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Article

# Impaired Glucocorticoid Receptor Signaling Aggravates Lung Injury after Hemorrhagic Shock

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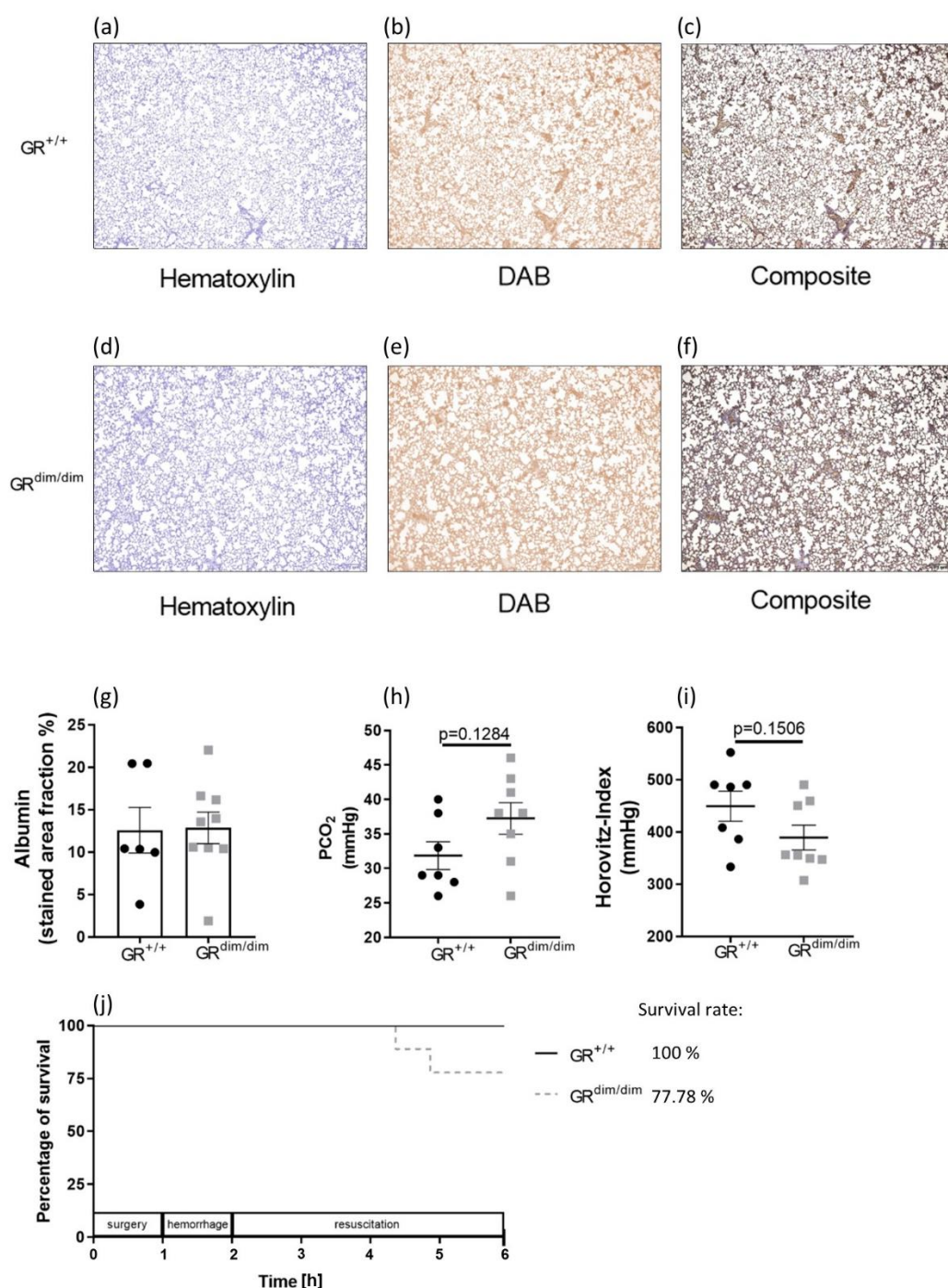
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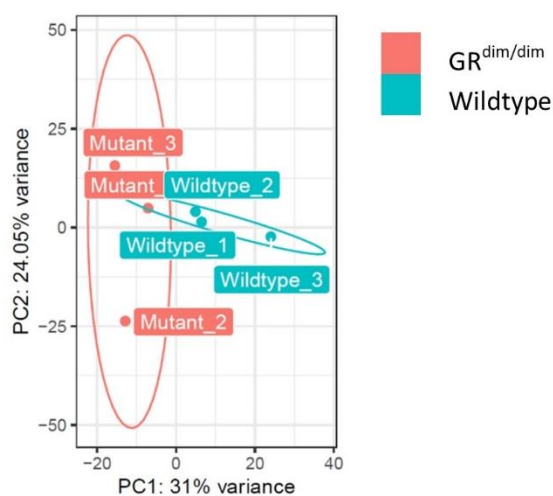
† These authors have contributed equally to this work.

## Supplementaries

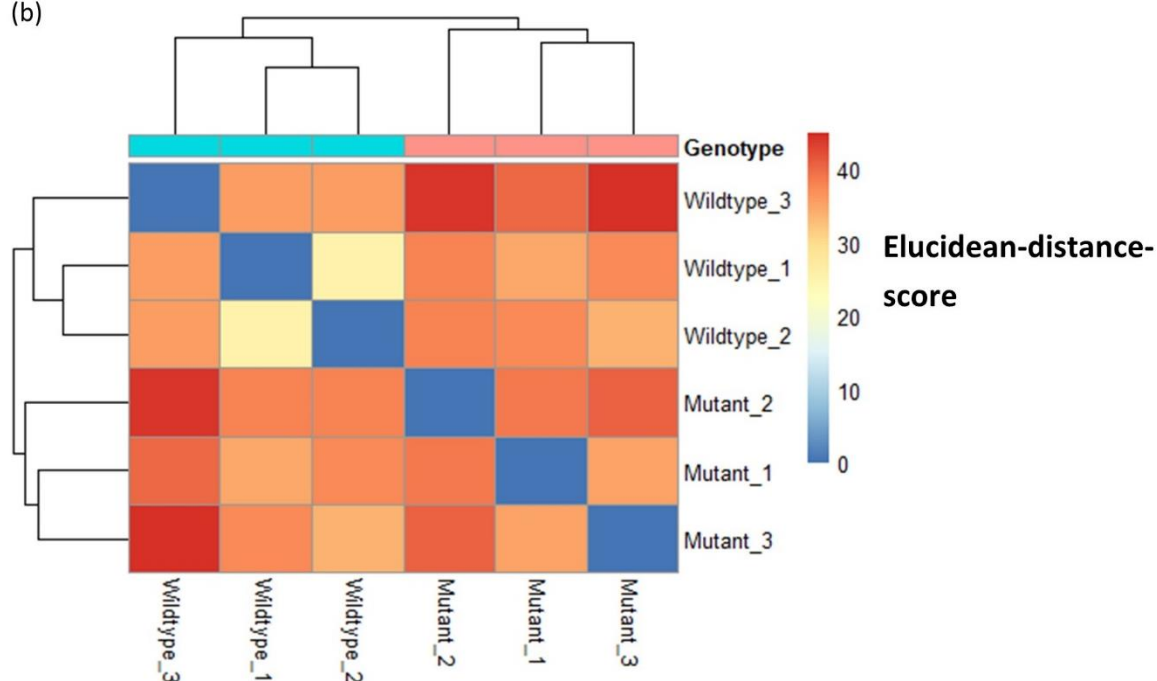


**Figure S1:** Vascular permeability, blood gas analysis and survival of  $GR^{dim/dim}$  mice and  $GR^{+/+}$  mice after HS in MICU. (a) Representative hematoxylin counter-stained lung slice of  $GR^{+/+}$  mouse. (b) DAB signal for albumin detection on lung slice from  $GR^{+/+}$  mouse. (c) Overlay of hematoxylin and DAB staining as composite of  $GR^{+/+}$  mouse. Below, representative pictures of lung slice from a  $GR^{dim/dim}$  mouse with (d) hematoxylin staining, (e) DAB staining and (f) composite. All pictures were taken with 4× magnification. (g) Stained area fraction of albumin was not different between lung slices of  $GR^{dim/dim}$  mice and  $GR^{+/+}$  mice. (h) HS led to trends of enhanced arterial partial carbon dioxide pressure ( $PCO_2$ ) in  $GR^{dim/dim}$  mice compared to  $GR^{+/+}$ . (i)  $GR^{dim/dim}$  mice showed tendencies of a reduced Horovitz-Index (ratio of inhaled oxygen fraction to arterial partial oxygen pressure) after HS compared to  $GR^{+/+}$ . (j) 77.78 % of  $GR^{dim/dim}$  mice survived at the end of the experiment while all  $GR^{+/+}$  mice survived. Significance was analyzed with unpaired Mann-Whitney and indicated according to \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

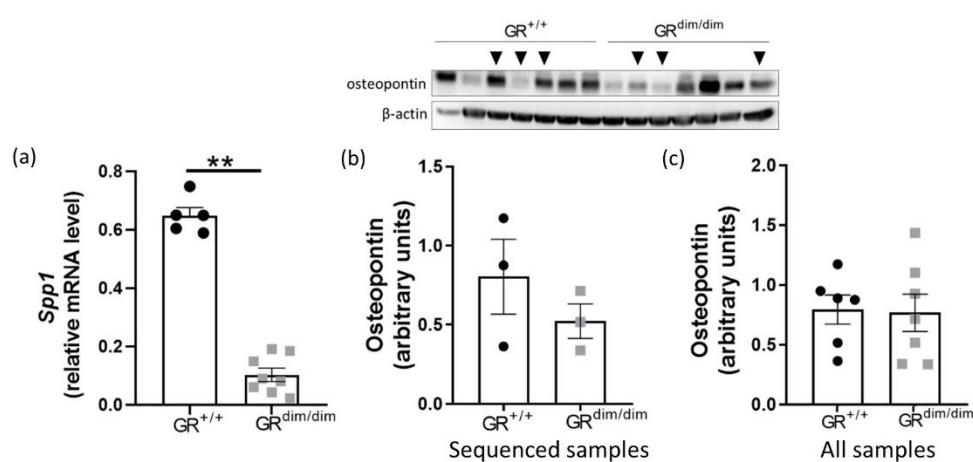
(a)



(b)



**Figure S2:** Quality assessment of RNA-sequencing results. (a) PCA blot showing clustering of the samples according to the genotype for their variance (PCA1 and PCA2). (b) Heatmap of euclidean-distance-clustering in a sample-to-sample comparison indicating least (blue) to most (red) differences in gene expression profile. Dendrograms cluster the samples according to their similarities.



**Figure S3:**  $GR^{dim/dim}$  mice showed reduced RNA but not protein level of osteopontin in the lung after HS. (a) qRT-PCR showed significantly reduced *Spp1* expression in lung samples of  $GR^{dim/dim}$  mice compared to lung samples of  $GR^{+/+}$  mice after HS. (b) Analysis of the lung tissue samples that were analyzed by RNA-sequencing (indicated by arrow heads in the blot) showed trends towards reduced protein level of osteopontin (OPN), the gene product of *Spp1*, in  $GR^{dim/dim}$  lungs compared to  $GR^{+/+}$  after HS. (c) Western blot of lungs samples from all  $GR^{dim/dim}$  and  $GR^{+/+}$  mice after HS showed no genotype dependent differences of OPN. Data points shown in (b) are included in (c). Significance was analyzed with unpaired Mann-Whitney and indicated according to \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S2.** Concentration of cytokines in BAL of GR<sup>+/+</sup> (n=6-7) and GR<sup>dim/dim</sup> (n=8-9) mice after hemorrhagic shock with subsequent resuscitation (4 h) at the end of experiment. \*p<0.05 vs. GR<sup>+/+</sup> calculated with Mann-Whitney t-test. Data is shown as mean (SEM).

Cytokine in BAL [pg/ml]	GR <sup>+/+</sup>	GR <sup>dim/dim</sup>
Interleukine-1 alpha (IL-1 $\alpha$ )	9.7 (0.47)	9.3 (0.70)
Interleukine-2 (IL-2)	7.7 (0.64)	7.5 (0.77)
Interleukine-3 (IL-3)	1.0 (0.11)	1.6 (0.18)
Interleukine-4 (IL-4)	1.8 (0.22)	1.5 (0.079)
Interleukine-5 (IL-5)	6.1 (0.95)	7.7 (0.93)
Interleukine-6 (IL-6)	544 (105)	777 (98)
Interleukine-9 (IL-9)	6.9 (1.0)	9.8 (0.52)
Interleukine-10 (IL-10)	4.0 (0.53)	4.7 (0.68)
Interleukine-12 p40 (IL-12 p40)	16 (1.6)	13 (1.0)
Interleukine-12 p70 (IL-12 p70)	48 (5.0)	54 (3.8)
Interleukine-13 (IL-13)	20 (0.73)	<b>28 (3.0)*</b>
Interleukine-17 (IL-17)	3.6 (0.16)	4.3 (0.29)
Eotaxin	93 (13)	144 (24)
Granulocyte Colony-stimulating Factor (G-CSF)	107 (10)	<b>204 (25)*</b>
Granulocyte-macrophage Colony-stimulating Factor (GM-CSF)	23 (1.4)	30 (2.0)
Interferon gamma (INF- $\gamma$ )	3.1 (0.37)	3.4 (0.24)
Keratinocyte Chemoattractant (KC)	225 (31)	<b>422 (49)*</b>
Monocyte Chemoattractant Protein-1 alpha (MCP-1 $\alpha$ )	12 (1.3)	15 (2.5)
Monocyte Chemoattractant Protein-1 beta (MCP-1 $\beta$ )	23 (5.2)	20 (3.3)
Regulated And Normal T-cell Expressed and Secreted (RANTES)	4.3 (0.44)	5.5 (0.74)
Tumor Necrosis Factor alpha (TNF- $\alpha$ )	17 (1.4)	24 (2.2)