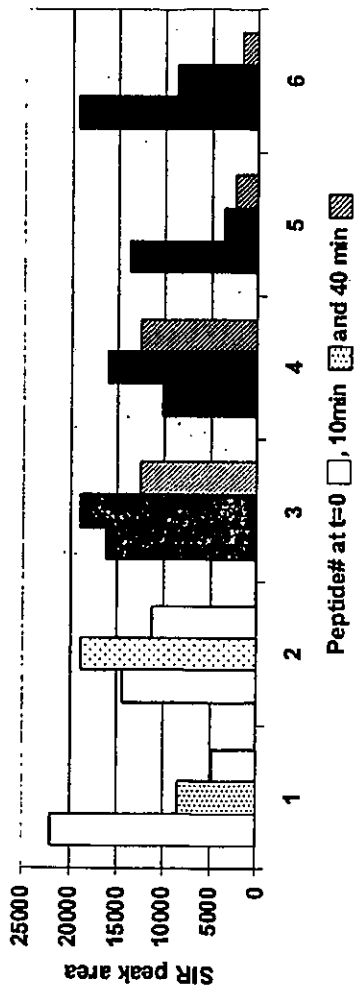
Minimal pattern conferring sensibility to proteases (Prot K)



ID#	Pattern	Sequence (N-term to C-term)	Protease sensitivity
1-	all L-	RRRRRRRRR (reference sequence)	+
2-	all D-	rrrrrrrrr	-
3-	D/L-	rRrRrRrRr	-
4-	DD/LL-	rrRRrrRRr	-
5-	DDD/LLL-	rrrRRRrrr	+
6-	DDDD/LLLL-	rrrRRRRrr	+

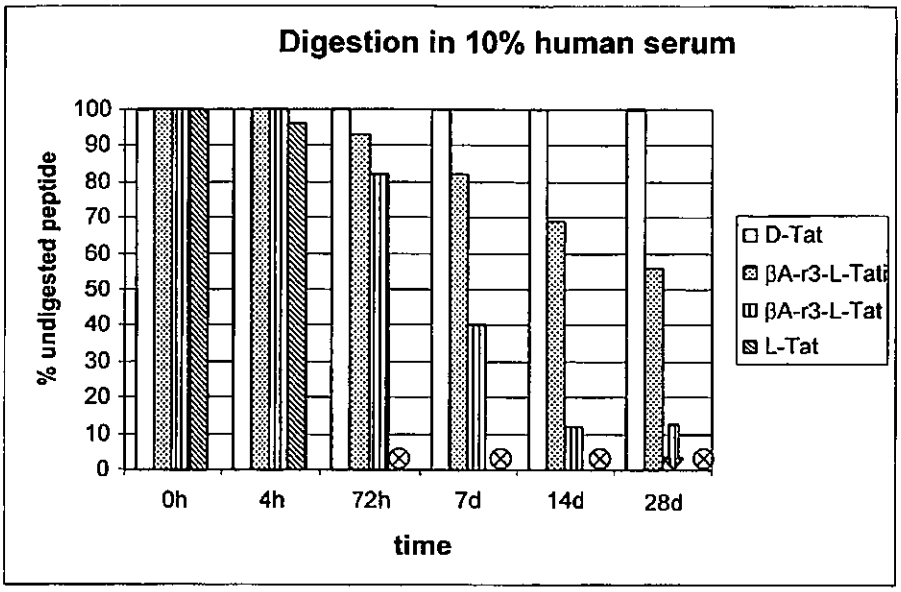
Fig. 1

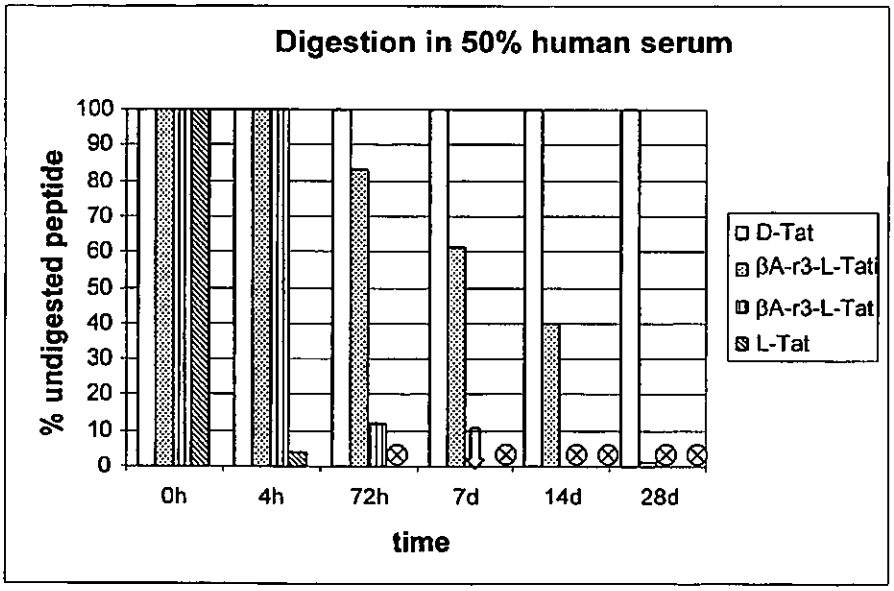
Comparison of transporters motifs of D/L-TAT derivatives (9 a.a.)

Name	Sequence	MW	pI
L-TATi	RKKRRQRRR	1339.6	12.7
r3-TAT	rKKRrQRRr	1339.6	12.7
r3-TATi	rRRQrRKKr	1339.6	12.7
D-TAT	rrrqrrkkr	1339.6	12.7

Fig. 2

Stability of new transporters in Human Serum

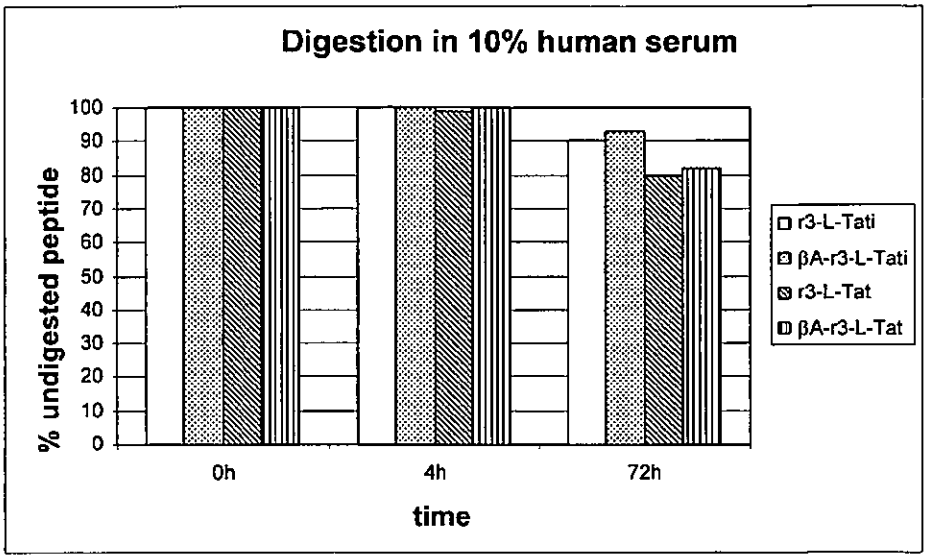




⊗ = fully digested Tat (M_w < 300)
⇩ = Tat fragments (M_w > 300)

Fig. 3

N-terminal protection (β-Ala) does not confer improved stability



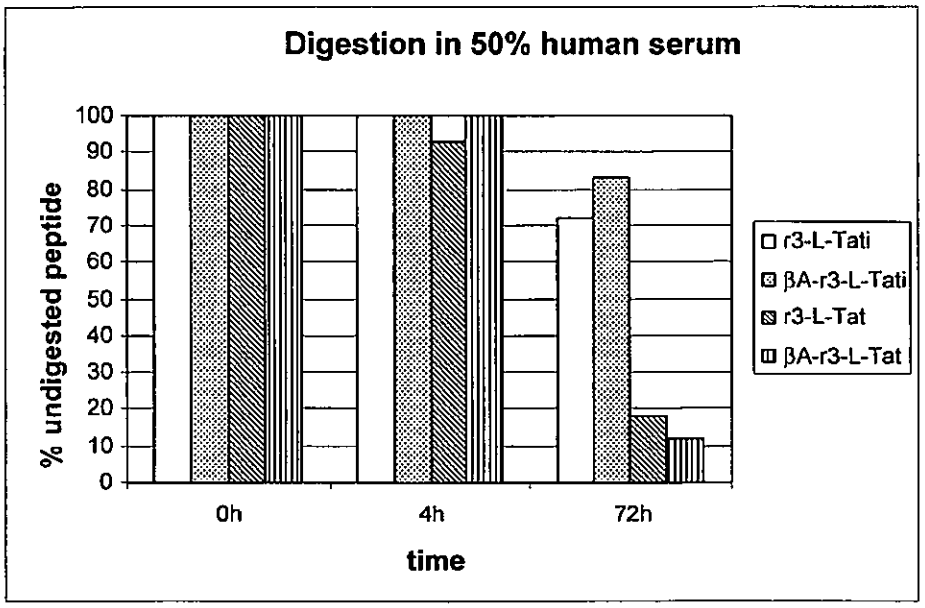


Fig. 4

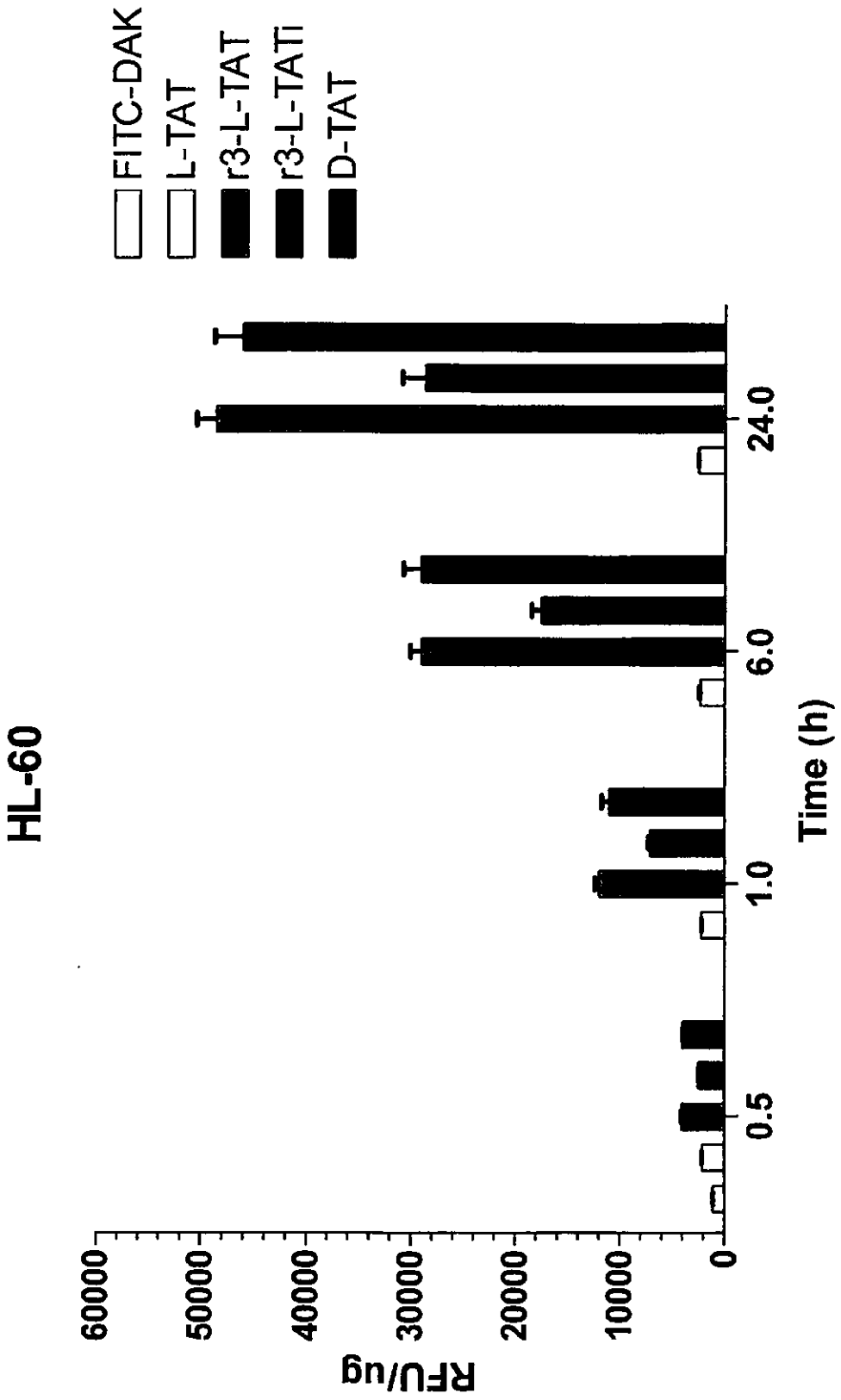

FITC-DAK
L-TAT
r3-L-TAT
r3-L-TATi
D-TAT
HL-60
RFU/ug
60000
50000
40000
30000
20000
10000
0
0.5
1.0
6.0
24.0
Time (h)

Fig. 5

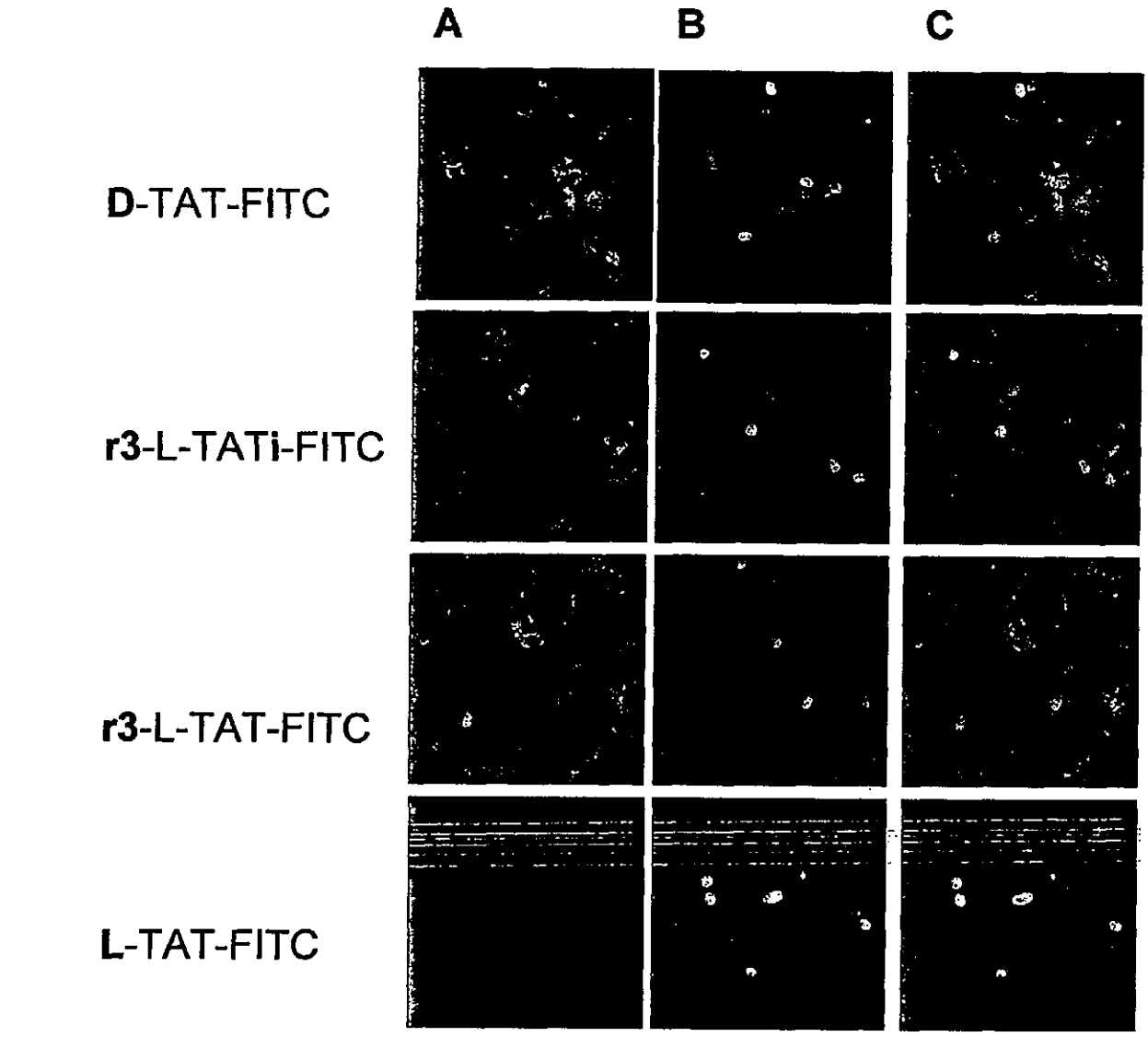

Cortical neurons – 24h
A
B
C
D-TAT-FITC
r3-L-TATi-FITC
r3-L-TAT-FITC
L-TAT-FITC

Fig. 6

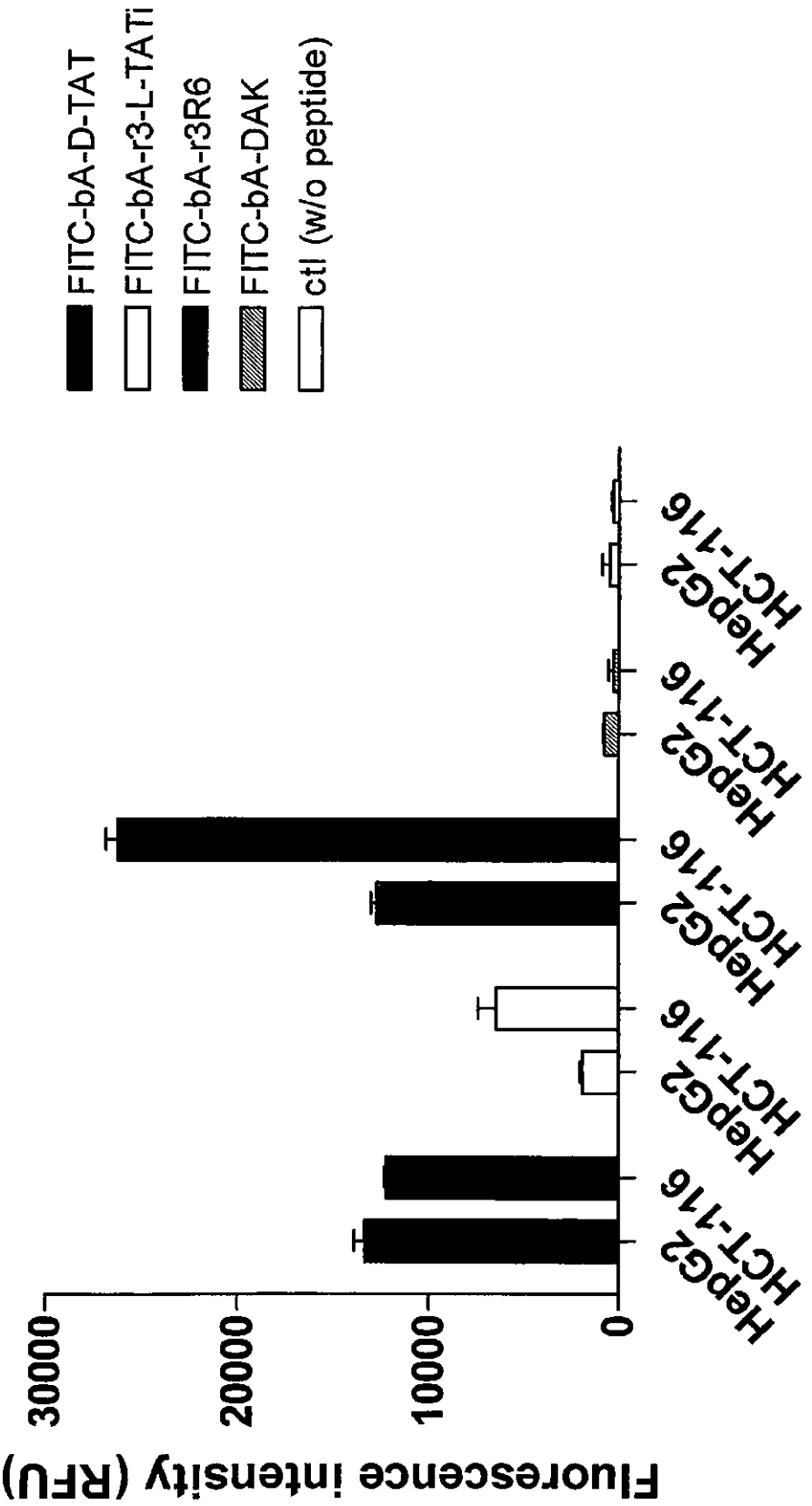

FITC-bA-D-TAT
FITC-bA-r3-L-TATi
FITC-bA-r3R6
FITC-bA-DAK
ctl (w/o peptide)
HepG2
HCT-116
HepG2
HCT-116
HepG2
HCT-116
HepG2
HCT-116
HepG2
HCT-116
0
10000
20000
30000
Fluorescence intensity (RFU)

Fig. 7

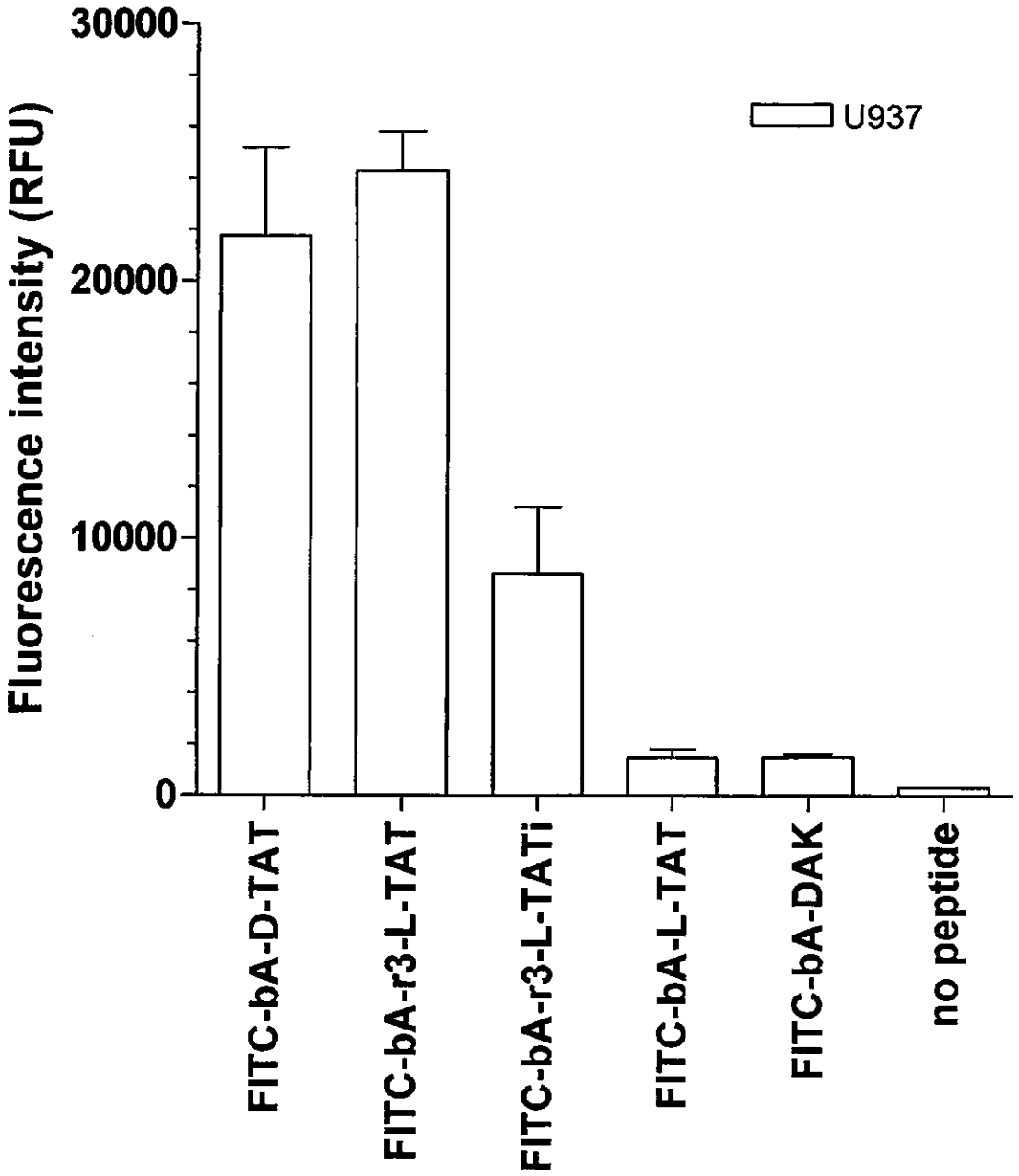

30000
20000
10000
0
Fluorescence intensity (RFU)
U937
FITC-bA-D-TAT
FITC-bA-r3-L-TAT
FITC-bA-r3-L-TATi
FITC-bA-L-TAT
FITC-bA-DAK
no peptide

Fig. 8

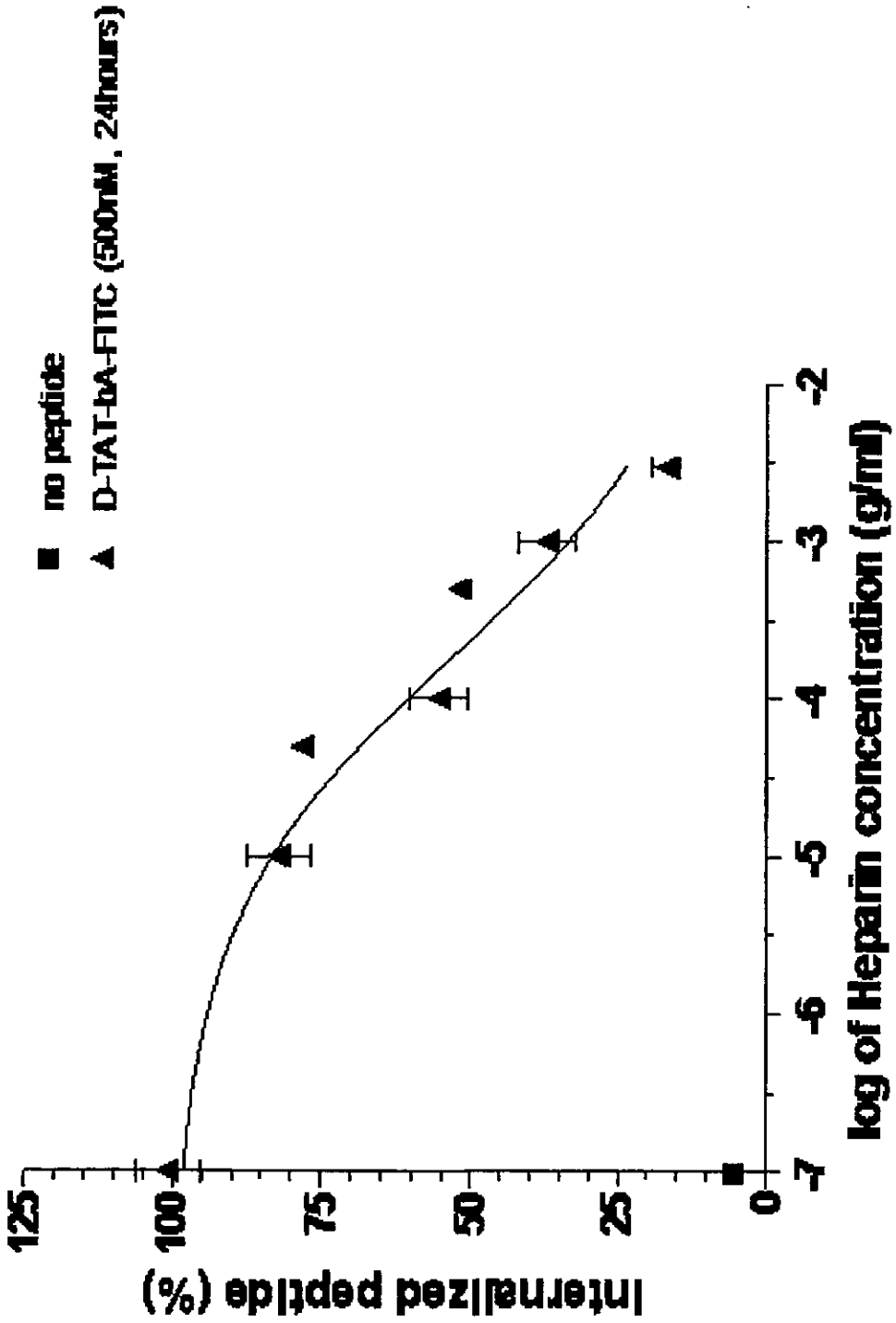

no peptide
D-TAT-bA-FITC (500nM, 24hours)
Internalized peptide (%)
125
100
75
50
25
0
-2
-3
-4
-5
-6
-7
log of Heparin concentration (g/ml)

Fig. 9

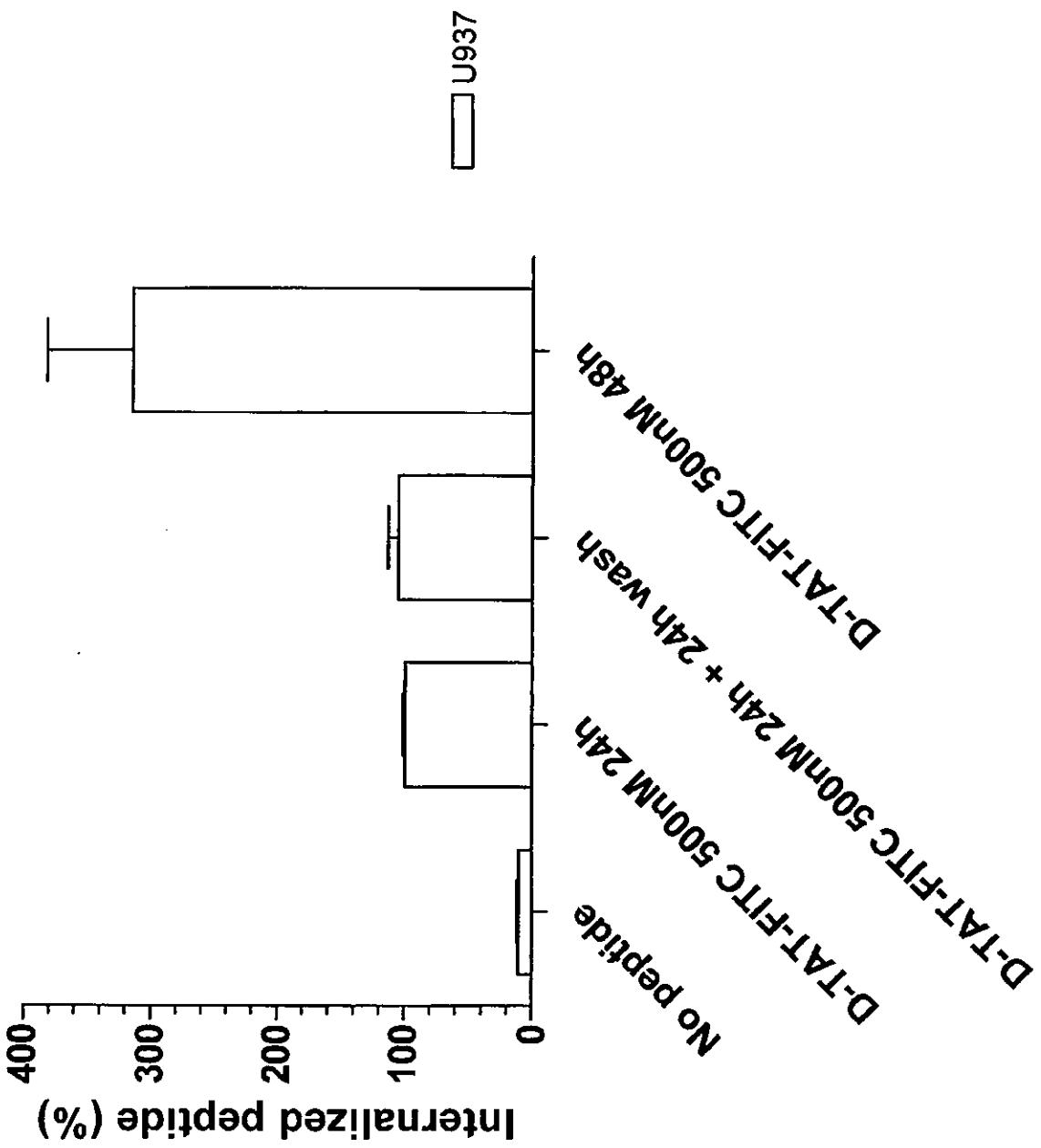

U937
No peptide
D-TAT-FITC 500nM 24h
D-TAT-FITC 500nM 24h + 24h wash
D-TAT-FITC 500nM 48h
0
100
200
300
400
Internalized peptide (%)

Fig. 10

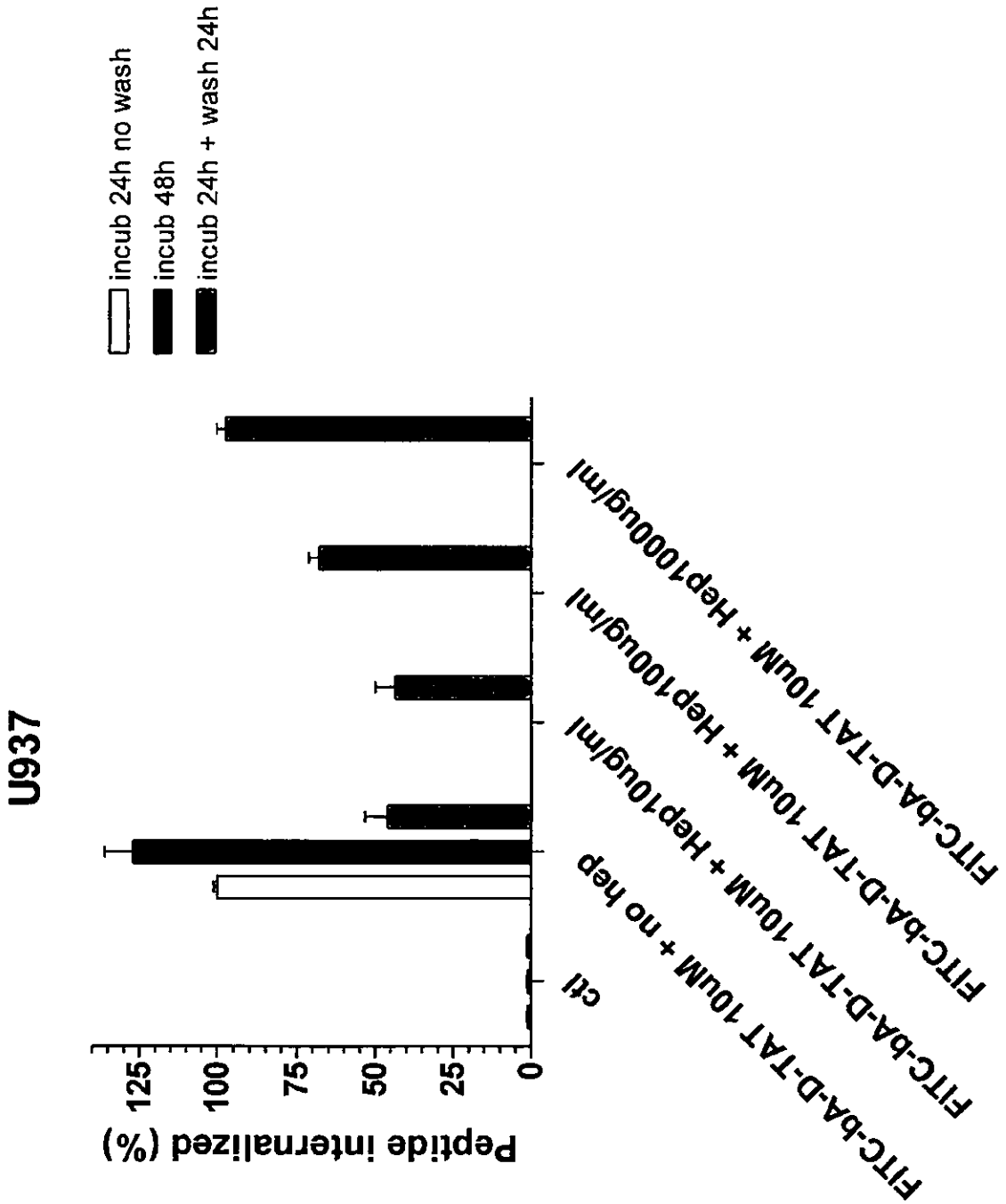

incub 24h no wash
incub 48h
incub 24h + wash 24h
U937
Peptide internalized (%)
0
25
50
75
100
125
ctl
FITC-bA-D-TAT 10uM + no hep
FITC-bA-D-TAT 10uM + Hep10ug/ml
FITC-bA-D-TAT 10uM + Hep100ug/ml
FITC-bA-D-TAT 10uM + Hep1000ug/ml

Fig. 11

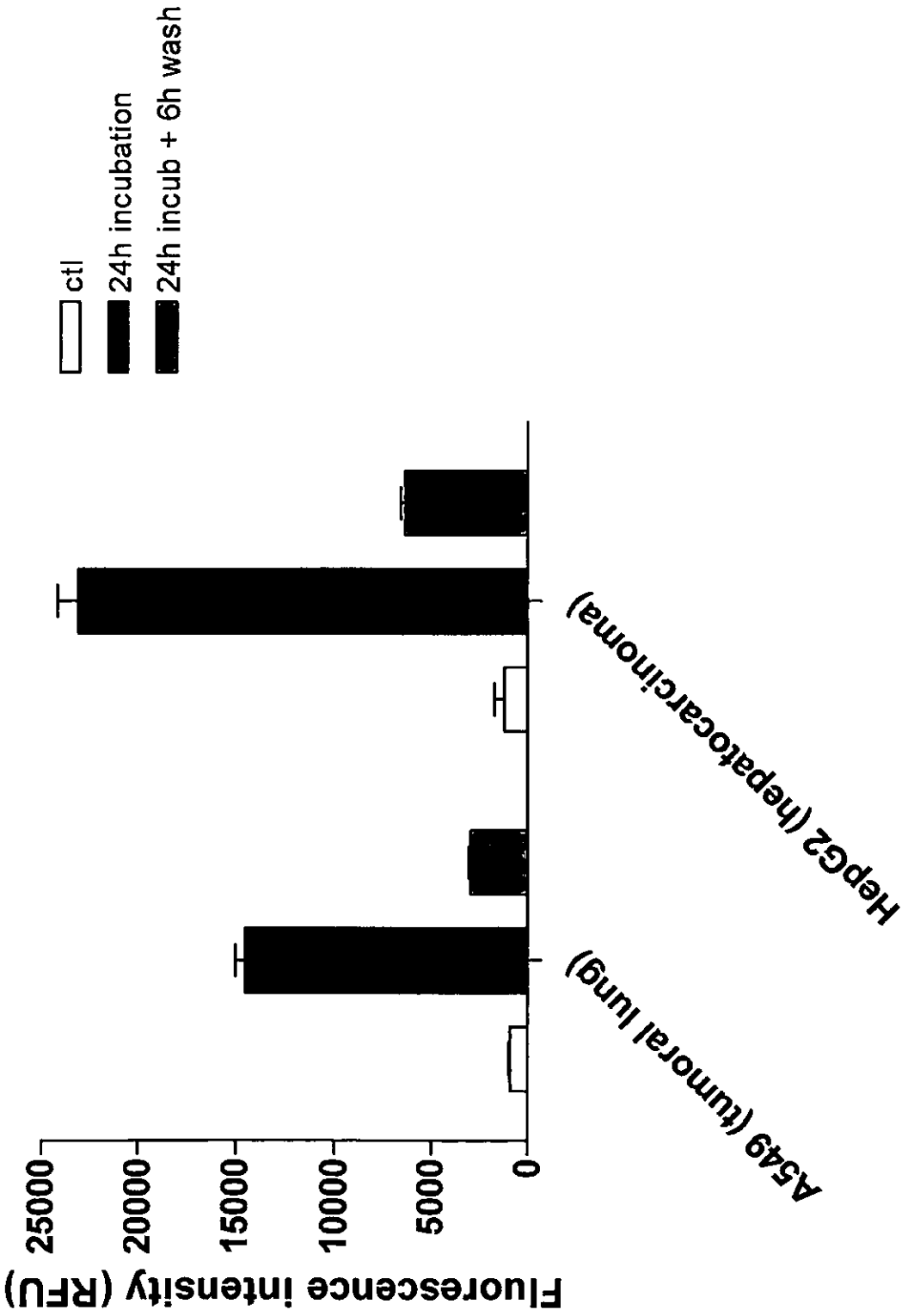

ctl
24h incubation
24h incub + 6h wash
Fluorescence intensity (RFU)
25000
20000
15000
10000
5000
0
A549 (tumoral lung)
HepG2 (hepatocarcinoma)

Fig. 12

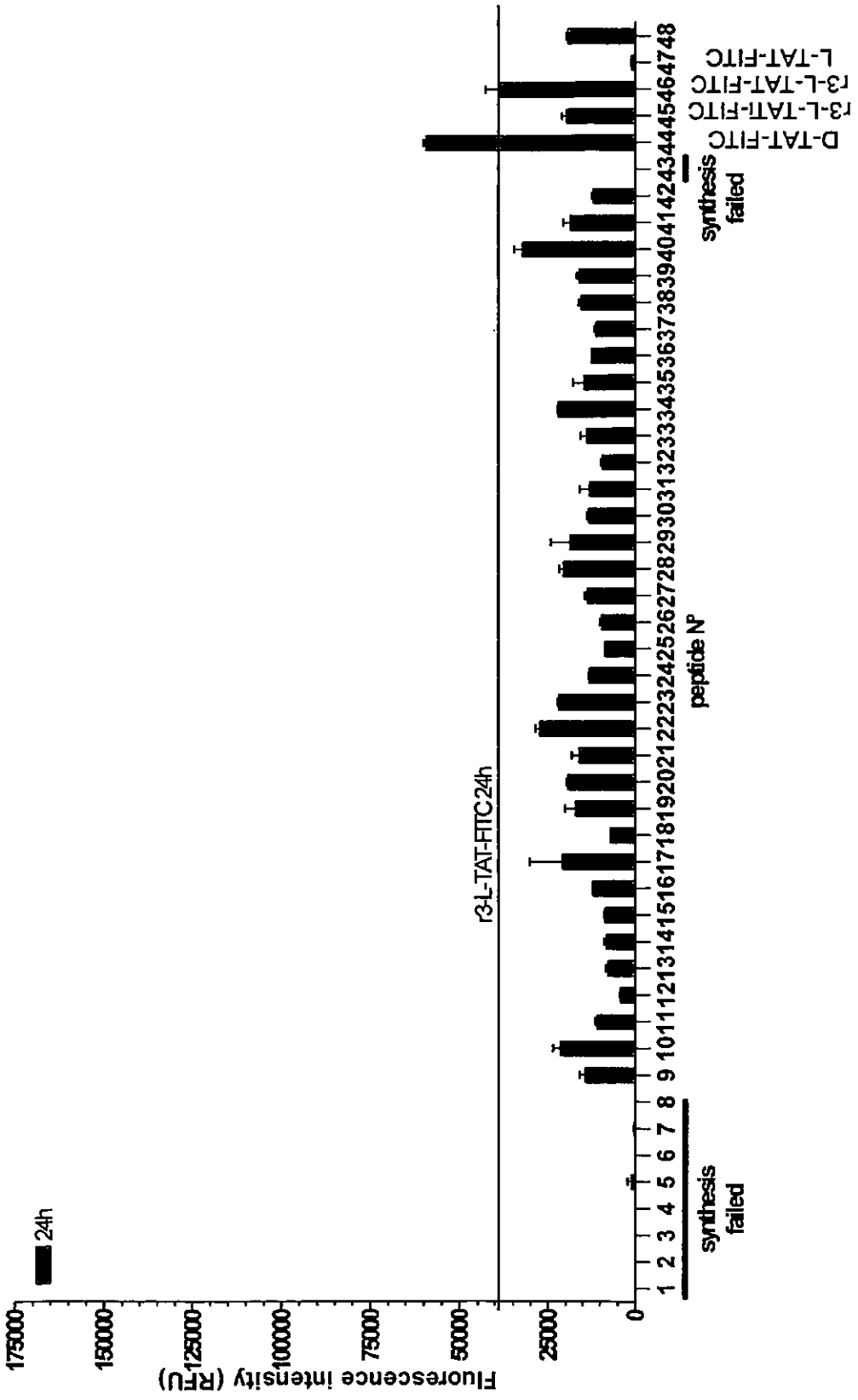

Fluorescence intensity (RFU)
175000
150000
125000
100000
75000
50000
25000
0
24h
r3-L-TAT-FITC24h
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
peptide N°
synthesis failed
synthesis failed
D-TAT-FITC
r3-L-TAT-FITC
r3-L-TAT-FITC
L-TAT-FITC

Fig. 13A

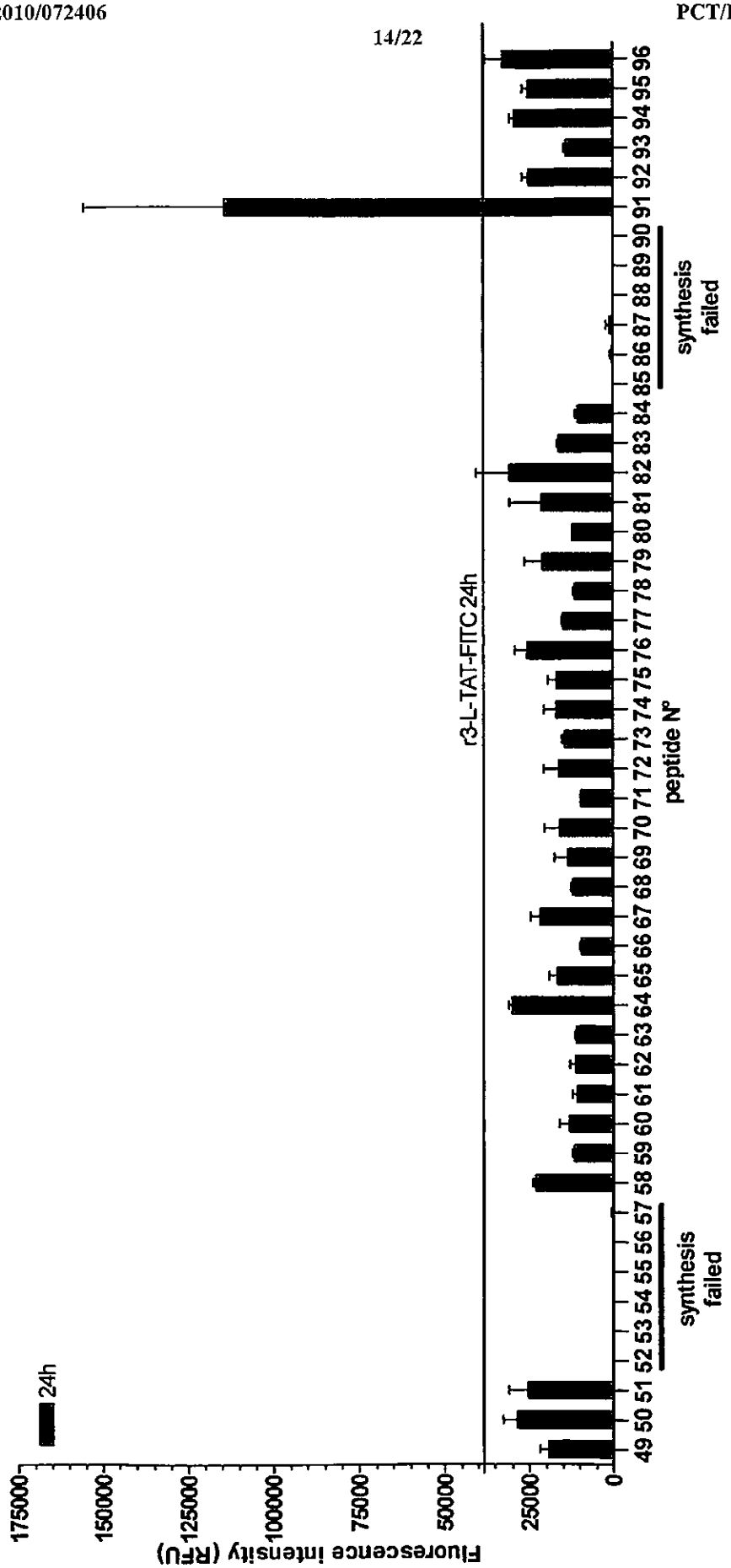

Fluorescence intensity (RFU)
175000
150000
125000
100000
75000
50000
25000
0
24h
r3-L-TAT-FITC 24h
peptide N°
49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96
synthesis failed
synthesis failed

Fig. 13B

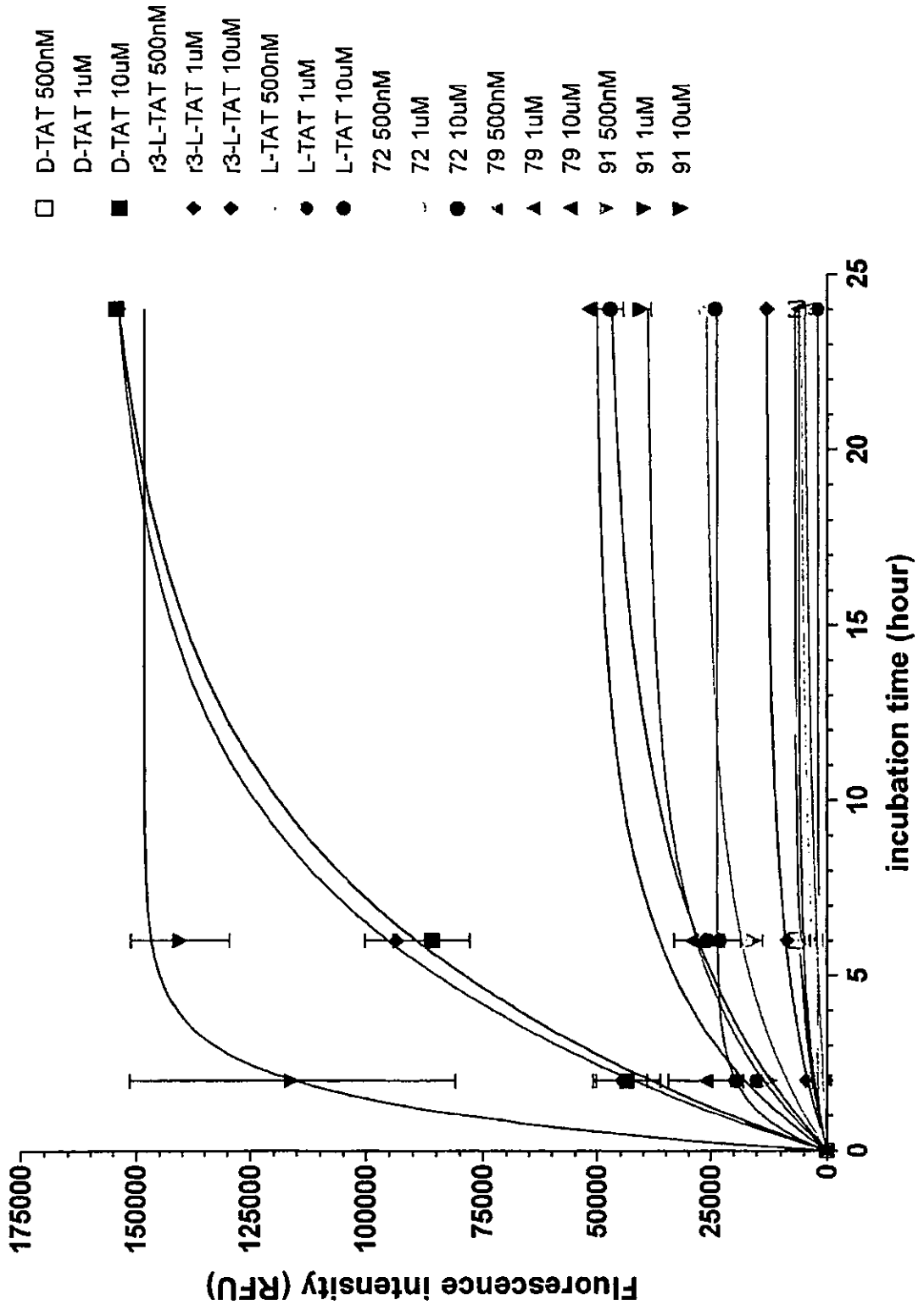

D-TAT 500nM
D-TAT 1uM
D-TAT 10uM
r3-L-TAT 500nM
r3-L-TAT 1uM
r3-L-TAT 10uM
L-TAT 500nM
L-TAT 1uM
L-TAT 10uM
72 500nM
72 1uM
72 10uM
79 500nM
79 1uM
79 10uM
91 500nM
91 1uM
91 10uM
Fluorescence intensity (RFU)
175000
150000
125000
100000
75000
50000
25000
0
0
5
10
15
20
25
incubation time (hour)

Fig. 14A

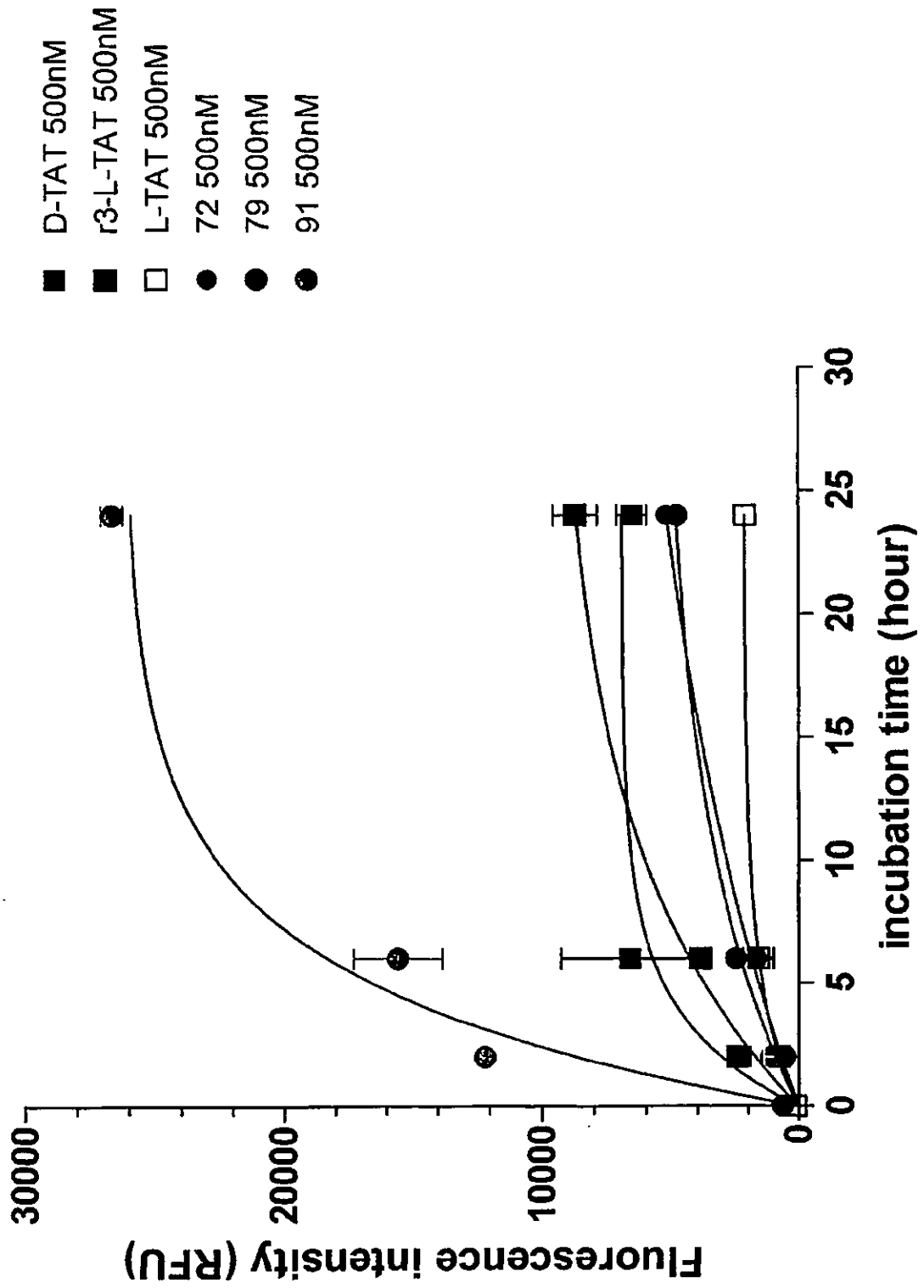

D-TAT 500nM
r3-L-TAT 500nM
L-TAT 500nM
72 500nM
79 500nM
91 500nM
Fluorescence intensity (RFU)
30000
20000
10000
0
0
5
10
15
20
25
30
incubation time (hour)

Fig. 14B

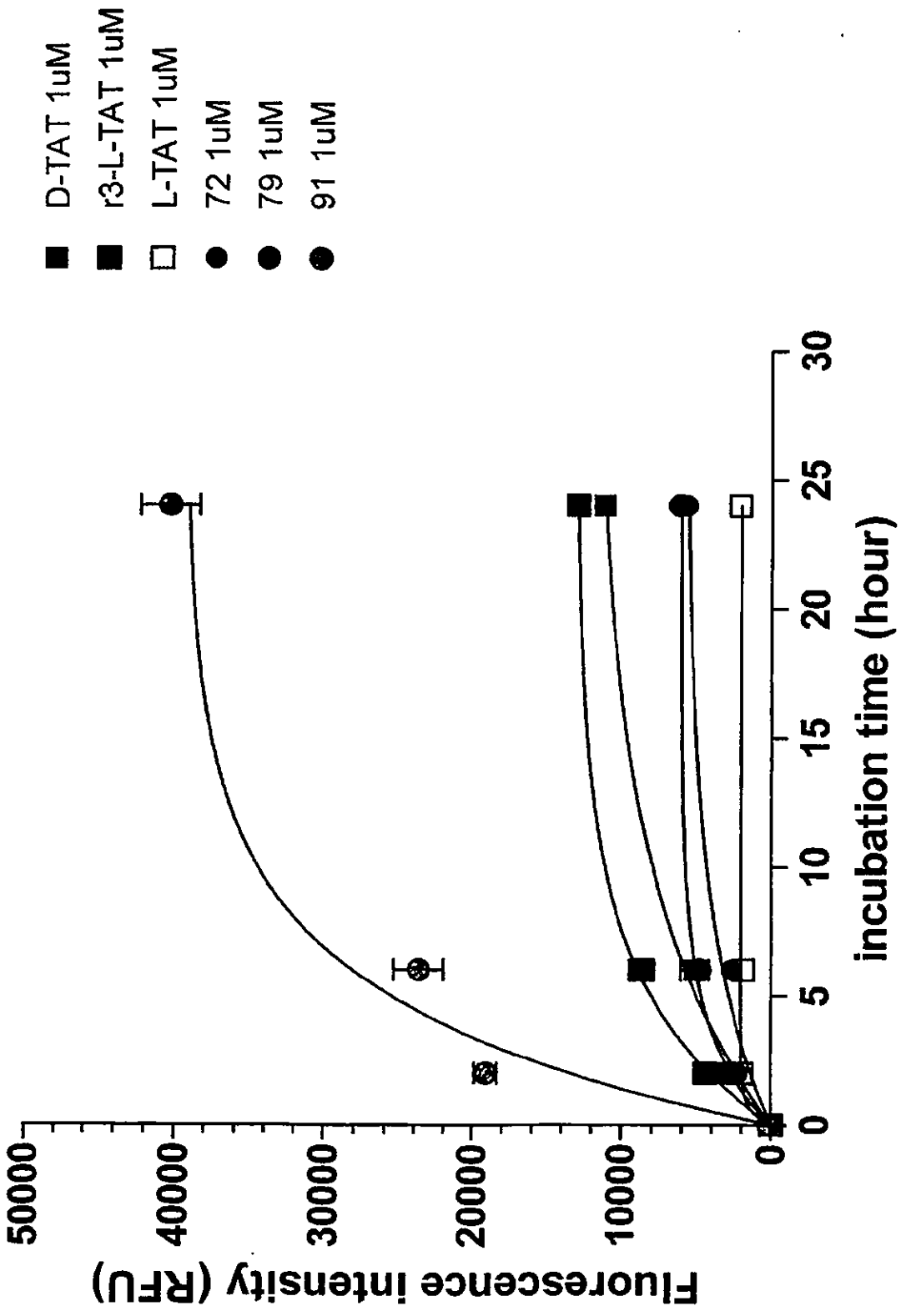

D-TAT 1uM
r3-L-TAT 1uM
L-TAT 1uM
72 1uM
79 1uM
91 1uM
Fluorescence intensity (RFU)
50000
40000
30000
20000
10000
0
0
5
10
15
20
25
30
incubation time (hour)

Fig. 14C

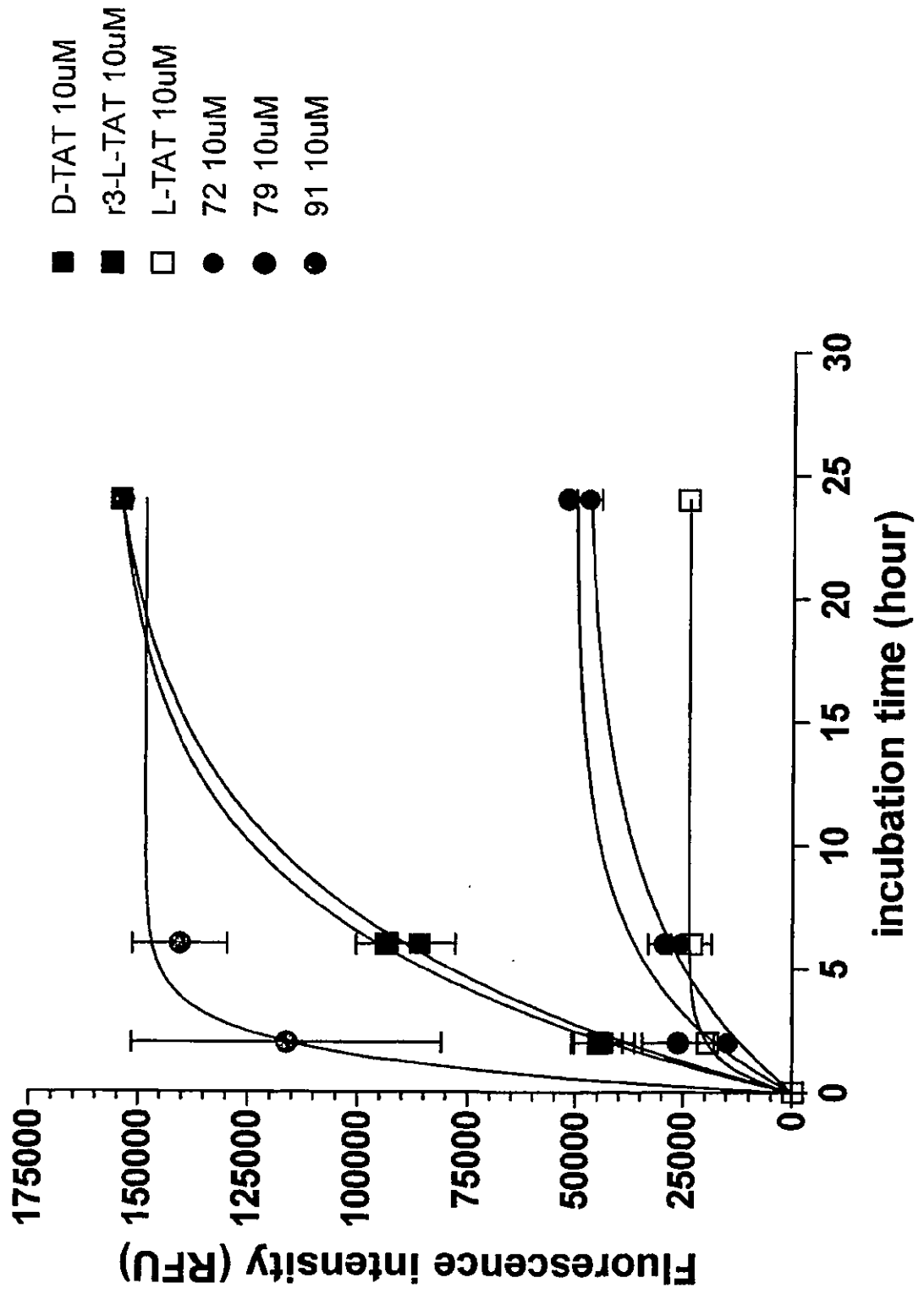

D-TAT 10uM
r3-L-TAT 10uM
L-TAT 10uM
72 10uM
79 10uM
91 10uM
Fluorescence intensity (RFU)
175000
150000
125000
100000
75000
50000
25000
0
0
5
10
15
20
25
30
incubation time (hour)

Fig. 14D

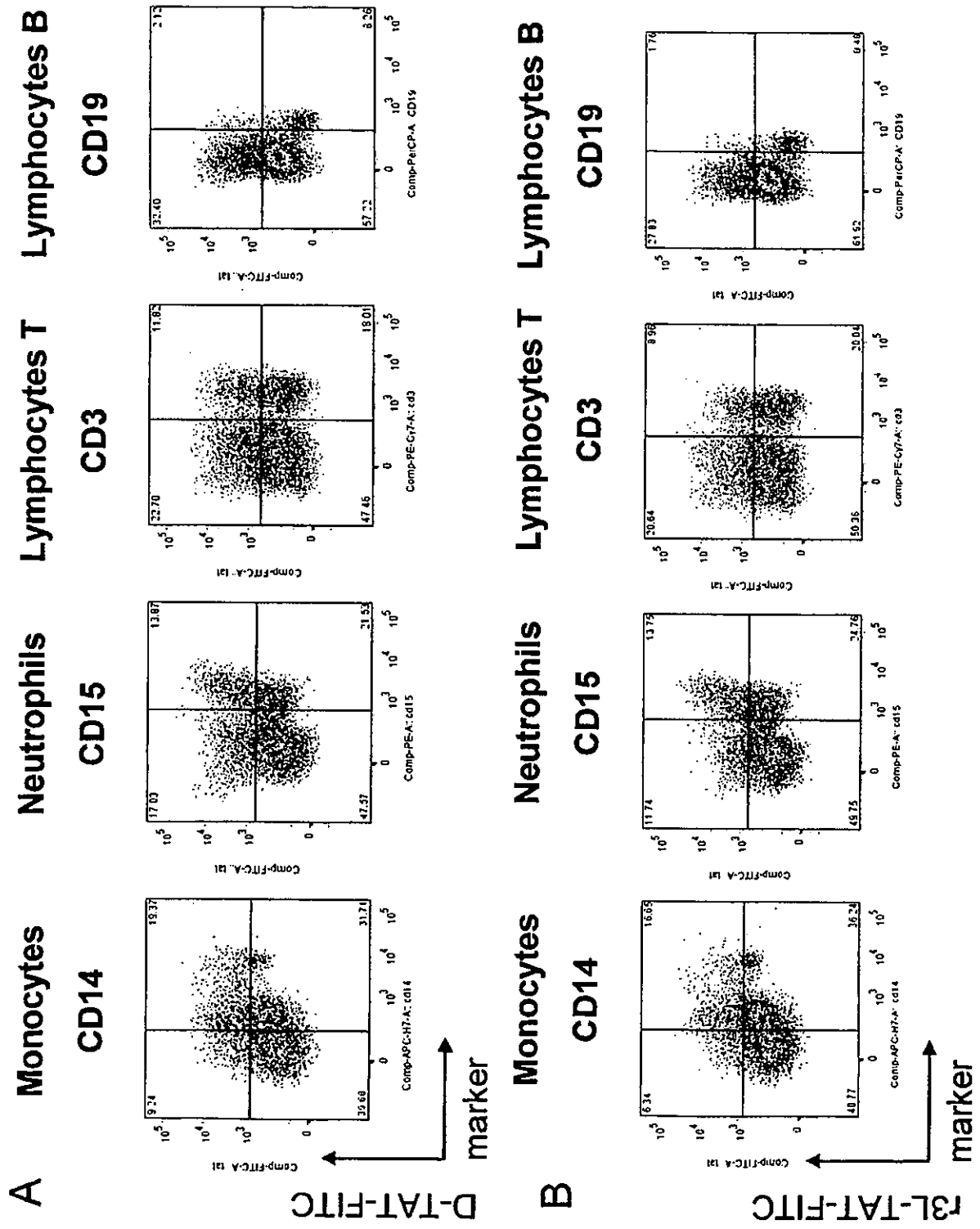
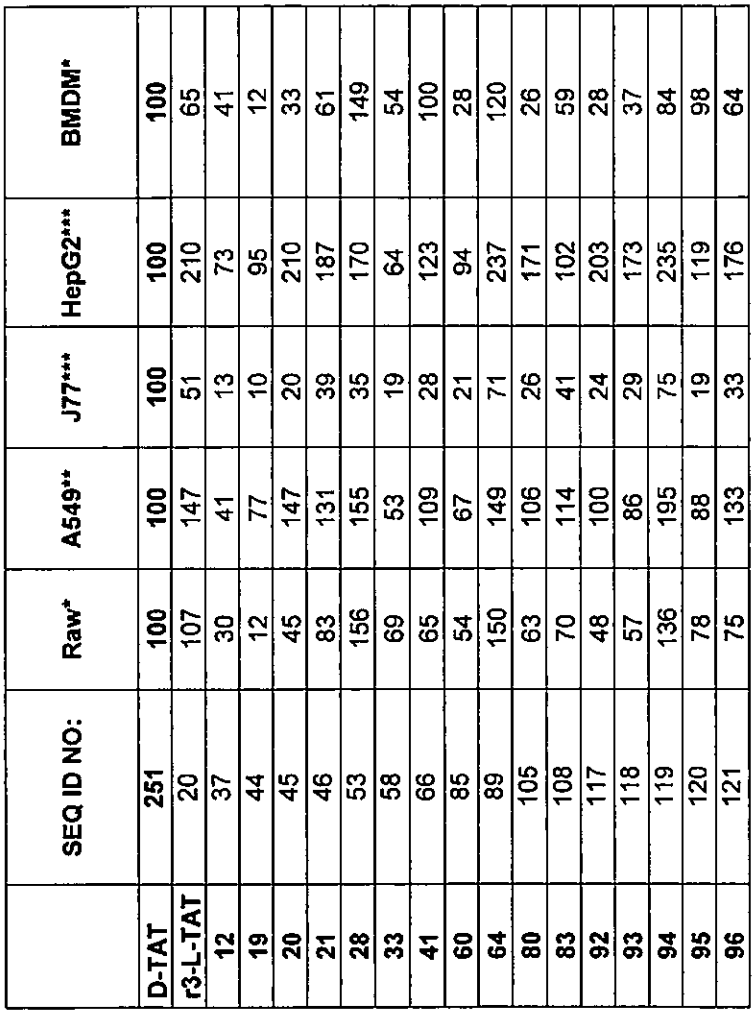

A
Monocytes
CD14
Neutrophils
CD15
Lymphocytes T
CD3
Lymphocytes B
CD19
D-TAT-FITC
marker
B
Monocytes
CD14
Neutrophils
CD15
Lymphocytes T
CD3
Lymphocytes B
CD19
rL-TAT-FITC
marker

Fig. 15

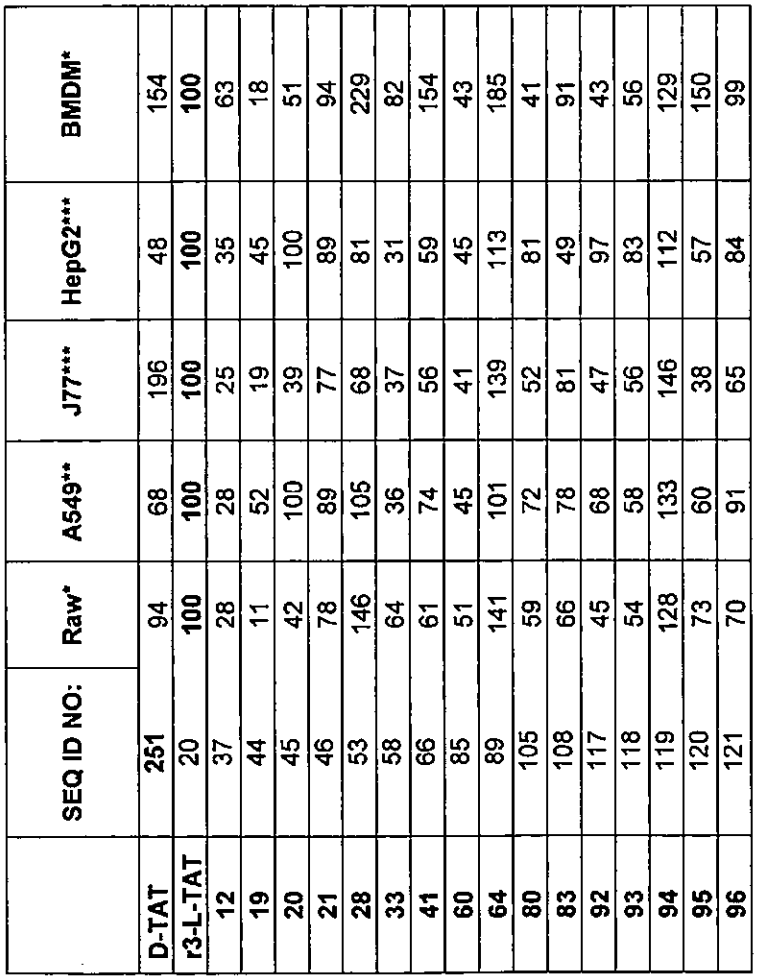
	Monocytes	Neutrophils	T lympho.	B lympho.
D-TAT	3510.30	1698.16	1551.66	1323.95
r3L-TAT	2952.56	1518.37	995.46	1051.82

Fig. 16



	SEQ ID NO:	Raw*	A549**	J77***	HepG2***	BMDM*
D-TAT	251	100	100	100	100	100
r3-L-TAT	20	107	147	51	210	65
12	37	30	41	13	73	41
19	44	12	77	10	95	12
20	45	45	147	20	210	33
21	46	83	131	39	187	61
28	53	156	155	35	170	149
33	58	69	53	19	64	54
41	66	65	109	28	123	100
60	85	54	67	21	94	28
64	89	150	149	71	237	120
80	105	63	106	26	171	26
83	108	70	114	41	102	59
92	117	48	100	24	203	28
93	118	57	86	29	173	37
94	119	136	195	75	235	84
95	120	78	88	19	119	98
96	121	75	133	33	176	64

Fig. 17



	SEQ ID NO:	Raw*	A549**	J77***	HepG2***	BMDM*
D-TAT	251	94	68	196	48	154
r3-L-TAT	20	100	100	100	100	100
12	37	28	28	25	35	63
19	44	11	52	19	45	18
20	45	42	100	39	100	51
21	46	78	89	77	89	94
28	53	146	105	68	81	229
33	58	64	36	37	31	82
41	66	61	74	56	59	154
60	85	51	45	41	45	43
64	89	141	101	139	113	185
80	105	59	72	52	81	41
83	108	66	78	81	49	91
92	117	45	68	47	97	43
93	118	54	58	56	83	56
94	119	128	133	146	112	129
95	120	73	60	38	57	150
96	121	70	91	65	84	99

Fig. 18