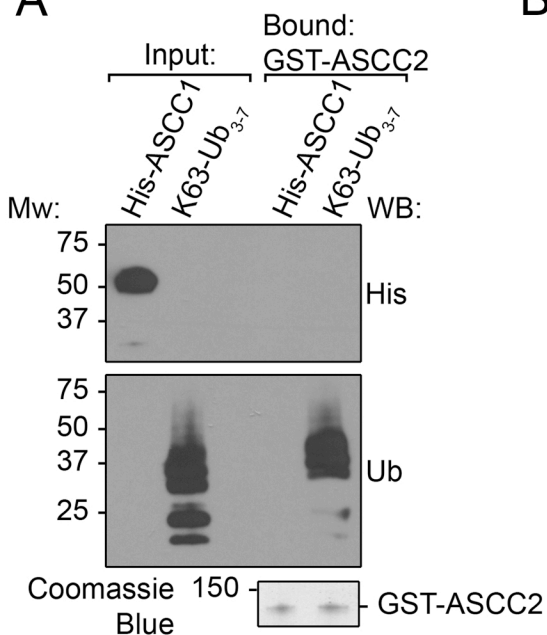
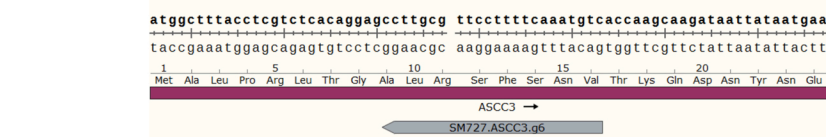


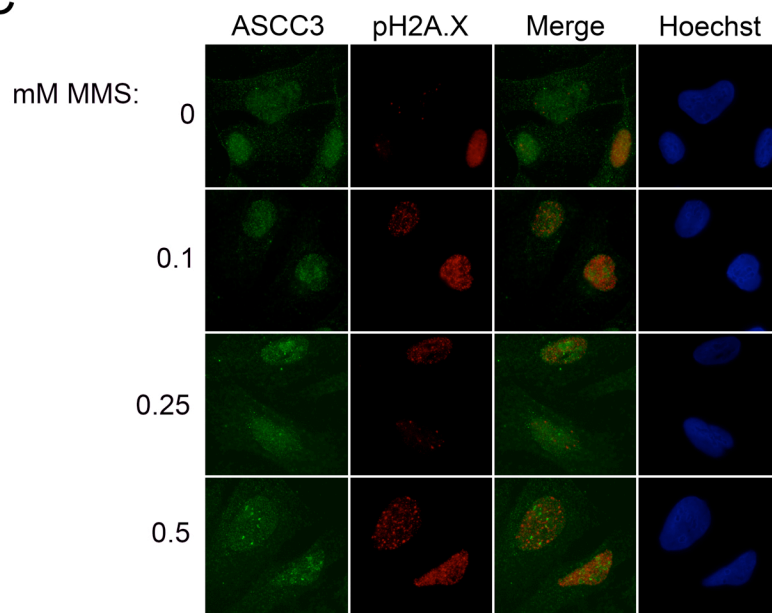
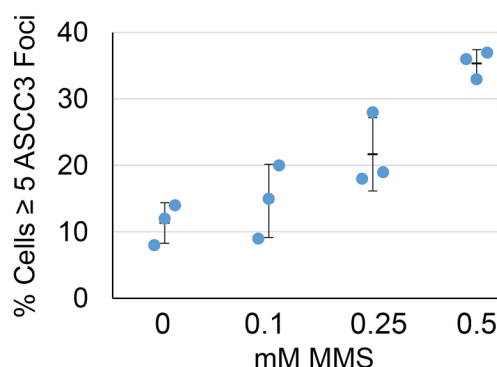
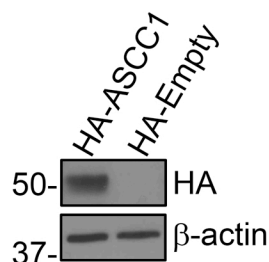
Antibodies used in this study

Protein/Antigen:	Manufacturer (catalog no.):	Application (Dilution):
ASCC3 (Rabbit)	(Described in Dango, et. al 2011)	IF (1:500) IP (1:100) WB (1:2500, 1:3000)
6x-His (Mouse)	Abcam (ab18184)	WB (1:2500)
ASCC1 (Mouse)	Abcam (ab67919)	WB (1:1250, 1:2500)
ASCC1 (Mouse)	Abcam (ab88792)	WB (1:1250)
ASCC2 (Rabbit)	Bethyl (A304-020A)	IP (1:200), WB (1:2500)
Flag (Mouse)	Sigma (F3165)	WB (1:2500)
HA (Mouse)	BioLegend (901501)	IF (1:250, 1:300) WB (1:2500, 1:5000)
HA (Rabbit)	Santa Cruz (sc-805)	WB (1:2500)
HA (Rabbit)	Abcam (ab9110)	WB (1:2500)
IgG (Rabbit)	Santa Cruz (sc-2027)	IP (1:200)
LSD1 (Mouse)	Santa Cruz (sc-53875)	WB (1:2500)
LSD1 (Rabbit)	Active Motif (39186)	WB (1:2500)
pH2A.X (Mouse)	Abcam (ab26350)	IF (1:2000)
PRP8 (Rabbit)	Bethyl (A303-922A)	IF (1:200, 1:400, 1:600)
Ub (P4D1) (Mouse)	Santa Crus (sc-8017)	WB (1:5000)
β -actin HRP (Mouse)	Sigma (A3854)	WB (1:2500, 1:5000)

Table S1. All antibodies used in this study with concentrations noted. The antibodies were produced in either rabbit or mouse. Applications include immunofluorescence microscopy (IF), Immunoprecipitation (IP) and Western blot (WB).

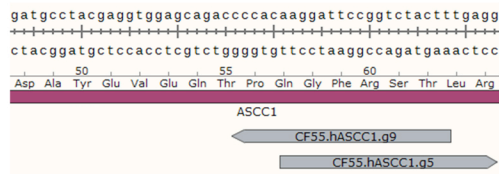
A**B**

	Total Reads:	WT Reads	#1 Indel:	#1 Reads:	#2 Indel:	#2 Reads:	#3 Indel:	#3 Reads:	#4 Indel:	#4 Reads:
ASCC3 KO	947	0 (0.0%)	-7	489 (51.6%)	-1	453 (47.8%)	-2	3 (0.3%)	-8	2 (0.2%)

C**D****E****F**

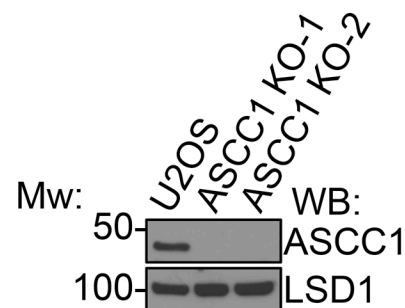
Supporting Information S1. (A) GST-ASCC2 was immobilized onto glutathione-Sepharose and incubated with His-ASCC1 or K63-Ub₃₋₇. After washing, the bound material was analyzed by SDS-PAGE and Western blot using anti-His antibody, anti-Ub antibody or by Coomassie Blue staining. **(B)** PC-3 ASCC3 knockout was generated using CRISPR/Cas9 technology. **(C)** U2OS WT cells were treated with the indicated doses of MMS for 6 hours as shown. Cells were processed for immunofluorescence using anti-ASCC3 and anti-pH2A.X antibodies, with Hoechst as the nuclear counterstain. **(D)** Quantification of **(C)**. **(E)** Whole cell lysates from U2OS WT cells expressing HA-tagged ASCC1 or empty vector were analyzed by Western blot. **(F)** Whole cell lysate from U2OS WT cells expressing HA-tagged ASCC1 were untreated or treated with MMS (0.5 mM) for 6 hours and analyzed by Western blotting.

A

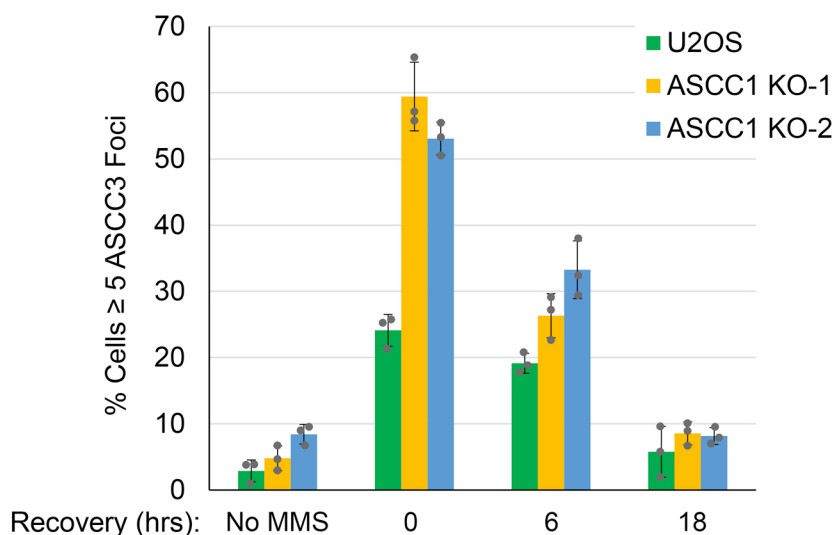


	Total Reads:	WT Reads	#1		#2		#3		#4	
			Indel:	Reads:	Indel:	Reads:	Indel:	Reads:	Indel:	Reads:
KO-1	5281	0 (0.0%)	-67	3428 (64.9%)	-1	1826 (34.6%)	-2	19 (0.4%)	-68	6 (0.1%)
KO-2	6106	0 (0.0%)	-58	2071 (33.9%)	-31	1587 (26.0%)	-28	1340 (21.9%)	1	1030 (16.9%)

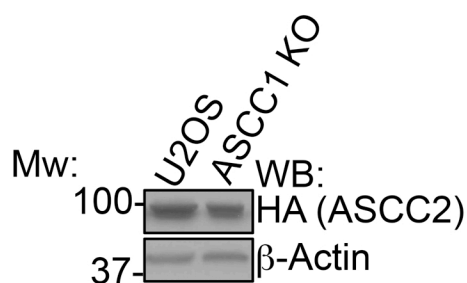
B



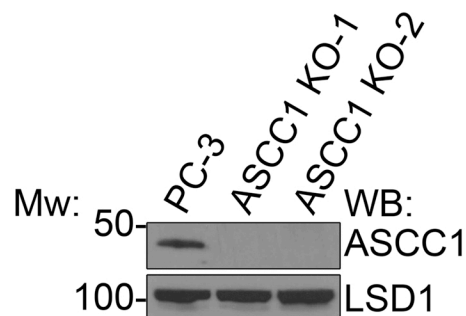
C



D

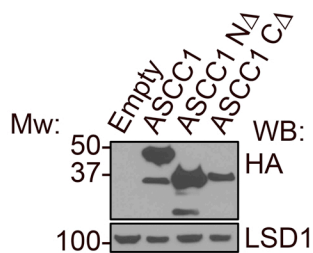


E



Supporting Information S2. (A) U2OS ASCC1 knockout was generated using CRISPR/Cas9 technology. **(B)** Whole cell lysate from WT and two U2OS ASCC1 KO clones were analyzed by Western blotting. **(C)** Quantification of ASCC3 foci in WT and ASCC1 KO cells treated with MMS (0.5 mM) for 6 hours at indicated recovery times. N=3 biological replicates of 100 cells and error bars indicate \pm S.D. of the mean. **(D)** Whole cell lysates from U2OS WT and ASCC1 KO cells expressing HA-tagged ASCC2 were analyzed by Western blotting. **(E)** Whole cell lysate from WT and two PC-3 ASCC1 KO clones were analyzed by Western blotting.

A



B

h.sapiens_ASCC1 131- QPFTHTLAFFLNEVEVQEGF-----LRFQEEVLAKCSM
h.sapiens_AKAP18γ 84- QP-NYFLSIPITNKEIIKGIKILQNAIIQQDERLAKAMV

 DHGVDSISIFQNPKKLILIGMLVLISEEIIQQTCEMLQQCKEEFINDISGGKPLEVEMAGIE
 SDG-----SFTIILLVMQLINEDFVNIGIDALLEK-PFIEELLQGGKHLTLFPQGIG

 YMNDDPGMVDVLYAKVHMKDGSNRIQELVDRVLERFCASGLIVKEWNSVKLHAIVM-----
 TFGNQGVGV----KLAEGDHVNSLLEIAETANRTFQEKILVGSRSFKPILLFMKLSKSP

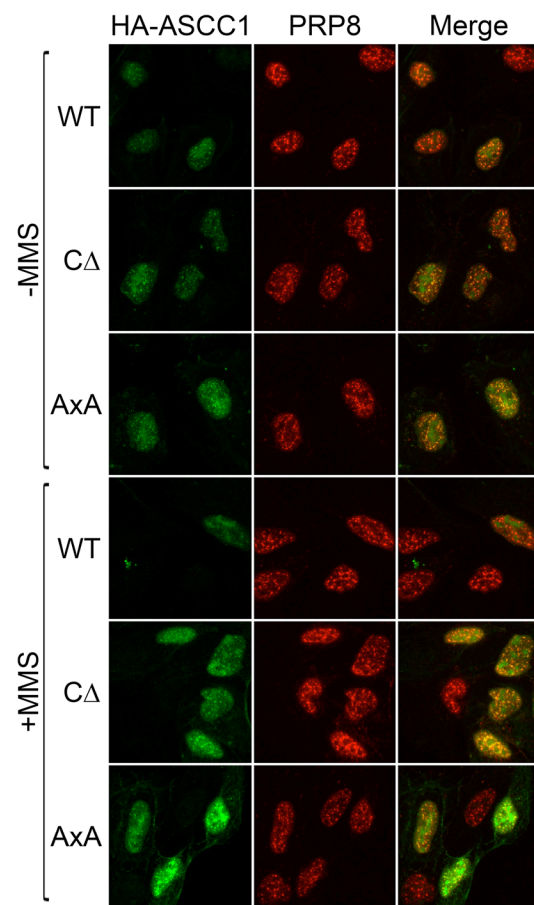
 ----NTLFRKDPNAEGRNLYTAEGKYIFKERESFDGRNLIKLFENFYFGSLKLSNIHISOR
 WLRKNGVKKIDPD-----LYEKT-----ISHR

 FTVDSFGNYASCGQIDFS -357
 EGEEILYRIDLCMLKKK -269

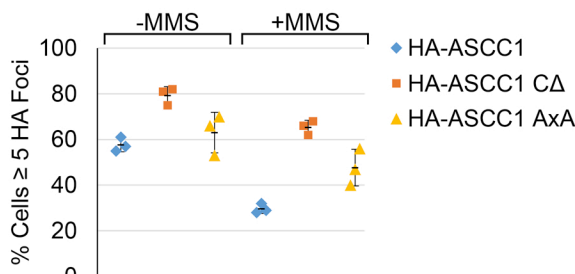
C

h.sapiens_ASCC1 196- TCEMLQQCKEEFINDISGG
h.sapiens_DNA ligase 1 564- TCEYKYDGQRAQHHALEGG
t.thermophilus_DNA ligase 114- TVEHHKVDGLSVNLYYEEGV
e.coli_RNA ligase 40- VHTEKLDGENNCLNRYGVF
e.coli_DNA ligase 111- CCEHLKLDGLAVSLYENGV

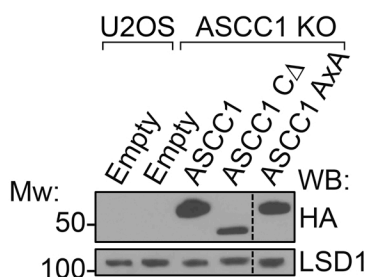
D



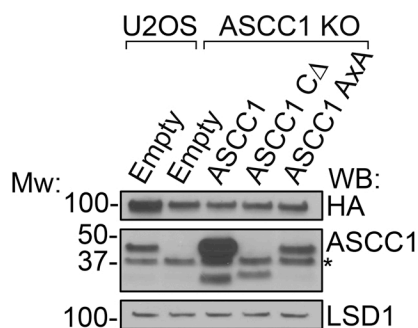
E



F



G

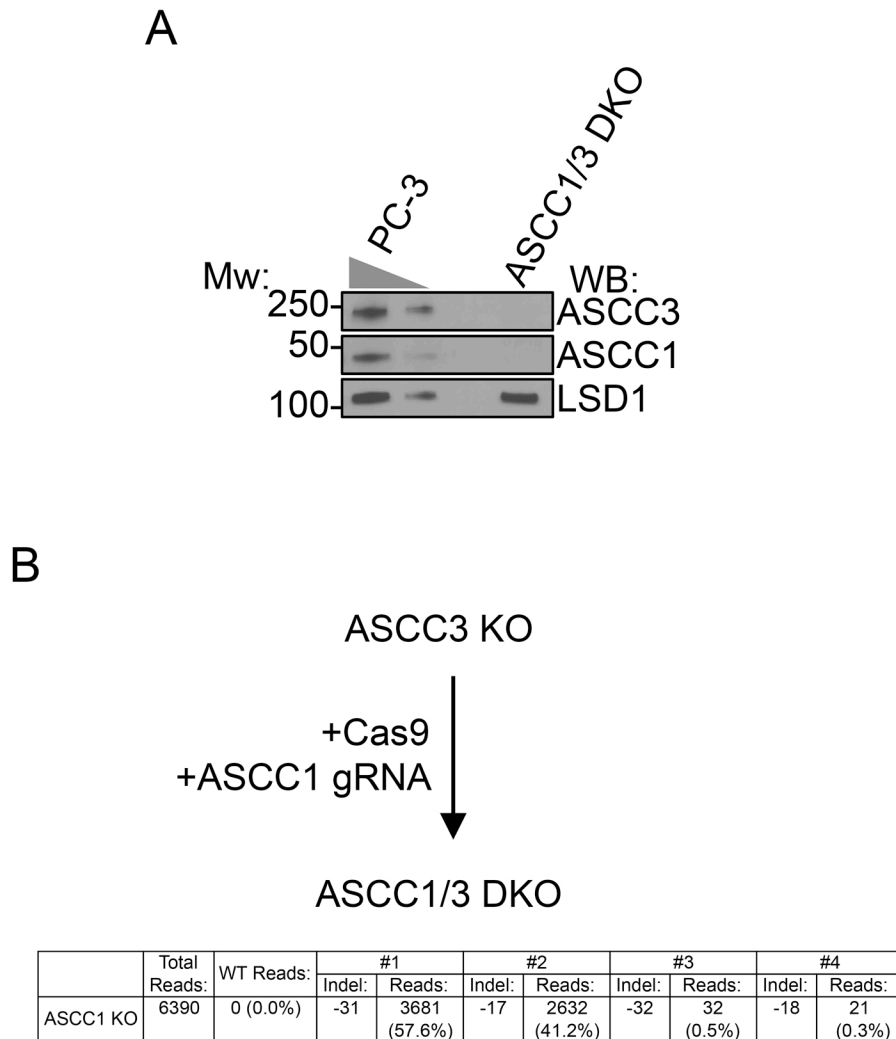


Supporting Information S3. (A) Whole cell lysates from U2OS cells expressing HA-tagged ASCC1 or indicated ASCC1 deletions were collected and expression was analyzed by Western blotting as shown.

(B) Sequence alignment of ASCC1 and AKAP18. HxT motifs highlighted in green. Conserved residues highlighted in blue. Bolded residues are important for ligase activity. R-loop is underscored. **(C)**

Sequence alignment of conserved KxDG sequence motif found in DNA and RNA ligases. The active site lysine is shown in red. **(D)** U2OS ASCC1 KO cells expressing HA-tagged ASCC1 or indicated ASCC1 mutant were untreated or treated with MMS (0.5 mM) for 6 hours as indicated. Cells were processed for immunofluorescence using anti-HA and anti-PRP8 antibodies, with Hoechst as the nuclear counterstain.

(E) Quantification of **(D)**. **(F)** Whole cell lysates from U2OS WT and ASCC1 KO cells expressing HA-tagged ASCC1 FL or indicated ASCC1 mutant were analyzed by Western blotting. **(G)** Whole cell lysates from U2OS WT and ASCC1 KO cells expressing HA-tagged ASCC2 and untagged ASCC1 FL or indicated ASCC1 mutant were analyzed by Western blotting. Asterisk (*) indicates Ig heavy chain.



Supporting Information S4. (A) Whole cell lysates from PC-3 cells, as well as the PC-3 ASCC1/3 double-knockout (DKO) cells, were collected and analyzed by Western blotting as shown. **(B)** Strategy for creating PC-3 ASCC1/3 DKO cells using CRISPR/Cas9 technology. Deep sequencing results are shown at the bottom.