OMTN, Volume 13

Supplemental Information

Ablation of a Single N-Glycosylation Site in Human FSTL 1 Induces Cardiomyocyte Proliferation and Cardiac Regeneration

Ajit Magadum, Neha Singh, Ann Anu Kurian, Mohammad Tofael Kabir Sharkar, Elena Chepurko, and Lior Zangi

Supplemental Information

Table of Contents

Figure S1. modRNA open reading frame and translated amino-acid sequences used for this study. The start codon (green) stop codon (red) and wild-type and mutated glycosylation sites (yellow) are indicated on the DNA sequence.

Luciferase (modRNA control)

ATGGCCGATGCTAAGAACATTAAGAAGGGCCCTGCTCCCTTCTACCCTCTGG

AGGATGGCACCGCTGGCGAGCAGCTGCACAAGGCCATGAAGAGGTATGCCC

TGGTGCCTGGCACCATTGCCTTCACCGATGCCCACATTGAGGTGGACATCACC

TATGCCGAGTACTTCGAGATGTCTGTGCGCCTGGCCGAGGCCATGAAGAGGT

ACGGCCTGAACACCAACCACCGCATCGTGGTGTGCTCTGAGAACTCTCTGCA

GTTCTTCATGCCAGTGCTGGGCGCCCTGTTCATCGGAGTGGCCGTGGCCCCTG

CTAACGACATTTACAACGAGCGCGAGCTGCTGAACAGCATGGGCATTTCTCA

GCCTACCGTGGTGTTCGTGTCTAAGAAGGGCCTGCAGAAGATCCTGAACGTG

CAGAAGAAGCTGCCTATCATCCAGAAGATCATCATCATGGACTCTAAGACCG

ACTACCAGGGCTTCCAGAGCATGTACACATTCGTGACATCTCATCTGCCTCCT

GGCTTCAACGAGTACGACTTCGTGCCAGAGTCTTTCGACAGGGACAAAACCA

TTGCCCTGATCATGAACAGCTCTGGGTCTACCGGCCTGCCTAAGGGCGTGGC

CCTGCCTCATCGCACCGCCTGTGTGCGCTTCTCTCACGCCCGCGACCCTATTT

TCGGCAACCAGATCATCCCCGACACCGCTATTCTGAGCGTGGTGCCATTCCAC

CACGGCTTCGGCATGTTCACCACCCTGGGCTACCTGATTTGCGGCTTTCGGGT

GGTGCTGATGTACCGCTTCGAGGAGGAGCTGTTCCTGCGCAGCCTGCAAGAC

TACAAAATTCAGTCTGCCCTGCTGGTGCCAACCCTGTTCAGCTTCTTCGCTAA

GAGCACCCTGATCGACAAGTACGACCTGTCTAACCTGCACGAGATTGCCTCT

GGCGGCGCCCCACTGTCTAAGGAGGTGGGCGAAGCCGTGGCCAAGCGCTTTC

ATCTGCCAGGCATCCGCCAGGGCTACGGCCTGACCGAGACAACCAGCGCCAT

TCTGATTACCCCAGAGGGCGACGACAAGCCTGGCGCCGTGGGCAAGGTGGTG

CCATTCTTCGAGGCCAAGGTGGTGGACCTGGACACCGGCAAGACCCTGGGAG

TGAACCAGCGCGGCGAGCTGTGTGTGCGCGGCCCTATGATTATGTCCGGCTA

CGTGAATAACCCTGAGGCCACAAACGCCCTGATCGACAAGGACGGCTGGCTG

CACTCTGGCGACATTGCCTACTGGGACGAGGACGAGCACTTCTTCATCGTGG

ACCGCCTGAAGTCTCTGATCAAGTACAAGGGCTACCAGGTGGCCCCAGCCGA

GCTGGAGTCTATCCTGCTGCAGCACCCTAACATTTTCGACGCCGGAGTGGCC

GGCCTGCCCGACGACGATGCCGGCGAGCTGCCTGCCGCCGTCGTCGTGCTGG

AACACGGCAAGACCATGACCGAGAAGGAGATCGTGGACTATGTGGCCAGCC

AGGTGACAACCGCCAAGAAGCTGCGCGGCGGAGTGGTGTTCGTGGACGAGG

TGCCCAAGGGCCTGACCGGCAAGCTGGACGCCCGCAAGATCCGCGAGATCCT

GATCAAGGCTAAGAAAGGCGGCAAGATCGCCGTGTAA

Met A D A K N I K K G P A P F Y P L E D G T A G E Q L H K A M K R Y A L V P G

T I A F T D A H I E V D I T Y A E Y F E M SV R LA E A M K R Y G L N T N H R I

V V C S E N S L Q F F M P V L G A L F I G V A V A P A N D I Y N E R E L L N S M

G I S Q P T V V F V S K K G L Q K I L N V Q K K L P I I Q K I I I M D S K T D Y Q

G F Q S M Y T F V T S H L P P G F N E Y D F V P E S F D R D K T I A L I M N S S

G S T G L P K G V A L P H R TA C V R F S H A R D P I F G N Q II P D T A I L S V

V P F H H G F G M F T T L G Y L I C G F R V V L M Y R F E E E L F L R S L Q D Y

K I Q S A L L V P T L F SF F A K S T L I D K Y DL S N L H E I A S G G A P L S K E

V G E A V A K R F H L P G I R Q G Y G L T E T T S A I L I T P E G D D K P G A V

G K V V P F F E A K V V D L D T G K T L G V N Q R G E L C V R G P M I M S G

Y V N N P E A T N A L I D K D G W L H SG D I A Y W D E D E H F F I V D R L K S

L I K Y K G Y Q V A P A E L E S I L L Q H P N I F D A G V A G L P D D D A G E L

P A A V V V L E H G K T M T E K E I V D Y V A S Q V T T A K K L R G G V V F V

D E V P K G L T G K L D A R K I R E I L I K A K K G G K I A V

Human Fstl1

ATGTGGAAACGCTGGCTCGCGCTCGCGCTCGCGCTGGTGGCGGTCGCCTGGG

TCCGCGCCGAGGAAGAGCTAAGGAGCAAATCCAAGATCTGTGCCAATGTGTT

TTGTGGAGCCGGCCGGGAATGTGCAGTCACAGAGAAAGGGGAACCCACCTGT

CTCTGCATTGAGCAATGCAAACCTCACAAGAGGCCTGTGTGTGGCAGTAATG

GCAAGACCTACCTCAACCACTGTGAACTGCATCGAGATGCCTGCCTCACTGG

ATCCAAAATCCAGGTTGATTACGATGGACACTGCAAAGAGAAGAAATCCGTA

AGTCCATCTGCCAGCCCAGTTGTTTGCTATCAGTCCAACCGTGATGAGCTCCG

ACGTCGCATCATCCAGTGGCTGGAAGCTGAGATCATTCCAGATGGCTGGTTCT

CTAAAGGCAGCAACTACAGTGAAATCCTAGACAAGTATTTTAAGAACTTTGA

TAATGGTGATTCTCGCCTGGACTCCAGTGAATTCCTGAAGTTTGTGGAACAGA

ATGAAACTGCCATCAATATTACAACGTATCCAGACCAGGAGAACAACAAGTT

GCTTAGGGGACTCTGTGTTGATGCTCTCATTGAACTGTCTGATGAAAATGCTG

ATTGGAAACTCAGCTTCCAAGAGTTTCTCAAGTGCCTCAACCCATCTTTCAAC

CCTCCTGAGAAGAAGTGTGCCCTGGAGGATGAAACGTATGCAGATGGAGCTG

AGACCGAGGTGGACTGTAACCGCTGTGTCTGTGCCTGTGGAAATTGGGTCTG

TACAGCCATGACCTGTGACGGAAAGAATCAGAAGGGGGCCCAGACCCAGAC

AGAGGAGGAGATGACCAGATATGTCCAGGAGCTCCAAAAGCATCAGGAAAC

AGCTGAAAAGACCAAGAGAGTGAGCACCAAAGAGATCTAA

Met W K R W L A L A L A L V A V A W V R A E E E L R S K S K I C A N V F C G

A G R E C A V T E K G E P T C L C I E Q C K P H K R P V C G S N G K T Y L N H

C E L H R D A C L T G S K I Q V D Y D G H C K E K K S V S P S A S P V V C Y Q

S N R D E L R R R I I Q W L E A E I I P D G W F S K G S N Y S E I L D K Y F K N F

D N G D S R L D S S E F L K F V E Q N E T A I N I T T Y P D Q E N N K L L R G L

C V D A L I E L S D E N A D W K L S F Q E F L K C L N P S F N P P E K K C A L E

D E T Y A D G A E T E V D C N R C V C A C G N W V C T A Met T C D G K N Q K

G A Q T Q T E E E MetT R Y V Q E L Q K H Q E T A E K T K R V S T K E I

Mutant 1 (Human Fstl1 N180Q)

ATGTGGAAACGCTGGCTCGCGCTCGCGCTCGCGCTGGTGGCGGTCGCCTGGG

TCCGCGCCGAGGAAGAGCTAAGGAGCAAATCCAAGATCTGTGCCAATGTGTT

TTGTGGAGCCGGCCGGGAATGTGCAGTCACAGAGAAAGGGGAACCCACCTGT

CTCTGCATTGAGCAATGCAAACCTCACAAGAGGCCTGTGTGTGGCAGTAATG

GCAAGACCTACCTCAACCACTGTGAACTGCATCGAGATGCCTGCCTCACTGG

ATCCAAAATCCAGGTTGATTACGATGGACACTGCAAAGAGAAGAAATCCGTA

AGTCCATCTGCCAGCCCAGTTGTTTGCTATCAGTCCAACCGTGATGAGCTCCG

ACGTCGCATCATCCAGTGGCTGGAAGCTGAGATCATTCCAGATGGCTGGTTCT

CTAAAGGCAGCAACTACAGTGAAATCCTAGACAAGTATTTTAAGAACTTTGA

TAATGGTGATTCTCGCCTGGACTCCAGTGAATTCCTGAAGTTTGTGGAACAGA

ATGAAACTGCCATCCAGATTACAACGTATCCAGACCAGGAGAACAACAAGTT

GCTTAGGGGACTCTGTGTTGATGCTCTCATTGAACTGTCTGATGAAAATGCTG

ATTGGAAACTCAGCTTCCAAGAGTTTCTCAAGTGCCTCAACCCATCTTTCAAC

CCTCCTGAGAAGAAGTGTGCCCTGGAGGATGAAACGTATGCAGATGGAGCTG

AGACCGAGGTGGACTGTAACCGCTGTGTCTGTGCCTGTGGAAATTGGGTCTG

TACAGCCATGACCTGTGACGGAAAGAATCAGAAGGGGGCCCAGACCCAGAC

AGAGGAGGAGATGACCAGATATGTCCAGGAGCTCCAAAAGCATCAGGAAAC

AGCTGAAAAGACCAAGAGAGTGAGCACCAAAGAGATCTAA

Met W K R W L A L A L A L V A V A W V R A E E E L R S K S K I C A N V F C G

A G R E C A V T E K G E P T C L C I E Q C K P H K R P V C G S N G K T Y L N H

C E L H R D A C L T G S K I Q V D Y D G H C K E K K S V S P S A S P V V C Y Q

S N R D E L R R R I I Q W L E A E I I P D G W F S K G S N Y S E I L D K Y F K N F

D N G D S R L D S S E F L K F V E Q N E T A I **Q** I T T Y P D Q E N N K L L R G L

C V D A L I E L S D E N A D W K L S F Q E F L K C L N P S F N P P E K K C A L E

D E T Y A D G A E T E V D C N R C V C A C G N W V C T A Met T C D G K N Q K

G A Q T Q T E E EMet T R Y V Q E L Q K H Q E T A E K T K R V S T K E I

Mutant 2 (Human Fstl1 N175Q)

ATGTGGAAACGCTGGCTCGCGCTCGCGCTCGCGCTGGTGGCGGTCGCCTGGG

TCCGCGCCGAGGAAGAGCTAAGGAGCAAATCCAAGATCTGTGCCAATGTGTT

TTGTGGAGCCGGCCGGGAATGTGCAGTCACAGAGAAAGGGGAACCCACCTGT

CTCTGCATTGAGCAATGCAAACCTCACAAGAGGCCTGTGTGTGGCAGTAATG

GCAAGACCTACCTCAACCACTGTGAACTGCATCGAGATGCCTGCCTCACTGG

ATCCAAAATCCAGGTTGATTACGATGGACACTGCAAAGAGAAGAAATCCGTA

AGTCCATCTGCCAGCCCAGTTGTTTGCTATCAGTCCAACCGTGATGAGCTCCG

ACGTCGCATCATCCAGTGGCTGGAAGCTGAGATCATTCCAGATGGCTGGTTCT

CTAAAGGCAGCAACTACAGTGAAATCCTAGACAAGTATTTTAAGAACTTTGA

TAATGGTGATTCTCGCCTGGACTCCAGTGAATTCCTGAAGTTTGTGGAACAGC

AGGAAACTGCCATCAATATTACAACGTATCCAGACCAGGAGAACAACAAGTT

GCTTAGGGGACTCTGTGTTGATGCTCTCATTGAACTGTCTGATGAAAATGCTG

ATTGGAAACTCAGCTTCCAAGAGTTTCTCAAGTGCCTCAACCCATCTTTCAAC

CCTCCTGAGAAGAAGTGTGCCCTGGAGGATGAAACGTATGCAGATGGAGCTG

AGACCGAGGTGGACTGTAACCGCTGTGTCTGTGCCTGTGGAAATTGGGTCTG

TACAGCCATGACCTGTGACGGAAAGAATCAGAAGGGGGCCCAGACCCAGAC

AGAGGAGGAGATGACCAGATATGTCCAGGAGCTCCAAAAGCATCAGGAAAC

AGCTGAAAAGACCAAGAGAGTGAGCACCAAAGAGATCTAA

Met W K R W L A L A L A L V A V A W V R A E E E L R S K S K I C A N V F C G

A G R E C A V T E K G E P T C L C I E Q C K P H K R P V C G S N G K T Y L N H

C E L H R D A C L T G S K I Q V D Y D G H C K E K K S V S P S A S P V V C Y Q

S N R D E L R R R I I Q W L E A E I I P D G W F S K G S N Y S E I L D K Y F K N F

D N G D S R L D S S E F L K F V E Q **Q** E T A I N I T T Y P D Q E N N K L L R G L

C V D A L I E L S D E N A D W K L S F Q E F L K C L N P S F N P P E K K C A L E

D E T Y A D G A E T E V D C N R C V C A C G N W V C T A Met T C D G K N Q K

G A Q T Q T E E EMet T R Y V Q E L Q K H Q E T A E K T K R V S T K E I

Mutant 3 (Human Fstl1 N144Q)

ATGTGGAAACGCTGGCTCGCGCTCGCGCTCGCGCTGGTGGCGGTCGCCTGGG

TCCGCGCCGAGGAAGAGCTAAGGAGCAAATCCAAGATCTGTGCCAATGTGTT

TTGTGGAGCCGGCCGGGAATGTGCAGTCACAGAGAAAGGGGAACCCACCTGT

CTCTGCATTGAGCAATGCAAACCTCACAAGAGGCCTGTGTGTGGCAGTAATG

GCAAGACCTACCTCAACCACTGTGAACTGCATCGAGATGCCTGCCTCACTGG

ATCCAAAATCCAGGTTGATTACGATGGACACTGCAAAGAGAAGAAATCCGTA

AGTCCATCTGCCAGCCCAGTTGTTTGCTATCAGTCCAACCGTGATGAGCTCCG

ACGTCGCATCATCCAGTGGCTGGAAGCTGAGATCATTCCAGATGGCTGGTTCT

CTAAAGGCAGCCAGTACAGTGAAATCCTAGACAAGTATTTTAAGAACTTTGA

TAATGGTGATTCTCGCCTGGACTCCAGTGAATTCCTGAAGTTTGTGGAACAGA

ATGAAACTGCCATCAATATTACAACGTATCCAGACCAGGAGAACAACAAGTT

GCTTAGGGGACTCTGTGTTGATGCTCTCATTGAACTGTCTGATGAAAATGCTG

ATTGGAAACTCAGCTTCCAAGAGTTTCTCAAGTGCCTCAACCCATCTTTCAAC

CCTCCTGAGAAGAAGTGTGCCCTGGAGGATGAAACGTATGCAGATGGAGCTG

AGACCGAGGTGGACTGTAACCGCTGTGTCTGTGCCTGTGGAAATTGGGTCTG

TACAGCCATGACCTGTGACGGAAAGAATCAGAAGGGGGCCCAGACCCAGAC

AGAGGAGGAGATGACCAGATATGTCCAGGAGCTCCAAAAGCATCAGGAAAC

AGCTGAAAAGACCAAGAGAGTGAGCACCAAAGAGATCTAA

Met W K R W L A L A L A L V A V A W V R A E E E L R S K S K I C A N V F C G

A G R E C A V T E K G E P T C L C I E Q C K P H K R P V C G S N G K T Y L N H

C E L H R D A C L T G S K I Q V D Y D G H C K E K K S V S P S A S P V V C Y Q

S N R D E L R R R I I Q W L E A E I I P D G W F S K G S **Q** Y S E I L D K Y F K N F

D N G D S R L D S S E F L K F V E Q N E T A I **N** I T T Y P D Q E N N K L L R G L

C V D A L I E L S D E N A D W K L S F Q E F L K C L N P S F N P P E K K C A L E

D E T Y A D G A E T E V D C N R C V C A C G N W V C T A Met T C D G K N Q K

G A Q T Q T E E EMet T R Y V Q E L Q K H Q E T A E K T K R V S T K E I

Mutant (1-3) (Human Fstl1 N180Q, N175Q, N144Q)

ATGTGGAAACGCTGGCTCGCGCTCGCGCTCGCGCTGGTGGCGGTCGCCTGGG

TCCGCGCCGAGGAAGAGCTAAGGAGCAAATCCAAGATCTGTGCCAATGTGTT

TTGTGGAGCCGGCCGGGAATGTGCAGTCACAGAGAAAGGGGAACCCACCTGT

CTCTGCATTGAGCAATGCAAACCTCACAAGAGGCCTGTGTGTGGCAGTAATG

GCAAGACCTACCTCAACCACTGTGAACTGCATCGAGATGCCTGCCTCACTGG

ATCCAAAATCCAGGTTGATTACGATGGACACTGCAAAGAGAAGAAATCCGTA

AGTCCATCTGCCAGCCCAGTTGTTTGCTATCAGTCCAACCGTGATGAGCTCCG

ACGTCGCATCATCCAGTGGCTGGAAGCTGAGATCATTCCAGATGGCTGGTTCT

CTAAAGGCAGCCAGTACAGTGAAATCCTAGACAAGTATTTTAAGAACTTTGA

TAATGGTGATTCTCGCCTGGACTCCAGTGAATTCCTGAAGTTTGTGGAACAGC

AGGAAACTGCCATCCAGATTACAACGTATCCAGACCAGGAGAACAACAAGTT

GCTTAGGGGACTCTGTGTTGATGCTCTCATTGAACTGTCTGATGAAAATGCTG

ATTGGAAACTCAGCTTCCAAGAGTTTCTCAAGTGCCTCAACCCATCTTTCAAC

CCTCCTGAGAAGAAGTGTGCCCTGGAGGATGAAACGTATGCAGATGGAGCTG

AGACCGAGGTGGACTGTAACCGCTGTGTCTGTGCCTGTGGAAATTGGGTCTG

TACAGCCATGACCTGTGACGGAAAGAATCAGAAGGGGGCCCAGACCCAGAC

AGAGGAGGAGATGACCAGATATGTCCAGGAGCTCCAAAAGCATCAGGAAAC

AGCTGAAAAGACCAAGAGAGTGAGCACCAAAGAGATCTAA

Met W K R W L A L A L A L V A V A W V R A E E E L R S K S K I C A N V F C G

A G R E C A V T E K G E P T C L C I E Q C K P H K R P V C G S N G K T Y L N H

C E L H R D A C L T G S K I Q V D Y D G H C K E K K S V S P S A S P V V C Y Q

S N R D E L R R R I I Q W L E A E I I P D G W F S K G S **Q** Y S E I L D K Y F K N F

D N G D S R L D S S E F L K F V E Q **Q** E T A I **Q** I T T Y P D Q E N N K L L R G L

C V D A L I E L S D E N A D W K L S F Q E F L K C L N P S F N P P E K K C A L E

D E T Y A D G A E T E V D C N R C V C A C G N W V C T A Met T C D G K N Q K

G A Q T Q T E E EMet T R Y V Q E L Q K H Q E T A E K T K R V S T K E I

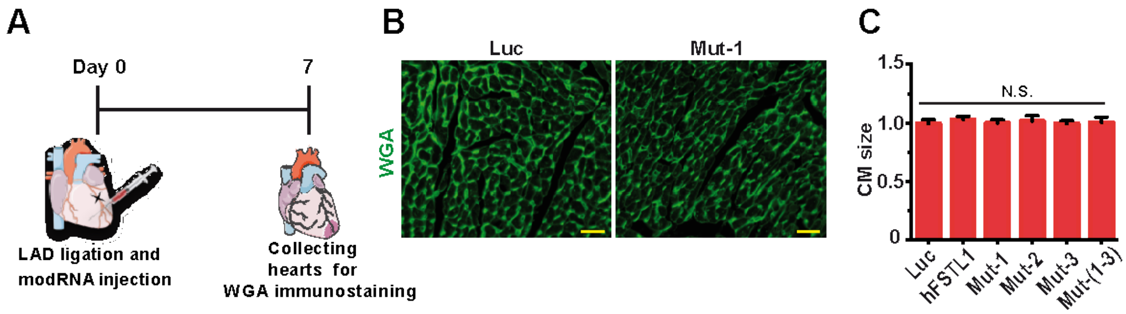
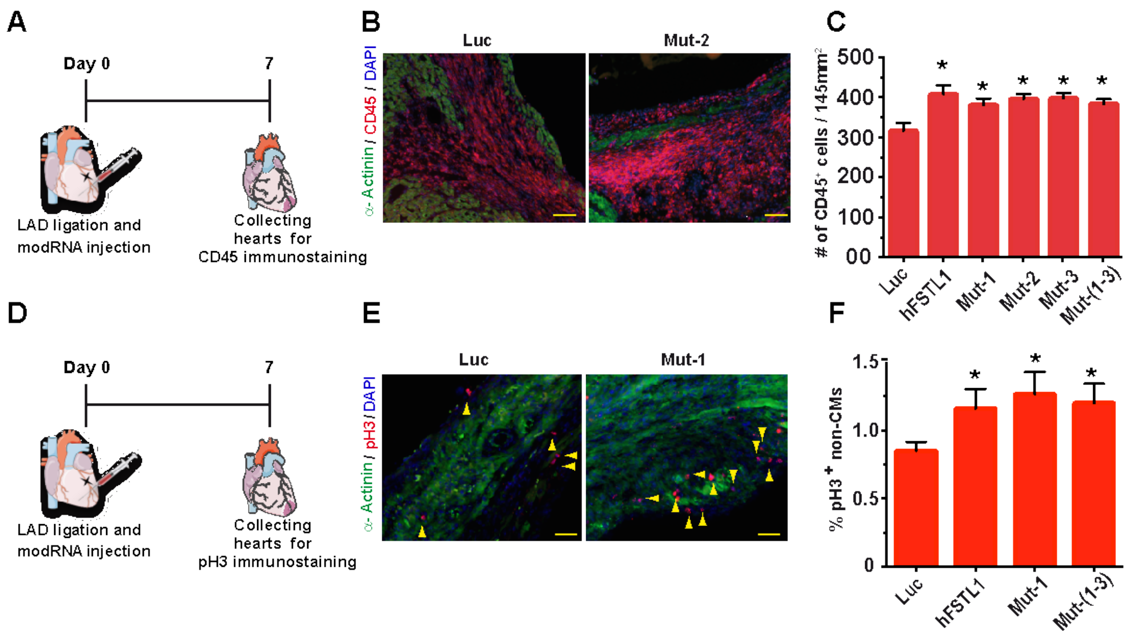


Figure S2. Neither hFSTL1 or N-glycosylation-deficient hFSTL1 modRNAs cause CM hypertrophy in a mouse model of MI. **A.** Experimental timeline for evaluation of the effects of the different hFSTL1 modRNAs with mutations affecting the various N-glycosylation sites on CM size *in vivo* seven days post MI. **B.** Representative images of wheatgerm agglutinin (WGA) staining to evaluate CM size 28 days after LAD ligation and treatment with hFSTL1 modRNAs with mutations of the different N-glycosylation sites. **C.** Quantification of the experiment described in B. Representative results of two independent experiments are shown; N.S. not significant, *n*=5, one-way ANOVA with Bonferroni correction for multiple testing, scale bar = 50 µm.



<u>Figure S3. hFSTL1 or N-glycosylation-deficient hFSTL1 enhances the immune response in a mouse model of MI.</u> **A.** Experimental timeline for evaluation of the effect of the different mutant hFSTL1 modRNAs on $CD45^+$ cell (leukocyte) infiltration into the left ventricle *in vivo*, seven days post MI. **B.** Representative images of $CD45^+$ cell infiltration into the left ventricle seven days post MI and treatment with Luc (modRNA control) or Mut-2 modRNAs. **C.** Quantification of the experiment described in A. **D.** Experimental timeline for evaluation of the effect of the different mutant hFSTL1 modRNAs on the PH3 proliferation marker in non-CMs in the left ventricle seven days post MI. **E.** Representative images of $PH3^+$ non-CMs cells in the left ventricle seven days post MI and treatment with Luc (modRNA control) or Mut-1 modRNAs **F.** Quantification of the experiment described in D. Representative results from two independent experiments are shown. *, $P<0.05$. $n=7$. One-way ANOVA with Bonferroni correction for multiple testing, scale bar = 50 μm.

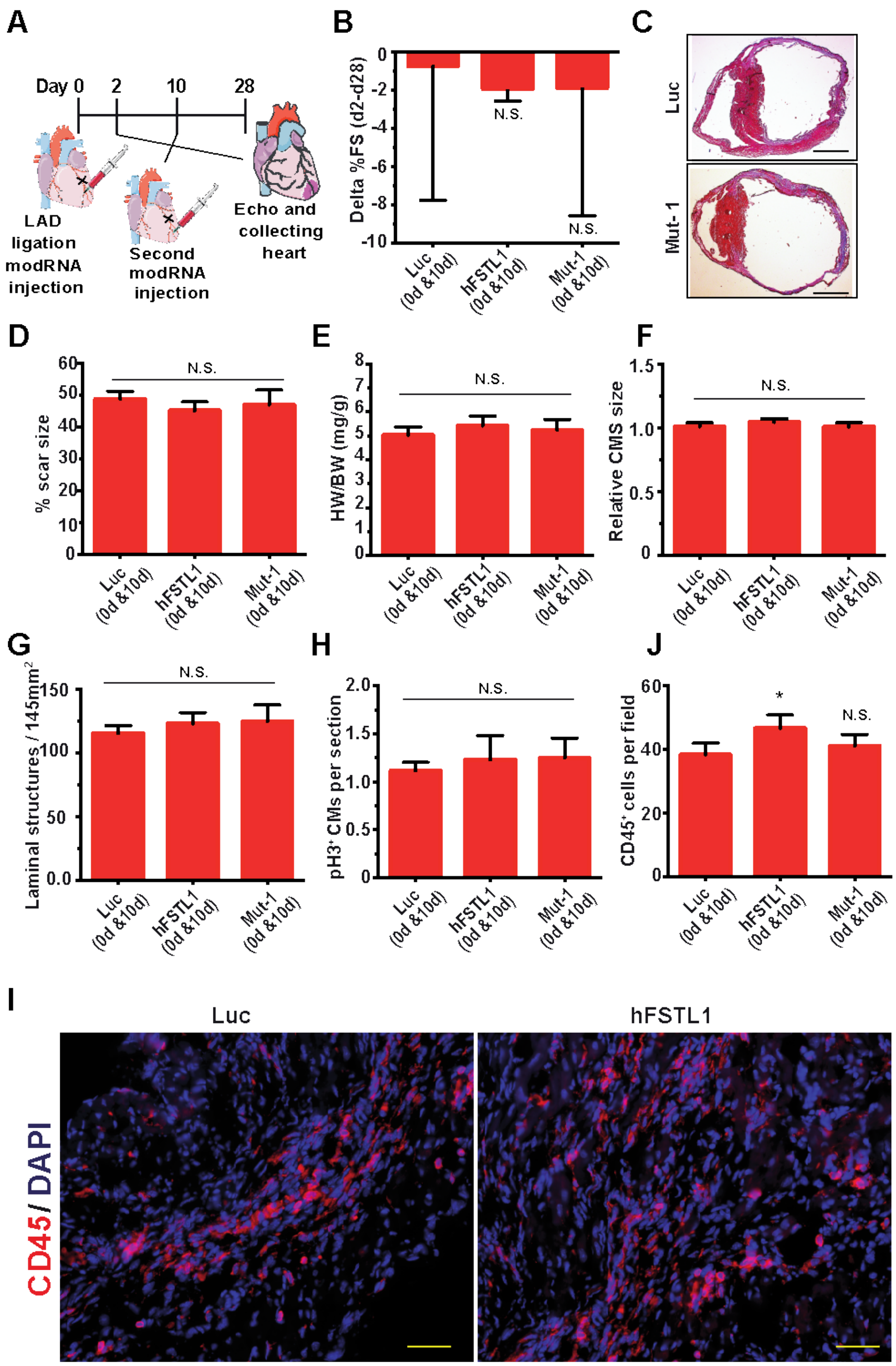


Figure S4. Double hFSTL1 Mut-1 (N180Q) modRNA administration, immediately and 10 days post MI, does not improve cardiac function and outcome post MI

A. Experimental timeline for the evaluation of cardiac function after the delivery on days 0 and 10 post MI of Luc (modRNA control), hFSTL1 or mutated hFSTL1 (at a single N-glycosylation site: N180Q, Mut-1) modRNAs. **B.** Echo evaluation of the change in % fractional shortening between day 2 (baseline) and day 28 post-MI. **C.** Representative

Masson trichrome staining to evaluate scar size 28 days post-MI. **D-I**. Quantification of scar size (**D**), heart weight-to-body weight ratio (**E**), CM size (**F**), and capillary density (**G**) PH3 expression in CMs (**H**), representive image of CD45 cells in the left ventricle after treatment with Luc or hFSTL1 modRNAs(**I**) and quantification of CD45 cells in the left ventricle 28 days post treatment. Representative results for two independent experiments are shown; *, P<0.05, N.S., not significant. n=4. One-way ANOVA with Bonferroni correction for multiple testing, Scale bar = 1 mm (C) and 50 μm (I).

2. Supplemental Tables

Table S1: Antibodies used in this study

Antigen	Dilution	Company	Catalog number
FSTL1	1:100	R&D Systems	AF1694
BrdU	1:200	Abcam	ab6326
Aurora B	1:200	BD Transduction	611082
α-Actinin	1:100	Abcam	ab9465
Ki-67	1:100	Abcam	ab16667
PH3	1:100	Millipore	06-570
Troponin I	1:50	Santa Cruz Biotechnology	SC-15368
CD45	1:100	BD Pharmingen	550539
CD31	1:100	R&D Biosystems	AF3628
WGA	1:50	Life Technologies	W11261