## Supplemental data for manuscript # MOLPHARM-AR-2020-000184

## Cysteine modification by ebselen reduces the stability and cellular levels of 14-3-3 proteins

Kai Waløen, Jung K.C. Kunwar, Elisa D. Vecchia, Sunil Pandey, Norbert Gasparik, Anne Døskeland, Sudarshan Patil, Rune Kleppe, Jozef Hritz, William H.J. Norton, Aurora Martinez, and Jan Haavik

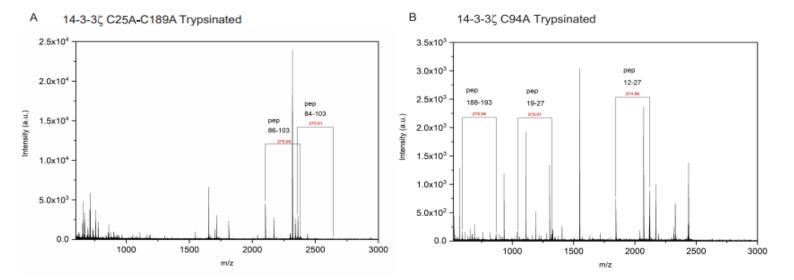
ID	Drug name	Formula	Therapeutic class/	$\Delta T_m$ at 400
			therapeutic effect	μΜ
				(∘C)
1	Fulvestrant*	CH <sub>3</sub> OH	Endocrinology/	-23.6
			Antineoplastic	
		HO F		
2	Cefoxitin	Г, ОН <sub>3</sub> С <sub>О Н</sub>	Metabolism/	-17.3
			Antibacterial	
3	Thimerosal		Infectiology/	-11.7
5	minerosa		Antiseptic	-11.7
4	Rabeprazole <sup>1</sup>		Metabolism/	-11.4
		N, OH,C, O	Antiulcer	
5	Mercaptopurine		Immunology/	-10.8
		Ň	Immunosuppressant	
		N S		
6	Thioguanosine		Metabolism/ Antineoplastic	-9.2
		HO OH NYN NH <sub>2</sub>		
7	Tenatoprazole <sup>1</sup>		Metabolism/	-8.6
			Antiulcer	
		н,с∕ ≫		
8	Altrenogest*	H <sub>3</sub> C <sup>OH</sup>	Endocrinology/	-8.4
			Progestogen	
		0 H		
9	Nifedipine <sup>2</sup>	H <sub>1</sub> C- <sup>O</sup> H <sub>1</sub> C <sup>-</sup> CH <sub>3</sub>	Cardiovascular/	-7.2
			Antianginal	
10	Pinaverium <sup>2</sup>	ис <sup>СН</sup> ,	Neuromuscular/	-6.7
		H <sub>3</sub> C CH <sub>3</sub> H	Antispastic	
		ó, (,) CH₃ o		
11	Ebselen		Metabolism/	-7.0
		N-Se	Anti-inflammatory	
		0 \_/		

12	Lansoprazole <sup>1</sup>	$ \begin{array}{c} & 0 & N \\ & & & & \\ & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & $	Metabolism/ Antiulcer	-6.0
13	Cefaclor		Infectiology/ Antibacterial	-6.0
14	Nisoldipine <sup>2</sup>	$H_{3}C \sim O$ $H_{3}C \sim N$ $H_{3}C \sim O$ $H_{3}C \sim O$ $H_{3}C \sim O$ $H_{3}C \sim O$ $H_{3}C \sim O$ $CH_{3}$ $CH_{3}$	Cardiovascular/ Antianginal	-5.8
15	Carbenoxolone <sup>*,3</sup>		Metabolism/ Antiulcer	-5.1

Supplemental Table 1. Small-molecule drug hits obtained from the primary DSF screen. The midpoint melting temperature ( $T_m$ ) of 14-3-3 $\zeta$  with 4% DMSO was 61.1 ± 0.5°C, and 15 hits that caused I $\Delta T_m I \ge 10 \times SD$  (5.1°C), used as a cut-off value, were selected for concentration dependent studies. Based on its consistent concentration-dependent effect and reduced toxicity, ebselen was selected for further studies. \*Steroids. <sup>1</sup>Proton pump inhibitor. <sup>2</sup>Calcium channel blocker <sup>3</sup>Probable calcium channel blocker

<pre>1 epsilon -MDDREDLUYQANLAEQAERTDEMA2NKVACMOVELTVEENNLLSVAYKNVIGARRASHRIISIEQKENKGOEDKL 2 sigma -MURRQLUVQANLAEQAERTDEMAANKVACHELGELS ZEENLLSVAYKNVUGARRASHRVISIEQKENKGOEDKL 4 eta -MGDRQLLQRARLAEQAERTDEMAANKVATELNEPLSNEENNLLSVAYKNVUGARRASHRVISIEQKENKGOEKKI 4 eta -MGDRQLLQRARLAEQAERTDEMAANKVATELNEPLSNEENNLLSVAYKNVUGARRASHRVISIEQKTSADGNEKKI 5 theta -MEKTELIQKAKLAEQAERTDEMAANKVATELNEPLSNEENNLLSVAYKNVUGARRASHRVISIEQKTSADGNEKKI 6 beta MTMEKSELUQKAKLAEQAERTDEMAANKVATEQGHELSNEENNLLSVAYKNVUGARRASHRVISIEQKTERNEKKQ 1 . 1 . 1</pre>			1	[	80
3 gamma       -MVDREQLVQKARLABQAERYDDMAAAMKAVTELNEPLSNEERNLLSVATKNVVQABRSSMRVISSIEQKTAADQREKKI         4 eta       -MEXTELIQKARLABQAERYDDMASAMKAVTELNEPLSNEERNLLSVATKNVVQABRSSMRVISSIEQKT-DTSDKKL         6 beta       -MEXTELIQKARLABQAERYDDMAAMKAVTEQGHELSNEERNLLSVATKNVVQABRSSMRVISSIEQKT-DTSDKKL         6 beta       MINDKSELVQKARLABQAERYDDMAAMKAVTEQGHELSNEERNLLSVATKNVVQABRSSMRVISSIEQKT-DSNKKL         7 zeta       -MEXTELIQKARLABQAERYDDMAAMKAVTEQGHELSNEERNLLSVATKNVVQABRSSMRVISSIEQKT-EBNEKKQ         1       1	1	epsilon		-MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEENKGGEDKL	
<pre>4 etaMGDREQLLQRARLABQAERYDDMASAMKAVTELNEPLSNEDRNLLSVAYKNVVGARRSSNRVISSIBQKTDTSDKKL 5 thetaMEKTELIQKAKLABQAERYDDMASAMKAVTEQGAELSNEERNLLSVAYKNVVGARRSSNRVISSIBQKTDTSDKKL 6 betaMEKNELVQKAKLABQAERYDDMAAMKAVTEQGAELSNEERNLLSVAYKNVVGARRSSNRVISSIBQKTEXNEKKQ 7 zetaMDKNELVQKAKLABQAERYDDMAAMKAVTEQGAELSNEERNLLSVAYKNVVGARRSSNRVVSSIBQKTEXNEKKQ 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1</pre>	2	sigma		MERASLIQKAKLAEQAERYEDMAAFMKGAVEKGEELS <mark>C</mark> EERNLLSVAYKNVVGGQRAAWRVLSSIEQKSNEEGSEEKG	
S theta      MEKTELIQKAKLAEQAERYDDMATOMKAVTEQGAELSNEERNLLSVAYKNVVGGRRSAMRVISSIEQKTDISDKKL         6 beta      MEKTELIQKAKLAEQAERYDDMAAMKAVTEQGAELSNEERNLLSVAYKNVVGGRRSAMRVISSIEQKTERNEKKQ         7 zeta      MEKNELVQKAKLAEQAERYDDMAAMKAVTEQGAELSNEERNLLSVAYKNVVGGRRSAMRVISSIEQKTERNEKKQ         1       1       .       1         1       1       .       1         1       1       .       1         2 sigma       1       .       1         3 gamma       EMVRAYREKIEKELEAVQODVLSLLDNYLIKMGSETQYESKVFYIKMKGDYRYLAEVATGGNDRKRAAENSUVAYKAASD         9 devrayrekieketeavoduvLsLDSKLIKEGEVVDESKVFYIKMKGDYRYLAEVATGGRAFKNVVESEKASVARAB         2 sigma       QUIKDYREKVESELASIGTTVLELLDKYLIKMGDYRYLAEVATGGNDRKRATVVESEKKNSVVEAREBAAYKEAFE         5 theta       QUIKDYREKVESELASIGTTVLELLDKYLIKMKGDYRYLAEVAGGDRKQTIDNSQQAYQEAFE         6 beta       QUKEYREKIEAELQDIONDVLELLDKYLIPNATQESKVFYLKMKGDYRYLAEVAGDDKKGIVDSQQAYQEAFE         1       2       .         2 eta       QUAREYREKIEAELQDIONDVLELLDKYLIPNATQESKVFYLKMKGDYRYLAEVAGDDKKGIVDSQQAYQEAFE         1       2       .       .         2 sigma       ISKKEMPTNPIKIETELDIONDVLELLDKYLIPNATQESKVFYLKMKGDYRYLAEVAGDKKGIVDSQQAYQEAFE         2 ieta       IAMTELPPTHPIRIGLALNPSVYYELIANPACHLAKAAFDDAIAELDTINEDSYKDSTLIMQLLRNNLTMYSDQD         3 gamma       ISKKEMPTNPIRIGLALNPSVYYELIANSPEAA	3	gamma		-MVDREQLVQKARLAEQAERYDDMAAAMKNVTELNEPLSNEERNLLSVAYKNVVGARRSSWRVISSIEQKTSADGNEKKI	
6       beta       MTMDKSELVQKAKLAEQAERYDDMAAAMKAVTEQGHELSNEERNLLSVAYKNVVGARRSSNRVISSIEQKTERNEKKQ         7       zeta      MDKNELVQKAKLAEQAERYDDMAAAMKAVTEQGHELSNEERNLLSVAYKNVVGARRSSNRVVSSIEQKTEGAEKKQ         1       1       1       .       160         1       1       .       1       .       160         1       1       .       .       160         1       1       .       .       160         1       1       .       .       160         1       1       .       .       160         1       1       .       .       160         1       1       .       .       160         1       1       .       .       160         2       .       .       .       160         1       .       1       .       .       160         2       .       .       .       .       160         3       gamma       EMVRAYREKIEKELETVONDULSLLOKYLINAGOTYKYLLAVAGDYRYLAEVAGEDRKGINSVESEKAYEANKAPE       240         1       .       2       .       .       240         1       .       2       .       .       .	4	eta		-MGDREQLLQRARLAEQAERYDDMASAMKAVTELNEPLSNEDRNLLSVAYKNVVGARRSSWRVISSIEQKTMADGNEKKL	
7     zeta    MCKNELVQKAKLAEQAERYDDMAACMKSVTEQGAELSNEERNLLSVAYKNVVGARRSSWRVVSSIEQKTEGAEKKQ       1     1	5	theta		MEKTELIQKAKLAEQAERYDDMATCMKAVTEQGAELSNEERNLLSVAYKNVVGGRRSAWRVISSIEQKTDTSDKKL	
1       .1       .1	6	beta		MTMDKSELVQKAKLAEQAERYDDMAAAMKAVTEQGHELSNEERNLLSVAYKNVVGARRSSWRVISSIEQKTERNEKKQ	
<pre>1 epsilon KMIREYRQMVETELKLIGCDILDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEPATGNDRKEAAENSLVAYKAASD 2 sigma PEVREYREKVETELQGVCDTVLGLLDSHLIKERGDAESRVFYLKMKGDYTRYLAEVATGDDKKRIIDSARSATQEAMD 3 gamma EMVRAYREKIEKELEAVCODVLSLLDNYLIKNGSETQYESKVFYLKMKGDYYRYLAEVATGDDKRAITUNESSEKAYSEAHE 4 eta EKVKAYREKIEKELETVCNDVLSLLDKYLINNCNDFQTESKVFYLKMKGDYTRYLAEVATGDRKATUVESSEKAYSEAHE 5 theta QLIKDYREKVESELRSICTTVLELLDKYLIANATNPESKVFYLKMKGDYTRYLAEVACGDDRKQTIDNSQGAYQEAFD 6 beta QMGREYREKIETELRDICNDVLELLDKYLIPNATQFSKVFYLKMKGDYFRYLAEVACGDDRKQTVSNSQQAYQEAFE 7 zeta QMAREYREKIETELRDICNDVLELLDKYLIPNASQAESKVFYLKMKGDYTRYLAEVACGDDRKQTVSNSQQAYQEAFE 2 sigma 1</pre>	7	zeta		MDKNELVQKAKLAEQAERYDDMAACMKSVTEQGAELSNEERNLLSVAYKNVVGARRSSWRVVSSIEQKTEGAEKKQ	
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2 sigma       PEVREYREKVETELOGVCDTVLGLLDSHLIKEAGDAESRVFYLKMKGDYYRYLAEVATGDDKKRIIDSARSAYQEAMD         3 gamma       EMVRATREKIEKELEAVCODVLSLLDNYLIKMCSETOYESKVFYLKMKGDYYRYLAEVATGERRATVVESSEKAYSEAHE         4 eta       EKVKAYREKIEKELETVCNDVLSLLDKFLIKMCNDFOYESKVFYLKMKGDYYRYLAEVASGEKKNSVVEASEAAYKEAFE         5 theta       QLIKOYREKVESELRSICTTVLELLDKYLIKMCNDFOYESKVFYLKMKGDYFRYLAEVASGEKKNSVVEASEAAYKEAFE         6 beta       QMGKEYREKIEAELQDICNDVLSLLDKFLIKMCNDFOYESKVFYLKMKGDYFRYLAEVASGEKKNSVVEASEAAYKEAFE         7 zeta       QMGKEYREKIEAELQDICNDVLSLLEKFLIPNATOPESKVFYLKMKGDYFRYLSEVASGDNKOTTVSNSQQAYQEAFE         1 epsilon       2			1	. 1	160
3 gamma       EMVRAYREK IEKELEAVCODVLSLLDNYL I KNČSETOYESKVFYLKMKGDYYRYLAEVATGEKRATVVESSEKAYSEAHE         4 eta       EKVKAYREK IEKELETVCNOVLSLLDKYL IKNČNDFOYESKVFYLKMKGDYRYLAEVAGGEKRATVVESSEKAYSEAHE         5 theta       QLIKDYREKVESELRS I CTTVLELLDKYL IKNČNDFOYESKVFYLKMKGDYRYLAEVAGGEDRKOT IDNSQGAYQEAPE         6 beta       QMGKEYREK IEAELQDI CNOVLELLDKYL INATNPESKVFYLKMKGDYRYLAEVAGGDDRKOT IDNSQGAYQEAPE         7 zeta       QMGKEYREK IEAELQDI CNOVLELLDKYL IPNATQPESKVFYLKMKGDYRYLAEVAAGDDKKGI VDOSQAYQEAPE         1	1	epsilon		KMIREYR@MVETELKLICCDILDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEPATGNDRKEAAENSLVAYKAASD	
<pre>4 eta EKVKAYREKIEKELETVCNDVLSLLDKPLIKNCNDPQYESKVPYLKMKGDYYRYLAEVASGEKKNSVVEASEAAYKEAFE 5 theta OLIKDYREKVESELRSIGTTVLELLDKYLIANATNPESKVPYLKMKGDYFRYLAEVASGEDRKQTIDNSQGAYQEAFE 6 beta OMGKEYREKIEAELQDICNDVLSLLEKPLIPNASQAESKVPYLKMKGDYFRYLAEVASGDDKKGIVDQSQQAYQEAFE 7 zeta 0 MAREYREKIETELRDICNDVLSLLEKPLIPNASQAESKVPYLKMKGDYYRYLAEVASGDDKKGIVDQSQQAYQEAFE 1</pre>	2	sigma		PEVREYREKVETELQGVCDTVLGLLDSHLIKEAGDAESRVFYLKMKGDYYRYLAEVATGDDKKRIIDSARSAYQEAMD	
5       theta       QLIKDYREKVESELRSIGTTVLELLDKYLIANATNPESKVFYLKMKGDYFRYLAEVAGGDDRKQTIDNSQGAYQEAFD         6       beta       QMGKEYREKIEAELQDICNDVLELLDKYLIPNATQPESKVFYLKMKGDYFRYLAEVAGDDRKQTIDNSQQAYQEAFE         7       zeta       QMAREYREKIETELRDICNDVLELLDKYLIPNASQAESKVFYLKMKGDYFRYLAEVAGDDRKQTIDNSQQAYQEAFE         1       .	3	gamma		emvrayrekiekeleav <mark>o</mark> odvlslldnylikn <mark>o</mark> setoyeskvfylkmkgdyyrylaevatgekratvvessekayseahe	
6 beta 7 zeta QMGREYREK I BAELQDI CNDVLELLDKYLI I PNATQPESKVFYI KMKGDYFRYLSEVASGDNKQTTVSNSQQAYQEAFE 7 zeta QMAREYREK I BELRDI CNDVLSLLEKFLI I PNASQAESKVFYI KMKGDYFRYLSEVASGDNKQTTVSNSQQAYQEAFE 1	4	eta		EKVKAYREKIEKELETV <mark>C</mark> NDVLSLLDKFLIKN <mark>C</mark> NDFQYESKVFYLKMKGDYYRYLAEVASGEKKNSVVEASEAAYKEAFE	
7 zeta       QMAREYREKIETELRDICADVLSLLEKFLIPNASQAESKVFYLKMKGDYYRYLAEVAAGDDKKGIVDQSQQAYQEAFE         1	5	theta		QLIKDYREKVESELRSI <mark>C</mark> TTVLELLDKYLIANATNPESKVFYLKMKGDYFRYLAEVA <mark>C</mark> GDDRKQTIDNSQGAYQEAFD	
1	6	beta		QMGKEYREKIEAELQDI <mark>C</mark> NDVLELLDKYLIPNATQPESKVFYLKMKGDYFRYLSEVASGDNKQTTVSNSQQAYQEAFE	
1 epsilon       IAMTELPPTHPIRLGLALNFSVFYYEILNSPDRACKLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDMQG         2 sigma       ISKEMPPTNPIRLGLALNFSVFYYEILNSPDRACKLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDMQG         3 gamma       ISKEMQPTHPIRLGLALNFSVFYYEIONAPEQACHLAKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQD         4 eta       ISKEMQPTHPIRLGLALNFSVFYYEIQNAPEQACHLAKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQD         5 theta       ISKEMQPTHPIRLGLALNFSVFYYEIQNAPEQACHLAKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQD         6 beta       ISKKEMQPTHPIRLGLALNFSVFYYEINNPELACTLAKTAFDEAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDAGG         7 zeta       ISKKEMQPTHPIRLGLALNFSVFYYEINNPELACTLAKTAFDEAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDTQG         1       :       ] 258         1 epsilon       DGEEQNKEALQDVEDENQ         2 sigma       EEGGEAPQEPQS         3 gamma       DDGGECONN	7	zeta		QMAREYREK IETELRD I <mark>C</mark> NDVLSLLEKFL I PNASQAESKVFYLKMKGDYYRYLAEVAAGDDKKG I VDQSQQAYQEAFE	
1 epsilon       IAMTELPPTHPIRLGLALNFSVFYYEILNSPDRACKLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDMQG         2 sigma       ISKEMPPTNPIRLGLALNFSVFYYEILNSPDRACKLAKAAFDDAIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDMQG         3 gamma       ISKEMPPTNPIRLGLALNFSVFYYEIONAPPQACHLAKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQD         4 eta       ISKEMQPTHPIRLGLALNFSVFYYEIQNAPPQACHLAKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQD         5 theta       ISKEMQPTHPIRLGLALNFSVFYYEIQNAPPQACHLAKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQD         6 beta       ISKKEMQPTHPIRLGLALNFSVFYYEINNPELACTLAKTAFDEAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDSAG         7 zeta       ISKKEMQPTHPIRLGLALNFSVFYYEINNPELACTLAKTAFDEAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDTQG         1       :       ] 258         1 epsilon       DGEEQNKEALQDVEDENQ         2 sigma       EEGGEAPQEPQS         3 gamma       DDGGECONN					
2 sigma       ISKKEMPPTNPIRLGLALNFSVFHYEIANSPEEAISLAKTTFDEAMADLHTLSEDSYKDSTLIMQLLRDNLTLWTADNAG         3 gamma       ISKEMMQPTHPIRLGLALNFSVFHYEIQNAPEQACHLAKTAFDDAIAELDTINEDSYKDSTLIMQLLRDNLTLWTADNAG         4 eta       ISKEMQPTHPIRLGLALNFSVFYYEIQNAPEQACHLAKTAFDDAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDQQD         5 theta       ISKEMQPTHPIRLGLALNFSVFYYEIQNAPEQACHLAKTAFDEAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDQQD         5 theta       ISKKEMQPTHPIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDAGG         7 zeta       ISKKEMQPTHPIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTINEESYKDSTLIMQLLRDNLTLWTSDTQG         1       :       ] 258         1 epsilon       DGEEQNKEALQDVEDENQ         2 sigma       EEGGEAPQEPQS         3 gamma       DDGGECON			1		240
3 gamma       ISKEHMQPTHPIRLGLALNYSVPYYEIQNAPEQACHLAKTAFDDAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDQQD         4 eta       ISKEMQPTHPIRLGLALNYSVPYYEIQNAPEQACLLAKQAPDDAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDQQD         5 theta       ISKKEMQPTHPIRLGLALNYSVPYYEIQNAPEQACLLAKQAPDDAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDQQD         6 beta       ISKKEMQPTHPIRLGLALNYSVPYYEILNNPELACTLAKTAFDEAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDSAG         7 zeta       ISKKEMQPTHPIRLGLALNYSVPYYEILNSPEKACSLAKTAFDEAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDTQG         1       :       ] 258         1       :       ] 258         2 sigma       EEGGEAPQEPQS         3 gamma       DDGGEGNKEALQDVEDENQ         4 eta       EEAGEGN         5 theta       EEGGEAPQEPQS         6 beta       DEGDAGEGEN	1				
4 eta     ISKEQMOPTHPIRLGLALNFSVFYYEIQNAPEQACLLAKQAFDDAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDQQD       5 theta     ISKKEMQPTHPIRLGLALNFSVFYYEILNNPELACTLAKTAFDEAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDSAG       6 beta     ISKKEMQPTHPIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTINEESYKDSTLIMQLLRDNLTLWTSDSAG       7 zeta     ISKKEMQPTHPIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTINEESYKDSTLIMQLLRDNLTLWTSDTQG       1     :     ] 258       1 epsilon     DGEEQNKEALQDVEDENQ       2 sigma     EEGGEAPQEPQS       3 gamma     DDGGEGNN	2				
5 theta       ISKKEMQPTHPIRLGLALNFSVFYYEILNNPELACTLAKTAFDEAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDSAG         6 beta       ISKKEMQPTHPIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTINEDSYKDSTLIMQLLRDNLTLWTSDSAG         7 zeta       ISKKEMQPTHPIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTINEESYKDSTLIMQLLRDNLTLWTSDTQG         1       :       ] 258         1 epsilon       DGEEQNKEALQDVEDENQ         2 sigma       EEGGEAPQEPQS         3 gamma       DDGGECONN	3	-			
6 beta ISKKEMQPTHFIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTINEESYKDSTLINQLLRDNLTLWTSENQG 7 zeta ISKKEMQPTHFIRLGLALNFSVFYYEILNSPEKACSLAKTAFDEAIAELDTLSEESYKDSTLINQLLRDNLTLWTSENQG 1 : ] 258 1 epsilon DGEEQNKEALQDVEDENQ 2 sigma EEGGEAPQEPQS 3 gamma DDGGECON	4				
7 zeta ISKKEMQPTHPIRLGLALNPSVFYYEILNSPEKACSLAKTAFDEAIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDTQG 1 : ] 258 1 epsilon DGEEQNKEALQDVEDENQ 2 sigma EEGGEAPQEPQS 3 gamma DDGGEGNN 4 eta EEGGAAPGEPQ	5			·····	
1       :       ] 258         1       epsilon       DGEEQNKEALQDVEDENQ         2       sigma       EEGGEAPQEPQS         3       gamma       DDGGEGNN         4       eta       EEGCAAEGAEN         5       theta       EECDAAEGAEN	6			• • • • • • • • • • • • • • • • • • • •	
1 epsilon DGEEQNKEALQDVEDENQ 2 sigma EEGGEAPQEPQS 3 gamma DDGGECNN 4 eta EEAGEGN 5 theta EECDAREGAEN 6 beta DEGDAGEGEN	7	zeta		I SKKEMQPTHPIRLGLALNPSVPYYEI LNSPEKA <mark>C</mark> SLAKTAFDEA I AELDTLSEESYKDSTLIM <u>O</u> LLRDNLTLWTSDTQG	
1 epsilon DGEEQNKEALQDVEDENQ 2 sigma EEGGEAPQEPQS 3 gamma DDGGECNN 4 eta EEAGEGN 5 theta EECDAAEGAEN 6 beta DEGDAGEGEN					
2 sigma EEGGEAPQEPQS 3 gamma DDGGEGNN 4 eta EEGGEAPQEPQS 5 theta EECDAREGAEN 6 beta DEGDAGEGEN			1		
3 gamma DDGGEGNN 4 eta EEAGEGN 5 theta EECDAAEGAEN 6 beta DEGDAGEGEN	-	*			
4 eta     EEAGEGN       5 theta     EECDAAEGAEN       6 beta     DEGDAGEGEN	2				
5 theta EECDARGAEN	3	-			
6 beta DEGDAGEGEN	1				
	6				
	7				
		2004		DERENGOUGH	

Supplemental Figure S1. Amino acid sequences of the seven isoforms of 14-3-3 are presented. The cysteine residues are highlighted in yellow.



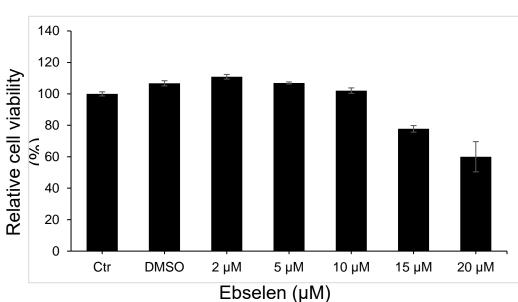
Supplemental Figure S2. MALDI-TOF MS/MS peptide analysis of trypsin digested 1433ζ mutant constructs. a) 1433ζ-C25A-C189A and b) 1433ζ-C94A, after ebselen treatment. Peptides containing cysteines in tagged and untagged form (peptides with a mass shift of 275 Da) are denoted. In a) the figure shows two peptides involving ebselen tagging of C94 in very small amounts. In b) the figure shows a peptide involving C189 and two peptides involving C25.

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**Supplementary Figure S3.** Viability of SHSY5Y cells treated with increasing concentration of ebselen and ebselen oxide. A Cell Titer-Blue assay was performed to examine cell viability of SHSY5Y cells treated with increasing concentration of ebselen and ebselen oxide. The cells were plated in 96-well plates at the density of 25000 cells per well and 5 h later were treated with different concentration of ebselen, ebselen oxide and DMSO (0.05%). Cells were then incubated for 16 h and then 20  $\mu$ L of Cell Titre Blue reagent was added to each well. The plates were incubated for 2hrs before the fluorescence ( $\lambda$ =590) was measured with Victor 3 1420 Multilabel counter plate reader. A, Cell images using ebselen, B, relative cell viability using ebselen, C Cell images using ebselen oxide D, relative cell viability with ebselen oxide. Statistical analyses by 2-tailed Student's t test. (\*) p< 0.05, (\*\*) p < 0.005, (\*\*\*) p < 0.001, (\*\*\*\*) p < 0.0001. All data are presented as mean ± SEM.

Ebselen Control 2µM 10µM 20µM 0.05% DMSO 5µM 15µM

В



Α

Ebselen oxide

