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Supplemental information

mRNA-based therapy proves superior to the standard of care for treating hereditary tyrosinemia 1 in a mouse model

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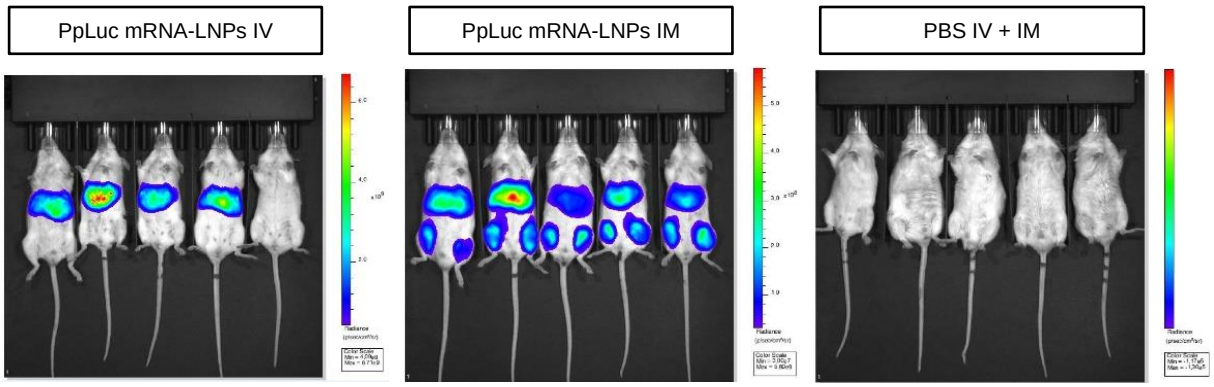
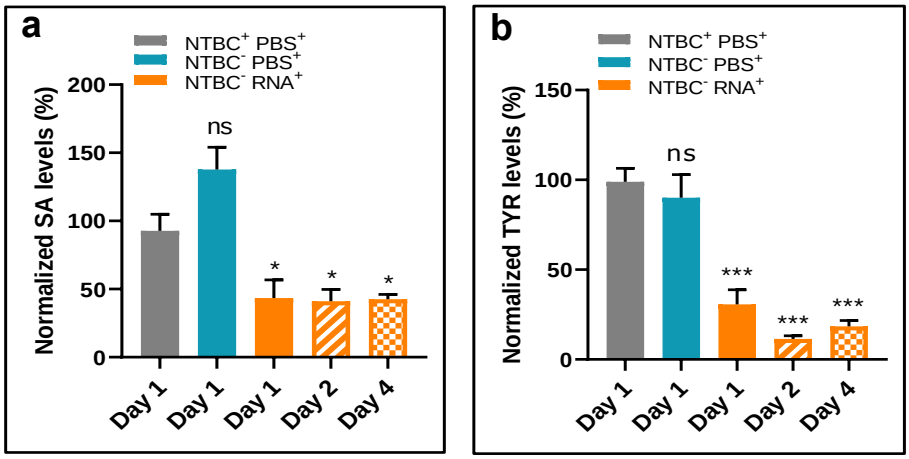
Supplemental Information

Supplemental Figure 1



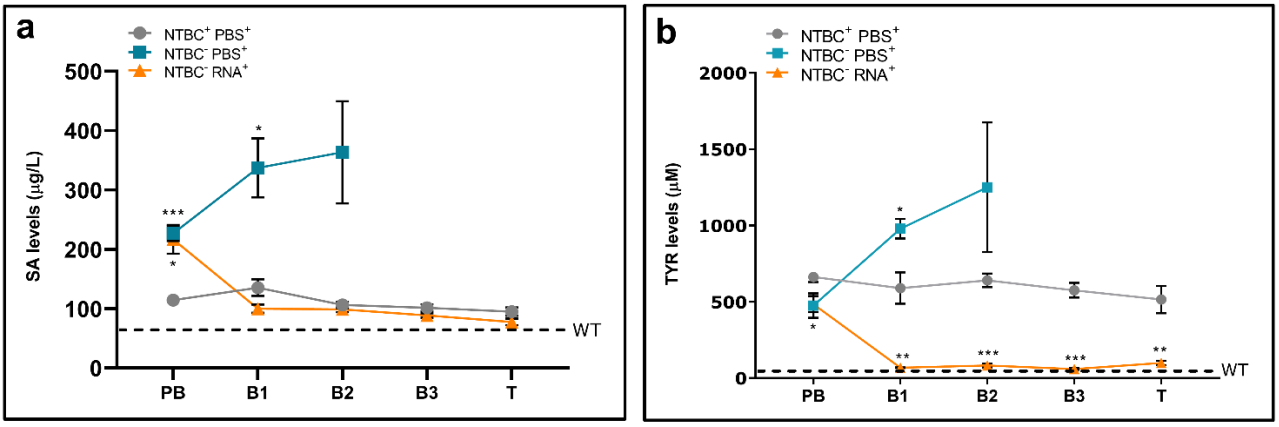
Supplemental Figure 1. *PpLuc* mRNA-LNPs induced luciferase expression in *Fah*-deficient mice. *In vivo* imaging of *Fah*-deficient mice 6 hours after a single injection of PpLuc mRNA-LNPs IV (left panel) or IM (center panel). Mice injected with PBS IV and IM are shown in the right panel. Note: Mouse number 5 in the left panel was not injected with luciferin substrate.

Supplemental Figure 2



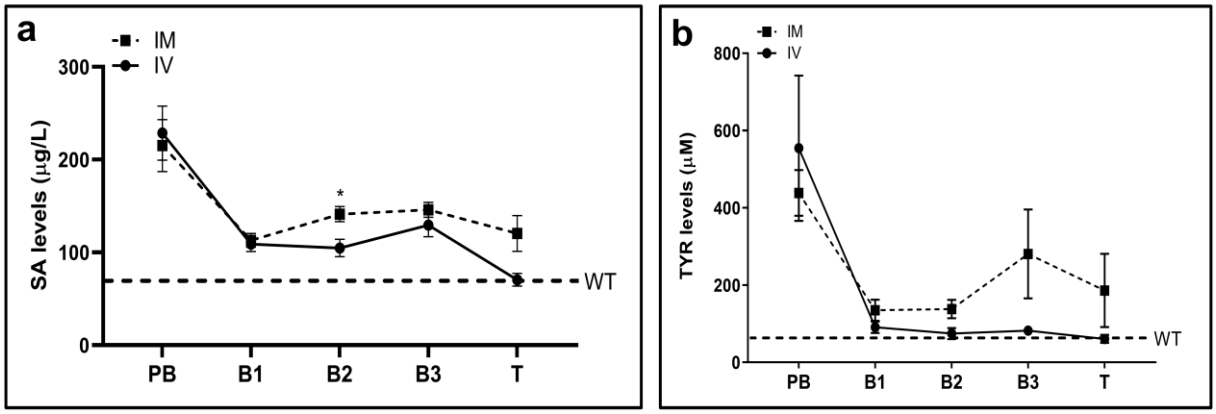
Supplemental Figure 2. Reduction in serum succinylacetone and tyrosine levels in *Fah*-deficient mice after single IV injection of *FAH* mRNA-LNPs. Normalized serum **(a)** succinylacetone (SA) and **(b)** tyrosine (TYR) levels in *Fah*-deficient mice at 1, 2, and 4 days after single IV injection. Dashed lines indicate metabolite levels in WT mouse serum. Data are the means +/- SEM. Significance tested versus NTBC^{+} PBS^{+}: *P<0.05; ***P<0.001; ns P>0.05 (two-tailed Student´s t-test).

Supplemental Figure 3



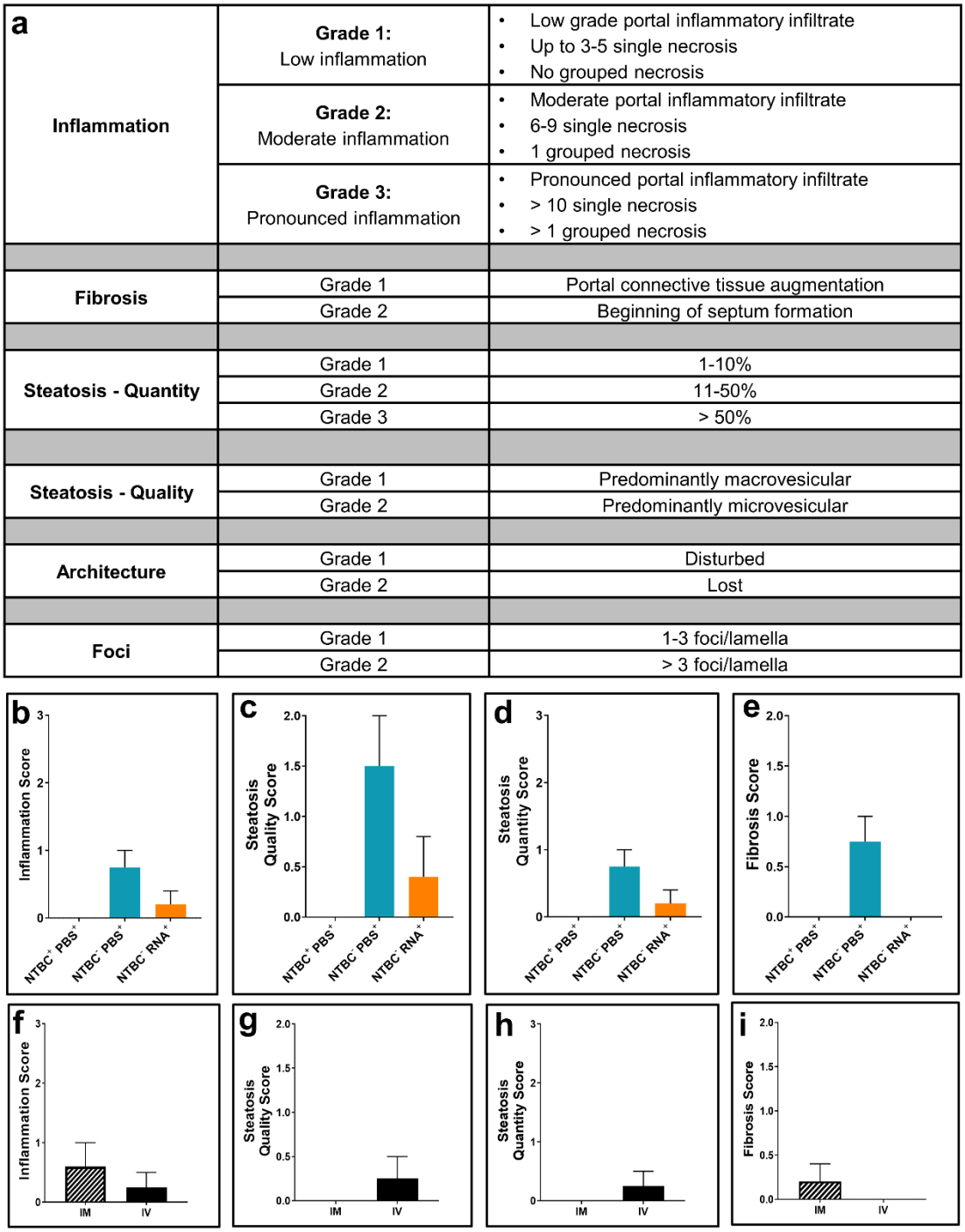
Supplemental Figure 3. Repeated IV injections of *FAH* mRNA-LNPs reduced pathologic increases in succinylacetone and tyrosine in *Fah*-deficient mice. *Fah*-deficient mice, +/- NTBC supplementation, were injected IV 5 times every 5 days with *FAH* mRNA-LNPs. Absolute serum **(a)** succinylacetone (SA) and **(b)** tyrosine (TYR) levels were determined at times indicated. Dashed lines indicate metabolite levels in WT mouse serum. Data are the means +/- SEM. **P*<0.05; ***P*<0.01; ****P*<0.001; ns *P*>0.05 for all other comparisons, not indicated (two-tailed Student´s *t*-test).

Supplemental Figure 4



Supplemental Figure 4. Reduced succinylacetone and tyrosine levels in *Fah*-deficient mouse serum after repeated IV or IM injections of *FAH* mRNA-LNPs. *Fah*-deficient mice, on a NTBC-free diet 5 days prior injection, were injected IV or IM with *FAH* mRNA-LNPs 5 times every 5 days. Absolute serum **(a)** succinylacetone (SA) and **(b)** tyrosine (TYR) levels were determined at times indicated. Dashed lines indicate metabolite levels in WT mouse serum. Data are the mean +/- SEM. P values were obtained from two-tailed Student´s t-test. * P<0,05; all other comparisons are not significant P>0,05 (not indicated).

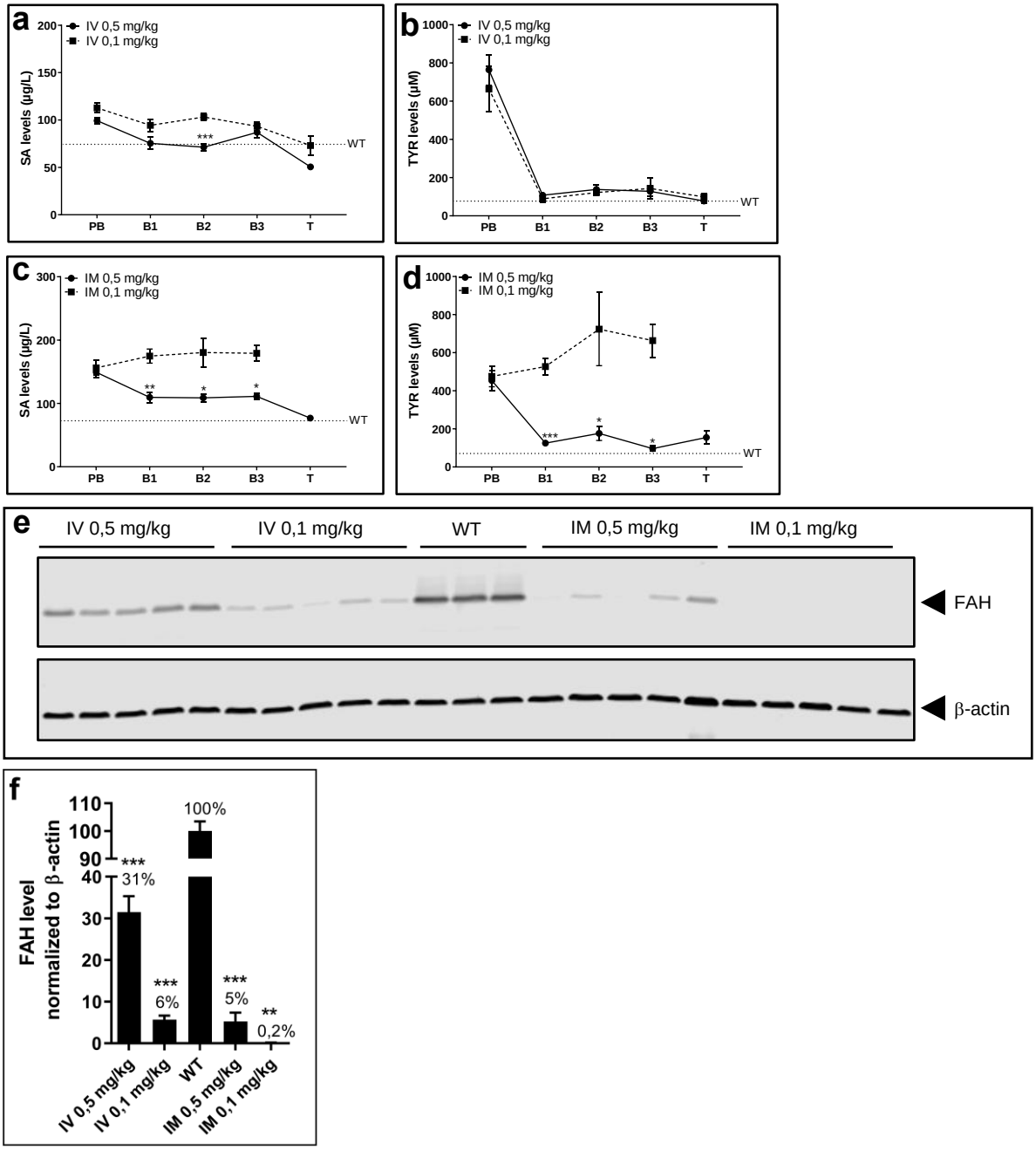
Supplemental Figure 5



a		
Inflammation	**Grade 1:** Low inflammation	• Low grade portal inflammatory infiltrate • Up to 3-5 single necrosis • No grouped necrosis
	Grade 2: Moderate inflammation	• Moderate portal inflammatory infiltrate • 6-9 single necrosis • 1 grouped necrosis
	Grade 3: Pronounced inflammation	• Pronounced portal inflammatory infiltrate • > 10 single necrosis • > 1 grouped necrosis
Fibrosis	Grade 1	Portal connective tissue augmentation
	Grade 2	Beginning of septum formation
Steatosis - Quantity	Grade 1	1-10%
	Grade 2	11-50%
	Grade 3	> 50%
Steatosis - Quality	Grade 1	Predominantly macrovesicular
	Grade 2	Predominantly microvesicular
Architecture	Grade 1	Disturbed
	Grade 2	Lost
Foci	Grade 1	1-3 foci/lamella
	Grade 2	> 3 foci/lamella

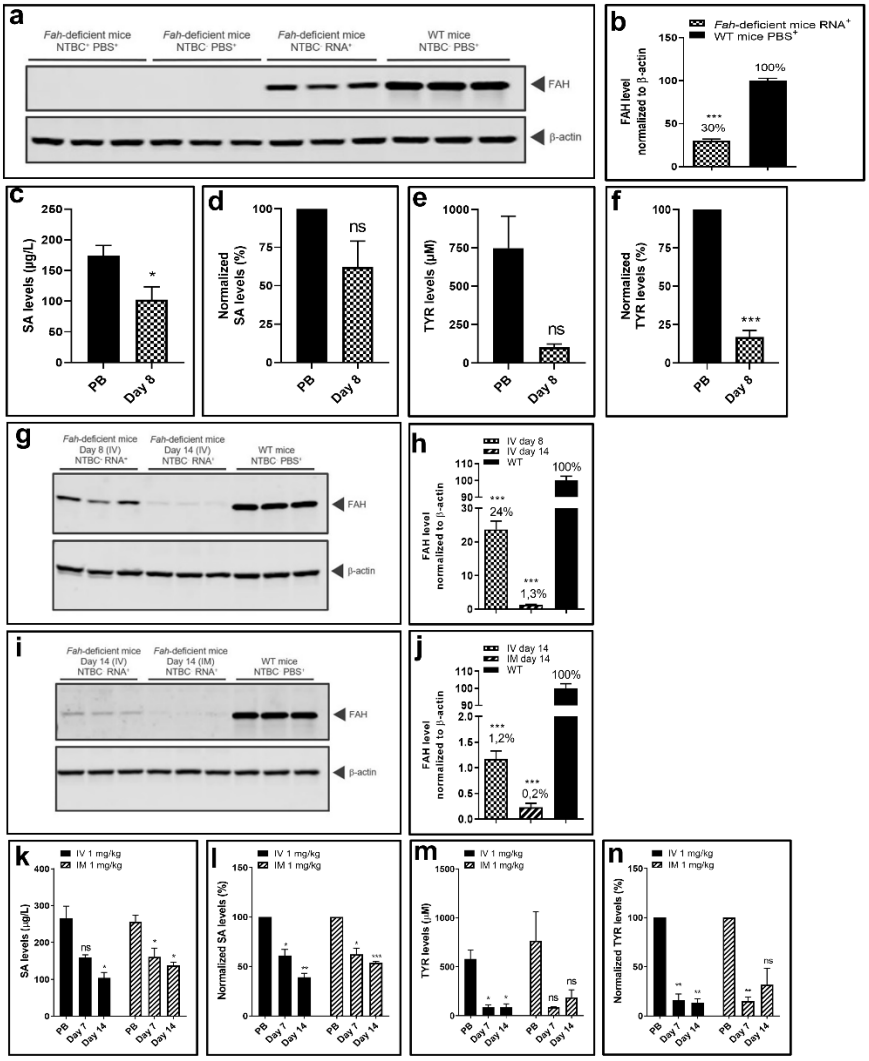
Supplemental Figure 5. Histological analysis of paraffin-embedded *Fah*-deficient mouse livers after repeated IV and IM injections of *FAH* mRNA-LNPs. (a) Scaling system used for evaluating liver histopathology. *Fah*-deficient mouse livers dissected on day of termination after repeated IV injections of 1 mg/kg *FAH* mRNA-LNPs were analyzed and compared to $NTBC^{+}PBS^{+}$ and $NTBC^{-}PBS^{+}$ controls. **(b)** Quantified inflammation score. **(c)** Quality steatosis score **(d)** Quantified steatosis score. **(e)** Fibrosis score. Histological analysis comparing *Fah*-deficient mouse livers dissected on day 21 after repeated IV or IM injections of 1 mg/kg *FAH* mRNA-LNPs. **(f)** Quantified inflammation score. **(g)** Quality steatosis score. **(h)** Quantified steatosis score. **(i)** Fibrosis score.

Supplemental Figure 6



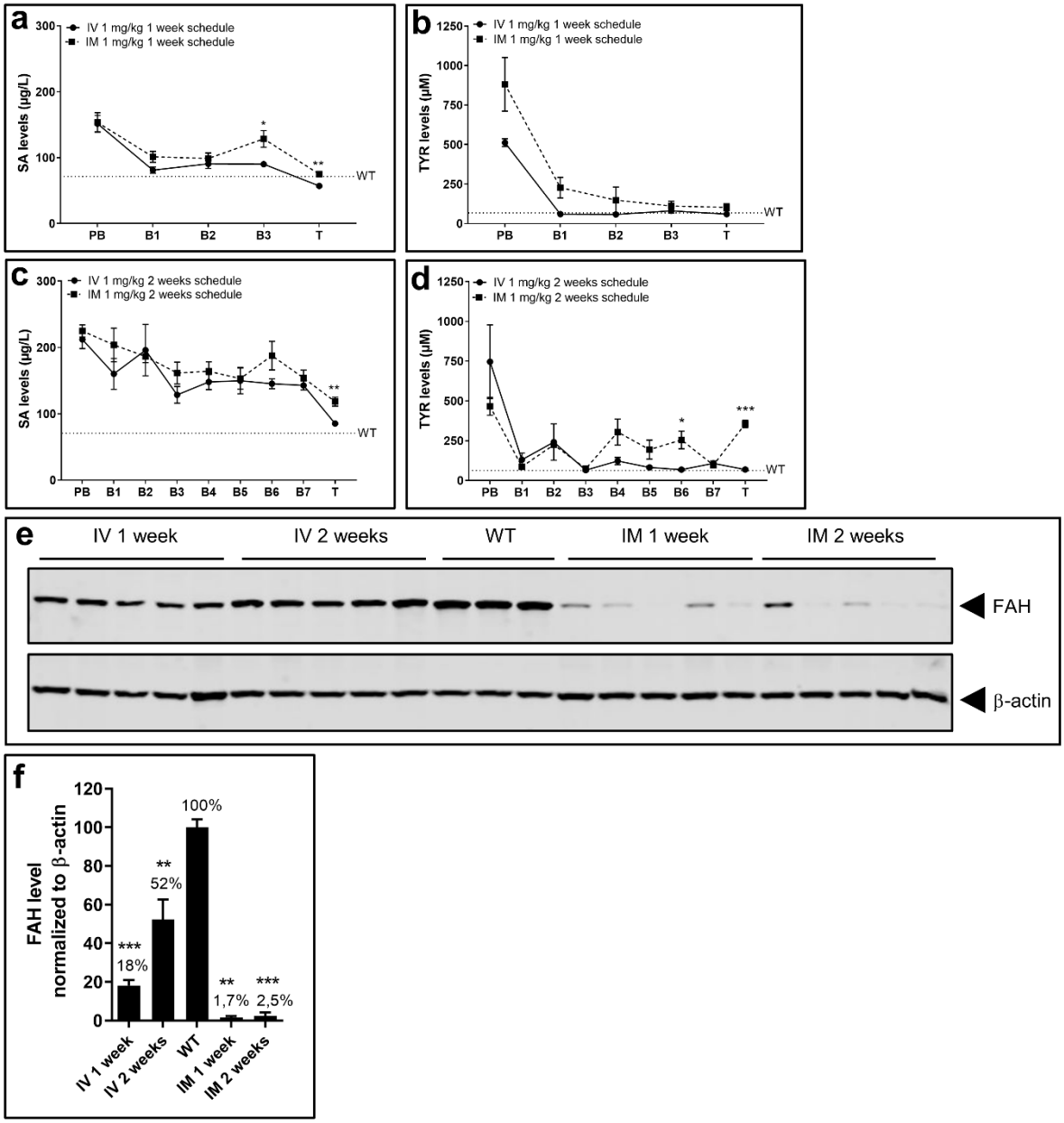
Supplemental Figure 6. Dose finding studies (0,5 mg/kg versus 0,1 mg/kg) in *Fah*-deficient mice upon repeated IV and IM injections of *FAH* mRNA-LNPs. Mice were injected with *FAH* mRNA-LNPs using doses and routes indicated. Absolute serum **(a,c)** succinylacetone (SA) and **(b,d)** tyrosine (TYR) levels were determined at times indicated. Dotted lines indicate SA and TYR levels in WT mouse serum. **(e)** Western blot analysis of FAH protein in livers dissected at termination from WT and *Fah*-deficient mice using doses and routes indicated. **(f)** Quantitation of FAH protein in livers of *FAH* mRNA-LNPs-treated and WT mice normalized to β-actin loading control; FAH protein in WT mouse livers normalized to 100%. Data are the means +/- SEM. Values are significantly different from WT control: **P*<0.05; ***P*<0.01; ****P*<0.001 (two-tailed Student´s *t*-test).

Supplemental Figure 7



Supplemental Figure 7. Therapeutic efficacy of a single *FAH* mRNA-LNPs injection extends up to 14 days. **(a)** Western blot analysis of FAH protein in livers of *Fah*-deficient mice after single IV injection with 1 mg/kg *FAH* mRNA-LNPs compared to FAH protein in livers of PBS-injected *Fah*-deficient mice +/- NTBC supplementation. Mice were terminated on day 8 (n = 3 livers/condition). **(b)** Quantitation of FAH protein in *Fah*-deficient mouse livers on day 8 following single IV injection of *FAH* mRNA-LNPs (**c)** Absolute serum succinylacetone (SA) levels prior to treatment (PB) and 8 days after single *FAH* mRNA-LNPs injection. **(d)** SA levels normalized to PB levels. **(e)** Absolute serum tyrosine (TYR) levels prior to treatment and 8 days after single IV injection of *FAH* mRNA-LNPs. **(f)** TYR levels normalized to PB levels. **(g)** Western blot analysis of FAH protein in livers of *Fah*-deficient mice on days 8 and 14 after single IV injection. *FAH* mRNA-LNPs injection compared to WT mice injected with PBS (n = 3 mice/condition). **(h)** Quantitation of FAH protein in livers on days 8 and 14 after single IV injection of *FAH* mRNA-LNPs. **(i)** Western blot analysis of FAH protein in livers of *Fah*-deficient mice on day 14 after single IV or IM injection of *FAH* mRNA-LNPs (n = 3 mice/condition). **(j)** Quantitation of FAH protein in livers of *Fah*-deficient mice on day 14 following a single IV or IM injection of *FAH* mRNA-LNPs. **(k)** Absolute serum SA levels in *Fah*-deficient mice prior to treatment (PB), and on days 7 and 14 after a single injection of *FAH* mRNA-LNPs IV or IM. **(l)** SA levels normalized to PB values in *Fah*-deficient mouse serum after a single IV or IM injection of *FAH* mRNA-LNPs. Serum was collected one day before injection (PB), and on days 7 and 14 following injection (n = 3 mice/condition). **(m)** Absolute serum TYR levels prior to treatment (PB), and on days 7 and 14 after single IV or IM injection. **(n)** TYR levels normalized to PB values after single IV or IM injection of *FAH* mRNA-LNPs. Serum was collected one day before injection (PB), and on days 7 and 14 following injection (n = 3 mice/condition). Data are the means +/- SEM. Significantly different: **P*<0.05; ***P*<0.01; ****P*<0.001; ns, not significant *P*>0.05 (two-tailed Student´s *t*-test). For Western blot analysis, FAH protein in *Fah*-deficient mouse livers was compared to endogenous FAH protein in livers of WT mice; β-actin served as loading control. FAH protein in WT mouse livers was normalized to 100%. WT mice were terminated on day 1 after single IV injection and their livers collected to serve as a reference of endogenous FAH protein in mouse liver.

Supplemental Figure 8



Supplemental Figure 8. Interval finding studies (1-week versus 2-weeks) for repeated IV and IM injections of *FAH* mRNA-LNPs in *Fah*-deficient mice. *FAH* mRNA-LNPs were injected at 1-week intervals and absolute serum **(a)** succinylacetone (SA) and **(b)** tyrosine (TYR) levels were quantified at pre-bleeding time point, at intermediate bleeding time points B1-3, and at termination. *FAH* mRNA-LNPs were injected at 2-week intervals and absolute serum **(c)** SA and **(d)** TYR levels were quantified at times indicated. Dotted lines indicate SA and TYR levels in WT mouse serum. **(e)** Livers were dissected at termination and FAH protein in livers of *Fah*-deficient mice after repeated IV and IM injection with *FAH* mRNA-LNPs was determined by Western blot analysis: comparison of 1-week and 2-week intervals. **(f)** Quantitation of FAH liver protein by Western blot analysis, normalized to β-actin loading control and compared to FAH quantified in WT mouse livers (normalized to 100%). Data are the means +/- SEM. Significantly different: **P*<0.05; ***P*<0.01; ****P*<0.001 (two-tailed Student´s t-test).