Supplemental Material

Quantitative real-time PCR primer sequences

Mouse

***Arp* forward** – TCA TCC AGC TGT TTG ACA A, ***Arp* reverse** – ATT GCG GAC ACC CTG TAG GAA G. ***Muc5ac* forward**-GTG GTG GAA ACT GAC ATT GG, ***Muc5ac* reverse** - CAT CAA AGT TCC CAC ACA GG. ***IL-5* forward** -TCC TTG CAG TGT GAA TGA GAG, ***IL-5* reverse -** CCC TGA TAC CTG AAT AAC ATC CC. ***IL-4* forward -** TCT TTA GGC TTT CCA GGA AGT C, ***IL-4* reverse -**GAG CTG CAG AGA CTC TTT CG. ***Eotaxin* forward -** CAC TTC CTT CAC CTC CCA GGT GC, ***Eotaxin* reverse-** CCC ACT TCT TCT TGG GGT CAG CA.

Human

***GAPDH* forward-** CAA TGA CCC CTT CAT TGA CC, ***GAPDH* reverse -** GAC AAG CTT CCC GTT CTC AG. ***IL-5* forward-** GAG GAT GCT TCT GCA TTT GAG TTT G, ***IL-5* reverse -** GTC AAT TTT CTT TAT TAA GGA CAA G. ***IL-1β* forward-**GAA CAA GTC ATC CTC ATT GCC, ***IL-1β* reverse-**CAG CCA ATC TTC ATT GCT CAA G. ***TSLP* forward-** AGG AGA ATC ACC AGT GGC AAA T**.** ***TSLP* reverse-** GCA CAG ATA TCC TTG GCC AGT T**.** ***Eotaxin* forward-** TTC GGC TAT ACG TAA CAG GG**.** ***Eotaxin* reverse-** TCG ATG CAA CGA GTG ATG AG**.** ***NLRP3* forward-** AGC TCA GGC TTT TCT TCT TGA**.** ***NLRP3* reverse-** GAT GTT CTG TGA AGT GAA GTG CTG AAA C**.**

Mt-CaMKIIN-HA Immunoblot

HAEC were grown as described and infected with empty (Mt-GFP) or Mt-CaMKIIN (10MOI) virus for 48 hr. Cytosolic and mitochondrial fractions were isolated, and cell lysates were prepared in RIPA buffer with phophatase and protease inhibitors. The proteins were separated by SDS-PAGE and transferred to a PVDF membrane. The membrane was then

incubated with primary antibodies against HA (1:500, cat. MMS101P-500, Covance), GAPDH (1:2000, cat. 2118S, Cell Signaling) or COXIV (1:2000, cat. 4850, Cell Signaling) followed by incubation with the appropriate HRP-conjugated anti-rabbit (1:1000, cat. sc-2004, Bio-Rad) IgG secondary antibody.

Mt-CaMKIIN-HA Immunofluorescence

HAEC were grown and infected with Mt-CaMKIIN (10MOI) virus for 48 hr. The mitochondrial localization of staining was confirmed by colocalizing with mitoTracker green (50nM, cat. M7514, Thermo Fisher Scientific) and HA antibody (red, Covance). Cells were then imaged using a LSM 510 confocal microscope (Carl Zeiss).

Mitochondrial O2 measurements

HAEC were infected and treated as described previously. After IL-13 exposure, cells were lysed at 4 °C in a mitochondrial isolation buffer (70 mM sucrose, 210 mM mannitol, 5 mM Hepes, 1 mM EGTA, 0.5% BSA, pH 7.2) and then centrifuged at 4 °C twice at 600 × *g*, and supernatant was collected. A final spin of 10,000 × *g* at 4 °C was performed. The pellet was resuspended in mitochondrial assay buffer (70 mM sucrose, 210 mM mannitol, 10 mM KH_2PO_4, 5 mM $MgCl_2$, 2 mM Hepes, 1 mM EGTA, 0.2% BSA, 10 mM succinic acid, 2 µM rotenone, pH 7.2). Mitochondria were plated on a 96-well Seahorse Extracellular Flux Analyzer plate (Seahorse Biosciences) at a density of 0.5 µg/well. The plate centrifuged at 770 × *g* at 4 °C to ensure mitochondria stably present in the bottom of each well. The mitochondria assay buffer was replaced with prewarmed assay buffer. The addition of ADP (4 mM), oligomycin (2.5 µg/mL), FCCP (4 µM), and antimycin A (4 µM) were added sequentially with OCR measurements taken between each substrate addition.

Cell viability

HAEC were grown in KSFM media and early passages were treated with adenovirus expressing Mt-CaMKIIN or Mt-GFP for 48 hrs (10 MOI). Cells were trypsinized and 100µl HAEC were incubated at room temperature for 3 minutes with an equal volume of 0.4% (w/v) trypan blue solution (cat. 15250061, ThermoFisher). Cells were counted using a hemocytometer and light microscope. Viable and nonviable cells were recorded separately and the means of independent experiments, n =3, 3 replicates/n) were analyzed.

MitoTEMPO Treatment

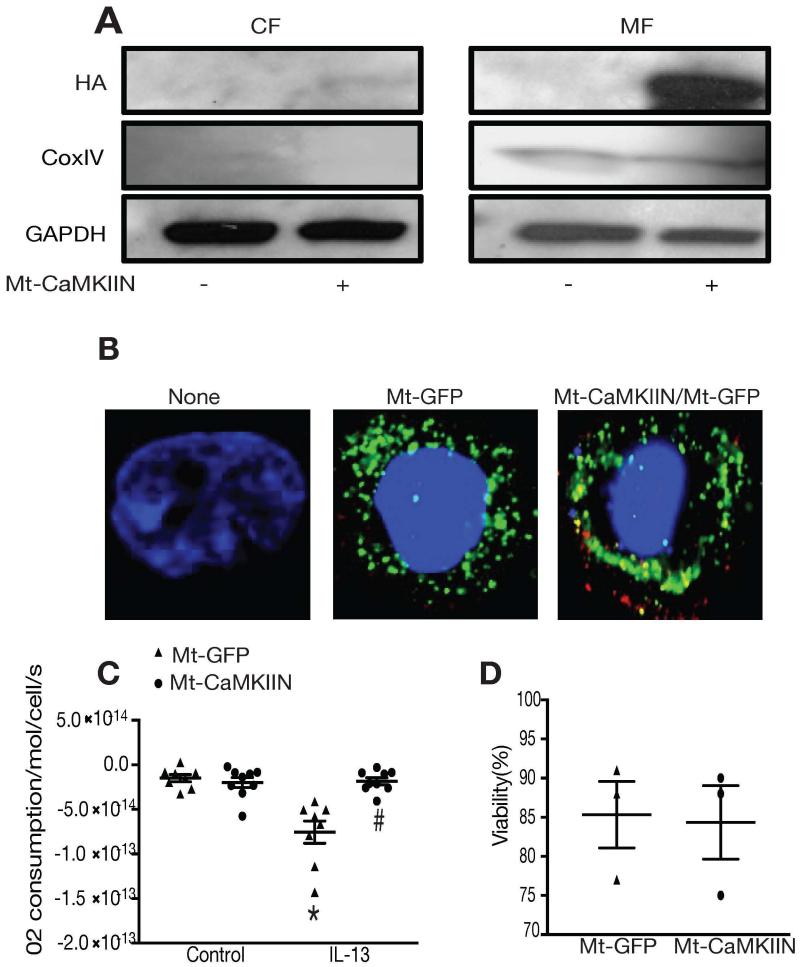
Triphenyl phosphate (TPP) or MitoTEMPO (Enzo Life Sciences, Farmingdale, NY) was delivered *in vivo* using a micro-osmotic minipump (Alzet, Cupertino, CA). Control animals were implanted with saline-filled minipumps. The pumps were implanted 3 days before OVA challenge and delivered drug at a dose of 0.7 mg/kg/d, as previously described (1, 2).

Animals

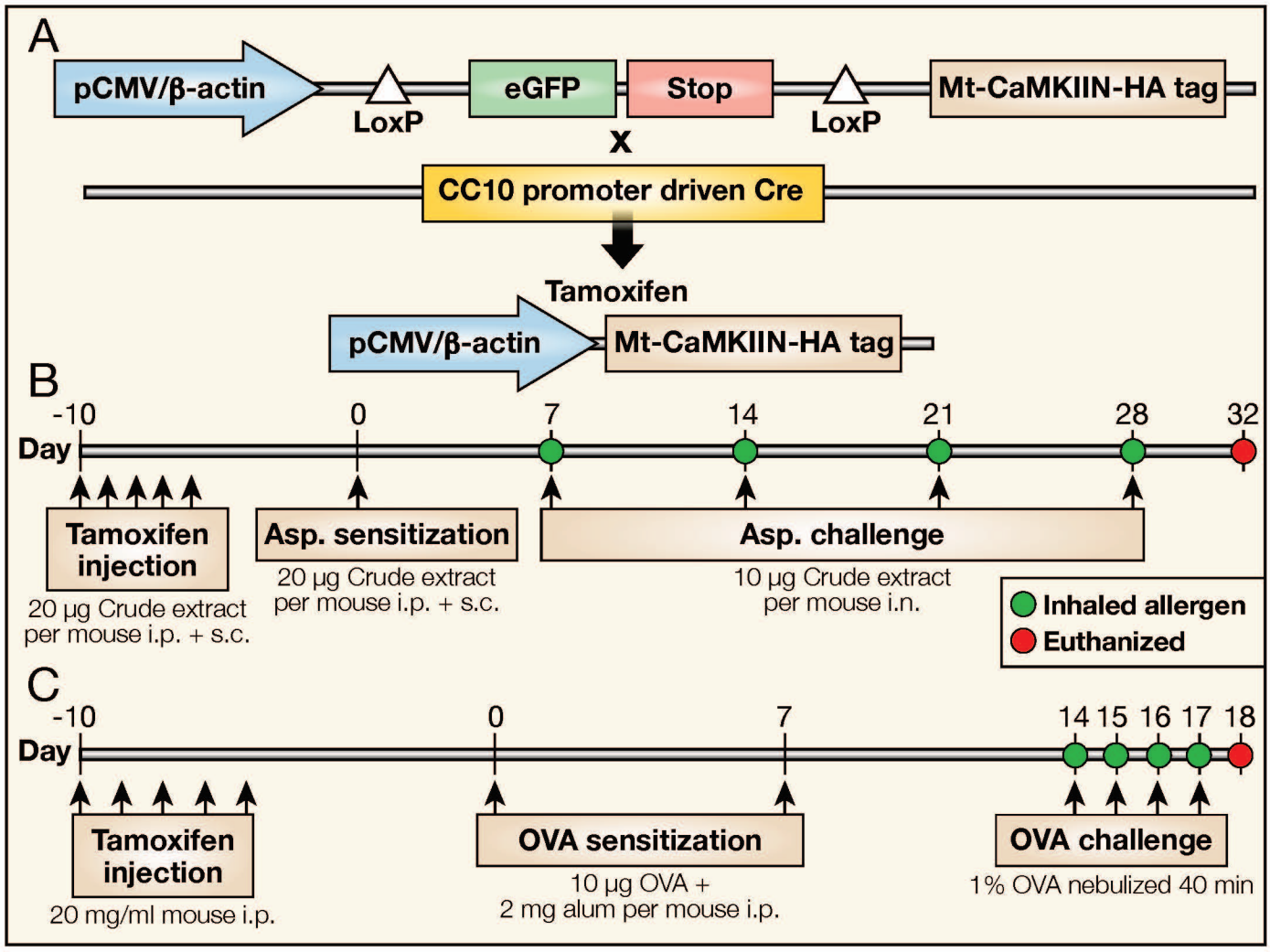
All animal care and housing requirements of the National Institutes of Health Committee on Care and Use of Laboratory Animals were followed. All protocols were reviewed and approved by the University of Iowa Animal Care and Use Committee.

References

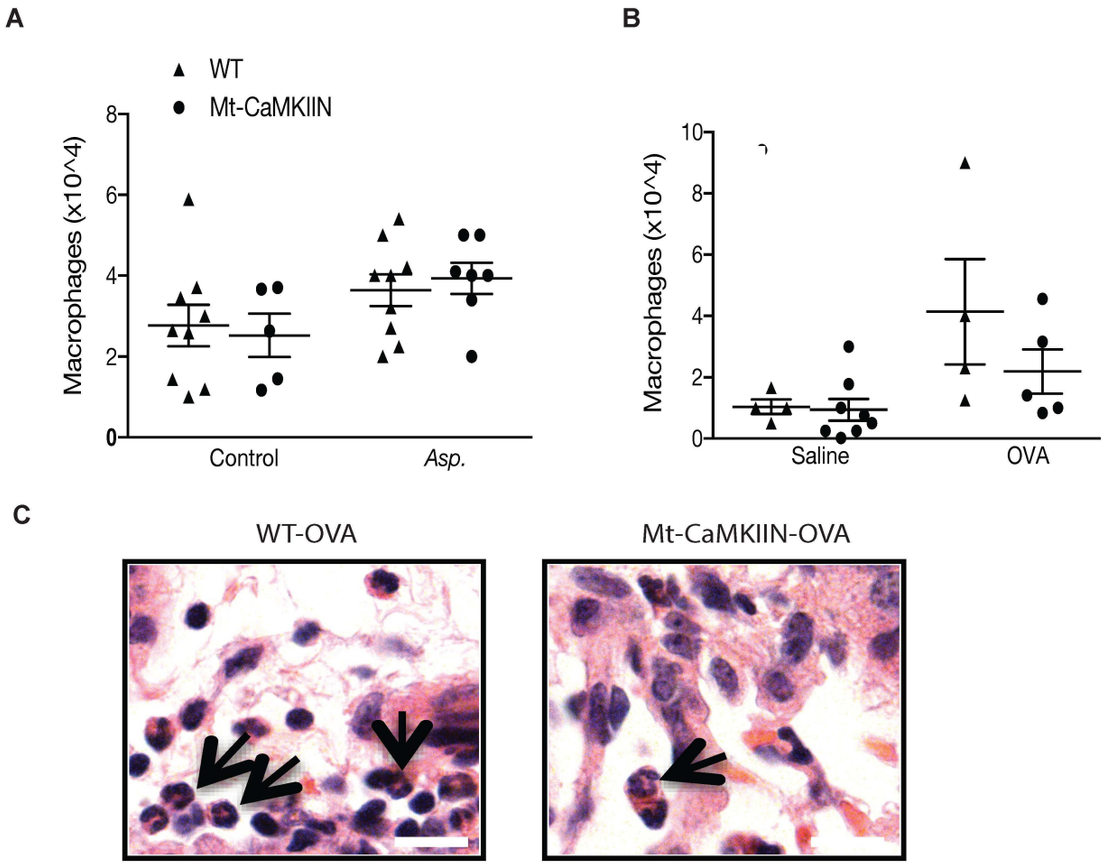
1. Jaffer OA, Carter AB, Sanders PN, Dibbern ME, Winters CJ, Murthy S, Ryan AJ, Rokita AG, Prasad AM, Zabner J, et al. Mitochondrial-targeted antioxidant therapy decreases transforming growth factor-beta-mediated collagen production in a murine asthma model. *Am J Respir Cell Mol Biol.* 2015;52(1):106-15.
2. Dikalova AE, Bikineyeva AT, Budzyn K, Nazarewicz RR, McCann L, Lewis W, Harrison DG, and Dikalov SI. Therapeutic targeting of mitochondrial superoxide in hypertension. *Circ Res.* 2010;107(1):106-16.



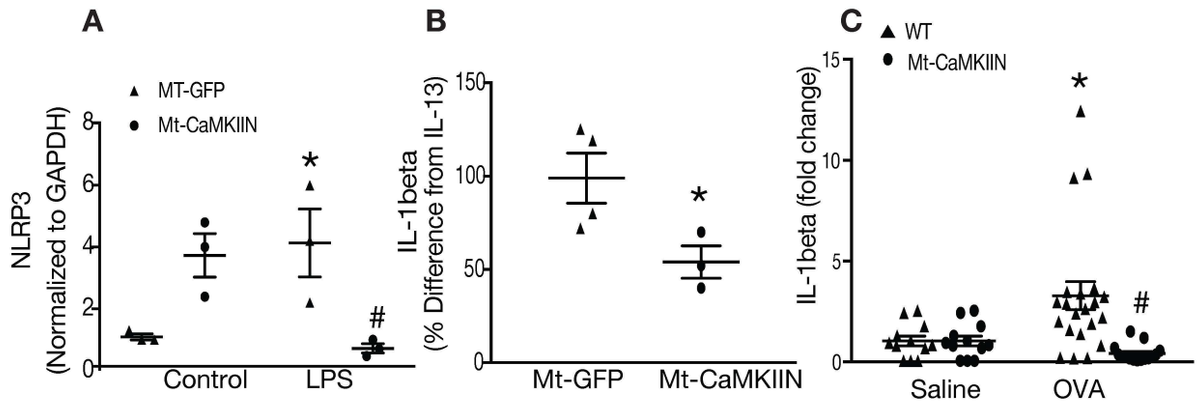
Supplemental Figure 1. (A) Mt-CaMKIIN is detected in mitochondria of adenovirus-infected HAEC . HAEC were infected with adenovirus expressing Mt-CaMKIIN or Mt-GFP control (10 MOI) for 48 h. MF = Mitochondrial Fraction; CF = Cytoplasmic Fraction.Immunoblots for HA-Tag of Mt-CaMKIIN, Cox IV (mitochondrial marker) and GAPDH (cytoplasmic marker). (B) HAEC were infected with nothing, adenovirus expressing Mt-GFP, adenoviruses Mt-GFP and Mt-CaMKIIN (10MOI). Cells were visualized by immunofluorescence for GFP (green), HA-tagged Mt-CaMKIIN (red) and nuclei (blue) and then images were merged. Scale bars are 100μM. Overlap between GFP and HA (signal represented as yellow). (C) HAEC were infected with adenovirus expressing Mt-CaMKIIN or Mt-GFP control (10 MOI) for 48 h. Oxygen consumption rate was measured (n = 3 independent experiments).* $p < 0.05$ versus control; # $p < 0.05$ versus GFP-IL-13.(D) HAEC were infected with adenovirus expressing Mt-CaMKIIN or Mt-GFP control (10 MOI) for 48 h. Cell viability was calculated by trypan blue exclusion assay (n= 3 independent experiments). Analysis using Student's 2 tailed t test.



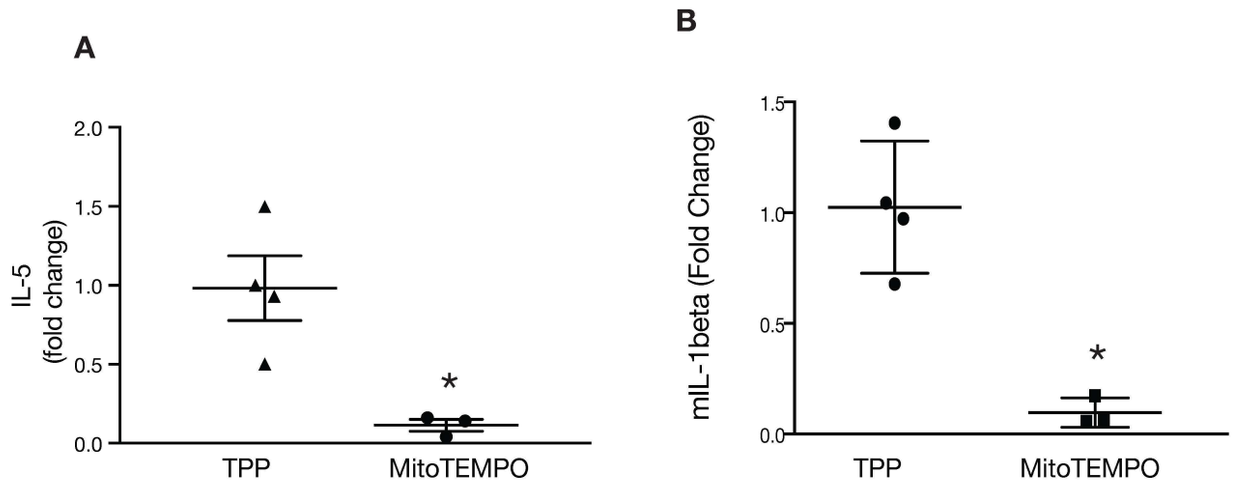
Supplemental Figure 2. Experimental models of allergic asthma in mice. (A) Schematic depiction of generation of Mt-CaMKIIN Tg mice. (B) Experimental protocol for *Aspergillus*-allergen challenge. (C) Experimental protocol for OVA-sensitization.



Supplemental Figure 3. Total macrophage numbers from A) *Asp.* and B) OVA-treated mice (N = 9 WT and 5 Mt-CaMKIIN control mice and 7-9 *Asp*-treated mice. For OVA N = 4 WT and 8 Mt-CaMKIIN control mice ,4 OVA-treated WT mice,and 6 OVA-treated Mt-CaMKIIN mice), Triangle: WT mice, circle: Mt-CaMKIIN mice.
C) Interstitial eosinophilia is reduced in OVA-treated Mt-CaMKIIN mice. Representative H&E staining showing eosinophils in the lung interstitium of OVA-treated WT and Mt-CaMKIIN mice (×100, 1mm, arrows point to eosinophils).



Supplemental Figure 4. Epithelial Mt-CaMKII inhibition reduces mRNA expression of NF-κB-dependent markers. Triangles: Mt-GFP; circles: Mt-CaMKIIN. qRT-PCR of (A) NLRP3-HAEC were infected with adenovirus containing Mt-GFP or Mt-CaMKIIN and treated with control or LPS (5μg/ml) for 24 hrs. GAPDH, loading control (n= 3 independent experiments). mRNA was quantified by RT-PCR and normalized to GAPDH. (B) HAEC were infected with adenovirus containing Mt-GFP or Mt-CaMKIIN and treated with control or IL-13 (10ng/ml) for 48 hrs. Control cells treated with media alone had no detectable expression . mRNA was quantified by RT-PCR, normalized to GAPDH and then represented as percent difference GFP-IL-13 versus Mt-CaMKIIN-IL-13 . (n = 3 independent experiments).(C) qRT-PCR for IL-1beta in lungs from OVA-challenged WT or Mt-CaMKIIN mice (triangle and circle respectively; n = 4 controls and 7 -OVA-treated mice). Normalized to ARP control. Data was analyzed by ANOVA with Tukey post hoc test.
* $p < 0.05$ vs. control; # $p < 0.05$ vs. WT/Mt-GFP-treated.



Supplemental Figure 5. MitoTEMPO reduces OVA-mediated IL-5 and IL-1beta mRNA expression. qRT-PCR from lungs of mice infused with either Triphenyl phosphate (TPP) control or MItoTEMPO and challenged with OVA (n = 4 mice/group) (A) IL-5 mRNA; (B) IL-1beta mRNA. Data normalized to TPP OVA-challenged mice. Student's two tailed t-test.
* $p < 0.05$ versus TPP-OVA.

	Atopic asthmatics	Normal controls
Gender, M/F	2/7	4/2
Age	30.2±5.9 (23-39)	58±6.7 (47-67)
Race		
White	8	4
African American	1	2
Family history of atopy and asthma	9	0
Baseline spirometry (%predicted)		
FVC	101±15.6 (83-123)	110.5±11 (92-128)
FEV1	100±14.5 (79-126)	105±9.9 (89-122)
FEV1/FVC	90±8.3 (75-104)	91.5±9.6 (79-106)
Drugs (daily or as needed)		
Short-acting β_2-agonist	9	0
Long-acting β_2-agonist	1	0
Inhaled corticosteroids	1	0
Nasal corticosteroids	2	0
Leukotriene modifier	1	0
Antihistamine	3	0

Table 1. Demographic data, history of atopy and asthma and asthma medication use.
FVC- forced vital capacity
FEV1% - forced expiratory volume in 1 second