SUPPLEMENTAL EXPERIMENTAL PROCEDURES, FIGURES AND TABLES FOR ALBERSHARDT ET AL

Reagents and Antibodies

BH3 mimetics were obtained as follows: ABT-737 from Abbott Laboratories (Abbott Park, IL); GX15-070 from Gemin X Pharmaceuticals (Malvern, PA); apogossypol from NCI/CTEP (Bethesda, MD); gossypol, HA14-1, and 2-methoxy-antimycin A_3 from Enzo Life Sciences Inc. (Farmingdale, NY); S1 was synthesized according to a previously published method (1). Bortezomib was purchased from LC Laboratories (Woburn, MA), Hoechst 33342 from Molecular Probes (Eugene, OR), zVAD-fmk from Enzyme Systems Products, Inc. (Livermore, CA), and digitonin from Sigma (St. Louis, MO).

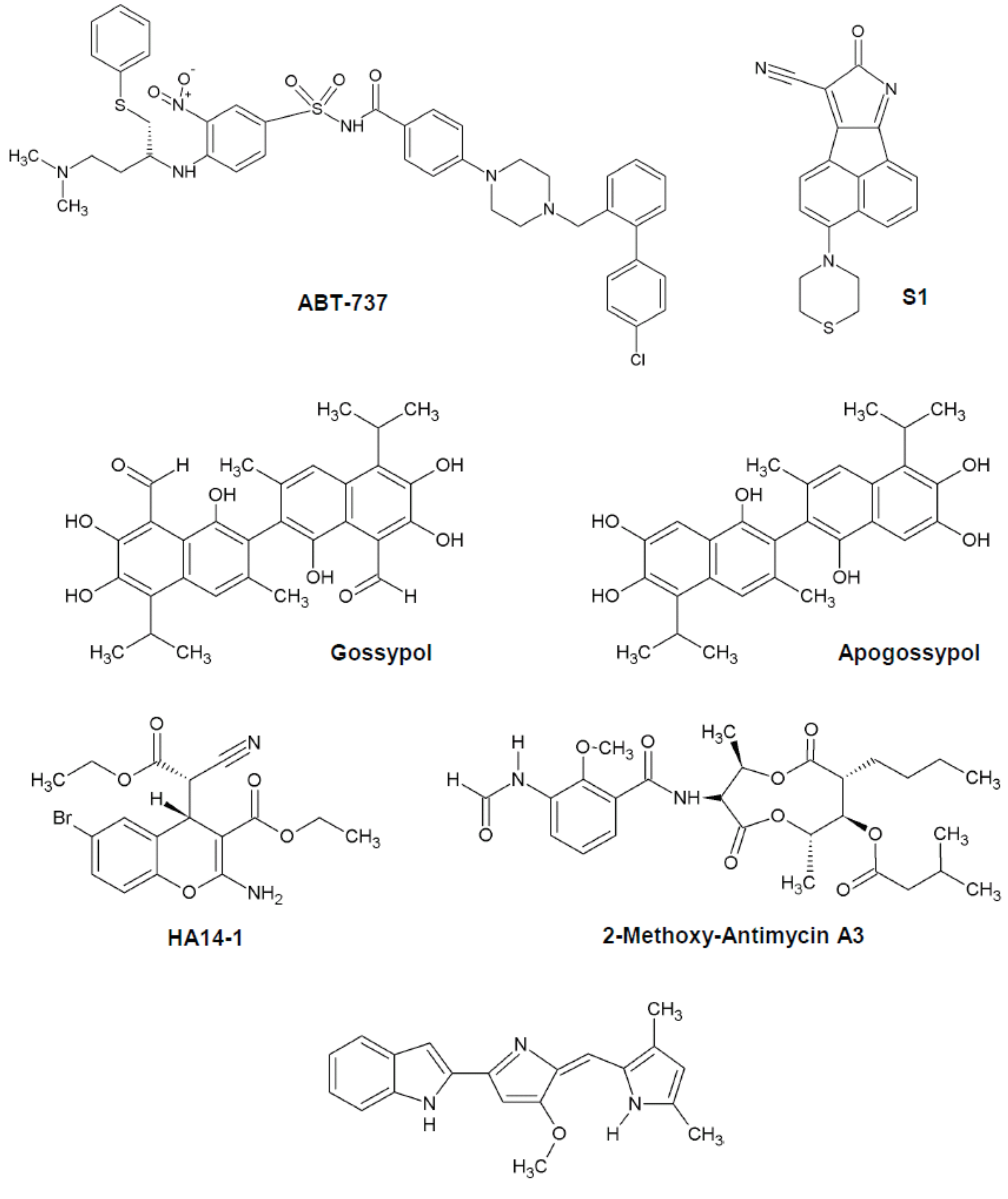
Antibodies were obtained as follows: mouse anti-BAD (C-7), goat anti-PUMA (N-19), rabbit anti-BAX (N-20), rabbit anti-ATF4 (H-290), rabbit anti-ATF3 (C-19) and rabbit anti-c-Myc (N-262) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA); mouse anti-NOXA and rabbit anti-BIM from Calbiochem (San Diego, CA); goat anti-BID from R&D Systems (Minneapolis, MN); rabbit anti-BAK from Millipore (Billerica, MA); mouse anti-BCL2 from Dako (Carpinteria, CA); mouse anti-BCL-X_L from Zymed Laboratories (San Francisco, CA); mouse anti-MCL1 from BD Pharmingen (San Diego, CA); rabbit anti-phospho-eIF2α (D9G8) and rabbit anti-PARP from Cell Signaling Technology (Beverly, MA); and mouse anti-tubulin from Sigma. Secondary antibodies were purchased from BioRad (Hercules, CA).

Primers and Thermal Cycling Conditions for Quantitative Reverse Transcription PCR Analysis

Primer sequences were as follows:
MCL1 (F: 5’-TCAAAAACGAAGACGATGTGA-3’, R: 5’-CAAAGGCACCAAAAGAAATGA -3’),
NOXA (F: 5’-AAGAAGGCGCGCAAGAAC-3’, R: 5’-TCCTGAGCAGAAGAGTT TGG-3’),
ATF3 (F: 5’-CTCCTGGGTCACTGGTGTTT-3’, R: 5’-GTCGCCTCTTTTTCCTTTCA-3’),
GAPDH (F: 5’-CTCAGACACCATGGGGAAGGTGA-3',
R: 5'-ATGATCTTGAGGCTGTTGTCATA-3').

Thermal cycling was carried out by DNA Engine Opticon^{TM} (BioRad), using the following conditions. MCL1 and NOXA: 40 cycles at 94°C for 30 sec, 50°C for 30 sec, 72°C for 15 sec; ATF3: 40 cycles at 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min; and GADPH: 30 cycles at 94°C for 30 sec, 56.7°C for 30 sec, 72°C for 1 min.

1. Zhang, Z., Jin, L., Qian, X., Wei, M., Wang, Y., Wang, J., Yang, Y., Xu, Q., Xu, Y., and Liu, F. (2007) *Chembiochem* **8**, 113-121



ABT-737

S1

Gossypol

Apogossypol

HA14-1

2-Methoxy-Antimycin A3

GX15-070

FIGURE S1. **Chemical Structures of Putative BH3 Mimetics.**

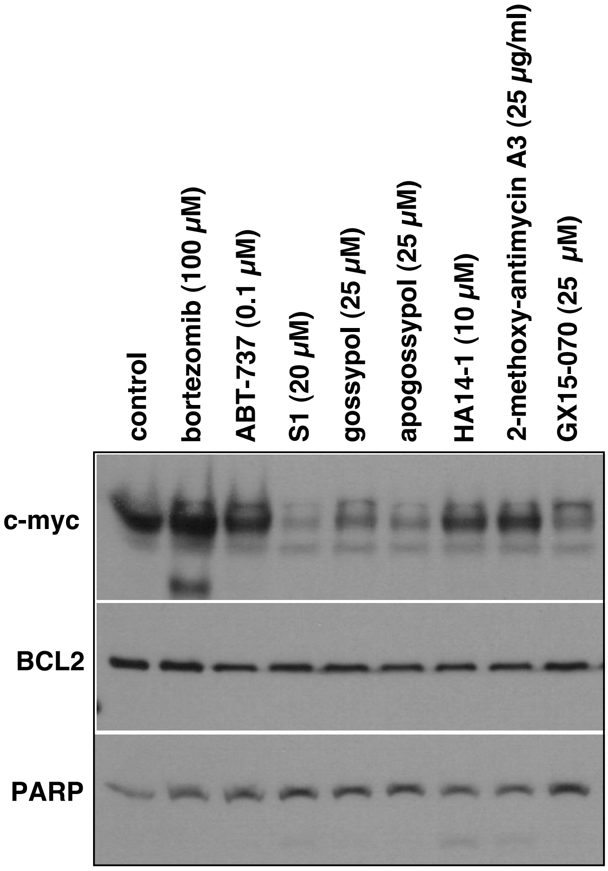


FIGURE S2. **Impact of BH3 Mimetics on c-Myc Expression.** NB4 cells were incubated with the indicated compounds for 6 h, then analyzed for expression of c-Myc, BCL2 and PARP (the latter two as loading controls). While bortezomib slightly induces c-myc expression, ABT-737 has little impact. Most of the other "BH3 mimetics" reduce expression consistent with activation of eIF2α and inhibition of translation.

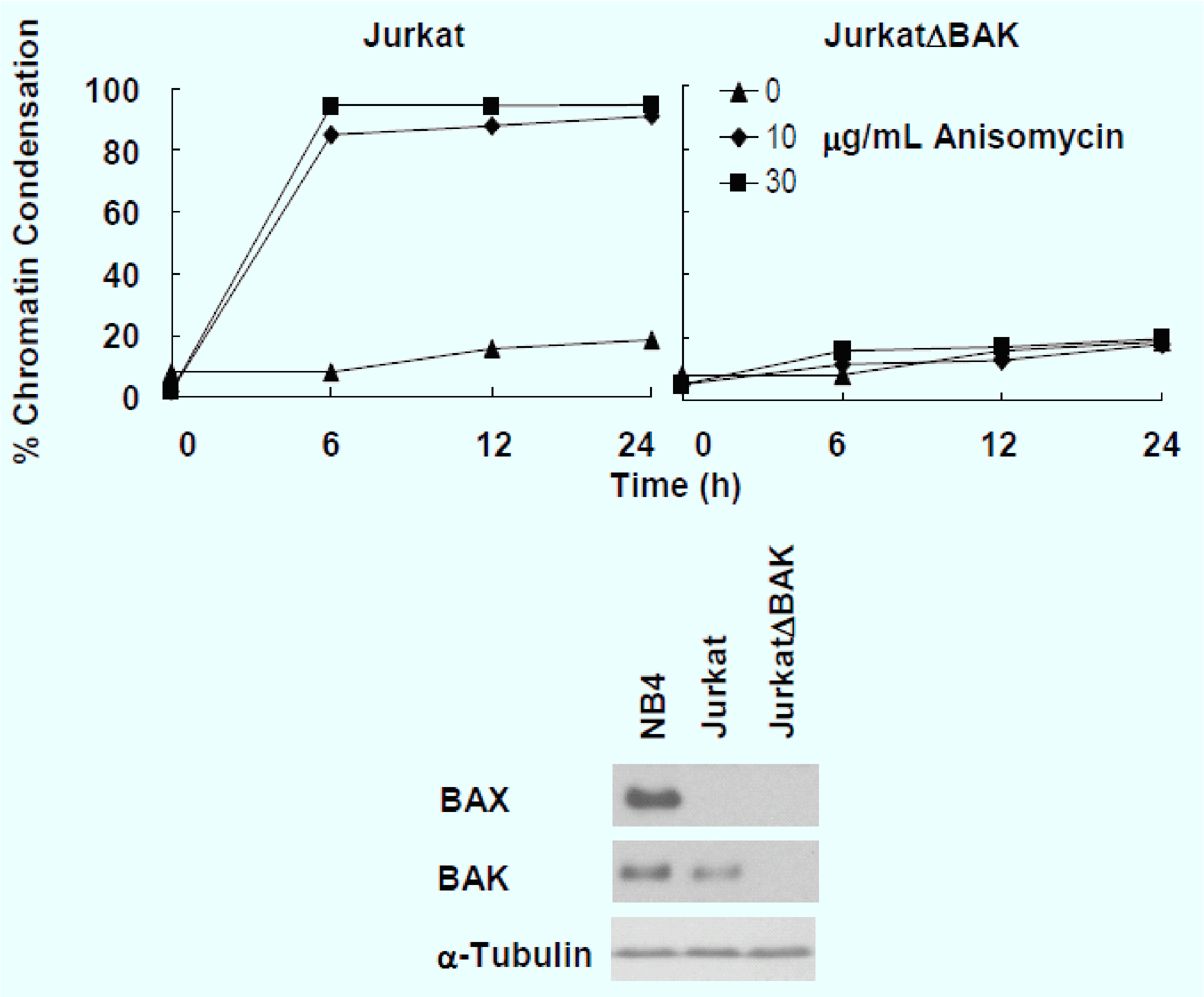


FIGURE S3. **Characterization of Wild-Type Jurkat and JurkatΔBAK Cells.** Wild-type Jurkat and JurkatΔBAK cells were incubated with anisomycin for 0-24 h, stained with Hoechst 33342, and scored for chromatin condensation. Untreated cells were lysed and probed for BAX and BAK, using NB4 cell lysate as a positive control.

Table S1. **Genes Induced by Gossypol and S1.** NB4 cells were incubated with 20 µM gossypol or 20 µM S1 for 6 h and analyzed for gene expression. Numbers reflect average of two independent experiments. Stress-induced genes discussed in the text are highlighted. PMAIP1=NOXA; some genes are represented several times. A, Genes induced more than 2-fold by both gossypol and S1. B, Genes induced more than 2-fold by gossypol but not S1. C, Genes induced more than 2-fold by S1 but not gossypol.

A. Genes Induced More Than 2-Fold by Both Gossypol and S1

Gene Symbol	Control average	Gossypol average	S1 average	Gossypol fold induction	S1 fold induction
KLF2	221	2231	562	10.1	2.5
ATF3	279	1762	1499	6.3	5.4
DDIT4	2367	14854	11309	6.3	4.8
IL8	2008	12563	47976	6.3	23.9
STC2	300	1750	1124	5.8	3.7
DDIT3	562	2932	9435	5.2	16.8
EGR1	381	1737	1070	4.6	2.8
IL8	6370	24730	55002	3.9	8.6
CEBPB	2491	9530	13459	3.8	5.4
KLF6	435	1636	978	3.8	2.3
TRIB3	5022	18862	20503	3.8	4.1
PPP1R15A	1739	6305	11213	3.6	6.4
CCL3	158	558	571	3.5	3.6
KLF6	710	2427	1460	3.4	2.1
CCL3L3	244	828	813	3.4	3.3
PMAIP1	669	2242	2528	3.4	3.8
ARL4	405	1296	1022	3.2	2.5
NFKBIZ	346	1103	4387	3.2	12.7
VEGFA	392	1199	917	3.1	2.3
GADD45A	720	2193	3395	3.0	4.7
ADM2	256	710	989	2.8	3.9
KCNG1	784	2166	5099	2.8	6.5
GADD45A	1661	4361	6184	2.6	3.7
NFIL3	519	1305	1518	2.5	2.9
CTH	175	395	1195	2.3	6.8
PSAT1	3261	7244	9430	2.2	2.9
PRDM13	145	320	342	2.2	2.4
PHLDA1	516	1103	5487	2.1	10.6
PCK2	658	1401	2963	2.1	4.5
MAFB	411	872	2616	2.1	6.4
NUDT18	200	418	526	2.1	2.6
RELB	159	332	485	2.1	3.0
PMAIP1	225	448	536	2.0	2.4
CCL3L3	180	359	386	2.0	2.1
ABCA1	827	1643	2794	2.0	3.4
C6ORF48	196	383	526	2.0	2.7

B. Genes induced more than 2-fold by gossypol but not S1

Gene Symbol	Control average	Gossypol average	S1 average	Gossypol fold induction	S1 fold induction
TNFAIP3	290	1303	512	4.5	1.8
TSC22D3	324	1295	465	4.0	1.4
JUN	1065	4126	1412	3.9	1.3
TSC22D3	494	1642	627	3.3	1.3
PTGER4	885	2458	866	2.8	1.0
HNRPDL	3325	7505	4793	2.3	1.4
TFAP2C	185	416	241	2.3	1.3
SC4MOL	2549	5699	1454	2.2	0.6
LOC643031	6098	13134	10799	2.2	1.8
TIPARP	1433	2995	2460	2.1	1.7
HMGCS1	5846	11858	2923	2.0	0.5
TNFAIP3	290	1303	512	4.5	1.8

C. Genes induced more than 2-fold by S1 but not gossypol

Gene Symbol	Control average	Gossypol average	S1 average	Gossypol fold induction	S1 fold induction
OSGIN1	197	257	4125	1.3	21.0
RGC32	764	1065	7160	1.4	9.4
IER3	244	463	2148	1.9	8.8
LOC338758	341	647	2690	1.9	7.9
HYPE	337	254	2589	0.8	7.7
HERPUD1	1689	1888	12710	1.1	7.5
HERPUD1	1282	1355	9210	1.1	7.2
GCLM	1177	919	8267	0.8	7.0
SRXN1	1006	583	7012	0.6	7.0
C1ORF24	1027	1540	6804	1.5	6.6
GCLM	213	176	1397	0.8	6.5
TNFRSF10B	528	807	3223	1.5	6.1
DUSP6	432	437	2582	1.0	6.0
DNAJB9	946	655	5416	0.7	5.7
TSPYL2	323	277	1778	0.9	5.5
MAP1LC3B	1509	2373	8304	1.6	5.5
NAB2	273	233	1457	0.9	5.3
STX5A	647	497	3385	0.8	5.2
KIAA0746	170	191	878	1.1	5.2
C1ORF24	1868	2559	9530	1.4	5.1
PBEF1	681	750	3335	1.1	4.9
ME1	572	749	2755	1.3	4.8
PBEF1	422	470	2008	1.1	4.8
TSPYL2	291	231	1343	0.8	4.6
CCPG1	953	1684	4377	1.8	4.6
CDKN1A	708	1059	3205	1.5	4.5
SMOX	199	332	897	1.7	4.5
SERPINB8	478	547	2084	1.1	4.4
TXNRD1	2261	1491	9782	0.7	4.3
TGIF1	966	1796	4010	1.9	4.1
HYOU1	910	693	3770	0.8	4.1
FTHL12	5375	4716	22173	0.9	4.1

FTHL11	711	631	2921	0.9	4.1
SERPINB8	476	552	1936	1.2	4.1
ITPR1	342	335	1382	1.0	4.0
TXNRD1	4225	2908	17005	0.7	4.0
CDK5RAP2	312	304	1240	1.0	4.0
CBX4	645	896	2556	1.4	4.0
OSCAR	425	505	1659	1.2	3.9
CDK2AP2	374	265	1460	0.7	3.9
BTG1	3936	7203	15286	1.8	3.9
SLC3A2	2056	3857	7975	1.9	3.9
NRP1	409	398	1561	1.0	3.8
CYP2R1	679	881	2578	1.3	3.8
ARRDC4	207	281	783	1.4	3.8
KRCC1	451	714	1635	1.6	3.6
CARS	836	1583	3031	1.9	3.6
TGIF1	1542	2802	5549	1.8	3.6
JAG1	801	1419	2882	1.8	3.6
ATP2B1	250	315	884	1.3	3.5
STX5A	204	175	699	0.9	3.4
FTHL12	3348	3350	11386	1.0	3.4
ITPKA	775	975	2593	1.3	3.3
PTPDC1	193	241	644	1.2	3.3
GOT1	4819	7238	16071	1.5	3.3
VLDLR	971	1787	3182	1.8	3.3
ANXA1	1746	2126	5717	1.2	3.3
GFPT1	569	559	1862	1.0	3.3
PPAPDC1B	292	314	952	1.1	3.3
PGD	4692	4651	15185	1.0	3.2
TXNIP	1948	3123	6294	1.6	3.2
ASNS	7689	14650	24728	1.9	3.2
RFFL	354	398	1125	1.1	3.2
DUSP5	322	372	1019	1.2	3.2
LOC647650	201	313	633	1.6	3.2
FBXL13	284	303	891	1.1	3.1
LMO4	530	823	1660	1.6	3.1
NFE2L1	846	1032	2600	1.2	3.1
ADFP	1469	1953	4507	1.3	3.1
TUFT1	180	276	550	1.5	3.0
BASP1	7715	8334	23460	1.1	3.0
UCHL1	510	396	1545	0.8	3.0
FKBP14	372	393	1128	1.1	3.0
MXD1	621	910	1869	1.5	3.0
ADFP	390	550	1163	1.4	3.0
CSNK2A2	1275	1472	3799	1.2	3.0
TMEM22	281	261	831	0.9	3.0
SLC3A2	3981	6727	11736	1.7	2.9
SLC3A2	918	1355	2705	1.5	2.9
EMP1	201	346	588	1.7	2.9
CLEC5A	209	194	596	0.9	2.8
LOC646463	268	331	764	1.2	2.8
NRP1	165	222	465	1.4	2.8
SERTAD1	252	388	711	1.5	2.8
EIF1	6765	10132	19109	1.5	2.8

SYVN1	1467	833	4135	0.6	2.8
KRCC1	268	367	748	1.4	2.8
YPEL5	338	354	944	1.0	2.8
ASNS	998	1563	2755	1.6	2.8
DUSP6	1182	1101	3252	0.9	2.8
ISGF3G	332	321	913	1.0	2.7
RNF185	158	165	431	1.0	2.7
ADAM17	271	306	739	1.1	2.7
ASF1A	1206	890	3269	0.7	2.7
CCPG1	156	223	421	1.4	2.7
DFNA5	176	171	475	1.0	2.7
CHGB	355	296	936	0.8	2.6
P2RX4	747	1074	1960	1.4	2.6
CYLD	199	223	520	1.1	2.6
RIT1	185	173	485	0.9	2.6
CNIH3	253	273	659	1.1	2.6
UBE2H	262	363	683	1.4	2.6
SLC6A9	146	200	380	1.4	2.6
TRIM5	386	293	994	0.8	2.6
DNAJC3	182	157	469	0.9	2.6
EPB41L5	324	316	831	1.0	2.6
FTHL7	22055	20321	56563	0.9	2.6
PRNP	1696	1401	4283	0.8	2.5
TSPYL2	221	190	557	0.9	2.5
C10ORF32	610	724	1527	1.2	2.5
FAM100B	175	148	438	0.8	2.5
HS.143018	168	223	416	1.3	2.5
GAB2	738	876	1831	1.2	2.5
SDF2L1	8161	3202	20238	0.4	2.5
HS.355933	632	963	1567	1.5	2.5
GARS	10632	13589	26295	1.3	2.5
ARMET	8892	5241	21979	0.6	2.5
MLPH	149	180	368	1.2	2.5
TMEM154	338	585	832	1.7	2.5
ADORA2B	595	533	1433	0.9	2.4
TIGA1	5829	9558	14021	1.6	2.4
LOC653344	226	266	534	1.2	2.4
RN7SK	196	259	463	1.3	2.4
IL1B	165	187	389	1.1	2.4
CMTM8	183	193	430	1.1	2.4
WARS	1943	1990	4561	1.0	2.3
PGM3	393	332	921	0.8	2.3
AXUD1	580	932	1343	1.6	2.3
ELL2	171	192	394	1.1	2.3
FAM130A1	255	430	589	1.7	2.3
STK19	667	942	1537	1.4	2.3
KIAA0286	915	771	2087	0.8	2.3
FAM107B	1342	1547	3040	1.2	2.3
NRIP3	2602	2426	5888	0.9	2.3
SLC22A15	771	989	1735	1.3	2.3
SSR2	326	316	728	1.0	2.2
TLE4	514	490	1145	1.0	2.2
NEIL3	1172	971	2608	0.8	2.2

ZMYM5	304	445	674	1.5	2.2
PPP1R3F	274	272	608	1.0	2.2
PGM3	273	243	595	0.9	2.2
ERRFI1	646	1063	1401	1.6	2.2
HBEGF	814	1168	1764	1.4	2.2
UBE2H	265	293	572	1.1	2.2
STK40	2487	2191	5355	0.9	2.2
MAX	244	463	524	1.9	2.1
STK19	1083	1351	2320	1.2	2.1
MCTP1	357	386	765	1.1	2.1
SDCBP	823	854	1761	1.0	2.1
PNPLA8	245	248	517	1.0	2.1
C16ORF52	318	350	670	1.1	2.1
FTL	21183	19086	44701	0.9	2.1
DGKQ	736	888	1551	1.2	2.1
SNX5	1646	2333	3457	1.4	2.1
H1F0	668	883	1403	1.3	2.1
GPR84	1620	1664	3393	1.0	2.1
HBP1	212	219	442	1.0	2.1
HLA-E	886	875	1845	1.0	2.1
PRMT1	364	578	757	1.6	2.1
FLT4	212	268	440	1.3	2.1
PSCD2	1786	1909	3707	1.1	2.1
MTHFD2	10917	15091	22554	1.4	2.1
FTL	24753	21908	50821	0.9	2.1
LIMS1	393	392	803	1.0	2.0
SDC4	322	379	650	1.2	2.0
LOC196752	226	207	455	0.9	2.0
ARF4	850	596	1698	0.7	2.0
SLC25A36	588	505	1174	0.9	2.0