Science Advances
AAAS

Supplementary Materials for

ATM deficiency confers specific therapeutic vulnerabilities in bladder cancer

Yuzhen Zhou et al.

Corresponding author: Kent W. Mouw, kent_mouw@dfci.harvard.edu

Sci. Adv. **9**, eadg2263 (2023)
DOI: 10.1126/sciadv.adg2263

The PDF file includes:

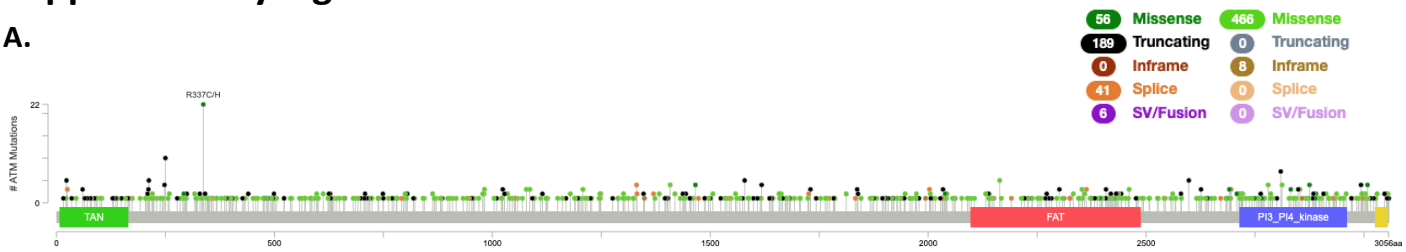
Figs S1 to S9
Legends for tables S1 to S5
References

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S5

Supplementary Figure 1

A.



B.

R337
R1466
R2832
N2875
I2888L
L2890
R3008

Supplementary Figure 1: ATM alterations in bladder cancer and other tumor types. A. Location of ATM mutations in The Cancer Genome Atlas (TCGA) pan-cancer cohort (n = 10,967 tumors). Alterations are color-coded by type. Data was accessed and visualized using the cBioPortal interface (www.cbioportal.org). B. Seven amino acid positions have been identified as ATM missense mutational hotspots (www.cancerhotspots.org).

Supplementary Figure 2

TCGA Case ID	Alteration	Mutation type	Germline/ Somatic	LOH	
TCGA-FD-A3N6	M1|	Startloss	Germline	No	Truncating mutations
TCGA-DK-A6AW	R250X	Stopgain	Somatic	No	
TCGA-FD-A43Y	S403X	Stopgain	Somatic	Yes	
TCGA-UY-A8OD	Q781X	Stopgain	Somatic	Yes	
TCGA-GV-A40E	W1026Lfs	Frameshift ins.	Somatic	Yes	
TCGA-XF-AAMG	Q1276X	Stopgain	Somatic	No	
TCGA-4Z-AA84	R1466X	Stopgain	Somatic	No	
TCGA-BT-A42E	Q1636X	Stopgain	Somatic	Yes	
TCGA-E7-A5KF	Q1839X	Stopgain	Somatic	Yes	
TCGA-UY-A9PF	Q1839X	Stopgain	Somatic	No	
TCGA-DK-A6B5	E1978X	Stopgain	Germline	No	
TCGA-K4-A3WU	E1996fs	Frameshift ins.	Germline	No	
TCGA-FD-A43P	E2221X	Stopgain	Somatic	No	
TCGA-KQ-A41N	X792_splice	Splice site	Somatic	No	Splice site mutations
TCGA-E7-A8O8	E1249_splice	Splice site	Somatic	No	
TCGA-FD-A3B5	X2191_splice	Splice site	Somatic	Yes	
TCGA-GV-A6ZA	X2326_splice	Splice site	Somatic	Yes	
TCGA-GD-A6C6	X2643_splice	Splice site	Somatic	No	
TCGA-XF-AAME	I323V	Nonsyn. SNV	Somatic	Yes	Missense mutations
TCGA-UY-A9FP	E2039K	Nonsyn. SNV	Somatic	No	
TCGA-ZF-AA53	V2424G*	Nonsyn. SNV	Germline	Yes	
TCGA-FD-A5BY	R2832C*	Nonsyn. SNV	Germline	Yes	

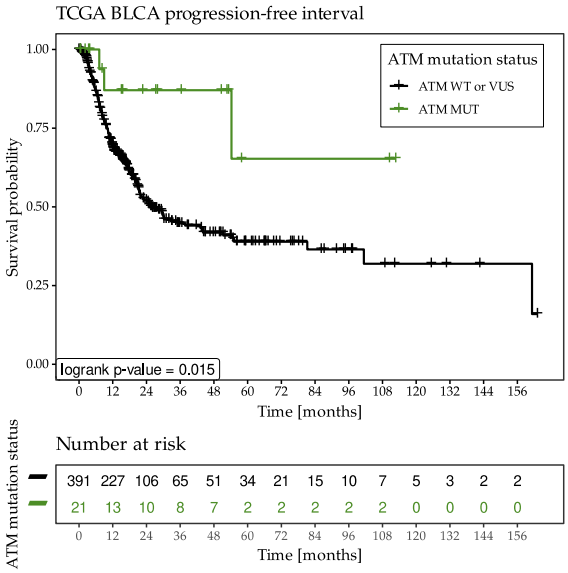
Supplementary Figure 2: Complete list of predicted deleterious ATM alterations in the bladder cancer (BLCA) The Cancer Genome Atlas (TCGA) cohort. See Methods for additional details regarding mutation calling. Cases are color-coded by alteration type (orange, truncating; pink, splice site; red, missense).

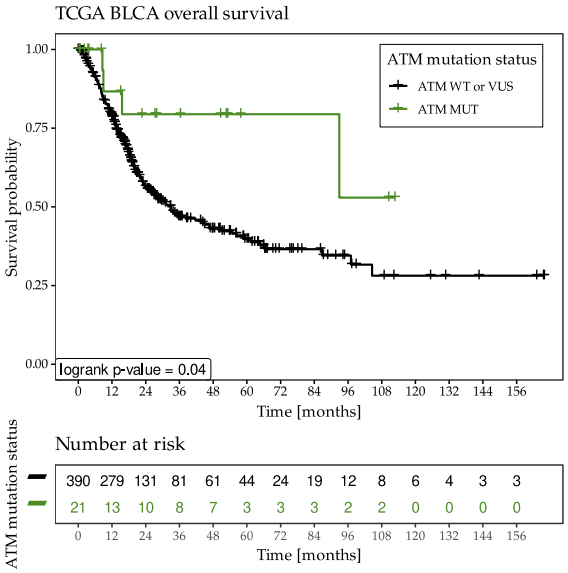
Supplementary Figure 3

	ATM-mutant (n=22)	ATM wild-type (n=384)
Age (mean ± s.d)	66 ± 12	68 ± 11
Sex		
Female	4 (4%)	104 (96%)
Male	17 (6%)	287 (94%)
Stage at diagnosis		
1	0 (0%)	1 (<1%)
2	10 (48%)	121 (31%)
3	7 (33%)	134 (35%)
4	4 (19%)	132 (34%)

Supplementary Figure 3: Clinical characteristics of ATM-altered and unaltered cases in TCGA bladder cancer (BLCA) cohort.

Supplementary Figure 4





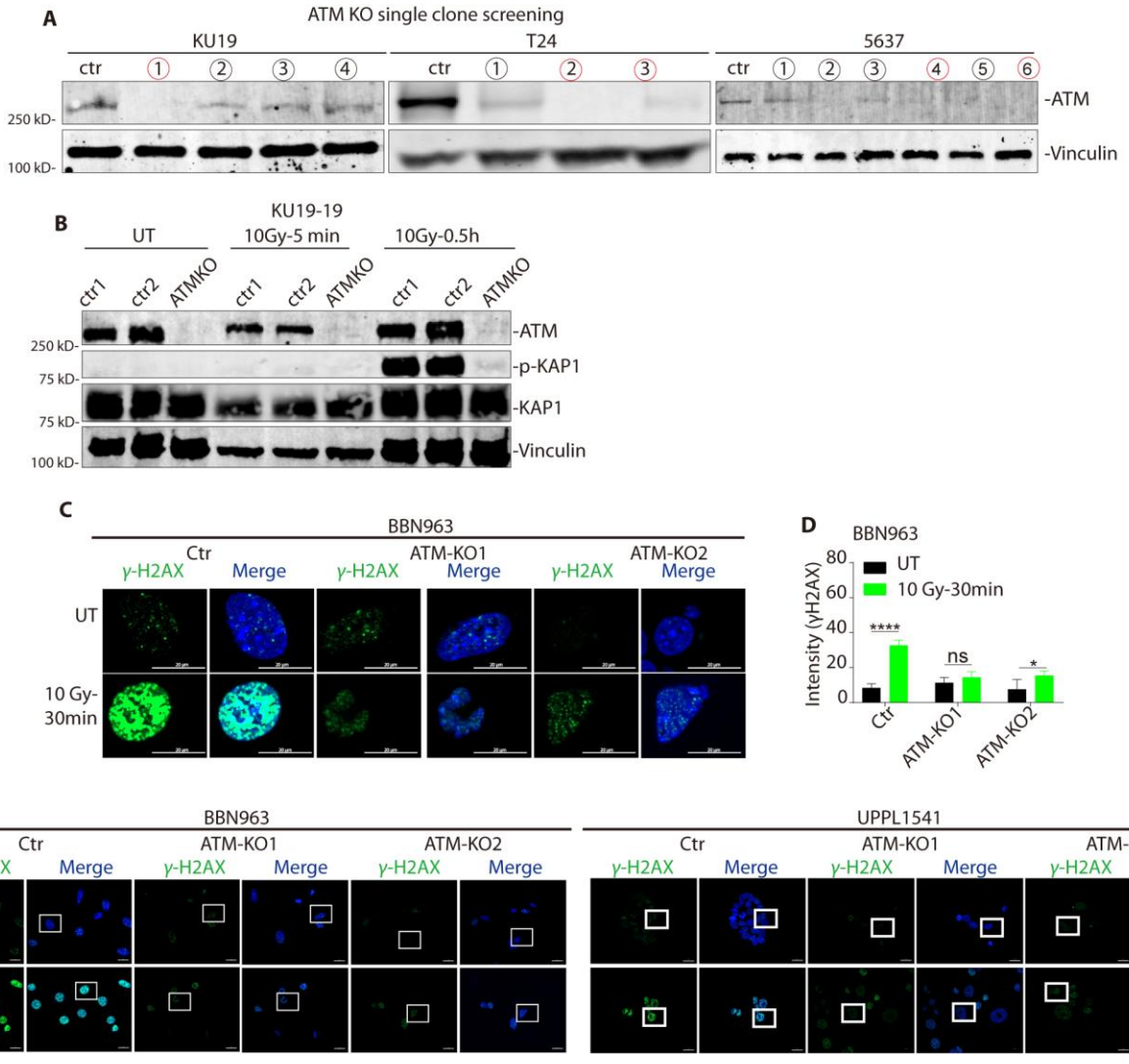
Supplementary Figure 4: Overall survival (OS) and progression-free survival (PFS) in ATM altered vs unaltered cases in the TCGA bladder cancer (BLCA) cohort. PFS and OS were significantly longer in ATM-altered compared to ATM-unaltered cases.

Supplementary Figure 5

Case No.	Alteration	Mutation type
1	R250X	stopgain
2	E343X	stopgain
3	E343Ifs*2	frameshift deletion
4	E390X	stopgain
5	Q513X	stopgain
6	K828Sfs*8	frameshift deletion
7	Q1017Rfs*5	frameshift deletion
8	Q1116X	stopgain
9	V1268*	stopgain
10	S1589X	stopgain
11	Q1636X	stopgain
12	R1730X	stopgain
13	I1792Nfs*9	frameshift insertion
14	E1991X	stopgain
15	E2157X	stopgain
16	Q2433X	stopgain
17	R2598X	stopgain
18	Q2615X	stopgain
19	W2960X	stopgain
20	R2547_S2549del	nonframeshift deletion
21	splice	splicing
22	splice	splicing
23	splice	splicing
24	splice	splicing
25	splice	splicing
26	P292L	nonsynonymous SNV
27	E2039K	nonsynonymous SNV
28	E2039K	nonsynonymous SNV
29	S2394L	nonsynonymous SNV
30	V2424G	nonsynonymous SNV
31	R2832C	nonsynonymous SNV
32	R3008C	nonsynonymous SNV

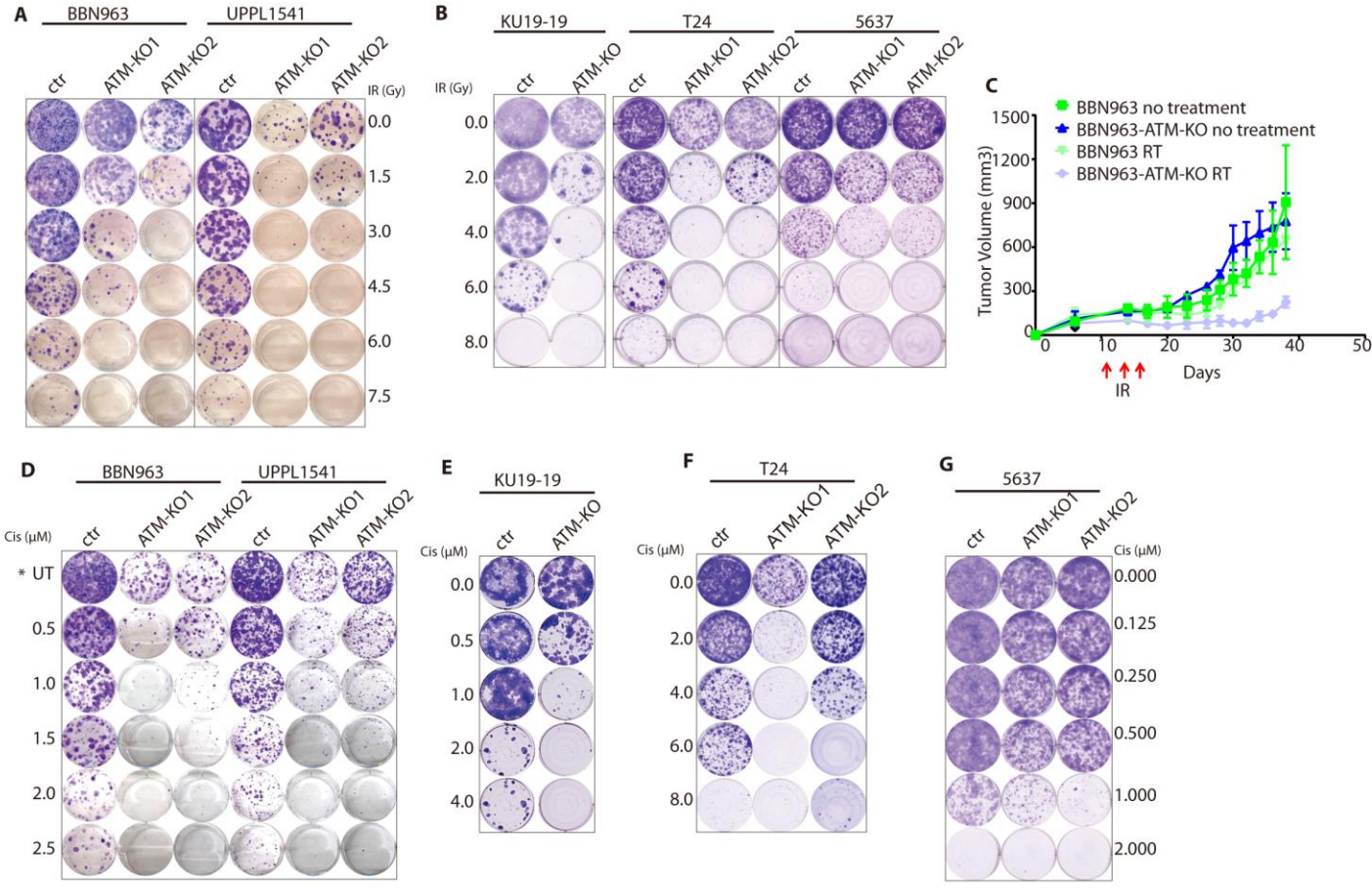
Supplementary Figure 5: Complete list of predicted deleterious ATM alterations in the Dana-Farber/Brigham & Women's Cancer Center (DFBWCC) urothelial tumor cohort. See Methods for additional details regarding mutation calling. Cases are color-coded by alteration type (orange, truncating; green, non-frameshift deletion; pink, splice site; red, missense).

Supplementary Figure 6



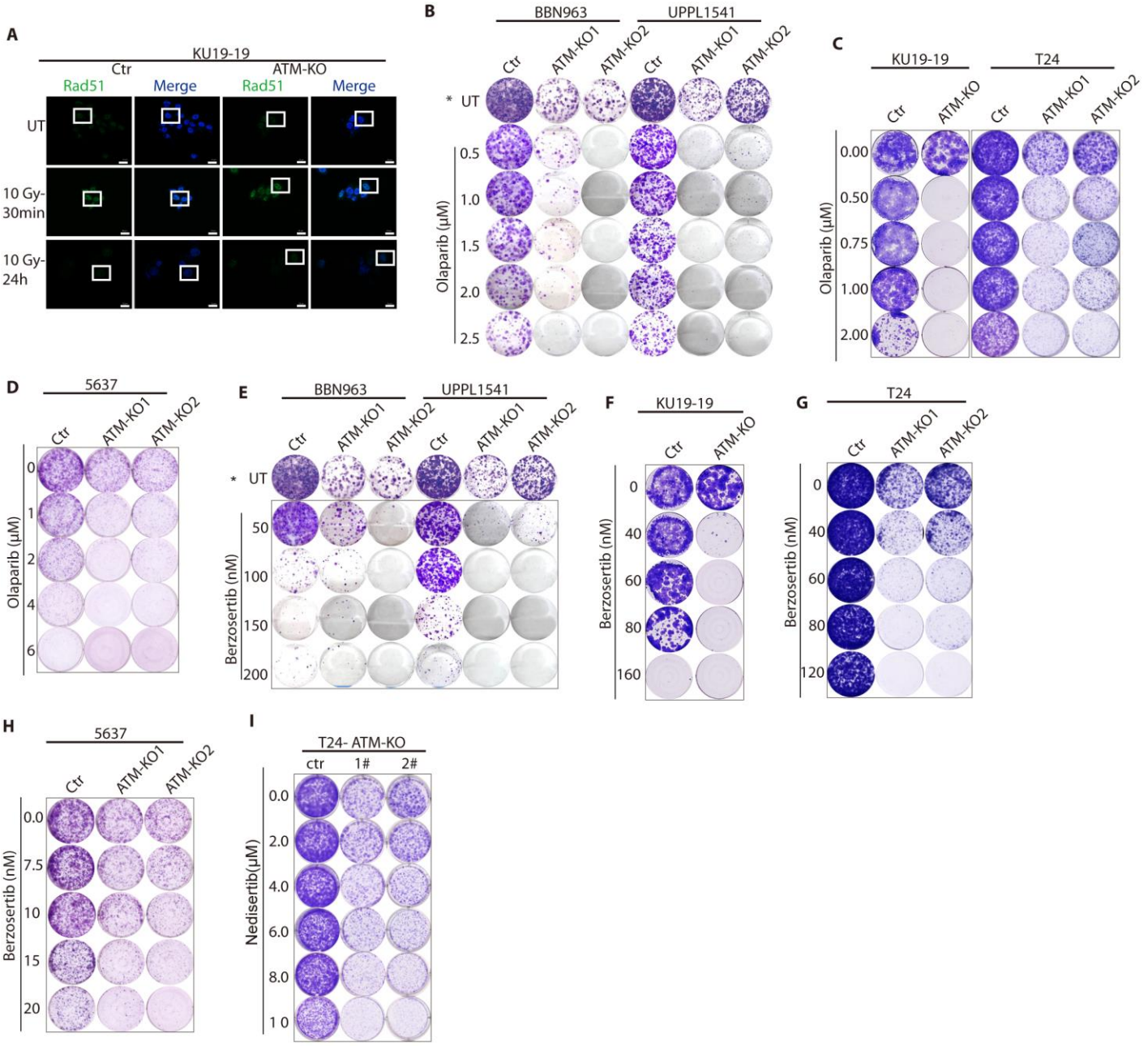
Supplementary Figure 6: ATM deletion in bladder cancer cell lines and DNA repair properties of ATM-deleted models. A. Following nucleofection of Cas9/sgRNA ribonucleoparticles, single cells were sorted and clones were expanded and then screened by ATM immunoblot to identify clones with loss of ATM protein expression. Clones with red circles were selected as ATM-deleted clones. B. KAP1 is a primary target of ATM phosphorylation following DNA damage and radiation-induced phosphorylation of KAP1 was markedly reduced in ATM-deleted vs WT ATM (control, 'ctr') KU19-19 bladder cancer cells. C. ATM phosphorylates histone H2A following DNA damage and radiation-induced γH2AX (Ser139) foci were significantly decreased in ATM-deleted compared to WT ATM BBN bladder cancer cells. D. Quantification of γH2AX (Ser139) foci from experiment represented in panel C. ****, $p<0.0001$. E. Full scale images (20X) of γH2AX (Ser139) foci in ATM-deleted and ATM WT BBN and UPPL cells. Cells in white boxes were amplified and displayed representatively in Figure 2C and and Supplementary Figure 6C.

Supplementary Figure 7



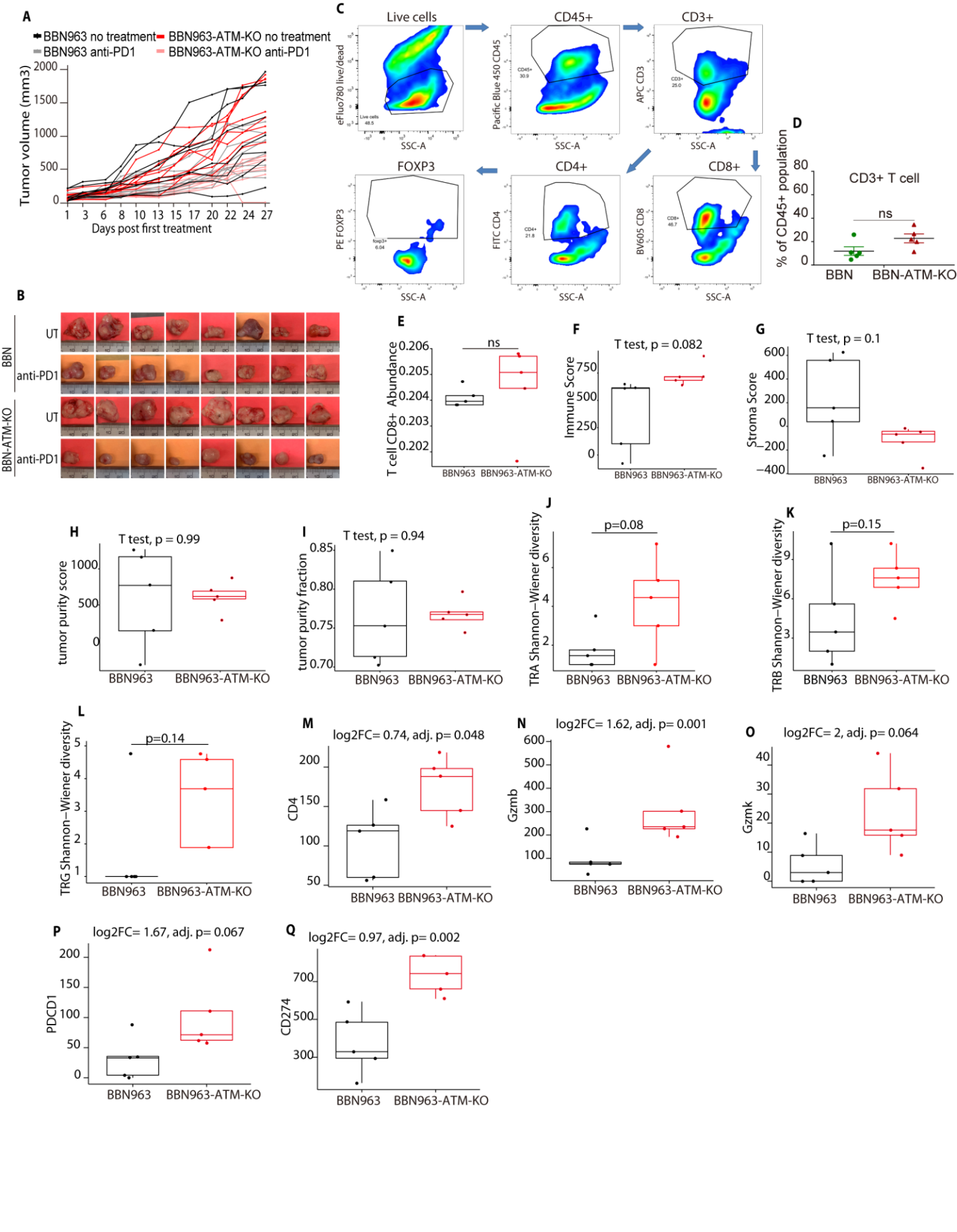
Supplementary Figure 7: ATM deletion increases sensitivity to DNA damaging agents. A-B. Representative images of crystal violet stained wells from cell survival assays following ionizing radiation. **C.** Xenograft growth curves for ATM-deleted and WT ATM BBN xenografts. The ATM-deleted and WT ATM models grow at a similar rate in untreated conditions (dark blue and dark green lines, respectively), but only the ATM-deleted xenograft model has growth delay following treatment with 3 Gy x 3 of tumor-directed radiation. Radiation treatments are denoted by red arrows. **D-G.** Representative images of crystal violet stained wells from cell survival assays following cisplatin treatment. For the BBN and UPPL cell lines, the same untreated ("UT") data was used for cisplatin, olaparib and berzosertib sensitivity assays because these three experiments were run in parallel.

Supplementary Figure 8



Supplementary Figure 8: ATM deletion increases sensitivity to DNA repair directed agents. A. Full scale images (20X) of Rad51 immunofluorescence in ATM-deleted and ATM WT KU19-19 cells. Cells in white boxes were amplified and displayed representatively in Figure 3A. B-C. Representative images of crystal violet stained wells from cell survival assays following treatment with the PARP inhibitor olaparib. D-H. Representative images of crystal violet stained wells from cell survival assays following treatment with the ATR inhibitor berzosertib. I. Representative images of crystal violet stained wells from cell survival assays following treatment with the DNA-PK inhibitor nedisertib. For the BBN and UPPL cell lines, the same untreated ("UT") data was used for cisplatin, olaparib and berzosertib sensitivity assays because these three experiments were run in parallel.

Supplementary Figure 9



Supplementary Figure 9: Impact of ATM loss on immune properties and anti-PD1 response. A. Individual xenograft growth curves for ATM-deleted and WT ATM BBN tumor-bearing mice treated with versus without anti-PD1 (n=8 mice per group). **B.** Photographs of harvested xenografts from all mice represented in panel A. **C.** Overview of T cell flow cytometry gating strategy. **D**. Flow cytometric analysis showed no significant difference in CD3+ T cell population in ATM-deleted versus WT ATM BBN tumors. **E.** RNA-seq based immune cell fraction estimation using TIMER revealed a trend towards increased CD8+ T cells in ATM-deleted compared to WT ATM xenografts. **F-I.** Immune deconvolution of RNA-seq data using ESTIMATE revealed a trend towards higher immune score in the ATM-deleted xenografts, higher stroma score in the WT ATM xenografts, and no difference in the tumor purity score or fraction. **J-L**. BBN-ATM-KO samples showed increased TRA, TRB and TRG diversity. **M-Q.** RNA-seq normalized counts for selected immune-related genes showed differences in expression levels of CD4, granzyme B (Gzmb), granzyme K (Gzmk), PD-1 (PDCD1), and PD-L1 (CD274) between WT and KO tumors with higher levels in the ATM KO tumors. P-values were adjusted using the Benjamini-Hochberg procedure; log2FC, log-transformed fold-change.

Supplementary Table 1: Genomic and clinical data for DFBWCC urothelial cohort.

Supplementary Table 2: Differential RNA expression data for BBN963 ATM WT and KO cell lines.

Supplementary Table 3: ATM IHC data for DFBWCC bladder cancer cohort.

Supplementary Table 4: ATM IHC data for MGH bladder cancer cohort.

Supplementary Table 5: Reagent details.

REFERENCES AND NOTES

1. Y. Shiloh, Y. Ziv. The ATM protein kinase: Regulating the cellular response to genotoxic stress, and more, *Nat. Rev. Mol. Cell Biol.* **14**, 197–210 (2013).

2. A. Ciccia, S. J. Elledge. The DNA damage response: Making it safe to play with knives, *Mol. Cell* **40**, 179–204 (2010).

3. C. Rothblum-Oviatt, J. Wright, M. A. Lefton-Greif, S. A. McGrath-Morrow, T. O. Crawford, H. M. Lederman Ataxia telangiectasia: A review, *Orphanet J. Rare Dis.* **11**, 159 (2016).

4. A. G. Robertson, J. Kim, H. Al-Ahmadie, J. Bellmunt, G. Guo, A. D. Cherniack, T. Hinoue, P. W. Laird, K. A. Hoadley, R. Akbani, M. A. A. Castro, E. A. Gibb, R. S. Kanchi, D. A. Gordenin, S. A. Shukla, F. Sanchez-Vega, D. E. Hansel, B. A. Czerniak, V. E. Reuter, X. Su, Benilton de Sa Carvalho, V. S. Chagas, K. L. Mungall, S. Sadeghi, C. S. Pedamallu, Y. Lu, L. J. Klimczak, J. Zhang, C. Choo, A. I. Ojesina, S. Bullman, K. M. Leraas, T. M. Lichtenberg, C. J. Wu, N. Schultz, G. Getz, M. Meyerson, G. B. Mills, D. J. Mc Conkey, TCGA Research Network, J. N. Weinstein, D. J. Kwiatkowski, S. P. Lerner, Comprehensive molecular characterization of muscle-invasive bladder cancer, *Cell* **171**, 540–556.e25 (2017).

5. M. Choi, T. Kipps, R. Kurzrock. ATM mutations in cancer: Therapeutic implications, *Mol. Cancer Ther.* **15**, 1781–91 (2016).

6. G. Iyer, A. V. Balar, M. I. Milowsky, B. H. Bochner, G. Dalbagni, S. M. Donat, H. W. Herr, W. C. Huang, S. S. Taneja, M. Woods, I. Ostrovnaya, H. al-Ahmadie, M. E. Arcila, J. C. Riches, A. Meier, C. Bourque, M. Shady, H. Won, T. L. Rose, W. Y. Kim, B. E. Kania, M. E. Boyd, C. K. Cipolla, A. M. Regazzi, D. Delbeau, A. S. McCoy, H. A. Vargas, M. F. Berger, D. B. Solit, J. E. Rosenberg, D. F. Bajorin Multicenter prospective phase II trial of neoadjuvant dose-dense gemcitabine plus cisplatin in patients with muscle-invasive bladder cancer, *J. Clin. Oncol.* **36**, 1949–1956 (2018).

7. E. R. Plimack, R. L. Dunbrack, T. A. Brennan, M. D. Andrake, Y. Zhou, I. G. Serebriiskii, M. Slifker, K. Alpaugh, E. Dulaimi, N. Palma, J. Hoffman-Censits, M. Bilusic, Y. N. Wong, A. Kutikov, R. Viterbo, R. E. Greenberg, D. Y. T. Chen, C. D. Lallas, E. J. Trabulsi, R.

Yelensky, D. J. McConkey, V. A. Miller, E. A. Golemis, E. A. Ross Defects in DNA repair genes predict response to neoadjuvant cisplatin-based chemotherapy in muscle-invasive bladder cancer, *Eur. Urol.* **68**, 959–967 (2015).

8. M. Y. Teo, R. M. Bambury, E. C. Zabor, E. Jordan, H. Al-Ahmadie, M. E. Boyd, N. Bouvier, S. A. Mullane, E. K. Cha, N. Roper, I. Ostrovnaya, D. M. Hyman, B. H. Bochner, M. E. Arcila, D. B. Solit, M. F. Berger, D. F. Bajorin, J. Bellmunt, G. Iyer, J. E. Rosenberg, DNA damage response and repair gene alterations are associated with improved survival in patients with platinum-treated advanced urothelial carcinoma, *Clin. Cancer Res.* **23**, 3610–3618 (2017).

9. E. M. Van Allen, K. W. Mouw, P. Kim, G. Iyer, N. Wagle, H. Al-Ahmadie, C. Zhu, I. Ostrovnaya, G. V. Kryukov, Kevin W O'Connor, J. Sfakianos, I. Garcia-Grossman, J. Kim, E. A. Guancial, R. Bambury, S. Bahl, N. Gupta, D. Farlow, A. Qu, S. Signoretti, J. A. Barletta, V. Reuter, J. Boehm, M. Lawrence, G. Getz, P. Kantoff, B. H. Bochner, T. K. Choueiri, D. F. Bajorin, D. B. Solit, S. Gabriel, A. D'Andrea, L. A. Garraway, J. E. Rosenberg, Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma, *Cancer Discov.* **4**, 1140–1153 (2014).

10. J. M. Cleary, A. J. Aguirre, G. I. Shapiro, A. D. D'Andrea. Biomarker-guided development of DNA repair inhibitors, *Mol. Cell* **78**, 1070–1085 (2020).

11. S. Carreira, N. Porta, S. Arce-Gallego, G. Seed, A. Llop-Guevara, D. Bianchini, P. Rescigno, A. Paschalis, C. Bertan, C. Baker, J. Goodall, S. Miranda, R. Riisnaes, I. Figueiredo, A. Ferreira, R. Pereira, M. Crespo, B. Gurel, D. Nava Rodrigues, S. J. Pettitt, W. Yuan, V. Serra, J. Rekowski, C. J. Lord, E. Hall, J. Mateo, J. S. de Bono Biomarkers associating with PARP inhibitor benefit in prostate cancer in the TOPARP-B Trial, *Cancer Discov.* **11**, 2812, 2827 (2021),.

12. P. Grivas, Y. Loriot, R. Morales-Barrera, M. Y. Teo, Y. Zakharia, S. Feyerabend, N. J. Vogelzang, E. Grande, N. Adra, A. Alva, A. Necchi, A. Rodriguez-Vida, S. Gupta, D. H. Josephs, S. Srinivas, K. Wride, D. Thomas, A. Simmons, A. Loehr, R. L. Dusek, D. Nepert, S. Chowdhury Efficacy and safety of rucaparib in previously treated, locally advanced or

metastatic urothelial carcinoma from a phase 2, open-label trial (ATLAS), *BMC Cancer* **21**, 593 (2021).

13. T. Powles, D. Carroll, S. Chowdhury, G. Gravis, F. Joly, J. Carles, A. Fléchon, P. Maroto, D. Petrylak, F. Rolland, N. Cook, A. V. Balar, S. S. Sridhar, M. D. Galsky, P. Grivas, A. Ravaud, R. Jones, J. Cosaert, D. Hodgson, I. Kozarewa, R. Mather, R. McEwen, F. Mercier, D. Landers An adaptive, biomarker-directed platform study of durvalumab in combination with targeted therapies in advanced urothelial cancer, *Nat. Med.* **27**, 793–801 (2021).

14. J. E. Rosenberg, S. H. Park, T. V. Dao, D. E. Castellano, J. R. Li, S. Mukherjee, K. Howells, H. Dry, M. C. Lanasa, R. Stewart, D. F. Bajorin BAYOU: A phase II, randomized, multicenter, double-blind, study of durvalumab (D) in combination with olaparib (O) for the first-line treatment of platinum-ineligible patients with unresectable, stage IV urothelial carcinoma (UC), *J. Clin. Oncol.* **40**, 437–437 (2022).

15. S. J. Crabb, S. A. Hussain, E. Soulis, S. Hinsley, L. Dempsey, A. Trevethan, Y. P. Song, J. Barber, J. A. Frew, J. Gale, G. Faust, S. J. Brock, U. B. McGovern, O. Parikh, D. Enting, S. Sundar, G. Ratnayake, K. Lees, T. Powles, R. J. Jones A randomized, double blind, biomarker selected, phase II clinical trial of maintenance PARP inhibition following chemotherapy for metastatic urothelial carcinoma (mUC): Final analysis of the ATLANTIS rucaparib arm, *J. Clin. Oncol.* **40**, 436–436 (2022).

16. K. W. Mouw, M. S. Goldberg, P. A. Konstantinopoulos, A. D. D'Andrea. DNA damage and repair biomarkers of immunotherapy response, *Cancer Discov.* **7**, 675–693 (2017).

17. R. Yi, A. Lin, M. Cao, A. Xu, P. Luo, J. Zhang ATM Mutations benefit bladder cancer patients treated with immune checkpoint inhibitors by acting on the tumor immune microenvironment, *Front. Genet.* **11**, 933 (2020).

18. M. Hu, M. Zhou, X. Bao, D. Pan, M. Jiao, X. Liu, F. Li, C. Y. Li ATM inhibition enhances cancer immunotherapy by promoting mtDNA leakage and cGAS/STING activation, *J. Clin. Invest.* **131**, e139333 (2021).

19. Q. Zhang, M. D. Green, X. Lang, J. Lazarus, J. D. Parsels, S. Wei, L. A. Parsels, J. Shi, N. Ramnath, D. R. Wahl, M. Pasca di Magliano, T. L. Frankel, I. Kryczek, Y. L. Lei, T. S. Lawrence, W. Zou, M. A. Morgan Inhibition of ATM increases interferon signaling and sensitizes pancreatic cancer to immune checkpoint blockade therapy, *Cancer Res.* **79**, 3940–3951 (2019).

20. M. Y. Teo, K. Seier, I. Ostrovnaya, A. M. Regazzi, B. E. Kania, M. M. Moran, C. K. Cipolla, M. J. Bluth, J. Chaim, H. al-Ahmadie, A. Snyder, M. I. Carlo, D. B. Solit, M. F. Berger, S. Funt, J. D. Wolchok, G. Iyer, D. F. Bajorin, M. K. Callahan, J. E. Rosenberg Alterations in DNA damage response and repair genes as potential marker of clinical benefit from PD-1/PD-L1 blockade in advanced urothelial cancers, *J. Clin. Oncol.* **36**, 1685–1694 (2018).

21. M. T. Chang, T. S. Bhattarai, A. M. Schram, C. M. Bielski, M. T. A. Donoghue, P. Jonsson, D. Chakravarty, S. Phillips, C. Kandoth, A. Penson, A. Gorelick, T. Shamu, S. Patel, C. Harris, J. J. Gao, S. O. Sumer, R. Kundra, P. Razavi, B. T. Li, D. N. Reales, N. D. Socci, G. Jayakumaran, A. Zehir, R. Benayed, M. E. Arcila, S. Chandarlapaty, M. Ladanyi, N. Schultz, J. Baselga, M. F. Berger, N. Rosen, D. B. Solit, D. M. Hyman, B. S. Taylor Accelerating discovery of functional mutant alleles in cancer, *Cancer Discov.* **8**, 174–183 (2018).

22. A. H. Nassar, R. Umeton, J. Kim, K. Lundgren, L. Harshman, E. M. van Allen, M. Preston, F. Dong, J. Bellmunt, K. W. Mouw, T. K. Choueiri, G. Sonpavde, D. J. Kwiatkowski Mutational analysis of 472 urothelial carcinoma across grades and anatomic sites, *Clin. Cancer Res.* **25**, 2458–2470 (2019).

23. P. Mullen. PARP cleavage as a means of assessing apoptosis, *Methods Mol. Med.* **88**, 171–181 (2004).

24. S. Rafiei, K. Fitzpatrick, D. Liu, M. Y. Cai, H. A. Elmarakeby, J. Park, C. Ricker, B. S. Kochupurakkal, A. D. Choudhury, W. C. Hahn, S. P. Balk, J. H. Hwang, E. M. van Allen, K. W. Mouw ATM loss confers greater sensitivity to atr inhibition than PARP inhibition in prostate cancer, *Cancer Res.* **80**, 2094–2100 (2020).

25. J. H. L. Fok, A. Ramos-Montoya, M. Vazquez-Chantada, P. W. G. Wijnhoven, V. Follia, N. James, P. M. Farrington, A. Karmokar, S. E. Willis, J. Cairns, J. Nikkilä, D. Beattie, G. M. Lamont, M. R. V. Finlay, J. Wilson, A. Smith, L. O. O'Connor, S. Ling, S. E. Fawell, M. J. O'Connor, S. J. Hollingsworth, E. Dean, F. W. Goldberg, B. R. Davies, E. B. Cadogan AZD7648 is a potent and selective DNA-PK inhibitor that enhances radiation, chemotherapy and olaparib activity, *Nat. Commun.* **10**, 5065 (2019).

26. A. J. Davis, B. P. Chen, D. J. Chen. DNA-PK: A dynamic enzyme in a versatile DSB repair pathway, *DNA Repair* **17**, 21–29 (2014).

27. A. Snyder, T. Nathanson, S. A. Funt, A. Ahuja, J. Buros Novik, M. D. Hellmann, E. Chang, B. A. Aksoy, H. al-Ahmadie, E. Yusko, M. Vignali, S. Benzeno, M. Boyd, M. Moran, G. Iyer, H. S. Robins, E. R. Mardis, T. Merghoub, J. Hammerbacher, J. E. Rosenberg, D. F. Bajorin Contribution of systemic and somatic factors to clinical response and resistance to PD-L1 blockade in urothelial cancer: An exploratory multi-omic analysis, *PLoS Med.* **14**, e1002309 (2017).

28. S. Mariathasan, S. J. Turley, D. Nickles, A. Castiglioni, K. Yuen, Y. Wang, E. E. Kadel III, H. Koeppen, J. L. Astarita, R. Cubas, S. Jhunjhunwala, R. Banchereau, Y. Yang, Y. Guan, C. Chalouni, J. Ziai, Y. Şenbabaoğlu, S. Santoro, D. Sheinson, J. Hung, J. M. Giltnane, A. A. Pierce, K. Mesh, S. Lianoglou, J. Riegler, R. A. D. Carano, P. Eriksson, M. Höglund, L. Somarriba, D. L. Halligan, M. S. van der Heijden, Y. Loriot, J. E. Rosenberg, L. Fong, I. Mellman, D. S. Chen, M. Green, C. Derleth, G. D. Fine, P. S. Hegde, R. Bourgon, T. Powles TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells, *Nature* **554**, 544–548 (2018).

29. A. V. Balar, M. D. Galsky, J. E. Rosenberg, T. Powles, D. P. Petrylak, J. Bellmunt, Y. Loriot, A. Necchi, J. Hoffman-Censits, J. L. Perez-Gracia, N. A. Dawson, M. van der Heijden, R. Dreicer, S. Srinivas, M. M. Retz, R. W. Joseph, A. Drakaki, U. N. Vaishampayan, S. S. Sridhar, D. I. Quinn, I. Durán, D. R. Shaffer, B. J. Eigl, P. D. Grivas, E. Y. Yu, S. Li, Kadel EE 3rd, Z. Boyd, R. Bourgon, P. S. Hegde, S. Mariathasan, A. Thåström, O. O. Abidoye, G. D. Fine, D. F. Bajorin, IMvigor210 Study Group Atezolizumab as first-

line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: A single-arm, multicentre, phase 2 trial, *Lancet* **389**, 67–76 (2017).

30. C. D. Carey, D. Gusenleitner, M. Lipschitz, M. G. M. Roemer, E. C. Stack, E. Gjini, X. Hu, R. Redd, G. J. Freeman, D. Neuberg, F. S. Hodi, X. S. Liu, M. A. Shipp, S. J. Rodig Topological analysis reveals a PD-L1-associated microenvironmental niche for Reed-Sternberg cells in Hodgkin lymphoma, *Blood* **130**, 2420–2430 (2017).

31. A. Neeb, N. Herranz, S. Arce-Gallego, S. Miranda, L. Buroni, W. Yuan, A. Athie, T. Casals, J. Carmichael, D. N. Rodrigues, B. Gurel, P. Rescigno, J. Rekowski, J. Welti, R. Riisnaes, V. Gil, J. Ning, V. Wagner, I. Casanova-Salas, S. Cordoba, N. Castro, M. D. Fenor de la Maza, G. Seed, K. Chandran, A. Ferreira, I. Figueiredo, C. Bertan, D. Bianchini, C. Aversa, A. Paschalis, M. Gonzalez, R. Morales-Barrera, C. Suarez, J. Carles, A. Swain, A. Sharp, J. Gil, V. Serra, C. Lord, S. Carreira, J. Mateo, J. S. de Bono Advanced prostate cancer with ATM loss: PARP and ATR inhibitors, *Eur. Urol.* **79**, 200–211 (2021).

32. H. Kaur, D. C. Salles, S. Murali, J. L. Hicks, M. Nguyen, C. C. Pritchard, A. M. de Marzo, J. S. Lanchbury, B. J. Trock, W. B. Isaacs, K. M. Timms, E. S. Antonarakis, T. L. Lotan Genomic and clinicopathologic characterization of atm-deficient prostate cancer, *Clin. Cancer Res.* **26**, 4869–4881 (2020).

33. R. M. Miller, C. Nworu, L. McKee, D. Balcerzak, L. Pham, J. Pugh, Y. Z. Liu, H. Gustafson, E. Marwah, T. Lamb, J. Clements Development of an immunohistochemical assay to detect the ataxia-telangiectasia mutated (ATM) protein in gastric carcinoma, *Appl. Immunohistochem. Mol. Morphol.* **28**, 303–310 (2020).

34. T. A. Yap, D. S. P. Tan, A. Terbuch, R. Caldwell, C. Guo, B. C. Goh, V. Heong, N. R. M. Haris, S. Bashir, Y. Drew, D. S. Hong, F. Meric-Bernstam, G. Wilkinson, J. Hreiki, A. M. Wengner, F. Bladt, A. Schlicker, M. Ludwig, Y. Zhou, L. Liu, S. Bordia, R. Plummer, E. Lagkadinou, J. S. de Bono First-in-human trial of the oral ataxia telangiectasia and RAD3-related (ATR) inhibitor BAY 1895344 in patients with advanced solid tumors, *Cancer Discov.* **11**, 80–91 (2021).

35. P. A. Konstantinopoulos, Alexandre André B. A. da Costa, Doga Gulhan, Elizabeth K. Lee, Su-Chun Cheng, Andrea E. Wahner Hendrickson, B. Kochupurakkal, D. L. Kolin, E. C. Kohn, J. F. Liu, E. H. Stover, J. Curtis, N. Tayob, M. Polak, D. Chowdhury, U. A. Matulonis, A. Färkkilä, Alan D. D'Andrea, G. I. Shapiro, A Replication stress biomarker is associated with response to gemcitabine versus combined gemcitabine and ATR inhibitor therapy in ovarian cancer, *Nat. Commun.* **12**, 5574 (2021).

36. N. J. Giacalone, W. U. Shipley, R. H. Clayman, A. Niemierko, M. Drumm, N. M. Heney, M. D. Michaelson, R. J. Lee, P. J. Saylor, M. F. Wszolek, A. S. Feldman, D. M. Dahl, A. L. Zietman, J. A. Efstathiou Long-term outcomes after bladder-preserving tri-modality therapy for patients with muscle-invasive bladder cancer: An updated analysis of the Massachusetts General Hospital experience, *Eur. Urol.* **71**, 952, 960 (2017).

37. D. J. Konieczkowski, J. A. Efstathiou, K. W. Mouw. Contemporary and emerging approaches to bladder-preserving trimodality therapy for muscle-invasive bladder cancer, *Hematol. Oncol. Clin. North Am.* **35**, 567–584 (2021).

38. S. C. Kamran, Y. Zhou, K. Otani, M. Drumm, Y. Otani, S. Wu, C.-L. Wu, A. S. Feldman, M. Wszolek, R. J. Lee, P. J. Saylor, J. Lennerz, E. Van Allen, H. Willers, T. S. Hong, Y. Liu, E. Davicioni, E. A. Gibb, W. U. Shipley, K. W. Mouw, J. A. Efstathiou, D. T. Miyamoto, Genomic tumor correlates of clinical outcomes following organ-sparing chemoradiation therapy for bladder cancer. *Clin. Cancer Res.* 10.1158/1078-0432.CCR-23-0792 (2023).

39. R. Russell, L. Perkhofer, S. Liebau, Q. Lin, A. Lechel, F. M. Feld, E. Hessmann, J. Gaedcke, M. Güthle, M. Zenke, D. Hartmann, G. von Figura, S. E. Weissinger, K. L. Rudolph, P. Möller, J. K. Lennerz, T. Seufferlein, M. Wagner, A. Kleger Loss of ATM accelerates pancreatic cancer formation and epithelial-mesenchymal transition, *Nat. Commun.* **6**, 7677 (2015).

40. M. Squatrito, C. W. Brennan, K. Helmy, J. T. Huse, J. H. Petrini, E. C. Holland Loss of ATM/Chk2/p53 pathway components accelerates tumor development and contributes to radiation resistance in gliomas, *Cancer Cell* **18**, 619–629 (2010).

41. L. Perkhofer, A. Schmitt, M. C. Romero Carrasco, M. Ihle, S. Hampp, D. A. Ruess, E. Hessmann, R. Russell, A. Lechel, N. Azoitei, Q. Lin, S. Liebau, M. Hohwieler, H. Bohnenberger, M. Lesina, H. Algül, L. Gieldon, E. Schröck, J. Gaedcke, M. Wagner, L. Wiesmüller, B. Sipos, T. Seufferlein, H. C. Reinhardt, P. O. Frappart, A. Kleger ATM deficiency generating genomic instability sensitizes pancreatic ductal adenocarcinoma cells to therapy-induced dna damage, *Cancer Res.* **77**, 5576–5590 (2017).

42. C. Wang, N. Jette, D. Moussienko, D. G. Bebb, S. P. Lees-Miller ATM-deficient colorectal cancer cells are sensitive to the PARP inhibitor olaparib, *Transl. Oncol.* **10**, 190–196 (2017).

43. K. L. Pitter, D. L. Casey, Y. C. Lu, M. Hannum, Z. Zhang, X. Song, I. Pecorari, B. M. Millan, J. Ma, R. M. Samstein, I. X. Pei, A. J. Khan, L. Z. Braunstein, L. G. T. Morris, C. A. Barker, A. Rimner, K. M. Alektiar, P. B. Romesser, C. H. Crane, J. Yahalom, M. J. Zelefsky, H. I. Scher, J. L. Bernstein, D. L. Mandelker, B. Weigelt, J. S. Reis-Filho, N. Y. Lee, S. N. Powell, T. A. Chan, N. Riaz, J. Setton, Pathogenic ATM mutations in cancer and a genetic basis for radiotherapeutic efficacy, *J. Natl. Cancer Inst.* **13**, 266–273 (2021).

44. J. A. Efstathiou, K. Bae, W. U. Shipley, D. S. Kaufman, M. P. Hagan, N. M. Heney, H. M. Sandler Late pelvic toxicity after bladder-sparing therapy in patients with invasive bladder cancer: RTOG 89-03, 95-06, 97-06, 99-06, *J. Clin. Oncol.* **27**, 4055–61 (2009).

45. P. Grivas. DNA damage response gene alterations in urothelial cancer: Ready for practice?, *Clin. Cancer Res.* **25**, 907–909 (2019).

46. M. D. Galsky, S. Daneshmand, K. G. Chan, T. B. Dorff, J. P. Cetnar, B. O Neil, A. D'souza, R. Mamtani, C. Kyriakopoulos, P. Garcia, S. Izadmehr, M. Yu, Q. Zhao, R. Mehrazin, S. C. Lewis, J. Sfakianos, S. K. Pal Phase 2 trial of gemcitabine, cisplatin, plus nivolumab with selective bladder sparing in patients with muscle- invasive bladder cancer (MIBC): HCRN GU 16-257, *J. Clin. Oncol.* **39**, 4503–4503 (2021).

47. D. M. Geynisman, P. Abbosh, E. A. Ross, M. R. Zibelman, P. Ghatalia, F. Anari, K. Ansel, J. R. Mark, L. Stamatakis, J. H. Hoffman-Censits, R. Viterbo, E. M. Horwitz, M. A. Hallman, R. K. Alpaugh, R. E. Greenberg, M. C. Smaldone, R. G. Uzzo, D. Chen, A. Kutikov, E. R.

Plimack A phase II trial of risk enabled therapy after initiating neoadjuvant chemotherapy for bladder cancer (RETAIN BLADDER): Interim analysis, *J. Clin. Oncol.* **39**, 397–397 (2021).

48. G. Balmus, D. Pilger, J. Coates, M. Demir, M. Sczaniecka-Clift, A. C. Barros, M. Woods, B. Fu, F. Yang, E. Chen, M. Ostermaier, T. Stankovic, H. Ponstingl, M. Herzog, K. Yusa, F. M. Martinez, S. T. Durant, Y. Galanty, P. Beli, D. J. Adams, A. Bradley, E. Metzakopian, J. V. Forment, S. P. Jackson ATM orchestrates the DNA-damage response to counter toxic non-homologous end-joining at broken replication forks, *Nat. Commun.* **10**, 87 (2019).

49. S. K. Pal, P. H. Frankel, A. Mortazavi, M. Milowsky, U. Vaishampayan, M. Parikh, Y. Lyou, P. Weng, R. Parikh, B. Teply, R. Dreicer, H. Emamekhoo, D. Michaelson, C. Hoimes, T. Zhang, S. Srinivas, W. Y. Kim, Y. Cui, E. Newman, P. N. Lara Jr Effect of cisplatin and gemcitabine with or without berzosertib in patients with advanced urothelial carcinoma: A phase 2 randomized clinical trial, *JAMA Oncol.* **7**, 1536–1543 (2021).

50. D. Y. Oh, S. S. Kwek, S. S. Raju, T. Li, E. McCarthy, E. Chow, D. Aran, A. Ilano, C. C. S. Pai, C. Rancan, K. Allaire, A. Burra, Y. Sun, M. H. Spitzer, S. Mangul, S. Porten, M. V. Meng, T. W. Friedlander, C. J. Ye, L. Fong Intratumoral CD4(+) T cells mediate anti-tumor cytotoxicity in human bladder cancer, *Cell* **181**, 1612.e13–1625.e13 (2020).

51. W. H. Weir, P. J. Mucha, W. Y. Kim. A bipartite graph-based expected networks approach identifies DDR genes not associated with TMB yet predictive of immune checkpoint blockade response, *Cell Rep Med* **3**, 100602 (2022).

52. K. Wang, M. Li, H. Hakonarson. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data, *Nucleic Acids Res.* **38**, e164 (2010).

53. Q. Li, K. Wang. InterVar: Clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines, *Am. J. Hum. Genet.* **100**, 267–280 (2017).

54. M. J. Landrum, J. M. Lee, M. Benson, G. R. Brown, C. Chao, S. Chitipiralla, B. Gu, J. Hart, D. Hoffman, W. Jang, K. Karapetyan, K. Katz, C. Liu, Z. Maddipatla, A. Malheiro, K. McDaniel, M. Ovetsky, G. Riley, G. Zhou, J. B. Holmes, B. L. Kattman, D. R. Maglott

ClinVar: Improving access to variant interpretations and supporting evidence, *Nucleic Acids Res.* **46**, D1062–D1067 (2018).

55. T. Li, J. Fu, Z. Zeng, D. Cohen, J. Li, Q. Chen, B. Li, X. S. Liu TIMER2.0 for analysis of tumor-infiltrating immune cells, *Nucleic Acids Res.* **48**, W509-W514 (2020).

56. L. M. Sholl, K. Do, P. Shivdasani, E. Cerami, et al. Institutional implementation of clinical tumor profiling on an unselected cancer population, *JCI Insight* **1**, e87062 (2016).

57. R. Saito, C. C. Smith, T. Utsumi, L. M. Bixby, J. Kardos, S. E. Wobker, K. G. Stewart, S. Chai, U. Manocha, K. M. Byrd, J. S. Damrauer, S. E. Williams, B. G. Vincent, W. Y. Kim Molecular subtype-specific immunocompetent models of high-grade urothelial carcinoma reveal differential neoantigen expression and response to immunotherapy, *Cancer Res.* **78**, 3954–3968 (2018).

58. P. A. Ewels, A. Peltzer, S. Fillinger, H. Patel, J. Alneberg, A. Wilm, M. U. Garcia, P. di Tommaso, S. Nahnsen The nf-core framework for community-curated bioinformatics pipelines, *Nat. Biotechnol.* **38**, 276–278 (2020).

59. R. Patro, G. Duggal, M. I. Love, R. A. Irizarry, C. Kingsford Salmon provides fast and bias-aware quantification of transcript expression, *Nat. Methods* **14**, 417–419 (2017).

60. M. I. Love, W. Huber, S. Anders. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, *Genome Biol.* **15**, 550 (2014).

61. T. Li, J. Fan, B. Wang, N. Traugh, Q. Chen, J. S. Liu, B. Li, X. S. Liu TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells, *Cancer Res.* **77**, e108-e110 (2017).

62. K. Yoshihara, M. Shahmoradgoli, E. Martinez, R. Vegesna, Inferring tumour purity and stromal and immune cell admixture from expression data, *Nat. Commun.* **4**, 2612 (2013).

63. D. A. Bolotin, S. Poslavsky, A. N. Davydov, F. E. Frenkel, L. Fanchi, O. I. Zolotareva, S. Hemmers, E. V. Putintseva, A. S. Obraztsova, M. Shugay, R. I. Ataullakhanov, A. Y.

Rudensky, T. N. Schumacher, D. M. Chudakov Antigen receptor repertoire profiling from RNA-seq data, *Nat. Biotechnol.* **35**, 908–911 (2017).