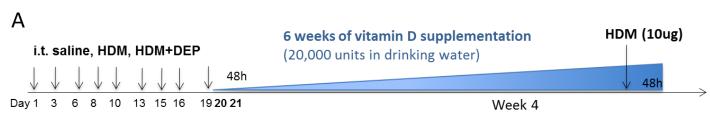
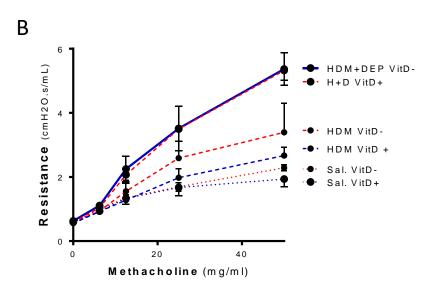
# Supplemental

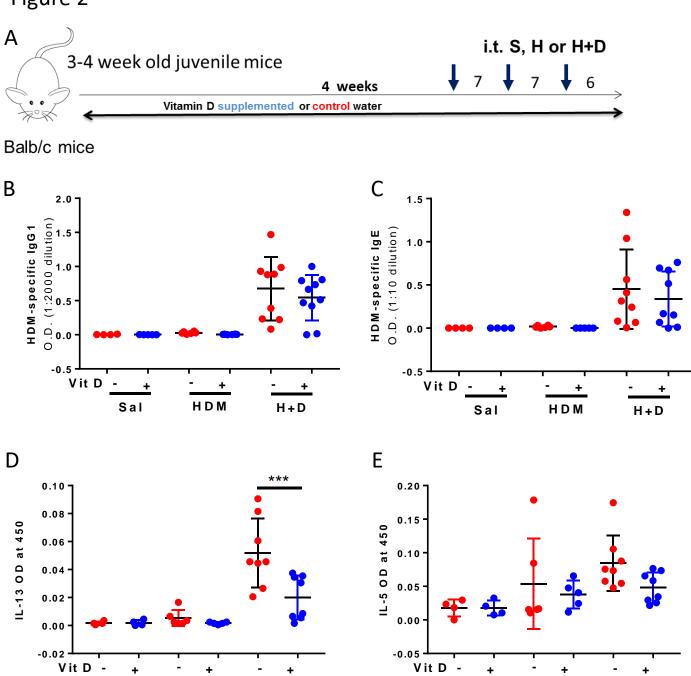






## Supplemental

Figure 2



HDM

Sal

H+D

HDM

Sal

H+D

#### 1 Online Repository Materials

### 2 Supplemental Methods

#### **Murine Models**

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- 4 To assess vitamin D as a treatment, BALB/c mice were challenged intratracheal with saline,
- 5 HDM, and H+D three times a week for three weeks to establish airway disease. Then a subset
- 6 of mice was given either control water or water supplemented with 20,000 IU cholecalciferol
- 7 (Sigma) ad libitum for 6 weeks (to attain sufficient vitamin D levels) prior to a single HDM (10µg)
- 8 intratracheal challenge. For the vitamin D sensitization experiment, mice were challenged once
- 9 a week for three weeks with saline, HDM, or HDM+DEP. Six days after the last intratracheal
- 10 challenge, lungs, spleen, and mediastinal lymph nodes were plated at 100,000 cells per well in
- triplicate and ex vivo stimulated with HDM (25µg) for six days prior to supernatant collection.

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**ELISA** 

- 14 Plasma HDM-specific IgE and IgG1 levels and supernatant IL-13 levels (free and IL-
- 13Rα2-bound) were assessed as previously described(13). IL-5 levels were determined
- using the IL-5 ELISA kit (Biolegend, San Diego, CA).

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- Supplemental Figure 1. Vitamin D restoration after established asthma had no impact on
- 20 AHR upon re-challenge with HDM.
- 21 (A) Protocol: BALB/c mice were exposed to saline, HDM, or HDM+DEP. A subset of mice were
- 22 placed on vitamin D supplemented water for 6 weeks to restore vitamin D levels. The mice then
- 23 received a single challenge of HDM (10µg). (B). Airway responsiveness was measured 48 hours
- 24 after the last challenge. (n=4-8 mice per group).

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Supplemental Figure 2. Vitamin D does not significantly impact sensitization.

(A) Protocol: Vitamin D supplemented or deficient BALB/c mice were exposed to saline, HDM, or HDM+DEP once a week for three weeks. Mice were sacrificed six days later and lung cells were *ex vivo* stimulated with HDM for 6 days prior to culture supernatant collection. (B) HDM specific IgG1 levels. (C) HDM specific IgE levels. (D) IL-13 O.D. levels (E) IL-5 O.D. levels .

\*\*\*\*p< 0.0001, \*\*\*p <.001, \*\*p <.01, \*p < 0.05 using 1-way ANOVA with Bonferroni's multiple

comparison test. (n=4-9 mice per group).