

Supplemental material

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Table S1: Blood biochemistry of 18-month-old WT and KO mouse. * $p < 0.05$, ** $p < 0.01$, mean \pm SD, two-tailed T-test. “CaSR” indicates concentration of N-terminal soluble CaSR / CaSR-fragment. Osmolality and CaSR were measured in male and female mice.

Parameter	Unit	WT	N	KO	N	<i>p</i> -value
Na ⁺	mmol / l	149.0 \pm 3.61	3	146.3 \pm 3.06	3	0.3837
K ⁺	mmol/l	10.64 \pm 0.61	3	11.88 \pm 2.53	3	0.4541
Cl ⁻	mmol / l	114.33 \pm 1.15	3	111.00 \pm 3.61	3	0.2020
Ca ²⁺	mmol / l	2.34 \pm 0.13	3	2.99 \pm 0.35	3	0.0401 *
Mg ²⁺	mmol / l	1.65 \pm 0.18	3	2.04 \pm 0.33	3	0.1369
FGF23	pg / ml	131.1 \pm 45.5	3	330.0 \pm 49.7	3	0.0069 **
α -Klotho	pg / ml	2005.9 \pm 1342.1	4	1873.0 \pm 1016.5	4	0.8797
Osmolality	mosmol/kg	289.2 \pm 11.7	5	288.1 \pm 5.6	7	0.8369
CaSR	ng / ml	2.17 \pm 0.98	5	2.03 \pm 0.78	7	0.7816

Table S2: Organ weights of 6 and 18-month-old animals. WT vs. KO, mean \pm SD, two-tailed T-test.

6-month-old animals						
Organ	Unit	WT	N	KO	N	p-value
Kidney	mg	235.0 \pm 32.3	7	232.9 \pm 23.8	7	0.8899
Liver	mg	1332.2 \pm 134.2	8	1445.6 \pm 190.9	7	0.2016
Stomach	mg	339.4 \pm 86.8	8	444.6 \pm 256.8	7	0.2938
Spleen	mg	88.1 \pm 31.3	8	99.1 \pm 34.3	6	0.5467
Heart	mg	148.8 \pm 31.0	8	145.4 \pm 17.7	6	0.8144
18-month-old animals						
Kidney	mg	313.1 \pm 37.6	6	313.0 \pm 12.9	4	0.9950
Liver	mg	2294.1 \pm 461.2	6	2399.8 \pm 107.9	4	0.6703
Stomach	mg	846.8 \pm 372.0	6	738.8 \pm 247.6	4	0.6268
Spleen	mg	129.1 \pm 52.6	6	101.6 \pm 24.8	4	0.3637
Heart	mg	201.8 \pm 20.4	5	231.9 \pm 8.4	4	0.0285 *

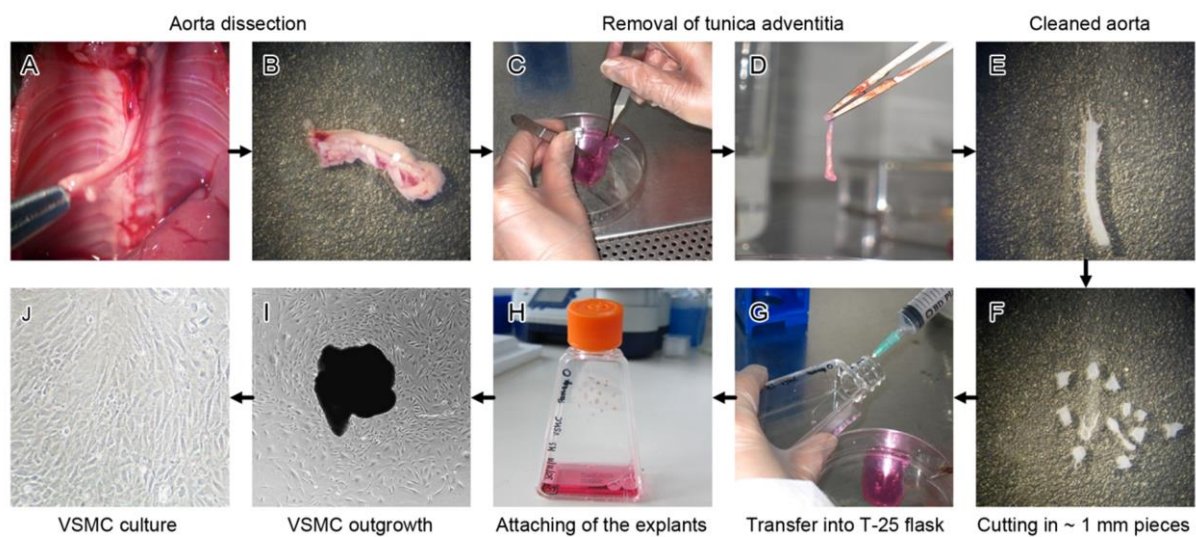


Figure S1: Generation of explant derived aortic VSMC. **A:** The thoracic aorta is dissected from the spine and **B:** removed to a Petri dish filled with sterile isolation medium where **C and D:** the vessel is cleared from tunica adventitia by gently pulling / scraping the connective tissue until **E:** only the semi-translucent tunica media remains. **F:** The vessel is then cut into small (ca. 1 mm) pieces that are then **G:** transferred into a T-25 cell culture flask by the use of a hypodermic needle. **H:** The flask is kept in an upright position at 37 °C for 10–15 minutes so that the explants are not in contact with medium and can attach firmly to the surface of the flask. 5 ml isolation medium is added, and the explants are kept at 37 °C / 95 % relative humidity (rh) / 5 % CO₂ for ca. 7 days after which the medium is changed. **I:** VSMC will start to grow out of the explants. **J:** After ca. 2-3 weeks, the explants are removed and the VSMC passaged to generate a monolayer of cells.

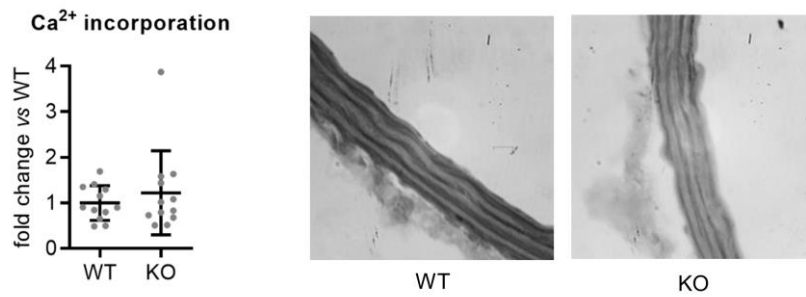


Figure S2: *Ex vivo* aortic calcification. Graph: Quantification of Ca²⁺ deposition in WT and KO aortas of 3-month-old mice. Mean±SD. Pictures: Alizarin Red S stainings of thoracic aorta sections from 12-month-old WT and KO animals incubated for 10 days in the presence of medium containing 1.8 mM Ca²⁺ and 3 mM Pi.

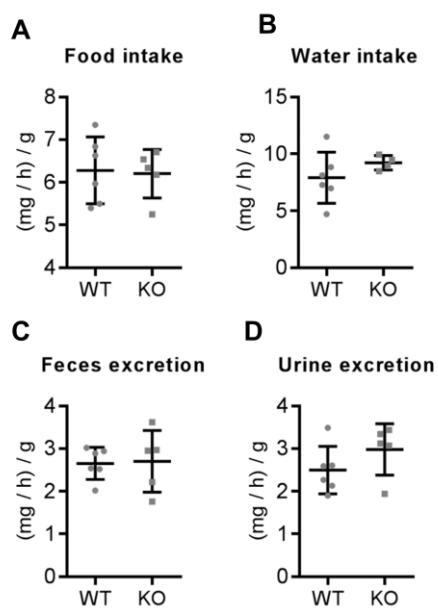


Figure S3: Metabolic cage studies of WT and KO mice. **A:** Food intake, **B:** Water intake, **C:** Feces excretion, **D:** Urine excretion. Data are shown as consumption (in mg) per h per g bodyweight. Mean±SD.

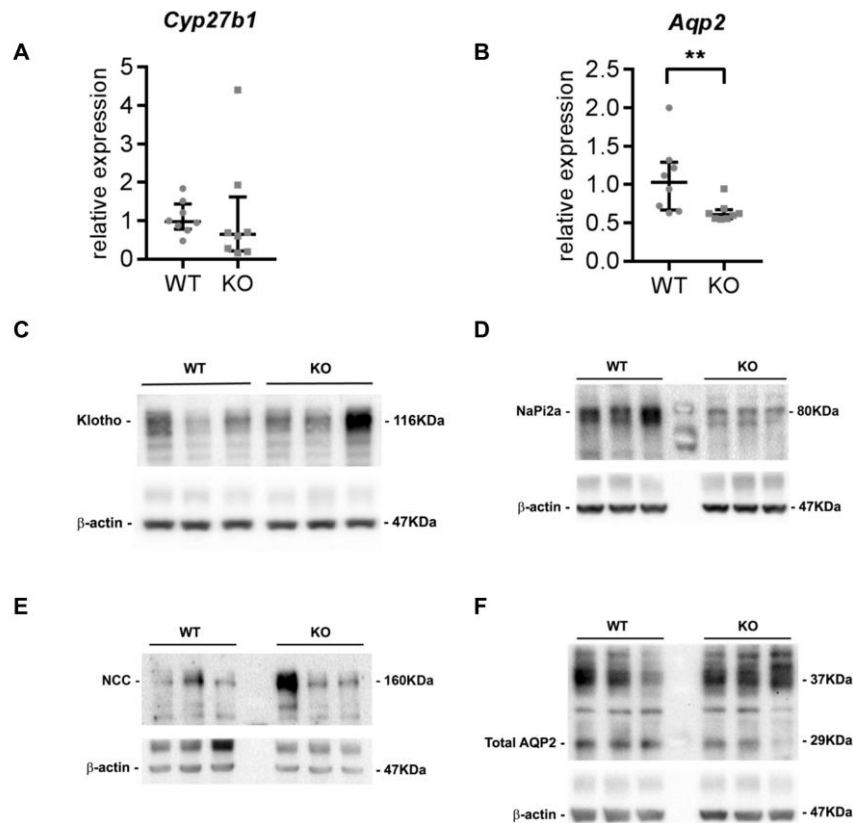


Figure S4: Supplemental kidney mRNA and protein expression. **A:** *Cyp27b1*, and **B:** *Aqp2* mRNA expression levels relative to calibrator (mean Δ CT WT). Representative Western blots showing **C:** Klotho, **D:** NaPi2a, **E:** NCC, **F:** AQP2 expression in kidneys from WT and KO mice.

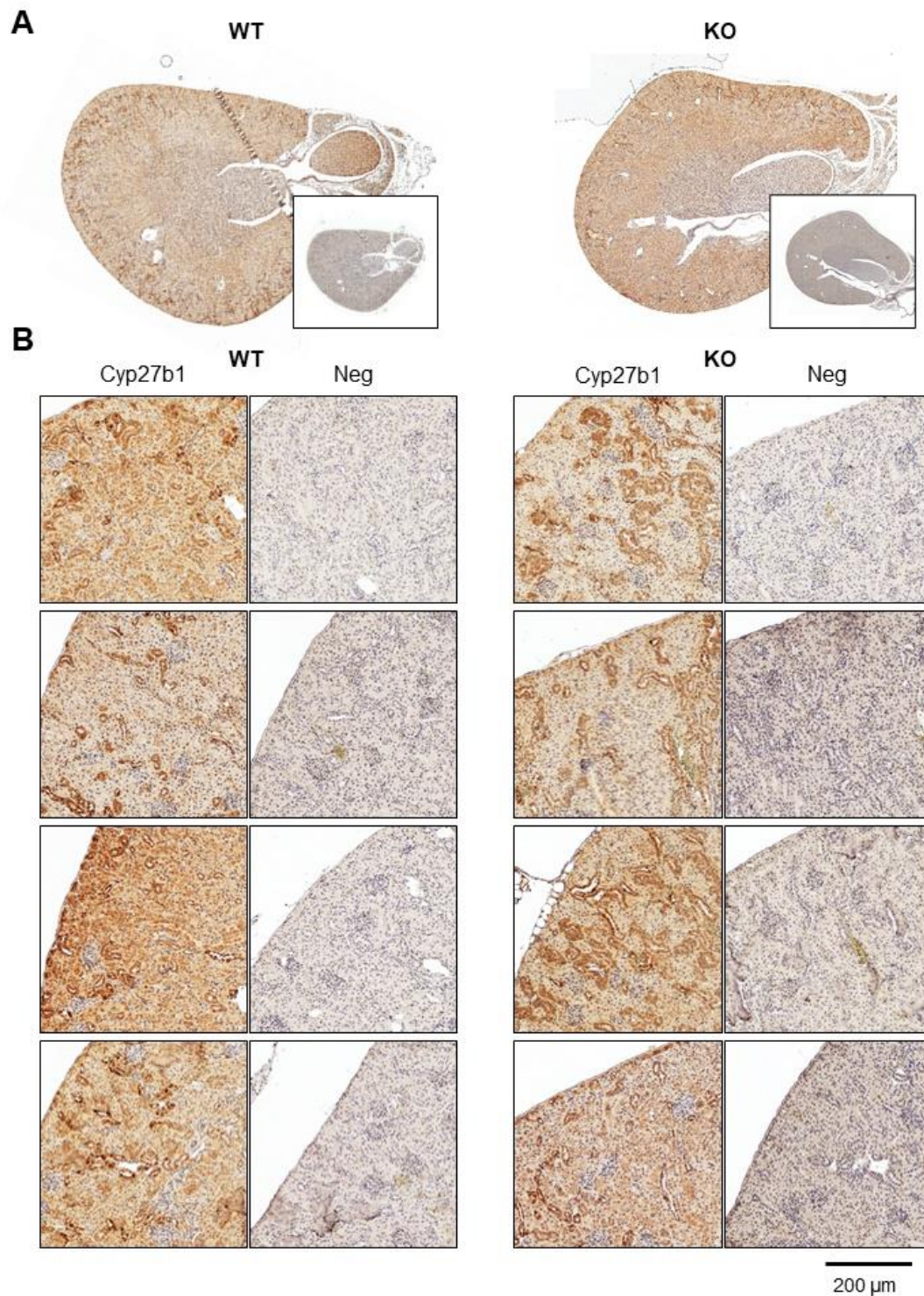


Figure S5: Immunohistochemistry stainings of Cyp27b1 in kidneys of WT and KO mice. Stainings were performed as described in the methods section for Cyp27b1 using the LSBio (Seattle, USA) rabbit anti-Cyp27b1 antibody at 1:1000 dilution. **A:** Overview of representative whole kidney sections stained for Cyp27b1. Insert: negative control. **B:** Cortex of N=4 WT and KO kidneys stained for Cyp27b1 and respective negative controls.

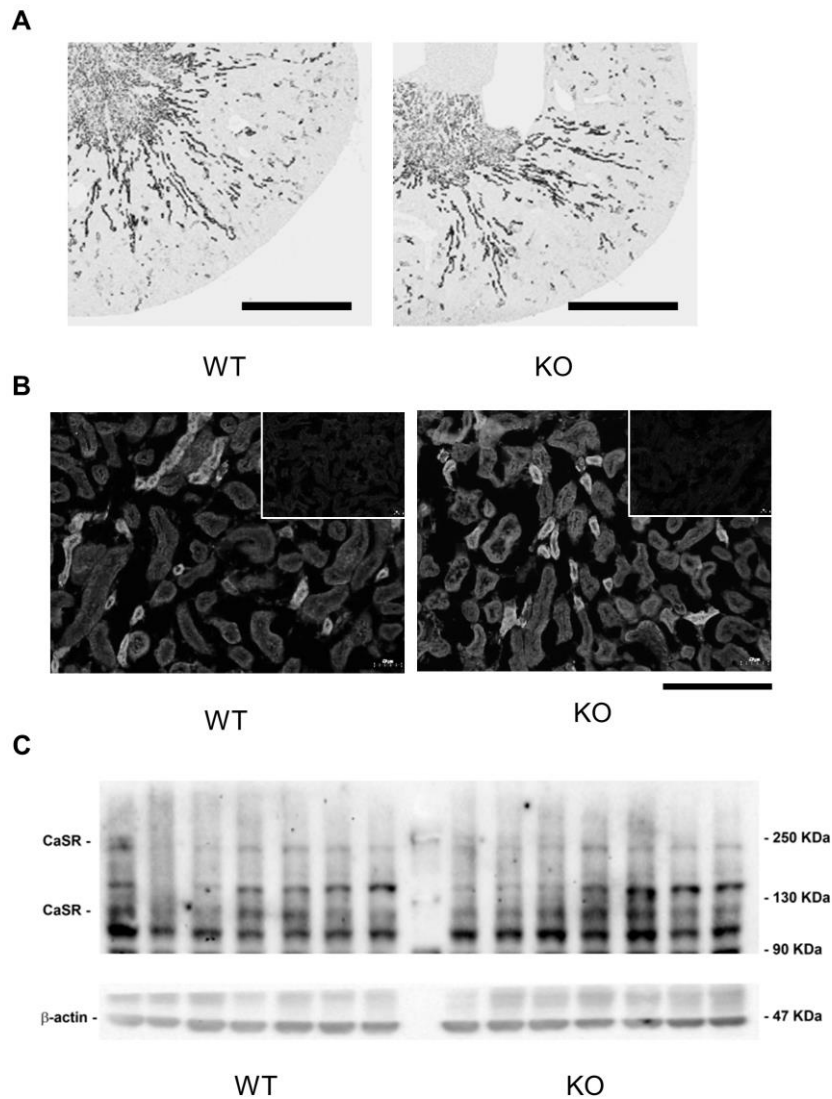


Figure S6: Supplemental kidney CaSR expression data and representative images from Figure 3. **A:** immunohistochemistry of CaSR expression pattern in WT and KO kidney sections. Scale bar = 1 mm. **B:** immunofluorescence analysis of CaSR expression levels in WT and KO kidneys used for quantitative immunofluorescence analysis. Scale bar = 200 μ m. **C:** representative Western blot for CaSR in the kidney (~120-150 kDa: monomer; 250 kDa: dimer).

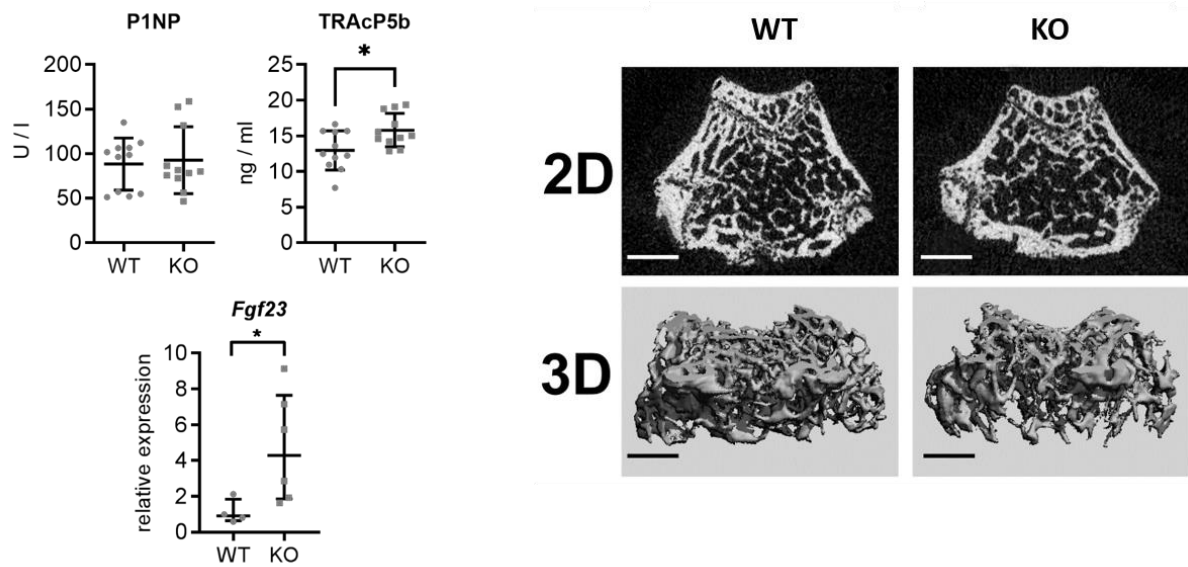


Figure S7: Plasma levels of bone metabolism markers procollagen type 1 (P1NP) and Tartrate-resistant acid phosphatase 5b (TRAcP5b), Fgf23 mRNA expression in bone, and μ CT. Bone metabolism markers: * $p < 0.05$, two tailed T-test; measured in male and female mice. RT-qPCR: * $p < 0.05$, Mann-Whitney test. μ CT: representative 2-dimension (2D) radiographs and 3-dimension (3D) reconstructed images from distal femurs of 3 months old KO and WT (control) littermates. The 2D radiographs were taken 100 μ m below the growth plate. Scale bar: 400 μ m

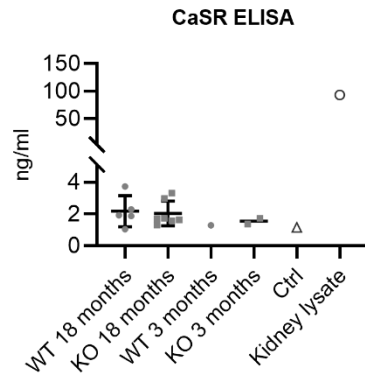


Figure S8: Serum levels of “soluble” CaSR / CaSR fragment. N = 5 (WT, 18 months), N = 7 (KO, 18 months), N = 3 (WT, 3 months), and N = 3 (KO, 3 months). Three of the 3-month samples, (2 WT, 1 KO) were below the detection range and are thus not included in the graph. An additional serum sample of a genetically non-modified 14 month-old mouse (“Ctrl”) was added for reference, which had a comparable level of CaSR in the serum. Finally, a sample of 100 mg / ml kidney lysate from a genetically non-modified mouse was tested as positive control.