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Supporting Information

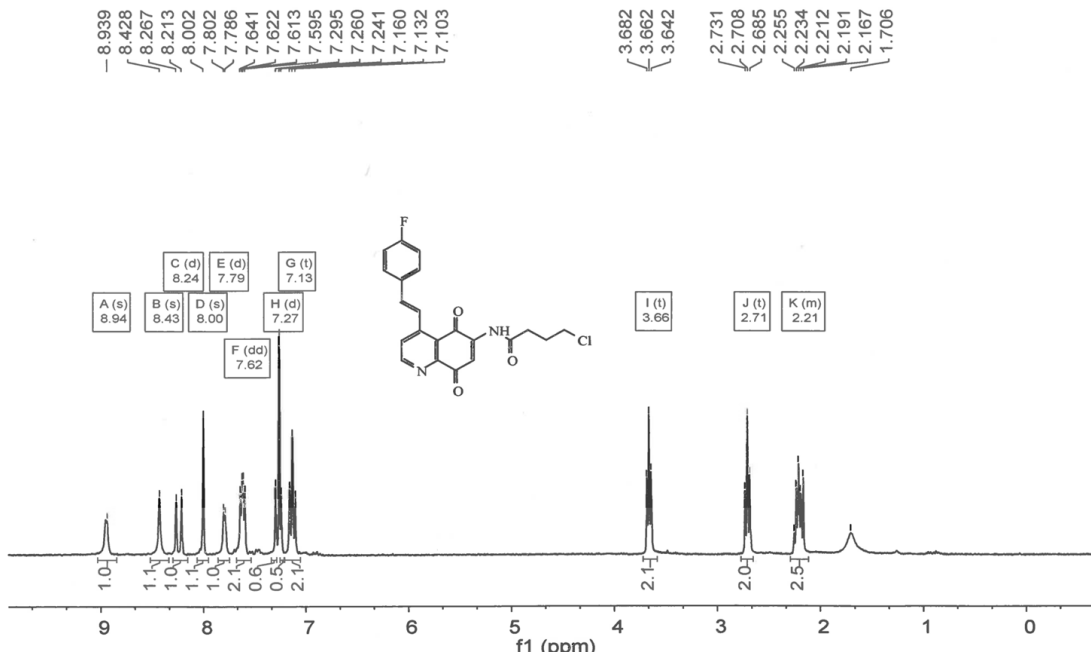
Discovery of Mitochondrial Transcription Inhibitors Active in Pancreatic Cancer Cells

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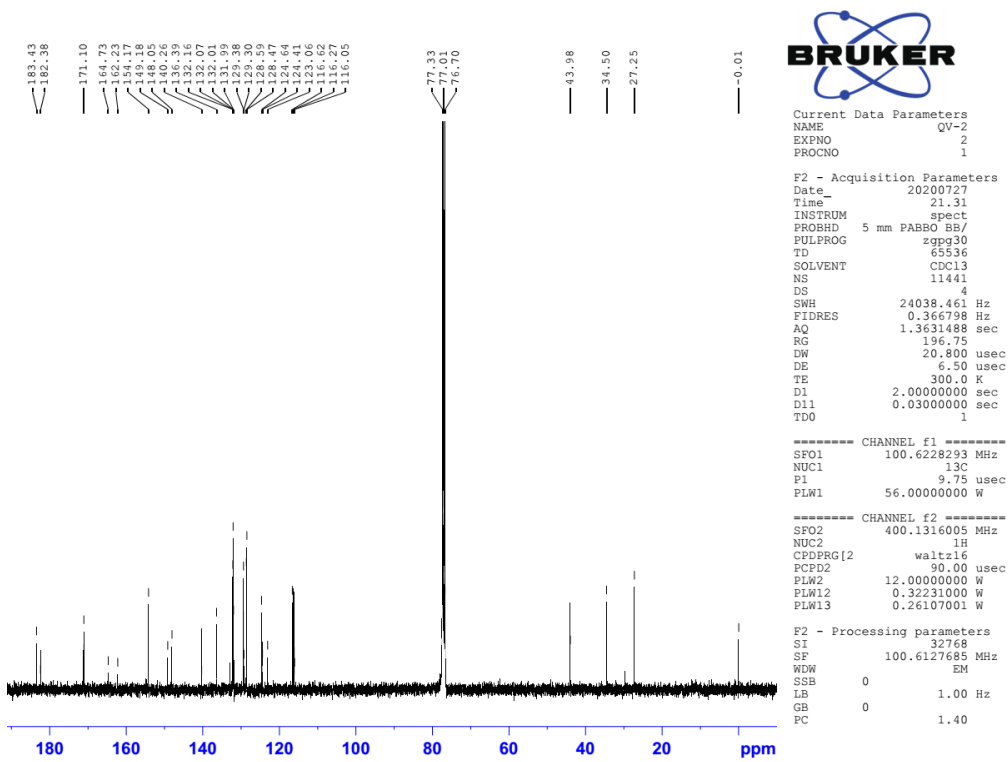
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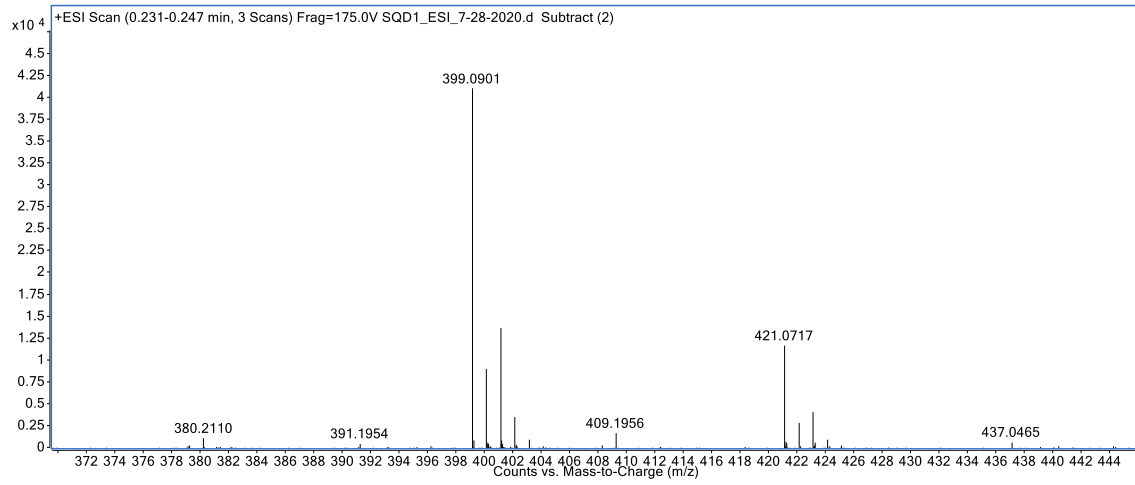
The NMR of SQD1



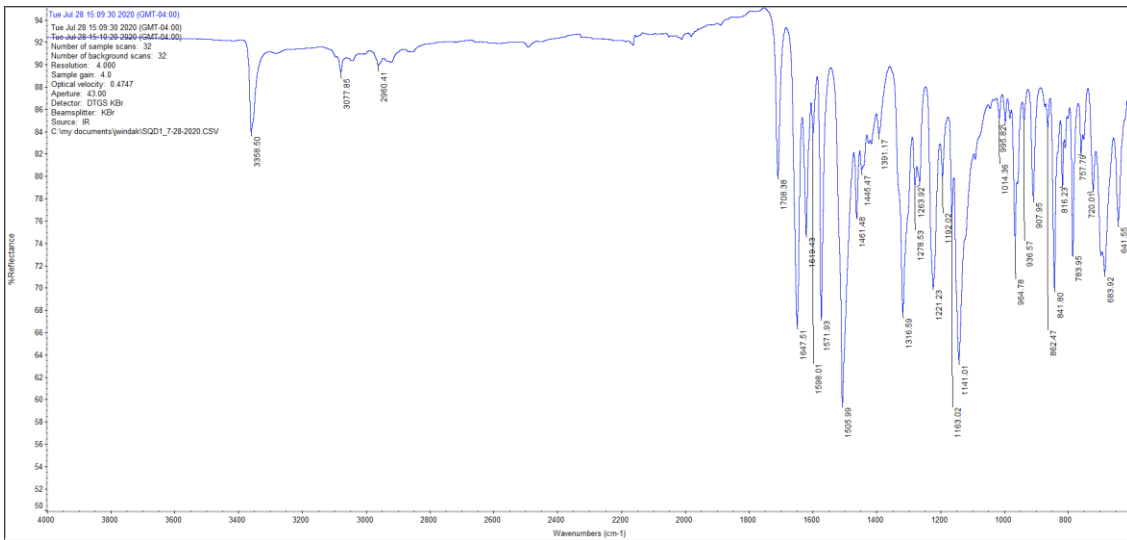
13C-NMR



HRMS



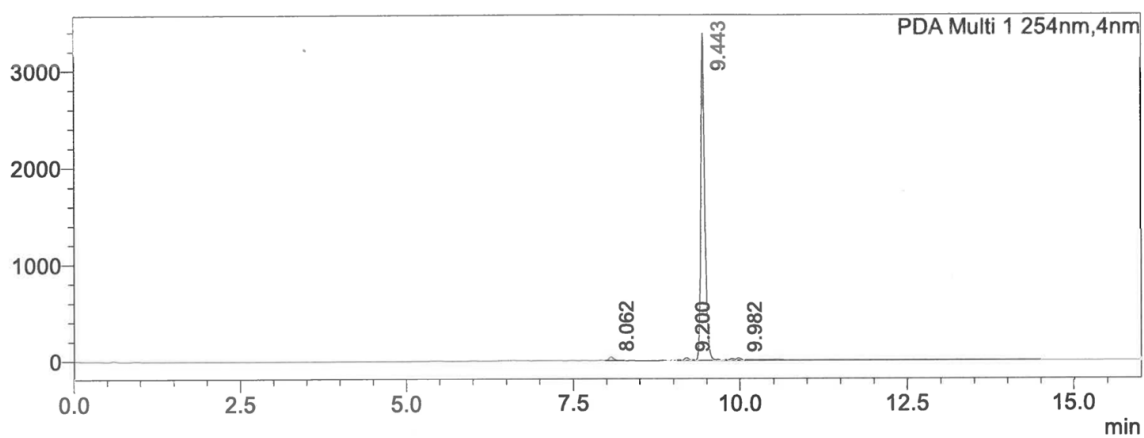
FTIR



The purity of SQD1

<LC-Chromatogram>

mAU



Peak Table

Peak#	Ret. Time	Area	Area%
1	8.062	203762	1.374
2	9.200	212655	1.434
3	9.443	14255323	96.126
4	9.982	158162	1.067
Total		14829901	100.000

Supplementary Table 1. The top 25 upregulated and downregulated protein-coding genes after Mito-Chlor and SQD1 treatment.

Mito-Chlor_51μM treatment				SQD1_5.4μM treatment			
up genes		down genes		up genes		down genes	
<i>Gene name</i>	FC	<i>Gene name</i>	FC	<i>Gene name</i>	FC	<i>Gene name</i>	FC
<i>HMOX1</i>	205.6	<i>TAS2R13</i>	0.07	<i>HMOX1</i>	315.4	<i>E2F8</i>	0.1
<i>CHAC1</i>	115.8	<i>TAS2R20</i>	0.07	<i>ANKRD37</i>	56.1	<i>NSL1</i>	0.11
<i>SLC7A11</i>	34.4	<i>CDK5RAP3</i>	0.1	<i>HK2</i>	53.2	<i>SLC25A12</i>	0.12
<i>OSGIN1</i>	25.7	<i>SPIDR</i>	0.11	<i>PFKFB4</i>	40.9	<i>UBE2T</i>	0.13
<i>CTH</i>	25.6	<i>CDKAL1</i>	0.11	<i>SCAND2P</i>	40.3	<i>MMS22L</i>	0.13
<i>FOSB</i>	25.3	<i>TAS2R13</i>	0.07	<i>ADM</i>	40	<i>GSTA4</i>	0.14
<i>DDIT4</i>	25.2	<i>TAS2R20</i>	0.07	<i>DDIT4</i>	37.1	<i>ATP23</i>	0.14
<i>TRIB3</i>	24.4	<i>CDK5RAP3</i>	0.1	<i>BNIP3L</i>	34.7	<i>TRMT1L</i>	0.15
<i>LMO4</i>	22.3	<i>SPIDR</i>	0.11	<i>CXCL8</i>	31.7	<i>POLE2</i>	0.15
<i>CDKN1A</i>	22.1	<i>CDKAL1</i>	0.11	<i>LUCAT1</i>	31	<i>HAUS4</i>	0.15
<i>SESN2</i>	21.6	<i>RGS3</i>	0.13	<i>BHLHE40</i>	30.2	<i>ETAA1</i>	0.15
<i>GABARAPL1</i>	20.6	<i>MSH3</i>	0.14	<i>MAP1LC3B</i>	29.6	<i>FMC1</i>	0.15
<i>BTG1</i>	20.5	<i>EXOC4</i>	0.14	<i>ENO2</i>	29.1	<i>RANP3</i>	0.15
<i>MAP1LC3B</i>	19.2	<i>RANBP17</i>	0.14	<i>C10orf10</i>	28.8	<i>SFXN2</i>	0.15
<i>ASNS</i>	18.6	<i>SCMH1</i>	0.14	<i>HSPA1B</i>	28.4	<i>ALG10</i>	0.15
<i>CEBPB</i>	18.3	<i>DPH6</i>	0.14	<i>HSPA1A</i>	28.3	<i>MTMR4</i>	0.15
<i>DDIT3</i>	18.2	<i>DYM</i>	0.15	<i>CSRNP1</i>	24.9	<i>NAPEPLD</i>	0.16
<i>HBEGF</i>	18	<i>FTO</i>	0.15	<i>UPRT</i>	24.6	<i>PHKB</i>	0.17
<i>TRIB1</i>	17.5	<i>CIT</i>	0.15	<i>JUN</i>	24.6	<i>ZNF66</i>	0.17
<i>SLFN5</i>	16.6	<i>ZNF407</i>	0.15	<i>HILPDA</i>	24.5	<i>MAP10</i>	0.17
<i>NDST2</i>	16.3	<i>TM7SF3</i>	0.15	<i>GABARAPL1</i>	24.1	<i>ZBED8</i>	0.17
<i>DNAJB4</i>	15.5	<i>TAS2R46</i>	0.15	<i>HSPH1</i>	22.3	<i>LAGE3P1</i>	0.17
<i>DUSP5</i>	14.4	<i>SKP2</i>	0.15	<i>PGK1</i>	22.1	<i>RWDD2B</i>	0.17
<i>CXCL8</i>	14.3	<i>TAS2R14</i>	0.15	<i>TRIB3</i>	21.3	<i>DPH6</i>	0.18
<i>PHGDH</i>	14.1	<i>LMNB1</i>	0.15	<i>FAM162A</i>	21	<i>PIGM</i>	0.18

Supplementary Table 2. Common top 20 gene sets in SQD1 and Mito-Chlortreated MIA PaCa-2 cells

(A) 7 common upregulated enriched gene sets. (B) 10 common downregulated enriched gene sets.

(A)

Common upregulated enriched gene sets

PODAR_RESPONSE_TO_ADAPHOSTIN_UP
CONCANNON_APOPTOSIS_BY_EPOXOMICIN_UP
HELLER_SILENCED_BY_METHYLATION_DN
GROSS_HYPOXIA_VIA_ELK3_DN
NAGASHIMA_NRG1_SIGNALING_UP
NAGASHIMA_EGF_SIGNALING_UP
ZWANG_CLASS_3_TRANSIENTLY_INDUCED_BY_EGF

(B)

Common downregulated enriched gene sets

KONG_E2F3_TARGETS
LEE_EARLY_T_LYMPHOCYTE_UP
GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIVIDING_DN
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_TURQUOISE_D
N
DUTERTRE ESTRADIOL_RESPONSE_24HR_UP
ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER
CHANG_CYCLING_GENES
GOBERT_OLIGODENDROCYTE_DIFFERENTIATION_UP
ZHANG_TLX_TARGETS_DN
FUJII_YBX1_TARGETS_DN

Supplementary Table 3. Top 25 (A) upregulated and (B) downregulated enriched gene sets in SQD1-treated MIA PaCa-2 cells.

(A)

NAME	SIZE	NES	FDR
NAGASHIMA_NRG1_SIGNALING_UP	126	3.22	0
ELVIDGE_HYPOXIA_UP	100	3.19	0
MENSE_HYPOXIA_UP	70	3.17	0
PODAR_RESPONSE_TO_ADAPHOSTIN_UP	111	3.15	0
ELVIDGE_HYPOXIA_BY_DMOG_UP	77	3.12	0
WINTER_HYPOXIA_METAGENE	141	3.05	0
CONCANNON_APOPTOSIS_BY_EPOXOMICIN_UP	167	2.99	0
GROSS_HYPOXIA_VIA_ELK3_AND_HIF1A_UP	103	2.99	0
GROSS_HYPOXIA_VIA_ELK3_DN	105	2.99	0
LEONARD_HYPOXIA	34	2.96	0
QI_HYPOXIA	83	2.95	0
ZWANG_CLASS_3_TRANSIENTLY_INDUCED_BY_EGF	155	2.93	0
NAGASHIMA_EGF_SIGNALING_UP	42	2.93	0
ELVIDGE_HIF1A_AND_HIF2A_TARGETS_DN	60	2.92	0
HARRIS_HYPOXIA	38	2.91	0
ELVIDGE_HIF1A_TARGETS_DN	49	2.88	0
HELLER_SILENCED_BY_METHYLATION_DN	60	2.88	0
PRAMOONJAGO_SOX4_TARGETS_UP	39	2.81	0
KIM_WT1_TARGETS_UP	153	2.80	0
ZWANG_CLASS_1_TRANSIENTLY_INDUCED_BY_EGF	308	2.80	0
GALINDO_IMMUNE_RESPONSE_TO_ENTEROTOXIN	57	2.79	0
KRIGE_AMINO_ACID_DEPRIVATION	20	2.79	0
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLI PIDS_BLUE_UP	105	2.79	0
PICCALUGA_ANGIOIMMUNOBLASTIC_LYMPHOMA_D N	106	2.78	0
KIM_WT1_TARGETS_8HR_UP	121	2.77	0

(B)

NAME	SIZE	NES	FDR
DUTERTRE ESTRADIOL_RESPONSE_24HR_UP	254	-2.88	0
ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER	124	-2.70	0
LEE_EARLY_T_LYMPHOCYTE_UP	74	-2.68	0
GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIVIDING_DN	75	-2.65	0
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPI	39	-2.62	0

DS_TURQUOISE_DN			
KONG_E2F3_TARGETS	86	-2.55	0
GOBERT_OLIGODENDROCYTE_DIFFERENTIATION_UP	440	-2.52	0
REACTOME_MEIOTIC_RECOMBINATION	59	-2.50	0
CHANG_CYCLING_GENES	127	-2.49	0
ZHANG_TLX_TARGETS_DN	81	-2.49	0
PYEON_HPVP_POSITIVE_TUMORS_UP	61	-2.49	0
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_24HR	105	-2.48	0
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	139	-2.48	0
REACTOME_MEIOSIS	77	-2.48	0
FUJII_YBX1_TARGETS_DN	171	-2.47	0
REACTOME_G2_M_CHECKPOINTS	40	-2.46	0
REN_BOUND_BY_E2F	58	-2.45	0
ZHAN_MULTIPLE_MYELOMA_PR_UP	40	-2.43	0
REACTOME_ACTIVATION_OF_ATR_IN_RESPONSE_TO_REPLICATION_STRESS	34	-2.40	0
KANG_DOXORUBICIN_RESISTANCE_UP	49	-2.40	0
ISHIDA_E2F_TARGETS	49	-2.40	0
REACTOME_CHROMOSOME_MAINTENANCE	92	-2.39	0
PID_FANCONI_PATHWAY	40	-2.36	0
REACTOME_DEPOSITION_OF_NEW_CENPA_CONTAINING_NUCLEOSOMES_AT_THE_CENTROMERE	50	-2.36	0

Supplementary Table 4. Top 25 (A) upregulated and (B) downregulated enriched gene sets in Mito-Chlor-treated MIA PaCa-2 cells.

(A)

NAME	SIZE	NES	FDR
PODAR_RESPONSE_TO_ADAPHOSTIN_UP	114	3.46	0
NAGASHIMA_NRG1_SIGNALING_UP	127	3.27	0
BLUM_RESPONSE_TO_SALIRASIB_UP	211	3.08	0
ZHANG_RESPONSE_TO_IKK_INHIBITOR_AND_TNF_UP	128	3.08	0
PICCALUGA_ANGIOIMMUNOBLASTIC_LYMPHOMA_DN	106	3.06	0
HELLER_SILENCED_BY_METHYLATION_DN	63	3.02	0
CONCANNON_APOPTOSIS_BY_EPOXOMICIN_UP	168	2.99	0
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPID_S_BLUE_UP	106	2.92	0
GROSS_HYPOXIA_VIA_ELK3_DN	105	2.91	0
KRIGE_AMINO_ACID_DEPRIVATION	21	2.90	0
NAGASHIMA_EGF_SIGNALING_UP	43	2.88	0
TIEN_INTESTINE_PROBIOTICS_24HR_DN	181	2.87	0
PHONG_TNF_TARGETS_UP	44	2.85	0
ZWANG_CLASS_3_TRANSIENTLY_INDUCED_BY_EGF	151	2.83	0
BURTON_ADIPOGENESIS_PEAK_AT_2HR	39	2.82	0
GERY_CEBP_TARGETS	73	2.80	0
MISSIAGLIA_REGULATED_BY_METHYLATION_UP	75	2.78	0
MITSIADES_RESPONSE_TO_APLIDIN_UP	311	2.78	0
CHEN_LVAD_SUPPORT_OF_FAILING_HEART_UP	54	2.74	0
PHONG_TNF_RESPONSE_VIA_P38_PARTIAL	110	2.70	0
WINTER_HYPOXIA_METAGENE	141	2.70	0
GROSS_HYPOXIA_VIA_ELK3_AND_HIF1A_UP	100	2.70	0
LEONARD_HYPOXIA	34	2.69	0
BOQUEST_STEM_CELL_CULTURED_VS_FRESH_UP	183	2.69	0
PRAMOONJAGO_SOX4_TARGETS_UP	37	2.69	0

(B)

NAME	SIZE	NES	FDR
DUTERTRE ESTRADIOL_RESPONSE_24HR_UP	256	-2.77	0
VILLANUEVA_LIVER_CANCER_KRT19_UP	139	-2.62	0
GOBERT_OLIGODENDROCYTE_DIFFERENTIATION_UP	448	-2.61	0
ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER	124	-2.60	0
ZHANG_TLX_TARGETS_DN	81	-2.54	0
CHANG_CYCLING_GENES	125	-2.51	0

ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_6HR	76	-2.51	0
ZHANG_TLX_TARGETS_60HR_DN	239	-2.46	0
ZHANG_TLX_TARGETS_36HR_DN	169	-2.44	0
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHO LIPIDS_TURQUOISE_DN	42	-2.40	0
FUJII_YBX1_TARGETS_DN	171	-2.40	0
PUJANA_BRCA2_PCC_NETWORK	355	-2.39	0
VERNELL_RETINOBLASTOMA_PATHWAY_UP	63	-2.38	0
CHIANG_LIVER_CANCER_SUBCLASS_PROLIFERATI ON_UP	125	-2.37	0
LEE_EARLY_T_LYMPHOCYTE_UP	76	-2.37	0
FLORIO_NEOCORTEX_BASAL_RADIAL_GLIA_DN	144	-2.26	2.74E-05
ISHIDA_E2F_TARGETS	49	-2.27	2.85E-05
CROONQUIST_IL6_DEPRIVATION_DN	90	-2.28	2.97E-05
KONG_E2F3_TARGETS	86	-2.29	3.10E-05
GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIV IDING_DN	75	-2.30	3.24E-05
REN_BOUND_BY_E2F	59	-2.30	3.40E-05
ZHAN_MULTIPLE_MYELOMA_PR_UP	40	-2.30	3.57E-05
KAUFFMANN_MELANOMA_RELAPSE_UP	58	-2.32	3.76E-05
PYEON_HPV_POSITIVE_TUMORS_UP	63	-2.33	3.96E-05

Supplementary Table 5. Top 25 (A) upregulated and (B) downregulated enriched gene sets in chlorambucil-treated MIA PaCa-2 cells.

(A)

NAME	SIZE	NES	FDR
KRIGE_AMINO_ACID_DEPRIVATION	21	2.46	0
DIRMEIER_LMP1_RESPONSE_EARLY	42	2.45	0
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_BLUE_UP	106	2.38	0
PODAR_RESPONSE_TO_ADAPHOSTIN_UP	114	2.34	0
PACHER_TARGETS_OF_IGF1_AND_IGF2_UP	20	2.29	0
HELLER_SILENCED_BY_METHYLATION_DN	61	2.21	8.20E-04
SCHMIDT_POR_TARGETS_IN_LIMB_BUD_UP	20	2.16	0.00106
SAGIV_CD24_TARGETS_DN	25	2.07	0.00710
PICCALUGA_ANGIOIMMUNOBLASTIC_LYMPHOMA_DN	107	2.05	0.00893
DACOSTA_UV_RESPONSE_VIA_ERCC3_COMMON_UP	60	2.05	0.00955
REACTOME_CHOLESTEROL_BIOSYNTHESIS	20	2.02	0.0114
SMIRNOV_RESPONSE_TO_IR_2HR_UP	41	2.01	0.0121
NAGASHIMA_NRG1_SIGNALING_UP	126	2.00	0.0127
UZONYI_RESPONSE_TO_LEUKOTRIENE_AND_THROMBIN	18	1.97	0.0162
TIAN_TNF_SIGNALING_NOT_VIA_NFKB	18	1.97	0.0170
SESTO_RESPONSE_TO_UV_C0	91	1.93	0.0226
CEBALLOS_TARGETS_OF_TP53_AND_MYC_UP	18	1.93	0.0228
ZHAN_MULTIPLE_MYELOMA_CD1_UP	18	1.94	0.0232
ADDYA_ERYTHROID_DIFFERENTIATION_BY_HEMIN	50	1.89	0.0288
NAGASHIMA_EGF_SIGNALING_UP	42	1.89	0.0289
AMIT_SERUM_RESPONSE_60_MCF10A	38	1.89	0.0297
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_YELLOW_UP	28	1.89	0.0299
ZHU_CMV_8_HR_UP	28	1.87	0.0303
WATTEL_AUTONOMOUS_THYROID_ADENOMA_DN	25	1.88	0.0304
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_MAGENTA_UP	23	1.90	0.0304

(B)

NAME	SIZE	NES	FDR
DACOSTA_UV_RESPONSE_VIA_ERCC3_COMMON_DN	416	-2.49	0
MARTINEZ_RESPONSE_TO TRABECTEDIN	34	-2.38	0
FISCHER_G2_M_CELL_CYCLE	207	-2.18	3.66E-04
GENTILE_UV_LOW_DOSE_DN	43	-2.15	4.38E-04

DAZARD_UV_RESPONSE_CLUSTER_G6	118	-2.15	5.48E-04
FLORIO_NEOCORTEX_BASAL_RADIAL_GLIA_DN	147	-2.07	0.00348
WHITFIELD_CELL_CYCLE_G2	148	-2.03	0.00532
IZADPANAHA_STEM_CELL_ADIPOSE_VS_BONE_DN	40	-2.02	0.00711
AMUNDSON_GAMMA_RADIATION_RESPONSE	38	-2.01	0.00730
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOL			
IPIDS_TURQUOISE_DN	44	-1.98	0.0103
HUTTMANN_B_CLL_POOR_SURVIVAL_DN	30	-1.99	0.0105
SHEPARD_BMYB_TARGETS	42	-1.97	0.0106
DACOSTA_UV_RESPONSE_VIA_ERCC3_TTD_DN	56	-1.98	0.0107
REICHERT_MITOSIS_LIN9_TARGETS	27	-1.96	0.0136
MIKKELSEN_IPS_WITH_HCP_H3K27ME3	16	-1.95	0.0144
DUTERTRE ESTRADIOL_RESPONSE_6HR_DN	35	-1.94	0.0150
BROWNE_HCMV_INFECTION_12HR_DN	56	-1.94	0.0154
DAZARD_RESPONSE_TO_UV_NHEK_DN	255	-1.93	0.0191
REACTOME_SIGNALING_BY_RHO_GTPASES	61	-1.92	0.0197
REACTOME_RNA_POL_I_PROMOTER_OPENING	45	-1.91	0.0230
NAKAYAMA_SOFT_TISSUE_TUMORS_PCA2_UP	53	-1.90	0.0254
KEGG_FOCAL_ADHESION	104	-1.89	0.0258
BROWNE_HCMV_INFECTION_20HR_DN	60	-1.90	0.0265
ODONNELL_TFRC_TARGETS_DN	98	-1.90	0.0274

MTT assay

Cytotoxicity of compounds was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were seeded in 96-well tissue culture plates at a density of 2.5×10^3 cells per well and allowed to adhere overnight. Cells were subsequently continuously treated with drugs for 72 h. Then, MTT solution was added to each well to give a final concentration of 0.3 mg mL^{-1} . The cells were incubated with MTT for 3 h. DMSO was added after removal of the media and the plates were shaken at room temperature for 15 min before measuring the absorption of soluble purple formazan at $\lambda_{\text{max}} = 570 \text{ nm}$ using microplate reader (Molecular Devices). IC_{50} values were calculated by a non-linear regression using GraphPad Prism (Version 6.0). At least three independent experiments were conducted.

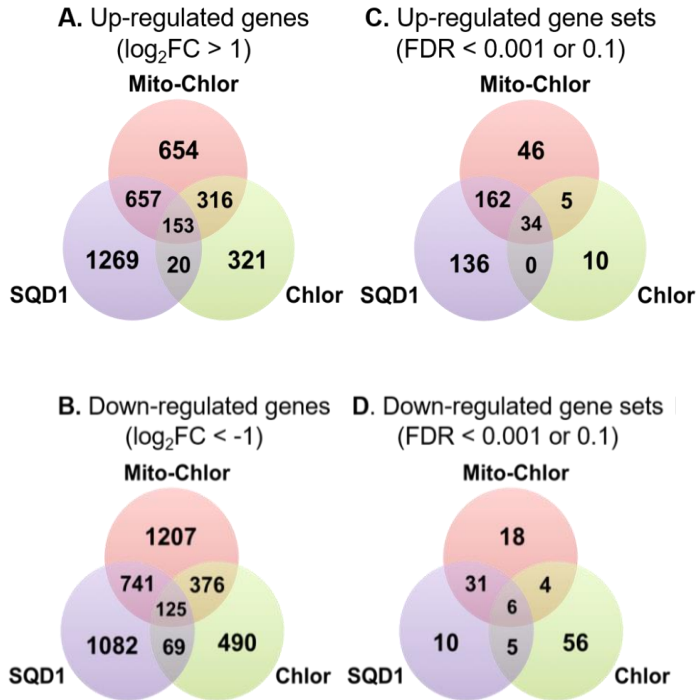
Supplementary Table 6. Cytotoxicity (IC_{50}^a , μM) of SQD1 in a panel of cancer cell lines.

	MDA-MB-231	MDA-MB-468	T-47D	HUCCT1	TFK1	BxPC-3	PANC-1	KYSE-410	KYSE-70
SQD1	3.2 ± 0.9	1.1 ± 0.2	1.2 ± 0.2	1.5 ± 0.6	5.6 ± 2.6	2.4 ± 0.2	1.1 ± 0.1	3.5 ± 0.1	2.9 ± 0.3

^a IC_{50} data are shown as mean \pm standard deviation from three independent experiments.

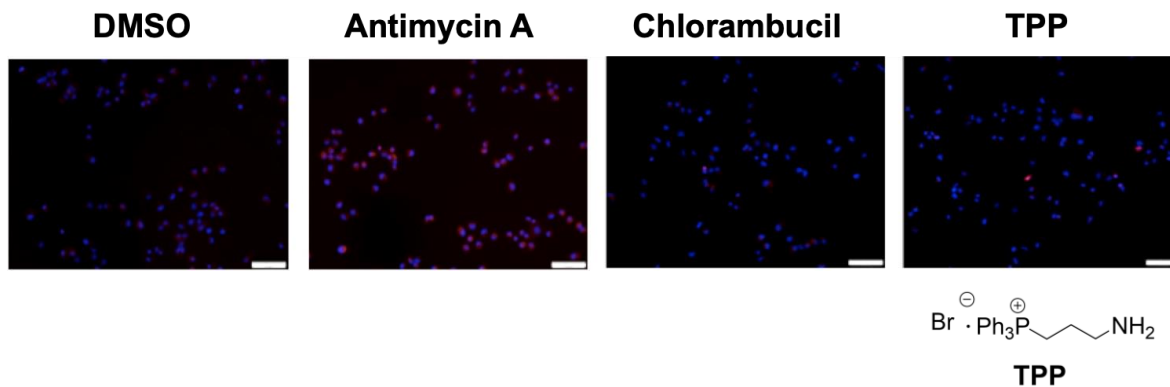
Supplementary Figure 1. Mito-Chlor-treated sample has more genes and gene sets in common with SQD1-treated sample than with chlorambucil-treated sample.

Chlor, chlorambucil. (A-B) Comparisons of upregulated and downregulated genes. (C-D) Comparisons of upregulated and downregulated gene sets.



Supplementary Figure 2. Chlorambucil and TPP did not induce mtROS in the MIA PaCa-2 cells.

Chlorambucil (233 μM), TPP (51 μM). MitoSOX dye was used at 2 μM . Objective: 20x.



Supplementary Figure 3. Measurement of cell survival and cell death in compound-treated MIA PaCa-2 cells.

(A) Cell morphology changes and percentage of live cells after 24h **SQD1** treatment. (B) Cleaved PARP expression in response to 24h treatment by **SQD1**, Mito-Chlor and H₂O₂. (C-D) Cell morphology changes and percentage of live cells after 4 h H₂O₂ treatment.

