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## Calcium signaling and $\beta$ 2-adrenergic receptors regulate 1-nitropyrene induced CXCL8 responses in BEAS-2B cells.

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1 Title:

2 **Calcium Signaling and  $\beta$ 2-Adrenergic Receptors Regulate 1-Nitropyrene Induced**  
3 **CXCL8 Responses in BEAS-2B Cells**

4

5

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28

1 **Abstract**

2 Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are widespread environmental  
3 pollutants, generated from reactions between PAHs and nitrogen oxides during combustion  
4 processes. In the present study we have investigated the mechanisms of CXCL8 (IL-8)  
5 responses induced by 1-nitropyrene (1-NP) in human bronchial epithelial BEAS-2B cells,  
6 with focus on the possible importance of  $\text{Ca}^{2+}$ -signaling and activation of  $\beta$ 2-adrenergic  
7 receptors ( $\beta$ 2AR).  $\text{Ca}^{2+}$ -chelator treatment obliterated 1-NP-induced CXCL8 (IL-8) responses.  
8 1-NP at 10  $\mu\text{M}$  (but not 1  $\mu\text{M}$ ) induced a rapid and sustained increase in intracellular  $\text{Ca}^{2+}$ -  
9 levels ( $[\text{Ca}^{2+}]_i$ ). The early but not the later, sustained phase of 1-NP-induced  $[\text{Ca}^{2+}]_i$  was  
10 suppressed by beta-blocker treatment (carazolol). Moreover, inhibition of  $\beta$ 2AR by blocking-  
11 antibody, beta-blocker treatment (ICI 118551) or siRNA transfection attenuated CXCL8  
12 responses induced by 1-NP. The results confirm that PAHs may induce  $\text{Ca}^{2+}$ -signaling also in  
13 BEAS-2B cells, at least partly through activation of  $\beta$ 2AR, and suggest that both  $\beta$ 2AR- and  
14  $\text{Ca}^{2+}$ -signaling may be involved in 1-NP-induced CXCL8 responses in bronchial epithelial  
15 cells.

16  
17 **Key words:** Lung; Inflammation; Chemokines; Polycyclic Aromatic Hydrocarbons; Calcium;  
18 Adrenergic Receptors.

1

2 **Introduction**

3 Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are ubiquitous air pollutants associated  
4 with combustion particles, in particular diesel exhaust particles. Nitro-PAHs have long been  
5 considered among the main contributors to the mutagenic effects of DEP (Hayakawa et al.,  
6 1997; Scheepers et al., 1995). Nitro-PAHs may also exhibit considerable pro-inflammatory  
7 potential, by inducing cytokine and chemokine responses in epithelial lung cells (Øvrevik et  
8 al., 2010; Øvrevik et al., 2009; Park and Park, 2009; Pei et al., 2002).

9

10 Previous studies have shown that 1-nitropyrene (1-NP) and 3-nitrofluoranthene (3-NF) are  
11 particularly potent inducers of the neutrophil attracting chemokine CXCL8 (IL-8), compared  
12 to their amine counterparts (1-AP and 3-AF), un-substituted pyrene or benzo[*a*]pyrene  
13 (B[*a*]P) (Øvrevik et al., 2010; Øvrevik et al., 2009; Øvrevik et al., 2013). This marked effect  
14 of 1-NP and 3-NF on CXCL8 induction seems to be independent of aryl-hydrocarbon  
15 receptor (AhR) activation, although it appears to involve formation of reactive oxygen species  
16 (ROS) and/ or reactive electrophilic metabolites at least partly formed via CYP-mediated  
17 metabolism (Øvrevik et al., 2010; Øvrevik et al., 2013).

18

19 Of notice, it has recently been shown that certain PAHs may induce calcium signaling in  
20 endothelial cells through an aryl-hydrocarbon receptor (AhR)-independent mechanism  
21 (Mayati et al., 2011). The effects were reported to be due to direct PAH-mediated activation  
22 of  $\beta$ 2-adrenergic receptors ( $\beta$ 2AR), leading to a G protein/adenylyl cyclase/cyclic-AMP-  
23 mediated calcium release from the endoplasmic reticulum (Mayati et al., 2012). Pyrene  
24 seemed to be a particular potent inducer of this pathway and caused considerably stronger  
25 calcium response in endothelial cells compared to other PAHs including B[*a*]P, chrysene and

1 benzo[*e*]pyrene (Mayati et al., 2011). Besides, we previously showed that the cell-permeable  
2 calcium chelator BAPTA-AM attenuated CXCL8 responses in BEAS-2B cells by a panel of  
3 environmental pollutants, including 1-NP (Øvrevik et al., 2011). Thus, in the present study we  
4 have investigated the 1-NP-induced effects on intracellular calcium levels in relation to  
5 CXCL8 responses in BEAS-2B cells, in order to test the hypothesis that  $\beta$ 2AR-mediated  
6 calcium-signaling may be a central mechanism for the pro-inflammatory effects of nitro-  
7 PAHs. Our results suggest that 1-NP is able to induce  $\beta$ 2AR-mediated calcium responses in  
8 BEAS-2B cells, and that both  $\beta$ 2AR- and  $\text{Ca}^{2+}$ -signaling are involved in 1-NP-induced  
9 CXCL8 responses. However, 1-NP also appeared to affect intracellular calcium levels  
10 through additional mechanisms, and  $\beta$ 2AR-mediated  $\text{Ca}^{2+}$ -signaling alone may not be  
11 sufficient for CXCL8-induction.

12

13

## 14 **Materials and methods**

### 15 *Reagents*

16 B[*a*]P, 1-NP, dimethyl sulphoxide (DMSO), ICI 118551 and carazolol were purchased from  
17 Sigma-Aldrich (St. Louis, MO, USA). LHC-9 cell culture medium and fura-2 acetoxymethyl  
18 ester (Fura-2-AM) were from Invitrogen (Carlsbad, CA, USA). Cytokine ELISA assay for  
19 CXCL8 (Human IL-8 Cytoset) was purchased from Biosource International (Camarillo, CA,  
20 USA). Antibodies against  $\beta$ 2AR (sc-569) were from Santa Cruz Biotechnology (Santa Cruz,  
21 CA, USA), whereas antibodies against  $\beta$ -actin were from Sigma-Aldrich. Short interfering  
22 RNA (siRNA) against  $\beta$ 2AR (sc-35147) with corresponding non-targeting control siRNA (sc-  
23 37007) were from Santa Cruz Biotechnology (CA, USA). All other chemicals used were  
24 purchased from commercial sources at the highest purity available.

25

1 *Cell cultures and exposures*

2 The BEAS-2B cell line, a SV40 hybrid (Ad12SV40) transformed human bronchial epithelial  
3 cell line, was from European Collection of Cell Cultures (ECACC, Salisbury, UK). Cells  
4 were maintained in LHC-9 medium in collagen-coated (PureCol™, Inamed Biomaterials,  
5 Fremont, CA, USA) flasks in a humidified atmosphere at 37°C with 5% CO<sub>2</sub>, and passaged  
6 twice per week. Prior to exposure, cells were plated in 12-well culture dishes, grown to near  
7 confluence in serum-free LHC-9 medium and exposed to PAHs and inhibitors as described  
8 elsewhere. Controls for 1-NP -exposed cells were treated with vehicle (DMSO) only. DMSO  
9 concentrations in all samples were below 0.5%.

10

11 *Chemokine release*

12 CXCL8 protein levels in cell-supernatants were determined by ELISA (Biosource  
13 International, Camarillo, CA, USA), as described elsewhere (Øvrevik et al., 2010).  
14 Absorbance was measured using a plate reader (TECAN Sunrise, Phoenix Research Products,  
15 Hayward, CA, USA) complete with software (Magellan V 1.10).

16

17 *Gene silencing by siRNA*

18 Cells were reverse-transfected with siRNAs against  $\beta$ 2AR or non-targeting siRNAs, using  
19 HiPerFect transfection reagent as recommended by the manufacturer (Qiagen, Germany: Fast-  
20 Forward protocol for adherent cells). SiRNAs and HiPerFect were mixed by vortexing in  
21 LHC-9 medium, incubated at room temperature (5-10 min) to form transfection complexes,  
22 and added drop-wise to the cell cultures (100  $\mu$ l/well) immediately after seeding (at a final  
23 siRNA concentration of 10 nM and 2.75  $\mu$ l of HiPerFect in a total of 1 ml growth medium).  
24 Gene silencing was monitored by measuring  $\beta$ 2AR protein levels by Western blotting.

25

### 1 *Calcium measurements*

2 Variations in intracellular  $\text{Ca}^{2+}$  concentrations ( $[\text{Ca}^{2+}]_i$ ) were analyzed by  
3 microspectrofluorimetry using the acetoxymethyl ester form of the  $\text{Ca}^{2+}$ -sensitive probe Fura-  
4 2, , as previously reported (Le et al., 2002; N'Diaye et al., 2006). Cells were then submitted  
5 alternatively to 340 and 380 nm excitation wavelengths and the fluorescence from the trapped  
6 dye was measured at 510 nm. The F340/F380 ratio, i.e., ratio of fluorescence intensities after  
7 excitation at 340 nm and 380 nm, respectively, was used to estimate  $[\text{Ca}^{2+}]_i$ . To avoid  
8 potential problems with autofluorescence, PAH-concentrations were restricted to 10  $\mu\text{M}$  in  
9 the  $[\text{Ca}^{2+}]_i$ -assay.

10

### 11 *Statistical analysis*

12 Statistical significance was evaluated by GraphPad Prism software (GraphPad Software Inc.,  
13 San Diego, CA, USA), using analysis of variance (ANOVA) with Bonferroni post-test.

14

15

## 16 **Results**

### 17 *Role of calcium signaling in 1-NP-induced CXCL8 responses in BEAS-2B cells*

18 We have previously shown that 1-NP induces a concentration-dependent increase in CXCL8  
19 release from BEAS-2B cells from 10  $\mu\text{M}$  (Øvrevik et al., 2010; Øvrevik et al., 2013). In line  
20 with this, we observed that 1-NP induced a strong increase in CXCL8 gene expression and  
21 protein release in BEAS-2B cells (Fig 1 A and B). To assess the role of  $\text{Ca}^{2+}$ -signaling in 1-  
22 NP-induced CXCL8 responses, BEAS-2B cell were pre-treated with the cell permeable  $\text{Ca}^{2+}$ -  
23 chelator BAPTA-AM for 30 min prior to 1-NP exposure for 18 h. BAPTA-AM completely  
24 blocked the 1-NP-induced CXCL8-response in BEAS-2B cells without affecting basal

1 CXCL8 levels (Fig. 1B). Thus, 1-NP-induced CXCL8 responses seemed to depend on  
2 intracellular  $\text{Ca}^{2+}$ -levels in BEAS-2B cells.

3

4 We next assessed whether 1-NP could affect intracellular  $\text{Ca}^{2+}$ -concentrations ( $[\text{Ca}^{2+}]_i$ ) in  
5 BEAS-2B cells. Cells were loaded with the  $\text{Ca}^{2+}$ -sensitive fluorescent probe Fura-2 prior to 1-  
6 NP exposure, and changes in  $[\text{Ca}^{2+}]_i$  were assessed by microspectrofluorimetry. As seen from  
7 Fig 2A, 10  $\mu\text{M}$  1-NP induced a time-dependent increase in  $[\text{Ca}^{2+}]_i$  in BEAS-2B cells. The  
8 kinetics of 1-NP-induced  $[\text{Ca}^{2+}]_i$  in BEAS-2B cells resembled that reported for B[a]P in  
9 endothelial HMEC cells (Mayati et al., 2011; Mayati et al., 2012) with a small, transient peak  
10 around 5 min after exposure, followed by a gradual increase that reached maximum after  
11 about half an hour exposure. Moreover, it has recently been reported that PAHs may induce  
12 increases in  $[\text{Ca}^{2+}]_i$  in HMEC cells through direct activation of  $\beta 2\text{AR}$  (Mayati et al., 2012).  
13 Thus, to assess whether similar mechanisms could be involved in the present PAH-induced  
14  $\text{Ca}^{2+}$  response, BEAS-2B cells were pre-incubated with the  $\beta$ -blocker carazolol for 30 min  
15 prior to exposure with 10  $\mu\text{M}$  1-NP. Carazolol almost completely blocked the early phases of  
16 1-NP induced increases in  $[\text{Ca}^{2+}]_i$  (Fig. 2B). However, 1-NP induced a sustained increase in  
17  $[\text{Ca}^{2+}]_i$  lasting at least up to 6 h (Fig. S1A, online supplementary materials). The later phases  
18 of 1-NP-induced (from 45 min to 6 h) seemed unaffected by Carazolol treatment (Fig. 2B).

19

20 Of notice, previous studies have shown that PAHs such as B[a]P and un-substituted pyrene  
21 may stimulate  $\beta 2\text{AR}$ -mediated  $[\text{Ca}^{2+}]_i$  already at 1  $\mu\text{M}$  concentration (Mayati et al., 2011;  
22 Mayati et al., 2012). In line with this we observed that 1  $\mu\text{M}$  B[a]P was able to induce  
23 increased  $[\text{Ca}^{2+}]_i$  in BEAS-2B cells. However, 1-NP failed to affect  $\text{Ca}^{2+}$  signaling at this low  
24 concentration. Moreover, at 10  $\mu\text{M}$  B[a]P appeared to induce considerably higher effects on  
25  $[\text{Ca}^{2+}]_i$  compared to 1-NP (Fig S1B and C, online supplementary materials). Thus, although 1-

1 NP was able to stimulate  $\beta$ 2AR-induced  $[Ca^{2+}]_i$ , it appeared to be a less potent activator  
2 compared to other PAHs.

3

#### 4 *Role of $\beta$ 2AR in 1-NP-induced CXCL8 responses in BEAS-2B cells*

5 Next we wanted to assess whether  $\beta$ 2AR could be involved in nitro-PAH-induced CXCL8  
6 responses in BEAS-2B cells. Therefore we pre-incubated the cells with a  $\beta$ 2AR-blocking  
7 antibody (Mayati et al., 2012) for 30 min prior to exposure with 1-NP. The  $\beta$ 2AR-blocking  
8 antibody suppressed 1-NP-induced CXCL8 responses by approximately 50% at both tested  
9 concentrations (Fig 3A). To further examine the role of  $\beta$ 2AR in 1-NP-induced CXCL8  
10 responses, we assess the effects of the selective  $\beta$ 2AR-antagonist ICI 118551 and of silencing  
11  $\beta$ 2AR by siRNA (si $\beta$ 2AR). Both ICI treatment and transfection with si $\beta$ 2AR resulted in a  
12 partial, but statistically significant suppression of 1-NP-induced CXCL8 (Fig 3B and C),  
13 comparable to the effect obtained with the  $\beta$ 2AR-blocking antibody. Thus  $\beta$ 2AR-signaling  
14 seemed to be involved in 1-NP-induced chemokine responses.

15

16

#### 17 **Discussion**

18 In the present study we have assessed the mechanisms of the 1-NP induced CXCL8 response  
19 with emphasis on the importance of  $Ca^{2+}$ -signaling and the  $\beta$ 2AR-receptor. Previously,  $Ca^{2+}$ -  
20 chelation was found to suppress CXCL8 in BEAS-2B cells by a variety of different air  
21 pollution constituents including 1-NP (Øvrevik et al., 2011). In coherence with this, the  
22 present results confirmed that the cell-permeable  $Ca^{2+}$ -chelator BAPTA-AM almost  
23 completely block 1-NP-induced CXCL8. Of interest, PAHs may increase  $[Ca^{2+}]_i$  in  
24 endothelial cells through AhR-independent mechanisms (Mayati et al., 2011). Recent findings  
25 suggest that this effect may be regulated through PAH-mediated binding and activation of the

1  $\beta$ 2AR (Mayati et al., 2012). In support of this, we observed that 1-NP (10  $\mu$ M) exposure  
2 induced an increase in  $[Ca^{2+}]_i$  in BEAS-2B cells that could be suppressed by beta-blocker  
3 treatment. Thus, PAH-induced  $Ca^{2+}$ -signaling through  $\beta$ 2AR-activation may extend to several  
4 cell types. However, only the early phase of the  $Ca^{2+}$ -response seemed to depend on signaling  
5 through  $\beta$ -adrenergic receptors, as beta-blocker treatment had no effect on the 1-NP induced  
6 increase in  $[Ca^{2+}]_i$  after 40 min exposure. Thus it is conceivable that 1-NP may induce  $Ca^{2+}$ -  
7 signaling through multiple mechanisms.

8

9 Interference with  $\beta$ 2AR-signalling by use of blocking antibodies, a pharmacological beta-  
10 blocker, or by silencing  $\beta$ 2AR-expression by siRNA-transfection, led to a suppression of 1-  
11 NP-induced CXCL8 responses. Thus 1-NP-induced CXCL8 release in BEAS-2B cells  
12 seemed at least partly dependent on  $\beta$ 2AR-activation. In line with this, the  $\beta$ 2AR agonists  
13 salbutamol and salmeterol have been found to enhance CXCL8 and interleukin-6 (IL-6)  
14 responses by IL-1 $\beta$  or virus infections in BEAS-2B cells and primary human bronchial  
15 epithelial cells (Edwards et al., 2007; Holden et al., 2010). However, salbutamol and  
16 salmeterol had no effect on CXCL8 or IL-6 responses alone, suggesting that  $\beta$ 2AR-signalling  
17 alone may be insufficient for activation of cytokine/chemokine responses. Indeed, B[a]P  
18 appeared to be a more potent inducer of  $\beta$ 2AR-dependent  $[Ca^{2+}]_i$ -responses than 1-NP, but is  
19 nevertheless unable to induce CXCL8 or other chemokines in BEAS-2B cells (Øvrevik et al.,  
20 2010; Øvrevik et al., 2013). Thus, additional 1-NP-induced mechanisms are likely required  
21 for the response. This notion is supported by previous findings suggesting that 1-NP-induced  
22 CXCL8 responses also involve metabolic activation by CYP-enzymes and possibly ROS  
23 (Øvrevik et al., 2013).

24

1 While  $\beta$ 2AR-interference only partly attenuated 1-NP-induced CXCL8 release,  $\text{Ca}^{2+}$ -chelation  
2 by BAPTA-AM completely abrogated the CXCL8 response. It should therefore be considered  
3 that the role of  $\beta$ 2AR in 1-NP-induced CXCL8-release could be linked to other signaling  
4 mechanisms such as cAMP or  $\beta$ -arrestin. Indeed, salbutamol-induced exacerbation of virus-  
5 induced IL-6 responses in BEAS-2B cells seemed to be mediated through a cAMP-dependent  
6 mechanism (Edwards et al., 2007). If so, the effects of BAPTA-AM on 1-NP-induced CXCL8  
7 may be also related to interference with the later, sustained,  $\beta$ 2AR-independent  $[\text{Ca}^{2+}]_i$ -  
8 response.

9

10 The strong CXCL8-induction by 1-NP seems predominately to occur at high concentrations  
11 ( $\geq 10 \mu\text{M}$ ) in BEAS-2B cells (Øvrevik et al., 2013). However, we recently observed that low  
12 concentrations of 1-NP (1  $\mu\text{M}$ ; giving no cytokine release alone) potentiated CXCL8-  
13 responses induced by priming the cells with a Toll-like receptor 3-agonist. Similar effects  
14 were observed with low concentrations of 1-AP and un-substituted pyrene (Øvrevik et al.,  
15 2013). Since 1-NP may exacerbate CXCL8 responses at concentrations that are insufficient to  
16 induce  $\beta$ 2AR-dependent  $[\text{Ca}^{2+}]_i$ , other cellular targets seem to exist that are more sensitive  
17 towards 1-NP than the  $\beta$ 2AR. Such low-concentration targets may be more important  
18 scenarios of real-life exposure to pyrene and pyrene-derivatives. Other PAHs may have  
19 considerably higher affinity for  $\beta$ 2AR. As reported in the present study and elsewhere (Mayati  
20 et al., 2012), B[a]P may induce  $\beta$ 2AR-dependent  $[\text{Ca}^{2+}]_i$ -responses already at 1  $\mu\text{M}$ . In fact,  
21 the affinity of B[a]P ( $K_d = 10 \text{ nM}$ ) appears to be among the highest reported for  $\beta$ 2AR  
22 ligands (Mayati et al., 2012). However, it should also be noted that 1-NP concentrations in  
23 diesel exhaust particles may be up to 10-fold higher than the concentration of B[a]P  
24 (Totlandsdal et al., 2012; Totlandsdal et al., 2014).

25

1 In extension of previous observations from endothelial cells (Mayati et al., 2011; Mayati et  
2 al., 2012), the present results suggest that PAHs may induce increased  $[Ca^{2+}]_i$  through  $\beta$ 2AR-  
3 activation also in epithelial lung cells. Moreover,  $\beta$ 2AR-signalling and  $[Ca^{2+}]_i$  appeared to be  
4 involved in the regulation of 1-NP-induced CXCL8 in the BEAS-2B cells. Although other yet  
5 unidentified cellular targets may be more sensitive to 1-NP exposure,  $\beta$ 2AR appears to be a  
6 highly sensitive for other PAHs (Mayati et al., 2012). PAHs have also been reported to impair  
7  $\beta$ 2AR-function and interfere with asthma treatment (Factor et al., 2011). Thus, further studies  
8 are warranted to clarify the role of  $\beta$ 2AR in PAH-induced responses and its possible  
9 implications in lung toxicity.

10

11

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15

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2 **Figure legends**

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4 **FIGURE 1. CXCL8 gene expression and protein release in 1-NP-exposed BEAS-2B cells,**  
5 **and role of Ca<sup>2+</sup>-chelation by BAPTA-AM.** Cells were exposed to 20  $\mu$ M 1-NP or vehicle  
6 (DMSO) alone. CXCL8 gene expression was measured after 6 h by real-time PCR (A). Cells  
7 were pre-incubated with 10  $\mu$ M of the cell-permeable Ca<sup>2+</sup>-chelator BAPTA-AM for 30 min  
8 prior to exposure to 20  $\mu$ M 1-NP or vehicle (DMSO) alone for 18 h (B). The figure depicts  
9 mean  $\pm$  SEM of three independent experiments. \*Significantly different from unexposed  
10 controls ( $P < 0.05$ ); †Significant down-regulation by chelator-treatment ( $P < 0.05$ ).

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12 **FIGURE 2. Intracellular Ca<sup>2+</sup>-levels and role of  $\beta$ 2AR-signaling in 1-NP exposed BEAS-**  
13 **2B cells.** Cells were pre-incubated with 10  $\mu$ M of the  $\beta$ -blocker carazolol (Cara) prior to  
14 incubation with 10  $\mu$ M 1-NP or vehicle (DMSO) alone (A and B). Intracellular Ca<sup>2+</sup>-  
15 concentrations ( $[Ca^{2+}]_i$ ) were measured by incubation with the Ca<sup>2+</sup>-sensitive probe Fura-2  
16 and the ratio of fluorescence intensities after excitation at 340 nm and 380 nm, respectively,  
17 was used to estimate  $[Ca^{2+}]_i$ . Figure A depicts the mean of normalized  $[Ca^{2+}]_i$  from two  
18 independent continuous recordings, while figure B depicts the mean  $\pm$  SEM of three  
19 independent experiments after 6 h (B) exposure.

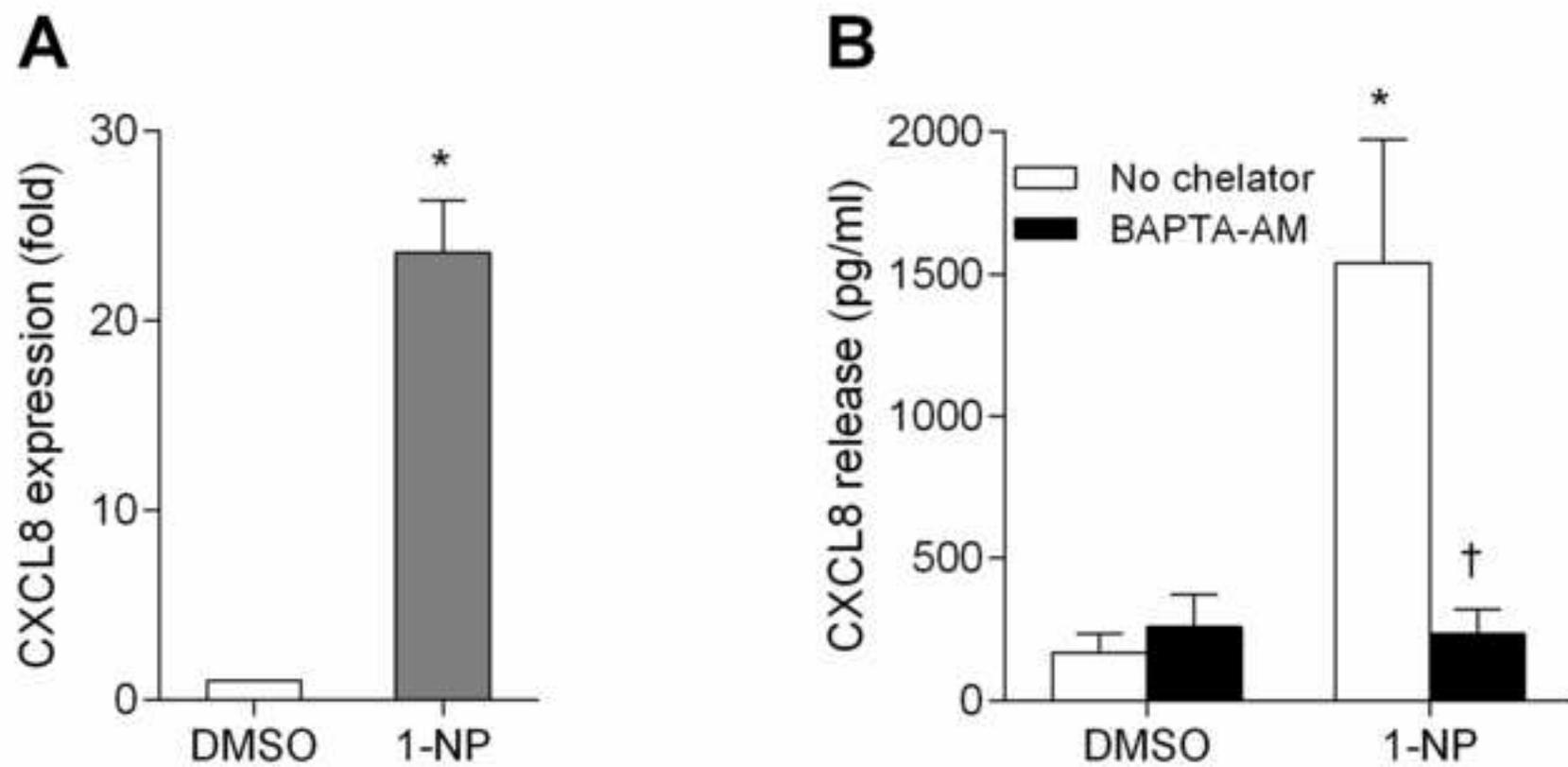
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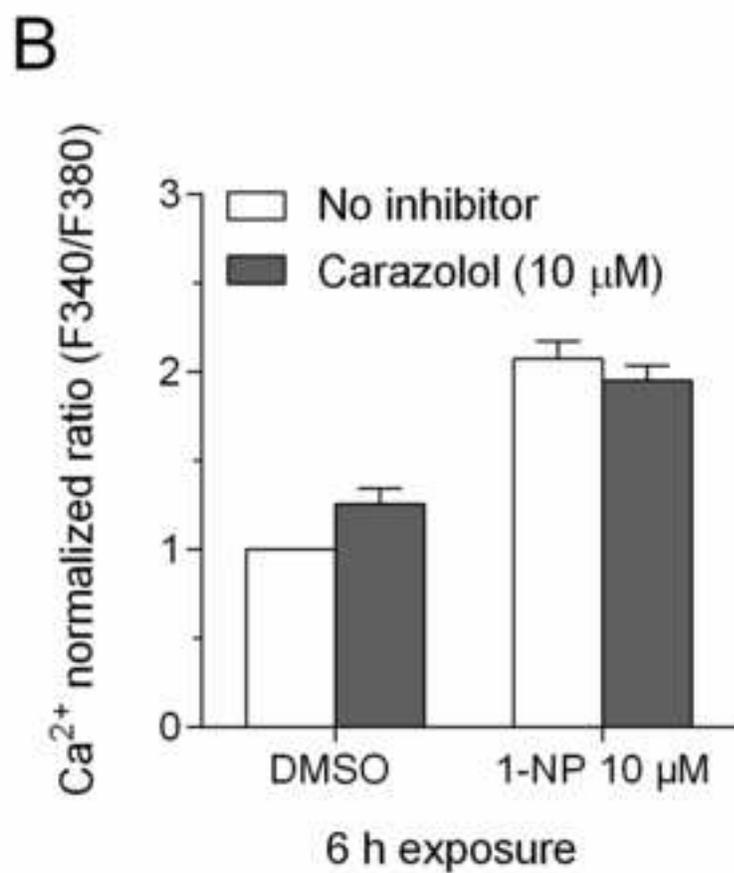
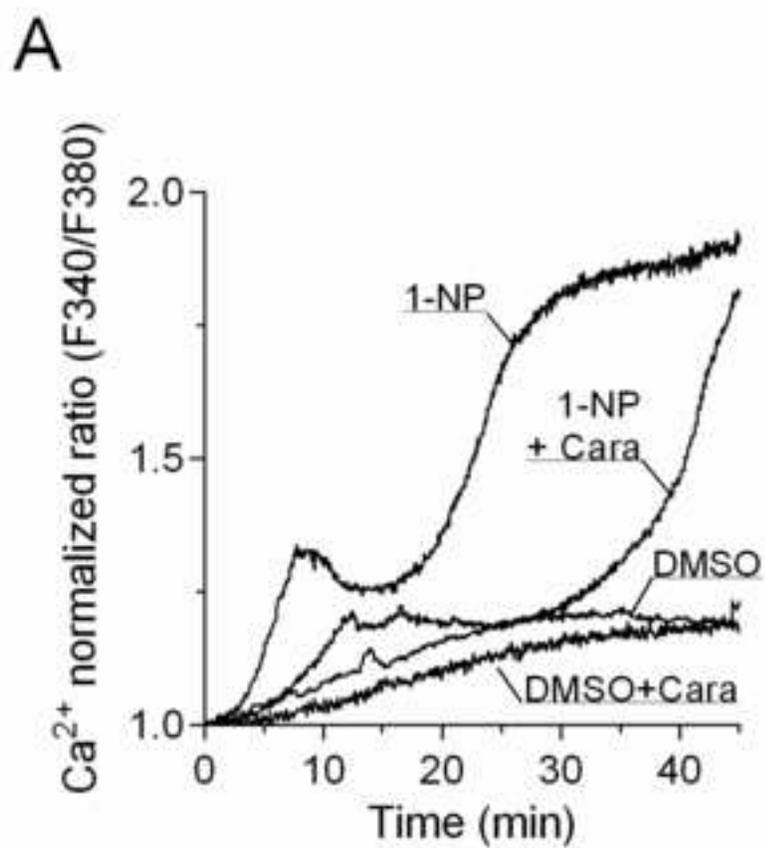
21 **FIGURE 3. Role of  $\beta$ 2AR-signaling in CXCL8-responses in 1-NP-exposed BEAS-2B**  
22 **cells.** Cells were pre-incubated for 30 min with a  $\beta$ 2AR-blocking antibody (A) or the  $\beta$ -  
23 blocker ICI 118551 (B), or transfected with siRNA against  $\beta$ 2AR (si $\beta$ 2AR) or non-targeting  
24 control siRNA (siNT) (C), prior to exposure with 20  $\mu$ M 1-NP or vehicle (DMSO) alone for  
25 18 h. CXCL8 release were measured by ELISA. Efficiency of  $\beta$ 2AR knock-down by siRNA

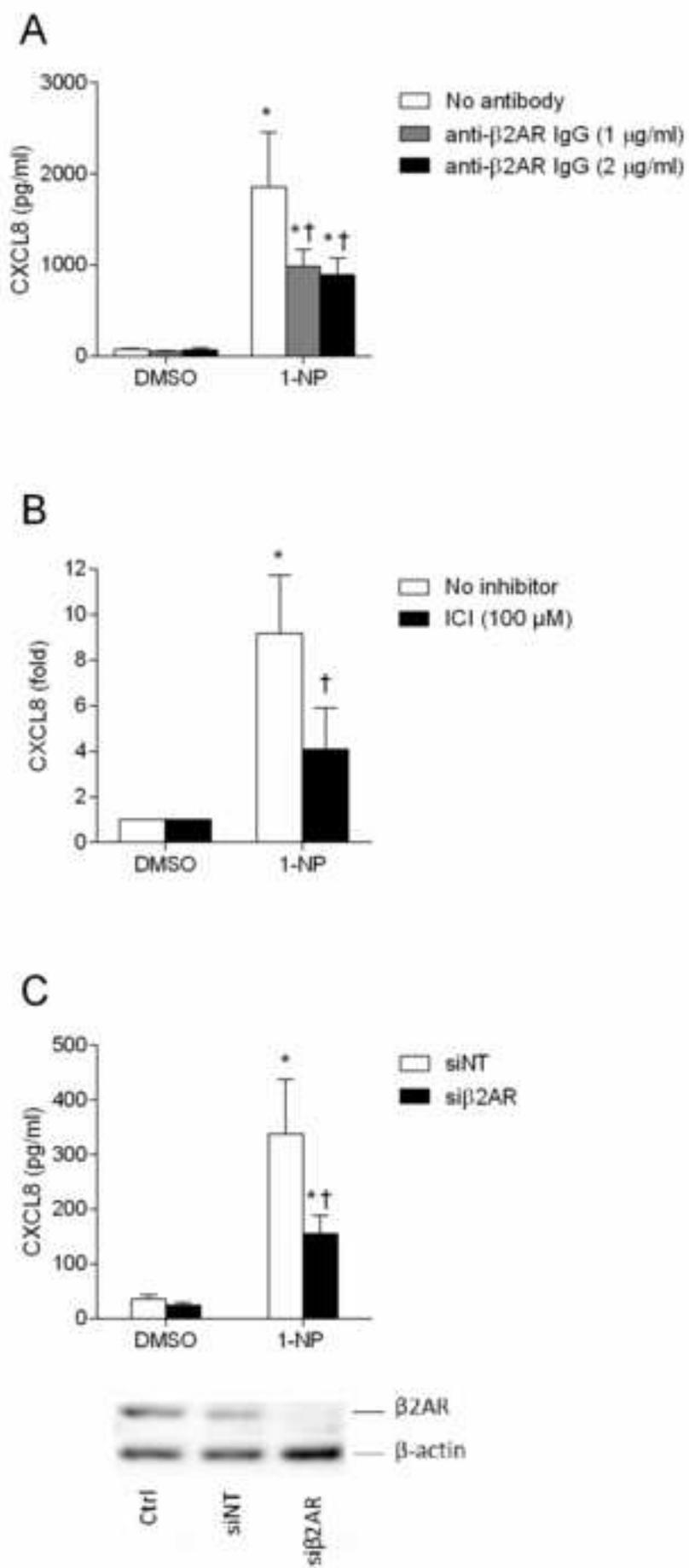
1 was assessed by Western blotting (C). The figures depict mean  $\pm$  SEM of three or more  
2 independent experiments. \*Significantly different from unexposed controls ( $P < 0.05$ );  
3 †Significant down-regulation by inhibitor/antibody/siRNA treatment ( $P < 0.05$ ).

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2 **Highlights**

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- 4 • We examined mechanisms of 1-NP induced CXCL8 responses in BEAS-2B cells
  - 5 • Treatment with a Ca<sup>2+</sup>-chelator abrogated the 1-NP-induced CXCL8 response
  - 6 • 1-NP induced a rapid increase in intracellular Ca<sup>2+</sup>-levels
  - 7 • Beta-blocker treatment suppressed the 1-NP-induced Ca<sup>2+</sup>-responses
  - 8 • Inhibition of  $\beta$ 2AR suppressed 1-NP-induced CXCL8 responses

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