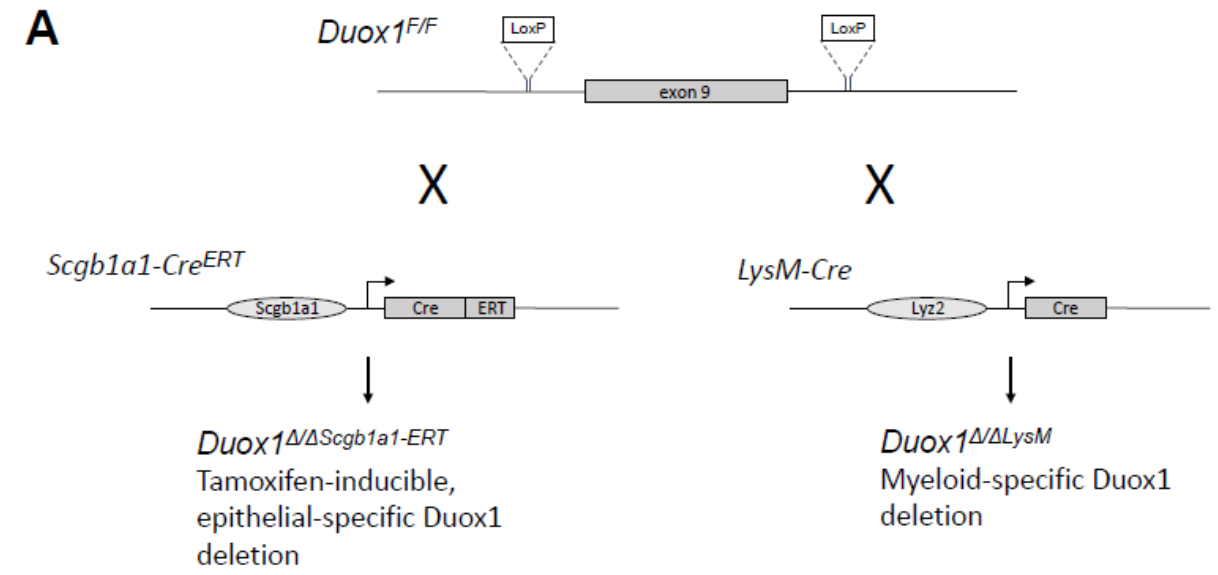
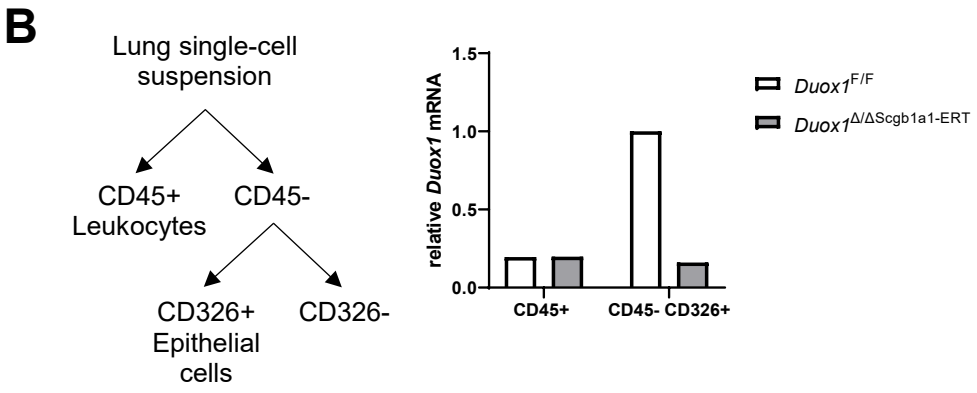
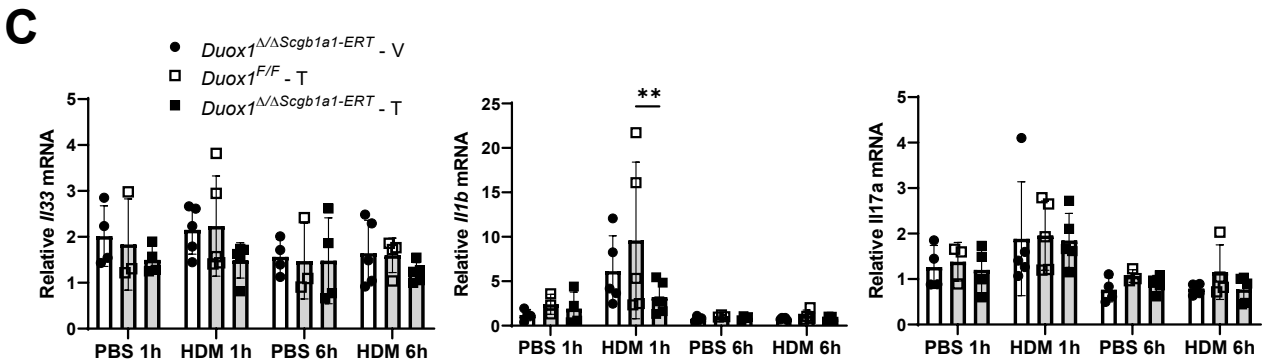
Supplementary Figures

Macrophage-intrinsic DUOX1 contributes to type 2 inflammation and mucus metaplasia during allergic airway disease

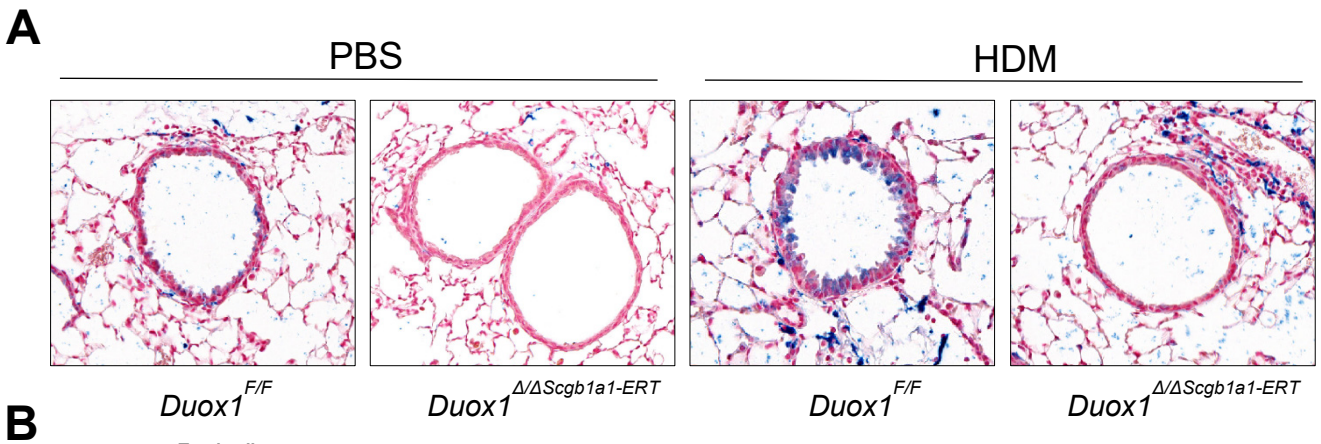
Carolyn R. Morris, Aida Habibovic, Christopher M. Dustin, Caspar Schiffers, Miao-Chong Lin, Jennifer L. Ather, Yvonne M.W. Janssen-Heininger, Matthew E. Poynter, Olaf Utermohlen, Martin Krönke, and Albert van der Vliet

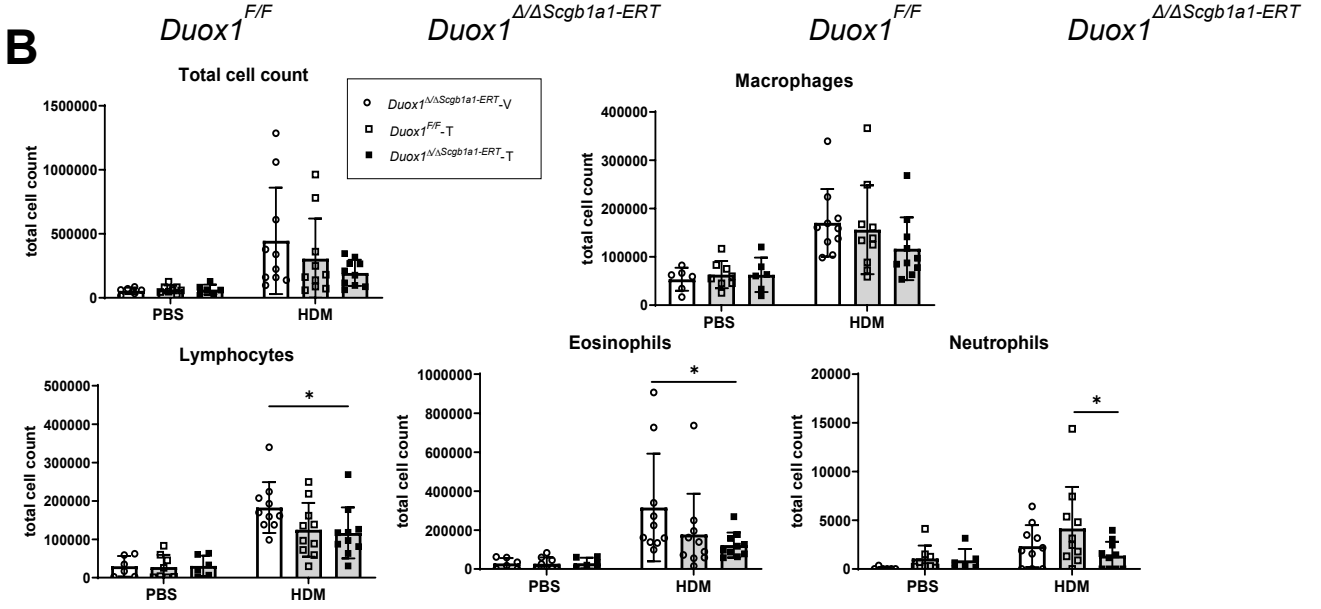


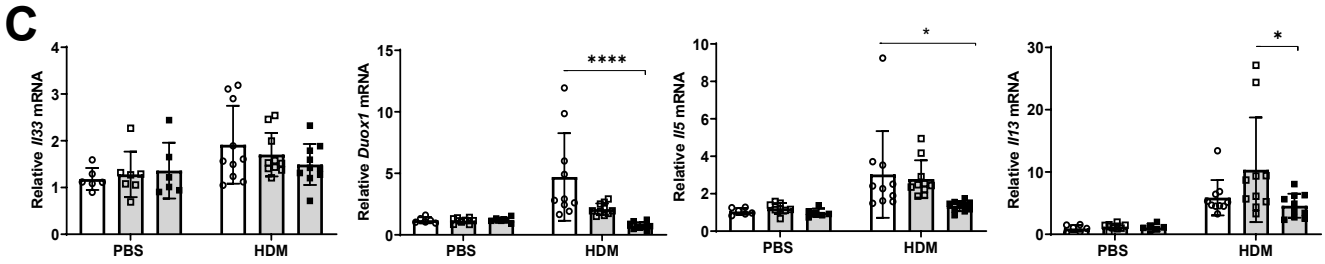


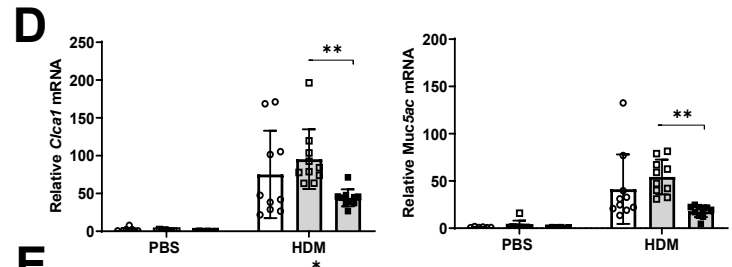


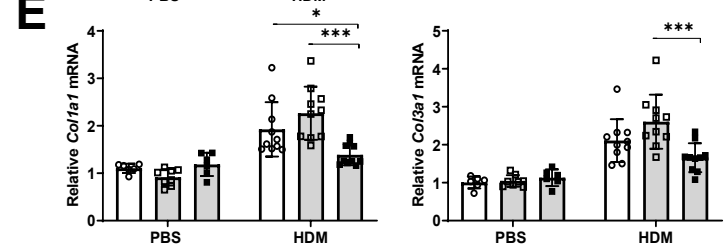
Supplementary Figure 1: (**A**) Schematic illustration of mouse lines used in this study. (**B**) Confirmation of selective *Duox1* ablation in CD326+ epithelial cells from tamoxifen-treated *Duox1*$^{\Delta/\Delta Scgb1a1\text{-}ERT}$ mice. Average results of duplicate analyses are shown. (**C**) RT-PCR analysis of *Il1b*, *Il33,* and *Il17a* mRNA in lung tissues from *Duox1*$^{F/F}$ or *Duox1*$^{\Delta/\Delta Scgb1a1\text{-}ERT}$ mice, pretreated with either tamoxifen (T) or vehicle control (V), following acute intranasal HDM challenge. **: p<0.01.

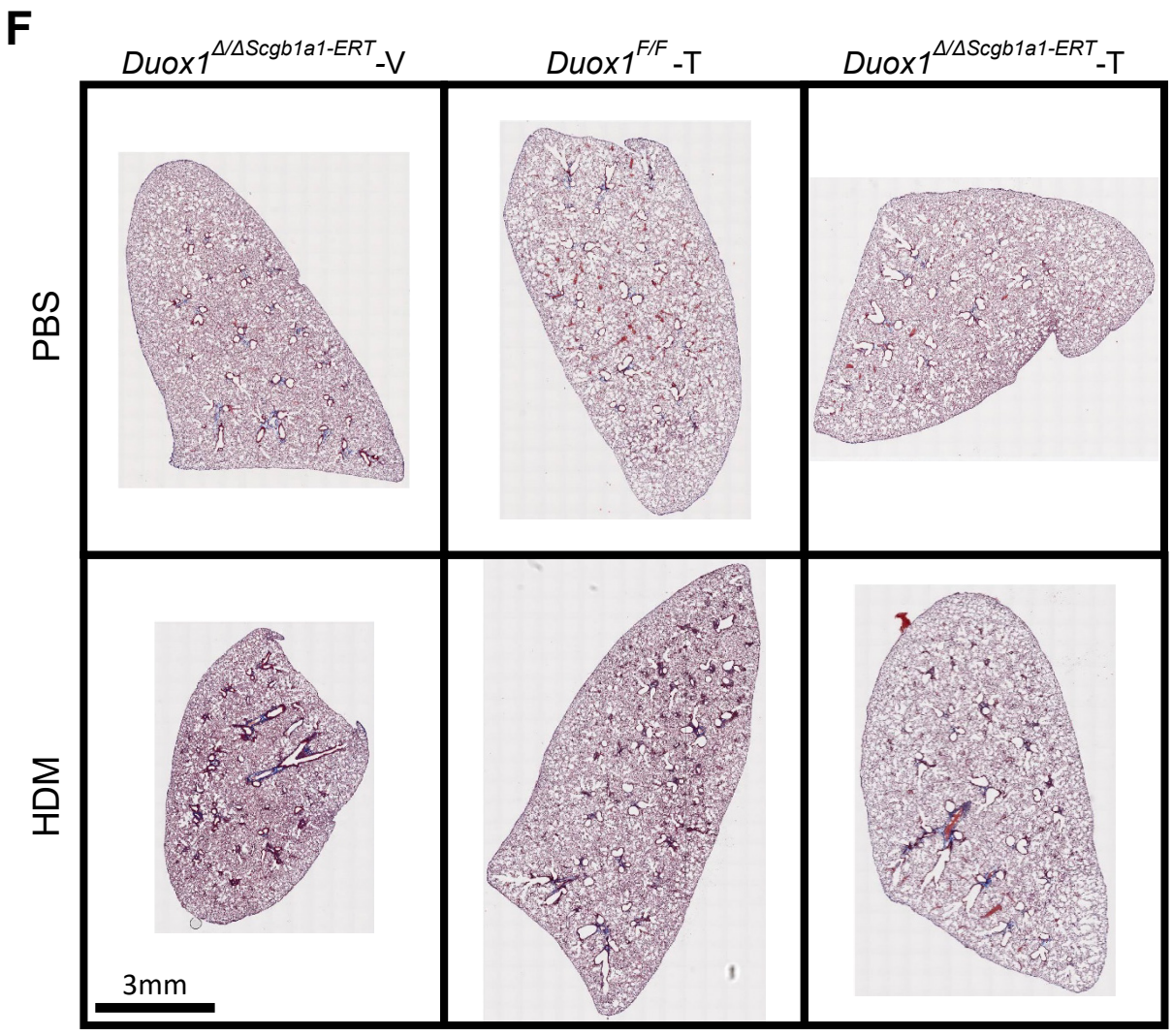

A
PBS
HDM
Duox1F/F
Duox1Δ/ΔScgb1a1-ERT
Duox1F/F
Duox1Δ/ΔScgb1a1-ERT


B
Total cell count
Duox1Δ/ΔScgb1a1-ERT-V
Duox1F/F-T
Duox1Δ/ΔScgb1a1-ERT-T
total cell count
PBS
HDM
Macrophages
Lymphocytes
Eosinophils
Neutrophils
*

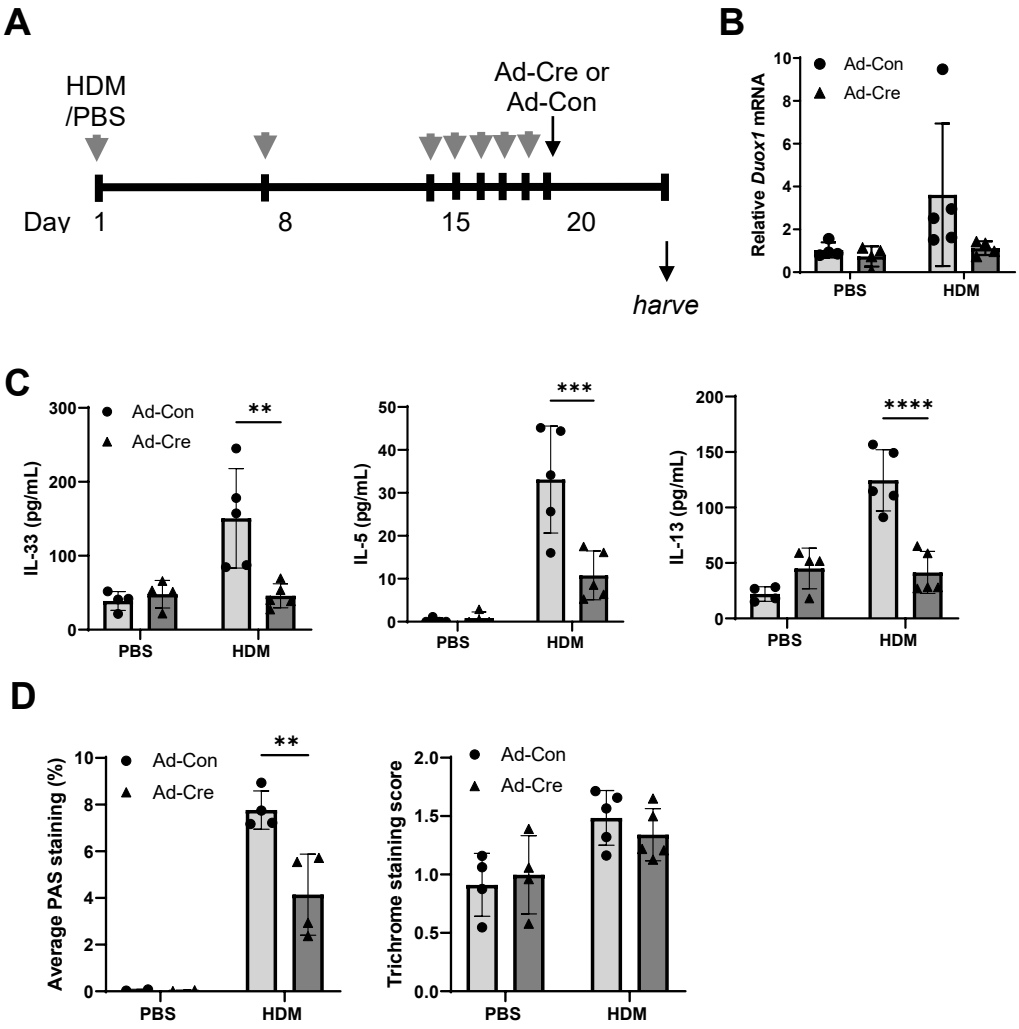

C
Relative Il33 mRNA
Relative Duox1 mRNA
Relative Il5 mRNA
Relative Il13 mRNA
PBS
HDM
****
*


D
Relative Clca1 mRNA
Relative Muc5ac mRNA
PBS
HDM
**

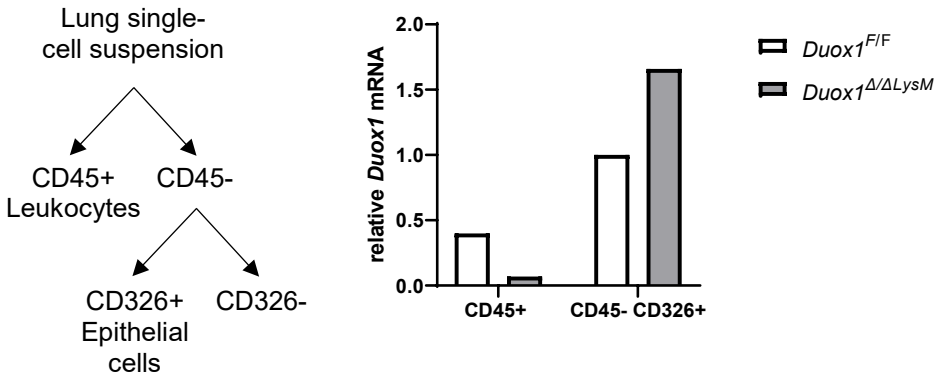

E
Relative Col1a1 mRNA
Relative Col3a1 mRNA
PBS
HDM
*
***



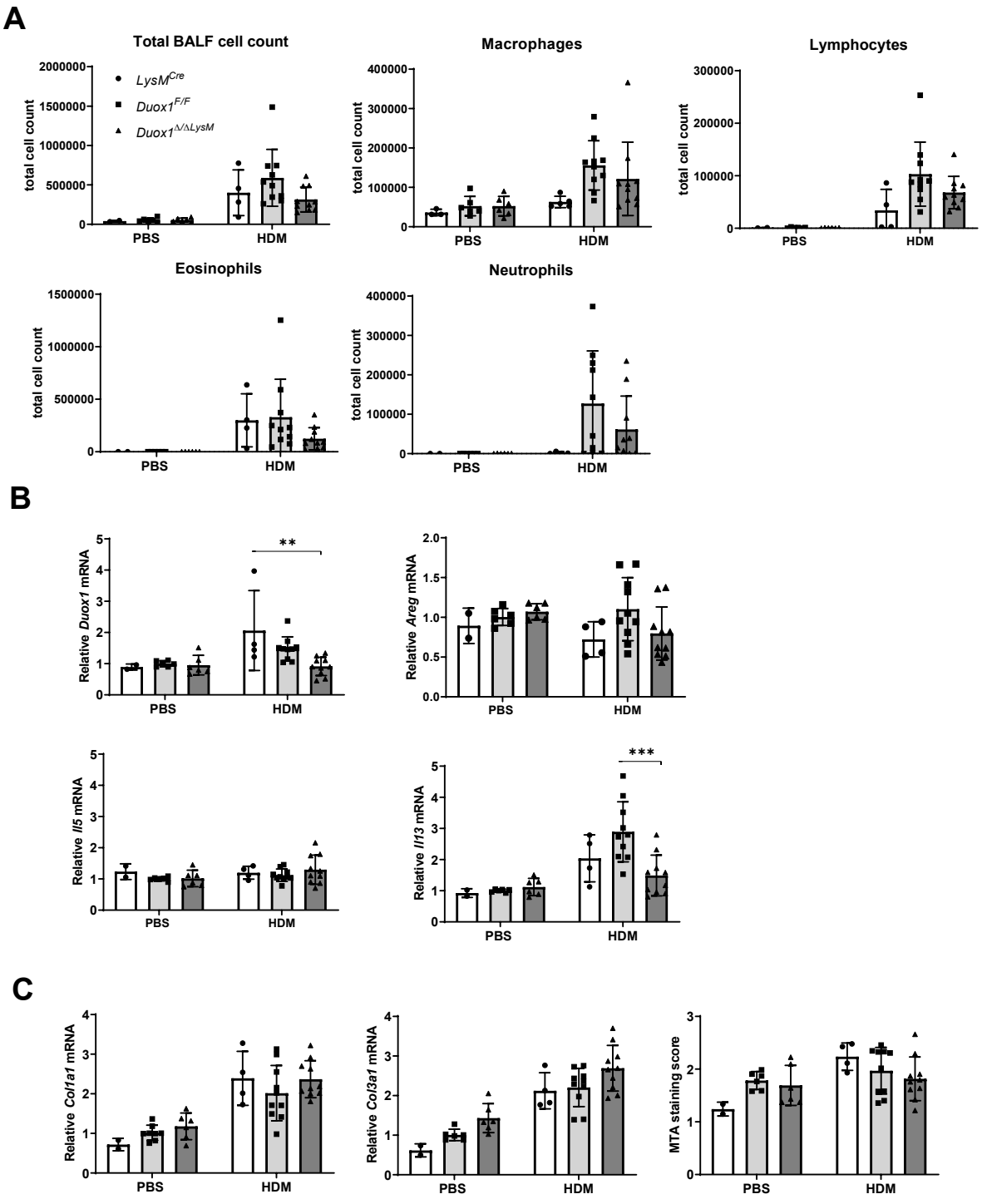
Supplementary Figure 2: Epithelial DUOX1 ablation attenuates type 2 inflammation and airway remodeling during repeated HDM challenge. *Duox1*$^{F/F}$ or *Duox1*$^{\Delta/\Delta Scgb1a1\text{-}ERT}$ mice pretreated with either tamoxifen (T) or vehicle control (V) were subjected to repeated intranasal HDM or PBS. (**A**) Immunohistochemical analysis of DUOX1 protein in tamoxifen-treated *Duox1*$^{F/F}$ or *Duox1*$^{\Delta/\Delta Scgb1a1\text{-}ERT}$ mice following repeated PBS or HDM challenge, revealing selective ablation of DUOX1 within the epithelium. (**B**) Quantification of BAL inflammatory cell types. (**C-E**) Lung tissue analysis of mRNA levels of genes related to type 2 inflammation (**C**), mucus metaplasia (**D**), and collagen production (**E**). (**F**) Whole slide images of representative full lung sections following Masson's trichrome staining. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$.



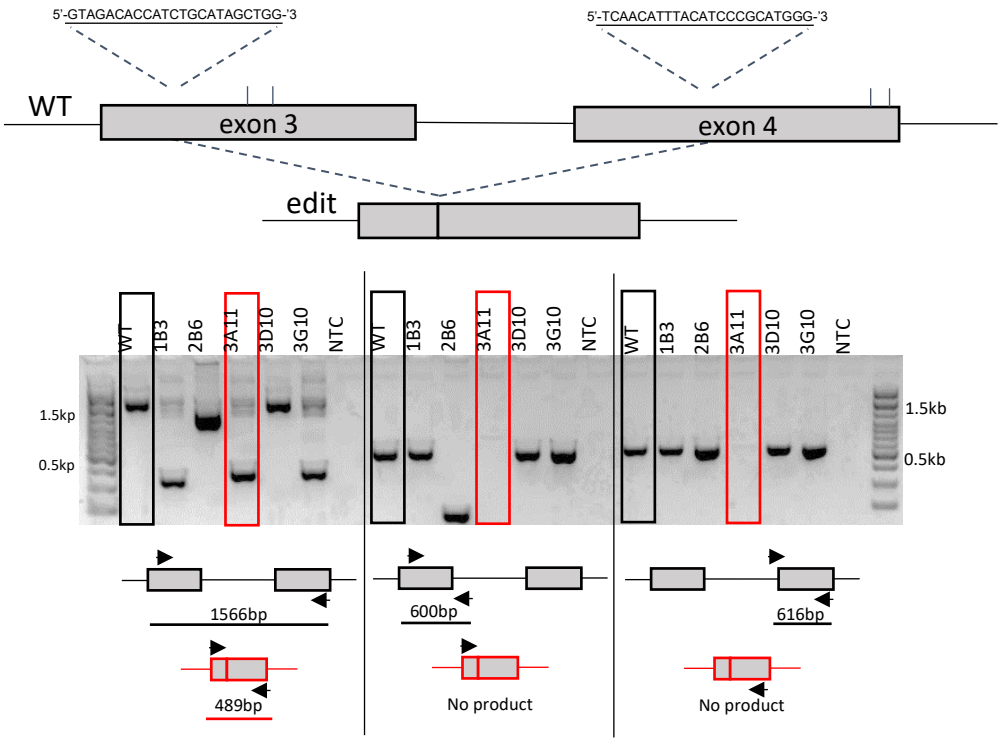
Supplementary Figure 3: DUOX1 ablation during ongoing allergic airway inflammation promotes resolution of inflammation and remodeling. (**A**) Schematic of experimental design and timeline of HDM and Adeno-Cre administration. (**B**) Analysis of lung tissue *Duox1* mRNA expression. (**C**) ELISA analysis of type cytokines in BAL fluids. (**D**) Quantification of PAS staining and Masson's trichrome staining. **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$.



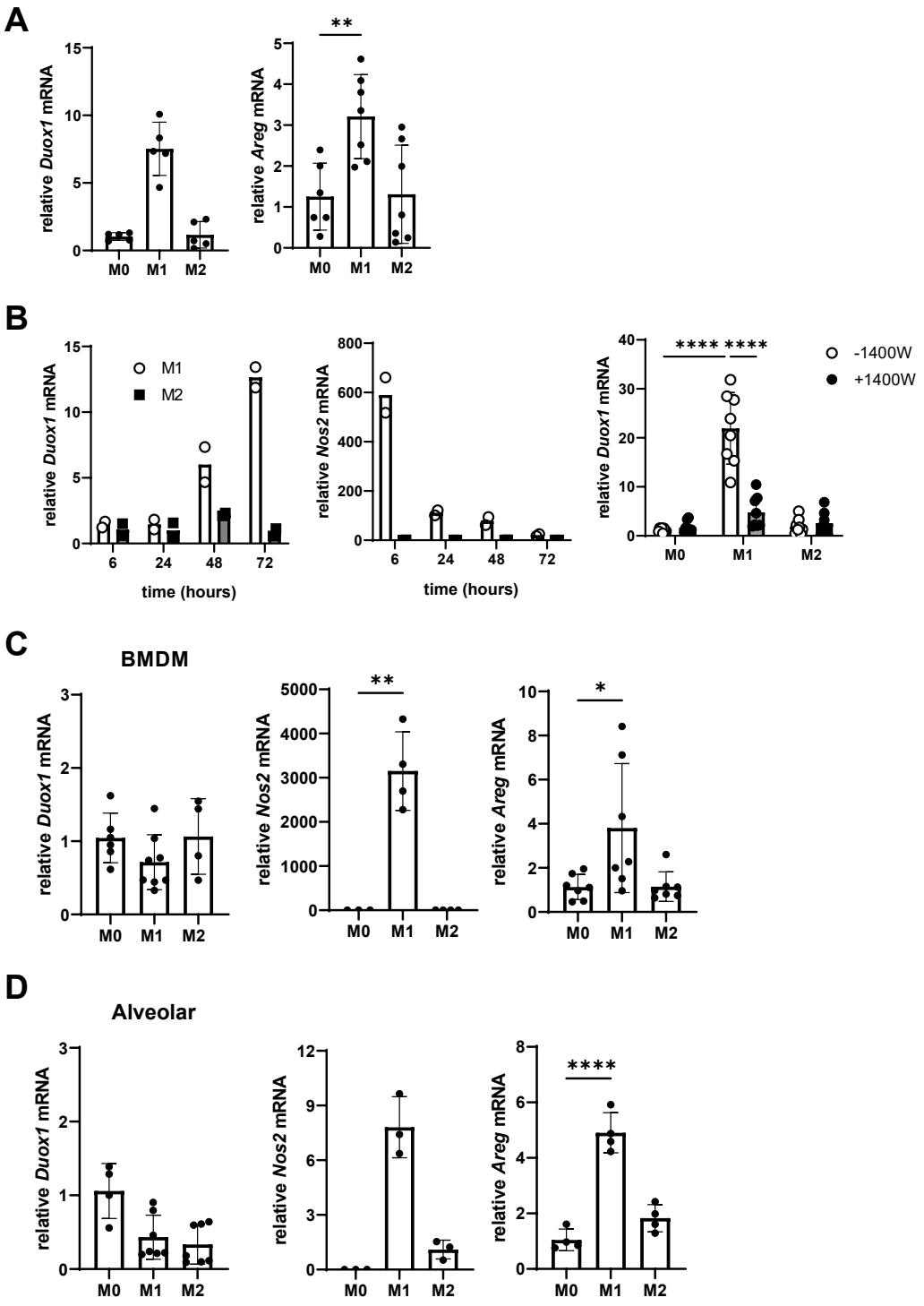
Supplementary Figure 4: Analysis of selective *Duox1* ablation in CD45+ cells but not CD326+ epithelial cells from *Duox1*$^{\Delta/\Delta LysM}$ mice. Average results of duplicate analyses are shown.



Supplementary Figure 5: Selective ablation of myeloid DUOX1 attenuates type 2 inflammation and mucus metaplasia during chronic HDM challenge. *Duox1*$^{\Delta/\Delta LysM}$ mice and *LysM*$^{Cre+/-}$ or *Duox1*$^{F/F}$ as controls, were subjected to repeated challenge with HDM or PBS, and BAL fluid were analysed for inflammatory cell counts (**A**), and lung tissues were analyzed for mRNA levels of genes related to type 2 inflammation (**B**). (**C**) Lung tissue analysis of mRNA levels of collagen genes or Masson's trichrome staining. **: p<0.01; ***: p<0.001.



Supplementary Figure 6: Schematic of *Duox1* editing and screening in MH-S cell clones.



Supplementary Figure 7: Impact of M1/M2 polarization on macrophage *Duox1* expression. MH-S cells were subjected to M1 or M2 polarization for 48 hrs (**A**) or indicated times (**B**), and analyzed for mRNA expression of *Duox1* or M1 markers *Nos2* or *Areg*. (**B**, right panel) Impact of NOS2 inhibition by 1400W on *Duox1* mRNA during 72-hr M1 polarization. (**C,D**) Impact of 48-hr M1/M2 polarization of mouse bone marrow-derived macrophaged (BMDM) or alveolar macrophages on *Duox1*, *Nos2* or *Areg*. *: p<0.05; **: p<0.01; ****: p<0.0001.