SUPPORTING INFORMATION MATERIALS

Measurement of endogenous angiotensin metabolites and ACE2 activity in mouse lung tissue

Mice were perfused with ice-cold PBS, lungs were immediately removed, snap-frozen in liquid nitrogen and stored at -80 °C. Frozen lung segments (30 - 90 mg) were homogenized under liquid nitrogen. The frozen tissue powder was dissolved at 100 mg/ml in 6 mol/L aqueous guanidinium chloride supplemented with 1% (v/v) trifluoroacetic acid (both from Sigma-Aldrich, Munich, Germany) by cooled sonication using a 2 mm microtip (Sonics and Materials, Newton, NJ). Stable isotope-labeled internal standards for individual angiotensin metabolites were added to tissue homogenates at 200 pg/mL. The samples then underwent C-18-based solid-phase-extraction and were subjected to LC-MS/MS analysis using a reversed-phase analytical column operating in line with a Xevo TQ-S triple quadrupole mass spectrometer (Waters). Internal standards were used to correct for peptide and steroid recovery of the sample preparation procedure for each analyte in each individual sample. Analyte concentrations are reported in [fmol/g] and are calculated considering the corresponding response factors determined in appropriate calibration curves, on condition that integrated signals exceeded a signal-to-noise ratio of 10.

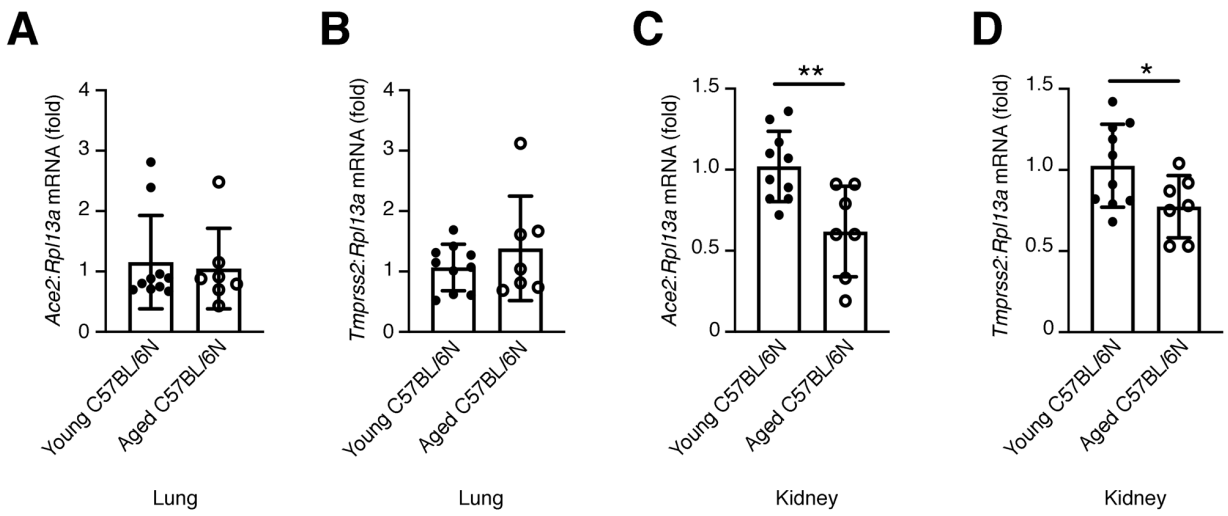
Measurement of ACE2 activity in mouse lung tissue

Frozen lung segments were homogenized in PBS using low-energy sonication. Angiotensin formation rates were determined in protein-normalized tissue homogenates after spiking the samples with Ang II (100 ng/µg protein) ex vivo followed by incubation at 37 °C in the presence

or absence of the ACE2 inhibitor MLN-4760 at 10 µmol/L (Merck-Millipore, Darmstadt, Germany). An aminopeptidase inhibitor cocktail mixture (bestatin, amastatin and apstatin; each 10 µmol/L), Z-pro-prolinal (20 µmol/L) and lisinopril (10 µmol/L (all three from Sigma-Aldrich) were added to all samples for substrate (Ang II) and product (Ang 1-7) stabilization. Following incubation, samples were stabilized, spiked with stable isotope-labelled internal standards for angiotensin metabolites (Ang II, Ang 1-7) and assayed as described previously for endogenous angiotensin quantification (see above). Specific activity of ACE2 was calculated by determining the inhibitor sensitive fraction (solvent minus inhibitor) of Ang 1-7 formation and is given in [(pmol/liter / µg protein)/h].

SUPPORTING INFORMATION FIGURE LEGENDS

Supporting Information Figure 1. *Ace2* and *Tmprss2* mRNA levels are reduced in the kidneys of aged male C57BL/6N mice (44 weeks of age, n = 7), in comparison to young male C57BL/6N mice (9 weeks, n = 10) of age. qRT-PCR for lung *Ace2* (A) and *Tmprss2* (B) and kidney *Ace2* (C) and *Tmprss2* (D). Values are mean ± S.D.. **P* < 0.05, ***P* < 0.01 by two-tailed Student's *t* test.



Supporting Information Figure 1.