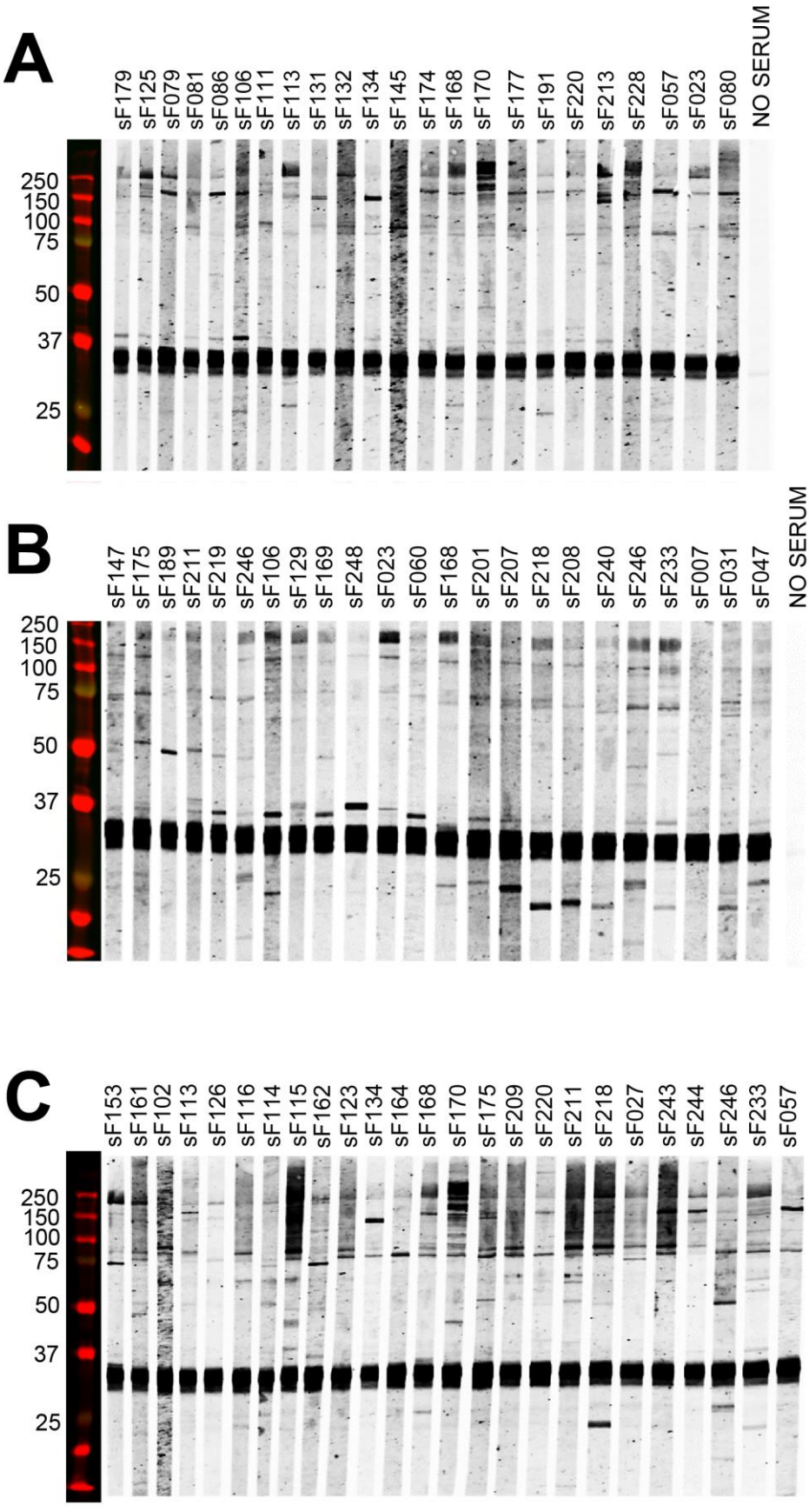
Supplementary Data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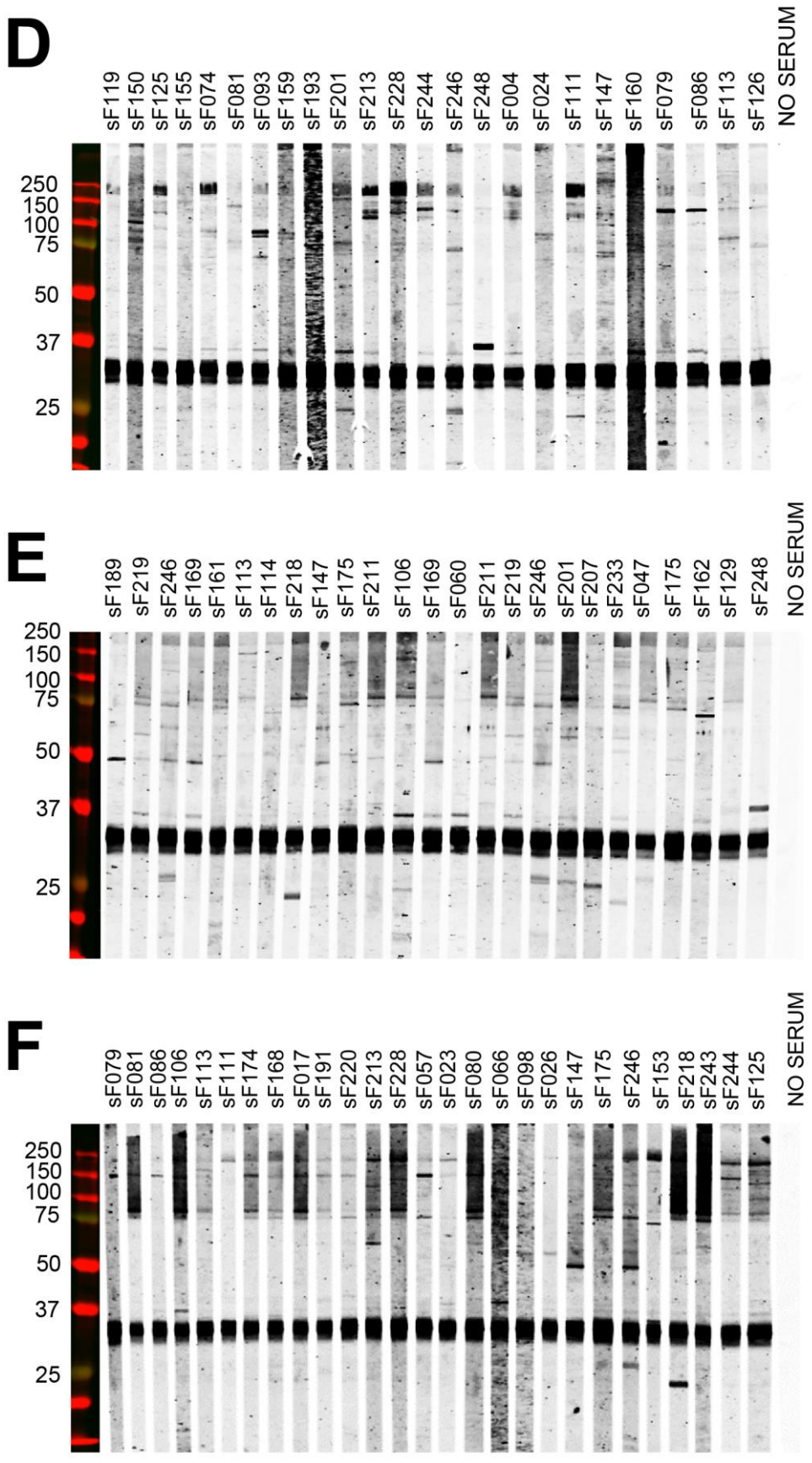
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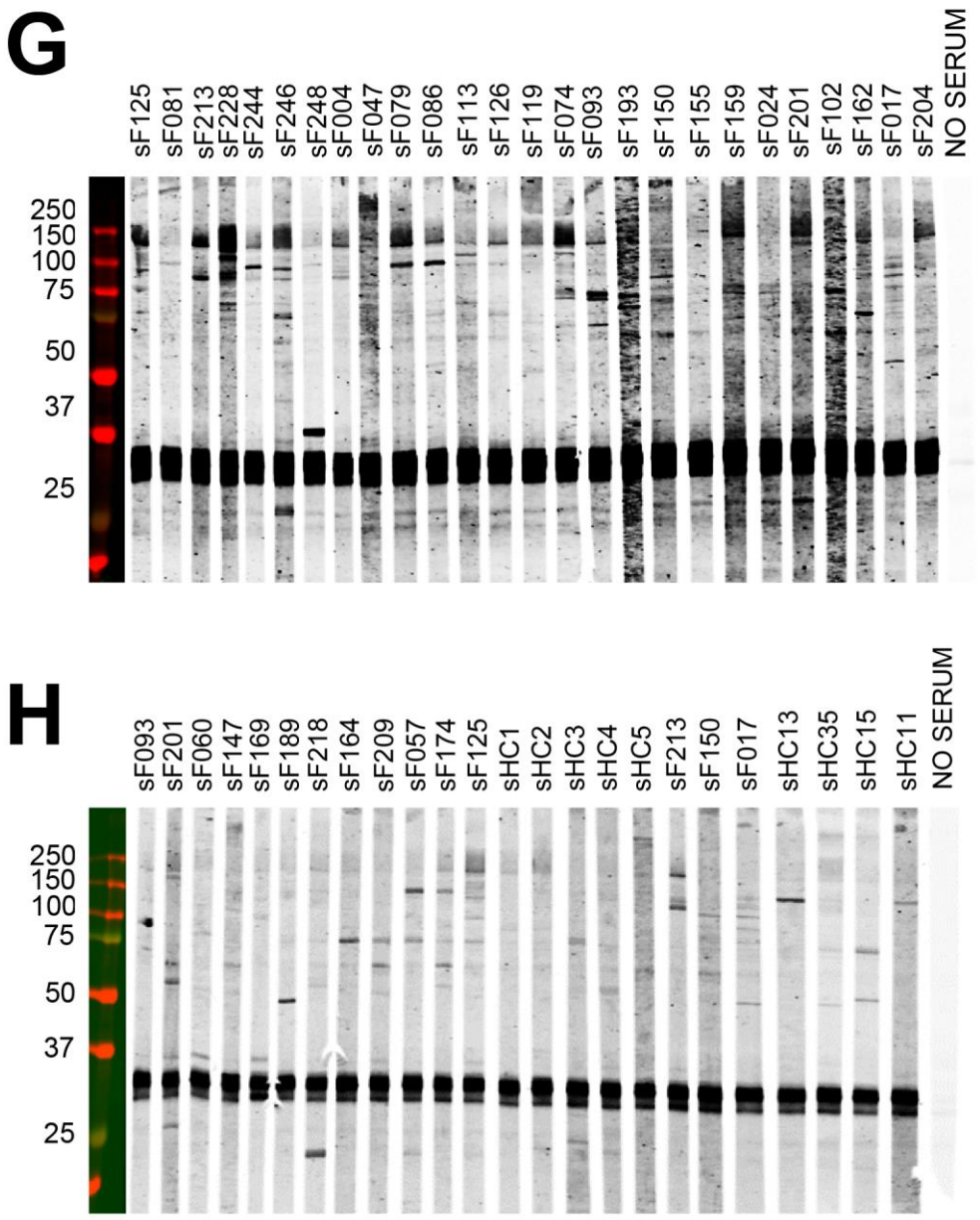
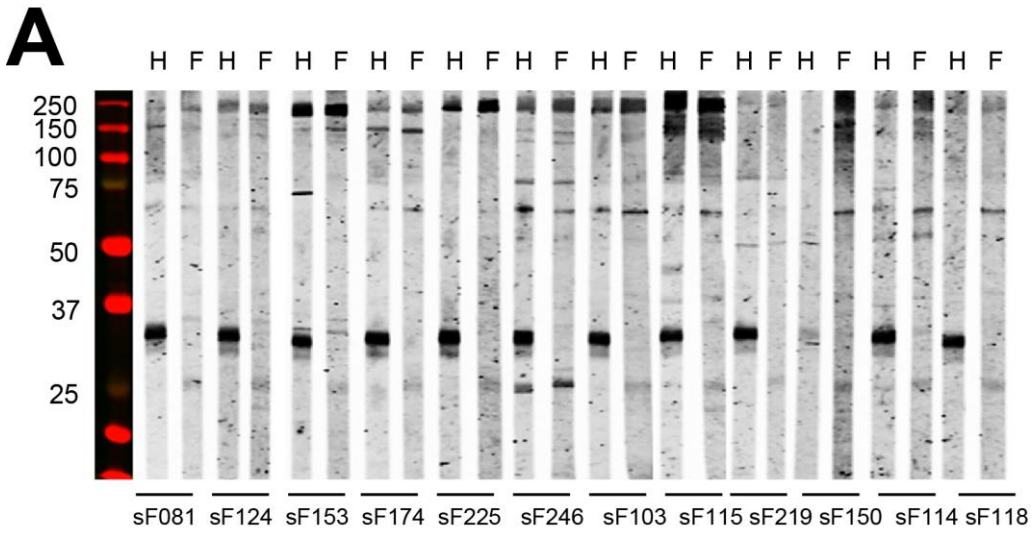


Supplementary Figure 1. Reactivity of FSHD patient sera and HC sera with healthy human skeletal muscle protein extract. Healthy human skeletal muscle lysate was separated by SDS-PAGE and transferred to nitrocellulose membranes. Blot strips were incubated with FSHD patient sera (A–H) and healthy control sera (H), and bound antibodies were visualized by IRDye-labeled secondary antibodies. Patient sera are coded as sF followed by a number, and healthy

control sera as sHC followed by a number. The left side of each panel indicates the position of molecular weight markers.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100
75
50
37
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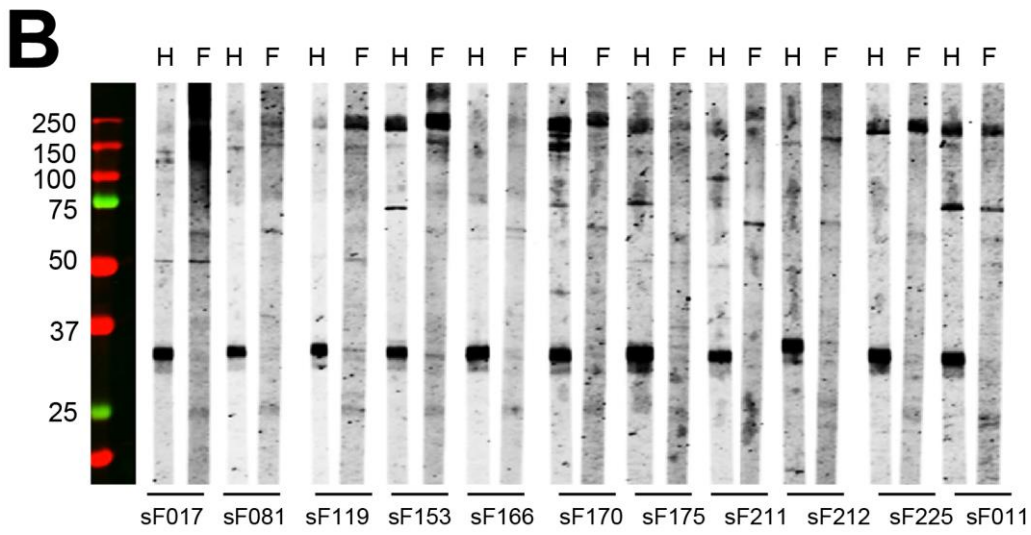
B
H F H F H F H F H F H F H F H F H F H F H F H F
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75
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37
25
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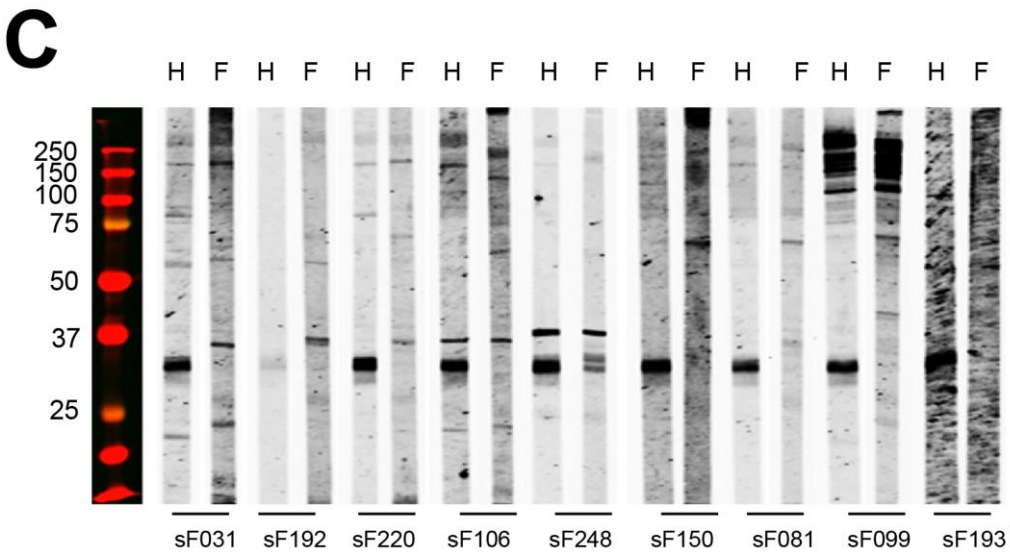
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75
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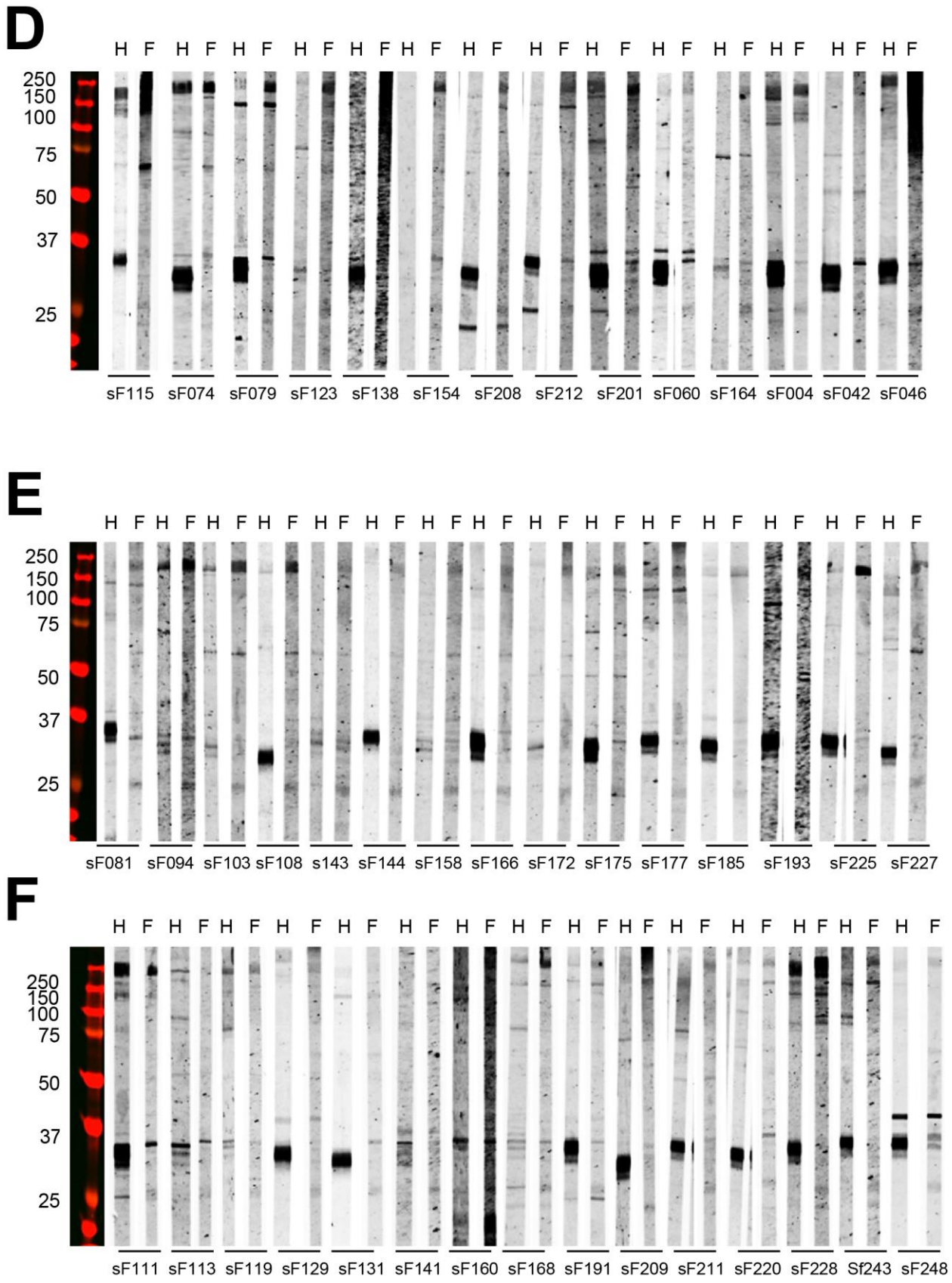
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150
100
75
50
37
25
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E
H F H F H F H F H F H F H F H F H F H F H F H F H F H F H F
250
150
100
75
50
37
25
sF081 sF094 sF103 sF108 s143 sF144 sF158 sF166 sF172 sF175 sF177 sF185 sF193 sF225 sF227
F
H F H F H F H F H F H F H F H F H F H F H F H F H F H F H F
250
150
100
75
50
37
25
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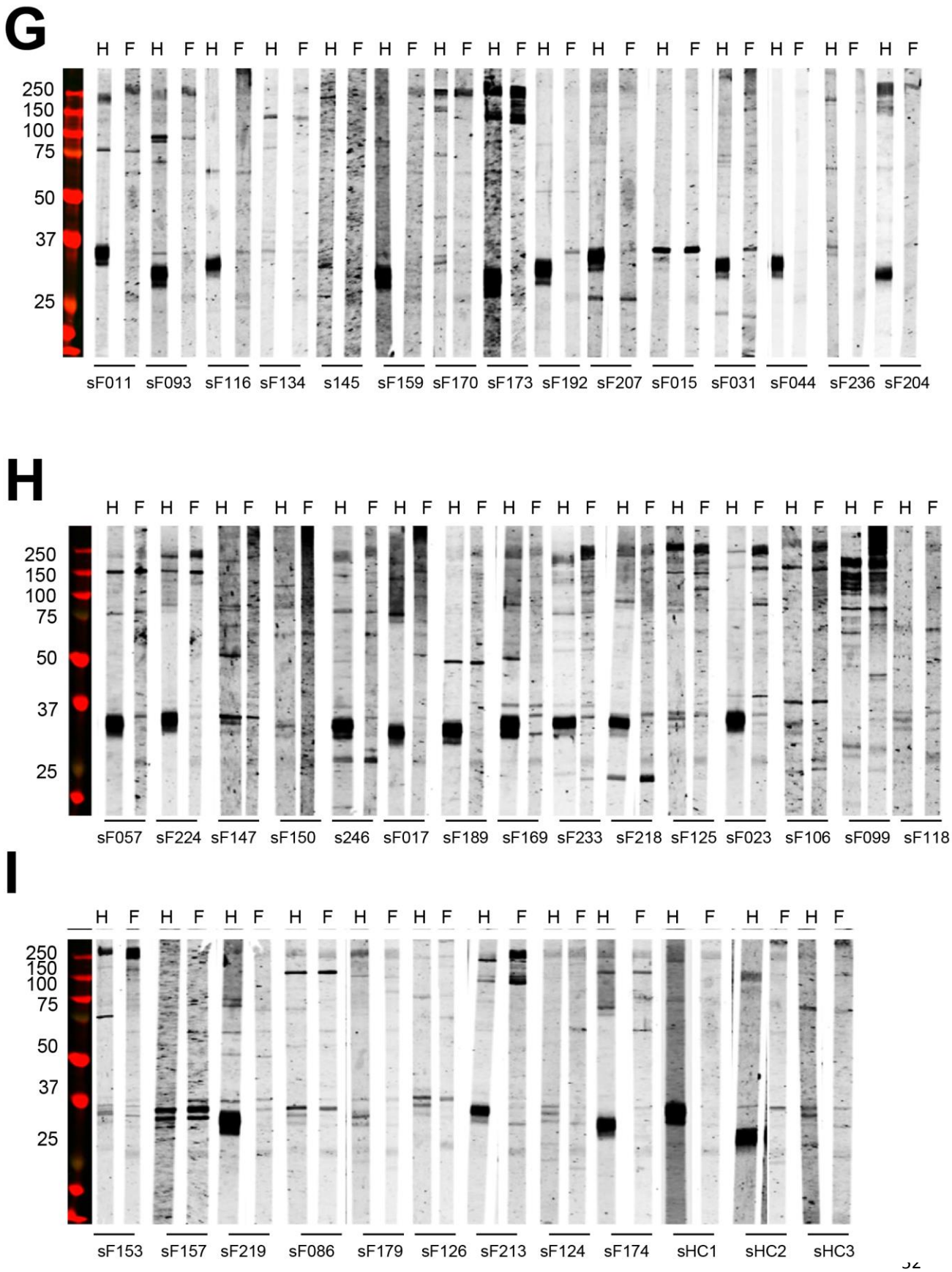
G
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150
100
75
50
37
25
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H
H F H F H F H F H F H F H F H F H F H F H F H F H F H F H F
250
150
100
75
50
37
25
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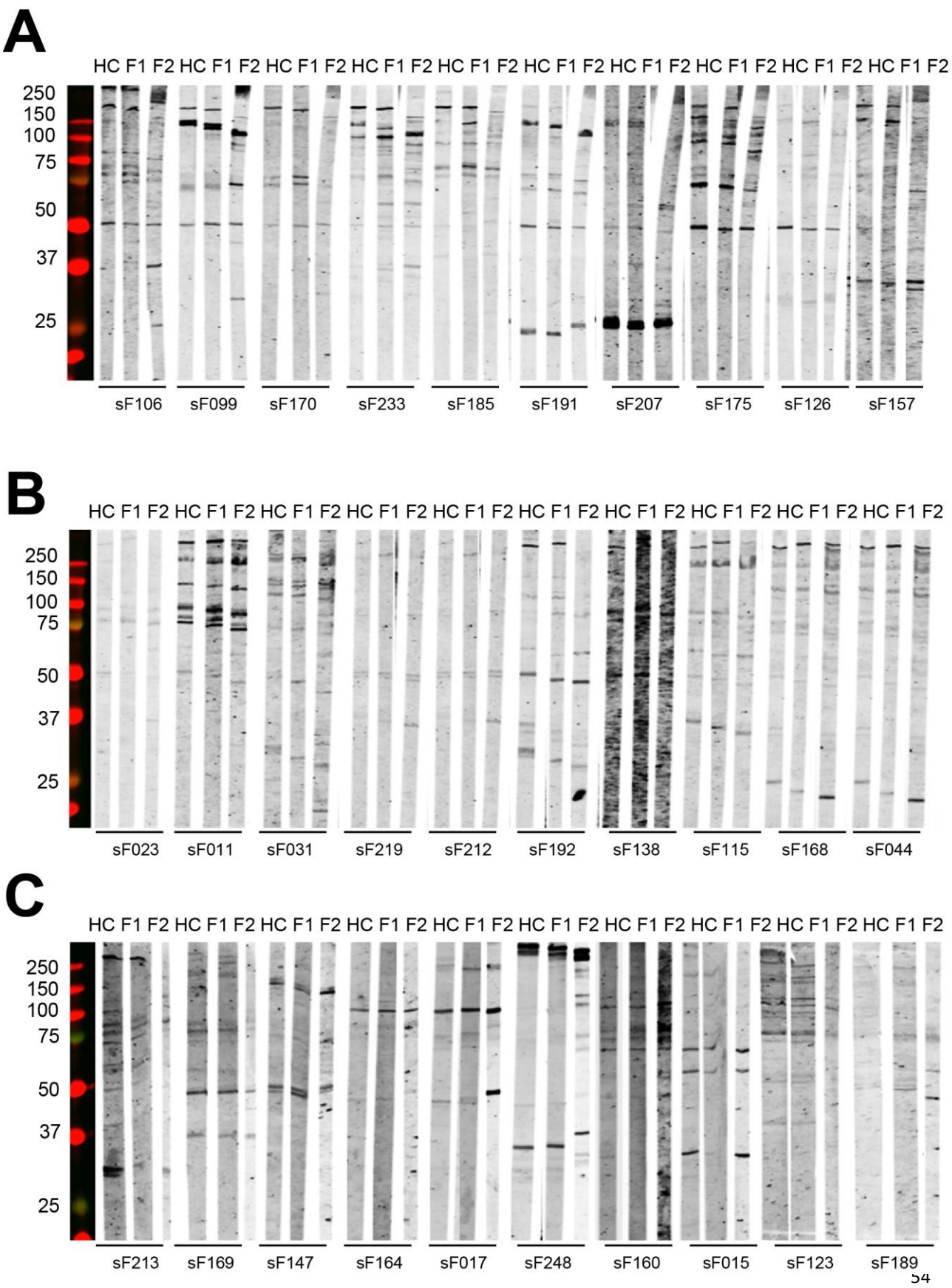
I
H F H F H F H F H F H F H F H F H F H F H F H F H F
250
150
100
75
50
37
25
sF153 sF157 sF219 sF086 sF179 sF126 sF213 sF124 sF174 sHC1 sHC2 sHC3

Supplementary Figure 2. Reactivity of FSHD patient sera and HC sera with FSHD quadriceps muscle protein extract. FSHD muscle lysate (F) and, as a reference an extract from healthy muscles (H), were separated by SDS-PAGE and transferred to nitrocellulose membranes. H and F blot strips were incubated with FSHD patient sera (sF) (A-I) and with healthy control sera (sHC) (I).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150
100
75
50
37
25
sF106 sF099 sF170 sF233 sF185 sF191 sF207 sF175 sF126 sF157
B
HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2
250
150
100
75
50
37
25
sF023 sF011 sF031 sF219 sF212 sF192 sF138 sF115 sF168 sF044
C
HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2 HC F1 F2
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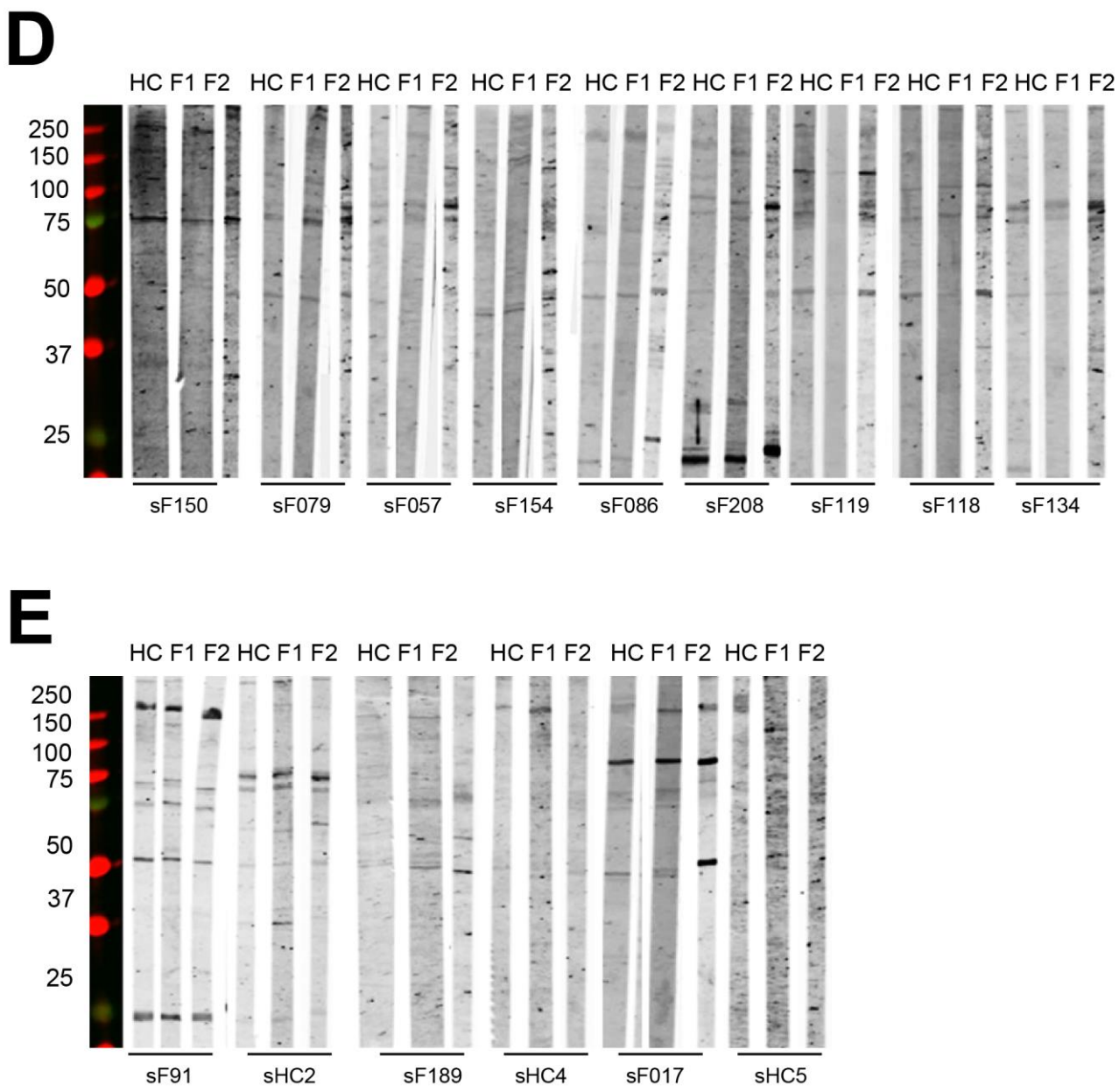
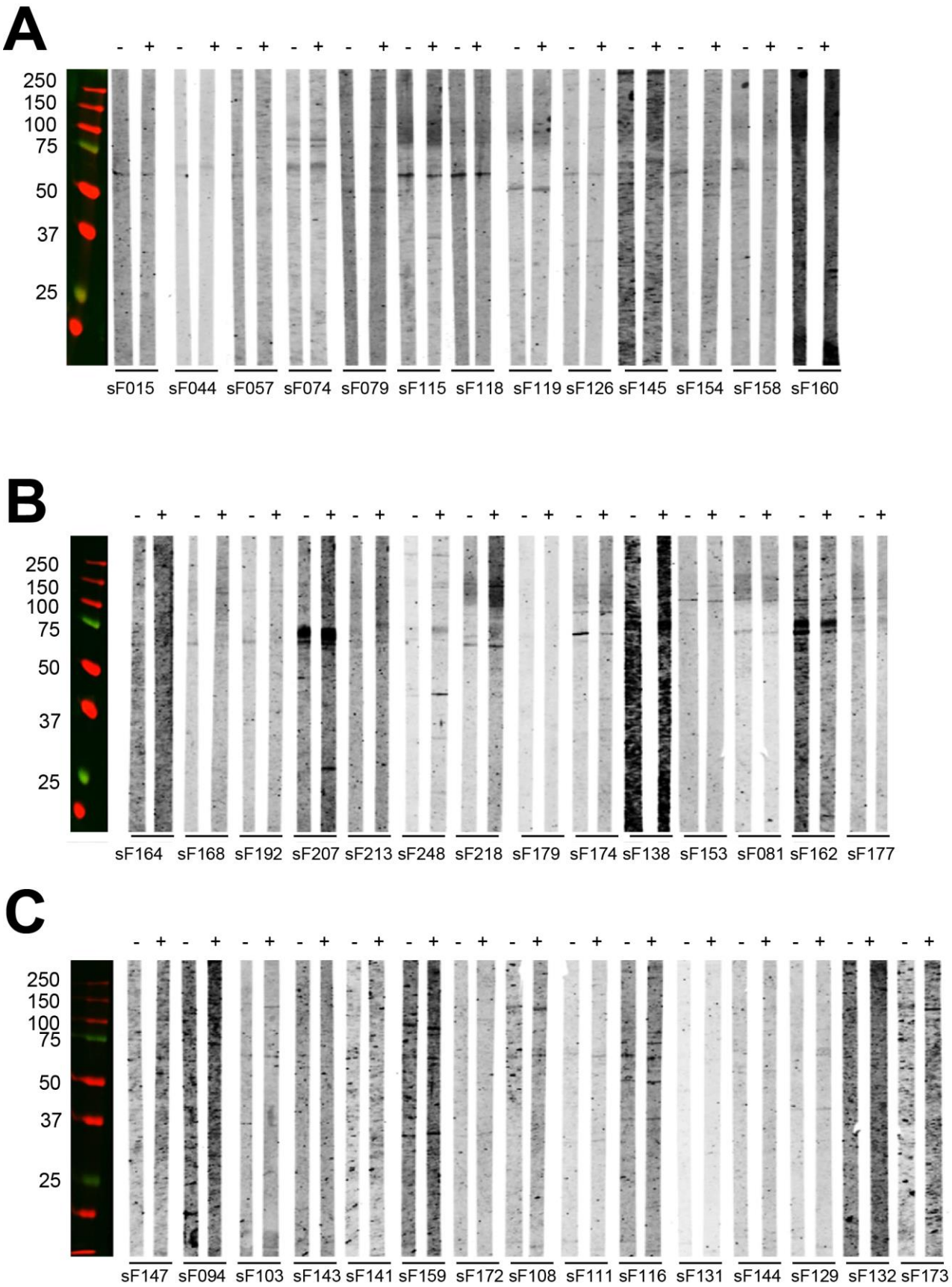


Supplementary Figure 3. Reactivity of FSHD patient sera and HC sera with FSHD myotubes protein extract. Healthy control myotubes protein extract (HC), FSHD1 myotubes protein extract (F1), and FSHD2 myotubes protein extract (F2) were separated by SDS-PAGE and transferred to nitrocellulose membranes. Blot strips were incubated with FSHD patient sera (sF) (A-E) and with healthy control sera (sHC) (E). On the left of each panel the positions of molecular weight markers are indicated.


A
- + - + - + - + - + - + - + - + - + - + - + - + - +
250
150
100
75
50
37
25
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B
- + - + - + - + - + - + - + - + - + - + - + - + - + - +
250
150
100
75
50
37
25
sF164 sF168 sF192 sF207 sF213 sF248 sF218 sF179 sF174 sF138 sF153 sF081 sF162 sF177
C
- + - + - + - + - + - + - + - + - + - + - + - + - + - + - +
250
150
100
75
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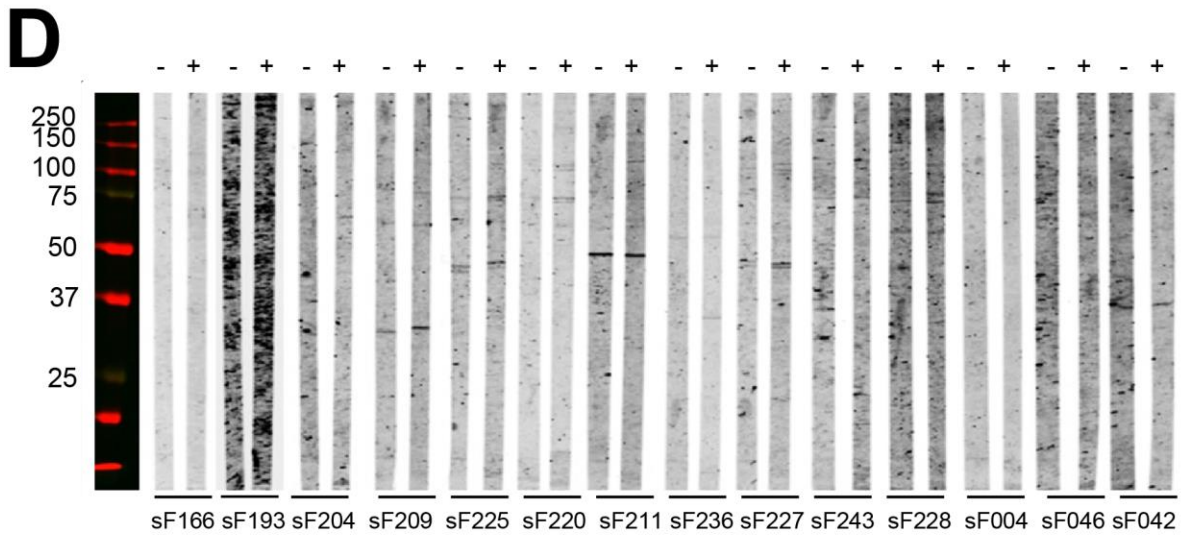
D
- + - + - + - + - + - + - + - + - + - + - + - + - + - +
250
150
100
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37
25
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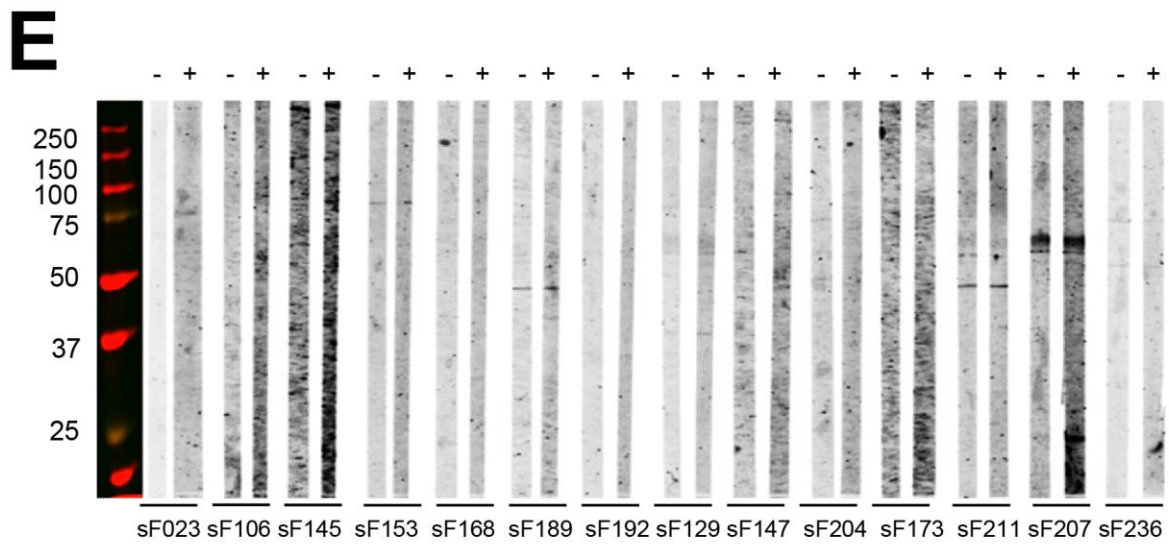
E
- + - + - + - + - + - + - + - + - + - + - + - + - + - +
250
150
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50
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25
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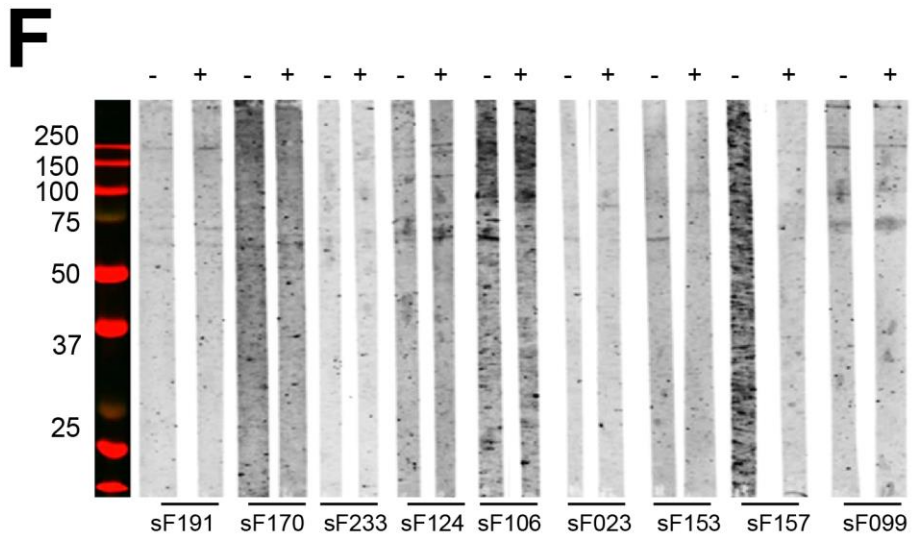
F
- + - + - + - + - + - + - + - + - + - +
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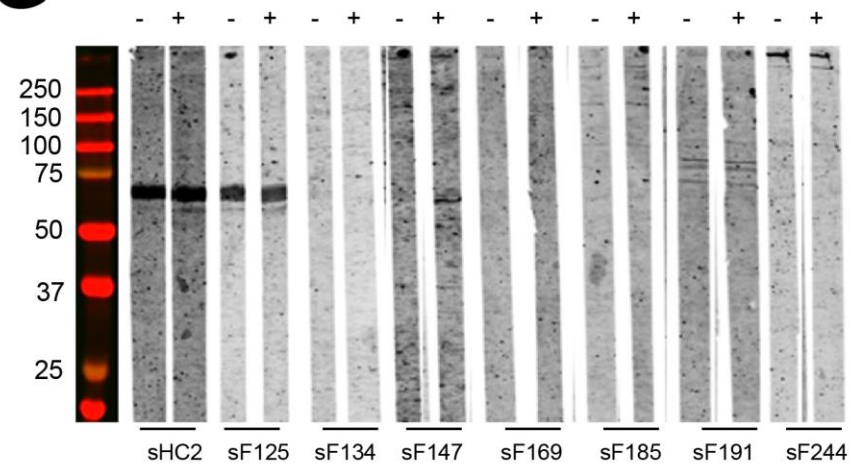
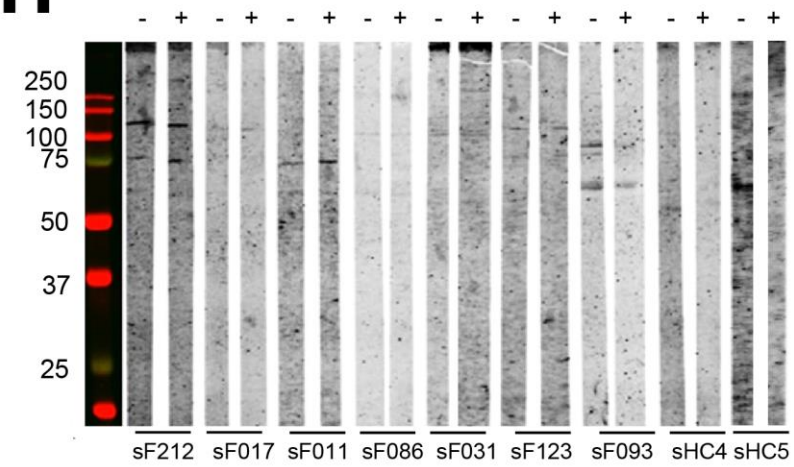
150
100
75
50
37
25
sF191 sF170 sF233 sF124 sF106 sF023 sF153 sF157 sF099

G


- + - + - + - + - + - + - + - +
250
150
100
75
50
37
25
sHC2 sF125 sF134 sF147 sF169 sF185 sF191 sF244

H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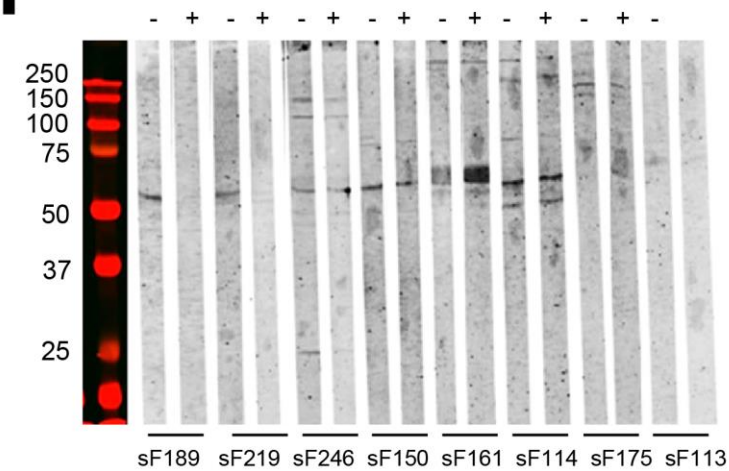
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50
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25
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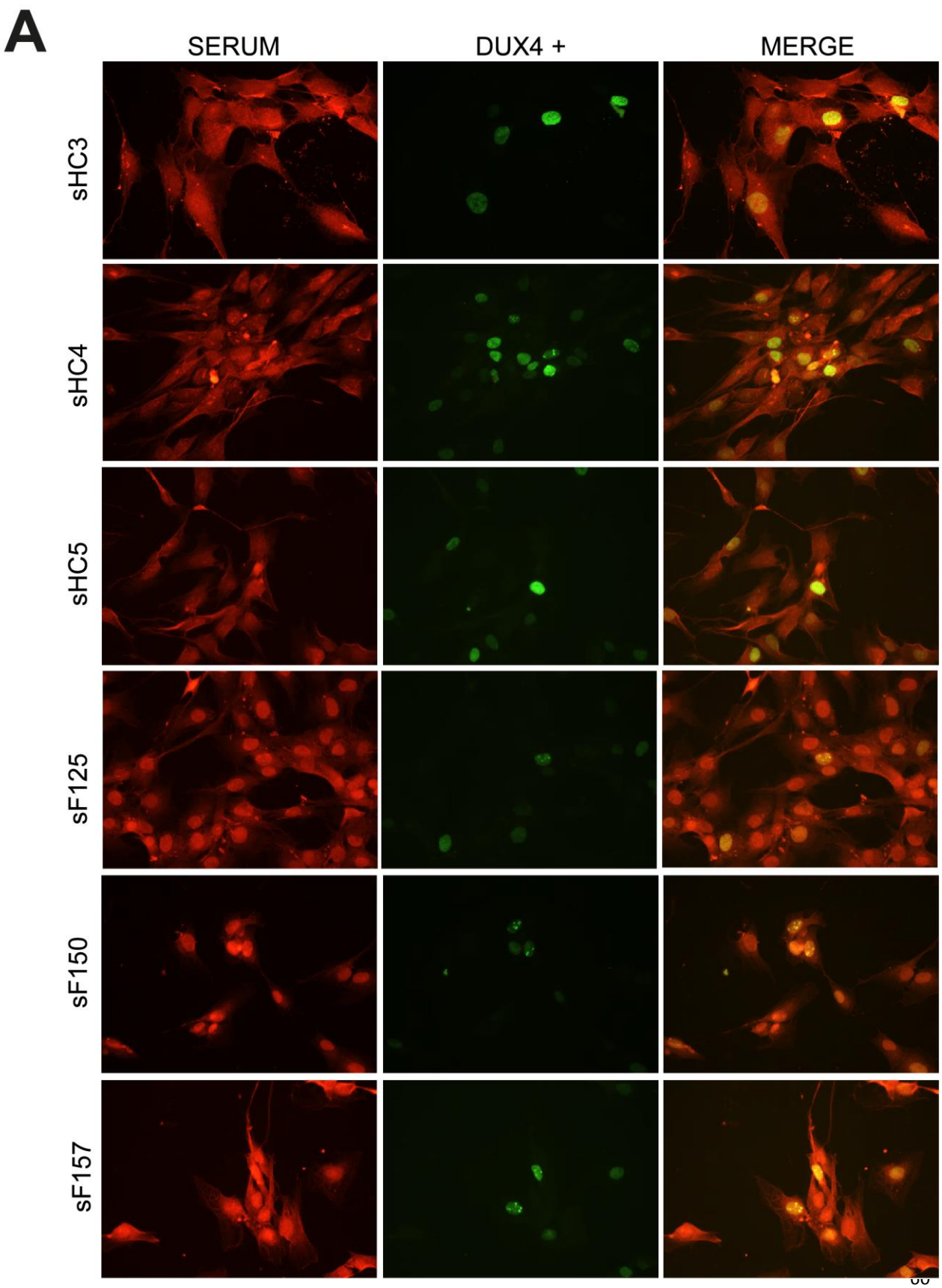
Supplementary Figure 4. Reactivity of sera with DUX4-inducible myoblasts protein extract. DUX4-expressing myoblast protein extract (+) and control myoblast protein extract (-) were separated by SDS-PAGE and transferred to nitrocellulose membranes. Blot strips were incubated with FSHD patient sera (sF) (A-I). On the left the positions of molecular weight markers are indicated.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DUX4 +
MERGE
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sHC4
sHC5
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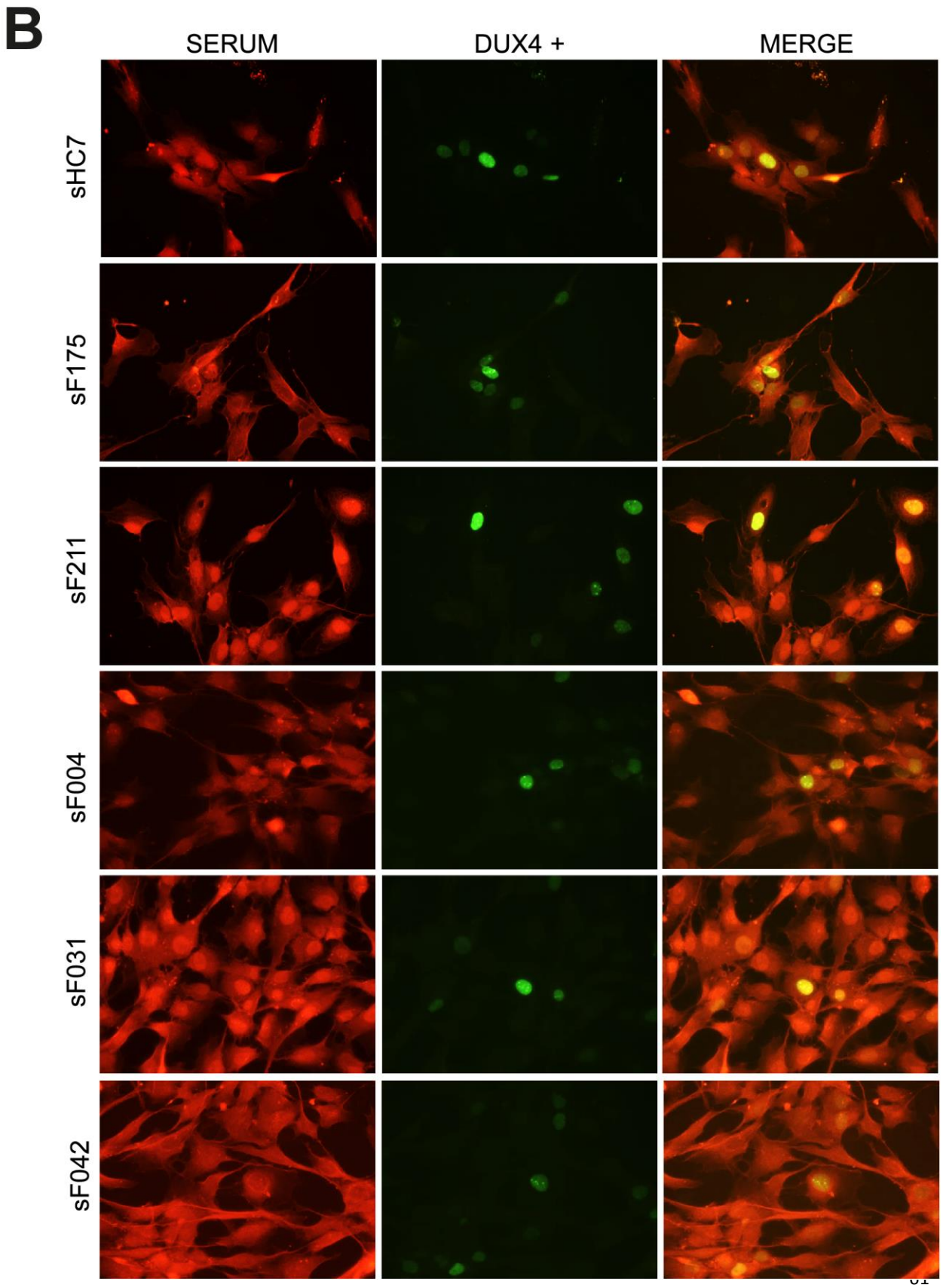
sF150
sF157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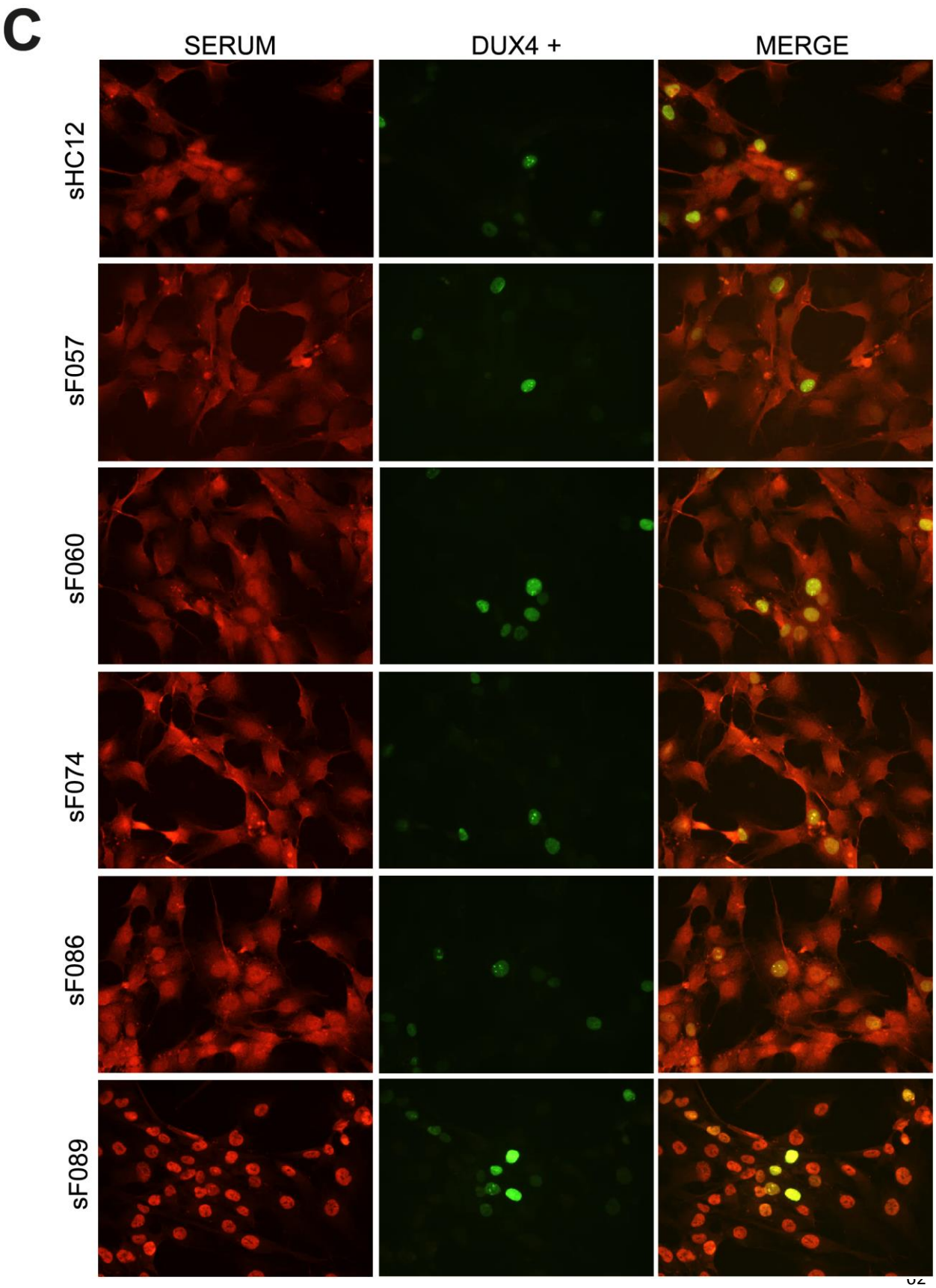
DUX4 +
MERGE
sHC7
sF175
sF211
sF004
sF031
sF042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DUX4 +
MERGE
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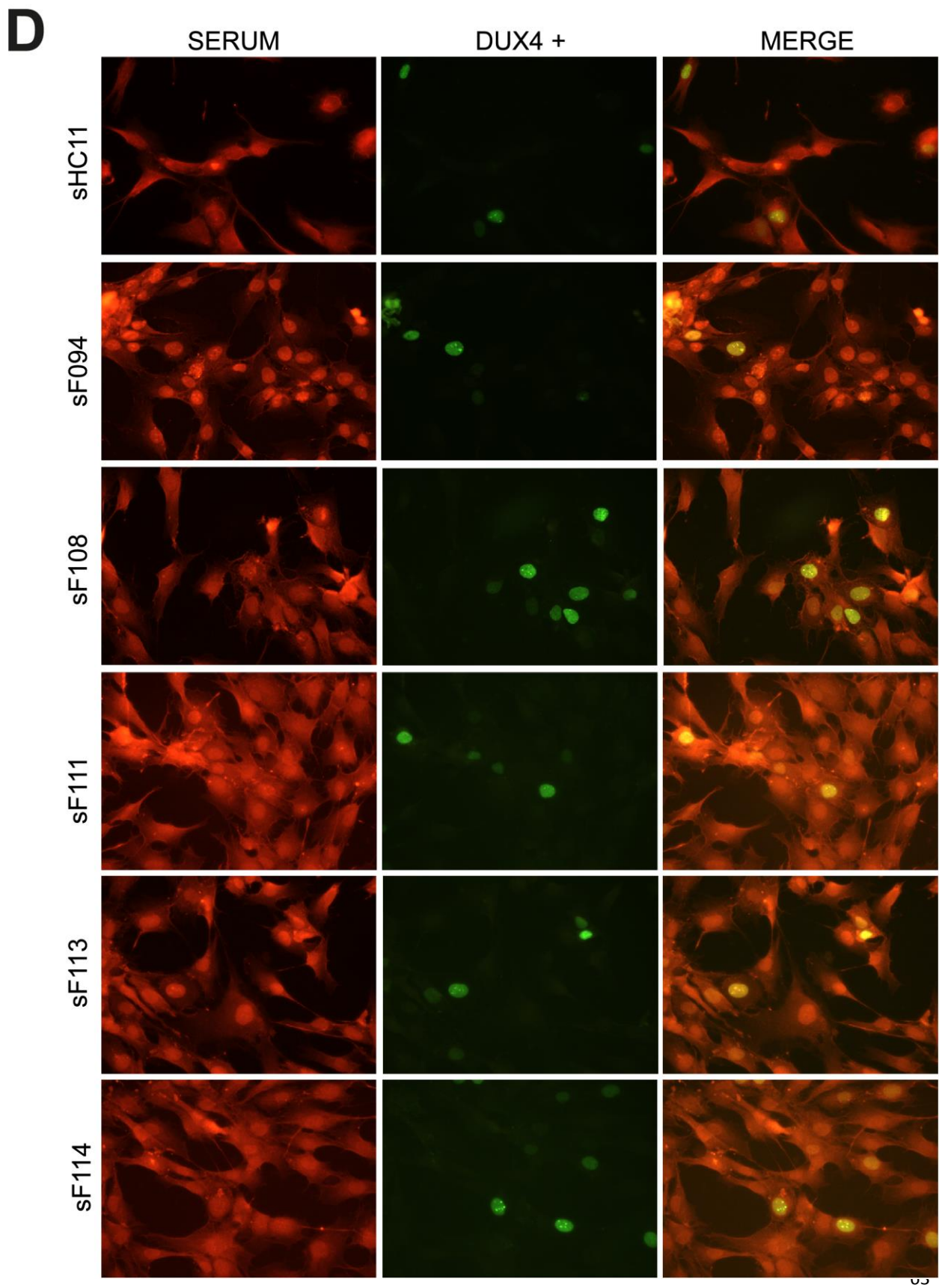
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DUX4 +
MERGE
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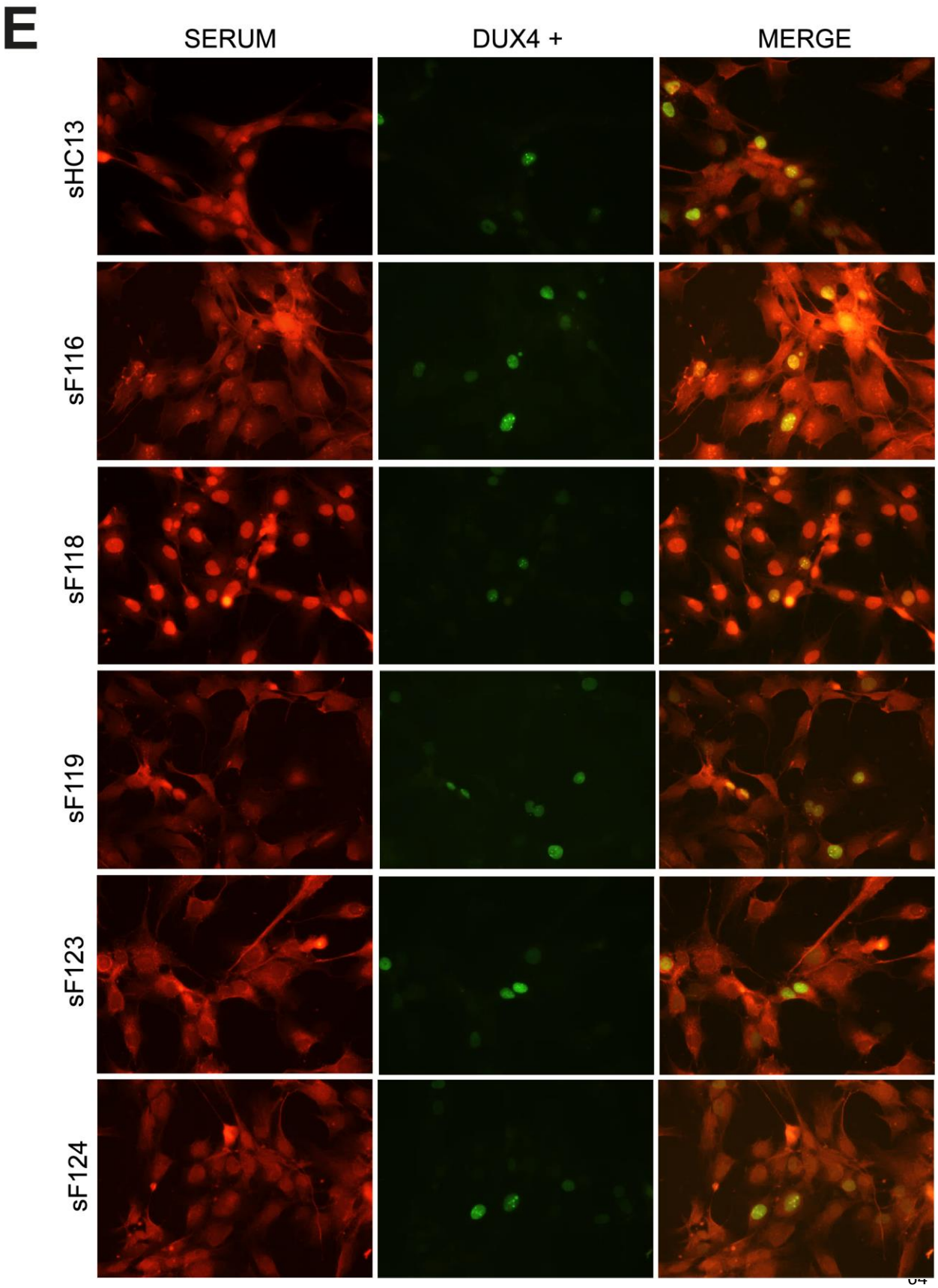
sF111
sF113
sF114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DUX4 +
MERGE
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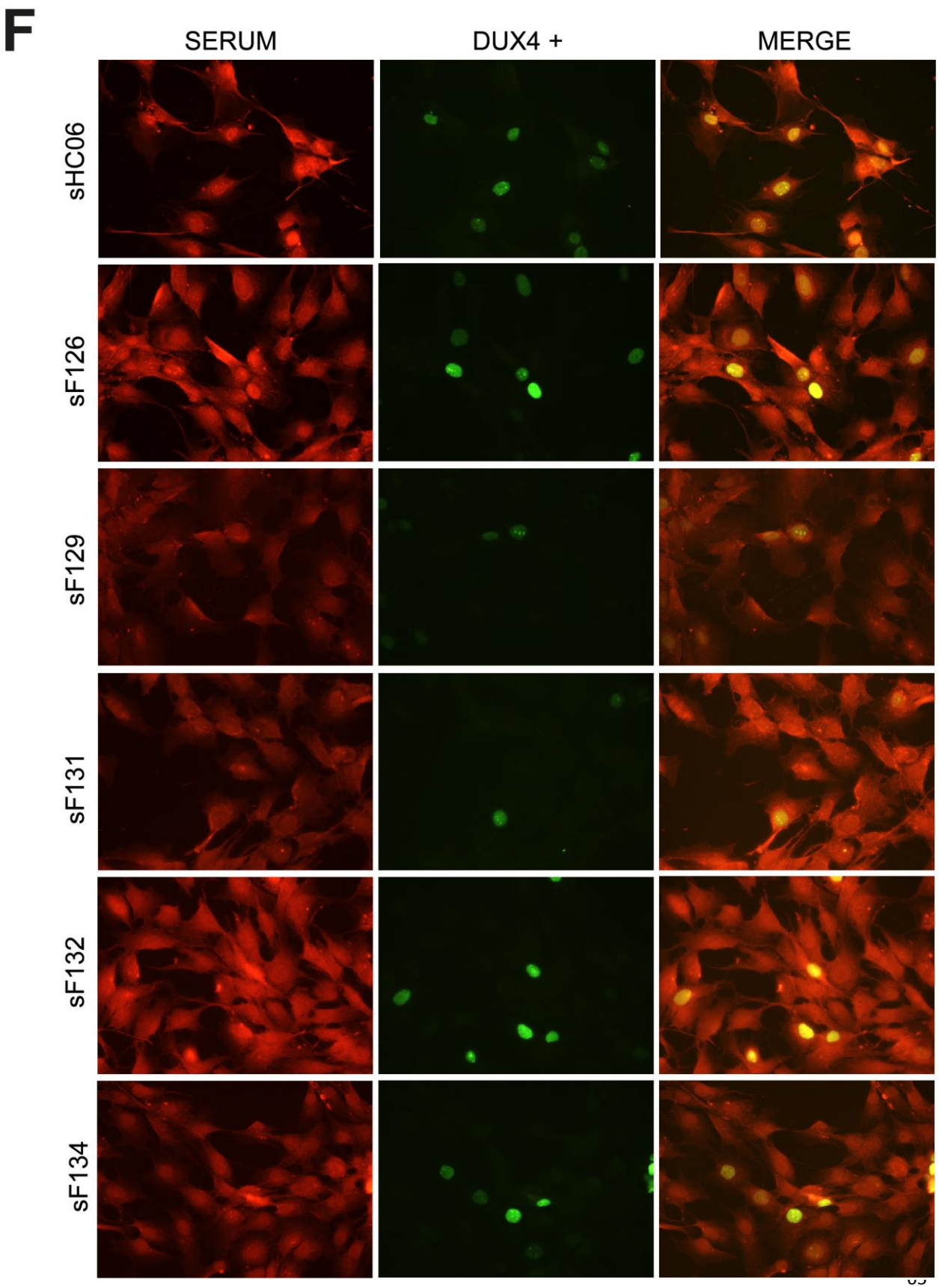
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DUX4 +
MERGE
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sF131
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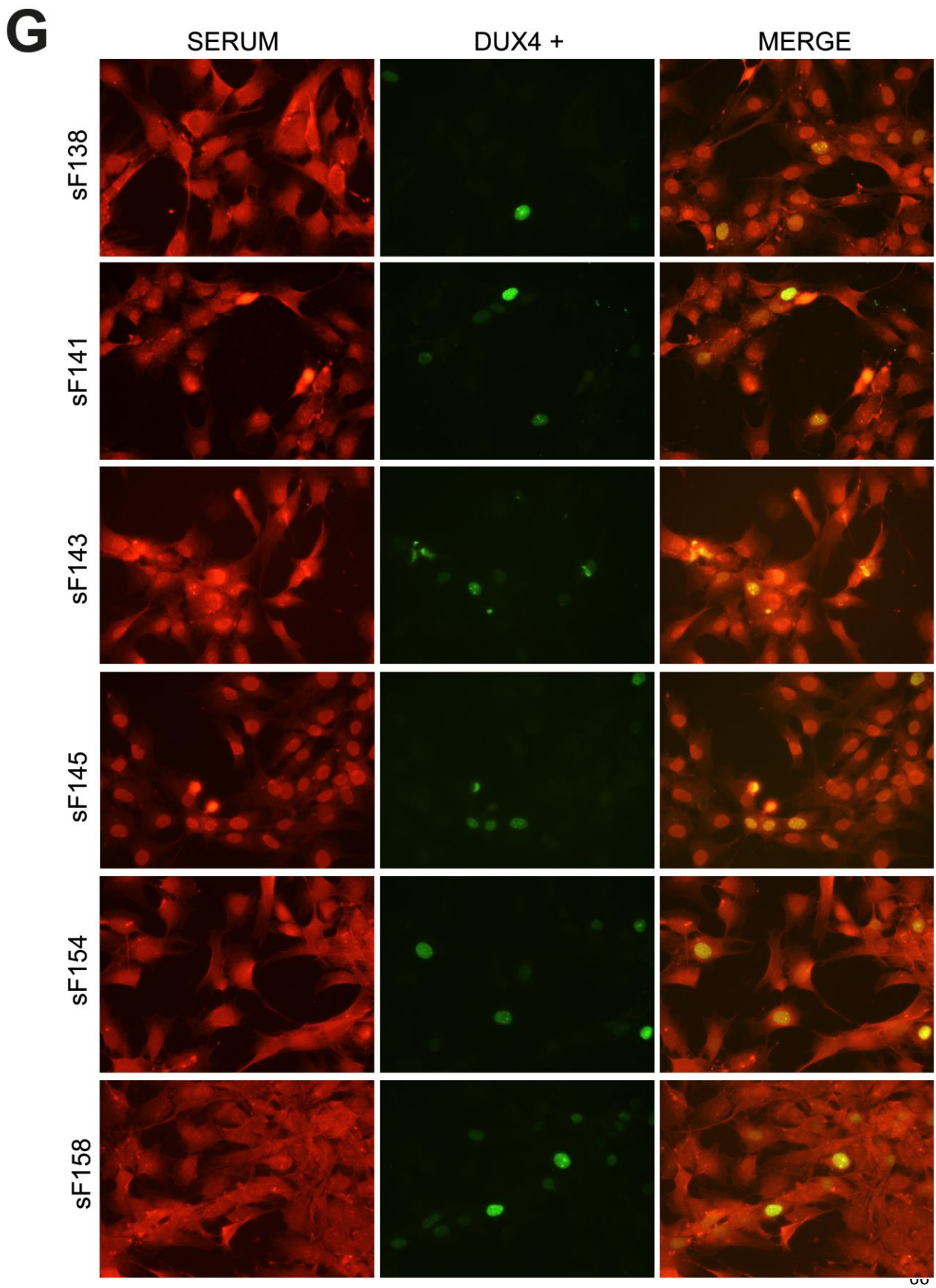
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SERUM
DUX4 +
MERGE
sF138
sF141
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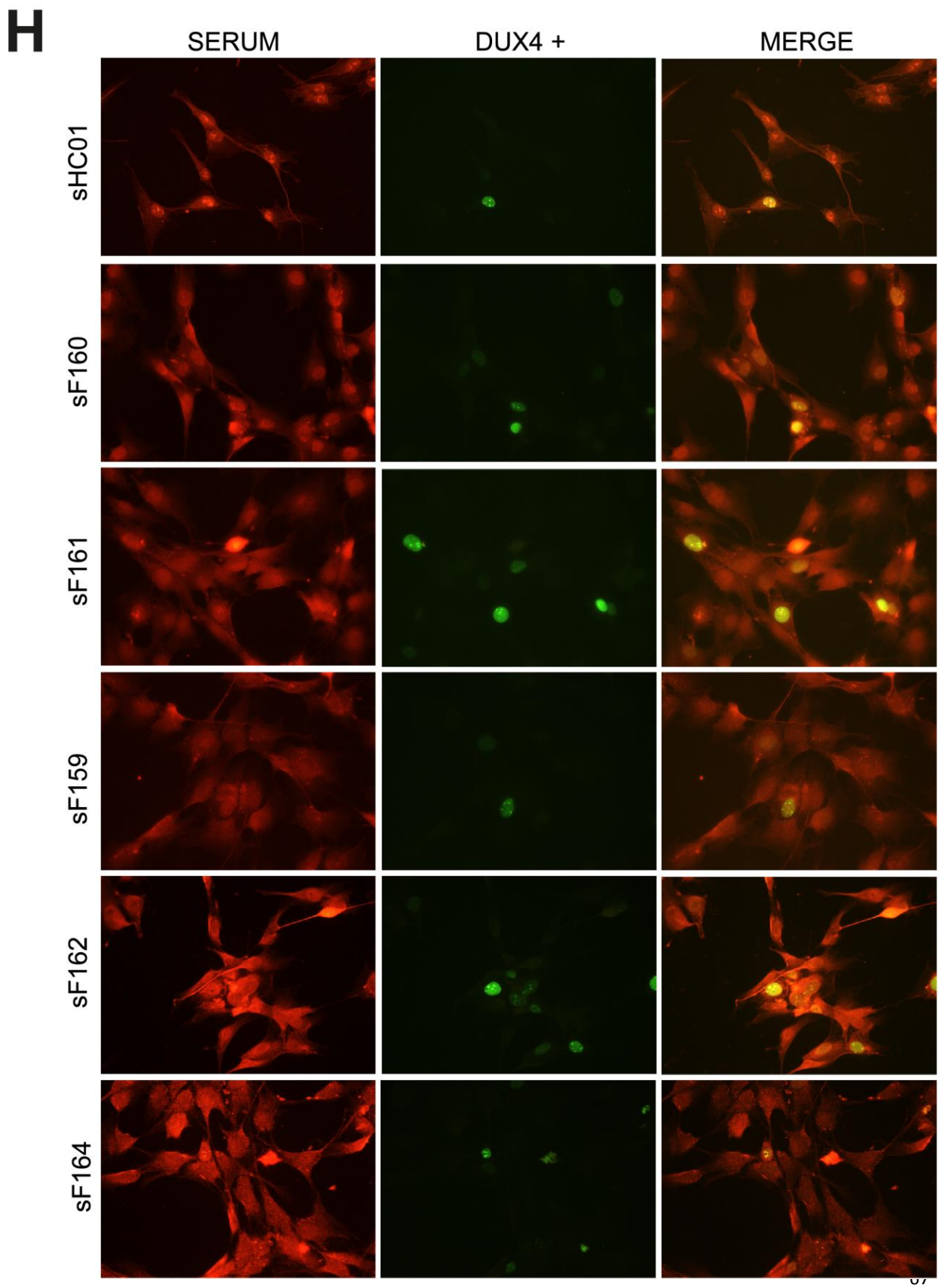
sF154
sF158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DUX4 +
MERGE
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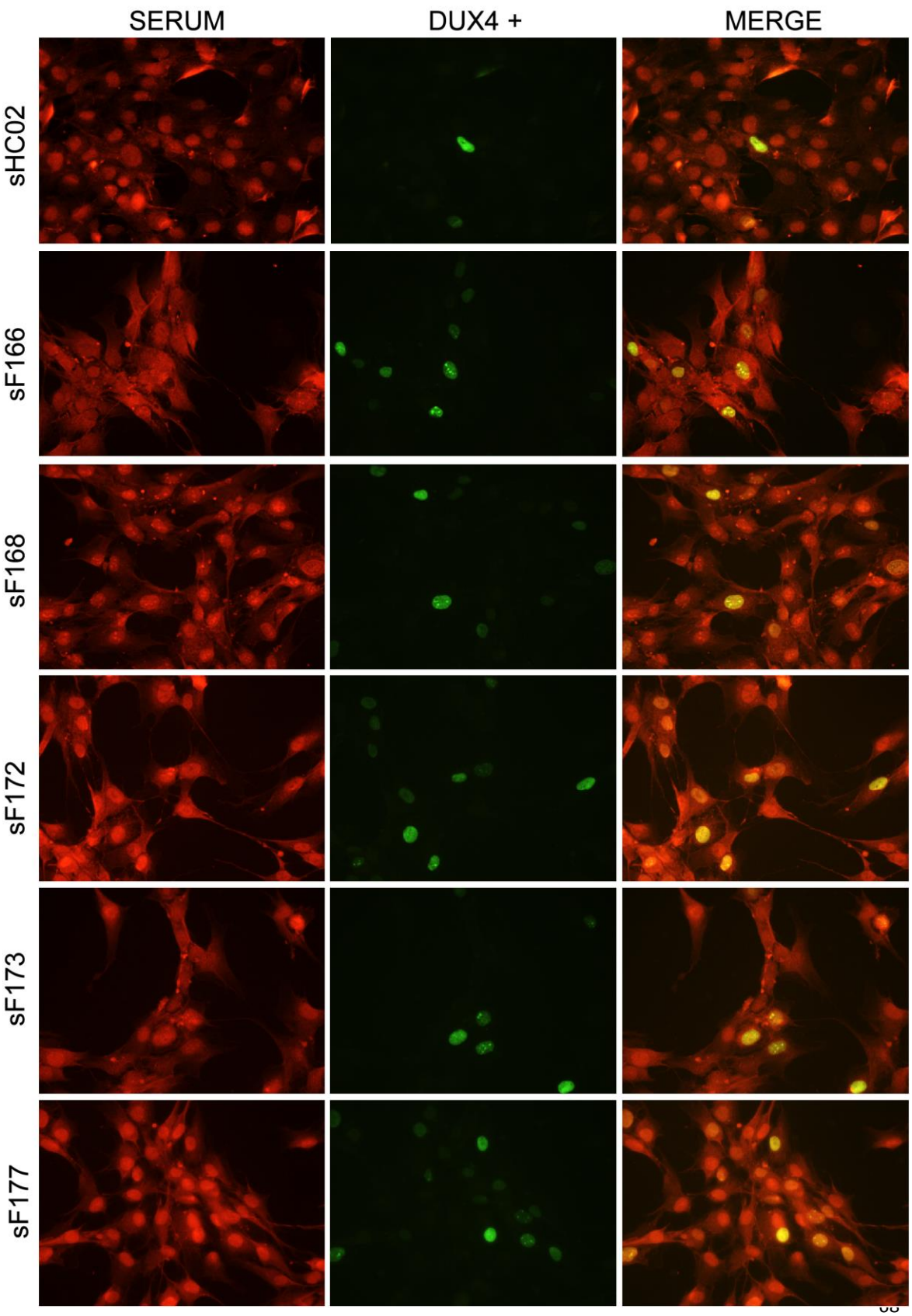
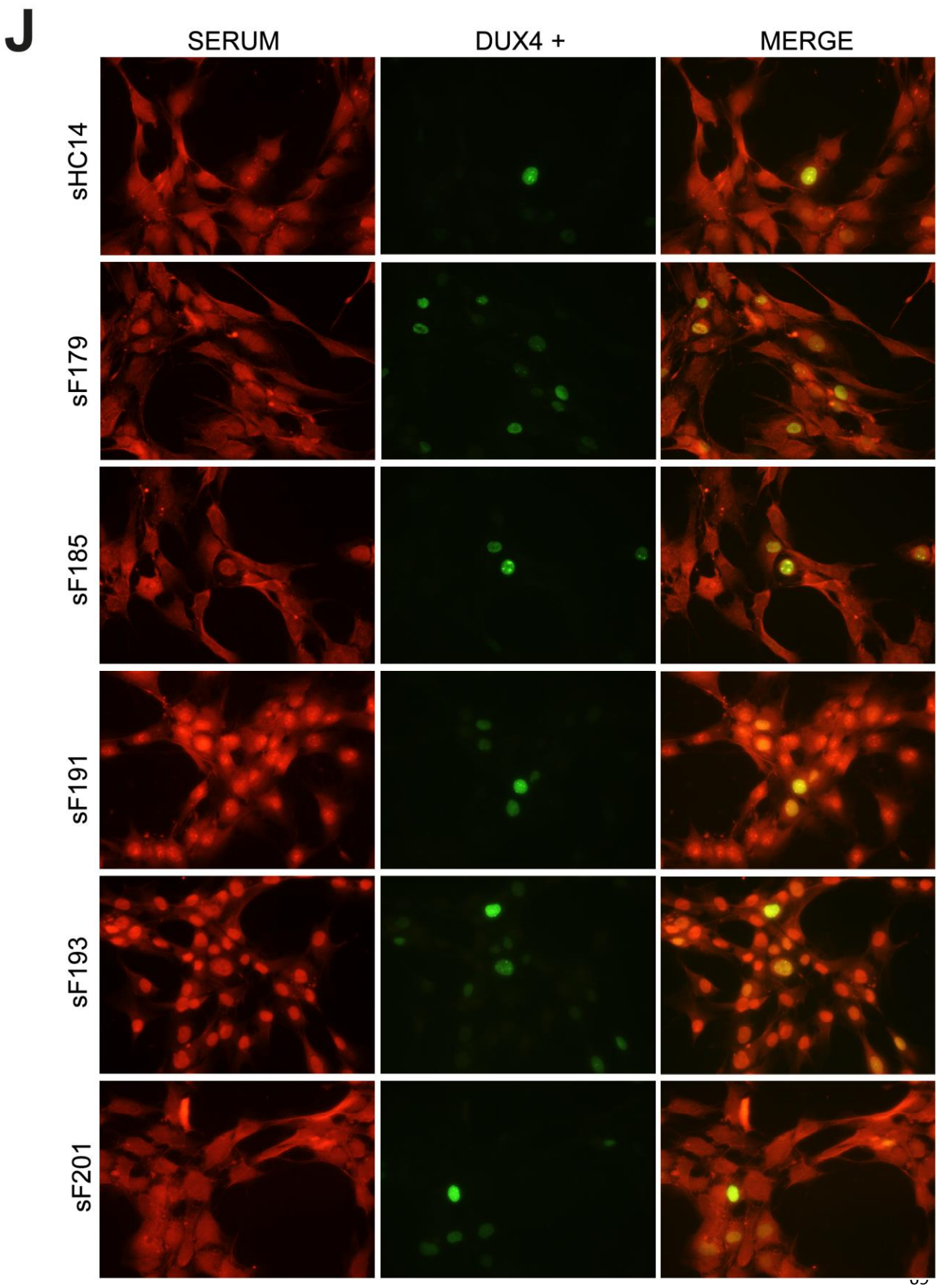
sF159
sF162
sF164

I

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DUX4 +
MERGE
sHC14
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sF185
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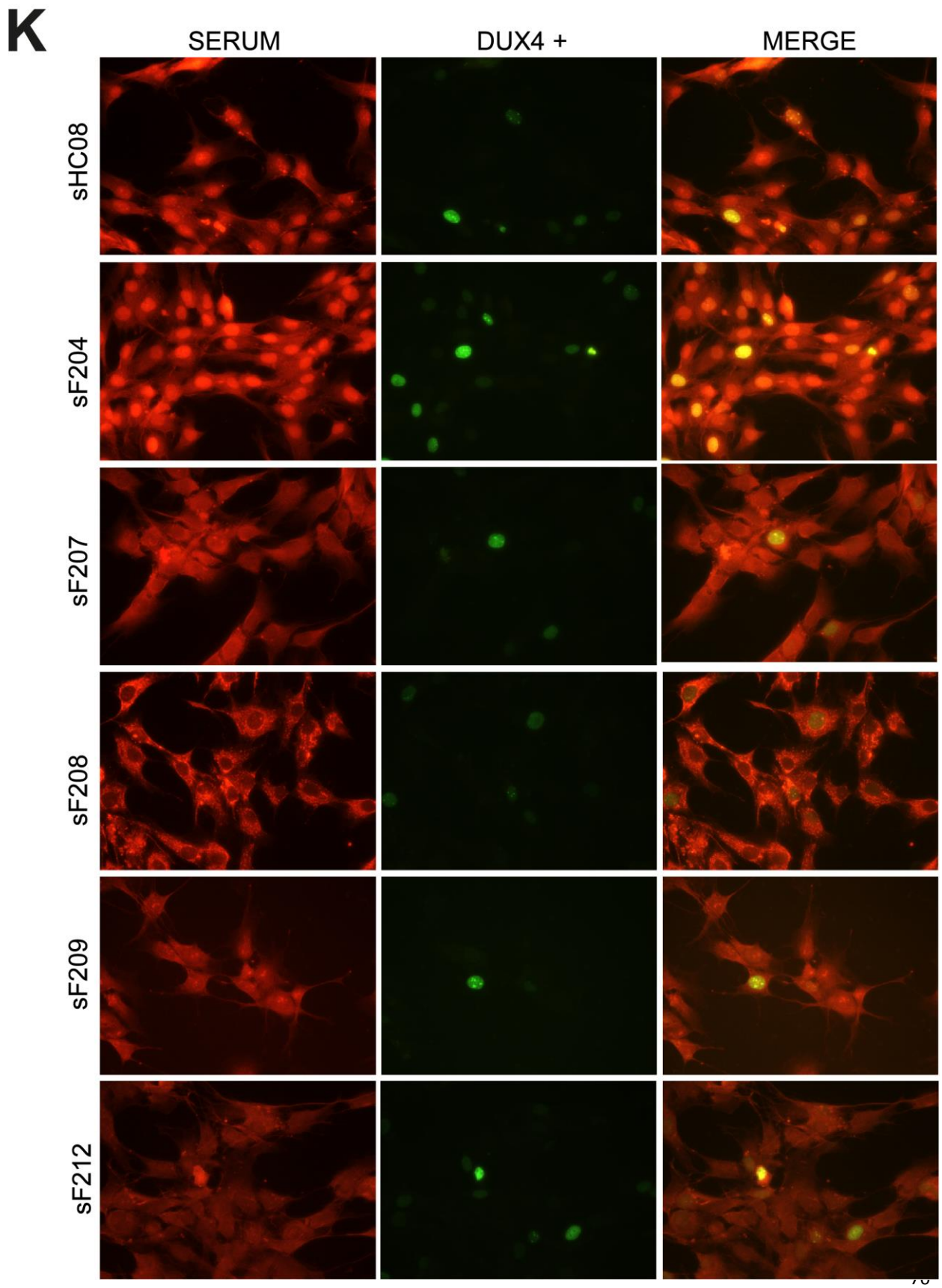
sF193
sF201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MERGE
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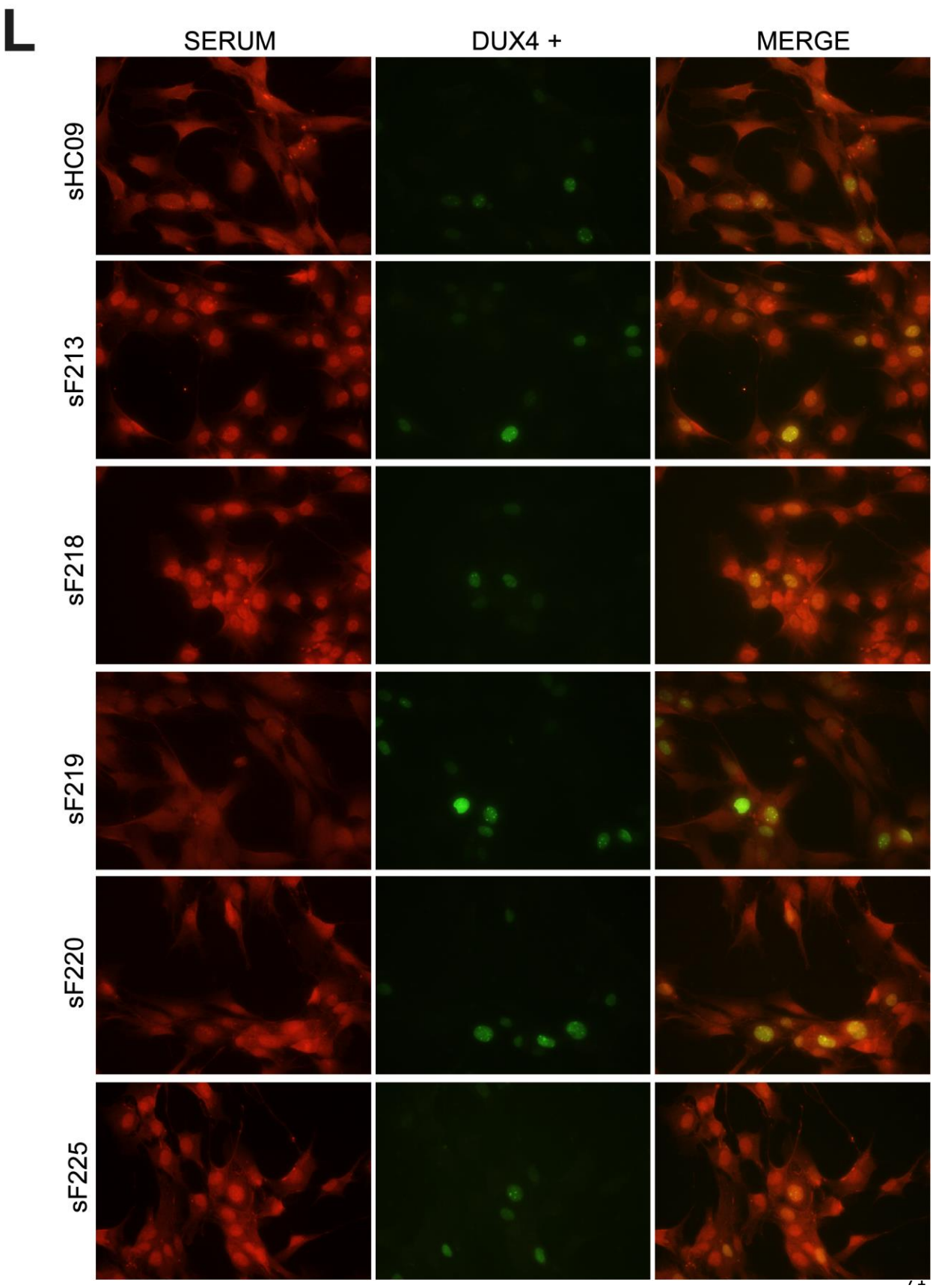
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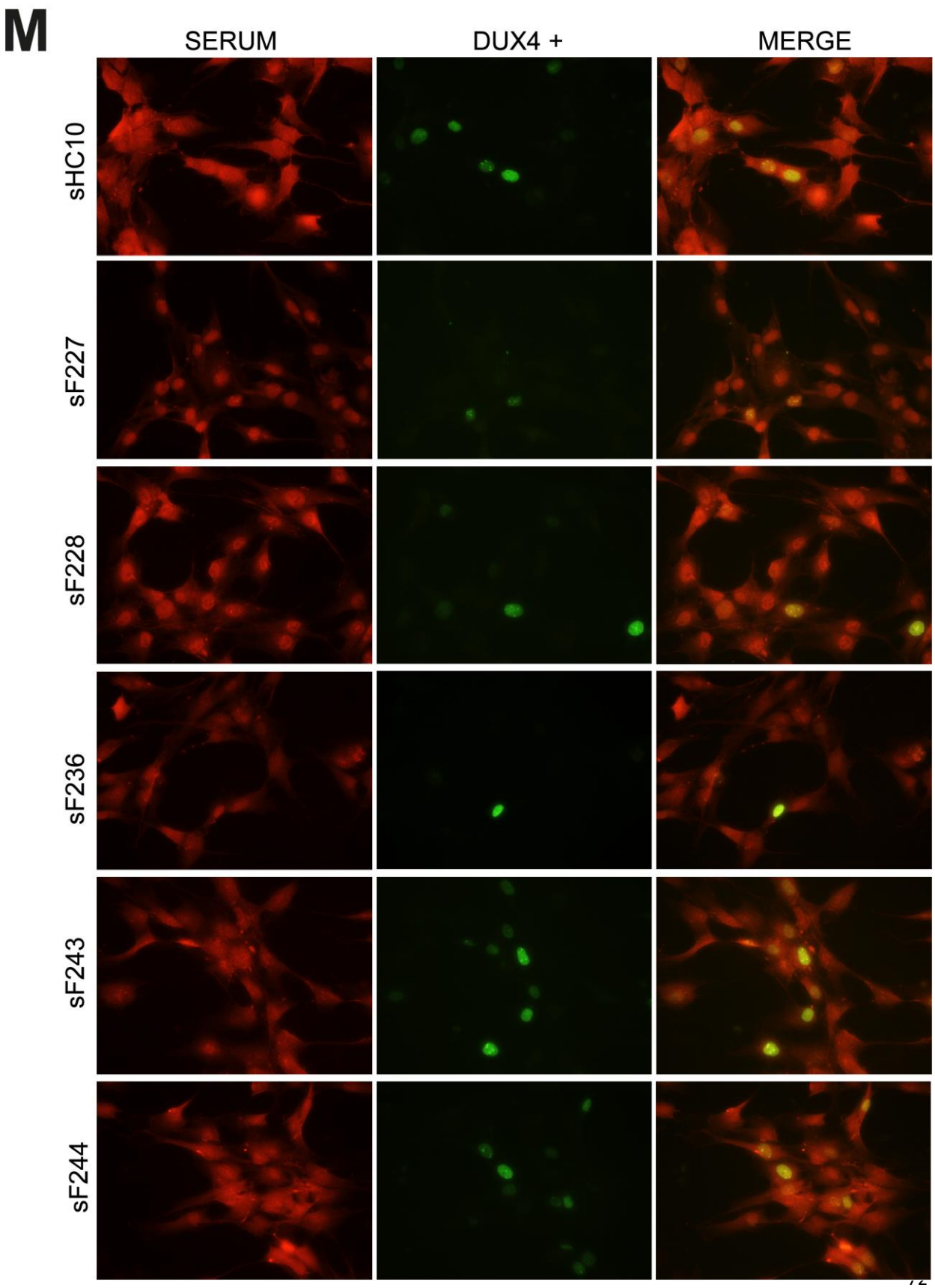
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MERGE
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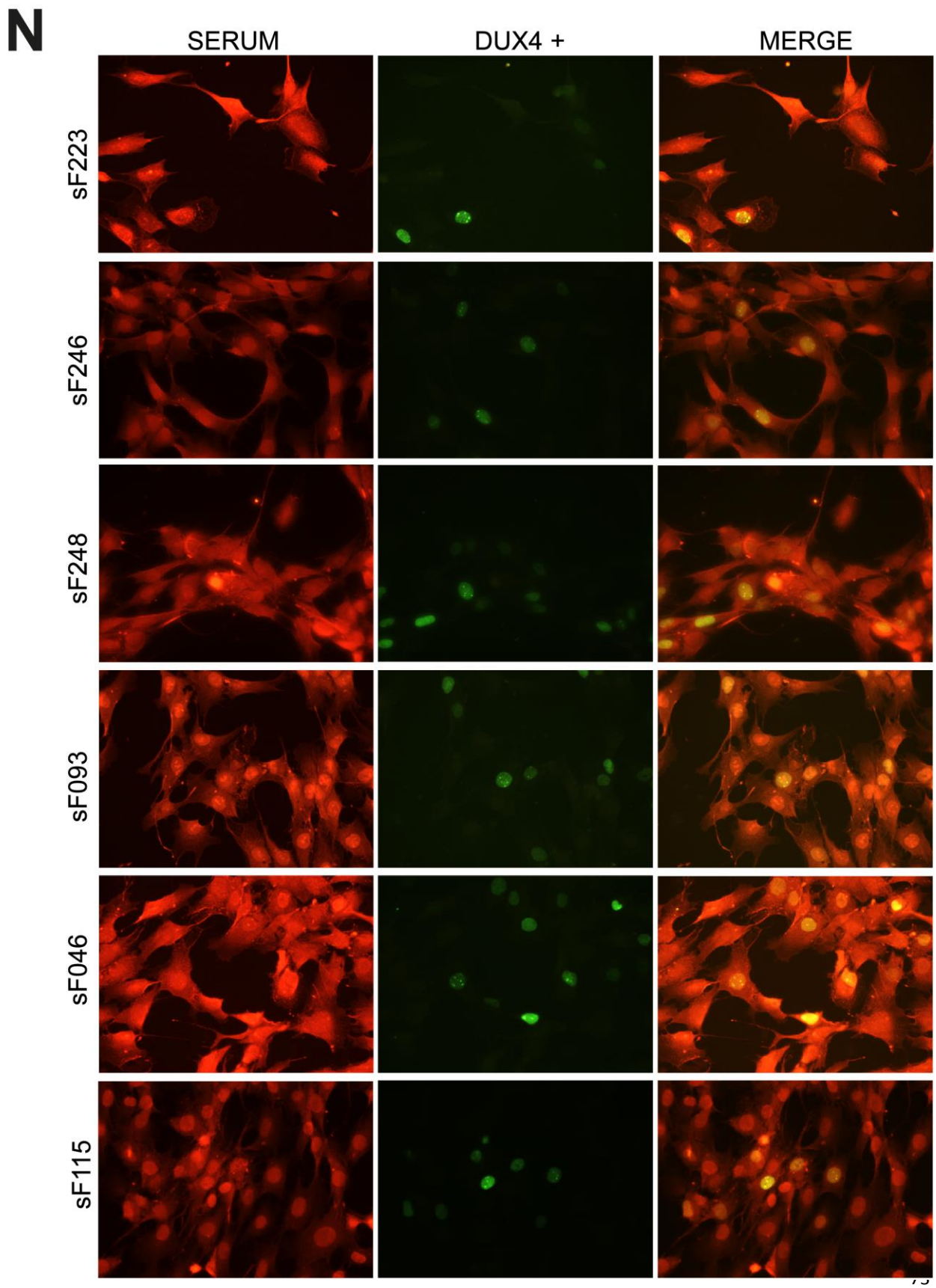
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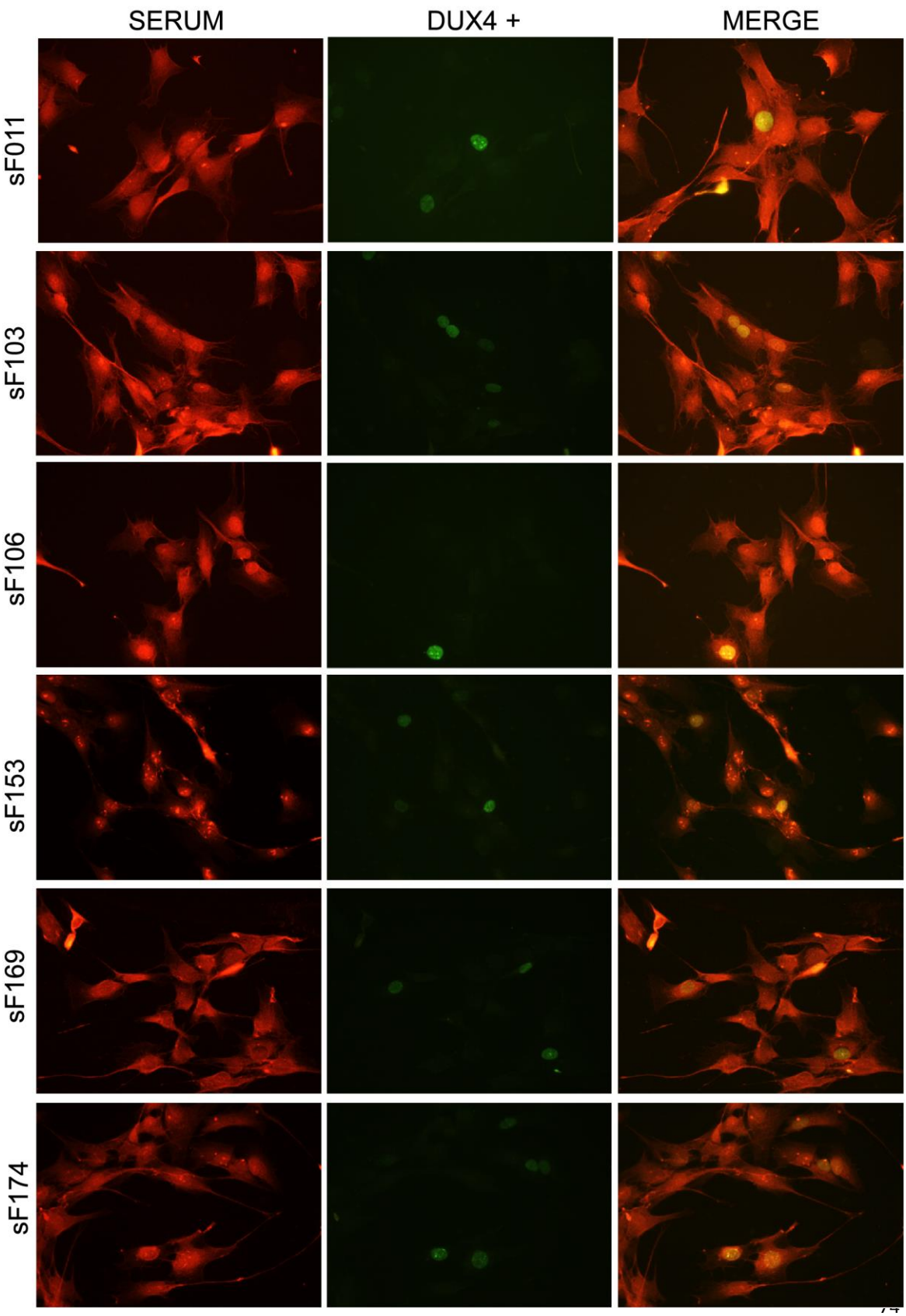
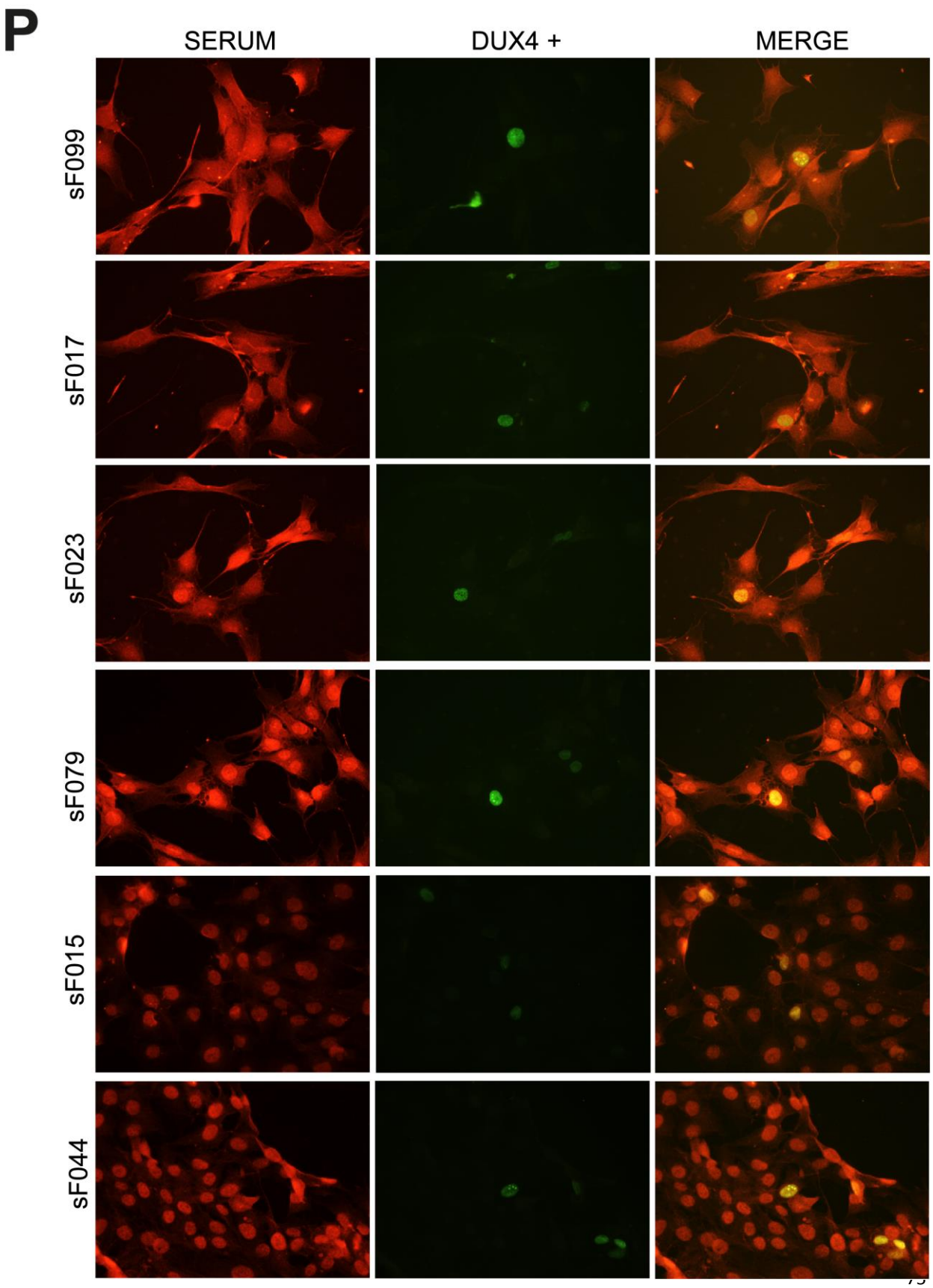
O




P
SERUM
DUX4 +
MERGE
sF099
sF017
sF023
sF079
sF015
sF044

Supplementary Figure 5. Antibody reactivity in FSHD patient sera assessed by indirect immunofluorescence. DUX4-expressing myoblasts were fixed and incubated with either patient sera (A-P, left panels) or control sera (A-M, left panels) and with an anti-DUX4 antibody (middle panels). Bound antibodies were visualized by fluorescent secondary antibodies. Merged images are shown in the right panels. sF= FSHD serum. sHC= healthy control serum. Scale bar: 20 µm.