Supplemental information

Alternatively spliced isoforms reveal a novel type of PTB domain in CCM2 protein

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Suppl. Table 1. **Primers for the identification and amplification of various CCM2 isoforms**. The primers are listed in three groups, RACE primers, nested primers, and full-length CCM2 amplification primers. The sequence, direction, and intragenic location of each primer are also listed.

Suppl. Table 2. **Primers for the quantitative PCR (qPCR) for the detection of differentially expressed CCM2 isoforms**. The primer pairs starting exon1 and spanning into other downstream exon (exon 2, 3, etc.) junctions were used for detecting A group isoforms; primer pairs starting exon1A and spanning into other following exon (exon2, 3, etc.) junctions for detecting B group isoforms, respectively. The sequence, qPCR product size, potentially covered isoform(s) and optimized annealing temperature of each primer pair are listed. The primer sets A-100 (spanning exon1-exon2 junction and within exon 2) and B200 (spanning exon1A-exon2 junction and within exon 2) represent a collection of comparable isoforms from A and B group, respectively; while the primer sets A-102 (spanning exon1-exon3 junction and exon4-exon7) and B210 (spanning exon1A-exon3 junction and exon4-exon7) for distinguishing a specific pair of isoforms (CCM2-102, CCM2-210). Allele-specific primer sets for V5 tag and neomycin were used for isoform-specific qPCR.

Suppl. Table 3. **The length and distribution of functional domains along the protein structure of the representative isoforms of CCM2**. Total 9 distinct functional domains were identified by search NCBI CDD database. The phosphotyrosine-binding domain (PTB, or phosphotyrosine interaction - PI domain) is listed first due to its interaction with

NPXY motif of CCM1 and β1 integrin cytoplasmic tail. Besides PTB domain, there are additional 8 functional domains: DUF1722 (unknown function) that is partially overlapped with reported LD motif and global domain predicted in this report (Fig. 3D) in the middle of CCM2; SSL2 (DNA or RNA helicase); N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase (both protein methyltransferase and tRNA (guanine-N7-)-methyltransferase activities with ATPase activity); GATase1-like (glutamine metabolic process); tbpA (transmembrane movement); excinuclease ABC subunit A (DNA mismatch repair process); flagellar hook-associated protein L (cellular ciliary or flagellar motility), and PRK07168 (bifunctional uroporphyrinogen-III methyltransferase and/or uroporphyrinogen-III synthase activity). PTB, DUF1722 and SSL2 domains are most abundant domains among all CCM2 isoforms. aa -- amino acid residues. *CCM2-100 isoform is originally reported as canonical CCM2 protein with ten- coding exons.

Suppl. Table 4. **Identification of essential cores of atypical PTB domain in CCM2**. The sequence of exon 6 along with newly identified adjacent exon 6A and exon 6B of CCM2 are compared. Neither their sequences nor their tertiary structure (Fig) share any similarity to the PTB domain in CCM2, by comparing exons with PTB domain; while exon 6 and exon 6A were defined by their specific interaction with various NPXY motifs in cytoplasmic domains of representative β-integrins and CCM1. The LD-like sequence within exon 6 is underlined/Latinized (consensus: LDxLLxxL, Red colored letter: present identical to LD-like sequence; Green colored letter present conserved amino acids to LD-like sequence), which does not share any notable homologs or similarities to the sequence of exon 6A of CCM2, but share highly similar tertiary structure (Fig. 7).

Suppl. Fig. 1. **Relative expression profiling of endogenous CCM2 isoforms in brain tissues and cell lines by qPCR**. The expression levels of CCM2 isoforms ($2^{-\Delta CT}$) were presented with bar plots, while β-actin was taken as an inference gene. qPCR primer set information for CCM2 isoforms was provided in Suppl. Table 2. A). For Brain tissues: CB, Cerebellum; CC, Cerebral Cortex; CR, Corpus Callosum; DM, Dura Matter; FL, Frontal Lobe; HC, Hippocampus; IS, Insula; MO, Medulla Oblongata; NA, Nucleus Accumbens; OP, Occipital Pole; PG, Paracentral Gyrus; PL, Parietal Lobe; PN, Pons; SC, Spinal Cord; SN, Substantia Nigra; TL, Temporal Lobe. B). For endothelial cell lines: HU, HUVEC; HA, HAEC; HE, HMVEC. For cancer cell lines: MD, MDA231; K5, K562; L6, HL60; U9, U937; C3, PC-3; J1, RAJ1; JU, JURKAT; 29, 293T; LA, HELA.

Suppl. Fig. 2. **Differentiated cellular compartmentations among different CCM2 isoforms in various cell lines**. Each CCM2 isoform was transiently transfected and images show the subcellular localization of CCM2 isoforms with /without treatment of leptomycin. Cell lines: A. Hela cells, B. HUVEC cells, C. HMVEC cells; and D, hCMEC/D3 cells.

Suppl. Fig. 3. **Original digital images at a lower exposure of Western blots in Figures 3 and 6.** Protein lysates were separated by SDS-PAGE using Criterion Precast gels (Bio-Rad), specifically TGX gels for Fig. 3, 4-15% Tris-HCL gels for Fig. 6A and 16.5% Tris-Tricine gels for Fig. 6B and 6C. In Fig. 6B and 6C, all target proteins were tagged with epitopes and labeled with radioactive with S^{35}.

Suppl. Table 1

PRIMER	SEQUENCE	DIRECTION	LOCATION
RACE primers:			
MGC41F	CTCTGTCACCTTCTCATGGGCTTTCTT	**FORWARD**	**intra-exon:2**
MGC42F	CAGGCTTCTTGCCCTTCTTGCCC	**FORWARD**	**inter-exon:1-2**
MGC-6A-F1	AGCCACGTGACCTCTTCCACAGTCA	**FORWARD**	**intra-exon:6A**
MGC-6A-F2	TTCCTTTGACTGTCAGCTTCCTCT	**FORWARD**	**intra-exon:6A**
MGC-6A-R1	AAATAGGGAGATGACCCTGGA	**REVERSE**	**intra-exon:6A**
MGC-6A-R2	TTTGGGTGGGCTCAATGTGATT	**REVERSE**	**intra-exon:6A**
MGC5'RACE-3	GGGAGATGACCCTGGAGAATTTGGGTG	**REVERSE**	**intra-exon:6A**
MGC5'RACE-1	GCCCAGCGCCTCAATGTCGCTGCTGATG	**REVERSE**	**intra-exon:10**
MGC5'RACE-2	CGAAGTGCTGGCTGTCCTTCTCAGGG	**REVERSE**	**intra-exon:10**
MGC5'RACE-4	GGAGGGATTCAGGTATCCTGGTATGGACG	**REVERSE**	**intra-exon:3**
MGC5'RACE-5	CTCAGCTACTCAGGAGGCTGAGGC	**REVERSE**	**intra-exon:2B**
MGC5'RACE-6	CGAAGGGTGGAAACACGCGACAAGGC	**REVERSE**	**intra-exon:1B**
MGC5'RACE-7	CACACCTGCAGTGCCTTGGGATC	**REVERSE**	**intra-exon:1D**
MGC5'RACE-8	CTAGCTGCATTCTCCATGCAAAACATTGAG	**REVERSE**	**intra-exon:1C**
MGC5'RACE-10	GGAGAGATTCTGGTTCCTCCTCTGCCG	**REVERSE**	**intra-exon:1A**
MGC200-F2	CAGAGGGAGGGCTAACATCTGGCCATA	**FORWARD**	**intra-exon:1A**
MGC5'RACE-11	GCTCAGCAGTCTGTCTGGCTCGACGCG	**REVERSE**	**intra-exon:2**
Nested primers:			
MGC1A-R1	TCATTCTCCATGGAATACCCTGTGTG	**REVERSE**	**intra-exon:1A**
MGC1A-R2	TTCCTCCTCTGCCGACAGCTACTATGCAT	**REVERSE**	**intra-exon:1A**
MGC1B-F1	CATGAGTCCTGGAGGCAGGTAGAGGC	**FORWARD**	**intra-exon:1B**
MGC1B-F2	CACCTGGGAATGGAGTTATCTCT	**FORWARD**	**intra-exon:1B**
MGC1B-R1	GTTTGCAGTGCTTGCTCAAAGGCAG	**REVERSE**	**intra-exon:1B**
MGC1B-R2	AGATAACTCCATTCCCAGGTGGGGCTT	**REVERSE**	**intra-exon:1B**
MGC1C-F1	CTTTTTAGCACTTTCTCCAGAGT	**FORWARD**	**intra-exon:1C**
MGC1C-F2	ATGTTTTGCATGGAGAATGCAGC	**FORWARD**	**intra-exon:1C**
MGC1D-F1	CTGTTTCTTGGAGTCCACATCT	**FORWARD**	**intra-exon:1D**
MGC1D-F2	GGATGATCCTCGTTTTGCTGGGAGTA	**FORWARD**	**intra-exon:1D**
MGC1D-R1	TCACACCTGCAGTGCCTTGGGATCT	**REVERSE**	**intra-exon:1D**
MGC1D-R2	CTACTCCCAGCAAAACGAGGATCATCC	**REVERSE**	**intra-exon:1D**
MGC1E-F1	TTTGCCTTGAAGAAGTGAGCTTG	**FORWARD**	**intra-exon:1B**
MGC1E-F2	ATGAGTCCTGGAGGCAGGTAGAG	**FORWARD**	**intra-exon:1B**
MGC1E-R1	TGCAGTGCTTGCTCAAAGGCAGCAAGAT	**REVERSE**	**intra-exon:1B**
MGC1E-R2	CTCTACCTGCCTCCAGGACTCATGAGT	**REVERSE**	**intra-exon:1B**
MGC2B-F1	ATGGAGTTTTGCTCTTGTTGCCCA	**FORWARD**	**intra-exon:2B**
MGC2B-F2	CAGTCTTGGCTCACCACAACCTC	**FORWARD**	**intra-exon:2B**
MGC223-F1	ATGCAAAGCTGCTTCCCTGAATC	**FORWARD**	**inter-exon:3-8**
MGC142-F1	ATGCTGACGCTGCGCACCAAGCTGTCA	**FORWARD**	**inter-exon:8-9**
MGC-EX6A-F1	CCAGCCACGTGACCTCTTCCA	**FORWARD**	**intra-exon:6A**
MGC-EX4-F1	AGAGCCCACCAGCTTCCGGGA	**FORWARD**	**intra-exon:4**
MGC-EX7-F1	ATGACTCTTCTACAAAAGTGGA	**FORWARD**	**intra-exon:7**
MGC-EX6-R1	CGCTGTGCAGGGACAGGTGGT	**REVERSE**	**intra-exon:6**
MGC-EX4-R1	CTGTCTTCAGGACCACCAGGTGT	**REVERSE**	**intra-exon:4**
MGC-EX2-R1	CTTTACCTCCTTCTCAATATAGT	**REVERSE**	**intra-exon:2**
Full-length:			
MGCF1	ATGGAAGAGGAGGGCAAGAA	**FORWARD**	**CCM2-A (Start codon)**
MGCR1	GCTGAGTCCTGGTCCATGCT	**REVERSE**	**HUMAN MGC**
MGCR2	TGCTGAGTCCTGGTCCAT	**REVERSE**	**HUMAN MGC**
MCGR3	TGCTGAGTCCTGGTCCATGCT	**REVERSE**	**HUMAN MGC**
MGCF-001	ATGCATAGTAGCTGTCGGCAGA	**FORWARD**	**CCM2-B (Start codon)**
MGC200-F1	ATGCATAGTAGCTGTCGGCAGAGGAGGA	**FORWARD**	**CCM2-B (Start codon)**

Suppl. Table 2

RT-PCR Code	Primer	Sequence	Product Size (bp)	Representig Isoform(s)	Anneling Temp (C)
A-100	MGC-EXB1-2-F1	GGCAAGAAGCCTGGAATTGT	**98**	**100, 103, 104,115, 116, 117**	**65**
	MGC-EX2-R1	CGCCTCTCTGTCACCTTCTC		**118, 119, 402, 404, 602**	
A-101	MGC-EXB1-3-F1	GGGCAAGAAGTATTTAGGTCAG	**103**	**101, 102, 120, 401, 601, 603**	**60**
	MGC-EXB3-4-R1	GTGGGCTCTCTTTGCATTGT			
A-102	MGC-EXB1-3-F1	GGGCAAGAAGTATTTAGGTCAG	**291**	**102**	**65**
	MGC-EXB4-7-R1	GTAGAAGAGTCATCTGTCTTCAGGA			
B-213	MGC-EXB2-3-F1	GGAGGTAAAGTATTTAGGTCAG	**291**	**117, 203, 404**	**65**
	MGC-EXB4-7-R1	GTAGAAGAGTCATCTGTCTTCAGGA			
A-117	MGC-EXB4-7-R1	GTAGAAGAGTCATCTGTCTTCAGGA	**148**	**102, 117, 203, 404, 210**	**60**
	MGC-EX4-F3	TCAGCCTGTCTGCGTACAAC			
B-204	MGC-EXB1A-2-F1	GGAGAATGAGCCTGGAATTG	**193**	**204**	**60**
	MGC-EXB2-4-R1	GTGGGCTCTCTTTACCTCCTT			
B-209	MGC-EXB1A-8-F1	GAGAATGAGCTGCTTCCCTGA	**146**	**209**	**65**
	MGC-EX9-R1	CCTGTGATGACAGCTTGGTG			
B-210	MGC-EXB1A-3-F1	GGAGAATGAGTATTTAGGTCAG	**291**	**210**	**55**
	MGC-EXB4-7-R1	GTAGAAGAGTCATCTGTCTTCAGGA			
B-213	MGC-EXB6-7as01-F1	TGCACAGCGATGACTCTTCT	**89**	**213**	**60**
	MGC-EX10as04-R1	GCCAAAGCTGTCAGTGATGA			
A-401	MGC-EXB1-3-F1	GGGCAAGAAGTATTTAGGTCAG	**138**	**401**	**60**
	MGC-EXB-4as01-10as01-R1	ATGTGGTGCTCCTGAGTCAA			
A-116	MGC-EXB1-2-F1	GGCAAGAAGCCTGGAATTGT	**193**	**116**	**60**
	MGC-EXB2-6-R2	CGCAGCGACCTTTACCTCCTT			
B-200	MGC-EXB1A-2-F1	GGAGAATGAGCCTGGAATTG	**120**	**200, 203, 204, 206**	**65**
	MGC-EX2-R2	AACACCACAGTGTGCAGAGG		**207, 208,213, 216,**	
B-201	MGC-EXB3-4-R1	GTGGGCTCTCTTTGCATTGT	**103**	**201, 202, 210, 214**	**60**
	MGC-EXB1A-3-F2	CCATGGAGAATGAGTATTTAGGTCAG			
A-402	MGC-EXB3-4as02-F2	CAAAGAGAGCCCACCAGCTT	**104**	**402**	**65**
	MGC-EXB4as02-10as02-R2	CTCATCCCCGTCCCTCCA			
A-404	MGC-EXB9-10as04-F2	GTTCCTGCTGCTTGGTCTGA	**87**	**404**	**65**
	MGC-EXB10as04-10as05-R2	CCATCATGGCCAATGGTCT			
AB-6A	MGC-EXB6A-7-F2	CTCCCTATTTCAAGATGACTCTTCTAC	**103**	**EX6A**	**60**
	MGC-EX8-R2	ACCACCCACATCCACAGATT			
CCM3	PDCD102F1	ATGAGGATGACAATGGAAGAGATGAAG	**307**	**PDCD10**	**65**
	PDCD101R2	GGTCTTGGAATTCTGGCTCTGGTCGTTC			
V5	EX10V5-F1	CCAGCACTTCGAGAACTTCC	**265**	**V5**	**65**
	EX10V5-R1	ATCTGCAGAATTGCCCTTtg			
Neo	Neo-F1	CTGAATGAACTGCAGGACGA	**172**	**Neo**	**65**
	Neo-R1	ATACTTTCTCGGCAGGAGCA			

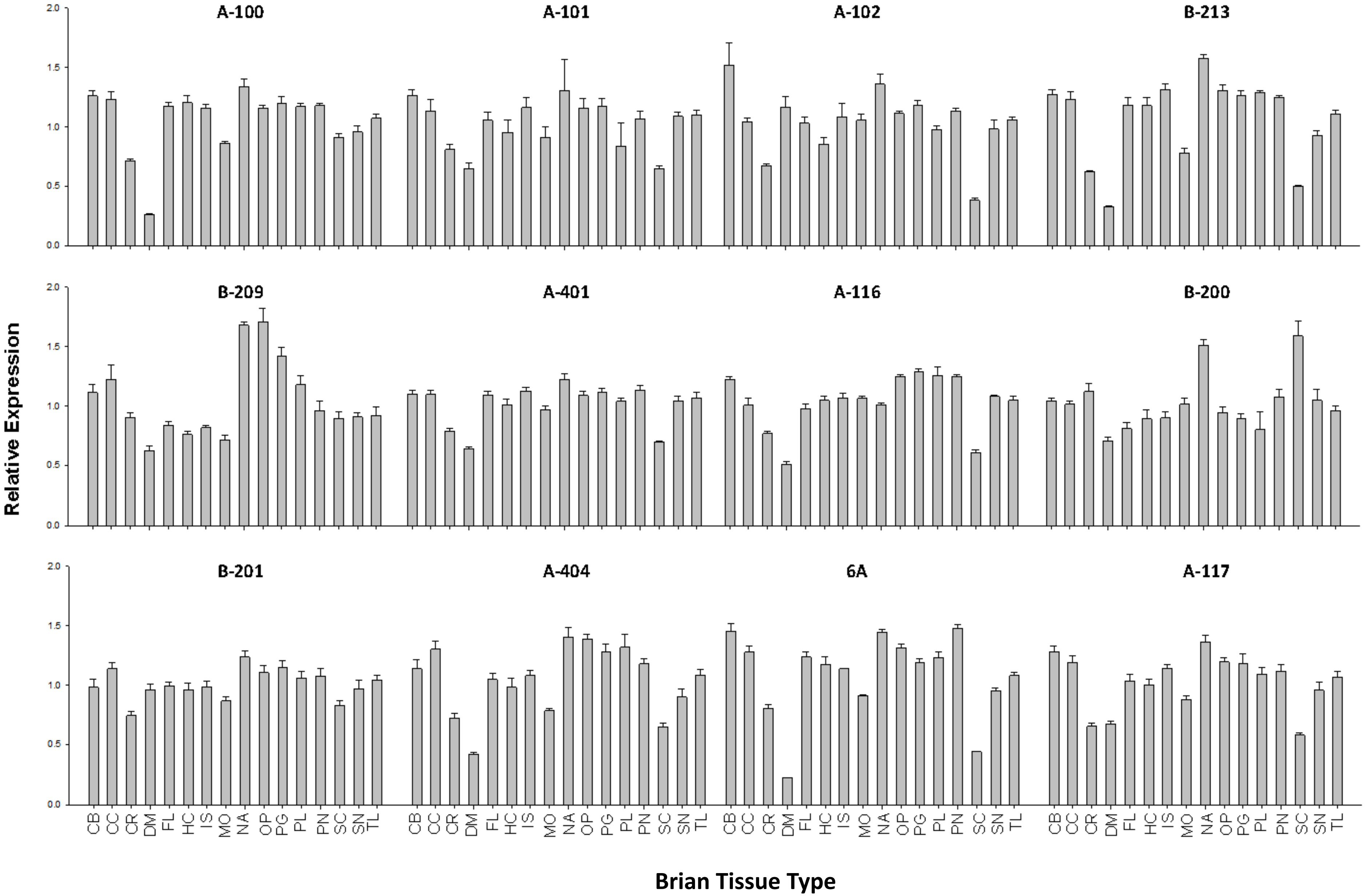
Suppl. Table 3

Isoform identifier	Length (aa)	PTB domain	Domain-2	Domain-3	Domain-4
CCM2-100*	444	61-221: PTB (161aa)	244-361:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-101	386	1-163:PTB (162aa)	186-303:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-102	295	1-143:PTB (143aa)	95-212:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-107	251	NO PTB	51-168:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-109	142	NO PTB	1-59:Protein of unknown function (DUF1722) (59 aa)	SSL2: DNA or RNA helicases of superfamily II	Excinuclease ABC subunit A
CCM2-116	309	NO PTB	1-59:Protein of unknown function (DUF1722) (59 aa)	SSL2: DNA or RNA helicases of superfamily II	Flagellar hook-associated protein L
CCM2-117	353	60-201: PTB (142aa)	153-270:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-402	153	NO PTB	4-153:N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase		
CCM2-404	334	60-201: PTB (142aa)		SSL2: DNA or RNA helicases of superfamily II	
CCM2-600	485	60-221: PTB (162aa)	285-402:Protein of unknown function (DUF1722)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-601	427	1-163: PTB (163aa)	227-344:Protein of unknown function (DUF1722)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-602	188	60-188: PTB (129aa)	44-188: Type 1 glutamine amidotransferase (GATase1)-like domain		
CCM2-200	465	81-242: PTB (162aa)	265-382:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-201	407	22-184: PTB (163aa)	207-324:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	1-238: Thiamin transporter substrate binding subunit
CCM2-203	374	81-222: PTB (142aa)	174-291:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-204	437	52-214: PTB (163aa)	237-354:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	1-187:tRNA pseudouridine synthase C
CCM2-206	506	81-242: PTB (162aa)	306-423:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-210	316	22-164: PTB (143aa)	116-233:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-212	272	NO PTB	72-189:Protein of unknown function (DUF1722) (118 aa)	SSL2: DNA or RNA helicases of superfamily II	
CCM2-213	354	81-242: PTB (162aa)	1-296:tbpA (thiamin transporter substrate binding subunit)(296aa)		
CCM2-216	415	81-242: PTB (162aa)	215-333:Protein of unknown function (DUF1722) (119 aa)	SSL2: DNA or RNA helicases of superfamily II	PRK07168

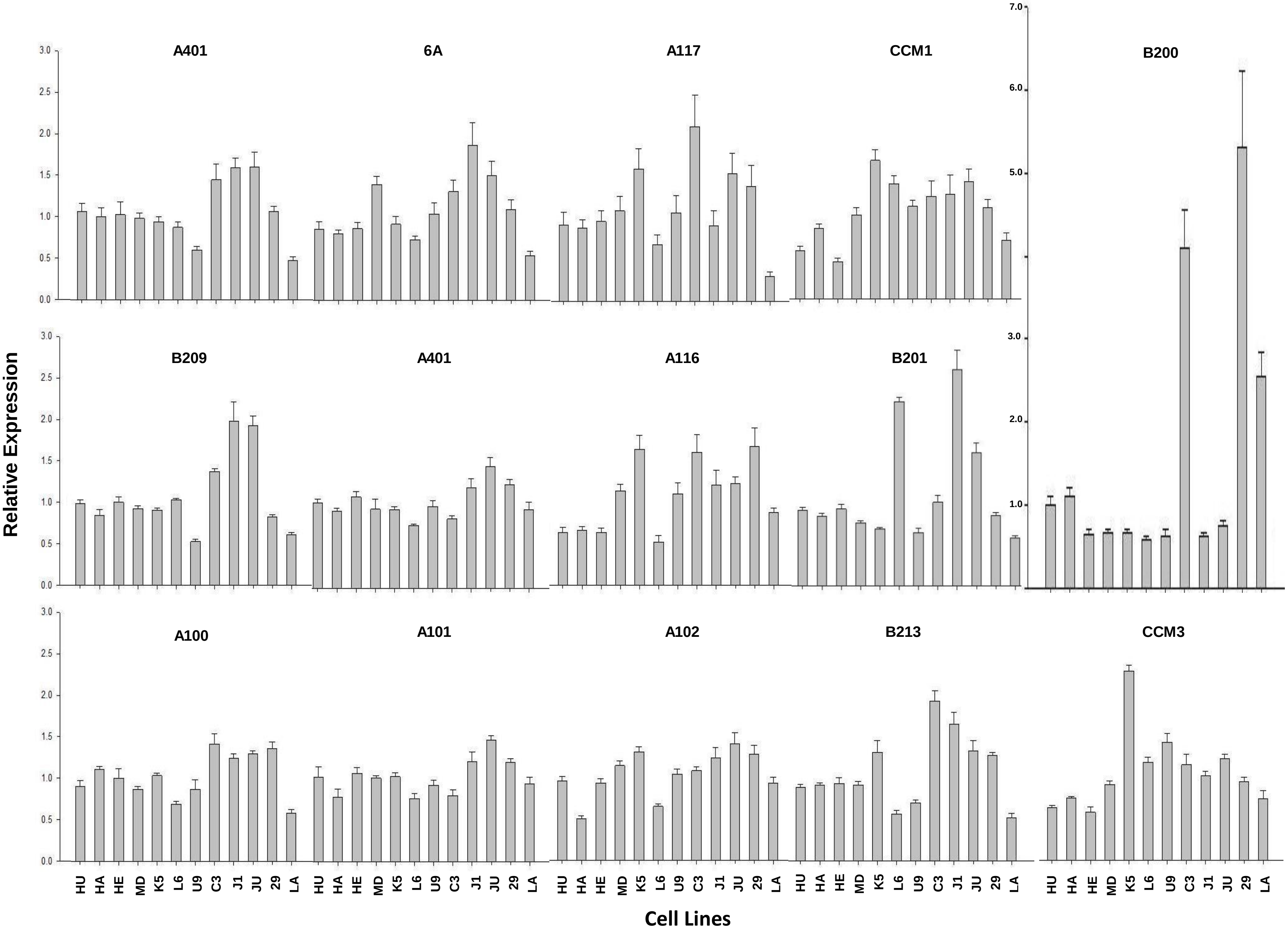
Suppl. Table 4

Motif	**Sequence**	**Length** (aa)	***Domains***
CCM2-Exon6	**VAAEELCCLLGQVFQVVYTE*STIDFLDRAIFDGAST*PTHHLSLHS**	**45**	**No putative conserved domain** **No relatipnship with PTB 1**
Isoform	*CCM2-107, CCCM2-116, CCM2-212*		
CCM2-Exon6A	**ASHVTSSTVTSSFDCQLPLHFRTLAITLSPPKFSRVISLFQ**	**41**	**No putative conserved domain** **No relatipnship with PTB 1**
Isoform	*CCM2-600, CCM2-601, CCM2-606, CCM2-607,CCM2-206*		
CCM2-Exon6B	**DHGQTHGKLLRLPWEASWQWWSSGRWGRMEGTPELSLPAWPTQHFQE**	**47**	**No putative conserved domain** **No relatipnship with PTB 1**
Isoform	*CCM2-610*		

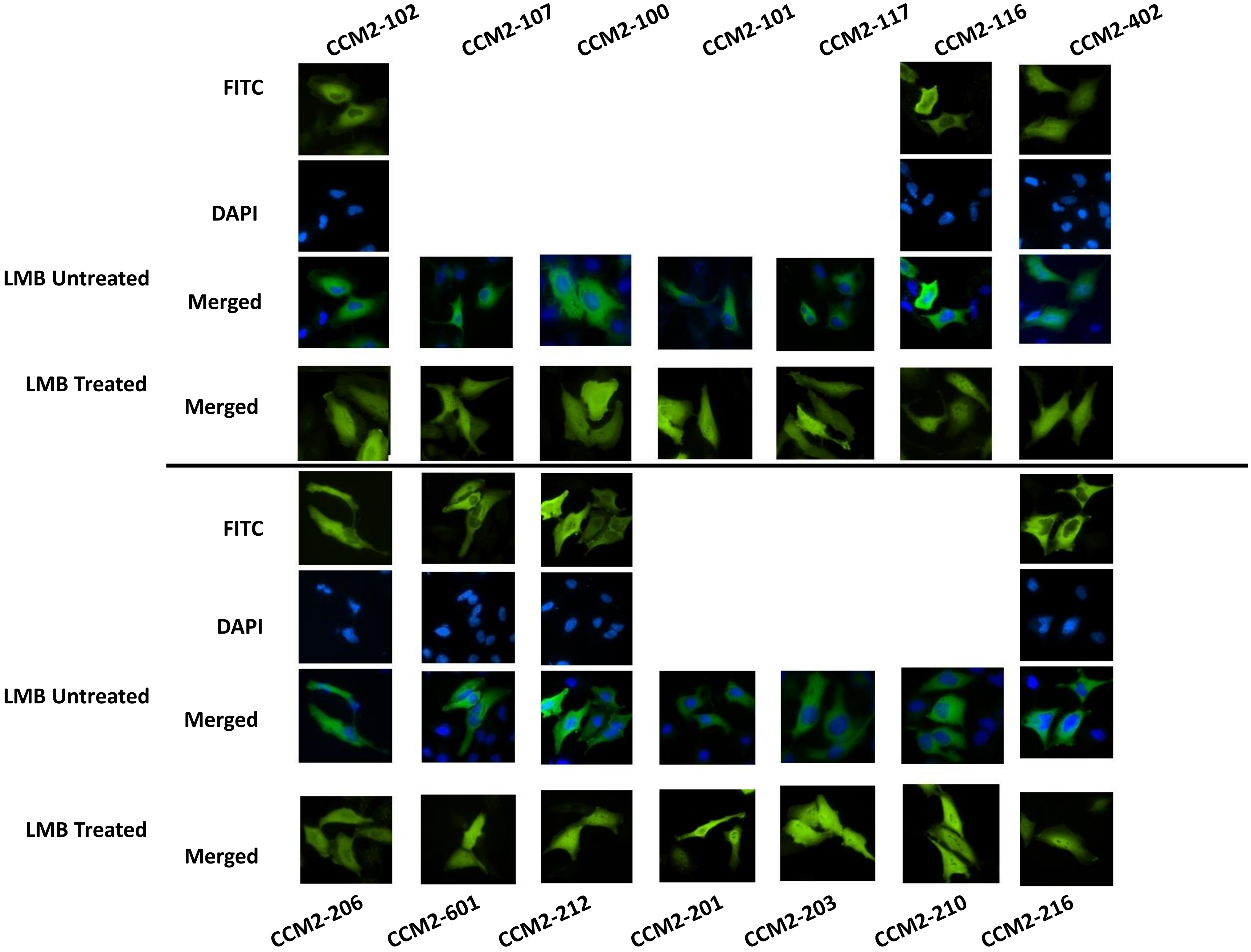
Suppl. Fig. 1A Brain



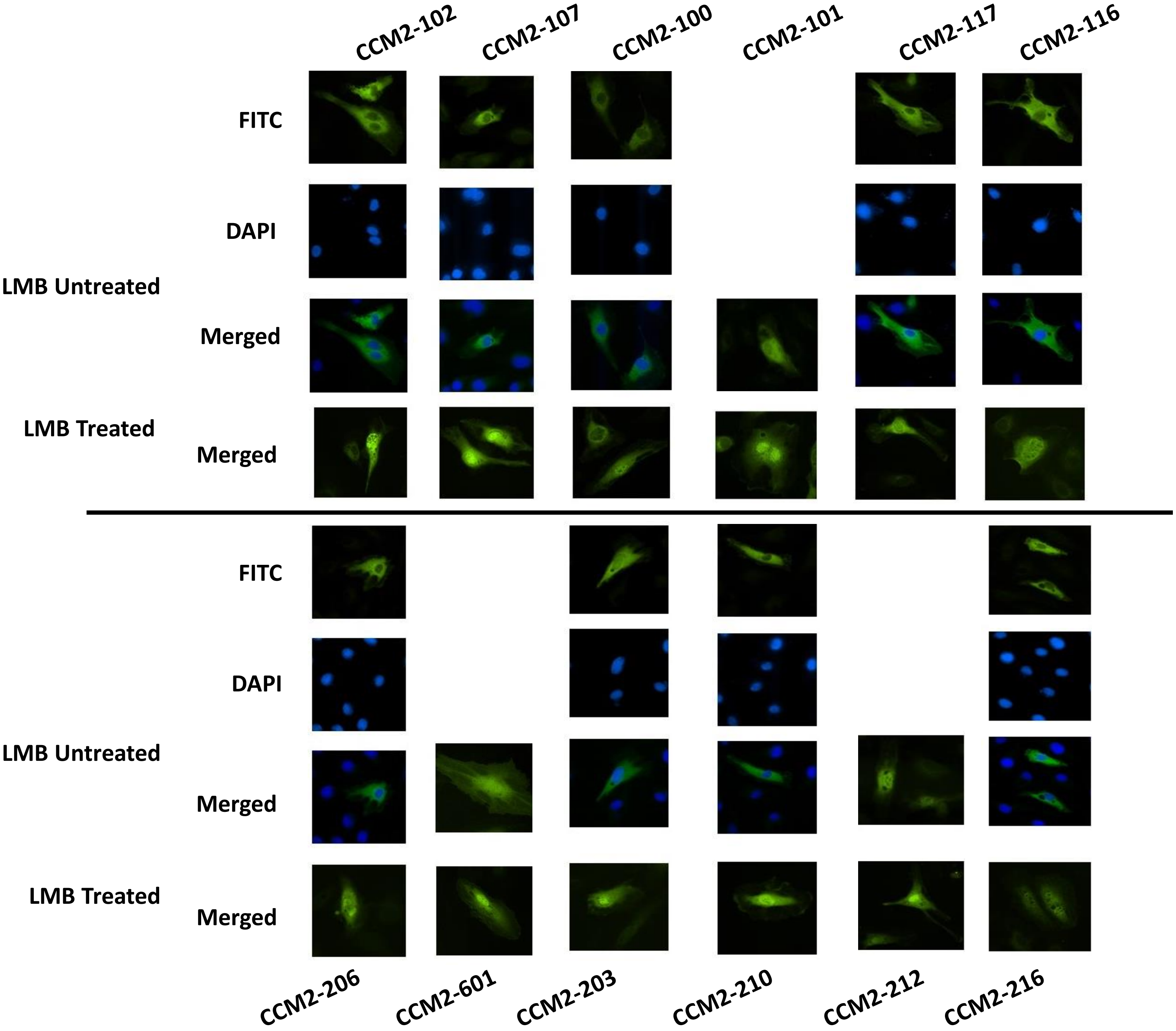
Suppl. Fig. 1B Cell



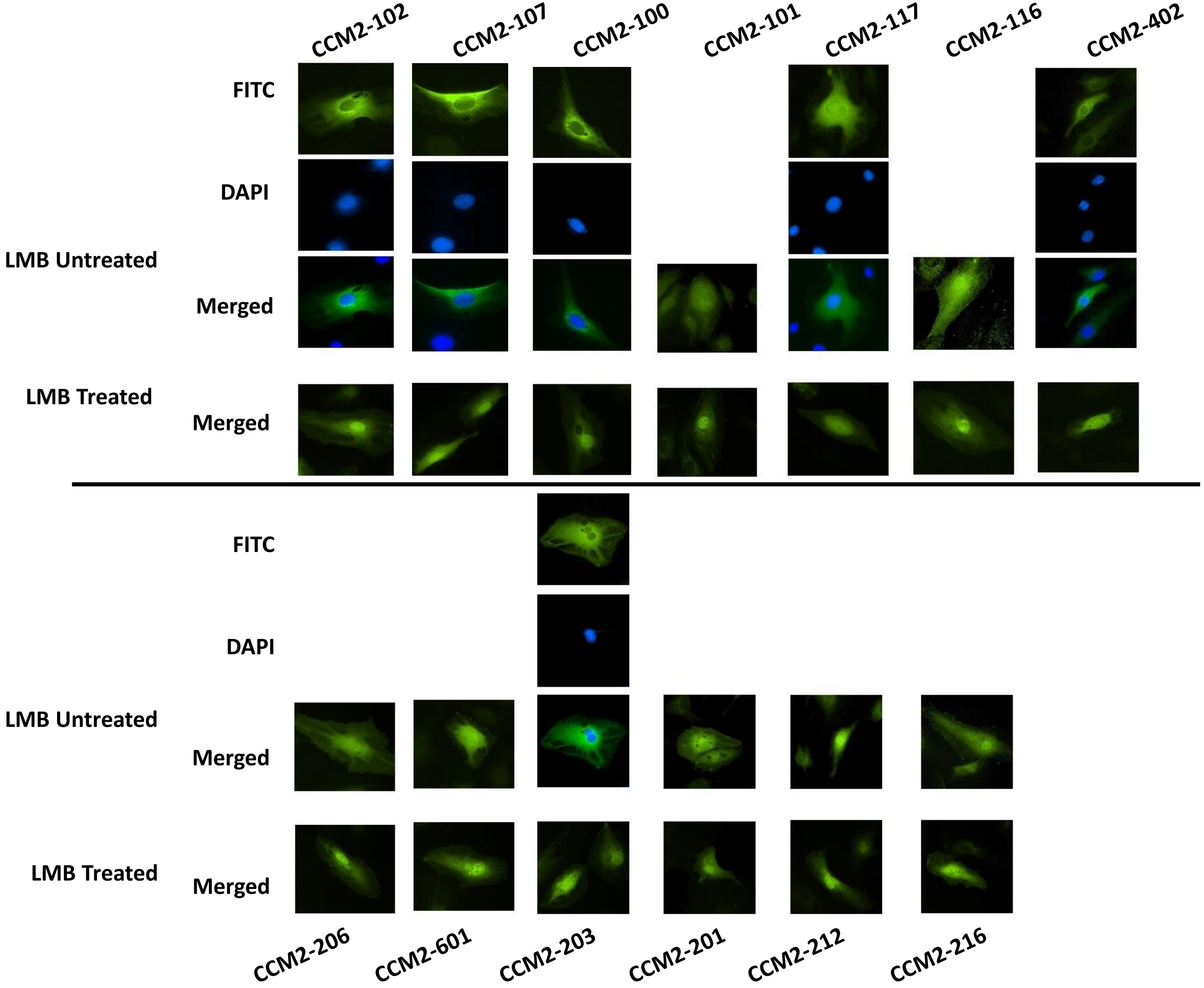
Suppl. Fig. 2A HeLa



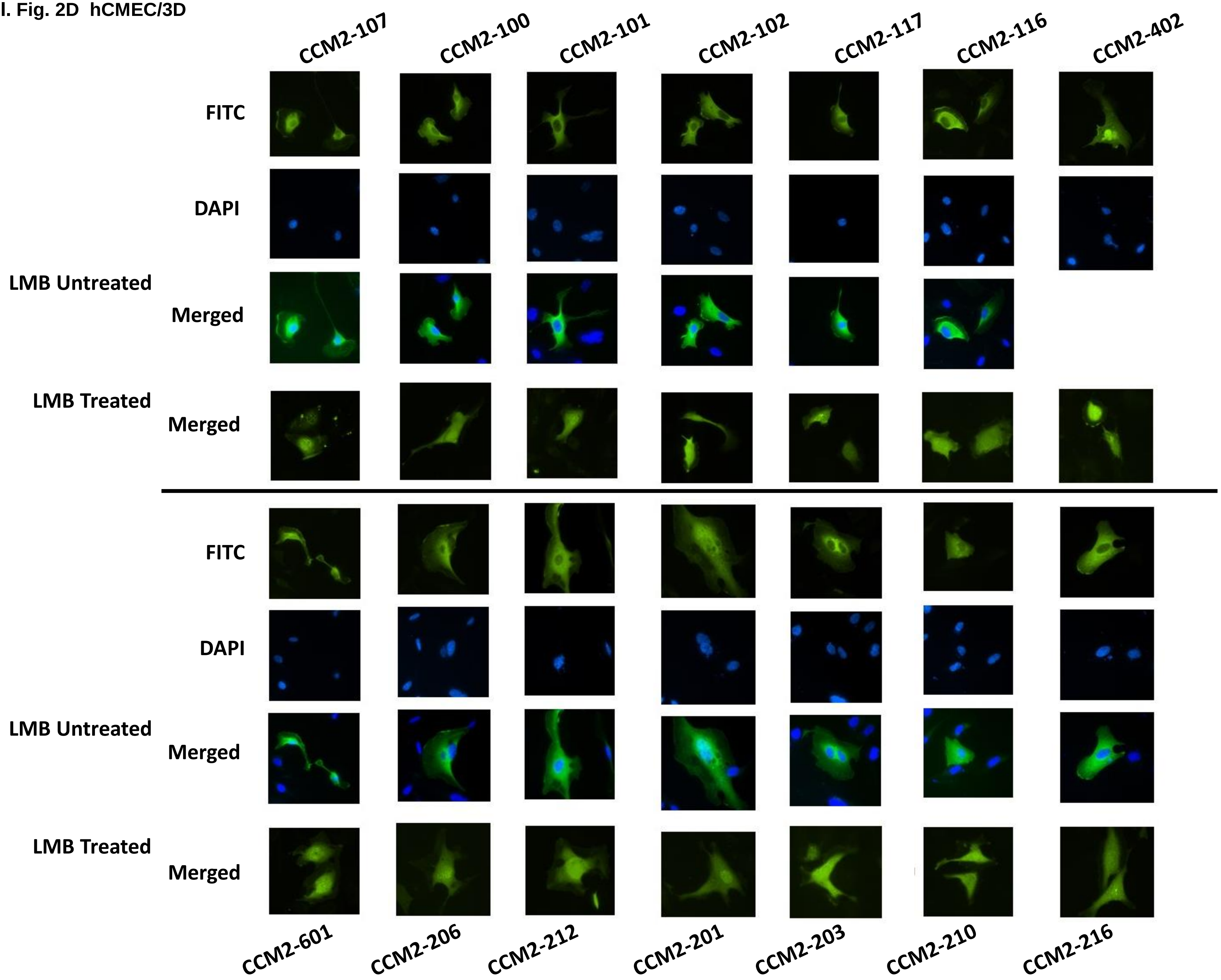
Suppl. Fig. 2B HUVEC



Suppl. Fig. 2C HMVEC



Suppl. Fig. 2D hCMEC/3D



3B1

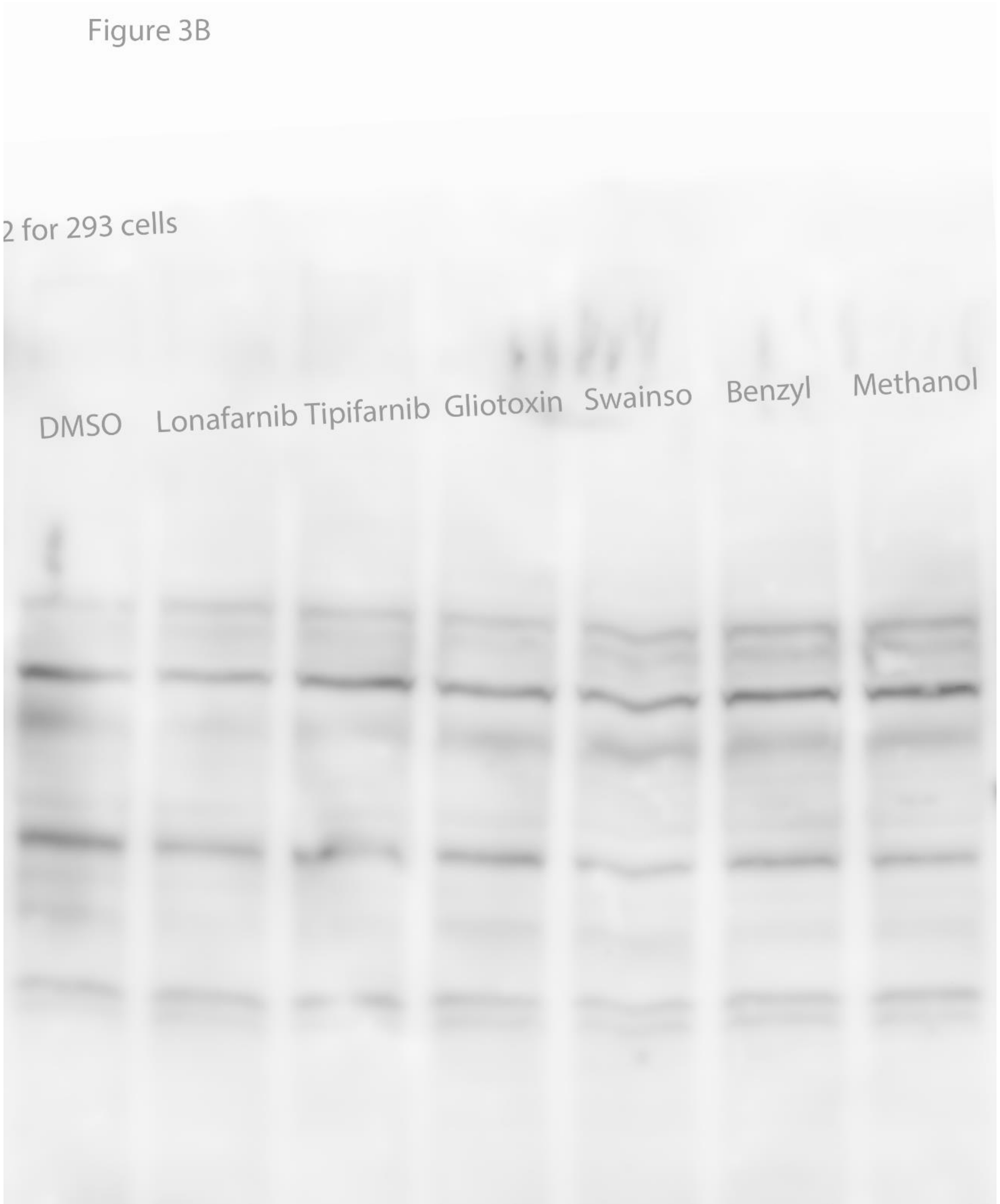


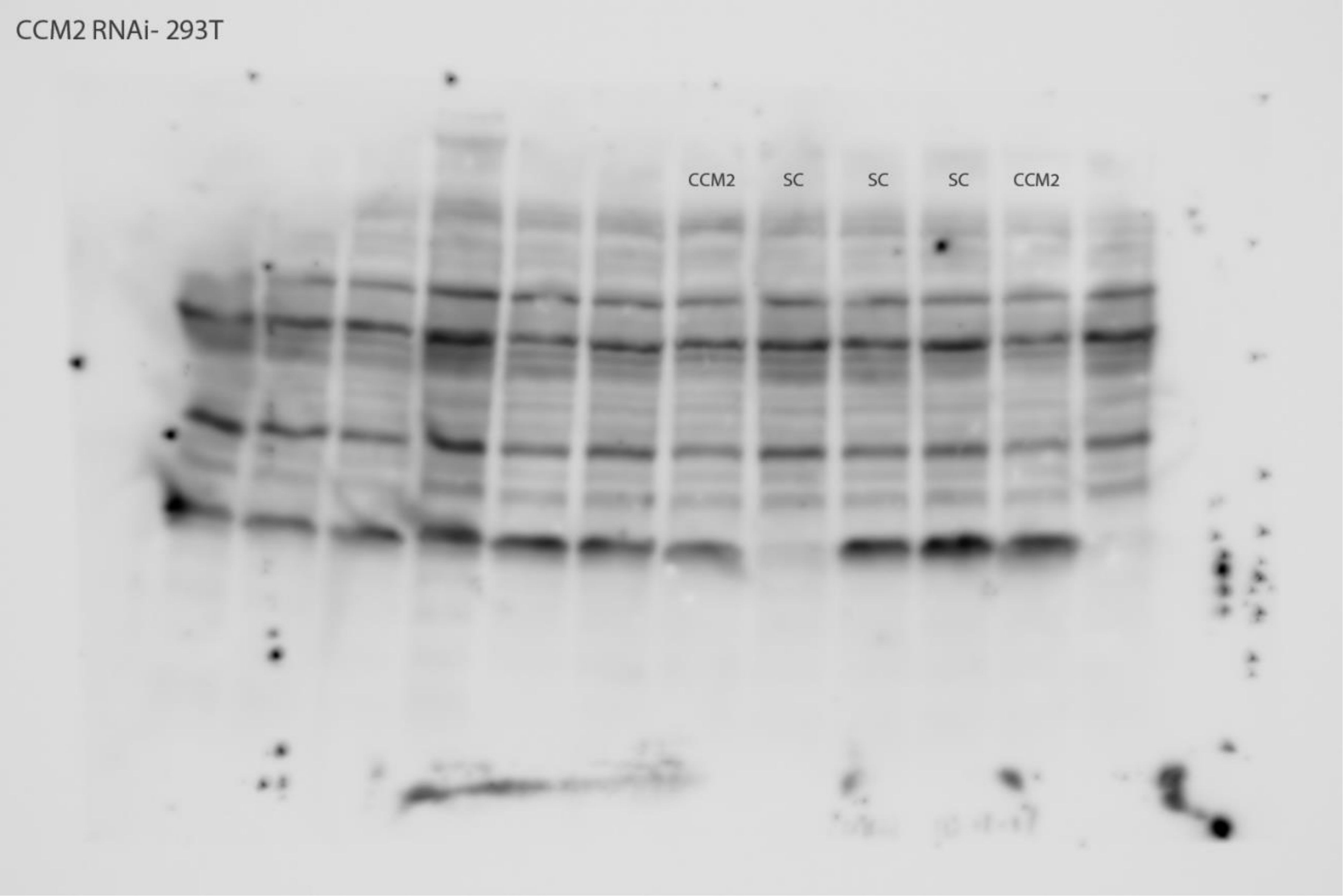
Figure 3B
2 for 293 cells
DMSO
Lonafarnib
Tipifarnib
Gliotoxin
Swainso
Benzyl
Methanol

3B2

Fig.3B. Actinin control

for 293 cells

3C1



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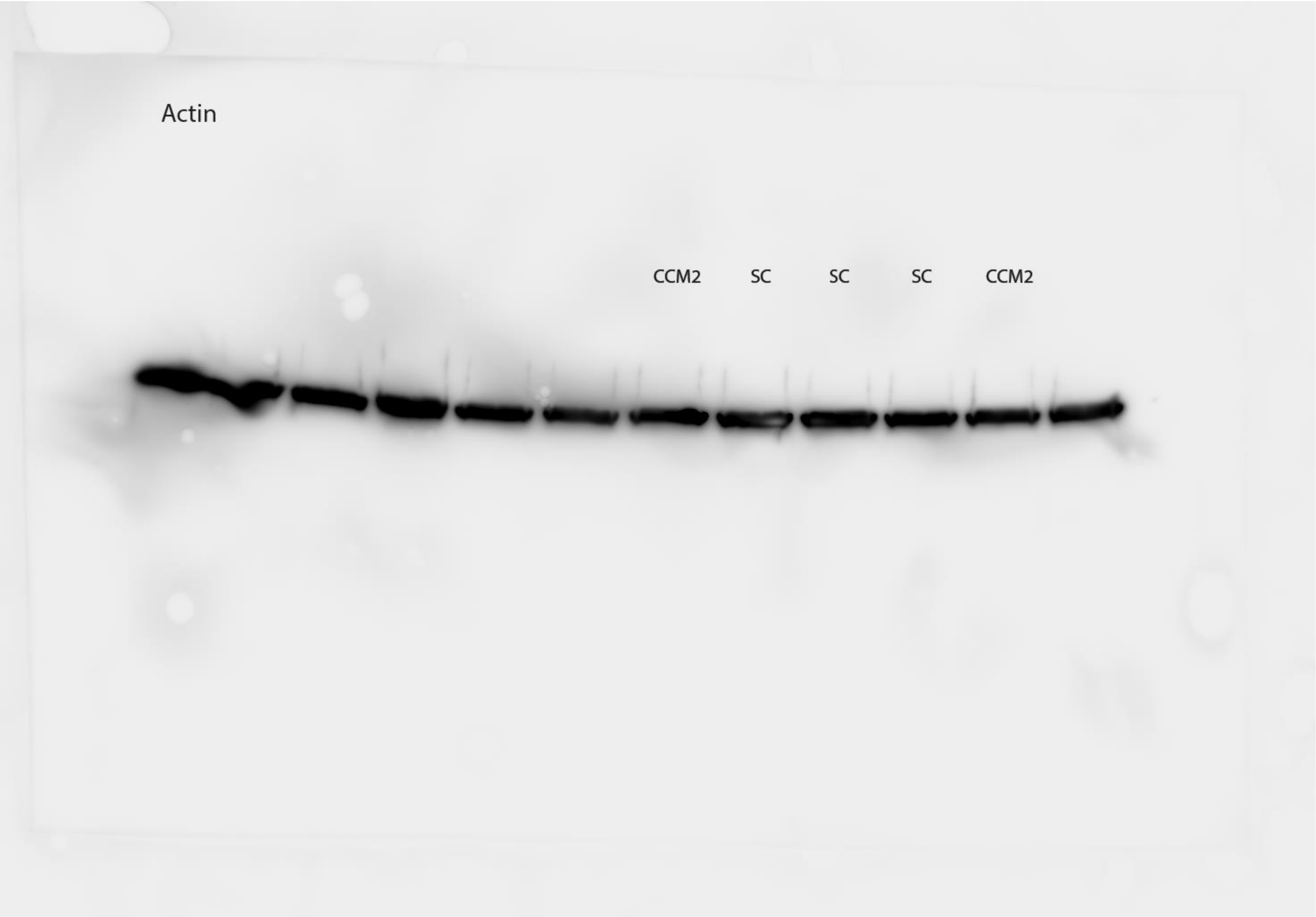

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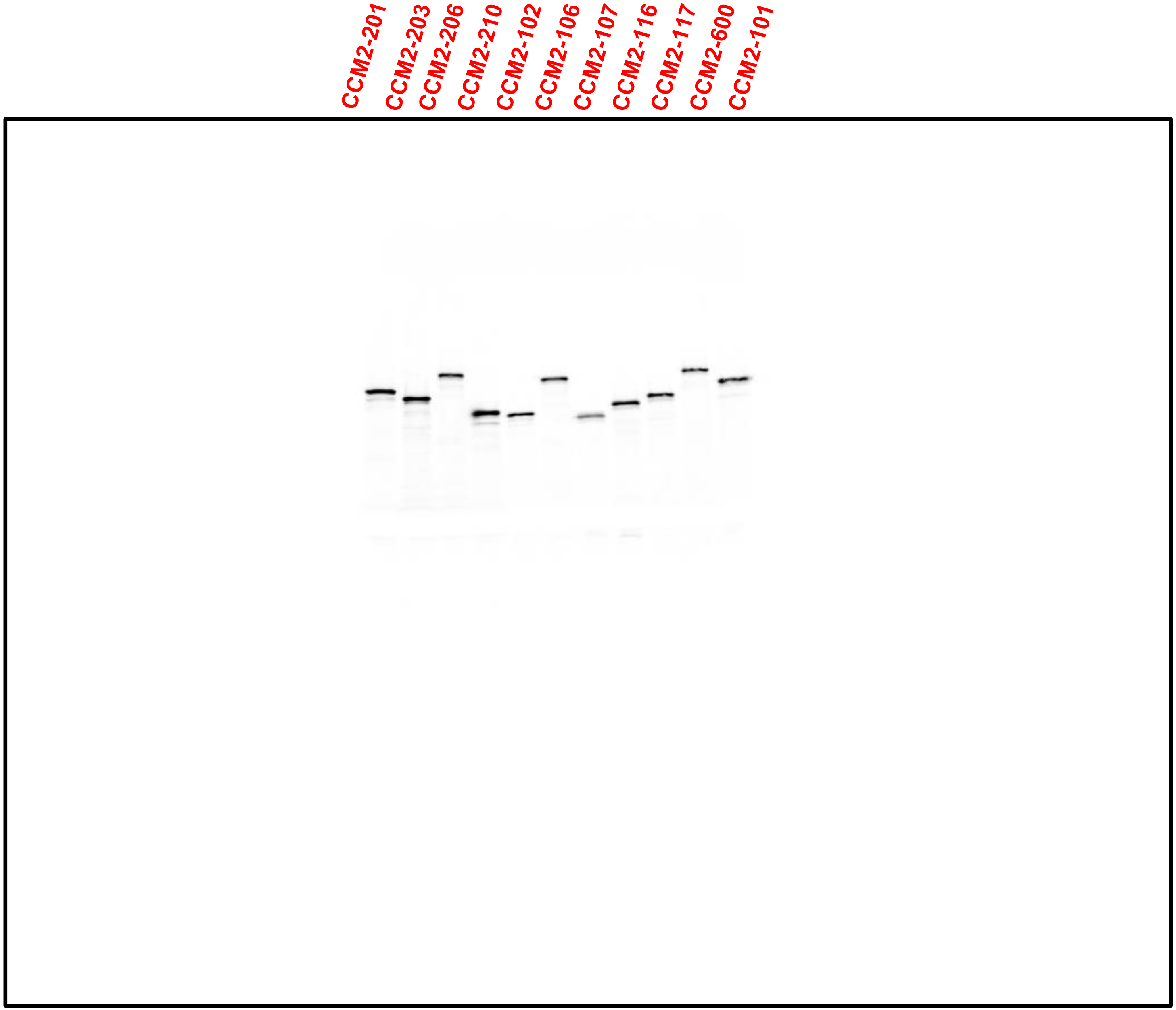
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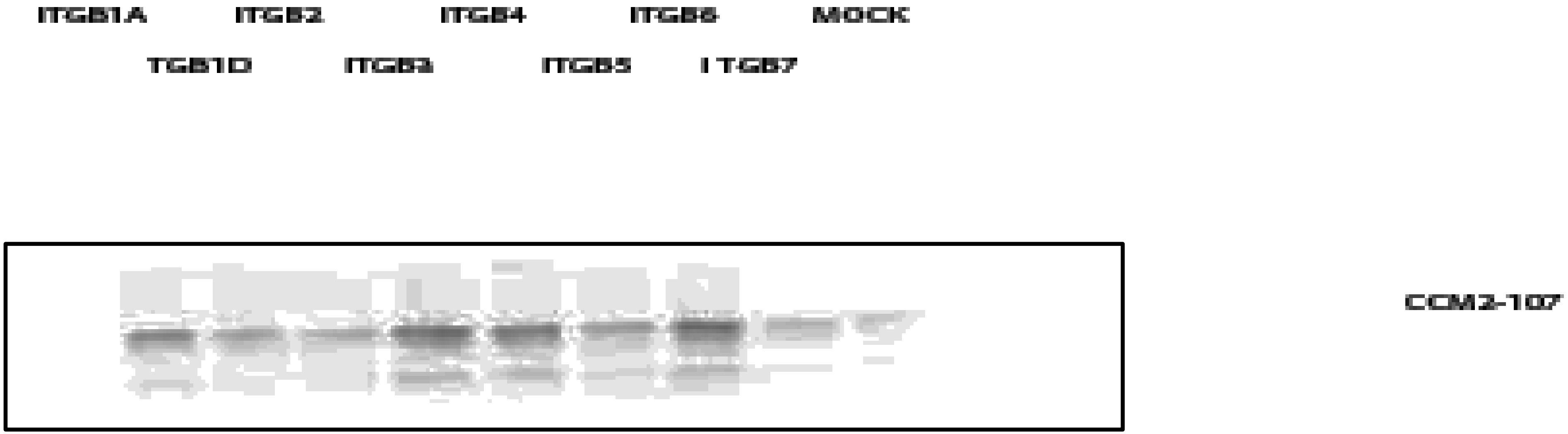
6A1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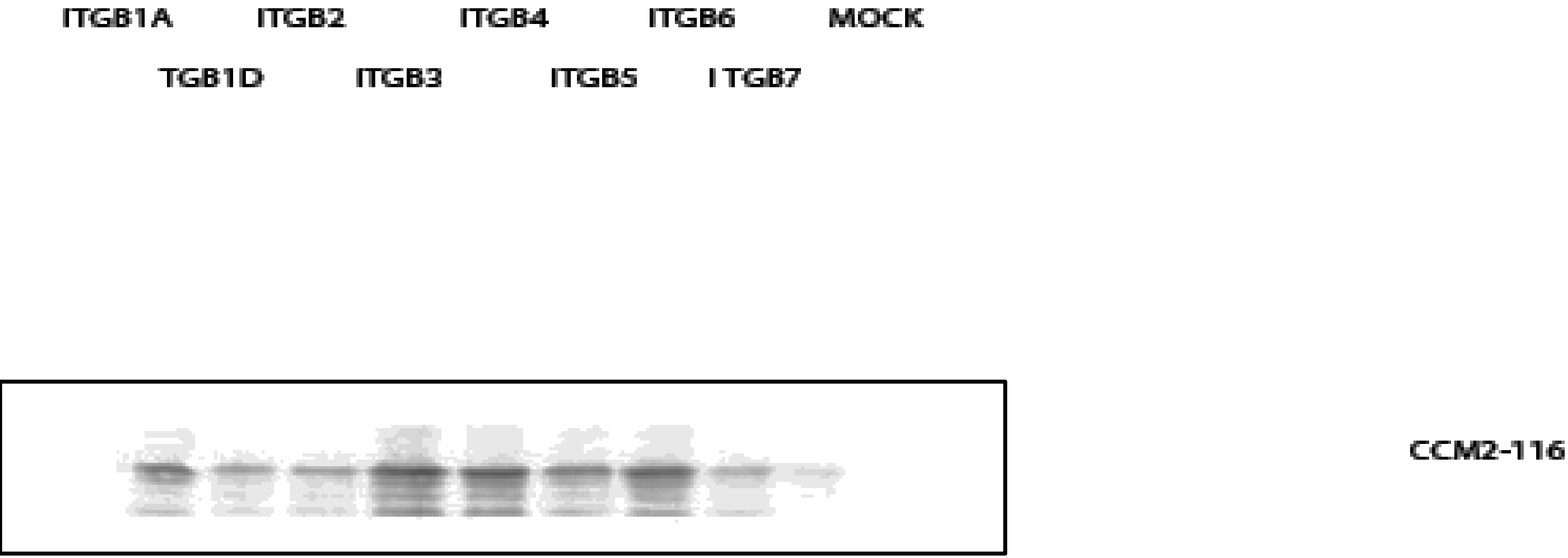
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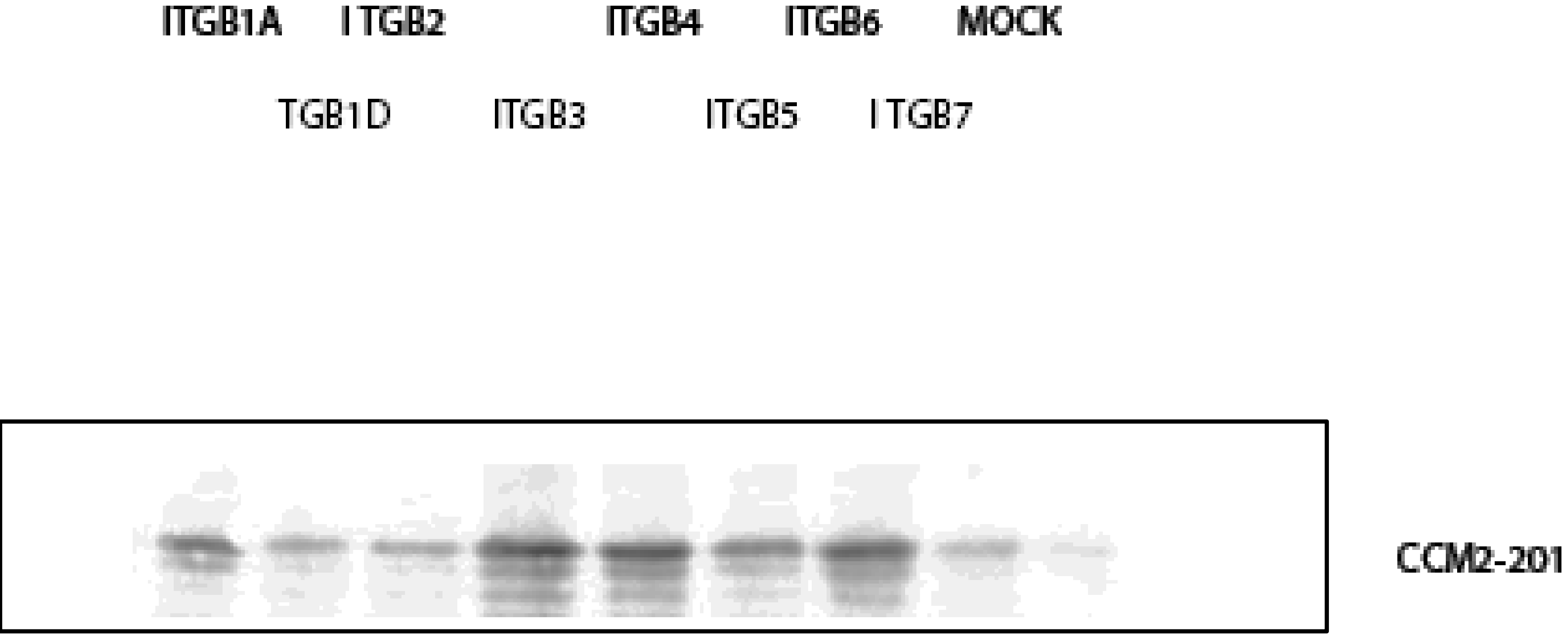
6A3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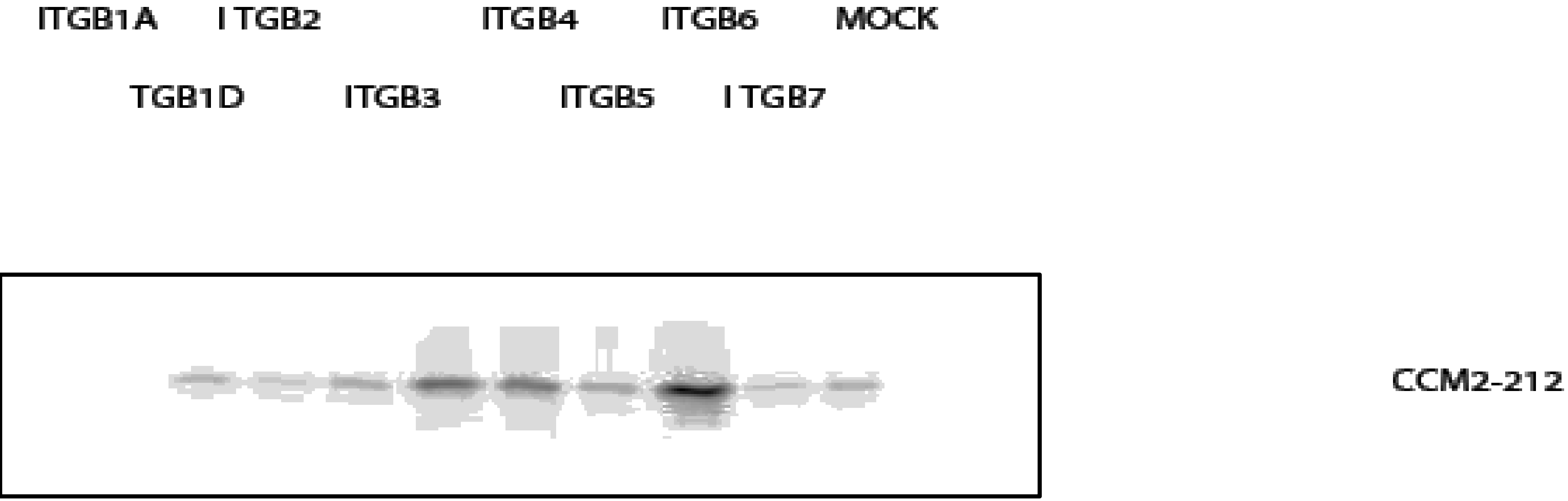
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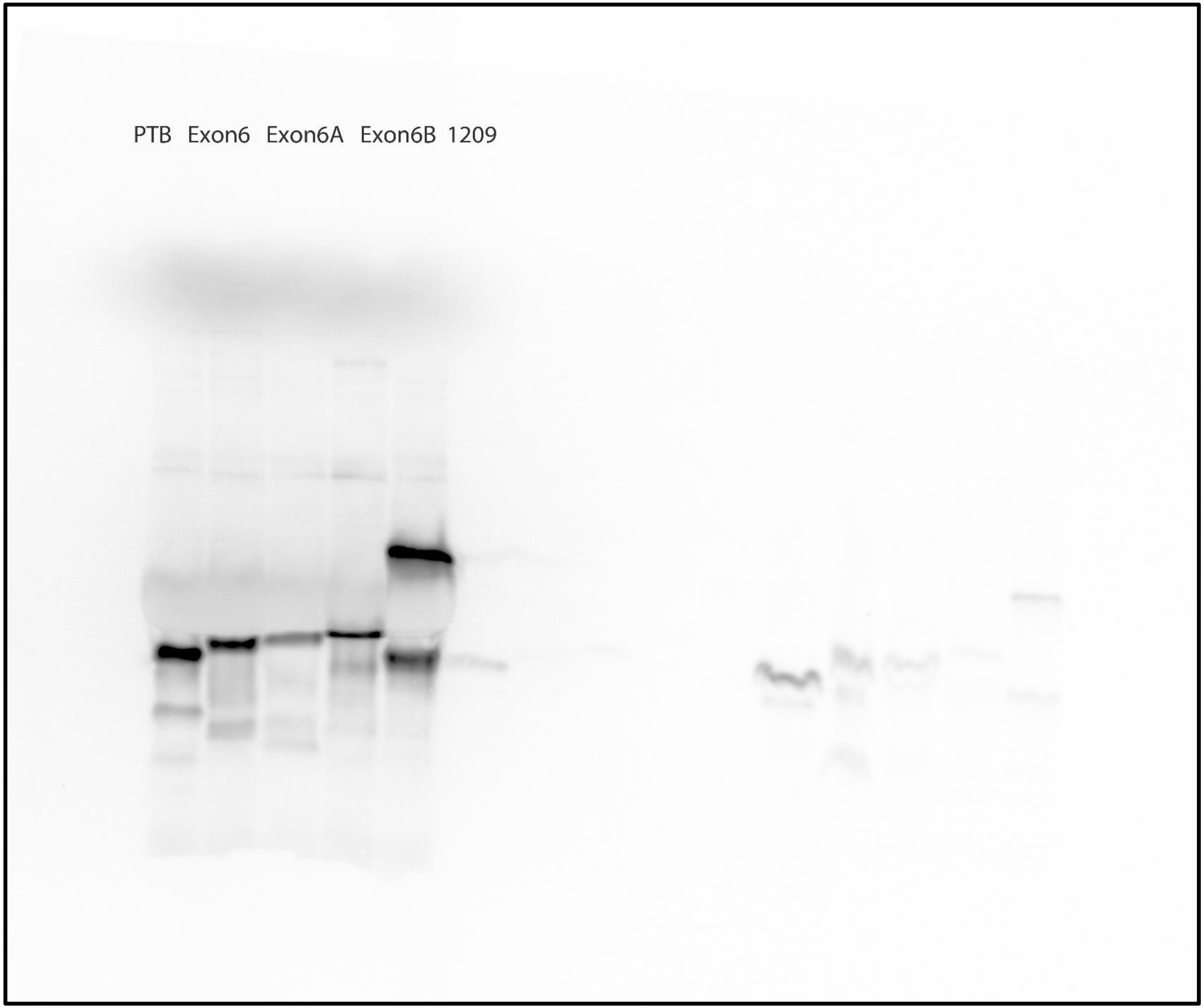
6B1



6B2


K5
ITGB3
ITGB2
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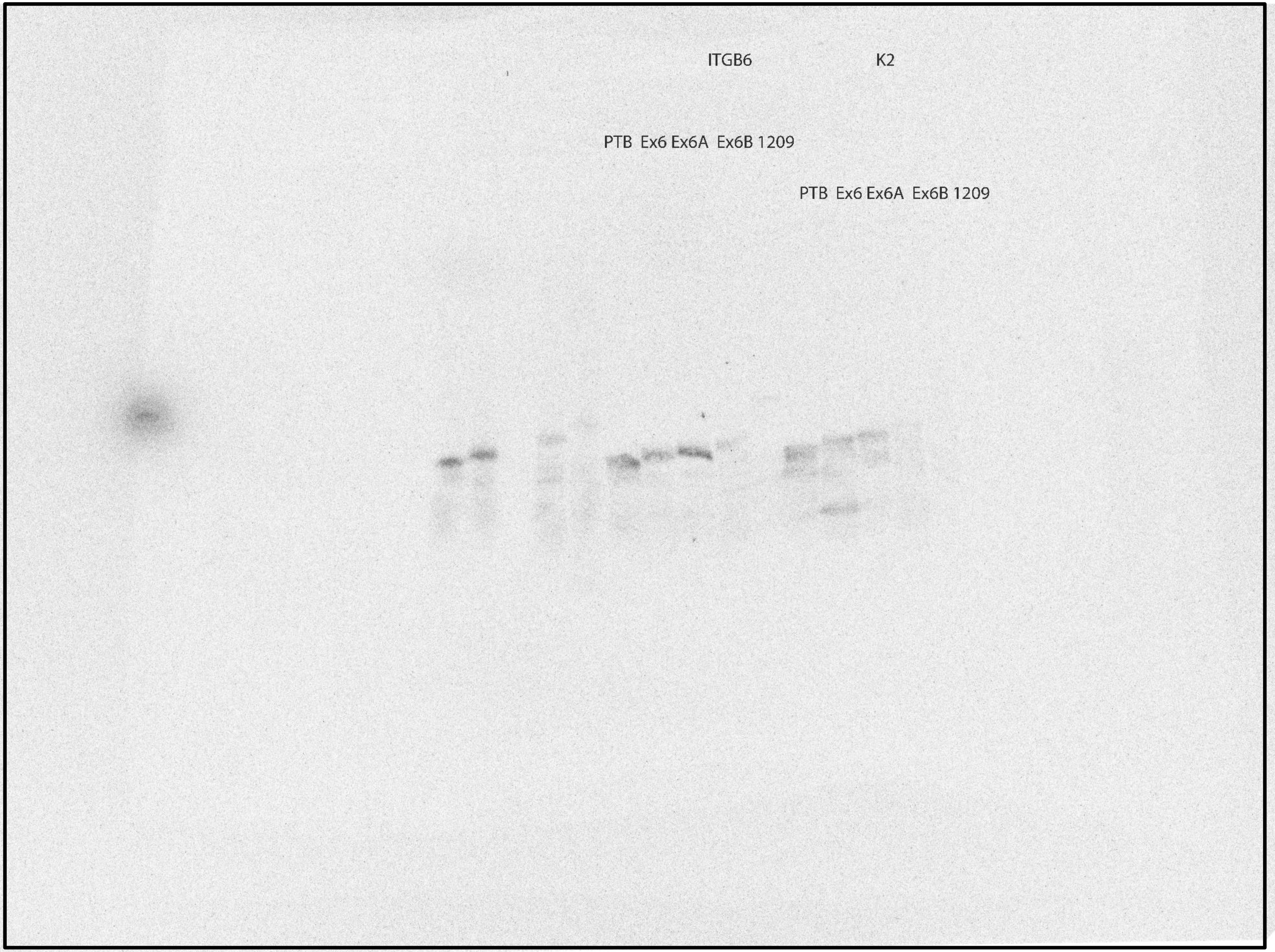
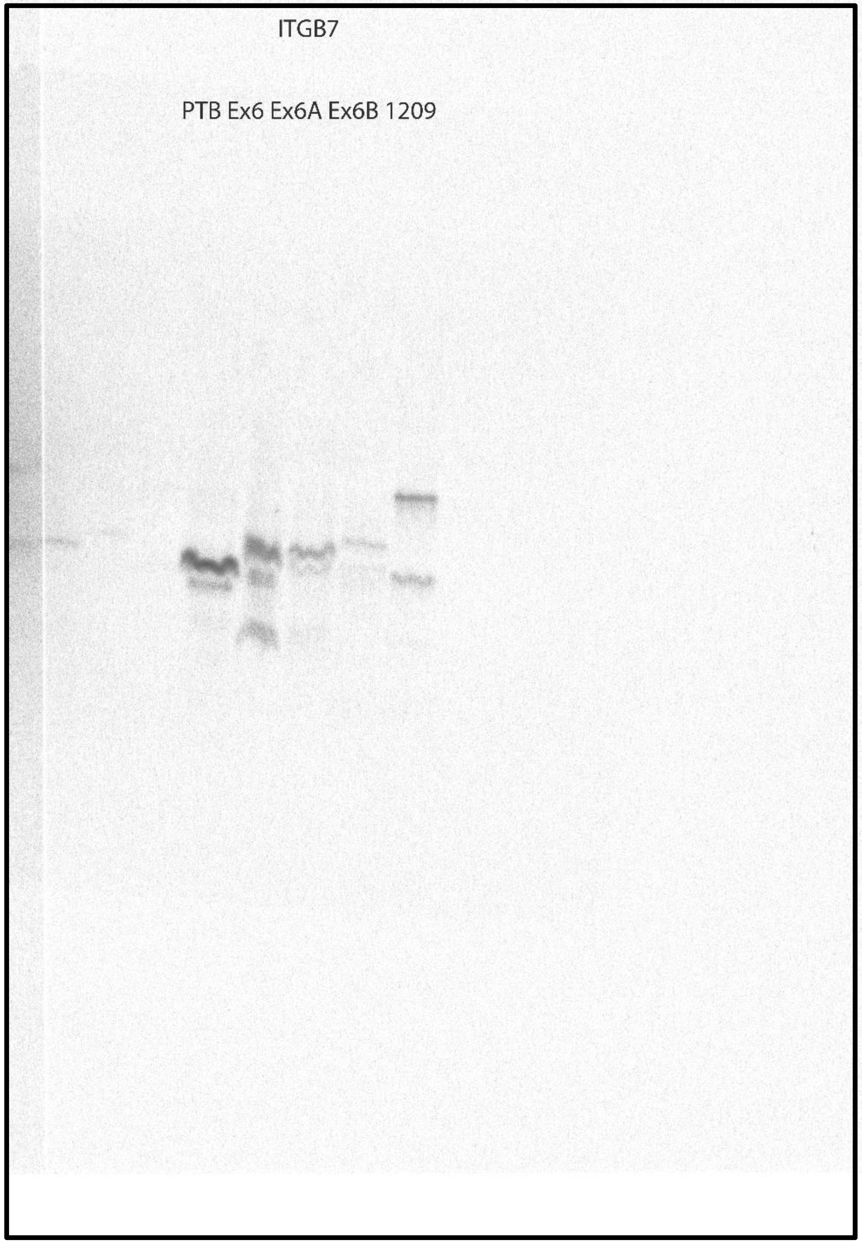
PTB Ex6 Ex6A Ex6B 1209
PTB Ex6 Ex6A Ex6B 1209

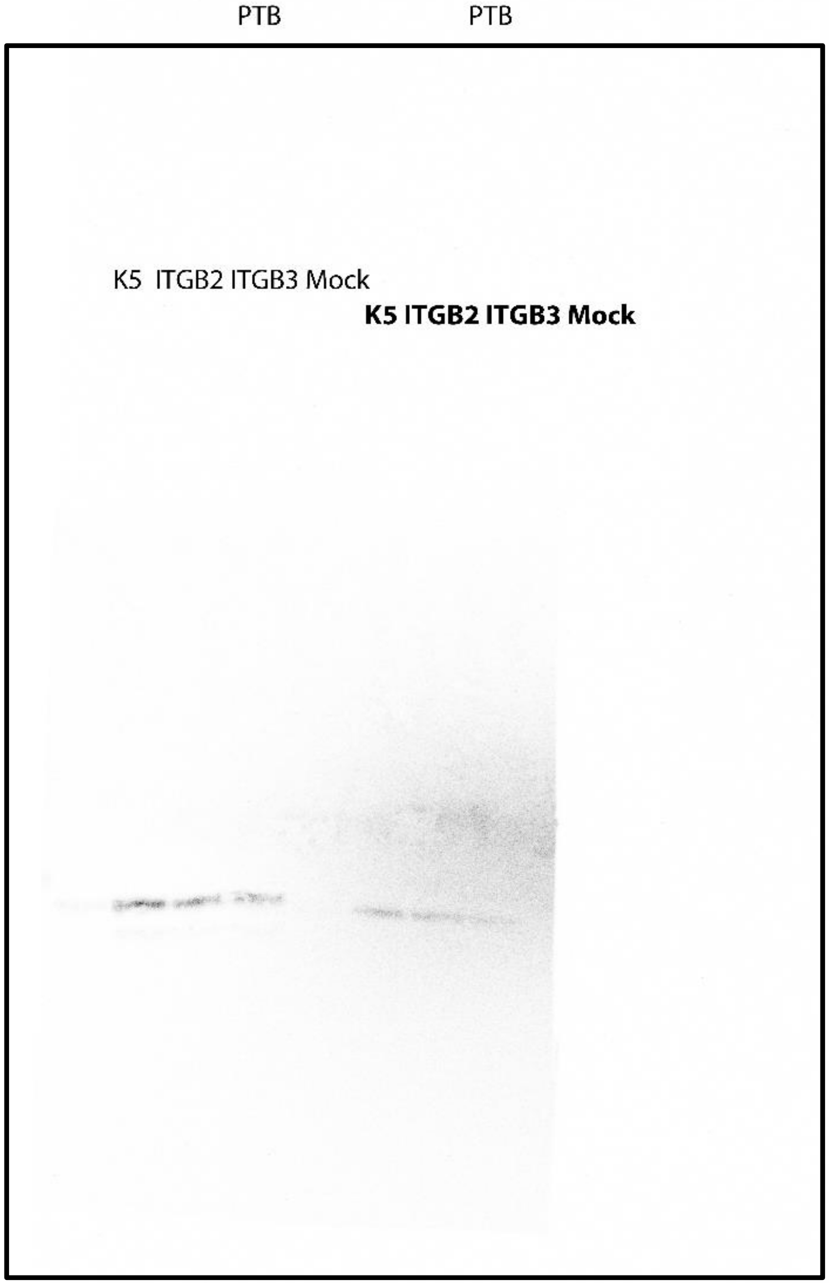
6B3

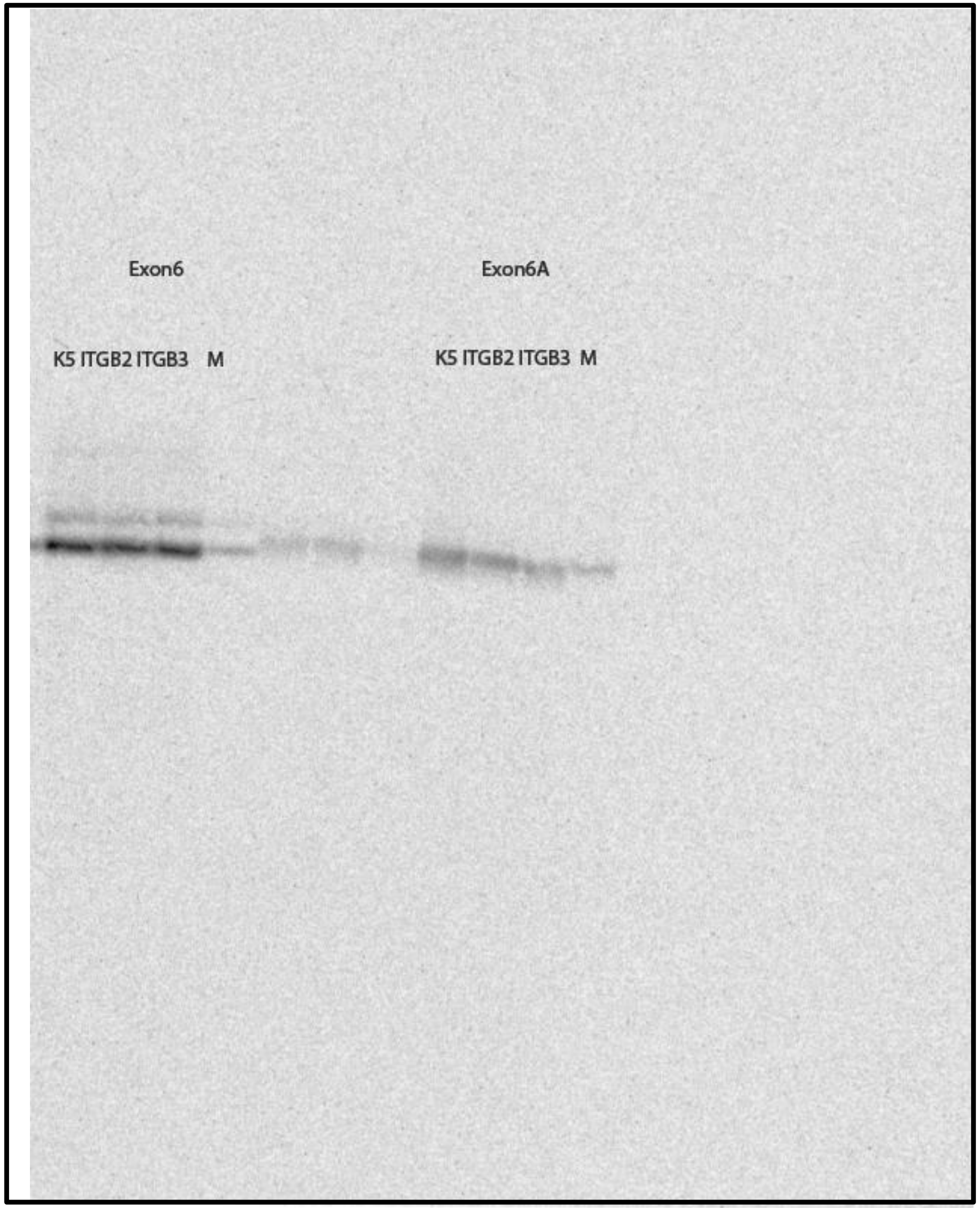


6B4



6C1




Exon6
K5 ITGB2 ITGB3 M
Exon6A
K5 ITGB2 ITGB3 M