after reinjection (lanes 6 through 9). The mitochondrial loading control shows approximately the same intensity in each sample. (d) Mut expression in kidney extracts prepared from *Mut*$^{+/-}$ and *Mut*$^{-/-}$ mice after systemic re-administration of rAAV9-CBA-mMut at 1 year of life. The animals are the same group studied in (c). 30 μg of clarified whole kidney extracts were analyzed for transgene expression by western blotting in exactly the same fashion as for the liver extracts. Immunoreactive Mut enzyme is present in the untreated *Mut*$^{+/-}$ extract, but not in an untreated *Mut*$^{-/-}$ animal (lane 3) or *Mut*$^{-/-}$ mouse that was studied 1 year after receiving a single neonatal injection of rAAV9-CBA-mMut (lane 4). However, in the *Mut*$^{-/-}$ mice that received a second injection of rAAV9-CBA-mMut 1 year after birth, immunoreactive enzyme was detected 1 month (lane 5) and 2 months (lanes 6, 7 and 9) after retreatment. The mitochondrial loading control shows approximately the same intensity in each sample.

Supplemental figures:

Figure S1: Growth after systemic re-administration.

rAAV9-CBA-mMut treated *Mut*$^{-/-}$ mice were reinjected at 1 year of age (n=8) and compared to similarly treated *Mut*$^{+/-}$ diet and gender-matched littermates (n=9). The graph depicts the percent weight at day 14 and 60 post rAAV9 re-administration. Treated *Mut*$^{-/-}$ mice re-injected with rAAV9-CBA-mMut did not show significant growth improvement compared to *Mut*$^{+/-}$ diet and gender-matched littermates (*** $p< 0.001$). Error bars represent plus and minus one standard deviation.

Figure S2: Renal histology.

Histology (hematoxylin and eosin stain) of kidney from a *Mut*$^{+/-}$ control animal (*a*) and an rAAV9-CBA-mMut treated *Mut*$^{-/-}$ knockout littermate sacrificed at 12 months of age (*b*). Both had received a single intrahepatic injection in the neonatal period. Histological findings were also normal in the other rAAV9-CBA-mMut treated *Mut*$^{-/-}$ mice studied 1 month after re-administration of rAAV9-CBA-mMut (n=4, data not presented). Renal histology from older, untreated *Mut*$^{-/-}$ animals were not available for this study but have been previously described (see Reference 34, Figure 2)

Figure S3: Liver function tests before and after readministration.

The level of plasma alanine transaminase (ALT) (a) and aspartate transaminase (AST) (b) concentrations were measured to ascertain whether rAAV9 gene delivery was associated with transaminitis. The horizontal line represents the mean ALT or AST plasma concentration for each group. rAAV9-CBA-mMut *Mut*$^{-/-}$ mice displayed similar ALT and AST concentrations at 1 year of life and 2 months after re-administration of rAAV9-CBA-mMut. These concentrations were not significantly different between untreated or treated *Mut*$^{+/-}$ mice. One rAAV9-CBA-mMut treated *Mut*$^{-/-}$ mouse in this study displayed high ALT and AST concentrations that increased after reinjection. Results are reported as units per liter (U/L).