

## Supplementary Materials

### Development of an RNAi therapeutic for alpha-1-antitrypsin liver disease

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## Supplementary Data

### Development of RNAi therapeutic ARC-AAT

Potential RNAi trigger sequences that target the human *SERPINA1* mRNA and cross-react with the cynomolgus monkey homolog were identified. These sequences were evaluated *in silico* for potential off-target risk of the 17-nucleotide antisense strand (AS) sequences (positions 2-18). Potential trigger sequences with a seed region (positions 2-7) identical to a miRNA seed region were excluded from consideration, as were sequences with fewer than two mismatches to any expressed human gene in GenBank.

Candidate RNAi trigger sequences were synthesized with 2'-O-methyl or 2'-fluoro modifications of ribonucleotides for enhanced stability and reduced risk of innate immune stimulation. They were evaluated in the human hepatocarcinoma cell line Hep3B. EC<sub>50</sub> curves were generated for five RNAi triggers that exhibited at least 80% AAT KD (Table S1, Fig. S1). The most potent of these, RNAi-1, targeted nucleotide positions 1142-1160 in the AAT mRNA and had an EC<sub>50</sub> of 0.01 nM.

The five most efficacious sequences (RNAi-1 to RNAi-5) were further chemically modified for evaluation *in vivo* as chol-RNAi that were delivered to hepatocytes in the liver of PiZ mice transgenic for the human Z-AAT gene by intravenous (IV) co-injection of the hepatotropic chol-RNAi with the hepatocyte-targeted endosomal release excipient EX1. The chol-RNAi and EX1 were separate but co-injected molecules, allowing these components to be individually optimized. Initial studies in mice were performed with 8 mg/kg EX1 and 8 mg/kg chol-RNAi to determine maximal KD potential. The RNAi-1 sequence that was most efficacious *in vitro* was also the most efficacious *in vivo* in PiZ mice (data not shown).

Two modifications of the RNAi-1 sequence for *in vivo* evaluation were RNAi-6 and RNAi-7 (Table S1). RNAi-7 had the same sequence and modifications as RNAi-6 except for the presence of a single unlocked nucleobase analog (UNA) in the antisense strand. PiZ mice given one injection of 6 mg/kg EX1 and 12 mg/kg RNAi-6 or RNAi-7 had similarly reduced Z-AAT mRNA one week after the injection, 97% KD from RNAi-6 and 98% KD from RNAi-7 (Fig. S2 A, B). However, the mRNA reduction from RNAi-7 was more durable than that of RNAi-6. Maximal reduction of circulating Z-AAT protein (96%) was also similar between RNAi-6 and RNAi-7, but the reduction was substantially more durable from RNAi-7 containing the UNA (Fig. S2 A, B). Plasma Z-AAT was 93% reduced two weeks after mice were dosed with RNAi-7 but only 52% reduced in those dosed with RNAi-6.

The greater inhibitory activity of RNAi-7 compared to RNAi-6 was also observed in nonhuman primates (NHP). The nadir of serum AAT reduction ten days after injection of 6 mg/kg RNAi-6 with 3 mg/kg EX1 into NHP was 82% (Fig. S2 C). Using the same amounts of RNAi trigger and excipient, RNAi-7 reduced serum AAT by 88.5% and the nadir was on day 29. Six weeks after the injection, serum AAT

was almost back to baseline in animals that received RNAi-6, but 76% reduced in those that received RNAi-7.

The greater duration of RNAi-7 compared to RNAi-6 was identified as being due to increased phosphorylated antisense strand stability in the cytosol of hepatocytes. RNAi-6 and RNAi-7 were synthesized without a 5' phosphate (5'P) on the 5 prime-most nucleotide of the AS. When these chol-RNAi triggers are released from endosomes through the action of EX1, they become 5' phosphorylated in the cytoplasm (Trubetsky et al 2016). The amounts of AS that were full-length (FL) with or without a 5'P, as well as the total number of AS that were 15 bases or longer, were measured using a fluorescence-based hybridization and chromatographic assay. Maximal delivery of RNAi-6 and RNAi-7 to the liver was measured 1 hr post injection of 12 mg/kg chol-RNAi with 6 mg/kg EX1, at which time approximately 10% of the FL AS contained a 5'P that indicated its presence in the cytosol ( $12.3 \pm 0.8\%$  for RNAi-6 and  $9.8 \pm 0.7\%$  for RNAi-7; Fig. S3). Immunoprecipitation of the RNA-induced silencing complex (RISC) through the argonaut 2 subunit (AGO2) from liver homogenates of PiZ mice 1 hr post-injection indicated that more of the RNAi-7 AS ( $0.012 \pm 0.003\%$ ) than RNAi-6 AS ( $0.007 \pm 0.001\%$ ) taken up into the liver was in RISC. The nonphosphorylated (noP) form of both triggers decayed more rapidly than the 5'P triggers, as the noP AS forms had not escaped the endosomes, and by day 8 nearly all the remaining trigger was 5'P FL AS ( $90.7 \pm 2.2\%$  of RNAi-6 and  $92.7 \pm 1.4\%$  of RNAi-7; Fig. S3). The 5'P FL AS of RNAi-6 decayed more rapidly than RNAi-7, explaining the more limited duration of RNAi-6 activity (Fig. S2 A, B and Fig. S3). On day 8,  $18.5 \pm 2.3\%$  of the total 5'P FL AS of RNAi-7 in the liver was in RISC. As expected by the lack of KD activity in the absence of EX1, no RNAi-7 AS was in RISC when RNAi-7 was injected without EX1 (data not shown). RNAi-7 was selected as the active pharmaceutical ingredient (API) of AATD therapeutic ARC-AAT.

AAT KD in mice depended on the doses of both the API and EX1, the greater effect being from the EX1 dose (Fig. S4 A). EX1, however, was thought to be the component that would be dose-limiting in humans due to its membrane-disruptive potential. Primates were more sensitive to the EX1 dose than mice. A 2:1 weight/weight ratio of API to EX1 was selected to comprise ARC-AAT because NHP studies showed more consistent response to the trigger delivered at this ratio. In NHP co-injected with 1.5 mg/kg EX1 and 0.75, 1.5 or 3 mg/kg ARC-AAT API, KD was more consistent in animals that received 3 mg/kg API than in those given the lower doses (Fig. S4 B). This 2:1 ratio modestly increased the consistency of KD in PiZ mice given a low dose of EX1 as well (data not shown). The API alone even at a dose as high as 24 mg/kg was not able to reduce AAT in cynomolgus monkeys (Fig. S5).

### **Development of RNAi therapeutic ARO-AAT**

A similar *in silico* approach was used to select candidate sequences for ARO-AAT as for ARC-AAT. These were synthesized with chemical modifications of ribonucleotides for enhanced stability and reduced risk of innate immune stimulation. In addition, the NAG ligand for the asialoglycoprotein receptor was synthesized onto the 5-prime end of the sense strand, allowing for subcutaneous rather than intravenous administration. The NAG ligand directs the trigger to hepatocytes in the liver. Two days

after dosing rodents, measurable trigger was detected only in liver and kidney. The amount in the kidney was approximately one order of magnitude less than that in liver.

RNAi triggers were evaluated for KD activity and durability of response in the PiZ mouse model and in NHP (data not shown). In light of our experience highlighting the importance of trigger stability for ARC-AAT, sequences with suitable KD activity were further chemically modified in multiple iterations of nucleotide chemistries, NAG ligands, and attachment chemistries to yield not only maximal KD but also highly durable KD (data not shown). ARO-AAT was selected as the drug candidate.

A dose response study with ARO-AAT was conducted in PiZ mice to assess a correlation between the reduction of Z-AAT mRNA and the corresponding amount of ARO-AAT AS in the liver (Fig. S15). Each animal received a single SC injection of 1, 2, 4 or 8 mg/kg ARO-AAT or saline as a control. Plasma Z-AAT protein was reduced  $75.0 \pm 3.1\%$ ,  $83.8 \pm 3.8\%$ ,  $96.2 \pm 0.3\%$ , and  $97.5 \pm 0.3\%$  at the day 15 nadir following injection of 1, 2, 4 or 8 mg/kg ARO-AAT, respectively (Fig. S15A). Three mice from each dose level were euthanized at weekly intervals to measure the amount of Z-AAT mRNA and AS in the liver (Fig. S15 B-D). The corresponding day 15 reductions of Z-AAT mRNA were somewhat less than plasma AAT: 58.1%, 76.5%, 83.9% and 94.5% for 1, 2, 4 and 8 mg/kg ARO-AAT, respectively. Comparing the reduction of mRNA with the amount of RNAi AS in each animal and at each time point demonstrated a close dose exposure-response relationship. The consistency of this relationship irrespective of the initial dose and the time point demonstrate that mRNA KD depended directly on the amount of RNAi AS present in the liver.

### **Globules are extruded into the hepatic veins**

As previously described in another strain of PiZ mice (1), we also observed that Z-AAT globules were relatively small, numerous and panlobular in the hepatocytes of young adult PiZ mice, present in the region of the portal triads, around central veins, and in hepatocytes occupying the areas between these two structures (Fig. S12A). As noted in our manuscript, globules were rarely observed in the periportal parenchyma (i.e. around portal triads) of 64-69-week old PiZ mice (Fig. S12 B, C). In these old mice, globules were much larger and persistently present in hepatocytes around terminal central veins and in zones 2-3 of hepatic units (see (2) for reference to liver architecture). This pattern appeared more pronounced 32 weeks later in both the saline-injected control group and the ARC-AAT-injected group (Fig. S12 D, E). The quantity of large globules that had been observed in 64-69-week old PiZ mice was significantly decreased in the 23-month old saline group mice and ARC-AAT-treated mice.

Increasing size of globules in PiZ mice that are over a year old, compared to younger mice, can be explained by the pathophysiology of the disease, but changes we noted in the globule distribution pattern and the decreased numbers of globules in both groups of 23-month old mice motivated us to look more closely for a plausible explanation. We suggest there is one parsimonious explanation for both features and this is based on the “liver streaming” model. Rudnick et al. previously demonstrated that globule-devoid hepatocytes proliferated in response to signals produced by sick, globule-stressed hepatocytes

(3). Chronic damage to hepatocytes due to the accumulated Z-AAT protein and likely reduced metabolic capacity eventually result in death of these hepatocytes. Large cell-free globules have been observed in the liver parenchyma (4). Our morphological evidence in young and old PiZ mice support a pathway whereby globule-containing hepatocytes and cell-free Z-AAT globules exit the liver via terminal hepatic veins (Fig. S12). The dead hepatocytes are replaced by a new population of hepatocytes not producing Z-AAT. Indeed, we observed a population of hepatocytes that stained negatively for human AAT in the liver of an 11-month old PiZ mouse (Fig. S6). Decreased globules in aged PiZ mice were previously described and are believed to be due to modification of pathways affecting the disposal of Z-AAT (1, 5).

It is well-established that in normal human and rodent livers apoptotic hepatocytes are preferentially in the perivenular or pericentral area, zone 3 of the Rappaport hepatic unit (6-8). Based on these observations, the “streaming liver” model was proposed, in which hepatocytes originate in the periportal area (zone 1) and as they mature they “stream” out toward the perivenular region (zone 3) where aged hepatocytes die by apoptosis (9, 10). Although some studies reported observations that contradict the “streaming” model (11-13), the overwhelming evidence supports a model in which the main source of cells for hepatocyte renewal in non-hepatectomized livers are the cells of the canals of Hering and the immediate periportal hepatocytes (14-17) and hepatocytes undergoing apoptosis are more often in zone 3 of the hepatic unit (6-8), the latter described particularly in stressed mouse livers (18).

## Supplementary Methods

*In vitro screening of siRNAs.* Candidate sequences identified by *in silico* analysis were initially evaluated as chemically modified siRNAs in Hep3B cells plated in 96-well format. Each siRNA was transfected at concentrations ranging from 0.001-1nM, in triplicate, using Lipofectamine RNAiMax (Life Technologies, 0.3 $\mu$ L/well). Twenty-four hours post-transfection, cells were lysed and cDNA generated (TaqMan Cells-to-CT Gene Expression kit, Life Technologies). AAT gene KD was assessed by qRT-PCR with TaqMan chemistry-based assays (Life Technologies) for human AAT (Assay ID: Hs01097800\_m1), normalized to the endogenous control, human cyclophilin A (PPIA, 4326316E). EC<sub>50</sub> curves were generated from the knockdown at each concentration.

*Quantitation of RNAi triggers.* Liver tissues for RNAi-6 and RNAi-7 (ARC-AAT API) AS analysis were pulverized while frozen, then mixed with Affymetrix Lysis Buffer (Affymetrix Lysis Solution and nuclease-free water at 1:2 ratio and 50  $\mu$ g/ml proteinase K) and sonicated to result in tissue lysates. Tissue lysates for ARO-AAT AS analysis were prepared by placing frozen liver tissue into tubes containing Lysis Mixture A (dry material to assist homogenization, MP Bio) and then adding complete lysis buffer and homogenizing. Homogenized samples were centrifuged for 10 min at 4°C, 21.2 x g. The supernatant was the tissue lysate. Tissue lysates prepared either way were incubated at 65°C for 30 min. Further dilution of tissue lysates and serial dilutions of RNAi trigger standards with or without a 5-prime phosphate were prepared in complete lysis buffer. SDS was precipitated from the diluted standards and

tissue lysates by adding 10  $\mu$ l of 3M KCl to 100  $\mu$ l diluted tissue lysates or standards, incubating 10 min on ice, then centrifuging for 15 min at 4°C, 2,700 x g. Quantitation of AS was performed with the resulting supernatant. Sequence-specific peptide nucleic acid (PNA) probes containing the fluorescent Atto425 label separated from the PNA chain by a diethylene glycol linker were designed to bind to the full-length AS. The PNA probe for the ARC-AAT API was Atto425-OO-ATATCATCACCAAGTTCC and for ARO-AAT was Atto425-OO- CGTTTAGGCATGTTTAACA (PNA Bio, Thousand Oaks, CA). Hybridization of the probe to AS was performed with 40  $\mu$ L sample supernatant and 160  $\mu$ L hybridization buffer [60 mM Tris-HCl pH 8.0/acetonitrile (60:40; v/v)] containing 250 pmol PNA probe by incubating in a thermal cycler at 95°C for 15 min and then gradually reducing the temperature to 54°C over 15 min. Thereafter hybridization mixtures were stored at 4°C until analysis. These 200  $\mu$ L hybridization mixtures were transferred to a 96-deep well plate containing 20  $\mu$ L of 10% CHAPS in H<sub>2</sub>O-Glycerol (2:1; v/v) in each well. Samples of 50  $\mu$ L injection volumes were drawn from this plate and loaded onto a DNAPac PA-100 4 x 250 mm analytical column (#DX043010; Fisher Scientific, Pittsburgh, PA) through an autosampler for ion exchange HPLC analysis using a Shimadzu HPLC system equipped with an LC-20AT pump, SIL-20AC autosampler, RF-10AxI fluorescence detector, and a CTO-20Ac column oven (Shimadzu Scientific Instruments, Columbia, MD). Analysis was carried out at a flow rate of 1 ml/min with a column oven temperature of 60°C. A gradient elution using mobile phase A (10 mM Tris-HCl pH 7, 100 mM NaCl, 30% acetonitrile) and mobile phase B (10 mM Tris-HCl pH 7, 900 mM NaCl, 30% acetonitrile) was used. Fluorescence detection was set to 436 nm excitation and 484 nm emission with a medium gain setting of 4. Concentrations of analytes eluted in the relevant range were calculated using a multi-point external standard calibration curve. Calibration curves were generated with PNA-hybridized full-length antisense strand. The lower limit of quantitation was 1 ng/ml.

*Evaluation of apoptotic hepatocytes in PiZ mouse liver.* Apoptotic hepatocytes were stained with an *In Situ* Cell Death Detection Kit (#11684795910, Roche) to label DNA strand breaks (TUNEL technology) in formalin-fixed paraffin-embedded liver tissue according to the manufacturer's directions and with the following specifications. Tissue was dewaxed and rehydrated. Cells were permeabilized with 0.1% Triton X-100 for 20 min at ambient temperature. Tissue was then washed with phosphate-buffered saline (PBS) three times for 3 min. The TUNEL reaction mixture was added to the tissue, or just the labeling solution for the negative control, and incubated in a humidity tray at 37°C for 60 min in the dark. Tissue was again washed with PBS three times for 3 min. A coverslip was added with Vector Hardset Mounting Media + DAPI. The TUNEL stain was imaged on a Zeiss LSM710 Confocal Microscope.

*Cynomolgus monkey procedures.* NHP studies were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and were approved by either the University of Wisconsin - Madison or the BASi Institutional Animal Care and Use Committees. Cynomolgus monkeys (*Macaca fascicularis*) were obtained from commercial vendors. They were provided water *ad libitum* and fed monkey diet daily. Animals were either restrained or sedated with

ketamine (up to 7 mg/kg, IM)/dexmedetomidine (up to 0.03 mg/kg, IM) for injections and blood collection. Cholesterol-RNAi triggers with EX1, ARC-AAT, and ARC-AAT components (API and/or EX1) were injected intravenously via a peripheral vein at dose volumes of 2 mL/kg and a targeted rate of approximately 3 min. ARC-AAT consisted of a vial of lyophilized EX1 that was solubilized with the ARC-AAT API in liquid solution. Vehicle (0.9% sodium chloride containing 7 mM bicarbonate/3 mM carbonate, pH 9.2-9.5) was used to dilute solutions for IV injection. After injection, animals that had been sedated were administered the dexmedetomidine reversal drug atipamezole (0.10 to 0.25 mg/kg, IM).

*Cynomolgus monkey serum AAT analysis.* Serum AAT concentration was determined using an immunoturbidometric assay as described in the manufacturer's method description (Tina-quant alpha-1 antitrypsin, version 2) for the Cobas Integra 400 Plus. The concentration of AAT in samples and controls were automatically calculated using a six-point calibration curve. Two levels of controls were analyzed and verified prior to analyzing each set of samples. All samples were automatically pre-diluted with diluent (0.9% NaCl) by the instrument using a standard dilution ratio but samples that had calculated values below the lower limit of detection (LOD) were rerun with a reduced predilution. Pre-dilutions and calculations of concentration were automatically performed by the instrument and reported in grams per liter (g/L). The LOD for this assay was 0.06 g/L. A value of 0.06 g/L was used in calculations if a sample was <0.06 g/L.

*Clinical trial with ARC-AAT.* Multi-center, randomized, placebo-controlled, double-blind, single dose-escalation, first-in-human, phase I study (clinical trial NCT02363946) was conducted to evaluate the safety, tolerability, pharmacokinetics and effect on circulating AAT of the investigational product ARC-AAT (19). Male and female healthy volunteer subjects and PiZZ patients with alpha-1 antitrypsin deficiency were 18-50 years old. Only normal volunteer data are shown in this manuscript. Single dose cohorts (2 placebo: 4 active) were IV-infused with 0.38 to 8 mg/kg ARC-AAT. All subjects were treated with an oral antihistamine 2 hours prior to infusion. Quantitative AAT measurement of the subjects' serum were performed with the ARCHITECT® cil6200™, Roche Cobas® c501, or the BN™ II System as previously described (19).

*AROAT1001 clinical study design.* AROAT1001 (clinical trial NCT03362242) is a phase 1 single and multiple dose-escalating study to evaluate the safety, tolerability, pharmacokinetics and effect of ARO-AAT on serum alpha-1 antitrypsin levels in adult normal health volunteers (NHVs). This first in human study is being conducted at a single phase 1 facility in New Zealand. The study primary objective is to determine the incidence and frequency of adverse events possibly or probably related to treatment as a measure of the safety and tolerability of ARO-AAT using escalating single doses and escalating multiple doses in NHVs.

Secondary objectives include:

- To evaluate the single-dose and multi-dose pharmacokinetics of ARO-AAT in NHV.

- To determine the reduction in serum alpha-1 antitrypsin (AAT) in response to ARO-AAT as a measure of drug activity.

Exploratory objectives include:

- To evaluate the effect of single doses of ARO-AAT on cytokines (Cytokine panel A: interleukin-6 [IL-6], monocyte chemoattractant protein-1 [MCP-1], tumor necrosis factor-alpha [TNF-alpha], interleukin-8 [IL-8], interleukin-1beta [IL-1beta], interferon alpha [IFN alpha], IL-10, IL-12 [p40], IL-12 [p70], macrophage inflammatory protein-1alpha [Mip-1alpha]) in NHV.
- To evaluate the effect of single escalating doses of ARO-AAT on complement factors Bb, CH50, C5a, C4a, and C3a in NHV.
- To collect plasma samples in NHV for subsequent metabolite identification (reported in a separate report outside of this study).
- To collect urine samples in NHV for subsequent determination of urinary excretion and metabolite identification (reported in a separate report outside of this study).

NHV adult male and female subjects, aged 18-52 years with BMI between 19.0 and 35.0 kg/m<sup>2</sup> enrolled sequentially into a total of 7 cohorts, Cohorts 1 through 4 were randomized to receive ARO-AAT or placebo (4 active: 4 placebo) at a single dose of 35 mg (Cohort 1) followed by multiple escalating doses of 100 mg (Cohort 2), 200 mg (Cohort 3), and 300 mg (Cohort 4) administered as a subcutaneous injection. Cohorts 2b, 3b and 4b were open label consisting of 4 subjects receiving single-doses of 100, 200, and 300 mg ARO-AAT (Fig. S17).

Sentinel dosing was included for each new dose level. Dose escalation required approval by the Data Safety Committee (DSC) based on all cumulative available safety data for prior cohorts, and through at least Day 15 of the current cohort. Starting at Cohort 2, the DSC also voted to approve return of all subjects in the most advanced cohort (Cohorts 2, 3 and 4) to receive two additional doses of ARO-AAT or placebo. All doses in multi-dose Cohorts 2, 3 and 4 were administered every 28 days. DSC decisions were based on all aggregate safety data available including all data available at least through Day 15 of the current cohort as shown in Figure S17. Subjects who withdrew from the study prior to their End of Study visit, for reasons other than an adverse event, were replaced. A total of 45 NHV subjects are enrolled in the study which includes one replacement subject in Cohort 3.

*Inclusion Criteria.* To be eligible for randomization, participants were required to meet all the following inclusion criteria:

1. Male or female healthy volunteers 18-55 years of age
2. Able and willing to provide written informed consent prior to the performance of any study specific procedures
3. Participants with a BMI between 19.0 and 35.0 kg/m<sup>2</sup>, inclusive
4. A 12-lead ECG at Screening and pre-dose assessment that, in the opinion of the PI, had no abnormalities that compromised participant's safety in this study
5. Non-nursing females



6. Non-smoker for at least one year with current non-smoking status confirmed by urine cotinine at screening (with repeat cotinine test required for initial positive result)
7. Normal lung function (or not clinically significant based on PI's assessment) based on spirometry (FEV<sub>1</sub>) at screening conducted as per ATS-ERS criteria
8. Participants using highly effective, double barrier contraception (both male and female partners) during the study and for 3 months following the dose of ARO-AAT. Males were prohibited from donating sperm for at least 3 months post-dose of the last study treatment. Male partners of female participants and female partners of male participants were also required to use contraception, if they were of childbearing potential. Females of childbearing potential were required to have a negative urine pregnancy test at Screening and on Day 1 (pre-dose). Females not of childbearing potential were required to be post-menopausal (defined as cessation of regular menstrual periods for at least 12 months), confirmed by follicle-stimulating hormone (FSH) level in the post-menopausal reference range.
  - Using twice the normal protection of birth control by using a condom AND one other form of the following:
    - Birth control pills (The Pill)
    - Depot or injectable birth control
    - IUD (Intrauterine Device)
    - Birth Control Patch (e.g., Othro Evra)
    - NuvaRing®
    - Surgical sterilization. i.e., tubal ligation or hysterectomy for women or vasectomy for men or other forms of surgical sterilization

Rhythm methods were not considered as highly effective methods of birth control. Subject abstinence for the duration of the study and three months after the dose of ARO-AAT was acceptable only when this method was in alignment with the normal lifestyle of the patient.
9. Participants who were willing and able to comply with all study assessments and adhere to the protocol schedule
10. Suitable venous access for blood sampling required
11. No abnormal finding of clinical relevance at the Screening evaluation
12. AST, ALT and Creatinine levels ≤ upper limit of normal at Screening
13. AAT level at or above the lower end of the reference range (above or equal to 16.6 microM or 90 mg/dL) at Screening visit

#### *Exclusion Criteria*

1. Female subjects with a positive pregnancy test or lactating.
2. Acute signs of hepatitis (e.g., moderate fever, jaundice, nausea, vomiting, abdominal pain) at Screening or at baseline
3. Use within the last 14 days or an anticipated requirement for anticoagulants (aspirin was acceptable)

4. Use of prescription medication within 7 days prior to administration of study treatment that in the opinion of the PI or the Sponsor would interfere with study conduct. Topical products without systemic absorption, OTC and prescription pain medication or hormonal contraceptives (females) were acceptable.
5. A history of poorly controlled autoimmune disease or any history of autoimmune hepatitis
6. Human immunodeficiency virus infection, as shown by the presence of anti-HIV antibody (seropositive)
7. Seropositive for HBV or HCV
8. Uncontrolled hypertension defined as blood pressure > 170/100 mmHg at screening confirmed by repeat
9. A history of torsades de pointes, ventricular rhythm disturbances (e.g., ventricular tachycardia or fibrillation), pathologic symptomatic bradycardia, 2<sup>nd</sup> degree or 3<sup>rd</sup> degree heart block, congenital long QT syndrome, prolonged QT interval due to medications, or new elevation or depression in the part of an ECG immediately following the QRS complex and merging into the T wave (ST segment) or new pathologic inverted T waves, or new pathologic Q waves on ECG that were deemed clinically significant in the opinion of the PI. Subjects with a history of atrial arrhythmias were to be discussed with the Sponsor Medical Monitor and CRO Medical Monitor.
10. A family history of congenital long QT syndrome, Brugada syndrome or unexplained sudden cardiac death
11. Taking medications known to prolong the QTc interval (the length of washout from a medication known to prolong the QTc interval was determined by the PI depending on the medication's half-life)
12. Symptomatic heart failure (per NYHA guidelines), unstable angina, myocardial infarction, severe cardiovascular disease (ejection fraction < 20%, transient ischemic attack (TIA) or cerebrovascular accident (CVA) within 6 months prior to study entry
13. History of malignancy within the last 5 years except for adequately treated basal cell carcinoma, squamous cell skin cancer, superficial bladder tumors, or in situ cervical cancer. Participants with other curatively treated malignancies who had no evidence of metastatic disease and >2 years disease-free could be entered following approval by the Sponsor Medical Monitor
14. History of major surgery within 3 months of Screening
15. Regular use of alcohol within one month prior to the Screening visit (i.e., more than fourteen units of alcohol per week [1 Unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol])
16. Had evidence of severe systemic acute inflammation, sepsis, or hemolysis
17. Recent (within 3 months) use of illicit drugs (such as cocaine, phencyclidine [PCP], MDMA,) or positive test for such drugs of abuse at screening. Subjects who were on prescription medications that caused a positive result on urine drug screen were eligible based on PI discretion. Subjects with a positive urine drug screen for opioids, cannabinoids or benzodiazepines could be eligible based on PI discretion.
18. Use of an investigational agent or device within 30 days prior to dosing or current participation in an investigational study

19. Had any clinically significant history or presence of poorly controlled or decompensated neurological, endocrine, cardiovascular, pulmonary, hematological, immunologic, psychiatric, metabolic, or other uncontrolled systemic disease, that could affect participation in the study
20. Any recent (within last 35 days) transfusion of fresh frozen plasma, platelets, or packed red blood cells, or anticipated need for transfusion during the study period
21. Any concomitant medical or psychiatric condition or social situation that would make it difficult to comply with protocol requirements or put the participant at additional safety risk
22. Had a history of coagulopathy or thromboembolic disease (including deep vein thrombosis and pulmonary embolism) or stroke within 6 months of baseline, and/or concurrent anticoagulant medication(s).
23. Subjects with any of the following laboratory abnormalities:
  - a. International normalized ratio (INR)  $> 1.5 \times$  ULN at Screening
  - b. Platelets  $<$  lower limit of normal at Screening
24. Participants who were unable to return for all scheduled study visits
25. Had any other condition that, in the opinion of the PI, would render the subject unsuitable for enrollment, or could interfere with his/her participation in the study
26. Use of dietary and/or herbal supplements (e.g., Milk Thistle, Burdock Root, S-Adenosyl-methionine [e.g., S-AMe, Gumbaral, Samyr, Adomet, Heptral) that had the potential to interfere with liver metabolism within 7 days prior to Screening
27. Use of any drugs known to induce or inhibit hepatic drug metabolism (examples of inducers: rifampin, barbiturates, carbamazepine, phenytoin, chronic systemic glucocorticoids; examples of inhibitors: diltiazem, macrolides, imidazoles, neuroleptics, rosuvastatin, verapamil, fluoroquinolones,) within 7 days prior to administration of study treatment
28. Blood donation (500 mL) within 7 days prior to study treatment administration. Donation or loss of whole blood (excluding the volume of blood that would be drawn during the Screening procedures of this study) prior to administration of the study treatment as follows: 50 mL to 499 mL of whole blood within 30 days, or more than 499 mL of whole blood within 56 days prior to study treatment administration

**Table S1. RNAi triggers**

RNAi trigger ID	Antisense strand sequence 5' → 3'	Sense strand sequence 5' → 3'
RNAi-1	dTGfgAfaCfuUfgGfuGfaUfgAfuAfudTsdT	AfuAfuCfaUfcAfcCfaAfgUfuCfcAf(inv dT)
RNAi-2	dTGfaUfcAfuAfgGfuUfcCfaGfuAfadTsdT	UfuAfcUfgGfaAfcCfuAfuGfaUfcAf(inv dT)
RNAi-3	dTAfcAfgCfcUfuAfuGfcAfcGfgCfcdTsdT	GfgCfcGfuGfcAfuAfaGfgCfuGfuAf(inv dT)
RNAi-4	dTUfcGfaUfgGfuCfaGfcAfcAfgCfcdTsdT	GfgCfuGfuGfcUfgAfcCfaUfcGfaAf(inv dT)
RNAi-5	dTCfaAfaGfgGfuUfuGfuUfgAfaCfudTsdT	AfgUfuCfaAfcAfaAfcCfcUfuUfgAf(inv dT)
RNAi-6	dTGfgAfaCfuUfgGfuGfaUfgAfuAfudTsdT	(Chol-TEG)uAuAfuAfuCfaUfcAfcCfaAfgUfuCfcAf(inv dT)
RNAi-7	dTGfgAfaCfU <sub>una</sub> UfgGfuGfaUfgAfuAfudTsdT	(Chol-TEG)uAuAfuAfuCfaUfcAfcCfaAfgUfuCfcAf(inv dT)
ARO-AAT	usGfsuUfaAfacaugCfcUfaAfaCfcGfsu	(NAG37)s(invAb)sagcguuuuGfGfCfauguuuuacac(invAb)

dT, deoxythymidine; small letter, 2'-O-methyl substitution; f, 2'-Fluoro substitution; s, phosphorothioate bond; inv dT, inverted deoxythymidine; una, unlocked nucleobase analog; Chol-TEG, cholesterol-conjugated moiety synthesized at the 5'-end of the oligomers using the 1-Dimethoxytrityloxy-3-O-(N-cholesteryl-3-aminopropyl)-triethyleneglycol-glycerol-2-O-(2-cyanoethyl)-(N,N,-diisopropyl)-phosphoramidite (Glen Research, Sterling, VA, USA); invAb, inverted abasic; NAG37 is a proprietary targeting group for the asialoglycoprotein receptor whose chemical structure and synthesis may be found in International Patent Application Publication Nos. WO 2018/132432 and WO 2018/044350.

**Table S2. TaqMan gene expression assays**

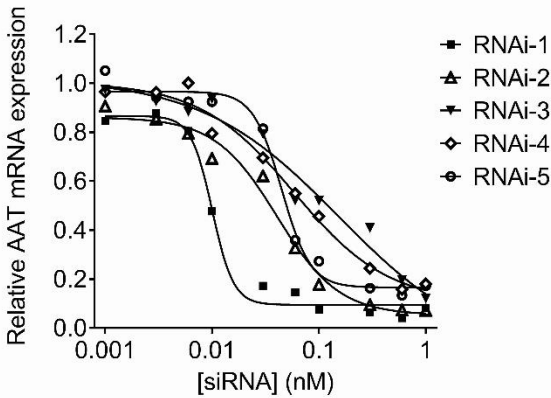
<b>Gene</b>	<b>Species</b>	<b>Assay ID (Thermo Fisher Scientific)</b>
<i>SERPINA1</i> (AAT)	Human	Hs00165475_m1
<i>COL1A1</i>	Mouse	Mm00801666_g1
<i>COL1A2</i>	Mouse	Mm00483888_m1
<i>COL3A1</i>	Mouse	Mm00802300_m1
<i>MMP2</i>	Mouse	Mm00439498_m1
<i>MMP12</i>	Mouse	Mm00500554_m1
<i>MMP13</i>	Mouse	Mm00439491_m1
<i>TIMP1</i>	Mouse	Mm01341361_m1
<i>NUPR1</i>	Mouse	Mm00498104_m1
<i>GPNMB</i>	Mouse	Mm01328587_m1
<i>LGALS3</i>	Mouse	Mm00802901_m1
<i>GSTM3</i>	Mouse	Mm00833923_m1
<i>CBR3</i>	Mouse	Mm00557339_m1
<i>ACTA2 (ASMA)</i>	Mouse	Mm00725412_s1
<i>GAPDH</i>	Rhesus monkey, identical to cynomolgus monkey and human	ID Rh02621745_g1
<i>SERPINA1</i> (AAT)	Cynomolgus monkey	Custom assay Probe: FAM- TGGTCAGCACAGCCTTATGCACG-MGBNFQ Forward primer: ACTAAGGTCTTCAGCAATGGG Reverse primer: GCTTCAGTCCCTTTCTCATCG

**Table S3. AEs reported in the AROAAT1001 Phase 1 study**

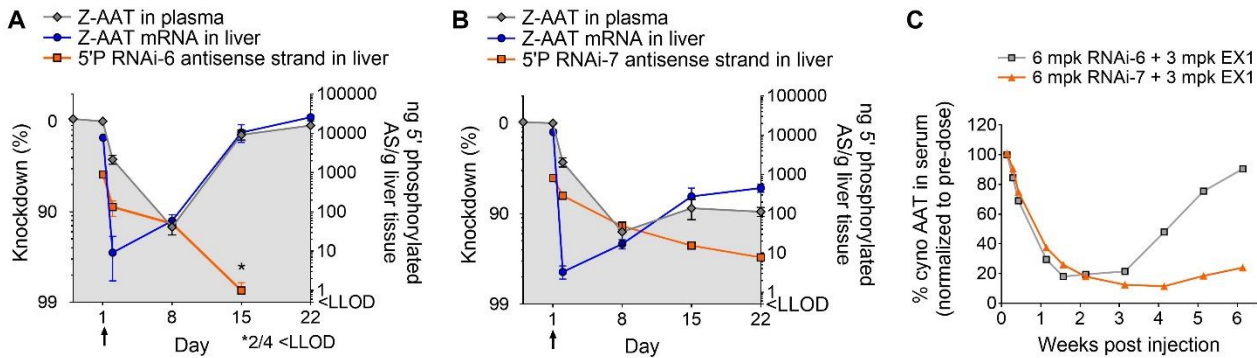
<u>AROAAAT1001 AE Preferred Terms (AEs reported in &gt;1 subject)</u>	<u>Cohort 1 35 mg single dose n = 4</u>	<u>Cohort 2 100 mg x3 doses n = 4</u>	<u>Cohort 2B 100 mg single dose n = 4</u>	<u>Cohort 3 200 mg x3 doses n = 4</u>	<u>Cohort 3B 200 mg single dose n = 4</u>	<u>Cohort 4 300 mg x3 doses n = 4</u>	<u>Cohort 4B 300 mg single dose n = 4</u>	<u>ARO-AAT All Cohorts n = 28</u>	<u>PBO All Cohorts n = 17</u>
Abdominal pain/Discomfort			1	2			2	5	1
Nausea	1			2			2	5	1
Vomiting		1	1	1				3	1
Injection site erythema, Injection site pain, Injection site bruising			2	1	1	1	1	6	0
Pyrexia								0	2
Gastroenteritis								0	2
Upper respiratory tract infection, Viral Upper respiratory tract infection		2	1	1	2	2	3	11	4
Arthropod bite	1							1	1
Vascular access site bruising, hematoma, pain, swelling	2	1	1		2	1		7	4
Forced expiratory volume decreased		1						1	2
Back pain		1						1	2
Musculoskeletal chest pain			1					1	1
Dizziness	1			1			1	3	1
Headache	2	1		2	1	1	2	9	2
Cough			1			1	1	3	3
Epistaxis		1		1				2	0

Nasal congestion								0	2
Oropharyngeal pain	1	1	1		1	1		5	3
Rhinorrhea		1	2	1				4	3
<b>Total AEs occurring more than once:</b>								<b>67</b>	<b>35</b>

**Figure S1. *In vitro* EC<sub>50</sub> comparison of AAT RNAi triggers.** EC<sub>50</sub> values for AAT mRNA reduction by the 5 most potent AAT RNAi triggers were determined in the Hep3B human hepatocyte cell line.

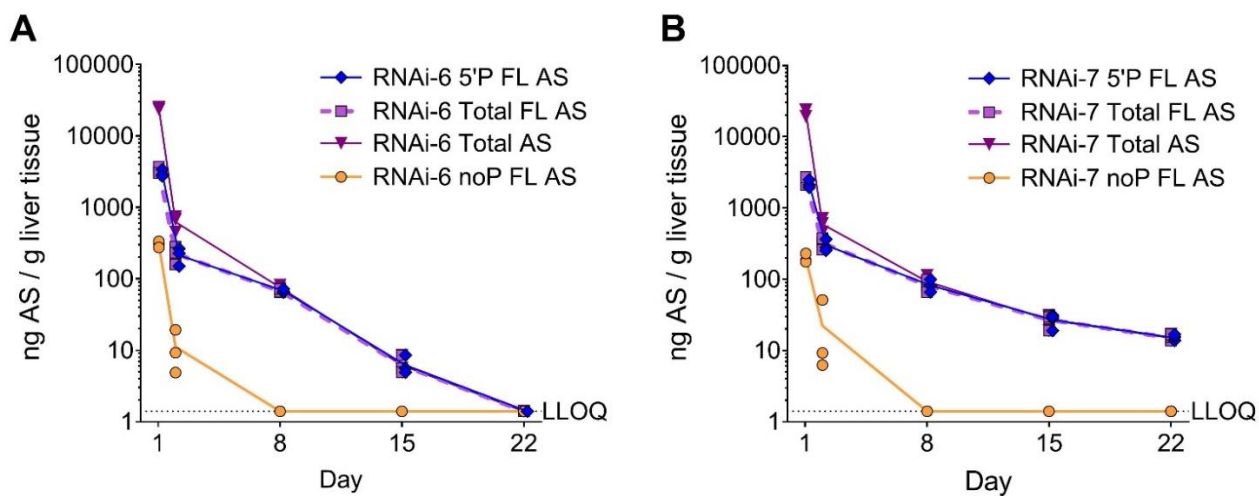


**Figure S2. Inhibition activity of RNAi triggers with and without UNA modification in PiZ mice and cynomolgus monkeys.** PiZ mice were given a single IV injection of 6 mg/kg EX1 and 12 mg/kg RNAi-6 (A) or RNAi-7 (B) on day 1. Plasma was collected at the indicated times and Z-AAT protein measured. Mice were euthanized and amounts of Z-AAT mRNA and the antisense strands of RNAi-6 or RNAi-7 in homogenized liver tissue were quantified, n = 3-4 for each time point. Knockdown of plasma Z-AAT protein and liver Z-AAT mRNA (left axis) were compared to the amount of 5' phosphorylated full-length antisense strand in liver (right axis). (C) Cynomolgus monkeys (n = 2) were given a single IV injection of 3 mg/kg EX1 and 6 mg/kg of either RNAi-6 or RNAi-7. Serum was collected at the indicated times and endogenous AAT protein measured. Reduction is shown relative to pre-dose as the mean ± SEM.

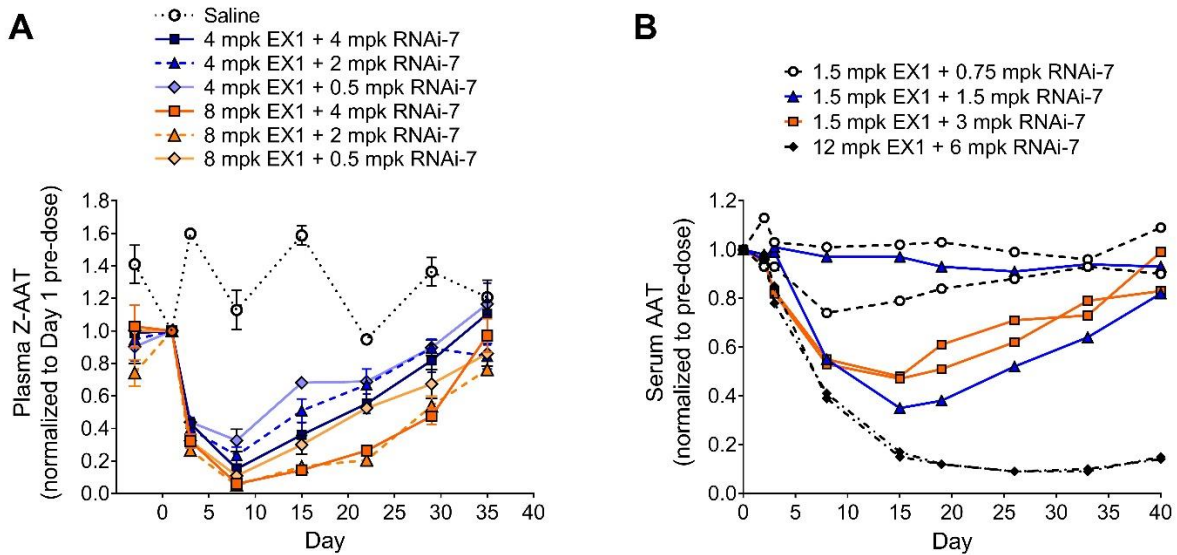




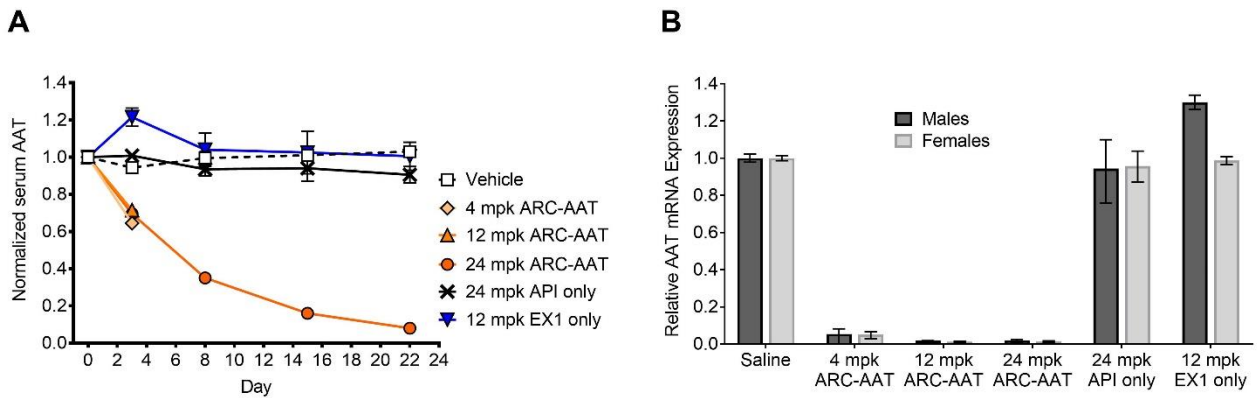
**Figure S3. Time course of RNAi-6 and RNAi-7 antisense strands in liver.** Wild-type mice were given a single IV injection of 6 mg/kg EX1 and 12 mg/kg RNAi-6 (**A**) or RNAi-7 (**B**) on day 1. Liver tissue from mice euthanized at 1 hr, 24 hr (day 2), and days 8, 15 and 22 was homogenized and the amount of RNAi-6 or RNAi-7 antisense strand that was full-length and 5' phosphorylated (5' FL AS) or nonphosphorylated (noP FL AS), together comprising the total FL AS, as well as the total amount of measured AS including metabolites are shown for each liver.



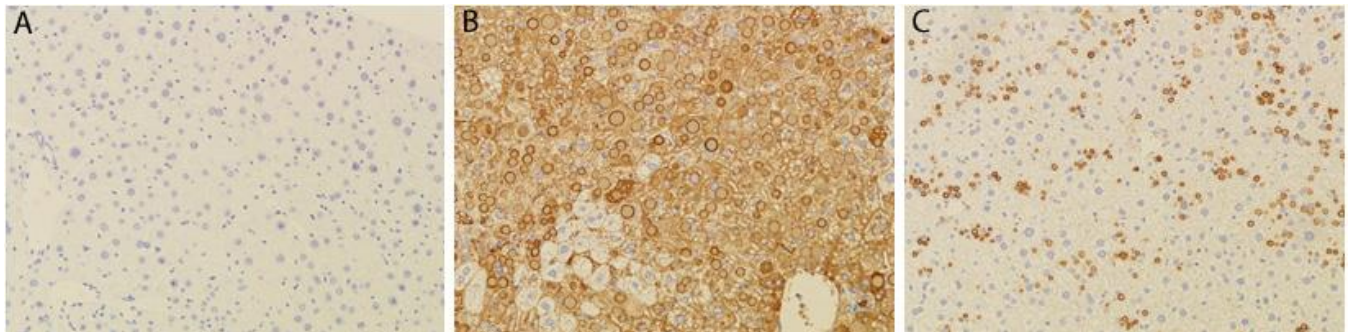
**Figure S4. Variations in the RNAi trigger: excipient ratio in PiZ mice and monkeys.** (A) PiZ mice (n = 4) were given a single IV injection of 4 or 8 mg/kg EX1 along with 0.5 to 4 mg/kg RNAi-7. Plasma was collected at the indicated times and Z-AAT protein measured, shown normalized to pre-dose (mean ± SEM). Control animals were injected with saline. (B) Cynomolgus monkeys (n = 2) were given a single IV injection of 1.5 mg/kg EX1 and 0.75 – 6 mg/kg RNAi-7. Serum was collected at the indicated times and endogenous AAT protein measured. Reduction is shown relative to pre-dose for individual animals.



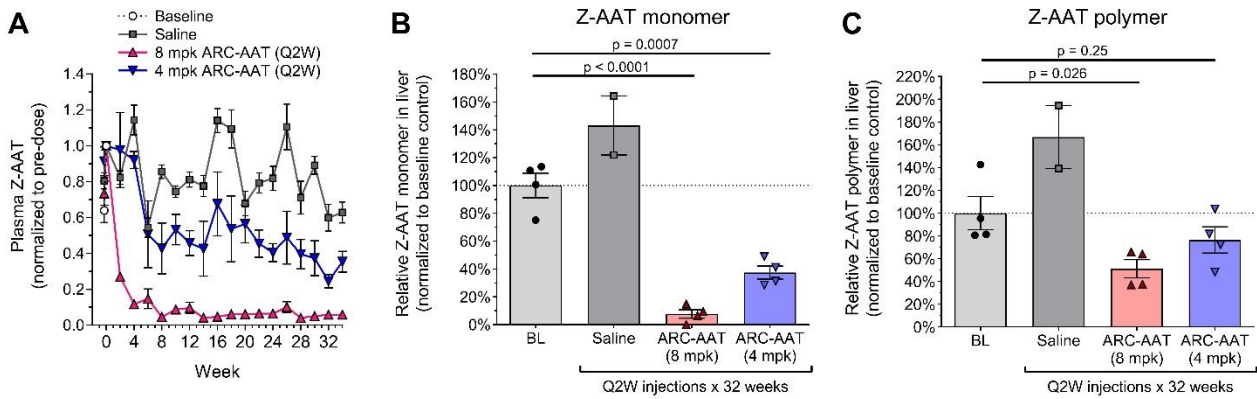
**Figure S5. RNAi activity of ARC-AAT required the excipient EX1.** Male and female cynomolgus monkeys (NHP, n = 4-6 for each sex) were given a single IV injection of vehicle alone, ARC-AAT (4 mg/kg, 12 mg/kg or 24 mg/kg), 24 mg/kg ARC-AAT API alone (API alone) or 12 mg/kg EX1 alone. **(A)** Serum was collected at the indicated times and endogenous AAT protein measured. Reduction is shown relative to pre-dose for the males, mean  $\pm$  SEM. **(B)** NHP were euthanized on Day 3 (48 hours post-injection) for mRNA measurement: one animal of each sex from the vehicle alone and the EX1 alone groups and 3 animals of each sex from the rest of the groups. Liver AAT mRNA expression is shown by sex normalized to that in animals injected with vehicle alone.



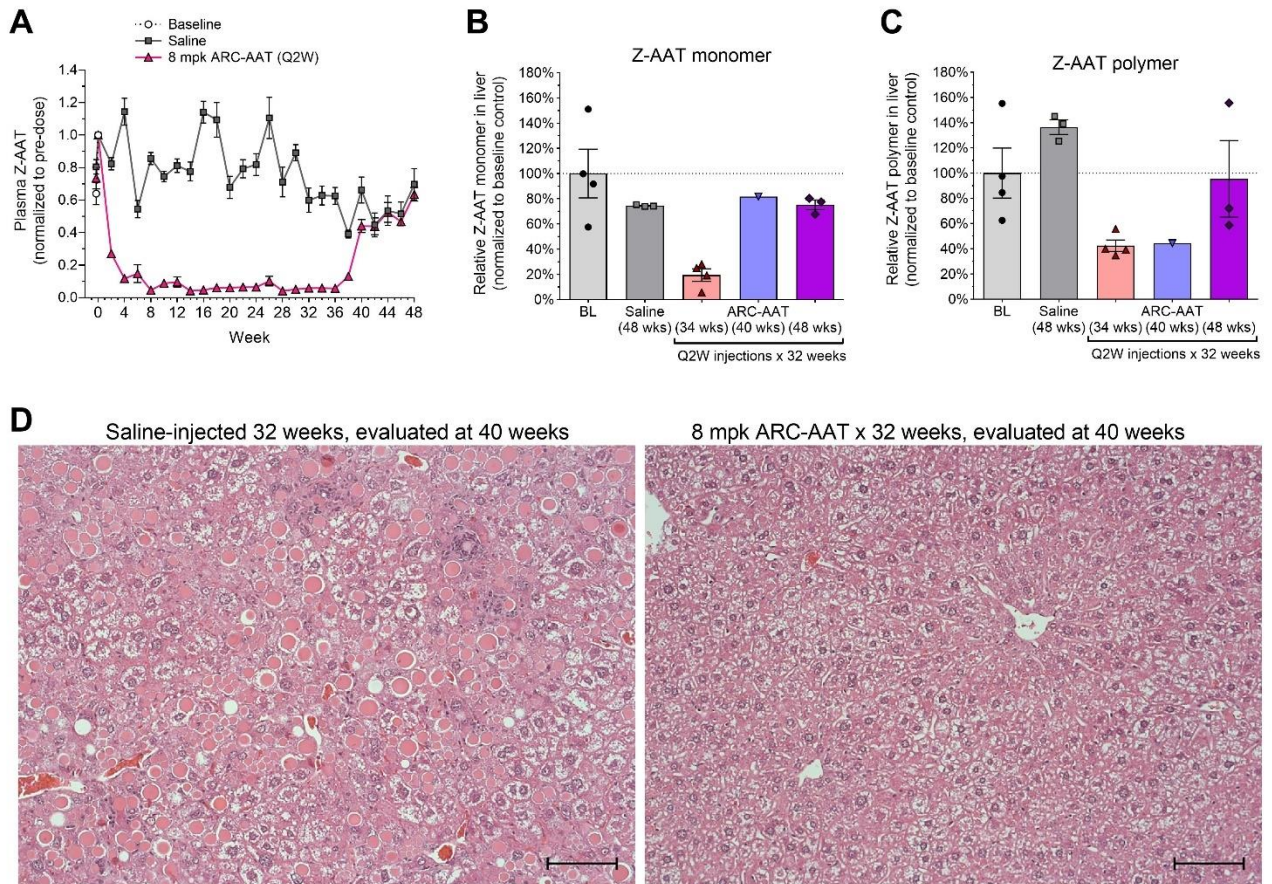
**Figure S6. Liver Z-AAT protein remaining in ARC-AAT-treated mice is in globular form.** Paraffin-embedded liver specimens from eleven-month old wild-type mouse **(A)**, saline group PiZ mouse **(B)** and PiZ mouse treated for 33 weeks with 8 mg/kg ARC-AAT **(C)** were stained with primary antibodies to human AAT and with rabbit anti-goat horseradish peroxidase-conjugated secondary antibodies.



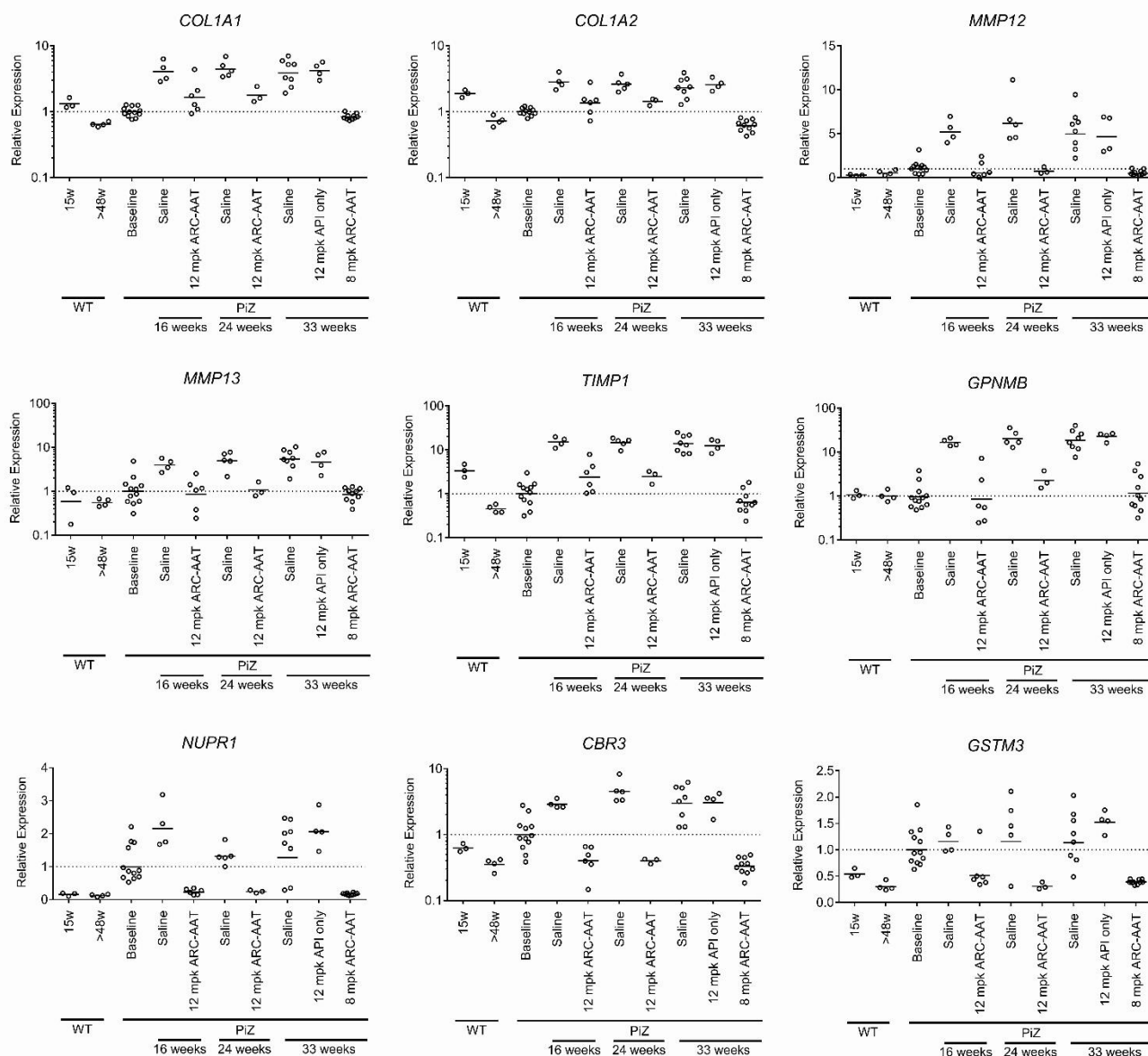
**Figure S7. Dose-dependency of liver Z-AAT protein reduction.** Male PiZ mice that were 11-17 weeks old at baseline were given 17 biweekly IV injections of 4 or 8 mg/kg ARC-AAT or saline. **(A)** Plasma was collected biweekly prior to dosing and Z-AAT protein measured, shown normalized to pre-dose. **(B, C)** Mice were euthanized at baseline (BL) or after 32 weeks of 4 mg/kg or 8 mg/kg ARC-AAT treatment (n = 4). The monomeric and polymeric Z-AAT protein in the liver were measured by semi-quantitative Western blotting, shown as mean  $\pm$  SEM.



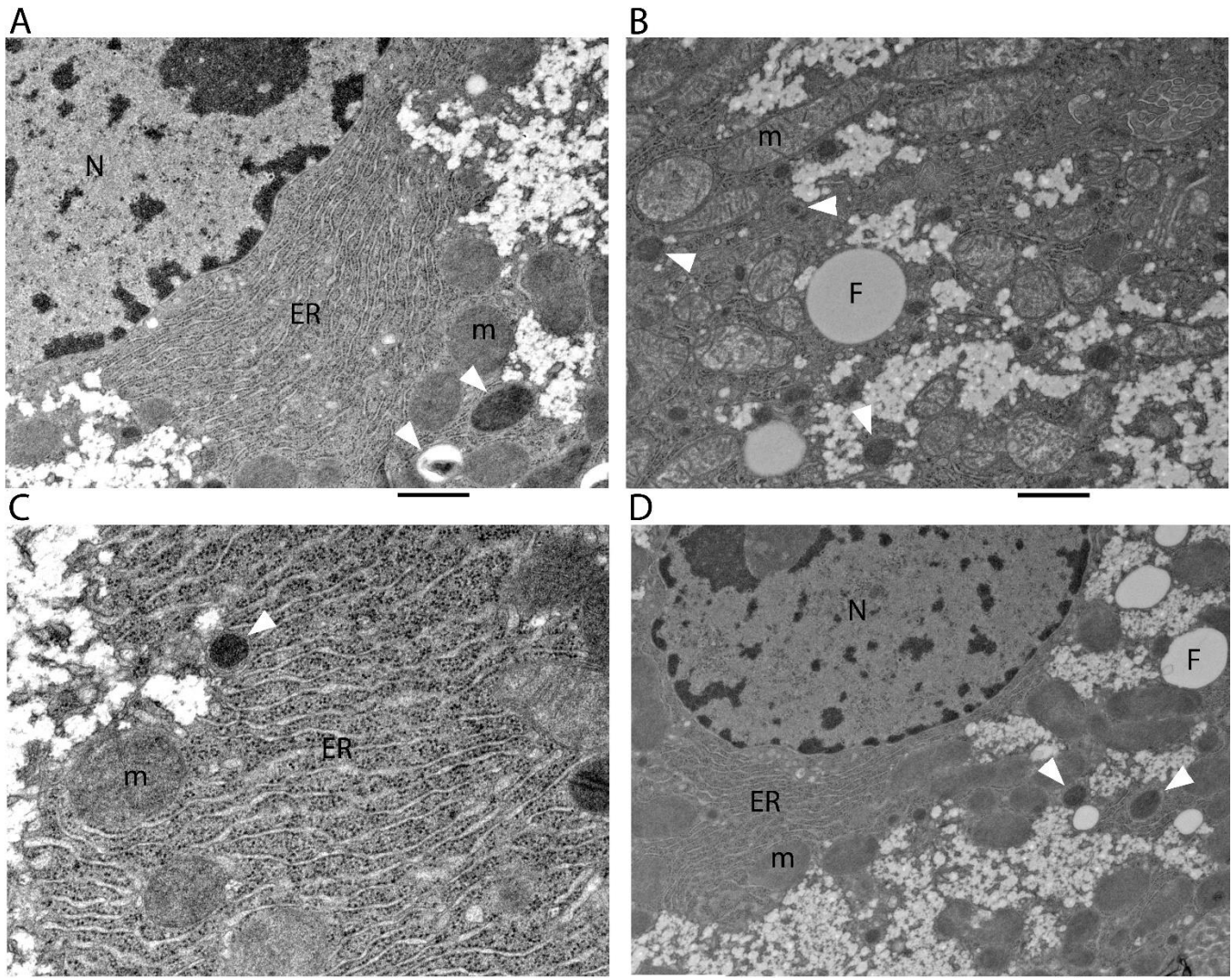
**Figure S8. Prolonged benefit of liver Z-AAT protein reduction.** Male PiZ mice that were 11-17 weeks old at baseline were given 17 biweekly IV injections of 8 mg/kg ARC-AAT or saline and evaluated up to 16 weeks after the final dose. Means are shown with SEM. **(A)** Plasma was collected at the indicated times and Z-AAT protein measured, shown normalized to pre-dose. **(B-D)** Mice were euthanized at baseline (BL) or 2, 8 or 16 weeks after final injection of 8 mg/kg ARC-AAT treatment at weeks 34, 40 or 48, respectively. The monomeric **(B)** and polymeric **(C)** Z-AAT proteins in the liver were measured by semi-quantitative Western blotting. **(D)** H&E stained liver sections of saline injected and 8 mg/kg ARC-AAT injected mice 8 weeks after the final injection. Scale bar represents 20  $\mu$ m.



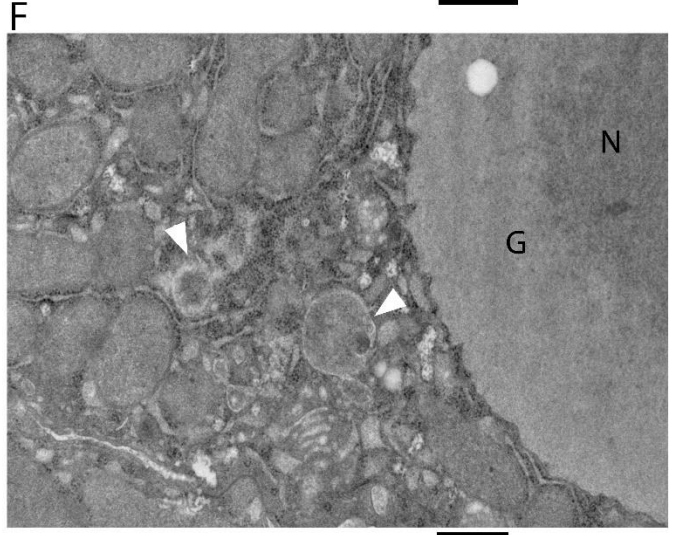
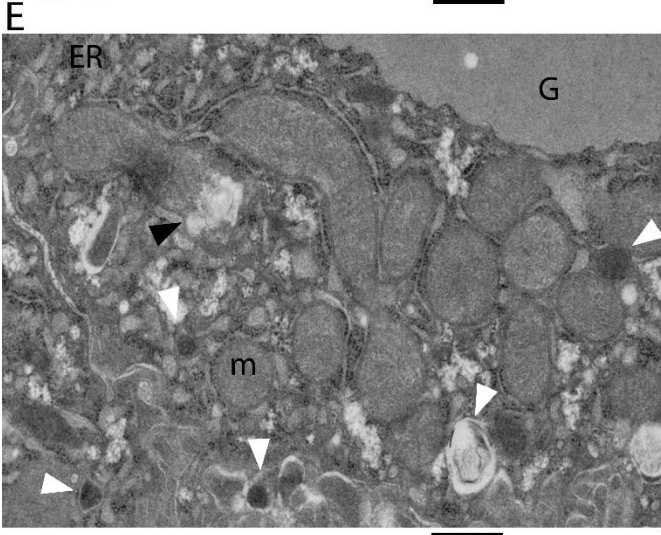
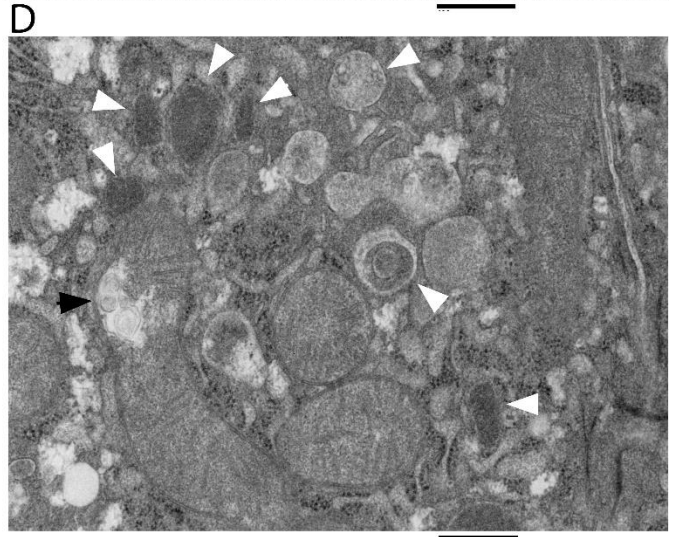
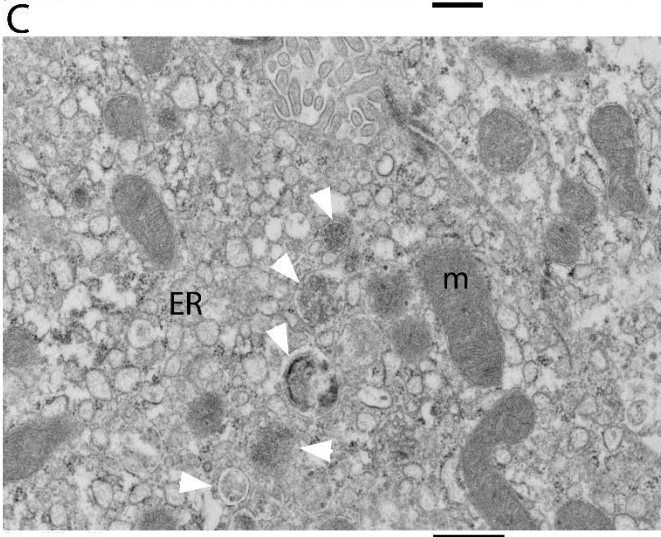
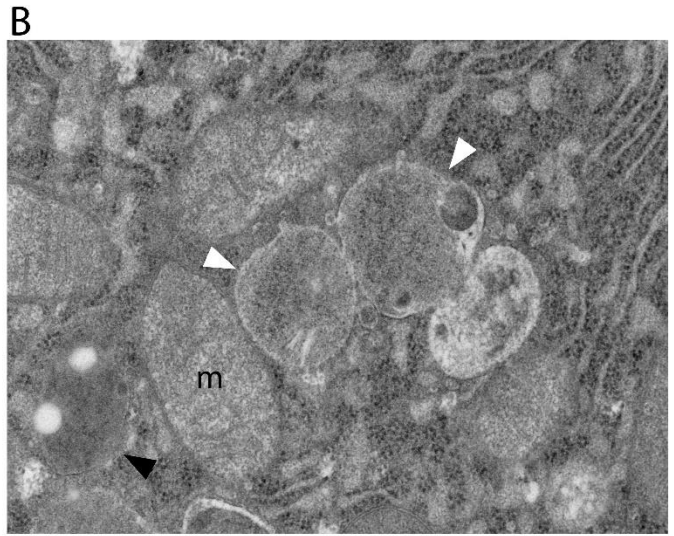
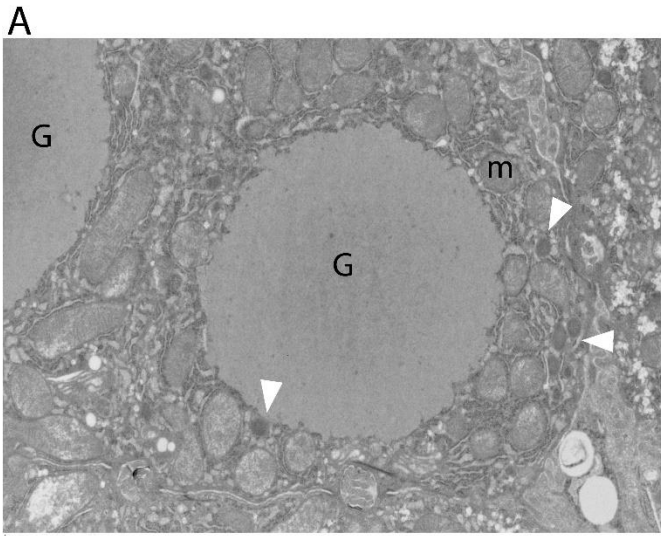
**Figure S9. ARC-AAT treatment prevented or normalized expression of PiZ disease-associated genes.** Expression of genes associated with fibrosis (*COL1A1*, *COL1A2*, *MMP12*, *MMP13*, *TIMP1*); stress, autophagy and apoptosis (*GPMB* and *NUPR1*); and redox-regulation (*CBR3*, *GSTM3*) were measured in livers of adult male PiZ mice (11-17-weeks old at baseline) that were given IV injections Q2W of 8 mg/kg or 12 mg/kg ARC-AAT, 12 mg/kg ARC-AAT API alone, or saline (n = 3-12) and euthanized for evaluation two weeks after the final injection, which was 16, 24 or 33 weeks from start of study. Untreated baseline mice were euthanized at start of study. Gene expression in wild-type C57Bl/6 mice that were young adults (15 weeks old) or 48-78 weeks old (>48wk) is shown for comparison.



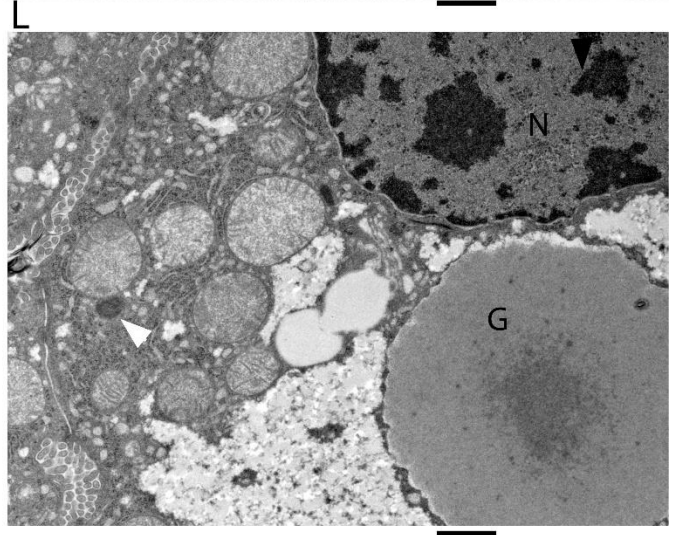
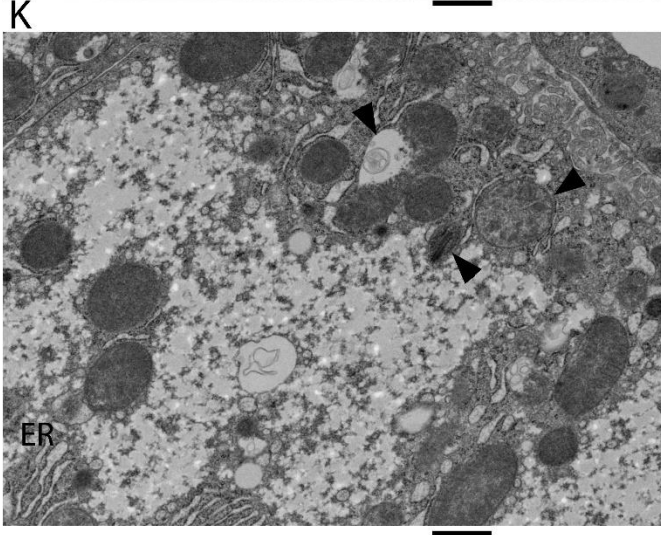
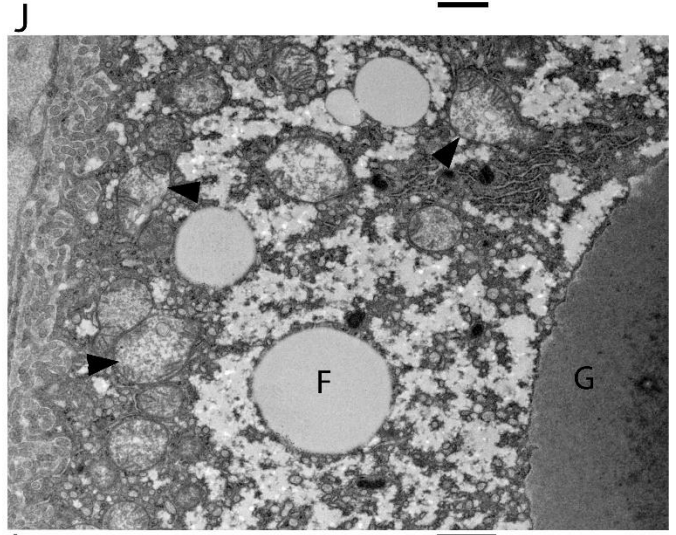
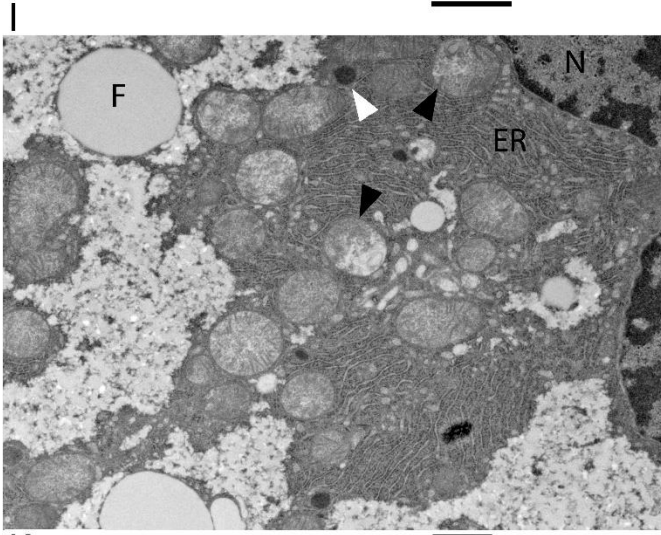
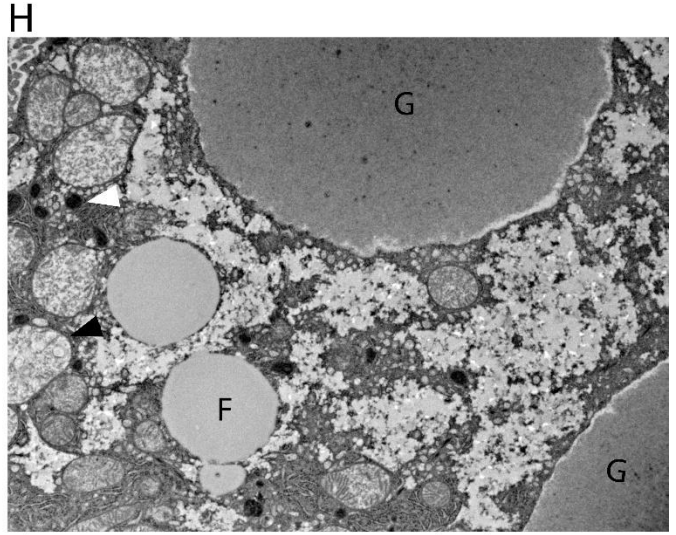
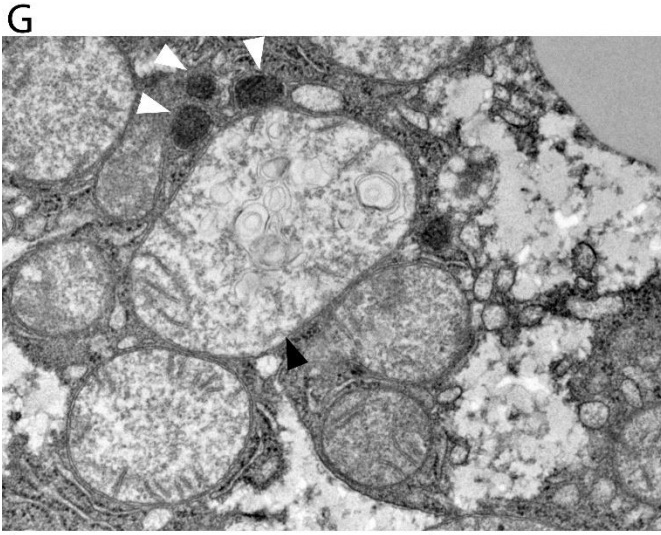
**Figure S10. Hepatocyte ultrastructure of wild-type mouse.** Electron microscope images of hepatocytes in liver tissue from a naïve wild-type mouse, 11 months old (**A-D**). ER, endoplasmic reticulum; F, microvesicular fat; m, healthy mitochondrion; N, nucleus; white arrowheads, autophagosomes. Scale bars = 1  $\mu$ m in **A, B** and **D**; 500 nm in **C**.



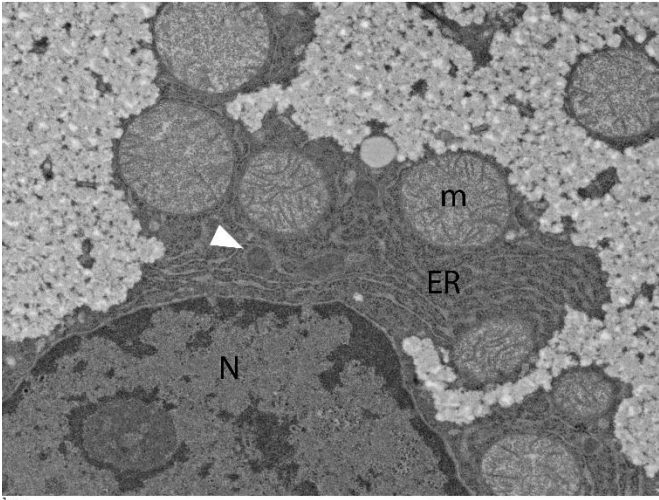
**Figure S11. Hepatocyte ultrastructure of PiZ mice.** Electron microscope images of hepatocytes in liver tissue from a PiZ mouse at baseline, 3 months old (**A - F**); PiZ mouse IV-injected with saline (Q2W) for 31 weeks, 11 months old (**G - L**); and PiZ mouse IV-injected (Q2W) with 8 mg/kg ARC-AAT for 31 weeks, 11 months old (**M - R**). ER, endoplasmic reticulum; G, globule; m, healthy mitochondrion; N, nucleus; black arrowheads, damaged mitochondria; white arrowheads, autophagosomes. Scale bars = 1  $\mu$ m in **A, H-N, P-R**; 600 nm in **C, E, F**; 500 nm in **D, G** and **O**; and 400 nm in **B**.



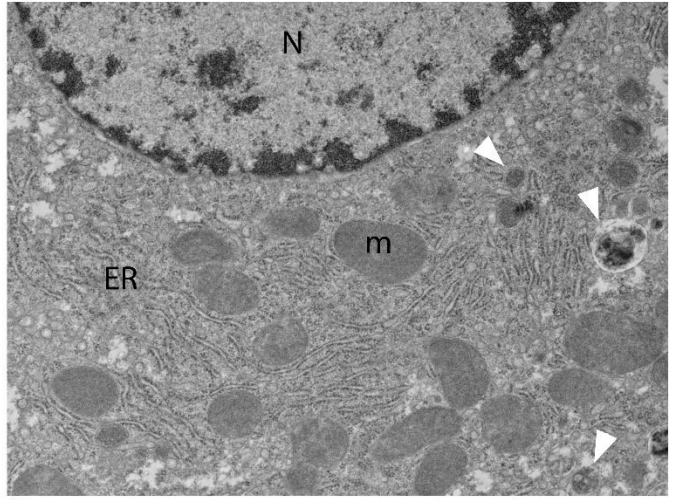




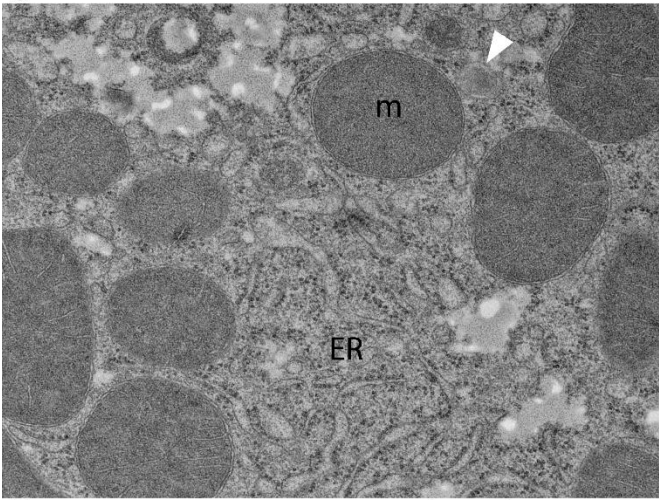
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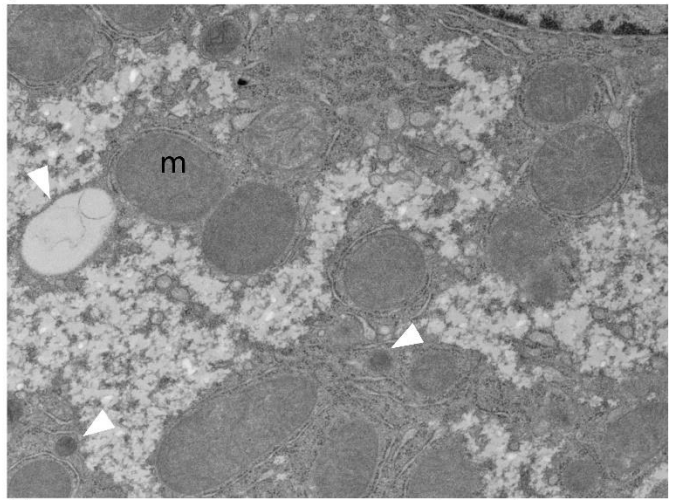
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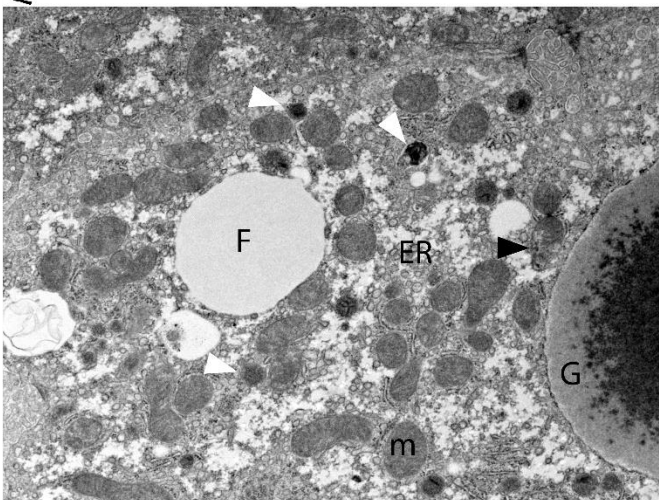
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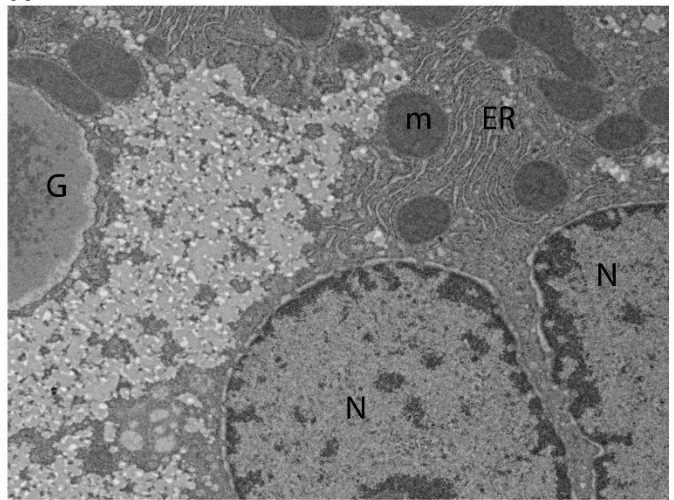
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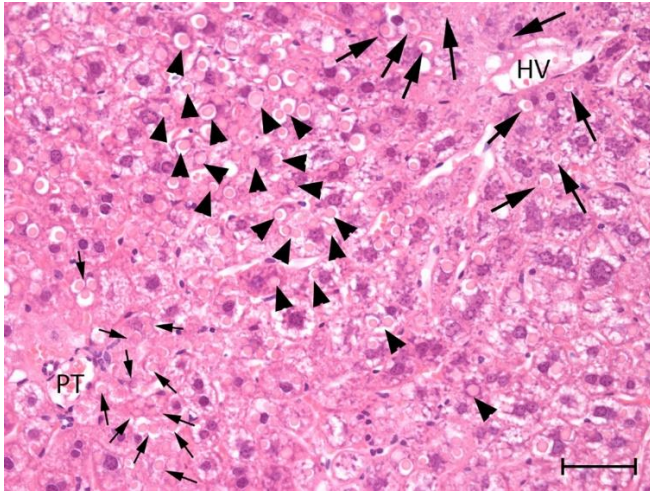


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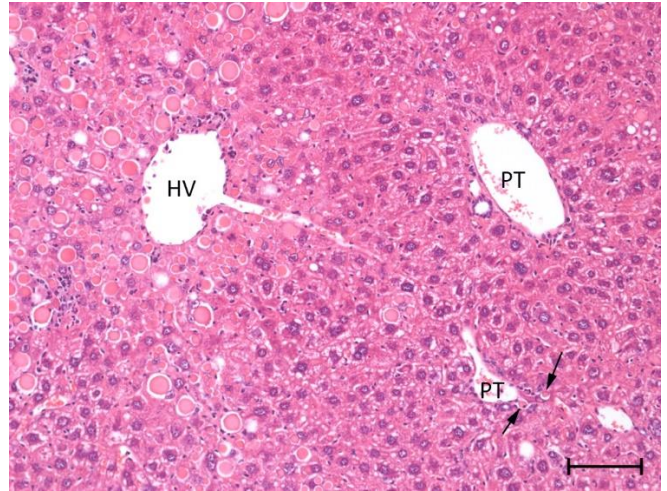


**Figure S12. Clearance of globules in old PiZ mice.** Male PiZ mouse paraffin-embedded liver specimens from animals of varying ages were stained with H&E, PAS-D with hematoxylin, or with fluorescent stain. **(A)** 14-week old untreated mouse has panlobular globules; small arrows, globules in the periportal region (PT, portal triad), part of hepatic unit zone 1; arrowheads, globules in zone 2; large arrows, globules in proximity to the hepatic vein (HV), part of zone 3. **(B, C)** 68-week old untreated mouse #277 has large globules proximal to HV and in zone 2; rare, small globules near PT (arrows in **B**). The few PAS-D-stained globules in 88-week old saline-injected control mouse #315 liver (**D**) and in liver of 90-week old mouse #290 treated with 8 mg/kg ARC-AAT for 32 weeks (**E**) are in zones 2-3. **(F-H)** Apoptotic cells proximal to the hepatic vein in untreated mouse #277. **(F)** Arrows indicate apoptotic hepatocytes in H&E-stained liver section. **(G, H)** Confocal microscopy imaging shows TUNEL stain of apoptotic cells (red fluorescence), DAPI-stained nuclei (blue) and tissue structure is defined by Differential Interference Contrast microscopy imaging; arrowheads, apoptotic hepatocytes; arrows, apoptotic infiltrating cells; white asterisks, globules; green asterisks, globules adjunct to the hepatic vein lumen. Extrusion of globules into hepatic veins in untreated baseline 67-week old mouse #280 (**I**) and 65-week old mouse #295 (**J**); saline-injected 91-week old mouse #281 (**K**) and 88-week old mouse #315 (**L**); ARC-AAT-treated 90-week old mouse #291 (**M**) and 91-week old mouse #282 (**N, O**). H&E stain: panels A, B, F, I-O. Scale bar = 50  $\mu\text{m}$  in A, N; 100  $\mu\text{m}$  in B-E; 20  $\mu\text{m}$  in F, I-M, O

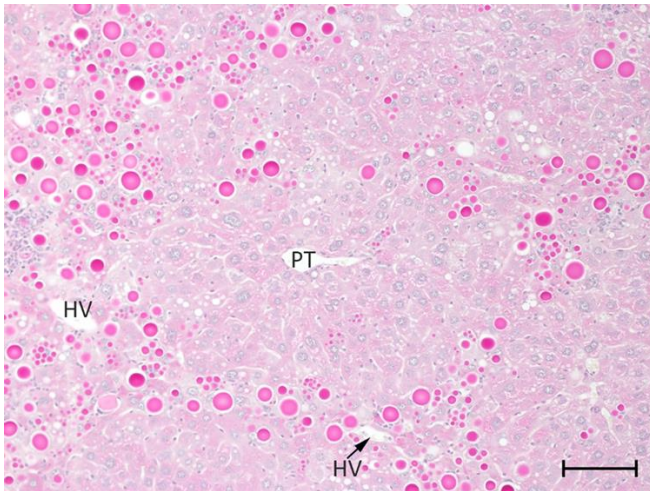
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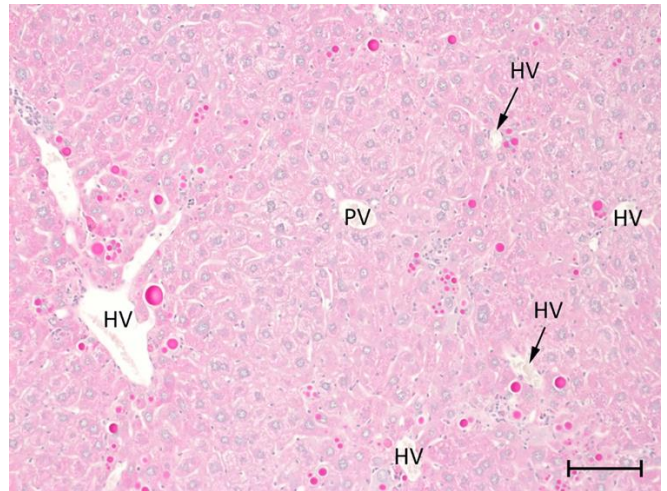
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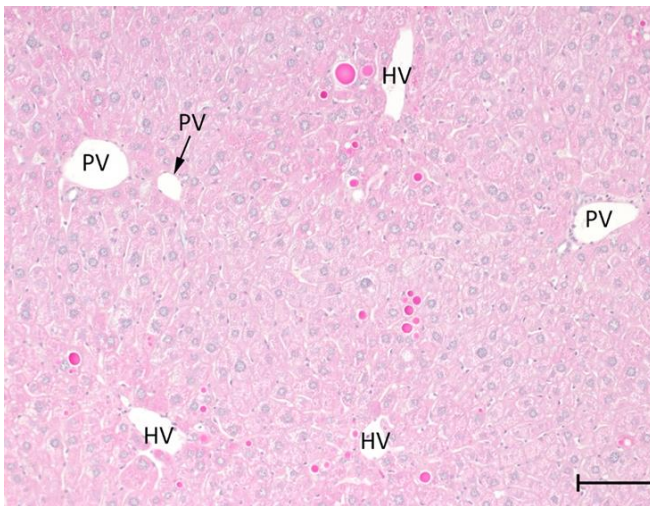
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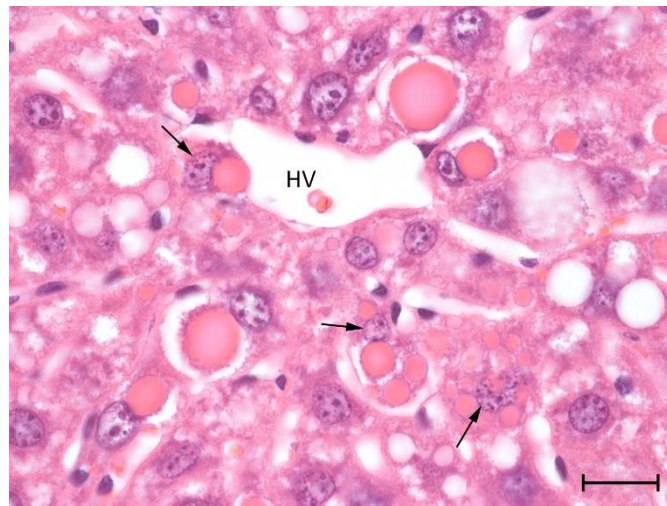
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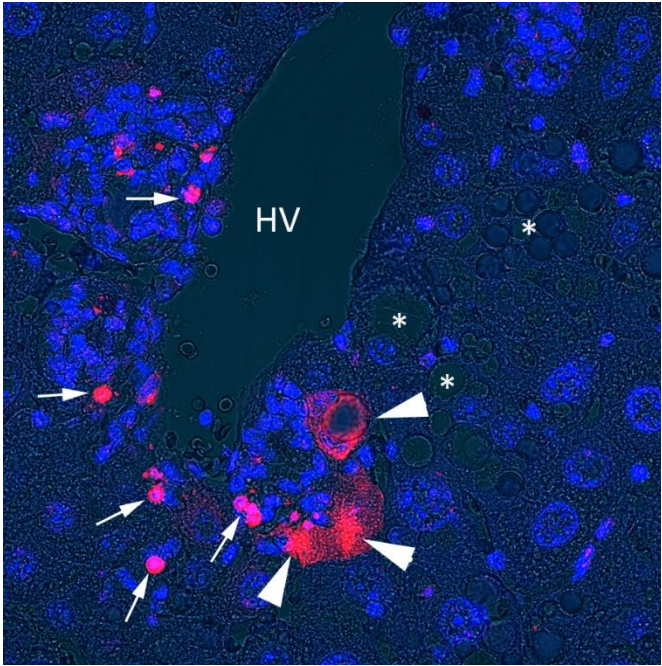
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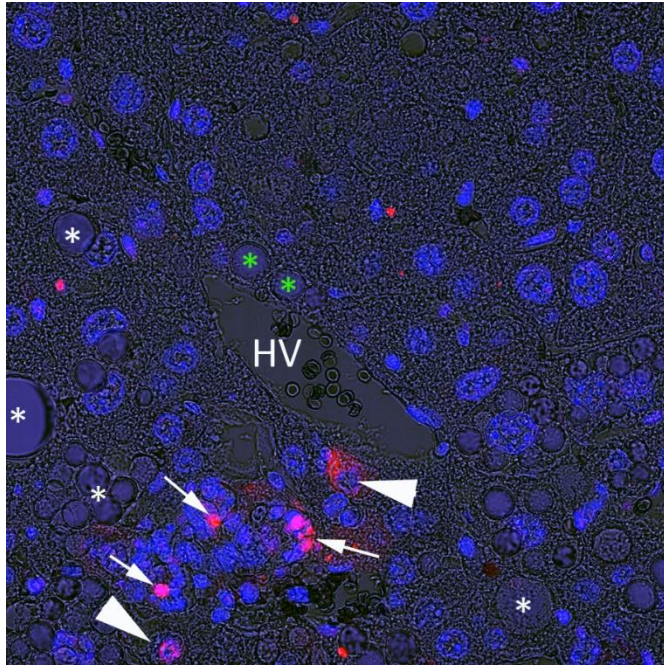
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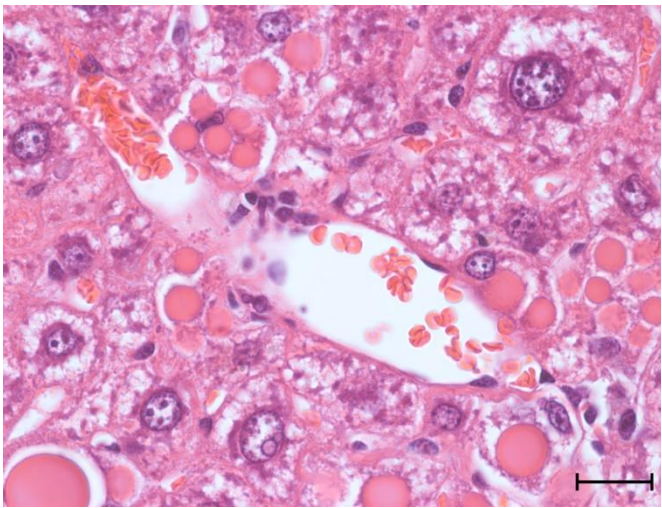
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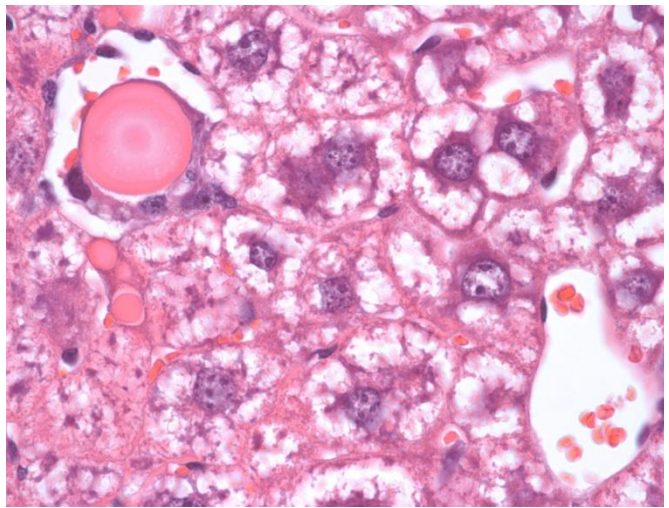
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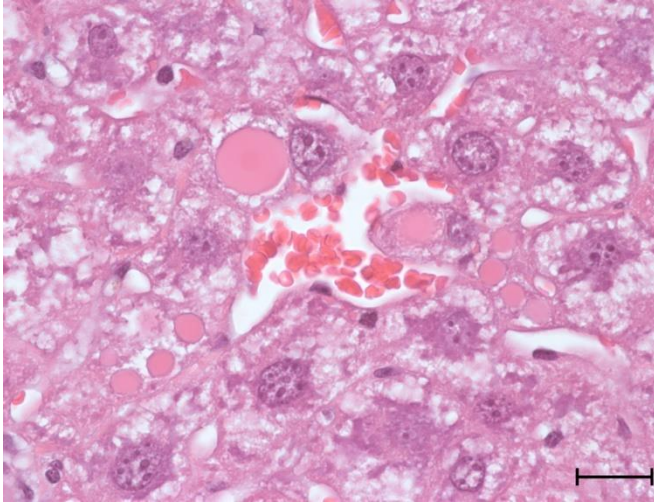
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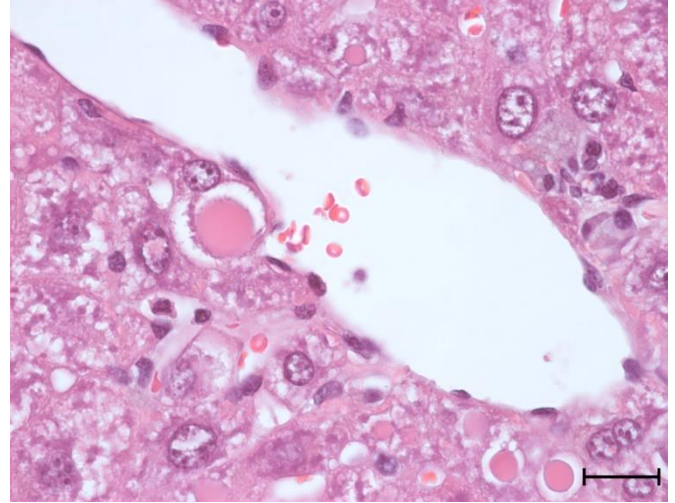
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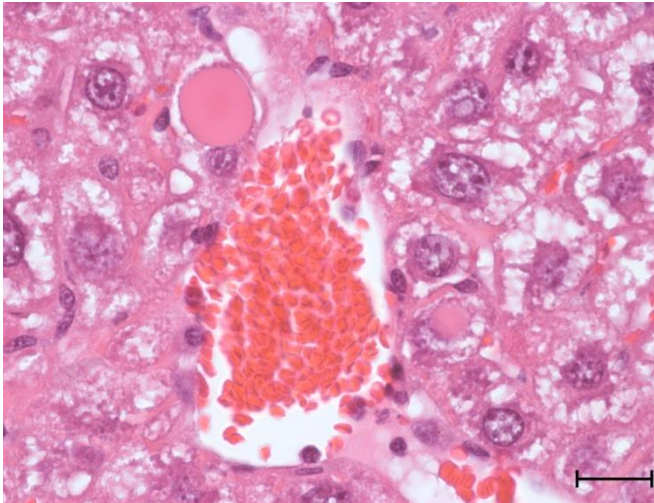
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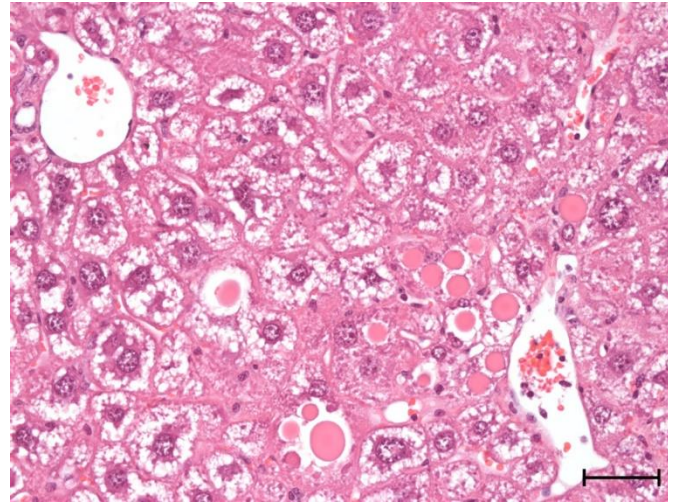
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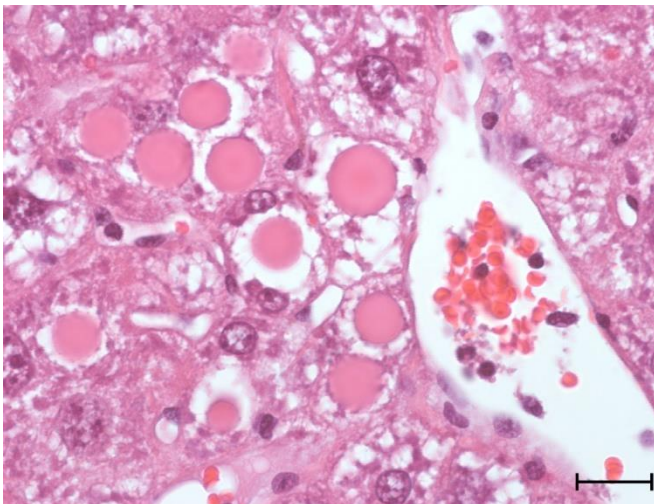
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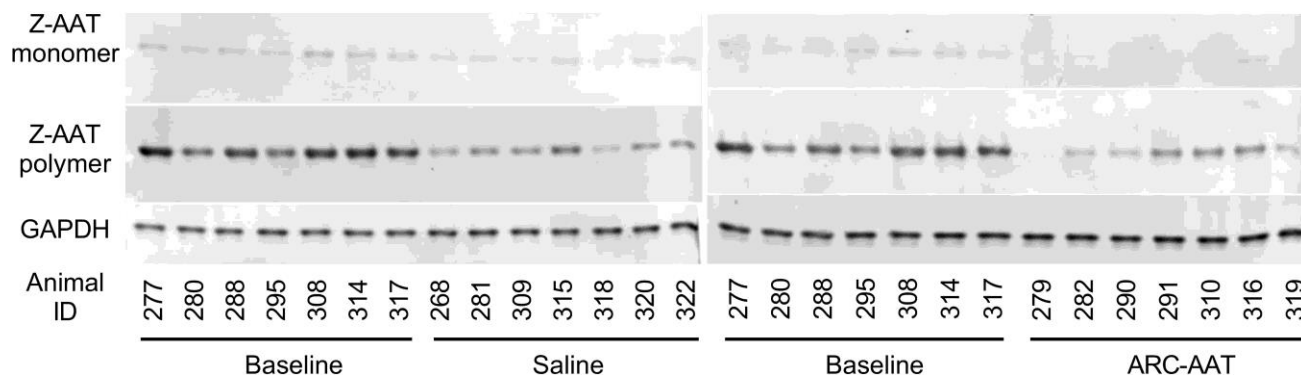
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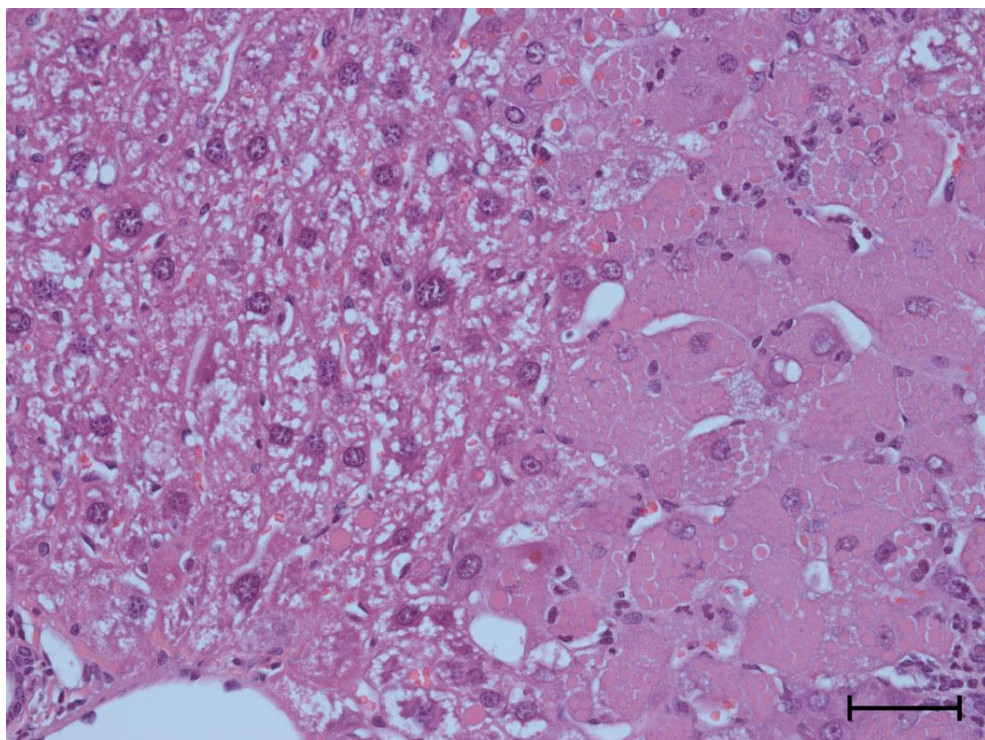
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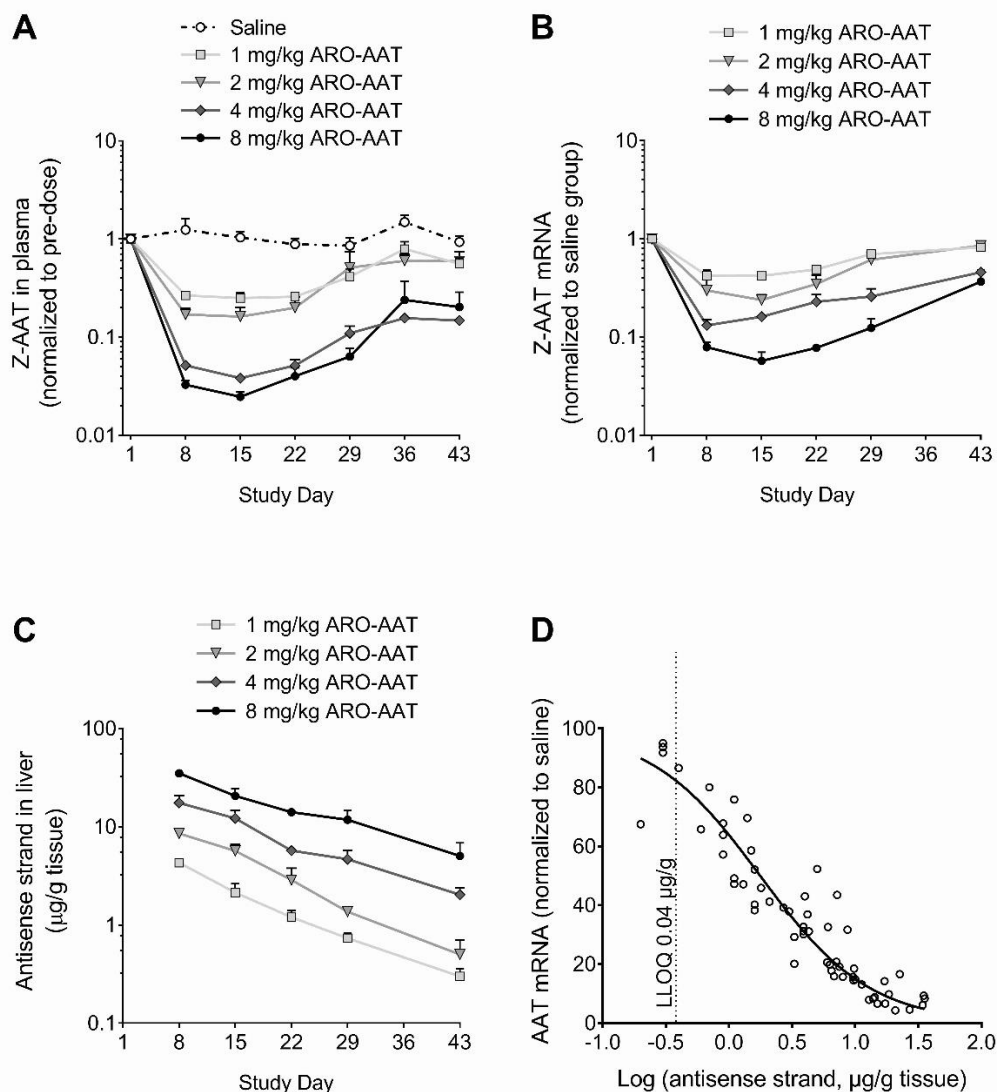
**Figure S13. Reduction of monomeric and polymeric Z-AAT in aged PiZ mice.** Male PiZ mice 64-69 weeks old were euthanized at baseline or given sixteen Q2W injections of saline or 8 mg/kg ARC-AAT and euthanized after 32 weeks. The amounts of monomeric and polymeric Z-AAT protein in liver lysates were measured by semi-quantitative Western blotting of available specimens. Loading control GAPDH is shown for the soluble fraction. Animals with identification numbers 317, 281, 309, 315, 318, 320, 322, 279, 291 had neoplastic transformations.



**Figure S14. Dysplastic hepatocytes overloaded with globules.** A 64-week old male PiZ mouse (ID #310) was given sixteen Q2W IV injections of 8 mg/kg ARC-AAT and euthanized two weeks after the final injection. Liver sections were stained with H&E. Liver tissue on the left half of the image has a normal appearance. Dysplastic hepatocytes on the right side are abnormally large and overloaded with numerous globules. Scale bar = 50  $\mu$ m

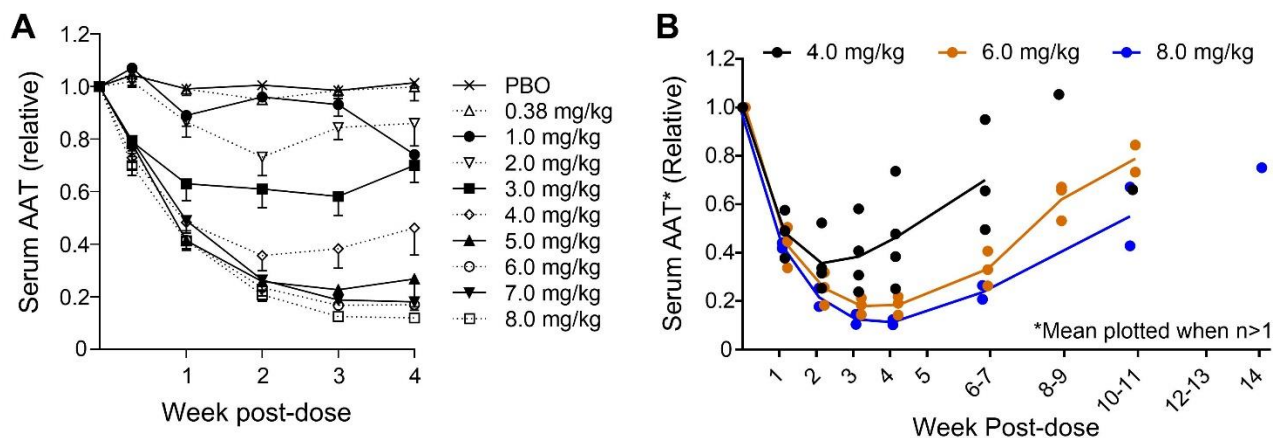


**Figure S15. Dose exposure-response relationship of ARO-AAT in PiZ mice.** Groups of PiZ mice were given a single SC injection of 1, 2, 4 or 8 mg/kg ARO-AAT (n = 15) or saline (n = 4). At weekly intervals, plasma Z-AAT was measured, shown as the mean + SEM (A). Three animals per group were euthanized at each time point to measure the amounts of Z-AAT mRNA in the liver, shown as the geometric mean + SEM relative to the saline group that was euthanized on day 43 (B) and the amounts of ARO-AAT antisense strand in the liver (C). The percentage of Z-AAT mRNA expression in each liver sample was graphed as a function of the quantity of antisense strand in the same sample to visualize the dose-exposure relationship (D). The lower limit of quantitation (LLOQ) was 0.04  $\mu\text{g}$  antisense strand/gram liver tissue.





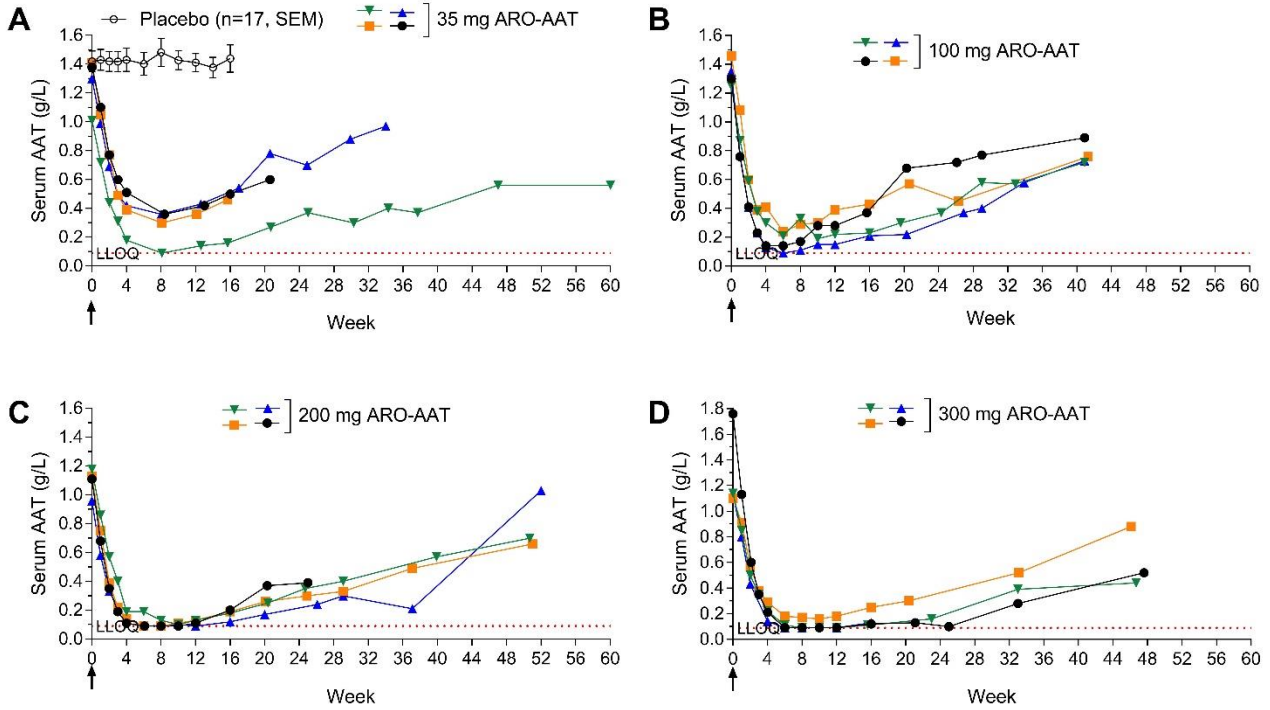
**Figure S16. Dose-responsive serum AAT reductions and duration of effect after ARC-AAT administration in human volunteers.** A double-blind first-in-human clinical trial Part A randomized 54 healthy volunteers into single dose cohorts, 2 placebo: 4 active (19). Subjects were IV-infused with a single dose of 0.38 to 8 mg/kg ARC-AAT (n = 3-4). Serum was collected at the indicated times and AAT protein measured, shown relative to pre-study as the means with SEM (A). The duration of response was assessed in subjects given a single dose of 4, 6 or 8 mg/kg ARC-AAT (B). The means are shown for time points with more than one data point. This figure was re-produced from the original paper by permission.



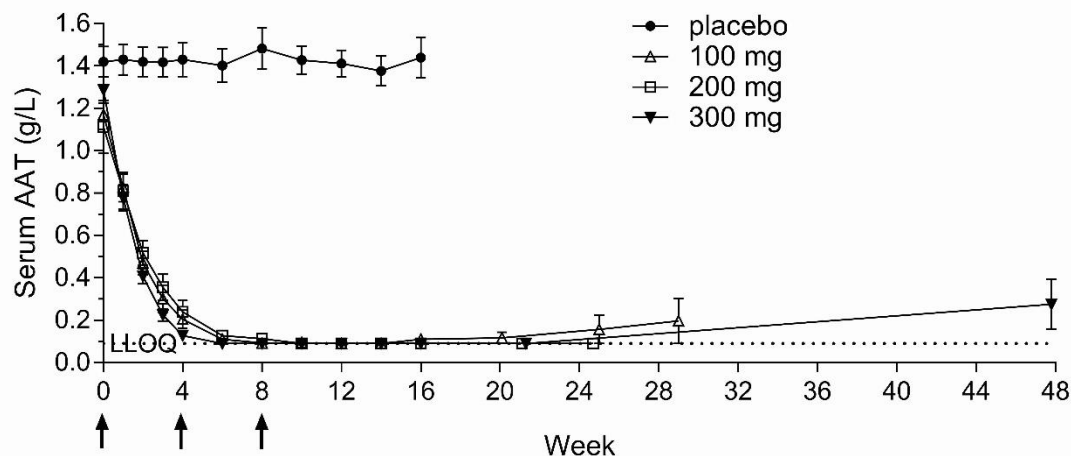
**Figure S17. AROAAT1001 study design and dose escalation schedule**

Single Dose Normal Healthy Volunteers (Cohorts 1, 2, 3, 4 are double blind; Cohorts 2b, 3b, 4b are open label)			Multi-dose Normal Healthy Volunteer (double blind)
Cohort	Dose (Day 1)	Day 15 safety evaluation	Dose Regimen
Cohort 1	35 mg		N/A
Cohort 2/2b	100 mg		100 mg dosed on Day 29, 57/ Cohort 2b: single dose only (Day 1)
Cohort 3/3b	200 mg		200 mg dosed on Day 29, 57/ Cohort 3b: single dose only (Day 1)
Cohort 4/4b	300 mg		300 mg dosed on Day 29, 57/ Cohort 4b: single dose only (Day 1)

**Figure S18. Single dose cohorts of human volunteers dosed with ARO-AAT in AROAAT1001.** Healthy subjects were given a single SC injection of 100 mg, 200 mg, or 300 mg ARO-AAT (n = 4) or placebo (n = 17). Serum was collected at the indicated times and AAT measured. Mean  $\pm$  SEM is shown for the placebo group and serum AAT for individual subjects is shown for those dosed with ARO-AAT. AAT LLOQ was 0.09 g/L.



**Figure S19. Multi-dose cohorts of human volunteers dosed with ARO-AAT.** Healthy subjects were given SC injections of 100, 200, or 300 mg ARO-AAT or placebo on Days 1, 29 and 57: placebo (n = 17 through week 4, then n = 13); 100 mg (n = 4 through week 8, then n = 3 through week 29); 200 mg (n = 4 through week 8, then n = 3 through week 25); 300 mg (n = 4). Serum was collected at the indicated times and AAT measured, shown as mean  $\pm$  SEM. LLOQ is 0.09 g/L.



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