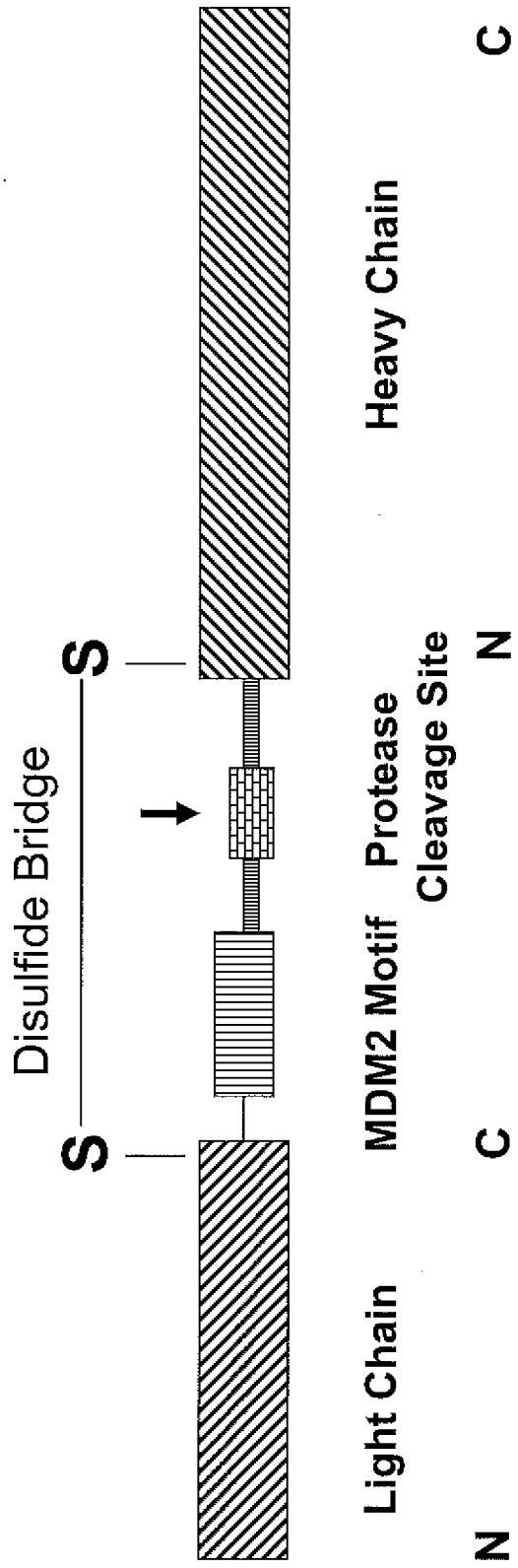

Heavy Chain
C
N
Protease
Cleavage Site
MDM2 Motif
Disulfide Bridge
S
S
Light Chain
C
N

Fig. 1

Fig. 2

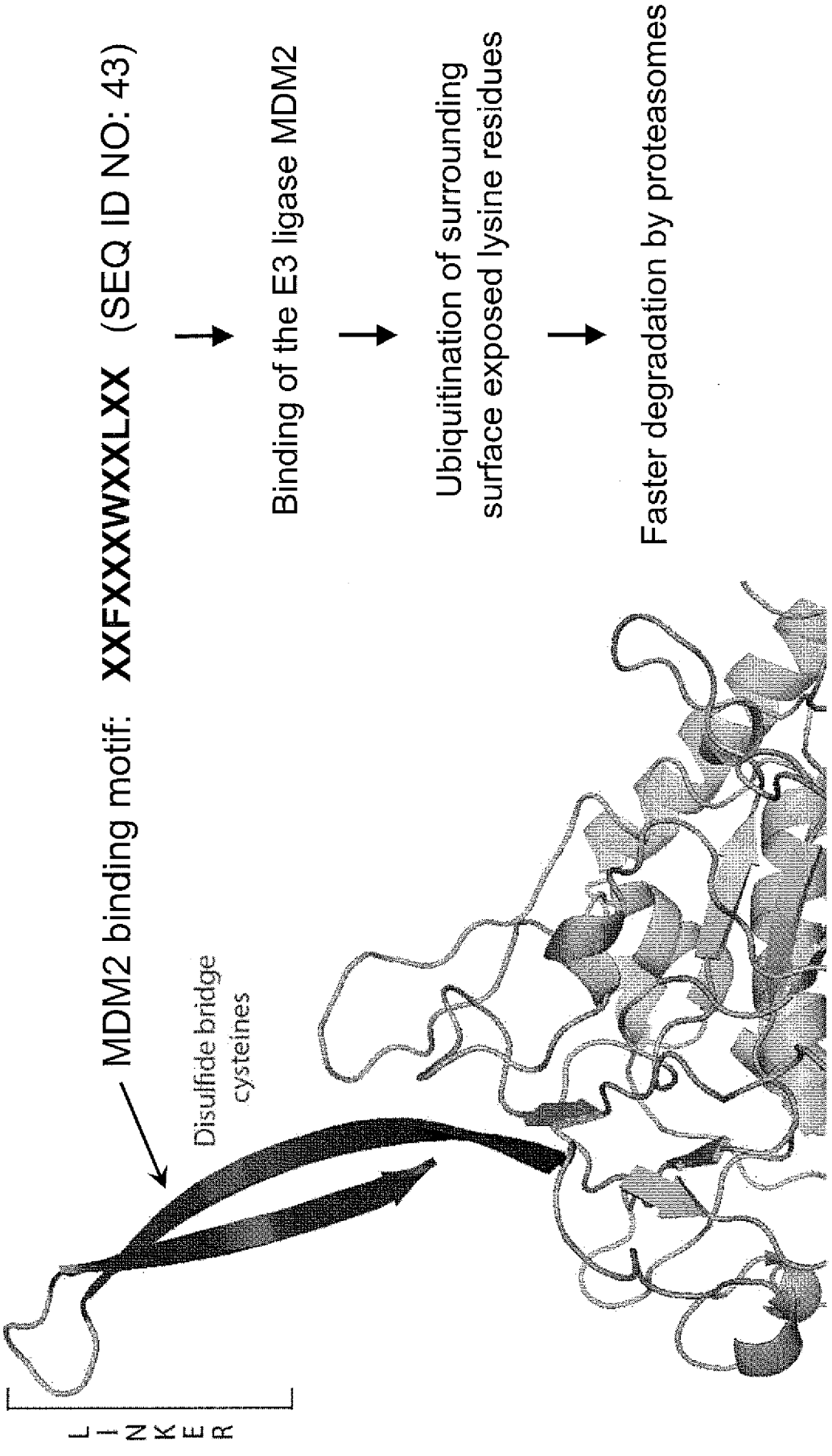

MDM2 binding motif: XXFXXXWXXLXX (SEQ ID NO: 43)
Binding of the E3 ligase MDM2
Ubiquitination of surrounding surface exposed lysine residues
Faster degradation by proteasomes
Disulfide bridge cysteines
LINKER

Fig. 3

BoNT/E-MDM2 mutants											SEQ ID NO.:	Comparison with BoNT/E-MDM2 (SEQ ID NO. 52)
Q27	E28	Q53	N72	N378	N379	V382	N383	L391	R394	T400		
		X	X	X	X				X	X	57	**Reduced duration of biological effect**
			X						X	X	64	no effect
		X								X	60	no effect
			X						X		63	no effect
		X		X	X						58	**Reduced duration of biological effect**
				X	X						66	no effect
				X							65	no effect
					X						76	no effect
				X	X				X		74	no effect
			X	X	X						61	**Reduced duration of biological effect**
				X	X					X	75	**Reduced duration of biological effect**
X	X										53	no effect
X	X	X									54	no effect
X	X		X								55	no effect
				X	X	X					67	no effect
				X	X			X			73	no effect
				X	X		X				71	n.d.
				X	X	X	X				68	n.d.
				X	X	X		X			70	n.d.
				X	X		X	X			72	n.d.
				X	X	X	X	X			69	n.d.
		X	X	X	X						56	n.d.
			X	X	X					X	62	n.d.
		X		X	X					X	59	n.d.
		X	X	X	X					X	77	n.d.

Fig. 4

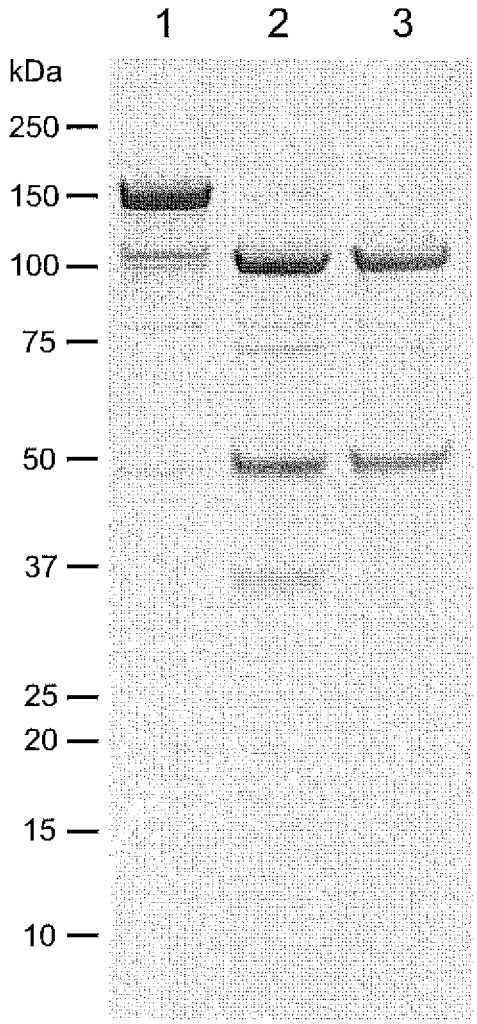


Fig. 5

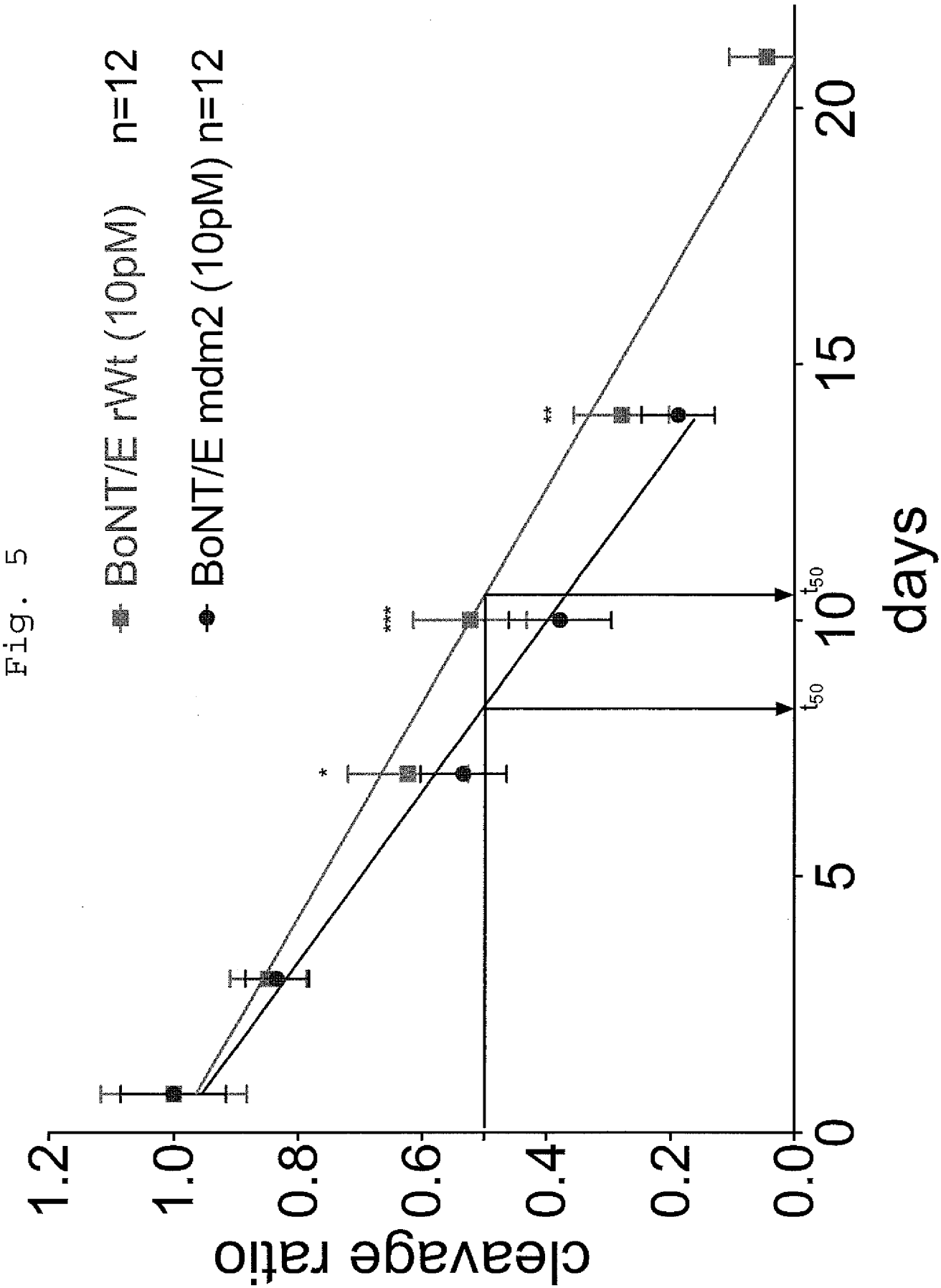

BoNT/E rWt (10pM) n=12
BoNT/E mdm2 (10pM) n=12
cleavage ratio
days
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
5
10
15
20
t_{50}
t_{50}
*
***
**

Fig. 6

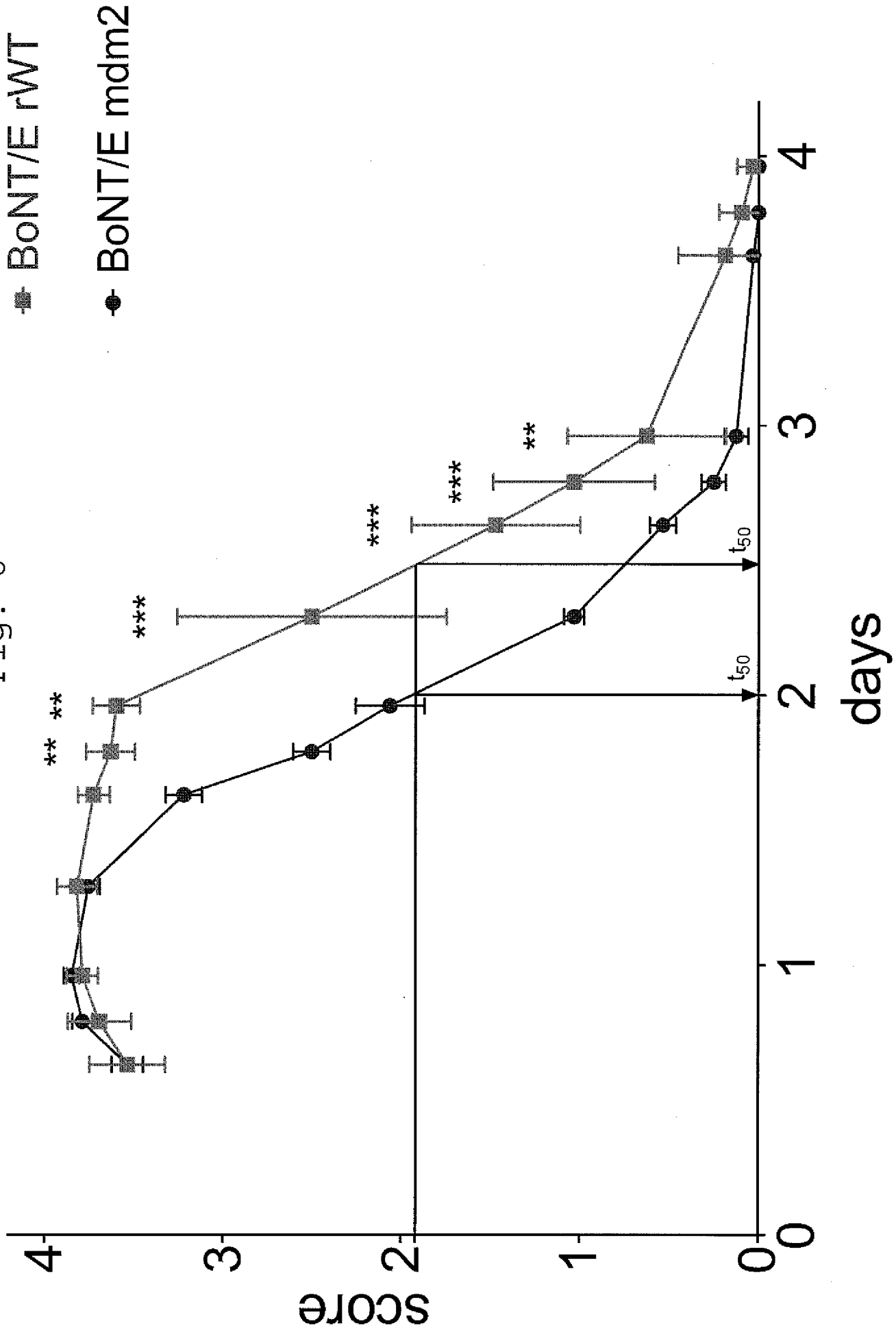


Fig. 7

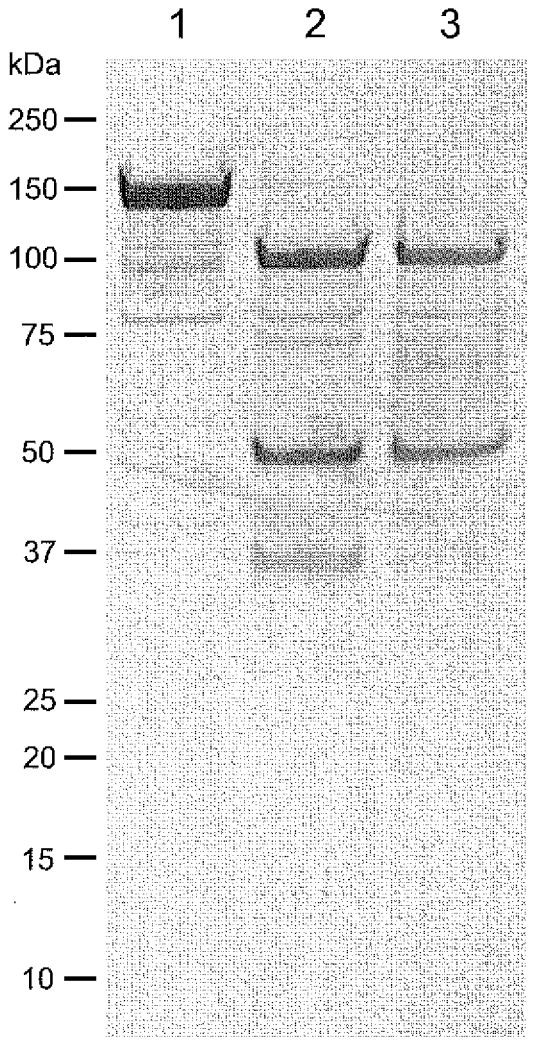


Fig. 8

