

SUPPORTING INFORMATION

**Selective ALDH3A1 inhibition by benzimidazole analogs
increase mafosfamide sensitivity in cancer cells**

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Figure S1. Sequence alignment of human ALDH1A1 and sheep ALDH1A1 highlighting the conserved residues located in active site. Residues in the active site are 100% identical in both human and sheep. Trp177, which is crucial for CB7 binding is highlighted in blue which is also identical between sheep and human ALDH1A1.

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sheep      MSSSAMPDVPAPLTLNLQFKYTKIFINNEWHSSVSGKKFPVFNPAATEEKLCEVEEGDKEDV 60
human      MSSSGTDLPLVLLTDLKIQYTKIFINNEWHDSVSGKKFPVFNPAATEEELCQVEEGDKEDV 60
          ****. **:*. **:*.:*****.*****:****:*****

sheep      DKA VKAARQAFQIGSPWRTMDASERGRLLNKLADLIERDRLLLATMEAMNGGKLF SNAYL 120
human      DKA VKAARQAFQIGSPWRTMDASERGRLLYKLADLIERDRLLLATMESMNGGKLYS NAYL 120
          *****:*****:*****:*****

sheep      MDLGGCIKTLRYCAGWADKIQGRTIPMDGNFFTYTRSEPVGVCGQIIPWNFPLLMFLWKI 180
human      NDLAGCIKTLRYCAGWADKIQGRTIPIDGNFFTYTRHEPIGVCGQIIPWNFPLVMLIWKI 180
          *.*****:***** **:*****:***:***

sheep      GPALSCGNTVVVKPAEQTPLTALHMGS LIKEAGFPPGVVNI VPGYGPTAGAAISSHMDVD 240
human      GPALSCGNTVVVKPAEQTPLTALHVAS LIKEAGFPPGVVNI VPGYGPTAGAAISSHMDID 240
          *****:*****:*****:*****

sheep      KVAFTGSTEVGKLIKEAAGKSNLKRVSLELG GKSPCIVFADADLDNAVEFAHQGVFYHQG 300
human      KVAFTGSTEVGKLIKEAAGKSNLKRVTLELG GKSPCIVLADADLDNAVEFAHHGVFYHQG 300
          *****:*****:*****:*****

sheep      QCCI AASRLFVEESIYDEFVRRSVERAKKYVLGNPLTPGVSQGPQIDKEQYKILD LIES 360
human      QCCI AASRIFVEESIYDEFVRRSVERAKKYILGNPLTPGVTQGPQIDKEQYDKILD LIES 360
          *****:*****:*****:*****

sheep      GKKEGAKLECGGGPWGNKGYFIQPTVFS DVTDDMRIAKEEIFGPVQQIMKFKSLDDVIKR 420
human      GKKEGAKLECGGGPWGNKGYFVQPTVFS NVTDEMRIAKEEIFGPVQQIMKFKSLDDVIKR 420
          *****:*****:***:*****

sheep      ANNTFYGLSAGIFTNDIDKAITVSSALQSGTVWVNCYSV VSAQCF FGGFKMSGNGRELGE 480
human      ANNTFYGLSAGVFTKDIDKAITISSALQAGTVWVNCYGV VSAQCF FGGFKMSGNGRELGE 480
          *****:***:*****:*****:*****:*****

sheep      YGFHEYTEVKTVTIKISQKNS 501
human      YGFHEYTEVKTVTKISQKNS 501
          *****:*****

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Figure S2. Multiple sequence alignment in the region of W177 of ALDH1A1 and the mature form of ALDH2.

ALDH2	GDF---FSYTRHEPV-GVCGQII PWNFPLLMQAWKLGPALA	201
ALDH1A1	GNF---FTYTRHEPI-GVCGQII PWNFPLVMLIWKIGPALS	185
ALDH1A2	GDY---FTFTRHEPI-GVCGQII PWNFPLLMFAWKIAPALC	202
ALDH1B1	GQH---FCFTRHEPV-GVCGQII PWNFPLVMQGWKLAPALA	201
ALDH1A3	DNV---VCFTRHEPI-GVCGAITPWNFPLMLVWKLAPALC	196
ALDH9A1	GGG---FGYTRREPL-GVCVGIGAWNYPFQIASWKSAPALA	196
ALDH8A1	LGC---MHYTVRAPV-GVAGLISPWNLPLYLLTWKIAPAMA	170
ALDH1L1	NRN---LTLTRKEPV-GVCGIII PWNYPMLMLSWKTAACLA	599
ALDH1L2	NRN---LTFTKKEPL-GVCAIII PWNYPMLMLAWKSAACLA	610
ALDH5A1	KDR---RALVLKQPI-GVAAVITPWNFPSAMITRKVGAALA	220
ALDH6A1	KDM---DLYSYRLPL-GVCAGIAPFNFPAMI PLWMFPMAMV	201
ALDH7A1	-GH---ALIEQWNPV-GLVGIITAFNFPVAVYGWNNAIAMI	210
ALDH3A1	Q---GDELYIHSEPL-GVVLVIGTWNYPFNLTIQPMVGAIA	130
ALDH3A2	M---LDEAYIQPQPL-GVVLII GAWNYPFVLTIQPLIGAIA	127
ALDH3B1	LATQLDSAFIRKEPF-GLVLI IAPWNYPLNLTLPVPLVGALA	130
ALDH4A1	PPS---TNSTVYRGLEGFVAAISP FNFTAIGGNLAGAPALM	226
ALDH3B2	LFMKLDSVFIWKEPF-GLVLI IAPWNYPLNLTLPVLLVGALA	49
ALDH16A1	-QE---EALAGWEPM-GVIGLILPPTFSFLEMMWRICPALA	191
ALDH18A1	KNL---ELEQVTVPI-GVLLVI--FESRPDCLPQVAALAI A	496

Figure S3. ALDH3A1 expression in A549, SF767, and CCD-13Lu cells. Lysates from various cancer cell lines (A549, SF767, and CCD-13Lu) were examined for ALDH3A1 expression. Purified recombinant His-tagged ALDH3A1 protein is taken as positive control and GAPDH serves as a loading control. Purified recombinant ALDH3A1 shows a slightly higher band because it is His-tagged and travels slowly on SDS-PAGE gel.

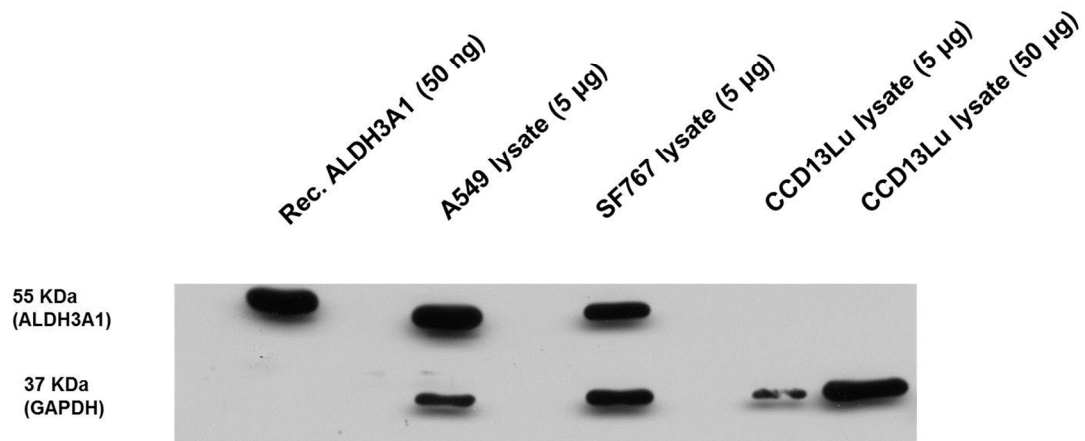


Figure S4. ALDH1A1 expression in A549, SF767, and CCD-13Lu cells. Lysates from various cancer cell lines (A549, SF767, and CCD-13Lu) were examined for ALDH1A1 expression. Purified recombinant ALDH1A1 protein is taken as positive control and GAPDH serves as a loading control.

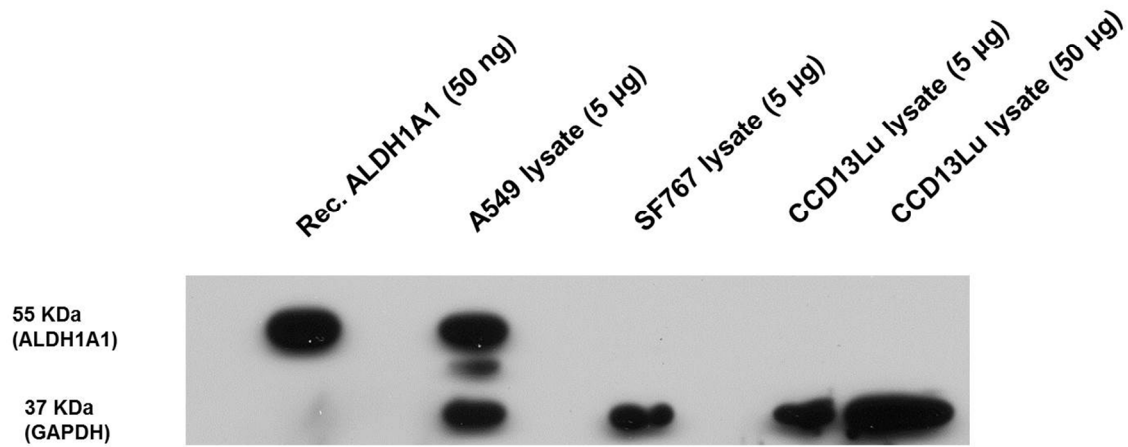


Figure S5. Cross reactivity of ALDH1A1 antibody. Figure shows the cross reactivity of ALDH1A1 antibody, Abcam (ab-23375) with other ALDH subtypes. Human ALDH1 isoforms (ALDH1A1, ALDH1A2, ALDH1A3, and ALDH1B1) and ALDH2 were purified in the lab and were provided by Lanmin Zhai. Each lane was loaded with between 50 and 70 ng of the purified recombinant human ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1 and ALDH2 isoenzymes respectively and was detected using ALDH1A1 antibody.

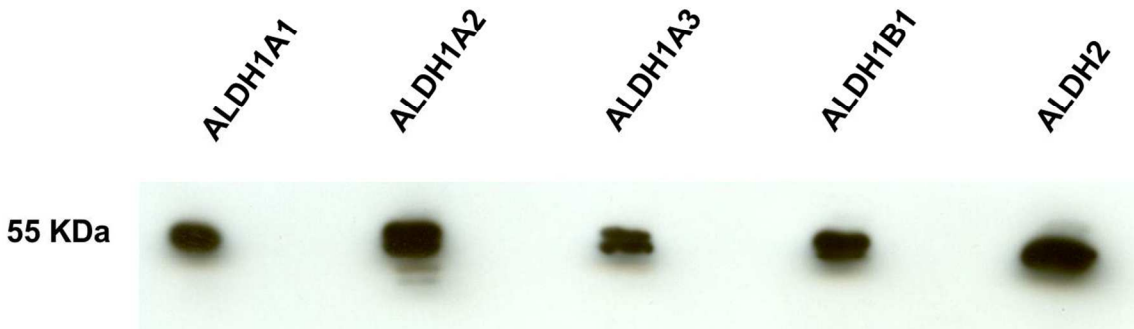


Table S1. Compounds discussed in this study with their respective vendors and corresponding catalog numbers.

Compounds	Vendors with catalog numbers
A3	(Chembridge Corp.- 5172826)
A5	(Chembridge Corp.- 5172831)
A6	(Chembridge Corp.-5175600)
A10	(Chembridge Corp.-5215982)
A13	(Chembridge Corp.-5231103)
A16	(Chembridge Corp.- 5243439)
A20	(Chembridge Corp.-5260321)
A21	(Chembridge Corp.-5264371)
A22	(Chembridge Corp.-5284379)
A24	(Chembridge Corp.- 5510049)
A30	(Chembridge Corp.-5607189)
A38	(Chembridge Corp.-5648440)
A39	(Chembridge Corp.-5651872)
A40	(Vitas M. Laboratories.- STK354007)
A47	(Chembridge Corp.-6104618)
A53	(Chembridge Corp.-6382505)
A62	(Chembridge Corp.-7224032)
A64	(Chembridge Corp.-7289639)
A67	(Chembridge Corp.- 7567094)
A70	(Chembridge Corp.-7928260)
B27	(Vitas M. Laboratories.- STK454495)
B36	(Chemdiv Corp. 6529-0359)
B37	(Chemdiv Corp. K783-5471)
CB7	(Chembridge Corp.- 5613645)