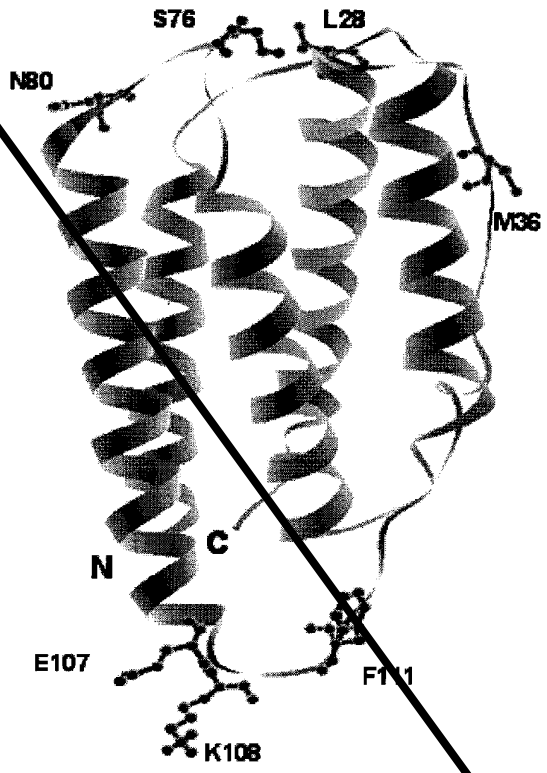
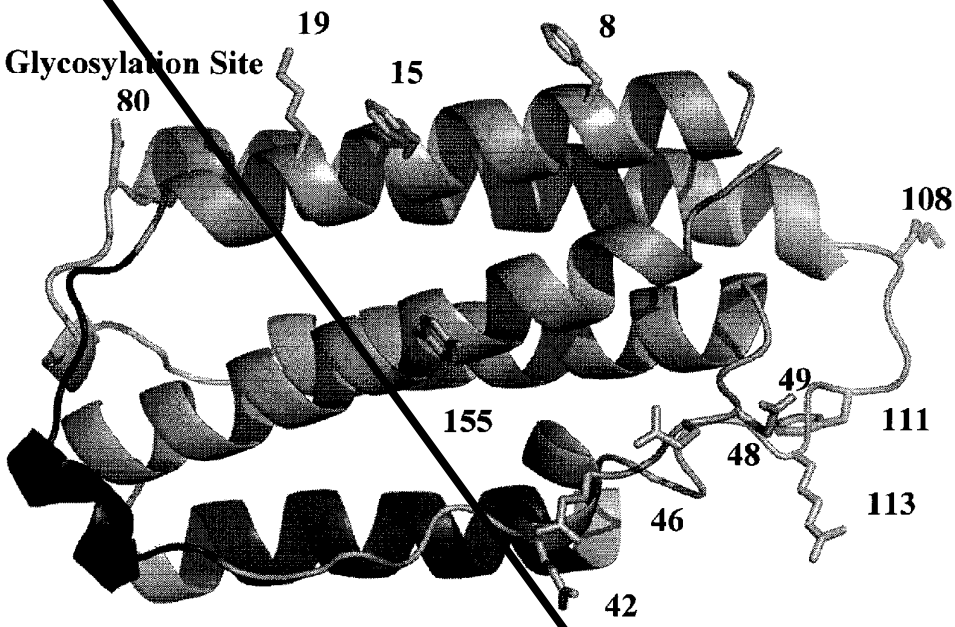
Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
Applicant:	AMBRX, INC.
Appl. No.:	Not Assigned
Atty:	John W. Wallen, III Telephone: (858) 875-2403
Docket No.:	AMBX-0136.00PCT

Figure 1



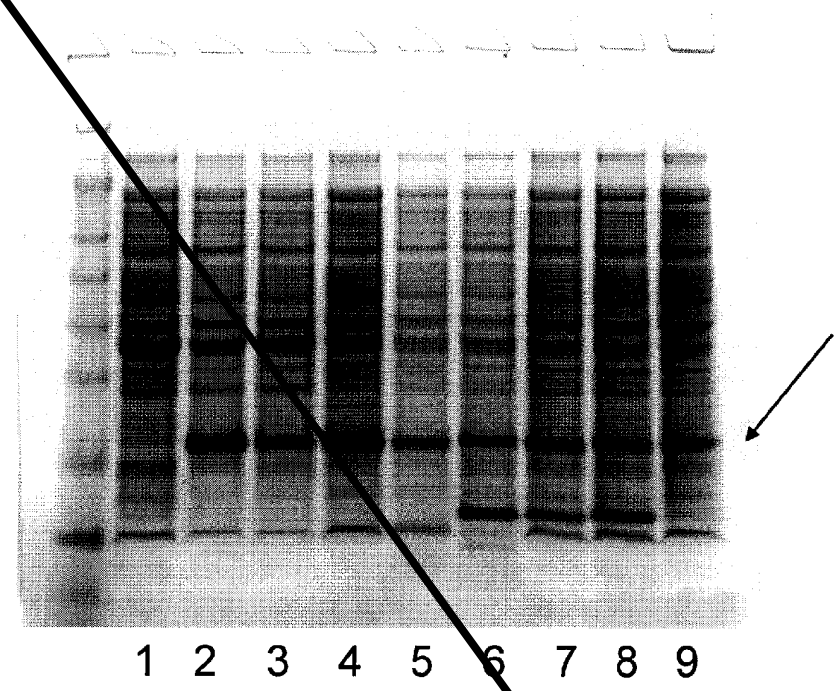
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Figure 2



Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
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Docket No.:	AMBX-0136.00PCT

Figure 3



Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
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Appl. No.:	Not Assigned
Atty:	John W. Wallen, III Telephone: (858) 875-2403
Docket No.:	AMBX-0136.00PCT

Figure 4

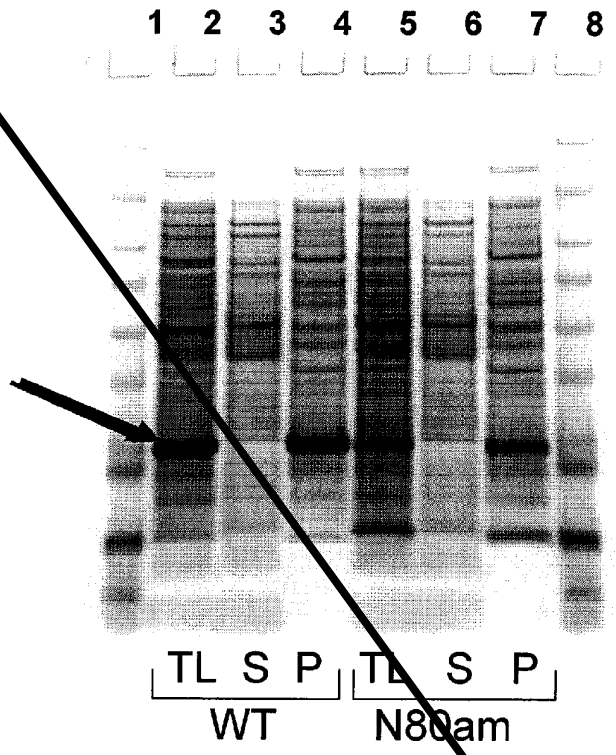
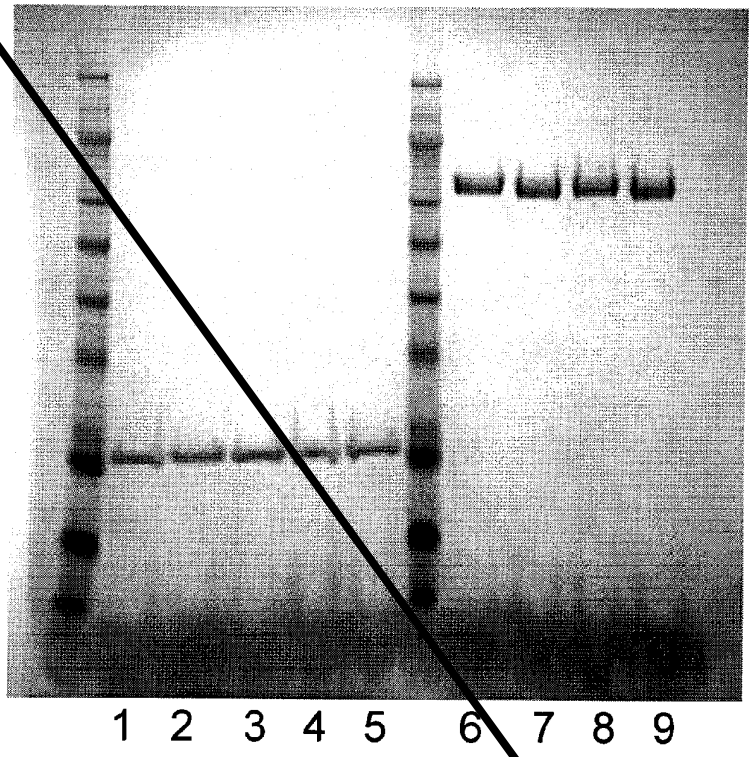


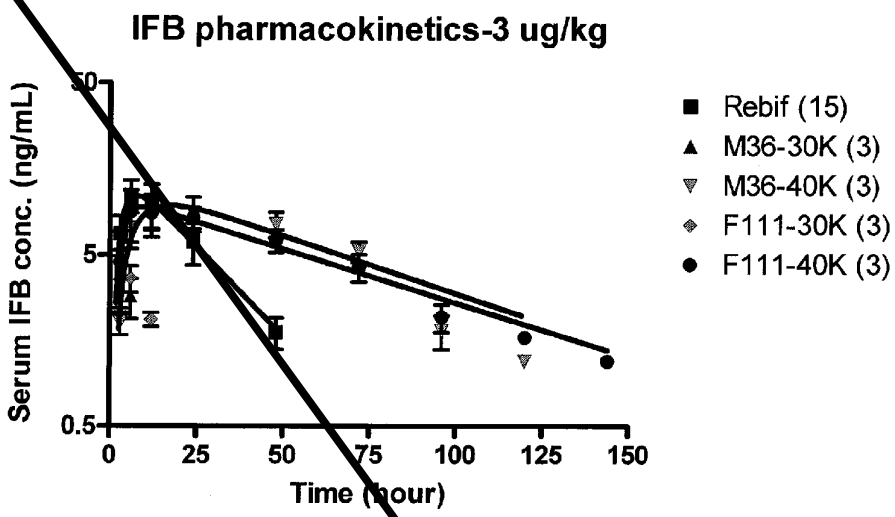
Figure 5



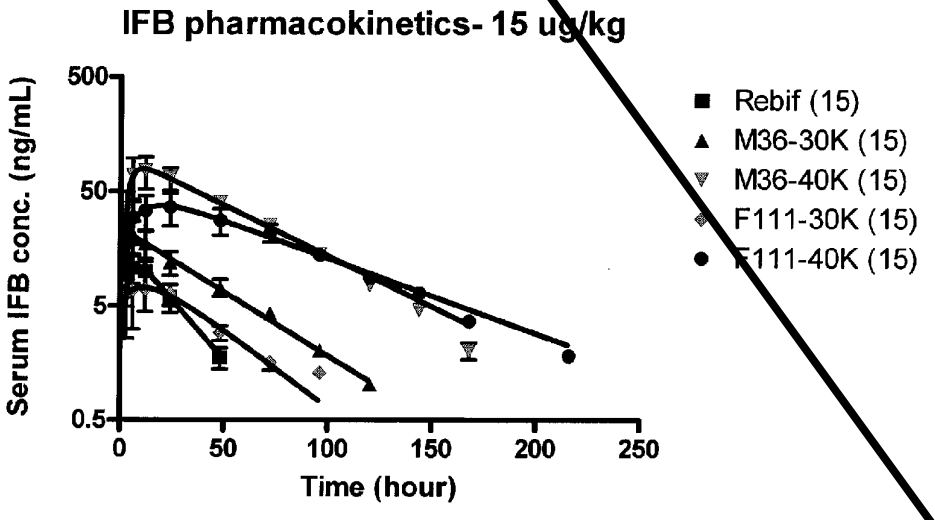
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Figure 6

A



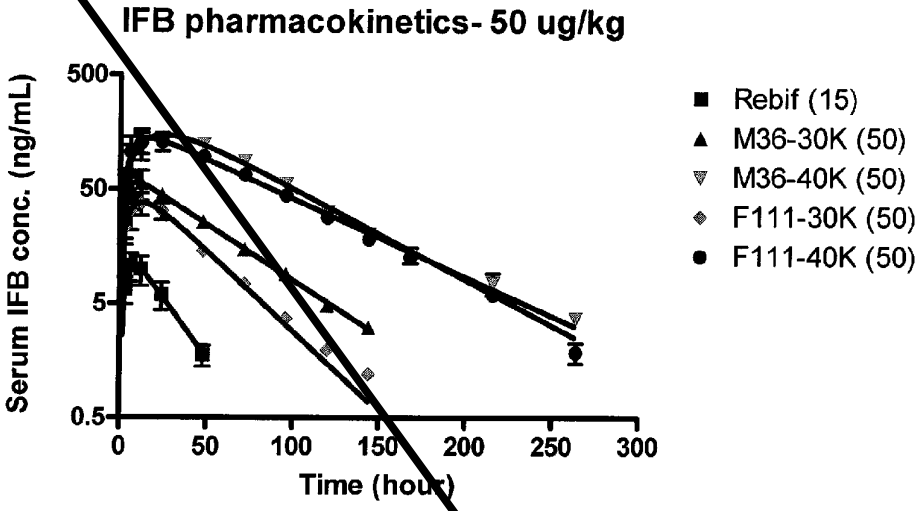
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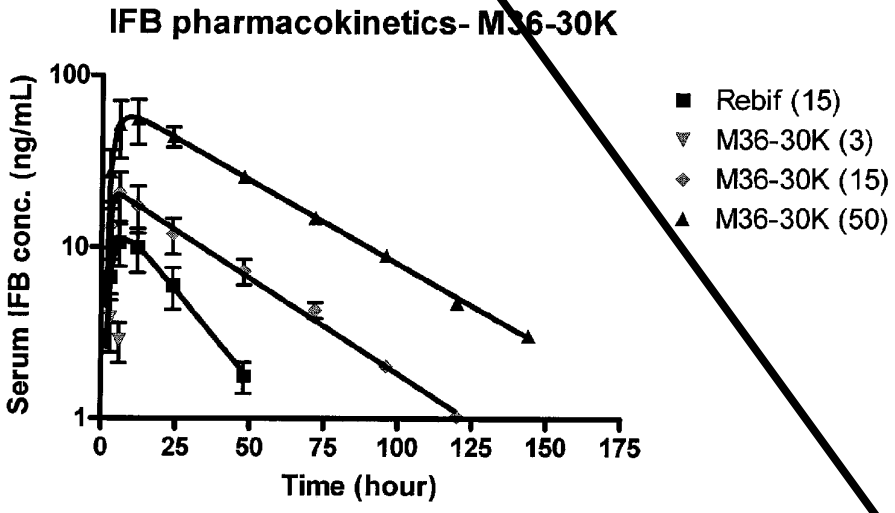
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Docket No.:	AMBX-0136.00PCT

Figure 7

A



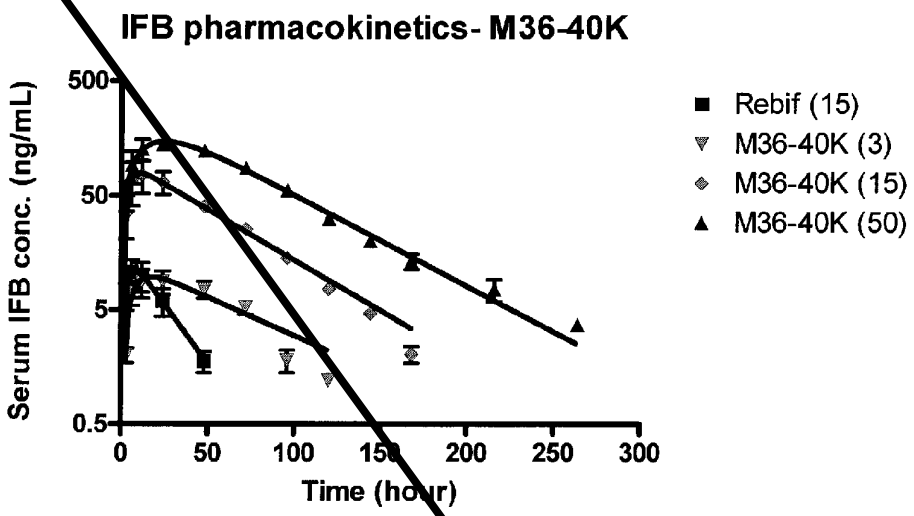
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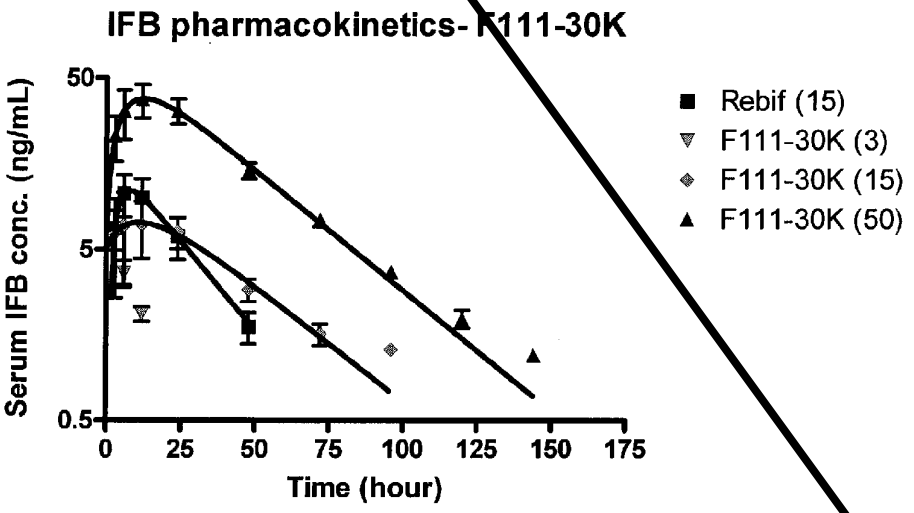
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Figure 8

A



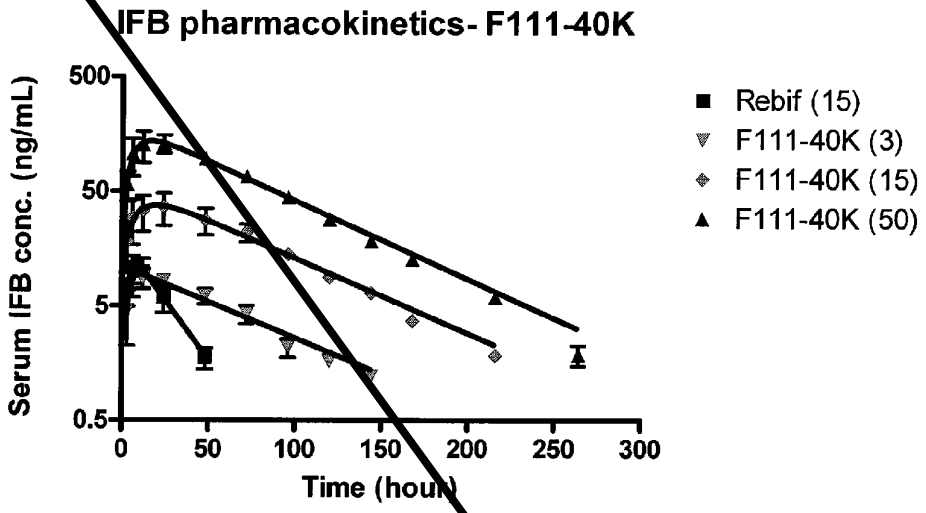
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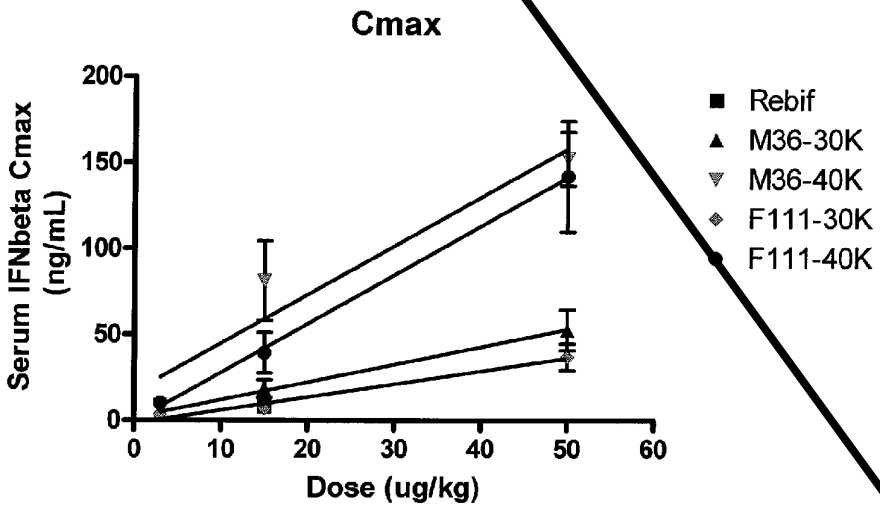
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Figure 9

A



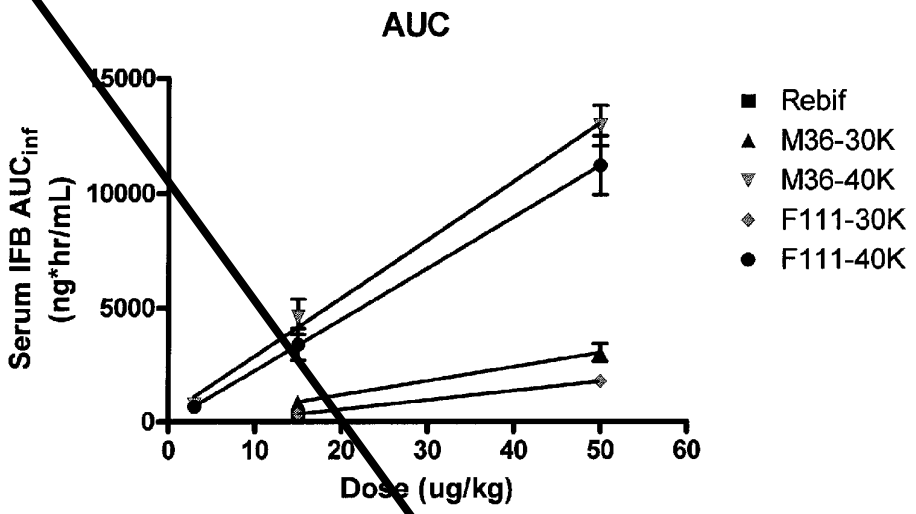
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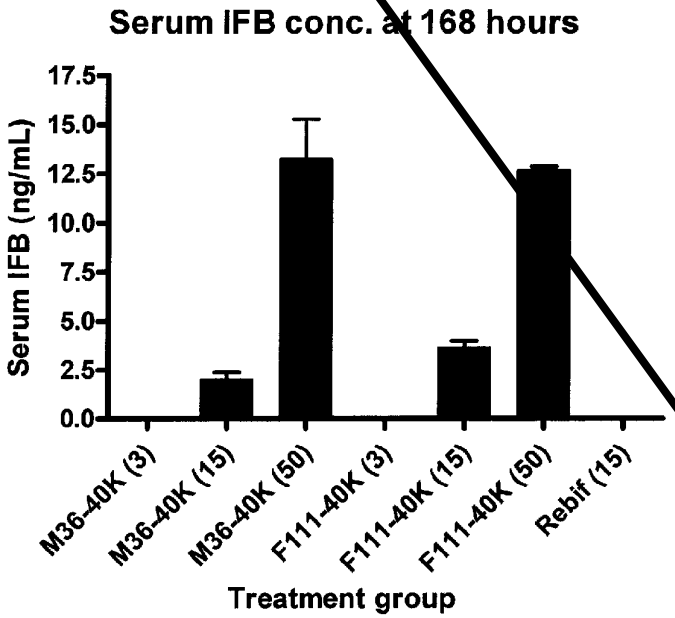
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Figure 10

A



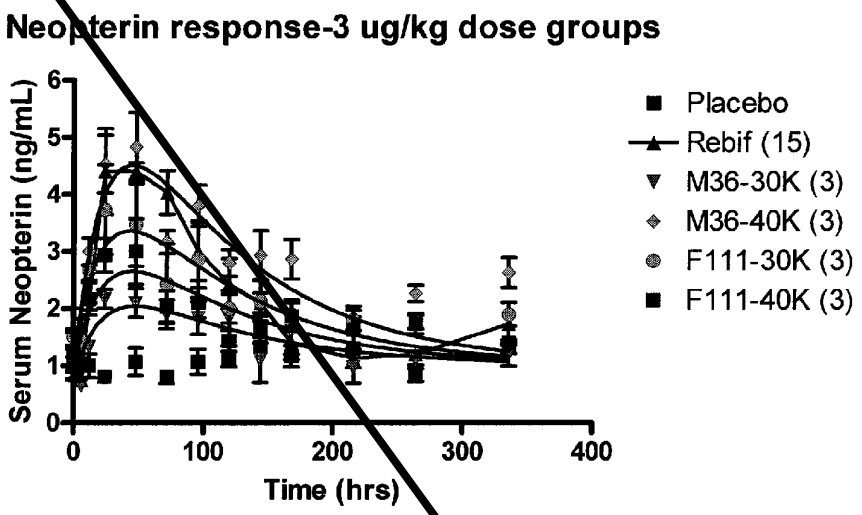
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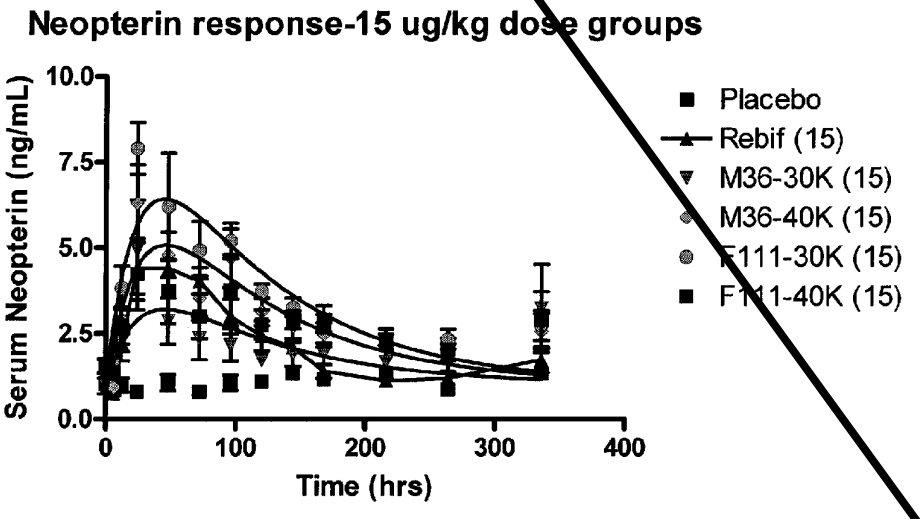
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Figure 11

A



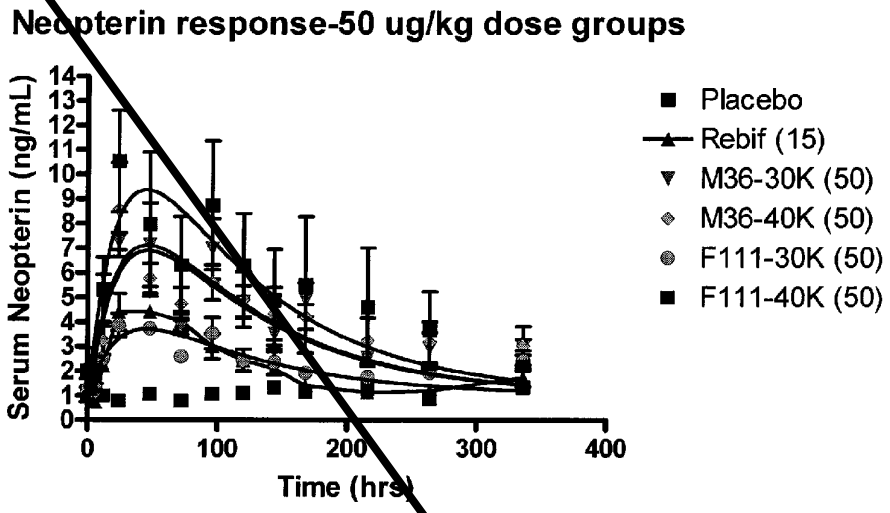
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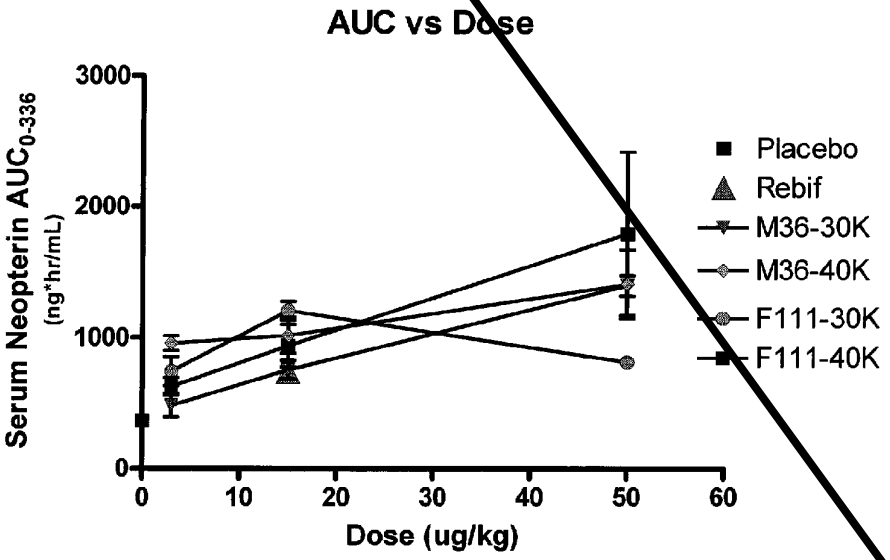
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Figure 12

A

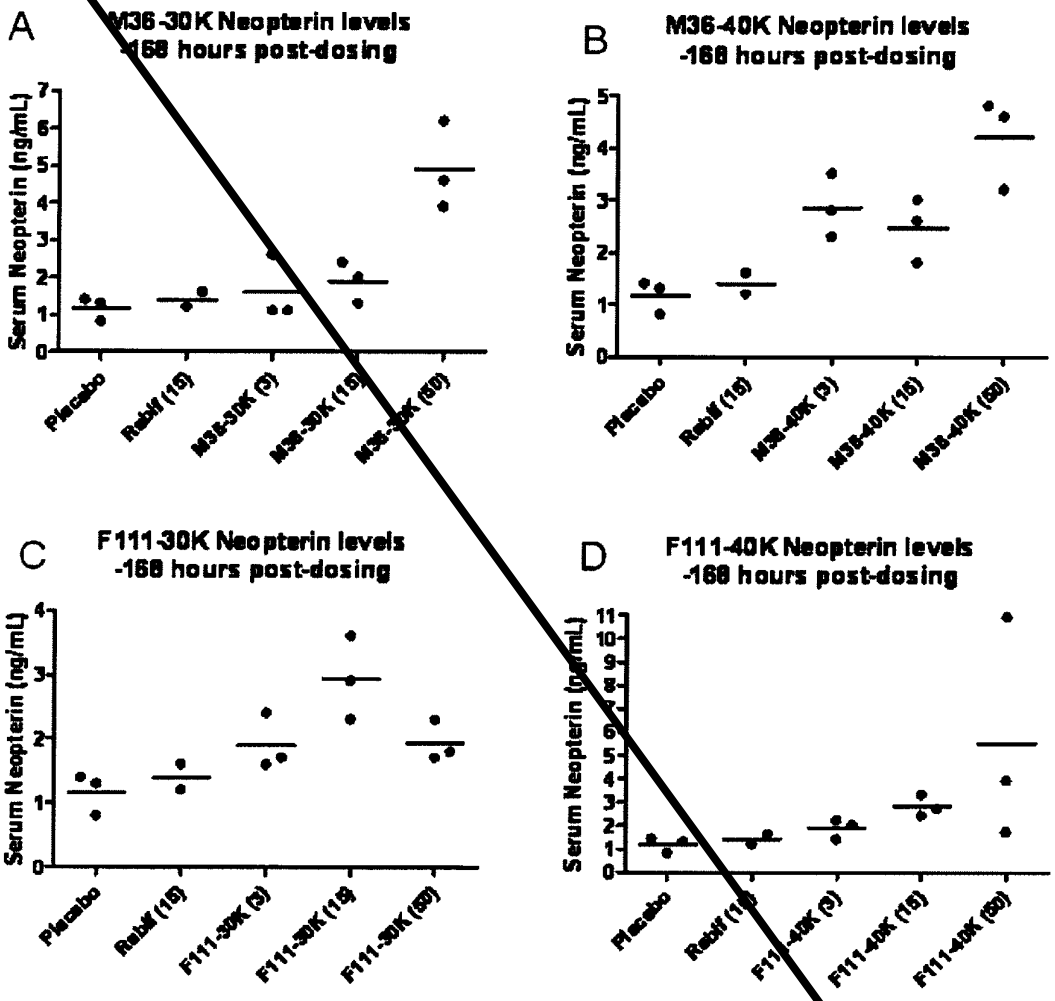


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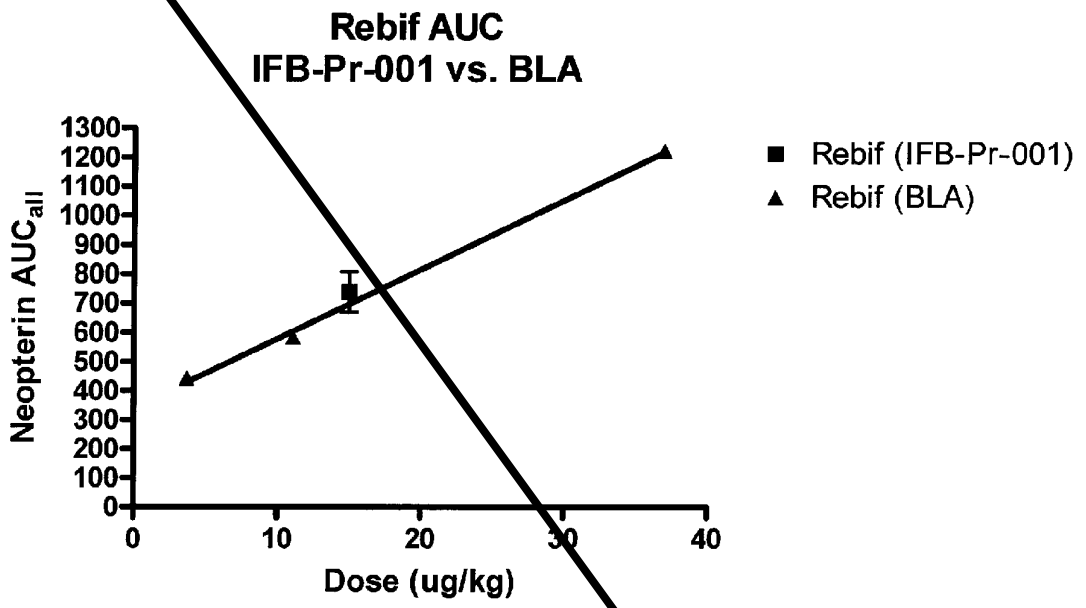
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Figure 13



Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
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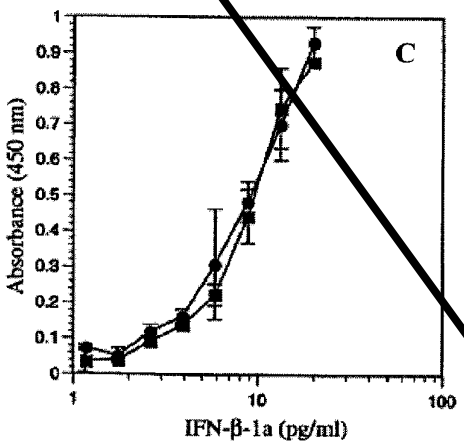
Figure 14

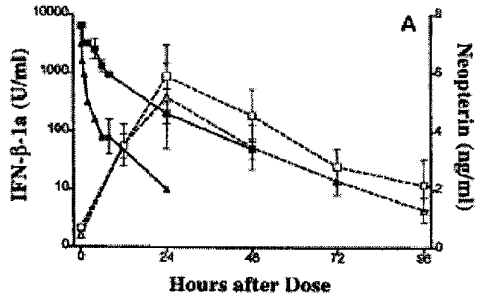


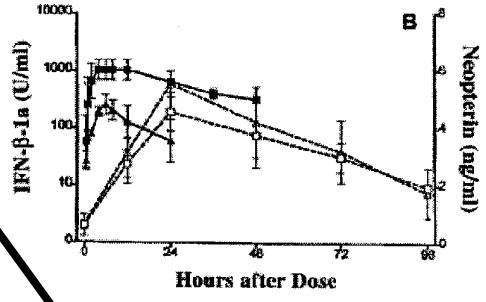
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Figure 15

	Antiviral IC50 (pg/mL)	Antiviral IC50 (pg/mL)
WT human	11	5.7
NtermPEG-20	11	11.9



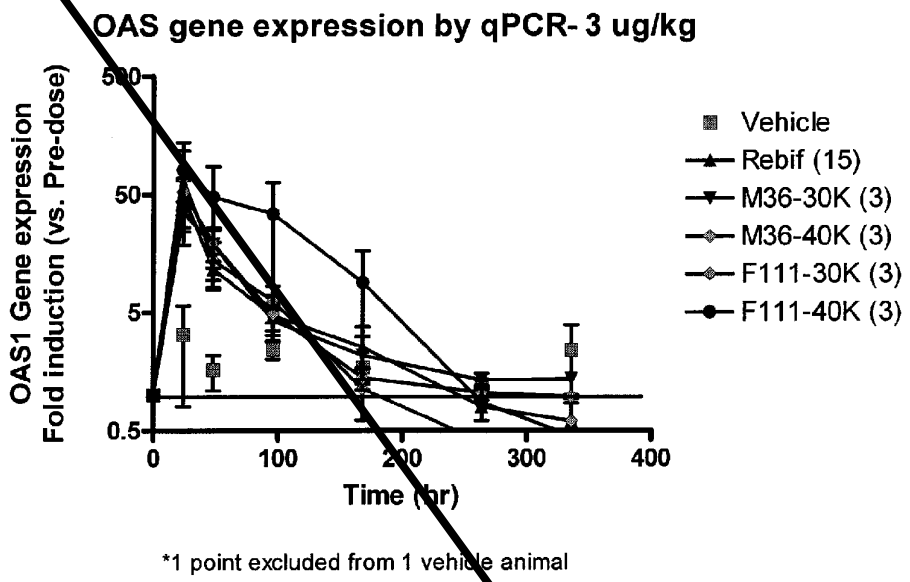




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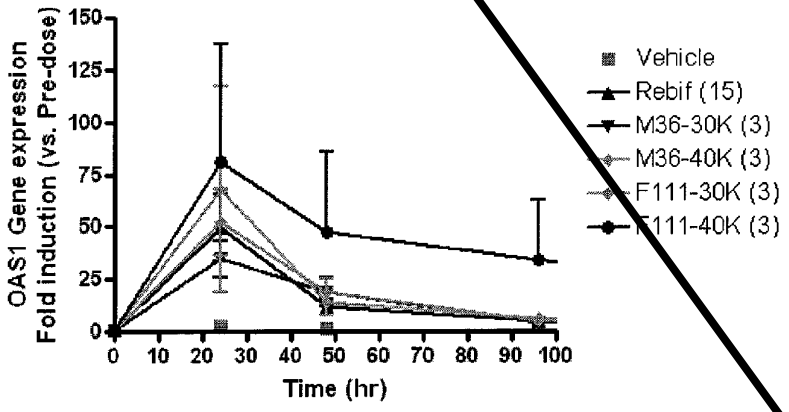
Figure 16

A



*1 point excluded from 1 vehicle animal

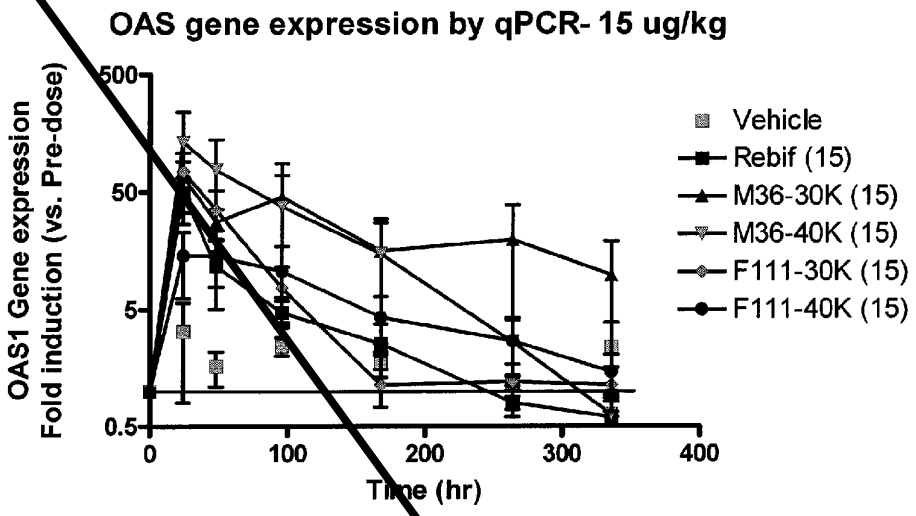
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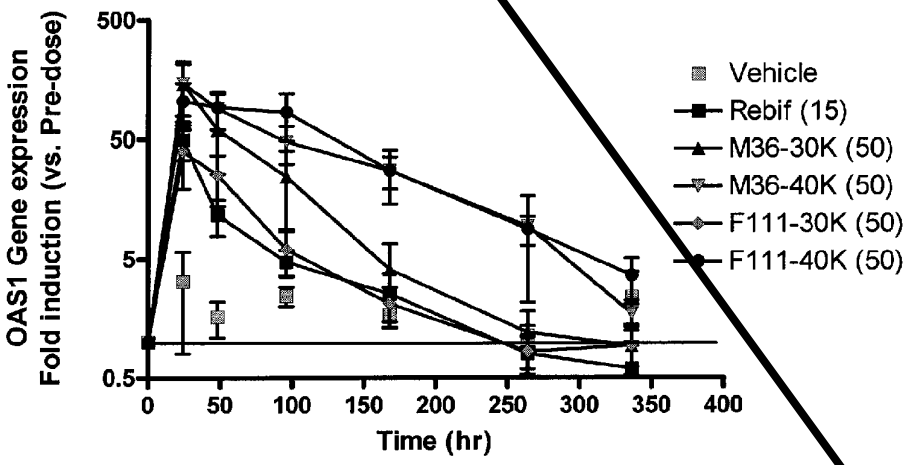
Figure 17

A



*1 point excluded from 1 vehicle animal

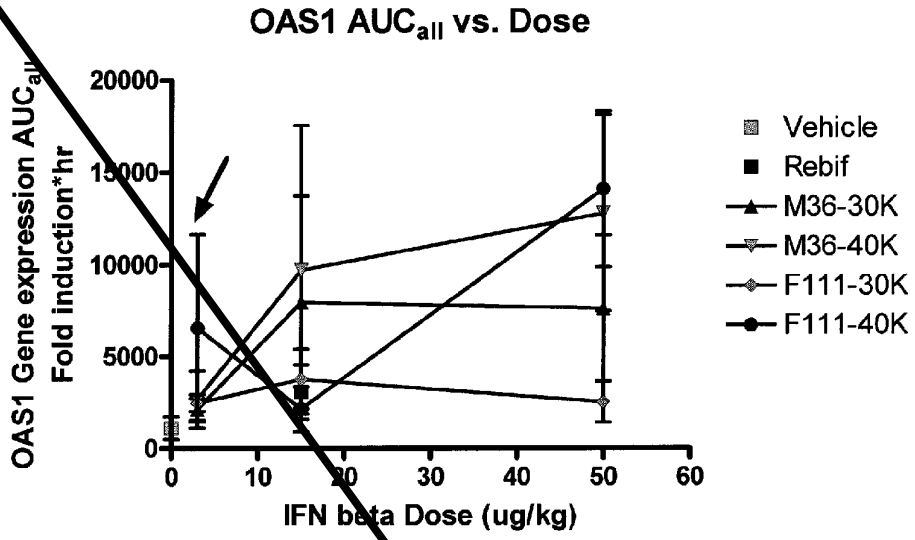
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*1 point excluded from 1 vehicle animal

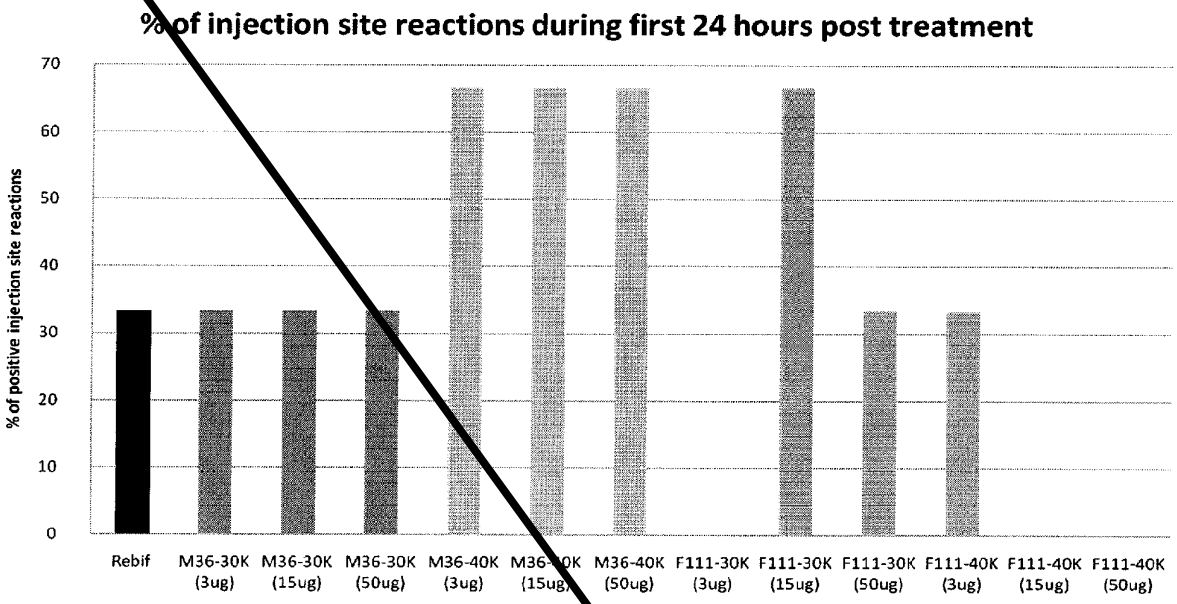
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Figure 18



Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
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Figure 19



Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
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Docket No.:	AMBX-0136.00PCT

Figure 20

Treatment Group	Albumin	Alkaline Phosphatase (ALP)	Alanine Aminotransferase (ALT)	Aspartate Transaminase (AST)	Total Bilirubin	Calcium	Total Cholesterol	Creatine Kinase (CK)	Creatine	Gamma Glutamyl Transaminase (GGT)
Rebif	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-30K (3ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-30K (15ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-30K (50ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-40K (3ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-40K (15ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-40K (50ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-30K (3ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-30K (15ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-30K (50ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-40K (3ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-40K (15ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-40K (50ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

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Figure 21

Treatment Group	Inorganic Phosphorus	Total Protein (TP)	Triglyceride	Blood Urea Nitrogen (BUN)	Sodium	Potassium	Chloride	Globulin	Albumin:Globulin Ratio
Rebif	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-30K (3ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-30K (15ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-30K (50ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-40K (3ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-40K (15ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
M36-40K (50ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-30K (3ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-30K (15ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-30K (50ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-40K (3ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-40K (15ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
F111-40K (50ug)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

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Figure 22

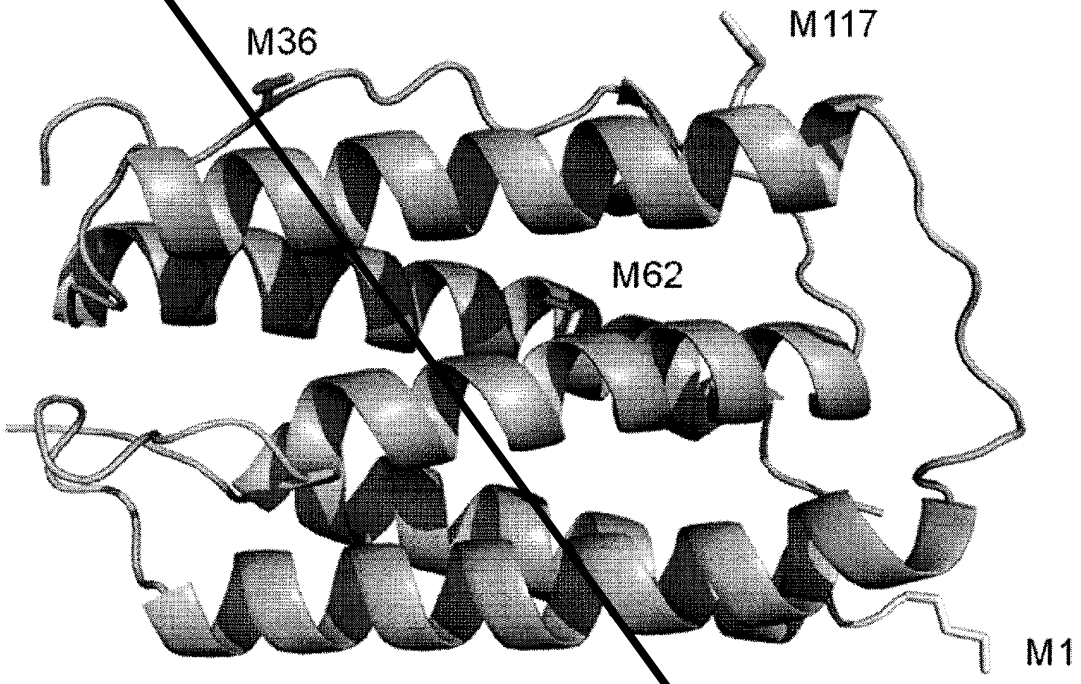
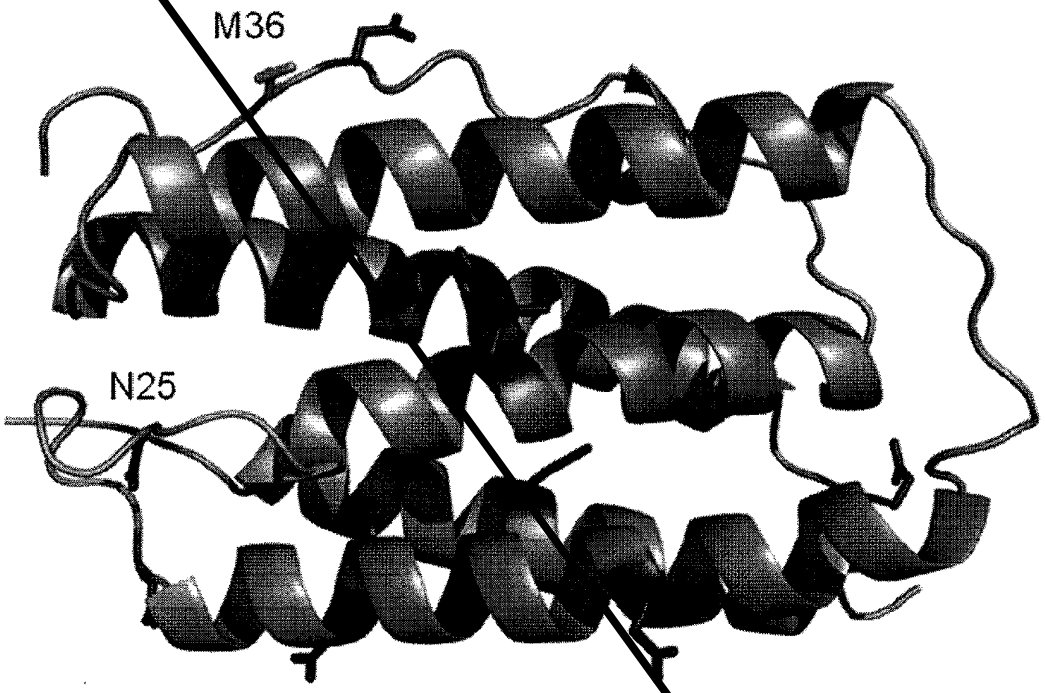
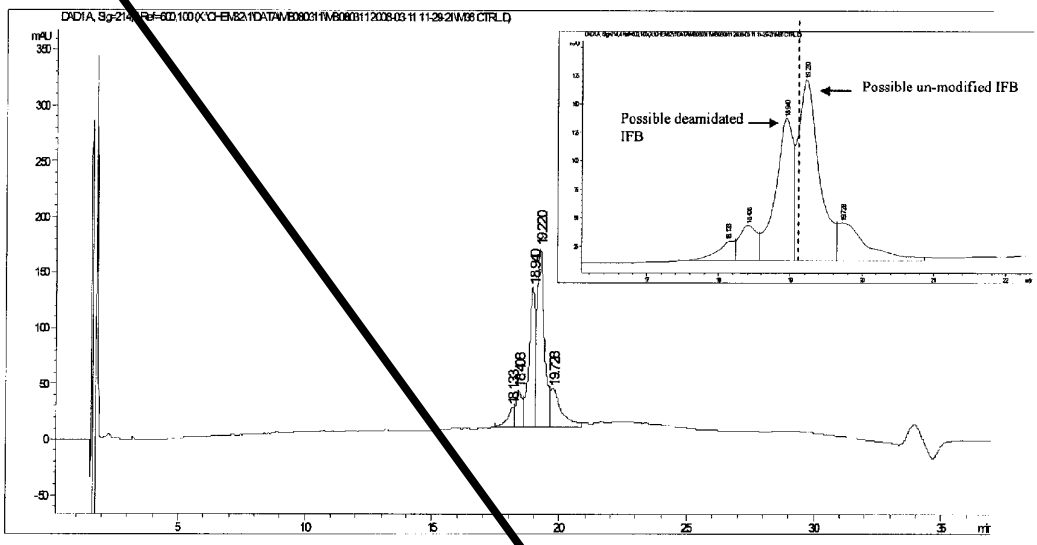


Figure 23



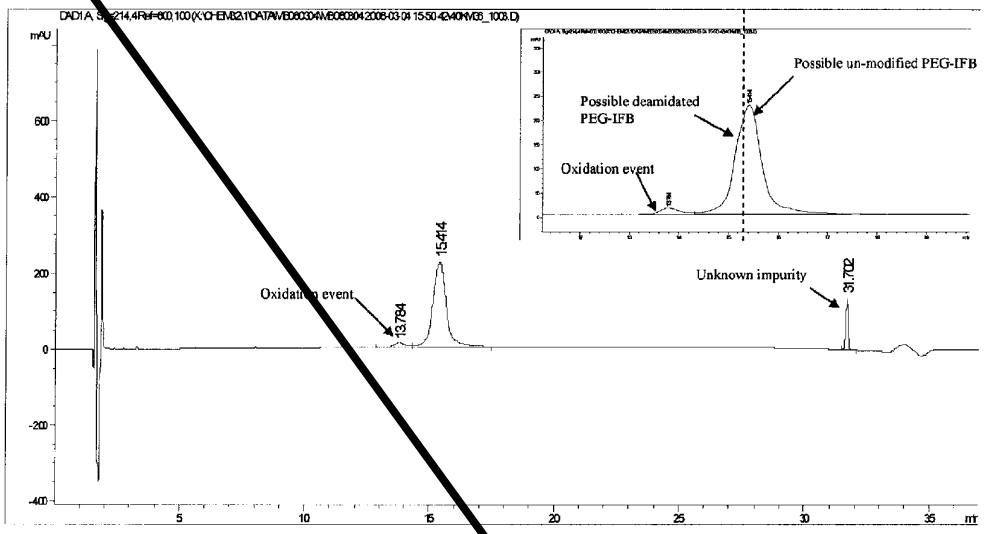
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Atty:	John W. Wallen, III Telephone: (858) 875-2403
Docket No.:	AMBX-0136.00PCT

Figure 24



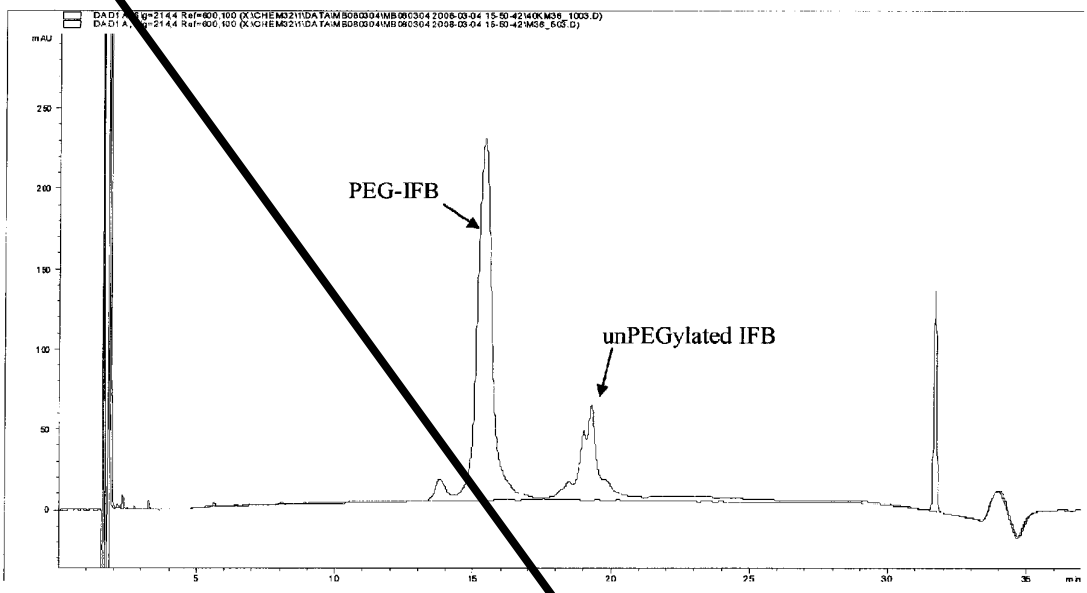
Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
Applicant:	AMBRX, INC.
Appl. No.:	Not Assigned
Atty:	John W. Wallen, III Telephone: (858) 875-2403
Docket No.:	AMBX-0136.00PCT

Figure 25



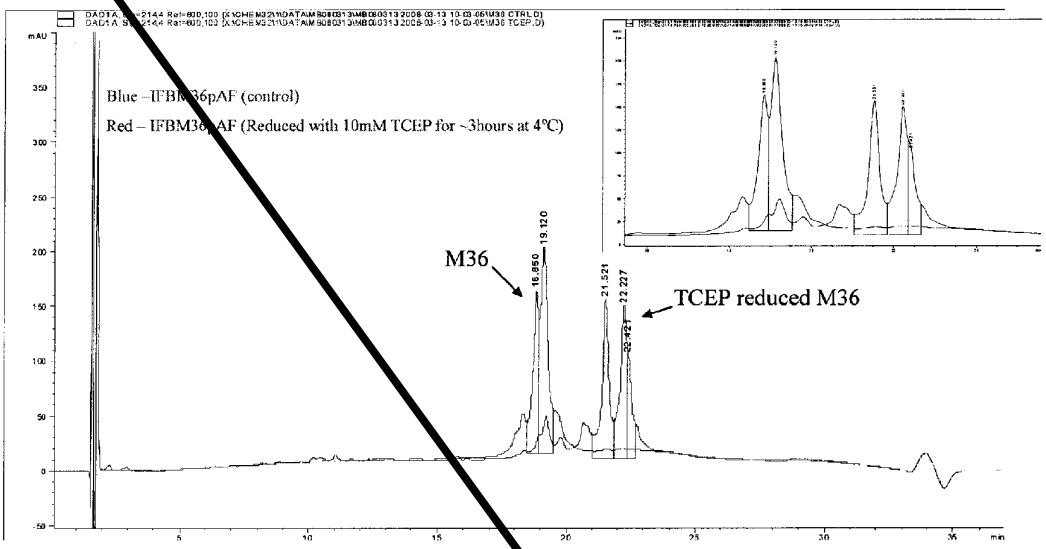
Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
Applicant:	AMBRX, INC.
Appl. No.:	Not Assigned
Atty:	John W. Wallen, III Telephone: (858) 875-2403
Docket No.:	AMBX-0136.00PCT

Figure 26



Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
Applicant:	AMBRX, INC.
Appl. No.:	Not Assigned
Atty:	John W. Wallen, III Telephone: (858) 875-2403
Docket No.:	AMBX-0136.00PCT

Figure 27



Title:	MODIFIED INTERFERON BETA POLYPEPTIDES AND THEIR USES
Applicant:	AMBRX, INC.
Appl. No.:	Not Assigned
Atty:	John W. Wallen, III Telephone: (858) 875-2403
Docket No.:	AMBX-0136.00PCT

Figure 28

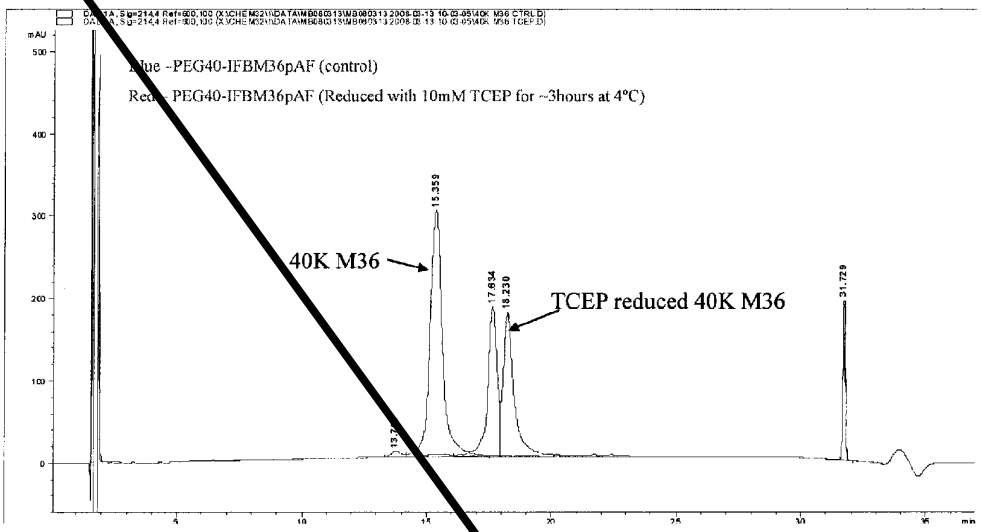


Figure 1

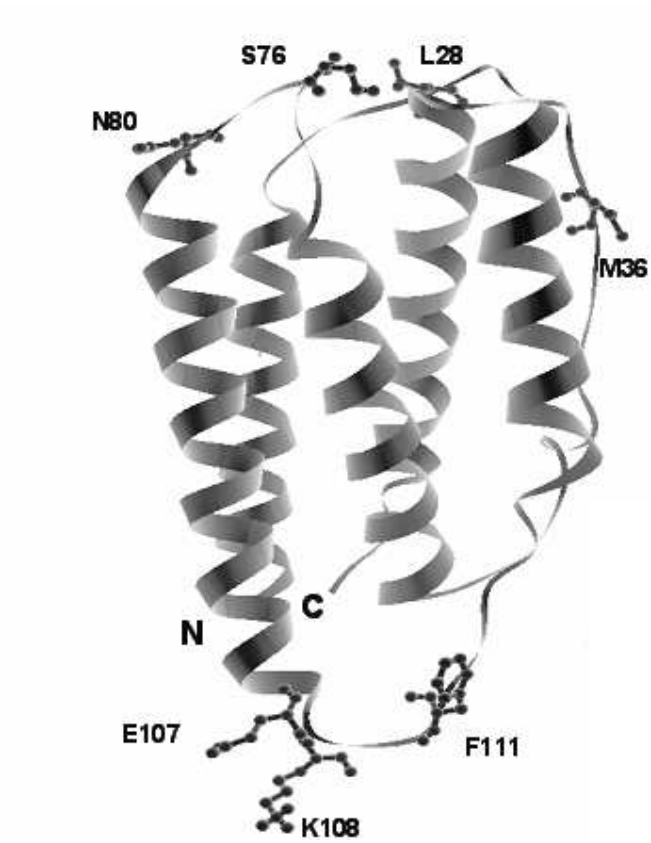


Figure 2

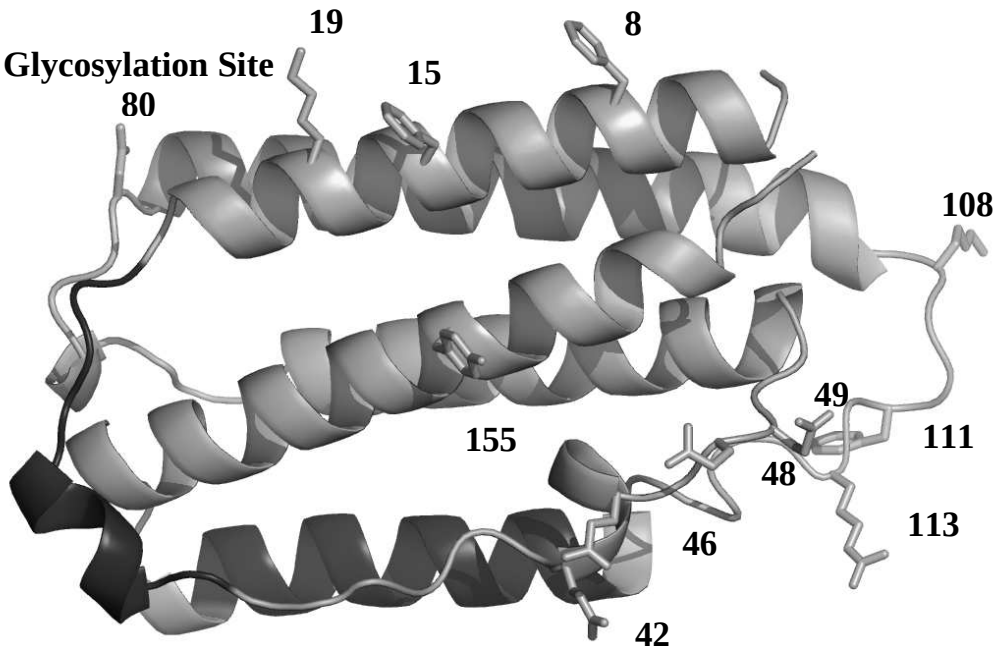


Figure 3

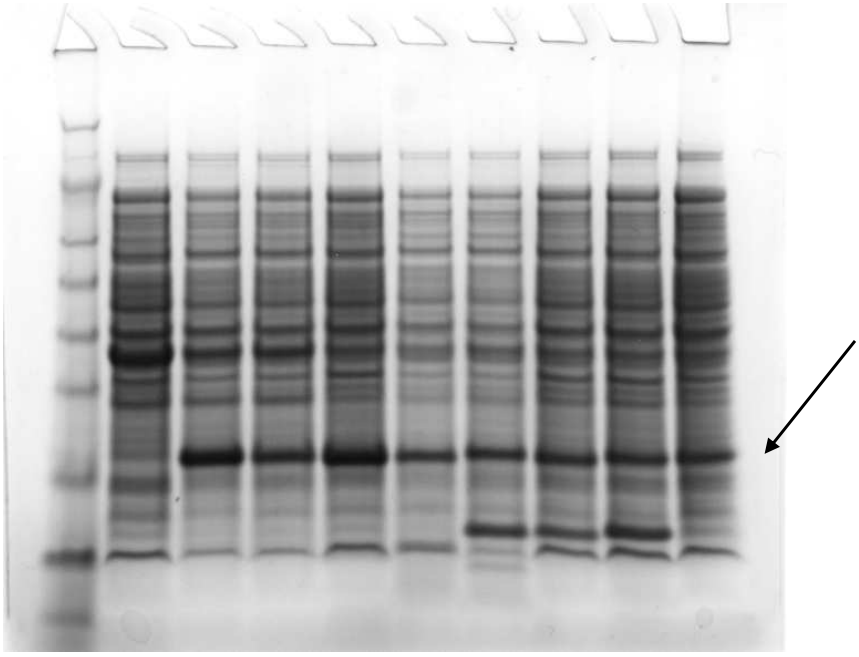


Figure 4

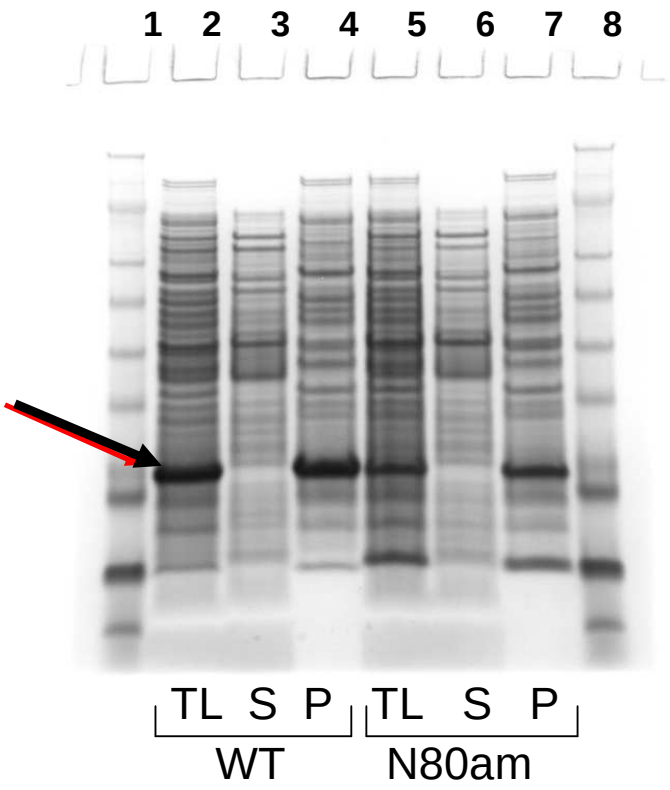


Figure 5

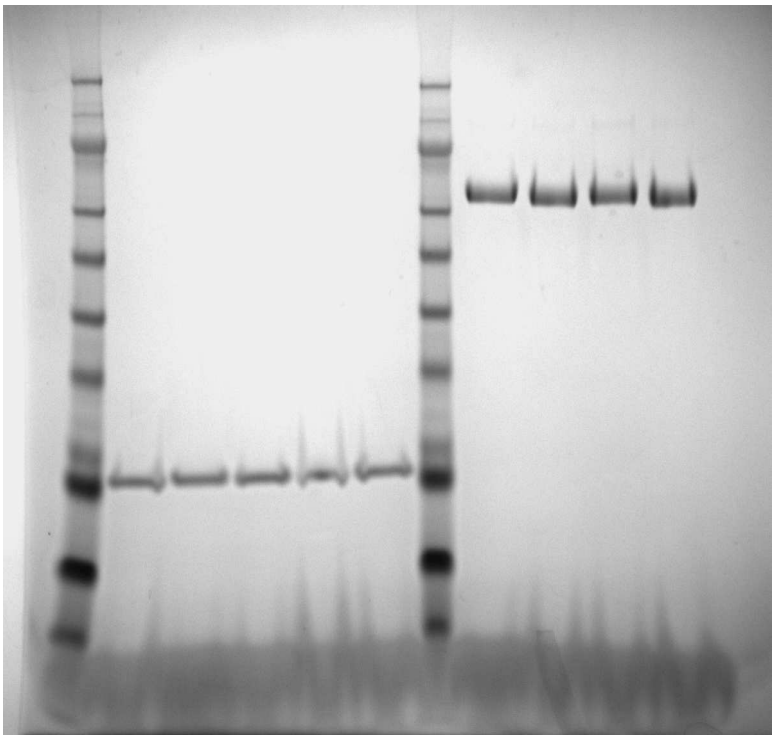
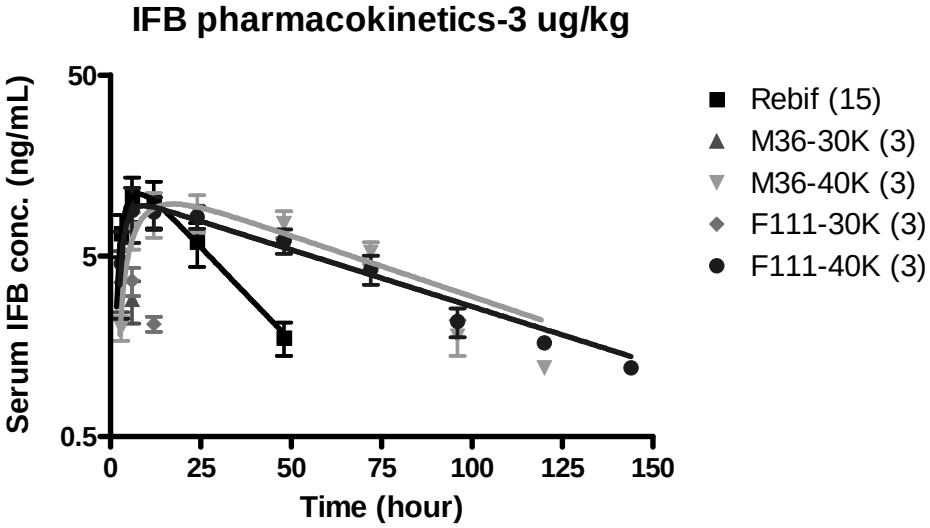


Figure 6

A


IFB pharmacokinetics-3 ug/kg
Serum IFB conc. (ng/mL)
50
5
0.5
0
25
50
75
100
125
150
Time (hour)
Rebif (15)
M36-30K (3)
M36-40K (3)
F111-30K (3)
F111-40K (3)

B

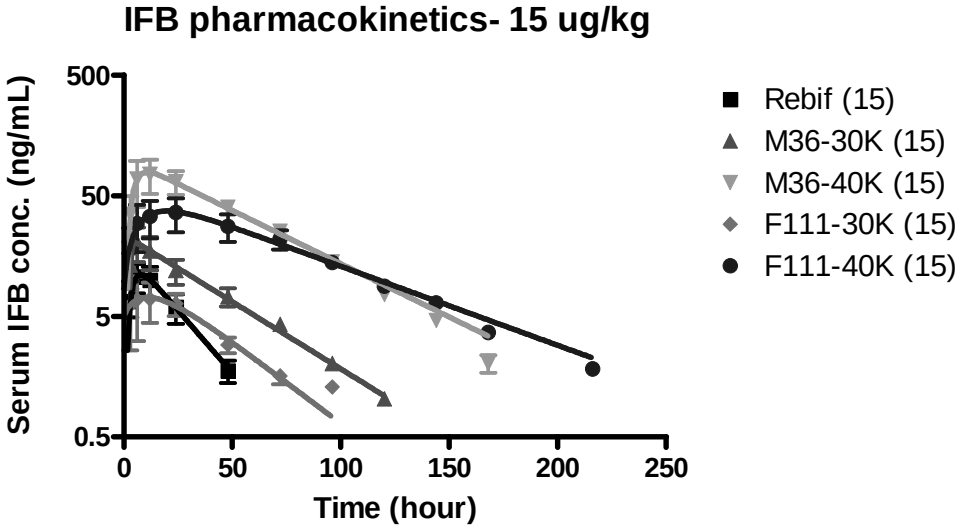
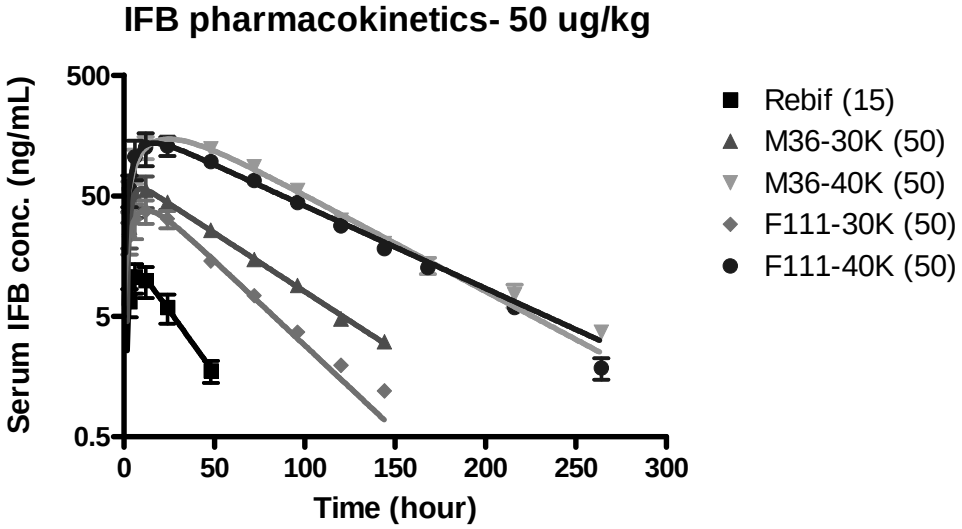

IFB pharmacokinetics- 15 ug/kg
Serum IFB conc. (ng/mL)
500
50
5
0.5
0
50
100
150
200
250
Time (hour)
Rebif (15)
M36-30K (15)
M36-40K (15)
F111-30K (15)
F111-40K (15)

Figure 7

A


IFB pharmacokinetics- 50 ug/kg
Serum IFB conc. (ng/mL)
500
50
5
0.5
0
50
100
150
200
250
300
Time (hour)
Rebif (15)
M36-30K (50)
M36-40K (50)
F111-30K (50)
F111-40K (50)

B

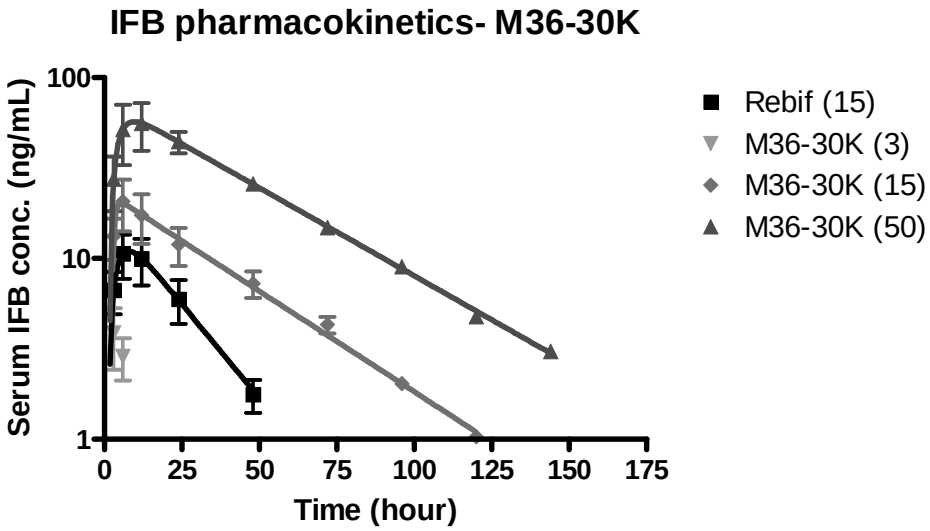
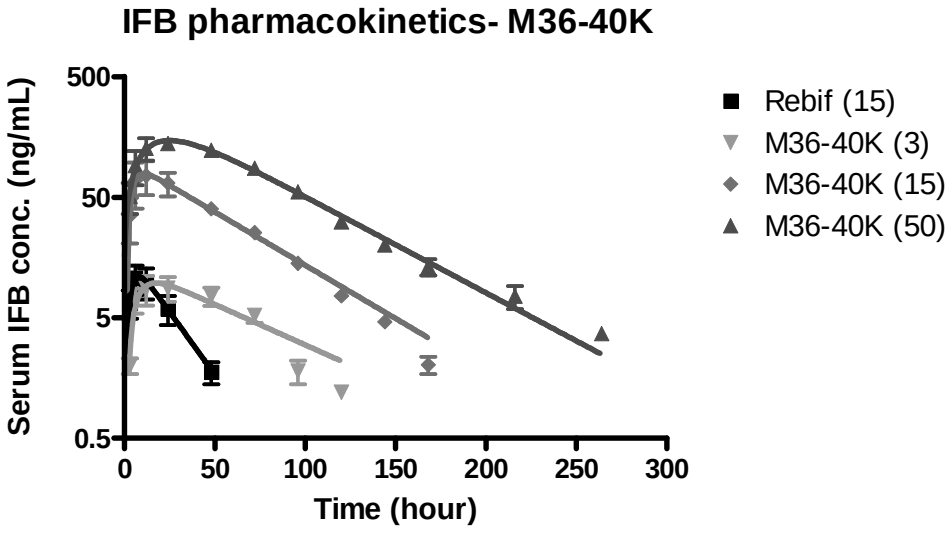

IFB pharmacokinetics- M36-30K
Serum IFB conc. (ng/mL)
100
10
1
0
25
50
75
100
125
150
175
Time (hour)
Rebif (15)
M36-30K (3)
M36-30K (15)
M36-30K (50)

Figure 8

A


IFB pharmacokinetics- M36-40K
Serum IFB conc. (ng/mL)
500
50
5
0.5
0
50
100
150
200
250
300
Time (hour)
Rebif (15)
M36-40K (3)
M36-40K (15)
M36-40K (50)

B

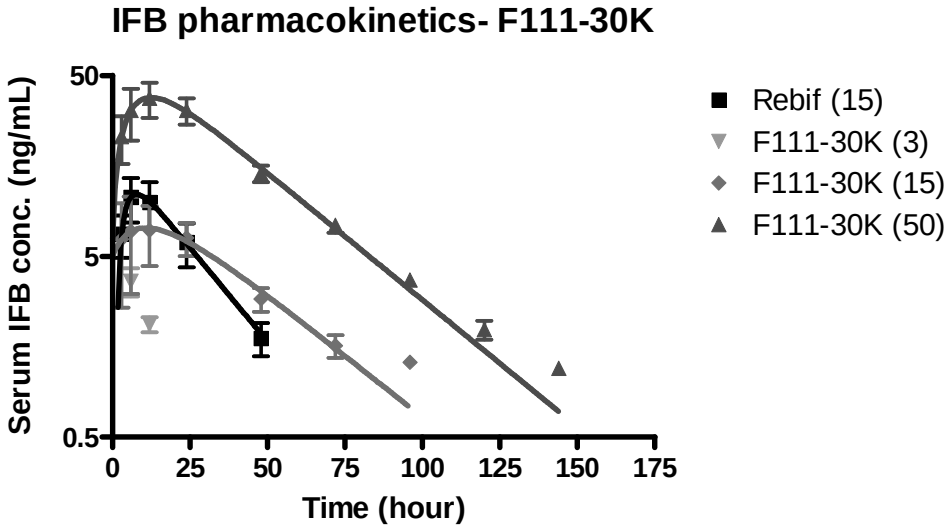
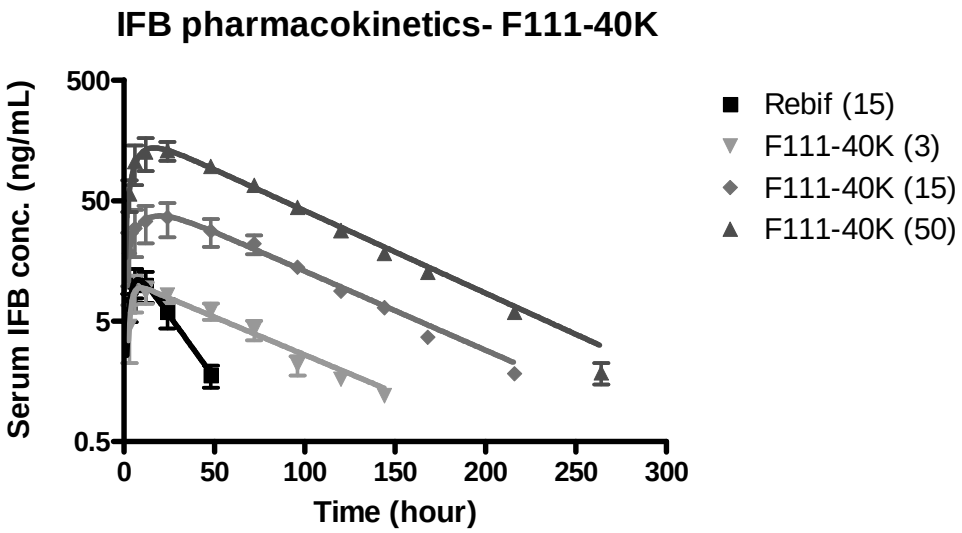

IFB pharmacokinetics- F111-30K
Serum IFB conc. (ng/mL)
50
5
0.5
0
25
50
75
100
125
150
175
Time (hour)
Rebif (15)
F111-30K (3)
F111-30K (15)
F111-30K (50)

Figure 9

A


IFB pharmacokinetics- F111-40K
Serum IFB conc. (ng/mL)
500
50
5
0.5
0
50
100
150
200
250
300
Time (hour)
Rebif (15)
F111-40K (3)
F111-40K (15)
F111-40K (50)

B

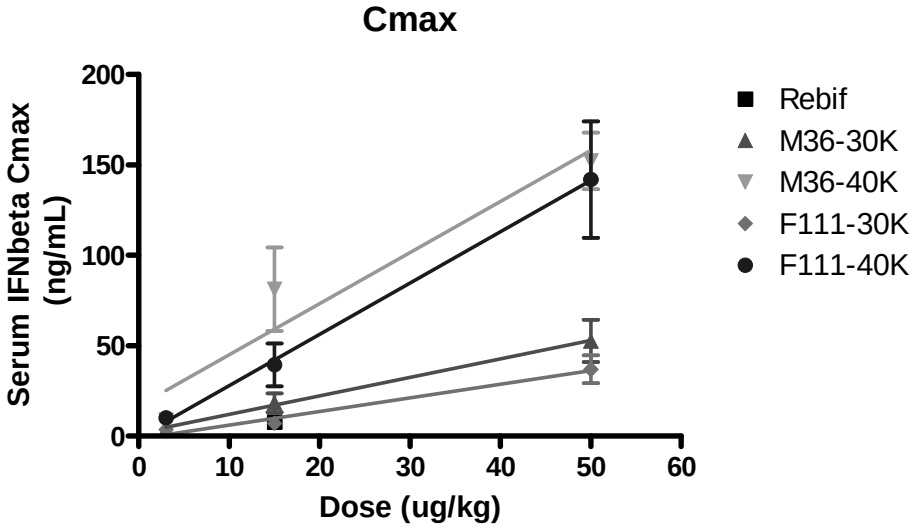
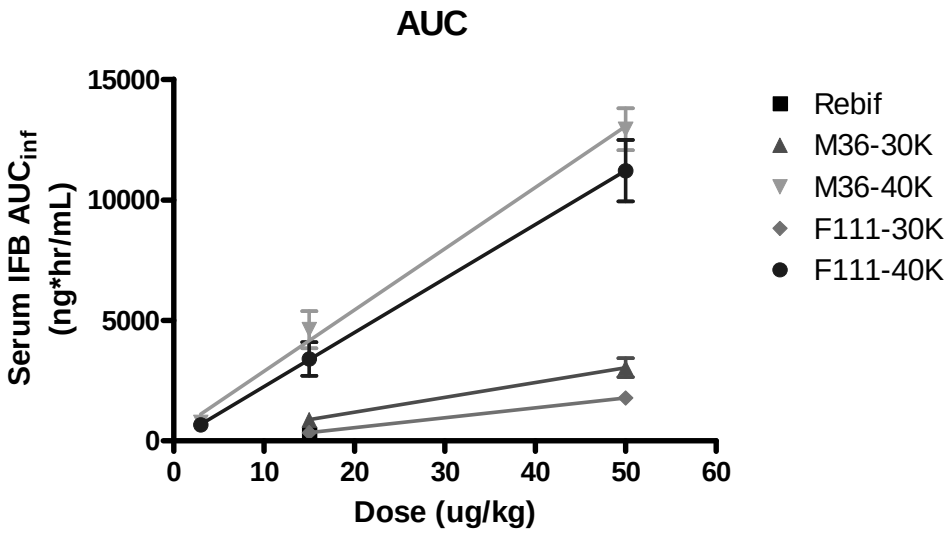

Cmax
Serum IFNbeta Cmax (ng/mL)
200
150
100
50
0
0
10
20
30
40
50
60
Dose (ug/kg)
Rebif
M36-30K
M36-40K
F111-30K
F111-40K

Figure 10

A


AUC
Serum IFB AUC$_{inf}$ (ng*hr/mL)
15000
10000
5000
0
0
10
20
30
40
50
60
Dose (ug/kg)
Rebif
M36-30K
M36-40K
F111-30K
F111-40K

B

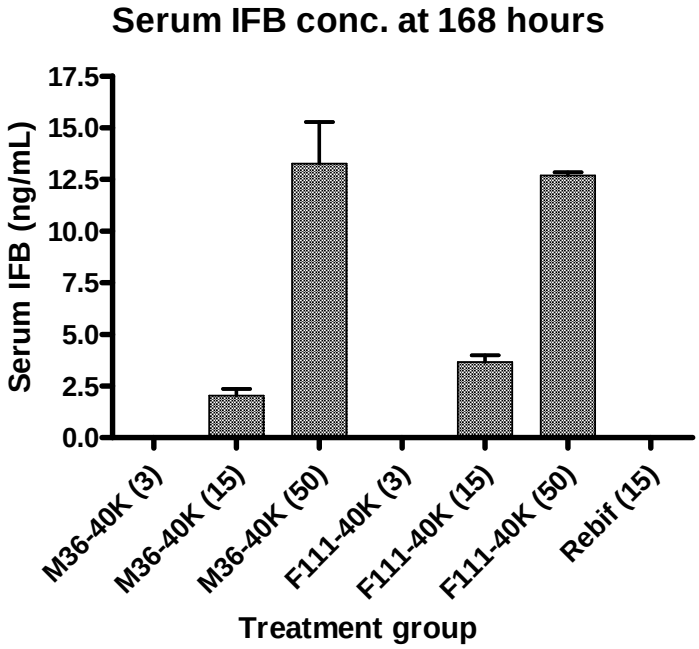
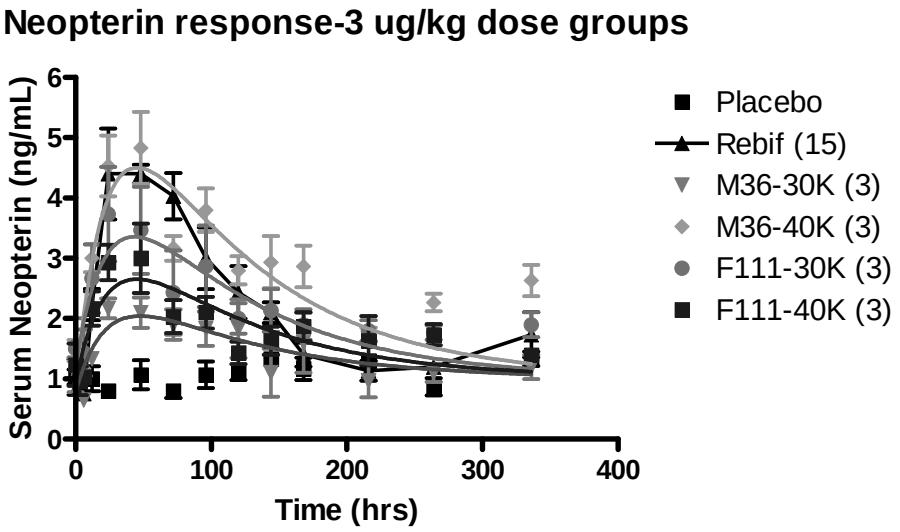

Serum IFB conc. at 168 hours
Serum IFB (ng/mL)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
M36-40K (3)
M36-40K (15)
M36-40K (50)
F111-40K (3)
F111-40K (15)
F111-40K (50)
Rebif (15)
Treatment group

Figure 11

A


Neopterin response-3 ug/kg dose groups
Serum Neopterin (ng/mL)
Time (hrs)
0
1
2
3
4
5
6
0
100
200
300
400
Placebo
Rebif (15)
M36-30K (3)
M36-40K (3)
F111-30K (3)
F111-40K (3)

B

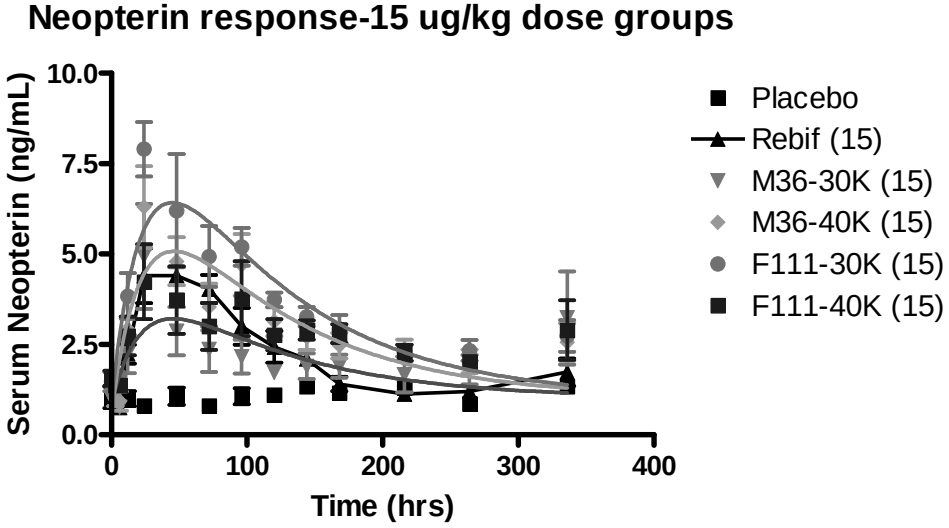
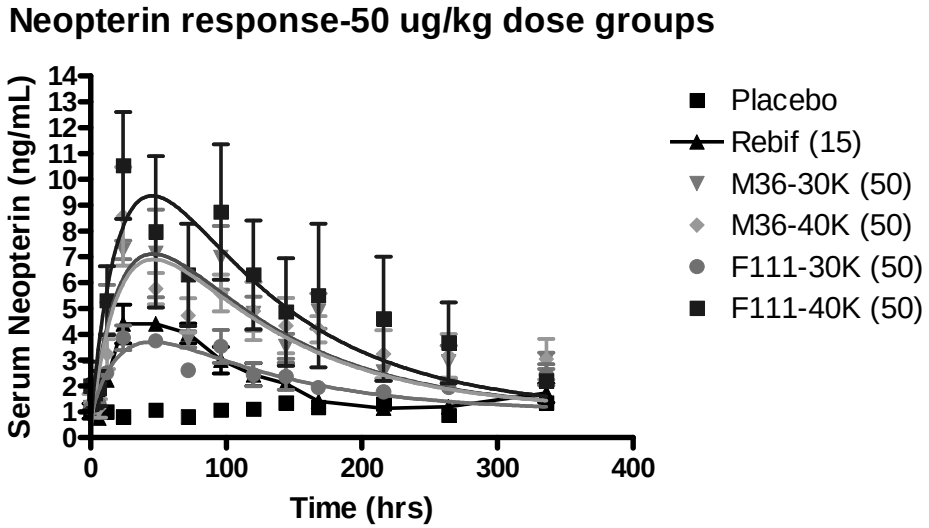

Neopterin response-15 ug/kg dose groups
Serum Neopterin (ng/mL)
Time (hrs)
0.0
2.5
5.0
7.5
10.0
0
100
200
300
400
Placebo
Rebif (15)
M36-30K (15)
M36-40K (15)
F111-30K (15)
F111-40K (15)

Figure 12

A


Neopterin response-50 ug/kg dose groups
Serum Neopterin (ng/mL)
Time (hrs)
Placebo
Rebif (15)
M36-30K (50)
M36-40K (50)
F111-30K (50)
F111-40K (50)

B

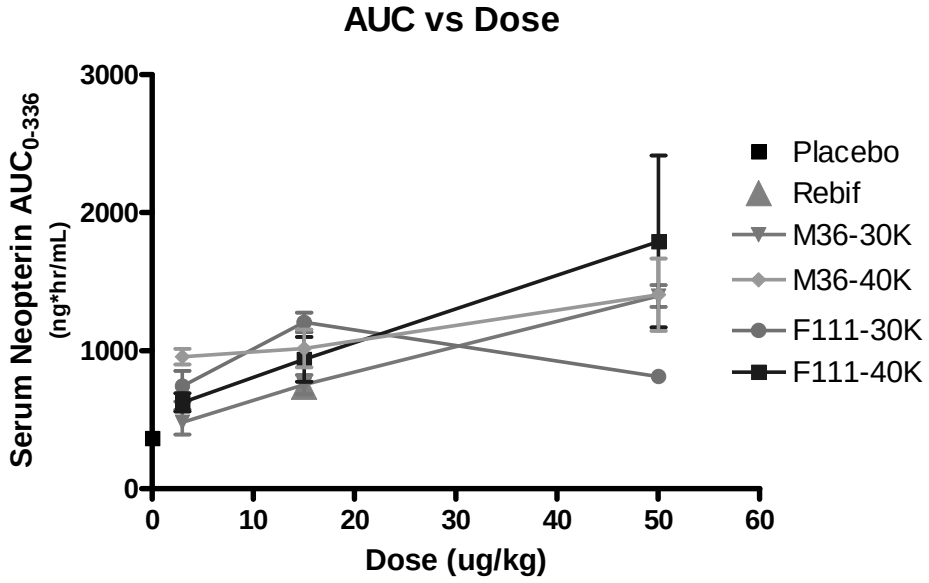

AUC vs Dose
Serum Neopterin AUC0-336
(ng*hr/mL)
Dose (ug/kg)
Placebo
Rebif
M36-30K
M36-40K
F111-30K
F111-40K

Figure 13

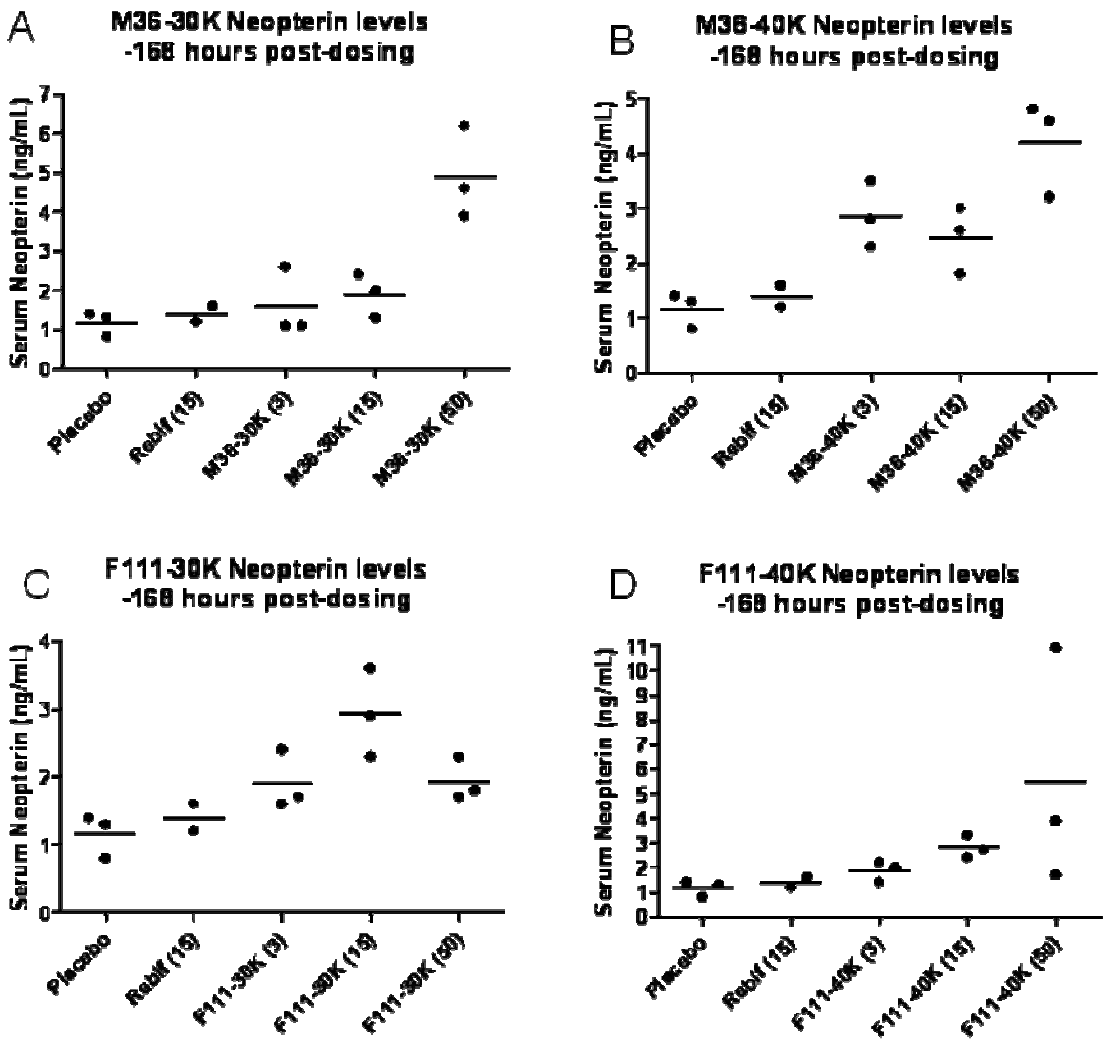

A
M36-30K Neopterin levels
-168 hours post-dosing
Serum Neopterin (ng/mL)
Placebo
Rebif (15)
M36-30K (3)
M36-30K (15)
M36-30K (50)
B
M36-40K Neopterin levels
-168 hours post-dosing
Serum Neopterin (ng/mL)
Placebo
Rebif (15)
M36-40K (3)
M36-40K (15)
M36-40K (50)
C
F111-30K Neopterin levels
-168 hours post-dosing
Serum Neopterin (ng/mL)
Placebo
Rebif (15)
F111-30K (3)
F111-30K (15)
F111-30K (50)
D
F111-40K Neopterin levels
-168 hours post-dosing
Serum Neopterin (ng/mL)
Placebo
Rebif (15)
F111-40K (3)
F111-40K (15)
F111-40K (50)

Figure 14

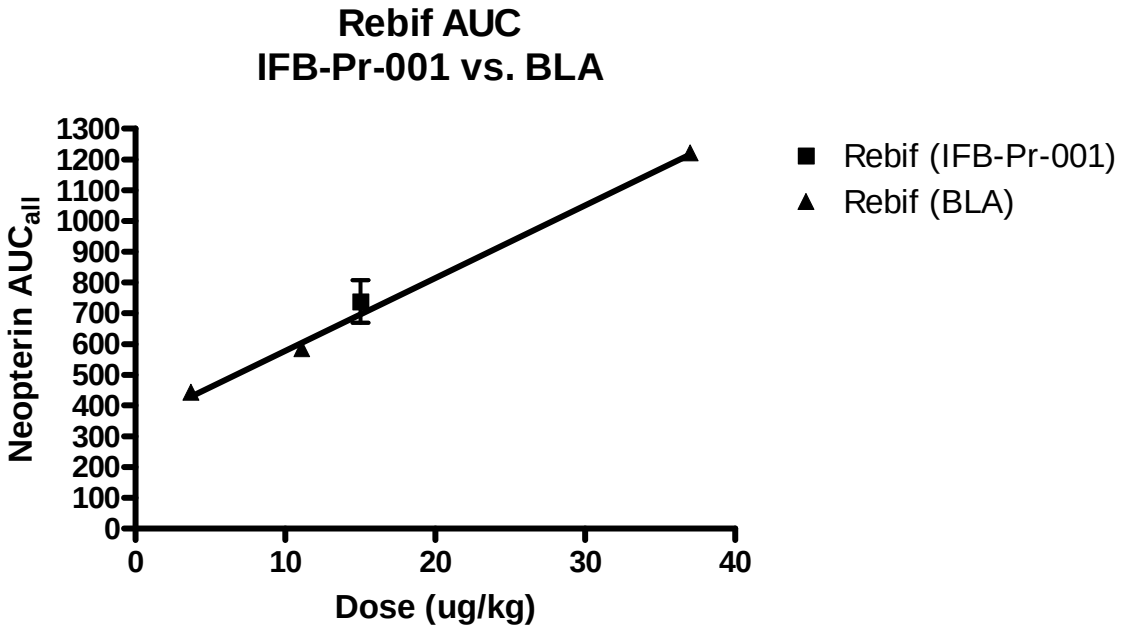
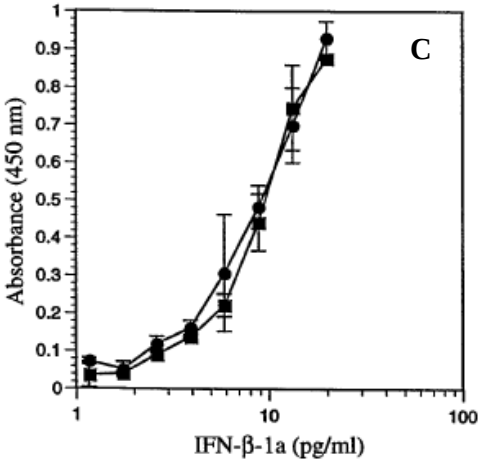
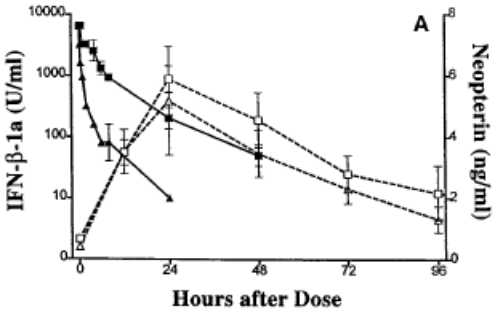

Rebif AUC
IFB-Pr-001 vs. BLA
Neopterin AUCall
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
Dose (ug/kg)
Rebif (IFB-Pr-001)
Rebif (BLA)

Figure 15

	Antiviral IC50 (pg/mL)	Antiviral IC50 (pg/mL)
WT human	11	5.7
NtermPEG-20	11	11.9





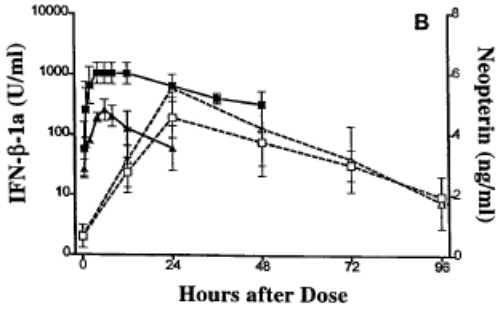
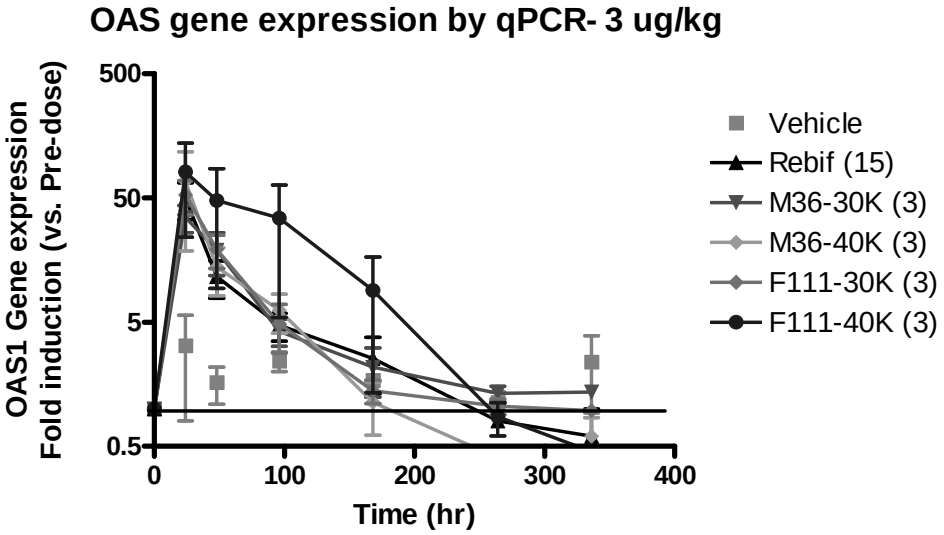


Figure 16

A



*1 point excluded from 1 vehicle animal

B

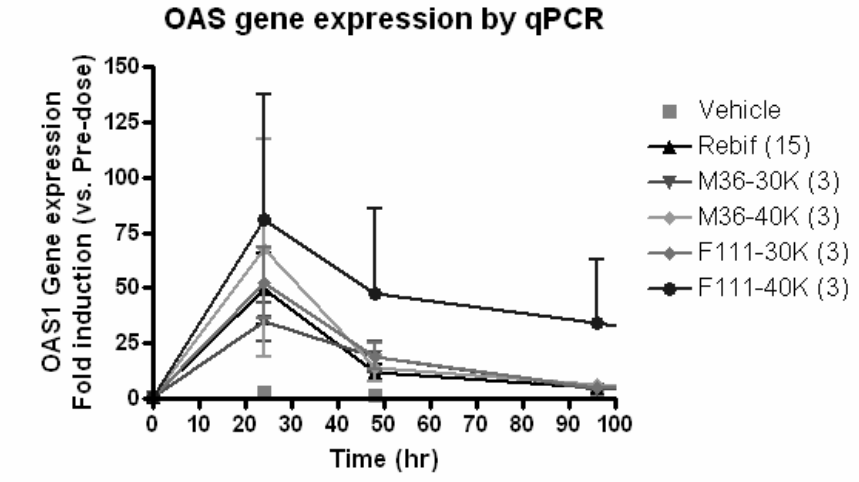
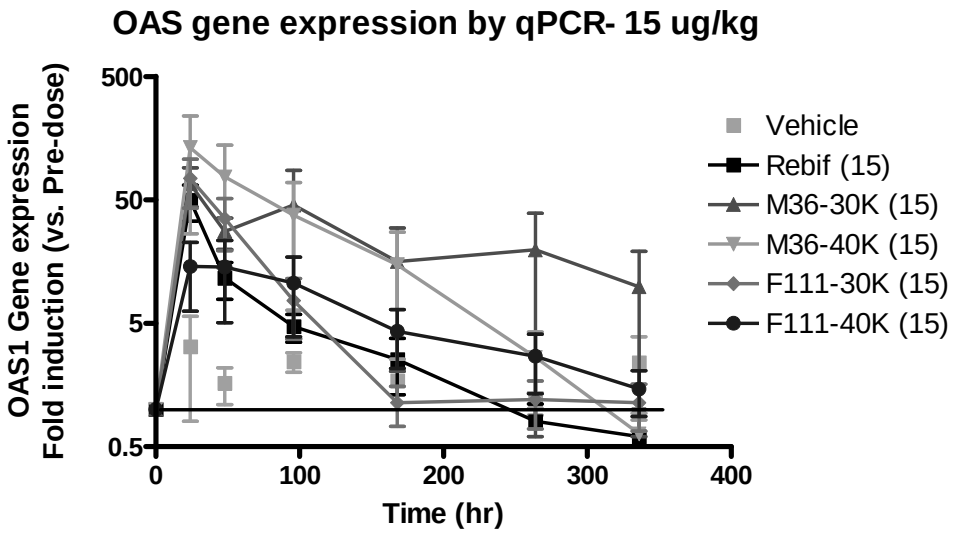


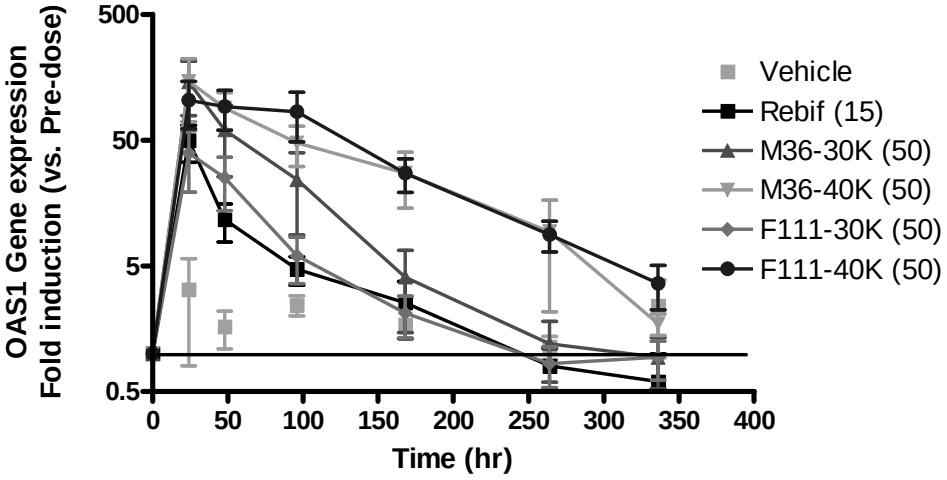
Figure 17

A



*1 point excluded from 1 vehicle animal

B



*1 point excluded from 1 vehicle animal

Figure 18

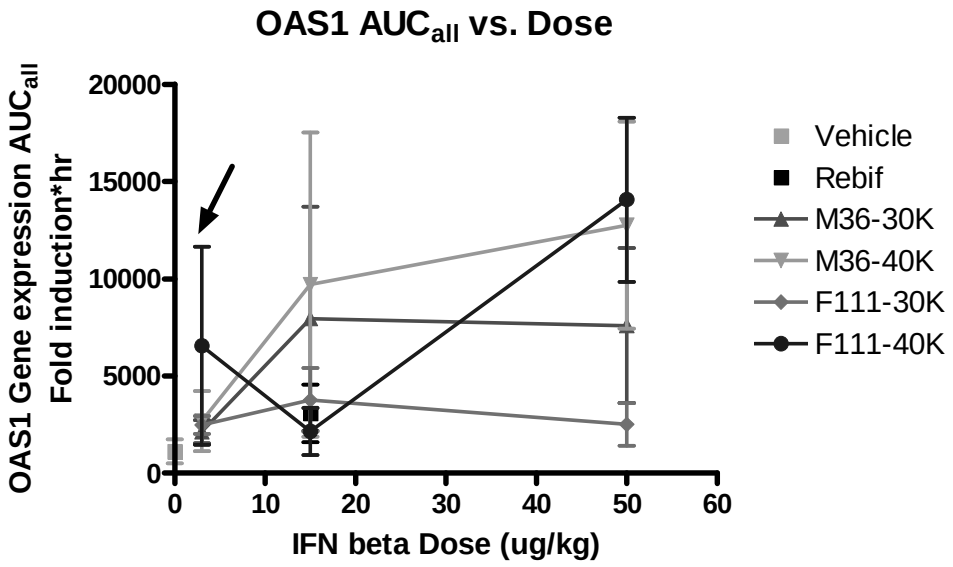

OAS1 AUCall vs. Dose
OAS1 Gene expression AUCall
Fold induction*hr
20000
15000
10000
5000
0
0
10
20
30
40
50
60
IFN beta Dose (ug/kg)
Vehicle
Rebif
M36-30K
M36-40K
F111-30K
F111-40K

Figure 19

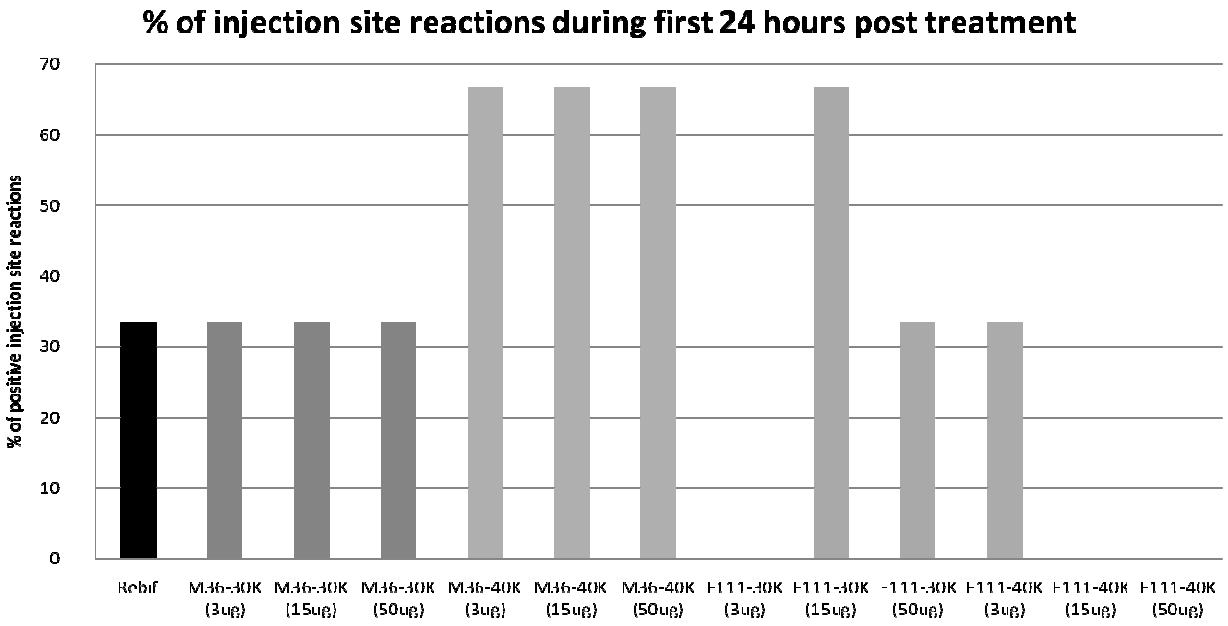

% of injection site reactions during first 24 hours post treatment
% of positive injection site reactions
70
60
50
40
30
20
10
0
Rebif
M36-40K (3uℊ)
M36-40K (15uℊ)
M36-40K (50uℊ)
M46-40K (3uℊ)
M46-40K (15uℊ)
M46-40K (50uℊ)
H111-40K (3uℊ)
H111-40K (15uℊ)
H111-40K (50uℊ)
H111-40K (3uℊ)
H111-40K (15uℊ)
H111-40K (50uℊ)

Figure 20

Treatment Group	Albumin	Alkaline Phosphatase (ALP)	Alanine Aminotransferase (ALT)	Aspartate Transaminase (AST)	Total Bilirubin	Calcium	Total Cholesterol	Creatine Kinase (CK)	Creatine	Gamma Glutamyl Transaminase (GGT)
Rebif	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-30K (3ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-30K (15ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-30K (50ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-40K (3ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-40K (15ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-40K (50ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-30K (3ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-30K (15ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-30K (50ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-40K (3ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-40K (15ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-40K (50ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**

Figure 21

Treatment Group	Inorganic Phosphorus	Total Protein (TP)	Triglyceride	Blood Urea Nitrogen (BUN)	Sodium	Potassium	Chloride	Globulin	Albumin:Globulin Ratio
Rebif	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-30K (3ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-30K (15ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-30K (50ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-40K (3ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-40K (15ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
M36-40K (50ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-30K (3ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-30K (15ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-30K (50ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-40K (3ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-40K (15ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**
F111-40K (50ug)	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**

Figure 22

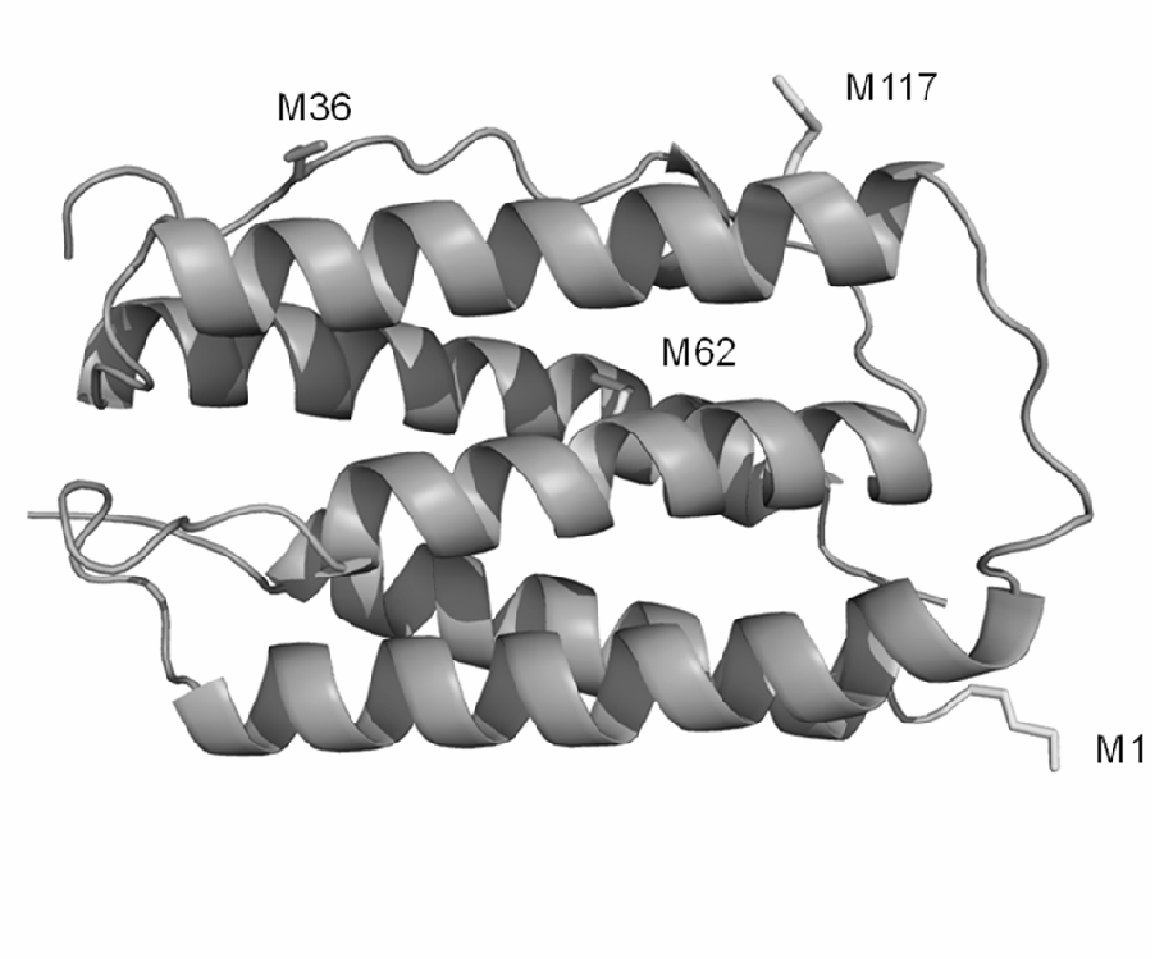


Figure 23

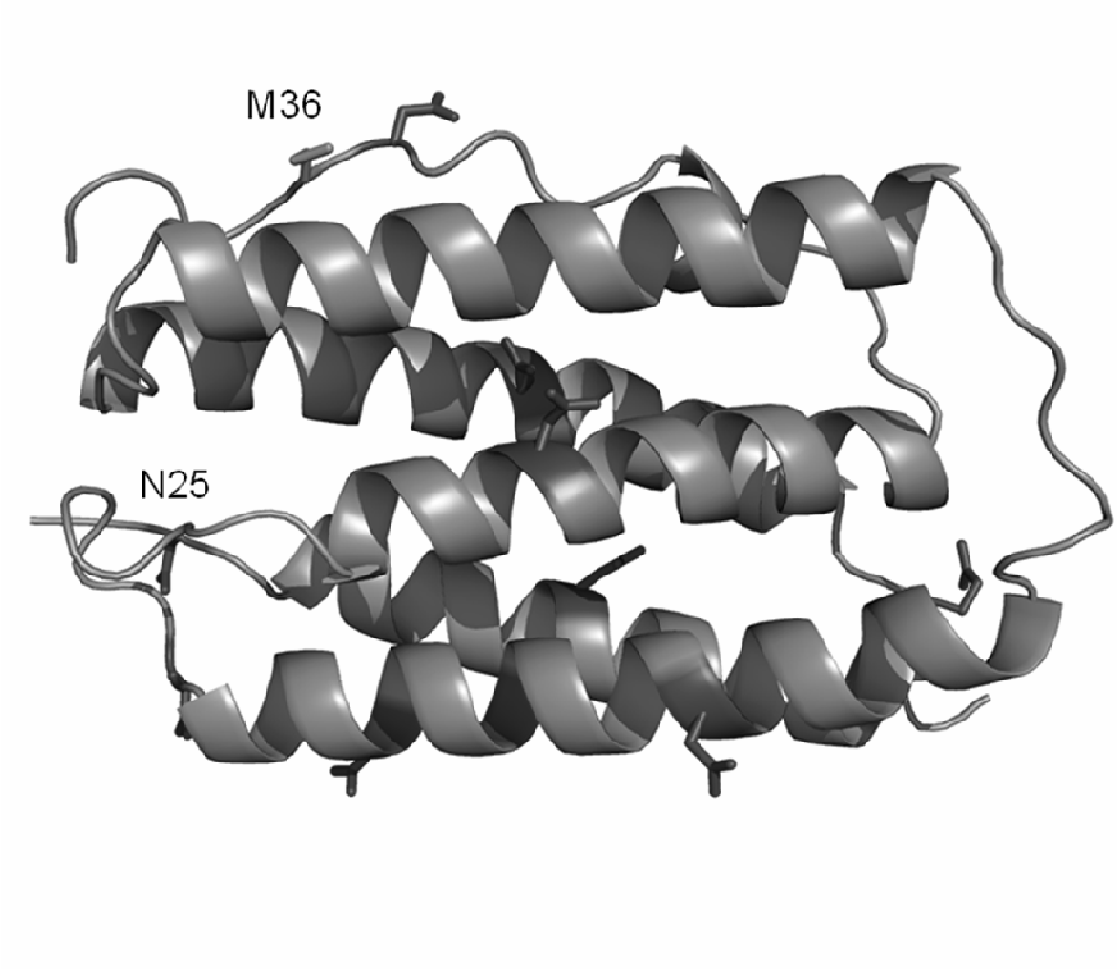


Figure 24

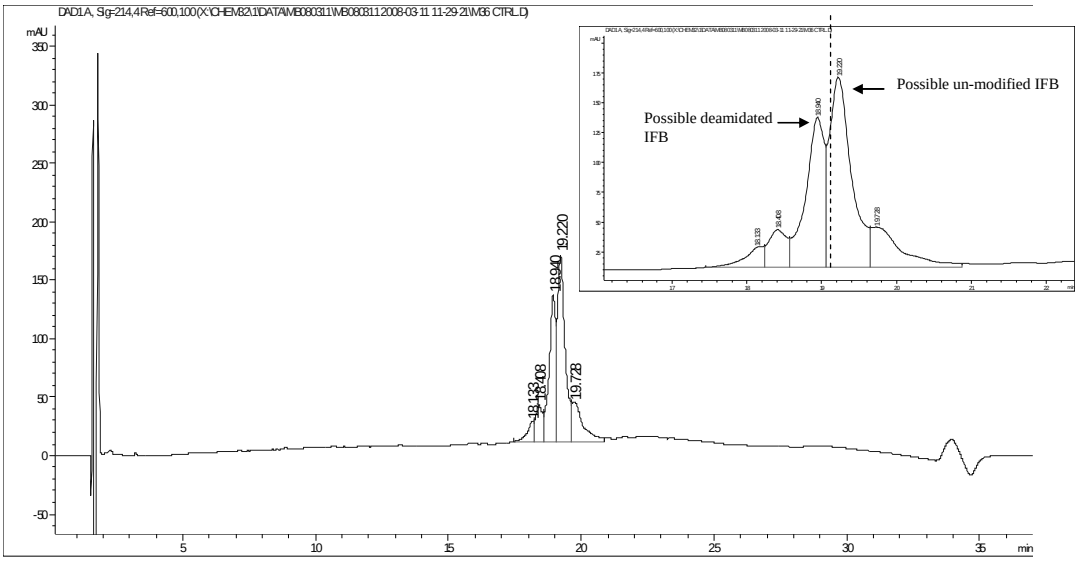

DAD1A, Sig=214,4 Ref=600,100 (X:\CHEM32\1\DATA\MB080311\MB080311 2008-03-11 11-29-21\M66 CTRL.D)
mAU
350
300
250
200
150
100
50
0
-50
5
10
15
20
25
30
35
min
18.133
18.408
18.940
19.220
19.728
Possible deamidated IFB
Possible un-modified IFB

Figure 25

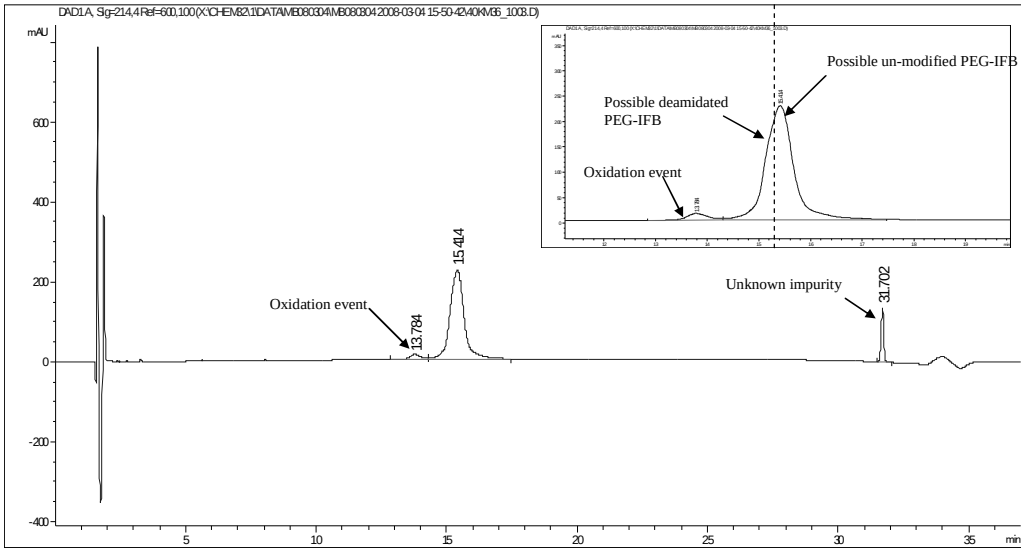

DAD1A, Sig=214,4 Ref=600,100 (X:\CHEM32\1\DATA\MB080304\MB080304 2008-03-04 15-50-42\40KM66_1003.D)
mAU
600
400
200
0
-200
-400
5
10
15
20
25
30
35
min
Oxidation event
13.784
15.414
Unknown impurity
31.702
Possible un-modified PEG-IFB
Possible deamidated
PEG-IFB
Oxidation event

Figure 26

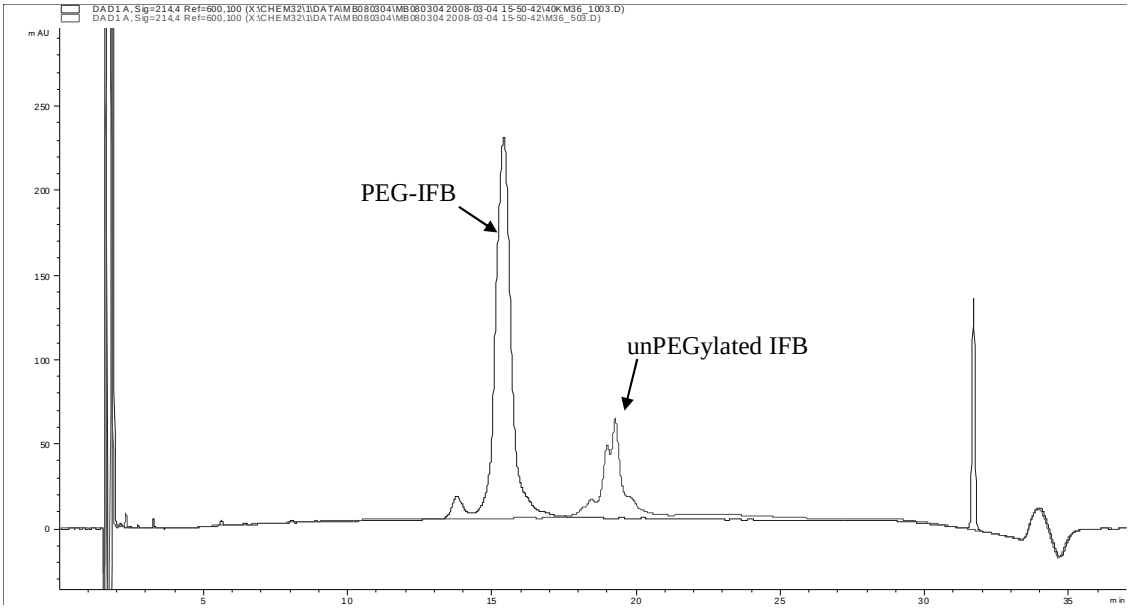

DAD1 A, Sig=214,4 Ref=600,100 (X:\CHEM32\1\DATA\MB080304\MB080304 2008-03-04 15-50-42\40KM36_1003.D)
DAD1 A, Sig=214,4 Ref=600,100 (X:\CHEM32\1\DATA\MB080304\MB080304 2008-03-04 15-50-42\M36_503.D)
m AU
250
200
150
100
50
0
PEG-IFB
unPEGylated IFB
5
10
15
20
25
30
35
min

Figure 27

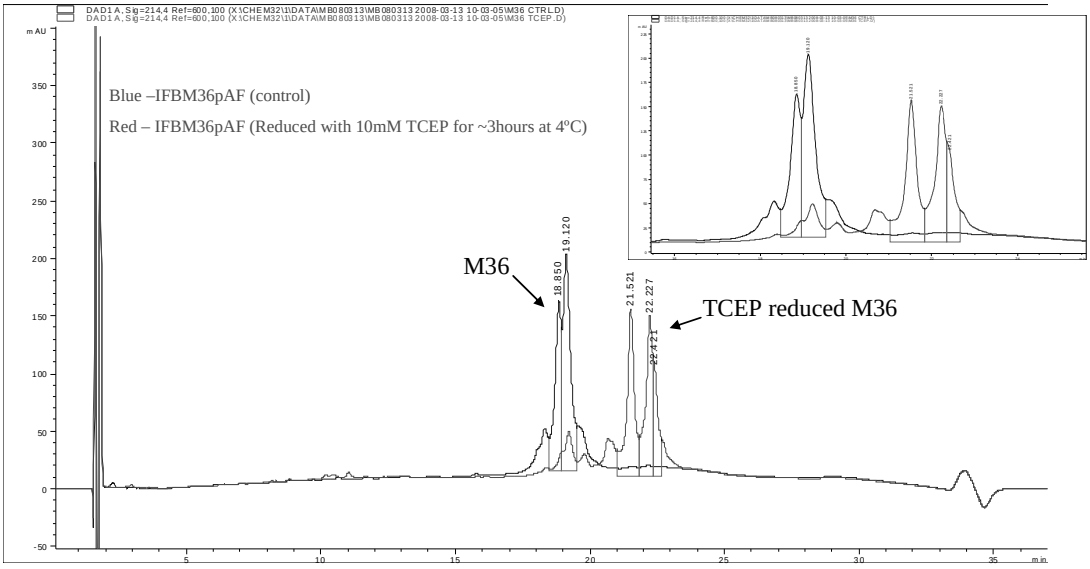
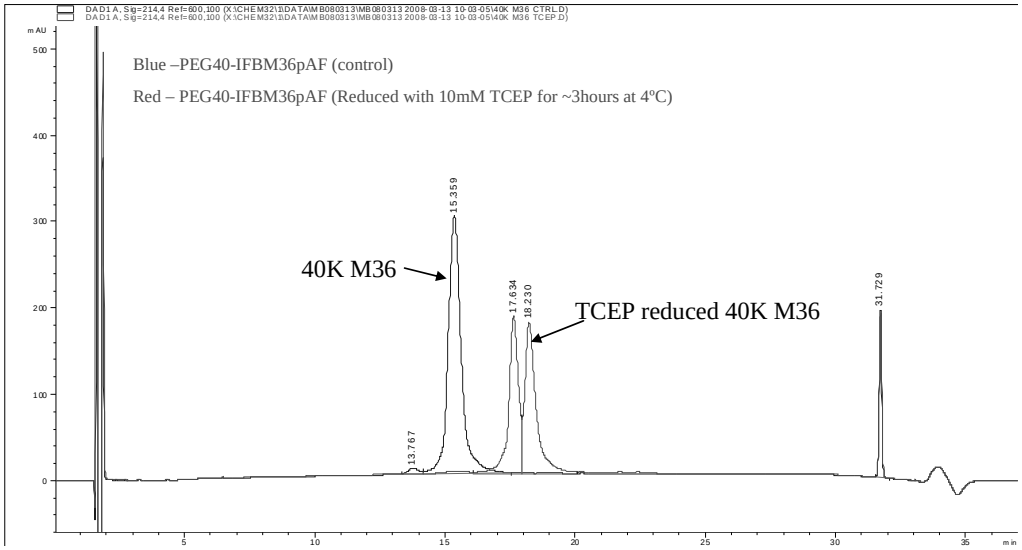

DAD1 A, Sig=214,4 Ref=600,100 (X:\CHEM32\1\DATA\MB080313\MB080313 2008-03-13 10-03-05\M36 CTRLD)
DAD1 A, Sig=214,4 Ref=600,100 (X:\CHEM32\1\DATA\MB080313\MB080313 2008-03-13 10-03-05\M36 TCEP.D)
mAU
Blue –IFBM36pAF (control)
Red – IFBM36pAF (Reduced with 10mM TCEP for ~3hours at 4ºC)
M36
TCEP reduced M36
18.850
19.120
21.521
22.227
min

Figure 28


DAD1 A, Sig=214,4 Ref=600,100 (X:\CHEM32\1\DATA\MB080313\MB080313 2008-03-13 10-03-05\40K M36 CTRL.D)
DAD1 A, Sig=214,4 Ref=600,100 (X:\CHEM32\1\DATA\MB080313\MB080313 2008-03-13 10-03-05\40K M36 TCEP.D)
mAU
Blue –PEG40-IFBM36pAF (control)
Red – PEG40-IFBM36pAF (Reduced with 10mM TCEP for ~3hours at 4ºC)
40K M36
TCEP reduced 40K M36
13.767
15.359
17.634
18.230
31.729
min