Fig. S4

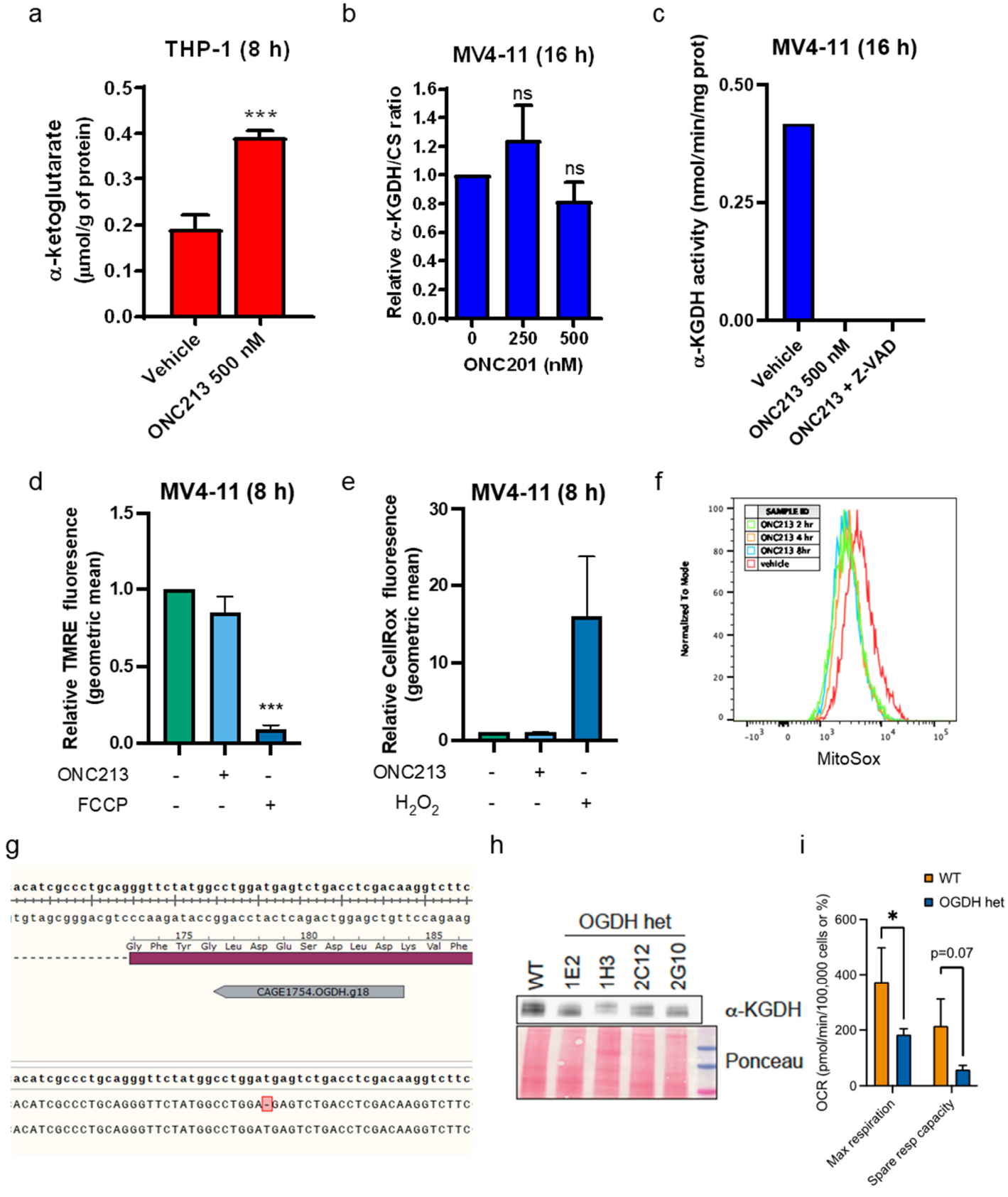
a
THP-1 (8 h)
α-ketoglutarate (µmol/g of protein)
0.5
0.4
0.3
0.2
0.1
0.0
***
Vehicle
ONC213 500 nM
b
MV4-11 (16 h)
Relative α-KGDH/CS ratio
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ns
ns
0
250
500
ONC201 (nM)
c
MV4-11 (16 h)
α-KGDH activity (nmol/min/mg prot)
0.50
0.25
0.00
Vehicle
ONC213 500 nM
ONC213 + Z-VAD
d
MV4-11 (8 h)
Relative TMRE fluoresence (geometric mean)
1.5
1.0
0.5
0.0
***
ONC213 - + -
FCCP - - +
e
MV4-11 (8 h)
Relative CellRox fluoresence (geometric mean)
30
20
10
0
ONC213 - + -
H2O2 - - +
f
SAMPLE ID
ONC213 2 hr
ONC213 4 hr
ONC213 8hr
vehicle
Normalized To Mode
100
80
60
40
20
0
-10^3
0
10^3
10^4
10^5
MitoSox
g
acatcgccctgcagggttctatggcctggatgagtctgacctcgacaaggtcttc
tgtagcgggacgtcccaagataccggacctactcagactggagctgttccagaag
175
180
185
Gly Phe Tyr Gly Leu Asp Glu Ser Asp Leu Asp Lys Val Phe
CAGE1754.OGDH.g18
acatcgccctgcagggttctatggcctggatgagtctgacctcgacaaggtcttc
ACATCGCCCTGCAGGGTTCTATGGCCTGGA-GAGTCTGACCTCGACAAGGTCTTC
ACATCGCCCTGCAGGGTTCTATGGCCTGGATGAGTCTGACCTCGACAAGGTCTTC
h
OGDH het
WT
1E2
1H3
2C12
2G10
α-KGDH
Ponceau
i
WT
OGDH het
OCR (pmol/min/100,000 cells or %)
600
400
200
0
*
p=0.07
Max respiration
Spare resp capacity

Fig. S4. ONC213 but not ONC201 inhibits the TCA cycle and α-KGDH. a. THP-1 cells were treated with vehicle or 500 nM ONC213 for 8 h. Cell pellet metabolites were quantitatively profiled using the LC-MS/MS–based targeted metabolomics platform. Data were analyzed using www.MetaboAnalyst.ca, version 4.0. α-ketoglutarate is graphed as mean ± SEM. *** P <0.001. **b**. MV4-11 cells were treated with vehicle or ONC201 for 16 h. α-ketoglutarate dehydrogenase (α-KGDH) and citrate synthase (CS) activities were measured. The ratio of α-KGDH activity to CS activity was determined and normalized to the vehicle control. ns, not significant. **c**. MV4-11 cells were treated with vehicle, 500 nM ONC213, or the combination of ONC213 and the pan-caspase inhibitor Z-VAD-FMK for 16 h. α-KGDH activity was measured and normalized to whole cell protein. **d**, MV4-11 cells were treated with vehicle or 250 nM ONC213 for 8 h. As a positive control, MV4-11 cells were treated with 10 μM FCCP for 10 minutes prior to TMRE staining. Cells were stained with 100 nM TMRE for 30 minutes, collected, and washed with PBS. Flow cytometry was used to measure TMRE fluorescence. Geometric mean was calculated using FlowJo version 10.8.1. Relative TMRE fluorescence was determined with respect to the vehicle treated group. There was no significant difference in mitochondrial membrane potential in vehicle compared to ONC213 treated cells. **e**. MV4-11 cells were treated with vehicle or 250 nM ONC213 for 8 h. MV4-11 cells treated with 10 uM Hydrogen Peroxide for 15 minutes prior to Invitrogen CellROX Deep Red staining were used as positive controls. Cells were stained with 5 uM CellROX for 20 minutes, collected, and washed with PBS. Flow cytometry was used to measure CellROX fluorescence. Geometric mean was calculated using FlowJo version 10.8.1. Relative CellROX fluorescence was determined with respect to the vehicle treated group. There was no significant difference in vehicle or ONC213 ROS as measured by CellROX. **f**. MV4-11 cells were treated with vehicle for 8 h or 250 nM ONC213 for 2, 4, or 8 h. Cells were stained with 5 uM MitoSox for 30 minutes. Samples were collected, washed with PBS, and flow cytometry was used to measure MitoSox fluorescence. **g**. Next generation sequencing of an isolated *OGDH* g18 clone showed a heterozygous out-of-frame deletion. All 6 clones were heterozygous. **h**. Parental or *OGDH* g18 cells were harvested and cell lysates analyzed for a-KGDH levels. **i**. Parental or *OGDH* g18 cells were analyzed for mitochondrial respiration using the Seahorse bioanalyzer. * p<0.05.