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Supplemental information

A kinome-wide CRISPR screen identifies CK1α as a target to overcome enzalutamide resistance of prostate cancer

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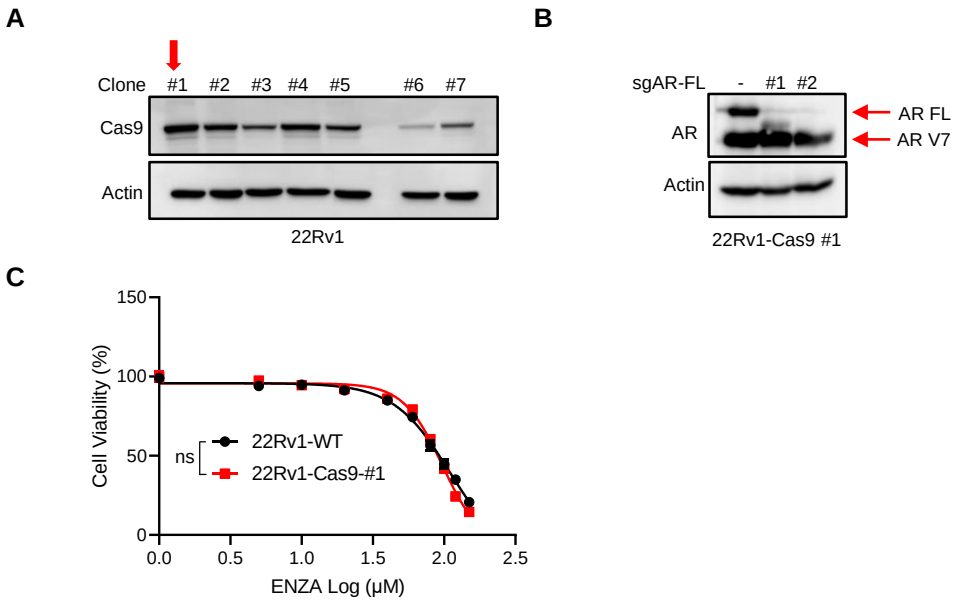
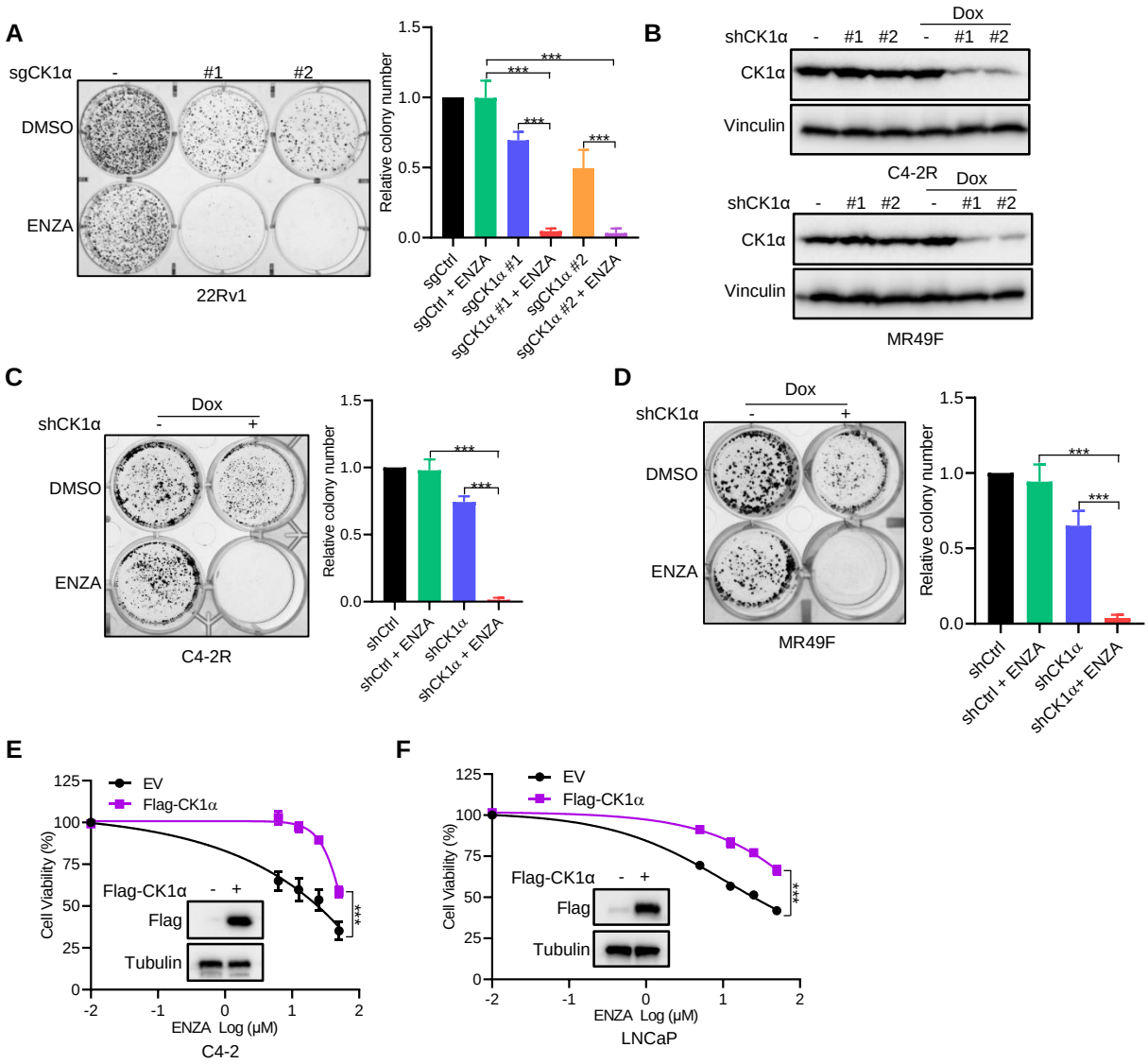


Supplementary Figure S1. Generation of a stable cell line expressing Cas9 in 22Rv1 cells, Related to Figure 1

(**A**) IB analysis of WCL derived from various clones of 22Rv1 cells stably expressing Cas9 (22Rv1-Cas9).

(**B**) IB analysis of WCL derived from 22Rv1-Cas9 #1 in (A) expressing sgCtrl or two sgRNAs against AR-full length (AR-FL).

(**C**) *In vitro* proliferation of 22Rv1-Cas9 #1 in (A) or parental 22Rv1 cells was detected by AquaBluer assay after the indicated concentrations of ENZA treatment for 72 hrs.



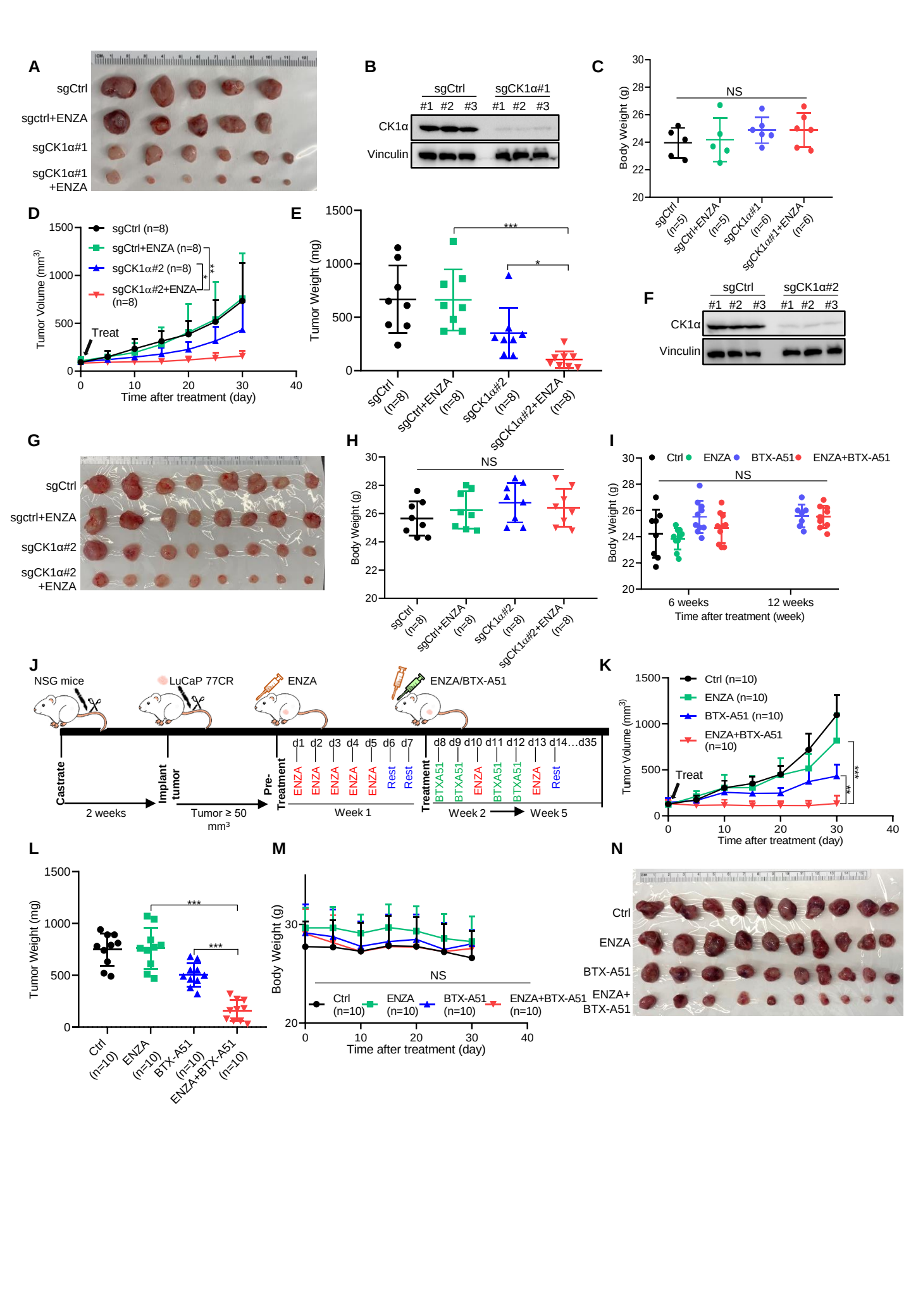
Supplementary Figure S2. CK1α is a therapeutic target to overcome ENZA resistance, Related to Figure 2

(**A**) *In vitro* proliferation of 22Rv1 cells expressing sgCtrl or two sgRNAs against CK1α was determined by colony formation assay after 20 μM ENZA treatment for 3 weeks. The colonies were analyzed and quantified by Image J.

(**B**) IB analysis of WCL of C4-2R or MR49F cells expressing shCtrl or shCK1α treated with or without 100 ng ml^{-1} Dox for 48 hrs.

(**C** and **D**) Colony formation assay of C4-2R (C) or MR49F (D) cells expressing shCtrl or shCK1α were pre-treated with 100 ng ml^{-1} Dox for 48 hrs to induce CK1α knockdown and then treated with 20 μM ENZA for 3 weeks. The colonies were analyzed and quantified by Image J.

(**E** and **F**) *In vitro* proliferation of C4-2 (E) or LNCaP (F) cells stably expressing empty vector (EV) or Flag-CK1α was determined by AquaBluer assay after treatment for 72 hrs with the indicated concentrations of ENZA. Insets: Immunoblot analysis of whole cell lysates from C4-2 (E) or LNCaP (F) cells expressing EV or Flag-CK1α.


A
sgCtrl
sgctrl+ENZA
sgCK1α#1
sgCK1α#1 +ENZA
B
sgCtrl
sgCK1α#1
#1 #2 #3 #1 #2 #3
CK1α
Vinculin
C
Body Weight (g)
NS
sgCtrl (n=5)
sgCtrl+ENZA (n=5)
sgCK1α#1 (n=6)
sgCK1α#1+ENZA (n=6)
D
sgCtrl (n=8)
sgCtrl+ENZA (n=8)
sgCK1α#2 (n=8)
sgCK1α#2+ENZA (n=8)
Tumor Volume (mm3)
Treat
Time after treatment (day)
E
Tumor Weight (mg)
sgCtrl (n=8)
sgCtrl+ENZA (n=8)
sgCK1α#2 (n=8)
sgCK1α#2+ENZA (n=8)
F
sgCtrl
sgCK1α#2
#1 #2 #3 #1 #2 #3
CK1α
Vinculin
G
sgCtrl
sgctrl+ENZA
sgCK1α#2
sgCK1α#2 +ENZA
H
Body Weight (g)
NS
I
Ctrl
ENZA
BTX-A51
ENZA+BTX-A51
NS
6 weeks
12 weeks
Time after treatment (week)
J
NSG mice
LuCaP 77CR
ENZA
ENZA/BTX-A51
Castrate
2 weeks
Implant tumor
Tumor ≥ 50 mm3
Pre-Treatment
d1 d2 d3 d4 d5 d6 d7
ENZA ENZA ENZA ENZA ENZA Rest Rest
Week 1
Treatment
d8 d9 d10 d11 d12 d13 d14…d35
BTXA51 BTXA51 ENZA BTXA51 BTXA51 ENZA Rest
Week 2
Week 5
K
Ctrl (n=10)
ENZA (n=10)
BTX-A51 (n=10)
ENZA+BTX-A51 (n=10)
Tumor Volume (mm3)
Treat
Time after treatment (day)
L
Tumor Weight (mg)
Ctrl (n=10)
ENZA (n=10)
BTX-A51 (n=10)
ENZA+BTX-A51 (n=10)
M
Body Weight (g)
NS
Ctrl (n=10)
ENZA (n=10)
BTX-A51 (n=10)
ENZA+BTX-A51 (n=10)
Time after treatment (day)
N
Ctrl
ENZA
BTX-A51
ENZA+ BTX-A51

Supplementary Figure S3. Targeting CK1α to overcome ENZA resistance, Related to Figure 2

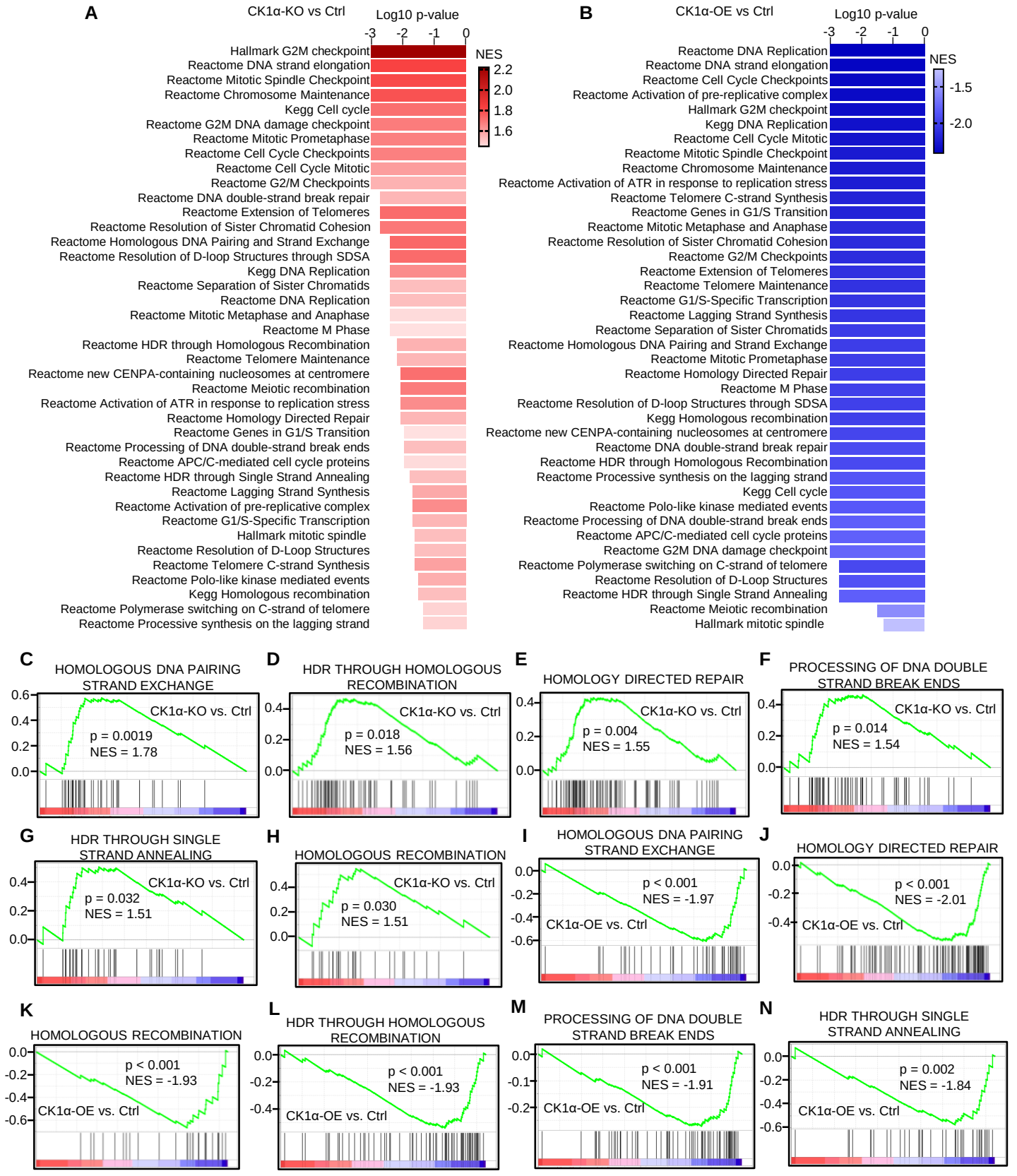
(**A** and **C**) *In vivo* 22Rv1 (sgCtrl or sgCK1α#1) xenograft assay. Mice with 22Rv1 xenograft tumors were treated with vehicle or ENZA for 5 weeks. Tumors were harvested and photographed (A) and body weights (C) were shown as mean ± SEM.

(**B** and **F**) Immunoblot analysis of whole cell lysates from 22Rv1 xenograft tumors (n=3 for each group).

(**D**-**H**) *In vivo* 22Rv1 (sgCtrl or sgCK1α#2) xenograft assay. 22Rv1 (sgCtrl or sgCK1α#2) cells were injected into the flanks of pre-castrated male nude mice (n=8 for each group). When the tumors reached 100 mm^3, the mice were treated with vehicle or ENZA (20 mg kg^{-1} by oral gavage daily, 5 days on, and 2 days off for 4 weeks). Tumor volume (D) and tumor weight (E) were shown as mean ± SEM. Tumors were harvested and photographed (G) and body weights (H) were shown as mean ± SEM.

(**I**) Body weight of mice from *in vivo* xenograft of LuCaP 35CR PDX was shown as mean ± SEM.

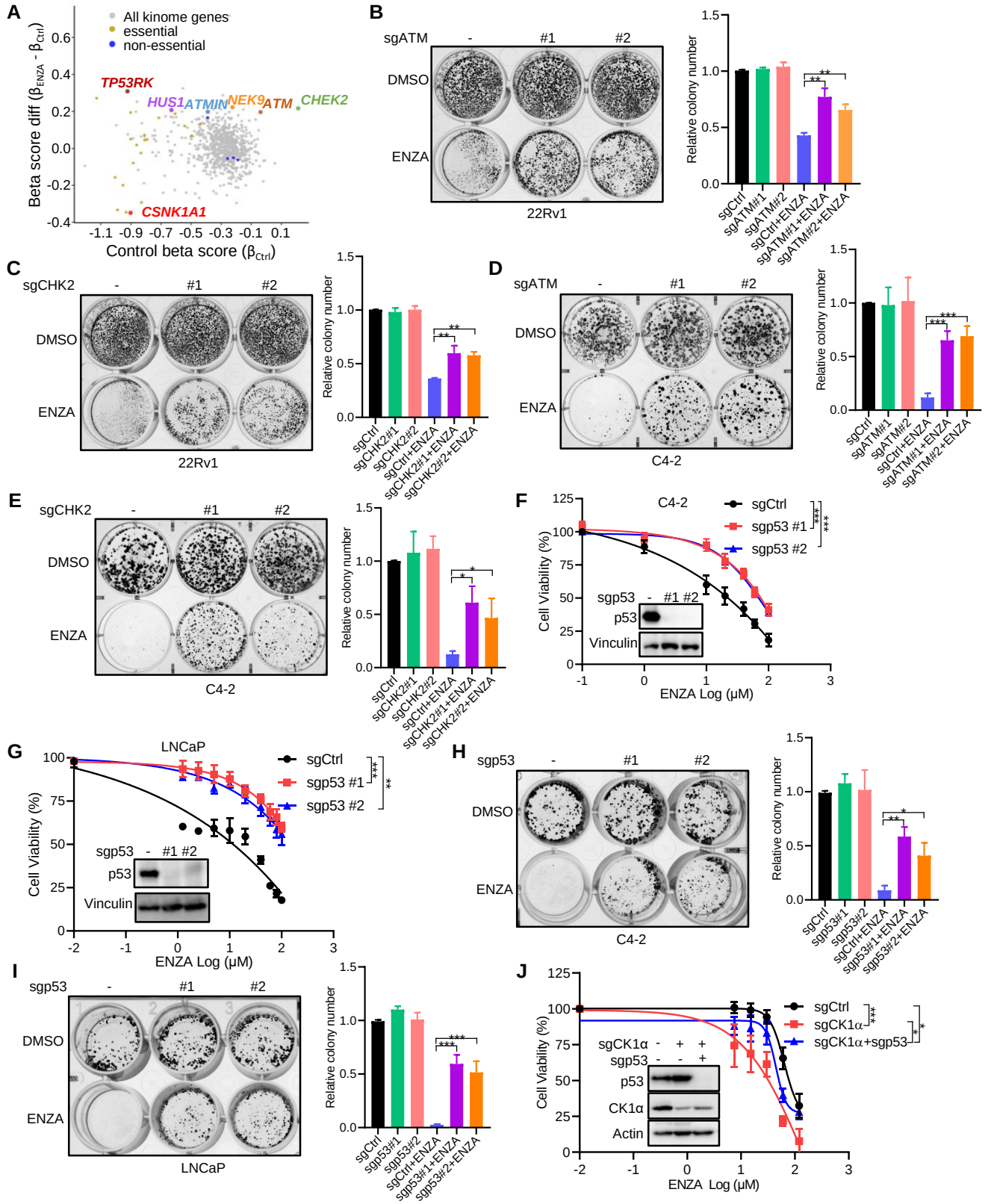
(**J**-**N**) *In vivo* LuCaP 77CR PDX xenograft assay (n=10 for each group). (J) A schema showing the experiment design of LuCaP 77CR PDX xenograft. LuCaP 77CR tumor bits were implanted into the flanks of pre-castrated NSG mice. When the tumors reached 50 mm^3, the mice were pre-treated with ENZA (50 mg kg^{-1}, by oral gavage daily, 5 days on, 2 days off, for one week) and then treated with Vehicle or BTX-A51 (5 mg kg^{-1}, by oral gavage, two days on and one day off) or ENZA (20 mg kg^{-1}, by oral gavage every 3 days) or the combination for the indicated time. Tumor volume (K), tumor weight (L) and body weight (M) were shown as mean ± SEM and tumors were photographed (N).



Supplementary Figure S4. CK1α regulates DNA damage response signaling, Related to Figure 3

(**A** and **B**) Bar plots showing that gene sets related to DNA damage-response (DDR) are positively regulated in CK1α KO versus Ctrl (A) but are negatively regulated in CK1α OE versus Ctrl (B). p = 0.001 if p value is less than or equal to 0.001. NES, normalized enrichment score.

(**C-N**) GSEA showing the enrichment of DSB response-related gene sets in CK1α KO versus Ctrl (C-H) or CK1α OE versus Ctrl (I-N).


A
All kinome genes
essential
non-essential
TP53RK
HUS1
ATMIN
NEK9
ATM
CHEK2
CSNK1A1
Beta score diff (βENZA - βCtrl)
Control beta score (βCtrl)
B
sgATM
DMSO
ENZA
22Rv1
Relative colony number
sgCtrl
sgATM#1
sgATM#2
sgCtrl+ENZA
sgATM#1+ENZA
sgATM#2+ENZA
C
sgCHK2
22Rv1
sgCHK2#1
sgCHK2#2
sgCHK2#1+ENZA
sgCHK2#2+ENZA
D
sgATM
C4-2
E
sgCHK2
C4-2
F
C4-2
sgCtrl
sgp53 #1
sgp53 #2
Cell Viability (%)
ENZA Log (μM)
sgp53
p53
Vinculin
G
LNCaP
H
sgp53
I
sgp53
LNCaP
sgp53#1
sgp53#2
sgCtrl+ENZA
sgp53#1+ENZA
sgp53#2+ENZA
J
sgCK1α
sgCK1α+sgp53
CK1α
Actin

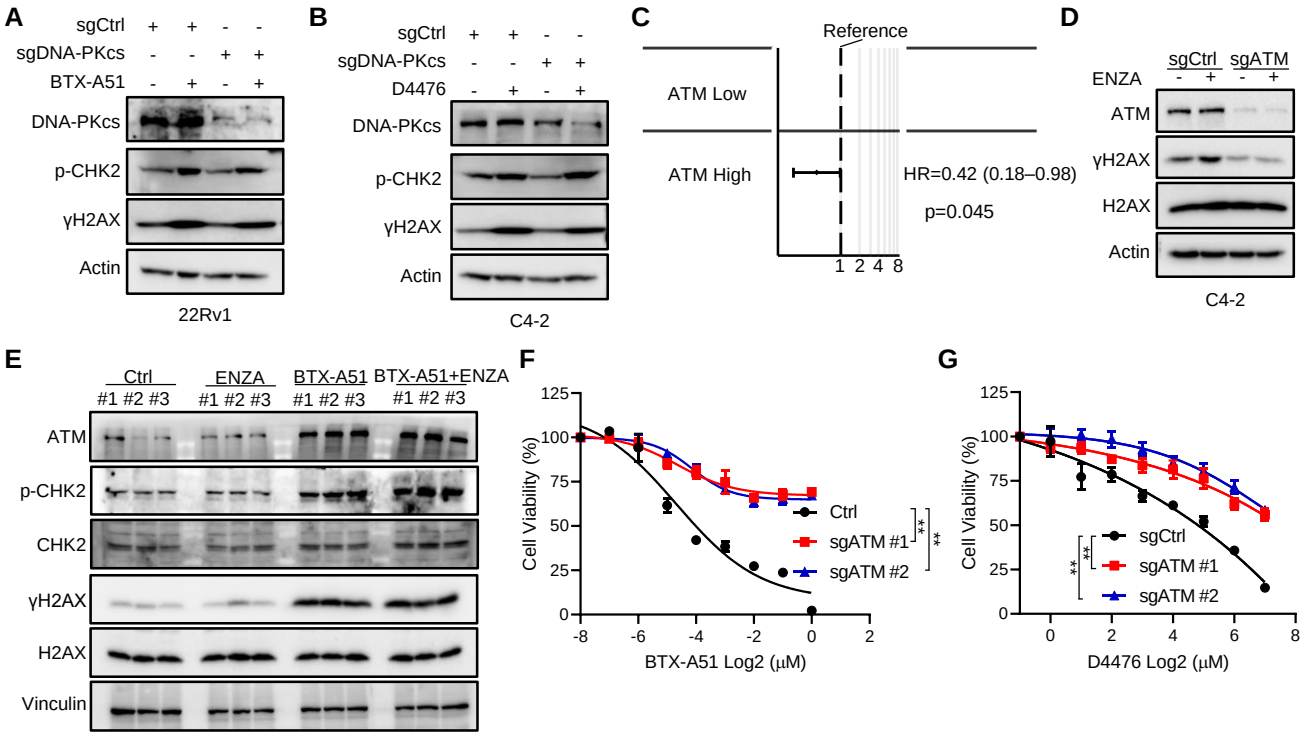
Supplementary Figure S5. DSB signaling is related to ENZA response, Related to Figure 4

(**A**) Scatter plot showing gene differential beta score (subtracting the control beta score from the treatment beta score) versus gene beta score in control samples. Whereas *CSNK1A1* is the top negatively selected gene that mediates sensitivity to ENZA, *CHK2*, *ATM*, *NEK9*, *ATMIN*, *HUS1*, and *TP53RK* are the top positively selected genes that mediate resistance to ENZA. Genes in the kinome library that overlap with essential genes and non-essential genes previously reported are highlighted in yellow and blue, respectively.

(**B**-**E**) Colony formation assay of 22Rv1 cells (B and C) or C4-2 cells (D and E) expressing sgCtrl or two sgRNAs against ATM (B and D) or CHK2 (C and E) after ENZA (60 μM for 22Rv1 and 5 μM for C4-2) treatment for 3 weeks. The colonies were analyzed and quantified by Image J.

(**F**-**I**) *In vitro* proliferation of C4-2 (F and H) or LNCaP (G and I) cells expressing sgCtrl or two sgRNAs against p53 was determined by AquaBluer assay (F and G) after treatment for 72 hrs with the indicated concentrations of ENZA or colony formation assay (H and I) after treatment with 5 μM ENZA for 3 weeks. The colonies were analyzed and quantified by Image J.

(**J**) *In vitro* proliferation of 22Rv1 cells expressing sgCtrl or sgCK1α or sgCK1α and sgp53 was determined by AquaBluer assay after treatment for 72 hrs with the indicated concentrations of ENZA. Inset: Immunoblot analysis of whole cell lysates from 22Rv1 cells expressing sgCtrl or sgCK1α or sgCK1α and sgp53.



Supplementary Figure S6. ATM is involved in the ENZA response, Related to Figure 6

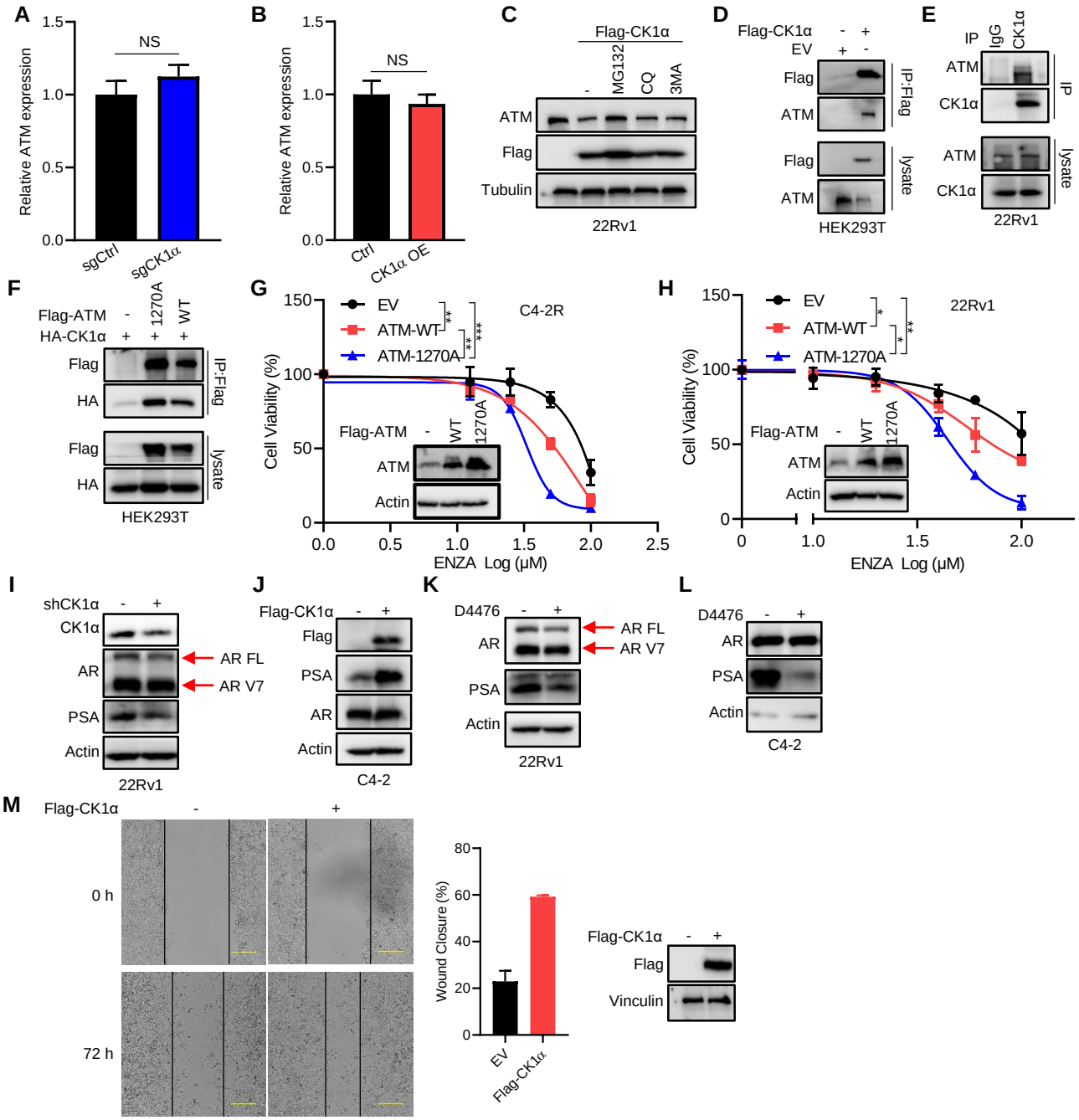
(**A** and **B**) IB analysis of WCL from 22Rv1 (A) or C4-2 (B) cells expressing sgCtrl or sgRNA against DNA-PKcs after treatment with 100 nM BTX-A51 (A) or 20 μM D4476 (B) for 24 hrs.

(**C**) Forest plot associated with the statistics based on Cox proportional hazards model to show ATM-high group displays longer efficacy of ENZA treatment compared to the group presenting with ATM-low levels (dichotomized as high versus low with 75% and 25% quantiles chosen as the cutoff). HR, hazard ratio.

(**D**) IB analysis of WCL from C4-2 cells expressing sgCtrl or sgATM after treatment with 40 μM ENZA for 24 hrs.

(**E**) IB analysis of WCL derived from LuCap 35CR PDX xenografts. n=3 for each group.

(**F** and **G**) *In vitro* proliferation of 22Rv1 cells expressing sgCtrl or two sgRNAs against ATM was determined by AquaBluer assay after treatment for 72 hrs with the indicated concentrations of BTX-A51 (F) or D4476 (G).



Supplementary Figure S7. CK1α regulates ATM stability, Related to Figure 7

(**A** and **B**) Normalized ATM mRNA level from the RNA sequencing of 22Rv1 cells in the indicated groups: sgCtrl versus sgCK1α (A), Ctrl versus CK1α OE (B).

(**C**) 22Rv1 cells stably expressing EV or Flag-CK1α were treated with DMSO or 20 μM MG132 or 2 mM 3-methyladenine (3-MA) or 50 μM chloroquine (CQ) for 12 hrs and harvested for IB.

(**D**) HEK293T cells were transfected with Flag-CK1α or EV, and harvested for anti-Flag IP, followed by IB.

(**E**) IB of anti-CK1α immunoprecipitates and WCL derived from 22Rv1 cells.

(**F**) HEK293T cells were transfected with HA-CK1α and Flag-ATM (WT or 1270A), and harvested for anti-Flag IP, followed by IB.

(**G** and **H**) *In vitro* proliferation of C4-2R (G) or 22Rv1 (H) cells transfected with empty vector (EV) or Flag-ATM (WT or 1270A) was determined by AquaBluer assay after treatment for 72 hrs with the indicated concentrations of ENZA. Insets: IB analysis of whole cell lysates from C4-2R (G) or 22Rv1 (H) cells transfected with EV or Flag-ATM (WT or 1270A).

(**I** and **J**) IB analysis of whole cell lysates from 22Rv1 cells expressing shCtrl or shCK1α (I) or C4-2 cells expressing EV or Flag-CK1α (J).

(**K** and **L**) IB analysis of whole cell lysates from 22Rv1 (K) or C4-2 (L) cells treated with 20 μM D4476 for 24 hrs.

(**M**) Wound scratch assay of C4-2B cells expressing EV or Flag-CK1α, Scale bar, 500 μm. Wound closure was calculated and quantified by GraphPad. IB analysis of whole cell lysates from C4-2B cells expressing EV or Flag-CK1α.

Supplementary Table S2. Gene sets enriched in both CK1α-knockout and CK1α-overexpressing cells, Related to Figure 3

	CK1α KO vs. Ctrl up			CK1α OE vs. Ctrl down		
Gene set	**ES**	**NES**	**NOM p-val**	**ES**	**NES**	**NOM p-val**
HALLMARK_E2F_TARGETS	0.58	2.25	0	-0.66	-2.75	0
HALLMARK_G2M_CHECKPOINT	0.56	2.22	0	-0.55	-2.33	0
HALLMARK_MITOTIC_SPINDLE	0.35	1.35	0.022	-0.3	-1.26	0.049
HALLMARK_MYC_TARGETS_V1	0.36	1.42	0.002	-0.56	-2.32	0
KEGG_CELL_CYCLE	0.47	1.72	0	-0.49	-1.86	0
KEGG_DNA_REPLICATION	0.56	1.65	0.004	-0.75	-2.3	0
KEGG_HOMOLOGOUS_RECOMBINATION	0.54	1.53	0.033	-0.67	-1.99	0
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	0.41	1.49	0.006	-0.36	-1.38	0.011
REACTOME_ACTIVATION_OF_ATR_IN_RESPONSE_TO_REPLICATION_STRESS	0.55	1.65	0.008	-0.7	-2.22	0
REACTOME_ACTIVATION_OF_THE_PRE_REPLICATIVE_COMPLEX	0.56	1.65	0.02	-0.79	-2.35	0
REACTOME_APC_C_MEDIATED_DEGRADATION_OF_CELL_CYCLE_PROTEINS	0.42	1.46	0.012	-0.47	-1.79	0
REACTOME_CELL_CYCLE_CHECKPOINTS	0.42	1.68	0	-0.55	-2.37	0
REACTOME_CELL_CYCLE_MITOTIC	0.38	1.61	0	-0.5	-2.29	0
REACTOME_CHROMOSOME_MAINTENANCE	0.52	1.79	0	-0.59	-2.24	0
REACTOME_DEPOSITION_OF_NEW_CENPA_CONTAINING_NUCLEOSOMES_AT_THE_CENTROMERE	0.61	1.72	0.008	-0.65	-1.96	0
REACTOME_DNA_DOUBLE_STRAND_BREAK_REPAIR	0.42	1.55	0.002	-0.54	-1.92	0
REACTOME_DNA_REPLICATION	0.42	1.53	0.004	-0.62	-2.41	0
REACTOME_DNA_STRAND_ELONGATION	0.63	1.83	0	-0.78	-2.38	0
REACTOME_EXTENSION_OF_TELOMERES	0.55	1.73	0.002	-0.62	-2.09	0
REACTOME_FORMATION_OF_THE_CORNIFIED_ENVELOPE	0.64	1.96	0	-0.53	-1.73	0.005
REACTOME_G1_S_SPECIFIC_TRANSCRIPTION	0.55	1.55	0.021	-0.7	-2.07	0
REACTOME_G2_M_CHECKPOINTS	0.42	1.56	0	-0.54	-2.12	0
REACTOME_G2_M_DNA_DAMAGE_CHECKPOINT	0.51	1.69	0	-0.51	-1.76	0
REACTOME_HDR_THROUGH_HOMOLOGOUS_RECOMBINATION_HRR	0.46	1.56	0.006	-0.54	-1.93	0
REACTOME_HDR_THROUGH_SINGLE_STRAND_ANNEALING_SSA	0.5	1.53	0.017	-0.57	-1.8	0.002
REACTOME_HOMOLOGOUS_DNA_PAIRING_AND_STRAND_EXCHANGE	0.57	1.74	0.004	-0.61	-2.01	0
REACTOME_HOMOLOGY_DIRECTED_REPAIR	0.43	1.55	0.008	-0.53	-2.01	0
REACTOME_KERATINIZATION	0.63	1.97	0	-0.53	-1.72	0.002
REACTOME_LAGGING_STRAND_SYNTHESIS	0.61	1.58	0.019	-0.76	-2.06	0
REACTOME_M_PHASE	0.35	1.45	0.004	-0.45	-1.99	0
REACTOME_MEIOTIC_RECOMBINATION	0.62	1.69	0.008	-0.54	-1.55	0.031
REACTOME_MITOTIC_G1_PHASE_AND_G1_S_TRANSITION	0.38	1.45	0.011	-0.55	-2.18	0
REACTOME_MITOTIC_METAPHASE_AND_ANAPHASE	0.37	1.46	0.004	-0.5	-2.13	0
REACTOME_MITOTIC_PROMETAPHASE	0.43	1.69	0	-0.48	-2.01	0
REACTOME_MITOTIC_SPINDLE_CHECKPOINT	0.5	1.81	0	-0.59	-2.24	0
REACTOME_POLO_LIKE_KINASE_MEDIATED_EVENTS	0.63	1.57	0.029	-0.7	-1.84	0
REACTOME_POLYMERASE_SWITCHING_ON_THE_C_STRAND_OF_THE_TELOMERE	0.54	1.48	0.041	-0.68	-1.94	0.002
REACTOME_PROCESSING_OF_DNA_DOUBLE_STRAND_BREAK_ENDS	0.46	1.53	0.012	-0.45	-1.8	0
REACTOME_PROCESSIVE_SYNTHESIS_ON_THE_LAGGING_STRAND	0.6	1.48	0.044	-0.74	-1.86	0
REACTOME_RESOLUTION_OF_D_LOOP_STRUCTURES	0.52	1.53	0.023	-0.61	-1.88	0.002
REACTOME_RESOLUTION_OF_D_LOOP_STRUCTURES_THROUGH_SYNTHESIS_DEPENDENT_STRAND_ANN	0.63	1.73	0.004	-0.68	-1.99	0
REACTOME_RESOLUTION_OF_SISTER_CHROMATID_COHESION	0.46	1.7	0.002	-0.55	-2.13	0
REACTOME_RHO_GTPASE_EFFECTORS	0.31	1.26	0.026	-0.39	-1.65	0
REACTOME_RHO_GTPASES_ACTIVATE_FORMINS	0.41	1.54	0.004	-0.52	-2.04	0
REACTOME_SEPARATION_OF_SISTER_CHROMATIDS	0.4	1.53	0.004	-0.49	-2.02	0
REACTOME_TELOMERE_C_STRAND_LAGGING_STRAND_SYNTHESIS	0.55	1.6	0.026	-0.71	-2.18	0
REACTOME_TELOMERE_MAINTENANCE	0.47	1.55	0.006	-0.59	-2.08	0

	CK1α OE vs. Ctrl up			CK1α KO vs. Ctrl down		
Gene set	**ES**	**NES**	**NOM p-val**	**ES**	**NES**	**NOM p-val**
KEGG_ABC_TRANSPORTERS	0.59	1.71	0.002	-0.51	-1.46	0.035
KEGG_RETINOL_METABOLISM	0.61	1.76	0.005	-0.56	-1.58	0.006
KEGG_STARCH_AND_SUCROSE_METABOLISM	0.57	1.66	0.015	-0.64	-1.87	0.002
REACTOME_BIOLOGICAL_OXIDATIONS	0.44	1.6	0.003	-0.44	-1.64	0