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Supplemental information

ImmTOR nanoparticles enhance AAV transgene expression after initial and repeat dosing in a mouse model of methylmalonic acidemia

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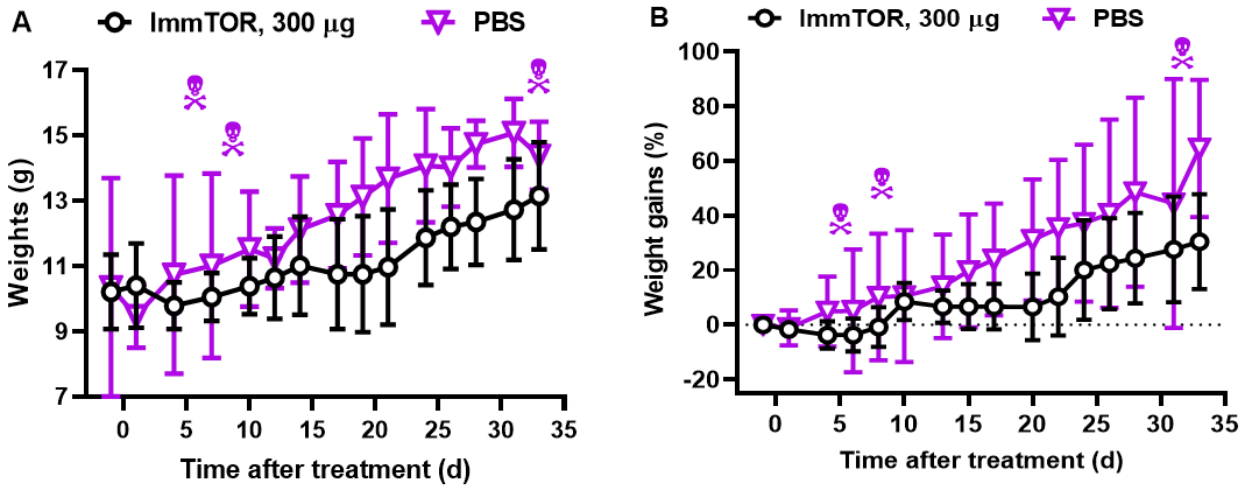
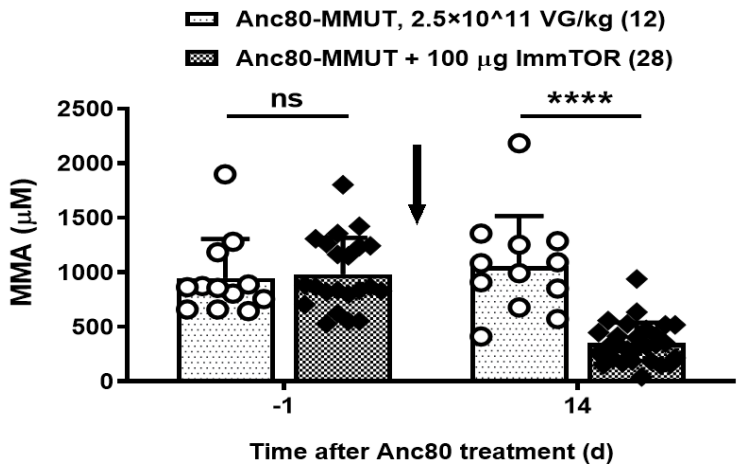
Supplementary Materials

Supplementary Table S1. Absolute levels of plasma MMA (pMMA) at 14 days after treatment with Anc80-MMUT, ImmTOR and dexamethasone (Dex-P). Averages ± SD are shown for each group. Number of subjects in each group is shown in parentheses.

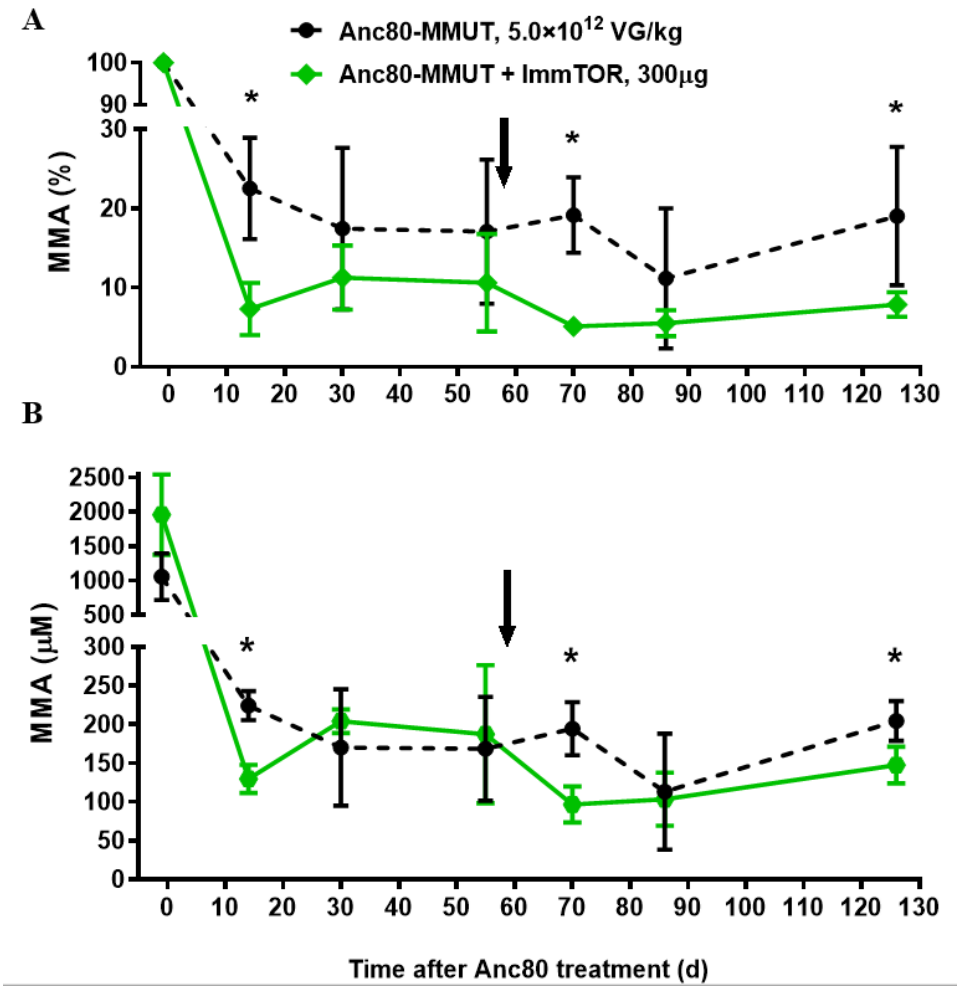
Treatment	Anc80-MMUT (15)	Anc80-MMUT (15)	Anc80-MMUT (12)	Anc80-MMUT (7)	Anc80-MMUT (7)
AAV	2.5E12 vg/kg	2.5E12 vg/kg	2.5E12 vg/kg	2.5E12 vg/kg	2.5E12 vg/kg
ImmTOR	None	100 µg	300 µg	None	300 µg
Dexp-P	None	None	None	10 mg/kg	10 mg/kg
pMMA	228 ± 98 µM	127 ± 39 µM	159 ± 80 µM	246 ± 107 µM	100 ± 23 µM



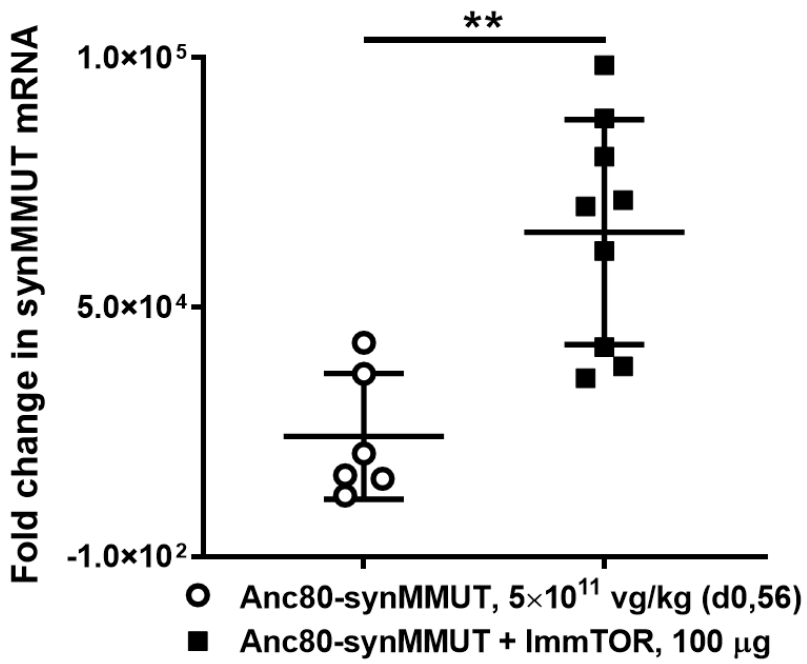
Supplementary Figure S1. Weight gains in MCK mice treated with ImmTOR or mock-treated. Absolute weights (**A**) or weight gains in % vs. pre-injection weight (**B**) are shown with times of mouse deaths in mock-treated group indicated by crossbones sign.



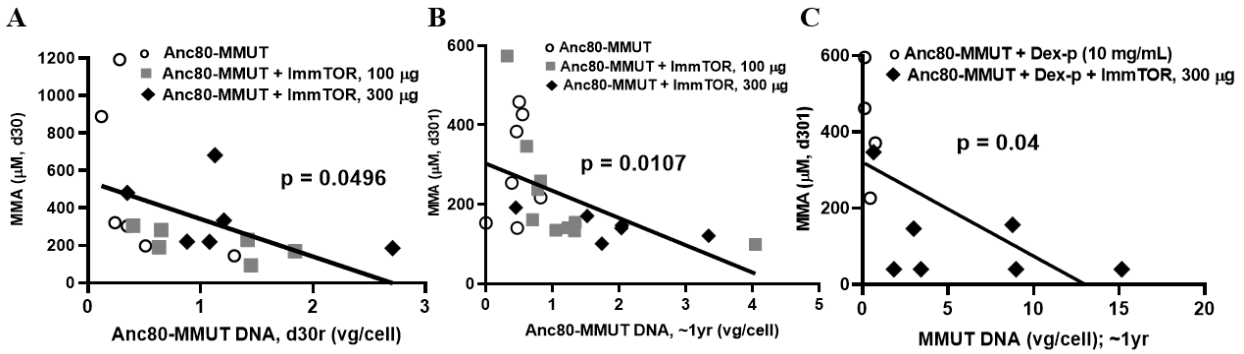
Supplementary Figure S2. ImmTOR provides added benefit at subtherapeutic doses of Anc80-MMUT. *Mmut*$^{-/-}$; Tg$^{\text{INS-MCK-}Mmut}$ mice at 14 days of age were treated once with Anc80-MMUT (2.5×10^{11} vg/kg) either alone or combined with ImmTOR (100 μg). Plasma methylmalonic acid (pMMA) levels before the treatment (day -1) and at 14 days after a single Anc80-MMUT administration are shown with numbers of mice in each group indicated in parentheses. Statistical significance is indicated (ns – not significant, **** – $p < 0.0001$).



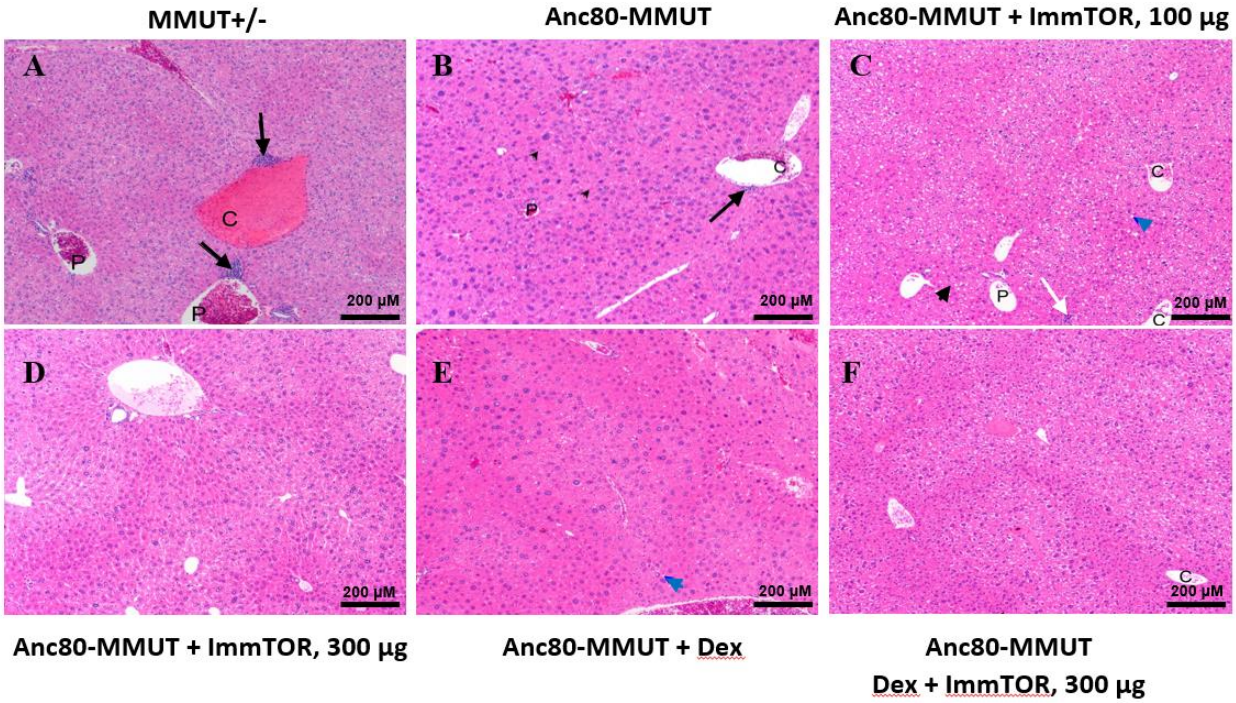
Supplementary Figure S3. Co-administration of high therapeutic dose of Anc80-MMUT with ImmTOR elevates transgene activity. Anc80-MMUT (5.0×10^{12} vg/kg) alone or combined with 300 µg of ImmTOR was injected twice on days 0 and 56 in groups of 3-4 mice. Plasma MMA levels are shown for the duration of the study (126 days after initial treatment) as % vs. pre-treatment levels as 100% (**A**) or in absolute numbers (**B**). Time-points with statistically significant (* – $p<0.05$) differences vs. group not receiving ImmTOR are indicated. Day 56 repeat injection is indicated by arrow. Averages with SD are shown.



Supplementary Figure S4. AAV-driven *MMUT* mRNA expression after repeated injections with Anc80-MMUT combined with ImmTOR. *synMMUT* mRNA levels in livers of wild-type C57BL/6 female mice after two (days 0 and 56) injections with Anc80-synMMUT with or without 100 μg of ImmTOR as measured by fold increase (GAPDH-normalized) over untreated baseline 43 days after repeat vector dose (day 99 after initial treatment). Statistical significance (** – $p<0.01$) is shown. Averages with SD are shown.

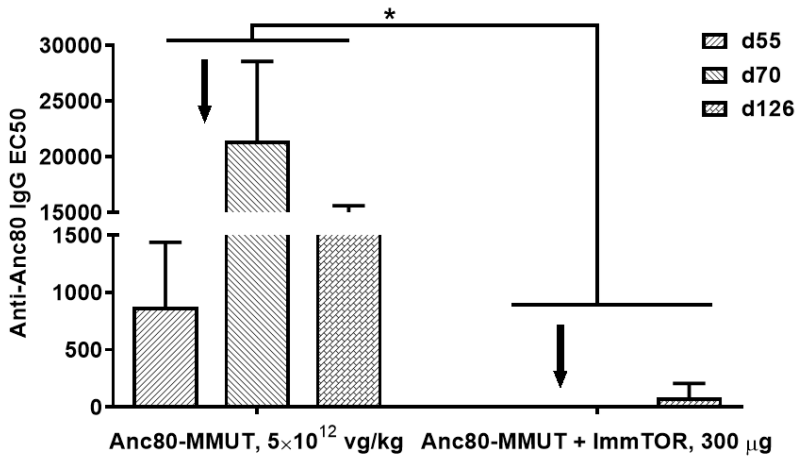


Supplementary Figure S5. Inverse correlation of short-term and long-term AAV transduction efficacy and serum levels of methylmalonic acid in individual studies. **A.** Viral DNA copy numbers and serum levels of methylmalonic acid have been measured on day 30 following a single injection with Anc80-MMUT alone or combined with 100 or 300 µg ImmTOR. Data shown in Figs. 1B and 2A are used for analysis. **B, C.** Viral DNA copy numbers and serum levels of methylmalonic acid have been measured 1 year after two doses of Anc80-MMUT administered alone or combined with 100 or 300 µg ImmTOR. Data from Figs. 4A and 4C (**B**) or from Figs. 5A and 5C (**C**) are used for analysis. P value of correlation is shown for each graph.



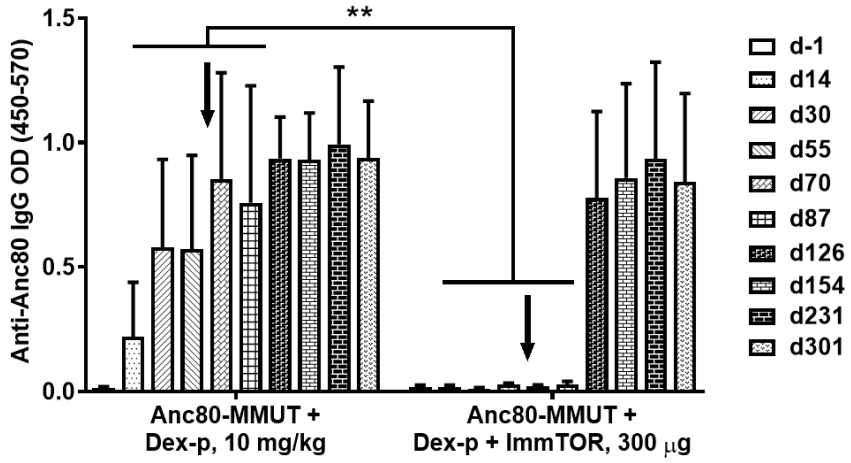
Supplementary Figure S6. Long-term alleviation of hepatic pathology by Anc80-MMUT and ImmTOR. Representative photomicrographs of liver sections from heterozygous *Mmut*$^{+/-}$ (**A**),

and Anc80-MMUT-treated *Mmut*$^{-/-}$; Tg$^{INS\text{-}MCK\text{-}Mmut}$ mice (**B-F**) are shown (40×). Anc80-MMUT (days 0 and 56) was used alone (B) or combined with 100 µg (**C**) or 300 µg (**D**) of ImmTOR, combined with dexamethasone (**E**) or dexamethasone and 300 µg ImmTOR (**F**). Hematoxylin and eosin staining of liver sections from 1-year old mice are shown, scale bars: 200 µm. Heterozygous control (**A**) shows small aggregates of perivascular mononuclear cells (black arrows) adjacent to both portal tracts (P) and central veins (C). Livers from mice treated Anc80-MMUT (**B**, **E**) showed minimal mononuclear cell infiltration (black arrow) accompanied by a few necrotic hepatocytes as well as low numbers of hepatocytes with intracytoplasmic inclusions (black arrowheads) or vacuoles (blue arrowheads). Mice co-treated with low dose ImmTOR (**C**) shown minimal, random mixed cell inflammation (white arrows), hepatocyte vacuolization (blue arrowheads), and hepatocyte intracytoplasmic inclusions (black arrowheads), while in mice co-treated with high dose ImmTOR (**D**, **F**) histologic findings were minimal to absent.



Supplementary Figure S7. Co-administration of high therapeutic dose of Anc80-MMUT with ImmTOR prevents IgG antibody formation. Anc80-MMUT (5.0×10^{12} vg/kg) alone or combined with 300 µg of ImmTOR was injected twice on days 0 and 56 in groups of 3-4 *Mmut*$^{-/-}$

; $Tg^{INS\text{-}MCK\text{-}Mmut}$ mice. EC_{50} of Anc80 IgG in serum was measured at times indicated. Time-points with statistically significant (* – $p<0.05$) differences between groups are indicated. Day 56 repeat injection is indicated by arrow. Averages with SD are shown.



Supplementary Figure S8. Co-administration of high therapeutic dose of Anc80-MMUT with ImmTOR and dexamethasone prevents IgG antibody formation, while dexamethasone alone is ineffective. Anc80-MMUT (2.5×10^{12} vg/kg) alone or combined with 300 µg of ImmTOR was injected twice on days 0 and 56 in groups of 5-7 $Mmut^{-/-}$; $Tg^{INS\text{-}MCK\text{-}Mmut}$ mice (see Fig. 5). Both groups also received 10 mg/ml dexamethasone concurrently with Anc80 inoculation. Anc80 IgG in serum was measured at times indicated and presented as top OD. Time-points with statistically significant (** – $p<0.01$) differences between groups receiving and not receiving ImmTOR are indicated. Day 56 repeat injection is indicated by arrow. Averages with SD are shown.