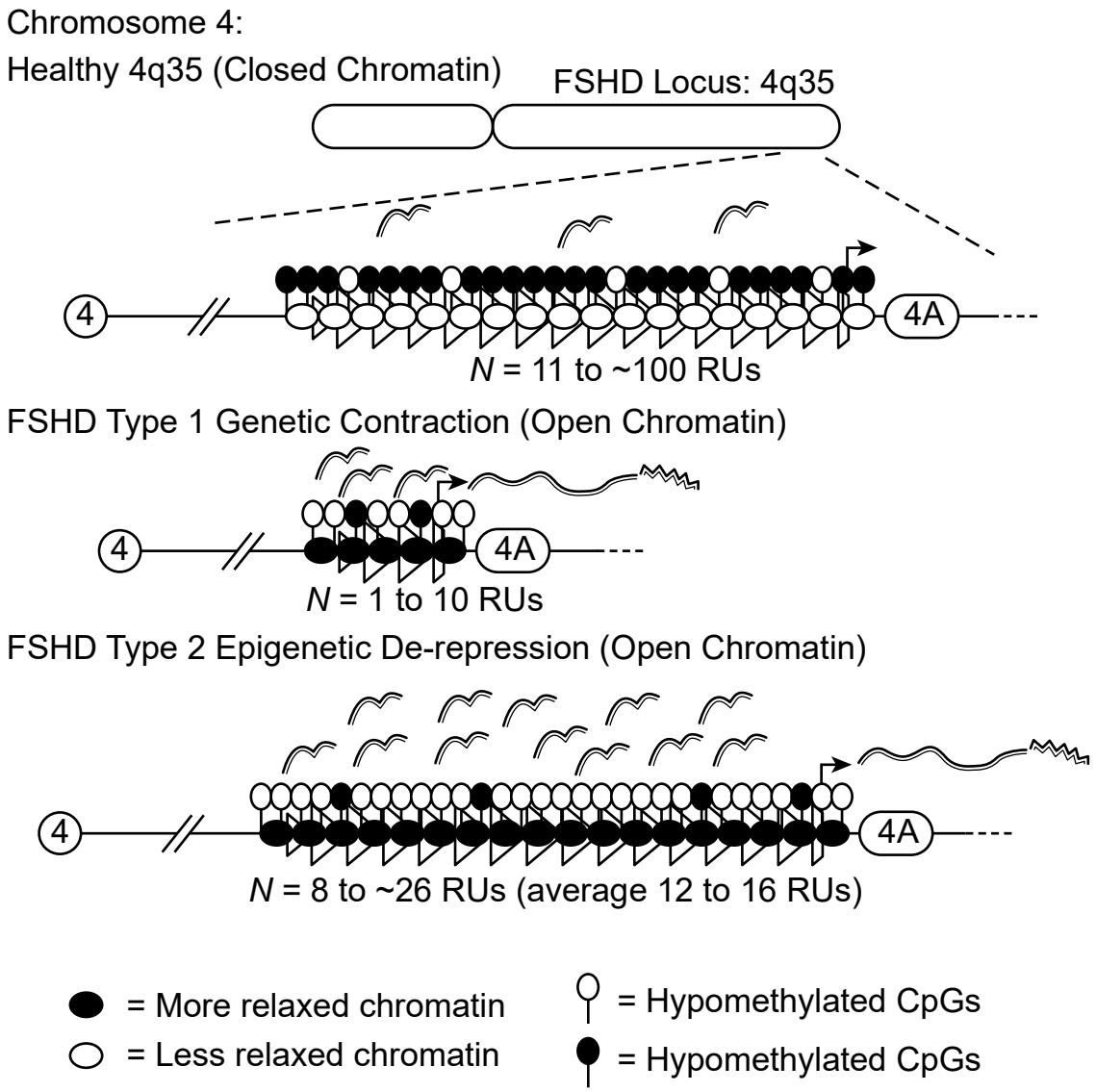

Chromosome 4:
Healthy 4q35 (Closed Chromatin)
FSHD Locus: 4q35
4
4A
N = 11 to ~100 RUs
FSHD Type 1 Genetic Contraction (Open Chromatin)
4
4A
N = 1 to 10 RUs
FSHD Type 2 Epigenetic De-repression (Open Chromatin)
4
4A
N = 8 to ~26 RUs (average 12 to 16 RUs)
= More relaxed chromatin
= Less relaxed chromatin
= Hypomethylated CpGs
= Hypomethylated CpGs

FIG. 1

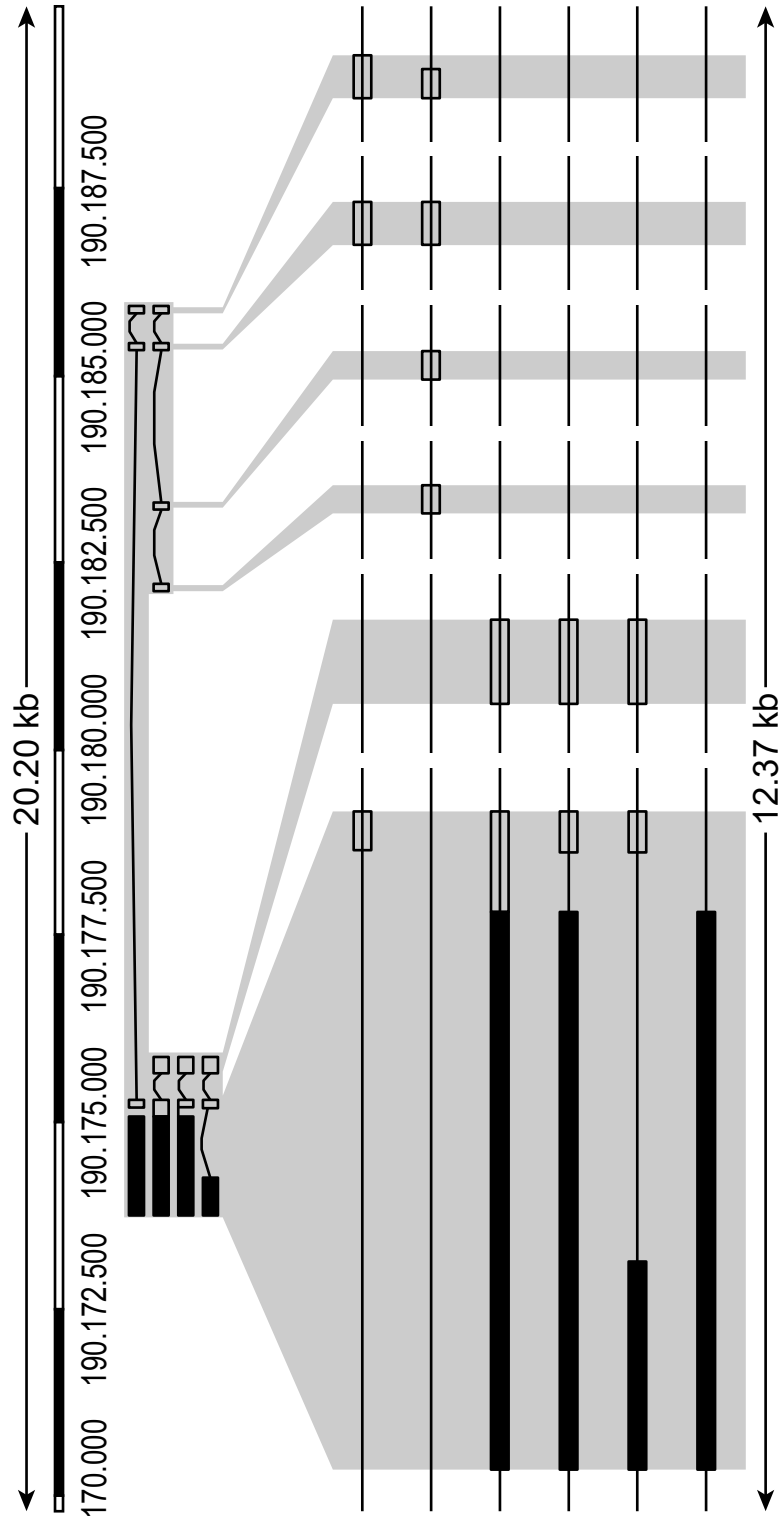

20.20 kb
190.170.000
190.172.500
190.175.000
190.177.500
190.180.000
190.182.500
190.185.000
190.187.500
Genes
(Comprehensiv...
ENST00000563716.5
DUX4-201
ENST00000564366.1
DUX4-202
ENST00000565211.1
DUX4-203
ENST00000569241.5
DUX4-204
ENST00000570263.5
DUX4-205
ENST00000616166.1
DUX4-206
12.37 kb

FIG. 2

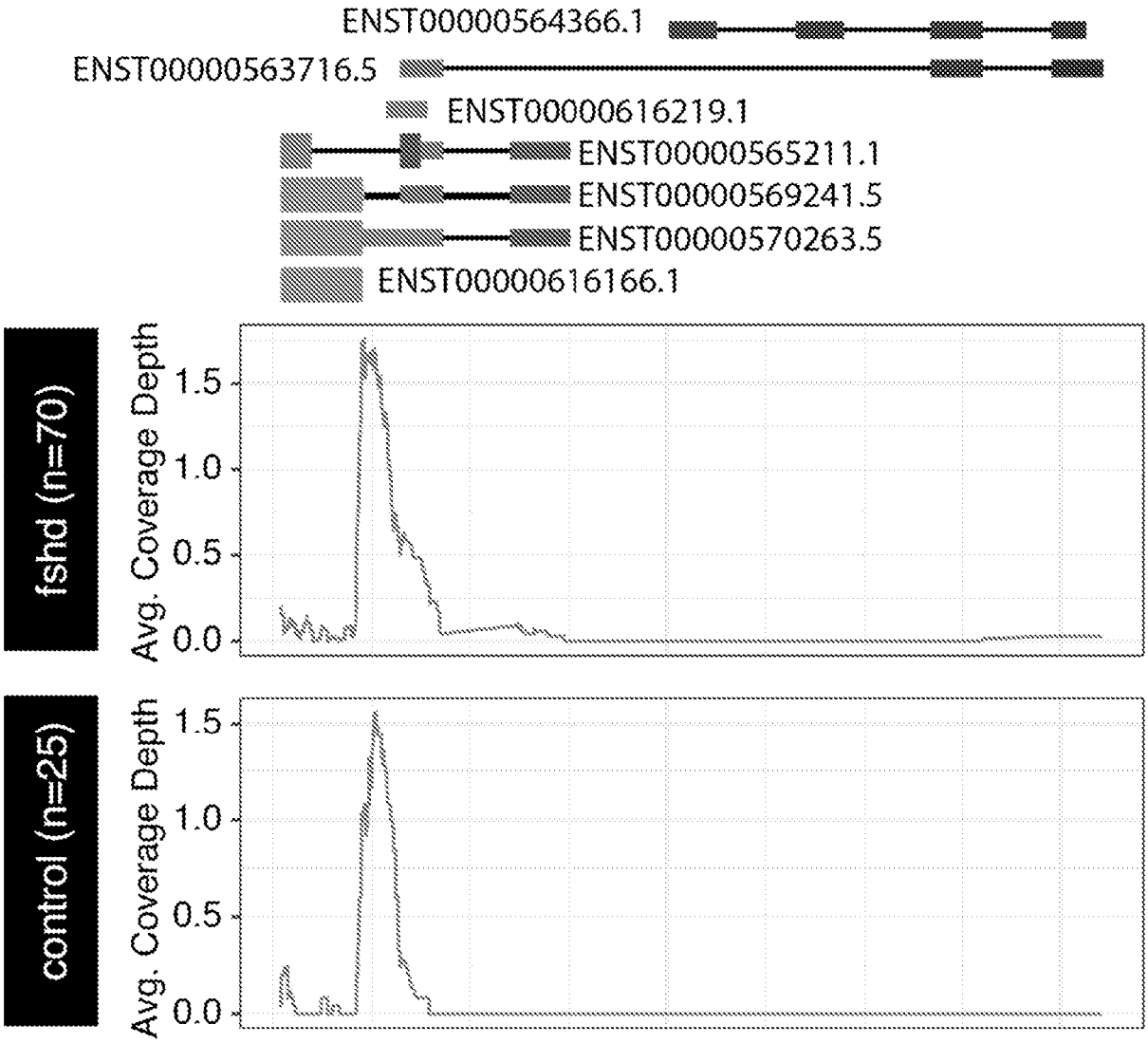

ENST00000564366.1
ENST00000563716.5
ENST00000616219.1
ENST00000565211.1
ENST00000569241.5
ENST00000570263.5
ENST00000616166.1
fshd (n=70)
Avg. Coverage Depth
1.5
1.0
0.5
0.0
control (n=25)
Avg. Coverage Depth
1.5
1.0
0.5
0.0

FIG. 3

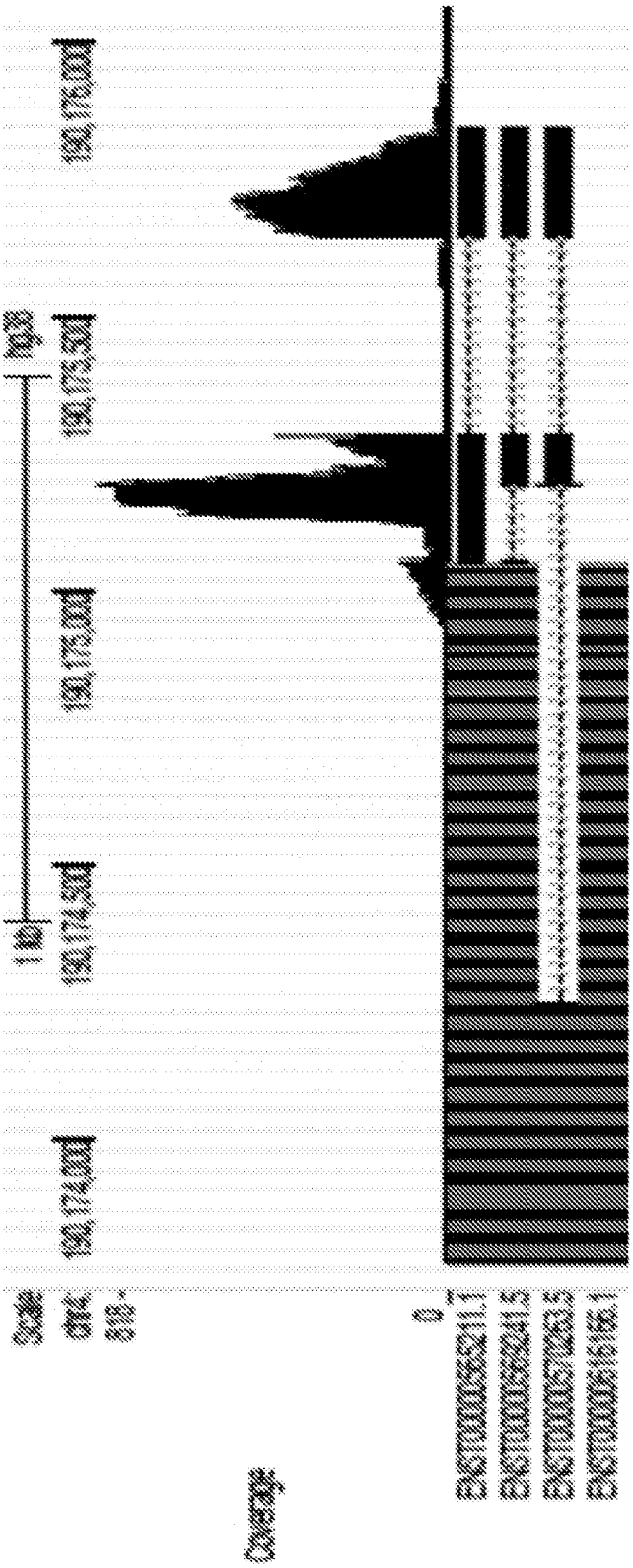

Coverage
Scale
chr4
kb -
190,174,000
1 kb
190,174,500
190,175,000
bp
190,175,500
190,176,000
0 -
ENSMUSG00000028311.1
ENSMUSG00000028415
ENSMUSG00000071263.5
ENSMUSG00000116166.1

FIG. 4

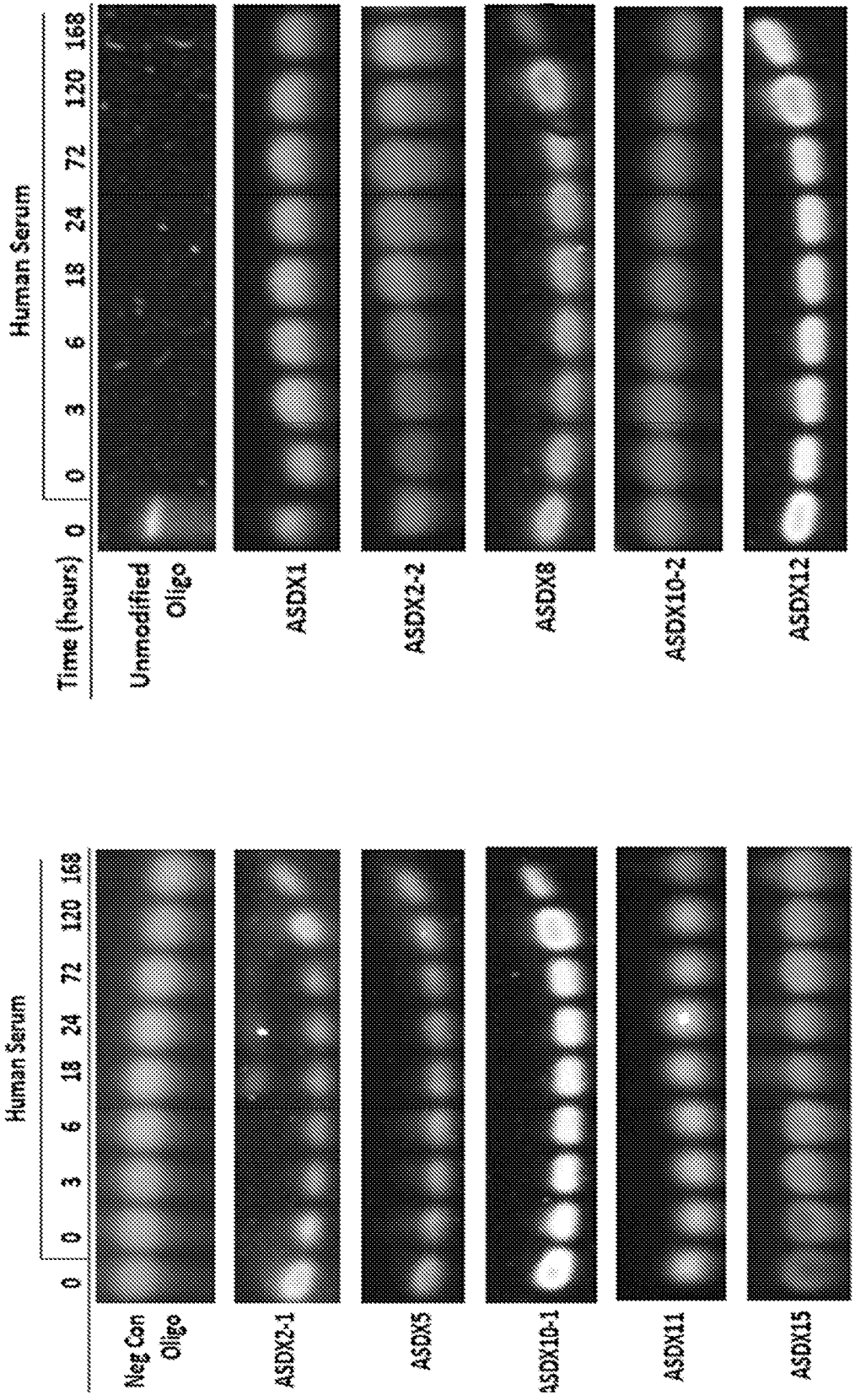

Human Serum
Time (hours)
0
0
3
6
18
24
72
120
168
Unmodified Oligo
ASDX1
ASDX2-2
ASDX8
ASDX10-2
ASDX12
Human Serum
0
0
3
6
18
24
72
120
168
Neg Con Oligo
ASDX2-1
ASDX5
ASDX10-1
ASDX11
ASDX15

FIG. 5

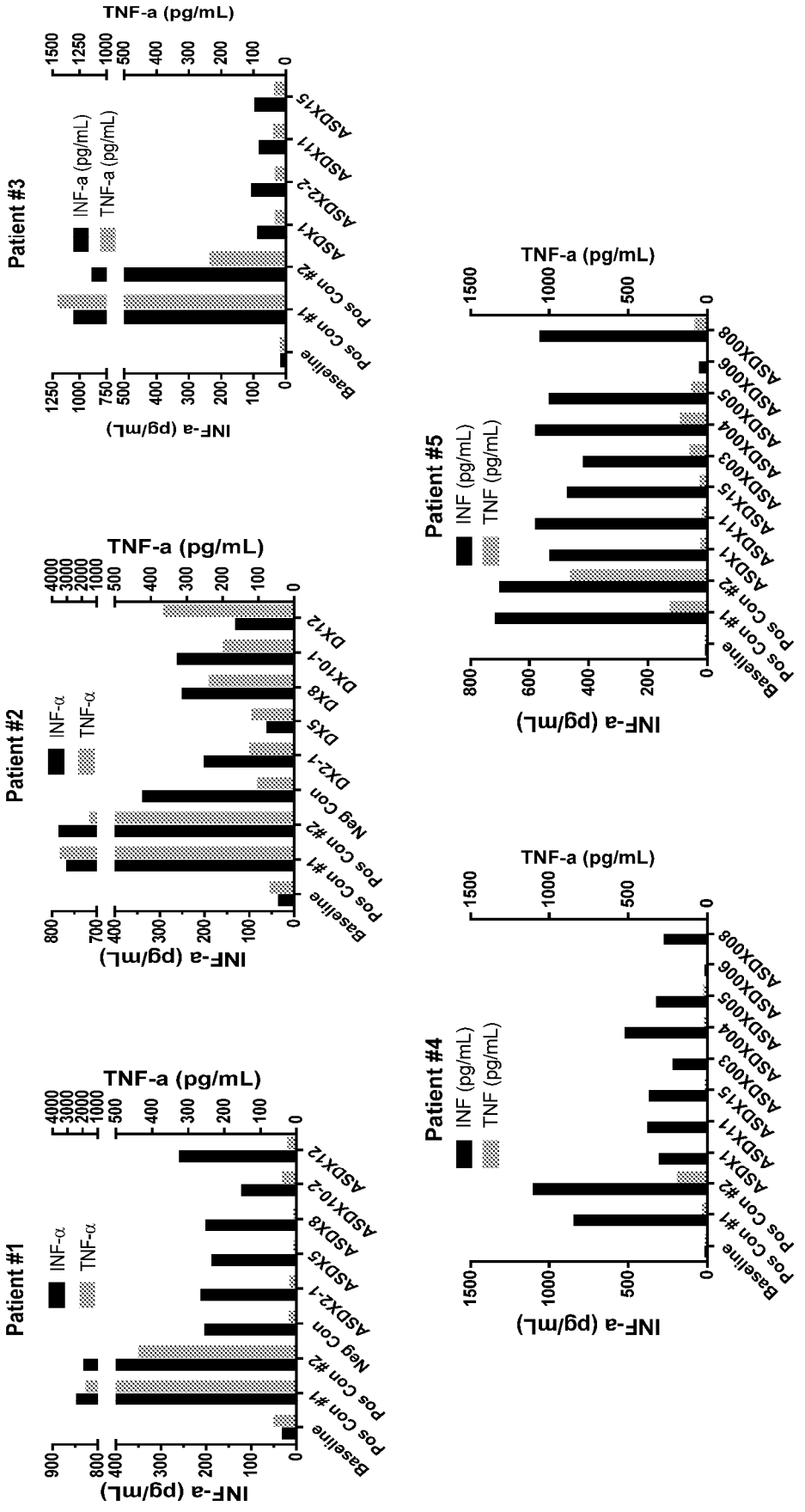
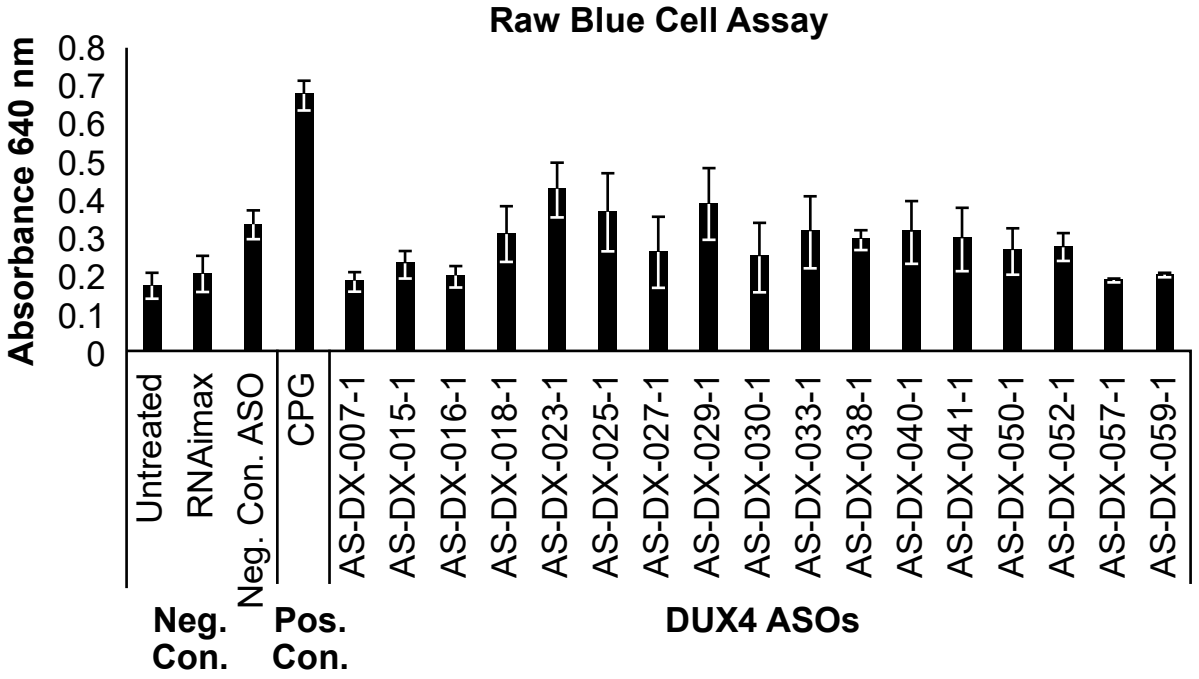

Patient #1
INF-α
TNF-α
INF-a (pg/mL)
TNF-a (pg/mL)
Baseline
Pos Con #1
Pos Con #2
Neg Con
ASDX2-1
ASDX5
ASDX8
ASDX10-2
ASDX12
Patient #2
INF-α
TNF-α
INF-a (pg/mL)
TNF-a (pg/mL)
Baseline
Pos Con #1
Pos Con #2
Neg Con
DX2-1
DX5
DX8
DX10-1
DX12
Patient #3
INF-a (pg/mL)
TNF-a (pg/mL)
INF-a (pg/mL)
TNF-a (pg/mL)
Baseline
Pos Con #1
Pos Con #2
ASDX1
ASDX2-2
ASDX11
ASDX15
Patient #4
INF (pg/mL)
TNF (pg/mL)
INF-a (pg/mL)
TNF-a (pg/mL)
Baseline
Pos Con #1
Pos Con #2
ASDX1
ASDX11
ASDX15
ASDX003
ASDX004
ASDX005
ASDX006
ASDX008
Patient #5
INF (pg/mL)
TNF (pg/mL)
INF-a (pg/mL)
TNF-a (pg/mL)
Baseline
Pos Con #1
Pos Con #2
ASDX1
ASDX11
ASDX15
ASDX003
ASDX004
ASDX005
ASDX006
ASDX008

FIG. 6A


Raw Blue Cell Assay
Absorbance 640 nm
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Untreated
RNAimax
Neg. Con. ASO
CPG
AS-DX-007-1
AS-DX-015-1
AS-DX-016-1
AS-DX-018-1
AS-DX-023-1
AS-DX-025-1
AS-DX-027-1
AS-DX-029-1
AS-DX-030-1
AS-DX-033-1
AS-DX-038-1
AS-DX-040-1
AS-DX-041-1
AS-DX-050-1
AS-DX-052-1
AS-DX-057-1
AS-DX-059-1
Neg. Con.
Pos. Con.
DUX4 ASOs

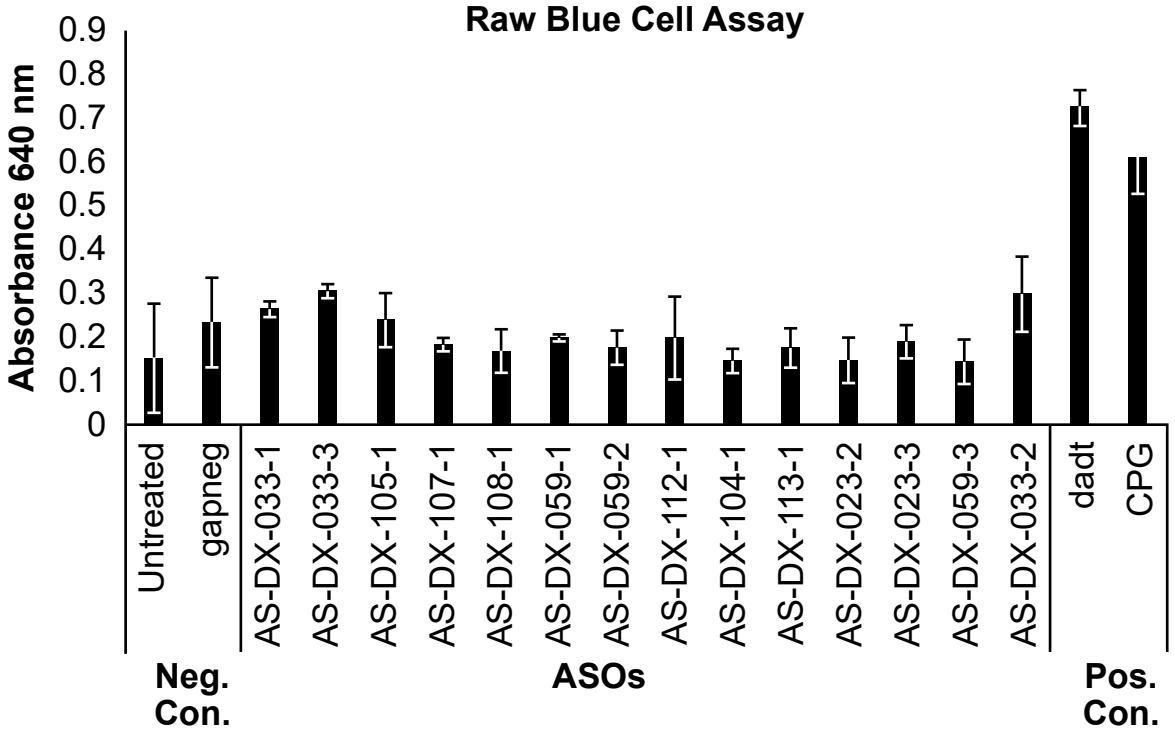

Raw Blue Cell Assay
Absorbance 640 nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Untreated
gapneg
AS-DX-033-1
AS-DX-033-3
AS-DX-105-1
AS-DX-107-1
AS-DX-108-1
AS-DX-059-1
AS-DX-059-2
AS-DX-112-1
AS-DX-104-1
AS-DX-113-1
AS-DX-023-2
AS-DX-023-3
AS-DX-059-3
AS-DX-033-2
dadt
CPG
Neg. Con.
ASOs
Pos. Con.

FIG. 6B

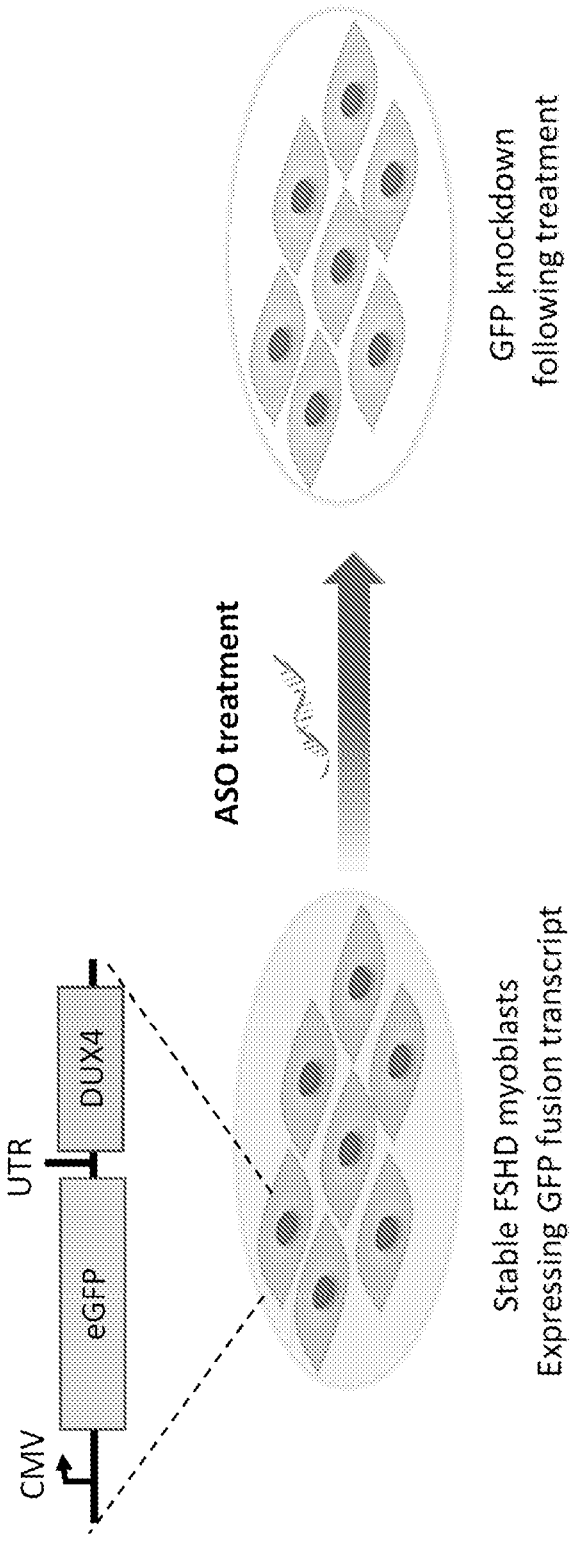

CMV
eGFP
UTR
DUX4
Stable FSHD myoblasts
Expressing GFP fusion transcript
ASO treatment
GFP knockdown
following treatment

FIG. 7

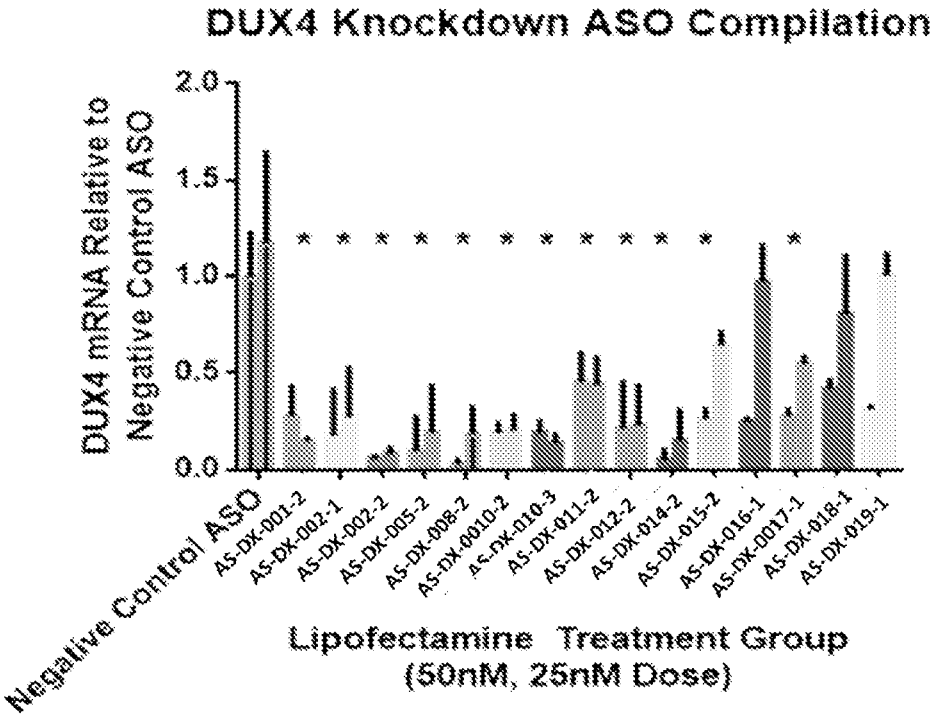
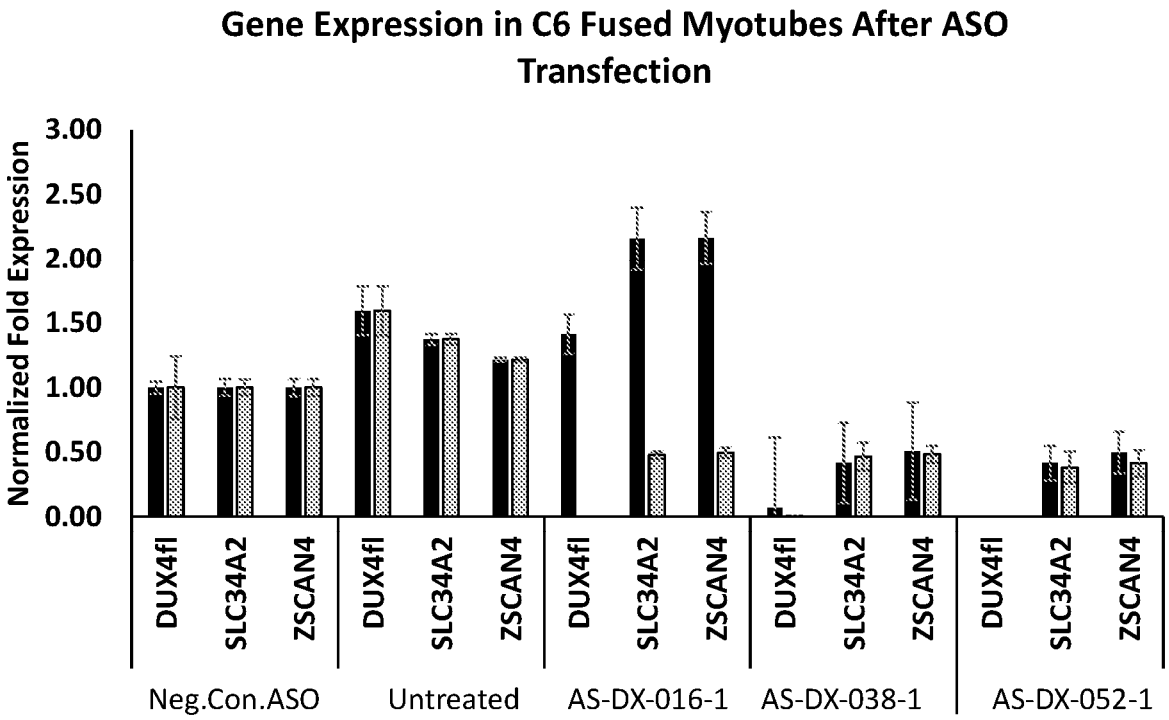

DUX4 Knockdown ASO Compilation
DUX4 mRNA Relative to Negative Control ASO
2.0
1.5
1.0
0.5
0.0
Negative Control ASO
AS-DX-001-2
AS-DX-002-1
AS-DX-002-2
AS-DX-005-2
AS-DX-008-2
AS-DX-0010-2
AS-DX-010-3
AS-DX-011-2
AS-DX-012-2
AS-DX-014-2
AS-DX-015-2
AS-DX-016-1
AS-DX-0017-1
AS-DX-018-1
AS-DX-019-1
Lipofectamine Treatment Group
(50nM, 25nM Dose)

FIG. 8A


Gene Expression in C6 Fused Myotubes After ASO Transfection
Normalized Fold Expression
3.00
2.50
2.00
1.50
1.00
0.50
0.00
DUX4fl
SLC34A2
ZSCAN4
Neg.Con.ASO
Untreated
AS-DX-016-1
AS-DX-038-1
AS-DX-052-1

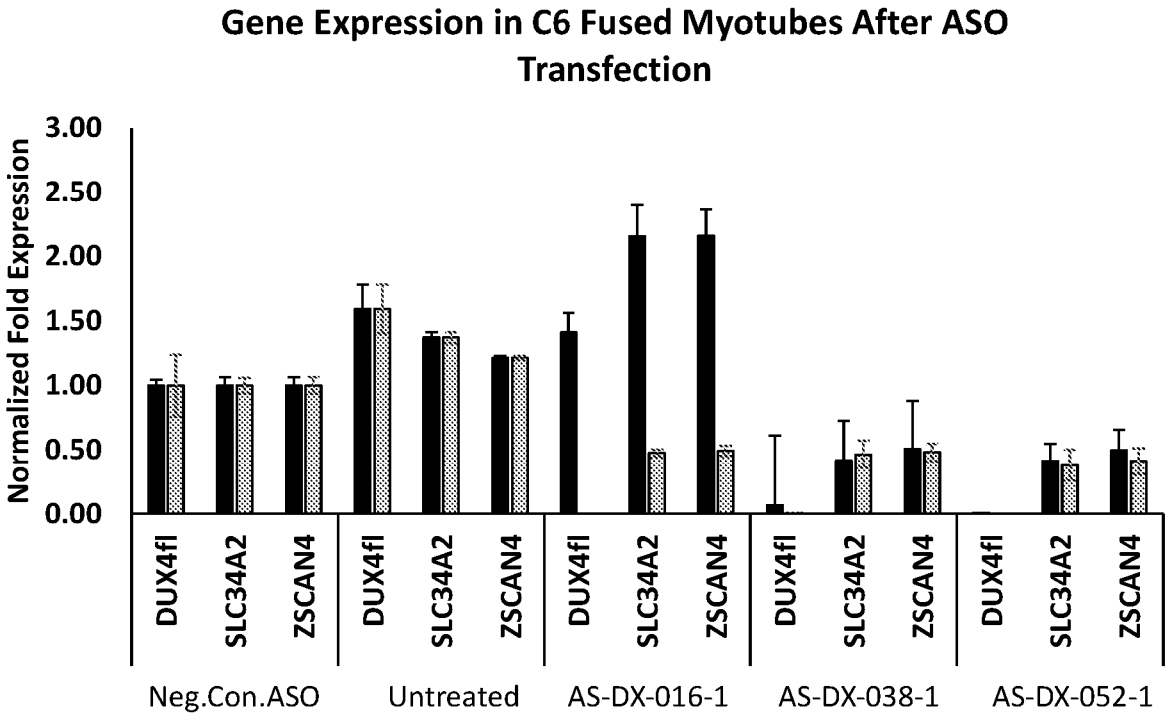

Gene Expression in C6 Fused Myotubes After ASO Transfection
Normalized Fold Expression
3.00
2.50
2.00
1.50
1.00
0.50
0.00
DUX4fl
SLC34A2
ZSCAN4
Neg.Con.ASO
Untreated
AS-DX-016-1
AS-DX-038-1
AS-DX-052-1

FIG8B

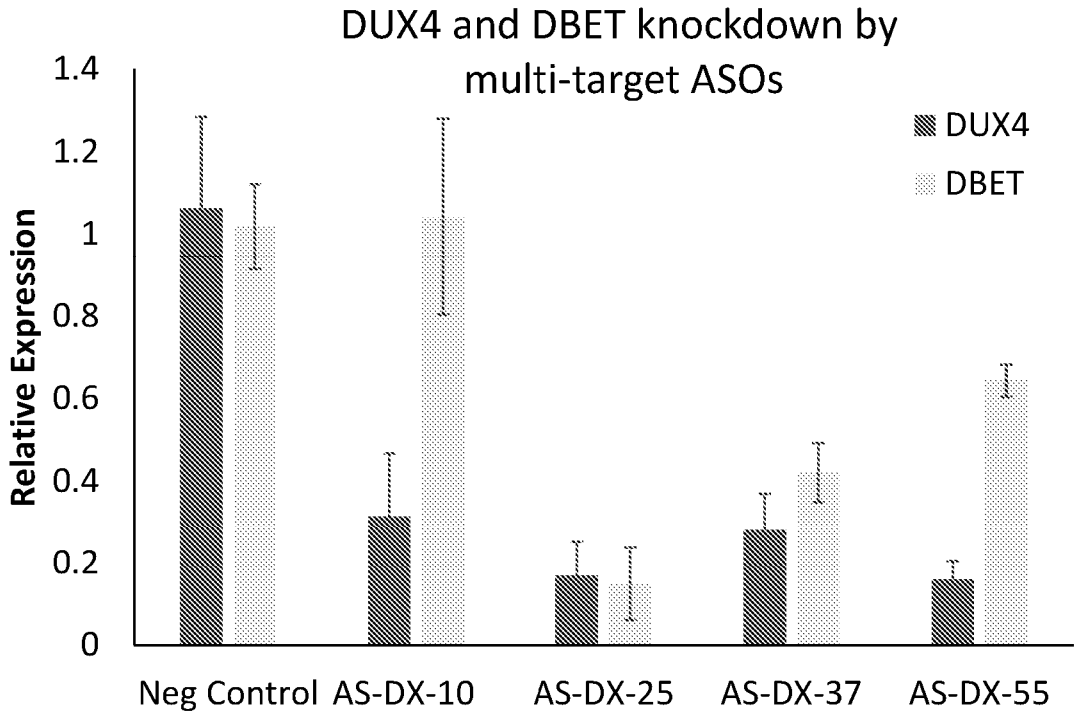

DUX4 and DBET knockdown by multi-target ASOs
DUX4
DBET
Relative Expression
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Neg Control
AS-DX-10
AS-DX-25
AS-DX-37
AS-DX-55

FIG. 9

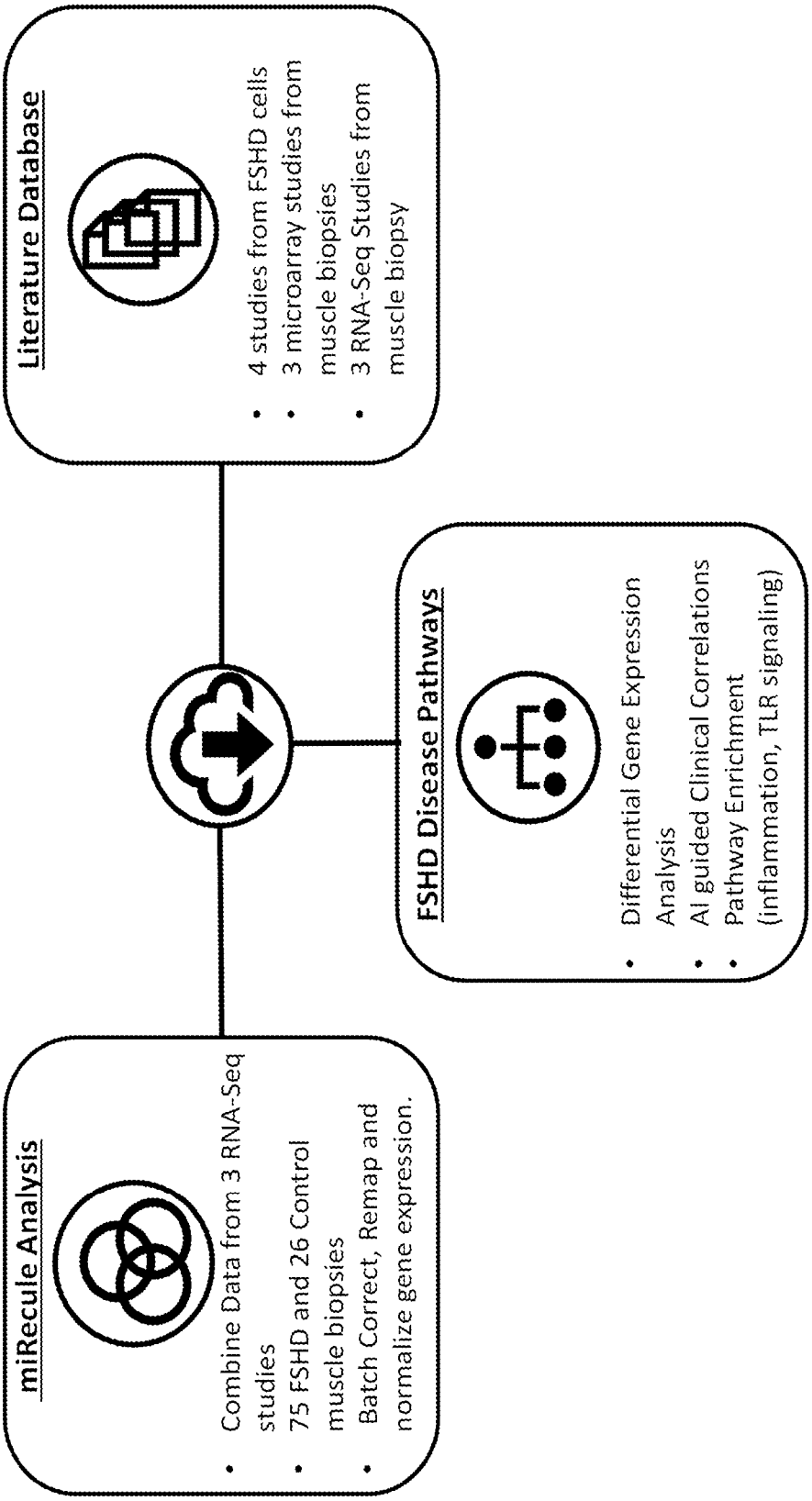

Literature Database
4 studies from FSHD cells
3 microarray studies from muscle biopsies
3 RNA-Seq Studies from muscle biopsy
FSHD Disease Pathways
Differential Gene Expression Analysis
AI guided Clinical Correlations
Pathway Enrichment (inflammation, TLR signaling)
miRecule Analysis
Combine Data from 3 RNA-Seq studies
75 FSHD and 26 Control muscle biopsies
Batch Correct, Remap and normalize gene expression.

FIG. 10

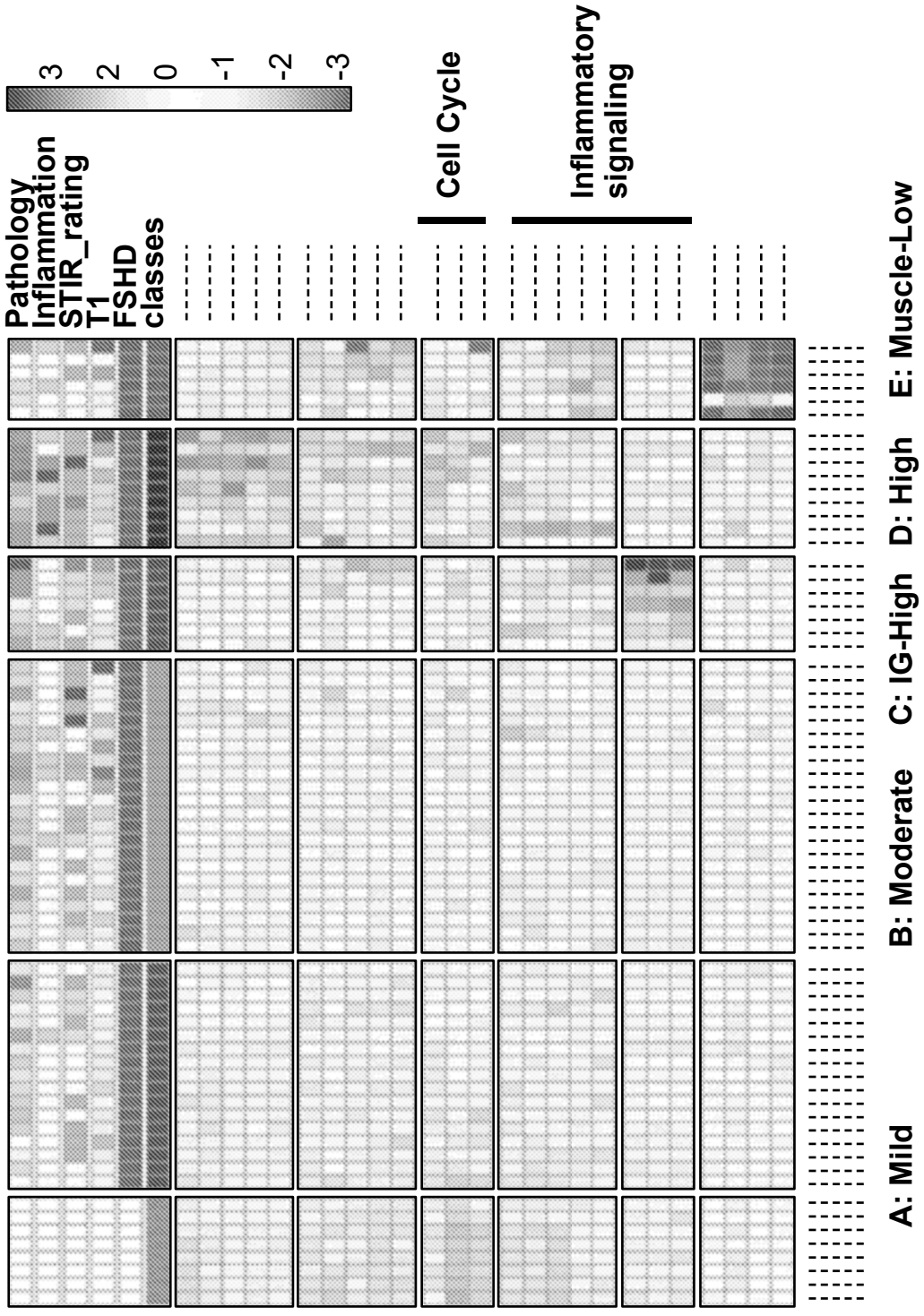

3
2
0
-1
-2
-3
Pathology
Inflammation
STIR_rating
T1
FSHD
classes
Cell Cycle
Inflammatory signaling
A: Mild
B: Moderate
C: IG-High
D: High
E: Muscle-Low

FIG. 11

A

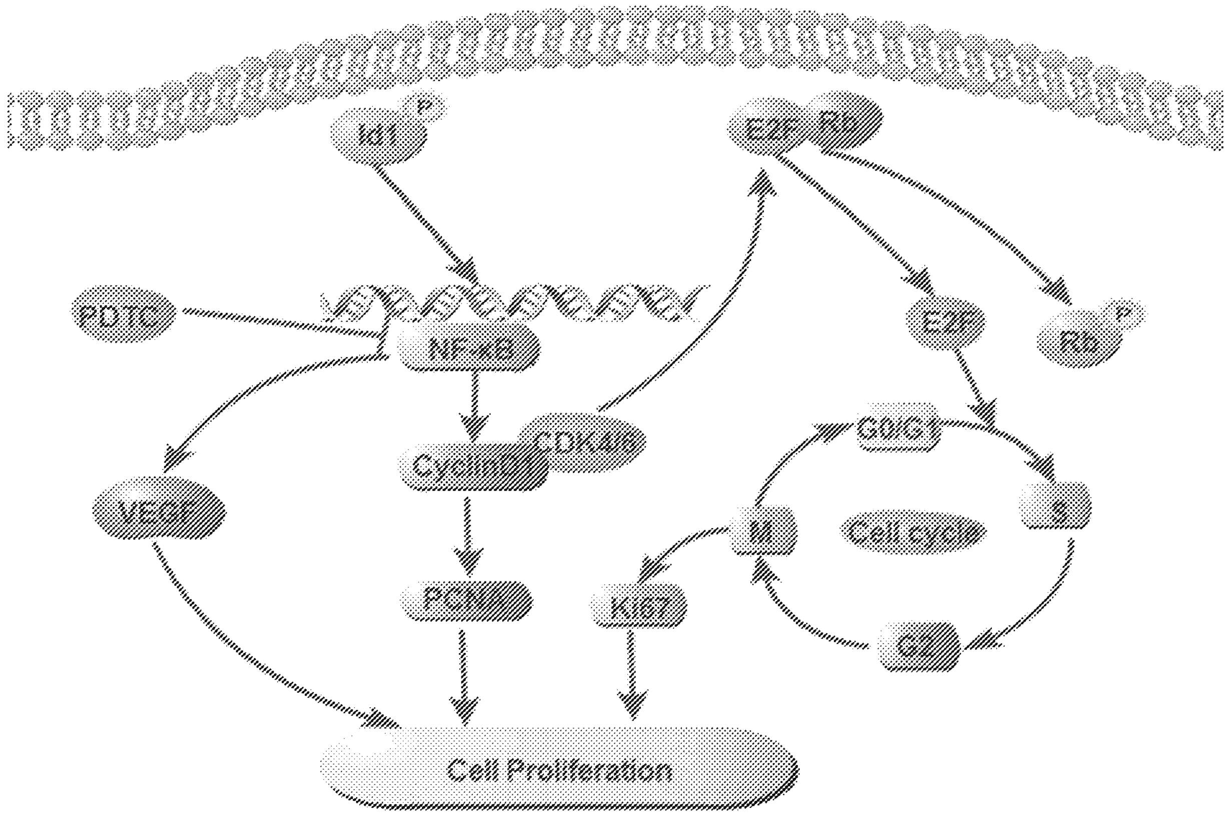

Id1
P
E2F
Rb
PDTC
NF-κB
E2F
Rb
P
CDK4/6
G0/G1
Cyclin
VEGF
S
M
Cell cycle
PCN
Ki67
G2
Cell Proliferation

FIG. 12A

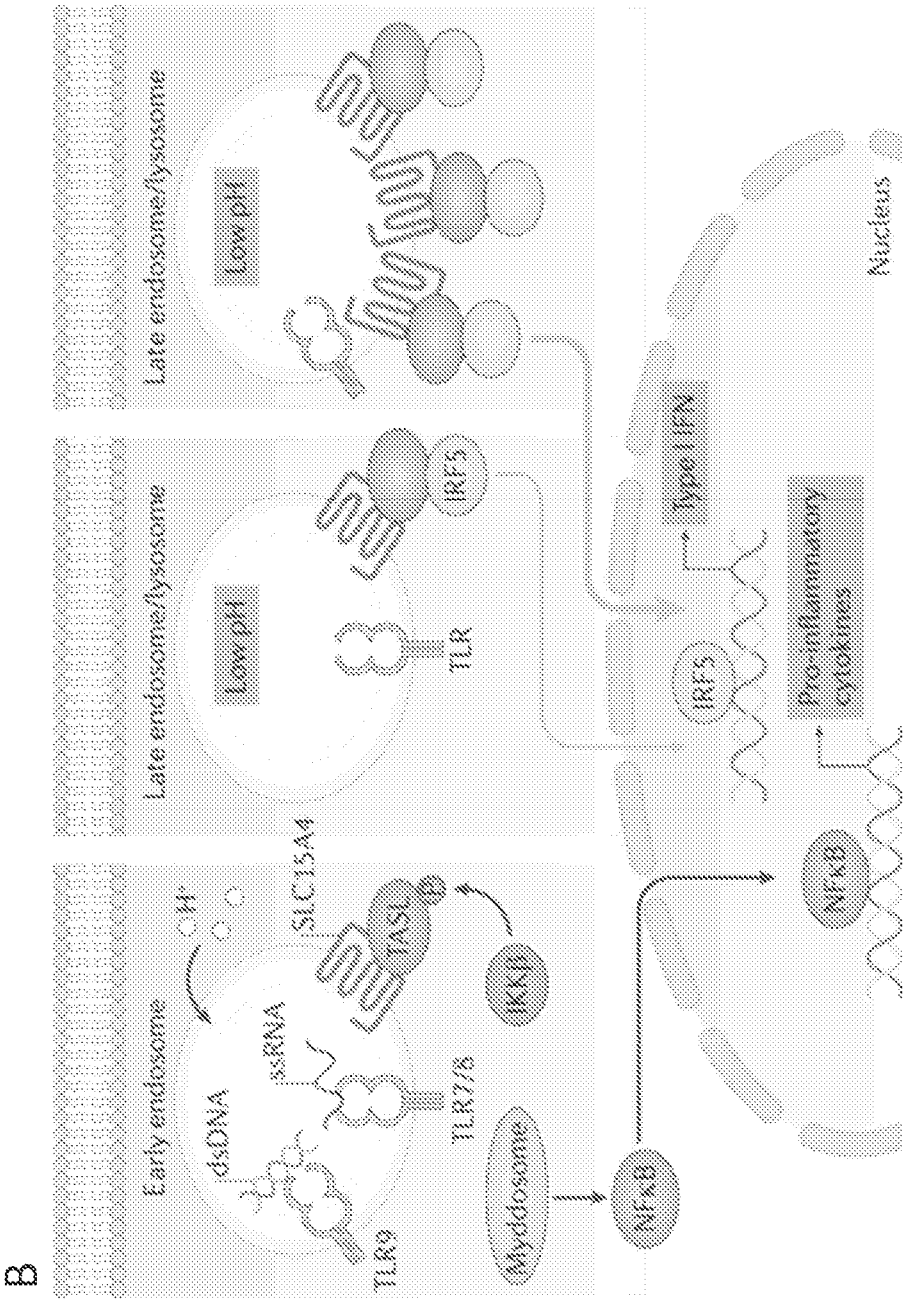
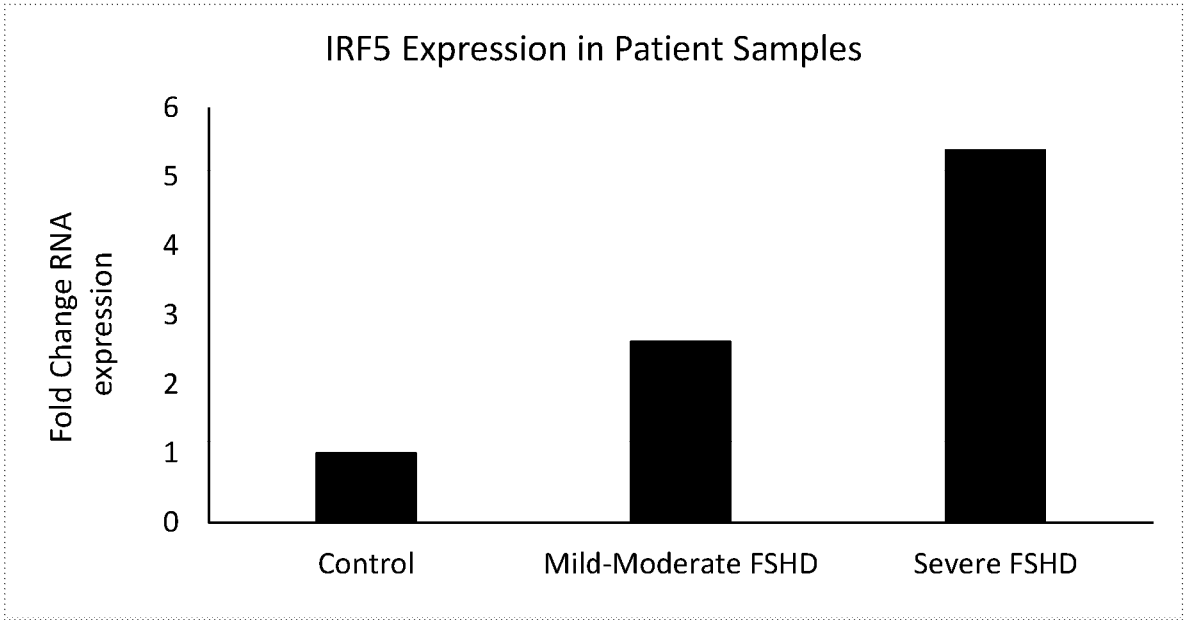

B
Early endosome
Late endosome/lysosome
Low pH
dsDNA
ssRNA
H+
SLC15A4
TLR9
TLR7/8
TLR
IRF5
IKKβ
Myddosome
NFκB
Type I IFN
IRF5
Pro-inflammatory cytokines
NFκB
Nucleus

FIG. 12B


IRF5 Expression in Patient Samples
Fold Change RNA expression
0
1
2
3
4
5
6
Control
Mild-Moderate FSHD
Severe FSHD

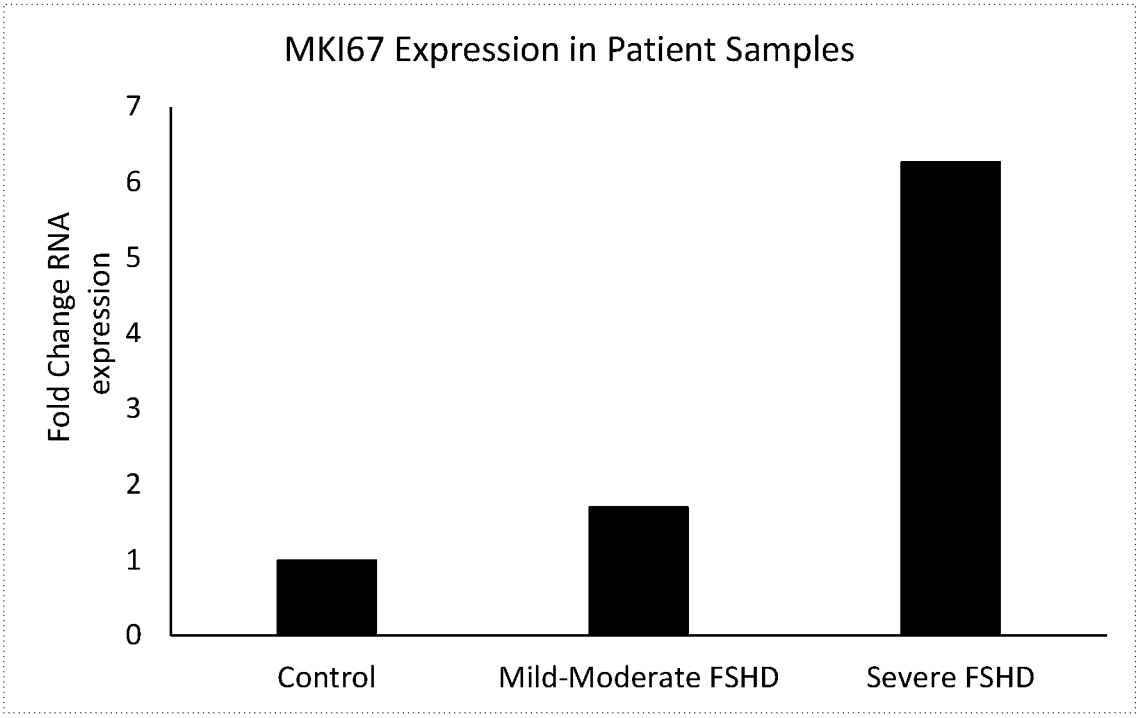

MKI67 Expression in Patient Samples
Fold Change RNA expression
0
1
2
3
4
5
6
7
Control
Mild-Moderate FSHD
Severe FSHD

FIG. 13

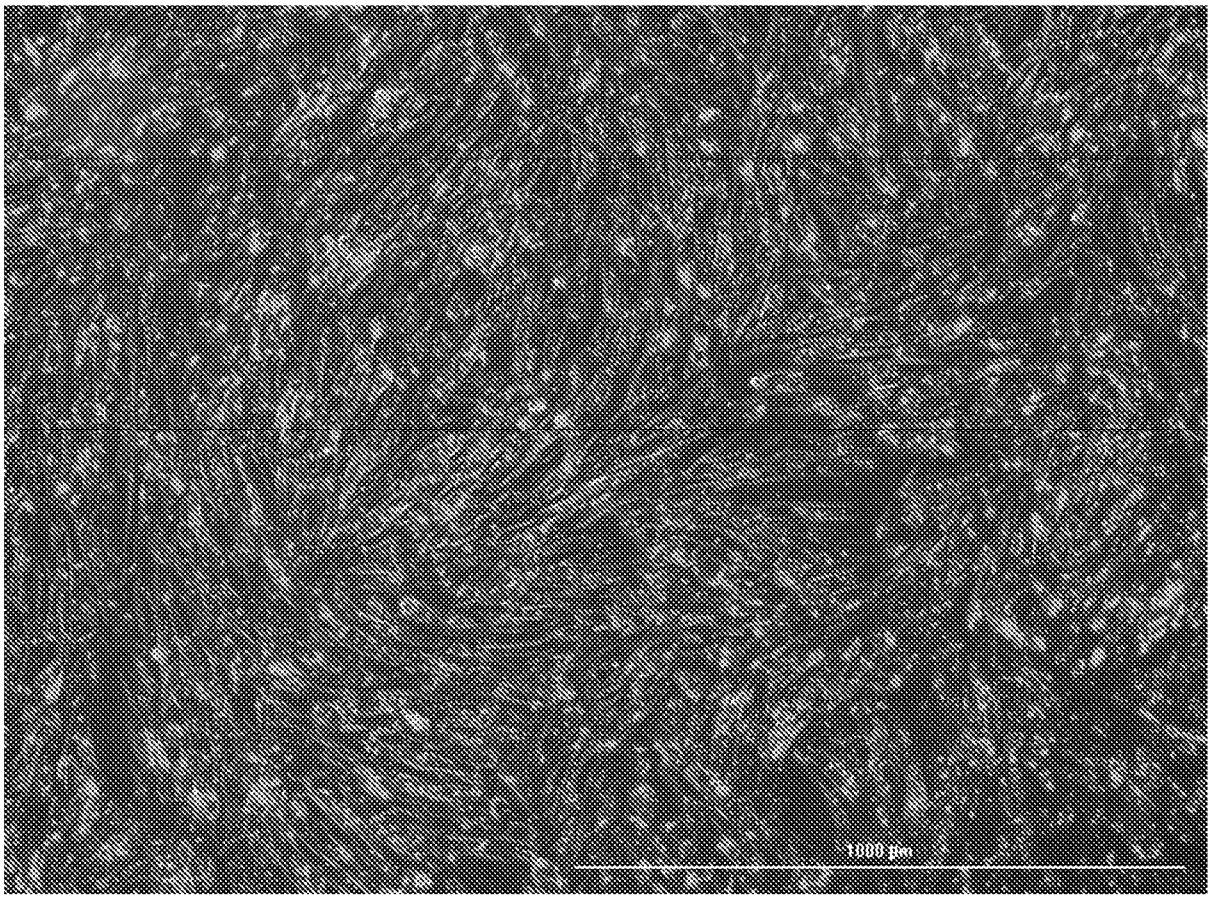


FIG. 14A

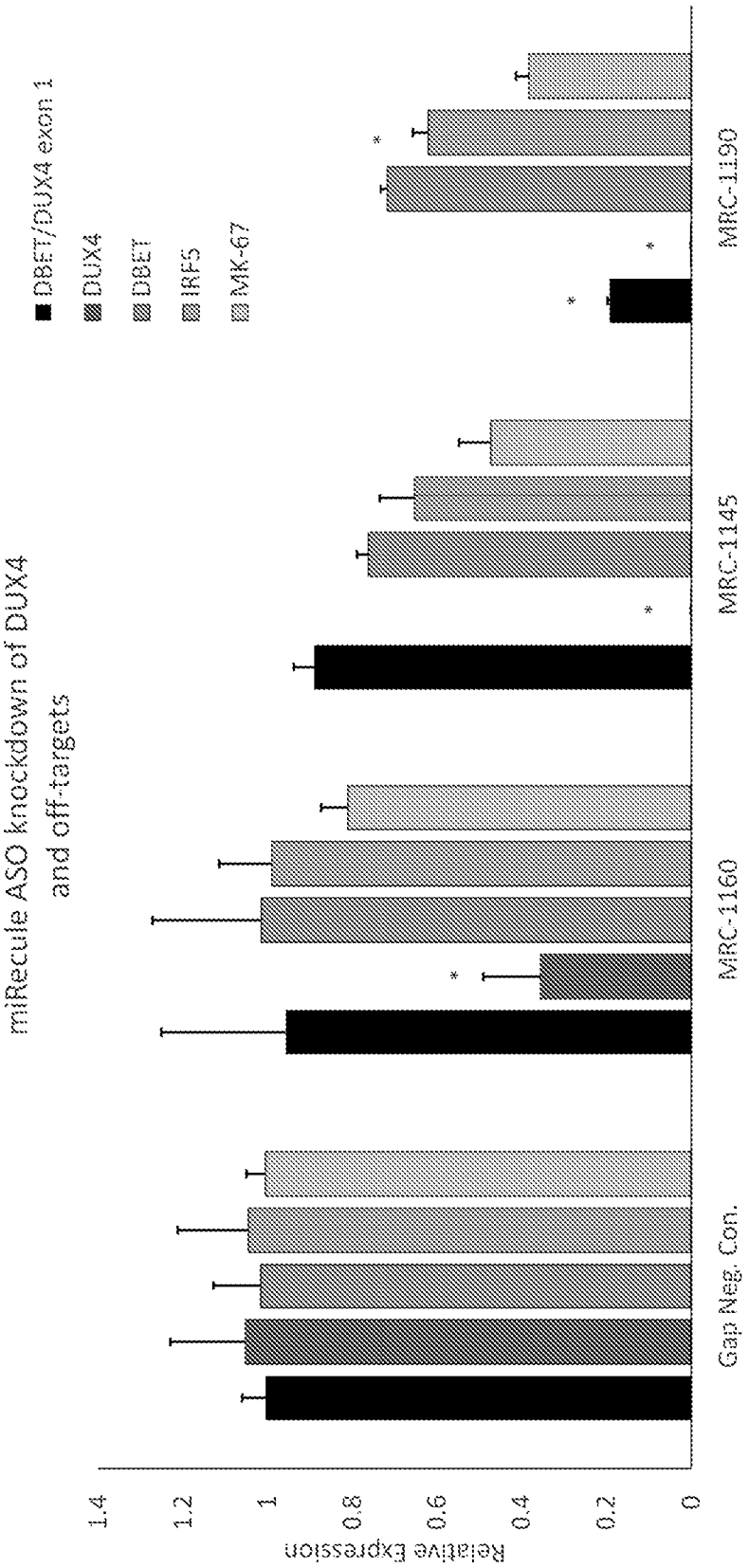

miRecule ASO knockdown of DUX4
and off-targets
DBET/DUX4 exon 1
DUX4
DBET
IRF5
MK-67
Relative Expression
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Gap
Neg. Con.
MRC-1160
MRC-1145
MRC-1190
*

FIG. 14B

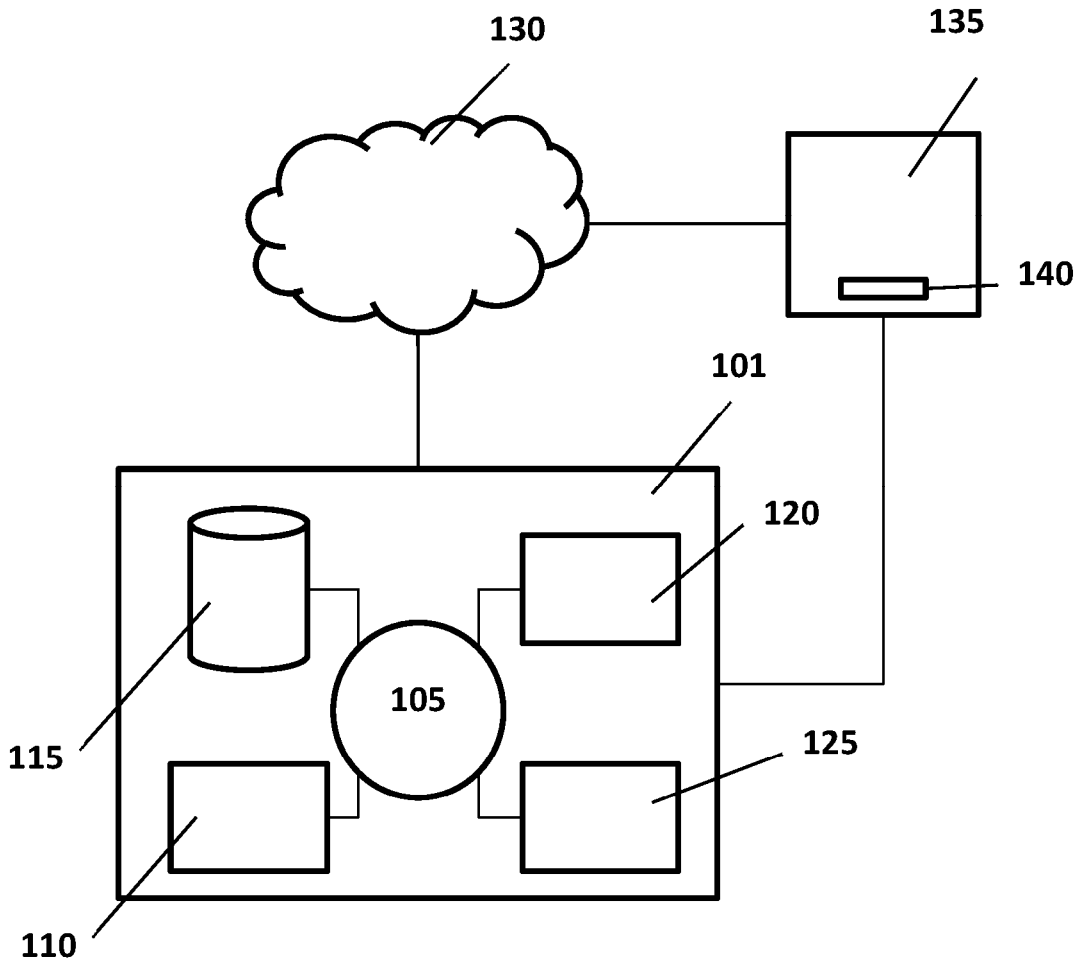


FIG. 15

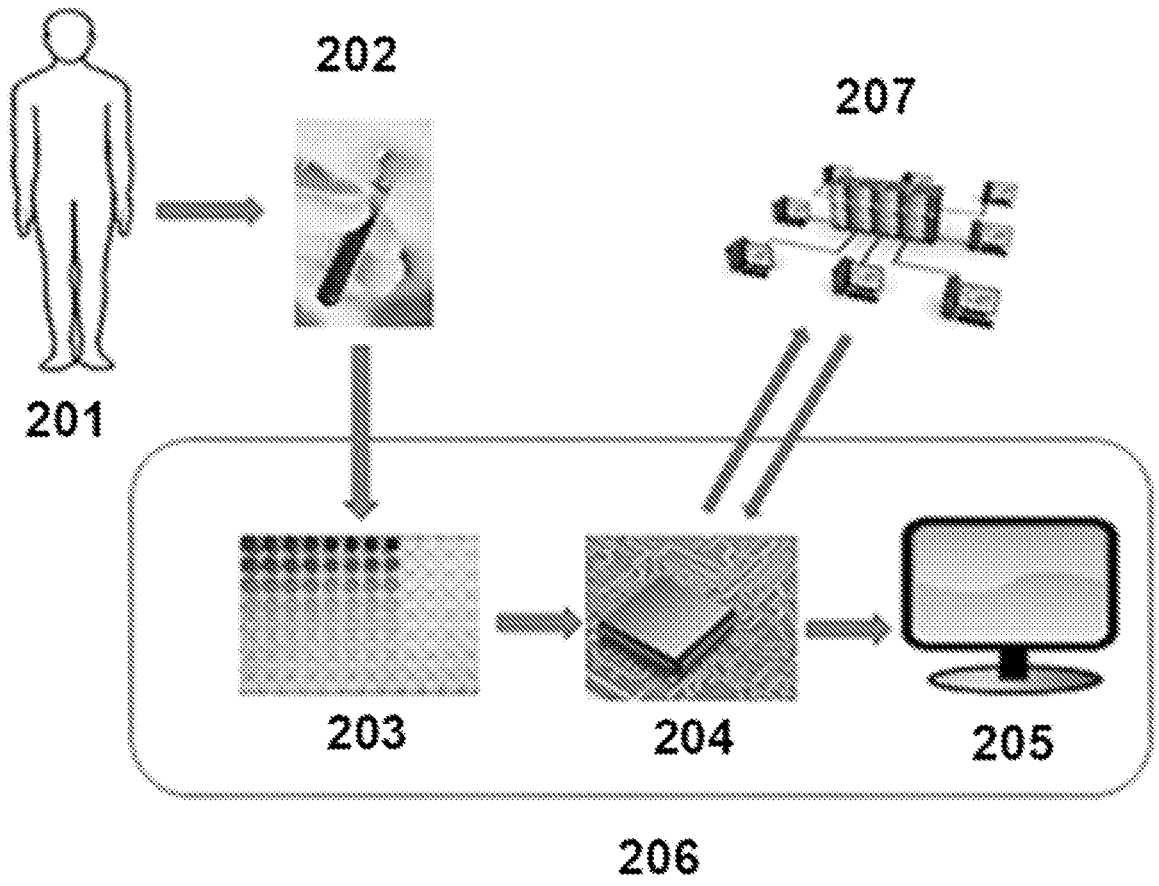


FIG. 16