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Supplemental Information

Allele-Selective Knockdown of *MYH7*

Using Antisense Oligonucleotides

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Figure S1

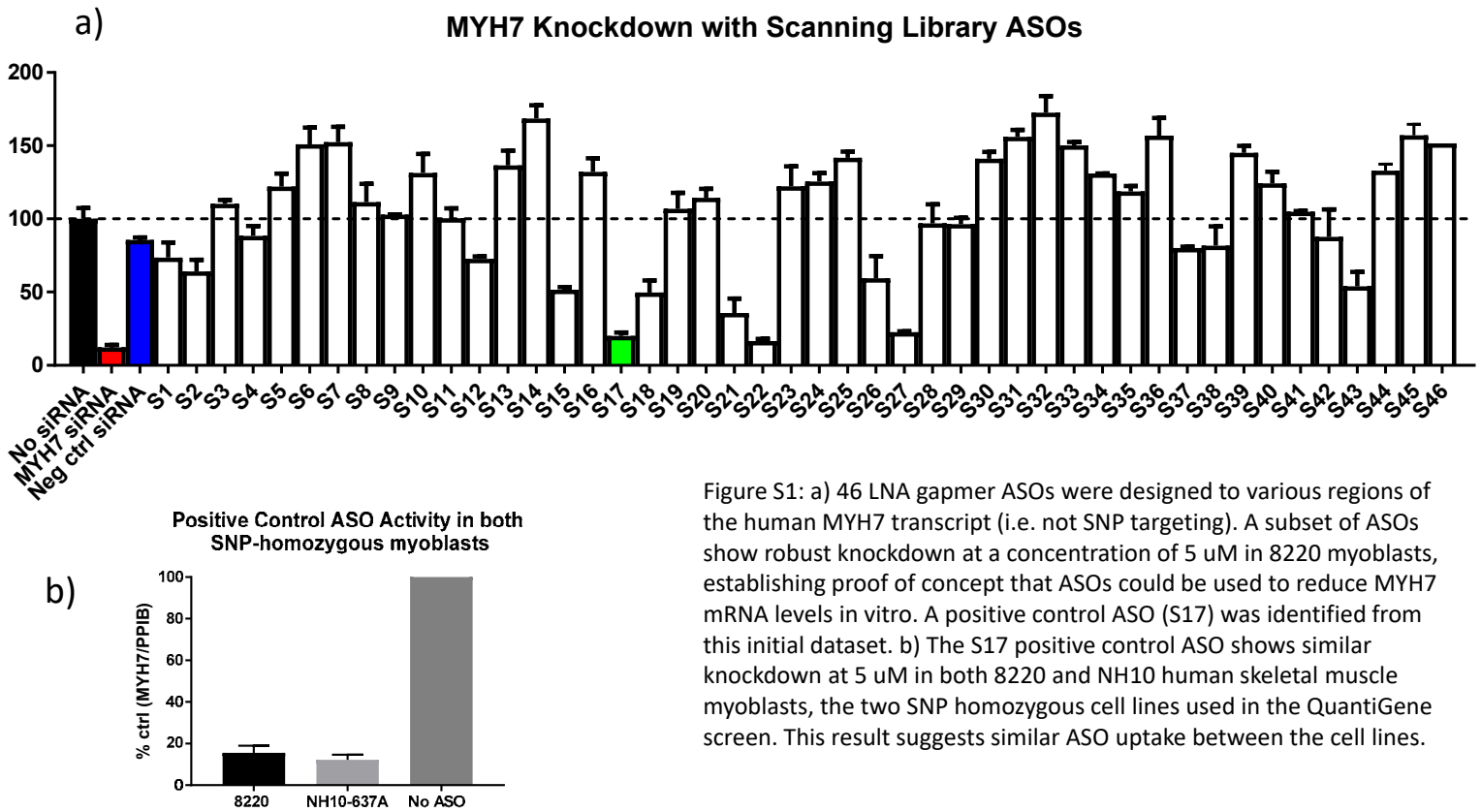


Figure S1: a) 46 LNA gapmer ASOs were designed to various regions of the human MYH7 transcript (i.e. not SNP targeting). A subset of ASOs show robust knockdown at a concentration of 5 uM in 8220 myoblasts, establishing proof of concept that ASOs could be used to reduce MYH7 mRNA levels in vitro. A positive control ASO (S17) was identified from this initial dataset. b) The S17 positive control ASO shows similar knockdown at 5 uM in both 8220 and NH10 human skeletal muscle myoblasts, the two SNP homozygous cell lines used in the QuantiGene screen. This result suggests similar ASO uptake between the cell lines.

Figure S2a – A250 (C) redesigns

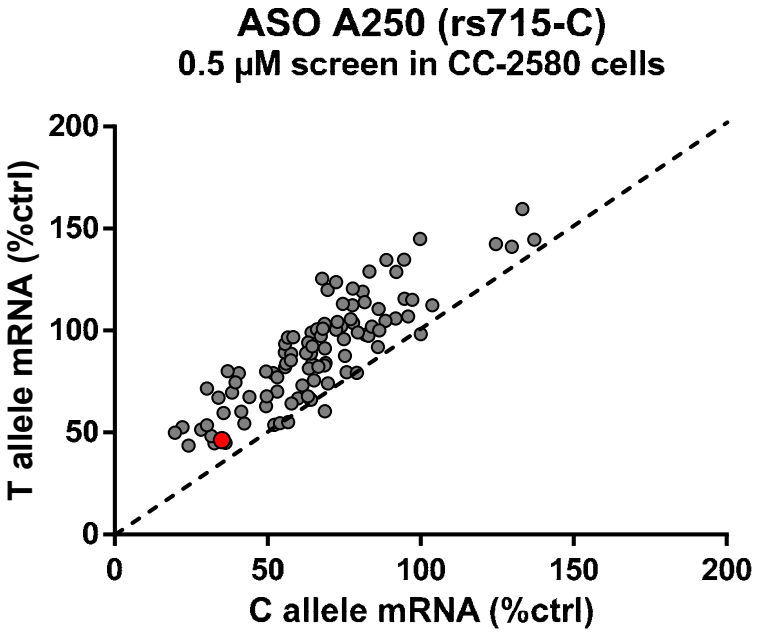


Figure S2a: 102 ASOs were redesigned based on the A250 sequence, which targets the rs715-C allele (TCagcttggcgatgATCT; LNA uppercase, DNA lowercase). The primary sequence was maintained, but the distribution of LNA and DNA bases was varied. SNP-matched (C allele) and SNP-mismatched (T allele) knockdown at 0.5 uM is shown. Data points lying above the dotted line indicate stronger C allele knockdown. A250 is shown in red.

Figure S2b – A270 (T) redesigns

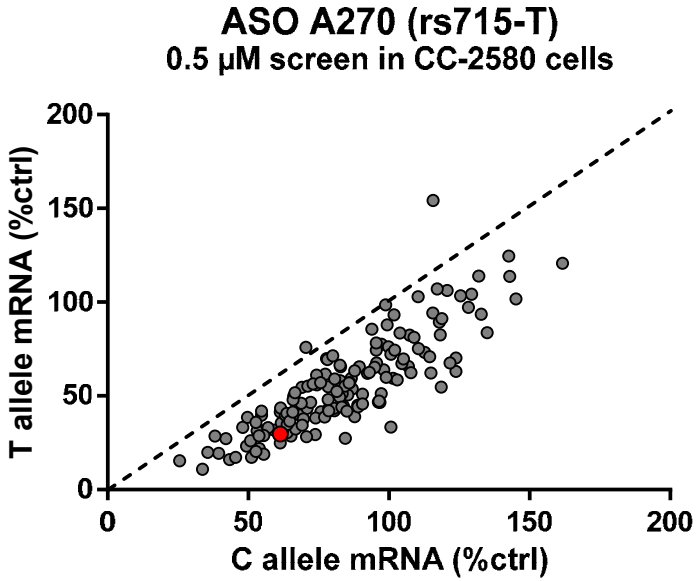


Figure S2b: 162 ASOs were redesigned based on the A270 sequence, which targets the rs715-T allele (CTtggcaatgatctcATCC; LNA uppercase, DNA lowercase). The primary sequence was maintained, but the distribution of LNA and DNA bases was varied. SNP-matched (T allele) and SNP-mismatched (C allele) knockdown at 0.5 uM is shown. Data points lying below the dotted line indicate stronger T allele knockdown. A270 is shown in red.

Figure S2c – A249 (C) redesigns

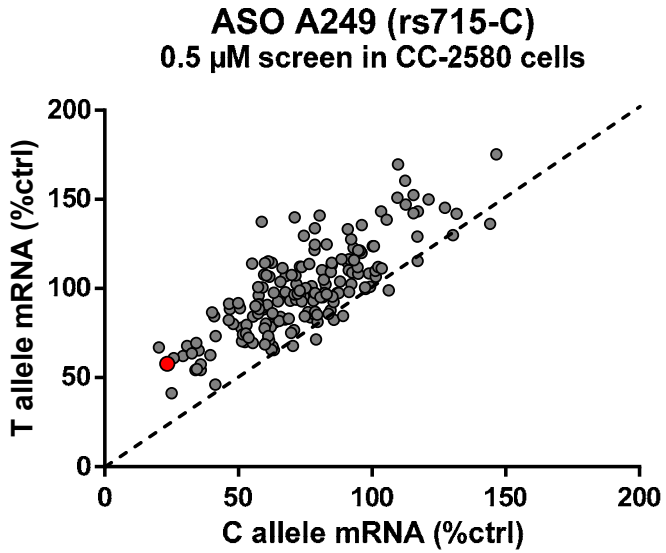
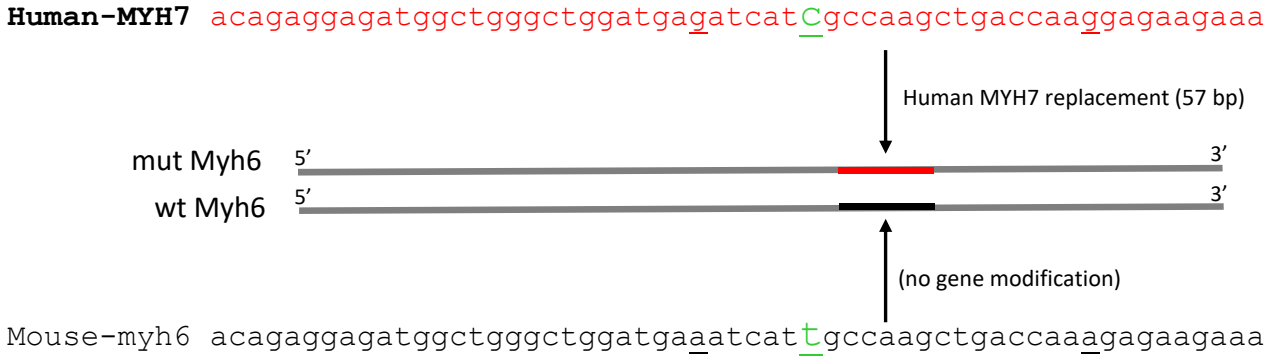


Figure S2c: 189 ASOs were redesigned based on the A249 sequence, which targets the rs715-C allele (CAGcttggcgatgatCT; LNA uppercase, DNA lowercase). The primary sequence was maintained, but the distribution of LNA and DNA bases was varied. SNP-matched (C allele) and SNP-mismatched (T allele) knockdown at 0.5 uM is shown. Data points lying above the dotted line indicate stronger C allele knockdown. A249 is shown in red.

Figure S3



ASO	Sequence	Reverse Complement
A249	CAGcttggcgatgatCT	agatcatcgccaagctg
B44	TCAGcttggcgatgaTCT	agatcatcgccaagctga
B55	TCaGcttggcgatgaTCT	agatcatcgccaagctga
B56	TCAGcttggcgatgatCT	agatcatcgccaagctga
B82	TCagCttggcgatgaTCT	agatcatcgccaagctga

Uppercase: LNA
Lowercase: DNA

Figure S3: A small section of human MYH7 containing the rs715-C SNP and flanking sequence was inserted into one allele of the mouse Myh6 gene. The SNP base is shown in green. The other Myh6 allele was unchanged. This insertion of human sequence did not affect amino acid sequence. Five ASOs targeting the rs715-C allele were tested in these partially humanized mice. Because of the presence of an additional mismatch between human MYH7 and mouse Myh6 near the SNP position, all ASOs have two basepair mismatches between their template sequence and the WT Myh6 sequence.

Figure S4 (1/2)

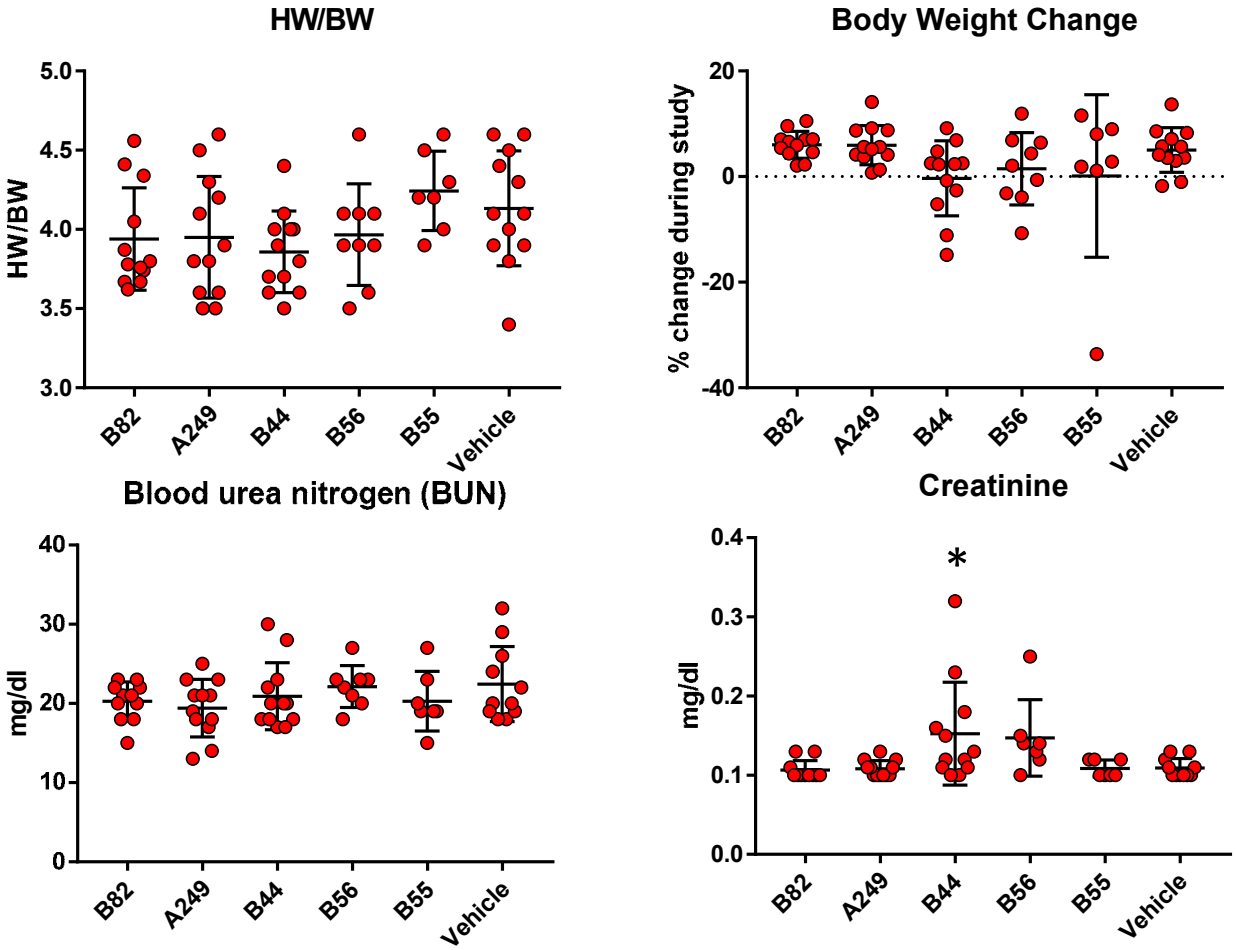

HW/BW
HW/BW
5.0
4.5
4.0
3.5
3.0
B82
A249
B44
B56
B55
Vehicle
Body Weight Change
% change during study
20
0
-20
-40
B82
A249
B44
B56
B55
Vehicle
Blood urea nitrogen (BUN)
mg/dl
40
30
20
10
0
B82
A249
B44
B56
B55
Vehicle
Creatinine
mg/dl
0.4
0.3
0.2
0.1
0.0
*
B82
A249
B44
B56
B55
Vehicle

Figure S4 (2/2)

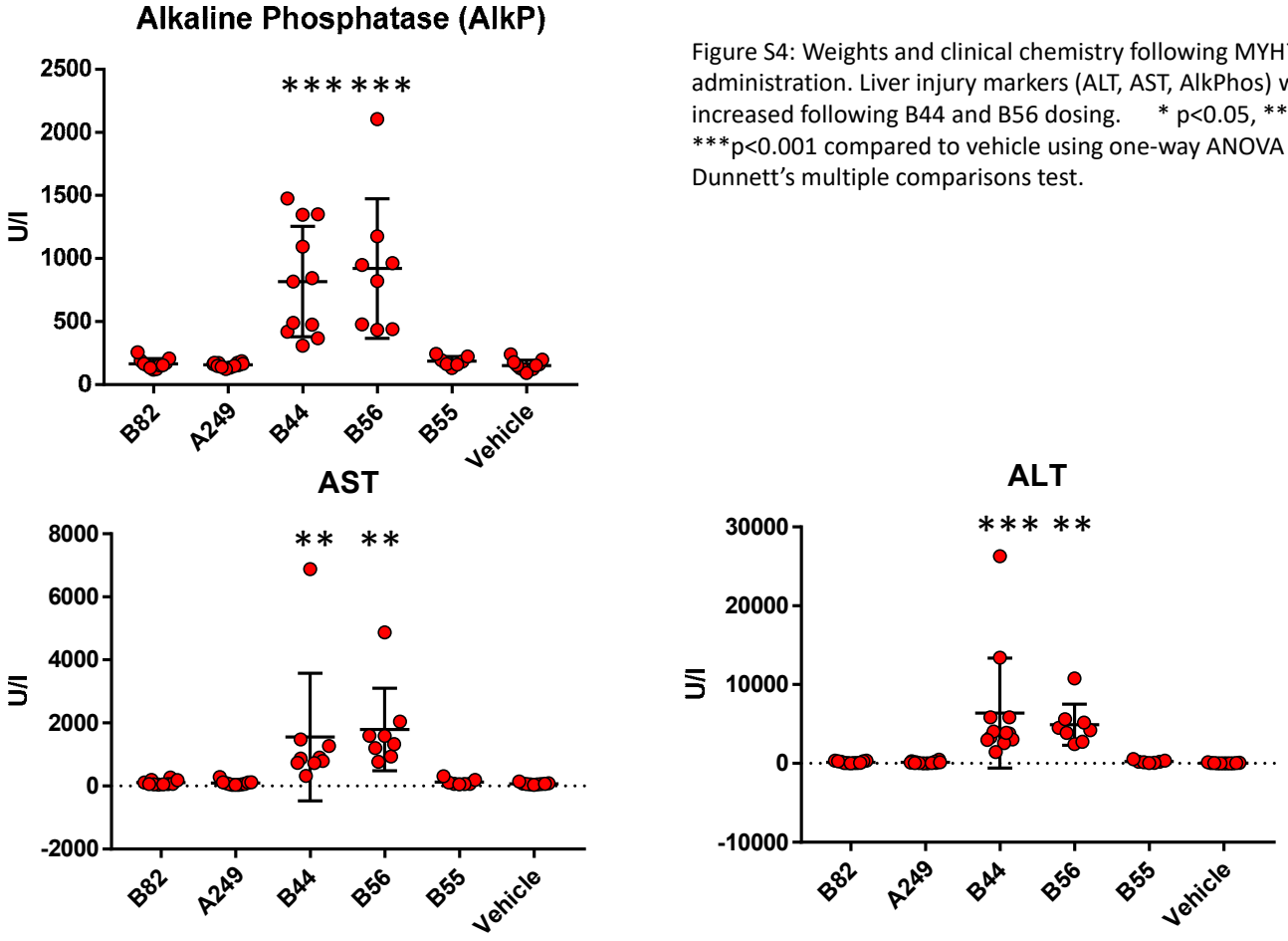


Figure S4: Weights and clinical chemistry following MYH7 ASO administration. Liver injury markers (ALT, AST, AlkPhos) were increased following B44 and B56 dosing. * p<0.05, **p<0.01, ***p<0.001 compared to vehicle using one-way ANOVA and Dunnett's multiple comparisons test.