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Supplemental Information

Increased expression or activation of TRPML1 reduces hepatic storage of toxic Z alpha-1 antitrypsin

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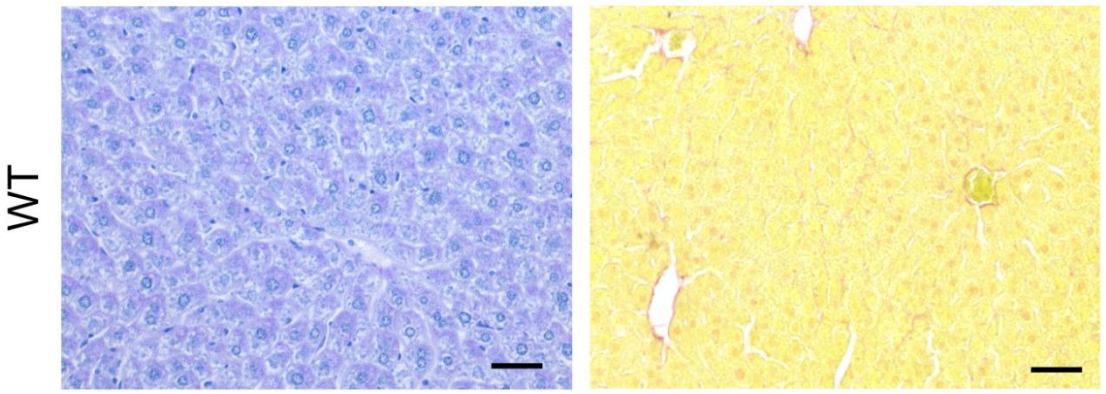

Figure S1. PAS staining and Sirius red in wild-type mice.

Liver PAS-D (left panel) and Sirius Red (right panel) stainings of C57BL/6 wild-type (WT) mice of 13 weeks of age.

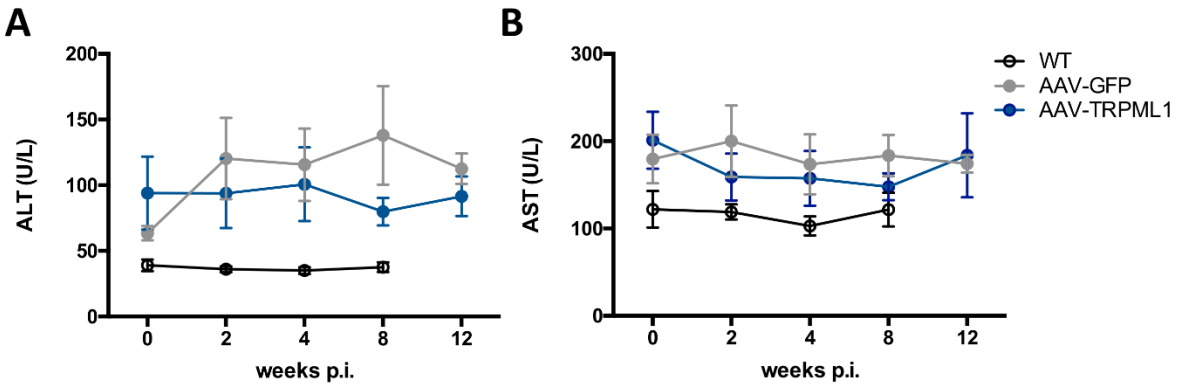

Figure S2. Hepatic *TRPML1* gene transfer is not associated with increases in trasaminases.

Serum activities of ALT (**A**) and AST (**B**) in PiZ mice injected with AAV-GFP or AAV-TRPML1 (n=7 per group). Control wild-type (WT) mice (n=4) are also shown. Data are shown as mean ± standard error.

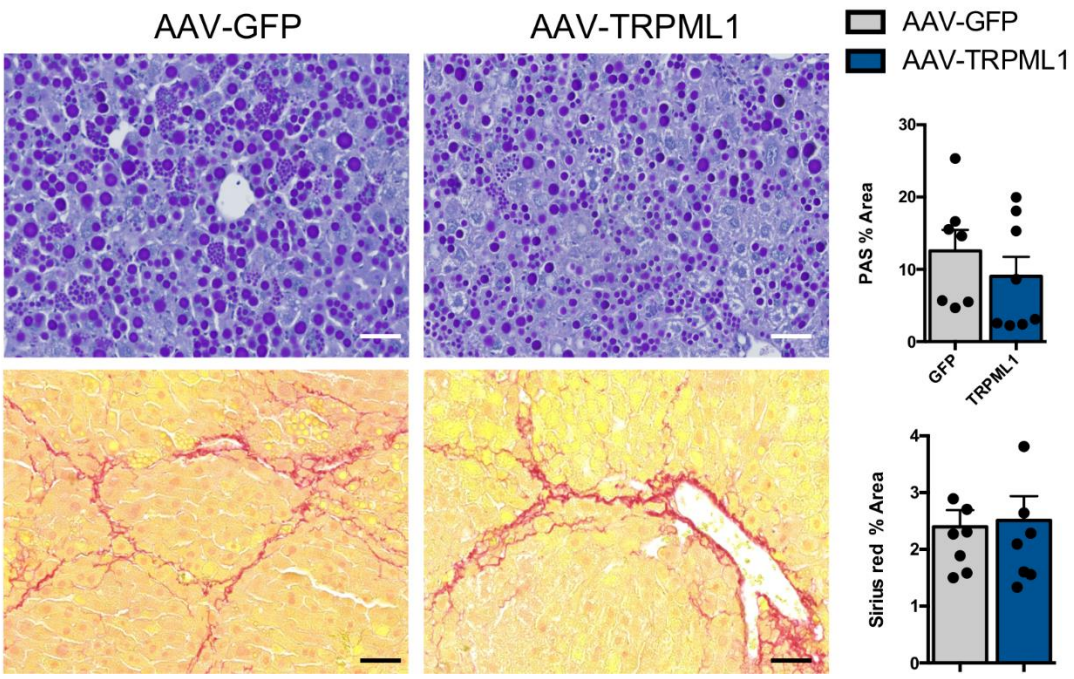

Figure S3. Hepatic *TRPML1* gene transfer in 16-week-old PiZ mice.

Liver PAS-D (upper panels) and Sirius Red (lower panels) stainings of PiZ mice injected at 16-weeks of age and analyzed 12-weeks post-injection with relative quantifications (n=7 AAV-GFP, n=8 AAV-TRPML1). Data are shown as mean ± standard error.

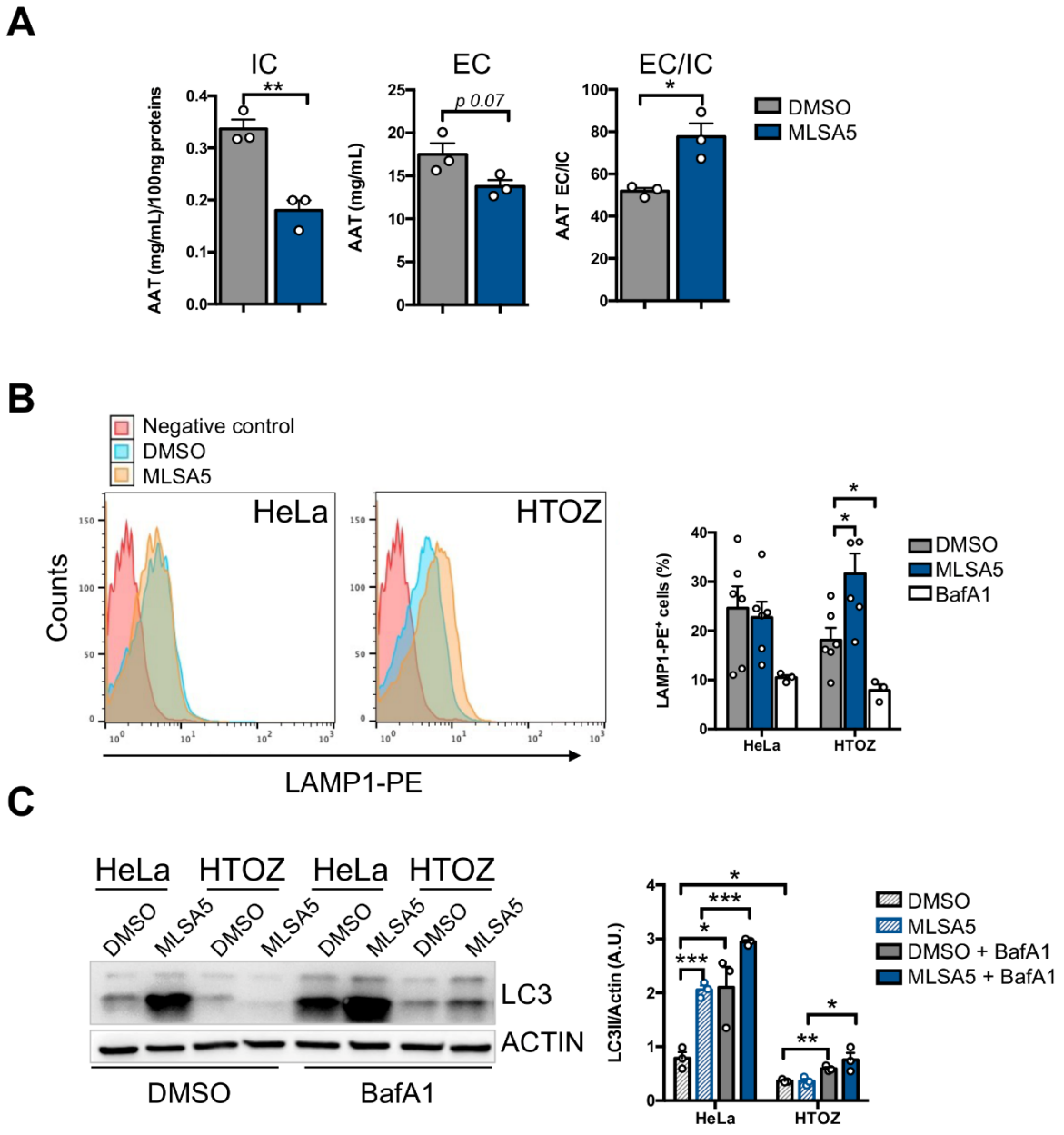

Figure S4. Activation of TRPML1 induces release of AAT in the media.

A. Intracellular (IC), extracellular (EC) and EC/IC ratio of AAT in HTO/Z cells treated with 10 μM ML-SA5 for 24 hours (n=3 per group). **B.** FACS analysis for LAMP1 on the plasma membrane of HeLa and H/TOZ cells incubated with 10 μM ML-SA5 for 30 minutes and relative quantifications (n=6 for DMSO, n=5 for ML-SA5, n=3 for BafilomycinA1-treated cells). **C.** Representative immunoblots for LC3 in HeLa and HTO/Z cells treated with 10 μM ML-SA5 for 24 hours and relative quantifications (n=3 per group). Cells were treated with BafilomycinA1 or DMSO for 4 hours before harvesting. Data are shown as mean ± standard error. Student's *t*-test: *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001. *Abbreviations: BafA1=BafilomycinA1; IC= Intracellular; EC= extracellular.*

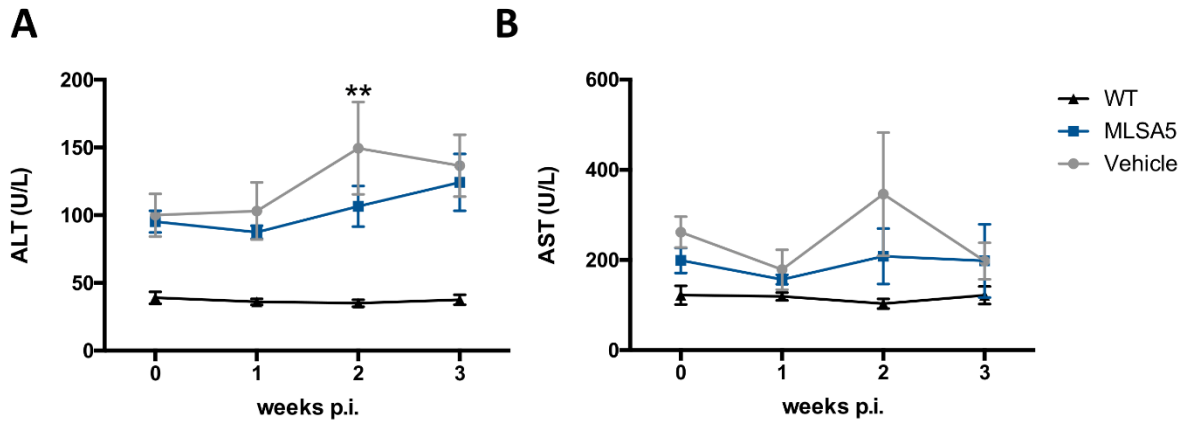

Figure S5. Pharmacological activation of TRPML1 by ML-SA5 is not associated with an increase in transaminases.

Serum activities of ALT and AST in PiZ mice treated with vehicle or ML-SA5 (n=7 per group). Control wild-type (WT) mice (n=4; the same shown in Fig. EV1) are also shown. Data are shown as mean ± standard error. Student's *t*-test: **p-value < 0.01.

Table S1. *Primary antibodies used for immunoblots and stainings.*

Antigen	**Species in which the Ab was raised**	**Source**	**Code**
AAT	Rabbit	Dako	A0012
AAT polymer (2C1)	Mouse	Hycult biotech	HYC-HM2289
β-actin	Mouse	Invitrogen	AM4302
GAPDH	Mouse	Santa Cruz	sc-32233
P62/SQSTM1	Mouse	Abnova	H00008878
LAMP1	Rat	Santa Cruz	sc19992
Laminin2	Rat	Sigma-Aldrich	L0663
Na-K ATPase	Rabbit	Abcam	ab7671
TFEB	Rabbit	Bethyl	A303-673A
H3	Rabbit	Cell Signaling	9715
LC3	Rabbit	Cell Signaling	2978
Myc-Tag	Rabbit	Cell signaling	2278
LAMP1-PE	Rat	Invitrogen	12-1071-82

Table S2. *Primers for real time PCR.*

Gene	**Species**		**Sequence (5'→ 3')**
TRPML1	mouse	forward	CTGACCCCCAATCCTGGGTAT
		reverse	GGCCCGGAACTTGTCACAT
TRPML1	human	forward	GAGTCCCTGCGACAAGTTTC
		reverse	TGTTCTTCCCGGAATGTC
Beta2 microglobulin	mouse	forward	TGGTGCTTGTCTCACTGACC
		reverse	GTATGTTCGGCTTCCCATTC