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**Supplemental information**

**Systemic gene therapy using an AAV44.9 vector  
rescues a neonatal lethal mouse model  
of propionic acidemia**

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**Table S1. Relative hepatic and cardiac PCCA protein levels**

Genotype	Liver	Heart
<i>Pcca</i> <sup>+/+</sup> (n=2)	100±2.4	100±20.3
<i>Pcca</i> <sup>-/-</sup> (n=2)	ND	ND
<i>Pcca</i> <sup>-/-</sup> + AAV44.9 (n=3)	44.7±4.4	214.3±18.9

PCCA protein levels from Figure 4D shown as a mean percentage±SEM of *Pcca*<sup>+/+</sup> wildtype PCCA protein levels after normalization with ACTB (liver) or GAPDH (heart).

A.

**NM\_144844.2 Mus musculus *Pcca* var1 with c.398\_401delAAGC mutation**

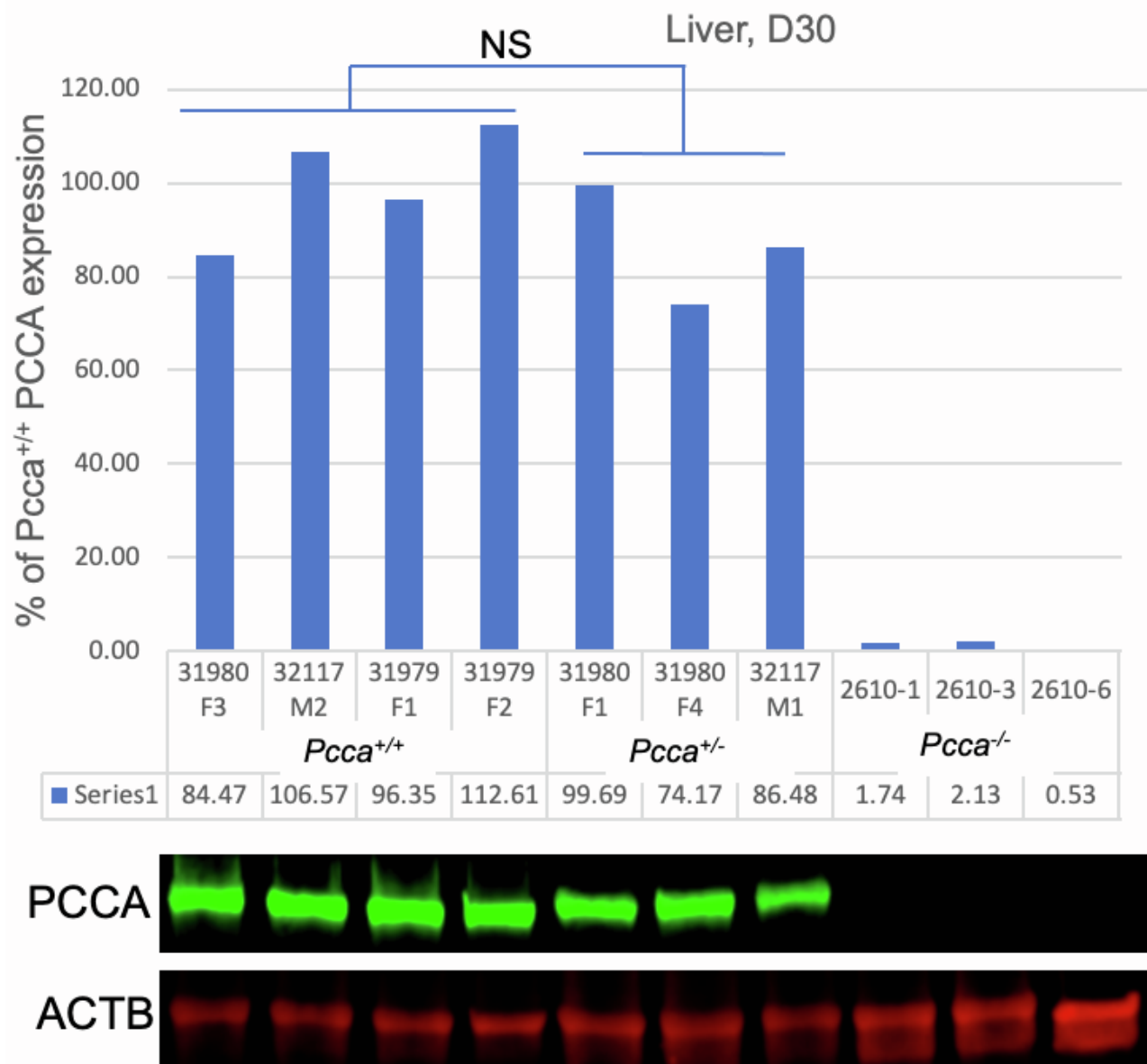
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B.

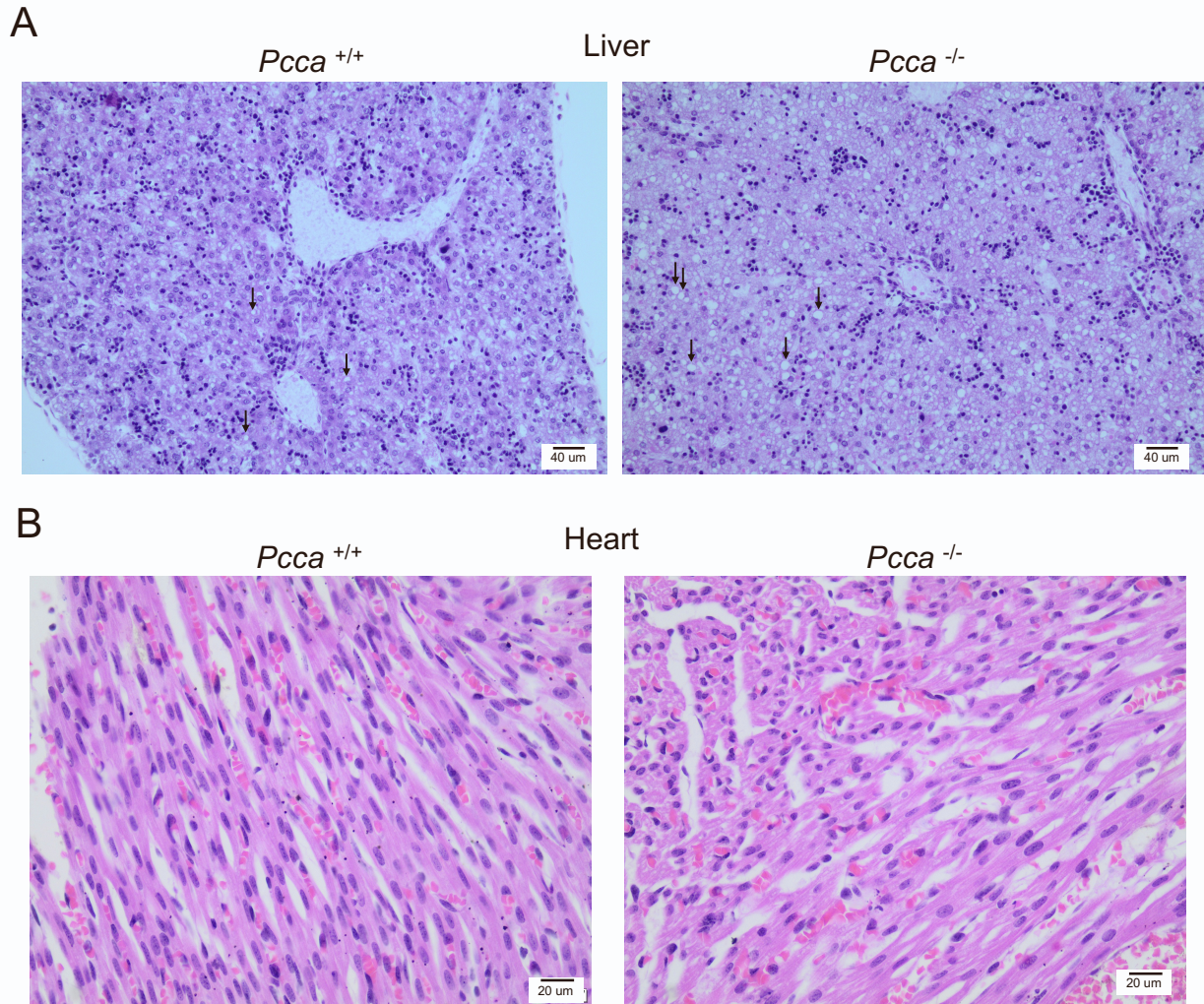
**Predicted PCCA protein p.Gln133Leufs\*41**

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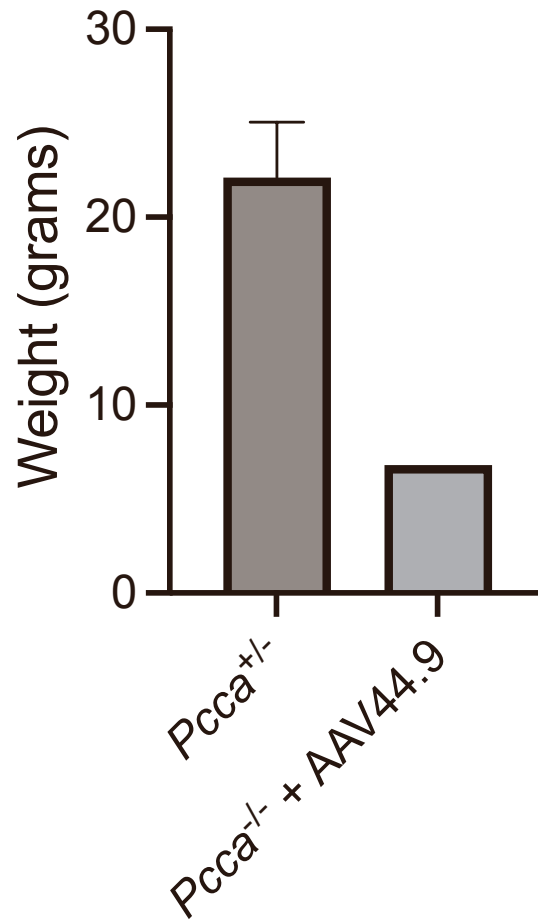
**Figure S1. DNA and Protein Sequence of new murine *Pcca* PA disease allele A. cDNA sequence of NM\_144844.2 Mus musculus *Pcca* var1 with c.398\_401delAAGC mutation B. Predicted amino acid of sequence of PCCA p.Gln133Leufs\*41**



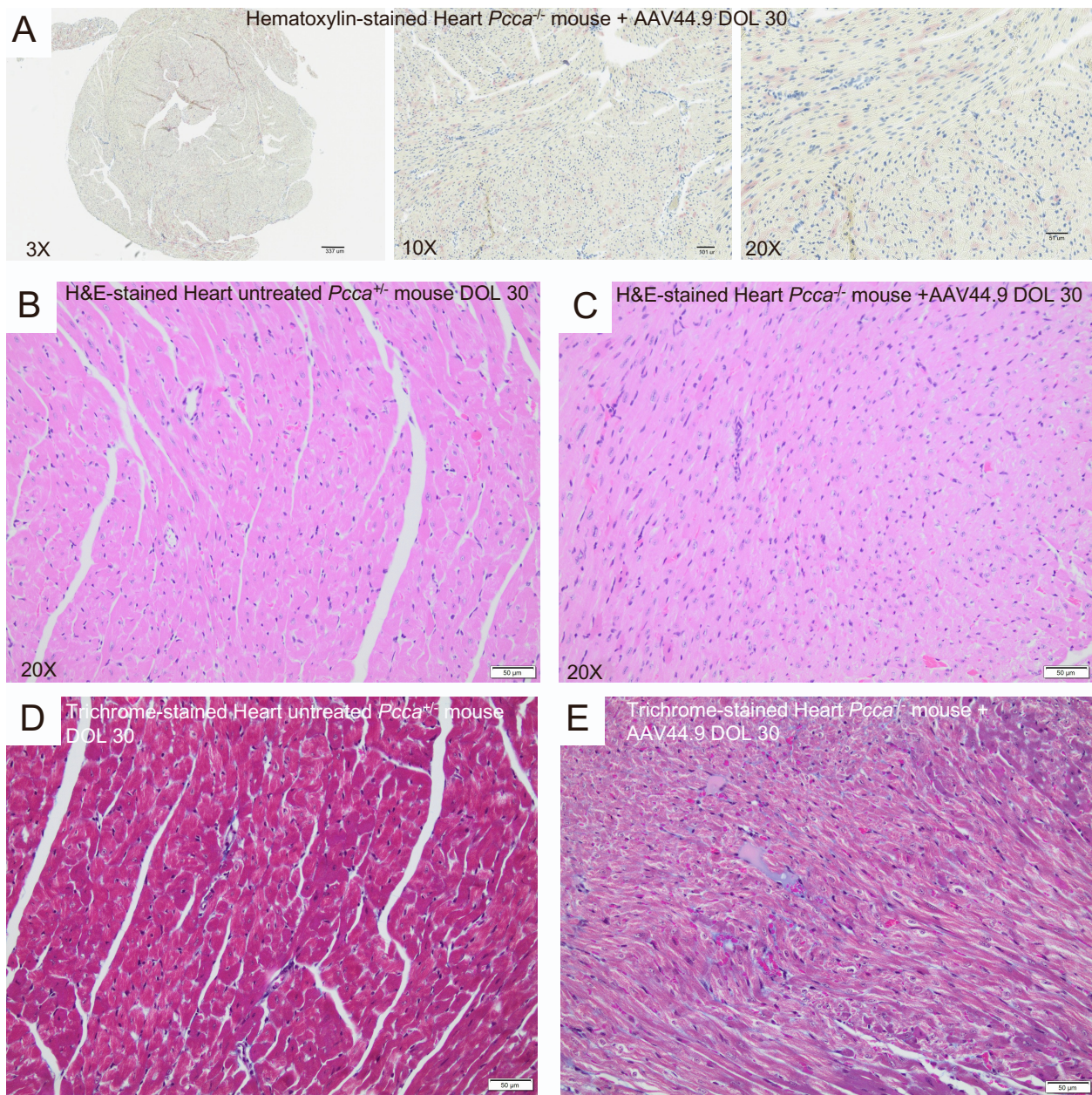
**Figure S2.** Immunoblot of 50  $\mu$ g hepatic protein from a *Pcca*<sup>+/+</sup>, *Pcca*<sup>+/-</sup> and a *Pcca*<sup>-/-</sup> newborn pups for the PCCA protein using ACTB as a loading control. The amount of PCCA protein expression from the immunoblot was used to determine the percent of the wildtype PCCA expression after normalization with the loading control using ImageJ software



**Figure S3. Liver and heart histopathology.** **A.** Liver (10X) H&E stained neonatal *Pcca*<sup>-/-</sup> mice (untreated day of life 1) have diffuse mixed microvesicular and macrovesicular lipidoses (see as clear vacuoles indicated by arrows) compared to *Pcca*<sup>+/+</sup> mice. **B.** Heart (20X) H&E stained tissues from *Pcca*<sup>-/-</sup> and *Pcca*<sup>+/+</sup> mice (untreated day of life 1). No notable changes in the *Pcca*<sup>-/-</sup> mice were noted.



**Figure S4. Weight of AAV44.9 treated litter mates.** *Pcca*<sup>+/-</sup> (n=2) and a *Pcca*<sup>-/-</sup> (n=1) AAV44.9 treated male littermates at 2 months of age.



**Figure S5. Cardiac histology.** **A.** Hematoxylin-stained heart from a AAV44.9 treated *Pcca*<sup>-/-</sup> mice DOL 30 Heart 3X, 10X and 20X (left to right). **B.** H&E-stained heart DOL 30 wildtype (*Pcca*<sup>+/-</sup>) untreated control 20X. **C.** H&E-stained heart DOL 30 *Pcca*<sup>-/-</sup> AAV44.9 treated mouse. **D.** Trichrome DOL 30 wildtype (*Pcca*<sup>+/-</sup>) untreated control. **E.** Masson's Trichrome stained heart DOL 30 *Pcca*<sup>-/-</sup> AAV44.9 treated mouse. No notable changes in the *Pcca*<sup>-/-</sup> mice were noted in any of the stains. *Pcca*<sup>-/-</sup> mouse was treated with a dose of 1e11vg at DOL1. DOL (Day of life)