Table S3. Peptides Derived from Caspase Cleavage Sites Identified by N-CLAP Approach after Drug Treatment of Jurkat T-Cells

Ĥ	DIC D	J. I (срис	ics Derived	1110111	aspase Cleavage Sites Identified by N-	CLAI A	pproaci	laitei		Tcatine	III OI JU	II Nat I -		1	1			
#	Score	Fwd- Rev Score	SPI %	N-terminal Modification	Start AA Position	Sequence Map	Modification	m/z Measured (Da)	MH ⁺ Matched (Da)	MH ⁺ Mass Shift (Da)	MH ⁺ Error (ppm)	Protein MW (Da)	Accession #	Protein Name	4 AAs Before Cleavage Sites	Cleavage Site	Sample	Refs. Reported	Site Report (Y/N)
1	15.47			HSCH ₂ CH ₂ CO-		$(G)VTHTV PI\YEG\Y/A/L/P/HAIL/R(L)$		746.7372	2238.174	0.0231			P60709	Actin, cytoplasmic 1	DSGD	157	treated	1	N
2	9.89	5.6	66.6	HSCH ₂ CH ₂ CO-	266	(M)SK/P/DL/T/A/ALR(D)	K:PITC	647.818	1294.628	0.0005	0.4	53536	P17661	Desmin *	VEMD	264	treated	2	Y
3	13.96	13.96	78.9	HSCH₂CH₂CO-	8	(F)L/GQDSDGG\S\EE\V V L/T/PAE/L/IE/R(L)		1201.5707	2402.139	-0.005	-2.1	26047	Q9BRT9	DNA replication complex GINS protein SLD5	EEVD	6	treated	N/A	N
4	12.69	12.69	61.2	HSCH ₂ CH ₂ CO-	95	(S)LKE A L T/Y/D/G/AL/L/GDR(S)	K:PITC	929.4416	1857.887	-0.0114	-6.2	27385.2	Q15056	Eukaryotic translation initiation factor 4H	DEVD	93	treated	3	Y
5	12.78	-0.16	71.1	HSCH ₂ CH ₂ CO-	137	(L)S/P/P/PQ/AA/R(R)		456.2223	911.44	-0.003	-3.3	23410.1	P63118	HERV-H_3q26 provirus ancestral Gag polyprotein **	DPSD	135	treated	N/A	N
				HSCH ₂ CH ₂ CO-		$(G) ASA \setminus E \setminus T \setminus E \mid PWA \setminus A \mid A \mid V \mid PPEWV \mid PII \mid QQDIQ \mid SQR(K)$			3205.562	-0.017	-5.3	119409.3		Large proline-rich protein BAT3	DEQD	1001	treated	4	Y
7	14.28	6.81	79.4	HSCH ₂ CH ₂ CO-	1162	(T)T/A A/Q/Q/E/L/R(T)		502.7451	1004.483	0	0	228940.2	P35580	Myosin-10 ***	DTLD	1160	treated	N/A	N
				HSCH₂CH₂CO-		$(G)LV E T P\T/G/Y/I/E/S/L/PR(V)$			1662.841		0.8		P55209	Nucleosome assembly protein 1-like 1	ERLD	57	treated	3, 5	Y
9	19.34	10.84	89.1	HSCH ₂ CH ₂ CO-	520	(D)II V N W/V/N/E/T/L/R(E)		722.8809	1444.762	-0.0072	-5	70289.7	P13796	Plastin-2	KVND	518	treated	N/A	N
10	15.01	7.43	81.5	HSCH ₂ CH ₂ CO-	16	(A)L/P\Y/F/D/QGY/E/A/PGVR(E)		850.3974	1699.778	0.0091	5.3	26131.6	O75934	Pre-mRNA-splicing factor SPF27	VVVD	14	treated	N/A	N
11	17.41	17.41	78.9	HSCH ₂ CH ₂ CO-	195	(N)GDV\C\QDC\I/Q/M/V/T/D/I/Q/T/A/VR(T)		728.3306	2182.96	0.0173	7.9	58113.1	P07602	1 /1 1	QPKD	193	both	N/A	N
12	15.19	15.19	79.1	HSCH ₂ CH ₂ CO-	140	(N)EE\A\A\LLHEEAT\M/T I/E/E/L/L/T/R(Y)		796.3898	2387.147	0.008	3.3	59271.9	O15355	Protein phosphatase 1G	DEDD	138	treated	N/A	N
13	2 20.63	20.63	98.8	HSCH ₂ CH ₂ CO-	586	(A)A E L V E T I A A T A R E		666.8489	1332.683	0.0077	5.8	86707.4	Q6P996	Pyridoxal-dependent decarboxylase domain-containing protein 1	DNVD	584	treated	N/A	N
14	10.13	10 13	01.2	HSCH ₂ CH ₂ CO-	7	(G)DD\S\L Y PI/A/VL/I/D/E/LR(N)		910.4593	1810 015	0.0033	-1.8	65309	P30153	Serine/threonine- protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	AAAD	5	treated	6	N
14	17.13	17.13	71.2	113C112CH2CU-	1	(O)DD/D E I II/N VEIUDIE/ER(N)		710.4373	1017.713	-0.0033	-1.0	05507	1 30133	Splicing factor U2AF	ллли	J	исакси	U	14
15	20.87	20.87	94.7	HSCH ₂ CH ₂ CO-	130	$(G)L\backslash A\backslash V T PT/PV/P/V/V/GSQMT/R(Q)$		614.3298	1840.966	0.0089	4.8	53501.3	P26368	65 kDa subunit	MTPD	128	treated	3	Y
16				HSCH ₂ CH ₂ CO-		(S)I E A N V E/S/SEV/HV E/R(A)		843.393	1685.78	-0.0012	-0.7	31642.1	Q86Y82	Syntaxin-12	DLID	217	treated	3	Y
				HSCH ₂ CH ₂ CO-		(S)I E A N V E/N/A/E/V/HVQQANQQLSR(A)		822.4052		0.0173	7		O15400	Syntaxin-7	DVID	204	treated	7	Y
18	15.32	6.81	85.6	HSCH ₂ CH ₂ CO-	154	(A)GL D A L S/S/II/S/R(Q)		610.3203	1219.635	-0.0018	-1.5	26906.8	Q9UNK0	Syntaxin-8	QEQD	152	treated	8	N
19	19.45	19.45	90.6	HSCH₂CH₂CO-	8	(Q)Q Q\T T/N/T V E/E/PL/D/LI/R(L)		922.9606	1844.906	0.0081	4.4	11845.5	P62310	U6 snRNA-associated Sm-like protein LSm3	DDVD	6	treated	3	Y
	10.63			HSCH ₂ CH ₂ CO-		(A)SS/T/VP/SLCTEAR(A)		669.8173	1338.603		18.3		Q96NJ1		GFPD	52	treated	N/A	N
21	18.70	18.7	93.1	HSCH ₂ CH ₂ CO-	159	$(Q)IG\SE I I E E L/G/E/Q/R(D)$		780.8819	1560.757	-0.0009	-0.6	26688.6	Q9UEU0	Vesicle transport	TETD	157	treated	N/A	N

															through interaction with t-SNAREs homolog 1B					
2	2 2	14.00	14	77.3	HSCH ₂ CH ₂ CO-	87	$(F)SL\backslash A\backslash D\backslash A I N/T E/FK N/TR(T)$	K:PITC	901.9211	1802.82	0.0149	8.3	53651.9	P08670	Vimentin	DSVD	85	treated	7, 9, 10	Y
2	3 2	12.05	5.23	73.1	HSCH ₂ CH ₂ CO-	259	$(V)DV\S K/P/D/L/TAAL/R(D)$	K:PITC	754.8691	1508.724	0.0073	4.9	53651.9	P08670	Vimentin	VQID	257	treated	7, 9, 10	Y

^{*}This peptide is the same one as the N-CLAP peptide (261-270) generated from vimentin which is cleaved by caspases at the position of 259. Here only Desmin is listed. This peptide has a lower score. Because this caspase-cleavage site has been previously identified (Ref. 2), we included this peptide in our list.

References:

- 1. Chen Z, Naito M, Mashima T, & Tsuruo T (1996) Activation of actin-cleavable interleukin 1beta-converting enzyme (ICE) family protease CPP-32 during chemotherapeutic agent-induced apoptosis in ovarian carcinoma cells. *Cancer Res* 56:5224-5229.
- 2. Chen F, Chang R, Trivedi M, Capetanaki Y, & Cryns VL (2003) Caspase proteolysis of desmin produces a dominant-negative inhibitor of intermediate filaments and promotes apoptosis. *J Biol Chem* 278:6848-6853.
- 3. Van Damme P, et al. (2005) Caspase-specific and nonspecific in vivo protein processing during Fas-induced apoptosis. Nat Methods 2:771-777.
- 4. Wu YH, Shih SF, & Lin JY (2004) Ricin triggers apoptotic morphological changes through caspase-3 cleavage of BAT3. J Biol Chem 279(18):19264-19275.
- 5. Thiede B, Treumann A, Kretschmer A, Sohlke J, & Rudel T (2005) Shotgun proteome analysis of protein cleavage in apoptotic cells. *Proteomics* 5:2123-2130.
- 6. Santoro MF, et al. (1998) Regulation of protein phosphatase 2A activity by caspase-3 during apoptosis. J Biol Chem 273:13119-13128.
- 7. Mahrus S, et al. (2008) Global sequencing of proteolytic cleavage sites in apoptosis by specific labeling of protein N termini. Cell 134:866-876.
- 8. Wong SH, Santambrogio L, & Strominger JL (2004) Caspases and nitric oxide broadly regulate dendritic cell maturation and surface expression of class II MHC proteins. *Proc Natl Acad Sci U S A* 101:17783-17788.
- 9. Byun Y, et al. (2001) Caspase cleavage of vimentin disrupts intermediate filaments and promotes apoptosis. Cell Death Differ 8:443-450.
- 10. Yang X, et al. (2005) Cleavage of p53-vimentin complex enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of rheumatoid arthritis synovial fibroblasts. Am J Pathol 167:705-719.

^{**} This peptide has a low For-Rev Score. Since its homologue, HERV-K_5q33.3 provirus ancestral Gag polyprotein, is cleaved by caspases and the cleavage site is conserved in HERV-H_3q26, we included this cleavage site as a potential caspase cleavage site.

^{***} This peptide is the same one as the N-CLAP peptide (1155-1162) generated from Myosin-9 which is cleaved by caspases at the position of 1153. Here only Myosin-10 is listed.