Human Alpha-L-iduronidase (huIDUA)

```
→Signal                    -->Alpha-L-iduronidase
        10         20         30         40         50
MRPLRPRAAL LALLASLLAA PPVAPAEAPH LVHVDAARAL WPLRRFWRST
        60         70         80         90        100
GFCPPLPHSQ ADQYVLSWDQ QLNLAYVGAV PHRGIKQVRT HWLLELVTTR
       110        120        130        140        150
GSTGRGLSYN FTHLDGYLDL LRENQLLPGF ELMGSASGHF TDFEDKQQVF
       160        170        180        190        200
EWKDLVSSLA RRYIGRYGLA HVSKWNFETW NEPDHHDFDN VSMTMQGFLN
       210        220        230        240        250
YYDACSEGLR AASPALRLGG PGDSFHTPPR SPLSWGLLRH CHDGTNFFTG
       260        270        280        290        300
EAGVRLDYIS LHRKGARSSI SILEQEKVVA QQIRQLFPKF ADTPIYNDEA
       310        320        330        340        350
DPLVGWSLPQ PWRADVTYAA MVVKVIAQHQ NLLLANTTSA FPYALLSNDN
                          N-372 req'd for binding & activity
       360        370        380        390        400
AFLSYHPHPF AQRTLTARFQ VNNTRPPHVQ LLRKPVLTAM GLLALLDEEQ
       410        420        430        440        450
LWAEVSQAGT VLDSNHTVGV LASAHRPQGP ADAWRAAVLI YASDDTRAHP
       460        470        480        490        500
NRSVAVTLRL RGVPPGPGLV YVTRYLDNGL CSPDGEWRRL GRPVFPTAEQ
                                                Disulfide
       510        520        530        540        550
FRRMRAAEDP VAAAPRPLPA GGRLTLRPAL RLPSLLLVHV CARPEKPPGQ
                              Bond
       560        570        580        590        600
VTRLRALPLT QGQLVLVWSD EHVGSKCLWT YEIQFSQDGK AYTPVSRKPS
       610        620        630        640        650
TFNLFVFSPD TGAVSGSYRV RALDYWARPG PFSDPVPYLE VPVPRGPPSP

GNP
```

N = N-linked glycosylation site (GlcNac...)
Y = Y-sulfation site
C = Disulfide Bond

FIG. 1

Presequence
human
dog
cow
mouse
rat
platypus
chicken
xenopus
zebrafish
sea_urchin
ciona
fruitfly
human res#

FIG. 2

FIG. 2 continued

```
                 :::***  :::::: *.
human            VQLLRKPVLTAWGLLALLDEEQLWAEVSQAGTVLDSHT--VGVLASAHRPQ--GPADAWRAAVLIYASDDTRAHPTRSVAVTLRLRGVPPGPG------  468
dog              VQLLRKPVLTAWALLALLDGRQLWAEVSRGGTVLDSHT--VGVLASAHLPA--GPRDAWRATVLLYASDDTRAHAARAVPVTLRLLGVPRGPG------  467
cow              VQLLRKPVLTAWALLALLDGEQLWAEVSRGGTVLDSHT--VGVLASVHRPT--GPADAWRATVLVYGSDDTRAHARTVPLQLSLQGVPPGPE------  467
mouse            VQLLRKPVLTVWGLWALLDGEQLWAEVSKAGAVLDSHT--VGVLASTHHPE--GSAAAWSTTVLIYTSDDTHAHPTHSIPVTLRLRGVPPGLD------  467
rat              VQLLRKPVLTVWGLWALLDGEQLWAEVLKAGAVLDSHT--VGVLASTHHPE--GPSEAWRTTVLIYTSDDTHIHPTDSIPVILHLRGVPPGLE------  467
platypus         VQIVRKPVLTVWGLLALLGEKQISANVYIDQEEMDSHT--IGVLASSHEPTELNPSDSWQATVLVYASDDNRTSSY-SSFVTIRLANFPKFYE------  472
chicken          VQWVRKPVLTVWGLLALLGEKQIFAEVNSSEGESTQNT--FGVLASVHTPSELQPSDSWQATLLWYSSEDNRTSSY-ISTITVNATHFPKLRE------  456
xenopus          VQWVRKPVLTVWGFLALLGEVQWFTEVLWTDANARNTNI--IGAIGSTHEPEKGEKSDSWQSTILCYASDDNKTSSV-INSITVNWTNFPKSKD------  455
zebrafish        VQILRKPVLTVWGLLALLGETQVQAEVS------DWAS--VGLLASVHVPQVPFSADSWQSSVLIYNSRDNQTSTS-DDVISLRITGLPQRQG------  463
sea_urchin       VQTIKKPVLSIWGLLSLLGEQQVEVDDLSYRVKGGAVSAGDVDVLATIIHTPTQSRGSDSWQASILIHNSADTDNVTGTDSIQLEVGGVNPALTTNVADLA  475
ciona            VFLVKKPVLIVWGLLSKLGNVQLKQVIKYNQQQSRIGGISSVCMDCRTTEISTVIYNSENVEVTEKITKVNIEYSTAENTPLKPFKTYGNDTIR------  473
fruitfly         SQLVQKPVYAALGWLAKLGTRAADVEWNVWDTKHSVQVLR----------TVSGGLGGPGQYWATIFLSPEEAGPKWTAFHHKYTLNWSIAN------  453
                 380.....390.....400.....410.....420.....430.....440.....450.....460.....

                                  :  :  .*  *.  . * :*.          :  :  .  * .:* :*
human            --LVYVTRYLDNGLCSPDGEWRRLGRPVFPTAEQFRRWRAAEDP--VAAAPRPLPAGGRLTLRPALRLPSLLLVHVCARPEKPPGQVTRLRALPLTQGQL  564
dog              --LVYVTLALDNPRCSPHGEWQRLGRPVFPTAEEFRRWRAAEDP--VAEAPRPFPASGRLTLSVELRLPSLLLLHVCARPEKPPGPVTRLRALPLTRGQV  563
cow              --VVFVELYVDNWLCNPYGEWRRLGWPVFPSAAQFRRWRAAEDP--AAVKPRSFPSSGRLTLRPPLALPSLLLVHVCARPEEPPGQVTRLRALPLTRGQL  563
mouse            --LVYIVLYLDNQLSSPYSAWQHWGQPVFPSAEQFRRWRWVEDP--VAEAPRPFPARGRLTLHRKLPVPSLLLVHVCTRPLKPPGQVSRLRALPLTHGQL  563
rat              --LVYVVLYLDNQLSSPYSAWQHWGQPVFPSAEQFRQWRWVEDP--VAEAPRPFPARGRLTLYRKLPVPSLWLVHLCARPLEPPGQVSRLRALPLTRGQL  563
platypus         --LVYVAYYLDNHLTSPYLQWKNLGRPDFPSVKQFQRWRAAEDP--VVWGPLPFPKDGRLIFKRELPLPSVFLIHVCARPHLPPNQVNEVRVIPLTRGQI  568
chicken          --PVYVTYYLDNNQTNPYLQWKKLGSPDFPSAKQFQQIRDAEDP--VVTGPLPFPESGTLTLKQDFPIPSVFLIHICSRPSSVPDQVTGVHFIPLTKGQV  552
xenopus          --LVYVTYYLDNNLTNPFMEWQRVGSPDFPTIKQFQQIRQWEDP--IRKGPIAFPKDGNWFLKVQLPIPSVFLIHICEKPHSPPNKVTGLHFLPLTKGQV  551
zebrafish        --LWYVTHYWDNNVTNPFKLWESWSRPDYPTAQQFTQWRNQEDP--QTDGPLPVPSDGSLWLKKSLPIPSVLLVHICAQSEEWPNQVNGLRFISITKGQV  559
sea_urchin       GDLVYVEYLLDNAHGNPSAFWRKYDSPVYPTRQQFKEWRQNQEP--VRIVGPKTFTPPSWTFSISLNKPGVILVHICAKSASAPEKVTGLRLHQYTASDV  573
ciona            ------HWFCKIDNVLTNPYKVWQAVGSPSFPTPALFTKLQIASES--HCTEPEPFTTTR----FNLDLRLPSVVLHQICWKPTQQLIWPSELHLHKVSNNIV  564
fruitfly         -ESAFVTELLVPKETDPYYIWQQAGSPAYPNATLREAWRRAQAPRLYKTGPIWQYNSELVINSASIPLPWAWLLRVCSASWPKLRRPQQLSIAEVTQREV  552
                  470.....480.....490.....500.....510 .....520.....530.....540.....550.....560.....
```

FIG. 2 continued

```
             : * :     ::*: :::  :                  .     :    . .                * *:.  :: :    .  *
human        VLVWSDEHVGSKCLWTYEIQFSQDGK-------AYTPVSRKPSTFNLFVFSPD-------TGAVSGSYRVRALDYWARPGPFSDPVPYLEVPVPRGPPSPGN 652
dog          LLVWSDERVGSKCLWTYEIQFSADGE-------VYTPISRKPSTFNLFVFSPE-------SAVTSGSYRVRAVDYWARPGPFSTRVHYVEVPAPSGPPRPSD 651
cow          LLVWSDERVGSKCLWTYEIQFCPEGG-------VFAPISRKPSTFNLFVFSPD-------TAVVSGSYRVCAVDYWARPGPFSSPVRYLEAPAS---------- 643
mouse        ILVWSDERVGSKCLWTYEIQFSQKGE-------EYAPINRRPSTFNLFVFSPD-------TAVVSGSYRVRALDYWARPGPFSDPVTYLDVPAS---------- 643
rat          VLVWSDEHVGSKCLWTYEIQFSQKGE-------VYAPINRRPSTFNLFVFSPD-------TAVVSGFYRVRAVDYWARPGPFSDPVTYLDTPAS---------- 643
platypus     AIAWSDSCVNSKCLKTYEVEFSPNGK-------SYKRINVKDSTFNLCVYSPD-------NTSVSGFYKVRAIDYWGRPGLFSIPVRYTEATE----------- 647
chicken      VVLWDDGCVNSKCIKTFEVEFSPNGK-------VYKRINAKDTIFTLYVYSP--------GSSVSGFYRVRAIDYWGKAGLSSVPVEYVEALK----------- 630
xenopus      AVIWSDKHIRSRCLKTFEVEESENGS-------TYSRINKKDTIFTLFIYSPE-------TAIVSGSYRVRTINYWDIFGPYSLPQQYTEKMD----------- 630
zebrafish    LIIWKDHCVGTKCIKTYEVEFSRDES-------TFLRINHRDTIFTYFTYSPE-------SLEVSGFYRARAVDYWGRNGEYSVTEEYSENR------------ 637
sea_urchin   LVIWDDSNITTRCILTYEAEYSSTGSE------GTYKRINTVDLIFTAFVYSPDQEKKGASSLISGWYRVRAVDYWNRAGLYSNPIQLQTQTPFT---------- 662
ciona        LLKWSDSKITTKCIMTYNVEFARDGR-------NFQTVNTFKVLFNSFAHIACSDG-CVSNTVYGSTRVQALDFWGRPGQYSKVMKHSSK------------- 646
fruitfly     FISWMEHPKSTQCLLSYEVWFKERDNLGRSADWMLISQGWHLPYPSFQYAPGD-------KGSVNGFYKVRGVDVFNETSPYSQIVEYLEL------------- 636
             ...570.......580.......590       .......600.......610      .......620.......630.......640.......650..

human        P---  653
dog          CERC  655
cow          ----  643
mouse        ----  643
rat          ----  643
platypus     ----  647
chicken      ----  630
xenopus      ----  603
```

FIG. 2 continued

Mutation	RMSD (Å)	Number of affected atoms		ASA ($Å^2$)	Phenotype	Reference	Clinical form	Mut
		Main chain	Side chain					
G51D	0.114	256	293	0	Severe	[22]	Hurler	W402X
A75T	0.037	35	36	1.9	Severe	[23]	Hurler	Not Available
T103P	0.017	6	8	57	Severe	[24]	Hurler	Not Available
A160D	0.069	65	79	1.6	Severe	[25]	Severe	A160D
F177S	0.017	4	8	4.8	Severe	[26]	Hurler	F177S
E178K	0.124	244	338	0.3	Severe	[25]	Severe	c.134-145del12
T179R	0.137	316	356	0.4	Severe	[27]	Hurler	T179R
E182K	0.261	809	991	27.4	Severe	[28]	Severe	W402X
E182D	0.017	0	4	27.4	Severe	[27]	Hurler	C53X
P183R	0.184	380	410	0.1	Severe	[25]	Severe	A327P
G208V	0.094	129	143	0	Severe	[29]	Hurler	G208V
G208D	0.068	66	89	0	Severe	[30]	Hurler	Q70X
L218P	0.040	28	38	0.5	Severe	[22]	Hurler	W402X
L237R	0.096	148	180	0.1	Severe	[27]	Hurler	c.973-4GNA
L238R	0.068	109	105	0.5	Severe	[27]	Hurler	c.883dupC
I270S	0.009	0	5	44.7	Severe	[31]	Hurler	R628X
D315Y	0.075	120	147	8.6	Severe	[30]	Hurler	Q70X
A327P	0.031	15	12	0	Severe	[22]	Hurler	W402X
D349N	0.038	32	57	7.4	Severe	[32]	Severe	Q70X
R363C	0.074	118	144	14.5	Severe	[33]	Hurler/Scheie[b]	F602I
T366P	0.034	22	29	0.3	Severe	[34]	Hurler	T366P
P385R	0.125	246	239	1	Severe	[24]	Hurler	A327P
T388R	0.155	343	406	2.9	Severe	[35]	Hurler	W402X
R489P	0.047	28	37	164.5	Severe	[22]	Hurler	W402X
P533L	0.177	341	341	1.3	Severe	[36]	Severe	Q70X
V620F	0.078	150	153	0.7	Severe	[37]	Hurler	V620F

FIG. 3

R628P	0.107	119	134	58	Severe	[38]	Severe	A327P
P533R	0.140	300	279	1.3	Severe-intermediate	[39, 24]	Hurler, Hurler/Scheie	P533R
H33P	0.059	83	96	73.9	Intermediate	[27]	Hurler/Scheie	c.1402+2TNG
A75P	0.039	32	28	1.9	Intermediate	[36]	Intermediate	Q70X
H82P	0.051	50	70	70.7	Intermediate	[40]	Intermediate	**W402X**
L238Q	0.015	0	10	0.5	Intermediate	[33]	Hurler/Scheie	**W402X**
G265R	0.144	238	271	1.7	Intermediate	[33]	Hurler/Scheie	**W402X**
L346R	0.066	101	147	2.9	Intermediate	[41]	Hurler/Scheie	L346R
R363H	0.029	20	48	14.5	Intermediate	[42]	Hurler/Scheie	c.1190-1GNA
T364M	0.069	76	91	0	Intermediate	[43]	Hurler/Scheie	T364M
A436P	0.052	63	39	3.2	Intermediate	[24]	Hurler/Scheie	A436P
P496L	0.055	52	53	0.1	Intermediate	[44]	Hurler/Scheie	A75T
L535F	0.055	48	69	0	Intermediate	[24]	Hurler/Scheie	**W402X**
F602I	0.006	0	1	2.6	Intermediate	[33]	Hurler/Scheie[b]	R363C
R619G	0.010	3	8	60.5	Intermediate	[45]	Hurler/Scheie	R619G
L490P	0.082	40	67	24.1	Attenuated-intermediate	[44, 24]	Hurler/Scheie, Scheie	L490P
Y76C	0.018	14	12	0	Attenuated	[24]	Scheie	Q70X
R89Q	0.048	79	124	0	Attenuated	[32]	Mild	**W402X**
D203N	0.024	14	16	0	Attenuated	[46]	Scheie	Q60X
C205Y	0.000	1	0	0.8	Attenuated	[29]	Scheie	Y167X
H240R	0.051	59	73	10.5	Attenuated	[29]	Scheie	**W402X**
E276K	0.143	449	558	3.5	Attenuated	[47, 48]	Scheie	E276K
A319V	0.082	129	109	0	Attenuated	[29]	Scheie	A319V
N348K	0.095	175	213	11.6	Attenuated	[24]	Scheie	c.1727+5GNC

FIG. 3 continued

N350I	0.038	40	34	3.8	Attenuated	[38]	Slow progression	c.134-145del12
T374N	0.023	17	18	43	Attenuated	[49]	Attenuated	c.252insC
Q380R	0.114	179	199	0	Attenuated	[32]	Mild	R628P
R383H	0.042	34	61	0.1	Attenuated	[50]	Attenuated	R383H
R492P	0.051	13	34	153.4	Attenuated	[44]	Scheie	Q70X
L578Q	0.007	1	4	0.7	Attenuated	[51]	Scheie	P533R
S633L	0.050	51	69	7.9	Attenuated	[29]	Scheie	R633L

The mutations localized in the Ig-like domain and adjacent region are shown in bold/shaded.

[a] Description of the clinical form of the patients is according to the original papers.

[b] An expression study revealed that F602I-IDUA exhibited residual enzyme activity, but not in the case of R363C-IDUA [34].

FIG. 3 continued

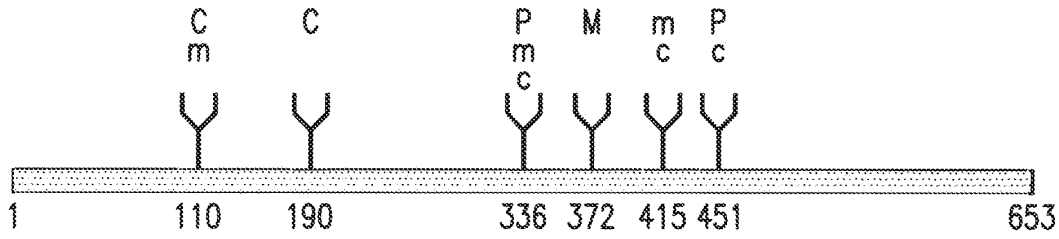


FIG. 4

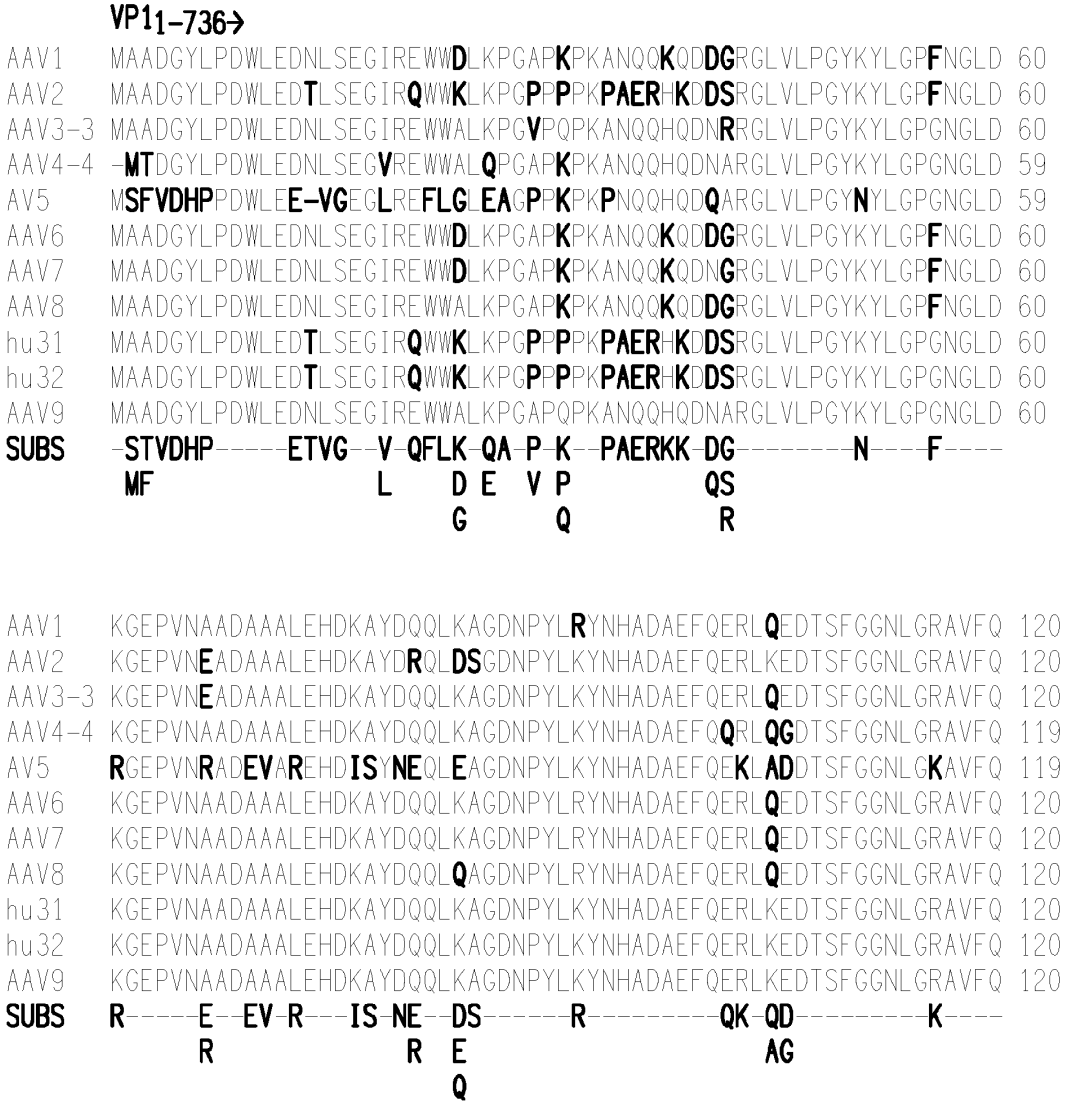

VP1 1-736→
AAV1 MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLD 60
AAV2 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD 60
AAV3-3 MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGYKYLGPGNGLD 60
AAV4-4 -MTDGYLPDWLEDNLSEGVREWWALQPGAPKPKANQQHQDNARGLVLPGYKYLGPGNGLD 59
AV5 MSFVDHPPDWLEE-VGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLD 59
AAV6 MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLD 60
AAV7 MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFNGLD 60
AAV8 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLD 60
hu31 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPGNGLD 60
hu32 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPGNGLD 60
AAV9 MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD 60
SUBS -STVDHP-----ETVG--V-QFLK-QA-P-K--PAERKK-DG--------N---F----
MF L D E V P QS
G Q R
AAV1 KGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQ 120
AAV2 KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ 120
AAV3-3 KGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLQEDTSFGGNLGRAVFQ 120
AAV4-4 KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQQRLQGDTSFGGNLGRAVFQ 119
AV5 RGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQ 119
AAV6 KGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQ 120
AAV7 KGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQ 120
AAV8 KGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQ 120
hu31 KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ 120
hu32 KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ 120
AAV9 KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ 120
SUBS R-----E--EV-R---IS-NE--DS------R---------QK-QD---------K----
R R E AG
Q

FIG. 5

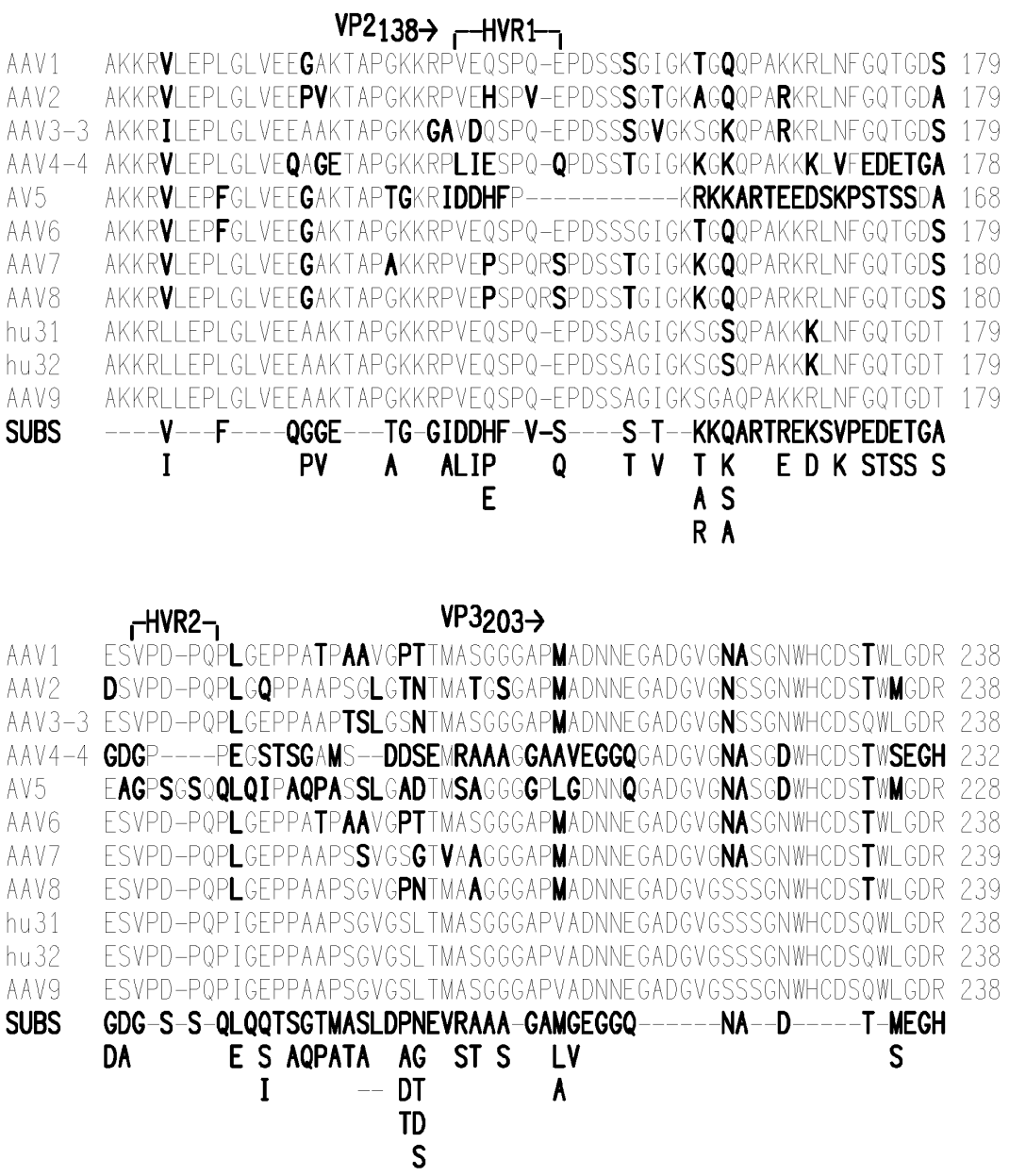


FIG. 5 continued

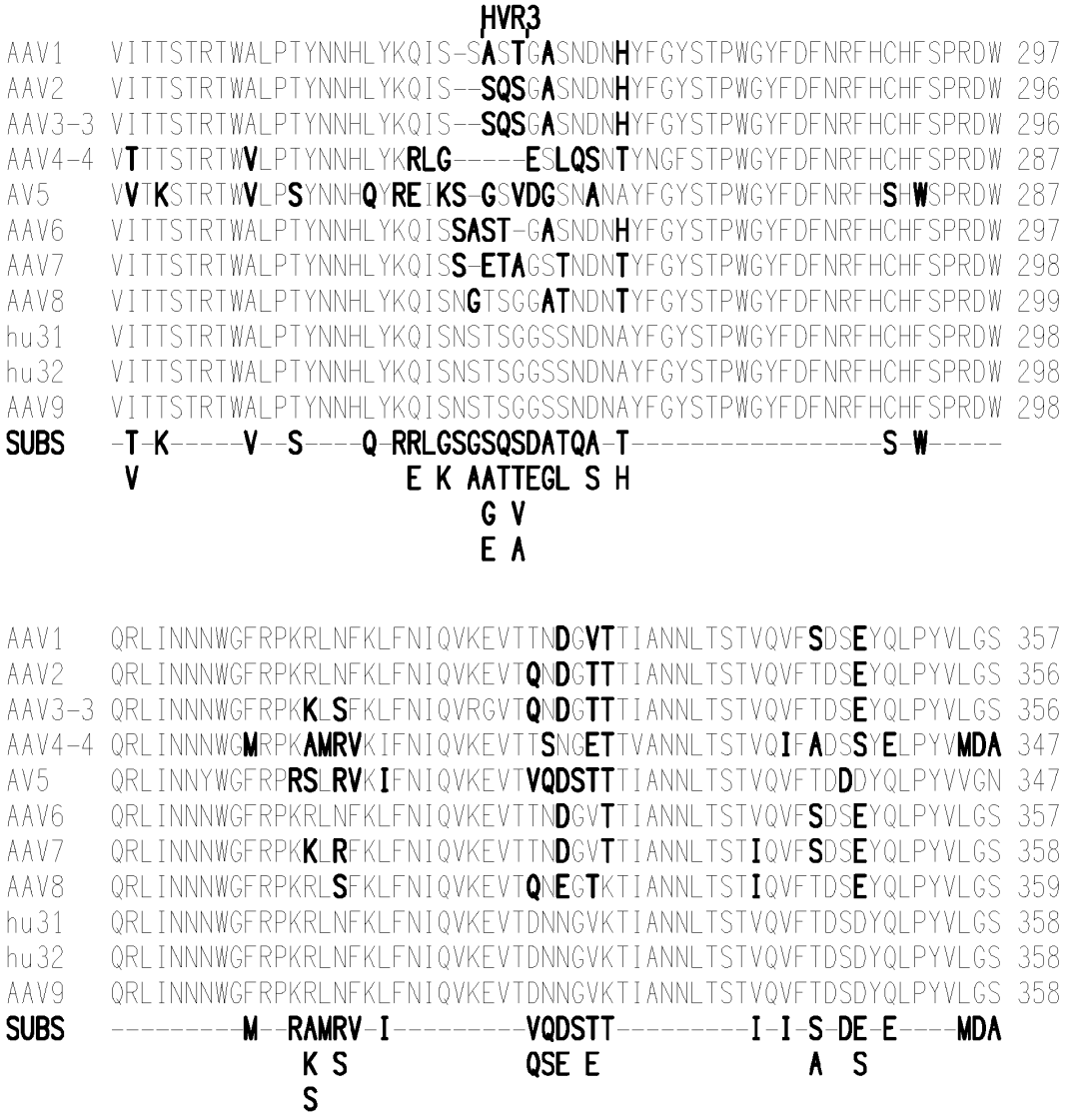


FIG. 5 continued

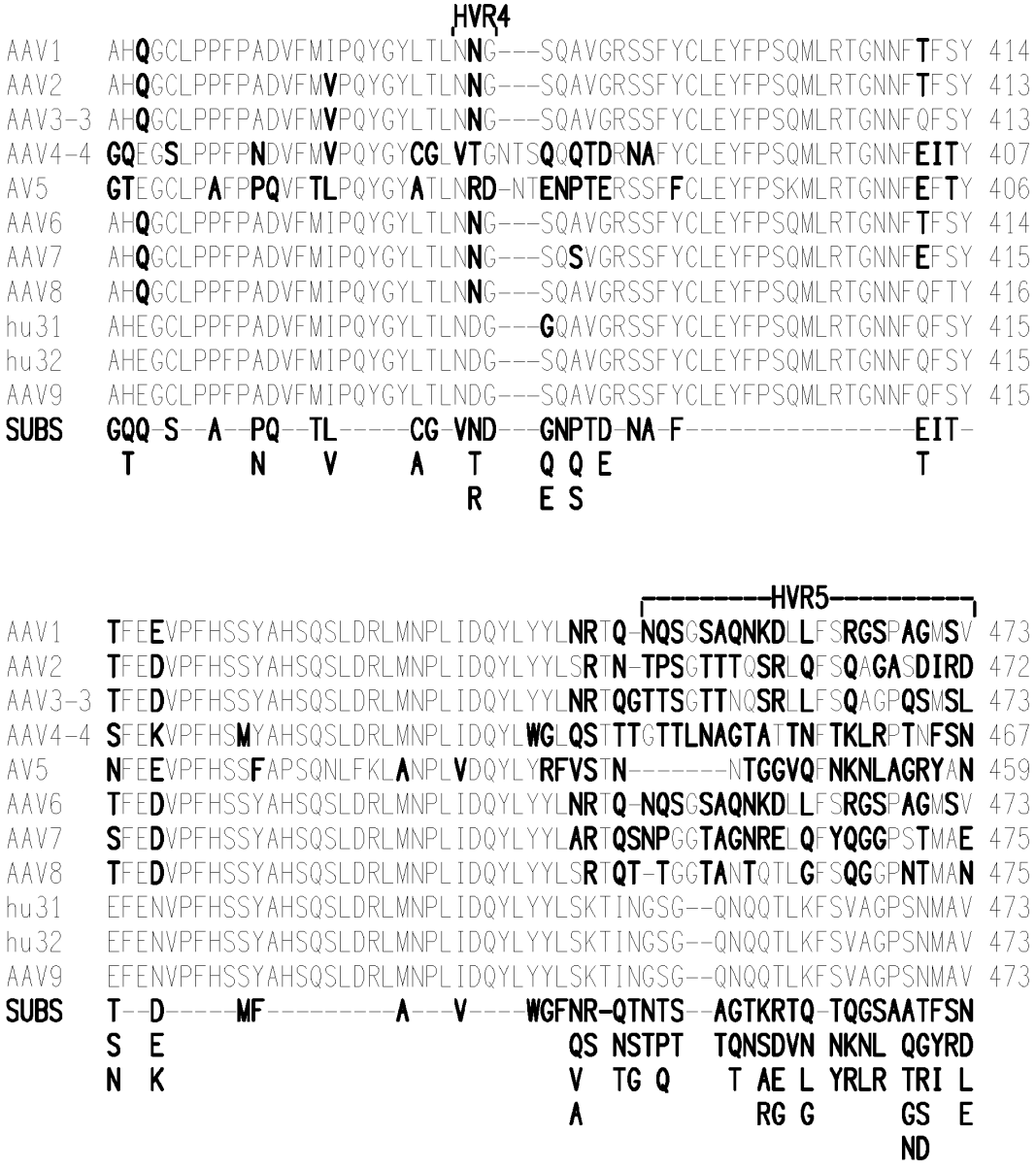


FIG. 5 continued

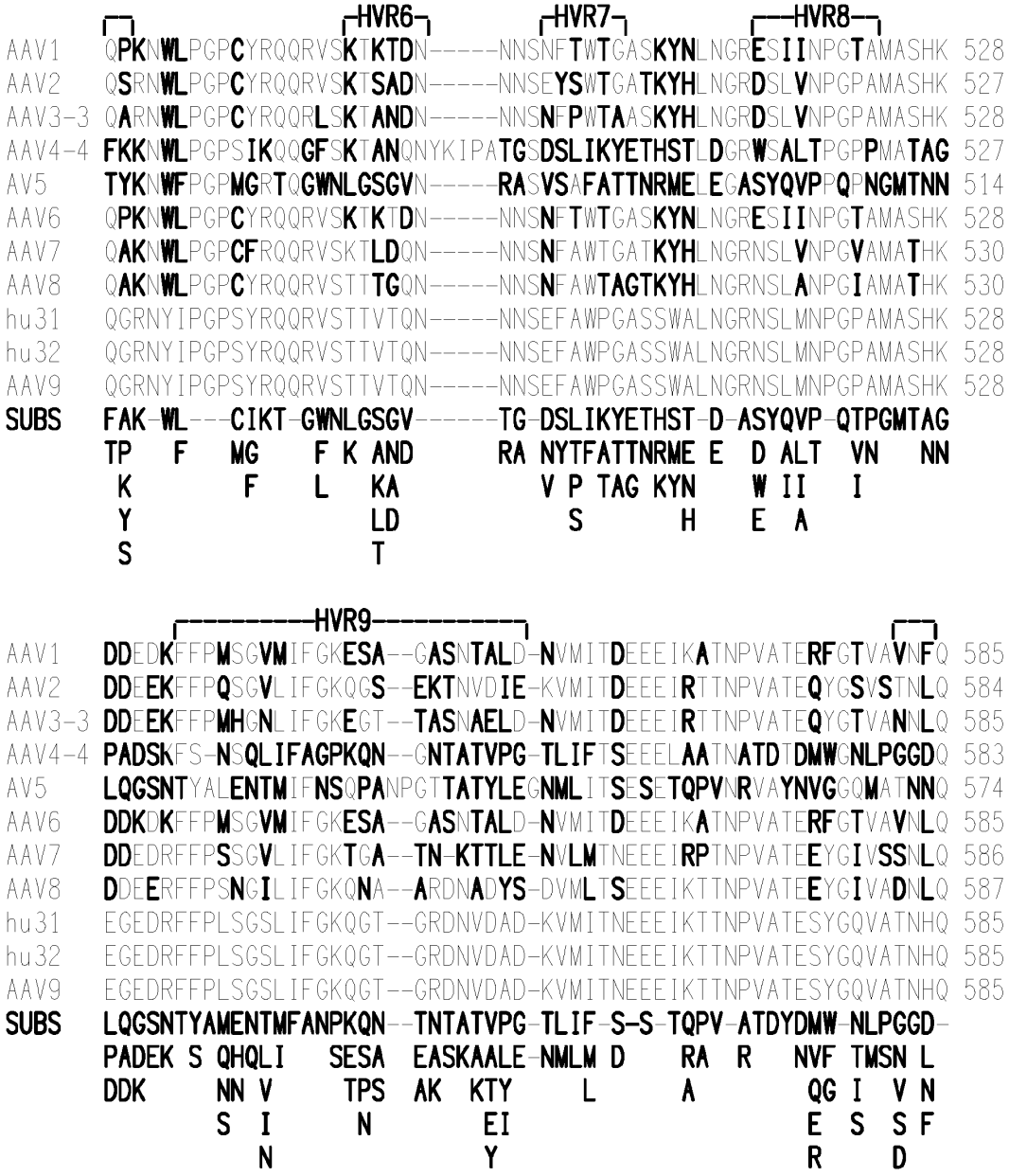


FIG. 5 continued

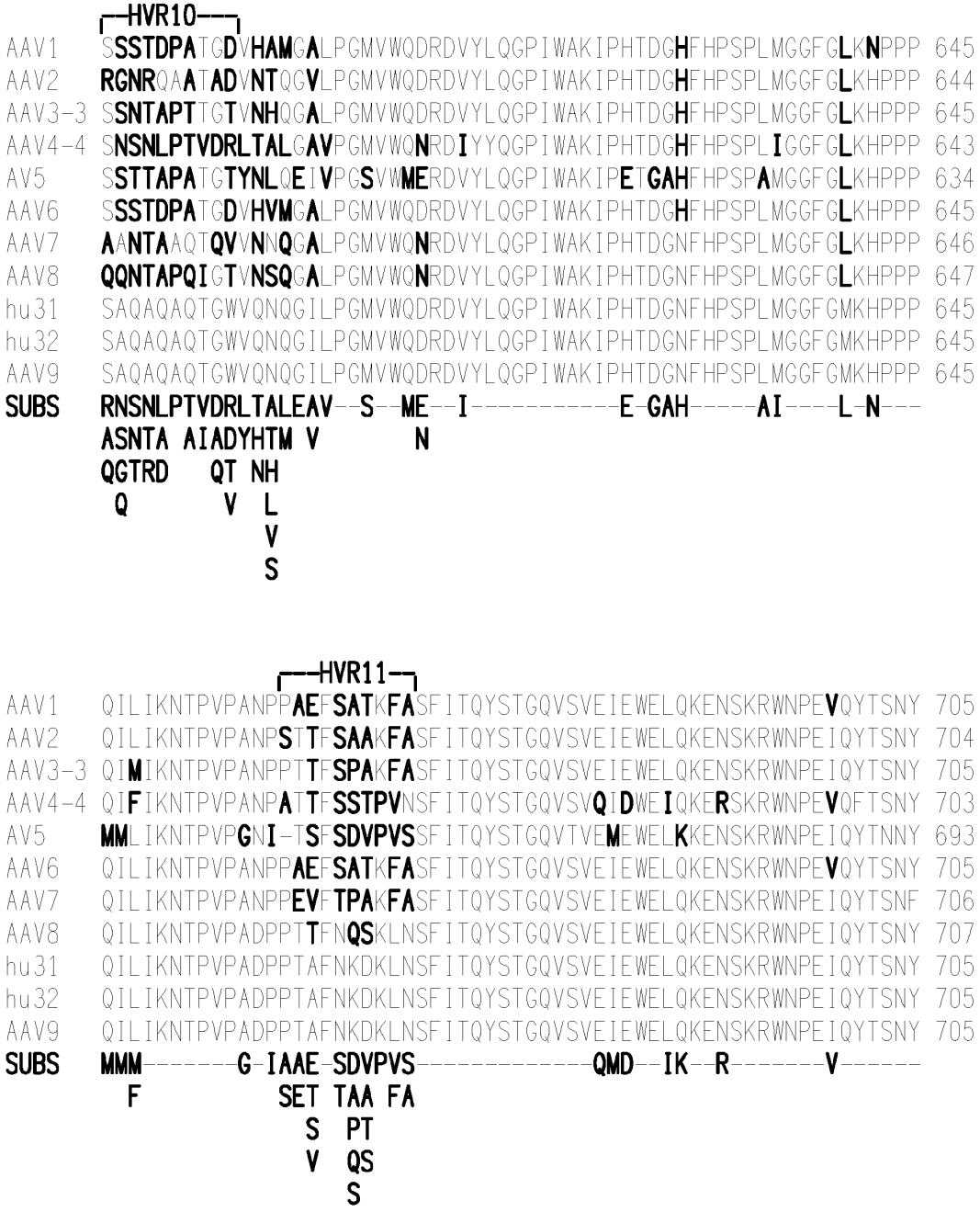


FIG. 5 continued

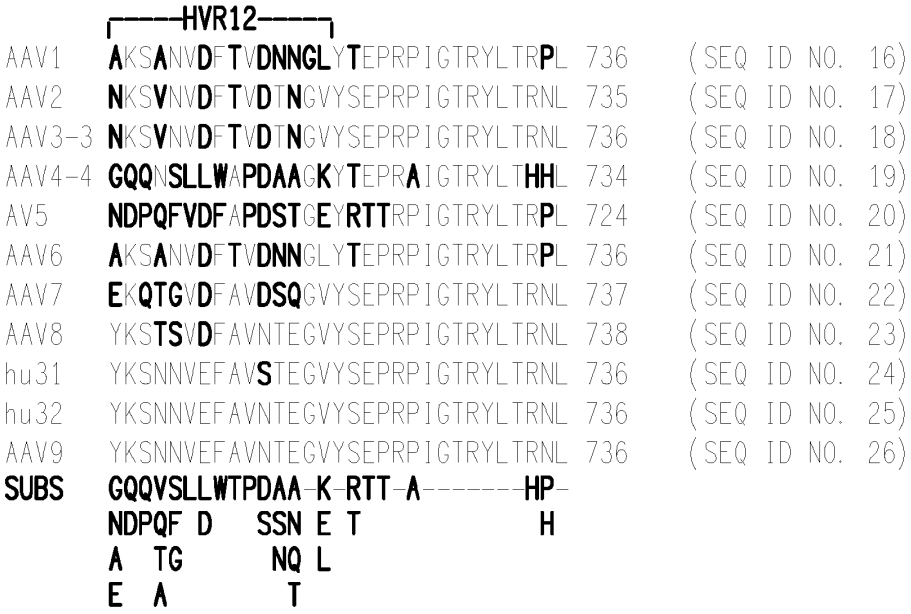


FIG. 5 continued