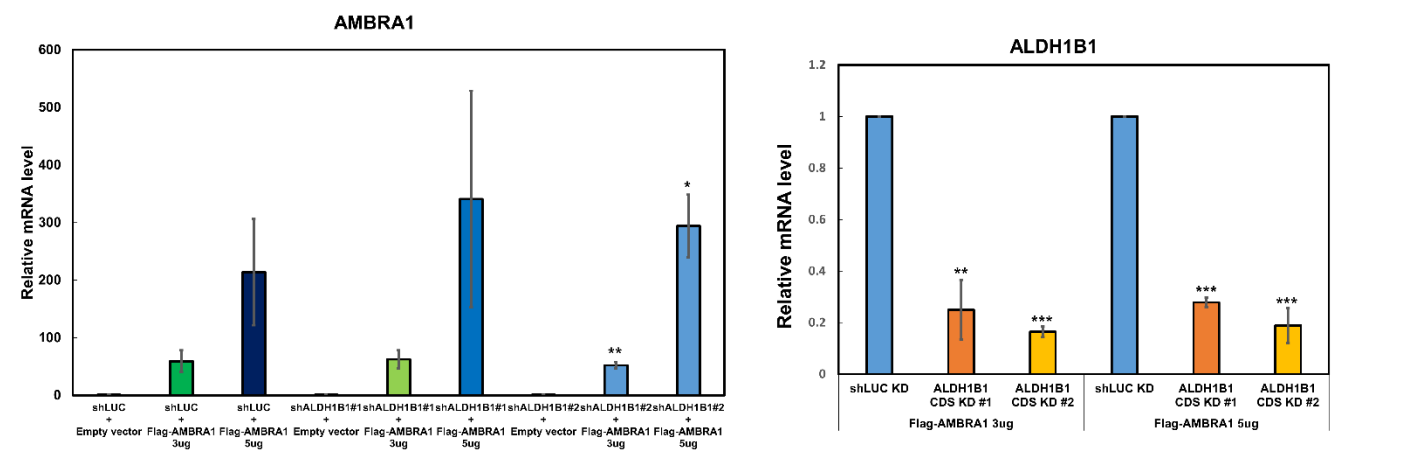


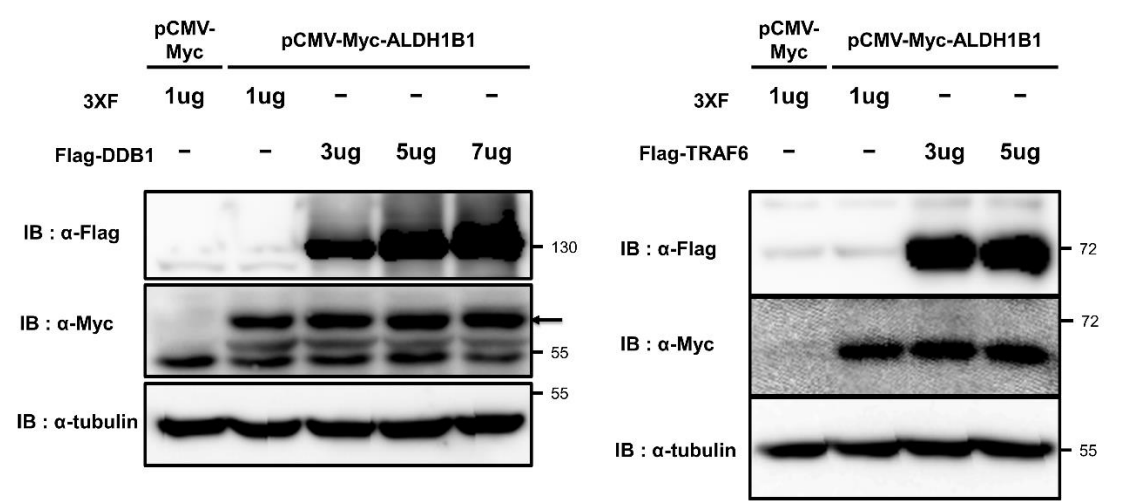
AMBRA1 negatively regulates the function of ALDH1B1, a Cancer Stem Cell Marker, by Controlling its Ubiquitination

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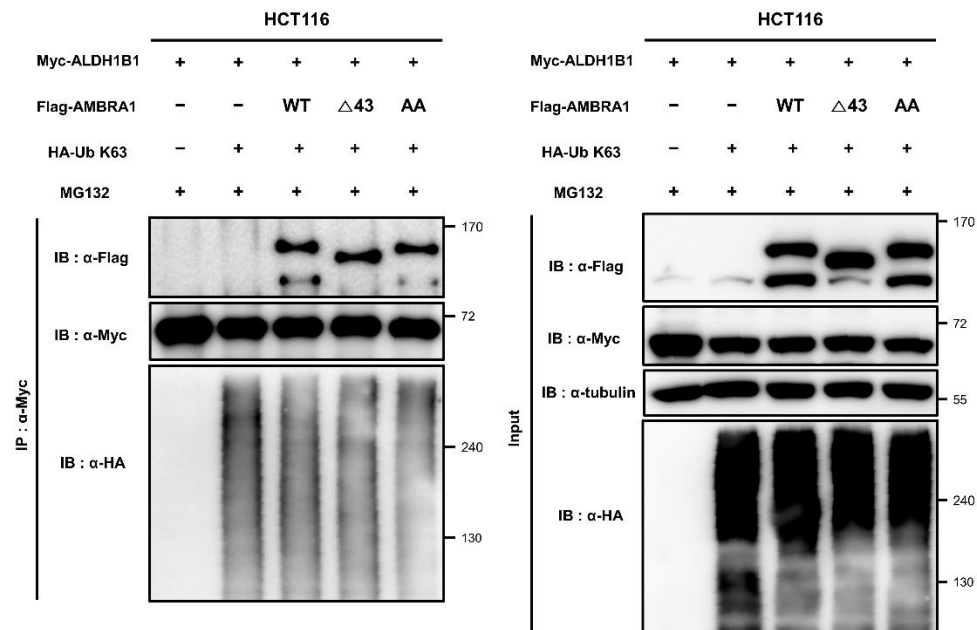
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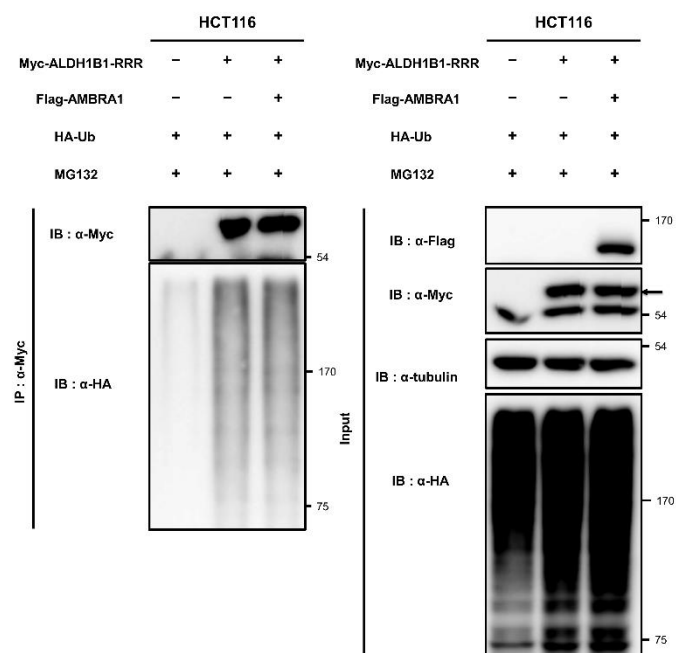
Supplementary Figure S1. Efficiencies of AMBRA1 overexpression and *ALDH1B1* knockdown were confirmed by RT-qPCR (corresponds to Figure 3) (*n* = 3).



Supplementary Figure S2. Protein level of Myc-ALDH1B1 after overexpressing Flag-DDB1 and Flag-TRAF6. Indicated amounts of Flag-DDB1 or Flag-TRAF6 were transfected into pCMV-Myc-ALDH1B1 HCT116 cells and the protein level of Myc-ALDH1B1 was investigated by western blot analysis. α-Tubulin was detected as a loading control (*n* = 3).



Supplementary Figure S3. K63-mediated ubiquitination of Myc-ALDH1B1 as investigated by co-immunoprecipitation. Myc-ALDH1B1 and Flag-AMBRA1 WT, $\Delta 43$, AA were cotransfected with HA-Ub K63 into HCT116 cells, which were treated with 10 μ M MG-132 for 6 h. K63-linked ubiquitination of Myc-ALDH1B1 was detected by immunoprecipitation with anti-c-Myc agarose affinity gel antibody ($n = 3$).



Supplementary Figure S4. The ubiquitination of Myc-ALDH1B1-RRR (Myc-ALDH1B1 K506R, K511R, K515R) was analyzed after ectopic expression of AMBRA1. As shown in Fig. 4A, we observed an increase in the ubiquitination of wild-type Myc-ALDH1B1 by AMBRA1. In contrast, immunoprecipitation and immunoblot analysis confirmed that

potential K27- and K33-linked ubiquitination-defective ALDH1B1 interacted with a similar amount of HA-Ub before and after AMBRA1 overexpression ($n = 3$).

Supplementary Table S1. Primer sequences used for cloning

Gene	Primer sequence (5' to 3')	Accession no.
AMBRA1	F : GCGGATATCATGAAGGTTGTCCCAGAA R : GCGATCGATCTACCTGTTCCGTGGTTC	NM_001367471.1
ALDH1B1	F : ATAGATATCATGCTGCGCTTCCTGGCA R : GCGGTCGACTTACGAGTTCTTCTGAGG	NM_000692.5
DDB1	F : ATAGATATCATGTCGTACAACCTACGTGGTA R : ATAGTCGACCTAATGGATCCGAGTTAGCTC	NM_001923.5
TRAF6	F : GCGAAGCTTATGAGTCTGCTAAACTGT R : ATAGTCGACCTATACCCCTGCATCAGT	NM_145803.3

Supplementary Table S2. Primer sequences used for mutagenesis

Mutation	Primer sequence (5' to 3')	Type of mutation
AMBRA1 Δ43	F : GCGGATATCATGGGCAAGAGAGTAGAACTG R : GCGATCGATCTACCTGTTCCGTGGTTC	Deletion
AMBRA1 E620A	F : AGTTGCCACCTCTCGCGCGGACTGAGGGCCA R : TGGCCCTCAGTCCGCGCGAGAGGTGGCAACT	Point mutation
AMBRA1 E683A	F : AGGATATGCCTGAGGCGAGCTCTGAGGAGGA R : TCCTCCTCAGAGC31merTCGCCTCAGGCATATCCT	Point mutation

Supplementary Table S3. Primer sequences used for small hairpin RNA(shRNA)-based knockdown system

Gene	Primer sequence (5' to 3')	Target region	Accession no.
AMBRA1	F : CCGG GTCCACGCTCTACCTTCTTAT CTCGAG ATAAGAAGGTAGAGCGTGGAC TTTTGT R : AATTCAAAAA GTCCACGCTCTACCTTCTTAT CTCGAG ATAAGAAGGTAGAGCGTGGAC	CDS (coding sequence)	NM_001367471.1
	F : CCGG GCATGTGGACTCTTAACTGTA CTCGAG TACAGTTAAGAGTCCACATGC TTTTGT R: AATTCAAAAA GCATGTGGACTCTTAACTGTA CTCGAG TACAGTTAAGAGTCCACATGC	3'-UTR (3' Untranslated Region)	
ALDH1B1	F : CCGG GAATCCATCTACAATGAGTTT CTCGAG AAACTCATTGTAGATGGATTC TTTTGT R : AATTCAAAAA GAATCCATCTACAATGAGTTT CTCGAG AAACTCATTGTAGATGGATTC	CDS	NM_000692.5
	F : CCGG TCGAGAGAACCGTGGAGAAAG CTCGAG CTTTCTCCACGGTTCTCTCGA TTTTGT R : AATTCAAAAA TCGAGAGAACCGTGGAGAAAG CTCGAG CTTTCTCCACGGTTCTCTCGA	CDS	

Supplementary Table S4. Primer sequences used for real-time PCR

Gene	Primer sequence (5' to 3')	Accession no.
GAPDH	F : TGATGACATCAAGAAGGTGGTGAAG R : TCCTTGGAGGCCATGTGGGCCAT	NM_001289746.2
β -actin	F : GCCCTGAGGCACTCTTCCA R : CCAGGGCAGTGATCTCCTTCT	NM_001101
AMBRA1	F : GAAATGGGAGGGCAAGAGA R : TCACATGGGTGGAGGCTAA	NM_001367471.1
ALDH1B1	F : AGAGGGCCAACAACACCA R : CCCTCCCGTTTCCAGATT	NM_000692.5
PTEN	F : TGTTCACTGGCGGAAGTTG R : TCGTGTGGGTCTGAATTG	NM_001304718.2
CTNNB1	F : AGTTGATGGGCTGCCAGAT R : TCAAACCAGGCCAGCTGAT	NM_001904.4
LGR5	F : CCTGTCCTTGCCTGTGCT R : CCACCCTGAGCAACATCC	NM_003667.4
HIF1a	F : GGCAGCAACGACACAGAA R : TTGAGTGCAGGGTCAGCA	NM_001530.4
ALDH1A3	F : TCTCGACAAAGCCCTGAAGT R : GGCCAAAGCGTATTCACCTA	NM_000693.4
CD24	F : CCCACGCAGATTTATTCCAG R : ACCACGAAGAGACTGGCTGT	NM_013230.3
SOX4	F : AAGTACCGGCCAGGAAG R : CTGCCACCGACCTTGTCT	NM_003107