Supplemental Digital Content 2

table 1. Ventilatory parameters and arterial blood gases at BASELINE

	$\Delta P,L_{LOW}$		$\Delta P,L_{MEAN}$			$\Delta P,L_{HIGH}$	
$\Delta P,L$ (cmH_2O)	**7.5**	**8.5**	**10**	**10**	**9.2**	**12**	**12**
V_T (ml/kg)	**6**	**6**	**13**	**6**	**6**	**22**	**6**
PEEP (cmH_2O)	**3**	**5.5**	**3**	**9.5**	**7.5**	**3**	**11**
Pplat,rs (cmH_2O)	**11**	**14**	**14**	**20**	**17**	**17**	**24**
$\Delta P,L$ (cmH_2O)	7.4 ± 1.2	8.0 ± 1.3	6.6 ± 0.6	7.6 ± 0.9	8.4 ± 1.0	6.8 ± 0.8	6.9 ± 0.8
Est,rs (cmH_2O)	3.9 ± 0.7	4.2 ± 0.6	3.5 ± 0.3	4.1 ± 0.4	4.4 ± 0.5	3.6 ± 0.2	3.6 ± 0.5
V_T (ml/kg)	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
PEEP (cmH_2O)	3.1 ± 0.2	3.5 ± 0.2	3.5 ± 0.4	3.6 ± 0.2	3.5 ± 0.2	3.1 ± 0.4	3.1 ± 0.2
Pplat,rs (cmH_2O)	11.3 ± 1.2	12.6 ± 1.4	11 ± 0.8	12.1 ± 0.7	13.0 ± 0.1	10.6 ± 0.8	11 ± 0.8
RR (bpm)	75 ± 7.4	78 ± 6.9	77 ± 5.4	77 ± 5.2	75 ± 6.8	77 ± 6.4	74 ± 7.1
pHa	7.3 ± 2.4	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.3	7.4 ± 0.1	7.2 ± 0.1	7.3 ± 0.1
PaO_2 (mmHg)	164 ± 67	168 ± 66	174 ± 63	161 ± 53	182 ± 98	170 ± 73	183 ± 50
$PaCO_2$ (mmHg)	52 ± 9.4	41 ± 12	56 ± 9.4	53 ± 2.9	43 ± 7.9	56 ± 8.2	50 ± 11
MAP (mmHg)	81.8 ± 12.6	89.2 ± 14.3	80.7 ± 10.4	89.2 ± 18.2	83.2 ± 16.6	90.7 ± 12.0	75.0 ± 6.5

Values are expressed as mean ± SD of 6 animals per group. One-way ANOVA followed by Bonferroni post-hoc test. ΔP,L: transpulmonary driving pressure; Est,rs: respiratory system static elastance; V_T: tidal volume; PEEP: positive end-expiratory pressure; Pplat,rs: respiratory system plateau pressure; RR: respiratory rate; pHa: arterial pH; PaO_2: arterial oxygen partial pressure; $PaCO_2$: arterial carbon dioxide partial pressure; MAP: mean arterial pressure. Dashed lines represent Pplat,rs similar to $\Delta P,L_{MEAN}$ and $\Delta P,L_{HIGH}$ at high V_T (13 ml/kg [Pplat,rs = 14 cmH_2O] and 22 ml/kg [Pplat,rs = 17 cmH_2O]). For this purpose, V_T was kept low (6 ml/kg) and PEEP was adjusted for the level of Pplat,rs. Gas exchange was evaluated at PEEP = 3 cmH_2O and FiO_2 = 1.0 in all groups.

table 2. Mechanical and hemodynamic parameters at INITIAL and END

	$\Delta P,L_{LOW}$		$\Delta P,L_{MEAN}$			$\Delta P,L_{HIGH}$	
$\Delta P,L$ (cmH_2O)	**7.5**	**8.5**	**10**	**10**	**9.2**	**12**	**12**
V_T (ml/kg)	**6**	**6**	**13**	**6**	**6**	**22**	**6**
PEEP (cmH_2O)	**3**	**5.5**	**3**	**9.5**	**7.5**	**3**	**11**
Pplat,rs (cmH_2O)	**11**	**14**	**14**	**20**	**17**	**17**	**24**
$\Delta P,L$ (cmH_2O)							
INITIAL	7.5 ± 1.3	8.4 ± 1.0	10 ± 0.6*	9.7 ± 0.4*	9.2 ± 0.4*	12.1 ± 0.4*§	12.3 ± 0.8*§
END	6.8 ± 1.2	7.6 ± 1.2	9.6 ± 0.3*	9.4 ± 0.7	7.9 ± 0.8	11.7 ± 0.6*	12.4 ± 1.3*§
Est,rs (cmH_2O)							
INITIAL	3.9 ± 0.7	4.3 ± 0.5	2.6 ± 0.3†	5.4 ± 0.3*‡	4.6 ± 0.2	2.0 ± 0.3**	6.0 ± 0.8*##
END	3.7 ± 0.7	3.8 ± 0.6	2.7 ± 0.3†	5.0 ± 0.6*‡	4.1 ± 0.1	1.8 ± 0.3**	6.2 ± 0.7*##
V_T (ml/kg)							
INITIAL	6 ± 0	6 ± 0	13 ± 1	6 ± 0‡	6 ± 0	22 ± 3**	6 ± 0##
END	6 ± 0	6 ± 0	12 ± 1	6 ± 0‡	6 ± 0	24 ± 4**	6 ± 0##
PEEP (cmH_2O)							
INITIAL	3.1 ± 0.2	5.5 ± 1.0	3.3 ± 0.4	9.3 ± 1.1*‡	7.5 ± 0.6	3.1 ± 0.6	11.2 ± 0.9*##
END	3.3 ± 0.5	5.9 ± 1.2	3.1 ± 0.1	9.6 ± 1.1*‡	8.1 ± 0.5	3.1 ± 0.2	12.5 ± 1.7*##§
Pplat,rs (cmH_2O)							
INITIAL	11.4 ± 1.3	15.0 ± 0.4*	14.2 ± 0.8*	20.0 ± 1.3*‡	17.5 ± 0.5*†	17.1 ± 1.1*	24.1 ± 1.0*##§
END	11.4 ± 1.4	14.3 ± 0.6	13.5 ± 0.4*	19.0 ± 1.4*‡	17.0 ± 0.4*	16.7 ± 0.5*	25.7 ± 2.6*##§
RR (bpm)							
INITIAL	76 ± 9	77 ± 7	39 ± 3	82 ± 4‡	75 ± 7	23 ± 3**	78 ± 7##
END	76 ± 9	77 ± 7	40 ± 5	82 ± 4‡	75 ± 7	21 ± 2**	78 ± 7##
MAP (mmHg)							
INITIAL	73.8 ± 8.7	82.8 ± 8.8	84.8 ± 13.6†	63.2 ± 3.7	78.8 ± 10.0	66.8 ± 12.3	66.8 ± 12.3

END	85.7 ± 24.9	70.0 ± 25.2	105.7 ± 19.6	81.2 ± 9.1	77.7 ± 13.4	76.7 ± 15.8	76.7 ± 15.8

Values are expressed as mean ± SD of 6 animals per group. One-way ANOVA followed by Bonferroni post-hoc test. **vs.* V_T6-PEEP3; †*vs.* V_T6-PEEP5.5; ***vs.* V_T6-PEEP7.5; ‡*vs.* V_T13-PEEP3; §*vs.* V_T6-PEEP9.5; ##*vs.* V_T22-PEEP3. ΔP,L: transpulmonary driving pressure; Est,rs: respiratory system static elastance; V_T: tidal volume; PEEP: positive end-expiratory pressure; Pplat,rs: respiratory system plateau pressure; RR: respiratory rate; MAP: mean arterial pressure. Dashed lines represent Pplat,rs similar to $\Delta P,L_{MEAN}$ and $\Delta P,L_{HIGH}$ at high V_T (13 ml/kg [Pplat,rs = 14 cmH_2O] and 22 ml/kg [Pplat,rs = 17 cmH_2O]). For this purpose, V_T was kept low (6 ml/kg) and PEEP was adjusted for the level of Pplat,rs. Gas exchange was evaluated at PEEP = 3 cmH_2O and FiO_2 = 1.0 in all groups.

table 3. Semi-quantitative analysis of electron microscopy

	$\Delta P,L_{LOW}$		$\Delta P,L_{MEAN}$			$\Delta P,L_{HIGH}$	
$\Delta P,L$ (cmH_2O)	**7.5**	**8.5**	**10**	**10**	**9.2**	**12**	**12**
V_T (ml/kg)	**6**	**6**	**13**	**6**	**6**	**22**	**6**
PEEP (cmH_2O)	**3**	**5.5**	**3**	**9.5**	**7.5**	**3**	**11**
Pplat,rs (cmH_2O)	**11**	**14**	**14**	**20**	**17**	**17**	**24**
ACM	3.0 (2.0-3.0)	3.0 (2.5-3.0)	3.0 (3.0-4.0)	4.0 (3.5-4.0)	3.0 (3.0-3.5)	2.0 (2.0-2.5)	2.0 (1.0-2.0) §
PI	3.0 (2.5-3.0)	2.0 (2.0-3.0)	2.0 (2.0-2.5)	3.0 (3.0-4.0)	3.0 (3.0-3.0)	2.0 (2.0-2.0)	2.0 (1.0-2.0)**§
PII	3.0 (2.5-3.0)	2.0 (2.0-3.0)	3.0 (2.0-3.0)	3.0 (3.0-3.5)	3.0 (3.0-3.5)	2.0 (1.5-2.0)	2.0 (1.0-2.0) **§
Interstitial edema	3.0 (2.0-3.0)	3.0 (2.0-3.5)	3.0 (2.5-3.5)	4.0 (3.5-4.0)	3.0 (2.0-3.5)	2.0 (1.5-2.0)	1.0 (1.0-1.5) §

Semi-quantitative analysis of lung electron microscopy. Pathologic findings were graded on a five-point, semi-quantitative, severity-based scoring system as follows: 0 = normal lung parenchyma, 1 = changes in 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% of the examined tissue. Values expressed as median (interquartile range) of 5 rats per group. One-way ANOVA on ranks followed by Bonferroni post-hoc test. ** *vs.* V_T6-PEEP7.5; § *vs.* V_T6-PEEP9.5. V_T: tidal volume. PEEP: positive end-expiratory pressures Pplat,rs: respiratory system plateau pressure. ACM: alveolar-capillary membrane. PI and PII: types I and II epithelial cells. Dashed lines represent additional groups in which V_T = 6 ml/kg was applied and PEEP adjusted to similar Pplat,rs achieved when $\Delta P,L_{MEAN}$ and $\Delta P,L_{HIGH}$ were associated with high V_T (13 ml/kg [Pplat,rs = 14 cmH_2O] and 22 ml/kg [Pplat,rs = 17 cmH_2O]).

table 4. Correlation between mechanical, morphometric, and biochemical parameters in all groups

	IL-6	RAGE	Amphiregulin	PCIII	Alveolar hyperinflation (%)	Alveolar collapse (%)
$\Delta P,L$ (cmH_2O)	r = 0.256 p = 0.189	r = -0.281 p = 0.148	r = 0.353 p = 0.065	r = -0.211 p = 0.281	r = 0.731 p < 0.001	r = -0.415 p = 0.028
Pplat,rs (cmH_2O)	r = 0.511 p = 0.005	r = -0.055 p = 0.780	r = 0.654 p< 0.001	r = 0.097 p = 0.621	r = 0.745 p < 0.001	r = -0.767 p < 0.001
PEEP (cmH_2O)	r = 0.583 p = 0.001	r = 0.091 p = 0.643	r = 0.619 p< 0.005	r = 0.036 p = 0.018	r = 0.497 p = 0.007	r = -0.806 p < 0.001
V_T (ml/kg)	r = -0.104 p = 0.600	r = -0.155 p = 0.432	r = -0.057 p = 0.771	r = -0.341 p = 0.075	r = 0.178 p = 0.363	r = 0.278 p = 0.151

Pearson's correlations of transpulmonary driving pressure (ΔP, L), respiratory system plateau pressure (Pplat,rs), positive-end expiratory pressure (PEEP), and tidal volume (V_T) with IL-6, RAGE, amphiregulin, and PCIII mRNA expressions and alveolar hyperinflation and collapse. The r value represents the correlation coefficient, and p, the respective p-value. Statistical significance was accepted at $p < 0.05$.