

LIVER DETARGETED CAPSIDS

FIELD OF DISCLOSURE

This disclosure relates to viral capsids.

BACKGROUND

5 Recombinant AAVs (rAAVs) are the most commonly used delivery vehicles for gene therapy and gene editing. Nonetheless, rAAVs that contain natural capsid variants have limited cell tropism. Indeed, rAAVs used today mainly infect the liver after systemic delivery. Further, the transduction efficiency of conventional rAAVs in other cell-types, tissues, and organs by these conventional rAAVs with natural capsid variants is limited.

10 Therefore, AAV-mediated polynucleotide delivery for diseased that affect cells, tissues, and organs other than the liver, such as the central nervous system) typically requires an injection of a large dose of virus (typically about 2×10^{14} vg/kg), which often results in liver toxicity. Furthermore, because large doses are required when using conventional rAAVs, manufacturing sufficient amounts of a therapeutic rAAV needed to dose adult patients is

15 extremely challenging. Additionally, due to differences in gene expression and physiology, mouse and primate models respond differently to viral capsids. Transduction efficiency of different virus particles varies between different species, and as a result, preclinical studies in mice often do not accurately reflect results in primates, including humans. As such there exists a need for improved rAAVs for use in the treatment of various genetic diseases.

SUMMARY

20 The present invention provides novel capsid protein variants for viral vectors that detarget liver tissue and target skeletal muscle and heart tissue at the same time. Aspects of the present invention provide adeno-associated virus (AAV) vector's comprising a capsid protein comprising the amino acid sequence RGDR. In the capsid protein, RGDR may be

25 inserted after amino acid 455 in reference to an AAV9 capsid or equivalent position in another AAV capsid. AAV vectors may comprise the amino acid sequence $X_1NX_2X_3X_4RGDRX_5X_6L$, wherein X_1 , X_2 , X_3 , X_4 , X_5 , and X_6 may be any amino acid.

In aspects of the invention, X₁ may be an amino acid selected from the group consisting of: A, I, F, G, H, L, M, Q, S, T, V. In preferred aspects of the invention, X₁ may be an amino acid selected from the group consisting of: A, I, L, M, S, V.

5 In aspects of the invention, X₂ may be an amino acid selected from the group consisting of A, G, S, T, Y.

In aspects of the invention, X₃ may be an amino acid selected from the group consisting of S, N, G, P. In preferred aspects of the invention, X₃ may be S.

In aspects of the invention, X₄ may be an amino acid selected from the group consisting of A, G, H, I, M, S, T, V.

10 In aspects of the invention, X₅ may be an amino acid selected from the group consisting of A, G, Q.

In aspects of the invention, X₆ may be an amino acid selected from the group consisting of A, I, L, M, N, Y. In preferred aspects of the invention, X₆ may be selected from the group consisting of A, S, and Y.

15 X₁ may be located at amino acid 451, X₂ located at amino acid 453, X₃ located at amino acid 454, X₄ is located amino acid 455, and RGDRX₅X₆L inserted after amino acid 455 in reference to an AAV9 capsid or equivalent position in another AAV capsid.

In aspects of the invention, two amino acids from X₁, X₂, X₃, and X₄ are wild type amino acids in reference to an AAV9 capsid or equivalent position in another AAV capsid
20 and two amino acids from X₁, X₂, X₃, and X₄ are not wild type amino acids.

For example, capsid proteins variants of the invention may comprise a sequence as set forth in Table 1a. Notably, capsid protein variants on the invention comprise deletions, substitutions, and/or insertions relative to wild-type viral vector capsids.

In aspects of the invention, the capsid protein comprises an amino acid sequence
25 selected from Table 1a and the amino acid sequence is in hypervariable region IV (HVR IV) relative to wild-type AAV9. The capsid protein variant may comprise substitutions at amino acids 451-455 relative to a wild-type AAV9 vector capsid. For example, the substitutions at amino acids 451-455 relative to a wild-type AAV9 vector capsid may be an amino acid sequence selected from column 1 of Table 1b. In aspects of the invention, the capsid protein
30 variant may further comprise an insert. For example, the capsid protein may comprise a 7-

mer insert selected from column 2 of Table 1b. The insert may be in the location after amino acid 455 relative to a wild-type AAV9 vector.

Advantageously, viral vectors comprising an amino acid sequence of the invention exhibit muscle tropism as compared to a wild-type AAV vector.

5 In aspects of the invention, the capsid protein further comprises a deletion of G267 in reference to an AAV9 capsid or equivalent position in another AAV capsid. Advantageously, the vector may exhibit reduced liver tropism as compared to a wild-type AAV vector.

As described, for the HVR IV variants, the 5 amino acids upstream are at positions 451-455, shown in column 1 of Table 1b. The 7-mer insert for HVR IV variants starts with
10 "RGD" and is inserted after amino acid 455, shown in column 2 of Table 1b.

Table 1a: HVR IV Capsid Variants

Amino Acid Sequence	SEQ ID NO:
ANASARGDRAAL	2
ANASARGDRGSL	3
ANASARGDRGYL	4
ANASARGDRQAL	5
ANASARGDRQSL	6
ANASARGDRQYL	7
ANASGRGDRAAL	8
ANASGRGDRASL	9
ANASGRGDRAYL	10
ANASGRGDRGAL	11
ANASGRGDRGSL	12
ANASGRGDRGYL	13
ANASGRGDRQAL	14
ANASGRGDRQSL	15
ANASGRGDRQYL	16
ANASMRGDRAAL	17
ANASMRGDRASL	18
ANASMRGDRAYL	19
ANASMRGDRQAL	20
ANASMRGDRQSL	21
ANASSRGDRGSL	22
ANASSRGDRGYL	23

Amino Acid Sequence	SEQ ID NO:
ANASSRGDRQAL	24
ANASSRGDRQSL	25
ANASSRGDRQYL	26
ANASTRGDRASL	27
ANASTRGDRAYL	28
ANASTRGDRGAL	29
ANASTRGDRGSL	30
ANASTRGDRGYL	31
ANASTRGDRQAL	32
ANASTRGDRQSL	33
ANASTRGDRQYL	34
ANASVRGDRASL	35
ANASVRGDRGYL	36
ANASVRGDRQAL	37
ANASVRGDRQSL	38
ANASVRGDRQYL	39
ANGNFRGDRQNL	40
ANGNVRGDRGML	41
ANGSARGDRAYL	42
ANGSARGDRGSL	43
ANGSARGDRGYL	44
ANGSARGDRQAL	45
ANGSARGDRQYL	46
ANGSGRGDRAAL	47

Amino Acid Sequence	SEQ ID NO:
ANGSGRGDRAYL	48
ANGSGRGDRGAL	49
ANGSGRGDRGSL	50
ANGSGRGDRGYL	51
ANGSGRGDRQAL	52
ANGSGRGDRQSL	53
ANGSGRGDRQYL	54
ANGSMRGDRGYL	55
ANGSMRGDRQAL	56
ANGSMRGDRQSL	57
ANGSMRGDRQYL	58
ANGSSRGDRAYL	59
ANGSSRGDRGSL	60
ANGSSRGDRGYL	61
ANGSSRGDRQAL	62
ANGSSRGDRQSL	63
ANGSSRGDRQYL	64
ANGSTRGDRAAL	65
ANGSTRGDRAYL	66
ANGSTRGDRGAL	67
ANGSTRGDRGYL	68
ANGSTRGDRQAL	69
ANGSTRGDRQSL	70
ANGSTRGDRQYL	71
ANGSVRGDRAYL	72
ANGSVRGDRGYL	73
ANGSVRGDRQAL	74
ANGSVRGDRQYL	75
ANSGMRGDRGAL	76
ANSSARGDRGAL	77
ANSSARGDRGSL	78
ANSSARGDRGYL	79
ANSSARGDRQAL	80
ANSSARGDRQSL	81
ANSSARGDRQYL	82
ANSSGRGDRAAL	83
ANSSGRGDRASL	84
ANSSGRGDRAYL	85

Amino Acid Sequence	SEQ ID NO:
ANSSGRGDRGAL	86
ANSSGRGDRGSL	87
ANSSGRGDRGYL	88
ANSSGRGDRQAL	89
ANSSGRGDRQSL	90
ANSSGRGDRQYL	91
ANSSMRGDRAYL	92
ANSSMRGDRQAL	93
ANSSMRGDRQSL	94
ANSSMRGDRQYL	95
ANSSSRGDRGSL	96
ANSSSRGDRGYL	97
ANSSSRGDRQAL	98
ANSSSRGDRQSL	99
ANSSSRGDRQYL	100
ANSSTRGDRAAL	101
ANSSTRGDRGSL	102
ANSSTRGDRQAL	103
ANSSTRGDRQSL	104
ANSSTRGDRQYL	105
ANSSVRGDRAYL	106
ANSSVRGDRGYL	107
ANSSVRGDRQAL	108
ANSSVRGDRQSL	109
ANSSVRGDRQYL	110
ANTSARGDRAYL	111
ANTSARGDRGAL	112
ANTSARGDRGSL	113
ANTSARGDRGYL	114
ANTSARGDRQAL	115
ANTSARGDRQYL	116
ANTSGRGDRASL	117
ANTSGRGDRGAL	118
ANTSGRGDRGSL	119
ANTSGRGDRGYL	120
ANTSGRGDRQAL	121
ANTSGRGDRQSL	122
ANTSGRGDRQYL	123

Amino Acid Sequence	SEQ ID NO:
ANTSMRGDRAYL	124
ANTSMRGDRQAL	125
ANTSMRGDRQSL	126
ANTSMRGDRQYL	127
ANTSSRGDRGSL	128
ANTSSRGDRQAL	129
ANTSSRGDRQYL	130
ANTSTRGDRAAL	131
ANTSTRGDRQAL	132
ANTSTRGDRQSL	133
ANTSTRGDRQYL	134
ANTSVRGDRAYL	135
ANTSVRGDRGSL	136
ANTSVRGDRQAL	137
ANTSVRGDRQSL	138
ANTSVRGDRQYL	139
ANYPHRGDRGAL	140
ANYSARGDRAAL	141
ANYSARGDRASL	142
ANYSARGDRGAL	143
ANYSARGDRGSL	144
ANYSARGDRQAL	145
ANYSARGDRQSL	146
ANYSARGDRQYL	147
ANYSARGDRASL	148
ANYSARGDRGAL	149
ANYSARGDRGSL	150
ANYSARGDRQAL	151
ANYSARGDRQSL	152
ANYSARGDRQYL	153
ANYSMRGDRAAL	154
ANYSMRGDRASL	155
ANYSMRGDRAYL	156
ANYSMRGDRQAL	157
ANYSMRGDRQSL	158
ANYSSRGDRGAL	159
ANYSSRGDRGSL	160
ANYSSRGDRQAL	161

Amino Acid Sequence	SEQ ID NO:
ANYSSRGDRQSL	162
ANYSSRGDRQYL	163
ANYSTRGDRQAL	164
ANYSTRGDRQSL	165
ANYSTRGDRQYL	166
ANYSVRGDRQAL	167
ANYSVRGDRQSL	168
ANYSVRGDRQYL	169
FNSGMRGDRGAL	170
GNSGMRGDRGAL	171
HNSGMRGDRGAL	172
LNASARGDRAAL	173
LNASARGDRASL	174
LNASARGDRAYL	175
LNASARGDRGAL	176
LNASARGDRGSL	177
LNASARGDRGYL	178
LNASARGDRQAL	179
LNASARGDRQSL	180
LNASARGDRQYL	181
LNASGRGDRAAL	182
LNASGRGDRASL	183
LNASGRGDRAYL	184
LNASGRGDRGAL	185
LNASGRGDRGSL	186
LNASGRGDRGYL	187
LNASGRGDRQAL	188
LNASGRGDRQSL	189
LNASGRGDRQYL	190
LNASIRGDRQLL	191
LNASMRGDRAAL	192
LNASMRGDRASL	193
LNASMRGDRAYL	194
LNASMRGDRGAL	195
LNASMRGDRGSL	196
LNASMRGDRGYL	197
LNASMRGDRQAL	198
LNASMRGDRQSL	199

Amino Acid Sequence	SEQ ID NO:
LNASMRGDRQYL	200
LNASSRGDRAAL	201
LNASSRGDRASL	202
LNASSRGDRGAL	203
LNASSRGDRGSL	204
LNASSRGDRQAL	205
LNASSRGDRQSL	206
LNASSRGDRQYL	207
LNASTRGDRAAL	208
LNASTRGDRASL	209
LNASTRGDRGAL	210
LNASTRGDRGSL	211
LNASTRGDRGYL	212
LNASTRGDRQAL	213
LNASTRGDRQSL	214
LNASTRGDRQYL	215
LNASVRGDRAAL	216
LNASVRGDRASL	217
LNASVRGDRGAL	218
LNASVRGDRGSL	219
LNASVRGDRQAL	220
LNASVRGDRQSL	221
LNASVRGDRQYL	222
LNGNFRGDRQNL	223
LNGNVRGDRGML	224
LNGSARGDRAAL	225
LNGSARGDRAYL	226
LNGSARGDRGSL	227
LNGSARGDRGYL	228
LNGSARGDRQAL	229
LNGSARGDRQSL	230
LNGSARGDRQYL	231
LNGSGRGDRAAL	232
LNGSGRGDRAYL	233
LNGSGRGDRGAL	234
LNGSGRGDRGSL	235
LNGSGRGDRGYL	236
LNGSGRGDRQAL	237

Amino Acid Sequence	SEQ ID NO:
LNGSGRGDRQYL	238
LNGSMRGDRASL	239
LNGSMRGDRGYL	240
LNGSMRGDRQAL	241
LNGSMRGDRQSL	242
LNGSMRGDRQYL	243
LNGSSRGDRAAL	244
LNGSSRGDRAYL	245
LNGSSRGDRGAL	246
LNGSSRGDRQYL	247
LNGSTRGDRAAL	248
LNGSTRGDRASL	249
LNGSTRGDRAYL	250
LNGSTRGDRGAL	251
LNGSTRGDRGSL	252
LNGSTRGDRGYL	253
LNGSTRGDRQAL	254
LNGSTRGDRQSL	255
LNGSTRGDRQYL	256
LNGSVRGDRASL	257
LNGSVRGDRAYL	258
LNGSVRGDRGYL	259
LNGSVRGDRQAL	260
LNGSVRGDRQSL	261
LNGSVRGDRQYL	262
LNSSMRGDRGAL	263
LNSSARGDRASL	264
LNSSARGDRAYL	265
LNSSARGDRGAL	266
LNSSARGDRGIL	267
LNSSARGDRGSL	268
LNSSARGDRGYL	269
LNSSARGDRQAL	270
LNSSARGDRQSL	271
LNSSARGDRQYL	272
LNSSGRGDRAAL	273
LNSSGRGDRASL	274
LNSSGRGDRAYL	275

Amino Acid Sequence	SEQ ID NO:
LNSSGRGDRGAL	276
LNSSGRGDRGSL	277
LNSSGRGDRGYL	278
LNSSGRGDRQAL	279
LNSSGRGDRQSL	280
LNSSGRGDRQYL	281
LNSSMRGDRAAL	282
LNSSMRGDRASL	283
LNSSMRGDRAYL	284
LNSSMRGDRGAL	285
LNSSMRGDRGSL	286
LNSSMRGDRQAL	287
LNSSMRGDRQSL	288
LNSSMRGDRQYL	289
LNSSSRGDRAAL	290
LNSSSRGDRGAL	291
LNSSSRGDRGSL	292
LNSSSRGDRQAL	293
LNSSSRGDRQSL	294
LNSSSRGDRQYL	295
LNSSTRGDRAAL	296
LNSSTRGDRASL	297
LNSSTRGDRGAL	298
LNSSTRGDRGSL	299
LNSSTRGDRQAL	300
LNSSTRGDRQSL	301
LNSSTRGDRQYL	302
LNSSVRGDRAAL	303
LNSSVRGDRASL	304
LNSSVRGDRGAL	305
LNSSVRGDRGSL	306
LNSSVRGDRGYL	307
LNSSVRGDRQAL	308
LNSSVRGDRQSL	309
LNSSVRGDRQYL	310
LNTSARGDRAAL	311
LNTSARGDRASL	312
LNTSARGDRAYL	313

Amino Acid Sequence	SEQ ID NO:
LNTSARGDREGAL	314
LNTSARGDRGSL	315
LNTSARGDRGYL	316
LNTSARGDRQAL	317
LNTSARGDRQSL	318
LNTSARGDRQYL	319
LNTSGRGDRAAL	320
LNTSGRGDRASL	321
LNTSGRGDRAYL	322
LNTSGRGDRGAL	323
LNTSGRGDRGSL	324
LNTSGRGDRGYL	325
LNTSGRGDRQAL	326
LNTSGRGDRQSL	327
LNTSGRGDRQYL	328
LNTSMRGDRAAL	329
LNTSMRGDRASL	330
LNTSMRGDRAYL	331
LNTSMRGDRGAL	332
LNTSMRGDRGSL	333
LNTSMRGDRQAL	334
LNTSMRGDRQSL	335
LNTSMRGDRQYL	336
LNTSSRGDRAAL	337
LNTSSRGDRGSL	338
LNTSSRGDRQAL	339
LNTSSRGDRQSL	340
LNTSSRGDRQYL	341
LNTSTRGDRAAL	342
LNTSTRGDRASL	343
LNTSTRGDRGAL	344
LNTSTRGDRGSL	345
LNTSTRGDRQAL	346
LNTSTRGDRQSL	347
LNTSTRGDRQYL	348
LNTSVRGDRAAL	349
LNTSVRGDRASL	350
LNTSVRGDRAYL	351

Amino Acid Sequence	SEQ ID NO:
LNTSVRGDRGAL	352
LNTSVRGDRGSL	353
LNTSVRGDRQAL	354
LNTSVRGDRQSL	355
LNYPHRGDRGAL	356
LNYSARGDRGAL	357
LNYSARGDRGSL	358
LNYSARGDRQAL	359
LNYSARGDRQSL	360
LNYSARGDRQYL	361
LNYSGRGDRAAL	362
LNYSGRGDRASL	363
LNYSGRGDRGAL	364
LNYSGRGDRGSL	365
LNYSGRGDRQAL	366
LNYSGRGDRQSL	367
LNYSGRGDRQYL	368
LNYSMRGDRQAL	369
LNYSMRGDRQSL	370
LNYSRRGDRGAL	371
LNYSRRGDRGSL	372
LNYSRRGDRQAL	373
LNYSRRGDRQSL	374
LNYSRRGDRQYL	375
LNYSRGRDRAAL	376
LNYSRGRGDRGAL	377
LNYSRGRGDRGSL	378
LNYSRGRGDRQAL	379
LNYSRGRGDRQSL	380
LNYSRGRGDRQYL	381
LNYSVRGDRQAL	382
LNYSVRGDRQSL	383
MNASARGDRAAL	384
MNASARGDRAYL	385
MNASARGDRGSL	386
MNASARGDRGYL	387
MNASARGDRQAL	388
MNASARGDRQSL	389

Amino Acid Sequence	SEQ ID NO:
MNASARGDRQYL	390
MNASGRGDRAAL	391
MNASGRGDRASL	392
MNASGRGDRAYL	393
MNASGRGDRGSL	394
MNASGRGDRQAL	395
MNASGRGDRQSL	396
MNASGRGDRQYL	397
MNASIRGDRQLL	398
MNASMRGDRAAL	399
MNASMRGDRAYL	400
MNASMRGDRGAL	401
MNASMRGDRQAL	402
MNASMRGDRQSL	403
MNASSRGDRAAL	404
MNASTRGDRAAL	405
MNASTRGDRASL	406
MNASTRGDRAYL	407
MNASTRGDRGAL	408
MNASTRGDRGSL	409
MNASTRGDRQAL	410
MNASTRGDRQSL	411
MNASTRGDRQYL	412
MNASVRGDRAAL	413
MNASVRGDRASL	414
MNASVRGDRAYL	415
MNASVRGDRQAL	416
MNASVRGDRQSL	417
MNASVRGDRQYL	418
MNGNFRGDRQNL	419
MNGSARGDRAYL	420
MNGSARGDRGYL	421
MNGSARGDRQAL	422
MNGSARGDRQSL	423
MNGSARGDRQYL	424
MNGSGRGDRAAL	425
MNGSGRGDRAYL	426
MNGSGRGDRGAL	427

Amino Acid Sequence	SEQ ID NO:
MNGSGRGDRGSL	428
MNGSGRGDRGYL	429
MNGSGRGDRQAL	430
MNGSGRGDRQSL	431
MNGSGRGDRQYL	432
MNGSMRGDRQAL	433
MNGSMRGDRQSL	434
MNGSMRGDRQYL	435
MNGSSRGDRQYL	436
MNGSTRGDRAYL	437
MNGSTRGDRGAL	438
MNGSTRGDRGYL	439
MNGSTRGDRQAL	440
MNGSTRGDRQSL	441
MNGSTRGDRQYL	442
MNGSVRGDRGYL	443
MNGSVRGDRQAL	444
MNGSVRGDRQYL	445
MNSGMRGDRGAL	446
MNSSARGDRAAL	447
MNSSARGDRAYL	448
MNSSARGDRGSL	449
MNSSARGDRGYL	450
MNSSARGDRQAL	451
MNSSARGDRQSL	452
MNSSARGDRQYL	453
MNSSGRGDRAAL	454
MNSSGRGDRASL	455
MNSSGRGDRAYL	456
MNSSGRGDRGAL	457
MNSSGRGDRGSL	458
MNSSGRGDRQAL	459
MNSSGRGDRQSL	460
MNSSGRGDRQYL	461
MNSSMRGDRAAL	462
MNSSMRGDRASL	463
MNSSMRGDRAYL	464
MNSSMRGDRGSL	465

Amino Acid Sequence	SEQ ID NO:
MNSSMRGDRQAL	466
MNSSMRGDRQSL	467
MNSSMRGDRQYL	468
MNSSSRGDRAAL	469
MNSSTRGDRAAL	470
MNSSTRGDRASL	471
MNSSTRGDRQAL	472
MNSSTRGDRQSL	473
MNSSTRGDRQYL	474
MNSSVRGDRAAL	475
MNSSVRGDRAYL	476
MNSSVRGDRQAL	477
MNSSVRGDRQSL	478
MNSSVRGDRQYL	479
MNTSARGDRAYL	480
MNTSGRGDRAAL	481
MNTSGRGDRQAL	482
MNTSGRGDRQYL	483
MNTSMRGDRAYL	484
MNTSTRGDRQYL	485
MNYPHRGDRGAL	486
MNYSARGDRAAL	487
MNYSARGDRGYL	488
MNYSARGDRQAL	489
MNYSARGDRQSL	490
MNYSARGDRQYL	491
MNYSGRGDRAAL	492
MNYSGRGDRASL	493
MNYSGRGDRQAL	494
MNYSGRGDRQSL	495
MNYSSRGDRQAL	496
MNYSTRGDRAAL	497
MNYSTRGDRQAL	498
MNYSTRGDRQSL	499
MNYSTRGDRQYL	500
NNSGMRGDRGAL	501
QNSGMRGDRGAL	502
SNASARGDRAAL	503

Amino Acid Sequence	SEQ ID NO:
SNASARGDRASL	504
SNASARGDRAYL	505
SNASARGDRGAL	506
SNASARGDRGSL	507
SNASARGDRGYL	508
SNASARGDRQAL	509
SNASARGDRQSL	510
SNASARGDRQYL	511
SNASGRGDRAAL	512
SNASGRGDRASL	513
SNASGRGDRAYL	514
SNASGRGDRGAL	515
SNASGRGDRGSL	516
SNASGRGDRGYL	517
SNASGRGDRQAL	518
SNASGRGDRQSL	519
SNASGRGDRQYL	520
SNASIRGDRQLL	521
SNASMRGDRAAL	522
SNASMRGDRASL	523
SNASMRGDRGAL	524
SNASMRGDRGSL	525
SNASMRGDRQAL	526
SNASMRGDRQSL	527
SNASSRGDRAAL	528
SNASSRGDRASL	529
SNASSRGDRAYL	530
SNASSRGDRGAL	531
SNASSRGDRGSL	532
SNASSRGDRQAL	533
SNASSRGDRQSL	534
SNASSRGDRQYL	535
SNASTRGDRAAL	536
SNASTRGDRASL	537
SNASTRGDRAYL	538
SNASTRGDRGAL	539
SNASTRGDRGSL	540
SNASTRGDRGYL	541

Amino Acid Sequence	SEQ ID NO:
SNASTRGDRQAL	542
SNASTRGDRQSL	543
SNASTRGDRQYL	544
SNASVRGDRAAL	545
SNASVRGDRASL	546
SNASVRGDRAYL	547
SNASVRGDRGSL	548
SNASVRGDRGYL	549
SNASVRGDRQAL	550
SNASVRGDRQSL	551
SNASVRGDRQYL	552
SNGNFRGDRQNL	553
SNGNVRGDRGML	554
SNGSARGDRGYL	555
SNGSARGDRQAL	556
SNGSARGDRQSL	557
SNGSARGDRQYL	558
SNGSGRGDRASL	559
SNGSGRGDRAYL	560
SNGSGRGDRGAL	561
SNGSGRGDRGSL	562
SNGSGRGDRGYL	563
SNGSGRGDRQAL	564
SNGSGRGDRQSL	565
SNGSGRGDRQYL	566
SNGSMRGDRAYL	567
SNGSMRGDRGYL	568
SNGSMRGDRQAL	569
SNGSMRGDRQSL	570
SNGSMRGDRQYL	571
SNGSSRGDRGYL	572
SNGSSRGDRQYL	573
SNGSTRGDRAAL	574
SNGSTRGDRASL	575
SNGSTRGDRGAL	576
SNGSTRGDRGYL	577
SNGSTRGDRQAL	578
SNGSTRGDRQSL	579

Amino Acid Sequence	SEQ ID NO:
SNGSTRGDRQYL	580
SNGSVRGDRGYL	581
SNGSVRGDRQAL	582
SNGSVRGDRQSL	583
SNGSVRGDRQYL	584
SNSGMRGDRGAL	585
SNSSARGDRAYL	586
SNSSARGDRGAL	587
SNSSARGDRGIL	588
SNSSARGDRGSL	589
SNSSARGDRGYL	590
SNSSARGDRQAL	591
SNSSARGDRQSL	592
SNSSARGDRQYL	593
SNSSGRGDRAAL	594
SNSSGRGDRAYL	595
SNSSGRGDRGAL	596
SNSSGRGDRGSL	597
SNSSGRGDRGYL	598
SNSSGRGDRQAL	599
SNSSGRGDRQSL	600
SNSSGRGDRQYL	601
SNSSMRGDRAAL	602
SNSSMRGDRASL	603
SNSSMRGDRAYL	604
SNSSMRGDRGSL	605
SNSSMRGDRQAL	606
SNSSMRGDRQSL	607
SNSSMRGDRQYL	608
SNSSSRGDRAAL	609
SNSSSRGDRGSL	610
SNSSSRGDRQAL	611
SNSSSRGDRQSL	612
SNSSSRGDRQYL	613
SNSSTRGDRAAL	614
SNSSTRGDRASL	615
SNSSTRGDRGSL	616
SNSSTRGDRGYL	617

Amino Acid Sequence	SEQ ID NO:
SNSSTRGDRQAL	618
SNSSTRGDRQSL	619
SNSSTRGDRQYL	620
SNSSVRGDRQAL	621
SNSSVRGDRQSL	622
SNSSVRGDRQYL	623
SNTSARGDRAAL	624
SNTSARGDRGAL	625
SNTSARGDRGSL	626
SNTSARGDRGYL	627
SNTSARGDRQAL	628
SNTSARGDRQSL	629
SNTSARGDRQYL	630
SNTSGRGDRAAL	631
SNTSGRGDRASL	632
SNTSGRGDRGAL	633
SNTSGRGDRGSL	634
SNTSGRGDRGYL	635
SNTSGRGDRQAL	636
SNTSGRGDRQSL	637
SNTSGRGDRQYL	638
SNTSMRGDRAYL	639
SNTSMRGDRGSL	640
SNTSMRGDRQAL	641
SNTSMRGDRQSL	642
SNTSSRGDRQAL	643
SNTSTRGDRQAL	644
SNTSTRGDRQSL	645
SNTSTRGDRQYL	646
SNTSVRGDRAYL	647
SNTSVRGDRQAL	648
SNTSVRGDRQSL	649
SNTSVRGDRQYL	650
SNYPHRGDRGAL	651
SNYSARGDRAAL	652
SNYSARGDRASL	653
SNYSARGDRGAL	654
SNYSARGDRGSL	655

Amino Acid Sequence	SEQ ID NO:
SNYSARGDRQAL	656
SNYSARGDRQSL	657
SNYSARGDRQYL	658
SNYSGRGDRAAL	659
SNYSGRGDRGAL	660
SNYSGRGDRGSL	661
SNYSGRGDRQAL	662
SNYSGRGDRQSL	663
SNYSMRGDRAAL	664
SNYSMRGDRASL	665
SNYSMRGDRGSL	666
SNYSMRGDRQAL	667
SNYSMRGDRQSL	668
SNYSSRGDRGAL	669
SNYSSRGDRGSL	670
SNYSSRGDRQAL	671
SNYSSRGDRQSL	672
SNYSTRGDRAAL	673
SNYSTRGDRGAL	674
SNYSTRGDRGSL	675
SNYSTRGDRQAL	676
SNYSTRGDRQSL	677
SNYSTRGDRQYL	678
SNYSVRGDRQAL	679
SNYSVRGDRQSL	680
SNYSVRGDRQYL	681
TNSGMRGDRGAL	682
VNASARGDRAAL	683
VNASARGDRGAL	684
VNASARGDRGSL	685
VNASARGDRGYL	686
VNASARGDRQAL	687
VNASARGDRQYL	688
VNASGRGDRAAL	689
VNASGRGDRAYL	690
VNASGRGDRGAL	691
VNASGRGDRGSL	692
VNASGRGDRGYL	693

Amino Acid Sequence	SEQ ID NO:
VNASGRGDRQAL	694
VNASGRGDRQYL	695
VNASMRGDRAYL	696
VNASMRGDRQAL	697
VNASMRGDRQSL	698
VNASSRGDRGSL	699
VNASSRGDRQAL	700
VNASSRGDRQYL	701
VNASTRGDRAAL	702
VNASTRGDRGAL	703
VNASTRGDRGSL	704
VNASTRGDRGYL	705
VNASTRGDRQAL	706
VNASTRGDRQSL	707
VNASTRGDRQYL	708
VNASVRGDRGSL	709
VNASVRGDRGYL	710
VNASVRGDRQAL	711
VNASVRGDRQSL	712
VNASVRGDRQYL	713
VNGNFRGDRQNL	714
VNGNVRGDRGML	715
VNGSARGDRAYL	716
VNGSARGDRGAL	717
VNGSARGDRGSL	718
VNGSARGDRGYL	719
VNGSARGDRQAL	720
VNGSARGDRQSL	721
VNGSARGDRQYL	722
VNGSGRGDRAAL	723
VNGSGRGDRASL	724
VNGSGRGDRAYL	725
VNGSGRGDRGAL	726
VNGSGRGDRGSL	727
VNGSGRGDRGYL	728
VNGSGRGDRQAL	729
VNGSGRGDRQSL	730
VNGSGRGDRQYL	731

Amino Acid Sequence	SEQ ID NO:
VNGSMRGDRGYL	732
VNGSMRGDRQAL	733
VNGSMRGDRQSL	734
VNGSMRGDRQYL	735
VNGSSRGDRAYL	736
VNGSSRGDRGAL	737
VNGSSRGDRGSL	738
VNGSSRGDRQAL	739
VNGSSRGDRQSL	740
VNGSSRGDRQYL	741
VNGSTRGDRAAL	742
VNGSTRGDRAYL	743
VNGSTRGDRGAL	744
VNGSTRGDRGYL	745
VNGSTRGDRQAL	746
VNGSTRGDRQSL	747
VNGSTRGDRQYL	748
VNGSVRGDRAYL	749
VNGSVRGDRQAL	750
VNGSVRGDRQSL	751
VNGSVRGDRQYL	752
VNSGMRGDRGAL	753
VNSSARGDRGAL	754
VNSSARGDRGSL	755
VNSSARGDRGYL	756
VNSSARGDRQAL	757
VNSSARGDRQSL	758
VNSSARGDRQYL	759
VNSSGRGDRAAL	760
VNSSGRGDRAYL	761
VNSSGRGDRGAL	762
VNSSGRGDRQAL	763
VNSSGRGDRQSL	764
VNSSGRGDRQYL	765
VNSSMRGDRAYL	766
VNSSMRGDRQAL	767
VNSSMRGDRQSL	768
VNSSMRGDRQYL	769

Amino Acid Sequence	SEQ ID NO:
VNSSSRGDRGSL	770
VNSSSRGDRQAL	771
VNSSSRGDRQSL	772
VNSSTRGDRGAL	773
VNSSTRGDRQAL	774
VNSSTRGDRQSL	775
VNSSTRGDRQYL	776
VNSSVRGDRAYL	777
VNSSVRGDRQAL	778
VNSSVRGDRQSL	779
VNTSARGDRAYL	780
VNTSGRGDRAAL	781
VNTSGRGDRGAL	782
VNTSGRGDRGSL	783
VNTSGRGDRGYL	784
VNTSGRGDRQAL	785
VNTSGRGDRQSL	786
VNTSGRGDRQYL	787
VNTSTRGDRAYL	788
VNYPHRGDRGAL	789
VNYSARGDRGAL	790
VNYSARGDRGSL	791
VNYSARGDRQAL	792
VNYSARGDRQSL	793
VNYSGRGDRAAL	794
VNYSGRGDRGAL	795
VNYSGRGDRQAL	796
VNYSGRGDRQSL	797
VNYSGRGDRQYL	798
VNYSSRGDRGSL	799
VNYSSRGDRGYL	800

Table 1b: Split Amino Acid Sequences from

Table 1a

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
ANASA	801	RGDRAAL	1600
ANASA	802	RGDRGSL	1601
ANASA	803	RGDRGYL	1602
ANASA	804	RGDRQAL	1603
ANASA	805	RGDRQSL	1604
ANASA	806	RGDRQYL	1605
ANASG	807	RGDRAAL	1606
ANASG	808	RGDRASL	1607
ANASG	809	RGDRAYL	1608
ANASG	810	RGDRGAL	1609
ANASG	811	RGDRGSL	1610
ANASG	812	RGDRGYL	1611
ANASG	813	RGDRQAL	1612
ANASG	814	RGDRQSL	1613
ANASG	815	RGDRQYL	1614
ANASM	816	RGDRAAL	1615
ANASM	817	RGDRASL	1616
ANASM	818	RGDRAYL	1617
ANASM	819	RGDRQAL	1618
ANASM	820	RGDRQSL	1619
ANASS	821	RGDRGSL	1620
ANASS	822	RGDRGYL	1621
ANASS	823	RGDRQAL	1622
ANASS	824	RGDRQSL	1623
ANASS	825	RGDRQYL	1624
ANAST	826	RGDRASL	1625
ANAST	827	RGDRAYL	1626
ANAST	828	RGDRGAL	1627
ANAST	829	RGDRGSL	1628
ANAST	830	RGDRGYL	1629
ANAST	831	RGDRQAL	1630
ANAST	832	RGDRQSL	1631
ANAST	833	RGDRQYL	1632
ANASV	834	RGDRASL	1633
ANASV	835	RGDRGYL	1634
ANASV	836	RGDRQAL	1635

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
ANASV	837	RGDRQSL	1636
ANASV	838	RGDRQYL	1637
ANGNF	839	RGDRQNL	1638
ANGNV	840	RGDRGML	1639
ANGSA	841	RGDRAYL	1640
ANGSA	842	RGDRGSL	1641
ANGSA	843	RGDRGYL	1642
ANGSA	844	RGDRQAL	1643
ANGSA	845	RGDRQYL	1644
ANGSG	846	RGDRAAL	1645
ANGSG	847	RGDRAYL	1646
ANGSG	848	RGDRGAL	1647
ANGSG	849	RGDRGSL	1648
ANGSG	850	RGDRGYL	1649
ANGSG	851	RGDRQAL	1650
ANGSG	852	RGDRQSL	1651
ANGSG	853	RGDRQYL	1652
ANGSM	854	RGDRGYL	1653
ANGSM	855	RGDRQAL	1654
ANGSM	856	RGDRQSL	1655
ANGSM	857	RGDRQYL	1656
ANGSS	858	RGDRAYL	1657
ANGSS	859	RGDRGSL	1658
ANGSS	860	RGDRGYL	1659
ANGSS	861	RGDRQAL	1660
ANGSS	862	RGDRQSL	1661
ANGSS	863	RGDRQYL	1662
ANGST	864	RGDRAAL	1663
ANGST	865	RGDRAYL	1664
ANGST	866	RGDRGAL	1665
ANGST	867	RGDRGYL	1666
ANGST	868	RGDRQAL	1667
ANGST	869	RGDRQSL	1668
ANGST	870	RGDRQYL	1669
ANGSV	871	RGDRAYL	1670
ANGSV	872	RGDRGYL	1671
ANGSV	873	RGDRQAL	1672
ANGSV	874	RGDRQYL	1673
ANSKM	875	RGDRGAL	1674

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
ANSSA	876	RGDRGAL	1675
ANSSA	877	RGDRGSL	1676
ANSSA	878	RGDRGYL	1677
ANSSA	879	RGDRQAL	1678
ANSSA	880	RGDRQSL	1679
ANSSA	881	RGDRQYL	1680
ANSSG	882	RGDRAAL	1681
ANSSG	883	RGDRASL	1682
ANSSG	884	RGDRAYL	1683
ANSSG	885	RGDRGAL	1684
ANSSG	886	RGDRGSL	1685
ANSSG	887	RGDRGYL	1686
ANSSG	888	RGDRQAL	1687
ANSSG	889	RGDRQSL	1688
ANSSG	890	RGDRQYL	1689
ANSSM	891	RGDRAYL	1690
ANSSM	892	RGDRQAL	1691
ANSSM	893	RGDRQSL	1692
ANSSM	894	RGDRQYL	1693
ANSSS	895	RGDRGSL	1694
ANSSS	896	RGDRGYL	1695
ANSSS	897	RGDRQAL	1696
ANSSS	898	RGDRQSL	1697
ANSSS	899	RGDRQYL	1698
ANSST	900	RGDRAAL	1699
ANSST	901	RGDRGSL	1700
ANSST	902	RGDRQAL	1701
ANSST	903	RGDRQSL	1702
ANSST	904	RGDRQYL	1703
ANSSV	905	RGDRAYL	1704
ANSSV	906	RGDRGYL	1705
ANSSV	907	RGDRQAL	1706
ANSSV	908	RGDRQSL	1707
ANSSV	909	RGDRQYL	1708
ANTSA	910	RGDRAYL	1709
ANTSA	911	RGDRGAL	1710
ANTSA	912	RGDRGSL	1711
ANTSA	913	RGDRGYL	1712
ANTSA	914	RGDRQAL	1713

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
ANTSA	915	RGDRQYL	1714
ANTSG	916	RGDRASL	1715
ANTSG	917	RGDRGAL	1716
ANTSG	918	RGDRGSL	1717
ANTSG	919	RGDRGYL	1718
ANTSG	920	RGDRQAL	1719
ANTSG	921	RGDRQSL	1720
ANTSG	922	RGDRQYL	1721
ANTSM	923	RGDRAYL	1722
ANTSM	924	RGDRQAL	1723
ANTSM	925	RGDRQSL	1724
ANTSM	926	RGDRQYL	1725
ANTSS	927	RGDRGSL	1726
ANTSS	928	RGDRQAL	1727
ANTSS	929	RGDRQYL	1728
ANTST	930	RGDRAAL	1729
ANTST	931	RGDRQAL	1730
ANTST	932	RGDRQSL	1731
ANTST	933	RGDRQYL	1732
ANTSV	934	RGDRAYL	1733
ANTSV	935	RGDRGSL	1734
ANTSV	936	RGDRQAL	1735
ANTSV	937	RGDRQSL	1736
ANTSV	938	RGDRQYL	1737
ANYPH	939	RGDRGAL	1738
ANYSA	940	RGDRAAL	1739
ANYSA	941	RGDRASL	1740
ANYSA	942	RGDRGAL	1741
ANYSA	943	RGDRGSL	1742
ANYSA	944	RGDRQAL	1743
ANYSA	945	RGDRQSL	1744
ANYSG	946	RGDRAAL	1745
ANYSG	947	RGDRASL	1746
ANYSG	948	RGDRGAL	1747
ANYSG	949	RGDRGSL	1748
ANYSG	950	RGDRQAL	1749
ANYSG	951	RGDRQSL	1750
ANYSG	952	RGDRQYL	1751
ANYSM	953	RGDRAAL	1752

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
ANYSM	954	RGDRASL	1753
ANYSM	955	RGDRAYL	1754
ANYSM	956	RGDRQAL	1755
ANYSM	957	RGDRQSL	1756
ANYSS	958	RGDRGAL	1757
ANYSS	959	RGDRGSL	1758
ANYSS	960	RGDRQAL	1759
ANYSS	961	RGDRQSL	1760
ANYSS	962	RGDRQYL	1761
ANYST	963	RGDRQAL	1762
ANYST	964	RGDRQSL	1763
ANYST	965	RGDRQYL	1764
ANYSV	966	RGDRQAL	1765
ANYSV	967	RGDRQSL	1766
ANYSV	968	RGDRQYL	1767
FNSGM	969	RGDRGAL	1768
GNSGM	970	RGDRGAL	1769
HNSGM	971	RGDRGAL	1770
LNASA	972	RGDRAAL	1771
LNASA	973	RGDRASL	1772
LNASA	974	RGDRAYL	1773
LNASA	975	RGDRGAL	1774
LNASA	976	RGDRGSL	1775
LNASA	977	RGDRGYL	1776
LNASA	978	RGDRQAL	1777
LNASA	979	RGDRQSL	1778
LNASA	980	RGDRQYL	1779
LNASG	981	RGDRAAL	1780
LNASG	982	RGDRASL	1781
LNASG	983	RGDRAYL	1782
LNASG	984	RGDRGAL	1783
LNASG	985	RGDRGSL	1784
LNASG	986	RGDRGYL	1785
LNASG	987	RGDRQAL	1786
LNASG	988	RGDRQSL	1787
LNASG	989	RGDRQYL	1788
LNASI	990	RGDRQLL	1789
LNASM	991	RGDRAAL	1790
LNASM	992	RGDRASL	1791

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
LNASM	993	RGDRAYL	1792
LNASM	994	RGDRGAL	1793
LNASM	995	RGDRGSL	1794
LNASM	996	RGDRGYL	1795
LNASM	997	RGDRQAL	1796
LNASM	998	RGDRQSL	1797
LNASM	999	RGDRQYL	1798
LNASS	1000	RGDRAAL	1799
LNASS	1001	RGDRASL	1800
LNASS	1002	RGDRGAL	1801
LNASS	1003	RGDRGSL	1802
LNASS	1004	RGDRQAL	1803
LNASS	1005	RGDRQSL	1804
LNASS	1006	RGDRQYL	1805
LNAST	1007	RGDRAAL	1806
LNAST	1008	RGDRASL	1807
LNAST	1009	RGDRGAL	1808
LNAST	1010	RGDRGSL	1809
LNAST	1011	RGDRGYL	1810
LNAST	1012	RGDRQAL	1811
LNAST	1013	RGDRQSL	1812
LNAST	1014	RGDRQYL	1813
LNASV	1015	RGDRAAL	1814
LNASV	1016	RGDRASL	1815
LNASV	1017	RGDRGAL	1816
LNASV	1018	RGDRGSL	1817
LNASV	1019	RGDRQAL	1818
LNASV	1020	RGDRQSL	1819
LNASV	1021	RGDRQYL	1820
LNGNF	1022	RGDRQNL	1821
LNGNV	1023	RGDRGML	1822
LNGSA	1024	RGDRAAL	1823
LNGSA	1025	RGDRAYL	1824
LNGSA	1026	RGDRGSL	1825
LNGSA	1027	RGDRGYL	1826
LNGSA	1028	RGDRQAL	1827
LNGSA	1029	RGDRQSL	1828
LNGSA	1030	RGDRQYL	1829
LNGSG	1031	RGDRAAL	1830

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
LNGSG	1032	RGDRAYL	1831
LNGSG	1033	RGDRGAL	1832
LNGSG	1034	RGDRGSL	1833
LNGSG	1035	RGDRGYL	1834
LNGSG	1036	RGDRQAL	1835
LNGSG	1037	RGDRQYL	1836
LNGSM	1038	RGDRASL	1837
LNGSM	1039	RGDRGYL	1838
LNGSM	1040	RGDRQAL	1839
LNGSM	1041	RGDRQSL	1840
LNGSM	1042	RGDRQYL	1841
LNGSS	1043	RGDRAAL	1842
LNGSS	1044	RGDRAYL	1843
LNGSS	1045	RGDRGAL	1844
LNGSS	1046	RGDRQYL	1845
LNGST	1047	RGDRAAL	1846
LNGST	1048	RGDRASL	1847
LNGST	1049	RGDRAYL	1848
LNGST	1050	RGDRGAL	1849
LNGST	1051	RGDRGSL	1850
LNGST	1052	RGDRGYL	1851
LNGST	1053	RGDRQAL	1852
LNGST	1054	RGDRQSL	1853
LNGST	1055	RGDRQYL	1854
LNGSV	1056	RGDRASL	1855
LNGSV	1057	RGDRAYL	1856
LNGSV	1058	RGDRGYL	1857
LNGSV	1059	RGDRQAL	1858
LNGSV	1060	RGDRQSL	1859
LNGSV	1061	RGDRQYL	1860
LNSGM	1062	RGDRGAL	1861
LNSSA	1063	RGDRASL	1862
LNSSA	1064	RGDRAYL	1863
LNSSA	1065	RGDRGAL	1864
LNSSA	1066	RGDRGIL	1865
LNSSA	1067	RGDRGSL	1866
LNSSA	1068	RGDRGYL	1867
LNSSA	1069	RGDRQAL	1868
LNSSA	1070	RGDRQSL	1869

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
LNSSA	1071	RGDRQYL	1870
LNSSG	1072	RGDRAAL	1871
LNSSG	1073	RGDRASL	1872
LNSSG	1074	RGDRAYL	1873
LNSSG	1075	RGDRGAL	1874
LNSSG	1076	RGDRGSL	1875
LNSSG	1077	RGDRGYL	1876
LNSSG	1078	RGDRQAL	1877
LNSSG	1079	RGDRQSL	1878
LNSSG	1080	RGDRQYL	1879
LNSSM	1081	RGDRAAL	1880
LNSSM	1082	RGDRASL	1881
LNSSM	1083	RGDRAYL	1882
LNSSM	1084	RGDRGAL	1883
LNSSM	1085	RGDRGSL	1884
LNSSM	1086	RGDRQAL	1885
LNSSM	1087	RGDRQSL	1886
LNSSM	1088	RGDRQYL	1887
LNSSS	1089	RGDRAAL	1888
LNSSS	1090	RGDRGAL	1889
LNSSS	1091	RGDRGSL	1890
LNSSS	1092	RGDRQAL	1891
LNSSS	1093	RGDRQSL	1892
LNSSS	1094	RGDRQYL	1893
LNSST	1095	RGDRAAL	1894
LNSST	1096	RGDRASL	1895
LNSST	1097	RGDRGAL	1896
LNSST	1098	RGDRGSL	1897
LNSST	1099	RGDRQAL	1898
LNSST	1100	RGDRQSL	1899
LNSST	1101	RGDRQYL	1900
LNSSV	1102	RGDRAAL	1901
LNSSV	1103	RGDRASL	1902
LNSSV	1104	RGDRGAL	1903
LNSSV	1105	RGDRGSL	1904
LNSSV	1106	RGDRGYL	1905
LNSSV	1107	RGDRQAL	1906
LNSSV	1108	RGDRQSL	1907
LNSSV	1109	RGDRQYL	1908

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
LNTSA	1110	RGDRAAL	1909
LNTSA	1111	RGDRASL	1910
LNTSA	1112	RGDRAYL	1911
LNTSA	1113	RGDRGAL	1912
LNTSA	1114	RGDRGSL	1913
LNTSA	1115	RGDRGYL	1914
LNTSA	1116	RGDRQAL	1915
LNTSA	1117	RGDRQSL	1916
LNTSA	1118	RGDRQYL	1917
LNTSG	1119	RGDRAAL	1918
LNTSG	1120	RGDRASL	1919
LNTSG	1121	RGDRAYL	1920
LNTSG	1122	RGDRGAL	1921
LNTSG	1123	RGDRGSL	1922
LNTSG	1124	RGDRGYL	1923
LNTSG	1125	RGDRQAL	1924
LNTSG	1126	RGDRQSL	1925
LNTSG	1127	RGDRQYL	1926
LNTSM	1128	RGDRAAL	1927
LNTSM	1129	RGDRASL	1928
LNTSM	1130	RGDRAYL	1929
LNTSM	1131	RGDRGAL	1930
LNTSM	1132	RGDRGSL	1931
LNTSM	1133	RGDRQAL	1932
LNTSM	1134	RGDRQSL	1933
LNTSM	1135	RGDRQYL	1934
LNTSS	1136	RGDRAAL	1935
LNTSS	1137	RGDRGSL	1936
LNTSS	1138	RGDRQAL	1937
LNTSS	1139	RGDRQSL	1938
LNTSS	1140	RGDRQYL	1939
LNTST	1141	RGDRAAL	1940
LNTST	1142	RGDRASL	1941
LNTST	1143	RGDRGAL	1942
LNTST	1144	RGDRGSL	1943
LNTST	1145	RGDRQAL	1944
LNTST	1146	RGDRQSL	1945
LNTST	1147	RGDRQYL	1946
LNTSV	1148	RGDRAAL	1947

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
LNTSV	1149	RGDRASL	1948
LNTSV	1150	RGDRAYL	1949
LNTSV	1151	RGDRGAL	1950
LNTSV	1152	RGDRGSL	1951
LNTSV	1153	RGDRQAL	1952
LNTSV	1154	RGDRQSL	1953
LNYPH	1155	RGDRGAL	1954
LNYS A	1156	RGDRGAL	1955
LNYS A	1157	RGDRGSL	1956
LNYS A	1158	RGDRQAL	1957
LNYS A	1159	RGDRQSL	1958
LNYS A	1160	RGDRQYL	1959
LNYS G	1161	RGDRAAL	1960
LNYS G	1162	RGDRASL	1961
LNYS G	1163	RGDRGAL	1962
LNYS G	1164	RGDRGSL	1963
LNYS G	1165	RGDRQAL	1964
LNYS G	1166	RGDRQSL	1965
LNYS G	1167	RGDRQYL	1966
LNYS M	1168	RGDRQAL	1967
LNYS M	1169	RGDRQSL	1968
LNYS S	1170	RGDRGAL	1969
LNYS S	1171	RGDRGSL	1970
LNYS S	1172	RGDRQAL	1971
LNYS S	1173	RGDRQSL	1972
LNYS S	1174	RGDRQYL	1973
LNYS T	1175	RGDRASL	1974
LNYS T	1176	RGDRGAL	1975
LNYS T	1177	RGDRGSL	1976
LNYS T	1178	RGDRQAL	1977
LNYS T	1179	RGDRQSL	1978
LNYS T	1180	RGDRQYL	1979
LNYS V	1181	RGDRQAL	1980
LNYS V	1182	RGDRQSL	1981
MNASA	1183	RGDRAAL	1982
MNASA	1184	RGDRAYL	1983
MNASA	1185	RGDRGSL	1984
MNASA	1186	RGDRGYL	1985
MNASA	1187	RGDRQAL	1986

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
MNASA	1188	RGDRQSL	1987
MNASA	1189	RGDRQYL	1988
MNASG	1190	RGDRAAL	1989
MNASG	1191	RGDRASL	1990
MNASG	1192	RGDRAYL	1991
MNASG	1193	RGDRGSL	1992
MNASG	1194	RGDRQAL	1993
MNASG	1195	RGDRQSL	1994
MNASG	1196	RGDRQYL	1995
MNASI	1197	RGDRQLL	1996
MNASM	1198	RGDRAAL	1997
MNASM	1199	RGDRAYL	1998
MNASM	1200	RGDRGAL	1999
MNASM	1201	RGDRQAL	2000
MNASM	1202	RGDRQSL	2001
MNASS	1203	RGDRAAL	2002
MNAST	1204	RGDRAAL	2003
MNAST	1205	RGDRASL	2004
MNAST	1206	RGDRAYL	2005
MNAST	1207	RGDRGAL	2006
MNAST	1208	RGDRGSL	2007
MNAST	1209	RGDRQAL	2008
MNAST	1210	RGDRQSL	2009
MNAST	1211	RGDRQYL	2010
MNASV	1212	RGDRAAL	2011
MNASV	1213	RGDRASL	2012
MNASV	1214	RGDRAYL	2013
MNASV	1215	RGDRQAL	2014
MNASV	1216	RGDRQSL	2015
MNASV	1217	RGDRQYL	2016
MNGNF	1218	RGDRQNL	2017
MNGSA	1219	RGDRAYL	2018
MNGSA	1220	RGDRGYL	2019
MNGSA	1221	RGDRQAL	2020
MNGSA	1222	RGDRQSL	2021
MNGSA	1223	RGDRQYL	2022
MNGSG	1224	RGDRAAL	2023
MNGSG	1225	RGDRAYL	2024
MNGSG	1226	RGDRGAL	2025

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
MNGSG	1227	RGDRGSL	2026
MNGSG	1228	RGDRGYL	2027
MNGSG	1229	RGDRQAL	2028
MNGSG	1230	RGDRQSL	2029
MNGSG	1231	RGDRQYL	2030
MNGSM	1232	RGDRQAL	2031
MNGSM	1233	RGDRQSL	2032
MNGSM	1234	RGDRQYL	2033
MNGSS	1235	RGDRQYL	2034
MNGST	1236	RGDRAYL	2035
MNGST	1237	RGDRGAL	2036
MNGST	1238	RGDRGYL	2037
MNGST	1239	RGDRQAL	2038
MNGST	1240	RGDRQSL	2039
MNGST	1241	RGDRQYL	2040
MNGSV	1242	RGDRGYL	2041
MNGSV	1243	RGDRQAL	2042
MNGSV	1244	RGDRQYL	2043
MNSGM	1245	RGDRGAL	2044
MNSSA	1246	RGDRAAL	2045
MNSSA	1247	RGDRAYL	2046
MNSSA	1248	RGDRGSL	2047
MNSSA	1249	RGDRGYL	2048
MNSSA	1250	RGDRQAL	2049
MNSSA	1251	RGDRQSL	2050
MNSSA	1252	RGDRQYL	2051
MNSSG	1253	RGDRAAL	2052
MNSSG	1254	RGDRASL	2053
MNSSG	1255	RGDRAYL	2054
MNSSG	1256	RGDRGAL	2055
MNSSG	1257	RGDRGSL	2056
MNSSG	1258	RGDRQAL	2057
MNSSG	1259	RGDRQSL	2058
MNSSG	1260	RGDRQYL	2059
MNSSM	1261	RGDRAAL	2060
MNSSM	1262	RGDRASL	2061
MNSSM	1263	RGDRAYL	2062
MNSSM	1264	RGDRGSL	2063
MNSSM	1265	RGDRQAL	2064

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
MNSSM	1266	RGDRQSL	2065
MNSSM	1267	RGDRQYL	2066
MNSSS	1268	RGDRAAL	2067
MNSST	1269	RGDRAAL	2068
MNSST	1270	RGDRASL	2069
MNSST	1271	RGDRQAL	2070
MNSST	1272	RGDRQSL	2071
MNSST	1273	RGDRQYL	2072
MNSSV	1274	RGDRAAL	2073
MNSSV	1275	RGDRAYL	2074
MNSSV	1276	RGDRQAL	2075
MNSSV	1277	RGDRQSL	2076
MNSSV	1278	RGDRQYL	2077
MNTSA	1279	RGDRAYL	2078
MNTSG	1280	RGDRAAL	2079
MNTSG	1281	RGDRQAL	2080
MNTSG	1282	RGDRQYL	2081
MNTSM	1283	RGDRAYL	2082
MNTST	1284	RGDRQYL	2083
MNYPH	1285	RGDRGAL	2084
MNYSA	1286	RGDRAAL	2085
MNYSA	1287	RGDRGYL	2086
MNYSA	1288	RGDRQAL	2087
MNYSA	1289	RGDRQSL	2088
MNYSG	1290	RGDRAAL	2089
MNYSG	1291	RGDRASL	2090
MNYSG	1292	RGDRGSL	2091
MNYSG	1293	RGDRQAL	2092
MNYSG	1294	RGDRQSL	2093
MNYSS	1295	RGDRQAL	2094
MNYST	1296	RGDRAAL	2095
MNYST	1297	RGDRQAL	2096
MNYST	1298	RGDRQSL	2097
MNYST	1299	RGDRQYL	2098
NNSGM	1300	RGDRGAL	2099
QNSGM	1301	RGDRGAL	2100
SNASA	1302	RGDRAAL	2101
SNASA	1303	RGDRASL	2102
SNASA	1304	RGDRAYL	2103

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
SNASA	1305	RGDRGAL	2104
SNASA	1306	RGDRGSL	2105
SNASA	1307	RGDRGYL	2106
SNASA	1308	RGDRQAL	2107
SNASA	1309	RGDRQSL	2108
SNASA	1310	RGDRQYL	2109
SNASG	1311	RGDRAAL	2110
SNASG	1312	RGDRASL	2111
SNASG	1313	RGDRAYL	2112
SNASG	1314	RGDRGAL	2113
SNASG	1315	RGDRGSL	2114
SNASG	1316	RGDRGYL	2115
SNASG	1317	RGDRQAL	2116
SNASG	1318	RGDRQSL	2117
SNASG	1319	RGDRQYL	2118
SNASI	1320	RGDRQLL	2119
SNASM	1321	RGDRAAL	2120
SNASM	1322	RGDRASL	2121
SNASM	1323	RGDRGAL	2122
SNASM	1324	RGDRGSL	2123
SNASM	1325	RGDRQAL	2124
SNASM	1326	RGDRQSL	2125
SNASS	1327	RGDRAAL	2126
SNASS	1328	RGDRASL	2127
SNASS	1329	RGDRAYL	2128
SNASS	1330	RGDRGAL	2129
SNASS	1331	RGDRGSL	2130
SNASS	1332	RGDRQAL	2131
SNASS	1333	RGDRQSL	2132
SNASS	1334	RGDRQYL	2133
SNAST	1335	RGDRAAL	2134
SNAST	1336	RGDRASL	2135
SNAST	1337	RGDRAYL	2136
SNAST	1338	RGDRGAL	2137
SNAST	1339	RGDRGSL	2138
SNAST	1340	RGDRGYL	2139
SNAST	1341	RGDRQAL	2140
SNAST	1342	RGDRQSL	2141
SNAST	1343	RGDRQYL	2142

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
SNASV	1344	RGDRAAL	2143
SNASV	1345	RGDRASL	2144
SNASV	1346	RGDRAYL	2145
SNASV	1347	RGDRGSL	2146
SNASV	1348	RGDRGYL	2147
SNASV	1349	RGDRQAL	2148
SNASV	1350	RGDRQSL	2149
SNASV	1351	RGDRQYL	2150
SNGNF	1352	RGDRQNL	2151
SNGNV	1353	RGDRGML	2152
SNGSA	1354	RGDRGYL	2153
SNGSA	1355	RGDRQAL	2154
SNGSA	1356	RGDRQSL	2155
SNGSA	1357	RGDRQYL	2156
SNGSG	1358	RGDRASL	2157
SNGSG	1359	RGDRAYL	2158
SNGSG	1360	RGDRGAL	2159
SNGSG	1361	RGDRGSL	2160
SNGSG	1362	RGDRGYL	2161
SNGSG	1363	RGDRQAL	2162
SNGSG	1364	RGDRQSL	2163
SNGSG	1365	RGDRQYL	2164
SNGSM	1366	RGDRAYL	2165
SNGSM	1367	RGDRGYL	2166
SNGSM	1368	RGDRQAL	2167
SNGSM	1369	RGDRQSL	2168
SNGSM	1370	RGDRQYL	2169
SNGSS	1371	RGDRGYL	2170
SNGSS	1372	RGDRQYL	2171
SNGST	1373	RGDRAAL	2172
SNGST	1374	RGDRASL	2173
SNGST	1375	RGDRGAL	2174
SNGST	1376	RGDRGYL	2175
SNGST	1377	RGDRQAL	2176
SNGST	1378	RGDRQSL	2177
SNGST	1379	RGDRQYL	2178
SNGSV	1380	RGDRGYL	2179
SNGSV	1381	RGDRQAL	2180
SNGSV	1382	RGDRQSL	2181

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
SNGSV	1383	RGDRQYL	2182
SNSGM	1384	RGDRGAL	2183
SNSSA	1385	RGDRAYL	2184
SNSSA	1386	RGDRGAL	2185
SNSSA	1387	RGDRGIL	2186
SNSSA	1388	RGDRGSL	2187
SNSSA	1389	RGDRGYL	2188
SNSSA	1390	RGDRQAL	2189
SNSSA	1391	RGDRQSL	2190
SNSSA	1392	RGDRQYL	2191
SNSSG	1393	RGDRAAL	2192
SNSSG	1394	RGDRAYL	2193
SNSSG	1395	RGDRGAL	2194
SNSSG	1396	RGDRGSL	2195
SNSSG	1397	RGDRGYL	2196
SNSSG	1398	RGDRQAL	2197
SNSSG	1399	RGDRQSL	2198
SNSSG	1400	RGDRQYL	2199
SNSSM	1401	RGDRAAL	2200
SNSSM	1402	RGDRASL	2201
SNSSM	1403	RGDRAYL	2202
SNSSM	1404	RGDRGSL	2203
SNSSM	1405	RGDRQAL	2204
SNSSM	1406	RGDRQSL	2205
SNSSM	1407	RGDRQYL	2206
SNSSS	1408	RGDRAAL	2207
SNSSS	1409	RGDRGSL	2208
SNSSS	1410	RGDRQAL	2209
SNSSS	1411	RGDRQSL	2210
SNSSS	1412	RGDRQYL	2211
SNSST	1413	RGDRAAL	2212
SNSST	1414	RGDRASL	2213
SNSST	1415	RGDRGSL	2214
SNSST	1416	RGDRGYL	2215
SNSST	1417	RGDRQAL	2216
SNSST	1418	RGDRQSL	2217
SNSST	1419	RGDRQYL	2218
SNSSV	1420	RGDRQAL	2219
SNSSV	1421	RGDRQSL	2220

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
SNSSV	1422	RGDRQYL	2221
SNTSA	1423	RGDRAAL	2222
SNTSA	1424	RGDRGAL	2223
SNTSA	1425	RGDRGSL	2224
SNTSA	1426	RGDRGYL	2225
SNTSA	1427	RGDRQAL	2226
SNTSA	1428	RGDRQSL	2227
SNTSA	1429	RGDRQYL	2228
SNTSG	1430	RGDRAAL	2229
SNTSG	1431	RGDRASL	2230
SNTSG	1432	RGDRGAL	2231
SNTSG	1433	RGDRGSL	2232
SNTSG	1434	RGDRGYL	2233
SNTSG	1435	RGDRQAL	2234
SNTSG	1436	RGDRQSL	2235
SNTSG	1437	RGDRQYL	2236
SNTSM	1438	RGDRAYL	2237
SNTSM	1439	RGDRGSL	2238
SNTSM	1440	RGDRQAL	2239
SNTSM	1441	RGDRQSL	2240
SNTSS	1442	RGDRQAL	2241
SNTST	1443	RGDRQAL	2242
SNTST	1444	RGDRQSL	2243
SNTST	1445	RGDRQYL	2244
SNTSV	1446	RGDRAYL	2245
SNTSV	1447	RGDRQAL	2246
SNTSV	1448	RGDRQSL	2247
SNTSV	1449	RGDRQYL	2248
SNYPH	1450	RGDRGAL	2249
SNYSA	1451	RGDRAAL	2250
SNYSA	1452	RGDRASL	2251
SNYSA	1453	RGDRGAL	2252
SNYSA	1454	RGDRGSL	2253
SNYSA	1455	RGDRQAL	2254
SNYSA	1456	RGDRQSL	2255
SNYSA	1457	RGDRQYL	2256
SNYSG	1458	RGDRAAL	2257
SNYSG	1459	RGDRGAL	2258
SNYSG	1460	RGDRGSL	2259

5 aa subs-titution	SEQ ID NO	7 aa insert	SEQ ID NO
SNYSG	1461	RGDRQAL	2260
SNYSG	1462	RGDRQSL	2261
SNYSM	1463	RGDRAAL	2262
SNYSM	1464	RGDRASL	2263
SNYSM	1465	RGDRGSL	2264
SNYSM	1466	RGDRQAL	2265
SNYSM	1467	RGDRQSL	2266
SNYSS	1468	RGDRGAL	2267
SNYSS	1469	RGDRGSL	2268
SNYSS	1470	RGDRQAL	2269
SNYSS	1471	RGDRQSL	2270
SNYST	1472	RGDRAAL	2271
SNYST	1473	RGDRGAL	2272
SNYST	1474	RGDRGSL	2273
SNYST	1475	RGDRQAL	2274
SNYST	1476	RGDRQSL	2275
SNYST	1477	RGDRQYL	2276
SNYSV	1478	RGDRQAL	2277
SNYSV	1479	RGDRQSL	2278
SNYSV	1480	RGDRQYL	2279
TNSGM	1481	RGDRGAL	2280
VNASA	1482	RGDRAAL	2281
VNASA	1483	RGDRGAL	2282
VNASA	1484	RGDRGSL	2283
VNASA	1485	RGDRGYL	2284
VNASA	1486	RGDRQAL	2285
VNASA	1487	RGDRQYL	2286
VNASG	1488	RGDRAAL	2287
VNASG	1489	RGDRAYL	2288
VNASG	1490	RGDRGAL	2289
VNASG	1491	RGDRGSL	2290
VNASG	1492	RGDRGYL	2291
VNASG	1493	RGDRQAL	2292
VNASG	1494	RGDRQYL	2293
VNASM	1495	RGDRAYL	2294
VNASM	1496	RGDRQAL	2295
VNASM	1497	RGDRQSL	2296
VNASS	1498	RGDRGSL	2297
VNASS	1499	RGDRQAL	2298

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
VNASS	1500	RGDRQYL	2299
VNAST	1501	RGDRAAL	2300
VNAST	1502	RGDRGAL	2301
VNAST	1503	RGDRGSL	2302
VNAST	1504	RGDRGYL	2303
VNAST	1505	RGDRQAL	2304
VNAST	1506	RGDRQSL	2305
VNAST	1507	RGDRQYL	2306
VNASV	1508	RGDRGSL	2307
VNASV	1509	RGDRGYL	2308
VNASV	1510	RGDRQAL	2309
VNASV	1511	RGDRQSL	2310
VNASV	1512	RGDRQYL	2311
VNGNF	1513	RGDRQNL	2312
VNGNV	1514	RGDRGML	2313
VNGSA	1515	RGDRAYL	2314
VNGSA	1516	RGDRGAL	2315
VNGSA	1517	RGDRGSL	2316
VNGSA	1518	RGDRGYL	2317
VNGSA	1519	RGDRQAL	2318
VNGSA	1520	RGDRQSL	2319
VNGSA	1521	RGDRQYL	2320
VNGSG	1522	RGDRAAL	2321
VNGSG	1523	RGDRASL	2322
VNGSG	1524	RGDRAYL	2323
VNGSG	1525	RGDRGAL	2324
VNGSG	1526	RGDRGSL	2325
VNGSG	1527	RGDRGYL	2326
VNGSG	1528	RGDRQAL	2327
VNGSG	1529	RGDRQSL	2328
VNGSG	1530	RGDRQYL	2329
VNGSM	1531	RGDRGYL	2330
VNGSM	1532	RGDRQAL	2331
VNGSM	1533	RGDRQSL	2332
VNGSM	1534	RGDRQYL	2333
VNGSS	1535	RGDRAYL	2334
VNGSS	1536	RGDRGAL	2335
VNGSS	1537	RGDRGSL	2336
VNGSS	1538	RGDRQAL	2337

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
VNGSS	1539	RGDRQSL	2338
VNGSS	1540	RGDRQYL	2339
VNGST	1541	RGDRAAL	2340
VNGST	1542	RGDRAYL	2341
VNGST	1543	RGDRGAL	2342
VNGST	1544	RGDRGYL	2343
VNGST	1545	RGDRQAL	2344
VNGST	1546	RGDRQSL	2345
VNGST	1547	RGDRQYL	2346
VNGSV	1548	RGDRAYL	2347
VNGSV	1549	RGDRQAL	2348
VNGSV	1550	RGDRQSL	2349
VNGSV	1551	RGDRQYL	2350
VNSGM	1552	RGDRGAL	2351
VNSSA	1553	RGDRGAL	2352
VNSSA	1554	RGDRGSL	2353
VNSSA	1555	RGDRGYL	2354
VNSSA	1556	RGDRQAL	2355
VNSSA	1557	RGDRQSL	2356
VNSSA	1558	RGDRQYL	2357
VNSSG	1559	RGDRAAL	2358
VNSSG	1560	RGDRAYL	2359
VNSSG	1561	RGDRGAL	2360
VNSSG	1562	RGDRQAL	2361
VNSSG	1563	RGDRQSL	2362
VNSSG	1564	RGDRQYL	2363
VNSSM	1565	RGDRAYL	2364
VNSSM	1566	RGDRQAL	2365
VNSSM	1567	RGDRQSL	2366
VNSSM	1568	RGDRQYL	2367
VNSSS	1569	RGDRGSL	2368
VNSSS	1570	RGDRQAL	2369
VNSSS	1571	RGDRQSL	2370
VNSST	1572	RGDRGAL	2371
VNSST	1573	RGDRQAL	2372
VNSST	1574	RGDRQSL	2373
VNSST	1575	RGDRQYL	2374
VNSSV	1576	RGDRAYL	2375
VNSSV	1577	RGDRQAL	2376

5 aa subs- titution	SEQ ID NO	7 aa insert	SEQ ID NO
VNSSV	1578	RGDRQSL	2377
VNTSA	1579	RGDRAYL	2378
VNTSG	1580	RGDRAAL	2379
VNTSG	1581	RGDRGAL	2380
VNTSG	1582	RGDRGSL	2381
VNTSG	1583	RGDRGYL	2382
VNTSG	1584	RGDRQAL	2383
VNTSG	1585	RGDRQSL	2384
VNTSG	1586	RGDRQYL	2385
VNTST	1587	RGDRAYL	2386
VNYPH	1588	RGDRGAL	2387
VNYSA	1589	RGDRGAL	2388
VNYSA	1590	RGDRGSL	2389
VNYSA	1591	RGDRQAL	2390
VNYSA	1592	RGDRQSL	2391
VNYSG	1593	RGDRAAL	2392
VNYSG	1594	RGDRGAL	2393
VNYSG	1595	RGDRQAL	2394
VNYSG	1596	RGDRQSL	2395
VNYSG	1597	RGDRQYL	2396
VNYSS	1598	RGDRGSL	2397
VNYSS	1599	RGDRGYL	2398

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic of the novel platform of the invention.

FIG. 2 is a schematic of the novel capsid platform of the invention.

5 **FIG. 3A** show images of fluorescence in tissues following administration of capsids of the invention.

FIG. 3B shows graphs of transgene mRNA in tissues following administration of capsids of the invention.

FIG. 4A-B show graphs of relative luminescence in mouse and human primary myotubules following administration of capsids of the invention.

10 **FIG. 5A-5B** shows graphs of transduction percentage in mouse and human primary myotubules following administration of capsids of the invention.

FIG. 6 is a graph of probability of survival following capsid and transgene administration in Mtm1 knockout mice.

15 **FIG. 7** is an image of MTM1 levels in non-human primate skeletal muscle following capsid and transgene administration.

FIG. 8 shows images of fluorescence in tissues following administration of capsids of the invention.

FIG. 9A-B show mRNA transgene expression levels in NHP skeletal muscle following administration of capsids of the invention.

20 **FIG. 10A-B** show mRNA transgene expression levels in NHP cardiac muscle following administration of capsids of the invention.

FIG. 11A-B show mRNA transgene expression levels in NHP liver following administration of capsids of the invention.

25 **FIG. 12A-B** show mRNA transgene expression levels in NHP dorsal root ganglia (DRG) following administration of capsids of the invention.

30 **FIG. 1-25** are Appendix A providing results from capsids of the invention (MyoAAV-LD 6).

DETAILED DESCRIPTION

The present invention provides novel capsid protein variants for viral vectors that detarget liver tissue and target skeletal muscle and heart tissue at the same time. Aspects of the present invention provide adeno-associated virus (AAV) vector's comprising a capsid protein comprising the amino acid sequence RGDR. In the capsid protein, RGDR may be inserted after amino acid 455 in reference to an AAV9 capsid or equivalent position in another AAV capsid. For example, capsid proteins variants of the invention may comprise a sequence as set forth in Table 1.

10 Adeno Associated Virus Vectors

AAVs are particularly appropriate viral vectors for delivery of genetic material into mammalian cells. AAVs are not known to cause disease in mammals and cause a very mild immune response. Additionally, AAVs are able to infect cells in multiple stages whether at rest or in a phase of the cell replication cycle. Advantageously, AAV DNA is not regularly inserted into the host's genome at random sites, reducing the oncogenic properties of this vector.

AAVs have been engineered to deliver a variety of treatments, especially for genetic disorders caused by single nucleotide polymorphisms ("SNP"). Genetic diseases that have been studied in conjunction with AAV vectors include Cystic fibrosis, hemophilia, arthritis, macular degeneration, muscular dystrophy, Parkinson's disease, congestive heart failure, and Alzheimer's disease. The AAV can be used as a vector to deliver engineered nucleic acid to a host and utilize the host's own ribosomes to transcribe that nucleic acid into the desired proteins. See, e.g., West et al., Virology 160:38-47 (1987); U.S. Pat. No. 4,797,368; WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); and Muzyczka, J. Clin. Invest. 94:1351 (1994). AAVs have some deficiency in their replication and/or pathogenicity and thus can be safer than adenoviral vectors. In some embodiments, the AAV can integrate into a specific site on chromosome 19 of a human cell with no observable side effects. In some embodiments, the capacity of the AAV vector, system thereof, and/or AAV particles can be up to about 4.7 kb. The AAV vector or system thereof can include one or more engineered capsid polynucleotides described herein.

AAVs are small, replication-defective, nonenveloped viruses that infect humans and other primate species and have a linear single-stranded DNA genome. Naturally occurring AAV serotypes exhibit liver tropism. As a result, transfection of non-liver tissue with traditional AAV vectors is impeded by the virus's natural liver tropism. Moreover, because the liver acts to break down substances delivered to a subject, transfection of non-liver tissue with unmodified AAV vectors requires higher dosing to provide sufficient viral load to overcome the liver and reach non-liver tissue. More than 30 naturally occurring serotypes of AAV are available. Many natural variants in the AAV capsid exist. AAV serotypes include, but are not limited to, AAV serotypes AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV 12, AAV13. AAVs may be engineered using conventional molecular biology techniques, making it possible to optimize these particles, for example, for cell specific delivery, for minimizing immunogenicity, for tuning stability and particle lifetime, for efficient degradation, for accurate delivery to the nucleus. AAV vectors can be specifically targeted to one or more types of cells by choosing the appropriate combination of AAV serotype, promoter, and delivery method.

Previous approaches to identify AAV sequences correlated with tropism have relied upon the comparison of highly related extant serotypes with distinct characteristics, random domain swaps between unrelated serotypes, or consideration of higher-order structure, to identify motifs that define liver tropism. For example, mapping determinants of AAV tropism have been carried out by comparing highly related serotypes. One such example is the single-amino acid change (E531K) between AAV1 and AAV6 that improves murine liver transduction in AAV1. See Wu et al. (2006) J. Virol., 80(22):11393-7, incorporated by reference herein. Another example is a reciprocal domain swap between AAV2 and AAV8 that alters tropism, but fails to define any robust specific tissue-targeting motifs. See Raupp et al. (201) J. Virol., 86(17):9396-408, incorporated by reference herein. Further, global consideration of structure has only highlighted gross differences between better- or worse-liver-transducers that are more observational than useful in practice. Nam et al (2007) J. Virol., 81(22):12260-71.

AAVs exhibiting modified tissue tropism that may be used with the present invention are described in U.S. Patent No. 9,695,220, U.S. Patent No. 9,719,070; U.S. Patent No. 10,119,125; U.S. Patent No. 10,526,584; U.S. Patent Application Publication No. 2018-

0369414; U.S. Patent Application Publication No. 2020-0123504; U.S. Patent Application Publication No. 2020-0318082; PCT International Patent Application Publication No. WO 2015/054653; PCT International Patent Application Publication No. WO 2016/179496; PCT International Patent Application Publication No. WO 2017/100791; and PCT International Patent Application Publication No. WO 2019/217911, the entirety of the contents of each of which are incorporated by reference herein.

The AAV vector or system thereof may include one or more regulatory molecules, such as promoters, enhancers, repressors and the like. In some embodiments, the AAV vector or system thereof can include one or more polynucleotides that can encode one or more regulatory proteins. In some embodiments, the one or more regulatory proteins can be selected from Rep78, Rep68, Rep52, Rep40, variants thereof, and combinations thereof. In some embodiments, the muscle specific promoter can drive expression of an engineered AAV capsid polynucleotide.

The AAV vector or system thereof can include one or more polynucleotides that can encode one or more capsid proteins, such as the engineered AAV capsid proteins described elsewhere herein. The engineered capsid proteins can be capable of assembling into a protein shell (an engineered capsid) of the AAV virus particle. The engineered capsid can have a cell-, tissue-, and/or organ-specific tropism.

The AAV vector or system thereof can be configured to produce AAV particles having a specific serotype. In some embodiments, the serotype can be AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-8, AAV-9 or any combinations thereof. In some embodiments, the AAV can be AAV1, AAV-2, AAV-5, AAV-9 or any combination thereof. One can select the AAV of the AAV with regard to the cells to be targeted; e.g., one can select AAV serotypes 1, 2, 5, 9 or a hybrid capsid AAV-1, AAV-2, AAV-5, AAV-9 or any combination thereof for targeting brain and/or neuronal cells; and one can select AAV-4 for targeting cardiac tissue; and one can select AAV-8 for delivery to the liver. Thus, in some embodiments, an AAV vector or system thereof capable of producing AAV particles capable of targeting the brain and/or neuronal cells can be configured to generate AAV particles having serotypes 1, 2, 5 or a hybrid capsid AAV-1, AAV-2, AAV-5 or any combination thereof. In some embodiments, an AAV vector or system thereof capable of producing AAV particles capable of targeting cardiac tissue can be configured to generate an AAV particle

having an AAV-4 serotype. In some embodiments, an AAV vector or system thereof capable of producing AAV particles capable of targeting the liver can be configured to generate an AAV having an AAV-8 serotype. See also Srivastava. 2017. Curr. Opin. Virol. 21:75-80.

It will be appreciated that while the different serotypes can provide some level of cell, tissue, and/or organ specificity, each serotype still is multi-tropic and thus can result in tissue-toxicity if using that serotype to target a tissue that the serotype is less efficient in transducing. Thus, in addition to achieving some tissue targeting capacity via selecting an AAV of a particular serotype, it will be appreciated that the tropism of the AAV serotype can be modified by an engineered AAV capsid described herein. As described elsewhere herein, variants of wild-type AAV of any serotype can be generated via a method described herein and determined to have a particular cell-specific tropism, which can be the same or different as that of the reference wild-type AAV serotype. In some embodiments, the cell, tissue, and/or specificity of the wild-type serotype can be enhanced (e.g., made more selective or specific for a particular cell type that the serotype is already biased towards). For example, wild-type AAV-9 is biased towards muscle and brain in humans (see e.g., Srivastava. 2017. Curr. Opin. Virol. 21:75-80.) By including an engineered AAV capsid and/or capsid protein variant of wild-type AAV-9 as described herein, the tropism for nervous cells might be reduced or eliminated and/or the muscle specificity increased such that the nervous specificity appears reduced in comparison, thus enhancing the specificity for muscle as compared to the wild-type AAV-9. As previously mentioned, inclusion of an engineered capsid and/or capsid protein variant of a wild-type AAV serotype can have a different tropism than the wild-type reference AAV serotype. For example, an engineered AAV capsid and/or capsid protein variant of AAV-9 can have specificity for a tissue other than muscle or brain in humans.

In some embodiments, the AAV vector is a hybrid AAV vector or system thereof. Hybrid AAVs are AAVs that include genomes with elements from one serotype that are packaged into a capsid derived from at least one different serotype. For example, if it is the rAAV2/5 that is to be produced, and if the production method is based on the helper-free, transient transfection method discussed above, the 1st plasmid and the 3rd plasmid (the adeno helper plasmid) will be the same as discussed for rAAV2 production. However, the 2nd plasmid, the pRepCap will be different. In this plasmid, called pRep2/Cap5, the Rep

gene is still derived from AAV2, while the Cap gene is derived from AAV5. The production scheme is the same as the above-mentioned approach for AAV2 production. The resulting rAAV is called rAAV2/5, in which the genome is based on recombinant AAV2, while the capsid is based on AAV5. It is assumed the cell or tissue-tropism displayed by this AAV2/5 hybrid virus should be the same as that of AAV5. It will be appreciated that wild-type hybrid AAV particles suffer the same specificity issues as with the non-hybrid wild-type serotypes previously discussed.

Advantages achieved by the wild-type based hybrid AAV systems can be combined with the increased and customizable cell-specificity that can be achieved with the engineered AAV capsids can be combined by generating a hybrid AAV that can include an engineered AAV capsid described elsewhere herein. It will be appreciated that hybrid AAVs can contain an engineered AAV capsid containing a genome with elements from a different serotype than the reference wild-type serotype that the engineered AAV capsid is a variant of. For example, a hybrid AAV can be produced that includes an engineered AAV capsid that is a variant of an AAV-9 serotype that is used to package a genome that contains components (e.g., rep elements) from an AAV-2 serotype. As with wild-type based hybrid AAVs previously discussed, the tropism of the resulting AAV particle will be that of the engineered AAV capsid.

In some embodiments, the AAV vector or system thereof is configured as a “gutless” vector, similar to that described in connection with a retroviral vector. In some embodiments, the “gutless” AAV vector or system thereof can have the cis-acting viral DNA elements involved in genome amplification and packaging in linkage with the heterologous sequences of interest (e.g., the engineered AAV capsid polynucleotide(s)).

The vectors described herein can be constructed using any suitable process or technique. In some embodiments, one or more suitable recombination and/or cloning methods or techniques can be used to the vector(s) described herein. Suitable recombination and/or cloning techniques and/or methods can include, but not limited to, those described in U.S. Application publication No. US 2004-0171156 A1. Other suitable methods and techniques are described elsewhere herein.

Construction of recombinant AAV vectors are described in a number of publications, including U.S. Pat. No. 5,173,414; Tratschin et al., Mol. Cell. Biol. 5:3251-3260 (1985);

Tratschin, et al., Mol. Cell. Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samulski et al., J. Virol. 63:03822-3828 (1989). Any of the techniques and/or methods can be used and/or adapted for constructing an AAV or other vector described herein. AAV vectors are discussed elsewhere herein.

5 In some embodiments, the vector can have one or more insertion sites, such as a restriction endonuclease recognition sequence (also referred to as a “cloning site”). In some embodiments, one or more insertion sites (e.g., about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertion sites) are located upstream and/or downstream of one or more sequence elements of one or more vectors.

10 Delivery vehicles, vectors, particles, nanoparticles, formulations and components thereof for expression of one or more elements of a engineered AAV capsid system described herein are as used in the foregoing documents, such as International Patent Application Publications WO WO 2021/050974 and WO 2021/077000 and PCT International Application No. PCT/US2021/042812, the contents of which are incorporated by reference
15 herein.

 Additional AAV vectors are described in International Patent Application Publication WO 2019/2071632, the contents of which are incorporated by reference herein.

 Further AAV vectors are described in International Patent Application Publications WO 2020/086881 and WO 2020/235543, the contents of each of which are incorporated by
20 reference herein.

 Further AAV vectors are described in International Patent Application Publications WO 2005/033321; WO 2006/110689; WO 2007/127264; WO 2008/027084; WO 2009/073103; WO 2009/073104; WO 2009/105084; WO 2009/134681; WO 2009/136977; WO 2010/051367; WO 2010/138675; WO 2001/038187; WO 2012/112832; WO
25 2015/054653; WO 2016/179496; WO 2017/100791; WO 2017/019994; WO 2018/209154; WO 2019/067982; WO 2019/195701; WO 2019/217911; WO 2020/041498; WO 2020/210839; U.S. Patent No. 7,906,111; U.S. Patent No. 9,737,618; U.S. Patent No. 10,265,417; U.S. Patent No. 10,485,883; U.S. Patent No. 10,695,441; U.S. Patent No. 10,722,598; U.S. Patent No. 8,999,678; U.S. Patent No. 10,301,648; U.S. Patent No.
30 10,626,415; U.S. Patent No. 9,198,984; U.S. Patent No. 10,155,931; U.S. Patent No. 8,524,219; U.S. Patent No. 9,206,238; U.S. Patent No. 8,685,387; U.S. Patent No. 9,359,618;

U.S. Patent No. 8,231,880; U.S. Patent No. 8,470,310; U.S. Patent No. 9,597,363; U.S. Patent No. 8,940,290; U.S. Patent No. 9,593,346; U.S. Patent No. 10,501,757; U.S. Patent No. 10,786,568; U.S. Patent No. 10,973,928; U.S. Patent No. 10,519,198; U.S. Patent No. 8,846,031; U.S. Patent No. 9,617,561; U.S. Patent No. 9,884,071; U.S. Patent No. 10,406,173; U.S. Patent No. 9,596,220; U.S. Patent No. 9,719,010; U.S. Patent No. 10,117,125; U.S. Patent No. 10,526,584; U.S. Patent No. 10,881,548; U.S. Patent No. 10,738,087; U.S. Patent Publication No. 2011-023353; U.S. Patent Publication No. 2019-0015527; U.S. Patent Publication No. 2020-155704; U.S. Patent Publication No. 2017-0191079; U.S. Patent Publication No. 2019-0218574; U.S. Patent Publication No. 2020-0208176; U.S. Patent Publication No. 2020-0325491; U.S. Patent Publication No. 2019-0055523; U.S. Patent Publication No. 2020-0385689; U.S. Patent Publication No. 2009-0317417; U.S. Patent Publication No. 2016-0051603; U.S. Patent Publication No. 2016-00244783; U.S. Patent Publication No. 2017-0183636; U.S. Patent Publication No. 2020-0263201; U.S. Patent Publication No. 2020-0101099; U.S. Patent Publication No. 2020-0318082; U.S. Patent Publication No. 2018-0369414; U.S. Patent Publication No. 2019-0330278; U.S. Patent Publication No. 2020-0231986, the contents of each of which are incorporated by reference herein.

Capsid Protein

The capsid protein is the shell or coating of the virus that enables its delivery into the host. Without the protein, the nucleic acids would be destroyed by the host without entering into the host cells and beginning transcription and translation. The capsid protein may be in the natural conformation of a naturally occurring AAV, or it may be modified.

In certain example embodiments, the AAV capsid protein is an engineered AAV capsid protein having reduced or eliminated uptake in a non-muscle cell as compared to a corresponding wild-type AAV capsid polypeptide.

In some embodiments, the engineered AAV capsid encoding polynucleotide can be included in a polynucleotide that is configured to be an AAV genome donor in an AAV vector system that can be used to generate engineered AAV particles described elsewhere herein. In some embodiments, the engineered AAV capsid encoding polynucleotide can be operably coupled to a poly adenylation tail. In some embodiments, the poly adenylation tail

can be an SV40 poly adenylation tail. In some embodiments, the AAV capsid encoding polynucleotide can be operably coupled to a promoter. In some embodiments, the promoter can be a tissue specific promoter. In some embodiments, the tissue specific promoter is specific for muscle (e.g., cardiac, skeletal, and/or smooth muscle), neurons and supporting
5 cells (e.g., astrocytes, glial cells, Schwann cells, etc.), fat, spleen, liver, kidney, immune cells, spinal fluid cells, synovial fluid cells, skin cells, cartilage, tendons, connective tissue, bone, pancreas, adrenal gland, blood cell, bone marrow cells, placenta, endothelial cells, and combinations thereof. In some embodiments, the promoter can be a constitutive promoter. Suitable tissue specific promoters and constitutive promoters are discussed elsewhere herein
10 and are generally known in the art and can be commercially available. Suitable muscle specific promoters include, but are not limited to CK8, MHCK7, Myoglobin promoter (Mb), Desmin promoter, muscle creatine kinase promoter (MCK) and variants thereof, and SPc5-12 synthetic promoter.

Described herein are various embodiments of engineered viral capsids, such as adeno-
15 associated virus (AAV) capsids, that can be engineered to confer cell-specific tropism, such as muscle specific tropism, to an engineered viral particle. Engineered viral capsids can be lentiviral, retroviral, adenoviral, or AAV capsids. The engineered capsids can be included in an engineered virus particle (e.g., an engineered lentiviral, retroviral, adenoviral, or AAV virus particle), and can confer cell-specific tropism, reduced immunogenicity, or both to the
20 engineered viral particle. The engineered viral capsids described herein can include one or more engineered viral capsid proteins described herein. The engineered viral capsids described herein can include one or more engineered viral capsid proteins described herein that can contain a muscle-specific targeting moiety containing or composed of an n-mer motif described elsewhere herein.

25 The engineered viral capsid and/or capsid proteins can be encoded by one or more engineered viral capsid polynucleotides. In some embodiments, the engineered viral capsid polynucleotide is an engineered AAV capsid polynucleotide, engineered lentiviral capsid polynucleotide, engineered retroviral capsid polynucleotide, or engineered adenovirus capsid polynucleotide. In some embodiments, an engineered viral capsid polynucleotide (e.g., an
30 engineered AAV capsid polynucleotide, engineered lentiviral capsid polynucleotide, engineered retroviral capsid polynucleotide, or engineered adenovirus capsid polynucleotide)

can include a 3' polyadenylation signal. The polyadenylation signal can be an SV40 polyadenylation signal.

The engineered viral capsids can be variants of wild-type viral capsid. For example, in some embodiments, the engineered AAV capsids can be variants of wild-type AAV capsids. In some embodiments, the wild-type AAV capsids can be composed of VP1, VP2, VP3 capsid proteins or a combination thereof. In other words, the engineered AAV capsids can include one or more variants of a wild-type VP1, wild-type VP2, and/or wild-type VP3 capsid proteins. In some embodiments, the serotype of the reference wild-type AAV capsid can be AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-8, AAV-9 or any combination thereof. In some embodiments, the serotype of the wild-type AAV capsid can be AAV-9. The engineered AAV capsids can have a different tropism than that of the reference wild-type AAV capsid.

The engineered viral capsid can contain 1-60 engineered capsid proteins. In some embodiments, the engineered viral capsids can contain 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, or 60 engineered capsid proteins. In some embodiments, the engineered viral capsid can contain 0-59 wild-type viral capsid proteins. In some embodiments, the engineered viral capsid can contain 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, 53, 54, 55, 56, 57, 58, or 59 wild-type viral capsid proteins.

In some embodiments, the engineered AAV capsid can contain 1-60 engineered capsid proteins. In some embodiments, the engineered AAV capsids can contain 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, or 60 engineered capsid proteins. In some embodiments, the engineered AAV capsid can contain 0-59 wild-type AAV capsid proteins. In some embodiments, the engineered AAV capsid can contain 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, 53, 54, 55, 56, 57, 58, or 59 wild-type AAV capsid proteins.

In some embodiments, the engineered viral capsid protein can have an *n*-mer amino acid motif, where *n* can be at least 3 amino acids. In some embodiments, *n* can be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids. In some embodiments, an engineered AAV capsid can have a 6-mer or 7-mer amino acid motif. In some embodiments, the *n*-mer amino acid motif can be inserted between two amino acids in the wild-type viral protein (VP) (or capsid protein). In some embodiments, the *n*-mer motif can be inserted between two amino acids in a variable amino acid region in a viral capsid protein.

In some embodiments, the *n*-mer motif can be inserted between two amino acids in a variable amino acid region in an AAV capsid protein. The core of each wild-type AAV viral protein contains an eight-stranded beta-barrel motif (betaB to betaI) and an alpha-helix (alphaA) that are conserved in autonomous parvovirus capsids (see e.g., DiMattia et al. 2012. J. Virol. 86(12):6947-6958). Structural variable regions (VRs) occur in the surface loops that connect the beta-strands, which cluster to produce local variations in the capsid surface. AAVs have 12 variable regions (also referred to as hypervariable regions) (see e.g., Weitzman and Linden. 2011. "Adeno-Associated Virus Biology." In Snyder, R.O., Moullier, P. (eds.) Totowa, NJ: Humana Press). In some embodiments, one or more *n*-mer motifs can be inserted between two amino acids in one or more of the 12 variable regions in the wild-type AAV capsid proteins. In some embodiments, the one or more *n*-mer motifs can be each be inserted between two amino acids in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-III, VR-IX, VR-X, VR-XI, VR-XII, or a combination thereof. In some embodiments, the *n*-mer can be inserted between two amino acids in the VR-III of a capsid protein. In some embodiments, the engineered capsid can have an *n*-mer inserted between any two contiguous amino acids between amino acids 262 and 269, between any two contiguous amino acids between amino acids 327 and 332, between any two contiguous amino acids between amino acids 382 and 386, between any two contiguous amino acids between amino acids 452 and 460, between any two contiguous amino acids between amino acids 488 and 505, between any two contiguous amino acids between amino acids 545 and 558, between any two contiguous amino acids between amino acids 581 and 593, between any two contiguous amino acids between amino acids 704 and 714 of an AAV9 viral protein. In some embodiments, the engineered capsid can have an *n*-mer inserted between amino acids 588 and 589 of an AAV9 viral protein. In some embodiments, the engineered capsid

can have a 7-mer motif inserted between amino acids 588 and 589 of an AAV9 viral protein. In other embodiments, the motif inserted is a 10-mer motif, with replacement of amino acids 586-88 and an insertion before 589. SEQ ID NO. 1 is a reference AAV9 capsid sequence for at least referencing the insertion sites discussed above. It will be appreciated that *n*-mers can be inserted in analogous positions in AAV viral proteins of other serotypes. In some embodiments as previously discussed, the *n*-mer(s) can be inserted between any two contiguous amino acids within the AAV viral protein and in some embodiments the insertion is made in a variable region.

In some embodiments, the first 1, 2, 3, or 4 amino acids of an *n*-mer motif can replace 1, 2, 3, or 4 amino acids of a polypeptide into which it is inserted and preceding the insertion site. In some embodiments, the amino acids of the *n*-mer motif that replace 1 or more amino acids of the polypeptide into which the *n*-mer motif is inserted come before or immediately before an "RGD" in an *n*-mer motif. For example, in one or more of the 10-mer inserts, the first three amino acids shown can replace 1-3 amino acids into a polypeptide to which they may be inserted. Using an AAV as another non-limiting example, one or more of the *n*-mer motifs can be inserted into e.g., and AAV9 capsid polypeptide between amino acids 588 and 589 and the insert can replace amino acids 586, 587, and 588 such that the amino acid immediately preceding the *n*-mer motif after insertion is residue 585. It will be appreciated that this principle can apply in any other insertion context and is not necessarily limited to insertion between residues 588 and 589 of an AAV9 capsid or equivalent position in another AAV capsid. It will further be appreciated that in some embodiments, no amino acids in the polypeptide into which the *n*-mer motif is inserted are replaced by the *n*-mer motif.

In some embodiments, the AAV capsids or other viral capsids or compositions can be muscle-specific. In some embodiments, muscle-specificity of the engineered AAV or other viral capsid or other composition is conferred by a muscle specific *n*-mer motif incorporated in the engineered AAV or other viral capsid or other composition described herein. While not intending to be bound by theory, it is believed that the *n*-mer motif confers a 3D structure to or within a domain or region of the engineered AAV capsid or other viral capsid or other composition such that the interaction of the viral particle or other composition containing the engineered AAV capsid or other viral capsid or other composition described herein has increased or improved interactions (e.g., increased affinity) with a cell surface receptor

and/or other molecule on the surface of a muscle cell. In some embodiments, the cell surface receptor is AAV receptor (AAVR). In some embodiments, the cell surface receptor is a muscle cell specific AAV receptor. In some embodiments, the cell surface receptor or other molecule is a cell surface receptor or other molecule selectively expressed on the surface of a muscle cell. In some embodiments, the cell surface receptor or molecule is an integrin or dimer thereof. In some embodiments, the cell surface receptor or molecule is an Vb6 integrin heterodimer.

In some embodiments, a muscle specific engineered viral particle or other composition described herein containing the muscle-specific capsid, n-mer motif, or muscle-specific targeting moiety described herein can have an increased uptake, delivery rate, transduction rate, efficiency, amount, or a combination thereof in a muscle cell as compared to other cells types and/or other virus particles (including but not limited to AAVs) and other compositions that do not contain the muscle-specific n-mer motif of the present invention.

First- and second-generation muscle specific AAV capsids were developed using a muscle specific promoter and the resulting capsid libraries were screened in mice and non-human primates as described elsewhere herein and/or in e.g., U.S. Provisional Application Serial Nos. 62/899,453, 62/916,207, 63/018,454, 63/242,008, and No. 63/345,14.

EXPERIMENTAL DETAILS

Co-evolution of liver de-targeting and muscle targeting features resulted in development of MyoAAV-LD capsids in NHPs, resulting in “6th generation” capsids terms MyoAAV-LD 6.

First generation muscle gene therapies are not optimized for efficacy and safety.

Naturally occurring AAV capsids do not transduce muscle effectively.

Capsids used in first-generation gene therapies (e.g. AAV8, AAV9, AAVrh74) are mainly sequestered in the liver after systemic administration, requiring very high doses to be effective in muscle disease.

High doses cause toxicity. Serious side effects have occurred with high dose gene therapies, including liver injury, and an acute blood disorder called thrombotic microangiopathy that can cause kidney injury.

Cargoes lack effective and selective regulatory elements. First generation AAV muscle gene therapies do not use highly optimized skeletal muscle and cardiac regulatory elements, potential for off-target toxicity.

More potent and tissue selective capsids are required to treat muscle and heart disease safely and effectively.

Novel capsids

A novel platform was utilized comprising a capsid and gene expression regulation technologies to address critical challenges in muscle gene therapies.

FIG. 1 is a schematic of the novel platform of the invention. Machine learning driven cardiac and skeletal muscle specific regulatory elements (enhancer-promoter) were developed. Tissue specific miRNA mediated transgene silencing was developed. Machine learning driven capsid directed evolution was utilized to engineer muscle and heart tropic, liver de-targeted capsids.

Capsid development

FIG. 2 is a schematic of the novel capsid platform of the invention. Directed evolution of AAV capsids leveraged *in vivo* expression of transgene RNA. A diverse first-round virus library was designed based on the capsid 3D structure. Machine learning-based design was used for a second-round capsid library. Stringent mRNA-based selection was conducted of capsid variants. Potent capsid variants in any specific or cell type of interest (non-human primates, mice) was conducted.

The platform identified RGD-containing MyoAAV class of skeletal muscle and cardiotropic capsids in mice and non-human primates.

FIG. 3A shows images of fluorescence in tissues following administration of capsids of the invention.

FIG. 3B shows graphs of transgene mRNA in tissues following administration of capsids of the invention.

5E+13 vg/kg of MyoAAV1A- or AAV9-EGFP was administered to tissues harvested 14 days post IV administration from adult C57BL/6 mice. The platform was successful in

identifying a class of skeletal muscle and cardiotropic capsids. MyoAAV class capsids showed unprecedented targeting to skeletal muscle and heart.

FIG. 4A-B show graphs of relative luminescence in mouse and human primary myotubules following administration of capsids of the invention.

5 **FIG. 5A-5B** shows graphs of transduction percentage in mouse and human primary myotubules following administration of capsids of the invention.

Muscle tropism of MyoAAV was conserved across species and dependent on αV -containing integrin heterodimers. The conserved mechanism of transduction provides confidence in translating potency to humans.

10

Generation of MyoAAV capsids

MyoAAV capsid generation led to a more potent muscle-tropic gene therapy candidate for the treatment of XLMTM (KT430).

15 **FIG. 6** is a graph of probability of survival following capsid and transgene administration in Mtm1 knockout (KO) mice. KT430 reversed XLMTM disease phenotype at very low doses ($<3 \times 10^{12}$ vg/mg) in Mtm1 KO mice.

FIG. 7 is an image of MTM1 levels in non-human primate skeletal muscle following capsid and transgene administration. KT430 produced supra-physiological MTM1 levels in NHP skeletal muscle at 1×10^{13} vg/kg.

20

6th generation capsids

Additional modifications to AAV capsids enabled directed evolution of liver-de-targeted and muscle-tropic capsid variants.

25 **FIG. 8** shows images of fluorescence in tissues following administration of capsids of the invention.

Novel capsids (termed “6th generation”) Myo-AAV-LD capsids were generated. Barcoded transgenes (CBh-hFXN) were packaged into different novel capsid variants. The pool of capsid variants were injected into 4 Cyno Macaques and expression of transgene from each capsid variant was quantified by sequencing the barcodes 4 weeks after injection.

30 **FIG. 9A-B** show mRNA transgene expression levels in NHP skeletal muscle following administration of capsids of the invention.

FIG. 10A-B show mRNA transgene expression levels in NHP cardiac muscle following administration of capsids of the invention.

FIG. 11A-B show mRNA transgene expression levels in NHP liver following administration of capsids of the invention.

5 **FIG. 12A-B** show mRNA transgene expression levels in NHP dorsal root ganglia (DRG) following administration of capsids of the invention.

Capsids of the invention outperform in transducing NHL skeletal muscle versus naturally occurring and previously engineered capsids.

10 Capsids of the invention outperform in transducing NHL cardiac muscle versus naturally occurring and previously engineered capsids.

Capsids of the invention outperform were de-targeted from the liver at the DNA level in NHPs compared to naturally occurring capsids.

Capsids of the invention outperform were de-targeted from DRG in NHPs compared to naturally occurring capsids.

15 The top skeletal muscle tropic liver de-targeted MyoAAV-LD capsid, MyoAAV-LD 6.1, transduced NHP skeletal muscles ~20 times more effectively and was ~10 times de-targeted from the liver compared to AAV9.

20 The top cardiotropic liver de-targeted MyoAAV-LD capsid, MyoAAV-LD 6.9, transduced NHP cardiac muscle ~11 times more effectively and was ~6 times de-targeted from the liver compared to AAV9.

Discussion

25 Coevolution of liver de-targeting and muscle targeting features resulted in the development of MyoAAV-LD capsids in NHPs. The top skeletal muscle tropic liver de-targeted MyoAAV-LD capsid, MyoAAV-LD 6.1, transduced NHP skeletal muscles ~20 times more effectively and was ~10 times de-targeted from the liver compared to AAV9.

The top cardiotropic liver de-targeted MyoAAV-LD capsid, MyoAAV-LD 6.9, transduced NHP cardiac muscle ~11 times more effectively and was ~6 times de-targeted from the liver compared to AAV9.

30

Incorporation by Reference

References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made throughout this disclosure. All such documents are hereby incorporated herein by reference in their entirety
5 for all purposes.

Equivalents

Various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including references to the scientific and patent
10 literature cited herein. The subject matter herein contains important information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

CLAIMS

We claim:

1. An adeno-associated virus (AAV) vector comprising:
a capsid protein comprising the amino acid sequence RGDR.
2. The AAV vector of claim 1, wherein the amino acid sequence RGDR is inserted after amino acid 455 in reference to an AAV9 capsid
3. The AAV vector of claim 1, wherein
the capsid protein comprises the amino acid sequence $X_1NX_2X_3X_4RGDRX_5X_6L$,
wherein
 - X_1 is selected from among any amino acid;
 - X_2 is selected from among any amino acid;
 - X_3 is selected from among any amino acid;
 - X_4 is selected from among any amino acid;
 - X_5 is selected from among any amino acid; and
 - X_6 is selected from among any amino acid.
4. The AAV vector of claim 3, wherein X_1 is located at amino acid 451, X_2 is located at amino acid 453, X_3 is located at amino acid 454, X_4 is located amino acid 455, and $RGDRX_5X_6L$ is inserted after amino acid 455 in reference to an AAV9 capsid or equivalent position in another AAV capsid.
5. The AAV vector of claim 4, wherein X_1 is an amino acid selected from the group consisting of: A, I, F, G, H, L, M, Q, S, T, V.
6. The AAV vector of claim 5, wherein X_1 is an amino acid selected from the group consisting of: A, I, L, S, V.

7. The AAV vector of claim 4, wherein X_2 is an amino acid selected from the group consisting of A, G, S, T, Y.
8. The AAV vector of claim 4, wherein X_3 is an amino acid selected from the group consisting of S, N, G, P.
9. The AAV vector of claim 8, wherein X_3 is S.
10. The AAV vector of claim 4, wherein X_4 is an amino acid selected from the group consisting of A, G, H, I, M, S, T, V.
11. The AAV vector of claim 4, wherein X_5 is an amino acid selected from the group consisting of A, G, Q.
12. The AAV vector of claim 4, wherein X_6 is an amino acid selected from the group consisting of A, I, L, M, N, Y.
13. The AAV vector of claim 12, wherein X_6 may be selected from the group consisting of A, S, and Y.
14. The AAV vector of claim 4, wherein two amino acids from X_1 , X_2 , X_3 , and X_4 are wild type amino acids in reference to an AAV9 capsid or equivalent position in another AAV capsid and two amino acids from X_1 , X_2 , X_3 , and X_4 are not wild type amino acids.
15. The adeno-associated virus (AAV) vector of claim 1, wherein the capsid protein comprises an amino acid sequence selected from Tables 1a.
16. The AAV vector of claim 15, wherein the amino acid sequence selected from Table 1a is in hypervariable region IV (HVR IV) relative to wild-type AAV9.

17. The AAV vector of claim 16, wherein the capsid protein comprises substitutions at amino acids 451-455 relative to a wild-type AAV9 vector capsid.
18. The AAV vector of claim 17, wherein the substitutions at amino acids 451-455 relative to a wild-type AAV9 vector capsid are substituted with an amino acid sequence selected from column 1 of Table 1b.
19. The AAV vector of claim 18, wherein the capsid protein comprises a 7-mer insert selected from column 2 of Table 1b.
20. The AAV vector of claim 19, wherein the 7-mer insert is inserted after amino acid 455 relative to a wild-type AAV9 vector.
21. The AAV vector of claim 1, wherein the AAV vector exhibits increased muscle tropism as compared to a wild-type AAV vector.
22. The AAV vector of claim 1, wherein the capsid protein further comprises a deletion of the G267 in reference to an AAV9 capsid or equivalent position in another AAV capsid.
23. The AAV vector of claim 22, wherein the vector exhibits reduced liver tropism as compared to a wild-type AAV vector.