Supplemental Figures

Figure I. **Renin inhibition did not alter renal angiotensinogen, ACE, ACE2, AT1a and AT2 receptor mRNA.** Examples show mRNA abundances of angiotensinogen (AGT), angiotensin-converting enzyme (ACE), ACE2, AT1a receptors, AT2 receptors, and β-actin detected by RT-PCR and gel electrophoresis. The lanes were run on the same gel but were noncontiguous.

Figure II. **Renin inhibition reduced atherosclerotic lesion development was not solely attributable to systolic blood pressure.** Linear regression analyses were performed to assess the relationship between lesion size and systolic blood pressure while controlling for aliskiren dosage. (**A**) While 74% of the variation in (log-transformed) aortic arch lesion size is accounted for by the combination of aliskiren dosage and systolic blood pressure, the contribution of the latter is insignificant ($P = 0.32$); (**B**) While 57% of the variation in (log-transformed) aortic root lesion size is accounted for by the combination of aliskiren dosage and systolic blood pressure, the contribution of the latter is insignificant ($P = 0.22$).

Figure III. **Cultured macrophages expressed all the components of the classic angiotensin peptide synthesis pathway, and renin deficiency in macrophages did not alter cathepsin D mRNA abundance.** (**A**) Angiotensinogen, renin, and ACE mRNAs were expressed in cultured macrophages. Examples of positive controls (liver, kidney, and lung) and macrophages for angiotensinogen (AGT), renin, and ACE are shown. To demonstrate the specificity of renin signal, kidney and macrophages from

renin wild type (renin +/+, left lane) and renin deficient mice (renin -/-, right lane) are included. The lanes were run on the same gel but were noncontiguous. (**B**) Cultured macrophages secreted angiotensinogen. Western blotting analysis of mouse plasma revealed two specific bands of 52 and 64 kDa. In contrast, media from cultured macrophages contained a single protein of 64 kDa. (**C**) Renin deficiency did not change the mRNA abundance of cathepsin D in macrophages. Cathepsin D mRNA abundance in mouse macrophages (cathepsin D/β-actin ratio) was quantified by RT-PCR (*n* = 5/group). Data are mean ± SEM. Examples show mRNA band by gel electrophoresis.

Figure IV. **Renin deficiency did not result in overt changes in accumulation of modified lipoproteins.** Cultured peritoneal macrophages from renin +/+ or -/- mice were incubated in serum-free DMEM in the presence of acetylated LDL (20 μg/ml) for 24 hours. Neutral lipids stained with Oil Red O (**A**), or cellular cholesterol was extracted and measured by GC-MS (**B**). Data are mean ± SEM (*n* = 4/group). Original magnification (**A**) = 400x.

Figure V. **AngII did not induce VCAM-1 in endothelial cells.** HUVECs were incubated in the presence of vehicle, TNFα 25 ng/ml, or AngII 10^{-6} M for 18 hours and Western blotting of VCAM-1 and β-actin were then performed. Western blotting analysis revealed a specific band of 100 kD for VCAM-1 only in HUVECs in the presence of TNFα.

Figure I

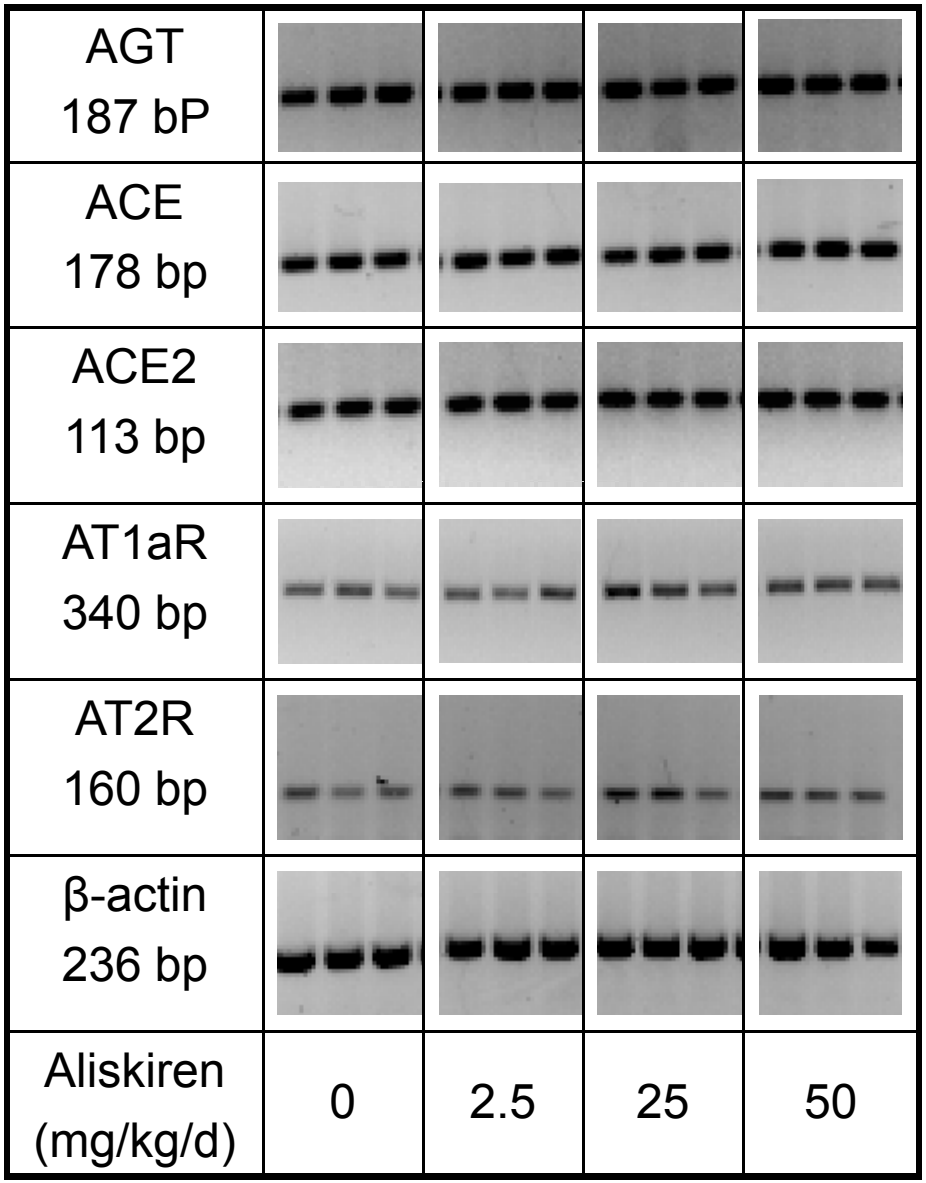

AGT
187 bP
ACE
178 bp
ACE2
113 bp
AT1aR
340 bp
AT2R
160 bp
β-actin
236 bp
Aliskiren
(mg/kg/d)
0
2.5
25
50

Figure II

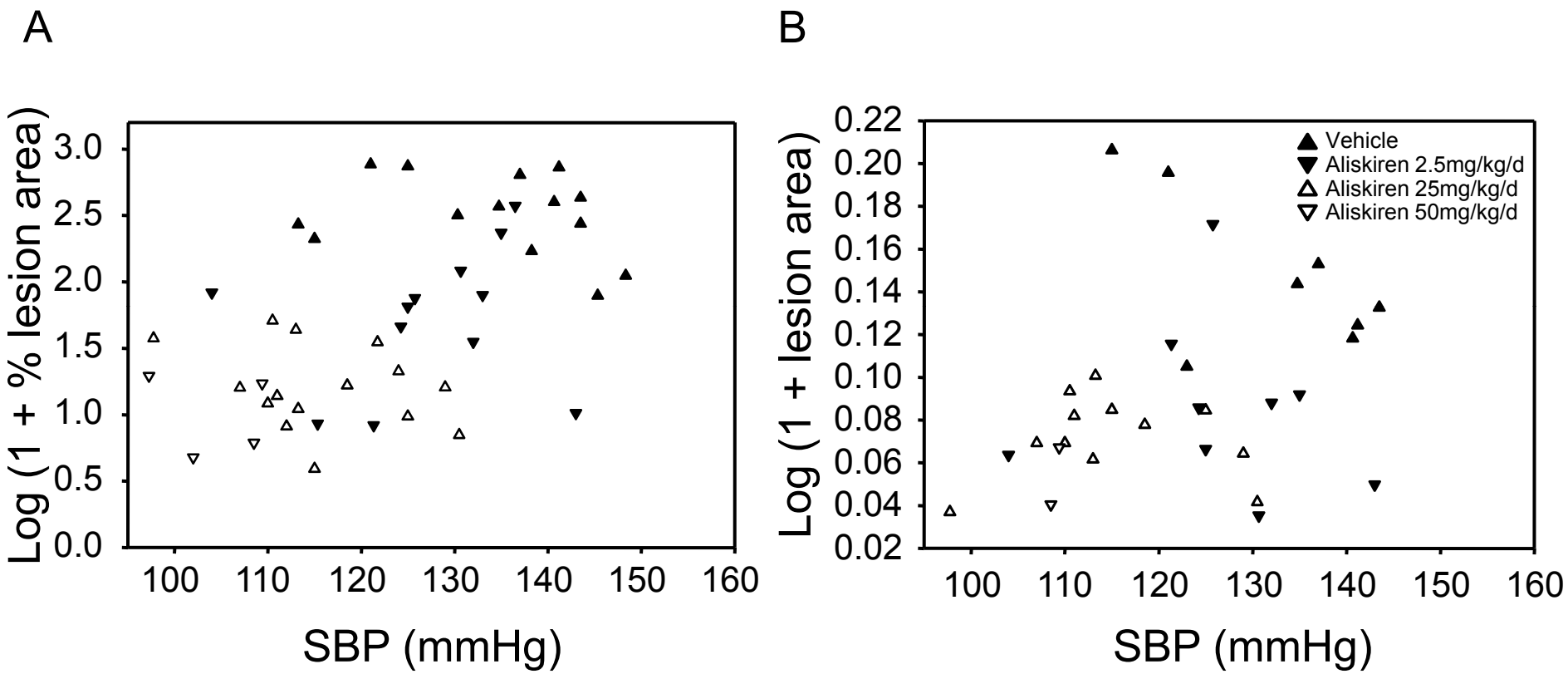
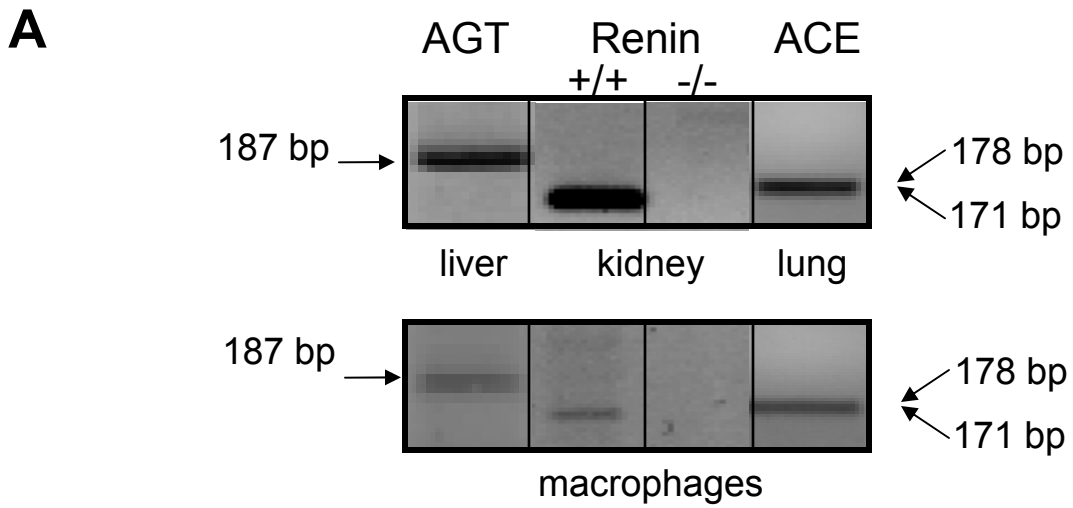
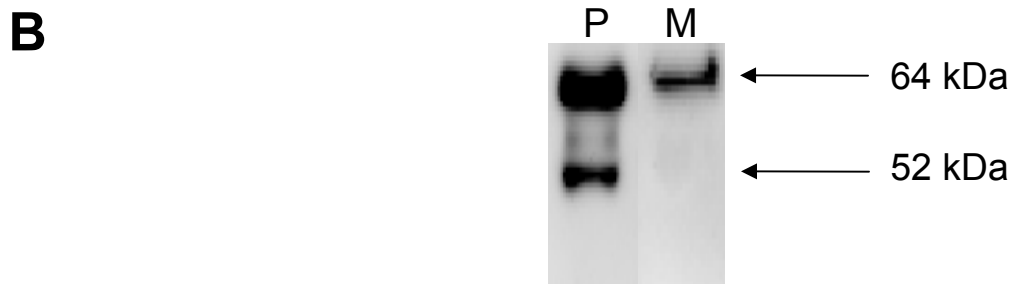
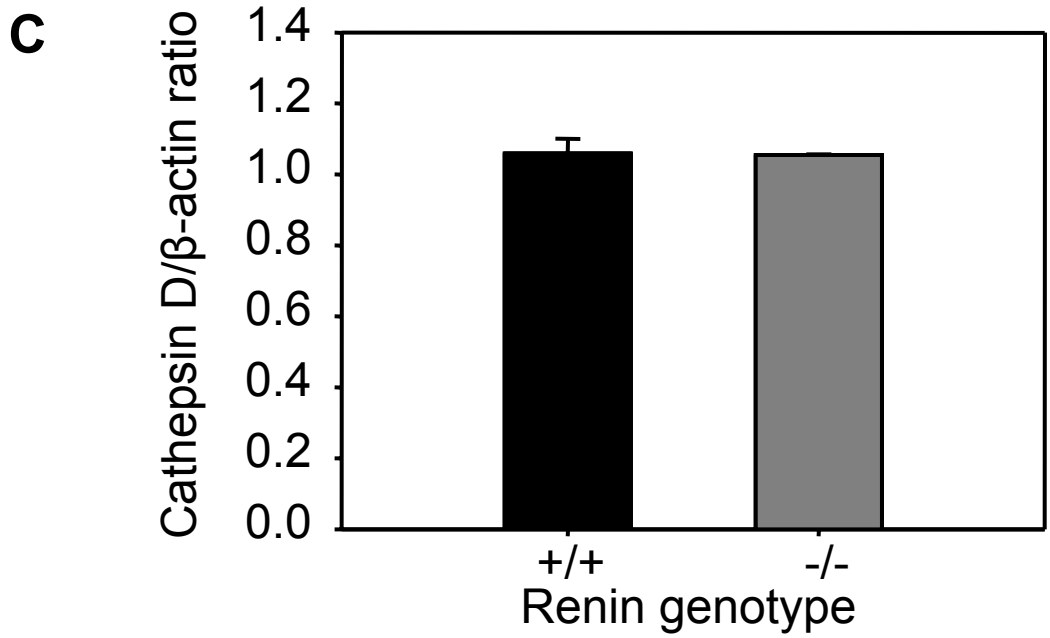

A
B
Log (1 + % lesion area)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
110
120
130
140
150
160
SBP (mmHg)
Log (1 + lesion area)
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
Vehicle
Aliskiren 2.5mg/kg/d
Aliskiren 25mg/kg/d
Aliskiren 50mg/kg/d

Figure III



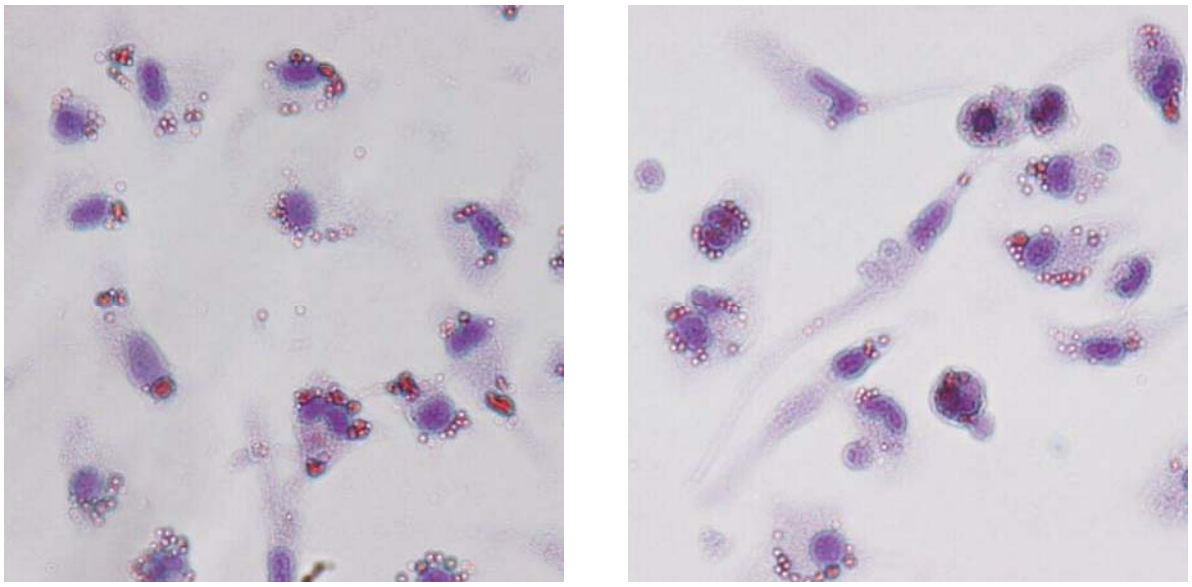




Cathepsin D 199 bp		
Renin Genotype	+/+	-/-

Figure IV

A


+/+
-/-
Renin genotype

B

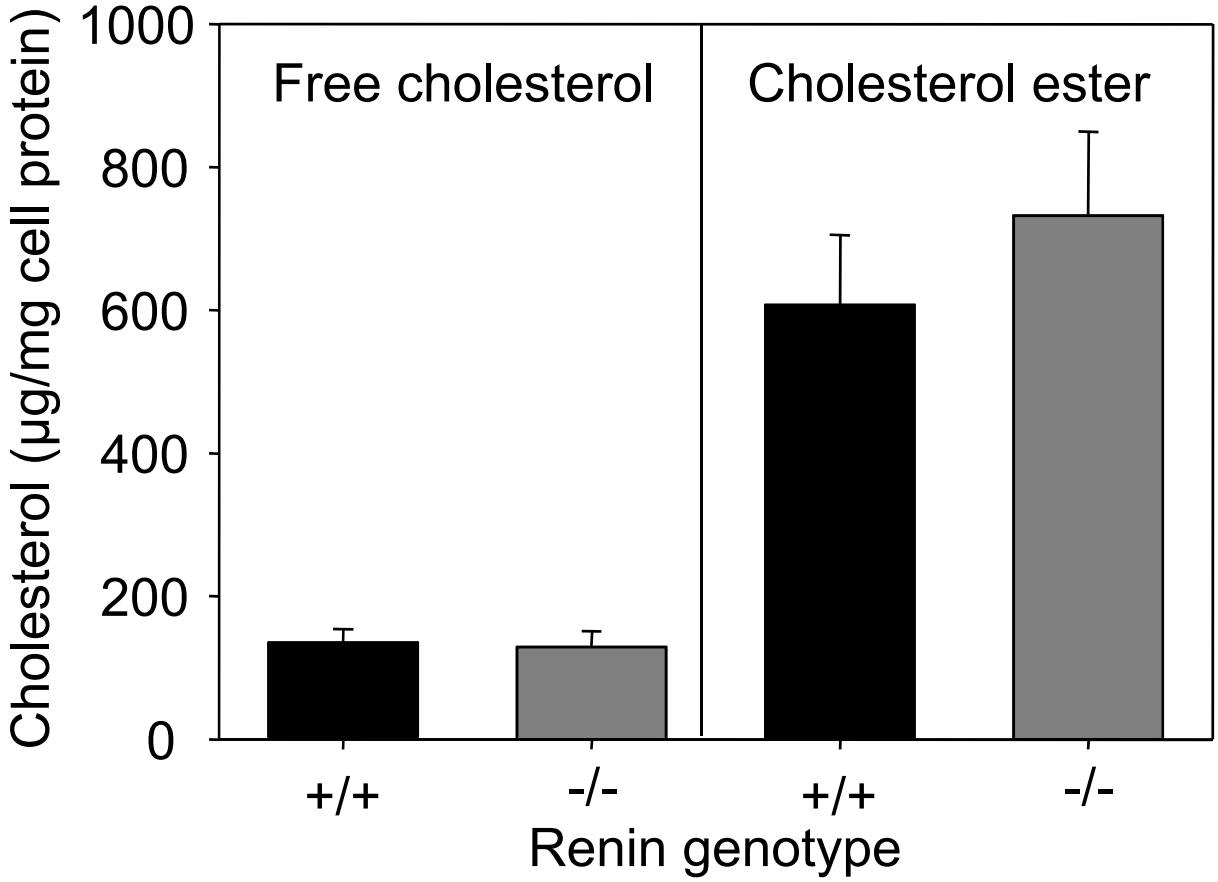

Cholesterol (µg/mg cell protein)
1000
800
600
400
200
0
Free cholesterol
Cholesterol ester
+/+
-/-
+/+
-/-
Renin genotype

Figure V