

# Supplementary Materials: Carnosol and Related Substances Modulate Chemokine and Cytokine Production in Macrophages and Chondrocytes

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**Table S1.** Gene expression levels in unstimulated and LPS-stimulated murine macrophages RAW264.7.

| Gene                   | Type of Expression | Time | Unstimulated Cells<br>$\Delta CT \pm stdev^2$ | LPS Stimulated Cells<br>$\Delta CT \pm stdev^2$ | Fold Change <sup>1</sup> |
|------------------------|--------------------|------|---|---|--------------------------|
| COX-2                  | Intermediate       | 4 h  | 15.81 ± 0.40                                  | 9.75 ± 0.10                                     | 48                       |
| iNOS                   | Intermediate       | 4 h  | 16.43 ± 0.19                                  | 10.75 ± 0.08                                    | 52                       |
| TNF- $\alpha$          | High               | 4 h  | 13.39 ± 0.05                                  | 9.10 ± 0.07                                     | 20                       |
| IL-1 $\alpha$          | Low                | 4 h  | 22.90 ± 0.25                                  | 10.26 ± 0.08                                    | 6650                     |
| IL-1 $\beta$           | Low                | 4 h  | 31.92 ± 0.23                                  | 20.68 ± 0.25                                    | 1260                     |
| IL-6                   | Low                | 4 h  | 29.83 ± 1.97                                  | 16.44 ± 0.18                                    | 10800                    |
| IL-10                  | Low                | 4 h  | 26.34 ± 0.82                                  | 23.64 ± 0.36                                    | 6                        |
| PGES                   | Low                | 4 h  | 24.17 ± 0.26                                  | 23.11 ± 0.04                                    | 2                        |
| PGDS                   | Intermediate       | 4 h  | 17.12 ± 0.04                                  | 18.23 ± 0.19                                    | 0.5                      |
| MMP-9                  | High               | 4 h  | 14.03 ± 0.04                                  | 11.52 ± 0.12                                    | 6                        |
| CCL4/MIP-1 $\beta$     | High               | 4 h  | 14.42 ± 0.29                                  | 8.86 ± 0.14                                     | 190                      |
| CCL5/RANTES            | Intermediate       | 4 h  | 19.30 ± 0.16                                  | 10.50 ± 0.12                                    | 450                      |
| CXCL10/IP-10           | Intermediate       | 4 h  | 19.26 ± 0.11                                  | 11.07 ± 0.40                                    | 290                      |
| NF- $\kappa$ B1        | High               | 4 h  | 13.83 ± 0.08                                  | 11.56 ± 0.10                                    | 5                        |
| NF- $\kappa$ B49       | High               | 4 h  | 14.97 ± 0.17                                  | 12.92 ± 0.13                                    | 4                        |
| NF- $\kappa$ Bp65      | High               | 4 h  | 13.01 ± 0.04                                  | 13.12 ± 0.04                                    | 1                        |
| I- $\kappa$ B $\alpha$ | High               | 4 h  | 13.73 ± 0.21                                  | 11.57 ± 0.08                                    | 5                        |
| CD14                   | High               | 4 h  | 14.05 ± 0.16                                  | 12.81 ± 0.10                                    | 0.4                      |

<sup>1</sup>:  $2^{-\Delta CT}$ ;  $\Delta CT$  refers to the  $\Delta CT$  of unstimulated minus LPS-treated cells. <sup>2</sup>: (threshold cycles for 18S rRNA [internal standard]—threshold cycles for genes of)  $\pm$  st.dev. Cells were cultured for 4 h in the absence or presence of 1  $\mu$ g/mL LPS. Gene expression levels were measured by quantitative real time PCR and classified as ‘high’ ( $\Delta CT < 15$ ), ‘intermediate’ (( $\Delta CT$  15–25) and ‘low’ (( $\Delta CT > 25$ )).

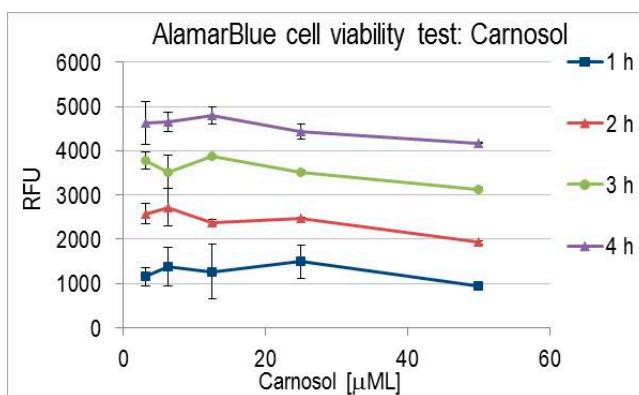
**Table S2.** Comparison of gene expression levels in unstimulated and IL-1 $\beta$ -stimulated SW1353 and primary chondrocytes NHAC-kn.

| Gene          | Time | SW1353                                    |  | NHAC-kn                                   |  |
|---------------|------|---|--|---|--|
|               |      | Unstimulated<br>$\Delta CT \pm st.dev.^1$ | IL-1 $\beta$ Stimulated<br>$\Delta CT \pm st.dev.^1$ | Unstimulated<br>$\Delta CT \pm st.dev.^1$ | IL-1 $\beta$ Stimulated<br>$\Delta CT \pm st.dev.^1$ |
| COX-2         | 4 h  | 22.76 ± 0.87                              | 19.30 ± 0.33   | 16.76 ± 0.09                              | 9.80 ± 0.18  |
| iNOS          | 4 h  | 25.70 ± 0.64                              | 19.13 ± 0.24   | nd  | nd   |
| TNF- $\alpha$ | 4 h  | 25.59 ± 1.07                              | 14.19 ± 0.22   | 28.98 ± 0.63                              | 15.70 ± 0.25   |
| IL-1 $\alpha$ | 4 h  | 23.19 ± 0.48                              | 17.97 ± 0.18   | 26.86 ± 0.27                              | 17.30 ± 0.32   |
| IL-1 $\beta$  | 4 h  | nd  | nd   | 19.57 ± 0.20                              | 9.08 ± 0.10  |
| IL-6          | 4 h  | 24.36 ± 1.85                              | 13.62 ± 0.34   | 20.66 ± 0.17                              | 8.69 ± 0.10  |
| IL-8          | 4 h  | 24.79 ± 0.82                              | 10.06 ± 0.15   | 19.62 ± 0.06                              | 9.10 ± 0.17  |
| MMP-1         | 4 h  | 18.67 ± 0.12                              | 15.75 ± 0.27   | 20.43 ± 0.16                              | 14.28 ± 0.27   |
| MMP-2         | 4 h  | 10.41 ± 0.65                              | 10.50 ± 0.46   | 12.65 ± 0.22                              | 12.60 ± 0.12   |
| MMP-3         | 4 h  | 16.73 ± 0.20                              | 11.38 ± 0.07   | 19.40 ± 0.14                              | 10.41 ± 0.14   |
| MMP-9         | 4 h  | 18.10 ± 0.19                              | 16.03 ± 0.11   | 20.16 ± 0.25                              | 19.16 ± 0.26   |
| MMP-13        | 4 h  | 19.53 ± 0.65                              | 14.09 ± 0.43   | 20.82 ± 0.21                              | 19.09 ± 0.35   |
| MIP-2         | 4 h  | nd  | nd   | 19.41 ± 0.19                              | 9.54 ± 0.16  |
| MIP-3         | 4 h  | nd  | nd   | 18.97 ± 0.13                              | 9.68 ± 0.12  |
| CCL5/RANTES   | 4 h  | 26.44 ± 0.01                              | 16.50 ± 0.49   | 18.50 ± 0.18                              | 12.37 ± 0.20   |

**Table S2.** *Cont.*

| Gene         | Time | SW1353  |   | NHAC-kn   |   |
|--------------|------|---|---|---|---|
|              |      | Unstimulated<br>$\Delta C_T \pm \text{st.dev.}^1$ | IL-1b Stimulated<br>$\Delta C_T \pm \text{st.dev.}^1$ | Unstimulated<br>$\Delta C_T \pm \text{st.dev.}^1$ | IL-1b Stimulated<br>$\Delta C_T \pm \text{st.dev.}^1$ |
| CXCL10/IP-10 | 4 h  | 25.84 ± 0.28                                      | 14.35 ± 0.26  | 31.56 ± 2.32                                      | 20.64 ± 0.51  |
| TIMP-1       | 4 h  | 10.33 ± 0.10                                      | 10.25 ± 0.31  | 8.39 ± 0.23                                       | 7.84 ± 0.10   |
| COL2A1       | 4 h  | 20.33 ± 0.66                                      | 20.95 ± 0.82  | 22.34 ± 0.51                                      | 22.15 ± 0.27  |
| Aggrecan     | 4 h  | 16.38 ± 0.12                                      | 15.49 ± 0.36  | 13.39 ± 0.18                                      | 13.59 ± 0.14  |
| ADAMTS-4     | 4 h  | 23.31 ± 0.73                                      | 21.81 ± 0.36  | 21.98 ± 0.12                                      | 19.38 ± 0.45  |
| LIF          | 4 h  | 18.63 ± 0.28                                      | 13.59 ± 0.32  | 16.77 ± 0.15                                      | 10.86 ± 0.16  |
| ADAMTS-5     | 4 h  | 19.08 ± 0.87                                      | 19.50 ± 1.13  | 13.73 ± 0.20                                      | 13.38 ± 0.15  |

<sup>1</sup>: (threshold cycles for 18S rRNA [internal standard]—threshold cycles for genes of) ± st.dev.



**Figure S1.** Freshly isolated PBLs were incubated with the indicated amounts of carnosol for 1–4 h. Using the Alamar Blue® cell viability test according to the manufacturers' instructions (ThermoFischer Scientific), the produced fluorescence was measured at the indicated time points. RFU, relative fluorescence units. In the absence of carnosol, cells produced similar RFU values as those observed in the presence of 3.125 to 25 μM carnosol.