Figure S8

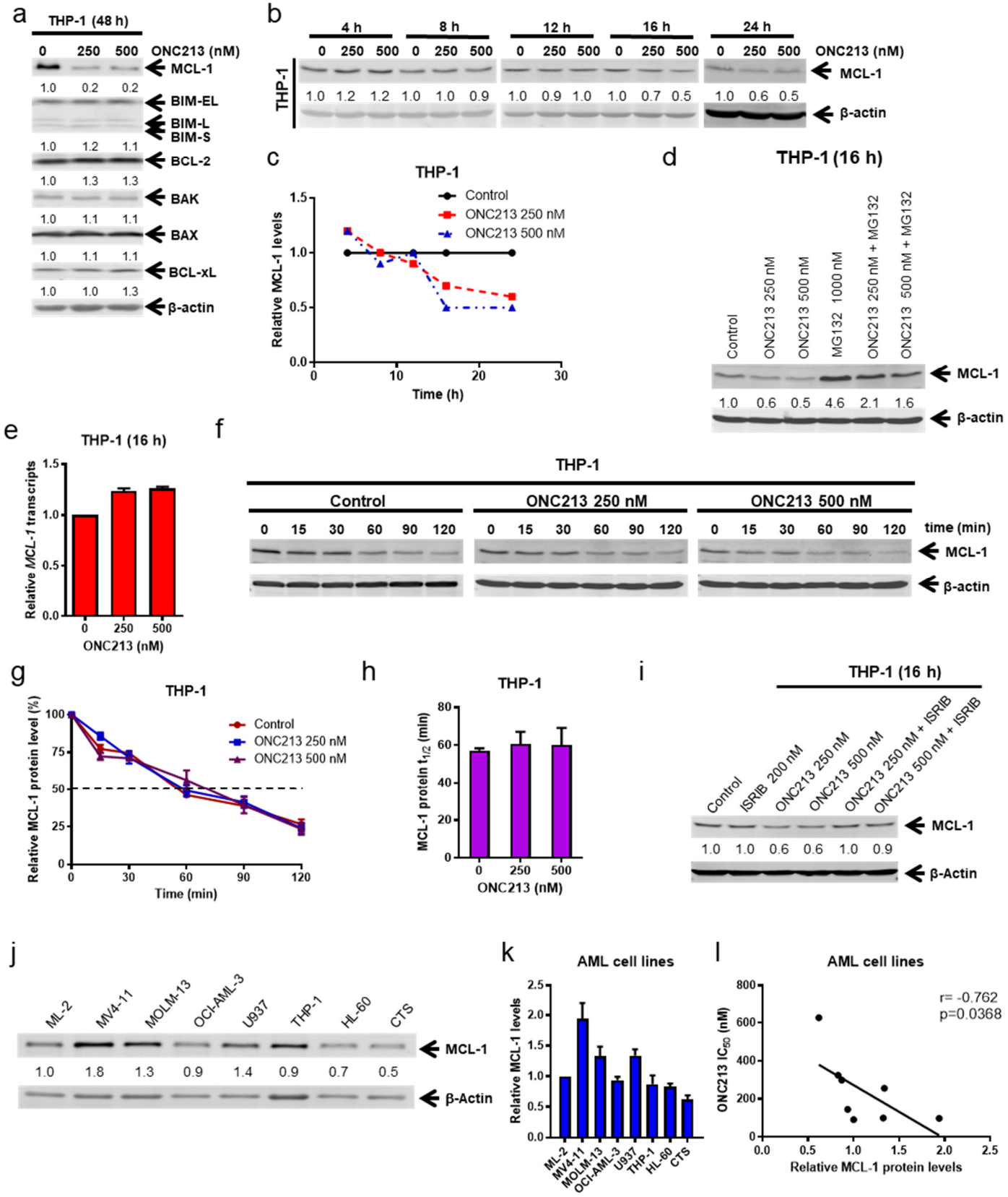
a
THP-1 (48 h)
0 250 500 ONC213 (nM)
MCL-1
1.0 0.2 0.2
BIM-EL
BIM-L
BIM-S
1.0 1.2 1.1
BCL-2
1.0 1.3 1.3
BAK
1.0 1.1 1.1
BAX
1.0 1.1 1.1
BCL-xL
1.0 1.0 1.3
β-actin
b
4 h 8 h 12 h 16 h 24 h
0 250 500 0 250 500 0 250 500 0 250 500 0 250 500 ONC213 (nM)
THP-1
MCL-1
1.0 1.2 1.2 1.0 1.0 0.9 1.0 0.9 1.0 1.0 0.7 0.5 1.0 0.6 0.5
β-actin
c
THP-1
Control
ONC213 250 nM
ONC213 500 nM
Relative MCL-1 levels
Time (h)
d
THP-1 (16 h)
Control
ONC213 250 nM
ONC213 500 nM
MG132 1000 nM
ONC213 250 nM + MG132
ONC213 500 nM + MG132
MCL-1
1.0 0.6 0.5 4.6 2.1 1.6
β-actin
e
THP-1 (16 h)
Relative MCL-1 transcripts
ONC213 (nM)
f
THP-1
Control
ONC213 250 nM
ONC213 500 nM
0 15 30 60 90 120 0 15 30 60 90 120 0 15 30 60 90 120 time (min)
MCL-1
β-actin
g
THP-1
Control
ONC213 250 nM
ONC213 500 nM
Relative MCL-1 protein level (%)
Time (min)
h
THP-1
MCL-1 protein t1/2 (min)
ONC213 (nM)
i
THP-1 (16 h)
Control
ISRIB 200 nM
ONC213 250 nM
ONC213 500 nM
ONC213 250 nM + ISRIB
ONC213 500 nM + ISRIB
MCL-1
1.0 1.0 0.6 0.6 1.0 0.9
β-Actin
j
ML-2
MV4-11
MOLM-13
OCI-AML-3
U937
THP-1
HL-60
CTS
MCL-1
1.0 1.8 1.3 0.9 1.4 0.9 0.7 0.5
β-Actin
k
AML cell lines
Relative MCL-1 levels
ML-2
MV4-11
MOLM-13
OCI-AML-3
U937
THP-1
HL-60
CTS
l
AML cell lines
r= -0.762
p=0.0368
ONC213 IC50 (nM)
Relative MCL-1 protein levels

Fig. S8. ONC213 suppresses MCL-1 in THP-1 cells through inhibition of protein synthesis. a-c. Whole cell lysates from THP-1 cells were treated with vehicle, 250 nM or 500 nM ONC213 for up to 48 h were analyzed by western blotting and probed with the indicated antibodies. The fold changes for the densitometry measurements, normalized to β-actin and then compared to vehicle control, are indicated below the corresponding blots. Relative MCL-1 protein levels shown in panel b are graphed in panel c. **d**. THP-1 cells were treated with vehicle, ONC213, MG132, or ONC213 + MG132 for 16 h. Whole cell lysates were analyzed by western blotting and probed with the indicated antibodies. Fold changes for the densitometry measurements, normalized to β-actin and then compared to vehicle control, are indicated. **e**. THP-1 cells were treated with vehicle or ONC213 at the indicated concentrations for 16 h. Total RNA was extracted, and real-time RT-PCR was performed. The relative changes in *MCL-1* transcripts, normalized to *GAPDH*, in comparison to control samples were quantified. The results represent the mean of three independent experiments, with fold changes calculated by the comparative Ct method. **f-h**. THP-1 cells were treated with vehicle, 250 nM, or 500 nM ONC213 for 16 h, washed, and then treated with 10 μg/mL cycloheximide for up to 2 h. Western blots were generated by using whole cell lysates. Representative blots are shown in panel f. The fold changes for densitometry measurements, normalized to β-actin and then compared to vehicle control, are graphed in panel g. MCL-1 protein half-life was calculated using GraphPad Prism 9.0 and graphed in panel h. **i**. THP-1 cells were treated with vehicle, ISRIB (integrated stress response inhibitor), ONC213, or ISRIB in combination with ONC213 for 16 h. Whole cell lysates were analyzed by western blotting. The fold changes for densitometry measurements, normalized to β-actin and then compared to vehicle control are indicated. **j-l**. Whole cell lysates from AML cell lines were analyzed by western blot. Representative western blots are shown. The fold changes for the densitometry measurements, normalized to β-actin and then compared to ML-2, are indicated. Normalized densitometry measurements are shown in panel k (cell line data was generated from three independent experiments). Relative MCL-1 protein levels were graphed against ONC213 IC_{50}s in panel l. The relationship between protein levels and IC_{50}s was determined by nonparametric Spearman rank correlation coefficient.