Supplementary Figure Legends

Figure S1. Gene set enrichment analysis (GSEA) of GBM cells treated with romidepsin. GBM14 cells were treated with 2.5 nM romidepsin for 24h and were submitted for microarray analysis followed by GSEA. NES: normalized enrichment score, FDR-q-value (n=2).

Figure S2. A synergistic interaction of imipridones and panobinostat to reduce GBM cell viability. GBM12, GBM43, U251, GBM61, GL261, and astrocytes cells were treated with ONC201, ONC206, or ONC212 (1 µM and 10 µM), panobinostat (50 nM and 200 nM) or the combination of both for 72h and cellular viability analysis was performed (n=4-5). Statistical significance was assessed by ANOVA coupled with Dunnett's multiple comparison test. * the statistical analysis was performed between imipridones and combination treatment, # the statistical analysis was performed between Pb/ Ro and the combination treatment. *p<0.05, ***/****p<0.001, $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#\#}$p<0.001.

Figure S3. A synergistic interaction of imipridones and romidepsin to reduce GBM cell viability. **A**, GBM12, GBM43, U251, GBM61, and astrocyte cells were treated with ONC201/ ONC206/ ONC212 (1 µM and 10 µM), romidepsin (1 nM and 2.5 nM) or the combination of both for 72h and cellular viability analysis was performed (n=4-5). **B**, A summary of the bliss analysis of all the cell lines. Statistical significance was assessed by ANOVA coupled with the Dunnett's multiple comparison test. * the statistical analysis was performed between imipridones and the combination treatment, # the statistical analysis was performed between Pb/ Ro and the combination treatment. **p<0.01, ****p<0.001, $^{\#\#}$p<0.01, $^{\#\#\#}$ / $^{\#\#\#\#}$p<0.001, n.s: not significant.

Figure S4. A synergistic interaction of imipridones and HDAC inhibitors to reduce GBM cell viability. **A**, Standard western blots of GBM14 and U251 cells treated with ONC206 in the presence of panobinostat or romidepsin for 24h. Histon H3 is used as a loading control. **B**,

Standard western blots of GBM14 and U251 cells treated with ONC206 in the presence of 0.2 μM panobinostat/ 2.5 nM romidepsin for 24h. Actin is used as a loading control. **C**, U251 and GBM14 cells were transfected with siRNA against HDAC1, HDAC2, or combination of both, were treated with 10 μM ONC201, ONC206, or ONC212 10 μM for 72h and cellular viability analysis was performed (n=4). **D**, Protein capillary electrophoresis of GBM14 and U251 cells transfected with siRNA against HDAC1, HDAC2, or combination of both. Vinculin is a loading control. **E**, Stable ClpP-wild type or ClpP-Y118A U251 cells were treated with panobinostat/ romidepsin for 72h and cellular viability analysis was performed. **F**, Stable ClpP-wild type or ClpP-Y118A U251 cells were transfected with siRNA against HDAC1, HDAC2, or combination of both and cellular viability analysis was performed. Shown are mean and SD (n=4). Statistical significance was assessed by two-tailed student's t-test. **p<0.01, ***/****p<0.001.

Figure S5. A synergistic interaction of imipridones (ONC201) and HDAC inhibitors to enhance GBM cell apoptosis like cell death. **A-D**, U251, GBM14, GBM12, and NCH644 cells were treated with 10 μM ONC201, 0.2 μM panobinostat/ 2.5 nM romidepsin or combinations of both and were labeled with Annexin V/ PI dye for flow cytometry analysis. The quantification of apoptotic cells is shown in (B, D). Statistical significance was assessed by ANOVA with Dunnett's multiple comparison test. ****p<0.001.

Figure S6. A synergistic interaction of imipridones (ONC206) and HDAC inhibitors to enhance GBM cell apoptosis like cell death. **A-D**, U251 and GBM12 cells were treated with 10 μM ONC206, 0.2 μM panobinostat/ 2.5 nM romidepsin or combinations and were labeled with Annexin V/ PI dye for flow cytometry analysis. The quantification of apoptotic cells is shown in (B, D). **E-H**, GBM61 cells were treated with 10 μM ONC206, 0.2 μM panobinostat/ 2.5 nM romidepsin or combinations of both and were labeled with Annexin V/ PI dye for flow cytometry analysis. The

quantification of apoptotic cells is shown in (F, H). Statistical significance was assessed by ANOVA coupled with the Dunnett's multiple comparison test. **p<0.01, ****p<0.001.

Figure S7. A synergistic interaction of imipridones (ONC212) and HDAC inhibitors to enhance GBM cell apoptosis like cell death. **A-D**, U251, GBM14, GBM12, and NCH644 cells were treated with 10 μM ONC212, 0.2 μM panobinostat/ 2.5 nM romidepsin or combinations of both and were labeled with Annexin V/ PI dye for flow cytometry analysis. The quantification of apoptotic cells is shown in (B, D). Statistical significance was assessed by ANOVA coupled with the Dunnett's multiple comparison test. ****p<0.001.

Figure S8. Activation of the ClpP protease along with inhibition of HDAC1/2 activates a cell death with apoptotic features that is partially caspase-dependent. **A**, U251 cells were treated with ONC206 and panobinostat/ romidepsin in the presence or absence of 10 μM z-VAD-FMK for 48h and were labeled with Annexin V/ PI dye for flow cytometry analysis. Shown is the quantification of apoptotic cells (n=3). **B, C,** U251 cells were treated with 10 μM ONC206 and 0.2 μM panobinostat/ 2.5 nM romidepsin for 48h and cell lysates were subjected for standard western blotting with the indicated antibodies. FL: full length. CF: cleavage fragment. The quantification is shown in (C) (n=3). Statistical significance was assessed by two-tailed student's t-test in (A) and by ANOVA with Dunnett's multiple comparison test in (C). * the statistical analysis was performed between ONC206 and the combination treatment, # the statistic analysis was performed between Pb/ Ro and combination treatment. ***/****p<0.001, ##p<0.01, ### / ####p<0.001.

Figure S9. Expression levels of anti-apoptotic Bcl-2 family proteins following exposures to imipridones and HDAC inhibitors in GBM cells. **A, B,** Standard western blots of Bcl2 family in GBM61 cells treated with 10 μM ONC206, 0.2 μM panobinostat/ 2.5 nM romidepsin, or the combination of both for 24h.

Figure S10. Activation of the ClpP protease along with inhibition of HDAC1/2 regulates the expression of anti-apoptotic Bcl-2 family members. **A**, **B** Real time PCR analysis of U251 and GBM14 cells treated with 10 μM ONC206, 0.2 μM panobinostat/ 2.5 nM romidepsin, or the combination of both for 24h. 18S is used as an internal control. Statistical significance was assessed by ANOVA coupled with the Dunnett's multiple comparison test. * the statistical analysis was performed between ONC206 and the combination treatment, # the statistical analysis was performed between Pb/ Ro and the combination treatment. *$p<0.05$, ***/****$p<0.001$, $^{\#}p<0.05$, $^{\#\#\#\#}p<0.001$. **C**, U251 and GBM14 cells were treated with siRNA against Bcl-xL or Mcl1, treated with 10 μM ONC206 in the presence or absence of 0.2 μM panobinostat/ 2.5 nM romidepsin for 72h and cellular viability analysis was performed. Shown are mean and SD (n=4). **D**, The confirmation of protein expression in U251 and GBM14 cells treated with siRNA against Bcl-xL or Mcl1. Statistical significance was assessed by two-tailed student's t-test. ***/****$p<0.001$.

Figure S11. Adenoviral mediated over-expression of Bcl-xL and Mcl-1 potently rescues from the reduction in cellular viability induced by imipridones and HDAC inhibitors treatment. **A**, U251 and GBM14 cells were transduced with empty vector (EV) or Mcl-1 adenovirus for 24h, treated with 10 μM ONC206 in the presence or absence of 0.2 μM panobinostat/ 2.5 nM romidepsin for 72h and cellular viability analysis was performed. Shown are mean and SD (n=4). **B**, The confirmation of protein expression in U251 and GBM14 cells transduced with empty vector or Mcl-1 adenovirus for 24h. **C**, U251 and GBM14 cells were transduced with empty vector (EV) or Bcl-xL adenovirus for 24h, treated with 10 μM ONC206 and 0.2 μM panobinostat/ 2.5 nM romidepsin for and cellular viability analysis was performed. Shown are mean and SD (n=4). **D**, The confirmation of protein expression in U251 and GBM14 cells transduced with empty vector (EV) or Bcl-xL adenovirus for 24h. **E**, **F**, U251 cells were transduced with empty vector (EV) or Mcl-1/Bcl-xL adenovirus for 24h, treated with 10 μM ONC206 in the presence or absence of 0.2 μM panobinostat/ 2.5 nM

romidepsin for 48h and were labeled with Annexin V/ PI dye for flow cytometry analysis. Shown are mean and SD (n=3). **G**, The confirmation of protein expression in NCH644 cells transduced with empty vector (EV) or Mcl-1/Bcl-xL adenovirus for 24h. Statistical significance was assessed by two-tailed student's t-test. ****p<0.001.

Figure S12. The combination treatment of imipridones and HDAC inhibitors interferes with oxidative energy metabolism in GBM cells. **A**, **B**, U251 and GBM14 cells were treated with 10 µM ONC206, 2.5 nM romidepsin/ 0.2 µM panobinostat or the combination for 24h and analyzed for oxygen consumption rate (OCR) by a mito stress assay on a Seahorse XFe24 device. OM: Oligomycin, F: FCCP, R/A: Rotenone/Antimycin. The graph (right panel) shows the OCR and coupled respiration level (n=4-5). Statistical significance was assessed by ANOVA coupled with Dunnett's multiple comparison test. *p<0.05, **p<0.01, ****p<0.001, n.s: not significant.

Figure S13. The combination treatment of imipridones and HDAC inhibitors rescue the effect of HDAC inhibitors treatment related to OXPHOS complex and fatty acid oxidation. **A**, **B**, Standard western blots of OXPHOS complexes in U251 and GBM14 cells treated with 10 µM ONC206/ONC212, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combinations for 24h. **C**, CHIP-qPCR (H3K27ac) of ACADSB in GBM14 cells treated with 10 µM ONC206, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination for 24h. **D**, CHIP-qPCR (H3K27ac) of three different promoter regions (PGC1α, PPARD and ACADSB) in U251 cells treated with 10 µM ONC206, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination of both for 24h. Shown are mean and SD (n=3). Statistical significance was assessed by ANOVA coupled with the Dunnett's multiple comparison test. *p<0.05, **p<0.01, ***/****p<0.001, n.s: not significant.

Figure S14. The mitochondrial protease, CLPP, determines response and resistance to the combination treatment of imipridones and HDAC inhibitors in GBM models. **A**, U251, GBM14,

and GBM12 cells were transfected with non-targeting or CLPP siRNA, treated with increasing concentration of ONC206 (from 0.2 µM to 200 µM) for 72h and cellular viability analysis was performed. **B**, U251 and GBM14 cells were transfected with non-targeting or CLPP siRNA, treated with increasing concentration of ONC212 (from 0.2 µM to 200 µM) for 72h and cellular viability analysis was performed (n=4-5). **C**, Western blot analysis of ClpP in U251, GBM14, and GBM12 cells transfected with non-targeting or CLPP siRNA. **D**, U251 and GBM14 cells were transfected with non-targeting or CLPP siRNA, treated with 10 µM ONC201/ ONC206/ ONC212, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination for 72h and cellular viability analysis was performed. Shown are means and SD (n=4). Statistical significance was assessed by two-tailed student's t-test. ***/****$p<0.001$.

Figure S15. The mitochondrial protease, CLPP, determines response and resistance to the combination treatment of imipridones and HDAC inhibitors to induce cell death. **A-C**, U251 cells were transfected with non-targeting or CLPP siRNA, treated with 10 µM ONC206, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination for 48h, and were labeled with Annexin V/ PI dye for flow cytometry analysis. The quantification of apoptotic cells was shown in (C). Shown are means and SD (n=3). **D**, Standard western blot of U251 cells were transfected with siRNA against CLPP, treated with 10 µM ONC206, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination of both for 24h. **E**, Standard western blots of stably transduced cells expressing ClpP-wild type or ClpP-D190A mutant treated with 10 µM ONC206, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination of both for 24h. Statistical significance was assessed by two-tailed student's t-test. *$p<0.05$, ***$p<0.001$.

Figure S16. The mitochondrial protease, CLPP, is the metabolic effect mediator of the combination treatment of imipridones and HDAC inhibitors. **A**, **B**, U251 cells were transfected with siRNA against CLPP, treated with 10 µM ONC206, 2.5 nM romidepsin and the combination of

both for 24h and analyzed for oxygen consumption rate (OCR) by a mito stress assay on a Seahorse XFe24 device. OM: Oligomycin, F: FCCP, R/A: Rotenone/Antimycin. The graph in (B) shows the OCR and coupled respiration level (n=4-5). **C-F**, Stably transduced cells expressing ClpP-wild type or ClpP-D190A mutant treated with 10 µM ONC206, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination for 24h were analyzed for oxygen consumption rate (OCR) by mito stress assay on a Seahorse XFe24 device. The graphs in (D, F) show the OCR and coupled respiration level (n=4-5). **G**, Standard western blot of OXPHOS complex in stably transduced U251 cells expressing ClpP-wild type or ClpP-D190A mutant treated with 10 µM ONC206, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination of both for 24h. Statistical significance was assessed by two-tailed student's t-test. ****p<0.001.

Figure S17. The mitochondrial protease, CLPP, is a key mediator of the metabolic effects induced by the combination treatment of imipridones and HDAC inhibitors. **A**, GBM14 cells were incubated in DMEM (devoid of phenol red, pyruvate and glutamine) supplemented with 25 mM U-^{13}C-glucose, 4 mM glutamine, and 1.5% dialyzed FBS in the presence of 10 µM ONC206, 2.5 nM romidepsin or the combination for 24h. Shown are the AMP and ATP level (n=3). **B**, U251 and GBM14 cells were treated with 10 µM ONC206, 0.2 µM panobinostat/ 2.5 nM romidepsin or the combination of both in the presence or absence of ATP for 72h and cellular viability analysis (DNA-content based assay) was performed (n=4). **C**, The protein expression level of Bcl2 family in U251 stably transduced cells expressing ClpP-wild type or ClpP-D190A mutant treated with 10 µM ONC206 and 0.2 µM panobinostat/ 2.5 nM romidepsin for 24h. Actin is used as a loading control. The expression of Mcl1 protein levels were quantified by using ImageJ (shown in cursive font). Statistical significance was assessed by two-tailed student's t-test. ***/****p<0.001.

Figure S18. The drug combination of imipridones and HDAC inhibitors does not exert organ toxicity. **A,** GBM12 cells were implanted into the subcutis of immunocompromised Nu/Nu mice

and were treated with vehicle, ONC206 (50 mg/kg), romidepsin (0.5 mg/kg), or combination treatment of both three times per week after tumors were established. Shown is the H&E staining of mice organs following vehicle or combination treatment. **B,** GBM12 cells were implanted into the right striatum of immunocompromised Nu/Nu mice and were treated with vehicle, ONC212 (50 mg/kg), panobinostat (5 mg/kg), or combination treatment of both two times per week after eight days post intracranial cell implantation. Shown is the H&E staining of mice organs following vehicle or combination treatment. **C**, Tumors from the experiment in Figure 6A were fixed and stained with TUNEL. The representative images show the TUNEL staining of the combination treatment of ONC206 and Romidepsin. The higher magnification was shown on the right and red arrows indicate the TUNEL staining in nuclei. The image is the TUNEL staining of the combination treatment of ONC206 and romidepsin that was shown in Figure 6F (same image) and a higher magnification was shown on the right to indicate the TUNEL staining in nuclei (red arrow).

Table S1: Primer sequences for real time PCR and chromatin immunoprecipitation qPCR

Name	**Sequence**
qPCR primer: hSDHA_F	GAGATGTGGTGTCTCGGTCCAT
qPCR primer: hSDHA_R	GCTGTCTCTGAAATGCCAGGCA
qPCR primer: hSDHB_F	GCAGTCCATAGAAGAGCGTGAG
qPCR primer: hSDHB_R	TGTCTCCGTTCCACCAGTAGCT
qPCR primer: hIDH3A_F	TCGGTGTGACACCAAGTGGCAA
qPCR primer: hIDH3A_R	TTCGCCATGTCCTTGCCTGCAA
qPCR primer: hPPARGC1A (PGC1α)_F	CCAAAGGATGCGCTCTCGTTCA
qPCR primer: hPPARGC1A (PGC1α)_R	CGGTGTCTGTAGTGGCTTGACT
qPCR primer: hPPARD_F	GGCTTCCACTACGGTGTTCATG
qPCR primer: hPPARD_R	CTGGCACTTGTTGCGGTTCTTC
qPCR primer: hACADSB_F	GCCACCTATTTGCCTCAGCTCA
qPCR primer: hACADSB_R	GCTCAGCACTGCTGATCCACAT
qPCR primer: hACADVL_F	TAGGAGAGGCAGGCAAACAGCT
qPCR primer: hACADVL_R	CACAGTGGCAAACTGCTCCAGA
ChIP qPCR primer: PGC1A promoter_F	CTGGGTGTGCGTCTGTTTG
ChIP qPCR primer: PGC1A promoter_R	CGGCGTGGTCTGATTTAGTG
ChIP qPCR primer: PPARD_F2	GCTACGTCCGCATCCCGCACC
ChIP qPCR primer: PPARD_R2	CGAGACGTCACCGCGCTGCTC
ChIP qPCR primer: ACADSB_F	CCGACTCCCTTCCCGCCCCC
ChIP qPCR primer: ACADSB_R	GGCCTCTCCGCTCTGCGCCTC

Figure S1

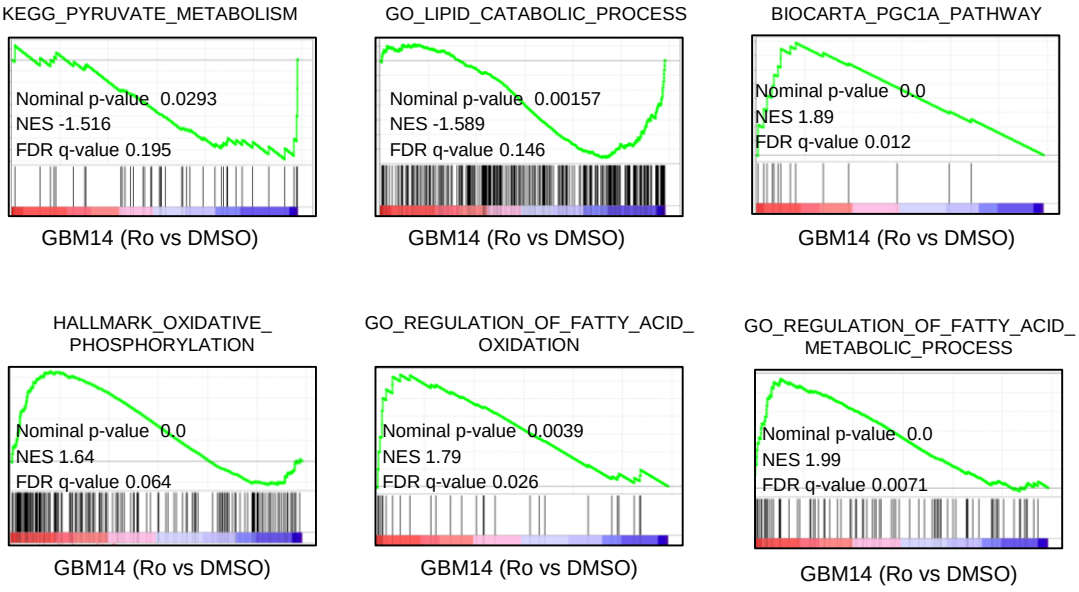

KEGG_PYRUVATE_METABOLISM
Nominal p-value 0.0293
NES -1.516
FDR q-value 0.195
GBM14 (Ro vs DMSO)
GO_LIPID_CATABOLIC_PROCESS
Nominal p-value 0.00157
NES -1.589
FDR q-value 0.146
GBM14 (Ro vs DMSO)
BIOCARTA_PGC1A_PATHWAY
Nominal p-value 0.0
NES 1.89
FDR q-value 0.012
GBM14 (Ro vs DMSO)
HALLMARK_OXIDATIVE_ PHOSPHORYLATION
Nominal p-value 0.0
NES 1.64
FDR q-value 0.064
GBM14 (Ro vs DMSO)
GO_REGULATION_OF_FATTY_ACID_ OXIDATION
Nominal p-value 0.0039
NES 1.79
FDR q-value 0.026
GBM14 (Ro vs DMSO)
GO_REGULATION_OF_FATTY_ACID_ METABOLIC_PROCESS
Nominal p-value 0.0
NES 1.99
FDR q-value 0.0071
GBM14 (Ro vs DMSO)

Figure S2

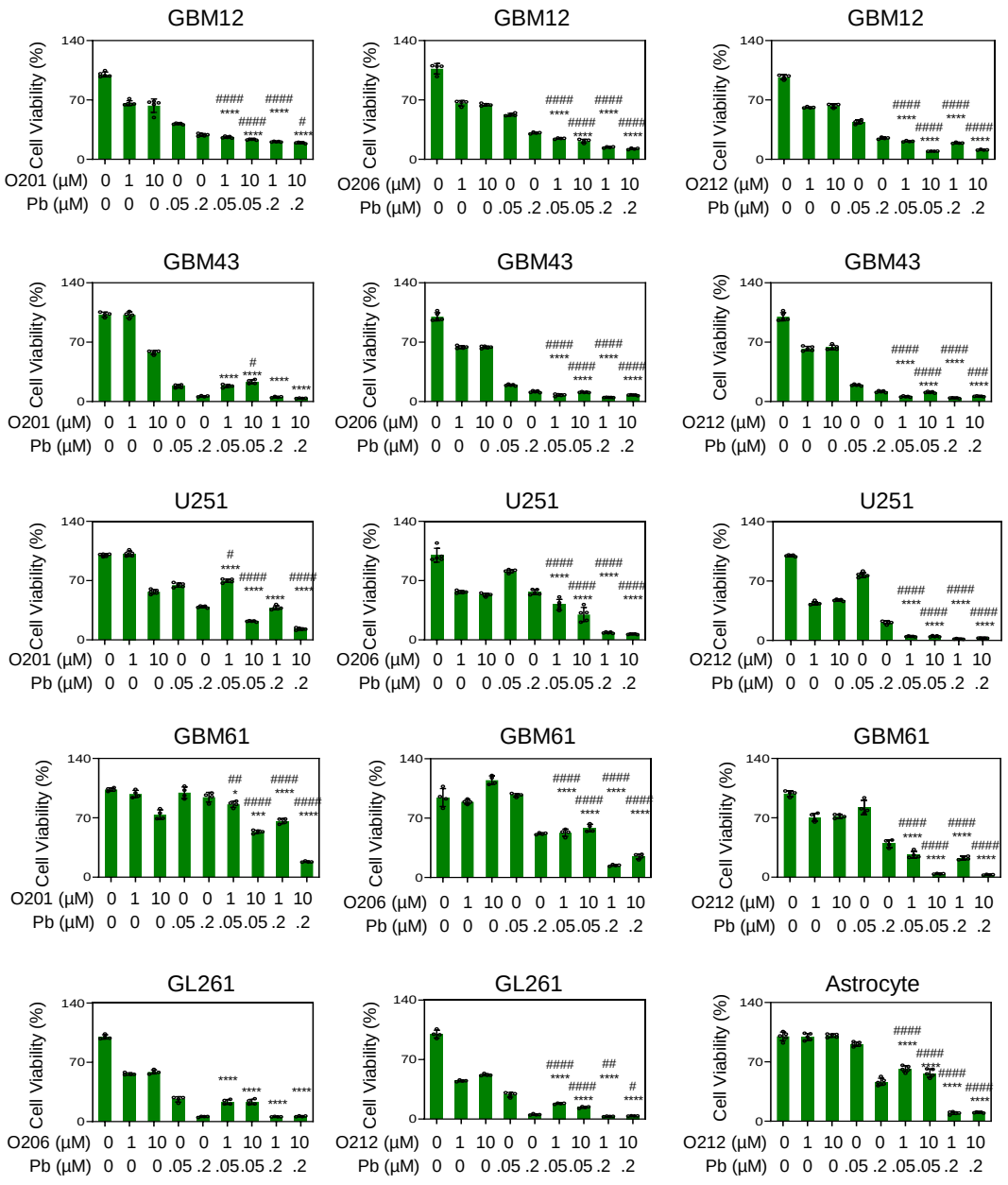
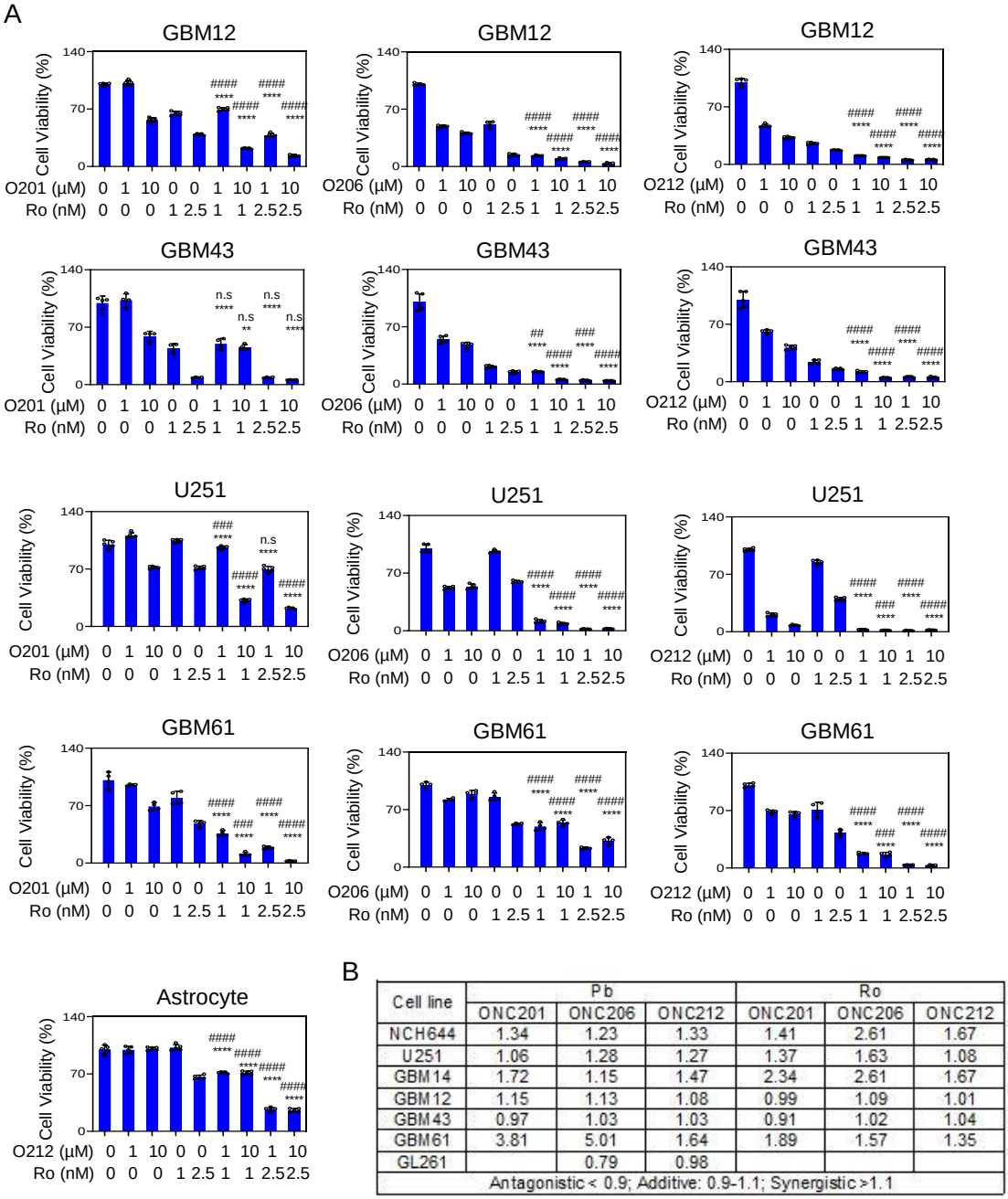

GBM12
GBM12
GBM12
GBM43
GBM43
GBM43
U251
U251
U251
GBM61
GBM61
GBM61
GL261
GL261
Astrocyte
Cell Viability (%)
O201 (μM) 0 1 10 0 0 1 10 1 10
O206 (μM) 0 1 10 0 0 1 10 1 10
O212 (μM) 0 1 10 0 0 1 10 1 10
Pb (μM) 0 0 0 .05 .2 .05 .05 .2 .2

Figure S3

A



B

Cell line	Pb			Ro		
	ONC201	ONC206	ONC212	ONC201	ONC206	ONC212
NCH644	1.34	1.23	1.33	1.41	2.61	1.67
U251	1.06	1.28	1.27	1.37	1.63	1.08
GBM14	1.72	1.15	1.47	2.34	2.61	1.67
GBM12	1.15	1.13	1.08	0.99	1.09	1.01
GBM43	0.97	1.03	1.03	0.91	1.02	1.04
GBM61	3.81	5.01	1.64	1.89	1.57	1.35
GL261		0.79	0.98			
Antagonistic < 0.9; Additive: 0.9-1.1; Synergistic >1.1						

Figure S4

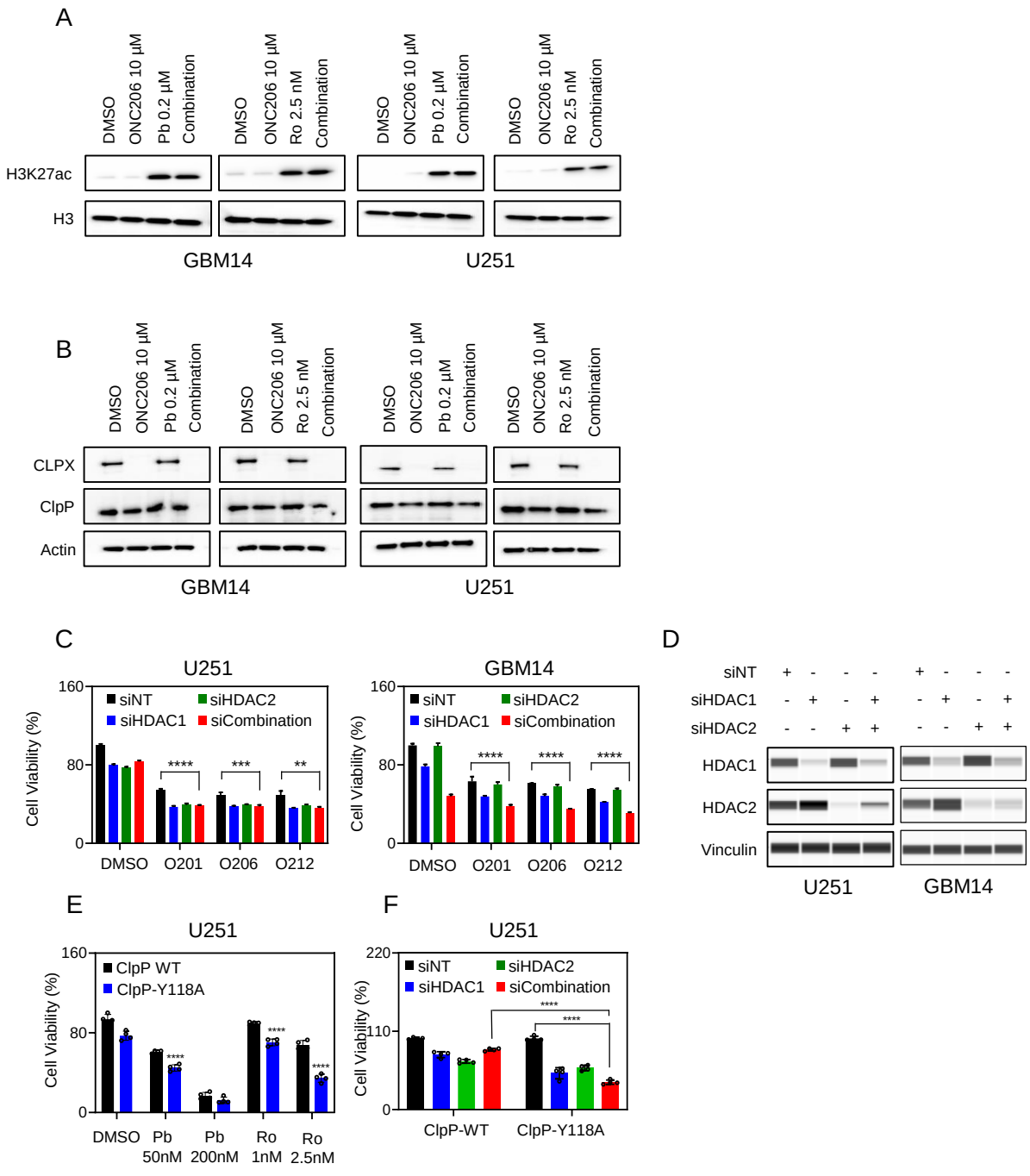

A
DMSO
ONC206 10 µM
Pb 0.2 µM
Ro 2.5 nM
Combination
H3K27ac
H3
GBM14
U251
B
CLPX
ClpP
Actin
C
U251
GBM14
siNT
siHDAC1
siHDAC2
siCombination
Cell Viability (%)
DMSO
O201
O206
O212
D
siNT
siHDAC1
siHDAC2
HDAC1
HDAC2
Vinculin
E
ClpP WT
ClpP-Y118A
Pb 50nM
Pb 200nM
Ro 1nM
Ro 2.5nM
F
ClpP-WT
ClpP-Y118A

Figure S5

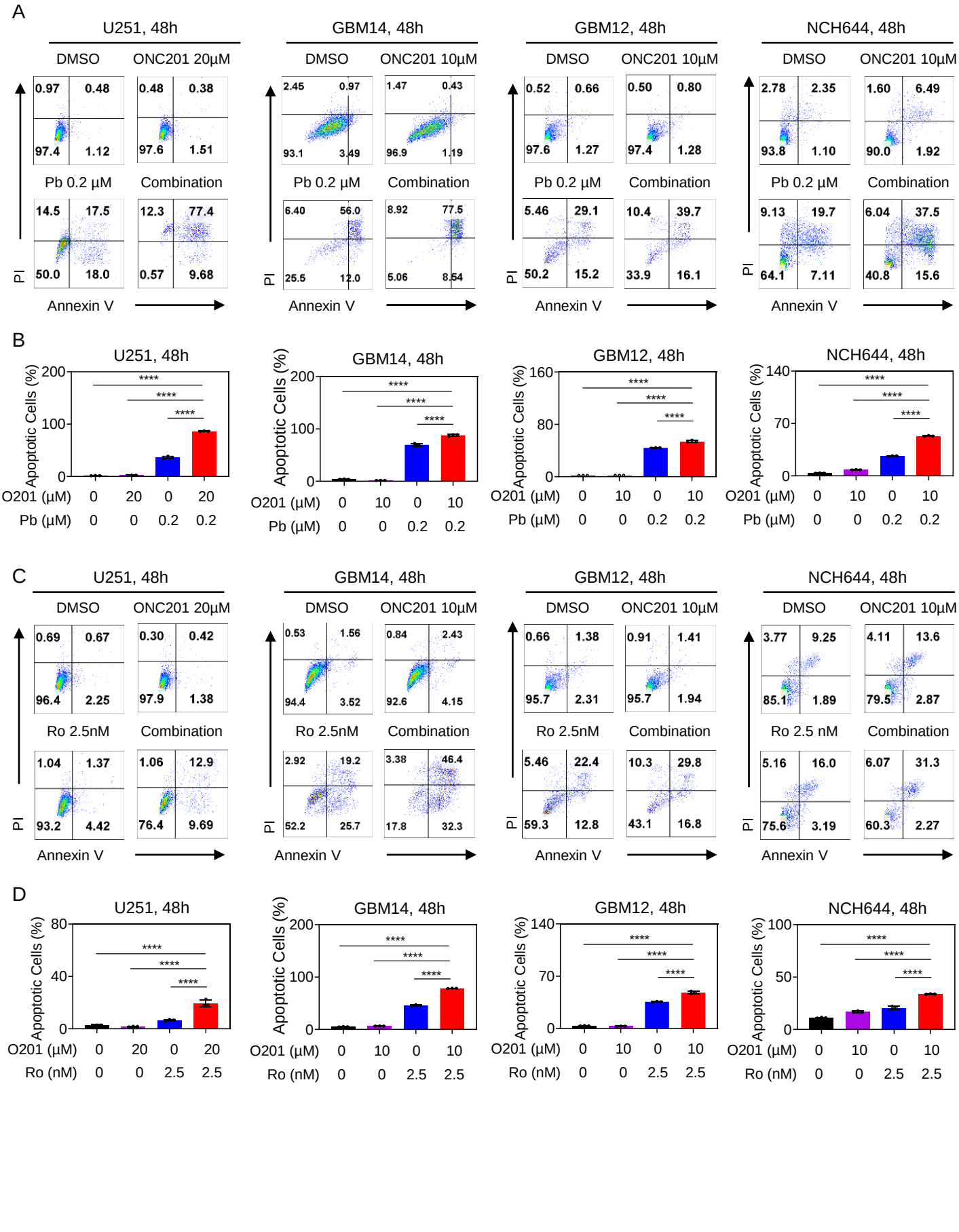

A
U251, 48h
GBM14, 48h
GBM12, 48h
NCH644, 48h
DMSO
ONC201 20μM
ONC201 10μM
Pb 0.2 μM
Combination
PI
Annexin V
B
Apoptotic Cells (%)
O201 (μM)
Pb (μM)
C
Ro 2.5nM
Ro 2.5 nM
D
Ro (nM)

Figure S6

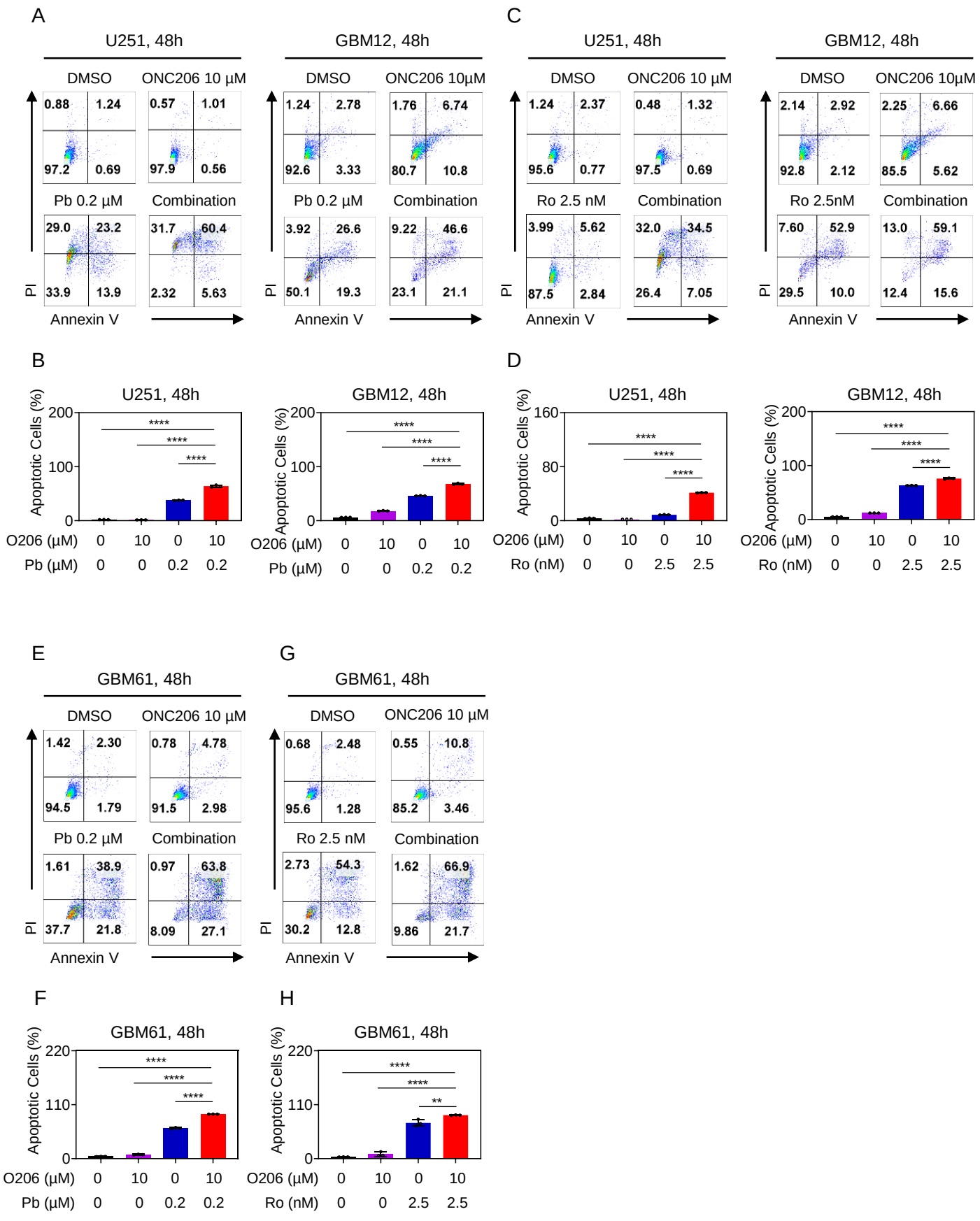

A
U251, 48h
GBM12, 48h
DMSO
ONC206 10 μM
ONC206 10μM
0.88
1.24
97.2
0.69
0.57
1.01
97.9
0.56
1.24
2.78
92.6
3.33
1.76
6.74
80.7
10.8
Pb 0.2 μM
Combination
29.0
23.2
33.9
13.9
31.7
60.4
2.32
5.63
3.92
26.6
50.1
19.3
9.22
46.6
23.1
21.1
PI
Annexin V
C
U251, 48h
GBM12, 48h
DMSO
ONC206 10 μM
ONC206 10μM
1.24
2.37
95.6
0.77
0.48
1.32
97.5
0.69
2.14
2.92
92.8
2.12
2.25
6.66
85.5
5.62
Ro 2.5 nM
Combination
Ro 2.5nM
Combination
3.99
5.62
87.5
2.84
32.0
34.5
26.4
7.05
7.60
52.9
29.5
10.0
13.0
59.1
12.4
15.6
PI
Annexin V
B
U251, 48h
GBM12, 48h
Apoptotic Cells (%)
200
100
0
****
****
****
O206 (μM)
0
10
0
10
Pb (μM)
0
0
0.2
0.2
D
U251, 48h
GBM12, 48h
Apoptotic Cells (%)
160
80
0
200
100
0
****
****
****
O206 (μM)
0
10
0
10
Ro (nM)
0
0
2.5
2.5
E
GBM61, 48h
DMSO
ONC206 10 μM
1.42
2.30
94.5
1.79
0.78
4.78
91.5
2.98
Pb 0.2 μM
Combination
1.61
38.9
37.7
21.8
0.97
63.8
8.09
27.1
PI
Annexin V
G
GBM61, 48h
DMSO
ONC206 10 μM
0.68
2.48
95.6
1.28
0.55
10.8
85.2
3.46
Ro 2.5 nM
Combination
2.73
54.3
30.2
12.8
1.62
66.9
9.86
21.7
PI
Annexin V
F
GBM61, 48h
Apoptotic Cells (%)
220
110
0
****
****
****
O206 (μM)
0
10
0
10
Pb (μM)
0
0
0.2
0.2
H
GBM61, 48h
Apoptotic Cells (%)
220
110
0
****
****
**
O206 (μM)
0
10
0
10
Ro (nM)
0
0
2.5
2.5

Figure S7

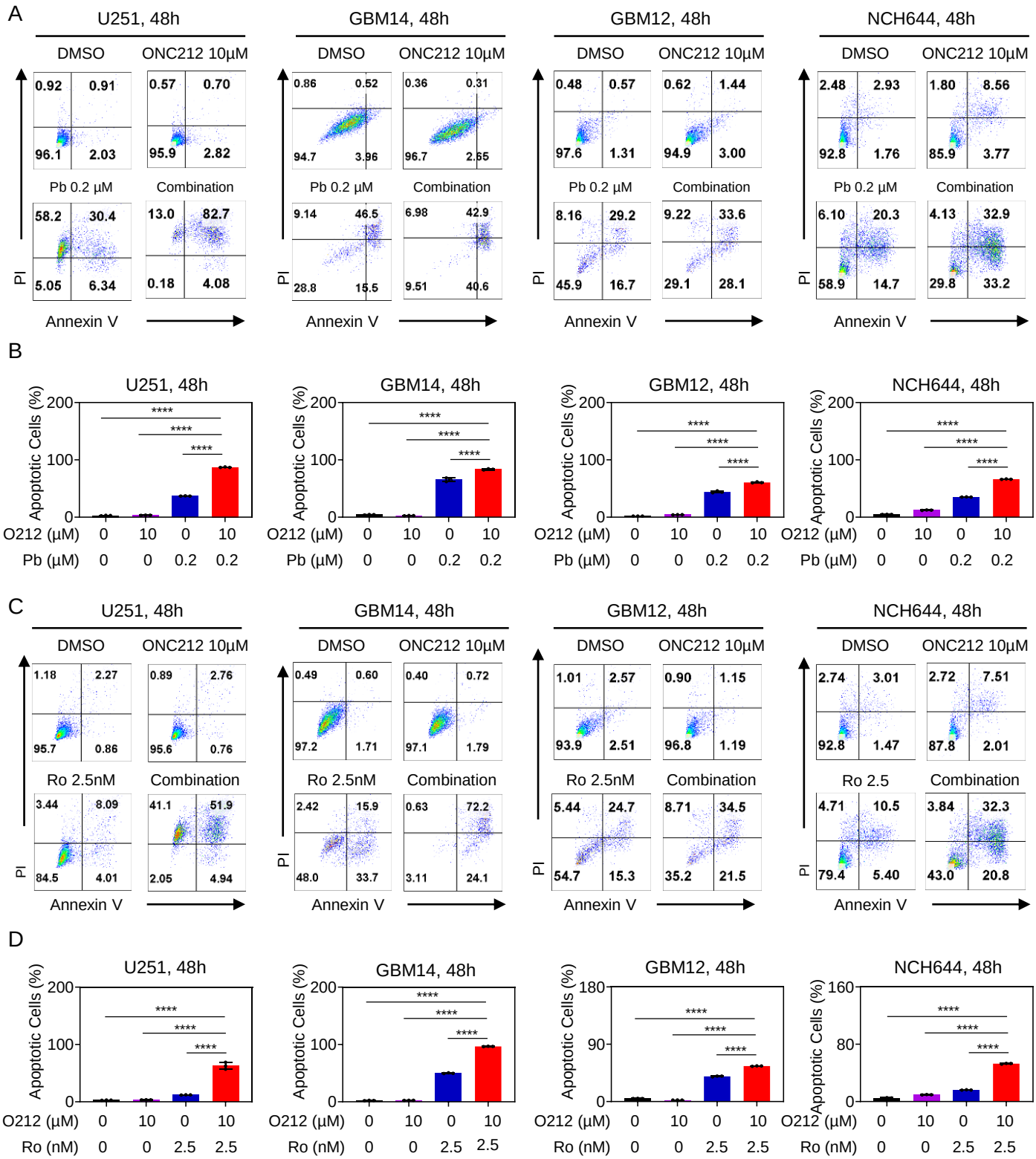

A
U251, 48h
GBM14, 48h
GBM12, 48h
NCH644, 48h
DMSO
ONC212 10μM
Pb 0.2 μM
Combination
PI
Annexin V
B
Apoptotic Cells (%)
O212 (μM)
Pb (μM)
C
Ro 2.5nM
Ro 2.5
D
Ro (nM)

Figure S8

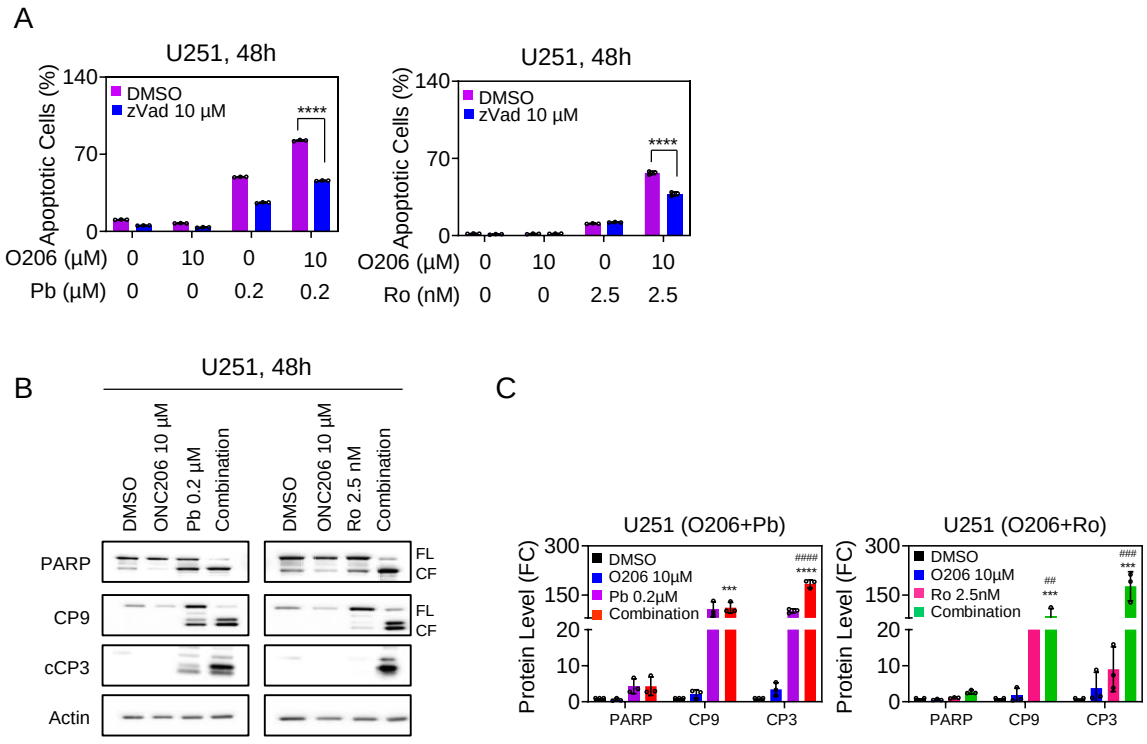

A
U251, 48h
Apoptotic Cells (%)
DMSO
zVad 10 µM
****
O206 (µM) 0 10 0 10
Pb (µM) 0 0 0.2 0.2
U251, 48h
Apoptotic Cells (%)
DMSO
zVad 10 µM
****
O206 (µM) 0 10 0 10
Ro (nM) 0 0 2.5 2.5
B
U251, 48h
DMSO
ONC206 10 µM
Pb 0.2 µM
Combination
DMSO
ONC206 10 µM
Ro 2.5 nM
Combination
PARP
CP9
cCP3
Actin
FL
CF
C
U251 (O206+Pb)
Protein Level (FC)
DMSO
O206 10µM
Pb 0.2µM
Combination
PARP
CP9
CP3
U251 (O206+Ro)
Protein Level (FC)
DMSO
O206 10µM
Ro 2.5nM
Combination
PARP
CP9
CP3

Figure S9

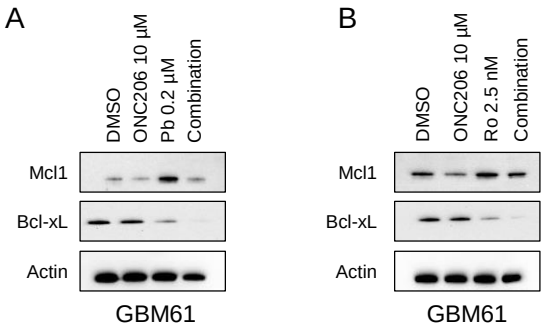

A
DMSO
ONC206 10 µM
Pb 0.2 µM
Combination
Mcl1
Bcl-xL
Actin
GBM61
B
DMSO
ONC206 10 µM
Ro 2.5 nM
Combination
Mcl1
Bcl-xL
Actin
GBM61

Figure S10

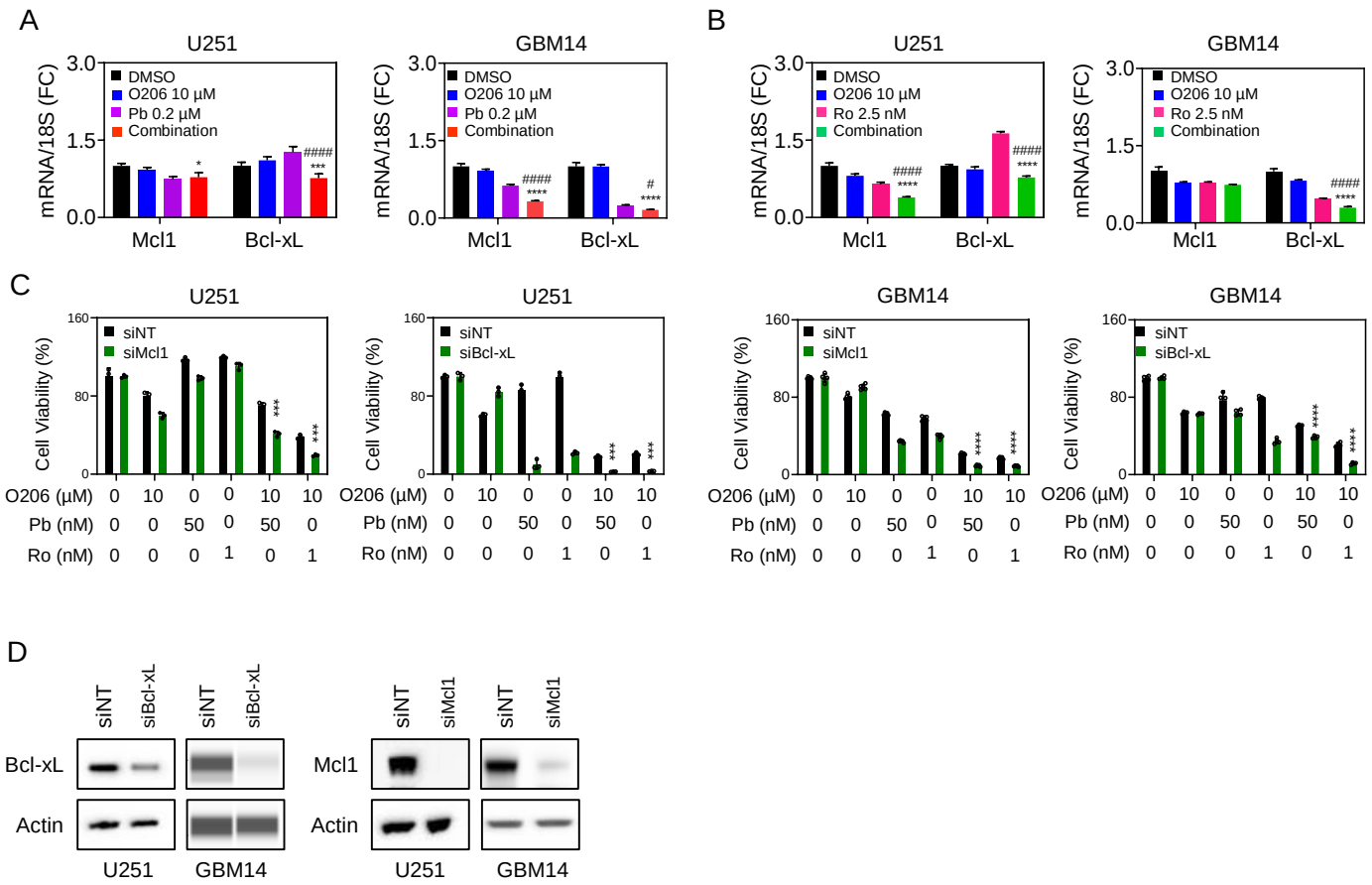

A
U251
GBM14
mRNA/18S (FC)
DMSO
O206 10 µM
Pb 0.2 µM
Combination
Mcl1
Bcl-xL
B
U251
GBM14
mRNA/18S (FC)
DMSO
O206 10 µM
Ro 2.5 nM
Combination
Mcl1
Bcl-xL
C
U251
U251
GBM14
GBM14
Cell Viability (%)
siNT
siMcl1
siBcl-xL
O206 (µM)
Pb (nM)
Ro (nM)
D
siNT
siBcl-xL
siMcl1
Bcl-xL
Mcl1
Actin
U251
GBM14

Figure S11

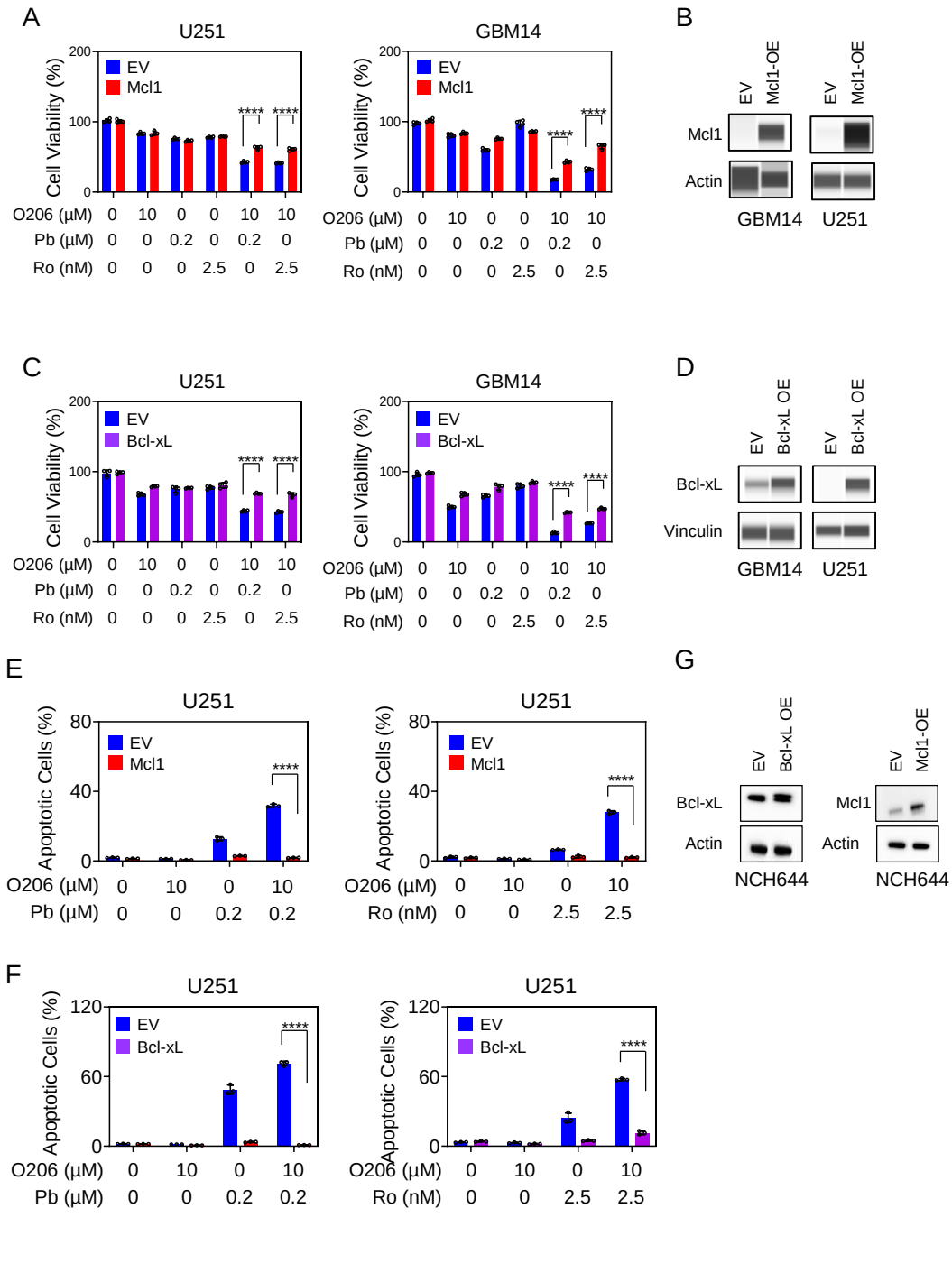

A
U251
GBM14
Cell Viability (%)
EV
Mcl1
O206 (μM)
Pb (μM)
Ro (nM)
B
EV
Mcl1-OE
Mcl1
Actin
GBM14
U251
C
Bcl-xL
D
Bcl-xL OE
Vinculin
E
Apoptotic Cells (%)
F
G
NCH644

Figure S12

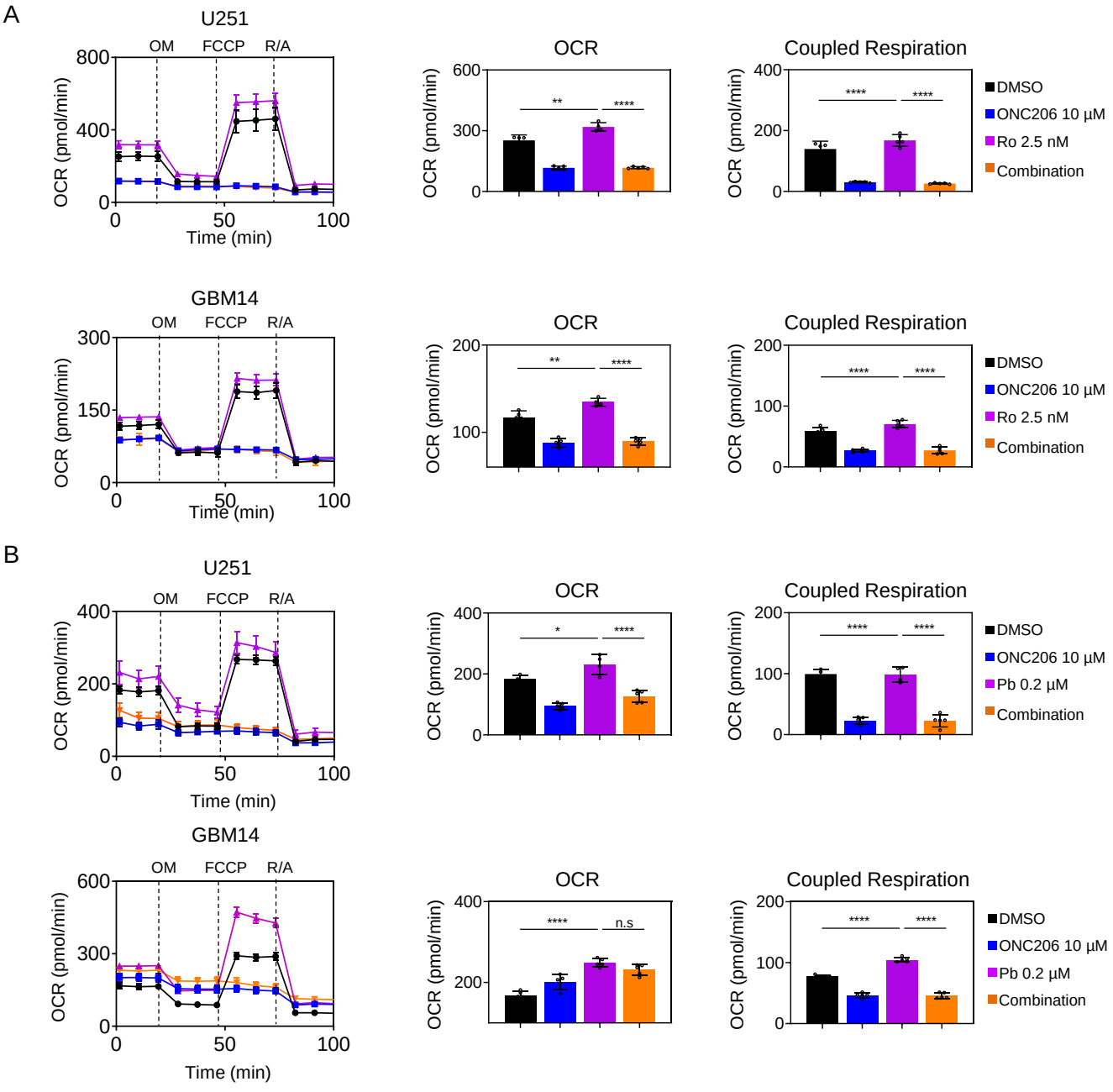

A
U251
OM
FCCP
R/A
OCR (pmol/min)
Time (min)
OCR
Coupled Respiration
DMSO
ONC206 10 µM
Ro 2.5 nM
Combination
GBM14
B
U251
Pb 0.2 µM
GBM14
n.s

Figure S13

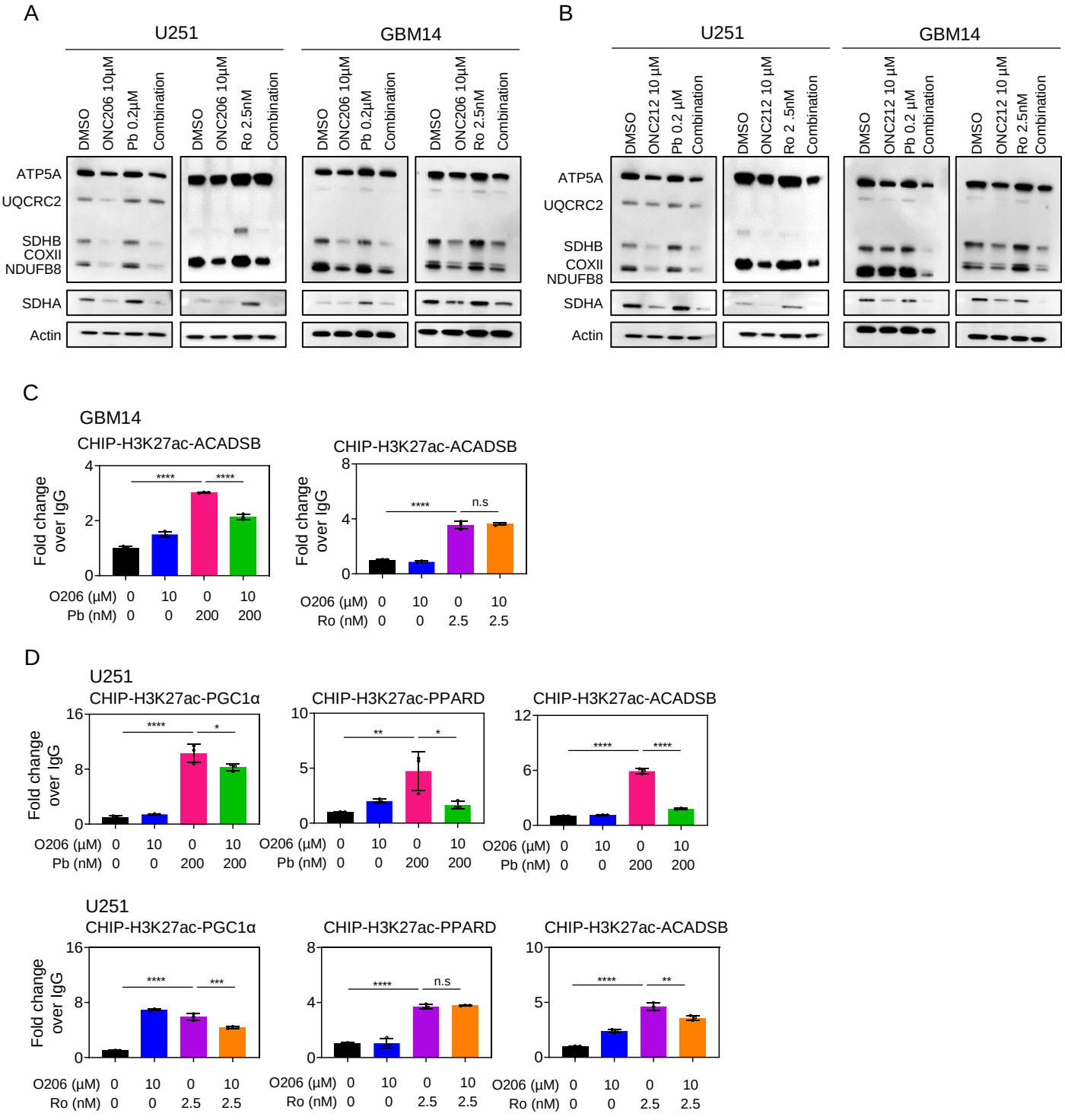

A
U251
GBM14
DMSO
ONC206 10μM
Pb 0.2μM
Ro 2.5nM
Combination
ATP5A
UQCRC2
SDHB
COXII
NDUFB8
SDHA
Actin
B
U251
GBM14
DMSO
ONC212 10 μM
Pb 0.2 μM
Ro 2 .5nM
Ro 2.5nM
Combination
ATP5A
UQCRC2
SDHB
COXII
NDUFB8
SDHA
Actin
C
GBM14
CHIP-H3K27ac-ACADSB
Fold change over IgG
O206 (μM) 0 10 0 10
Pb (nM) 0 0 200 200
CHIP-H3K27ac-ACADSB
Fold change over IgG
n.s
O206 (μM) 0 10 0 10
Ro (nM) 0 0 2.5 2.5
D
U251
CHIP-H3K27ac-PGC1α
CHIP-H3K27ac-PPARD
CHIP-H3K27ac-ACADSB
Fold change over IgG
O206 (μM) 0 10 0 10
Pb (nM) 0 0 200 200
U251
CHIP-H3K27ac-PGC1α
CHIP-H3K27ac-PPARD
CHIP-H3K27ac-ACADSB
Fold change over IgG
n.s
O206 (μM) 0 10 0 10
Ro (nM) 0 0 2.5 2.5

Figure S14

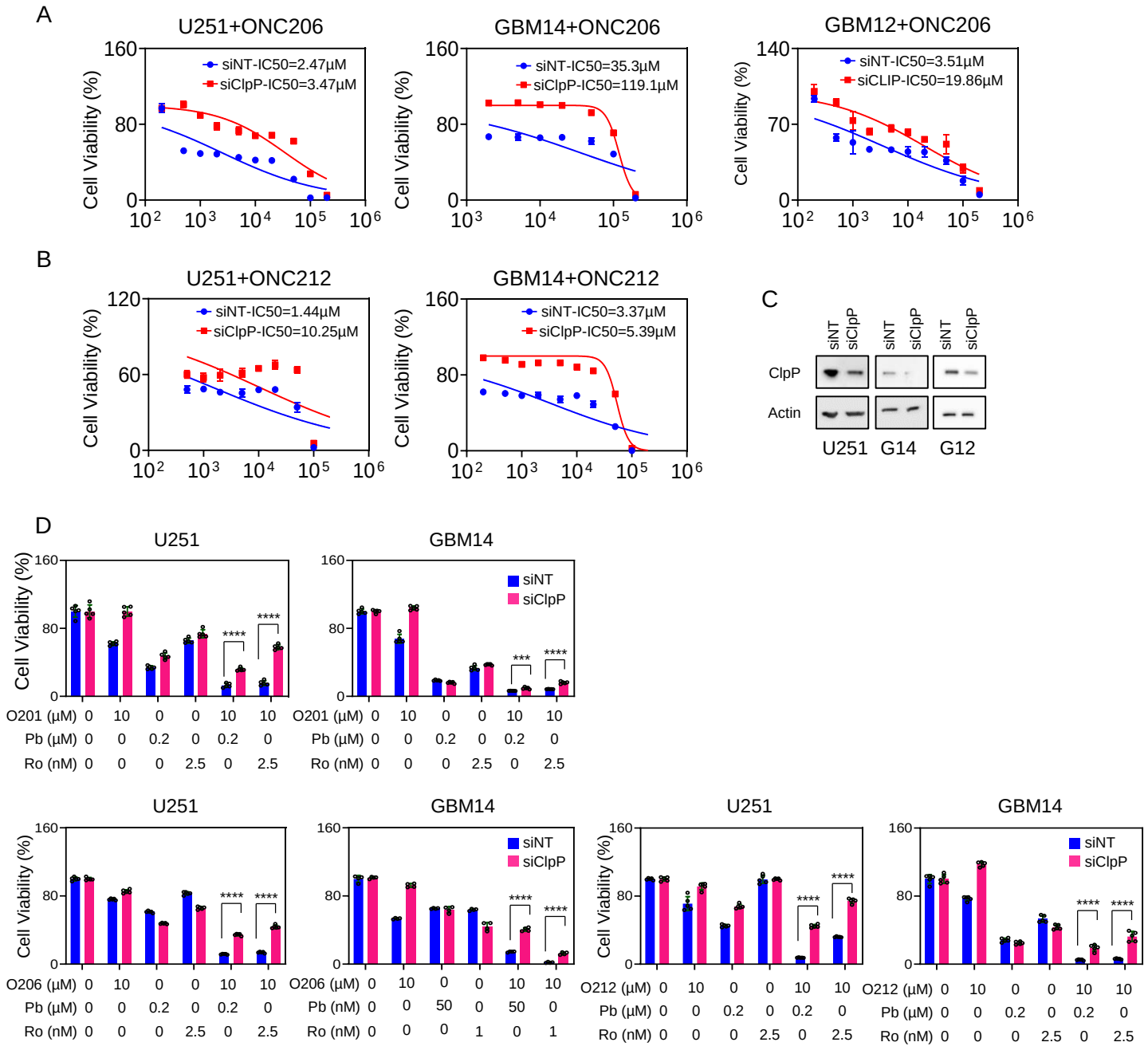

A
U251+ONC206
siNT-IC50=2.47μM
siClpP-IC50=3.47μM
GBM14+ONC206
siNT-IC50=35.3μM
siClpP-IC50=119.1μM
GBM12+ONC206
siNT-IC50=3.51μM
siCLIP-IC50=19.86μM
Cell Viability (%)
B
U251+ONC212
siNT-IC50=1.44μM
siClpP-IC50=10.25μM
GBM14+ONC212
siNT-IC50=3.37μM
siClpP-IC50=5.39μM
C
siNT
siClpP
ClpP
Actin
U251
G14
G12
D
U251
GBM14
siNT
siClpP
O201 (μM)
Pb (μM)
Ro (nM)
U251
GBM14
siNT
siClpP
O206 (μM)
Pb (μM)
Ro (nM)
U251
GBM14
siNT
siClpP
O212 (μM)
Pb (μM)
Ro (nM)

Figure S15

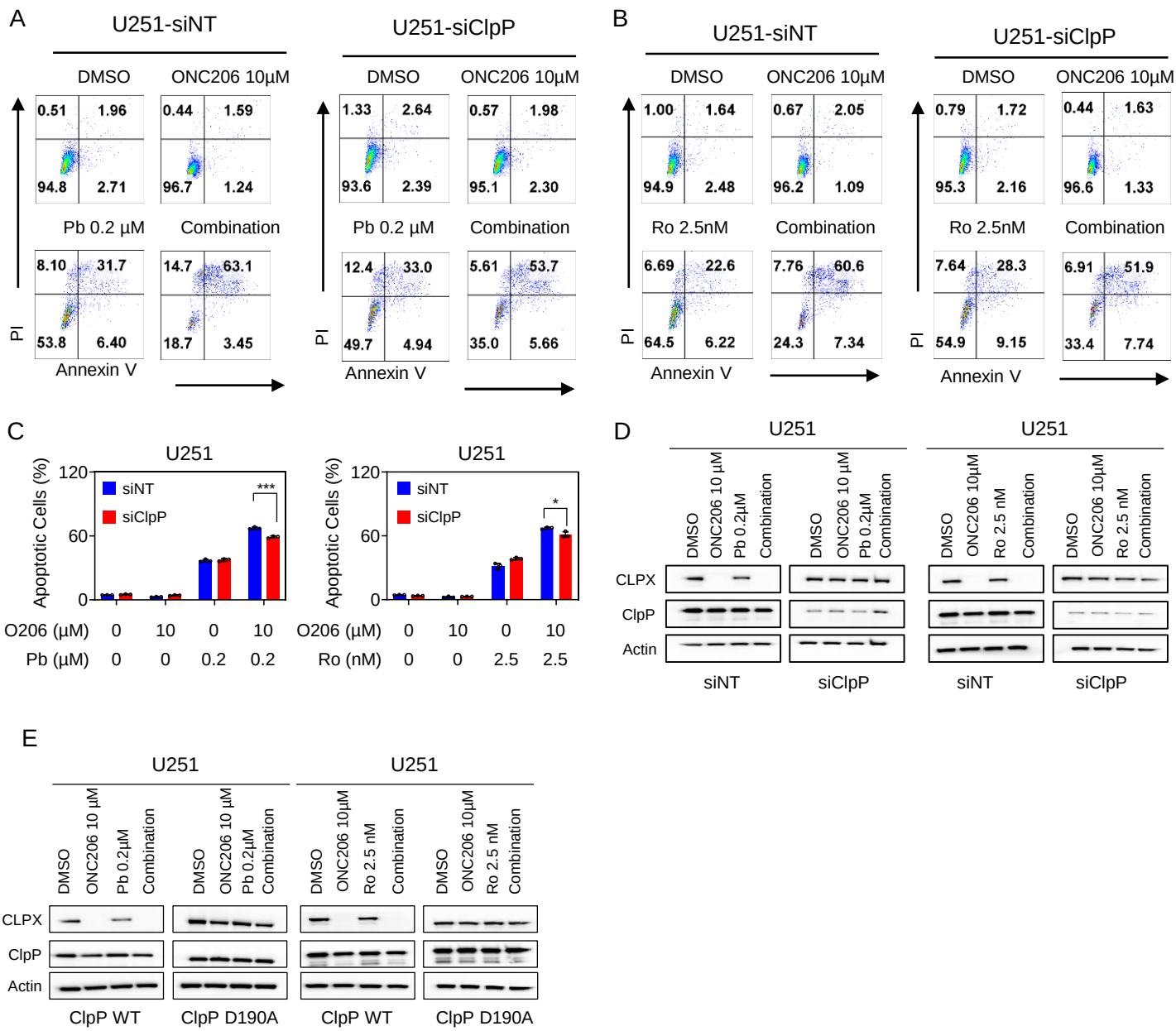

A
U251-siNT
U251-siClpP
DMSO
ONC206 10μM
Pb 0.2 μM
Combination
0.51
1.96
94.8
2.71
0.44
1.59
96.7
1.24
1.33
2.64
93.6
2.39
0.57
1.98
95.1
2.30
8.10
31.7
53.8
6.40
14.7
63.1
18.7
3.45
12.4
33.0
49.7
4.94
5.61
53.7
35.0
5.66
PI
Annexin V
B
U251-siNT
U251-siClpP
DMSO
ONC206 10μM
Ro 2.5nM
Combination
1.00
1.64
94.9
2.48
0.67
2.05
96.2
1.09
0.79
1.72
95.3
2.16
0.44
1.63
96.6
1.33
6.69
22.6
64.5
6.22
7.76
60.6
24.3
7.34
7.64
28.3
54.9
9.15
6.91
51.9
33.4
7.74
PI
Annexin V
C
U251
U251
Apoptotic Cells (%)
120
60
0
siNT
siClpP
***
*
O206 (μM) 0 10 0 10
Pb (μM) 0 0 0.2 0.2
O206 (μM) 0 10 0 10
Ro (nM) 0 0 2.5 2.5
D
U251
U251
DMSO
ONC206 10 μM
Pb 0.2μM
Combination
ONC206 10μM
Ro 2.5 nM
CLPX
ClpP
Actin
siNT
siClpP
siNT
siClpP
E
U251
U251
DMSO
ONC206 10 μM
Pb 0.2μM
Combination
ONC206 10μM
Ro 2.5 nM
CLPX
ClpP
Actin
ClpP WT
ClpP D190A
ClpP WT
ClpP D190A

Figure S16

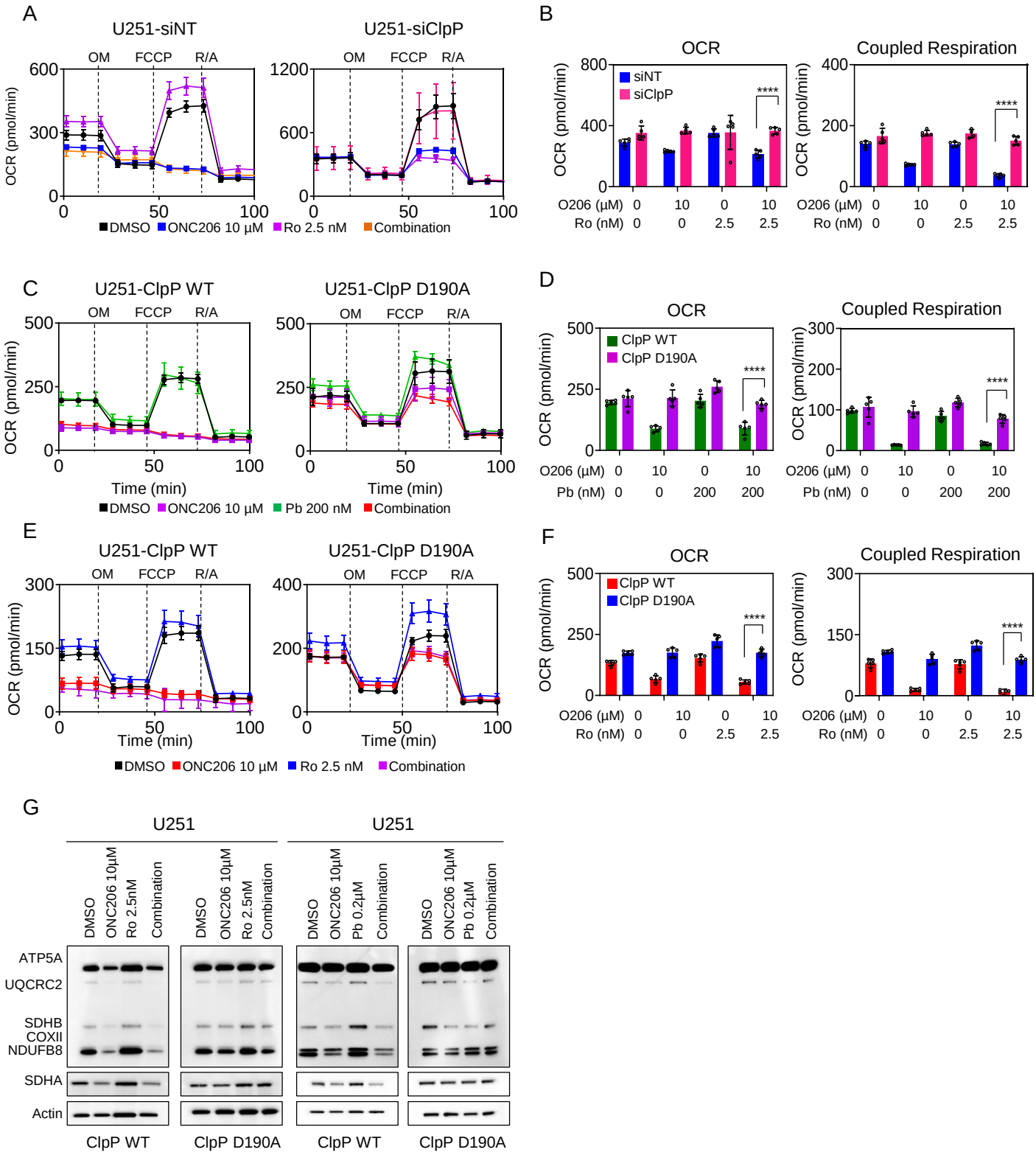

A
U251-siNT
U251-siClpP
OM
FCCP
R/A
OCR (pmol/min)
DMSO
ONC206 10 µM
Ro 2.5 nM
Combination
B
OCR
Coupled Respiration
siNT
siClpP
O206 (µM)
Ro (nM)
C
U251-ClpP WT
U251-ClpP D190A
Time (min)
Pb 200 nM
D
ClpP WT
ClpP D190A
Pb (nM)
E
F
G
U251
ATP5A
UQCRC2
SDHB
COXII
NDUFB8
SDHA
Actin

Figure S17

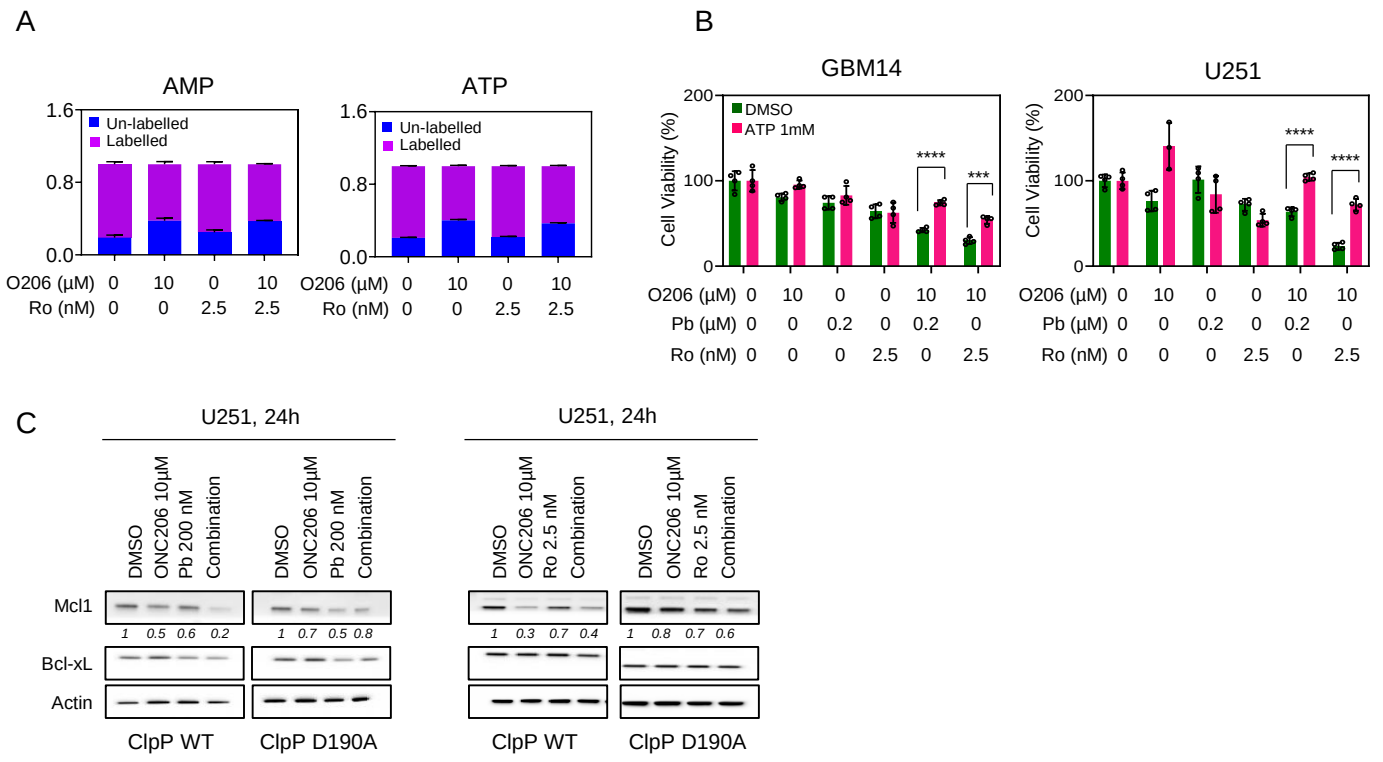
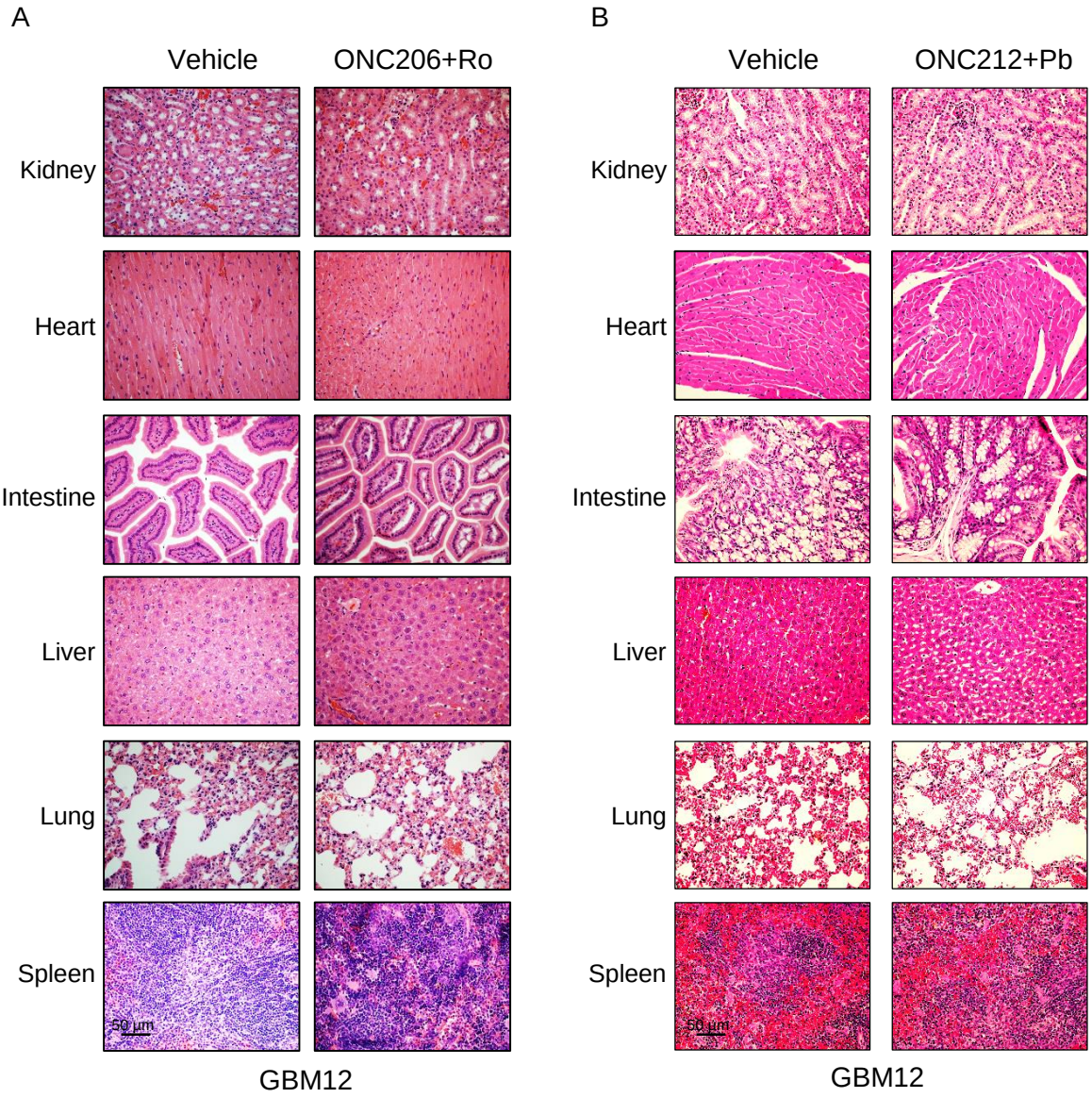
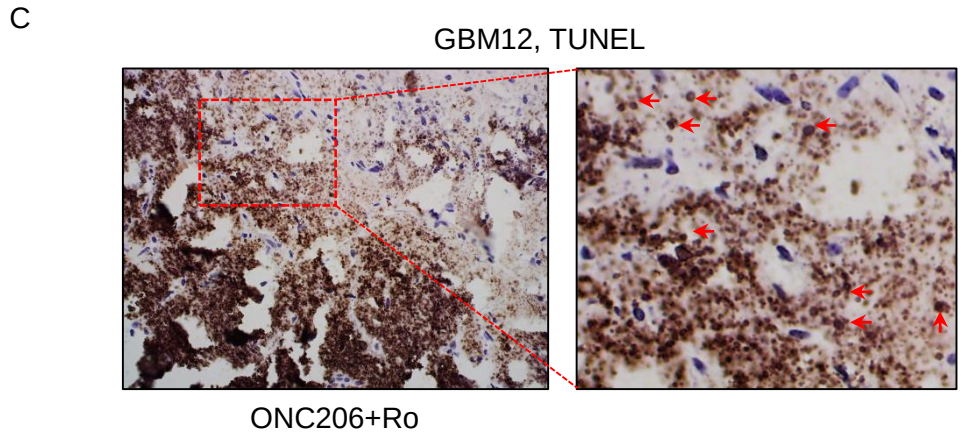

A
AMP
ATP
Un-labelled
Labelled
1.6
0.8
0.0
O206 (μM) 0 10 0 10
Ro (nM) 0 0 2.5 2.5
B
GBM14
U251
DMSO
ATP 1mM
Cell Viability (%)
200
100
0
****
***
O206 (μM) 0 10 0 0 10 10
Pb (μM) 0 0 0.2 0 0.2 0
Ro (nM) 0 0 0 2.5 0 2.5
C
U251, 24h
DMSO
ONC206 10μM
Pb 200 nM
Ro 2.5 nM
Combination
Mcl1
Bcl-xL
Actin
1 0.5 0.6 0.2
1 0.7 0.5 0.8
1 0.3 0.7 0.4
1 0.8 0.7 0.6
ClpP WT
ClpP D190A

Figure S18


A
Vehicle
ONC206+Ro
Kidney
Heart
Intestine
Liver
Lung
Spleen
50 μm
GBM12
B
Vehicle
ONC212+Pb
Kidney
Heart
Intestine
Liver
Lung
Spleen
50 μm
GBM12


C
GBM12, TUNEL
ONC206+Ro