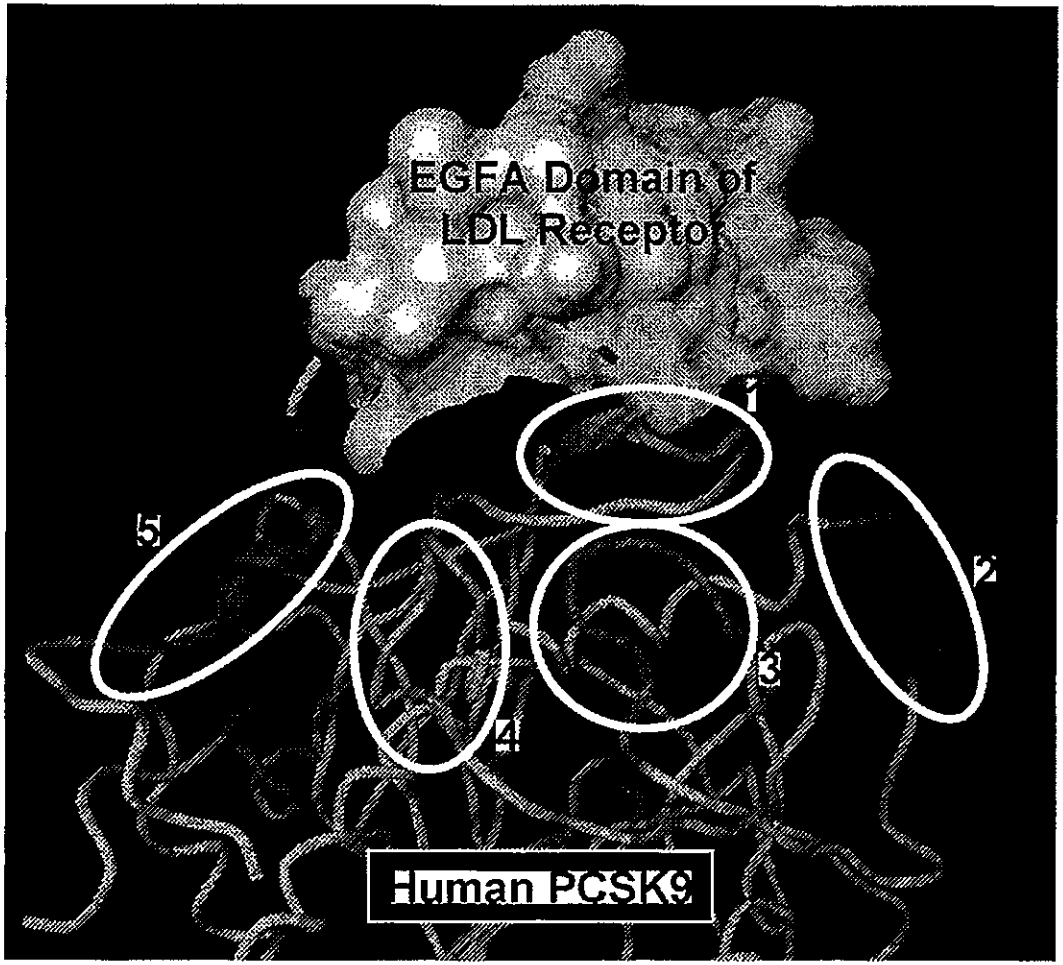
Figure 1



Peptide 1:
371-380: ASSDCSTCFV

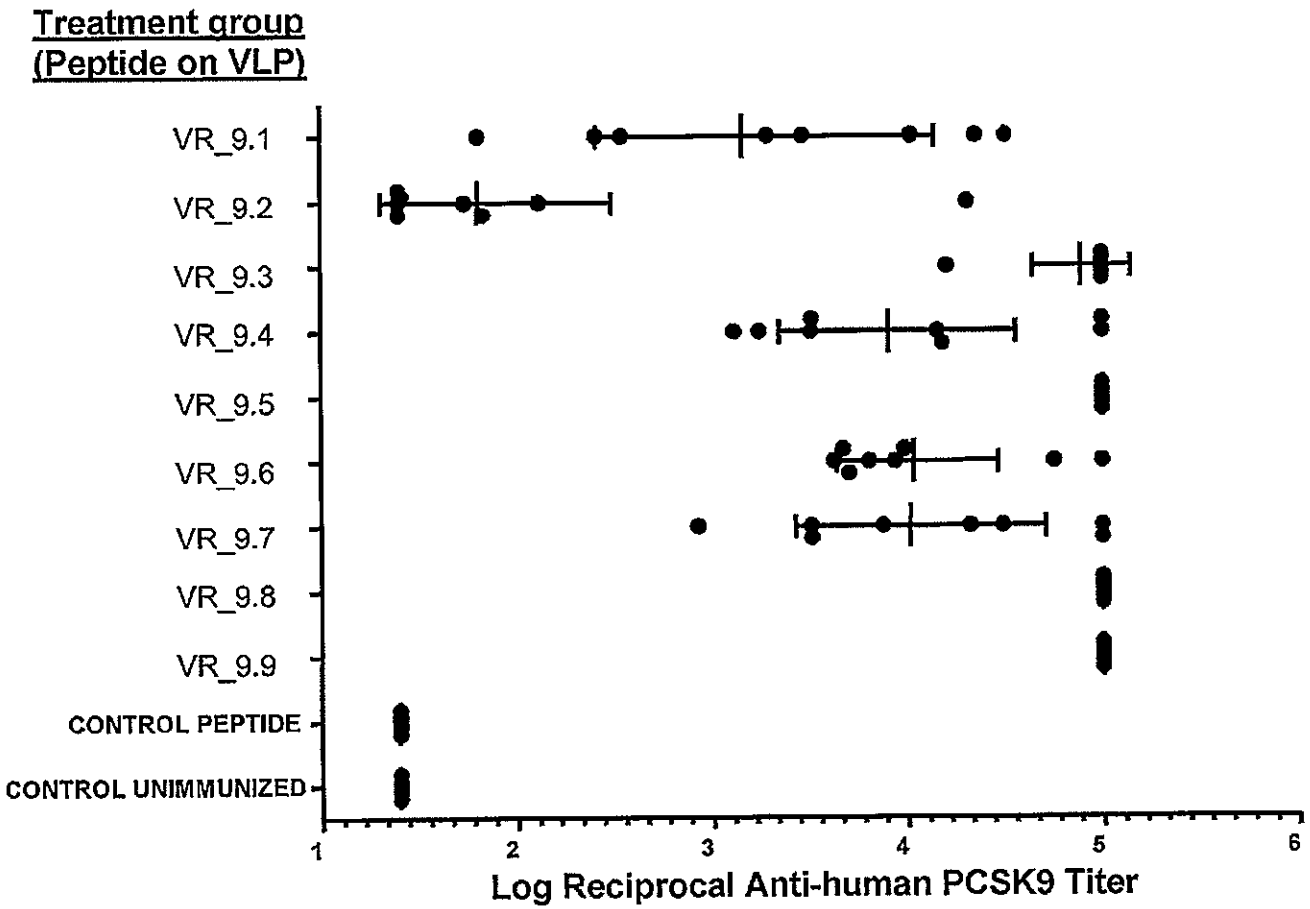
Peptide 2:
213-223: GTRFHRQASKC

Peptide 3:
191-200: SDHREIEGRV

Peptide 4:
235-245: SGRDAGVAKGA

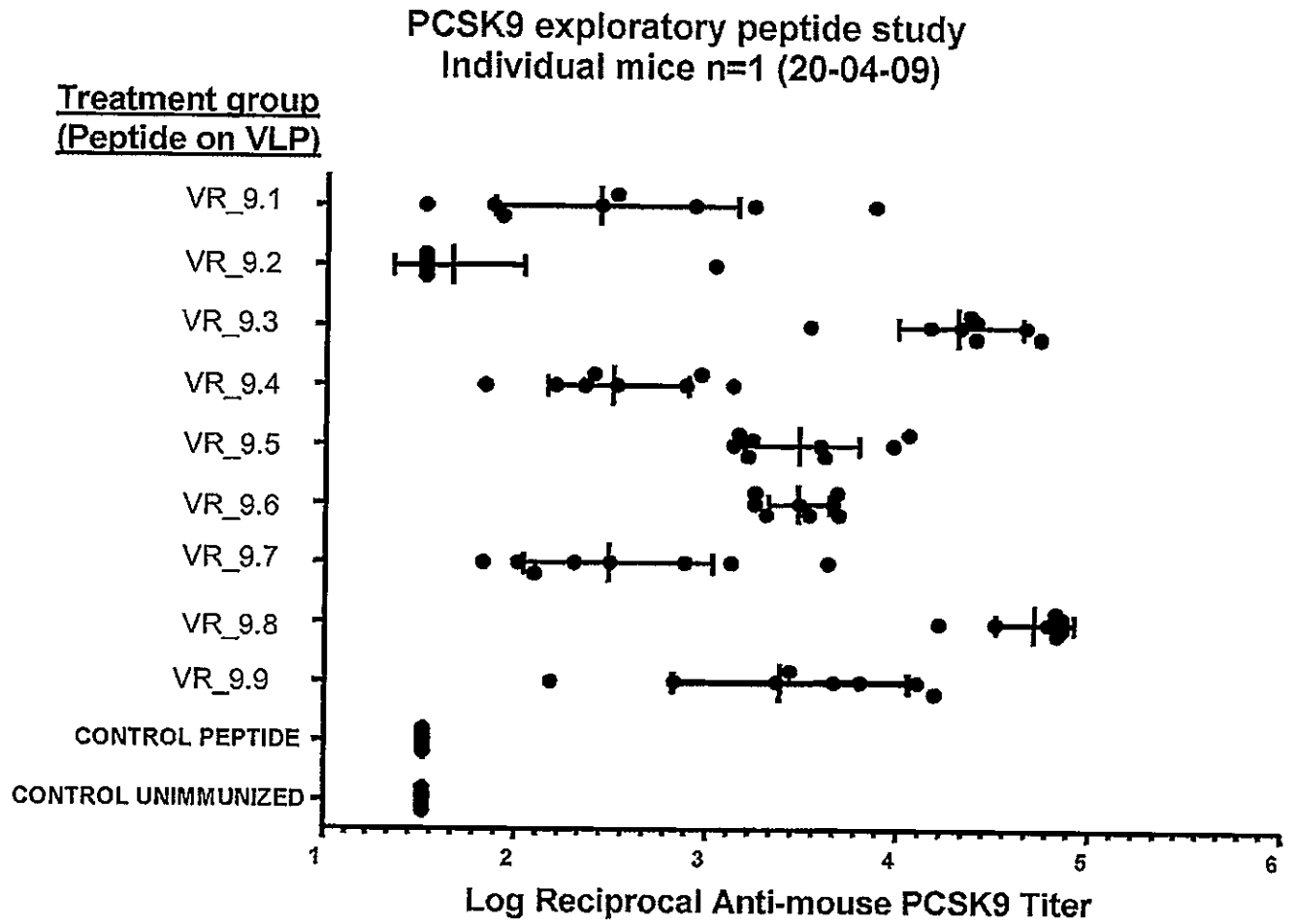
Peptide 5:
153-163: SIPWNLERITP

Figure 2



Treatment groups n=8. Each spot represents the reciprocal Ab titre for one mouse. Mean +/- SE are represented by lines

Figure 3



Treatment groups n=8. Each spot represents the reciprocal Ab titre for one mouse. Mean +/- SE are represented by lines

Figure 4

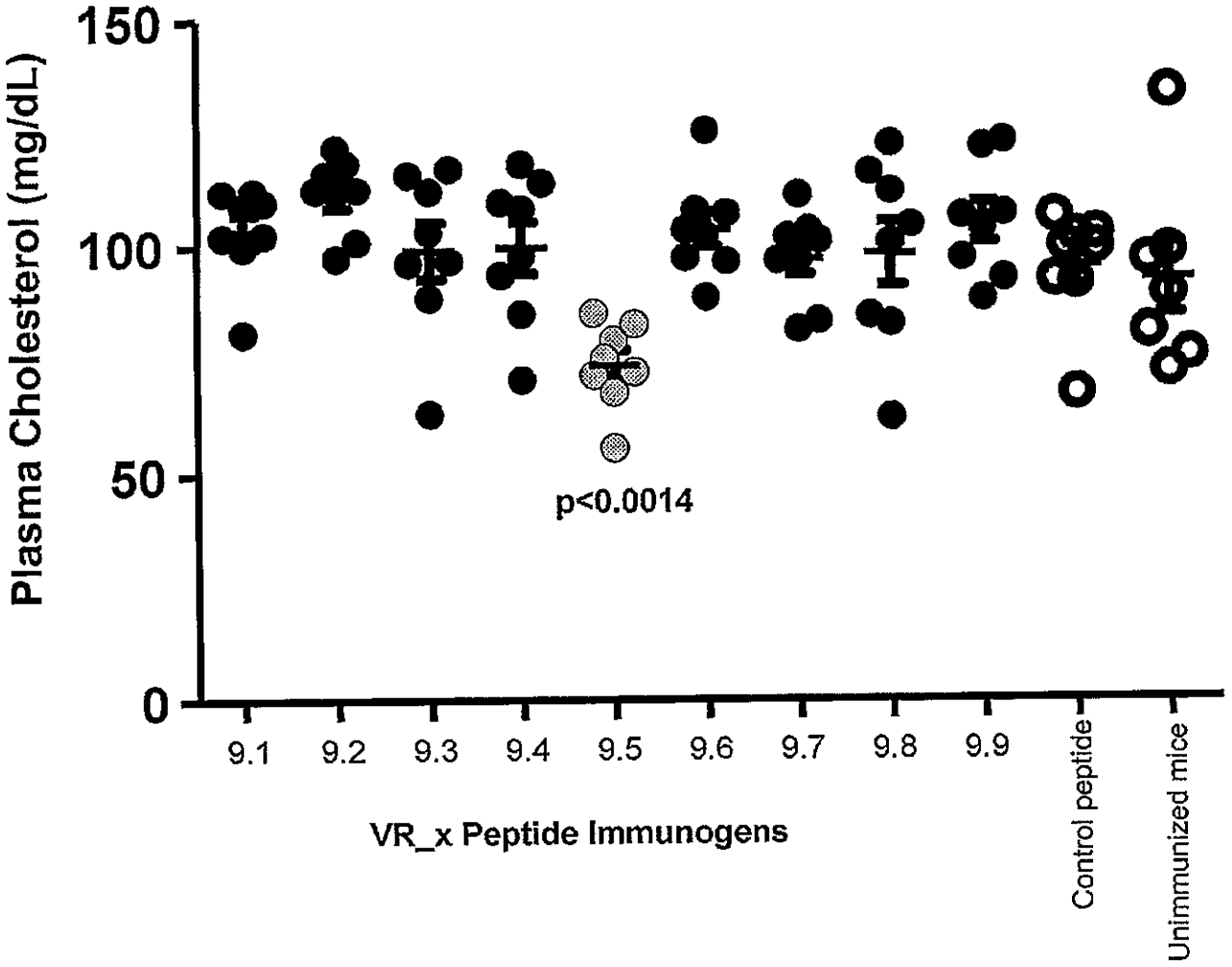

Plasma Cholesterol (mg/dL)
150
100
50
0
p<0.0014
9.1
9.2
9.3
9.4
9.5
9.6
9.7
9.8
9.9
Control peptide
Unimmunized mice
VR_x Peptide Immunogens

Figure 5

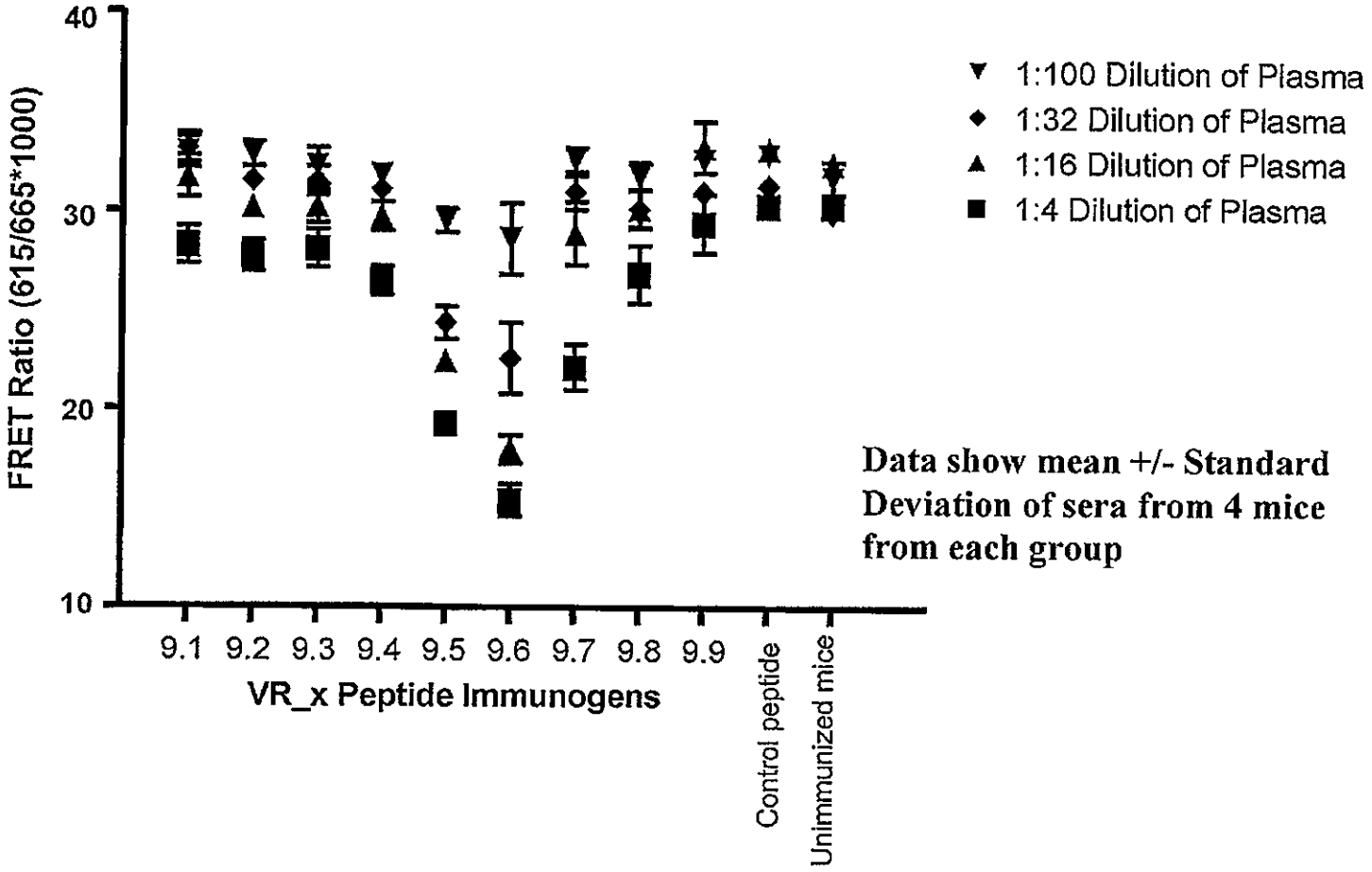

40
30
20
10
FRET Ratio (615/665*1000)
9.1
9.2
9.3
9.4
9.5
9.6
9.7
9.8
9.9
Control peptide
Unimmunized mice
VR_x Peptide Immunogens
1:100 Dilution of Plasma
1:32 Dilution of Plasma
1:16 Dilution of Plasma
1:4 Dilution of Plasma
Data show mean +/- Standard Deviation of sera from 4 mice from each group

Figure 6

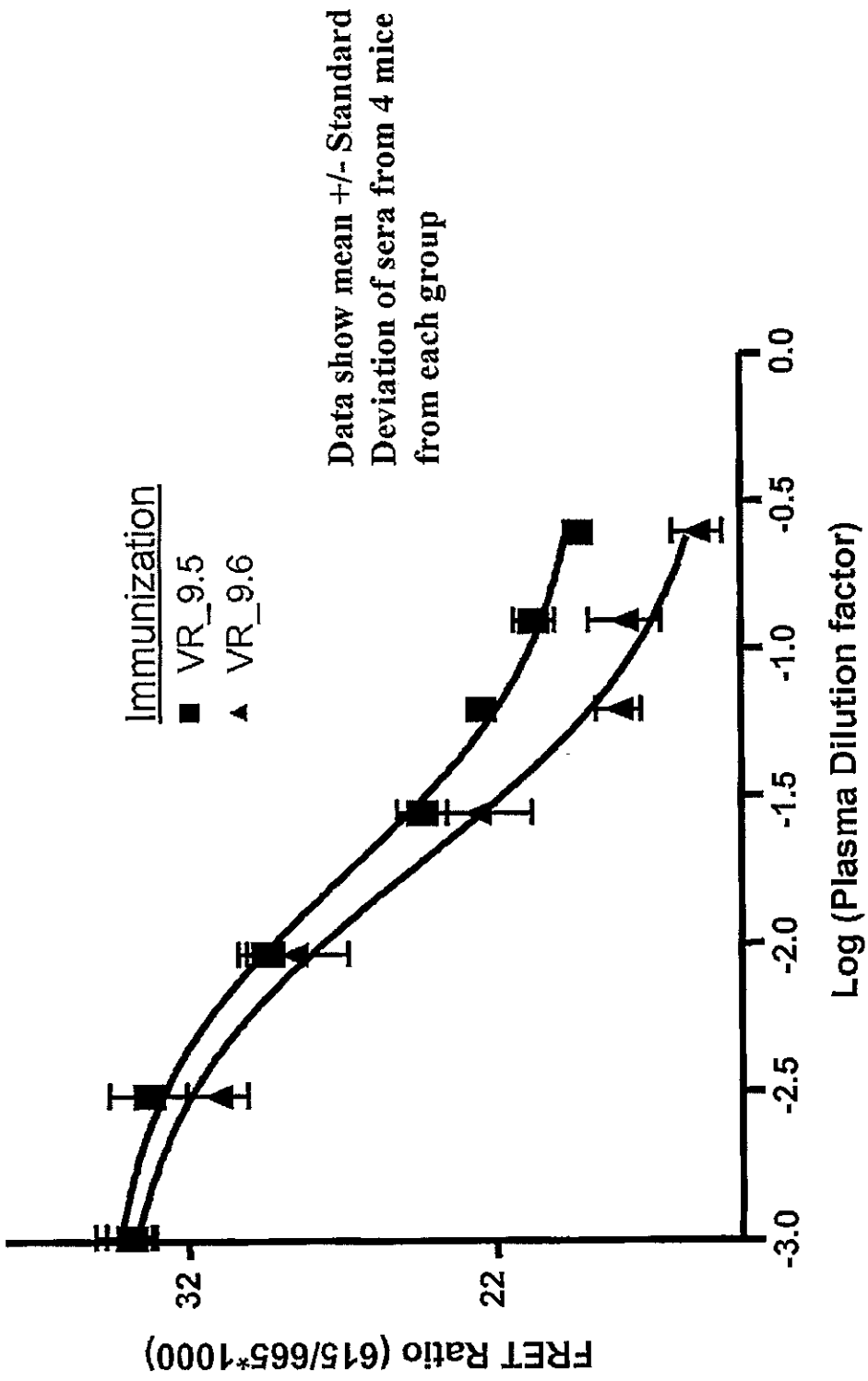
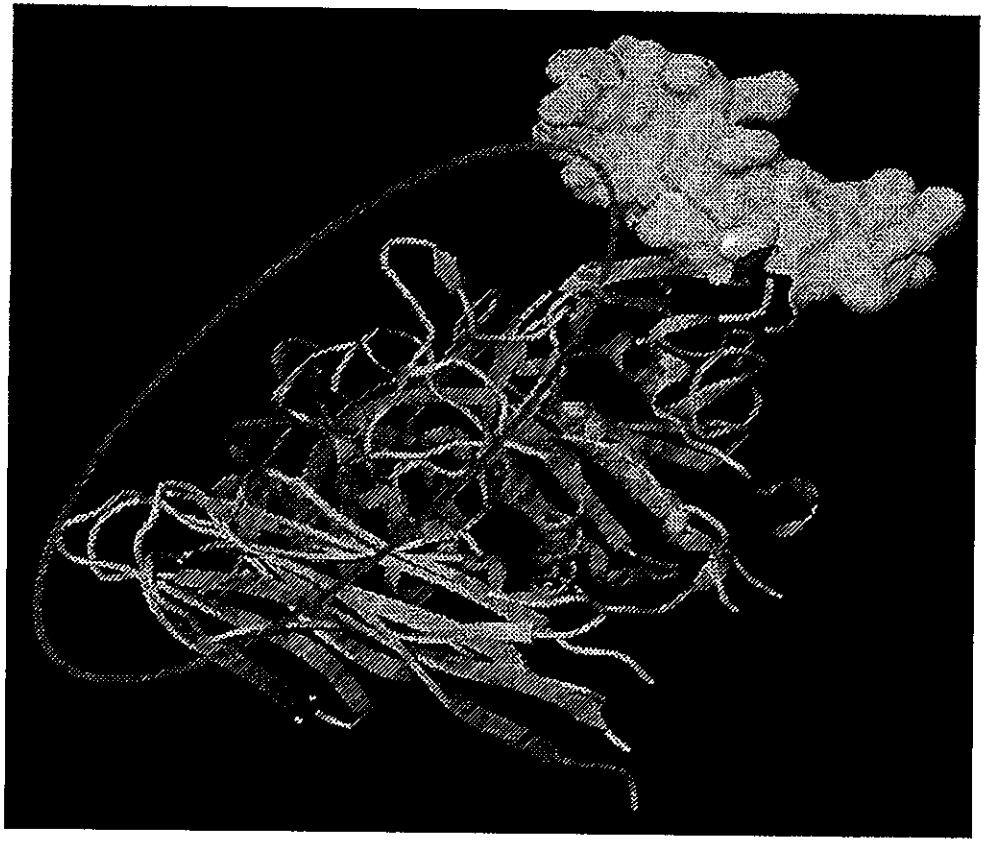
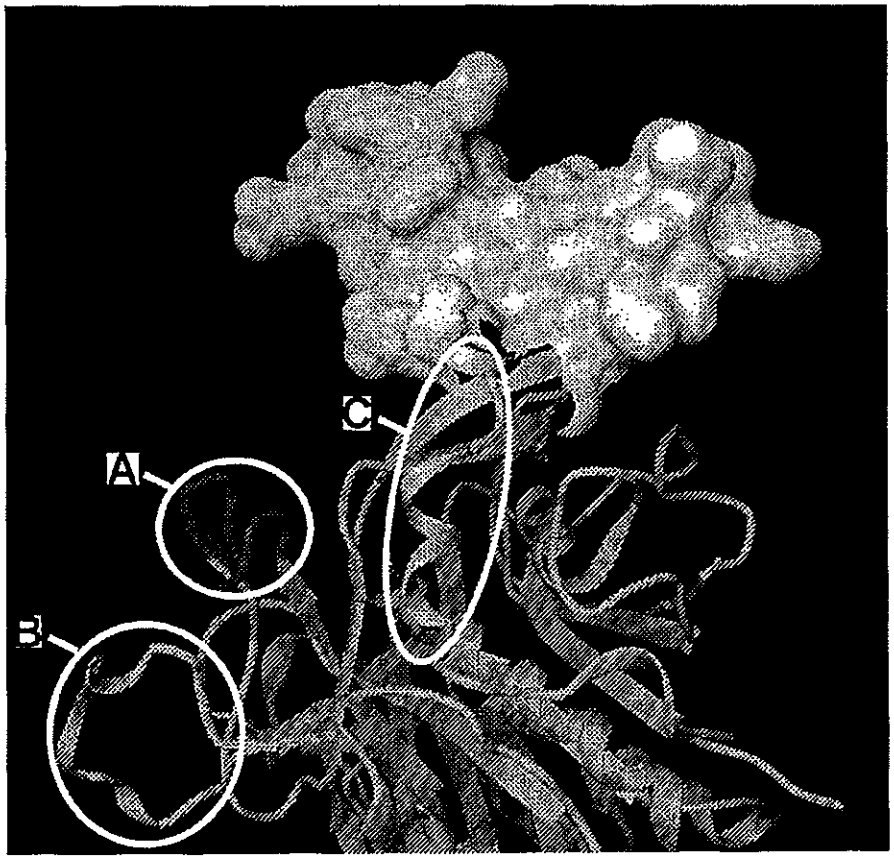

Data show mean +/- Standard Deviation of sera from 4 mice from each group
Immunization
VR_9.5
VR_9.6
FRET Ratio (615/665*1000)
32
22
Log (Plasma Dilution factor)
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0

Figure 7



Complex of PCSK9 (ribbons) and EGF-A (space fill). Potential regions of PCSK9 that may interact with domains of LDLR other than EGF-A are indicated by the elipse.

Figure 8



Complex of PCSK9 (ribbons) and EGF-A (space fill) with the amino acids corresponding to peptides VR_13/14 (A) and VR_15/16 (B) and VR_9.5 (C) are displayed.

Figure 9

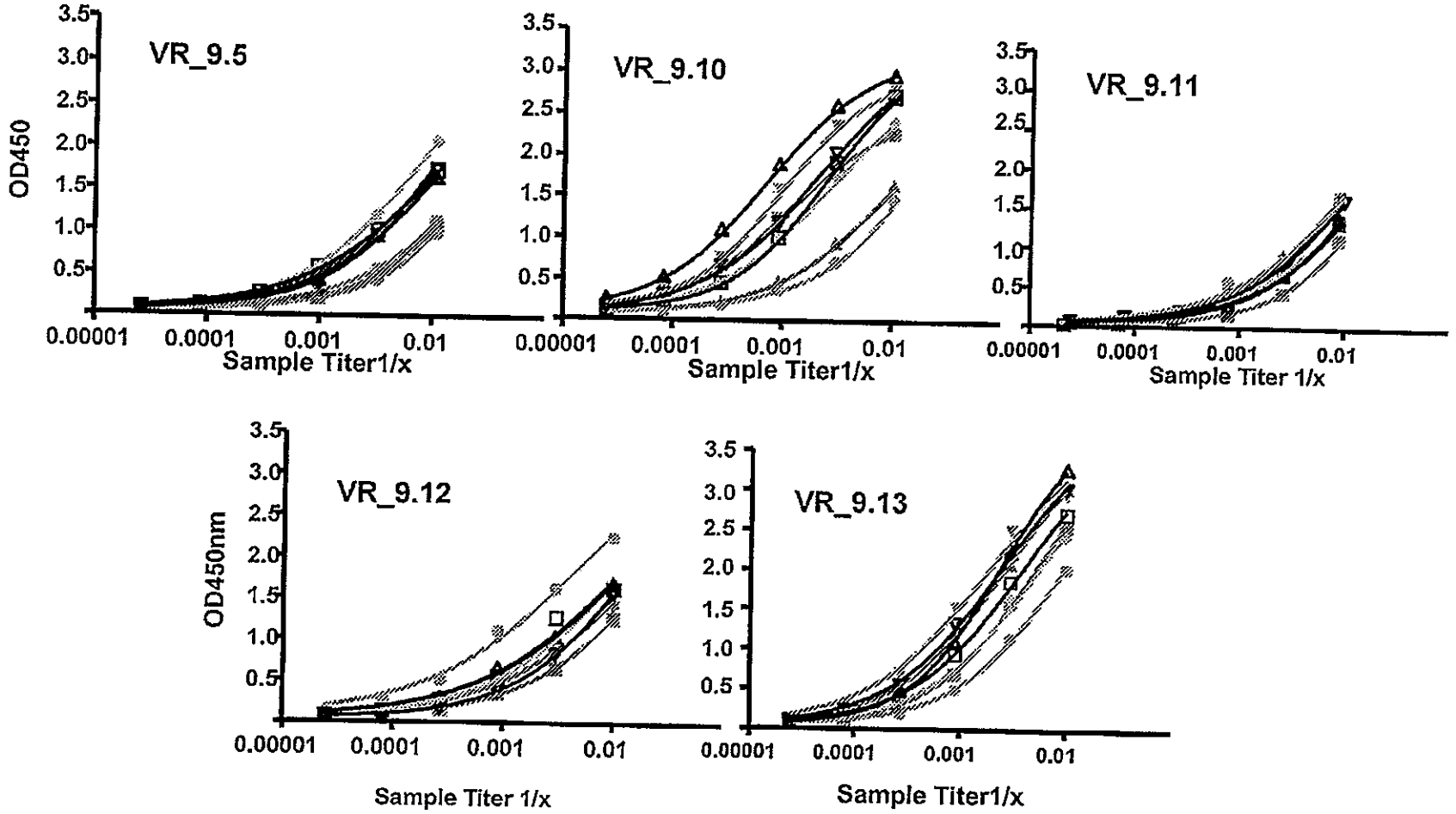

VR_9.5
VR_9.10
VR_9.11
VR_9.12
VR_9.13
OD450
OD450nm
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.00001
0.0001
0.001
0.01
Sample Titer1/x
Sample Titer 1/x

Figure 10

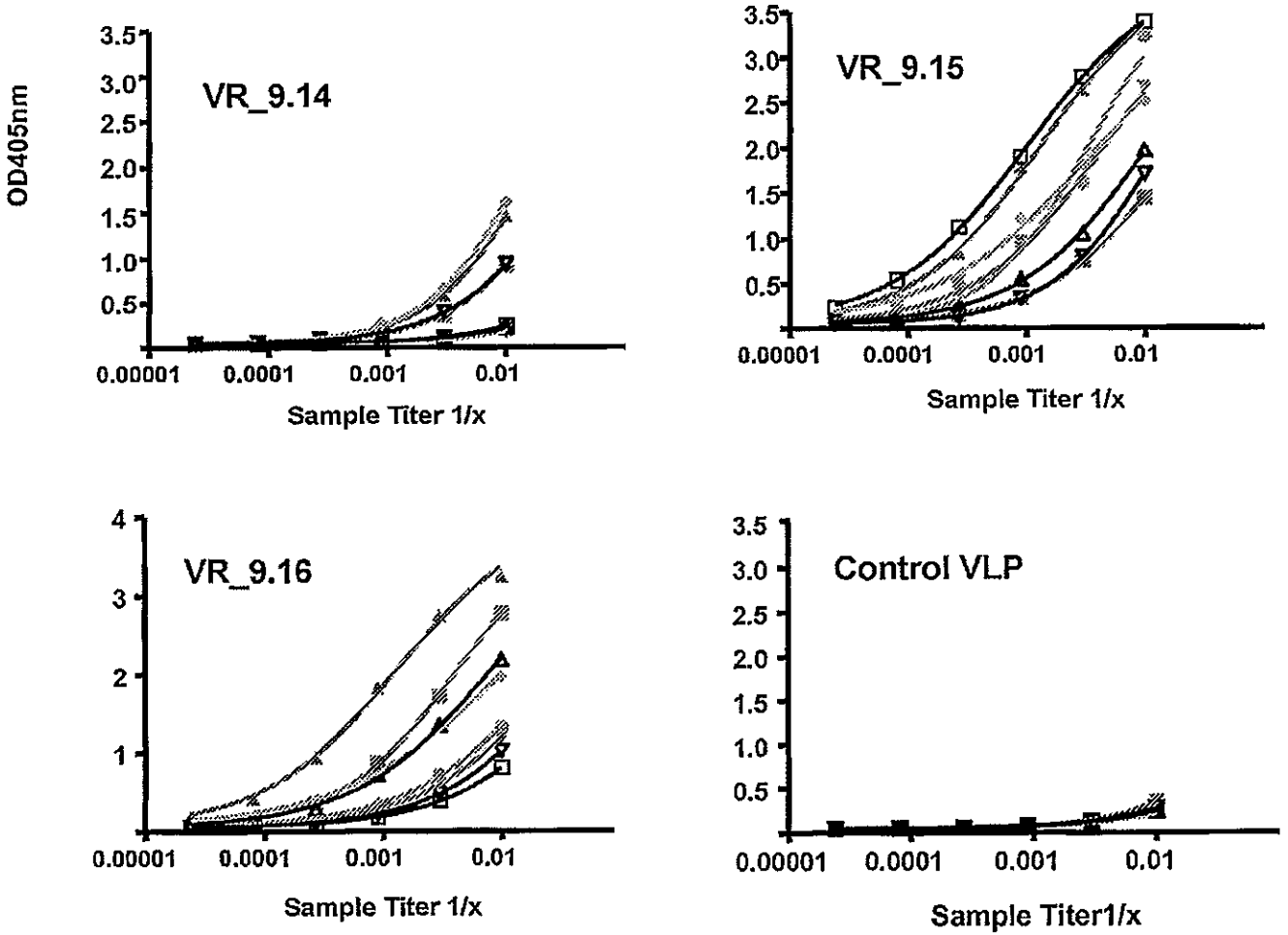

OD405nm
VR_9.14
VR_9.15
VR_9.16
Control VLP
Sample Titer 1/x
Sample Titer1/x
0.00001
0.0001
0.001
0.01

Figure 11

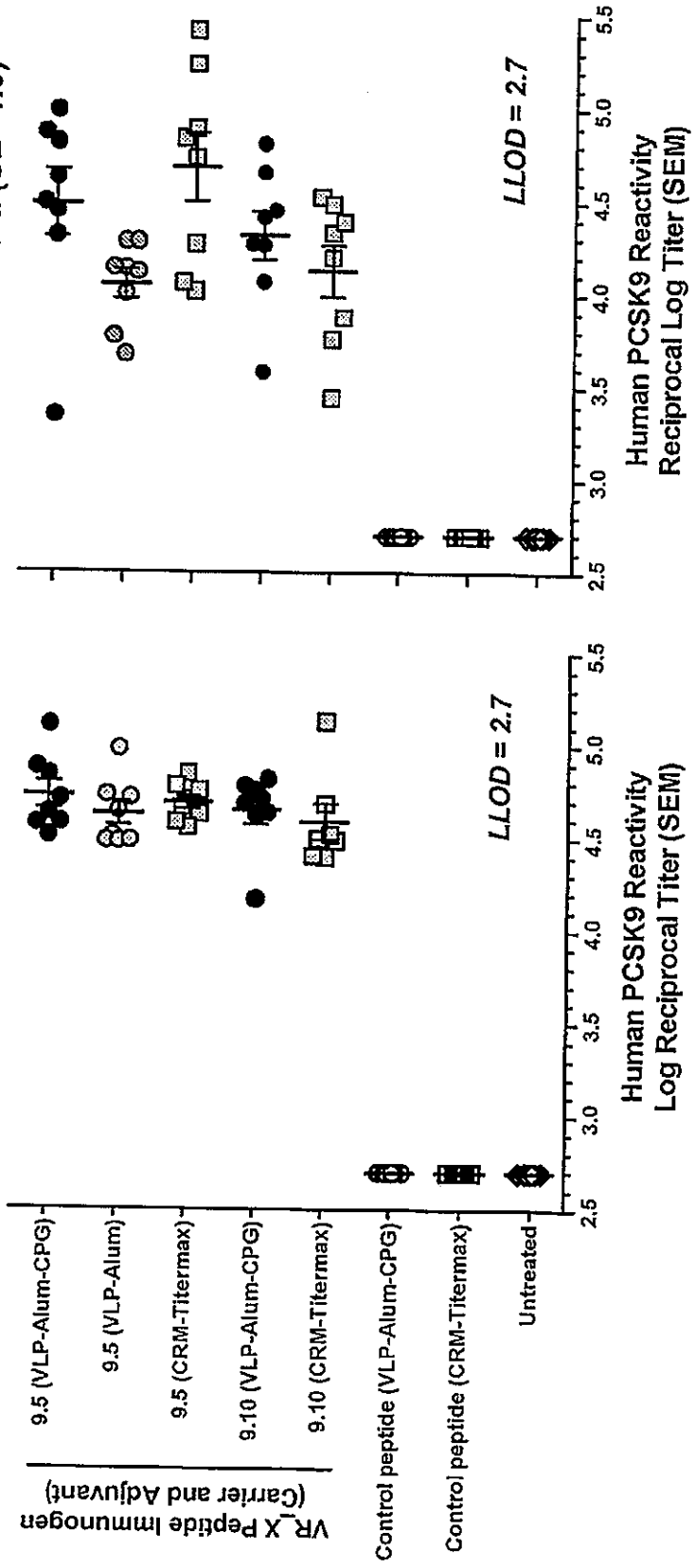

BALB/c Mouse Sera (OD=1.0)
C57BL/6 Mouse Sera (OD=1.0)
VR_X Peptide Immunogen (Carrier and Adjuvant)
9.5 (VLP-Alum-CPG)
9.5 (VLP-Alum)
9.5 (CRM-Titermax)
9.10 (VLP-Alum-CPG)
9.10 (CRM-Titermax)
Control peptide (VLP-Alum-CPG)
Control peptide (CRM-Titermax)
Untreated
LLOD = 2.7
2.5
3.0
3.5
4.0
4.5
5.0
5.5
Human PCSK9 Reactivity Log Reciprocal Titer (SEM)
Human PCSK9 Reactivity Reciprocal Log Titer (SEM)

Figure 12

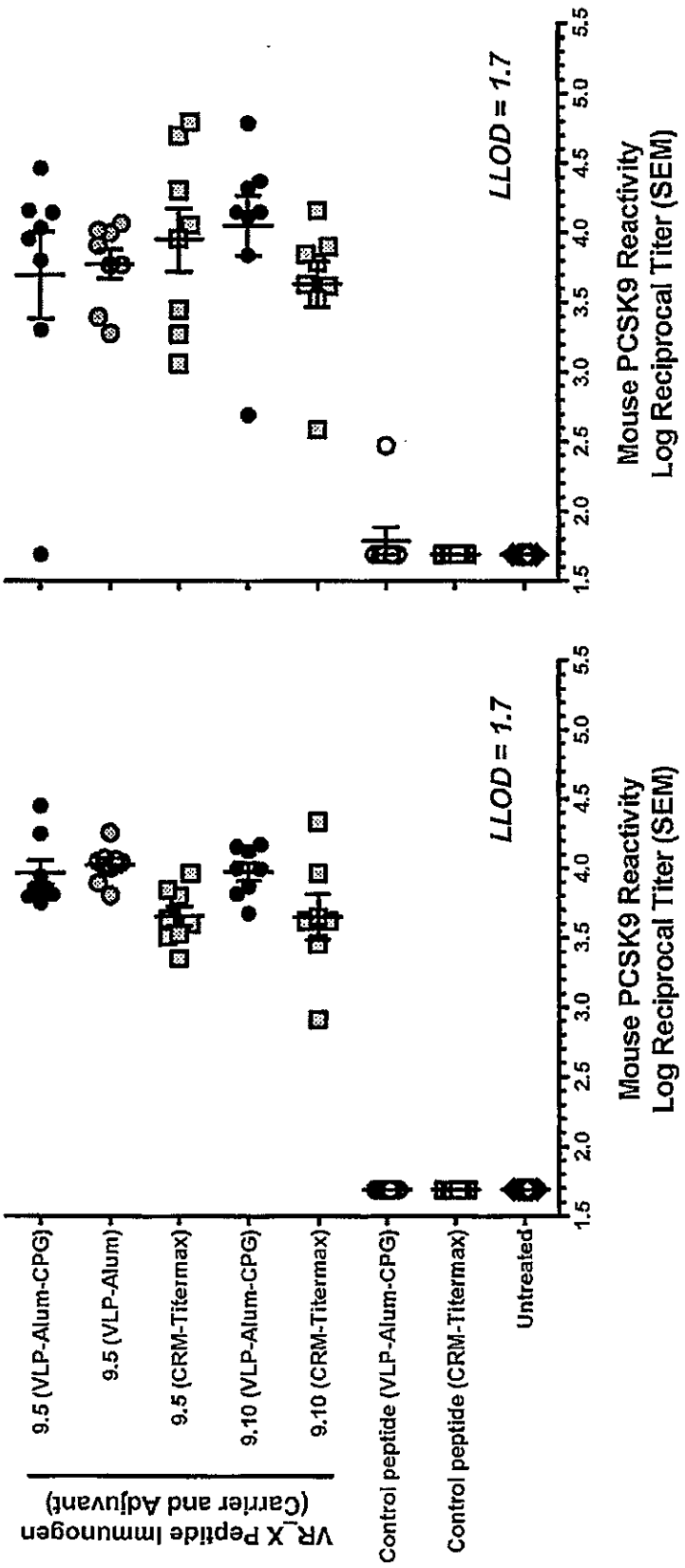

BALB/c Mouse Sera (OD = 0.5)
C57BL/6 Mouse Sera (OD = 0.5)
VR_X Peptide Immunogen (Carrier and Adjuvant)
9.5 (VLP-Alum-CPG)
9.5 (VLP-Alum)
9.5 (CRM-Titermax)
9.10 (VLP-Alum-CPG)
9.10 (CRM-Titermax)
Control peptide (VLP-Alum-CPG)
Control peptide (CRM-Titermax)
Untreated
LLOD = 1.7
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Mouse PCSK9 Reactivity
Log Reciprocal Titer (SEM)

Figure 13

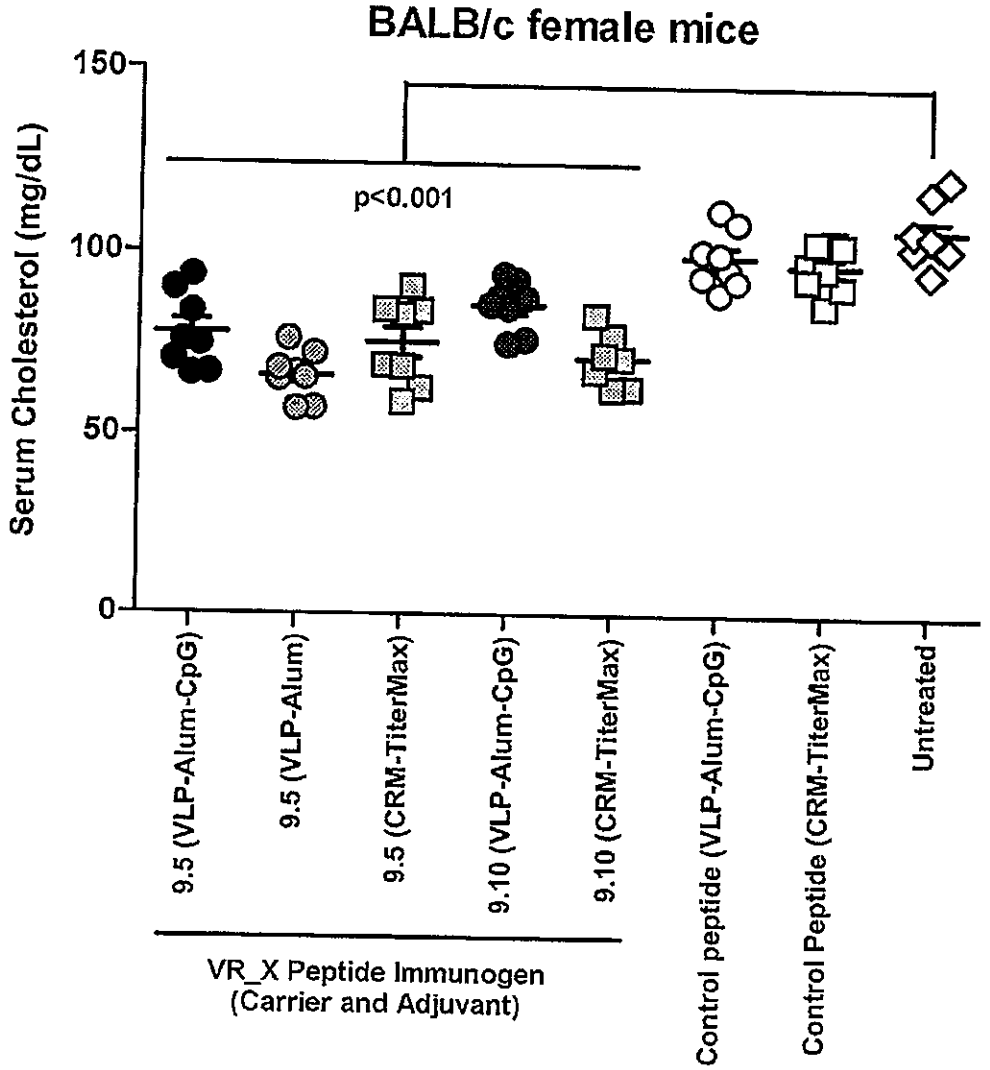

BALB/c female mice
Serum Cholesterol (mg/dL)
150
100
50
0
p<0.001
9.5 (VLP-Alum-CpG)
9.5 (VLP-Alum)
9.5 (CRM-TiterMax)
9.10 (VLP-Alum-CpG)
9.10 (CRM-TiterMax)
Control peptide (VLP-Alum-CpG)
Control Peptide (CRM-TiterMax)
Untreated
VR_X Peptide Immunogen
(Carrier and Adjuvant)

Figure 14

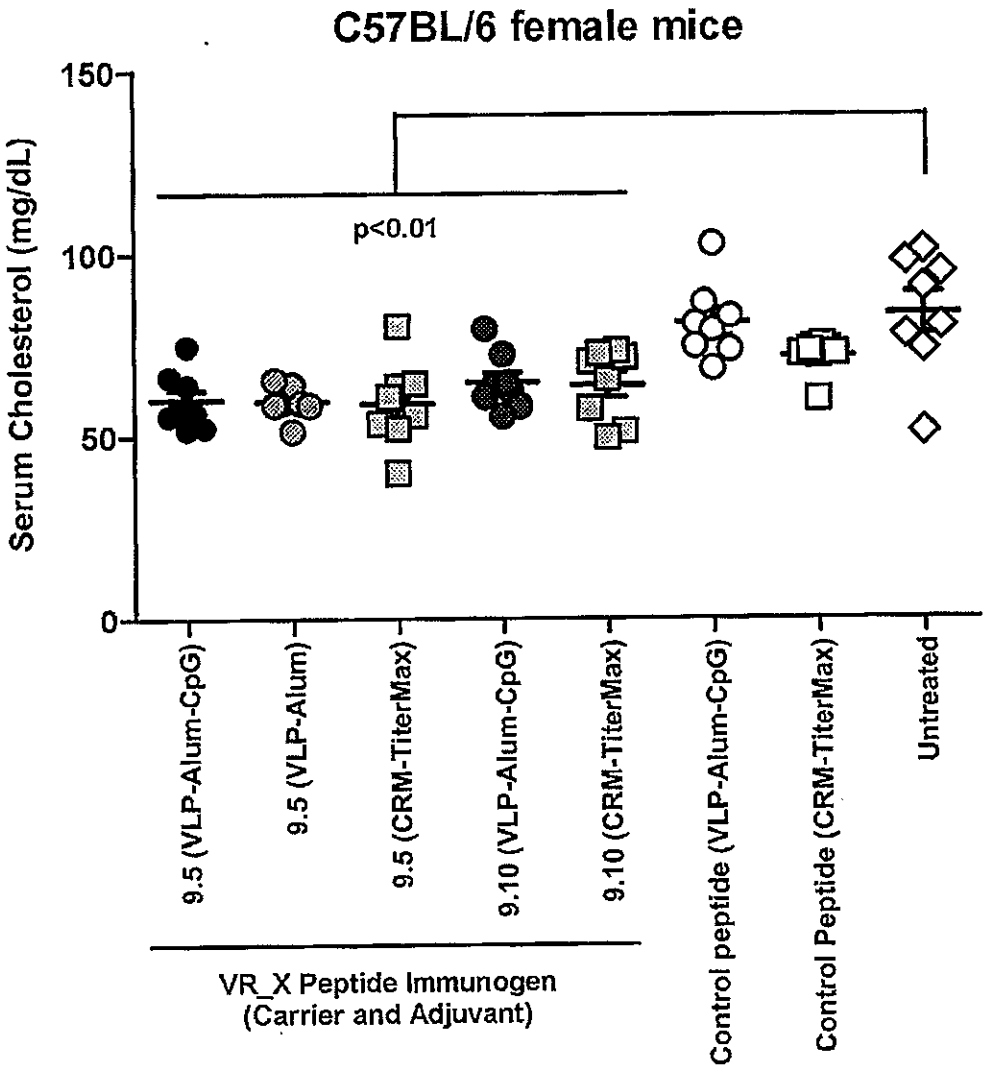

C57BL/6 female mice
Serum Cholesterol (mg/dL)
150
100
50
0
p<0.01
9.5 (VLP-Alum-CpG)
9.5 (VLP-Alum)
9.5 (CRM-TiterMax)
9.10 (VLP-Alum-CpG)
9.10 (CRM-TiterMax)
Control peptide (VLP-Alum-CpG)
Control Peptide (CRM-TiterMax)
Untreated
VR_X Peptide Immunogen
(Carrier and Adjuvant)

Figure 15

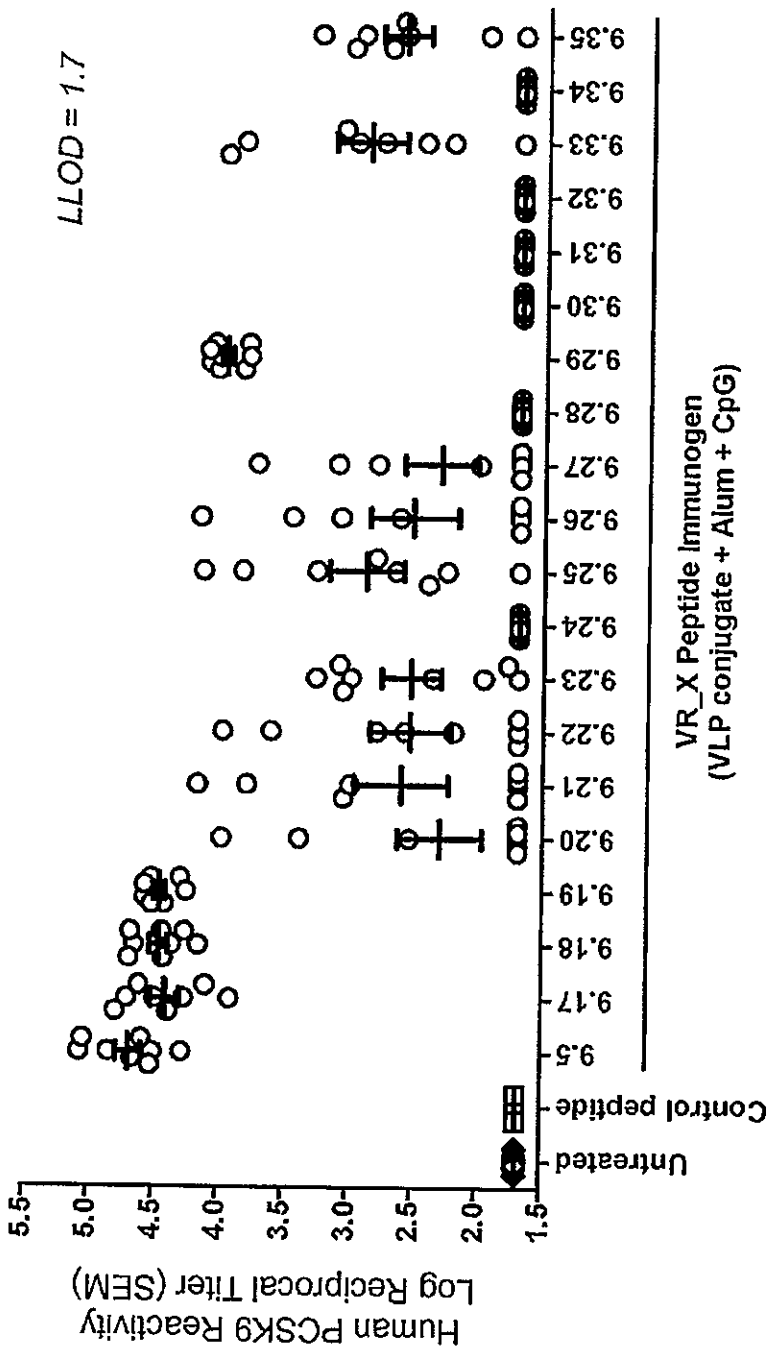

Anti-Human PCSK9 Humoral Responses
BALB/c Mouse Sera (OD=1.0)
LLOD = 1.7
Human PCSK9 Reactivity
Log Reciprocal Titer (SEM)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Untreated
Control peptide
9.5
9.17
9.18
9.19
9.20
9.21
9.22
9.23
9.24
9.25
9.26
9.27
9.28
9.29
9.30
9.31
9.32
9.33
9.34
9.35
VR_X Peptide Immunogen
(VLP conjugate + Alum + CpG)

Figure 16

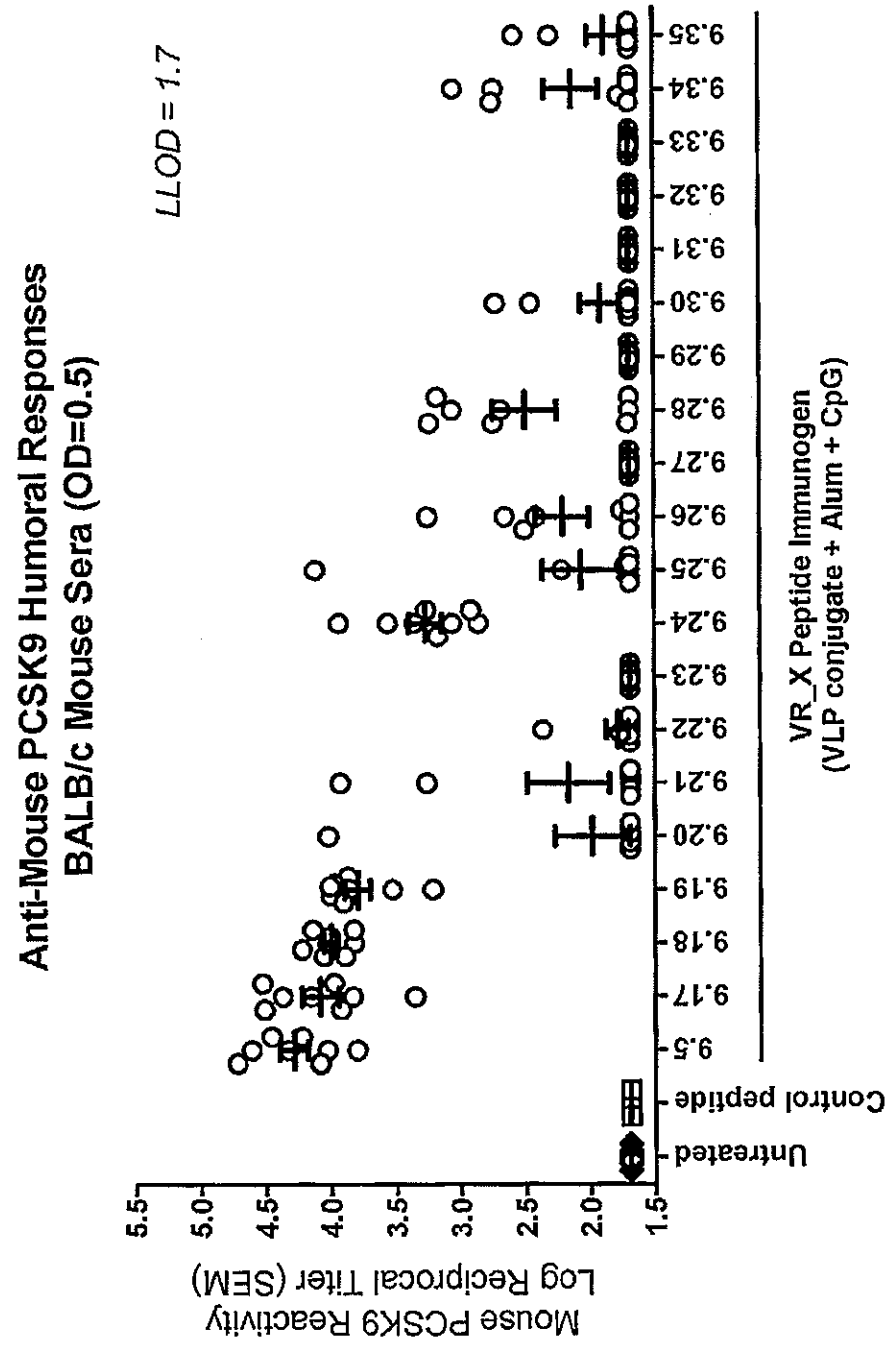

Anti-Mouse PCSK9 Humoral Responses
BALB/c Mouse Sera (OD=0.5)
LLOD = 1.7
Mouse PCSK9 Reactivity
Log Reciprocal Titer (SEM)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Untreated
Control peptide
9.5
9.17
9.18
9.19
9.20
9.21
9.22
9.23
9.24
9.25
9.26
9.27
9.28
9.29
9.30
9.31
9.32
9.33
9.34
9.35
VR_X Peptide Immunogen
(VLP conjugate + Alum + CpG)

Figure 17

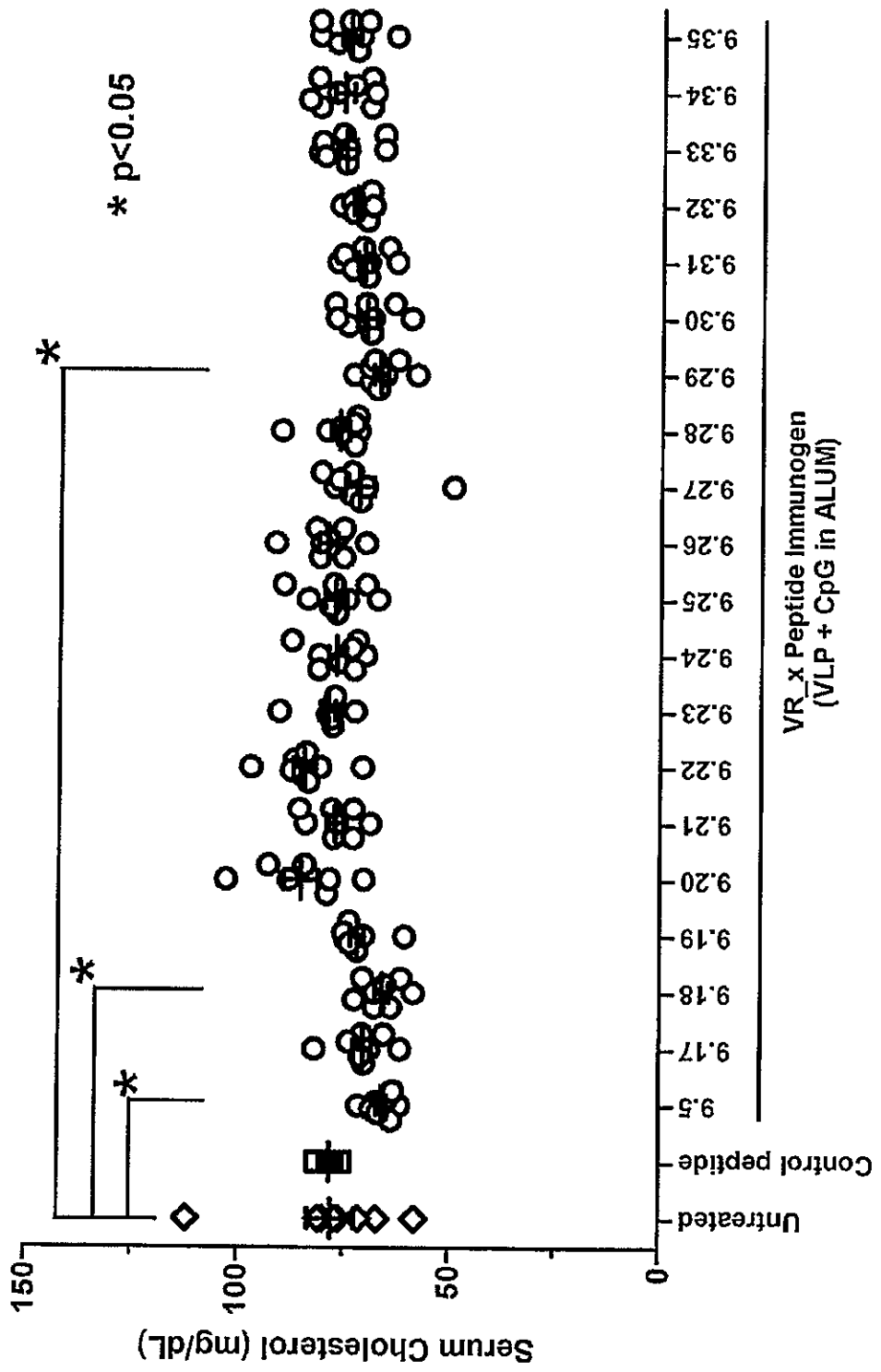

Serum Cholesterol (mg/dL)
150
100
50
0
* p<0.05
*
*
*
Untreated
Control peptide
9.5
9.17
9.18
9.19
9.20
9.21
9.22
9.23
9.24
9.25
9.26
9.27
9.28
9.29
9.30
9.31
9.32
9.33
9.34
9.35
VR_x Peptide Immunogen
(VLP + CpG in ALUM)

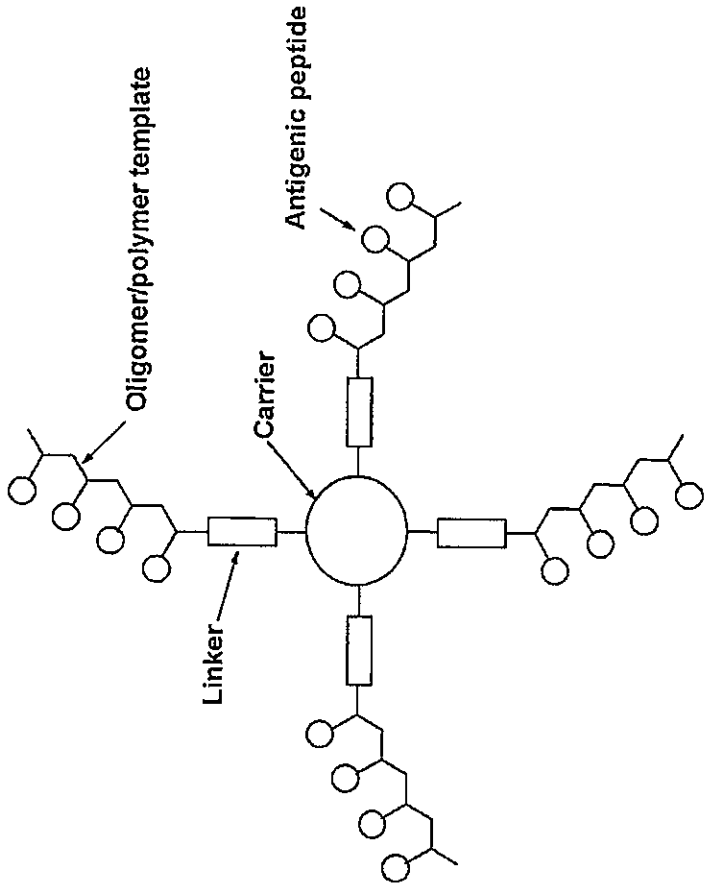
Figure 18



Complex of PCSK9 (ribbons) and EGF-A (space fill) with the amino acids corresponding to peptides VR_9.25 / VR_9.27 (A*), VR_9.29 (B), VR_9.31 (C), VR_9.33 (D), VR_9.35 (E), and VR_9.23 (F) are displayed.

*VR_9.25 and VR_9.27 are in an unresolved N-term region of PCSK9; highlighted residue is closest in sequence (aa Thr61).

Figure 19


Oligomer/polymer template
Carrier
Antigenic peptide
Linker