Supplmentary materials:

supplementary methods, 4 supplementary figures, and 1 supplementary table.

PMA-induced dissociation of Ku86 from the promoter causes transcriptional up-regulation of histamine H_1 receptor.

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Supplementary methods

Construction of the deletion mutants for promoter assay. The deletion mutant (MT) plasmids p1336, p1137, p960, p914, p398, p221, and p44 were constructed as follows: the cloned 2.1-kb fragment in p2029 was subcloned into pBluescript II vector (Stratagene) and serial deletion MT plasmids were PCR amplified using the primer sets summarized in Table S1. Resulting plasmids were digested with *Hind*III and *Kpn*I and ligated back into the pGL3-Basic vector. The deletion MT plasmids p170, p106, p85, and p65 were constructed as follows: a *Kpn*I site was inserted at the indicated sites in the p221 vector using the QuikChange II site-directed mutagenesis kit (Stratagene). Resulting plasmids were digested with *Kpn*I and self-ligated.

Preparation of nuclear extracts. HeLa cells in 100-mm dishes were serum-starved for 24 h, and then stimulated with 100 nM of PMA for 2 h in the same medium. After stimulation, the cells were harvested in PBS and centrifuged at 500 ×*g* for 3 min at 4°C, resuspended in 300 μl of buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, and 0.5 mM PMSF), and incubated on ice for 15 min. The cell suspension was then mixed with 0.6% NP-40, vortexed for 10 s, and

centrifuged at 500 ×*g* for 3 min at 4°C. After being washed with 300 μl of buffer A, the pellet was resuspended in 100 μl of nuclear lysis buffer [10% (v/v) glycerol, 20 mM HEPES, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 1 mM PMSF]. The cell suspension was vortexed 5 times for 1 min and incubated on ice for 15 min. After centrifugation at 15,000 ×g for 15 min, the resulting supernatant was designated as the nuclear extract. Protein concentration was determined by the Bradford method.

Immunoprecipitation assay. HeLa cells in 100-mm dishes were serum-starved for 24 h and stimulated with or without 100 nM of PMA for 1 h. The cells were harvested in PBS and whole cell extracts were prepared by sonication. rProtein A Sepharose Fast Flow beads (GE Healthcare) and normal mouse IgG (Santa Cruz) were added to the whole cell extracts and incubated for 30 min at 4°C. After centrifugation, aliquots were removed and immunoprecipited with antibody [Ku86(B-1) or PARP-1(F-2)]. In order to completely inhibit endogenous H1R signaling, HeLa cells were pretreated with 10 μM of rottlerin for 1 h before PMA stimulation. Proteins were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad). The membrane

was briefly rinsed in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and incubated for 1 h at room temperature in TBS-T containing 5% skim milk (Difco). The membrane was incubated overnight at 4°C with a primary antibody [Ku86(B-1) or PARP-1(F-2)]. HRP-conjugated goat anti-mouse IgG (Bio-Rad) was used as the secondary antibody, and proteins were visualized using an Immobilon Western Chemiluminescent HRP substrate (Millipore). For poly(ADP-ribisyl)ation study, HeLa cells in 150-mm dishes were serum-starved for 24 h and stimulated with or without 100 nM of PMA for the time indicated. The cells were harvested in lysis buffer [40 mM HEPES pH 7.5, 120 mM NaCl, 0.3% CHAPS, 1 mM EDTA, 1X Complete mini^{TM} protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA) and 1mM PARG inhibitor ADP-HPD (adenosine 5′-diphosphate (hydroxymethyl) pyrrolidinediol) (EMD Chemicals, Gibbstown, NJ, USA)] and whole cell extracts were prepared by sonication. Bio-Adembeads Protein A (Ademtech, Pessac France) and normal mouse IgG (Santa Cruz) were added to the whole cell extracts and incubated for 30 min at 4°C. After centrifugation, aliquots were removed and immunoprecipited with anti-pADPr antibody 10H (Tulip Biolabs, West Point, PA, USA). Proteins were

separated by 8% SDS-PAGE using WIDE RANGE Gel Preparation Buffer (4X) for PAGE and MOPS-SDS running buffer (50 mM Tris base, 50 mM MOPS, 1 mM EDTA, and 0.1% SDS) (nacalai tesque, Kyoto Japan).

Chromatin immunoprecipitation (ChIP) assay. HeLa cells in 60-mm culture plates were serum-starved for 24 h, and stimulated with or without 100 nM of PMA or 100 μM histamine for the times indicated. The cells were treated with 1% formaldehyde for 30 min at 4°C. The cross-linking reaction was terminated by the addition of 125 mM glycine for 5 min at room temperature. After cross-linking, the cells were harvested in 1 ml PBS containing Complete Mini Protease Inhibitor Cocktail (Roche) and centrifuged at 700 ×*g* for 5 min at 4°C. The pellets were resuspended in 0.3 ml of cell lysis buffer (10 mM HEPES-KOH, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and 0.5% NP-40) and centrifuged at 1400 × *g* for 5 min at 4°C. The resulting pellets were resuspended in 0.2 ml of nuclear lysis buffer (50 mM Tris-HCl, pH 8.1, 10 mM EDTA, and 1% SDS) and sonicated to generate chromatin fragments. The sheared chromatin fragments were collected by centrifugation at 13,000 × *g* for 10 min at 4°C and aliquots were diluted with 1.8 ml of dilution buffer (16.7 mM Tris-HCl, pH

8.1, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100, and 0.01% SDS) containing protease inhibitors. rProtein A Sepharose Fast Flow beads and sonicated salmon sperm DNA were added to the diluted aliquots and incubated for 1 h at 4°C. After centrifugation at 800 × *g* for 2 min at 4°C, 1% of aliquots were removed to serve as the positive input sample. The sheared chromatin immunoprecipitated with anti-Ku86 antibody or normal mouse IgG (Santa Cruz) was used as the negative control and was added to rProtein A Sepharose Fast Flow beads with sonicated salmon sperm DNA alone. The pellets were resuspended in 0.25 ml of elution buffer (0.1 M $NaHCO_3$ and 1% SDS) and 10 μl of 5 M NaCl and incubated overnight at 65°C. To remove proteins and RNA, 10 μl of 1 M Tris HCl, pH 6.5, 1.5 μl of 20 mg/ml proteinase K (Wako), 5 μl of EDTA, and 3 μl of 10 mg/ml RNase A (Wako) were added to the aliquots and incubated for 1 h at 45°C. The DNA fragments were purified using the QIAquick PCR Purification Kit (Qiagen) and analyzed by PCR (forward primer: 5′-GGGGTACCGAGGGTCTTTCCACTC-3′ and reverse primer: 5′-CCCTATTCCTTTAGAAGCCAGG-3′) to amplify a region B (−221/−44) within the H1R promoter. PCR conditions were as follows: denaturation at 94°C for 10 min and

35 cycles at 94°C for 30 s, 57.8°C for 30 s, and 72°C for 30 s. To exam the specificity of PCR, we performed the control experiment using a set of primers to amplify the region A promoter of H1R (forward primer: 5′-GGGAGTTTATCTAAACATCTATGCCC-3′ and reverse primer: 5′-CACCTCACCAGAACAGAACTATCC-3′). The PCR products were separated by electrophoresis on a 2% agarose gel and visualized after ethidium bromide staining. For the control, the extract was prepared from HeLa cells pretreated with 10 μM of rottlerin (Calbiochem, San Diego, CA) 1 h before PMA stimulation to inhibit endogenous H1R signaling.

Real-time Quantitative RT-PCR. HeLa cells cultured to 70% confluency in 6-well dishes were serum-starved for 24 h and treated with reagents 1 h before histamine or PMA stimulation. After a 3-h treatment with histamine or PMA, the cells were harvested with 700 μl of RNAiso Plus (Takara Bio Inc; Kyoto, Japan), mixed with 140 μl of chloroform, and centrifuged at 15,000 rpm for 15 min at 4 °C. The aqueous phase was collected, and RNA was precipitated by the addition of isopropyl alcohol. After centrifugation at 15,000 rpm for 15 min at 4 °C, the resulting RNA pellet was

washed with ice-cold 70% ethanol. Total RNA was resolved in 10 μl of diethylpyrocarbonate-treated water, and 5 μg of each RNA sample was used for the reverse transcription reaction. For animal study, rats were sacrificed 4 h after the final administration of histamine and nasal mucosa was removed from the nasal septum and collected in RNAlater (Applied Biosystems) until used. Then, nasal mucosa was homogenized using a Polytron (Model PT-K; Kinematica AG, Littau/Luzern, Switzerland) in 10 volumes of ice-cold RNAiso Plus and total RNA was isolated. RNA samples were reverse-transcribed to cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). TaqMan primers and the probe were designed using Primer Express (Applied Biosystems). Real-time PCR was conducted using a GeneAmp 7300 sequence detection system (Applied Biosystems). The sequences of the primers and TaqMan probe were as follows: forward primer for H1R, 5'-CAGAGGATCAGATGTTAGGTGATAGC-3'; reverse primer for H1R, 5'-AGCGGAGCCTCTTCCAAGTAA-3'; TaqMan probe, FAM-CTTCTCTCGAACGGACTCAGATACCACC-TAMRA. To standardize the starting material, the human GAPDH gene (Applied Biosystems) was used, and data

were expressed as the ratio of H1R mRNA to GAPDH mRNA. Amplicon size and reaction specificity were confirmed by agarose gel electrophoresis. Identities of the PCR products were verified by sequencing using a genetic analysis system (Beckman CEQ 8000; Beckman Coulter, Fullerton, CA).

Legends for supplementary figures

Supplementary Fig. S1. Ets-1, not NF-κB binds to putative binding site in the region A of the H1R promoter. **(**A), The nuclear extract (3 μg protein) was incubated with ^{32}P-labeled probe bearing NF-κB1, a putative NF-κB binding site. For competition studies, 200-fold molar excess of unlabeled WT (lane 3), mutant (lane 4), Ets-1 CON sequence (lane 5), or NF-κB CON sequence oligonucleotides were used. (B), The nuclear extract was incubated with ^{32}P-labeled probe with (lane 3) or without anti-Ets-1 (lane 2) antibody. The positions of the sequence-specific DNA–protein complex and the super-shifted band are shown as an arrow and an arrowhead, respectively.

Supplementary Fig. S2. Effect of DPQ on cell viability of HeLa cells. HeLa cells were treated with various concentrations of DPQ for 11 h, and their viability were determined using Cell Counting Kit-8 (DOJINDO, Kumamoto, Japan) according to the manufacturer's instructions. Data are presented as mean ± S.E.M. (n = 4). *, $p <$ 0.05 vs. control.

Supplementary Fig. S3. Histamine-induced c-Fos expression in HeLa cells. HeLa cells were serum-starved for 24 h, and then stimulated with histamine (10 μM). At the given time intervals after stimulation, total cell lysates were isolated and subjected to immunoblot analysis using aniti-c-Fos antibody (Santa Cruz).

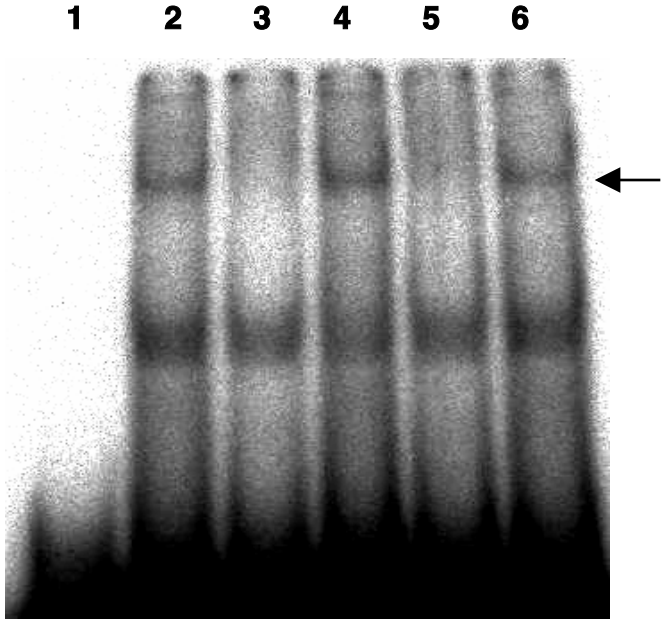
Supplementary Fig. S4. Intranasal application of histamine to normal (TDI-untreated) rats increased the H1R mRNA expression. Histamine (0.6-3 mg/day/rat) was administered bilaterally in the nasal cavity of rats for 1 week. Nasal mucosa was collected 4 h after the final administration of histamine, and the H1R mRNA levels were determined by real-time quantitative RT-PCR. Data are presented as mean ± S.E.M. (n = 4).

Supplementary Fig. S5. Epigallocatechin-3-O-gallate (EGCG) inhibits PKCδ Tyr^{311} phosphorylation. HeLa cells were stimulated with PMA (100 nM) for 10 min, and total cell lysates were isolated and subjected to immunoblot analysis. The cells were treated with EGCG (3.3 to 26 μM) for 24 h before stimulation with PMA.

Supplementary Table S1. Nucleotide sequences for primers used in this study.

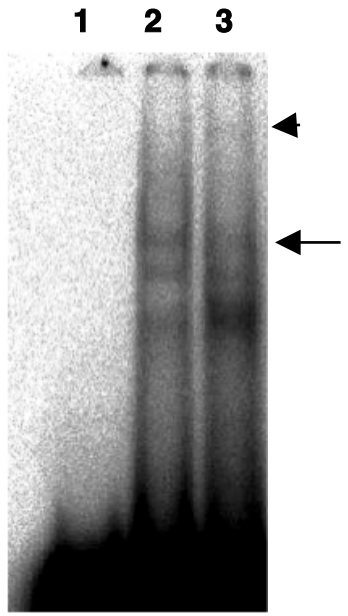
Oligonucleotide name	Sequence (position)	
For deletion mutants		
Forward primers		
p1336	5'- GGACTACTCCAAAGGCATAC- 3'	(-1336– -1307)
p1137	5'- TCTTCCGGTTTGAAAGGGAG- 3'	(-1137 – -1118)
p960	5'- CTTGAGTCAGTAAGCAACC- 3'	(-960 – -942)
p914	5'- TGTTCTGGTGAGGTGTGCTC- 3'	(-914 – -895)
p398	5'- GTTAACCCTTCATCCCACAG- 3'	(-398 – -379)
p221	5'- GTAAACCCCCGGGGTAAGAC- 3'	(-221 – -202)
p44	5'- AGTATGACCTGTCTGGCTTC- 3'	(-44 – -25)
Reverse primer		
	5'- CAGGAGTAAGAGCTCGGTAC-3'	
For EMSA		
AP-1 consensus	5'- CGCTTGATGAGTCAGCCGGAA -3'	
	3'- GCGAACTACTCAGTCGGCCTT -5'	
NF-κB consensus	5'-AGTTGAGGGGACTTTCCCAGGC -3'	
	3'- TCAACTCCCCTGAAAGGGTCCG -5’	
Ets-1 consensus	5'- GATCTCGAGCAGGAAGTTCGA -3'	
	3'- CTAGAGCTCGTCCTTCAAGCT -5'	

(A)



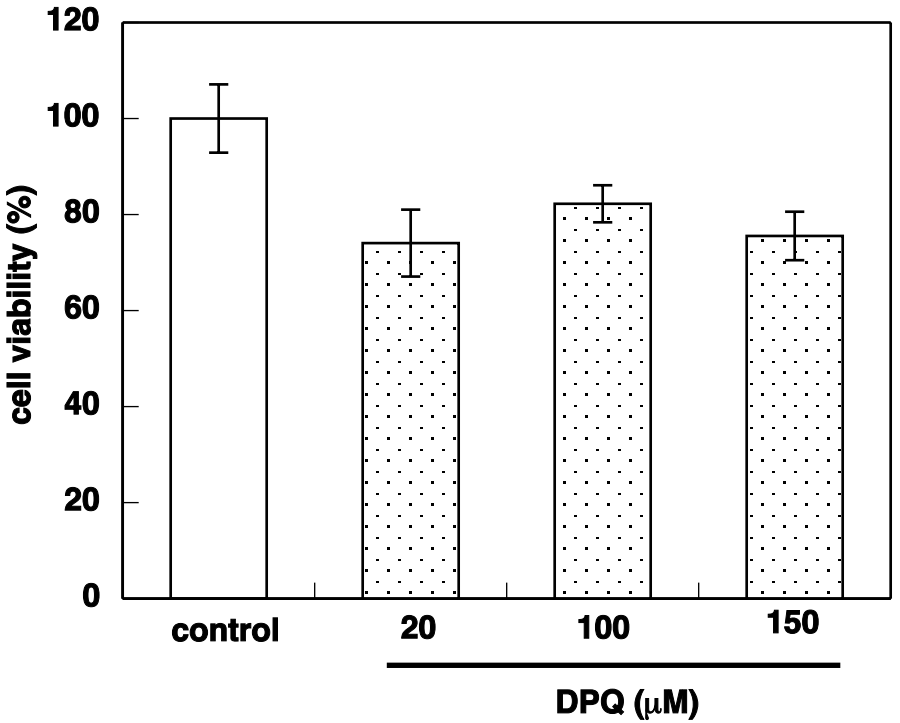
N.E.	−	+	+	+	+	+
WT competitor	−	−	+	−	−	−
mutant competitor	−	−	−	+	−	−
Ets-1consensus	−	−	−	−	+	−
NF-κB consensus	−	−	−	−	−	+

(B)

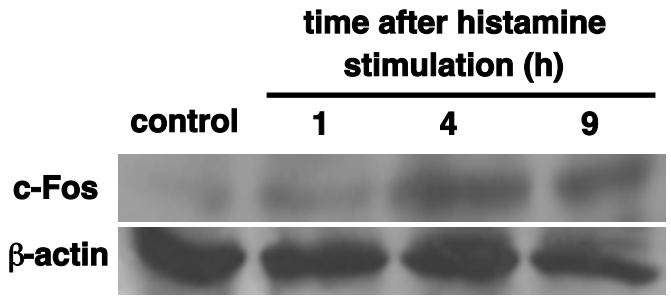


N.E.	−	+	+
Anti-Ets-1	−	−	+

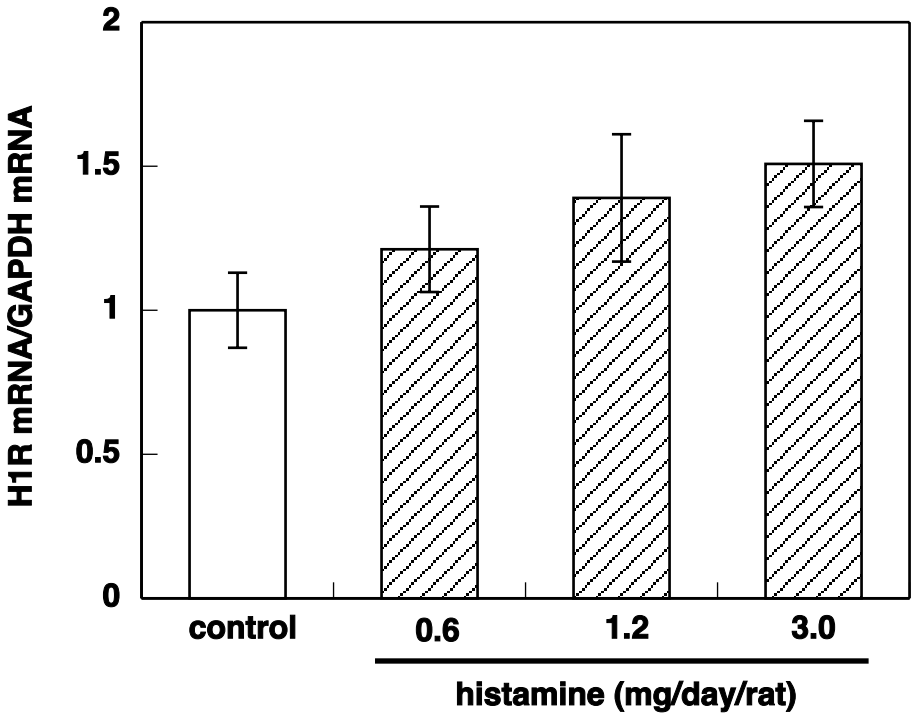
Mizuguchi et al. Supplementary Figure S1



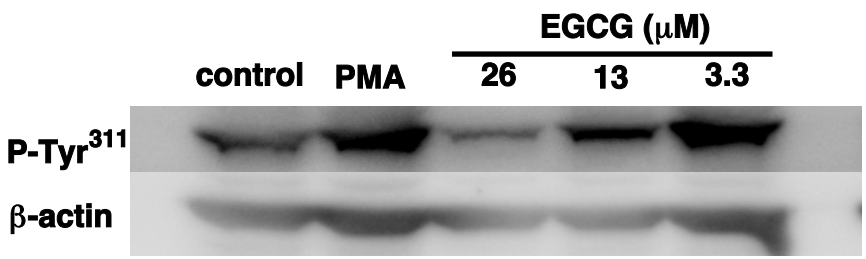
Mizuguchi et al. Supplementary Figure S2



Mizuguchi et al. Supplementary Figure S3



Mizuguchi et al. Supplementary Figure S4



Mizuguchi et al. Supplementary Figure S5