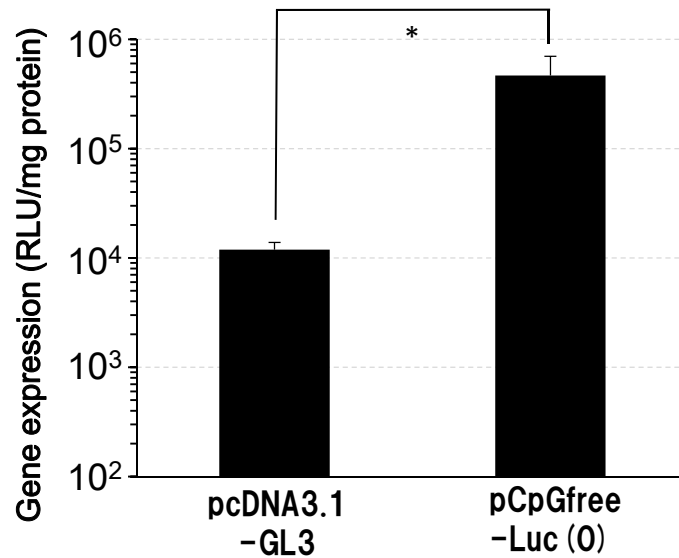




HOKKAIDO UNIVERSITY

Title	A neutral lipid envelope-type nanoparticle composed of a pH-activated and vitamin E-scaffold lipid-like material as a platform for a gene carrier targeting renal cell carcinoma
Author(s)	Akita, Hidetaka; Ishiba, Ryohei; Togashi, Ryohei et al.
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Supplemental Figure S1.

Effect of the type of pDNA on transgene expression in tumor tissue

The *in vivo* transgene expression in tissue was evaluated after the intravenous administration of the optimized LNP_{ssPalmE} (Figure 3) encapsulating pDNA with (pcDNA3.1-GL3) or without unmethylated CpG motifs (pCpGfree-Luc(0)). In pcDNA3.1-GL3, the expression of luciferase (GL3) was driven by the cytomegalovirus (CMV) promoter. On the other hand, in pCpGfree-Luc(0), luciferase expression is driven by the human elongation factor 1 α core promoter. Statistical analyses were performed by One-way ANOVA followed by Student's t-test (*; $p < 0.05$).

10 20 30 40 50 60
 ATGGTCAGCTGCTGGGACACCGCGGTCTTGCCTTACGCGCTGCTCGGGTGTCTGCTTCTC
 M V S C W D T A V L P Y A L L G C L L L
 70 80 90 100 110 120
 ACAGGATATGGCTCAGGTCGGAAGTTAAAGTGCCTGAAGTGAAGTTAAAGGCACCCAG
 T G Y G S G S K L K V P E L S L K G T Q
 130 140 150 160 170 180
 CATGTCATGCAAGCAGGCCAGACTCTCTTCTCAAGTGCAGAGGGAGGCAGCCACTCA
 H V M Q A G Q T L F L K C R G E A A H S
 190 200 210 220 230 240
 TGGTCTCTGCCCCACGACCGTGAGCCAGGAGGACAAAAGCTGAGCATCATCCCCATCG
 W S L P T V S Q E D K R L S I T P P S
 250 260 270 280 290 300
 GCCTGTGGGAGGAATAACAGGCAATCTGTCAGCACCTTGACCTTGACATGGCGCAGGCC
 A C G R N N R Q F C S T L T L D M A Q A
 310 320 330 340 350 360
 AACCCACGGGCTCTACACCTGTAGATACCTCCCTACATCTACTTCCGAAGAAAAGAAA
 N H T G L Y T C R Y L P T S T S K K K K
 370 380 390 400 410 420
 GCGGAATCTTCAATCTACATATTTGTTAGTGATGAGGAGTCCCTTCATAGAGATGCAC
 A E S S I Y I F V S D A G S P F I E M H
 430 440 450 460 470 480
 ACTGACATACCCAACTTGTGCACATGACGGAAGGAAGACAGCTCATCATCCCCGCGG
 T D I P K L V H M T E G R Q L I I P C R
 490 500 510 520 530 540
 GTGACGTACCCCAACGTACAGTACCCCTAAAAAGTTTCCATTTGATACTCTTACCCCT
 V T S P N V T V T L K K F P F D T L T P
 550 560 570 580 590 600
 GATGGGCAAGAATAACATGGGACAGTAGGAGAGGCTTTATAATGCAAAATGCAACGTAC
 D G Q R I T W D S R R G F I I A N A T Y
 610 620 630 640 650 660
 AAAGAGATAGGACTGCTGAACGTGCGAAGCCACCTGCAACGGGACCTGTACAGACAAC
 K E I G L L N C E A T V N G H L Y Q T N
 670 680 690 700 710 720
 TATCTGACCCATCGGCGAGACCAATACAATCCTAGATGTCCAAATACGCGCCGCGAGCCCA
 Y L T H R Q T N T I L D V Q I R P P S P
 730 740 750 760 770 780
 GTGAGACTGCTCCACGGGCGAGACTCTTGTCTCAACTGCACCGCCACACGGAGCTCAAT
 V R L L H G Q T L V L N C T A T T E L N
 790 800 810 820 830 840
 ACGAGGGTCAAAATGAGCTGGAATACCTGTGTAAGCACTAAGAGAGCATCTATAAGG
 T R V Q M S W N Y P G K A T K R A S I R
 850 860 870 880 890 900
 CAGCGGATTGACCGGAGCCATTCACACAACATGTGTCCACAGTGTCTTAAAGATCAAC
 Q R I D R S H S H N N V F H S V L K I N
 910 920 930 940 950 960
 AATGTGGAGACCGGAGACAAGGGGCTCTACACCTGTGCGGTGAAGAGTGGGTCTCGTTC
 N V E S R D K G L Y T C R V K S G S S F
 970 980 990 1000 1010 1020
 CAGTCTTTCAACACCTCCGTGCATGTGTATGAAAAGGATTTCATCAGTGTGAAACATCGG
 Q S F N T S V H V Y E K G F I S V K H R
 1030 1040 1050 1060 1070 1080
 AAGCAGCCGTGTCAGGAACACAGCAGGAGACCGTCTATCGGTGTCCATGAAAGTG
 K Q P V Q E T T A G R R S Y R L S M K V
 1090 1100 1110 1120 1130 1140
 AAGGCCCTCCCTCCCGAGAAATCGTATGGTTAAAGATGGCTCGCCTGCACATTGAAG
 K A F P S P E I V W L K D G S P A T L K
 1150 1160 1170 1180 1190 1200
 TCTGCTCGCTATTGGTACATGGCTACTCATTAAATATCAAGATGTGACAAACCGAGGAT
 S A R Y L V H G Y S L I I K D V T T E D
 1210 1220 1230 1240 1250 1260
 GCAGGGGACTATACGATCTTGTCTGGGCATAAAGCAGTCAAGGCTATTAAAAACCTCACT
 A G D Y T I L L G I K Q S R L F K N L T
 1270 1280 1290 1300 1310 1320
 GCCACTCTCATTGTAACGTGAAACCTCAGATCTACGAAAAGTCCGTGTCTCGCTTCCA
 A T L I V N V K P Q I Y E K S V S L P
 1330 1340 1350 1360 1370 1380
 AGCCCACTCTCTATCCGCTGGGCAGCAGACAAGTCTCTCACTTGCACCGTGTATGGCATC
 S P P L Y P L G S R Q V L T C T V Y G I
 1390 1400 1410 1420 1430 1440
 CCTCGGCCAACAATCACGTGGCTCTGGCACCCCTGTCCACACAATCACTCCAAAGAAAGG
 P R P T I T W L W H P C H H N H S K E R
 1450 1460 1470 1480 1490 1500
 TATGACTTCTGCAGTGAAGTGAAGAAATCCTTTATCCTGGATCCAGCAGCAACTTAGGA
 Y D F C T E N E E S F I L D P S S N L G
 1510 1520 1530 1540 1550 1560
 AACAGAAATTGAGAGCATCTCTCAGCGCATGACGGTCATAGAAGGAACAATAAGACGGTT
 N R I E S I S Q R M T V I E G T N K T V
 1570 1580 1590 1600 1610 1620
 AGCACATTGGTGGTGGCTGACTCTCAGACCCCTGGAATCTACAGCTGCCGGGCTTCAAT
 S T L V V A D S Q T P G I Y S C R A F N
 1630 1640 1650 1660 1670 1680
 AAAATAGGGACTGTGGAAAGAAACATAAAATTTATGTACAGATGTGCCGAATGGCTTT
 K I G T V E R N I K F Y V T D V F N G F
 1690 1700 1710 1720 1730 1740
 CACGTTTCCCTTGGAAAAGATGCCAGCGGAAGGAGAGGACCTGAAACTGTCTGTGGTCT
 H V S L E K M P A E G E D L K L S C V V
 1750 1760 1770 1780 1790 1800
 AATAAATTCCTGTACAGAGACATTACCTGGATTCTGCTACGGACAGTTAACAACAGAAC
 N K F L Y R D I T W I L L R T V N N R T
 1810 1820 1830 1840 1850 1860
 ATGCACCATAGTATACGCAAGCAAAAATGGCCACCCTCAAGATTACTCCATCACTCTG
 M H H S I S K Q K M A T T Q D Y S I T L
 1870 1880 1890 1900 1910 1920
 AACCTTGTCTAAGAACGTGTCTCTAGAAGACTCGGGCACCTTATGCGTGCAGAGCCAGG
 N L V I K N V S L E D S G T Y A C R A R
 1930 1940 1950 1960 1970 1980
 AACATATACACAGGGGAAGACATCCTTCGGAAGACAGAAGTTCTCGTTAGAGATTCCGAA
 N I Y T G E D I L R K T E V I V R D S E
 1990 2000 2010 2020 2030 2040
 GCGCCACACCTGCTTCAAAACCTCAGTGACTACGAGGTCTCCATCAGTGGCTCTACGACC
 A P H L L Q N L S D Y E V S I S G S T T
 2050 2060 2070 2080 2090 2100
 TTAGACTGTCAAGCTAGAGGTGTCCCGCGGCTCAGATCACTTGGTTCAAAAACAACCAC
 L D C Q A R G V P A P Q I T W F K N N H
 2110 2120 2130 2140 2150 2160
 AAAATACAACAAGAACCGGAATTATTTAGGACCAGGAACAGCAGCTGTGTTATTGAA
 K I Q Q E P G I I L G P G N S T L F I E
 2170 2180 2190 2200 2210 2220
 AGAGTCACAGAGGAGGATGAGGGTGTCTATAGGTGCCGAGCCACCAACAGAGGGGGCC
 R V T E E D E G V Y R C R A T N Q K G A
 GTGTAG
 V *

Supplemental Figure S2.

The sequence of the CpG-free sFlt-1 used in the present study

76 unmethylated CpG-motifs were deleted by replacing the codon to other one that can be translated to the same amines.