

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Gould DB, Phalan FC, van Mil SE, et al. Role of COL4A1 in small-vessel disease and hemorrhagic stroke. *N Engl J Med* 2006;354:1489-96.

## Supplemental Material Table of Contents

- 1) Supplemental text ‘Viability of surgically delivered pups’.
- 2) Supplemental text ‘Abnormalities of retinal and renal vasculature’.
- 3) Figure S1. Lung Histology.
- 4) Figure S2. Blood Pressure and Heart Rate.
- 5) Supplemental text references.

### **Viability of surgically delivered pups**

Viability for naturally born mutant pups was calculated by comparing the number of mutant pups surviving at P1 to the number of mutant pups born (24 out of 31). For the surgically derived/fostered cohort, pups were excluded from the calculation if the entire litter died (3 litters; 7 controls and 7 mutants). We reasoned that the foster mother rejected these litters. After exclusion of rejected litters, viability of surgically delivered mutant pups was normalized to the survival of wild-type pups after surgical delivery. Five out of 37 wild-type pups died at P0 for a survival rate of 86.5% after surgical delivery. Therefore, the number of mutant pups expected to survive surgical delivery was calculated as follows: 19 (mutant pups obtained after exclusion of lost litters) x .8649 (survival of wild-type pups after surgical delivery) x .7742 (survival of mutant pups from natural birth) = 12.72 pups. Instead, only 5 pups survived (Chi square 4.685 and P=0.03).

We previously suggested that perinatal cerebral hemorrhage contributes to perinatal death (1). Here we show that after rescue of perinatal cerebral hemorrhage by surgical delivery

of pups there is an unexpected trend towards increased perinatal mortality. The cyanosis and labored breathing in some newborn mutant pups is consistent with histological observation of compact lungs with few open terminal air spaces (Figure S1). Similarly, mice mutant for other BM components, laminin gamma 1 (*Lamc1*) (2) and nidogen 1 and 2 (3) died very soon after birth with cyanosis and highly condensed lungs. *Lamc1* mutant mice had marked reduction in mature prealveolar sacculi, mesenchymal thickening around terminal air spaces and abnormalities of alveolar basement membranes.

By necessity, surgical delivery of pups was performed 0.5-1 day before the time of natural birth. Because considerable lung maturation occurs during late gestational stages, *Col4a1* mutations could exacerbate the slight prematurity of these pups which might explain the increased perinatal death of surgically delivered pups. In humans, perinatal cyanosis was reported in a patient with familial porencephaly (4). Our data suggests that lung maturation could be of considerable medical importance for perinatal health/viability of individuals with *COL4A1* mutations.

### **Abnormalities of retinal and renal vasculature**

Central nervous system vasculature can be assessed *in vivo* in the retina (5). When observed by fluorescein angiography, the retinal vascular pattern was highly tortuous in mutant mice on the C57BL/6J genetic background compared to control littermates (Fig. 2A and B). Changing the genetic context had a profound effect on this phenotype. Mutant mice on a CASTB6F1 genetic background had retinal vasculature without obvious tortuosity (Fig. 2C). Thus, the phenotypic effect of *Col4a1* mutations is sensitive

to genetic context. Our data supports using retinal vessel appearance to predict the likelihood of cerebral vascular defects (5).

Proteinuria or hematuria are associated with retinal and cerebral defects in human families (6-8) and we reasoned that *Col4a1* mutations could cause glomerular defects. Type IV collagens are an important structural component of the glomerular basement membrane (GBM). The GBM is a specialized BM essential for the highly selective filtration of plasma in the kidney (9). Upon examination, the GBMs of mutant mice were focally disrupted (Fig. 2E) similar to focal disruptions observed in cerebral vascular BMs observed previously (1). To test if GBM disruptions have functional consequences, we assayed the level of urinary albumin. Up to 5 weeks of age, no mutant mice had detectable urinary albumin. However, almost all mutant mice tested between 5 and 10 weeks of age had micro-albuminuria (t-test;  $P < 0.0001$ ) (Fig. 2F). To test if the severity of albuminuria increased with age, we assayed mice aged for 2 years. At this age, all mutant mice had micro-albuminuria but the severity was not significantly different than in young mutant mice (Fig. 2G).

## Figure Legends

### Fig S1. Lung histology

To investigate why pups were still dying in the absence of severe cerebral hemorrhage, newborn cyanotic pups with labored breathing were analyzed. **A**, Control pups had normal large, inflated lungs with open terminal airspaces (arrows) whereas, **B**, lungs from

mutant mice appeared small and condensed with open proximal air spaces (asterisks) but few open terminal airspaces (arrows).

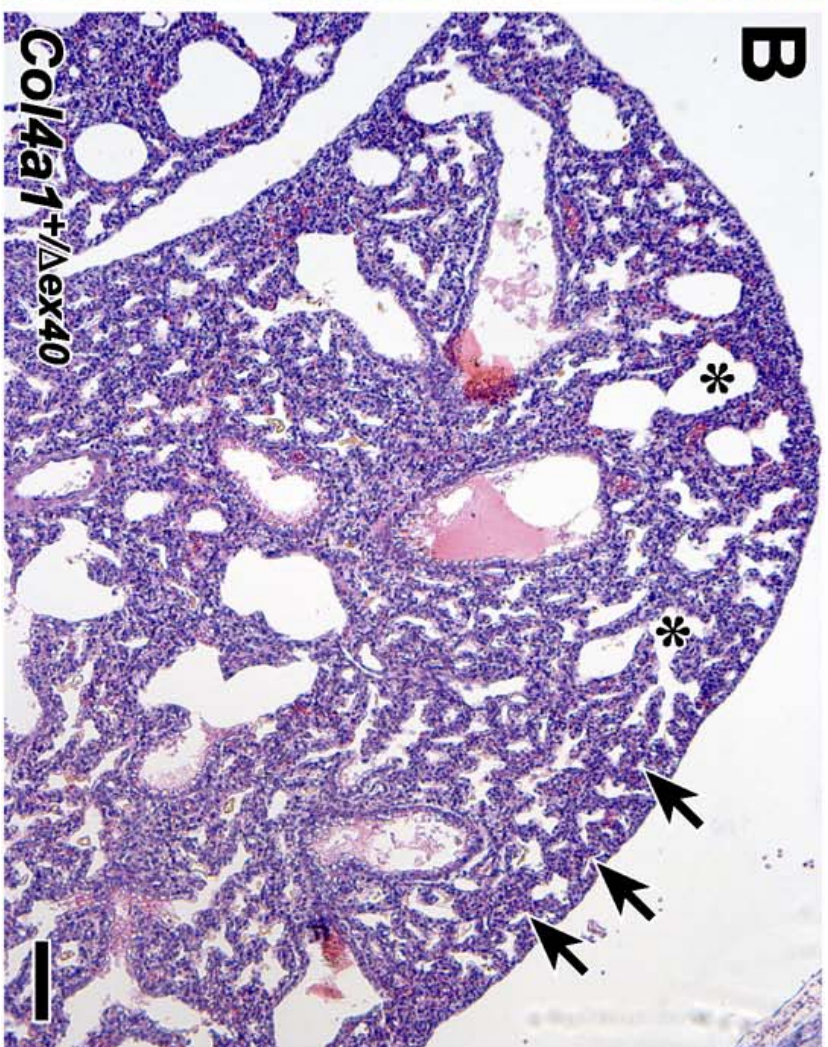
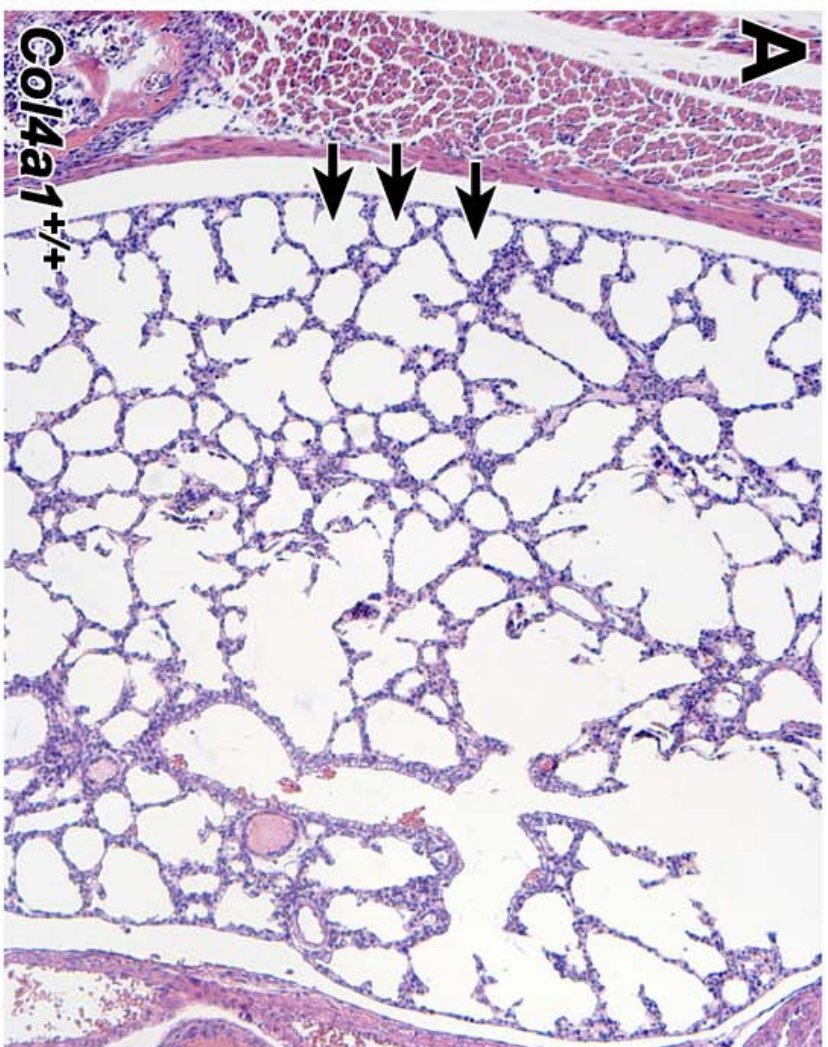
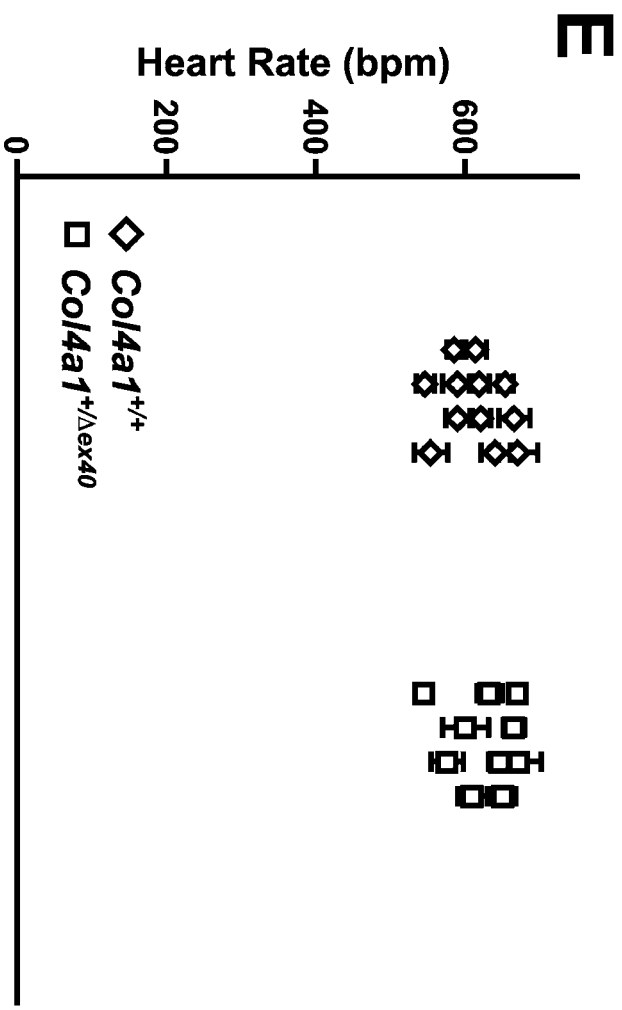
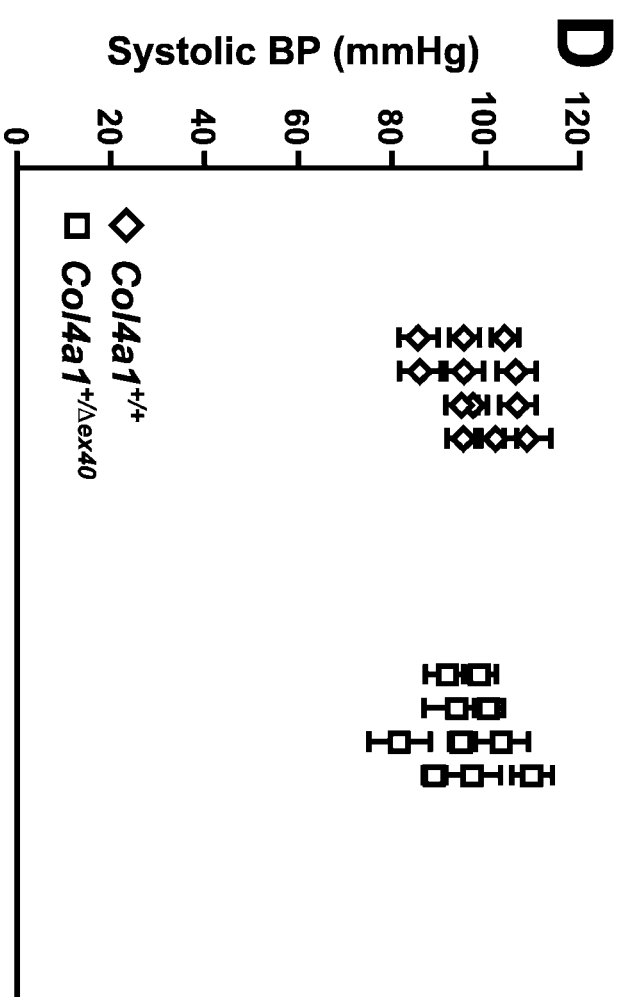


Fig S2. Blood Pressure and Heart Rate

**A and B,** Blood pressure and heart rate measurements revealed that mutant mice at 3 months of age (n=10) do not have differences in systolic blood pressure or heart rate compared to littermate controls (n=12). Each point indicates the mean  $\pm$  SEM for all readings of an individual mouse.



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