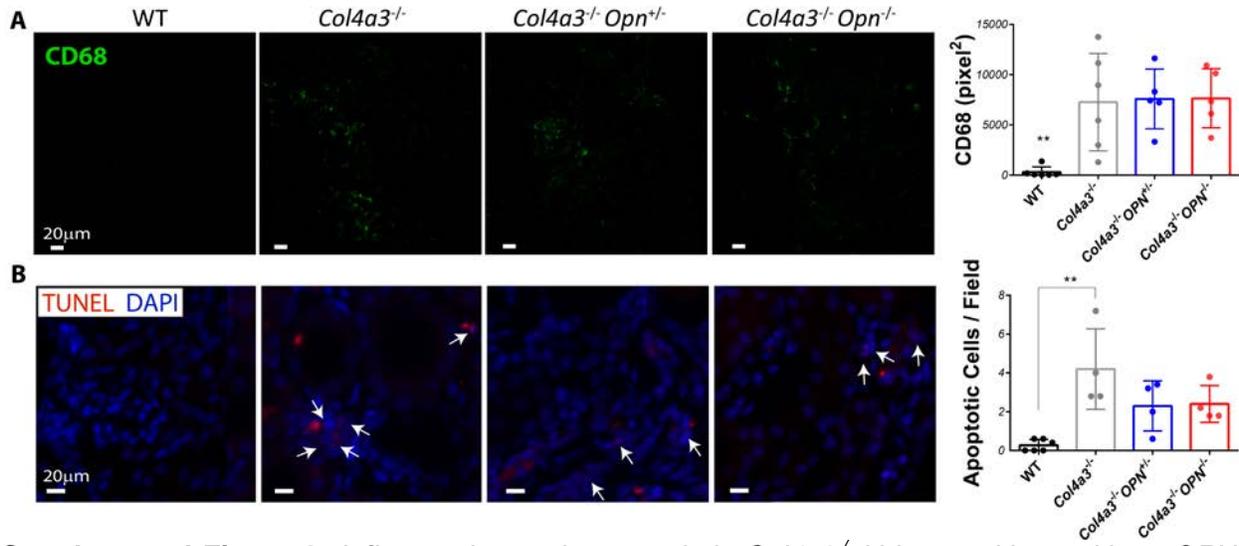
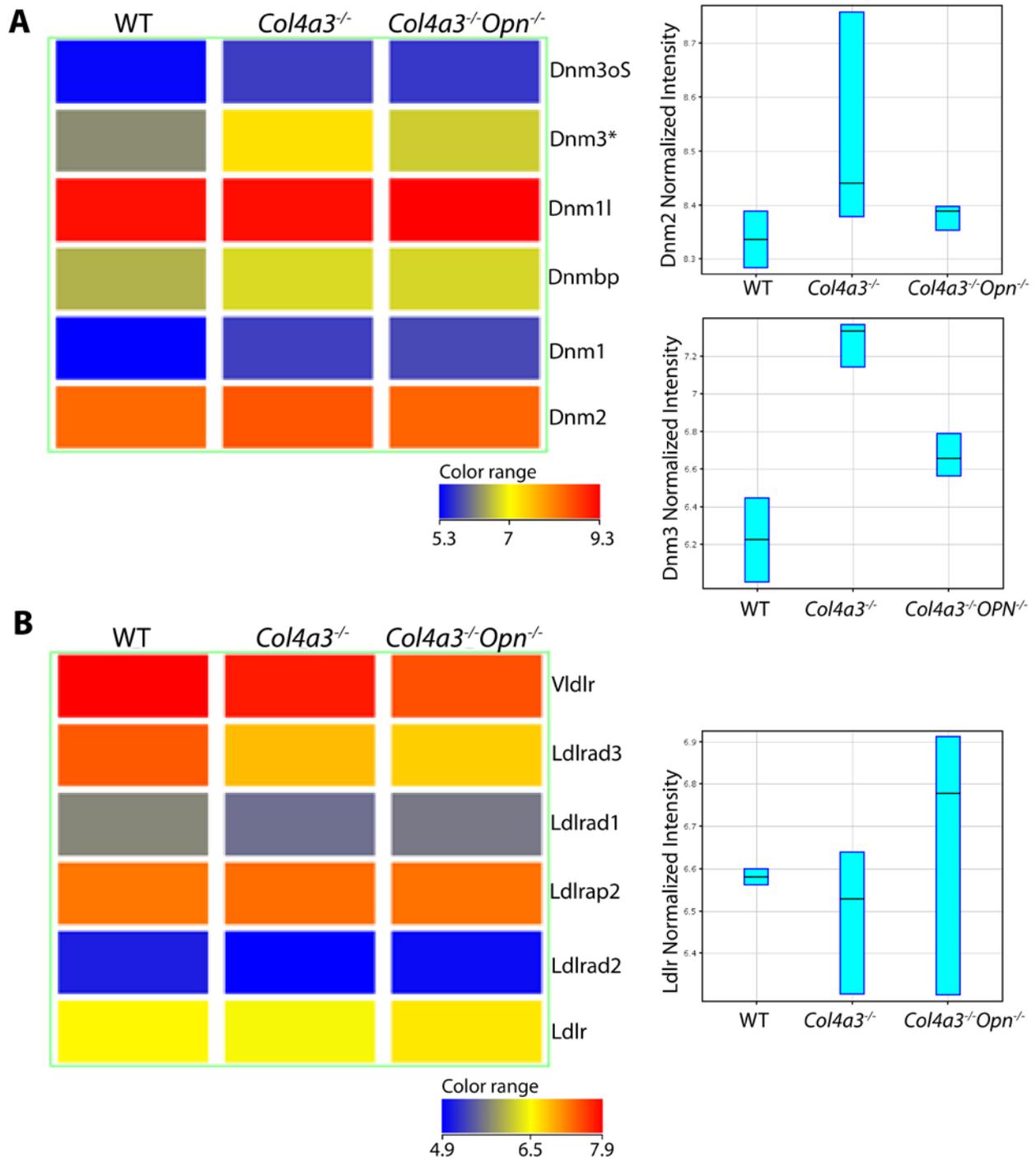


**Supplemental Figure 1.** OPN deficiency reduces free cholesterol deposition in the renal tubules of Alport mice relative to wild type (WT) mice. (A) Filipin staining and corresponding quantification reveal increased level of free cholesterol deposition in the renal tubules of Alport mice, and this deposition is reduced in Alport mice that are OPN deficient.  $N=5$  mice per group, and 5 images were quantified per mouse. Scale bar = 20  $\mu\text{m}$ . Data are mean  $\pm$  SEM. \* $P < 0.05$ ; \*\* $P < 0.01$  based on ANOVA with Tukey multiple correction. (B) Renal triglyceride levels in kidneys of non-fasted mice do not show differences between  $Col4a3^{-/-}$  and  $Col4a3^{-/-} OPN^{-/-}$  mice.  $N=4-8$  mice per group. TG = Triglycerides.

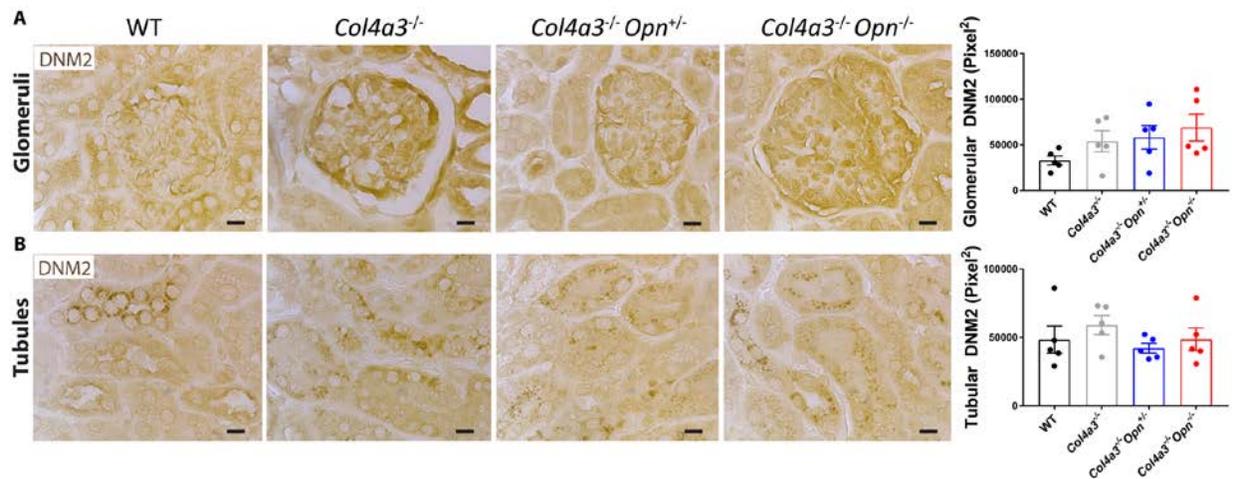


**Supplemental Figure 2. Inflammation and apoptosis in *Col4a3*<sup>-/-</sup> kidneys with or without OPN.** (A) CD68 expression is dramatically increased in kidneys of *Col4a3*<sup>-/-</sup> mice relative to wild type (WT) mice, and the elevated level of CD68 expression is retained in both *Col4a3*<sup>-/-</sup>Opn<sup>+/-</sup> and *Col4a3*<sup>-/-</sup>Opn<sup>-/-</sup> mouse kidneys. N=5-6 mice per group, and 5 images were quantified per mouse. Scale bar = 20  $\mu$ m. Data are mean $\pm$ SEM. \*\*P < 0.01 for WT relative to each of the other three groups based on ANOVA with Tukey multiple correction. (B) TUNEL staining (arrows) and corresponding quantification show apoptotic cells are significantly increased in *Col4a3*<sup>-/-</sup> but reduced in *Col4a3*<sup>-/-</sup>Opn<sup>+/-</sup> and *Col4a3*<sup>-/-</sup>Opn<sup>-/-</sup> mouse kidneys. DAPI/nuclear staining is shown in blue. N=4-6 mice per group, and 5 images were quantified per mouse. Data are mean $\pm$ SEM. \*\*P < 0.01 based on ANOVA with Tukey multiple correction.

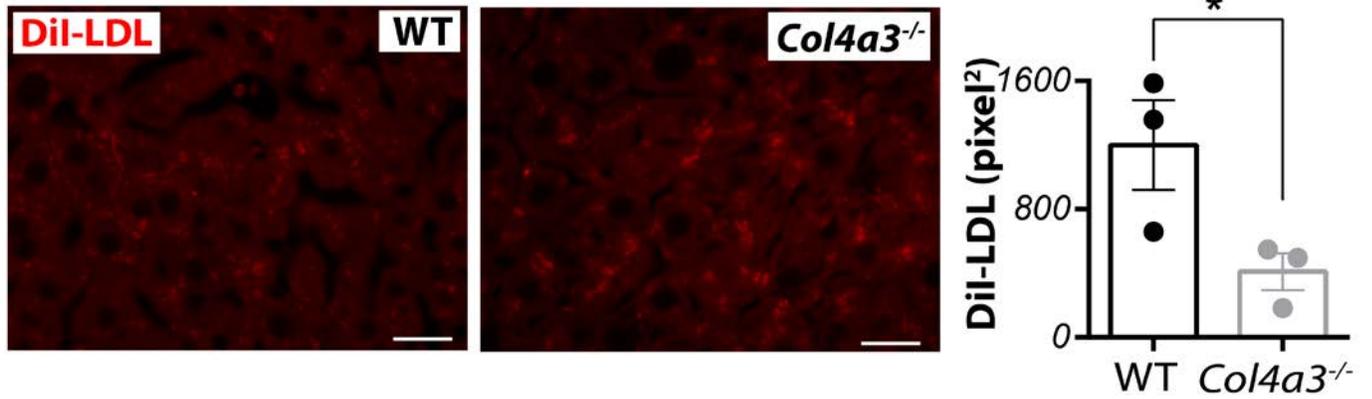


**Supplemental Figure 3.** Microarray gene expression of Dynamin and *Ldlr* genes in Alport kidneys. (A) Heatmap and box plot of Dynamin genes from microarray data show that *Dnm2* and *Dnm3* transcripts are increased in the kidneys of *Col4a3*<sup>-/-</sup> mice as compared to wild type (WT) mice, but only *Dnm3* transcripts are significantly ( $*p < 0.05$ ) reduced in *Col4a3*<sup>-/-</sup>*Opn*<sup>-/-</sup> mice using One Way ANOVA. (B) Heatmap and box plot of *Ldlr* genes from microarray data show

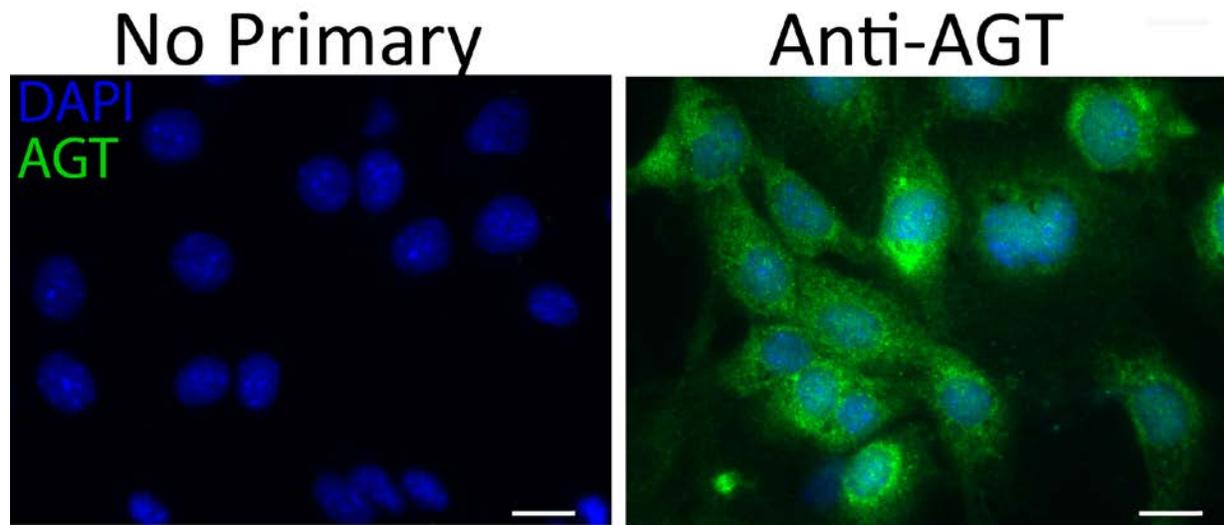
that *Ldlr* transcripts are not changed in the kidneys of *Col4a3<sup>-/-</sup> Opn<sup>-/-</sup>* mice compared to *Col4a3<sup>-/-</sup>* mice. N=2 WT, N=3 *Col4a3<sup>-/-</sup>* mice, N= 3 *Col4a3<sup>-/-</sup> OPN<sup>-/-</sup>* Alport mice.



**Supplemental Figure 4.** *OPN* deficiency does not alter *DNM2* protein expression in the Alport kidneys. Glomerular *DNM2* (A) and tubular *DNM2* (B) protein expression is not significantly changed in the *Col4a3<sup>-/-</sup> Opn<sup>+/-</sup>* or *Col4a3<sup>-/-</sup> Opn<sup>-/-</sup>* mouse kidneys relative to *Col4a3<sup>-/-</sup>* or wild type (WT) ones, as shown by immunohistochemistry and corresponding quantification. N=5 mice per group, and 5 images were quantified per mouse. Scale bar = 10  $\mu$ m. Data are mean  $\pm$  SEM. No statistical significance based on ANOVA with Tukey multiple correction.



**Supplemental Figure 5.** LDL cholesterol uptake by Alport livers. Intravenous infusion of DiI-LDL cholesterol in Alport mice shows a moderate increase (2.9 fold) in cholesterol uptake by Alport livers compared to wild type (WT) group. N= 3 mice per group, and 3 images were quantified per mouse. Scale bar = 20  $\mu$ m. Data are mean $\pm$ SEM. \*P < 0.05 based on student's t-test.



**Supplemental Figure 6.** *Characterization of primary renal tubular epithelial cells. Tubular epithelial protein Angiotensinogen (AGT) is substantially expressed in the isolated primary tubular cells as shown by immunostaining. Scale bar = 20  $\mu$ m.*

**Table 1-** Microarrays was performed on 2 wild type, 3 *Col4a3<sup>-/-</sup>* and 3 *Col4a3<sup>-/-</sup>Opn<sup>-/-</sup>* mous kidneys. Transcriptional alterations in wild type (WT) and *Col4a3<sup>-/-</sup>Opn<sup>-/-</sup>* relative to *Col4a3<sup>-/-</sup>* mice ( $p < 0.01$ ) using One Way ANOVA followed by Venn Diagram analysis.

Gene description	Gene symbol	Transcript cluster ID	RefSeq ID	Fold change WT vs <i>Col4a3<sup>-/-</sup></i>	Fold change <i>Col4a3<sup>-/-</sup>Opn<sup>-/-</sup></i> vs <i>Col4a3<sup>-/-</sup></i>
<i>Predicted gene, 23422</i>	<i>Dsec\GM23422</i>	17213976	<i>XM_002044602</i>	-2.0559	-1.7654
<i>RIKEN cDNA 9530091C08 gene</i>	<i>9530091C08Rik</i>	17518933	<i>NR_033299</i>	-2.2847	-1.7172
<i>Dynamin 3</i>	<i>Dnm3</i>	17228906	<i>NM_001038619</i>	-2.0809	-1.5276
<i>MicroRNA 24-1</i>	<i>miR24-1</i>	17288112	<i>NR_029496</i>	-1.7395	-1.6529
<i>Predicted gene, 20038</i>	<i>Gm20038</i>	17548135	N/A	-1.8419	-1.7043
<i>Predicted gene 12121</i>	<i>Gm12121</i>	17248406	<i>XR_872376.</i>	-2.1012	-1.6874
N/A	N/A	17434248	N/A	-3.3134	-1.5400
<i>RIKENcDNA B930095G15 gene</i>	<i>B930095G15Rik</i>	17302832	<i>XR_105643</i>	-1.9263	-1.5398
<i>Predicted gene 7945</i>	<i>Gm7945</i>	17305259	N/A	-1.8952	-1.5298
N/A	N/A	17424412	N/A	-3.3135	-1.5267
<i>RIKEN cDNA 1700054O19 gene</i>	<i>1700054O19Rik</i>	17304145	<i>XR_383299</i>	-2.4783	-1.5078

Supplemental Table II: Metabolic and Renal Parameters in Alport mice.					
Group/Parameter	WT	OPN <sup>-/-</sup>	Col4a3 <sup>-/-</sup>	Col4a3 <sup>-/-</sup> OPN <sup>+/-</sup>	Col4a3 <sup>-/-</sup> OPN <sup>-/-</sup>
<b>Body Weight (g)</b>	21.37±0.85 (N=32)	24.28±0.73* (N=42)	15.35±0.60****†††† (N=22)	19.63±0.79▲▲††† (N=13)	20.91±0.92▲▲▲ (N=33)
<b>Urine ALB/CRE ratio</b>	201.2±42.08 (N=21)	193.6±57.38 (N=19)	22488±4186****†††† (N=12)	13110±2250▲****†††† (N=21)	13411±2632****†††† (N=15)
<b>Plasma BUN (mg/dL)</b>	21.19±1.24 (N=21)	19.50±0.88 (N=18)	141.3±35.38**†† (N=24)	124.3±28.64*† (N=24)	73.21±25.39 (N=14)
<b>Plasma CRE (mg/dL)</b>	0.24±0.05 (N=20)	0.21±0.05 (N=16)	0.45±0.08 (N=20)	0.48±0.13 (N=20)	0.25±0.13 (N=11)
<b>MCHC (g/dL)</b>	28.78±0.5 (N=23)	27.6±0.6 (N=5)	25.27±1.38* (N=15)	29.89±0.92▲▲ (N=18)	27.64±0.76 (N=11)
<b>Plasma Galectin-3 (pg/mL)</b>	15595±2914 (N=9)	21479±2050 (N=10)	38806±5033* (N=17)	40299±5775* (N=15)	28559±5231 (N=7)
<b>Systolic Blood Pressure (mmHg)</b>	113.3±3.73 (N=11)	111.0±4.32 (N=11)	149.3±6.47****†††† (N=11)	116.2±3.95▲▲▲▲ (N=13)	123.1±2.78▲▲ (N=7)
<b>Diastolic Blood Pressure (mmHg)</b>	83.52±3.57 (N=11)	84.27±3.48 (N=11)	115.0±4.18****†††† (N=11)	90.08±3.95▲▲▲ (N=13)	92.3±2.85▲▲ (N=7)

Data are for 8-10 weeks old wild type (WT), OPN<sup>-/-</sup>, Col4a3<sup>-/-</sup>, Col4a3<sup>-/-</sup> OPN<sup>+/-</sup>, and Col4a3<sup>-/-</sup> OPN<sup>-/-</sup> littermates. All data are mean ± s.e.m. p-values are from ANOVA with Tukey multiple correction. \* = p ≤ 0.05 vs WT; \*\* = p ≤ 0.01 vs WT; † = p ≤ 0.05 vs OPN<sup>-/-</sup>; †† = p ≤ 0.01 vs OPN<sup>-/-</sup>. ▲ = p ≤ 0.05 vs Col4a3<sup>-/-</sup>; ▲▲ = p ≤ 0.01 vs Col4a3<sup>-/-</sup>.