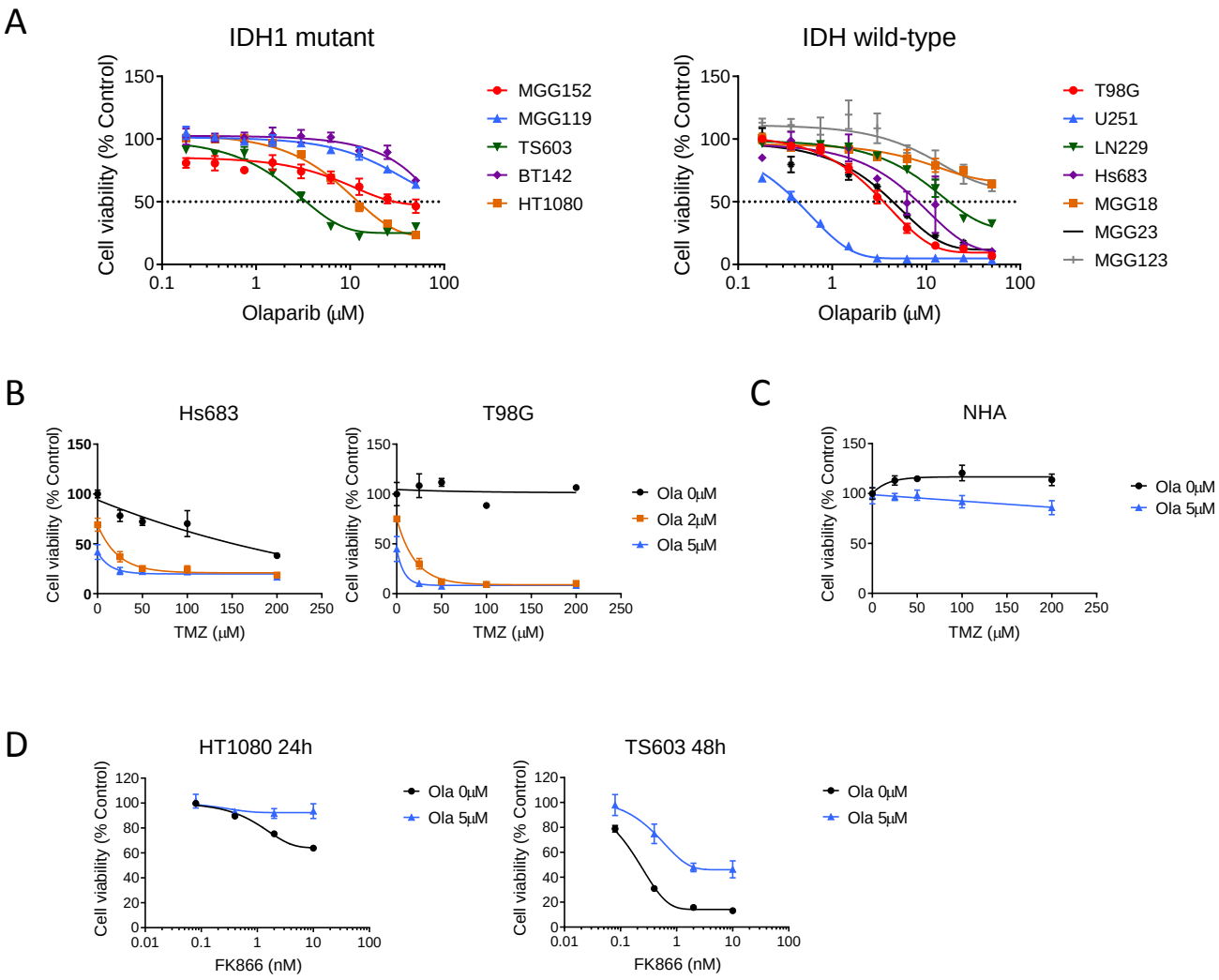
Supplementary Figure S1



Supplementary Figure S1 (Related to Figure 1)

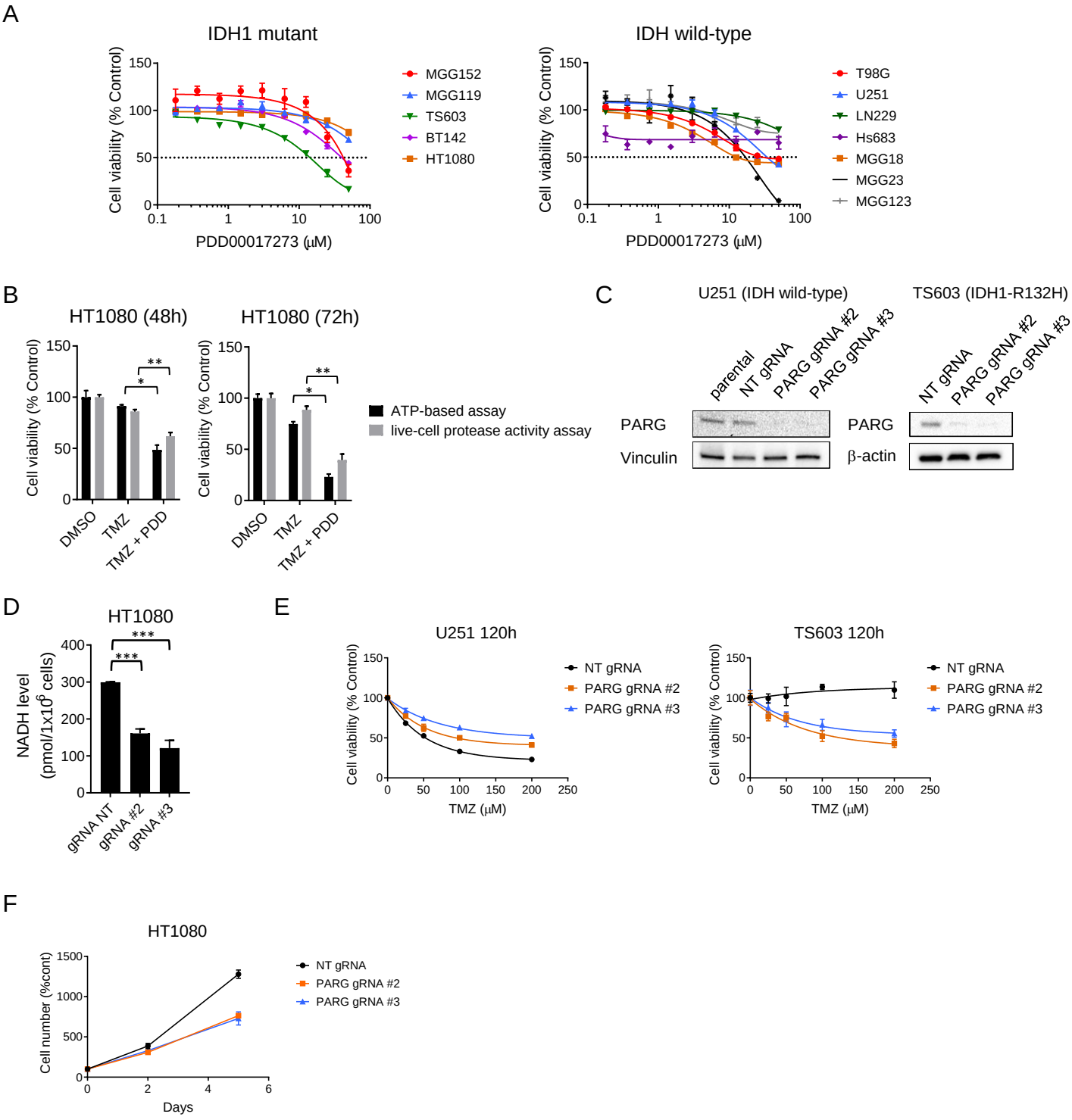
A. Cell viability assay (CellTiter-Glo) after 96-120h treatment with olaparib (Ola). Left panel shows IDH mutant cell lines: IDH1R132H in MGG152, MGG119, TS603, and BT142 (gliomas); IDH1R132C in HT1080 (fibrosarcoma). Right panel shows IDH wild-type glioblastoma cell lines.

B. Relative cell viability of Hs683 and T98G after 120-hour exposure to Ola (0 , 2, or 5 μM) with temozolomide (TMZ) at indicated concentrations.

C. Relative cell viability of Normal human astrocyte (NHA) after 120-hour exposure to Ola (0 or 5 μM) with TMZ at indicated concentrations.

D. Relative cell viability of HT1080 and TS603 after 24 or 48-hour exposure to Ola (0 or 5 μM) with FK866 at indicated concentrations.

Supplementary Figure S2



Supplementary Figure S2 (Related to Figure 2)

A. Cell viability assay (CellTiter-Glo) after 96-120h treatment with PDD00017273 (PDD). Left panel shows IDH mutant cell lines: IDH1R132H in MGG152, MGG119, TS603, BT142 (gliomas); IDH1R132C in HT1080 (fibrosarcoma). Right panel shows IDH wild-type glioblastoma cell lines.

B. Cell viability of HT1080 after treatment with PDD (2 μM), temozolomide (TMZ, 200 μM), or TMZ plus PDD measured by ATP-based CellTiter-Glo assay and the live cell protease activity assay at indicated time points. Bars, ± SEM; *$p < 0.05$ for difference between TMZ and TMZ plus PDD in ATP-based assay. **$p < 0.05$ for difference between TMZ and TMZ plus PDD in live cell protease activity assay.

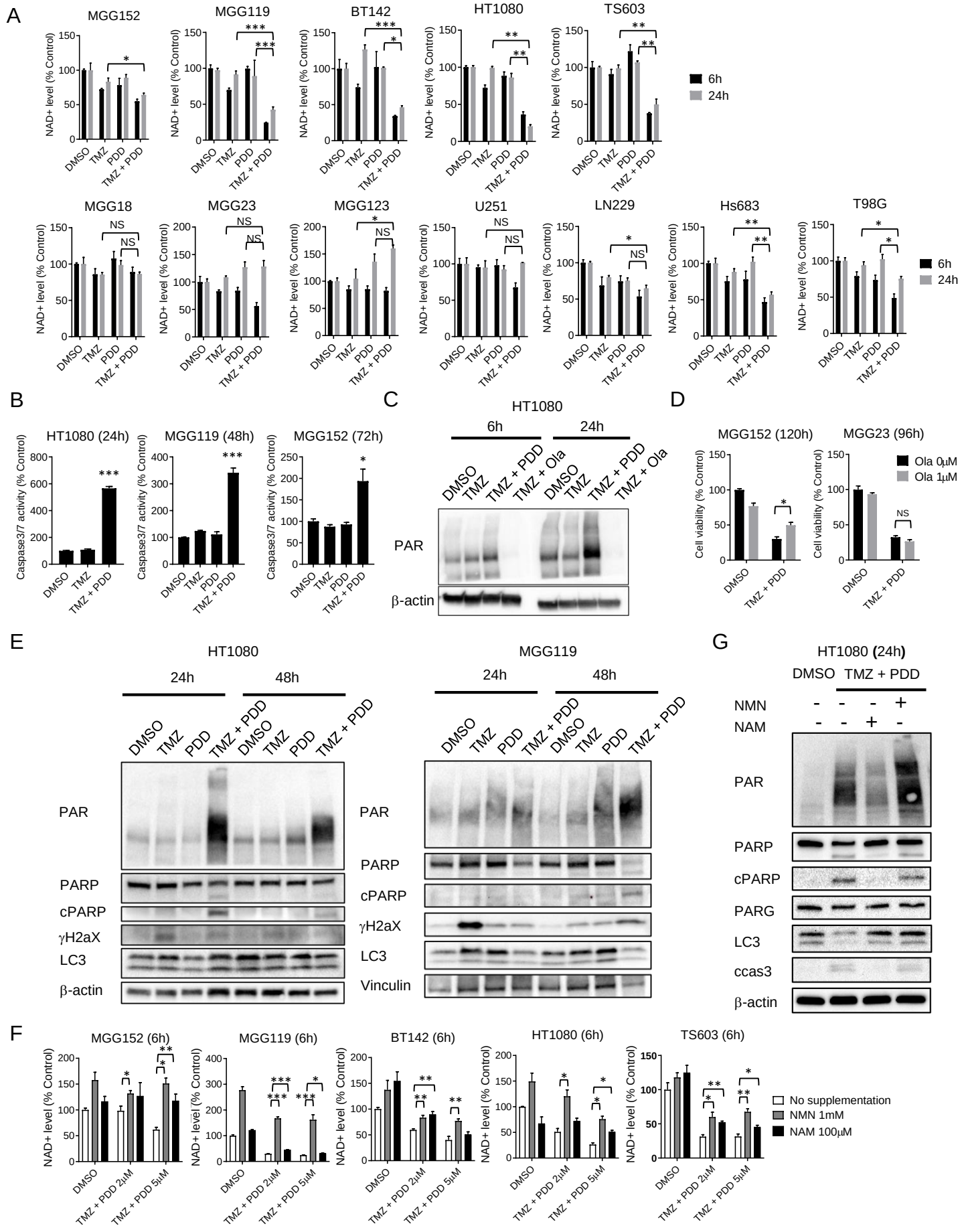
C. Western blot analysis of PARG in parental, non-targeting (NT), PARG gRNA #2 and #3-transduced HT1080 cells. Vinculin, loading control.

D. Quantitation of basal NADH levels in non-targeting (NT), PARG gRNA #2 and #3-transduced HT1080 cells. Bars, ± SEM; ***$p<0.001$.

E. Relative cell viability after 120-hour exposure to TMZ at indicated concentrations in NT, PARG gRNA #2 and #3-transduced U251 and TS603 cells.

F. Cell growth assay of NT, PARG gRNA #2 and #3–transduced HT1080 cells.

Supplementary Figure S3



Supplementary Figure S3 (Related to Figure 3)

A. Relative NAD+ levels in IDH1-mutant (upper row) and IDH wild-type (bottom row) lines with DMSO, temozolomide (TMZ, 200 μM), PDD00017273 (PDD, 5 μM), or TMZ plus PDD at 6 and 24 hours. Bars, ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001.

B. Caspase 3/7 activity assay with DMSO, TMZ (200 μM), PDD (5 μM), or TMZ plus PDD at 24 - 72 hours in HT1080, MGG119 and MGG152. In all panels: Bars, ± SEM; *p < 0.05, *** p < 0.01 for difference from TMZ alone.

C. Western blot analysis of PAR in HT1080 treated with DMSO, TMZ (200 μM), TMZ plus PDD (2 μM) or TMZ plus olaparib (Ola, 1 μM) for 6 and 24 hours. β-actin, loading control.

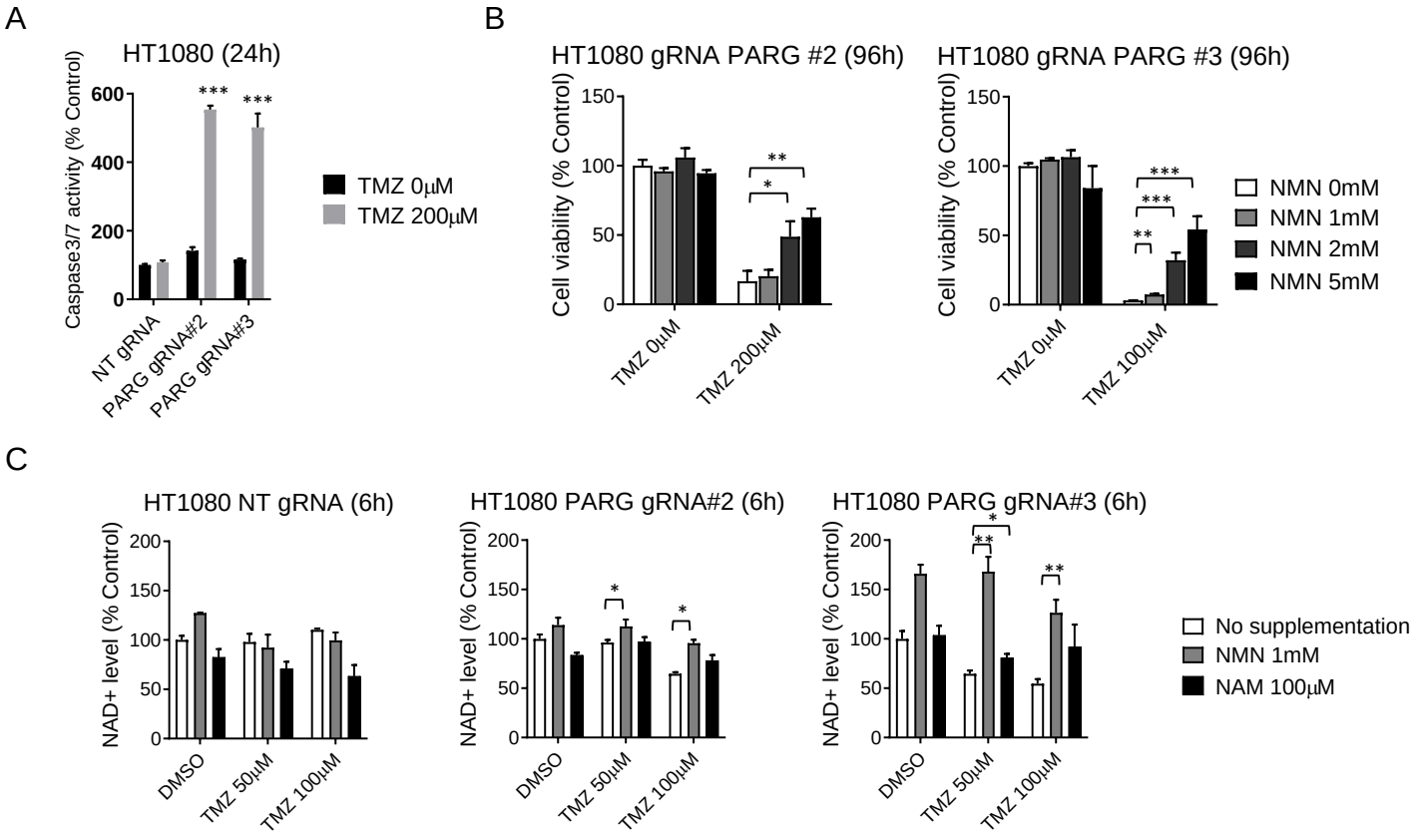
D. Relative cell viability of MGG152 (IDH-mutant) and MGG23 (IDH wild-type) cells after 96 or 120 hours exposure to DMSO or TMZ (200 μM) plus PDD (5 μM) with or without 1μM Ola. Bars, ± SEM; *p < 0.05

E. Western blot analysis of PAR, PARP, cleaved PARP, γH2AX and LC3 in HT1080 and MGG119 cells treated with DMSO, TMZ (200 μM), PDD (5 μM), and TMZ plus PDD for 24 and 48 hours. β-actin and vinculin are loading controls.

F. Relative NAD+ level of IDH mutant cells after 6h exposure to DMSO, TMZ (200 μM) plus PDD (2 μM), or TMZ (200 μM) plus PDD (5 μM) treated with no supplementation, NMN (1 mM), or NAM (100 μM). Bars, ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001.

G. Western blot analysis of PAR, PARP, cleaved PARP, PARG, LC3 and cleaved caspase3 in HT1080 cells treated with DMSO, and TMZ (200 μM) plus PDD (5 μM) with or without NMN (1 mM) or NAM (100 μM) for 24 hours. β-actin is loading control.

Supplementary Figure S4



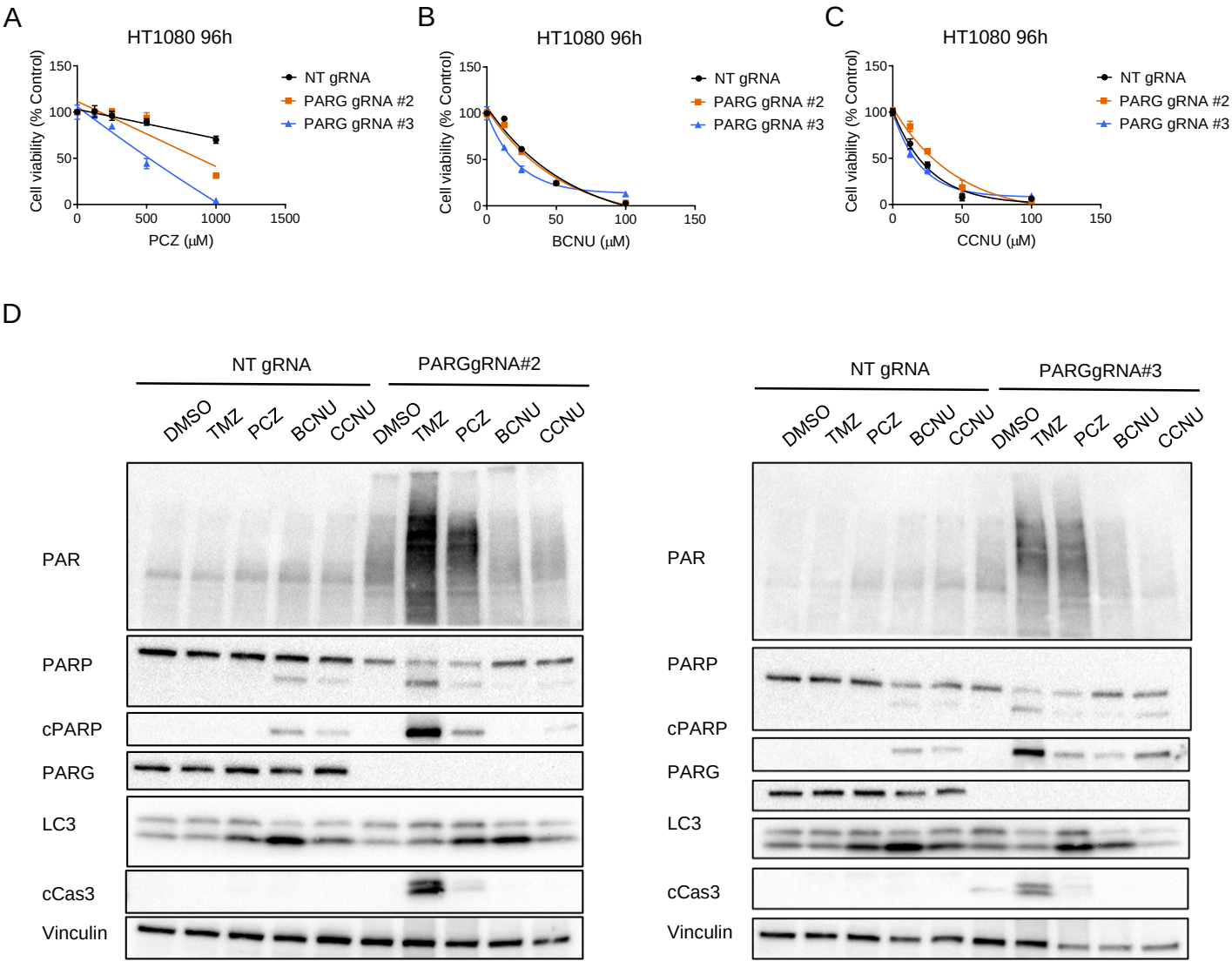
Supplementary Figure S4 (Related to Figure 3)

A. Caspase 3/7 assay with DMSO and temozolomide (TMZ, 200 μM) at 24 hours in non-targeting (NT), PARG gRNA #2 and #3–transduced HT1080 cells. Bars, ± SEM; ***p < 0.001 for difference from TMZ 0μM.

B. Relative cell viability of PARG gRNA #2 and #3-transduced HT1080 treated with TMZ (0, 100 or 200μM as indicated) with NMN at indicated concentrations. Bars, ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001.

C. Relative NAD+ level of HT1080 NT, PARG gRNA #2 and #3 cells after 6h exposure to DMSO, TMZ (50 μM or 100 μM) treated with no supplementation, NMN (1 mM), or NAM (100 μM). Bars, ± SEM; *p < 0.05, **p < 0.01.

Supplementary Figure S5

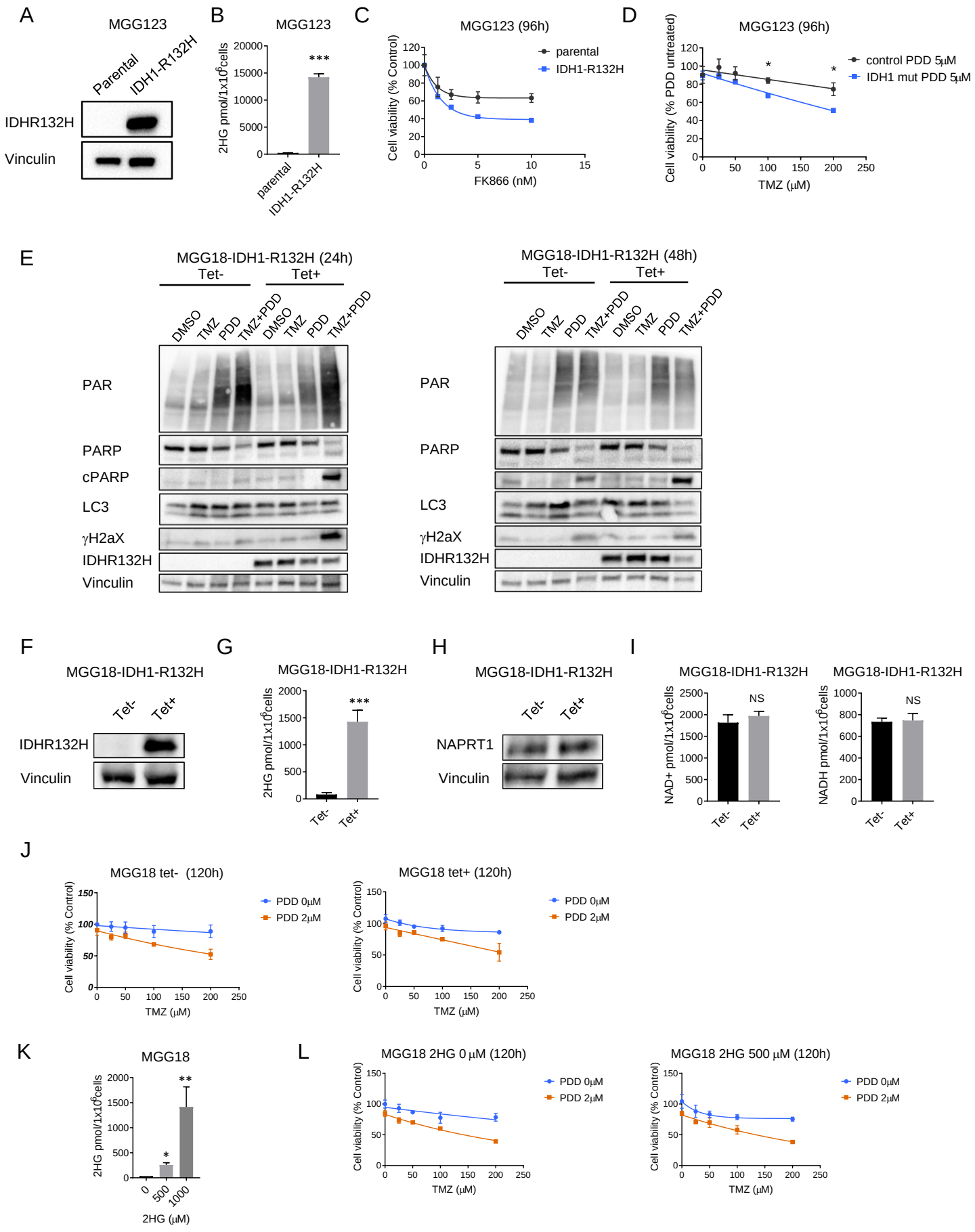


Supplementary Figure S5 (Related to Figure 3)

A-C. Relative cell viability after 96-hour exposure to procarbazine (PCZ, A), carmustine (BCNU, B) and lomustine (CCNU, C) at indicated concentrations in non-targeting (NT), PARG gRNA #2 and #3-transduced HT1080 cells.

D. Western blot analysis of PAR, PARP, cleaved PARP (cPARP), PARG, LC3 and cleaved caspase 3 (cCas3) in NT, PARG gRNA #2 and #3–transduced HT1080 cells treated with DMSO, temozolomide (TMZ, 200 μM), PCZ (500 μM), BCNU (25 μM) and CCNU (25 μM) for 24 hours. Vinculin is loading control.

Supplementary Figure S6



Supplementary Figure S6 (Related to Figure 4)

A. Western blot analysis of IDH1R132H stably-transfected MGG123 cells (at 3 months). Vinculin, loading control.

B. Quantification of 2-HG levels of MGG123 parental and IDH1R132H-MGG123 cells. ***p < 0.001.

C. Relative cell viability of parental and IDH1R132H-MGG123 treated with FK866.

D. Relative cell viability of parental and IDH1R132H-MGG123 treated with temozolomide (TMZ) and PDD00017273 (PDD). Parental and IDH1R132H-MGG123 were each compared to PDD untreated. *p < 0.05 for difference between parental and IDH1R132H-MGG123.

E. Western blot analysis of PAR, PARP, cleaved PARP, LC3, γH2AX and IDH1R132H in MGG18-IDH1-R132H cells (Tet - and Tet +) treated with DMSO, TMZ (200 μM), PDD (2 μM), or TMZ plus PDD for 24 and 48 hours. Vinculin is loading control.

F. Western blot analysis of IDH1R132H in tet-inducible MGG18-IDH1-R132H cells with or without 6-day exposure to 1 μg/mL doxycycline (Tet). Vinculin is loading control.

G. Quantification of 2-HG levels with or without 6-day exposure to Tet in MGG18-IDH1-R132H cells. ***p < 0.001.

H. Western blot analysis of NAPRT1 in MGG18-IDH1-R132H cells with or without 6-day Tet. Vinculin is loading control.

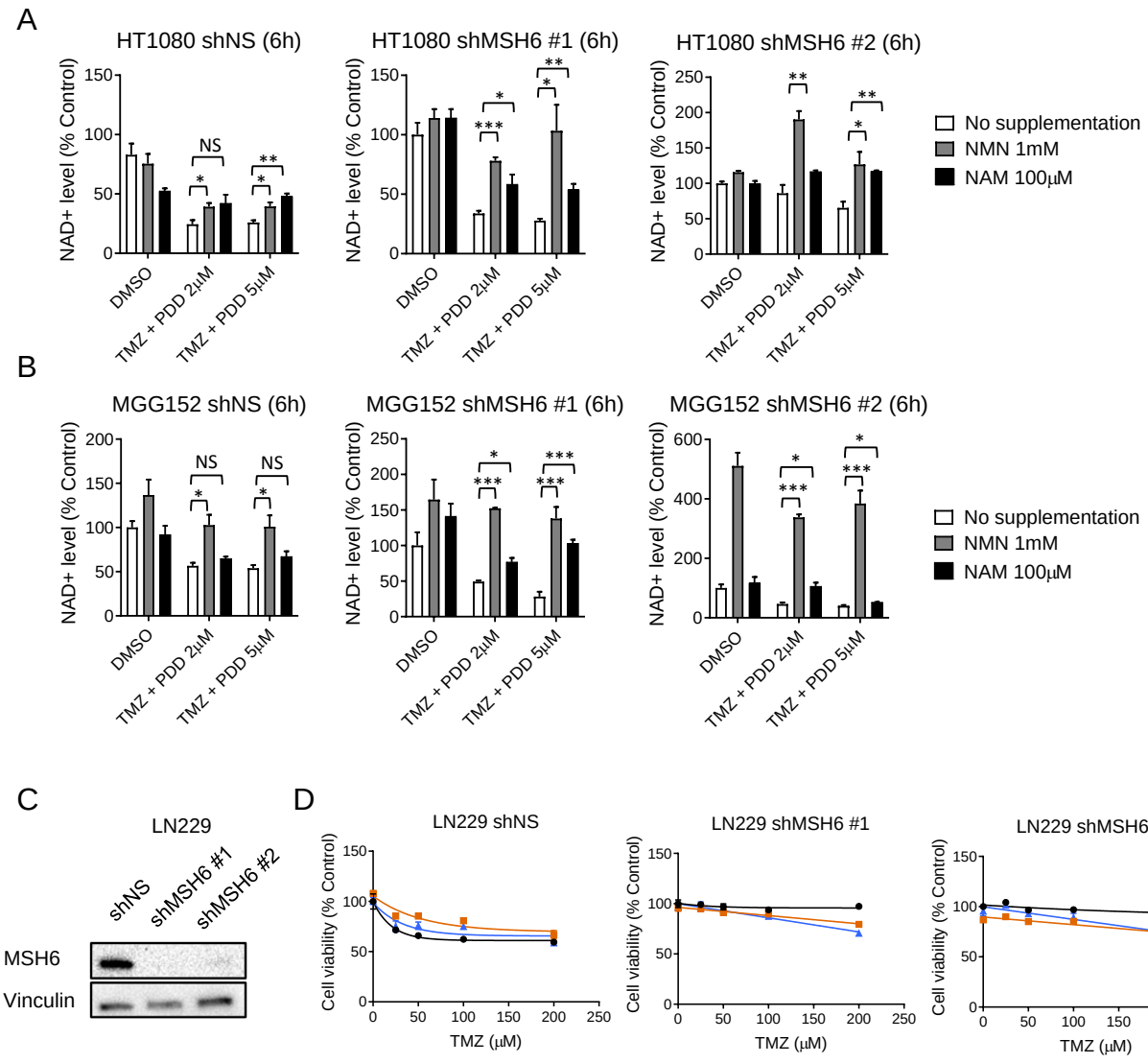
I. Quantitative NAD+ and NADH levels in MGG18-IDH1-R132H cells with or without 6-day Tet. NS, not significant.

J. Relative cell viability of MGG18-IDH1-R132H cells after 120-hour exposure to PDD (0 or 2 μM) with TMZ at indicated concentrations. Tet was used for 6 days in MGG18-IDH1-R132H line.

K. Quantification of 2-HG levels with octyl-2HG (0, 500 or 1000 μM) in parental MGG18 cells. *p < 0.05, **p < 0.01.

L. Relative cell viability of MGG18 cells with or without octyl-2HG (500 μM) after 120-hour exposure to PDD (0 or 2 μM) and TMZ at indicated concentrations.

Supplementary Figure S7



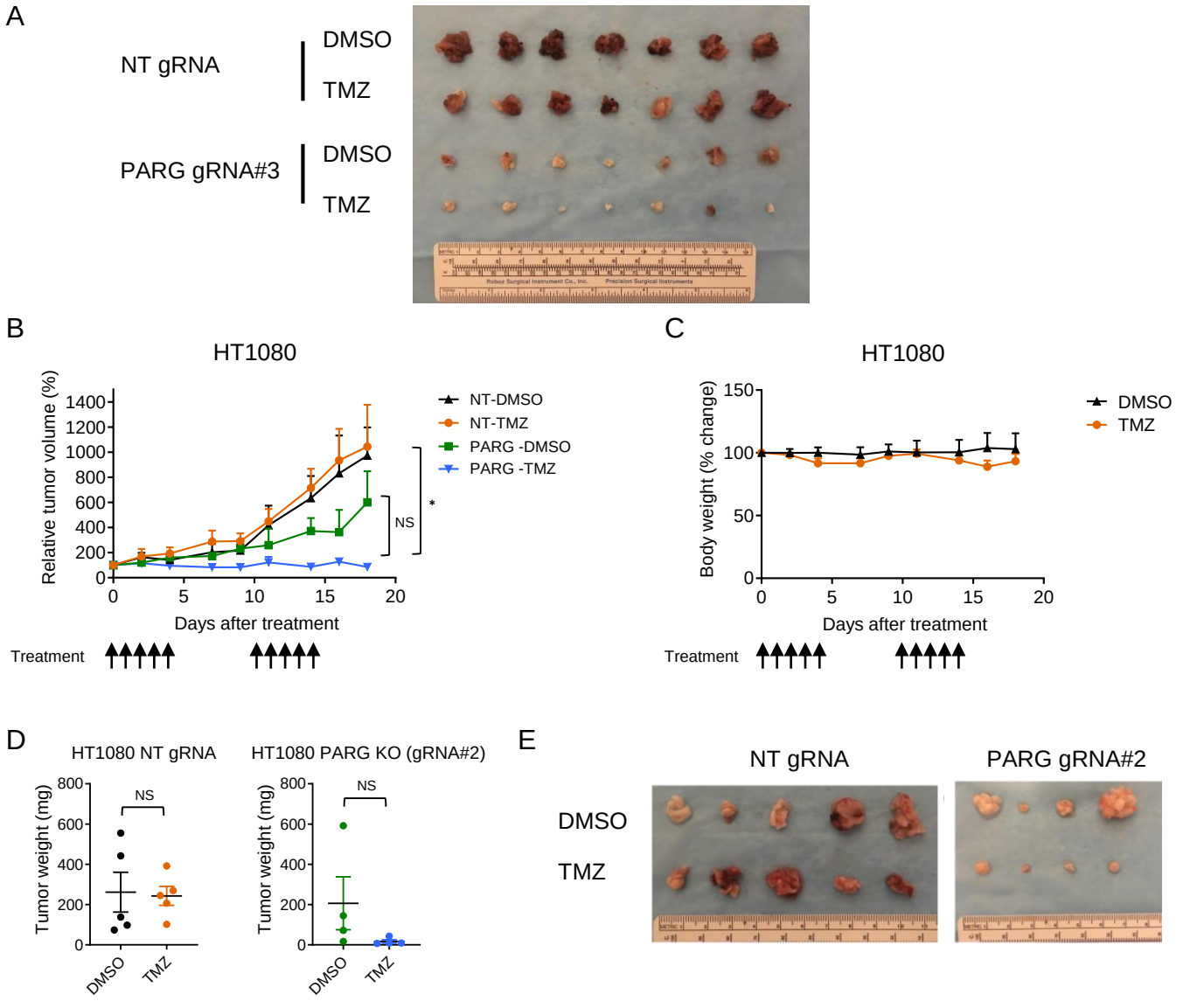
Supplementary Figure S7 (Related to Figure 5)

A. B. Relative NAD+ level in NS (non-silencing), MSH6 shRNA #1 and #2 HT1080 (A) cells and MGG152 (B) after 6h exposure to DMSO, temozolomide (TMZ, 200 μM) plus PDD00017273 (PDD, 2 μM), and TMZ, 200 μM plus PDD (5 μM) with no supplementation, NMN (1 mM), or NAM (100 μM). Bars, ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001.

C. Western blot analysis of MSH6 in NS and MSH6 shRNA (#1, 2)-transduced LN229 (IDH wild-type). Vinculin is loading control.

D. Relative cell viability of shNS, MSH6 shRNA #1 and #2 transduced LN229 cells after 120-hour exposure to PDD (0, 2, or 5 μM) and TMZ at indicated concentrations.

Supplementary Figure S8



Supplementary Figure S8 (Related to Figure 6)

A. Photographs of non-targeting (NT) gRNA and PARG gRNA#3 transduced tumors at day 18 after DMSO or temozolomide (TMZ) treatment.

B. Tumor growth curves in NT gRNA and PARG knockout (gRNA #2, PARG KO) HT1080 flank xenograft model. Animals were treated with DMSO (NT) or temozolomide (TMZ) (50 mg/kg i.p., 5 days/week x 2 cycles). Data are presented as mean tumor volume and SEM in each group. One PARG KO tumor in each group did not develop and was excluded. Arrows, time points when treatment doses were given. *p = 0.0452, TMZ in NT vs TMZ in PARG KO; NS, not significant (p = 0.1268), DMSO vs TMZ in PARG KO tumors on day 18, unpaired t test.

C. Mean body weight of mice with flank HT1080 tumors for each treatment groups (same experiment as B). Bars are +/- SEM. Mouse weight was measured three days a week. Arrows, the time points when treatment doses were given.

D. Weight of NT gRNA and PARG KO (gRNA #2) HT1080 tumors. Data are presented as mean tumor volume +/- SEM in each group. NS, p = 0.2443, DMSO vs TMZ in PARG KO tumors on day 18 (unpaired t test).

E. Photographs of NT gRNA and PARG gRNA#2 transduced tumors at day 18 after DMSO or TMZ treatment.