

SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Characterization of *Lamb2*^{-/-}; *Neph-Lamb2-S83R* transgenic mice.

(A) Founders were analyzed by immunofluorescence for LAMB2 expression and defined as “high” (left), “normal”, or “mosaic” (right) expressers. (B) LAMB2 co-localization with GBM nidogen was compared between 7-month-old *Lamb2*^{+/-} heterozygous nulls and *Lamb2*^{-/-}; *Neph-Lamb2-S83R* transgenic mice. Transgene-derived rat LAMB2 in *Lamb2*^{-/-}; *Neph-Lamb2-S83R* mice exhibited expression and co-localization with nidogen similar to endogenous mouse LAMB2. (C) LAMB1 localization was evaluated in 9-month-old *Lamb2*^{+/-} and *Lamb2*^{-/-}; *Neph-Lamb2-S83R* transgenic mice. LAMB1 remained restricted to the mesangium of *Lamb2*^{-/-}; *Neph-Lamb2-S83R* glomeruli, similar to *Lamb2*^{+/-} control. (D) Albuminuria was monitored by SDS-PAGE over a 13-month period in *Lamb2*^{-/-}; *Neph-Lamb2-S83R* mice and controls. Only mice with mosaic LAMB2-S83R expression exhibited nephrotic-range albuminuria by 9 months, which is likely due to the segmental lack of LAMB2 deposition in the GBM.

Supplemental Figure 2. LAMB2 and LAMB1 expression and localization in CRISPR/Cas9-mediated *Lamb2*^{S83R} mutant mice are normal.

(A) LAMB2 immunofluorescence revealed similar expression and co-localization with nidogen in the GBMs of *Lamb2*^{+/+} (10 months) and *Lamb2*^{S83R/S83R} (mimicking the patients genotype; 9 months) mice. (B) LAMB1 immunofluorescence revealed similar restriction to the mesangium in *Lamb2*^{+/+} (10 months) and *Lamb2*^{S83R/S83R} (9 months) mice, consistent with a lack of disease in *Lamb2*^{S83R/S83R} mice.

Supplemental Figure 3. Effects of LAMB2 loss on the Alport phenotype.

Electron micrographs demonstrate a typical Alport phenotype in 2.5-day old *Lamb2*^{+/-}; *Col4a3*^{-/-} mice, including segmental GBM splitting (asterisks). In contrast, the complete loss of LAMB2 in *Lamb2*^{-/-}; *Col4a3*^{-/-} mice has a dramatic effect on GBM architecture, including exaggerated GBM

splitting and GBM expansion and an almost complete lack of foot processes. GBM = glomerular basement membrane; Lu = capillary lumen.

Supplemental Figure 4. Localization of LAMB2 and LAMB1 in glomeruli of control, *Lamb2*^{+/+}; *Col4a3*^{-/-}, and *Lamb2*^{+/S83R}; *Col4a3*^{-/-} mice. (A) LAMB2 co-localizes with agrin in the GBM of *Lamb2*^{+/+}; *Col4a3*^{-/-}, and *Lamb2*^{+/S83R}; *Col4a3*^{-/-} mice (6 week-old tissues shown) at similar levels. (B) In 10 week-old mice, LAMB1 is restricted to the mesangium and absent from the glomerular capillary loops in control mice. In contrast, LAMB1 expression co-localizes with agrin in the *Lamb2*^{+/+}; *Col4a3*^{-/-} GBM as well as the *Lamb2*^{+/S83R}; *Col4a3*^{-/-} GBM. We observed increased GBM deposition of LAMB2 and LAMB1 in *Lamb2*^{+/S83R}; *Col4a3*^{-/-} mice versus *Lamb2*^{+/+}; *Col4a3*^{-/-} and control mice. n = 4-5.

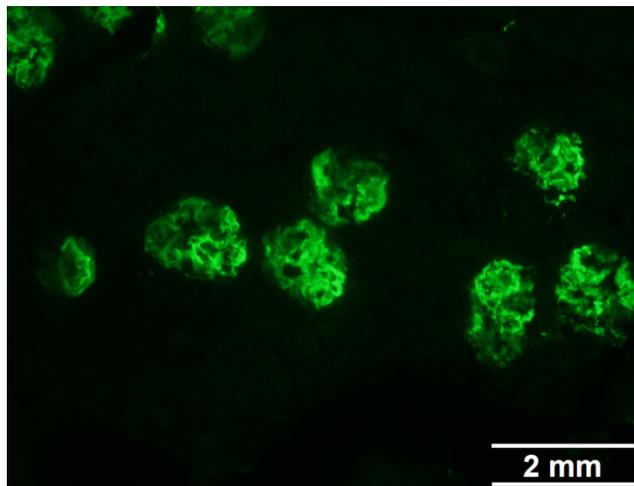
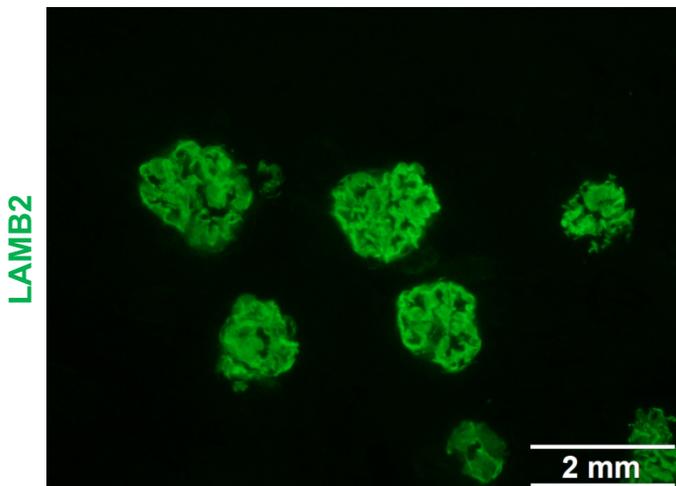
Supplemental Figure 5. Localization of LAMA2 in glomeruli of control, *Lamb2*^{+/+}; *Col4a3*^{-/-}, and *Lamb2*^{+/S83R}; *Col4a3*^{-/-} mice. LAMA2 is restricted to the mesangium in control mice (10 week-old tissue shown). *Lamb2*^{+/+}; *Col4a3*^{-/-} (10 week-old tissue shown) and *Lamb2*^{+/S83R}; *Col4a3*^{-/-} glomeruli (8 week-old tissue shown) exhibit similar LAMA2 localization in the GBM. n = 4-5.

Supplemental Figure 1

A

“High” *Neph-Lamb2-S83R*

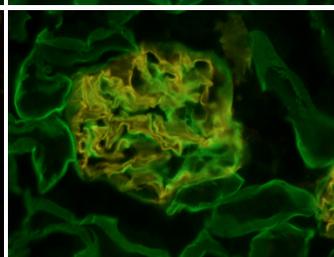
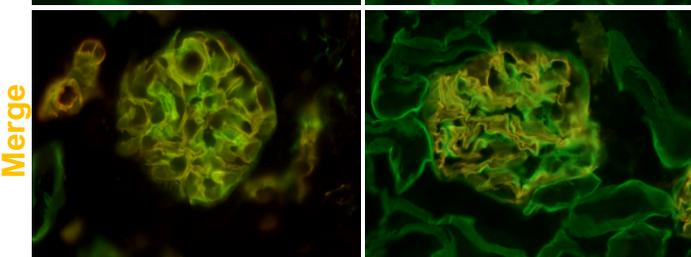
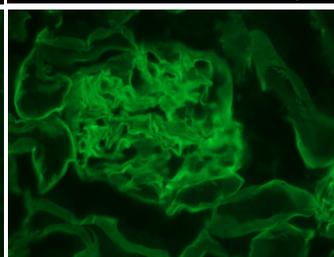
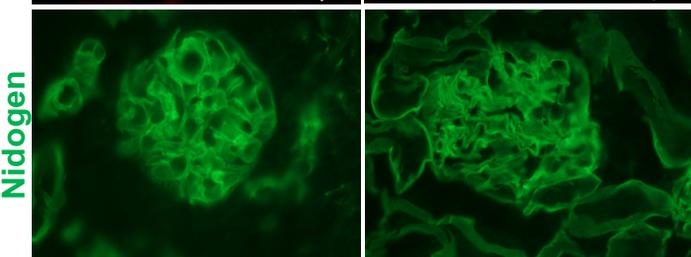
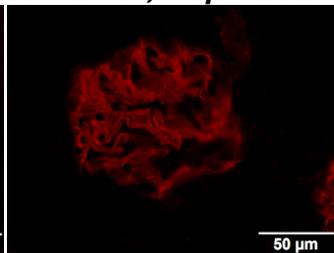
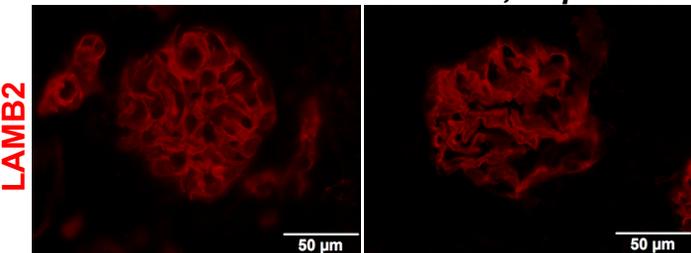
Mosaic *Neph-Lamb2-S83R*



B

Lamb2 +/-

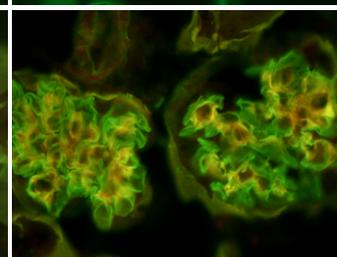
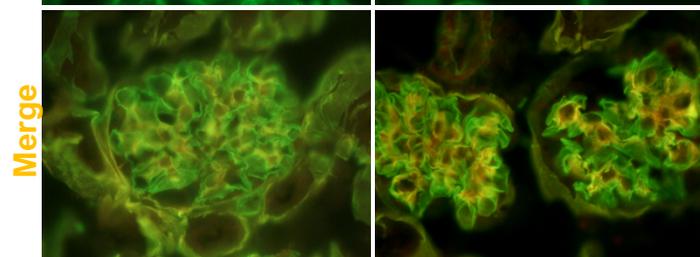
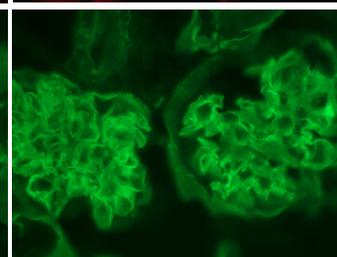
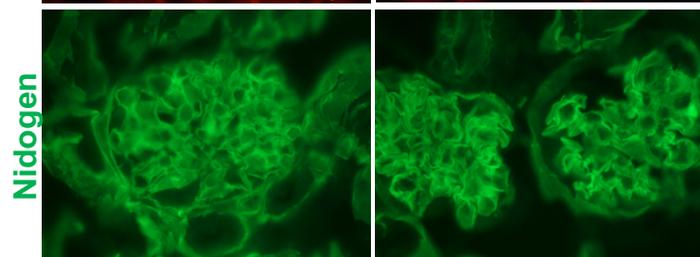
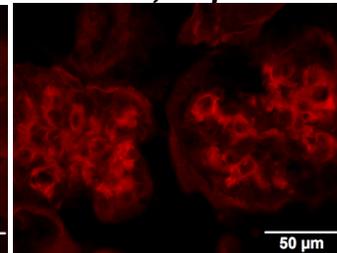
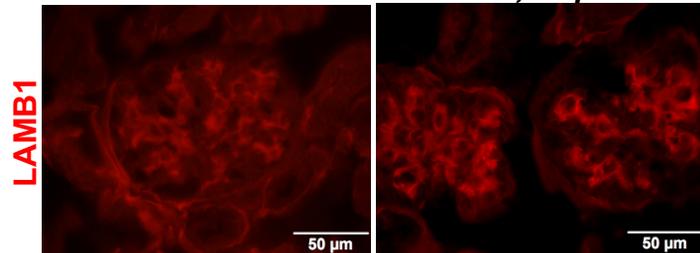
Lamb2 -/-; Neph-S83R



C

Lamb2 +/-

Lamb2 -/-; Neph-S83R



D

Albumin Std.		High			Mosaic	
1ug	5ug	+	-	-	+	-



9 mo. old

Albumin Std.		High			Mosaic		: Neph-S83R
1ug	5ug	+	-	-	+	-	



12 mo. old

Supplemental Figure 2

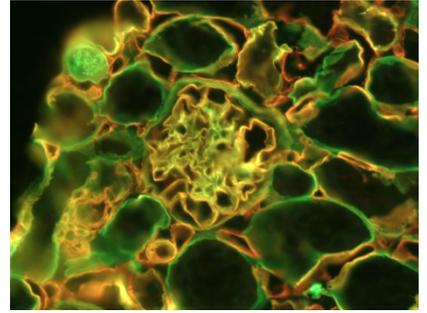
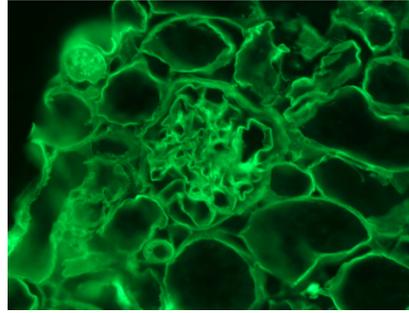
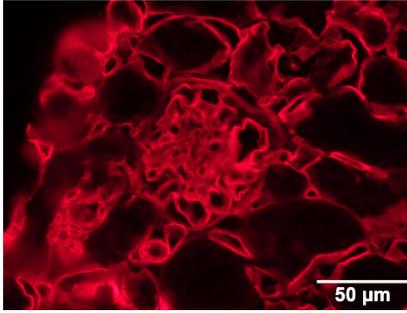
A

LAMB2

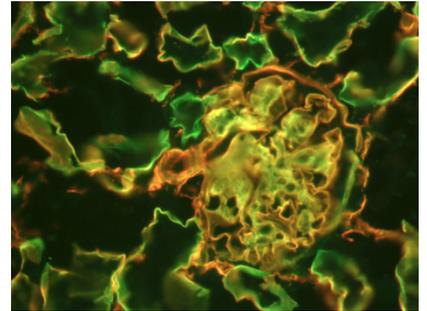
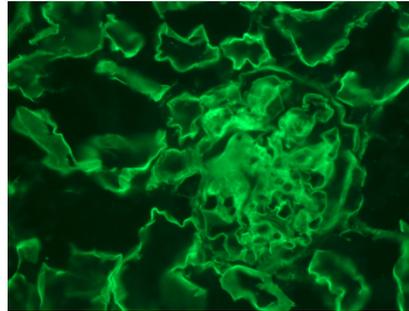
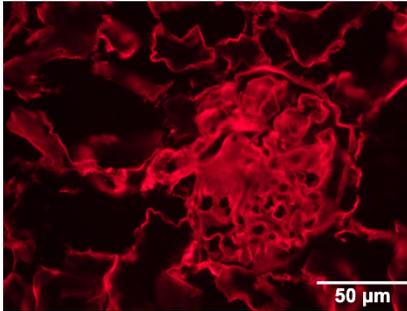
Nidogen

Merge

Lamb2 +/-



Lamb2 S83R/S83R



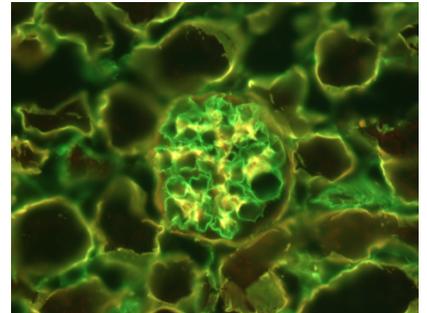
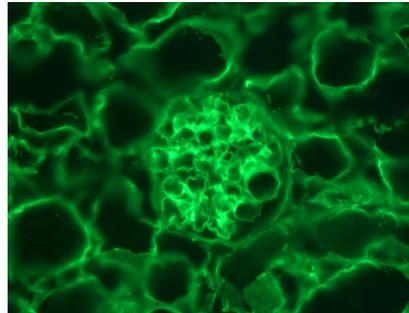
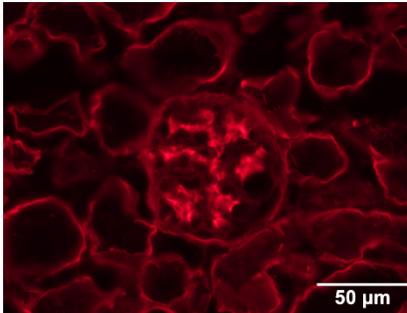
B

LAMB1

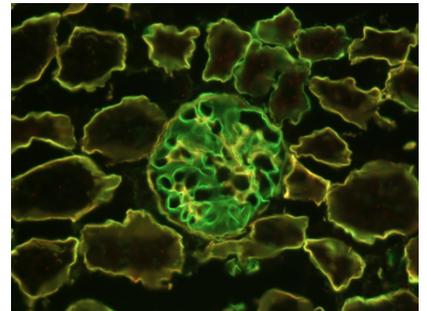
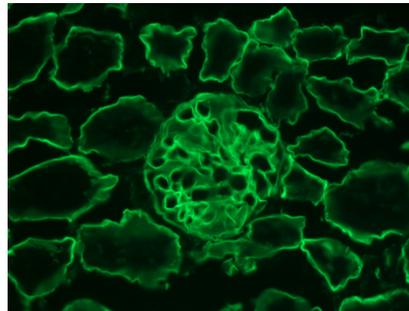
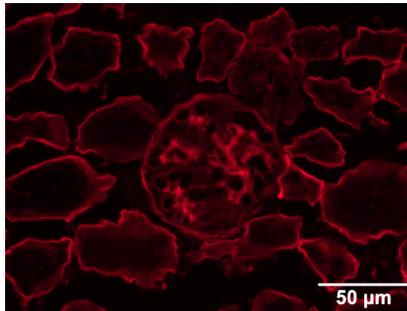
Nidogen

Merge

Lamb2 +/-



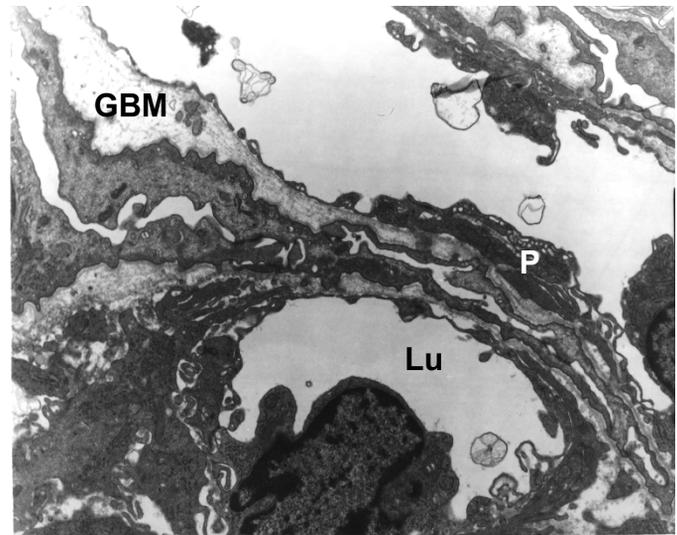
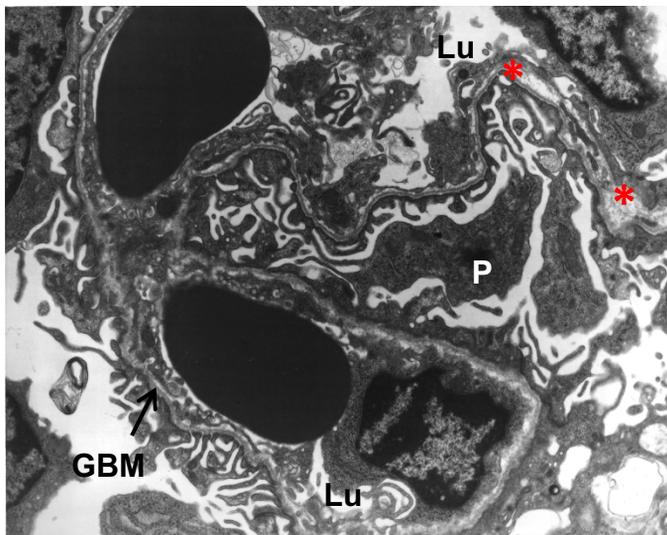
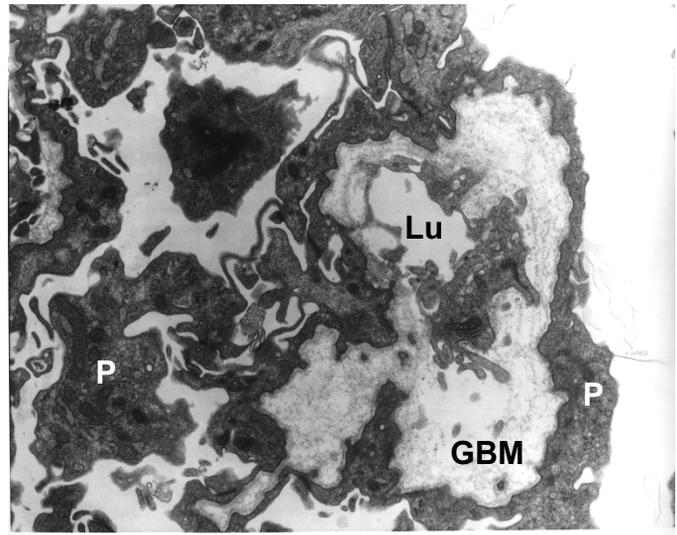
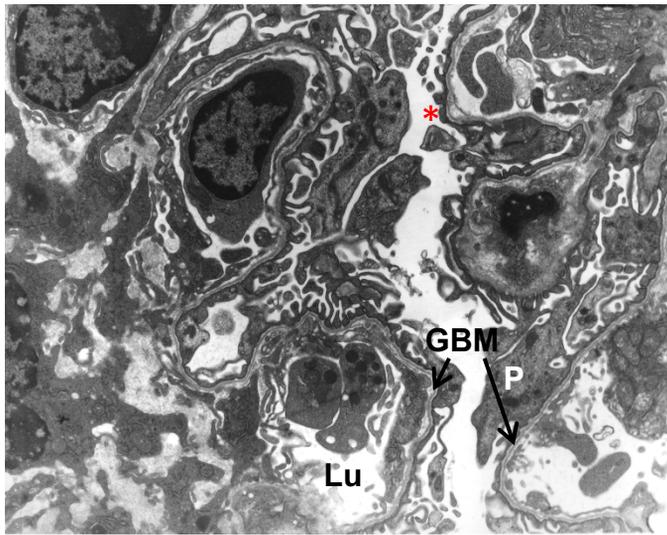
Lamb2 S83R/S83R



Supplemental Figure 3

Lamb2^{+/-}; *Col4a3*^{-/-}

Lamb2^{-/-}; *Col4a3*^{-/-}



Supplemental Figure 5

