Rose flavor compound β -damascone regulates dendritic cell-mediated immunoresponses by modulating the NRF2 pathway and ameliorates contact hypersensitivity

Running title: β -damascone suppresses DC activation

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32

33 **Abstract**

34 Numerous pharmacologically beneficial compounds have been isolated from natural
 35 products derived from plants, which are often characterized as phytochemicals and are
 36 used in flavors, spices, and fragrances. Here we show that β -damascone, a major
 37 ingredient of rose fragrance, was selected from an aroma library as a candidate
 38 compound that suppresses antigen-dependent T cell activation. β -Damascone inhibited
 39 dendritic cell (DC)-related responses, including DC-induced Th1 development, TLR
 40 ligand-induced production of inflammatory cytokines in DCs. β -Damascone treatment
 41 increased the levels of NRF2 protein and *Hmox1* mRNA in DCs. *Nrf2*^{-/-} DCs possessed
 42 Th1-induction activity and higher IL-12p40 production activity even in the presence of
 43 β -damascone in comparison with *Nrf2*^{+/-} DCs. Finally, we found that β -damascone
 44 intake suppressed ear swelling of contact hypersensitivity model mice. Taken together,
 45 we conclude that the rose aroma compound β -damascone, which suppresses
 46 DC-mediated immunoresponses by activating the NRF2 pathway, could be useful to
 47 ameliorate immunorelated diseases.

48

49 **Keywords:** β -damascone, dendritic cells, NRF2

50

51 **Abbreviations:** Ab; antibody, Ag; antigen, APCs; antigen presenting cells, BMDCs;
 52 bone marrow-derived dendritic cells, CHS; contact hypersensitivity, DCs; dendritic cells,
 53 DNCB; 2,4-dinitrochlorobenzene, DNFB; 2,3-dinitrofluorobenzene, NRF2;
 54 NF-E2-related factor 2, OVA; ovalbumin, p.o.; per os

55

56 **Introduction**

57 Since ancient times, humans have obtained pharmacological compounds from natural
58 products derived from plants and bacteria. Phytochemicals, which are produced in
59 plants to resist various stresses, including UV and insect pests, exhibit beneficial effects,
60 such as antioxidant, anticancer, and anti-inflammatory effects, on humans and mammals.
61 Because some phytochemicals are scenting and are used as flavors and fragrances, we
62 expected that unidentified immunomodulators could be found in aroma compounds.

63 Dendritic cells (DCs), the most typical antigen presenting cells (APCs), contribute to
64 both innate and adaptive immunity. Activation of DCs by pathogens leads to the release
65 of cytokines and chemokines from DCs, and DC-mediated expansion of
66 antigen-specific T cell clones. Therefore, DC activation is essential for host defense
67 against infection, but hyper and/or lasting activation of DC-mediated immunoresponses
68 causes inflammation and autoimmune diseases.

69 A number of studies regarding the immunomodulatory effects of phytochemicals have
70 been reported (Mainardi, Kapoor and Bielory 2009). Resveratrol is the most
71 well-known polyphenolic stilbenoid (Baur et al. 2006, Jang et al. 1997) and exhibits
72 effects on the prevention of inflammation-related diseases by suppressing TLR
73 signaling-induced expression of proinflammatory genes (Malaguarnera 2019).
74 Regarding scent phytochemicals, several food ingredients, including curcumin
75 (Kunnumakkara et al. 2017) and menthol (Oz et al. 2017), are expected to possess
76 therapeutic potential.

77 In the current study, we first performed screening to find novel immunomodulators by
78 analyzing the effect on APC-dependent T cell activation and selected β -damascone, a
79 major ingredient of rose fragrance, from approximately 150 kinds of aroma compounds.

80 Next, we investigated the molecular mechanisms by which β -damascone regulates
 81 antigen (Ag)-dependent T cell proliferation and found that β -damascone suppressed
 82 DC-mediated immunoresponses and that activation of the transcription factor
 83 NF-E2-related factor 2 (NRF2)-pathway in DCs by β -damascone is one of the causes.
 84 Finally, we confirmed the effect of β -damascone on immunoresponses *in vivo* using a
 85 contact hypersensitivity (CHS) mouse model. Taken together, these results indicate that
 86 β -damascone, which suppresses immunoresponses mainly targeting DCs, exhibits
 87 beneficial effects on the prevention and/or treatment of immunorelated diseases.

88

89 **Results**

90 **β -damascone was identified as an immunomodulator suppressing DC-mediated T** 91 **cell activation**

92 To find novel immunomodulators, we performed 2-step screening using an
93 aroma-compound library (**Supplementary Table SII**). By the 1st screening, in which
94 APC-dependent T cell proliferation was assayed, 22 candidates were selected from 150
95 compounds as immunosuppressors, and 3 compounds were passed through the 2nd
96 screening evaluating the suppressive effect on IL-2 release from OVA-pulsed OT-II
97 splenocytes (**Supplementary Figure S1**). Considering several factors, such as IC₅₀, we
98 finally selected #177 β -damascone (**Figure 1A**), a major component of rose aromas. In
99 accordance with preliminary observations in the abovementioned screening,
100 β -damascone suppressed OVA-induced IL-2 production from OT-II splenocytes (**Figure**
101 **1B**) and APC-dependent proliferation of CD4⁺ T cells (**Figure 1D**) in a dose-dependent
102 manner at concentrations much lower than those showing cytotoxicity (**Figure 1C**).
103 Although APC-independent T cell proliferation caused by stimulation with plate-coated
104 anti-CD3 and anti-CD28 Abs was moderately suppressed by β -damascone (**Figure 1E**),
105 considering that the development of Th1 cells induced by OVA-pulsed DCs (**Figure 1F**)
106 but not by CD3/CD28 stimulation (**Figure 1G**) was significantly suppressed by
107 β -damascone, we focused on the effect of β -damascone on the function of DCs.

108

109 **β -damascone suppressed LPS-induced activation of DCs**

110 We evaluated the effects of β -damascone on LPS-induced activation of DCs by
111 analyzing the cytokine expression levels and cell surface expression levels of
112 APC-related molecules in BMDCs. As shown in **Figure 2A**, β -damascone apparently

113 reduced the mRNA levels of IL-6, IL-12p40, and TNF- α in DCs, which was increased
 114 following LPS stimulation, in a dose-dependent manner. The concentrations of these
 115 inflammatory cytokines in the culture media of LPS-stimulated DCs were also
 116 decreased in the presence of β -damascone (**Figure 2B**), suggesting that suppression of
 117 the LPS-induced transactivation of cytokine genes by β -damascone was reflected in
 118 protein release. In addition, flow cytometric analysis revealed that LPS-induced
 119 upregulation of MHC class II and CD86 was also suppressed in β -damascone-treated
 120 DCs (**Figure 2C**). We also confirmed that the increased phosphorylation of I κ B in
 121 LPS-stimulated DCs was reduced by the pretreatment with β -damascone (**Figure 2D**).
 122 These results indicate that β -damascone exhibits a suppressive effect on LPS-induced
 123 inflammatory responses and antigen presentation activity in DCs.

125 **The effects of β -damascone on the activation of DCs via other TLRs**

126 The effects of β -damascone on the activation of DCs caused by stimulants other than
 127 LPS were examined by using polyI:C, R-848, and CpG. Quantification of mRNA levels
 128 (**Figure 3A-3C**) and determination of protein concentrations (**Figure 3D-3F**) revealed
 129 that the transactivation and subsequent protein production of IL-6, IL-12p40, TNF- α ,
 130 and IL-23p19 in BMDCs, which were induced by stimulation via TLR3, TLR7/8, or
 131 TLR9, were significantly suppressed by β -damascone. These results suggest that
 132 β -damascone exhibited suppressive effects against various TLR ligand-induced
 133 activation of DCs.

135 **Involvement of the NRF2 pathway in the suppressive effects of β -damascone on** 136 **DCs**

137 Some substances derived from plants, especially phytochemicals (e.g., sulforaphane),
138 often activate the NRF2 pathway by directly acting on Keap1, resulting in the induction
139 of antioxidant and anti-inflammatory responses (Kensler et al. 2013). To clarify whether
140 β -damascone activates the NRF2 pathway in DCs, we determined the NRF2 protein and
141 *Hmox1* mRNA levels in DB-treated DCs. As shown in **Figure 4A**, a Western blot
142 analysis revealed that the amount of NRF2 protein was increased in DCs in the presence
143 of β -damascone and peaked at 1 h after the addition of DB to the culture medium. The
144 mRNA levels of *Hmox1*, which is a target gene of NRF2, were markedly upregulated by
145 β -damascone much higher than those induced by LPS (**Figure 4B**). These results
146 showing the increase in NRF2 protein and *Hmox1* mRNA levels in β -damascone-treated
147 DCs suggest the possibility that β -damascone activates the NRF2 pathway in DCs. Then,
148 we investigated the roles of NRF2 in DB-mediated modification of DC function by
149 using *Nrf2*^{-/-} DCs. First, it was confirmed that the β -damascone-induced increase in
150 *Hmox1* mRNA levels in DCs was almost abolished by NRF2 deficiency (**Figure 4C**).
151 When OVA-pulsed *Nrf2*^{-/-} BMDCs were cocultured with OT-II CD4⁺ T cells under a
152 Th1-polarizing conditions, the suppression of Th1 development by β -damascone was
153 not observed, whereas control (*Nrf2*^{+/-}) DC-dependent Th1 development was
154 significantly suppressed in the presence of β -damascone (**Figure 4D**), as was the case
155 for WT BMDCs (**Figure 1E**). Furthermore, IL-12p40 release from LPS-stimulated DCs
156 tended to increase by *Nrf2* deficiency in DCs, and the suppressive effect of
157 β -damascone on IL-12p40 production was markedly reduced in *Nrf2*^{-/-} BMDCs
158 compared with that in *Nrf2*^{+/-} BMDCs (**Figure 4E**). These results demonstrate that DC
159 functions, including Th1 induction and IL-12 production, were suppressed by
160 β -damascone depending on activation of the NRF2 pathway in DCs.

161

162 **Oral administration of β -damascone ameliorates contact hypersensitivity in mice**

163 Finally, we investigated whether β -damascone, which inhibits DC-mediated
 164 inflammation in *in vitro* experiments, exhibits a biologically significant effect on
 165 immunorelated diseases by using CHS model mice (**Figure 5A**). First, we examined the
 166 effects of various doses of β -damascone on ear swelling symptoms in DNFB-treated
 167 mice and found that oral administration of β -damascone at 10 or 50 mg/kg per day from
 168 2 days before sensitization suppressed ear swelling (data not shown). Under optimized
 169 doses, the suppressive effect of β -damascone on ear swelling was observed not only
 170 after challenge but also after rechallenge (**Figure 5B**), accompanied by a tendency of
 171 reduced mRNA levels of *Il6* in the lesional skin (data not shown). These results indicate
 172 that orally administered β -damascone modulates DC-induced immunoresponses *in vivo*.

173

174

175 **Discussion**

176 In the current study, we identified β -damascone from approximately 150 compounds as
 177 a novel immunomodulator through 2-step screening monitoring of DC-mediated
 178 immunoresponses. By conducting *in vivo* experiments using a mouse model, we
 179 demonstrated that oral intake of β -damascone, which activates the NRF2 pathway,
 180 ameliorated CHS inflammation. NRF2 plays a role as a master regulator of antioxidant
 181 and anti-electrophilic responses by transactivating the genes encoding antioxidant
 182 enzymes and drug metabolizing enzymes. In a previous study using a
 183 2,4-dinitrochlorobenzene (DNCB)-induced CHS model, NRF2-deficient mice exhibited
 184 more severe inflammation accompanied by increased chemokine production and
 185 neutrophil recruitment in the skin than control mice (Helou et al. 2019). This
 186 observation that NRF2 deficiency deteriorates CHS may be coincident with our results,
 187 indicating the important roles of NRF2 in DC-mediated immune responses. In addition
 188 to CHS, various immunorelated inflammatory diseases and autoimmune diseases,
 189 including colitis and psoriasis, are reported to be exacerbated in NRF2 knockout mice
 190 (Khor et al. 2006, Ogawa et al. 2020, Yoh et al. 2001, Johnson et al. 2010). Although the
 191 effects of NRF2 deficiency are not restricted to DC function, because NRF2 is
 192 ubiquitously expressed, these studies of NRF2-deficient mice support the possibility
 193 that β -damascone could be useful for the prevention and/or treatment of inflammatory
 194 diseases and autoimmune diseases. We found that β -damascone significantly suppressed
 195 TLR7-mediated gene expression and protein production of IL-6, IL-12p40, and TNF- α
 196 in BMDCs (**Figure 3C and 3F**). Because a topical application of imiquimod (Schön and
 197 Schön 2007, Gilliet et al. 2004), a ligand of TLR7 and TLR8, causes psoriasis-like
 198 pathology in mouse skin through activation of dermal DCs and Langerhans cells (van

der Fits et al. 2009), we expect that β -damascone ameliorates psoriasis, even from the point of view of modification of DC function. We will evaluate the effects of β -damascone on various immunorelated diseases, such as psoriasis, inflammatory bowel disease, and multiple sclerosis using a mouse model in the near future.

Our results using *Nrf2*^{-/-} DCs indicated that the suppressive effects of β -damascone on IL-12 production in DCs and Th1 development activity of DCs are dependent on NRF2. In contrast, the suppressive effects of β -damascone on the production of TNF- α and IL-6 were still observed in *Nrf2*^{-/-} DCs, suggesting that β -damascone modulates DC function through additional target(s) other than NRF2. It has been reported that the induction of the proinflammatory cytokine genes, *Il1a*, *Il1b*, and *Il6* is inhibited by NRF2, which binds to these genes and subsequently inhibits the recruitment of RNA polymerase II to target genes in macrophages (Kobayashi et al. 2016). However, under our experimental conditions, we could not find a significant contribution of NRF2 in the suppression of the production of IL-6 and TNF- α in DCs. Further detailed analysis to clarify other target(s) of β -damascone could lead to the development of novel anti-inflammatory immunomodulators.

In the current study, we identified β -damascone as the most effective compound from an aroma library and confirmed that oral administration of β -damascone ameliorated CHS. Although we obtained β -damascone as a natural compound candidate of immunomodulator in the current study, there is room for improvement; briefly, β -damascone could be developed to a drug carrying stronger activity and lesser toxicity by chemical structural modification. Further research using an organic chemical approach considering β -damascone as a lead compound should be conducted to develop a novel immunomodulator.

224 **Materials and Methods**

225 ***Mice and cells***

226 C57BL/6 and Balb/c mice were purchased from Japan SLC (Hamamatsu, Japan), and
 227 OT-II mice were obtained from The Jackson Laboratory (USA). *Nrf2*^{-/-} mice were
 228 previously generated (Itoh et al. 1997). Mice were maintained under specific
 229 pathogen-free conditions. All animal experiments were performed in accordance with
 230 the guidelines of the Institutional Review Board of Tokyo University of Science. The
 231 current study was specifically approved by the Animal Care and Use Committees of
 232 Tokyo University of Science: K21004, K20005, K19006, K18006, K17009, and
 233 K17012. Bone marrow-derived DCs (BMDCs) were generated from whole BM cells by
 234 cultivation in RPMI-1640-based media supplemented with 20 ng/mL mGM-CSF
 235 (BioLegend, San Diego, CA, USA) as previously described (Kanada et al. 2011). Naïve
 236 CD4⁺ T cells were isolated from the spleen by using the MojoSort Mouse Naïve CD4⁺ T
 237 Cell Isolation Kit (#480040, BioLegend). CD4⁺ T cells isolated from the OT-II spleen
 238 were cocultured with BMDCs, which were generated from C57BL/6 mice and were
 239 preincubated with OVA peptide 323-339 (POV-3636-PI, Peptide Institute, Inc., Osaka,
 240 Japan). To stimulate CD4⁺ T cells with plate-coated antibodies (Abs), anti-CD3ε Ab
 241 (clone 145-2111C, BioLegend) and anti-CD28 Ab (clone 37.51, TONBO Bioscience)
 242 were used. For Th1 polarization, 10 ng/mL mIL-12 (PeproTech Inc., Rocky Hill, NJ,
 243 USA) and 10 µg/mL anti-IL-4 Ab (clone 11B11, BioLegend) were added to the culture
 244 media. Th2 polarization was induced with 20 ng/mL IL-4 (PeproTech) and 10 µg/mL
 245 anti-IL-12 Ab (clone C17.8, BioLegend).
 246 Damasone-β (#34059, Vigon International, East Stroudsburg, PA, USA) was diluted
 247 with DMSO. LPS (#L3024, Wako), poly-I:C (#P0913, Sigma), R848

(AG-CR1-3582-M005, AdipoGen, Liestal, Switzerland), and CpG (ODN1826, InvivoGen, San Diego, CA, USA) were used to stimulate BMDCs. DAPI (#11034-56, Nacalai Tesque Inc., Kyoto, Japan) was used to determine cell viability.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of mouse IL-2, IL-6, TNF- α , and IL-12p40 were determined by ELISA kits purchased from BioLegend (#431004, #431315, #430915, and #431604, respectively).

Flow cytometry

To analyze the proliferation of T cells, CFSE (eBioscience Inc., San Diego, CA, USA) was used. Cell surface MHC class II and CD86 on BMDCs were stained with anti-I-A/I-E-PerCP (clone M5/114.15.2, BioLegend) and anti-CD86-PE (clone GL-1, BioLegend), respectively. Intracellular IFN- γ and IL-4 were stained with anti-IFN- γ -PE/Cyanine7 (clone XMG1.2, BioLegend), and anti-IL-4-PE (clone 11B11, BioLegend), respectively, with anti-CD4-FITC (clone GK1.5, BioLegend) after treatment with the Fixation Buffer (#420801, BioLegend) and the Intracellular Staining Perm Wash Buffer (#421002, BioLegend). Fluorescence was detected by a MACS Quant Analyzer (Miltenyi Biotech) and analyzed with FlowJo (Tomy Digital Biology, Tokyo, Japan).

Western blot analysis

Western blot analysis was performed as previously described (Kitamura et al. 2012) with anti-phospho-I κ B α (Ser32) (clone 14D4, Cell Signaling), anti-NRF2 (clone D1Z9C,

272 Cell Signaling), and anti- β -actin (clone AC-15, Sigma-Aldrich) Abs.

273

274 ***Quantitative RT-PCR***

275 Total RNA was extracted from BMDCs using the ReliaPrep RNA Cell Miniprep System
276 (#Z6012, Promega, Madison, USA) and from the skin using ISOGEN (#311-07361,
277 Nippongene, Tokyo, Japan). Synthesis of cDNA, and quantitative PCR were performed
278 as previously described (Ito et al. 2021). The nucleotide sequences of the primer sets are
279 listed in **Supplementary Table SI**.

280

281 ***CHS model***

282 Mice were sensitized on shaved abdominal skin with 25 μ l 0.5% (w/v) DNFB in
283 acetone/olive oil (4:1) and were challenged with an application of 20 μ l 0.25% DNFB
284 on the ear at 5 days after sensitization. Ear thickness was measured by a caliper.

285

286 ***Statistical analysis***

287 To compare two samples, a two-tailed Student's t-test was used (Figure 1F, and 1G). To
288 compare more than two samples, one-way ANOVA-followed by Tukey's multiple
289 comparison test (Figure 1B, 1D, 1E, 2A, 2B, 3A-F, 4B-E, 5B, and 5C) or Dunnett's
290 multiple comparison test (Figure 2C) was used. *P* values < 0.05 were considered
291 significant.

292

293 **Authorship Contribution**

294 Contribution: N.K. performed experiments, analyzed data, and wrote the paper; H.O.
 295 performed experiments and analyzed data; K.N. analyzed data and wrote the paper;
 296 M.A. N.I. and T.Y. performed experiments; G.I. and M.Y. provided experimental tools;
 297 M.H. analyzed data and wrote the paper; C.N. designed research and wrote the paper.

298

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300

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305

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377

379 **Legends**

380 **Figure 1. Effects of β -damascone on DC-mediated activation of T cells.**

381 **A.** Structure of β -damascone.

382 **B.** The concentrations of IL-2 in the culture media of OT-II splenocytes. A total of 1.0×10^5 cells obtained from the whole spleen of OT-II were maintained in the presence or
383
384 absence of 500 ng OVA peptide and the indicated concentration of β -damascone (β -D)
385 in 200 μ L culture media for 72 h.

386 **C.** Cell viabilities of DCs and T cells in the presence of β -damascone. BMDCs and
387 naïve $CD4^+$ T cells were preincubated with the indicated concentrations of β -damascone
388 for 24 h. Cell viability was judged with DAPI staining.

389 **D.** Division of OT-II $CD4^+$ T cells cocultured with OVA-pulsed BMDCs. CFSE-labeled
390 OT-II $CD4^+$ T cells (5.0×10^4) were cocultured with or without C57BL/6 BMDCs (1.0×10^4), which were pretreated with 500 ng OVA peptide, in the presence or absence of the
391
392 indicated concentrations of β -damascone in 200 μ L of culture media for 72 h.

393 **E.** Division of $CD4^+$ T cells via stimulation with anti-CD3 and anti-CD28 Abs. $CD4^+$ T
394 cells, which were isolated from the spleen of C57BL/6 mice, were labeled with CFSE,
395 and then incubated with plate coated Abs as described in the Materials and Methods.

396 **F.** Effects of β -damascone on the frequencies of Th1 and Th2 cells developed under
397 polarization conditions in a DC-dependent manner. OT-II naïve $CD4^+$ T cells were
398 cocultured with OVA-pulsed C57BL/6 BMDCs under Th1 (top) or Th2 (bottom)
399 polarization conditions in the presence or absence of β -damascone (30 μ M) for 72 h as
400 described in the Materials and Methods section using a gating strategy shown in

401 **Supplementary Figure S2A.**

402 **G.** Frequencies of Th1 and Th2 cells induced under polarization conditions in an

403 APC-independent manner. C57BL/6 naïve T cells were stimulated with anti-CD3 and
 404 anti-CD28 Abs under Th1 (top) or Th2 (bottom) polarization conditions.
 405 The Tukey-Kramer test was used (**B, D, E, F and G**). *, $p < 0.05$, **, $p < 0/01$, n.s.; not
 406 significant.

407

408 **Figure 2. β -Damascone suppressed LPS-induced activation of DCs.**

409 **A.** Messenger RNA expression levels in BMDCs.

410 **B.** Concentrations of cytokines in the culture media of BMDCs.

411 **C.** Cell surface expression levels of MHC class II and CD86 on BMDCs.

412 **D.** A Western blot profile of phosphorylated I κ B.

413 BMDCs pretreated with 50 μ M β -damascone for 24 h were stimulated with 100 ng/mL
 414 LPS. The cells were harvested at 3 h and 24 h after LPS stimulation to determine
 415 mRNA levels (**A**) and cell surface protein expression levels (**C**), respectively, and
 416 culture media were collected at 24 h after stimulation to measure the concentration of
 417 cytokines (**B**). For Western blot analysis, cells at 1 h after LPS stimulation were lysed,
 418 and aliquots containing a total of 10 μ g protein were added to each lane (**D**). The data
 419 represent the mean $\pm$ SEM of three independent experiments (**A, B, and C**) performed
 420 in triplicate samples (**A and B**). A gating strategy of flowcytometric analysis of BMDCs
 421 is shown in **Supplementary Figure S2B**.

422 The Tukey-Kramer test (**A, and B**) or Dunnett's test (**C**) was used. *, $p < 0.05$, **, $p <$
 423 $0/01$.

424

425 **Figure 3. Suppressive effects of β -damascone on the stimulation of DCs by various**
 426 **TLR ligands.**

427 The effects of β -damascone (50 μ M) on mRNA expression levels (**A**, **B**, and **C**) and
428 protein release of cytokines (**D**, **E**, **F**) in BMDCs stimulated with polyI:C (**A** and **D**),
429 CpG (**B** and **E**), or R848 (**C** and **F**). The data represent the mean $\pm$ SEM of three
430 independent experiments performed in triplicate samples.

431 The Tukey-Kramer test was used. *, $p < 0.05$, **, $p < 0/01$.

432

433 **Figure 4. β -Damascone activated the NRF2 signaling in DCs, and NRF2 deficiency**
434 **reduced the suppressive effects of β -damascone.**

435 **A.** A Western blot profile showing NRF2 protein levels in BMDCs following treatment
436 with β -damascone. BMDCs were incubated in the presence of 50 μ M β -damascone for
437 the indicated hours.

438 **B.** The mRNA levels of *Hmox1* in BMDCs. BMDCs cultured in the presence or absence
439 of 50 μ M β -damascone for 24 h were incubated with or without 100 μ g/mL LPS for an
440 additional 3 h.

441 **C.** The mRNA levels of *Hmox1* in BMDCs generated from NRF2-deficient mice
442 (*Nrf2*^{-/-}) and control mice (*Nrf2*^{+/-}).

443 **D.** Th1 development induced by coculture with *Nrf2*^{-/-} BMDCs or control BMDCs.

444 **E.** LPS-induced cytokine production from *Nrf2*^{-/-} BMDCs and control BMDCs.

445 The data represent the mean $\pm$ SEM of three independent experiments performed in
446 triplicate samples. The Tukey-Kramer test was used. *, $p < 0.05$, **, $p < 0/01$, n.s.; not
447 significant.

448

449 **Figure 5. Oral administration of β -damascone ameliorated the pathology of CHS.**

450 **A.** Schematic of the oral administration schedule of β -damascone in the DNFB-induced
 451 CHS model. The indicated amounts of β -damascone in 200 μ l saline were orally
 452 administered every day. p.o.; per os.
 453 **B.** Ear swelling of CHS mice at the indicated time points.
 454 (Ear swelling) = (ear thickness after challenge) – (ear thickness before the first
 455 challenge)
 456 Data were pooled from 2 independent experiments. n = 9-10.

A)

B)

C)

D)

Kodama et al. Figure 3

A)

B)