Mechanisms responsible for the synergistic antileukemic interactions between ATR inhibition and cytarabine in acute myeloid leukemia cells

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Figure S1

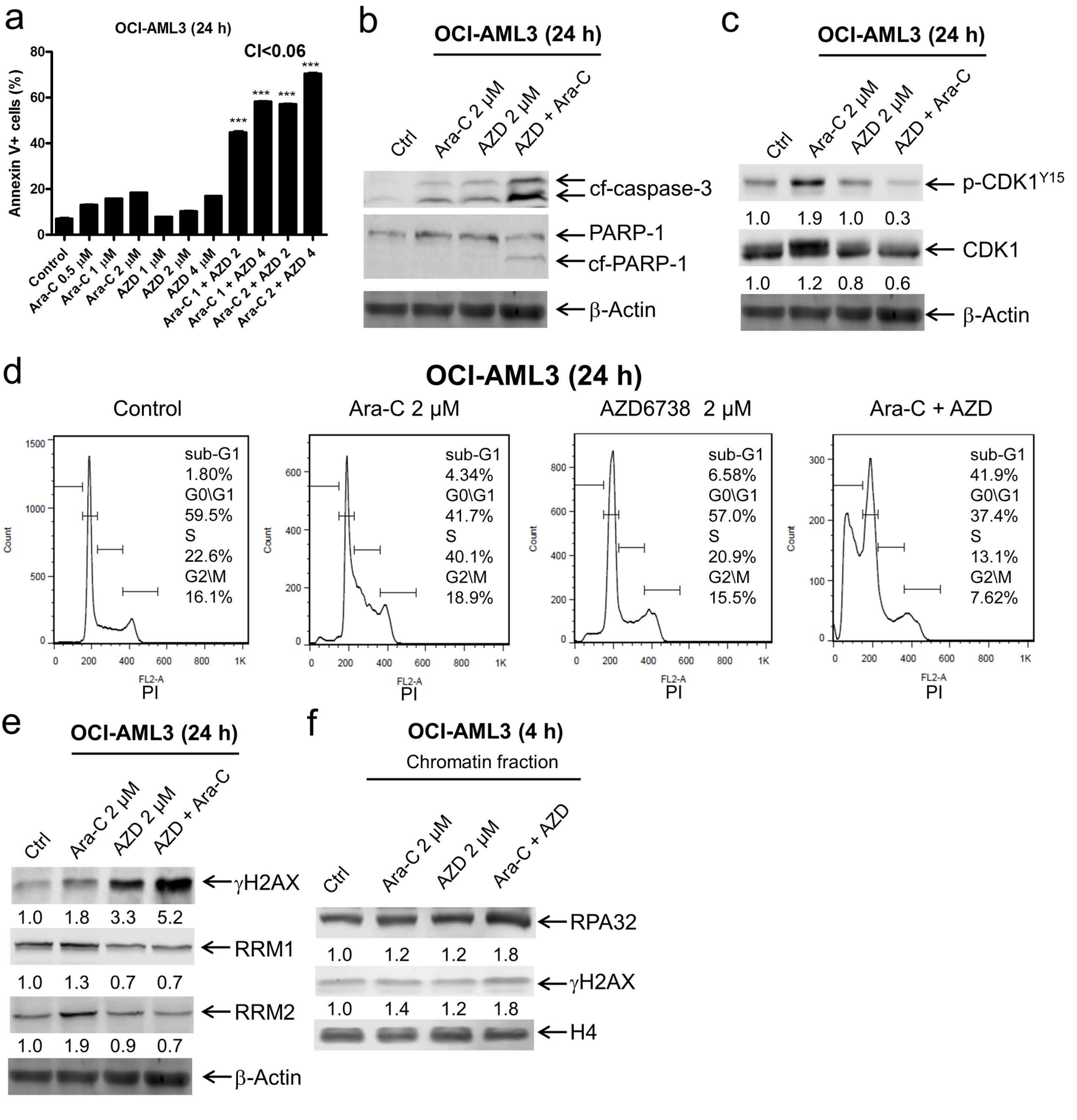
a
OCI-AML3 (24 h)
CI<0.06
Annexin V+ cells (%)
Control
Ara-C 0.5 µM
Ara-C 1 µM
Ara-C 2 µM
AZD 1 µM
AZD 2 µM
AZD 4 µM
Ara-C 1 + AZD 2
Ara-C 1 + AZD 4
Ara-C 2 + AZD 2
Ara-C 2 + AZD 4
b
OCI-AML3 (24 h)
Ctrl
Ara-C 2 µM
AZD 2 µM
AZD + Ara-C
cf-caspase-3
PARP-1
cf-PARP-1
β-Actin
c
OCI-AML3 (24 h)
p-CDK1Y15
CDK1
β-Actin
d
OCI-AML3 (24 h)
Control
Ara-C 2 µM
AZD6738 2 µM
Ara-C + AZD
sub-G1
G0\G1
S
G2\M
Count
PI
e
OCI-AML3 (24 h)
γH2AX
RRM1
RRM2
β-Actin
f
OCI-AML3 (4 h)
Chromatin fraction
RPA32
γH2AX
H4

Figure S1. AZD6738 (AZD) synergizes with cytarabine (ara-C) to induce apoptosis and proliferation inhibition in AML cells. (a) OCI-AML3 cells were treated with cytarabine and AZD6738, alone or in combination, for 24 h and then subjected to annexin V-FITC/PI staining and flow cytometry analyses. CI values were calculated using CompuSyn software. Combined drug treatments were compared to single drug treatment using 1-way ANOVA with Bonferroni post hoc test. ***indicates $p < 0.001$. (b and c) OCI-AML3 cells were treated with cytarabine and AZD-6738, alone or in combination, for 24 h. Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies. (d) OCI-AML3 cells were treated with cytarabine and AZ20, alone or in combination, for 24 h. Then the cells were fixed with ethanol and stained with PI for cell cycle analysis. (e) OCI-AML3 cells were treated with cytarabine and AZD6738, alone or in combination, for 24 h. Whole cell lysates were subjected to Western blotting and probed with the indicated antibodies. (f) OCI-AML3 cells were treated with cytarabine and AZ20, alone or in combination, for 4 h. Chromatin-bound RPA32 and γH2AX were analyzed by Western blotting. Densitometry measurements normalized to β-actin or histone H4 and then compared to control are presented.