<u>**Online Supplements**</u>

Smooth muscle cell-specific Fibronectin-EDA mediates Phenotypic Switching and Neointimal Hyperplasia

Manish Jain[1], Nirav Dhanesha[1], Prakash Doddapattar[1], Mehul R. Chorawala[1], Manasa K. Nayak[1], Anne Cornelissen[2], Liang Guo[2], Aloke V. Finn[2], Steven R. Lentz[1], and Anil K. Chauhan[1]

[1]Department of Internal Medicine, Division of Hematology/Oncology, University of Iowa, Iowa City, IA.

[2]CVPath Institute Inc., Gaithersburg, MD.

Materials

Online supplementary Table 1. Patient and Stent Characteristics

Characteristic	
No. of patients, n	6
No. of stents, n	7
Age, mean ± SD, y	70 ± 5
Male, n (%)	4 (66.67%)
Smokers, n (%)	0 (0%)
Hypertension, n (%)	4 (66.67%)
Hypercholesterolemia, n (%)	0 (0%)
Diabetes Mellitus, n (%)	2 (28.57%)
Indication for stenting, n (%) CAD ACS	5 (83.34%) 1 (16.67%)
Culprit artery, n (%) LAD LCX RCA	5 (71.43%) 2 (28.57%) 0
No. of diseased vessels, n (%) 1 2 3	1 (16.67%) 2 (28.57%) 3 (50%)
Type of stent, n (%) Multilink Multilink Duet	5 (71.43%) 2 (28.57%)
Duration of implant, mean ± SD, days	86.14 ± 24.72
Underlying pathology Plaque rupture, n (%) FA, n (%) fibrocalcific plaque, n (%)	1 (14.29%) 4 (57.14%) 2 (28.57%)

CAD, Coronary Artery Disease; ACS, Acute Coronary Syndrome; LAD, Left Anterior Descending; LCX, Left Circumflex Artery; RCA, Right Coronary Artery; FA, Fibroatheroma

Online supplementary Table 2: Complete blood counts from 8- 10 weeks old mice were obtained using automated veterinary hematology analyzer (Advia). Value are expressed as mean ± SEM. N= 5-6 mice/group. P= Non-significant versus control Fn-EDA$^{fl/fl}$ Apoe$^{-/-}$ mice

Complete blood count	Fn-EDA$^{fl/fl}$Apoe$^{-/-}$ BM→ Fn-EDA$^{fl/fl}$Apoe$^{-/-}$		Fn-EDA$^{fl/fl}$Apoe$^{-/-}$ BM→ Fn-EDA$^{fl/fl}$Tie2Cre^{+}Apoe$^{-/-}$		P Value
	Mean	SEM	Mean	SEM	
WBC (10^3/µL)	10.37	1.01	9.99	0.96	0.82
RBC (10^6/µL)	10.15	0.85	10.01	0.94	0.92
HGB (g/dL)	13.32	1.41	12.24	1.52	0.61
HCT (%)	52.56	4.40	52.02	4.84	0.94
PLT (10^3/µL)	1219	96	1159	103	0.71
Neutrophil (10^3/µL)	0.70	0.13	0.54	0.12	0.35
Lymphocytes (10^3/µL)	6.48	0.80	6.89	0.81	0.85
Monocytes (10^3/µL)	2.52	0.40	1.48	0.42	0.09

WBC, White Blood cells; RBC, Red Blood cells; HGB**,** Hemoglobin; HCT, Hematocrit; PLT, Platelet

Online supplementary Table 3. List of Antibodies

| Antibody	Company	Catalog	Species	Application
p-AKT1 (S473)	Cell Signaling	9018	Rabbit	WB
AKT (pan)	Cell Signaling	4691	Rabbit	WB
p-AKT2 (S474)	Cell Signaling	8599	Rabbit	WB
AKT2	Cell Signaling	3063	Rabbit	WB
p-mTOR (Ser2448)	Cell Signaling	2971	Rabbit	WB
mTOR	Cell Signaling	2972	Rabbit	WB
p-NFκB p65 (Ser536)	Cell Signaling	3033	Rabbit	WB
NFκB p65	Cell Signaling	8242	Rabbit	WB
p-FAK (Tyr397)	Cell Signaling	3283	Rabbit	WB
FAK	Cell Signaling	3285	Rabbit	WB
p-Src Family (Tyr416)	Cell Signaling	2101	Rabbit	WB
Src	Cell Signaling	2108	Rabbit	WB
p-ERK 1/2	Cell Signaling		Rabbit	WB
p-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	Cell Signaling	9101	Rabbit	WB
p44/42 MAPK (Erk1/2)	Cell Signaling	9102	Rabbit	WB
CD31	Abcam	Ab28364	Rabbit	IF
CD68	Abcam	Ab125212	Rabbit	IF
Anti-Fibronectin	Abcam	Ab2413	Rabbit	IF, WB
β-Actin	Abcam	Ab8227	Rabbit	WB
Osteopontin	Abcam	ab8448	Rabbit	IF, WB
SM22 alpha	Abcam	ab14106	Rabbit	IF, WB
Vimentin	Abcam	ab92547	Rabbit	IF, WB
Smooth Muscle Myosin Heavy Chain 11	Abcam	Ab683 / Ab82541	Mouse / Rabbit	IF / WB
TLR4	Abcam	ab22048	Mouse	IP, WB
BrdU	Abcam	Ab6326	Rat	IF
α-SMA	Sigma	A5228	Mouse	IF,
FN-3E2	Sigma	F6140	Mouse	IP, WB
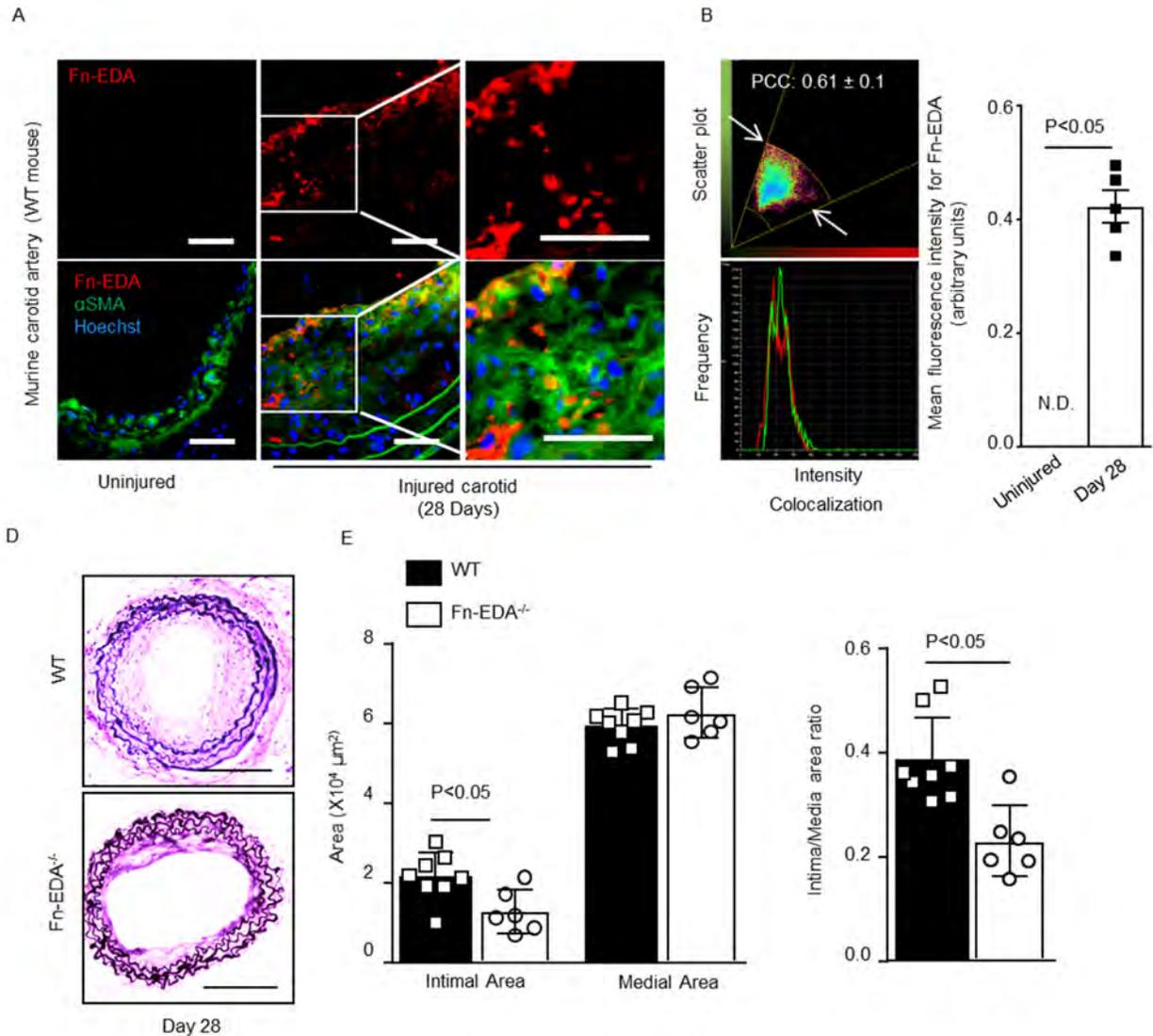
Figure S1. Expression and role of Fn-EDA in intimal hyperplasia. **(A)** Representative images showing double-immunostaining for Fn-EDA (red) and SMCs (green) in uninjured and injured carotid artery of WT mice harvested after 28 days of injury. N=5-6/group. Nuclei are counterstained with Hoechst (blue). Boxed region is magnified. Scale bar, 50 μm. **(B)** Representative scatter plot and intensity profile demonstrating colocalized pixels and pixel intensity for both channels (Fn-EDA-red, x-axis and SMC-green, y-axis) with Pearson's correlation coefficients (PCC). Colocalized pixels are defined as those whose intensity values for both channels fall within a preset range above the background intensity level (white arrows). **(C)** Quantification of the Fn-EDA-fluorescence intensity. **(D)** Representative photomicrographs of Verhoeff's Van Gieson stained carotid artery sections of Fn-EDA$^{-/-}$ and WT mice after 28 days of injury (N=6-8/group). Scale bar: 200 μm. **(E)** Quantification is showing intima area, media area and a ratio of intima to the media area. Each dot represents a single mouse. Values are represented as mean ± SEM. Statistical analysis: unpaired student t-test.
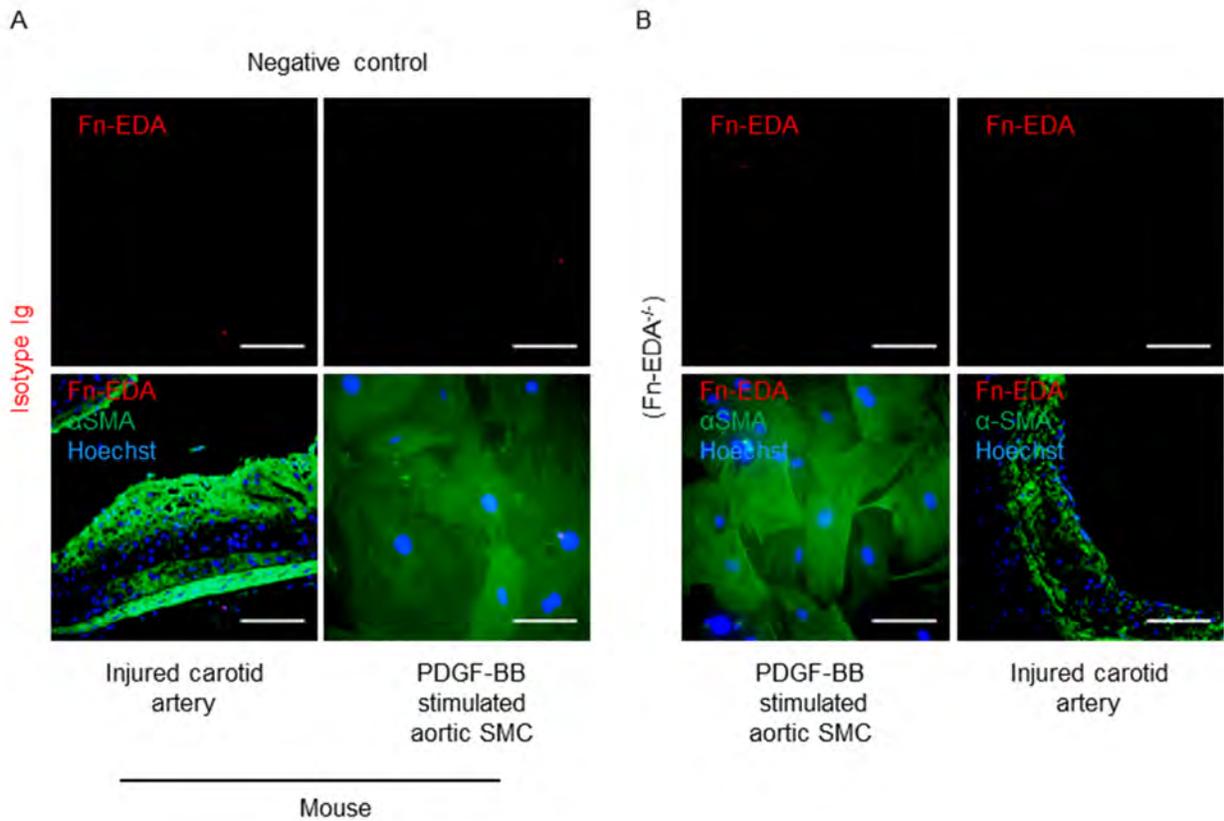
Figure S2. Negative controls. (A) Representative images of isotype controls for Fn-EDA (red) in injured carotid artery of Apoe$^{-/-}$ mice harvested after 28 days of injury and PDGF-BB stimulated SMCs from Apoe$^{-/-}$ mouse. N=5-6/group. SMC are stained with αSMA (green) and nuclei are counterstained with Hoechst (blue). Scale bar for arterial sections, 50 µm; for isolated SMC, 25 µm. **(B)** Fn-EDA was not detected in PDGF-BB stimulated SMCs or wire injured carotid artery sections of Fn-EDA$^{-/-}$Apoe$^{-/-}$ mice confirming specificity of anti-Fn-EDA Ig.
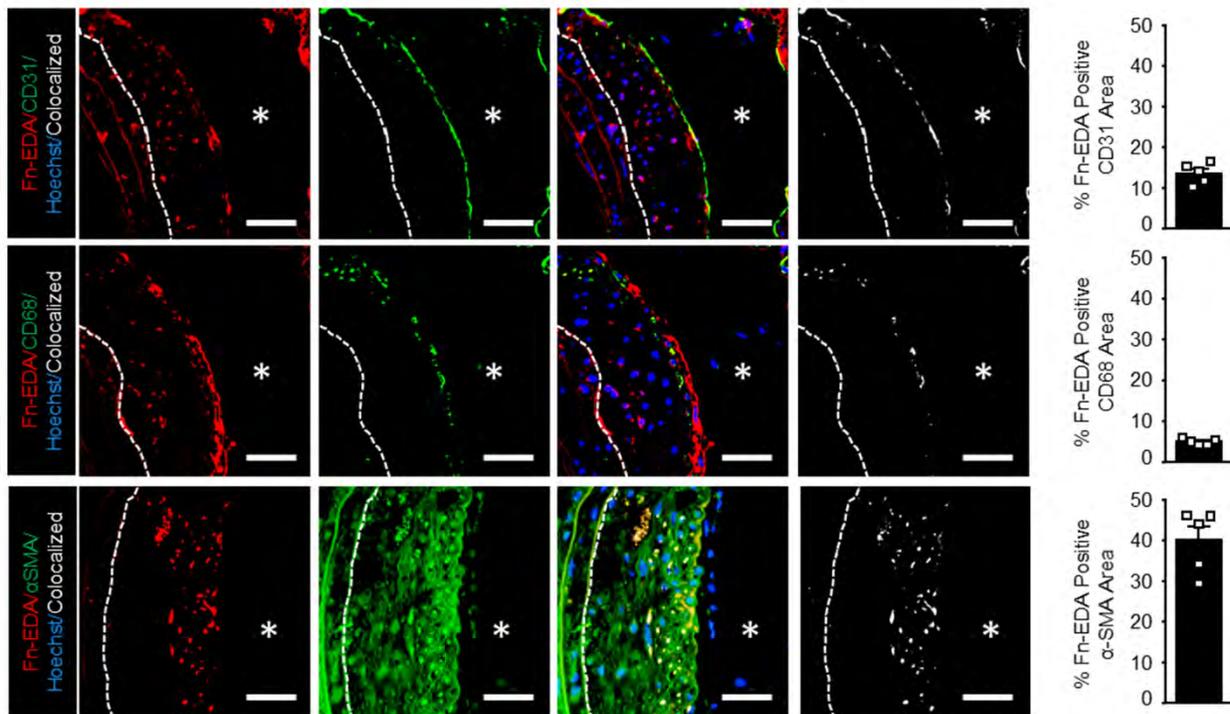
Figure S3. Cellular distribution of Fn-EDA in the neointima of Apoe^{-/-} mice. Representative images showing double-immunostaining of Fn-EDA (red) with endothelial cells (CD31, green, upper panel); macrophage (CD68, green, middle panel) and smooth muscle cells (αSMA, green, lower panel) in injured carotid artery of Apoe^{-/-} mice harvested after 28 days of injury. Nuclei are counterstained with Hoechst (blue). Scale bar, 50 μm. Dotted line separates external elastic lamina and neointima while * denotes lumen. Respective quantification in the right side is showing percentage of Fn-EDA positive endothelial cells, macrophages or smooth muscle cells. N=5-6/group. Values are represented as mean ± SEM.
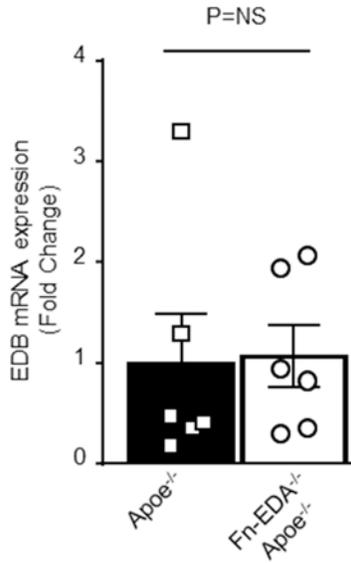
Figure S4. Effect of Fn-EDA deletion on Fn-EDB expression. Quiescent SMCs from Apoe$^{-/-}$ and Fn-EDA$^{-/-}$Apoe$^{-/-}$ were stimulated with PDGF-BB (20 ng/mL) for 24 hours and cells were processed for mRNA expression. Bar graph representing mRNA expression of Fn-EDB normalized to GAPDH and expressed as fold change (N=6/group). Values are represented as mean ± SEM. Statistical analysis: unpaired student t test.

A

Fibronectin

β-actin

#1 #2   #1 #2

Apoe⁻/⁻   Fn-EDA⁻/⁻
           Apoe⁻/⁻

P=NS

Fold change
(normalized to β-actin)

1.5

1.0

0.5

0.0

Apoe⁻/⁻   Fn-EDA⁻/⁻
           Apoe⁻/⁻

B

Apoe⁻/⁻

Fn-EDA⁻/⁻
Apoe⁻/⁻

Fibronectin
αSMA
Hoechst

PDGF-BB Stimulated VSMCs

P=NS

Mean fluorescence intensity (A.U.)

4

3

2

1

0

Apoe⁻/⁻   Fn-EDA⁻/⁻
           Apoe⁻/⁻

C

Apoe⁻/⁻

Fn-EDA⁻/⁻Apoe⁻/⁻

Fibronectin
αSMA
Hoechst

Injured carotid (28 Days)

P=NS

Mean fluorescence intensity (A.U.)

10

8

6

4

2

0

Apoe⁻/⁻   Fn-EDA⁻/⁻
           Apoe⁻/⁻

D

Quality of
Fibronectin Fibers

Fibronectin/αSMA/Hoechst

Apoe⁻/⁻

Fibronectin/αSMA/Hoechst

Fn-EDA⁻/⁻ Apoe⁻/⁻

Figure S5. Effect of Fn-EDA deletion on total Fn levels. Quiescent SMCs were stimulated with PDGF-BB (20 ng/mL) for 24 hours. **(A)** Western blotting and **(B)** immunofluorescence staining of total Fn (N=6/group). The right panel in A and B shows quantification of Fn expression (N=6 /group). Scale bar, 50 μm. **(C)** The left panels show representative double immunostaining for total Fn (red) and SMC (green) in carotid artery sections of in Apoe$^{-/-}$ and Fn-EDA$^{-/-}$Apoe$^{-/-}$ mice harvested after 28 days of wire injury. Scale bar: 50 μm. The right panel show quantification of total Fn expression (N=6/group). **(D)** Immunofluorescence analysis of Fn (red) and actin (αSMA, green) fibers. Nuclei are counterstained with Hoechst (blue). Values are expressed as mean ± SEM. Statistical analysis: unpaired student t test.
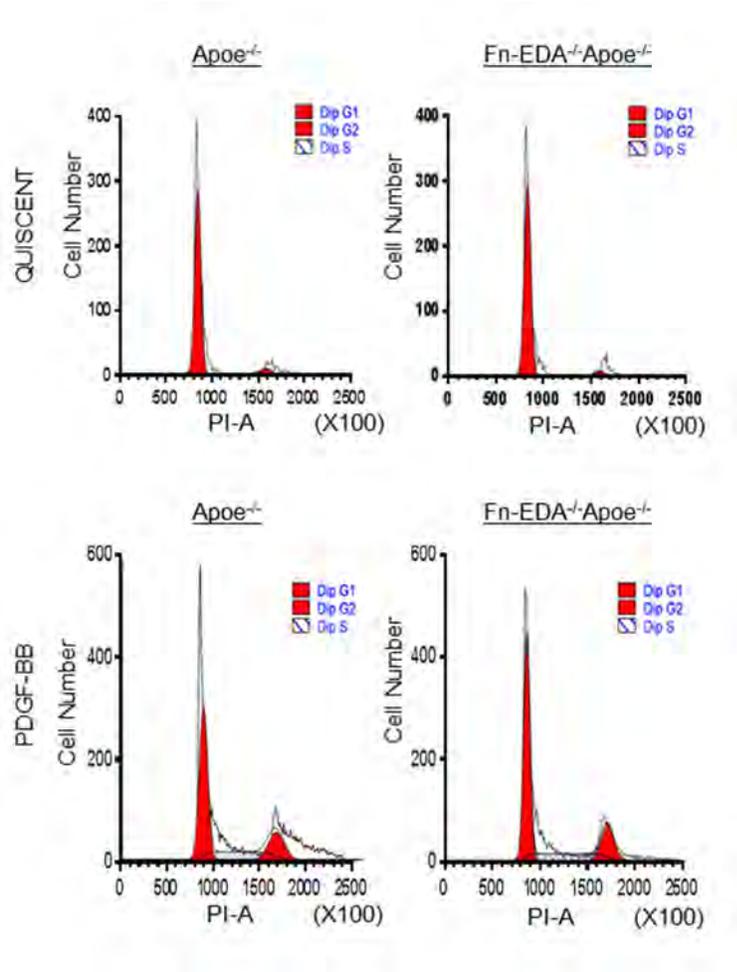
Figure S6: Cell cycle histograms. Serum-starved SMCs were stimulated with PDGF-BB for 24 hours. Representative DNA histograms of propidium iodide fluorescence in cells assessed by flow cytometry are shown.
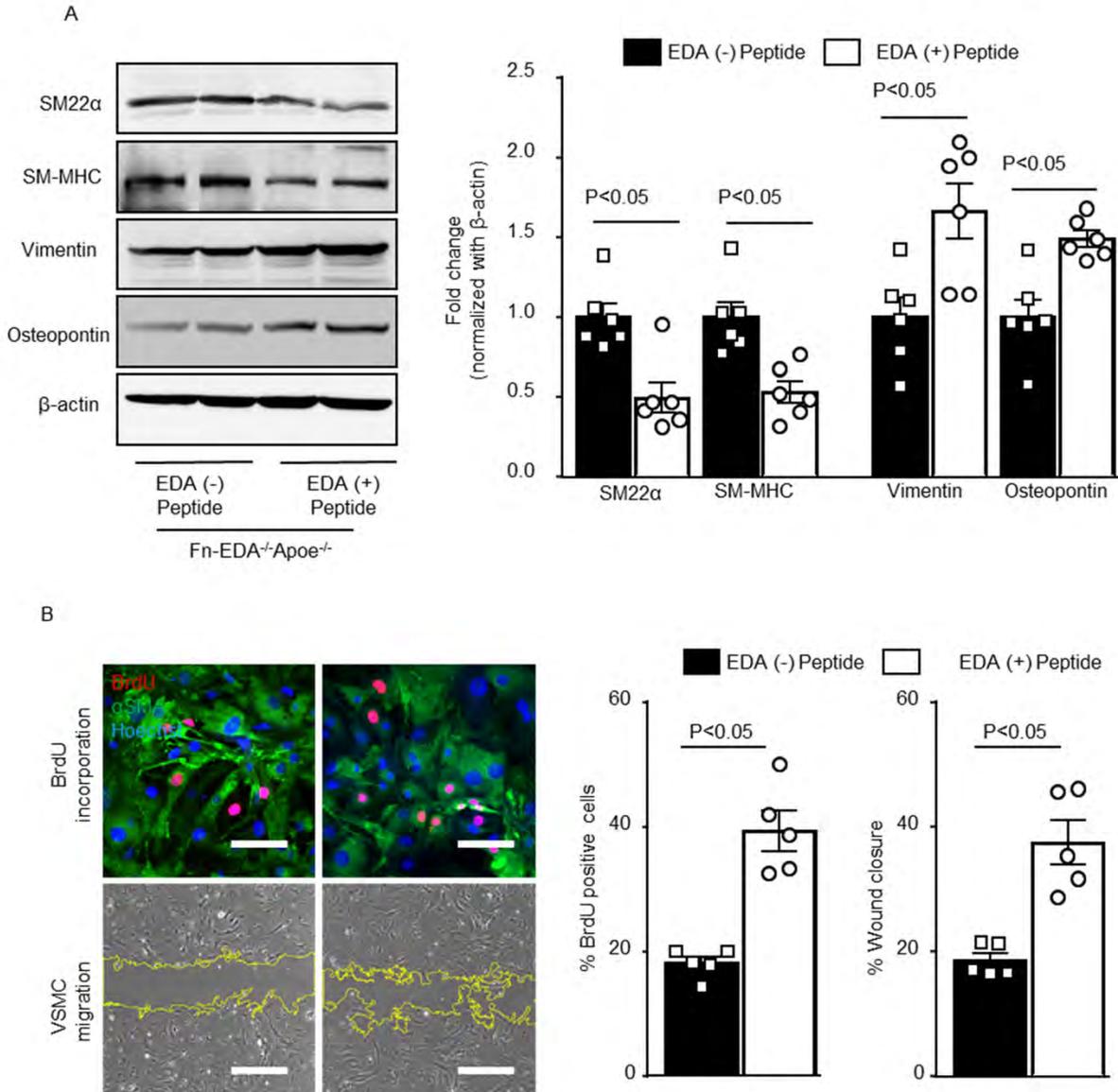
Figure S7: Fn-EDA peptide potentiates SMC phenotypic switching. Quiescent SMCs were exposed to 10 µg/ml of recombinant peptides containing (EDA+) or lacking (EDA-) for 24 hours. **(A)** Cells were processed for Western blotting. Representative immunoblots and densitometric analysis of contractile proteins [smooth muscle 22 alpha (SM22α), smooth muscle myosin heavy chain (SM-MHC)], and synthetic proteins (vimentin and osteopontin), N=6/group. All blots are with same biological samples. #1 and #2 represent two separate samples. **(B)** The upper left panels show representative images of SMC proliferation where BrdU-positive cells (red) were co-stained with αSMA (green) and Hoechst (blue). Scale bar: 50 μm. Lower left panels show representative phase-contrast images of SMC migration in the scratch assay. Scale bar: 500 µm. The middle and right panels show quantification of BrdU-positive cells (N=5/group) and migrated area (N=5). Values are expressed as mean ± SEM. Statistical analysis: unpaired student t-test.
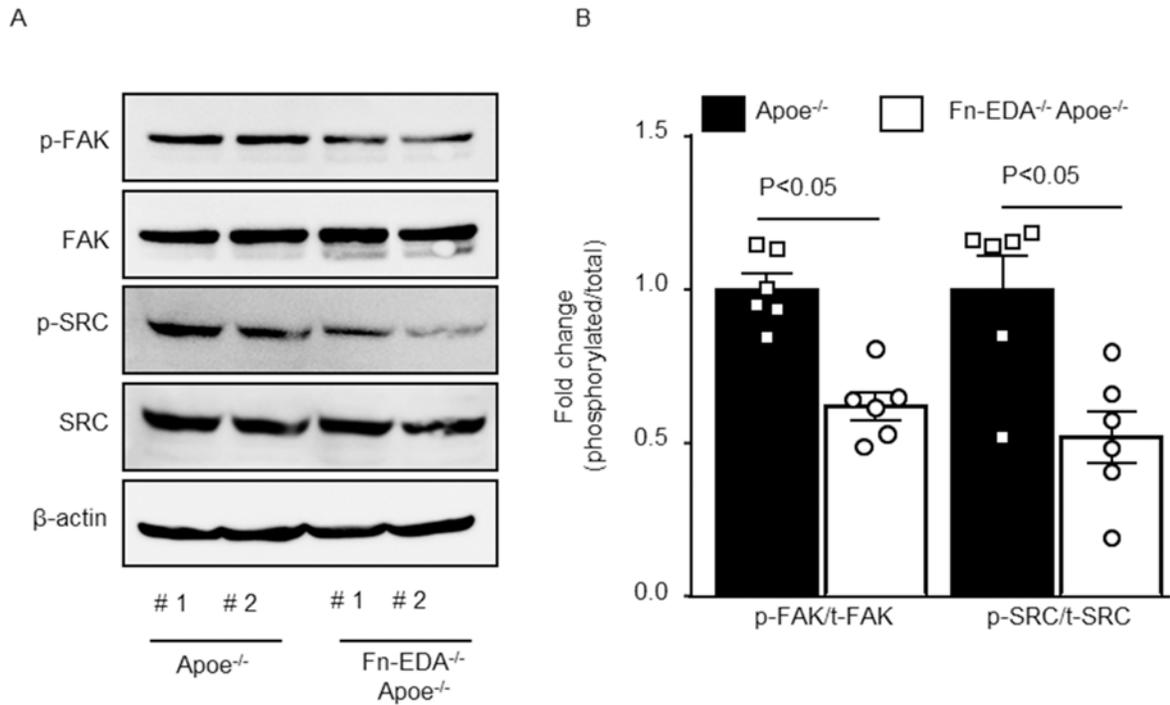
Figure S8. Defective Integrin signaling in Fn-EDA$^{-/-}$Apoe$^{-/-}$ mice. Quiescent SMCs from Apoe$^{-/-}$ and Fn-EDA$^{-/-}$Apoe$^{-/-}$ were stimulated with PDGF-BB (20 ng/ml) for 15 minutes and cells were processed for Western blotting. **(A, B)** Representative Immunoblots and densitometric analysis of FAK and Src (N=6/group). Blots for FAK and SRC are with same biological samples run in parallel. Values are expressed as mean ± SEM. Statistical analysis: unpaired student t-test.

A

pERK 1/2

ERK 1/2

β-actin

cFn (20 µg/ml) 0   15   30   60   180

Time (min)

B

BrdU incorporation

VSMC migration

cFn   (20 µg/ml)   -   +   +   +
U0126 (10 µM)   -   -   +   +

Fn-EDA$^{-/-}$Apoe$^{-/-}$ Fn-EDA$^{-/-}$
                                        TLR4$^{-/-}$ Apoe$^{-/-}$

Fn-EDA$^{-/-}$Apoe$^{-/-}$
Fn-EDA$^{-/-}$TLR4$^{-/-}$Apoe$^{-/-}$

% BrdU positive cells

cFn   (20 µg/ml)   -   +   +   +
U0126 (10 µM)   -   -   +   +

% Wound closure

cFn   (20 µg/ml)   -   +   +   +
U0126 (10 µM)   -   -   +   +
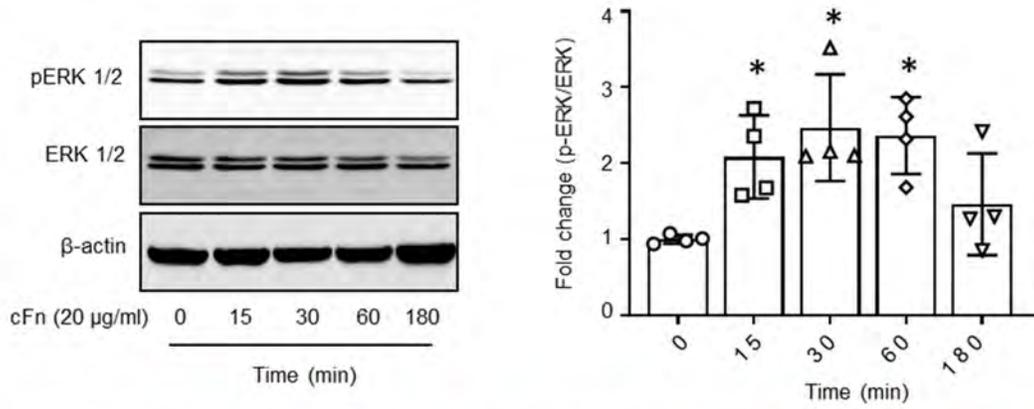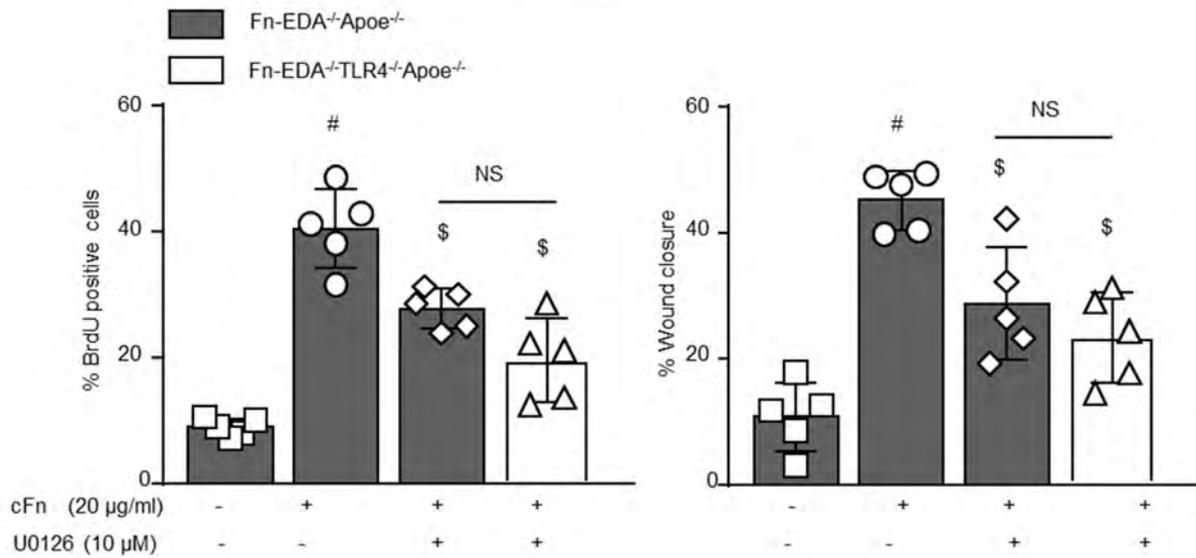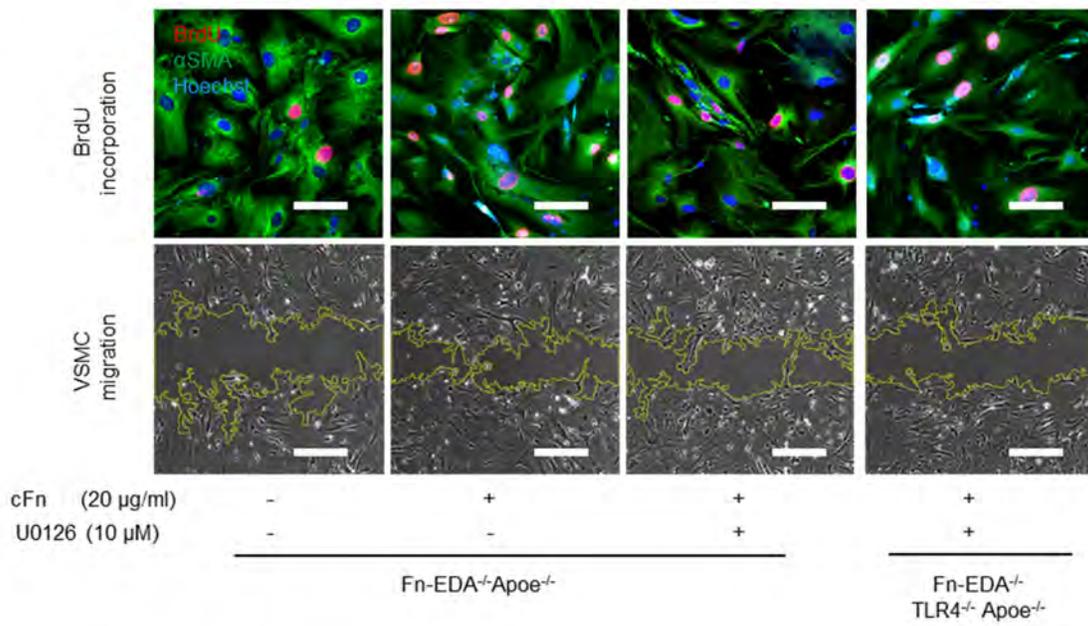
Figure S9: cFn promotes ERK dependent SMC proliferation and migration. Quiescent SMCs from Fn-EDA$^{-/-}$Apoe$^{-/-}$ were used **(A)** Cells were stimulated with PDGF-BB (20 ng/mL) for indicated time points and cells were processed for Western blotting. Representative Immunoblots and densitometric analysis of p-ERK 1/2, ERK 1/2 and β-actin (N=4/group). **(B)** Cells were pretreated with ERK inhibitor (U0126, 10 µM) for 30 minutes and then stimulated with either cFn-EDA (20 µg/ml) or plasma Fn lacking EDA (20 µg/ml) for 24 hours. The upper panel shows representative images of SMC proliferation and migration. The bottom panels show quantification (N=5/group). Values are expressed as mean ± SEM. Statistical analysis: One way ANOVA with Bonferroni post hoc test. *****P<0.05 vs. 0 hours, #p<0.05 vs. untreated EDA$^{-/-}$Apoe$^{-/-}$ cells, $^{\$}$P<0.05 vs. cFn-EDA stimulated cells. Scale bar, BrdU assay, 50 µm; migration assay, 500 µm. NS= Not significant**.**
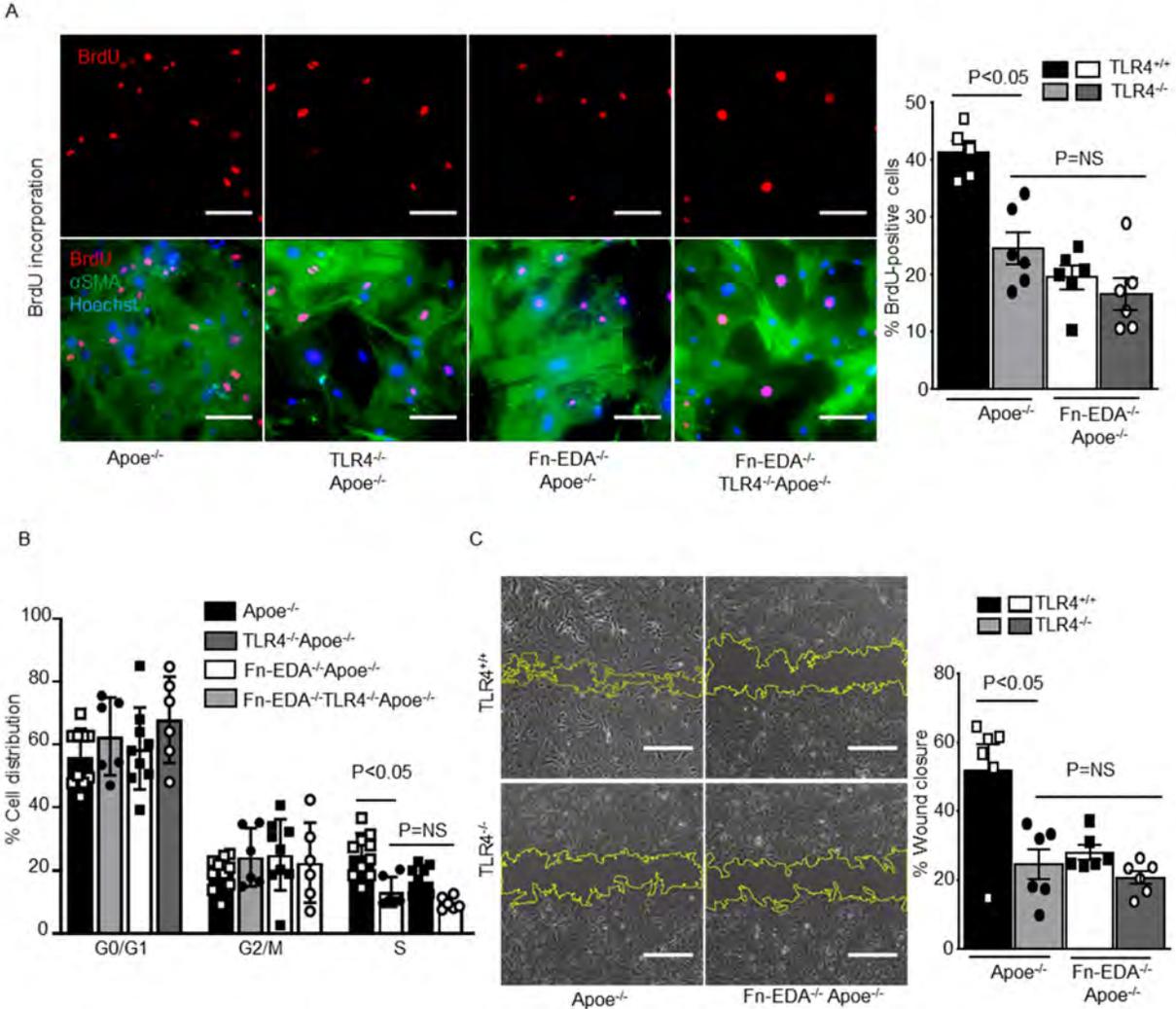
Figure S10: Fn-EDA promotes TLR4-dependent SMC proliferation. Quiescent SMCs from each genotype were stimulated with PDGF-BB for 24 hours. **(A)** The left panels show representative images of BrdU incorporation in SMCs. N=5-6/group. Scale bar: 50 μm. Right panel shows quantification of percent BrdU positive cells. **(B)** Bar diagram represents cell phase distribution in PDGF-BB stimulated SMCs from different genotypes (N=7-10). **(C)** The left panels show representative phase-contrast images of SMC migration in the scratch assay. The right panel shows quantification of the migrated area (N=6-8/group). Scale bar: 500 μm. Values are expressed as mean ± SEM. Statistical analysis: one way ANOVA with Bonferroni post hoc test. NS= Not significant.
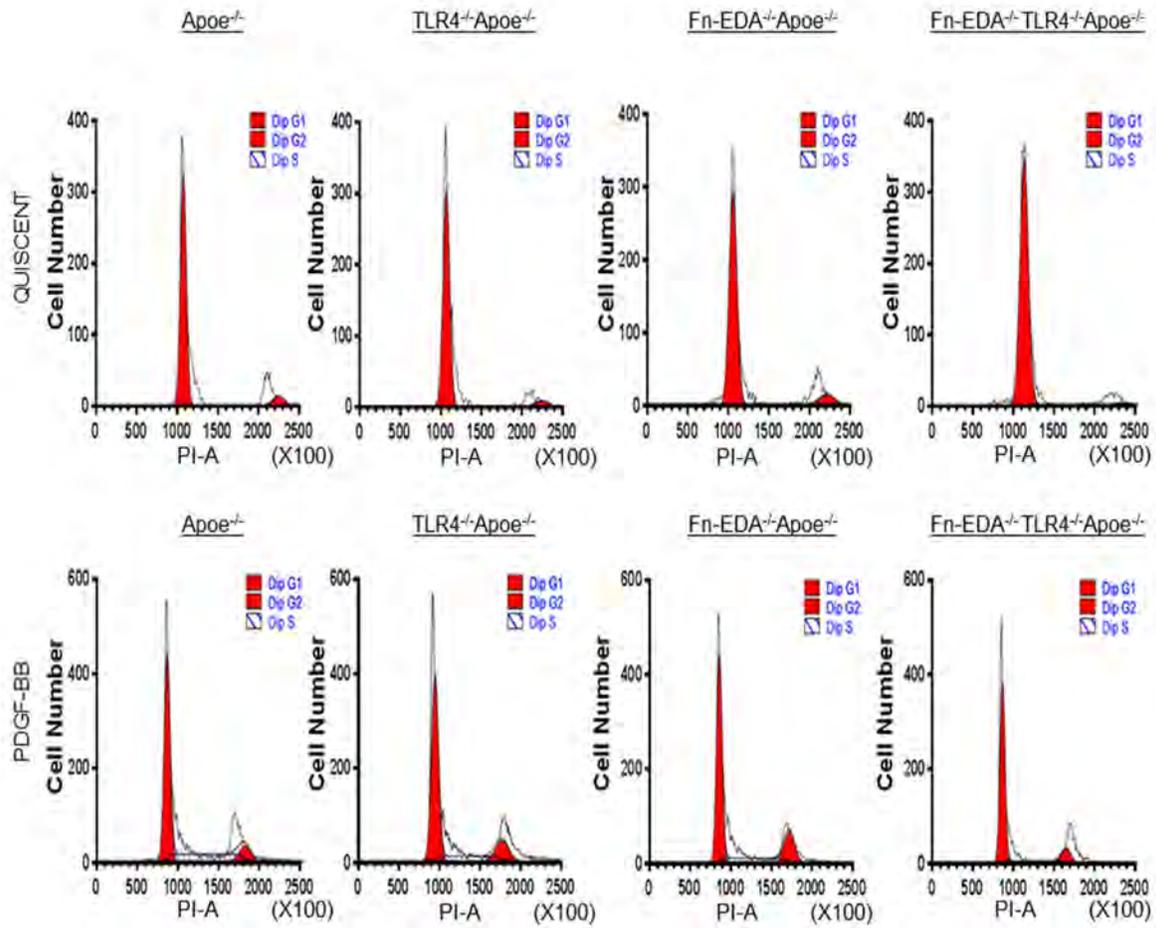
Figure S11: Cell cycle histograms. Serum-starved SMCs were stimulated with PDGF-BB for 24 hours. Representative DNA histograms of propidium iodide fluorescence in cells assessed by flow cytometry are shown.
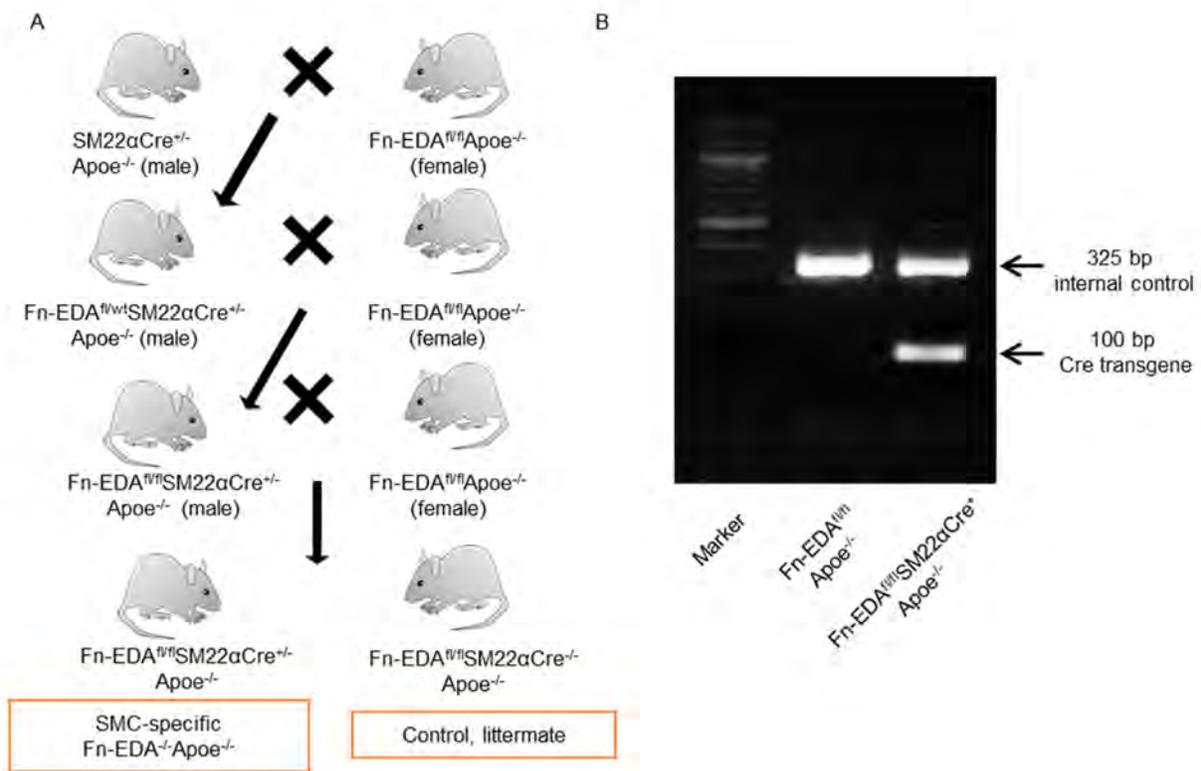
Figure S12. SMC-specific Fn-EDA deficient mice. (A) Schematic showing the strategy to generate SMC-specific Fn-EDA deficient mice. **(B)** Genomic PCR showing the presence of SM22αCre gene in Fn-EDA$^{fl/fl}$Apoe$^{-/-}$ mice.
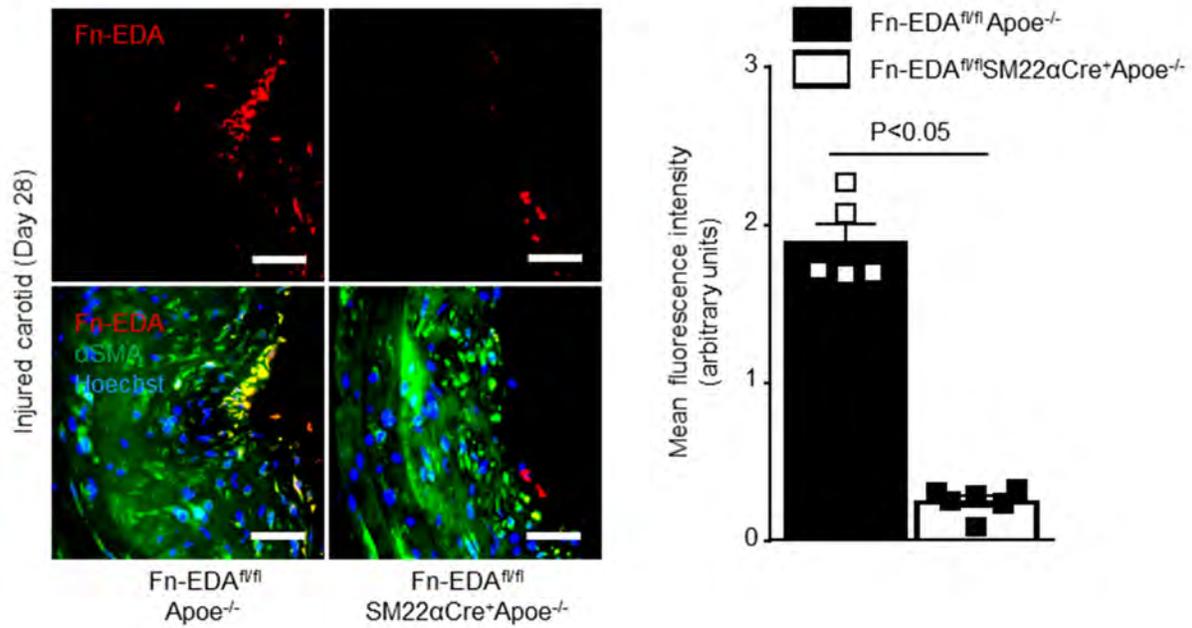
Figure S13. The left panels show representative double immunostaining for Fn-EDA (red) and SMC (green) in carotid artery sections of Fn-EDA$^{fl/fl}$Apoe$^{-/-}$ and Fn-EDA$^{fl/fl}$SM22αCre^{+}Apoe$^{-/-}$ mice harvested after 28 days of wire injury. The right panel show quantification of Fn-EDA (N=5-6/group). Scale bar: 50 μm. Values are expressed as mean ± SEM. Statistical analysis: unpaired student t test.

A

Male

Female

Apoe^{-/-}　　Fn-EDA^{fl/fl}Apoe^{-/-}

Female
Male
Apoe^{-/-}
Fn-EDA^{fl/fl}Apoe^{-/-}

Area (X10^4 μm^2)

9.0
7.5
6.0
4.5
3.0
1.5
0.0

Intima　　　Media

Intima/Media area ratio

1.2
0.9
0.6
0.3
0.0

B

BrdU

BrdU
αSMA
Hoechst

BrdU Incorporation

Apoe^{-/-}　　Fn-EDA^{fl/fl}Apoe^{-/-}　　Apoe^{-/-}　　Fn-EDA^{fl/fl}Apoe^{-/-}

Quiescent　　　　　　PDGF-BB

Apoe^{-/-}
Fn-EDA^{fl/fl}Apoe^{-/-}

% BrdU Positive cells

50
40
30
20
10
0

Quiescent　　PDGF-BB

C

Apoe^{-/-}
Fn-EDA^{fl/fl}Apoe^{-/-}

% Cell Distribution

100
80
60
30
20
10
0

G0/G1　　G2/M　　S
Quiescent

Apoe^{-/-}
Fn-EDA^{fl/fl}Apoe^{-/-}

% Cell Distribution

100
80
60
40
20
0

G0/G1　　G2/M　　S
PDGF-BB

D

Apoe^{-/-}

Fn-EDA^{fl/fl}Apoe^{-/-}

VSMC Migration

Time	0 hrs	24 hrs	24 hrs
PDGF - BB	-	+	+

Apoe^{-/-}
Fn-EDA^{fl/fl}Apoe^{-/-}

% Wound closure

80
60
40
20
0

Quiescent　　PDGF-BB

Figure S14. Constitutive expression of Fn-EDA does not further promote intimal hyperplasia. (**A**) Representative photomicrographs of Verhoeff's Van Gieson stained carotid artery sections of and Fn-EDA$^{fl/fl}$Apoe$^{-/-}$ mice and Apoe$^{-/-}$ mice after 28 days of injury (N=10/group). Right panel is showing quantification of intima area, media area and a ratio of intima to the media area. Each dot represents a single mouse. (**B**) Representative images and quantification of BrdU-positive cells (red) co-stained with αSMA (green) and Hoechst (blue). Scale bar: 50 μm. (**C**) Quiescent or PDGF-BB-stimulated aortic SMCs were stained with propidium iodide and cell cycle was analyzed using flow cytometry. The panels show quantitative data of cell cycle distribution in quiescent and PDGF-BB stimulated SMCs (N=6-10/group). (**D**) Representative phase-contrast images of SMC migration in the scratch assay. The right panel shows quantification of the migrated area (N=6-8/group). Scale bar: 500 μm. Values are expressed as mean ± SEM. Statistical analysis: unpaired student t-test.
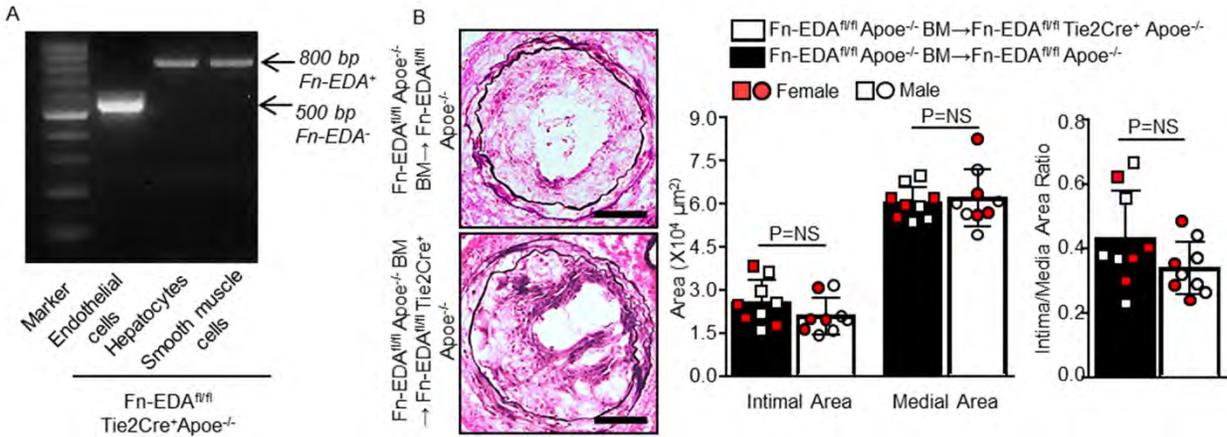
Figure S15. Endothelial cell-specific Fn-EDA deletion does not reduce neointimal hyperplasia. (A) RT-PCR confirmed the absence of Fn-EDA mRNA in endothelial cells but not in hepatocytes or SMCs of Fn-EDA$^{fl/fl}$Tie2Cre$^+$Apoe$^{-/-}$ mouse. (**B**) The left panels show representative photomicrographs of Verhoeff's Van Gieson stained carotid artery sections of male and female mice after 28 days of injury (N=4-5/group). Scale bar, 200 μm. The right panels (bar graphs) show quantification of intimal area, medial area and a ratio of intima to the media area. Each dot represents a single mouse. Values are expressed as mean ± SEM. Statistical analysis: unpaired student t-test. NS= Not significant.

Full unedited gel for Figure 3B-SM22α
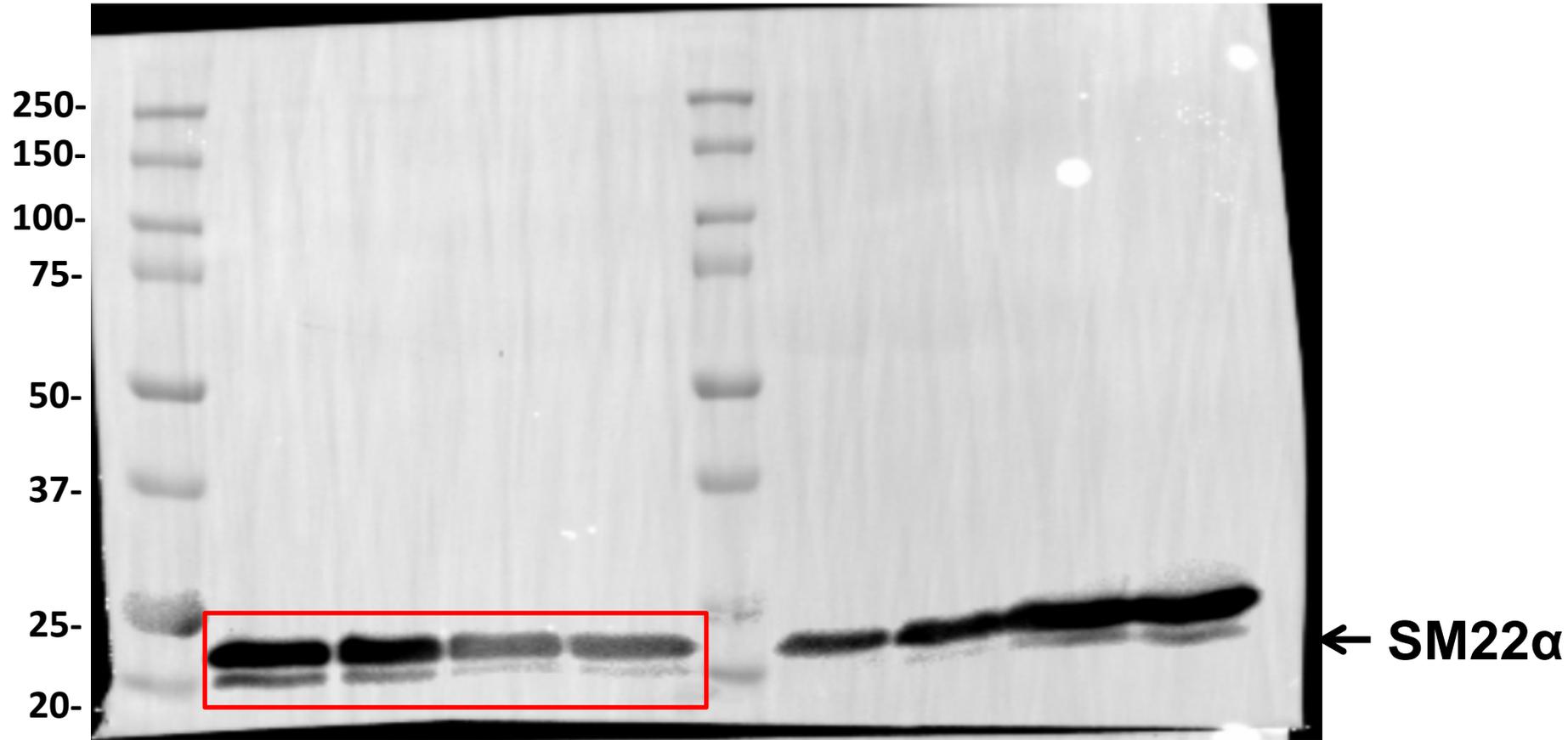
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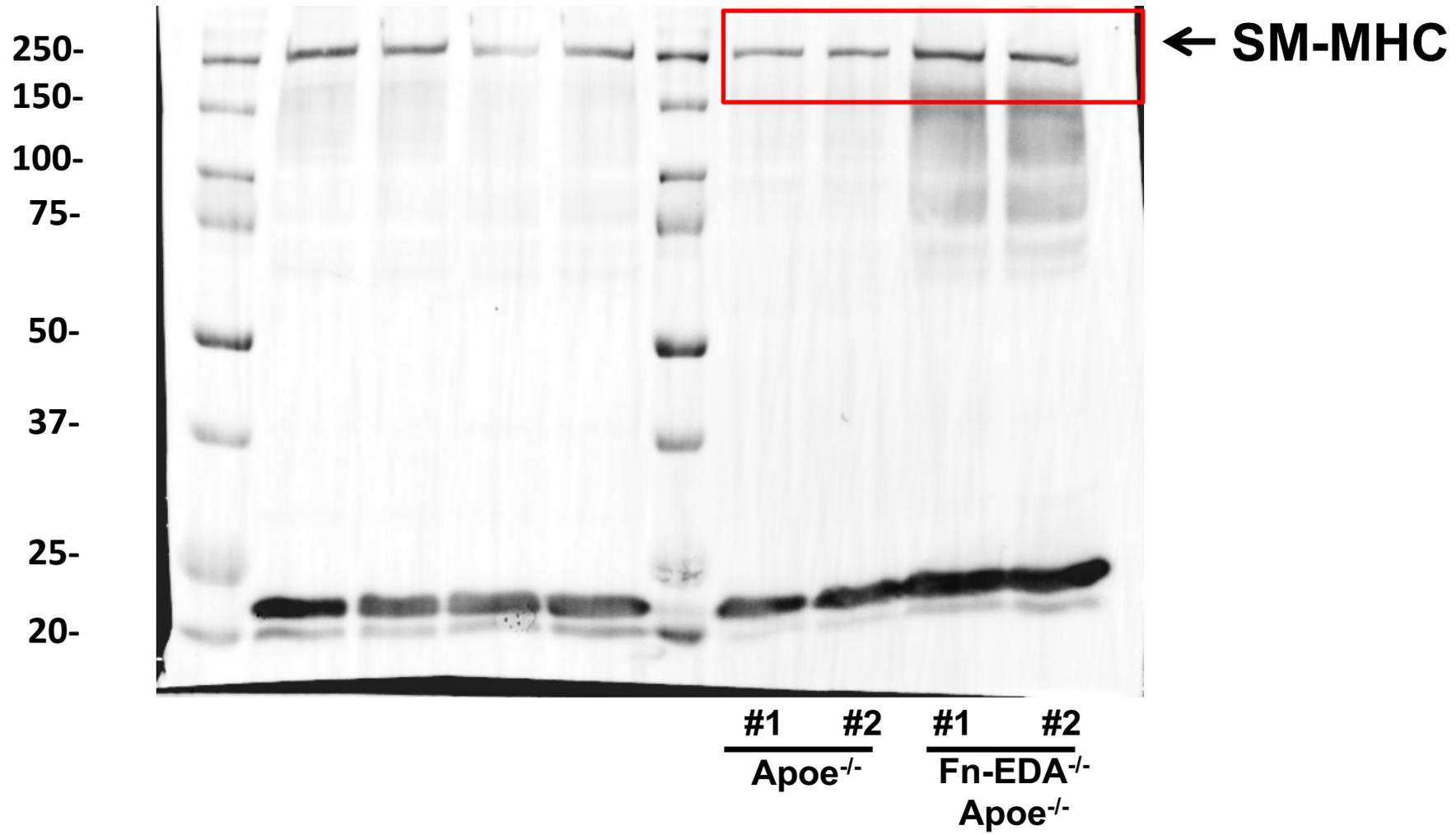
Full unedited gel for Figure 3B-Vimentin

250-
150-
100-
75-
50-
37-
25-
20-

← **Vimentin**

#2   #1   #2   #1
Fn-EDA$^{-/-}$ Apoe$^{-/-}$
Apoe$^{-/-}$
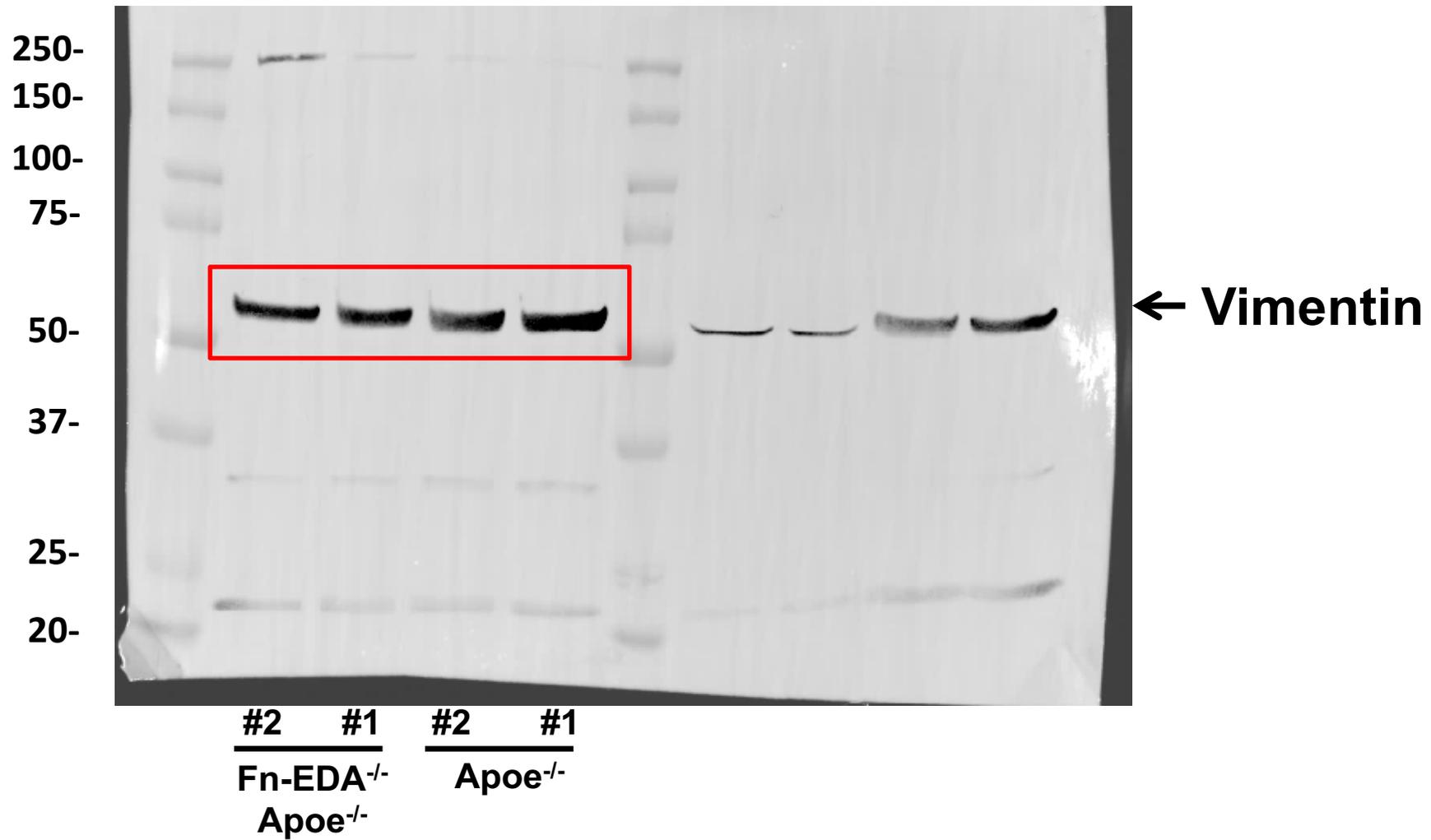
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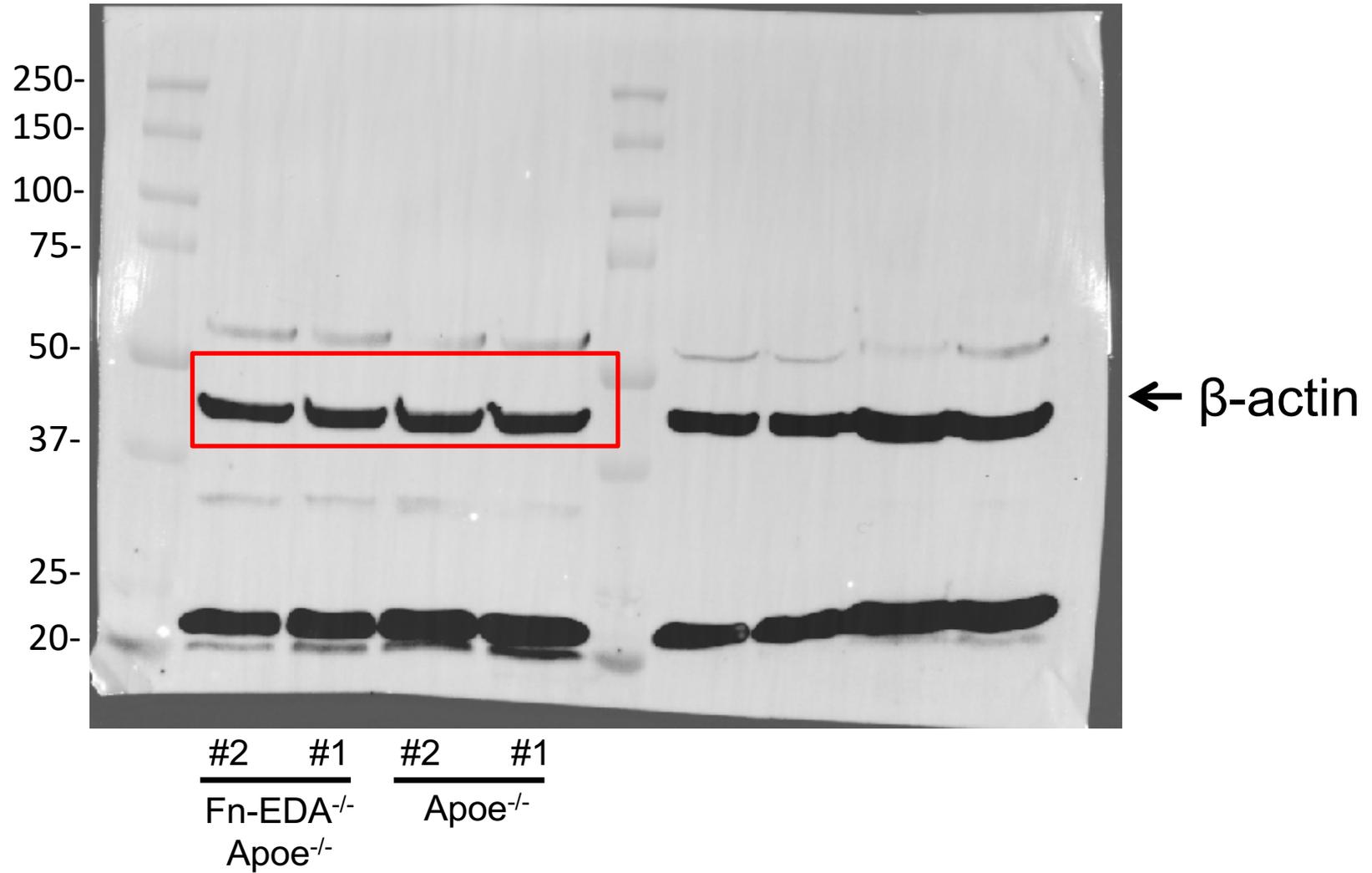
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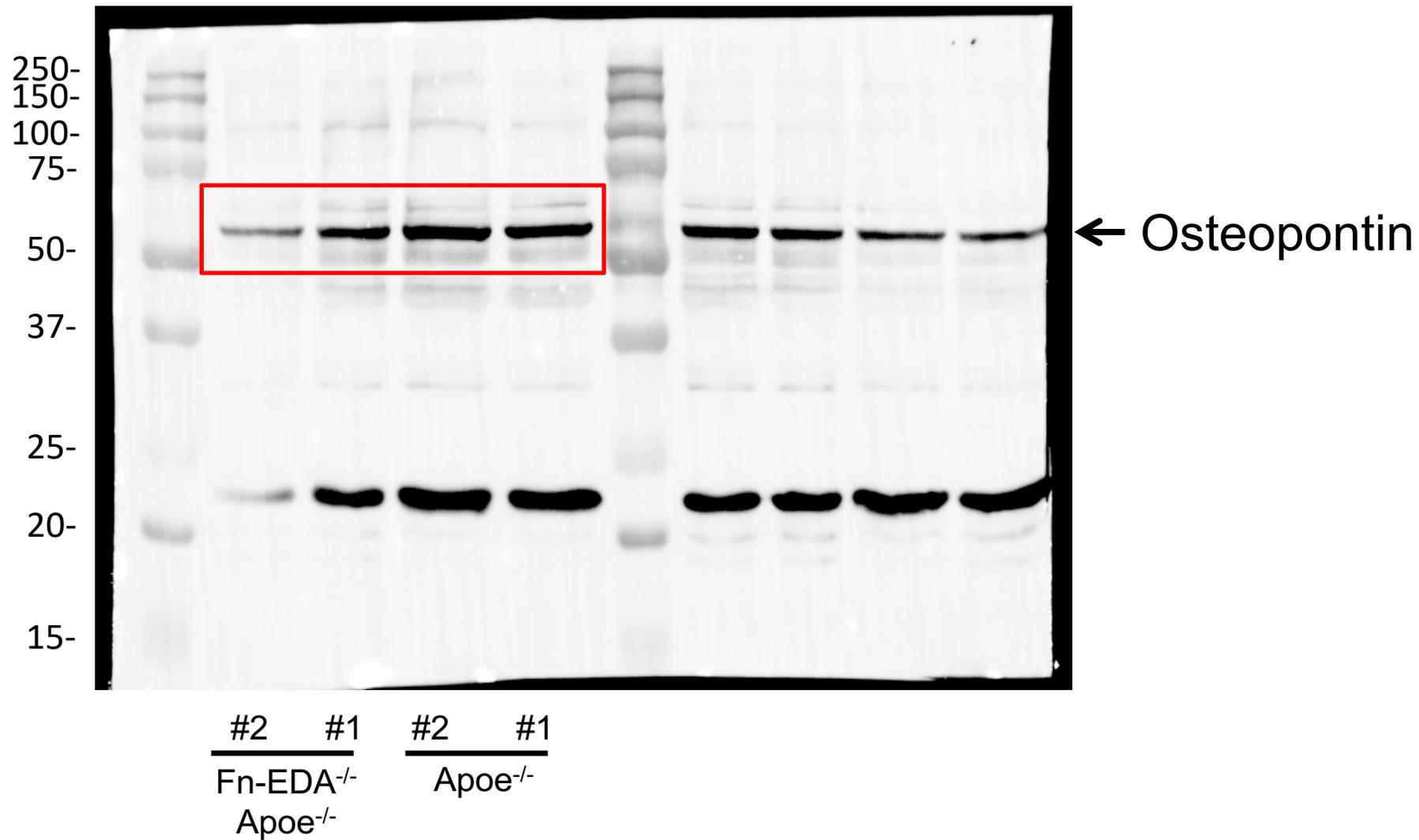
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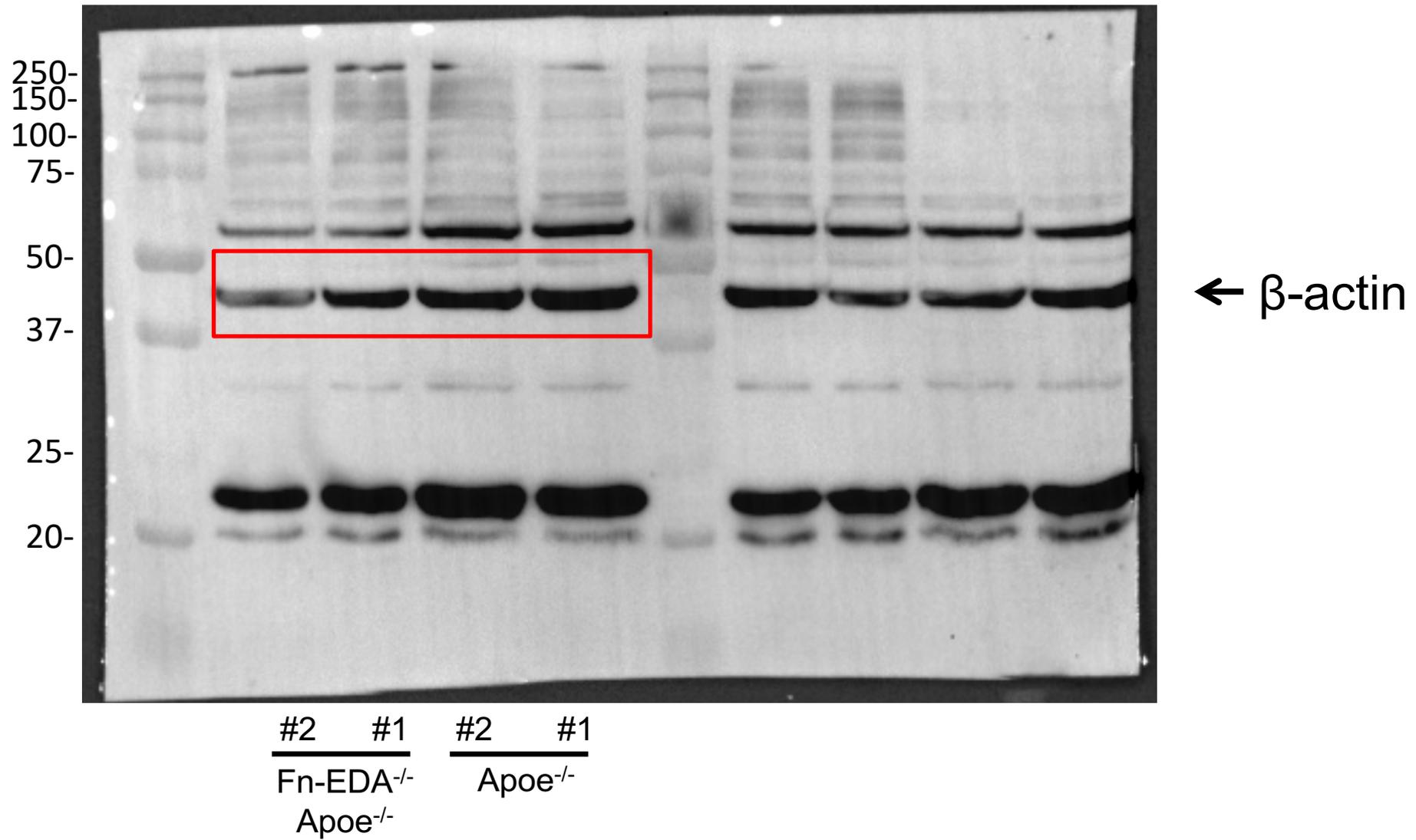
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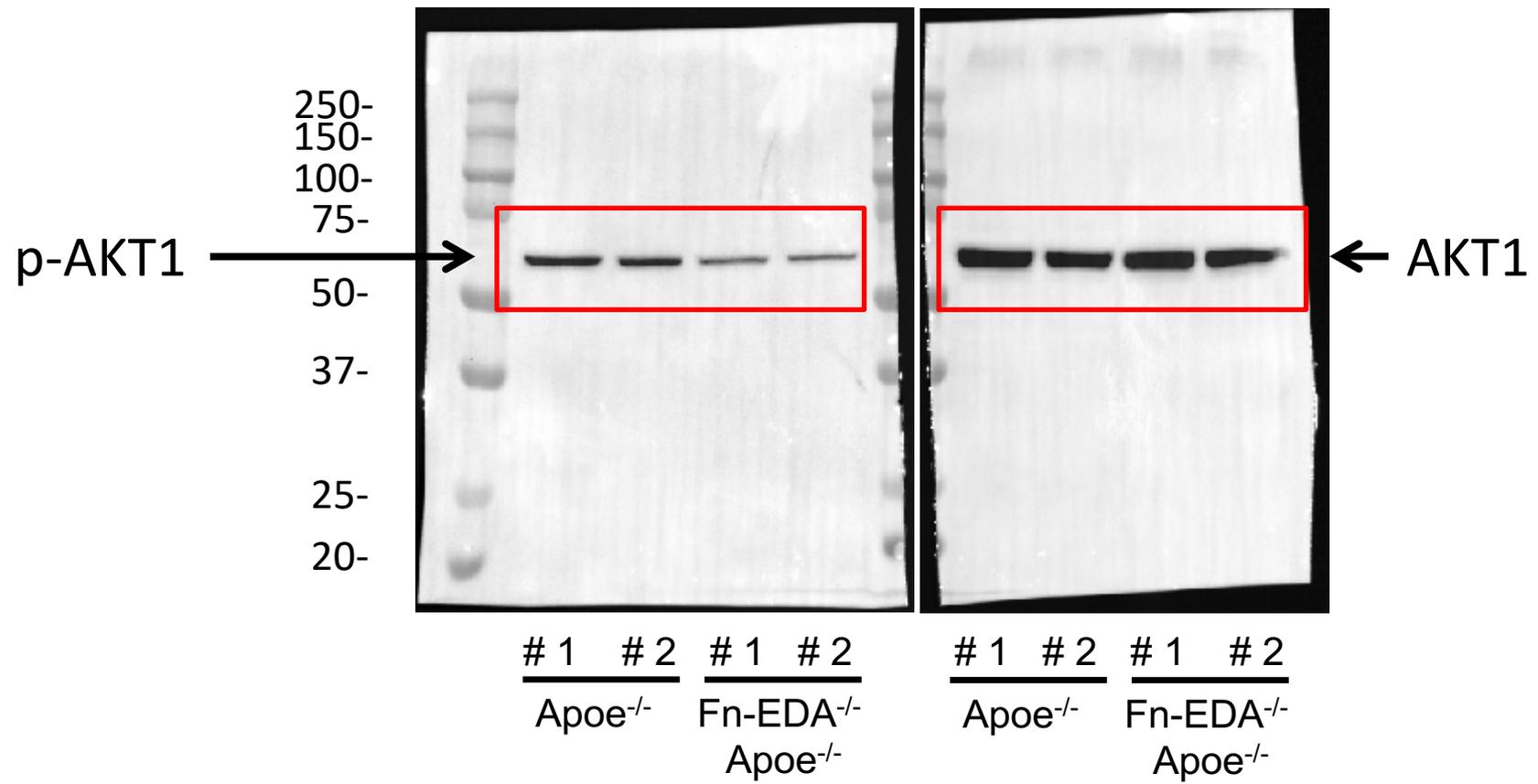
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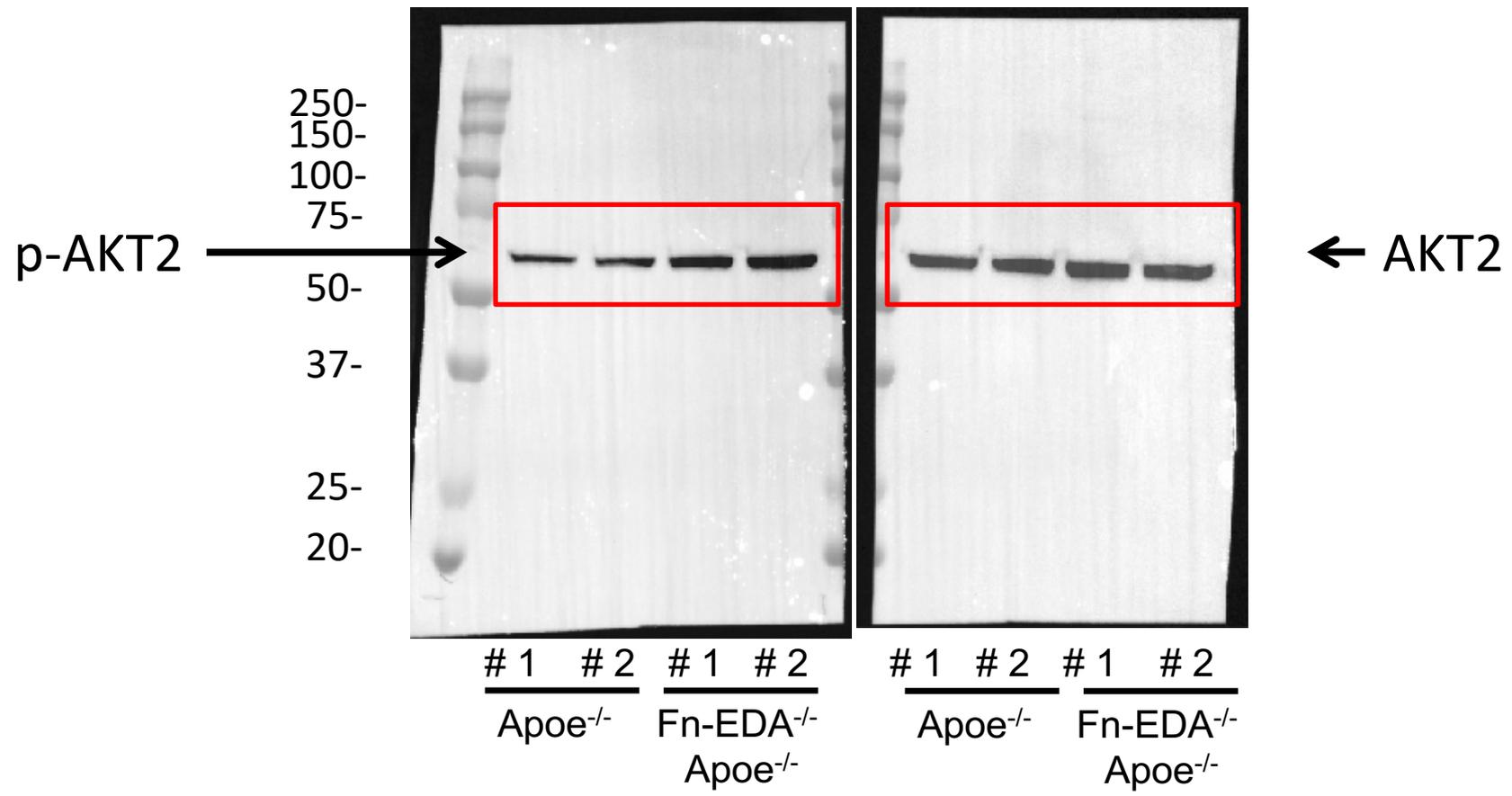
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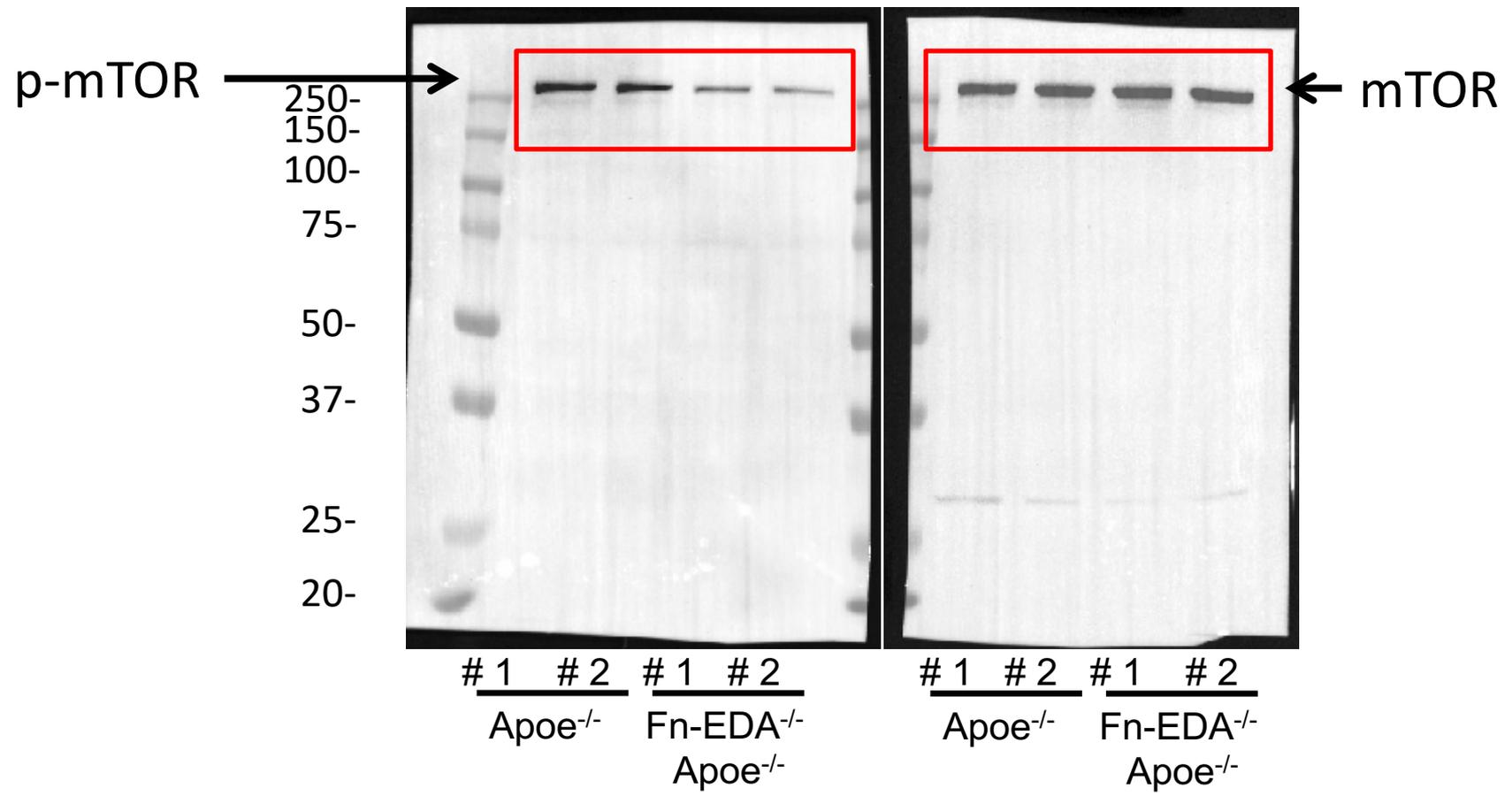
Full unedited gel for Figure 3C-p-NFκB (left) and Total NFκB (right)
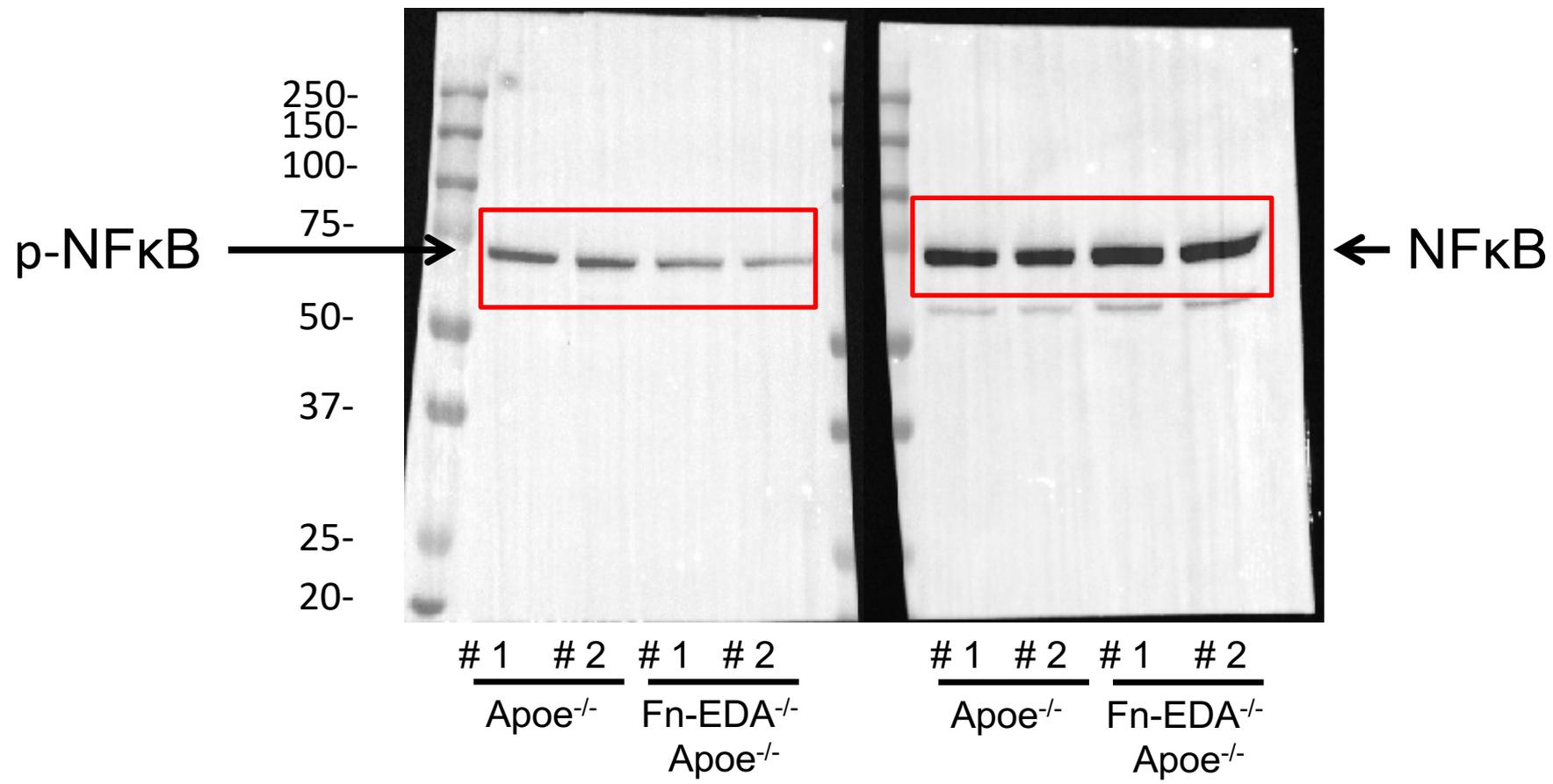
Full unedited gel for Figure 3C-β-actin
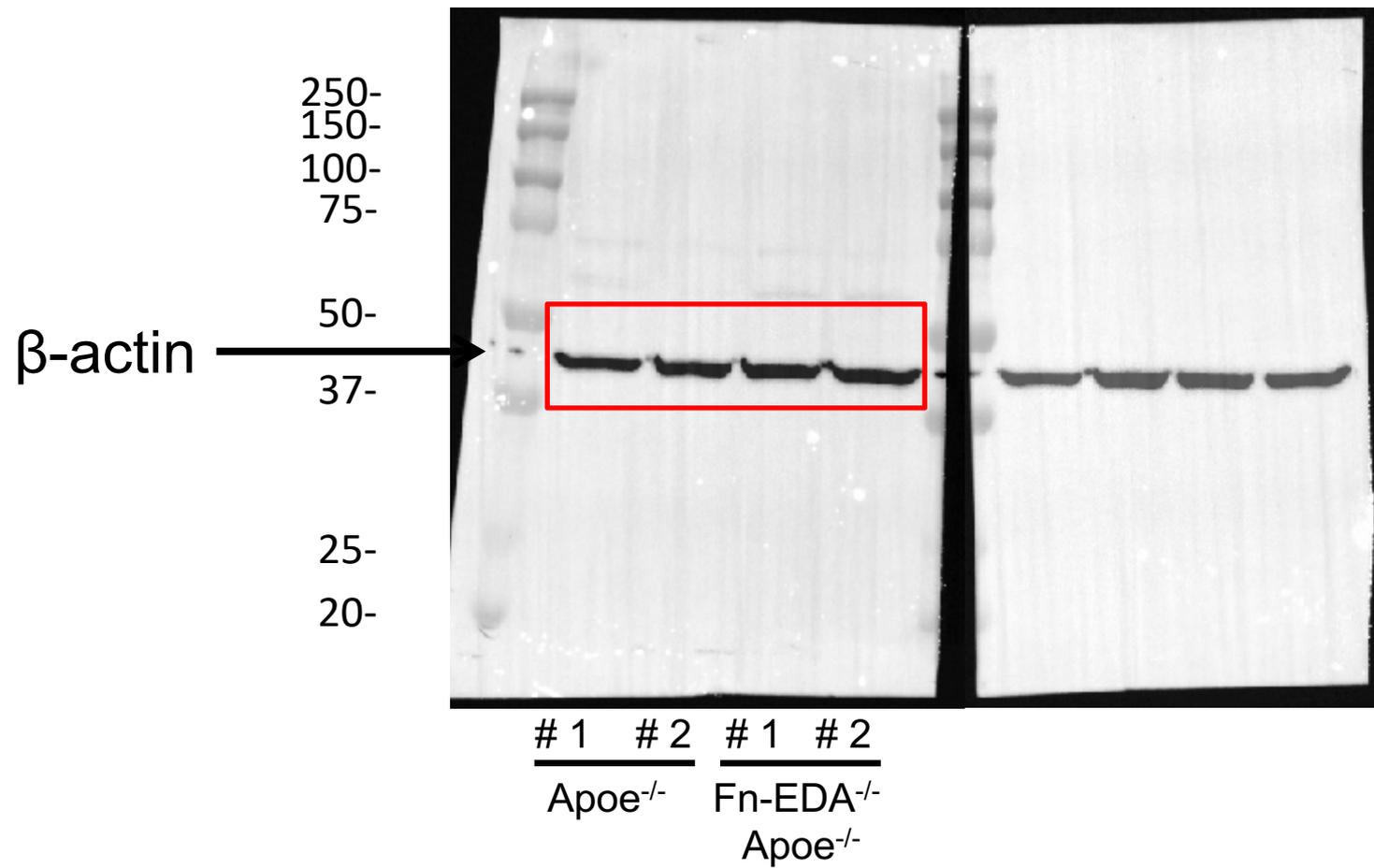
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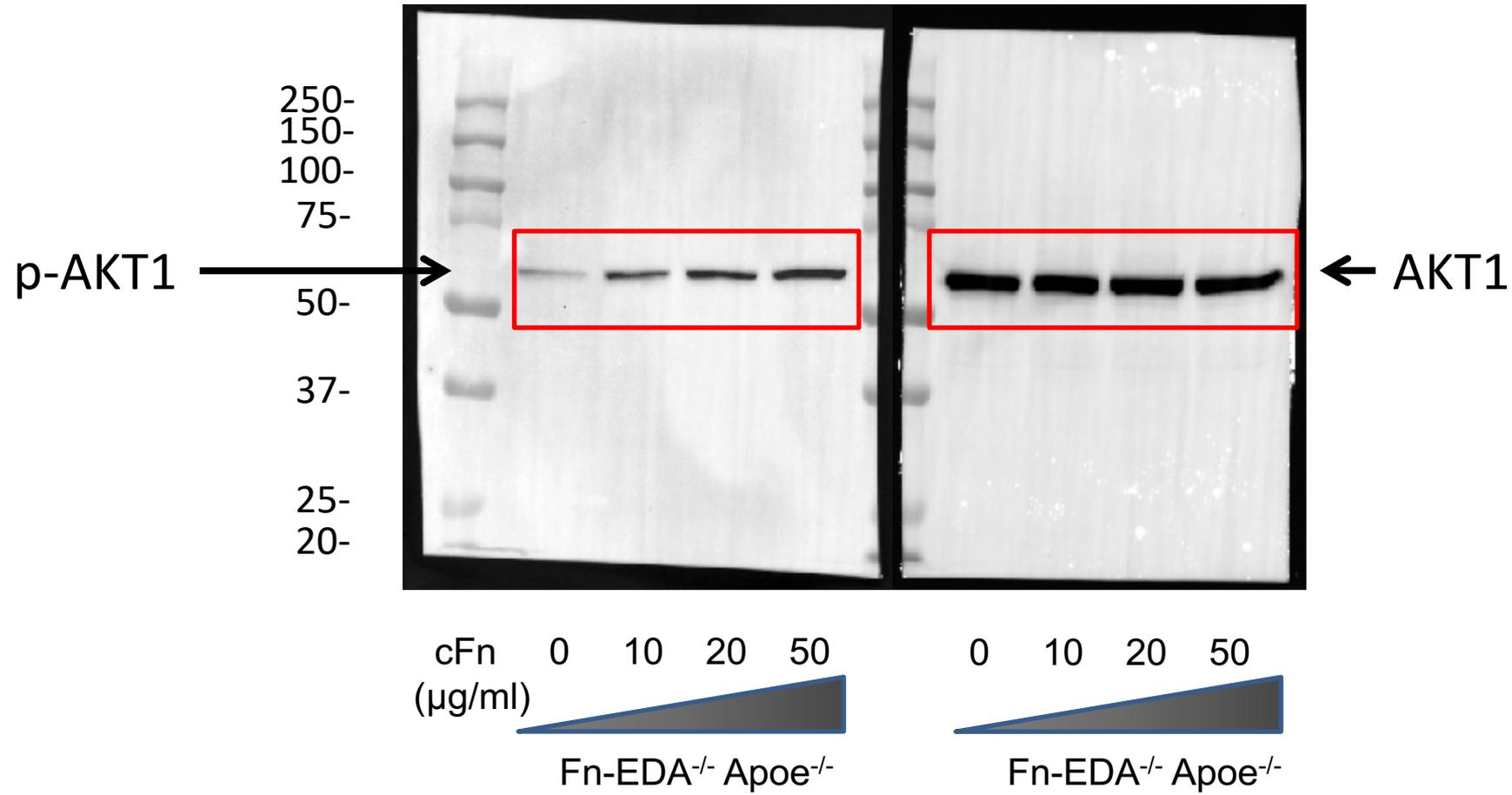
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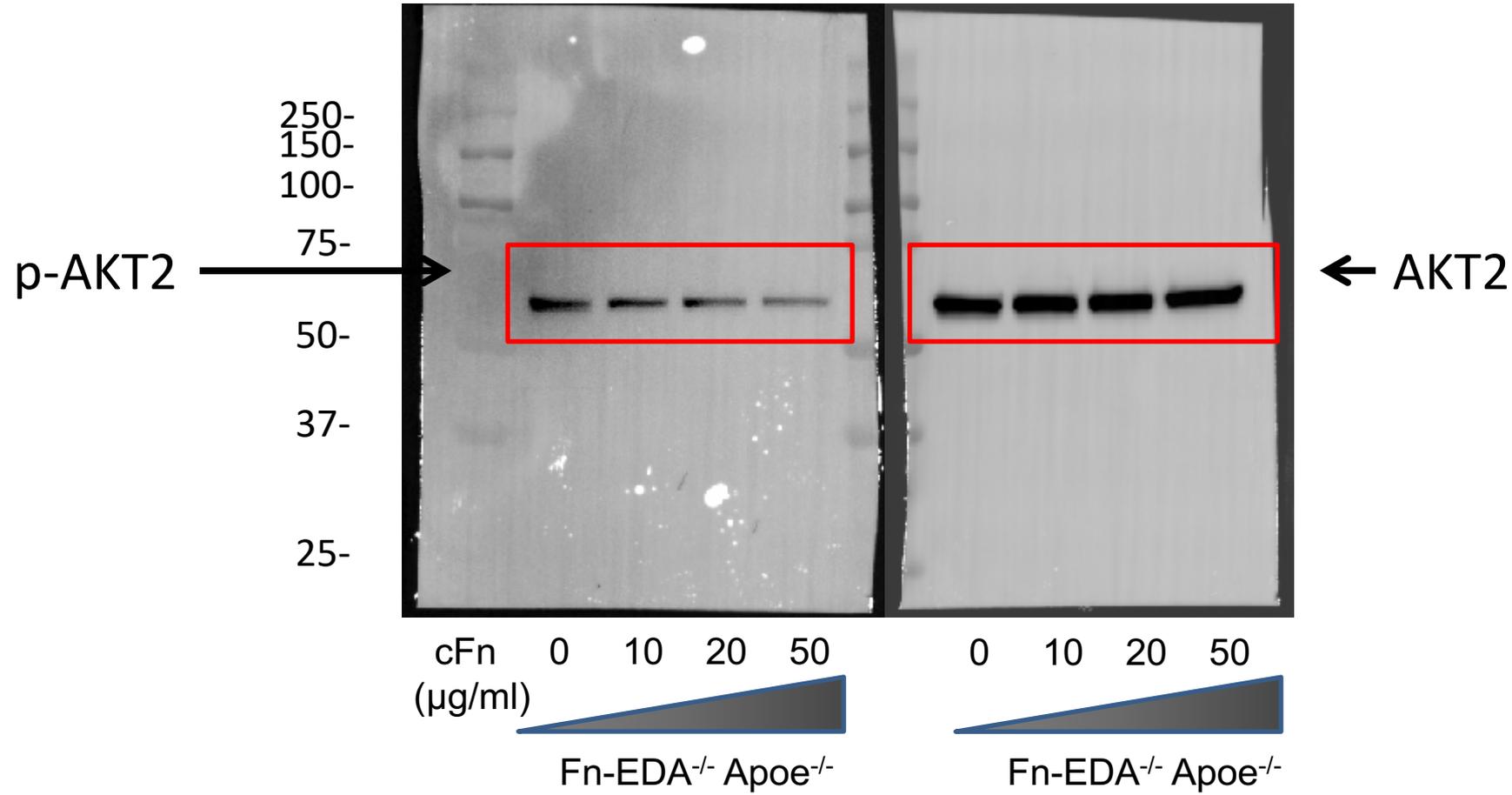
Full unedited gel for Figure 4A-p-mTOR (left) and Total mTOR (right)
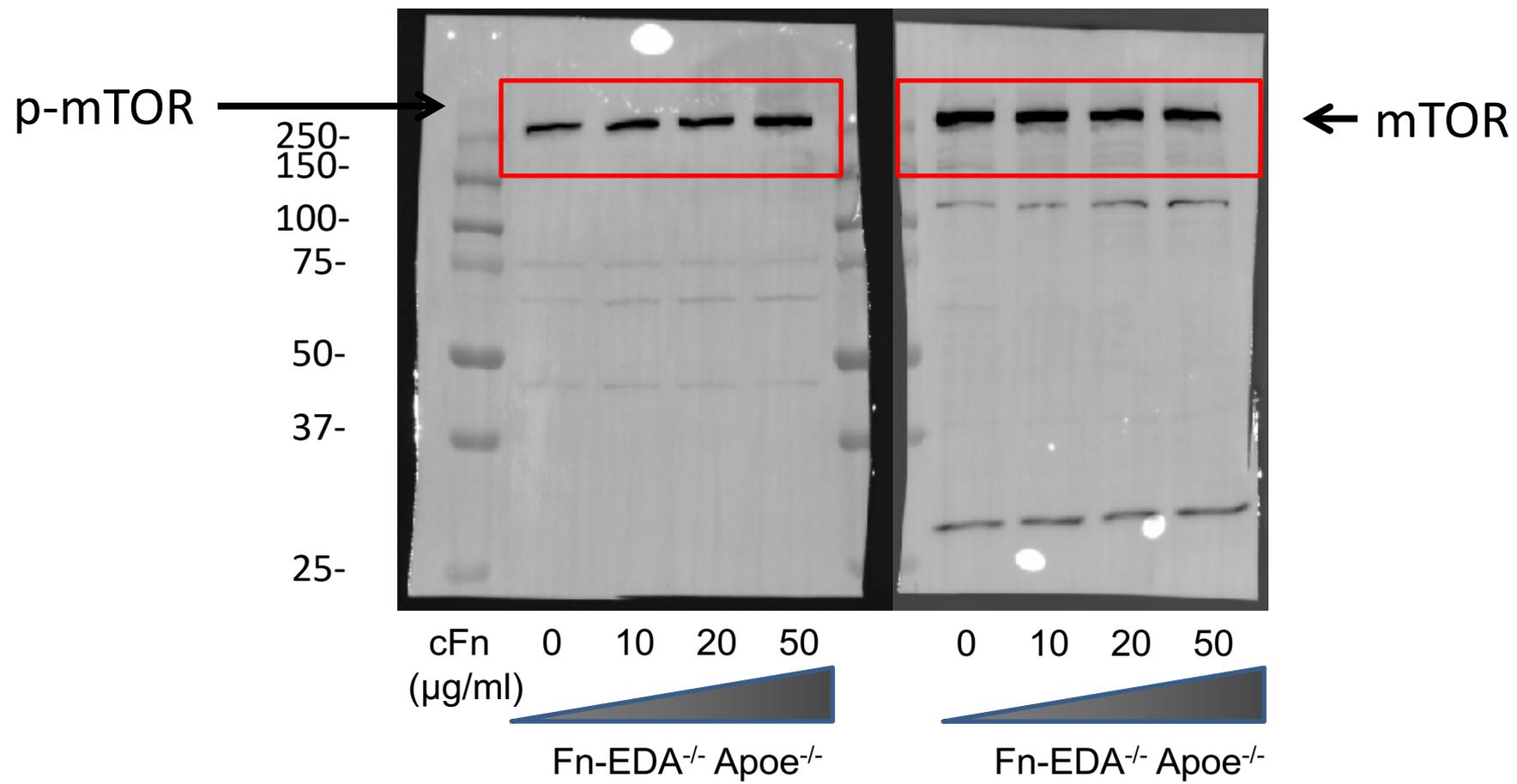
Full unedited gel for Figure 4A-p-NFκB (left) and Total NFκB (right)

Full unedited gel for Figure 4A β-actin

β-actin

250-
150-
100-
75-

50-

37-

25-

cFn   0   10   20   50
(μg/ml)

Fn-EDA$^{-/-}$ Apoe$^{-/-}$
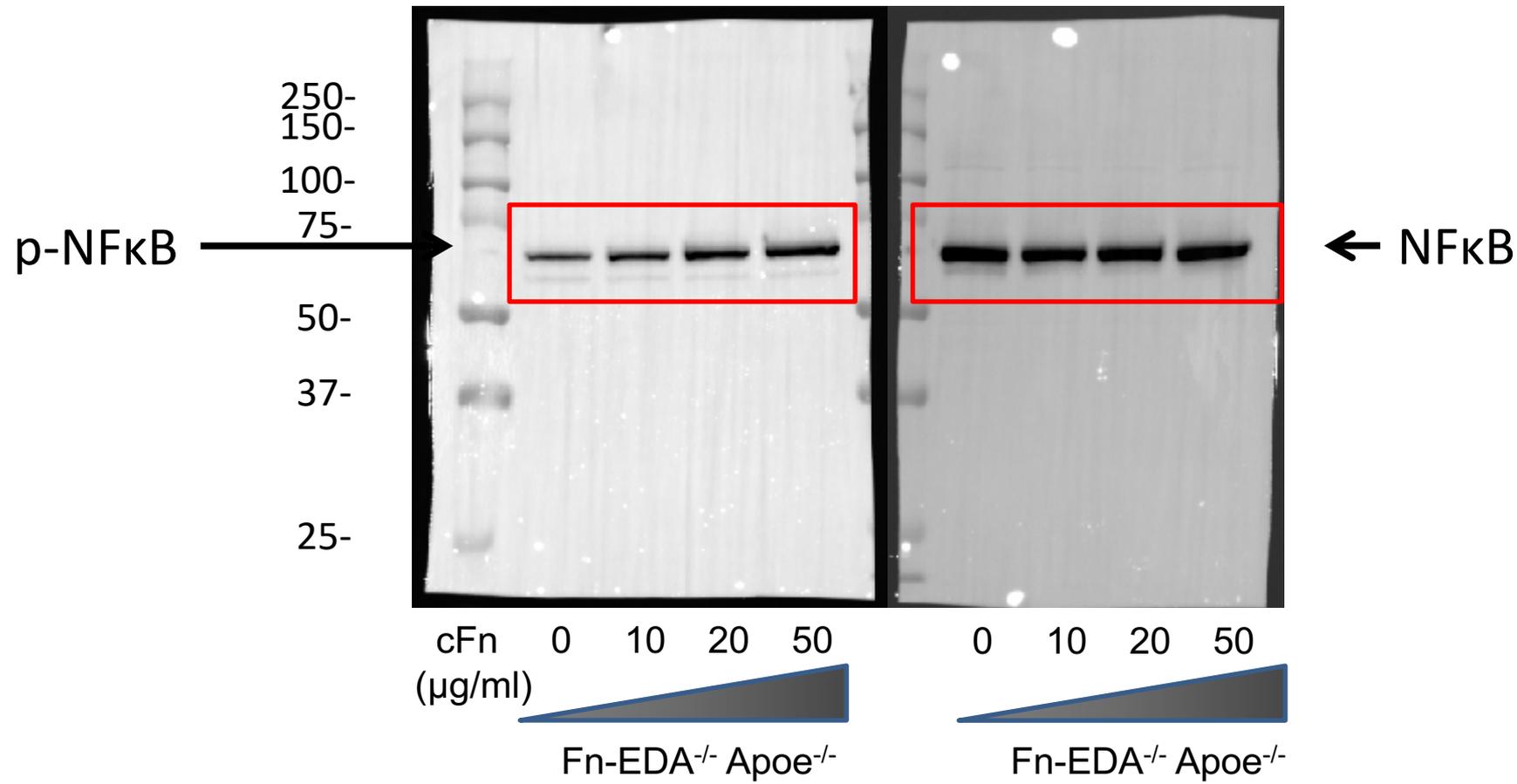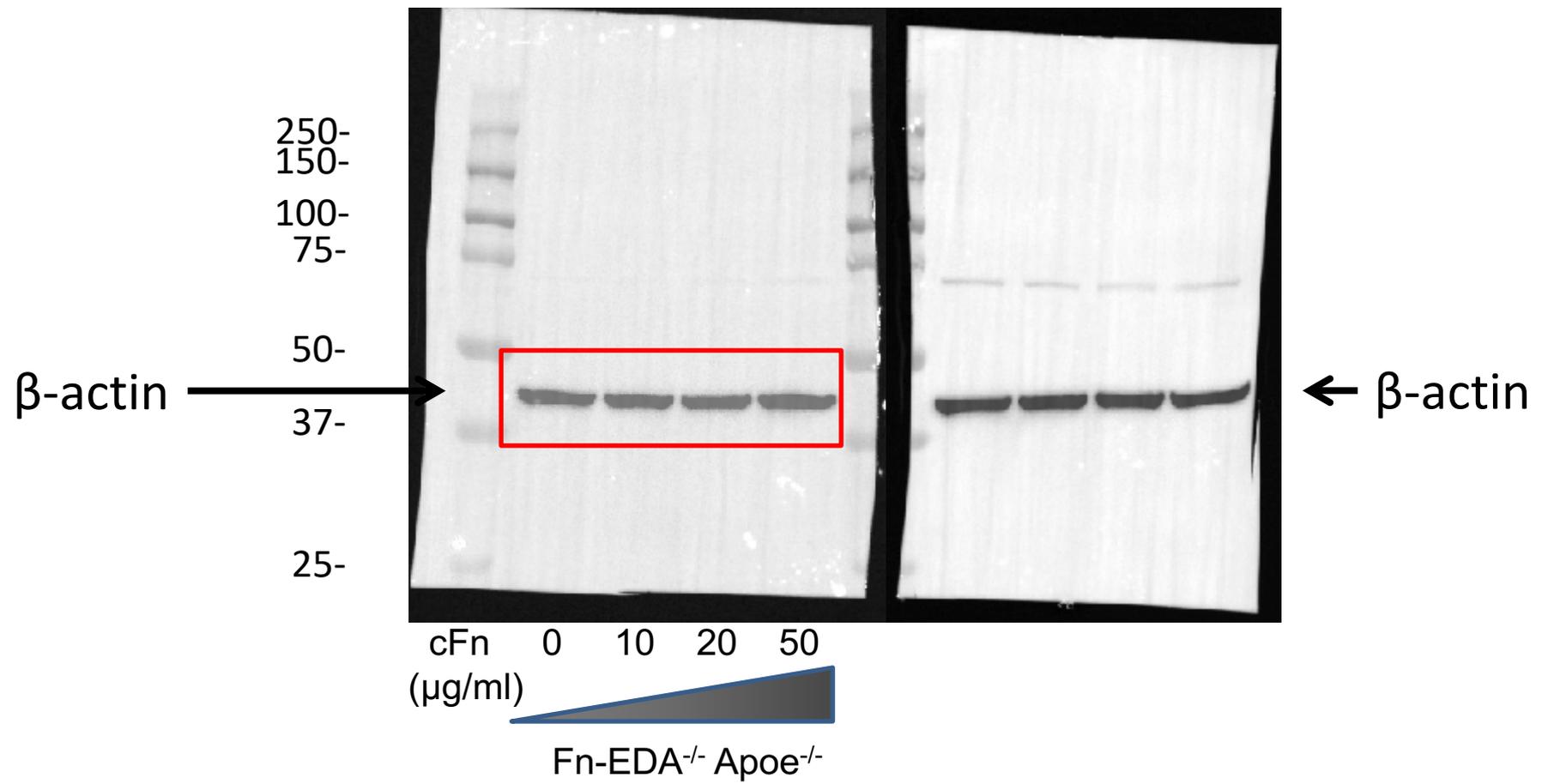
β-actin

Full unedited gel for Figure 4D-SM22α
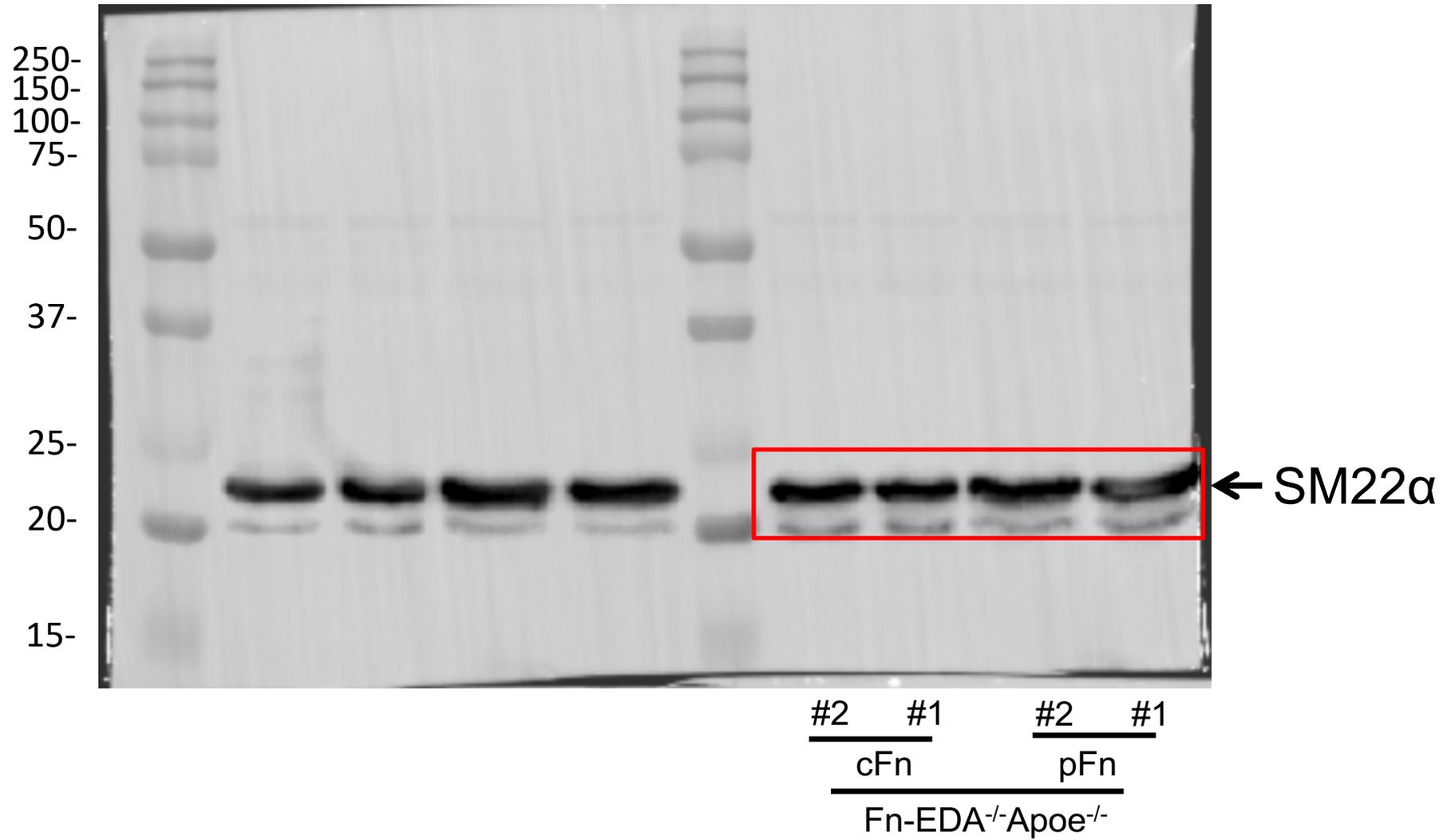
Full unedited gel for Figure 4D-Osteopontin

250-
150-
100-
75-

50-

37-

25-

20-

15-

← Osteopontin

#2 #1   #2 #1
___   ___
cFn   pFn
_____
Fn-EDA$^{-/-}$Apoe$^{-/-}$
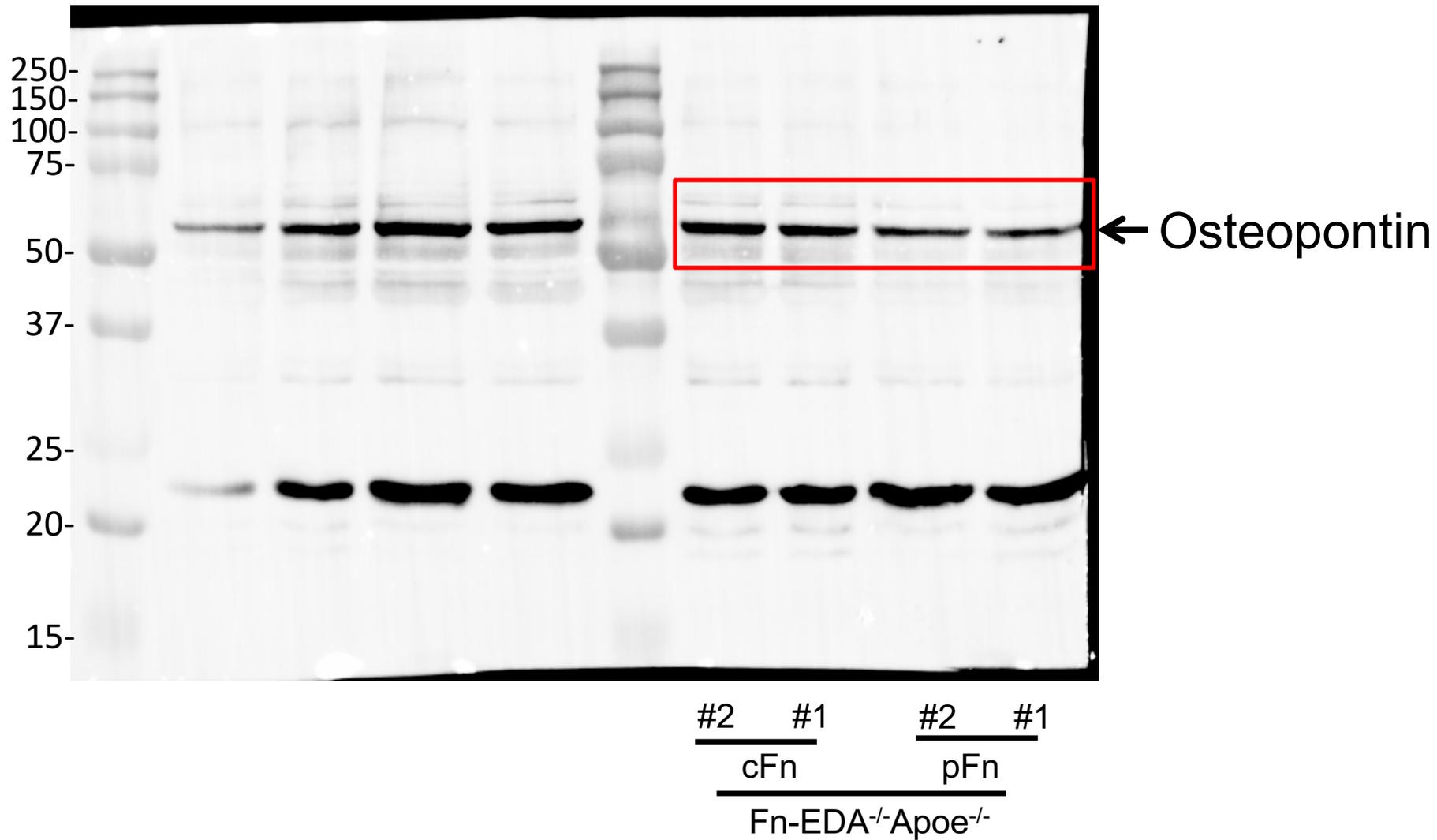
Full unedited gel for Figure 4D-β-actin

Full unedited gel for Figure 4D-SM-MHC

250-
150-
100-
75-
50-
37-
25-
20-
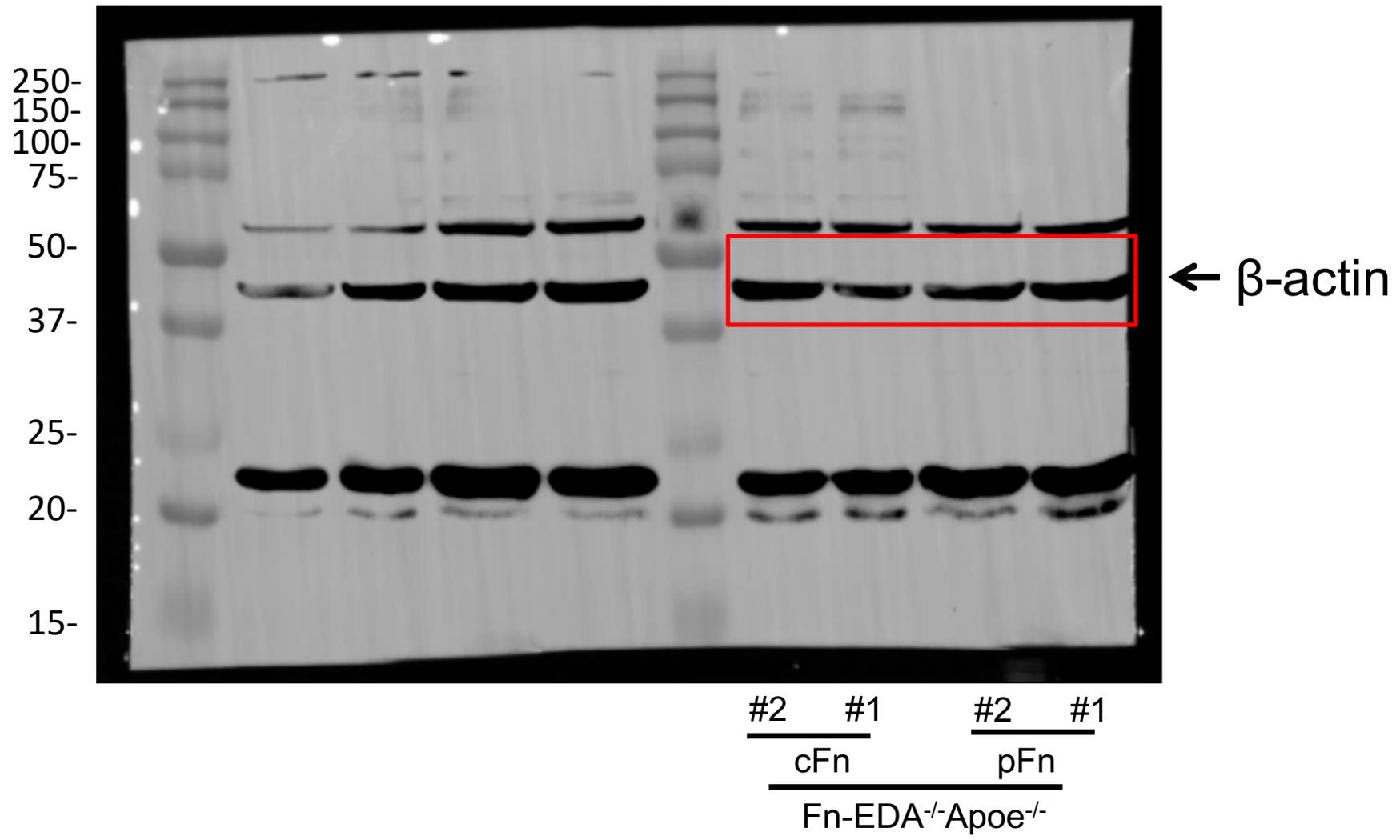
← SM-MHC

#2   #1   #2   #1
_____   _____
   cFn   pFn
_____
Fn-EDA$^{-/-}$Apoe$^{-/-}$
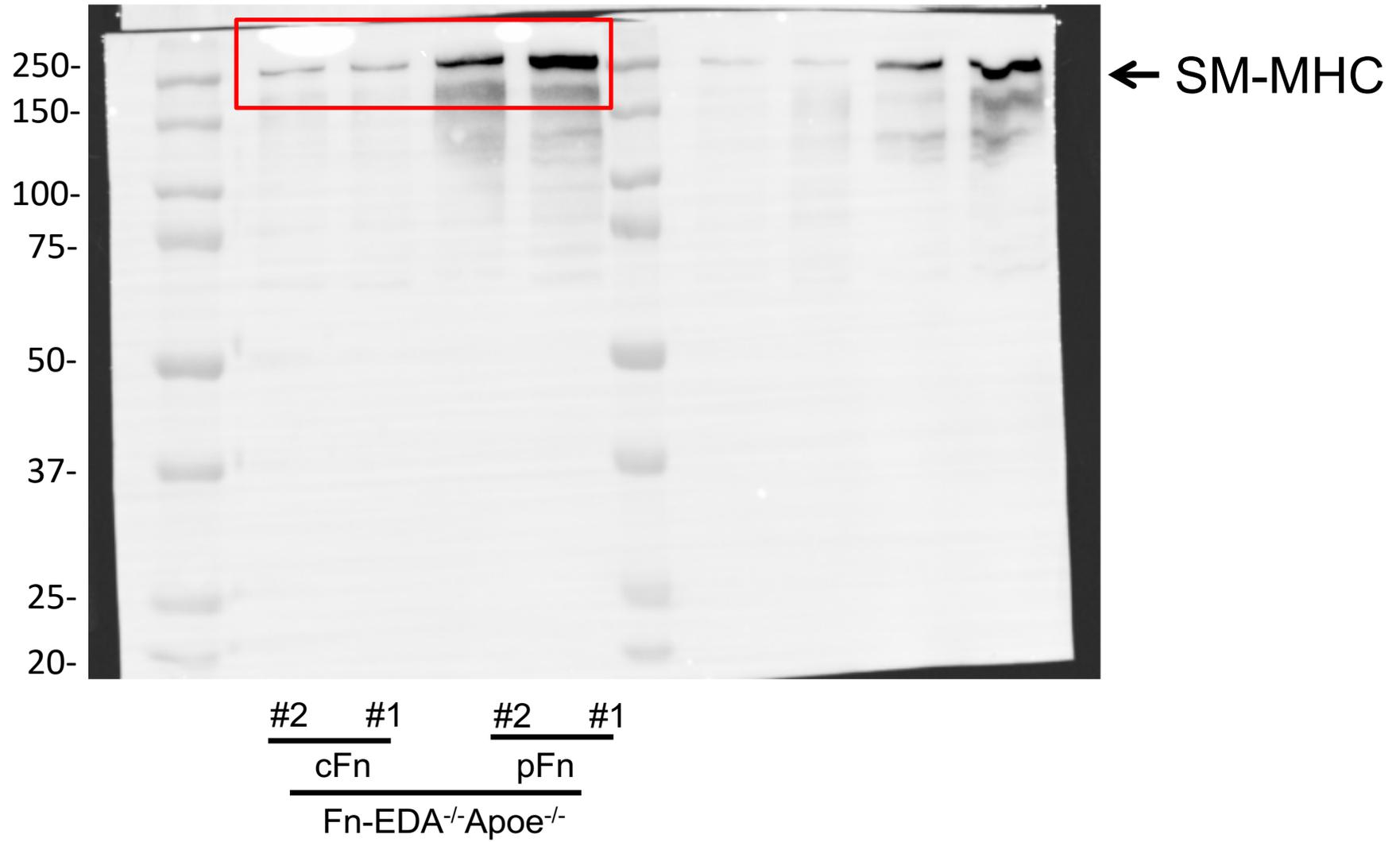
Full unedited gel for Figure 4D-Vimentin
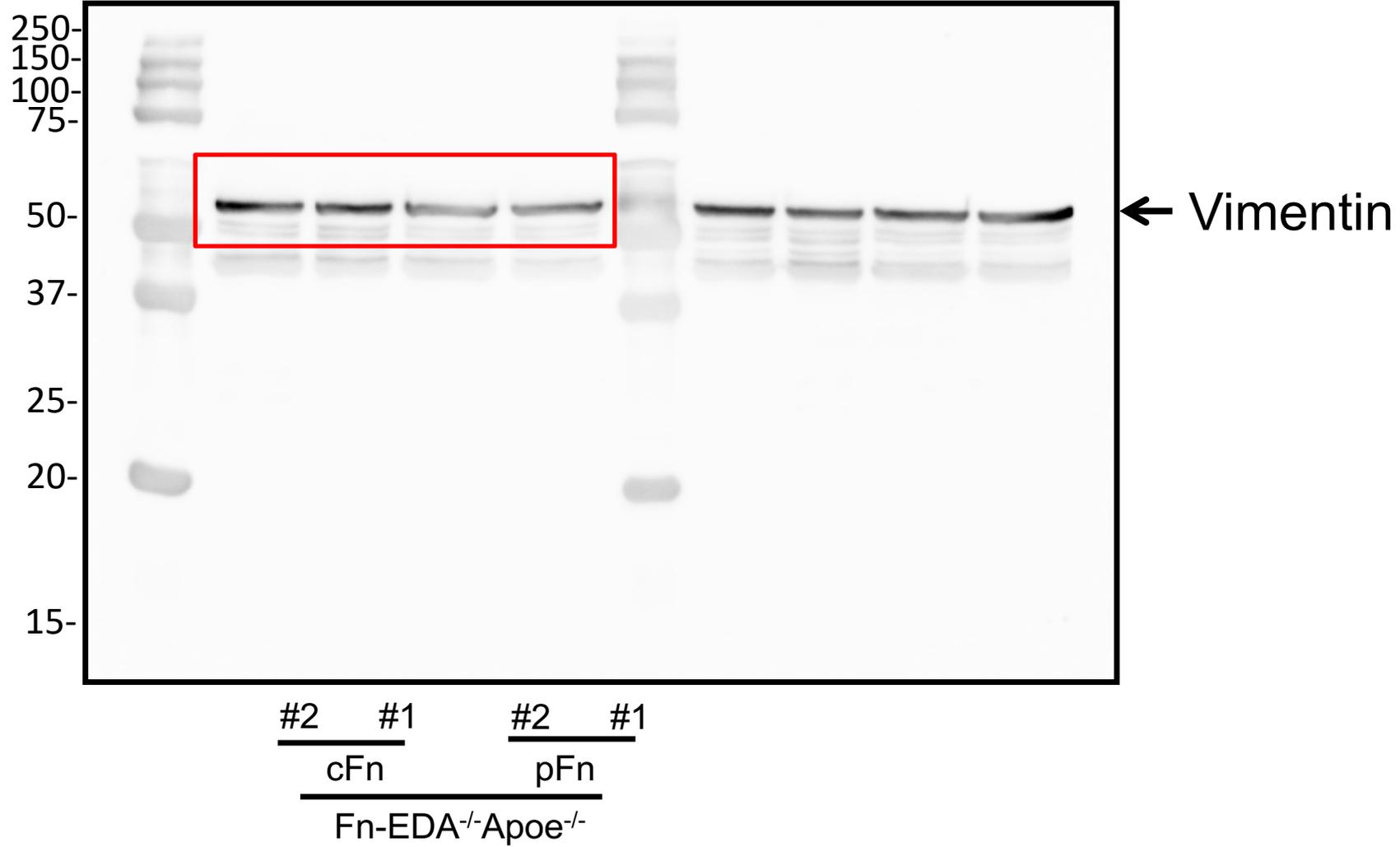
Full unedited gel for Figure 4D-β-actin
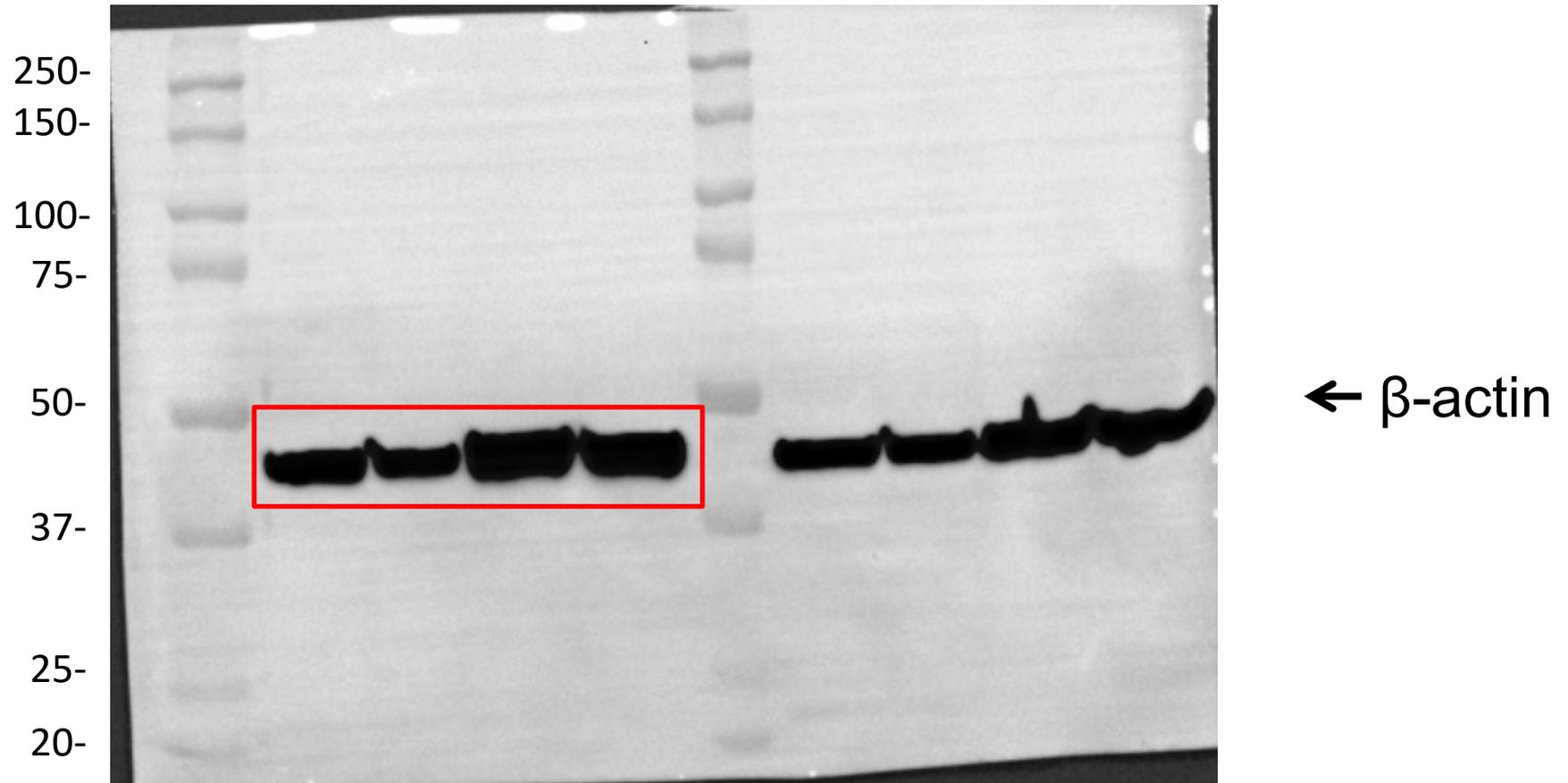
← β-actin

#2   #1   #2   #1
_____   _____
cFn   pFn
_____
Fn-EDA$^{-/-}$Apoe$^{-/-}$

Full unedited gel for Figure 5A-SM22α

Full unedited gel for Figure 5A-SM-MHC

250-
150-
100-
75-

50-

37-

25-

20-

← SM-MHC

cFn + RGDS Peptide
pFn + RGDS Peptide
cFn
pFn

Fn-EDA$^{-/-}$Apoe$^{-/-}$
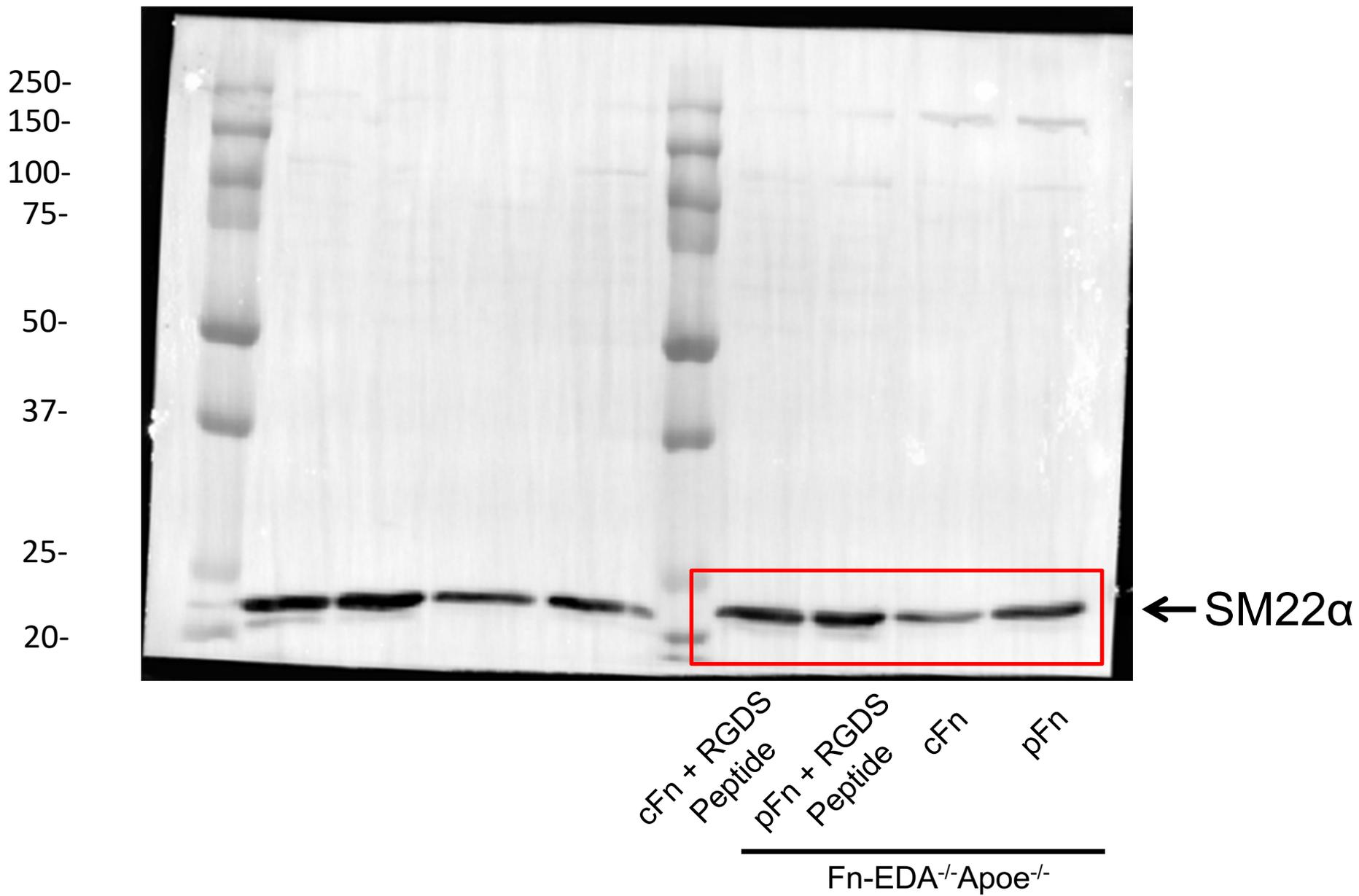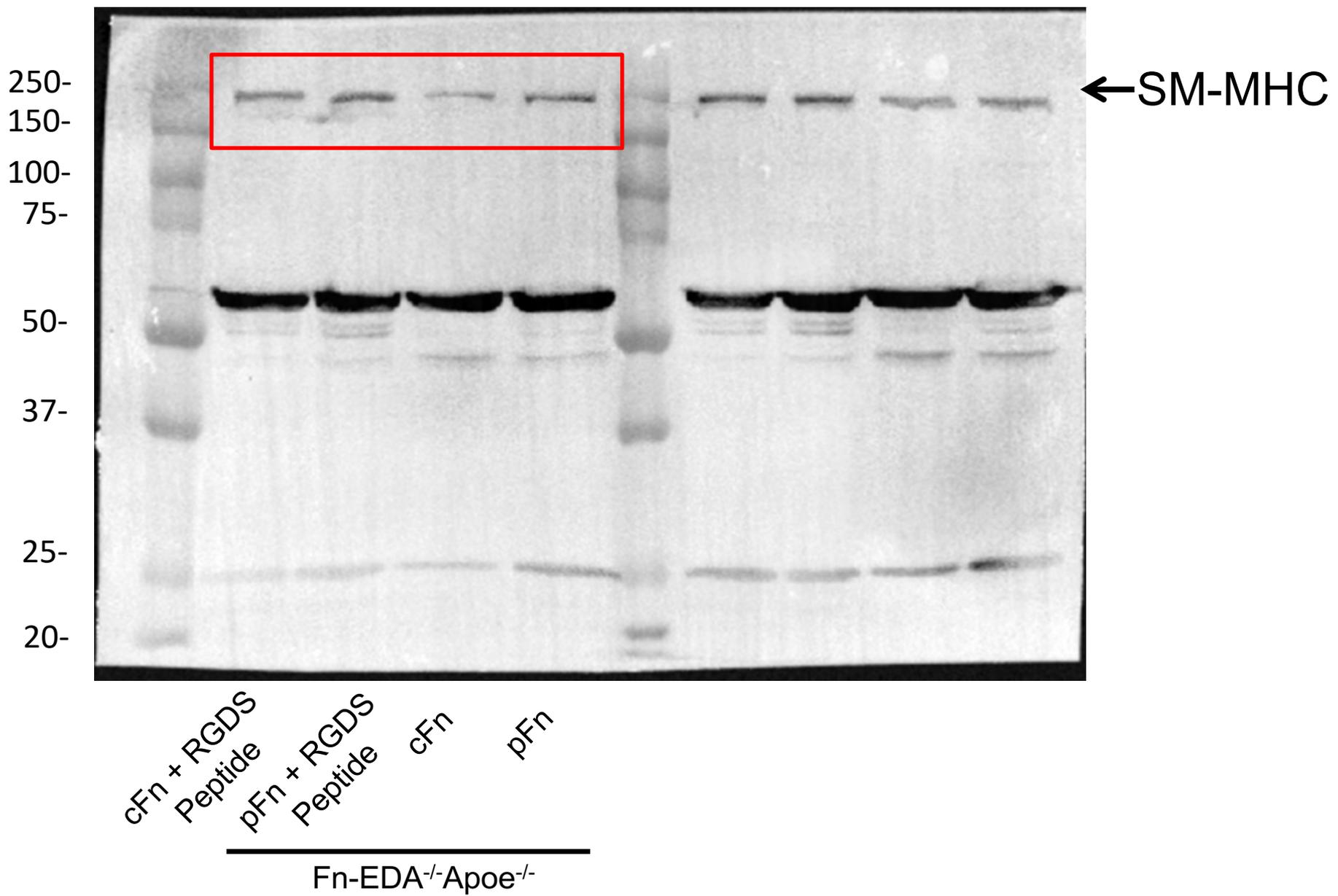
Full unedited gel for Figure 5A- Vimentin
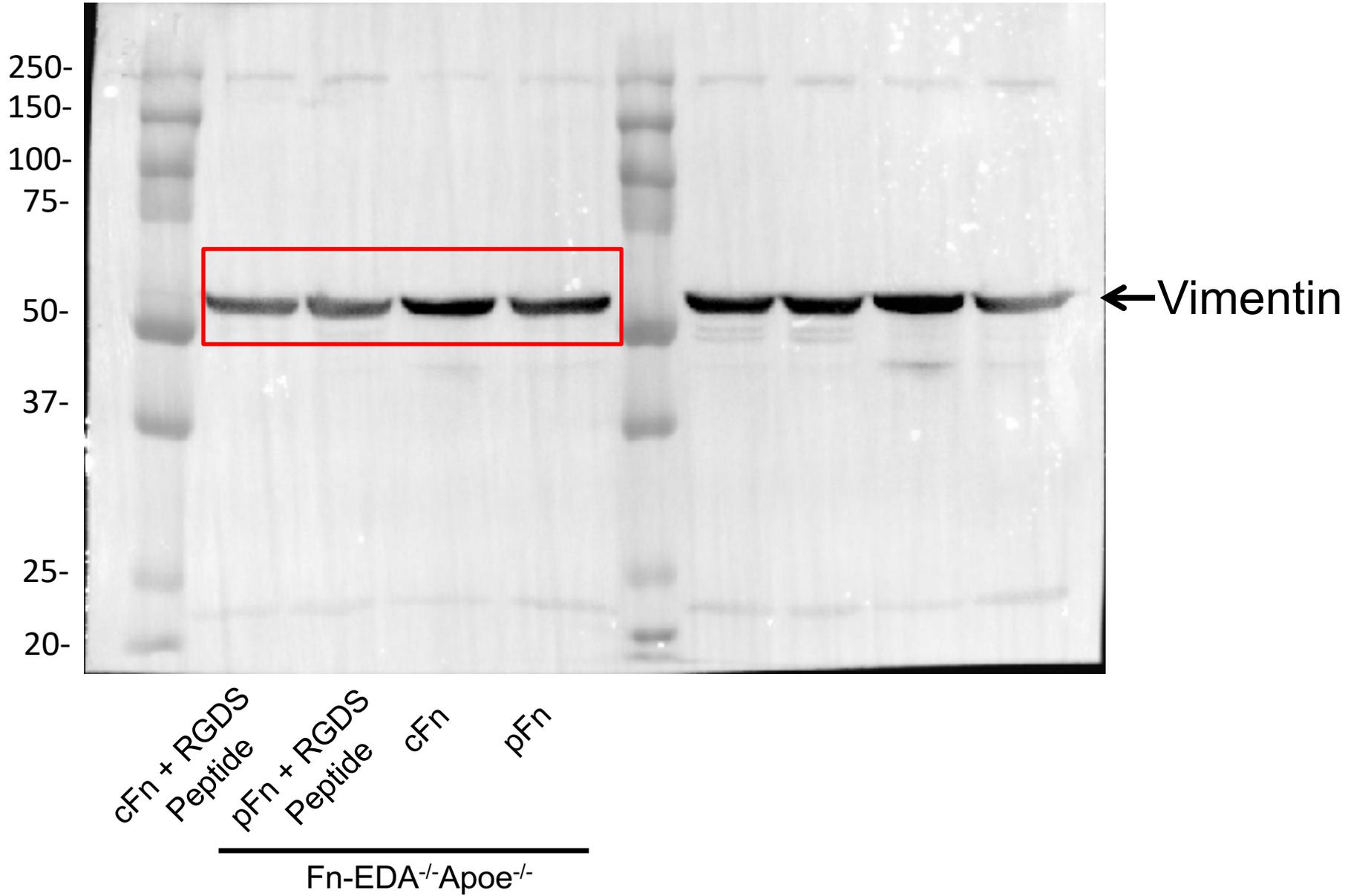
Full unedited gel for Figure 5A-Osteopontin

250-
150-
100-
75-
50-
37-
25-
20-

←Osteopontin

cFn + RGDS
Peptide

pFn + RGDS
Peptide

cFn

pFn

Fn-EDA$^{-/-}$Apoe$^{-/-}$
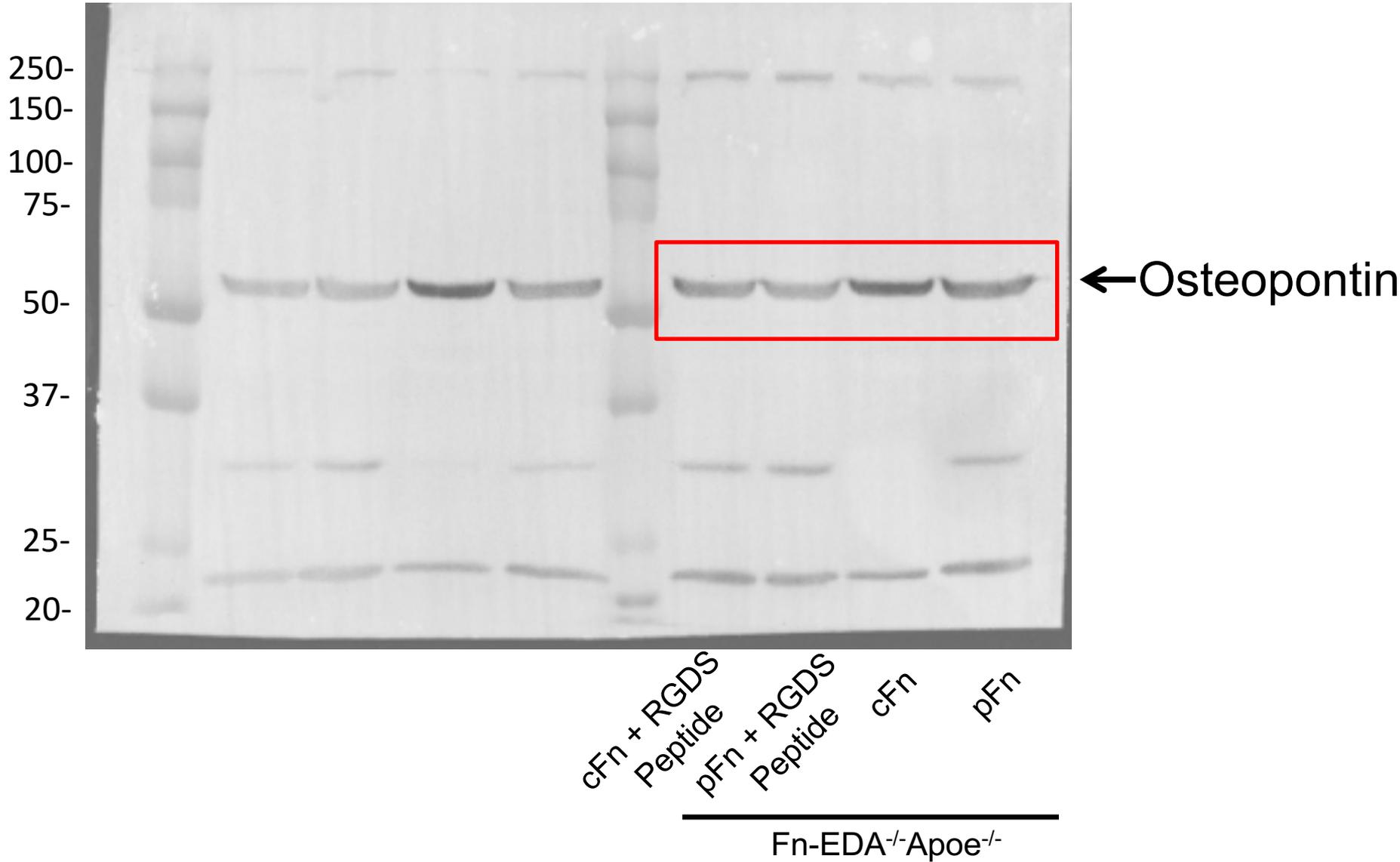
Full unedited gel for Figure 5A-β-actin
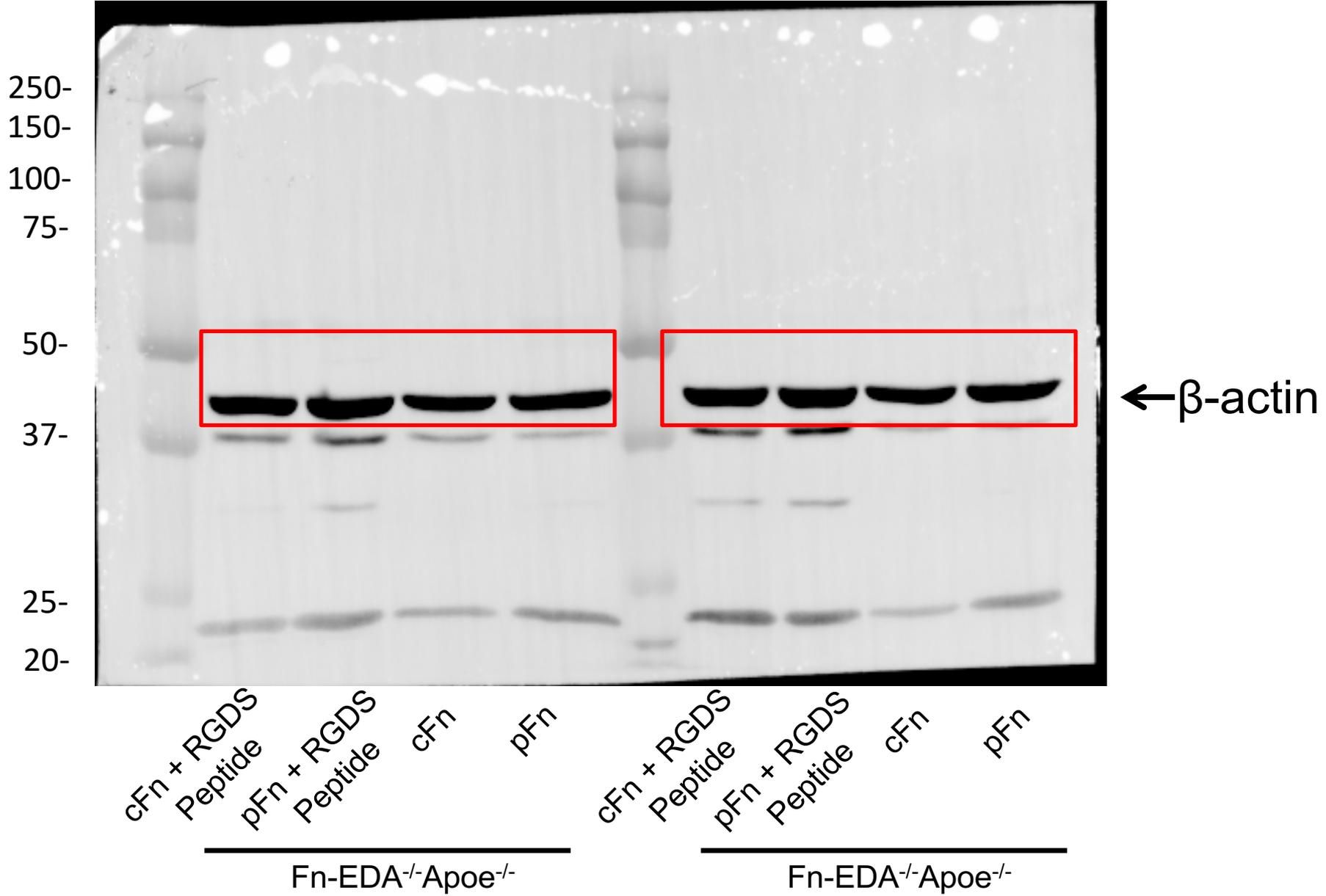
Full unedited gel for Figure 6A-Fn-EDA
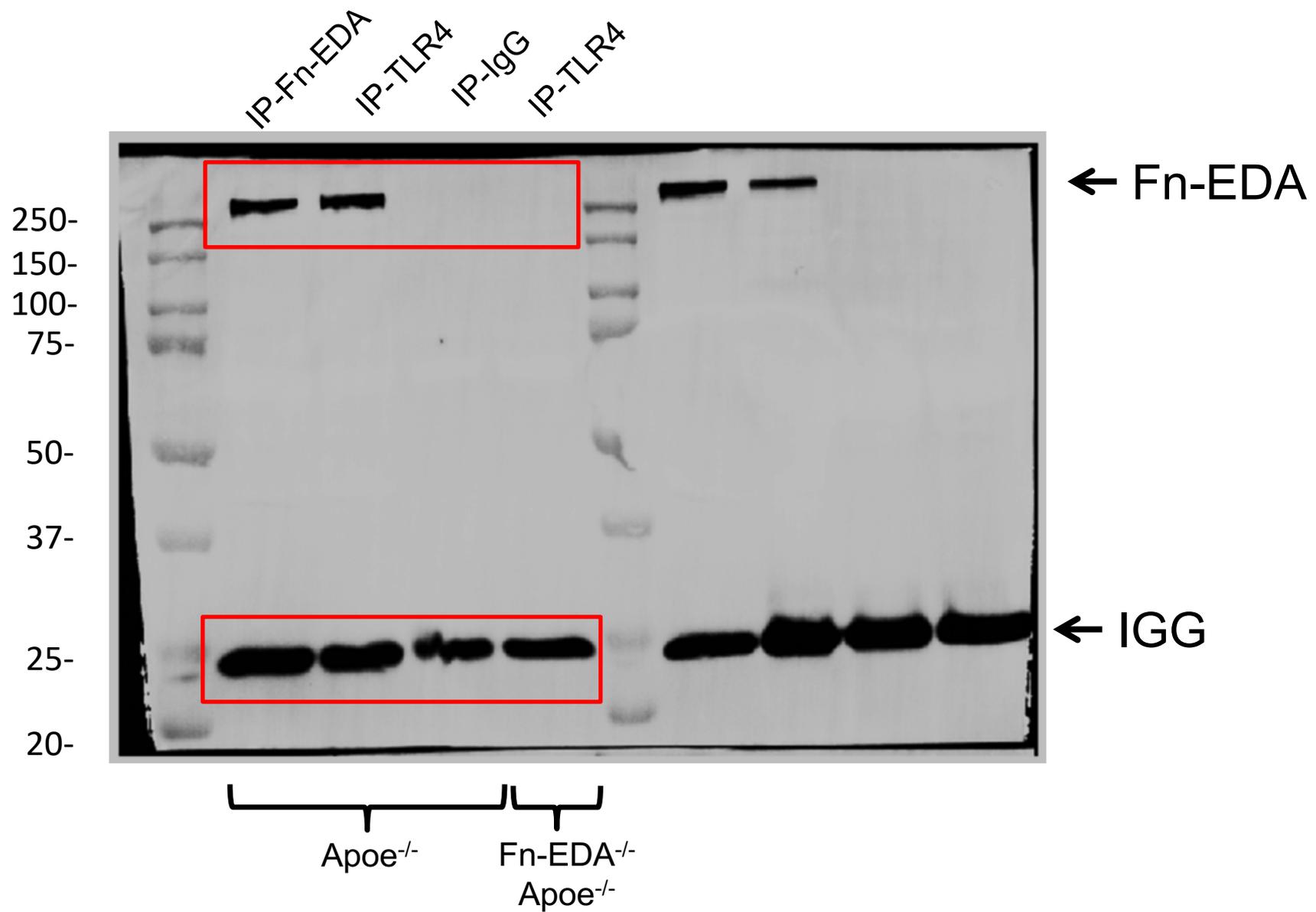
Full unedited gel for Figure 7A-p-AKT1

$\leftarrow$ p-AKT1

250-
150-
100-
75-
50-
37-
25-
20-

pFn cFn   pFn cFn

Fn-EDA$^{-/-}$ Fn-EDA$^{-/-}$
Apoe$^{-/-}$ TLR4$^{-/-}$ Apoe$^{-/-}$

Full unedited gel for Figure 7A-AKT1

250-
150-
100-
75-

50-

37-

25-

20-
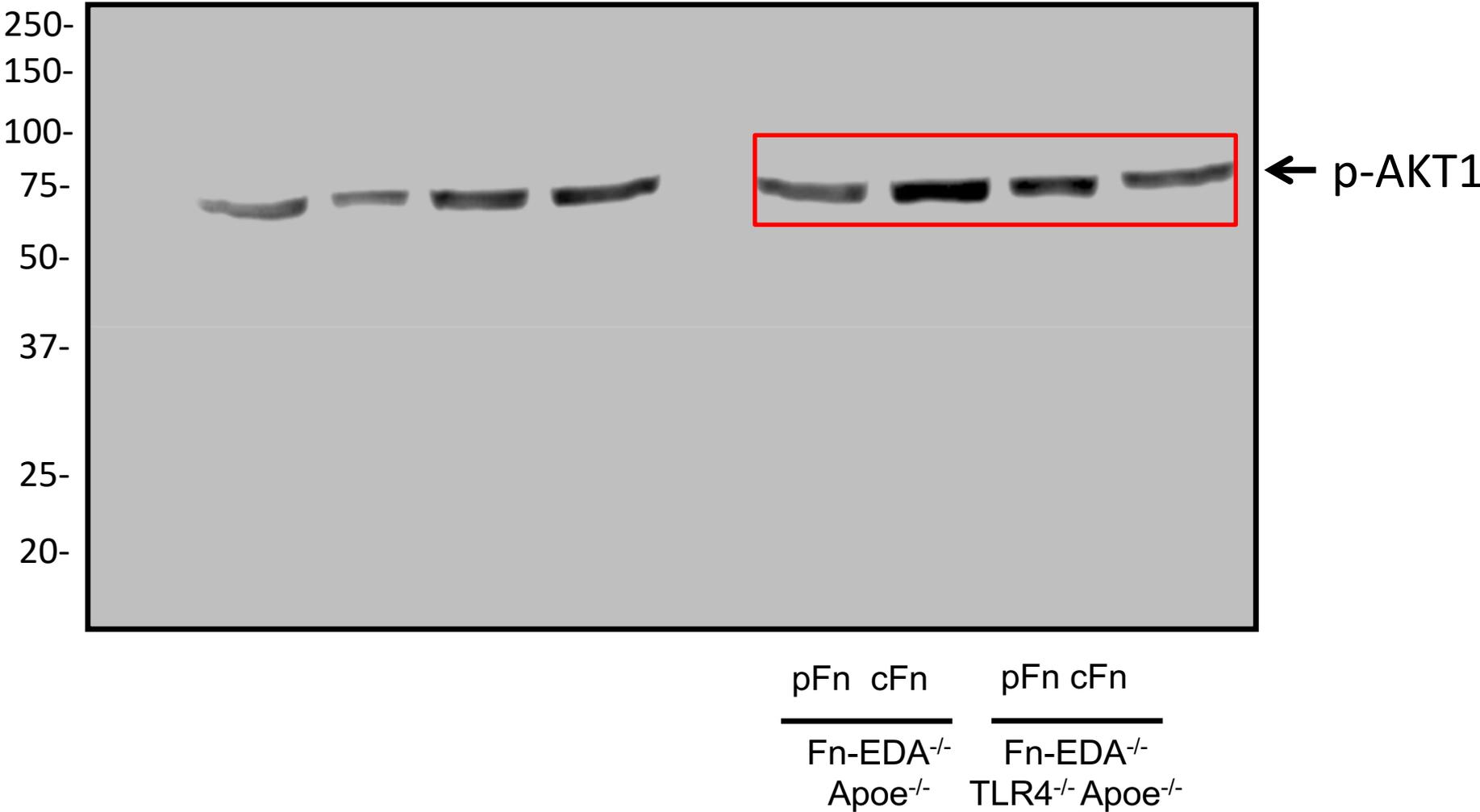
← AKT1

pFn cFn   pFn cFn

Fn-EDA$^{-/-}$ Fn-EDA$^{-/-}$
Apoe$^{-/-}$ TLR4$^{-/-}$ Apoe$^{-/-}$
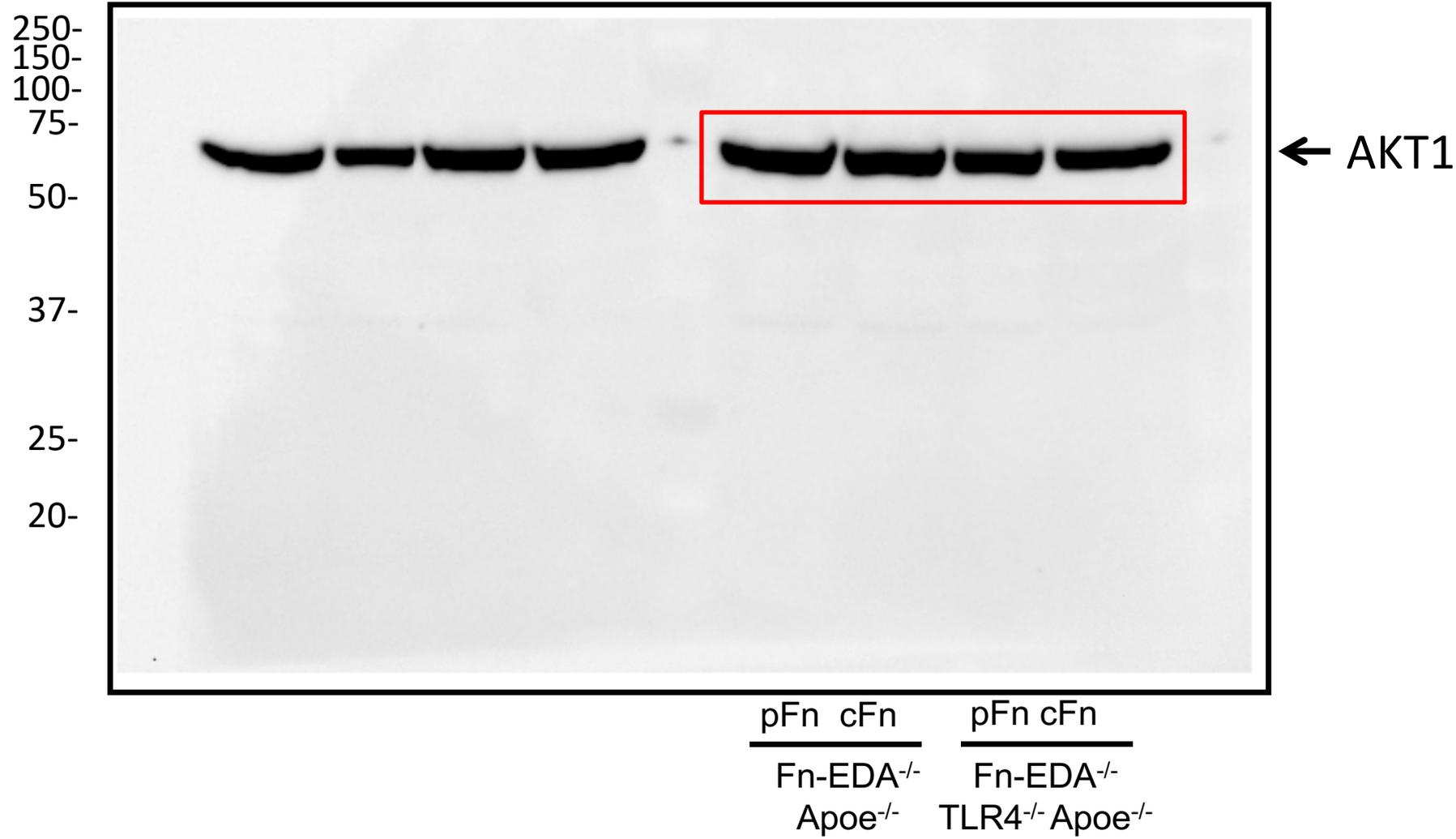
Full unedited gel for Figure 7A-p-NFκB

Full unedited gel for Figure 7A-NFκB

← NFκB

250-
150-
100-

75-

50-

37-

25-

20-
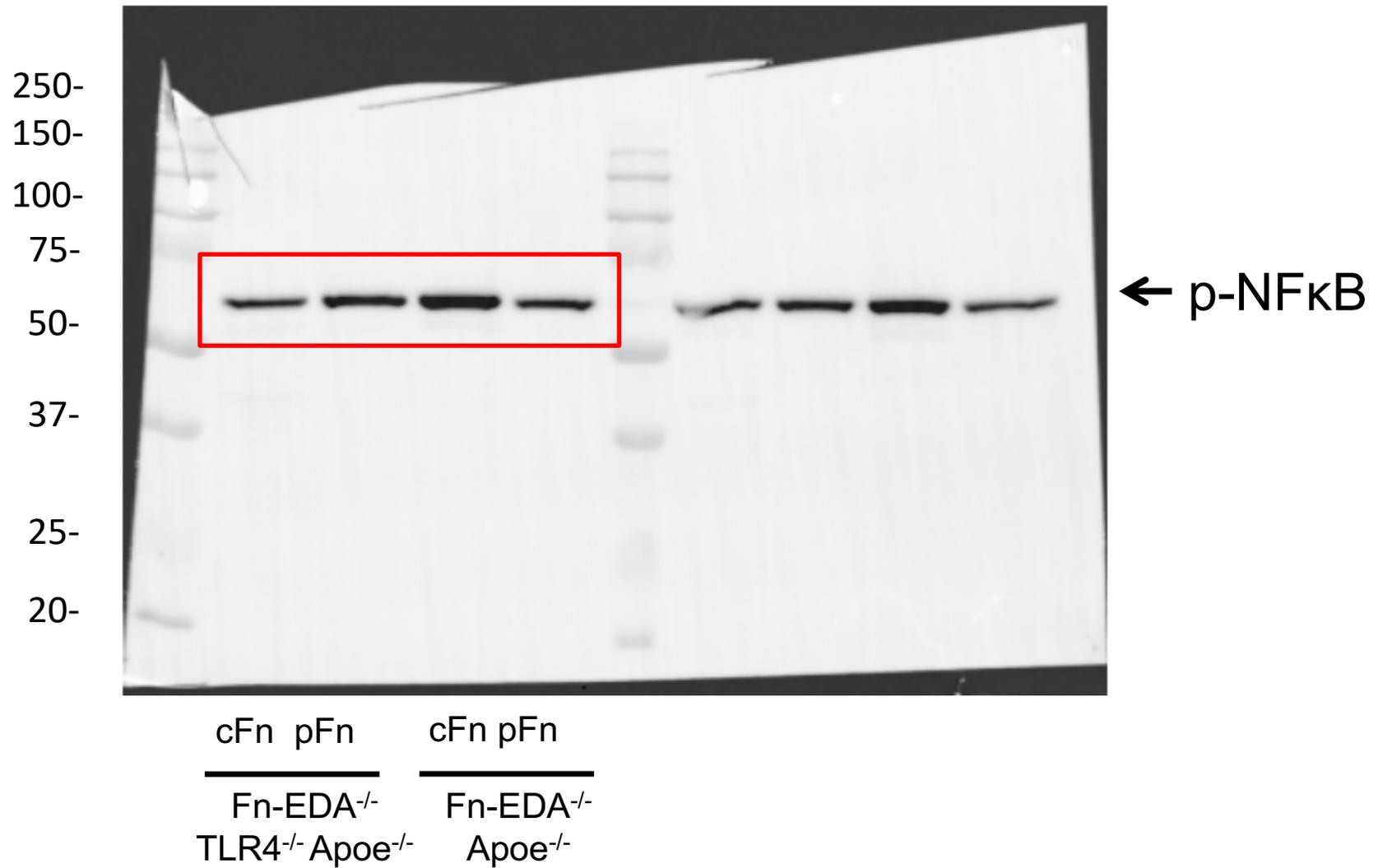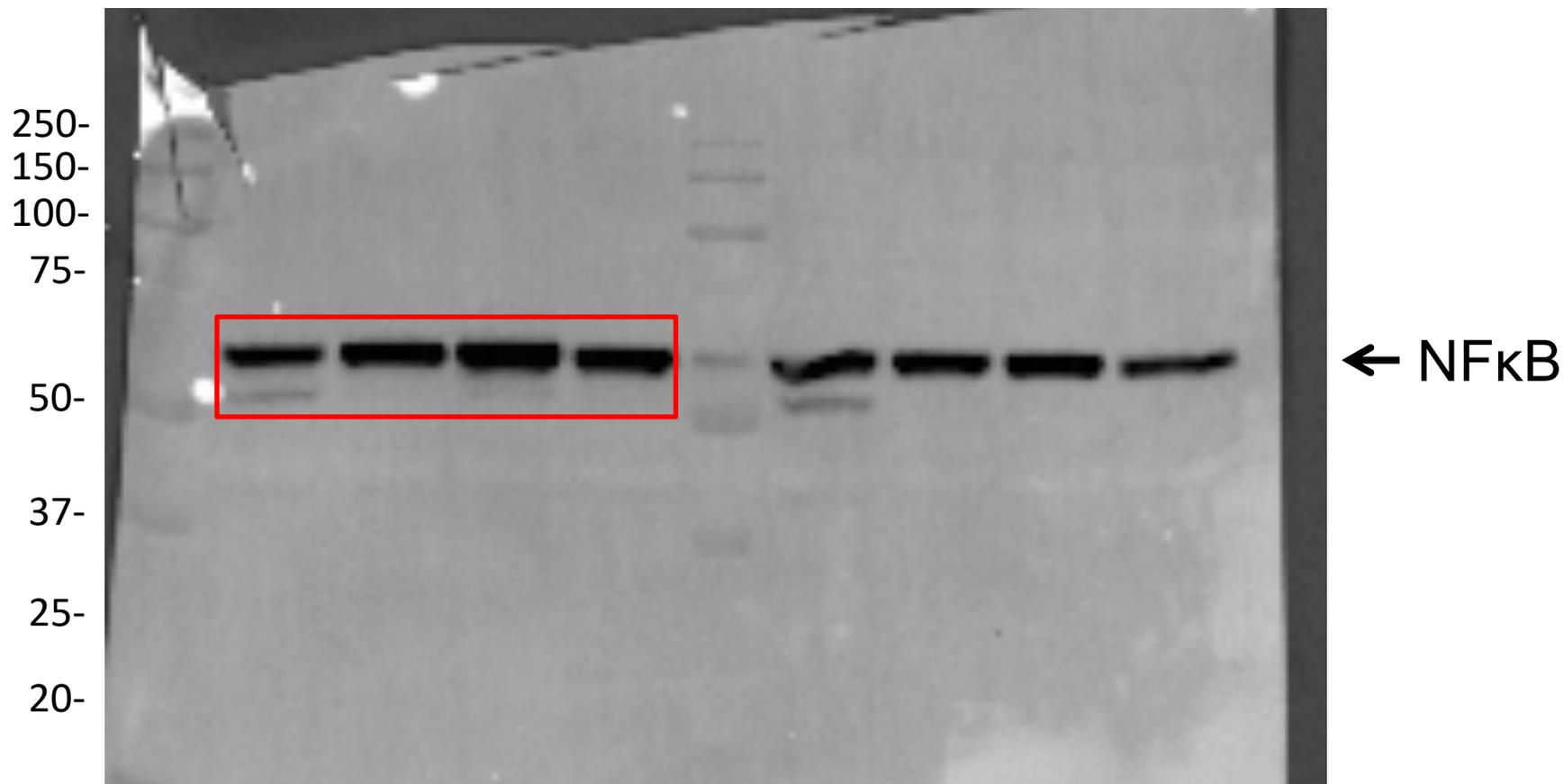
cFn pFn   cFn pFn

Fn-EDA$^{-/-}$ Fn-EDA$^{-/-}$
TLR4$^{-/-}$ Apoe$^{-/-}$ Apoe$^{-/-}$

Full unedited gel for Figure 7A-β-actin

Full unedited gel for Figure 7A-p-AKT2

250-
150-
100-
75-

50-

37-

25-

20-

15-

← p-AKT 2

cFn pFn   cFn pFn

Fn-EDA$^{-/-}$ Fn-EDA$^{-/-}$
TLR4$^{-/-}$ Apoe$^{-/-}$ Apoe$^{-/-}$
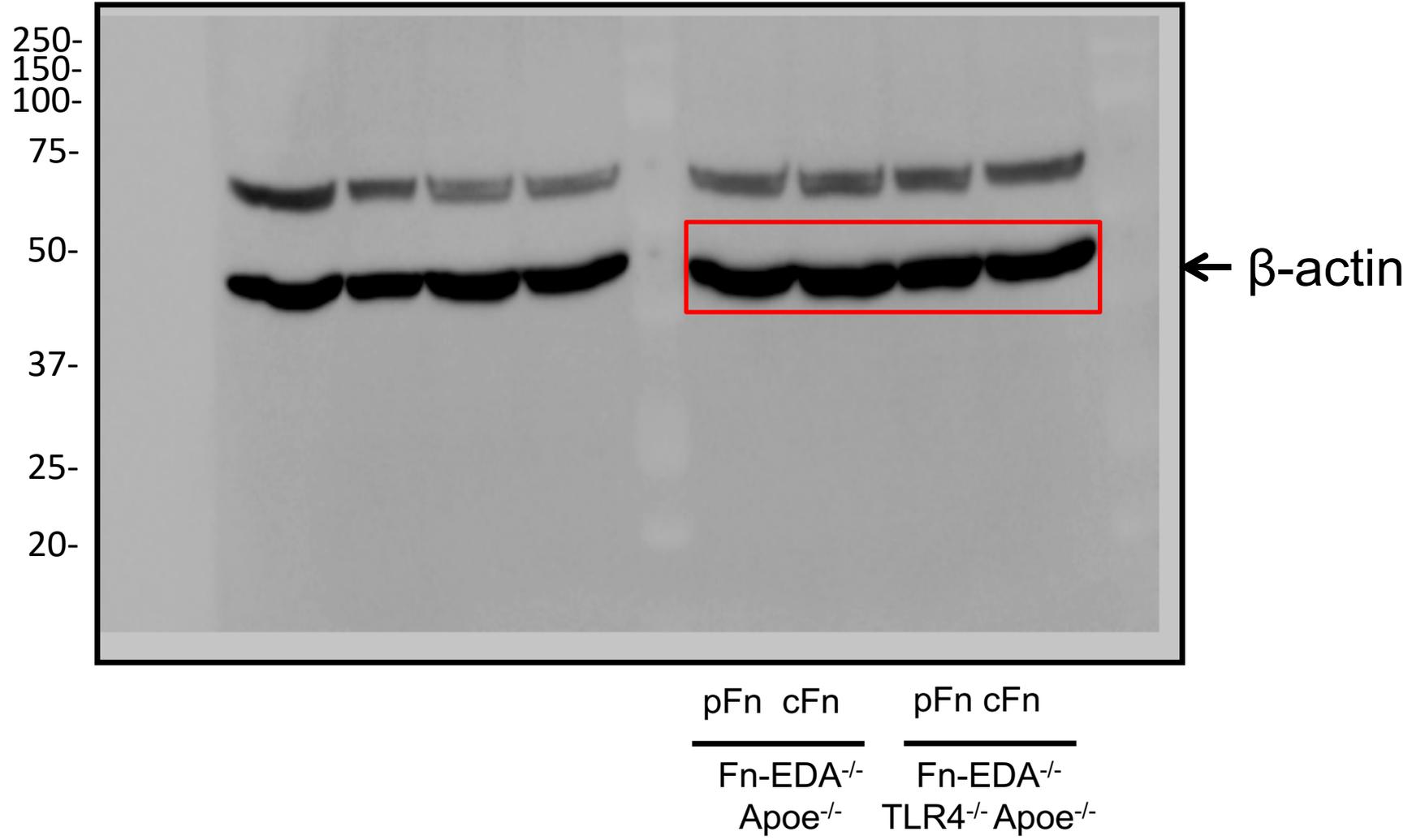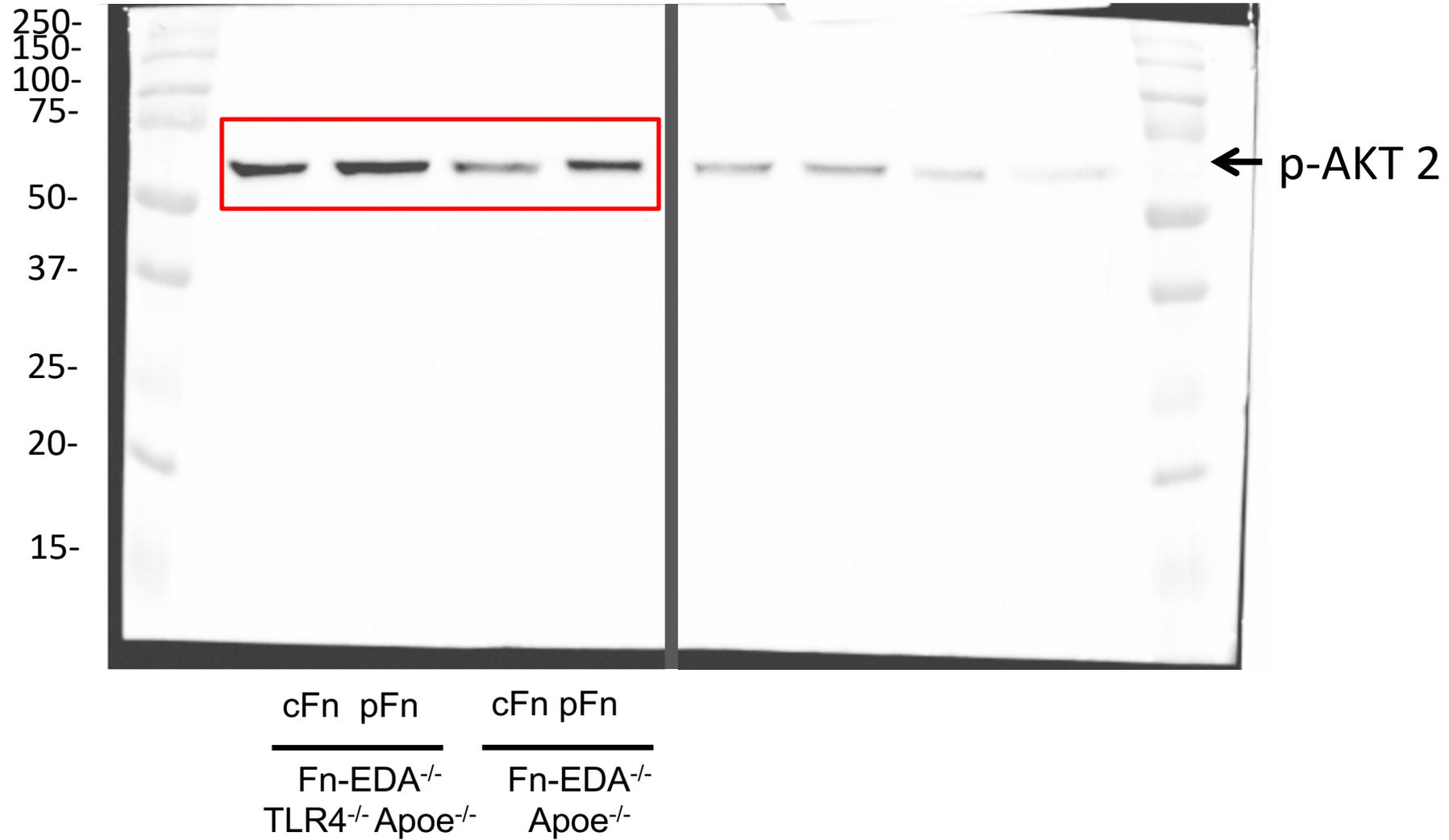
Full unedited gel for Figure 7A-AKT2

Full unedited gel for Figure 7A- β-actin
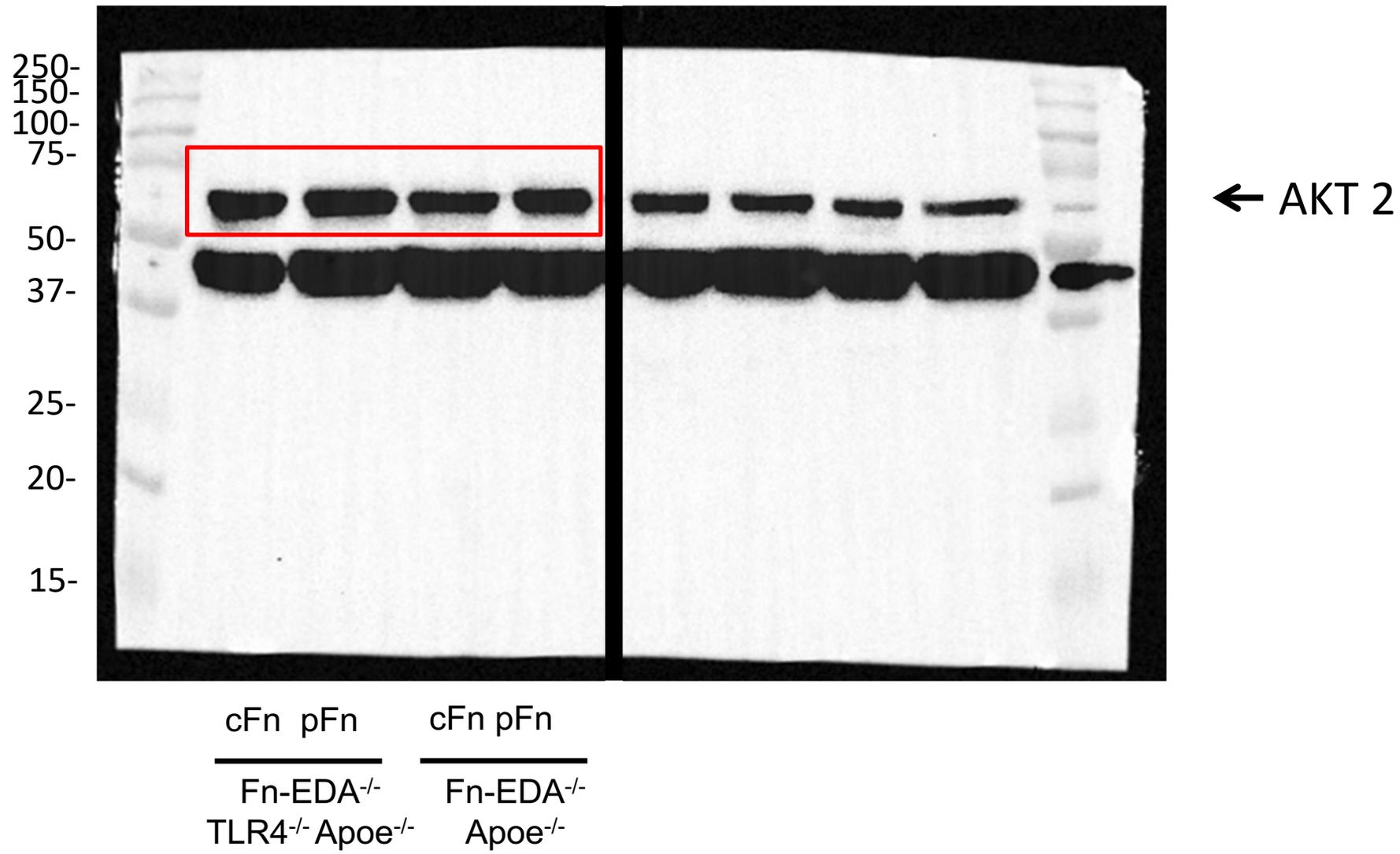
β-actin

cFn pFn   cFn pFn

Fn-EDA$^{-/-}$ Fn-EDA$^{-/-}$
TLR4$^{-/-}$ Apoe$^{-/-}$ Apoe$^{-/-}$
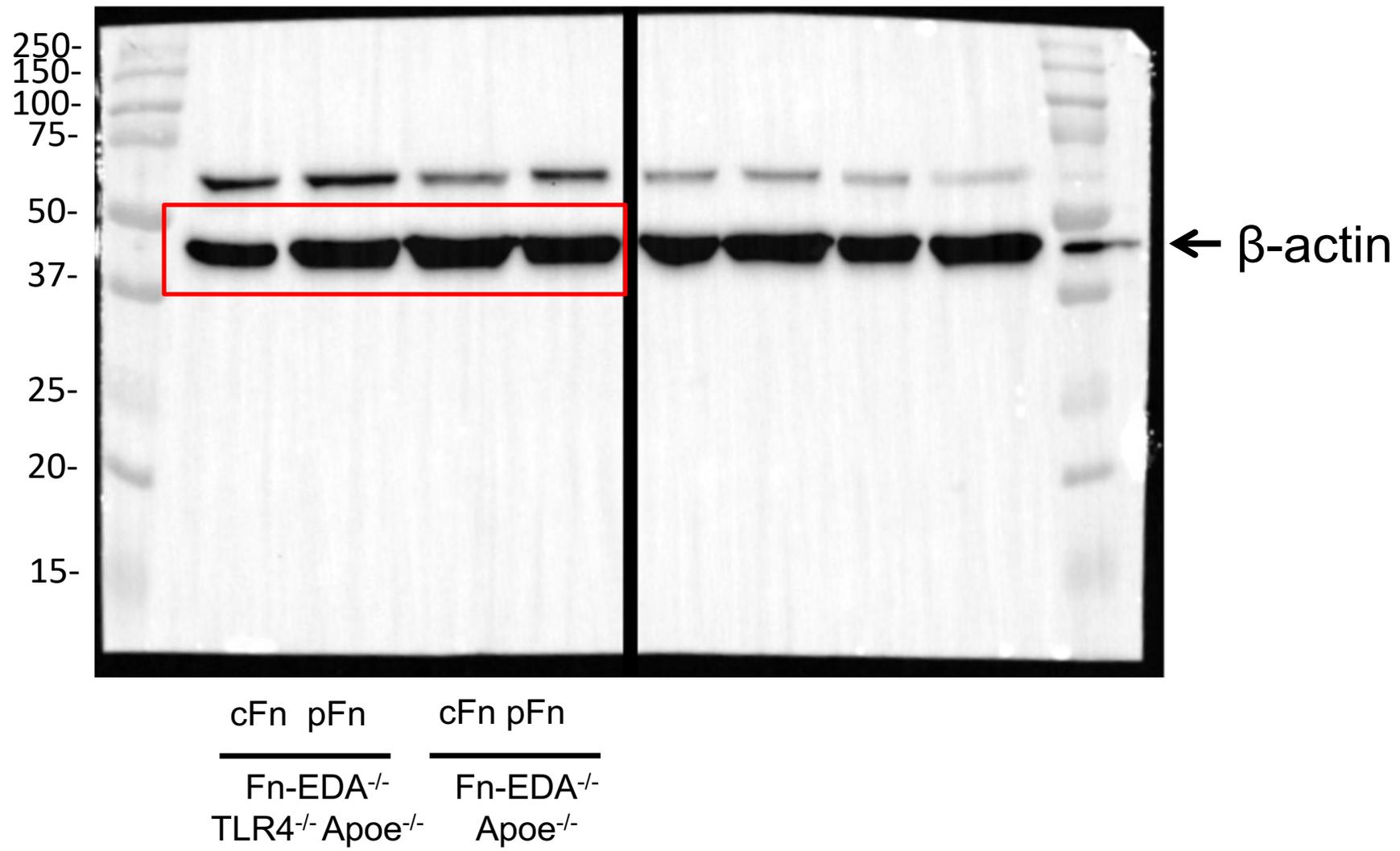
Full unedited gel for Figure 7A-p-mTOR
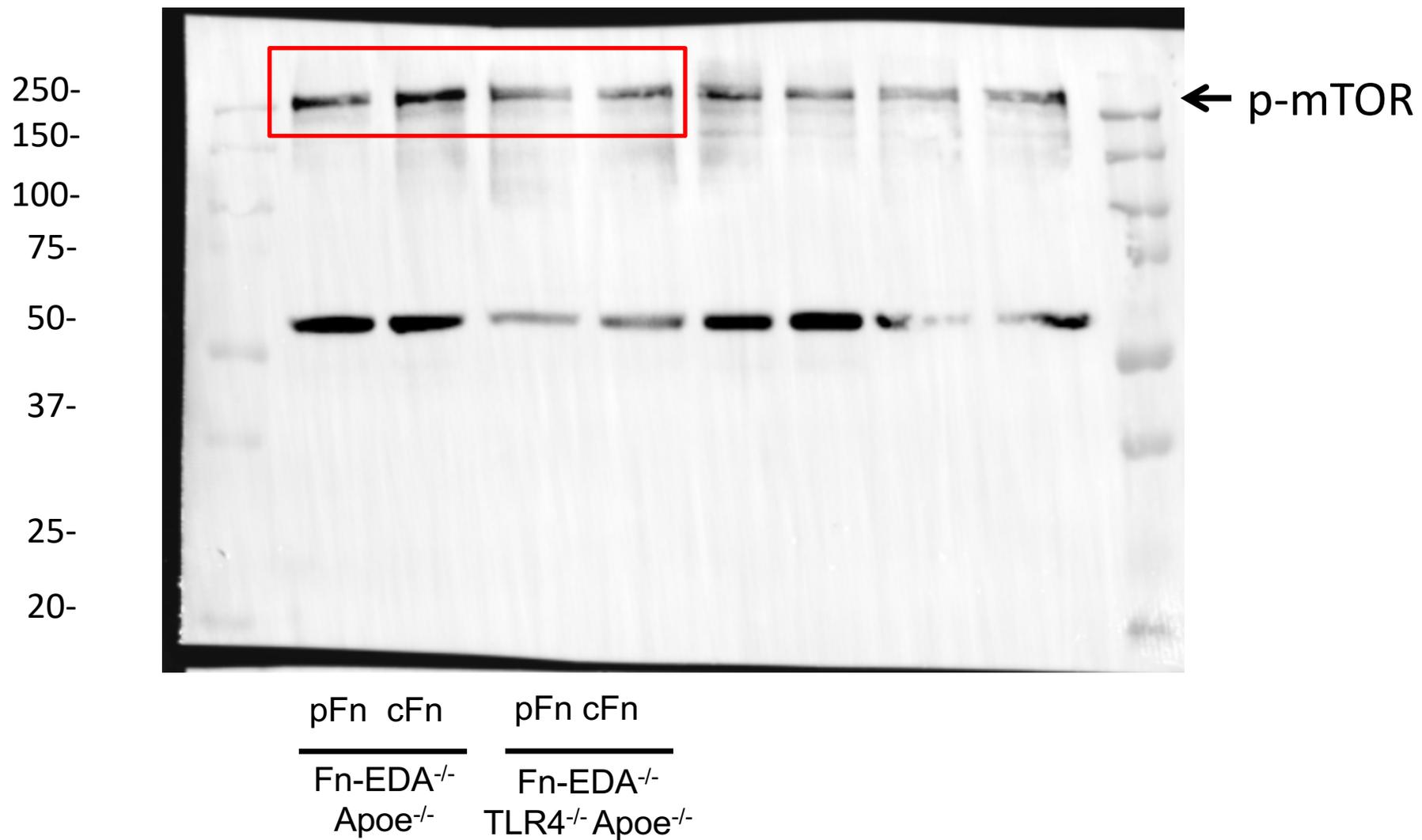
Full unedited gel for Figure 7A-mTOR
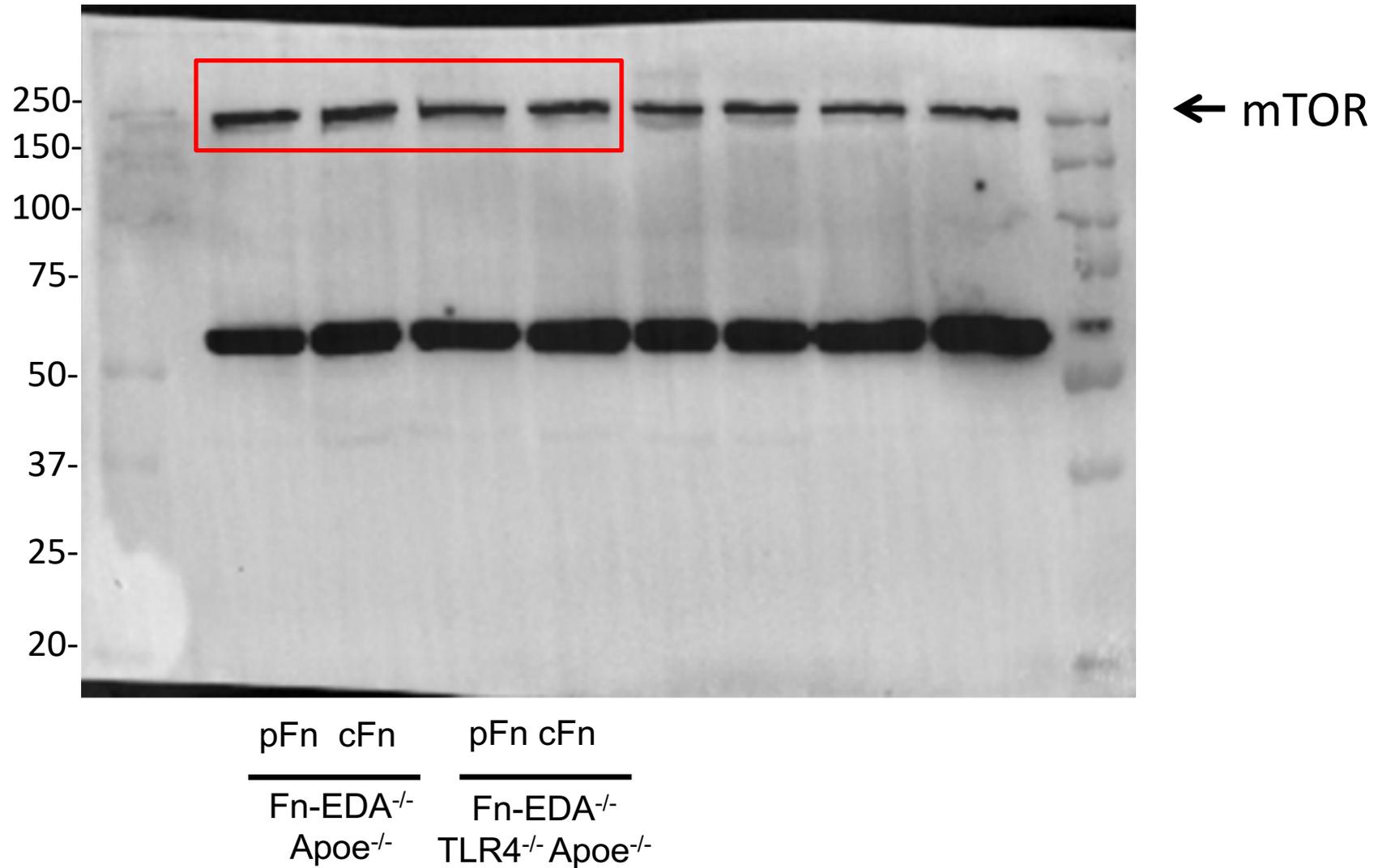
Full unedited gel for Figure 7A-β-actin

← β-actin
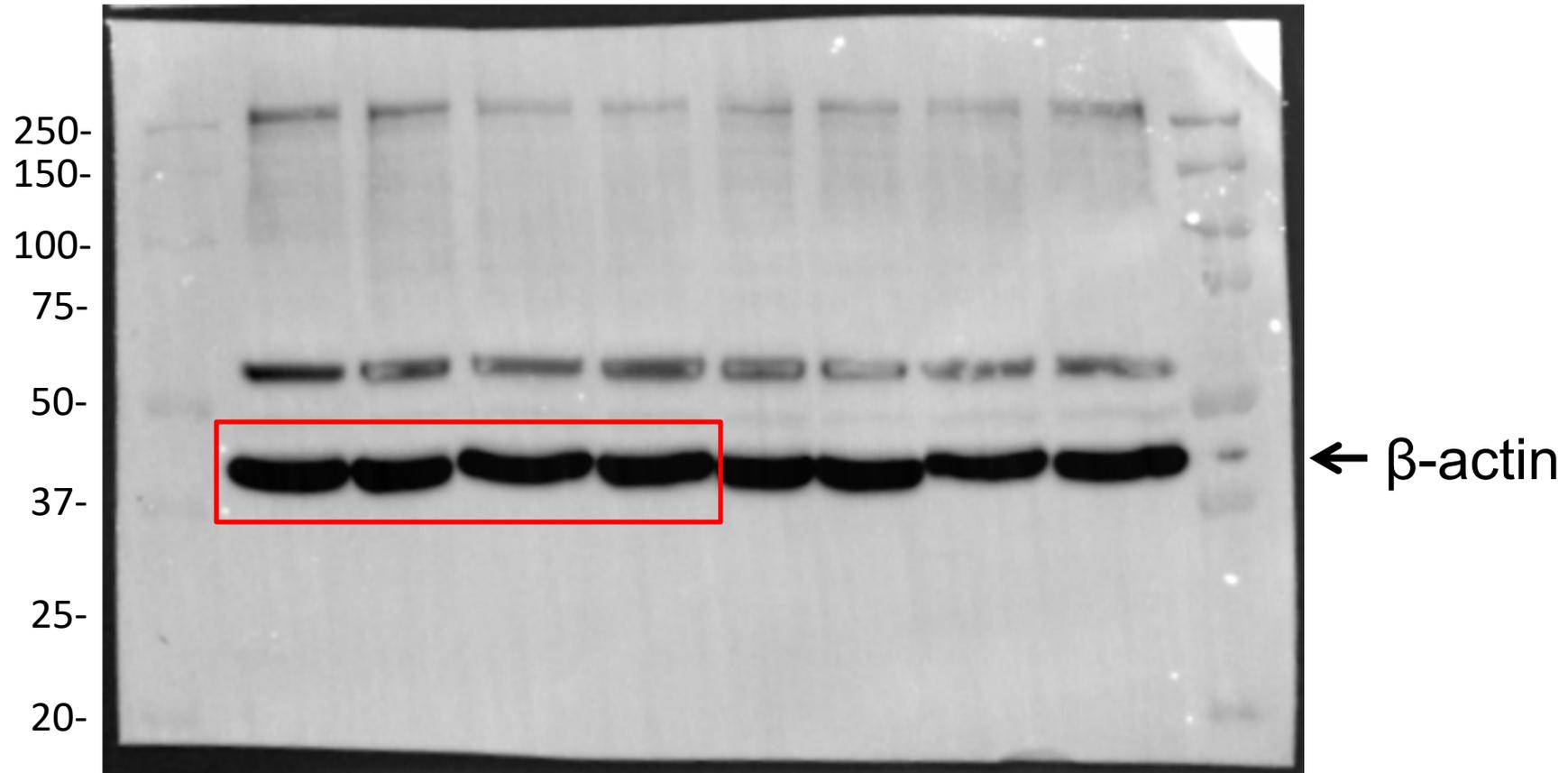
pFn cFn

pFn cFn

Fn-EDA$^{-/-}$
Apoe$^{-/-}$

Fn-EDA$^{-/-}$
TLR4$^{-/-}$ Apoe$^{-/-}$

Full unedited gel for Figure 9D-p-AKT1 (left) and Total AKT1 (right)
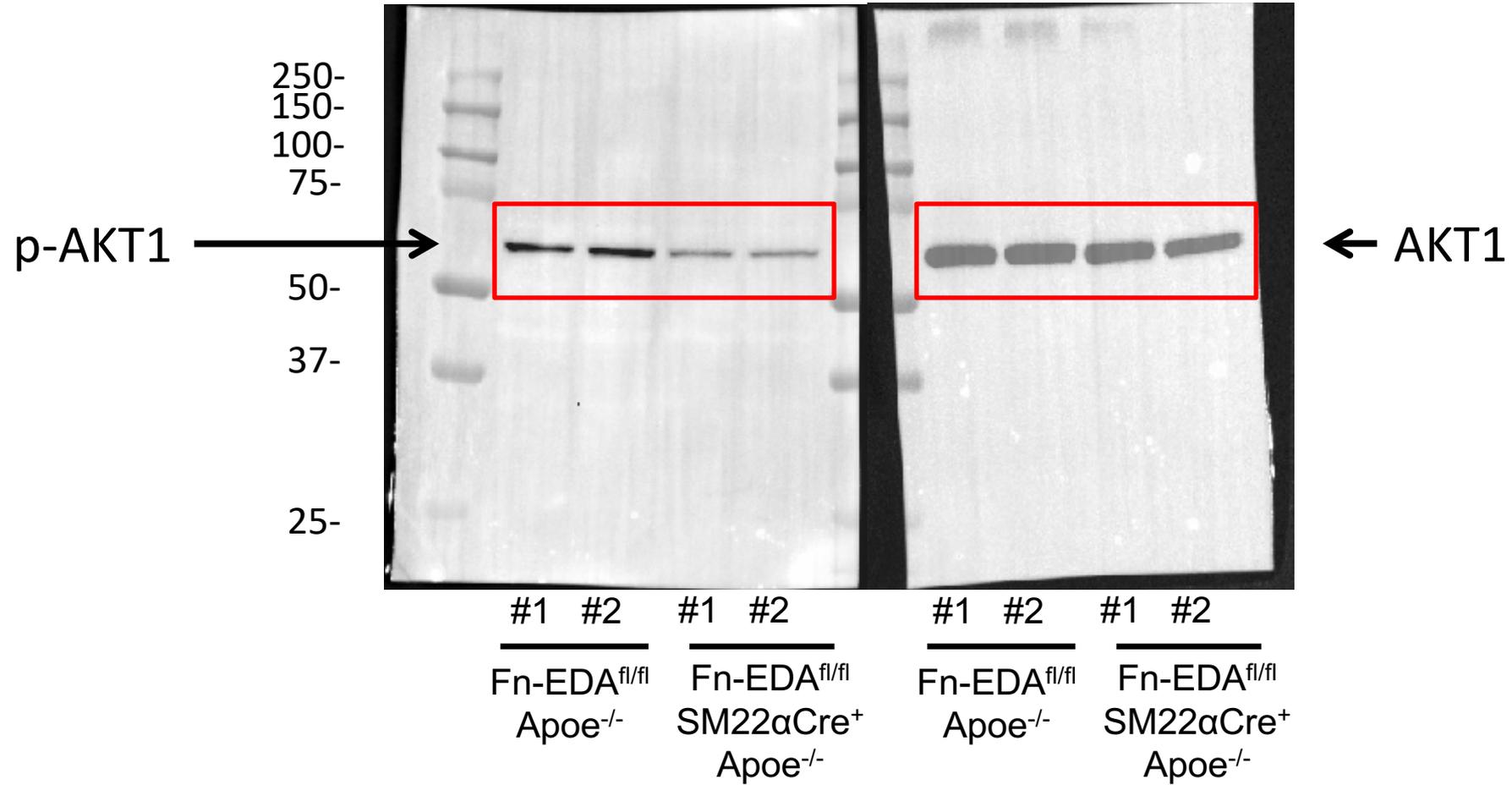
Full unedited gel for Figure 9D-p-AKT2 (left) and Total AKT2 (right)

p-AKT2

250-
150-
100-
75-

50-

37-

25-

AKT2

#1   #2   #1   #2   #1   #2   #1   #2

Fn-EDA$^{fl/fl}$ Fn-EDA$^{fl/fl}$ Fn-EDA$^{fl/fl}$ Fn-EDA$^{fl/fl}$
Apoe$^{-/-}$ SM22αCre^{+} Apoe$^{-/-}$ SM22αCre^{+}
            Apoe$^{-/-}$ Apoe$^{-/-}$
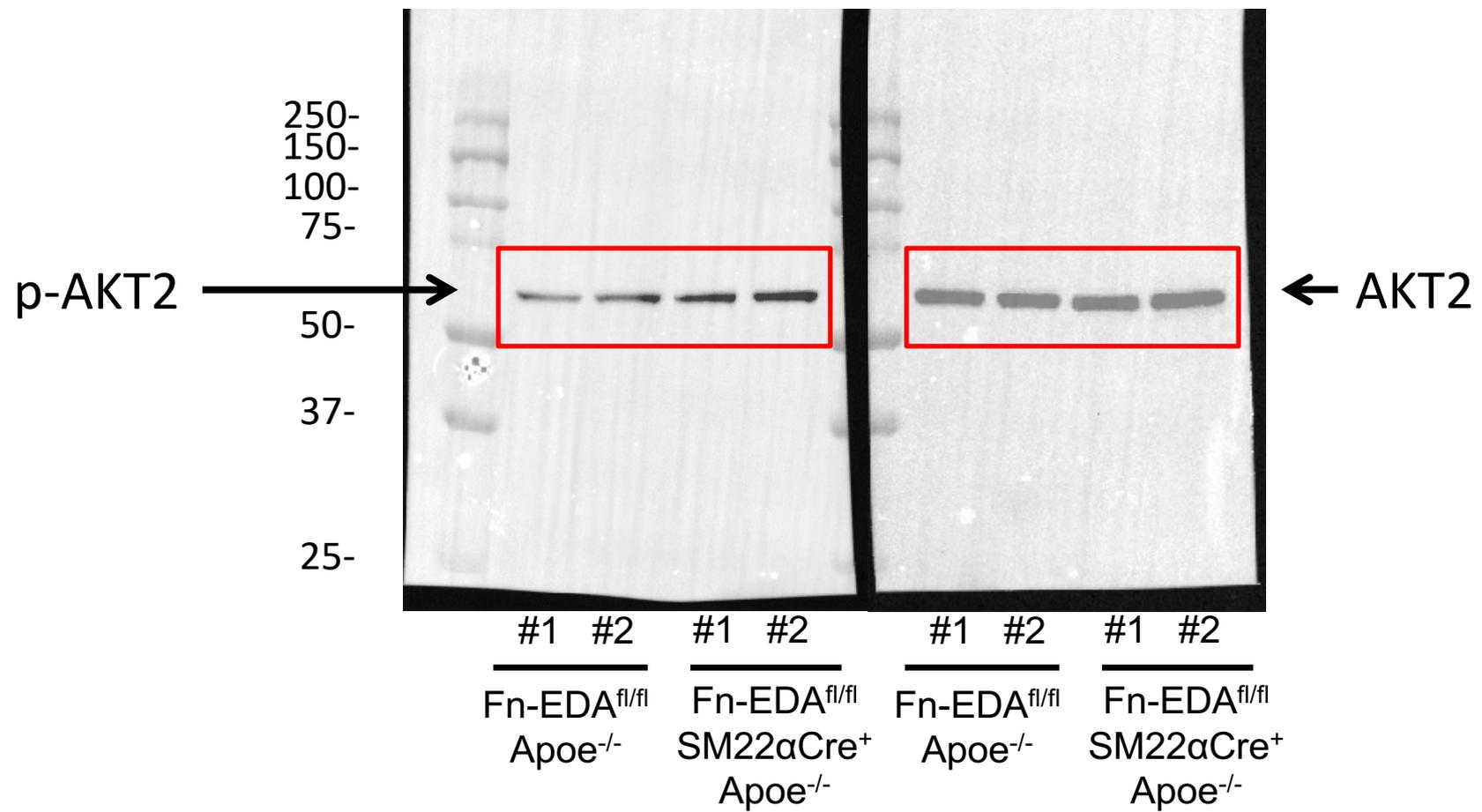
Full unedited gel for Figure 9D-p-mTOR (left) and Total mTOR (right)

p-mTOR

250-
150-
100-
75-

50-

37-

25-

20-

mTOR

#1 #2   #1 #2   #1 #2   #1 #2

Fn-EDA$^{fl/fl}$ Fn-EDA$^{fl/fl}$ Fn-EDA$^{fl/fl}$ Fn-EDA$^{fl/fl}$
Apoe$^{-/-}$ SM22αCre^{+} Apoe$^{-/-}$ SM22αCre^{+}
                Apoe$^{-/-}$ Apoe$^{-/-}$
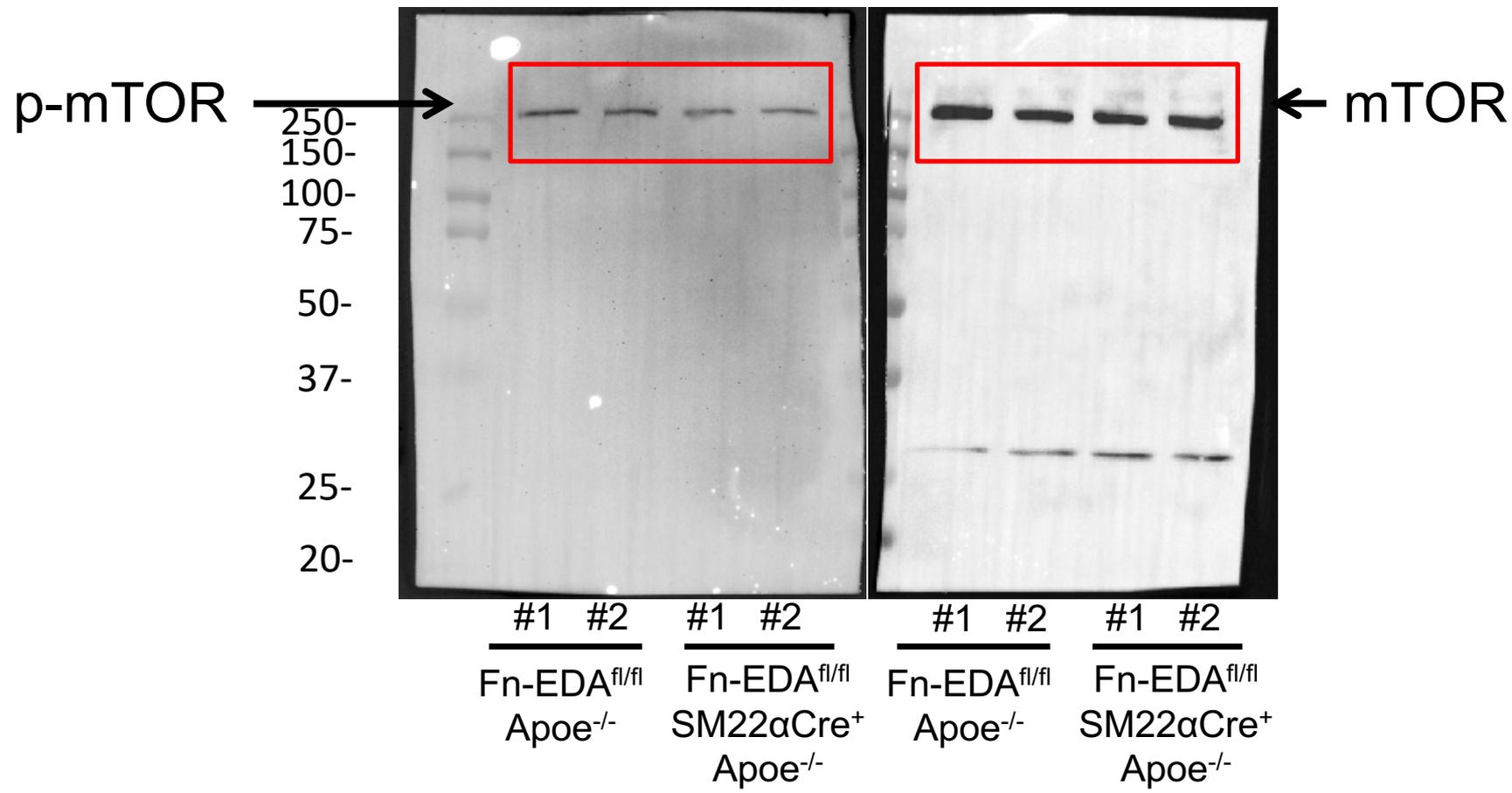
Full unedited gel for Figure 9D-p-NFκB (left) and Total NFκB (right)
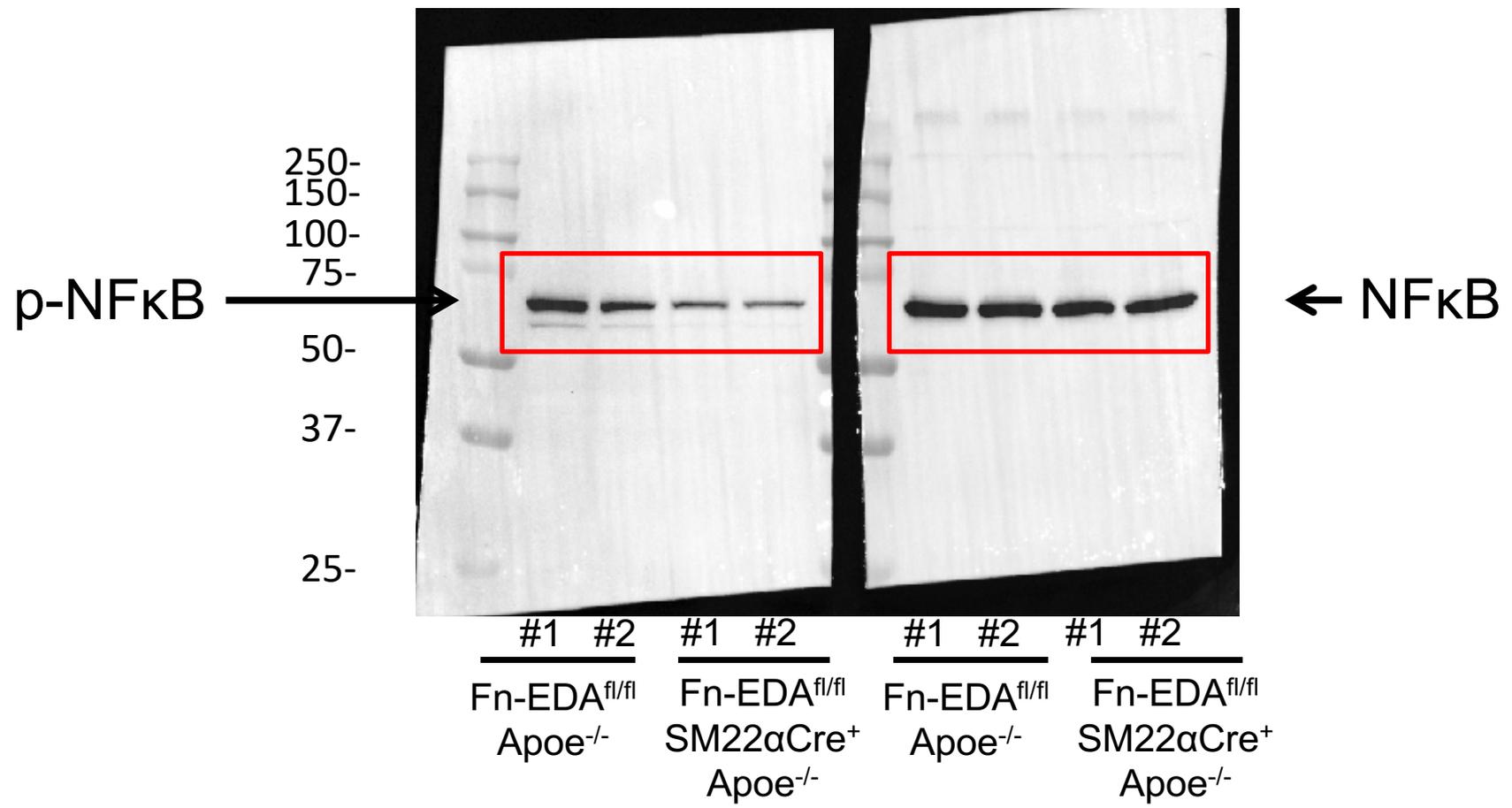
Full unedited gel for Figure 9D-β-actin

β-actin

250-
150-
100-
75-
50-
37-
25-

#1 #2   #1 #2   #1 #2   #1 #2

Fn-EDA$^{fl/fl}$ Fn-EDA$^{fl/fl}$
Apoe$^{-/-}$ SM22αCre$^+$
Apoe$^{-/-}$
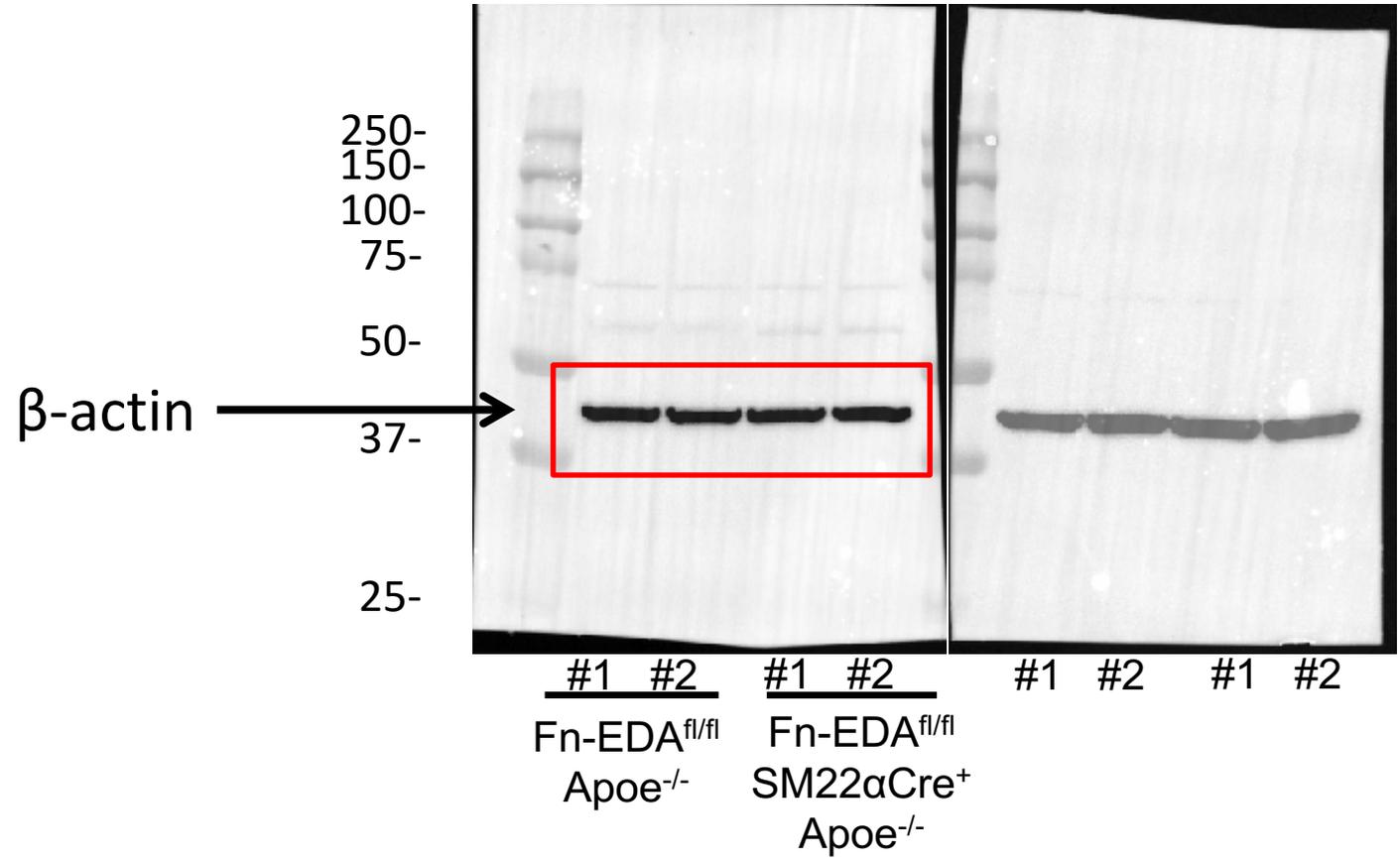
Full unedited gel for Figure 10C-SM22α
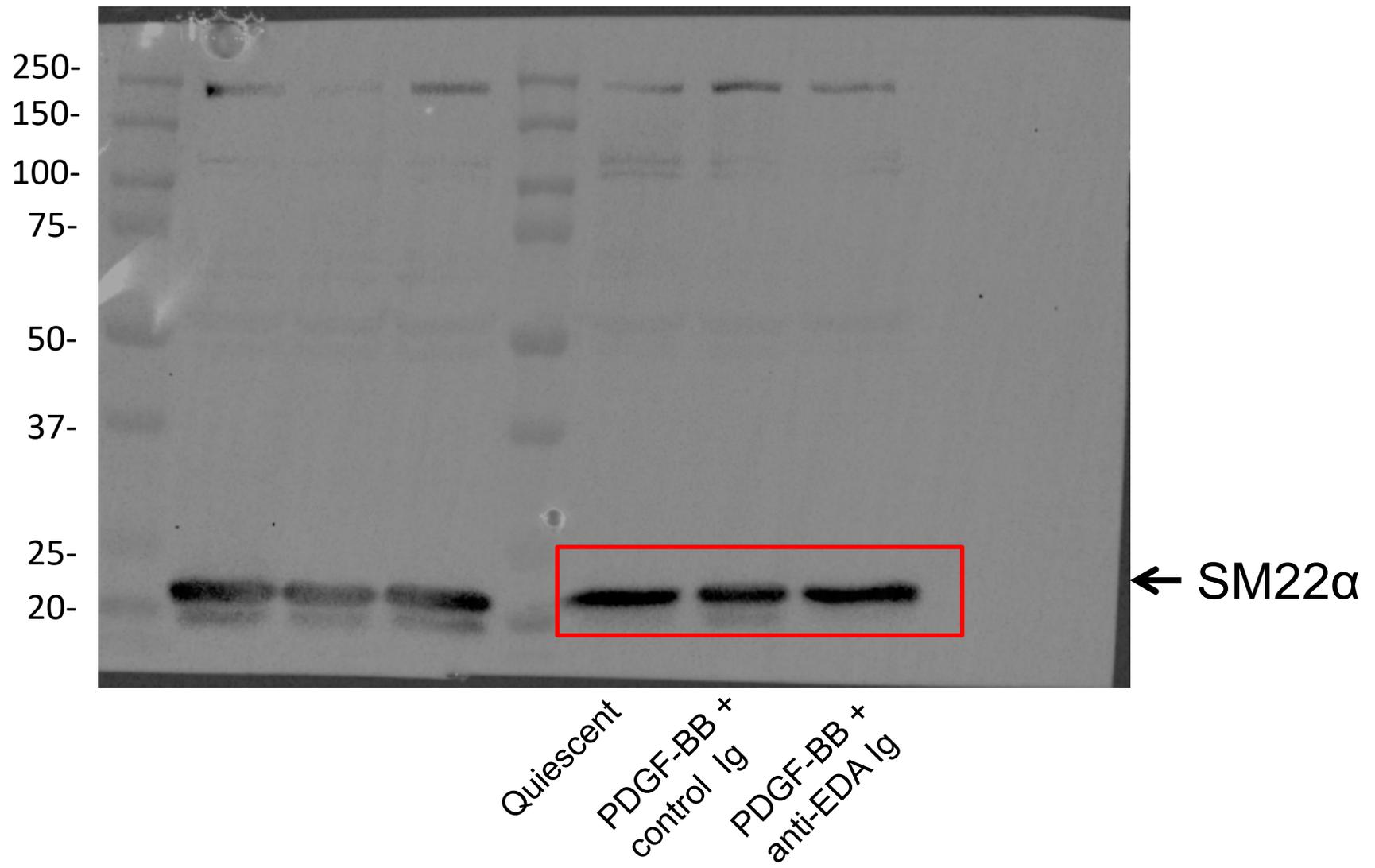
Full unedited gel for Figure 10C-Osteopontin
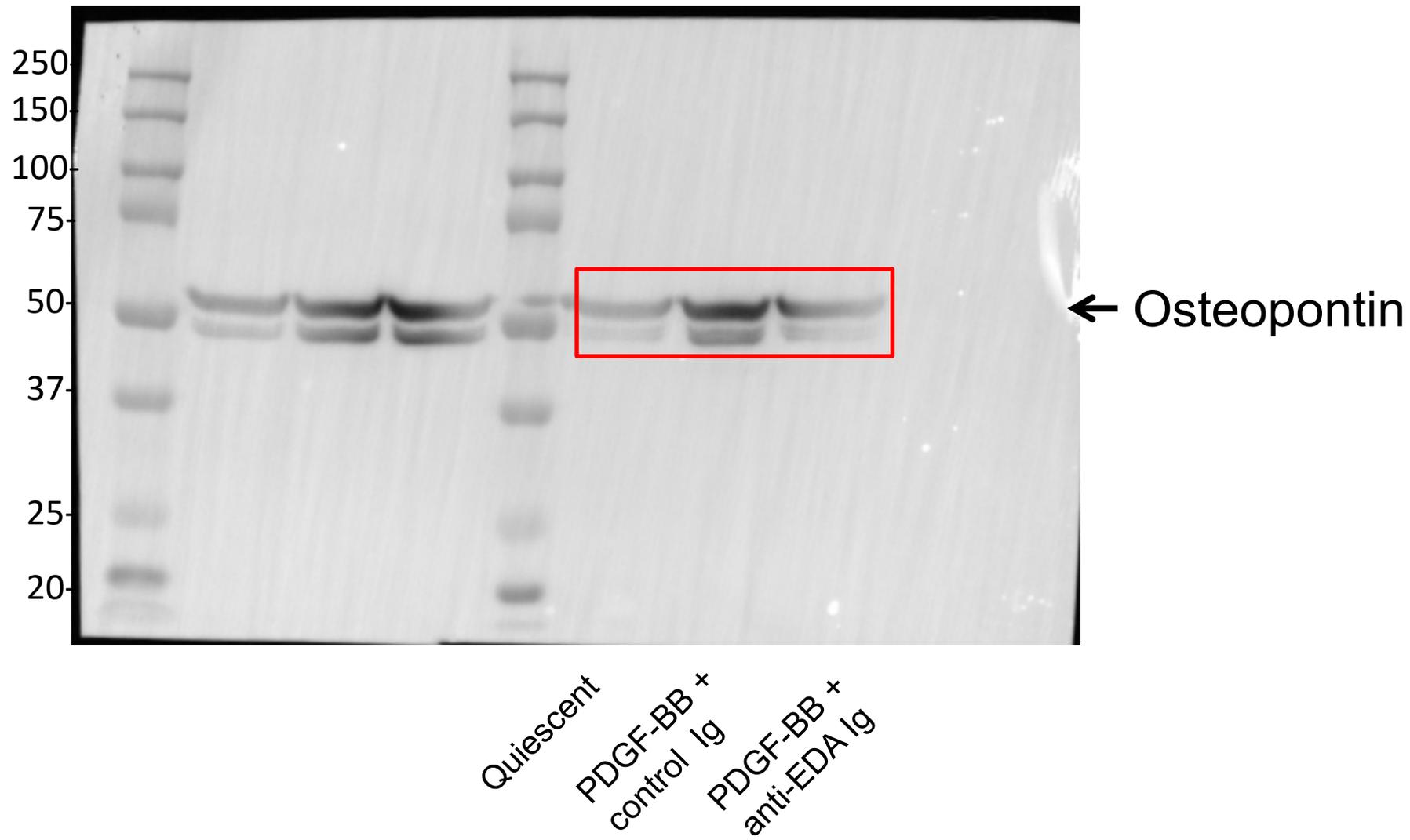
Full unedited gel for Figure 10C-SM-MHC
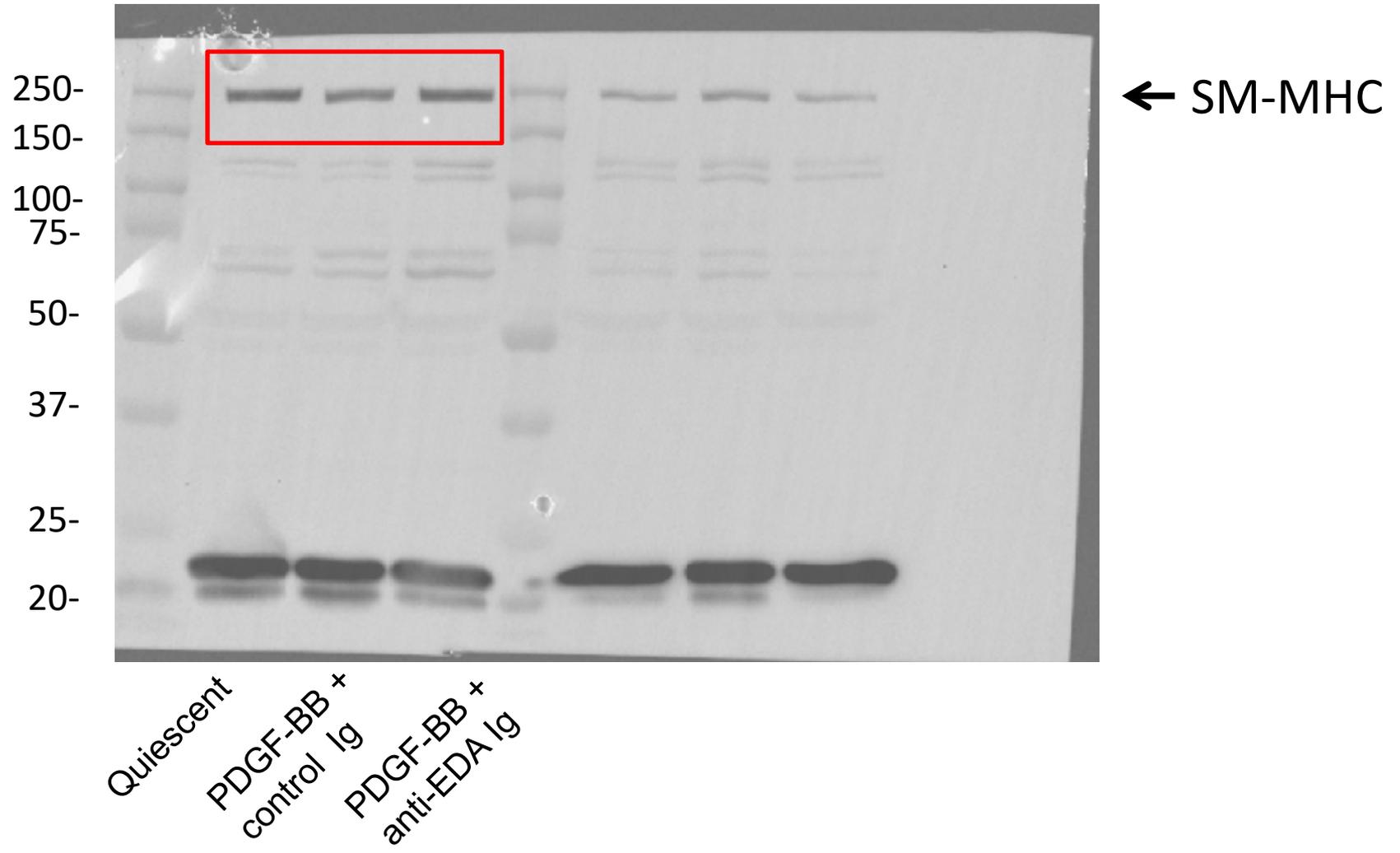
Full unedited gel for Figure 10C-Vimentin

← Vimentin

Quiescent

PDGF-BB +
control Ig

PDGF-BB +
anti-EDA Ig
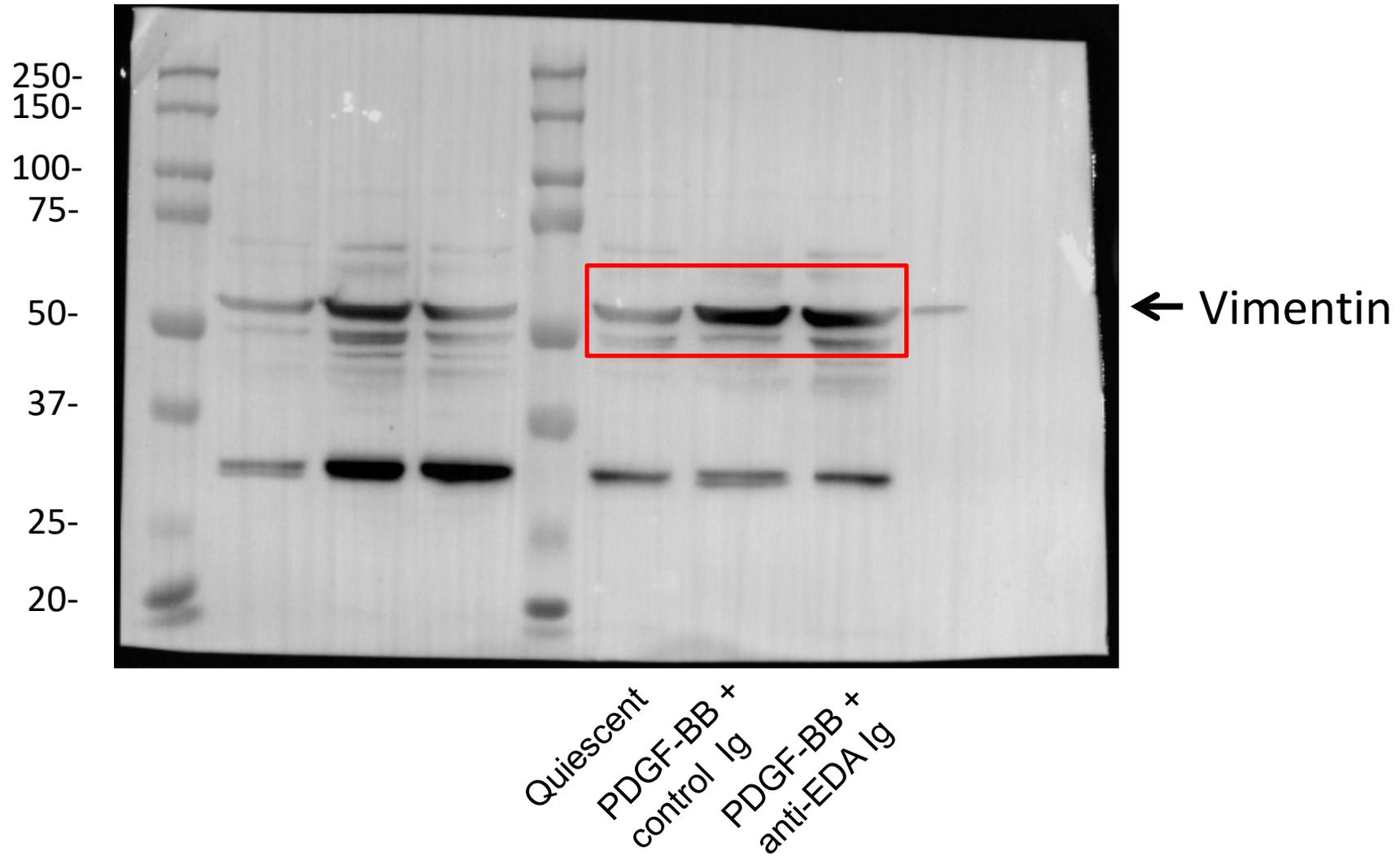
Full unedited gel for Figure 10C-β-actin
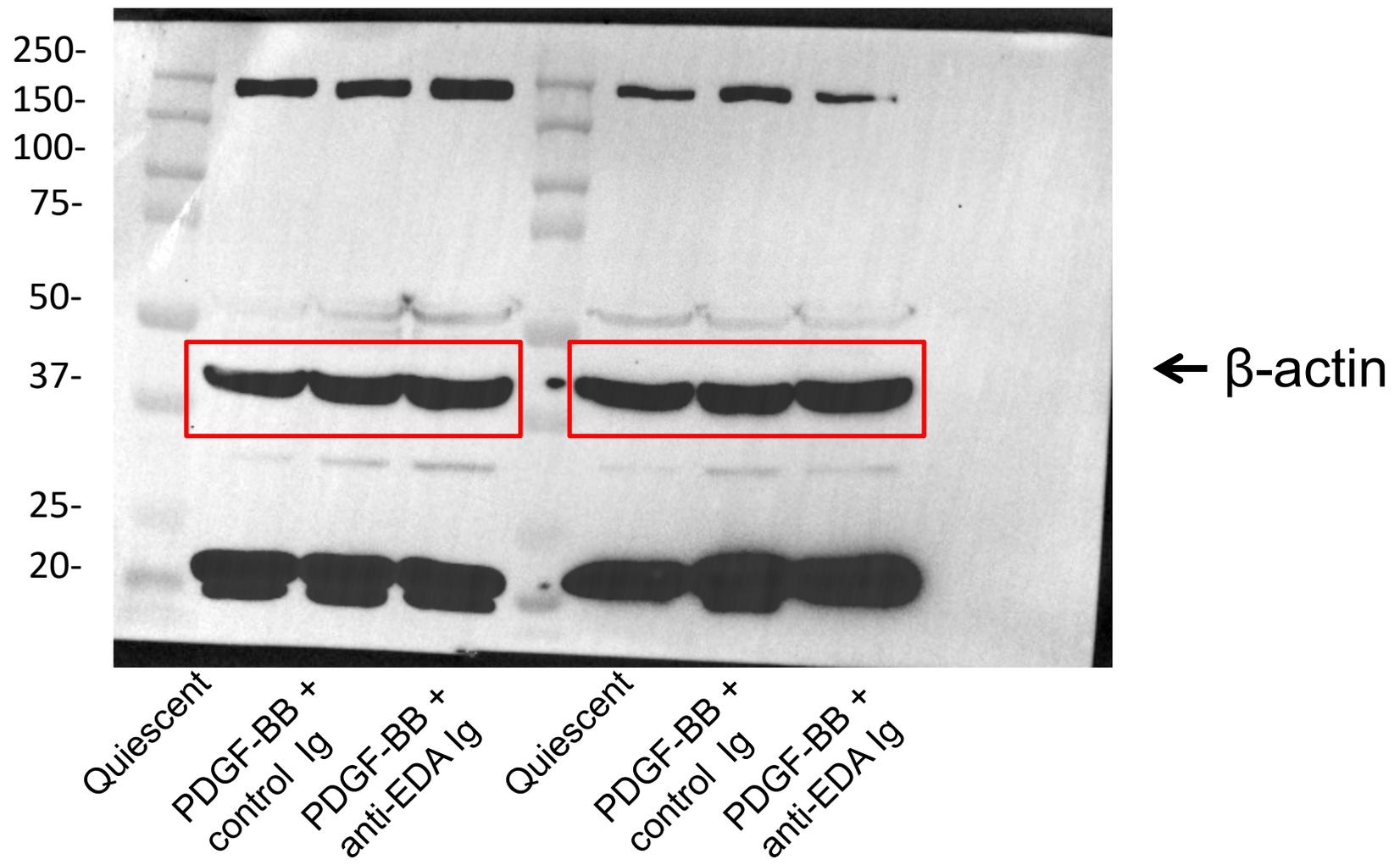
Full unedited gel for Figure 10E-p-AKT1
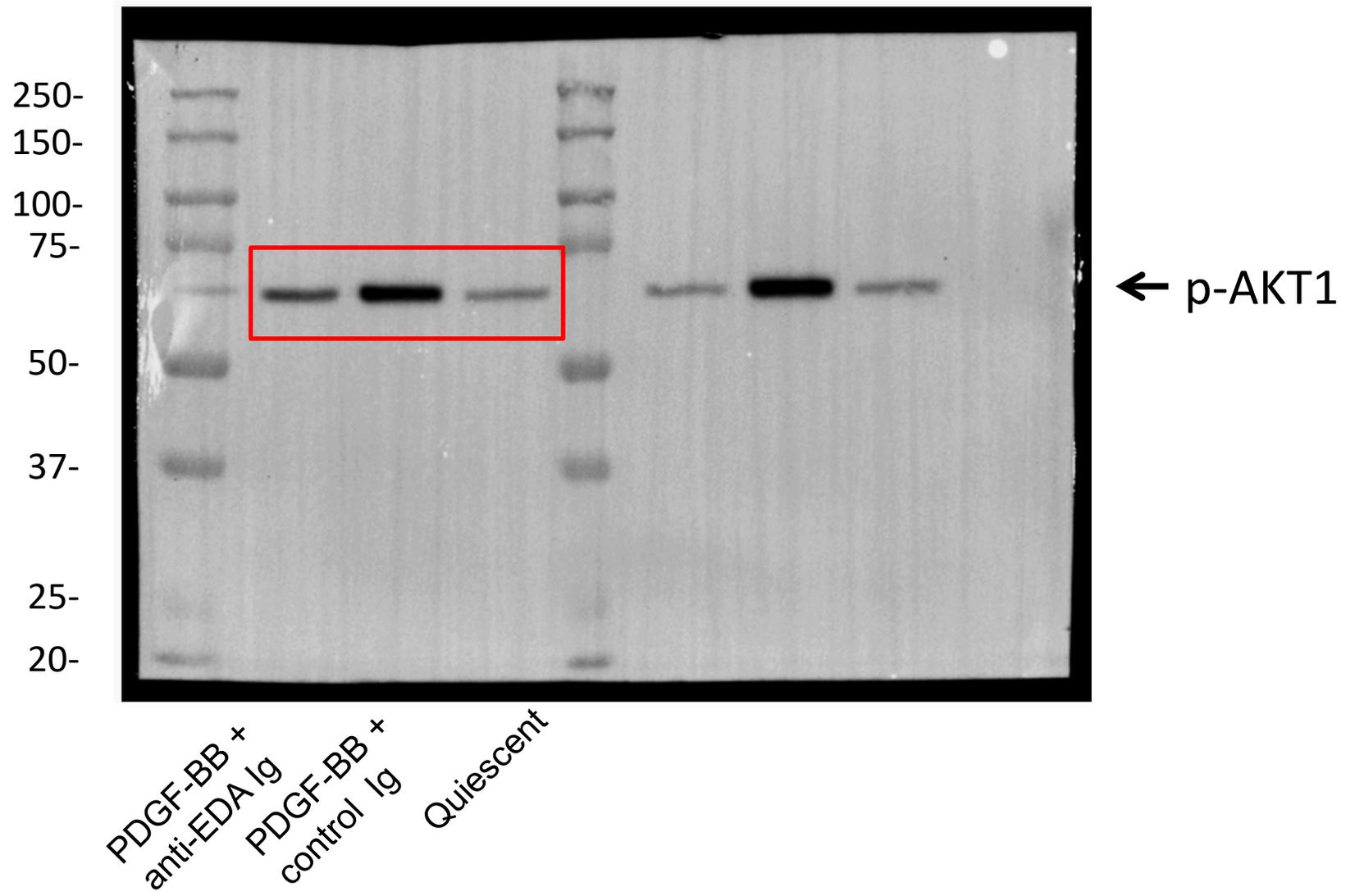
Full unedited gel for Figure 10E-AKT1
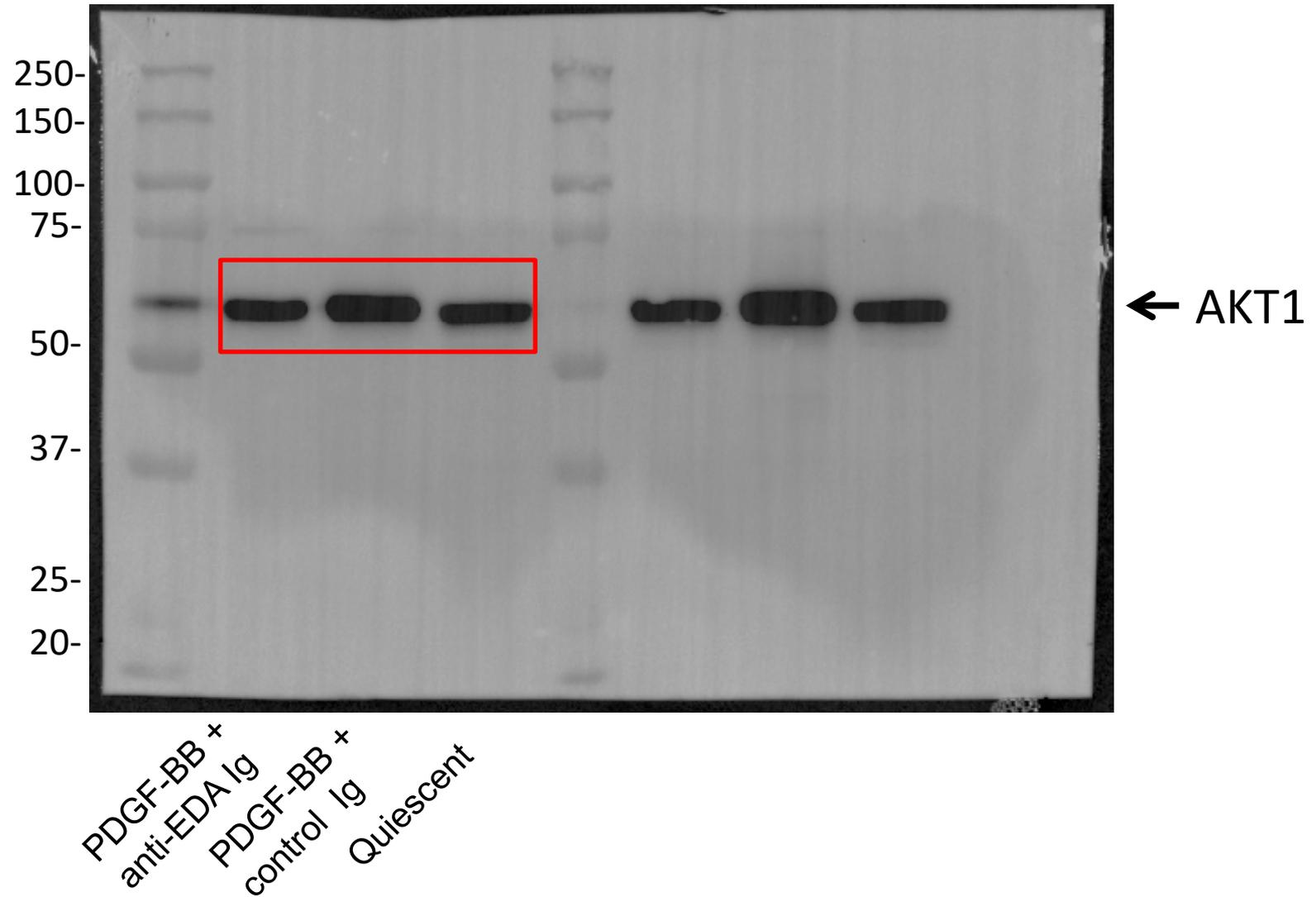
Full unedited gel for Figure 10E-p-mTOR

Full unedited gel for Figure 10E-mTOR

← mTOR

250-
150-

100-
75-

50-

37-

25-

20-

Quiescent

PDGF-BB +
control Ig

PDGF-BB +
anti-EDA Ig
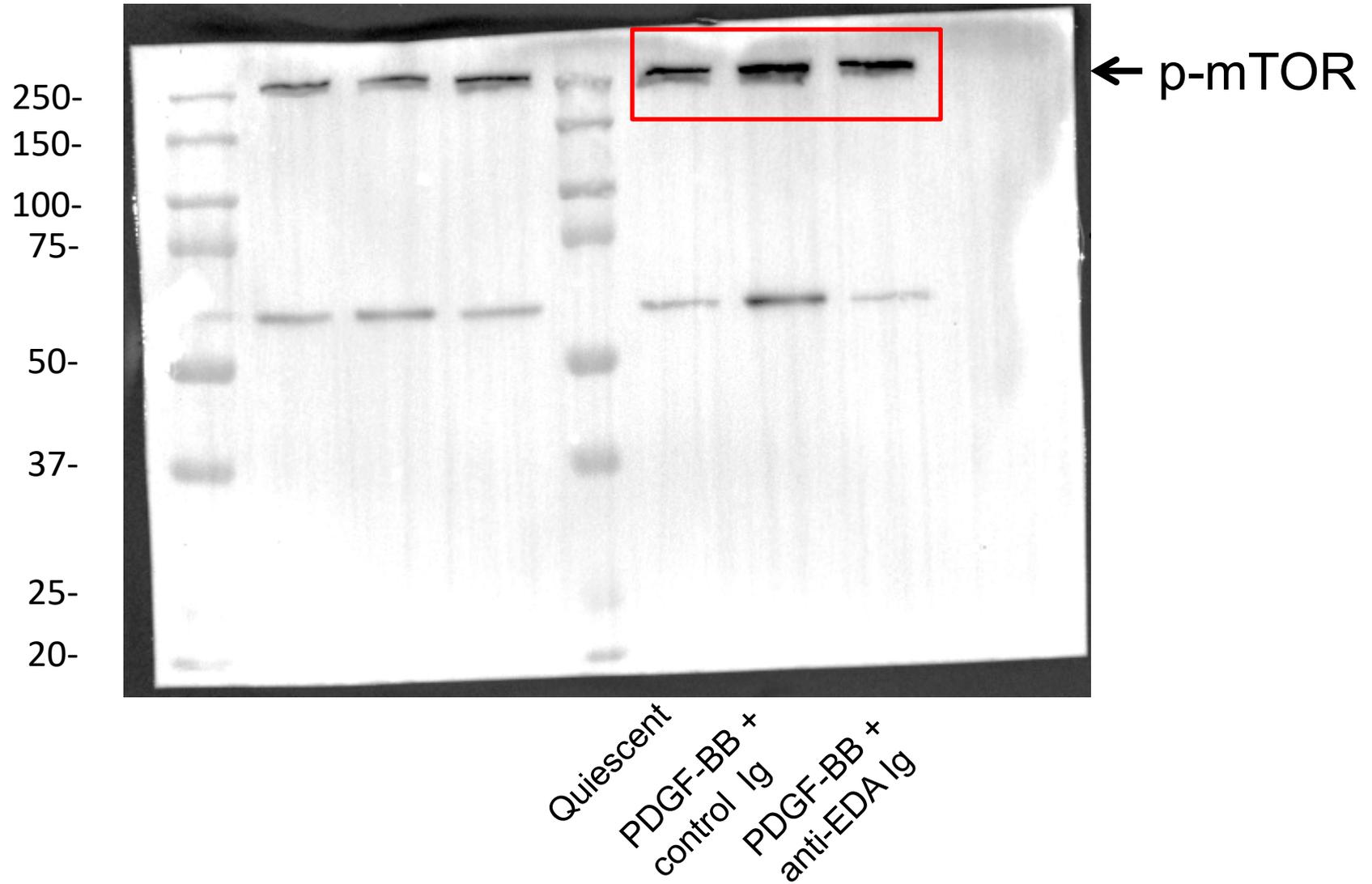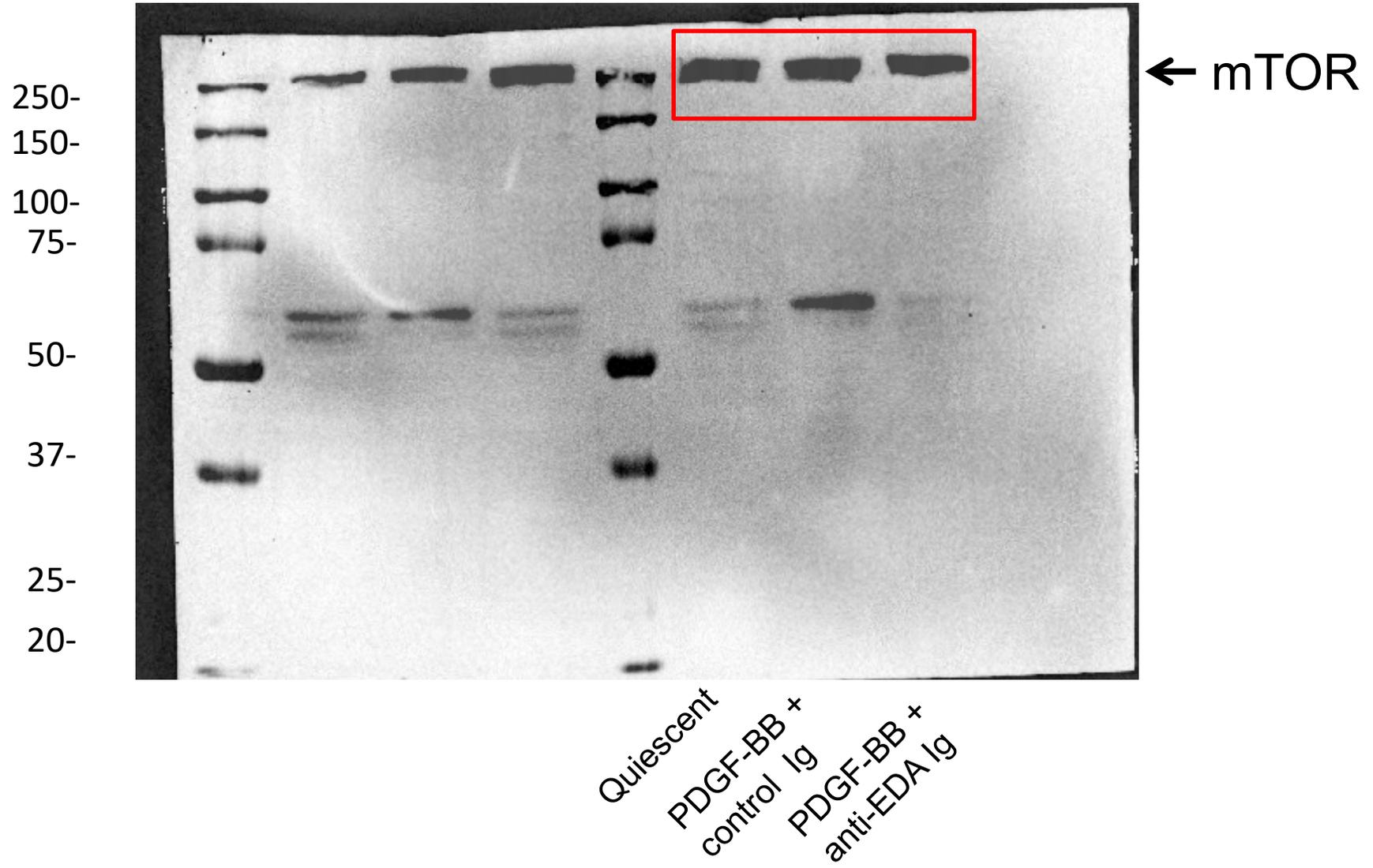
Full unedited gel for Figure 10E-p-NFκB
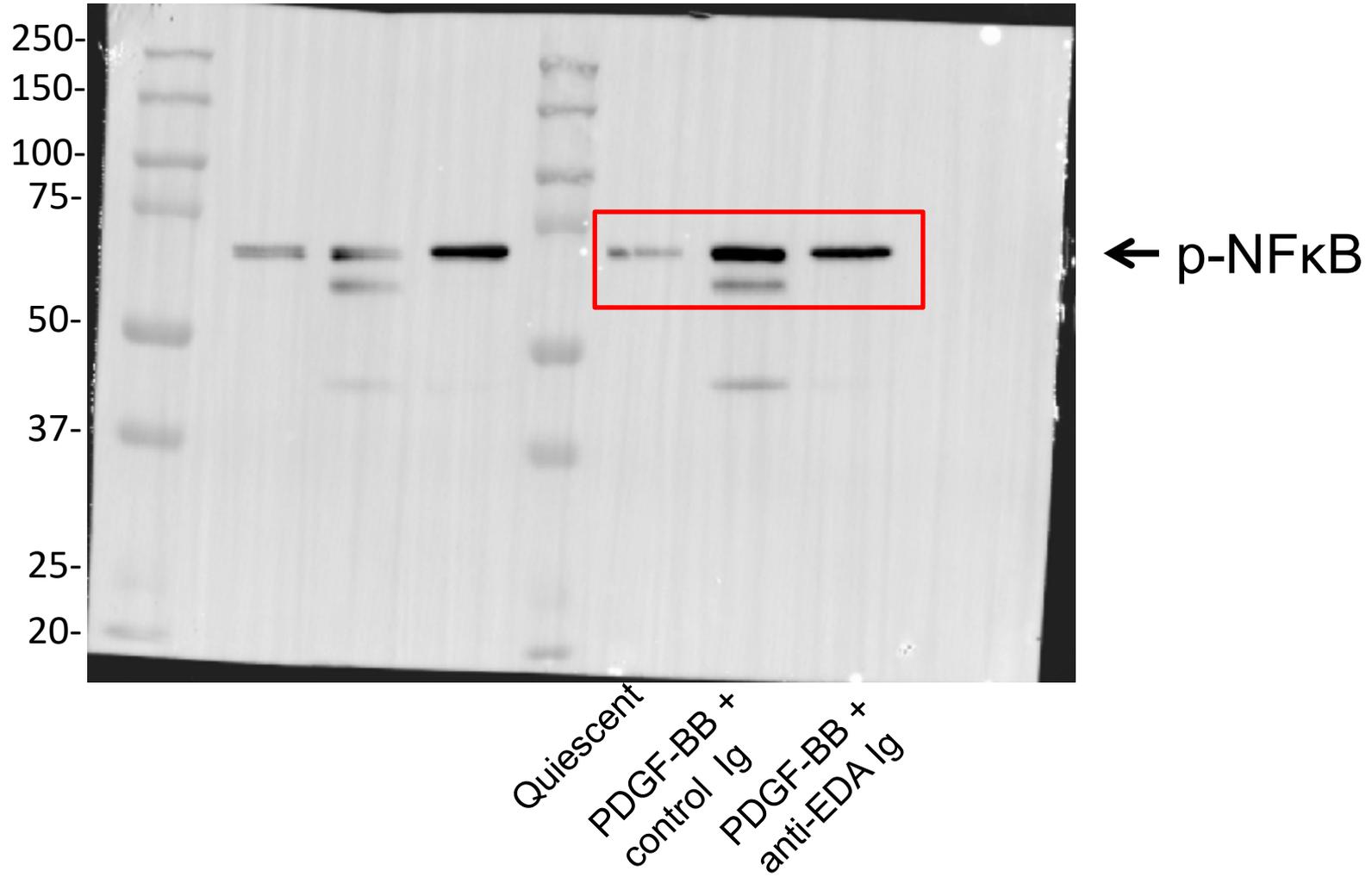
Full unedited gel for Figure 10E-NFκB
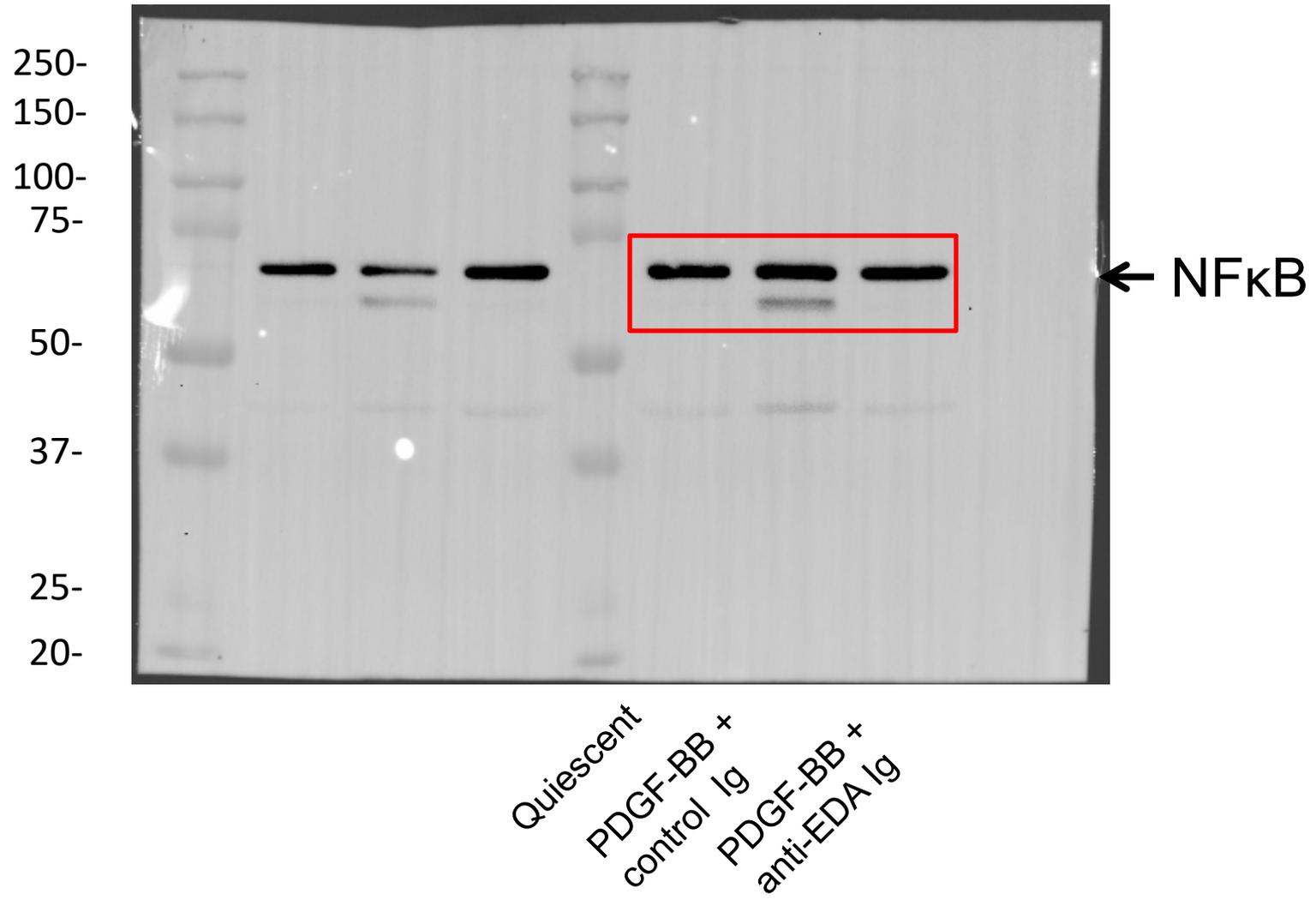
Full unedited gel for Figure 10E-β-actin
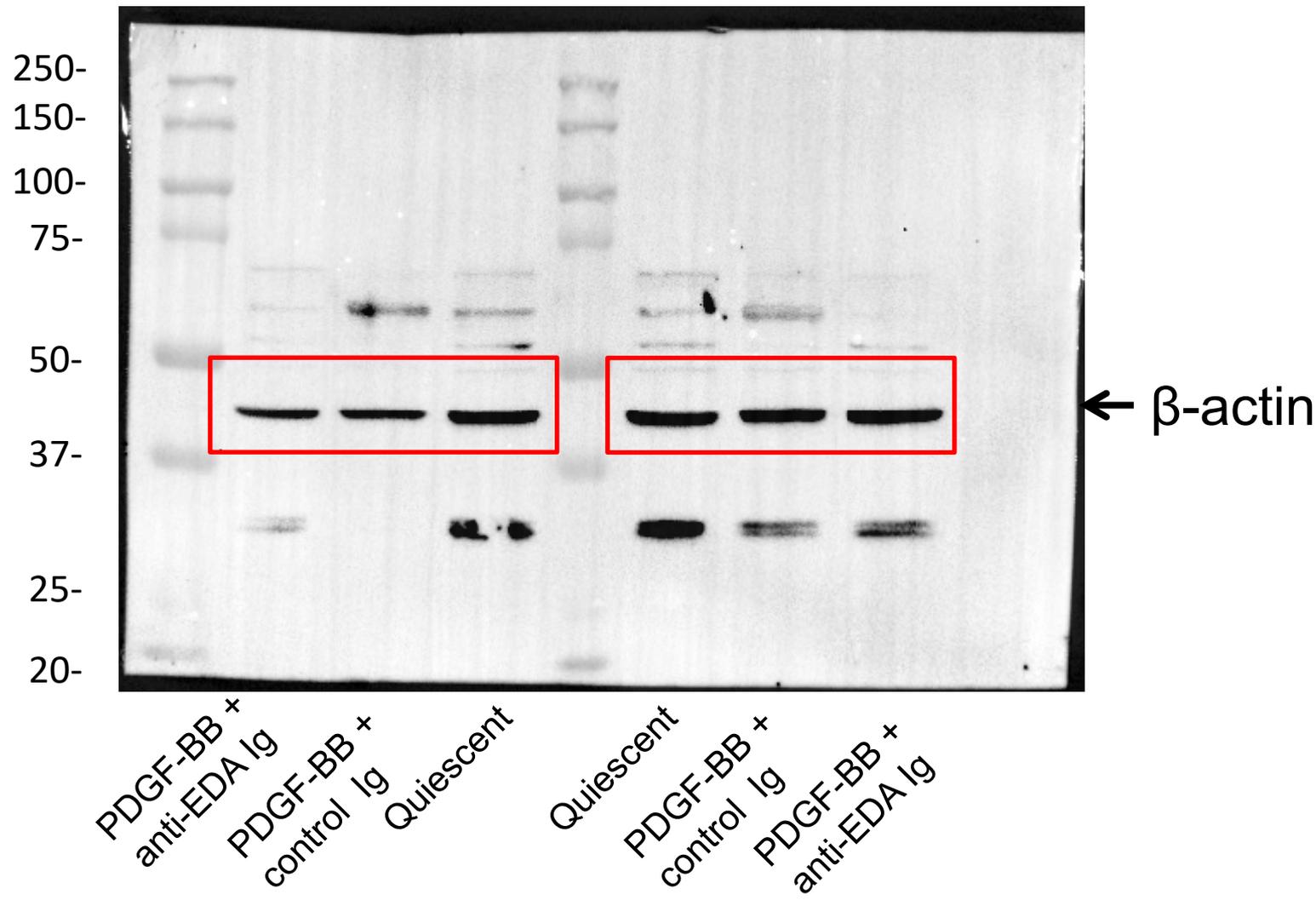
Full unedited gel for Supplemental Figure S5A- Fibronectin

← Fibronectin

250-
150-
100-
75-
50-
37-
25-
20-

#1   #2   #1   #2
Apoe$^{-/-}$ Fn-EDA$^{-/-}$
Apoe$^{-/-}$
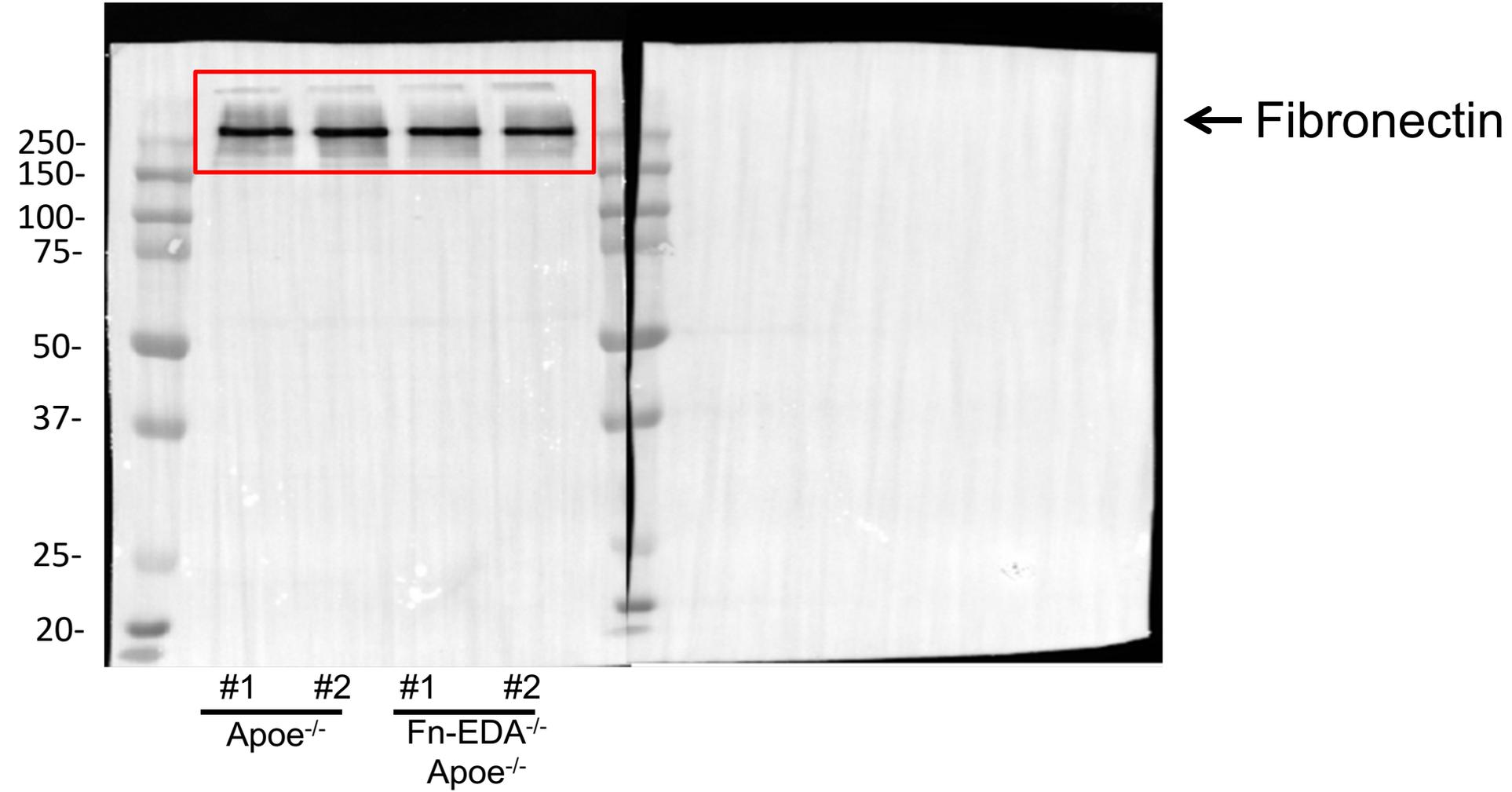
Full unedited gel for Supplemental Figure S5A-β-actin
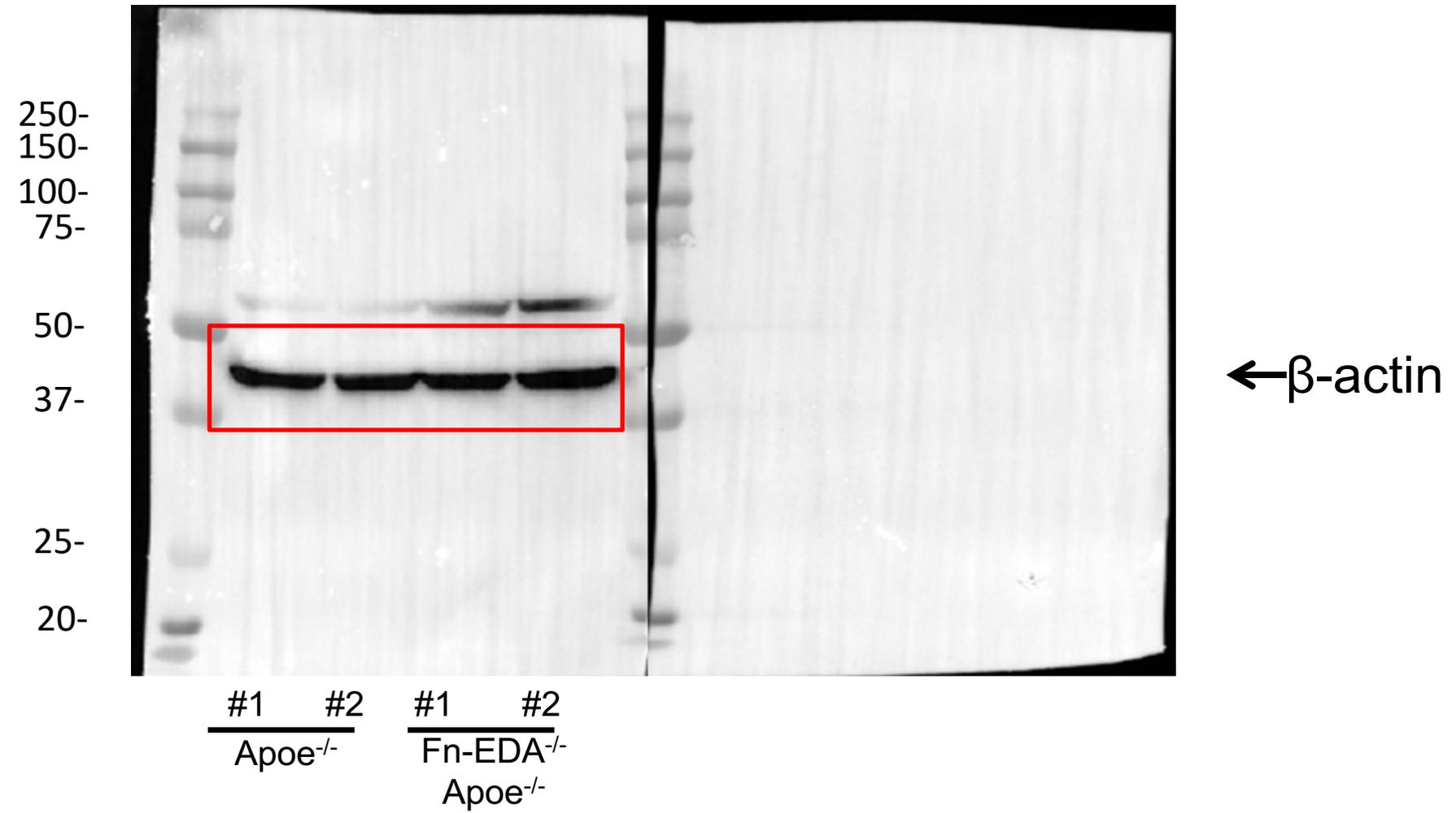
Full unedited gel for Supplemental Figure S7A-SM22α
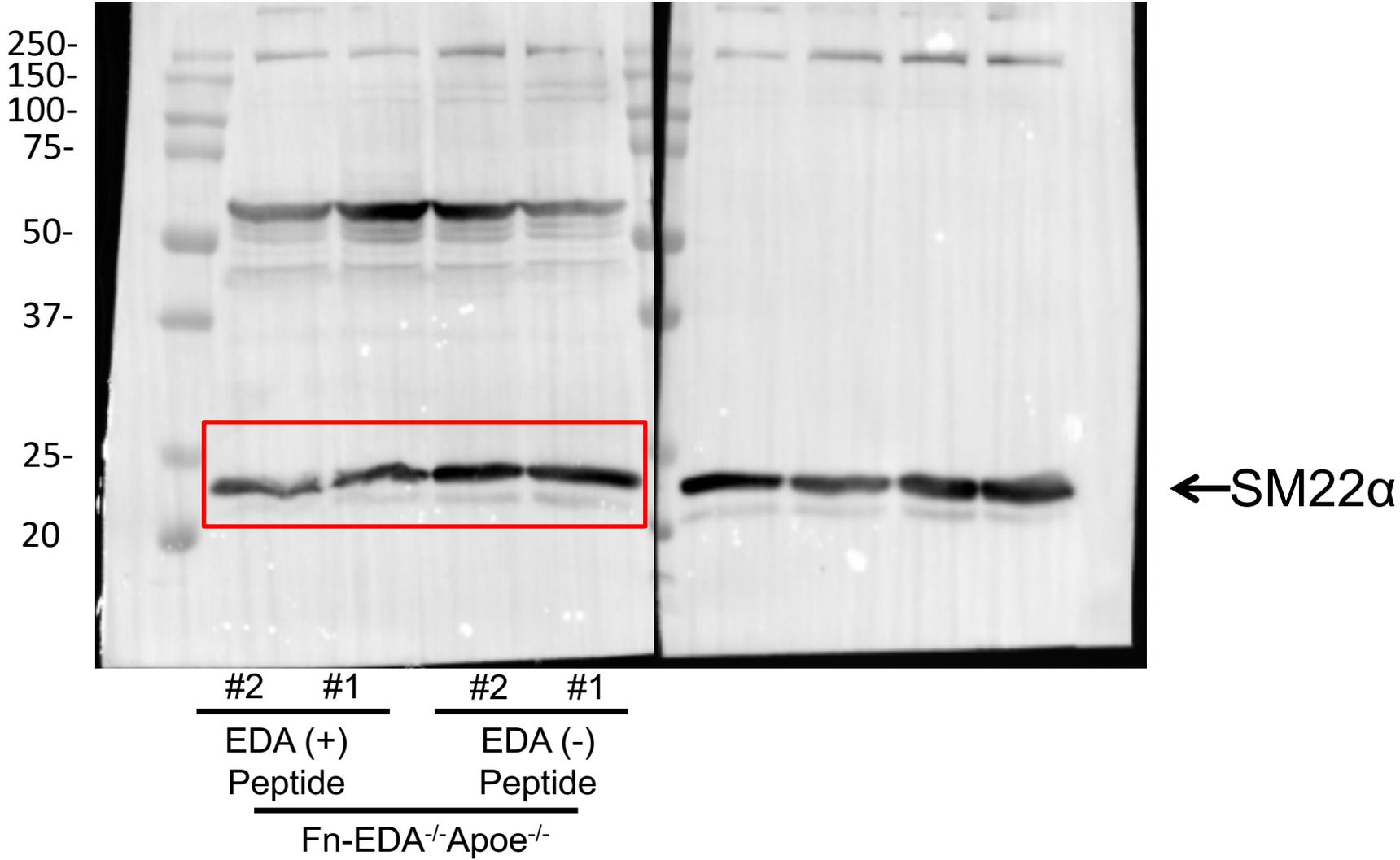
←SM22α

#2   #1   #2   #1
EDA (+)   EDA (-)
Peptide   Peptide
Fn-EDA$^{-/-}$Apoe$^{-/-}$

Full unedited gel for Supplemental Figure S7A- SM-MHC

←SM-MHC

250-
150-
100-
75-

50-

37-

25-

20

#2   #1   #2   #1

EDA (+)   EDA (-)
Peptide   Peptide

Fn-EDA$^{-/-}$Apoe$^{-/-}$
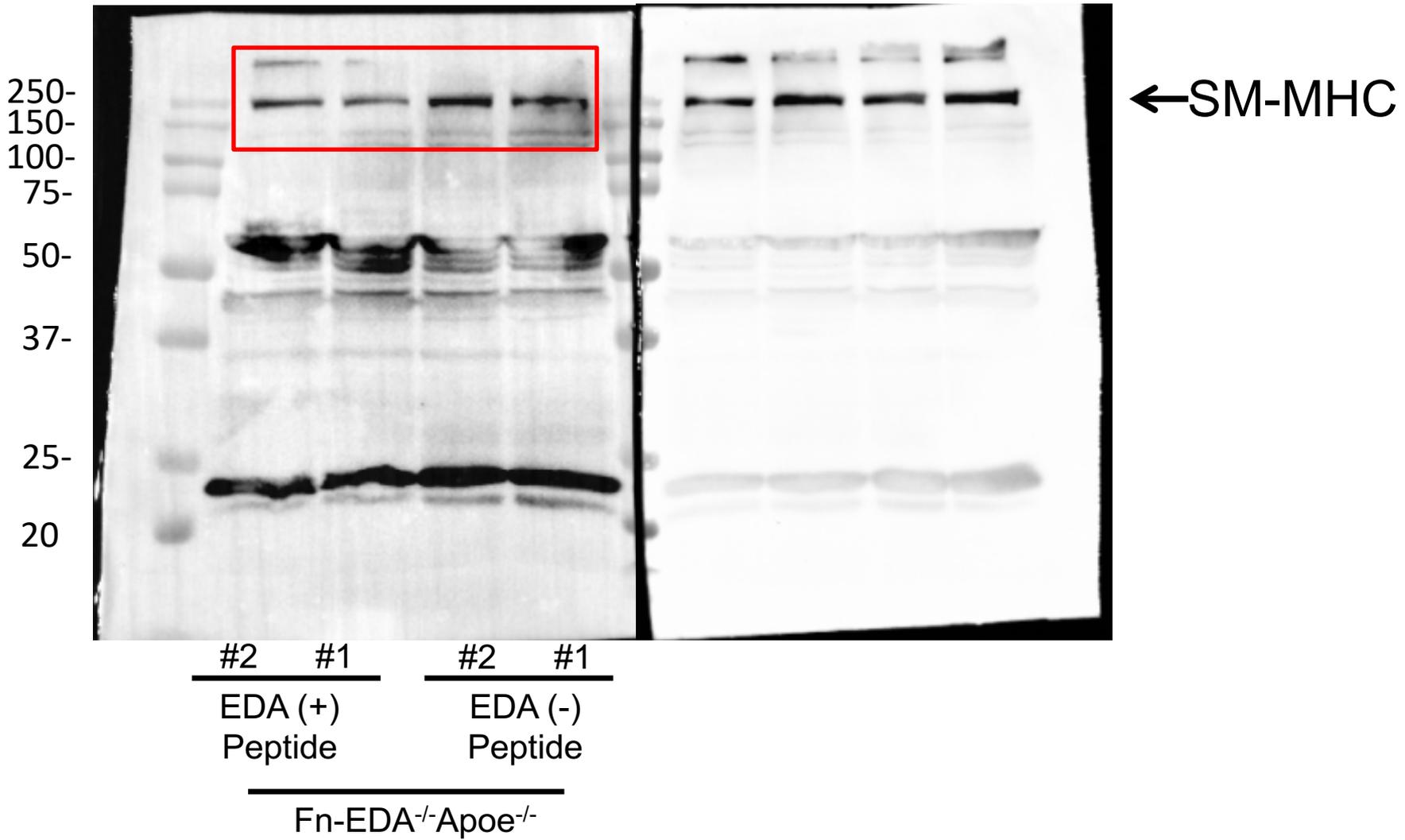
Full unedited gel for Supplemental Figure S7A- Vimentin

←Vimentin

250-
150-
100-
75-

50-

37-

25-

20

#2   #1   #2   #1

EDA (+)   EDA (-)
Peptide   Peptide

Fn-EDA$^{-/-}$Apoe$^{-/-}$
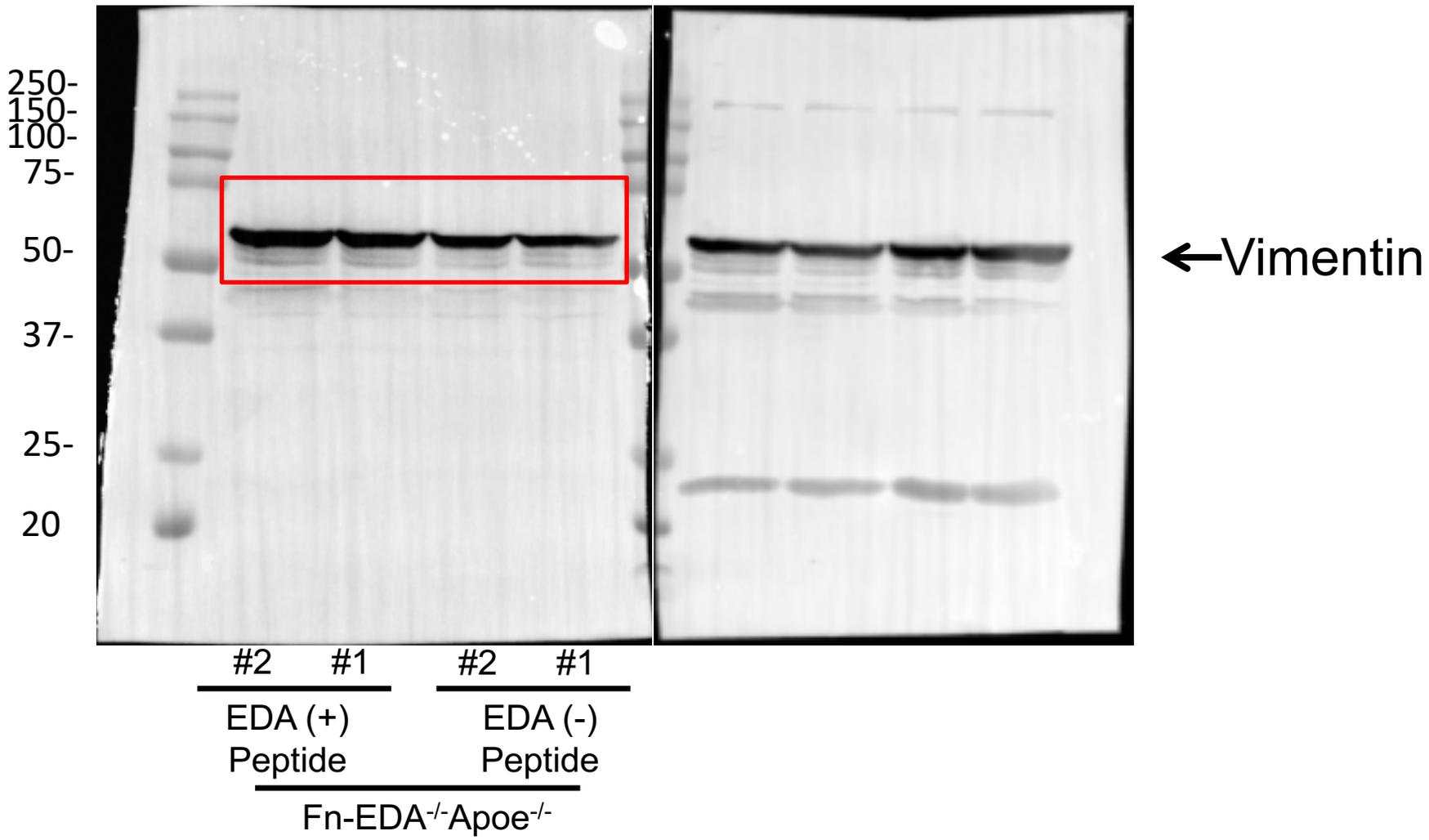
Full unedited gel for Supplemental Figure S7A- Osteopontin

←Osteopontin

250-
150-
100-
75-
50-
37-
25-
20

#2   #1   #2   #1
EDA (+)   EDA (-)
Peptide   Peptide

Fn-EDA$^{-/-}$Apoe$^{-/-}$
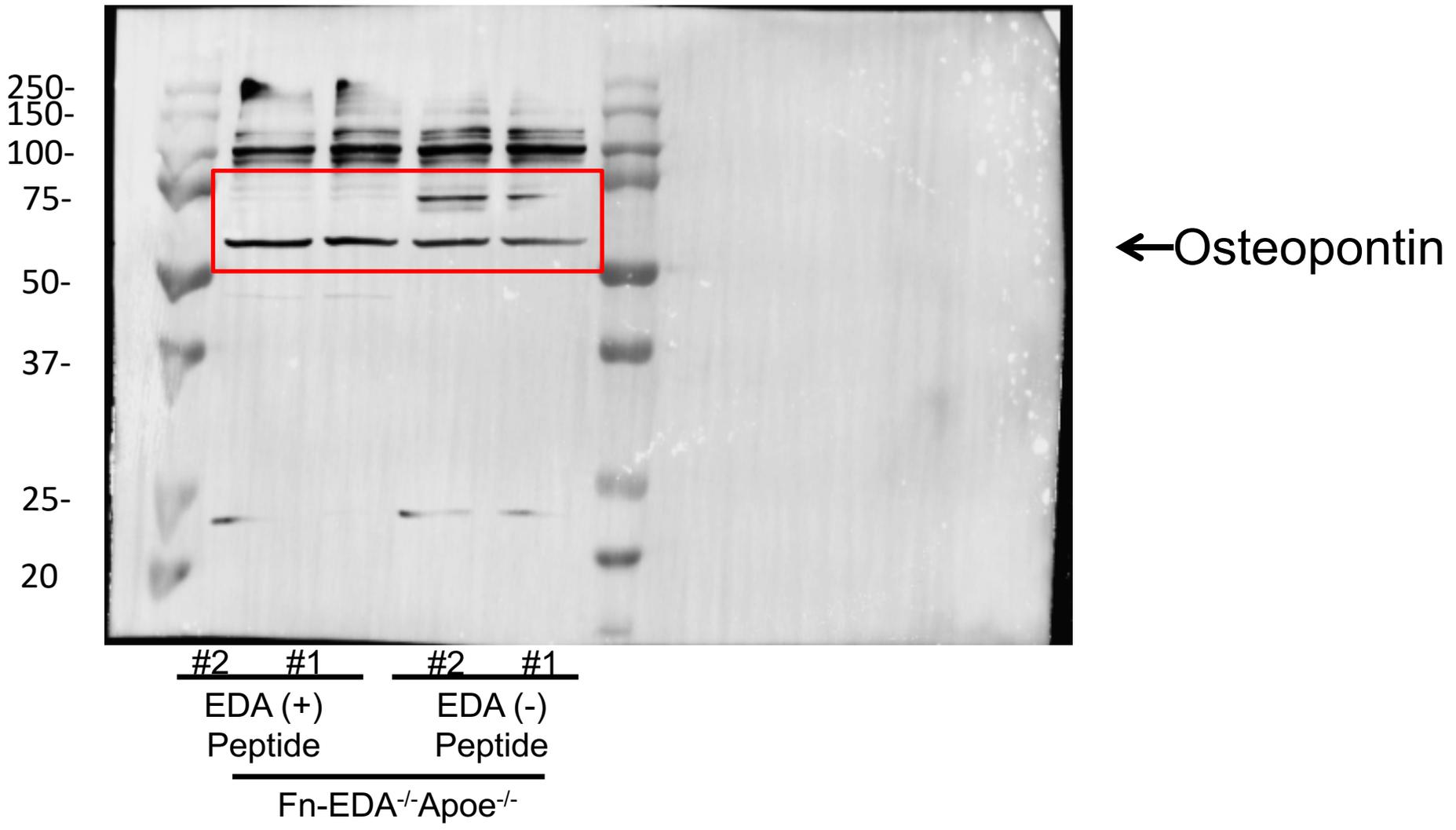
Full unedited gel for Supplemental Figure S7A-β-actin
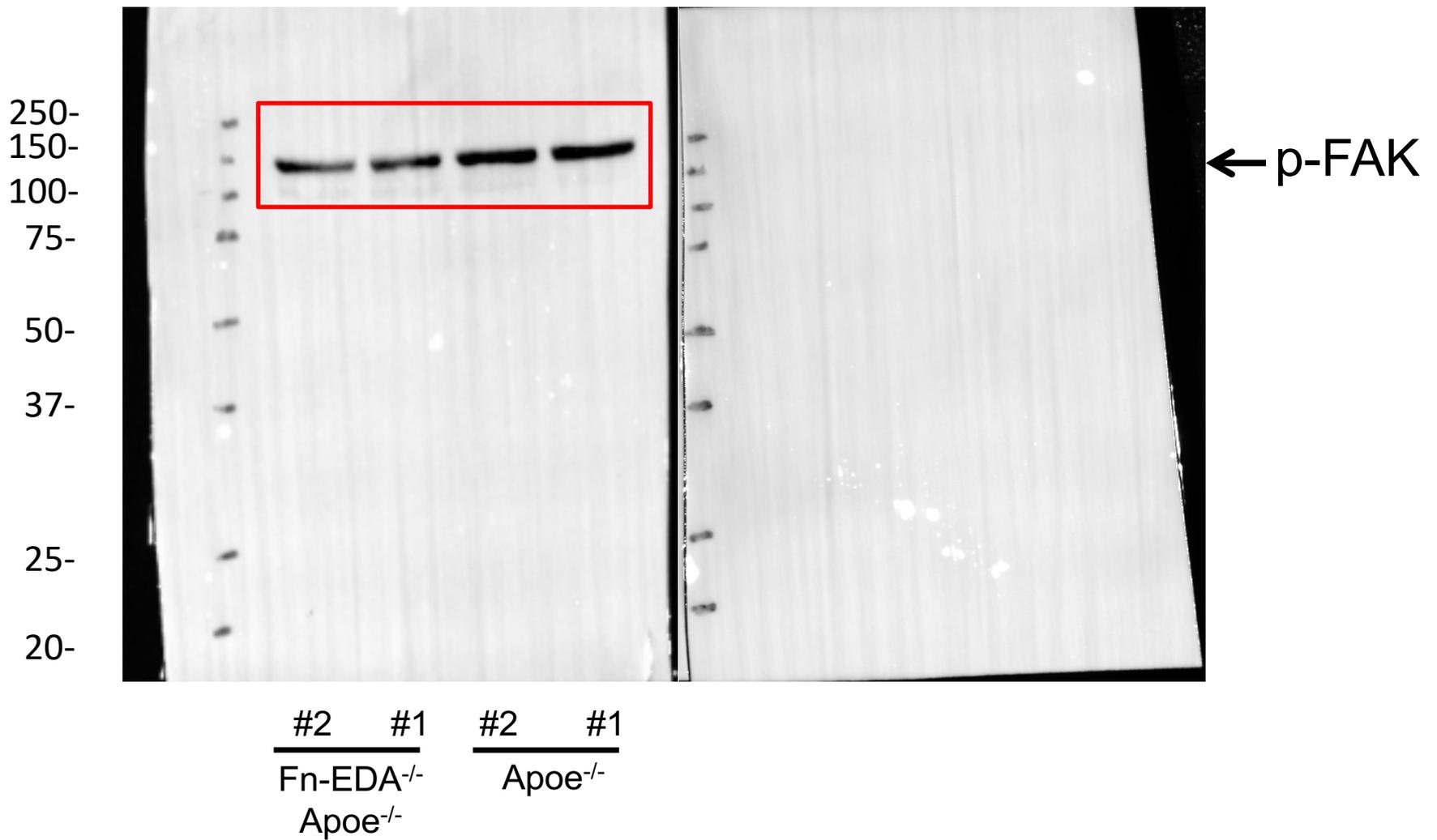
← β-actin

250-
150-
100-
75-

50-
37-

25-

20

#2   #1   #2   #1
EDA (+)   EDA (-)
Peptide   Peptide
Fn-EDA$^{-/-}$Apoe$^{-/-}$

Full unedited gel for Supplemental Figure S8A- p-FAK

Full unedited gel for Supplemental Figure S8A- FAK

← FAK

250-
150-
100-
75-
50-
37-
25-
20-

#2   #1   #2   #1
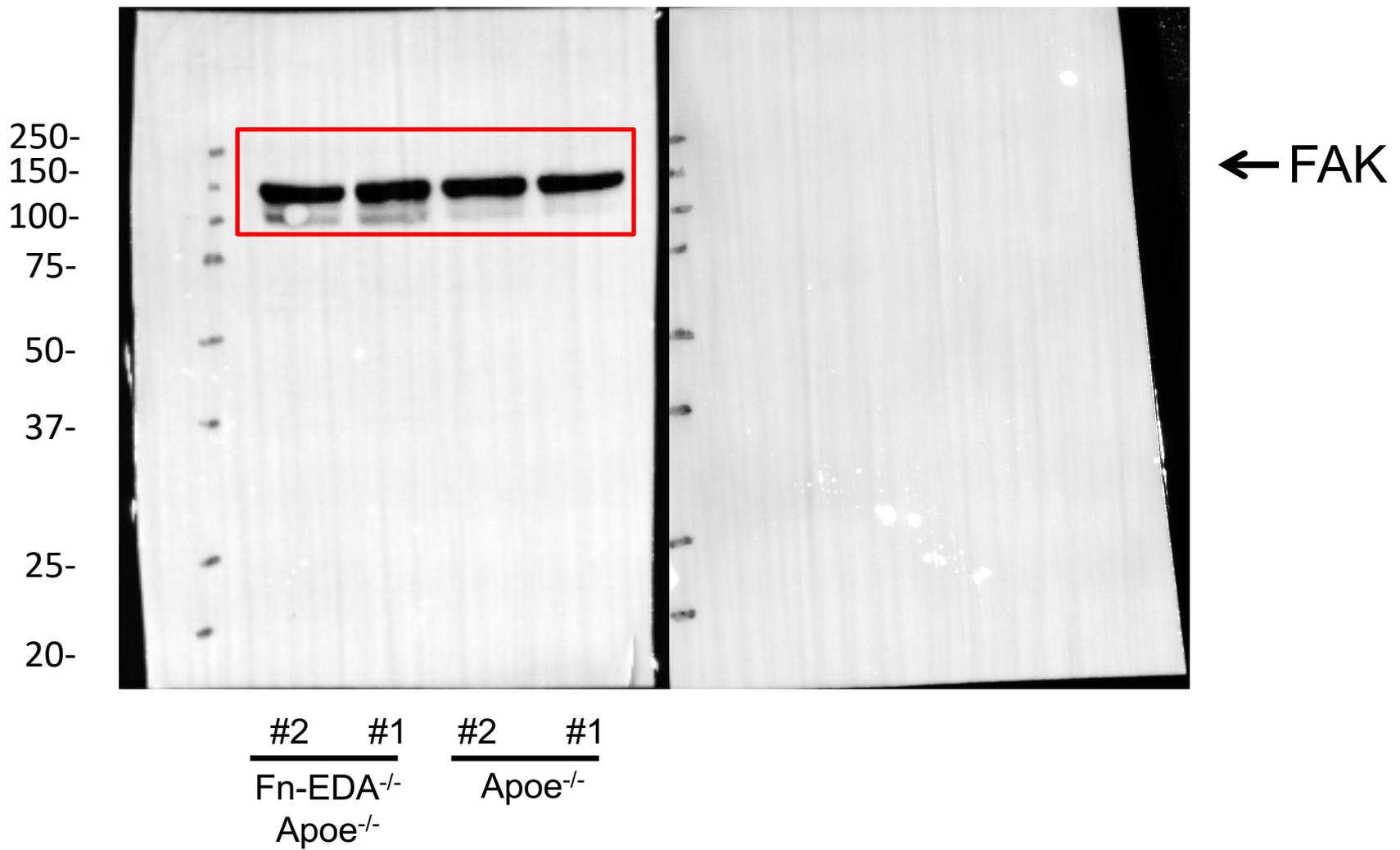
Fn-EDA$^{-/-}$ Apoe$^{-/-}$
Apoe$^{-/-}$

Full unedited gel for Supplemental Figure S8A-p-SRC
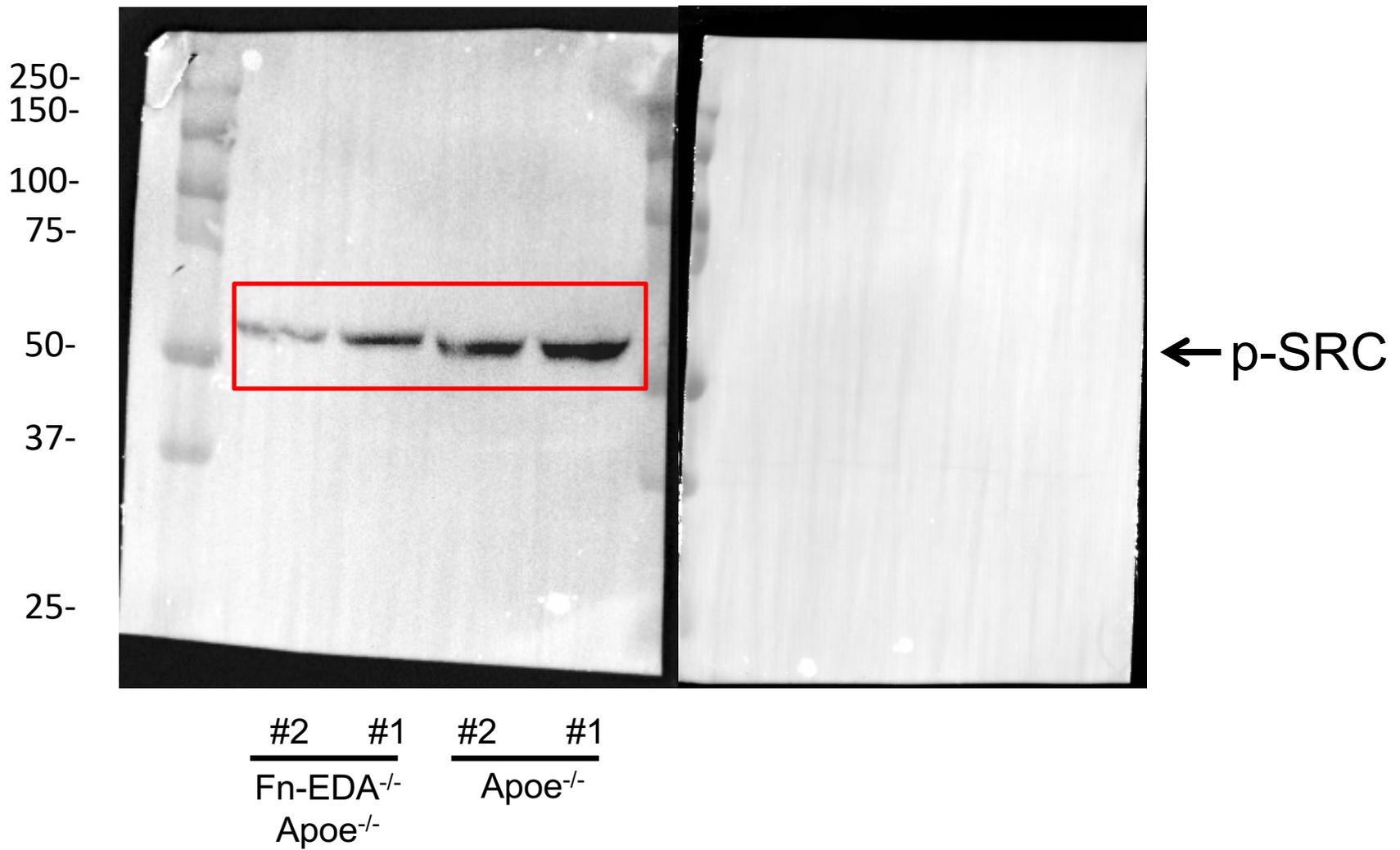
Full unedited gel for Supplemental Figure S8A-SRC
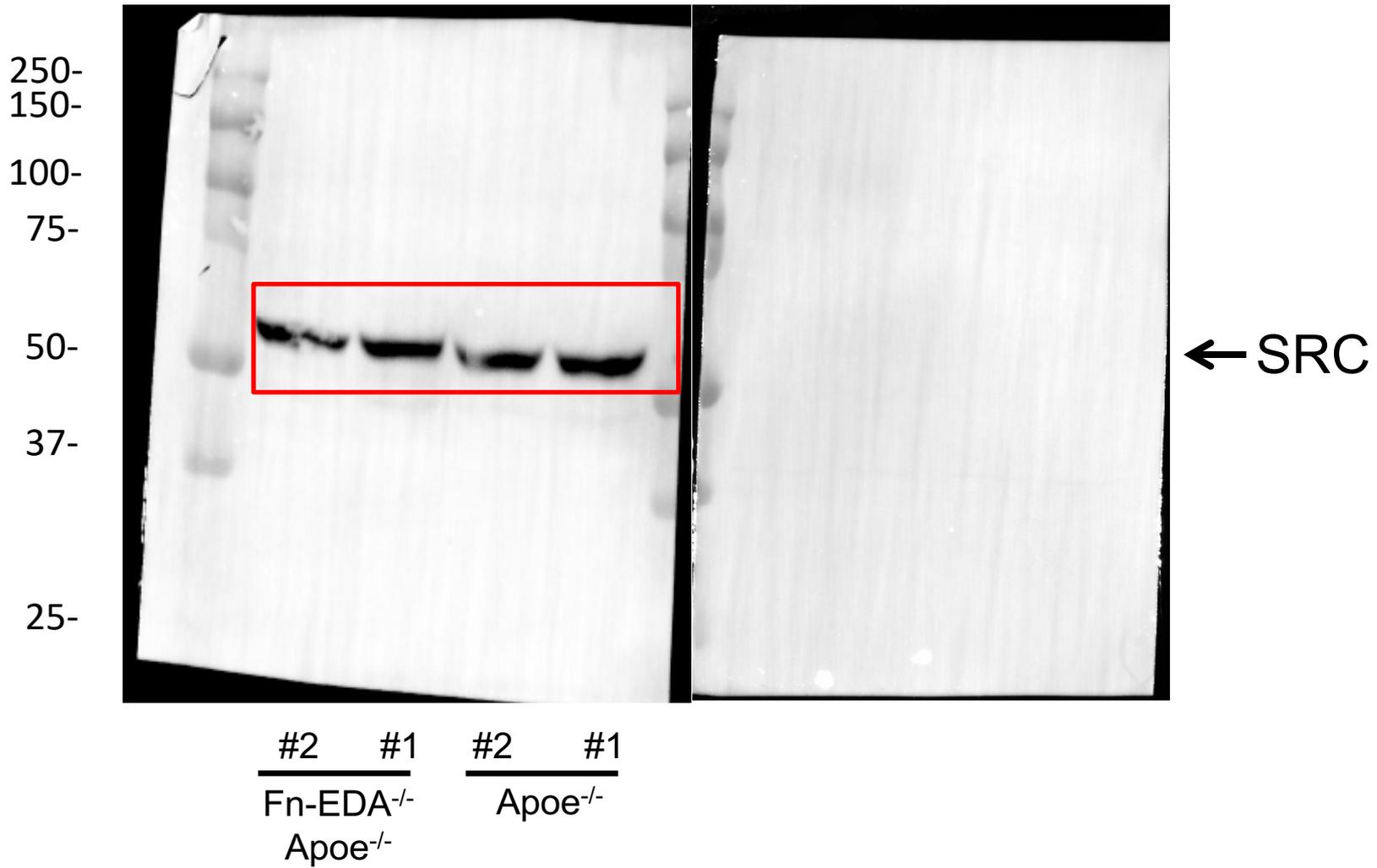
Full unedited gel for Supplemental Figure S8A-β-actin

250-
150-
100-
75-
50-
37-
25-
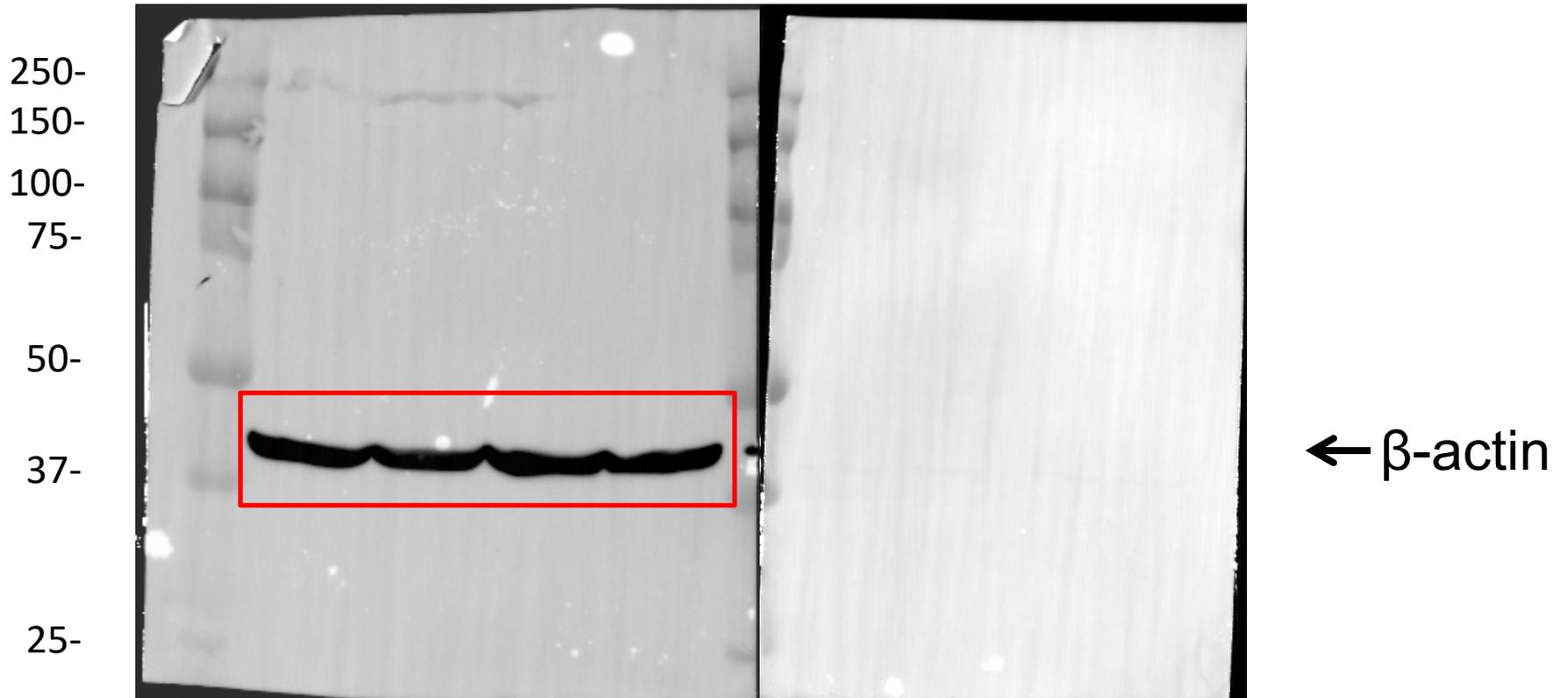
← β-actin

#2   #1   #2   #1
Fn-EDA⁻/⁻   Apoe⁻/⁻
Apoe⁻/⁻

Full unedited gel for Supplemental Figure S9A-p-ERK 1/2

Full unedited gel for Supplemental Figure S9A-ERK 1/2

←ERK 1/2

250-
150-
100-
75-

50-

37-

25-

20

cFn
(20 µg/ml)

0   15   30   60   180

Time (min)

Full unedited gel for Supplemental Figure S9A-β-actin

250-
150-
100-
75-

50-

37-

25-

20

←β-actin

cFn
(20 µg/ml)

0   15   30   60   180

Time (min)