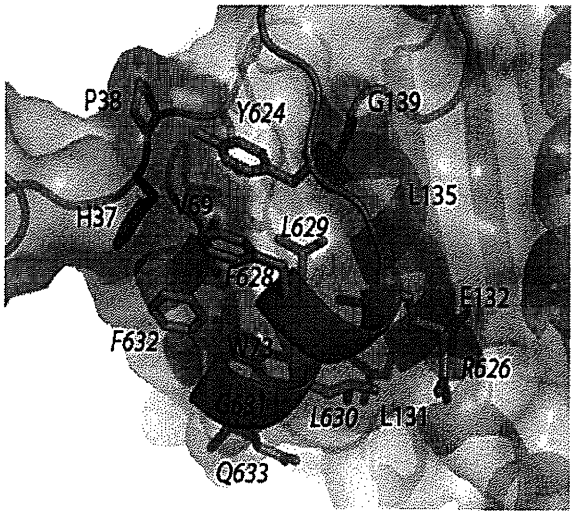
A



B

		624 629 634
eIF4G1		KKRYDREFLLGFQF-NH_2
Tr1		KKRYDREFLL-NH_2
Tr2		YDREFLLGFQF-NH_2
Tr3		YDREFLLGFQ-NH_2
Control		ADREFLLGFQF-NH_2
Tr2_AIB1		YXREFLLGFQF-NH_2
Tr2_AIB2		YDRXFLLGFQF-NH_2
Tr2_AIB3		YDREFLLXFQF-NH_2
Tr2_AIB4		YDREFLLGFXF-NH_2
Tr2_AIB3_ALA		YDREFLLAFQF-NH_2
Tr2_AIB3_A3C		YDREFLL3FQF-NH_2
Tr2_AIB3_A4C		YDREFLL4FQF-NH_2
Tr2_AIB3_A5C		YDREFLL5FQF-NH_2
Tr2_ALA_A5C1		YAREFLL5FQF-NH_2
Tr2_ALA_A5C2		YDAEFLL5FQF-NH_2
Tr2_ALA_A5C3		YDRAFLL5FQF-NH_2
Tr2_ALA_A5C4		YDREALL5FQF-NH_2
Tr2_ALA_A5C5		YDREFLL5AQF-NH_2
Tr2_ALA_A5C6		YDREFLL5FAF-NH_2
Tr2_ALA_A5C7		YDREFLL5FQA-NH_2
Tr2_me5_1		YDRE*LL5FQF-NH_2
Tr2_me5_2		YDREFLL5*QF-NH_2
Tr2_me5_3		YDREFLL5FQ*-NH_2
eIF4G1_me5		KKRYDRE*LL5FQF-NH_2
TAT	YGRKKRRQRRR-NH_2	
TAT_eIF4G1	YGRKKRRQRRRGTKKRYDREFLLGFQF-NH_2	
TAT_eIF4G1_Cntrl	YGRKKRRQRRRGTKKRADREFAAGFQF-NH_2	
TAT_eIF4G1_me5	YGRKKRRQRRRGTKKRYDRE*LL5FQF-NH_2	
TAT_eIF4G1_me5_Cntrl	YGRKKRRQRRRGTKKRADRE*AA5FQF-NH_2	
GT_eI4FG1		GTKKRYDREFLLGFQF-NH_2
GT_eI4FG1_Cntrl		GTKKRADREFAAGFQF-NH_2
GT_eIF4G1_me5		GTKKRYDRE*LL5FQF-NH_2
GT_eIF4G1_me5_Cntrl		GTKKRADRE*AA5FQF-NH_2
Conserved Motif		YXXXXLΦ

Figure 1

Minimal Motif	ΔT (°C)	Estimated K_d (nM)
eIF4G1	8.7	470
Tr1	1.7*	50160
Tr2	4.2	5960
Tr3	2.25*	35210
Control	0.65*	n/a
AIB scan for sites of helix stabilization		
Tr2_AIB1	1.32	29840
Tr2_AIB2	-	n/a
Tr2_AIB3	5.42	3050
Tr2_AIB4	1.52	25080
Replacement of Glycine by ALA and amino-cycloalkane-1-carboxylic acid derivatives		
Tr2_AIB3_ALA	5.60	2550
Tr2_AIB3_A3C	5.67	2500
Tr2_AIB3_A4C	5.80	2350
Tr2_AIB3_A5C	6.60	1450
Alanine scan of A5C substituted eIF4GI peptide.		
Tr2_Ala_A5C1	5.02	760
Tr2_Ala_A5C2	2.69	14300
Tr2_Ala_A5C3	4.22	1220
Tr2_Ala_A5C4	3.72	8090
Tr2_Ala_A5C5	3.65	8400
Tr2_Ala_A5C6	6.72	1370
Tr2_Ala_A5C7	5.48	2900
CA methlylation of Phe residue 628 dramatically increases the Affinity of Derivative Peptide		
Tr2_me5_1	8.8	360
Tr2_me5_2	NA	n/a
Tr2_me5_3	5.8	2400
Re-addition of the N-terminal to Residues 624-634 generates a High Affinity eIF4GI peptide derivative		
eIF4G1_me5	14.6	7

A

Peptides	K_d (nM)	ΔH (kCal $mol^{-1}K^{-1}$)	-TΔS (kCal $mol^{-1}K^{-1}$)
Tr2	1014.20±65.83	-10.95±0.13	-2.91
Tr2_me5_1	145.99±22.81	-16.07±0.2	-6.89
eIF4G1	147.93±22.81	-9.7±0.05	-0.58
eIF4G1_me5	9.43±2.57	-14.45±0.11	-3.69

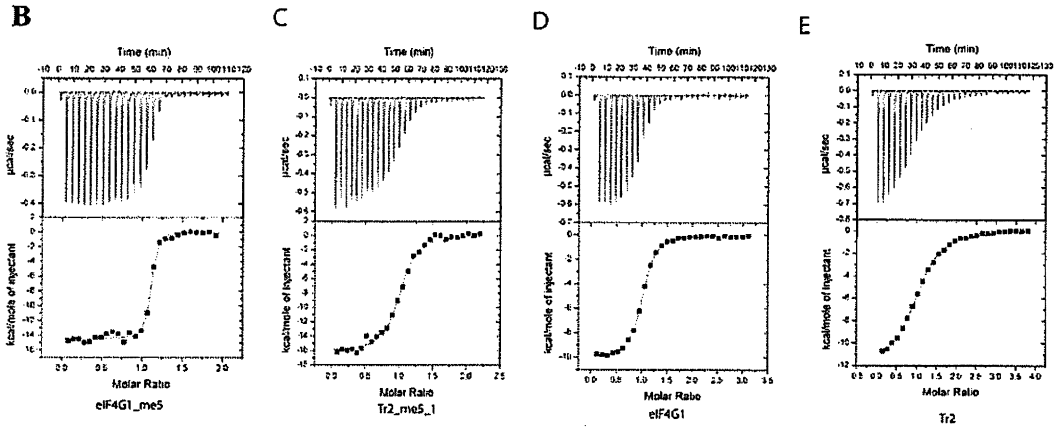


Figure 3

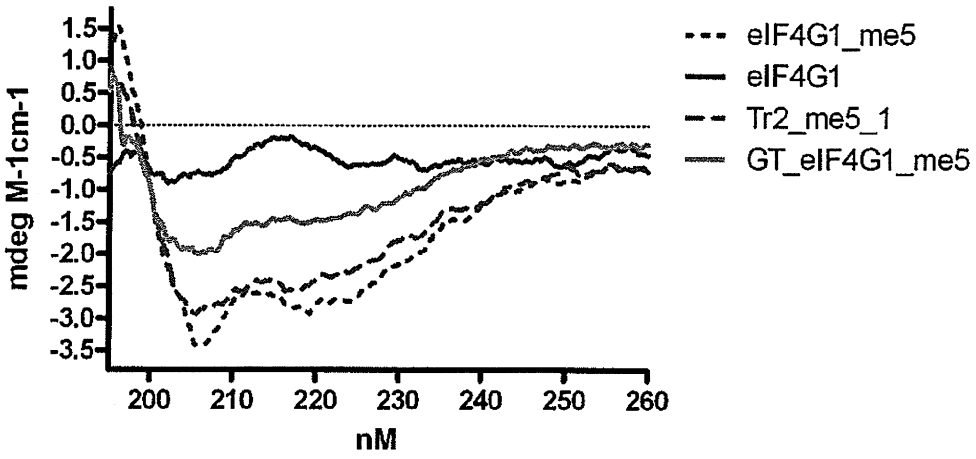


Figure 4A

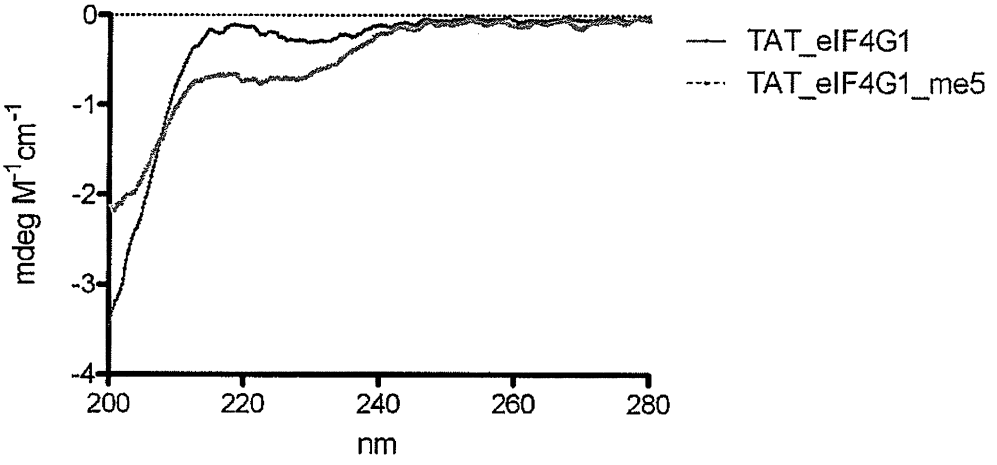


Figure 4B

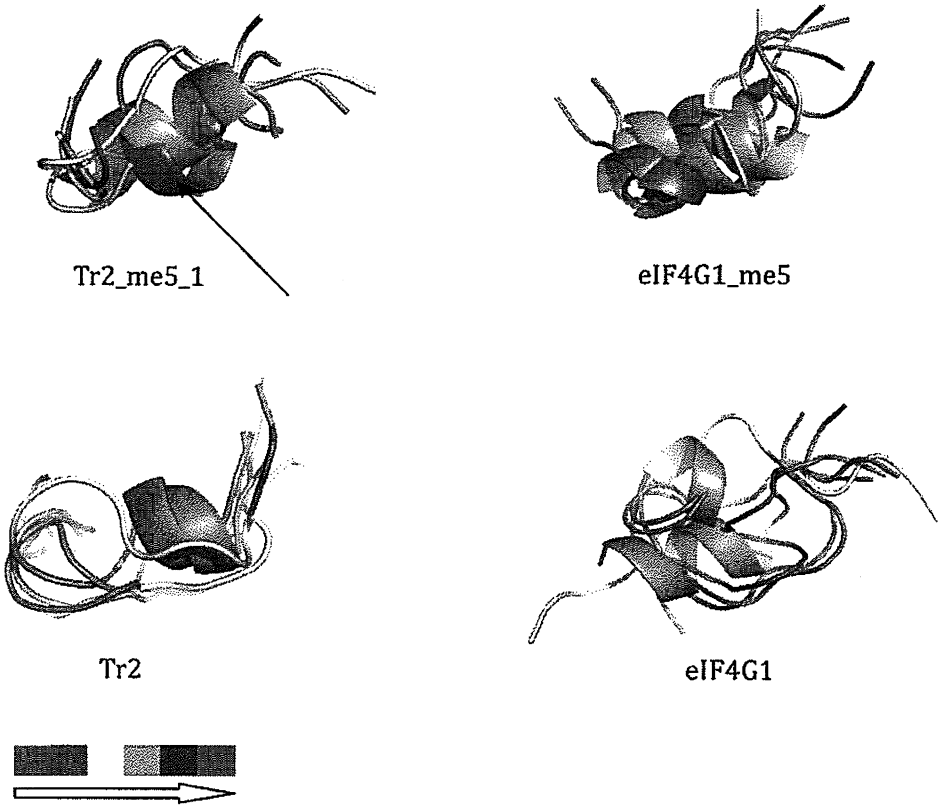


Figure 5

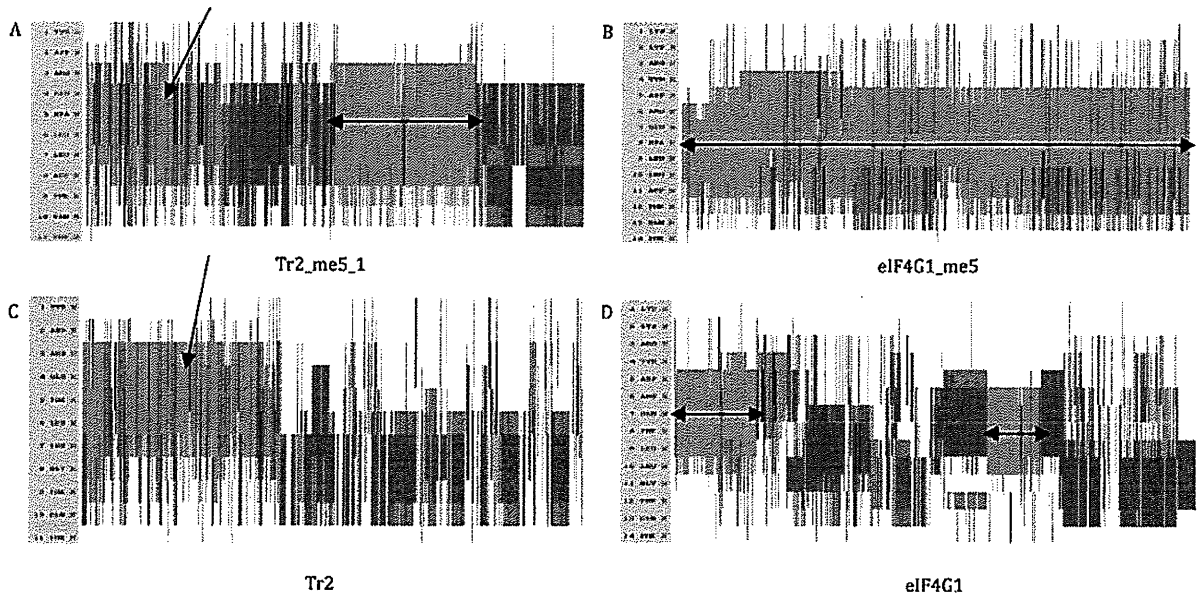


Figure 6

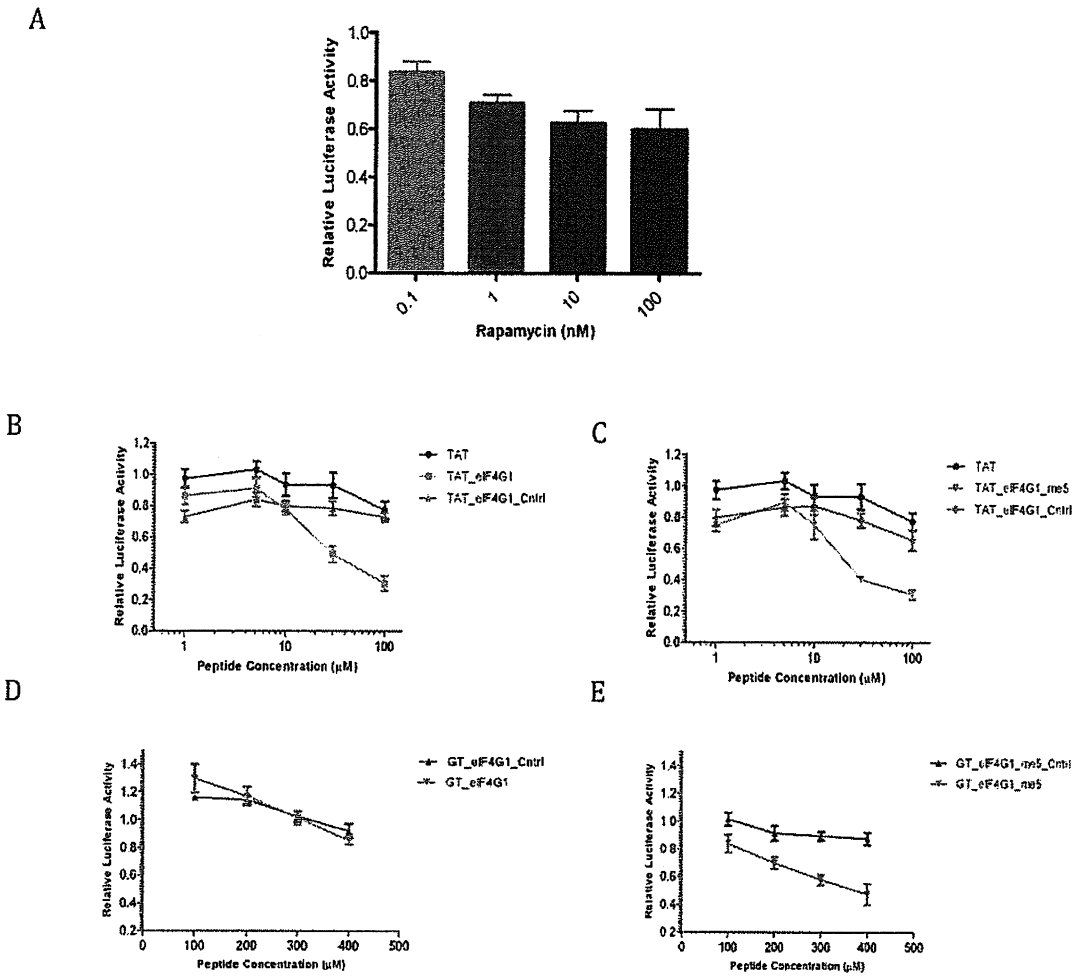

A
Relative Luciferase Activity
1.0
0.8
0.6
0.4
0.2
0.0
0.1
1
10
100
Rapamycin (nM)
B
Relative Luciferase Activity
Peptide Concentration (μM)
TAT
TAT_eIF4G1
TAT_eIF4G1_Cntrl
C
Relative Luciferase Activity
Peptide Concentration (μM)
TAT
TAT_eIF4G1_rre5
TAT_eIF4G1_Cntrl
D
Relative Luciferase Activity
Peptide Concentration (μM)
GT_eIF4G1_Cntrl
GT_eIF4G1
E
Relative Luciferase Activity
Peptide Concentration (μM)
GT_eIF4G1_rre5_Cntrl
GT_eIF4G1_rre5

Figure 7

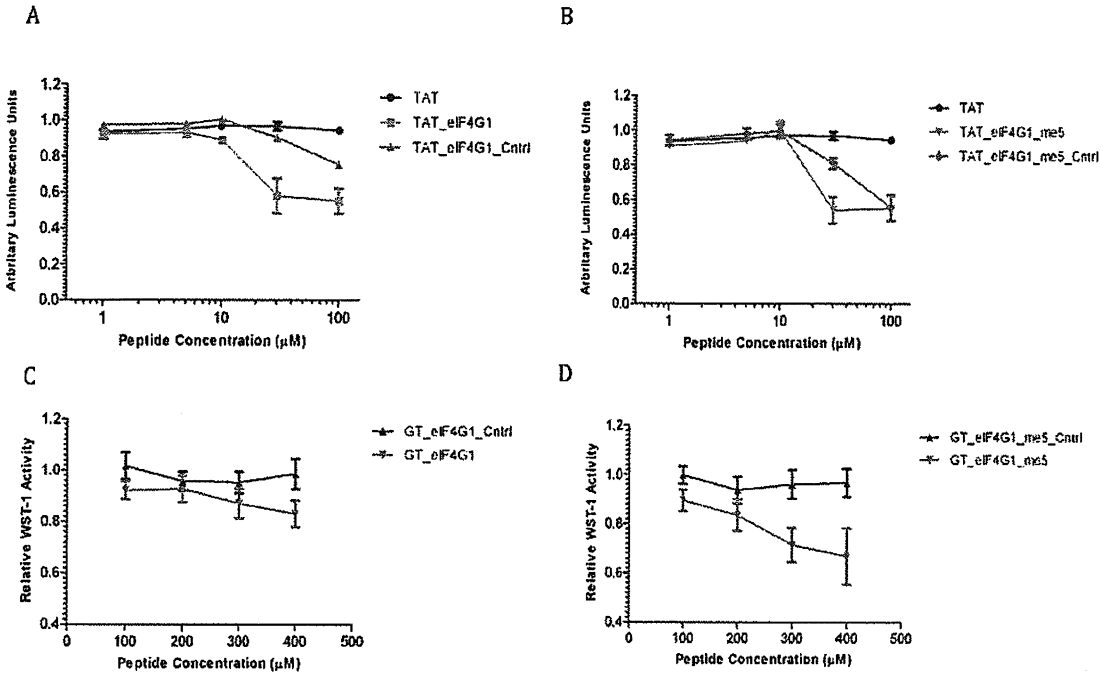

A
B
C
D
Arbitrary Luminescence Units
Peptide Concentration (μM)
TAT
TAT_eIF4G1
TAT_eIF4G1_Cntrl
TAT_eIF4G1_me5
TAT_eIF4G1_me5_Cntrl
Relative WST-1 Activity
GT_eIF4G1_Cntrl
GT_eIF4G1
GT_eIF4G1_me5_Cntrl
GT_eIF4G1_me5

Figure 8

	DMSO 0.1%	GT_eIF4G1_me5 (400μM)	GT_eIF4G1_me5_Cntrl (400μM)
G1 (%)	50.5 ±2.6	47.5±0.9 $^{(p=0.5493)}$	46.1±2.6$^{(p=0.3156)}$
S (%)	8.2± 0.9	2.2±0.1$^{(p=0.0065)}$	9.0±0.8$^{(p=0.4466)}$
G2 (%)	19.8±0.4	16.9±1.1$^{(p=0.0606)}$	27.2±3.6$^{(p=0.1034)}$
Sub G1 (%)	15.6±5.2	25.9±2.0$^{(p=0.0261)}$	11.1±2.8$^{(p=0.3967)}$

Figure 9

	TAT	**PBS Mock**
G1 (%)	40.1±0.6$^{(p=0.0013)}$	35.3±0.8
S (%)	16.8±1.12$^{(p=0.2077)}$	15.5±1.1
G2 (%)	13.3±1.3$^{(p=0.0017)}$	21.4±1.4
Sub G1 (%)	24.0±0.9$^{(p=0.1402)}$	21.8±1.9
	TAT_eIF4G1	**TAT_eIF4G1_Cntrl**
G1 (%)	34.7±2.8$^{(p=0.1793)}$	38.6±3.0
S (%)	5.5±0.2$^{(p<0.0001)}$	15.2±1.0
G2 (%)	5.5±0.4$^{(p=0.0002)}$	24.8±2.6
Sub G1 (%)	43.4±3.2$^{(p<0.0001)}$	15.2±1.0
	TAT_eIF4G1_me5	**TAT_eIF4G1_me5_Cntrl**
G1 (%)	17.0±0.7$^{(p<0.0001)}$	28.5±0.1
S (%)	2.6±0.3$^{(p<0.0001)}$	9.1±0.2
G2 (%)	3.2±0.1$^{(p<0.0001)}$	15.6±0.3
Sub G1 (%)	71.7±1.0$^{(p<0.0001)}$	40.1±0.8

Figure 10

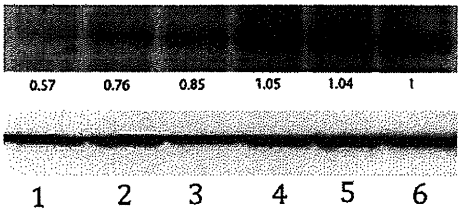


Figure 11

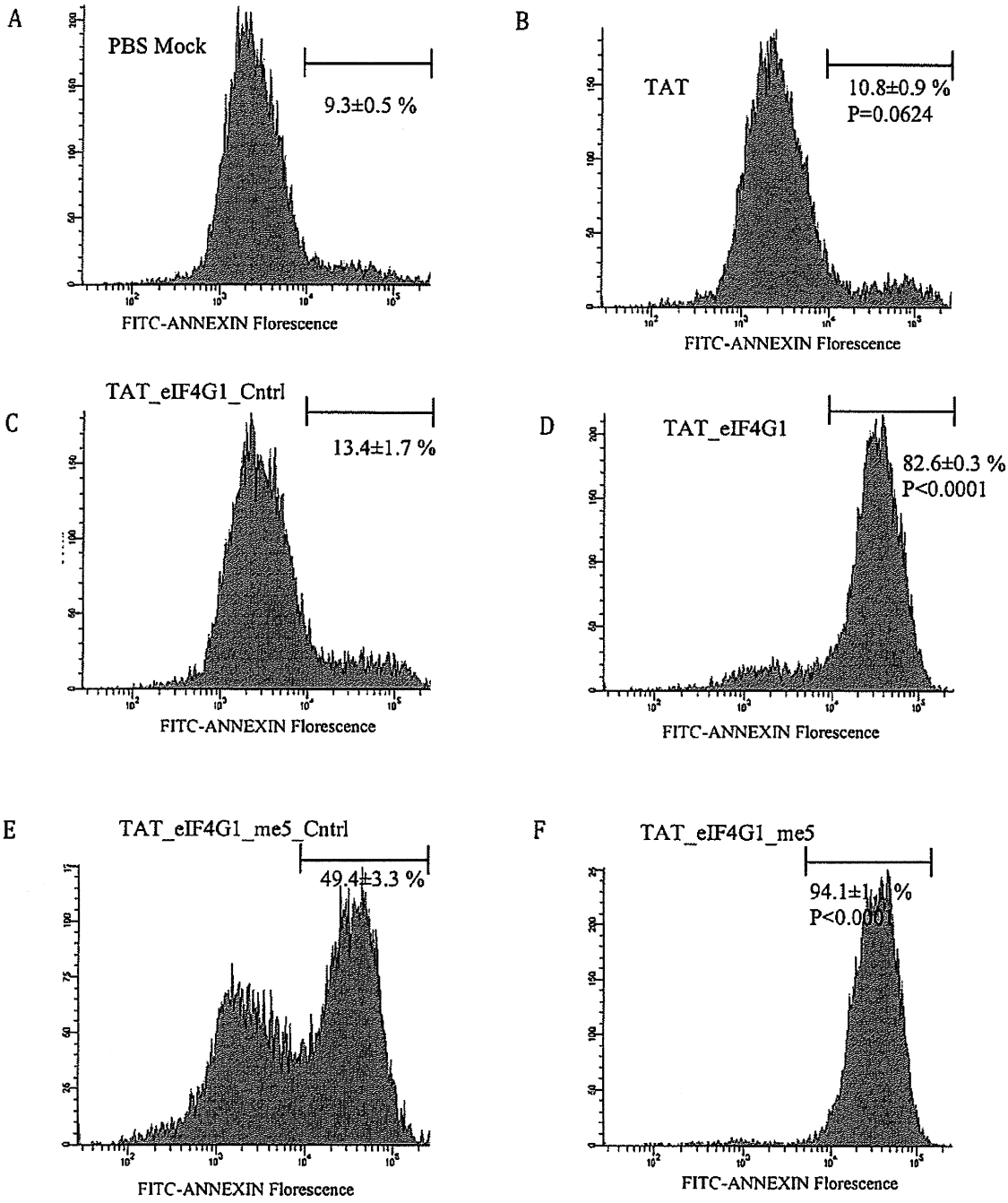

A
PBS Mock
9.3±0.5 %
FITC-ANNEXIN Florescence
B
TAT
10.8±0.9 %
P=0.0624
FITC-ANNEXIN Florescence
C
TAT_eIF4G1_Cntrl
13.4±1.7 %
FITC-ANNEXIN Florescence
D
TAT_eIF4G1
82.6±0.3 %
P<0.0001
FITC-ANNEXIN Florescence
E
TAT_eIF4G1_me5_Cntrl
49.4±3.3 %
FITC-ANNEXIN Florescence
F
TAT_eIF4G1_me5
94.1±1.1 %
P<0.0001
FITC-ANNEXIN Florescence

Figure 12

A

Peptides	K_d (nM)	ΔH (kCal $mol^{-1}K^{-1}$)	ΔS (Cal $mol^{-1}K^{-1}$)
TAT-eIF4G1	56.18±2.55	-5.52±0.06	15
TAT_eIF4GI_me5	10.17±8.60	-12.39±0.10	-5.69

B

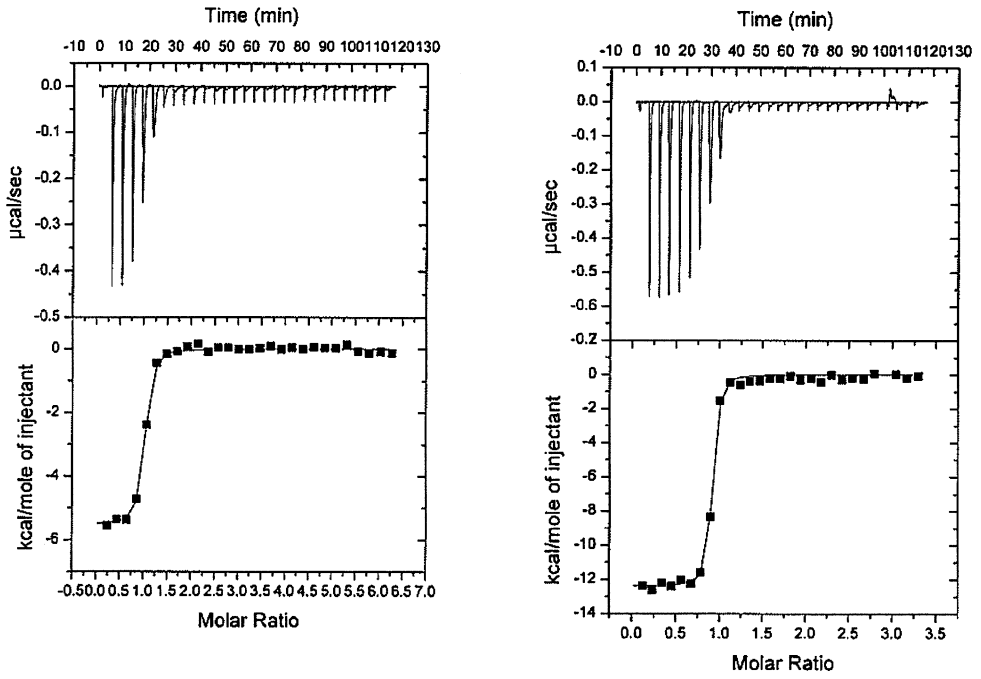


Figure 13

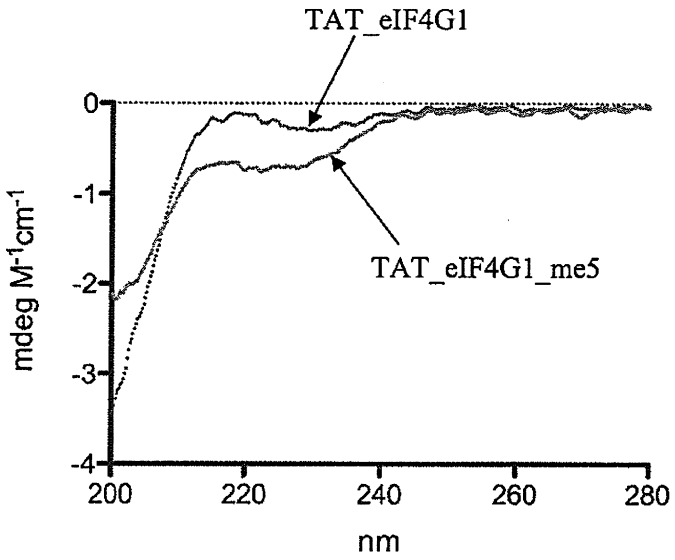
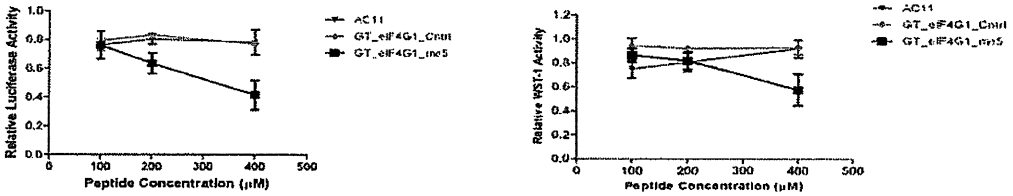
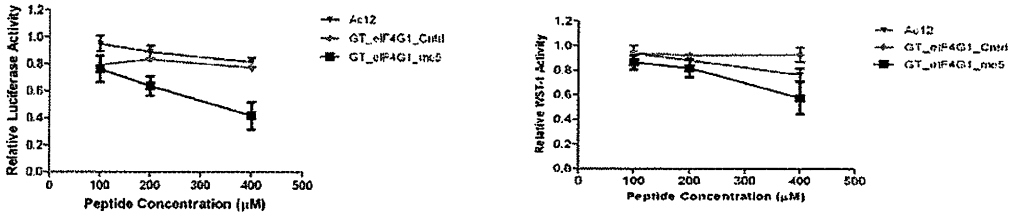

TAT_eIF4G1
TAT_eIF4G1_me5
0
-1
-2
-3
-4
mdeg M-1cm-1
200
220
240
260
280
nm

Figure 14

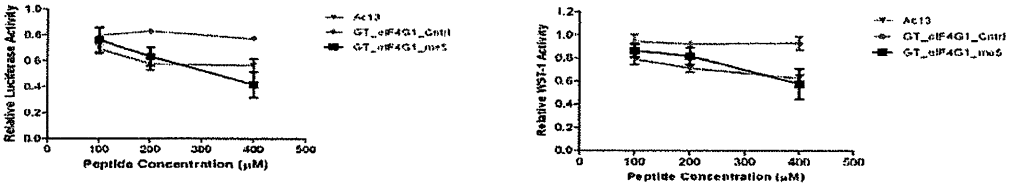
A



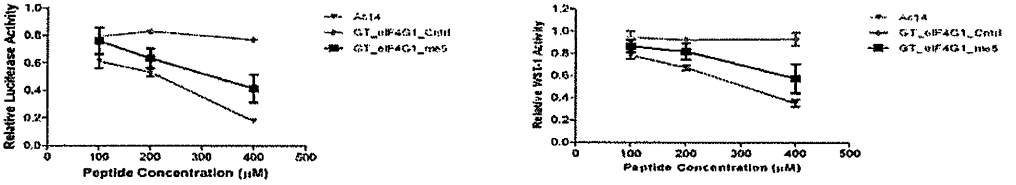
B



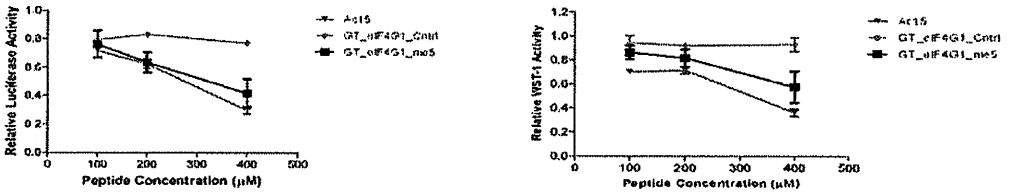
C



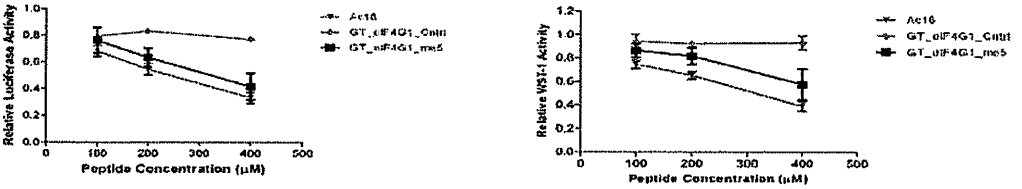
D



E



F



G

```
NH2-G-T-K-K-R-Y-D-R-E-*-L-L-5-F-Q-F-NH2 GT_eIF4G1_me5
NH2-G-T-K-K-R-A-D-R-E-*-A-A-5-F-Q-F-NH2 GT_eIF4G1_Cntrl
 Ac-G-T-K-K-R-Y-D-R-E-*-L-L-5-F-Q-F-NH2 AC16
   AC-T-K-K-R-Y-D-R-E-*-L-L-5-F-Q-F-NH2 Ac15
     AC-K-K-R-Y-D-R-E-*-L-L-5-F-Q-F-NH2 Ac14
       Ac-K-R-Y-D-R-E-*-L-L-5-F-Q-F-NH2 Ac13
         Ac-R-Y-D-R-E-*-L-L-5-F-Q-F-NH2 Ac12
           Ac-Y-D-R-E-*-L-L-5-F-Q-F-NH2 Ac11
```

*= C-alpha Methyl phenylalnine
5=Aminocyclopentanoic acid

Figure 15

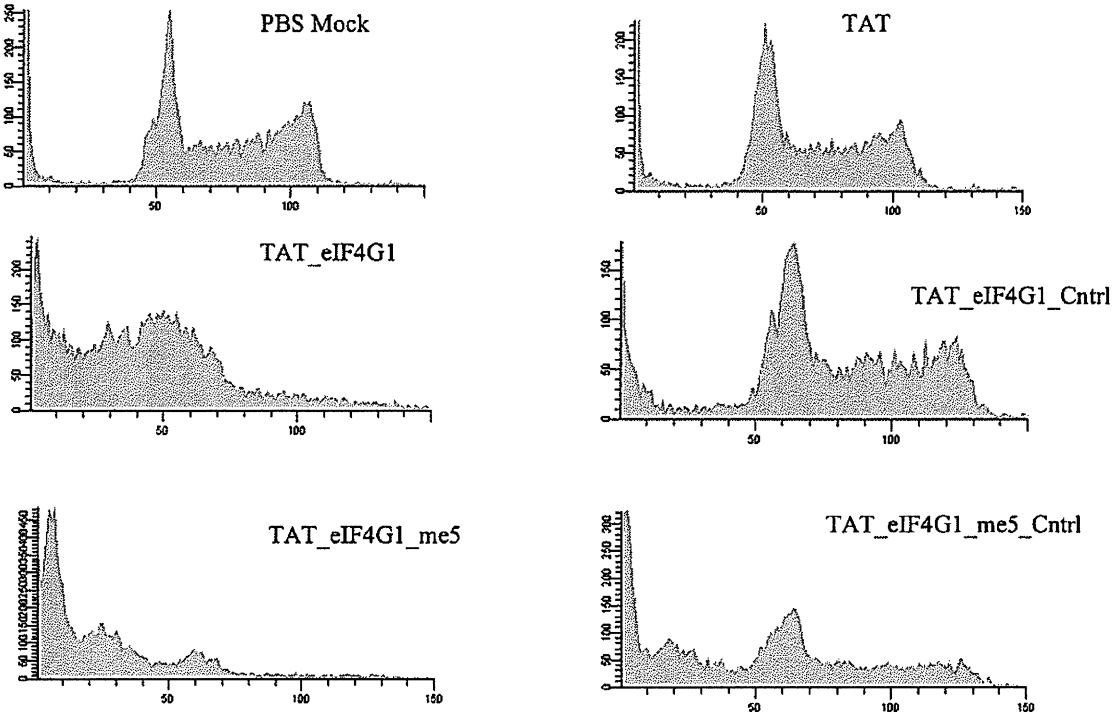

PBS Mock
TAT
TAT_eIF4G1
TAT_eIF4G1_Cntrl
TAT_eIF4G1_me5
TAT_eIF4G1_me5_Cntrl

Figure 16