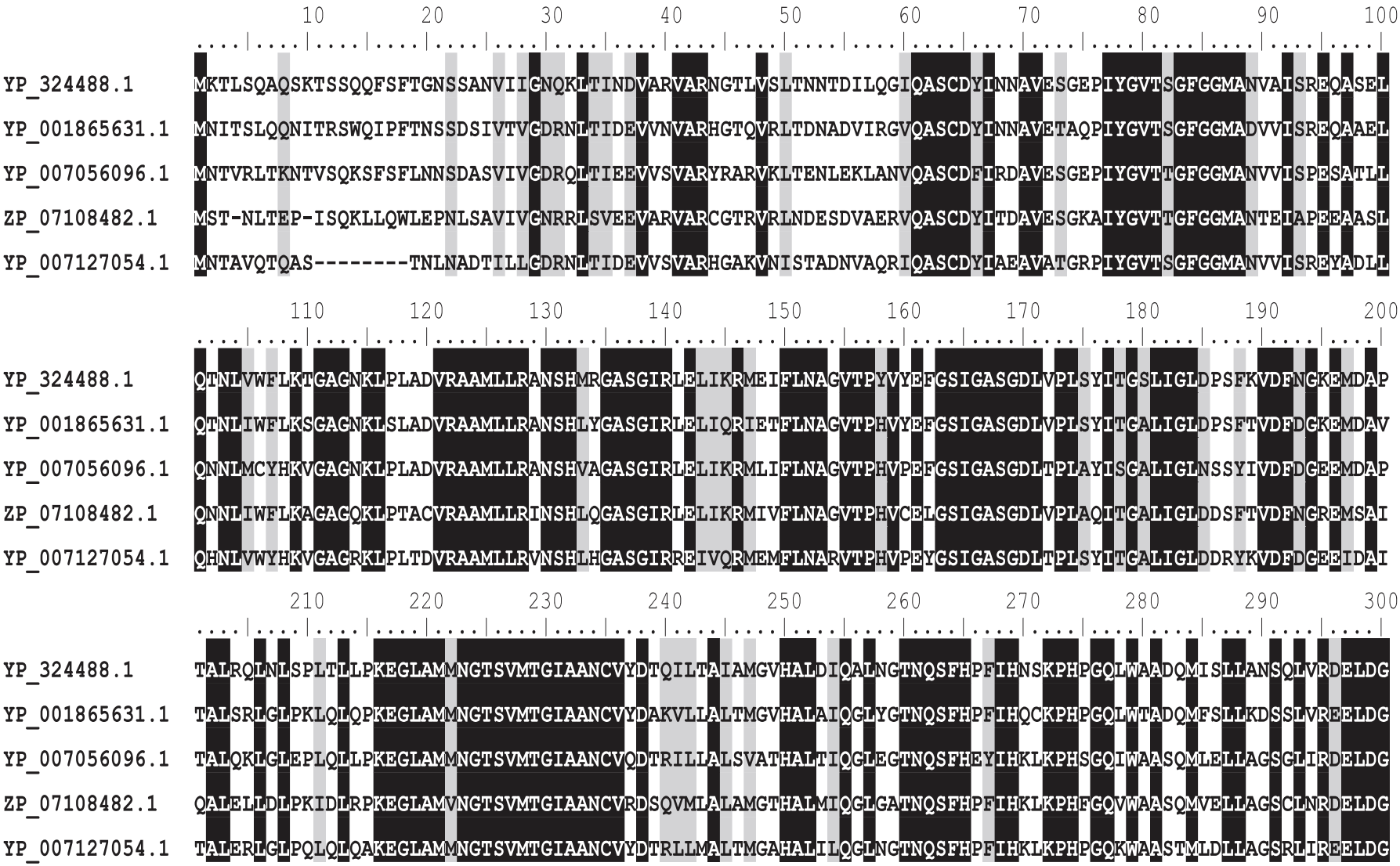

10 20 30 40 50 60 70 80 90 100
YP_324488.1 MKTLSQAQSKTSSQQFSFTGNSSANVIIGNQKLTINDVARVARNGTLVSLTNNTDILQGIQASCDYINNAVESGEPIYGVTSGFGGMANVAISREQASEL
YP_001865631.1 MNITSLQQNITRSWQIPFTNSSDSIVTVGDRNLTIDEVVNVARHGTQVRLTDNADVIRGVQASCDYINNAVETAQPIYGVTSGFGGMADVVISREQAAEL
YP_007056096.1 MNTVRLTKNTVSQKSFSFLNNSDASVIVGDRQLTIEEVVSVARYRARVKLTENLEKLANVQASCDFIRDAVESGEPIYGVTTGFGGMANVVISPESATLL
ZP_07108482.1 MST-NLTEP-ISQKLLQWLEPNLSAVIVGNRRLSVEEVARVARCGTRVRLNDESDVAERVQASCDYITDAVESGKAIYGVTTGFGGMANTEIAPEEAASL
YP_007127054.1 MNTAVQTQAS--------TNLNADTILLGDRNLTIDEVVSVARHGAKVNISTADNVAQRIQASCDYIAEAVATGRPIYGVTSGFGGMANVVISREYADLL
110 120 130 140 150 160 170 180 190 200
YP_324488.1 QTNLVWFLKTGAGNKLPLADVRAAMLLRANSHMRGASGIRLELIKRMEIFLNAGVTPYVYEFGSIGASGDLVPLSYITGSLIGLDPSFKVDFNGKEMDAP
YP_001865631.1 QTNLIWFLKSGAGNKLSLADVRAAMLLRANSHLYGASGIRLELIQRIETFLNAGVTPHVYEFGSIGASGDLVPLSYITGALIGLDPSFTVDFDGKEMDAV
YP_007056096.1 QNNLMCYHKVGAGNKLPLADVRAAMLLRANSHVAGASGIRLELIKRMLIFLNAGVTPHVPEFGSIGASGDLTPLAYISGALIGLNSSYIVDFDGEEMDAP
ZP_07108482.1 QNNLIWFLKAGAGQKLPTACVRAAMLLRINSHLQGASGIRLELIKRMIVFLNAGVTPHVCELGSIGASGDLVPLAQITGALIGLDDSFTVDFNGREMSAI
YP_007127054.1 QHNLVWYHKVGAGRKLPLTDVRAAMLLRVNSHLHGASGIRREIVQRMEMFLNARVTPHVPEYGSIGASGDLTPLSYITGALIGLDDRYKVDFDGEEIDAI
210 220 230 240 250 260 270 280 290 300
YP_324488.1 TALRQLNLSPLTLLPKEGLAMMNGTSVMTGIAANCVYDTQILTAIAMGVHALDIQALNGTNQSFHPFIHNSKPHPGQLWAADQMISLLANSQLVRDELDG
YP_001865631.1 TALSRLGLPKLQLQPKEGLAMMNGTSVMTGIAANCVYDAKVLLALTMGVHALAIQGLYGTNQSFHPFIHQCKPHPGQLWTADQMFSLLKDSSLVREELDG
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YP_007127054.1 TALERLGLPQLQLQAKEGLAMMNGTSVMTGIAANCVYDTRLLMALTMGAHALILQGLNGTNQSFHPFIHKLKPHPGQKWAASTMLDLLAGSRLIREELDG

FIG. 1

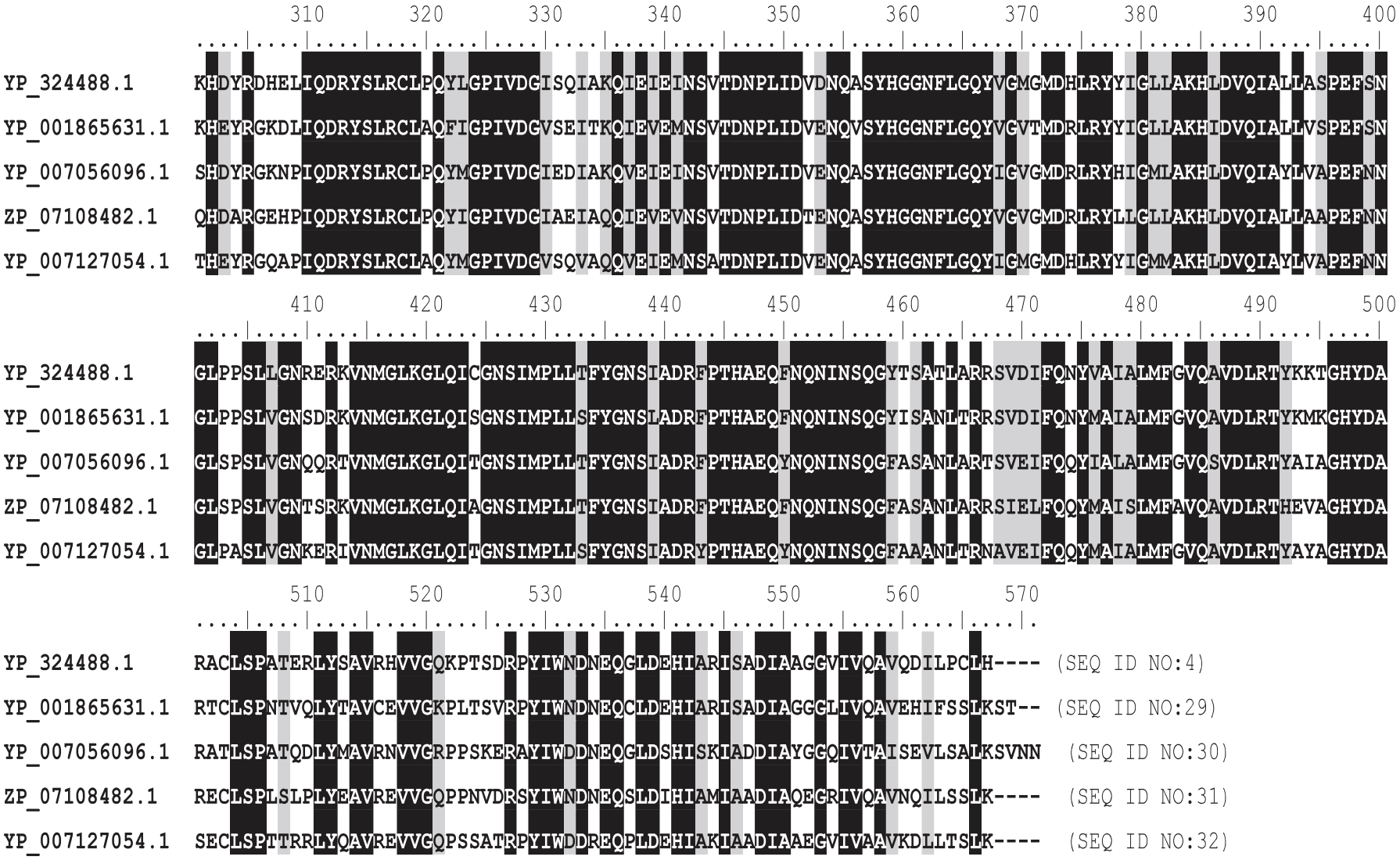

310 320 330 340 350 360 370 380 390 400
YP_324488.1 KHDYRDHELIQDRYSLRCLPQYLGPIVDGISQIAKQIEIEINSVTDNPLIDVDNQASYHGGNFLGQYVGMGMDHLRYYIGLLAKHLDVQIALLASPEFSN
YP_001865631.1 KHEYRGKDLIQDRYSLRCLAQFIGPIVDGVSEITKQIEVEMNSVTDNPLIDVENQVSYHGGNFLGQYVGVTMDRLRYYIGLLAKHIDVQIALLVSPEFSN
YP_007056096.1 SHDYRGKNPIQDRYSLRCLPQYMGPIVDGIEDIAKQVEIEINSVTDNPLIDVENQASYHGGNFLGQYIGVGMDRLRYHIGMLAKHLDVQIAYLVAPEFNN
ZP_07108482.1 QHDARGEHPIQDRYSLRCLPQYIGPIVDGIAEIAQQIEVEVNSVTDNPLIDTENQASYHGGNFLGQYVGVGMDRLRYLLGLLAKHLDVQIALLAAPEFNN
YP_007127054.1 THEYRGQAPIQDRYSLRCLAQYMGPIVDGVSQVAQQVEIEMNSATDNPLIDVENQASYHGGNFLGQYIGMGMDHLRYYIGMMAKHLDVQIAYLVAPEFNN
410 420 430 440 450 460 470 480 490 500
YP_324488.1 GLPPSLLGNRERKVNMGLKGLQICGNSIMPLLTFYGNSIADRFPTHAEQENQNINSQGYTSATLARRSVDIFQNYVAIALMEGVQAVDLRTYKKTGHYDA
YP_001865631.1 GLPPSLVGNSDRKVNMGLKGLQISGNSIMPLLSFYGNSLADRFPTHAEQENQNINSQGYISANLTRRSVDIFQNYMAIALMEGVQAVDLRTYKMKGHYDA
YP_007056096.1 GLSPSLVGNQQRTVNMGLKGLQITGNSIMPLLTFYGNSIADRFPTHAEQYNQNINSQGFASANLARTSVEIFQQYIALALMEGVQSVDLRTYAIAGHYDA
ZP_07108482.1 GLSPSLVGNTSRKVNMGLKGLQIAGNSIMPLLTFYGNSIADRFPTHAEQENQNINSQGFASANLARRSIELFQQYMAISLMEAVQAVDLRTHEVAGHYDA
YP_007127054.1 GLPASLVGNKERIVNMGLKGLQITGNSIMPLLSFYGNSIADRYPTHAEQYNQNINSQGFAAANLTRNAVEIFQQYMAIALMEGVQAVDLRTYAYAGHYDA
510 520 530 540 550 560 570
YP_324488.1 RACLSPATERLYSAVRHVVGQKPTSDRPYIWNDNEQGLDEHIARISADIAAGGVIVQAVQDILPCLH---- (SEQ ID NO:4)
YP_001865631.1 RTCLSPNTVQLYTAVCEVVGKPLTSVRPYIWNDNEQCLDEHIARISADIAGGGLIVQAVEHIFSSLKST-- (SEQ ID NO:29)
YP_007056096.1 RATLSPATQDLYMAVRNVVGRPPSKERAYIWDDNEQGLDSHISKIADDIAYGGQIVTAISEVLSALKSVNN (SEQ ID NO:30)
ZP_07108482.1 RECLSPLSLPLYEAVREVVGQPPNVDRSYIWNDNEQSLDIHIAMIAADIAQEGRIVQAVNQILSSLK---- (SEQ ID NO:31)
YP_007127054.1 SECLSPTTRRLYQAVREVVGQPSSATRPYIWDDREQPLDEHIAKIAADIAAEGVIVAAVKDLLTSLK---- (SEQ ID NO:32)

FIG. 1 (Cont.)

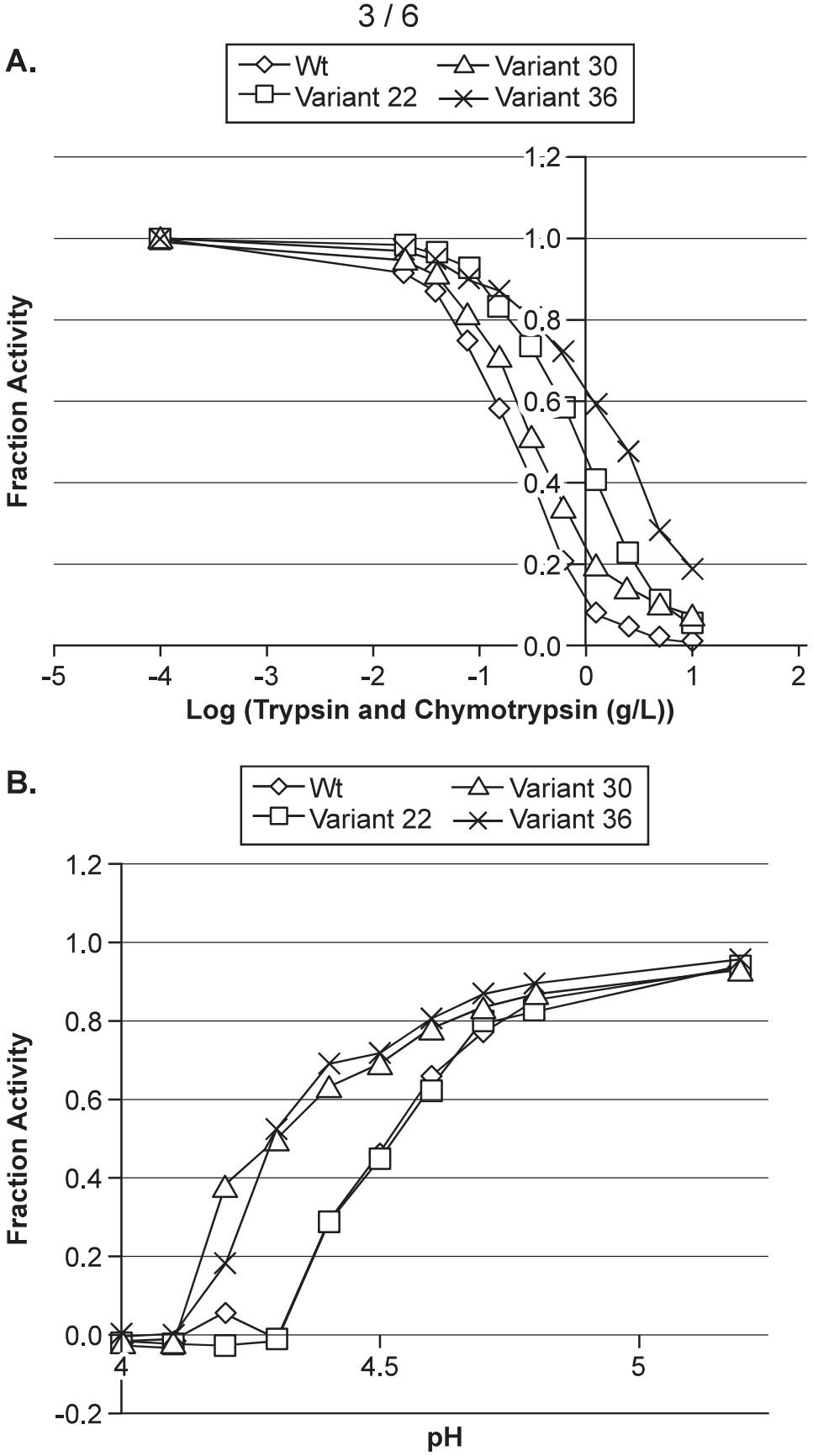

A.
Wt
Variant 30
Variant 22
Variant 36
Fraction Activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-5
-4
-3
-2
-1
0
1
2
Log (Trypsin and Chymotrypsin (g/L))
B.
Wt
Variant 30
Variant 22
Variant 36
Fraction Activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
4
4.5
5
pH

FIG. 2

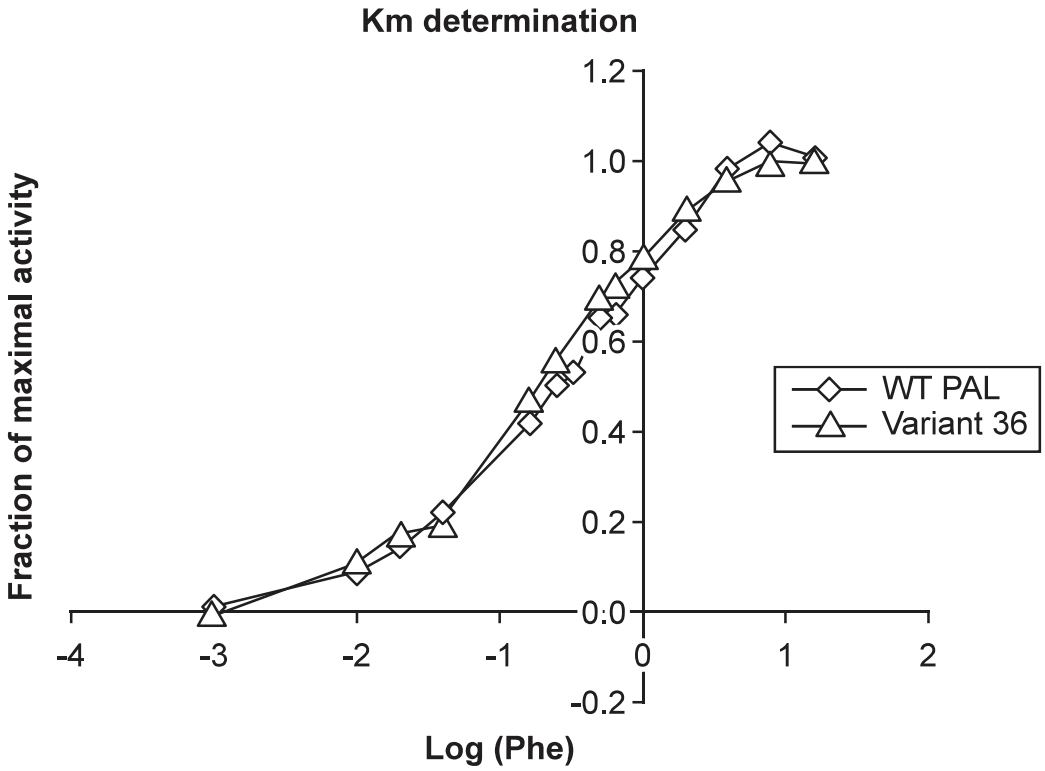

Km determination
Fraction of maximal activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-4
-3
-2
-1
0
1
2
Log (Phe)
WT PAL
Variant 36

FIG. 3

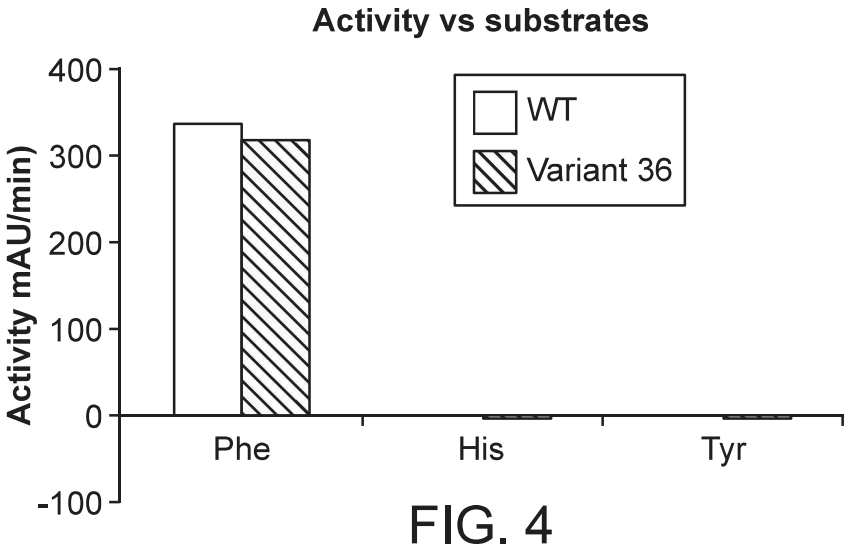

Activity vs substrates
Activity mAU/min)
400
300
200
100
0
-100
Phe
His
Tyr
WT
Variant 36

FIG. 4

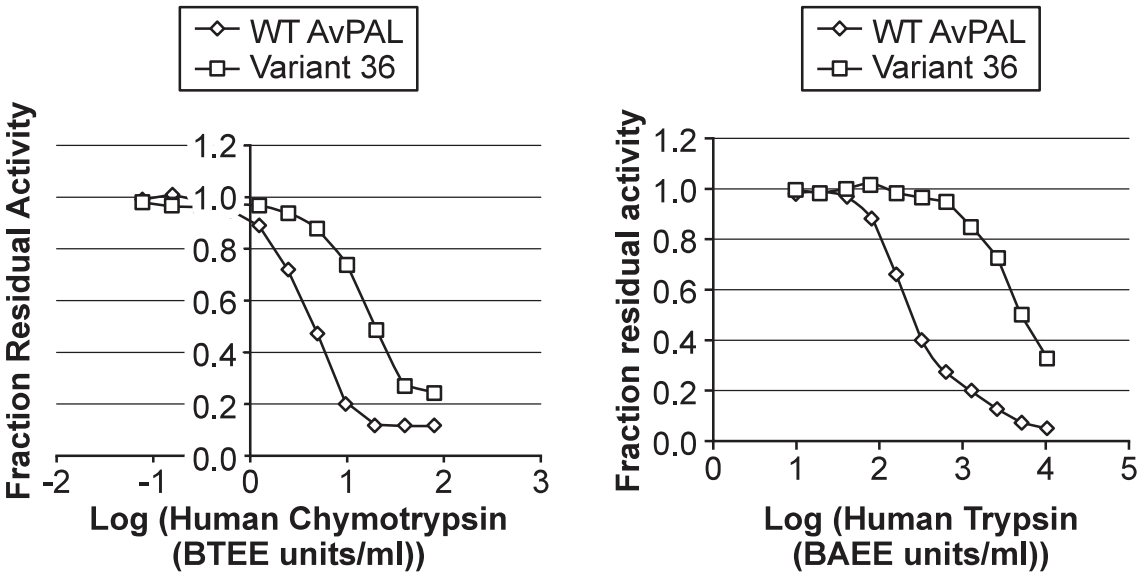

WT AvPAL
Variant 36
Fraction Residual Activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-2
-1
0
1
2
3
Log (Human Chymotrypsin (BTEE units/ml))
WT AvPAL
Variant 36
Fraction residual activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
Log (Human Trypsin (BAEE units/ml))

FIG. 5

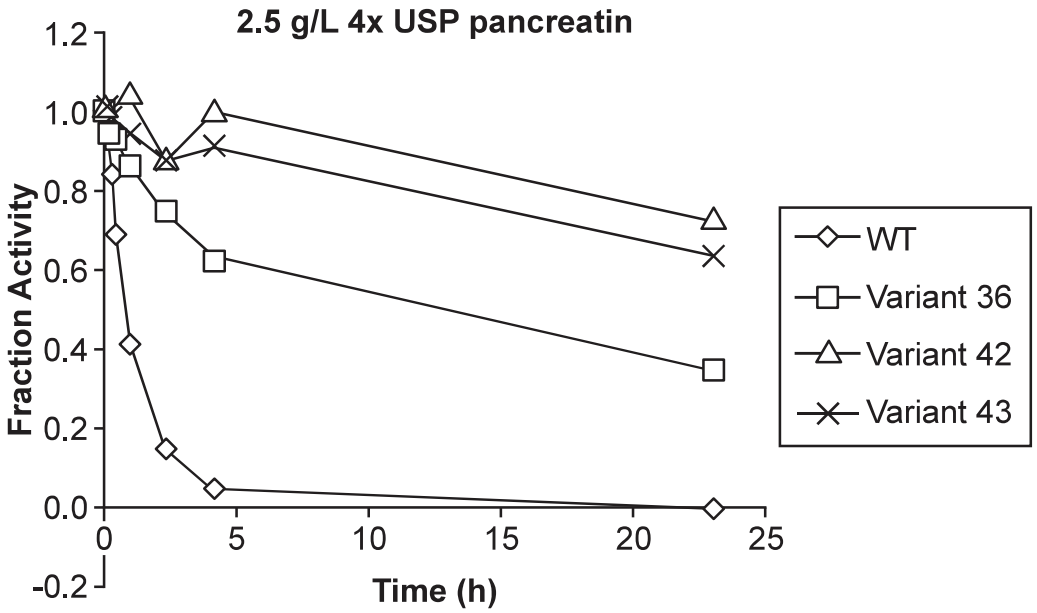

2.5 g/L 4x USP pancreatin
Fraction Activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
5
10
15
20
25
Time (h)
WT
Variant 36
Variant 42
Variant 43

FIG. 6

0 mM Sodium taurocholate
0.5 mM sodium taurocholate
8 mM sodium taurocholate
Fraction residual activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-3
-2
-1
0
1
2
Log Trypsin and Chymotrypsin (g/L)

FIG. 7

BSA
WT AvPAL
Variant 42
WT + Aprot
Phenylalanine level (micromolar)
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
Time post injection

FIG. 8