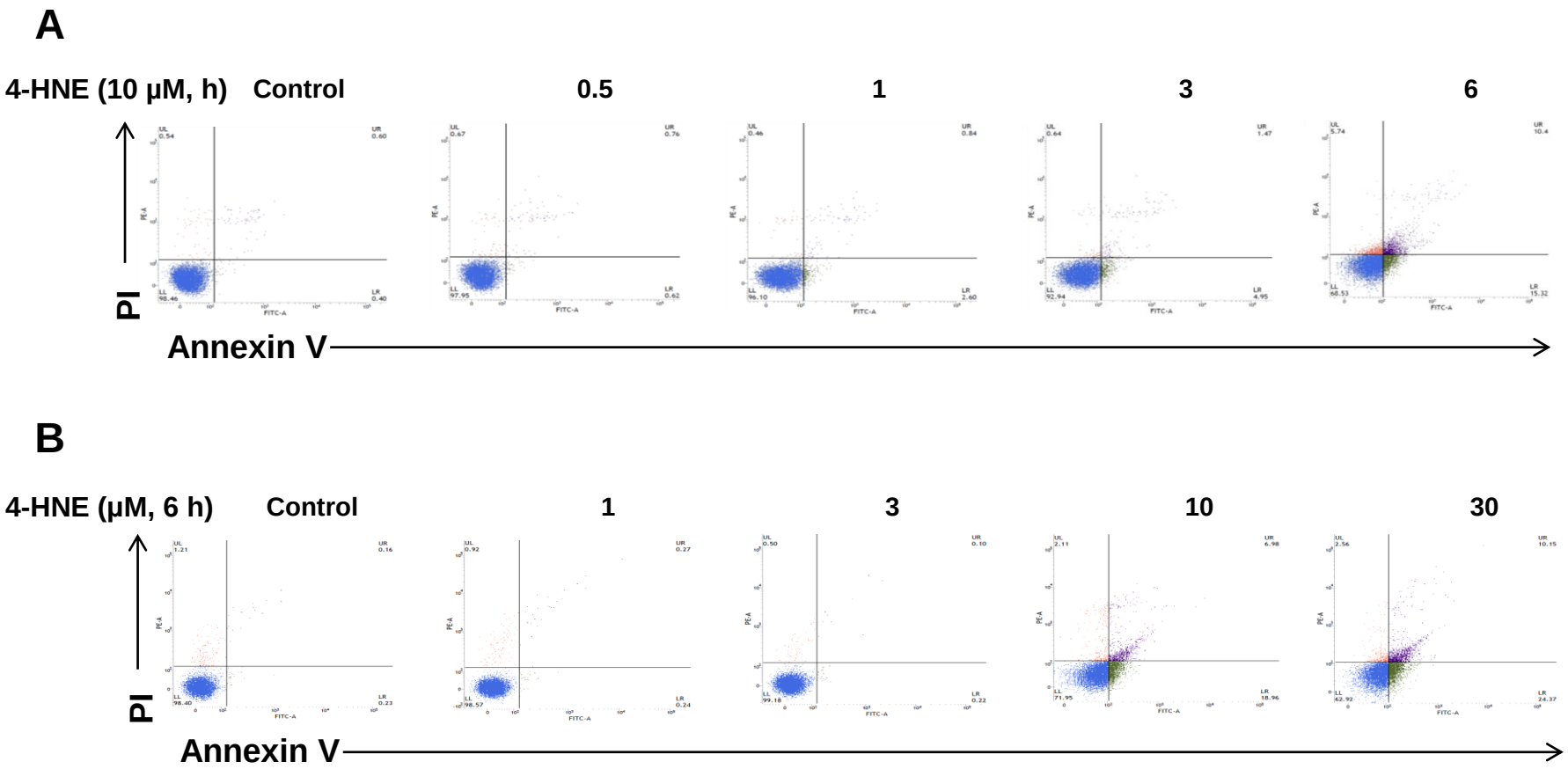
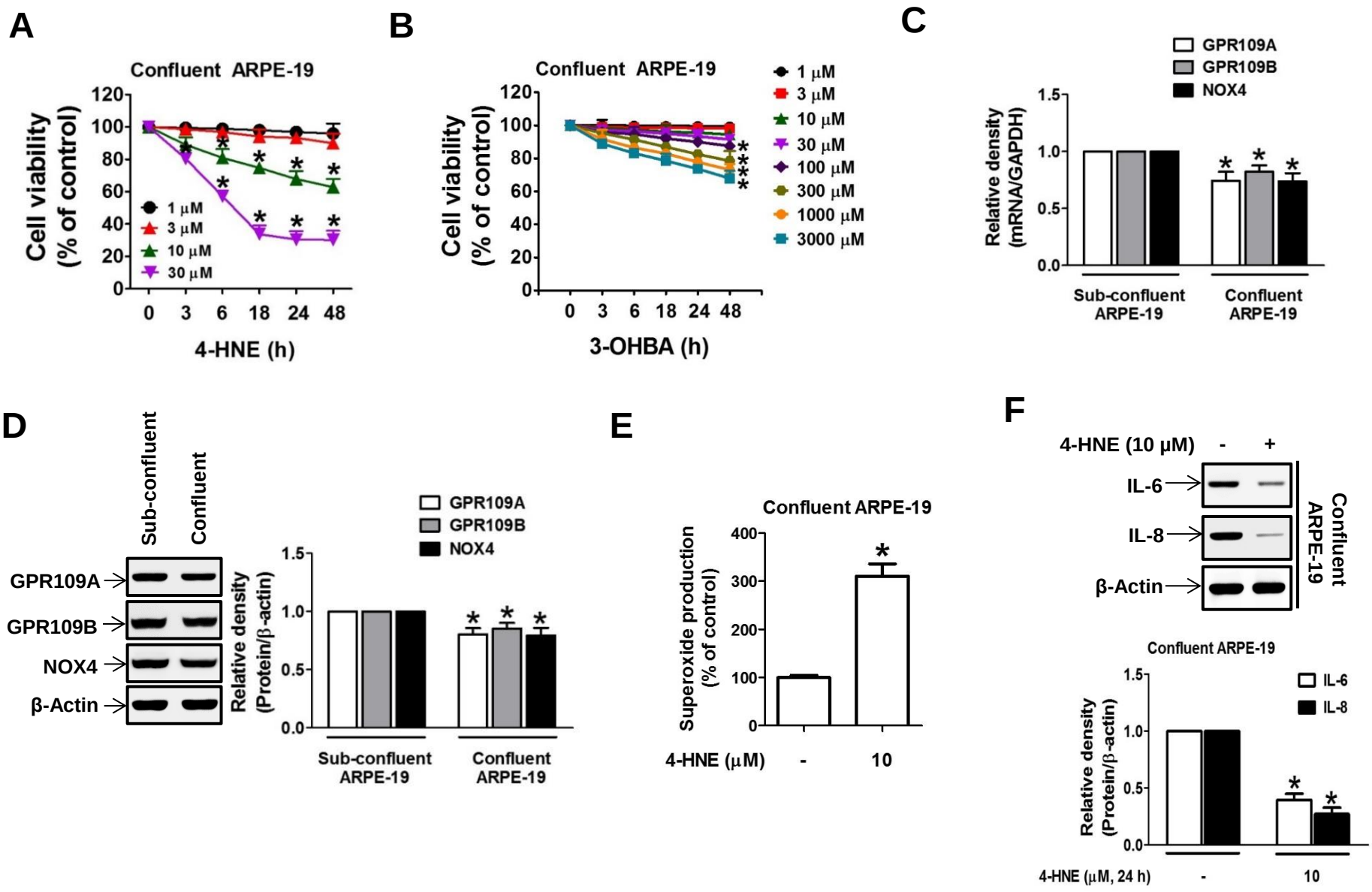
Supplementary Figure S1



Supplementary Figure S1

4-HNE induces ARPE-19 cell apoptosis in a concentration- and time-dependent manner. Annexin V-positive and propidium iodide-positive ARPE-19 cell populations were determined after the treatment with 4-HNE 10 μM at an indicated time points (A) and at an indicated concentrations of 4-HNE at 6 h (B) by using a flow cytometry.

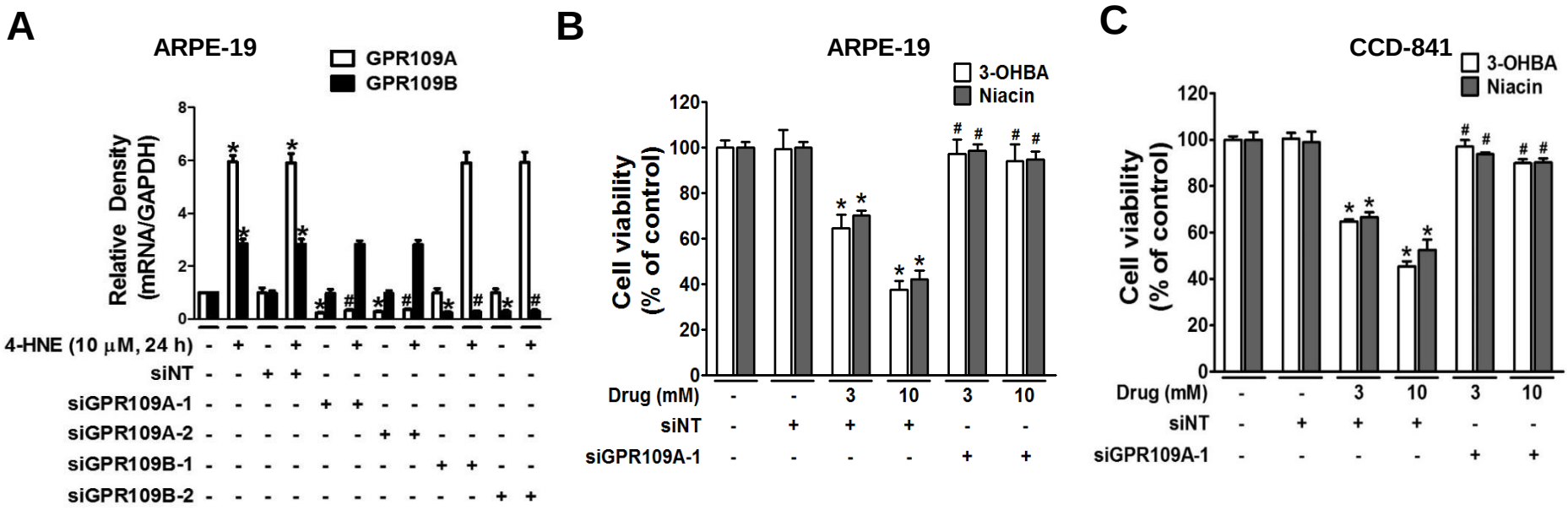
Supplementary Figure S2



Supplementary Figure S2

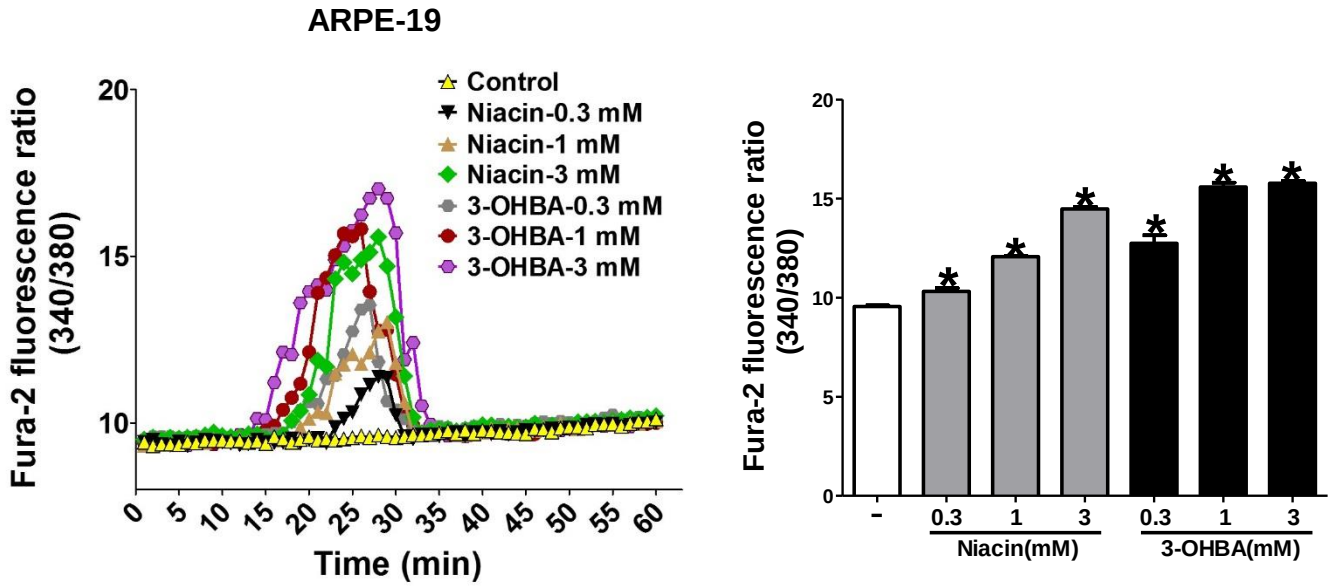
Reduced cytotoxicity and anti-inflammatory effect of 4-HNE in confluent ARPE-19 cells correlates with lower level expression of receptors and NOX4. After 24 hours of cell seeding at a density of $1*10^5$ cells/cm^2, cells were sub-confluent, whereas after 48 hours of cell seeding with same seeding density, cells were confluent. (A, E and F) Confluent cells were treated with or without 4-HNE at an indicated concentration for an indicated period of time and examined cytotoxicity (A), superoxide production (E) and Il-6 and IL-8 protein expressions (F). *$P < 0.05$ vs. vehicle-treated controls. (B) Confluent ARPE-19 cells were treated with or without 3-OHBA in a concentration and time-dependent manner and examined cytotoxicity. *$P < 0.05$ vs. vehicle-treated controls. (C, D) Comparison of basal mRNA (C) and protein (D) expressions in sub-confluent and confluent ARPE-19 cells. *$P < 0.05$ vs. sub-confluent ARPE-19 cells.

Supplementary Figure S3



Supplementary Figure S3

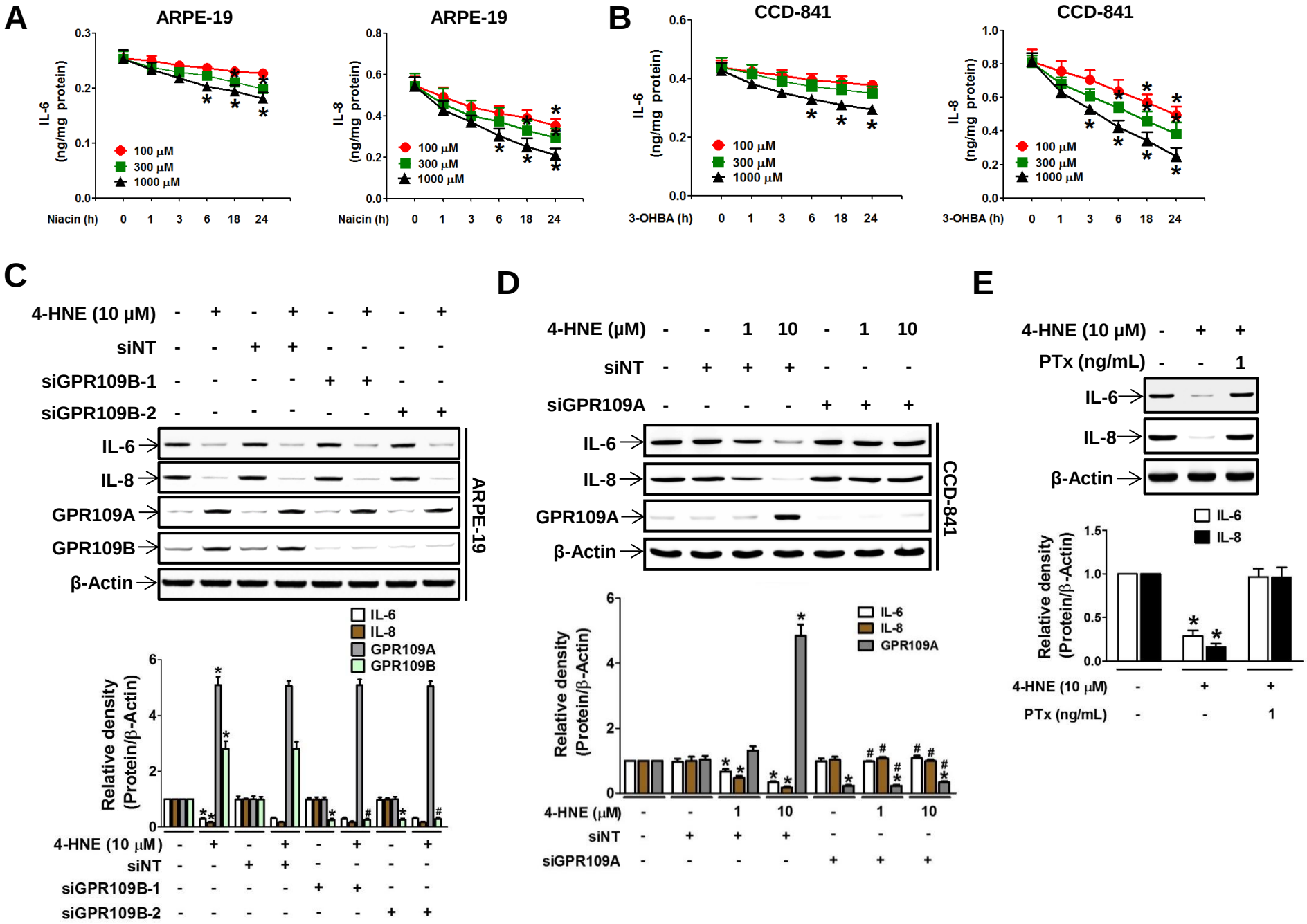
Niacin and 3-OHBA induces cell death in GPR109A–dependent manner. (A) Transfection efficiency of siRNA targeted against GPR109A or GPR109B was measured by qRT-PCR. After RNA extraction from the cells, cDNA was synthesized using the Goscript reverse transcription system (Promega Corporation, WI, USA). qRTPCR was performed using Quantitect Probe PCR kit (Qiagen, CA, USA) following the manufacturers protocol using probe PCR primers specific for GPR109A (Hs02341584_s1) or GPR109B (Hs02341102_s1) obtained from Applied Biosystems (Thermo Fisher Scientific corporation, CA, USA). *$P < 0.05$ vs. vehicle-treated controls. $^{\#}P < 0.05$ vs. 4-HNE-treated groups. (B and C) Cell viability was measured after the treatment with niacin or 3-OHBA in GPR109A siRNA-transfected ARPE-19 (B) and CCD-841 (C) cells. *$P < 0.05$ vs. vehicle-treated controls. $^{\#}P < 0.05$ vs. niacin or 3-OHBA-treated groups.



Supplementary Figure S4

Increase in intracellular Ca^{2+} levels after treatment of ARPE-19 cells with indicated concentrations of niacin or 3-OHBA. Bars represent means ± SEM from three independent experiments. *P < 0.05 vs. vehicle-treated controls.

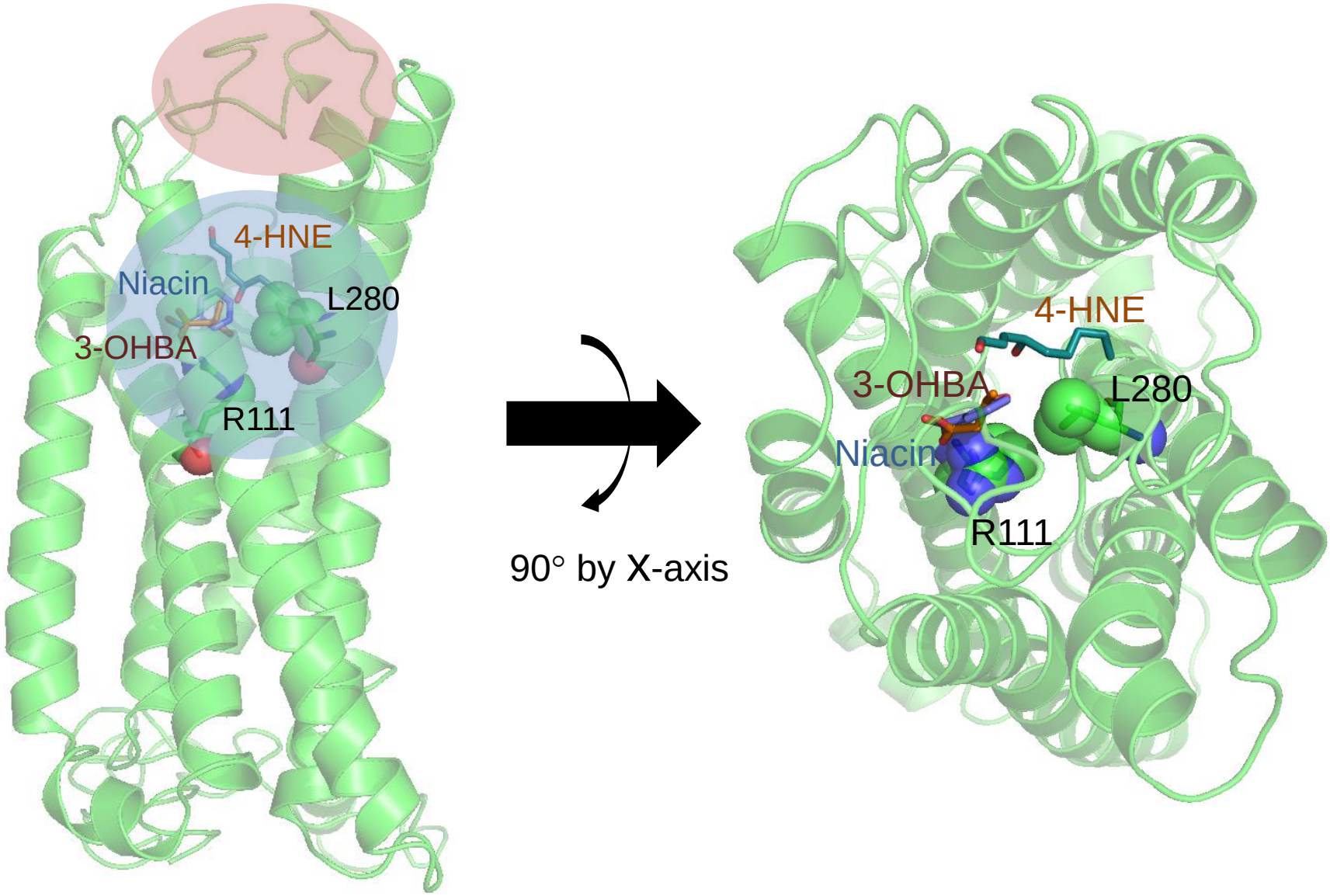
Supplementary Figure S5



Supplementary Figure S5

Niacin, 3-OHBA and 4-HNE inhibits the pro-inflammatory cytokines IL-6 and IL-8 expression. (A-B) Secretion of the IL-6 and IL-8 in ARPE-19 (A) and CCD-841 (B) cells was measured via ELISA. *P < 0.05 vs. vehicle-treated controls. (C and D) 4-HNE-induced expression of IL-6 and IL-8 was measured in GPR109B siRNA-transfected ARPE-19 cells (C) and GPR109A siRNA-transfected CCD-841 cells (D). *P < 0.05 vs. vehicle-treated controls $^{\#}P$ < 0.05 vs. 4-HNE-treated groups. (E) 4-HNE induced IL-6 and IL-8 protein expression was recovered by PTx treatment. *P < 0.05 vs. vehicle-treated controls.

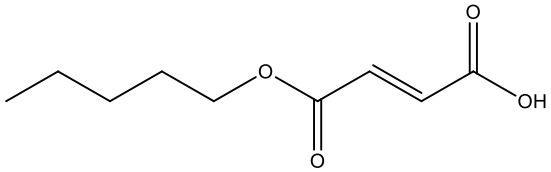
Supplementary Figure S6



Supplementary Figure S6

Model structure of GPR109A in complex with niacin, 3-OHBA, and 4-HNE. The structure was extracted from GPCR-I-TASSER server (https://zhanglab.ccmb.med.umich.edu/GPCR-HGmod/models/Q8TDS4) (Zhang et al., 2015). The predicted two ligand binding sites by FTMap (Kozakov et al., 2015) are circled in blue and orange colors. Arg-111 and Leu-280 that were identified as the critical residues by mutational studies are represented with spheres. The niacin, 3-OHBA, and 4-HNE were docked into the structure using Glide-XP (Friesner et al., 2006). The figure was prepared by Pymol (Schrodinger, 2010).

Supplementary Figure S7



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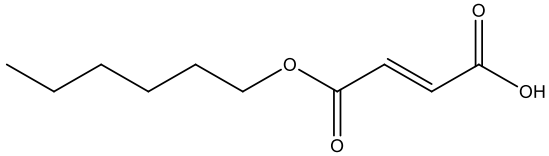

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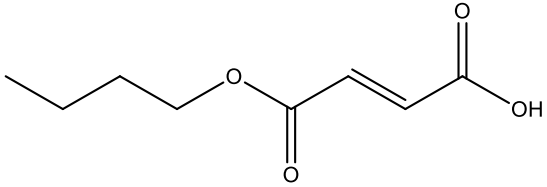

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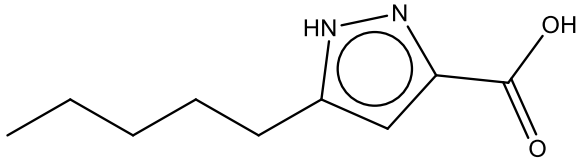

ZINC28824279 (0.20)

Supplementary Figure S7

The closest chemicals to 4-HNE of the known GPR109A agonists. ZINC IDs and parentheses enclosed corresponding Tanimoto coefficients are written.