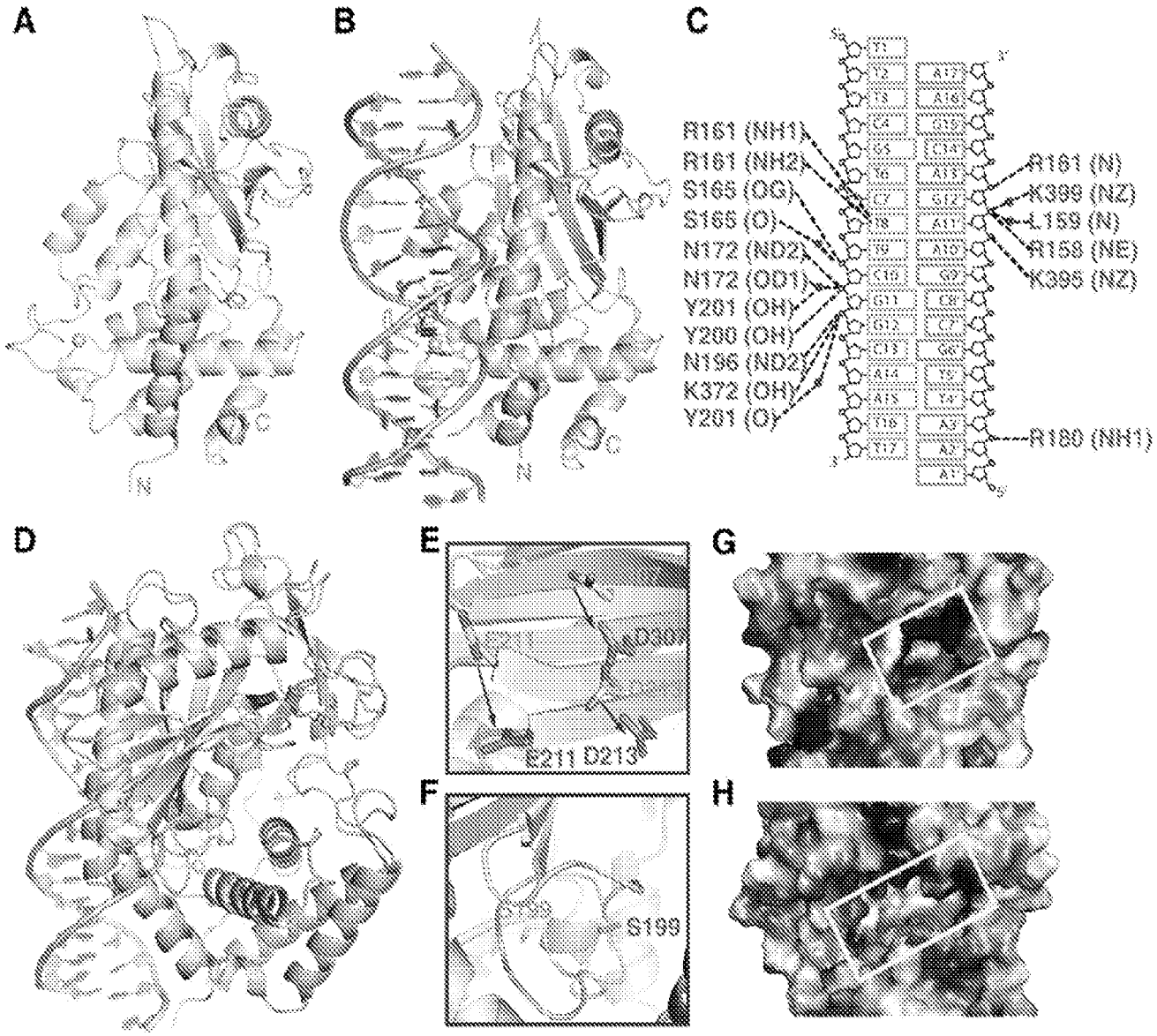

A
B
C
R161 (NH1)
R161 (NH2)
S165 (OG)
S165 (O)
N172 (ND2)
N172 (OD1)
Y201 (OH)
Y200 (OH)
N196 (ND2)
K372 (OH)
Y201 (O)
R161 (N)
K399 (NZ)
L159 (N)
R158 (NE)
K395 (NZ)
R180 (NH1)
D
E
E211 D213
F
S199
G
H

Figure 1

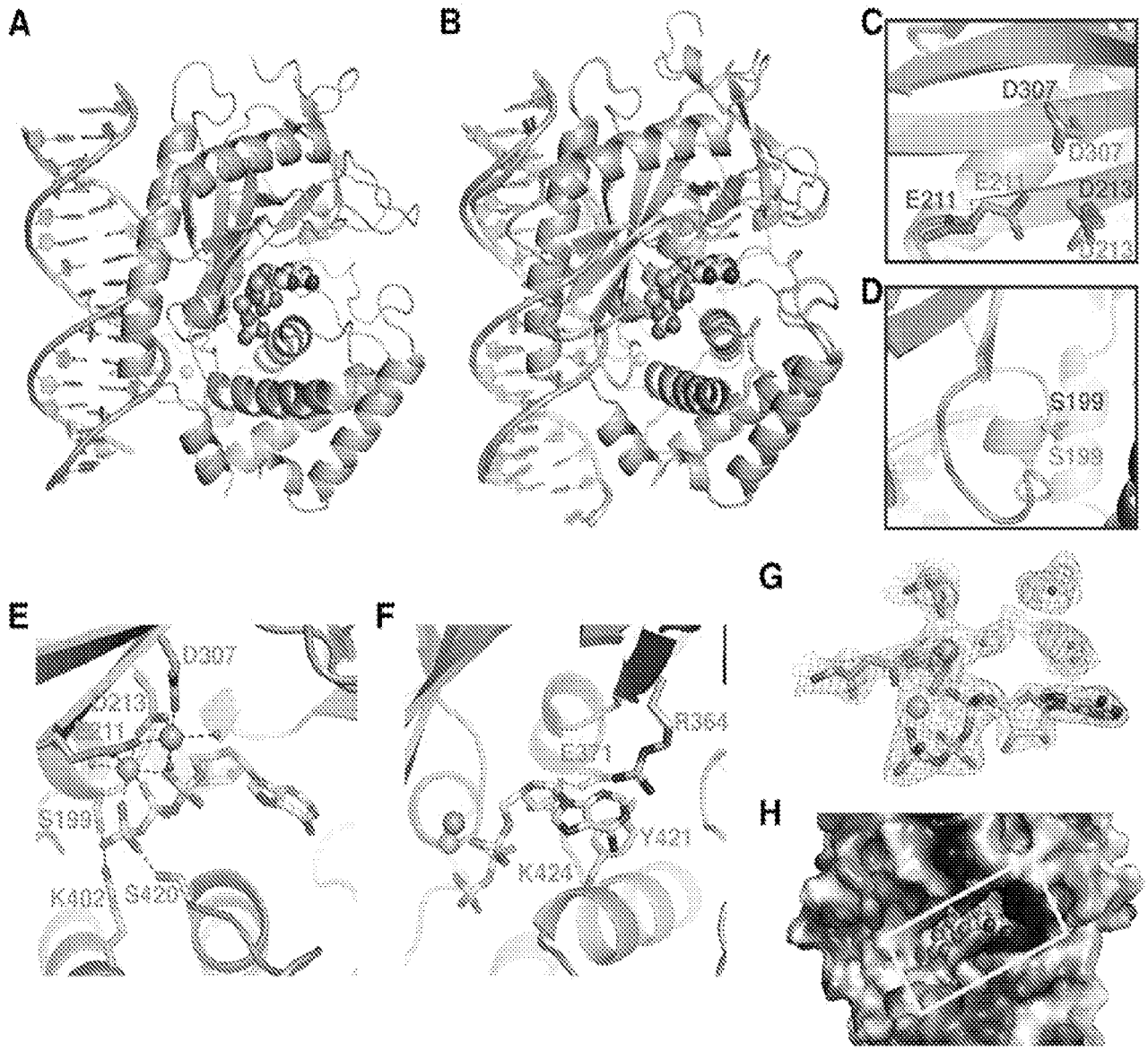

A
B
C
D307
D307
E211
E211
D213
D213
D
S195
S195
E
D307
D213
E211
S195
K402
S420
F
R364
E271
Y421
K424
G
H

Figure 2

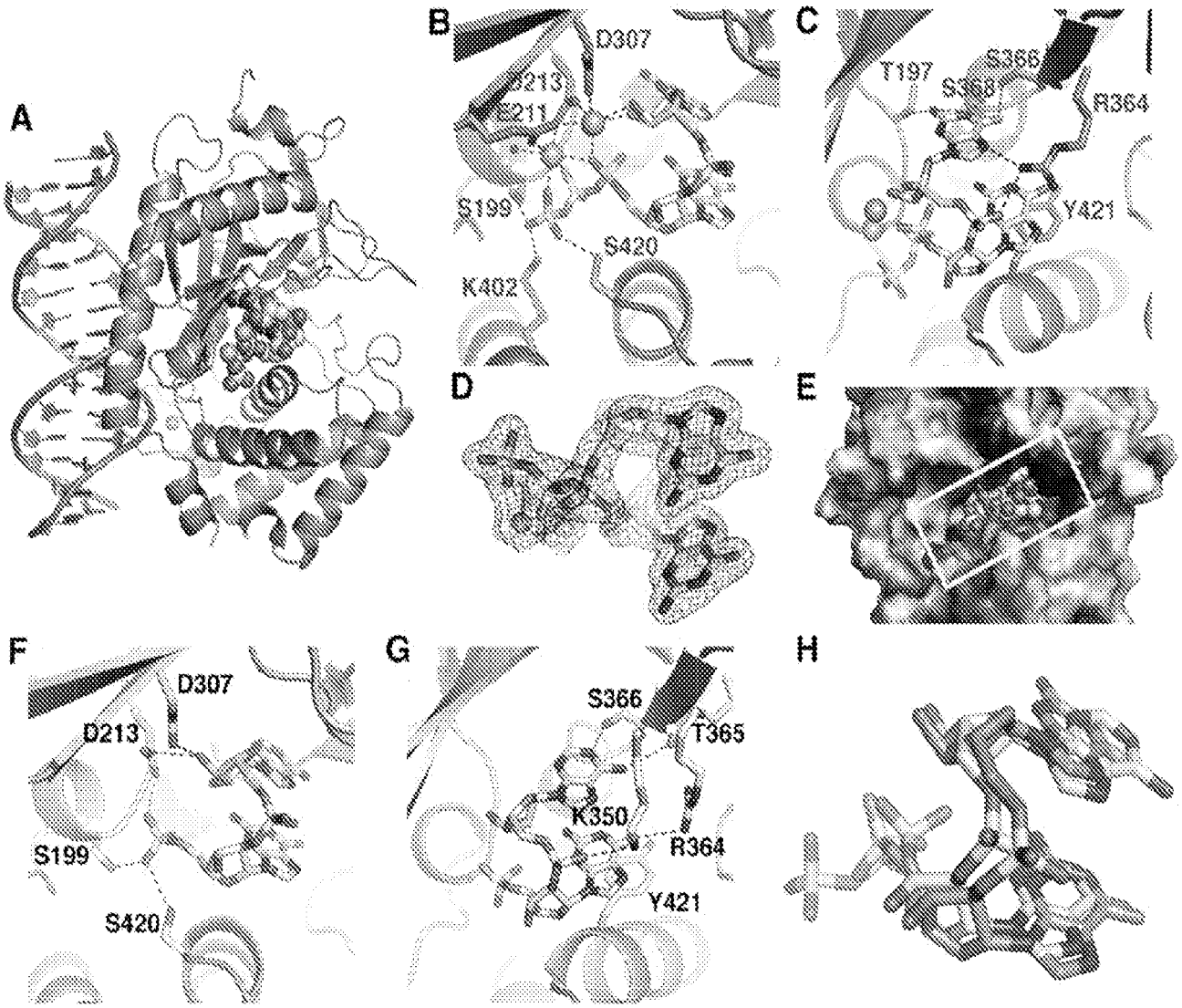

A
B
D307
D213
E211
S199
S420
K402
C
T197
S366
S368
R364
Y421
D
E
F
D307
D213
S199
S420
G
S366
T365
K350
R364
Y421
H

Figure 3

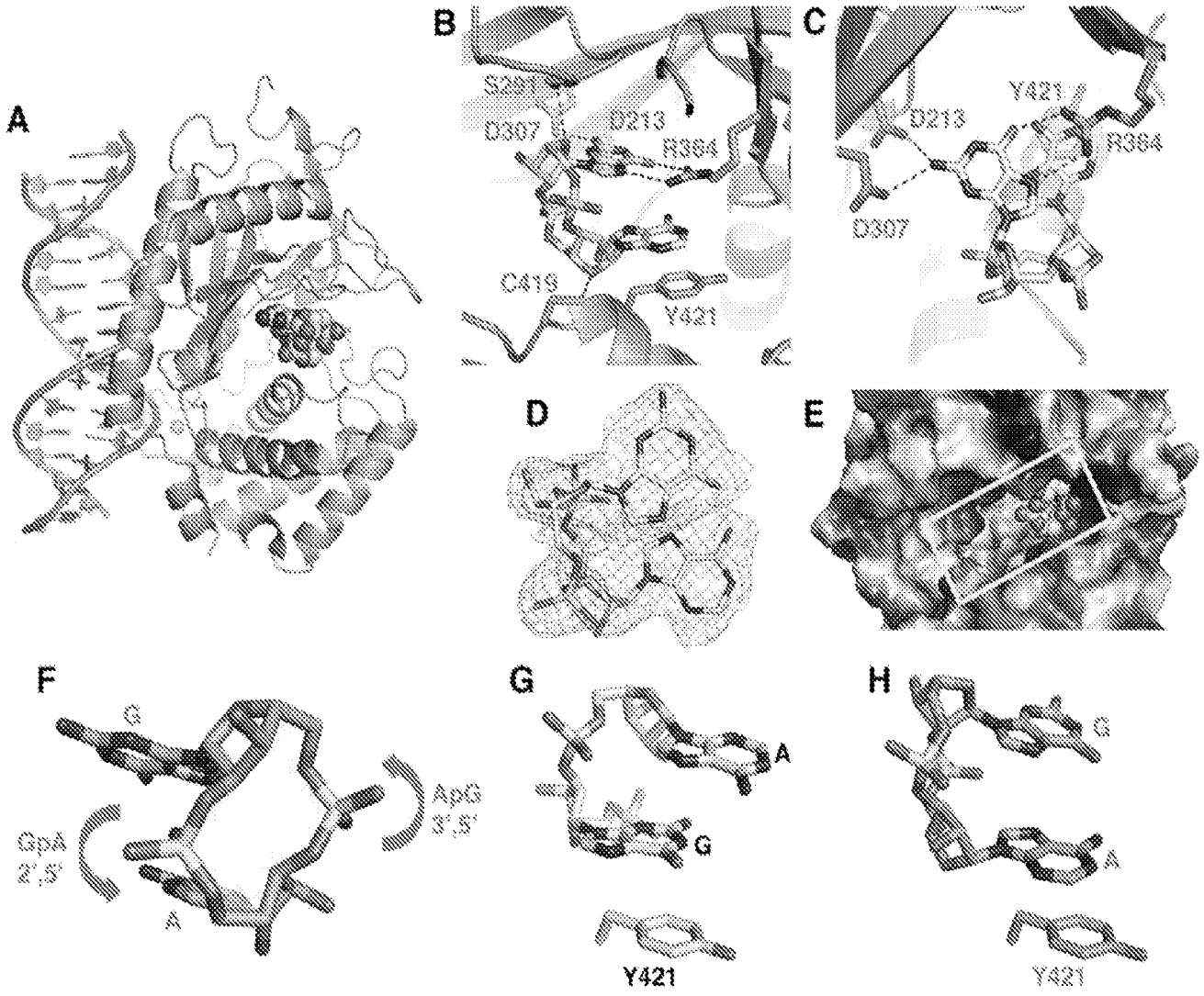

A
B
S281
D307
D213
R384
C419
Y421
C
Y421
D213
R384
D307
D
E
F
G
GpA
2',5'
ApG
3',5'
A
G
A
G
Y421
H
G
A
Y421

Figure 4

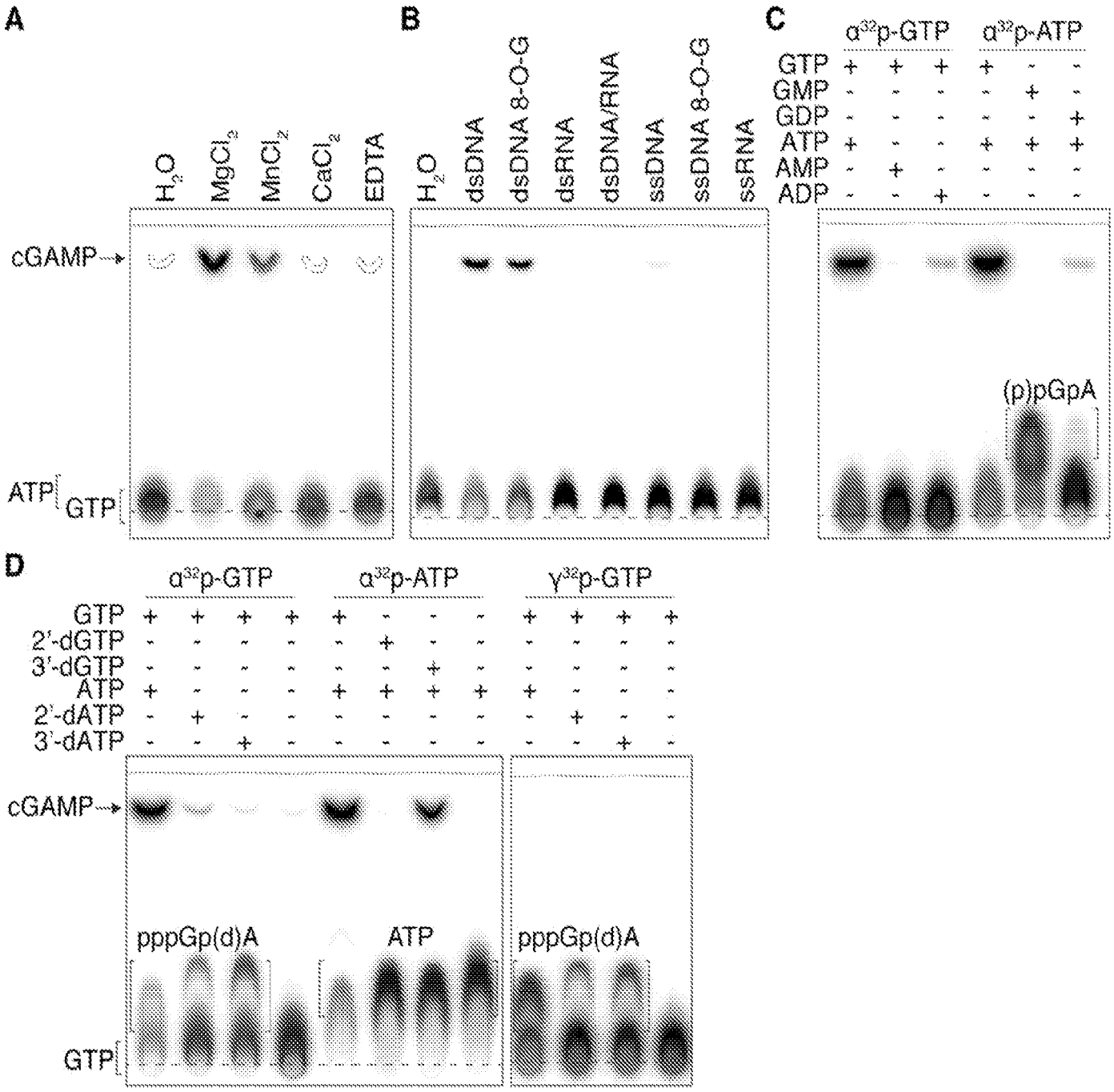

A
H2O
MgCl2
MnCl2
CaCl2
EDTA
cGAMP
ATP
GTP
B
H2O
dsDNA
dsDNA 8-O-G
dsRNA
dsDNA/RNA
ssDNA
ssDNA 8-O-G
ssRNA
C
α32p-GTP
α32p-ATP
GTP
GMP
GDP
ATP
AMP
ADP
(p)pGpA
D
α32p-GTP
α32p-ATP
γ32p-GTP
GTP
2'-dGTP
3'-dGTP
ATP
2'-dATP
3'-dATP
cGAMP
pppGp(d)A
ATP
pppGp(d)A
GTP

Figure 5

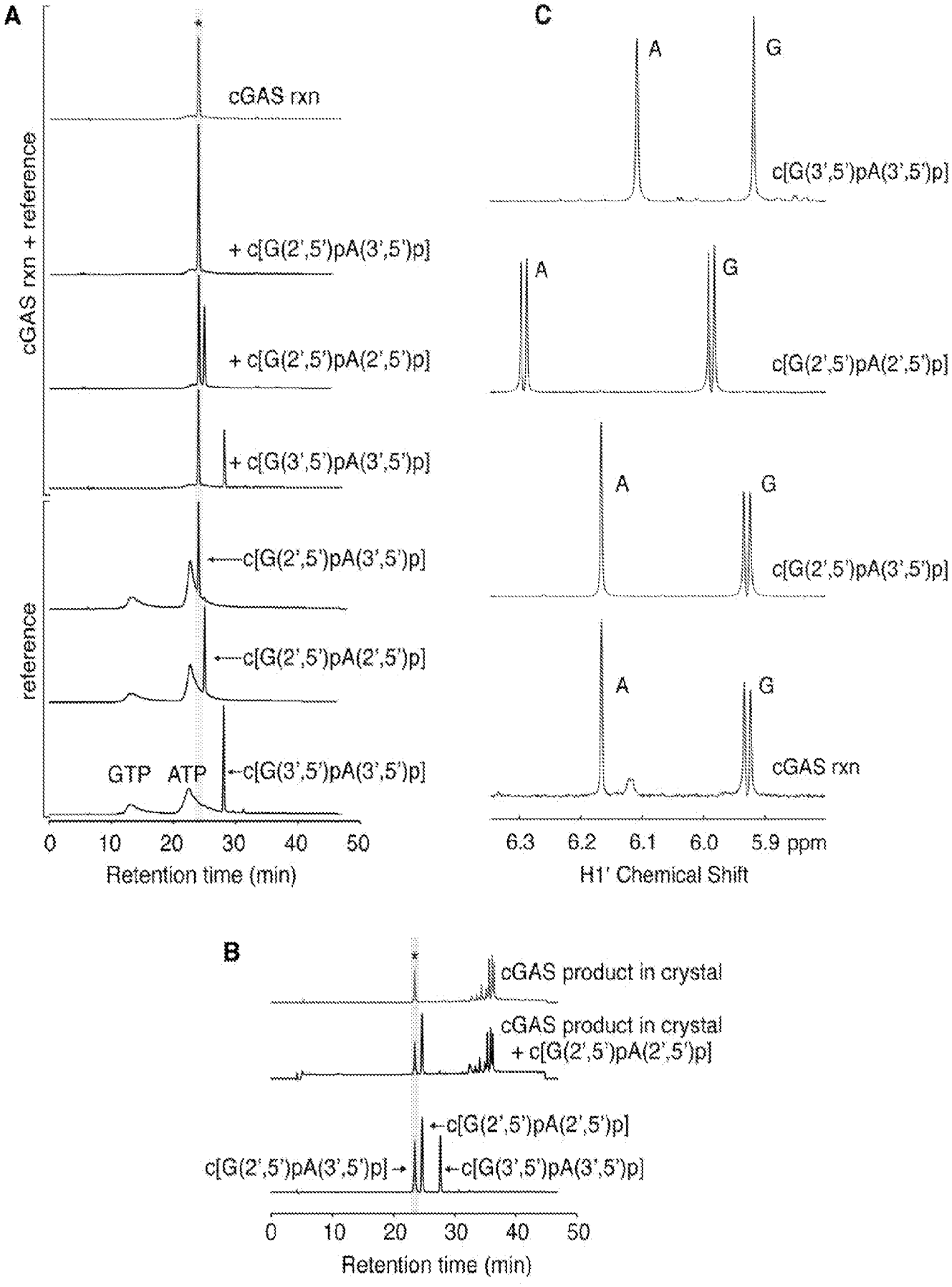

A
cGAS rxn + reference
cGAS rxn
+ c[G(2',5')pA(3',5')p]
+ c[G(2',5')pA(2',5')p]
+ c[G(3',5')pA(3',5')p]
reference
c[G(2',5')pA(3',5')p]
c[G(2',5')pA(2',5')p]
GTP ATP
c[G(3',5')pA(3',5')p]
0 10 20 30 40 50
Retention time (min)
C
A G
c[G(3',5')pA(3',5')p]
A G
c[G(2',5')pA(2',5')p]
A G
c[G(2',5')pA(3',5')p]
A G
cGAS rxn
6.3 6.2 6.1 6.0 5.9 ppm
H1' Chemical Shift
B
cGAS product in crystal
cGAS product in crystal
+ c[G(2',5')pA(2',5')p]
c[G(2',5')pA(2',5')p]
c[G(2',5')pA(3',5')p]
c[G(3',5')pA(3',5')p]
0 10 20 30 40 50
Retention time (min)

Figure 6

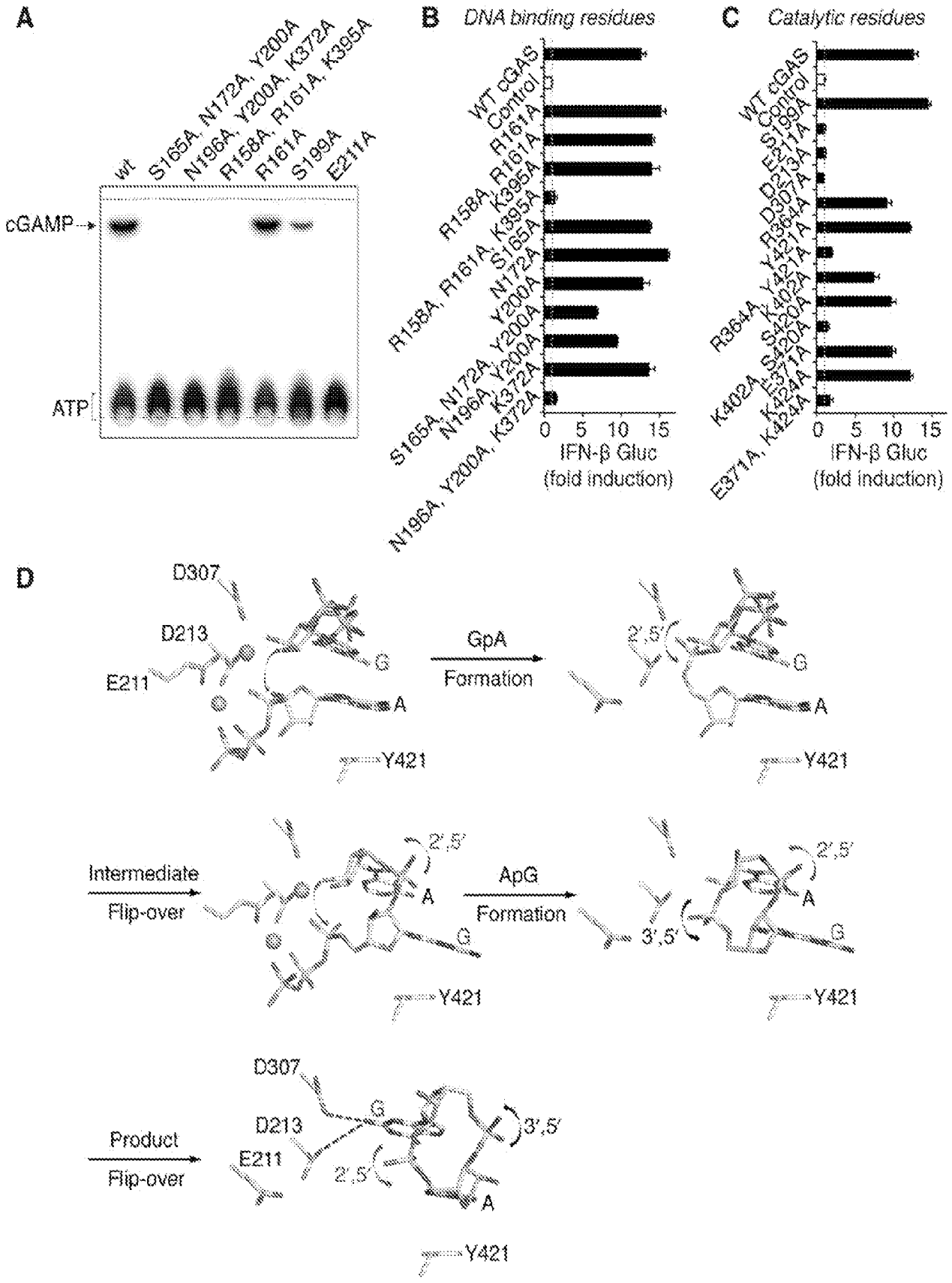

A
wt
S165A, N172A, Y200A
N196A, Y200A, K372A
R158A, R161A, K395A
R161A
S199A
E211A
cGAMP
ATP
B
DNA binding residues
WT cGAS
Control
R161A
R161A
R158A, R161A, K395A
K395A
S165A
N172A
Y200A
Y200A
Y200A
S165A, N172A, Y200A
N196A, Y200A, K372A
K372A
0 5 10 15
IFN-β Gluc
(fold induction)
C
Catalytic residues
WT cGAS
Control
S199A
E211A
D213A
D307A
R364A
Y421A
Y421A
K402A
S420A
S420A
E371A
K424A
E371A, K424A
0 5 10 15
IFN-β Gluc
(fold induction)
D
D307
D213
E211
G
A
Y421
GpA
Formation
2'5'
Intermediate
Flip-over
ApG
Formation
3'5'
Product
Flip-over

Figure 7

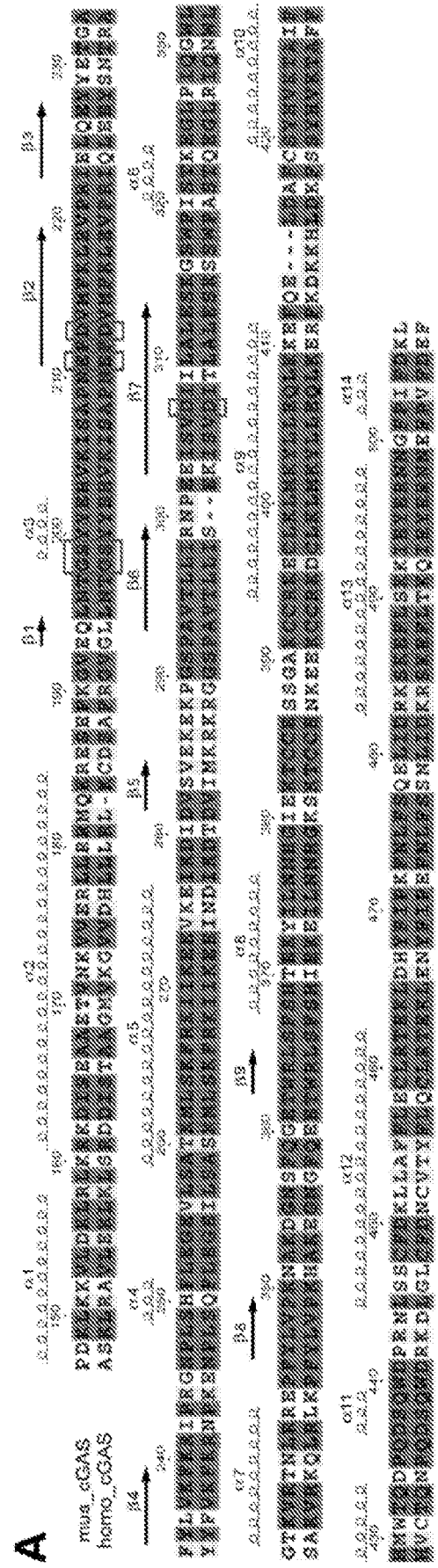


Figure S1A

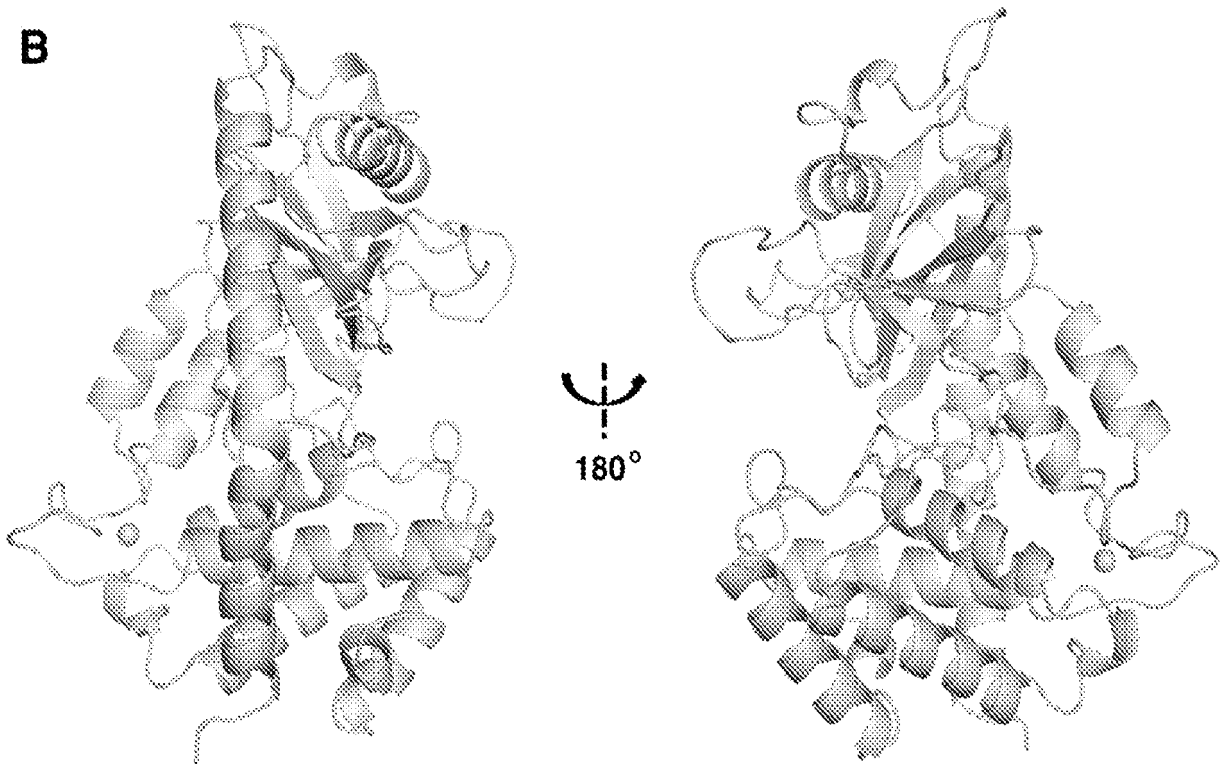


Figure S1B

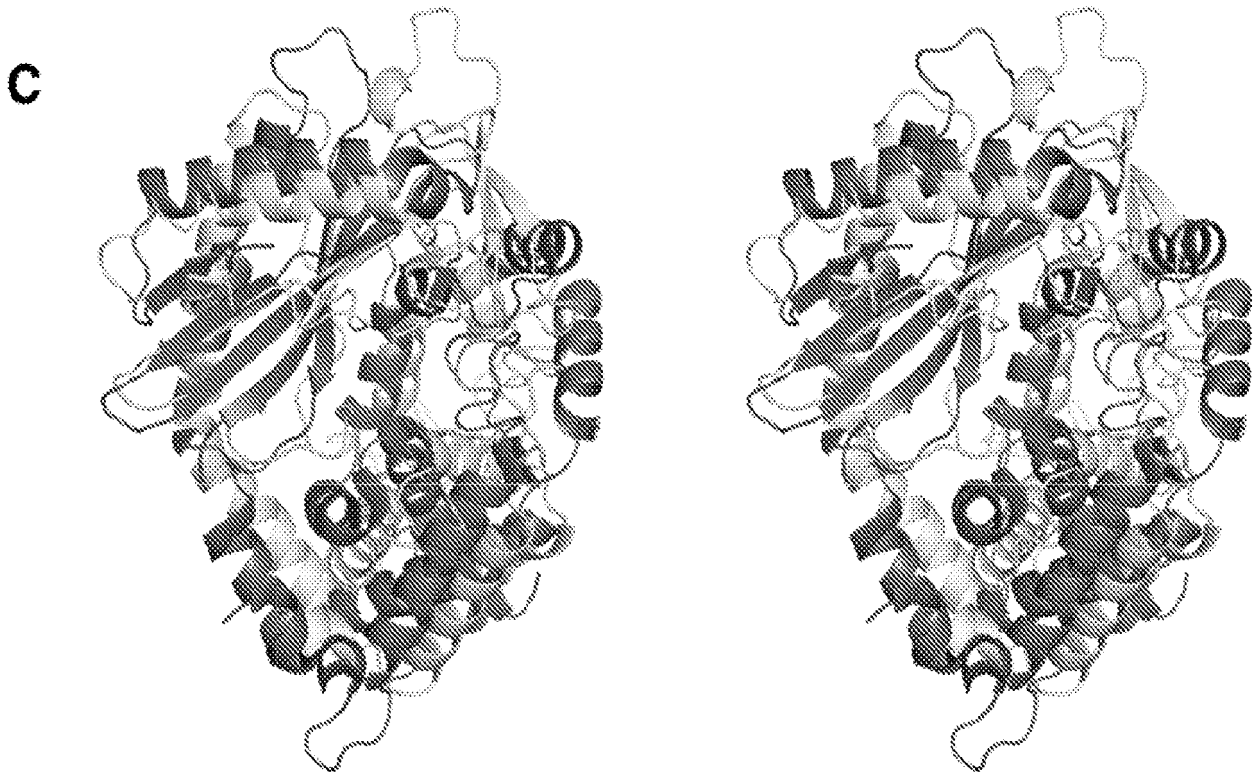


Figure S1C

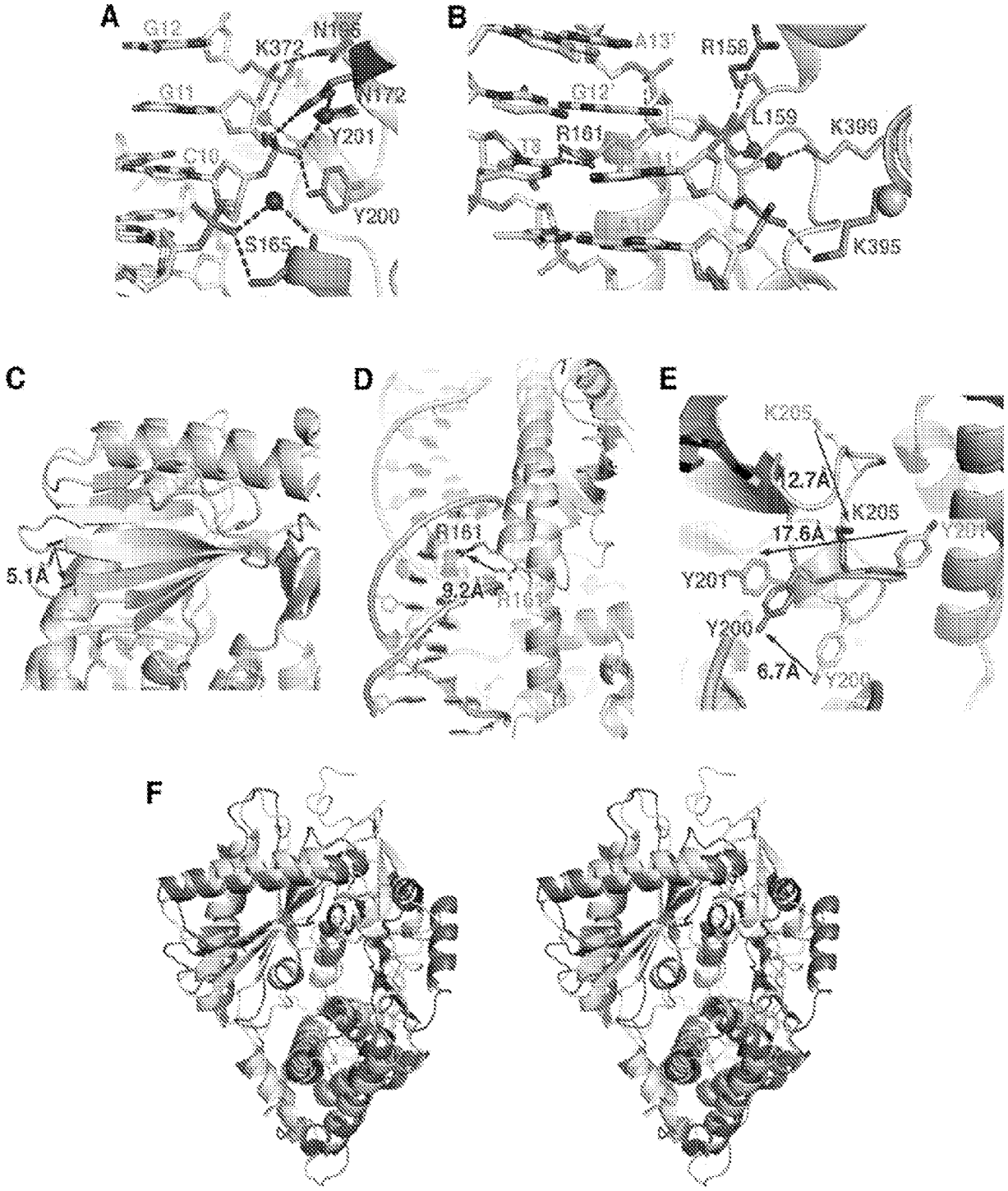

A
G12
K372
N116
G13
N172
Y201
C10
Y200
S165
B
A13
R158
G12
L159
R161
K399
K395
C
5.1Å
D
R161
3.2Å
E
K205
12.7Å
K205
17.6Å
Y201
Y201
Y200
6.7Å
F

Figure S2

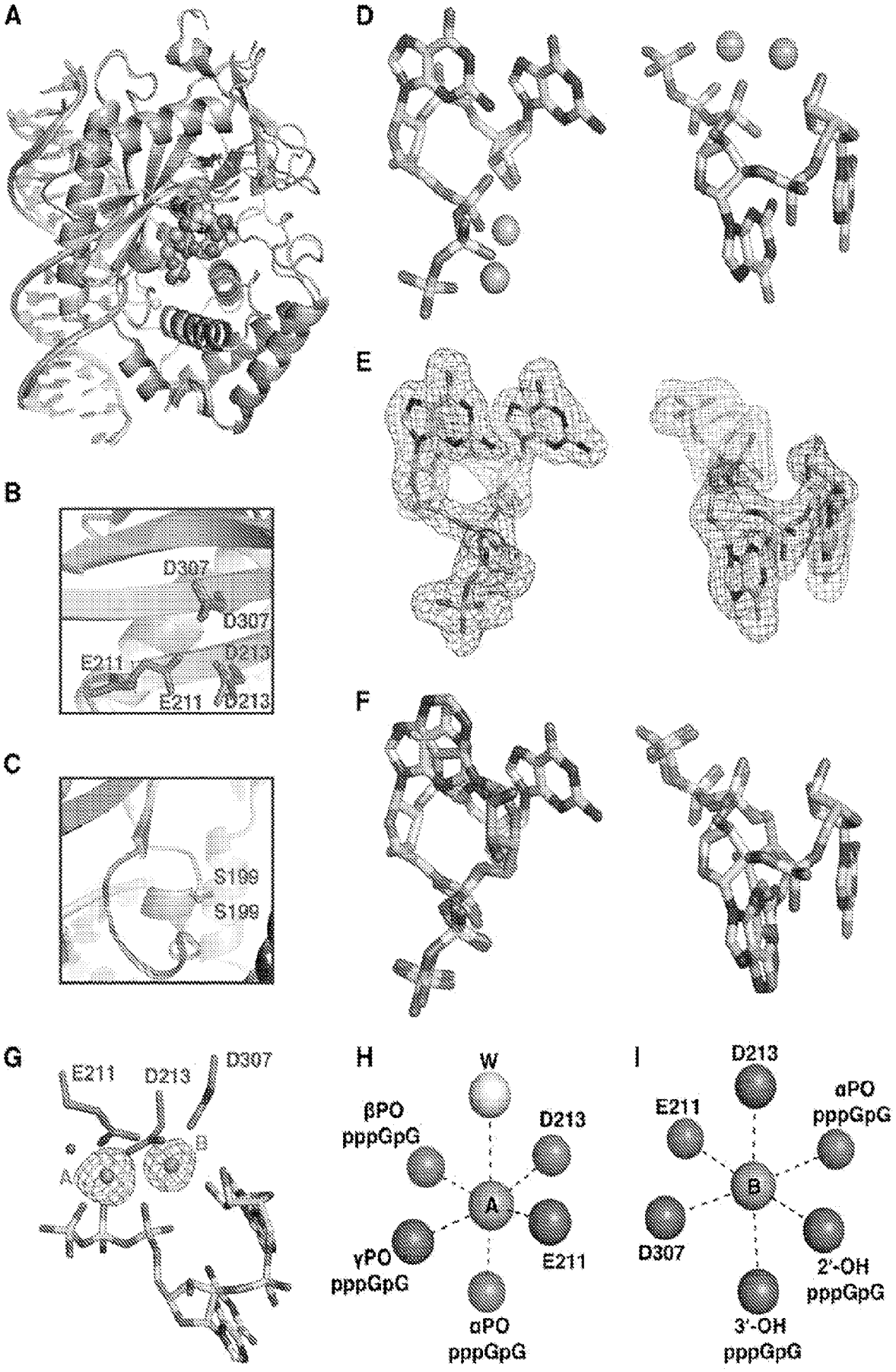

A
B
D307
D307
E211
D213
E211
D213
C
S199
S199
D
E
F
G
E211
D213
D307
A
B
H
W
βPO
pppGpG
D213
A
γPO
pppGpG
E211
αPO
pppGpG
I
D213
E211
αPO
pppGpG
B
D307
2'-OH
pppGpG
3'-OH
pppGpG

Figure S3

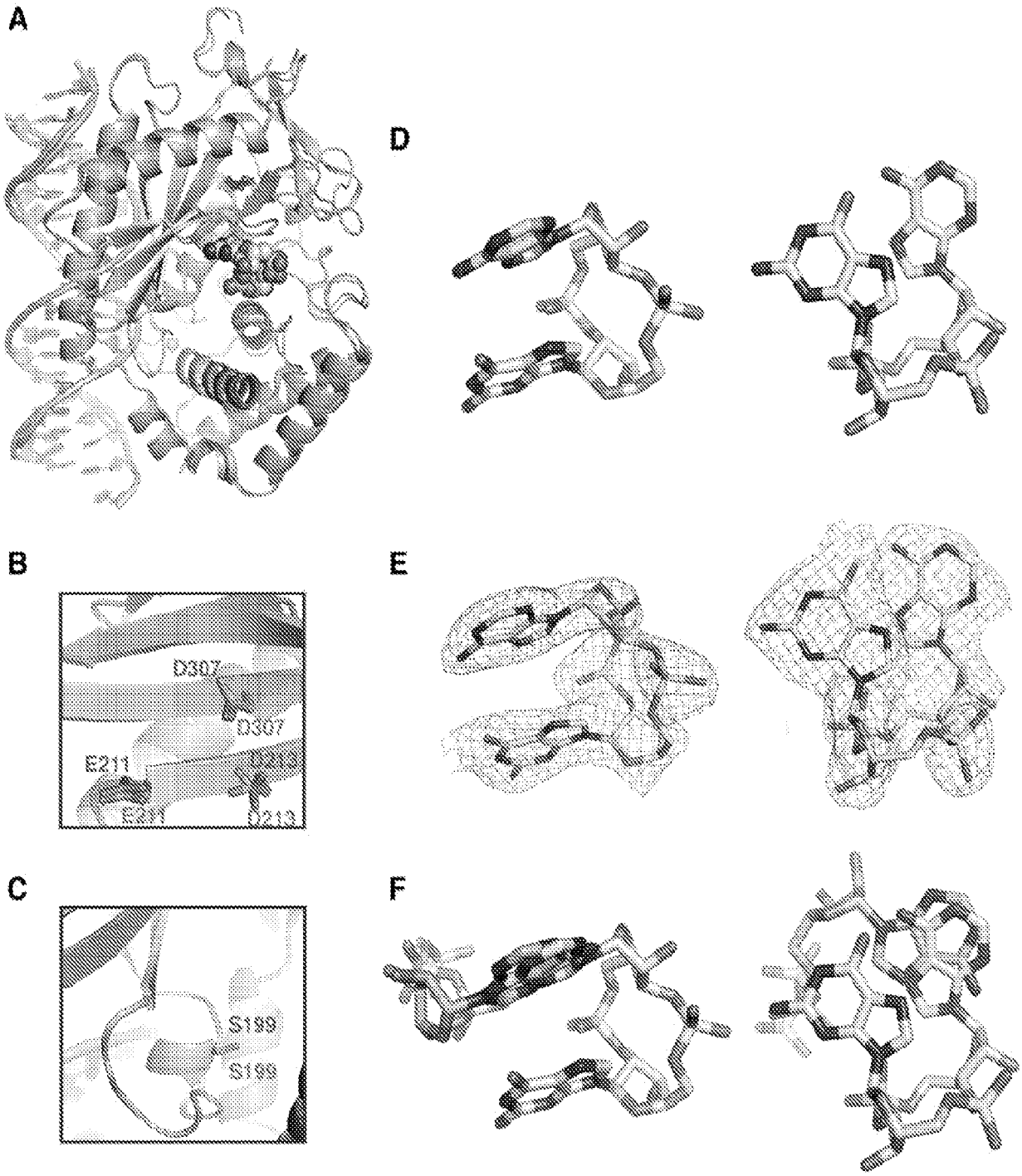
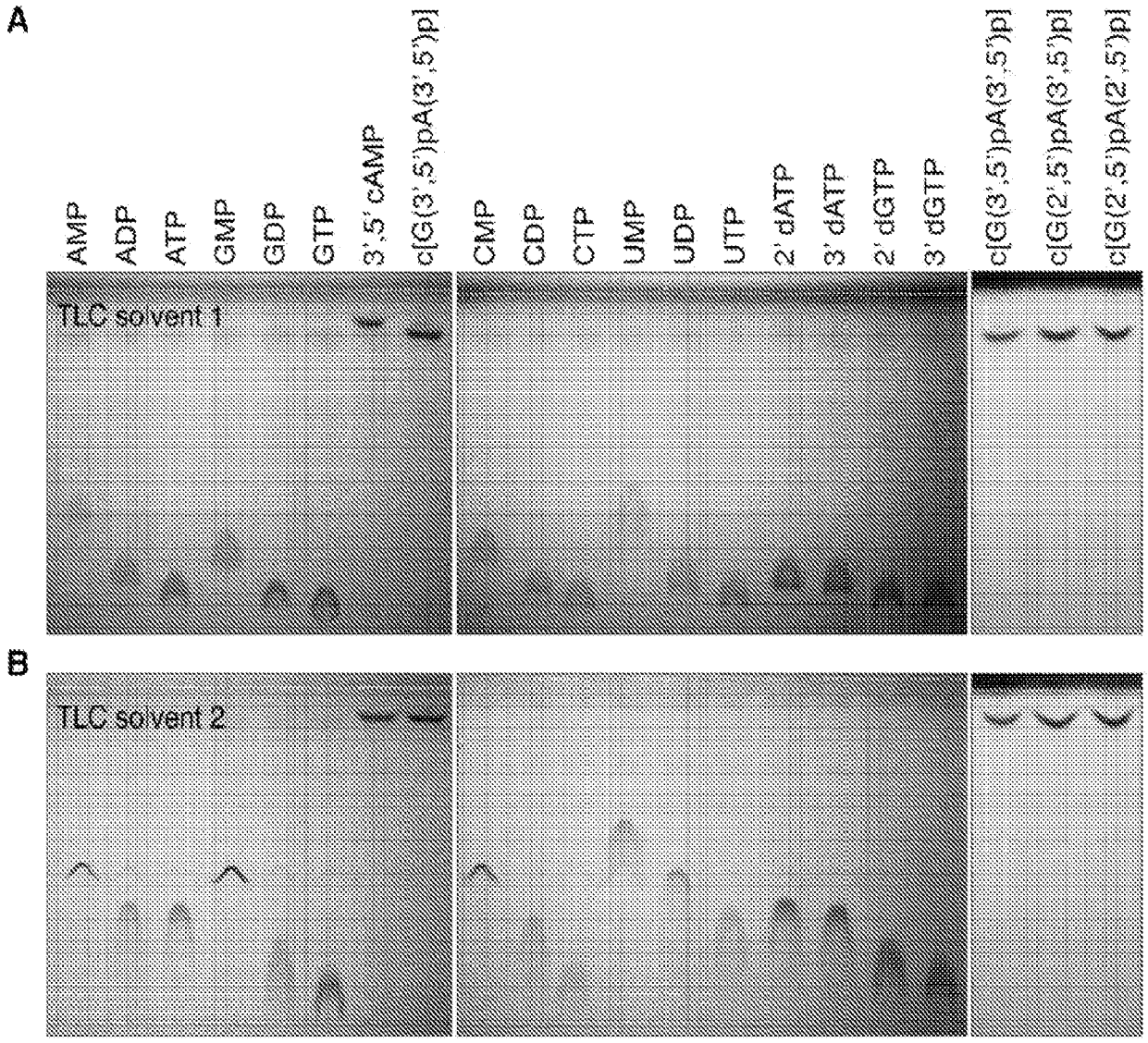


Figure S4



C

Rf values

	AMP	ADP	ATP	GMP	GDP	GTP	CMP	CDP	CTP	UMP	UDP	UTP	3',5' cAMP	c[G(3',5')pA(3',5')p]	2' dATP	3' dATP	2' dGTP	3' dGTP	c[G(2',5')pA(3',5')p]	c[G(2',5')pA(2',5')p]
Solvent 1	0.35	0.16	0.12	0.15	0.10	0.07	0.25	0.11	0.10	0.39	0.16	0.06	0.88	0.85	0.16	0.13	0.10	0.08	0.85	0.85
Solvent 2	0.48	0.37	0.35	0.46	0.25	0.15	0.44	0.29	0.19	0.54	0.41	0.32	0.89	0.89	0.36	0.32	0.25	0.17	0.89	0.89

Figure S5

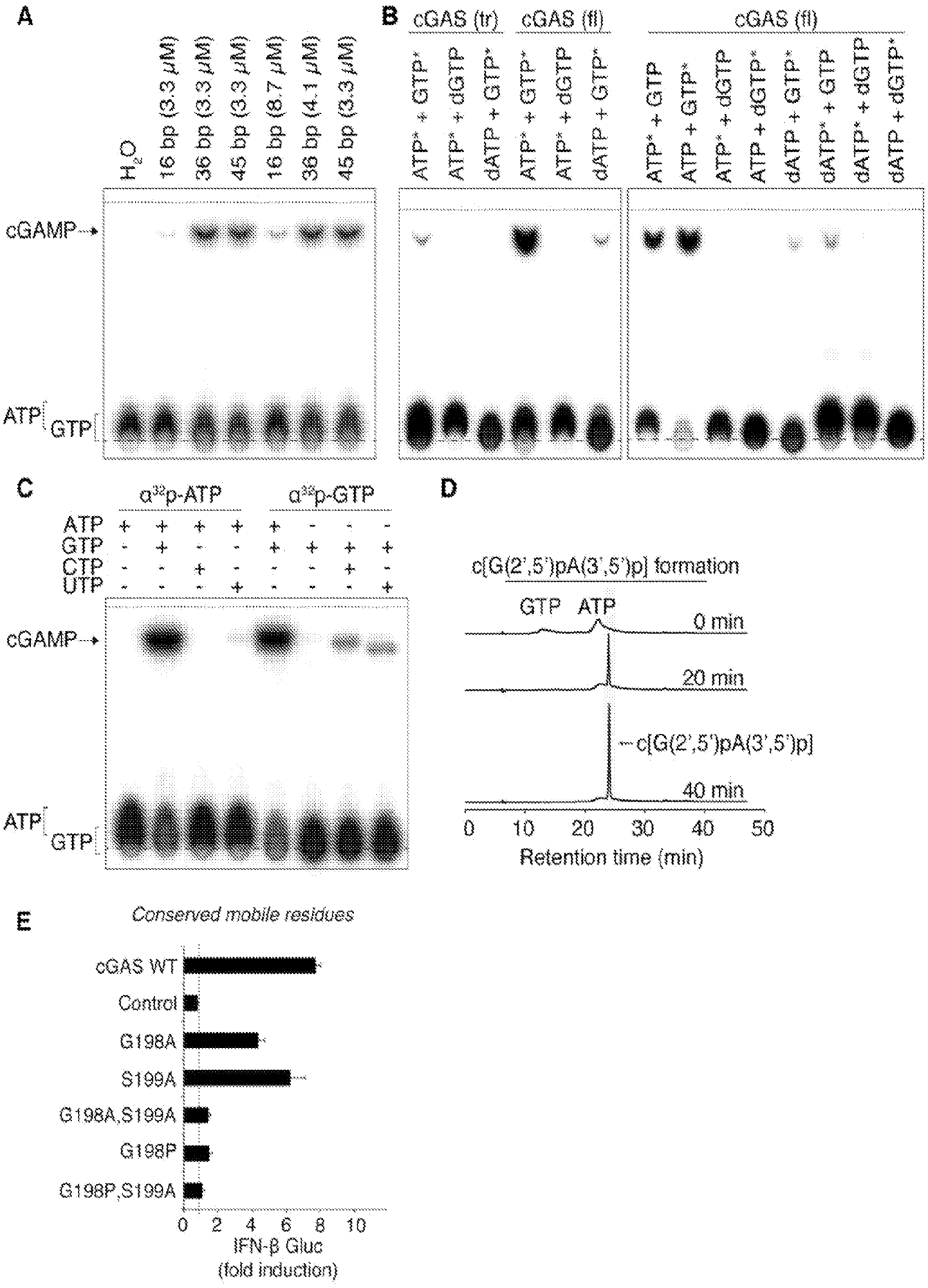

A
H2O
16 bp (3.3 μM)
36 bp (3.3 μM)
45 bp (3.3 μM)
16 bp (8.7 μM)
36 bp (4.1 μM)
45 bp (3.3 μM)
cGAMP
ATP
GTP
B
cGAS (tr)
cGAS (fl)
cGAS (fl)
ATP* + GTP*
ATP* + dGTP
dATP + GTP*
ATP* + GTP*
ATP* + dGTP
dATP + GTP*
ATP* + GTP
ATP + GTP*
ATP* + dGTP
ATP + dGTP*
dATP + GTP*
dATP* + GTP
dATP* + dGTP
dATP + dGTP*
C
α32p-ATP
α32p-GTP
ATP
GTP
CTP
UTP
cGAMP
ATP
GTP
D
c[G(2',5')pA(3',5')p] formation
GTP ATP
0 min
20 min
c[G(2',5')pA(3',5')p]
40 min
0 10 20 30 40 50
Retention time (min)
E
Conserved mobile residues
cGAS WT
Control
G198A
S199A
G198A,S199A
G198P
G198P,S199A
0 2 4 6 8 10
IFN-β Gluc
(fold induction)

Figure S6

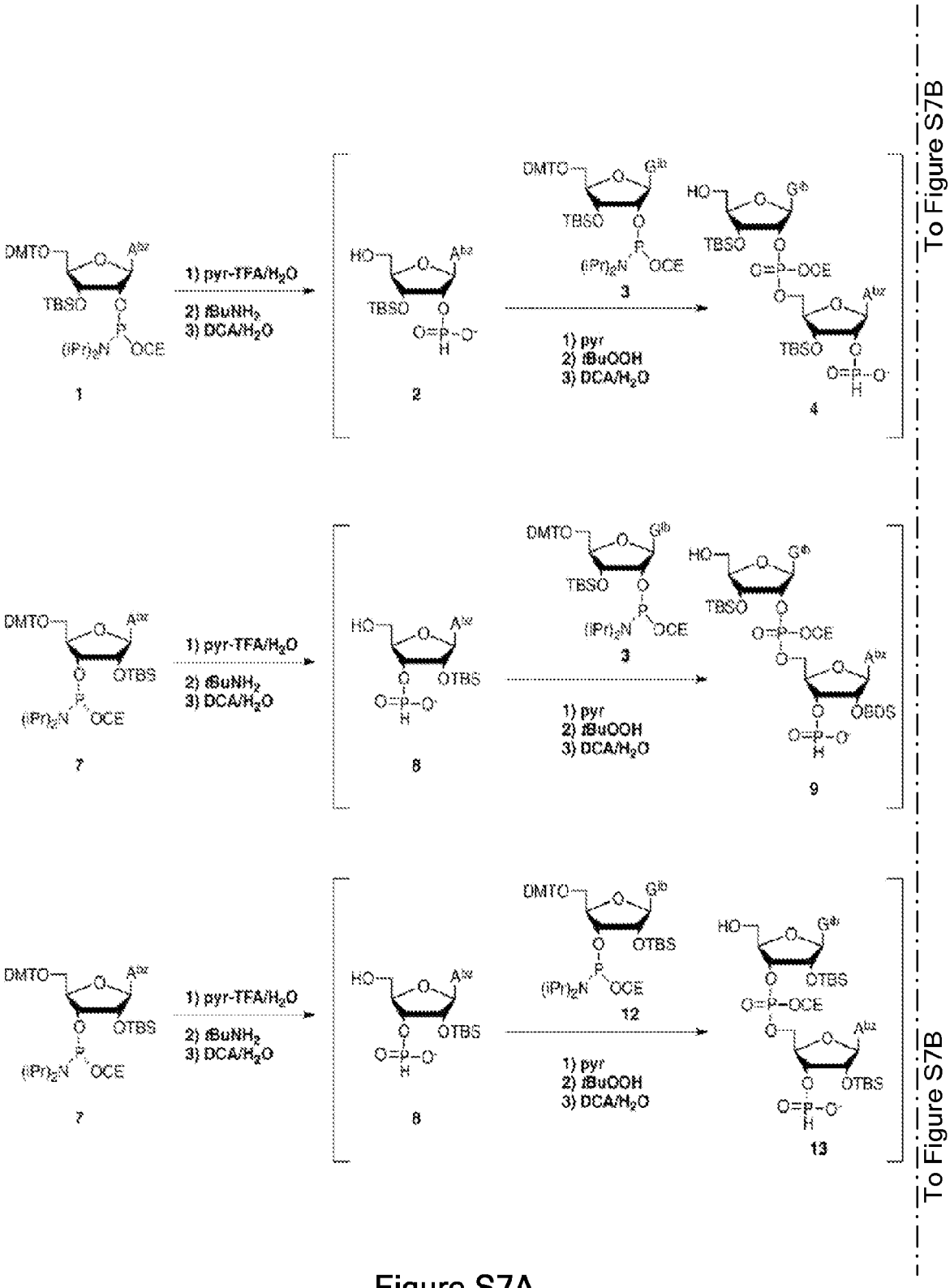
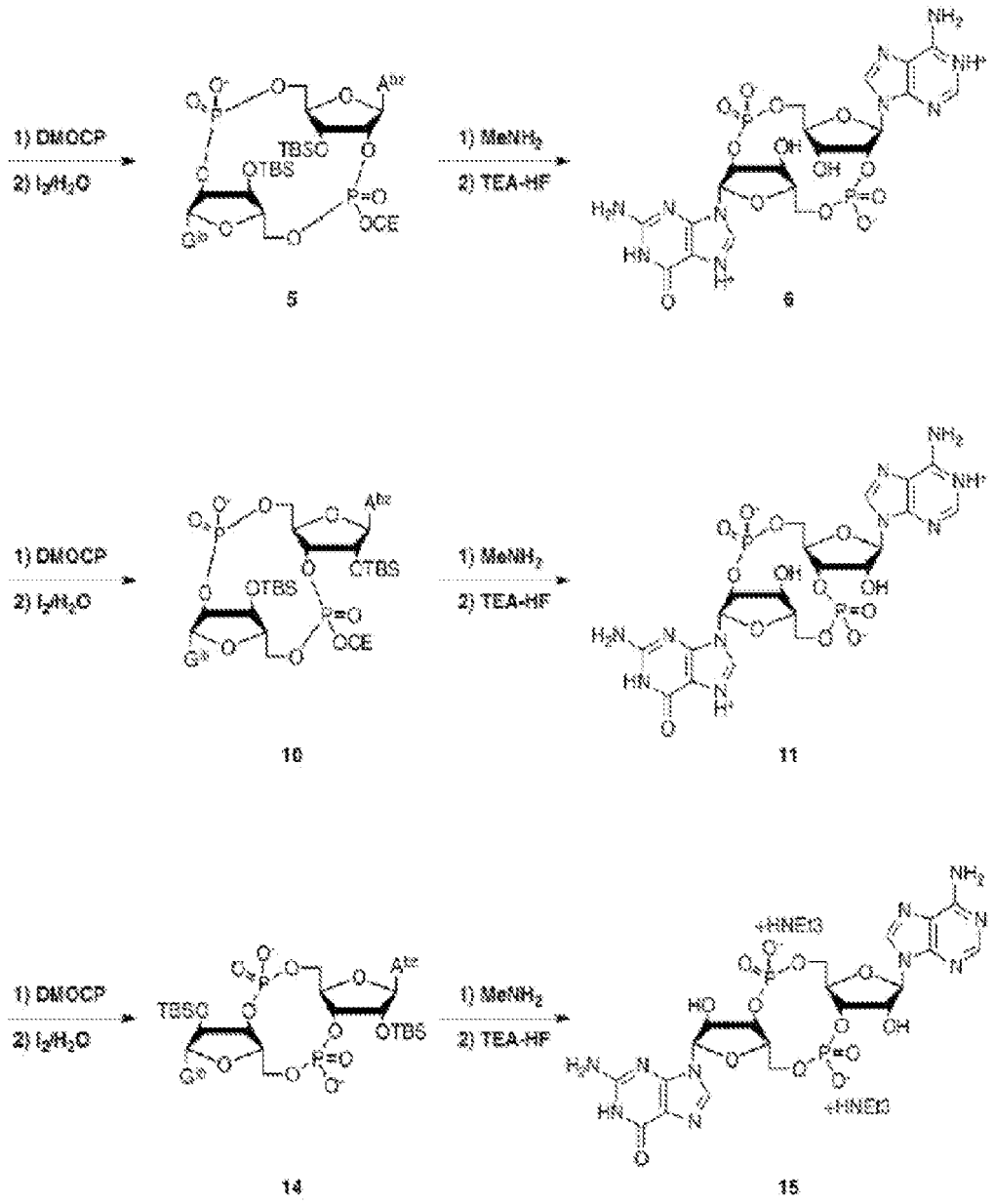

DMTO
TBSO
(iPr)2N
OCE
1) pyr-TFA/H2O
2) tBuNH2
3) DCA/H2O
1
2
3
1) pyr
2) tBuOOH
3) DCA/H2O
4
OTBS
7
8
9
12
13
OBDS
To Figure S7B
To Figure S7B

Figure S7A

From Figure S7A



From Figure S7A

Figure S7B

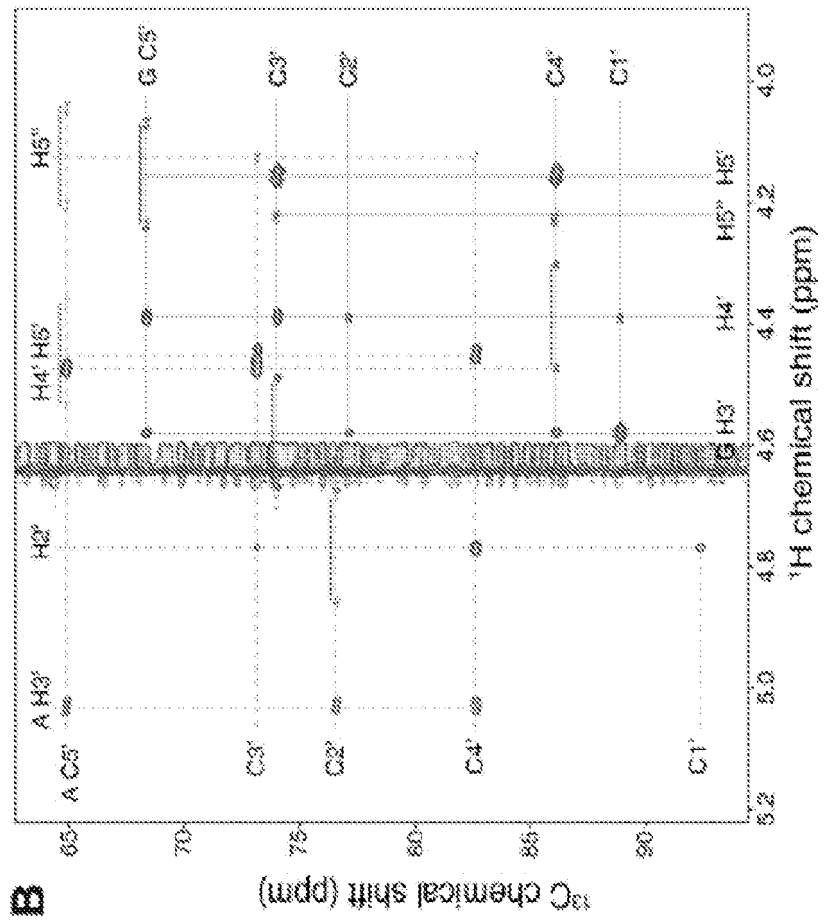


Figure S8B

Figure S8A

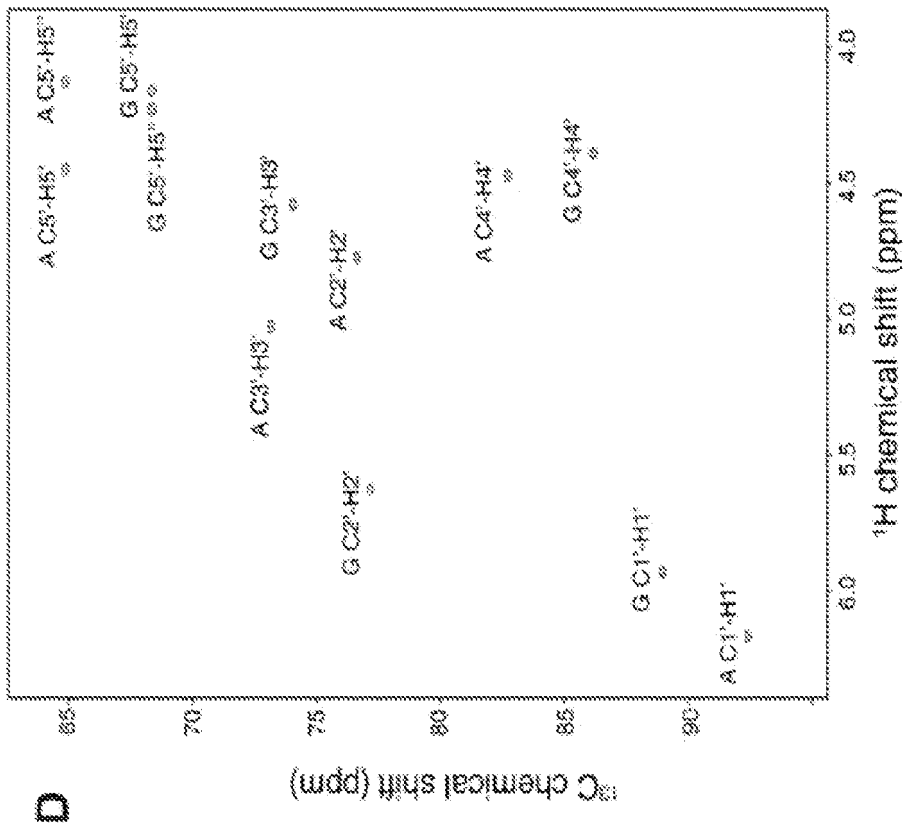


Figure S8D

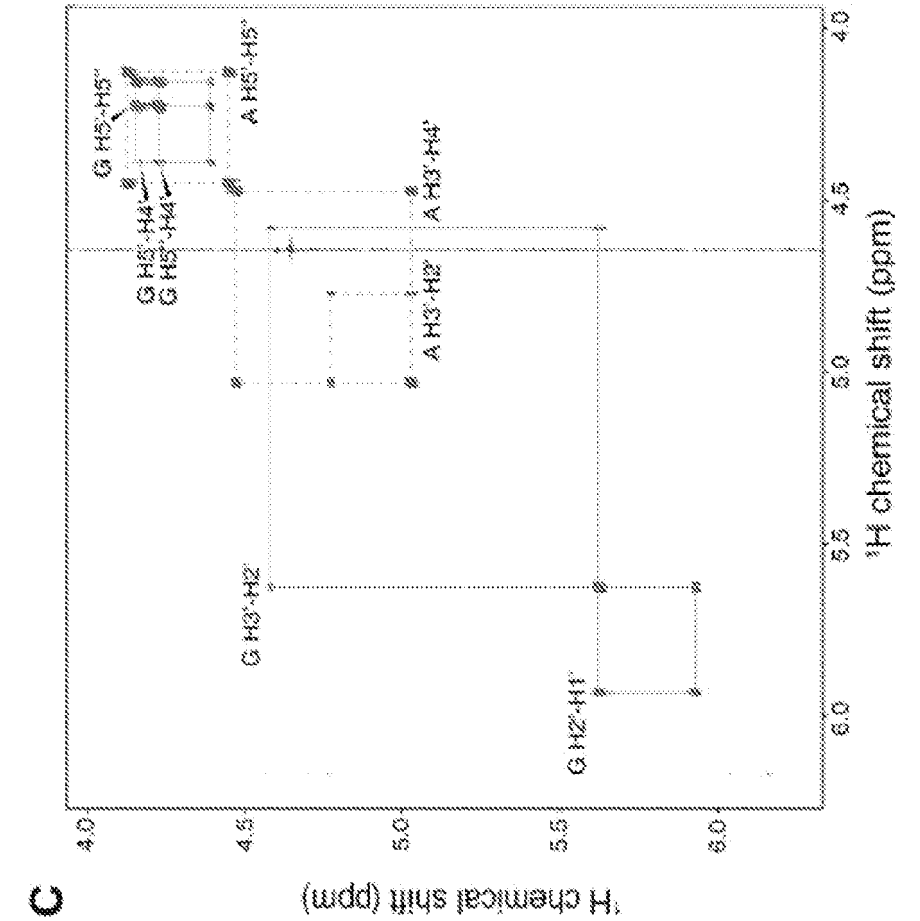


Figure S8C

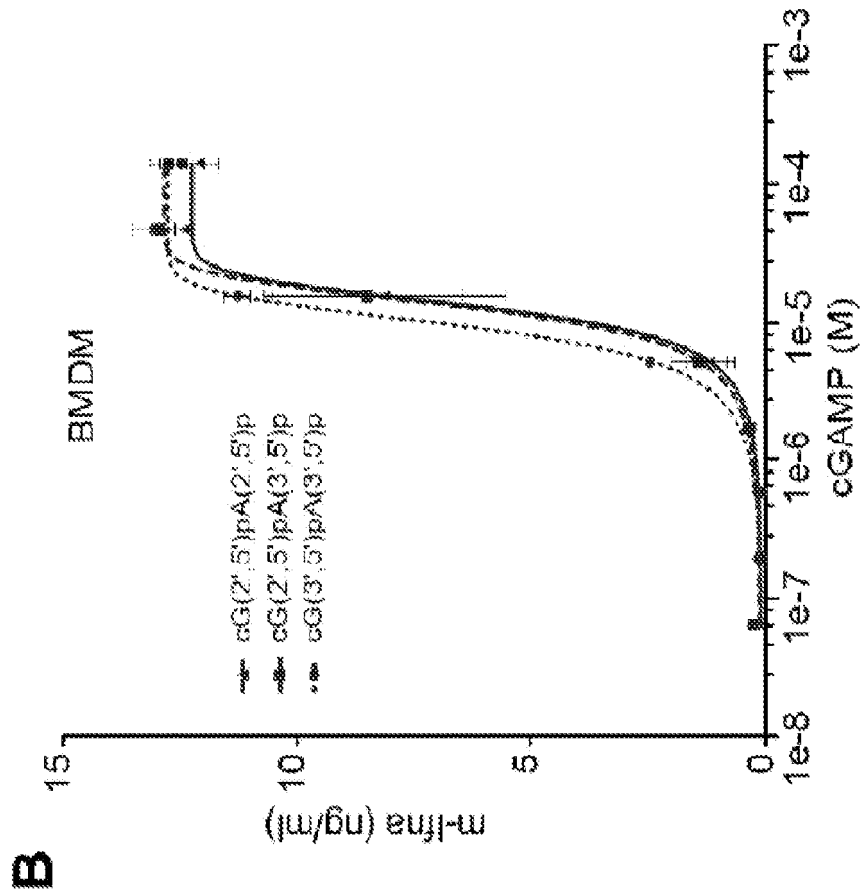


Figure S9B

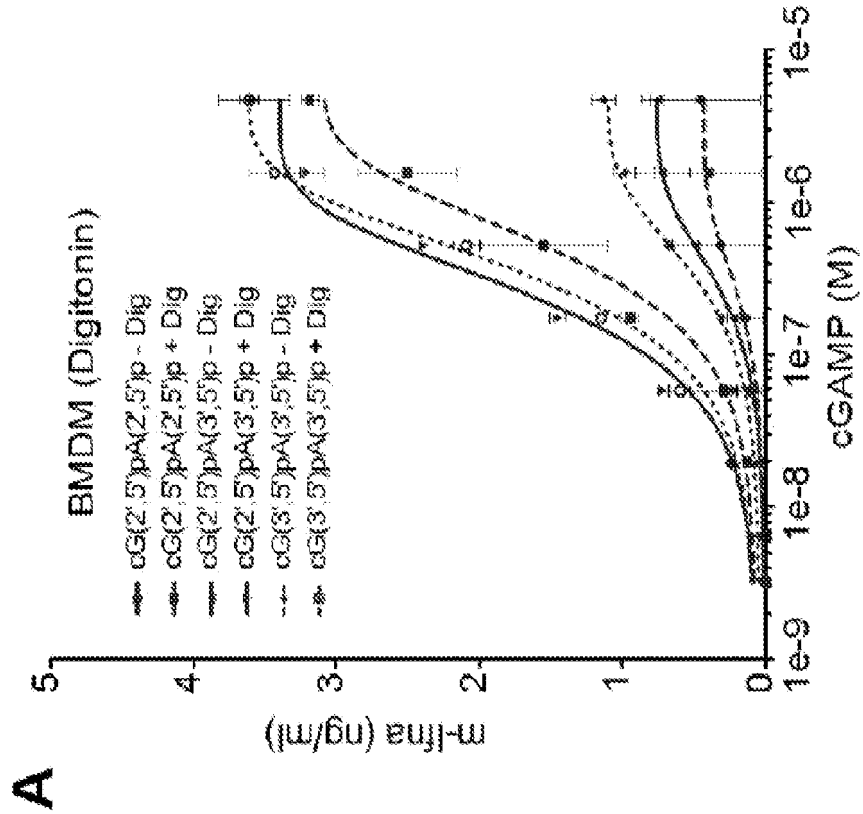


Figure S9A

C

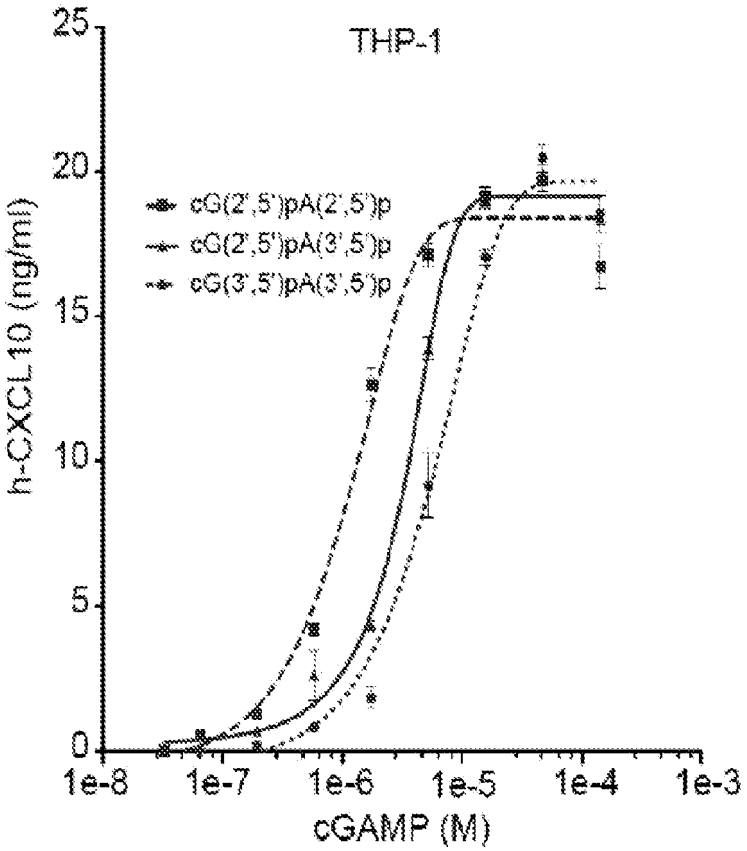


Figure S9C

D

BMDM (Digitonin)

	cG(2',5')pA(2',5')p	cG(2',5')pA(3',5')p	cG(3',5')pA(3',5')p
EC_{50} (μM)	0.80	0.29	0.42
95% CI (μM)	0.20 - 3.15	0.22 - 0.39	0.30 - 0.60

BMDM

EC_{50} (μM)	12.49	12.41	8.41
95% CI (μM)	9.65 - 16.17	10.12 - 15.22	7.69 - 9.20

THP-1

EC_{50} (μM)	1.17	3.35	5.66
95% CI (μM)	1.00 - 1.38	2.93 - 3.84	4.97 - 6.44

Figure S9D

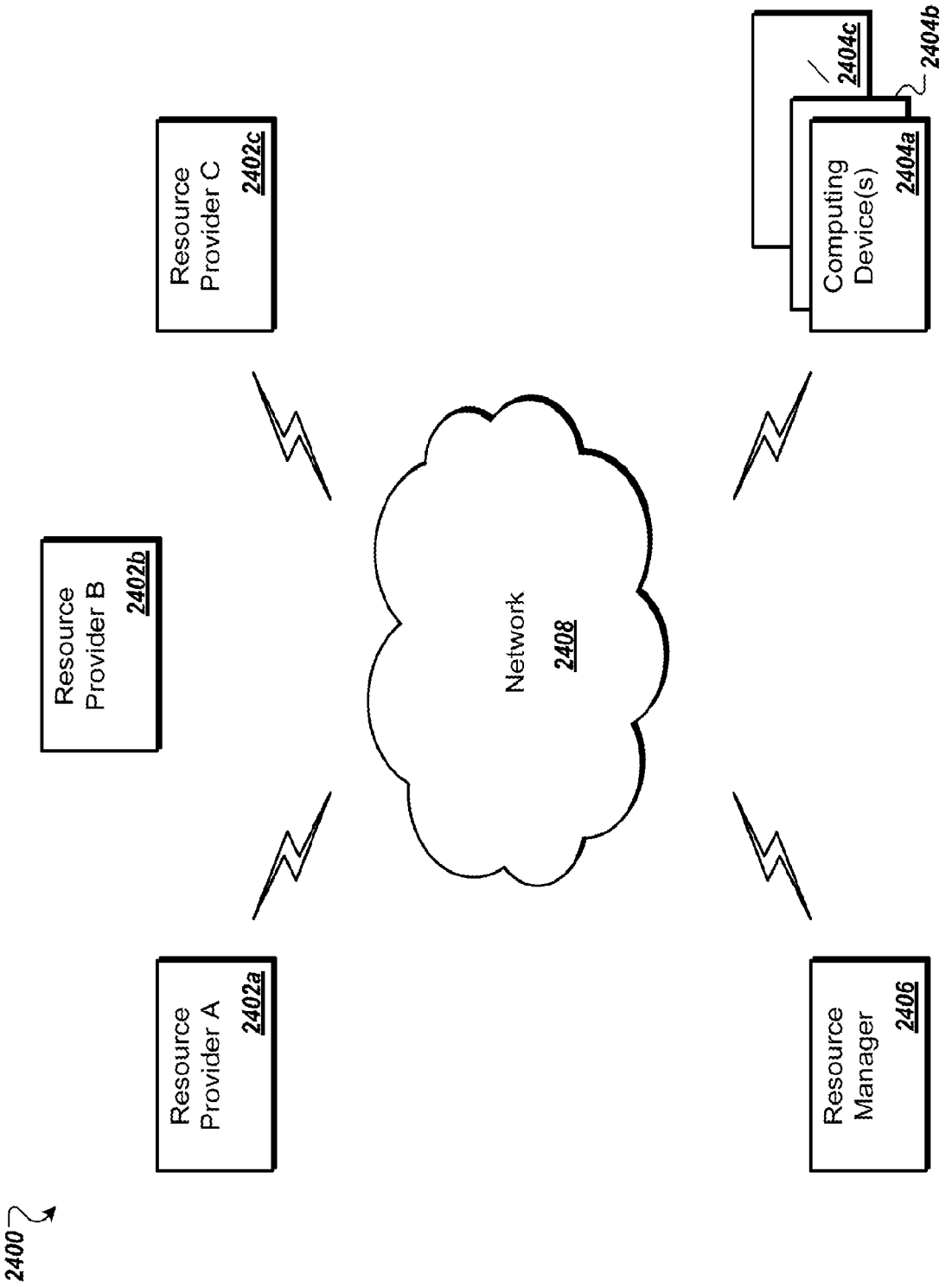

2400
Resource Provider A 2402a
Resource Provider B 2402b
Resource Provider C 2402c
Network 2408
Resource Manager 2406
Computing Device(s) 2404a
2404c
2404b

Figure S10

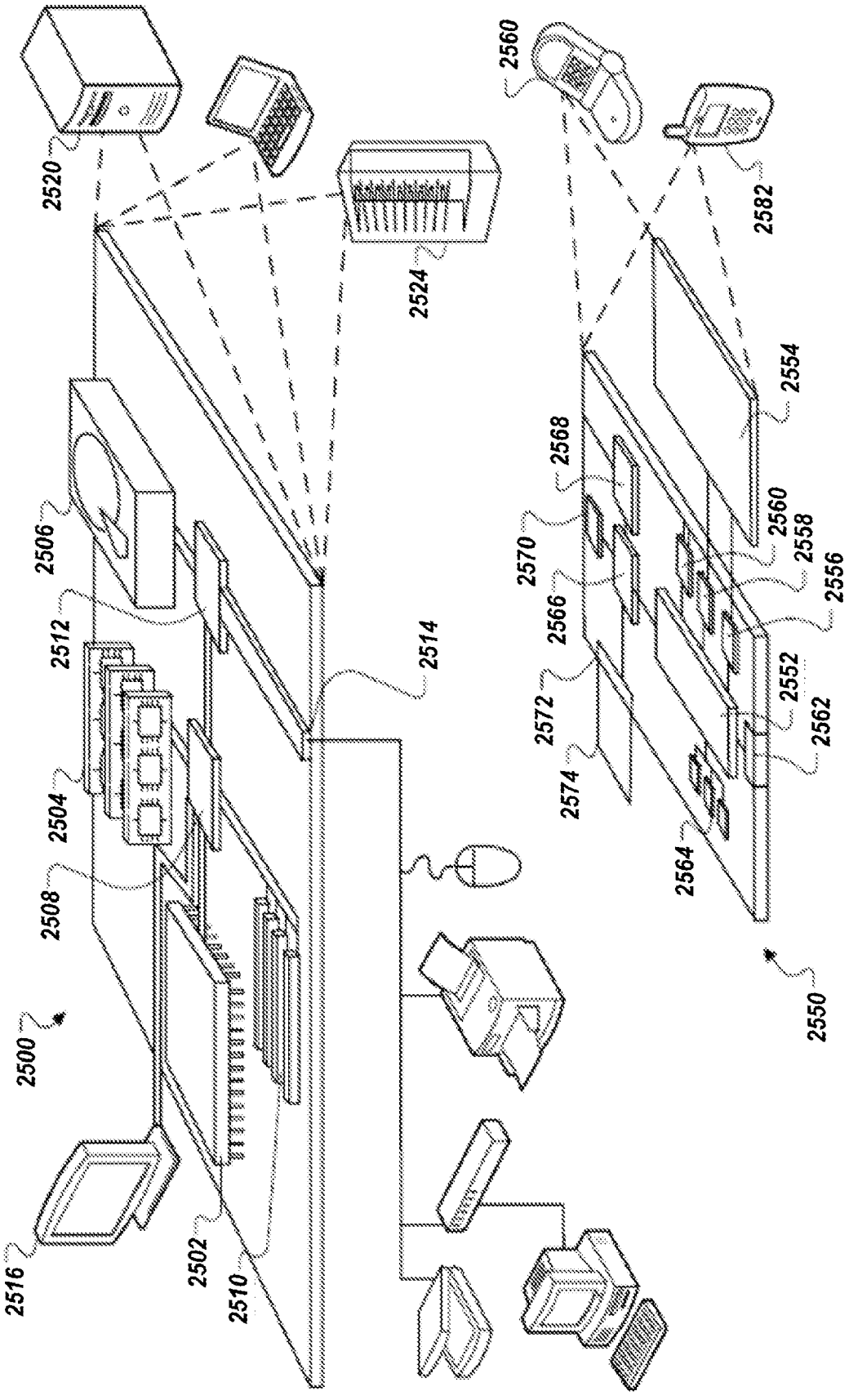

2520
2524
2560
2582
2506
2512
2514
2504
2508
2500
2516
2502
2510
2568
2554
2570
2560
2558
2566
2556
2572
2552
2562
2574
2564
2550

Figure S11