

## Online Supplementary Materials

### Oxidized CaMKII Promotes Asthma through the Activation of Mast Cells

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## **Materials and Methods**

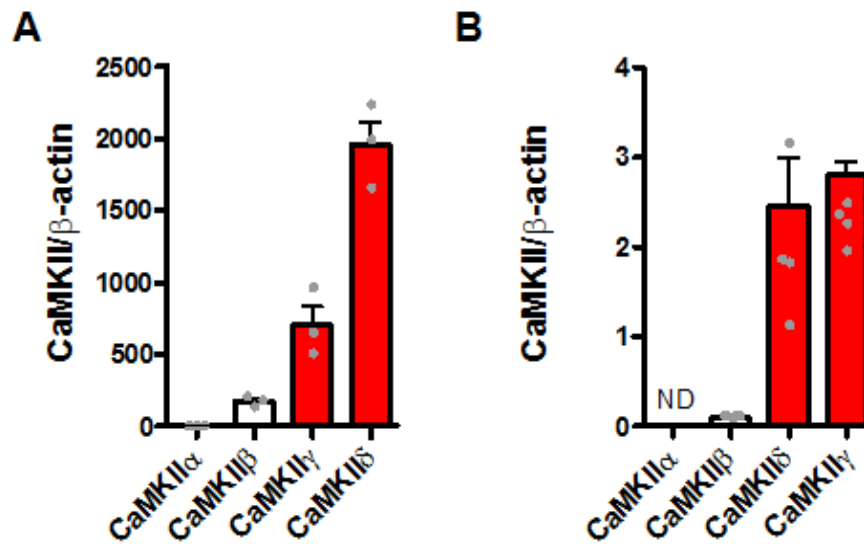
### **Immunocytochemistry and confocal microscopy**

**For staining mitochondria and nucleus:** The mitochondria and nucleus of living mouse primary mast cells were stained with 50 nM Mitotracker Red CMXRos and 250 ng/ml Hoechst 33342 (Invitrogen) for 20 min at 37°C/5% CO<sub>2</sub> in culture media. The cells were washed with fresh media for 3 times and further incubated in culture media for an additional 30 min to remove excess stains.

**For immunostaining of CaMKII Delta:** The mast cells were fixed with 3.8% v/v paraformaldehyde in PBS for 20 min at room temperature, and then washed 3 times with PBS. The cells were permeabilized with PBST (0.3% v/v TritonX-100) for 1 h, and were blocked with 1% w/v BSA in PBST for 1 h at room temperature. The cells were then incubated with primary antibody targeting to CaMKII $\delta$  (Proteintech, Cat: 15443-1-AP, 1:25 v/v) in PBST and 1% w/v BSA at 4°C overnight. The cells were then washed with PBST for 3 times to remove the excess antibody and incubated with Alexa Fluor 488 goat anti-rabbit (Invitrogen, Cat: A11034, 1:300 v/v) in PBST and 1% w/v BSA at room temperature for 1 h, and then washed with PBST for 3 times. The cells were then mounted to the glass slide with ProLong Diamond Antifade Mountant (Molecular Probes, P36961).

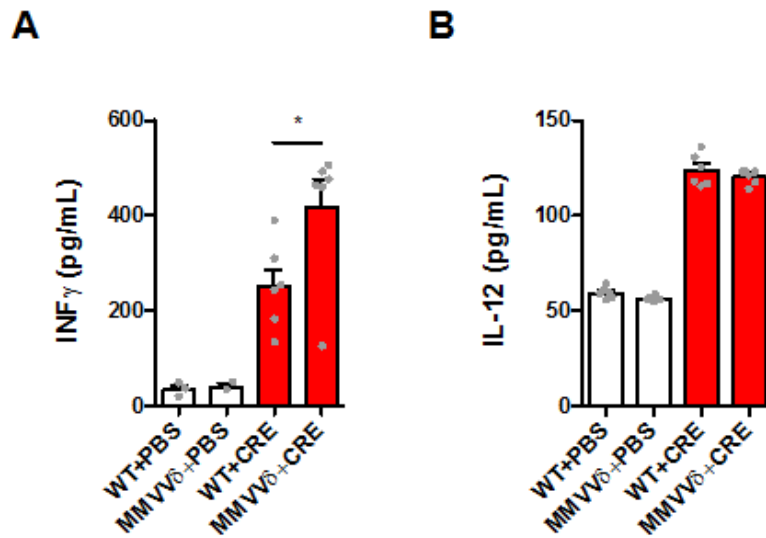
**Confocal microscopy:** Images were captured using a Cell Observer Z1 (Carl Zeiss) with a LSM780 scanner (Carl Zeiss) a 40X, NA 0.95 Corr Plan-Apochromat objective (Carl Zeiss), and then analyzed using Zen Black, Zen Blue or AxioVision 4.2 software (Carl Zeiss). Fluorescence signals of CaMKII $\delta$ , mitochondria and nucleus were visualized by fluorescence with excitation at 488, 561 and 405 nm, respectively.

## Supplementary Figure 1



**Figure S1. Expression profile of CaMKII isoforms in mouse lung tissues and BMMCs. (A-B)** Expression of CaMKII $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$  in mouse lung tissues (A, n=3) and BMMCs (B, n=3) from wild type mice was detected by qRT-PCR.

## Supplementary Figure 2

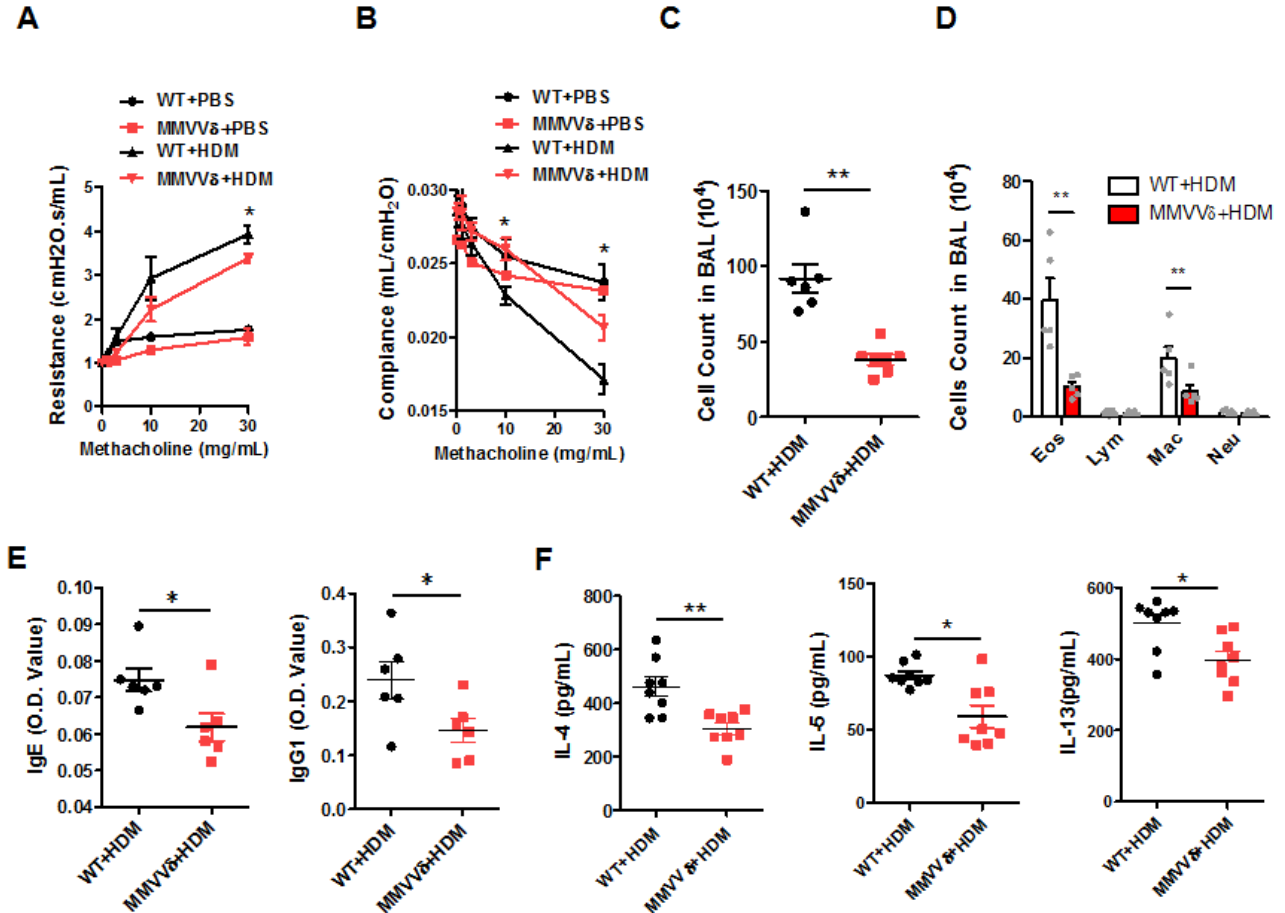


**Figure S2. Levels of IFN $\gamma$  and IL-12 in the BALs of WT and CaMKII MMVV $\delta$  mice. (A-B)**

Levels of IFN- $\gamma$  (A) and IL-12 (B) in the BAL fluids of PBS or cockroach allergen (CRE)-induced mouse model of asthma using WT (n=6/group) and MMVV $\delta$  (n=6/group) mice. Data are presented as mean  $\pm$ SEM, Student's t test of CRE-treated WT vs. MMVV $\delta$  mice and MMVV $\delta$  mice.

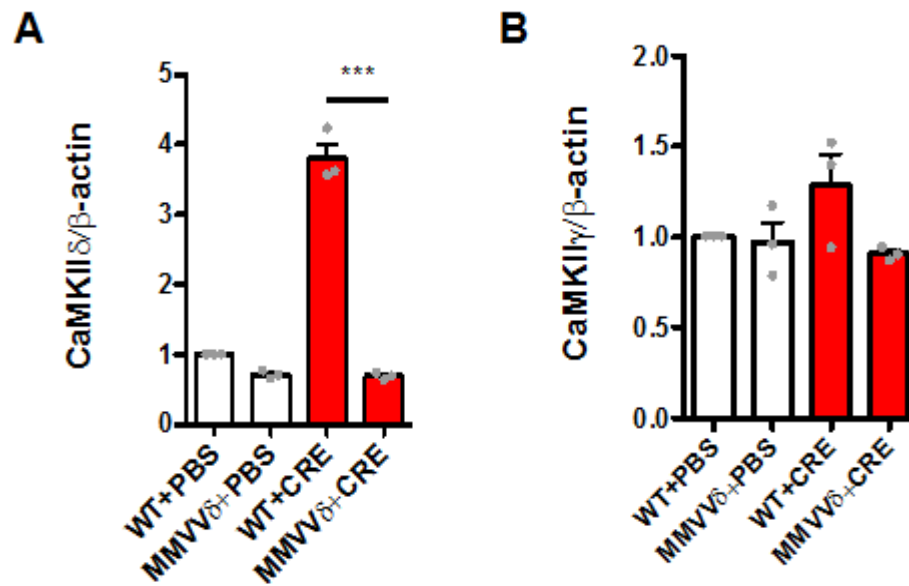
\* $P < 0.05$ .

Supplementary Figure 3



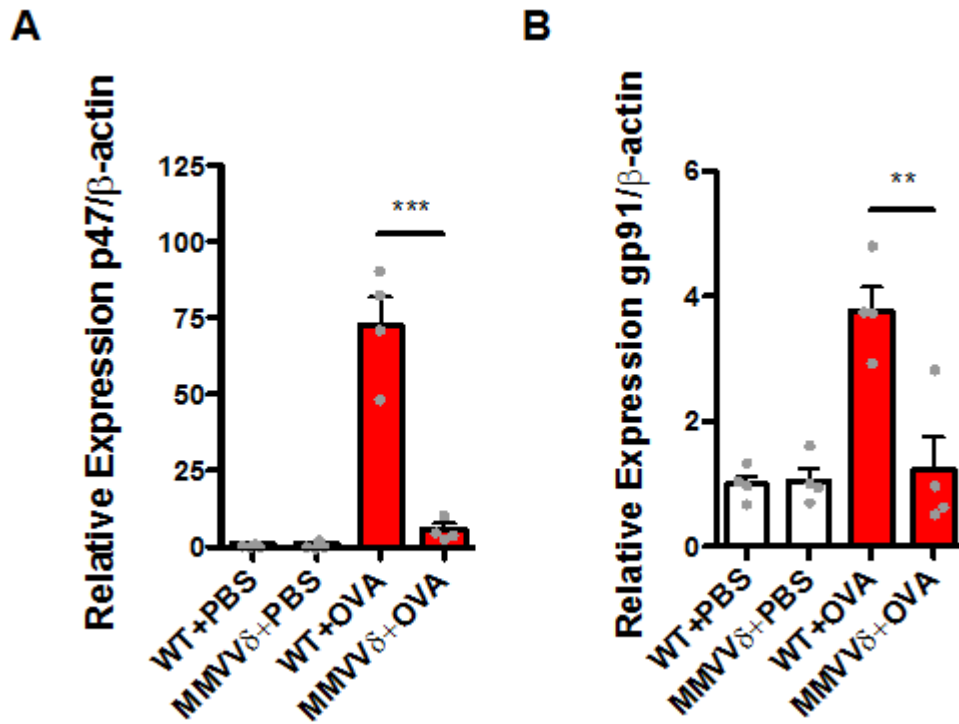
**Figure S3. MMV $\Delta$  mediates house dust mite (HDM)-induced lung inflammation. (A-B)** Lung resistance (A) and compliance (B) in response to increasing concentrations of methacholine using the forced oscillation technique (FlexiVent, SCIREQ) (4 mice/group). (C-D) Total (C) and differential cell counts (D) from the BAL fluids of HDM-challenged WT (n=6) and MMV $\Delta$  (n=6-8) mice. (E) Serum levels of HDE specific IgE (n=6) and IgG1 (n=6). (F) Levels of cytokines in BALs of HDM-challenged WT (n=6-8) and MMV $\Delta$  (n=6-8) mice. Data are presented as mean  $\pm$ SEM, Student's t test of HDM-treated WT vs. MMV $\Delta$  mice. \* $P$ <0.05, \*\* $P$ <0.01.

Supplementary Figure 4



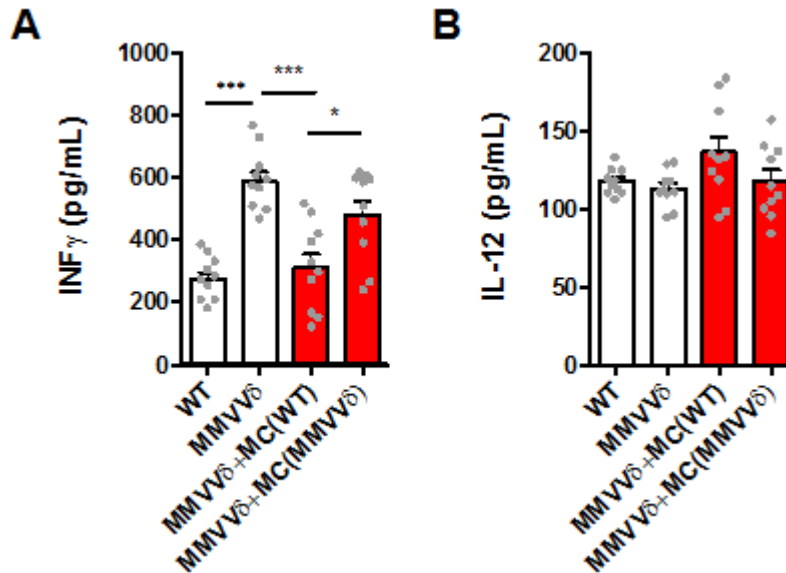
**Figure S4. Expression of CaMKII $\delta$  and  $\gamma$  in mouse lung tissues.** (A-B) Expression of CaMKII $\delta$  (A) and  $\gamma$  (B) in the lung tissues of WT (n=3) and MMVV $\delta$  (n=3) mice in cockroach allergen (CRE)-induced mouse model of asthma as detected by RT-PCR. Data are presented as mean  $\pm$ SEM, Student's t test of CRE-treated WT vs. MMVV $\delta$  mice. \*\*\* $P$ <0.001.

Supplementary Figure 5



**Figure S5.** Expression of p47 and gp91phox in OVA-activated WT and CaMKII MMVV $\Delta$  BMMCs. (A-B) Relative expression of p47 (A) and gp91phox (B) was quantified using qPCR from PBS or OVA-challenged WT (n=4) and MMVV $\Delta$  (n=4) BMMCs. Data are presented as mean  $\pm$ SEM, Student's t test of OVA-activated WT vs. MMVV $\Delta$  BMMCs. \*\* $P$ <0.01, \*\*\* $P$ <0.001.

## Supplementary Figure 6



**Figure S6. Levels of IFN- $\gamma$  and IL-12 in the BALs of CaMKII MMVV $\delta$  mice adoptively transferred BMMCs from wild type (WT) or MMVV $\delta$ . (A-B) Levels of IFN $\gamma$  (A) and IL-12 (B) in the BAL fluids of cockroach allergen (CRE)-induced mouse model of asthma using WT (n=6/group) and MMVV $\delta$  (n=6/group) mice with or without adoptively transferred BMMCs from WT or MMVV $\delta$ . Data are presented as mean  $\pm$ SEM, Student's t test of CRE-treated WT vs. MMVV $\delta$  mice and MMVV $\delta$  mice with vs without adoptively transferred BMMCs as well as adoptively transferred WT BMMCs vs. MMVV $\delta$  BMMCs. \* $P$ <0.05, \*\*\* $P$ <0.001.**