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Article

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Peptostreptococcus anaerobius mediates anti-PD1 therapy resistance and exacerbates colorectal cancer via myeloid-derived suppressor cells in mice

In the format provided by the authors and unedited

Supplementary Table 1 Antibodies used for Western-blot, Immunofluorescence staining and Flow cytometry in this Experiments.

Antibodies	Source	Identifier	Dilution
Anti-Integrin alpha 2	abcam	ab133557	1:1000
Anti-Integrin beta 1	abcam	ab179471	1:1000
Anti-NF-κB p65 (D14E12)	cell signaling technology	8242S	1:1000
Anti-Phospho-NF-κB p65 (Ser536)	cell signaling technology	3033S	1:1000
Anti-Beta-Actin (13E5)	cell signaling technology	4970S	1:1000
Anti-slamf4 (D5J9D)	cell signaling technology	54560	1:1000 (wb) 1:100 (pull down)
Anti-CD11b	abcam	ab133357	1:200
Alexa Fluor 555 Donkey anti-Mouse IgG (H+L)	thermofisher	A-31570	1:500
Alexa Fluor® 488 AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson Immuno	111-545-003 (X2)	1:500
normal mouse IgG	Santa Cruz	sc-2025	1:200
Brilliant Violet 605 anti-mouse CD45	biolegend	103140	1:100
Brilliant Violet 711 anti-mouse CD46	biolegend	103147	1:100
FITC anti-mouse CD3	eBioscience	11-0031-85	1:100
PE anti-mouse CD3	biolegend	100206	1:100
FITC anti-mouse CD4	biolegend	100510	1:100
PE-Cyanine5 anti-mouse CD4	biolegend	100409	1:100
Brilliant Violet 421 anti-mouse CD8	biolegend	100738	1:100
FITC anti-mouse IFNγ	biolegend	505806	1:100
PE-Cyanine5 anti-mouse TNF-α	biolegend	506324	1:100
FITC anti-mouse Gr-1	biolegend	108405	1:100
PE-Cyanine7 anti-mouse ly6g	biolegend	127618	1:100
PE-Cyanine7 anti-mouse Granzyme B	biolegend	396411	1:100
PE-Cyanine7 anti-mouse Arg1	eBioscience	25-3697-80	1:100
PI	eBioscience	BMS500PI	1:100
Brilliant Violet 421 anti-mouse PD-1 (CD279)	biolegend	135221	1:100
Brilliant Violet 421 anti-mouse Ly6C	biolegend	128032	1:100
purified anti-mouse CD16/32	biolegend	101302	1:100
PE anti-mouse iNOS	biolegend	696806	1:100
Alexa Fluor® 594 anti-mouse Ly-6G/Ly-6C (Gr-1)	biolegend	108448	1:50 (IF) 1:100 (FC)
PE-Cyanine5 anti-mouse CD11b	biolegend	101210	1:100
FITC anti-mouse CD12b	biolegend	101206	1:100

Supplementary Table 2 Chemicals, Recombinant Proteins and Kits used in this Experiments

Chemicals and kits	Sources	Identifier
Azoxymethane	Sigma-Aldrich	A5486
Dextran Sodium Sulfate	MP Biomedicals	MFCD00081551
Corning matrigel matrix	Corning	356231
Recombinant mouse GM-CSF	biolegend	576304
Recombinant mouse IL-6	biolegend	575704
CFSE	thermofisher	C1157
Dnase I, Grade II	Roche	10104159001
Collagenase IV	Roche	58001
InVivoPlus anti-mouse Ly6G	Bio XCell	BP0075-1
InVivoMab anti-mouse PD-1	Bio XCell	BE0146
Human Slamf4	Abclonal	RP00167
IC Fixation Buffer 125 mL	eBiosciences	00-8822-49
Permeabilization Buffer 10X	Invitrogen	00-8333-56
RBC Lysis Buffer (10X)	biolegend	420301
Recombinant mouse IL-2	biolegend	575404
Ionomycin	STEMCELL Technologies	73724
phorbol 12-myristate 13-acetate	abcam	ab120297
Human CXCL1 Elisa kit	abcam	ab190805
Mouse CXCL1 Elisa kit	abcam	ab216951
Human Chemokine Antibody Array	abcam	ab169812
Mouse MDSC (CD11b+Gr1+) Isolation Kit	STEMCELL Technologies	19867
Mouse T Cell Isolation Kit	STEMCELL Technologies	19851

Supplementary Table 3 Primers used for q-PCR

Gene	Oligonucleotides
mouse-b-actin-F	TGTTACCAACTGGGACGACATG
mouse-b-actin-R	CTGGATGGCTACGTACATGGCT
mouse-Slamf1-F	CAGAAATCAGGGCCTCAAGAG
mouse-Slamf1-R	CACTGGCATAAACTGTGGTGG
mouse-Slamf2-F	CCCAAGCCTTCCATAGAAATCAA
mouse-Slamf2-R	CCAAGTATAGTCAACATGCTGGT
mouse-Slamf3-F	TCAGGGATGCTAGGGGGTTC
mouse-Slamf3-R	TTCGCTGACTTTGAGTCTGCC
mouse-Slamf4-F	CTCGGGGCCATCATTTGTTTC
mouse-Slamf4-R	GCTAGAAGGGAGCTGAACATCA
mouse-Slamf6-F	TATGCACAAGTCACTCGTCCA
mouse-Slamf6-R	GGTTATAGCCGGTTAAAGCCAC
mouse-Tlr2-F	GCAAACGCTGTTCTGCTCAG
mouse-Tlr2-R	AGGCGTCTCCCTCTATTGTATT
mouse-Tlr4-F	ATGGCATGGCTTACACCACC
mouse-Tlr4-R	GAGGCCAATTTTGTCTCCACA
mouse-Tlr5-F	GCAGGATCATGGCATGTCAAC
mouse-Tlr5-R	ATCTGGGTGAGGTTACAGCCT
mouse-Tlr6-F	TGAGCCAAGACAGAAAACCCA
mouse-Tlr6-R	GGGACATGAGTAAGGTTCCTGTT
mouse-Tlr9-F	AATCCCTCATATCCCTGTCCC
mouse-Tlr9-R	GTTGCCGTCCATGAATAGGAAG
mouse-Cxcl1-F	CTGGGATTCACCTCAAGAACATC
mouse-Cxcl1-R	CAGGGTCAAGGCAAGCCTC
mouse-Arg1-F	CTCCAAGCCAAAGTCCTTAGAG
mouse-Arg1-R	AGGAGCTGTCATTAGGGACATC
mouse-inos-F	GGGCTGTCACGGAGATCA
mouse-inos-R	CCATGATGGTCACATTCTGC
P. anaerobius -F	CTGATTTGATGCTTGCATTA
P. anaerobius-R	AGCCCCGAAGGGAAGGTGTG

Supplementary Table 4 Mass Spectrometry Results of Bands Identified from 50-100kDa fraction from culture supernatant of *P. anaerobius*

Protein Group	Accession	Coverage(%)	Area	#Unique	#Spec	Mass	Description
1	A0A379CGB7|A0A379CGB7_9FIRM	92.98	6085644300 0	105	2856	80.864	N-acetylmuramoyl-L-alanine amidase LytC OS=Peptostreptococcus anaerobius OX=1261 GN=lytC_22 PE=4 SV=1
10	A0A379CFM5|A0A379CFM5_9FIRM	50	1007110961	33	46	81.972	N-acetylmuramoyl-L-alanine amidase LytC OS=Peptostreptococcus anaerobius OX=1261 GN=lytC_21 PE=4 SV=1
151	A0A6N7X0K1|A0A6N7X0K1_9FIRM	48.12	5657323	2	2	14.493	30S ribosomal protein S11 OS=Peptostreptococcus anaerobius OX=1261 GN=rpsK PE=3 SV=1
151	A0A135YUR7|A0A135YUR7_9FIRM	48.12	5657323	2	2	14.479.	30S ribosomal protein S11 OS=Peptostreptococcus anaerobius OX=1261 GN=rpsK PE=3 SV=1
151	D3MTE5|D3MTE5_9FIRM	48.12	5657323	2	2	14.479	30S ribosomal protein S11 OS=Peptostreptococcus anaerobius 653-L OX=596329 GN=rpsK PE=3 SV=1
23	A0A379CHV1|A0A379CHV1_9FIRM	41.11	57788176	19	24	67.248	Uncharacterized peptidase SA1530 OS=Peptostreptococcus anaerobius OX=1261 GN=NCTC11460_01965 PE=4 SV=1
28	A0A379CFM8|A0A379CFM8_9FIRM	40.31	42666476	16	20	62.369	Pyruvate kinase OS=Peptostreptococcus anaerobius OX=1261 GN=pyk PE=3 SV=1
28	D3MPK9|D3MPK9_9FIRM	40.31	42666476	16	20	62.392	Pyruvate kinase OS=Peptostreptococcus anaerobius 653-L OX=596329 GN=pyk PE=3 SV=1
28	A0A135YSW5|A0A135YSW5_9FIRM	40.31	42666476	16	20	62.366	Pyruvate kinase OS=Peptostreptococcus anaerobius OX=1261 GN=HMPREF3195_01071 PE=3 SV=1
2	D3MU98|D3MU98_9FIRM	38.11	629723580	8	951	80.903	Putative cell wall binding repeat 2 OS=Peptostreptococcus anaerobius 653-L OX=596329 GN=HMPREF0631_1404 PE=4 SV=1
7	A0A379CFB2|A0A379CFB2_9FIRM	33.3	51208652	25	34	120.494	N-acetylmuramoyl-L-alanine amidase LytC OS=Peptostreptococcus anaerobius OX=1261 GN=lytC_20 PE=4 SV=1
570	A0A379CEQ2|A0A379CEQ2_9FIRM	30.99	1518149.2	1	1	84.16	Barstar (Barnase inhibitor) OS=Peptostreptococcus anaerobius OX=1261 GN=NCTC11460_00474 PE=3 SV=1
40	A0A379CF33|A0A379CF33_9FIRM	24.01	23831450	11	15	81.537	N-acetylmuramoyl-L-alanine amidase LytC OS=Peptostreptococcus anaerobius OX=1261 GN=lytC_11 PE=4 SV=1
43	A0A379CHG0|A0A379CHG0_9FIRM	23.21	16648673	9	15	71.305	85 kDa vitronectin-binding protein OS=Peptostreptococcus anaerobius OX=1261 GN=fusA_2 PE=3 SV=1

Supplementary Table 5 siRNA Used in the Experiments.

Gene	Species	Company	Identifier
siCXCL1-1	Human	Invitrogen	s6215
siCXCL1-2	Human	Invitrogen	s6216
siCXCL3-1	Human	Invitrogen	s6221
siCXCL3-1	Human	Invitrogen	s6223
siITGA2	Human	Invitrogen	s7573
siITGB1	Human	GCACCAGCCCAUUUAGCUATT	

Fig 5a

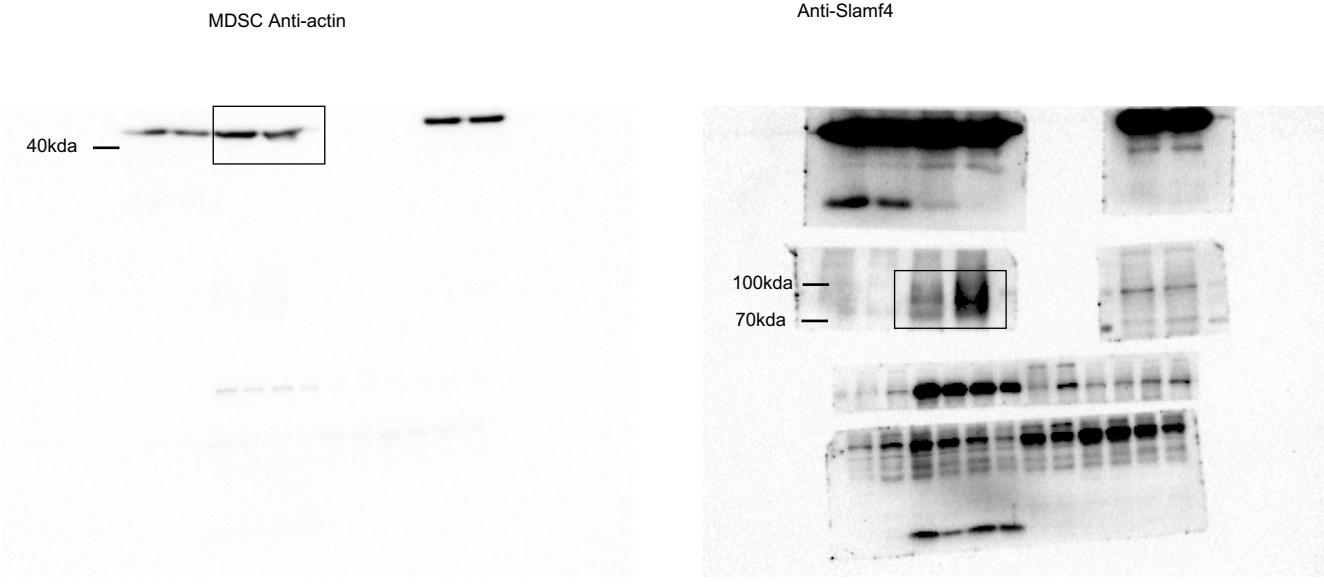

MDSC Anti-actin
40kda
Anti-Slamf4
100kda
70kda

Fig 5f

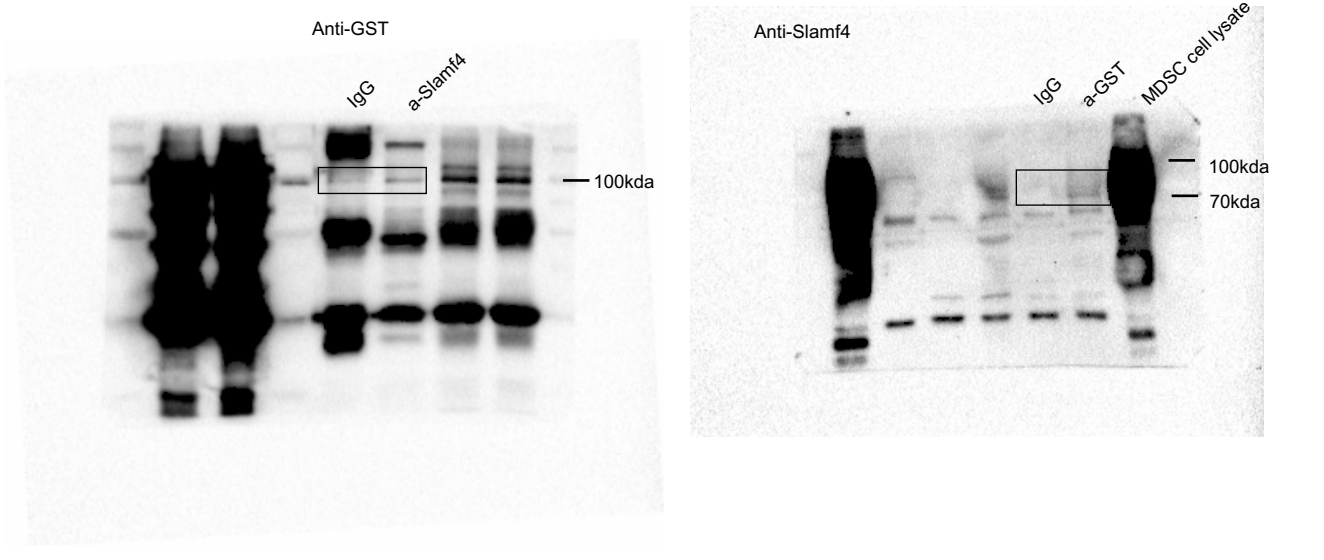
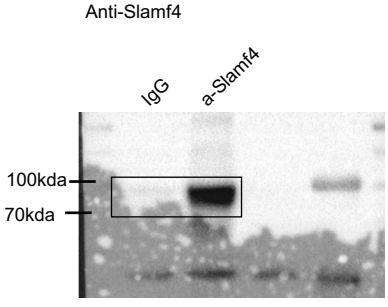
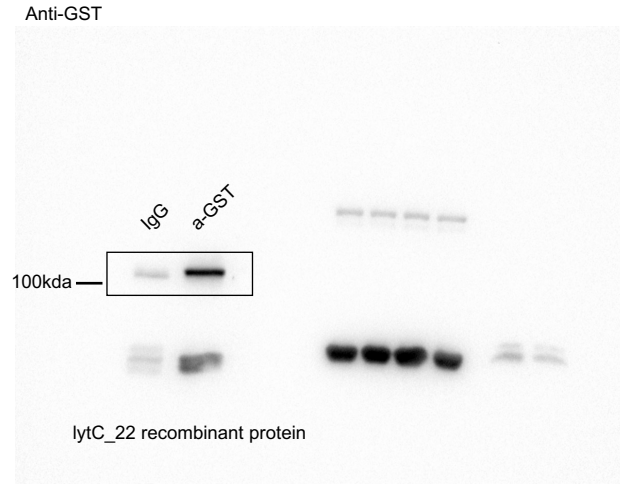
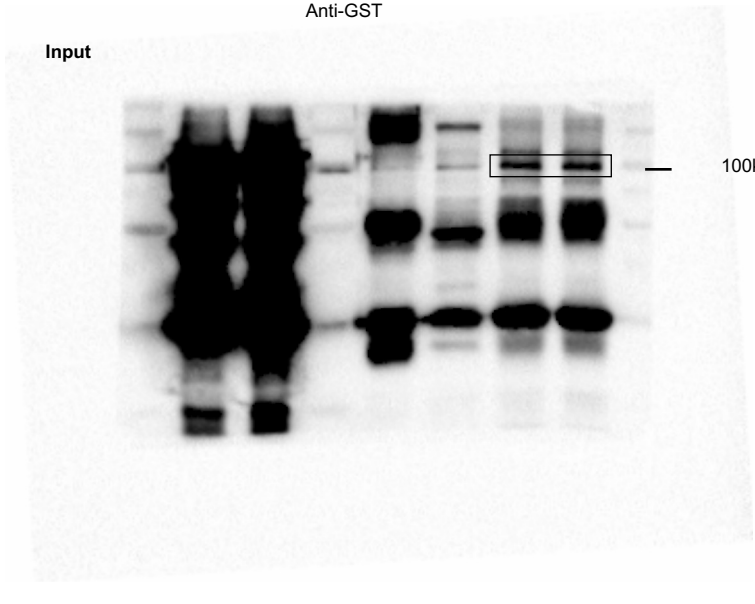

Anti-GST
IgG
a-Slamf4
100kda
Anti-Slamf4
IgG
a-GST
MDSC cell lysate
100kda
70kda

Fig 5f


Anti-Slamf4
IgG
a-Slamf4
100kda
70kda
MDSC cell lysate


Anti-GST
IgG
a-GST
100kda
lytC_22 recombinant protein


Anti-GST
Input
100kda

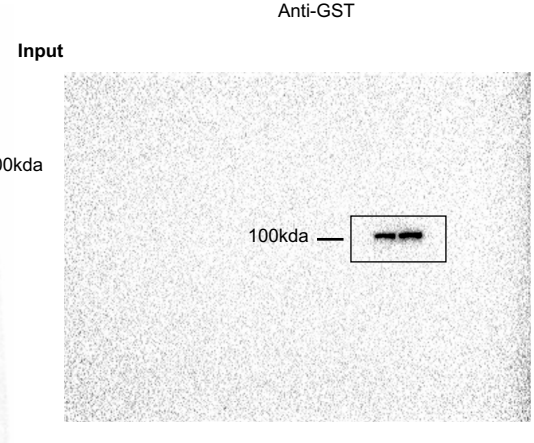
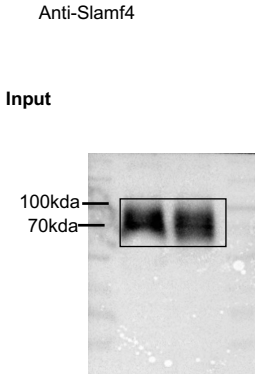

Anti-GST
Input
100kda

Fig 5f


Anti-Slamf4
Input
100kda
70kda
MDSC cell lysate

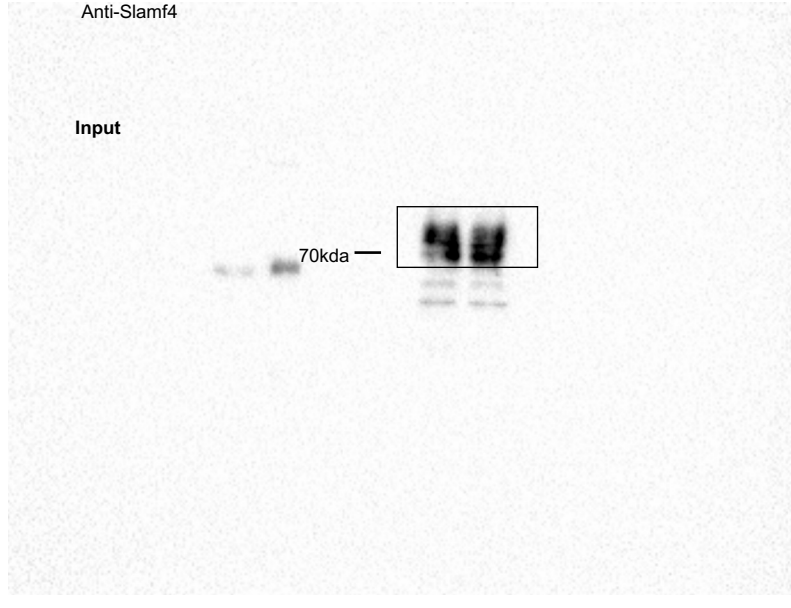
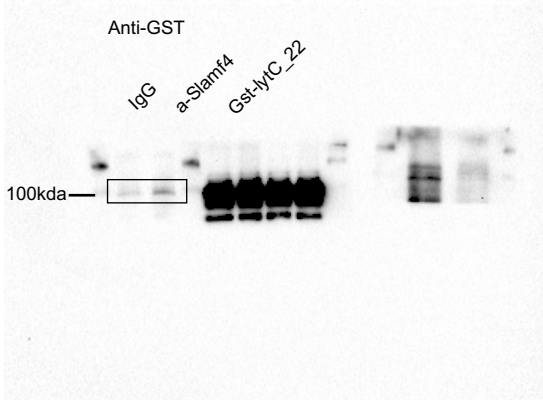
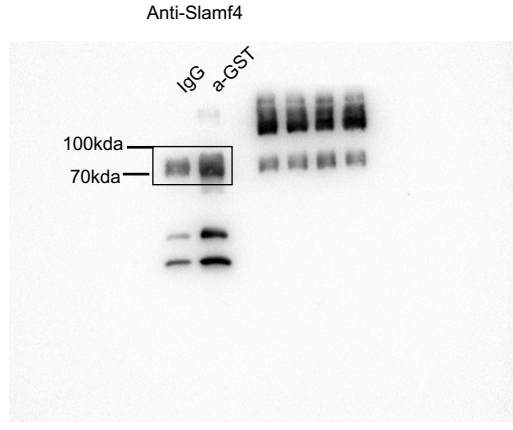
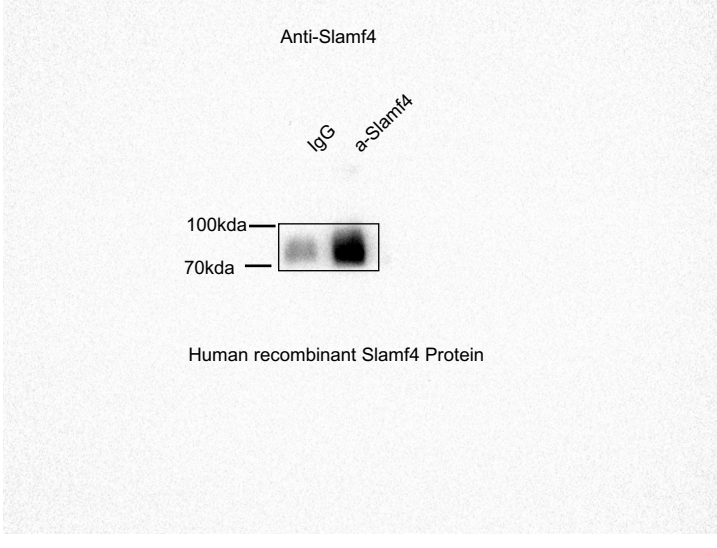

Anti-Slamf4
Input
70kda

Fig 5g


Anti-GST
IgG
a-Slamf4
Gst-lytC_22
100kda


Anti-Slamf4
IgG
a-GST
100kda
70kda


Anti-Slamf4
IgG
a-Slamf4
100kda
70kda
Human recombinant Slamf4 Protein

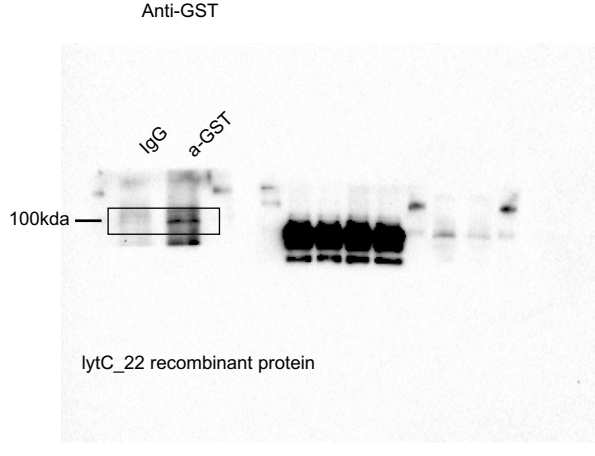
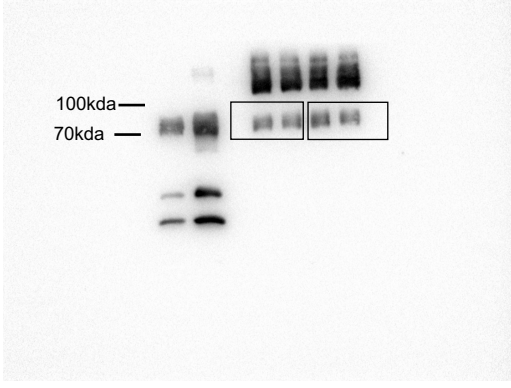

Anti-GST
IgG
a-GST
100kda
lytC_22 recombinant protein

Fig 5g

Input

Anti-Slamf4



Input

Anti-GST

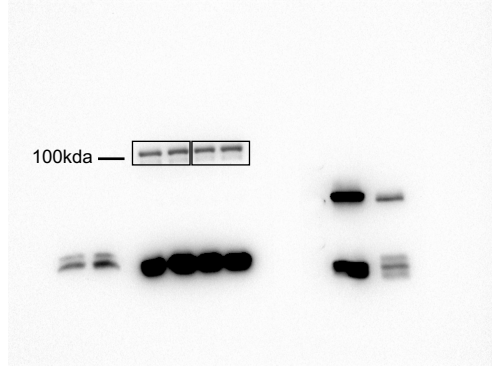
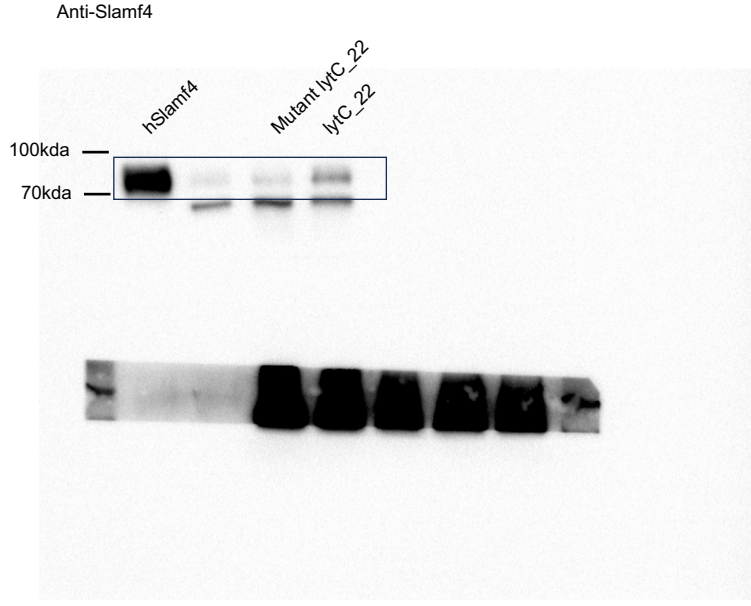
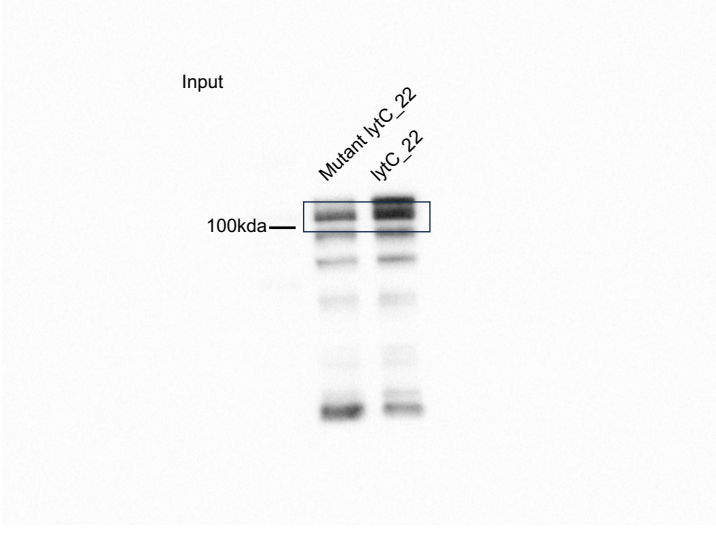


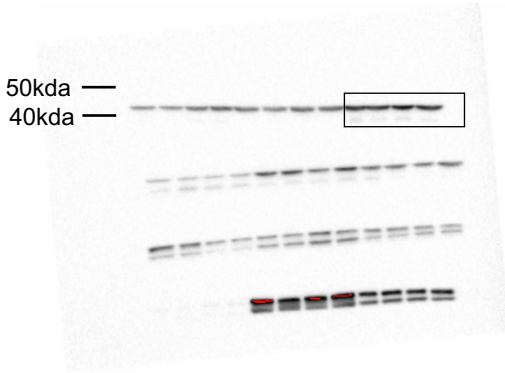
Fig 6b


Anti-Slamf4
hSlamf4
Mutant lytC_22
lytC_22
100kda
70kda

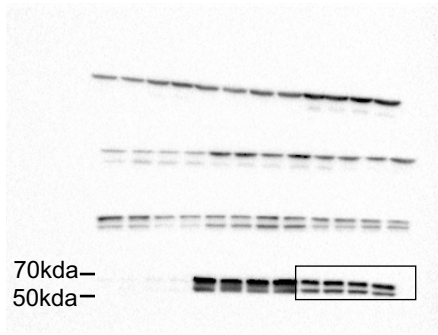

Input
Mutant lytC_22
lytC_22
100kda

Extended Figure 5a **HCT116**

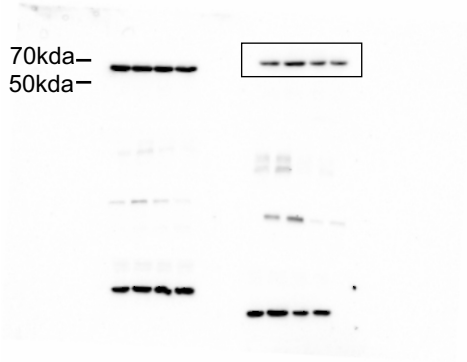
HCT116 Actin



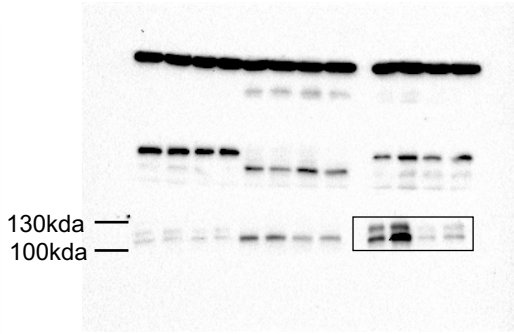
HCT116 NF-KB p65



HCT116 p-NF-KB p65



HCT116 Integrin $\beta 1$



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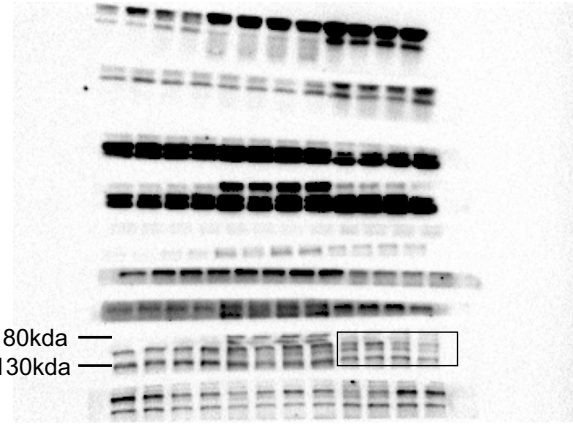
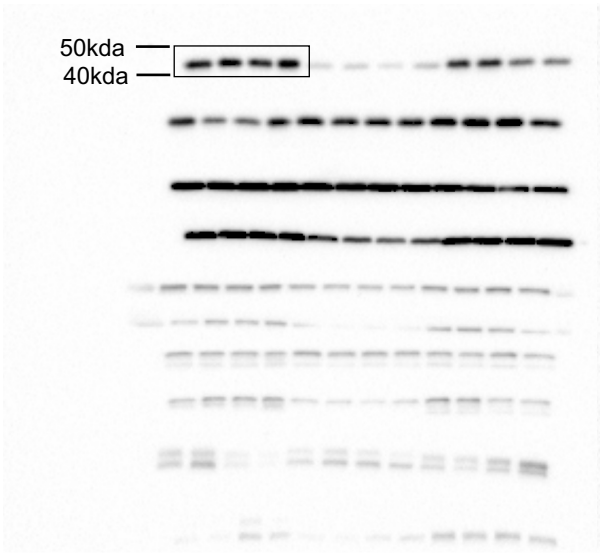


Extended Figure 5a

Caco-2 actin



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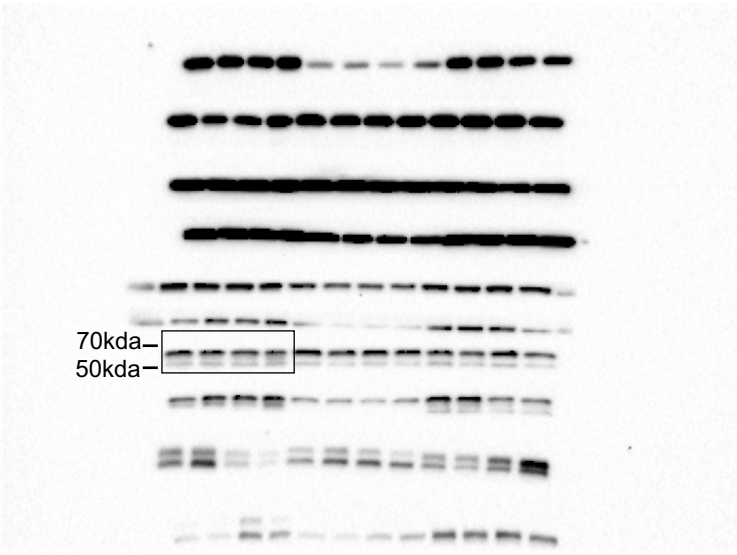
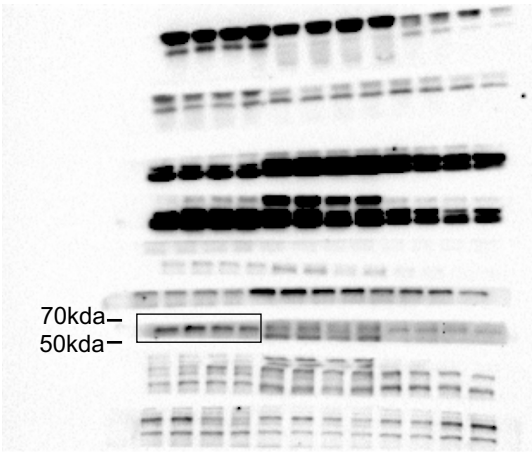


Caco-2 p-NF-KB p65



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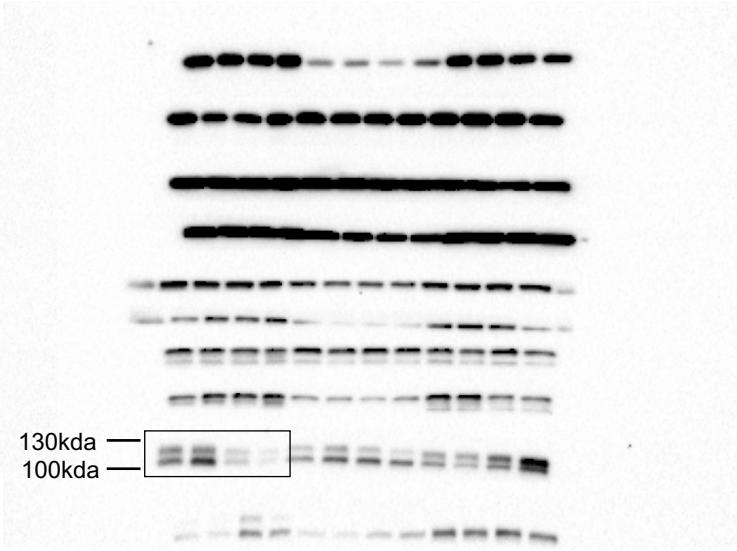

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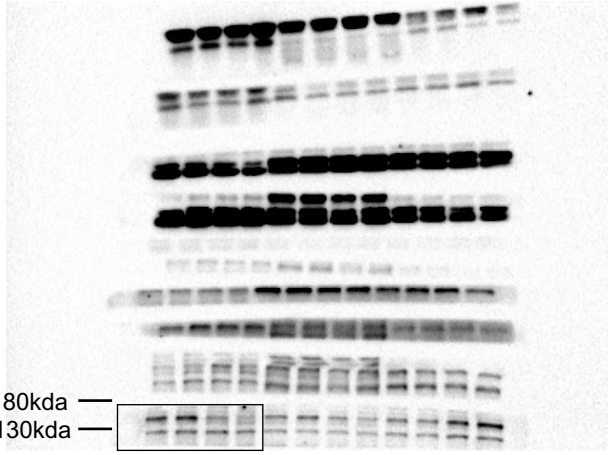
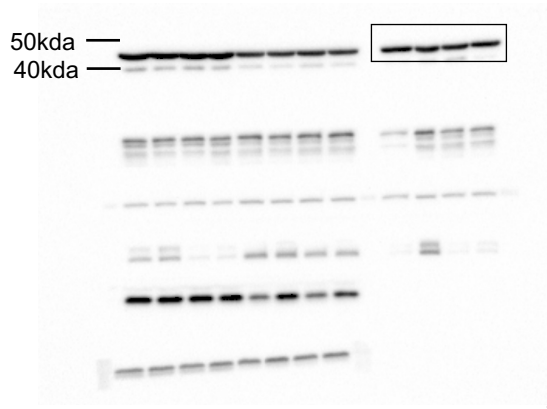


Extended Figure 5b

HCT116 actin



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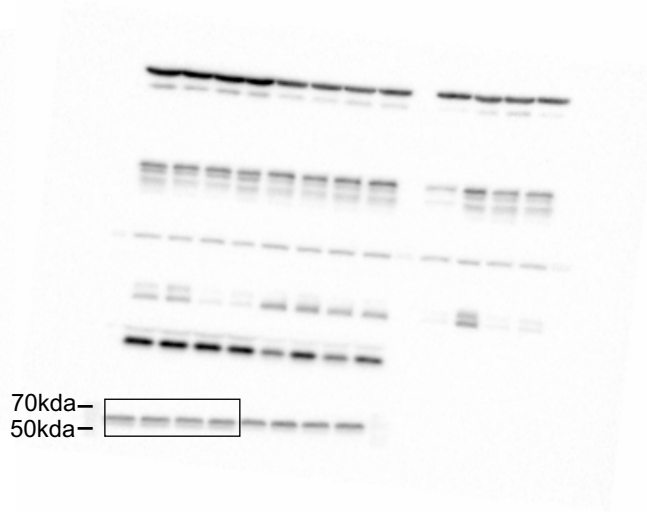

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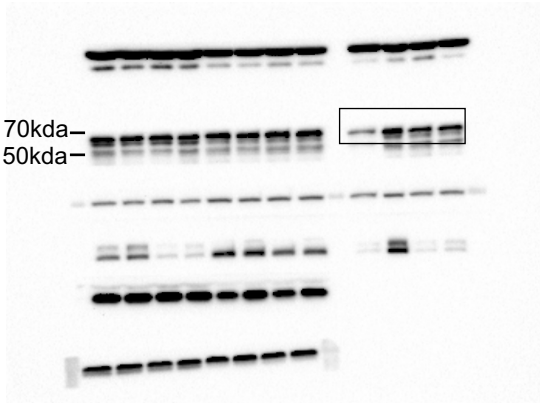
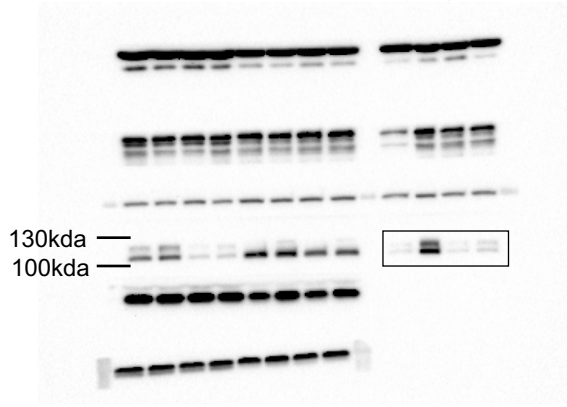


HCT116 Integrin $\beta 1$



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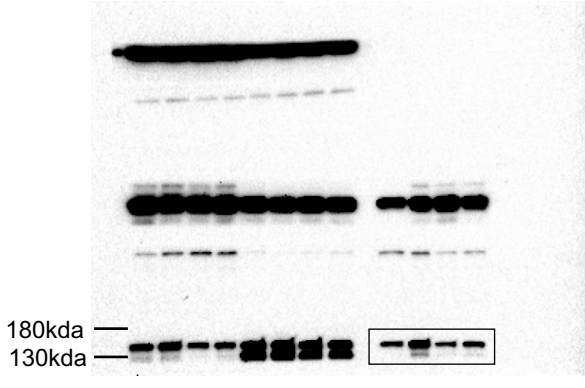
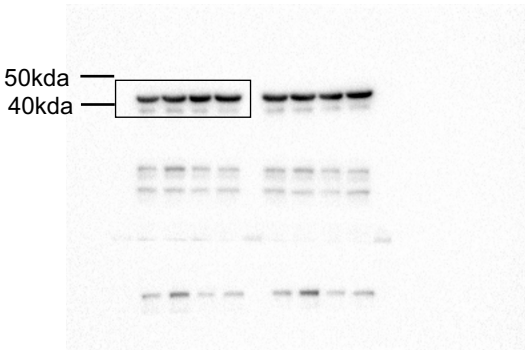


Extended Figure 5b

MC38 actin



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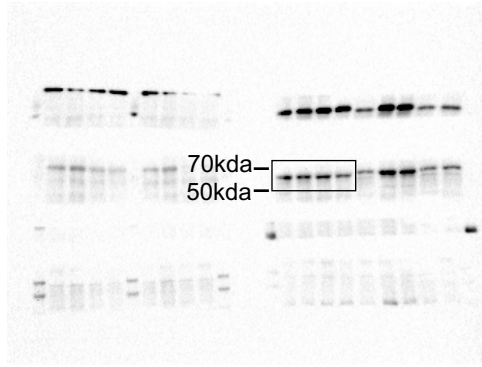

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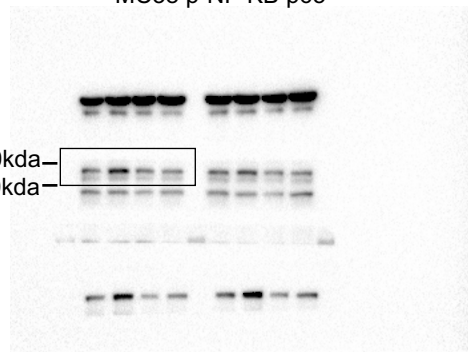
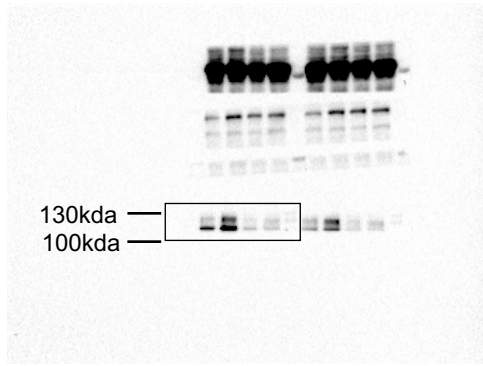


MC38 Integrin $\beta 1$



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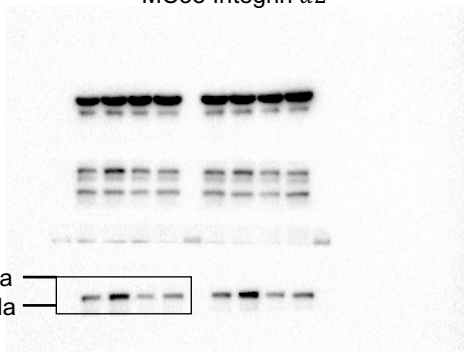
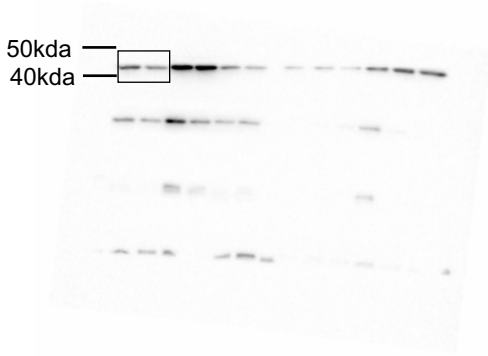
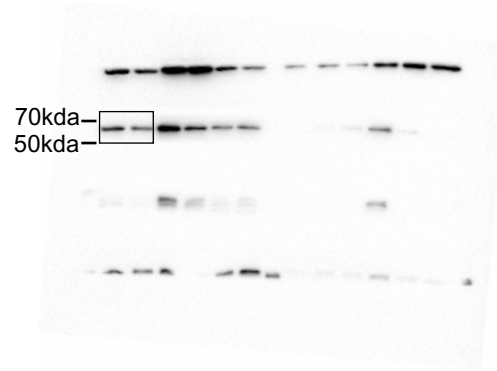


Extended Figure 5c

HCT116 Cyto-Actin



HCT116 Cyto-p-NF-KB p65



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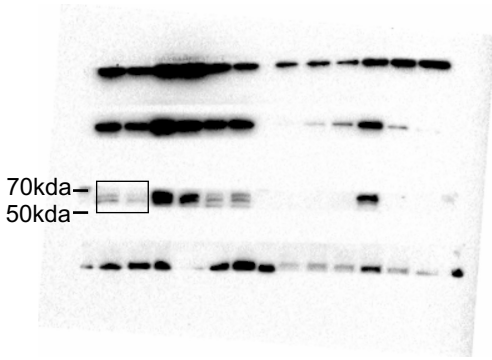
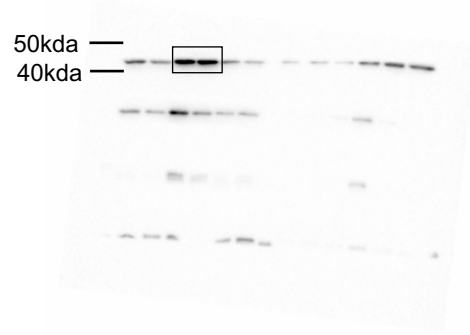
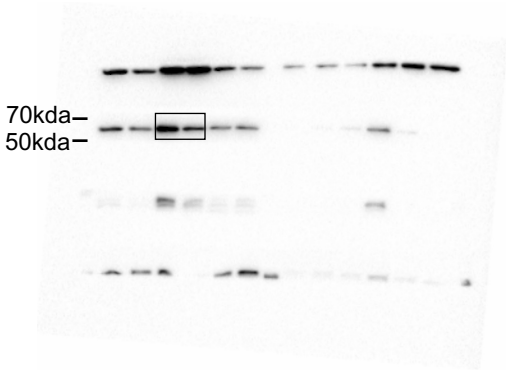


Caco-2 Cyto-Actin



Caco-2 Cyto-p-NF-KB p65



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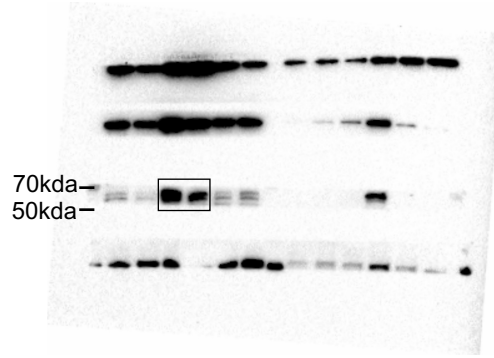
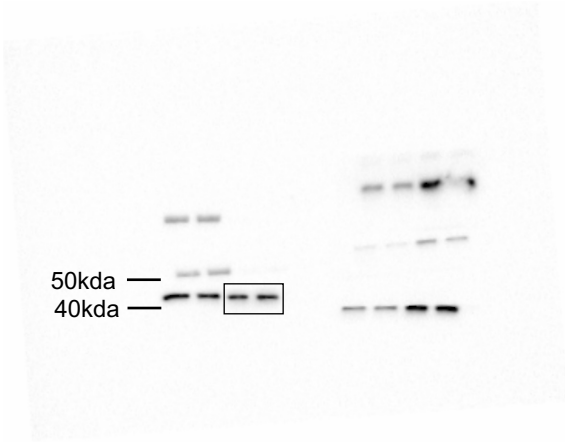


Extended Figure 5c

MC38 Cyto-Actin



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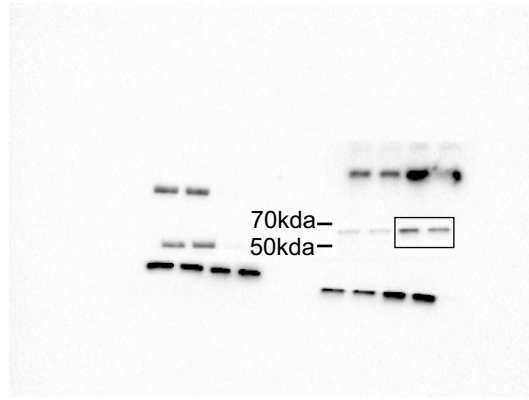
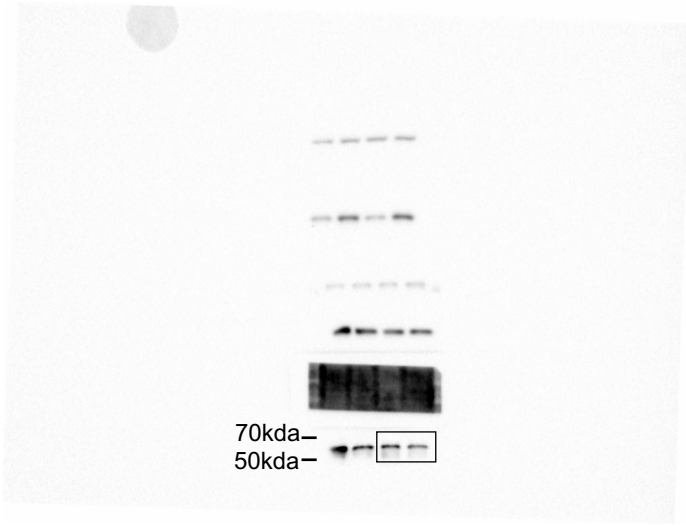
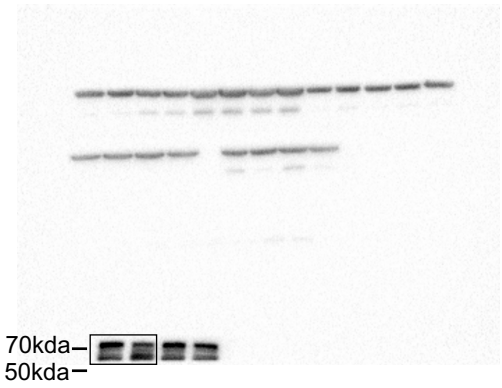


MC38 Cyto-p-NF-KB p65



Extended Figure 5c

HCT116 nu-Lamin A/C



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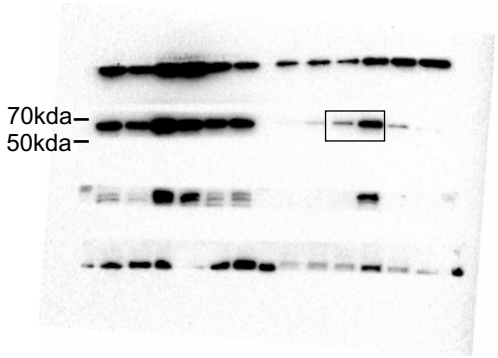

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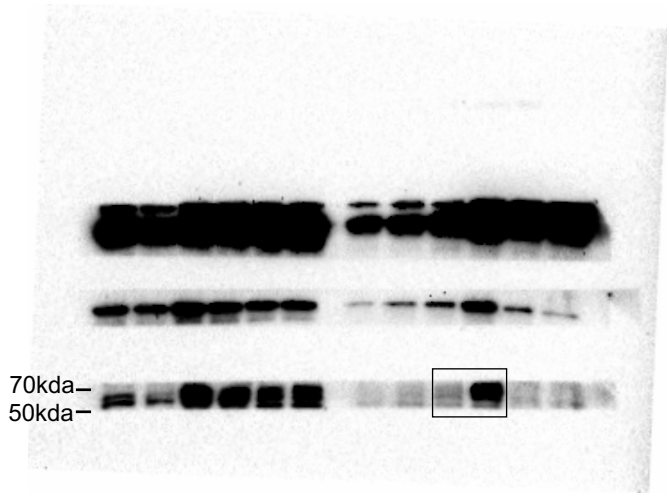
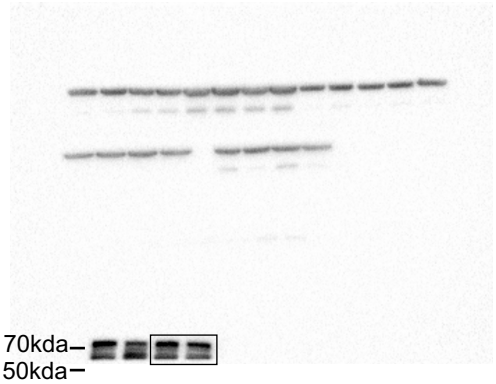
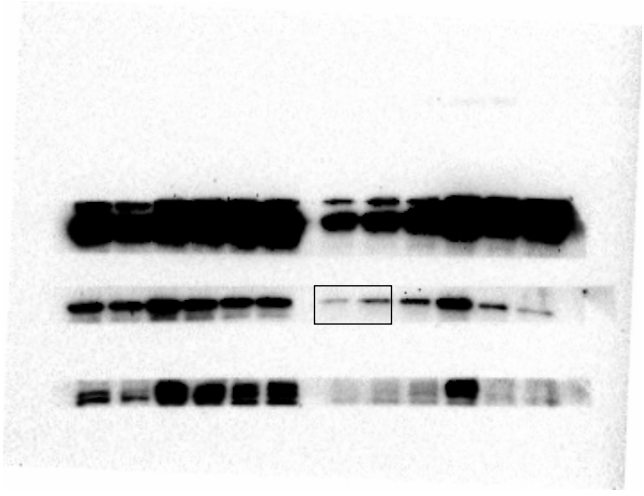


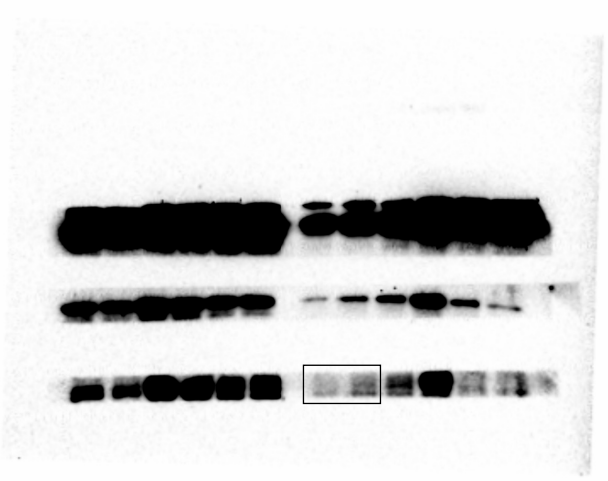
Caco-2 nu-Lamin A/C



Caco-2 nu-NF-KB p65

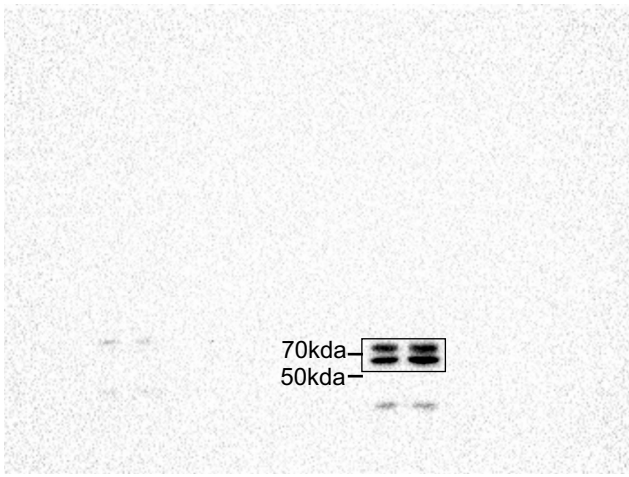


Caco-2 nu-p-NF-KB p65

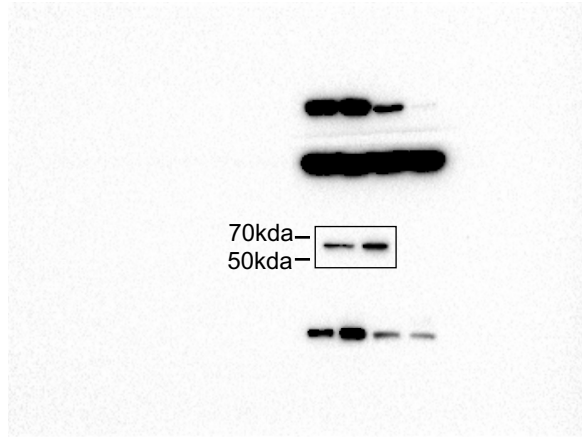


Extended Figure 5c

MC38 nu-lamin A/C



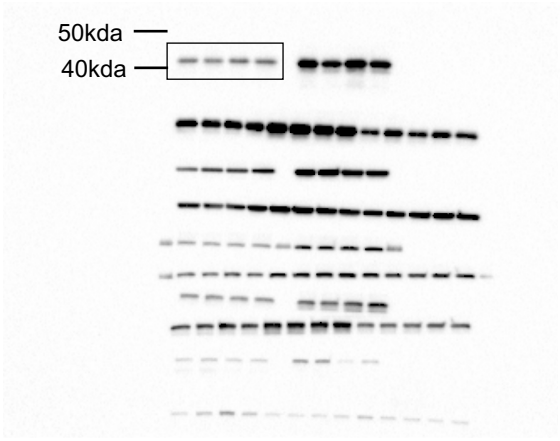
MC38 nu-NF-KB p65



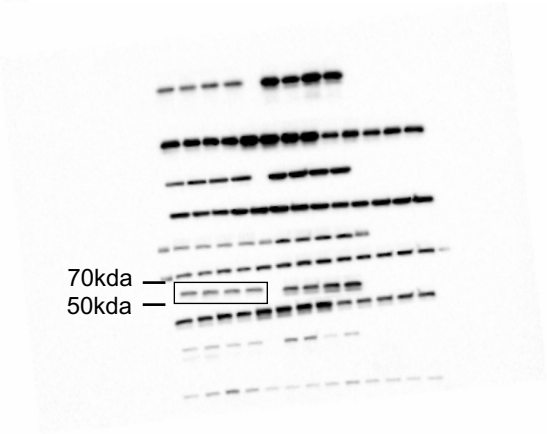
MC38 nu-p-NF-KB p65

Extended Figure 5d

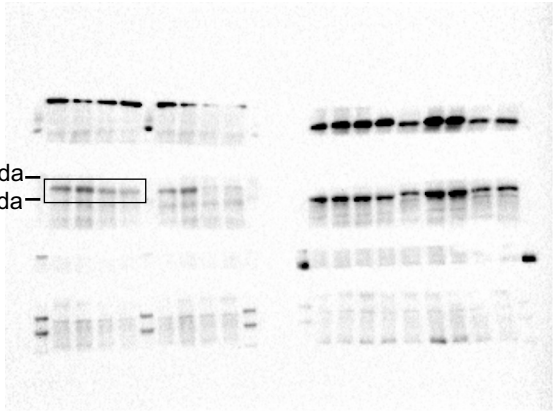
HCT116 actin



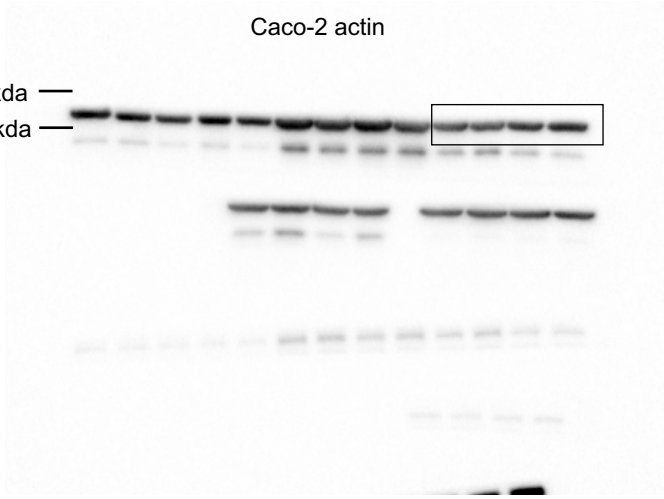
HCT116 NF-KB p65



HCT116 p-NF-KB p65



Caco-2 actin



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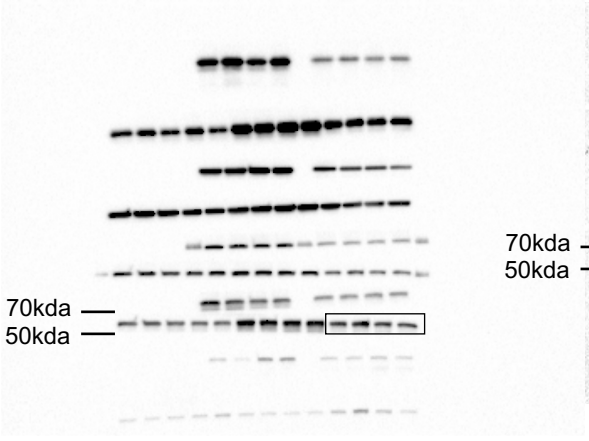
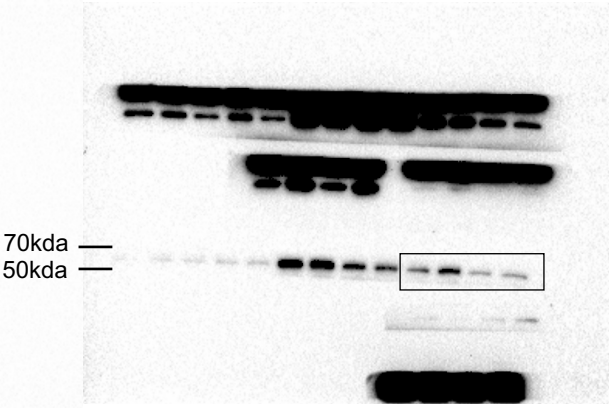


Caco-2 p-NF-KB p65

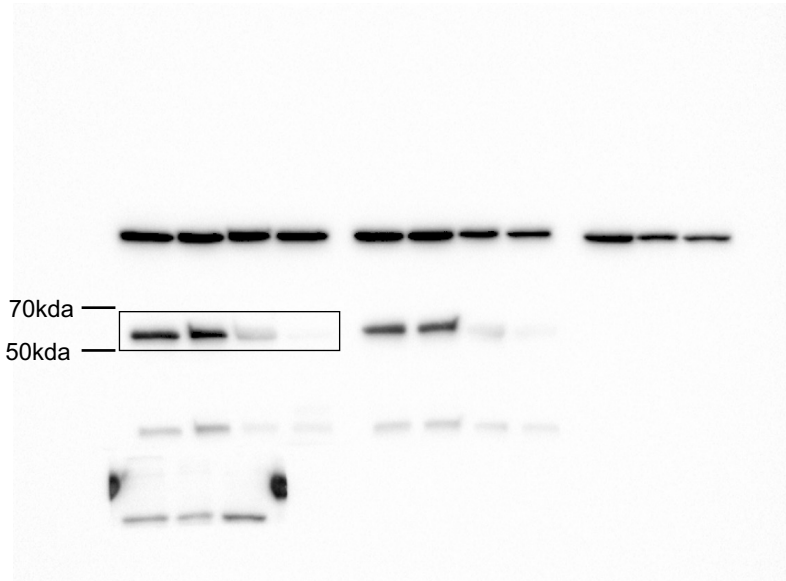


Extended Figure 5d

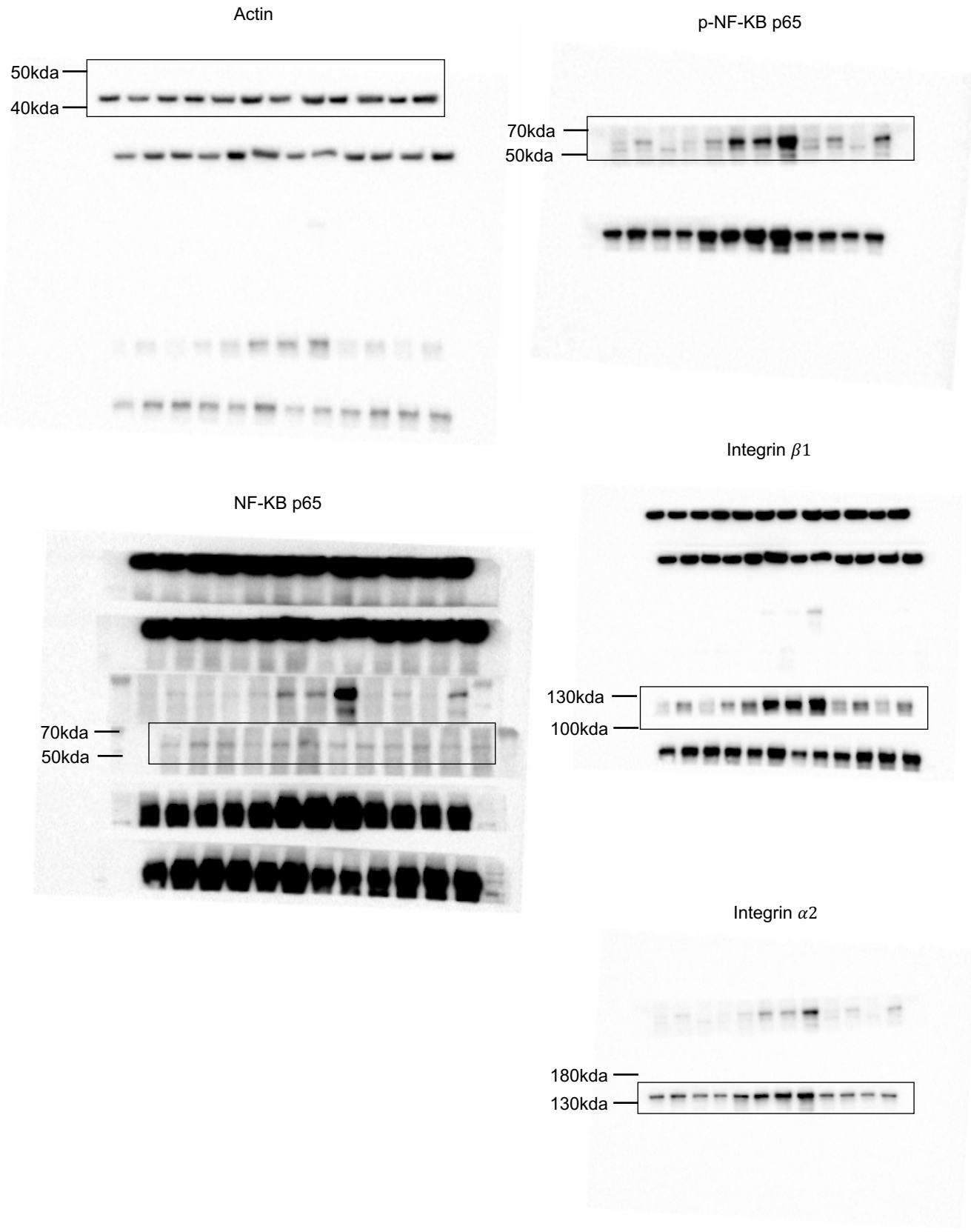
MC38 actin

MC38 NF-KB p65

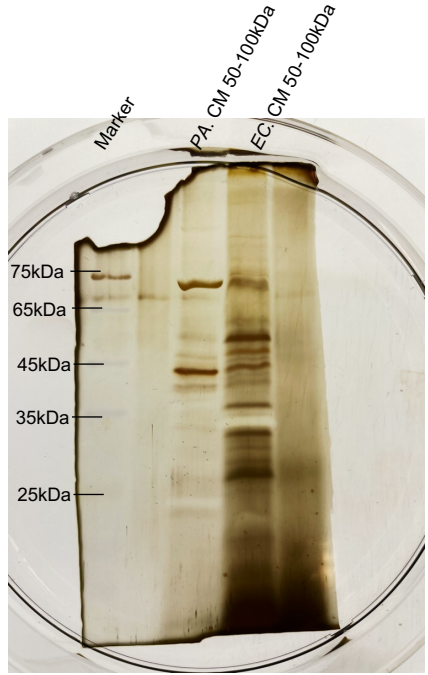
MC38 p-NF-KB p65



Extended Figure 5k



Extended Data Fig 7a



Extended Data Fig 8g