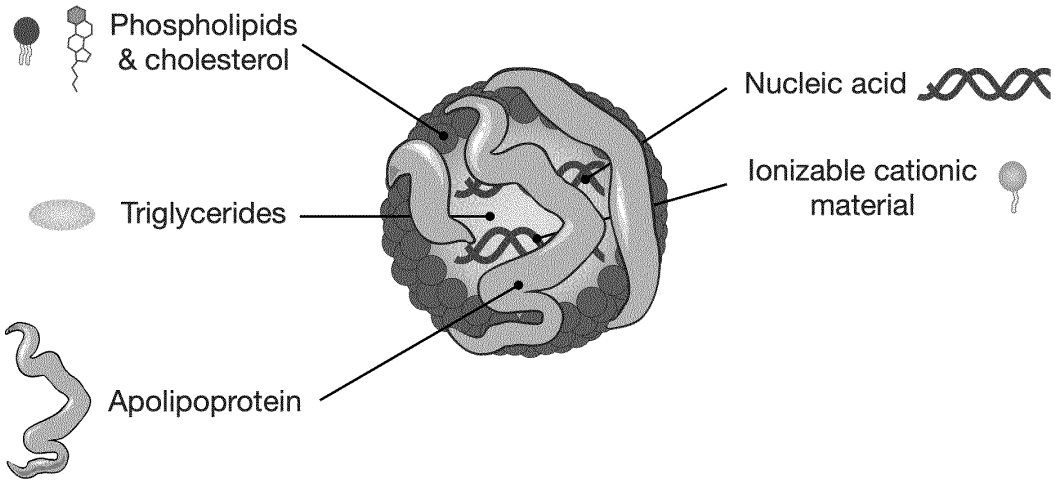
FIG. 1


Phospholipids
& cholesterol
Triglycerides
Apolipoprotein
Nucleic acid
Ionizable cationic
material

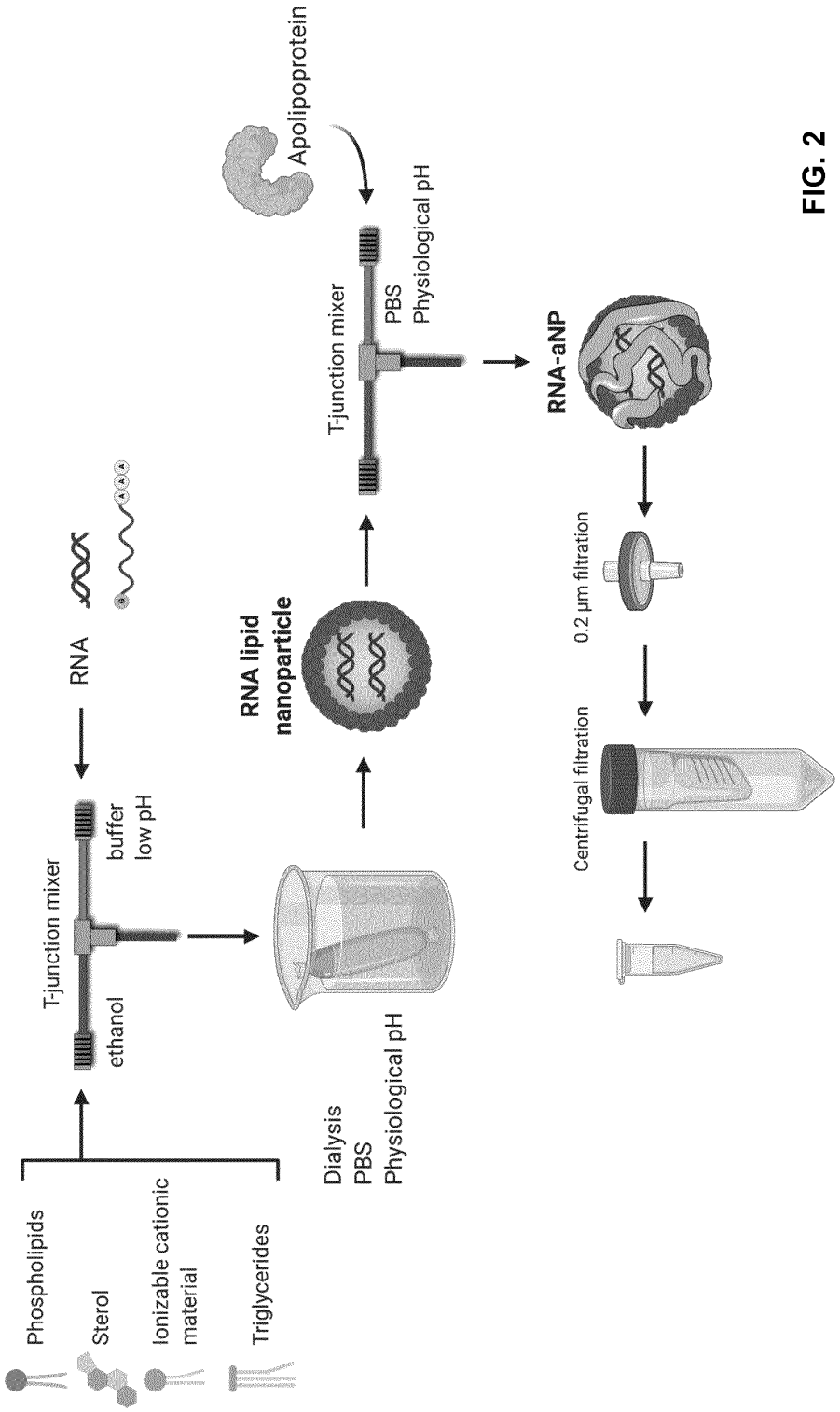
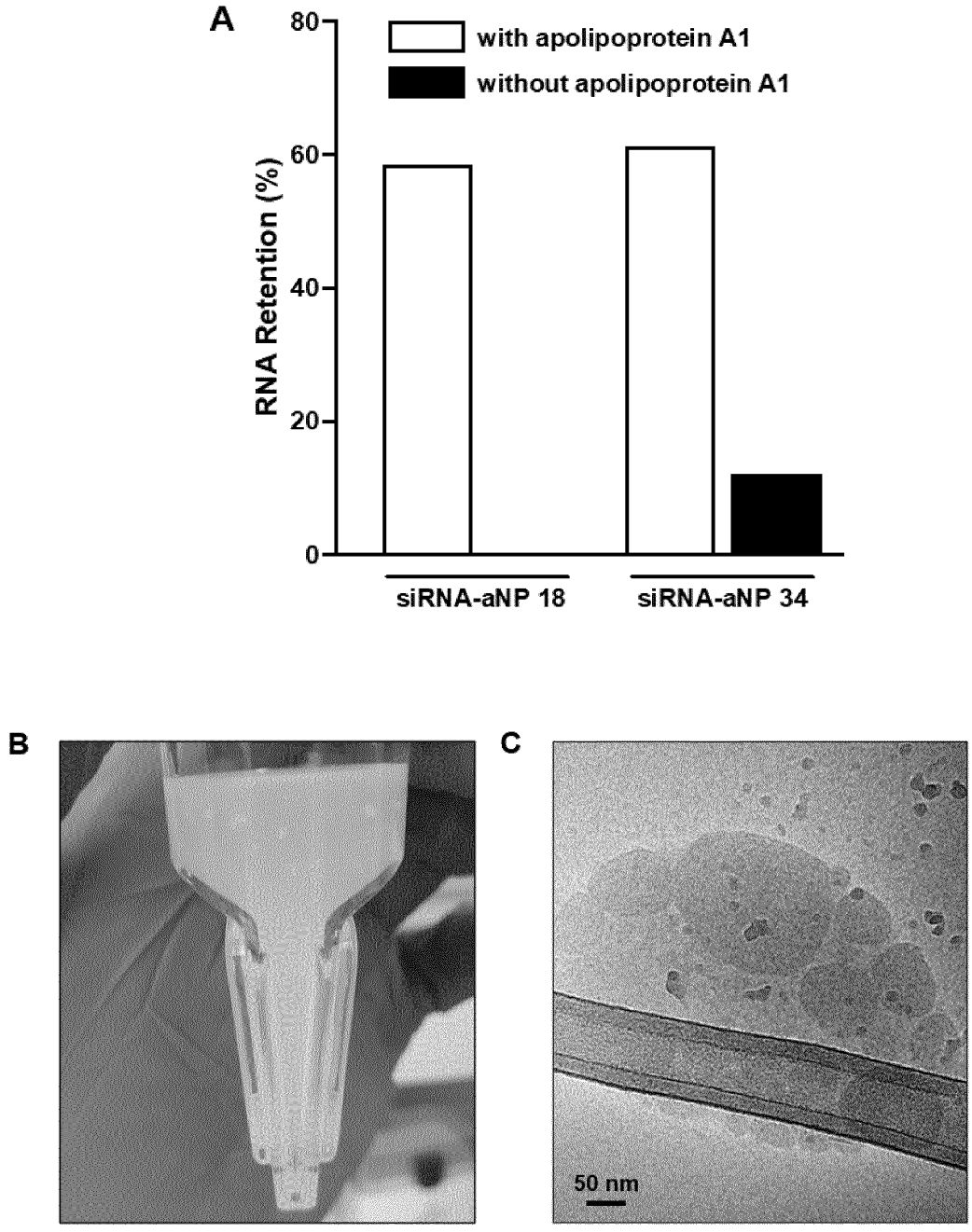

Phospholipids
Sterol
Ionizable cationic material
Triglycerides
T-junction mixer
ethanol
buffer low pH
RNA
Dialysis
PBS
Physiological pH
RNA lipid nanoparticle
Apolipoprotein
T-junction mixer
PBS
Physiological pH
RNA-aNP
0.2 µm filtration
Centrifugal filtration

FIG. 2

FIG. 3


A
80
60
40
20
0
RNA Retention (%)
with apolipoprotein A1
without apolipoprotein A1
siRNA-aNP 18
siRNA-aNP 34
B
C
50 nm

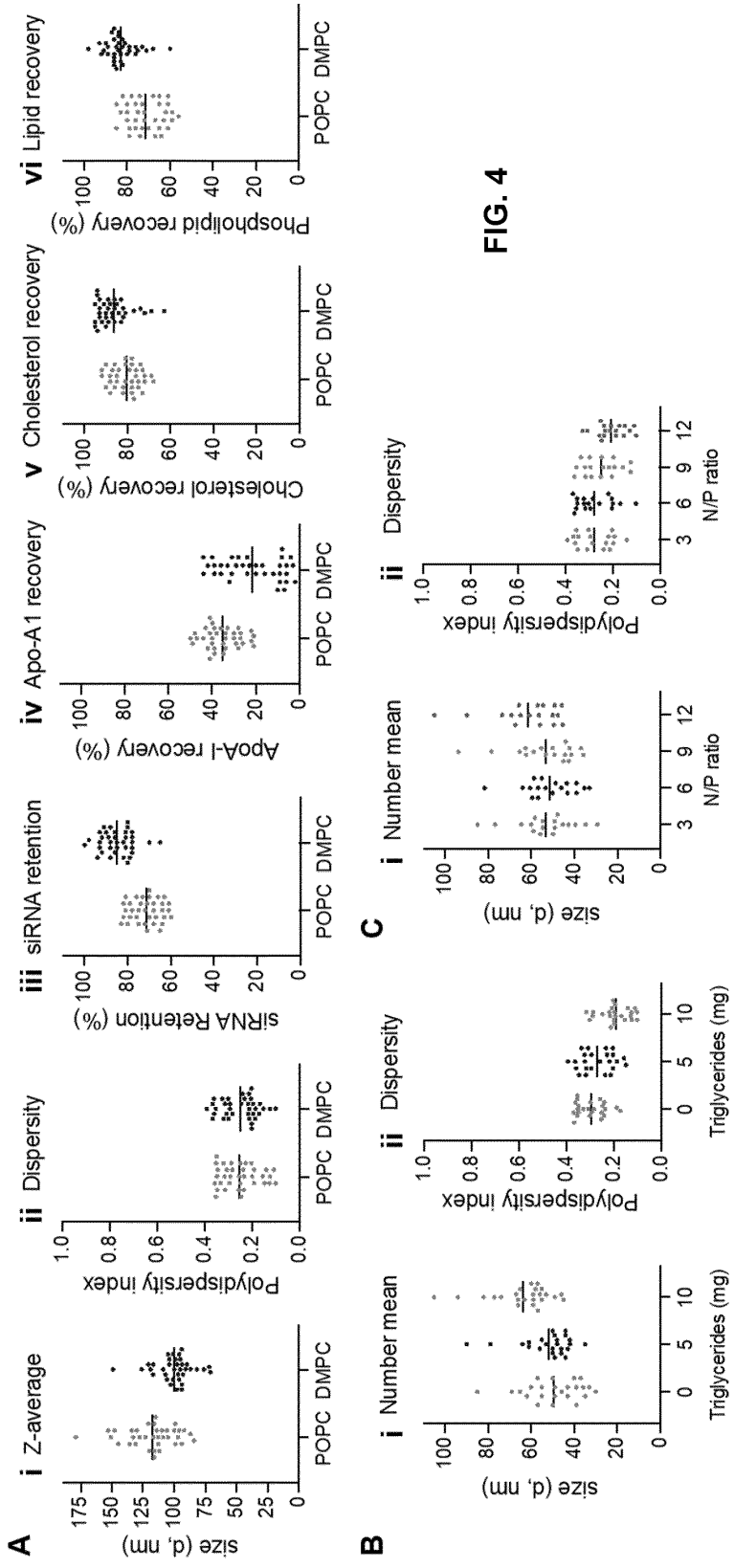

A
i Z-average
size (d, nm)
ii Dispersity
Polydispersity index
iii siRNA retention
siRNA Retention (%)
iv Apo-A1 recovery
ApoA-I recovery (%)
v Cholesterol recovery
Cholesterol recovery (%)
vi Lipid recovery
Phospholipid recovery (%)
POPC DMPC
B
i Number mean
size (d, nm)
ii Dispersity
Polydispersity index
Triglycerides (mg)
C
i Number mean
size (d, nm)
ii Dispersity
Polydispersity index
N/P ratio

FIG. 4

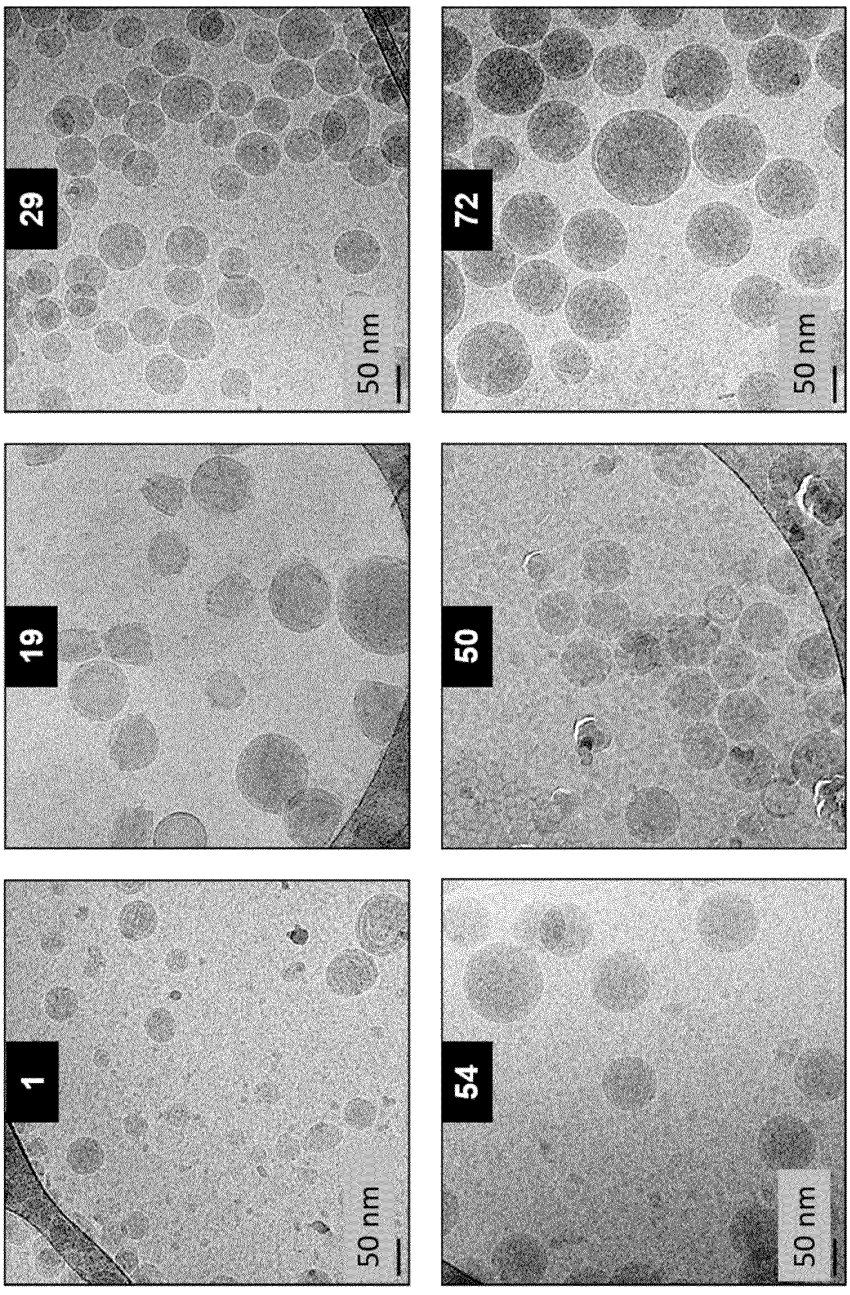


FIG. 5

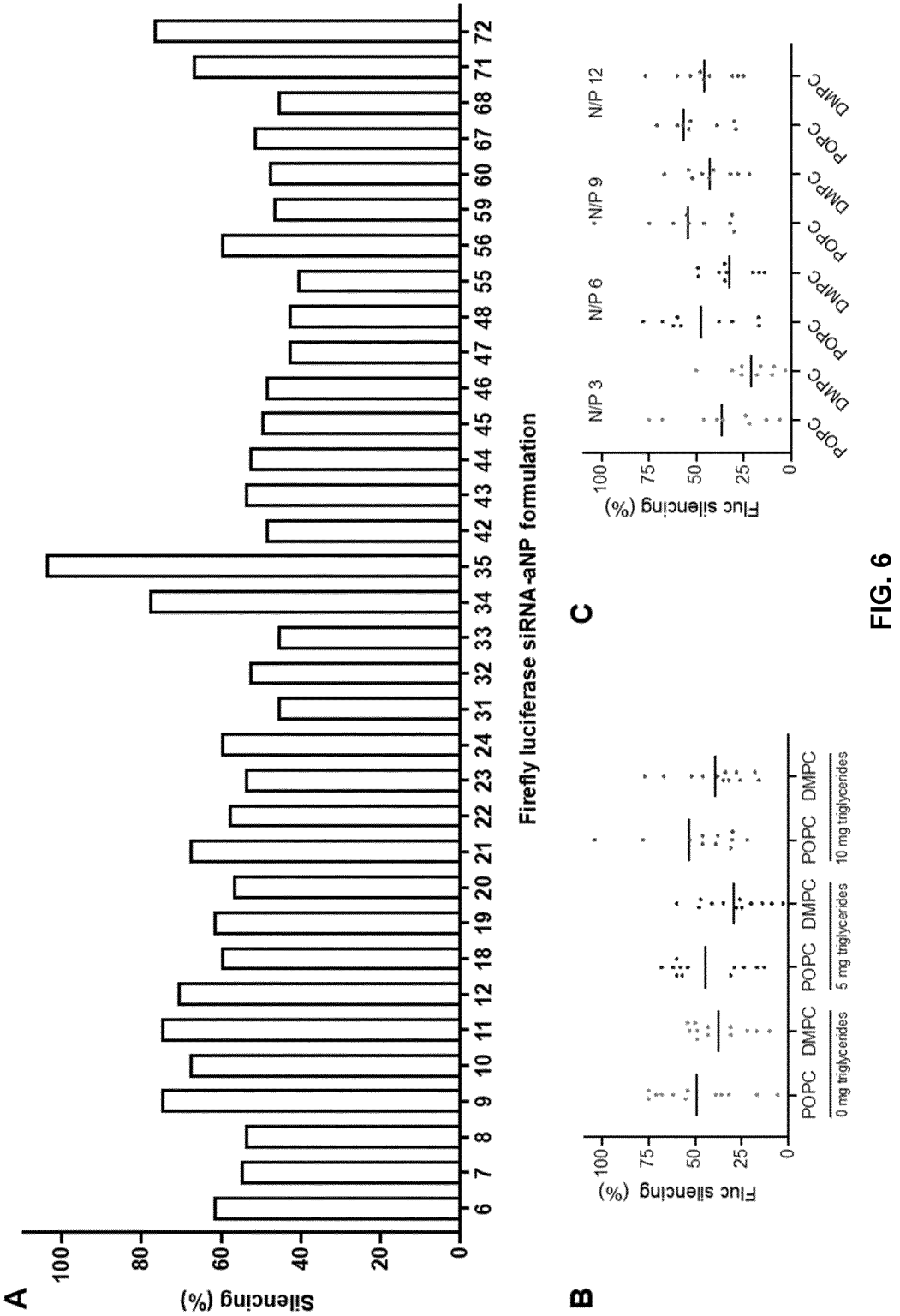

A
Silencing (%)
100
80
60
40
20
0
6 7 8 9 10 11 12 18 19 20 21 22 23 24 31 32 33 34 35 42 43 44 45 46 47 48 55 56 59 60 67 68 71 72
Firefly luciferase siRNA-aNP formulation
B
Fluc silencing (%)
100
75
50
25
0
POPC DMPC POPC DMPC POPC DMPC
0 mg triglycerides
5 mg triglycerides
10 mg triglycerides
C
Fluc silencing (%)
100
75
50
25
0
N/P 3
N/P 6
N/P 9
N/P 12
POPC DMPC POPC DMPC POPC DMPC POPC DMPC

FIG. 6

FIG. 7

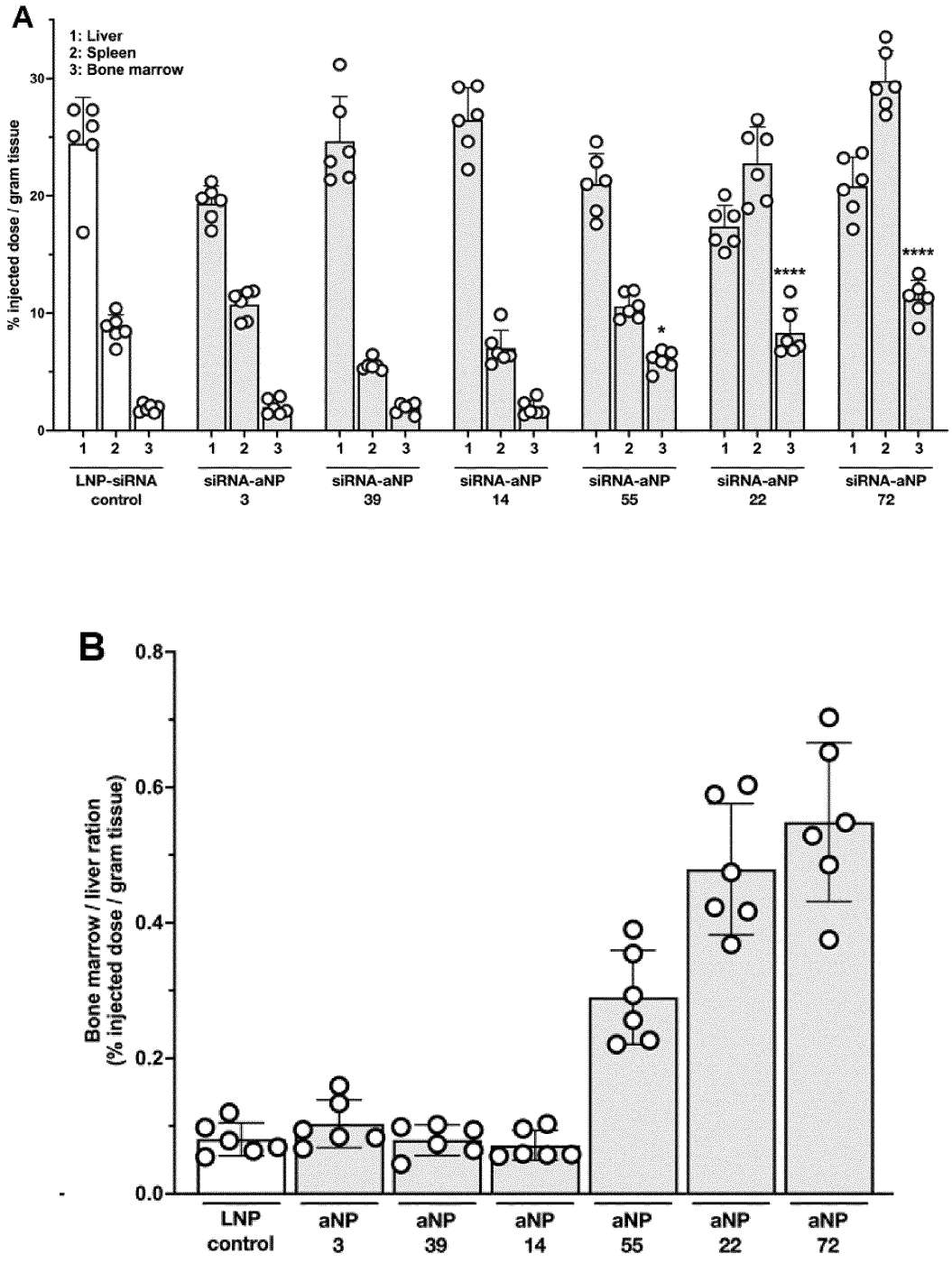

A
1: Liver
2: Spleen
3: Bone marrow
% injected dose / gram tissue
0
10
20
30
****
*
****
LNP-siRNA control
siRNA-aNP 3
siRNA-aNP 39
siRNA-aNP 14
siRNA-aNP 55
siRNA-aNP 22
siRNA-aNP 72
B
Bone marrow / liver ration (% injected dose / gram tissue)
0.0
0.2
0.4
0.6
0.8
LNP control
aNP 3
aNP 39
aNP 14
aNP 55
aNP 22
aNP 72

FIG. 8

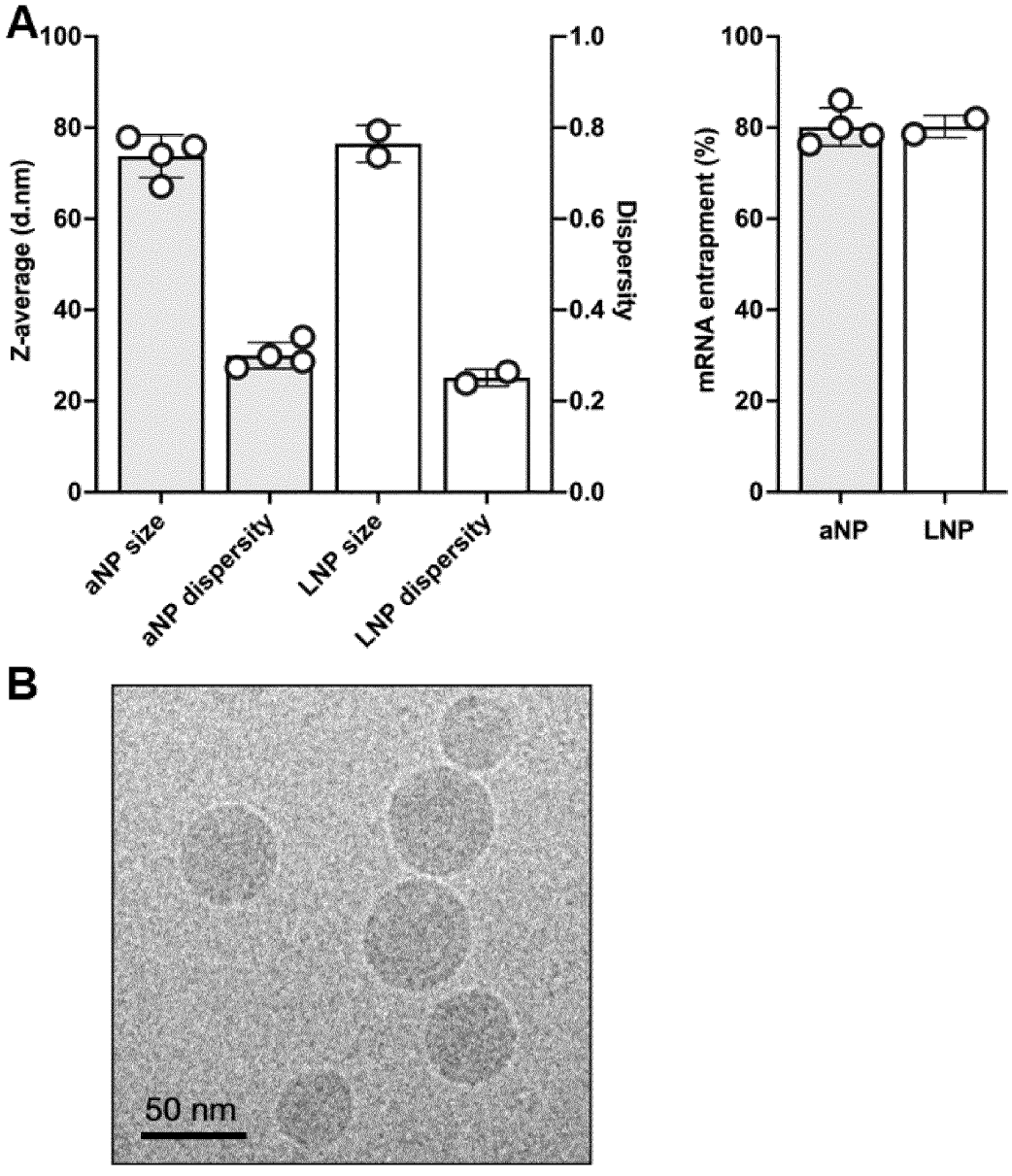
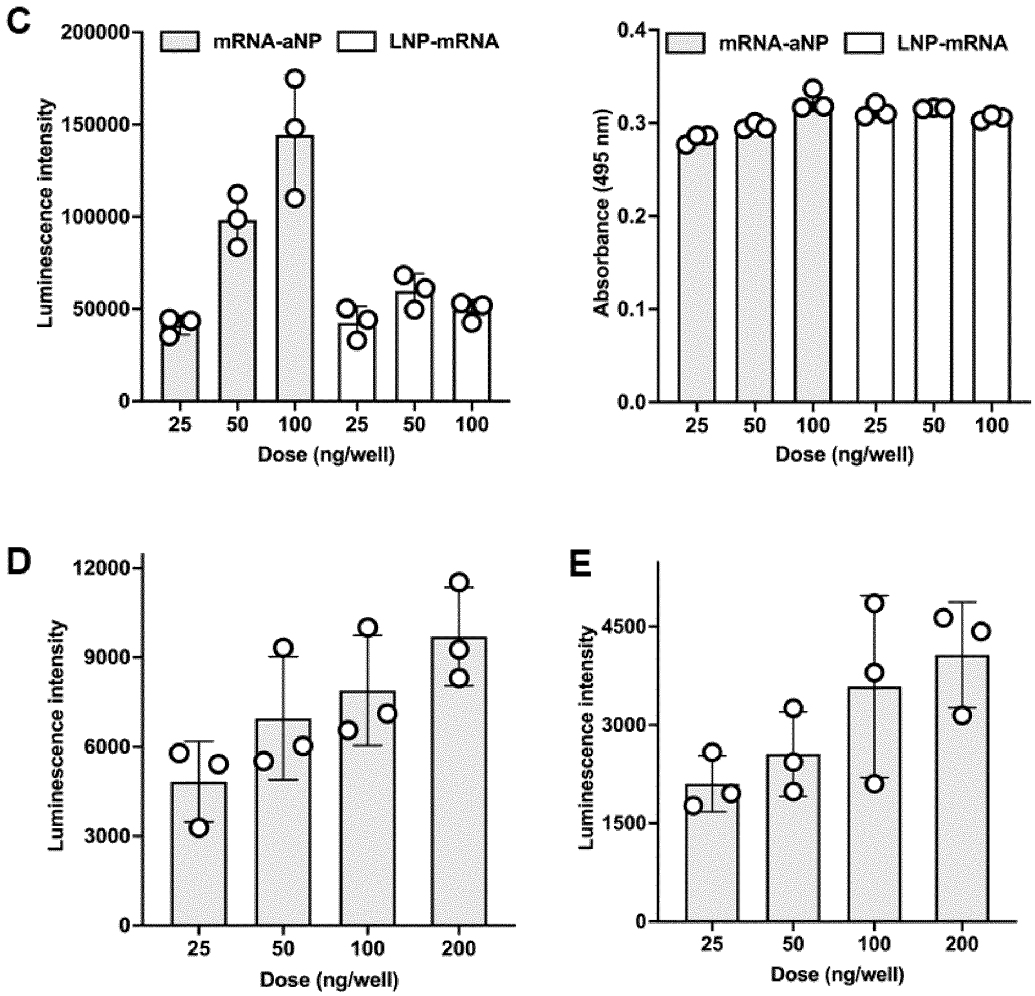

A
Z-average (d.nm)
Dispersity
100
80
60
40
20
0
1.0
0.8
0.6
0.4
0.2
0.0
aNP size
aNP dispersity
LNP size
LNP dispersity
mRNA entrapment (%)
aNP
LNP
B
50 nm

FIG. 8 (continued)



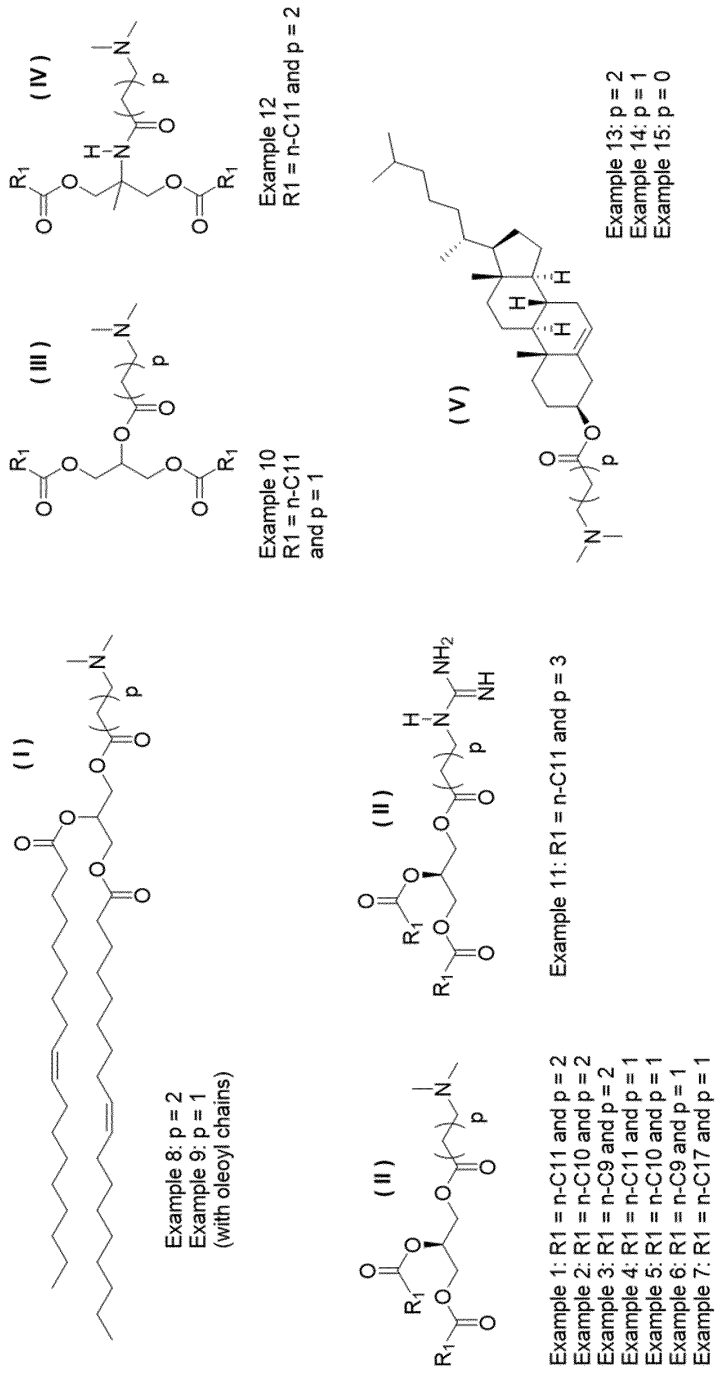

(I)
Example 8: p = 2
Example 9: p = 1
(with oleoyl chains)
(II)
Example 1: R1 = n-C11 and p = 2
Example 2: R1 = n-C10 and p = 2
Example 3: R1 = n-C9 and p = 2
Example 4: R1 = n-C11 and p = 1
Example 5: R1 = n-C10 and p = 1
Example 6: R1 = n-C9 and p = 1
Example 7: R1 = n-C17 and p = 1
(II)
Example 11: R1 = n-C11 and p = 3
(III)
Example 10
R1 = n-C11
and p = 1
(IV)
Example 12
R1 = n-C11 and p = 2
(V)
Example 13: p = 2
Example 14: p = 1
Example 15: p = 0

FIG. 9

FIG. 10

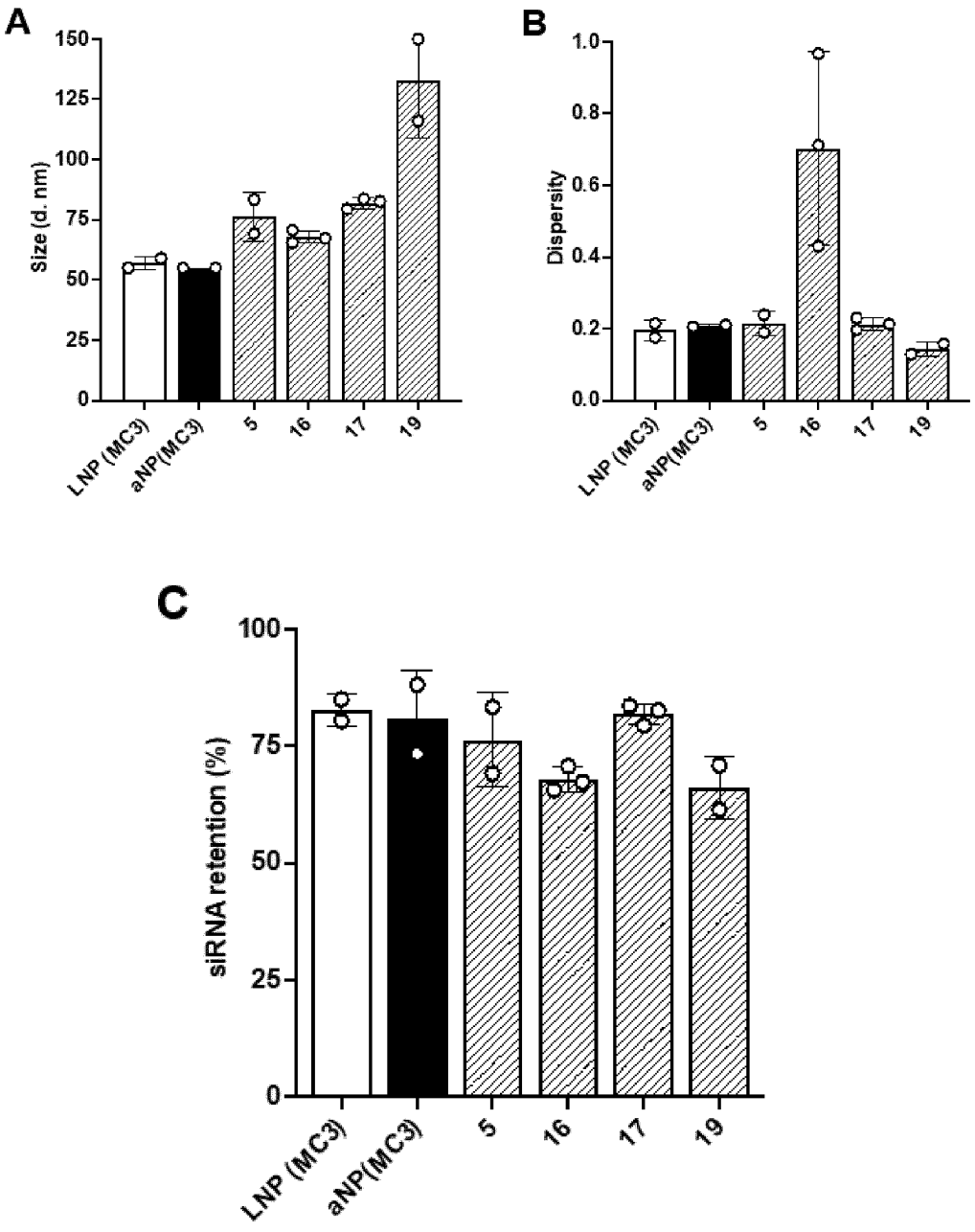

A
Size (d. nm)
150
125
100
75
50
25
0
LNP (MC3)
aNP(MC3)
5
16
17
19
B
Dispersity
1.0
0.8
0.6
0.4
0.2
0.0
LNP (MC3)
aNP(MC3)
5
16
17
19
C
siRNA retention (%)
100
75
50
25
0
LNP (MC3)
aNP(MC3)
5
16
17
19

Fig. 11

Table 1

Formulation #	Phospholipid	Phospholipid (mg)	Dlin-MC3-DMA (mg)	Cholesterol (mg)	Triglycerides (mg)	Apo A1 (mg)	siRNA (mg)
1	POPC	2.5	1.25	0.54	0	1	0.2
3	POPC	2.5	3.75	0.54	0	1	0.2
6	DMPC	2.5	2.5	0.54	0	1	0.2
7	DMPC	2.5	3.75	0.54	0	1	0.2
8	DMPC	2.5	5	0.54	0	1	0.2
9	POPC	2.5	1.25	1.35	0	1	0.2
10	POPC	2.5	2.5	1.35	0	1	0.2
11	POPC	2.5	3.75	1.35	0	1	0.2
12	POPC	2.5	5	1.35	0	1	0.2
14	DMPC	2.5	2.5	1.35	0	1	0.2
18	POPC	2.5	2.5	3.37	0	1	0.2
19	POPC	2.5	3.75	3.37	0	1	0.2
20	POPC	2.5	5	3.37	0	1	0.2
21	DMPC	2.5	1.25	3.37	0	1	0.2
22	DMPC	2.5	2.5	3.37	0	1	0.2
23	DMPC	2.5	3.75	3.37	0	1	0.2
24	DMPC	2.5	5	3.37	0	1	0.2
29	DMPC	2.5	1.25	0.54	5	1	0.2
31	DMPC	2.5	3.75	0.54	5	1	0.2
32	DMPC	2.5	5	0.54	5	1	0.2
33	POPC	2.5	1.25	1.35	5	1	0.2
34	POPC	2.5	2.5	1.35	5	1	0.2
35	POPC	2.5	3.75	1.35	5	1	0.2
39	DMPC	2.5	3.75	1.35	5	1	0.2
42	POPC	2.5	2.5	3.37	5	1	0.2
43	POPC	2.5	3.75	3.37	5	1	0.2
44	POPC	2.5	5	3.37	5	1	0.2
45	DMPC	2.5	1.25	3.37	5	1	0.2
46	DMPC	2.5	2.5	3.37	5	1	0.2
47	DMPC	2.5	3.75	3.37	5	1	0.2
48	DMPC	2.5	5	3.37	5	1	0.2
50	POPC	2.5	2.5	0.54	10	1	0.2
54	DMPC	2.5	2.5	0.54	10	1	0.2
55	DMPC	2.5	3.75	0.54	10	1	0.2
56	DMPC	2.5	5	0.54	10	1	0.2
59	POPC	2.5	3.75	1.35	10	1	0.2
60	POPC	2.5	5	1.35	10	1	0.2
67	POPC	2.5	3.75	3.37	10	1	0.2
68	POPC	2.5	5	3.37	10	1	0.2
71	DMPC	2.5	3.75	3.37	10	1	0.2
72	DMPC	2.5	5	3.37	10	1	0.2

Fig. 11 (continued)

Formulation #	Phospholipid (mol%)	Dlin-MC3-DMA	Cholesterol (mol %)	ApoA1 (mol%)	siRNA (mol%)	Triglycerides (mol%)
1	49%	29%	21%	0.50%	0.18%	0%
3	31%	55%	13%	0.32%	0.11%	0%
6	41%	43%	15%	0.37%	0.13%	0%
7	34%	53%	13%	0.31%	0.11%	0%
8	29%	60%	11%	0.26%	0.09%	0%
9	37%	22%	40%	0.38%	0.14%	0%
10	31%	36%	33%	0.31%	0.11%	0%
11	26%	46%	28%	0.26%	0.10%	0%
12	22%	53%	24%	0.23%	0.08%	0%
14	33%	35%	31%	0.30%	0.11%	0%
18	21%	24%	55%	0.21%	0.08%	0%
19	18%	33%	49%	0.19%	0.07%	0%
20	17%	39%	44%	0.17%	0.06%	0%
21	26%	14%	61%	0.23%	0.08%	0%
22	23%	24%	53%	0.21%	0.07%	0%
23	20%	32%	48%	0.18%	0.07%	0%
24	18%	38%	43%	0.17%	0.06%	0%
29	21%	11%	8%	0.19%	0.07%	60%
31	17%	27%	6%	0.16%	0.06%	49%
32	16%	33%	6%	0.14%	0.05%	45%
33	17%	10%	18%	0.17%	0.06%	55%
34	15%	18%	16%	0.16%	0.06%	50%
35	14%	25%	15%	0.14%	0.05%	46%
39	16%	25%	15%	0.14%	0.05%	45%
42	12%	15%	33%	0.13%	0.05%	40%
43	12%	20%	31%	0.12%	0.04%	37%
44	11%	26%	29%	0.11%	0.04%	35%
45	15%	8%	35%	0.13%	0.05%	42%
46	14%	14%	32%	0.12%	0.04%	39%
47	13%	20%	30%	0.12%	0.04%	37%
48	12%	25%	28%	0.11%	0.04%	34%
50	11%	13%	5%	0.11%	0.04%	71%
54	12%	13%	5%	0.11%	0.04%	70%
55	11%	18%	4%	0.10%	0.04%	66%
56	11%	23%	4%	0.10%	0.04%	62%
59	10%	17%	10%	0.10%	0.04%	63%
60	9%	22%	10%	0.09%	0.03%	59%
67	8%	15%	22%	0.09%	0.03%	54%
68	8%	19%	21%	0.08%	0.03%	52%
71	9%	15%	22%	0.08%	0.03%	54%
72	9%	19%	21%	0.08%	0.03%	51%