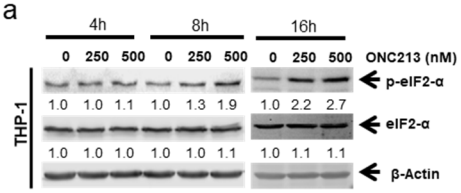
Fig. S7

a



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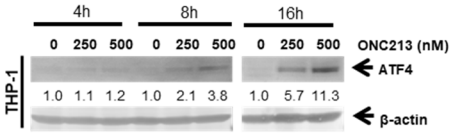
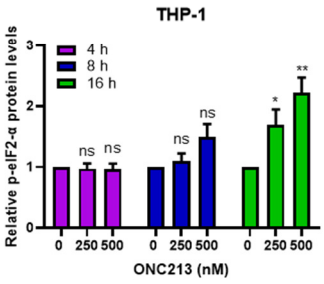
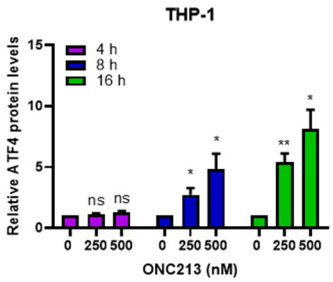






c

No enrichment of mTOR transcription targets

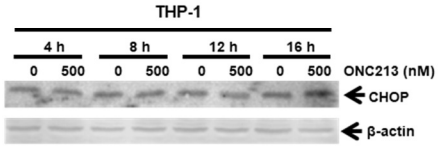
GeneSet	NES	FDR
HALLMARK_PI3K_AKT_MTOR_SIGNALING	1.01	0.770
KEGG_MTOR_SIGNALING_PATHWAY	0.96	0.797
BIOCARTA_MTOR_PATHWAY	-0.98	0.681

d

Enrichment of genes affecting translation

Term	P-value	Adjusted P-value	Genes	
Mitochondrial translation initiation Homo sapiens R-HSA-5368286	1.83E-18	2.99E-17	MRPL14	MRPS23
Mitochondrial translation termination Homo sapiens R-HSA-5419276	1.83E-18	2.99E-17	METTL17	NPEPL1
Mitochondrial translation elongation Homo sapiens R-HSA-5389840	1.83E-18	2.99E-17	CCDC106	NCOR1
Mitochondrial translation Homo sapiens R-HSA-5368287	3.78E-18	4.64E-17	NDUFA5	NDUFA6
Organelle biogenesis and maintenance Homo sapiens R-HSA-1852241	3.77E-14	3.69E-13	ERAL1	MRPS35
			MRPS18A	SDSL
			TMEM50A	MRPS15
			LRPAP1	MRPS18B
			MAMDC2	MRPL11
			ASPHD1	MRPL16
			TSPYL1	GNPAT
			MRPL28	MRPL51

e



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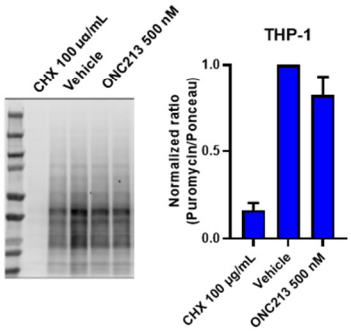
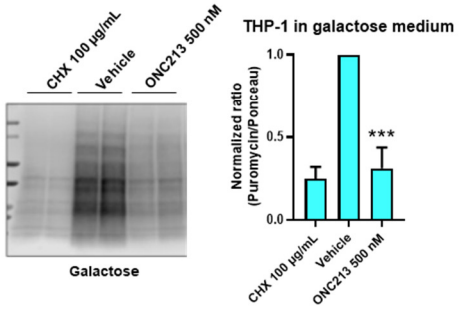


g



h

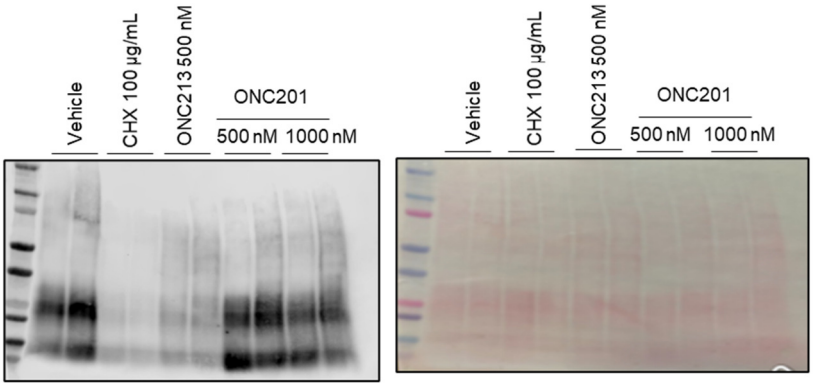


Fig. S7. ONC213 induces a mitochondrial stress gene expression signature and suppresses protein synthesis. a&b. THP-1 cells were treated with vehicle, 250 nM or 500 nM ONC213 for 4, 8, or 16 h. Whole cell lysates were analyzed by western blotting and probed with the indicated antibodies. The fold changes for densitometry measurements, normalized to β-actin and then compared to vehicle control at the same time point, are indicated below the corresponding blots (Upper panels). Densitometry results from 3 independent experiments are graphed and shown in the lower panels. ns, not significant; * $P<0.05$; ** $P<0.01$. **c**. GSEA (Gene set enrichment analysis) of mTOR (mammalian target of rapamycin) transcriptional targets using gene sets from HALLMARK, KEGG, and BIOCARTA compared to the top 100 upregulated genes by ONC213 in AML cell lines was performed. **d**. Enrichr analysis of the ONC213 signature genes affecting translation. **e**. THP-1 cells were treated with vehicle or 500 nM ONC213 for 4, 8, 12, or 16 h. Whole cell lysates were analyzed by western blotting and probed with the indicated antibodies. **f**. THP-1 cells were treated with vehicle, cycloheximide, or ONC213 for 16 h and then 1 μM puromycin was added for 30 min. Whole cell lysates were analyzed by western blot and probed with anti-puromycin antibody. One representative blot is shown (left panel). Densitometry measurements (normalized to vehicle control) from 2 independent experiments are graphed (right panel). **g**. THP-1 cells cultured in galactose-containing media were treated with vehicle, cycloheximide, or ONC213 for 16 h and then 1 μM puromycin was added for 30 min. Whole cell lysates were analyzed by and probed with anti-puromycin antibody. One representative blot is shown (left panel). Densitometry measurements (normalized to vehicle control) from 3 independent experiments are graphed and shown (right panel). *** $P<0.001$ compared to vehicle control. **h**. MV4-11 cells were treated with vehicle, cycloheximide (as a positive control for inhibition of protein synthesis), ONC213 (500 nM), or ONC201 (500 and 1000 nM) for 16 h and then analyzed by the puromycin pulse labeling assay, as described in the Methods. The left panel shows a western blot probed with the mouse anti-puromycin antibody. The right panel shows the same blot stained with Ponceau S as loading control.