
Predicted nucleotide and amino acid sequence encoded by MRIB1-NL-mIgG2a-TM Fusion Gene:
HindIII
NcoI
MetGly LeuGlnLys SerLeuIleLeu PheProLeu PhePheLeu
1 GTTAAGCTTG CCACCATGGG TCTCCAGAAG TCCTTCATTC TGTTTCCGCT GTTTTTCCTG
CAATTCGAAC GGTGGTACCC AGAGGTCTTC AGGAAGTAAG ACAAAGGCGA CAAAAAGGAC
SmaI
XmaI
AvaI
PstI
LeuLeuGlyTrp ValGlnPro SerProGly ArgGluSerAla AlaGlnLys PheGlnArg
61 CTGCTTGGAT GGGTCCAGCC TTCCCCGGGC AGGGAATCTG CAGCACAGAA GTTTCAGCGG
GACGAACCTA CCCAGGTCGG AAGGGGCCCG TCCCTTAGAC GTCGTGTCTT CAAAGTCGCC
BamHI
GlnHisMetAsp ProAspGly SerGlnIle AsnSerProThr TyrCysAsn GlnMetMet
121 CAGCACATGG ATCCAGATGG TTCCTCCATC AACAGCCCCA CCTACTGCAA CCAAATGATG
GTCGTGTACC TAGGTCTACC AAGGAGGTAG TTGTCGGGGT GGATGACGTT GGTTTACTAC
LysArgArgAsp MetThrAsn GlySerCys LysProValAsn ThrPheVal HisGluPro
181 AAACGCCGGG ATATGACAAA TGGGTCATGC AAGCCCGTGA ACACCTTCGT GCATGAGCCC
TTTGCGGCCC TATACTGTTT ACCCAGTACG TTCGGGCACT TGTGGAAGCA CGTACTCGGG
LeuAlaAspVal GlnAlaVal CysGlnGln GluAsnValThr CysLysAsn ArgLysSer
241 TTGGCAGATG TCCAGGCCGT CTGTCAGCAG GAAAATGTCA CCTGCAAGAA CAGGAAGAGC
AACCGTCTAC AGGTCCGGCA GACGAGGTCG CTTTTACAGT GGACGTTCTT GTCCTTCTCG
AsnCysTyrLys SerSerSer AlaLeuGln IleThrAspCys GlnLeuLys GlyAsnGln
301 AACTGCTACA AGAGCAGCTC TGCCCTGCAG ATCACTGACT GCCAGCTGAA GGGCAACTCC
TTGACGATGT TCTCGTCGAG ACGGGACGTC TAGTGACTGA CGGTCGACTT CCCGTTGAGG
LysTyrProAsn CysAspTyr LysThrThr GlnTyrGlnLys HisIleIle ValAlaCys
361 AAGTATCCCA ACTGTGACTA CAAAACCACT CAATACCAGA AGCACATCAT TGTGGCCTGT
TTCATAGGGT TGACACTGAT GTTTTGGTGA GTTATGGTCT TCGTGTAGTA ACACCGGACA
XhoI
AvaI
GlnGlyAsnPro TyrValPro ValHisPhe AspAlaThrVal LeuGluPro ArgGlyLeu
421 CAAGGTAACC CCTACGTACC AGTCCACTTT GATGCTACTG TGCTCGAGCC CAGAGGTCTC
GTTCCATTGG GGATGCATGG TCAGGTGAAA CTACGATGAC ACGAGCTCGG GTCTCCAGAG

Figure 1



Figure 1 (Con't)

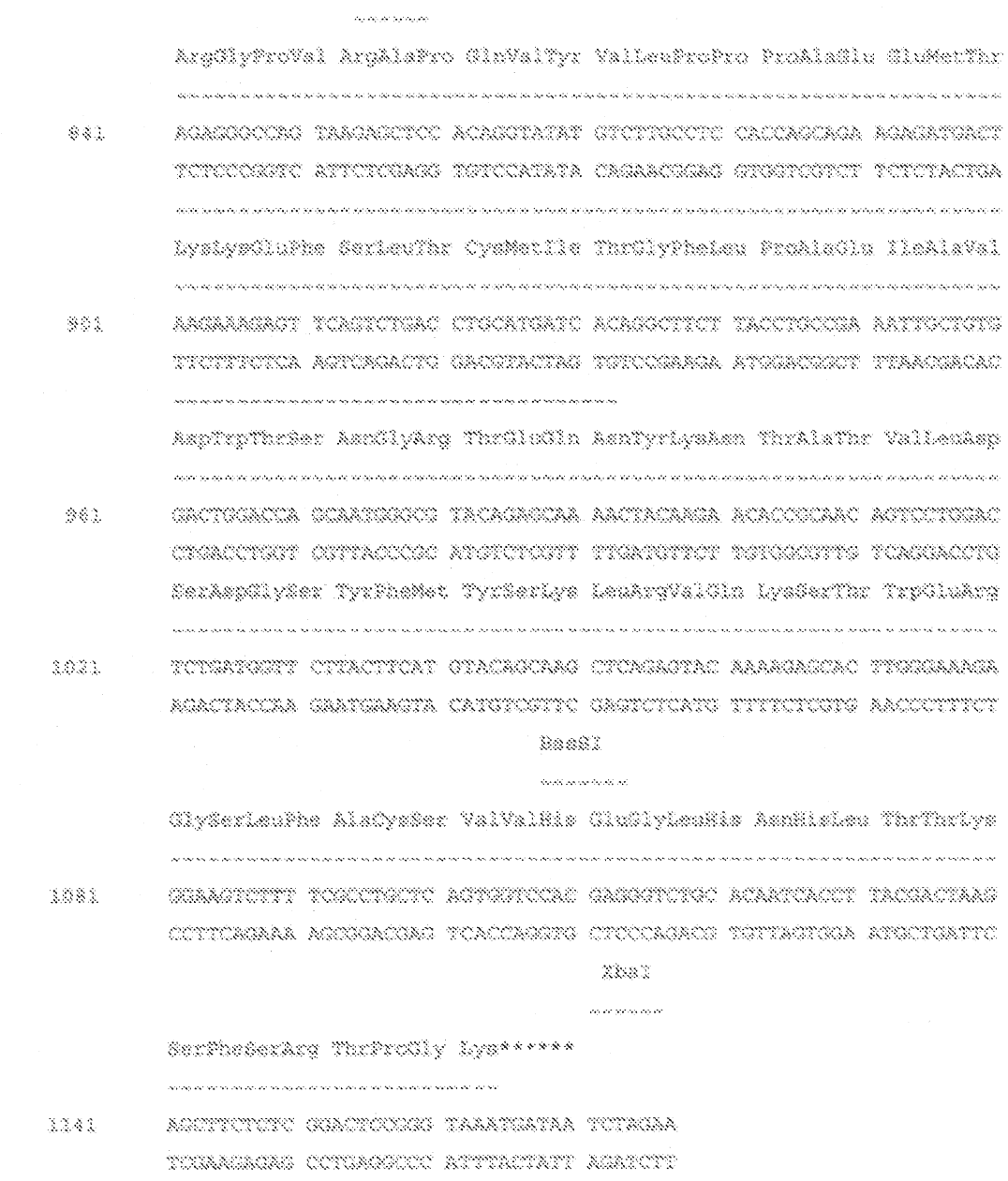


Figure 1 (Con't)

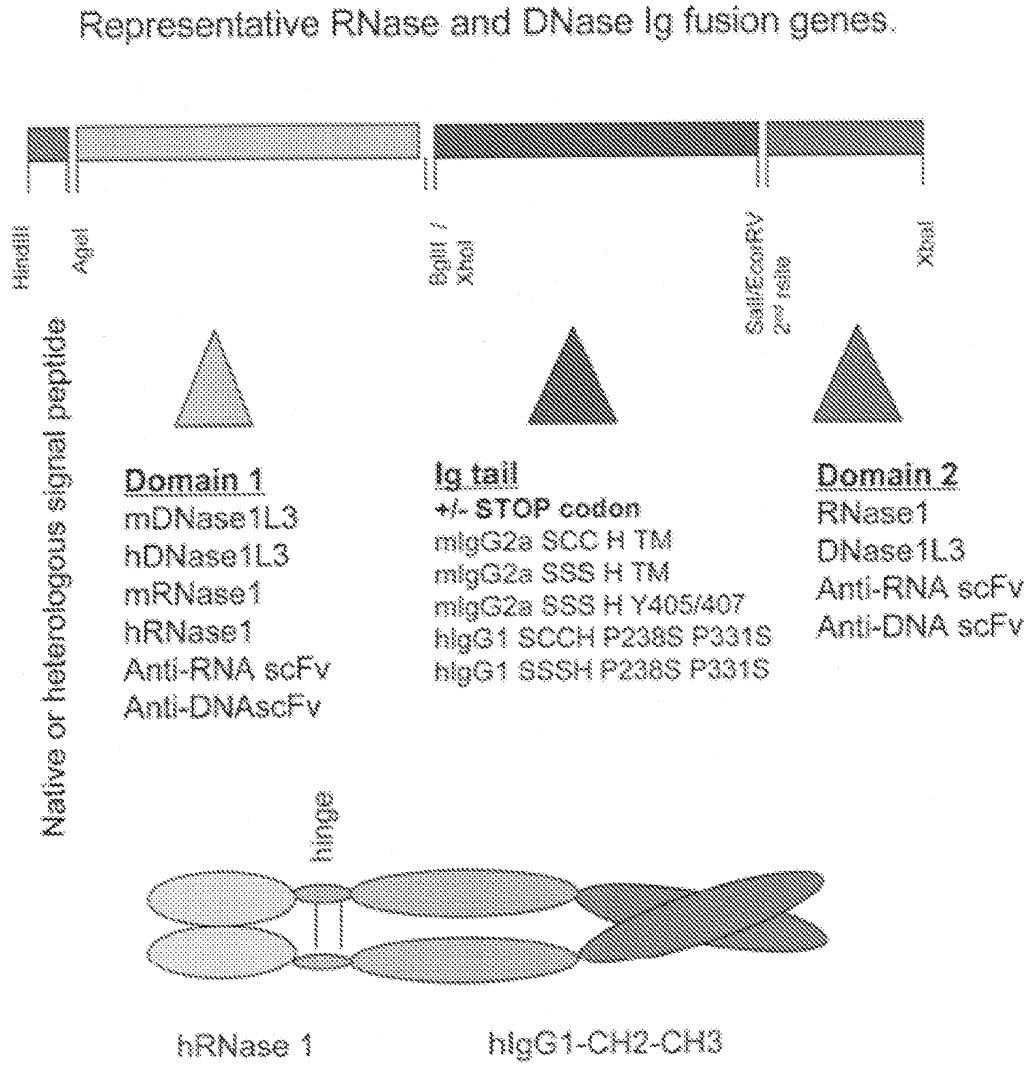

Representative RNase and DNase Ig fusion genes.
HindIII
AgeI
BglII / XhoI
SalI/EcoRV 2nd site
XbaI
Native or heterologous signal peptide
Domain 1
mDNase1L3
hDNase1L3
mRNase1
hRNase1
Anti-RNA scFv
Anti-DNAscFv
Ig tail
+/- STOP codon
mIgG2a SCC H TM
mIgG2a SSS H TM
mIgG2a SSS H Y405/407
hIgG1 SCCH P238S P331S
hIgG1 SSSH P238S P331S
Domain 2
RNase1
DNase1L3
Anti-RNA scFv
Anti-DNA scFv
hinge
hRNase 1
hIgG1-CH2-CH3

Figure 2

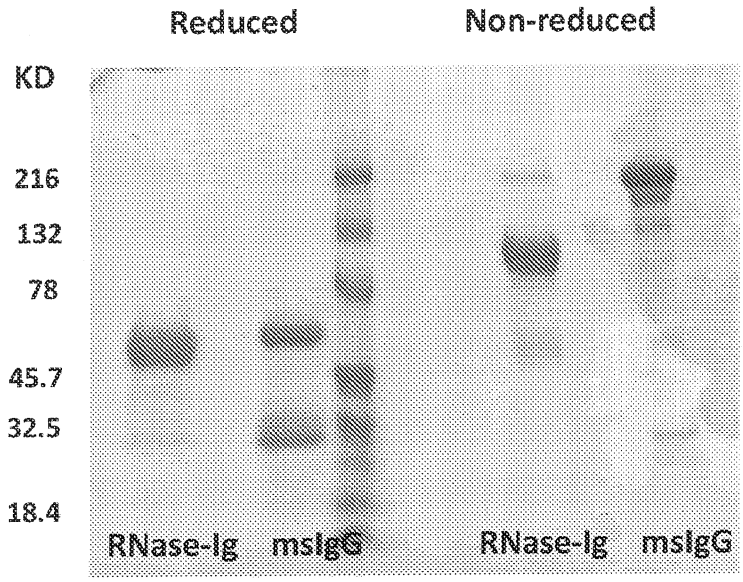

Reduced
Non-reduced
KD
216
132
78
45.7
32.5
18.4
RNase-Ig
msIgG
RNase-Ig
msIgG

Figure 3

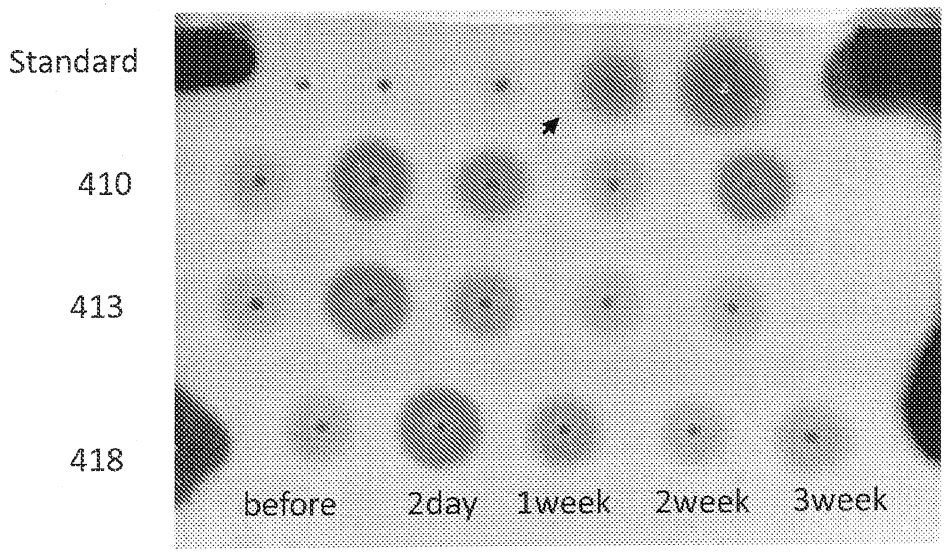


Figure 4

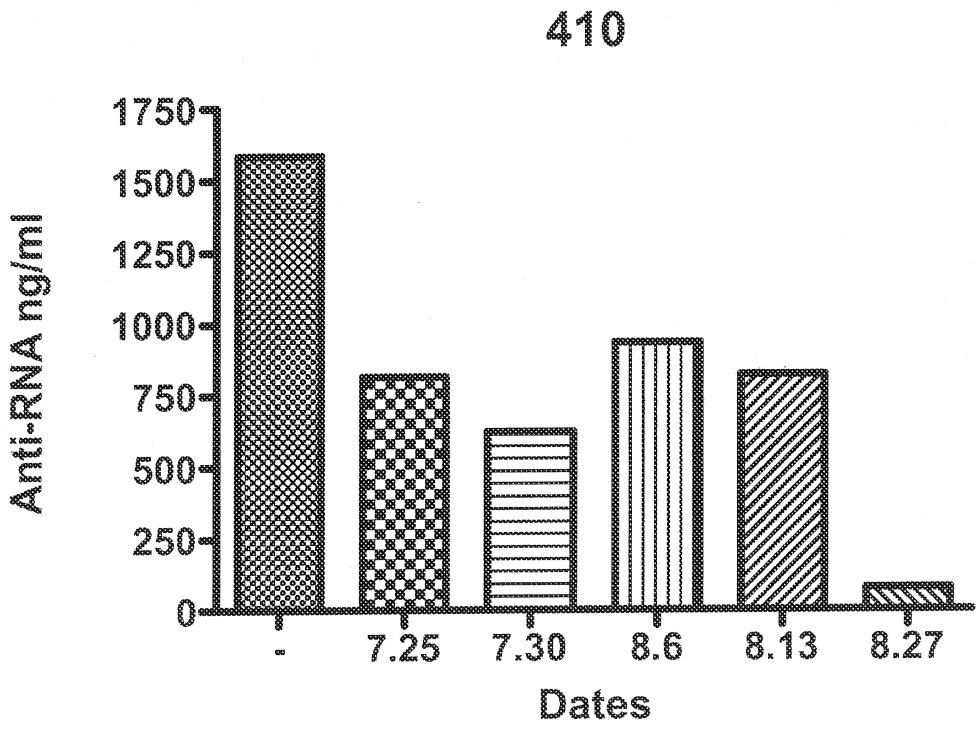

410
Anti-RNA ng/ml
1750
1500
1250
1000
750
500
250
0
-
7.25
7.30
8.6
8.13
8.27
Dates

Figure 5

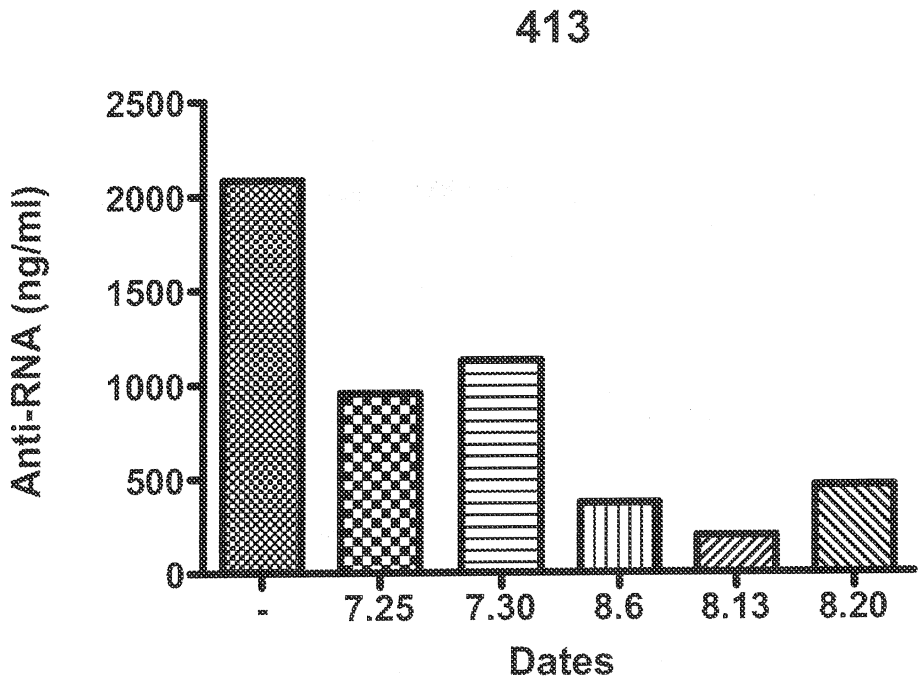

413
Anti-RNA (ng/ml)
2500
2000
1500
1000
500
0
-
7.25
7.30
8.6
8.13
8.20
Dates

Figure 6

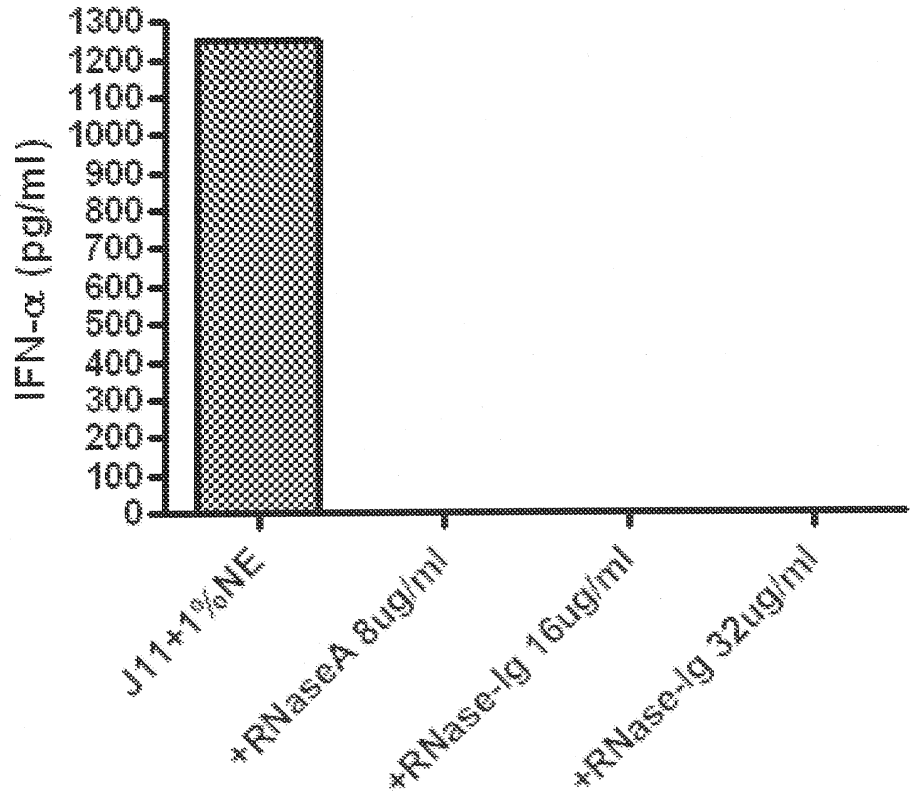

IFN-α (pg/ml)
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
J11+1%NE
+RNaseA 8ug/ml
+RNase-Ig 16ug/ml
+RNase-Ig 32ug/ml

Figure 7

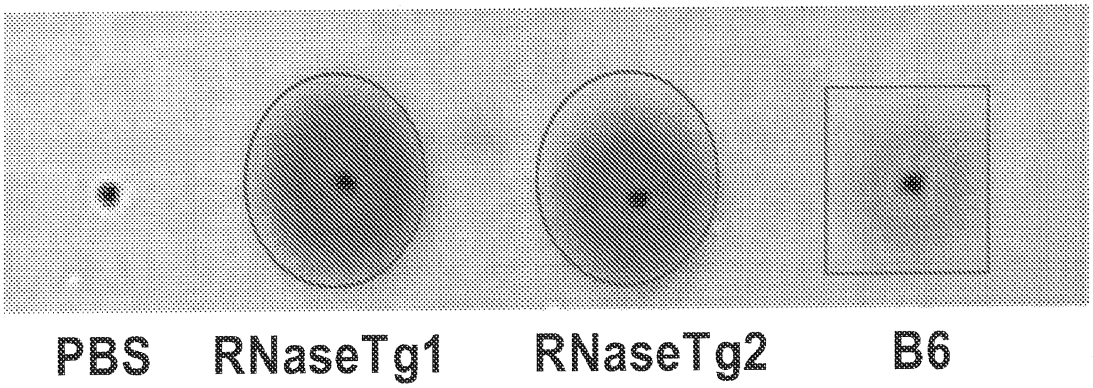


Figure 8

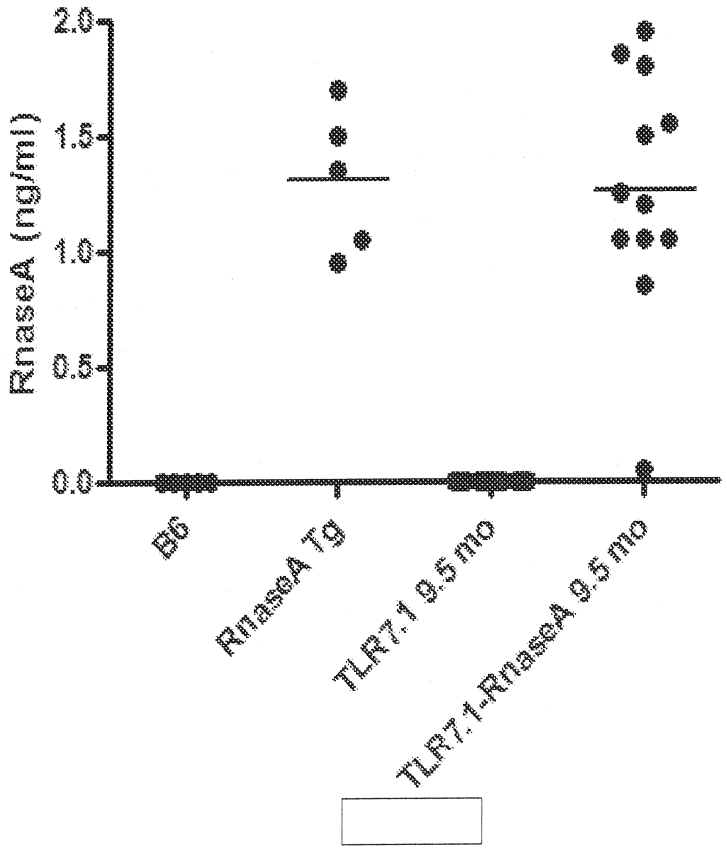

RnaseA (ng/ml)
2.0
1.5
1.0
0.5
0.0
B6
RnaseA Tg
TLR7.1 9.5 mo
TLR7.1-RnaseA 9.5 mo

Figure 9

TLR7.1-RnaseA:

Fraction of alive mice
TLR7.1
2 mo: 22/23
3 mo: 20/23
4 mo: 17/23
5 mo: 15/23
6 mo: 12/23
7 mo: 11/23
8 mo: 11/23
9 mo: 10/23
10 mo:9/23

Fraction of alive mice
TLR7.1-RNaseA
2 mo: 16/17
3 mo: 16/17
4 mo: 15/17
5 mo: 14/17
6 mo: 14/17
7 mo: 14/17
8 mo: 13/17
9 mo: 12/17
10 mo: 12/17

As of 10.14.10
P value: 0.0065

Survival proportion
TLR7.1: 40%
TLR7.1-RnaseA: 70.5%

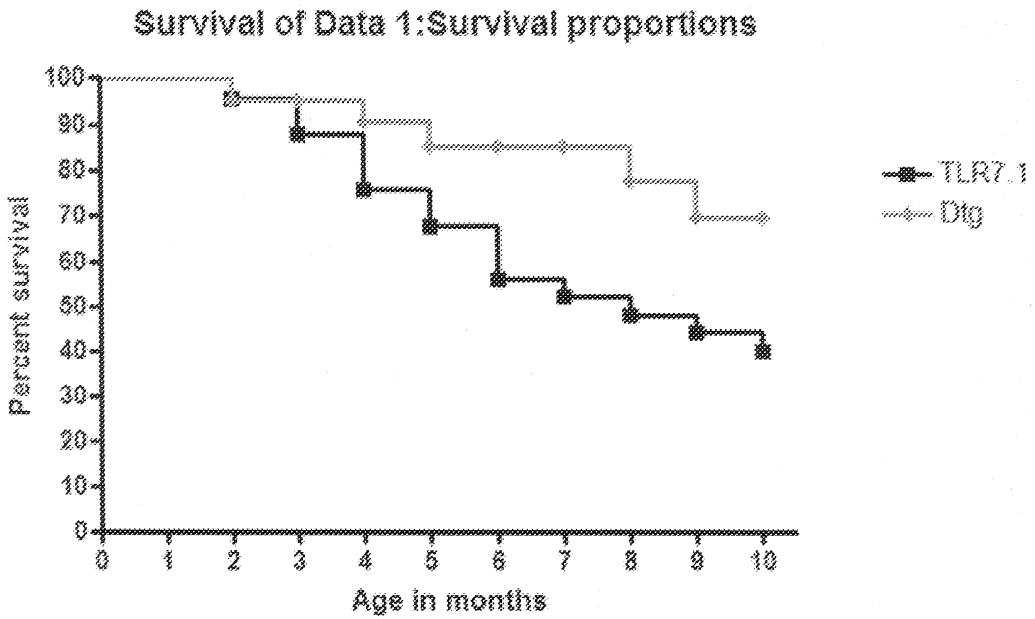


FIGURE 10

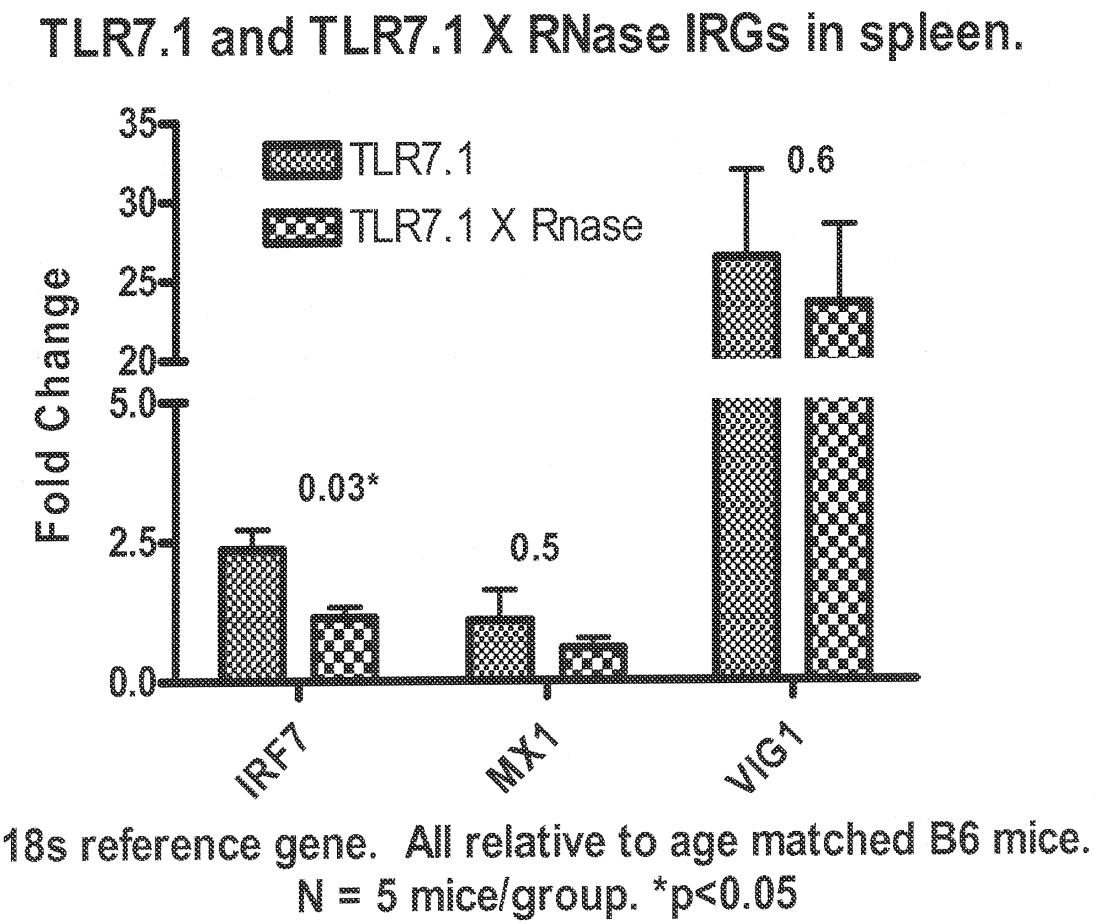
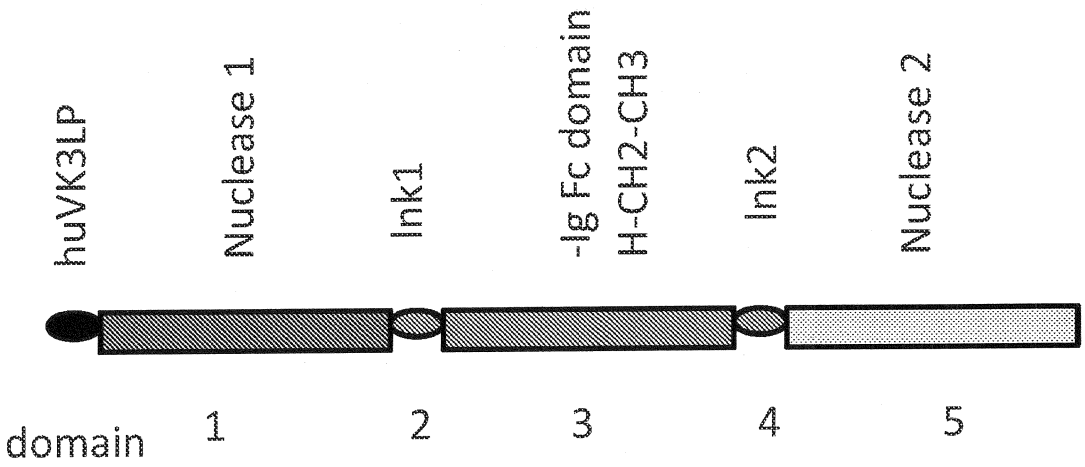

TLR7.1 and TLR7.1 X RNase IRGs in spleen.
Fold Change
35
30
25
20
5.0
2.5
0.0
TLR7.1
TLR7.1 X Rnase
0.03*
0.5
0.6
IRF7
MX1
VIG1
18s reference gene. All relative to age matched B6 mice.
N = 5 mice/group. *p<0.05

Figure 11



Nuclease 1 or 2	lnk 1 or 2	IgGFc
hRNase-WT	(gly4ser)3	SCCH-hIgG1WT
hRNase-G88D	(gly4ser)4	hIgG1-P238S
hDNase1 WT	(gly4ser)5	hIgG1-N297S
hDNAse1-A114F	NLG	hIgG1-P331S
hDNase1-G105R;A114F	none	mIgG2a-WT
hDNase1-G105R	(gly4ser)n	mIgG2c-WT
hTREX1-72AA		mIgG2A-C WT or MT
hDNase1L3		
hTREX1-lnk-hTREX1		

Figure 12

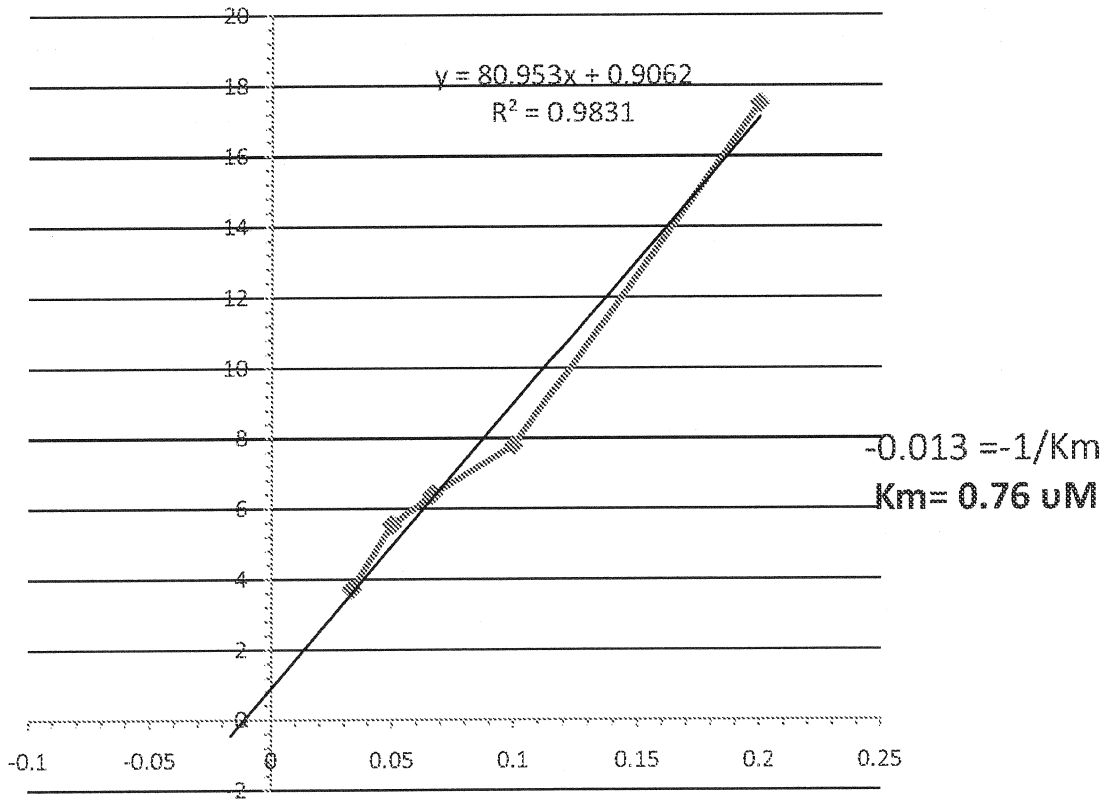

y = 80.953x + 0.9062
R² = 0.9831
20
18
16
14
12
10
8
6
4
2
0
-2
-0.1
-0.05
0
0.05
0.1
0.15
0.2
0.25
-0.013 =-1/Km
Km= 0.76 uM

Figure 13

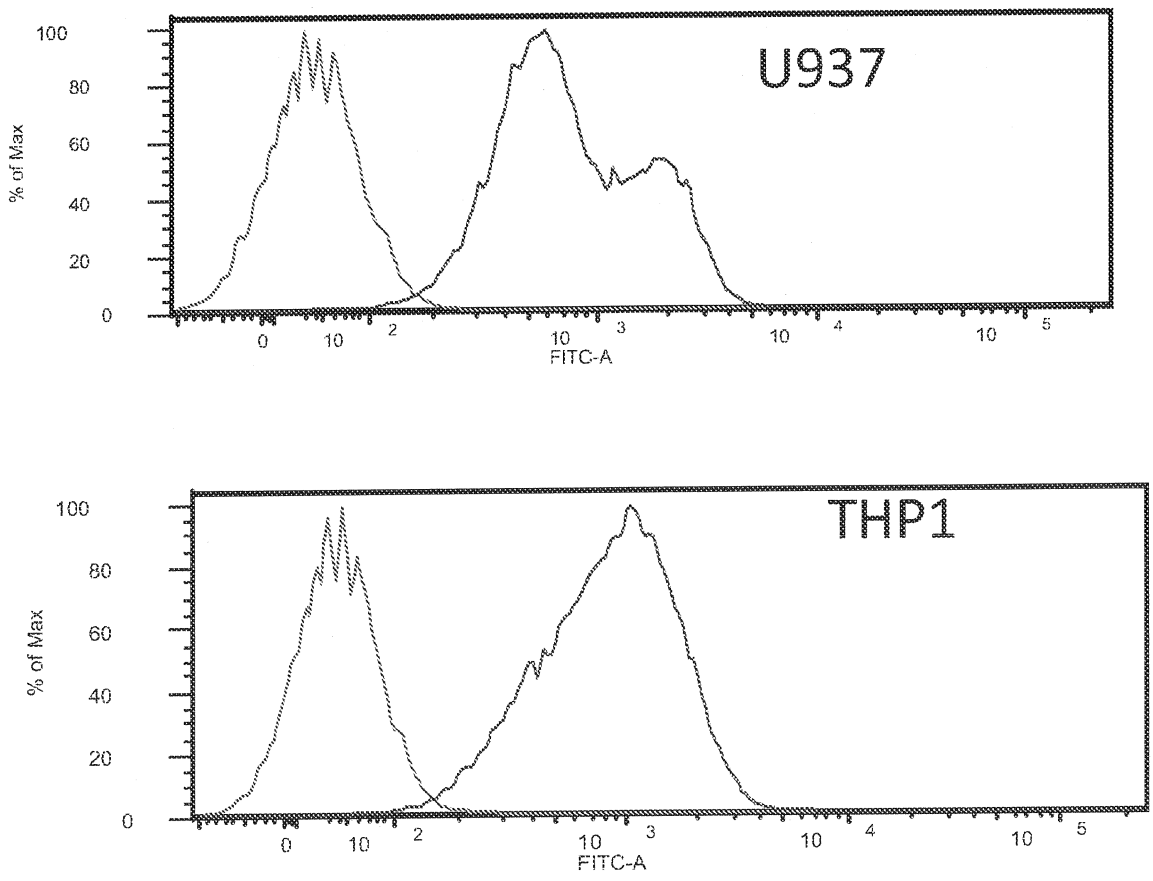

U937
% of Max
100
80
60
40
20
0
0
10
2
10
3
10
4
10
5
FITC-A
THP1
% of Max
100
80
60
40
20
0
0
10
2
10
3
10
4
10
5
FITC-A

Figure 14

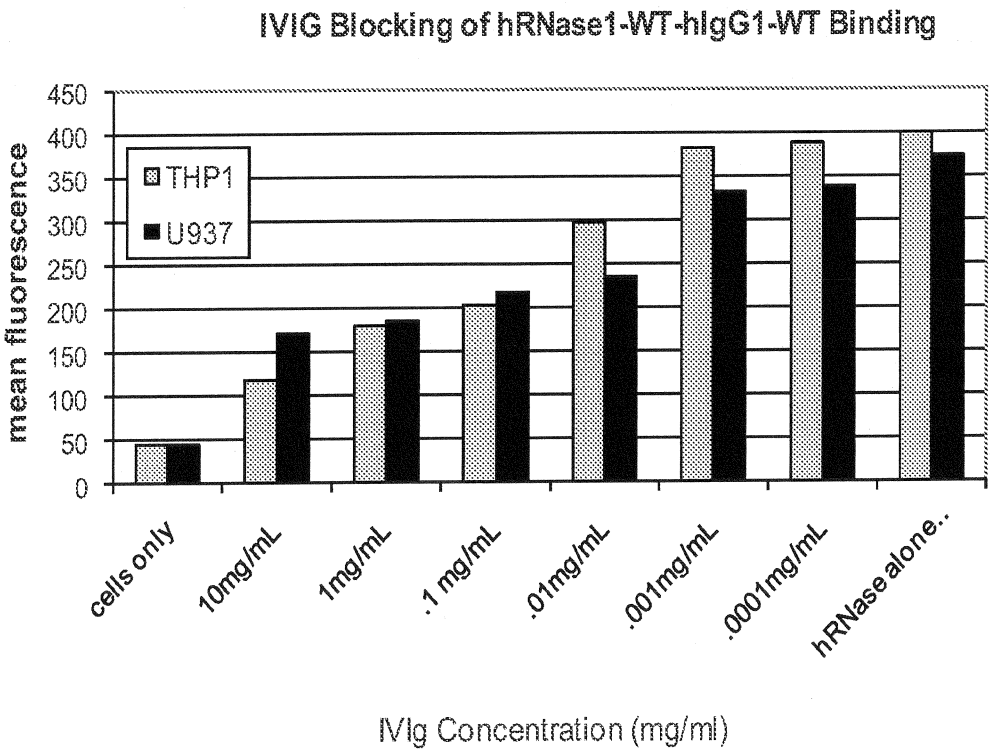
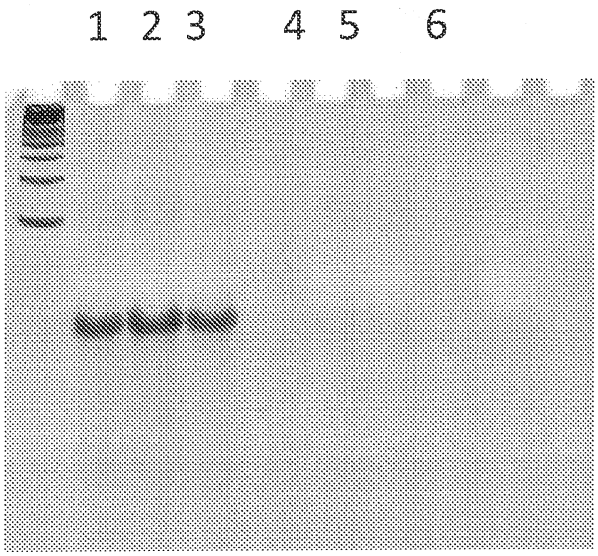
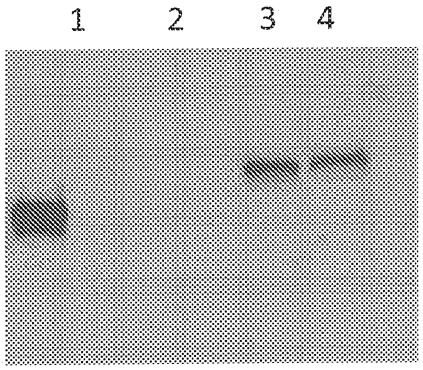

IVIG Blocking of hRNase1-WT-hIgG1-WT Binding
mean fluorescence
450
400
350
300
250
200
150
100
50
0
THP1
U937
cells only
10mg/mL
1mg/mL
.1 mg/mL
.01mg/mL
.001mg/mL
.0001mg/mL
hRNase alone..
IVIg Concentration (mg/ml)

Figure 15



1: BDCA2 transfected COS sup
2: Control without enzyme
3: Mock transfected COS sup
4: Trex1-(Gly4S)4-Ig transfected COS sup
5: Trex1-(Gly4S)5-Ig transfected COS sup
6: human TREX1 positive control

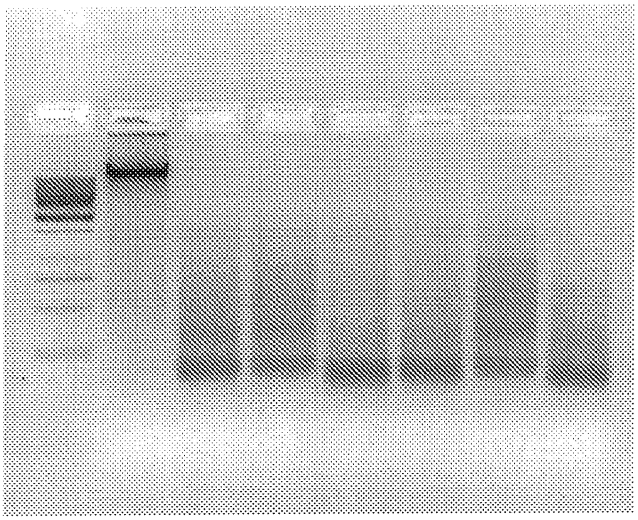
Figure 16



1: BDCA2-Ig transfected COS sup (1ml) used as positive control
2: Mock COS sup (1ml) used as negative control
3: Trex1-(Gly4S)4-Ig transfected COS sup (1ml)
4: Trex1-(Gly4S)5-Ig transfected COS sup (1ml)

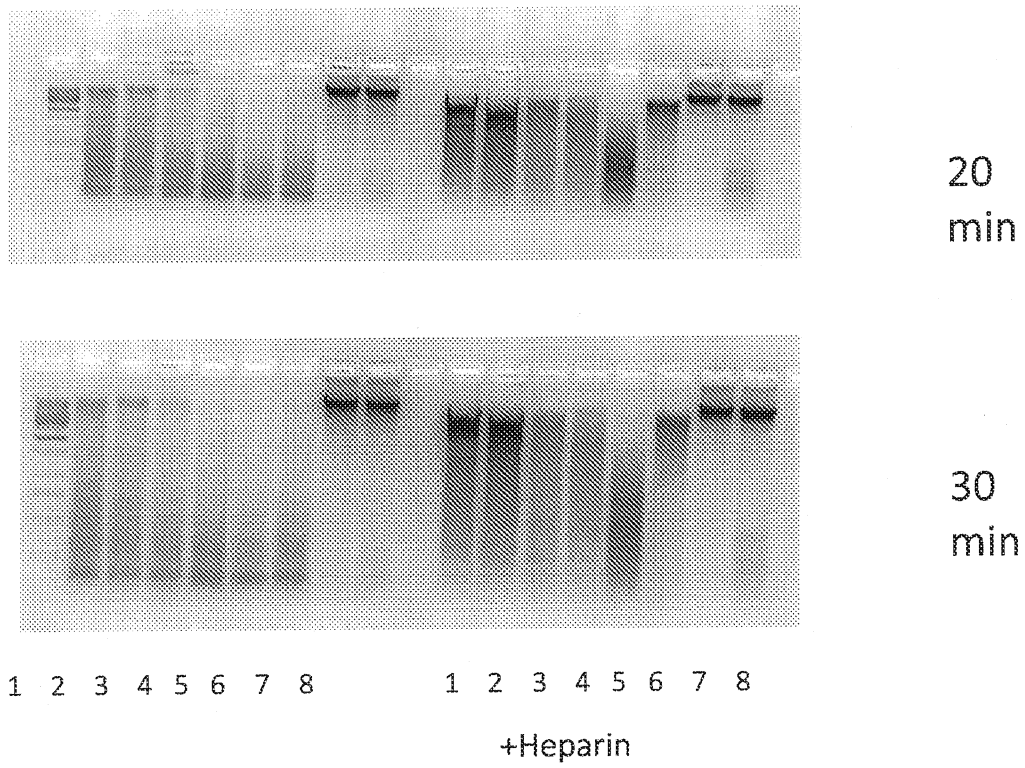
Figure 17

1 2 3 4 5 6 7



1.control
2.2A3(600nM MTX)
3.2A3(800nM MTX)
4.3A5(300nM MTX)
5.3A5(400nM MTX)
6.8H8(600nM MTX)
7.bulk CHO sup

Figure 18



1,2,3,4,5: mDNase1l3-L-Ig transfected COS sup 2ul, 5ul, 10ul, 15ul and 20ul
6: mDNase1l3-NL-Ig transfected COS sup 20ul
7: mRNase-Ig transfected COS sup 20ul
8: COS sup 20ul

Figure 19

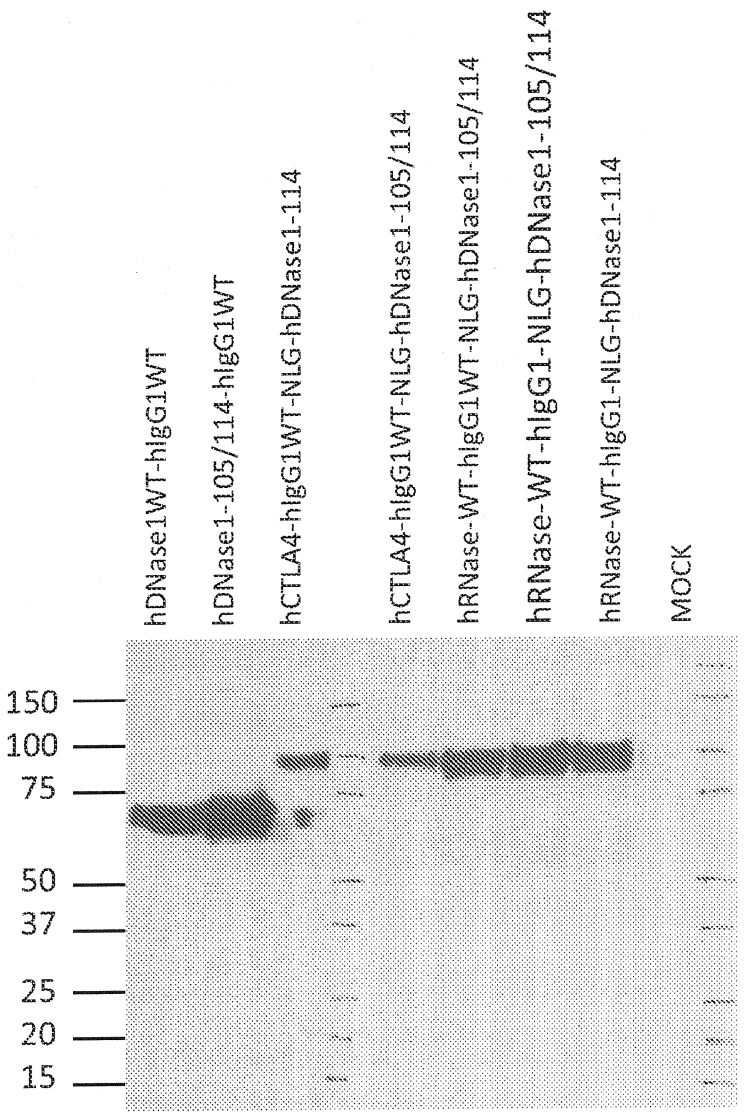
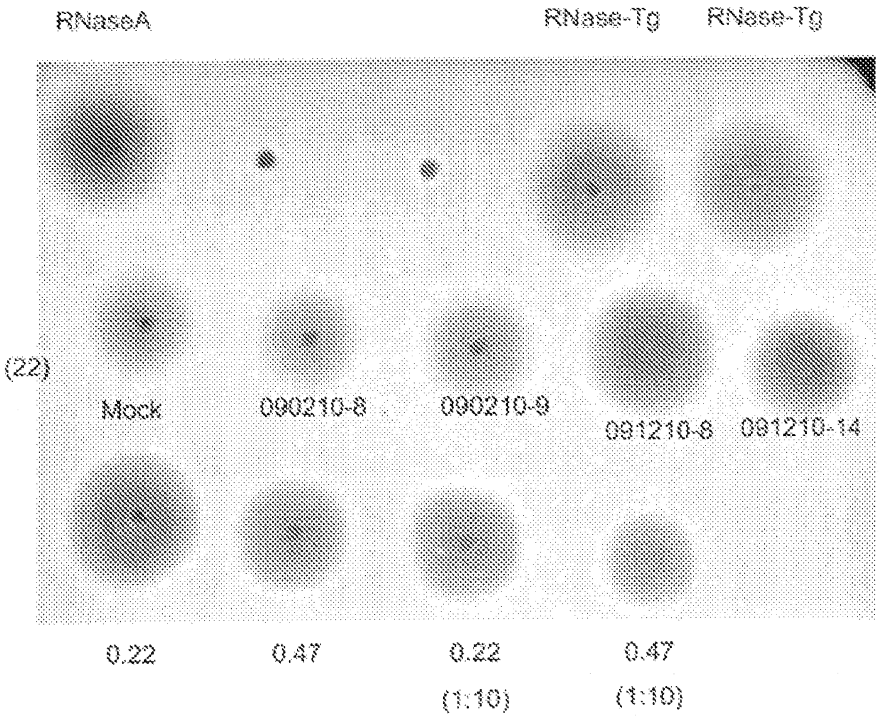

hDNase1WT-hIgG1WT
hDNase1-105/114-hIgG1WT
hCTLA4-hIgG1WT-NLG-hDNase1-114
hCTLA4-hIgG1WT-NLG-hDNase1-105/114
hRNase-WT-hIgG1WT-NLG-hDNase1-105/114
hRNase-WT-hIgG1-NLG-hDNase1-105/114
hRNase-WT-hIgG1-NLG-hDNase1-114
MOCK
150
100
75
50
37
25
20
15

Figure 20

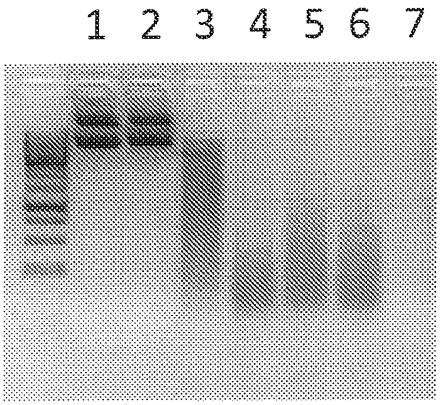


Mock
090210-8=DNaseWTIg
090210-9=DNase105/114Ig
091210-8=Rnase-Ig-DNase105/114
091210-14=Rnase-Ig-DNase114

0.22 purified mRNaseA-WT-mIgG2c-WT fusion protein
0.47 purified mRNaseA-WT-hmIgG2c-WT fusion protein

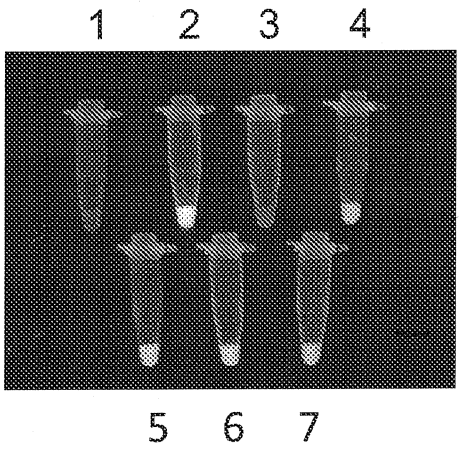
Figure 21

A. Gel analysis of Plasmid DNA Digestion



1: plasmid DNA mix
2: mock sup
3: 090210-8
4: 090210-9
5: 091210-8
6: 091210-14
7: DNAseI

B. DNase Alert Substrate digestion/UV visualization



1: neg. control ddH2O
2:DNase 1 (2 U)
3: mock transfected sup.
4: 090210-8
5: 090210-9
6: 091210-8
7: 091210-14

Figure 22

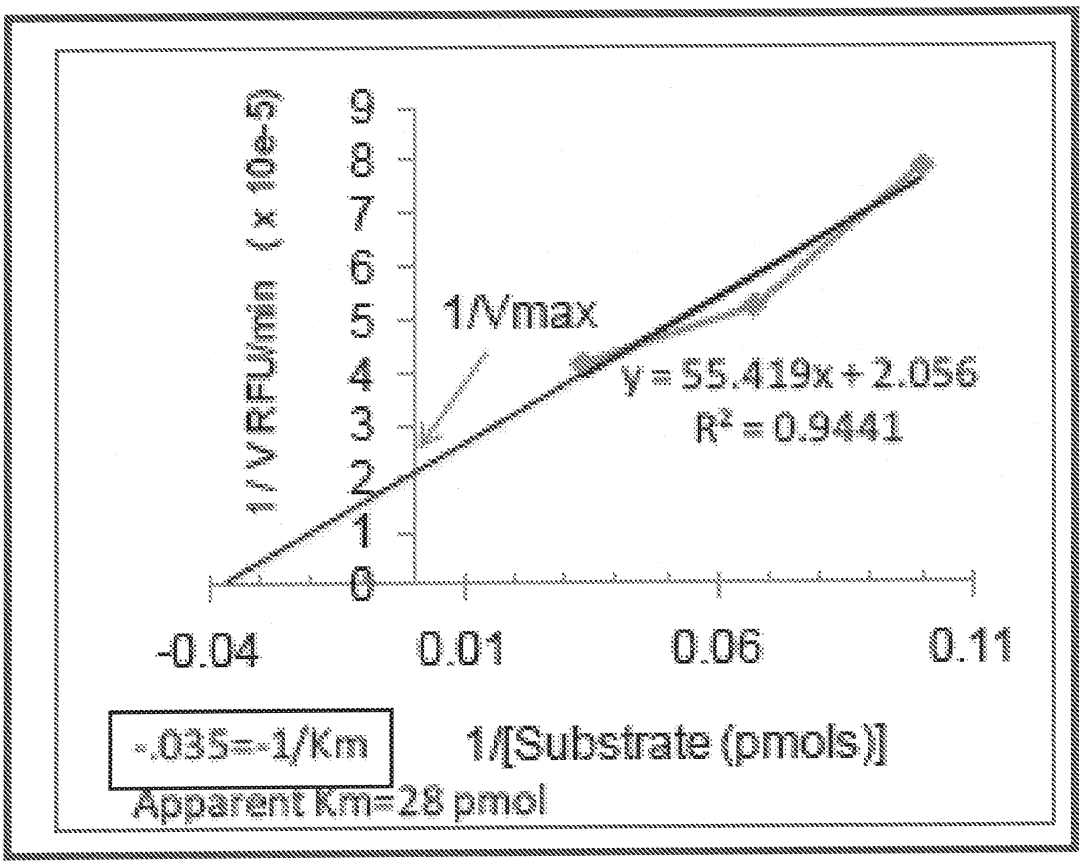

1 / V RFU/min (x 10e-5)
9
8
7
6
5
4
3
2
1
0
1/Vmax
y = 55.419x + 2.056
R² = 0.9441
-0.04
0.01
0.06
0.11
-.035=-1/Km
1/[Substrate (pmols)]
Apparent Km=28 pmol

Figure 23

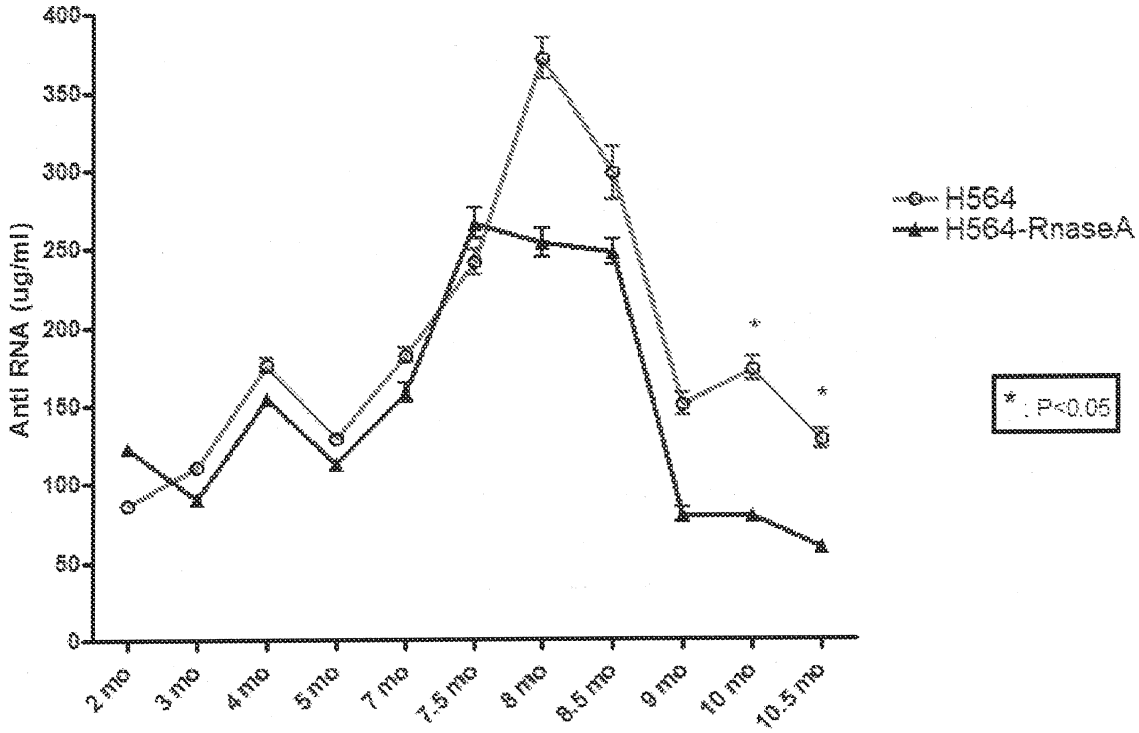

Anti RNA (ug/ml)
400
350
300
250
200
150
100
50
0
2 יום
3 יום
4 יום
5 יום
7 יום
7.5 יום
8 יום
8.5 יום
9 יום
10 יום
10.5 יום
H564
H564-RnaseA
*
*
*: P<0.05

Figure 24