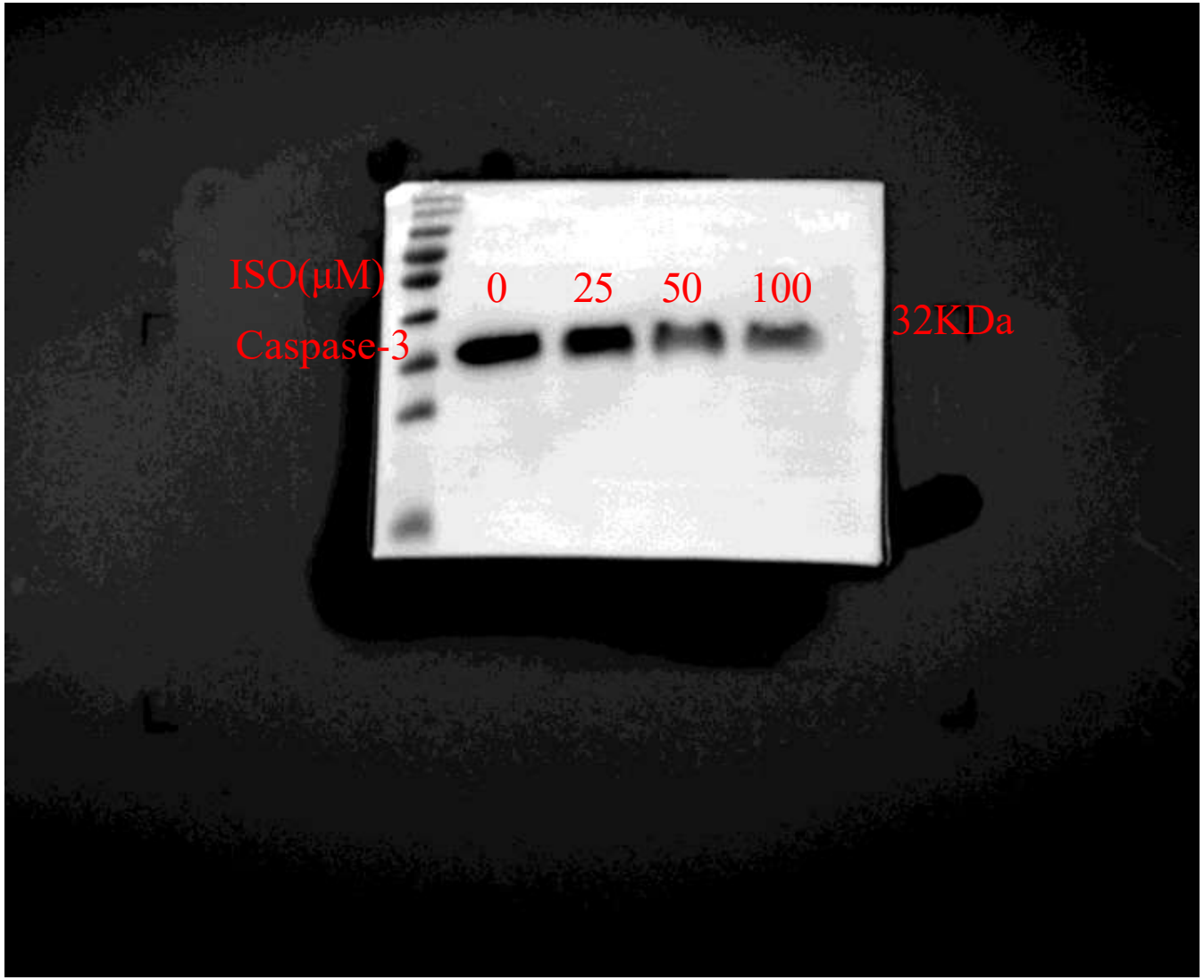
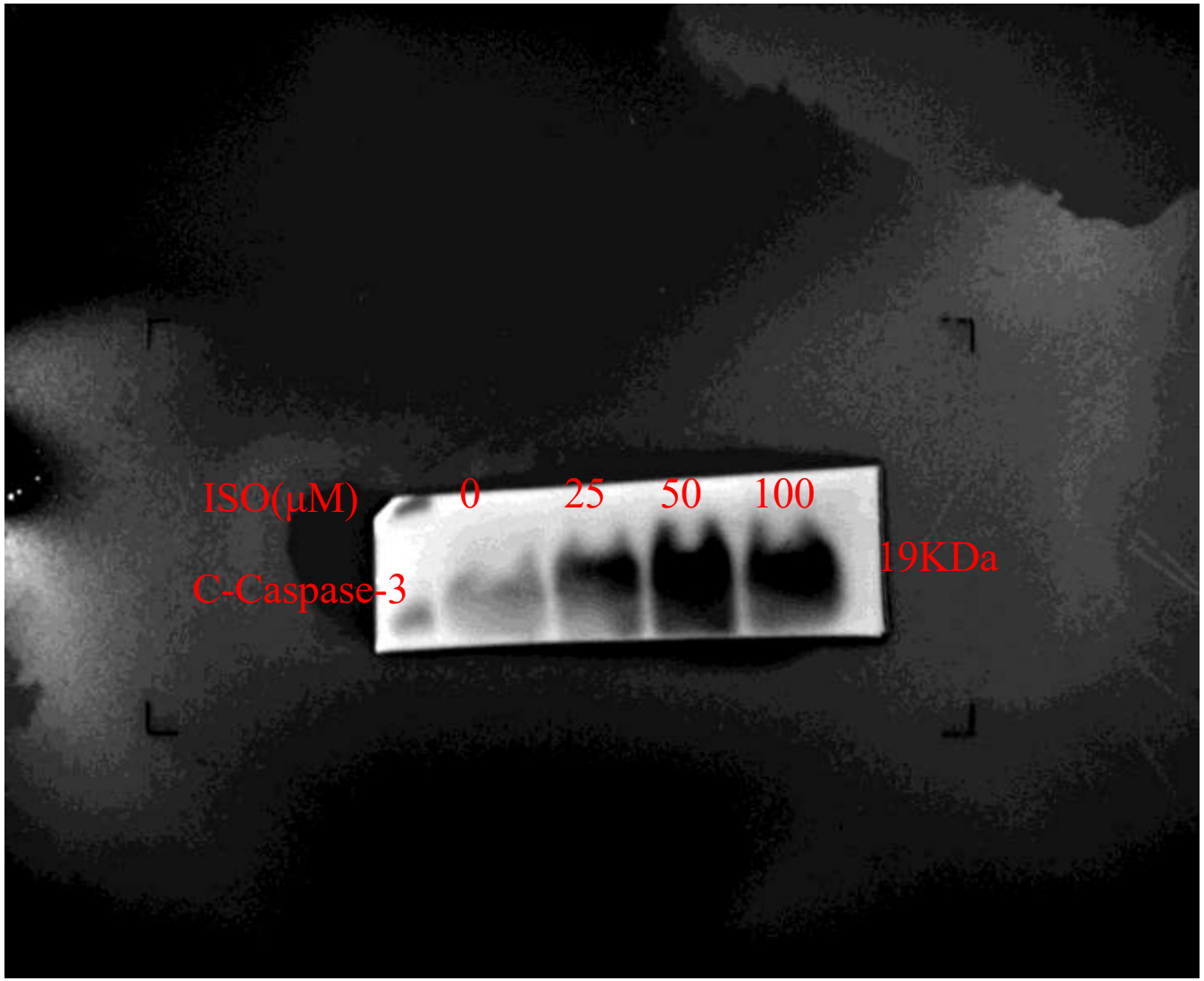
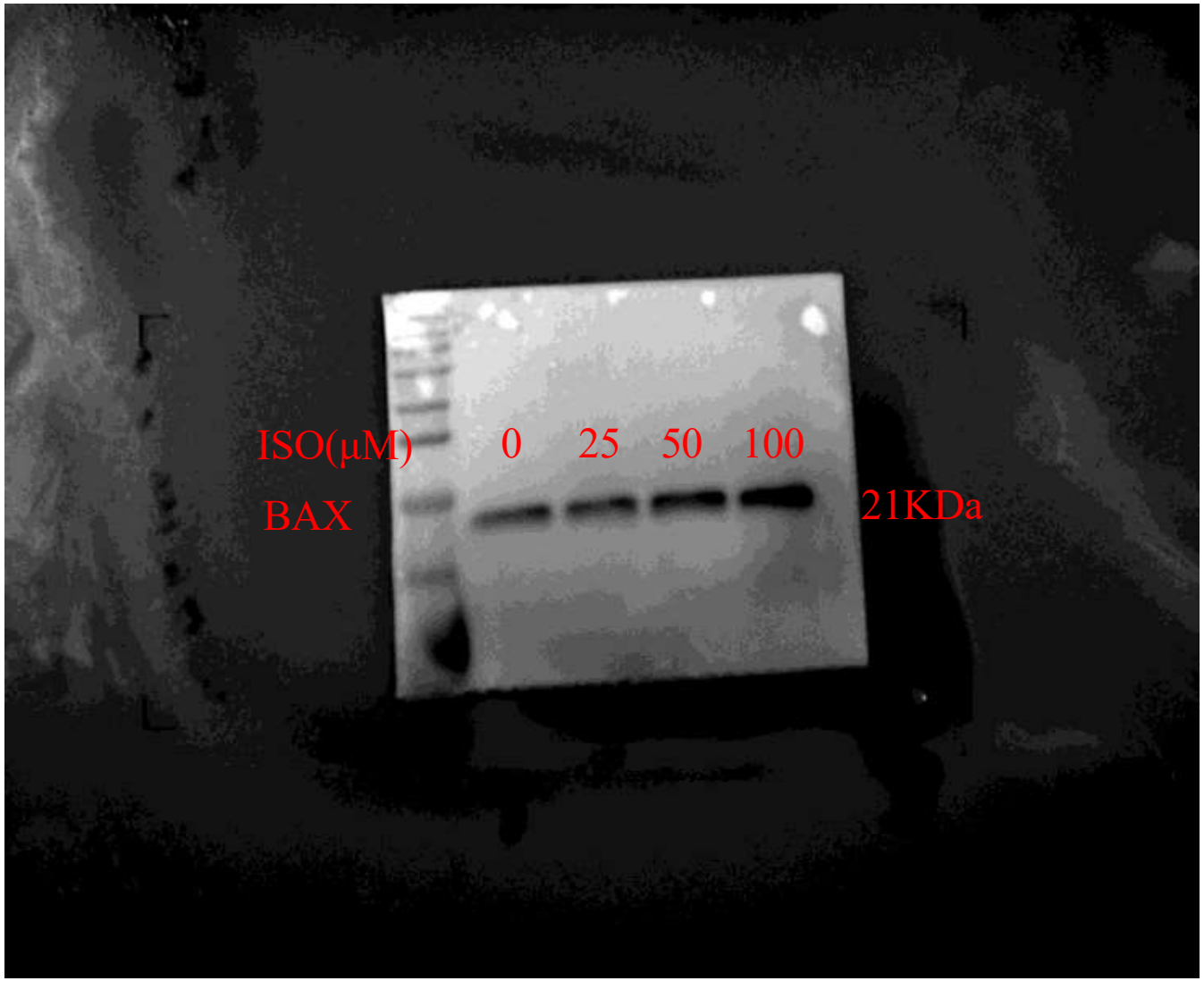
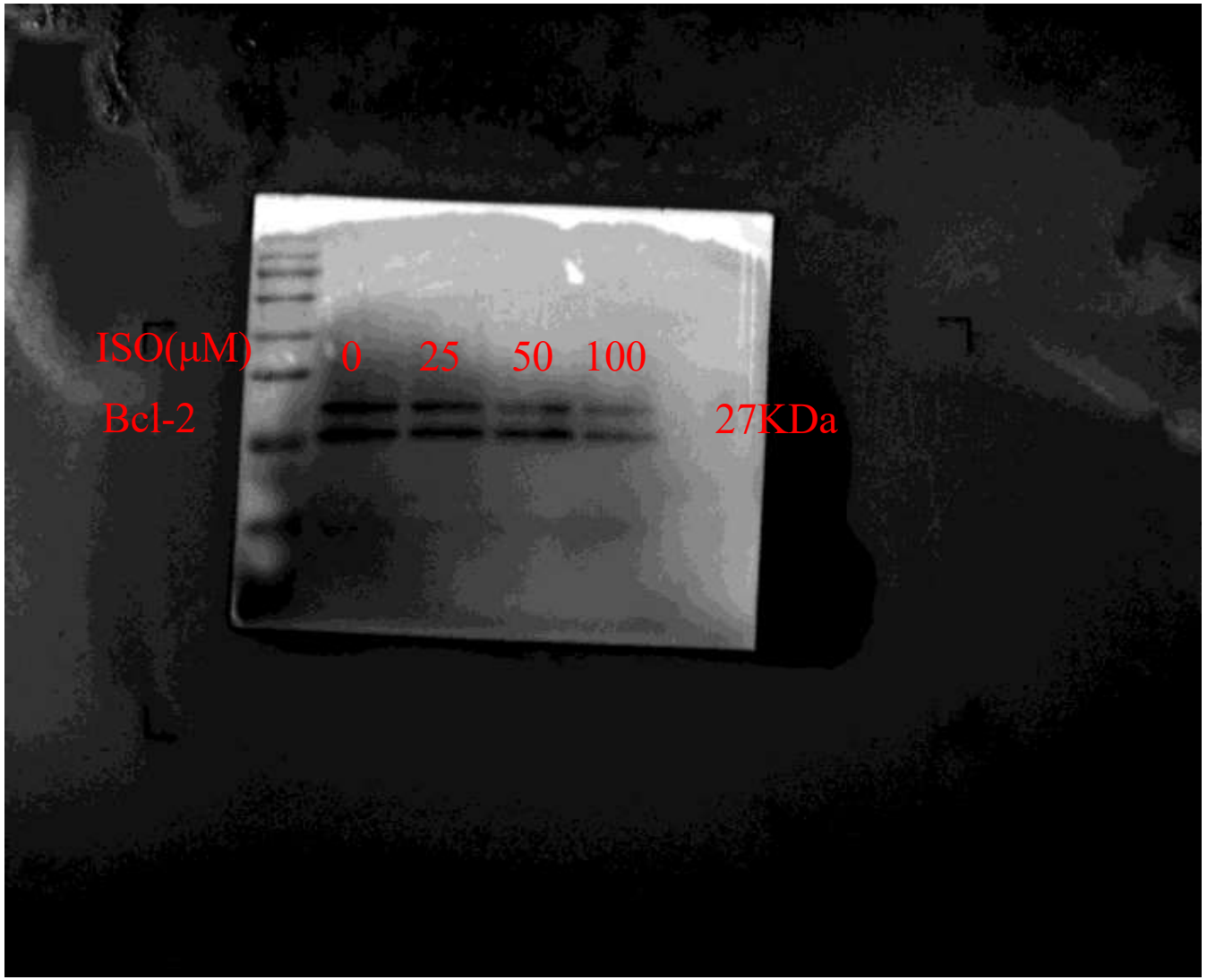
Figure 2. ISO promoted HSFBs apoptosis and induced cell cycle arrest

B. The expressions of apoptotic proteins in HSFBs and quantification of the apoptotic protein levels normalized to GAPDH.




ISO(μM)
0
25
50
100
C-Caspase-3
19KDa


ISO(μM)
0
25
50
100
BAX
21KDa


ISO(μM)
0
25
50
100
Bcl-2
27KDa

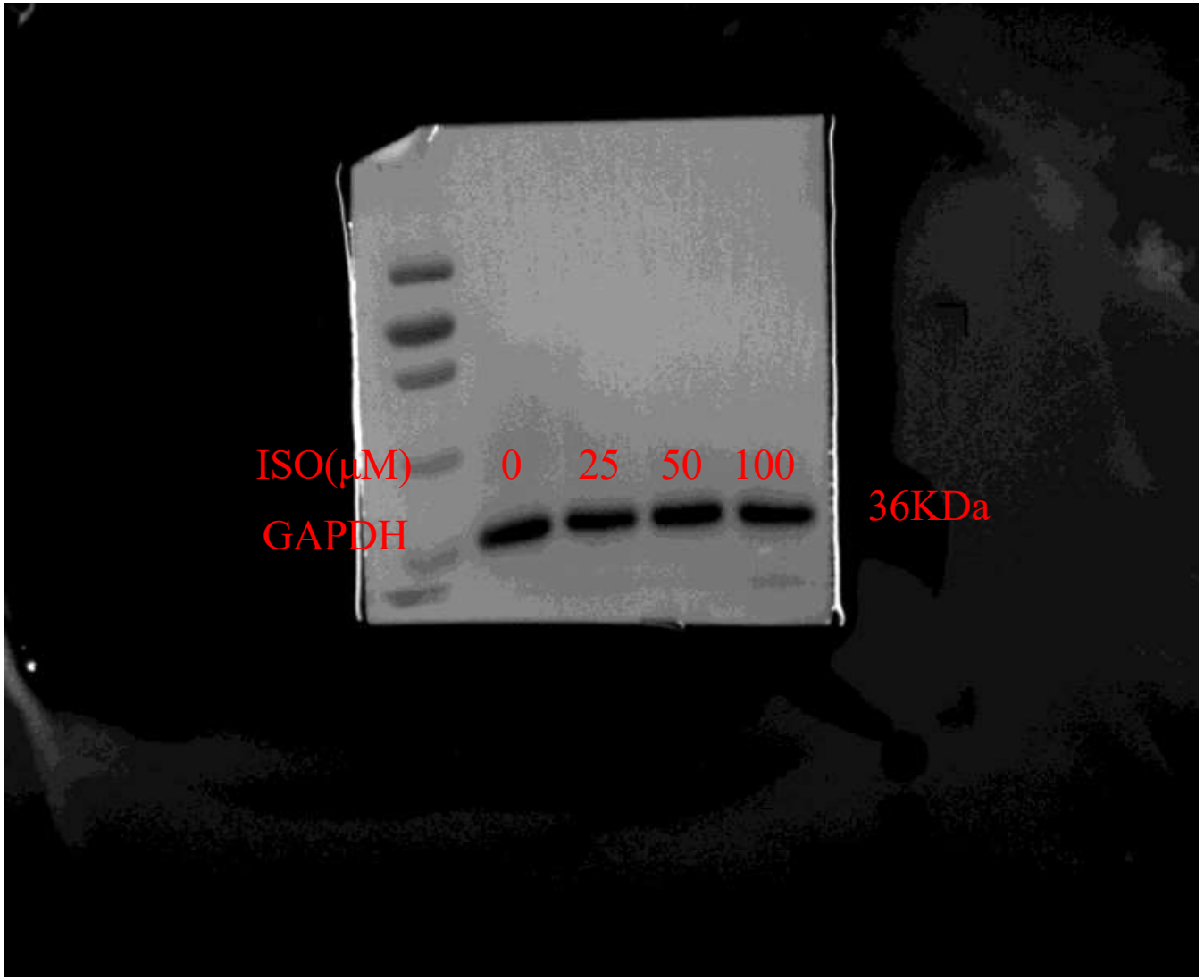
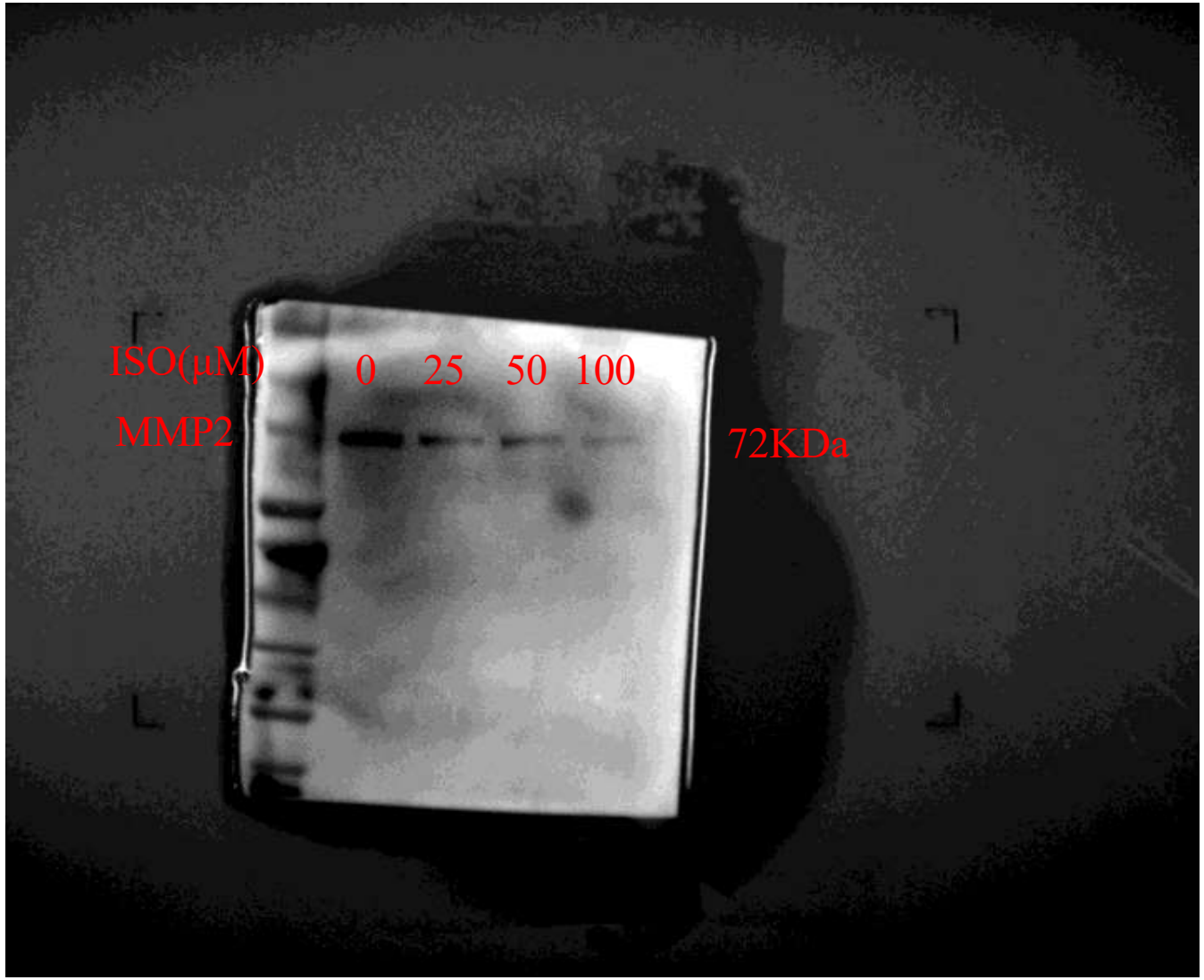
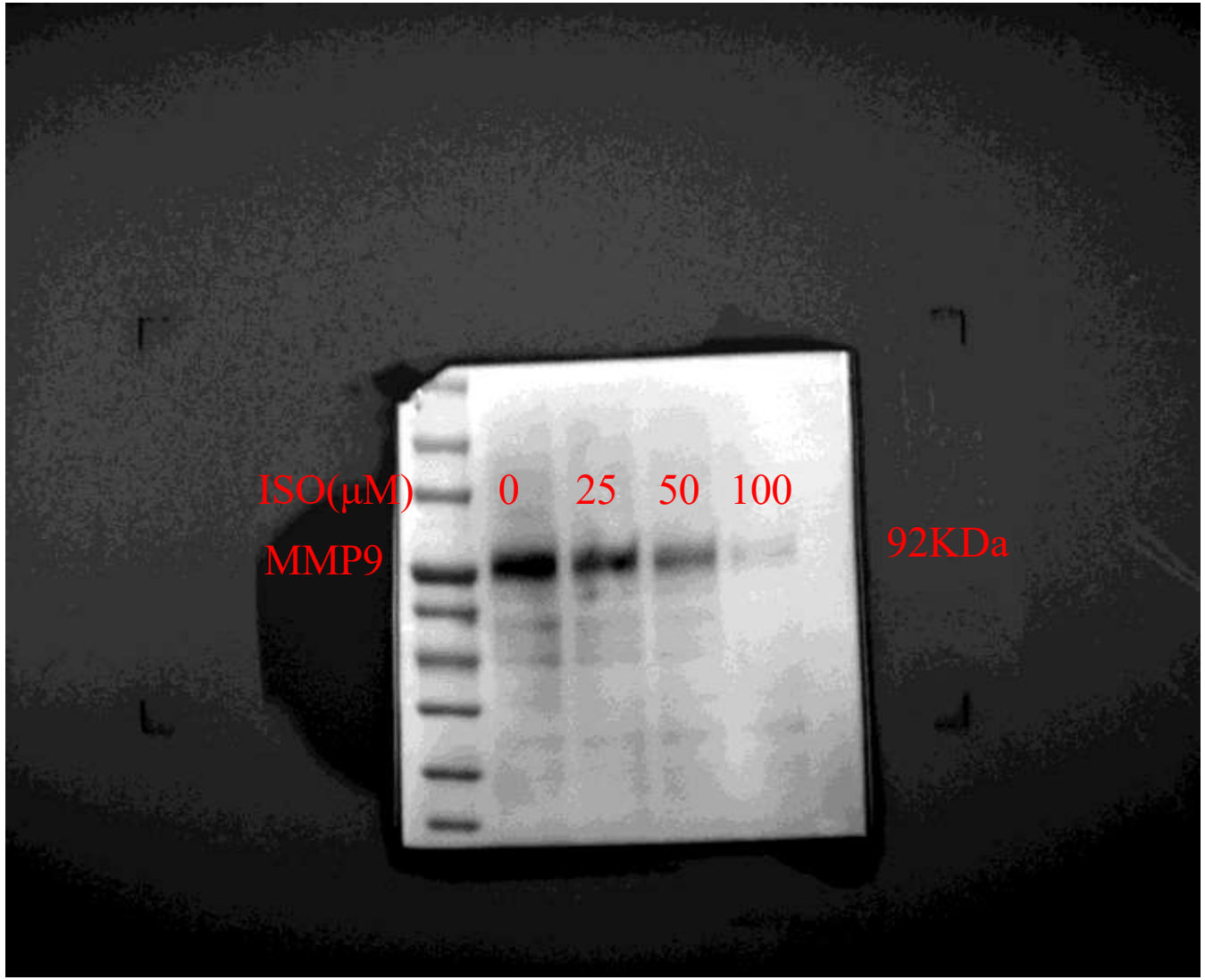

ISO(μM)
0
25
50
100
GAPDH
36KDa

Figure 3. ISO suppressed HSFBs migration.

E. Western blotting and quantification of MMP2 and MMP9 normalized to GAPDH.




ISO(μM)
0
25
50
100
MMP9
92KDa

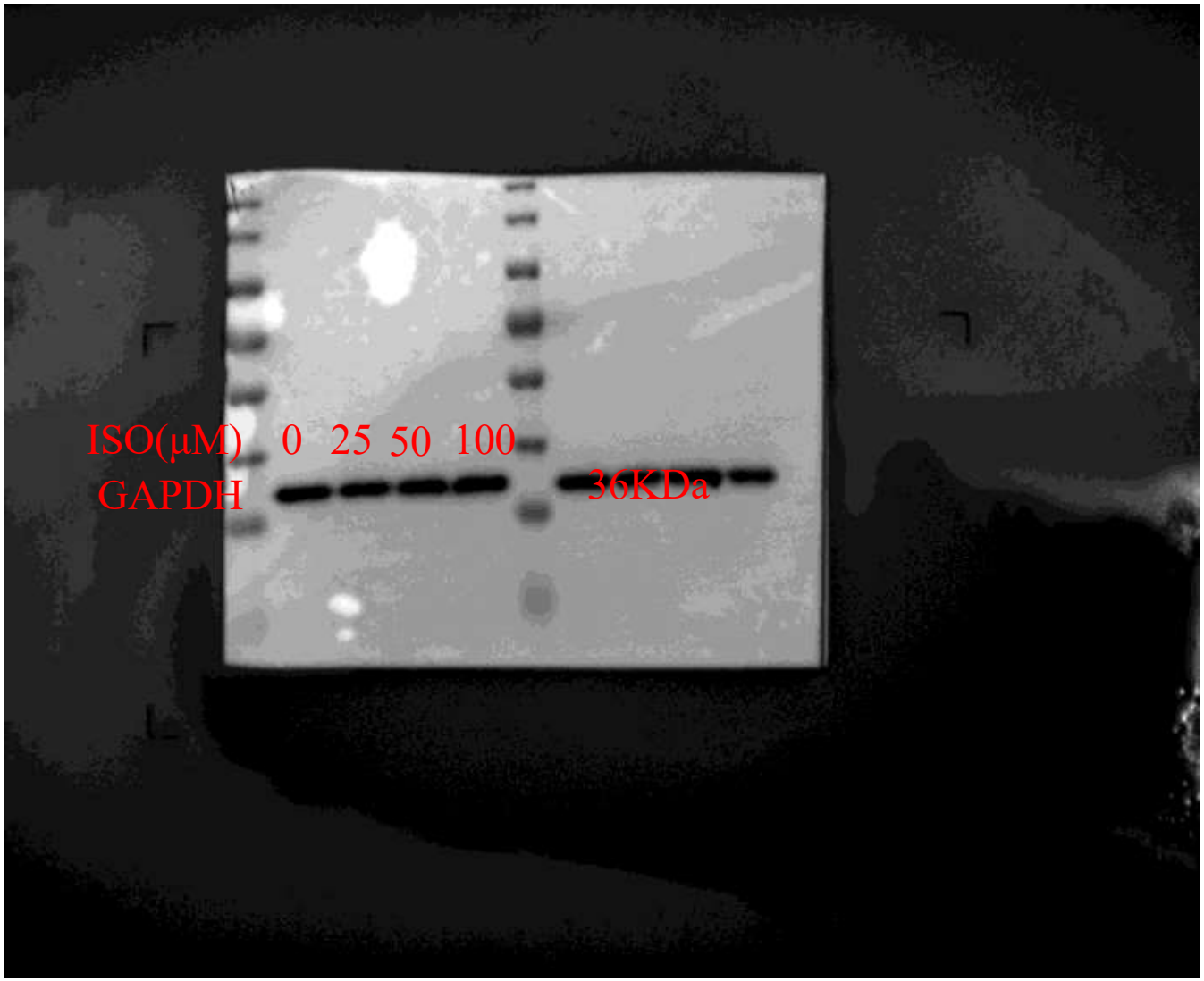
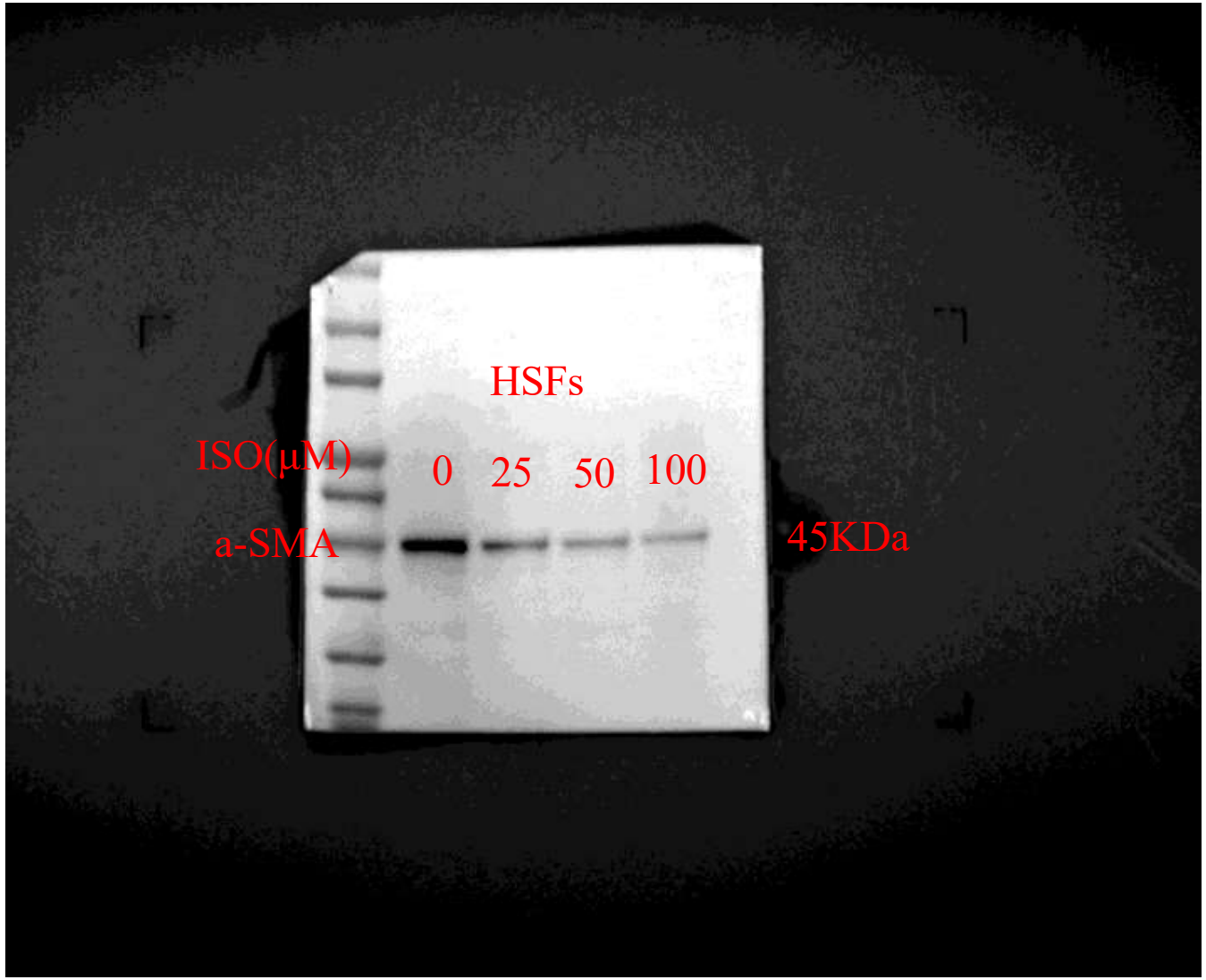
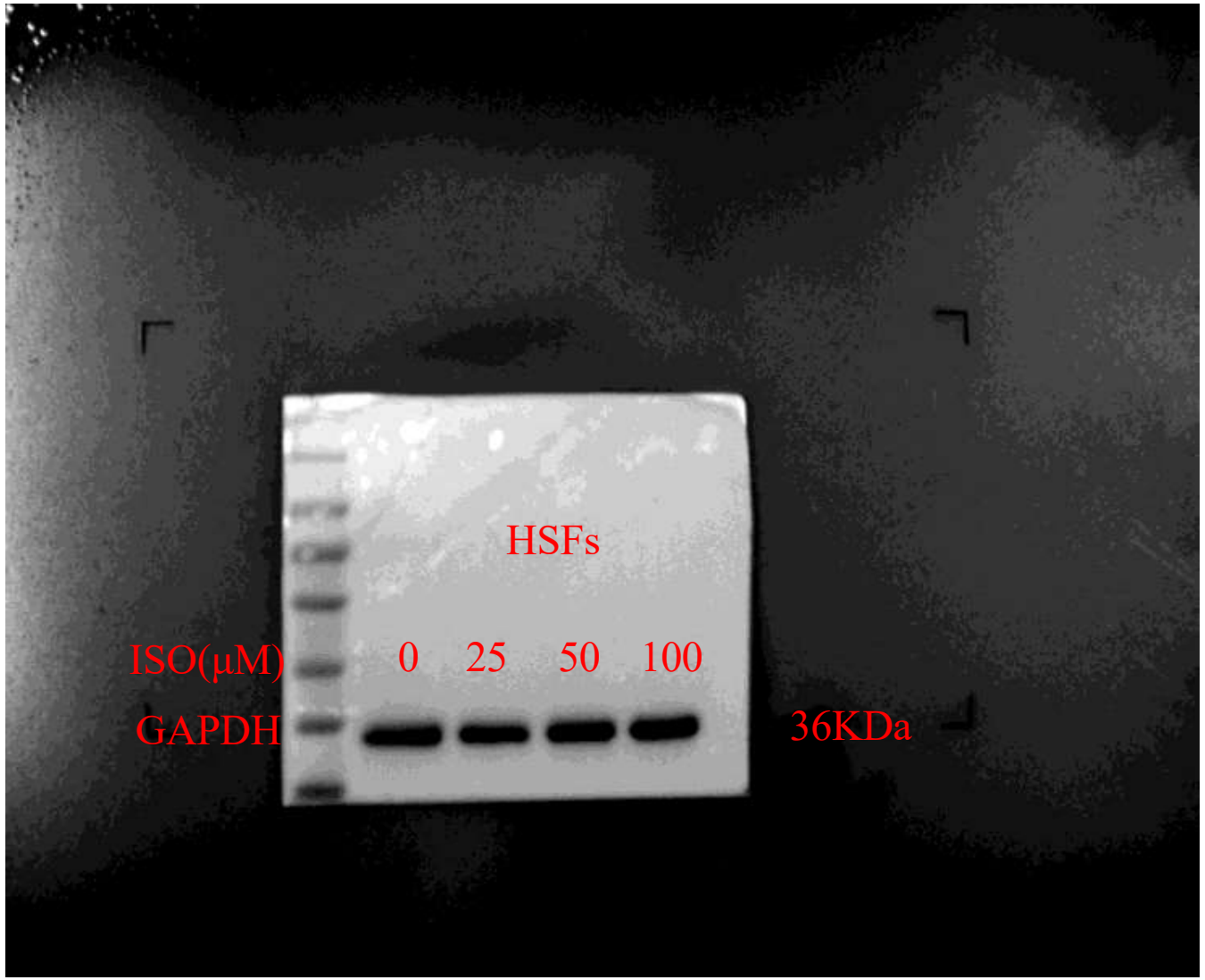
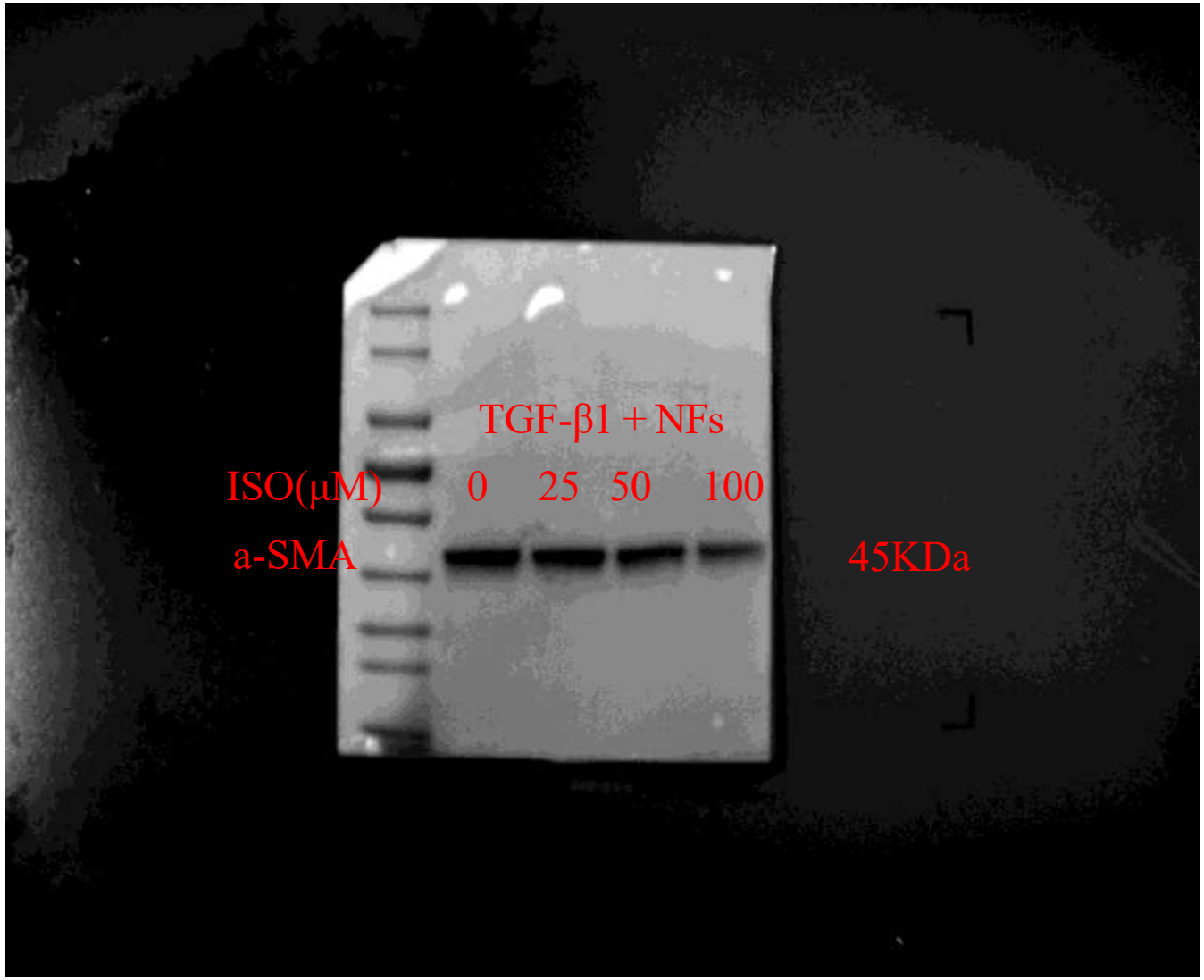

ISO(μM) 0 25 50 100
GAPDH
36KDa

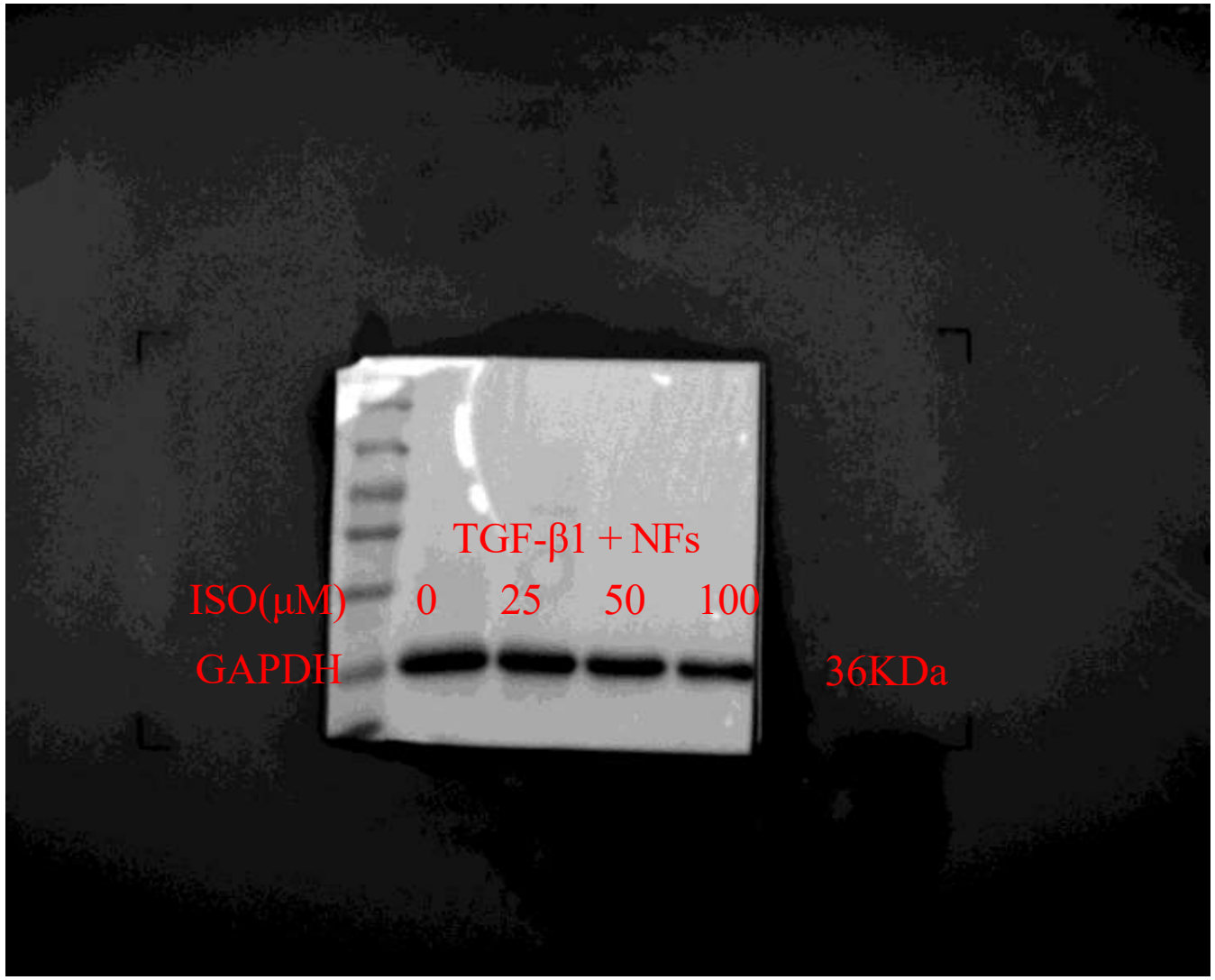
Figure 4. ISO inhibited the gel contraction, myofibroblast activation, and collagen expression.

C. Western blotting results of α-SMA in HSFBs and TGF-β1-stimulated NFBs after treatment with ISO (0, 25, 50, 100 μM) for 48 h and the quantification of the protein levels normalized to GAPDH.

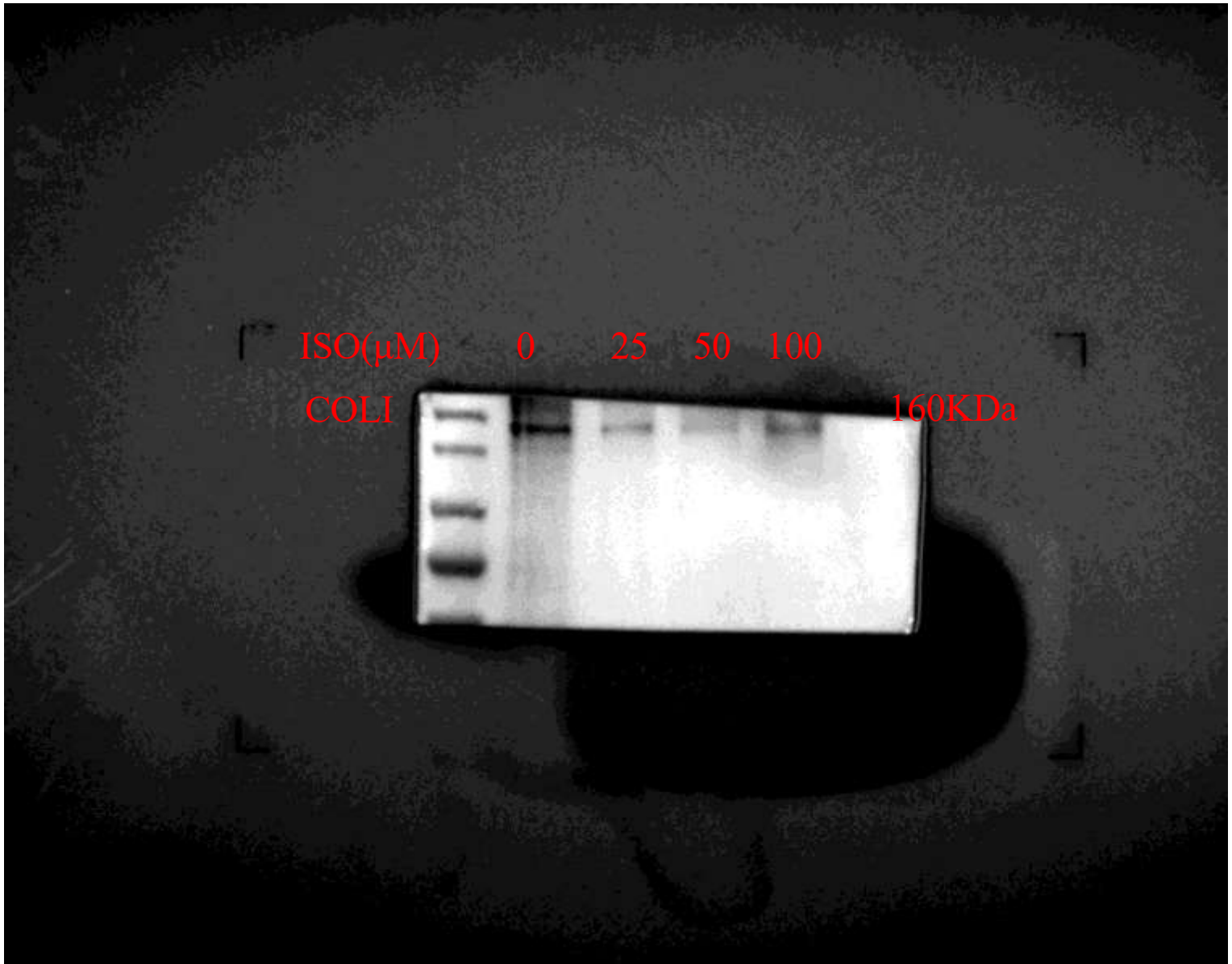


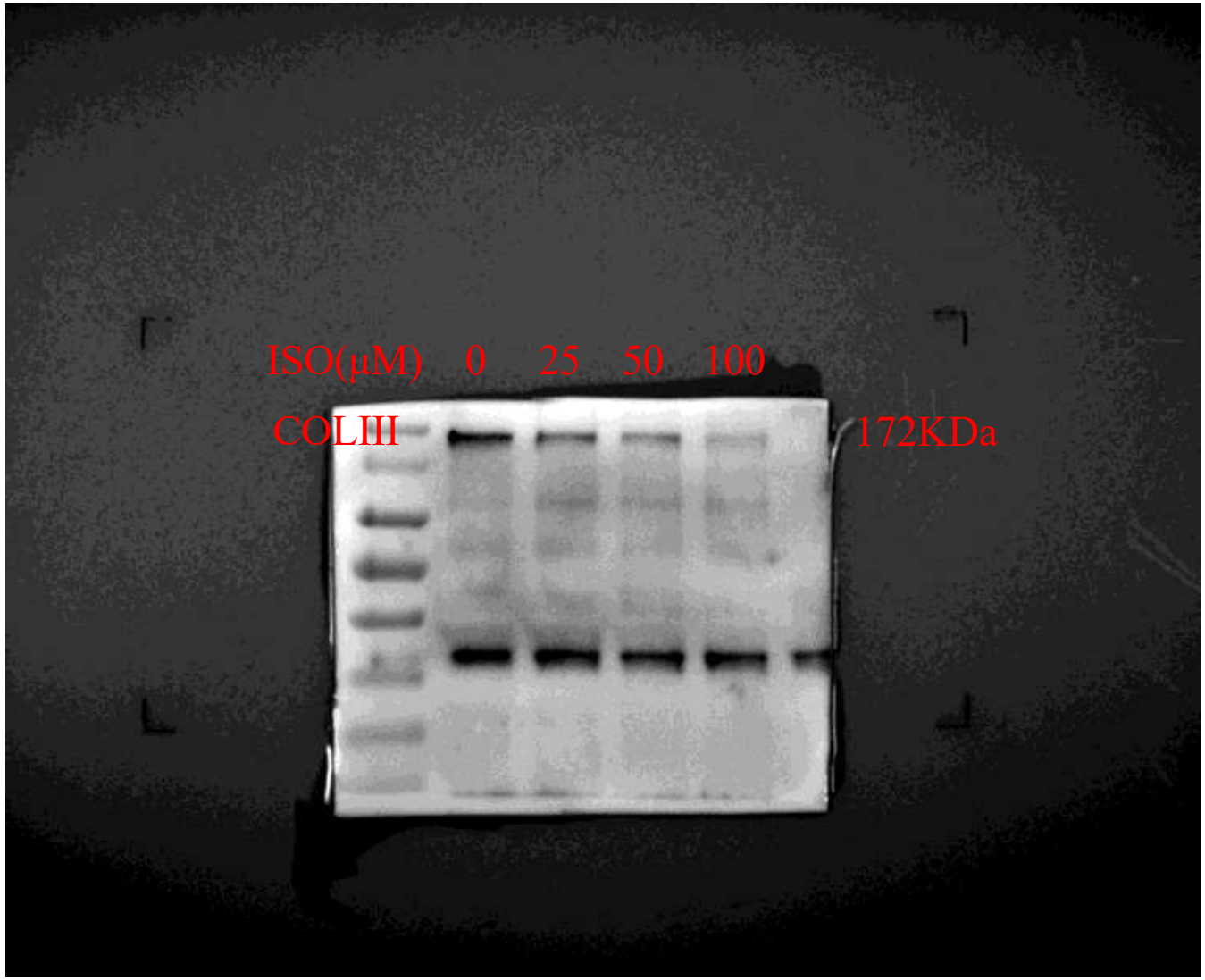

HSFs
ISO(μM)
0
25
50
100
GAPDH
36KDa


TGF-β1 + NFs
ISO(μM)
0
25
50
100
a-SMA
45KDa


TGF-β1 + NFs
ISO(μM)
0
25
50
100
GAPDH
36KDa

D. Western blotting results of COL I and COL III in HSFBs and the quantification of COL I and COL III normalized to GAPDH.




ISO(μM) 0 25 50 100
COLIII
172KDa

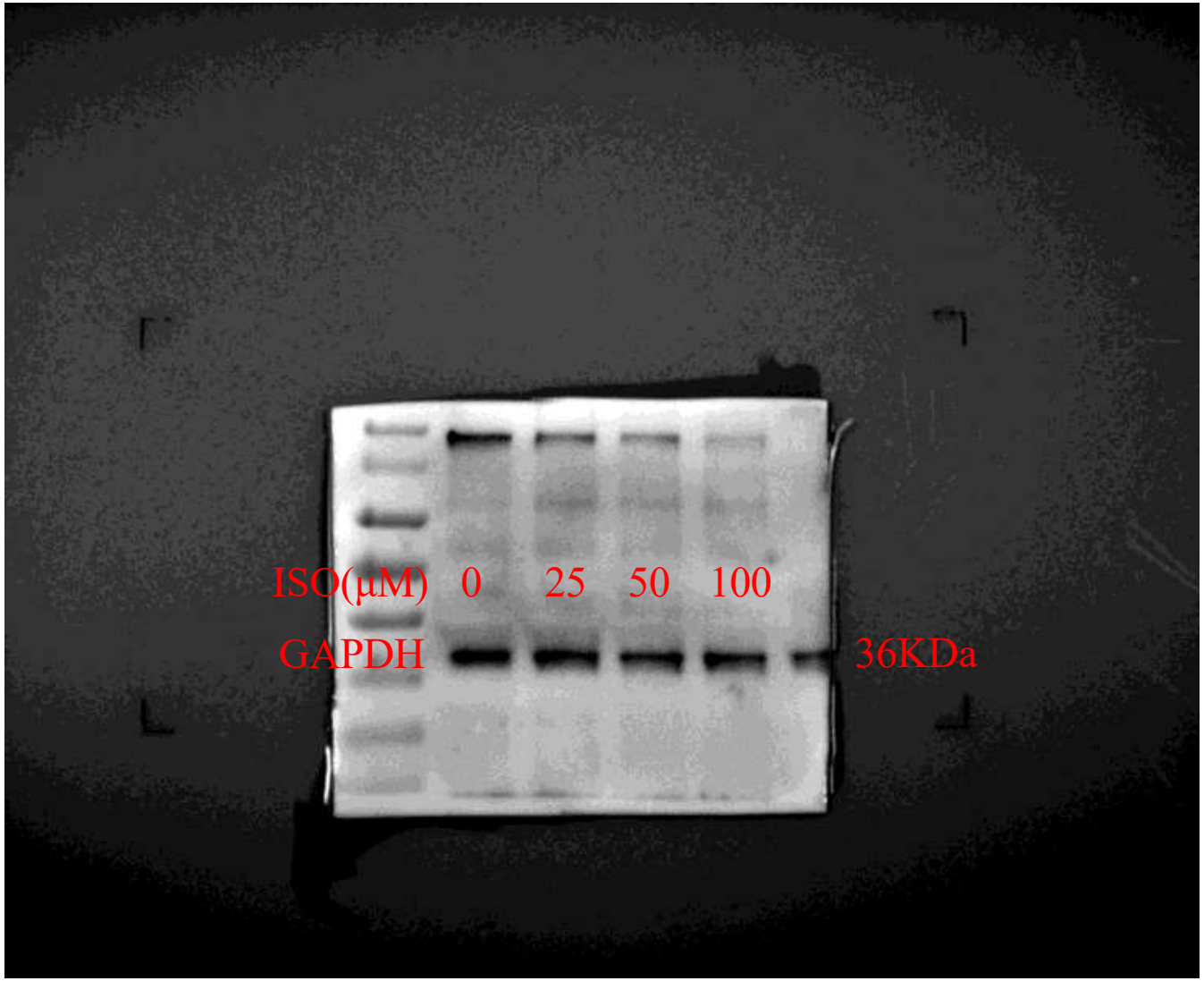
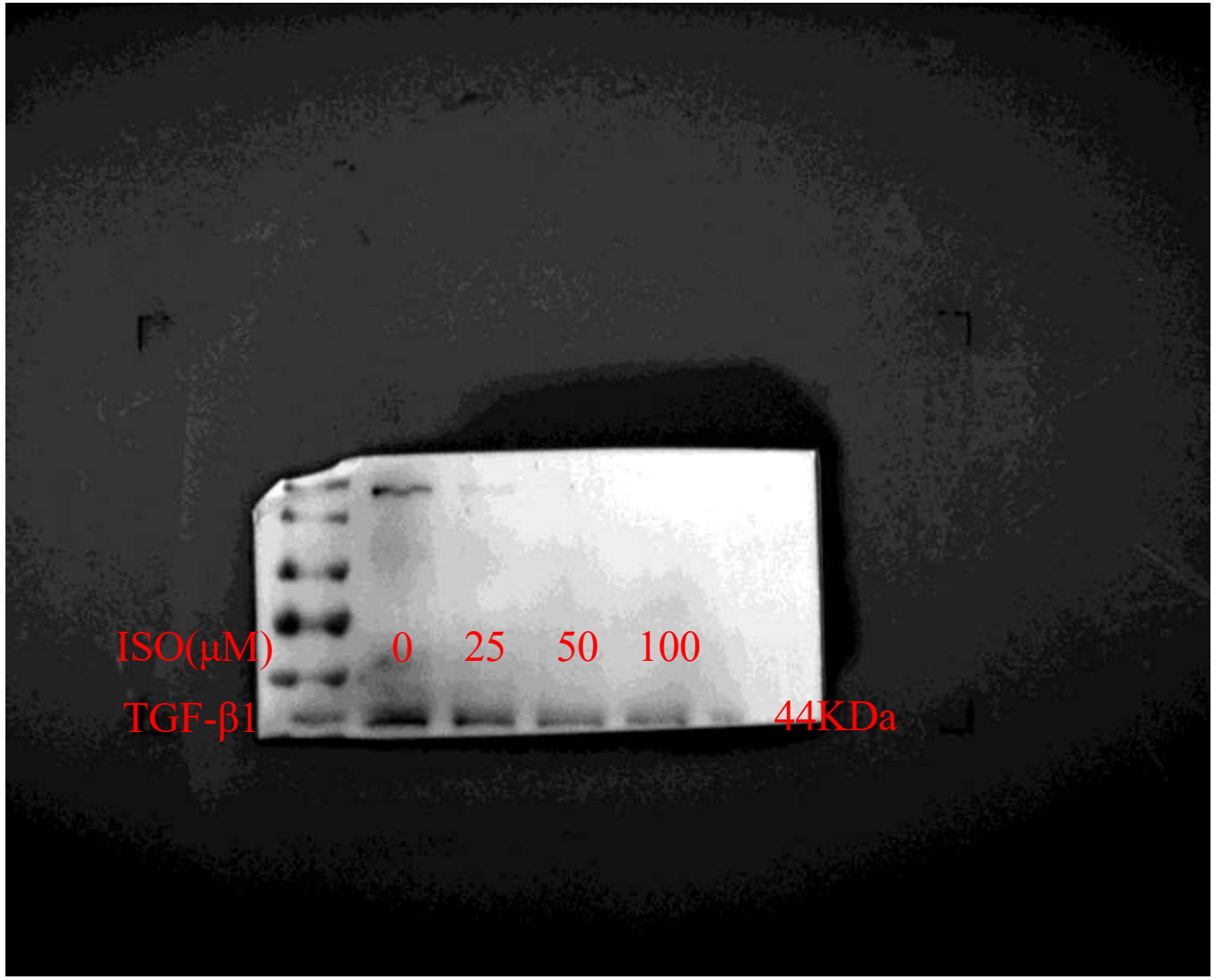
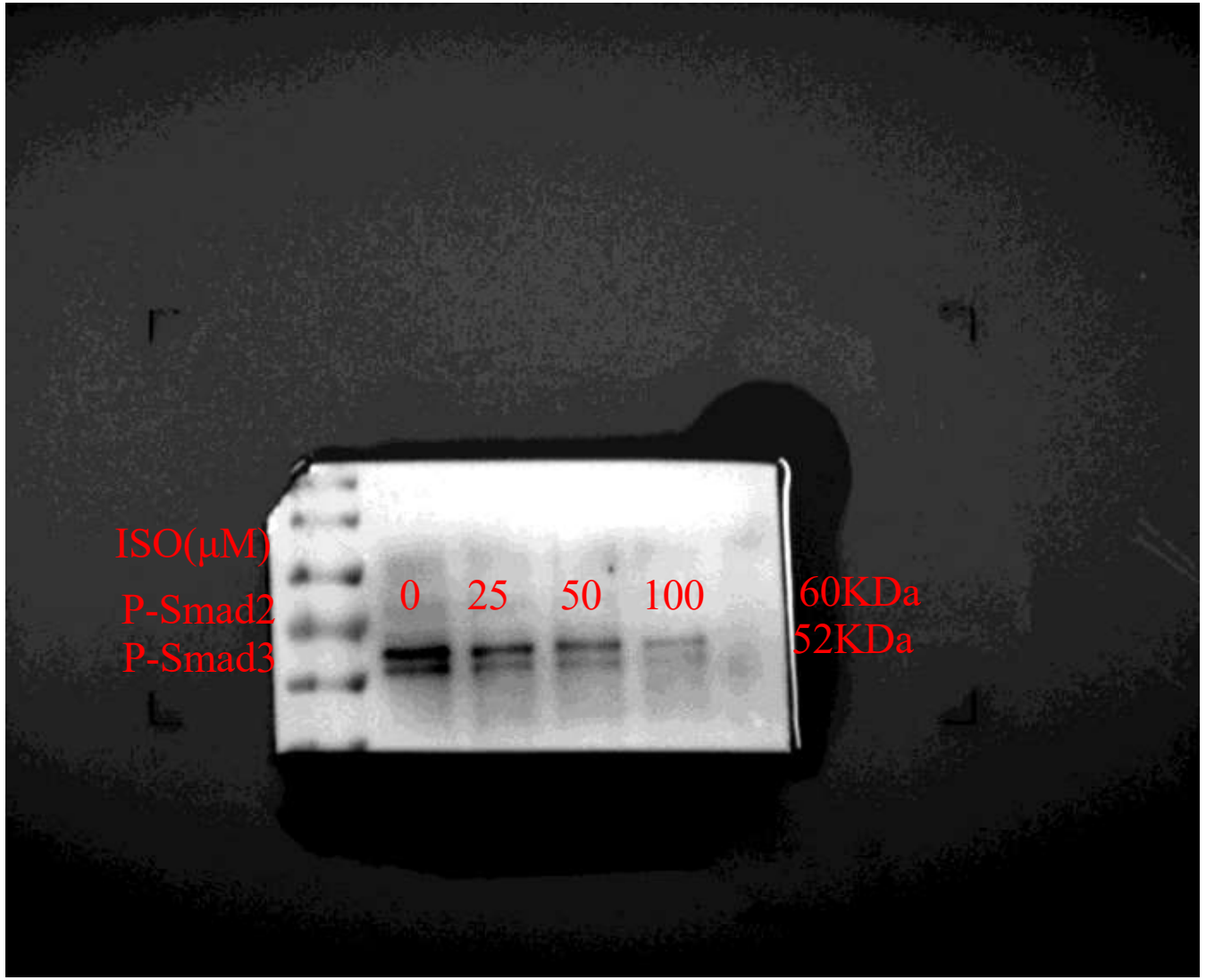
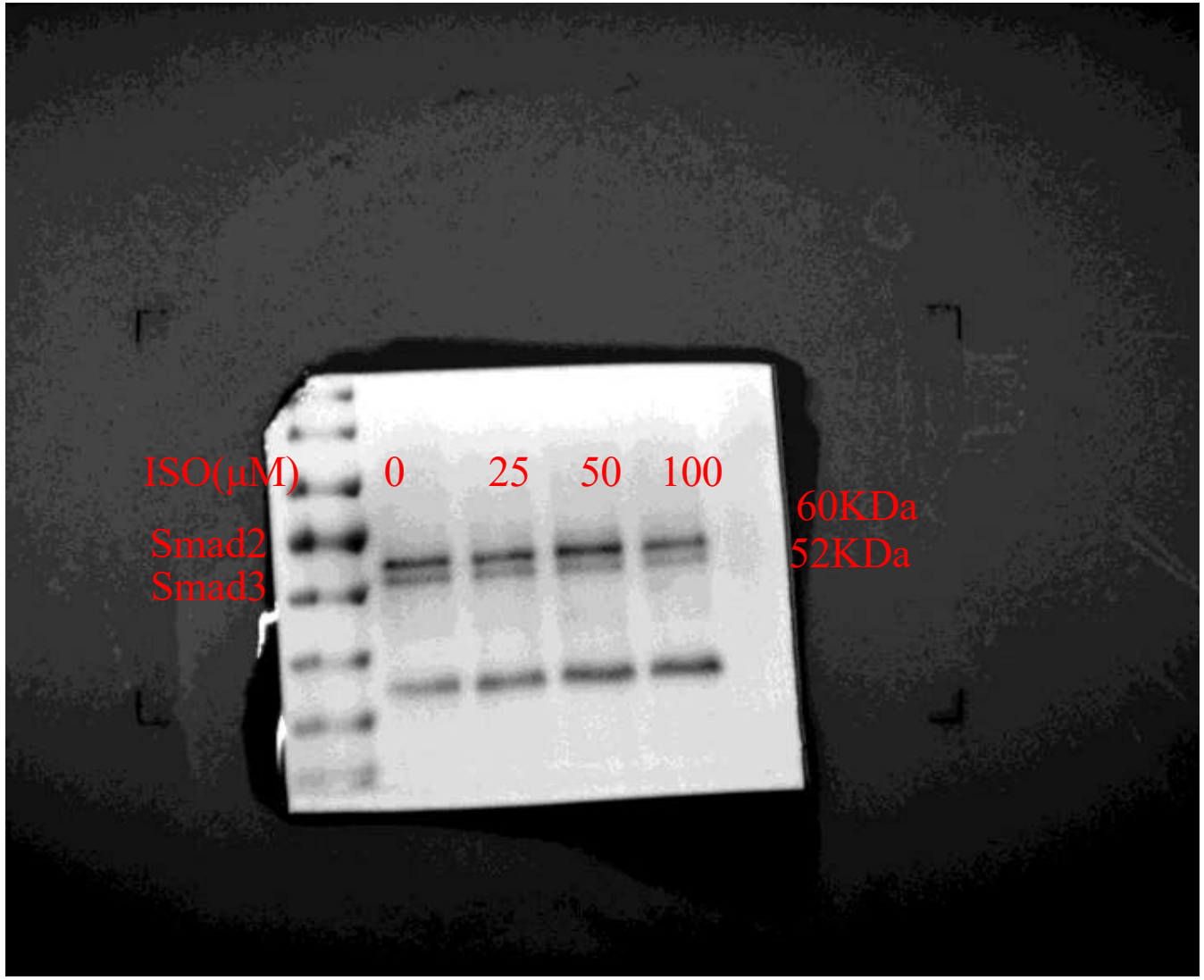

ISO(μM) 0 25 50 100
GAPDH
36KDa

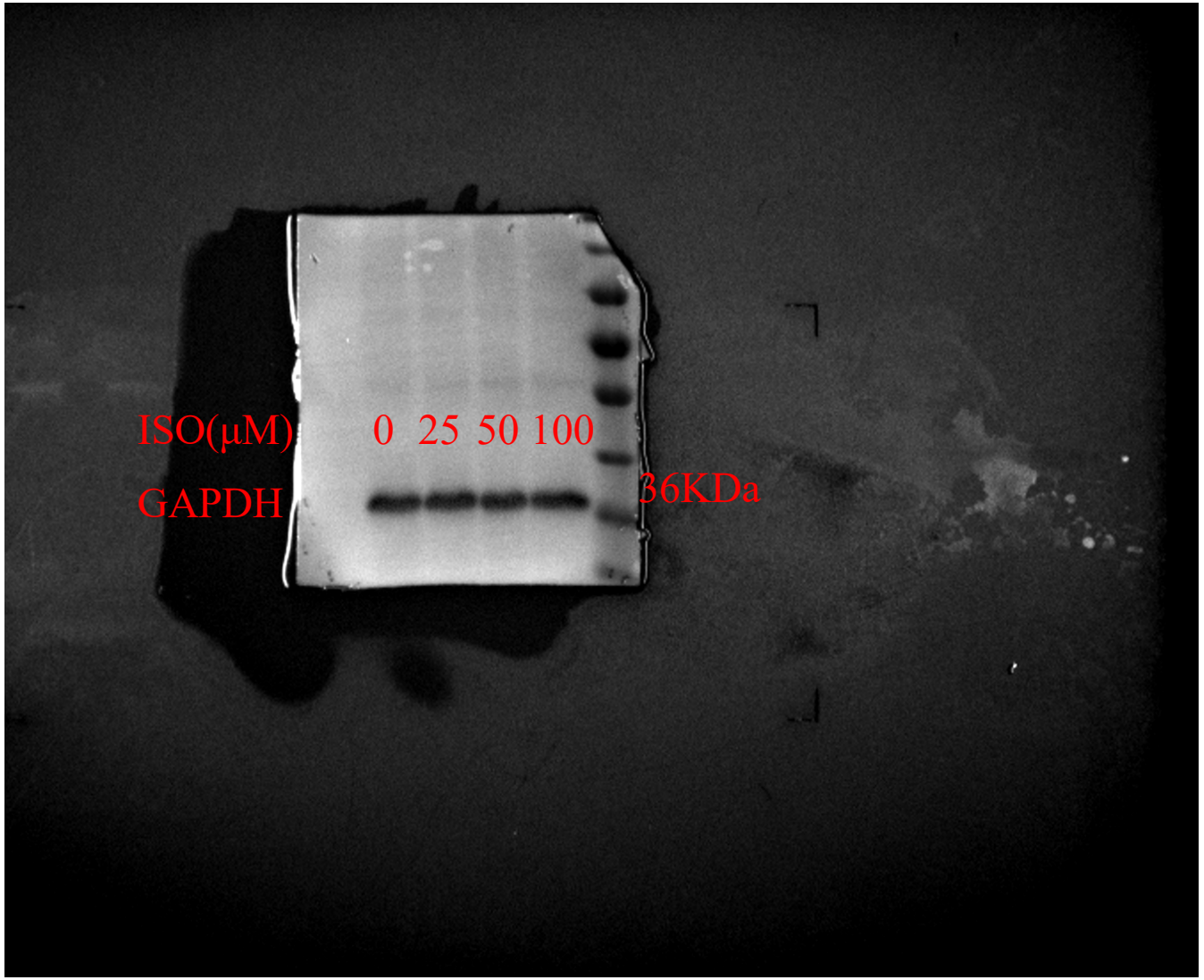
Figure 5. ISO inhibited ECM production by suppressing the TGF-β1/Smad signaling pathway

D. The protein levels of TGF-β1, phosphorylated Smad2 and Smad3, and total Smad2 and Smad3 detected by Western blotting and the quantification of protein levels normalized to GAPDH

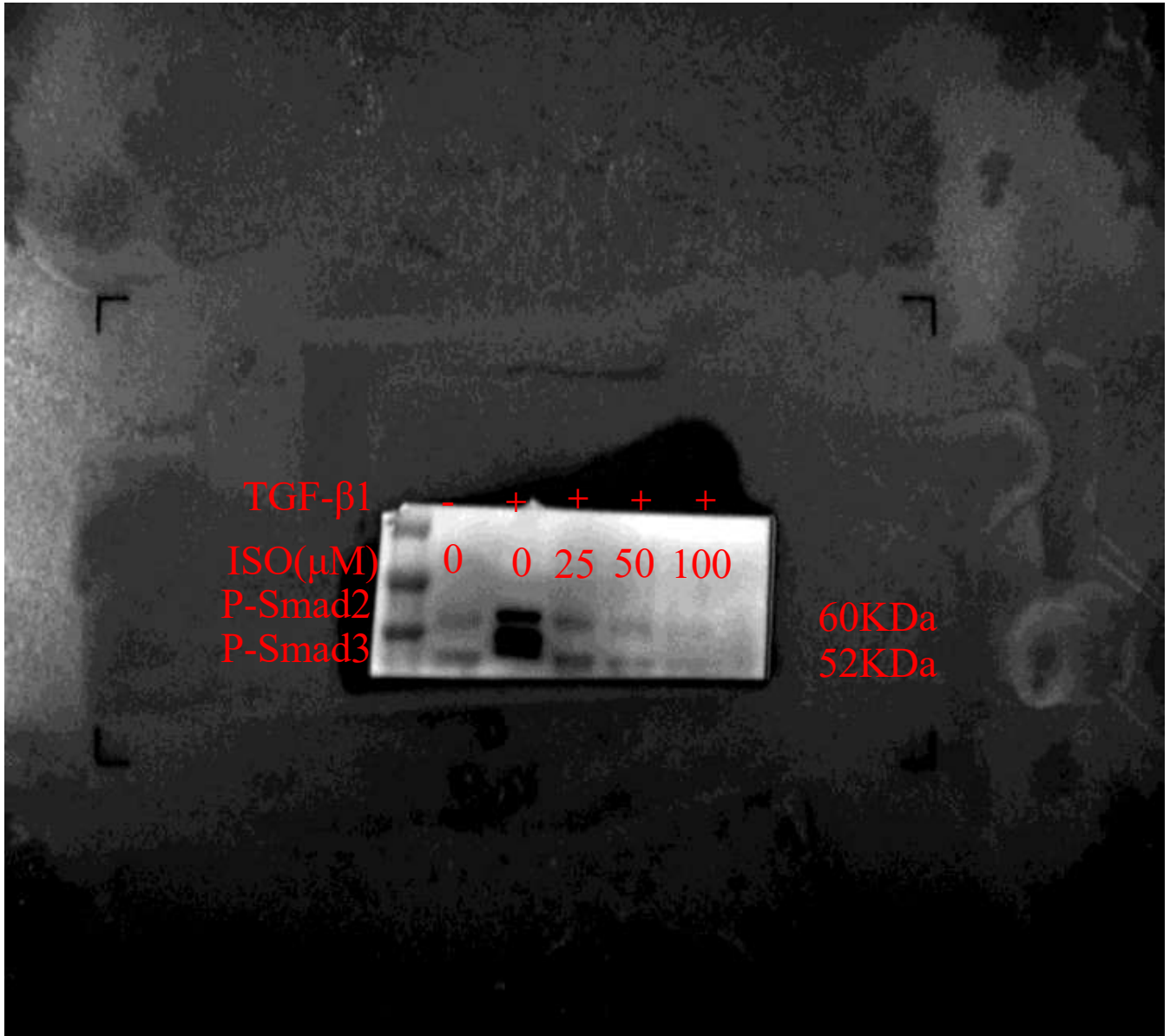


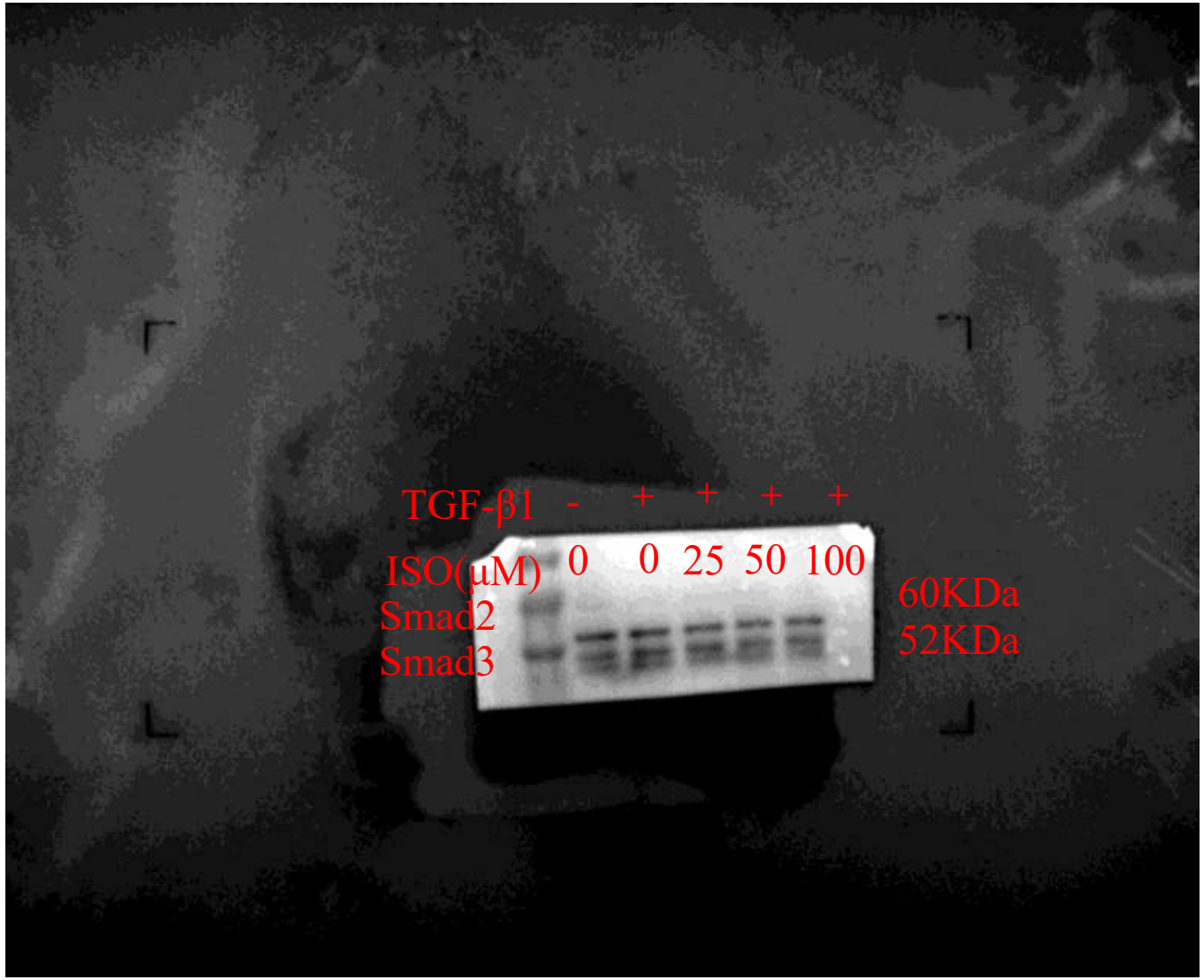

ISO(μM)
0
25
50
100
P-Smad2
P-Smad3
60KDa
52KDa

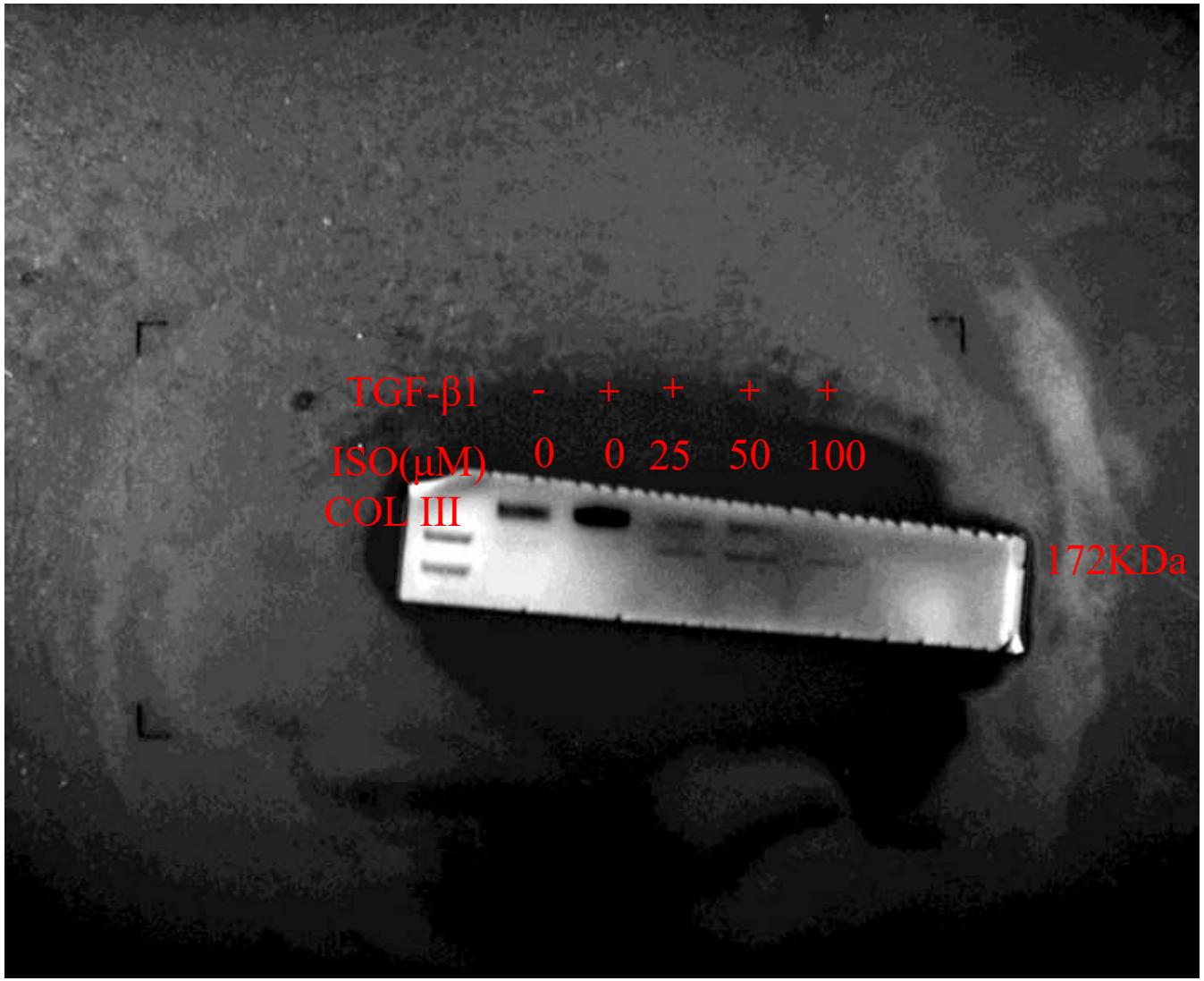

ISO(μM)
0
25
50
100
Smad2
Smad3
60KDa
52KDa

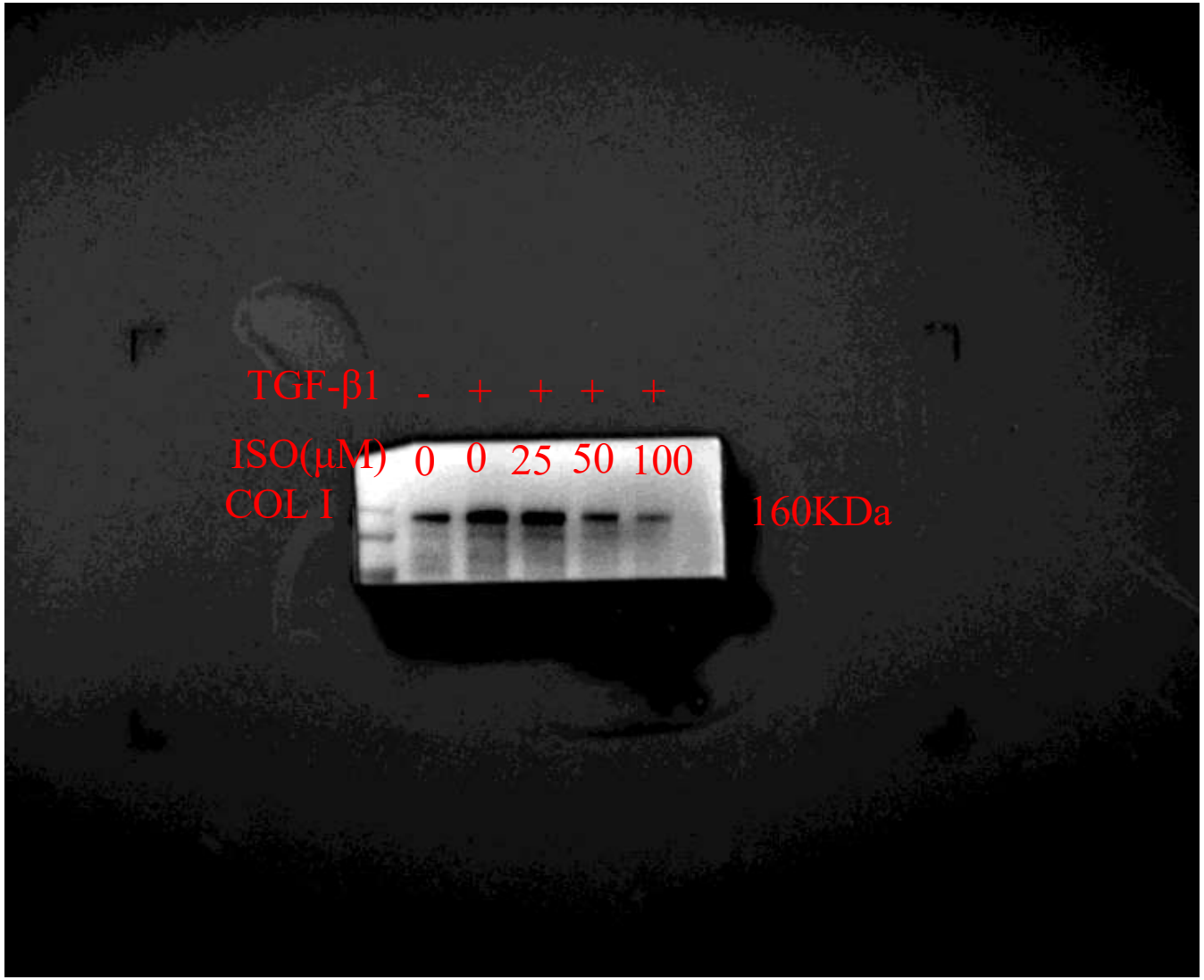

ISO(μM)
0 25 50 100
GAPDH
36KDa

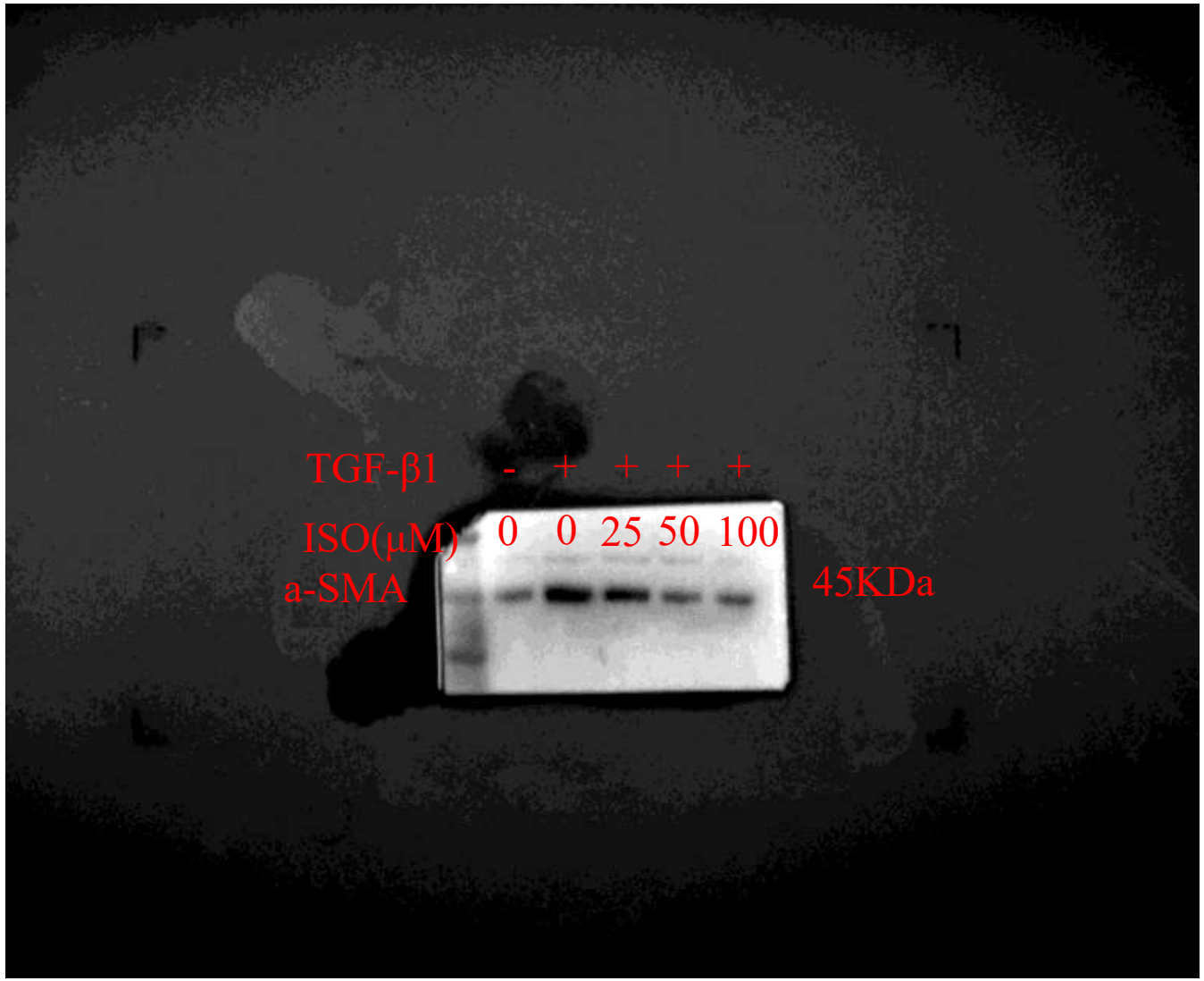
E. The protein levels of phosphorylated and total Smad2/3, COL I, COL III, and α-SMA assessed by Western blotting in HSFBs after treatment with ISO (0, 25, 50, 100 μM) + TGF-β1 (0, 10 ng/mL) for 48 h and the quantification of protein levels normalized to GAPDH.




TGF-β1 - + + + +
ISO(μM) 0 0 25 50 100
Smad2
Smad3
60KDa
52KDa


TGF-β1 - + + + +
ISO(μM) 0 0 25 50 100
COL III
172KDa


TGF-β1 - + + + +
ISO(μM) 0 0 25 50 100
COL I
160KDa


TGF-β1 - + + + +
ISO(μM) 0 0 25 50 100
a-SMA
45KDa

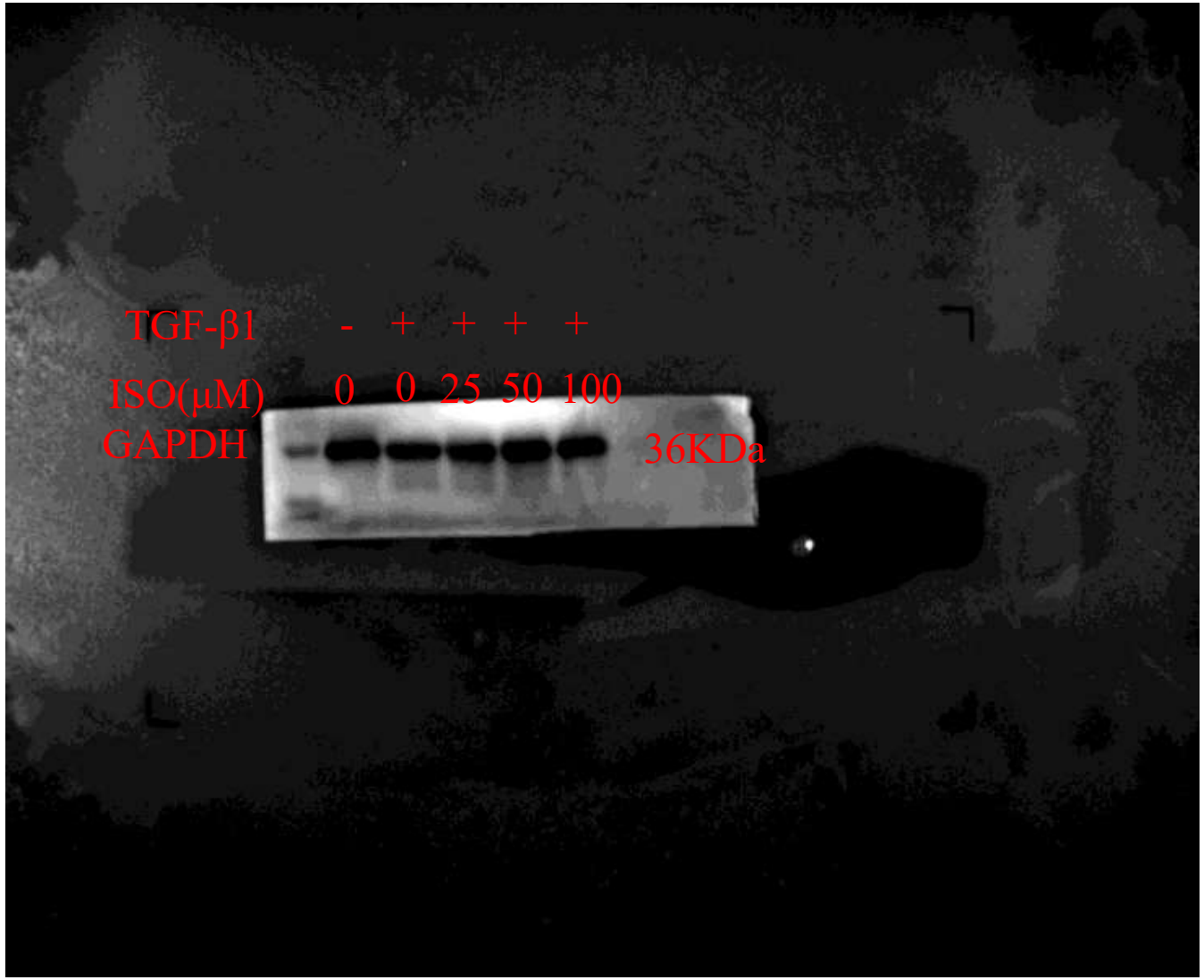
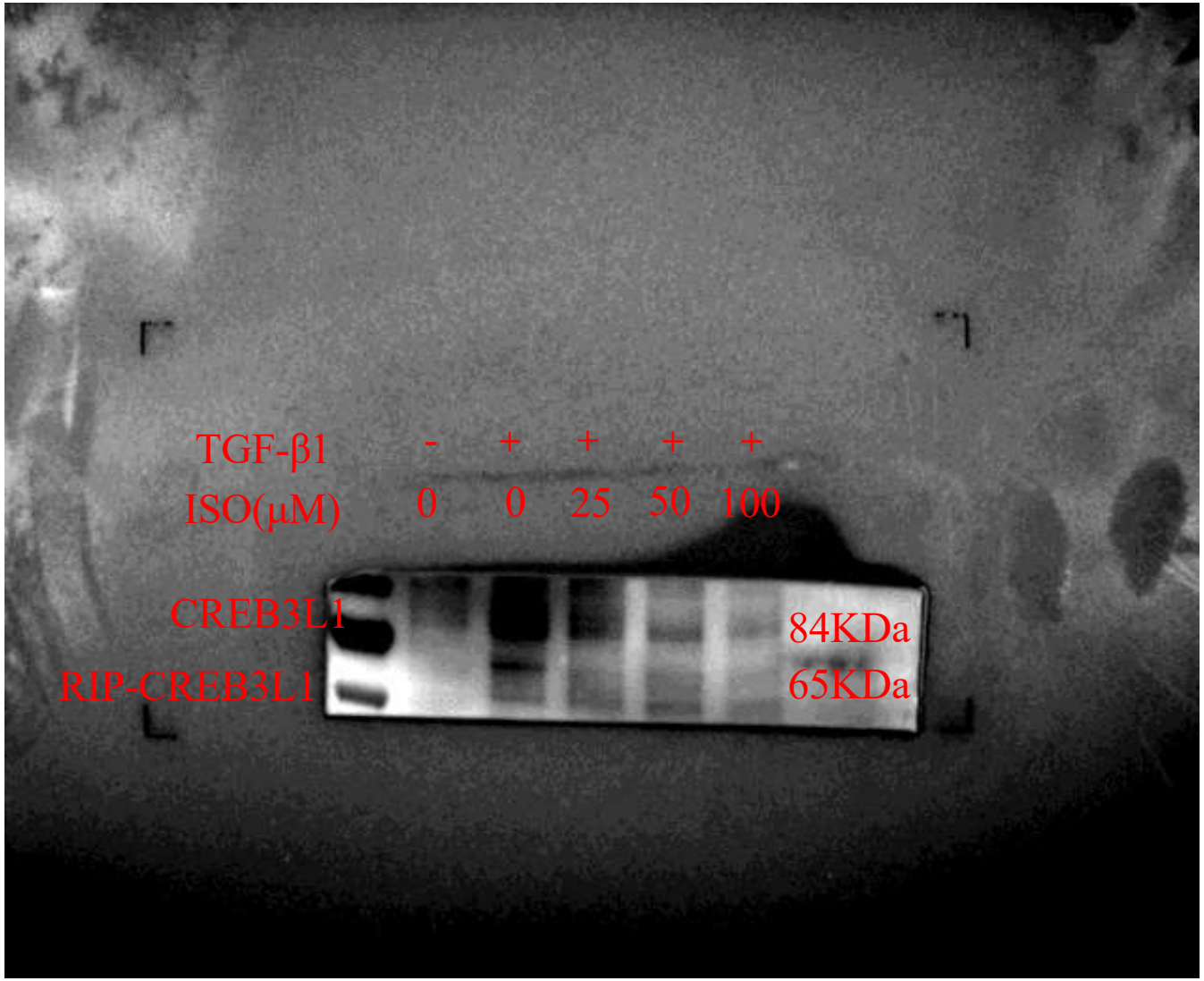
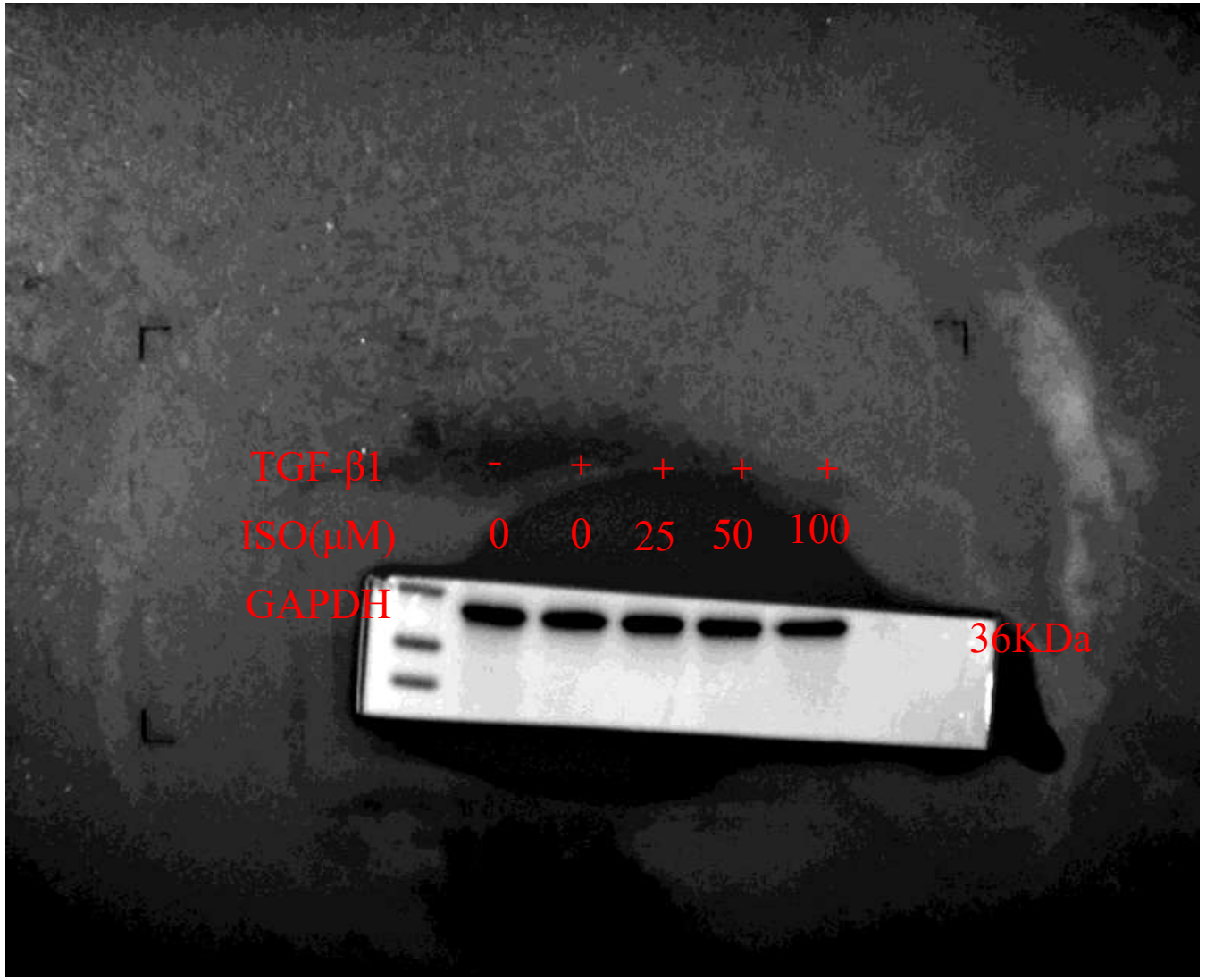

TGF-β1 - + + + +
ISO(μM) 0 0 25 50 100
GAPDH
36KDa

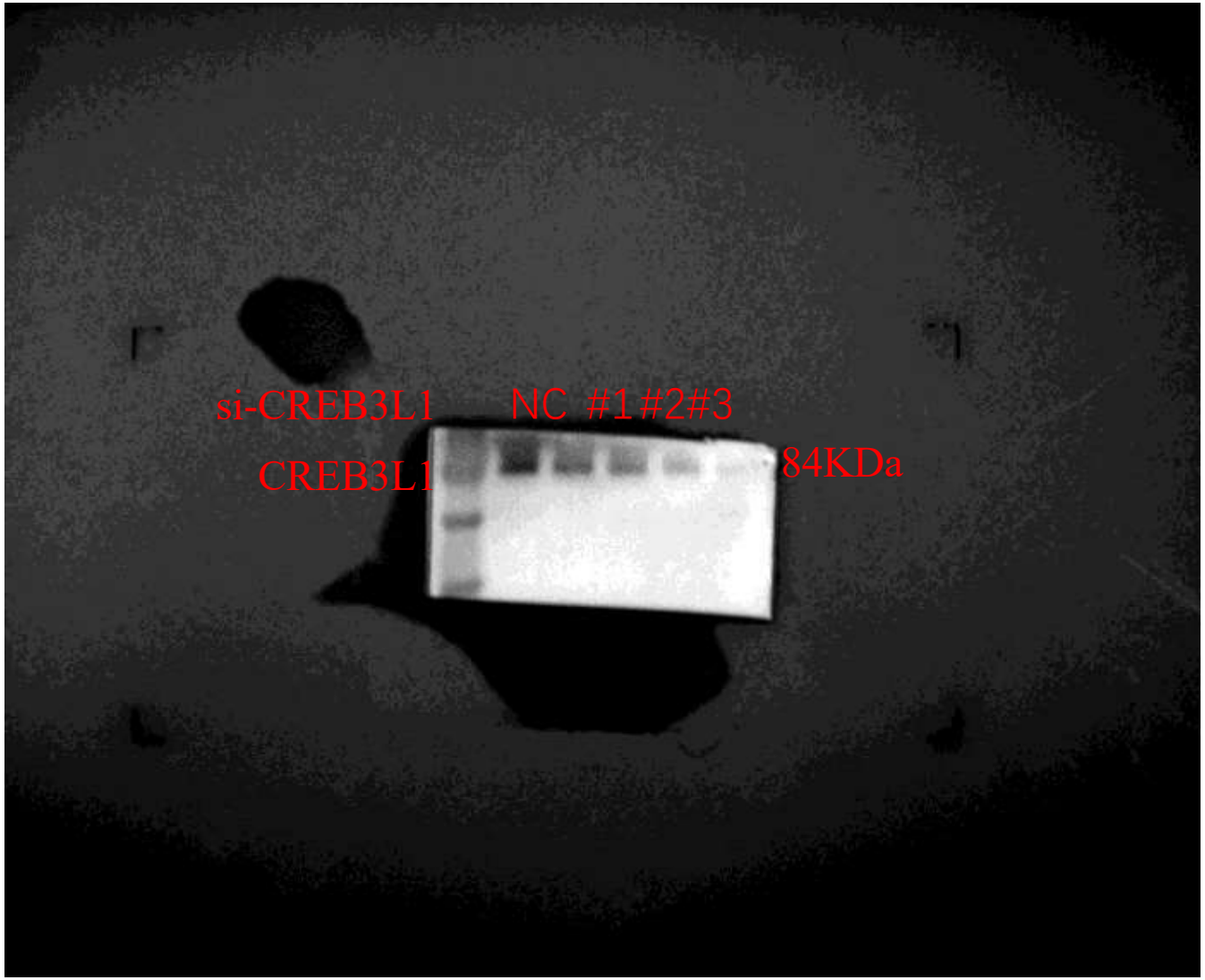
Figure 6. ISO inhibited ECM production by suppressing the TGF-β1/CREB3L1 signaling pathway.

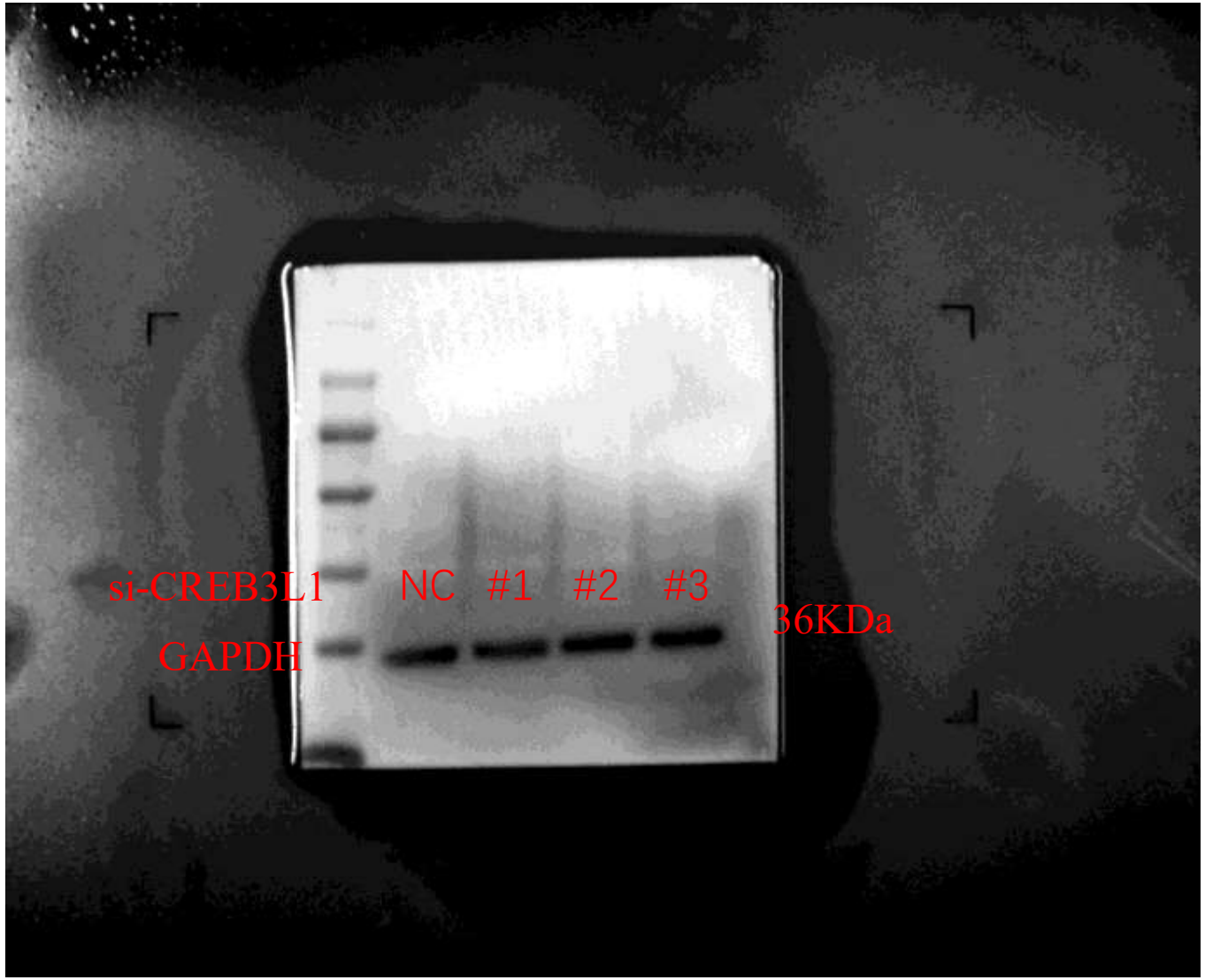
B. The protein levels of CREB3L1 and cleaved CREB3L1 detected by Western blotting after HSFBs were treated with ISO (0, 25, 50, 100 μM) + TGF-β1 (0, 10 ng/mL) for 48 h, and the quantification of the protein levels normalized to the GAPDH level.



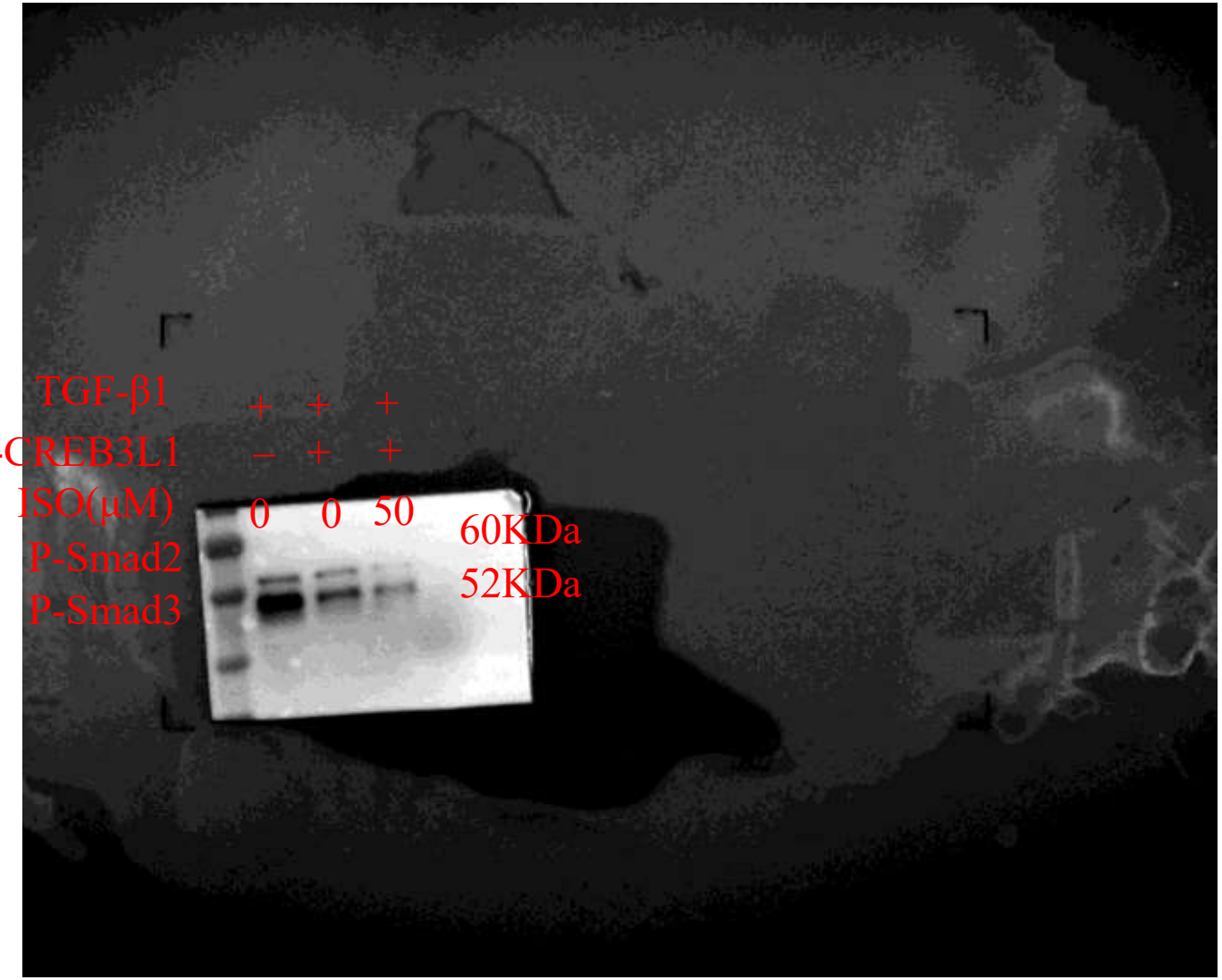

TGF-β1 - + + + +
ISO(μM) 0 0 25 50 100
GAPDH
36KDa

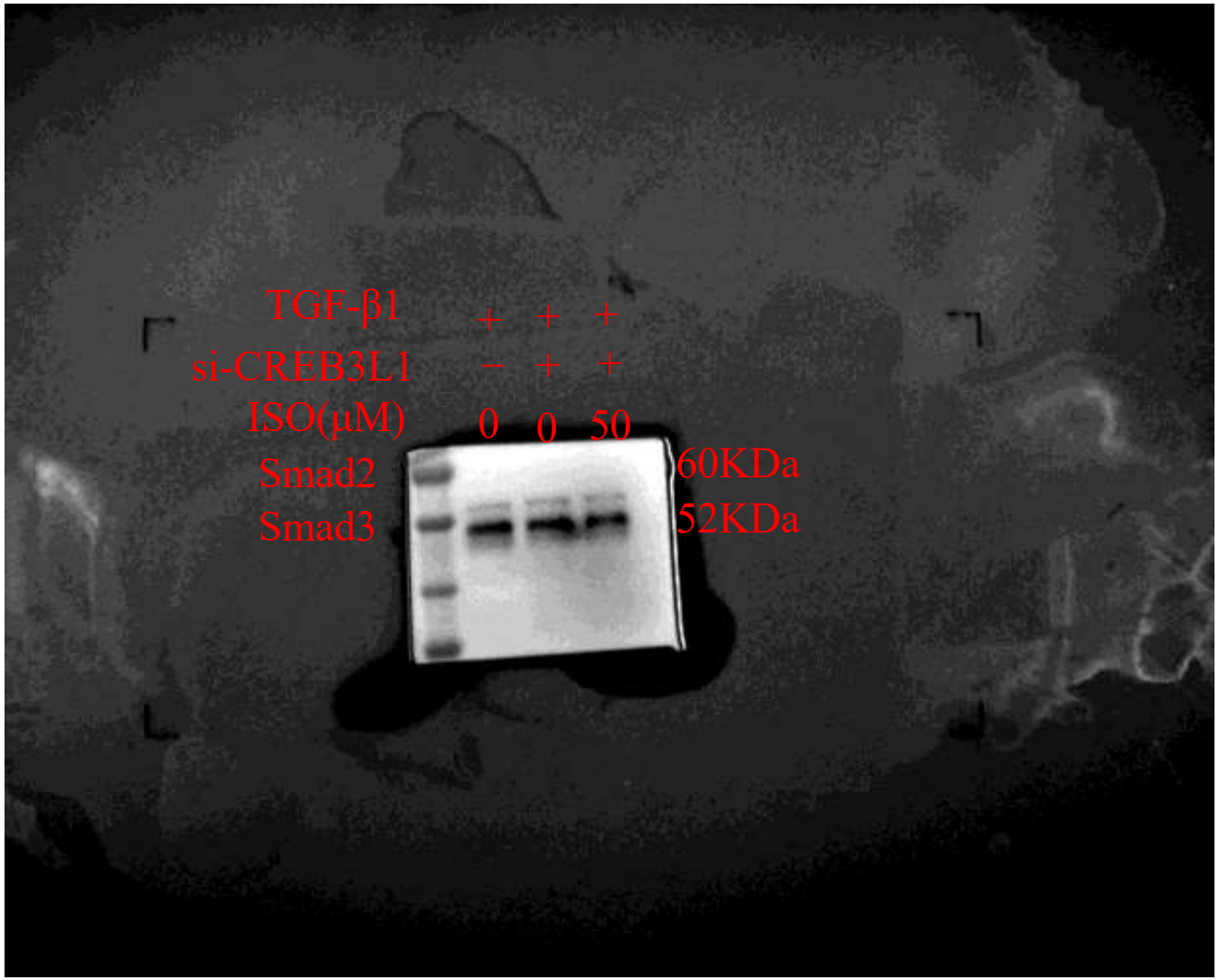
C. The protein levels of CREB3L1 detected by Western blotting after HSFBs were transfected with CREB3L1 siRNAs (si-CREB3L1#1, si-CREB3L1#2, and si-CREB3L1#3) and the quantification of the protein levels normalized to GAPDH.

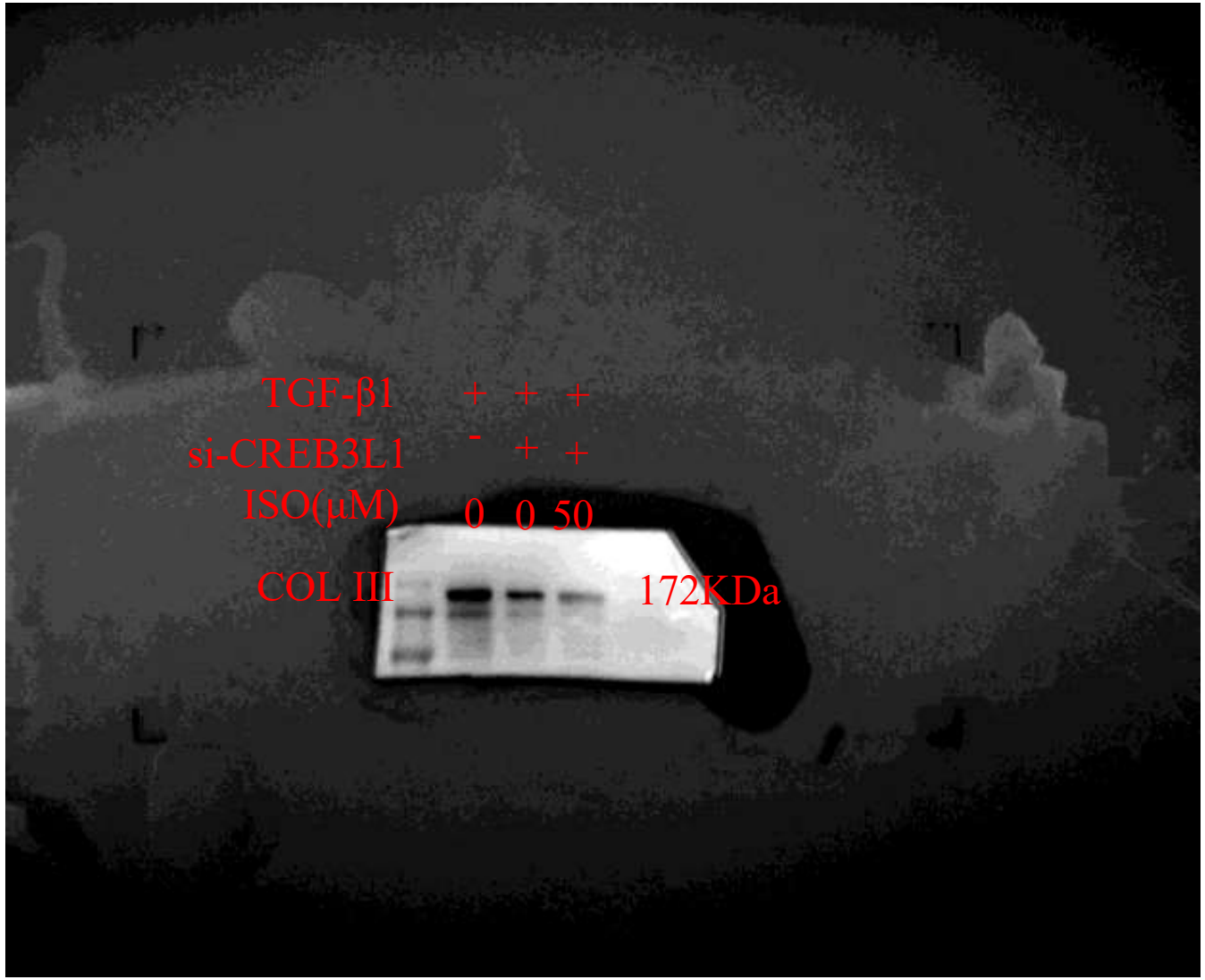


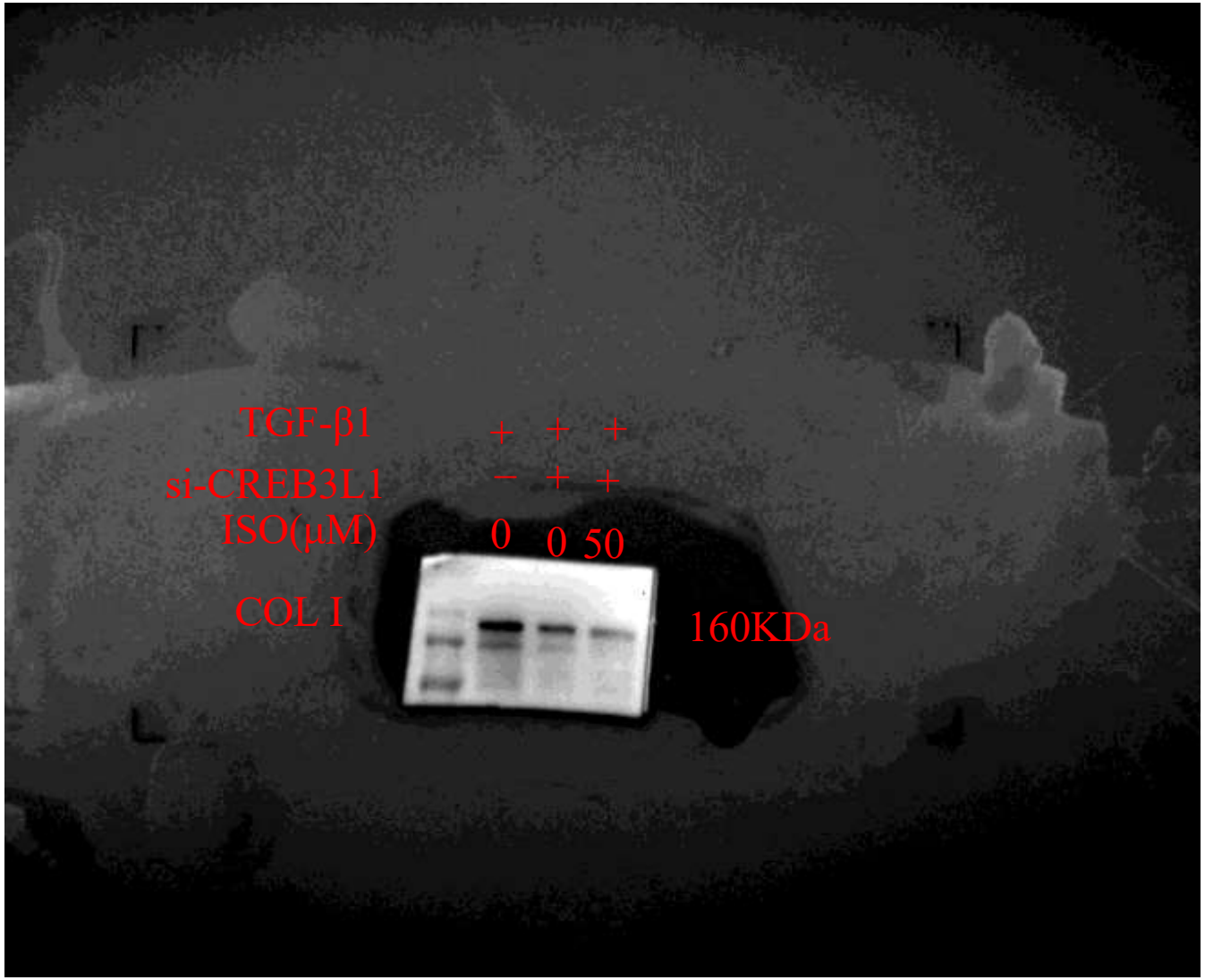

si-CREB3L1
NC
#1
#2
#3
GAPDH
36KDa

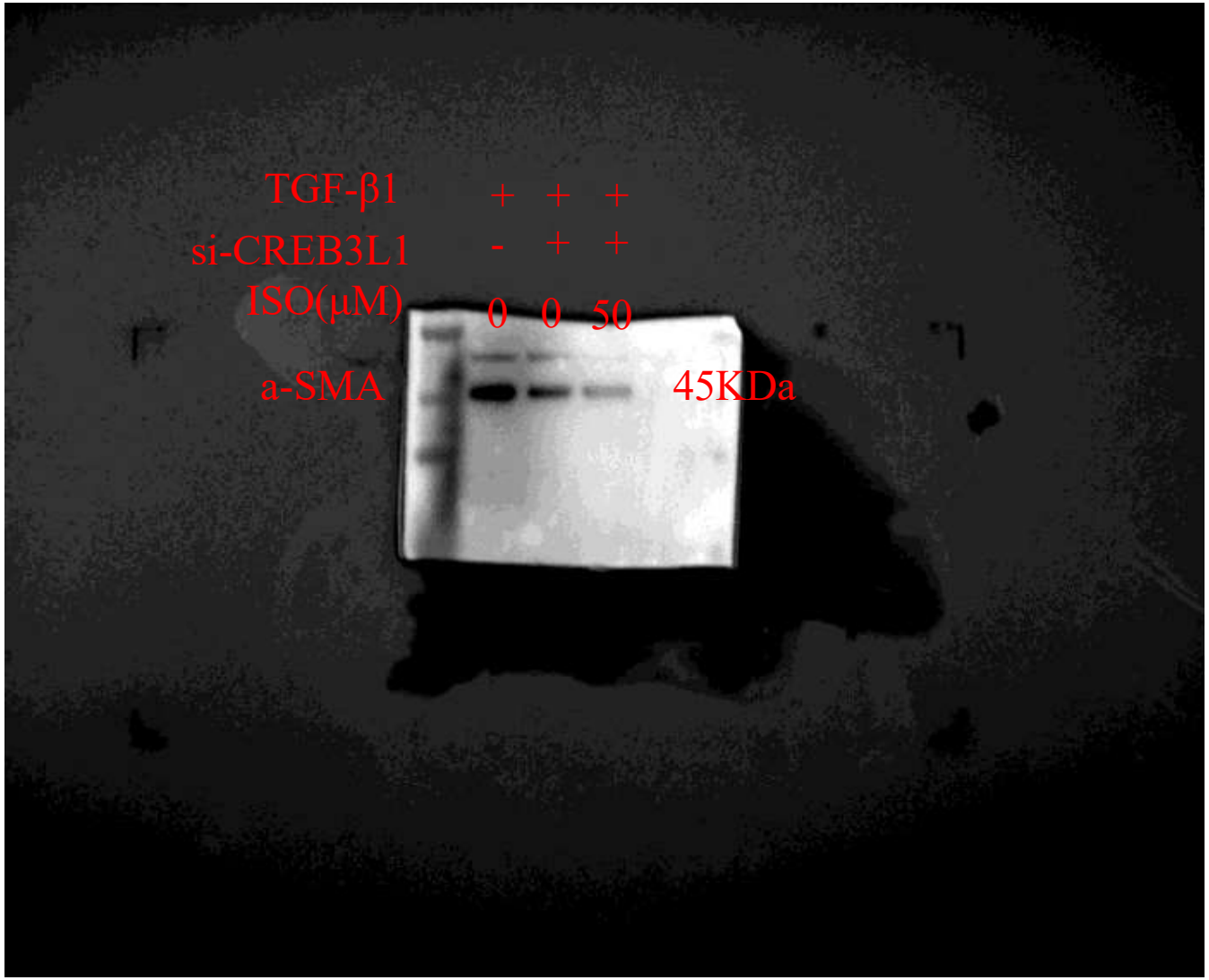
D. The protein levels of phosphorylated and total Smad2/3, COL I, COL III, and α-SMA in HFSBs treated with CREB3L1 siRNA or CREB3L1 siRNA combined with ISO (50 μM) for 48 h and the quantification of protein levels normalized to GAPDH. In this experiment, TGF-β1 (10 ng/mL) was added to induce the phosphorylation of Smad2/3 and the expression of COL I, COL III, and α-SMA.

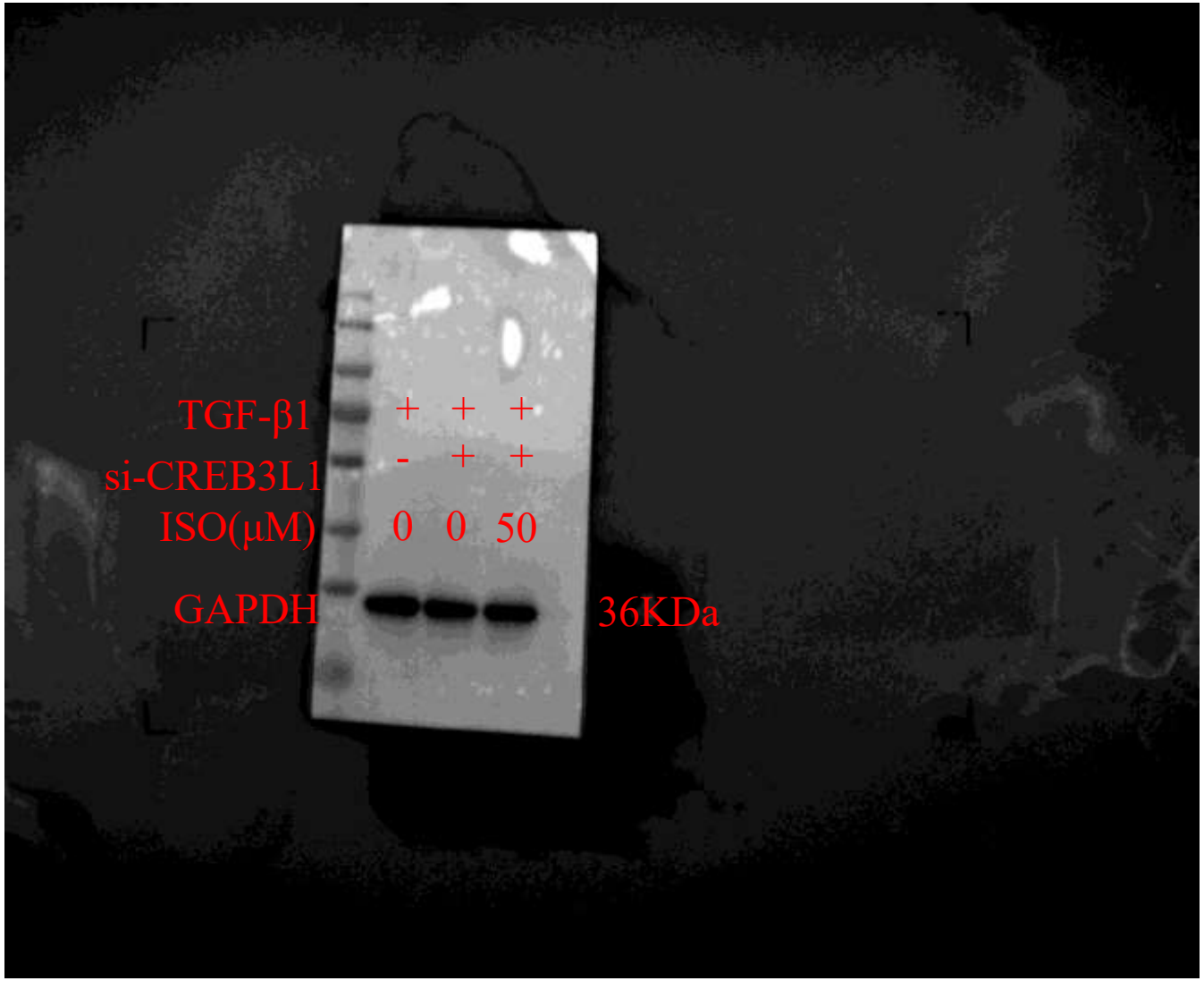



TGF-β1 + + +
si-CREB3L1 − + +
ISO(μM) 0 0 50
Smad2
Smad3
60KDa
52KDa


TGF-β1 + + +
si-CREB3L1 - + +
ISO(μM) 0 0 50
COL III
172KDa


TGF-β1 + + +
si-CREB3L1 − + +
ISO(μM) 0 0 50
COL I
160KDa


TGF-β1 + + +
si-CREB3L1 - + +
ISO(μM) 0 0 50
a-SMA
45KDa


TGF-β1 + + +
si-CREB3L1 - + +
ISO(μM) 0 0 50
GAPDH
36KDa