Supplementary Information

Chemical probe to identify the cellular targets of the reactive lipid metabolite 2-*trans*-hexadecenal

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I. Supplementary methods

Materials

Azide-biotin (AZ104) was purchased from Click Chemistry Tools. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP, C4706), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methy-l]amine (TBTA, 678937), Copper(II) sulfate pentahydrate were purchased from Sigma. Anti-HA (C29F4), anti-PARP (9532), anti-mouse secondary (7076S), anti-rabbit secondary (7074S) were purchased from Cell Signaling Technology. Anti-Bax 6A7 monoclonal antibodies (sc-23959), anti-Bax polyclonal antibodies (N-20, sc-493) were purchased from Santa Cruz Biotechnology. Anti-6X-His tag monoclonal (MA1-21315), Alexa Fluor 488 secondary antibody, streptavidin agarose beads (15942-050), DAPI (D1306), MitoTracker Deep Red FM were purchased from Invitrogen. Anti-Actin (ab6276) was purchased from Abcam. Streptavidin horseradish peroxidase conjugate (S911) was purchased from Life Technologies. Recombinant Human Bax fused with 6His tag at both N-terminus and C-terminus (BAX-6976H) was purchased from Creative BioMart. All chemicals were purchased from Sigma-Aldrich, Alfa Aesar, Fisher Scientific or Acros and were used as received. Azide-biotin was purchased from Click Chemistry Tools. Dess-Martin periodinane was purchased from Oakwood Chemicals. Anhydrous solvents were purchased from Sigma-Aldrich. The silica gel used in flash column chromatography was from Aldrich (Cat. 60737, pore size 60 Å, 230-400 mesh). The ^{1}H and ^{13}C NMR spectra were obtained on a Varian 500M spectrometer. Chemical shifts are reported in δ ppm values downfield from tetramethylsilane and *J* values are reporter in Hz.

Plasmid construction and site-directed mutagenesis

The full-length human HA-Bax was constructed by insertion of PCR-amplified DNA fragments into the EcoRI and XhoI sites of HA-pcDNA3.1. Various cysteine mutants of Bax were generated using the QuickChange mutagenesis kit (Agilent) according to the manufacturer's protocol. The sequences of the PCR are as follows:

Bax forward, 5'-GCATGCGAATTCATGGACGGGTCCGGGGAGCAG-3';

reverse, 5'-GCGCGCCTCGAGTCAGCCCATCTTCTTCCAGAT-3';

Bax C62S forward, 5'-ACCAAGAAGCTGAGCGAGTCTCTCAAGCGCATCGGGGAC-3';
reverse, 5'-GTCCCCGATGCGCTTGAGAGACTCGCTCAGCTTCTTGGT-3';

Bax C126S forward, 5'-CTGGTGCTCAAGGCCCTGTCCACCAAGGTGCCGGAACTG-3';
reverse, 5'- CAGTTCCGGCACCTTGGTGGACAGGGCCTTGAGCACCAG-3'.

Cell culture, transfection, metabolic labeling

HCT 116 WT and $Bax^{-/-}Bak^{-/-}$ cells were gift from Dr. Richard Youle and Dr. Lin Zhang. CHOK1 and CHOK1A cells were gifted by Dr. Zoeller. HCT116, CHOK1 and CHOK1A cells were grown at 37 °C with 5% CO_2. HCT116 cells were cultured in RPMI 1640 media, (Life Technologies) supplemented with 10% fetal bovine serum (FBS) (Thermo/Hyclone, Waltham, MA), 100U/mL penicillin and 100µg/mL streptomycin. CHO cells were grown in Ham's F-12 nutrient mixture (Life Technologies) supplemented with 10% fetal bovine serum (FBS) (Thermo/Hyclone, Waltham, MA), 100U/mL penicillin and 100µg/mL streptomycin.

Cell lysate preparation

HCT116 double knockout (DKO, $Bax^{-/-}/Bak^{-/-}$) cells were transfected with HA-Bax (WT) or C62S, C126S or C62/126S (2CS) mutant plasmid using polyethylenimine (PEI) according to manufactures protocol and further cultured for 48h for protein expression. Cells were washed once with cold PBS and lysed with lysis buffer containing 50 mM Hepes (pH=7.4), 0.5% NP-40, 1X Protease inhibitor (cOmplete EDTA-free protease inhibitors cocktail. Roche), and were incubated on ice for 15 min and then centrifuged at 15,000 *g* for 15 minutes to pellet the cell debris. The resulting supernatant was collected to a new tube and protein concentration was analyzed by the BCA method.

Probe labeling of Bax protein in cell lysate

Cell lysate (200 µg, 0.5 µg/µL.) was incubated with probe with the indicated concentrations and time. After incubation, 40 µL of click reagent mix was added to each sample and incubated for 1h at room temperature on bench top.

Preparation of click mix: 100 µM TBTA (75µL), 1 mM TCEP (30µL), 1 mM CuSO4 (30µL) and 100 µM biotin-azide (15µL).

After click reaction, proteins were precipitated with chilled MeOH (3.4 mL) at -80 °C overnight. The samples were then centrifuged at 17000 g for 15 min. Proteins were air dried and then re-dissolved in PBST with 1% SDS (50µL). Once protein was completely dissolved samples were further diluted with 950 µL of PBST. Streptavidin-agarose beads (40 µL, pre-washed 3 times with PBST) were added to the samples and samples were rotated at room temperature for 2 h. Then samples were centrifuged at 500 g for 2 min, and then washed with 3x PBST. After removing supernatant, the beads were eluted with 80ul elution buffer (95% Formamide + 10mM EDTA) and beads were boiled at 95 ^{0}C for five minutes. Then centrifuge the boiled beads at 500 g for 2 min and transfer the eluate to the fresh tube containing 20 µL of 6X SDS sample buffer. The samples were heated for 5 min at 95 ºC before being subjected to SDS-PAGE analysis (4–12% Bis-Tris polyacrylamide gel. MES running buffer (Invitrogen).

Immunoblotting

Proteins were transferred onto a polyvinylidene fluoride membranes (PVDF Millipore), and then the membrane was blocked in 5% nonfat dried milk in TBST solution for 1 h at room temperature, incubated with anti-HA primary antibody (C29F4, Cell Signaling Technology, diluted 1:2000 in 5% nonfat dried milk TBST solution) overnight at 4 °C, washed with TBST (3 times, 10 min each washing), and incubated with secondary anti-mouse antibody (1:1000 dilution in 5% nonfat dried milk TBST solution) for 1 h at RT. At last, the membrane was developed with ECL (Thermo).

Streptavidin beads pull-down and elution of labeled proteins for Mass Spectrometry

After click chemistry using azido-biotin, proteins were precipitated with chilled MeOH at -80 °C overnight. The samples were then centrifuged at 3500 g for 10 min, and washed twice with chilled MeOH. Proteins were air dried and then re-dissolved in resuspension buffer containing 1% SDS (50 mM Tris-HCl, pH 7.4,150 mM NaCl, 10 mM EDTA, 1% SDS) by sonication using a water bath sonicator. The samples were then diluted with 1 volume of 1% Brij97 buffer (50 mM Tris-HCl, pH 7.4,150 mM NaCl, 10 mM EDTA, 1% Brij97). Streptavidin-agarose beads were pre-washed 3 times with PBS, and then added to the samples. The resulted mixture was rotated at room temperature for 1.5 h, centrifuged at 2000 g for 2 min, and then washed with 3x 0.2 SDS in PBS and 1x 250 mM ammonium bicarbonate. After removing supernatant, the beads were incubated with 500 µL of 8M urea, 50 µL of 500 mM TCEP and 50 µL of 400 mM iodoacetamide for 40 min in the dark and then washed twice with 250 mM ammonium bicarbonate. The proteins bound on beads were digested with trypsin overnight, and the samples were submitted for Taplin Mass spectrometry for mass spec analysis.

Labeling experiment with recombinant Human His-Bax protein

Recombinant Human Bax fused with 6His tag at both N-terminus and C-terminus (50 ng, Bax-6976H, Creative Biomart) was incubated with 10 µM probe **1** or **2** in 50 µL of lysis buffer (50 mM Hepes (pH=7.4), 0.5% NP-40) for 1 h at 37 °C and subjected for Click reaction with 100 µM biotin azide, 1 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 100 µM tris((1-benzyl-1H-1,2,3-triazol-4-yl)methy-l)amine (TBTA) and 1 mM $CuSO_4$ for 1 h at room temperature. Reaction was terminated by the addition of 10 µL of 6X SDS-sample loading buffer (50 mM Tris-HCl, pH 6.8, 6% SDS, 48% glycerol, 0.03% bromphenol blue, 30 mM EDTA, 9% MeSH. The samples were heated for 5 min at 95 ºC before being subjected to SDS-PAGE analysis (4–12% Bis-Tris polyacrylamide gel, MES running buffer Invitrogen). The membrane was then washed five times with Tris-buffered saline (TBST)-0.05% Tween 20, incubated with Streptavidin horseradish peroxidase (HRP) (1 mg/mL, with 1:10,000 dilution in PBST) for 1 h at room temperature, and developed with ECL Western blotting detection reagents after washing with TBST (5 times, 10 min each washing). For anti-His western blot, the membrane was blocked in 5% nonfat dried milk in TBST solution for 1h at room

temperature, incubated with anti-His primary antibody (MA1-21315, Thermo Scientific, diluted 1:1000 in 5% BSA in TBST solution) overnight at 4 ºC, then washed with TBST (3 times, 10 min each washing), and incubated with secondary anti-mouse antibody (1:1000 dilution in 5% nonfat dried milk TBST solution) for 1 h at RT. At last, the membrane was developed with ECL Western blotting detection reagents after washed with TBST (3 x 10 min).

Labeling experiment with mass spectrometry using recombinant His-tagged Bax protein

Recombinant Human Bax fused with 6His tag at both N-terminus and C-terminus (200 ng) was treated with probe **2** (25 μM) in 40 μL HEPS buffer (pH 7.4) for 1 h at 37 ^{o}C. The resulted protein sample was subjected to overnight trypsin digestion (Standard trypsin digestion procedure). Samples were cleaned using C4-zip-tips and then analyzed by MALDI-TOF.

Bax conformational change assay

HA-Bax WT or mutant C62S, C126S or 2CS were transfected in HCT116 Bax/Bak DKO cells and then treated with indicated concentration of Probe 2 for 24h. The cells were lysed in Chaps lysis buffer (10 mM Hepes, pH7.4, 150mM NaCl, 1% Chaps) with freshly added protease inhibitors (cOmplete EDTA-free protease inhibitors cocktail. Roche). After incubation on ice for 10 min and centrifugation at 17 000 × g for 10 min at 4°C, then incubated overnight with anti-Bax 6A7 monoclonal antibodies (Santa Cruz Biotechnology). Then 10 μl of protein G Magnetic Beads (Thermo) were added and incubated at 4°C for 2 h. The beads were washed three times in Chaps lysis buffer. The whole-cell lysates and immunoprecipitates were boiled for 5 min at 95°C in SDS sample buffer. Immunoblotting was performed using anti-Bax polyclonal antibodies (Santa Cruz Biotechnology).

Immunocytochemistry

For the immunostaining, HCT116 Bax/Bak DKO cells were grown on coverslips in plates and transfected with HA-Bax or mutant C62S, C126S or 2CS plasmids. Mitochondria was stained with 100nM MitoTracker Deep Red FM (Invitrogen) at 37°C for 30 min. And then the cells were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. After washing with PBS three times, cells were blocked for 1 h with bovine serum albumin (BSA, 5%) in PBS containing 0.1% Triton X-100 (PBST). Next, the cells were incubated with HA antibodies diluted in 0.1% PBST containing 5% BSA at 4 °C overnight. The samples were washed for 5 min in 0.1% PBST three times. The cells were then incubated with Alexa Fluor 488 secondary antibodies (Invitrogen) for 30 min at room temperature. After final washing, samples were mounted with mounting medium with DAPI (Invitrogen). The samples were examined under a fluorescence confocal microscope (Axio Observer Z1, Zeiss).

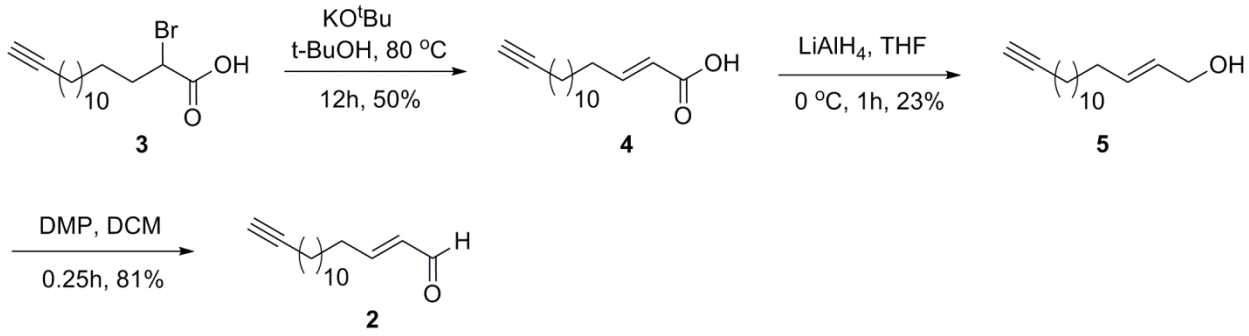
Apoptosis assay with CHO cells using probe 2

CHO cells were grown for 24 h on 6-well plate at 70% confluency. After 24 h, medium was aspirated and treated with fresh medium containing DMSO or probe **1** or probe **2** for 18h. For staining, cells were fixed and stained using crystal violet. For western blotting, cells were washed with cold PBS and lysed with RIPA buffer supplemented with protease inhibitors (Roche). Whole cell extracts were analyzed by SDS-PAGE and then immunoblotted with anti-PARP1 or actin antibodies.

Measurement of ROS level in Cells

HCT116 WT or Bax/Bak DKO cells were labeled with DCFDA (20 μM) for 30 min at 37 ºC. And then cultured an additional 6 h with Prove 2 or 4-HNE (50 μM). Cells were analyzed on a fluorescent plate reader (Perkin Elmer Envision 2104 Multilabel Reader).

2. Synthesis of (*E*)-Hexadec-2-en-15-ynal (Probe 2)



Synthesis of (*E*)-hexadec-2-en-15-ynoic acid 4

To a solution of 2-bromohexadec-15-ynoic acid **3** (90 mg, 0.27 mmol) in *tert*-Butyl alcohol (5 mL) was added potassium *tert*-butoxide (120 mg) and heated at 80 °C for 12h. The mixture was cooled to room temperature and diluted with 5 mL of ice cold water and acidified with 5N sulfuric acid. The organic layer was extracted with diethyl ether (3x10 mL), washed with brine and dried over sodium sulfate. Solvent was removed under vacuum and the crude residue was purified by flash column chromatography (FC) using mixtures of ethyl acetate/hexanes as the eluent to obtain the desired product (33.9 mg, 50%).

Synthesis of (*E*)-Hexadec-2-en-15-ynal (Probe 2)

To an ice cooled solution of (*E*)-hexadec-2-en-15-ynoic acid **4** (30 mg, 0.12 mmol) in anhydrous THF (3 mL) was added lithium aluminiumhydride (9 mg, 0.24 mmol). The reaction

was stirred for 0.5 h at room temperature. After completion, the reaction was quenched with sodium sulfate and stirred for 3 h at room temperature, and the solids were filtered. The filtrate was concentrated in vacuo and used in the next step immediately.

To a solution of (*E*)-hexadec-2-en-15-yn-1-ol **5** (10 mg, 0.042 mmol) in anhydrous dichloromethane (1 mL) was added Dess-martin periodinane (0.05 mmol, 18 mg) and stirred for 0.25 h at room temperature. After completion of the reaction, reaction mixture was filtered through silica gel pad using ethyl acetate. Solvent was removed under vacuum and the crude residue was purified by flash column chromatography (FC) using mixtures of ethyl acetate/hexanes as the eluent to obtain the desired product (8 mg, 81%).

^{1}H NMR (500 MHz, CDCl3) δ 9.37 (s, 1H), 6.68-7.17 (m, 1H). 5.95-6.04 (m, 1H), 2.03-2.25 (m, 4H), 1.80 (s, 1H), 1.40-1.43 (m, 6H), 1.1 (s, 12H).

II. Supplementary Figures

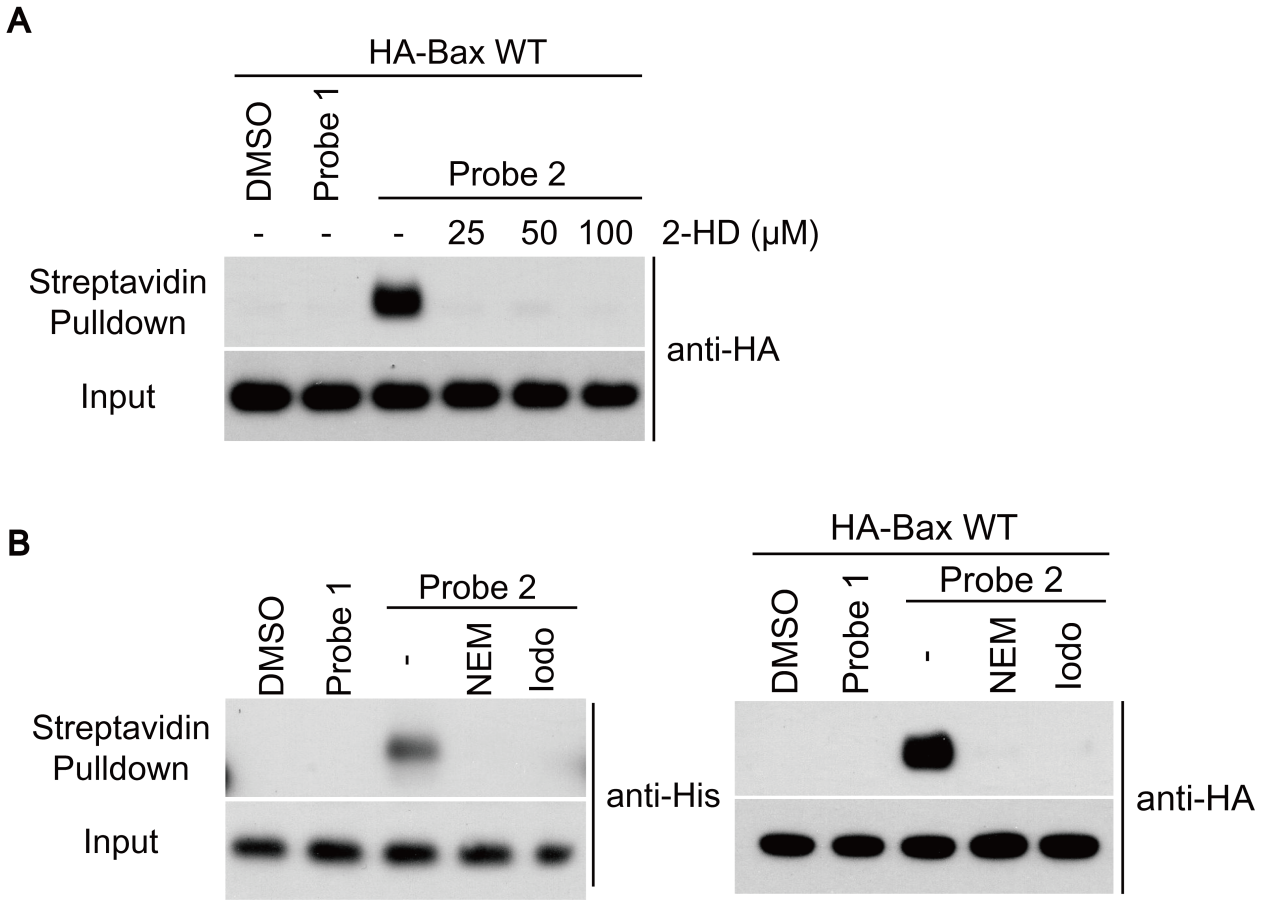


Figure S1. (A) Competition experiment with 2-HD displaces probe **2** in cells **(B)** Blocking of the Cysteine residues using NEM or Iodoacetamide in Bax abolished probe **2** labeling.

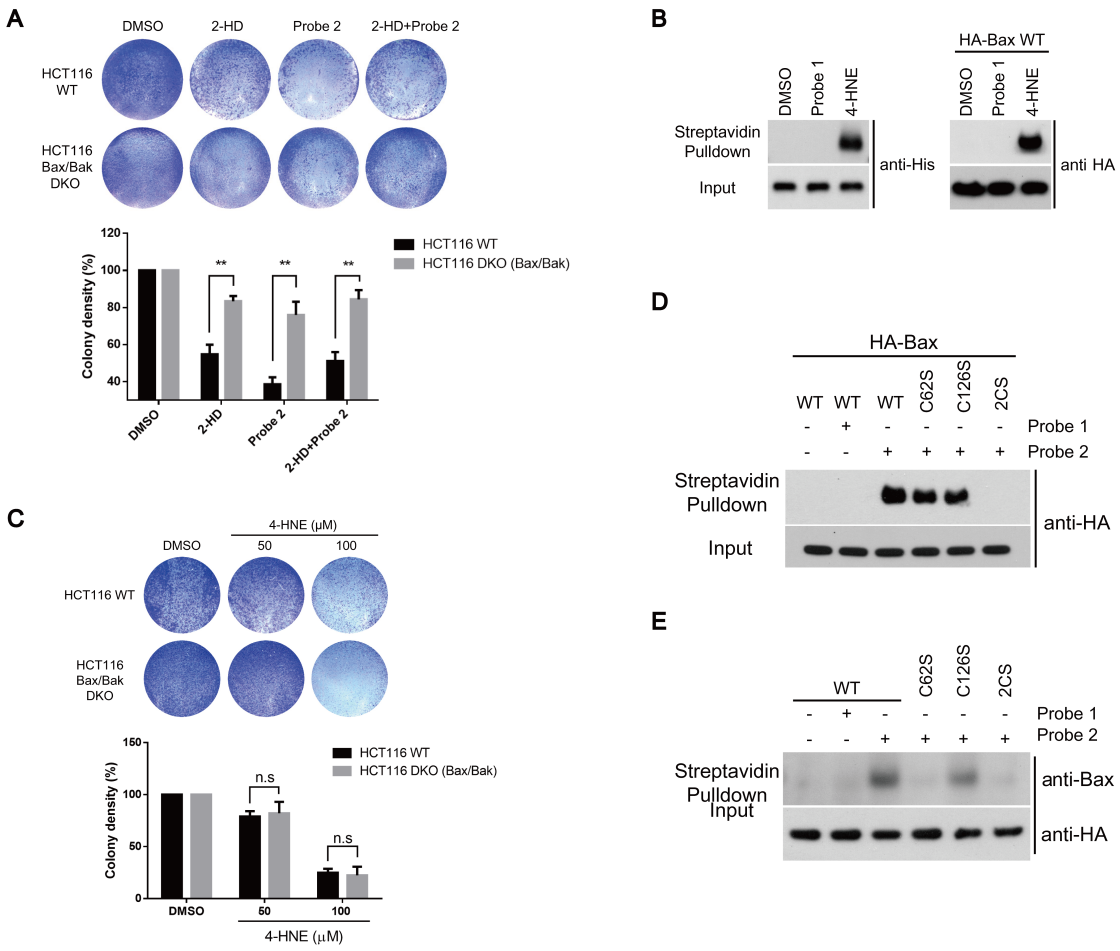


Figure S2. (A) Colony formation assay of HCT116 WT or Bax/Bak DKO cells with 2-HD or Probe **2** treatment. The colony density was quantified and shown in the bar graph. Data are represented as mean +/- SD, n=3, **, $P<0.01$. **(B)** Labeling of Bax by 4-HNE. (**C**) Colony formation assay of HCT116 WT or Bax/Bak DKO cells with 4-HNE treatment. The colony density was quantified and shown in the bar graph. Data are represented as mean +/- SD, n=3, n.s., not significant. (**D**) Labeling of Bax cysteine mutants by high concentration of probe **2** (50 μM). (**E**) Detection of labeled Bax using anti-Bax antibodies.

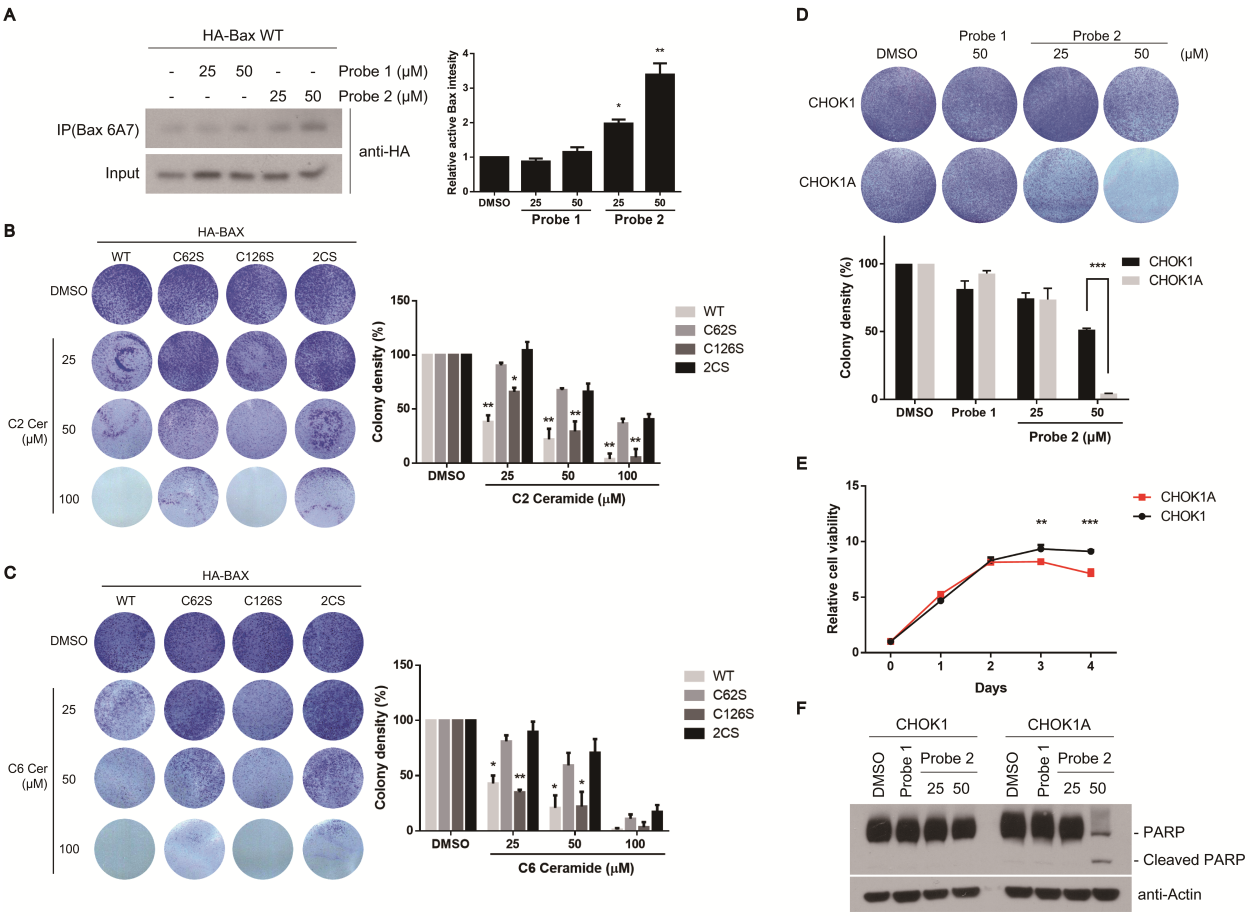


Figure S3. (**A**) Detection of the activated Bax upon Probe **2** treatment. Data are represented as mean +/- SD, n=3, *, P<0.05, **, P<0.01. **(B, C)** Colony formation assay in Bax WT or mutant transfected HCT116 Bax/Bak DKO cells treated with C2 or C6 ceramides. Data are represented as mean +/- SD, n=3, *, P<0.05, **, P<0.01. **(D)** Colony formation assay in aldehyde dehydrogenase ALDH3A2-deficient CHOK1A cells or wild type CHOK1 cells. Data are represented as mean +/- SD, n=3, ***, P<0.001. (**E**) Cell viability assay in WT CHOK1 cells and ALDH3A2-deficient CHOK1A cells. Data are represented as mean +/- SD, n=3, **, P<0.01, ***, P<0.001. (**F**) PARP cleavage assay by Probe **2** in CHOK1 or ALDH3A2-deficient CHOK1A cells.

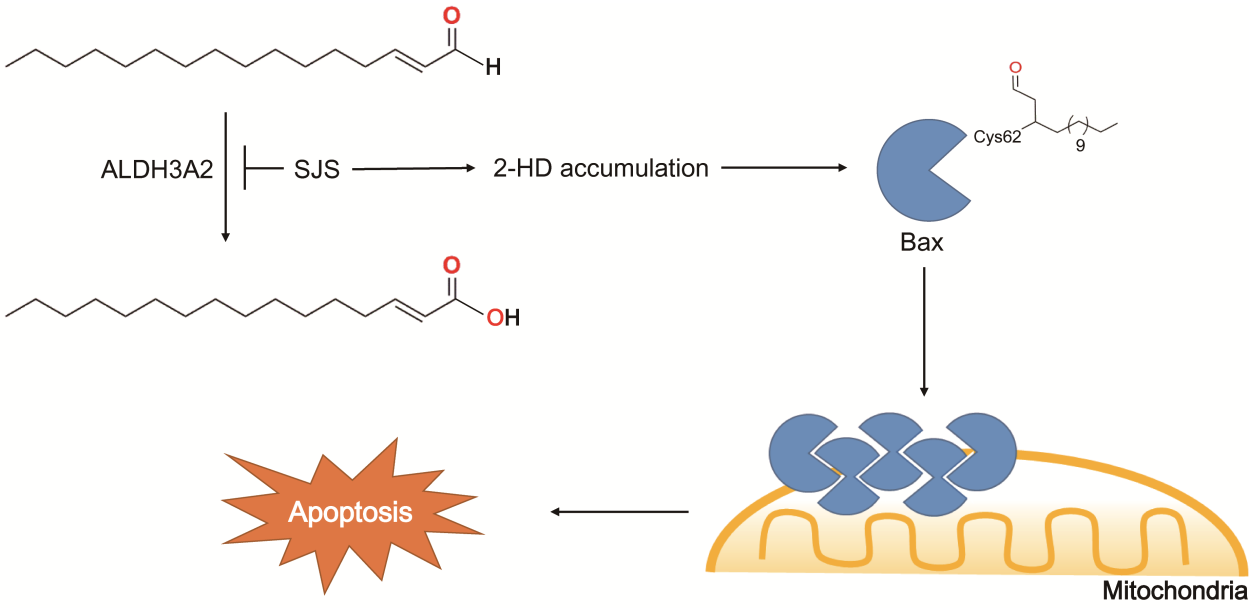


Figure S4. A scheme to show the proposed mechanism of 2-HD in inducing Bax-dependent apoptosis.

Table S1. List of proteins identified from Control 1 and Probe 2 labeled samples. The spectra counts of matched peptides are listed.

	Experiment 1		Experiment 2	
Proteins	Probe **1**	Probe **2**	Probe **1**	Probe **2**
AAAS_HUMAN	0	9	4	11
ABCB7_HUMAN	12	31	5	12
ABCD3_HUMAN	17	18	15	13
ABCE1_HUMAN	8	7	5	8
ABCF2_HUMAN	0	7	3	10
ABHDA_HUMAN	6	7	2	0
ACACA_HUMAN	63	13	78	12
ACD10_HUMAN	0	9	1	3
ACAD9_HUMAN	9	13	2	7
ACADM_HUMAN	7	6	4	5
ACADV_HUMAN	26	20	3	3
ACOT9_HUMAN	17	22	11	13
ACOX1_HUMAN	0	6	3	6
ACSL1_HUMAN	4	8	3	9
ACSL3_HUMAN	8	19	8	16
ACTB_HUMAN	24	34	79	82
ARP2_HUMAN	0	12	4	2
ADCK3_HUMAN	5	9	1	4
ADCK4_HUMAN	0	7	0	4
ADDA_HUMAN	5	12	1	5
ADRM1_HUMAN	0	5	2	2
AFG32_HUMAN	0	8	0	0
AGK_HUMAN	7	12	3	12
AHSA1_HUMAN	4	7	12	7
AIP_HUMAN	0	15	0	11
ALDR_HUMAN	5	9	3	4
AL1B1_HUMAN	6	10	5	10
ALDH2_HUMAN	19	25	14	18
AL3A2_HUMAN	4	9	6	6
AL9A1_HUMAN	6	12	7	2
ALDOA_HUMAN	24	26	38	27
ALG1_HUMAN	0	8	3	10

ALG2_HUMAN	0	4	0	7
ALG5_HUMAN	9	10	3	7
AMRA1_HUMAN	0	4	0	0
AMFR_HUMAN	5	7	4	8
ANKL2_HUMAN	10	0	7	9
AN32A_HUMAN	0	10	3	7
AN32B_HUMAN	0	4	3	5
AN32E_HUMAN	0	7	3	5
ANXA1_HUMAN	14	16	10	14
ANX11_HUMAN	5	7	4	3
ANXA2_HUMAN	6	9	14	18
ANXA4_HUMAN	0	6	0	0
ANXA5_HUMAN	13	21	11	18
ANXA6_HUMAN	12	21	1	5
AP1G1_HUMAN	0	4	3	3
AP2A1_HUMAN	0	8	4	5
AP2B1_HUMAN	12	6	8	13
AP2M1_HUMAN	0	5	3	1
AP2S1_HUMAN	0	4	0	0
AP3B1_HUMAN	6	12	8	8
AP3D1_HUMAN	11	6	11	8
APBB1_HUMAN	0	4	2	5
API5_HUMAN	6	8	5	6
APOO_HUMAN	6	11	0	4
APOOL_HUMAN	0	4	0	0
ARAF_HUMAN	0	6	1	4
ARF4_HUMAN	7	9	4	18
ARF5_HUMAN	4	8	2	4
ARFP1_HUMAN	0	15	1	14
ARFP2_HUMAN	0	15	3	9
ARHG2_HUMAN	0	4	2	3
ARH40_HUMAN	0	5	0	0
ARI1_HUMAN	0	4	2	3
ARL1_HUMAN	5	14	4	8
ARL3_HUMAN	4	0	0	2
AR6P1_HUMAN	4	31	0	3
ARL8A_HUMAN	5	11	3	6
ARMC1_HUMAN	0	8	2	2
ARM10_HUMAN	0	11	0	6

ARMC6_HUMAN	0	11	1	16
ARMX3_HUMAN	0	6	1	8
ARC1A_HUMAN	0	4	3	1
ARPC2_HUMAN	5	8	7	5
AS3MT_HUMAN	0	5	2	3
ASCC2_HUMAN	0	5	0	2
ASCC3_HUMAN	0	8	0	5
ASNA_HUMAN	6	9	0	7
ASSY_HUMAN	5	0	4	4
ATAD1_HUMAN	0	4	0	5
ATD3A_HUMAN	7	20	1	8
ATLA2_HUMAN	0	5	2	5
ATLA3_HUMAN	4	11	2	10
AT131_HUMAN	10	6	5	7
AT1A1_HUMAN	37	49	44	64
AT1B3_HUMAN	4	7	5	5
AT2B1_HUMAN	5	7	5	8
ATPA_HUMAN	24	36	26	24
ATPB_HUMAN	13	22	7	25
AT5F1_HUMAN	0	12	6	7
ATP5H_HUMAN	5	9	5	6
ATP5L_HUMAN	0	5	1	1
ATPF1_HUMAN	5	9	5	7
ATX10_HUMAN	8	24	13	48
AUP1_HUMAN	5	8	2	13
B3GA3_HUMAN	12	0	7	4
BAG5_HUMAN	0	4	0	4
BAG6_HUMAN	0	17	0	27
BAK_HUMAN	0	5	0	2
BAF_HUMAN	0	5	1	2
BAP18_HUMAN	0	5	0	0
BASP1_HUMAN	10	33	1	12
BAX_HUMAN	5	20	7	12
BAP31_HUMAN	25	10	37	12
BLMH_HUMAN	4	0	2	3
BPNT1_HUMAN	4	0	4	2
BCCIP_HUMAN	0	7	3	4
B2L12_HUMAN	0	4	0	4
BCS1_HUMAN	0	10	0	3

BET1_HUMAN	0	4	1	3
BID_HUMAN	0	5	2	0
BLMH_HUMAN	0	5	2	3
BIEA_HUMAN	0	4	6	3
BLVRB_HUMAN	0	4	0	0
BRX1_HUMAN	4	0	0	0
BTF3_HUMAN	4	0	2	2
BSDC1_HUMAN	0	4	0	3
BASI_HUMAN	0	10	0	6
BTAF1_HUMAN	0	7	2	6
BUB1_HUMAN	0	5	0	0
BYST_HUMAN	0	6	0	2
BZW1_HUMAN	18	61	4	33
BZW2_HUMAN	6	12	7	20
CJ076_HUMAN	0	5	0	4
CL073_HUMAN	0	5	0	0
CN166_HUMAN	4	7	6	6
CP080_HUMAN	5	0	0	0
CP062_HUMAN	0	5	0	3
C1QBP_HUMAN	7	5	6	7
CT072_HUMAN	0	4	0	0
CB018_HUMAN	6	28	0	0
CB029_HUMAN	0	10	0	0
CB043_HUMAN	0	4	0	3
CB047_HUMAN	0	5	0	2
CE051_HUMAN	0	6	2	11
CF047_HUMAN	0	5	0	2
CAB39_HUMAN	4	6	3	0
CACL1_HUMAN	0	8	0	8
CYBP_HUMAN	5	8	8	6
CADM1_HUMAN	0	8	0	6
CALR_HUMAN	23	21	31	38
CALU_HUMAN	0	8	2	5
CAMLG_HUMAN	0	4	1	2
CAND1_HUMAN	18	41	28	29
CAND2_HUMAN	0	4	0	6
CANT1_HUMAN	0	10	0	10
CALX_HUMAN	39	57	22	25
CAN1_HUMAN	17	9	8	7

CAN2_HUMAN	8	11	11	5
CAZA1_HUMAN	7	10	9	5
CARM1_HUMAN	5	0	2	0
CSKP_HUMAN	0	4	0	0
CASP3_HUMAN	0	4	0	0
CASP7_HUMAN	0	4	0	1
CASP8_HUMAN	0	4	0	0
CC134_HUMAN	0	6	0	3
CCD47_HUMAN	0	14	4	11
CCD51_HUMAN	0	5	0	6
CCNY_HUMAN	0	5	0	3
CD109_HUMAN	0	4	0	1
CD63_HUMAN	0	5	0	0
CDC23_HUMAN	0	5	0	3
CDC37_HUMAN	0	7	4	5
CDC45_HUMAN	0	5	0	5
CDIPT_HUMAN	0	8	2	8
CDK1_HUMAN	9	18	9	21
CDK2_HUMAN	0	11	5	7
CDK4_HUMAN	0	7	6	9
CDK5_HUMAN	0	7	2	4
CK5P3_HUMAN	0	10	1	8
CDKAL_HUMAN	0	4	0	2
CARF_HUMAN	0	9	2	1
CELF1_HUMAN	0	4	0	3
COF1_HUMAN	17	16	21	18
CHK2_HUMAN	0	4	0	2
CHERP_HUMAN	0	4	0	0
CHSTC_HUMAN	0	4	0	2
CTF18_HUMAN	0	4	0	3
CPIN1_HUMAN	4	16	3	5
CISD2_HUMAN	4	0	4	3
CKAP4_HUMAN	19	27	30	51
CKAP5_HUMAN	5	18	11	27
CLAP1_HUMAN	5	22	3	25
CLAP2_HUMAN	0	9	4	14
CLGN_HUMAN	0	11	0	5
CLIC1_HUMAN	0	11	11	14
CLPX_HUMAN	0	12	1	14

CLUS_HUMAN	0	5	1	9
CMBL_HUMAN	5	12	6	3
CNDP2_HUMAN	0	7	3	3
CNOT1_HUMAN	9	30	7	22
CNOTA_HUMAN	0	4	0	0
CNOT8_HUMAN	0	5	0	2
CN37_HUMAN	0	14	1	11
COA1_HUMAN	0	5	2	0
COG1_HUMAN	0	5	0	4
COG2_HUMAN	0	6	0	4
COG3_HUMAN	0	8	0	10
COG4_HUMAN	0	5	0	4
COG5_HUMAN	0	12	1	12
COG6_HUMAN	0	12	0	7
COG7_HUMAN	0	5	0	7
COG8_HUMAN	0	6	0	5
COMD4_HUMAN	0	4	1	7
COMD9_HUMAN	0	4	0	4
COPA_HUMAN	19	23	25	20
COPE_HUMAN	4	0	5	3
COPG1_HUMAN	14	18	15	25
COPG2_HUMAN	4	17	2	13
CSN2_HUMAN	0	4	3	2
CSN5_HUMAN	5	0	3	2
CSN6_HUMAN	0	6	0	4
COPZ1_HUMAN	0	5	1	4
COQ5_HUMAN	0	5	3	10
OQ7_HUMAN	0	8	0	2
COX15_HUMAN	0	12	0	5
COX41_HUMAN	0	15	1	5
COX5A_HUMAN	0	14	0	7
CX6B1_HUMAN	0	11	0	0
COX6C_HUMAN	0	6	0	0
CBPD_HUMAN	4	15	5	8
CPSF2_HUMAN	0	4	0	0
CPSF3_HUMAN	0	5	1	3
CPSF7_HUMAN	0	13	0	0
CPT2_HUMAN	8	16	4	9
CRKL_HUMAN	4	0	3	3

CSK_HUMAN	0	4	2	3
CSTF2_HUMAN	0	5	0	2
CTBP1_HUMAN	0	6	1	1
CTBP2_HUMAN	0	5	2	2
CTNA1_HUMAN	7	25	7	16
CTNL1_HUMAN	4	0	1	7
CTNB1_HUMAN	4	0	1	1
CTND1_HUMAN	0	18	0	13
CATC_HUMAN	0	4	1	1
CUL2_HUMAN	7	25	10	14
CUL3_HUMAN	0	6	2	4
CUTA_HUMAN	0	4	3	4
CXAR_HUMAN	0	6	0	0
CY1_HUMAN	5	8	0	2
CP20A_HUMAN	9	0	6	0
CYFP1_HUMAN	0	4	6	6
CP2U1_HUMAN	0	5	0	0
DAD1_HUMAN	0	4	7	7
DAG1_HUMAN	0	4	1	1
DGLB_HUMAN	0	5	0	3
DREB_HUMAN	7	11	6	7
DBNL_HUMAN	0	6	6	5
DCAF7_HUMAN	0	5	0	4
DCAKD_HUMAN	5	11	2	5
DCK_HUMAN	0	4	1	0
DCP1A_HUMAN	0	10	0	4
DCTN2_HUMAN	0	13	2	4
DD19A_HUMAN	0	5	3	6
DDX20_HUMAN	0	16	0	11
DDX24_HUMAN	0	4	0	3
DX39A_HUMAN	4	12	0	3
DX39B_HUMAN	0	6	7	9
DDX42_HUMAN	4	0	0	0
DDX46_HUMAN	9	0	28	13
DDX52_HUMAN	0	7	0	2
DDX56_HUMAN	0	4	0	0
DDX6_HUMAN	0	6	8	7
DECR_HUMAN	0	7	5	2
DERL1_HUMAN	0	5	3	3

DFFA_HUMAN	0	4	3	4
DRS7B_HUMAN	0	6	2	5
DIAP1_HUMAN	0	4	3	1
DIDO1_HUMAN	0	4	0	0
DNJA1_HUMAN	0	6	4	12
DNJA2_HUMAN	6	12	3	6
DNJA3_HUMAN	0	4	0	2
DNJB1_HUMAN	0	4	6	4
DJC10_HUMAN	0	4	0	3
DJC11_HUMAN	0	8	0	2
DJC25_HUMAN	0	4	0	4
DNJC5_HUMAN	0	5	0	2
DNM1L_HUMAN	15	23	16	23
DNMT1_HUMAN	0	4	0	1
DPM1_HUMAN	0	16	2	10
DPP3_HUMAN	7	15	10	10
DRG1_HUMAN	0	5	4	8
DSG2_HUMAN	0	9	0	8
DUS12_HUMAN	0	9	0	2
DUS9_HUMAN	0	6	0	8
DUT_HUMAN	5	9	7	6
DVL2_HUMAN	0	4	0	0
DYHC1_HUMAN	36	47	26	14
DC1L1_HUMAN	0	6	0	3
DYHC2_HUMAN	0	7	0	4
ECE1_HUMAN	0	5	0	2
ECH1_HUMAN	5	8	0	0
ECHD1_HUMAN	0	6	1	6
ECHM_HUMAN	7	12	7	6
ECI2_HUMAN	0	5	2	2
ECM29_HUMAN	36	49	27	65
EDC3_HUMAN	0	4	5	3
EDC4_HUMAN	10	31	5	16
EF1A1_HUMAN	37	42	142	116
EF1A2_HUMAN	0	5	2	3
EF1B_HUMAN	0	5	4	5
EF1G_HUMAN	12	21	41	45
EFNB1_HUMAN	0	9	0	4
E2AK2_HUMAN	0	10	5	15

EI2BG_HUMAN	0	9	3	10
EI2BG_HUMAN	0	8	3	10
EI2BE_HUMAN	0	7	0	4
EIF3B_HUMAN	9	16	11	11
EIF3C_HUMAN	13	15	22	14
EIF3E_HUMAN	17	22	11	17
EIF3F_HUMAN	0	10	11	16
ENOG_HUMAN	0	8	1	1
ENY2_HUMAN	0	7	0	2
EPIPL_HUMAN	0	11	0	2
ERAL1_HUMAN	0	7	0	3
ERAP1_HUMAN	0	12	3	6
ERCC2_HUMAN	0	6	0	0
ERGI3_HUMAN	0	7	0	2
ERO1A_HUMAN	0	7	3	7
ESYT2_HUMAN	0	11	2	15
ETFD_HUMAN	0	5	0	0
EXOC1_HUMAN	0	6	0	0
EXOC4_HUMAN	0	16	1	10
EXOC6_HUMAN	0	9	0	6
EXOC7_HUMAN	0	12	0	12
EXOC8_HUMAN	0	8	1	8
EXOSX_HUMAN	0	7	0	1
FADS2_HUMAN	0	12	2	8
FAF2_HUMAN	0	6	3	5
F1142_HUMAN	0	13	0	12
F134C_HUMAN	0	8	3	7
F16B1_HUMAN	0	8	0	2
F162A_HUMAN	0	8	0	3
F203A_HUMAN	5	14	0	0
FA49A_HUMAN	0	7	0	0
FA49B_HUMAN	0	13	0	8
FA54B_HUMAN	0	6	0	0
FACD2_HUMAN	0	7	2	4
FAKD2_HUMAN	0	7	0	7
FAKD5_HUMAN	0	9	0	7
FBRL_HUMAN	0	10	0	7
FDFT_HUMAN	0	8	0	10
FKBP5_HUMAN	0	10	4	5

GABPA_HUMAN	0	5	0	0
GAK_HUMAN	0	6	0	4
GAPD1_HUMAN	0	16	5	16
GCFC2_HUMAN	0	8	0	17
GEMI4_HUMAN	0	13	5	11
GEMI5_HUMAN	0	14	7	11
GHITM_HUMAN	0	9	0	10
GLMN_HUMAN	0	6	0	5
GLOD4_HUMAN	0	6	5	3
GOLP3_HUMAN	0	7	2	4
GOSR2_HUMAN	0	5	0	6
GRB2_HUMAN	0	6	0	1
GRWD1_HUMAN	0	6	4	7
GSTK1_HUMAN	0	14	1	5
HCFC1_HUMAN	0	10	5	3
HDAC2_HUMAN	0	5	1	2
HEAT2_HUMAN	0	17	0	0
HEAT3_HUMAN	0	5	0	5
HELZ_HUMAN	0	6	0	3
HINT1_HUMAN	0	5	0	3
HXK1_HUMAN	12	15	7	8
HM13_HUMAN	9	15	14	31
HMOX2_HUMAN	7	18	4	13
HNRPF_HUMAN	9	17	8	10
HNRH1_HUMAN	4	15	7	9
HNRPK_HUMAN	12	16	15	15
HNRPM_HUMAN	33	64	3	9
HNRDL_HUMAN	0	7	5	5
HPRT_HUMAN	6	10	10	8
HCD2_HUMAN	9	12	19	47
HS90A_HUMAN	17	27	63	49
HS90B_HUMAN	11	25	7	32
HSPB1_HUMAN	11	38	9	24
HS105_HUMAN	0	9	20	19
HUWE1_HUMAN	6	21	10	7
IDHC_HUMAN	4	12	4	11
IDHP_HUMAN	10	18	8	10
IDH3B_HUMAN	5	19	0	15
IDH3G_HUMAN	0	7	0	4

IFIT5_HUMAN	0	6	4	2
IMPA1_HUMAN	0	6	3	2
IMPA3_HUMAN	0	11	0	4
IMDH2_HUMAN	0	6	8	10
INF2_HUMAN	0	8	0	8
IPO5_HUMAN	17	29	22	36
IPO8_HUMAN	7	14	6	16
IQGA3_HUMAN	0	6	0	5
IRAK1_HUMAN	0	7	0	7
IST1_HUMAN	0	8	1	3
ITB1_HUMAN	5	17	6	23
KDM3B_HUMAN	0	12	1	3
KLC2_HUMAN	0	7	6	4
KLC3_HUMAN	0	12	0	6
LAS1L_HUMAN	0	8	0	6
LICH_HUMAN	0	6	0	0
LMAN1_HUMAN	0	13	3	16
LMF2_HUMAN	0	11	2	3
LGAT1_HUMAN	0	8	2	6
LRBA_HUMAN	0	11	2	8
LRP1_HUMAN	0	9	0	0
LPPRC_HUMAN	32	77	36	55
LRRC1_HUMAN	0	16	2	10
LYN_HUMAN	0	7	0	3
MAGT1_HUMAN	0	11	0	5
MK14_HUMAN	0	6	0	6
MK08_HUMAN	0	7	2	6
MAVS_HUMAN	0	10	4	12
MUC18_HUMAN	0	7	0	0
MCCB_HUMAN	6	13	16	7
MED27_HUMAN	0	9	0	5
MET7A_HUMAN	0	6	0	2
MPC1_HUMAN	0	6	0	0
MPC2_HUMAN	0	7	0	3
MPP2_HUMAN	0	6	1	0
MPP6_HUMAN	0	10	0	2
MSH2_HUMAN	0	12	5	16
MST4_HUMAN	0	7	0	0
MTDC_HUMAN	0	7	0	3

MTOR_HUMAN	0	27	0	23
MTX1_HUMAN	0	11	0	8
MTX2_HUMAN	0	7	0	2
MYCBP_HUMAN	0	7	0	0
MYO1B_HUMAN	0	10	0	6
MYO1C_HUMAN	0	10	0	8
MYO1D_HUMAN	0	9	0	1
NAA10_HUMAN	0	7	3	5
NAA15_HUMAN	6	10	13	7
NCAM1_HUMAN	0	9	0	0
NICA_HUMAN	0	10	2	9
NDRG1_HUMAN	0	8	4	8
NDUAA_HUMAN	0	9	0	5
NDUBA_HUMAN	0	7	0	4
NDUB8_HUMAN	0	7	0	4
NDUS3_HUMAN	0	8	0	6
NSDHL_HUMAN	7	14	2	6
NSF_HUMAN	5	12	6	21
NSUN2_HUMAN	29	44	23	18
NT5D2_HUMAN	10	37	3	12
NUCB2_HUMAN	0	8	0	2
NUDC3_HUMAN	0	10	2	5
NU188_HUMAN	0	11	0	7
NU188_HUMAN	0	10	0	7
NUP93_HUMAN	6	20	3	14
OAT_HUMAN	11	16	16	17
OCAD1_HUMAN	4	9	2	5
OSBP1_HUMAN	0	9	7	5
PA2G4_HUMAN	13	17	14	16
PACN3_HUMAN	7	0	5	4
PALM_HUMAN	0	5	5	4
PAPS2_HUMAN	6	0	0	3
PYC_HUMAN	70	19	106	24
PCCB_HUMAN	10	0	12	0
PCY1A_HUMAN	0	4	0	3
PDCD6_HUMAN	0	6	0	2
PDK3_HUMAN	4	12	4	9
PDK3_HUMAN	4	12	4	9
PDS5A_HUMAN	7	23	3	16

PDXD1_HUMAN	9	22	10	23
PEF1_HUMAN	0	6	0	7
PEX14_HUMAN	0	8	0	5
PEX19_HUMAN	0	6	0	8
PEX5_HUMAN	0	6	0	4
PGAM1_HUMAN	12	16	34	32
PGRC1_HUMAN	0	13	0	14
PHF6_HUMAN	0	7	4	1
P4K2A_HUMAN	0	9	2	6
PLPL6_HUMAN	0	4	1	2
PPID_HUMAN	0	16	0	16
PPME1_HUMAN	4	9	4	6
PTPA_HUMAN	0	7	2	3
PP4R1_HUMAN	0	11	2	8
PPT1_HUMAN	0	7	0	5
PRKDC_HUMAN	89	141	28	75
PRPF3_HUMAN	0	7	0	2
PRP6_HUMAN	0	19	3	8
PSN1_HUMAN	0	6	0	2
PSB7_HUMAN	0	7	9	9
PSD11_HUMAN	11	21	14	24
PSD12_HUMAN	7	20	10	23
PUM1_HUMAN	0	10	0	9
RAB14_HUMAN	5	12	8	7
RAB34_HUMAN	0	8	1	4
RBGPR_HUMAN	0	9	2	5
RAD50_HUMAN	0	12	0	1
RAGP1_HUMAN	0	20	12	21
RAVR1_HUMAN	0	9	4	1
RBM14_HUMAN	0	6	0	0
RBM4B_HUMAN	0	6	2	4
RCN2_HUMAN	0	8	3	7
RFC5_HUMAN	4	10	2	4
RGS10_HUMAN	0	6	0	0
RIF1_HUMAN	5	15	0	4
RN213_HUMAN	0	10	0	1
RFA2_HUMAN	0	6	0	2
RL19_HUMAN	0	6	28	24
RPN2_HUMAN	20	32	10	34

RCD1_HUMAN	0	6	1	3
RTN2_HUMAN	0	7	0	0
RTN4_HUMAN	8	18	9	35
SAC1_HUMAN	0	14	3	7
SMAG2_HUMAN	0	8	0	8
SCD5_HUMAN	0	6	0	4
S23IP_HUMAN	0	9	4	10
SC31A_HUMAN	7	21	3	12
SERPH_HUMAN	14	34	12	21
SFXN1_HUMAN	17	32	4	15
SFXN4_HUMAN	0	8	0	3
SG196_HUMAN	0	7	0	4
SGTA_HUMAN	13	43	2	5
SHLB1_HUMAN	0	10	7	19
SIN3A_HUMAN	0	6	0	0
S12A2_HUMAN	0	11	3	8
SNX4_HUMAN	0	7	2	7
SOAT1_HUMAN	0	13	3	5
SPCS2_HUMAN	0	13	3	6
SPG20_HUMAN	0	10	4	10
SPTC2_HUMAN	0	10	5	9
SRA1_HUMAN	0	11	0	0
SORCN_HUMAN	0	6	0	3
SRP68_HUMAN	6	15	8	15
SRPR_HUMAN	0	8	0	6
STX10_HUMAN	0	6	0	2
STX16_HUMAN	0	7	2	0
STX18_HUMAN	0	6	0	2
STX5_HUMAN	0	5	0	4
SUCB2_HUMAN	0	8	6	4
SURF4_HUMAN	0	12	9	24
SYMPK_HUMAN	6	24	0	5
TADBP_HUMAN	6	13	3	8
TBC15_HUMAN	4	16	2	12
TBCD5_HUMAN	0	6	0	0
TCF25_HUMAN	0	6	3	3
T11L1_HUMAN	0	11	0	4
TECR_HUMAN	4	16	7	16
TELO2_HUMAN	5	11	3	19

TES_HUMAN	0	6	2	1
TIM_HUMAN	0	6	1	6
TI23B_HUMAN	0	6	0	1
TIDC1_HUMAN	0	9	0	6
TMEDA_HUMAN	0	6	0	4
TMED2_HUMAN	0	7	0	5
T126A_HUMAN	0	6	0	6
TM165_HUMAN	0	6	4	7
TMOD3_HUMAN	0	12	0	4
TMX3_HUMAN	0	18	2	13
TNPO3_HUMAN	5	12	5	19
TOM34_HUMAN	0	9	4	7
TOM40_HUMAN	0	25	3	10
TOR1A_HUMAN	0	20	0	5
TOIP2_HUMAN	0	7	2	11
TPD52_HUMAN	0	6	0	1
TPD54_HUMAN	4	14	2	7
TPPC3_HUMAN	0	10	1	3
TRIPC_HUMAN	0	8	0	9
TTC19_HUMAN	0	7	0	0
TTI1_HUMAN	0	8	2	7
TTI2_HUMAN	0	8	0	6
TXND5_HUMAN	0	9	4	7
UBE2C_HUMAN	0	7	0	20
UB2L3_HUMAN	0	7	4	4
UBE2O_HUMAN	0	5	5	5
UBE3C_HUMAN	0	11	0	9
UBQL1_HUMAN	0	10	0	4
UBR4_HUMAN	0	26	8	12
UCHL5_HUMAN	4	17	5	9
UFL1_HUMAN	4	23	2	22
UN45A_HUMAN	7	23	9	25
UCRIL_HUMAN	0	6	2	0
UBP10_HUMAN	0	12	2	0
USP9X_HUMAN	10	48	19	36
VAMP7_HUMAN	0	6	3	6
VDAC1_HUMAN	18	44	9	17
VPS28_HUMAN	0	11	0	4
WAPL_HUMAN	4	14	0	1

XPO7_HUMAN	12	32	12	33
XXLT1_HUMAN	0	6	0	3
YIF1B_HUMAN	0	8	0	3
ZC3HF_HUMAN	0	6	4	4
ZDH13_HUMAN	4	7	0	4
ZW10_HUMAN	0	11	8	12