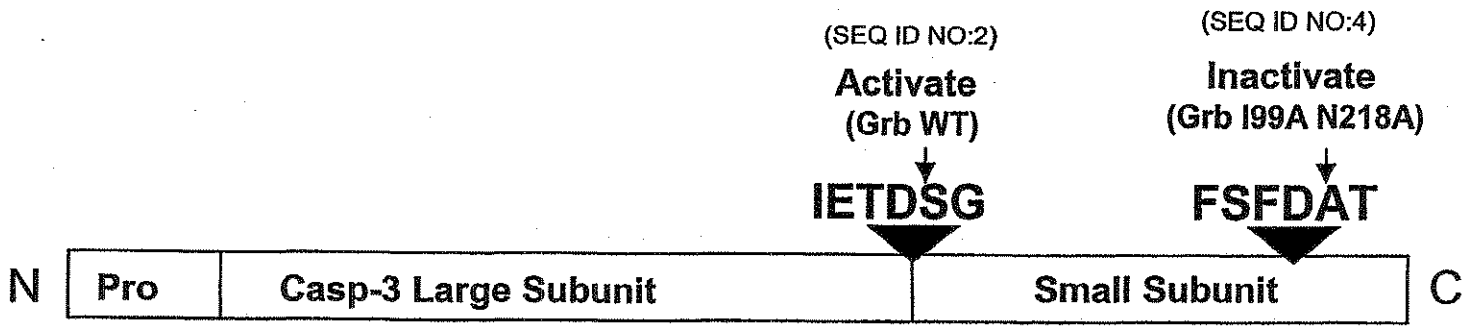


Large Subunit

MENTENSVDS KSIKNLEPKI IHGSESMDSG ISLDNSYKMD
YPEMGLCIII NNKNFHKSTG MTSRSGTDVD AANLRETFRN
LKYEVRNKND LTREEIVELM RDVSKEDHSK RSSFVCVLLS
HGEEGIIFGT NGPVDLKKIT NFFRGDRCRS LTGKPKLFII
QA**C**RGTELDC G**IETD**

Small Subunit

SGVDDDMACH KIPVDADFLY AYSTAPGYYS WRNSKDGSWF
IQSLCAMLKQ YADKLEFMHI LTRVNRKVAT EFES**FSFDAT**
FHAKKQIPCI VSMLTKELYF YH (SEQ ID NO:1)

Figure 1

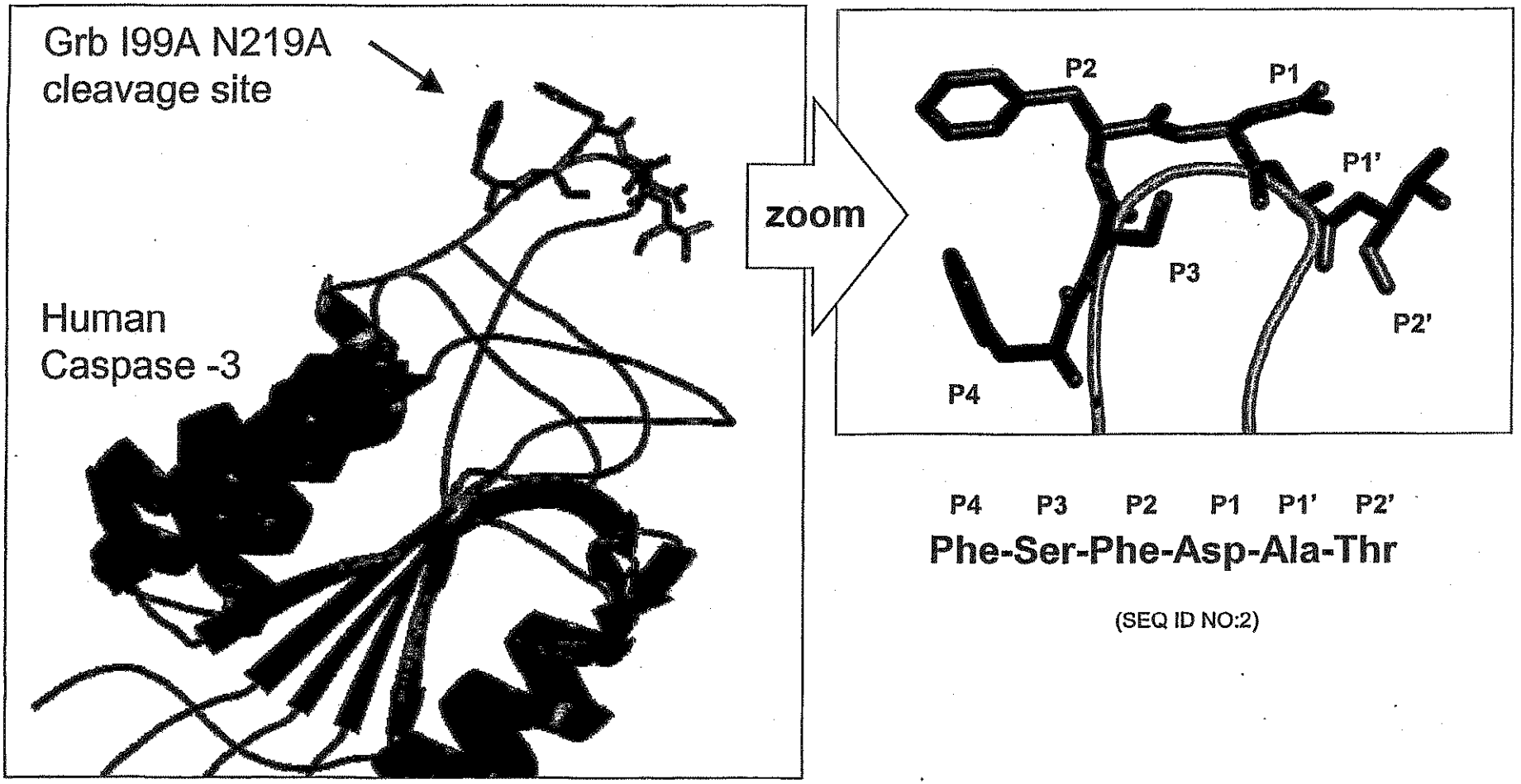
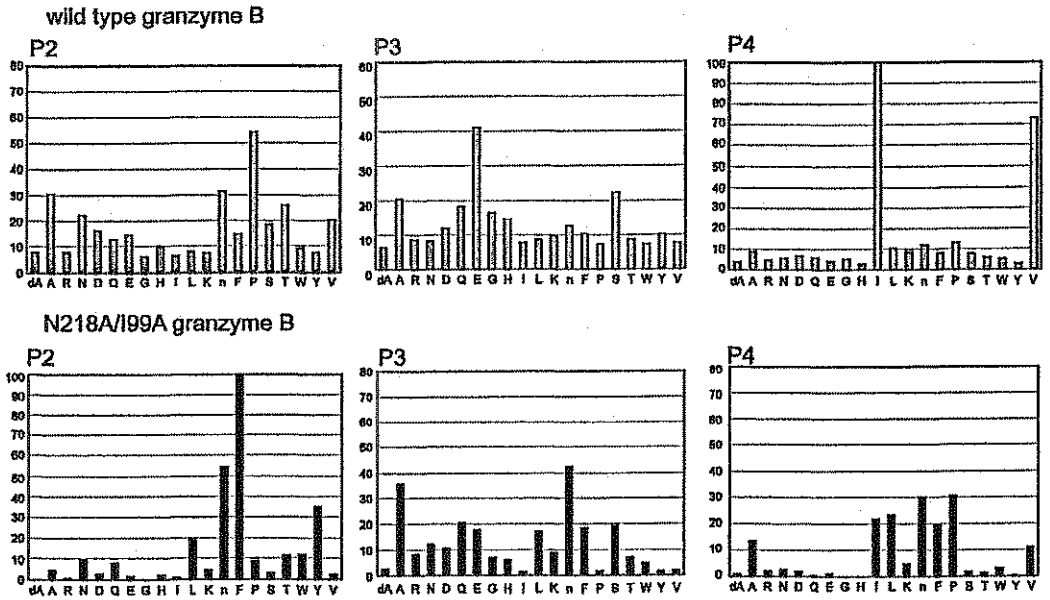

Grb I99A N219A
cleavage site
Human
Caspase -3
zoom
P2
P1
P1'
P3
P2'
P4
P4 P3 P2 P1 P1' P2'
Phe-Ser-Phe-Asp-Ala-Thr
(SEQ ID NO:2)

Figure 2

Figure 3

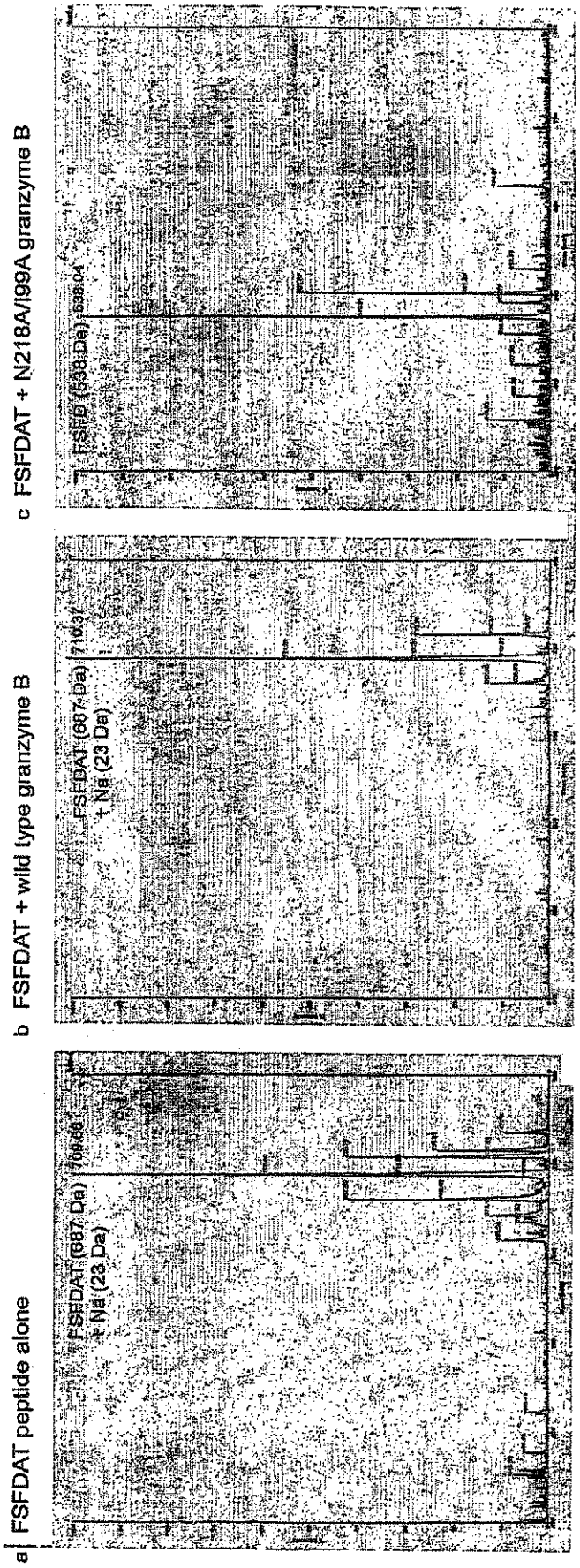
A



B

Kinetics:	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_{cat} (s^{-1})	K_m (μM^{-1})	
grB				
Ac-IE*P*D-AMC	3300 ± 500	1.2 ± 0.2	370 ± 70	> 1.25 X
Ac-IE*F*D-AMC	2600 ± 101	1.9 ± 0.7	750 ± 171	
N218A/I99A grB				
Ac-IE*P*D-AMC	32 ± 1.9	0.087 ± 0.005	2700 ± 590	> 5 X
Ac-IE*F*D-AMC	160 ± 14	0.26 ± 0.08	1600 ± 600	

Figure 4


a FSFDAT peptide alone
FSFDAT (687 Da) + Na (23 Da)
708.66
b FSFDAT + wild type granzyme B
FSFDAT (687 Da) + Na (23 Da)
710.37
c FSFDAT + N218A/I99A granzyme B
FSFD (538 Da)
538.04

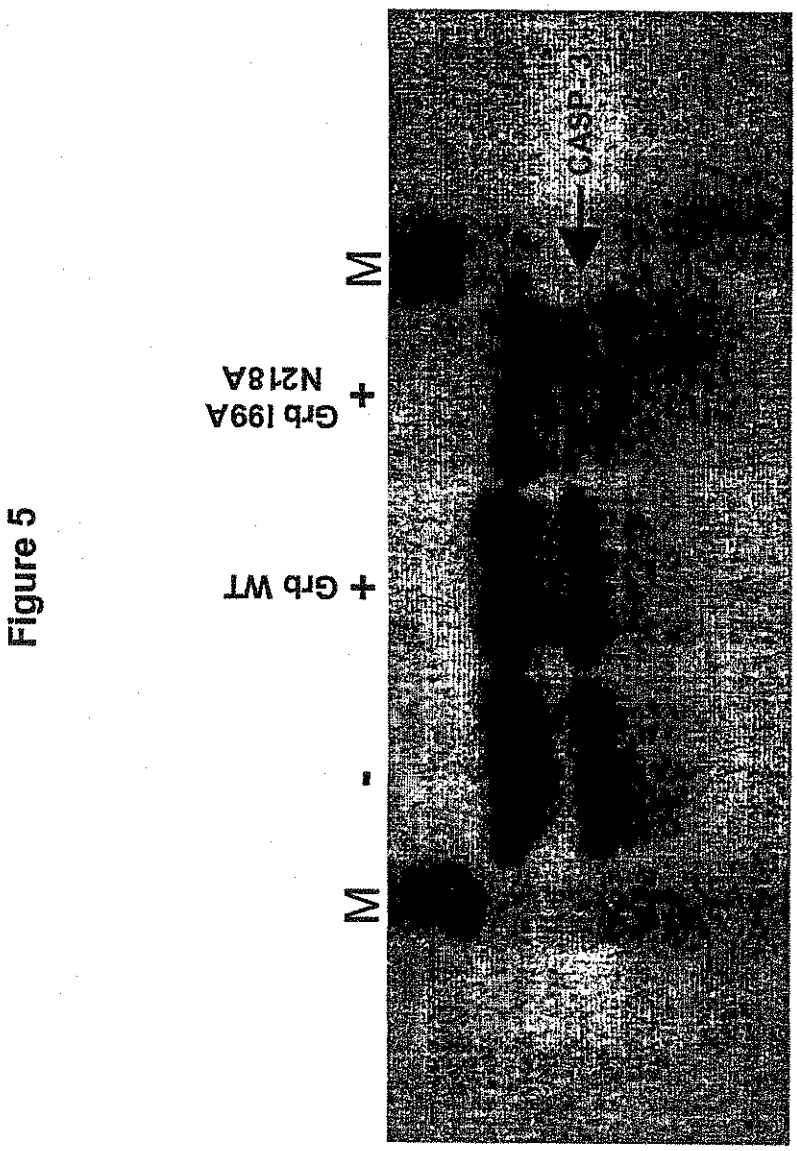
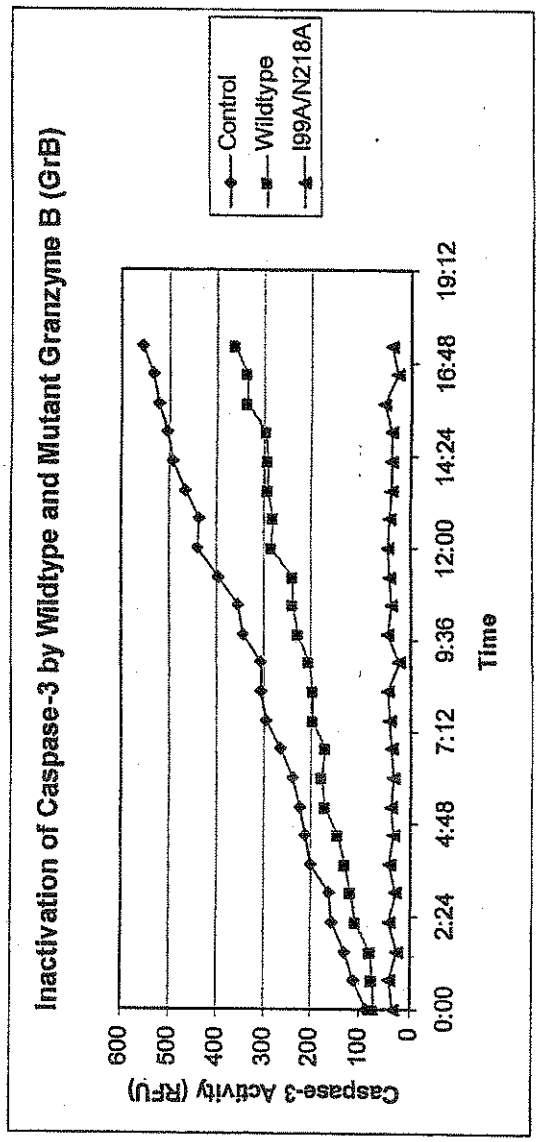


Figure 5

Figure 6

A


Inactivation of Caspase-3 by Wildtype and Mutant Granzyme B (GrB)
Caspase-3 Activity (RFU)
600
500
400
300
200
100
0
0:00
2:24
4:48
7:12
9:36
12:00
14:24
16:48
19:12
Time
Control
Wildtype
I99A/N218A

B

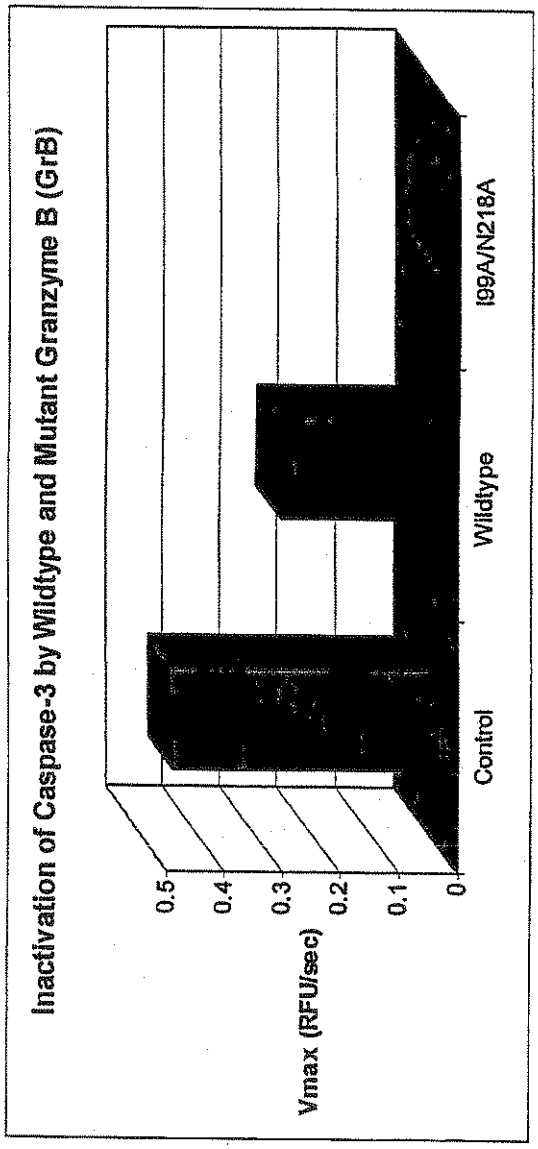
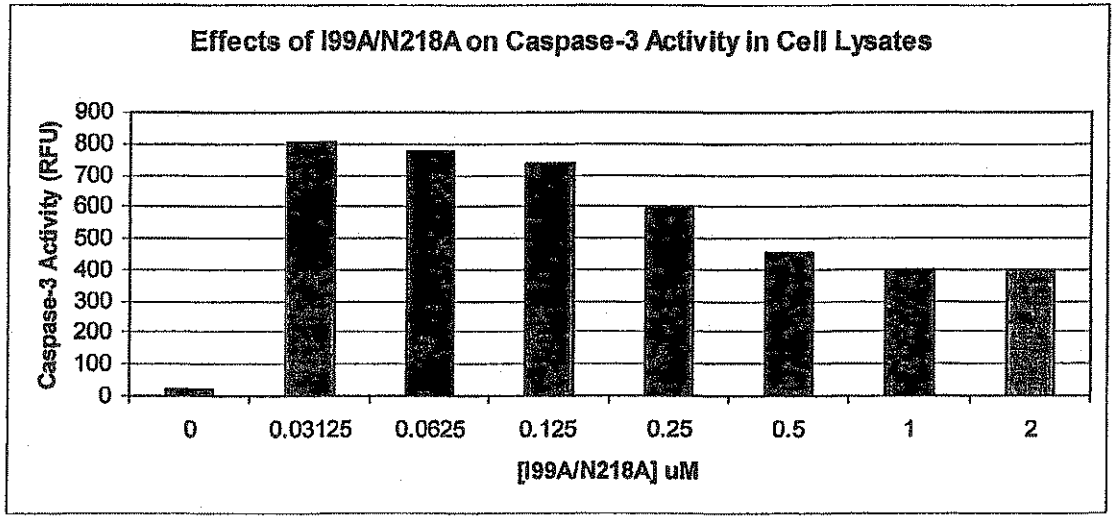
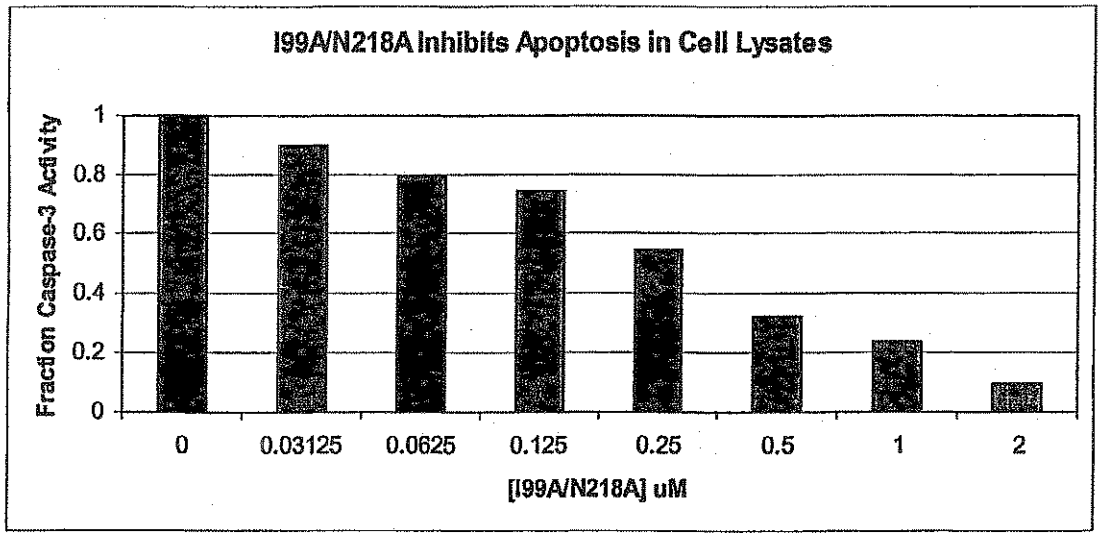

Inactivation of Caspase-3 by Wildtype and Mutant Granzyme B (GrB)
Vmax (RFU/sec)
0.5
0.4
0.3
0.2
0.1
0
Control
Wildtype
I99A/N218A

Figure 7

A


Effects of I99A/N218A on Caspase-3 Activity in Cell Lysates
Caspase-3 Activity (RFU)
900
800
700
600
500
400
300
200
100
0
0
0.03125
0.0625
0.125
0.25
0.5
1
2
[I99A/N218A] uM

B


I99A/N218A Inhibits Apoptosis in Cell Lysates
Fraction Caspase-3 Activity
1
0.8
0.6
0.4
0.2
0
0
0.03125
0.0625
0.125
0.25
0.5
1
2
[I99A/N218A] uM