SUPPLEMENTAL ONLINE DATA

TITLE

Properdin deficiency exacerbates C3 glomerulopathy in factor H-deficient mice

AUTHORS

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Affiliations

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3. Glomerular C9 reactivity in *CFH*$^{-/-}$.*P*$^{-/-}$ mice.
4. Glomerular C3 immunostaining using two monoclonal anti-C3 antibodies with differential reactivity to tissue-bound C3 fragments.
5. Tubulo-interstitial C3 immunostaining in wild-type, *P*$^{-/-}$, *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice and hepatic C3 staining in *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice.
6. Comparison of plasma C3 levels and glomerular C3 immunostaining in *CFH*$^{-/-}$, *CFH*$^{M/M}$, *CFH*$^{-/-}$.*P*$^{-/-}$ and *CFH*$^{M/M}$.*P*$^{-/-}$ mice.

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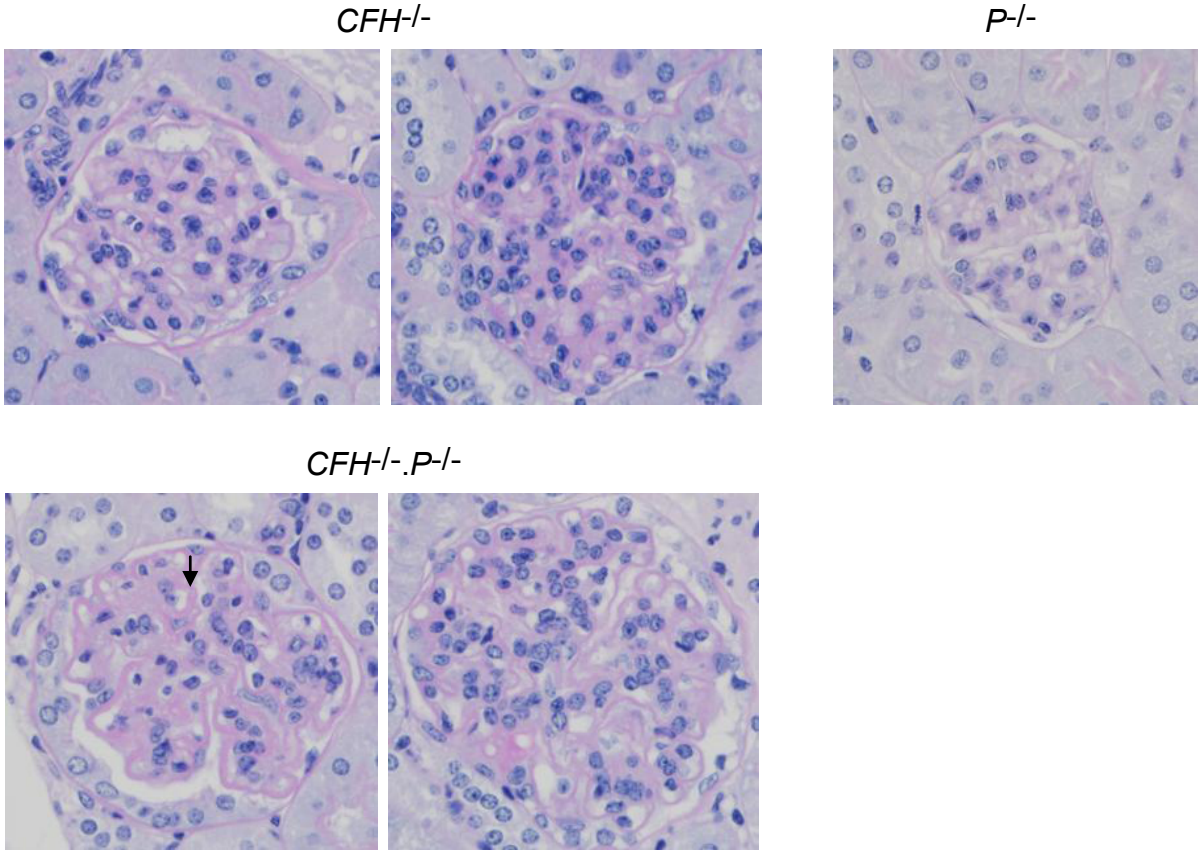

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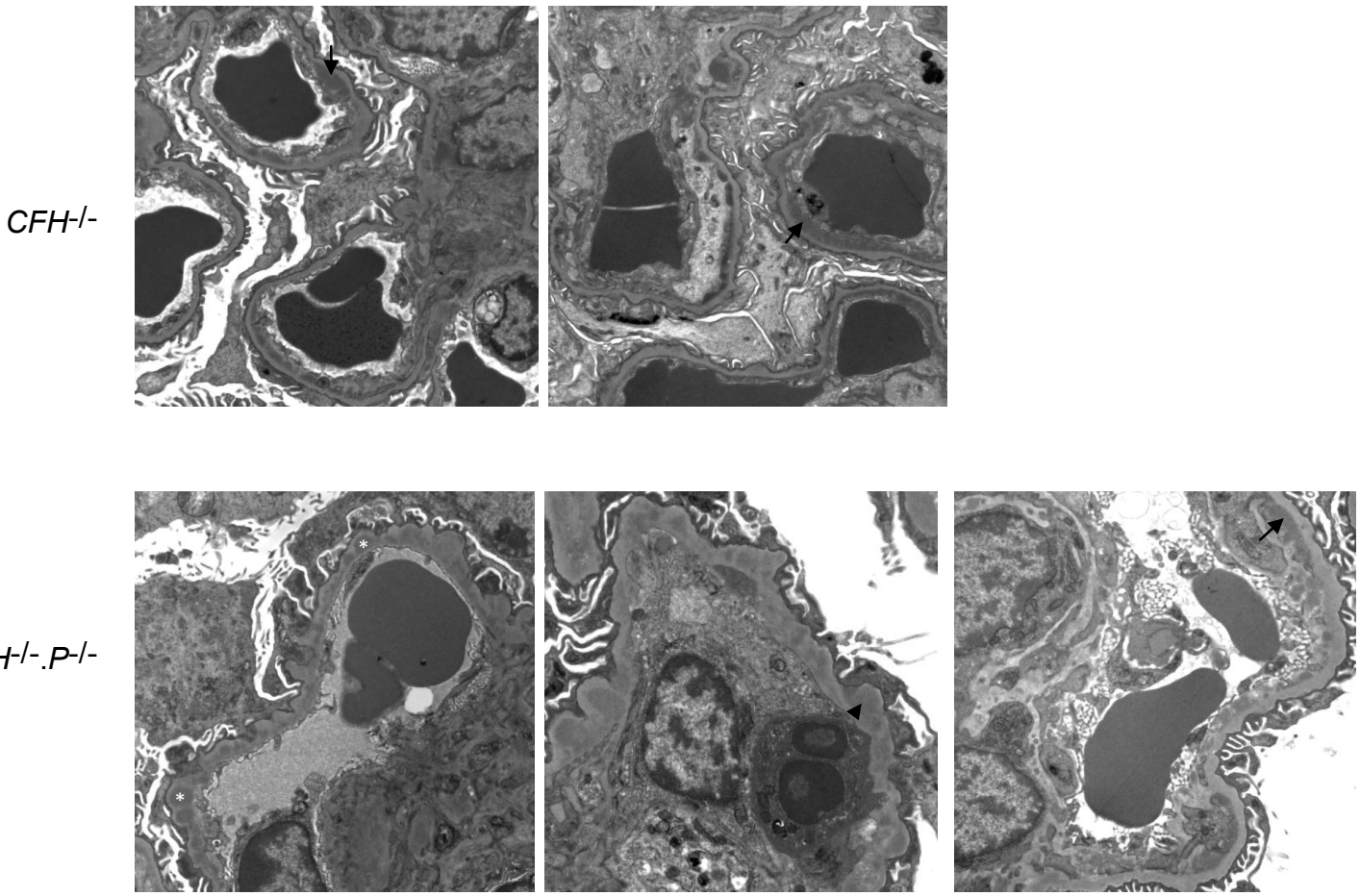

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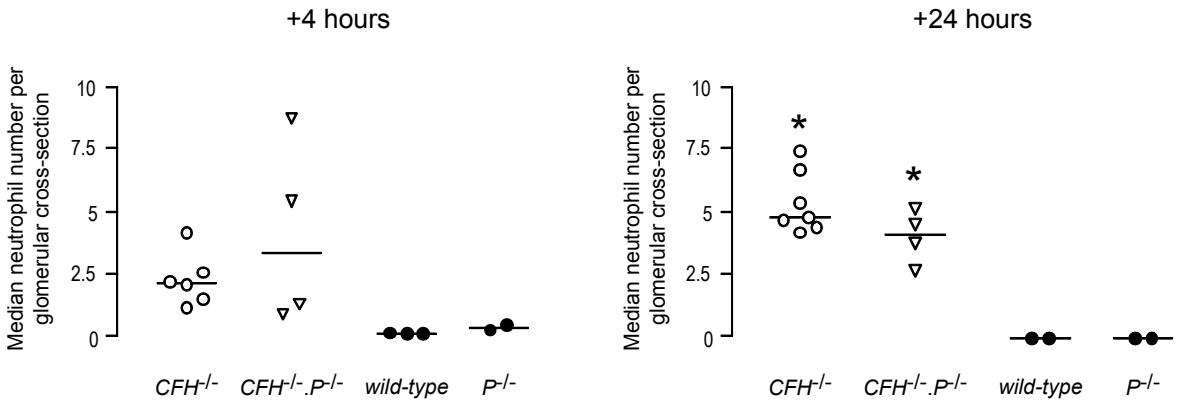
SUPPLEMENTAL FIGURE 1. Representative microscopic images of glomeruli from 38-week old *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice. (A) Light microscopy of PAS-stained glomerular sections. Glomerular hypercellularity is evident in *CFH*$^{-/-}$ animals. *CFH*$^{-/-}$.*P*$^{-/-}$ animals show more marked glomerular hypercellularity and capillary wall thickening with areas of capillary wall double contours (arrow) These changes were not observed in the *P*$^{-/-}$ mice in which the glomeruli appear normal. Original magnification x40 (B) Representative electron microscopic images of glomeruli from 38-week old *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice. Subendothelial electron-dense deposits (arrows) were evident in *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice. Electron-dense deposits along the subepithelial aspect (arrowhead) of the glomerular basement membrane were present in some of the *CFH*$^{-/-}$.*P*$^{-/-}$ and not in any of the *CFH*$^{-/-}$ mice. Some of the subepithelial deposits are incorporated in the basement membrane (asterisks)



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SUPPLEMENTAL FIGURE 2. **Glomerular neutrophil scores during heterologous NTN.** Median glomerular neutrophil number per glomerular cross-section at 4 and 24 hours following intravenous injection of nephrotoxic serum. Scores did not differ between *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice at either time point. Horizontal bars denote median values. *p<0.01 vs. either wild-type or *P*$^{-/-}$ groups, Bonferroni's Multiple Comparison Test.

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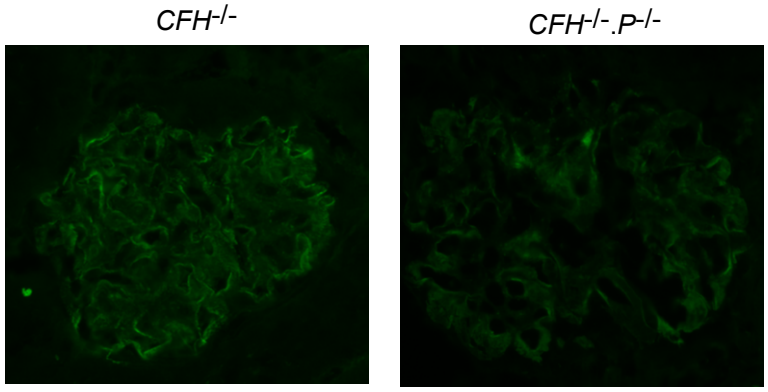

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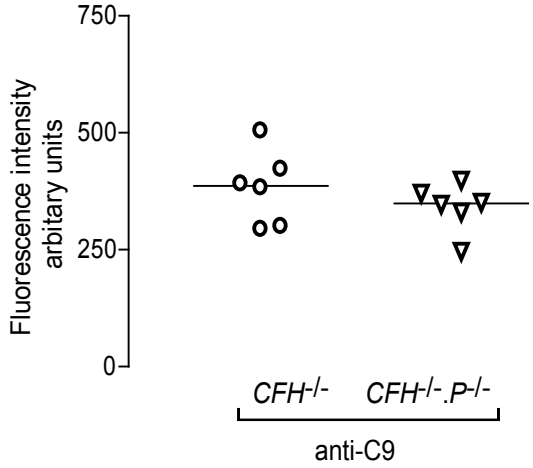

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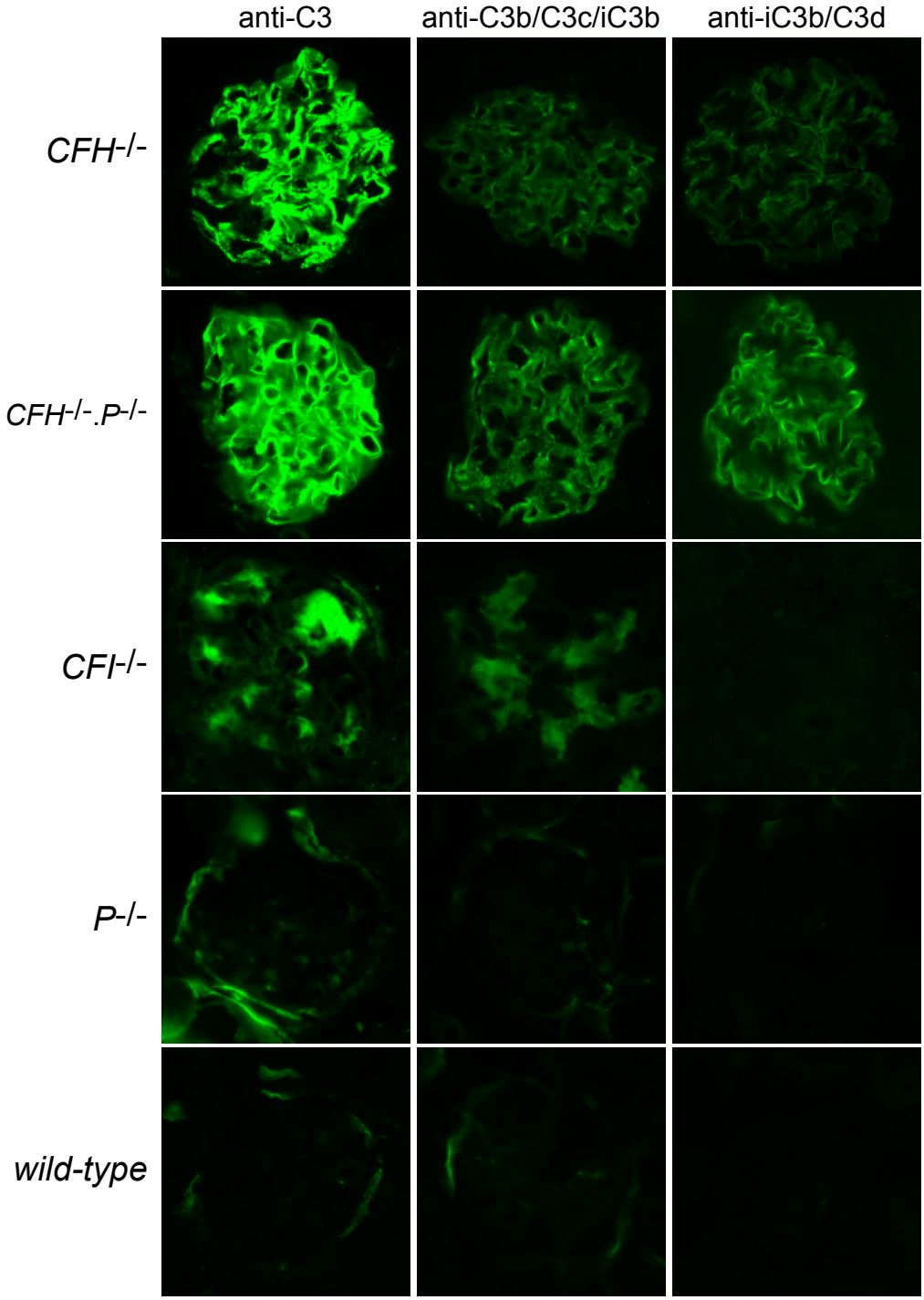
SUPPLEMENTAL FIGURE 3. **Glomerular C9 reactivity in *CFH*$^{-/-}$.*P*$^{-/-}$ mice.** (A) Representative images of glomerular C9 staining in *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice. Original magnification x40. (B) Quantitative immunofluorescence demonstrated that the median intensities did not differ between the two groups. Horizontal bars denote median values.



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SUPPLEMENTAL FIGURE 4. **Glomerular C3 immunostaining using two monoclonal anti-C3 antibodies with differential reactivity to tissue-bound C3 fragments.** Representative images of glomerular C3 staining in *CFH*$^{-/-}$, *CFH*$^{-/-}$.*P*$^{-/-}$, CFI-deficient mice (*CFI*$^{-/-}$), *P*$^{-/-}$ and wild-type animals using (i) goat anti-mouse C3 polyclonal antibody (anti-C3, at a dilution of 1:100), (ii) rat anti-mouse C3b/C3c/iC3b monoclonal antibody (anti-C3b/C3c/iC3b) and (iii) mouse anti-human iC3b/C3d monoclonal antibody (iC3b/C3d). Original magnification x40.

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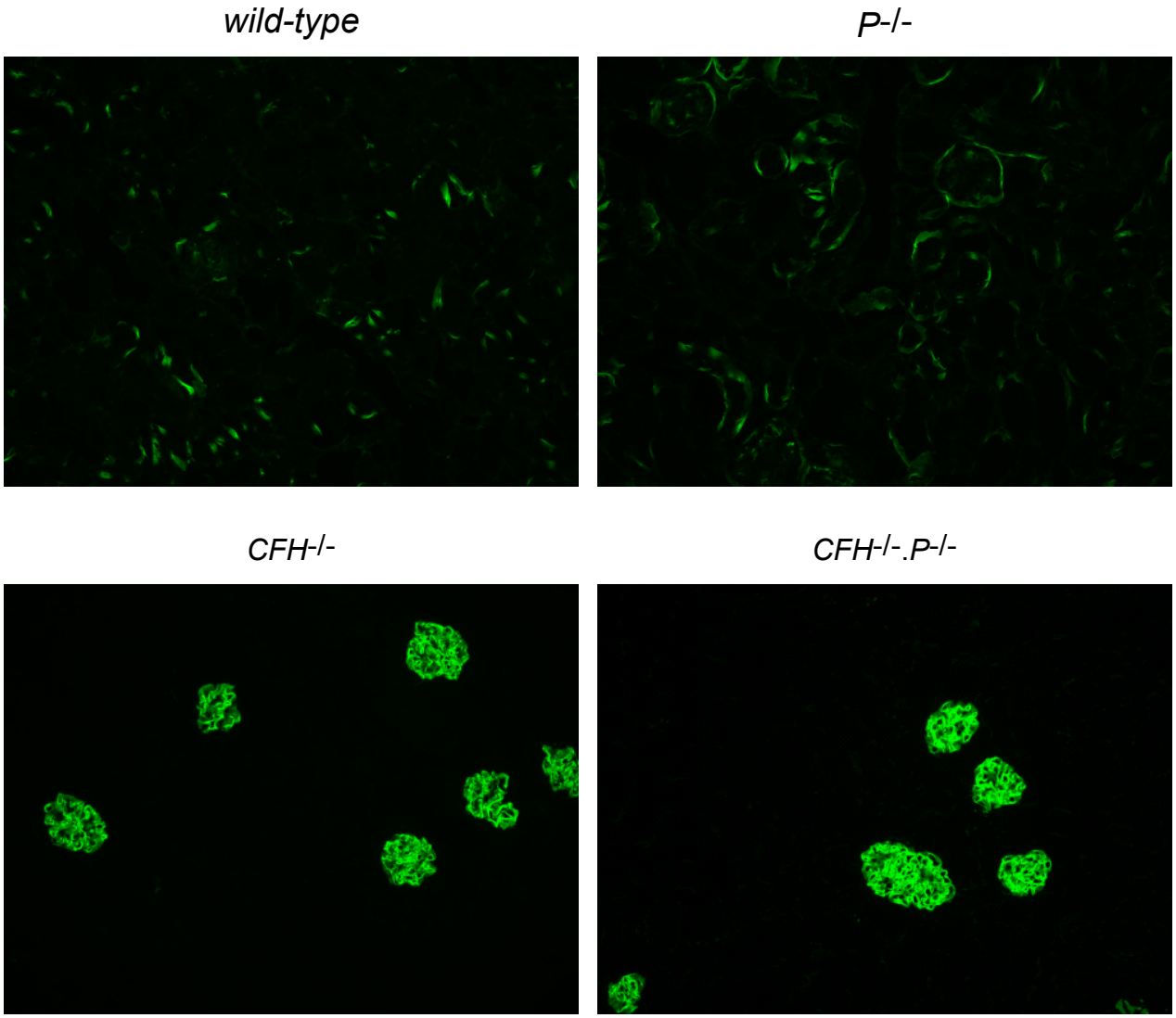

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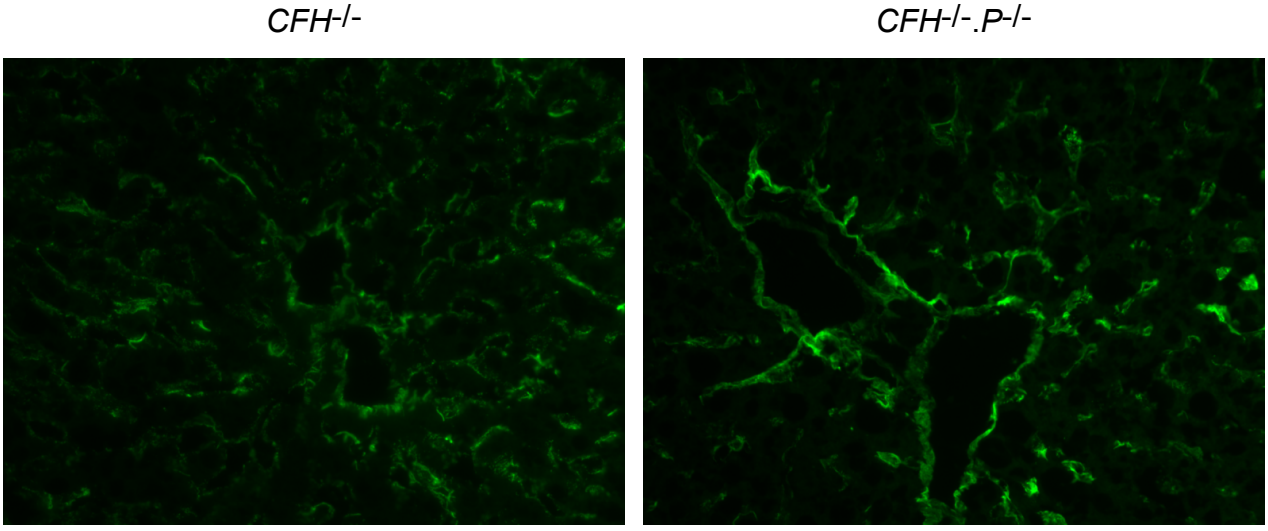

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SUPPLEMENTAL FIGURE 5. **(A) Tubulo-interstitial C3 immunostaining in wild-type, *P*$^{-/-}$, *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice.** Representative images of tubulo-interstitial C3 staining using a goat anti-mouse C3 polyclonal antibody (anti-C3) in wild-type, *P*$^{-/-}$, *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice. Tubulo-interstitial C3 staining was identical in wild-type and *P*$^{-/-}$ mice. As previously reported [4], tubulo-interstitial C3 staining was absent in *CFH*$^{-/-}$ mice. Similarly the staining was also absent in *CFH*$^{-/-}$.*P*$^{-/-}$ mice. Original magnification x20. **(B) Hepatic C3 staining in *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice.** Representative images of hepatic C3 staining using a goat anti-mouse C3 polyclonal antibody (anti-C3) in *CFH*$^{-/-}$ and *CFH*$^{-/-}$.*P*$^{-/-}$ mice. C3 reactivity is seen within hepatic sinusoids and the central veins to a comparable extent in both groups. Original magnification x40.

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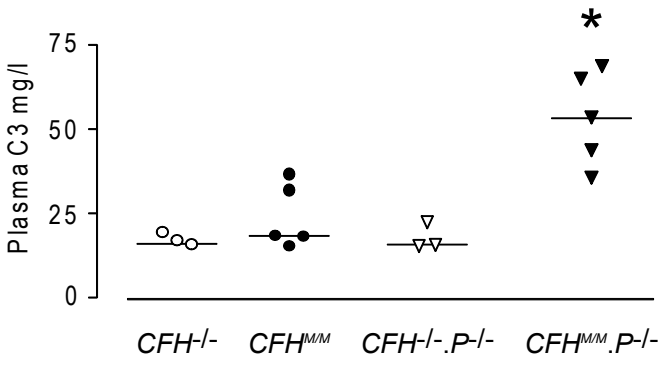

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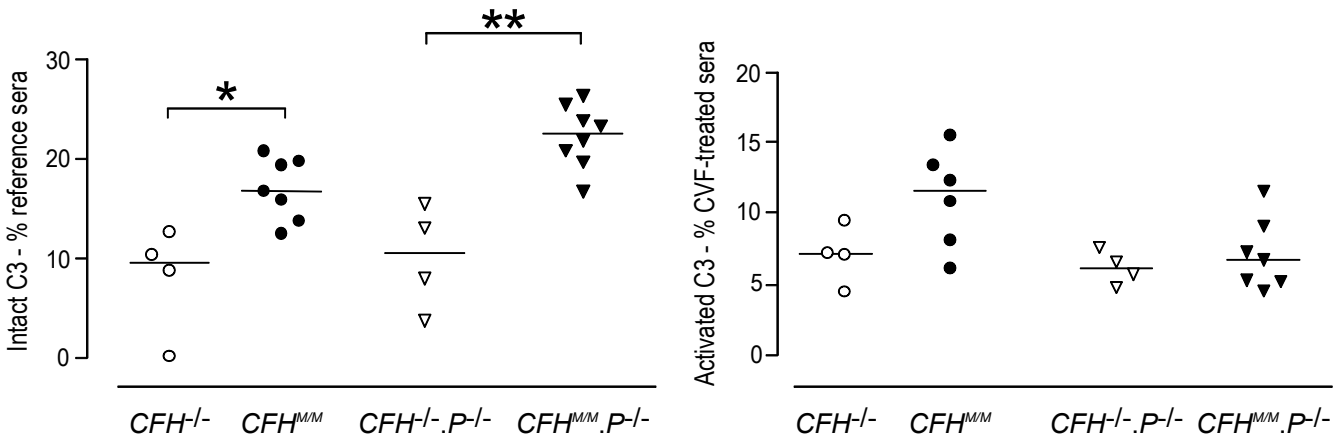

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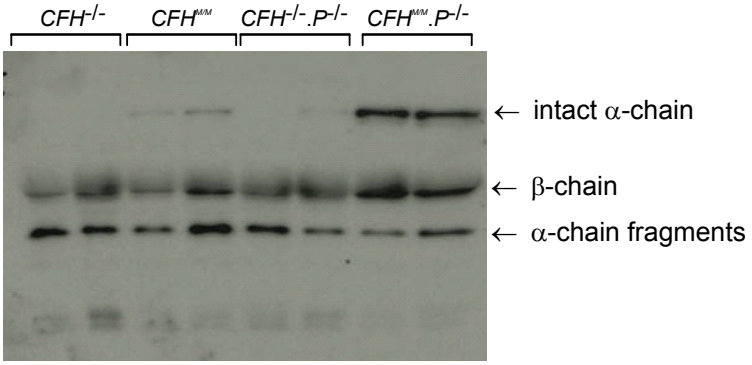

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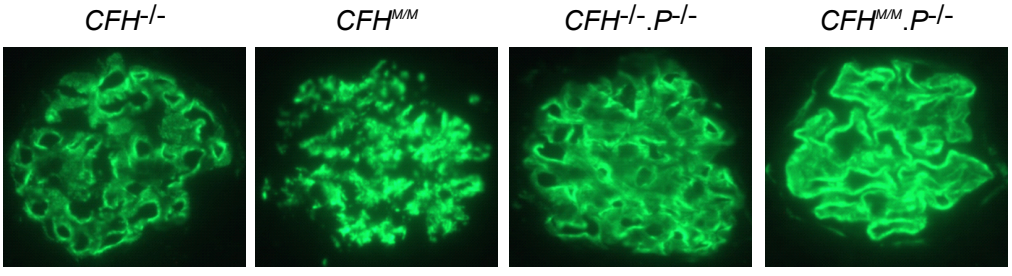

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SUPPLEMENTAL FIGURE 6. **Comparison of plasma C3 levels and glomerular C3 immunostaining in *CFH*$^{-/-}$, *CFH*$^{M/M}$, *CFH*$^{-/-}$.*P*$^{-/-}$ and *CFH*$^{M/M}$.*P*$^{-/-}$ mice**. (A) Plasma C3 levels were reduced in all four strains although the levels were significantly higher in the *CFH*$^{M/M}$.*P*$^{-/-}$ mice. * p<0.01 vs. *CFH*$^{-/-}$, *CFH*$^{M/M}$ or *CFH*$^{-/-}$.*P*$^{-/-}$ groups, Bonferroni's Multiple Comparison Test. Plasma C3 was measured by ELISA. Horizontal bars denote median values. (B) ELISA assays of intact and activated plasma C3 again showed that *CFH*$^{M/M}$.*P*$^{-/-}$ mice had the highest intact serum C3 levels. Values were normalized to reference samples as described in [23]. * p<0.01 and **p=0.004, Mann-Whitney test. CVF – Cobra venom factor. (C) Western blot for C3 under reducing conditions using sera from the four strains. Intact C3 α-chain was clearly seen in sera from *CFH*$^{M/M}$.*P*$^{-/-}$ mice and, with lower intensity, in sera from *CFH*$^{M/M}$ animals. One microlitre of EDTA plasma from the examined strains was loaded on the gel. (D) Representative images of glomerular C3 staining in *CFH*$^{-/-}$, *CFH*$^{M/M}$, *CFH*$^{-/-}$.*P*$^{-/-}$ and *CFH*$^{M/M}$.*P*$^{-/-}$ mice. *CFH*$^{M/M}$ animals had granular C3 glomerular staining pattern in contrast to the linear pattern seen in the other strains. Original magnification x40.