

Supplementary material

Address of correspondence: Barbara Schreier, Julius-Bernstein-Institut für Physiologie, Universität Halle-Wittenberg, Magdeburger Strasse 6, 06112 Halle (Saale), Germany, E-mail: barbara.schreier@medizin.uni-halle.de

Moderate inappropriately high aldosterone/NaCl constellation in mice: cardiovascular effects AND the role of cardiovascular epidermal growth factor receptor

Barbara Schreier¹, Sindy Rabe¹, Sabrina Winter¹, Stefanie Ruhs¹, Sigrid Mildenerberger¹, Bettina Schneider¹, Maria Sibilia², Michael Gotthardt³, Sabine Kempe⁴, Karsten Mäder⁴, Claudia Grossmann¹, Michael Gekle¹

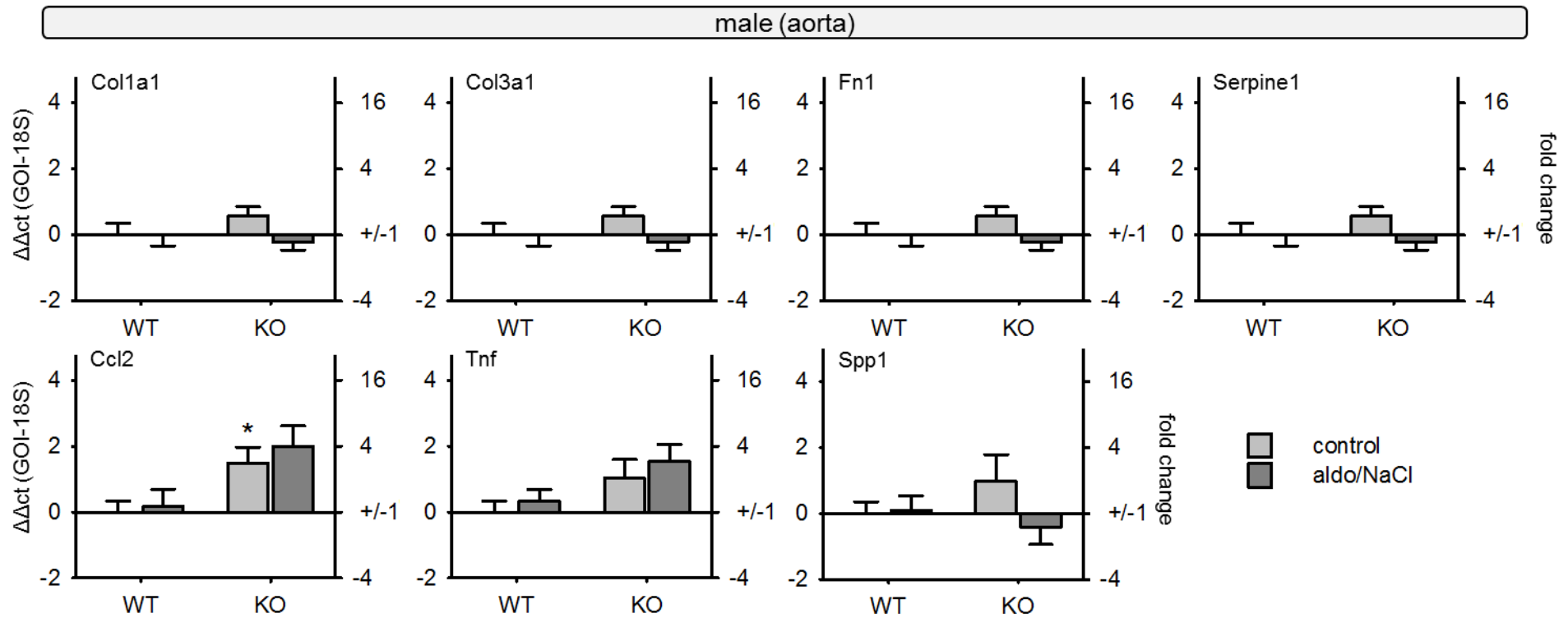
¹ Julius-Bernstein-Institute of Physiology, Medical Faculty, University of Halle-Wittenberg, Halle, Germany,

² Institute of Cancer Research, Medical University of Vienna, Vienna, Austria

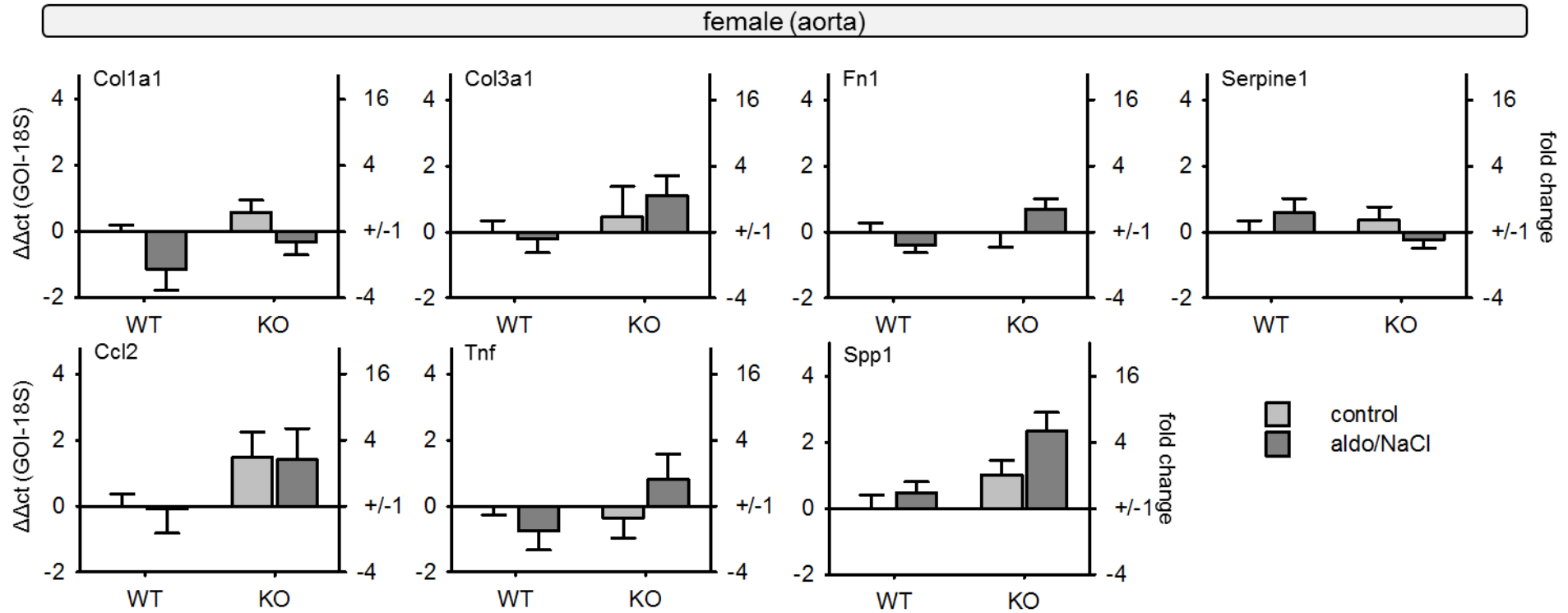
³ Max-Delbrück-Center for Molecular Medicine (MDC), Berlin-Buch, Germany

⁴ Institute of Pharmacy, Faculty of Natural Sciences 1, University of Halle-Wittenberg, Halle, Germany

Supplementary figure 1: Alteration of marker gene expression in aortas of male animals regarding genotype and treatment. After 28 days with or without aldo/NaCl treatment animals were sacrificed, the aortas dissected, freed from adjacent tissue and snap frozen in liquid nitrogen. Gene expression was analyzed according to the $-\Delta/\Delta\text{ct}$ - method normalizing the individual mRNA amount to 18S and the mean mRNA amount in the untreated wildtype. In each graph the $\Delta/\Delta\text{ct}$ amount is given on the left y-axis while the corresponding fold change in expression is given on the right y-axis. Data are given as mean \pm SEM, * $p < 0.05$ compared to respective wildtype, # $p < 0.05$ compared to respective control. N=6-11 animals/group.



Supplementary Figure 2: Alteration of marker gene expression in aortas of female animals regarding genotype and treatment. After 28 days with or without aldo/NaCl treatment animals were sacrificed, the aortas dissected, freed from adjacent tissue and snap frozen in liquid nitrogen. Gene expression was analyzed according to the $-\Delta/\Delta\text{ct}$ - method normalizing the individual mRNA amount to 18S and the mean mRNA amount in the untreated wildtype. In each graph the $\Delta/\Delta\text{ct}$ amount is given on the left y-axis while the corresponding fold change in expression is given on the right y-axis. Data are given as mean \pm SEM, * $p < 0.05$ compared to respective wildtype, # $p < 0.05$ compared to respective control. N=6-12 animals/group.



Supplementary Table 1: Primers used for qPCR.

Gen	Protein	Ref. Seq.	Sense Primer	Antisense Primer	Annealing Temp [°C]	Product [bp]
18 S		NR_003278.3	5'-GTTGGTGGAGCCGATTTGTCTGG	5'-AGGGCAGGGACTTAATCAACGC	64	346
Nppa	atrial natriuretic peptide	NM_008725.2	5'-GTGCGGTGCCAACACAGAT	5'-GCTTCCTCAGTCTGCTCACTCA	64	123
Nppb	b-type natriurtic peptide	NM_001287348.1	5'-CCAGTCTCCAGAGCAATTCAA	5'-AGCTGTCTCTGGGCCATTTTC	64	81
Myh6	alpha-myosin heavy chain	NM_001164171	5'-GCTGACAGATCGGGAGAATCAG	5'-CCCCTATGGCTGCAATGC	64	121
Myh7b	beta-myosin heavy chain	NM_001085378.2	5'-ACTCAAGCGGGAGAACAAGA	5'-ACCTGGGACAACCTCCAACCTG	64	213
Col1a1	collagen, type I, alpha 1	NM_007742	5'-ACATGTTTCAGCTTTGTGGACC	5'-TAGGCCATTGTGTATGCAGC	57	110
Col3a1	collagen, type III, alpha 1	NM_009930	5'-TGGTAGAAAGGACACAGAGGC	5'-TCCAACCTTACCCTTAGCACC	64	283
Fn1	fibronectin-1	NM_010233	5'-TTAAGCTCACATGCCAGTGC	5'-TCGTCATAGCACGTTGCTTC	57	208
Serpine1	plasminogen activator inhibitor-1	NM_008871	5'-GACACCCTCAGCATGTTTCATC	5'-AGGTTGCACTAAACATGTCAG	64	218
Tnf	tumor necrosis factor α	NM_013693	5'-CACACTCAGATCATCTTCTCAAAA	5'-GTAGACAAGGTACAACCCATCG	63	181
Tgfb1	transforming growth factor β	NM_011577	5'-TTTGGAGCCTGGACACACAG	5'-TGGACAACCTGCTCCACCTTG	63	156
Ccl2	monocyte chemoattractant protein-1	NM_011333	5'-AGGTCCCTGTCATGCTTCTG	5'-TCTGGACCCATTCTTCTTG	62	249
Spp1	secreted phosphoprotein 1	NM_009263	5'-ATTTGCTTTTGCCTGTTTGG	5'-TGGCTATAGGATCTGGGTGC	57	106
Nos1	nitric oxide synthase 1 (neuronal)	NM_008712	5'-AAGCCTATGCCAAGACCCTGTG	5'-GGGTGCCTCATCTCCATCAAAG	60	193
Nox4	NADPH oxidase 4	NM_015760	5'-CCTTGAAGTGAATGCAGCAA	5'-ACCACCTGAAACATGCAACA	57	220
Cybb	NADPH oxidase 2	NM_007807	5'-ACTGCGGAGAGTTTGAAGA	5'-GGTGATGACCACCTTTTGTCT	63	201
Cyba	NADPH oxidase subunit p22(phox)	NM_007806	5'-TCATGGGGCAGATCGAGT	5'-CCTGCAGCGATAGAGTAGGC	57	139