# Expanded View Figures

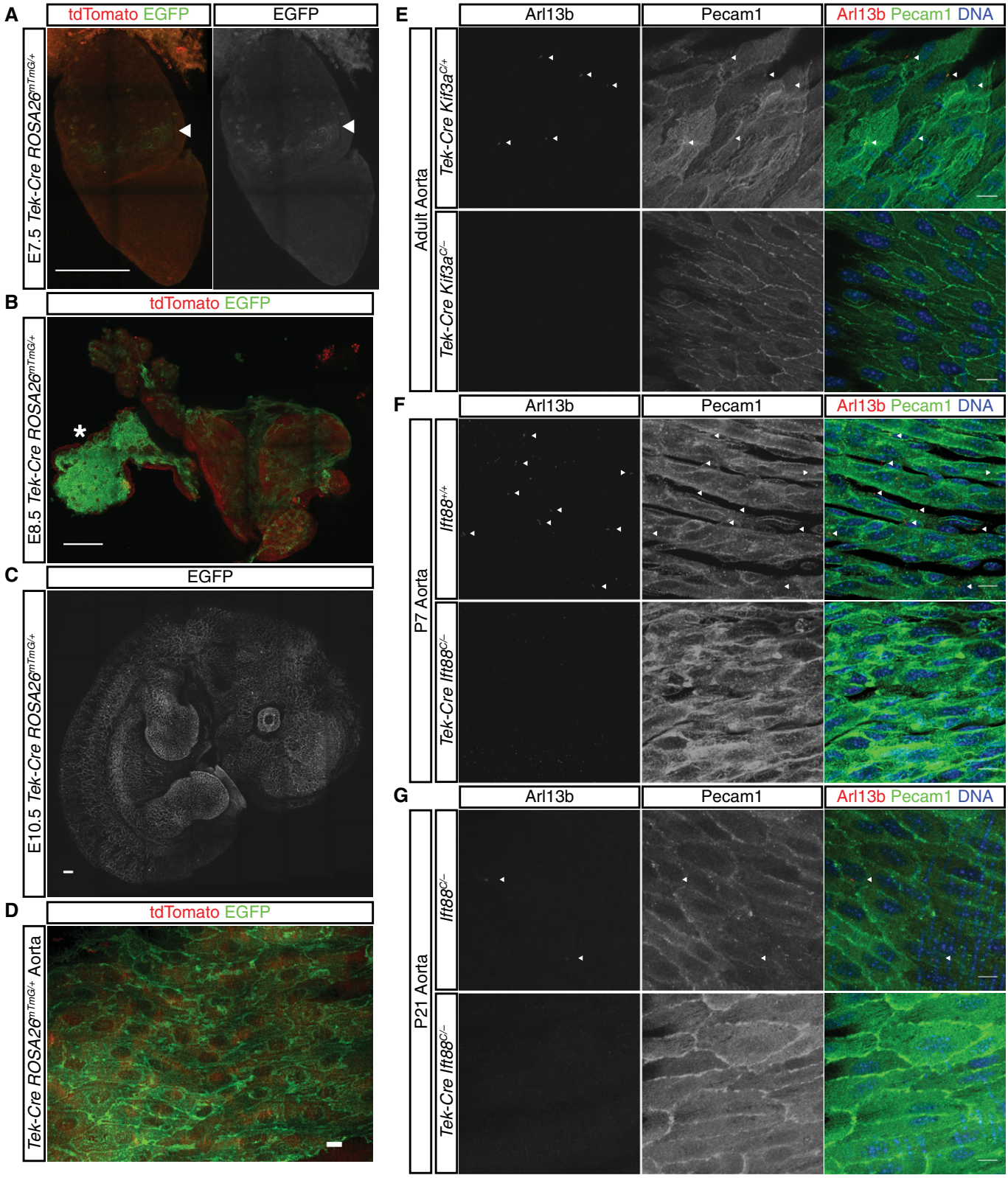


**Figure EV1.**

**Figure EV1. *Tek-Cre* mediates early, robust, and specific recombination in angioblasts.**

A–D *Tek-Cre* is active in the mouse embryo in *mTmG* reporter mice by (A) E7.5 and labeling persists at (B) E8.5, (C) E10.5, and (D) in the aorta of adult mice. The arrowheads in (A) indicate the initial site of recombination in extraembryonic mesoderm. The asterisk in (B) indicates the yolk sac.

E, F Primary cilia are efficiently removed from aortic endothelial cells by *Tek-Cre*-mediated removal of *Ift88* at (E) P5 and (F) P14.

G Primary cilia can also be ablated by *Tek-Cre*-mediated deletion of *Kif3a*. Endothelial cells and cilia are labeled by anti-Pecam1 staining (green) and anti-Arl13b staining (red).

Data information: Scale bars are 200 μm (A–C) and 10 μm (D–G). The arrowheads in (E–G) indicate primary cilia.

**A**

| *Tek-Cre Ift88*$^{+/-}$ x *Ift88*$^{C/C}$ | | | | | |
|---|---|---|---|---|---|
| | *Ift88*$^{C/+}$ | *Tek-Cre Ift88*$^{C/+}$ | *Ift88*$^{C/-}$ | *Tek-Cre Ift88*$^{C/-}$ | |
| Observed | 131 | 121 | 124 | 112 | |
| Expected | 122 | 122 | 122 | 122 | $P = 0.677$ ($\chi^2$ test) |

| *Tek-Cre Pkd2*$^{+/-}$ x *Pkd2*$^{C/C}$ | | | | | |
|---|---|---|---|---|---|
| | *Pkd2*$^{C/+}$ | *Tek-Cre Pkd2*$^{C/+}$ | *Pkd2*$^{C/-}$ | *Tek-Cre Pkd2*$^{C/-}$ | |
| Observed | 27 | 39 | 45 | 13 | |
| Expected | 31 | 31 | 31 | 31 | $P = 0.0002$ ($\chi^2$ test) |

| *Tek-Cre Kif3a*$^{+/-}$ x *Kif3a*$^{C/C}$ | | | | | |
|---|---|---|---|---|---|
| | *Kif3a*$^{C/+}$ | *Tek-Cre Kif3a*$^{C/+}$ | *Kif3a*$^{C/-}$ | *Tek-Cre Kif3a*$^{C/-}$ | |
| Observed | 78 | 91 | 80 | 75 | |
| Expected | 81 | 81 | 81 | 81 | $P = 0.614$ ($\chi^2$ test) |

| *Tek-Cre Kif3a*$^{C/C}$ *Pecam*$^{Gt/+}$ x *Kif3a*$^{C/C}$ *Pecam*$^{Gt/Gt}$ | | | | | |
|---|---|---|---|---|---|
| | *Kif3a*$^{C/C}$ *Pecam*$^{Gt/+}$ | *Tek-Cre Kif3a*$^{C/C}$ *Pecam*$^{Gt/+}$ | *Kif3a*$^{C/C}$ *Pecam*$^{Gt/Gt}$ | *Tek-Cre Kif3a*$^{C/C}$ *Pecam*$^{Gt/Gt}$ | |
| Observed | 4 | 7 | 4 | 2 | $P = 0.3926$ ($\chi^2$ test) |
| Expected | 4.25 | 4.25 | 4.25 | 4.25 | |

| *Tek-Cre Smo*$^{C/C}$ x *Smo*$^{C/C}$ | | | |
|---|---|---|---|
| | *Smo*$^{C/C}$ | *Tek-Cre Smo*$^{C/C}$ | |
| Observed | 55 | 56 | |
| Expected | 55.5 | 55.5 | $P > 0.999$ (2-tailed binomial test) |

**B**

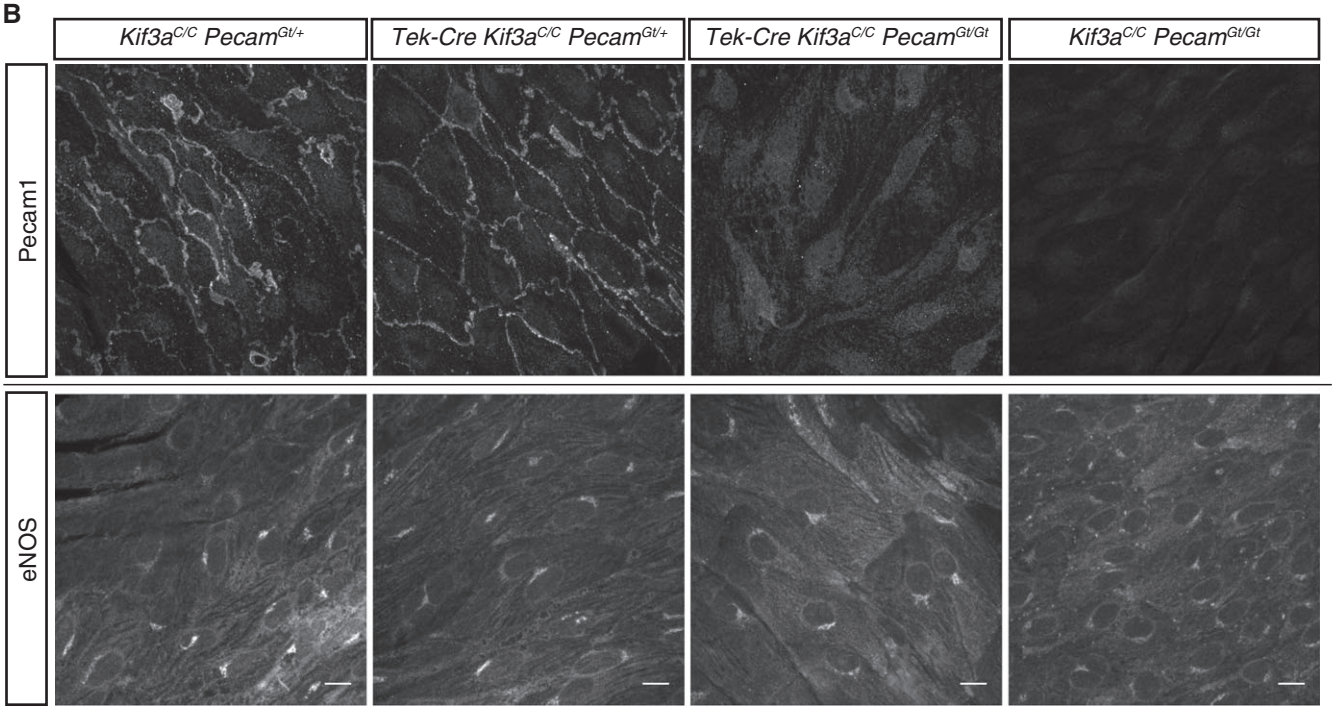


**Figure EV2. *Tek-Cre*-mediated depletion of *Ift88*, *Kif3a*, *Smo*, or *Kif3a* in a *Pecam*$^{Gt/Gt}$ background does not affect ratios at weaning, whereas *Tek-Cre Pkd2* mice are subviable.**

A Mice from the indicated crosses were weaned and genotyped at P21. Statistics (test indicated in table) suggest only *Pkd2* endothelial cell deletion results in subviability.

B Littermate *Tek-Cre Kif3a Pecam* mice of the indicated genotypes were sacrificed at P604 and stained for Pecam1 and eNOS (in different fields). Loss of Pecam1, EC cilia, or both did not affect aortic EC eNOS expression. Scale bars, 10 μm.

**Figure EV3. *Apoe*$^{-/-}$ mice lacking EC cilia show increased atherosclerosis at the aortic sinus.**

A The aortic sinuses from a cohort of male *Apoe*$^{-/-}$ mice with and without EC cilia were analyzed histologically with the indicated stains to determine plaque size and composition.

B Quantitation of the ORO$^{+}$ lesion area showed a trend for increased lesion size in mice lacking EC cilia (88% increase over controls, $P = 0.1338$, Student's two-tailed *t*-test, $n = 5$ mice each genotype). Black bars represent the mean and error bars are $\pm$ 1 SEM. Data points for sinuses in (A) are indicated in red.

C The aortic sinuses of female *Apoe*$^{-/-}$ mice with and without EC cilia were stained for CD68 and smooth muscle actin (SMA) following 12 weeks of high-fat, high-cholesterol diet. Scale bars, 250 μm.

D Quantitation of plaque composition from (C) showed no statistically significant differences between mice with and without EC cilia, although there was a trend for increased macrophage (CD68$^{+}$) area. Black bars represent the mean and error bars are $\pm$ 1 SEM. Data points for sinuses in (C) are indicated in red. Student's two-tailed *t*-test, $n = 5$ control and $n = 4$ mutant mice.

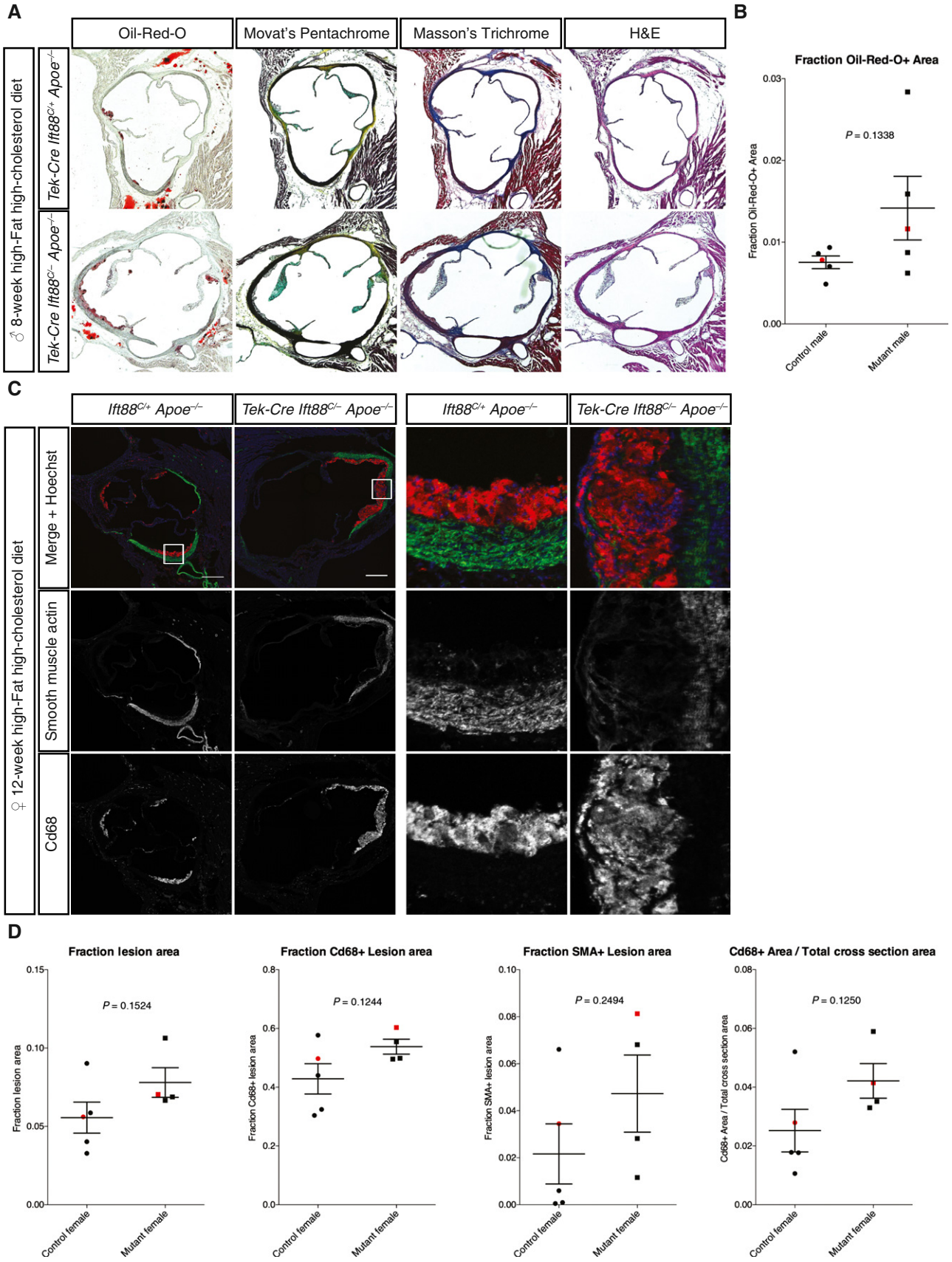


**Figure EV3.**

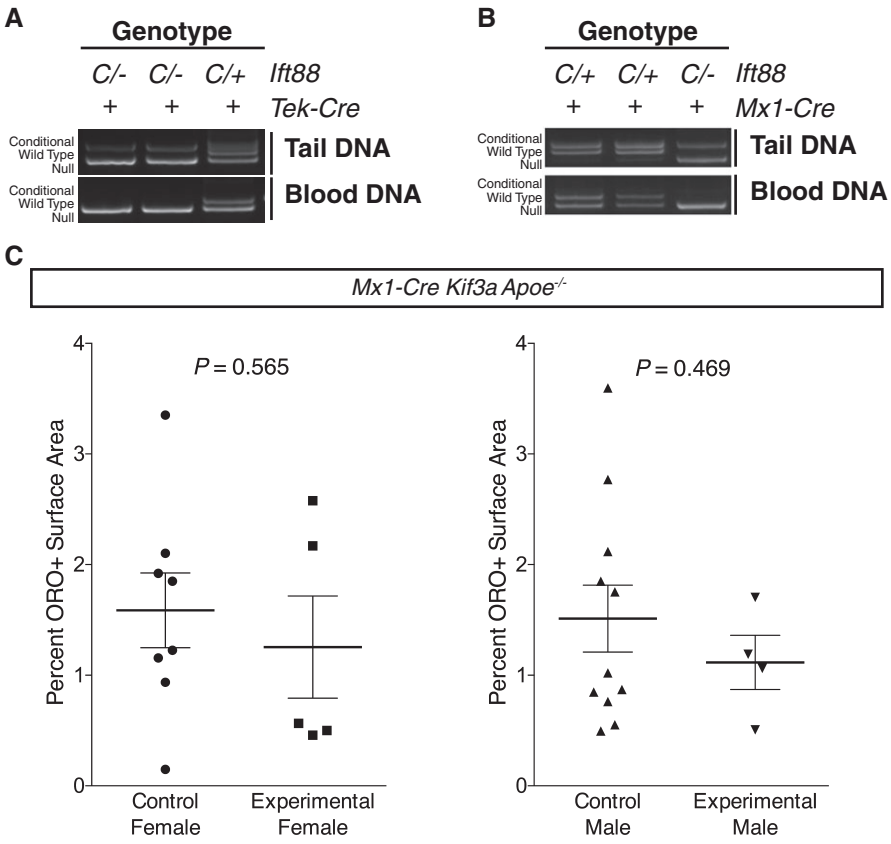


**Figure EV4. Extrahematopoietic, but not blood recombination, differs between *Tek-Cre* and *Mx1-Cre* mice and loss of *Kif3a* via *Mx1-Cre* does not affect atherosclerosis.**

A, B Tail and peripheral blood DNA was isolated from adult *Ift88* mice of the indicated genotypes carrying either (A) *Tek-Cre* or (B) *Mx1-Cre*. Genotyping for *Ift88* indicated total loss of the conditional allele in blood from both Cre lines. However, only *Tek-Cre* caused noticeable recombination in tail DNA, reflecting vascular recombination in the *Tek-Cre* but not *Mx1-Cre* lines. In each panel, the same 3 mice were assayed for blood and tail recombination and are presented in the same order. "C" indicates conditional allele, "+" indicates the wild-type allele, and "-" indicates the recombined null allele. The listed genotypes are the germ line genotypes of each mouse.

C Atherosclerosis was induced in control (*Mx1-Cre* $Kif3a^{C/+}$ $Apoe^{-/-}$, $Kif3a^{C/+}$ $Apoe^{-/-}$, $Kif3a^{C/-}$ $Apoe^{-/-}$) and mutant (*Mx1-Cre* $Kif3a^{C/-}$ $Apoe^{-/-}$) mice and the percent atherosclerosis assessed by oil red O staining. No difference was observed between control and experimental mice in males ($P$ = 0.4692, Student's two-tailed $t$-test, $n$ = 11 control and $n$ = 4 experimental mice) or females ($P$ = 0.5653, Student's two-tailed $t$-test, $n$ = 8 control and $n$ = 5 experimental mice). Horizontal bars are the mean and error bars are ± 1 SEM.

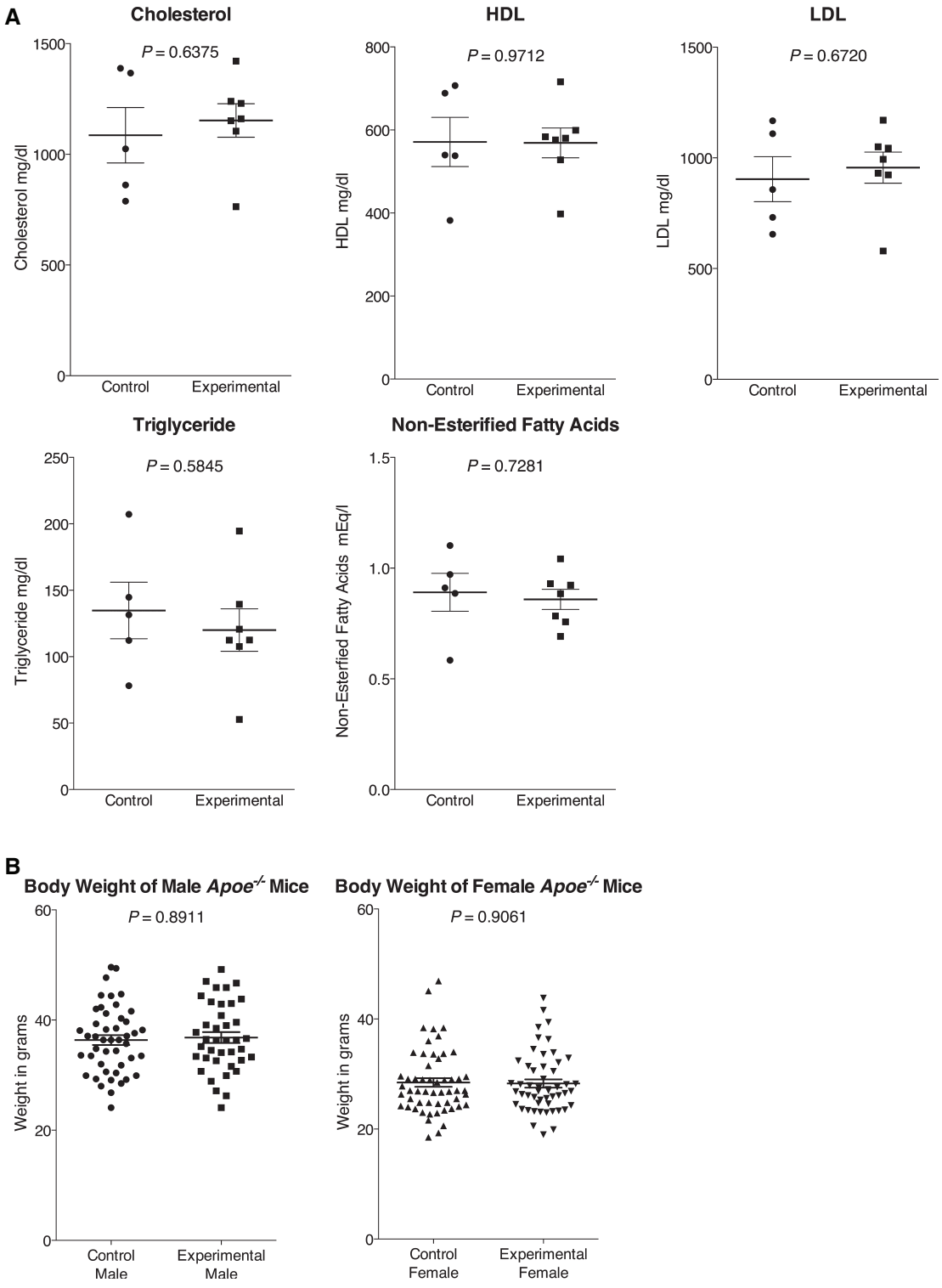


**Figure EV5. Serum lipid profiles and body weight are unchanged by loss of EC cilia in *Apoe*$^{-/-}$ mice.**

A Serum levels of cholesterol, HDL, LDL, triglyceride, and non-esterified fatty acids were measured in $n$ = 5 control (*Tek-Cre Ift88*$^{C/+}$ *Apoe*$^{-/-}$, *Ift88*$^{C/+}$ *Apoe*$^{-/-}$, *Ift88*$^{C/-}$ *Apoe*$^{-/-}$) and $n$ = 7 experimental (*Tek-Cre Ift88*$^{C/-}$ *Apoe*$^{-/-}$) female mice. Large horizontal bars are the mean and error bars are ± 1 SEM.

B There is no difference in body weight in *Apoe*$^{-/-}$ mice lacking EC cilia among males ($P$ = 0.8911, Kolmogorov–Smirnov test, $n$ = 46 control and $n$ = 39 mice) or females ($P$ = 0.9061, Kolmogorov–Smirnov test, $n$ = 55 control and $n$ = 52 mutant mice). These data include mice used for other experiments, hence the increased $n$. Horizontal bars are the mean and error bars are ± 1 SEM.