Supplementary Materials

Preventing the N-terminal processing of human interferon α-2b and its chimeric derivatives expressed in *Escherichia coli*

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7. Pseudotyped viral-particle generation
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Part A: Cloning, characterization of genes and encoded proteins

Section 1. Construction of chimeras and Met-Phe-derivatives of interferon (primer-pairs are in bold and plasmids bold-and-red)

Primary plasmids.

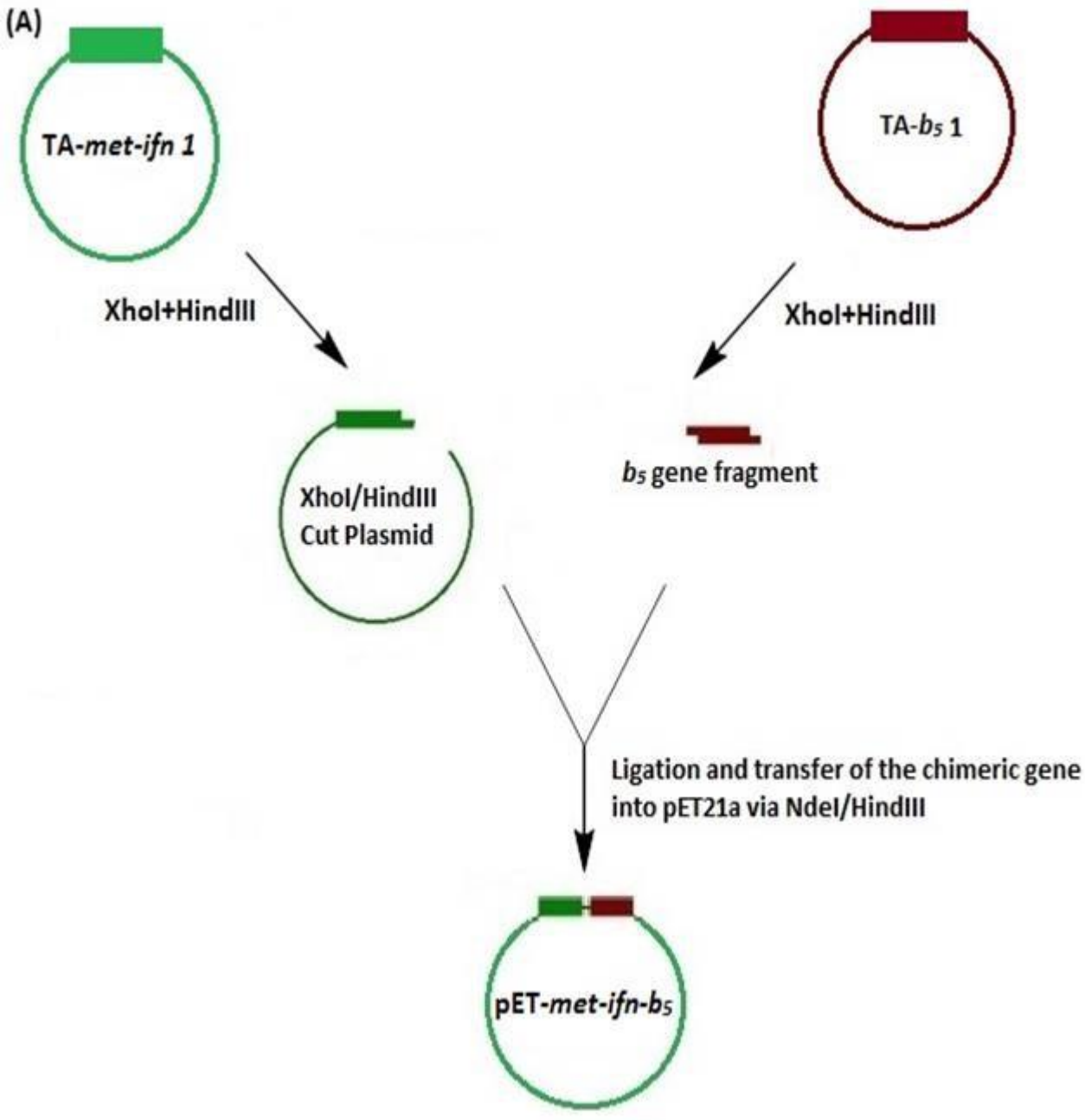
Polymerase Chain reaction was set up using reaction mixture described in Table S1. The gene for the soluble form of cytochrome b_5 was amplified, using the pair, T7-promoter primer/ChimR2 (Table S2), from a pT77 vector containing complete gene for cytochrome b_5 (protein with membrane anchoring domain). The latter template was a gift from Madam Monika Akhtar of Biological Sciences, University of Southampton, UK. The PCR product was subcloned into pET-21a to give **pET-*b_5*.** Using the latter as the template the b_5 gene was amplified with the primer pair ChimF2/ChimR2, which introduced a XhoI site upstream of the gene and HindIII downstream of the gene. The PCR product was then inserted into T/A vector (pTZ57R/T, Thermo Scientific®) to give **TA-*$b_5$1***. The complementary construct, **TA-*met-ifn 1***, in which interferon gene contained codons for N-terminal Met and its stop codon replaced by a XhoI site was constructed, using the primer pair ChimF1/ChimR1 and gene for interferon in **pET-21a** (**pET-*met-ifn*)**, as template. The latter plasmid has been described previously (Ahsan et al., 2014). The same plasmid was used as template to make another construct, **TA-m*et-ifn 2***, in which a XhoI site upstream of the interferon gene and HindIII site downstream of the gene was introduced using primer pair **Op-FwI/ Op-RvC**. **pET-*b_5*** was also used as template for the construction of **TA-*b_5 2***, in which b_5 gene was amplified using primer pair **T7-promoter primer/ Op-RvC**. In the resulting construct, T7-promoter primer retained the original NdeI site upstream of the gene and Op-RvC replaced the stop codon with XhoI restriction site downstream of the gene.

pET-*met-ifn-b_5-chimera* was constructed as follows: **AT-*b_5 1* was** digested with XhoI/ HindIII to produce the desired DNA fragment encoding b_5, ready to be ligated with the interferon gene. For the latter step, the plasmid **TA-*met-ifn 1*** was digested with XhoI and HindIII and into it ligated the cytochrome b_5 fragment. The chimeric fragment was then subcloned into pET-21a to give **pET-*met-ifn-b_5-chimera*** (Figure S1, A).

pET-*b_5-ifn-chimera* was constructed as follows: **TA-*met-ifn 2*** was digested with XhoI/ HindIII to produce the desired DNA fragment, m*et-ifn*, ready to be ligated with the cytochrome b_5 gene. For the latter step, the plasmid **TA- *b_5 2*** was digested with XhoI and HindIII and into it ligated the *Met-ifn* fragment. The chimeric fragment was then subcloned into **pET-21a** to give **pET-*b_5-ifn-chimera*** (Figure S1, B).

For **pET-*met-phe-ifn*** and **pET-*met-phe-ifn-b_5-chimera,*** the derivatives containing phenylalanine residue next to the first methionine, gene specific primers containing phenylalanine codon next to the ATG start codon were used. The *met-phe-ifn* gene was amplified with **Phe-F1/ Op-RvI** primer pair using **pET-*metifn*** as template. The *met-phe-ifn-b_5-chimera* was amplified with **Phe-F1/ChimR2** primer pair using **pET*met-ifn-b_5-chimera*** as template. These were then sub-cloned in pET-21a to give **pET-*met-phe-ifn*** and **pET-*met-phe-ifn-b_5***, respectively.

Section 2. Construction of chimeras



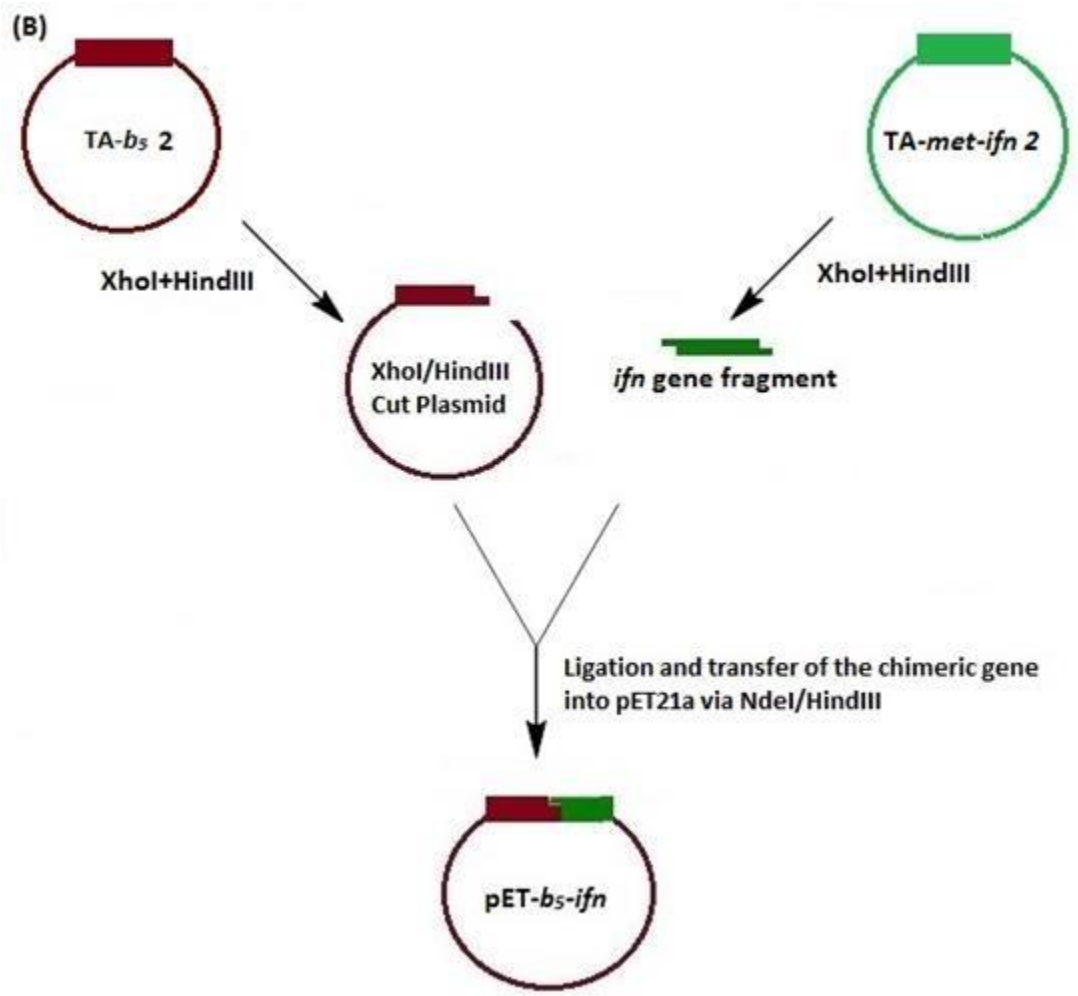


Figure S1. *A diagram summarizing the strategy for the construction of chimera involving human interferon α-2b (shown in green) and the soluble form of human cytochrome b5 (shown in red). A,* pET-*met-ifn-b5 (met-ifn is interferon gene containing codons for N-terminal methionine); B,* pET-*b5-ifn.*

Section 3. Polymerase chain reaction (PCR)

For all the PCR reactions used in this study, a 25 µl of reaction mixture per reaction was made. Each PCR reaction was set up with two replica reactions with two negative controls (one, without template, second without Taq polymerase). In each case the PCR master mix was prepared for 4 reactions with the following components:

Table S1. Reaction Mixture for PCR (Master Mix for 4 reactions (25 µl each)

Distilled H_2O	62 µl
10X Taq Buffer (+$MgCl_2$) 25 mM $MgCl_2$	10 µl (1X) 8 µl (2 mM)
2.5 mM dNTPs	10 µl (0.25 mM)
Primer I (100 µM) Primer II (100 µM)	2 µl (2 pmole/µl) 2 µl (2 pmole/µl)
DNA Template	5-10 ng (added after seperating control without template)
Taq Polymerase	1 µl per reaction (added to individual tubes)

Section 4. Primers and plasmids

Section 4. Primers and plasmids

Table S2. List of primers

Target Gene	Amplification of individual part	Primer Pair with Salient Features.
met-ifn-b_5-chimera	*ifn* gen	ChimF1: 5`-GAATTCC^ATATGTGTGATCTGCCTCAAACCCAC-3` ChimR1: 5`-CAGC^TCGAGTTCCTTACTTCTTAAAC-3`
	b_5 gene	ChimF2: 5`-GATC^TCGAGATGGCTGAACAGTCTGACGAAGC-3` ChimR2: 5`-AGTA^AGCTTTCAAGGTTCCGGAGGCTTGT-3`
b_5- ifn-chimera	b_5 gene	T7-promoter Primer : 5´- TAATACGACTCACTATAGGG- 3´ Op-RvC: 5`-C^TCGAGAGGTTCCGGAGGCTTGTTTA-3`
	ifn gene	Op-FwI: 5`-C^TCGAGTGTGACCTACCACAAACCCACAGCC-3` Op-RvI:5`-A^AGCTTTCATTACTCTTTAGATCTTAAACTTTCTTGC-3`
met-phe-ifn	*Met-phe-ifn*	Phe-F1: 5`-C^ATATGTTCTGTGACCTACCACAAACCCACAGCC-3` Op-RvI: 5`-A^AGCTTTCATTACTCTTTAGATCTTAAACTTTCTTGC-3`
met-phe-ifn-b_5-chimera	b_5 gene	Phe-F1: 5`-C^ATATGTTCTGTGACCTACCACAAACCCACAGCC-3` ChimR2: 5`-AGTA^AGCTTTCAAGGTTCCGGAGGCTTGT-3`

Key to the main features of the primers:

C^ATATG = NdeI restriction site
C^TCGAG = XhoI restriction site
A^AGCTT = HindIII restriction site
TTC = Codon introduced for Phenylalanine after initiator Methionine

Table S3 Description of engineered plasmid constructs.

Sr. No.	Description of the insert	Name of the construct
1	T/A plasmid with b_5-gene	TA-b_5*1*
2	T/A plasmid with *ifn* gene (with NdeI/XhoI restriction sites)	TA-*met-ifn 1*
3	T/A with b_5 gene (with XhoI/HindIII restriction sites)	TA-b_5*1*
4	T/A plasmid with *ifn* gene (with XhoI/HindIII restriction sites)	TA-m*et-ifn 2*
5	T/A plasmid with b_5 gene (with NdeI/XhoI restriction sites)	TA-b_5 *2*,
6	pET plasmid with b_5 gene	pET-b_5
7	pET plasmid with *met-ifn-*b_5*-chimera* gene	pET-*met-ifn-*b_5*chimera*
8	pET plasmid with b_5*-ifn-op-chimera* gene	pET-b_5*-ifn-chimera*
9	pET plasmid with *met-phe-ifn* gene	pET-*met-phe-ifn*
10	pET plasmid with *met-phe-ifn-*b_5*-chimera* gene	pET-*met-phe-ifn-*b_5*chimera*

***Section 5*. DNA sequences**

Figure S2. DNA sequences and encoded protein sequences for two of the key genes are shown below.

(a) Cytochrome b_5 (tail-less)

```
atggctgaacagtctgacgaagccgtgaagtactacaccctagaggagattcagaagcac
 M  A  E  Q  S  D  E  A  V  K  Y  Y  T  L  E  E  I  Q  K  H
aaccacagcaagagcacctggctgatcctgcaccacaaggtgtacgatttgaccaaattt
 N  H  S  K  S  T  W  L  I  L  H  H  K  V  Y  D  L  T  K  F
ctggaagagcatcctggtggggaagaagttttaagggaacaagctggaggtgacgctact
 L  E  E  H  P  G  G  E  E  V  L  R  E  Q  A  G  G  D  A  T
gagaactttgaggatgtcgggcactctacagatgccagggaaatgtccaaaacattcatc
 E  N  F  E  D  V  G  H  S  T  D  A  R  E  M  S  K  T  F  I
attggggagctccatccagatgacagaccaaagttaaacaagcctccggaaccttga
 I  G  E  L  H  P  D  D  R  P  K  L  N  K  P  P  E  P  -
```

Theoretical M_r (with N-terminal methionine cleaved) = 11137.4
Observed M_r (with N-terminal methionine cleaved) = 11137.6

(b) **Met-IFN-b_5-chimera** (**4** Figure 1). **Met-IFN** sequence, blue; **b_5** sequence, red; inter-gene linker, deep black.

```
atgtgtgatctgcctcaaacccacagcctgggtagcaggaggaccttgatgctcctggca
 M  C  D  L  P  Q  T  H  S  L  G  S  R  R  T  L  M  L  L  A
cagatgaggagaatctctcttttctcctgcttgaaggacagacatgactttggatttccc
 Q  M  R  R  I  S  L  F  S  C  L  K  D  R  H  D  F  G  F  P
caggaggagtttggcaaccagttccaaaaggctgaaaccatccctgtcctccatgagatg
 Q  E  E  F  G  N  Q  F  Q  K  A  E  T  I  P  V  L  H  E  M
atccagcagatcttcaatctcttcagcacaaaggactcatccgctgcttgggatgagacc
 I  Q  Q  I  F  N  L  F  S  T  K  D  S  S  A  A  W  D  E  T
ctcctagacaaattctacactgaactctaccagcagctgaatgacctggaagcctgtgtg
 L  L  D  K  F  Y  T  E  L  Y  Q  Q  L  N  D  L  E  A  C  V
atacaggggtgggggtgacagagactcccctgatgaaggaggactccattctggctgtg
 I  Q  G  V  G  V  T  E  T  P  L  M  K  E  D  S  I  L  A  V
aggaaatacttccaaagaatcactctctatctgaaagagaagaaatacagcccttgtgcc
 R  K  Y  F  Q  R  I  T  L  Y  L  K  E  K  K  Y  S  P  C  A
tgggaggttgtcagagcagaaatcatgagatcttttctttgtcaacaaacttgcaagaa
 W  E  V  V  R  A  E  I  M  R  S  F  S  L  S  T  N  L  Q  E
agtttaagaagtaaggaactcgagatggctgaacagtctgacgaagccgtgaagtactac
 S  L  R  S  K  E  L  E  M  A  E  Q  S  D  E  A  V  K  Y  Y
accctagaggagattcagaagcacaaccacagcaagagcacctggctgatcctgcaccac
 T  L  E  E  I  Q  K  H  N  H  S  K  S  T  W  L  I  L  H  H
aaggtgtacgatttgaccaaatttctggaagagcatcctggtggggaagaagttttaagg
 K  V  Y  D  L  T  K  F  L  E  E  H  P  G  G  E  E  V  L  R
gaacaagctggaggtgacgctactgagaactttgaggatgtcgggcactctacagatgcc
 E  Q  A  G  G  D  A  T  E  N  F  E  D  V  G  H  S  T  D  A
agggaaatgtccaaaacattcatcattggggagctccatccagatgacagaccaaagtta
 R  E  M  S  K  T  F  I  I  G  E  L  H  P  D  D  R  P  K  L
aacaagcctccggaaccttga
 N  K  P  P  E  P  -
```

Theoretical M_r (with two disulfide bridges) = 30889.0

Observed M_r (with two disulfide bridges) = 30889.2

Section 6. **Amino acid sequences of interferon derivatives (3-6, Figure 1) and of their tryptic peptides.**

(i) Data for Met-Phe-IFN (3 Figure 1):

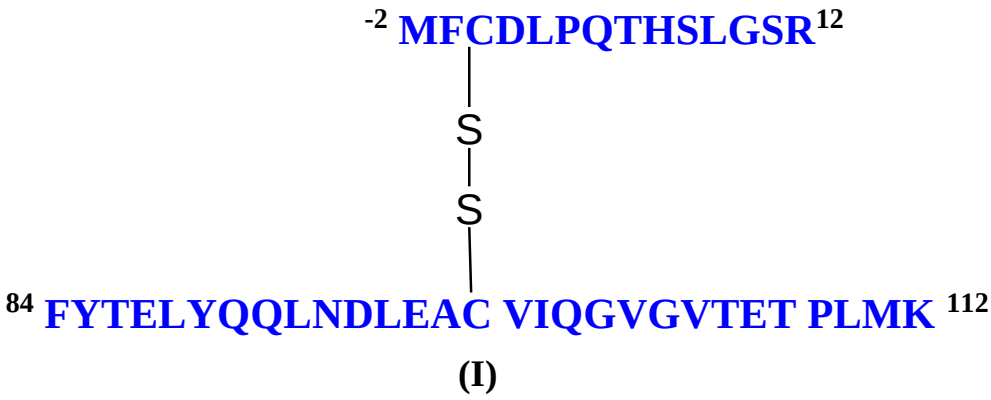
Amino acid sequence and M_r

MFCDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQE
EFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKF
YTELYQQLNDLEACVIQGVGVTETPLMKEDSILAVRKYFQRIT
LYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE.

Theoretical M_r (with two disulfide bridges) = 19543.4
Observed M_r (with two disulfide bridges) = 19542.7

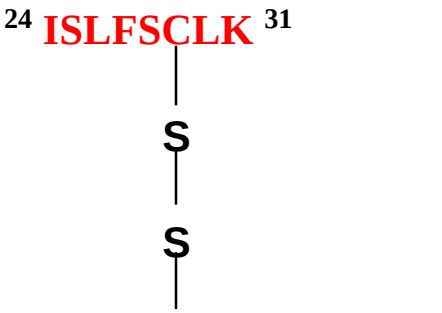
Table S4. Peptides from the tryptic digestion of Met-Phe-IFN showing monoisotopic masses as determined by ESI. The digestion was performed without reducing the disulphide bonds of the protein.

Peptide Name	Position	Peptide sequence	Theoretical Mass (a.m.u)	Observed Mass (a.m.u)	Difference
F1	(-)2-12	MFCDLPQTHSLGSR	1590.73	-	
F2	14-22	TLMLLAQMR	1075.59	1075.57	-0.02
F3	24-31	ISLFSCLK	909.5	-	
F4	34-49	HDFGFPQEEFGNQFQK	1953.86	1953.85	-0.01
F5	50-70	AETIPVLHEMIQQIFNLFSTK	2458.29	2458.25	-0.04
F6	71-83	DSSAAWDETLLDK	1449.66	1449.65	-0.01
F7	84-112	FYTELYQQLNDLEACVIQGVGVTETPLMK	3301.63	-	
F8	113-120	EDSILAVR	901.49	901.48	-0.01
F9	122-125	YFQR	612.3	-	-
F10	126-131	ITLYLK	749.47	749.46	-0.01
F11	135-144	YSPCAWEVVR	1208.56	-	
F12	145-149	AEIMR	618.32	618.47	0.15
F13	150-162	SFSLSTNLQESLR	1480.75	1480.74	-0.02
I	F1+F7	Peptides linked with disulphide bond	4890.35	4890.26	-0.1
II	F3+F11	Peptides linked with disulphide bond	2116.06	2116.02	-0.04



(I)

Monoisotopic mass (Theo: 4890.35; Obs:4890.26)

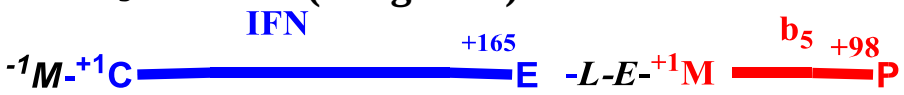


(II)

Monoisotopic mass (Theo: 2116.06; Obs:2116.02)

Figure S3a. Structures and monoisotopic masses (determined by ESI) of disulphide-bond containing peptides (I)) and (II) of Met-Phe-IFN.

(ii) Data for Met-IFN-b_5-chimera (4 Figure 1):



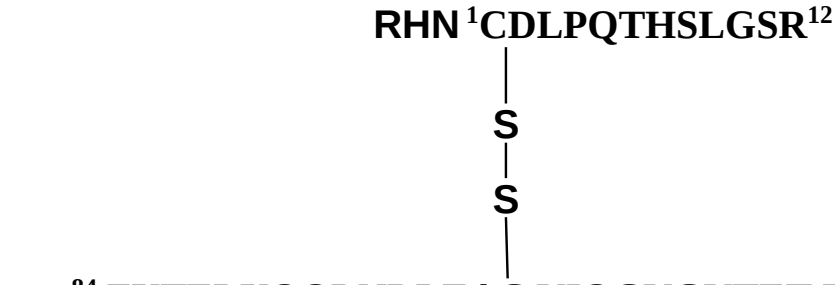
Amino acid sequence and M_r

MCDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQK
AETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQ
GVGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFS
LSTNLQESLRSKELEMAEQSDEAVKYYTLEEIQKHNHSKSTWLILHHKVY
DLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTD AREMSKTFII
GELHPDDRPK LNKPPEP

Theoretical M_r (with two disulfide bridges) = 30889.0
Observed M_r (with two disulfide bridges) = 30889.2

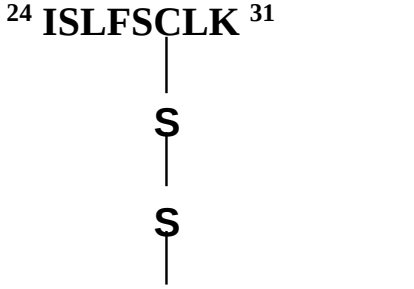
Table S5. Tryptic digestion of Met-IFN-b_5-chimera showing average values of $[M+H]^+$ ions. The digestion was performed without reducing the disulphide bonds of the protein. Masses below the structures are obtained by subtracting 1 from the $[M+H]^+$ ions in the table.

Peptide Name	Position	Peptide Sequence	$[M+H]^+$ ion mass	d $[M+H]^+$	Difference
PC0	1-12	CDLPQTHSLGSR	1314.4509	-	
PC1	(-)1-12	MCDLPQTHSLGSR	1445.6435	-	
PC2	14-22	TLMLLAQMR	1077.3808	1076.49	-0.8908
PC3	24-31	ISLFSCLK	911.1394	-	
PC4	34-49	HDFGFPQEEFGNQFQK	1956.0729	1955.597	-0.4759
PC5	50-70	AETIPVLHEMIQQIFNLFSTK	2460.886	-	
PC6	71-83	DSSAAWDETLLDK	1451.5218	-	
PC7	84-112	FYTELYQQLNDLEACVIQGVGVTETPLMK	3304.7898	-	
PC8	113-120	EDSILAVR	903.0153	-	
PC9	122-125	YFQR	613.6861	-	
PC10	126-131	ITLYLK	750.9487	-	
PC11	135-144	YSPCAWEVVR	1210.3852	1209.702	-0.6832
PC12	145-149	AEIMR	619.7491	-	
PC13	150-162	SFSLSTNLQESLR	1482.6255	-	
PC14	165-177	ELEMAEQSDEAVK	1479.5911	-	
PC15	178-186	YYTLEEIQK	1187.327	-	
PC16	187-191	HNHSK	622.6536	-	
PC17	192-200	STWLILHHK	1135.3463	-	
PC18	201-206	VYDLTK	738.8511	-	
PC19	207-219	FLEEHPGGEEVLR	1512.6544	1512.216	-0.4384
PC20	220-240	EQAGGDATENFEDVGHSTDAR	2207.1804	-	
PC21	245-258	TFIIGELHPDDRPK	1638.8559	1638.489	-0.3669
PC22	259-265	LNKPPEP	794.9182	-	
PC23	PC1+PC7		4747.4333	-	
III a	PC0+PC7	Fragments linked by disulphide bond	4616.2407	4616.494	0.2533
III b	(PC0+PC7)+42	Acetylated fragments linked by disulphide bond	4658.2407	4658.184	-0.0567
II	PC3+PC11	Fragments linked by disulphide bond	2118.5246	2118.061	-0.4636



(IIIa, R=H) ; **Average MALDI mass (Cal.:4615.2; Obs.: 4615.5)**

(IIIb, R= CH_3-CO); **Average MALDI mass (Cal.:4657.2; Obs.: 4657.2)**



(II)

Average MALDI mass (Cal.: 2117.5; Obs.:2117.3)

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Figure S3b. Structures and average MALDI masses of disulphide-bond containing peptides IIIa, IIIb and II of Met-IFN-b_5-chimera.

(iii) Data for Met-Phe-IFN-b_5-chimera (5 Figure 1):

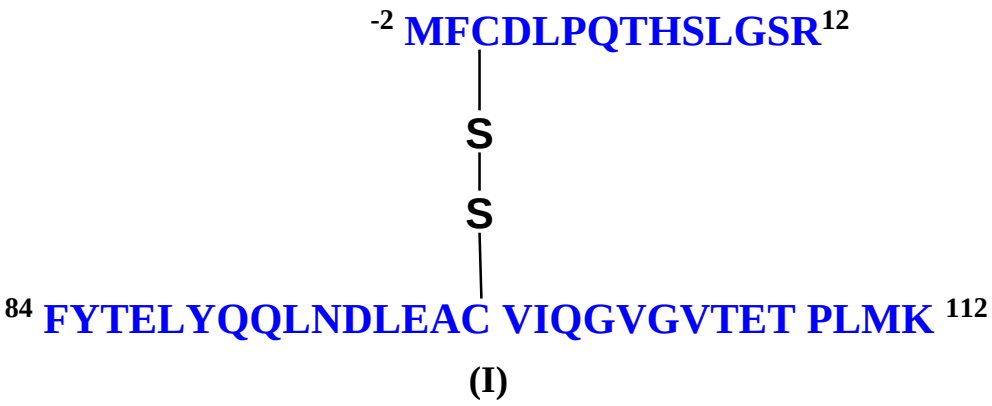
Amino acid sequence and M_r

MFCDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQF
QKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLE
ACVIQGVGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVR
AEIMRSFSLSTNLQESLRSKELEMAEQSDEAVKYYTLEEIQKHNHSKST
WLILHHKVYDLTKFLEEHPGGEEVLREQAGGDATENFEDVGHSTDARE
MSKTFIIGELHPDDRPKLNKPPEP

Theoretical M_r (with two disulfide bridges) = 31036.1
Observed M_r (with two disulfide bridges) =31036.2

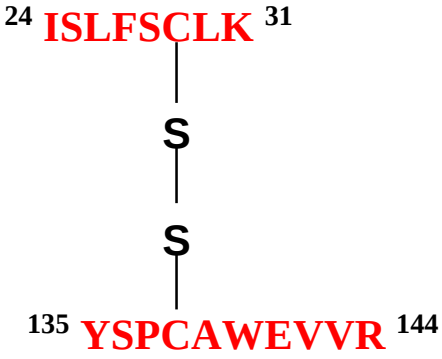
Table S6. Tryptic digestion of Met-Phe-IFN-b_5-chimera showing average values of $[M+H]^+$ ions. The digestion was performed without reducing the disulphide bonds of the protein. Masses below the structures are obtained by subtracting 1 from the $[M+H]^+$ ions in the Table.

Peptide Name	Position	Peptide sequence	Theoretical Mass $[M+H]^+$	Observed Mass $[M+H]^+$	Difference
FC1	(-)2-12	MFCDLPQTHSLGSR	1592.83	-	
FC2	14-22	TLMLLAQMR	1077.39	1076.58	-0.8
FC3	24-31	ISLFSCLK	911.15	-	-
FC4	34-49	HDFGFPQEEFGNQFQK	1956.08	1955.52	-0.56
FC5	50-70	AETIPVLHEMIQQIFNLFSTK	2460.89	2460.51	-0.38
FC6	71-83	DSSAAWDETLLDK	1451.53	-	-
FC7	84-112	FYTELYQQLNDLEACVIQGVGVTETPLMK	3304.79	-	-
FC8	113-120	EDSILAVR	903.02	-	-
FC9	122-125	YFQR	613.69	-	-
FC10	126-131	ITLYLK	750.95	-	-
FC11	135-144	YSPCAWEVVR	1210.39	-	-
FC12	145-149	AEIMR	619.75	-	-
FC13	150-162	SFSLSTNLQESLR	1482.63	-	-
FC14	165-177	ELEMAEQSDEAVK	1479.59	-	-
FC15	178-186	YYTLEEIQK	1187.33	-	-
FC16	187-191	HNHSK	622.66	-	-
FC17	192-200	STWLILHHK	1135.35	-	-
FC18	201-206	VYDLTK	738.85	-	-
FC19	207-219	FLEEHPGGEEVLR	1512.66	1512.23	-0.42
FC20	220-240	EQAGGDATENFEDVGHSTDAR	2207.18	2207.04	-0.14
FC21	245-258	TFIIGELHPDDRPK	1638.86	1638.52	-0.34
FC22	259-265	LNKPPEP	794.92	-	-
I	FC1+FC7	Peptides linked with disulphide bond	4894.62	4894.61	-0.01
II	FC3+FC11	Peptides linked with disulphide bond	2118.53	2118.5	-0.03



(I)

Average MALDI mass (Cal.: 4893.6; Obs.: 4893.6)

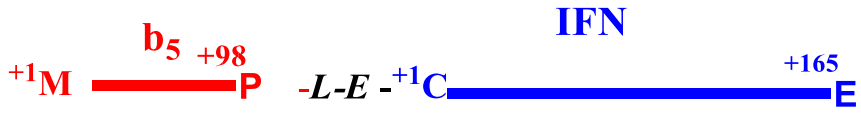


(II)

Average MALDI mass (Cal.: 2117.5; Obs.: 2117.5)

Figure S3c. Structures and average MALDI masses of disulphide bond containing peptides I and II of Met-Phe-IFN-b_5-chimera.

(iv) Data for b_5-IFN-chimera (6 Figure 1):



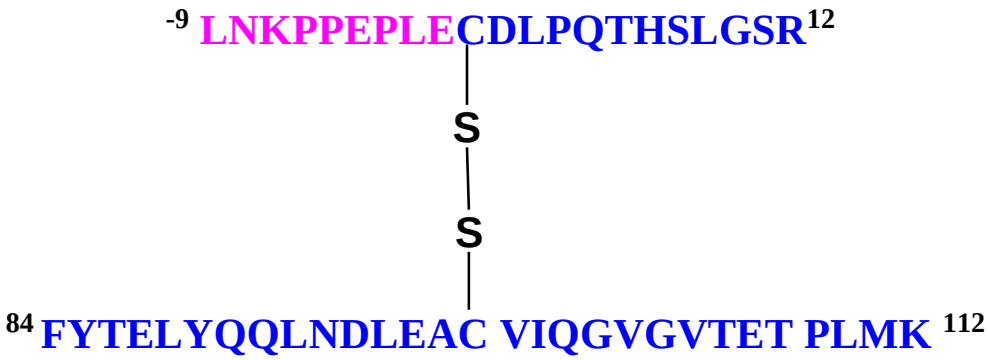
Amino acid sequence and M_r

MAEQSDEAVKYYTLEEIQKHNHSKSTWLILHHKVYDLTKFLEEHPGGEEV
LREQAGGDATENFEDVGHSTDAREMSKTFIIGELHPDDRPKLNKPPEPLEC
DLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAET
IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGV
GVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLS
TNLQESLRSKE

Theoretical M_r (with two disulfide bridges) = 30757.8
Observed M_r (with two disulfide bridges) = 30757.8

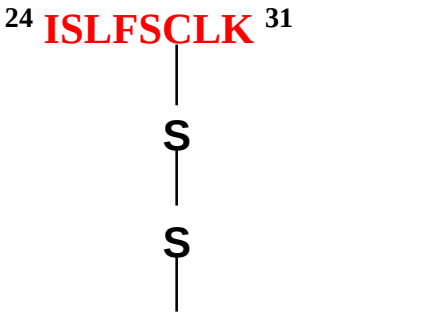
Table S7. Tryptic digestion of b_5-IFN-chimera showing average values of $[M+H]^+$ ions. The digestion was performed without reducing the disulphide bonds of the protein. Masses below the structures are obtained by subtracting 1 from the $[M+H]^+$ ions in the Table.

Peptide Name	Position	Peptide Sequence	[M+H]+	Observed [M+H]+	Difference
PO001	(-)100-(-)91	MAEQSDEAVK	1108.2	-	-
PO01	(-)99-(-)91	AEQSDEAVK	977.008	-	-
PO1	(-)98-(-)91	EQSDEAVK	905.929	-	-
PO2	(-)90-(-)82	YYTLEEIQK	1187.33	-	-
PO3	(-)81-(77)	HNHSK	622.654	-	-
PO4	(-)76-(-)68	STWLILHHK	1135.35	-	-
PO5	(-)67-62	VYDLTK	738.851	-	-
PO6	(-)61-49	FLEEHPGGEEVLR	1512.65	1512.315	-0.3394
PO7	(-)48-(-)28	EQAGGDATENFEDVGHSTDAR	2207.18	-	-
PO8	(-)23-(-)10	TFIIGELHPDDRPK	1638.86	1638.561	-0.2949
PO9	(-9)-12	LNKPPEPLECDLPQTHSLGSR	2332.63	-	-
PO10	14-22	TLMLLAQMR	1077.38	-	-
PO11	24-31	ISLFSCLK	911.139	-	-
PO12	34-49	HDFGFPQEEFGNQFQK	1956.07	1955.516	-0.5569
PO13	50-70	AETIPVLHEMIQQIFNLFSTK	2460.89	-	-
PO14	71-83	DSSAAWDETLLDK	1451.52	-	-
PO15	84-112	FYTELYQQLNDLEACVIQGVGVTETPLMK	3304.79	-	-
PO16	113-120	EDSILAVR	903.015	-	-
PO17	122-125	YFQR	613.686	-	-
PO18	126-131	ITLYLK	750.949	-	-
PO19	135-144	YSPCAWEVVR	1210.39	-	-
PO20	145-149	AEIMR	619.749	-	-
PO21	150-162	SFSLSTNLQESLR	1482.63	-	-
IV	PO9+PO15	Fragments linked by disulphide bond	5634.42	5634.429	0.01
II	PO11+PO19	Fragments linked by disulphide bond	2118.53	2118.095	-0.4296



(IV)

Average MALDI mass (Cal.: 5633.4; Obs:5633.4)



(II)

Average MALDI mass (Cal.: 2117.5 ; Obs.:2117.5)

Figure S3d. Structures and average MALDI masses of disulphide-bond containing peptides IV and II. The sequences in pink are from b_5 and the linker region b_5-IFN-chimera.

Section 7. Pseudotyped viral particle generation and determination of antiviral activity.

Pseudotyped viral particle

HEK-293T cells, 90 % confluent, grown in DMEM medium supplemented with 15% fetal bovine serum (FBS) from a 10 cm culture dish, were split 1/4 to dishes of similar size and incubated overnight at 37 °C with 10% CO_2 and 90% humidity. The following viral component plasmids and transfection reagents were mixed together and then added drop-wise:

I. 15 µl of TE buffer containing
   a. 1 µg of pMDG,
   b. 1 µg of p8.91 and
   c. 1.5 µg of pCSGW
II. 200 µl of Opti-MEM®
III. 10 µl of Fugene®)

Supernatant was collected 48 hours post transfection. Medium was replenished to the cells and incubated for another 24 hours. A new lot of supernatant was harvested next day (72 hours post transfection). The virus titer in supernatants containing packaged VSV-G pseudotyped HIV-1 vector was measured as described below.

Determination of VSV-G Pseudotyped HIV-1 vector infectious units

The number of infectious units per ml of the harvested virus solution was determined by infecting human monocytic leukemia cell line (Thp-1) with three-fold serially diluted 48 and 72 hours post transfection supernatants. Target cells expressing enhanced green fluorescent protein (eGFP) were the indicators of successful infection. Thp-1 cells were seeded, 10^5 per well in a six well plate, in RPMI-1640 medium containing 10% FBS and 1 µL of polybrene solution (Stock solution, 10 mg/ml) per ml of the medium. The above two supernatants, containing viral particles, were threefold serially diluted from 666 µL to 2.7 µL (see Table S7) and added to the respective wells (final volume in each cell was made upto 2ml). Cultures were incubated at 37 °C, 5% CO_2 for 72 hours. Cells were harvested 72 hours post infection and fixed with 4% para-formaldehyde solution in phosphate buffer saline (PBS). The latter suspensions (50-100 µL) were injected into an Accuri C6 flow cytometer to determine the side scattering height fluorescence levels (SSC-

H) in FL1 channel (510-nm/530-nm emission detection) setting with a gated sample size of 10,000 cells. A representative FACS (fluorescence activated cell sorting) plot is shown in Figure . Panel A represents total population of counted cells while panel B represents distribution of fluorescent (FL1-H > 10^4) and non-fluorescent cells. 23% of 10000 counted cells were fluorescent, hence GFP positive, falling in gated area P2 (Panel B, Figure S4. This indicated that 23% of the cells had become infected allowing calculation of the titer of the virus using the following formula.

$$\frac{(\%\text{age GFP positive cells}/100) \times \text{No. of cells transduced}}{\text{Volume of virus (ml)}}$$

The average titer in units/ml for both supernatants was found to be 0.7×10^6. These supernatants were used in the subsequent study of antiviral activity assays for recombinant proteins produced in this work and is descried in main body of the paper.

Met-IFN-b_5-chimera, **b_5-IFN-chimera**, **Met-Phe-IFN** and **Met-Phe-IFN-$b_5$chimera.**

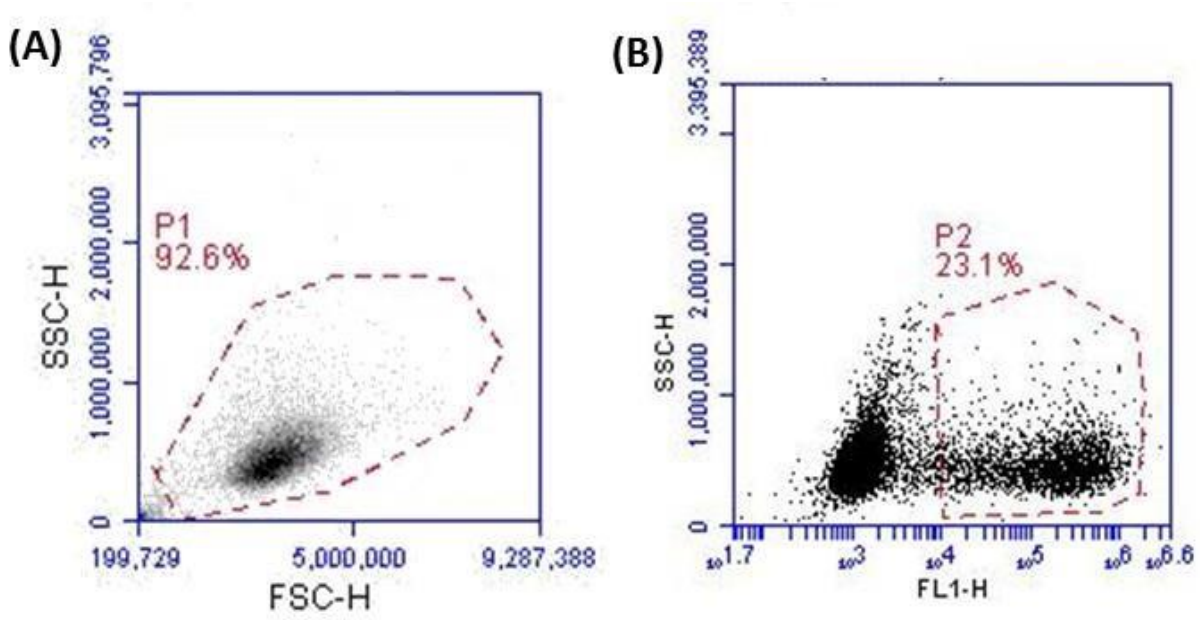


Figure S4 FACS analysis plot of an experiment.

Panel (A) shows the total population of cells counted with gated area P1 containing 10000 cells and Panel (B) shows the distribution of fluorescent (eGFP positive cells gated in P2) and non-fluorescent cells, in the gated population P1 from (A).

Table S8 Titration of infectious unit in packaged pseudotyped HIV-1 vector

In each case the final volume was made to 2 ml with the medium above.

Volume of viral vector supernatant (µL)	Supernatant 48 h post transfection (%age Infection)	Supernatant 72 h post transfection (%age Infection)
666	93.16%	83.08%
222	73.87%	83.98%
74	38.15%	42.03%
24.6	16.37%	17.36%
8.2	5.49%	6.17%
2.7	2.14%	1.88%

Infectivity units/ml = 0.7×10^6

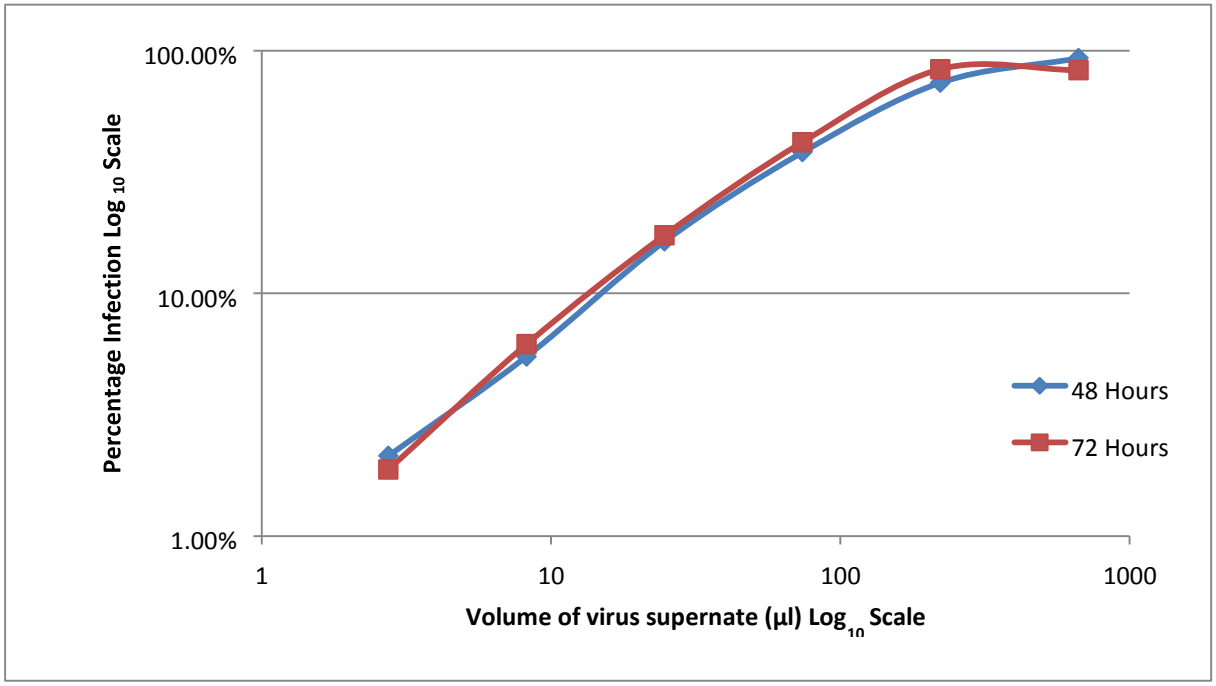


Figure S5 VSV-G pseudotyped HIV-1 vector titration in THP-1 cell line. Virus containing supernatants harvested 48 hours and 72 hours post transfection. The infectious units produced in both cases are very similar.