

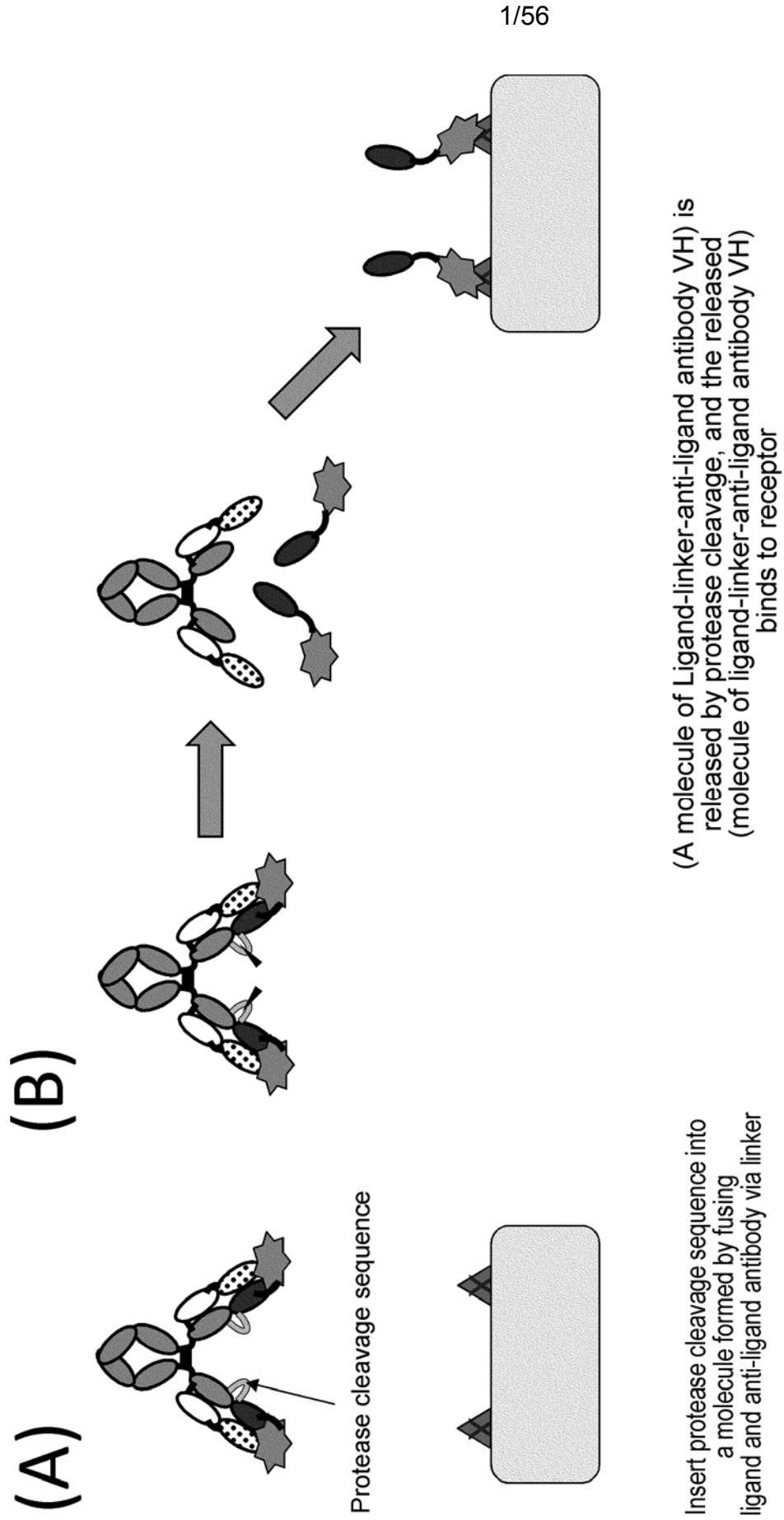
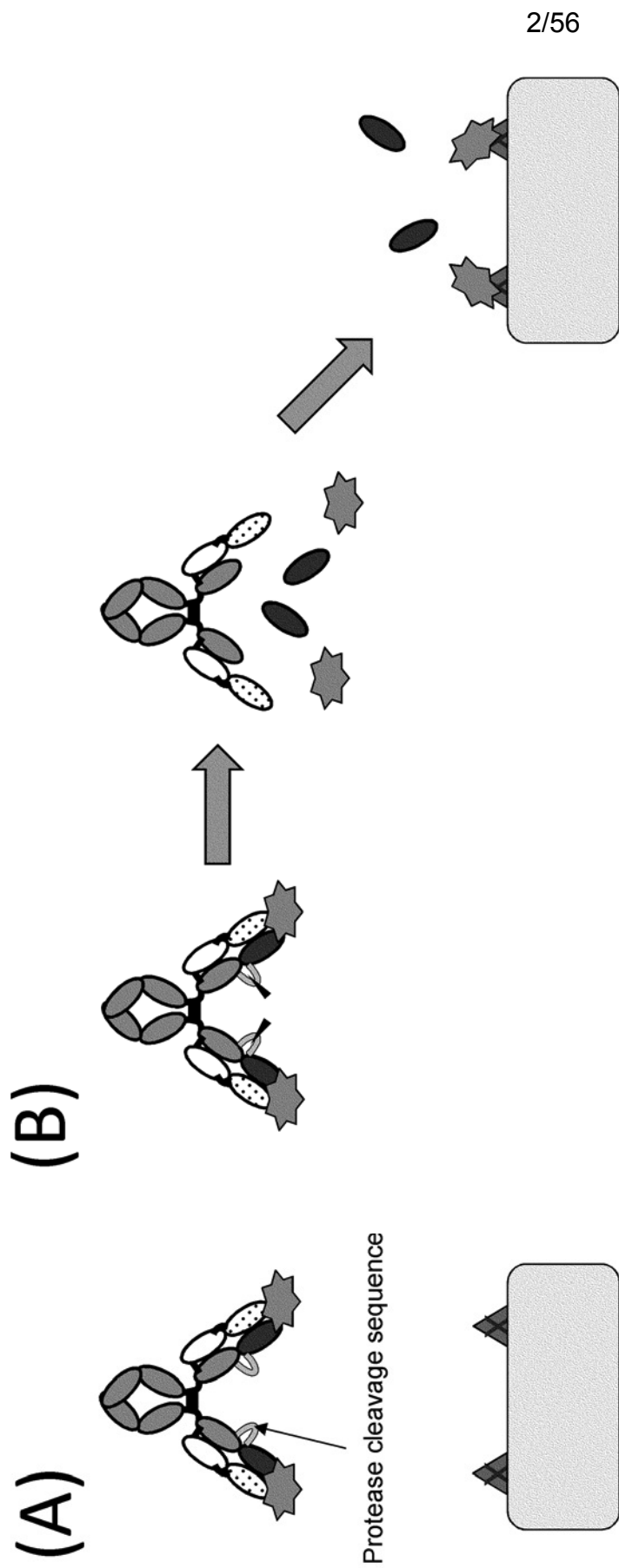


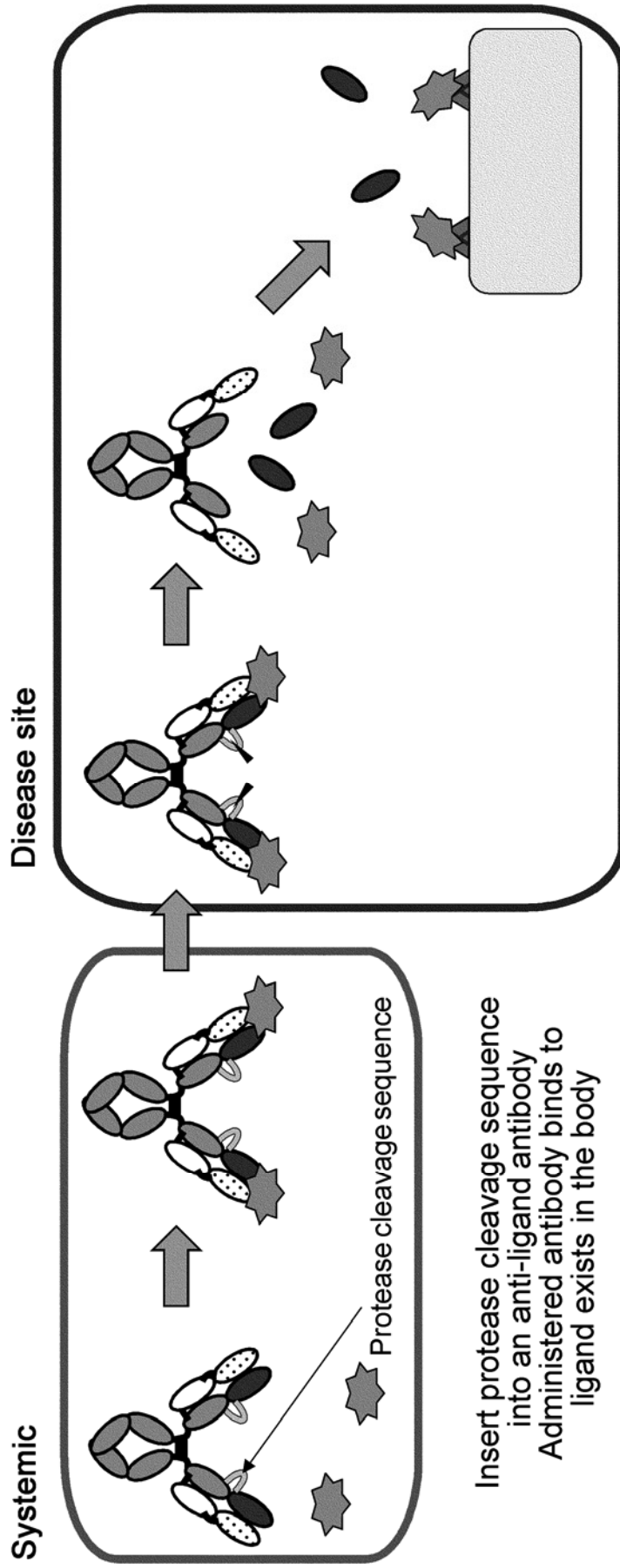
FIG. 1



Insert protease cleavage sequence
into an anti-ligand antibody

Ligand and VH molecule are released from
ligand-anti-ligand antibody complex by protease cleavage, and
the released ligand binds to receptor

FIG. 2



Ligand and VH molecule are released from
ligand-anti-ligand antibody complex by protease cleavage, and
the released ligand binds to receptor

FIG. 3

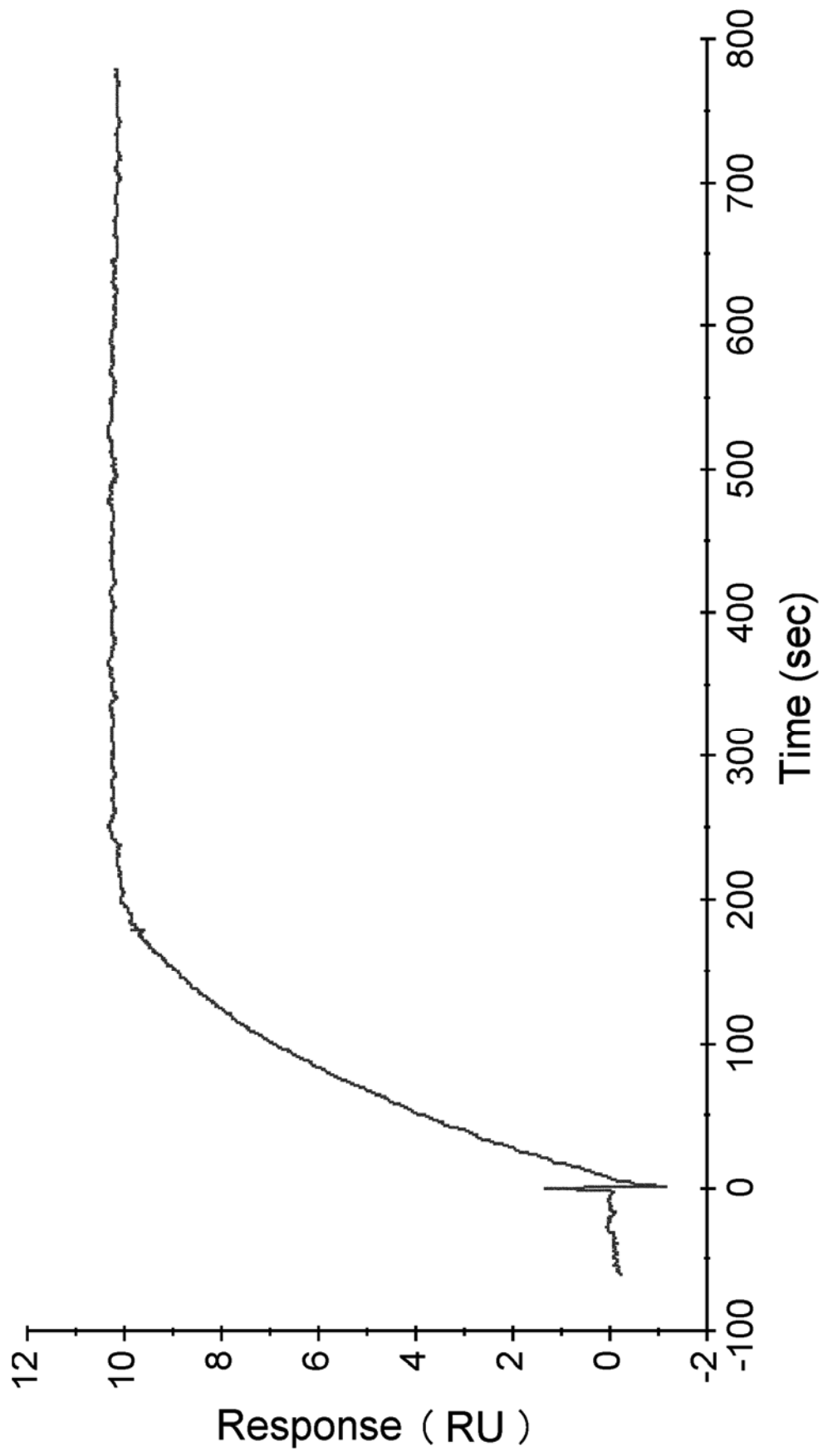
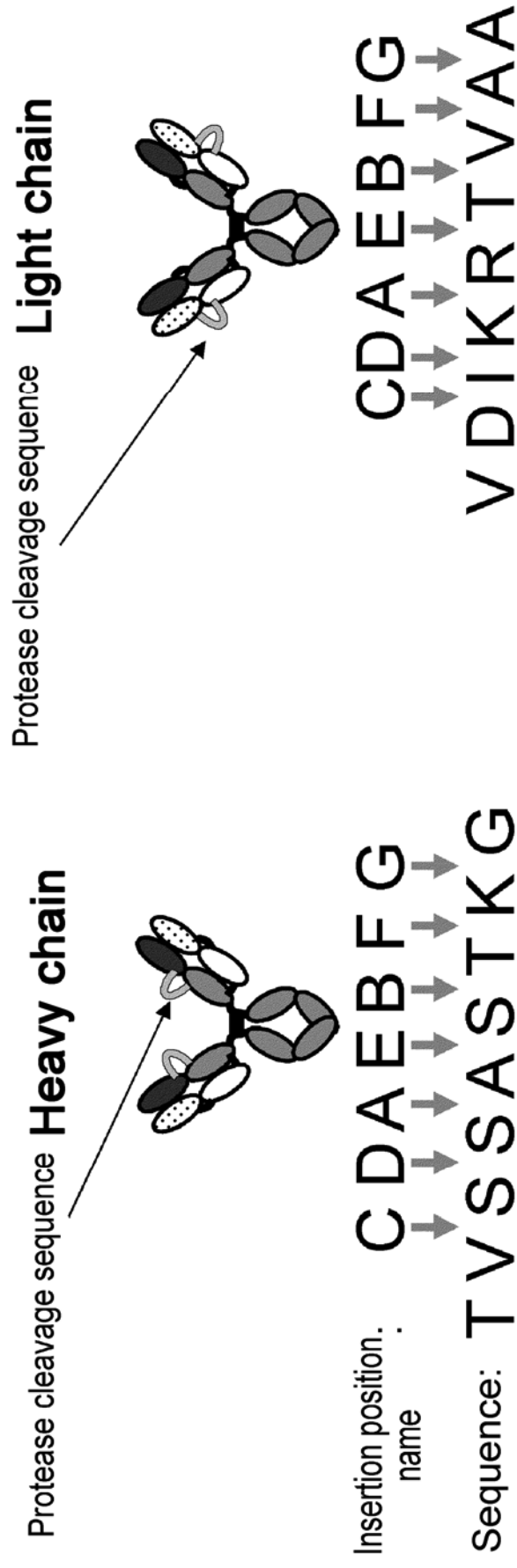


FIG. 4

(A)



Protease cleavage sequence was inserted at each position from insertion position names A to G in variable and constant regions

FIG. 5A

(B) Heavy chain

Heavy chain variant name	Protease cleavage sequence	insertion position	Inserted sequence
EEIVHA	TVSS [insert]	ASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHB	TVSSAS [insert]	TKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHC	TV [insert]	SSASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHD	TVS [insert]	SASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHE	TVSSA [insert]	STKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHF	TVSSAST [insert]	KGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHG	TVSSASTK [insert]	GP	LSGRSDNH (SEQ ID NO: 3)
EEIVHBG	TVSSAS [insert]	GKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHCG	TV [insert]	GSASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHDG	TVS [insert]	GASTKGP	LSGRSDNH (SEQ ID NO: 3)
EEIVHEG	TVSSA [insert]	GTKGP	LSGRSDNH (SEQ ID NO: 3)

FIG. 5B

(C) Light chain

Light chain variant name	Protease cleavage sequence insertion position	Inserted sequence
EEIVLA	VDIK [insert] RTVAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLB	VDIKRT [insert] VAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLC	VD [insert] IKRTVAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLD	VDI [insert] KRTVAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLE	VDIKR [insert] TVAAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLF	VDIKRTV [insert] AAP	LSGRSDNH (SEQ ID NO: 3)
EEIVLG	VDIKRTVA [insert] AP	LSGRSDNH (SEQ ID NO: 3)
EEIVLEG	VDIKR [insert] GVAAP	LSGRSDNH (SEQ ID NO: 3)

FIG. 5C

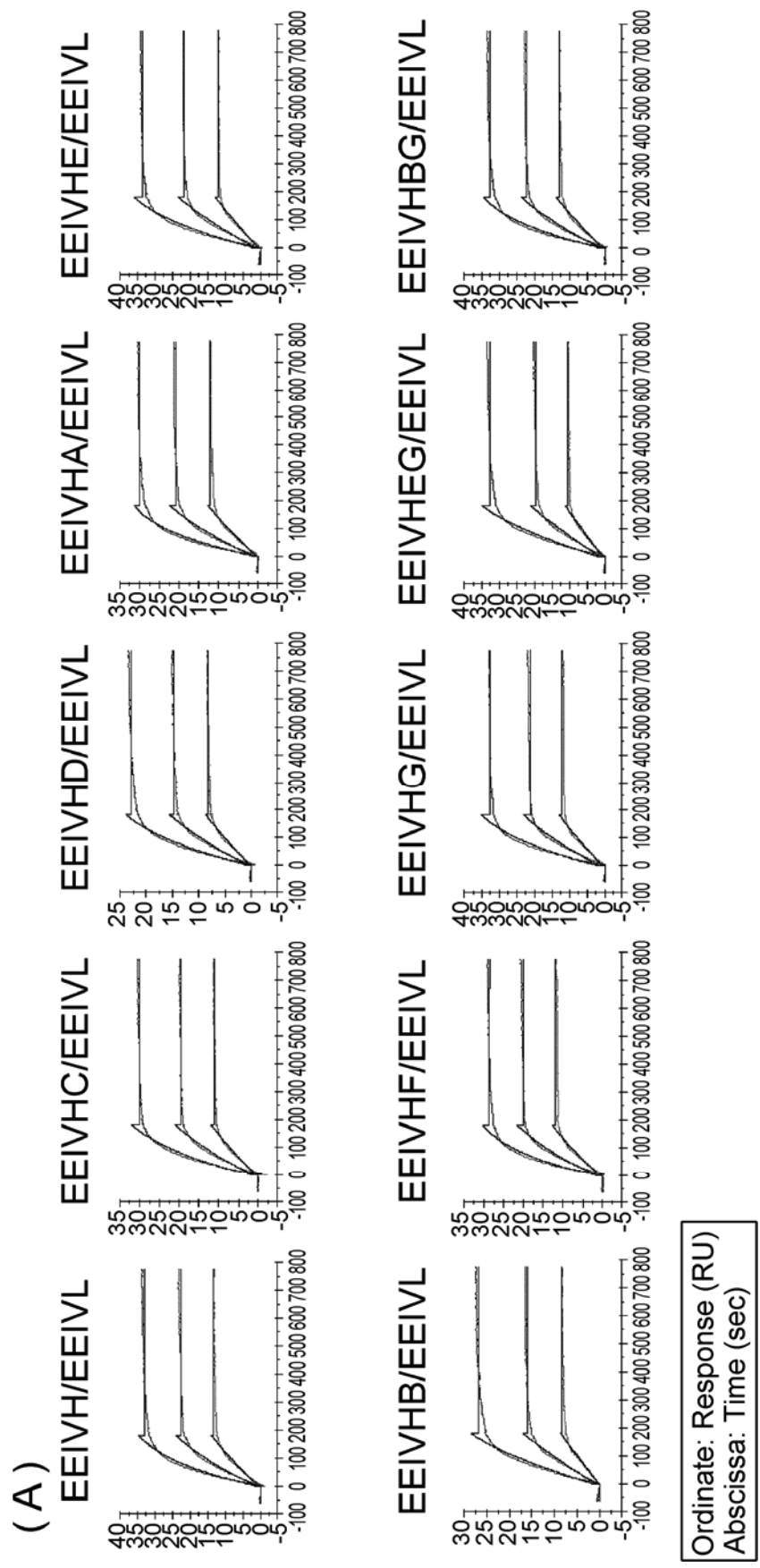


FIG. 6A

(B)

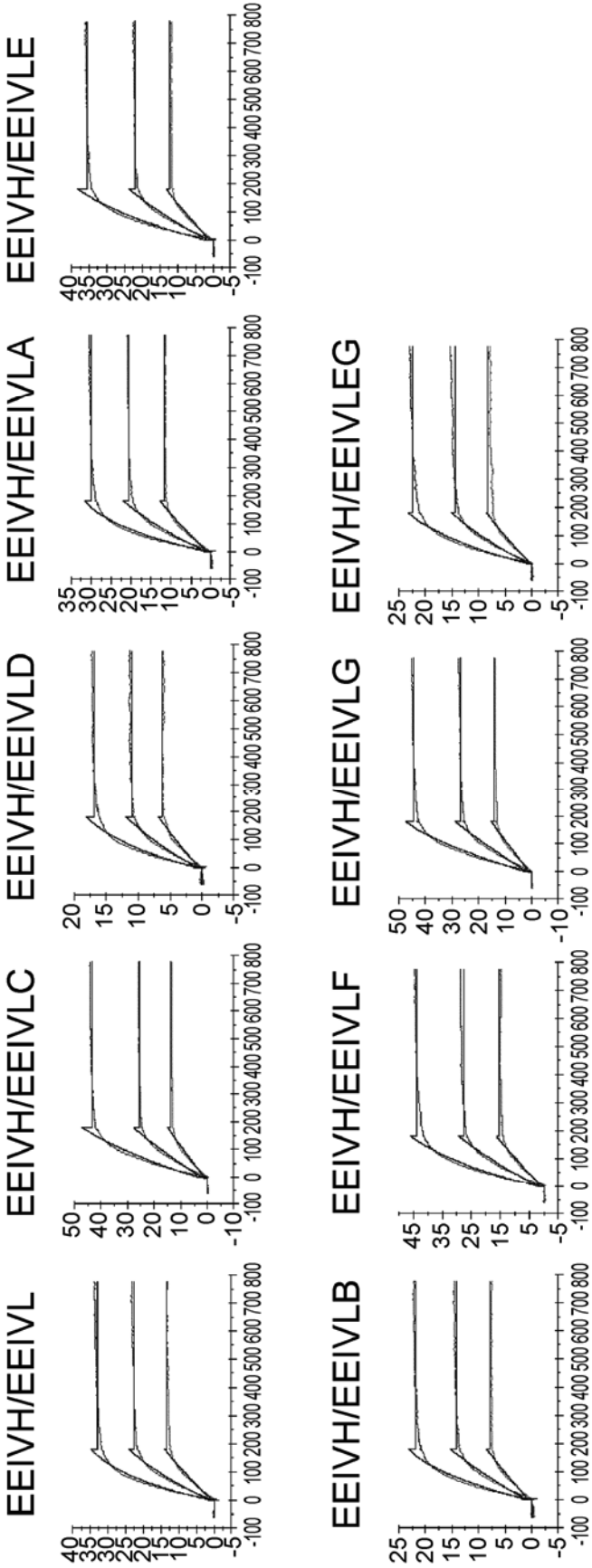


FIG. 6B

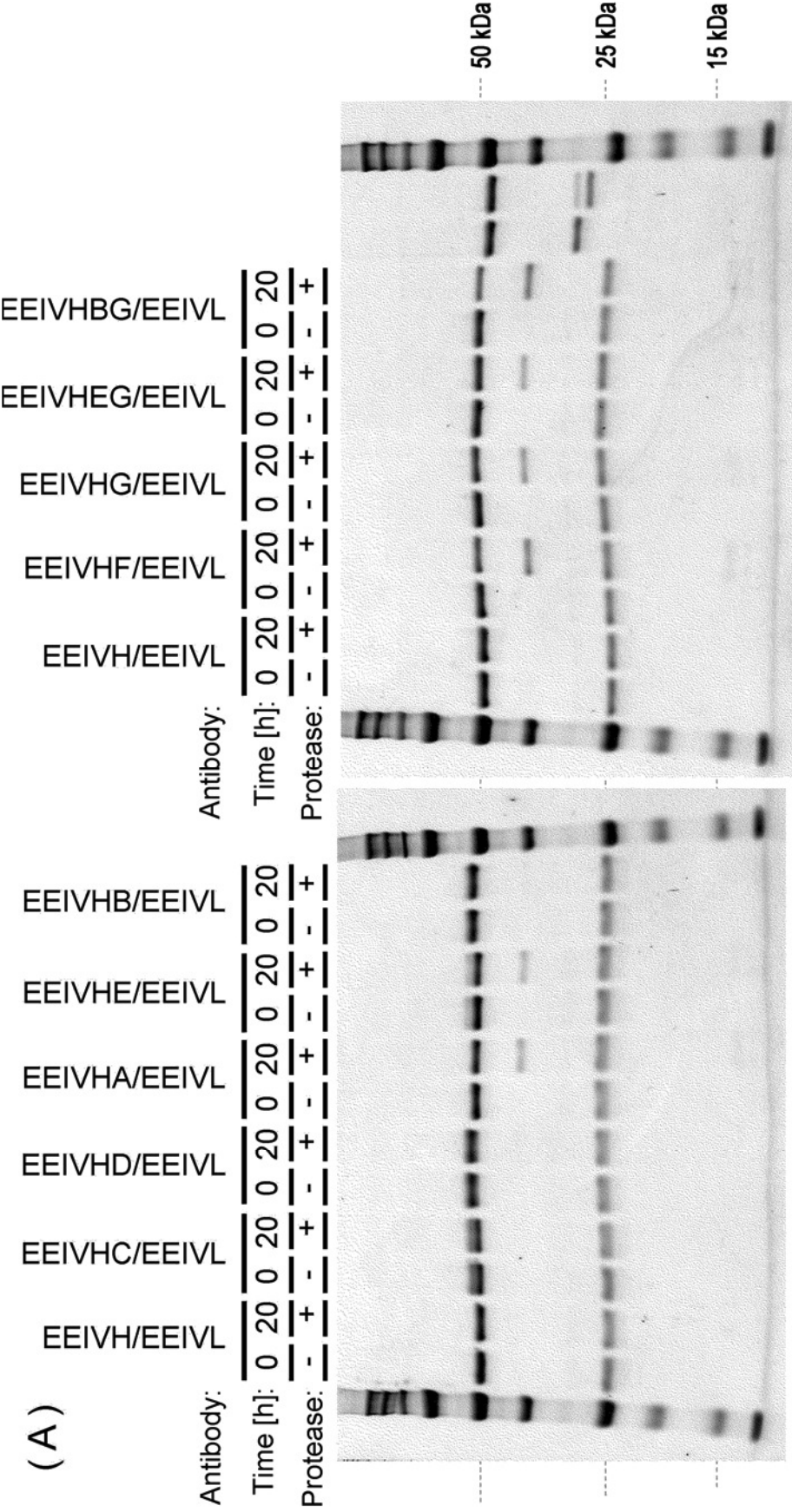


FIG. 7-1

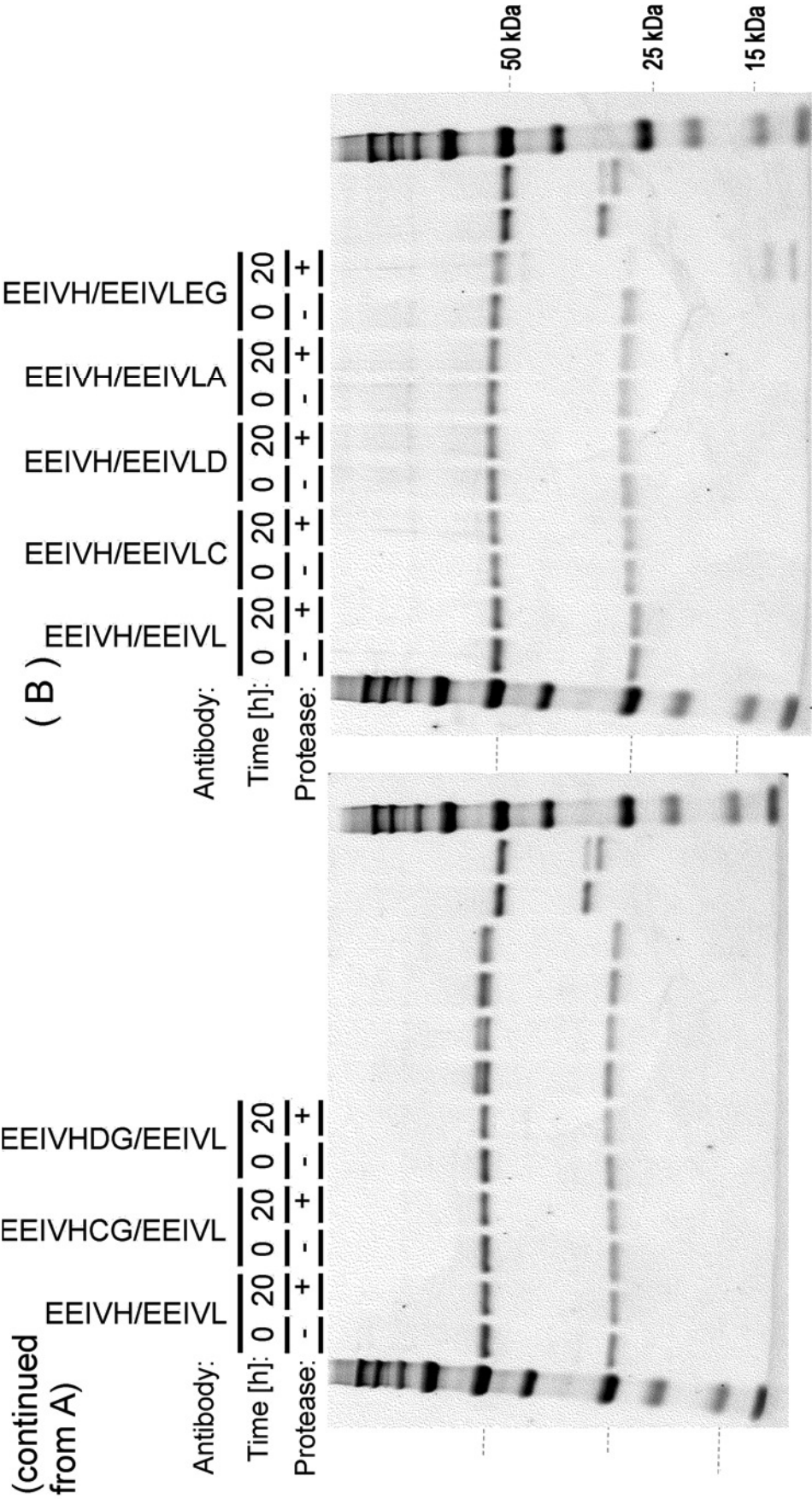


FIG. 7-2

(continued
from B)

EEIVH/EEIVLG	Antibody:		
EEIVH/EEIVLF	0	20	20
	-	+	-
	-	-	+

EEIVH/EEIVL	Antibody:		
	0	20	20
	-	-	+
	-	-	+

EEIVH/EEIVLE	Antibody:		
	0	20	20
	-	-	+
	-	-	+

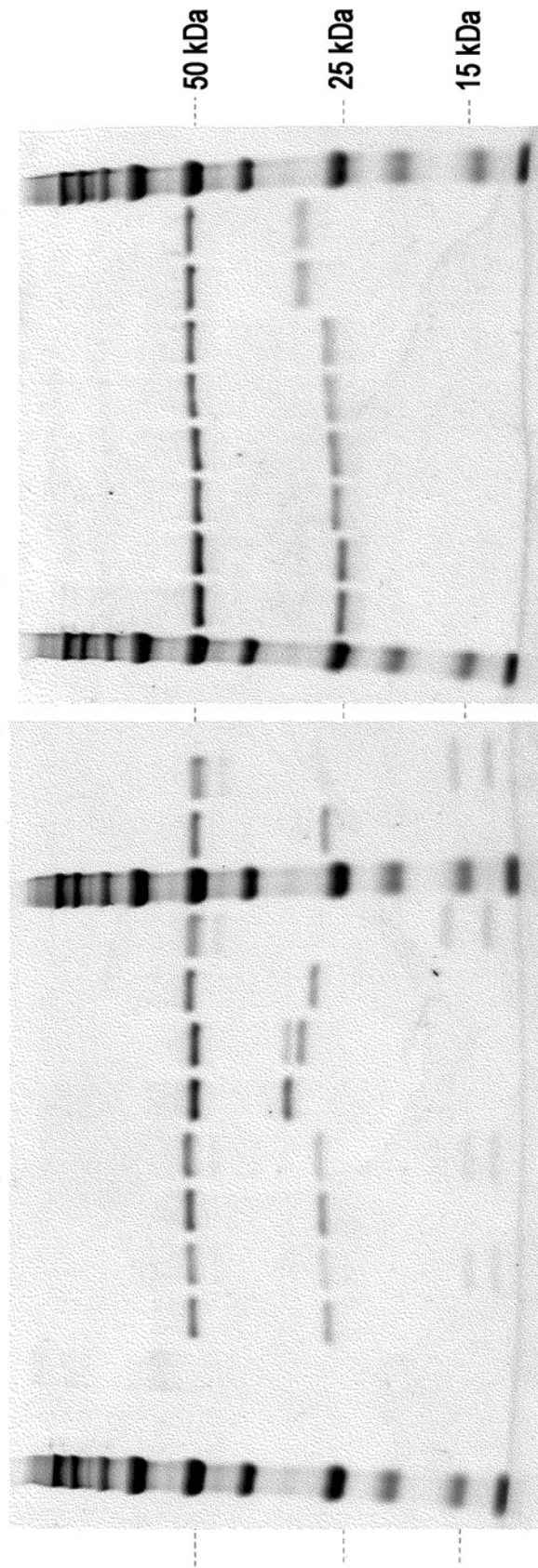


FIG. 7-3

Heavy chain variant name	Protease cleavage sequence	insertion position	Inserted sequence
EEIVHC	TV [insert]	SSASTKGP	LSGRSDNH (SEQ ID NO : 3)
EEIVHC002	TV [insert]	SSASTKGP	GLSGRSDNHG (SEQ ID NO : 28)
EEIVHC003	TV [insert]	SSASTKGP	SGLSGRSDNHGS (SEQ ID NO : 29)
EEIVHC004	TV [insert]	SSASTKGP	GSGLSGRSDNHGSS (SEQ ID NO : 30)
EEIVHC005	TV [insert]	SSASTKGP	GGSGLSGRSDNHGSSG (SEQ ID NO : 31)
EEIVHC006	TV [insert]	SSASTKGP	SGSGLSGRSDNHGSSGT (SEQ ID NO : 32)

FIG. 8

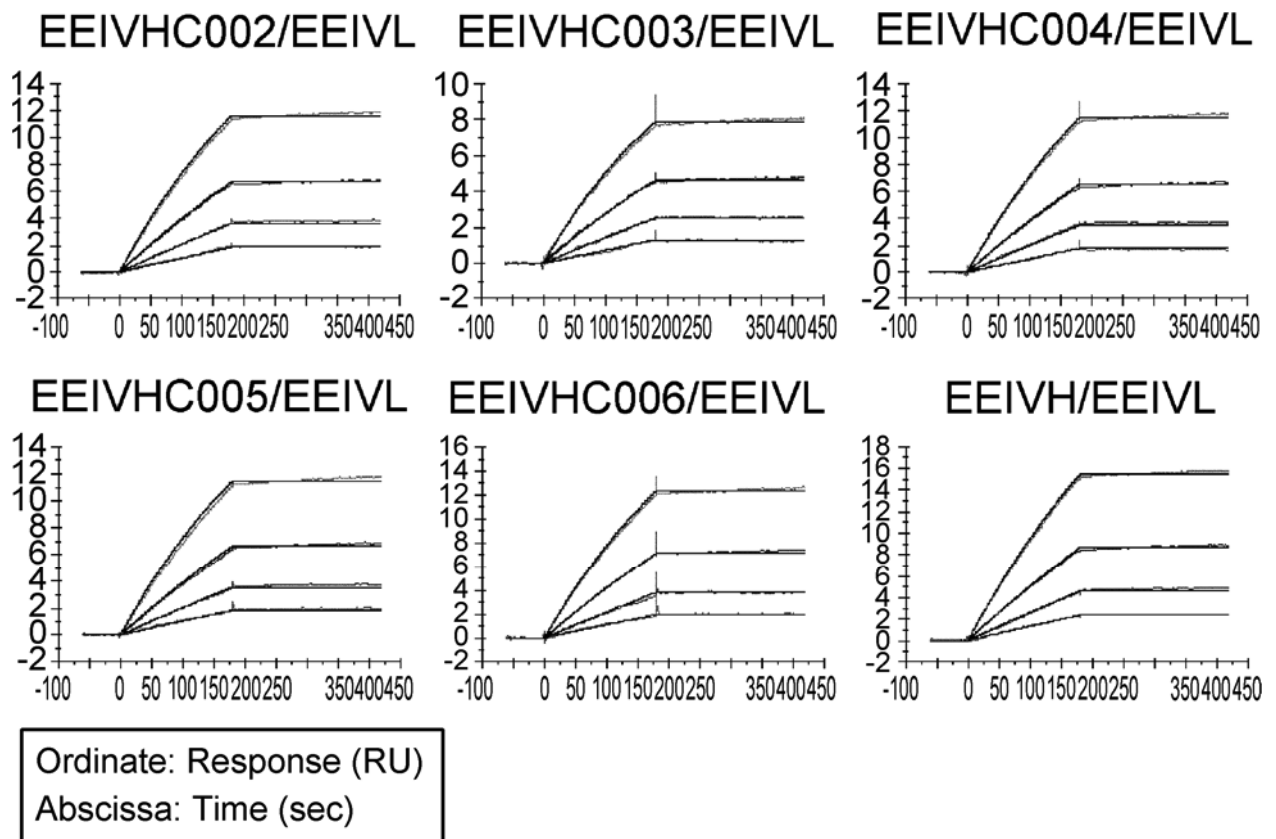


FIG. 9

(A)

Antibody:	EEIVHC/EEIVL				Antibody:	EEIVHC002/EEIVL				Antibody:	EEIVHC003/EEIVL					
Protease:	-	uPA	-	MT-SP1	Protease:	-	uPA	-	MT-SP1	Protease:	-	uPA	-	MT-SP1		
Time [h]:	0	2	20	0	2	20	0	2	20	Time [h]:	0	2	20	0	2	20

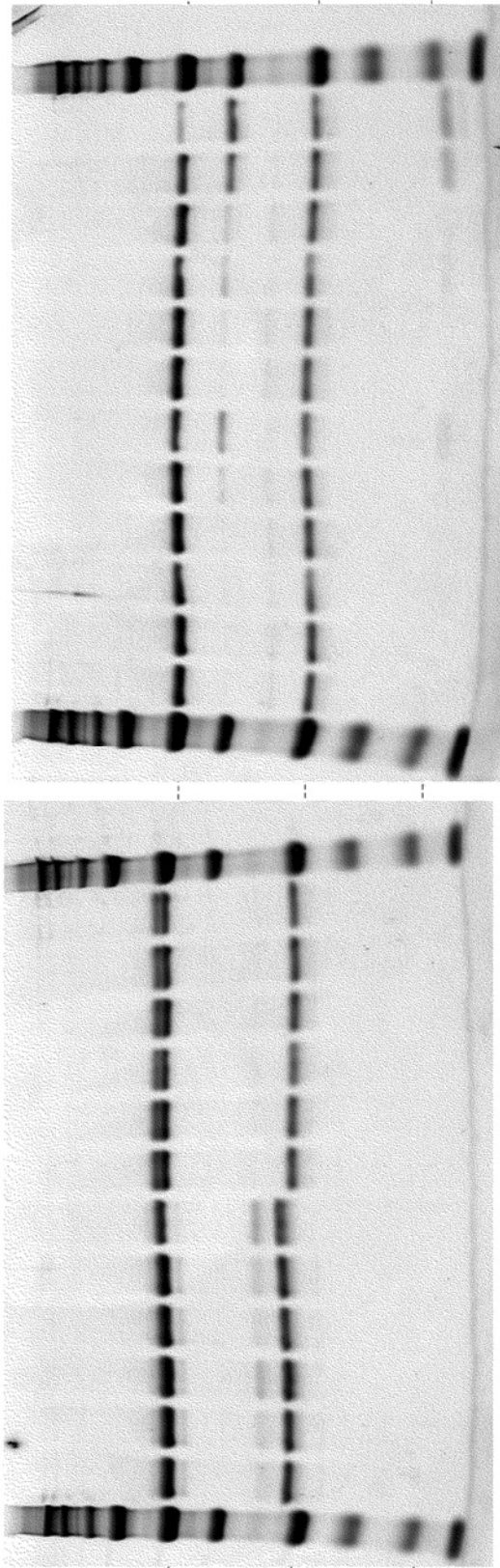


FIG. 10A

(B)

Antibody:	EEIVHC004/EEIVL				EEIVHC006/EEIVL				EEIVH/EEIVL			
Protease:	-	uPA	-	MT-SP1	-	uPA	-	MT-SP1	-	uPA	-	MT-SP1
Time [h]:	0	2	20	0	2	20	0	2	20	0	2	20

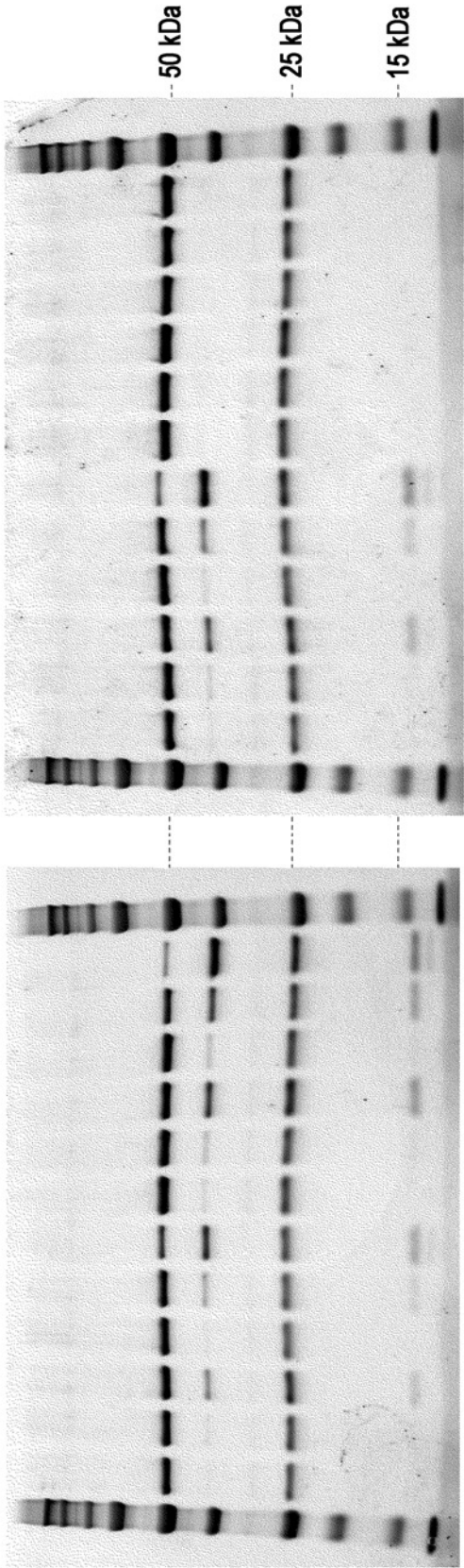


FIG. 10B

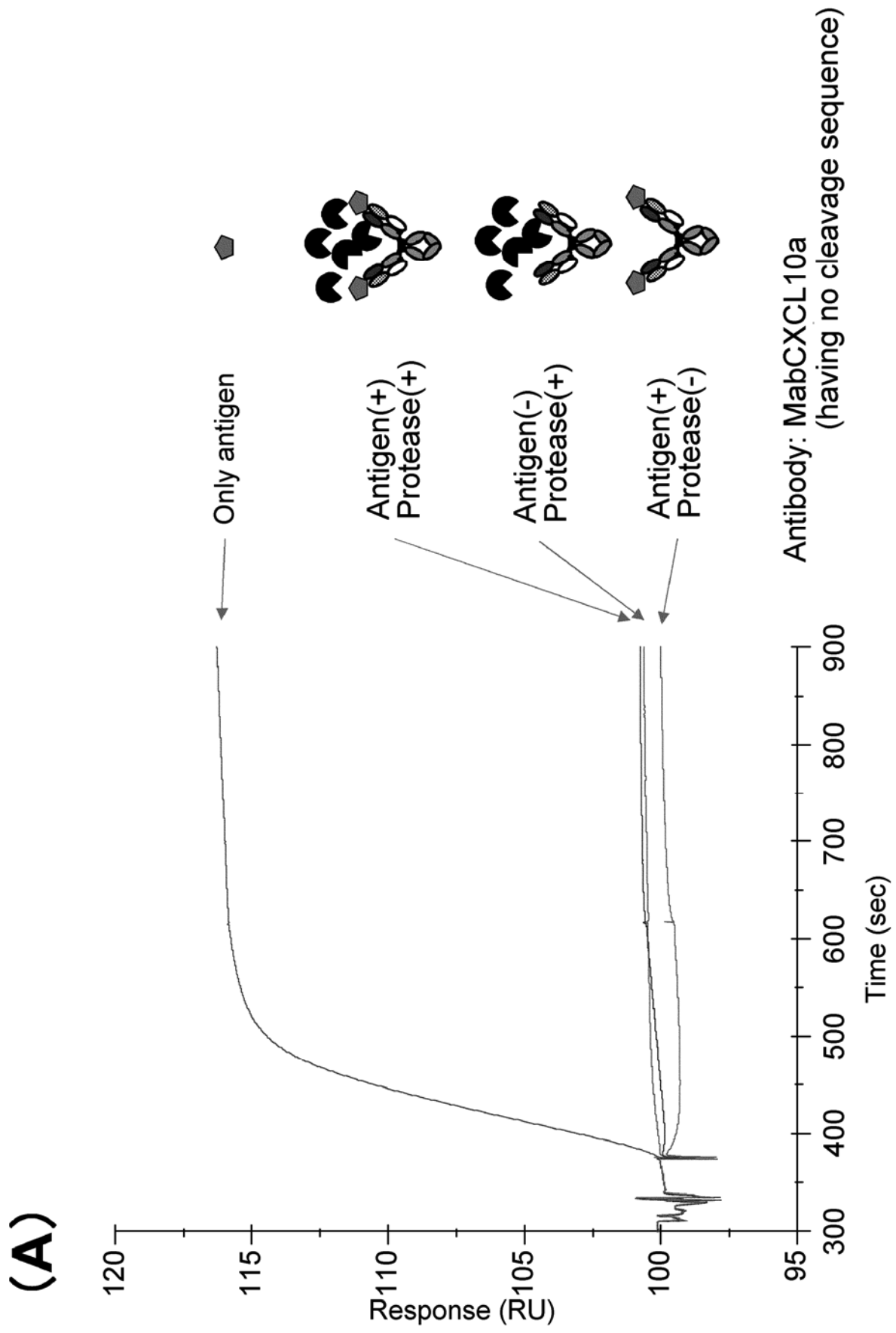


FIG. 11A

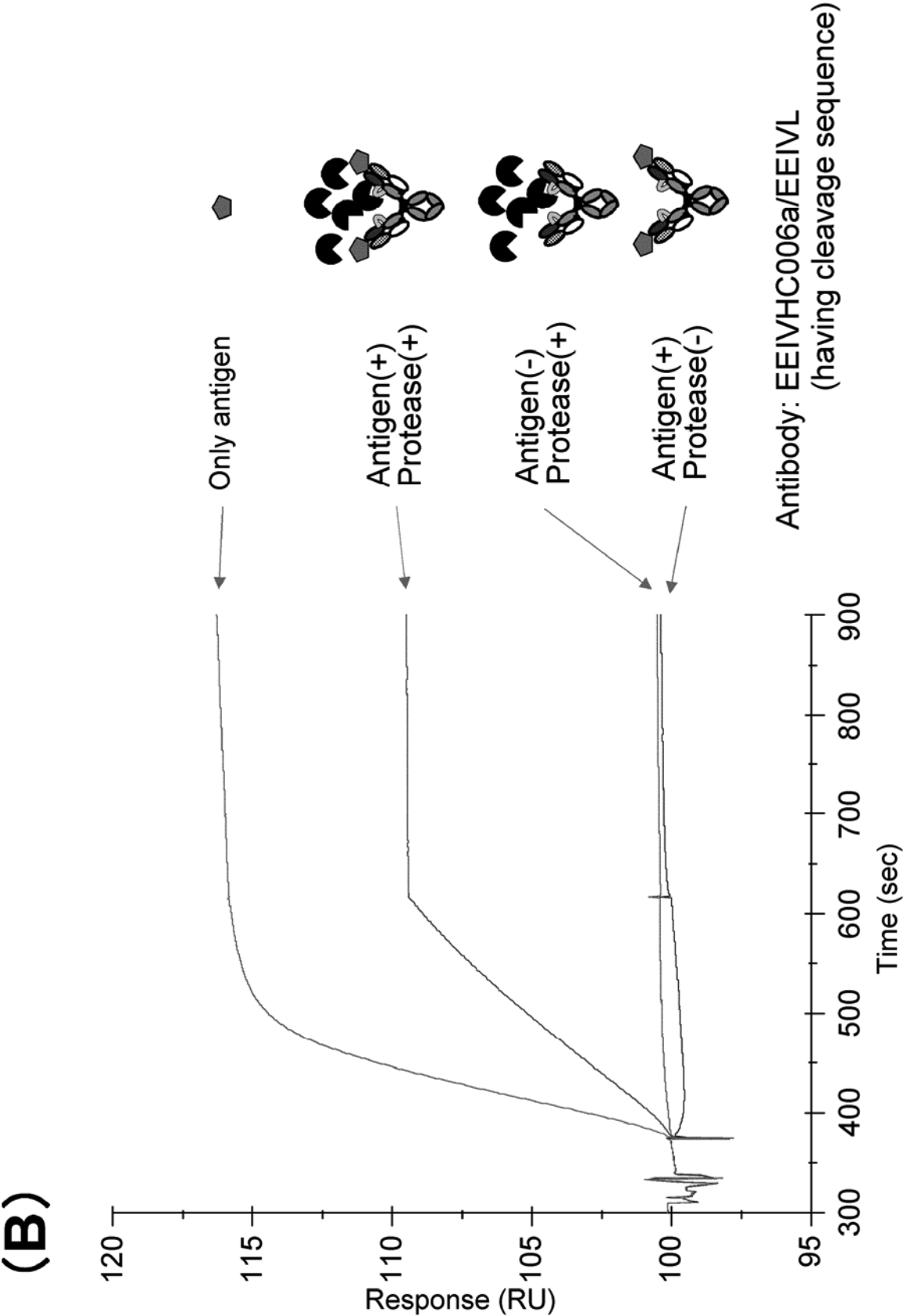


FIG. 11B

Heavy chain variant name	Insertion position and alteration position	Inserted sequence	Sequence after insertion and alteration
EESVHA009	TVSS [insert] A-STKGP	GLSGRSDNHGS (SEQ ID NO: 61)	TVSSGLSGRSDNHGS STKGP (SEQ ID NO : 62)
EESVHA012	TVSS [insert] A-STKGP	GGSLSGRSDNHGSSGT (SEQ ID NO : 63)	TVSSGGSLSGRSDNHGSS GTSTKGP (SEQ ID NO : 64)

FIG. 12

Antibody:		EESVHA012/EEIVL		EESVHA009/EEIVL	
Time [h]:		0	20	0	20
Protease:		-	U	M	-
		-	U	M	-

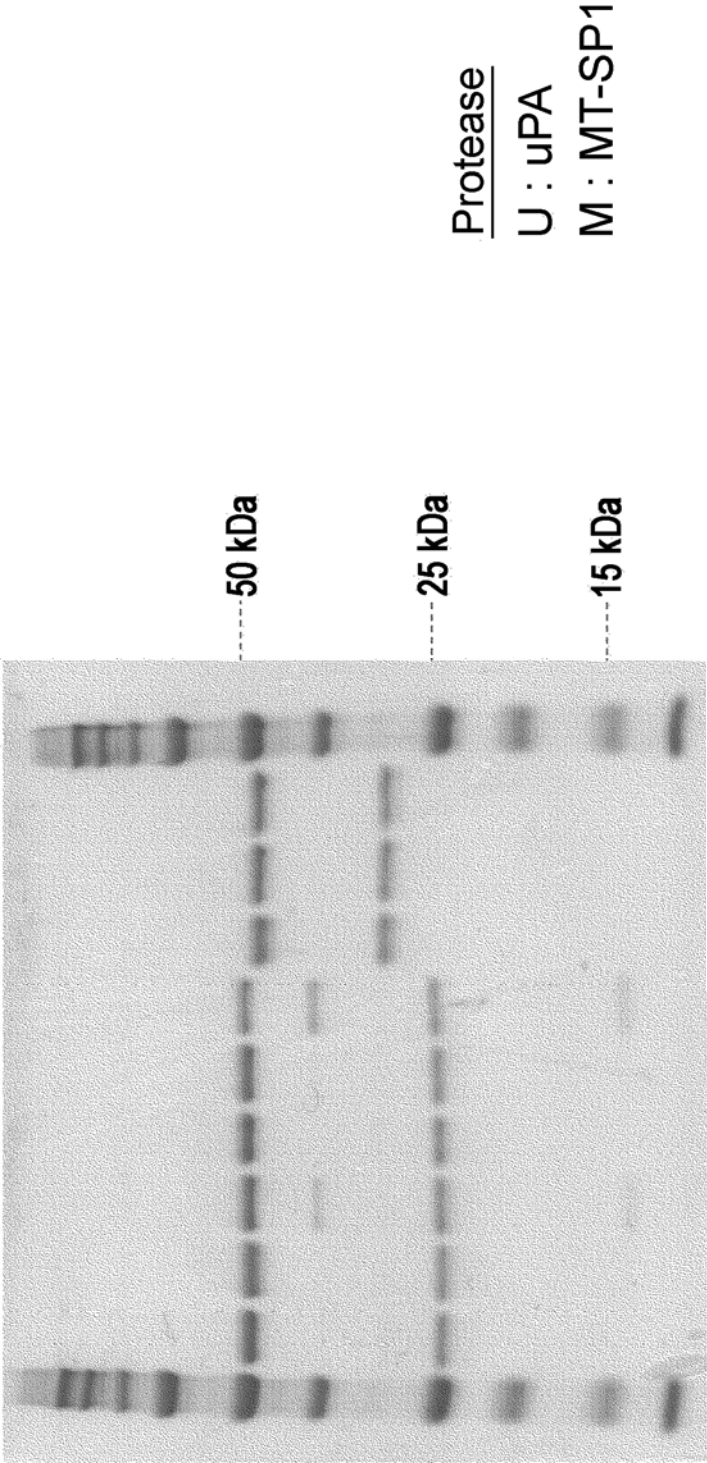


FIG. 13

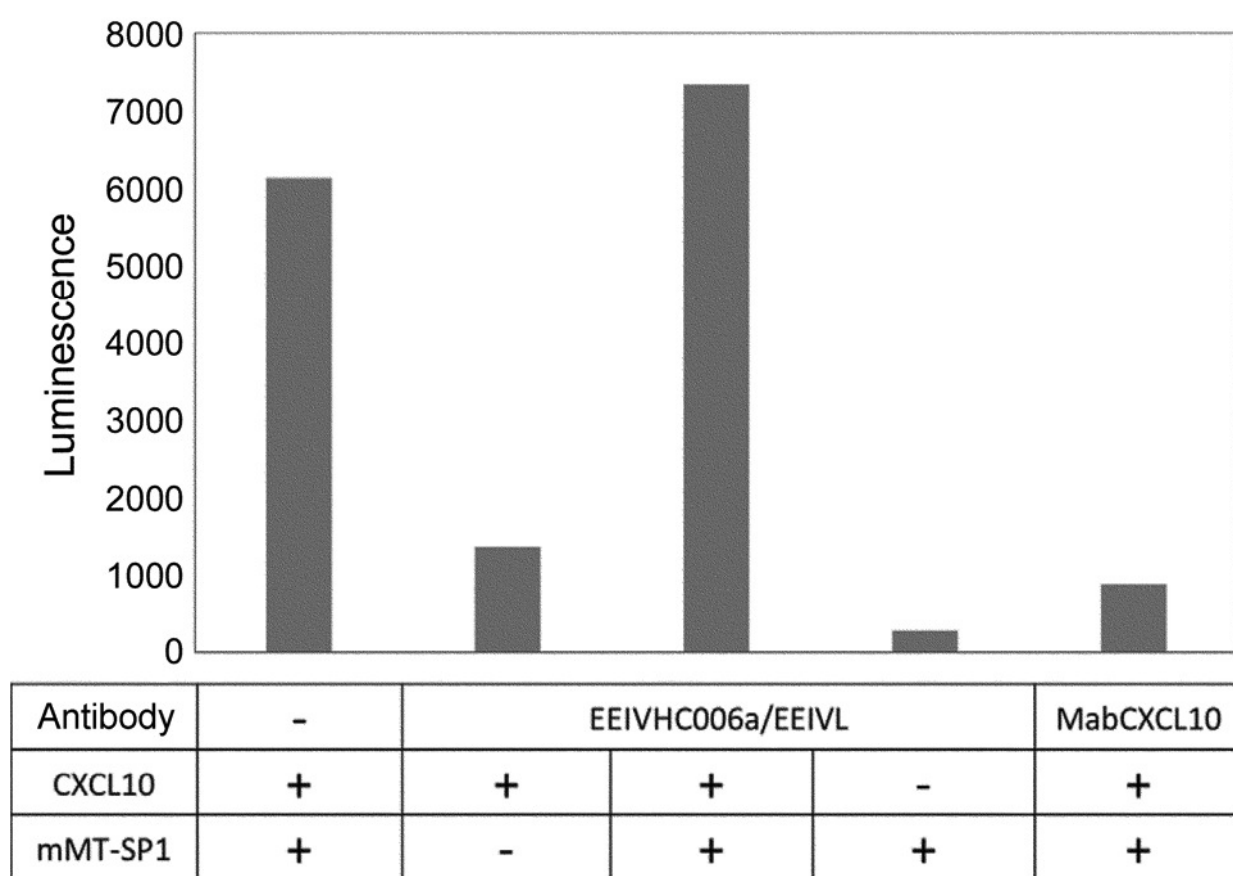


FIG. 14

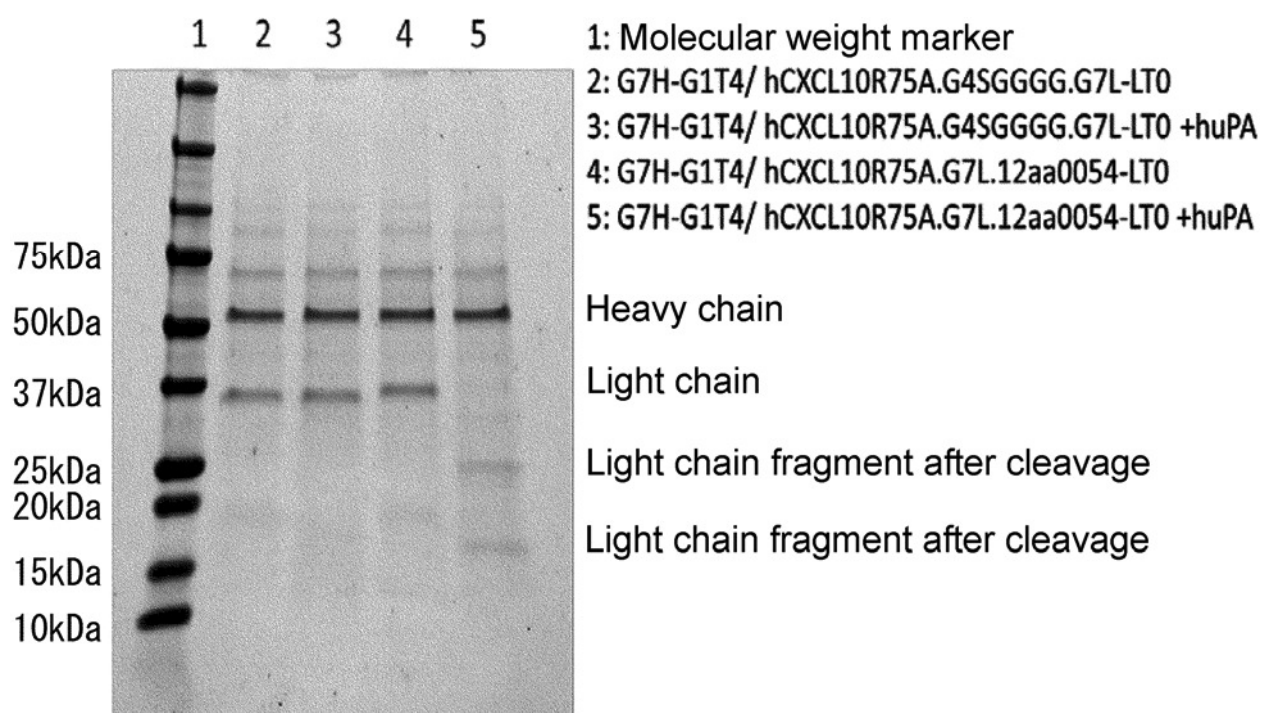


FIG. 15

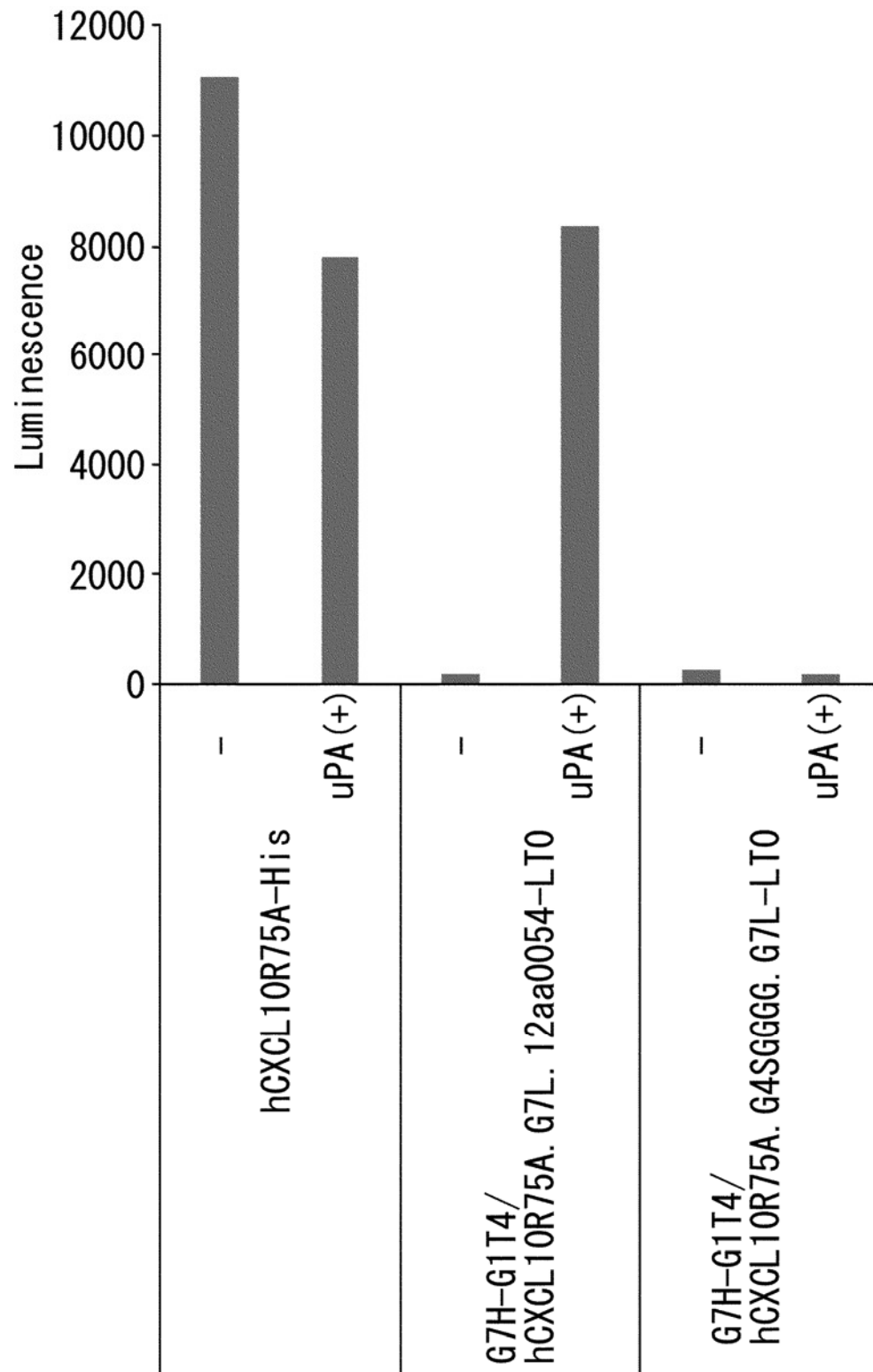


FIG. 16

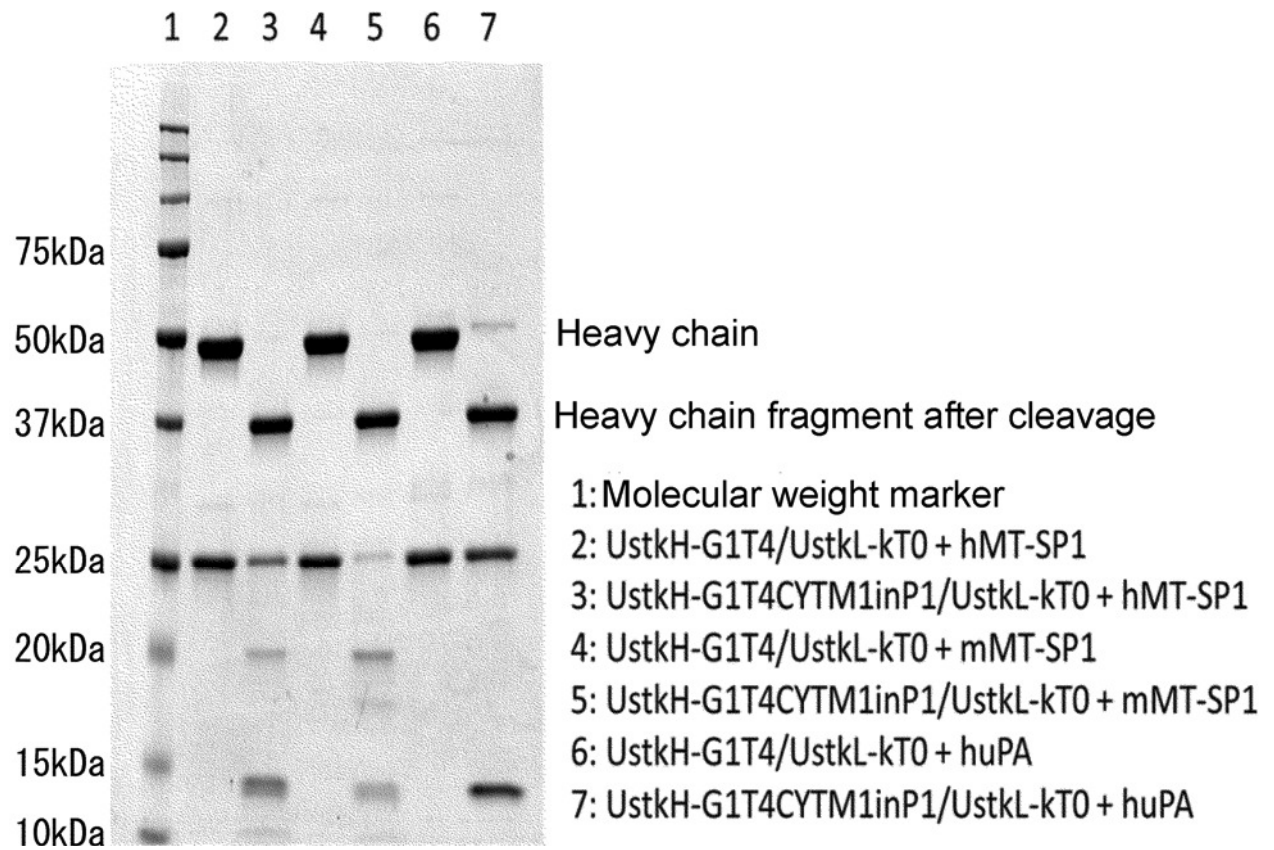


FIG. 17

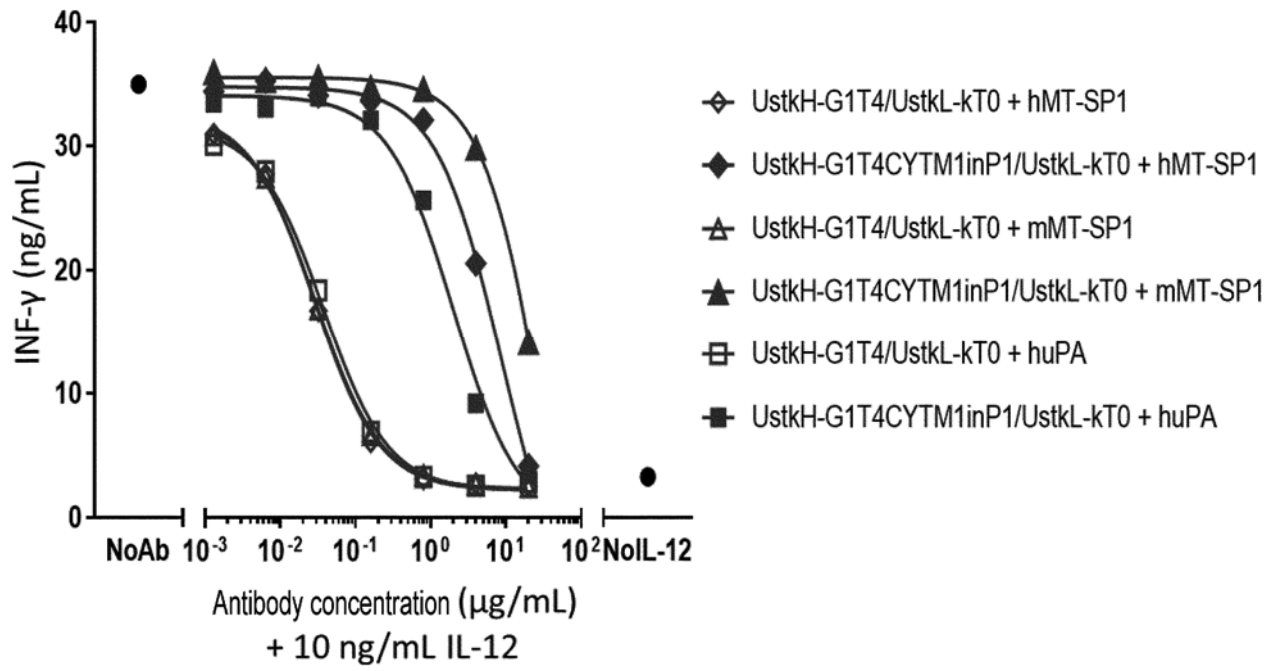


FIG. 18

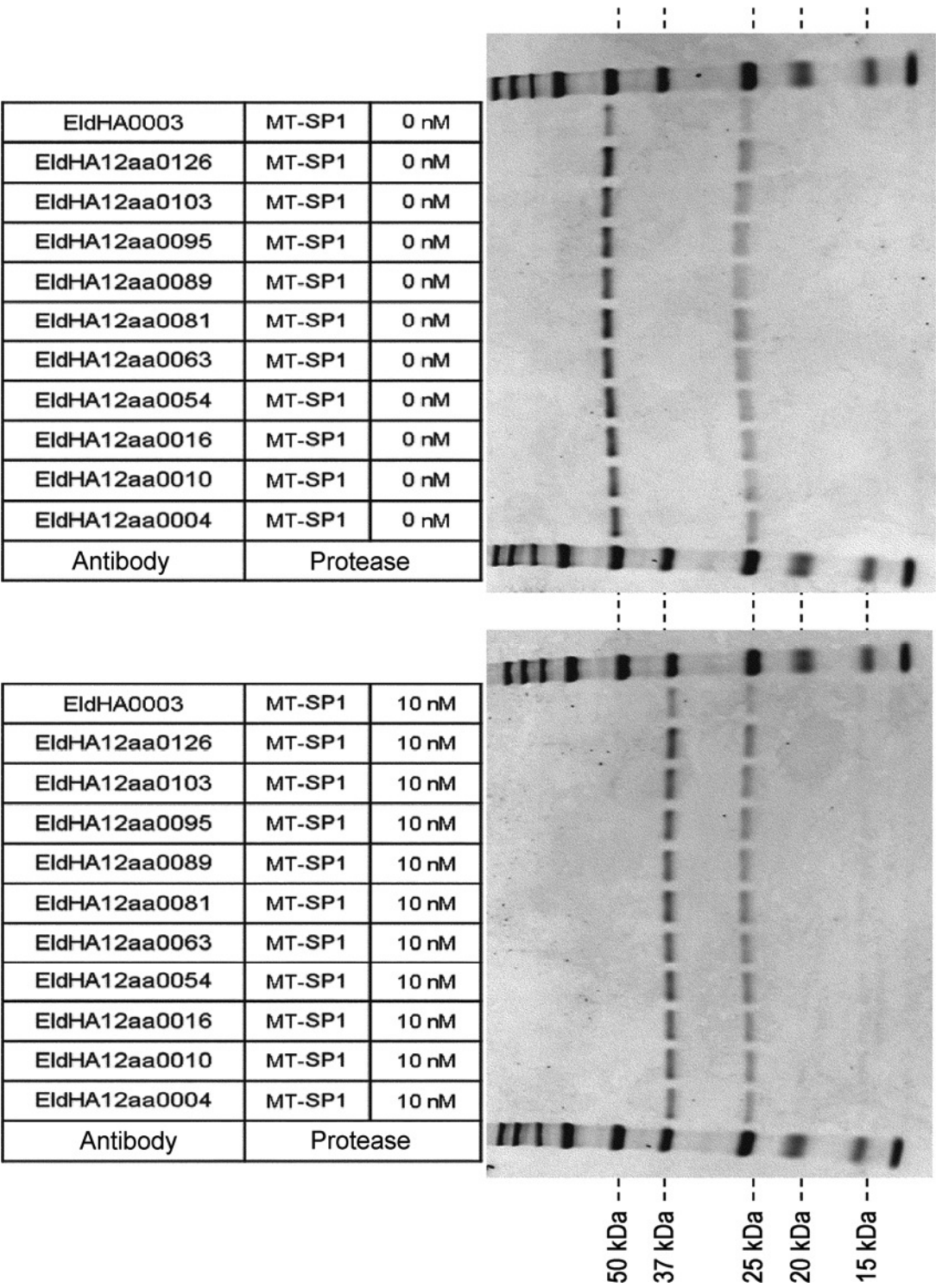


FIG. 19A

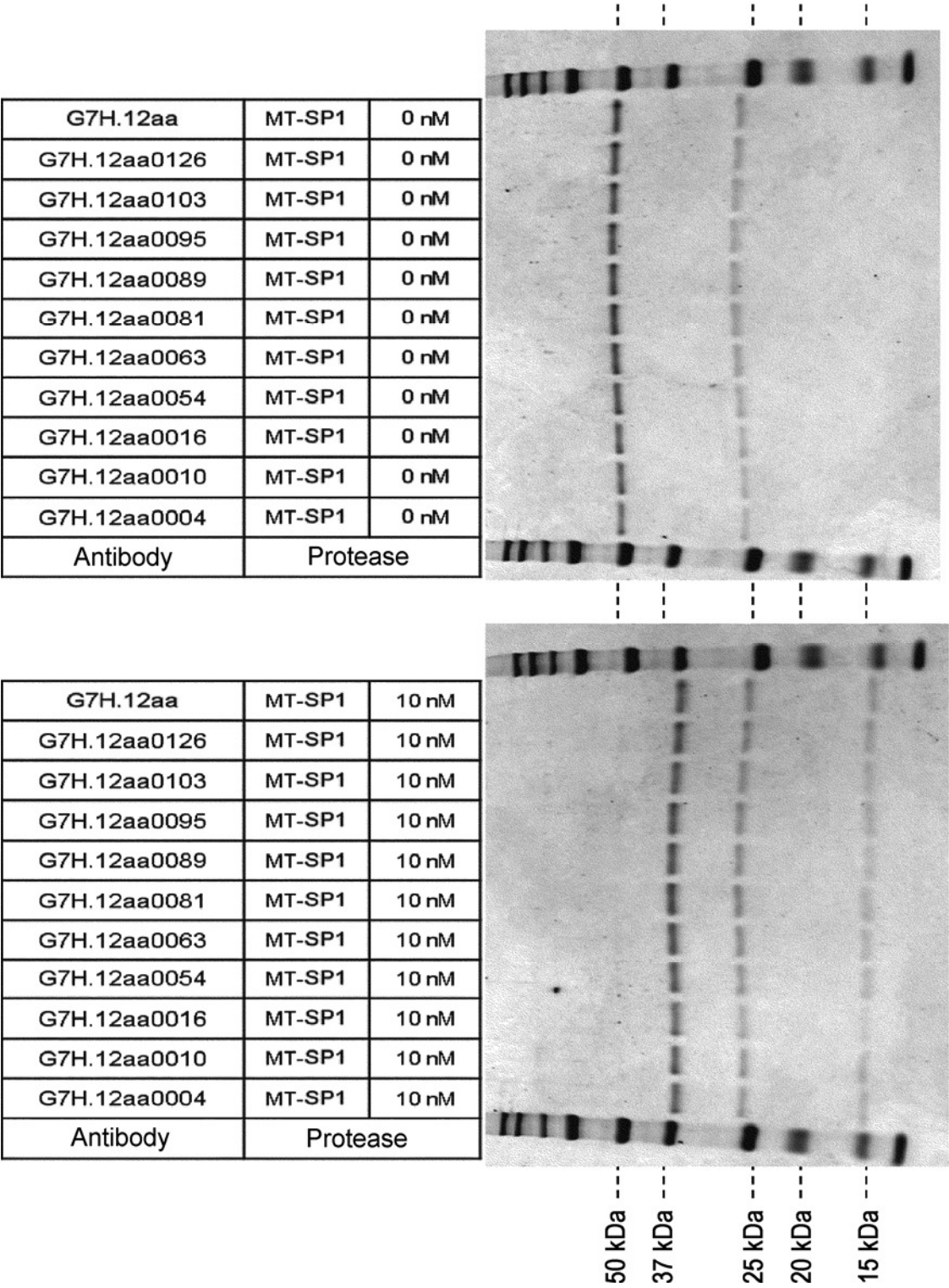


FIG. 19B

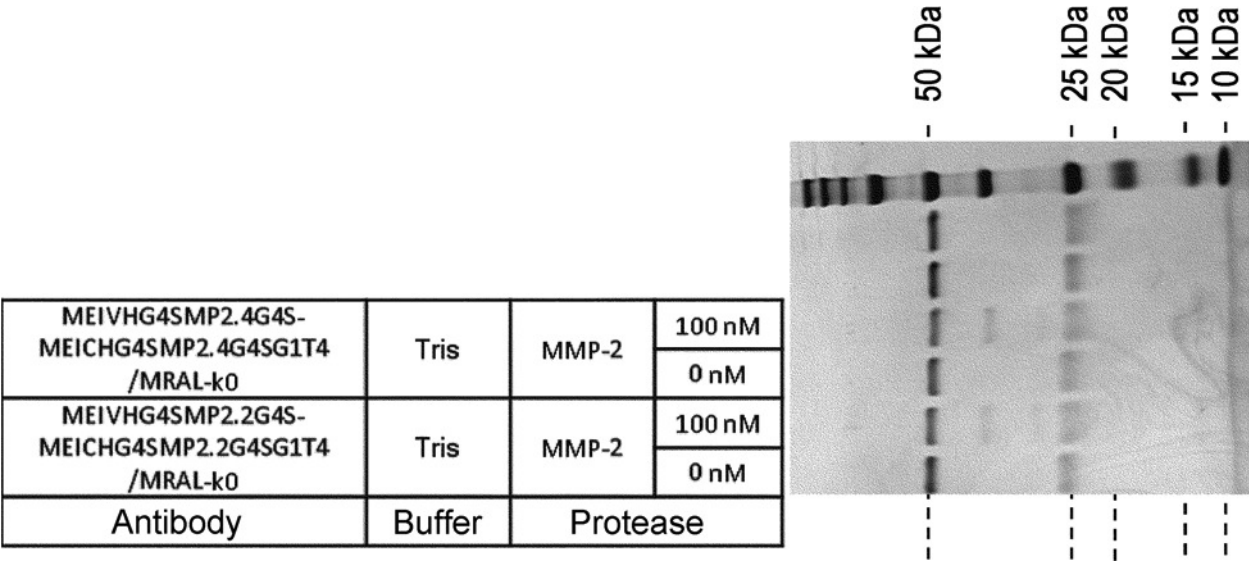


FIG. 20A

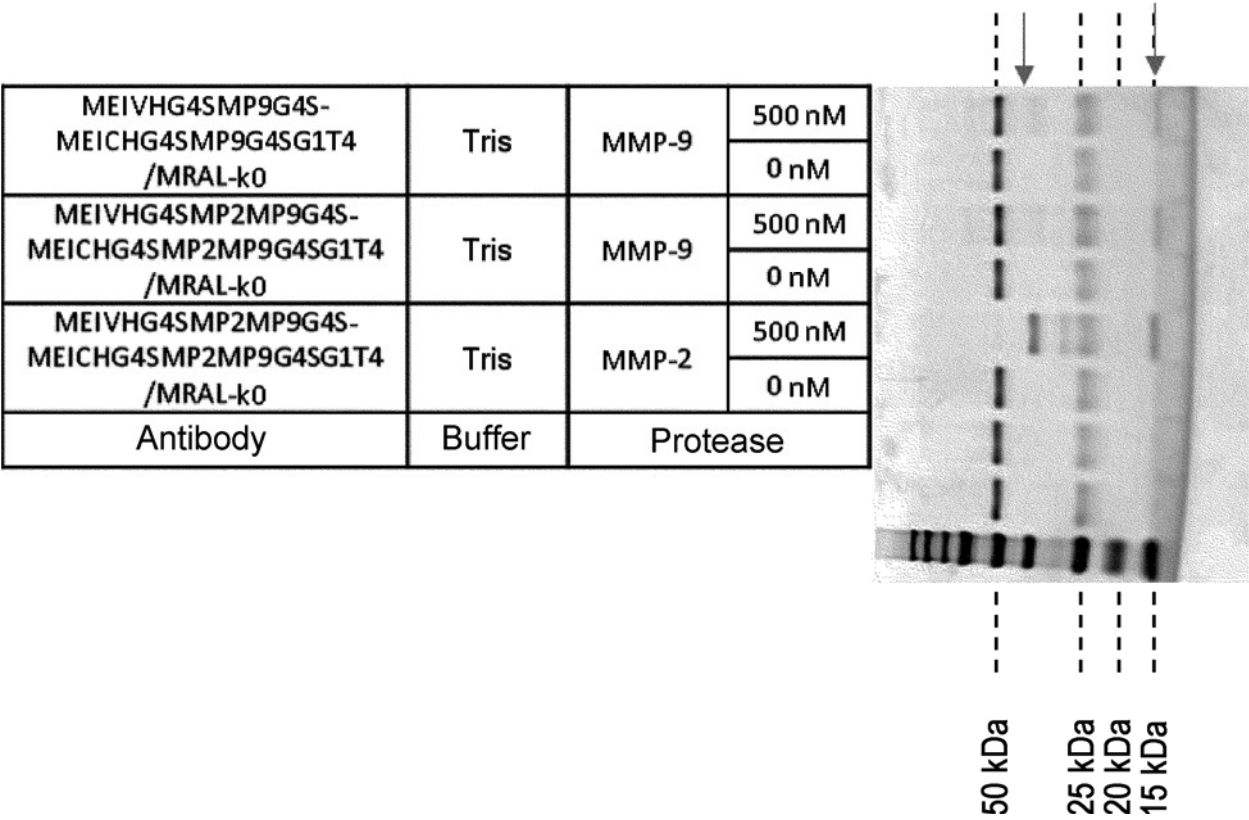


FIG. 20B

MEIVHMP7.2- MEIVHMP7.2G1T4/MRAL-k0	Assay buffer	MMP-7	50 nM
			0 nM
Sample	Buffer	Protease	
MEIVHMP2.3- MEIVHMP2.3G1T4/MRAL-k0	assay buffer	MMP-2	50 nM
			0 nM
Sample	Buffer	Protease	

MEIVHMP2.1- MEIVHMP2.1G1T4/MRAL-k0	Assay buffer	MMP-2	50 nM
			0 nM
Sample	Buffer	Protease	

MRAH-G1T4/MRAL-k0	assay buffer	MMP-9	50 nM
		MMP-7	50 nM
		MMP-2	50 nM
			0 nM
Sample	Buffer	Protease	



FIG. 21

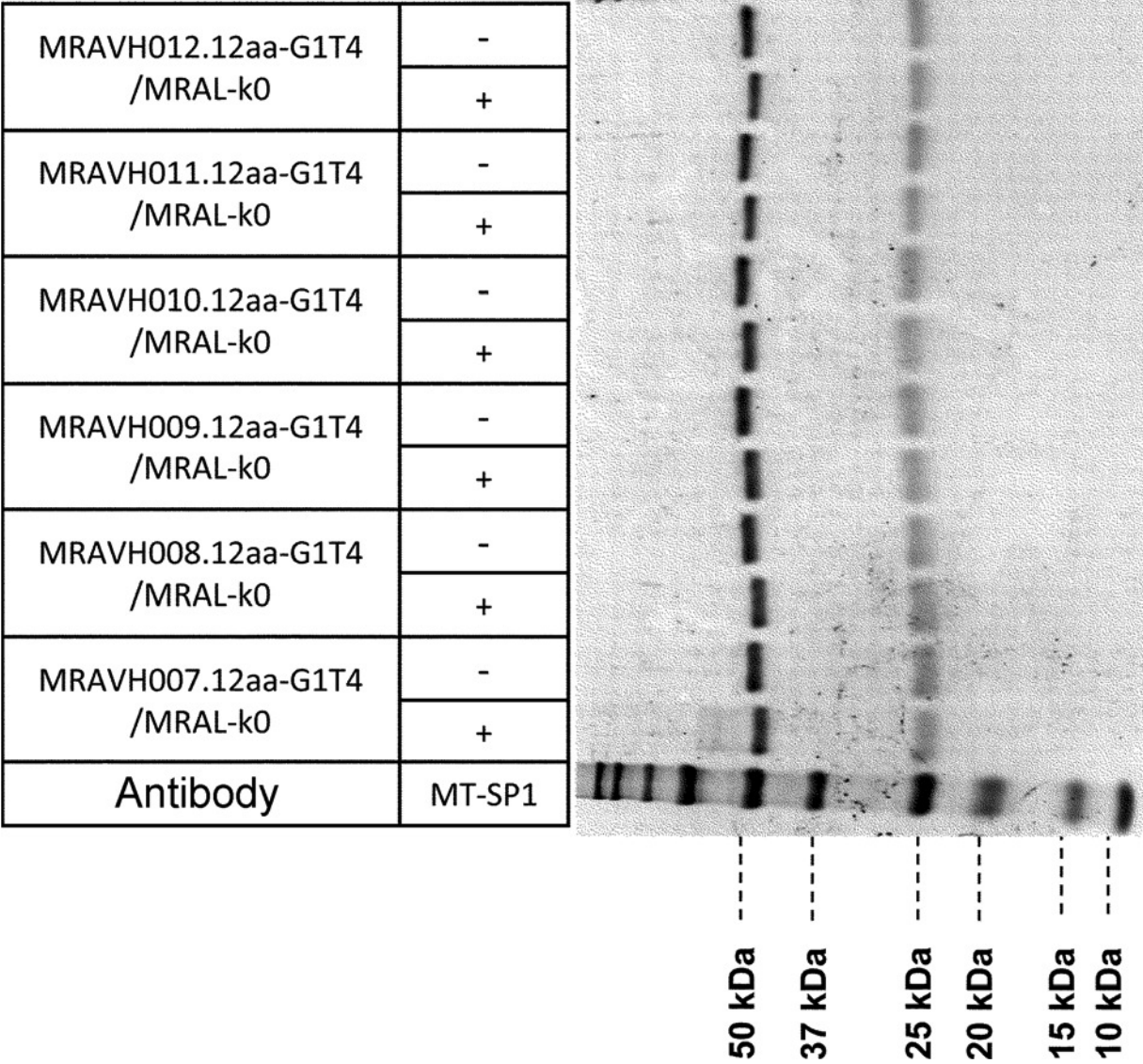


FIG. 22A

MRAVH043.12aa-G1T4 /MRAL-k0	-
	+
MRAVH042.12aa-G1T4 /MRAL-k0	-
	+
MRAVH041.12aa-G1T4 /MRAL-k0	-
	+
MRAVH015.12aa-G1T4 /MRAL-k0	-
	+
MRAVH014.12aa-G1T4 /MRAL-k0	-
	+
MRAVH013.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

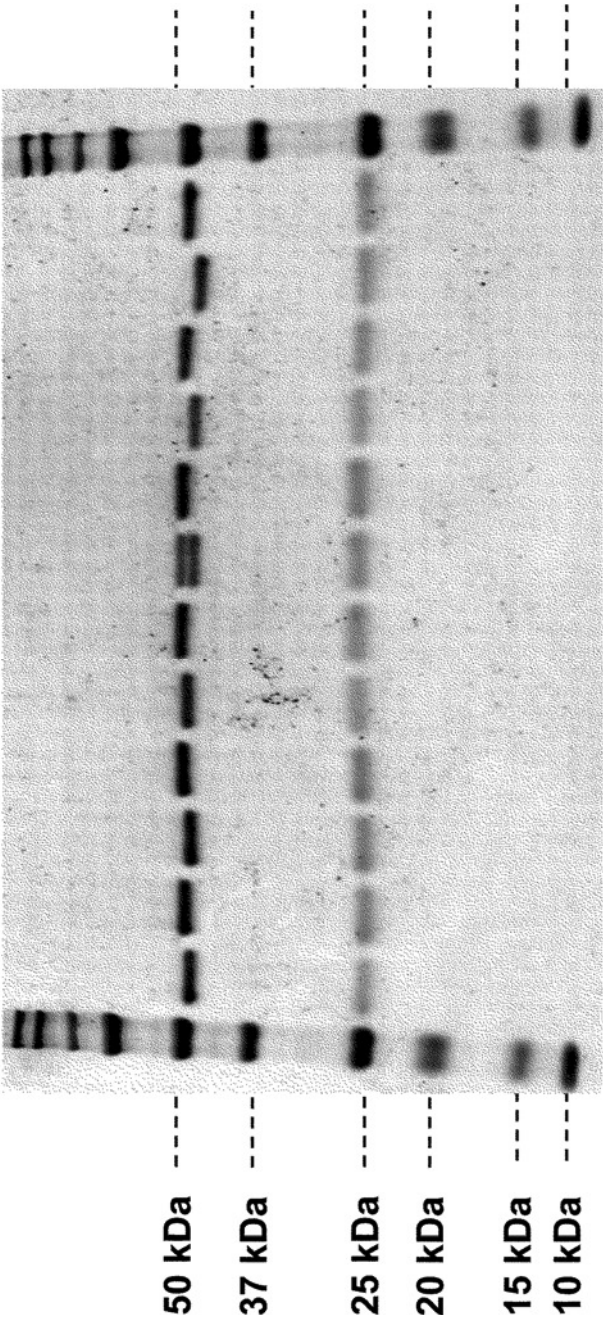


FIG. 22B

MRAVH058.12aa-G1T4 /MRAL-k0	-
	+
MRAVH057.12aa-G1T4 /MRAL-k0	-
	+
MRAVH056.12aa-G1T4 /MRAL-k0	-
	+
MRAVH046.12aa-G1T4 /MRAL-k0	-
	+
MRAVH045.12aa-G1T4 /MRAL-k0	-
	+
MRAVH044.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

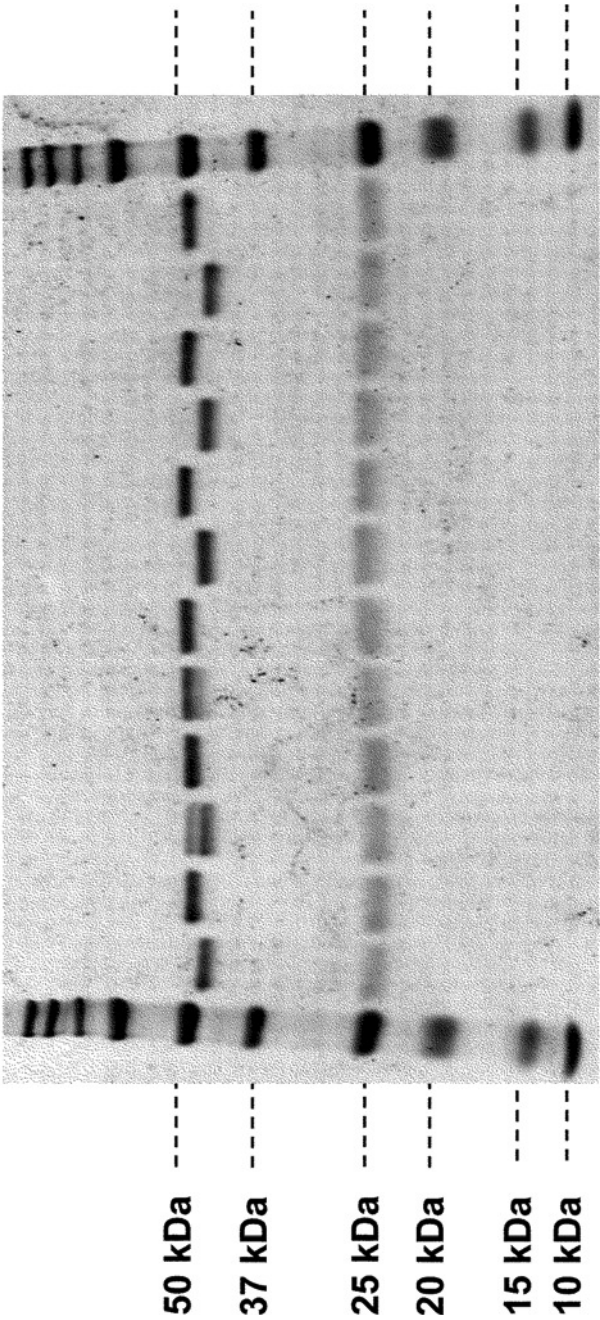


FIG. 22C

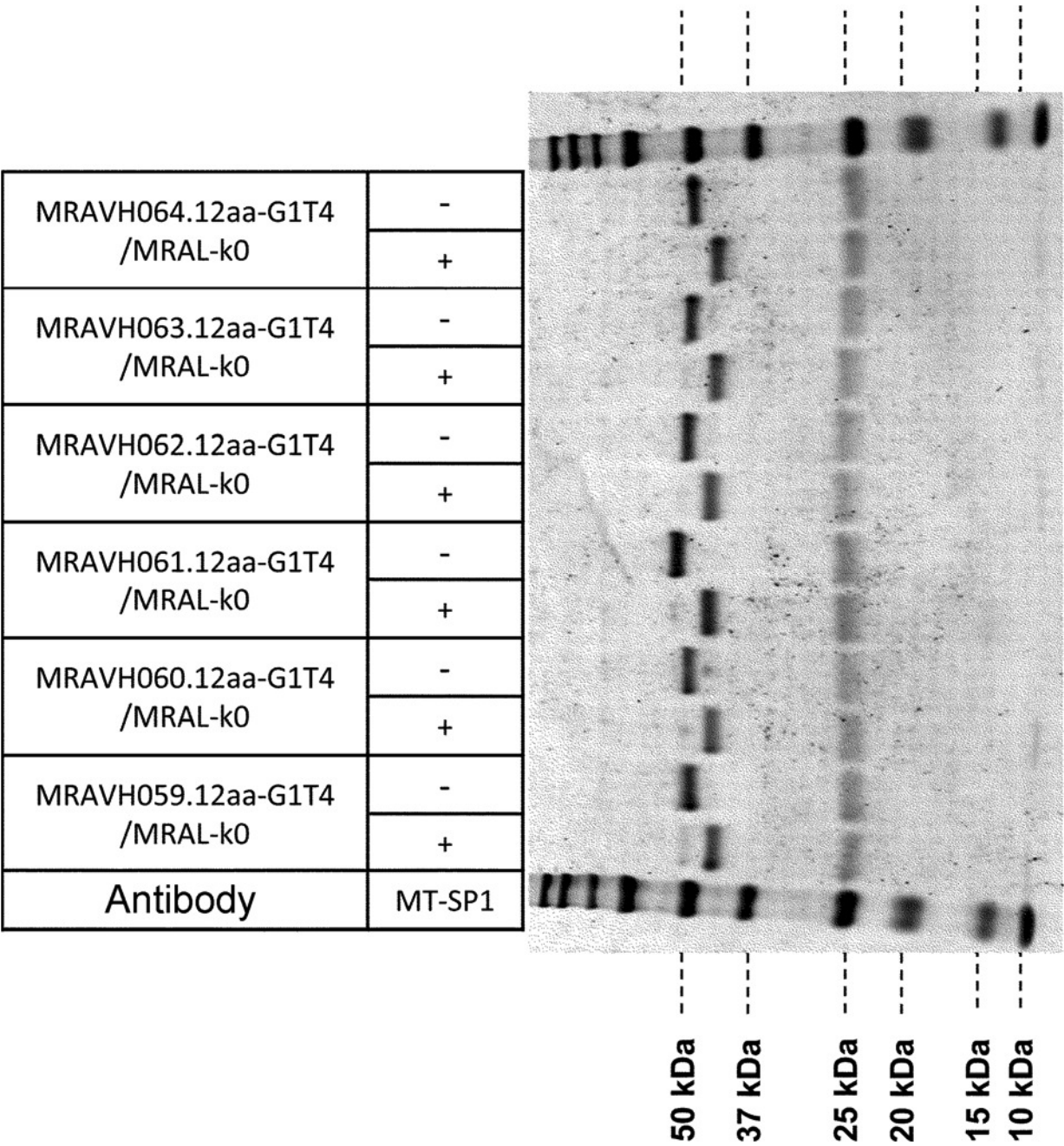


FIG. 22D

MRAVH075.12aa-G1T4 /MRAL-k0	-
	+
MRAVH074.12aa-G1T4 /MRAL-k0	-
	+
MRAVH068.12aa-G1T4 /MRAL-k0	-
	+
MRAVH067.12aa-G1T4 /MRAL-k0	-
	+
MRAVH066.12aa-G1T4 /MRAL-k0	-
	+
MRAVH065.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

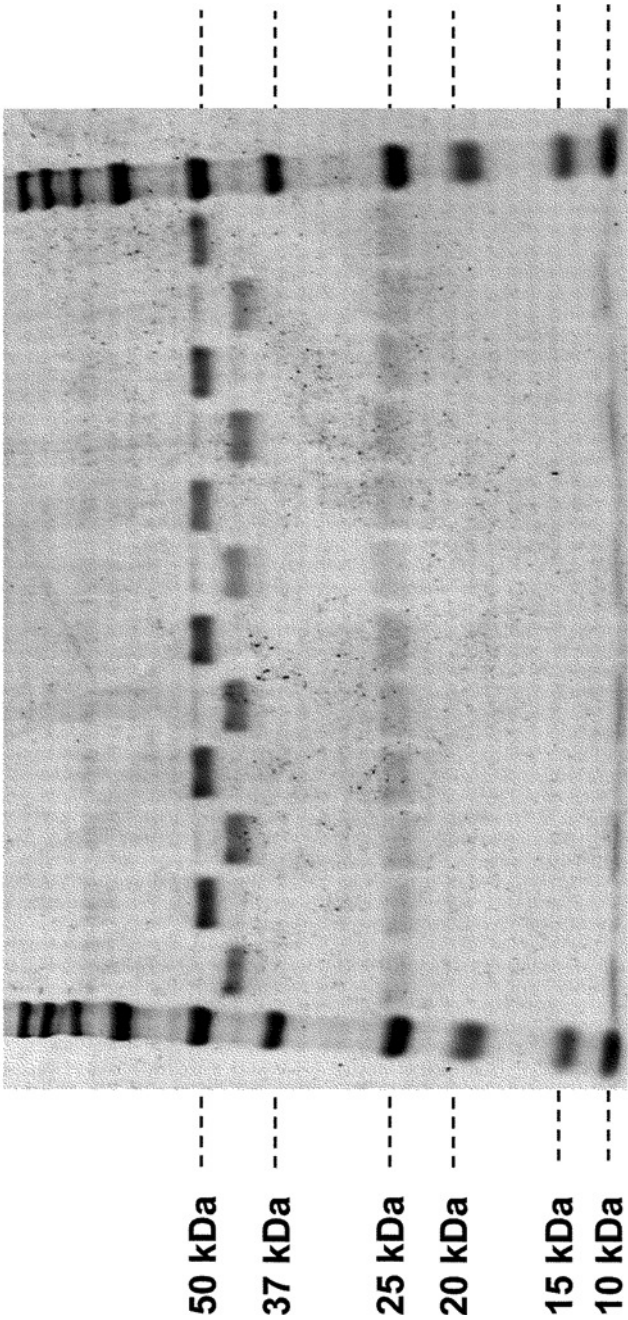


FIG. 22E

MRAVH101.12aa-G1T4 /MRAL-k0	-
	+
MRAVH088.12aa-G1T4 /MRAL-k0	-
	+
MRAVH087.12aa-G1T4 /MRAL-k0	-
	+
MRAVH078.12aa-G1T4 /MRAL-k0	-
	+
MRAVH077.12aa-G1T4 /MRAL-k0	-
	+
MRAVH076.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

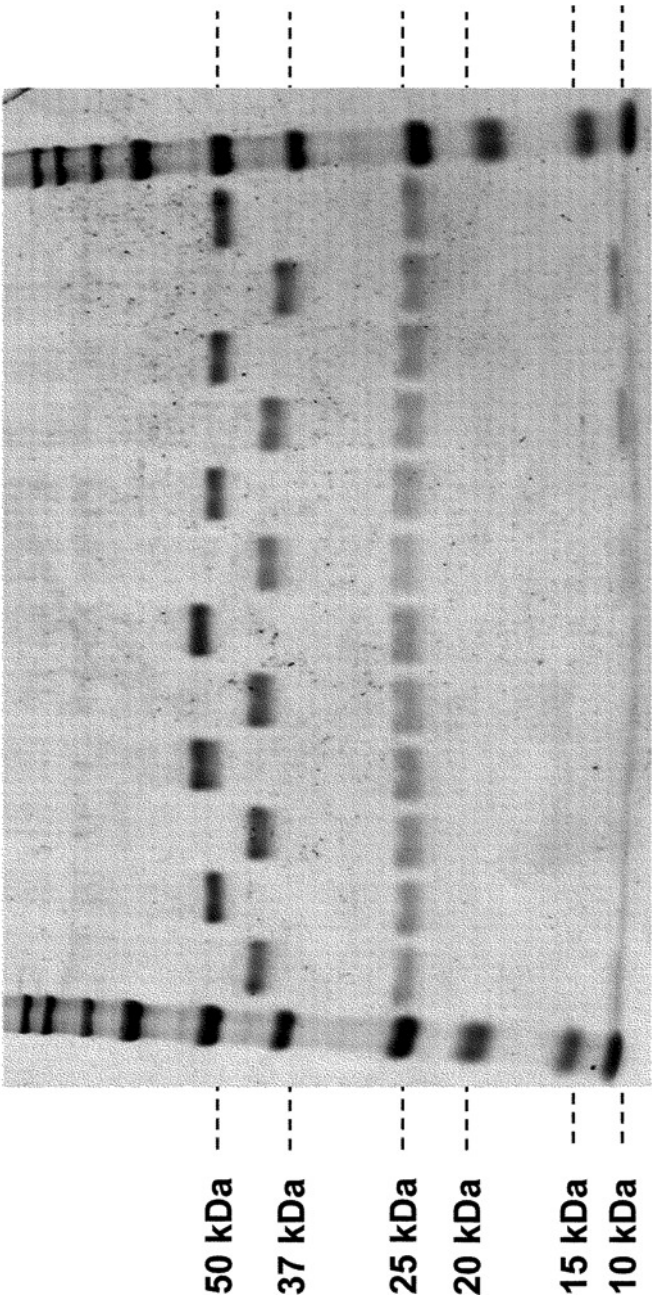


FIG. 22F

MRAVH113.12aa-G1T4 /MRAL-k0	-
	+
MRAVH112.12aa-G1T4 /MRAL-k0	-
	+
MRAVH111.12aa-G1T4 /MRAL-k0	-
	+
MRAVH110.12aa-G1T4 /MRAL-k0	-
	+
MRAVH109.12aa-G1T4 /MRAL-k0	-
	+
MRAVH102.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

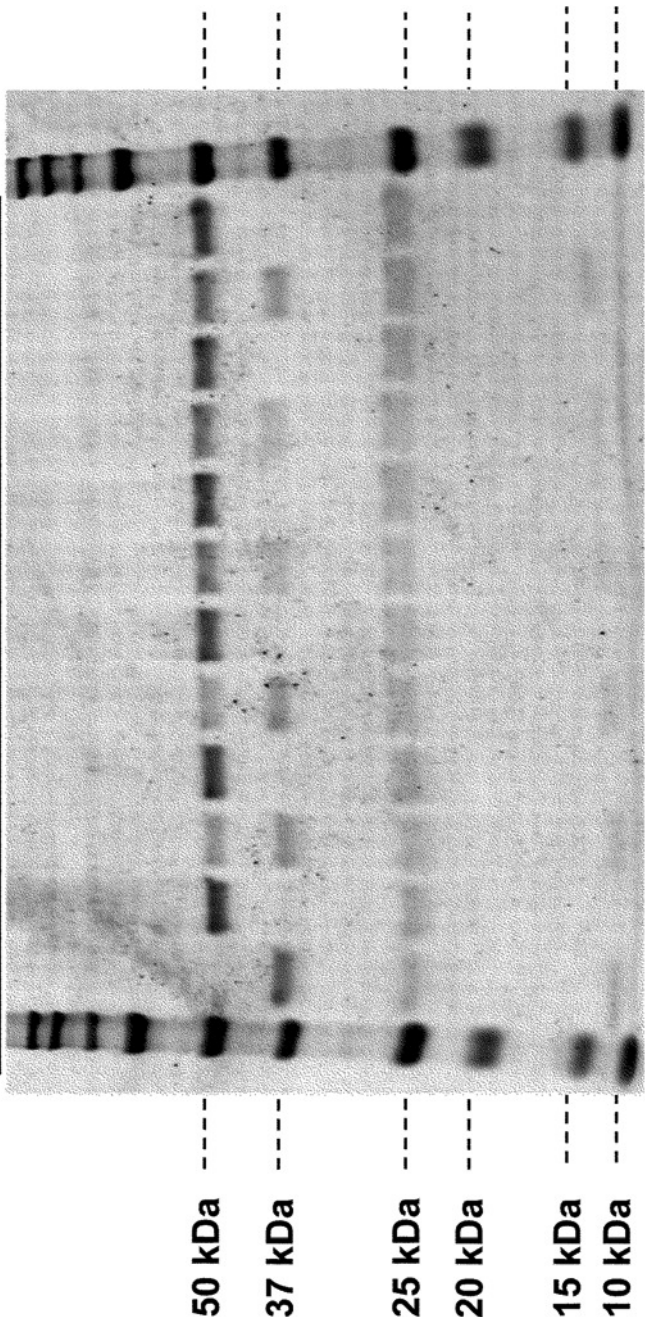


FIG. 22G

MRAVH115.12aa-G1T4 /MRAL-k0	-
	+
MRAVH114.12aa-G1T4 /MRAL-k0	-
	+
MRAVH100.12aa-G1T4 /MRAL-k0	-
	+
MRAVH099.12aa-G1T4 /MRAL-k0	-
	+
MRAVH089.12aa-G1T4 /MRAL-k0	-
	+
MRAVH069.12aa-G1T4 /MRAL-k0	-
	+
Antibody	MT-SP1

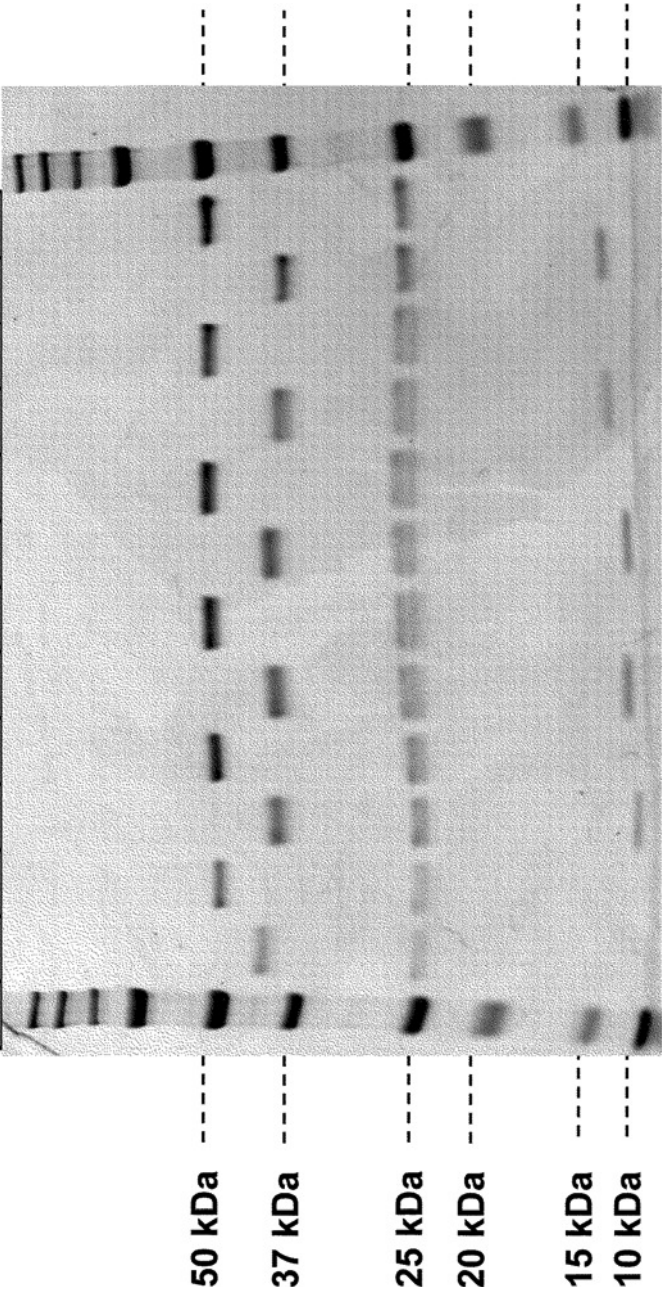


FIG. 22H

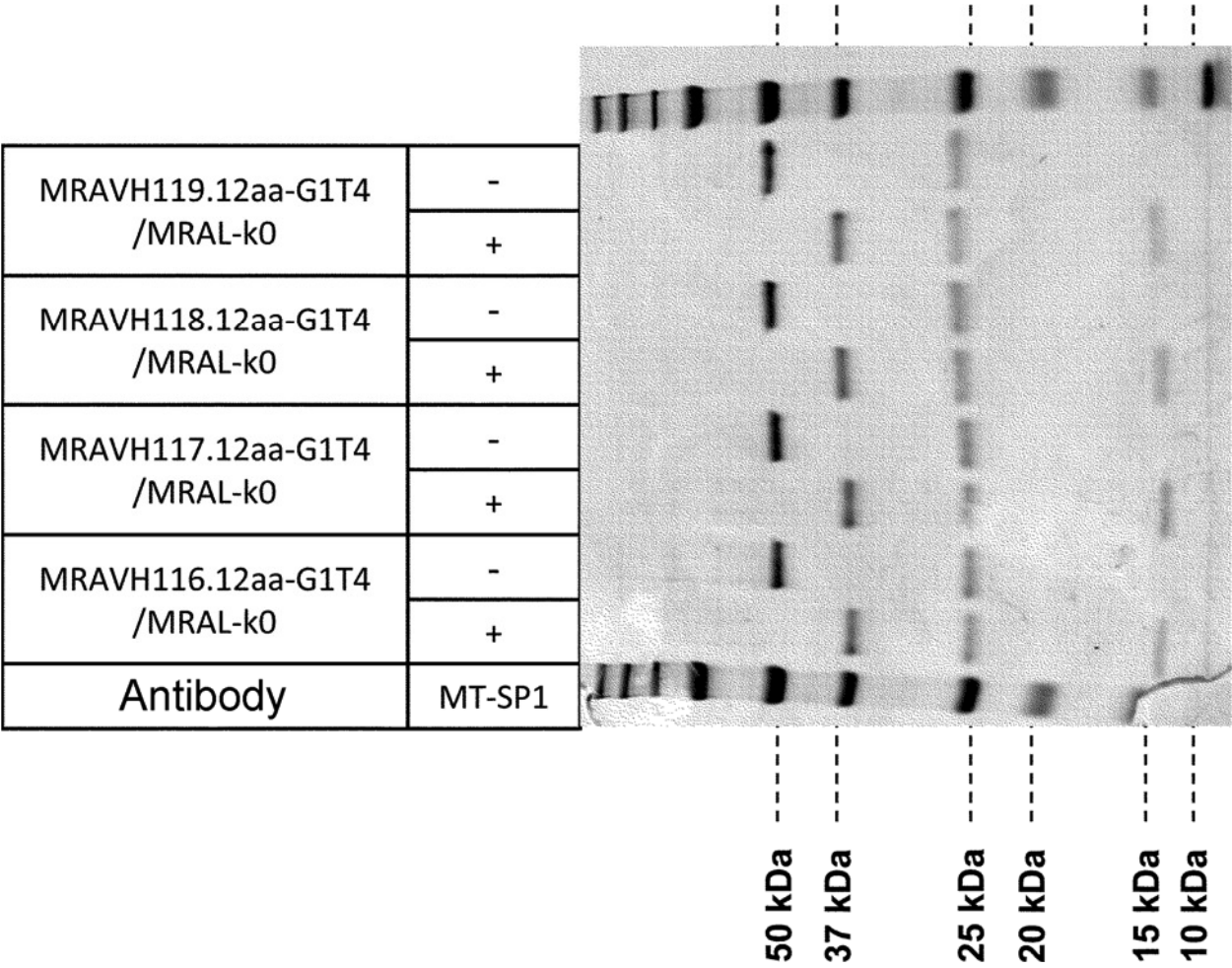


FIG. 22I

MRAH-G1T4.123.12aa /MRAL-k0	-
	+
MRAH-G1T4.122.12aa /MRAL-k0	-
	+
MRAH-G1T4.121.12aa /MRAL-k0	-
	+
MRAH-G1T4.120.12aa /MRAL-k0	-
	+
MRAH-G1T4.119.12aa /MRAL-k0	-
	+
MRAH-G1T4.118.12aa /MRAL-k0	-
	+
Antibody	MT-SP1

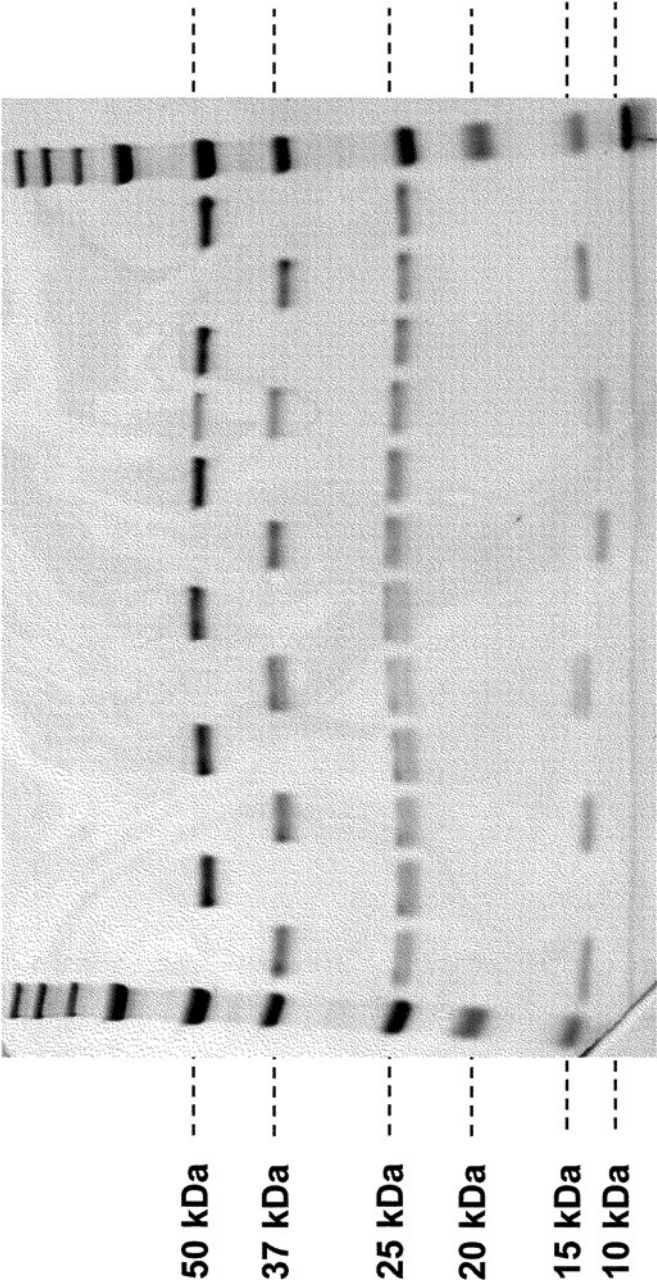


FIG. 23A

MRAH-G1T4.134.12aa /MRAL-k0	-
	+
MRAH-G1T4.132.12aa /MRAL-k0	-
	+
MRAH-G1T4.131.12aa /MRAL-k0	-
	+
MRAH-G1T4.130.12aa /MRAL-k0	-
	+
MRAH-G1T4.129.12aa /MRAL-k0	-
	+
MRAH-G1T4.124.12aa /MRAL-k0	-
	+
Antibody	MT-SP1

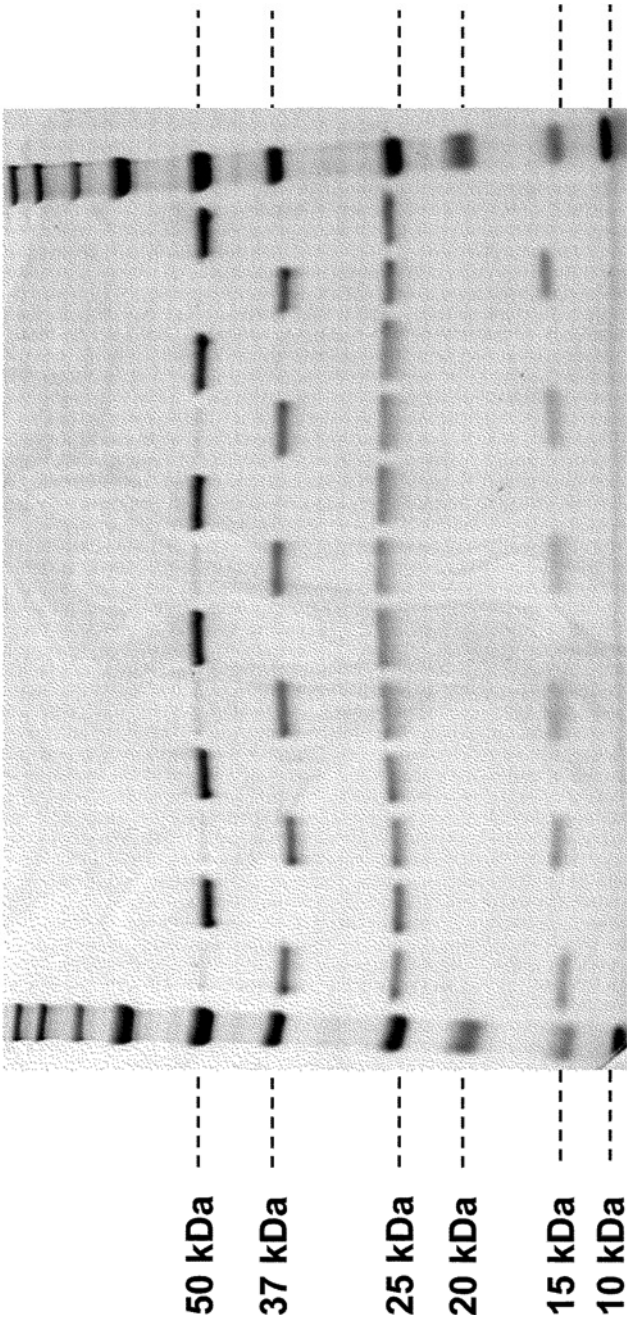


FIG. 23B

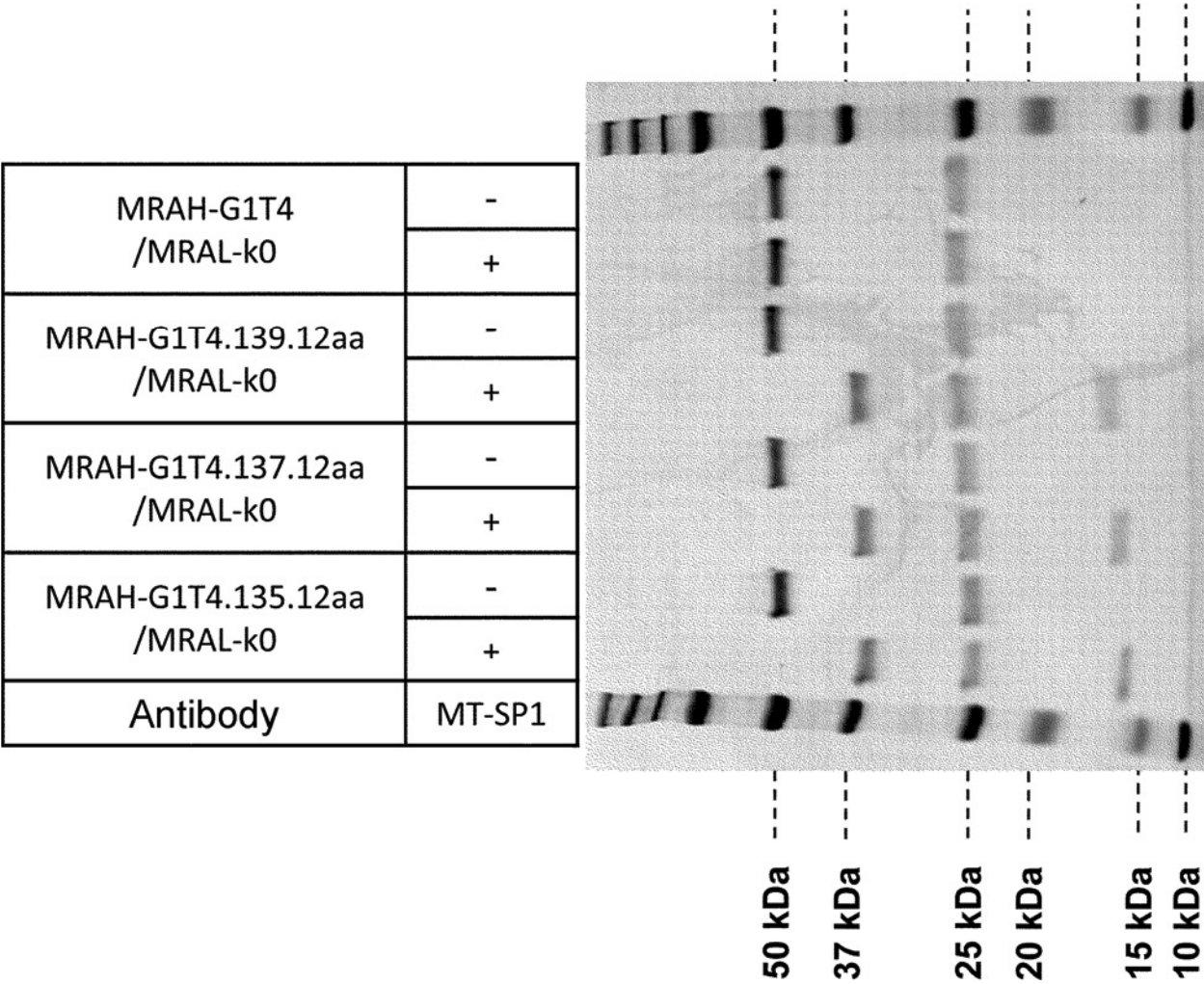


FIG. 23C

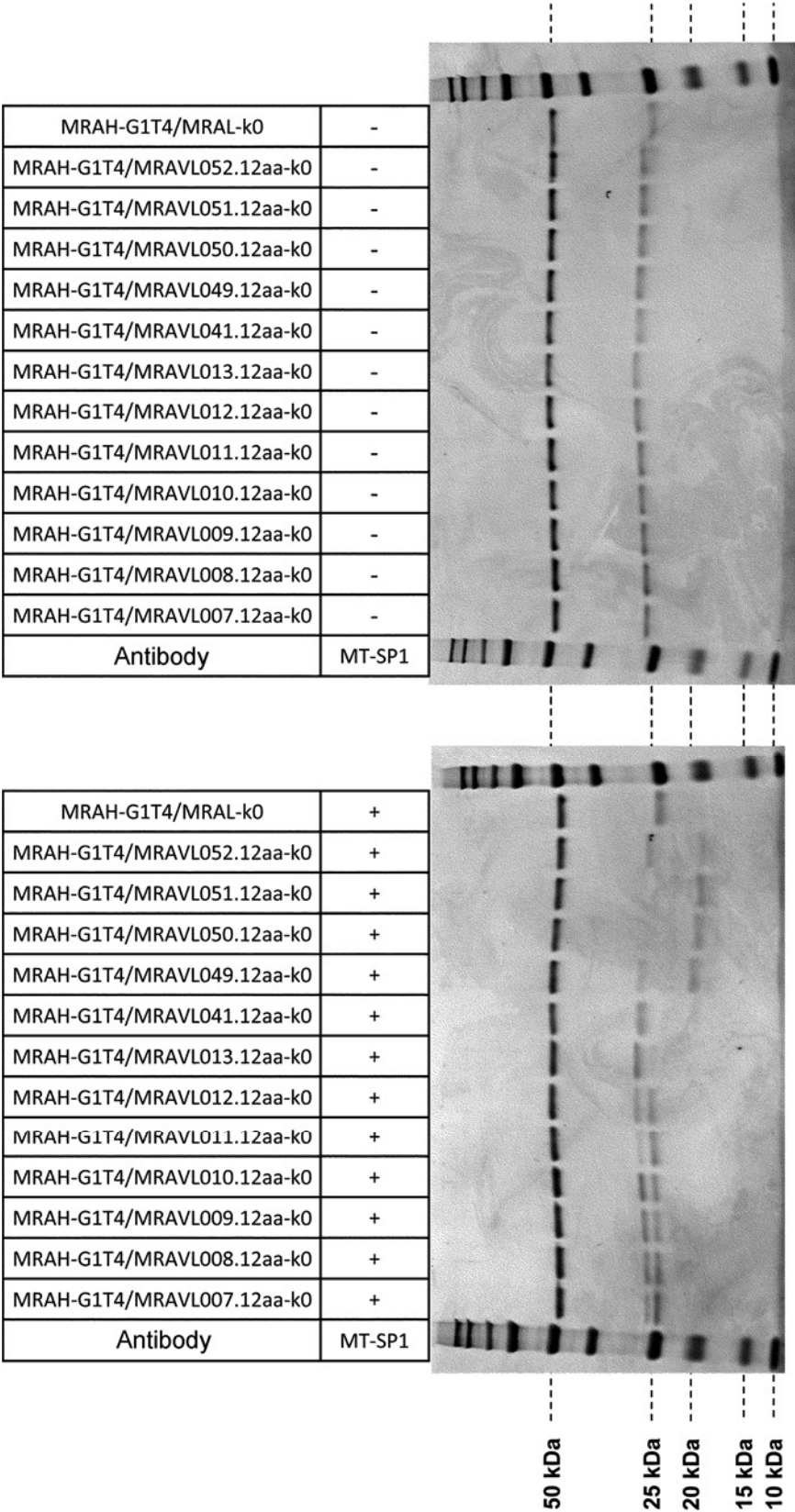


FIG. 24A

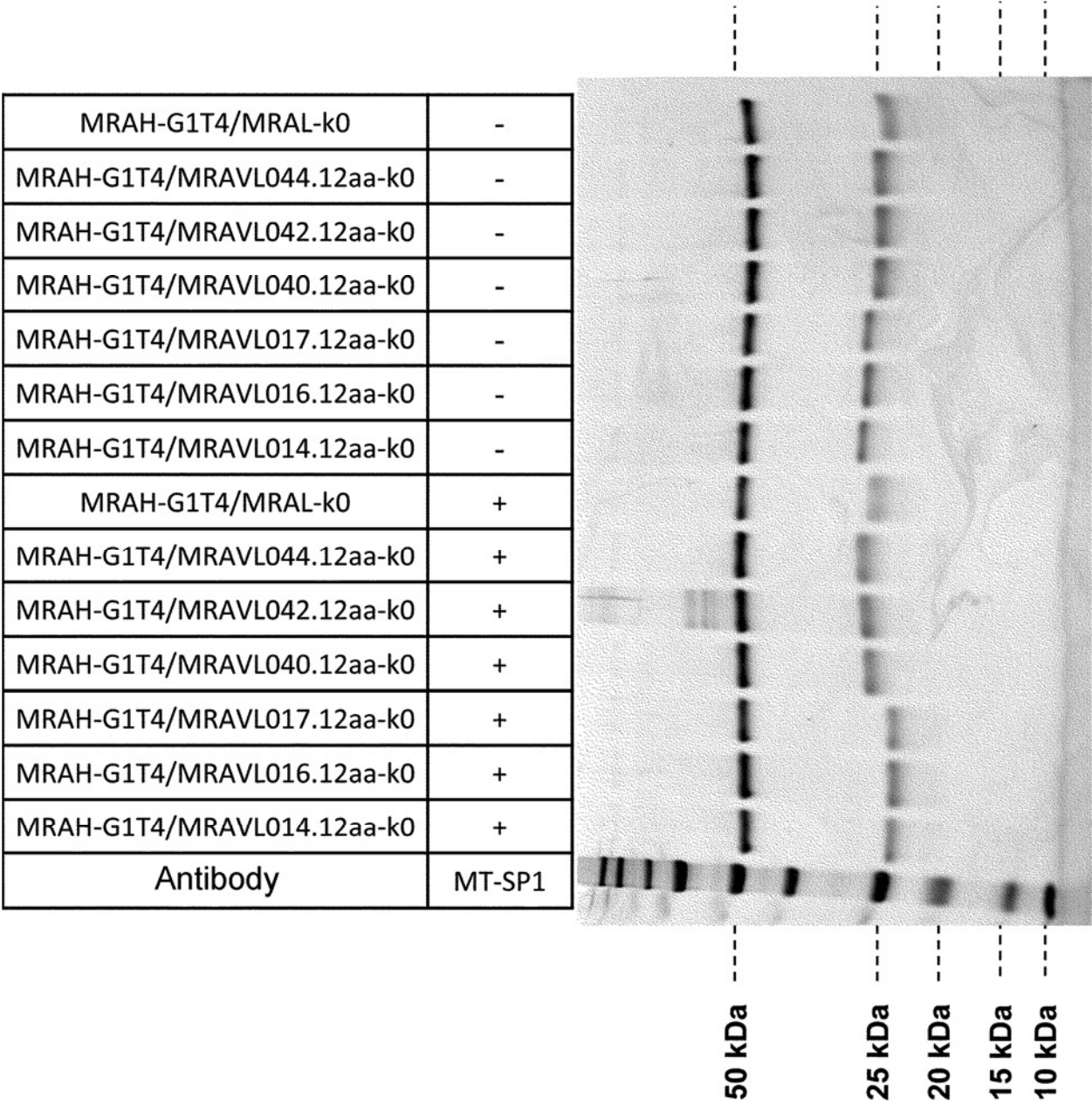
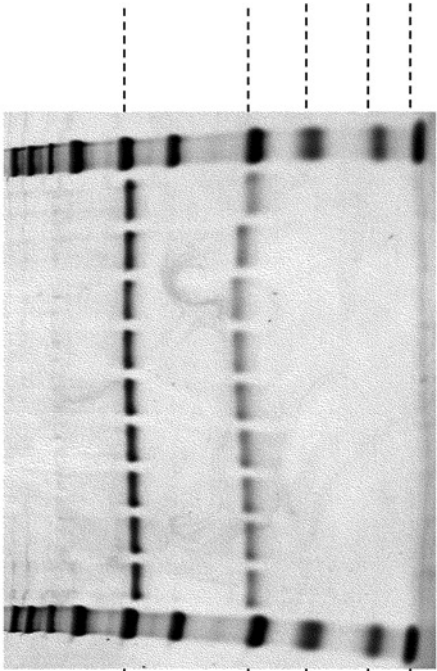
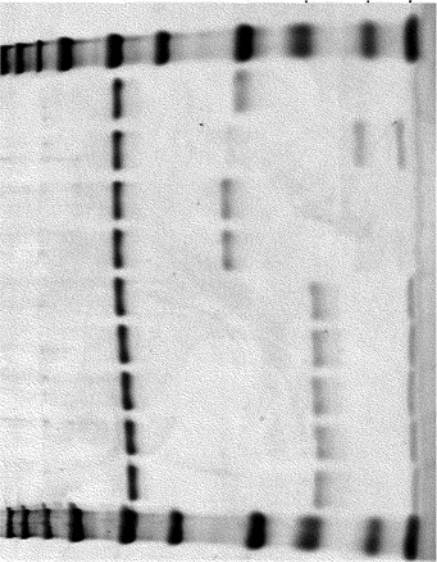


FIG. 24B

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAVL098.12aa-k0	-
MRAH-G1T4/MRAVL097.12aa-k0	-
MRAH-G1T4/MRAVL096.12aa-k0	-
MRAH-G1T4/MRAVL059.12aa-k0	-
MRAH-G1T4/MRAVL058.12aa-k0	-
MRAH-G1T4/MRAVL057.12aa-k0	-
MRAH-G1T4/MRAVL056.12aa-k0	-
MRAH-G1T4/MRAVL054.12aa-k0	-
Antibody	MT-SP1



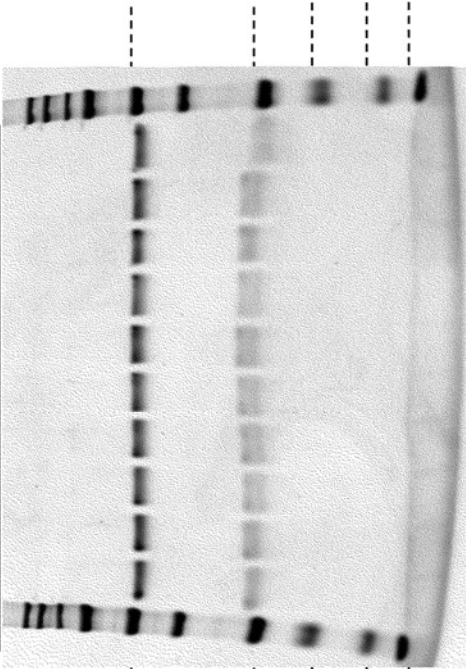
MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAVL098.12aa-k0	+
MRAH-G1T4/MRAVL097.12aa-k0	+
MRAH-G1T4/MRAVL096.12aa-k0	+
MRAH-G1T4/MRAVL059.12aa-k0	+
MRAH-G1T4/MRAVL058.12aa-k0	+
MRAH-G1T4/MRAVL057.12aa-k0	+
MRAH-G1T4/MRAVL056.12aa-k0	+
MRAH-G1T4/MRAVL054.12aa-k0	+
Antibody	MT-SP1



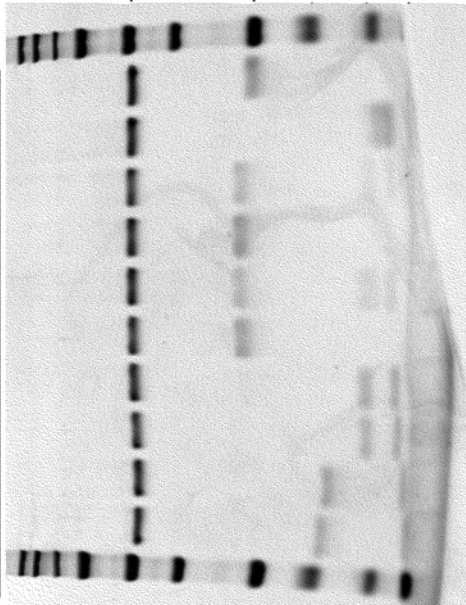
50 kDa
25 kDa
20 kDa
15 kDa
10 kDa

FIG. 24C

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAVL106.12aa-k0	-
MRAH-G1T4/MRAVL104.12aa-k0	-
MRAH-G1T4/MRAVL103.12aa-k0	-
MRAH-G1T4/MRAVL102.12aa-k0	-
MRAH-G1T4/MRAVL101.12aa-k0	-
MRAH-G1T4/MRAVL100.12aa-k0	-
MRAH-G1T4/MRAVL099.12aa-k0	-
MRAH-G1T4/MRAVL060.12aa-k0	-
MRAH-G1T4/MRAVL055.12aa-k0	-
Antibody	MT-SP1



MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAVL106.12aa-k0	+
MRAH-G1T4/MRAVL104.12aa-k0	+
MRAH-G1T4/MRAVL103.12aa-k0	+
MRAH-G1T4/MRAVL102.12aa-k0	+
MRAH-G1T4/MRAVL101.12aa-k0	+
MRAH-G1T4/MRAVL100.12aa-k0	+
MRAH-G1T4/MRAVL099.12aa-k0	+
MRAH-G1T4/MRAVL060.12aa-k0	+
MRAH-G1T4/MRAVL055.12aa-k0	+
Antibody	MT-SP1



50 kDa
25 kDa
20 kDa
15 kDa
10 kDa

FIG. 24D

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAVL107.12aa-k0	-
MRAH-G1T4/MRAVL105.12aa-k0	-
MRAH-G1T4/MRAVL053.12aa-k0	-
MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAVL107.12aa-k0	+
MRAH-G1T4/MRAVL105.12aa-k0	+
MRAH-G1T4/MRAVL053.12aa-k0	+
Antibody	MT-SP1

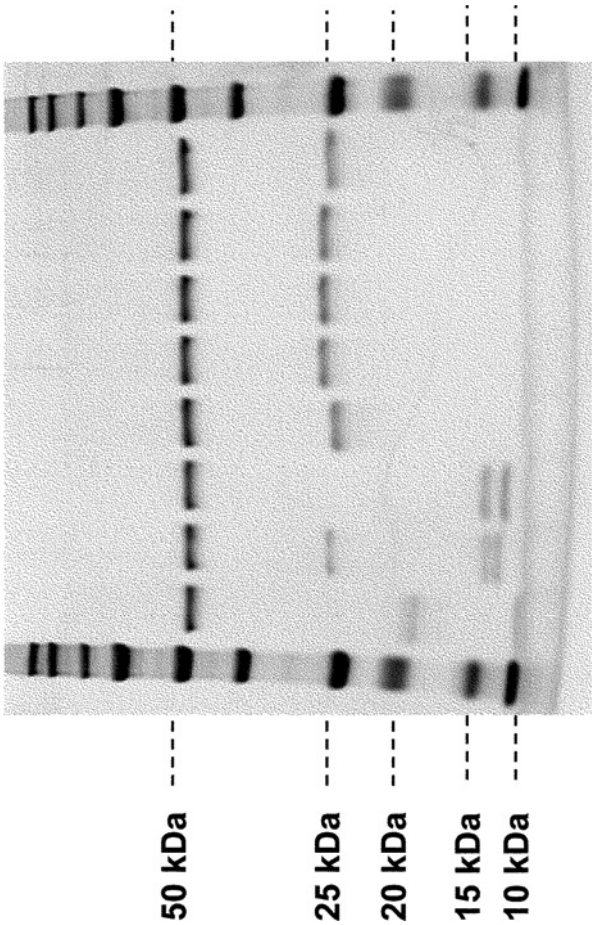
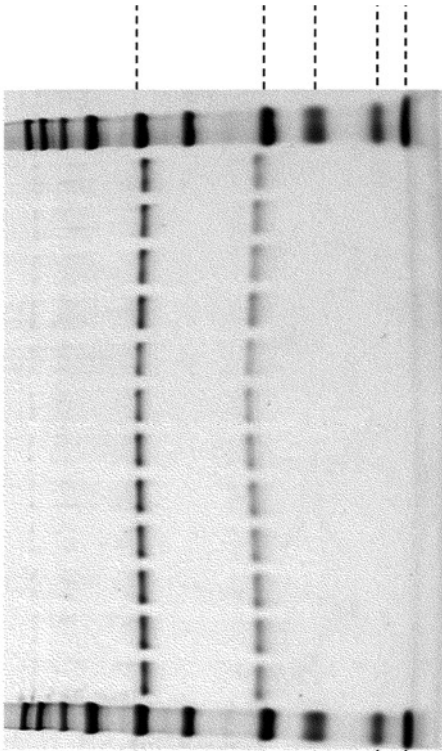


FIG. 24E

MRAH-G1T4/MRAL-k0.120.12aa	-
MRAH-G1T4/MRAL-k0.119.12aa	-
MRAH-G1T4/MRAL-k0.118.12aa	-
MRAH-G1T4/MRAL-k0.117.12aa	-
MRAH-G1T4/MRAL-k0.116.12aa	-
MRAH-G1T4/MRAL-k0.115.12aa	-
MRAH-G1T4/MRAL-k0.113.12aa	-
MRAH-G1T4/MRAL-k0.112.12aa	-
MRAH-G1T4/MRAL-k0.111.12aa	-
MRAH-G1T4/MRAL-k0.110.12aa	-
MRAH-G1T4/MRAL-k0.109.12aa	-
MRAH-G1T4/MRAL-k0.108.12aa	-
Antibody	MT-SP1



MRAH-G1T4/MRAL-k0.120.12aa	+
MRAH-G1T4/MRAL-k0.119.12aa	+
MRAH-G1T4/MRAL-k0.118.12aa	+
MRAH-G1T4/MRAL-k0.117.12aa	+
MRAH-G1T4/MRAL-k0.116.12aa	+
MRAH-G1T4/MRAL-k0.115.12aa	+
MRAH-G1T4/MRAL-k0.113.12aa	+
MRAH-G1T4/MRAL-k0.112.12aa	+
MRAH-G1T4/MRAL-k0.111.12aa	+
MRAH-G1T4/MRAL-k0.110.12aa	+
MRAH-G1T4/MRAL-k0.109.12aa	+
MRAH-G1T4/MRAL-k0.108.12aa	+
Antibody	MT-SP1

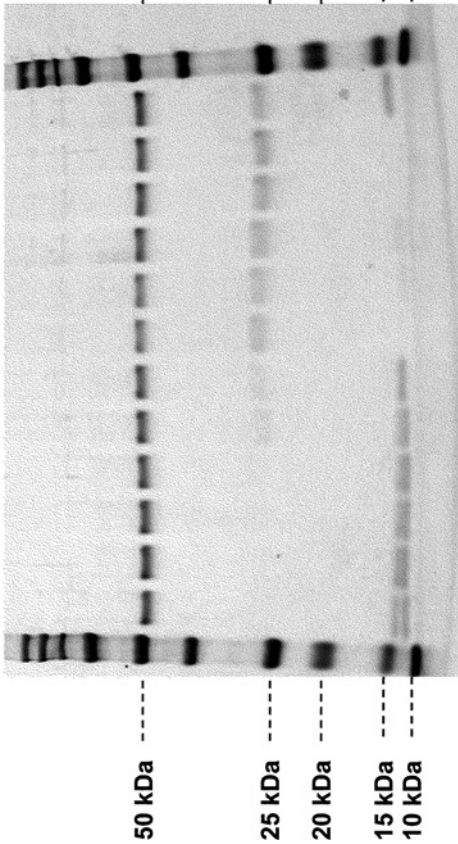
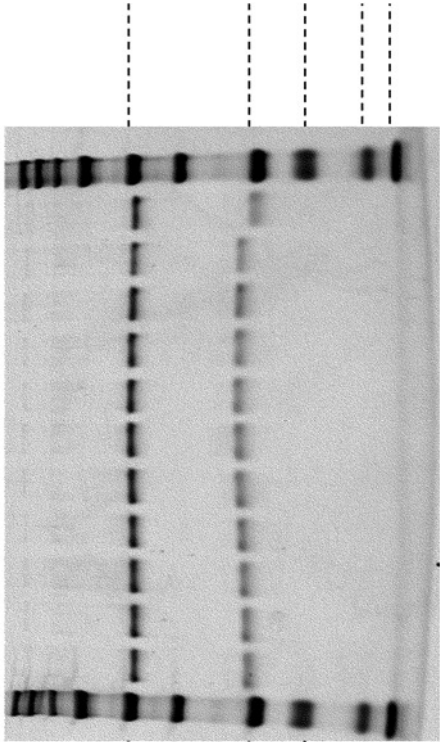
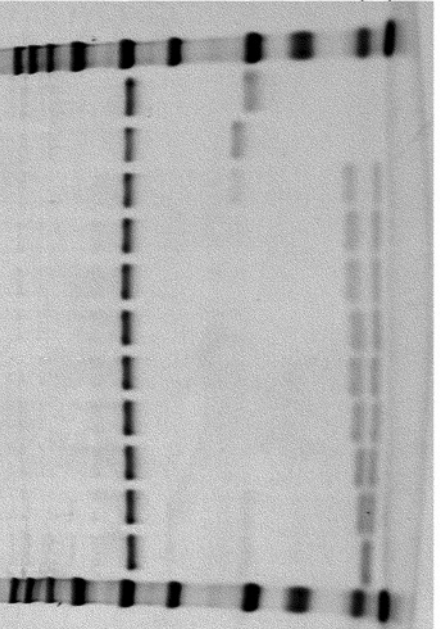


FIG. 25A

MRAH-G1T4/MRAL-k0	-
MRAH-G1T4/MRAL-k0.130.12aa	-
MRAH-G1T4/MRAL-k0.129.12aa	-
MRAH-G1T4/MRAL-k0.128.12aa	-
MRAH-G1T4/MRAL-k0.127.12aa	-
MRAH-G1T4/MRAL-k0.126.12aa	-
MRAH-G1T4/MRAL-k0.125.12aa	-
MRAH-G1T4/MRAL-k0.124.12aa	-
MRAH-G1T4/MRAL-k0.123.12aa	-
MRAH-G1T4/MRAL-k0.122.12aa	-
MRAH-G1T4/MRAL-k0.121.12aa	-
Antibody	MT-SP1



MRAH-G1T4/MRAL-k0	+
MRAH-G1T4/MRAL-k0.130.12aa	+
MRAH-G1T4/MRAL-k0.129.12aa	+
MRAH-G1T4/MRAL-k0.128.12aa	+
MRAH-G1T4/MRAL-k0.127.12aa	+
MRAH-G1T4/MRAL-k0.126.12aa	+
MRAH-G1T4/MRAL-k0.125.12aa	+
MRAH-G1T4/MRAL-k0.124.12aa	+
MRAH-G1T4/MRAL-k0.123.12aa	+
MRAH-G1T4/MRAL-k0.122.12aa	+
MRAH-G1T4/MRAL-k0.121.12aa	+
Antibody	MT-SP1



50 kDa
25 kDa
20 kDa
15 kDa
10 kDa

FIG. 25B

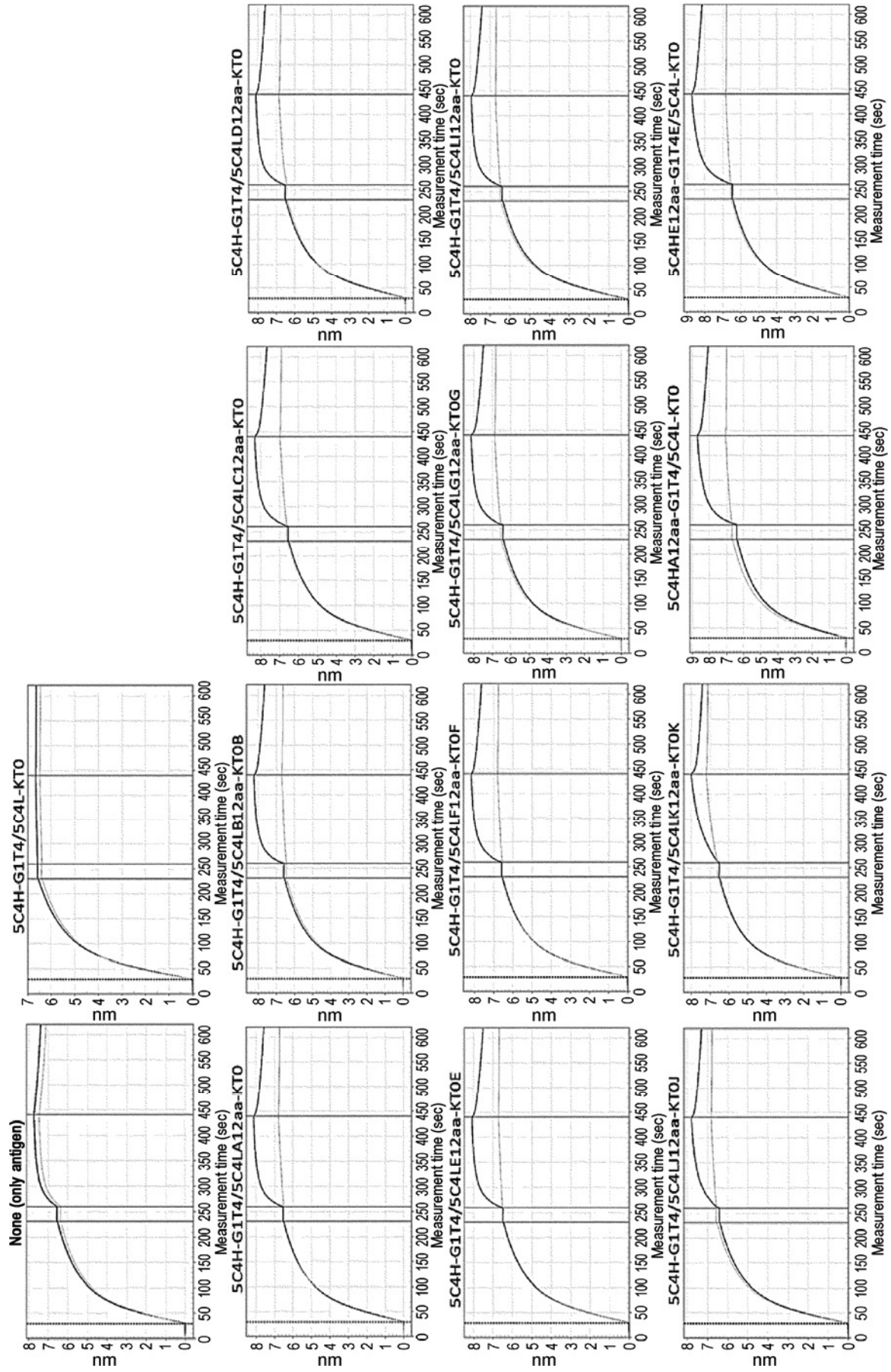


FIG. 26

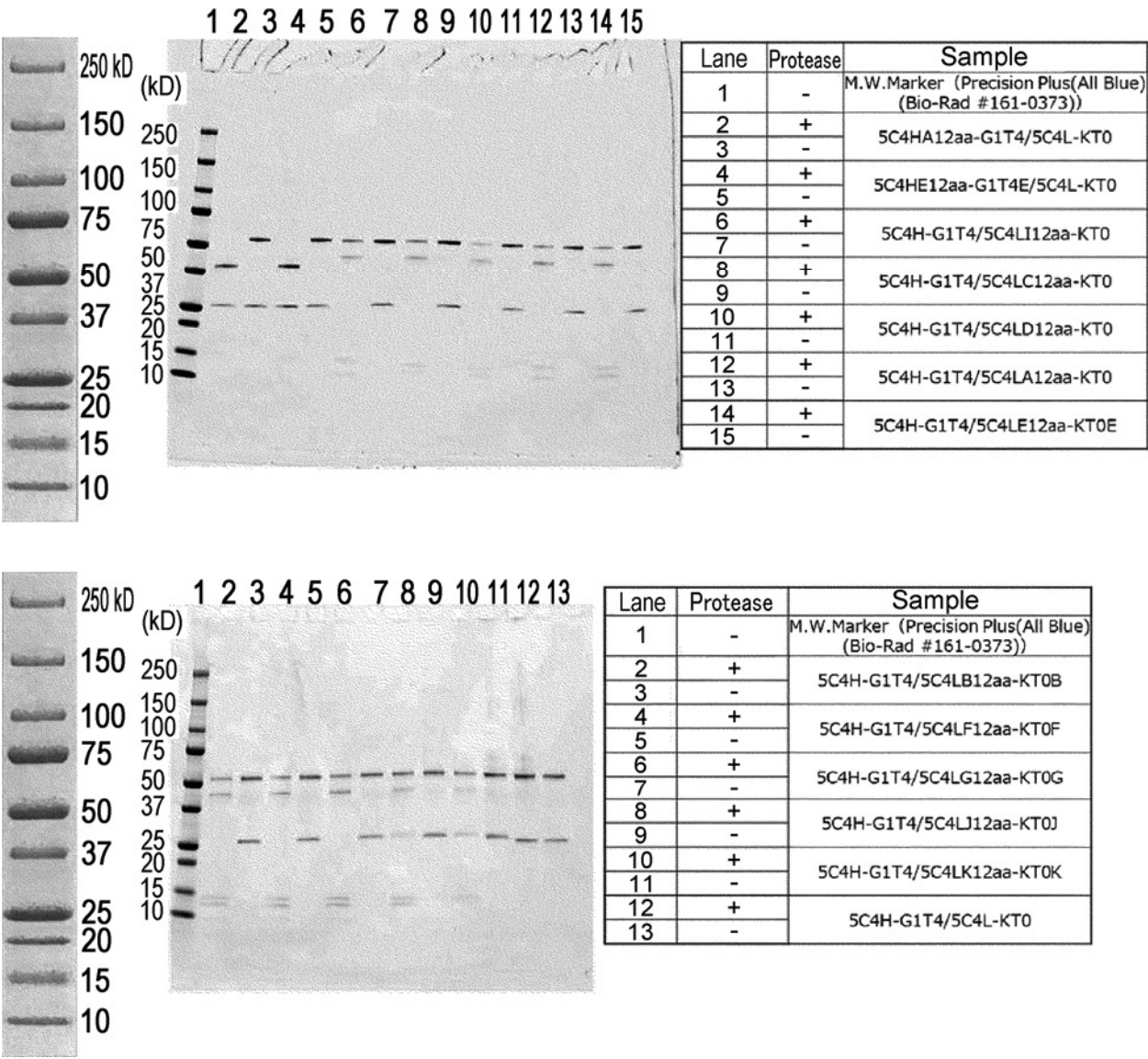


FIG. 27

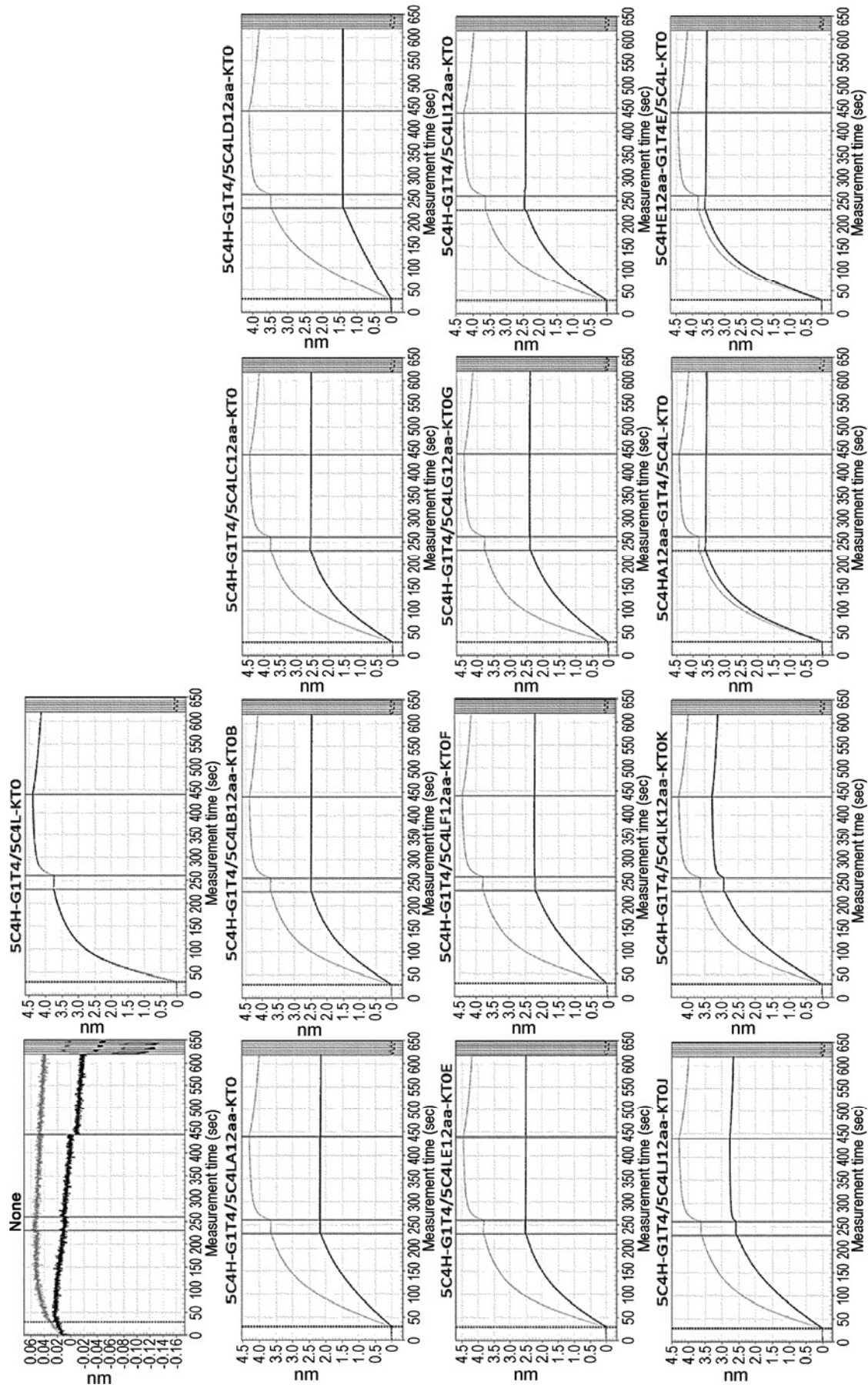


FIG. 28

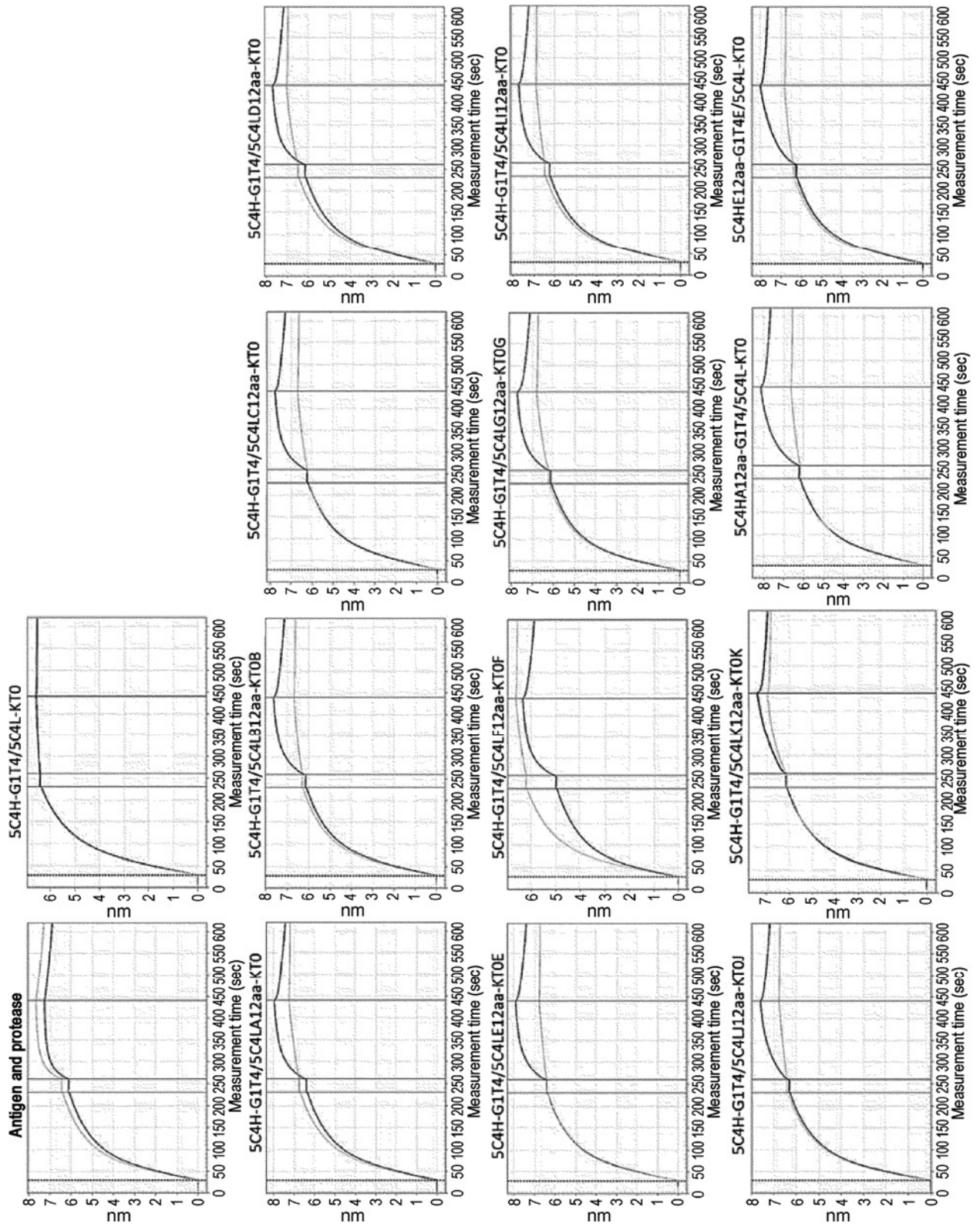


FIG. 29

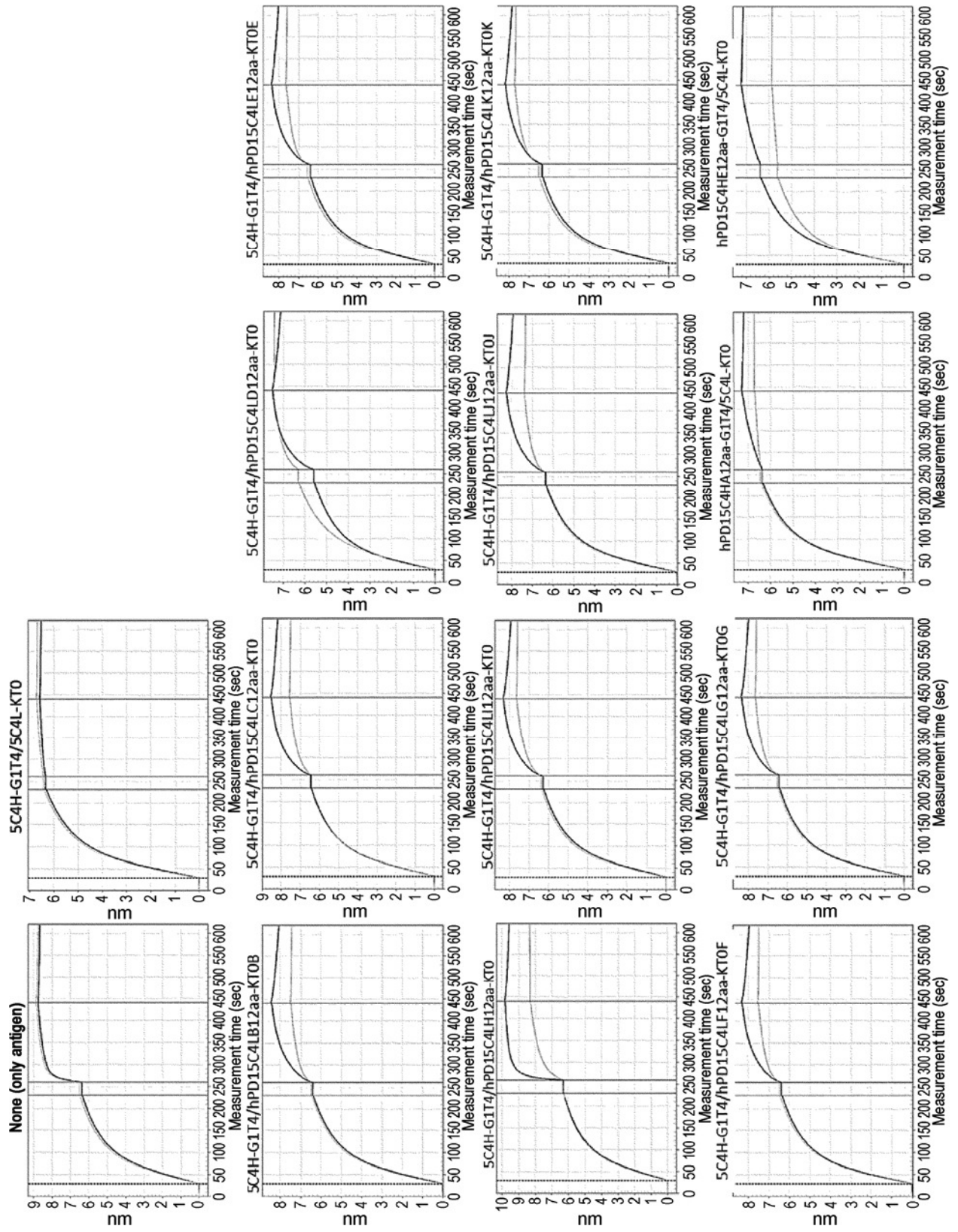


FIG. 30

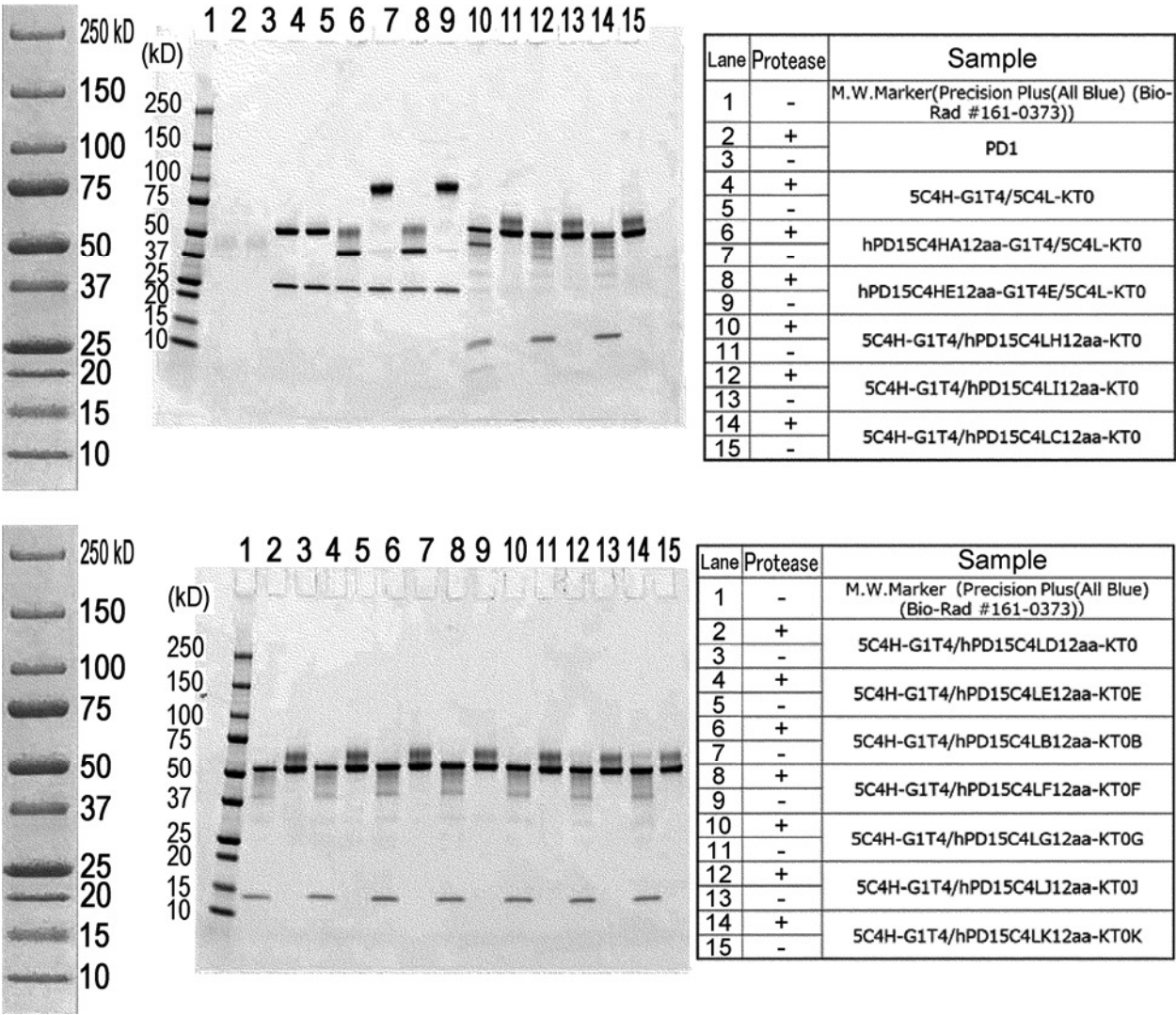


FIG. 31

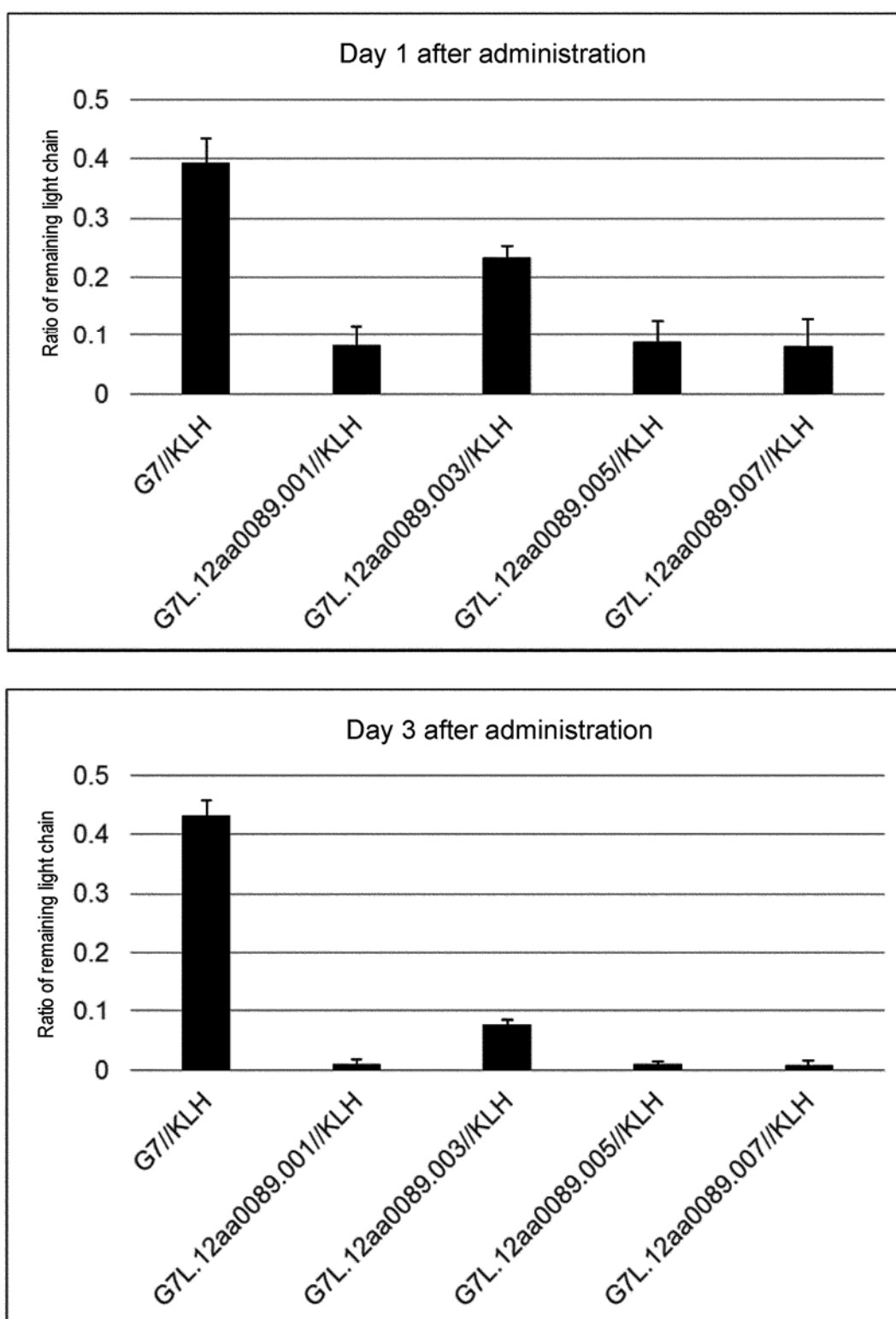


FIG. 32