SUPPLEMENTARY DATA

Polycomb repressive complex 2 is a critical mediator of allergic inflammation

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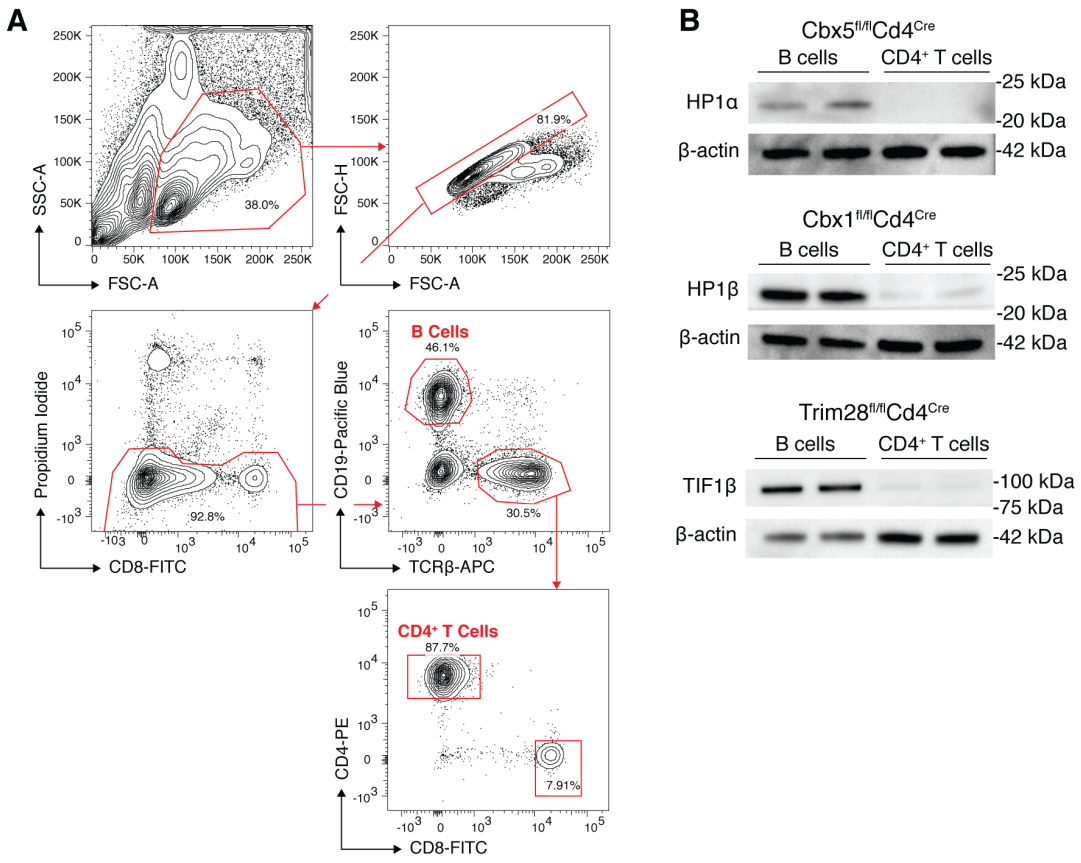
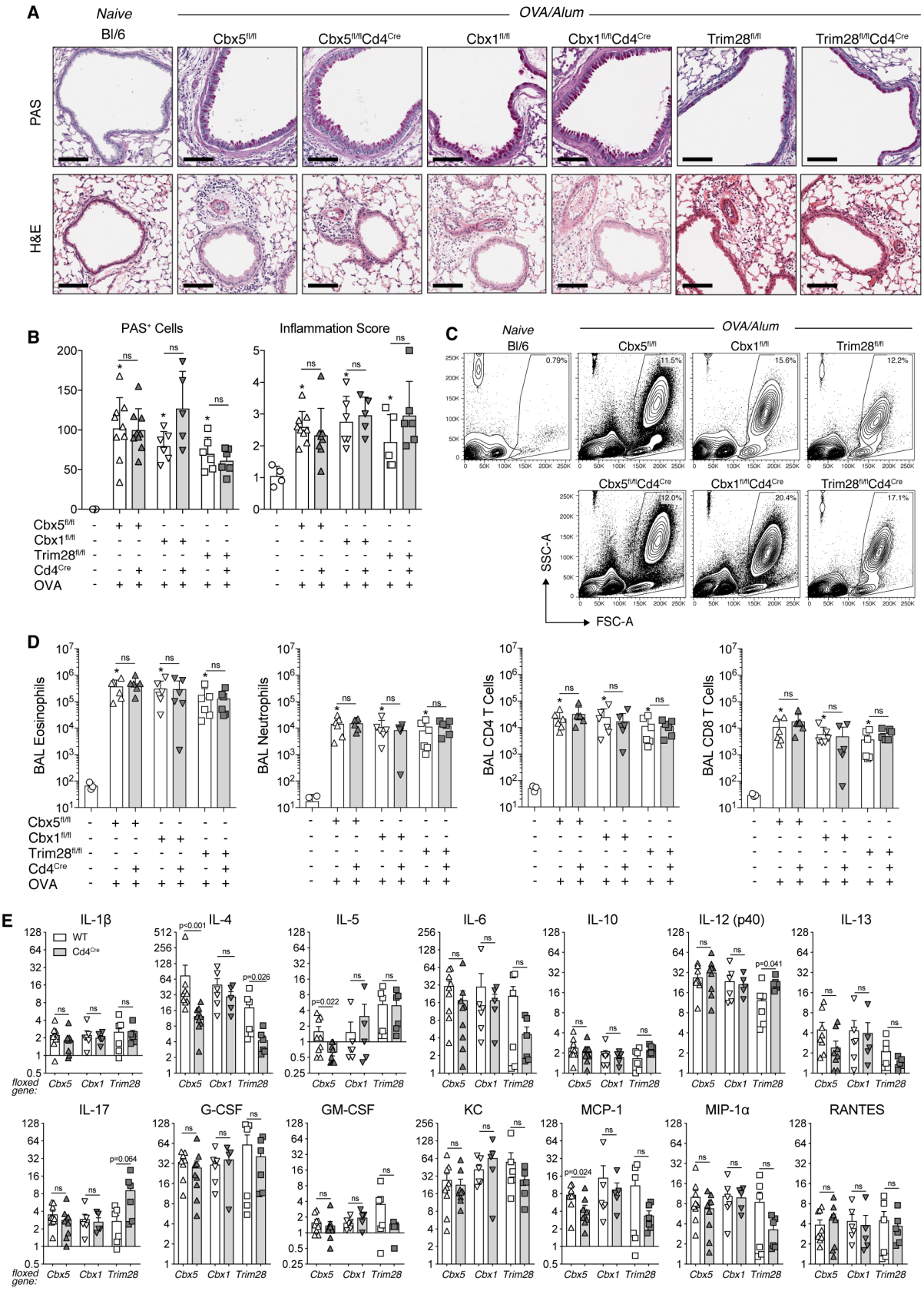


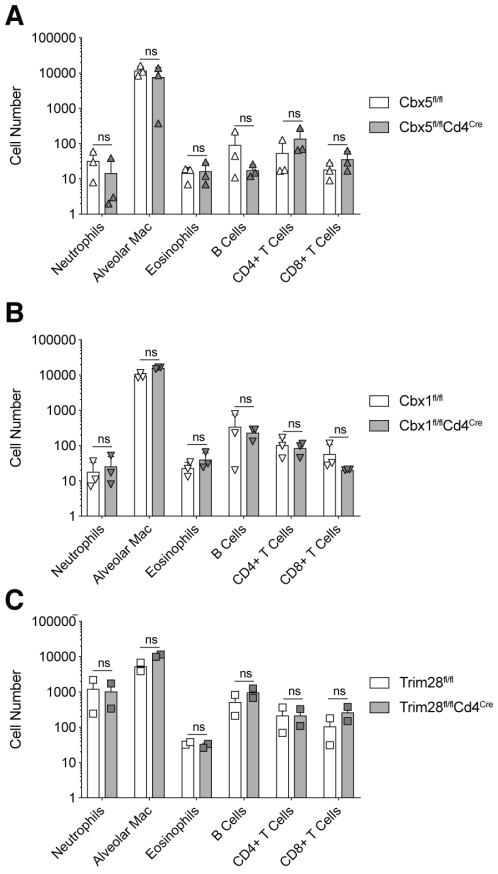
Supplementary Figure S1: *Cd4*-driven *cre* recombinase causes efficient deletion of *loxP*-flanked target gene products.

A) Fluorescence-activated cell sorting of splenic B cells and CD4$^+$ T cells. **B)** Western blot analysis from 2 representative *Cbx5*$^{fl/fl}$*Cd4*^{Cre}, *Cbx1*$^{fl/fl}$*Cd4*^{Cre} and *Trim28*$^{fl/fl}$*Cd4*^{Cre} mice showing efficient deletion of *Cbx5* (HP1α), *Cbx1* (HP1β) and *Trim28* (TIF1β) respectively in the T cell compartment.



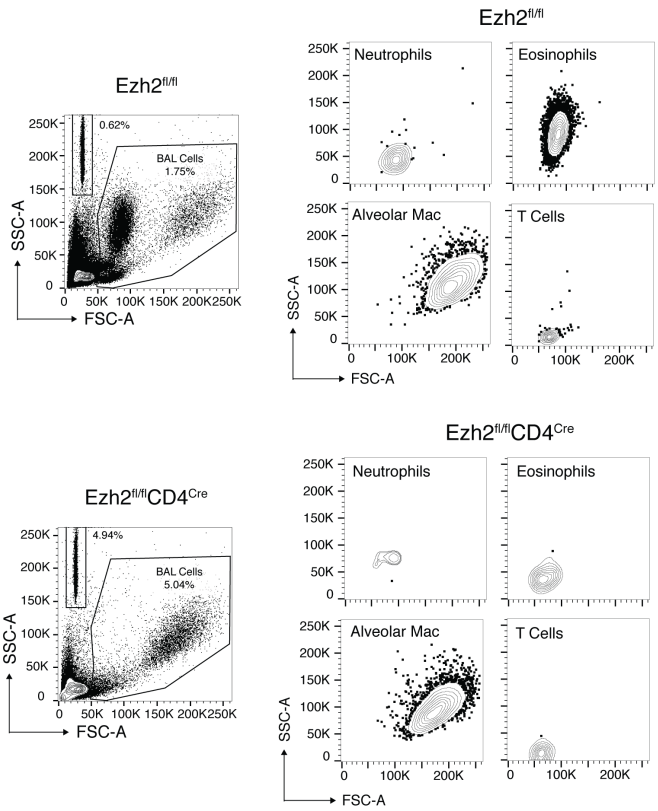
Supplementary Figure S2. Suv39h-HP1 family members are not required for development of allergic inflammation.

A) Histological analysis of the airways of *Cbx5*$^{fl/fl}$*Cd4*^{Cre}, *Cbx1*$^{fl/fl}$*Cd4*^{Cre} and *Trim28*$^{fl/fl}$*Cd4*^{Cre} and control floxed mice showing the frequencies of PAS^{+} cells and tissue inflammation following OVA-induced allergic inflammation. Un-exposed wildtype sections are shown for comparative purposes. Scale bar represents 100 μm. **B)** Quantification of histological analysis shown in **A**. Individual data points as well as Mean and SD are shown. Statistical significance was determined by Kruskal-Wallis test with Dunn's post-hoc comparing each floxed control group to un-exposed Bl/6 controls (*P<0.05) and corresponding Cd4^{Cre} mice. Group sizes are n=5 Bl/6, n=9 *Cbx5*$^{fl/fl}$ and *Cbx5*$^{fl/fl}$*Cd4*^{Cre}, n=6 *Cbx1*$^{fl/fl}$, *Cbx1*$^{fl/fl}$*Cd4*^{Cre}, *Trim28*$^{fl/fl}$ and *Trim28*$^{fl/fl}$*Cd4*^{Cre}. **C)** Representative flow cytometry analysis of bronchoalveolar lavage samples of indicated genotypes following OVA-induced allergic inflammation. **D)** Quantification of individual cell populations from flow cytometry shown in **c**. Eosinophils (CD11b^{+}, Siglec-F^{+}, CD11c^{-}), Neutrophils (CD11b^{Hi}, GR1^{Hi}), CD4^{+} T Cells (TCRβ^{+}, CD4^{+}, CD8^{-}), CD8^{+} T Cells (TCRβ^{+}, CD4^{-}, CD8^{+}). Individual data points as well as Mean and SD are shown. Statistical significance was determined as in **B**. Group sizes are n=4 Bl/6, n=6 all other groups. **E)** Bio-Plex® analysis of cytokine levels in BAL fluid in mice (group sizes as in **B**). Only cytokines induced more than 2-fold by OVA are shown. Mean and SEM together with individual data points are shown. Statistical analysis by Kruskal-Wallis test with Dunn's post-hoc.

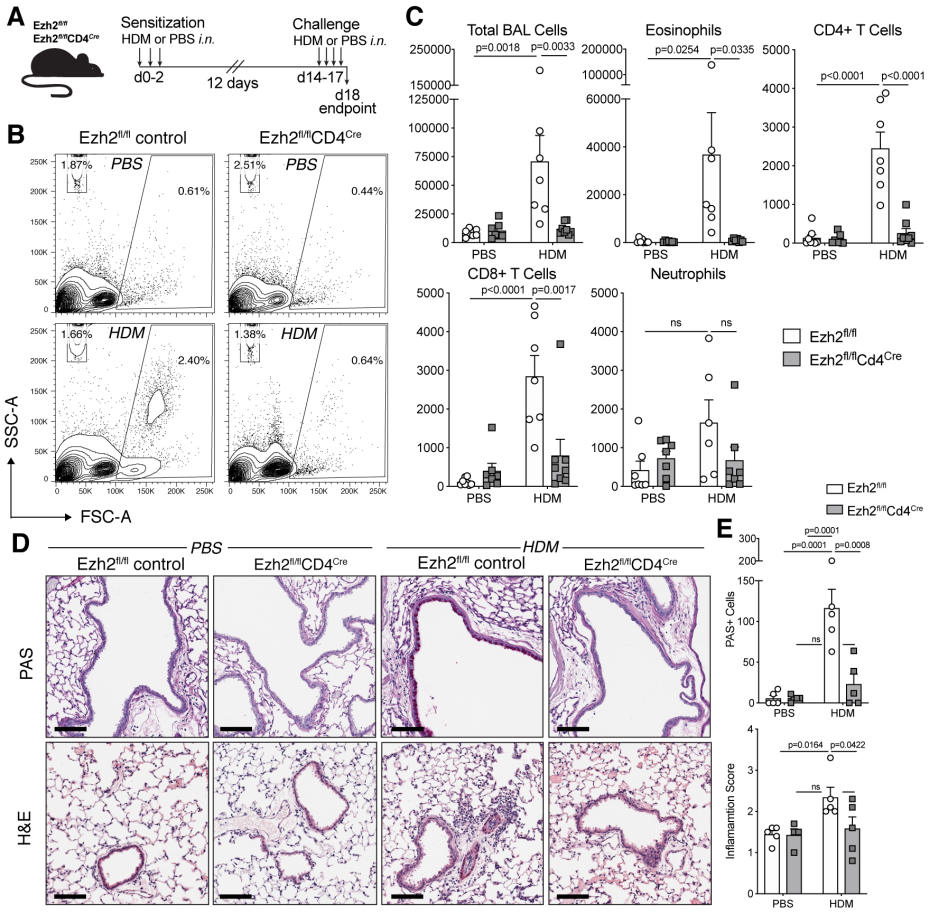


Supplementary Figure S3. Baseline effects of T-cell HP1α, HP1β or TIF1β deletion on lung inflammation levels in bronchoalveolar lavage samples.

Quantification of individual cell populations identified by flow cytometry are shown as individual data points as well as mean and SD for n=3 per group. Live cells (Sytox Blue^{-}) were further gated into individual subsets as follows: Eosinophils (CD11b^{+}, Siglec-F^{+}, CD11c^{-}); Alveolar Macrophages (CD11b^{+}, Siglec-F^{+}, CD11c^{+}); Neutrophils (CD11b^{Hi}, GR1^{Hi}); B Cells (CD11b^{-}, TCRβ^{-}, CD19^{+}); CD4 T Cells (TCRβ^{+}, CD4^{+}, CD8^{-}); CD8 T Cells (TCRβ^{+}, CD4^{-}, CD8^{+}). **A)** HP1α deletion and floxed controls. **B)** HP1β deletion and floxed controls. **C)** TIF1β deletion and floxed controls.



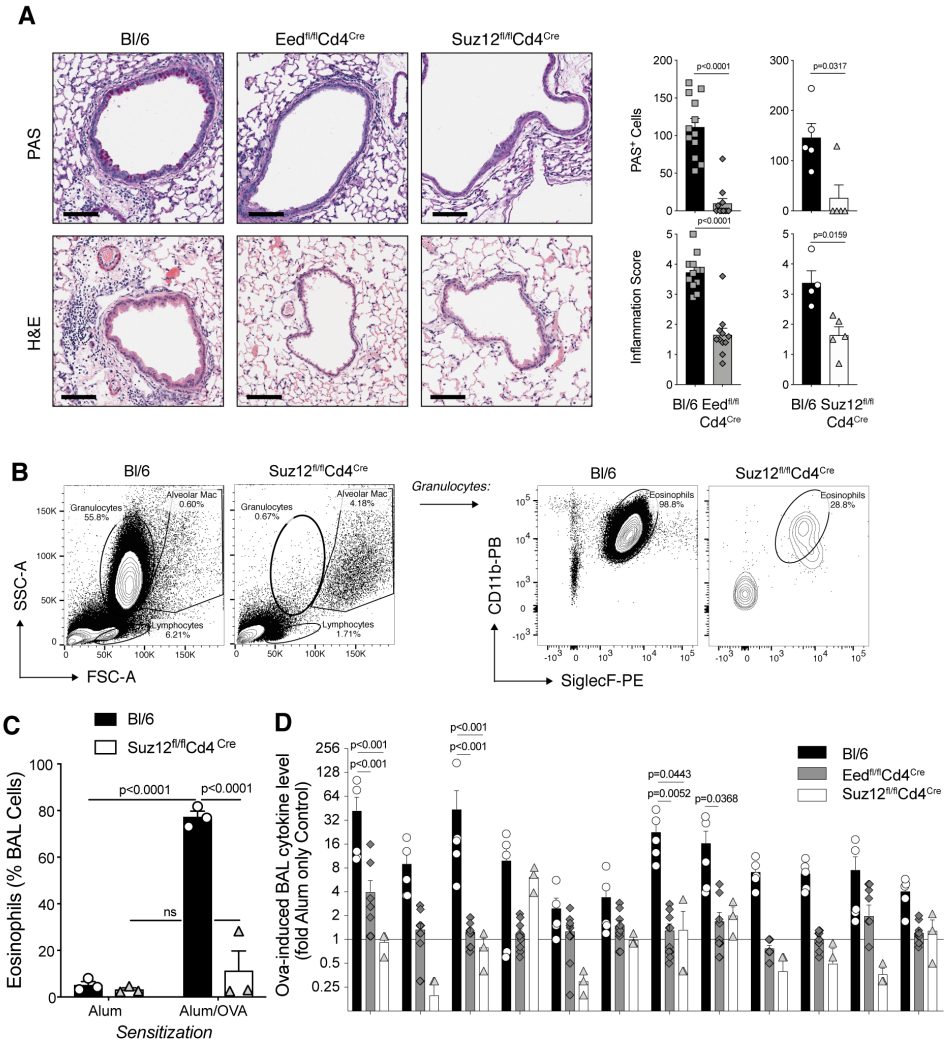
Supplementary Figure S4. Representative forward-scatter versus side-scatter flow cytometric plots of bronchoalveolar lavage (BAL) samples after gating individual leukocyte populations. Gating strategy can be seen in Figure 2c. Neutrophils are the SSC^{Mid} population gated by $CD11b^{Hi}GR1^{Hi}$; Eosinophils are the SSC^{Hi} population gated by $CD11b^{+}CD11c^{-}SiglecF^{+}$; Alveolar macrophages are the $FSC^{Hi}SSC^{Hi}$ population gated by $CD11b^{+}CD11c^{+}SiglecF^{+}$; T lymphocytes are the $FSC^{Lo}SSC^{Lo}$ population gated by $CD11b^{-}GR1^{-}TCR\beta^{+}$.



Supplementary Figure S5. Ezh2 deletion in T cell compartment protects against the development of HDM-induced allergic inflammation

A) Experimental protocol of house dust mite (HDM)-induced allergic inflammation. **B)** Flow cytometric analysis of bronchoalveolar lavage (BAL) fluid in *Ezh2*$^{fl/fl}$*Cd4*^{Cre} and control *Ezh2*$^{fl/fl}$ mice following house dust mite (HDM)-induced allergic inflammation (representative of n=7-8 per group pooled from 3 independent experiments). **C)** Quantification of total BAL cells (from **B.**) and individual leukocyte populations. Mean and SEM as well as individual data points is shown for n=8 (*Ezh2*$^{fl/fl}$/PBS and *Ezh2*$^{fl/fl}$*Cd4*^{Cre}/HDM groups), n=7 (*Ezh2*$^{fl/fl}$/HDM and *Ezh2*$^{fl/fl}$*Cd4*^{Cre}/PBS groups). Statistical analysis by 2-way ANOVA with Tukey's multiple comparisons test. **D)** Representative histological analysis of the airways of *Ezh2*$^{fl/fl}$*Cd4*^{Cre} and wildtype mice following HDM-induced allergic inflammation. Scale bar represents 100 µm.

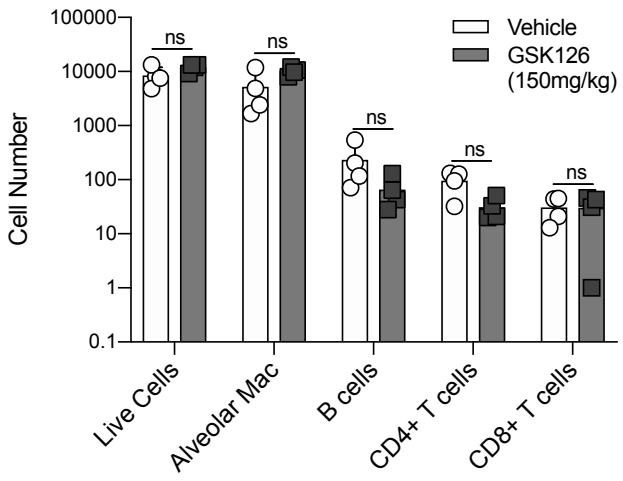
E) Quantification of histological analysis shown in **D.** Mean and SEM as well as individual data points shown for n=5 per group. Statistical analysis by 2-way ANOVA with Sidak's multiple comparisons test.



Supplementary Figure S6. Expression of PRC2 core components are critical for the development of allergic inflammation.

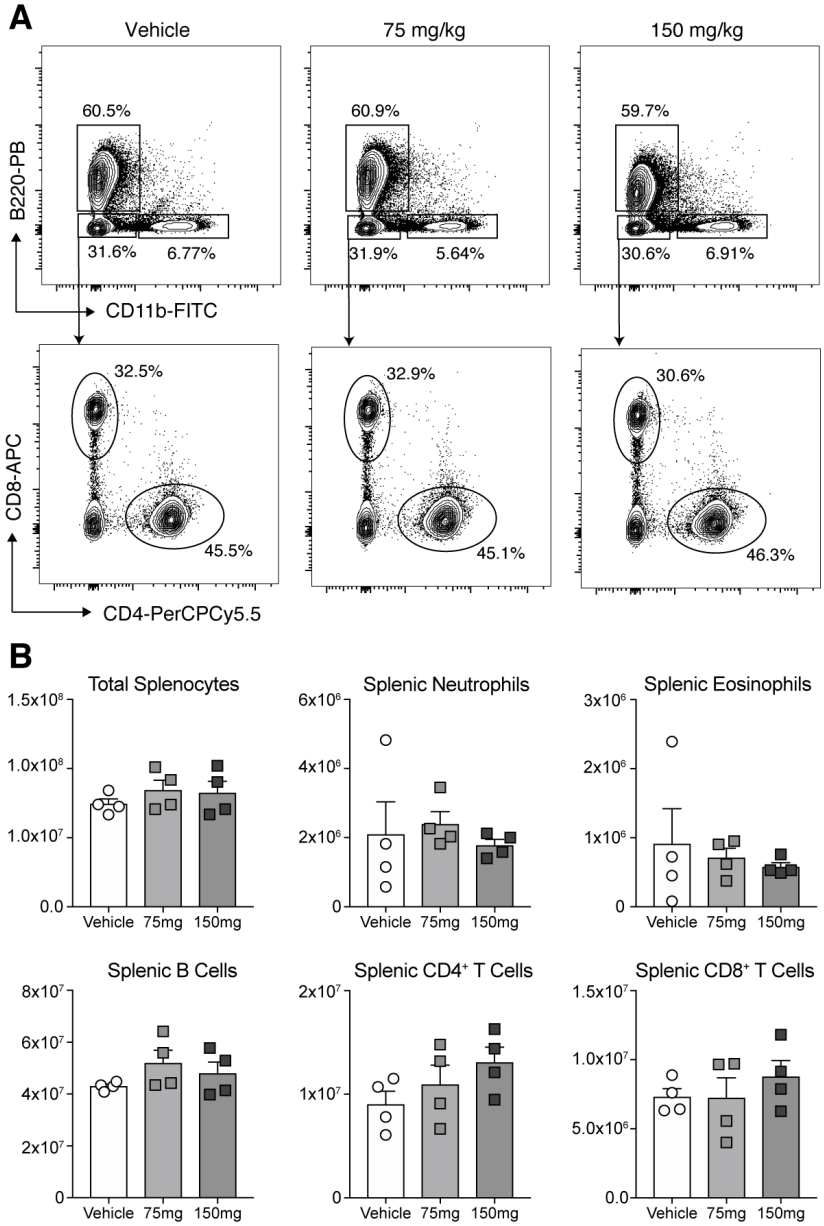
A) Histological analysis of the airways of *Eed*$^{fl/fl}$*Cd4*^{Cre}, *Suz12*$^{fl/fl}$*Cd4*^{Cre} and C57Bl/6 (Bl/6) wildtype mice following OVA challenge. Representative samples are shown on the left panels. The frequency of PAS^{+} cells and the Inflammation Score is shown on the right as individual data points together with mean and SEM. Group sizes are n=12 *Eed*$^{fl/fl}$*Cd4*^{Cre} with n=11 age-matched Bl/6 controls, n=5 *Suz12*$^{fl/fl}$*Cd4*^{Cre} with n=5 age-matched Bl/6 controls. Statistical significance was determined by Mann-Whitney U-test. **B)** Representative flow cytometric analysis of bronchoalveolar lavage (BAL) infiltrate in *Suz12*$^{fl/fl}$*Cd4*^{Cre} and wildtype Bl/6 mice following OVA-induced asthma. Populations corresponding to granulocytes, lymphocytes and alveolar macrophages are indicated. The frequency of eosinophils (CD11b^{+}, SiglecF^{+}) in the

granulocyte gate is depicted on the right. **C**) Grouped data from **B.** Mean and SEM shown for n=3 each group. Statistical analysis by 2-way ANOVA with Tukey's post-hoc. **D)** Bio-Plex® analysis of cytokine levels in BAL fluid in *Eed*$^{fl/fl}$*Cd4*^{Cre} (n=10), *Suz12*$^{fl/fl}$*Cd4*^{Cre} (n=3) and wildtype Bl/6 (n=5) mice following OVA-induced asthma. Only cytokines induced more than 2-fold by OVA are shown (individual values with mean and SEM) and statistical significance was determined by two-way ANOVA with Dunnet's post-hoc test.



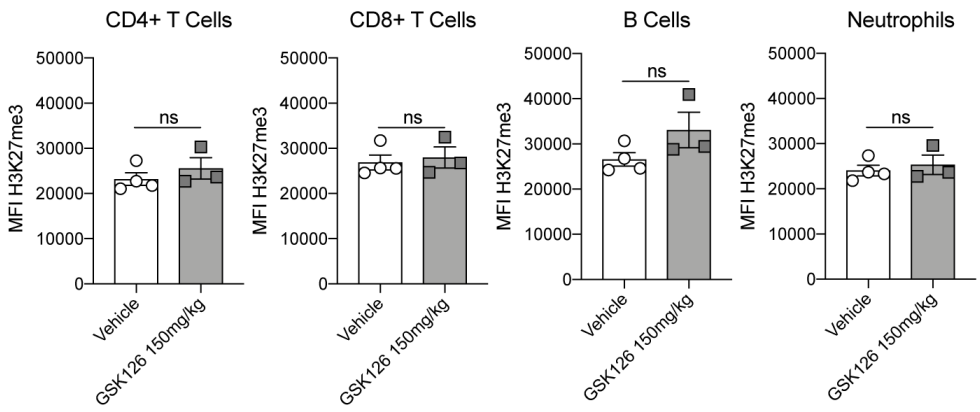
Supplementary Figure S7: Bronchoalveolar lavage cellularity from control C57Bl/6 mice treated for 4 days by oral administration of the Ezh2 inhibitor GSK126 (150mg/kg)

Data from flow cytometry presented as mean and SD together with individual data points from n=4 mice per group. Live cells (Sytox Blue^{-}) were further gated into individual subsets as follows: Alveolar Macrophages (CD11b^{+}, Siglec-F^{+}, CD11c^{+}); B Cells (CD11b^{-}, TCRβ^{-}, CD19^{+}); CD4 T Cells (TCRβ^{+}, CD4^{+}, CD8^{-}); CD8 T Cells (TCRβ^{+}, CD4^{-}, CD8^{+}). Data statistically analysed by 2-way ANOVA with Holm-Sidak post-hoc test.



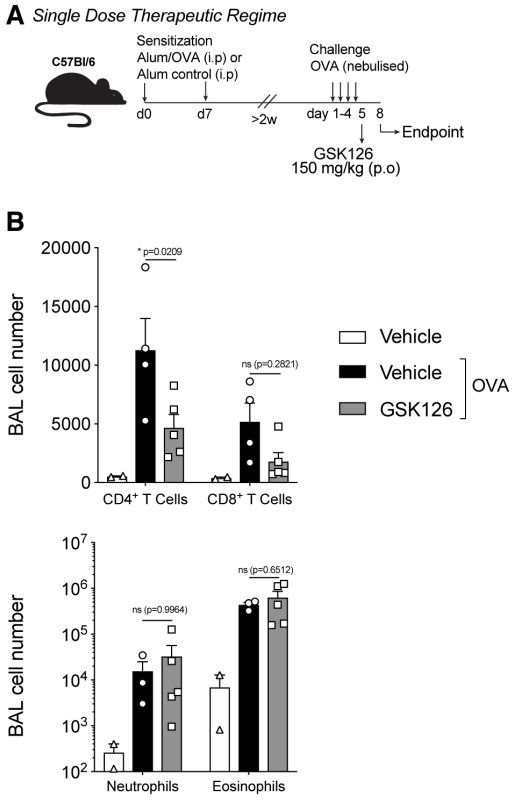
Supplementary Figure S8: Splenic cell numbers and proportions are unchanged following 4 days oral administration of the Ezh2 inhibitor GSK126

Flow cytometry analysis of splenic cell proportions in C57BL/6 mice following administration of GSK126 (75mg/kg and 150mg/kg) or vehicle control in OVA-induced asthma model (experimental protocol as per Figure 2b). **A)** Representative plots. **B)** Quantification of total splenocytes and individual leukocyte populations. Mean and SEM as well as individual data points are shown. n=4 each group. Data was statistically tested by 1-way ANOVA with Dunnet's post-hoc test.



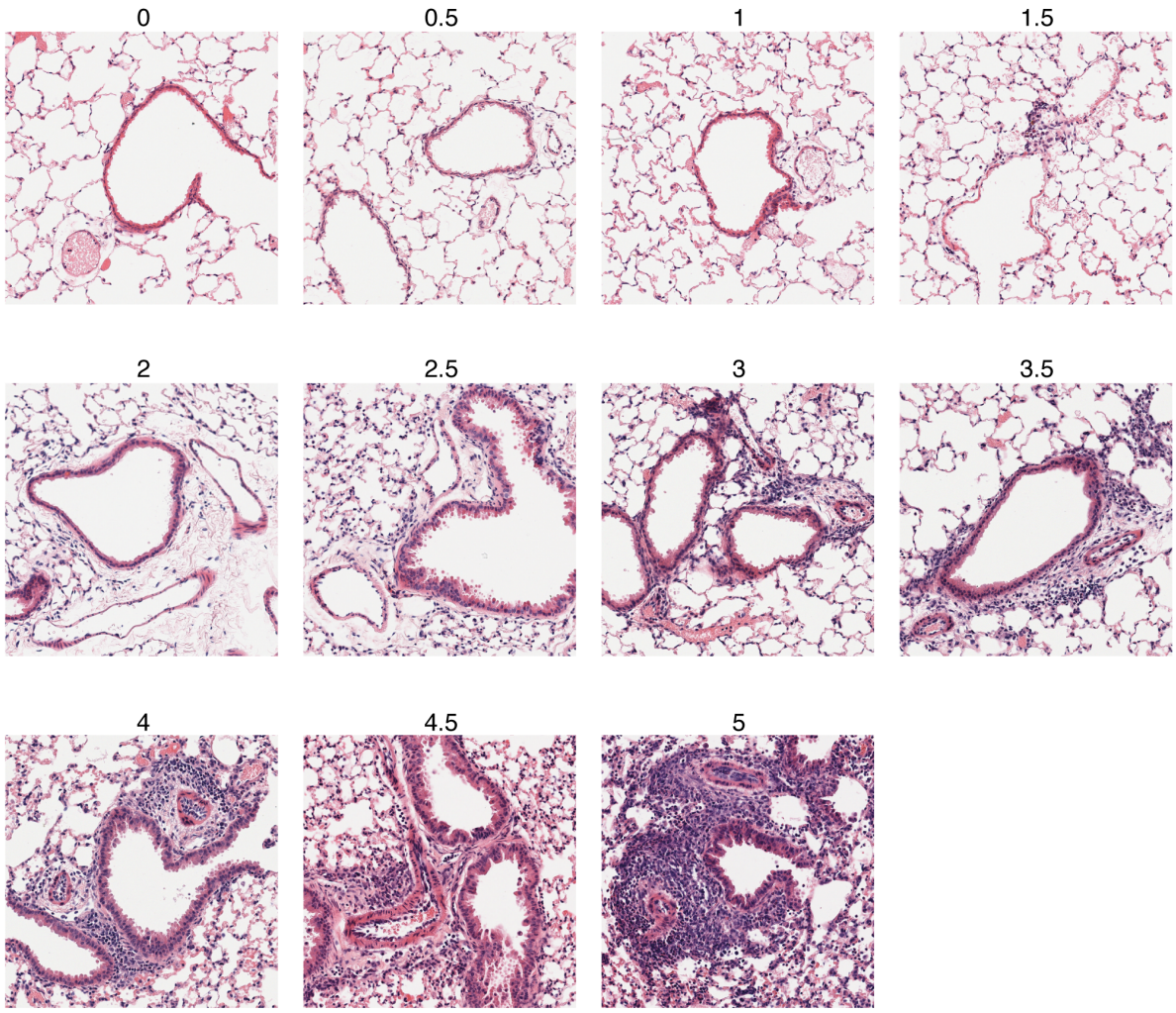
Supplementary Figure S9: 4 days oral administration of the Ezh2 inhibitor GSK126 does not alter H3K27me3 levels in splenic cell populations

Median fluorescence intensity (MFI) of H3K27me3-A647 stained splenic cells from C57BL/6 mice following administration of GSK126 (150mg/kg) by oral gavage. Mean and SEM as well as individual data points are shown n=4 (Vehicle) and n=3 (GSK126). Data was statistically tested by Mann-Whitney U-test.



Supplementary Figure S10: A single dose of GSK126 selectively reduces CD4$^+$ T cell numbers in the airways

A) Experimental protocol using one dose of Ezh2 inhibitor GSK126 (150mg/kg) in established inflammation. **B)** Bronchoalveolar lavage (BAL) cell numbers following single dose GSK126 or vehicle in established inflammation. CD4$^+$ and CD8$^+$ T cells were first gated by CD11b$^-$ GR1$^-$TCRβ$^+$. Neutrophils were gated by CD11b^{Hi}GR1^{Hi}. Eosinophils were gated by CD11b$^+$CD11c$^-$SiglecF$^+$. Mean and SEM together with individual data points are shown. n=2 Alum-sensitized vehicle treated controls shown for comparative purposes. OVA-sensitized groups were statistically compared by Student's t test. Group sizes were n=4 Vehicle and n=5 GSK126.



Supplementary Figure S11

Histological scoring chart to quantify leukocyte infiltration in H&E stained lung sections