

Supplementary Figures

FIGURE S1

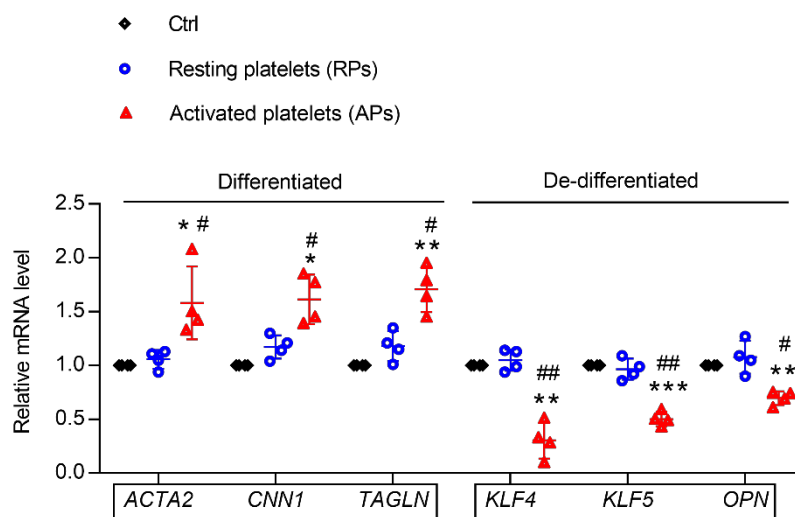


Figure S1. The effects of resting platelets or activated platelets on regulation of VSMCs phenotype. The expressions of markers for differentiation (*ACTA2*, *CNN1*, *TAGLN*) and dedifferentiation (*KLF4*, *KLF5*, *OPN*) in VSMCs (Ctrl) and in VSMCs after co-cultured with resting platelets (RPs) or activated platelets (APs) were determined by qRT-PCR. Data were presented as mean \pm SD (n = 4). *P<0.05, **P<0.01, ***P<0.001, vs Ctrl. #P<0.05, ##P<0.01, vs RPs. Statistical significance was determined using 1-way ANOVA followed by Tukey-Kramer multiple comparisons test.

FIGURE S2

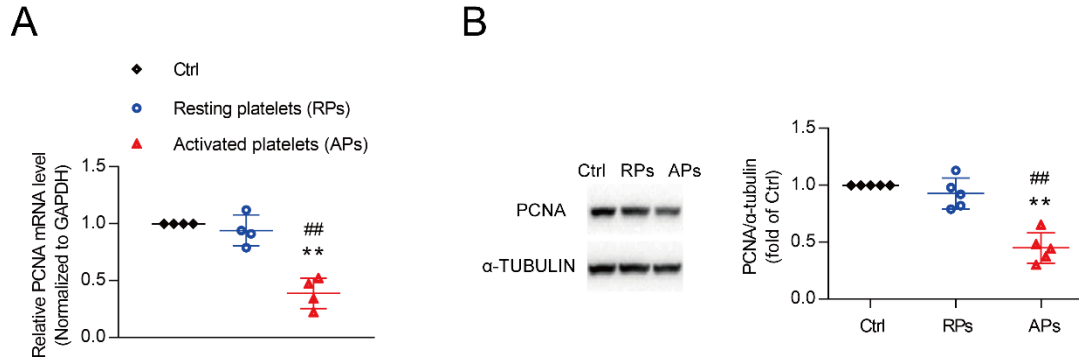


Figure S2. The effect of RPs and APs on PCNA expression in VSMCs. The expression of marker for proliferation (PCNA) in VSMCs (Ctrl) and in VSMCs after co-cultured with resting platelets (RPs) or activated platelets (APs) were determined by **(A)** qRT-PCR (n = 4) or **(B)** Western blot (n = 5), respectively. Data were presented as mean \pm SD. **P<0.01, vs Ctrl. ##P<0.01, vs RPs. Statistical significance was determined using 1-way ANOVA followed by Tukey-Kramer multiple comparisons test.

FIGURE S3

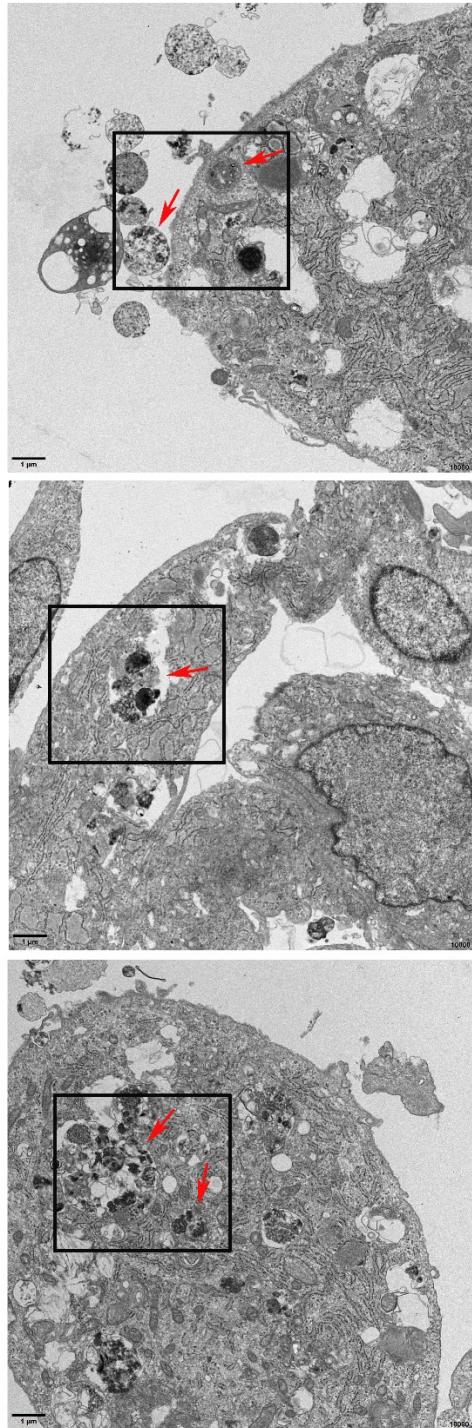


Figure S3. Transmission electron microscopy imaging of the process that platelets were internalized into the VSMCs. Platelets attached to the membrane of VSMCs, and then platelets are internalized into VSMCs. Finally, platelets are fused with lysosomes and lysed in VSMCs. The arrow indicates the entering and internalized platelets. Scale bar: 1 μm .

FIGURE S4

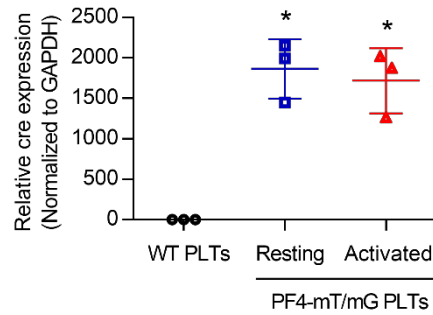


Figure S4. The expression of cre recombinase in platelets isolated from wildtype and PF4-mTmG mouse. The mRNA level of cre was detect by qRT-PCR in wildtype (WT) mice platelets (PLTs), resting PF4-mTmG mouse PLTs, and thrombin-activated PF4-mTmG mouse PLTs. Data were presented as mean \pm SD (n = 3). *P<0.05 vs WT PLTs. Statistical significance was determined using 1-way ANOVA followed by Tukey-Kramer multiple comparisons test.

FIGURE S5

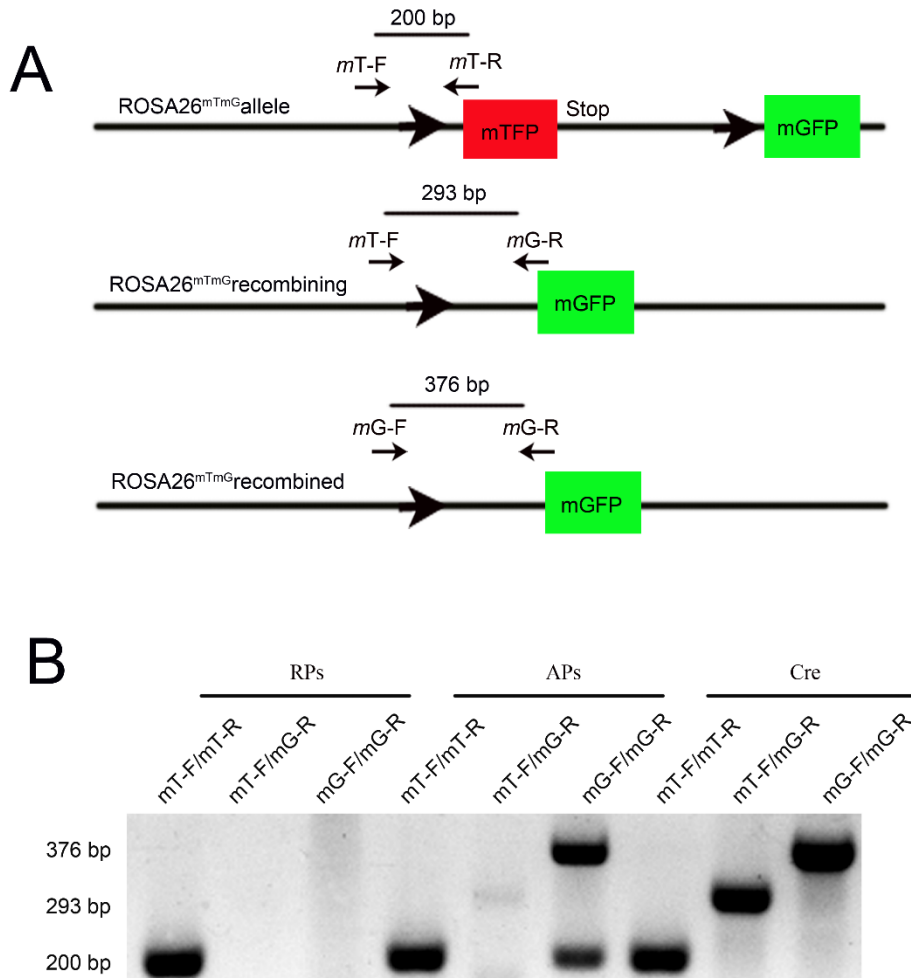


Figure S5. PF4-icre murine platelets mediated mTmG mouse VSMCs recombined after 48 hours co-culture. (A) A schematic diagram of the ROSA26mTmG allele before and after cre-recombination along with the locations of the PCR primers and the expected sizes of amplicons for non-recombined (200 bp), recombining (293 bp) and recombined (376 bp) alleles. **(B)** The representative blot showing PCR amplicons in VSMCs after co-cultured with RPs, APs and cre. RPs: resting platelets; APs: thrombin-activated platelets.

FIGURE S6

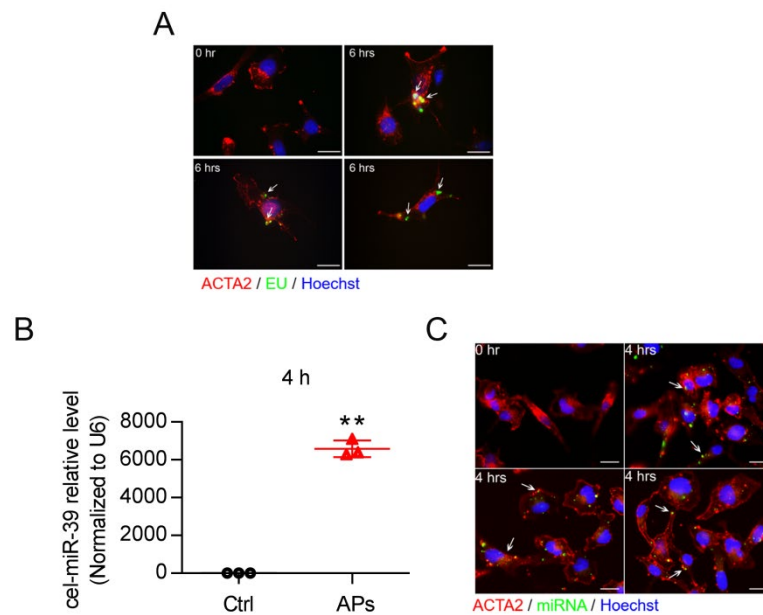


Figure S6. The platelet RNAs were incorporated and utilised in VSMCs after the horizontal transfer. (A) EU-labeled PLPs were incubated under serum-free conditions for 4 h with VSMCs (red, ACTA2). PLPs were generated from MEG-01 cells that had been exposed to EU. EU in PLPs was visualized using Click-iT RNA Alexa Fluor 488 (green). Scale bar: 20 μ m. **(B)** Relative quantity of cel-miR-39 in VSMCs co-cultured with activated platelets after transfection with cel-miR-39. Data were presented as mean \pm SD (n = 3). ***P<0.001 vs Ctrl. Statistical significance was determined using Parametric t-test. **(C)** Representative confocal image of VSMCs co-cultured with or without activated platelets after transfection with miR-Scr-FITC (green). Ctrl: control; APs: thrombin-activated platelets. Scale bar: 20 μ m.

FIGURE S7

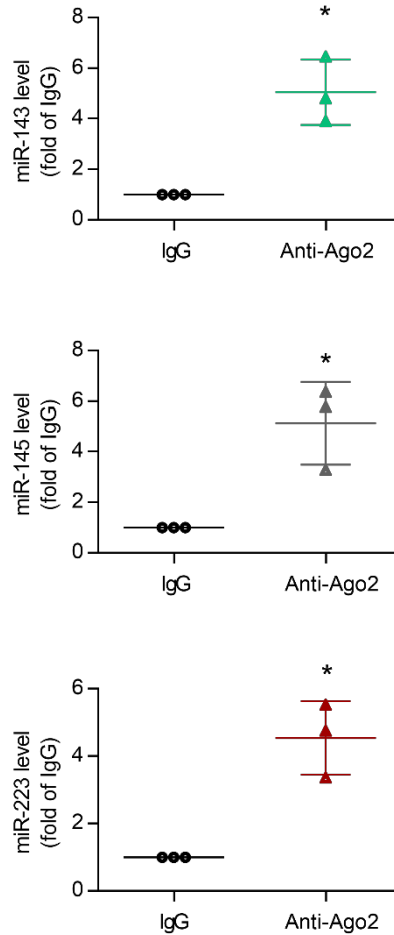


Figure S7. Platelets contained functional Ago2•miR-143/145/223 effector complexes. Protein extracts derived from the platelets were subjected to immunoprecipitation using anti-Ago2 antibody, followed by quantitative miR-143, miR-145 and miR-223 detection by qPCR. Data were presented as mean \pm SD (n = 3). *P<0.05 vs IgG. Statistical significance was determined using Parametric t-test.

FIGURE S8

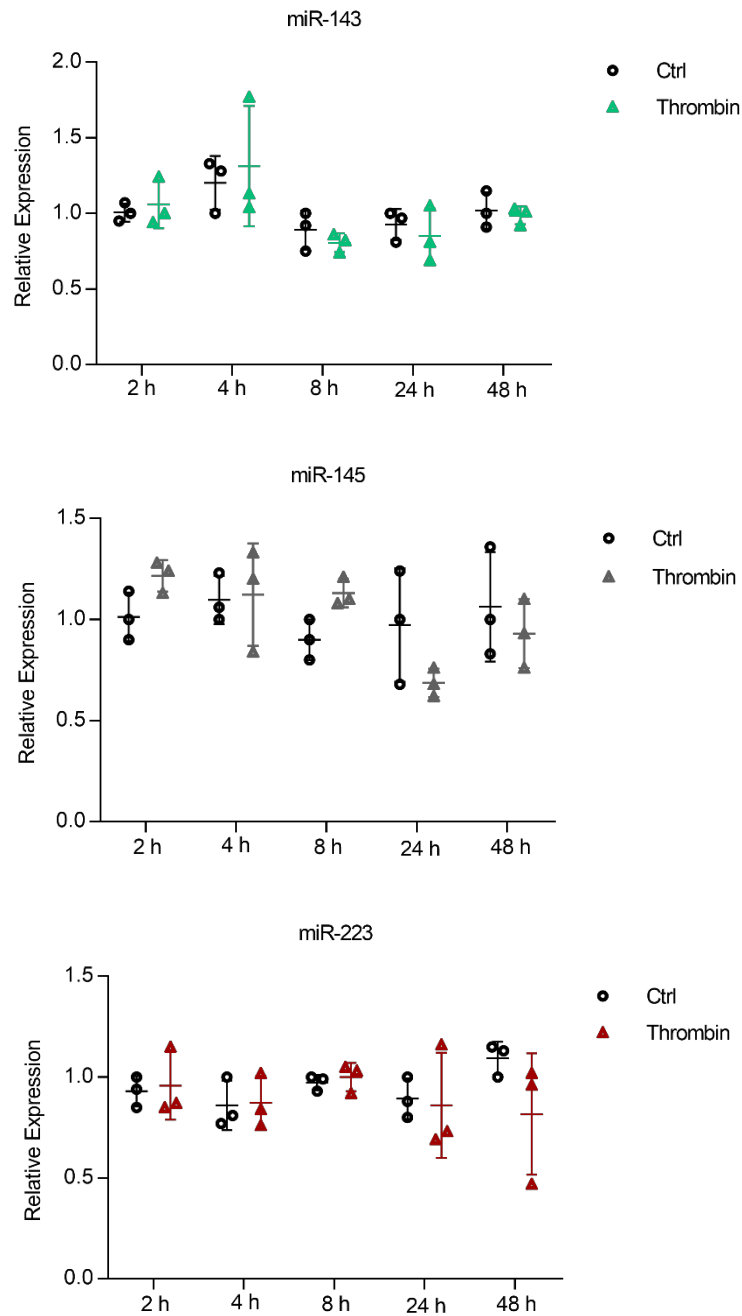


Figure S8. Thrombin did not change the levels of miRNAs in VSMCs. VSMCs were treated with or without 0.1 U/ml thrombin for 2, 4, 8, 24 or 48 hours. The levels of miR-143, miR-145 and miR-223 in VSMCs were detected by qRT-PCR. Data were presented as mean \pm SD (n = 3). Statistical significance was determined using Parametric t-test.

FIGURE S9

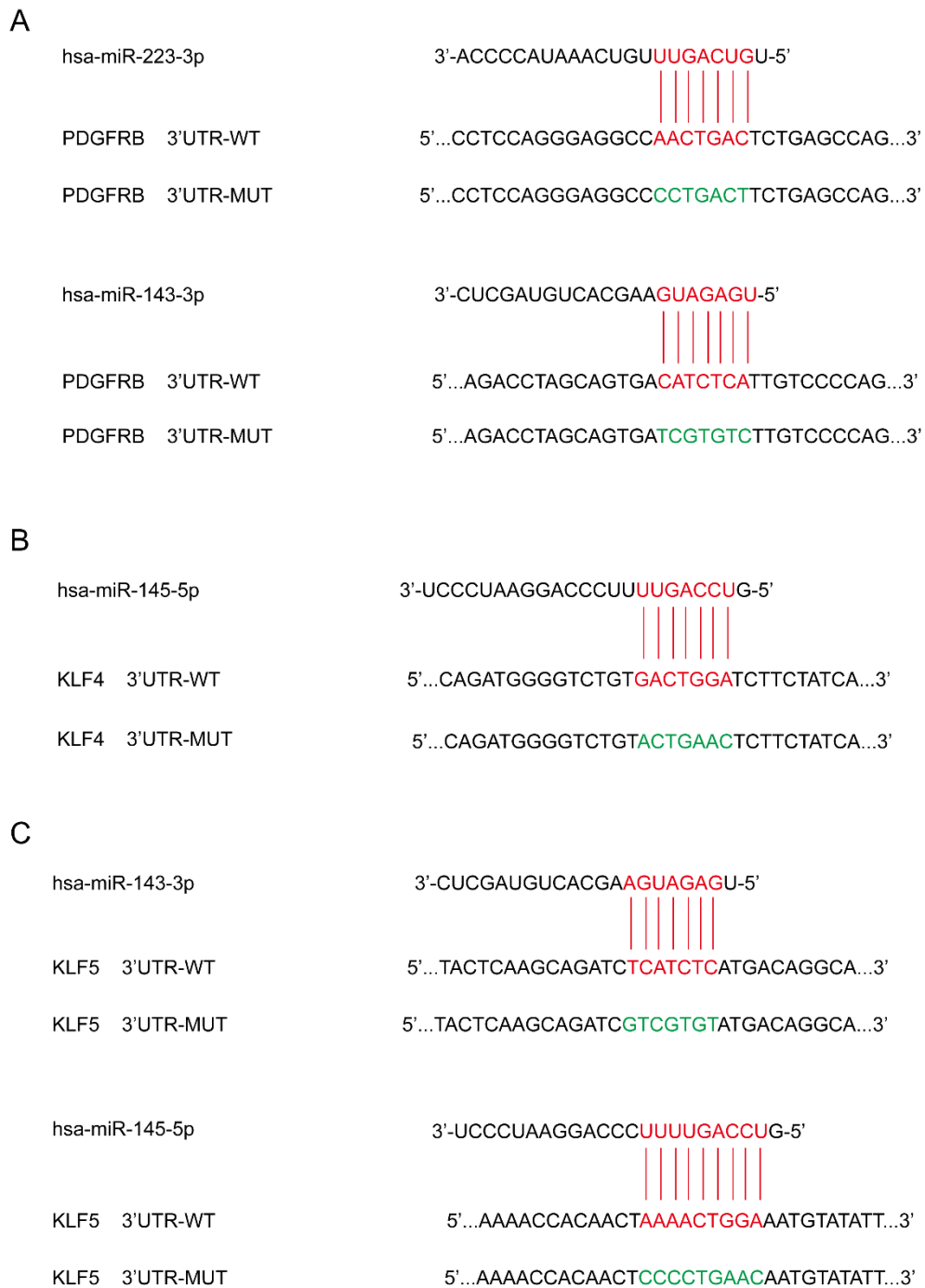


Figure S9. Schematic representation of PDGFR β , KLF4, KLF5 3'UTRs showing putative miRNA target site.

FIGURE S10

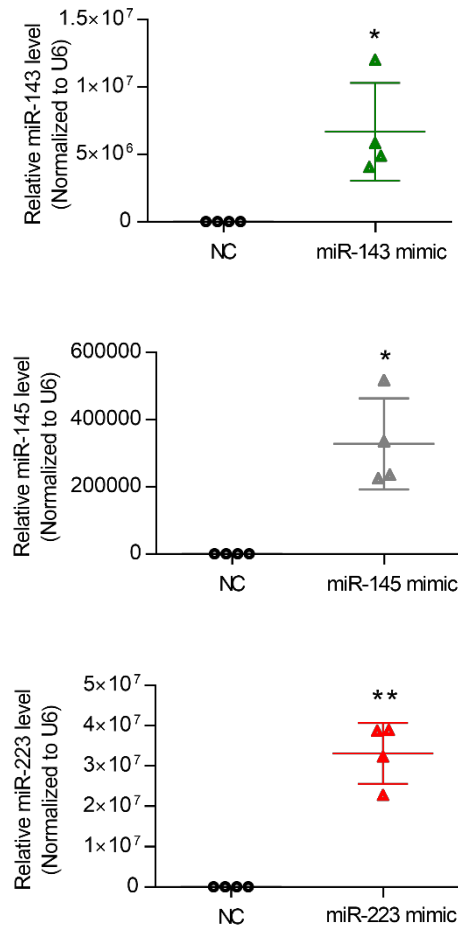


Figure S10. The levels of miR-143, miR-145 and miR-223 in VSMCs after transfection. VSMCs were transfected with miR-143, miR-145 and miR-223 mimic for 48 hours. The levels of miR-143, miR-145 and miR-223 were detected by qRT-PCR, respectively. Data were presented as mean \pm SD (n = 4). *P<0.05, **P<0.01 vs NC. Statistical significance was determined using Parametric t-test.

FIGURE S11

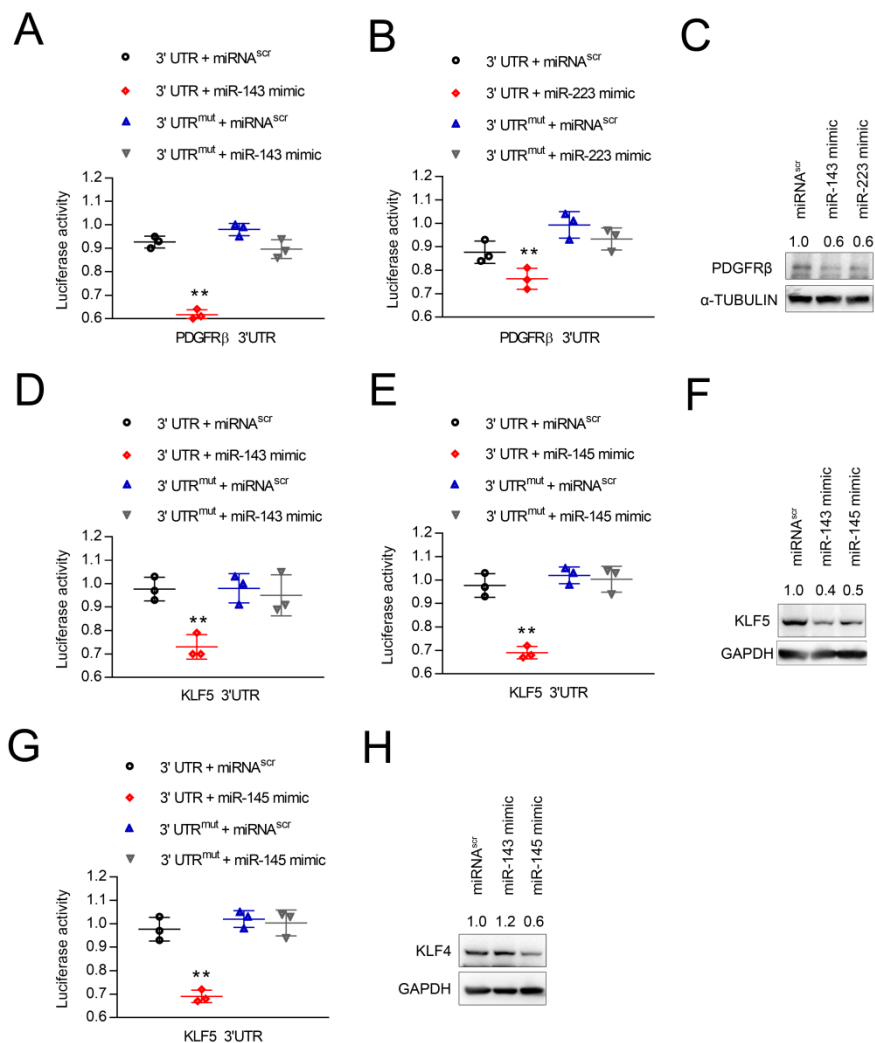


Figure S11. KLF4, KLF5 and PDGFR β were direct targets of miR-143, miR-145, and miR-223. (A-B) Luciferase activity assay in VSMCs following introduction of PDGFR β 3' UTR or mutant 3' UTR (mut) with or without miR-143 or miR-223 mimic (n = 3). (C) The expression of PDGFR β in VSMCs transfected with a scrambled (scr) miRNA, miR-143 mimic and miR-223 mimic (n = 6). (D-E) Luciferase activity assay in VSMCs following introduction of KLF5 3' UTR or mutant 3' UTR (mut) with or without miR-143 or miR-145 mimic (n = 3). (F) The expression of KLF5 in VSMCs transfected with a scrambled (scr) miRNA, miR-143 mimic and miR-145 mimic (n = 6). (G) Luciferase activity assay in VSMCs following introduction of KLF4 3' UTR or mutant 3' UTR (mut) with or without miR-145 mimic (n = 3). (H) The expression of KLF4 in VSMCs transfected with a scrambled (scr) miRNA, miR-143 mimic and miR-145 mimic (n = 6). Data were presented as mean \pm SD. **P<0.01, vs miRNA^{scr}. Statistical significance was determined using 1-way ANOVA followed by Tukey-Kramer multiple comparisons test.

FIGURE S12

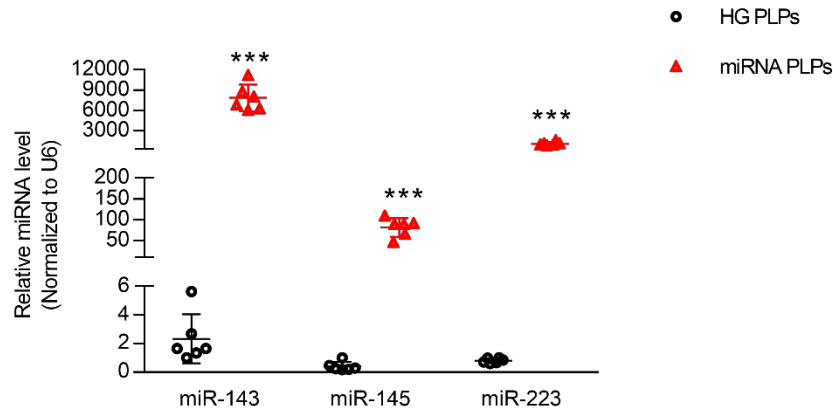


Figure S12. miR-143, miR-145, and miR-223 were overexpressed in high glucose treated MEG-01 cells-derived platelet-like particles (HG PLPs). High glucose (HG) treated MEG-01 cells were simultaneously transfected with miR-143, miR-145, and miR-223 at 100 nM by using lipofectamine RNAiMAX reagent (miRNA PLPs). After transfection, PLPs were collected from Thrombopoietin (TPO)-stimulated MEG-01 cells. The levels of miR-143, miR-145, and miR-223 in PLPs were detected by qRT-PCR, respectively. Data were presented as mean \pm SD (n = 6). ***P<0.001 vs HG PLPs. Statistical significance was determined using Parametric t-test.

Figure S13

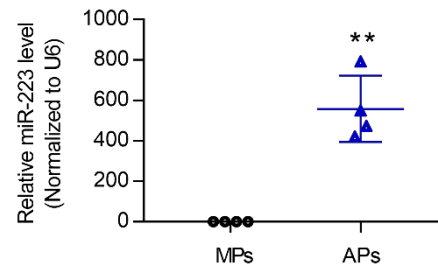


Figure S13. The level of miR-223 in VSMCs co-cultured with platelets-derived microparticles and activated platelets. VSMCs were co-cultured with platelets-derived microparticles (MPs) and activated platelets (APs) for 4 hours. The level of miR-223 in VSMCs was detected by qRT-PCR. MPs were in supernatant after removing thrombin-stimulated platelets. Data are presented as mean \pm SD (n = 4). ** $P < 0.01$ vs MPs. Statistical significance was determined using Parametric t-test.

Supplemental Tables

Table S1. Clinical characteristics of healthy subjects (HS) and patients with diabetes mellitus (DM)

	HS	DM	<i>P</i> value
	N=10	N=11	
Age (years \pm SD)	33.1 \pm 9.4	46.1 \pm 12.6	/
Gender (Males/Females)	6/4	8/3	/
BMI (kg/m ² \pm SD)	20.6 \pm 2.8	30.8 \pm 6.4*	< 0.05
Blood glucose (mg/dL \pm SD)	108.8 \pm 7.7	197.1 \pm 92.3*	< 0.05
HbA1c (% \pm SD)	5.1 \pm 0.7	7.8 \pm 1.1*	< 0.05
Systolic blood pressure (mmHg \pm SD)	116.1 \pm 8.5	129.9 \pm 14.5*	< 0.05
Diastolic blood pressure (mmHg \pm SD)	78.4 \pm 4.6	79.3 \pm 10.2	/
Hypertension (%)	0/10 (0%)	4/11 (36.4%)	/
CAD (%)	0/10 (0%)	2/11 (18.2%)	/

This Table represents typical differences observed between a diabetes mellitus group and our normal control. All data were expressed as mean \pm SD. The parametric t-test or Fisher's exact test was performed for comparisons of two groups. Analysis was performed with Prism software (GraphPad Software, Inc, La Jolla, CA). *A difference of $P < 0.05$ was considered significant.

Table S2: The sequences for miRNA mimic or inhibitor

Gene	sense (5'-3')	antisense (5'-3')
Negative control	UUCUCCGAACGUGUCA CGUTT	ACGUGACACGUUCG GAGAATT
hsa-miR-143 mimics	UGAGAUGAAGCACUGU AGCUC	GCUACAGUGCUUCA UCUCAUU
hsa-miR-145 mimics	GUCCAGUUUCCCAGG AAUCCCU	GGAUUCCUGGGAAA ACUGGACUU
hsa-miR-223 mimics	UGUCAGUUUGUCAAU ACCCCA	GGGUAUUUGACAAA CUGACAUU
cel-miR-39-3p mimics	UCACCGGGUGUAAAUC AGCUUG	AGCUGAUUUACACC CGGUGAUU

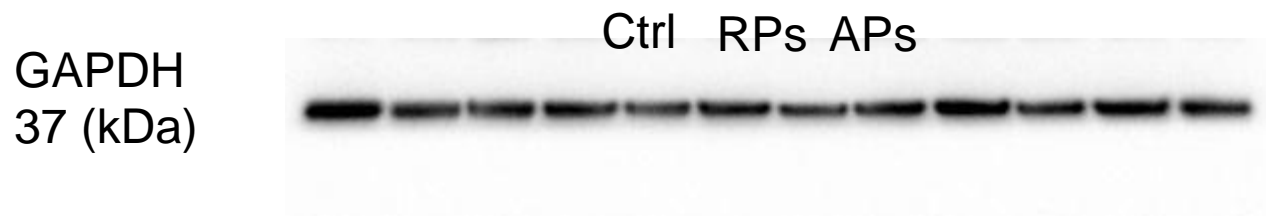
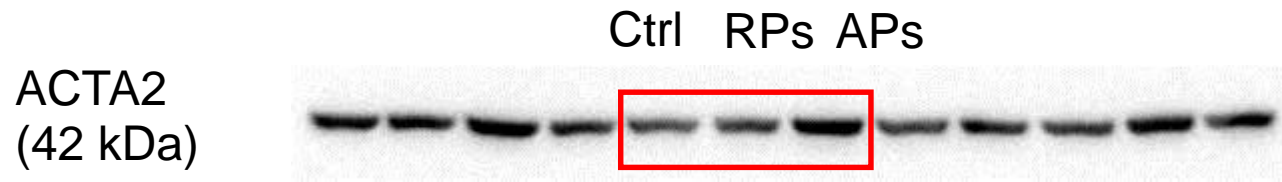
Table S3: Primers for miRNA PCR

Primer	Forward Sequence	Reverse Sequence
hsa-miR-143	CTGGCGTTGAGATGAAGC AC	CAGAGCAGGGTCCGAG GTA
hsa-miR-145	TGCCGAGTCCAGTTTTCC C	TATGGTTGTTACGAGT CCTTCAC
hsa-miR-223	GTTGCTCCTGTCAGTTTG TCAA	TATGGTTGTTACGACT CCTTCAC
mmu-miR-143	CTGGCGTTGAGATGAAGC AC	CAGAGCAGGGTCCGAG GTA
mmu-miR-145	TGCCGAGTCCAGTTTTCC C	TATGGTTGTTACGAGT CCTTCAC
mmu-mir-223	GTTGCTCCTGTCAGTTTG TCAA	TATGGTTGTTACGACT CCTTCAC
U6	ATTGGAACGATACAGAG AAGATT	GGAACGCTTCACGAATT TG
cel-miR-39-3p	CGTCGATCACCGGGTGTA AA	TATGGTTGTTCTGCTCT CTGTCTC

Table S4: Primers for mRNA PCR (Human)

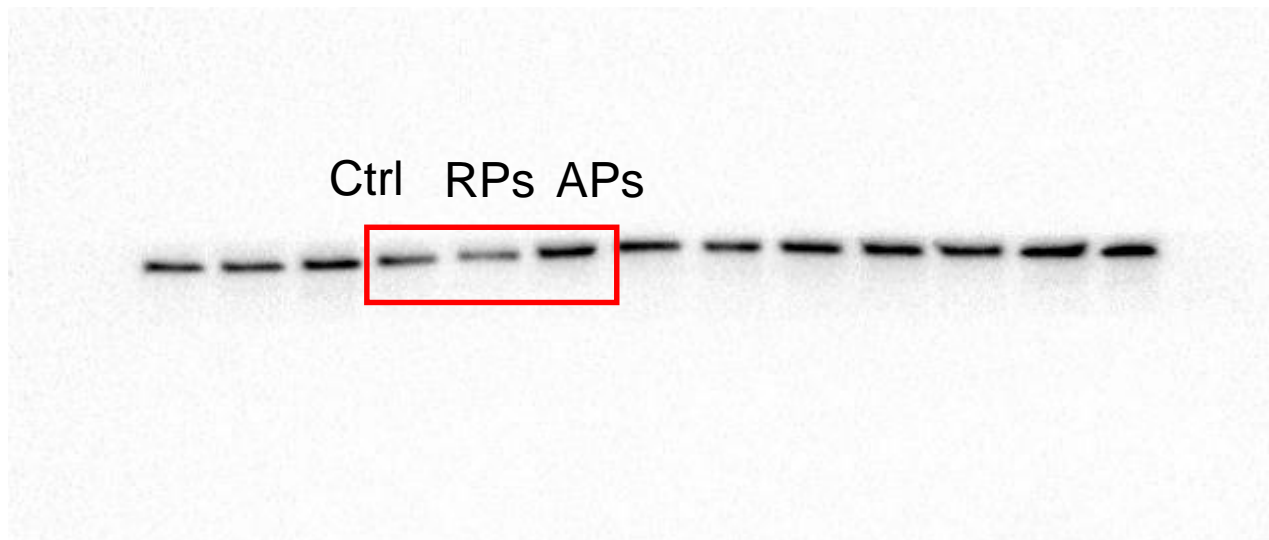
Primer	Forward Sequence	Reverse Sequence
ACTA2	GTGTTGCCCTGAAGAGCAT	GCTGGGACATTGAAAGT CTCA
CNN1	CTGTCAGCCGAGGTTAAGAAC	GAGGCCGTCCATGAAGT TGTT
TAGLN	CCGTGGAGATCCCAACTGG	CCATCTGAAGGCCAATG ACAT
KLF4	CCCACATGAAGCGACTTCCC	CAGGTCCAGGAGATCGT TGAA
KLF5	TCAGTCGTAGACCAGTTCTTCA	CTGGGATTTGTAGAGGC CAGT
OPN	GAAGTTTCGCAGACCTGACAT	GTATGCACCATTCAACT CCTCG
PCNA	CCTGCTGGGATATTAGCTCCA	CAGCGGTAGGTGTCGA AGC
GAPDH	CATGAGAAGTATGACAACAGC CT	AGTCCTTCCACGATACC AAAGT

Full uncut gels



Full unedited gel for Figure1A
Acquired with ChemiDoc (BioRad)

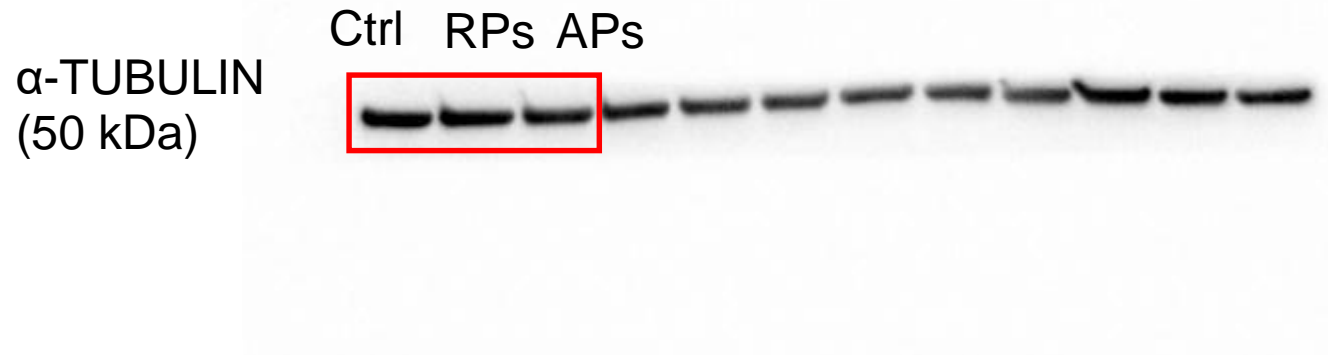
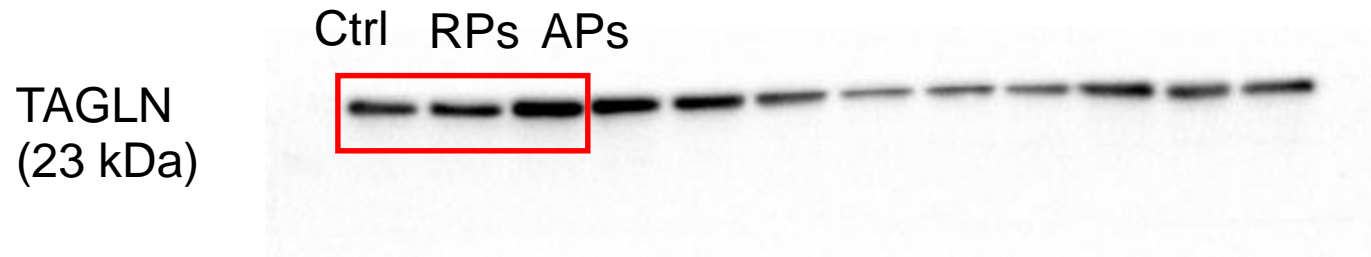
CNN1
(34 kDa)



α -TUBULIN
(50 kDa)

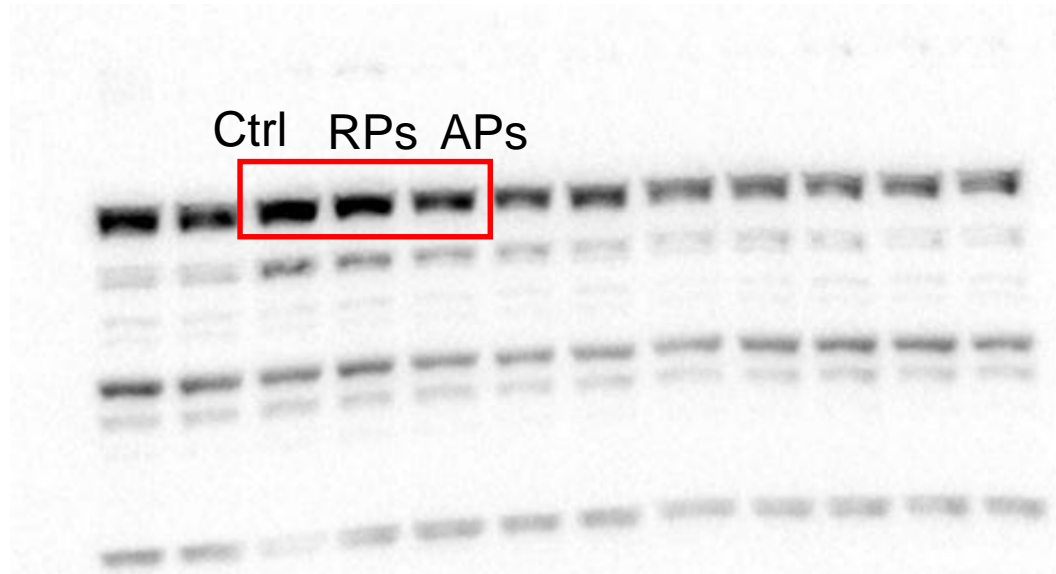


Full unedited gel for Figure1A
Acquired with ChemiDoc (BioRad)

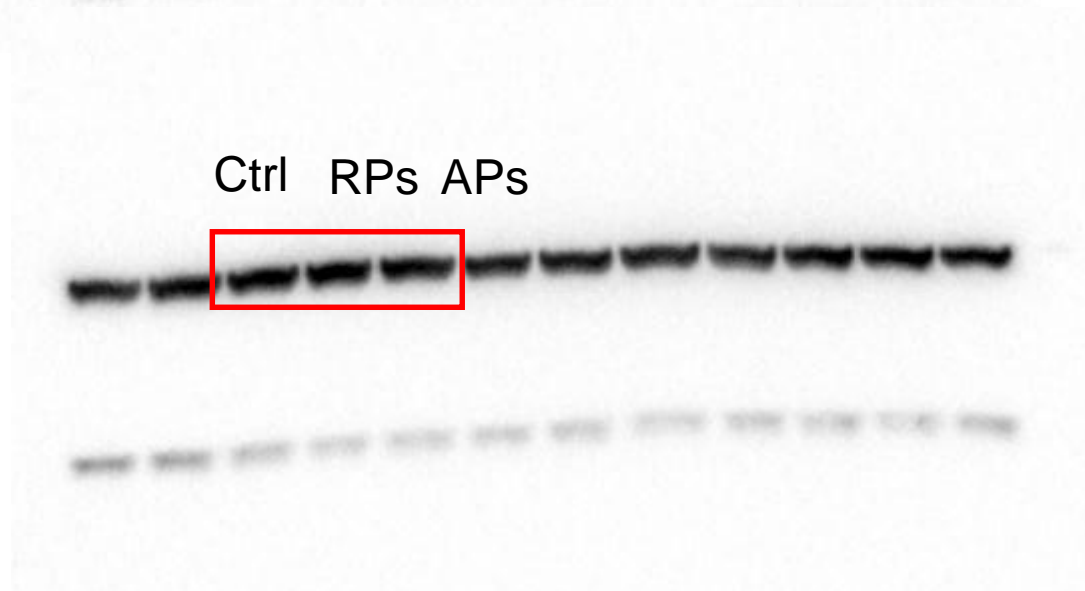


Full unedited gel for Figure1A
Acquired with ChemiDoc (BioRad)

KLF4 (55 kDa)



GAPDH
37 (kDa)

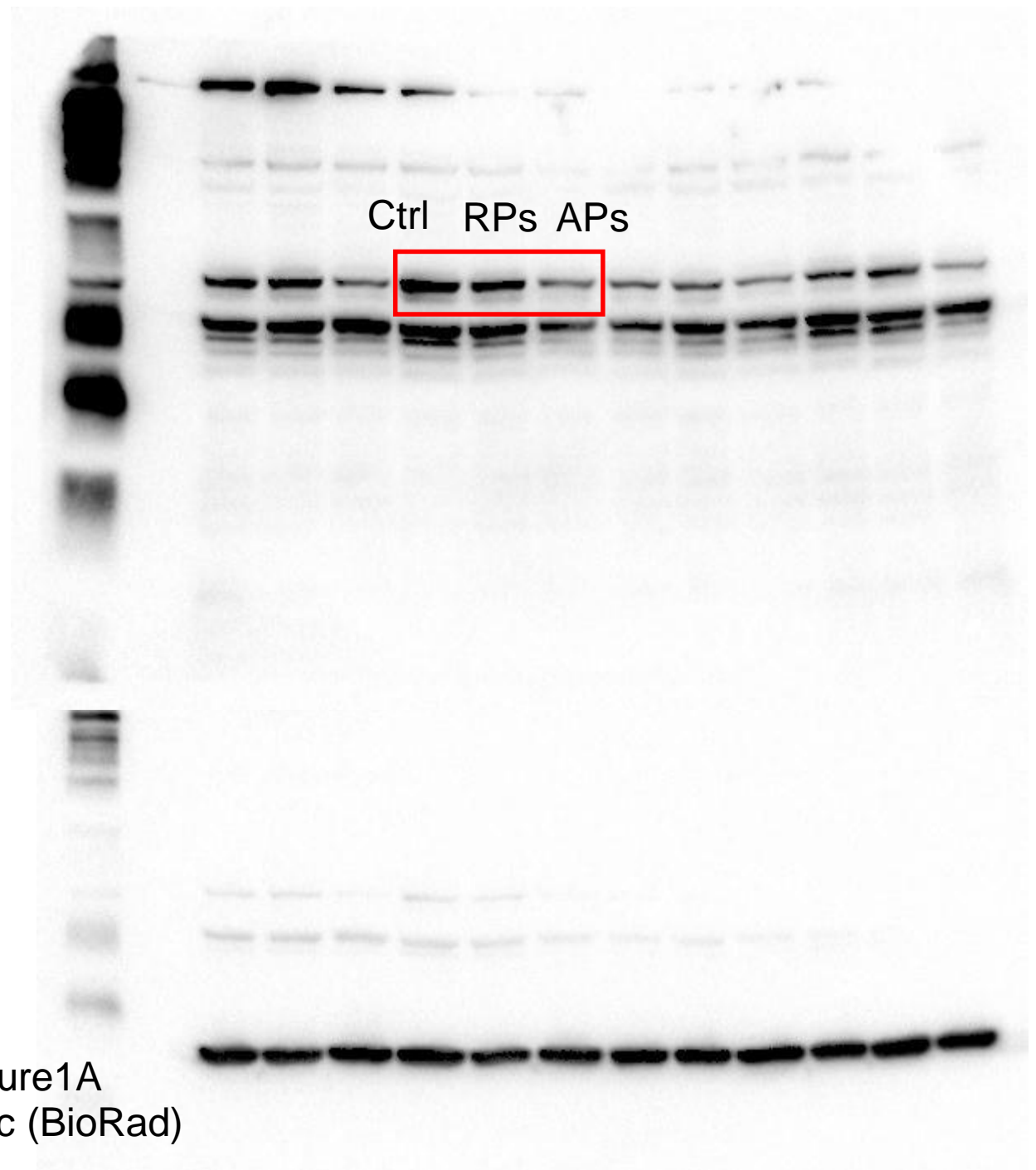


Full unedited gel for Figure1A
Acquired with ChemiDoc (BioRad)

KLF5 (51 kDa)

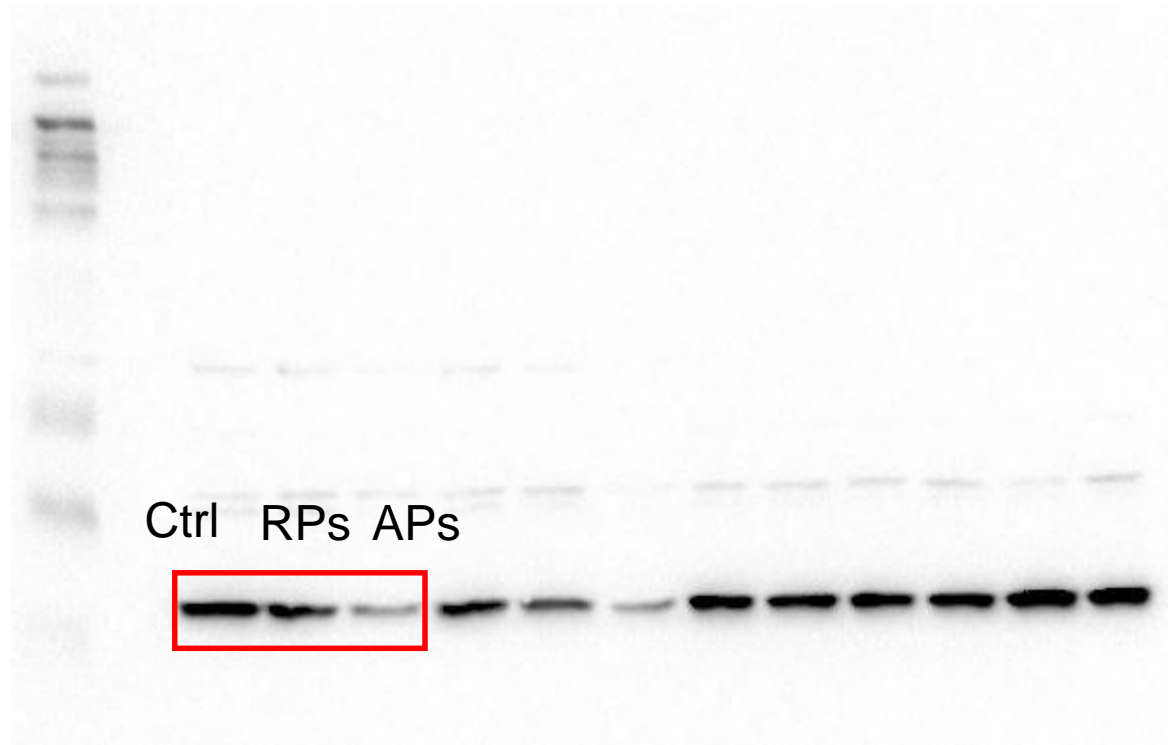
Ctrl RPs APs

GAPDH
37 (kDa)

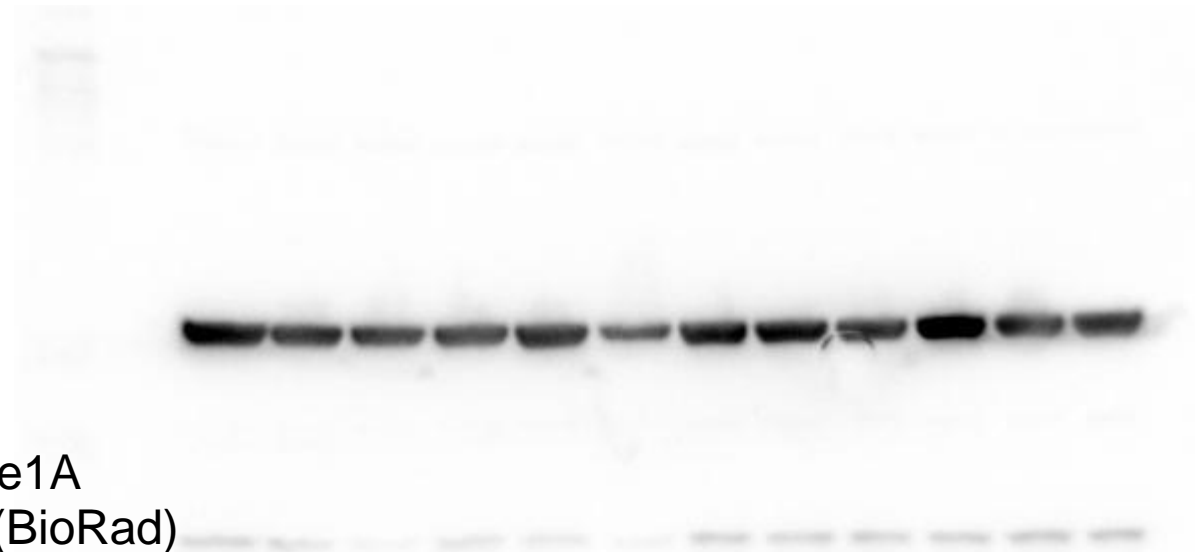


Full unedited gel for Figure 1A
Acquired with ChemiDoc (BioRad)

OPN (35 kDa)



α -TUBULIN
(50 kDa)



Full unedited gel for Figure 1A
Acquired with ChemiDoc (BioRad)

ACTA2
(42 kDa)

Ctrl

RPs

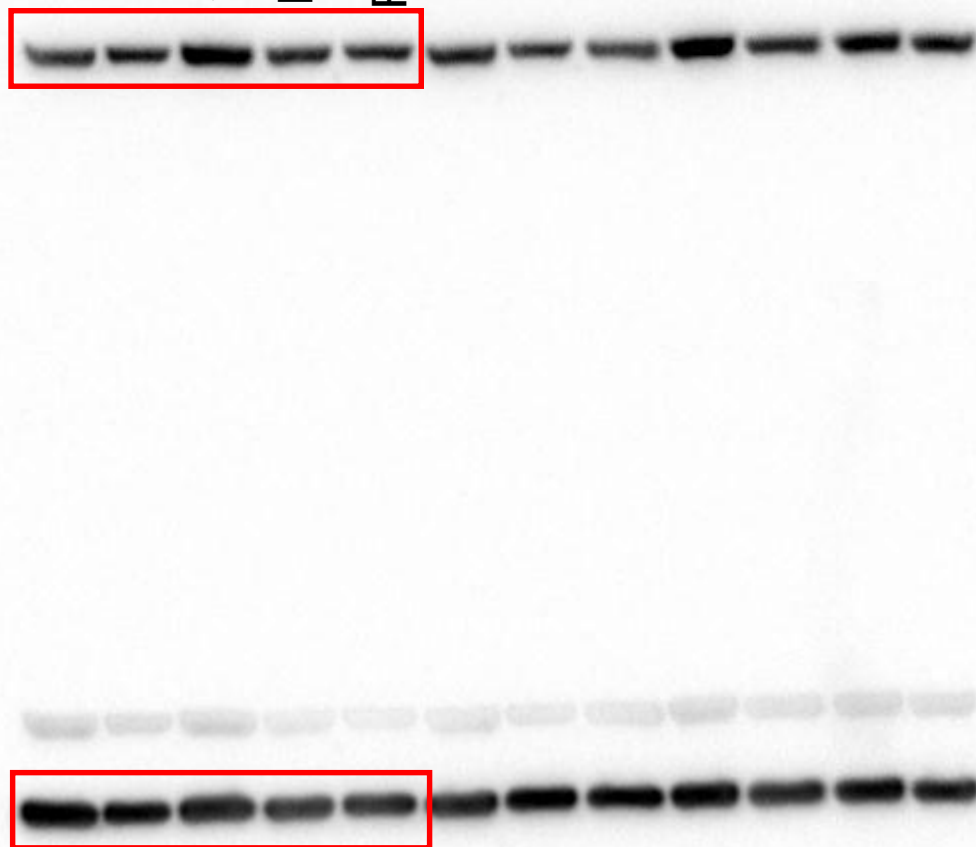
APs

Thrombin

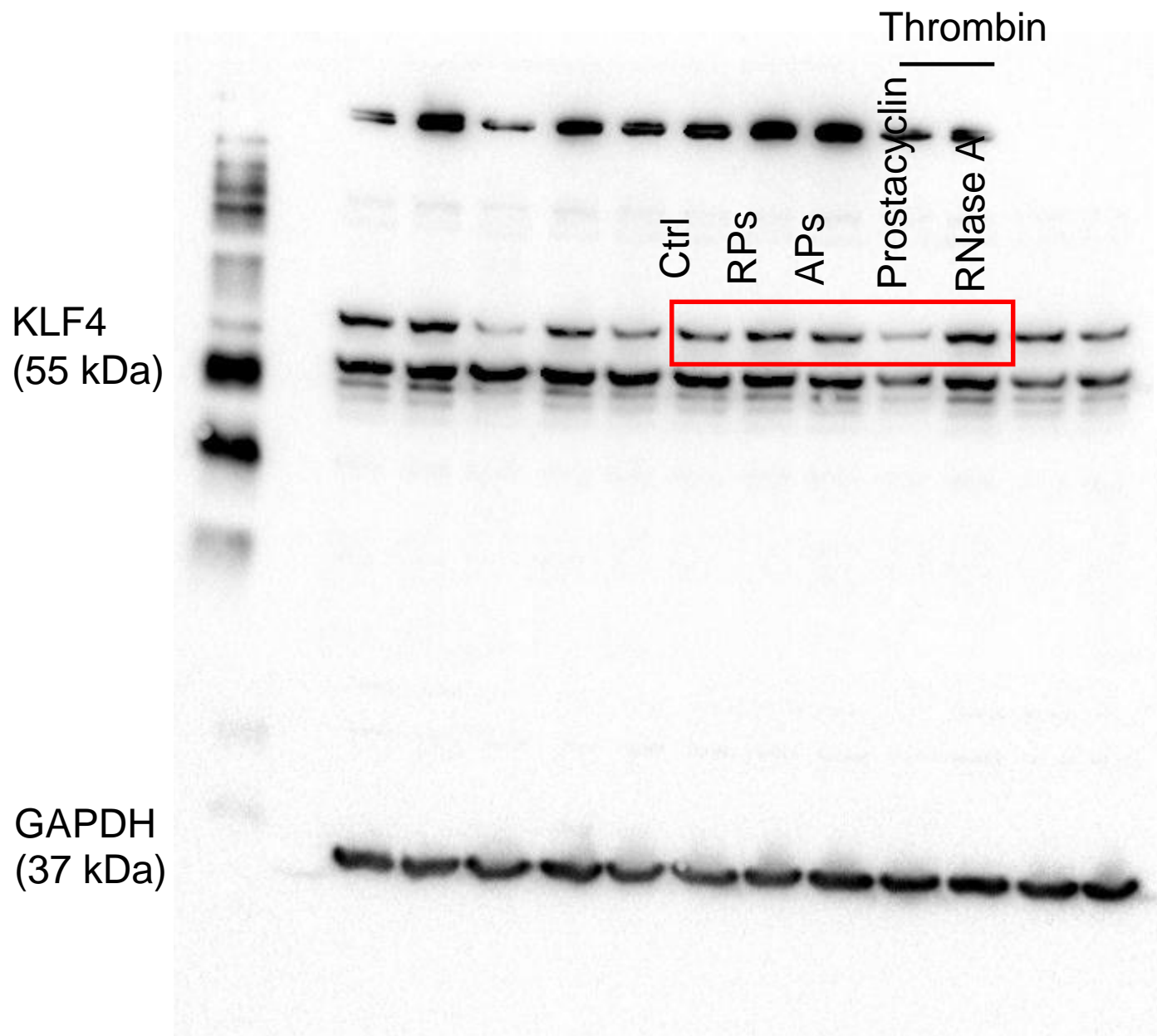
Prostacyclin

RNase A

GAPDH
37 (kDa)

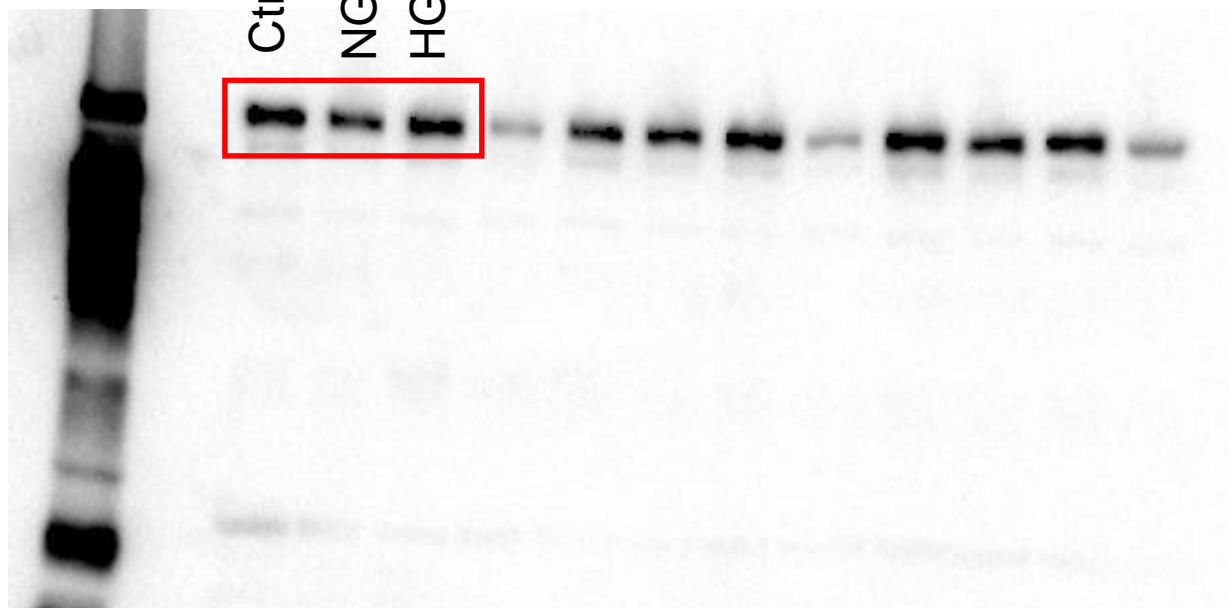


Full unedited gel for Figure 2D
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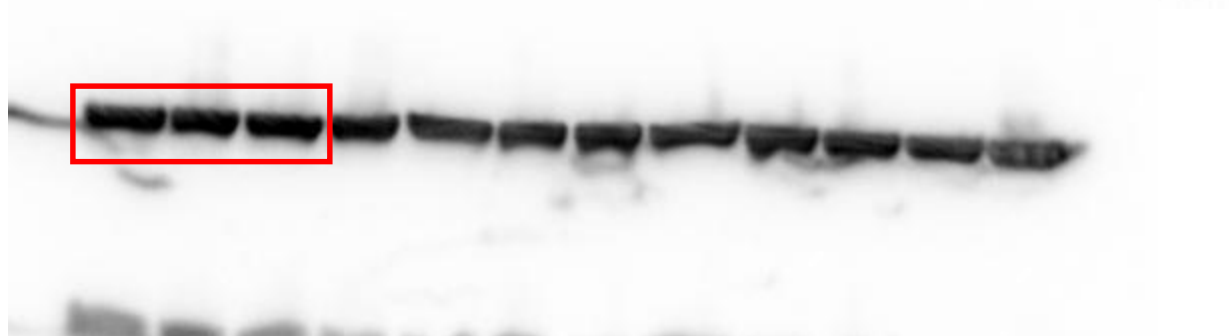


Full unedited gel for Figure 2E
Acquired with ChemiDoc (BioRad)

PDGFR β
(124 kDa)

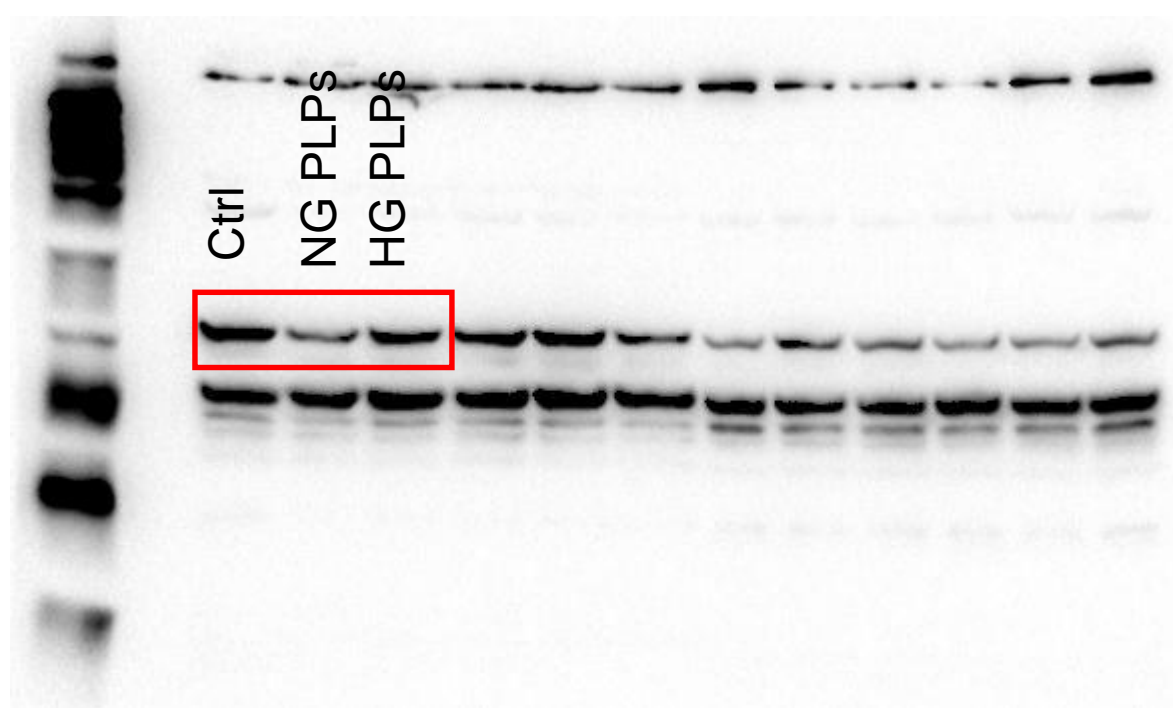


α -TUBULIN
(50 kDa)

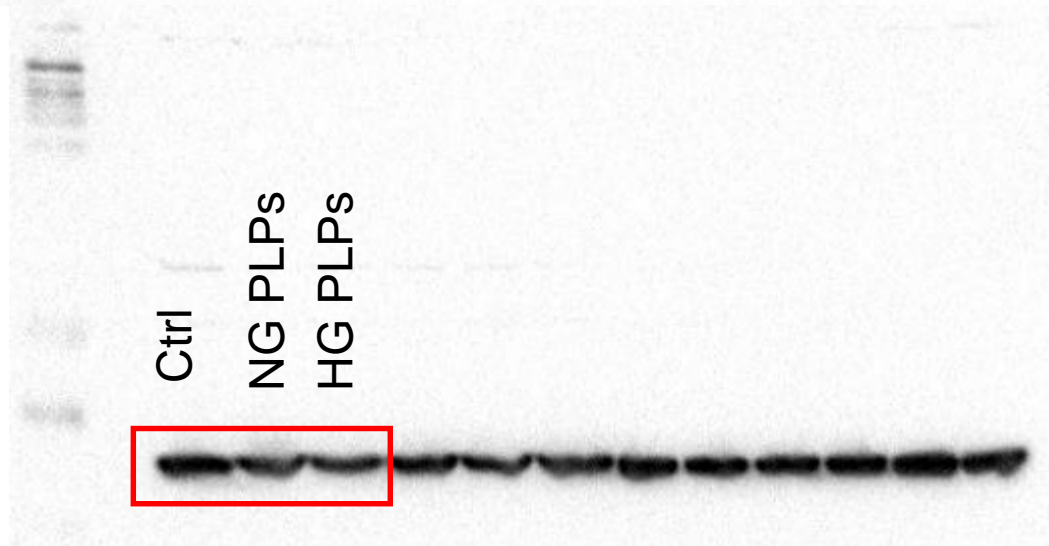


Full unedited gel for Figure 3B
Acquired with ChemiDoc (BioRad)

KLF4
(55 kDa)

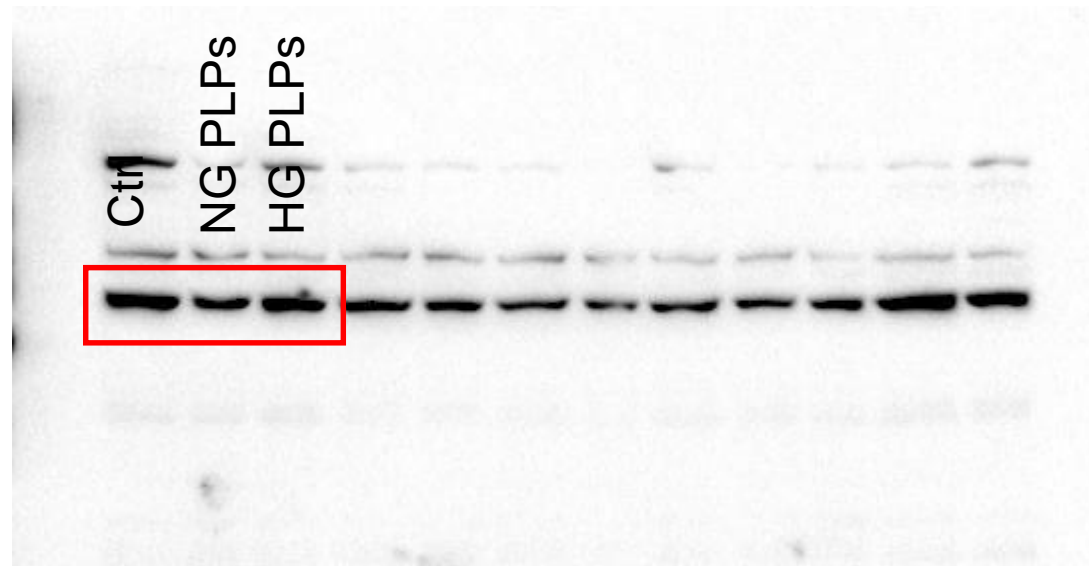


GAPDH
(37 kDa)

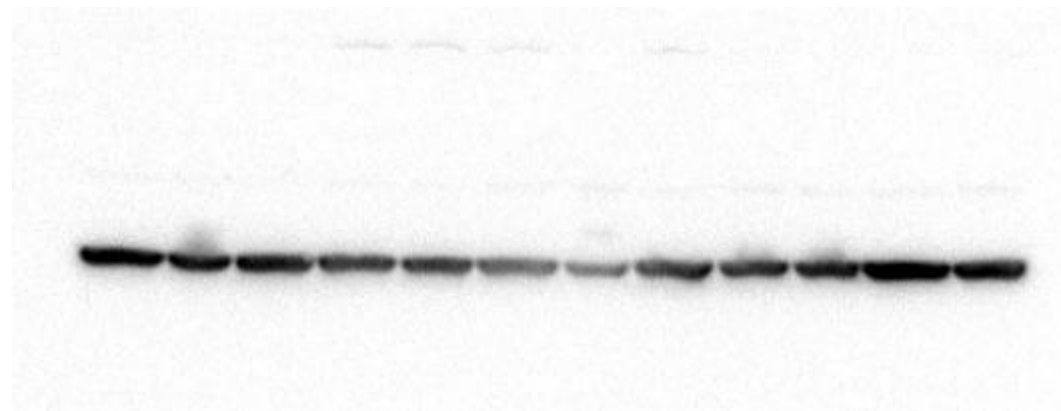


Full unedited gel for Figure 3B
Acquired with ChemiDoc (BioRad)

KLF5
(51 kDa)

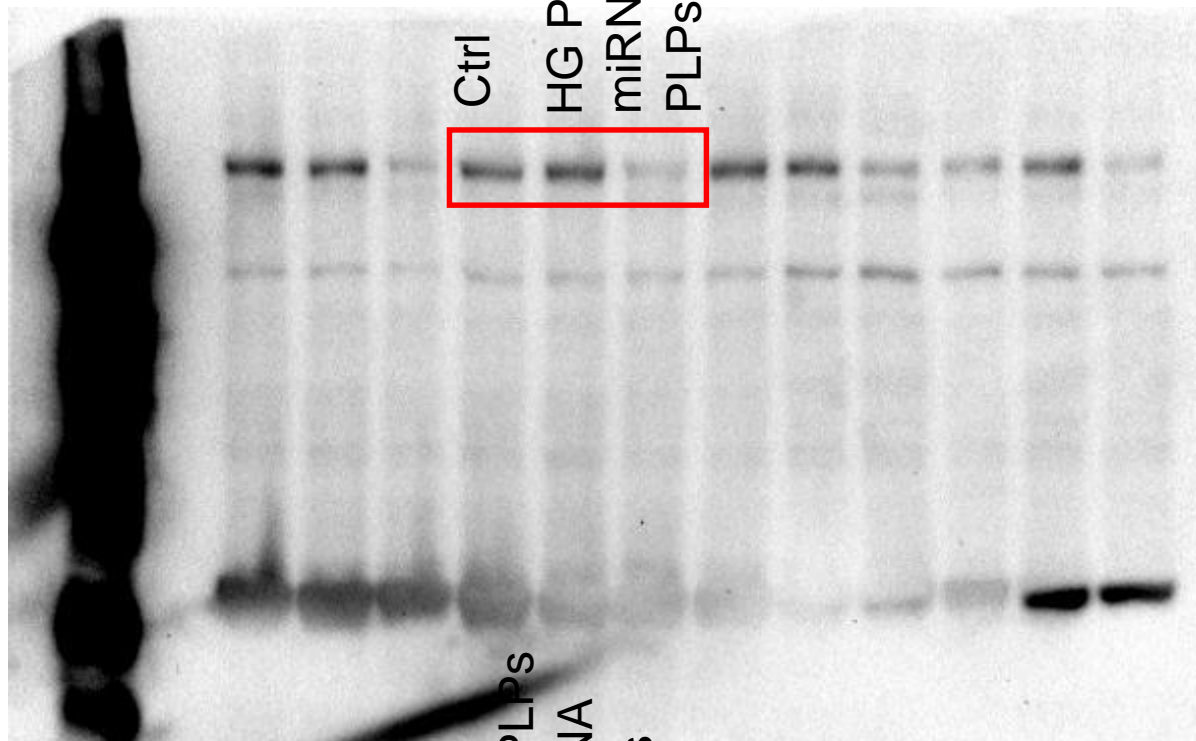


GAPDH
(37 kDa)

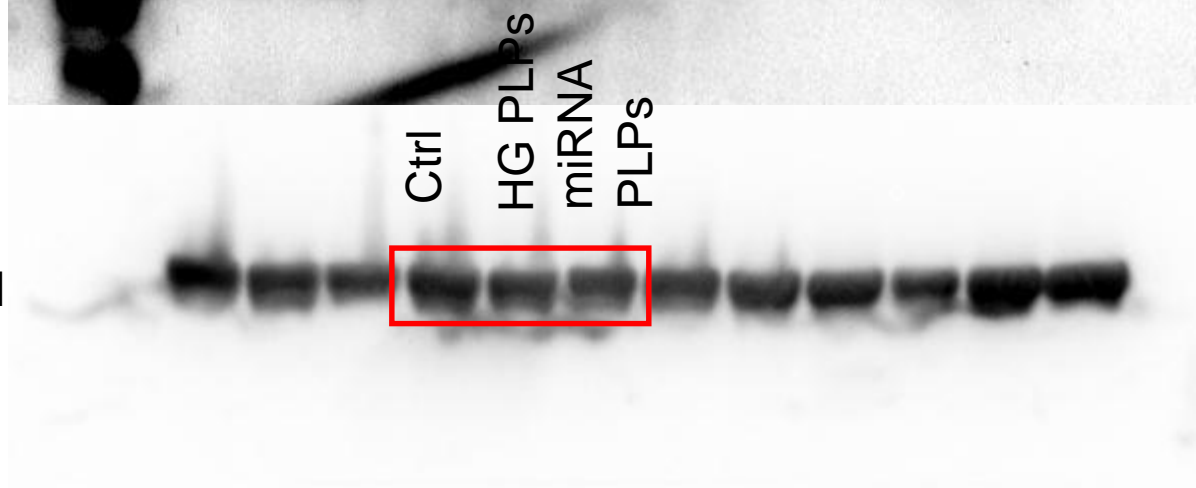


Full unedited gel for Figure 3B
Acquired with ChemiDoc (BioRad)

PDGFR β
(124 kDa)

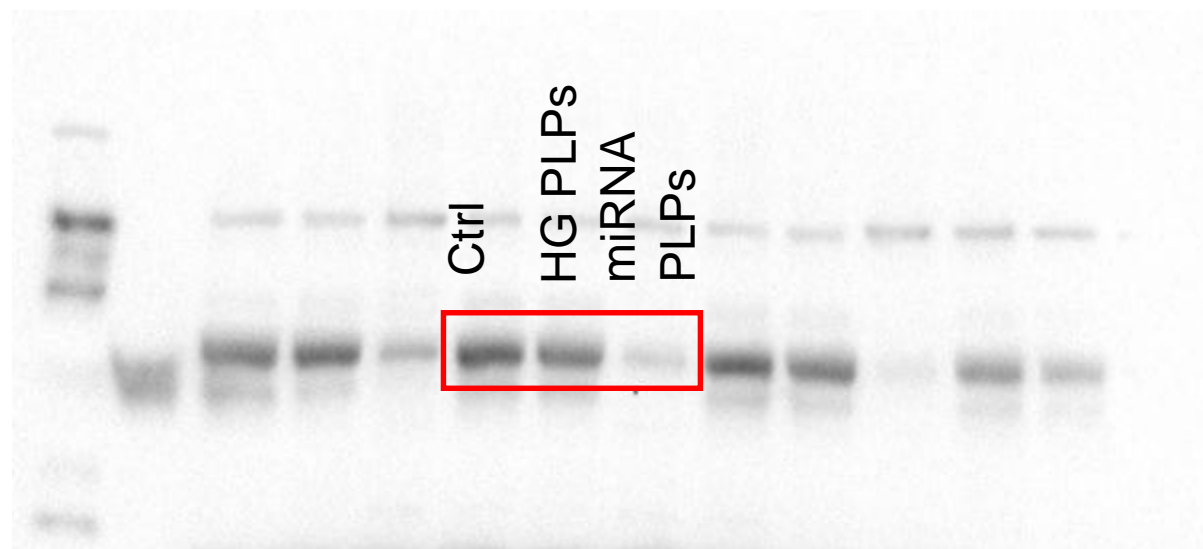


α -TUBULIN
(50 kDa)

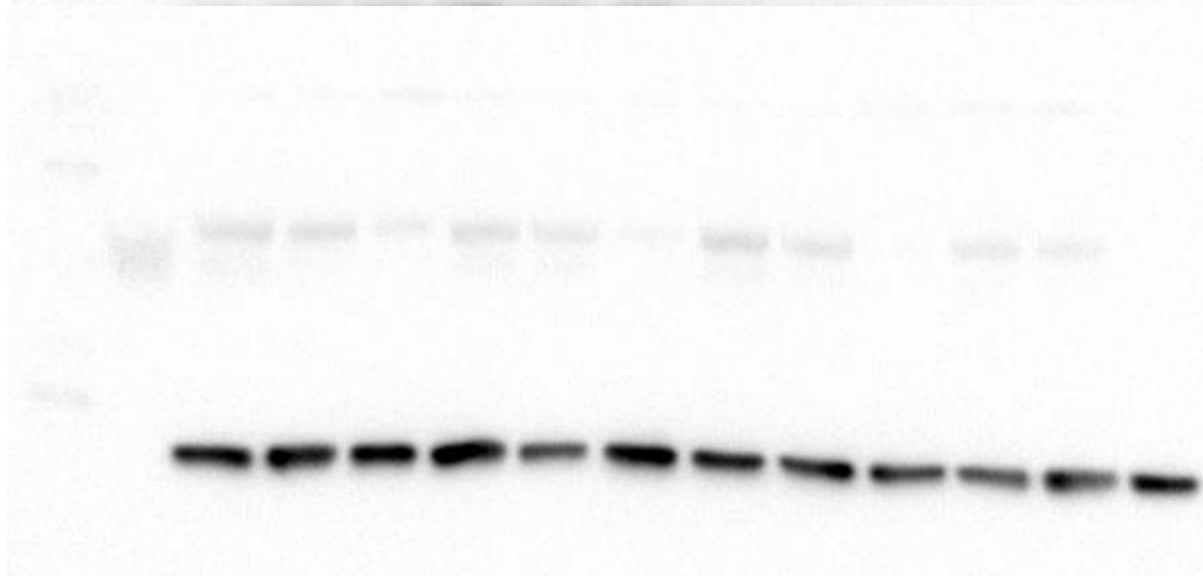


Full unedited gel for Figure 3C
Acquired with ChemiDoc (BioRad)

KLF4
(55 kDa)

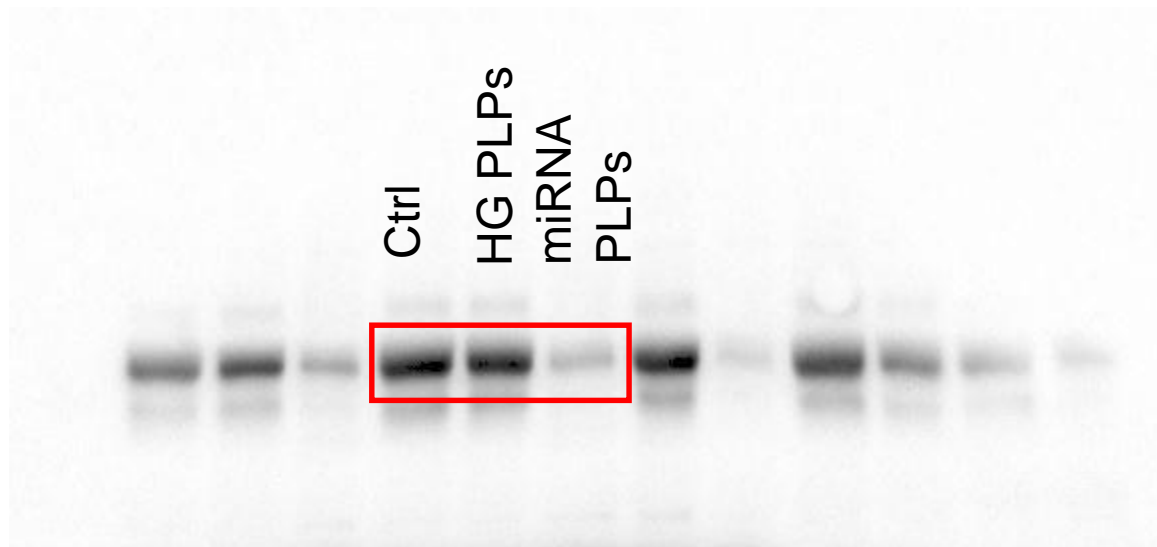


GAPDH
(37 kDa)

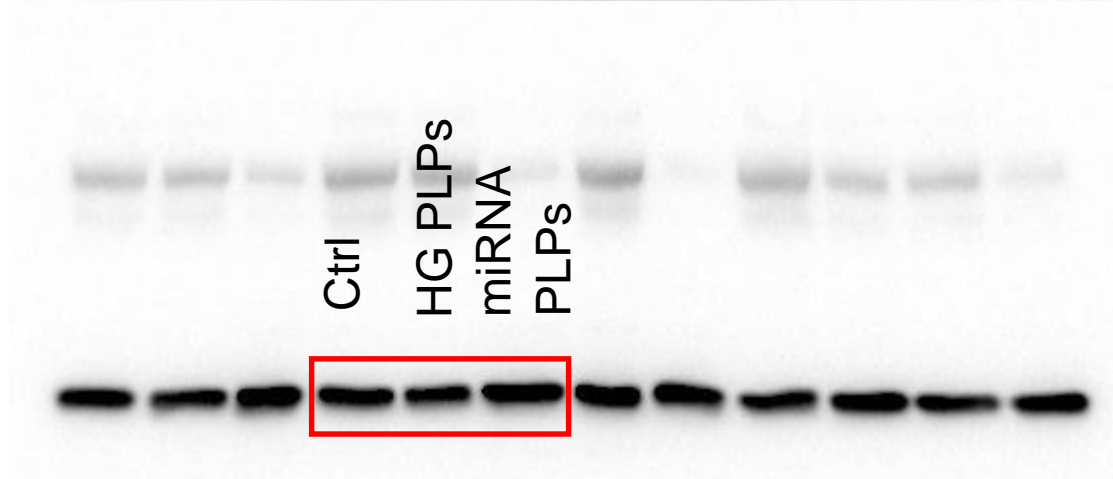


Full unedited gel for Figure 3C
Acquired with ChemiDoc (BioRad)

KLF5
(51 kDa)

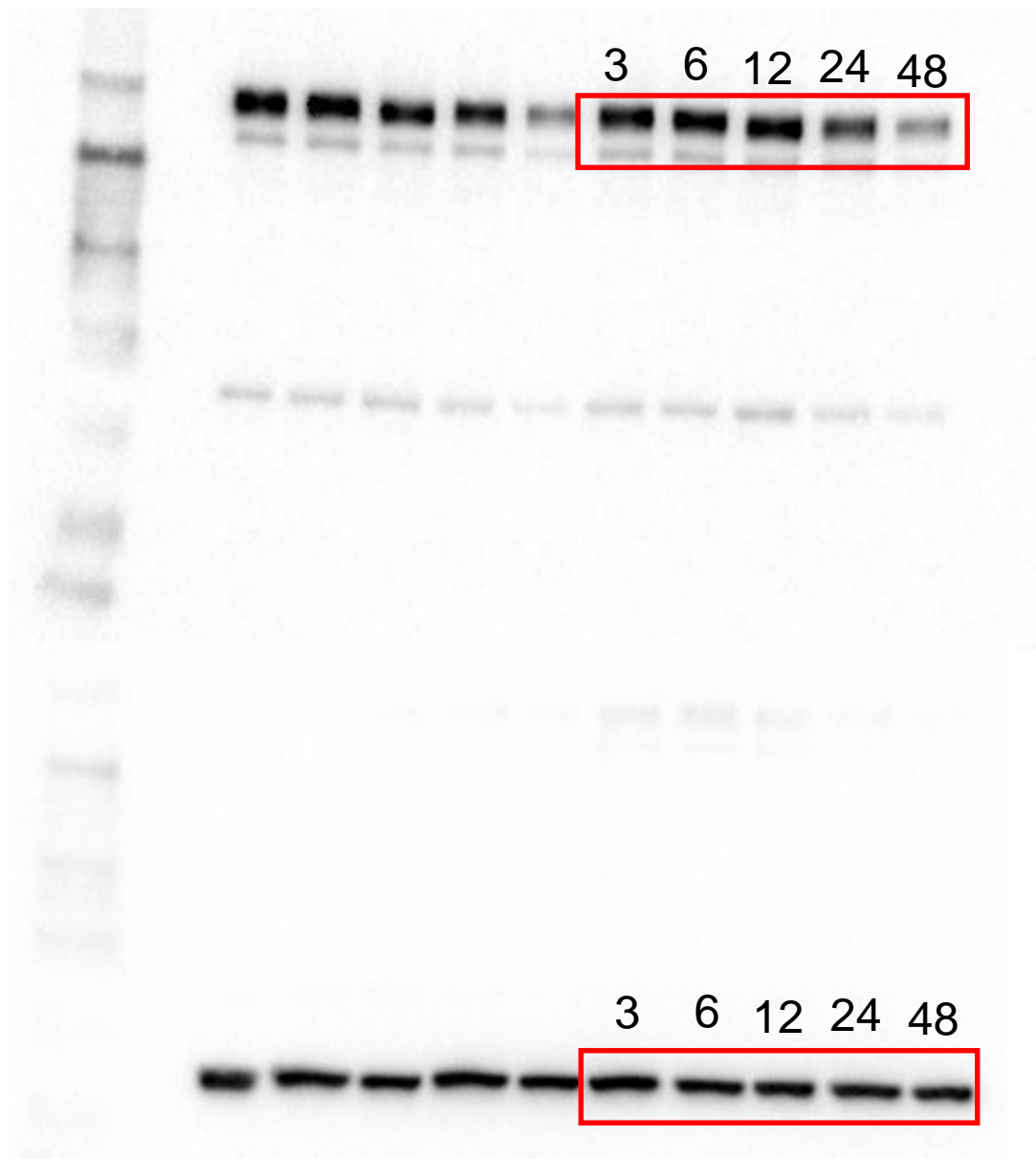


GAPDH
(37 kDa)



Full unedited gel for Figure 3C
Acquired with ChemiDoc (BioRad)

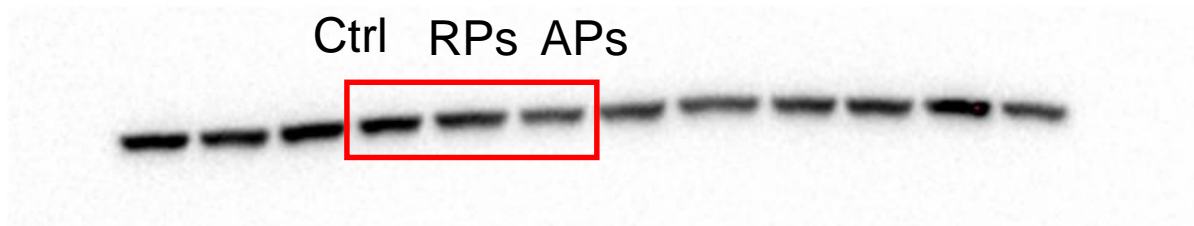
PDGFR β
(124 kDa)



α -TUBULIN
(50 kDa)

Full unedited gel for Figure 3D
Acquired with ChemiDoc (BioRad)

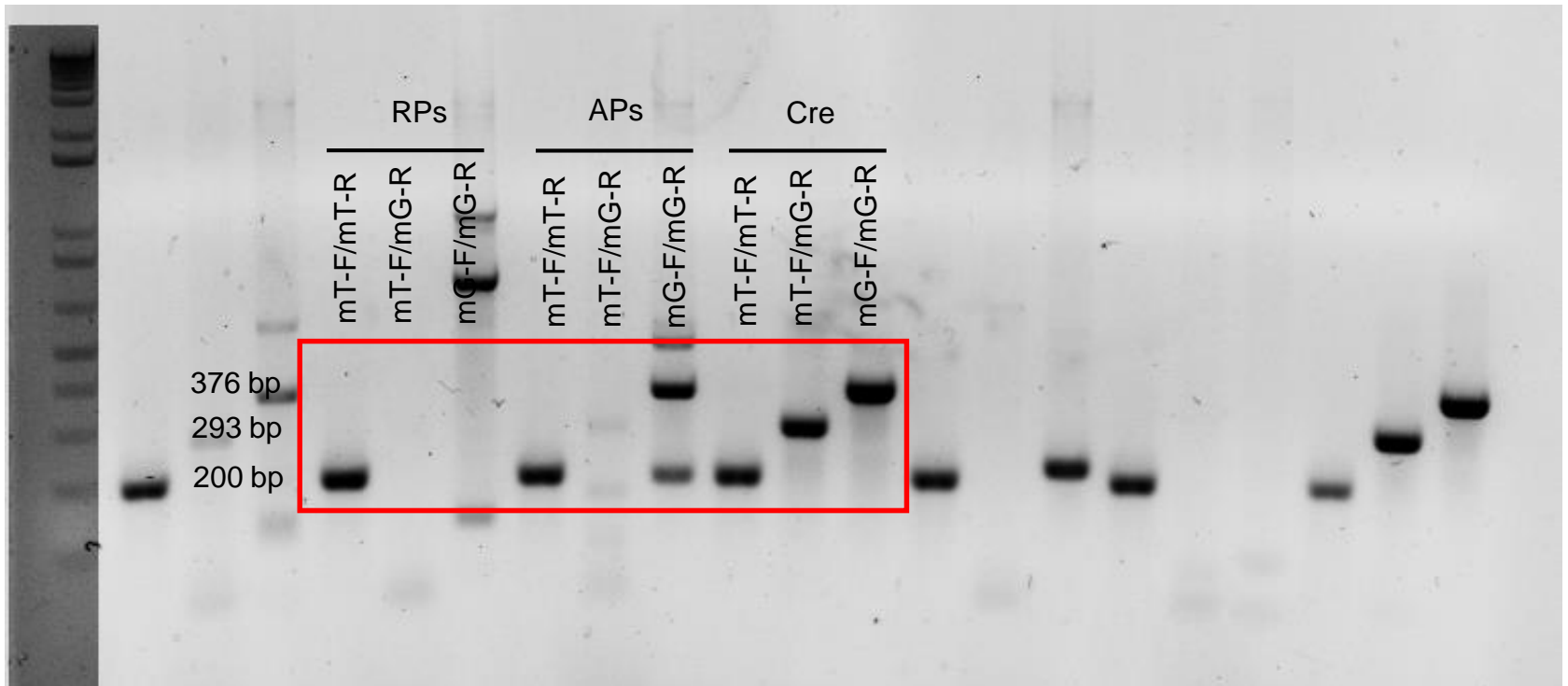
PCNA
(29 kDa)



α -TUBULIN
(50 kDa)

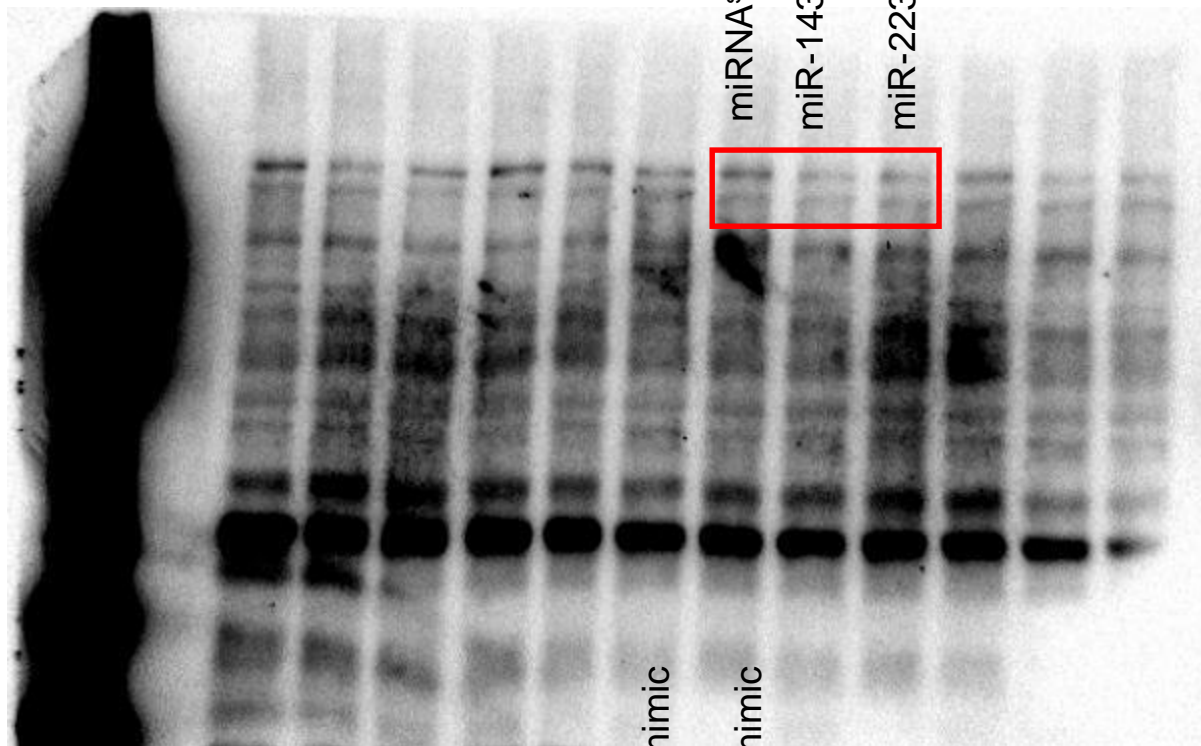


Full unedited gel for Figure S2B
Acquired with ChemiDoc (BioRad)

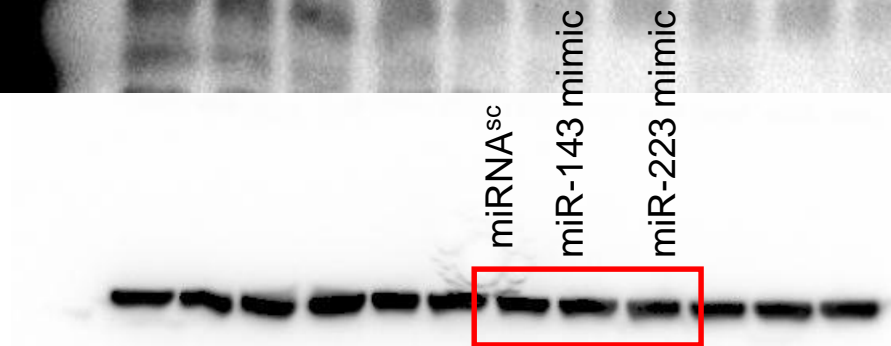


Full unedited gel for Figure S5B
Acquired with ChemiDoc (BioRad)

PDGFR β
(124 kDa)

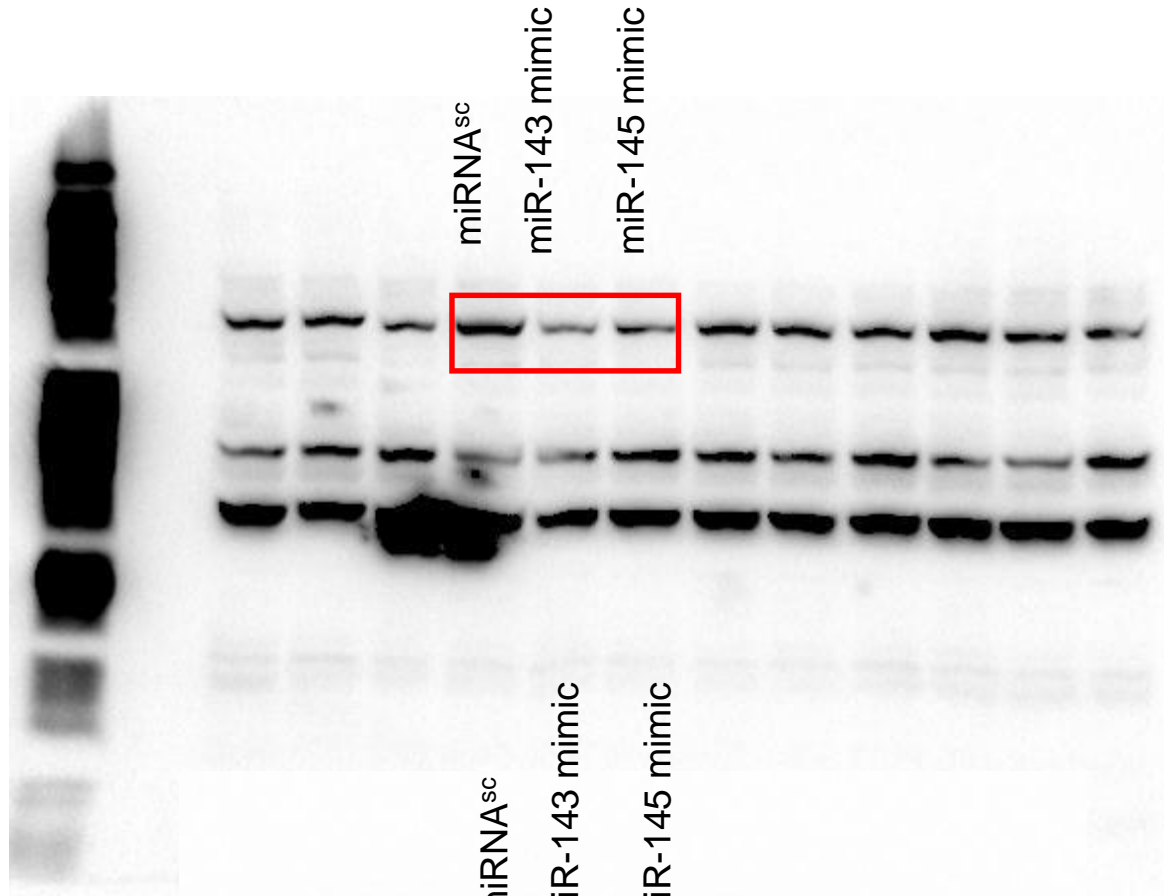


α -TUBULIN
(50 kDa)

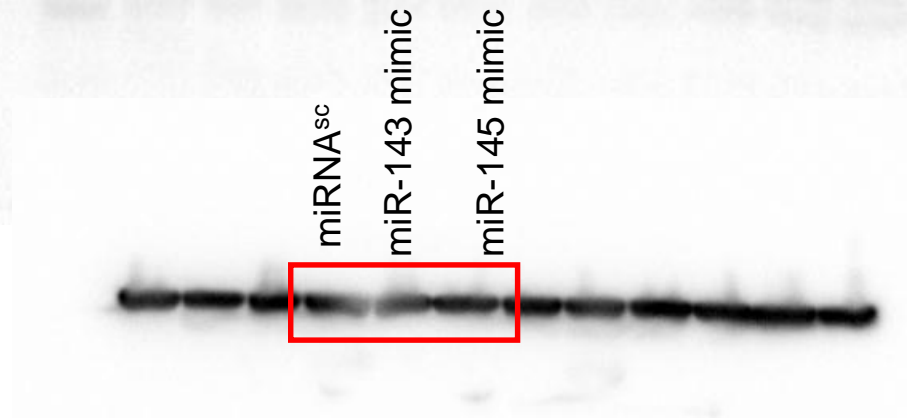


Full unedited gel for Figure S11C
Acquired with ChemiDoc (BioRad)

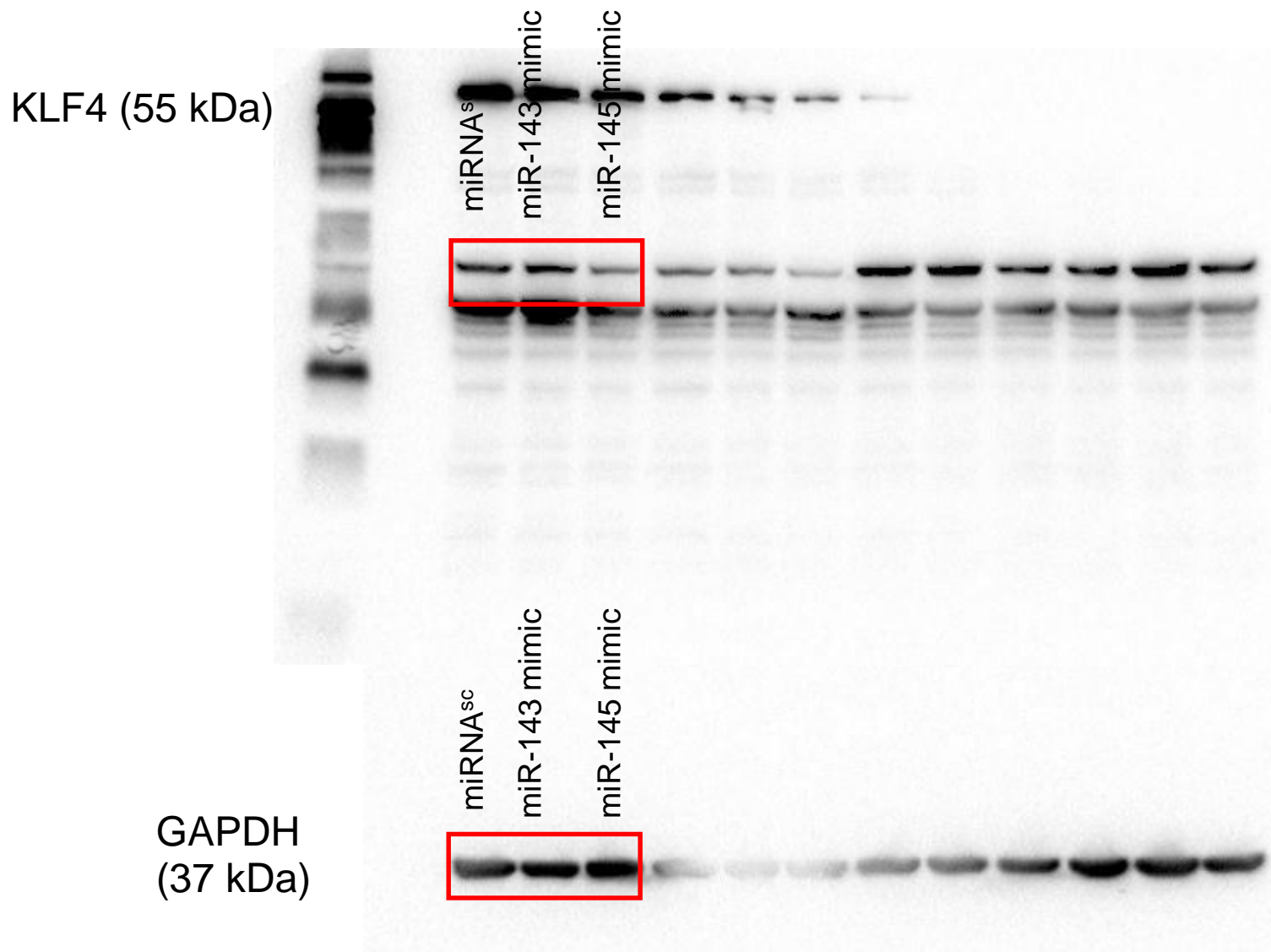
KLF5
(51 kDa)



GAPDH
(37 kDa)



Full unedited gel for Figure S11F
Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure S11H
Acquired with ChemiDoc (BioRad)