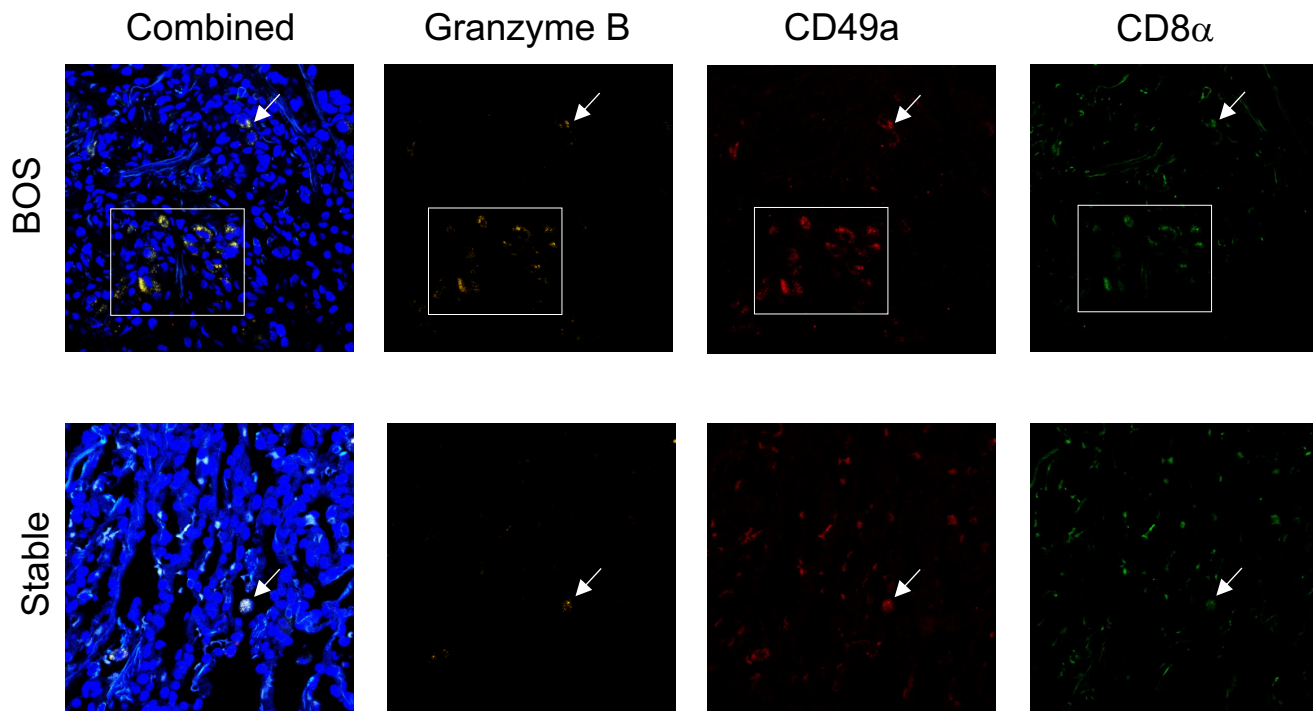


Fig. S11. Clusters of Gzmb⁺CD49a⁺CD8⁺ T cells in BOS subjects. Explant tissue from patients with BOS or core biopsies from patients without evidence of CLAD or antibody-mediated rejection (Stable) were stained with indicated antibodies. Images shown are representative stains from BOS (N=4) and stable recipients (N=6). The white box represents a cell cluster and arrows show a single cell.

Figure S12

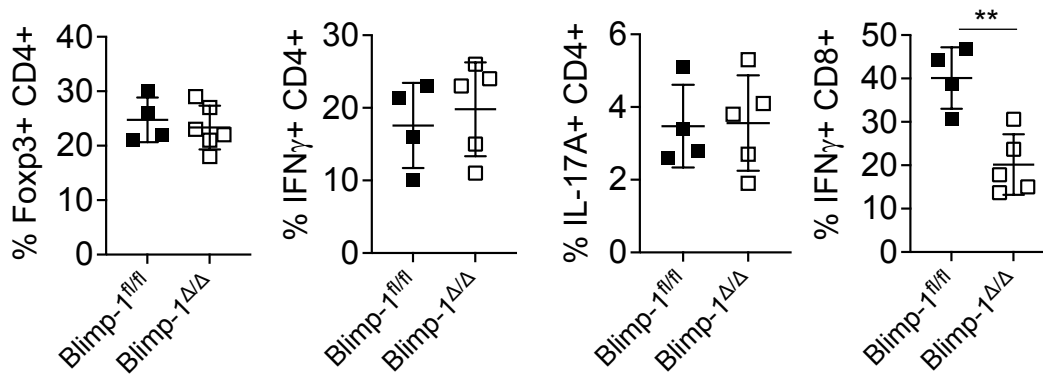


Fig. S12. CD8⁺ T cell-specific Blimp-1 deficiency reduces intragraft IFN- γ ⁺ CD8⁺ T cell accumulation. Flow cytometric analysis of indicated intragraft effector T cells in Blimp-1^{fl/fl} and Blimp-1^{ΔΔ} recipients (N=4/group). Data shown is mean percent abundance \pm S.D.; 2-sided Mann-Whitney U test; **p < 0.01.