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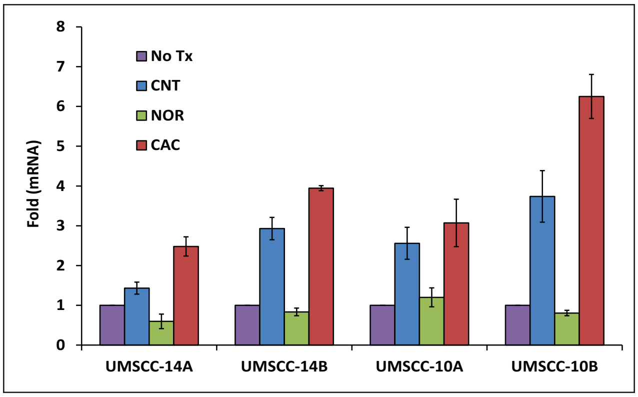
Cantharidins Induce ER Stress and a Terminal Unfolded Protein Response in OSCC

Y. Xi[1,2,*], D.M. Garshott[2,*], A.L. Brownell[2], G.H. Yoo[3], H.-S. Lin[3], T.L. Freeburg[3], N.G. Yoo[3], R.J. Kaufman[4], M.U. Callaghan[2], and A.M. Fribley[2,3,5]

Appendix



Appendix Figure 1. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis of CHOP messenger RNA transcripts in a panel of oral squamous cell carcinoma (OSCC) cell lines treated with 10 μM cantharidin, norcantharidin, and cantharidic acid for 6 h. For RT-qPCR, 18S ribosomal RNA was used as internal control, and error bars represent standard deviation of technical replicates.

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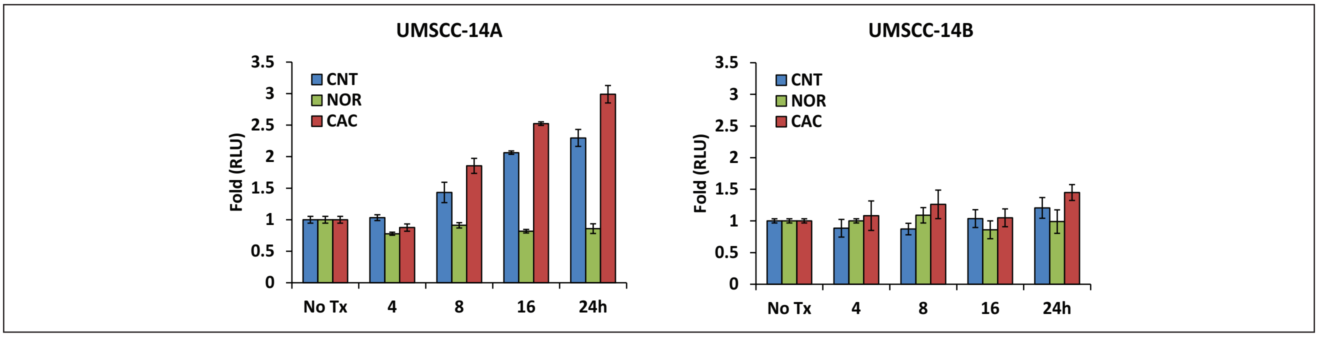
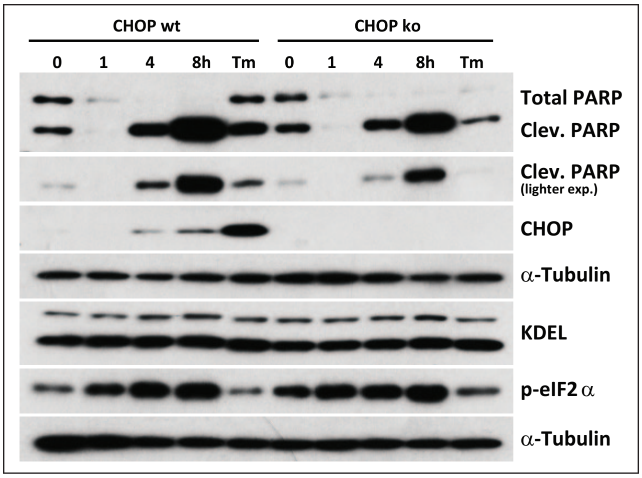
[1] The Key Laboratory of Pathiobiology, Ministry of Education, Norman Bethune College of Medicine, Jilin University, Changchun, China
[2] Carmen and Ann Adams Department of Pediatrics, Division of Hematology Oncology, Children's Hospital of Michigan, Detroit, MI, USA
[3] Department of Otolaryngology, Wayne State University and Karmanos Cancer Institute, Detroit, MI, USA
[4] Degenerative Diseases Program, Sanford|Burnham Medical Research Institute, La Jolla, CA and Department of Pharmacology, University of California Medical Center, La Jolla, CA, USA
[5] Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Detroit, MI, USA
[*] Authors contributing equally to this article.

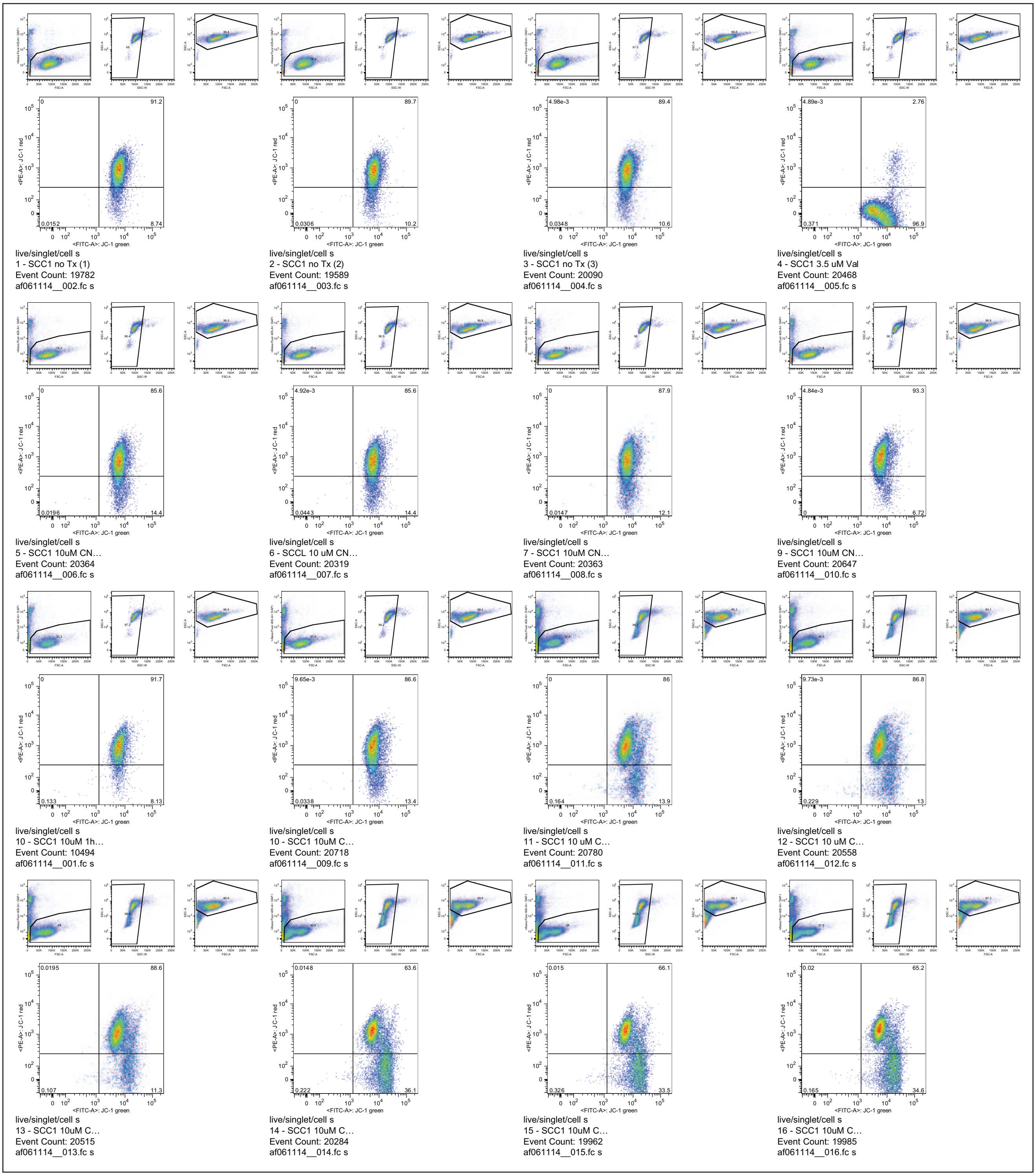
Corresponding Authors:
R.J. Kaufman, Degenerative Diseases Program, Sanford|Burnham Medical Research Institute, La Jolla, CA 92037 and Department of Pharmacology, University of California Medical Center, 10901 North Torrey Pines Road, rm 7103, La Jolla, CA 92037, USA.
Email: rkaufman@sanfordburnham.org
A.M. Fribley, Wayne State University School of Medicine 421 E. Canfield, rm 2228 Detroit, MI 48201, USA.
Email: afribley@med.wayne.edu



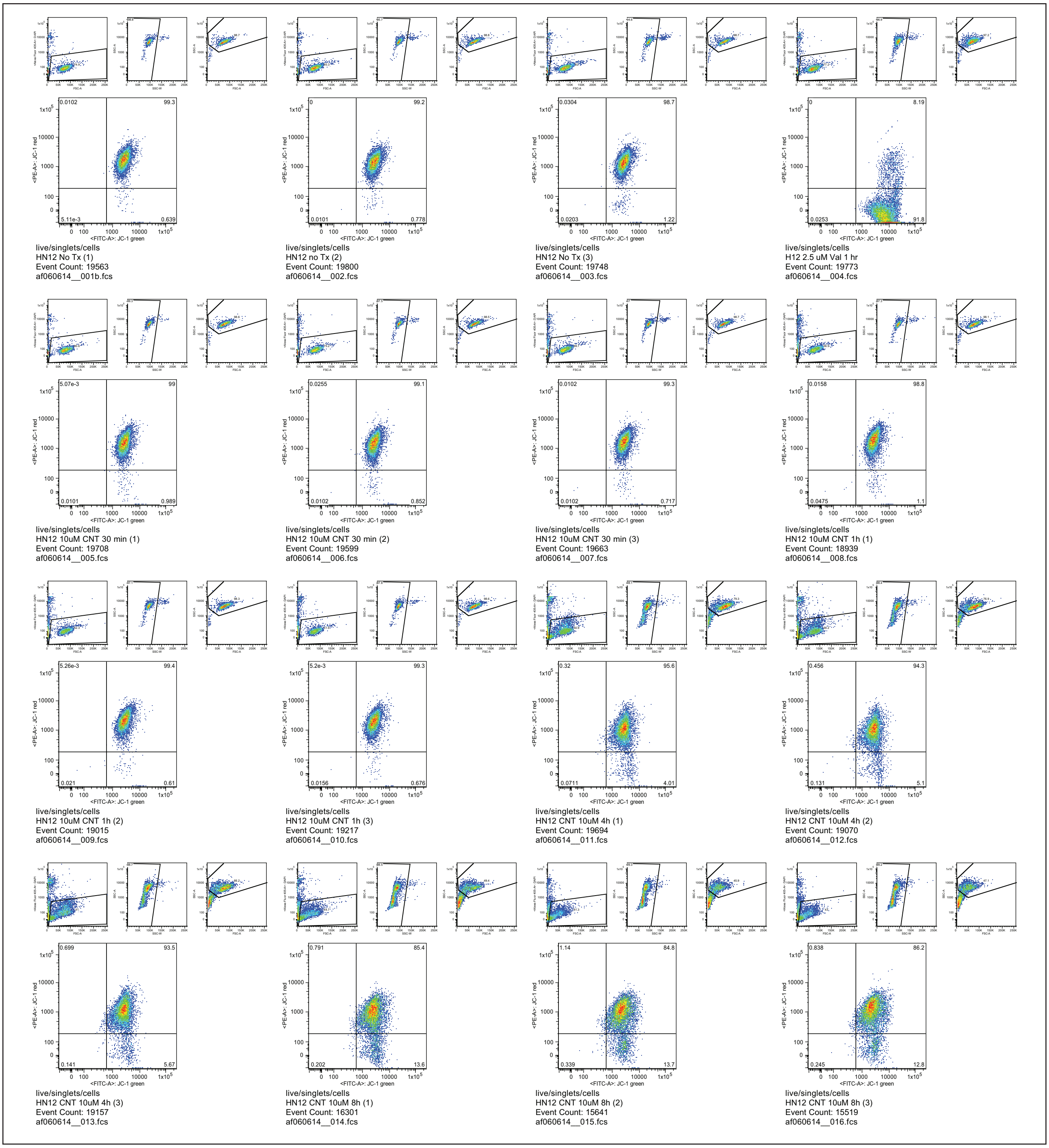
Appendix Figure 2. Luminescent caspase 3/7 enzymatic assay in 2 oral squamous cell carcinoma (OSCC) cell lines treated with 10 μM cantharidin, norcantharidin, and cantharidic acid for 4, 8, 16, and 24 h. Error bars represent standard deviation of technical replicates.



Appendix Figure 3. Immunoblot analysis of whole-cell lysates from *Chop* wt and knockout MEF with polyclonal antibodies for PARP, CHOP, KDEL (GRP78 and GRP94), and phospo-eIF2α.



Appendix Figure 4. Fluorescence-activated cell sorting data used to generate the graph in Figure 3C.



Appendix Figure 5. Fluorescence-activated cell sorting data used to generate the graph in Figure 3C.

Appendix Figure 6. Fluorescence-activated cell sorting data used to generate the graph in Figure 3C.

Appendix Table. RT2 Profiler Polymerase Chain Reaction Array Analysis of 86 Human Apoptotic Messenger RNA Transcripts, Performed and Analyzed According to the Manufacturer's Protocol, in UMSCC-1 Cells Treated with 10 µM Cantharidin for 4, 8, and 12 h.

		Fold Change		
Human Gene	Symbol	4	8	12h
C-abl oncogene 1, non-receptor tyrosine kinase	ABL1	1.0281	−1.09433	−1.71703
V-akt murine thymoma viral oncogene homolog 1	AKT1	1.057	1.0867	−1.77778
Apoptotic peptidase activating factor 1	APAF1	**−3.45781**	**2.2038**	−1.07181
BCL2-associated agonist of cell death	BAD	1.3104	−1.19746	**−2.49688**
BCL2-associated athanogene	BAG1	1.0281	1.3104	1.4241
BCL2-associated athanogene 3	BAG3	−1.07921	−1.1892	−1.74125
BCL2-associated athanogene 4	BAG4	**−7.57002**	−1.55836	**−2.04207**
BCL2-antagonist/killer 1	BAK1	1.2311	−1.54751	−1.89215
BCL2-associated X protein	BAX	−1.07921	−1.44383	−1.94515
B-cell CLL/lymphoma 10	BCL10	**−3.70645**	1.366	1.7901
B-cell CLL/lymphoma 2	BCL2	−1.08672	1.3851	1.1647
BCL2-related protein A1	BCL2A1	−1.6818	−1.17288	1.0867
BCL2-like 1	BCL2L1	−1.14863	−1.14077	−1.80245
BCL2-like 10 (apoptosis facilitator)	BCL2L10	−1.23977	**2.3457**	**3.8106**
BCL2-like 11 (apoptosis facilitator)	BCL2L11	−1.3947	1.815	1.7901
BCL2-like 2	BCL2L2	−1.10193	1.366	−1.4241
BCL2-associated transcription factor 1	BCLAF1	**−41.6667**	−1.20584	−1.65865
Bifunctional apoptosis regulator	BFAR	−1.6818	−1.40449	−1.82782
BH3 interacting domain death agonist	BID	1.0792	−1.12511	−1.69348
BCL2-interacting killer (apoptosis-inducing)	BIK	1.1892	1.9319	**2.6208**
NLR family, apoptosis inhibitory protein	NAIP	**−4.85673**	**2.2346**	1.1567
Baculoviral IAP repeat containing 2	BIRC2	**−16**	1.3472	−1.19746
Baculoviral IAP repeat containing 3	BIRC3	**−50.2513**	1.1975	−1.90512
X-linked inhibitor of apoptosis	XIAP	**−6.02047**	1.9453	−1.14077
Baculoviral IAP repeat containing 6	BIRC6	**−12.6422**	−1.16469	1.2311
Baculoviral IAP repeat containing 8	BIRC8	−1.18106	1.1567	2.1435
BCL2/adenovirus E1B 19kDa interacting protein 1	BNIP1	1.0718	−1.29232	**−3.2041**
BCL2/adenovirus E1B 19kDa interacting protein 2	BNIP2	**−9.38086**	1.3851	−1.12511
BCL2/adenovirus E1B 19kDa interacting protein 3	BNIP3	−1.38504	1.1329	1.0943
BCL2/adenovirus E1B 19kDa interacting protein 3-like	BNIP3L	**−2.92826**	1.7654	1.9185
V-raf murine sarcoma viral oncogene homolog B1	BRAF	**−2.84819**	1.7053	1.1408
Nucleotide-binding oligomerization domain containing 1	NOD1	−1.46413	**2.9079**	1.3287
Caspase recruitment domain family, member 6	CARD6	**−2.12857**	1.5801	1.4241
Caspase recruitment domain family, member 8	CARD8	**−7.1582**	1.5801	−1.00695
Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	CASP1	**−7.36377**	**−2**	−1.37552
Caspase 10, apoptosis-related cysteine peptidase	CASP10	**−3.50631**	−1.04243	**−3.41064**
Caspase 14, apoptosis-related cysteine peptidase	CASP14	−1.18106	1.1567	**2.1435**
Caspase 2, apoptosis-related cysteine peptidase	CASP2	−1.31044	−1.4339	−3.13873

(continued)

Appendix Table. (continued)

		Fold Change		
Human Gene	Symbol	4	8	12h
Caspase 3, apoptosis-related cysteine peptidase	CASP3	**−8.87311**	1.0943	−1.20584
Caspase 4, apoptosis-related cysteine peptidase	CASP4	**−2.25023**	−1.25707	−1.18106
Caspase 5, apoptosis-related cysteine peptidase	CASP5	−1.18106	1.1567	**2.1435**
Caspase 6, apoptosis-related cysteine peptidase	CASP6	−1.17288	−1.02103	−1.3947
Caspase 7, apoptosis-related cysteine peptidase	CASP7	−1.47427	1.4845	**−2.05634**
Caspase 8, apoptosis-related cysteine peptidase	CASP8	**−2.09952**	−1.14863	**−2.11416**
Caspase 9, apoptosis-related cysteine peptidase	CASP9	1.1173	1.7291	**2.042**
CD40 molecule, TNF receptor superfamily member 5	CD40	−1.14077	−1.02103	1.1096
CD40 ligand	CD40LG	−1.18106	1.1567	**2.1435**
CASP8 and FADD-like apoptosis regulator	CFLAR	**−2.05634**	−1.33797	**−2.56674**
Cell death-inducing DFFA-like effector a	CIDEA	1.2924	1.3566	**2.1435**
Cell death-inducing DFFA-like effector b	CIDEB	−1.01399	−1.37552	**−5.8548**
CASP2 and RIPK1 domain containing adaptor with death domain	CRADD	−1.3947	−1.51561	1.014
Death-associated protein kinase 1	DAPK1	−1.18106	1.8921	**7.0128**
DNA fragmentation factor, 45kDa, alpha polypeptide	DFFA	−1.32873	−1.38504	**−2.53165**
Fas (TNFRSF6)-associated via death domain	FADD	−1.02807	−1.14863	**−2.62055**
Fas (TNF receptor superfamily, member 6)	FAS	**−20.6612**	−1.3947	**−2.32937**
Fas ligand (TNF superfamily, member 6)	FASLG	−1.18106	1.1567	**2.1435**
Harakiri, BCL2 interacting protein (contains only BH3 domain)	HRK	1.1329	**2.0279**	**2.0139**
Insulin-like growth factor 1 receptor	IGF1R	−1.91865	1.1173	1.1408
Lymphotoxin alpha (TNF superfamily, member 1)	LTA	1.454	**4.0278**	**5.1337**
Lymphotoxin beta receptor (TNFR superfamily, member 3)	LTBR	−1.51561	−1.97239	**−2.49688**
Myeloid cell leukemia sequence 1 (BCL2-related)	MCL1	**−3.07409**	1.021	1.4241
Nucleolar protein 3 (apoptosis repressor with CARD domain)	NOL3	−1.53681	−1.20584	1.3104
PYD and CARD domain containing	PYCARD	1.1975	−1.46413	−1.61316
Receptor-interacting serine-threonine kinase 2	RIPK2	**−4.14079**	1.5801	−1.09433
Tumor necrosis factor	TNF	1.5476	**3.0525**	1.7171
Tumor necrosis factor receptor superfamily, member 10a	TNFRSF10A	1.1975	1.6358	**−2.88767**
Tumor necrosis factor receptor superfamily, member 10b	TNFRSF10B	1.4142	1.7411	−1.94515
Tumor necrosis factor receptor superfamily, member 11b	TNFRSF11B	**−4.14079**	−1	−1.63586
Tumor necrosis factor receptor superfamily, member 1A	TNFRSF1A	−1.4241	−1.60205	−1.82782
Tumor necrosis factor receptor superfamily, member 21	TNFRSF21	**−2.29727**	**−2.25023**	**−5.54017**
Tumor necrosis factor receptor superfamily, member 25	TNFRSF25	1.2311	**5.3889**	1.0353
CD27 molecule	CD27	−1.10951	**3.4581**	1.9053
Tumor necrosis factor receptor superfamily, member 9	TNFRSF9	1.9453	**9.8492**	**7.0128**
Tumor necrosis factor (ligand) superfamily, member 10	TNFSF10	**−12.5471**	1.1408	1.3379
CD70 molecule	CD70	1.3013	1.3013	−1.11732
Tumor necrosis factor (ligand) superfamily, member 8	TNFSF8	−1.18106	**5.2054**	**2.1435**
Tumor protein p53	TP53	−1.80245	1.007	**−2.75103**

(continued)

Appendix Table. (continued)

		Fold Change		
Human Gene	Symbol	4	8	12h
Tumor protein p53 binding protein, 2	TP53BP2	**–4.0833**	1.5157	−1.21418
Tumor protein p73	TP73	−1.18106	1.1567	**2.1435**
TNFRSF1A-associated via death domain	TRADD	−1.0353	−1.46413	−1.75316
TNF receptor-associated factor 2	TRAF2	1.3013	1.007	**–2.75103**
TNF receptor-associated factor 3	TRAF3	1.1173	1.2483	−1.56912
TNF receptor-associated factor 4	TRAF4	1.3104	1.1019	**–434.783**
Beta–2-microglobulin	B2M	**–2.11416**	−1.04976	−1.36612
Hypoxanthine phosphoribosyltransferase 1	HPRT1	**–4.31779**	−1.49477	−1.3947
Ribosomal protein L13a	RPL13A	−1	−1	−1
Glyceraldehyde–3-phosphate dehydrogenase	GAPDH	1.0644	−1.17288	−1.13289
Actin, beta	ACTB	1.1647	−1.26582	−1.89215
Human Genomic DNA Contamination	HGDC	−1.18106	1.1567	**2.1435**

Blue values represent genes increased ≥ 2 fold, and red values represent genes decreased ≥ to normalized controls, as described in the manufacturers' protocol.