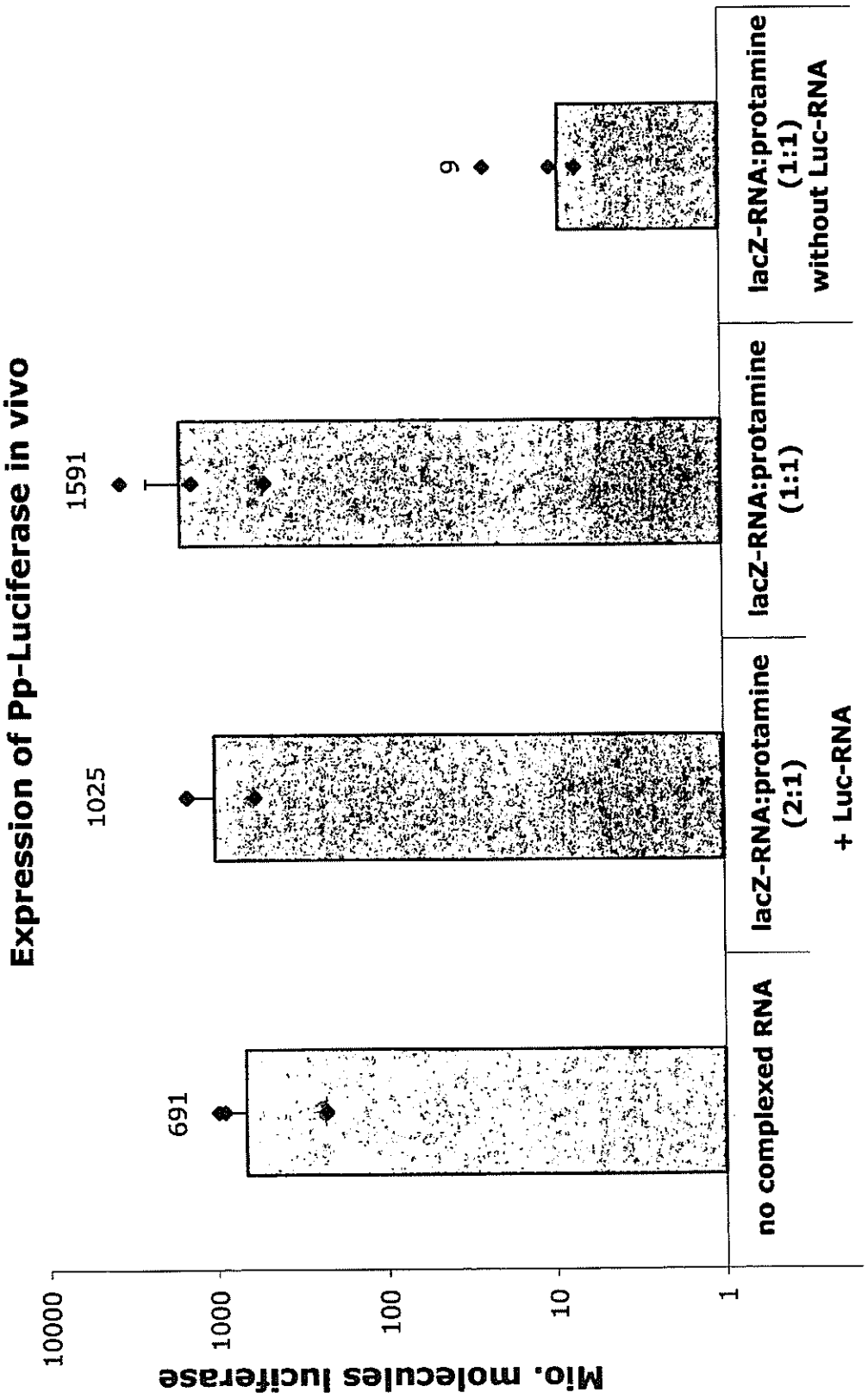

Expression of Pp-Luciferase in vivo
Mio. molecules luciferase
10000
1000
100
10
1
691
1025
1591
9
no complexed RNA
lacZ-RNA:protamine (2:1) + Luc-RNA
lacZ-RNA:protamine (1:1)
lacZ-RNA:protamine (1:1) without Luc-RNA

Figure 1

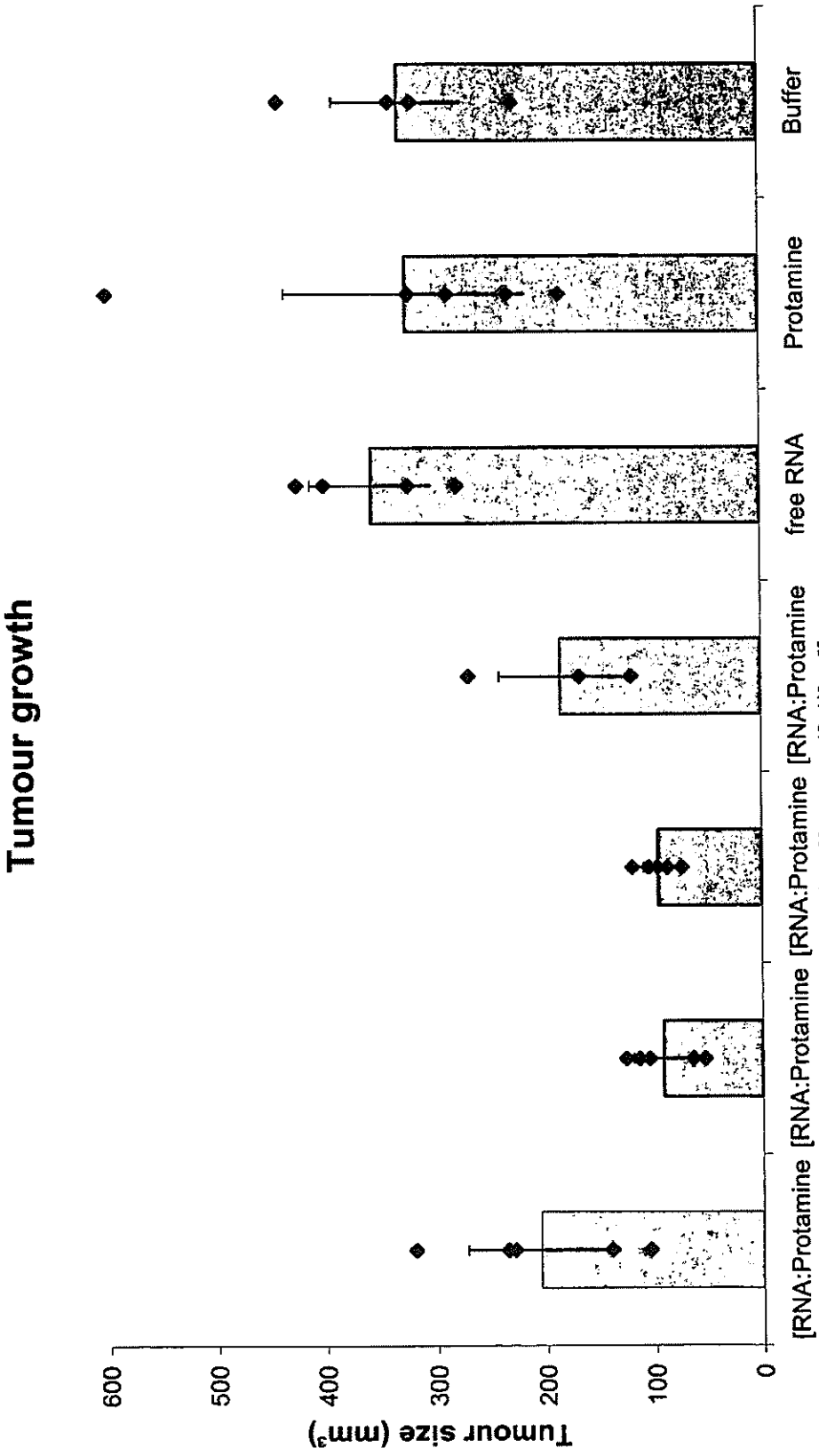

Tumour growth
Tumour size (mm³)
600
500
400
300
200
100
0
[RNA:Protamine (3:1)]
[RNA:Protamine (3:1)] + [free RNA] (1:1)
[RNA:Protamine (3:1)] + [free RNA] (1:4)
[RNA:Protamine (3:1)] + [free RNA] (1:8)
free RNA
Protamine
Buffer

Figure 2

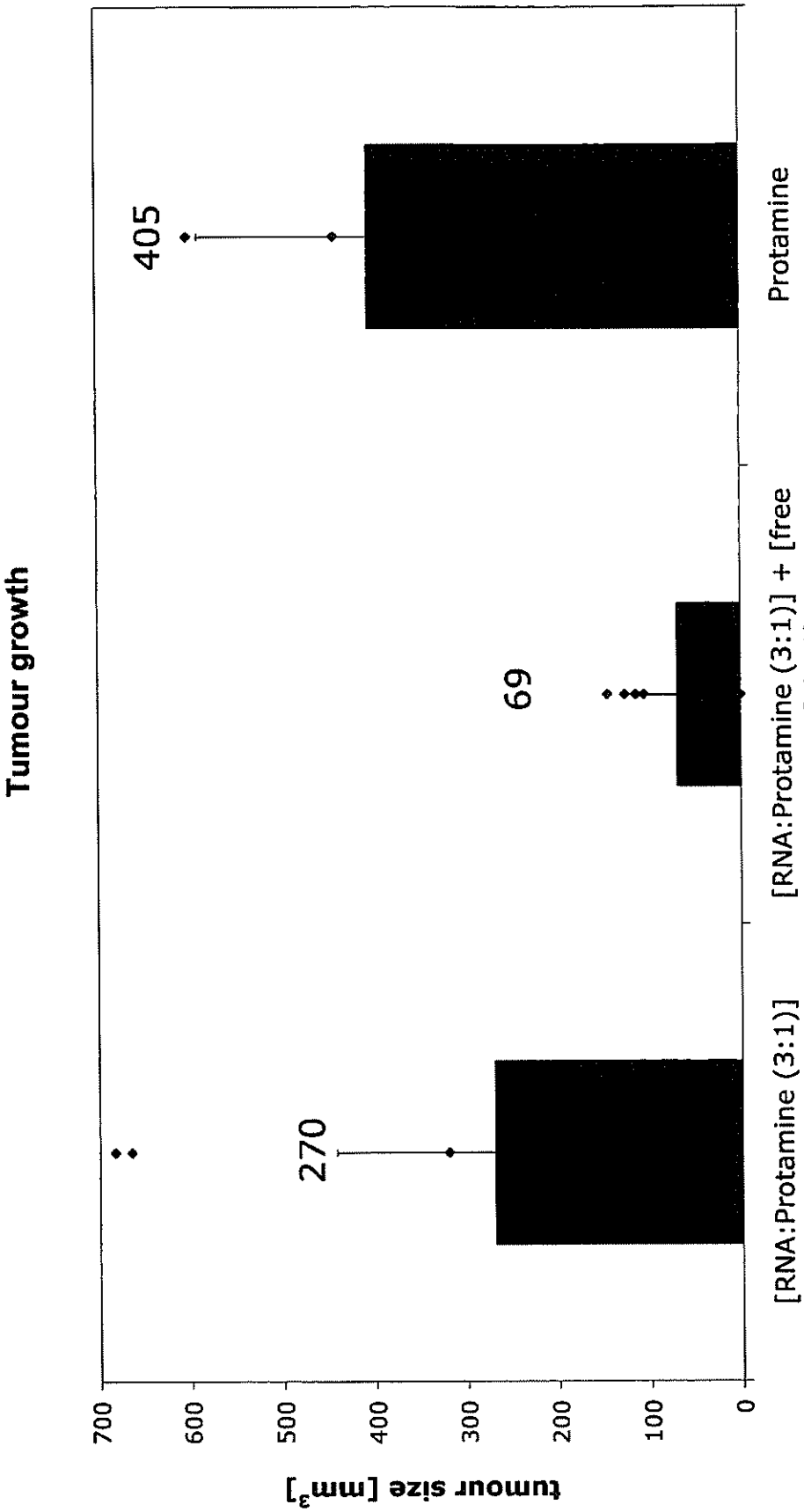

Tumour growth
tumour size [mm³]
700
600
500
400
300
200
100
0
270
69
405
[RNA:Protamine (3:1)]
[RNA:Protamine (3:1)] + [free RNA] (1:1)
Protamine

Figure 3

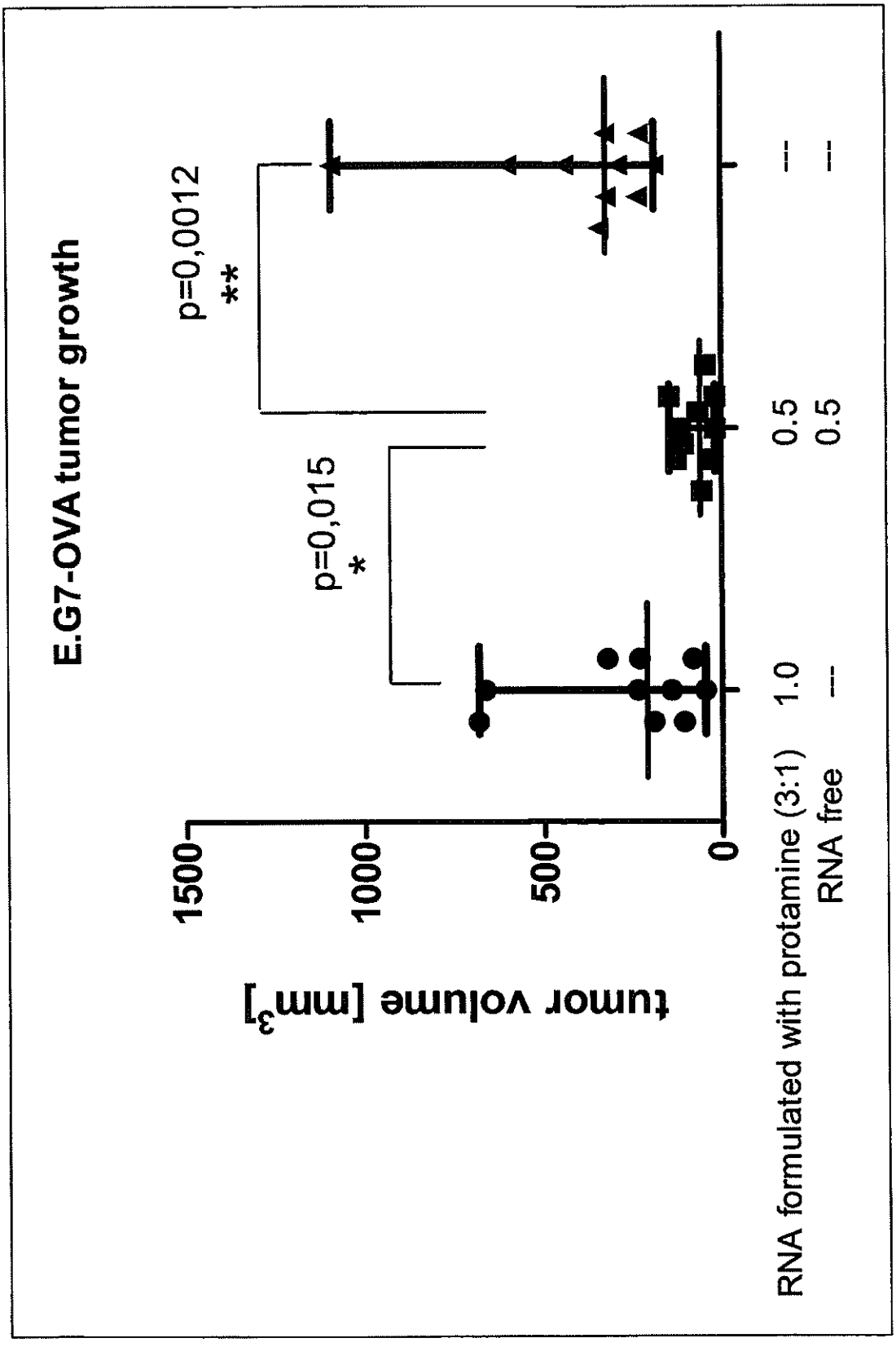

E.G7-OVA tumor growth
p=0,0012
**
p=0,015
*
1500
1000
500
0
tumor volume [mm³]
RNA formulated with protamine (3:1) 1.0 0.5
RNA free -- 0.5

Figure 4

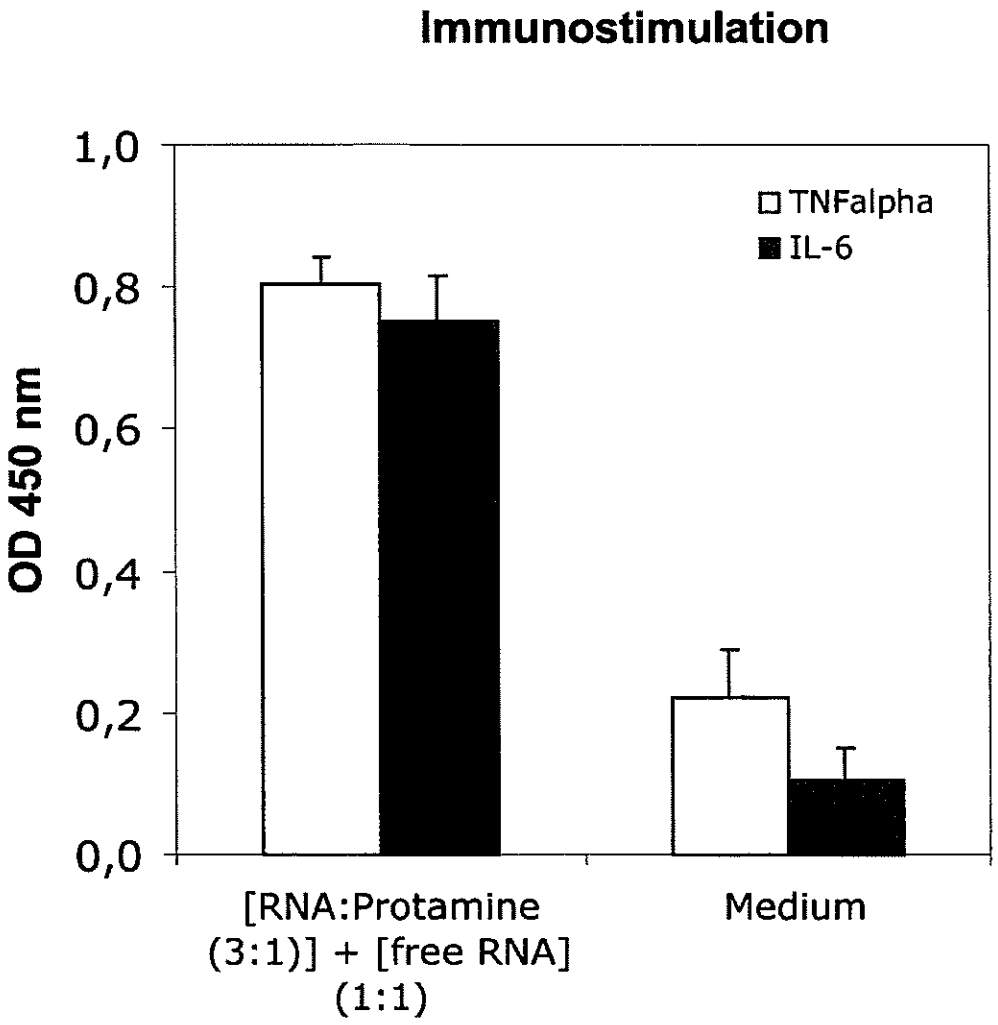

Immunostimulation
OD 450 nm
1,0
0,8
0,6
0,4
0,2
0,0
TNFalpha
IL-6
[RNA:Protamine (3:1)] + [free RNA] (1:1)
Medium

Figure 5

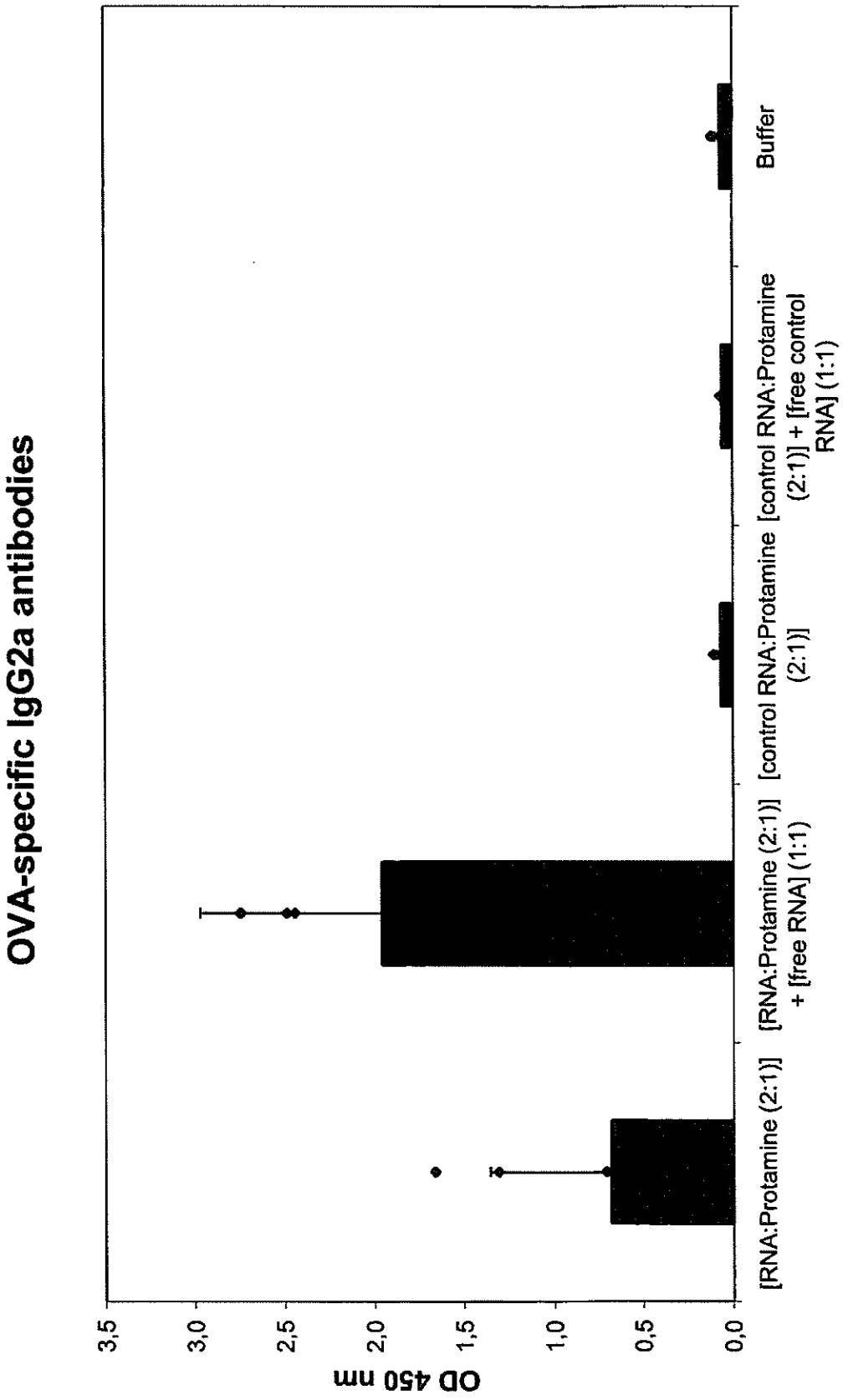

OVA-specific IgG2a antibodies
OD 450 nm
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
[RNA:Protamine (2:1)]
[RNA:Protamine (2:1)] + [free RNA] (1:1)
[control RNA:Protamine (2:1)]
[control RNA:Protamine (2:1)] + [free control RNA] (1:1)
Buffer

Figure 6

```
   1  GGGAGAAAGC UUACCAUGGG CAGCAUCGGG GCCGCGUCGA UGGAGUUCUG
  51  CUUCGACGUG UUCAAGGAGC UGAAGGUCCA CCACGCCAAC GAGAACAUCU
 101  UCUACUGCCC GAUCGCCAUC AUGAGCGCGC UCGCCAUGGU GUACCUGGGC
 151  GCCAAGGACA GCACCCGGAC GCAGAUCAAC AAGGUGGUCC GCUUCGACAA
 201  GCUGCCCGGC UUCGGGGACU CGAUCGAGGC GCAGUGCGGC ACCAGCGUGA
 251  ACGUGCACAG CUCGCUCCGG GACAUCCUGA ACCAGAUCAC CAAGCCGAAC
 301  GACGUCUACA GCUUCAGCCU GGCCUCGCGG CUCUACGCCG AGGAGCGCUA
 351  CCCGAUCCUG CCCGAGUACC UGCAGUGCGU GAAGGAGCUC UACCGGGGCG
 401  GGCUGGAGCC GAUCAACUUC CAGACGGCGG CCGACCAGGC CCGGGAGCUG
 451  AUCAACAGCU GGGUGGAGAG CCAGACCAAC GGCAUCAUCC GCAACGUCCU
 501  CCAGCCGUCG AGCGUGGACA GCCAGACCGC GAUGGUGCUG GUCAACGCCA
 551  UCGUGUUCAA GGGCCUGUGG GAGAAGACGU UCAAGGACGA GGACACCCAG
 601  GCCAUGCCCU UCCGGGUGAC CGAGCAGGAG UCGAAGCCGG UCCAGAUGAU
 651  GUACCAGAUC GGGCUCUUCC GGGUGGCGAG CAUGGCCAGC GAGAAGAUGA
 701  AGAUCCUGGA GCUGCCGUUC GCCUCGGGCA CGAUGAGCAU GCUCGUGCUG
 751  CUGCCCGACG AGGUCAGCGG CCUCGAGCAG CUGGAGUCGA UCAUCAACUU
 801  CGAGAAGCUG ACCGAGUGGA CCAGCAGCAA CGUGAUGGAG GAGCGCAAGA
 851  UCAAGGUGUA CCUCCCGCGG AUGAAGAUGG AGGAGAAGUA CAACCUGACG
 901  UCGGUCCUGA UGGCGAUGGG GAUCACCGAC GUGUUCAGCA GCUCGGCCAA
 951  CCUCAGCGGC AUCAGCUCGG CCGAGAGCCU GAAGAUCAGC CAGGCGGUGC
1001  ACGCCGCCCA CGCGGAGAUC AACGAGGCCG GCCGGGAGGU CGUGGGGUCG
1051  GCCGAGGCGG GCGUGGACGC CGCCAGCGUC AGCGAGGAGU UCCGCGCGGA
1101  CCACCCGUUC CUGUUCUGCA UCAAGCACAU CGCCACCAAC GCCGUGCUCU
1151  UCUUCGGCCG GUGCGUGUCG CCCUGACCAC UAGUUAUAAG ACUGACUAGC
1201  CCGAUGGGCC UCCCAACGGG CCCUCCUCCC CUCCUUGCAC CGAGAUUAAU
1251  AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
1301  AAAAAAAAAA AAAAUAUUCC CCCCCCCCCC CCCCCCCCCC CCCCCCCCUC
1351  UAGACAAUUG GAAUU
```

Figure 7

GGGAGACAAGCUUGGCAUUCCGGUACUGUUGGUAAAGCCACC*AUGGAAGACGCCAAAAACAU*
AAAGAAAGGCCCGGCGCCAUUCUAUCCGCUGGAAGAUGGAACCGCUGGAGAGCAACUGCAUA
AGGCUAUGAAGAGAUACGCCCUGGUUCCUGGAACAAUUGCUUUUACAGAUGCACAUAUCGAG
GUGGACAUCACUUACGCUGAGUACUUCGAAAUGUCCGUUCGGUUGGCAGAAGCUAUGAAACG
AUAUGGGCUGAAUACAAAUCACAGAAUCGUCGUAUGCAGUGAAAACUCUCUUCAAUUCUUUA
UGCCGGUGUUGGGCGCGUUAUUUAUCGGAGUUGCAGUUGCGCCCGCGAACGACAUUUAUAAU
GAACGUGAAUUGCUCAACAGUAUGGGCAUUUCGCAGCCUACCGUGGUGUUCGUUUCCAAAAA
GGGGUUGCAAAAAAUUUUGAACGUGCAAAAAAAGCUCCCAAUCAUCCAAAAAAUUAUUAUCA
UGGAUUCUAAAACGGAUUACCAGGGAUUUCAGUCGAUGUACACGUUCGUCACAUCUCAUCUA
CCUCCCGGUUUUAAUGAAUACGAUUUUGUGCCAGAGUCCUUCGAUAGGGACAAGACAAUUGC
ACUGAUCAUGAACUCCUCUGGAUCUACUGGUCUGCCUAAAGGUGUCGCUCUGCCUCAUAGAA
CUGCCUGCGUGAGAUUCUCGCAUGCCAGAGAUCCUAUUUUUGGCAAUCAAAUCAUUCCGGAU
ACUGCGAUUUUAAGUGUUGUUCCAUUCCAUCACGGUUUUGGAAUGUUUACUACACUCGGAUA
UUUGAUAUGUGGAUUUCGAGUCGUCUUAAUGUAUAGAUUUGAAGAAGAGCUGUUUCUGAGGA
GCCUUCAGGAUUACAAGAUUCAAAGUGCGCUGCUGGUGCCAACCCUAUUCUCCUUCUUCGCC
AAAAGCACUCUGAUUGACAAAUACGAUUUAUCUAAUUUACACGAAAUUGCUUCUGGUGGCGC
UCCCCUCUCUAAGGAAGUCGGGGAAGCGGUUGCCAAGAGGUUCCAUCUGCCAGGUAUCAGGC
AAGGAUAUGGGCUCACUGAGACUACAUCAGCUAUUCUGAUUACACCCGAGGGGGAUGAUAAA
CCGGGCGCGGUCGGUAAAGUUGUUCCAUUUUUUGAAGCGAAGGUUGUGGAUCUGGAUACCGG
GAAAACGCUGGGCGUUAAUCAAAGAGGCGAACUGUGUGUGAGAGGUCCUAUGAUUAUGUCCG
GUUAUGUAAACAAUCCGGAAGCGACCAACGCCUUGAUUGACAAGGAUGGAUGGCUACAUUCU
GGAGACAUAGCUUACUGGGACGAAGACGAACACUUCUUCAUCGUUGACCGCCUGAAGUCUCU
GAUUAAGUACAAAGGCUAUCAGGUGGCUCCCGCUGAAUUGGAAUCCAUCUUGCUCCAACACC
CCAACAUCUUCGACGCAGGUGUCGCAGGUCUUCCCGACGAUGACGCCGGUGAACUUCCCGCC
GCCGUUGUUGUUUUGGAGCACGGAAAGACGAUGACGGAAAAAGAGAUCGUGGAUUACGUCGC
CAGUCAAGUAACAACCGCGAAAAAGUUGCGCGGAGGAGUUGUGUUUGUGGACGAAGUACCGA
AAGGUCUUACCGGAAAACUCGACGCAAGAAAAAUCAGAGAGAUCCUCAUAAAGGCCAAGAAG
*GGCGGAAAGAUCGCCGUGUAA*UUCUAGU<u>AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA</u>
<u>AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA</u>CUGCAGGUCGACUCUAGAGGAUCCCC
<u>GGGUACCGAGCUCGAAUU</u>

Figure 8

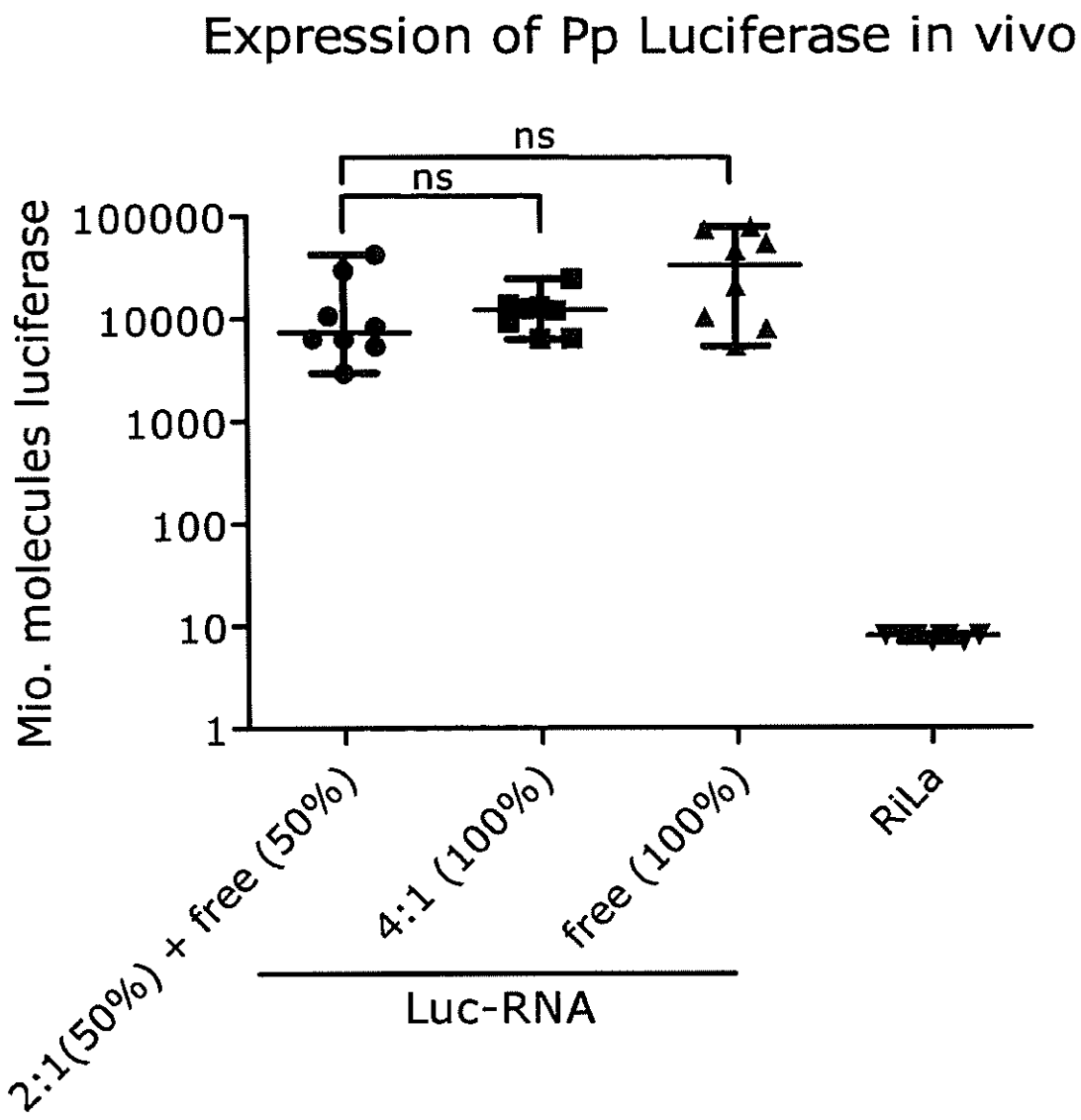

Expression of Pp Luciferase in vivo
ns
ns
Mio. molecules luciferase
100000
10000
1000
100
10
1
2:1(50%) + free (50%)
4:1 (100%)
free (100%)
RiLa
Luc-RNA

Figure 9

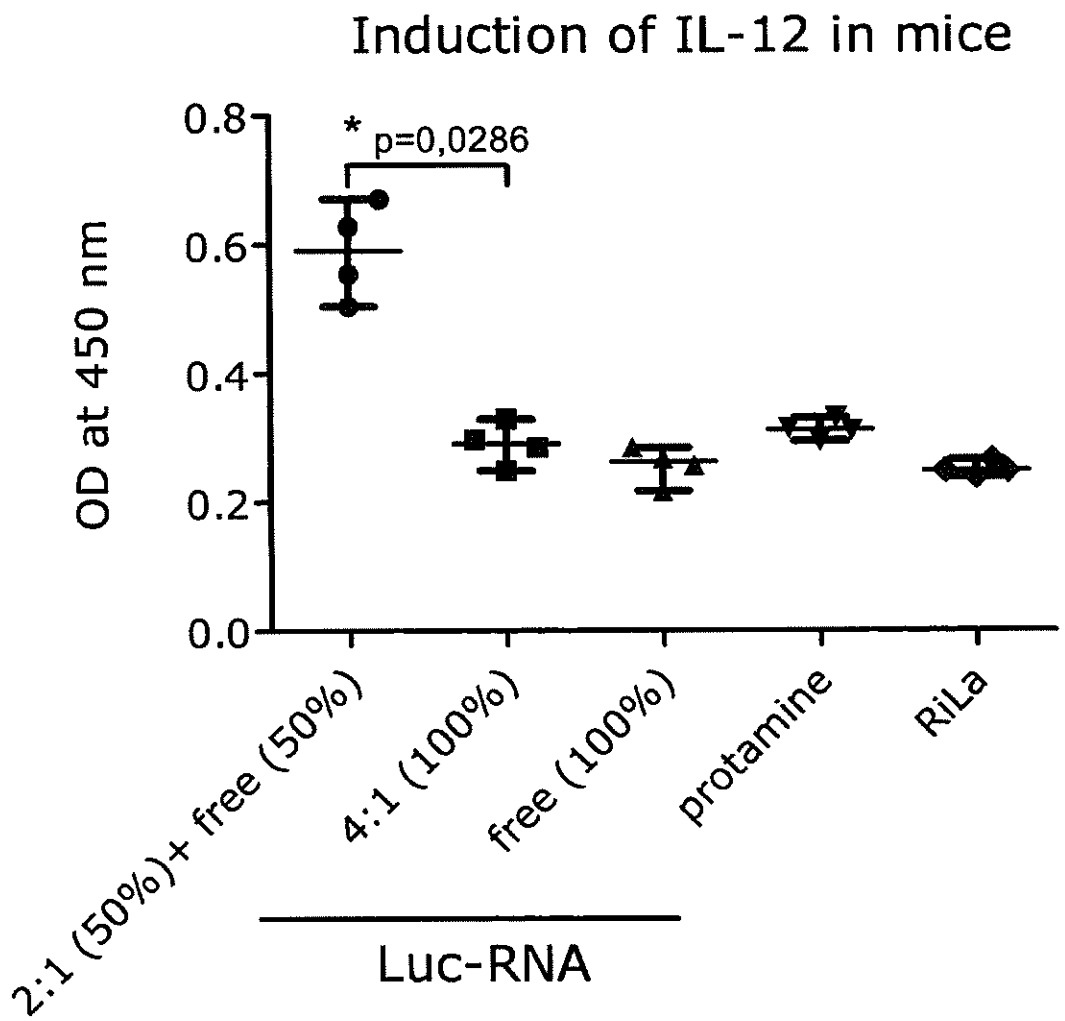

Induction of IL-12 in mice
OD at 450 nm
0.8
0.6
0.4
0.2
0.0
* p=0,0286
2:1 (50%)+ free (50%)
4:1 (100%)
free (100%)
protamine
RiLa
Luc-RNA

Figure 10

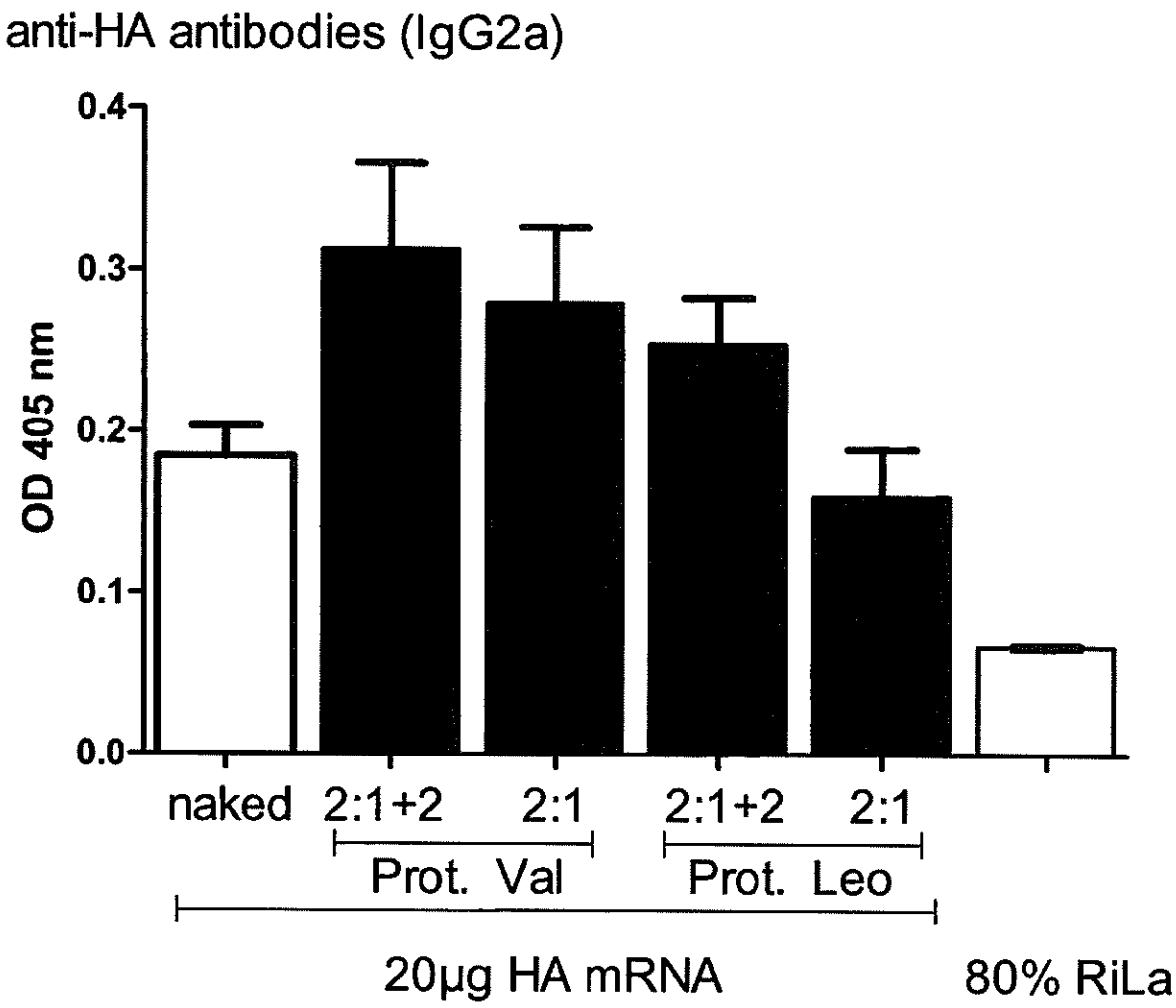

anti-HA antibodies (IgG2a)
OD 405 nm
0.4
0.3
0.2
0.1
0.0
naked
2:1+2
2:1
2:1+2
2:1
Prot. Val
Prot. Leo
20µg HA mRNA
80% RiLa

Figure 11

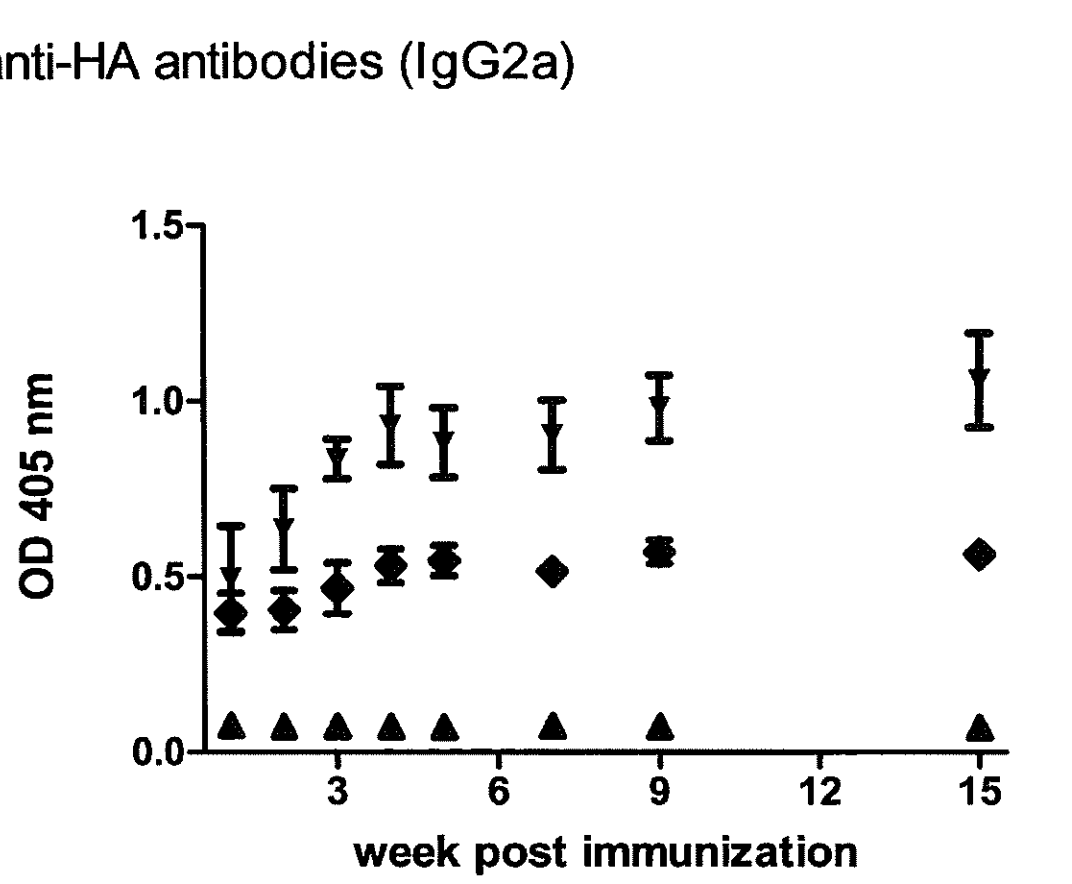

anti-HA antibodies (IgG2a)
OD 405 nm
1.5
1.0
0.5
0.0
3
6
9
12
15
week post immunization
complexed HA mRNA; RNA:Protamine 2:1 + free RNA (1:1)
naked HA mRNA
80% RiLa

Figure 12

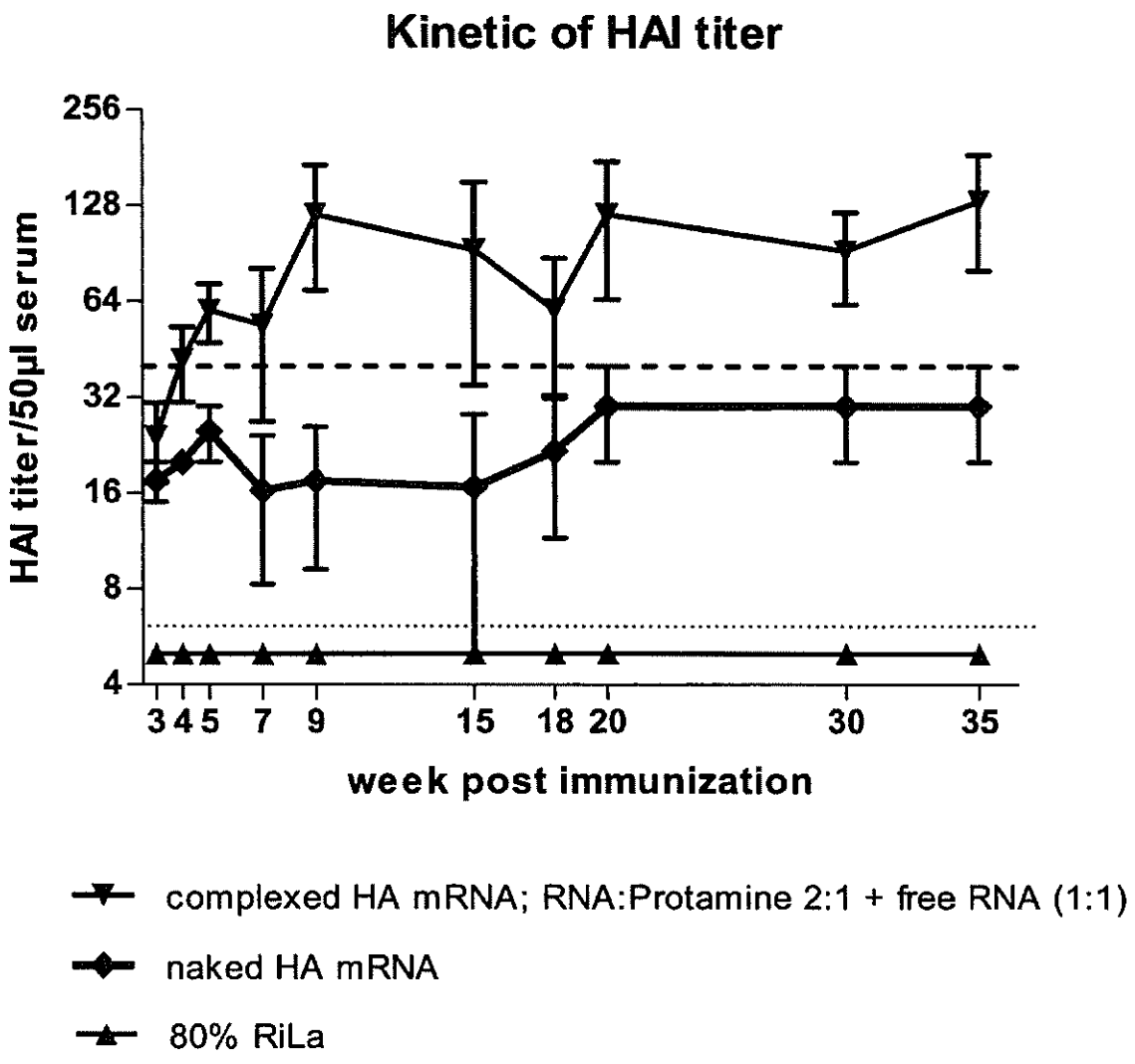

Kinetic of HAI titer
HAI titer/50µl serum
256
128
64
32
16
8
4
3 4 5 7 9 15 18 20 30 35
week post immunization
complexed HA mRNA; RNA:Protamine 2:1 + free RNA (1:1)
naked HA mRNA
80% RiLa

Figure 13

GGGAGAAAGCUUACCAUGAAGGCCAACCUGCUCGUGCUGCUGUGCGC
CCUCGCGGCCGCCGACGCCGACACCAUCUGCAUCGGCUACCACGCCA
ACAACAGCACCGACACGGUCGACACCGUGCUGGAGAAGAACGUGACC
GUCACCCACUCCGUGAACCUGCUCGAGGACAGCCACAACGGGAAGCU
GUGCCGGCUGAAGGGCAUCGCGCCCCUCCAGCUGGGGAAGUGCAACA
UCGCCGGCUGGCUGCUCGGGAACCCGGAGUGCGACCCCCUGCUGCCC
GUGCGCUCCUGGAGCUACAUCGUCGAGACGCCCAACUCCGAGAACGG
CAUCUGCUACCCGGGCGACUUCAUCGACUACGAGGAGCUCCGGGAGC
AGCUGAGCUCCGUGAGCUCCUUCGAGCGCUUCGAGAUCUUCCCCAAG
GAGAGCUCCUGGCCCAACCACAACACCAACGGGGUGACCGCCGCCUG
CAGCCACGAGGGCAAGUCCAGCUUCUACCGGAACCUGCUCUGGCUGA
CCGAGAAGGAGGGGUCCUACCCCAAGCUGAAGAACAGCUACGUCAAC
AAGAAGGGCAAGGAGGUGCUCGUGCUGUGGGGGAUCCACCACCCGCC
CAACUCCAAGGAGCAGCAGAACCUGUACCAGAACGAGAACGCGUACG
UCAGCGUGGUGACGUCCAACUACAACCGCCGGUUCACCCCCGAGAUC
GCCGAGCGCCCCAAGGUCCGGGACCAGGCCGGCCGCAUGAACUACUA
CUGGACCCUCCUGAAGCCGGGCGACACCAUCAUCUUCGAGGCCAACG
GGAACCUGAUCGCCCCGAUGUACGCGUUCGCCCUCAGCCGGGGCUUC
GGGAGCGGCAUCAUCACGUCCAACGCCAGCAUGCACGAGUGCAACAC
CAAGUGCCAGACCCCCCUGGGCGCCAUCAACUCCAGCCUGCCCUACC
AGAACAUCCACCCGGUGACCAUCGGGGAGUGCCCCAAGUACGUGCGC
UCCGCCAAGCUCCGGAUGGUCACGGGCCUGCGCAACAACCCCAGCAU
CCAGUCCCGGGGGCUGUUCGGCGCGAUCGCCGGGUUCAUCGAGGGCG
GCUGGACCGGGAUGAUCGACGGCUGGUACGGGUACCACCACCAGAAC
GAGCAGGGCAGCGGGUACGCCGCCGACCAGAAGUCCACCCAGAACGC
CAUCAACGGCAUCACCAACAAGGUGAACACGGUGAUCGAGAAGAUGA
ACAUCCAGUUCACCGCGGUCGGCAAGGAGUUCAACAAGCUCGAGAAG
CGCAUGGAGAACCUGAACAAGAAGGUGGACGACGGGUUCCUGGACAU
CUGGACCUACAACGCCGAGCUCCUGGUGCUGCUCGAGAACGAGCGGA
CCCUGGACUUCCACGACAGCAACGUCAAGAACCUGUACGAGAAGGUG
AAGUCCCAGCUCAAGAACAACGCCAAGGAGAUCGGCAACGGGUGCUU
CGAGUUCUACCACAAGUGCGACAACGAGUGCAUGGAGAGCGUCCGCA
ACGGCACGUACGACUACCCCAAGUACUCCGAGGAGAGCAAGCUGAAC
CGGGAGAAGGUGGACGGGGUGAAGCUGGAGUCCAUGGGCAUCUACCA
GAUCCUCGCCAUCUACAGCACCGUCGCCUCCAGCCUGGUGCUGCUGG
UGUCCCUCGGCGCGAUCAGCUUCUGGAUGUGCAGCAACGGGUCCCUG
CAGUGCCGCAUCUGCAUCUGACCACUAGUUAUAAGACUGACUAGCCC
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UAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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CCCCCUCUAGACAAUUGGAAUU

Figure 14