

Data Supplement

Integrin $\alpha 9$ regulates smooth muscle cell phenotype switching and vascular remodeling

Running title: *Jain et al.: Targeting integrin $\alpha 9$ inhibits neointimal hyperplasia.*

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Supplementary Tables

Complete blood count	$\alpha 9^{\text{fl/fl}}$	$\alpha 9^{\text{SMC-KO}}$
WBC ($10^3/\mu\text{L}$)	11 ± 2.9	10.5 ± 2.6
RBC ($10^6/\mu\text{L}$)	8.0 ± 2.0	8.6 ± 2.2
HGB (g/dL)	11.8 ± 2.9	12.2 ± 3.1
HCT (%)	38.2 ± 9.6	39.5 ± 9.9
Platelets ($10^3/\mu\text{L}$)	1008 ± 252	1034 ± 262
Neutrophil ($10^3/\mu\text{L}$)	0.2 ± 0.1	0.2 ± 0.1
Lymphocytes ($10^3/\mu\text{L}$)	7.1 ± 2.2	7.5 ± 2.1
Monocytes ($10^3/\mu\text{L}$)	1.8 ± 0.5	1.5 ± 0.4

Table S1: Complete blood counts from 8-10 weeks old mice were obtained using an automated veterinary hematology analyzer (ADVIA). Value are expressed as mean $\pm$ SEM. N= 5-6 mice/group. P= Non-significant versus control mice. WBC, White Blood cells; RBC, Red Blood cells; HGB, Hemoglobin; HCT, Hematocrit.

Target antigen	Vendor or Source	Cat#	Persistent ID / URL
Integrin α 9	Abcam	ab140599	https://www.abcam.com/integrin-alpha-9-antibody-epr9722-ab140599.html
Integrin β 1	Cell Signaling Technology	#4706	https://www.cellsignal.com/products/primary-antibodies/integrin-b1-antibody/4706
α -SMA	Sigma	#A5228	https://www.sigmaaldrich.com/catalog/product/sigma/a5228?lang=en&region=US
SM22 alpha	Abcam	ab14106	https://www.abcam.com/taglntransgelin-antibody-ab14106.html
Smooth Muscle Myosin Heavy Chain 11	Abcam	ab683 ab82541	https://www.abcam.com/smooth-muscle-myosin-heavy-chain-11-antibody-1g12-ab683.html https://www.abcam.com/smooth-muscle-myosin-heavy-chain-11-antibody-ab82541.html
Vimentin	Abcam	ab92547	https://www.abcam.com/vimentin-antibody-epr3776-cytoskeleton-marker-ab92547.html
Osteopontin	Abcam	ab8448	https://www.abcam.com/osteopontin-antibody-ab8448.html
BrdU	Abcam	ab6326	https://www.abcam.com/brdu-antibody-bu175-icrl-proliferation-marker-ab6326.html
Phospho-FAK (Tyr397)	Cell Signaling Technology	#8556	https://www.cellsignal.com/products/primary-antibodies/phospho-fak-tyr397-d20b1-rabbit-mab/8556?site-search-type=Products&N=4294956287&Ntt=8556p&fromPage=plp&requestid=1979083
FAK	Cell Signaling Technology	#3285	https://www.cellsignal.com/products/primary-antibodies/fak-antibody/3285?site-search-type=Products&N=4294956287&Ntt=3285s&fromPage=plp&requestid=1979244
Phospho-Src Family (Tyr416)	Cell Signaling Technology	#2101	https://www.cellsignal.com/products/primary-antibodies/phospho-src-family-tyr416-antibody/2101?site-search-type=Products&N=4294956287&Ntt=2101s&fromPage=plp&requestid=1979427
Src	Cell Signaling Technology	#2109	https://www.cellsignal.com/products/primary-antibodies/src-36d10-rabbit-mab/2109?site-search-type=Products&N=4294956287&Ntt=2109s&fromPage=plp&requestid=1979578
Phospho-p44/42	Cell Signaling Technology	#4370	https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370?site-search-type=Products&N=4294956287&Ntt=4370p&fromPage=plp&requestid=1979578

MAPK (Erk1/2)			type=Products&N=4294956287&Ntt=4370s&fromPage=plp&_requestid=1979840
p44/42 MAPK (Erk1/2)	Cell Signaling Technology	#4695	https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-137f5-rabbit-mab/4695?site-search-type=Products&N=4294956287&Ntt=4695s&fromPage=plp&_requestid=1984322
Phospho-p38 MAPK	Cell Signaling Technology	#9211	https://www.cellsignal.com/products/primary-antibodies/phospho-p38-mapk-thr180-tyr182-antibody/9211?site-search-type=Products&N=4294956287&Ntt=9211s&fromPage=plp&_requestid=1984437
p38 MAPK Antibody	Cell Signaling Technology	#9212	https://www.cellsignal.com/products/primary-antibodies/p38-mapk-antibody/9212
Phospho-GSK-3-beta (Ser9)	Cell Signaling Technology	#9322	https://www.cellsignal.com/products/primary-antibodies/phospho-gsk-3-beta-ser9-d3a4-rabbit-mab/9322
GSK-3β	Cell Signaling Technology	#9315	https://www.cellsignal.com/products/primary-antibodies/gsk-3b-27c10-rabbit-mab/9315
Non-phospho (Active) β-Catenin (Ser33/37/Thr41)	Cell Signaling Technology	#8814	https://www.cellsignal.com/products/primary-antibodies/non-phospho-active-b-catenin-ser33-37-thr41-antibody/4270?site-search-type=Products&N=4294956287&Ntt=4270s&fromPage=plp&_requestid=1984907
β-catenin	Cell Signaling Technology	#8480	https://www.cellsignal.com/products/primary-antibodies/b-catenin-d10a8-xp-rabbit-mab/8480
Lamin B1	Abcam	ab65986	https://www.abcam.com/lamin-b1-antibody-ab65986.html
GAPDH	Cell Signaling Technology	#2118	https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118
β-Actin	Abcam	ab8227	https://www.abcam.com/beta-actin-antibody-ab8227.html
goat anti-rat 488	Invitrogen	A11006	https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11006

goat anti-rabbit 546	Invitrogen	A11035	https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11035
goat anti-mouse 488	Invitrogen	A32723	https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32723
goat anti-rat 546	Invitrogen	A11081	https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11081
Goat-anti mouse	DAKO	P044701-02	https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/secondary-antibodies/goat-anti-mouse-immunoglobulins-hrp-(affinity-isolated)-153239
Goat-anti rabbit	DAKO	P048801-02	https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/secondary-antibodies/goat-anti-rabbit-immunoglobulins-hrp-(affinity-isolated)-153244

Table S2. List of Antibodies

Name	Sequence (from 5' to 3')
mouse MMP-2	Forward: GATGTCGCCCCTAAAACAGA Reverse: TGGTGTCTCTGGTCAAGGTCA
mouse MMP-9	Forward: AGACCTGAAAACCTCCAACCTCAC Reverse: TGTTATGATGGTCCCACCTTGAGGC
mouse c-Myc	Forward: AATCCTGTACCTCGTCCGATTCCA Reverse: TTTGCCTCTTCTCCACAGACACCA
mouse Cyclin D1	Forward: ACCTGGGCAGCCCCAACAAC Reverse: GGAGGCAGTCCGGGTCACACT
mouse GAPDH	Forward: ATGGTGAAGGTCGGTGTGAACG Reverse: CGCTCCTGGAAGATGGTGATGG

Table S3. RT-PCR primer sequences

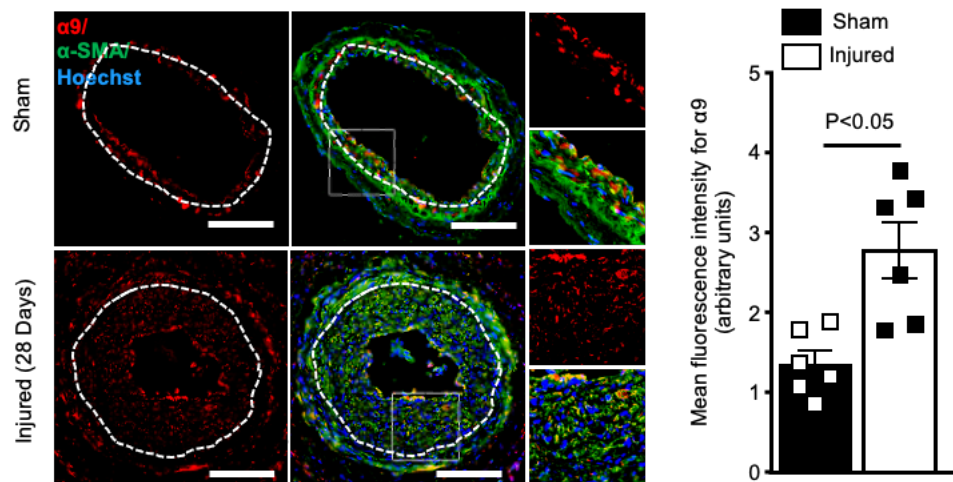


Figure S1. Integrin $\alpha 9$ expression at 28 days in a wire carotid injury model. The left panels show representative double immunostaining for $\alpha 9$ (red) and α SMA (green) in carotid artery sections of WT mice harvested after 28 days of wire injury. The right panel show quantification of $\alpha 9$ fluorescence intensity ($n = 6$). Scale bar: 200 μ m. Boxed regions are magnified. Values are expressed as mean $\pm$ SEM. Statistical analysis: unpaired student t-test.

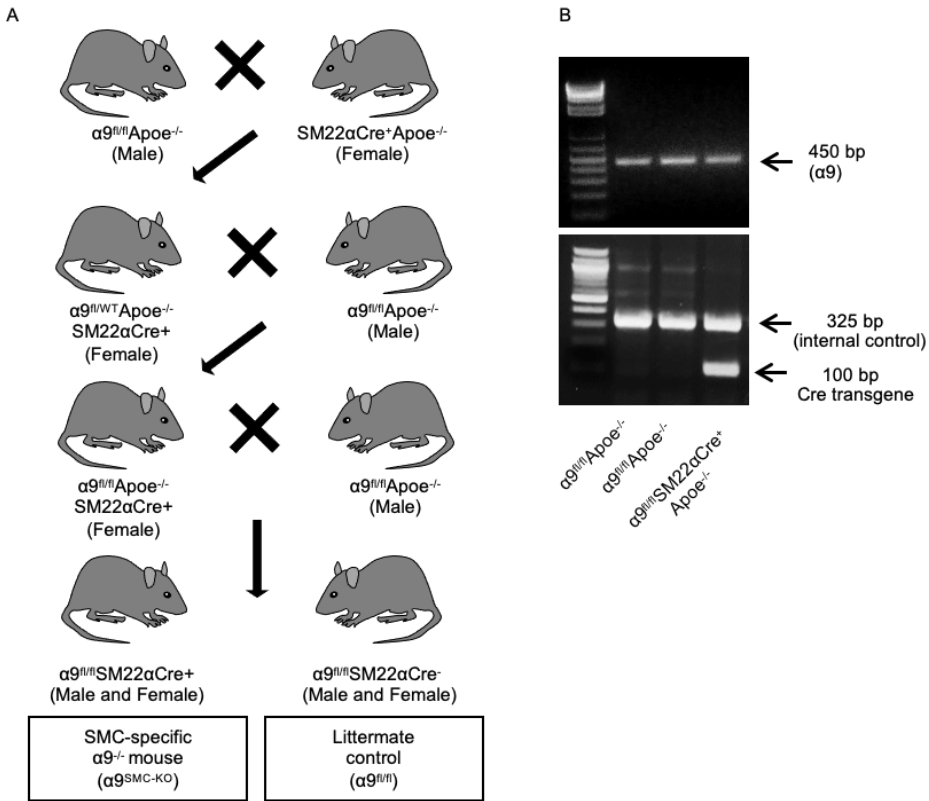


Figure S2. Characterization of SMC-specific $\alpha 9$ -deficient mice. (A) Schematic showing the strategy to generate SMC-specific $\alpha 9^{-/-}$ mice. (B) Genomic PCR showing the presence of Cre gene in $\alpha 9^{fl/fl} SM22\alpha Cre^{+/-}$ mice.

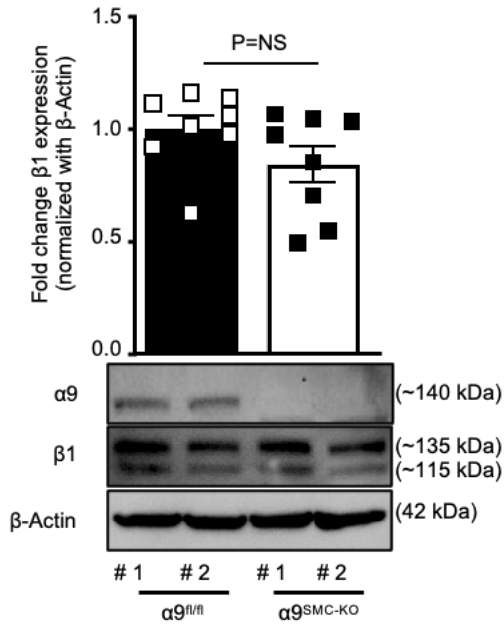


Figure S3. Genetic deletion of integrin $\alpha 9$ in smooth muscle cell does not change integrin $\beta 1$ expression. Western blot analysis of $\alpha 9$, $\beta 1$ and β -actin in SMCs isolated from $\alpha 9^{SMC-KO}$ and control $\alpha 9^{fl/fl}$ mice ($n = 8$). #1 and #2 represents samples from two individual mice. Values are expressed as mean $\pm$ SEM. Statistical analysis: unpaired student t-test.

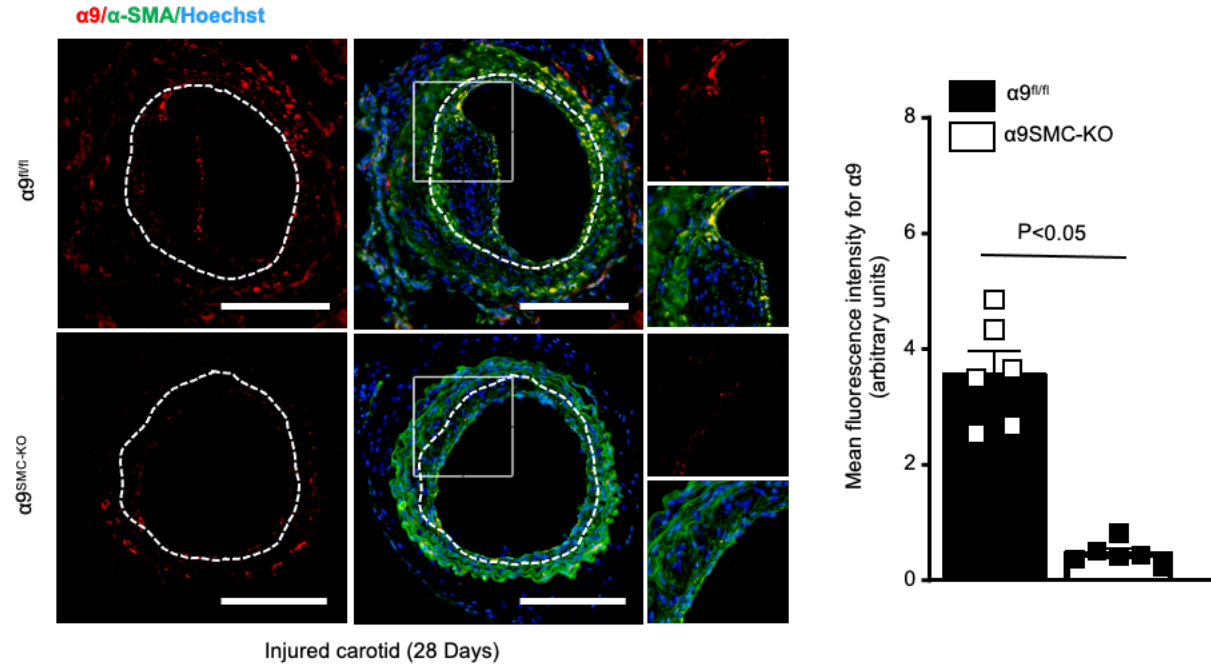


Figure S4. Integrin $\alpha 9$ expression at 28 days. The left panels show representative double immunostaining for $\alpha 9$ (red) and α SMA (green) in carotid artery sections of $\alpha 9^{fl/fl}$ and $\alpha 9^{SMC-KO}$ mice harvested after 28 days of wire injury. The right panel show quantification of $\alpha 9$ fluorescence intensity (n = 6). Scale bar: 200 μ m. Boxed regions are magnified. Values are expressed as mean $\pm$ SEM. Statistical analysis: unpaired student t-test.

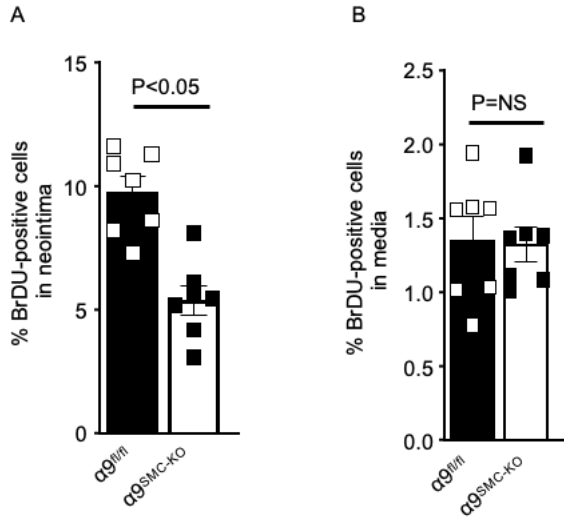


Figure S5. SMC-specific $\alpha 9$ deletion suppresses SMC proliferation *in vivo*. Bar graph representing quantification of percentage BrdU-positive cells in the **(A)** neointima and **(B)** media ($n = 7$). Values are expressed as mean $\pm$ SEM. Statistical analysis: unpaired Student's t-test.

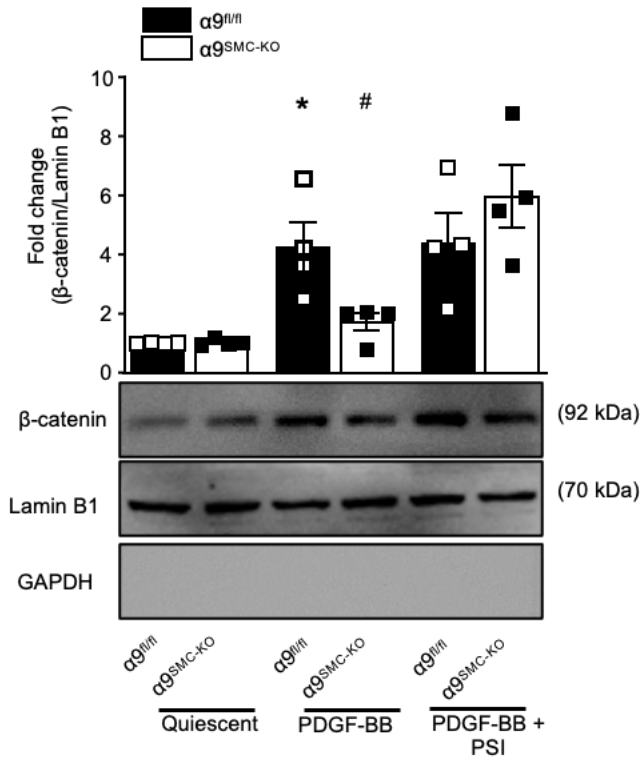


Figure S6. SMC-specific $\alpha 9$ promotes β -catenin degradation through proteasome pathway. Aortic SMCs isolated from $\alpha 9^{SMC-KO}$ and control $\alpha 9^{fl/fl}$ mice were serum-starved and stimulated with PDGF-BB for 6 h in the presence or absence of proteasome-specific inhibitor (PSI, 10 μ M). β -catenin and Lamin B1 were detected by immunoblotting in the nuclear extract (n = 4). Values are expressed as mean $\pm$ SEM. Statistical analysis: Two-way ANOVA followed by Fischer LSD test. *P < 0.05 vs. $\alpha 9^{fl/fl}$ quiescent SMCs; #P < 0.05 vs. $\alpha 9^{fl/fl}$ PDGF-BB treated SMCs.

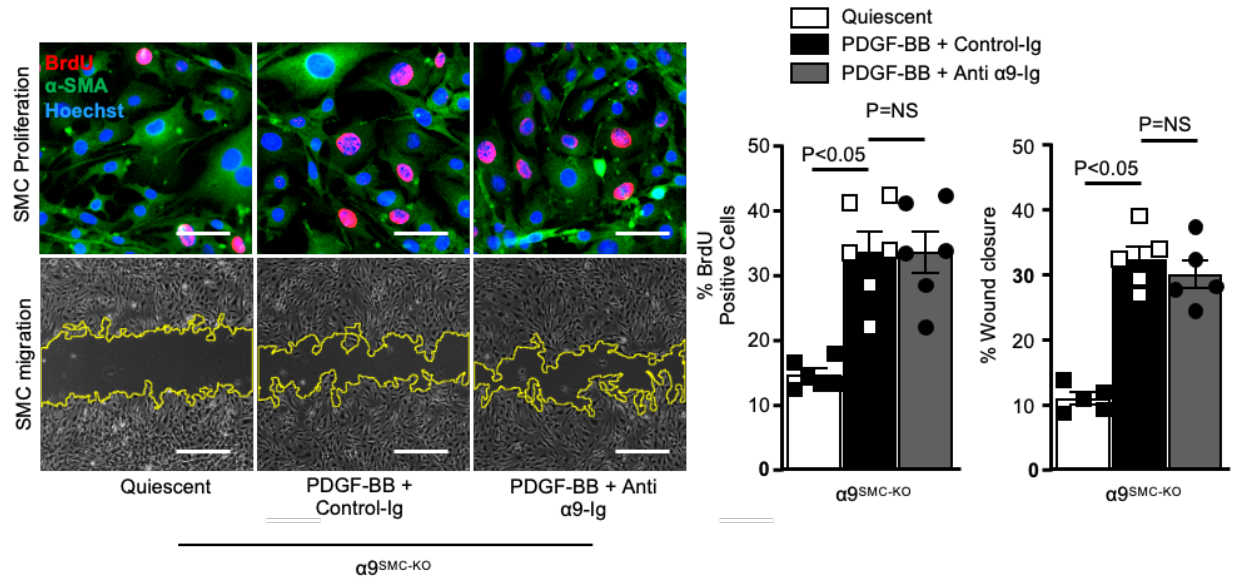


Figure S7. Anti-integrin $\alpha 9$ antibody does not have any off-target effect. Serum-starved SMCs from $\alpha 9^{\text{SMC-KO}}$ were pretreated with murine specific anti- $\alpha 9$ blocking antibody (clone 55A2C, 10 $\mu\text{g}/\text{ml}$) for 60 minutes and then stimulated with or without PDGF-BB for 24 h. The top panels show representative BrdU-positive cells co-stained with α SMA (green) and Hoechst (blue). Scale bars: 50 μm . The bottom panels show representative phase-contrast images of SMC migration in the scratch assay. Scale bars: 500 μm . The right panel shows the quantification of BrdU-positive cells to the total number of cells ($n = 6$) and migrated area ($n = 5$). Values are represented as mean $\pm$ SEM. Statistical analysis: One-way ANOVA with Bonferroni's post hoc test.

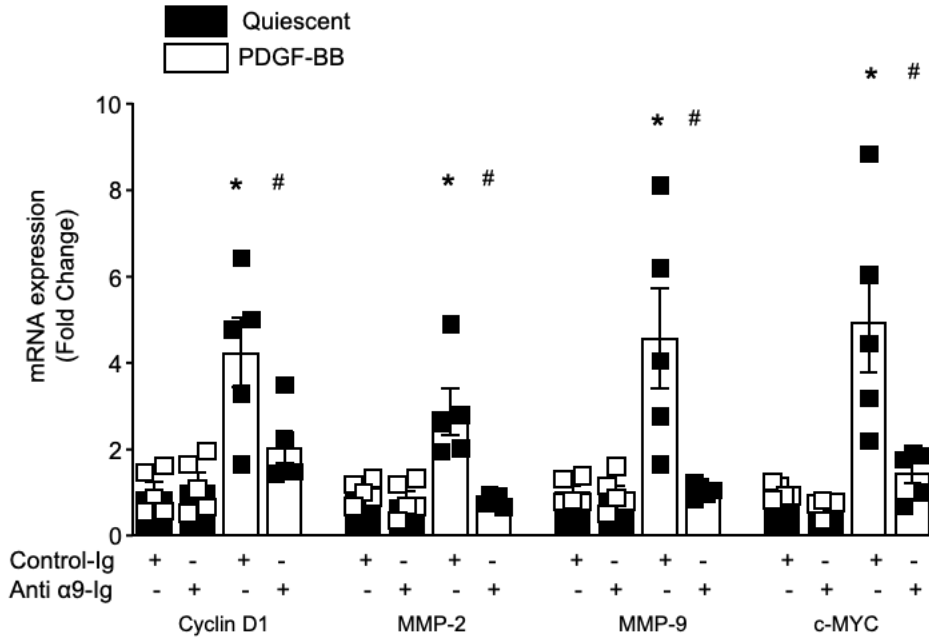


Figure S8. Treatment with anti-integrin $\alpha 9$ antibody suppresses transcription of β -catenin regulated genes. Serum-starved SMCs from WT mice were pretreated with murine specific anti- $\alpha 9$ blocking antibody (clone 55A2C, 10 μ g/ml) for 60 minutes and then stimulated with or without PDGF-BB for 24 h. Real-time quantitative PCR analysis of Cyclin D1, MMP-2, MMP-9 and cMYC ($n = 5$). Values are expressed as mean $\pm$ SEM. Statistical analysis: Two-way ANOVA followed by Fischer LSD test. One-way ANOVA with Bonferroni's post hoc test. * $P < 0.05$ vs. quiescent SMCs treated with control-Ig. # $P < 0.05$ vs. PDGF-BB stimulated SMCs treated with control-Ig.

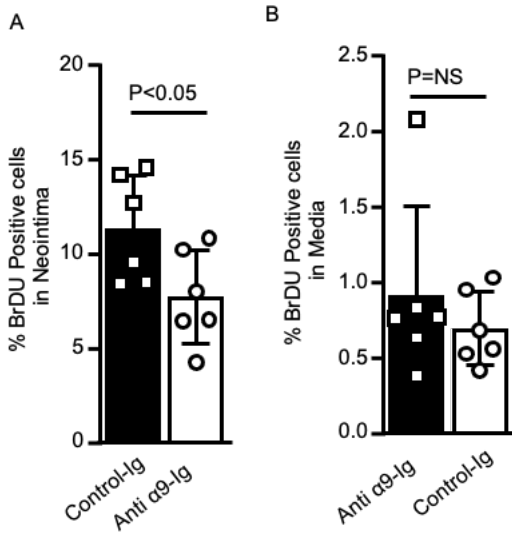


Figure S9. Infusion of anti-integrin $\alpha 9$ antibody reduces SMC proliferation *in vivo*. Bar graph representing quantification of percentage BrdU-positive cells in the (A) neointima and (B) media ($n = 6$) of WT mice treated with anti-integrin $\alpha 9$ antibody. Statistical analysis: Mann Whitney test.

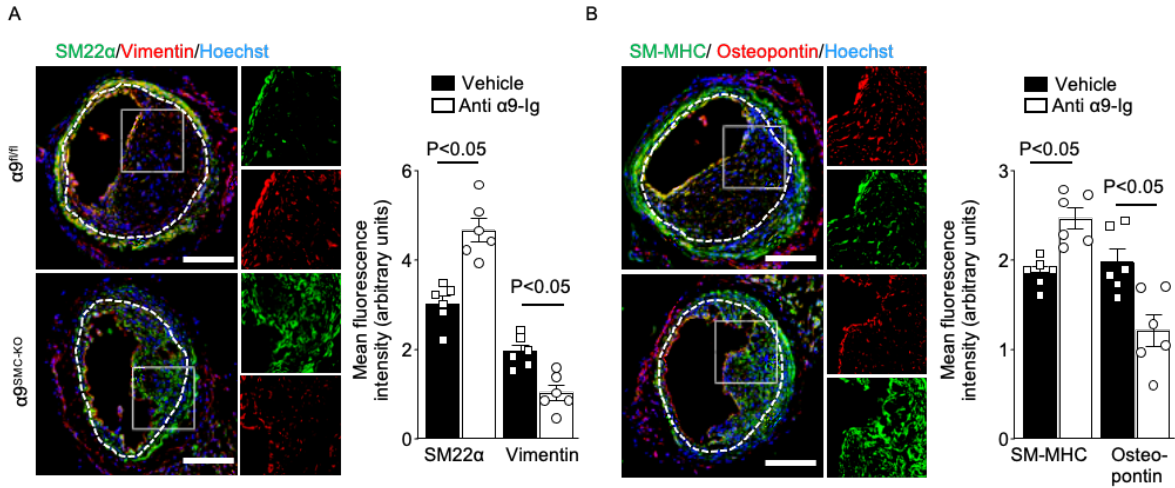
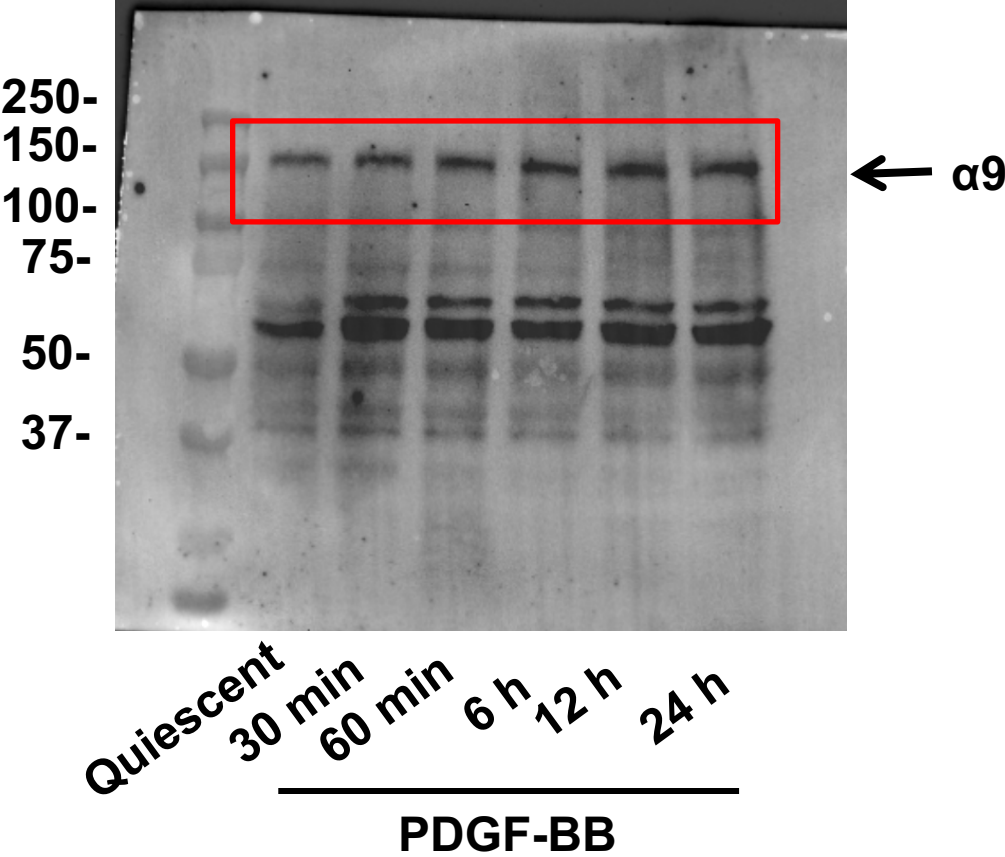
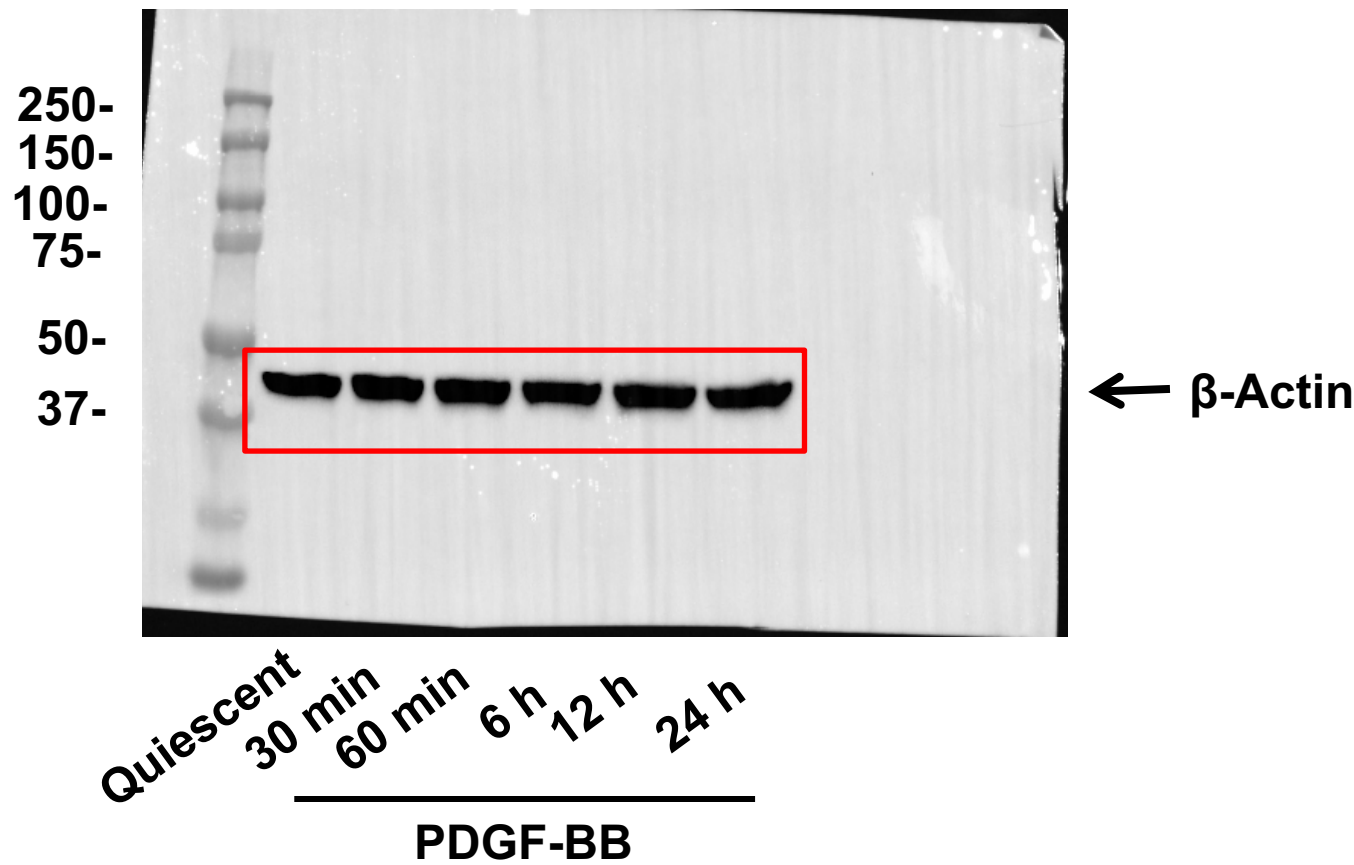


Figure S10. Infusion of anti-integrin $\alpha 9$ antibody modulates SMC phenotypic switching *in vivo*. Male WT mice were treated with 55A2C (200 $\mu\text{g}/\text{mouse}$) or control Ig. Wire injury was performed in the carotid artery after 60 minutes, and arteries were harvested after 28 days. **(A)** The left panels show representative immuno-stained carotid artery sections for SM22 α (green) and vimentin (red). The right panels show quantification of fluorescence intensity ($n = 6$). **(B)** The left panels show representative immuno-stained carotid artery sections for SM-MHC (green) and osteopontin (red). The right panels show quantification of fluorescence intensity ($n = 6$). Boxed regions are magnified. Scale bars: 200 μm . Statistical analysis: unpaired Student's t-test.

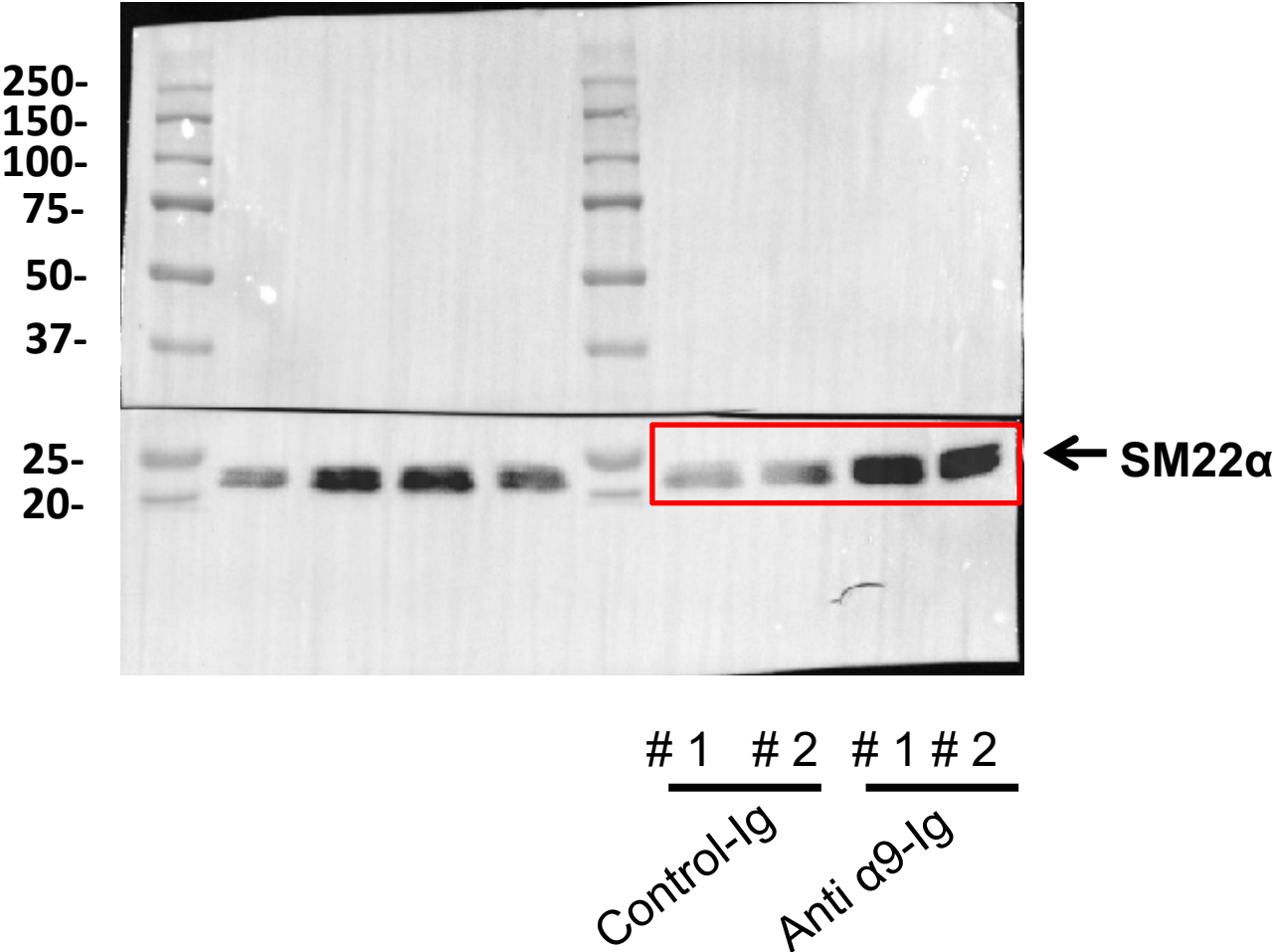
Full unedited gel for Figure 1A- $\alpha 9$



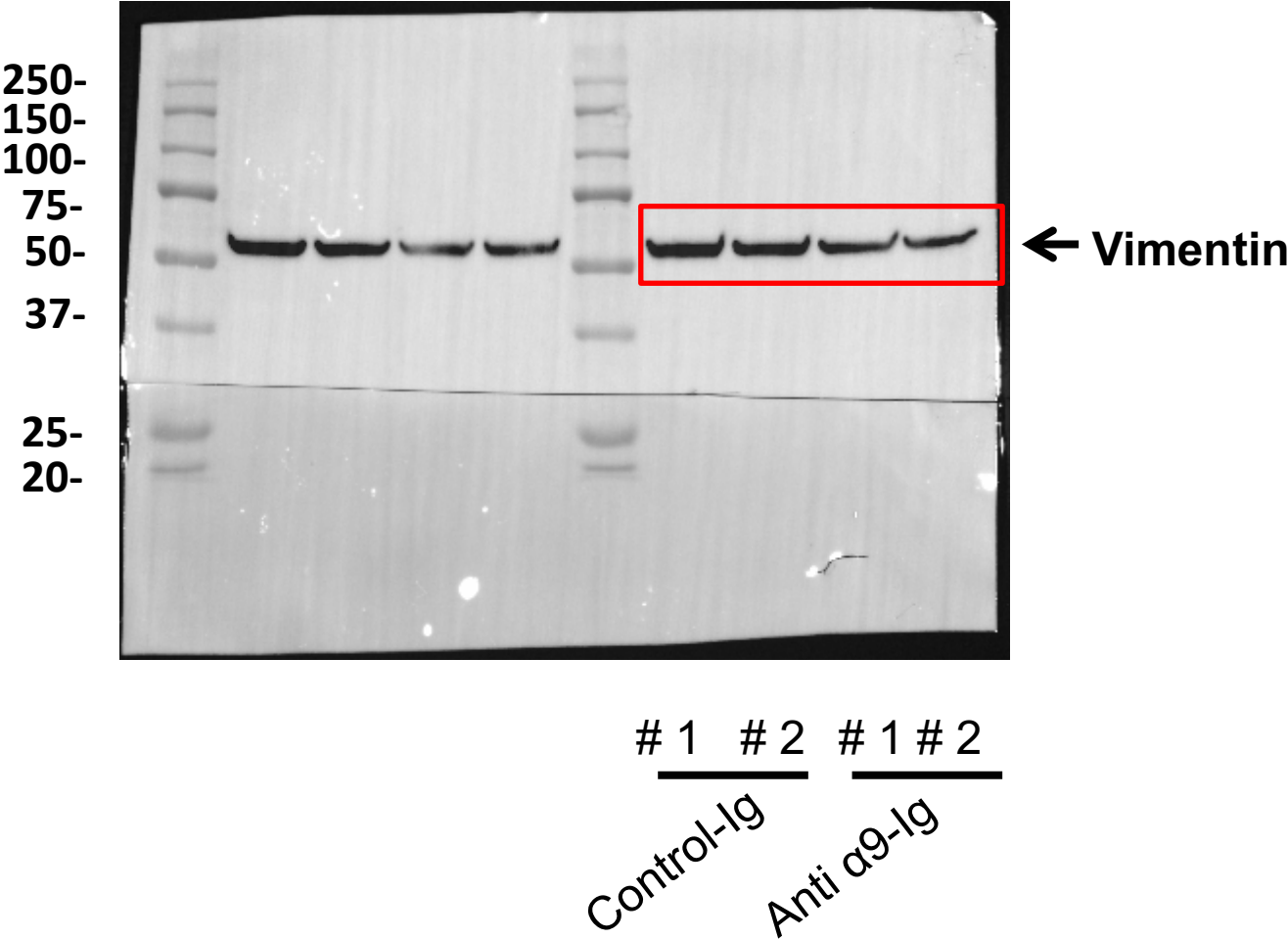
Full unedited gel for Figure 1A- β -Actin



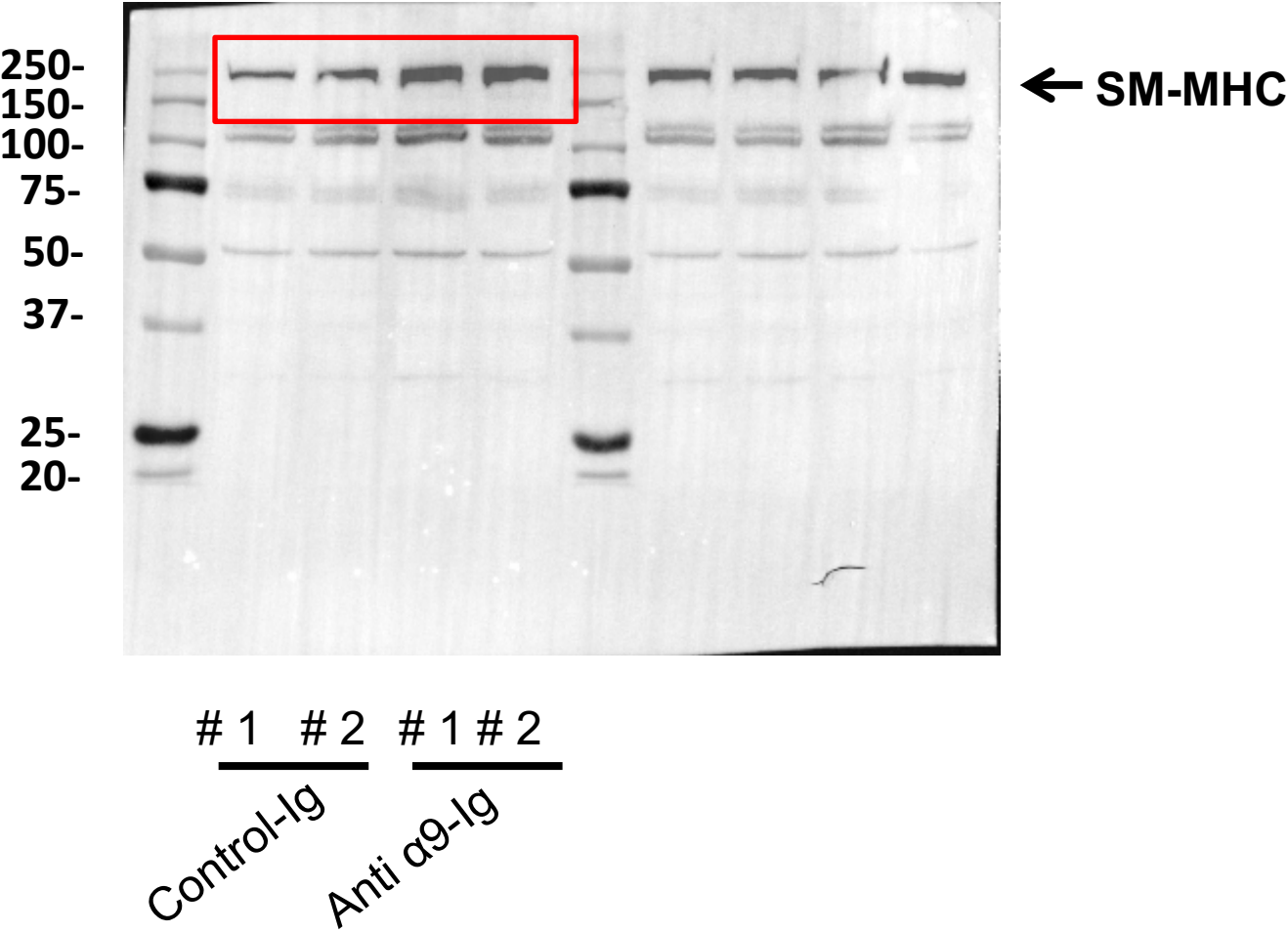
Full unedited gel for Figure 1D-SM22α



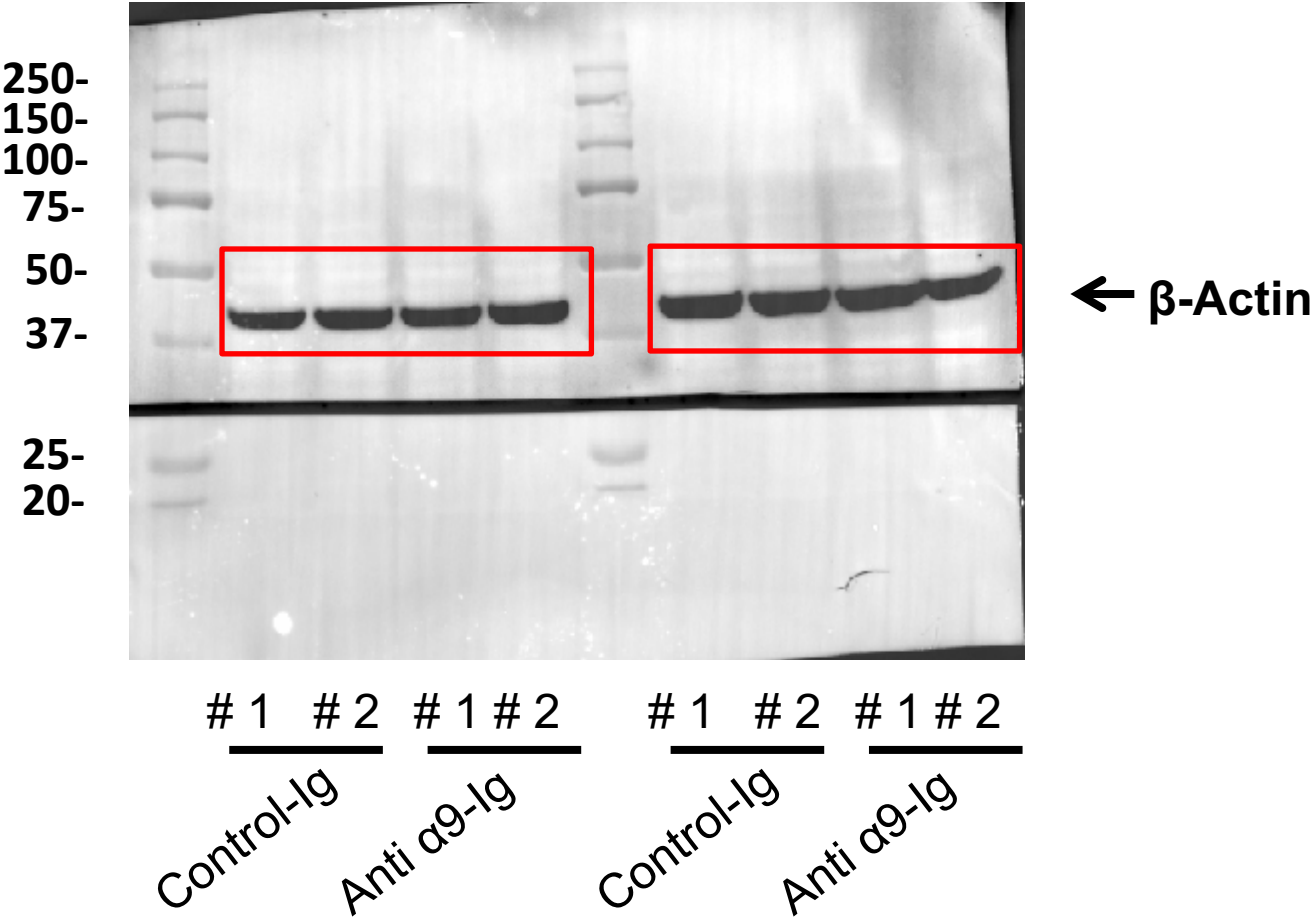
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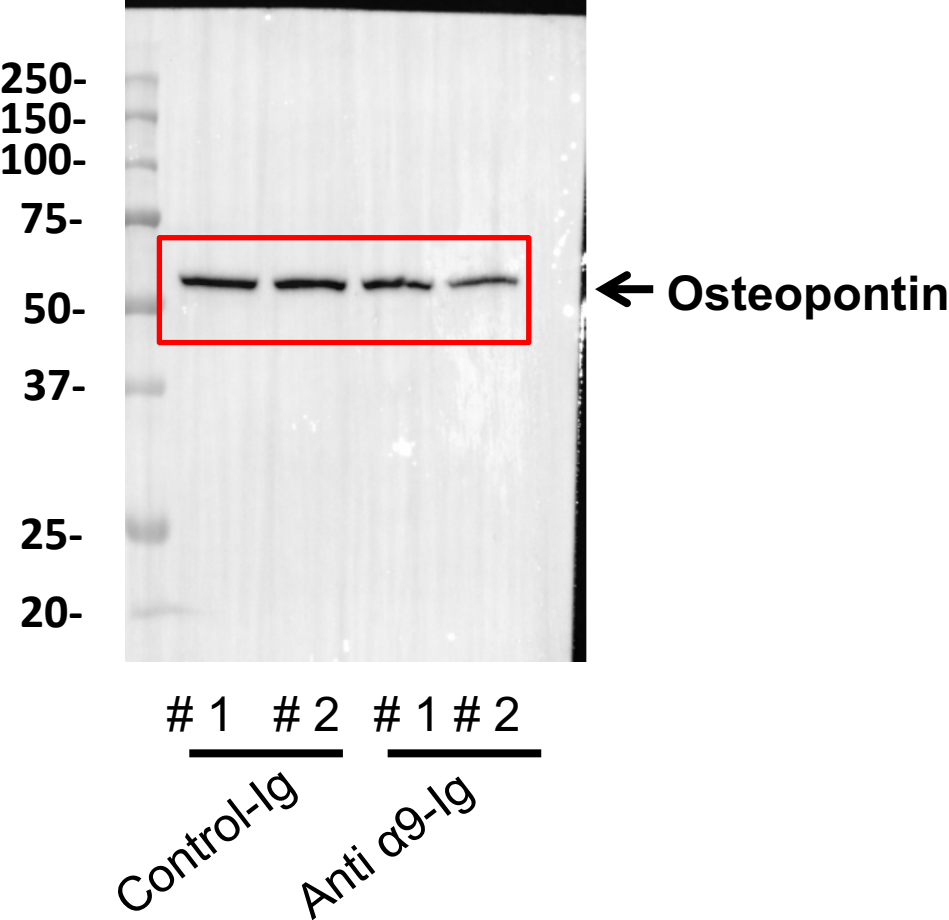
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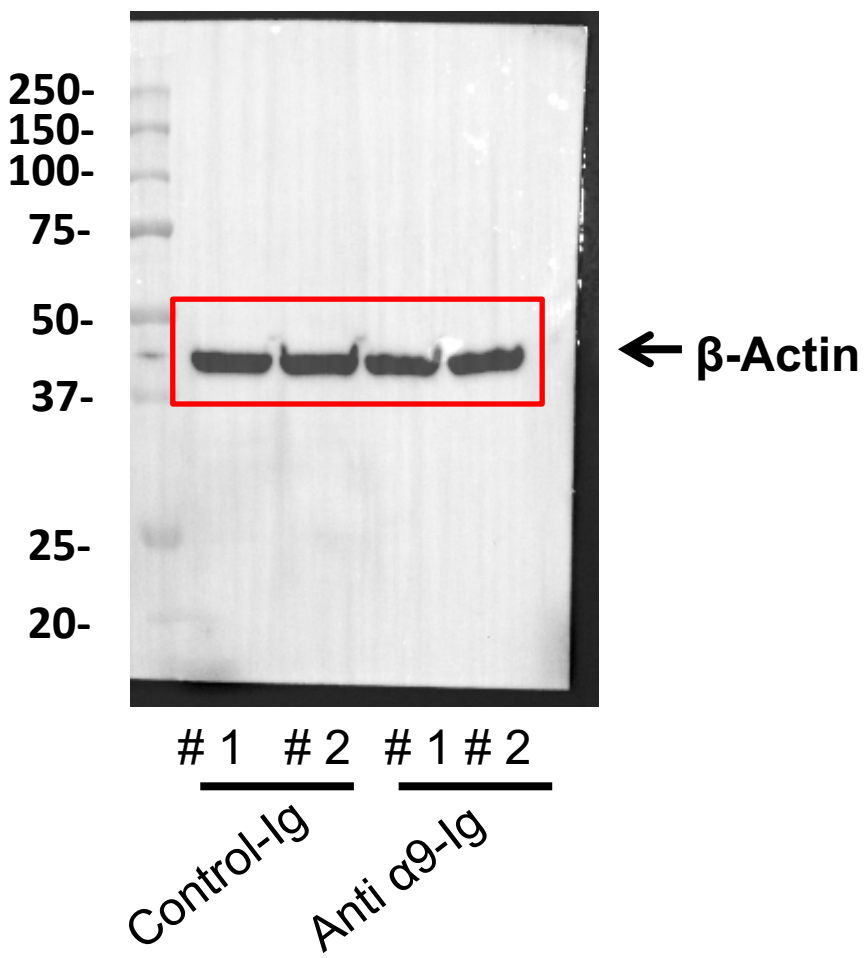
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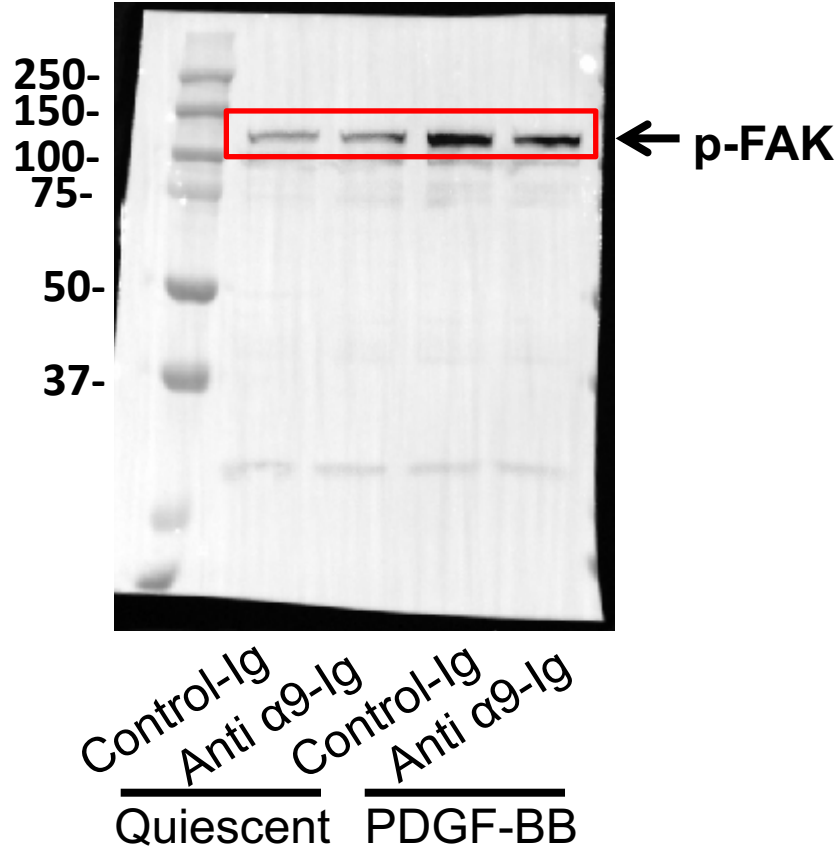
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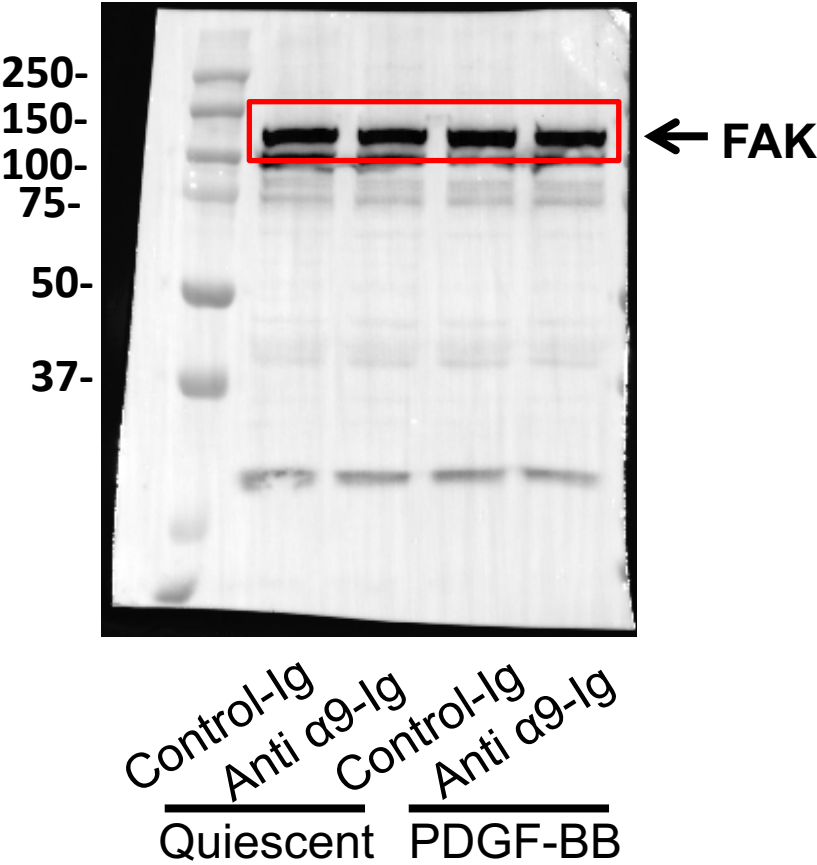
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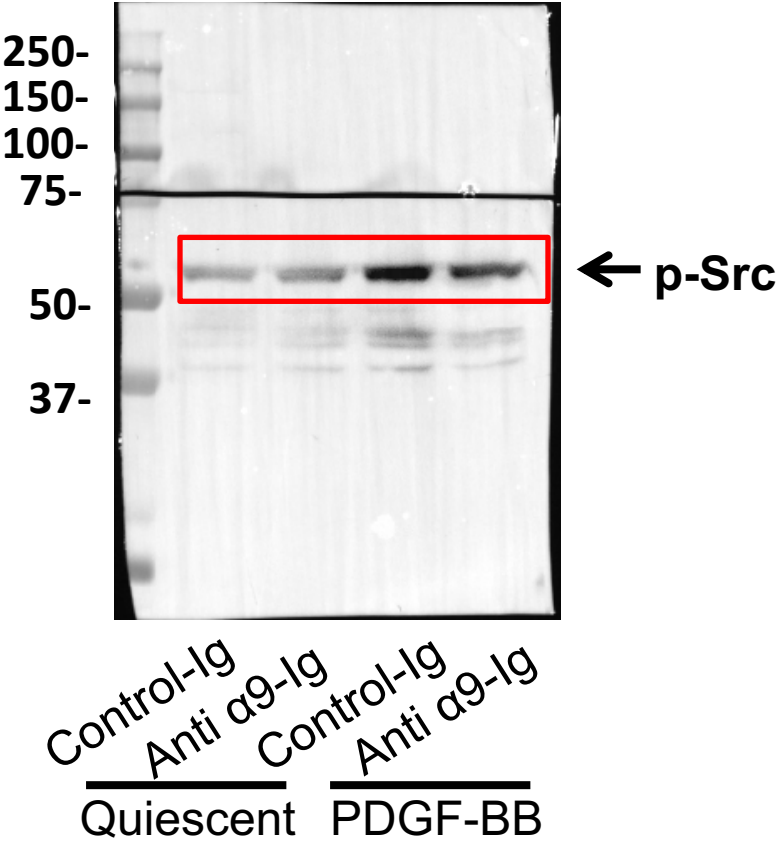
Full unedited gel for Figure 2A-p-FAK



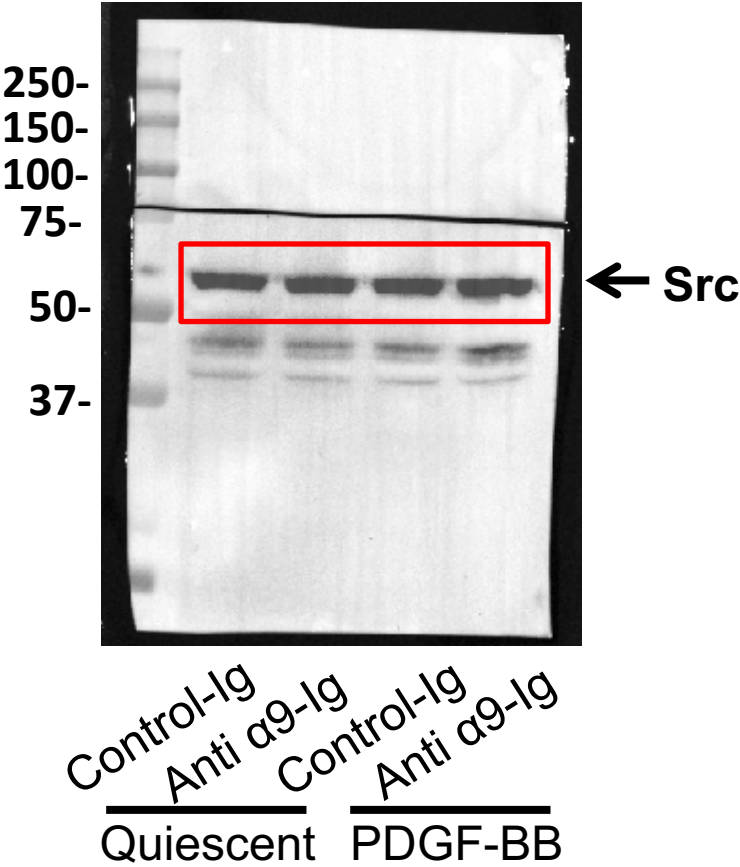
Full unedited gel for Figure 2A-FAK



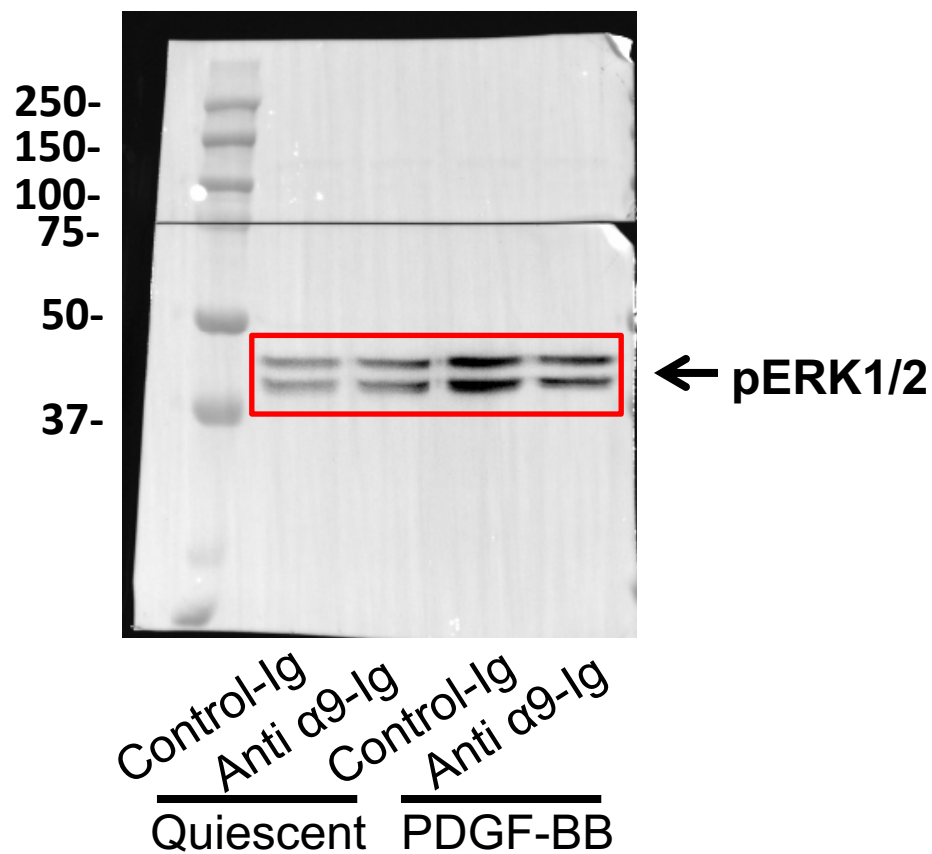
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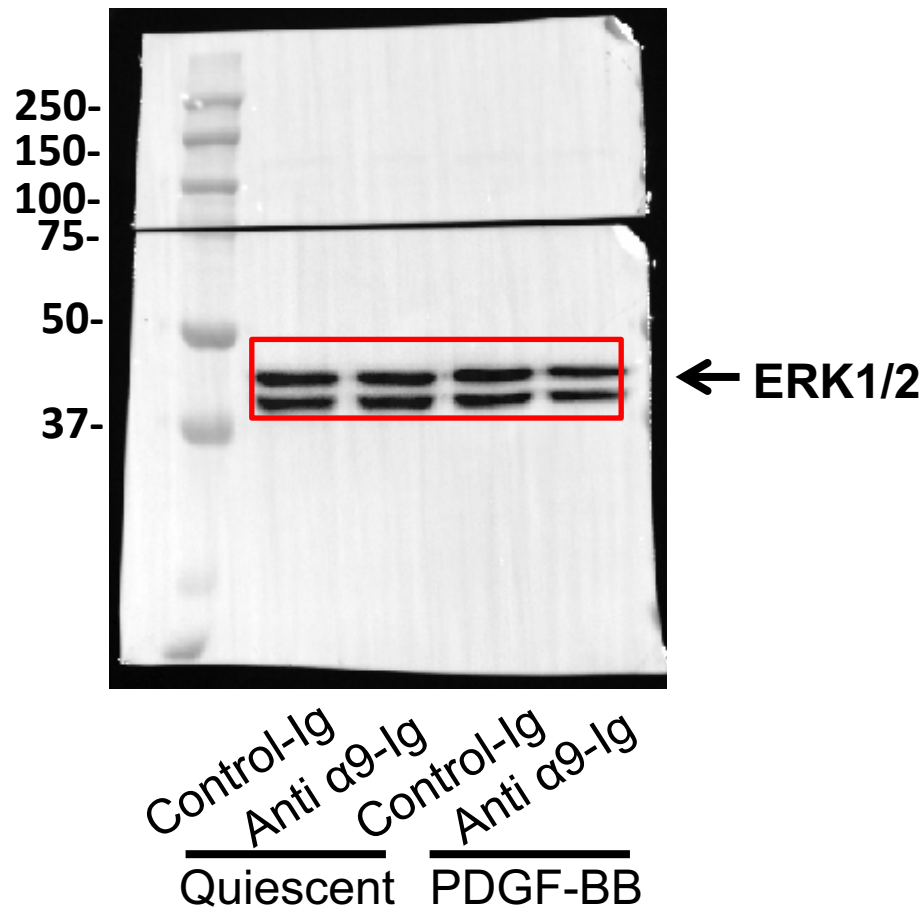
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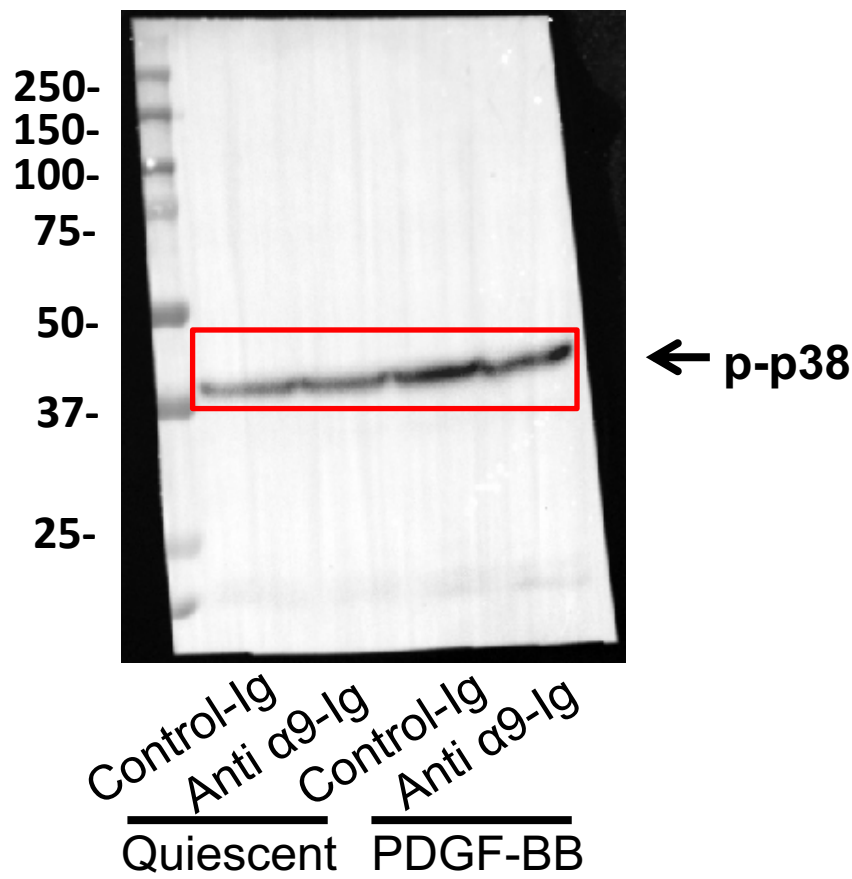
Full unedited gel for Figure 2A-pERK1/2



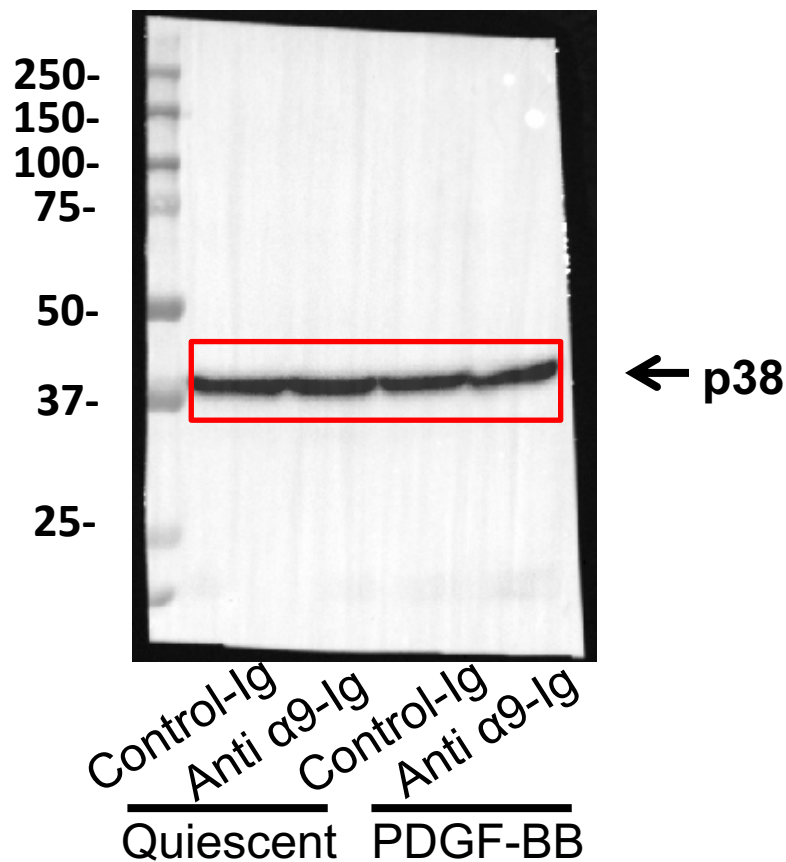
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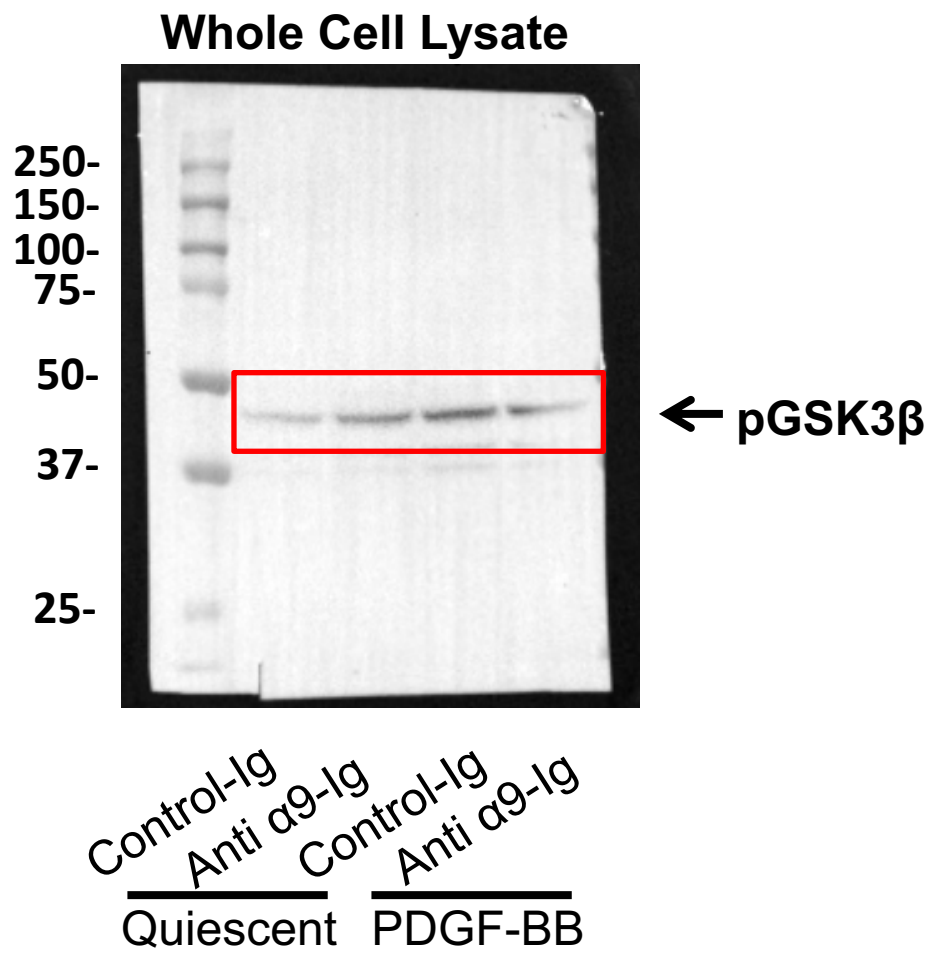
Full unedited gel for Figure 2A-p-p38



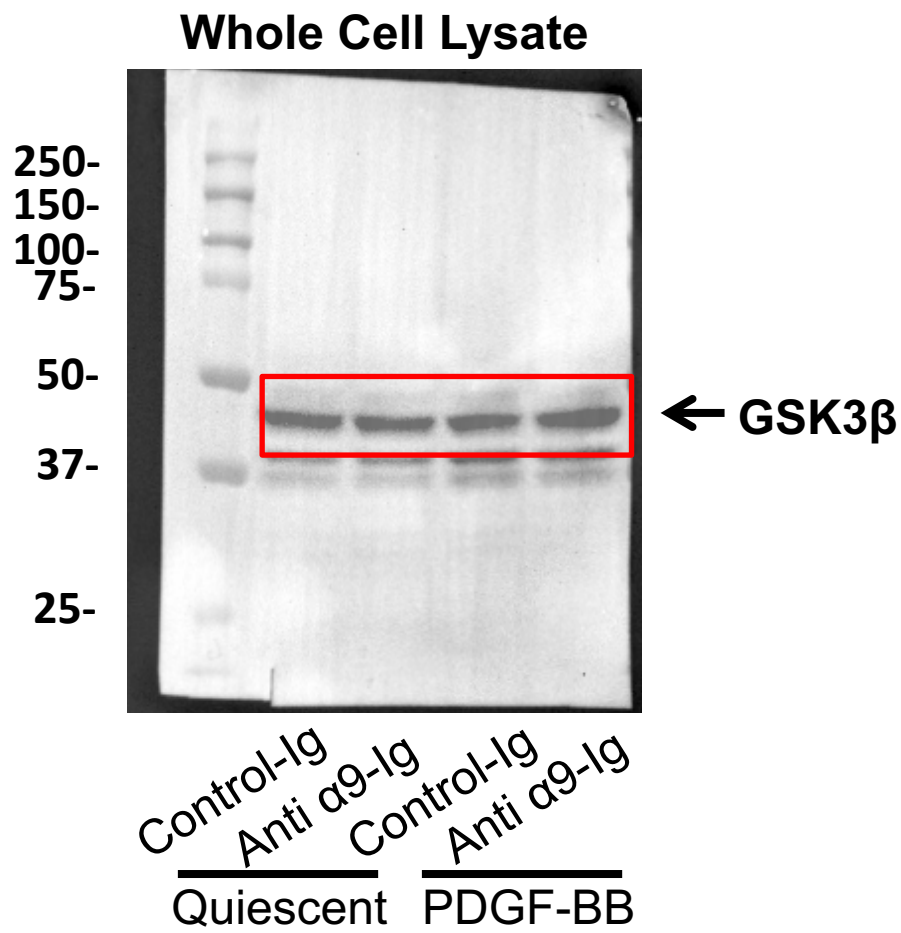
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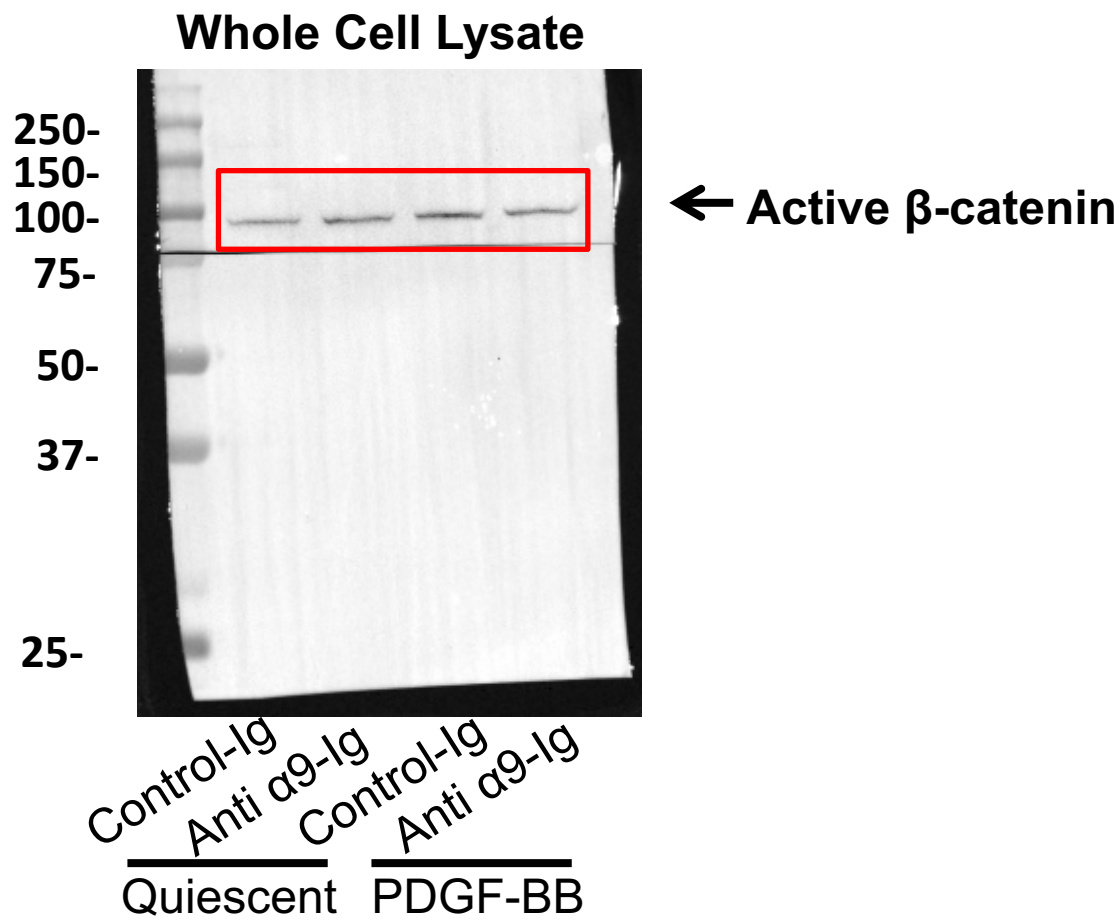
Full unedited gel for Figure 2B-pGSK3β



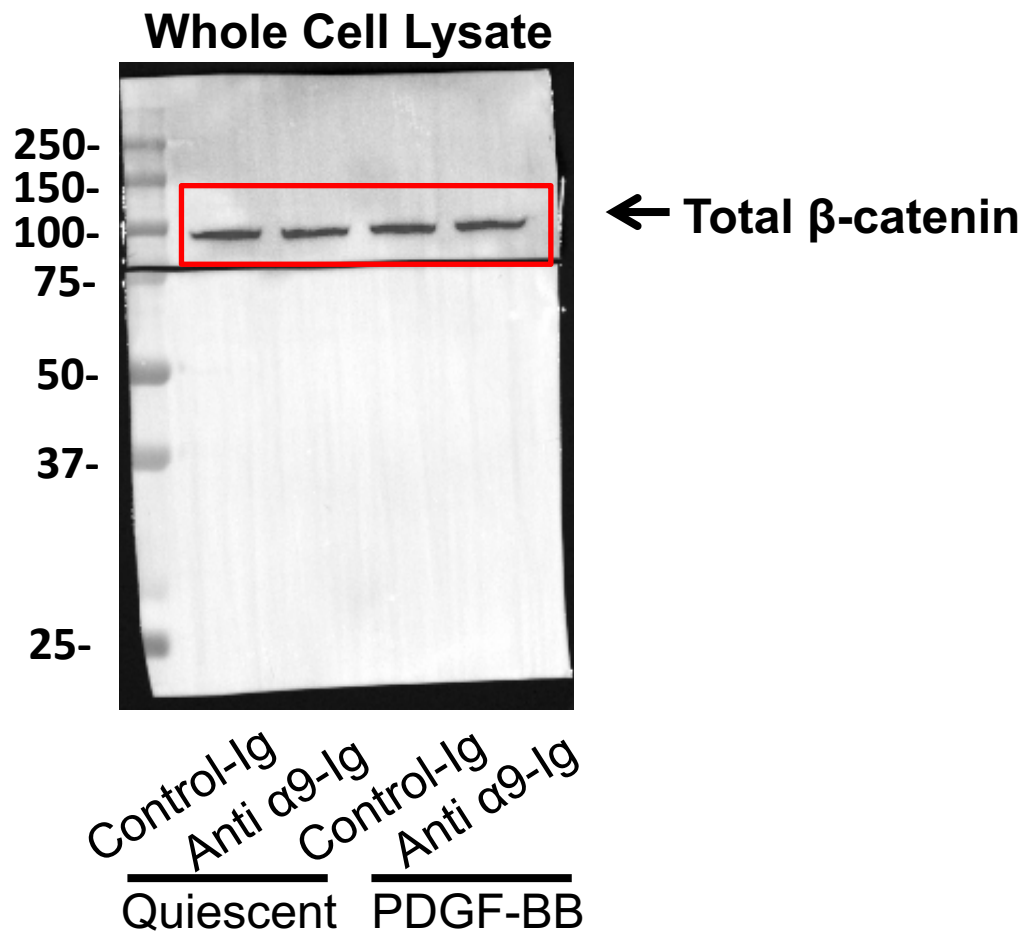
Full unedited gel for Figure 2B-GSK3β



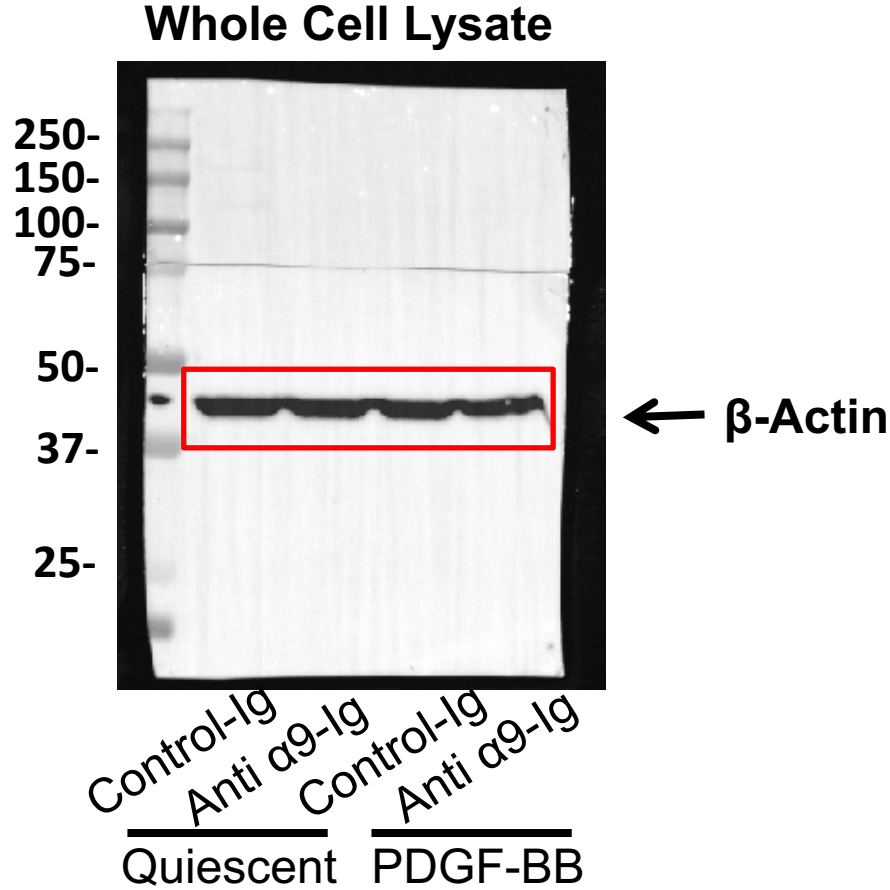
Full unedited gel for Figure 2B-Active β -catenin



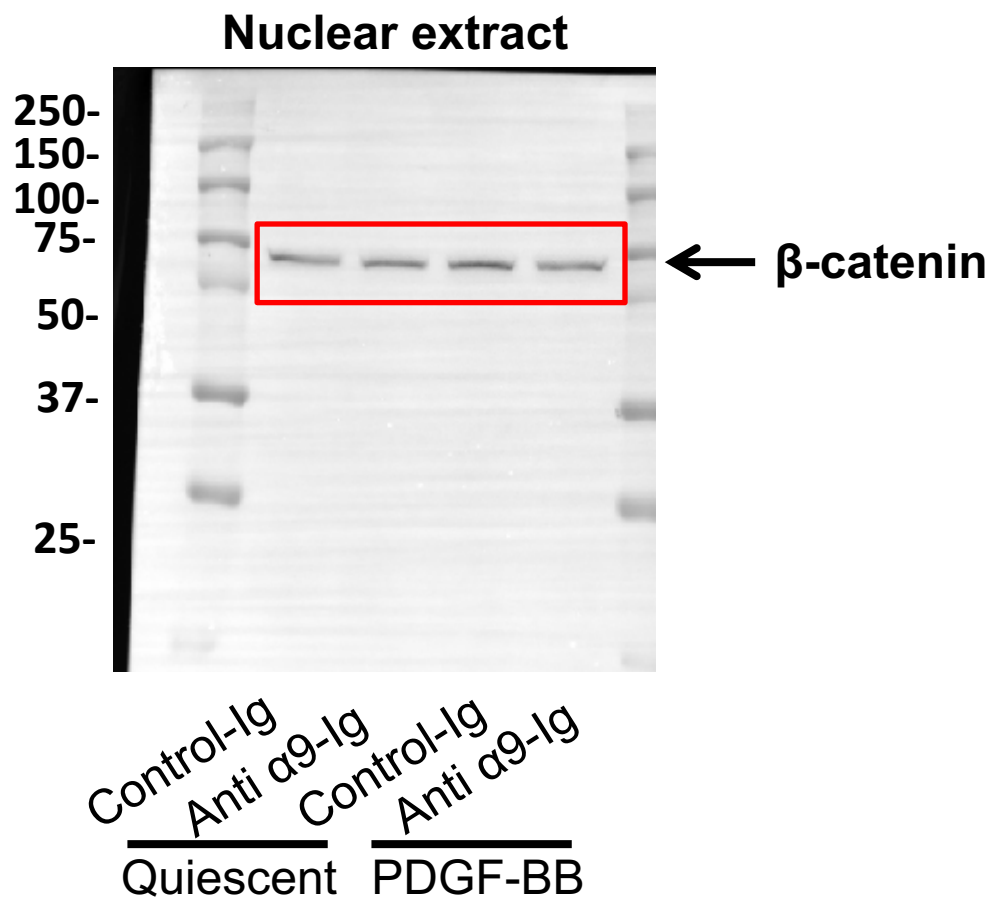
Full unedited gel for Figure 2B- Total β -catenin



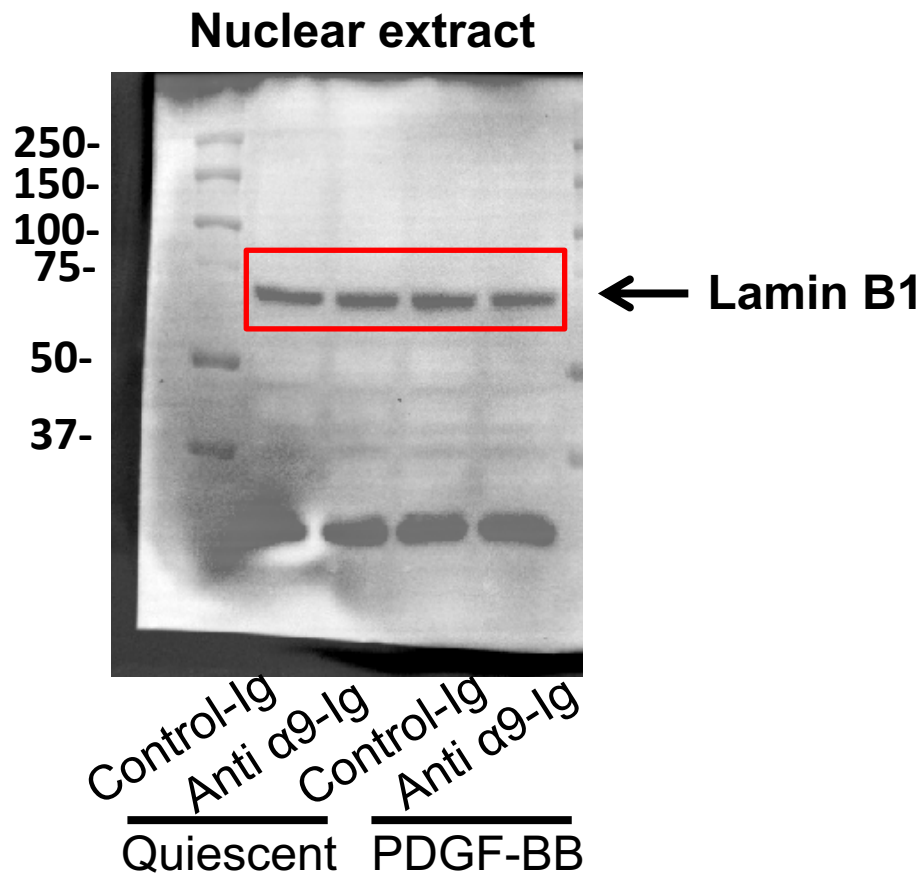
Full unedited gel for Figure 2B- β -Actin



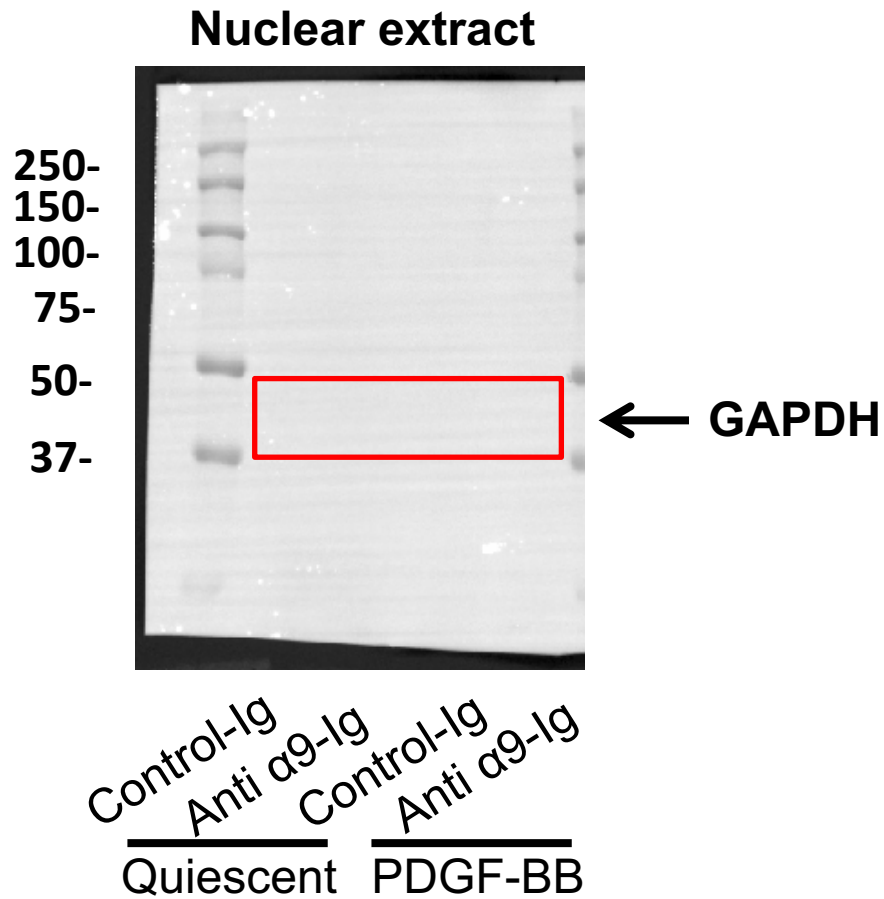
Full unedited gel for Figure 2B- β -catenin



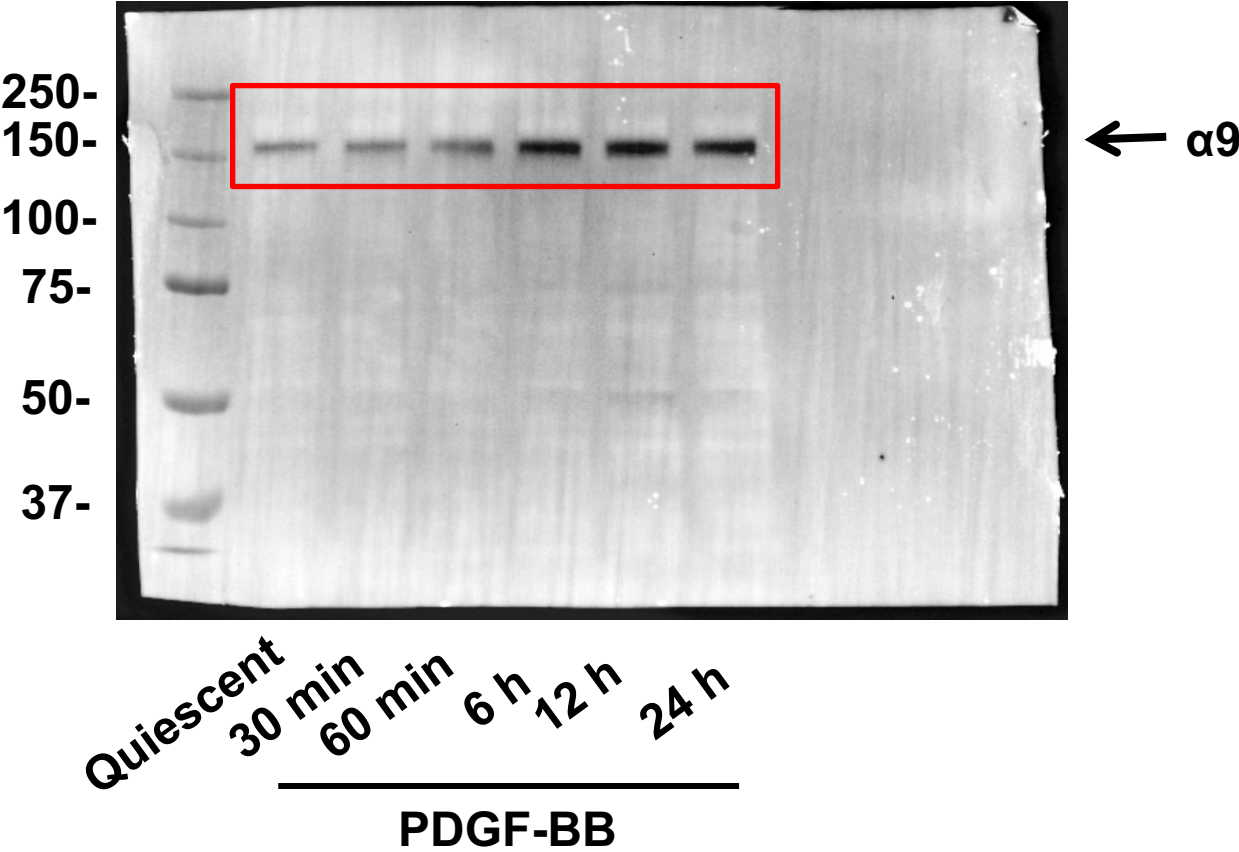
Full unedited gel for Figure 2B- Lamin B1



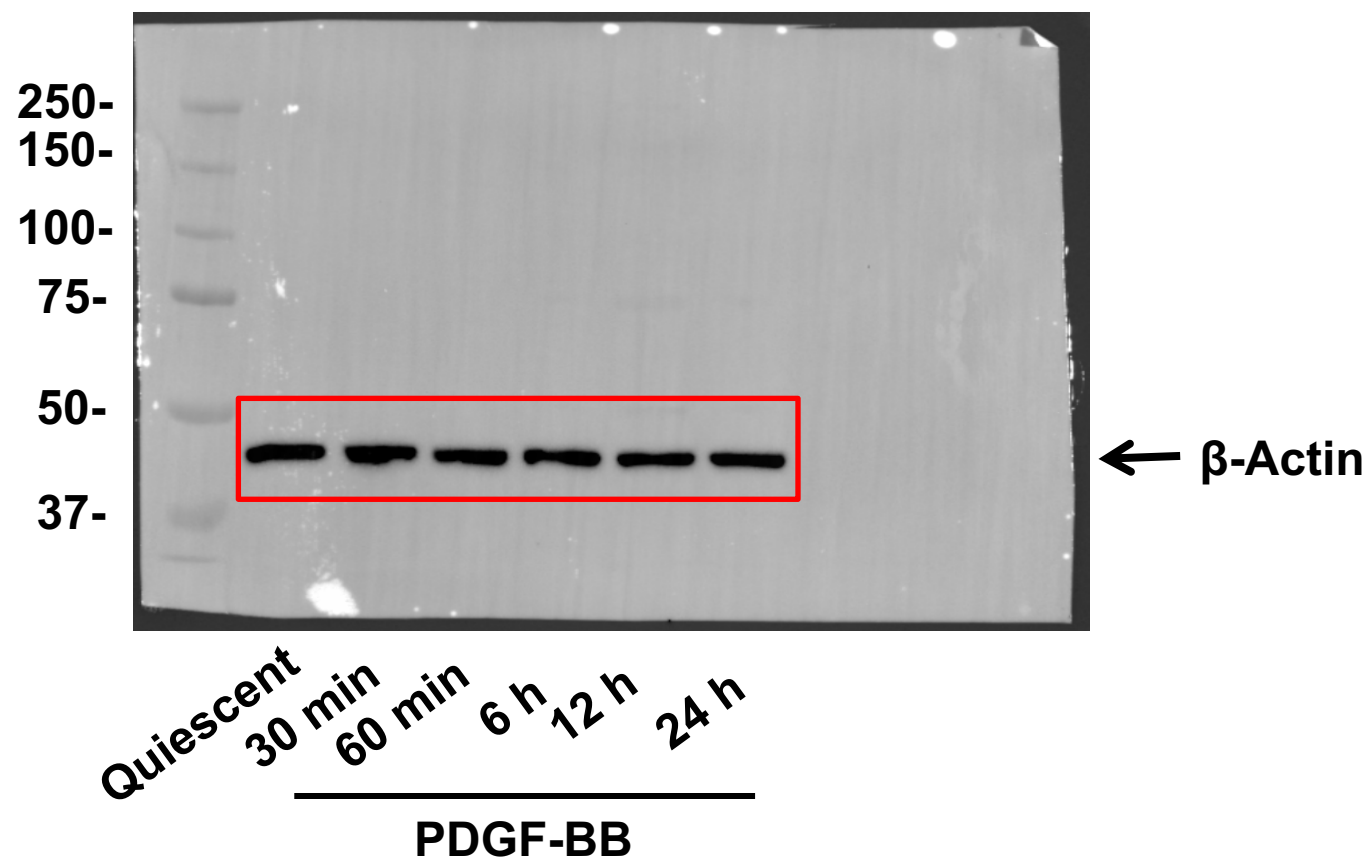
Full unedited gel for Figure 2B- GAPDH



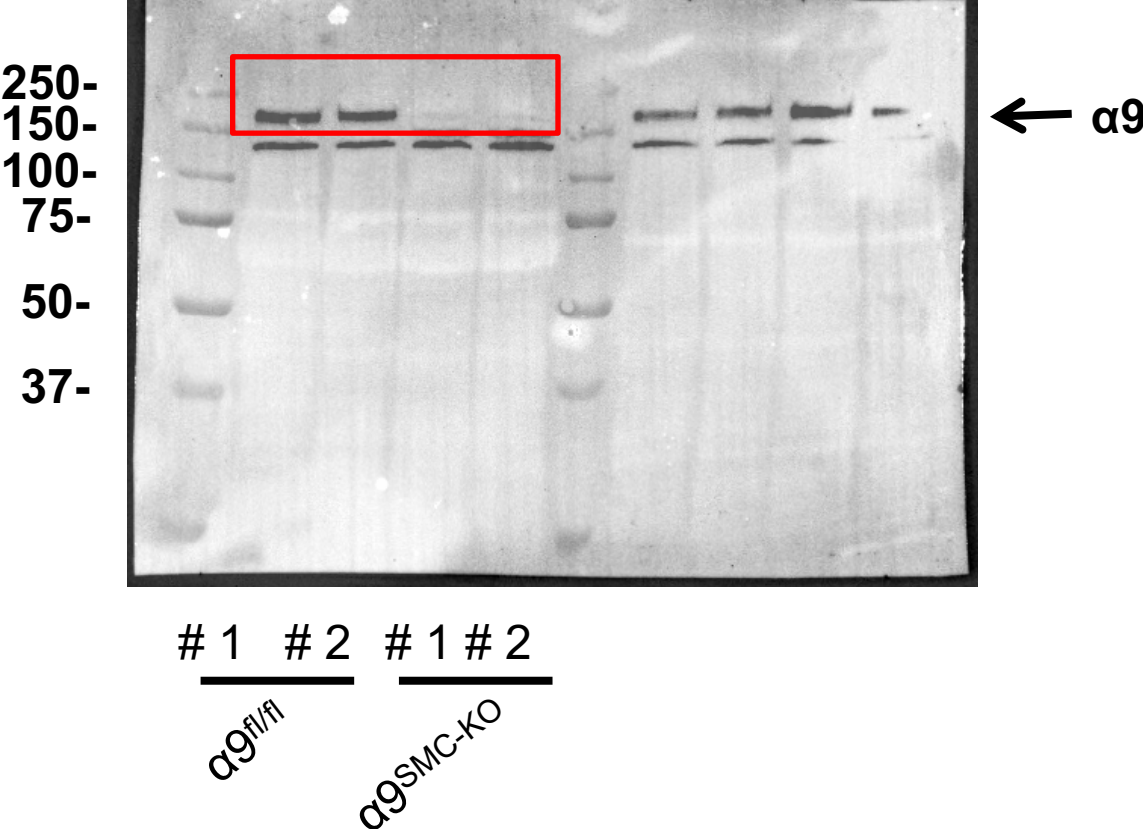
Full unedited gel for Figure 3A- Integrin $\alpha 9$



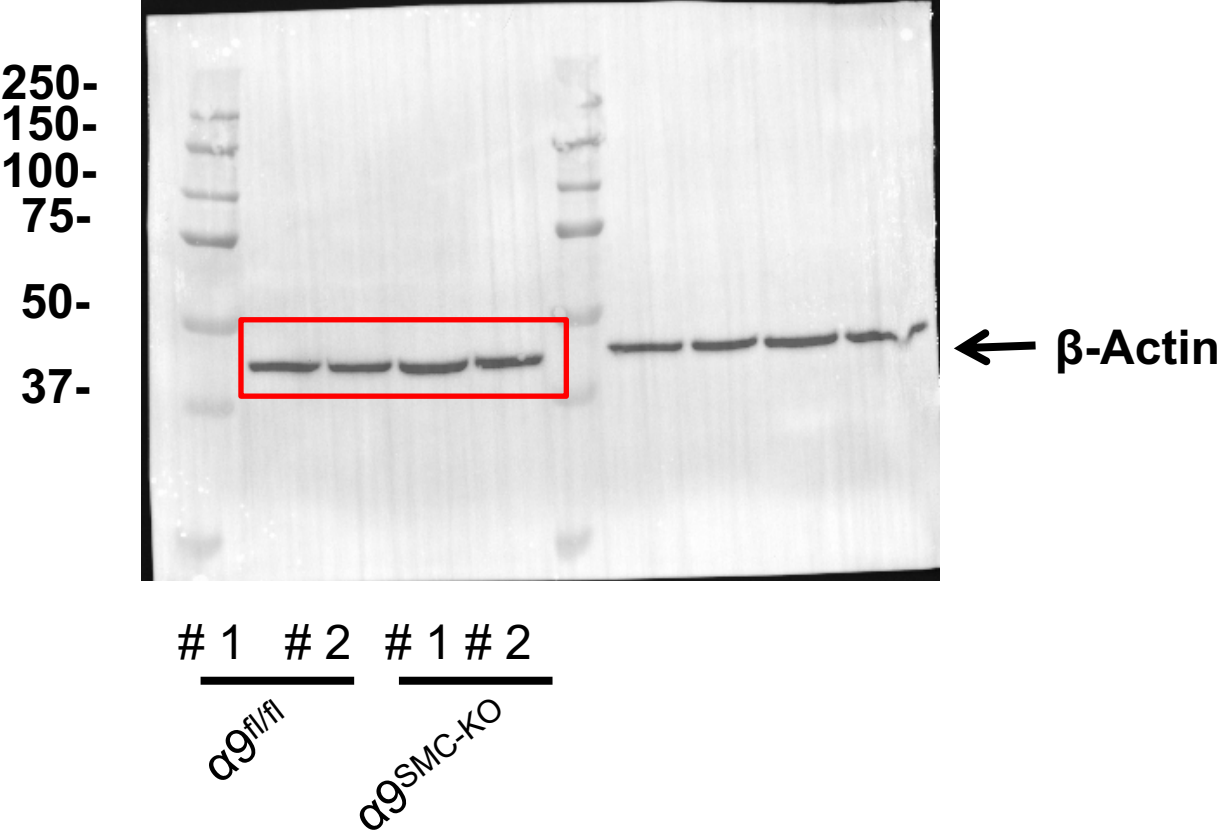
Full unedited gel for Figure 3A- β -Actin



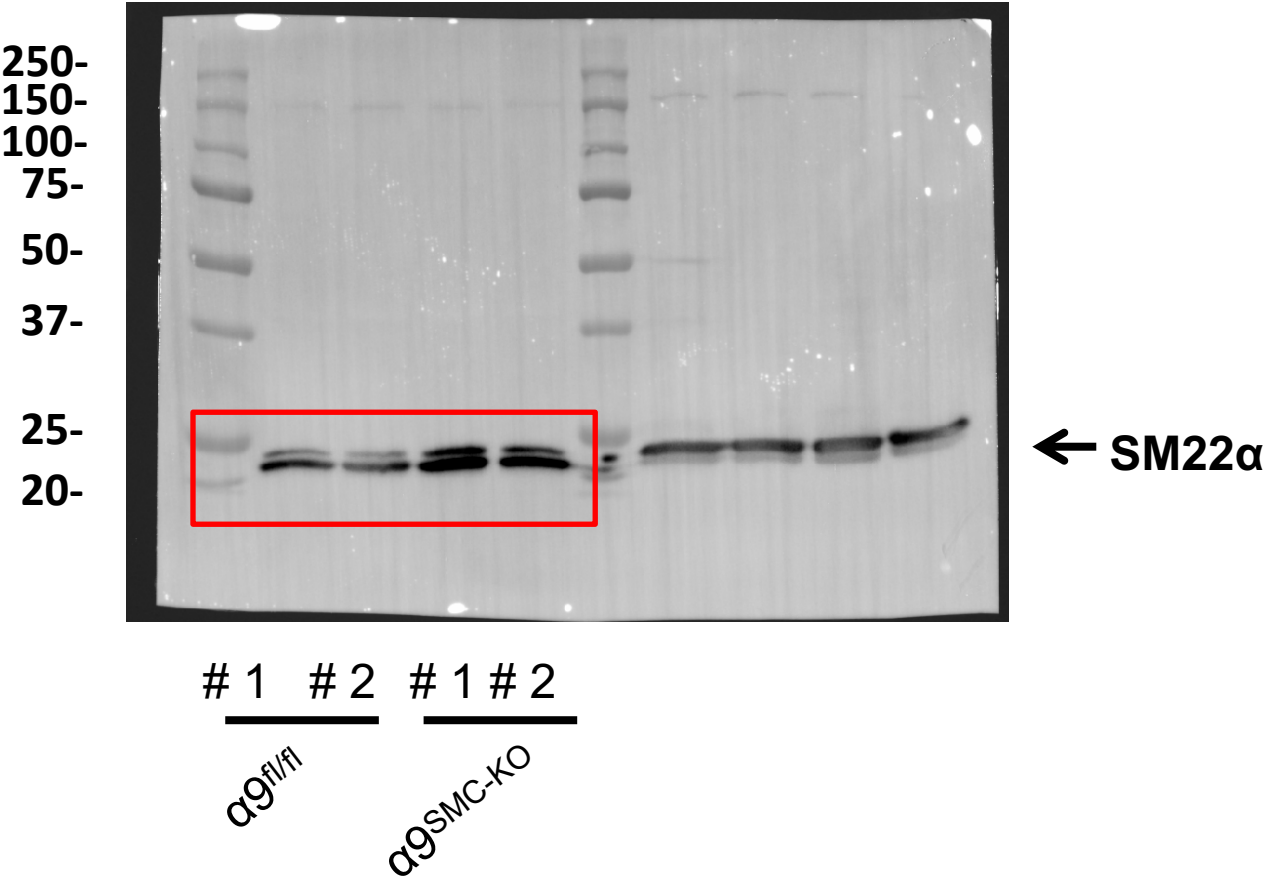
Full unedited gel for Figure 3C- Integrin $\alpha 9$



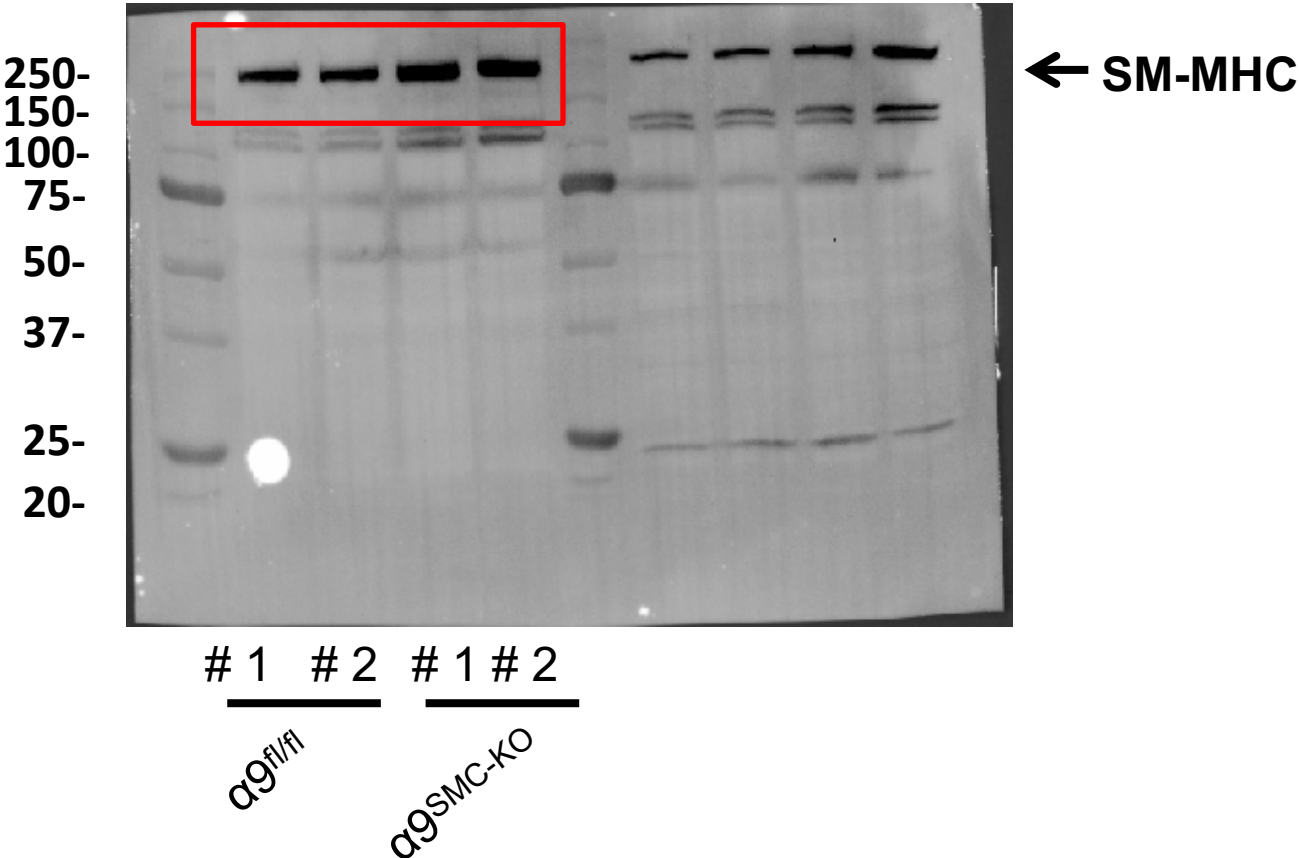
Full unedited gel for Figure 3C- β -Actin



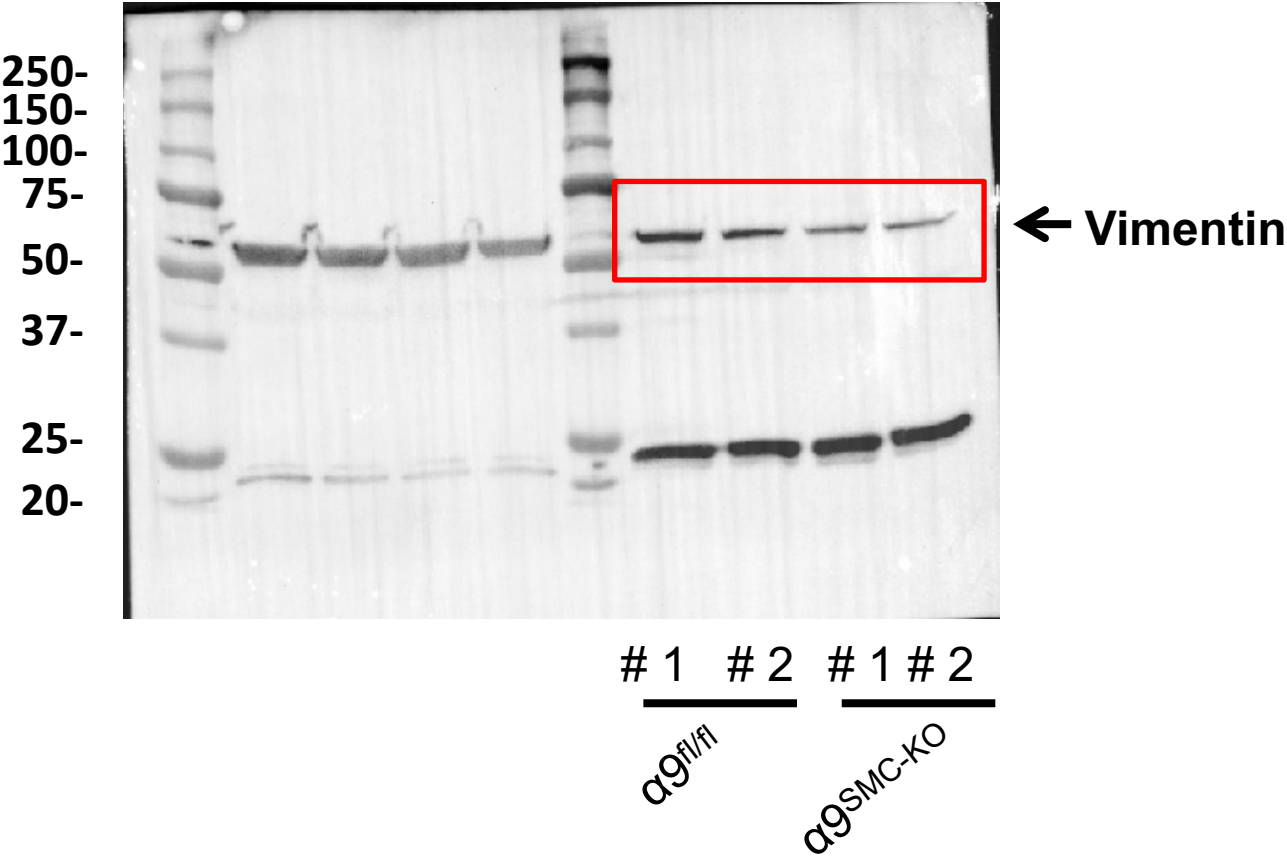
Full unedited gel for Figure 4B-SM22α



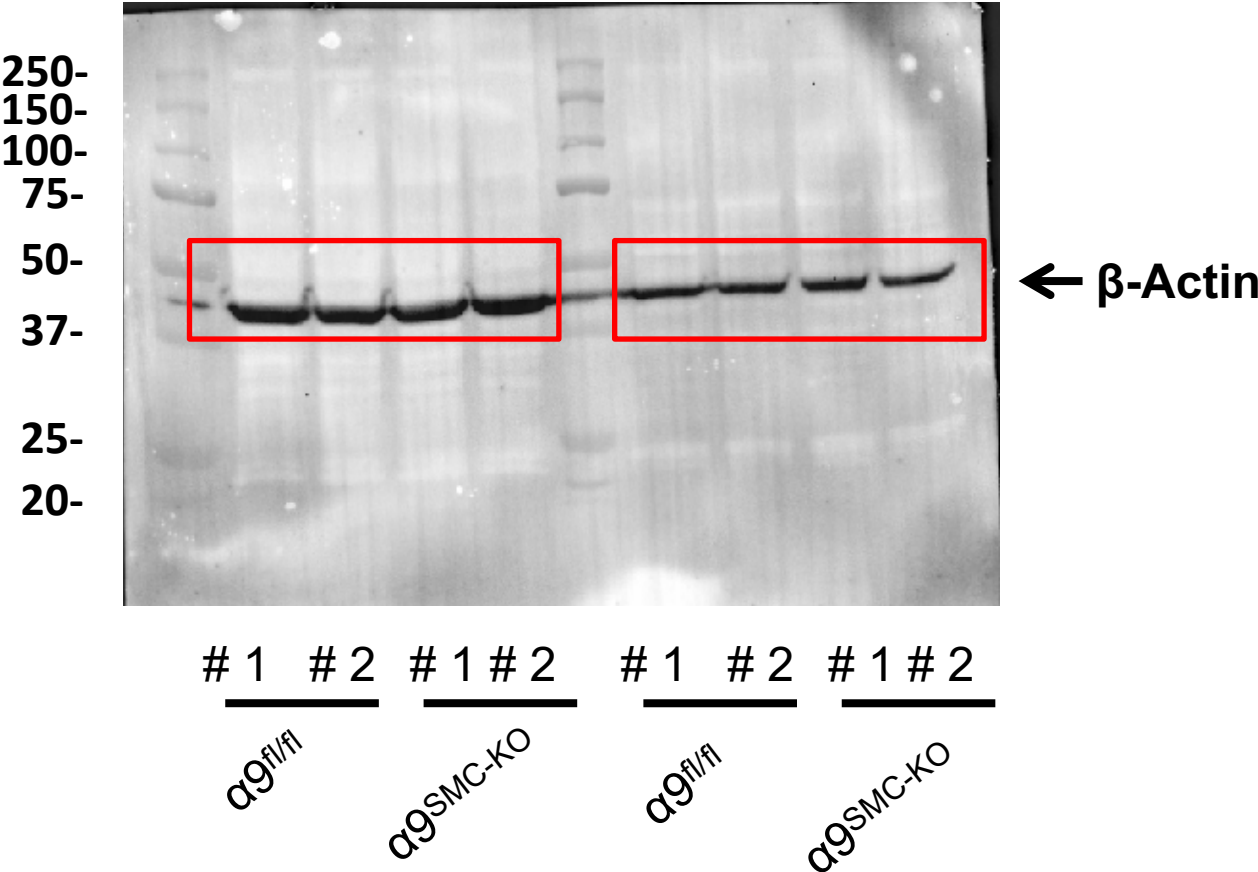
Full unedited gel for Figure 4B-SM-MHC



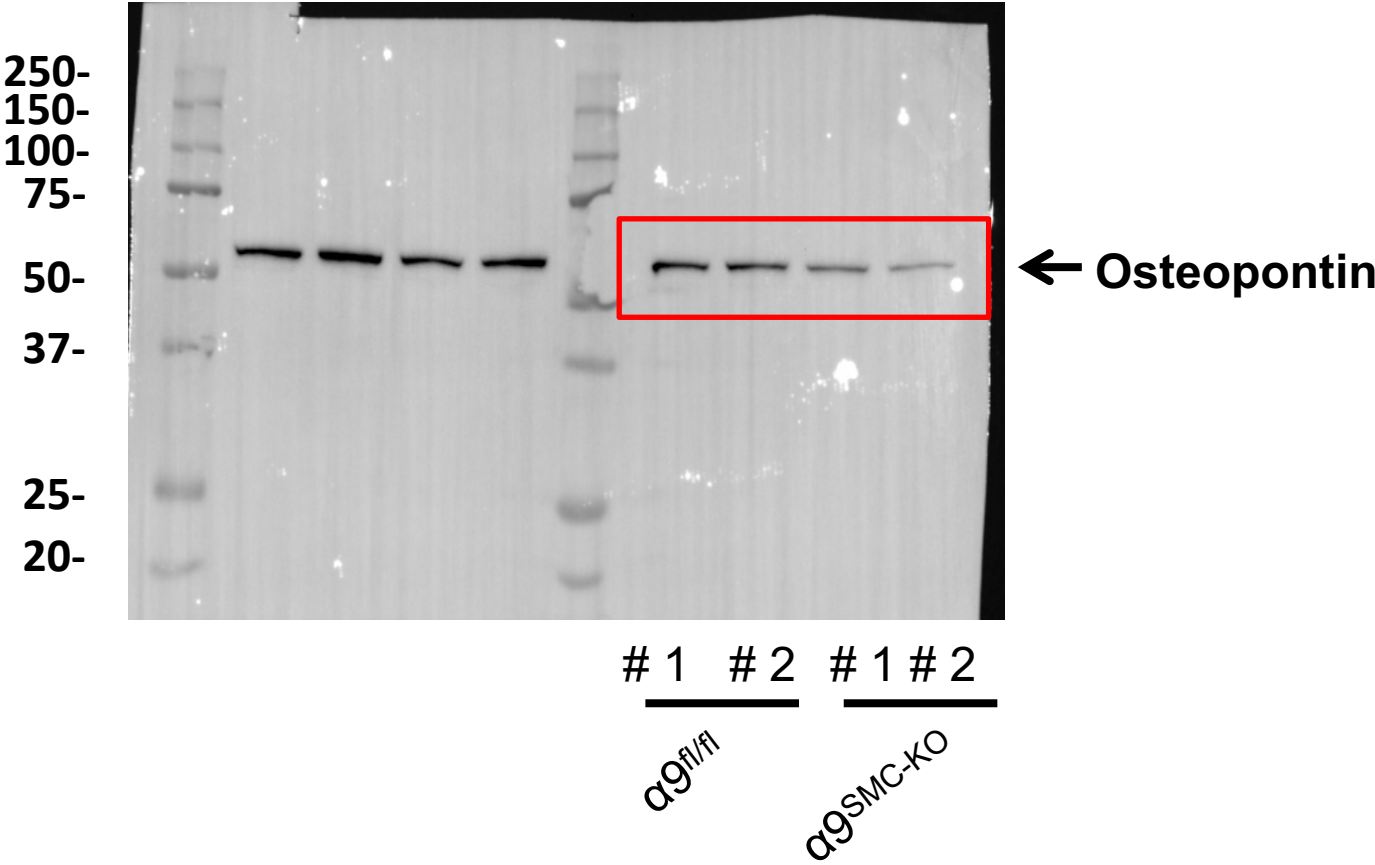
Full unedited gel for Figure 4B-Vimentin



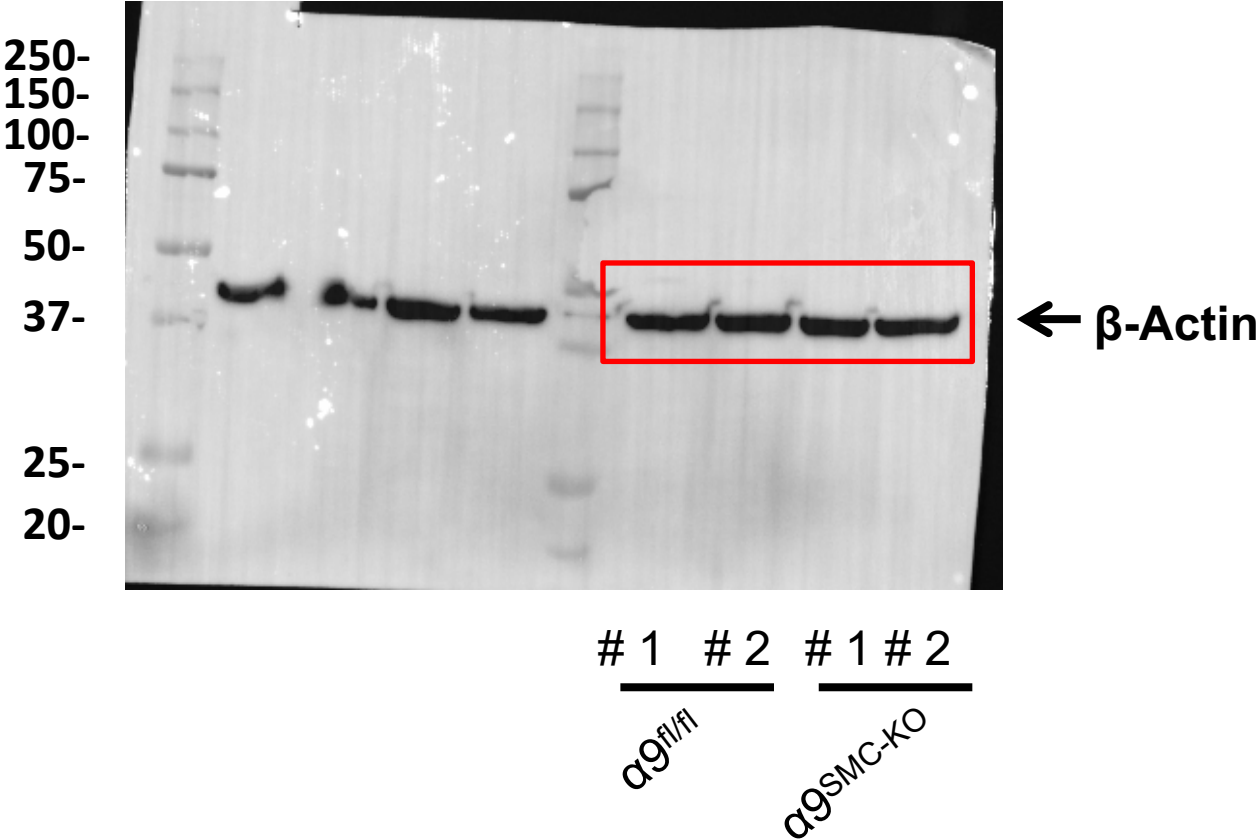
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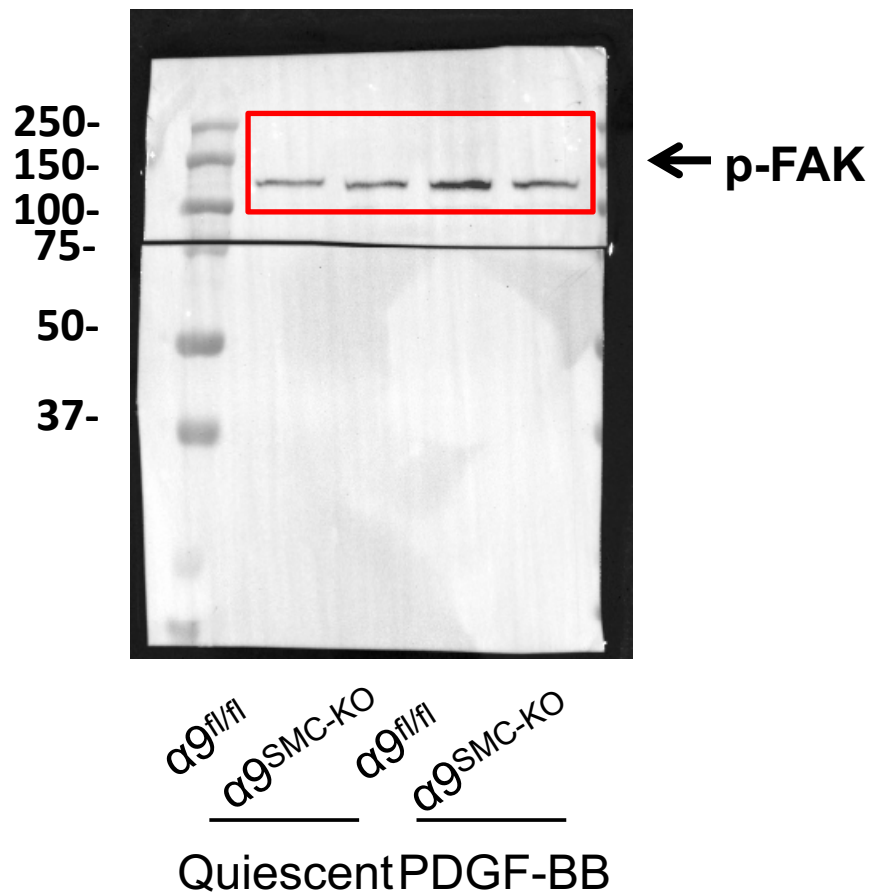
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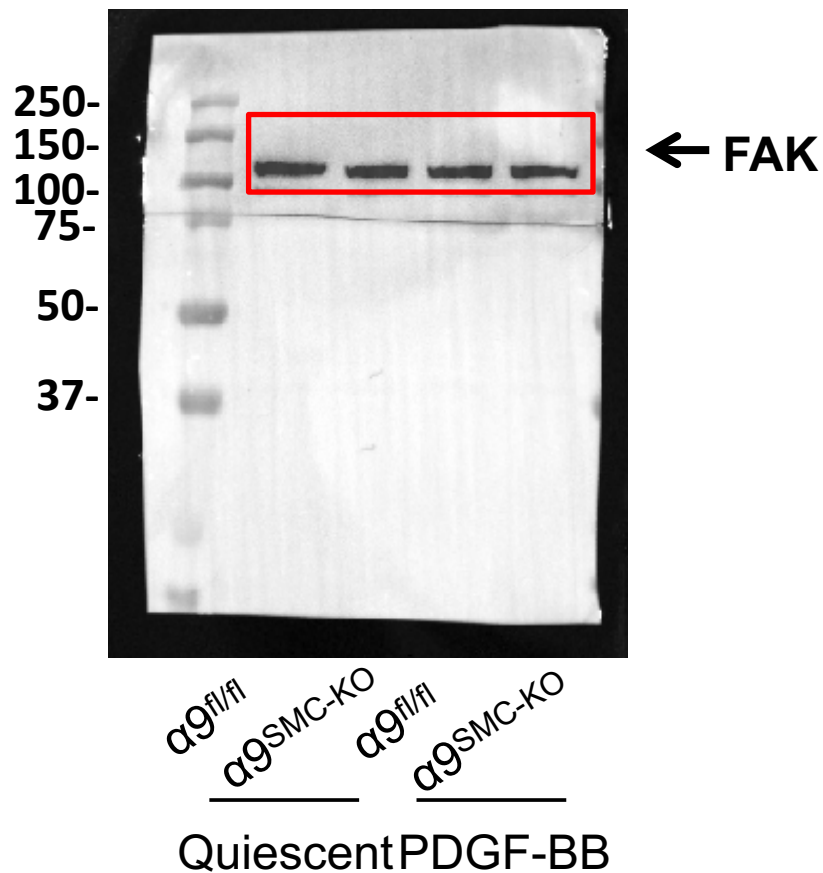
Full unedited gel for Figure 4B-β-Actin



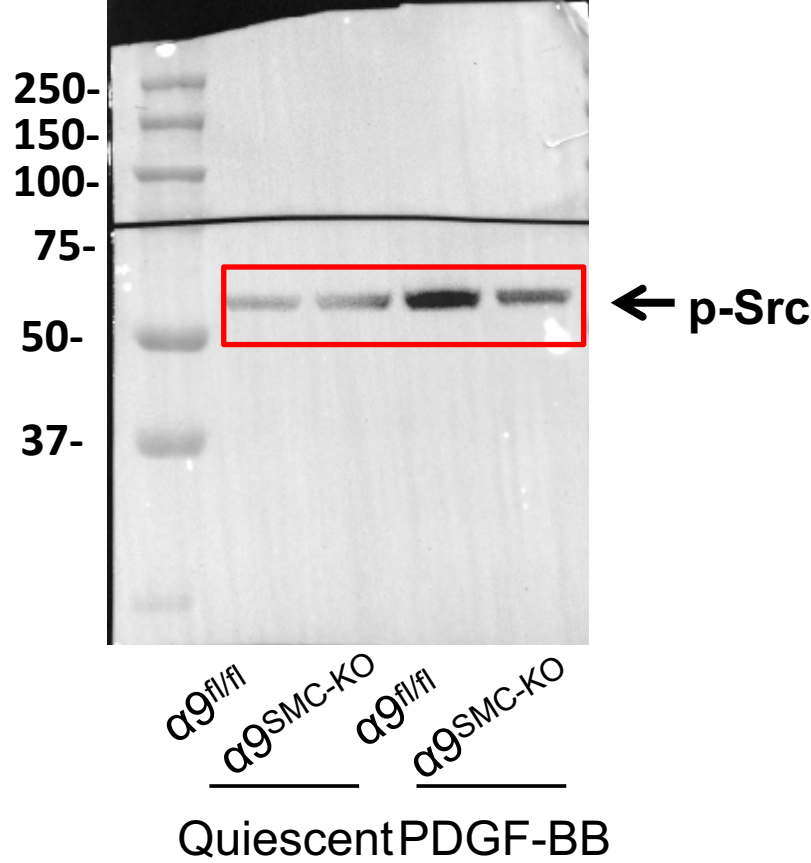
Full unedited gel for Figure 5A-p-FAK



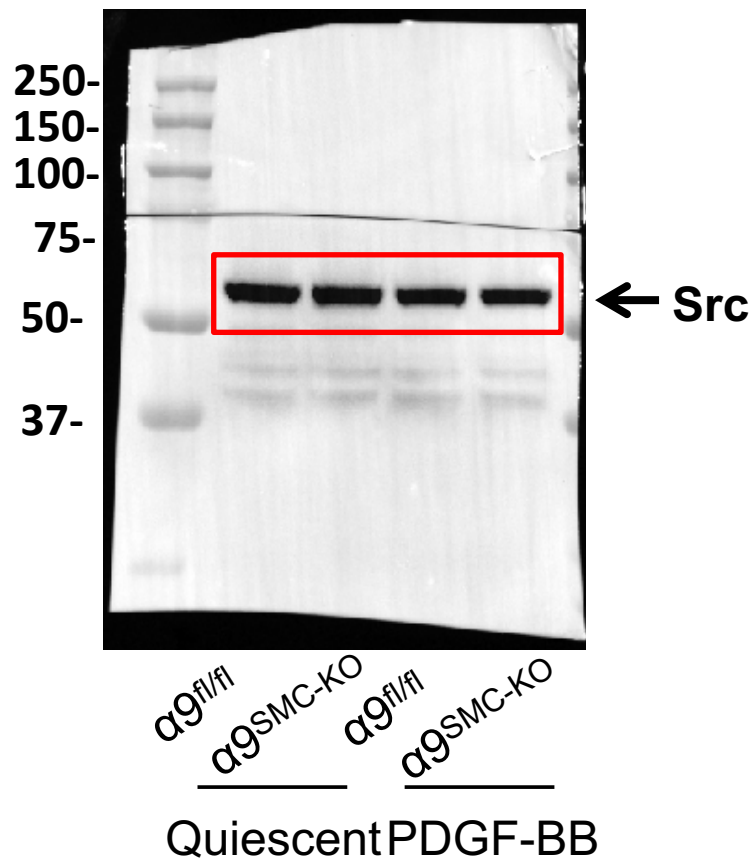
Full unedited gel for Figure 5A-FAK



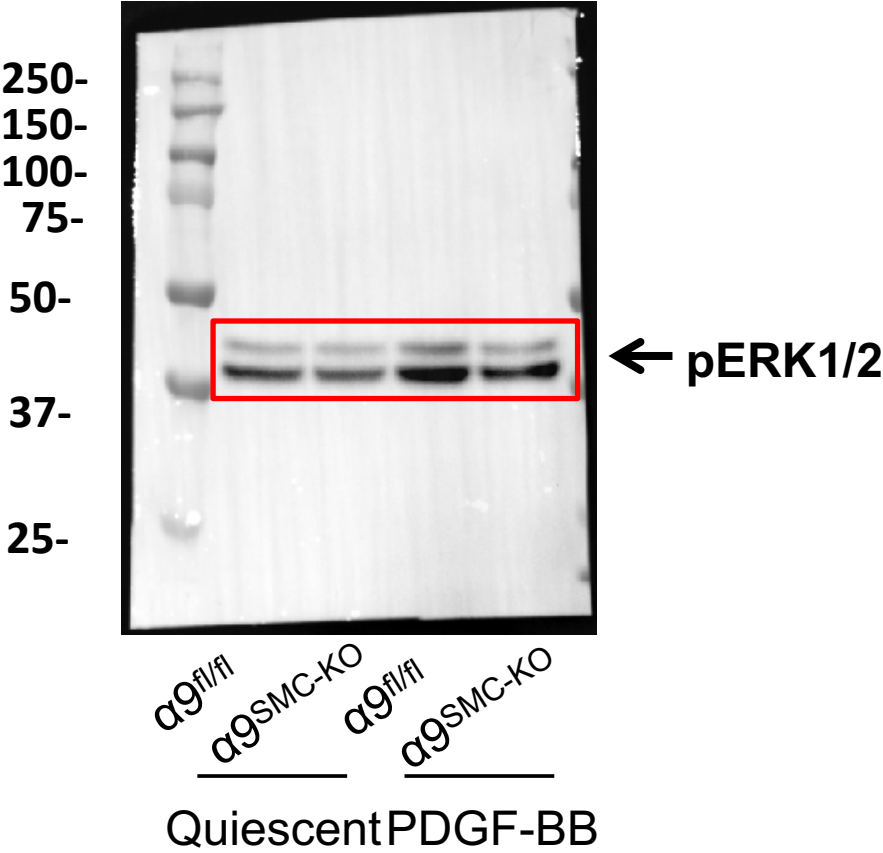
Full unedited gel for Figure 5A-p-Src



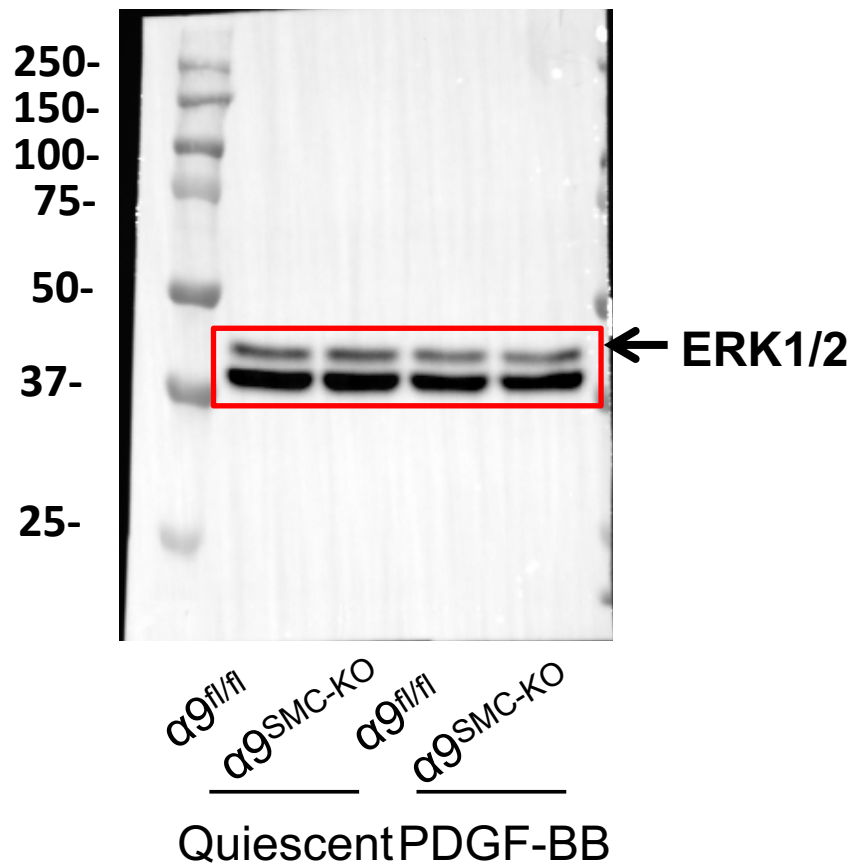
Full unedited gel for Figure 5A-Src



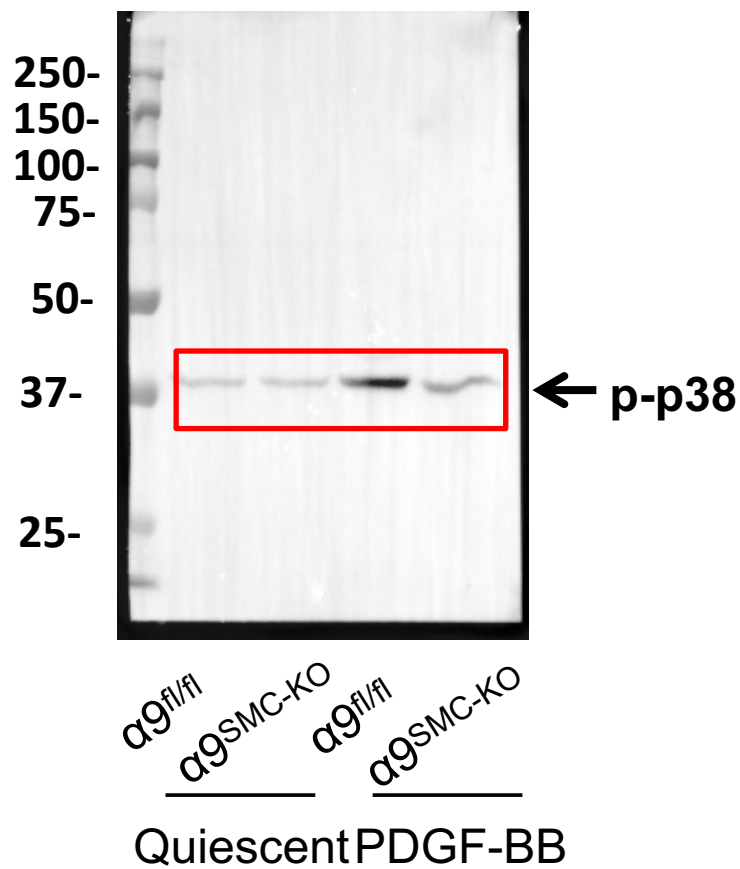
Full unedited gel for Figure 5A-pERK1/2



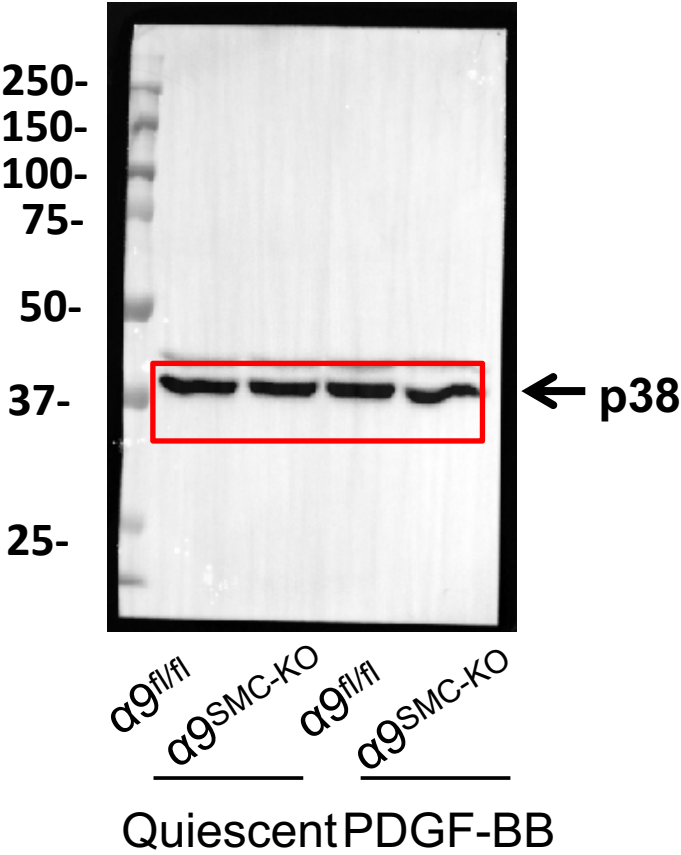
Full unedited gel for Figure 5A-ERK1/2



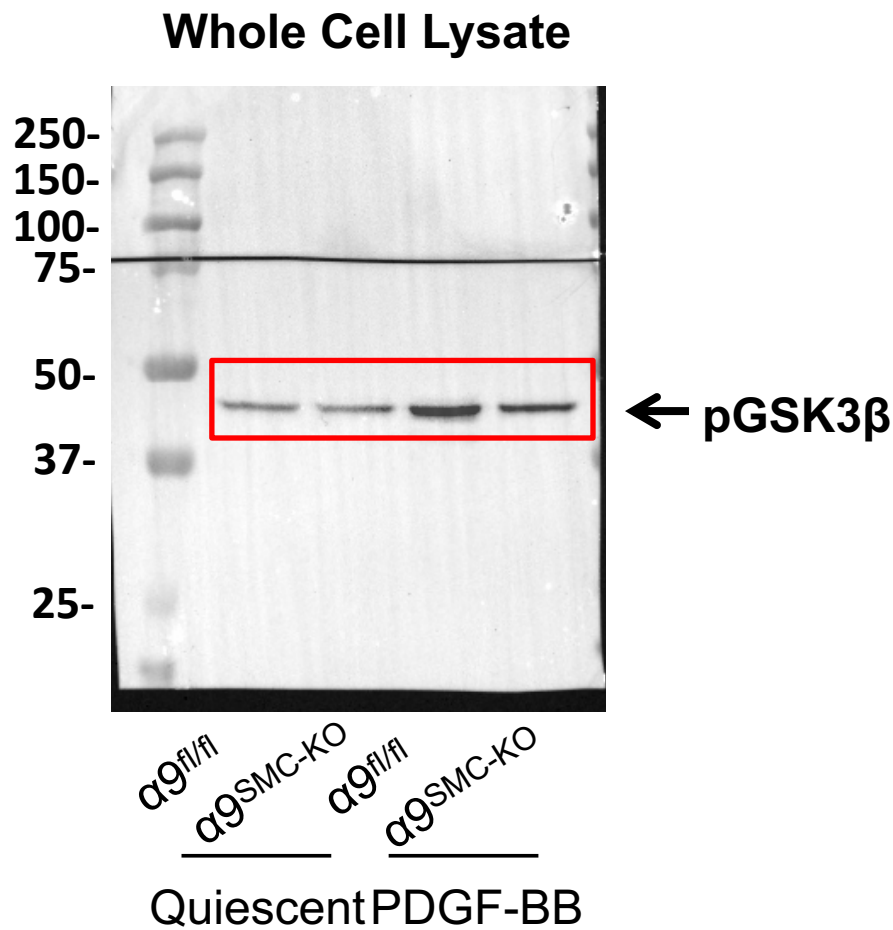
Full unedited gel for Figure 5A-p-p38



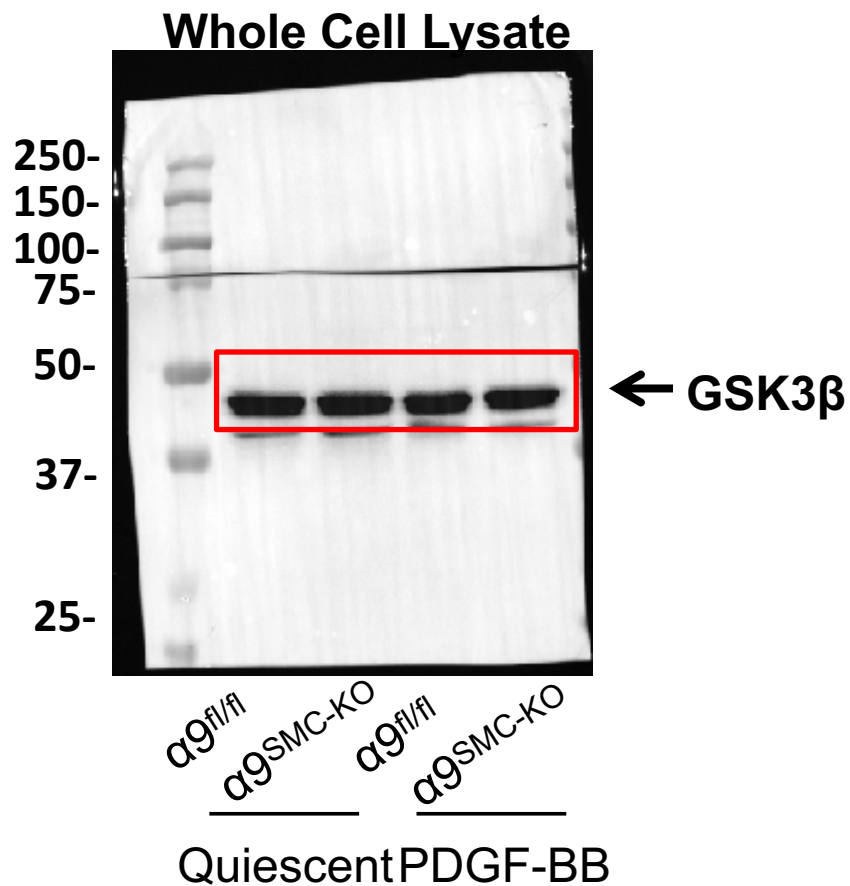
Full unedited gel for Figure 5A-p38



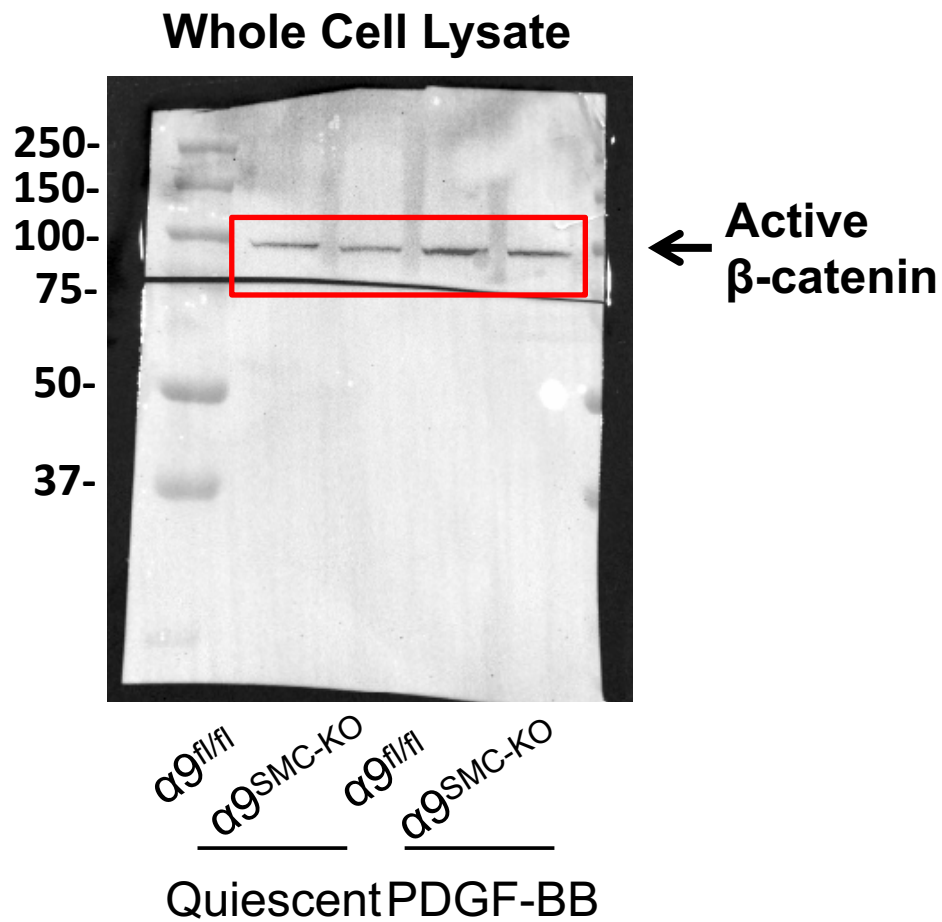
Full unedited gel for Figure 5B-pGSK3β



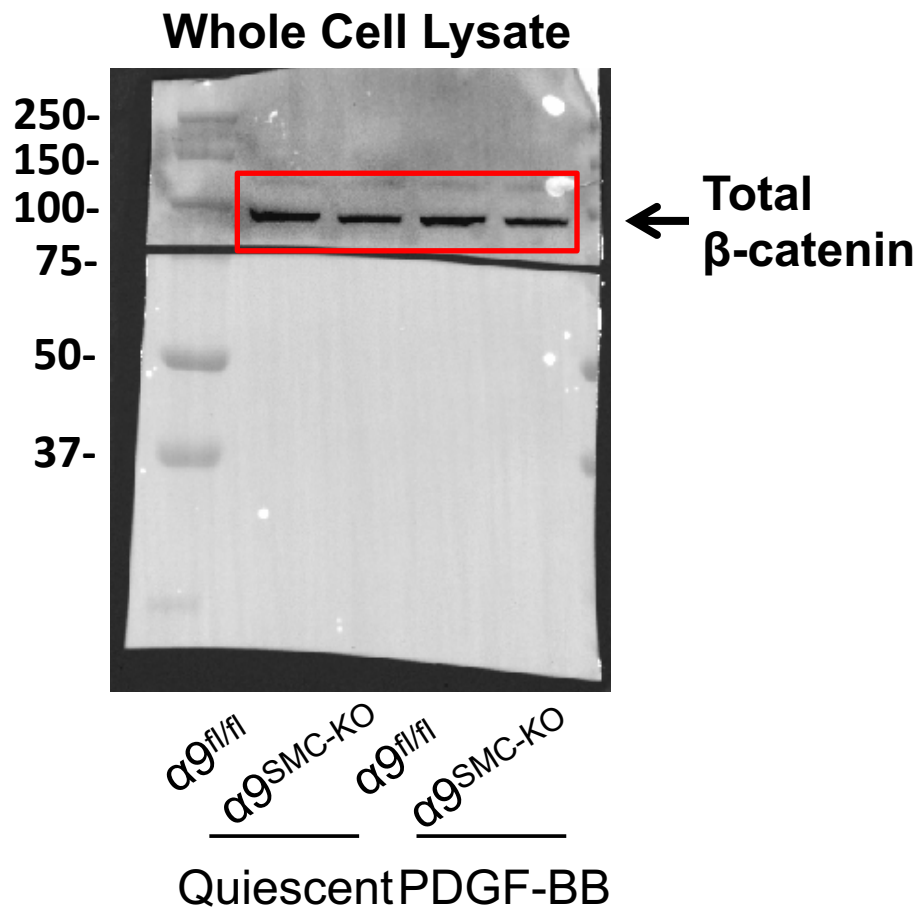
Full unedited gel for Figure 5B-GSK3β



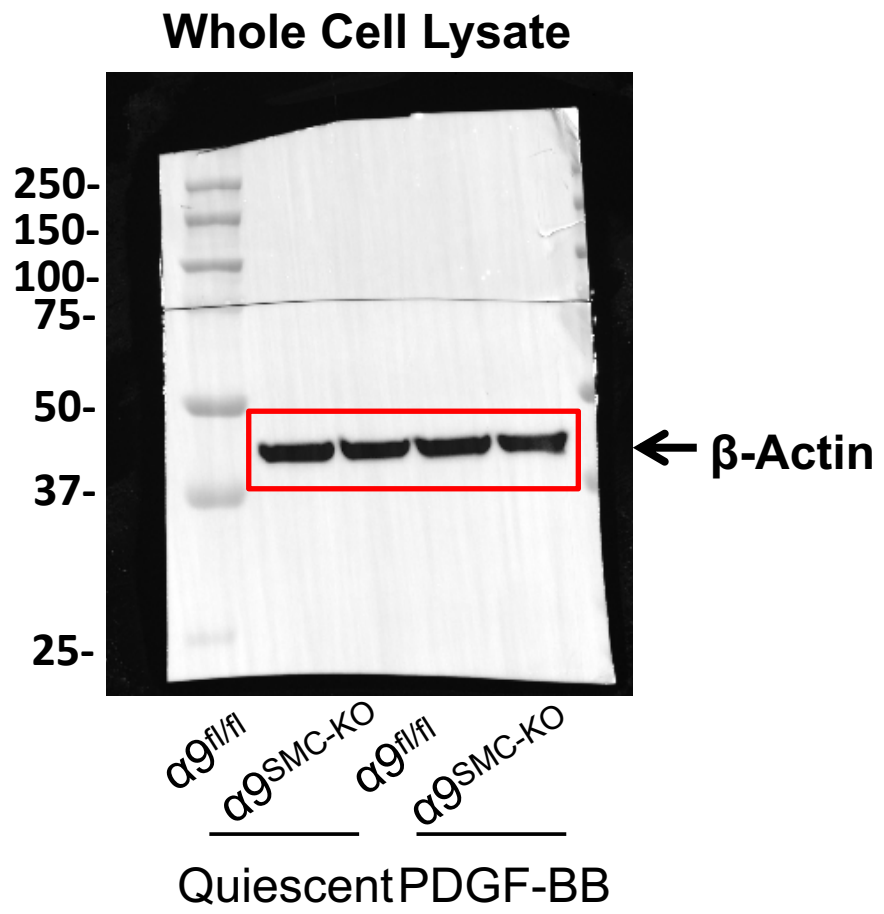
Full unedited gel for Figure 5B-Active β -catenin



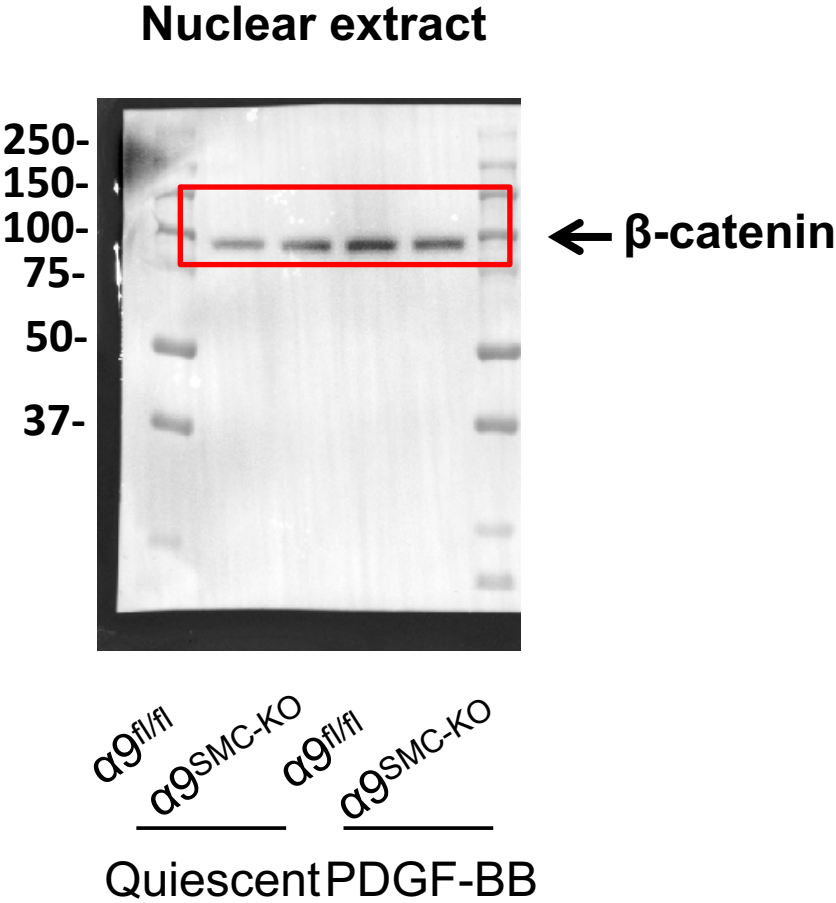
Full unedited gel for Figure 5B-Total β -catenin



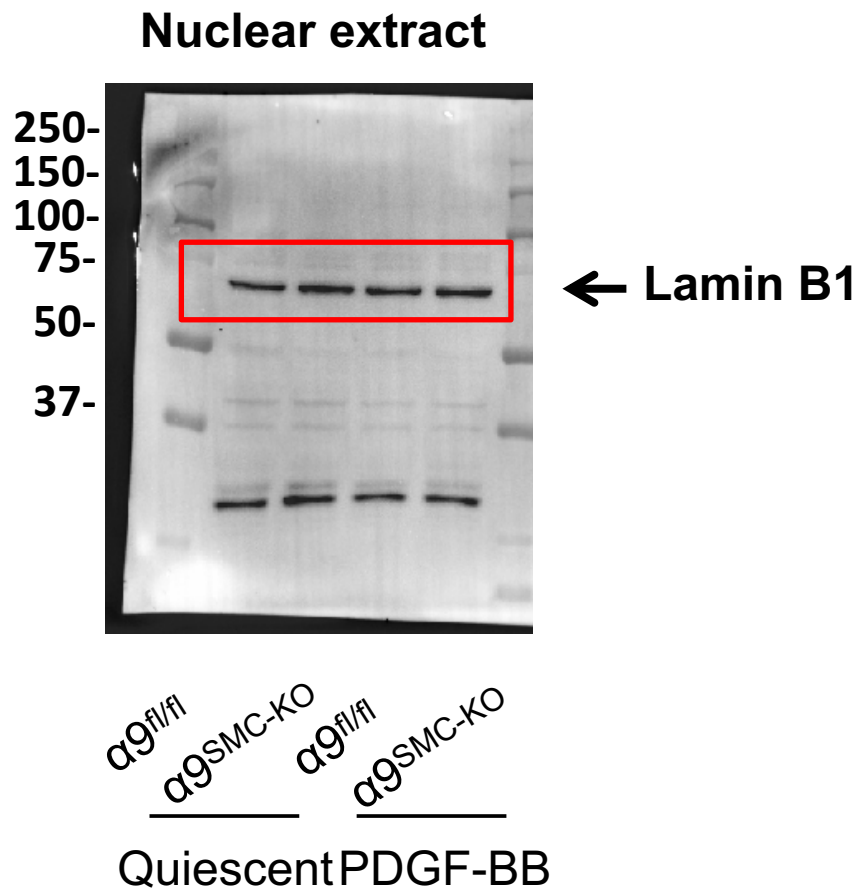
Full unedited gel for Figure 5B-β-Actin



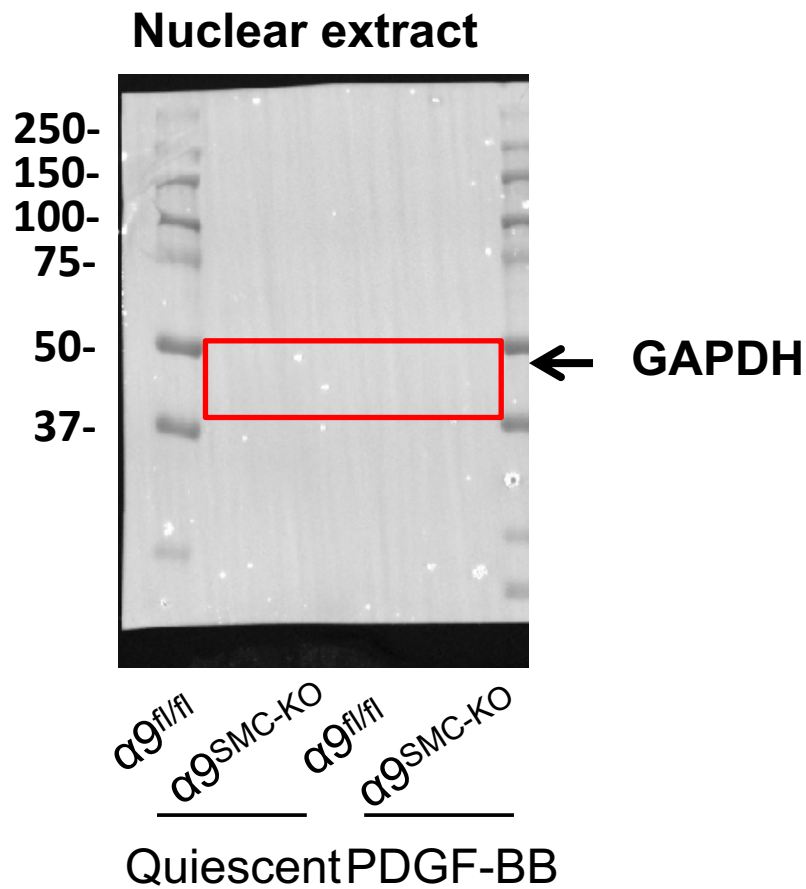
Full unedited gel for Figure 5C-β-catenin



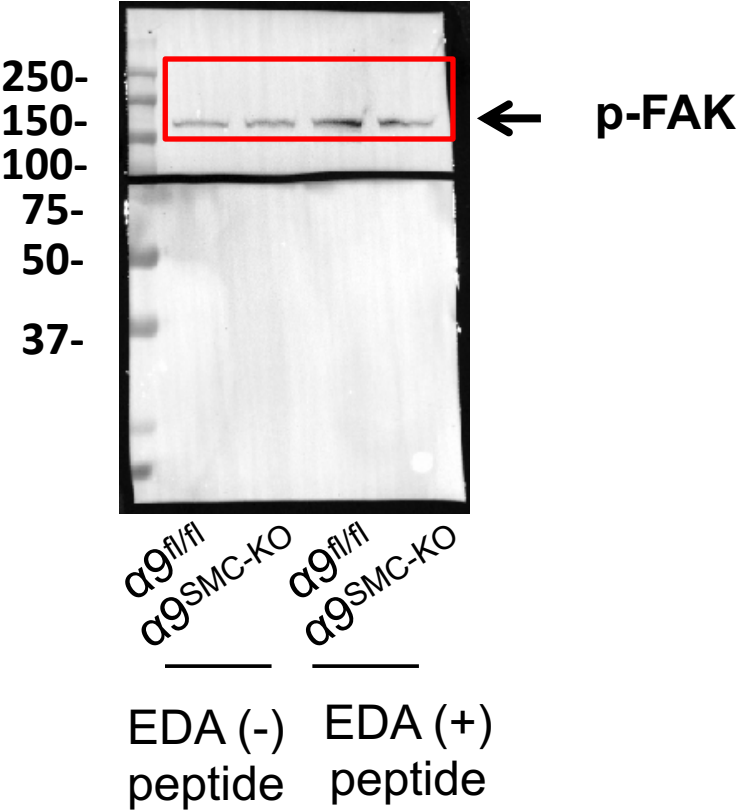
Full unedited gel for Figure 5C-Lamin B1



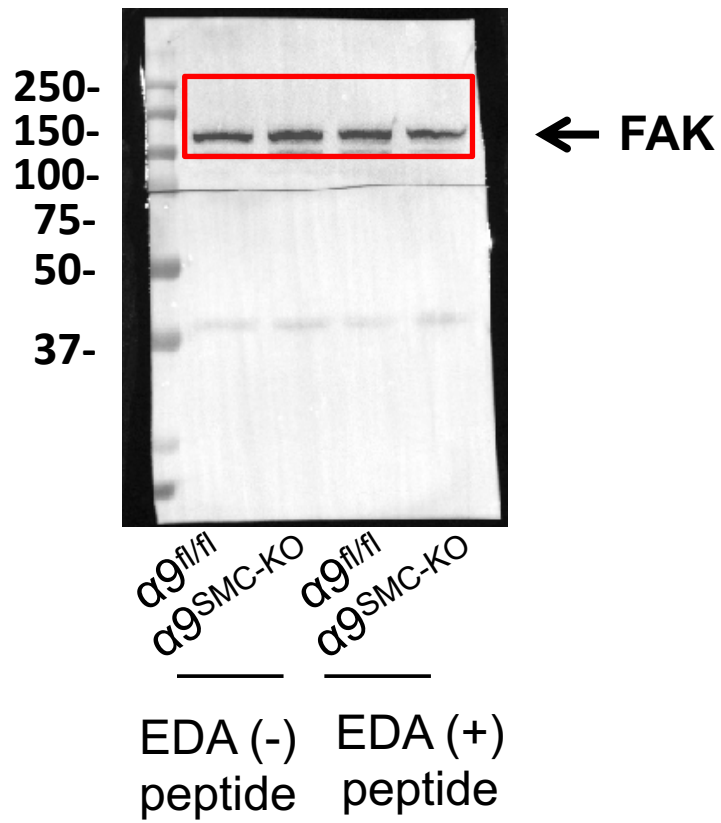
Full unedited gel for Figure 5C-GAPDH



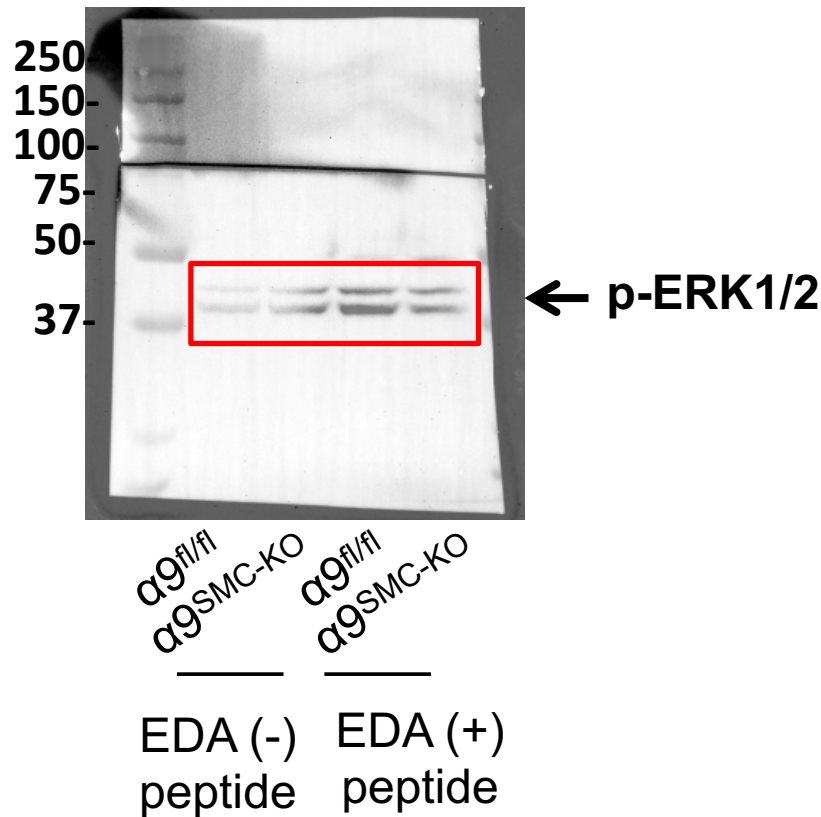
Full unedited gel for Figure 6C- p-FAK



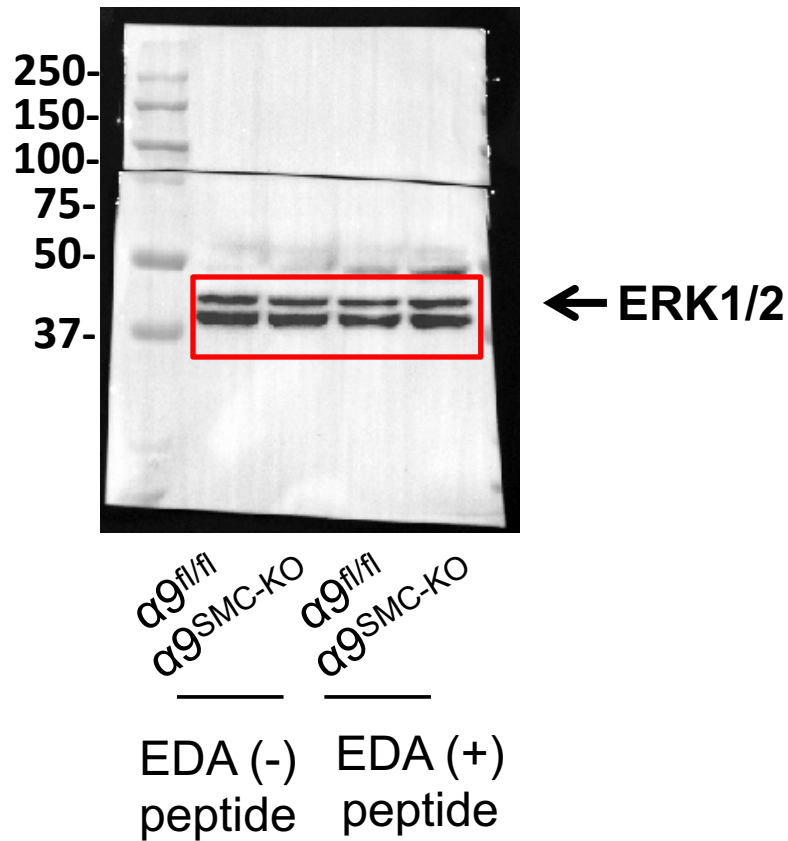
Full unedited gel for Figure 6C- FAK



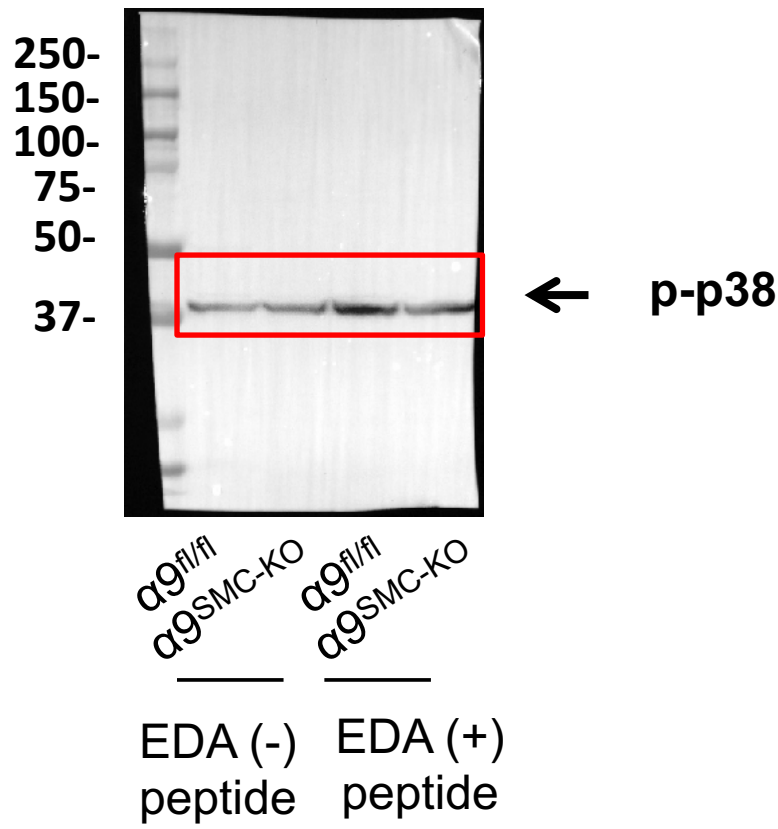
Full unedited gel for Figure 6C- p-ERK1/2



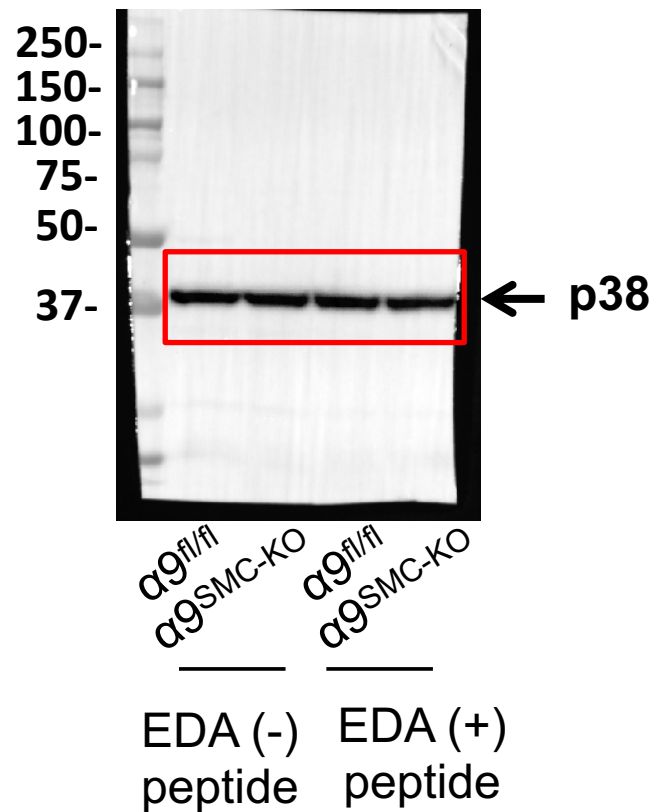
Full unedited gel for Figure 6C- ERK1/2



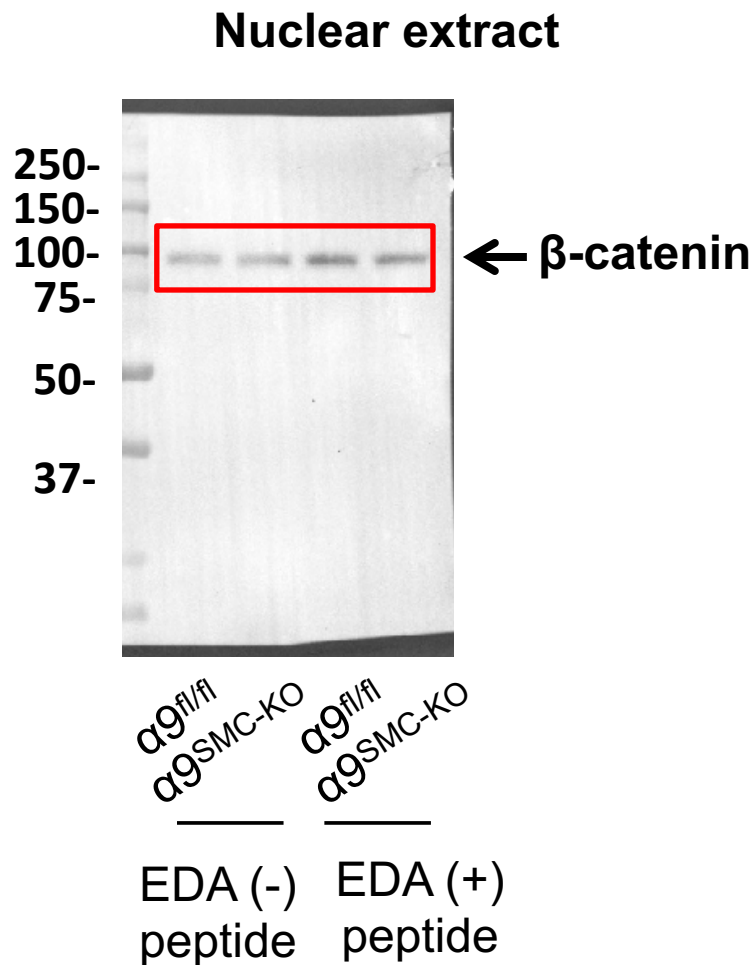
Full unedited gel for Figure 6C- p-p38



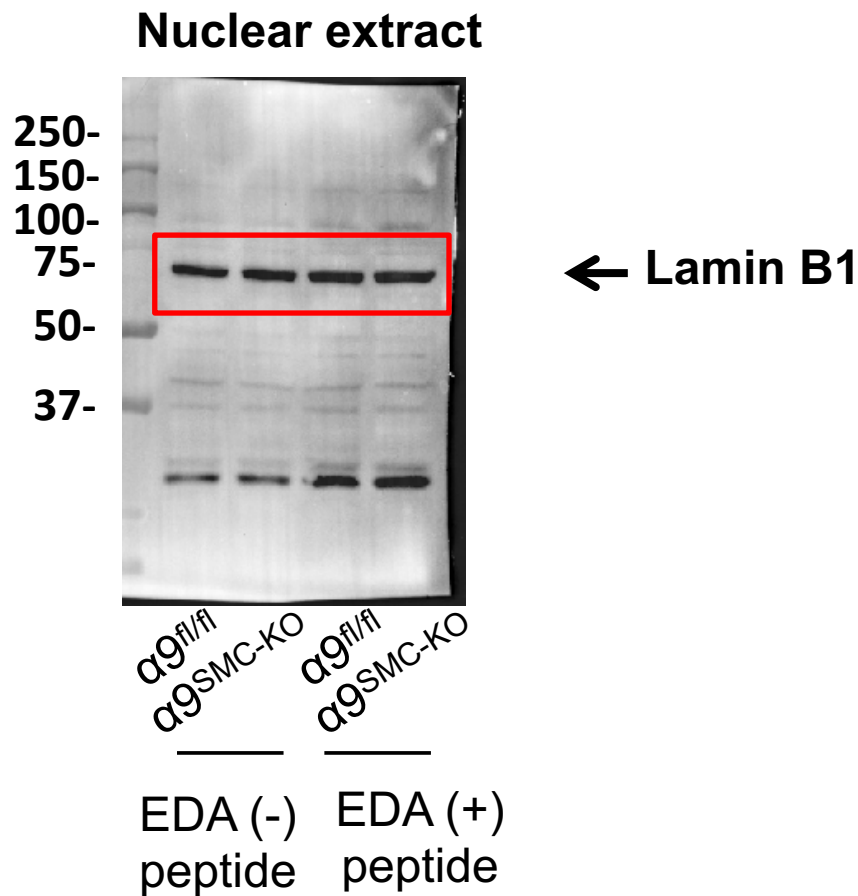
Full unedited gel for Figure 6C- p38



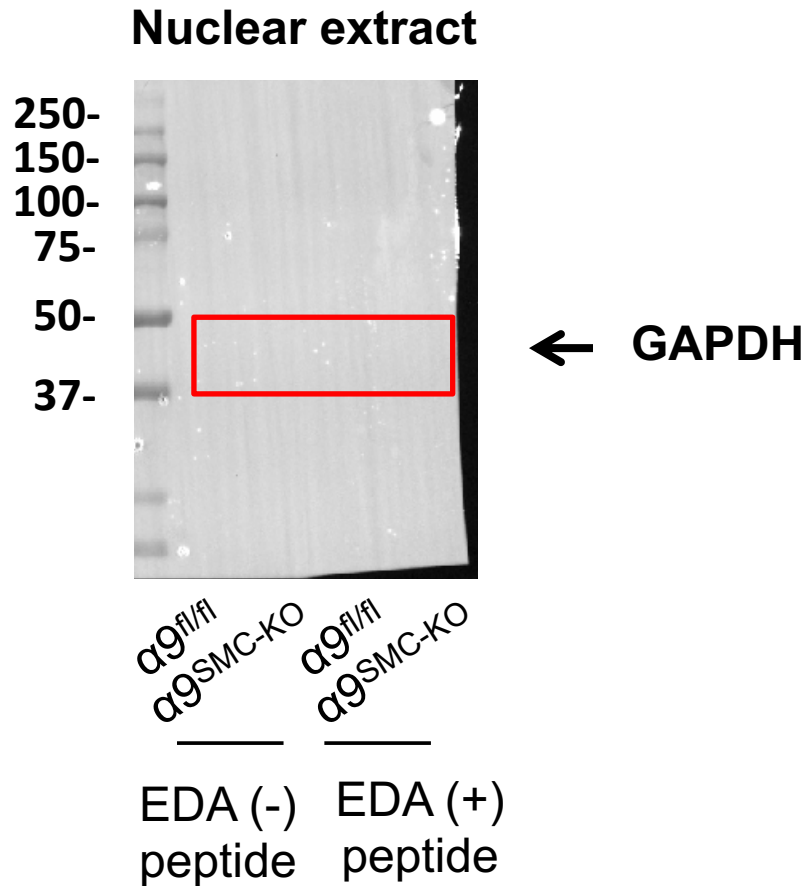
Full unedited gel for Figure 6C- β -catenin



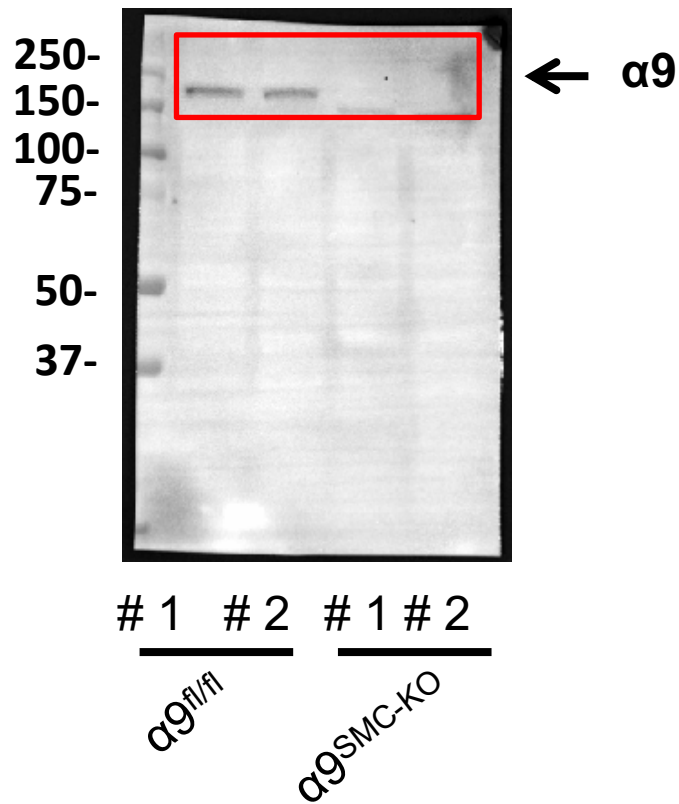
Full unedited gel for Figure 6C- Lamin B1



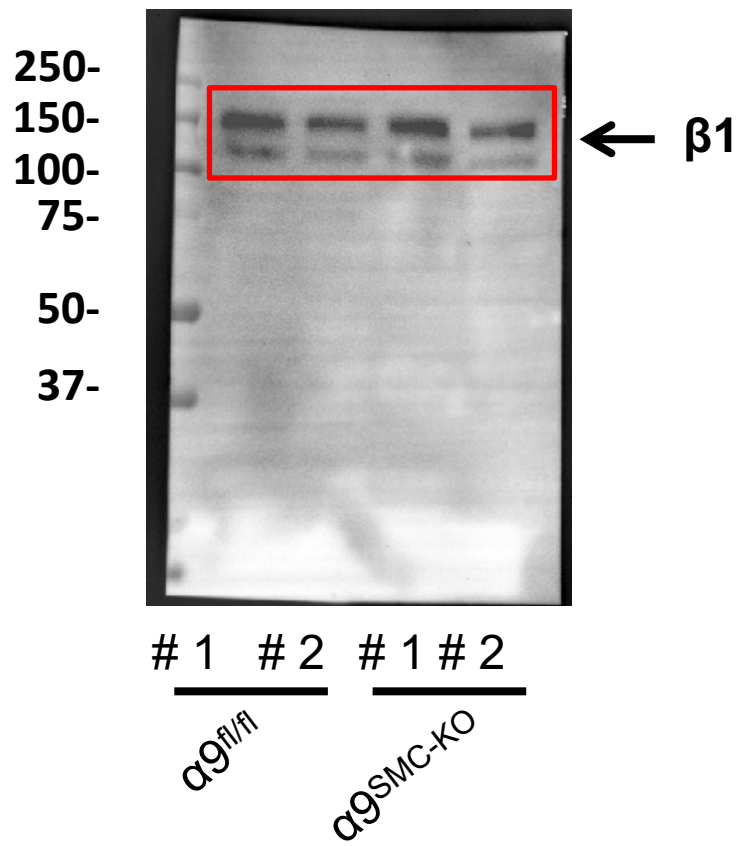
Full unedited gel for Figure 6C- GAPDH



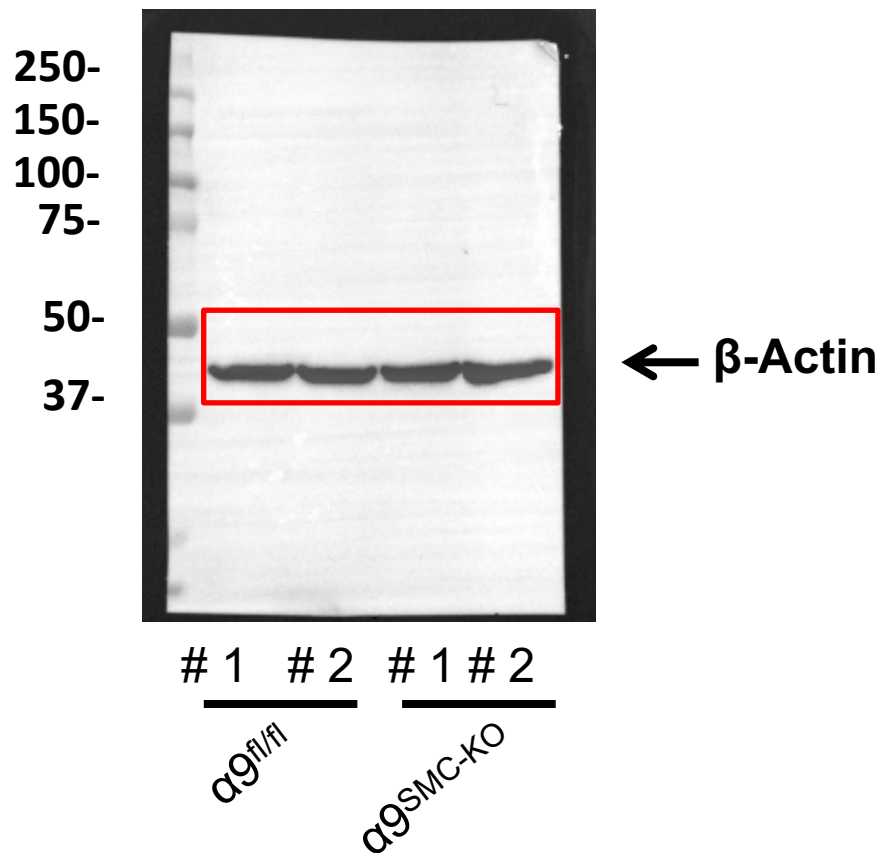
Full unedited gel for Supplementary Figure 3 –Integrin $\alpha 9$



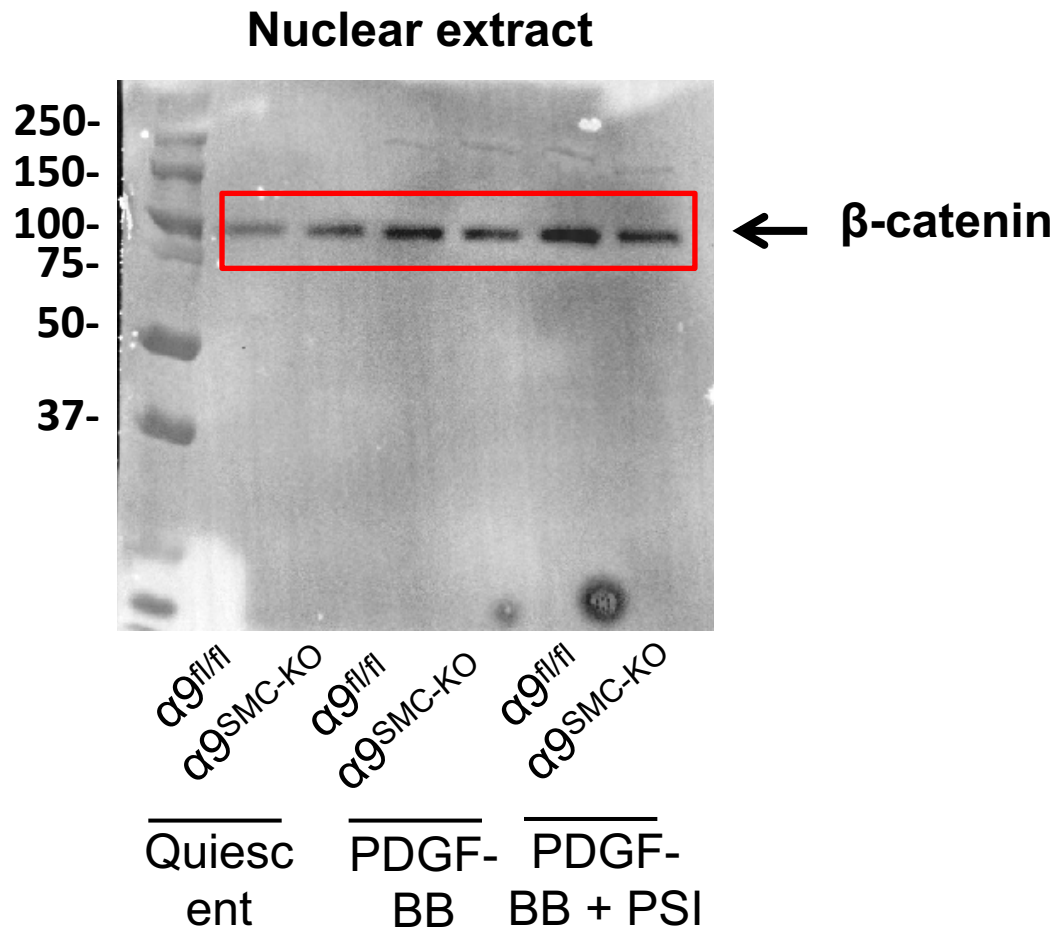
Full unedited gel for Supplementary Figure 3- Integrin $\beta 1$



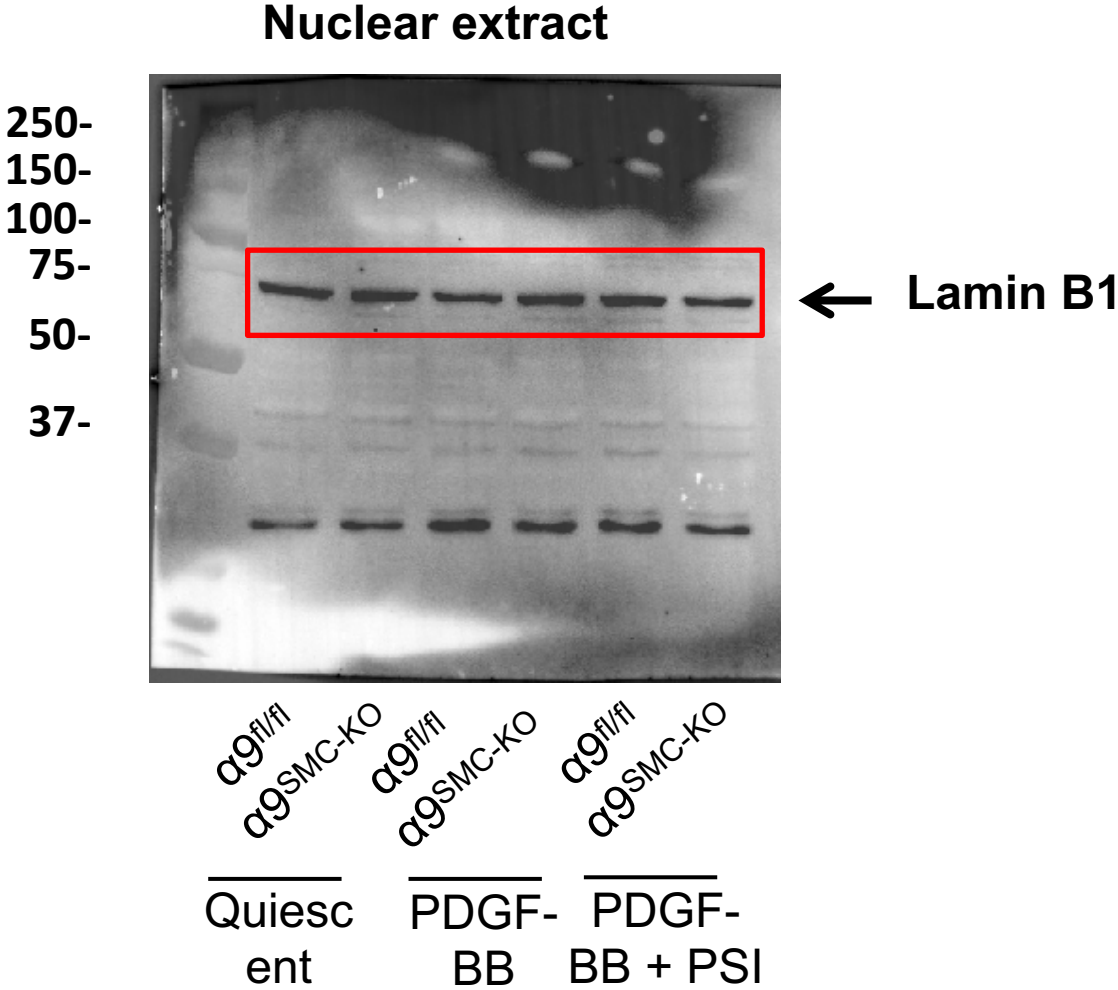
Full unedited gel for Supplementary Figure 3- β -Actin



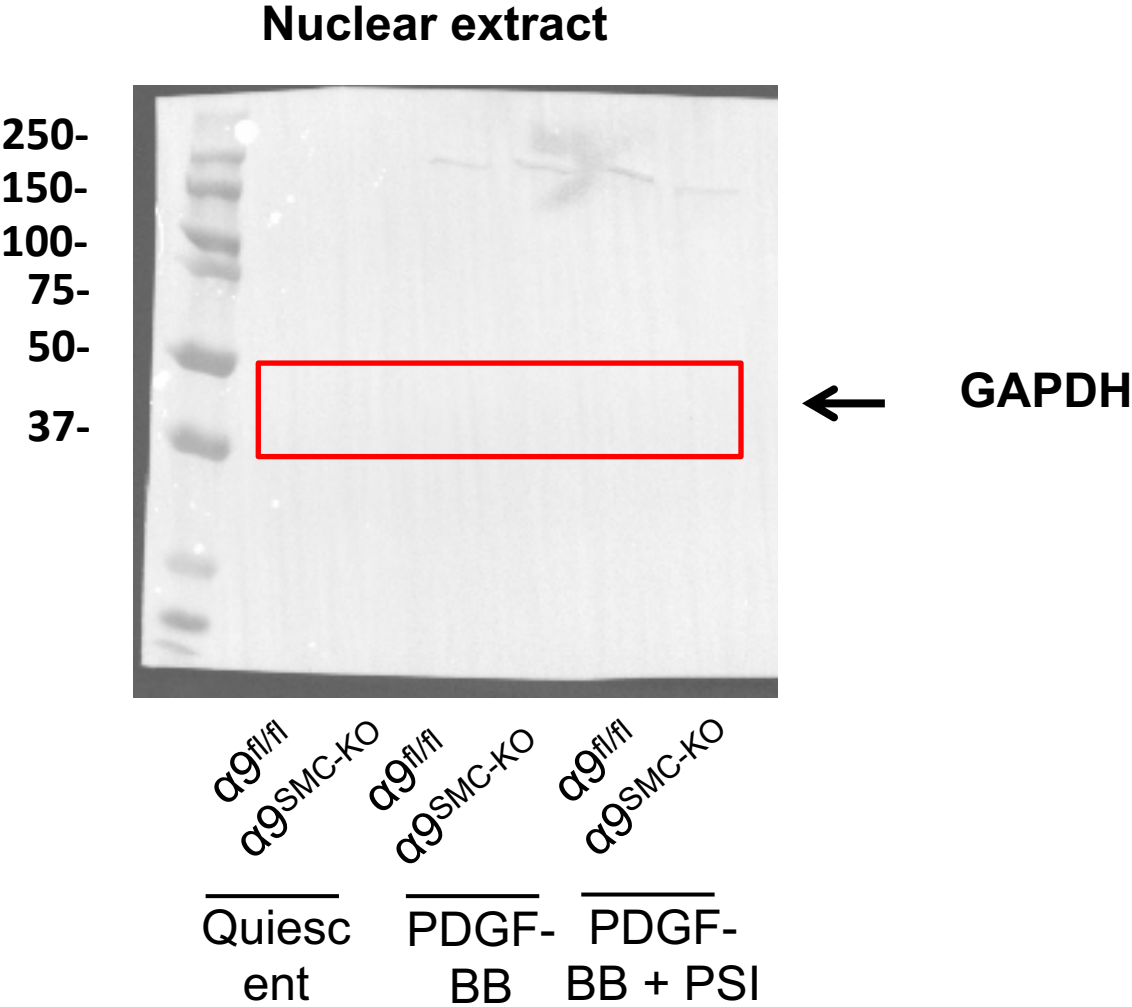
Full unedited gel for Supplementary Figure 6- β -catenin



Full unedited gel for Supplementary Figure 6 -Lamin B1



Full unedited gel for Supplementary Figure 6 -GAPDH



Full unedited gel for Supplementary Figure 6- β -Actin

