Supplemental Materials

Supplemental Figure Legends

Figure I. Novel multiplex PCR assay for genotyping CRISPR/Cas9 genome edited mice. (A) The same sequence shown in Figure 1 has the crRNA sequence (underlined), the CArG box (green), and the PAM sequence (blue box) indicated here. Sequence above represents the forward wildtype primer containing a 31 nucleotide extender sequence to vector DNA (F-wt), which bears little complementarity to mouse genomic DNA. Sequence below represents the forward mutant primer (F-mut) and the reverse primer used is shown at far right. (B) An agarose gel is shown that validates the PCR conditions (right) allowing for unambiguous discrimination between the wildtype allele (wt *Cnn1*) and the mutant allele (mut *Cnn1*) using diluted plasmids described above either alone or in a 1:1 ratio (wt + mut *Cnn1*). Note the doublet (asterisk) which reflects the size differential obtained with the two forward primers. This multiplex PCR assay is used for genotyping pups derived from interbreeding mutant CArG box mice.

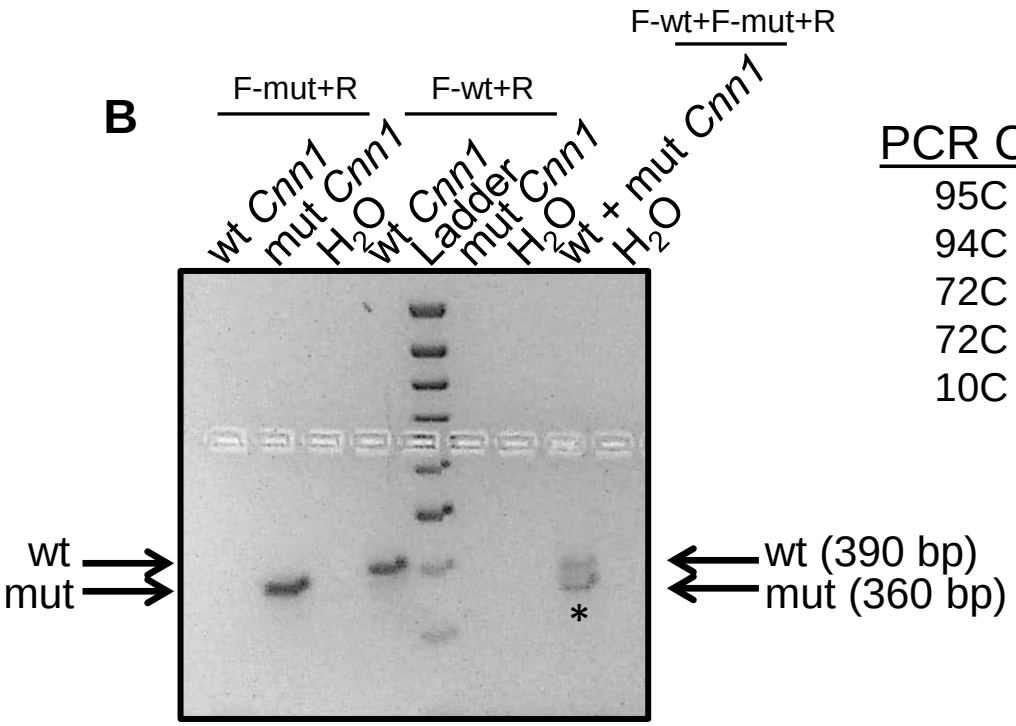
Figure II. Expression of *Cnn1* mRNA in biallelic targeted founder mouse. At left is an original genotype of the 3 targeted founder pups plus one wildtype pup using our multiplex PCR assay as described in Supplemental Figure I. The red arrow indicates the pup with both alleles carrying the CArG box mutation (note absence of upper wildtype band in multiplex PCR as well as PCR product using F-wt primer in bottom gel). At right is a quantitative RT-PCR experiment

showing a pronounced decrease in *Cnn1* mRNA in both aorta and bladder of this founder mouse as compared to a wildtype mouse aorta and bladder. The asterisks indicate statistically significant differences between the homozygous mutant and wildtype mouse tissues ($p<0.05$).

Figure III. Smooth muscle cell contractile gene/protein expression in CArG mutant mice. Quantitative RT-PCR (A) and Western blotting (B) of the indicated markers in aortic tissue showing virtually no *Cnn1* mRNA or CNN1 protein expression in the homozygous *Cnn1*$^{\Delta CArG/\Delta CarG}$ mutant mouse as compared to other markers of the smooth muscle cell lineage that are strictly dependent upon the CArG-SRF nucleoprotein complex. The differences are consistent with data in Figures 1-2 and represent results from an independent founder line.

Figure I

B



PCR Conditions

95C 3 mins x 1 cycle
94C 30 sec
72C 30 sec
x 30 cycles
72C 5 min x 1 cycle
10C Hold

A

ctgcaggaattcgatatcaagcttatcgata

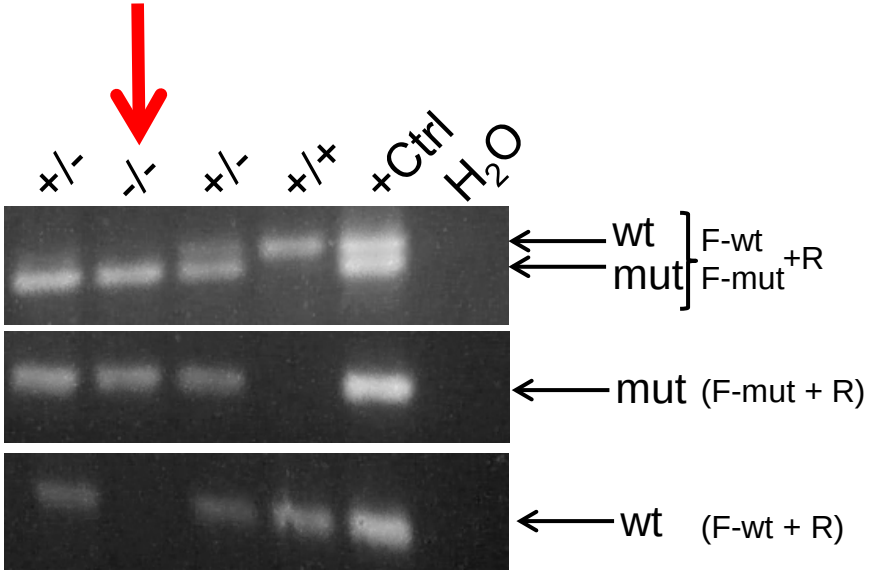
Forw-wt primer + ext (F-wt)

```
   gtcgcagaggagccgccgcgcct
agagtcgcagaggagccgccgcgccttataaggcggccttgggcag..ggaagatgggataagagctgaagcta
   gtcgcagaggagccgccgtcgac                        ccttctaccctattctcgacttcgat
```

Forw-mut primer (F-mut)

Rev-common primer (R)

Figure II



Founder mice

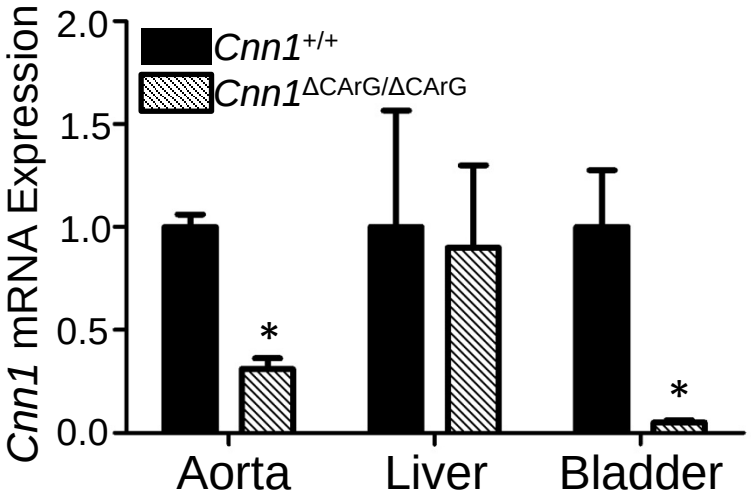
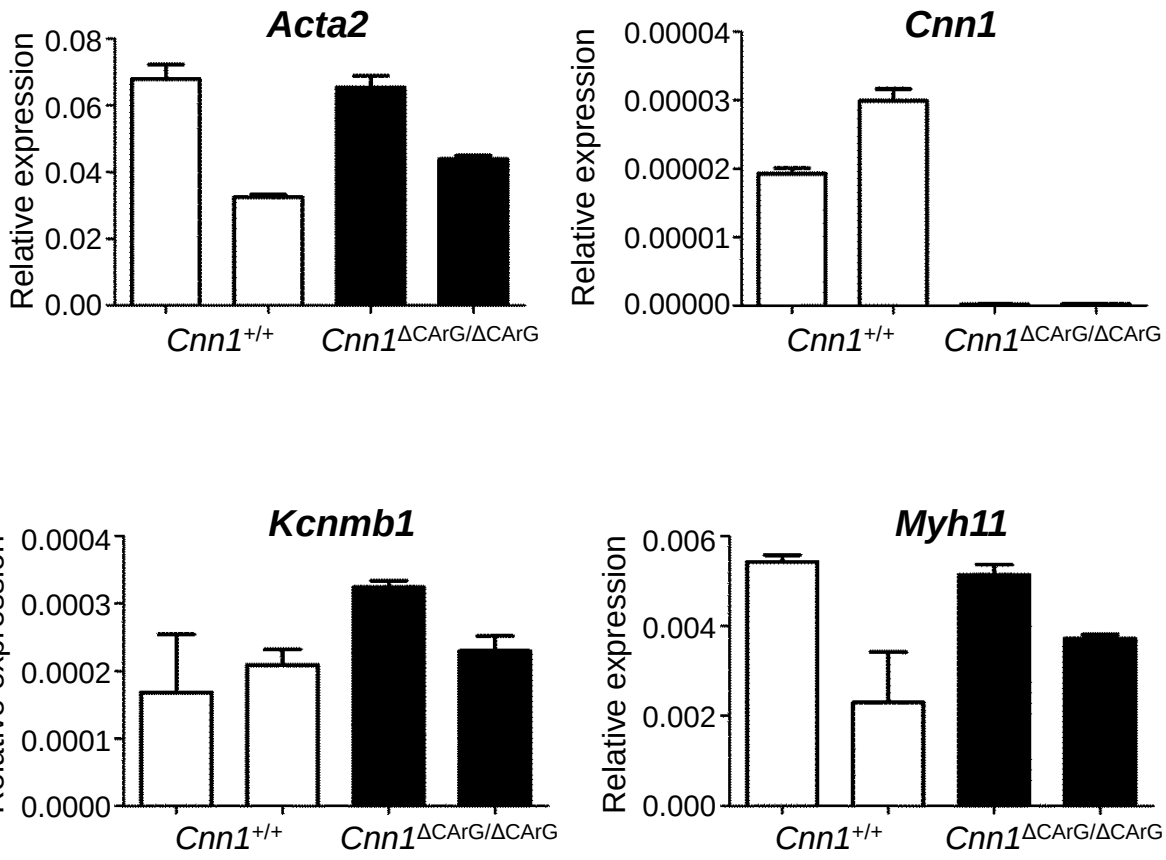
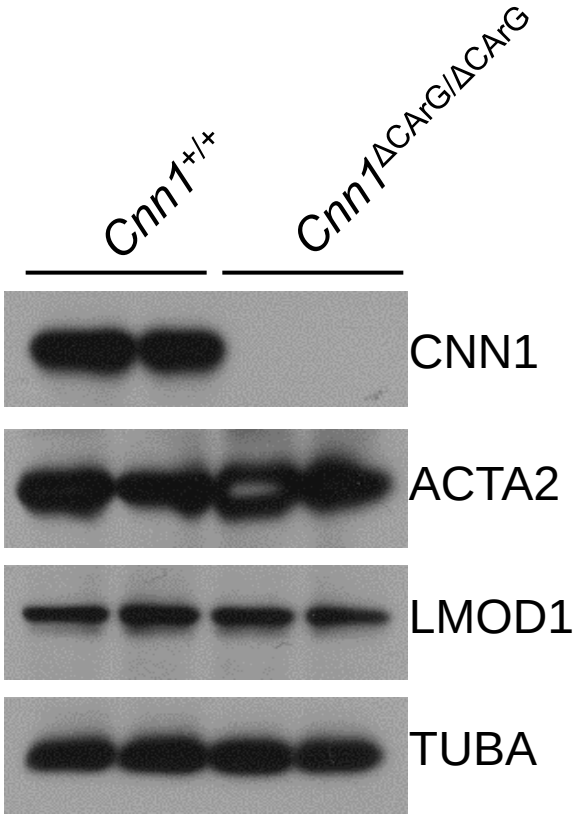


Figure III


Acta2
Relative expression
0.08
0.06
0.04
0.02
0.00
Cnn1+/+
Cnn1ΔCArG/ΔCArG
Cnn1
0.00004
0.00003
0.00002
0.00001
0.00000
Kcnmb1
0.0004
0.0003
0.0002
0.0001
0.0000
Myh11
0.006
0.004
0.002
0.000


Cnn1+/+
Cnn1ΔCArG/ΔCArG
CNN1
ACTA2
LMOD1
TUBA