Supplementary materials

Extracellular catalase had no effect on UVA-induced generation of intracellular ROS

To examine the possible effect of UVA irradiation on both buffer alone or on the probe CM-H_2DCFDA, buffer containing CM-H_2DCFDA (5 μM) was aliquoted to 96-well plate (150 μL / well) and fluorescence measured in a microplate reader (Infinite® F200 PRO, TECAN) with or without UVA irradiation (LED, 80 μW.cm^{-2}, 2 min) (excitation / emission at 485 / 535 nm). CM-H_2DCFDA fluorescence in buffer alone without UVA irradiation was minimal (Fig. S1***a***), UVA irradiation (LED 380 nm, 80 μW.cm^{-2}, 2 min) did not alter this fluorescence (Fig. S1***b***), neither did catalase (400 U. ml^{-1}) addition have any effect (Fig. S1***c***). CM-H_2DCFDA once taken up by RBL-2H3, fluorescence of the released DCFDA was brighter than CM-H_2DCFDA (Fig. S1***d***). DCFDA-loaded RBL-2H3 cells after irradiation with UVA (LED, 80 μW.cm^{-2}, 2 min) showed marked ROS production (Fig. S1***e***). Extracellualr catalase at 400 U. ml^{-1} had no effect on UVA-induced ROS generation in DCFDA-loaded RBL-2H3 cells (Fig. S1***f***). The above data indicate that UVA irradiation of buffer (without mast cells) does not generate H_2O_2; alternatively, UVA irradiation of the probe CM-H_2DCFDA does not generate ROS either. However, UVA irradiation of mast cells leads to marked ROS production, which is not altered by the addition of extracellular catalase (to remove hydrogen peroxide).

Specific siRNA down-regulated expression of gp91^{phox} (NOX2), p22^{phox}, p47^{phox} in RBL-2H3 mast cells as detected by Western blot

RBL-2H3 cells incubated with specific siRNA at 100 nM as indicated in Methods were harvested, washed twice in phosphate buffered saline. The cell pellet was re-suspended in lysis buffer, vortex mixed, and after centrifugation the supernatant (20 μg of protein of each cell lysate) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electroblotted onto PVDF membrane. The blotted membrane was washed 3 times in phosphate buffered saline /

Tween-20, blocked for 1 h in 5% non-fat milk, incubated with primary antibody at 4 °C overnight, followed by incubation with horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h, washed 3 times in phosphate buffered saline / Tween-20. Immunoreactive protein bands were detected with enhanced ECL kit in a chemiluminescence detector (Clinx, Shanghai, China). Western blot experiments revealed that specific siRNA down-regulated RBL-2H3 expression of gp91^{phox} (NOX2), p22^{phox}, p47^{phox} subunit proteins significantly (Fig. S2***A***), down to (26.32 ± 3.56) %, (56.08 ± 7.10) %, (56.25 ± 4.56) % of scramble controls respectively (Fig. S2***B***)($P < 0.05$, $N = 3$). Therefore data from Western blot further confirmed data from both real-time polymerase chain reaction (down regulated mRNA expression, Fig. 6***A***) and data from immunocytochemistry (down regulated subcellular protein localization, Fig. 6***B***).

Extracellular catalase had no effect on UVA-induced cytotolic calcium oscillations

To examine any possible effects of extracellular catalse on UVA-induced calcium oscillations, Fura-2 AM-loaded RBL-2H3 cells were irradiated with UVA (LED, 380 nm, 80 μW.cm^{-2}) in the absence (Fig. S3***a***) or presence (Fig. S3***b***) of catalase (400 U.ml^{-1}). Time-matched parallel experiments found that UVA irradiation induced regular calcium oscillations in perifused rat peritoneal mast cells (Fig. S3***a***)(peritoneal mast cells were used here because these cells tend to generate more regular and better-looking calcium oscillations than RBL-2H3). The addition of extracellular catalase (400 U. ml^{-1}) did not have any effect on UVA-induced calcium oscillations (Fig. S3***b***). Therefore extracellular catalase did not have any major impact on UVA-induced calcium oscillations in mast cells.

Supplementary figure legends

Figure S1. Extracellular catalase had no effect on UVA-induced intracellular ROS production. Buffer alone containing H_2DCFDA (***a-c***), H_2DCFDA-loaded RBL-2H3 cells (***d-f***) were monitored in 96-well plate in a microplate reader with (***b, c, e, f***) or without (***a, d***) UVA irradiation (LED, 380 nm, 80 μW.cm^{-2}, 2 min), with (***c, f***) or without (***a, b, d, e***) catalase (400 U.ml^{-1}) added 30 min before UVA irradiation. DCFDA fluorescence was plotted with time (mean ± SEM, $N = 3$).

Figure S2. Specific siRNA treatment of RBL-2H3 mast cells down-regulated NOX2, p22^{phox}, p47^{phox} protein expression as detected by Western blot. Cell lysate was subjected to SDS-PAGE and Western blot with actin as internal standard was done. (***A***) Both scramble controls (lanes 1, 3, 5) and specific siRNA (2, 4, 6)-treated RBL-2H3 mast cells were lyzed and Western blot performed. Panel (***A***) shows original images from representative experiments. In Panel (***B***) mean protein levels were normalized to scramble controls (as 1.0) and presented as mean ± SEM. Asterisk (*) indicates $P < 0.05$ ($N = 3$).

Figure S3. Extracellular catalase had no effect on UVA-induced cytosolic calcium oscillations. Fura-2-loaded rat peritoneal mast cells were perifused, UVA (LED, 380 nm, 80 μW.cm^{-2}) irradiated as indicated by the horizontal bars, in the absence (***a***) or presence (***b***) of extracellular catalse (400 U.ml^{-1}). Calcium tracings in each panel (obtained from one individual cell in the PMT-based system) are each representative of 3 independent experiments.

Figure S4. NOX2 plays an essential role in UVA-induced ROS generation, cytosolic calcium oscillation and mediator release in mast cells. UVA activates the prominent mast cell NADPH oxidase 2 (NOX2) to produce reactive oxygen species (ROS) via initial generation of superoxide anion ($O_2^{-\cdot}$). ROS oxidatively activates phospholipase C_γ (PLC_γ) to trigger eventual cytosolic calcium oscillations via the

PIP2-IP3 pathway. Increases in cytosolic calcium sequentially activate cytosolic phospholipase A_2 (PLA_2) and 5-lipoxygenase, leading to the release of leukotriene C4 (LTC4). Calcium increases also activate transcription factors such as NF-κB leading to the increased synthesis of IL-6.

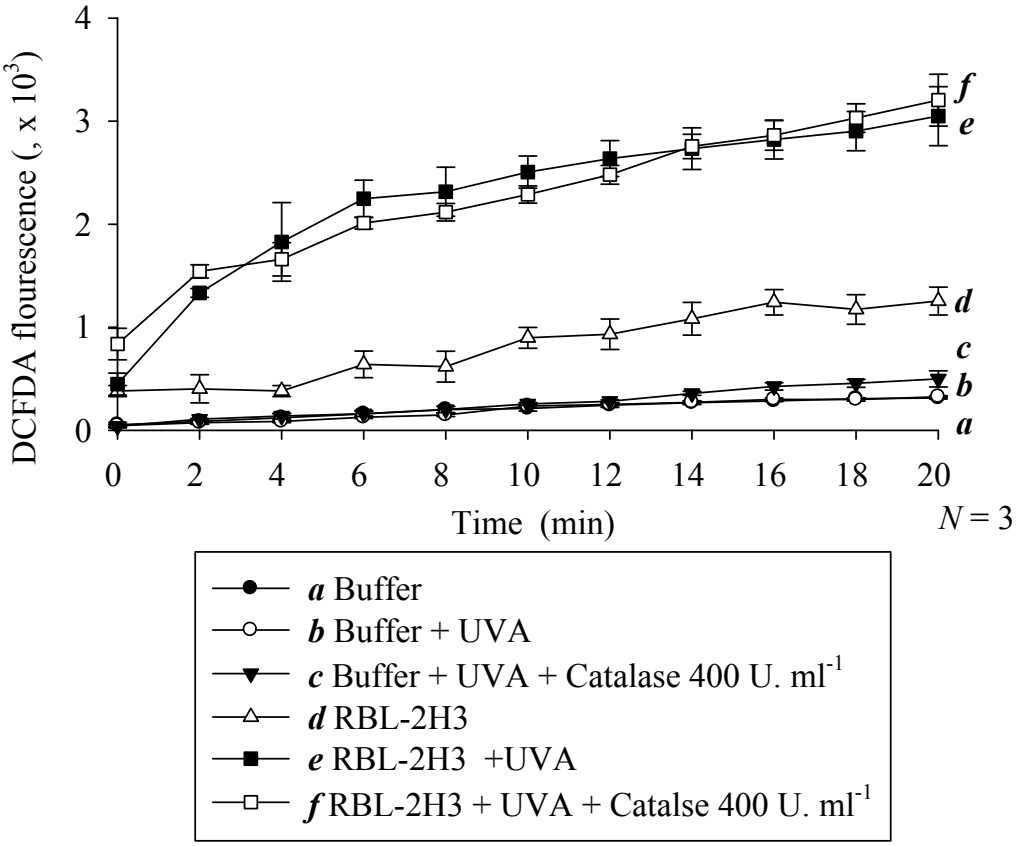
DCFDA flourescence (, x 10^3)
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
Time (min)
N = 3
f
e
d
c
b
a
a Buffer
b Buffer + UVA
c Buffer + UVA + Catalase 400 U. ml-1
d RBL-2H3
e RBL-2H3 +UVA
f RBL-2H3 + UVA + Catalse 400 U. ml-1

Figure S1

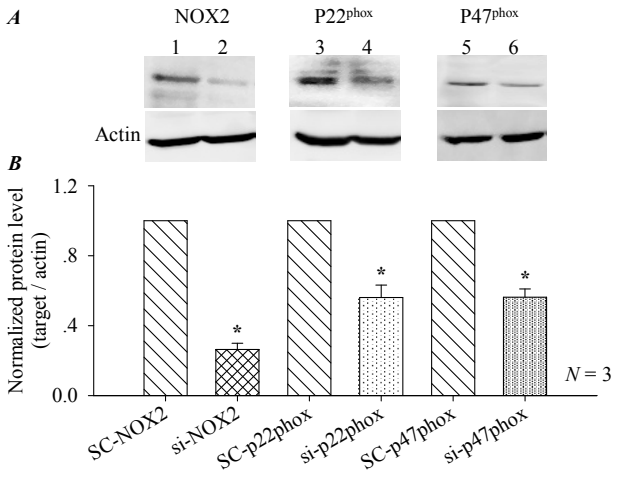
A
NOX2
P22phox
P47phox
1 2
3 4
5 6
Actin
B
Normalized protein level
(target / actin)
1.2
.8
.4
0.0
*
*
*
N = 3
SC-NOX2
si-NOX2
SC-p22phox
si-p22phox
SC-p47phox
si-p47phox

Figure S2

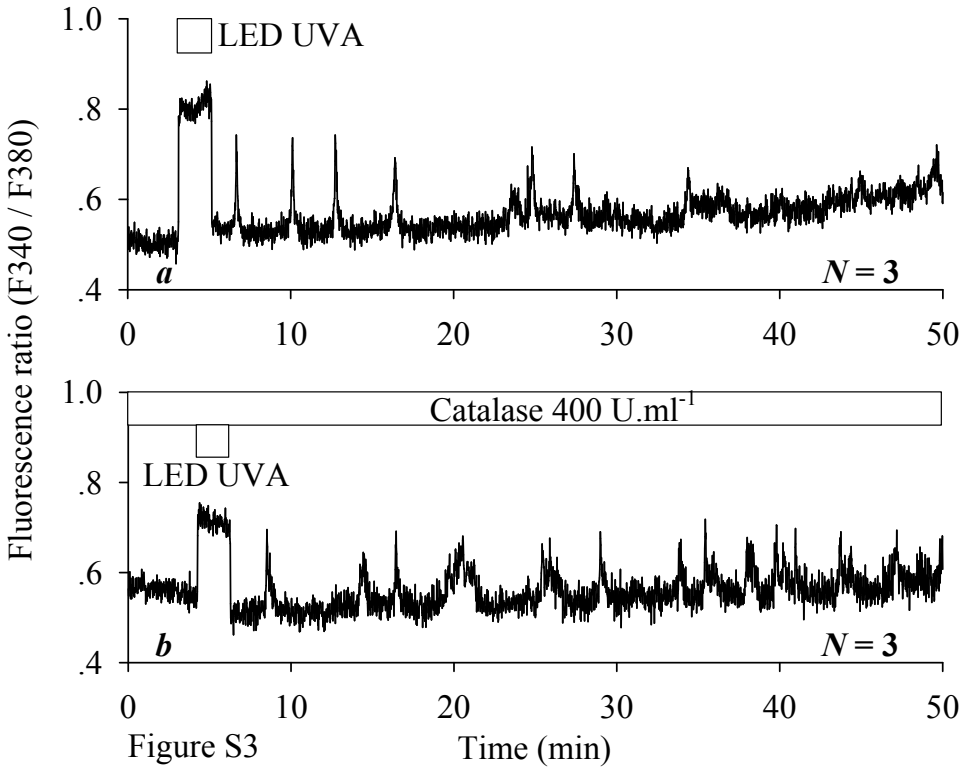
1.0
.8
.6
.4
LED UVA
a
N = 3
0
10
20
30
40
50
Fluorescence ratio (F340 / F380)
1.0
Catalase 400 U.ml-1
LED UVA
.8
.6
b
N = 3
.4
0
10
20
30
40
50
Time (min)

Figure S3

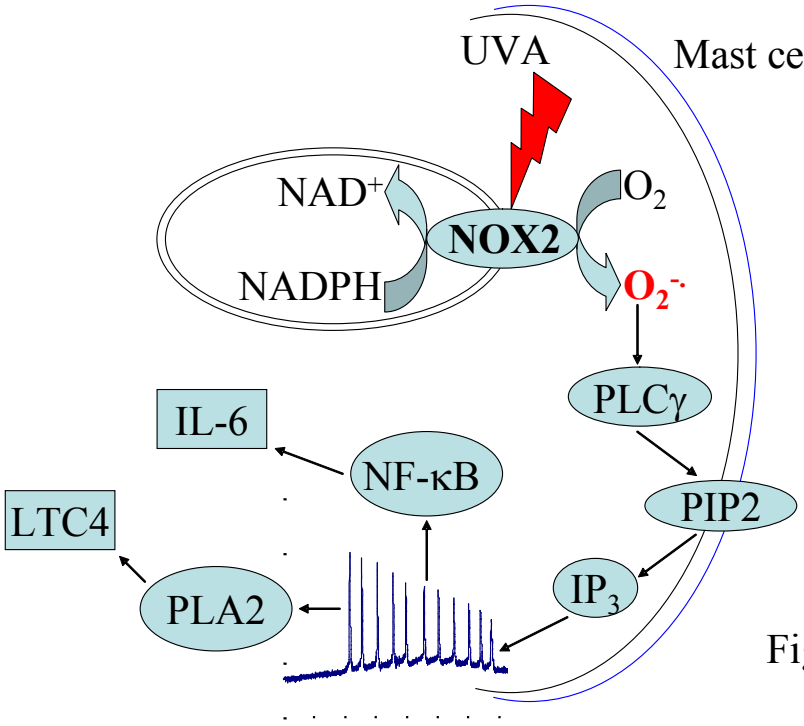
UVA
Mast cell
NAD+
NADPH
NOX2
O2
O2-·
PLCγ
IL-6
NF-κB
PIP2
LTC4
PLA2
IP3

Figure S4