

Blocking P2X Purinoceptor 4 Signalling Alleviates Cigarette Smoke-Induced Pulmonary Inflammation

Primer Sequences:

human *b2m*: Forward: 5'-GATAGTTAAGTGGGATCGAGACATG-3'
Reverse: 5'-AAAAGCAAGCAAGCAGAATTTGG-3'
Probe: 5'-FAM-AATGCGGCATCTTCAAACCTCCATGAT-Tamra-3'

human *P2RX4*: Forward: 5'-ATCCGCAGCCGCAAAGTG-3'
Reverse: 5'-CACGGAGTCAGTTTCCTGGTAG-3'
Probe: 5'-FAM-AATGCGGCATCTTCAAACCTCCATGAT-Tamra-3'

murine *b2m*: Forward: 5'-GCAGAGTTAAGCATGCCAGTAT-3'
Reverse: 5'-GGTTCAAATGAATCTTCAGAGCATC-3'
Probe: 5'-FAM-TGATGCTTGATCACATGTCTCGATCCCAGTA-Tamra-3'

murine *P2rx4*: Forward: 5'-AACACTTCTCAGCTTGGATTCCG-3'
Reverse: 5'-TTCACGGTGACGATCATGTTGG-3'
Probe: 5'-FAM-AGTTTTTCCTCCTGAGCTGGGACCACATAGTC-Tamra-3'

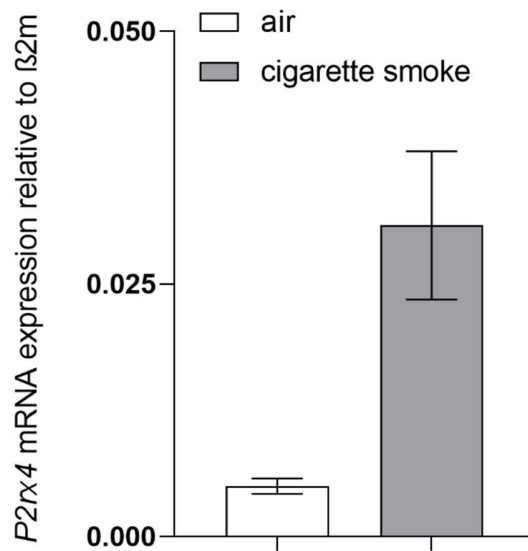
murine *P2rx7*: Forward: 5'-AAGTGGGTCTTGACATGATC-3'
Reverse: 5'-ATGCCTTTGACCTTGGTGTG-3'
Probe: 5'-FAM-TCTTTTCCTACATTAGCTTTGCTTTGGTGAGC-Tamra-3'

Supplementary Table 1: Descriptive statistics of BALF donors

	GOLD I & II	GOLD III & IV	Total COPD patients	Non-smokers
No. of Patients	9	6	15	15
Age in years (mean \pm SD)	62.2 \pm 9.3	69.0 \pm 5.3	64.9 \pm 8.6	36.9 \pm 11.3
Male in %	77.8	50.0	66.7	40.0
GOLD ratio	4 / 5	3 / 3	-	-

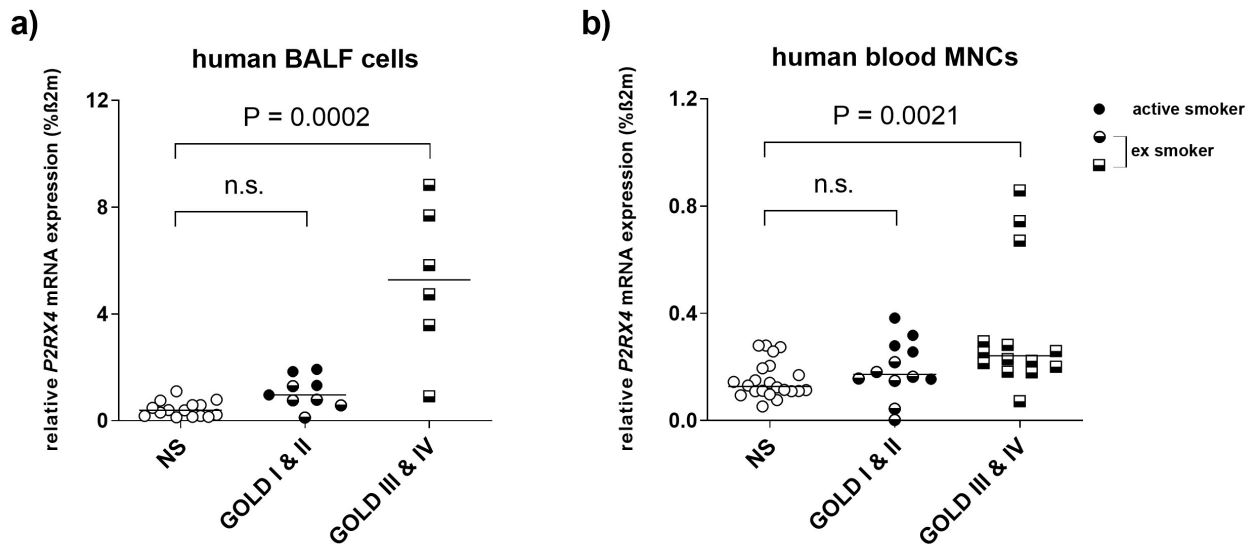
Supplementary Table 2: Descriptive statistics of MNCs donors

	GOLD I & II	GOLD III & IV	Total COPD patients	Non-smokers
No. of Patients	12	14	26	22
Age in years (mean \pm SD)	58.8 \pm 10.1	59.5 \pm 6.6	59.2 \pm 8.4	28.9 \pm 4.3
Male in %	83.3	42.9	61.5	50.0
GOLD ratio	5 / 7	9 / 5	-	-



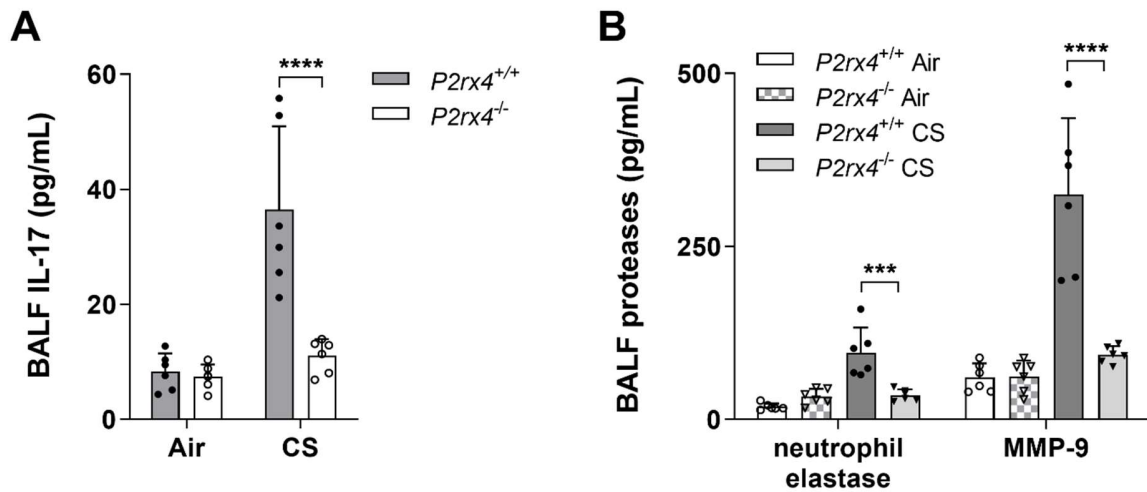
Supplementary Figure 1

Relative *P2rx4* expression in pooled BALF cells from 6 wild type mice per group. The *P2rx4* mRNA expression is quantified relative to $\beta 2$ microglobulin mRNA ($\beta 2m$) levels and presented as mean \pm SD of the triplicate assay.



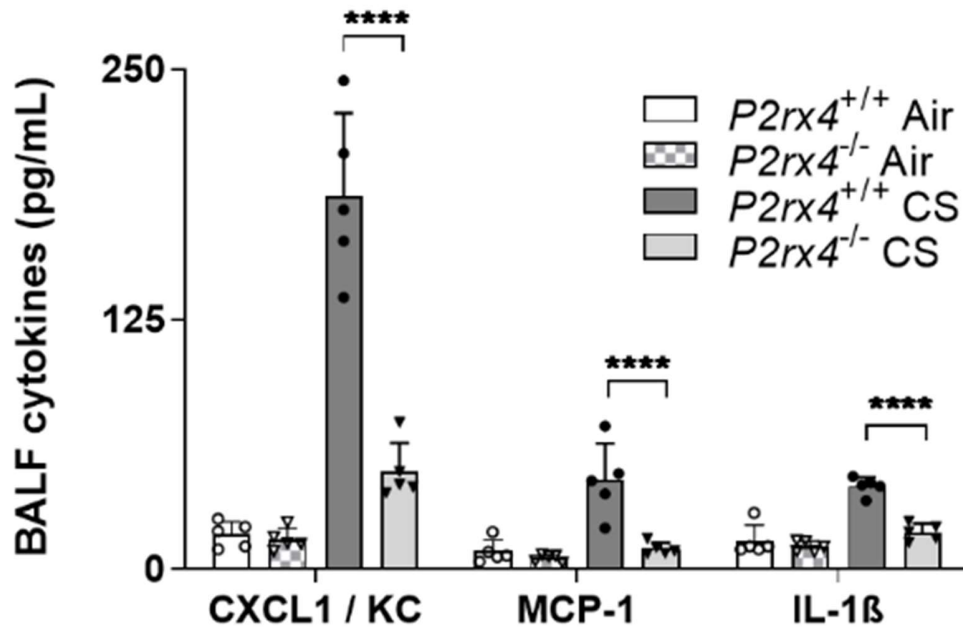
Supplementary Figure 2

Relative *P2RX4* expression in a) BALF cells and b) peripheral blood MNCs of COPD patients stratified for GOLD classification into mild-moderate (GOLD I & II) and severe (GOLD III & IV) and compared to healthy never-smoking individuals (NS). *P2X4* mRNA expression is quantified relative to $\beta 2$ microglobulin mRNA ($\beta 2m$) levels. Median expression levels are presented as horizontal lines. Each symbol represents a subject (BALF: n=6-15, monocytes: n=12-22). Shapes completely black represent current smokers and half-filled shapes represent the ex-smokers among the COPD patients. Data was analyzed using Kruskal-Wallis test with Dunn's post-hoc test to correct for multiple comparison. P is depicted in the corresponding graph and was considered statistical significant when ≤ 0.5 . n.s. = non-significant



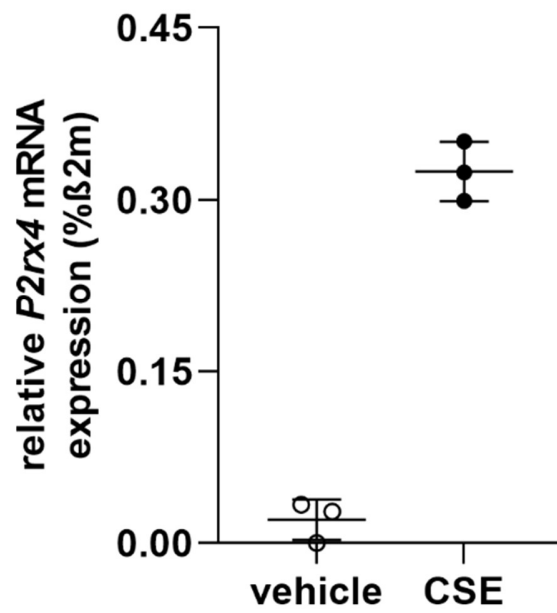
Supplementary Figure 3

Concentration of A) IL-17, B) neutrophil elastase and MMP-9 in the BALF of *P2rx4*-deficient mice (*P2rx4*^{-/-}) after cigarette smoke (CS) exposure for three consecutive days compared to wild type animals (*P2rx4*^{+/+}). Each animal is represented by an individual symbol (n=6). Data is presented as mean +SD and was analysed applying two-way ANOVA corrected for multiple comparison using Tukey test. *** P<0.001, **** P<0.0001



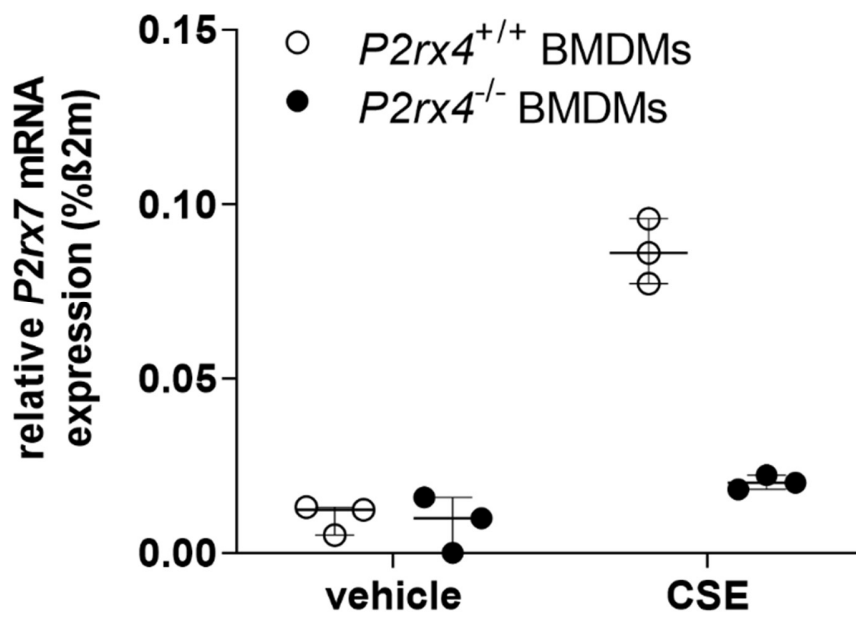
Supplementary Figure 4

Concentration of proinflammatory cytokines including CXCL1 / KC, IL-1 β and IL-6 in the BALF of $P2rx4$ -deficient mice ($P2rx4^{-/-}$) after exposure with the smoke of 5 cigarettes per day, five days a week for four months compared to wild type animals ($P2rx4^{+/+}$). Each animal is represented by an individual symbol (n=5). Data is presented as mean +SD and was analysed applying two-way ANOVA corrected for multiple comparison using Tukey test. **** P<0.0001



Supplementary Figure 5

P2rx4 expression quantified relative to $\beta 2$ microglobulin mRNA ($\beta 2m$) levels of bone marrow derived macrophages isolated from either wild type mice after vehicle or 5 % cigarette smoke extract stimulation. Mean \pm SD expression levels of the triplicate assay are presented as horizontal lines and the range is indicated by the error bars.



Supplementary Figure 6

P2rx7 expression quantified relative to β2 microglobulin mRNA (β2m) levels of bone marrow derived macrophages isolated from either *P2rx4*-deficient or wild type mice after vehicle or 5 % cigarette smoke extract stimulation. Mean ± SD expression levels of the triplicate assay are presented as horizontal lines and the range is indicated by the error bars.