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% CO₂ 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 CaMKIIδ (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 CaMKIIδ, mitochondria and nucleus were visualized by fluorescence with excitation at 488, 561 and 405 nm, respectively.
Figure S1. Expression profile of CaMKII isoforms in mouse lung tissues and BMMCs. (A-B)

Expression of CaMKIIα, β, δ and γ 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 IFNγ and IL-12 in the BALs of WT and CaMKII MMVVδ mice. (A-B)
Levels of IFN-γ (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 MMVVδ (n=6/group) mice. Data are presented as mean ±SEM, Student’s t test of CRE-treated WT vs. MMVVδ mice and MMVVδ mice.

*P<0.05.
Supplementary Figure 3

Figure S3. MMVVδ 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 MMVVδ (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 MMVVδ (n=6-8) mice. Data are presented as mean ±SEM, Student’s t test of HDM-treated WT vs. MMVVδ mice. *P<0.05, **P<0.01.
Supplementary Figure 4

Figure S4. Expression of CaMKIIδ and γ in mouse lung tissues. (A-B) Expression of CaMKIIδ (A) and γ (B) in the lung tissues of WT (n=3) and MMVVδ (n=3) mice in cockroach allergen (CRE)-induced mouse model of asthma as detected by RT-PCR. Data are presented as mean ±SEM, Student’s t test of CRE-treated WT vs. MMVVδ mice. ***P<0.001.
Figure S5. Expression of p47 and gp91phox in OVA-activated WT and CaMKII MMVVδ BMMCs. (A-B) Relative expression of p47 (A) and gp91phox (B) was quantified using qPCR from PBS or OVA-challenged WT (n=4) and MMVVδ (n=4) BMMCs. Data are presented as mean ±SEM, Student’s t test of OVA-activated WT vs. MMVVδ 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 ±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. *\(P<0.05\), ***\(P<0.001\).