Legends Supplemental Figures

Sup. Figure 1. *VILI in mice treated with specific A1AR (DPCPX) or A3AR (MRS1191) antagonists.* Age and gender matched mice (BL6/C57) treated with 1mg/kg i.p DPCPX (**A)** or 1mg/kg i.p. MRS1191 (**B**) or vehicle control 30 minutes prior to induction of anesthesia were exposed to VILI and survival times were determined. Mechanical ventilation was applied using pressure controlled settings (inspiratory pressure of 35 mbar, inspired oxygen concentration 100%, respiratory rate and inspiratory to expiratory ratio were adjusted to maintain normal pH) until a cardiac standstill was observed in the surface electrocardiogram. (**C, D**) Albumin concentration in the bronchoalveolar fluid was determined by murine ELISA. For this purpose, mice treated with DPCPX (**C**), MRS1191 (**D**) or vehicle were mechanically ventilated using pressure controlled ventilation with an inspired oxygen concentration of 100% for 180 minutes at 45 mbar. *Note: no significant changes of survival times or albumin leakage into the bronchoalveolar fluid after A1AR or A3AR antagonist treatment* (A-D: n=6).

Sup. Figure 2. *Influence of inspired oxygen concentration on A2BAR transcript levels during mechanical ventilation.* To investigate a potential influence of the fraction of inspired oxygen (FiO_2) on expression patterns of A2BARs, mice were mechanically ventilated (inspiratory pressure of 35 mbar, 100% oxygen) and A2BAR mRNA levels were determined by real-time RT-PCR. Different FiO_2 values are indicated. Results are derived from four animals in each condition.

Sup. Figure 3. *Influence of cystic fibrosis transmembrane conductance regulator (CFTR) inhibition on AFC during VILI.* To determine whether CFTR contributes to pulmonary fluid transport during VILI, we measured AFC using *A2BAR*$^{-/-}$ mice or age, sex and gender matched littermate controls mice (A2BAR$^{+/+}$). Mice were mechanically ventilated in a pressure-controlled setting at 45 mbar over indicated time periods (0 to 180 min). AFC was measured by instilling 300µl of isosmolar 0.9% NaCl solution with 5% bovine serum albumin. Mechanical ventilation was continued for 30 minutes and AFC was measured in the presence or absence of the CFTR inhibitor $CFTR_{inh\text{-}172}$ (10^{-6} M, 300 µl intratracheal).

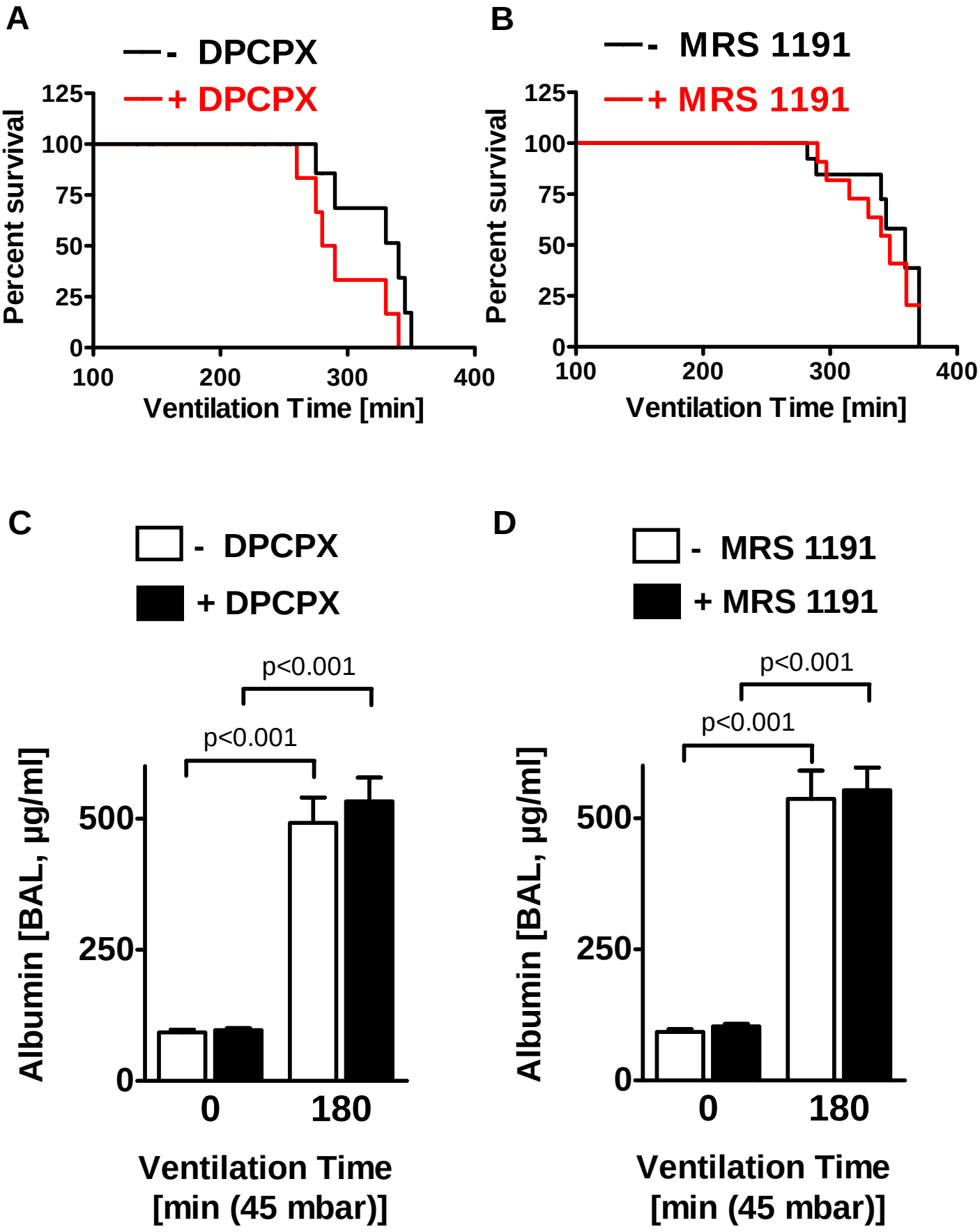
Sup. Figure 4. *Gas exchange and macroscopic appearance of mice exposed to VILI.* (**A**) Wildtype mice were mechanically ventilated [pressure controlled ventilation, inspired oxygen fraction (FiO2) of 1 (100% inspired oxygen)] for 0 or 360 minutes at an inspiratory pressure of 45 mbar using a positive end-experatory pressure (PEEP) of 5 mbar. To assess pulmonary gas exchange, blood gas analysis was performed by obtaining arterial blood via cardiac puncture. All results are presented as mean ± s.d and derived from six animals in each condition. *Note: During a ventilation period of 360 min, no significant changes in heart-rate or blood pressure were observed (see Supplemental Table 1 and 2). In contrast, pulmonary gas-exchange was significantly attenuated. (**B**)* Macroscopic images of lungs from mice exposed to VILI. Mice were mechanically ventilated (pressure controlled ventilation, 100% inspired oxygen) for 0 (upper panel) or 180 min (lower panel) at an inspiratory pressure of 45 mbar, PEEP 5 mbar. One of 4 representative macroscopic images is displayed.

Sup. Figure 5. *Repopulation of leukocytes in recipient mice after bone marrow transplantation.* Irradiated mice lacking CD45.1 received bone marrow cells from CD45.1-positive animals. Eight weeks after transplantation blood cells (B-cells, Neutrophils, CD4+ cells, CD8+ cells and Monocytes) were examined by FACS for CD45.1 and leukocyte markers. The percentage of specific cell lineages positive for the donor epitope CD45.1 is indicated at the top of each panel (CD45.1 negative specific cells "red circle", CD45.1 positive specific cells "blue circle").

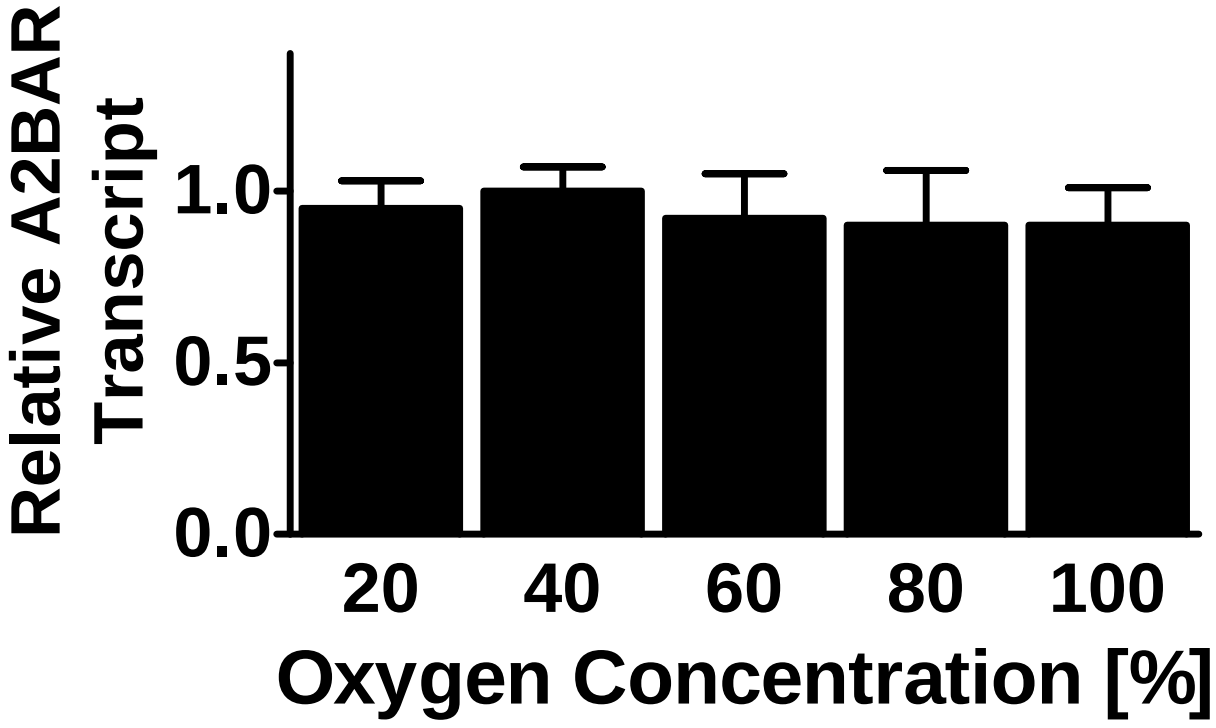
Supplemental Figure 6. *Repopulation of leukocytes in recipient mice after bone marrow transplantation.* Irradiated mice lacking CD45.1 received bone marrow cells from CD45.1-positive animals. Eight weeks after transplantation the percentage of CD45.1 positive cells in the different blood cell populations was examined by FACS. Data are presented as mean ± SD (n = 5).

Supplemental Figure 7. *Pulmonary histology and neutrophil content following bone-marrow transplantation (BMT).* **(A)** Eight weeks after transplantation, lungs were harvested and histological studies were performed (H&E staining, compared to sham operated control mice). **(B)** Mice (8–10 weeks of age, 20–25 g) were irradiated with a total dose of 12 Gy from a ^{137}Cs source. Immediately after irradiation, 10×10^6 bone marrow cells/recipient were injected in 0.3 ml 0.9% sodium chloride into the jugular vein. Four or 56 days after bone marrow transplantation (BMT), neutrophil accumulation in lungs using a myeloperoxidase assay was determined and compared to sham operated control mice (without radiation and without BMT; magnification x 400). *Note: no histological signs of inflammation or neutrophil accumulation 8 weeks after BMT. Consistent with white blood cell counts on day 4+ (Supplemental Table 3), myeloperoxidase was not detectable in lung tissue.*

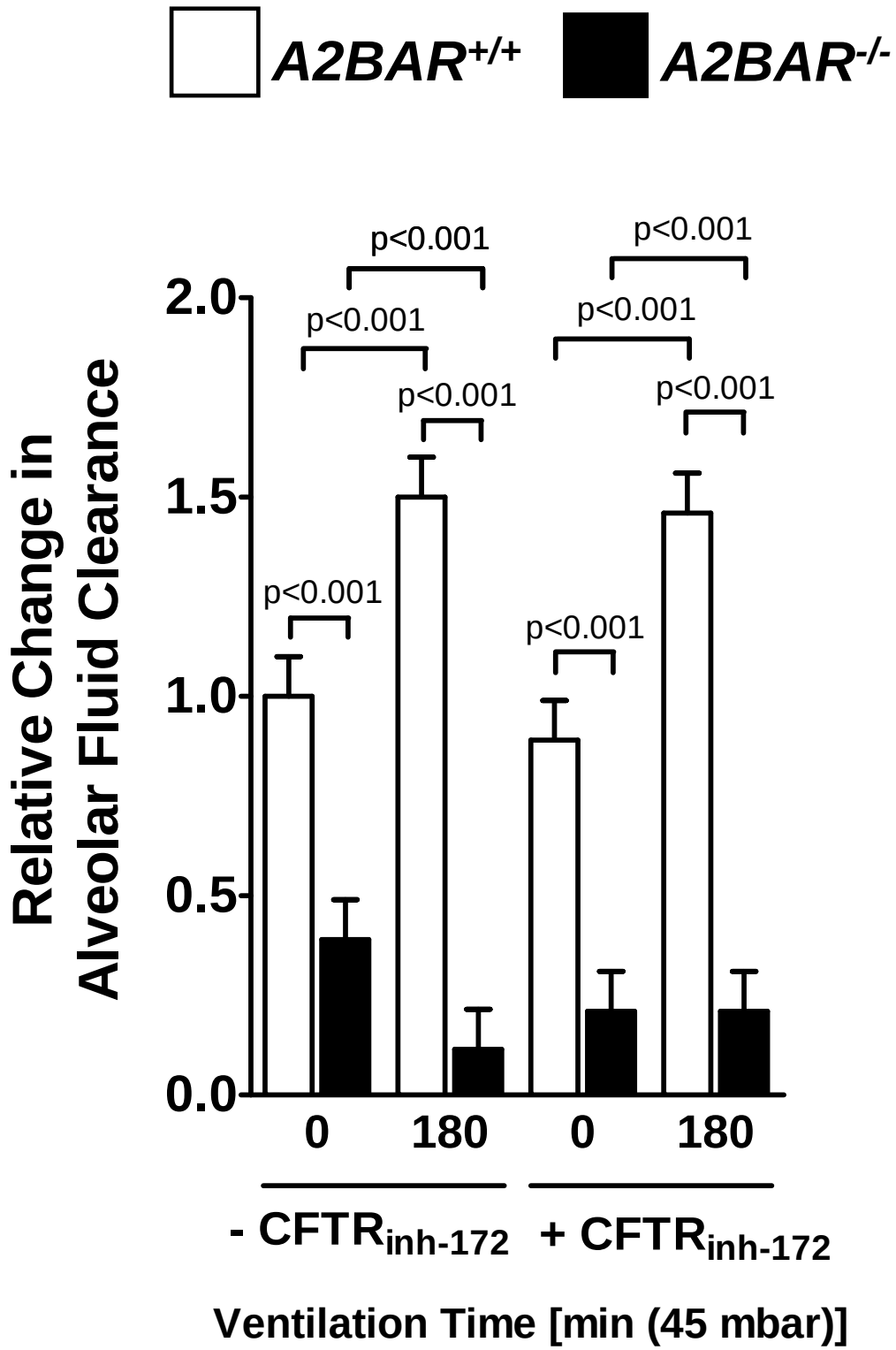
Sup. Figure 1


A
- DPCPX
+ DPCPX
Percent survival
125
100
75
50
25
0
100
200
300
400
Ventilation Time [min]
B
- MRS 1191
+ MRS 1191
Percent survival
125
100
75
50
25
0
100
200
300
400
Ventilation Time [min]
C
- DPCPX
+ DPCPX
p<0.001
p<0.001
Albumin [BAL, µg/ml]
500
250
0
0
180
Ventilation Time
[min (45 mbar)]
D
- MRS 1191
+ MRS 1191
p<0.001
p<0.001
Albumin [BAL, µg/ml]
500
250
0
0
180
Ventilation Time
[min (45 mbar)]

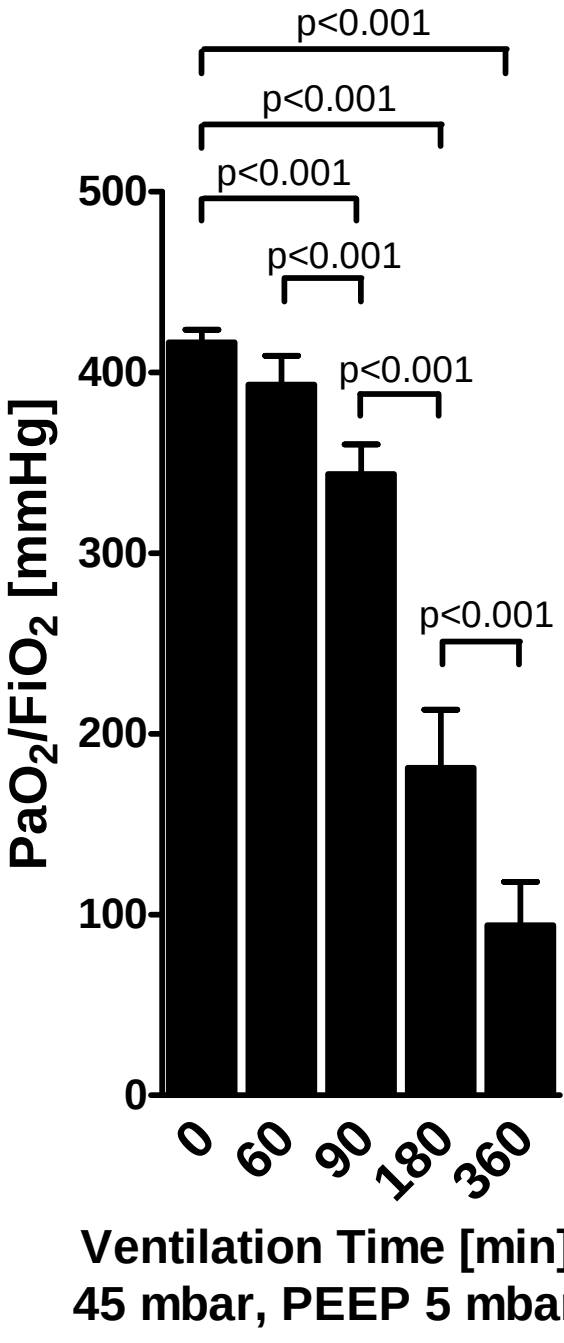
Sup. Figure 2


Relative A2BAR Transcript
1.0
0.5
0.0
20
40
60
80
100
Oxygen Concentration [%]

Sup. Figure 3

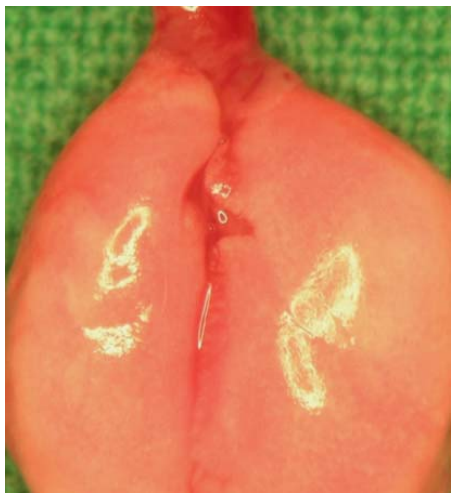

A2BAR+/+
A2BAR-/-
p<0.001
p<0.001
p<0.001
p<0.001
p<0.001
p<0.001
p<0.001
2.0
1.5
1.0
0.5
0.0
Relative Change in Alveolar Fluid Clearance
0
180
0
180
- CFTRinh-172
+ CFTRinh-172
Ventilation Time [min (45 mbar)]

A

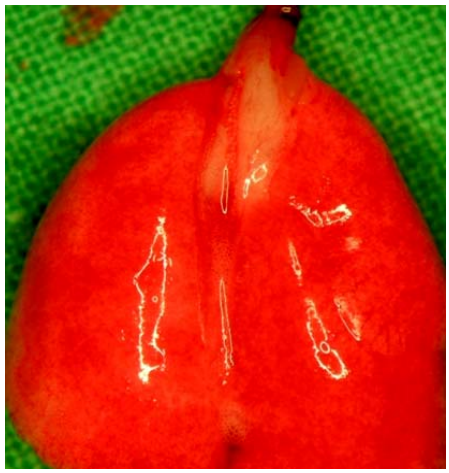


B

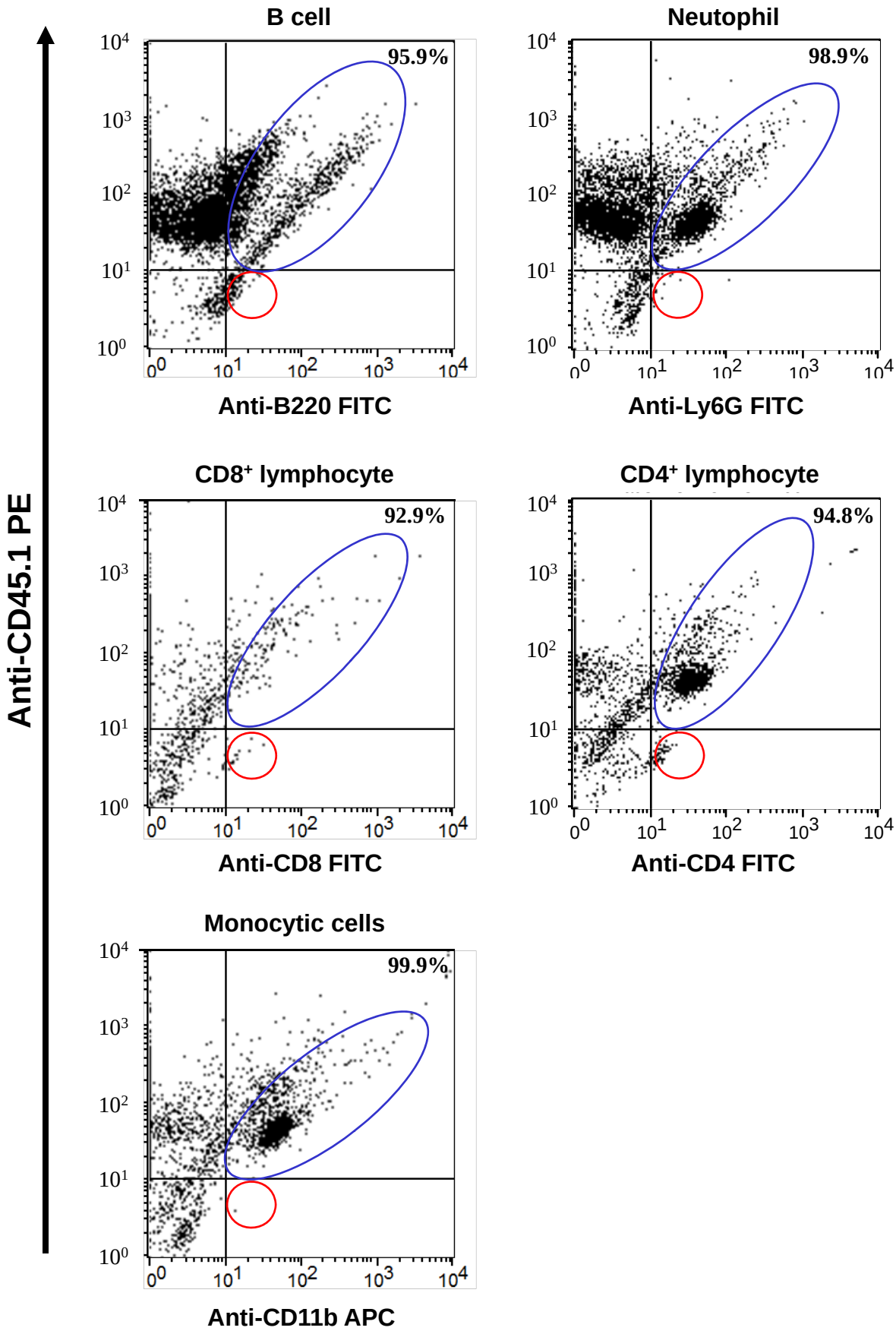
180 min Ventilation (15 mbar)



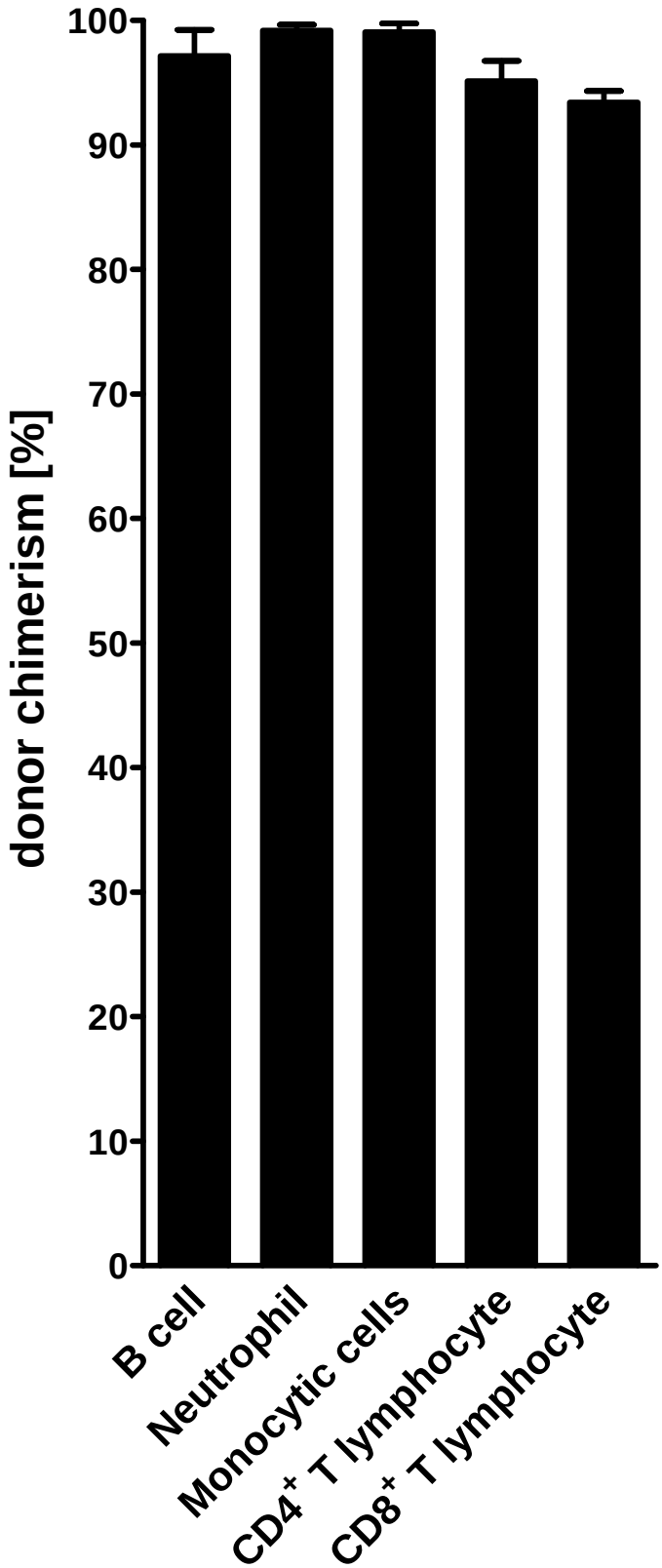
180 min Ventilation (45 mbar)



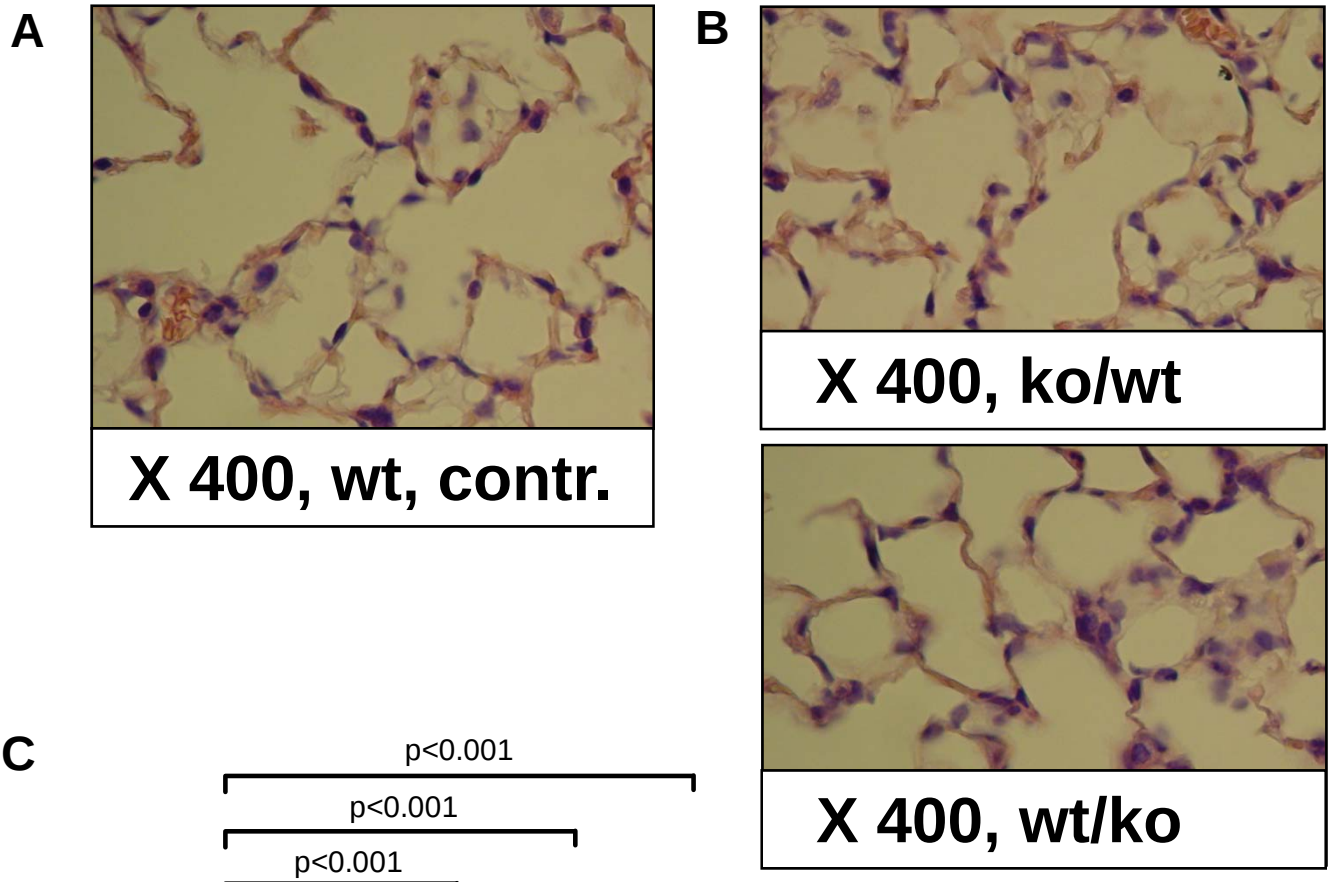
Sup. Figure 5


B cell
95.9%
Anti-B220 FITC
Neutophil
98.9%
Anti-Ly6G FITC
CD8⁺ lymphocyte
92.9%
Anti-CD8 FITC
CD4⁺ lymphocyte
94.8%
Anti-CD4 FITC
Monocytic cells
99.9%
Anti-CD11b APC
Anti-CD45.1 PE

Sup. Figure 6


donor chimerism [%]
100
90
80
70
60
50
40
30
20
10
0
B cell
Neutrophil
Monocytic cells
CD4+ T lymphocyte
CD8+ T lymphocyte

Sup. Figure 7


A
X 400, wt, contr.
B
X 400, ko/wt
X 400, wt/ko
C
p<0.001
p<0.001
p<0.001
p<0.001
Myeloperoxidase [ΔOD450nm/5 min]
0.10
0.05
0.00
n.d.
n.d.
n.d.
n.d.
day 4 after BMT
day 56 after BMT
control
wt/wt
wt/ko
ko/wt
ko/ko

Supplemental Table 1. Mean arterial pressure [mmHg]

	0 min	*60 min*	*90 min*	*180 min*	*360 min*
A2BAR*$^{+/+}$*(n=6)	115±10	90±10	85±5	80±5	70±5*
A2BAR*$^{-/-}$ *(n=6)	110±10	90±10	80±5	75±5	70±5*

* significant difference compared to anaesthesia induction $p<0.01$;

Supplemental Table 2. Heart Rate [beats/min]

	0 min	*60 min*	*90 min*	*180 min*	*360 min*
A2BAR*$^{+/+}$ *(n=6)	460±50	480±60	470±60	480±60	460±60
A2BAR*$^{-/-}$ *(n=6)	460±60	470±50	480±50	480±60	460±60

Supplemental Table 3.
White blood count (WBC) and red blood count (RBC)
after irridation and bone marrow transplantation (BMT)

	10^3/µl WBC	10^6/µl RBC
Days after BMT		
con	10,71	9,54
3	0,02	8,21
4	0,02	6,74
56	8,75	10,03
105	8,09	9,84