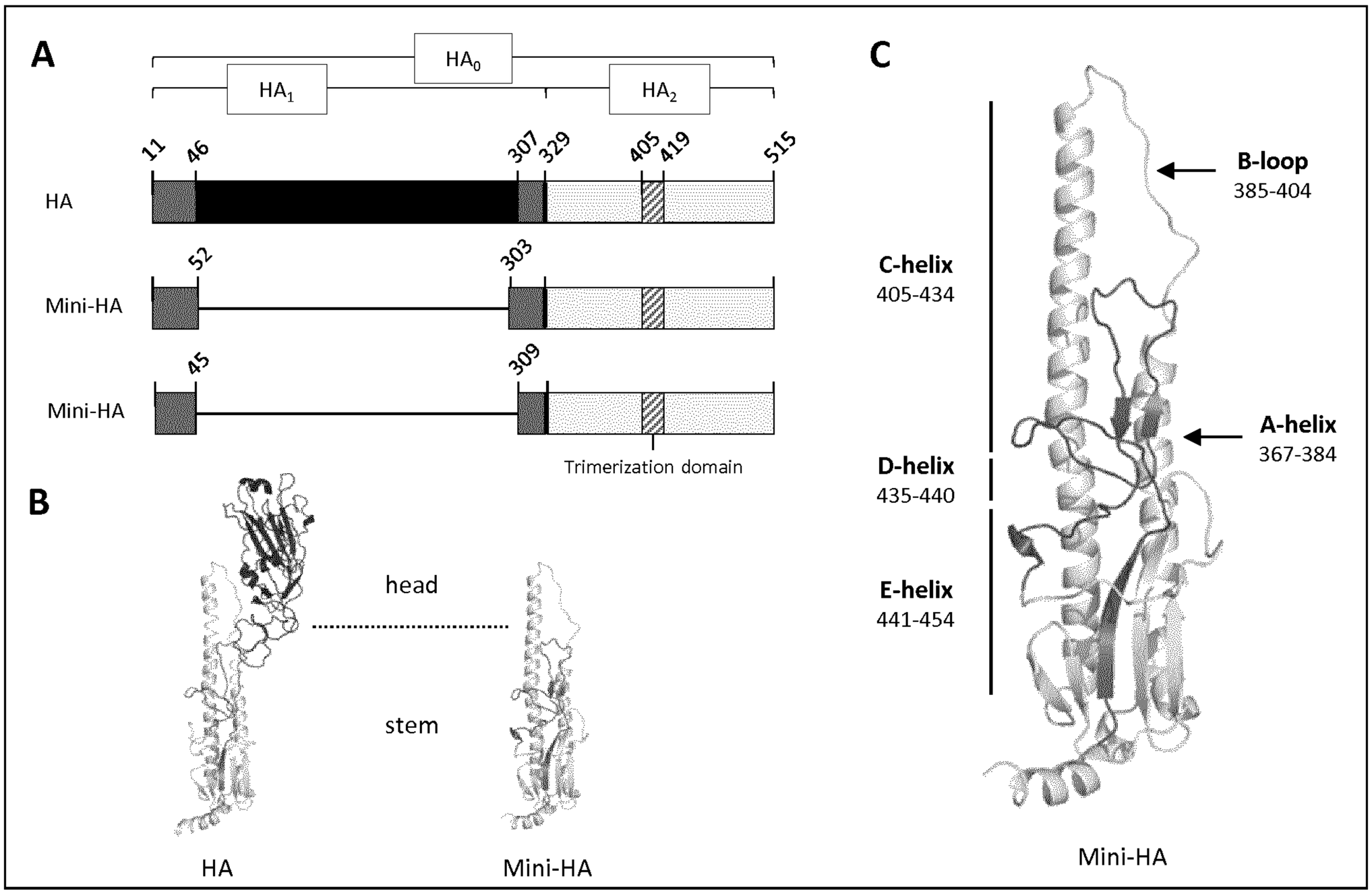

A
HA0
HA1
HA2
11
46
307
329
405
419
515
HA
52
303
Mini-HA
45
309
Mini-HA
Trimerization domain
B
head
stem
HA
Mini-HA
C
B-loop
385-404
C-helix
405-434
A-helix
367-384
D-helix
435-440
E-helix
441-454
Mini-HA

FIG. 1

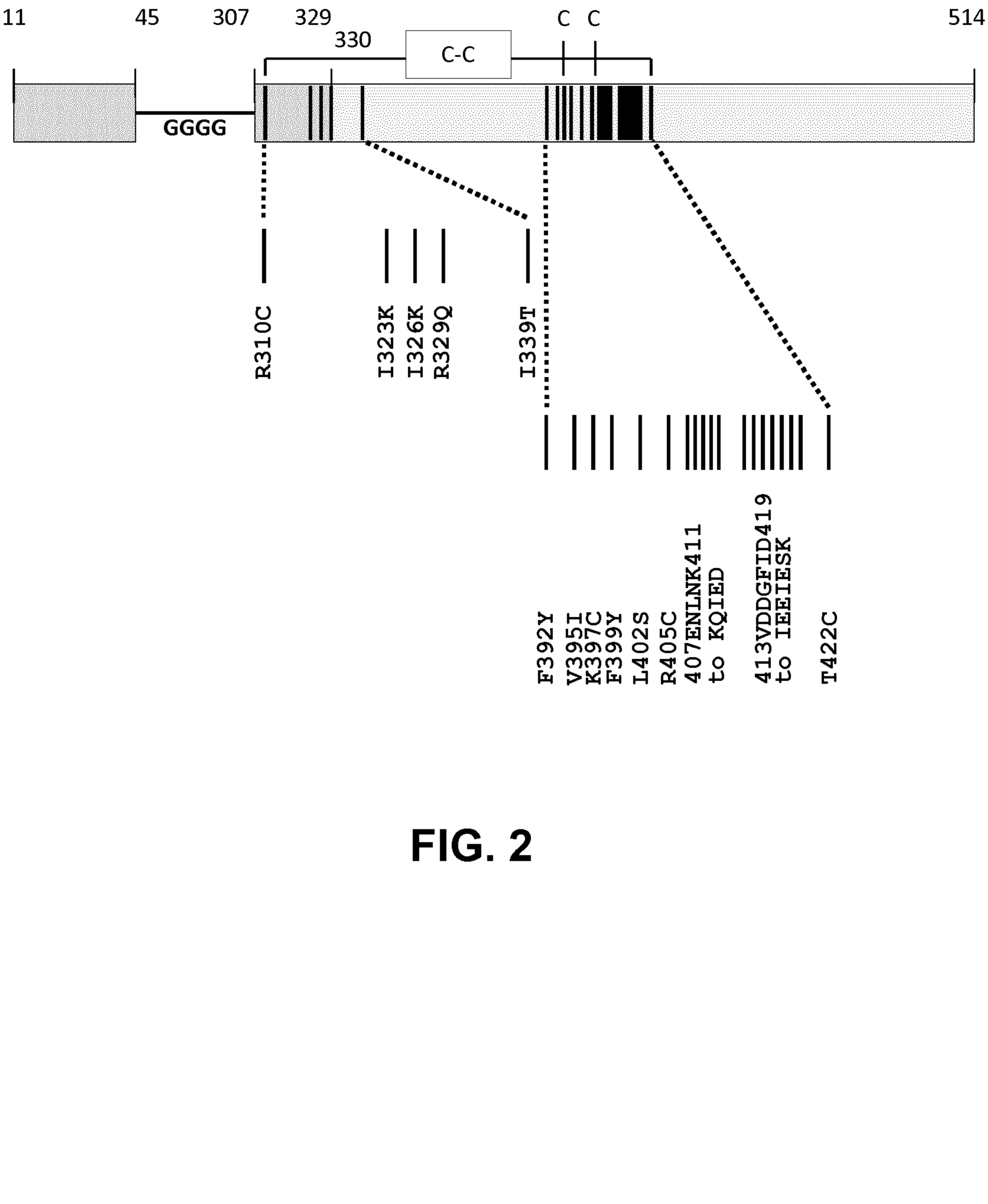

11
45
307
329
330
C-C
C C
514
GGGG
R310C
I323K
I326K
R329Q
I339T
F392Y
V395I
K397C
F399Y
L402S
R405C
407ENLNK411
to KQIED
413VDDGFID41
to IEEIESK
T422C

FIG. 2

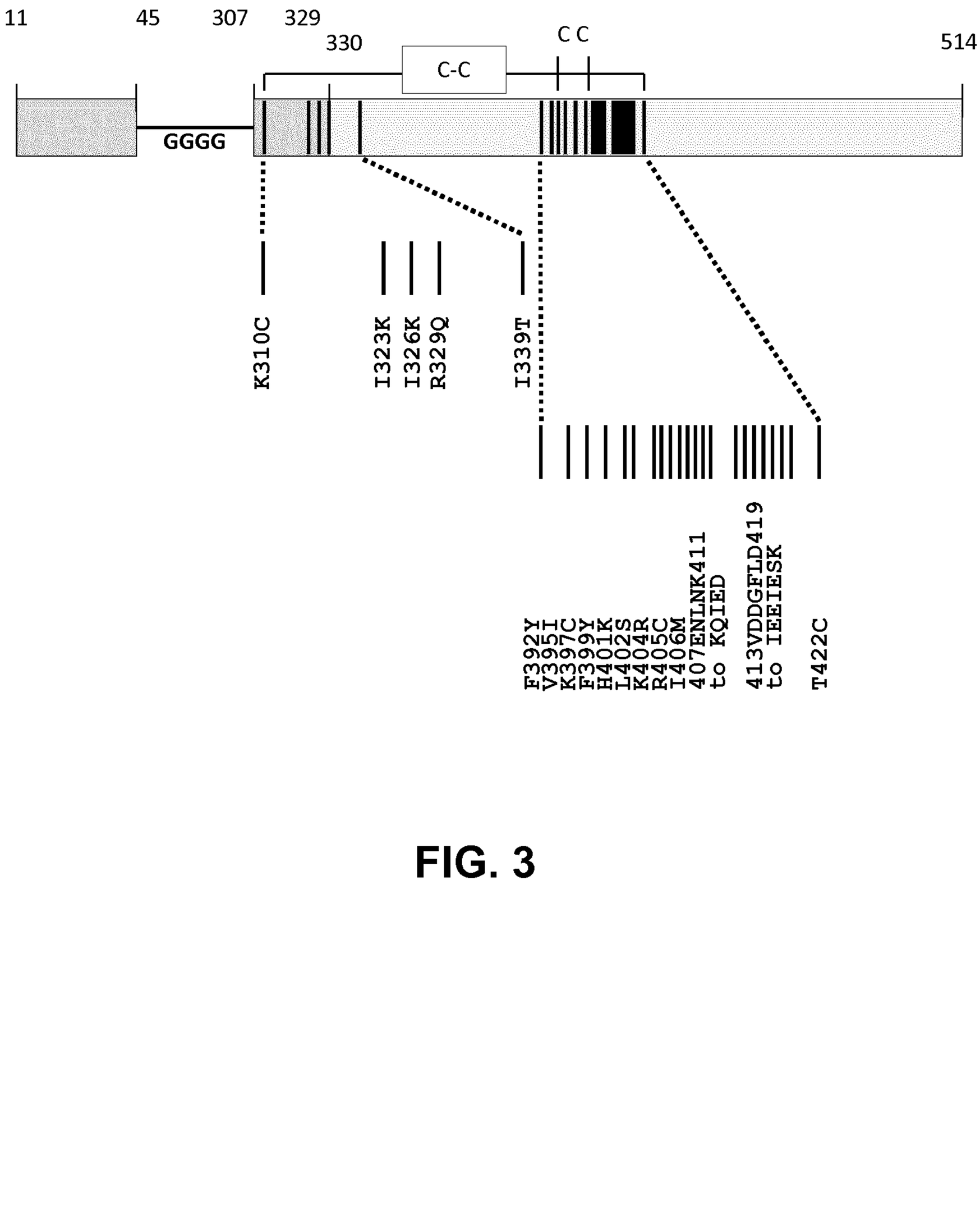

11
45
307
329
330
C C
514
C-C
GGGG
K310C
I323K
I326K
R329Q
I339T
F392Y
V395I
K397C
F399Y
H401K
L402S
K404R
R405C
I406M
407ENLNK411
to KQIED
413VDDGFLD419
to IEEIESK
T422C

FIG. 3

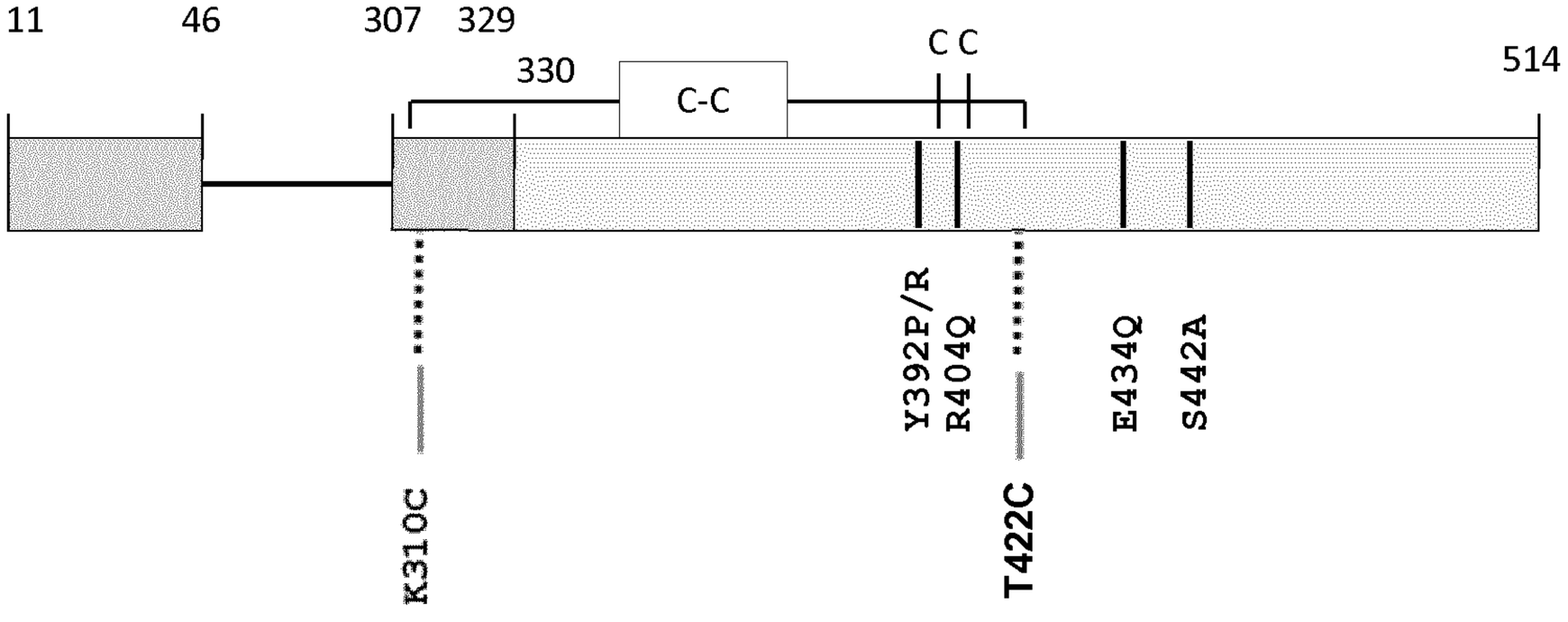
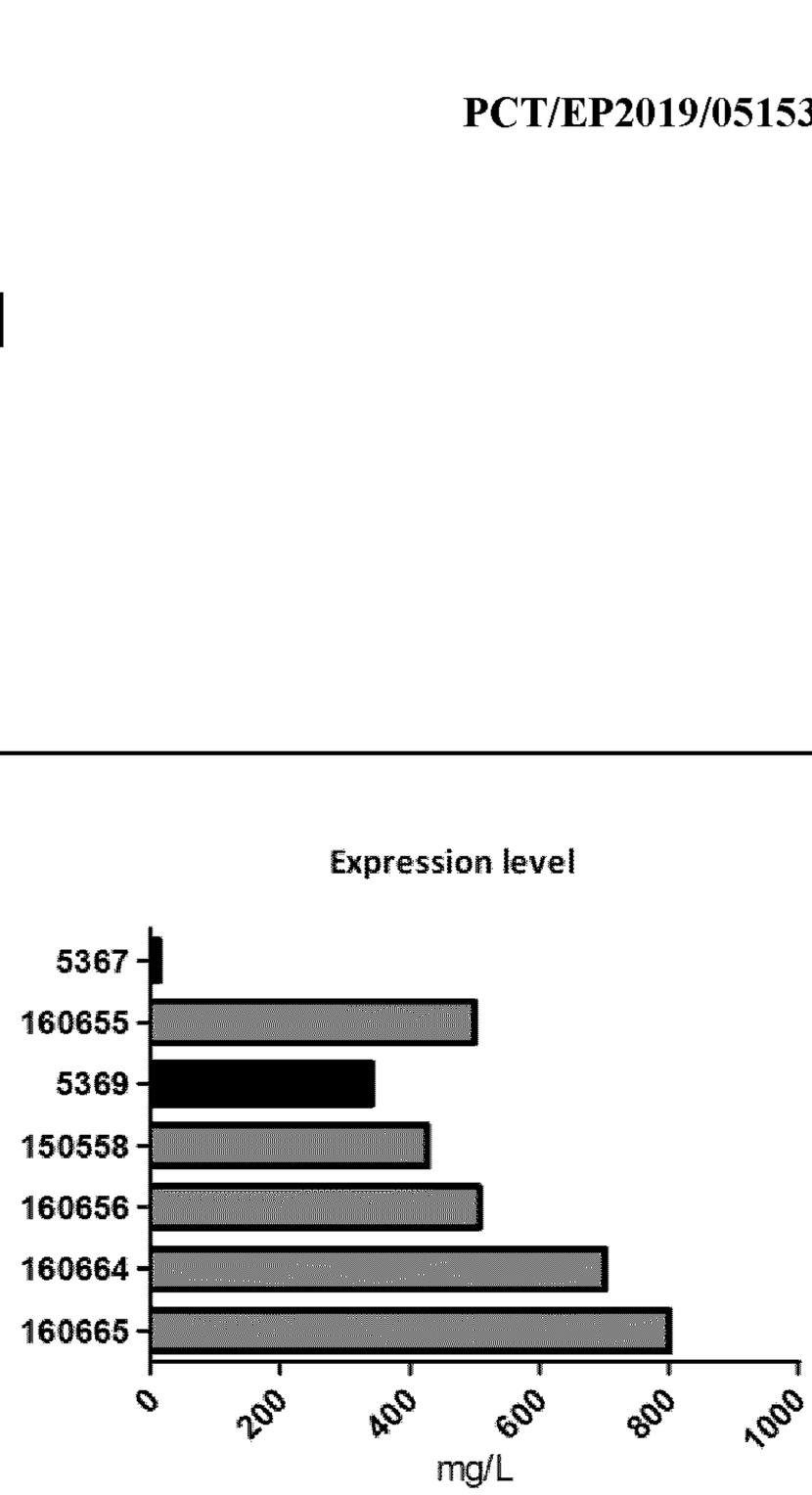

11
46
307
329
330
C-C
C C
514
K310C
Y392P/R
R404Q
T422C
E434Q
S442A

FIG. 4

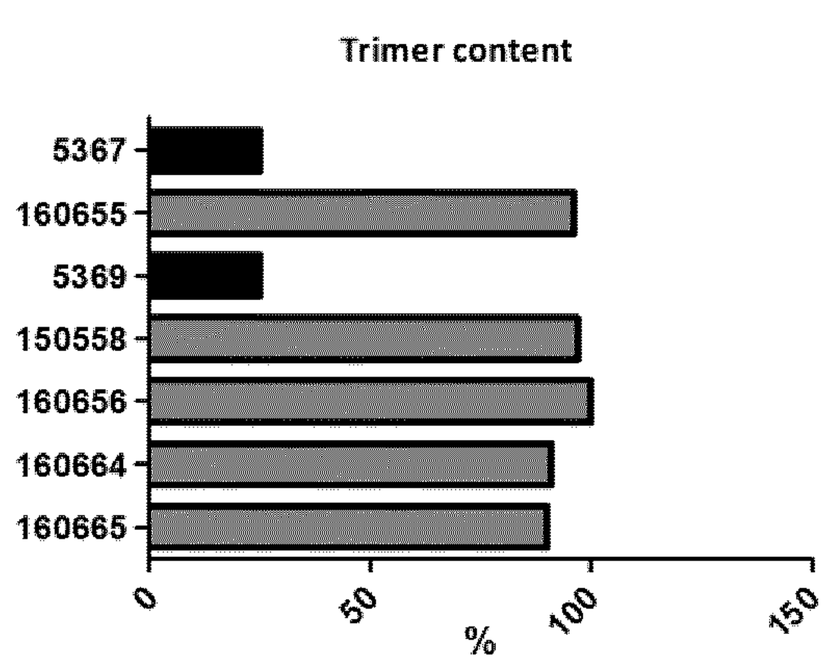
A

AA	329	392	404	434	438	442
WT	**R**	**F**	**R/K**	**E**	**D**	**S**
	Q	Y	R	E	D	S
	Q	P	Q	Q	D	A
	Q	Y	R	E	D	S
	Q	P	Q	Q	D	A
	Q	P	Q	Q	D	A
	Q	R	R	Q	D	S
	Q	P	R	Q	D	S



B

AA	329	392	404	434	438	442
WT	**R**	**F**	**R/K**	**E**	**D**	**S**
	Q	Y	R	E	D	S
	Q	P	Q	Q	D	A
	Q	Y	R	E	D	S
	Q	P	Q	Q	D	A
	Q	P	Q	Q	D	A
	Q	R	R	Q	D	S
	Q	P	R	Q	D	S



C

AA	329	392	404	434	438	442
WT	**R**	**F**	**R/K**	**E**	**D**	**S**
	Q	Y	R	E	D	S
	Q	P	Q	Q	D	A
	Q	R	R	Q	E	S
	R	R	R	Q	E	S
	R	R	R	Q	D	S
	R	Y	R	Q	E	S

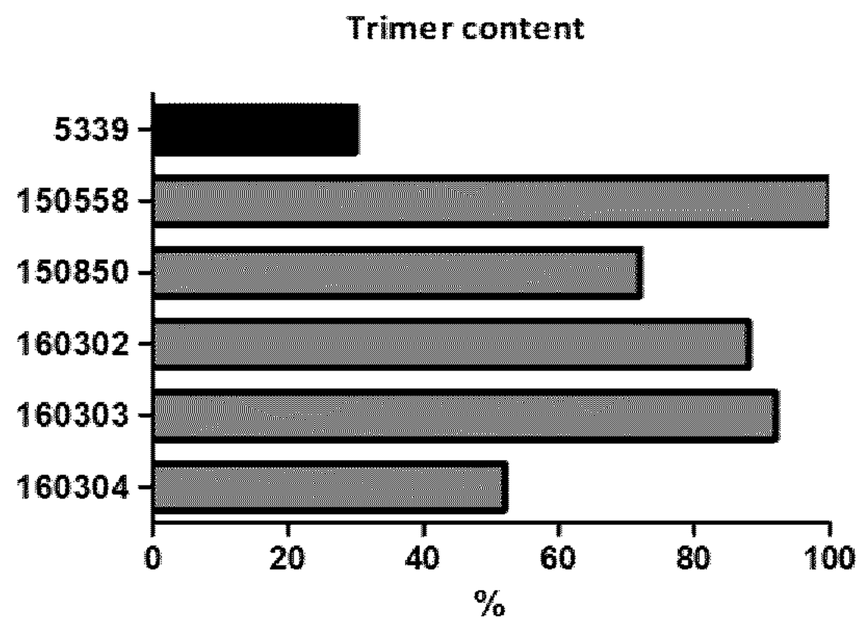


FIG. 5

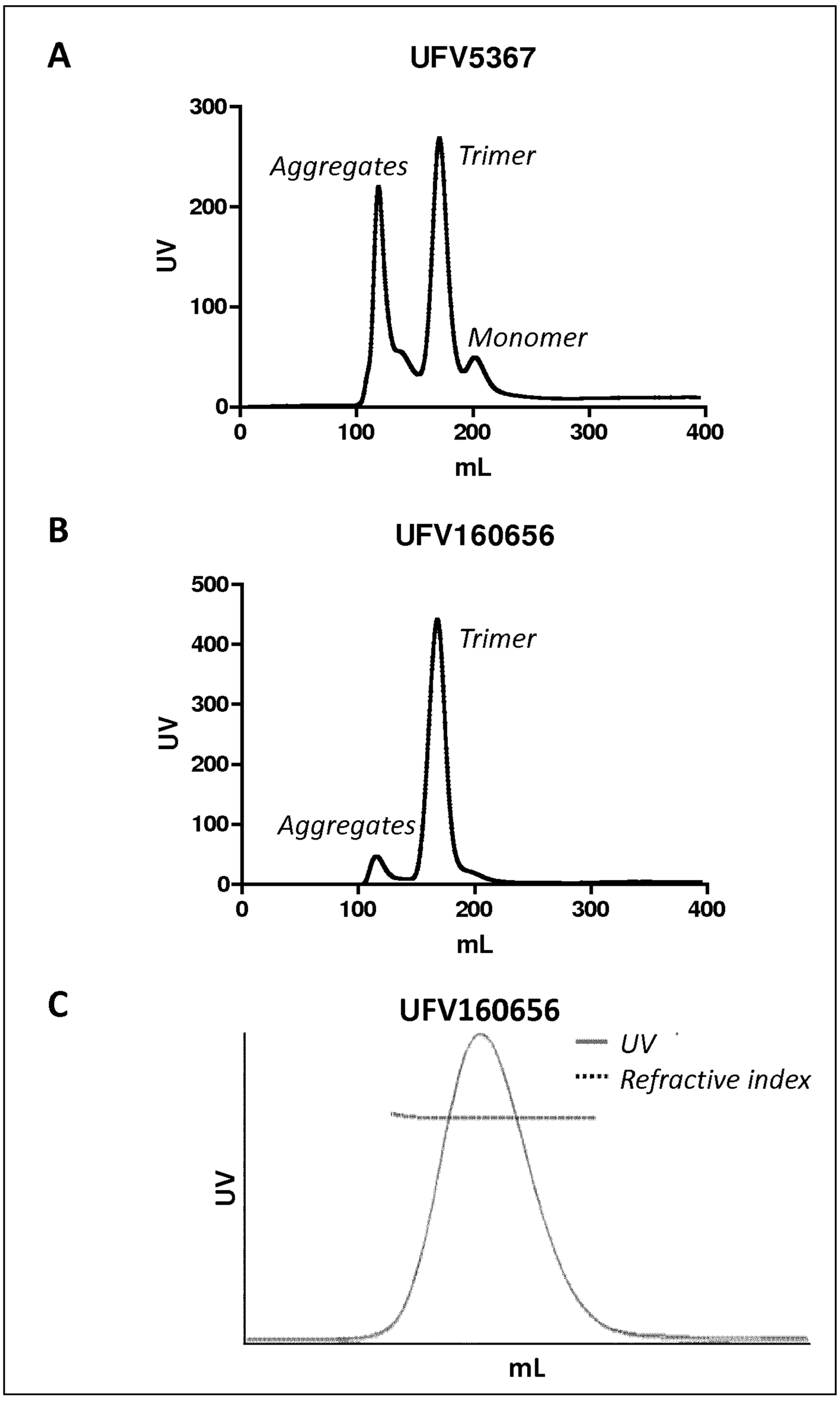
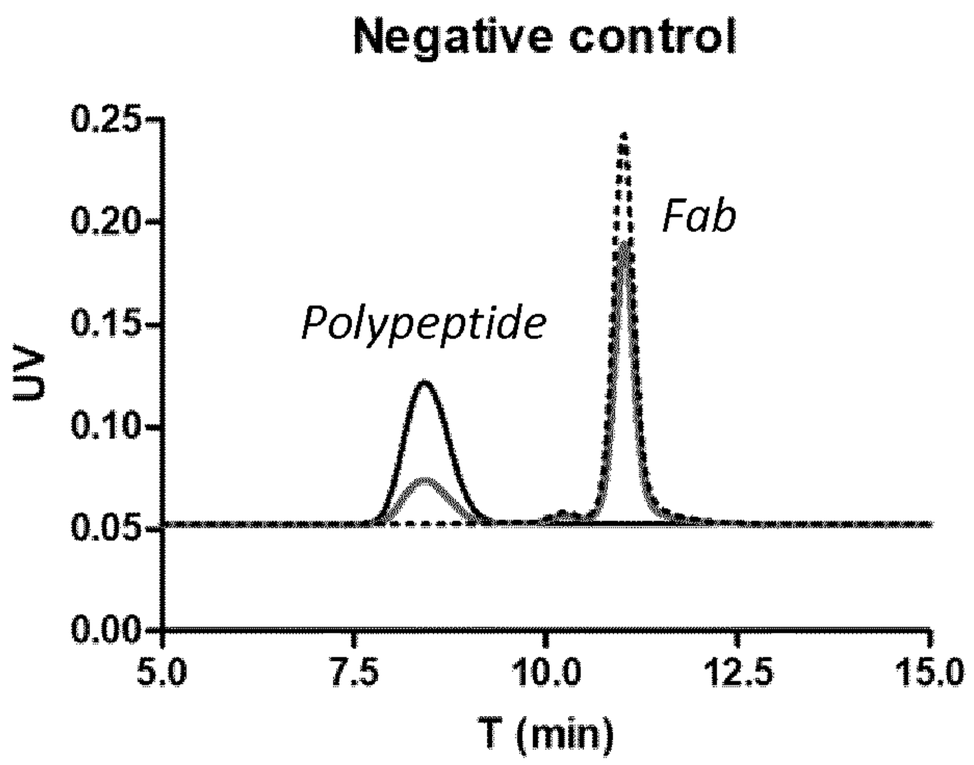
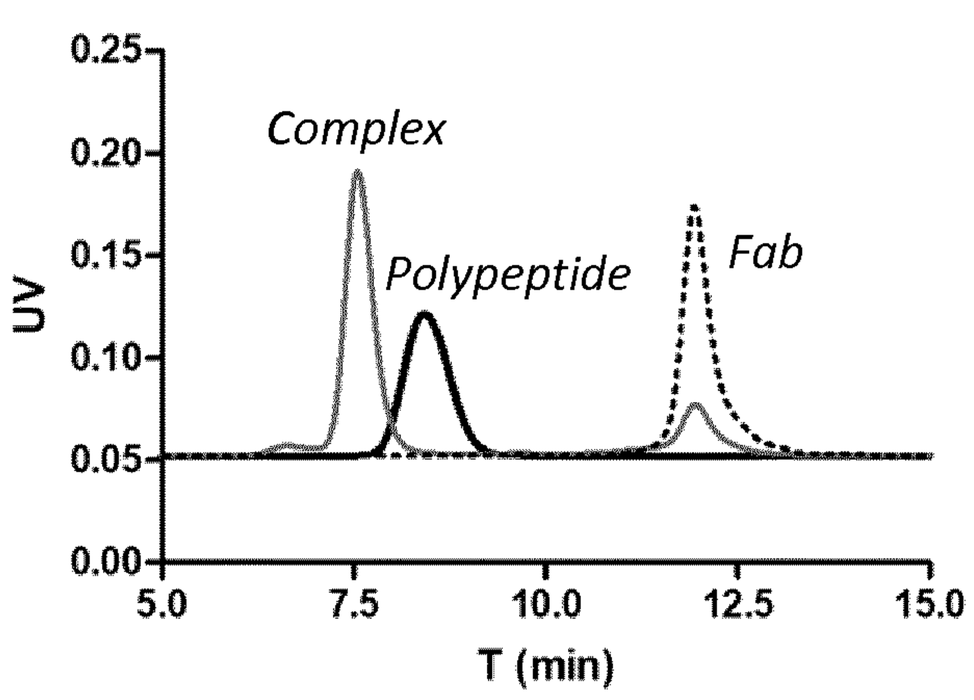

A
UFV5367
300
200
100
0
UV
Aggregates
Trimer
Monomer
0
100
200
300
400
mL
B
UFV160656
500
400
300
200
100
0
UV
Trimer
Aggregates
0
100
200
300
400
mL
C
UFV160656
UV
Refractive index
UV
mL

FIG. 6

A


Negative control
0.25
0.20
0.15
0.10
0.05
0.00
UV
Polypeptide
Fab
5.0
7.5
10.0
12.5
15.0
T (min)

B


Fab6261
0.25
0.20
0.15
0.10
0.05
0.00
UV
Complex
Polypeptide
Fab
5.0
7.5
10.0
12.5
15.0
T (min)

C

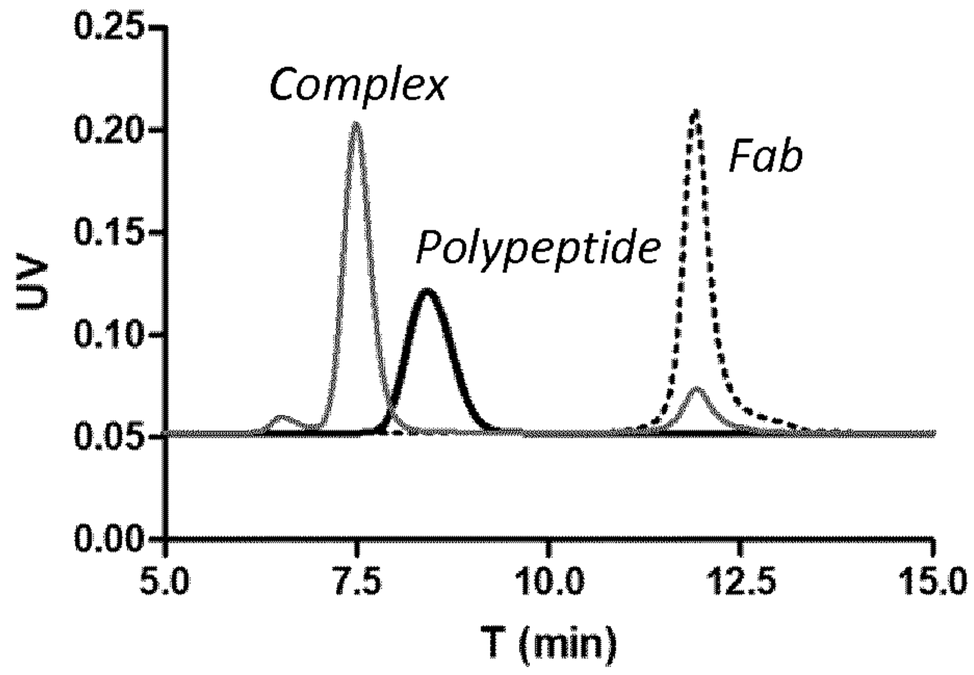

Fab9114
0.25
0.20
0.15
0.10
0.05
0.00
UV
Complex
Polypeptide
Fab
5.0
7.5
10.0
12.5
15.0
T (min)

FIG. 7

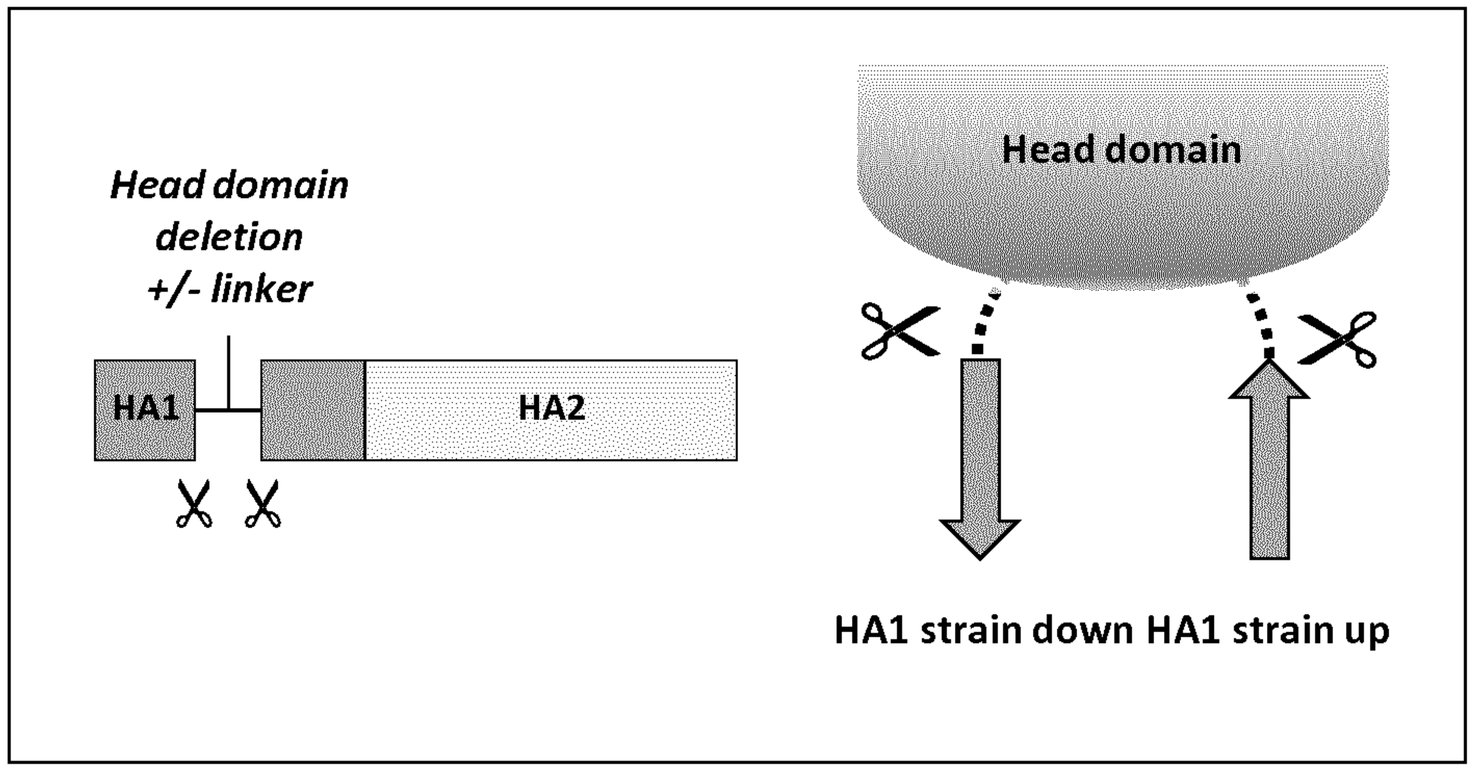

Head domain deletion +/- linker
HA1
HA2
Head domain
HA1 strain down
HA1 strain up

FIG. 8

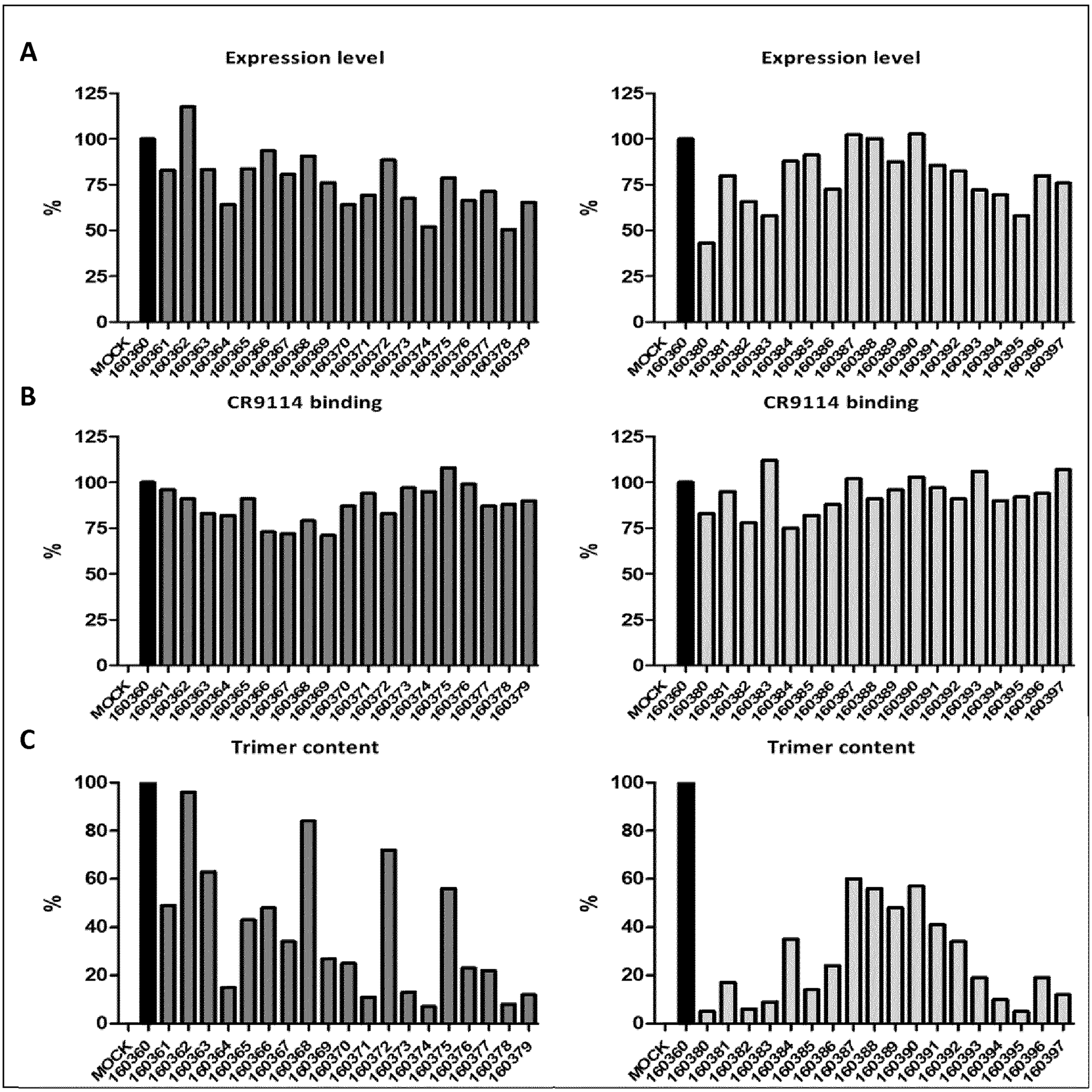

A
Expression level
%
MOCK
160360
160361
160362
160363
160364
160365
160366
160367
160368
160369
160370
160371
160372
160373
160374
160375
160376
160377
160378
160379
160380
160381
160382
160383
160384
160385
160386
160387
160388
160389
160390
160391
160392
160393
160394
160395
160396
160397
CR9114 binding
B
Trimer content
C

FIG. 9

FIG. 10

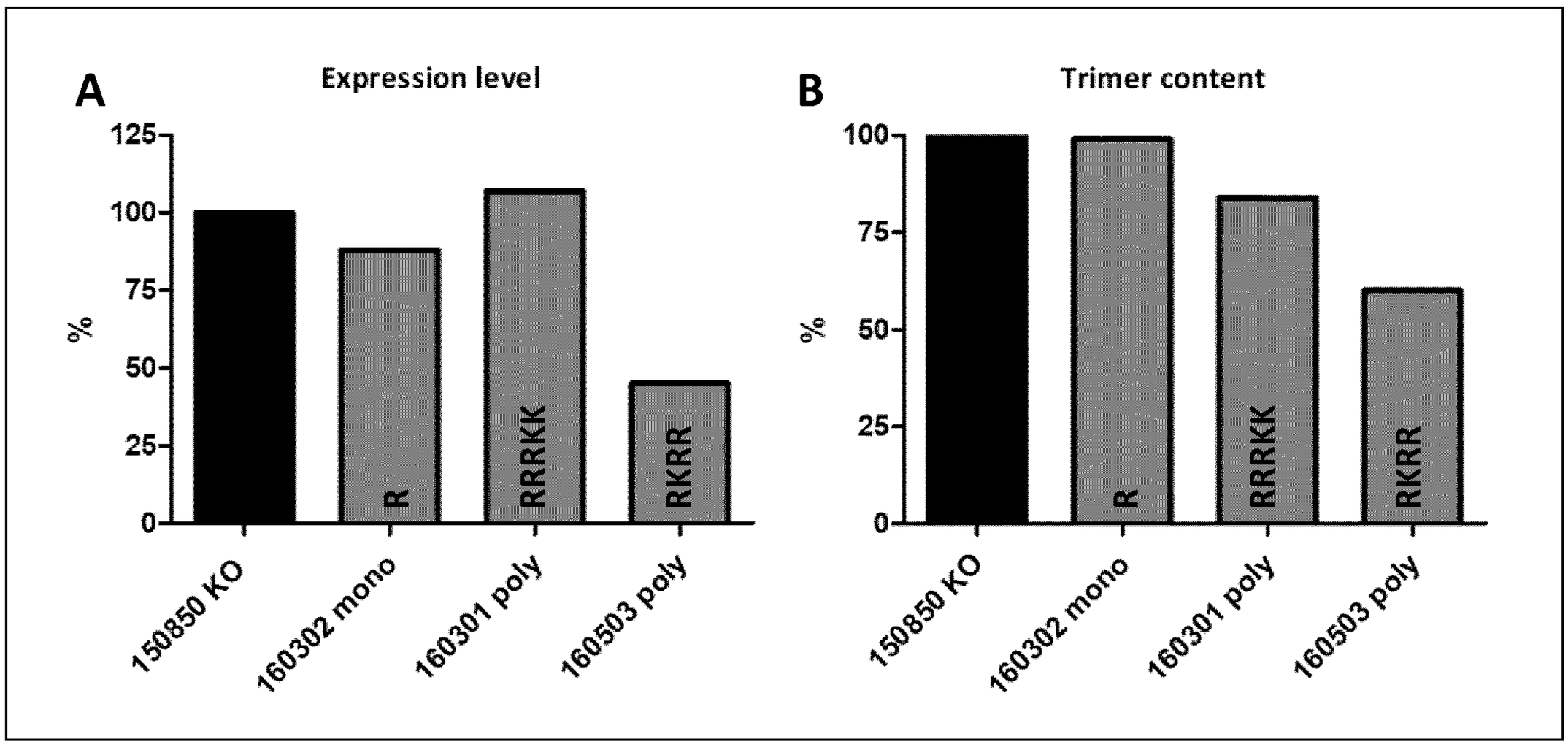
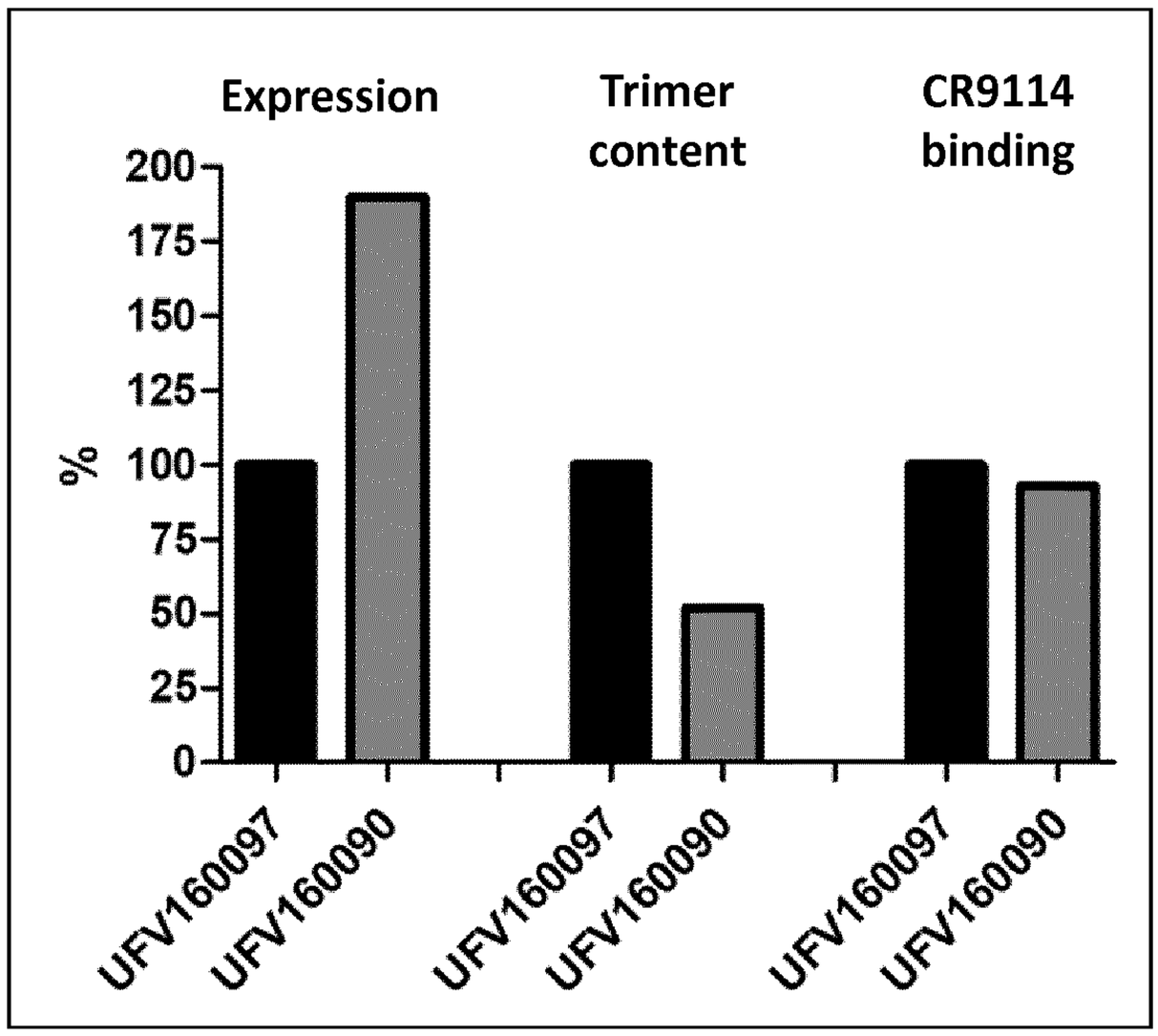

A
Expression level
%
125
100
75
50
25
0
R
RRRKK
RKRR
150850 KO
160302 mono
160301 poly
160503 poly
B
Trimer content
%
100
75
50
25
0
R
RRRKK
RKRR
150850 KO
160302 mono
160301 poly
160503 poly

FIG. 11


Expression
Trimer content
CR9114 binding
%
200
175
150
125
100
75
50
25
0
UFV160097
UFV160090
UFV160097
UFV160090
UFV160097
UFV160090

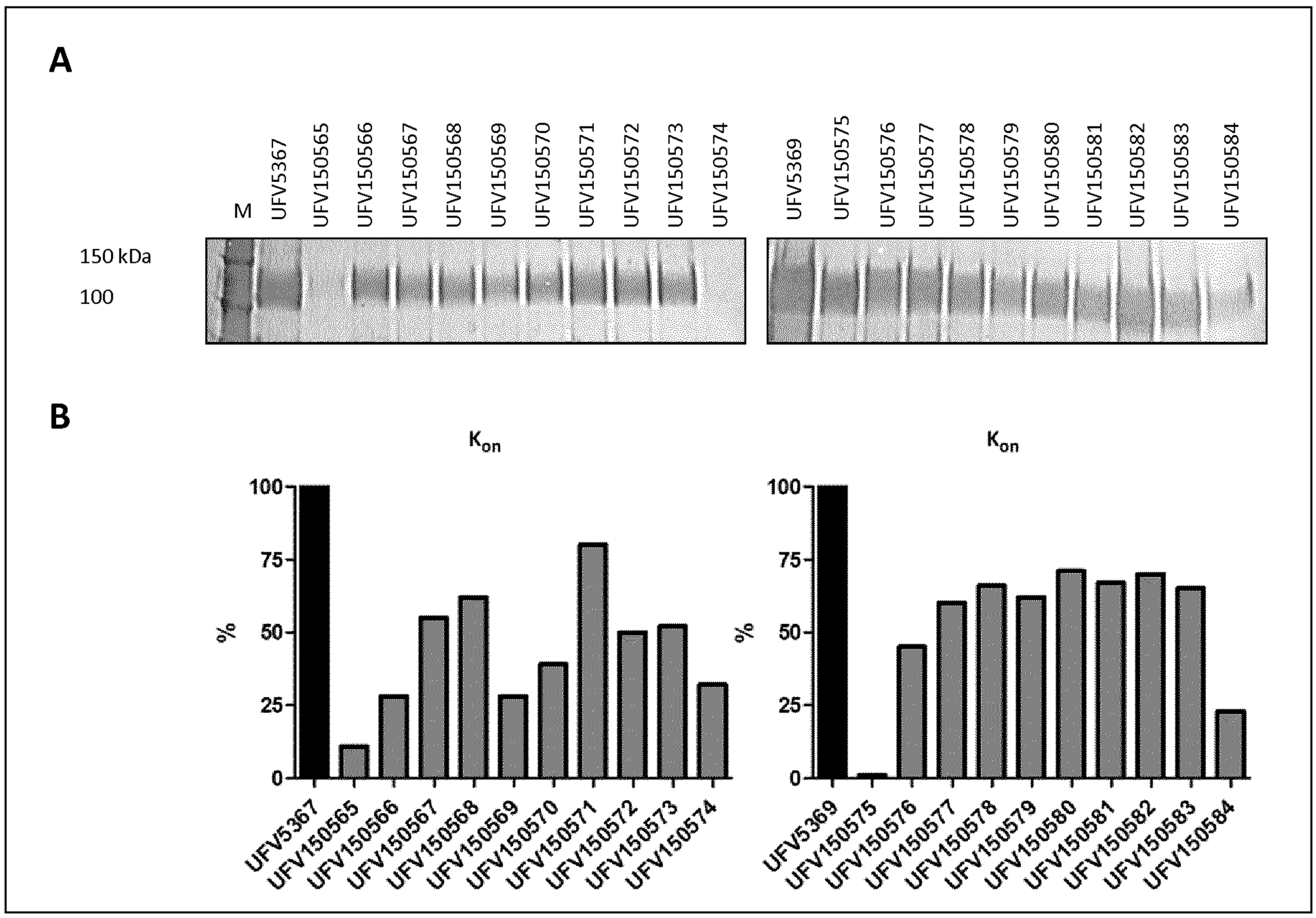

A
M
UFV5367
UFV150565
UFV150566
UFV150567
UFV150568
UFV150569
UFV150570
UFV150571
UFV150572
UFV150573
UFV150574
UFV5369
UFV150575
UFV150576
UFV150577
UFV150578
UFV150579
UFV150580
UFV150581
UFV150582
UFV150583
UFV150584
150 kDa
100
B
K_{on}
K_{on}
100
75
50
25
0
%
UFV5367
UFV150565
UFV150566
UFV150567
UFV150568
UFV150569
UFV150570
UFV150571
UFV150572
UFV150573
UFV150574
UFV5369
UFV150575
UFV150576
UFV150577
UFV150578
UFV150579
UFV150580
UFV150581
UFV150582
UFV150583
UFV150584

FIG. 12

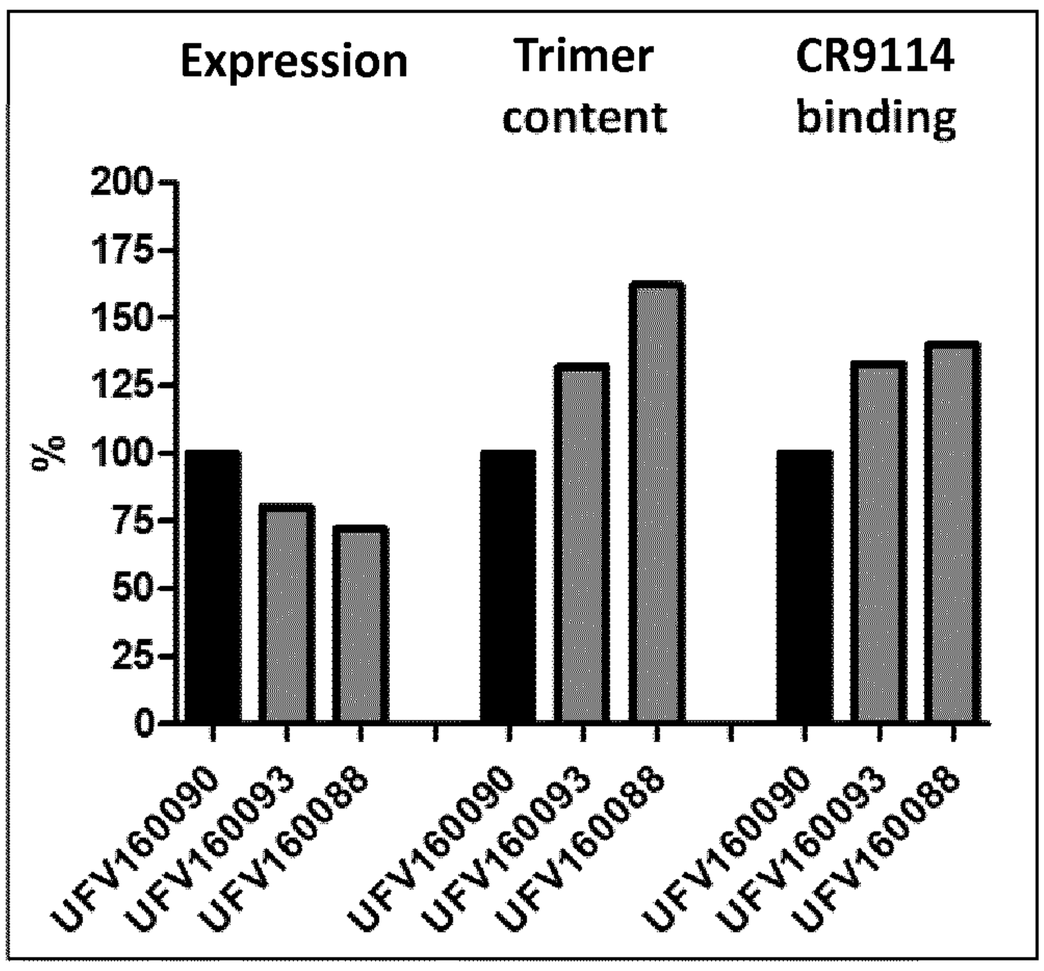

Expression
Trimer content
CR9114 binding
%
200
175
150
125
100
75
50
25
0
UFV160090
UFV160093
UFV160088
UFV160090
UFV160093
UFV160088
UFV160090
UFV160093
UFV160088

FIG. 13

	Signal peptide																	HA1																																																			
																																																					Cutting positions													HA1			
H3 numbering (Winter *et al.*)	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52			303	304	305	306	307	308	309	310
H1 A/California/07/09	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59			317	318	319	320	321	322	323	324
	M	K	A	I	L	V	V	L	L	Y	T	F	A	T	A	N	A	D	T	L	C	I	G	Y	H	A	N	N	S	T	D	T	V	D	T	V	L	E	K	N	V	T	V	T	H	S	V	N	L	L	E	D	K	H	N	G	K	L	C	-	-	G	K	C	P	K	Y	V	K
UFV160664	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	C

																			CS						Fusion peptide																																							A-Helix							
H3 numbering (Winter *et al.*)	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	329a	329b	329c	329d	329e	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374
H1 A/California/07/09	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343						344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388
	S	T	K	L	R	L	A	T	G	L	R	N	I	P	S	I	Q	S	R	-	-	-	-	-	G	L	F	G	A	I	A	G	F	I	E	G	G	W	T	G	M	V	D	G	W	Y	G	Y	H	H	Q	N	E	Q	G	S	G	Y	A	A	D	L	K	S	T	Q	N	A	I
UFV160664	.	.	.	.	.	.	.	.	.	.	.	.	K	.	.	K	.	.	Q	-	-	-	-	-	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	HA2																																																																				
											B-loop																				GCN4															C-Helix															D-helix						E-he		
H3 numbering (Winter *et al.*)	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443
H1 A/California/07/09	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457
	D	E	I	T	N	K	V	N	S	V	I	E	K	M	N	T	Q	F	T	A	V	G	K	E	F	N	H	L	E	K	R	I	E	N	L	N	K	K	V	D	D	G	F	L	D	I	W	T	Y	N	A	E	L	L	V	L	L	E	N	E	R	T	L	D	Y	H	D	S	N
UFV160664	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	I	.	C	.	Y	.	K	S	.	R	C	M	K	Q	I	E	D	.	I	E	E	I	E	S	K	.	.	C	.	.	.	.	.	.	.	.	.	.	.	Q	.	.	.	.	.	.	.	.	.

	lix																															G-helix													C-term helix																								
H3 numbering (Winter *et al.*)	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512
) H1 A/California/07/09	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526
	V	K	N	L	Y	E	K	V	R	S	Q	L	K	N	N	A	K	E	I	G	N	G	C	F	E	F	Y	H	K	C	D	N	T	C	M	E	S	V	K	N	G	T	Y	D	Y	P	K	Y	S	E	E	A	K	L	N	R	E	E	I	D	G	V	K	L	E	S	T	R	I
UFV160664	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

			Transmembrane domain																						Cytoplasmic domain														
H3 numbering (Winter *et al.*)	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	539b	540	541	542	543	544	545	546	547	548	549	550
H1 A/California/07/09	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565
	Y	Q	I	L	A	I	Y	S	T	V	A	S	S	L	V	L	V	V	S	L	G	A	I	S	F	W	M	C	S	N	G	S	L	Q	C	R	I	C	I
UFV160664	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

FIG. 14

FIG. 15

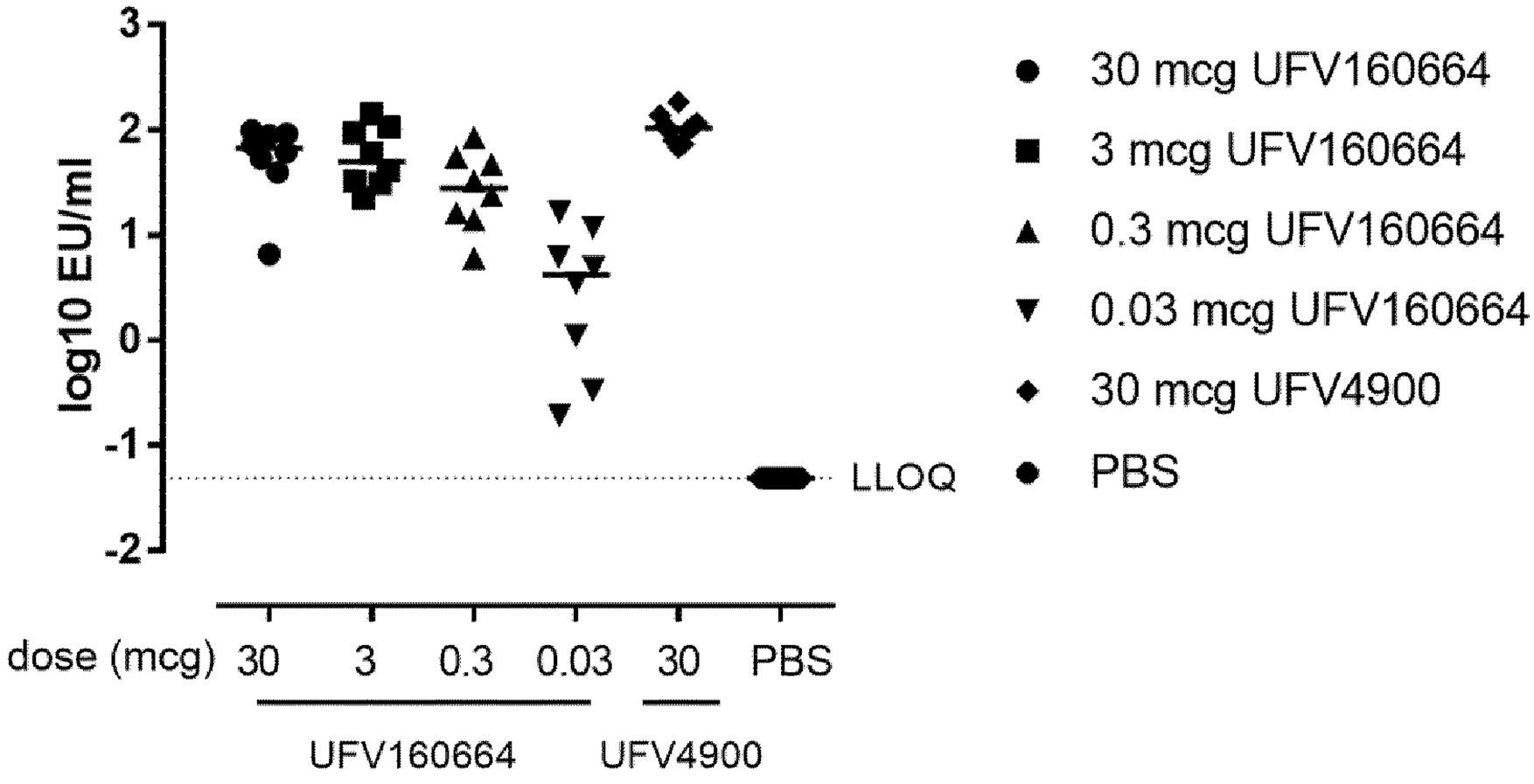

log10 EU/ml
3
2
1
0
-1
-2
LLOQ
dose (mcg)
30
3
0.3
0.03
30
PBS
UFV160664
UFV4900
30 mcg UFV160664
3 mcg UFV160664
0.3 mcg UFV160664
0.03 mcg UFV160664
30 mcg UFV4900
PBS

FIG. 16

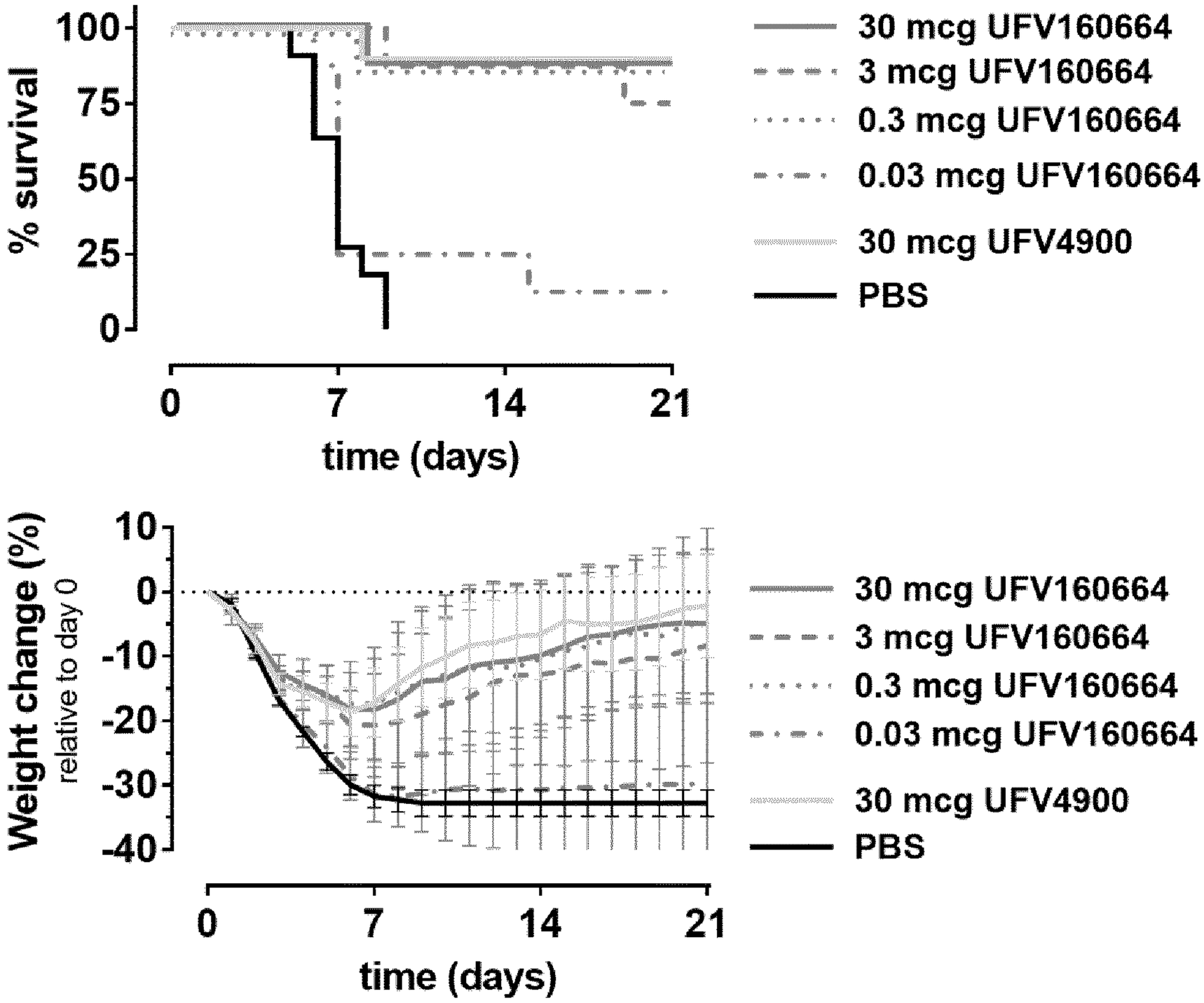

% survival
100
75
50
25
0
0
7
14
21
time (days)
30 mcg UFV160664
3 mcg UFV160664
0.3 mcg UFV160664
0.03 mcg UFV160664
30 mcg UFV4900
PBS
Weight change (%)
relative to day 0
10
0
-10
-20
-30
-40
0
7
14
21
time (days)
30 mcg UFV160664
3 mcg UFV160664
0.3 mcg UFV160664
0.03 mcg UFV160664
30 mcg UFV4900
PBS

FIG. 17

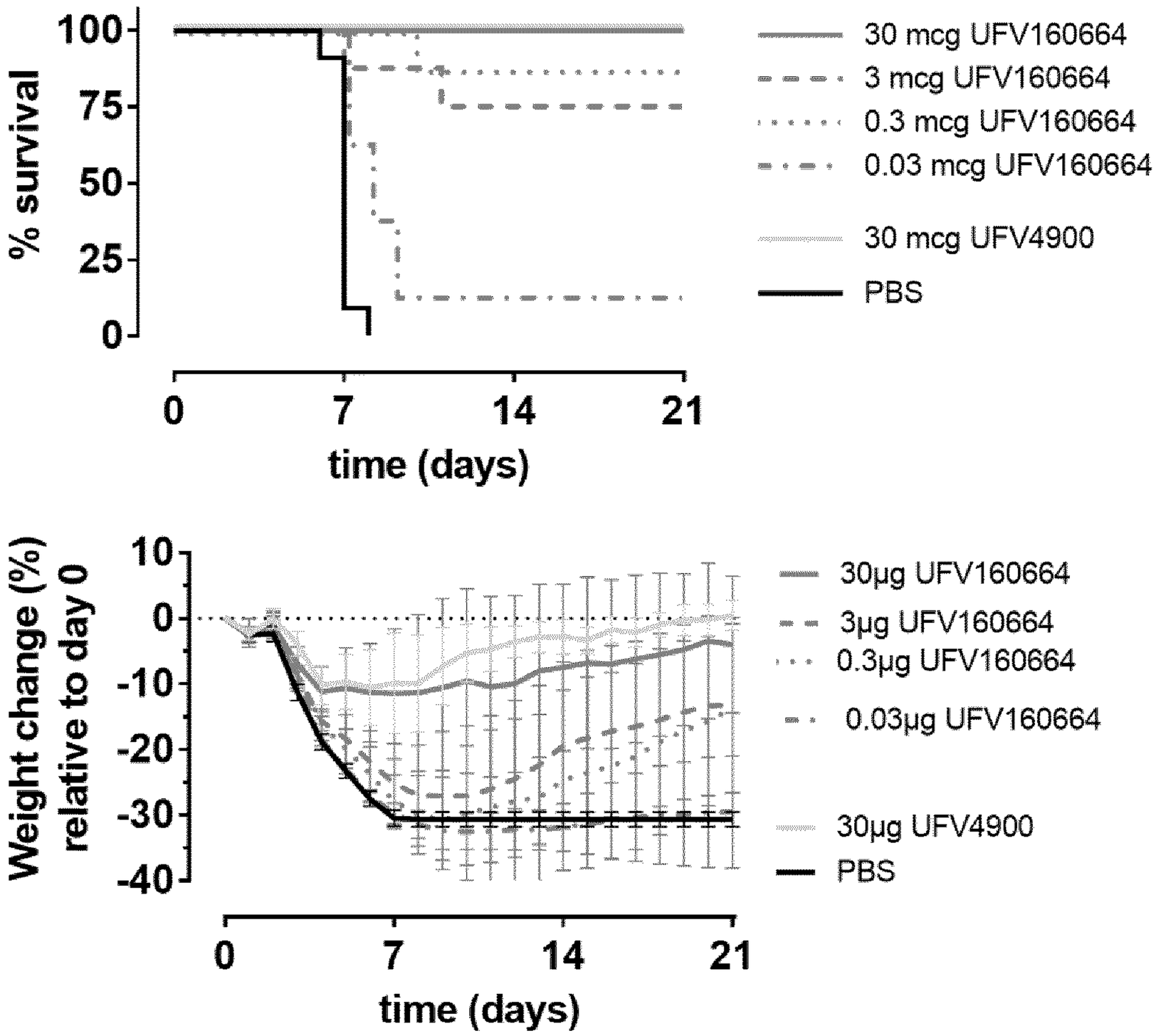

% survival
100
75
50
25
0
0
7
14
21
time (days)
30 mcg UFV160664
3 mcg UFV160664
0.3 mcg UFV160664
0.03 mcg UFV160664
30 mcg UFV4900
PBS
Weight change (%) relative to day 0
10
0
-10
-20
-30
-40
0
7
14
21
time (days)
30µg UFV160664
3µg UFV160664
0.3µg UFV160664
0.03µg UFV160664
30µg UFV4900
PBS

FIG. 18

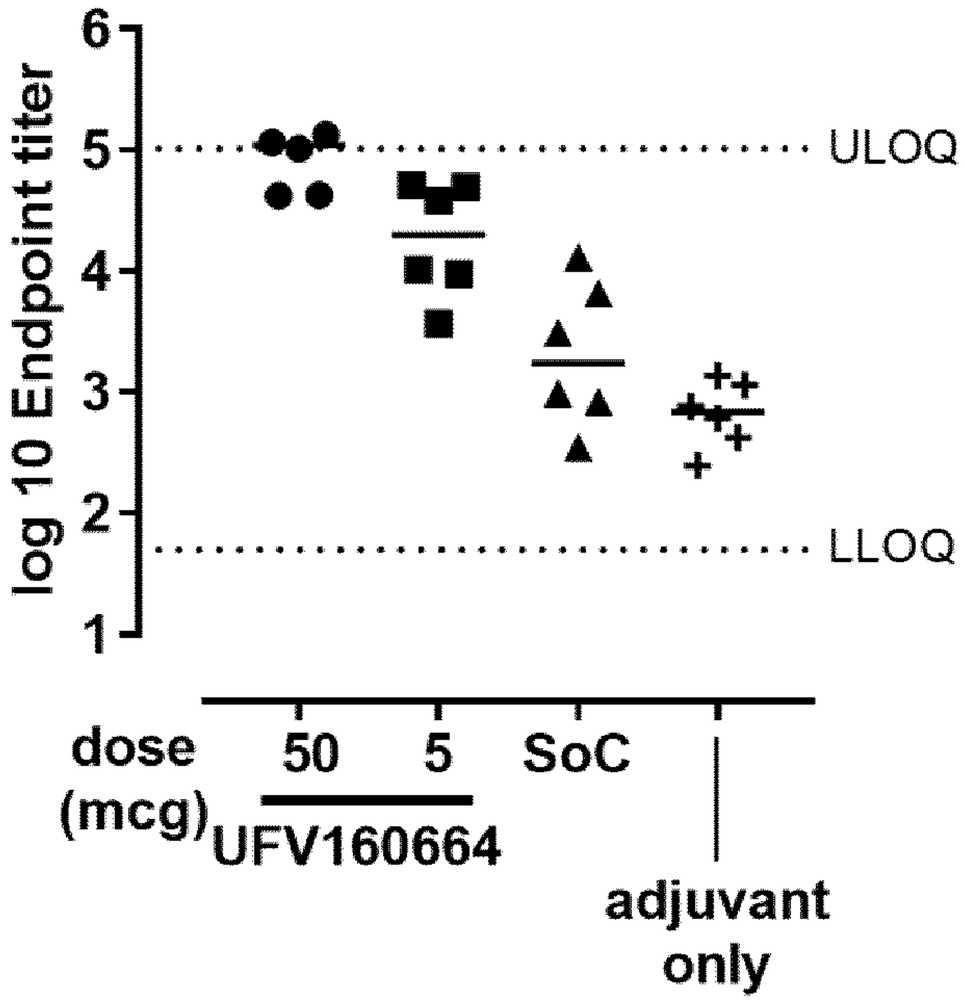

log 10 Endpoint titer
6
5
4
3
2
1
ULOQ
LLOQ
dose (mcg)
50
5
SoC
UFV160664
adjuvant only

FIG. 19

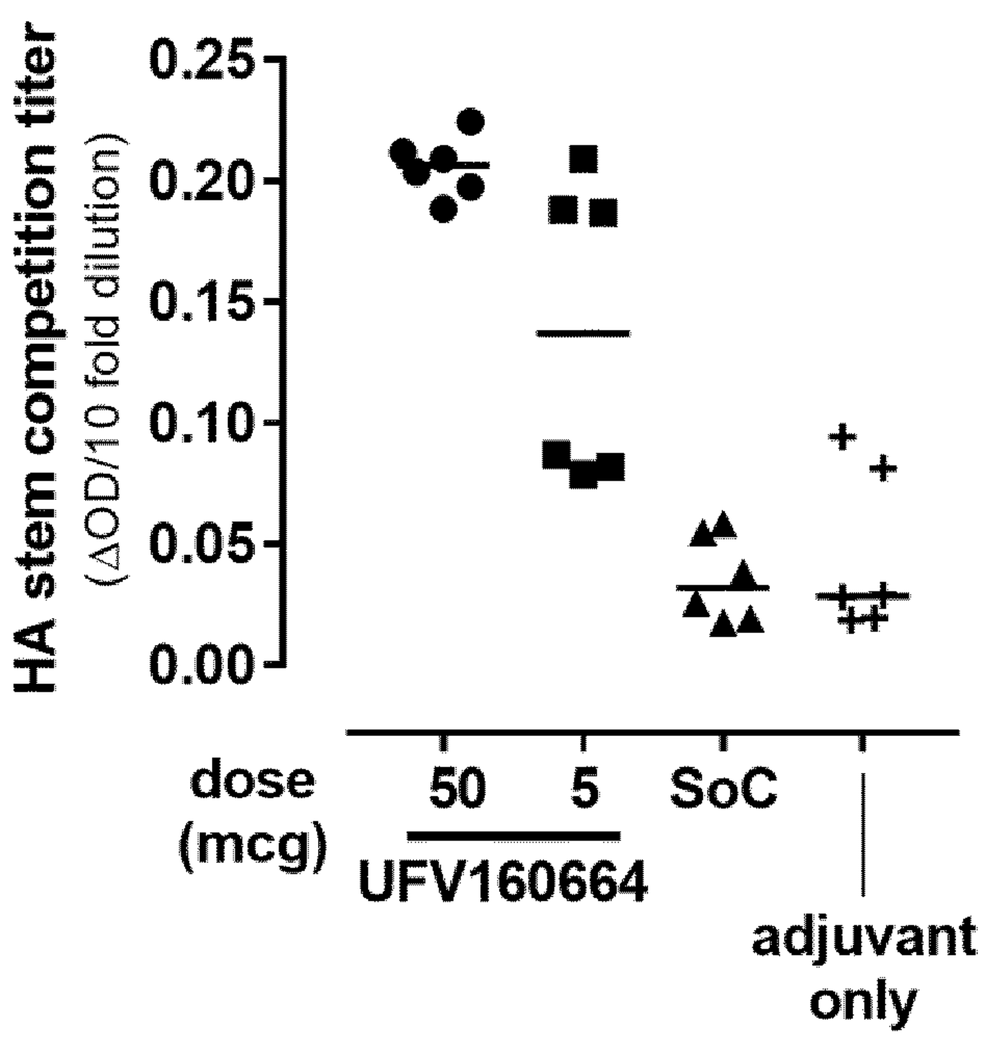
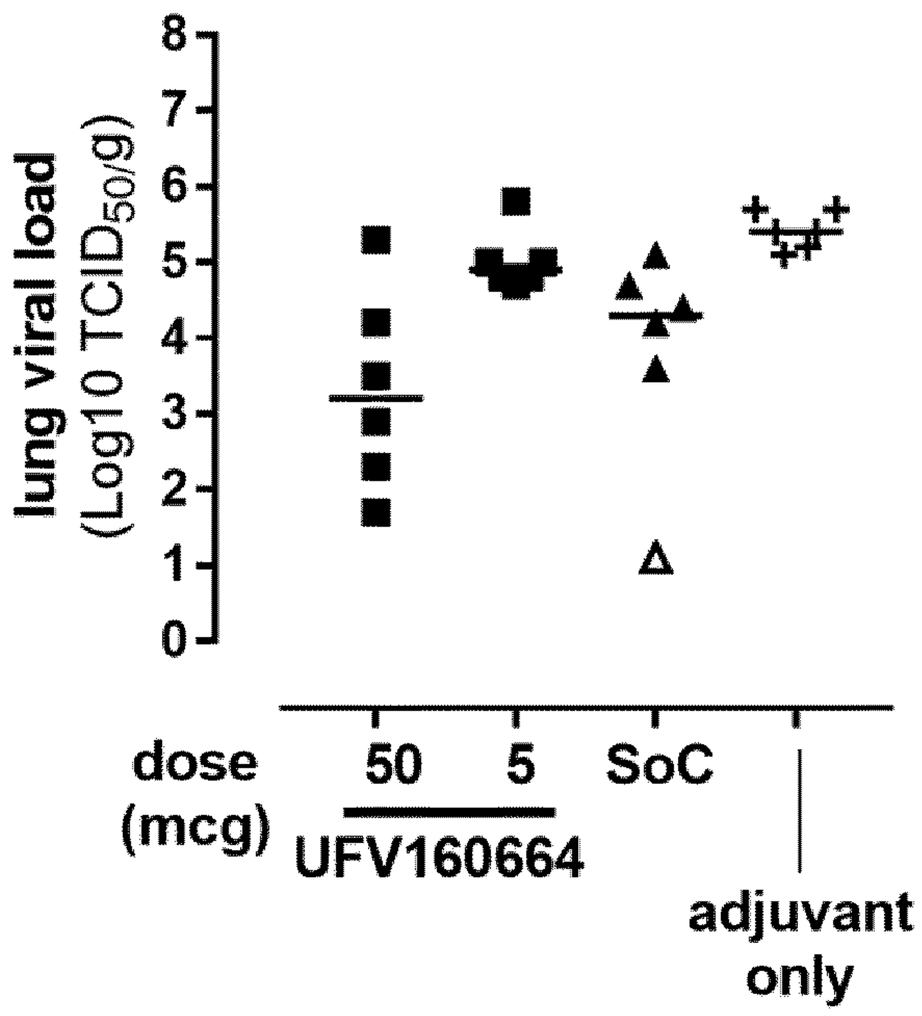

HA stem competition titer
(ΔOD/10 fold dilution)
0.25
0.20
0.15
0.10
0.05
0.00
dose
(mcg)
50
5
SoC
UFV160664
adjuvant
only

FIG. 20


lung viral load
(Log10 TCID50/g)
8
7
6
5
4
3
2
1
0
dose
(mcg)
50
5
SoC
UFV160664
adjuvant
only

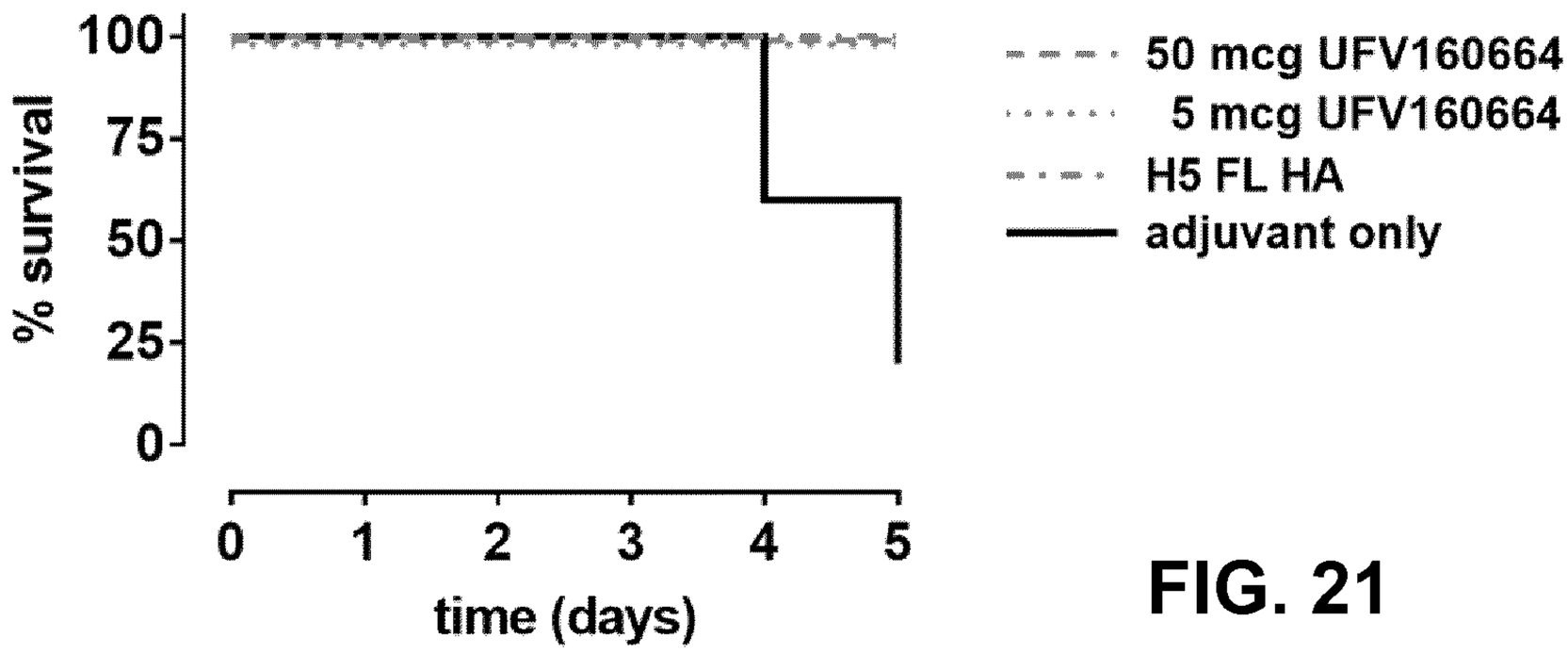


FIG. 21

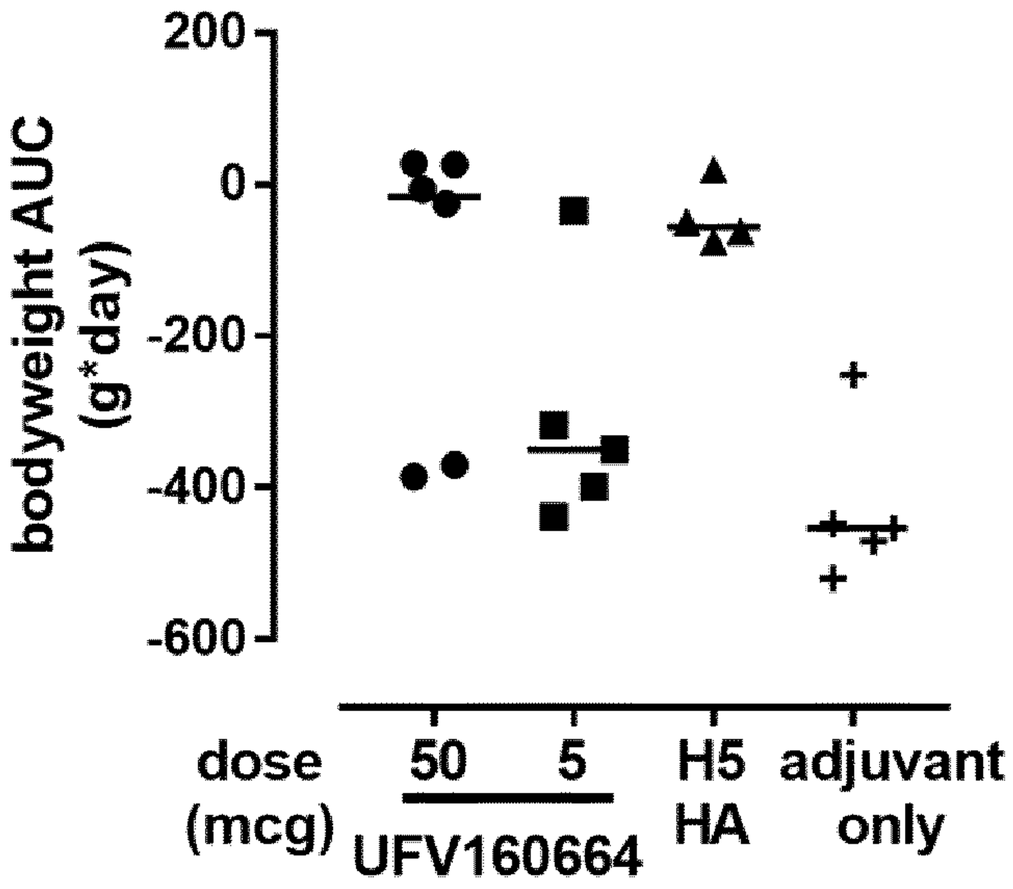


FIG. 22

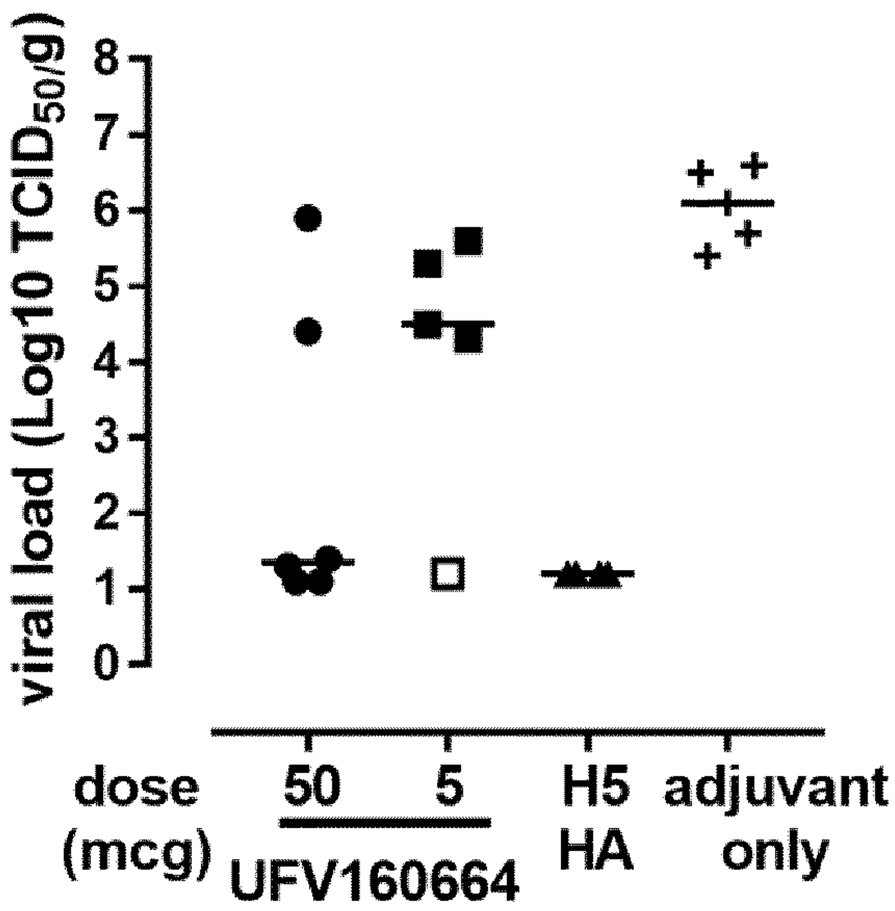


FIG. 23

FIG. 24

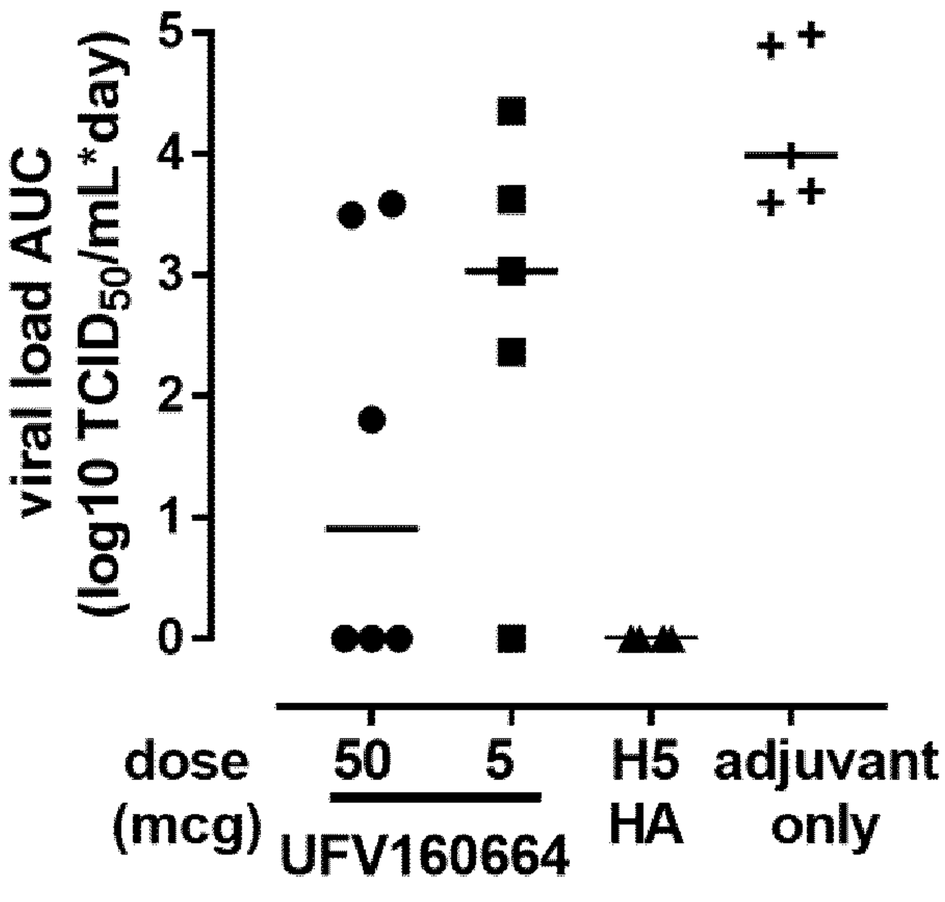

viral load AUC
(log10 TCID50/mL*day)
5
4
3
2
1
0
dose
(mcg)
50
5
UFV160664
H5
HA
adjuvant
only

FIG. 25

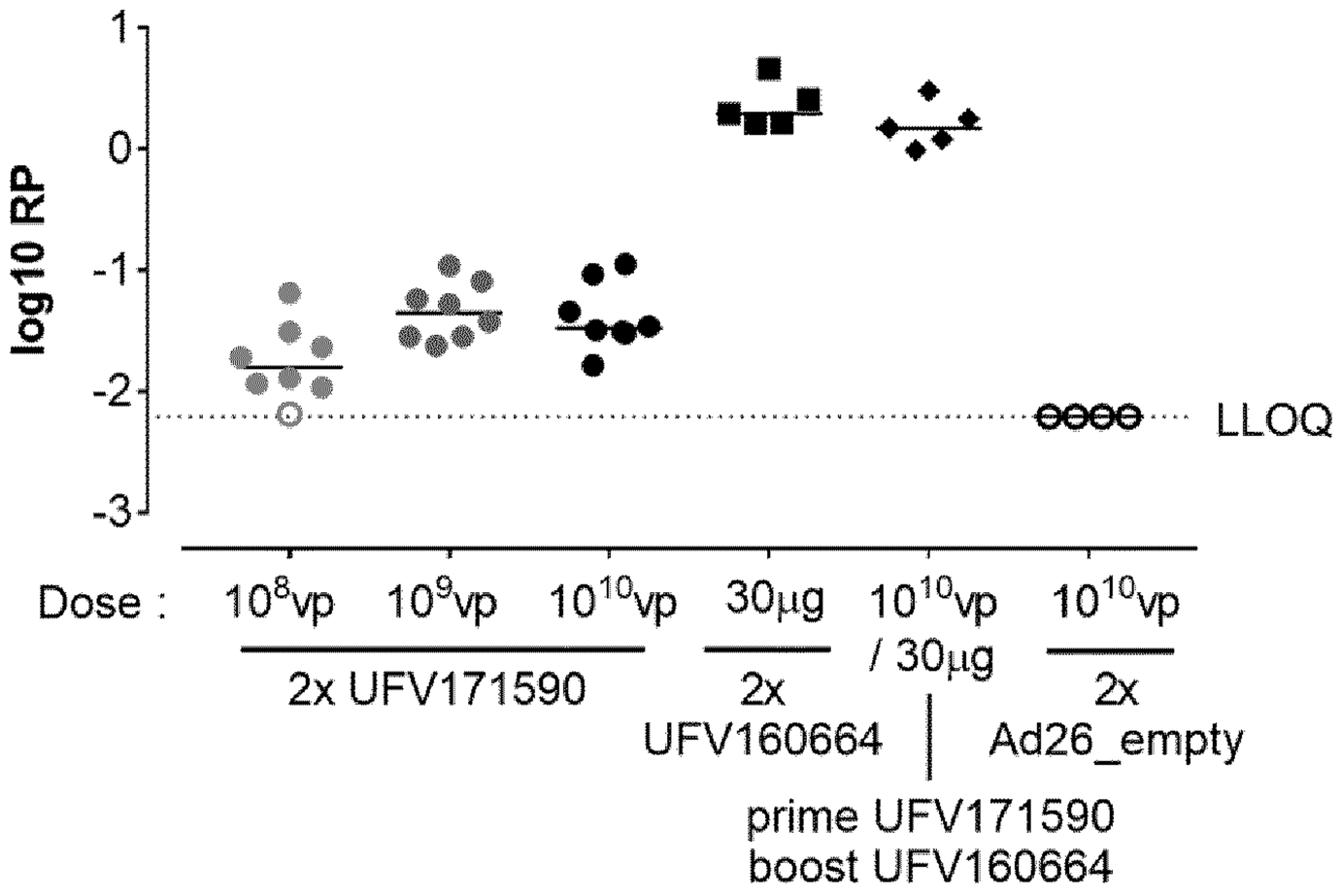

log10 RP
1
0
-1
-2
-3
LLOQ
Dose :
10^8vp
10^9vp
10^10vp
30µg
10^10vp
10^10vp
2x UFV171590
2x
UFV160664
/ 30µg
2x
Ad26_empty
prime UFV171590
boost UFV160664

FIG. 26

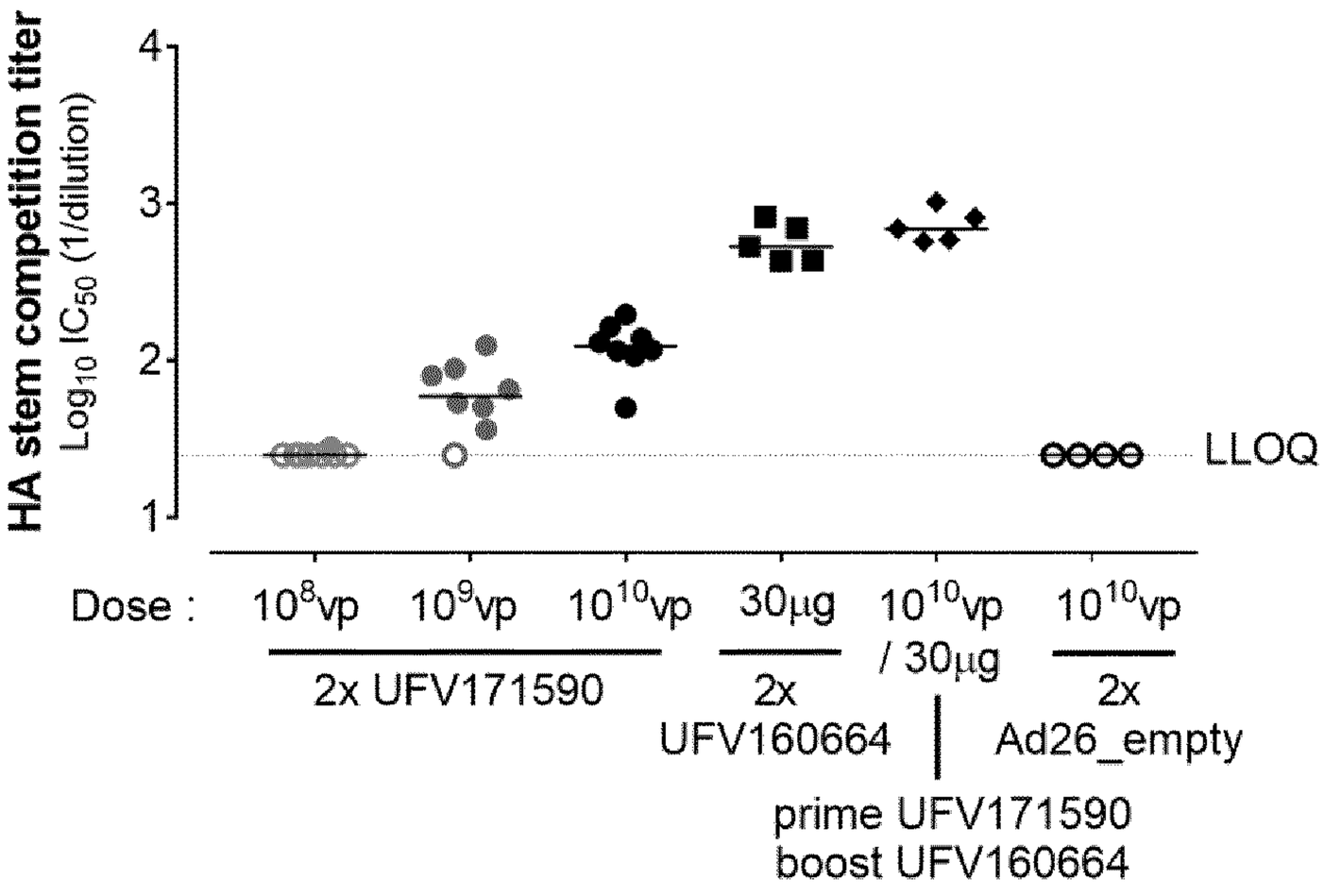

HA stem competition titer
Log10 IC50 (1/dilution)
4
3
2
1
LLOQ
Dose :
10^8vp
10^9vp
10^10vp
30µg
10^10vp / 30µg
10^10vp
2x UFV171590
2x UFV160664
2x Ad26_empty
prime UFV171590
boost UFV160664

FIG. 27

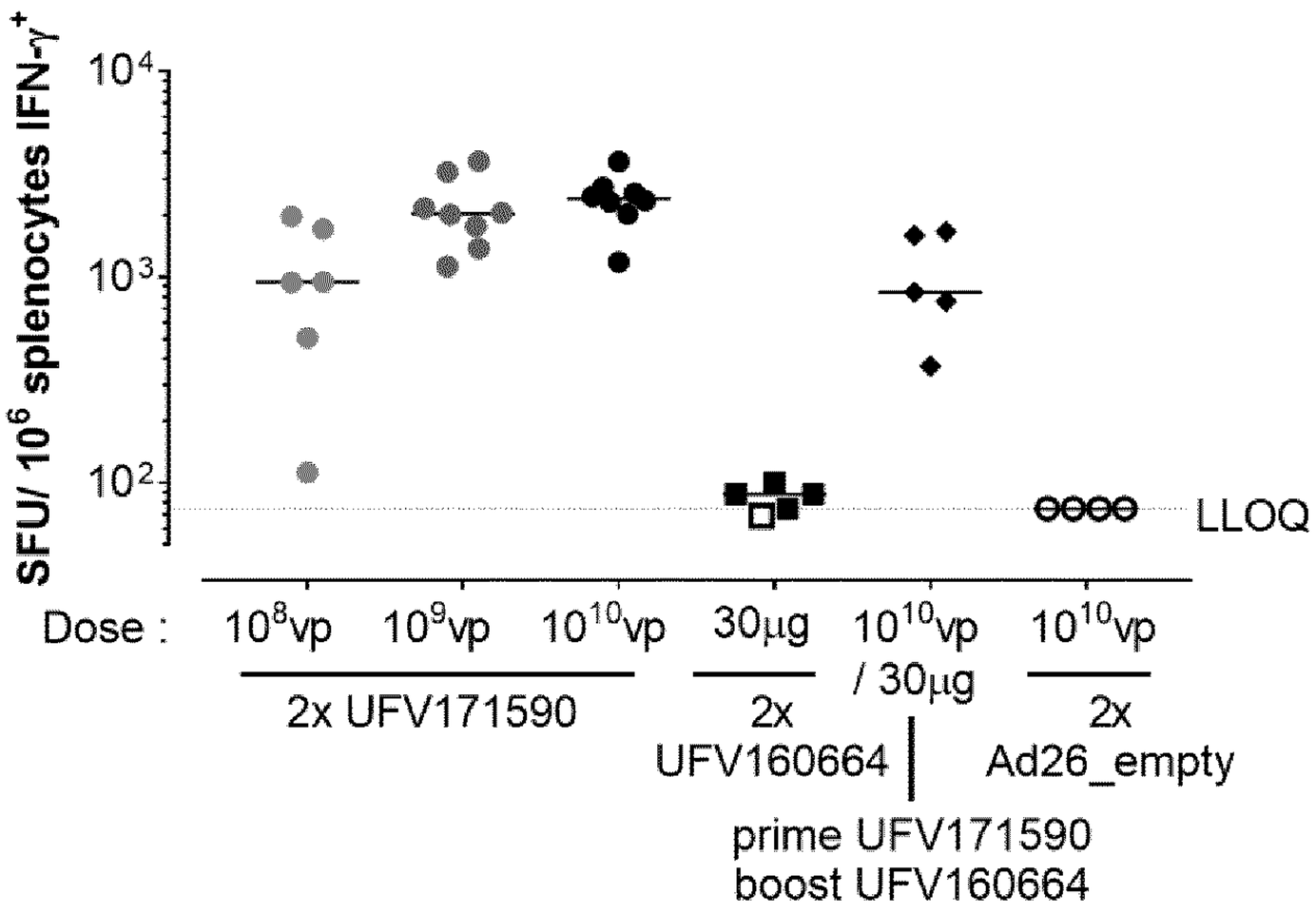
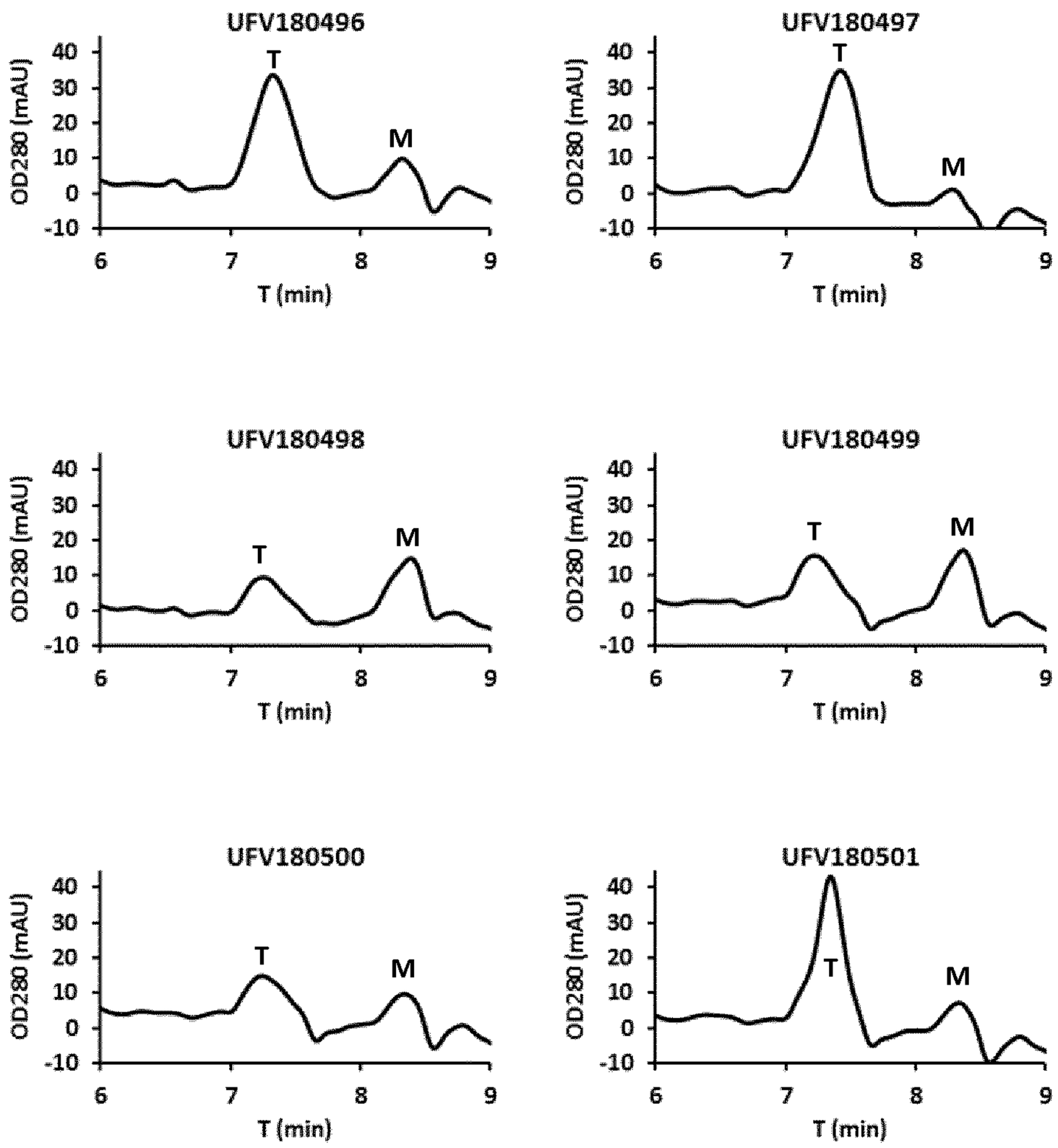

SFU/ 10^6 splenocytes IFN-γ+
10^4
10^3
10^2
LLOQ
Dose :
10^8vp
10^9vp
10^10vp
30µg
10^10vp / 30µg
10^10vp
2x UFV171590
2x UFV160664
2x Ad26_empty
prime UFV171590
boost UFV160664

FIG. 28

A

ID.	Strain	Expression
UFV180496	H1 A/California/07/09	375 mg/L
UFV180497	H1 A/Michigan/45/2015	367 mg/L
UFV180498	H1 A/Puerto Rico/8/1934	244 mg/L
UFV180499	H5 A/Hong Kong/156/97	133 mg/L
UFV180500	H5 A/Vietnam/1203/04	42 mg/L
UFV180501	H2 A/Singapore/1/57	229 mg/L

B



C

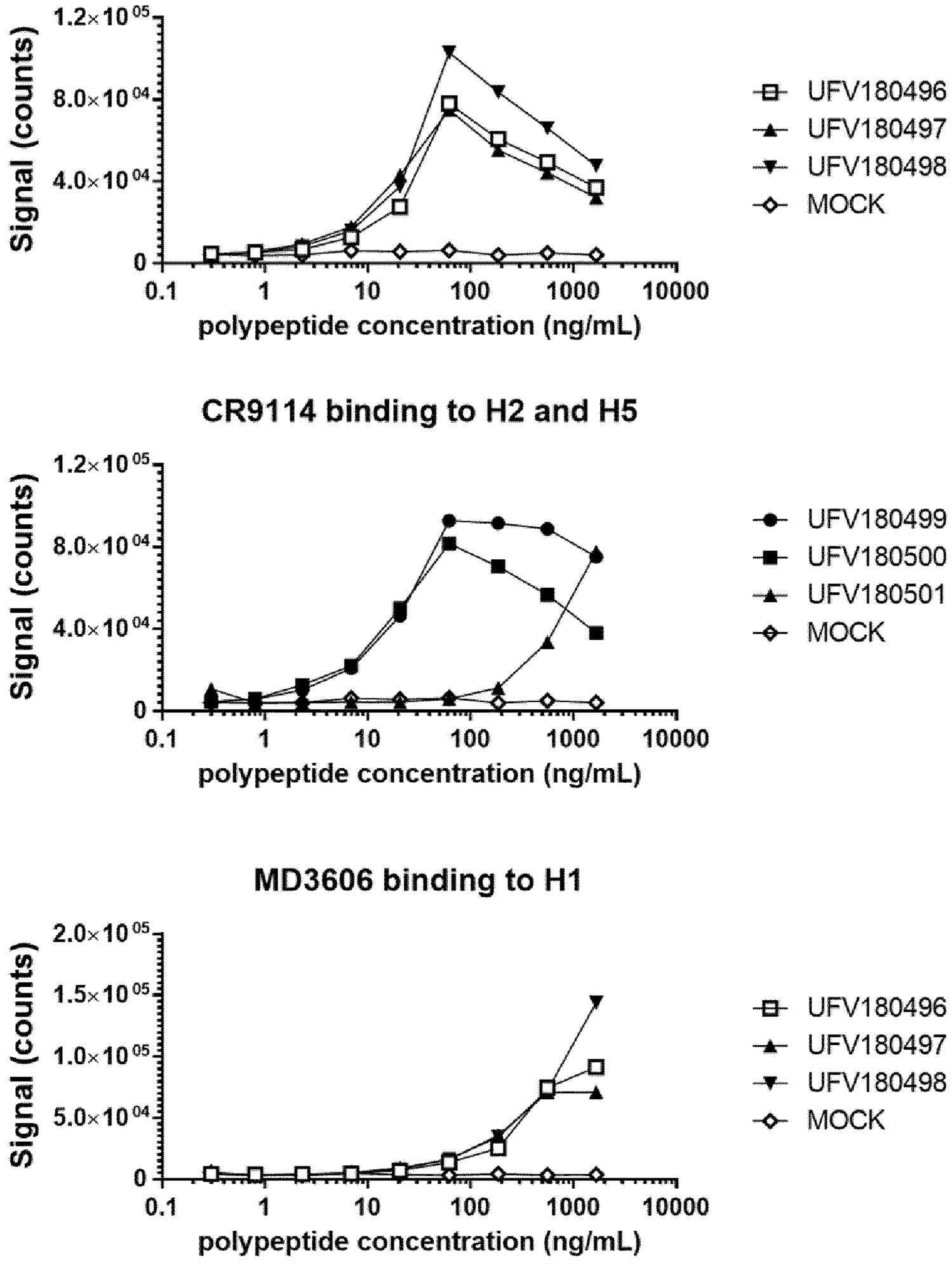


FIG. 28 - continued