# **Supplementary File for: Pre-exposure to hydrogen sulfide modulates the innate inflammatory response to organic dust**

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Supplemental figure and tables



Fig. S1: H2S and ODE exposure induced histopathological changes in the lungs H&E images are shown in the left and inflammatory score is shown in the right. Histopathological changes due to exposure to either H2S or ODE or co-exposure to H2S and ODE were not significantly different among any of the groups. Compared with controls (**a-c**), ODE exposure of mice did not induce any morphological changes in the blood vessels, bronchioles and septa except for the number of club cells (**b**). (Micrometer bar = 100 µm). Data (mean ± SEM, n = 5–6/group) analyzed with one-way ANOVA followed by Tukey’s post hoc test for multiple comparisons as compared with 0 ppm (TIF 92 MB)

**Table-1: Semi-quantitative evaluation of morphological changes in the lung tissue sections following H2S and ODE exposure and criteria for assigning scores**

|  |  |  |  |
| --- | --- | --- | --- |
| **Score** | **Peribronchiolar edema** | **Club cells**  **(per 100 µm)** | **Perivascular edema** |
| **1** | nil | <5 | upto 50 µm |
| **2** | 1-100 µm | 5-15/100 | 50-100 µm |
| **3** | 100-200 µm | 15-25/100 | 100-150 µm |
| **4** | >200 µm | >25 | >150 µm |

**Table-2: Primers used for RT-PCR**

|  |  |  |
| --- | --- | --- |
| **Human gene of interest** | **Primers** (5’ 3’) | |
| ***inos*** | **Forward** | GCTCTACACCTCCAATGTGACC |
| **Reverse** | CTGCCGAGATTTGAGCCTCATG |
| ***tlr2*** | **Forward** | CTTCACTCAGGAGCAGCAAGCA |
| **Reverse** | ACACCAGTGCTGTCCTGTGACA |
| ***tlr4*** | **Forward** | CCCTGAGGCATTTAGGCAGCTA |
| **Reverse** | AGGTAGAGAGGTGGCTTAGGCT |
| ***nfkbp65*** | **Forward** | CCAGACCAACAACAACCCCT |
| **Reverse** | TCACTCGGCAGATCTTGAGC |
| ***nrf2*** | **Forward** | CACATCCAGTCAGAAACCAGTGG |
| **Reverse** | GGAATGTCTGCGCCAAAAGCTG |

**Table-3: Primers used for RT-PCR**

|  |  |  |
| --- | --- | --- |
| Mouse Gene of interest | Primers (5’ 3’) | |
| *inos* | Forward | GAGACAGGGAAGTCTGAAGCAC |
| Reverse | CCAGCAGTAGTTGCTCCTCTTC |
| *tlr2* | Forward | ACAGCAAGGTCTTCCTGGTTCC |
| Reverse | GCTCCCTTACAGGCTGAGTTCT |
| *tlr4* | Forward | AGCTTCTCCAATTTTTCAGAACTTC |
| Reverse | TGAGAGGTGGTGTAAGCCATGC |
| *nfkbp65* | Forward | TCCTGTTCGAGTCTCCATGCAG |
| Reverse | GGTCTCATAGGTCCTTTTGCGC |
| *nrf2* | Forward | CAGCATAGAGCAGGACATGGAG |
| Reverse | GAACAGCGGTAGTATCAGCCAG |
| *vhl 1* | Forward | GTTTGTGCCATCCCTCAATGTCG |
| Reverse | ACCTGACGATGTCCAGTCTCCT |
| *hif-1α* | Forward | CCTGCACTGAATCAAGAGGTTGC |
| Reverse | CCATCAGAAGGACTTGCTGGCT |
| *claudin 1* | Forward | GGACTGTGGATGTCCTGCGTTT |
| Reverse | GCCAATTACCATCAAGGCTCGG |
| *claudin 3* | Forward | TCATCGTGGTGTCCATCCTGCT |
| Reverse | AGAGCCGCCAACAGGAAAAGCA |
| *claudin 5* | Forward | TGACTGCCTTCCTGGACCACAA |
| Reverse | CATACACCTTGCACTGCATGTGC |
| *claudin 18* | Forward | TGGTAGCATGGATGACTCTGCC |
| Reverse | GCTGTGGACATCCAGAAGTTGG |
| *ncf1* | Forward | GCTGACTACGAGAAGAGTTCGG |
| Reverse | CCTCGCTTTGTCTTCATCTGGC |
| *ncf2* | Forward | GCAGAAGAGCAGTTGGCATTGG |
| Reverse | CTGCCTCTCATTTGGACGGAAC |
| *ncf4* | Forward | CAAAGACCTGCTAGCGCTCATG |
| Reverse | CCACATCCTCATCTGACAGCAG |
| *hmgb1* | Forward | CCAAGAAGTGCTCAGAGAGGTG |
| Reverse | GTCCTTGAACTTCTTTTTGGTCTC |