BAP1 is a haploinsufficient tumor suppressor linking chronic pancreatitis to pancreatic cancer in mice

Stephanie Perkail, Jaclyn Andricovich, Yan Kai & Alexandros Tzatsos

Chronic pancreatitis represents a risk factor for the development of pancreatic cancer. We find that heterozygous loss of histone H2A lysine 119 deubiquitinase BAP1 (BRCA1 Associated Protein-1) associates with a history of chronic pancreatitis and occurs in 25% of pancreatic ductal adenocarcinomas and 40% of acinar cell carcinomas. Deletion or heterozygous loss of Bap1 in murine pancreata causes genomic instability, tissue damage, and pancreatitis with full penetrance. Concomitant expression of KrasG12D leads to predominantly intraductal papillary mucinous neoplasms and mucinous cystic neoplasms, while pancreatic intraepithelial neoplasias are rarely detected. These lesions progress to metastatic pancreatic cancer with high frequency. Lesions with histological features mimicking Acinar Cell Carcinomas are also observed in some tumors. Heterozygous mice also develop pancreatic cancer suggesting a haploinsufficient tumor suppressor role for BAP1. Mechanistically, BAP1 regulates genomic stability, in a catalytic independent manner, and its loss confers sensitivity to irradiation and platinum-based chemotherapy in pancreatic cancer.
Pancreatic ductal adenocarcinoma (PDA) is a lethal malignancy with a 5-year survival rate of 10%. Sequencing of pancreatic cancer genomes revealed clinically relevant molecular subtypes\(^1\)-\(^3\). Based on structural variations at the chromosomal level, PDA has been classified into four subtypes: stable, locally rearranged, scattered, and unstable\(^4\). The latter is driven by mutations in DNA-damage repair genes, such as \(BRCA1\), \(BRCA2\), \(ATM\), and \(PALB2\), and responds to treatments triggering DNA damage, such as platinum-based chemotherapy, poly (ADP-ribose) polymerase (PARP) inhibitors, and irradiation (IR)\(^5\)-\(^8\). Consistently, targeted inactivation of either \(Brca2\) or \(Atm\) in murine pancreata caused genomic instability and promoted aggressive cancer that was sensitive to IR\(^9\)-\(^10\). In early stages of PDA, the activation of DNA damage response (DDR) triggered by oncogenic Kras poses a barrier to cancer development. However, progressive failure of DNA repair pathways can facilitate malignant transformation and tumor evolution. Thus, further identification of molecular defects compromising genome integrity may indicate novel biomarkers of responsiveness to systemic chemotherapy and refine rational approaches for therapeutic intervention.

FOLFIRINOX (5-Fluorouracil, Leucovorin, Irinotecan, and Oxaliplatin), a platinum- and topoisomerase I inhibitor-containing combination therapy is emerging as a first-line treatment for patients with good performance status, who are diagnosed with PDA\(^11\). Intriguingly, although improved survival has been reported for patients with mutations in DNA repair genes\(^16\), the efficacy of FOLFIRINOX extends beyond the small percentage of cases exhibiting mutations of \(BRCA1/2\), \(PALB2\), and \(ATM\), indicating that defects in other genes regulating the DDR may contribute as well. This observation motivated us to analyze pancreatic cancer genomes for copy number alterations (CNAs) of genes implicated in DNA repair. We found that heterozygous loss of \(BAP1\) —but rarely mutation—occurs in a quarter of PDAs and 40% of acinar cell carcinomas (ACCs), a rare and distinct subtype of pancreatic cancer that infrequently harbors \(Kras\) mutations. \(BAP1\) is located on the short arm of chromosome 3 (3p21.1) where loss of heterozygosity (LOH) or deletion is the promoter located in the same cytogenetic band as \(BAP1\), as well as \(BRCA1\) and \(BRCA2\) which is also located on the arm of chromosome 3 (Chr 3p25.3). Although \(BAP1\) expression is not linked to a pancreatic cancer subtype\(^1\) (Supplementary Fig. 1c), in two independent pancreatic cancer cohorts\(^1,2\), patients with low expression of \(BAP1\) exhibited shorter median survival and a dismal prognosis (hazard ratio (HR) 4.94 with 95% confidence interval (95% CI) 2.62–10.79, Fig. 1c). Meta-analysis of additional studies confirmed that heterozygous loss, but rarely mutations or deletion, of \(BAP1\) is a common aberration in >25% of PDA patients\(^2,3,23\) (Supplementary Fig. 1d). Heterozygous loss of \(BAP1\) was also present in (a) 40% of ACC, which also exhibit copy number losses for several DNA repair genes\(^2,3,24\), and (b) 15–20% of pancreatic tumors arising from mucinous cystic neoplasms (MCNs), intraductal papillary mucinous neoplasms (IPMNs), and solid pseudopapillary neoplasms\(^2,3,25,26\) (Supplementary Fig. 1e, d). Consistently, most human PDA cell lines show heterozygous loss of \(BAP1\) and express reduced mRNA and protein compared with cell lines established from other malignancies (Supplementary Fig. 1f, g). Patients with loss of \(BAP1\) exhibited more than a twofold increase in the fraction of the genome carrying CNAs, but no difference in the mutational burden (Fig. 1d), and were associated with a history of chronic pancreatitis (Fig. 1e). The latter group also showed enrichment for CNAs but not mutations (Supplementary Fig. 1h). Thus, genomic instability due to \(BAP1\) deficiency may be associated to the development of chronic pancreatitis and pancreatic cancer.

Next, we stained human PDA tissue microarrays (TMAs) comprising specimens representing progression of premalignant lesions to overt cancer and metastatic disease, and stratified based on grade (well- vs. poorly differentiated, Fig. 1f), with a \(BAP1\) antibody that was validated for immunohistochemistry (IHC) in knockout mice (Supplementary Fig. 1i). In human and mouse normal pancreata, \(BAP1\) is expressed in islets and displays a mosaic pattern in acinar cells (Fig. 1f and Supplementary Fig. 1j). \(BAP1\) is expressed in acinar-to-ductal metaplasia and premalignant pancreatic intraepithelial neoplasias (PanINs), as well as in some cases of well-differentiated PDA. In contrast, \(BAP1\) was absent, or downregulated, in the majority of poorly differentiated (grade III) specimens, whereas a statistically significant inverse correlation was observed between \(BAP1\) staining and tumor grade, as well as progression to metastatic disease (Fig. 1f–g).

To study the expression of endogenous \(Bap1\) in a progression model of pancreatic cancer, we established a knockin reporter mouse carrying a lacZ cassette under the control of the endogenous \(Bap1\) promoter flanked byloxP sites to allow tissue-specific deletion following intercrossing with the \(Pdx1^{Cre}\) strain\(^27\) (Supplementary Fig. 1k). Early in life, \(Bap1^{lacZ/+}\) mice
showed widespread expression of β-galactosidase (Supplementary Fig. 1f). On the contrary, β-galactosidase in older mice was largely confined to islets with a weak mosaic staining of acinar and ductal cells. Consistent with the expression pattern observed in human (Fig. 1f), β-galactosidase staining in murine pancreata showed an increase in premalignant lesions driven by KrasG12D, but no staining was detected in overt cancer upon concomitant loss of Trp53 (Fig. 1h and IHC in Supplementary Fig. 1m).

**Loss of Bap1 induces DNA damage and chronic pancreatitis.** We generated pancreas-specific conditional knockout mice by sequentially crossing Bap1WT with β-ActinFLPe animals to sequentially crossing Bap1lacZ/+ with β-ActinFLPe animals to sequentially crossing Bap1lacZ/+ with β-ActinFLPe animals, but no
remove the Frt cassette and crossed the offspring to Pdx1<Cre> or Ptf1α<Cre> mice to delete Bap1 in pancreas progenitor cells (Supplementary Fig. 2a). Pancreatic-specific Bap1-null mice were born at the expected gender and genotype ratios. Genotyping confirmed recombination in genomic DNA isolated from the pancreas, but not the tail, and IHC confirmed the mosaic absence of Bap1 in the exocrine pancreas (Supplementary Fig. 2b and 1i). Islets retained expression of Bap1 and mice exhibited normal glucose homeostasis (Supplementary Fig. 2c, d). Histological analyses of knockout pancreata at 4 weeks of age revealed focal areas with loss of acinar architecture that over time evolved to variable hyperplastic, metaplastic, and connective tissue changes accompanied by obstruction of the ducts with eosinophilic zymogen material, which caused luminal dilation of acini and reactive proliferation (Fig. 2a and Supplementary Fig. 2e). Similar changes were also observed in heterozygous (Fig. 2a) and Bap1<lacZ+> pancreata (Supplementary Fig. 2f). IHC revealed a strong upregulation of H2A ubiquitinated at Lys119 (H2A.K119Ub), loss of acinar identity (Amylase), and expansion of Cytokeratin 17/19- and Sox9-positive duct-like cells (Fig. 2b). After 20 weeks of age, knockout out mice presented with atonic behavior, hunched posture, and poor grooming, rendering them moribund. Post-mortem examination revealed elevated levels of serum amylase and lipase (Fig. 2c), a reduction in pancreas size with loss of tissue integrity and a gelatinous appearance (Supplementary Fig. 2g). Microscopic findings included loss of acini, immune cell infiltration, fibrosis (Sirius Red staining), and saponification, indicative of tissue inflammation and damage (Fig. 2d-f). Consistently, IHC and flow cytometry confirmed infiltration by T cells (CD3+), dendritic cells (CD11c+), and macrophages (F4/80+ and CD11b+), but not B cells (B220+) (Supplementary Fig. 2h, i). The histologic changes—accumulation of insoluble eosinophilic material and inflammation—were reminiscent of chronic pancreatitis secondary to cystic fibrosis, a genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Cystic fibrosis patients exhibit higher risk for chronic pancreatitis and PDA. Cfr, which is primarily expressed in the duct epithelia and a very small percentage of acinar cells (Supplementary Fig. 2j), was downregulated in Bap1 heterozygotes and was absent in the expanded duct-like cells of Bap1-null pancreata (Fig. 2g).

We reasoned that BAP1 ablation might trigger chronic tissue damage and pancreatitis due to defective DDR. Indeed, we found a strong increase in the number of cells positive for H2A.X phosphorylated at Ser139 (H2A.XSer139, Fig. 3a), which occurs in response to DNA damage by ATM, ATR, or DNA-PK kinases and signals the recruitment of DNA-repair proteins. We also detected an increase of cells with apoptotic DNA fragmentation (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL), Fig. 3b) and cleaved Caspase-3 (Fig. 3c). At the molecular level, meta-analysis of gene expression profiles of acute pancreatitis in caerulein-treated mice revealed that Bap1 and other DNA repair genes (Brca1/2 and Atm) were upregulated within hours in response to tissue damage (Supplementary Fig. 3a, b). Transcriptional activation of the endogenous Bap1 locus was also confirmed in caerulein-treated Bap1<lacZ+> mice (Supplementary Fig. 3c), suggesting that DDR is part of the physiological response for tissue regeneration. Consistently, caerulein-treated Bap1 heterozygous and knockout mice showed defective tissue regeneration, persistent activation of DDR, immune cell infiltration, and ductal metaplasia (Supplementary Fig. 3d-f).

**BAP1 restrains Kras<GI2D>-driven pancreatic cancer.** Targeted activation of Kras<GI2D> in murine pancreas induces PanINs, which progress to cancer with a long latency. In contrast, caerulein-induced pancreatitis accelerates Kras<GI2D>-driven PanIN progression to cancer as early as 1 month post treatment, suggesting that tissue inflammation establishes a pro-oncogenic milieu. We found that deletion and heterozygous loss of Bap1 in Pdx1<Cre>; Kras<GI2D> and Ptf1α<Cre>Kras<GI2D> mice accelerated cancer progression and shortened survival (Fig. 4a). Macroscopic inspection of knockout pancreata revealed cystic lesions of variable size in all mice (Supplementary Fig. 4a). Histology resembled MCNs with the presence of large multilocular cysts surrounded by ovarian type stroma (Fig. 4b). In several mice, we also observed a spectrum of IPMN-like lesions or mixed MCN-IPMN histology with an intraductal proliferation of mucin-producing cells, the formation of papillae, and cystic dilatation of the pancreatic ducts (Fig. 4b). On the other hand, heterozygous tumors showed features of both PDA and cystic alterations. Interestingly, although they retained the wild-type allele, IHC showed mosaic staining and downregulation of Bap1 in cystic lesions (Supplementary Fig. 4b). Thus, Bap1 restrains Kras<GI2D>-driven cell transformation and results in a higher frequency of IPMN and MCN histology. The controlled Bap1-knockout pancreata exhibited increases in histone H2A.K119Ub, loss of acini (Amylase), and gain of duct-like fates (Cytokeratin 17/19 and Sox9) (Supplementary Fig. 4c, d). MCNs were variably positive for Muc1 and Muc5AC, which correlate with a poor prognosis in humans, and less frequently for Muc2, a marker of goblet cell metaplasia (Fig. 4c). IPMNs were more frequently positive and stained stronger for Muc1 and Muc5AC, and exhibited marked cytological atypia and a complex cribriform architecture similar to gastric-like and pancreatobiliary IPMNs. Serial sectioning and histological analyses of all knockout animals revealed tumor invasion of...
pancreatic lymph nodes and micro-metastases in the liver (Supplementary Fig. 4e). Although with slower kinetics, all heterozygous mice also presented with lymph node invasion and distal metastases in the liver. Lung metastases were also observed in two mice (Supplementary Fig. 4f).

To confirm these findings, we examined the tumor histology and pathology reports through the Cancer Digital Slide Archive (http://cancer.digitalslidearchive.net) and found that although the majority of KRAS mutant TCGA-PAAD patients with heterozygous loss of BAP1 harbored mutations of TP53 and developed PDA, 6/7 patients with wild-type TP53 presented with pancreatic cancer arising from IPMNs and MCNs (Fig. 4d and Supplementary Fig. 4g). Patients with heterozygous loss of BAP1 had sporadic mutations of unknown significance in BRCA1/2, ATM, and SF3B1—which are very rare in the TCGA-PAAD cohort, and were mutual exclusive with mutations of GNAS, which is a known driver of cystic pancreatic neoplasms,36,37 (Supplementary Fig. 4h). All patients with wild-type KRAS and
TP53 that harbor heterozygous loss of BAP1 presented with IPMNs and MCNs (Supplementary Fig. 4i). Last, the only patient carrying a truncating mutation of BAP1 and oncogenic KRAS presented with extensive parenchyma inflammation and metaplastic alterations (Supplementary Fig. 4j).

Exclusively in Ptf1αCre;Bap1KO mice, which recombine predominantly in the acinar compartment and to a lesser extent in ductal cells, we observed structures resembling normal acini with cells containing large nuclei, prominent nucleoli, and a finely granular cytoplasm that contained Periodic Acid–Schiff (PAS)-

Fig. 2 BAP1 loss induces pancreatitis. a H&E staining of pancreata from the indicated genotypes and ages. Red arrow points to an area with loss of acinar architecture and black arrows the accumulation of eosinophilic zymogen material and luminal dilation of acini. b IHC for the indicated markers in wild-type and Bap1-knockout mice at 8–10 weeks of age. The Pdx1Cre strain expresses Cre recombinase in a stochastic pattern causing mosaic histological alterations due to loss of Bap1. c Scatter dot plots (mean ± SEM) show serum amylase and lipase levels in 25–30-week-old mice of the indicated genotypes. (Amylase: WT n = 6, HET n = 5, and KO n = 5 mice. Lipase: WT n = 6, HET n = 6, and KO n = 6 mice). d H&E staining of 30-week-old pancreata of the indicated genotypes. Asterisks indicate saponification. LN, lymph node. e Scatter dot plot (mean ± SEM) showing the percentage of affected area in 30-week-old pancreata of the indicated genotypes (WT n = 6, HET = 5, KO n = 8 mice). f IHC for CD45 (top) and Sirius Red staining (bottom) showing leukocyte infiltration and collagen deposition, respectively, into the pancreatic parenchyma of 30-week-old wild-type and Bap1-deficient mice. Arrows point to ducts. In c and e, statistical significance was determined by one-way ANOVA with p-values shown on the top of each plot, followed by Tukey’s multiple comparison post-hoc test between groups. **p < 0.01; ***p < 0.001. Source data are provided as a Source Data file.

Fig. 3 BAP1 loss triggers a DNA damage response. a H2A.X phosphorylated at Ser139 (WT n = 3, HET n = 4, KO n = 6 mice), b TUNEL (WT n = 3, HET n = 4, KO n = 5 mice), and c cleaved Caspase-3 IHC (WT n = 3, HET n = 3, KO n = 6 mice) in 8–10-week-old mice of the indicated genotypes. Arrows indicate positively stained cells. Right: scatter dot plots showing the number of positive cells (mean ± SEM) per 0.1 mm² of tissue per mouse. Each dot represents a mouse. Filled and open circles indicate mice from the Pdx1Cre and Ptf1αCre cohorts, respectively. Mice lacking the Cre allele were used as control for both cohorts. Statistical significance was determined by one-way ANOVA with p-values shown on the top of each plot, followed by Tukey’s multiple comparison post-hoc test between groups. ns, nonsignificant; **p < 0.01; ***p < 0.001. Source data are provided as a Source Data file.
positive, Diastase-resistant (PAS-D) cytoplasmic zymogen granules, a feature of ACC. Consistently, these lesions lacked mucin, Cytokeratin 17/19, and Sox9 expression, but were positive for the acinar markers, Amylase and Trypsin-2 (Fig. 4e, top panel). Likewise, in Ptf1αCre;Bap1KO;KrasG12D mice we observed small areas emulating ACC adjacent to PDA with glandular histology formed by well-differentiated cells in monolayers with basally located nuclei and eosinophilic cytoplasm (Fig. 4e bottom panel and Supplementary Fig. 4k). Although cells were variably PAS-D positive, they were negative for ductal and acinar markers, and may represent atypical manifestations, consistent with the finding that a small percentage of ACC stain negative for acinar
markers^{38,39}. The only patient diagnosed with ACC in the TCGA-PAAD cohort harbored heterozygous loss of BAP1 but had wild-type KRAS and TP53 (Fig. 4d and Supplementary Fig. 4l). These data suggest that BAP1 loss may also play a role in ACC.

**Loss of Bap1 causes genomic instability.** To delineate the molecular pathways, we analyzed the transcriptomes of cell lines established from Pdx1^{Cre;Kras^{G12D}} wild-type and Bap1-null mice. Consistent with the aggressive behavior of tumors in vivo, Bap1-knockout cell lines proliferated faster in vitro (Supplementary Fig. 5a). Affymetrix exon level analysis and western blotting confirmed the recombination of floxed exons and the absence of Bap1 protein (Supplementary Fig. 5b, c). The expression of PR-DUB and PRCI/2 members were unaltered (Supplementary Fig. 5c, d). Principal component and Ingenuity Pathway (IPA) analyses revealed pronounced changes in gene expression and enrichment for pathways regulating cell identity and proliferation (epithelial-to-mesenchymal transition, transforming growth factor-β (TGF-β), and mitogen-activated protein kinase signaling), metabolism and redox homeostasis (AMPK/mammalian target of rapamycin and NRF2 signaling), and DDR (p53, telomerase, and ovarian cancer signaling), which is frequently driven by mutations in BRCA1/2 (Fig. 5a, b). Likewise, IPA inferred inhibition of TGF-β, BRG1/SMARCA4—a chromatin regulator frequently inactivated in IPMNs^{40}, and GATA6—a transcription factor that regulates pancreatic fate^{41}. Consistent with the cystic and mucinous histology of Bap1-null tumors, gene expression changes were concordant with activation of transcription factors that drive the maintenance of secretory epithelia such as estrogen receptor and SPDEF (SAM pointed domain containing ETS transcription factor)—a TGF-β-repressed transcription factor that regulates goblet cell differentiation^{42}. Finally, expression of Cfr showed a more than tenfold downregulation in the Affymetrix arrays and was confirmed by quantitative reverse transcriptase-PCR in cell lines established from knockout pancreata (Supplementary Fig. 5e).

Next, we profiled PANC1 cells that were exposed to IR in the context of BAP1 downregulation (sgBAP1) mediated by CRISPR/Cas9. IR or sgBAP1 alone caused concordant changes in gene expression (~30% of differentially expressed genes, DEGs), whereas simultaneous perturbation had an additive effect (Fig. 5c, d). IPA showed enrichment for pathways regulating Epithelial-Mesenchymal Transition (EMT), metabolic and redox homeostasis, and DDR, in particular upon exposure to IR in the context of BAP1 deletion (Fig. 5d). Consistently, human and murine BAP1-deficient pancreatic cancer cell lines showed a pronounced sensitivity and activation of ATM/ATR kinases and H2AX phosphorylation upon different means of DNA damage (Supplementary Fig. 5f, g). Although BAP1-knockout cells harbored increased H2AK119Ub, this modification was largely unaffected by DNA damage, suggesting the role of BAP1 in DDR may be independent of the deubiquitination activity. Indeed, reconstitution of knockout cells with wild-type or a catalytically inactive mutant of BAP1 (BAP1^{C91A}) revoked the sensitivity to IR (Supplementary Fig. 5h). We also observed that although sorted cells were expressing a similar amount of wild-type and mutant protein, BAP1 halted proliferation leading to counter-selection upon passaging (Supplementary Fig. 5i). In contrast, BAP1^{C91A} conferred a proliferative advantage and drove positive selection even when cells were plated at low densities. Mutations abolishing the deubiquitinase activity of BAP1 are not detected in PDA patients but are found in other malignancies and may confer an oncogenic potential. Interestingly, knockdown of BAP1 in heterozygous SW1990 and PANC1 had the opposite effect and slightly reduced cell proliferation (Supplementary Fig. 5j). Given that both cell lines harbor additional defects in genes regulating DNA repair (Supplementary Fig. S1f), complete loss of BAP1 may sensitize and render cells unable to cope with accumulation of excessive DNA damage.

DNA DSBs are lesions that must be repaired before cell division ensues. BAP1 is required for efficient HR^{21,22}, a function that has been attributed to its interaction with BRCA1^{43}. Indeed, by using a green fluorescent protein (GFP)-based reporter assay employing a recognition site for the rare-cutting I-SceI endonuclease for induction of DSBs^{44}, we found that loss of BAP1 caused a substantial reduction of GFP-positive cells, indicating loss of proper DNA repair (Supplementary Fig. 5k). Consistently, metaphase chromosome spreads revealed a spectrum of abnormalities in knockout cells—even in the absence of oncogenic Kras—including chromosome breaks, shattering, premature sister chromatid separation with incompletely condensed chromosomes, and aneuploidy, which were exacerbated upon exposure to IR (Fig. 5e, f). Kras^{G12D} cells exhibited normal karyotypes that were disrupted upon BAP1 ablation. Deletion of BAP1 in HEK293T cells and exposure to IR also led to pronounced chromosomal abnormalities (Fig. 5g), suggesting an essential role in maintaining genome integrity. Furthermore, we detected a defective expression of PALB2 foci, a scaffold protein in the BRCA1–PALB2–BRCA2 complex, following DNA damage induced by IR. No alterations were observed in 53BP1 foci formation, a protein that regulates non-homologous end joining (Supplementary Fig. 5l). Although we failed to detect a direct interaction with BRCA1, we found that BAP1 directly interacts with the tandem ankyrin domains of BARD1 (BRCA1-associated RING domain 1) (Fig. 5h), a protein that interacts with BRCA1 to regulate DNA repair mediated by HR and protection of nascent DNA at stalled replication forks^{45–47}. **BAP1 regulates the response to DNA damage.** To systematically study and causatively link epigenetic alterations with gene expression changes, we mapped the distribution of H3K4me3, H3K27me3, H2AK119Ub, and H2K120Ub in wild-type and knockout cells. Peak calling followed by K-means clustering and
genome-wide pairwise comparison of the normalized signal intensity over the transcription start site (TSS) revealed increases of repressive H2AK119Ub and H3K27me3 (cluster I in Fig. 6a and Supplementary Fig. 6a), particularly over DEGs (Fig. 6b). Besides genes driving pancreas cell fate (Sox17), Cluster I also contained members of TGF-β (Tgfβ1/2) and genes (Gnas and Rnf43) frequently deregulated in IPMNs and MCNs, as well as Cftr. In contrast, no significant alterations were observed in the distribution and signal intensity of H3K4me3 and H2BK120Ub, nor in H3K4me1 and H3K27ac that define poised and active super-enhancers, respectively (Supplementary Fig. 6b). Multiple promoters with a gain of H3K27me3 in knockout cells were pre-
marked with H3K4me3 and the de novo bivalent genes were enriched for pathways regulating cell-fate decisions and metabolic homeostasis, but not DDR (Fig. 6c). Loss of BAP1 increased the intensity of both H2AK119Ub and H3K27me3, and repressed the majority of DEG (66.2% downregulated vs. 33.8% upregulated, Fig. 6d). Hence, BAP1 primarily functions as a transcriptional coactivator by removing repressive H2AK119Ub.

Next, we mapped the genome-wide binding of BAP1 and distribution of H2AK119Ub in PANC1, which exhibits heterozygous loss of BAP1 (Supplementary Fig. 1e), as well as in immortal, but not transformed, human pancreatic ductal endothelial (HPDE) cells, in response to IR. K-means clustering revealed that BAP1 binds active chromatin enriched for H3K4me3, H3K36me3, and active RNA polymerase II phosphorylated at Ser5 (POLR2A Ser5), but is depleted for repressive H3K27me3 and H2AK119Ub (cluster II in Fig. 6e and Supplementary Fig. 6c). BAP1 and H2AK119Ub signal intensities were highly concordant in the two cell lines and largely unaffected by IR (Supplementary Fig. 6d, e), further supporting that gene expression changes triggered by IR are independent of the catalytic activity of BAP1. Consistently, IPA of genes bound by BAP1 showed significant overlap between PANC1 and HPDE, and enrichment for pathways and upstream regulators of DDR such as protein ubiquitination, ATM, and BRCA1 signaling pathways among others, particularly in response to DNA damage (Fig. 6e, f).

**BAP1 loss confers sensitivity to DNA damage treatments.** To test the response to DNA damage, murine wild-type, heterozygous, and knockout cells as well as human PDA cell lines treated with shBAP1 were exposed to cisplatin (a DNA cross-linker) and camptothecin (topoisomerase I inhibitor). We found that loss of BAP1 conferred an increased sensitivity (Fig. 7a and Supplementary Fig. 7a). Reconstitution of knockout cells with wild-type or mutant BAP1 Cry1 abolished sensitivity, suggesting a deubiquitinase independent function (Fig. 7b). On the other hand, knockout cells were resistant to gemcitabine, a nucleoside analog that is commonly used for treating pancreatic cancer (Supplementary Fig. 7b). Meta-analysis of pharmacogenomic data from the Cancer Cell Line Encyclopedia through the CellMinerCDB portal revealed that low BAP1 expression correlated with sensitivity to irinotecan (derivative of camptothecin) specifically in human pancreatic cancer cell lines and linear regression analysis among DNA repair genes highlighted BAP1 in explaining the response (Supplementary Fig. 7c, d). Similarly, BAP1-null mouse and human pancreatic cancer cell lines were sensitive to IR and failed to form colonies when plated at low densities (Supplementary Fig. 7e).

**Discussion**

Chronic pancreatitis is a risk factor for the development of pancreatic cancer and has been linked to alcohol intake, smoking, and poorly defined genetic factors. Unlike hereditary pancreatitis due to mutations in genes that regulate exocrine pancreas homeostasis, such as **CFTR**, somatic CNAs that predispose to chronic inflammation and pancreatitis remain unknown. We found that loss of BAP1 leads to the development of chronic pancreatitis in mice and is associated with poor prognosis in PDA patients. Notably, **Bra2** inactivation also promoted an inflammatory response and disrupted exocrine pancreas homeostasis in mice, and **BRCA1** downregulation has been documented in patients diagnosed with chronic pancreatitis. Likewise, DNA repair genes, including **Bap1**, are readily upregulated and required for pancreas regeneration in caerulein-treated mice. Although further proof is needed, these data suggest that defective DDR may be a common denominator in a subset of chronic pancreatitis cases and causatively linked to a higher risk for PDA.

Individuals with germline mutations in DNA repair genes have a higher risk of developing PDA. Although over 80% of familial pancreatic cancer cases harbor mutations in **BRCA1/2** and ATM, germline deletion and mutations of BAP1 have also been observed. On the other hand, somatic mutations of DNA repair genes are rare (<5% of patients), which is counter-intuitive given that recent sequencing studies showed a transcriptional signature of defective DDR in about 25% of PDA patients. We found that somatic heterozygous loss of BAP1 may account for this discrepancy, as it is the most frequent CNA among DNA repair genes occurring in over a quarter of PDA and 40% of **ACC**. Although LOH of additional genes within
Fig. 6 BAP1 binds active chromatin and regulates the response to DNA damage. 

a. Composite heatmap showing the K-means clustering (K = 6) of the genome-wide distribution and signal intensity of the indicated histone modifications in Pdx1Cre;KrasG12D wild-type and Bap1-knockout cells. Each horizontal line represents the normalized signal intensity for a gene over its transcription start site (TSS). A ±10 kb window is shown for each TSS. The grayscale bar shows the normalized RPKM.

b. Heatmap showing the signal intensity of the indicated histone modifications over the DEG (fold change > 1.4 and p < 0.05) in Pdx1Cre;KrasG12D wild-type and Bap1-knockout cells. The y axis shows the mean RPKM. The color bar represents DEG sorted based on the fold-change difference in gene expression between knockout and wild-type cells.

c. Composite heatmap showing the overlap of the indicated histone modifications in Pdx1Cre;KrasG12D wild-type and Bap1-knockout cells. Each horizontal line represents a gene. The grayscale bar shows the normalized RPKM.

d. Heatmap showing the signal intensity of the indicated histone modifications over the DEG (fold change > 1.4 and p < 0.05) in Pdx1Cre;KrasG12D wild-type and Bap1-knockout cells. The y axis shows the mean RPKM. The color bar represents DEG sorted based on the fold-change difference in gene expression between knockout and wild-type cells.

e. Composite heatmap showing the overlap of genes bound by BAPI in HPDE and PANC1 before and after IR (10 Gy). The grayscale bars show the normalized RPKM. The x axis corresponds to the raw binomial p-values.

f. Venn diagram showing the overlap of genes bound by BAPI in HPDE and PANC1 before and after IR (10 Gy). The x axis corresponds to the raw binomial p-values.
Fig. 7 BAP1 deficiency confers radio- and chemo-sensitivity. a Estimation of IC50 values for cisplatin (left) and camptothecin (right) for wild-type (n = 3), heterozygous (n = 3), and Bap1-knockout (n = 3) pancreatic cell lines independently established from the Pdx1Cre;KrasG12D cohort and assessed in triplicates. The average IC50 values (mean ± SEM) are shown. Statistical significance was determined by one-way ANOVA with p-values shown on the top of each plot. b Estimation of IC50 values for cisplatin (left) and camptothecin (right) for a Bap1-knockout pancreas cell line reconstituted with wild-type and BAP1C91A mutant. The graphs show cell viability assessed in triplicates (mean ± SEM) for the indicated concentrations of compounds. c, d 10–15-week-old mice of the indicated genotypes were exposed to 10 Gy of IR. Three days later, mice were killed and pancreata were stained for (c) H2AXSer139 (WT n = 4, HET n = 4, KO n = 5, and TP53KO n = 3 mice) and (d) TUNEL (WT n = 3, HET n = 3, KO n = 4, and TP53KO n = 3 mice). e 6–10-week-old mice of the indicated genotype (WT n = 3, HET n = 4, and KO n = 5 mice) were treated weekly for 4 weeks with 5 mg/kg cisplatin. Mice were killed and pancreata were stained for H2AXSer139. In c–e, the scatter dot plots show the number of positive cells (mean ± SEM) per 0.1 mm2 of tissue per mouse. Each dot represents a mouse. Black arrows point to positively stained cells. Filled and open circles indicate mice from the Pdx1Cre and Ptf1αCre cohorts, respectively. Statistical significance was determined by one-way ANOVA with p-values shown on the top of each plot, followed by Tukey’s multiple comparison post-hoc test between groups. ns, nonsignificant; ***p < 0.001. Source data are provided as a Source Data file.
Chr 3p21.1 may contribute to tumor suppression, and loss of BAP1 in pancreatic cancer may be secondary—rather than the cause of genomic instability—our mouse model suggests BAP1 ablation suffices to cause defective DNA repair, pancreatitis, and cooperates with oncogenic Kras to promote pancreatic cancer. Thus, downregulation of BAP1 alleviates a barrier to pancreatic cancer progression, but at the same time its low expression maintains basal DNA repair mechanisms protecting rapidly dividing cancer cells from the catastrophic consequences of uncontrolled accumulation of DNA damage. Although deletion of Bap1 in the context of KrasG12D induced MCNs and IPMNs, in Ptf1aCre<sup>-</sup>-knockout pancreata we also observed areas with features of acinar cell transformation. Although not yet comprehensive, human ACC exhibits frequent loss for BAP1 and other DNA repair genes in more than half of the cases<sup>24,25</sup>. Acinar tumors have been reported in murine pancreata with biallelic Brc2 mutations<sup>26</sup>. Altogether, defective DDR may drive ACC and simultaneous inactivation of multiple genes involved in HR may be required to phenocopy this rare pancreatic cancer subtype accurately.

Detecting biomarkers of responsiveness to chemotheraphy and IR can significantly improve treatment and clinical outcomes. BAP1-deficient pancreatic cancer showed numerical and structural chromosomal abnormalities and sensitivity to IR and compounds that cause replication fork stalling, such as cisplatin and camptothecin. Derivatives of cisplatin and camptothecin (oxaliplatin and irinotecan, respectively) are components of FOLFIRINOX which is emerging as a first-line treatment for pancreatic cancer<sup>21</sup>, whereas oxaliplatin showed efficacy as a single agent in ACC<sup>54</sup>. Although defective DDR is causative and permissive of pancreas oncogenesis, it may also uncover an Achilles heel to be exploited therapeutically. Mechanistically, BAP1 regulates the repair of DNA DSBs by HR and directly interacts with BAD1, a protein that forms a heterodimer with BRCA1 to regulate HR and stalled fork protection<sup>15–47</sup>. The latter is crucial in protecting stalled DNA replication forks in rapidly dividing cancer cells from nucleolytic degradation and may explain the sensitivity to platinum-based compounds and topoisomerase inhibitors<sup>55,56</sup>. Given that heterozygous loss of BAP1 is the most frequent somatic aberration among DNA repair genes in pancreatic cancer, our findings establish a rationale for evaluating BAP1 status to stratify patients who are likely to respond to FOLFIRINOX and radiotherapy.

**Methods**

**Study approval.** Mouse experiments were conducted under protocols A292, A293, and A308, which were reviewed and approved by the Institutional Animal Care and Use Committee of the George Washington University, Washington DC. Commercially available de-identified human TMAs were bought from the Cooperative Human Tissue Network (CHTN, which is funded by the National Cancer Institute; www.chtn.org) and Biomax (www.biomax.us/). The CHTN_Pancreas TMA contains a series of normal, premalignant (low- and high-grade PanIN), pancreatic cancer patients. Redundant patient specimens among the Biomax TMA and Use Committee of the George Washington University, Washington DC.

**Immunohistochemistry.** Pancreata and liver were formalin fixed, paraffin embedded, and sectioned on a Leica RM2165 microtome. Four-micrometer tissue sections were de-paraffinized in xylanes, rehydrated sequentially in ethanol, washed in 0.5% Triton X-100 in PBS, rinsed in water, and submerged in citrate buffer (pH 6.0) to unmask antigens using the Retriever 2100 (Aptum), according to manufacturer’s protocol. Then, slides were incubated with 3% H<sub>2</sub>O<sub>2</sub> to block endogenous peroxidase activity, washed, and blocked with 10% normal horse serum for 1 h. Primary antibodies were applied overnight at 4°C. The next day, slides were washed, incubated with biotinylated secondary antibodies for 1 h at room temperature, treated with Vectastain<sup>®</sup> Elite ABC-HP Kit for 30 min, developed with the DAB substrate kit (Vector Laboratories), counterstained with hematoxylin, and mounted with Dibutylphthalate Polystyrene Xylene (DPX). For BAP1 IHC, Buffer B (pH 8.0) was used for antigen retrieval. For MUC2 and MUC5AC IHC, the Vector M.O.M. Basic Immunodetection kit (Vector Laboratories, Burlingame, CA) was used according to the manufacturer’s protocol. For Sirius Red staining, slides were placed flat in a chamber, 200–300 μl of Sirius Red/Fast green stain was added to cover tissues, and stained for 30 min. Slides were thoroughly rinsed in water and briefly dehydrated through sequential alcohols, cleared in xylanes, and mounted with DPX. The PAS-D staining was performed at the pathology research facilities of the George Washington University (https://naph.webcast.gwu.edu). In situ hybridization for BAP1 nuclear staining was based on the intensity as “absent, weak, or strong” and determined with the Zen lite 2012 software. The chi-square test was used to determine whether there was a significant difference between the expected and observed frequencies in one or more categories. Supplementary Table 2 provides the catalog numbers and dilutions of antibodies.

**X-gal tissue staining.** Pancreata were rinsed in PBS and fixed in 4% paraformaldehyde for 1 h at room temperature, then washed once with PBS and twice in staining buffer (2 mM/L MgCl<sub>2</sub>, 0.01% Na-Deoxycholate, 0.02% NP-40 in PBS) for 15 min each at room temperature. Pancreata were stained overnight at 37°C in X-gal staining solution (1 mg/ml X-gal, 5 mM/L K<sub>4</sub>Fe(CN)<sub>6</sub>, and 5 mM/L K<sub>3</sub>Fe(CN)<sub>6</sub> in staining buffer), followed by three washes in PBS for 15 min each, fixed in 10% neutral buffered formalin overnight, and paraffin embedded. Four-micrometer sections were de-paraffinized in two changes of xylanes and rehydrated sequentially in ethanol. Sections were counterstained in nuclear fast red (Amresco), dehydrated sequentially in ethanol, cleared in two changes of xylanes, and mounted with DPX.

**TUNEL assay.** Click-iT<sup>™</sup> TUNEL Colorimetric IHC Detection Kit (Invitrogen) was used for detecting cell apoptosis according to the manufacturer’s protocol. Briefly, pancreatic tissue sections were de-paraffinized, rehydrated sequentially, washed with PBS, fixed at room temperature in 4% paraformaldehyde for 15 min, digested with proteinase K for 15 min, and finally fixed at room temperature in 4% paraformaldehyde for 5 min. The sections were incubated with the TdT reaction mixture at 37°C for 1 h in a humidity chamber. The TdT reaction was quenched by incubating the slides in 2x Sodium Sodium Citrate (SSC) buffer for 15 min and endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide for 5 min. The slides were then incubated in the TUNEL Colorimetric reaction cocktail at 37°C for 30 min in a humidity chamber. After developing with DAB, slides were counterstained in hematoxylin, dehydrated sequentially in ethanol, then cleared in two changes of xylanes, and mounted with DPX. Nuclei of cells stained brown were regarded as positive apoptotic cells by TUNEL staining.

**Histomorphometry.** All slides were photographed with a Zeiss Axioslab.A1 and AxiosCam ICS<sup>®</sup> camera, and analyzed with the Zen light 2012 software as indicated in the figure legends. To quantify the number of cells responding to different treatments sections were photographed at ≥20 or ×40 magnification as indicated in the figure legends, the tumor area was delimited with the ‘spine contour’ tool of the Zen light 2012 and calculated by the software. Data are presented as the number of positive cells per tumor area (mm<sup>2</sup>) ± SEM. The ‘spine contour’ tool was also employed to quantify the area of pancreas with signs of pancreatitis.

**Glucose, amylase, and lipase measurements.** Blood glucose levels (mg/dL) were quantified using the Bayer Contour Next One Glucose Meter. Mice were either fed ad libitum or fasted overnight. Levels of amylase and lipase in the blood were measured using as reference the ethylidenep-NP-G (catalog # MAK009, Sigma-
Aldrich) and glycerol (catalog # M0464, Sigma-Aldrich) substrates in kinetic activity assays, respectively. Serum (2 μL) was diluted in to 1:1 ratio with normal saline and mixed with either the amylase or lipase assay buffer to initiate the reaction. The increase in absorbance due to the release of p-nitrophenol and glycerol was monitored at 405 nm and 570 nm for amylase and lipase, respectively. Activity was recorded as nmole/min/ml and reported as Units/Liter (U/L). One unit of amylase is the amount of the enzyme that cleaves ethylenedine-pNP-G7 to generate 1.0 μmol of p-nitrophenol per minute at 25 °C. One unit of lipase is the amount of enzyme that will generate 1.0 μmol of glycerol from triglycerides per minute at 37 °C.

**Human and murine pancreatic cancer cell lines.** Human pancreatic cancer cell lines were bought from American Type Culture Collection and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. For murine cell lines, tumor-bearing pancreatic mice were killed, collagenase digested, and single-cell suspensions were plated on collagen-coated (rat tail type I) plate.40 Primary murine cell lines were maintained in DMEM/F12 without phenol red, 5 mg/mL d-Glucose, 12.1 mg/mL Nicotinamide, 100 mg/mL Cholera toxin, 5 μL/ML Insulin-Transferrin-Selenium Plus, 0.1 mg/mL soybean trypsin inhibitor, 20 ng/mL epidermal growth factor, 5% Nu-Serum IV culture supplement, 25 μg/mL bovine pituitary extract, 5 mM 3,5,3,5-tri-iodo-l-thyronine, 1 μM dexamethasone, 1% Pen/Strep, and plasmocin (InvivoGen). Two to three weeks later, collagen was removed and cells were expanded in 75% pancreatic media/25% complete DMEM. Early passage cell lines were used for all in vitro experiments and were maintained up to 2-3 passages, prior to freeze-triplicate in 12-well plates and passage to 1:2 or 1:4 ratios. Cells were counted with a Bio-Rad TC20 automatic cell counter. For reconstitution of murine Bap1 knockout pancreatic cancer cell lines retroviruses encoding BAP1-WT or BAP1-C91A (Addgene #81024 and #81025, respectively)62 were generated by transfecting C91A (Addgene, #81024 and #81025, respectively) targeting exon 2 or non-targeting guide RNA and homologous recombination repair assay. Wild-type and BAP1 knockout cells were transfected in duplicate with plasmids expressing the HR substrate composed of two differentially mutated GFP genes oriented as direct repeats (pDRGFP; Addgene #28475) and I-SceI endonuclease expression vector (pCBASceI; Addgene #28476) to activate expression of I-SceI. The cleaved DNA segments generated by I-SceI were inserted into the mutated GFP genes, which, when repaired by gene conversion, results in a functional GFP gene. Two days later, cells were collected for flow cytometry in a BD FACSCelesta to determine the percentage of GFP-positive cells. Data analyses were performed with FlowJo software (TreeStar). To adjust for different transfection efficiencies between cell lines, data were normalized by using a GFP reporter plasmid transfected in parallel.

**Gene expression analysis.** Total RNA was isolated using the RNeasy spin column kit (Qiagen) and quantified using a BioSpectrometer (Eppendorf). One hundred nanograms of RNA was used as input for the GeneChip® PrimeView™ (3′ IVT human array) and GeneChip® Mouse Gene 2.0 ST (mouse exon array) microarrays (Affymetrix). Synthesis, labeling, and purification of biotinylated complementary RNA and sense-stranded DNA targets were carried out according to manufacturer’s instructions using the GeneChip® 3′ IVT PLUS Reagent Kit and GeneChip® WT PLUS Reagent Kit, respectively. Hybridization was performed using a GeneChip® Hybridization Oven 640 overnight at 45 °C. Microarray washing and staining was performed on a GeneChip Fluidics Station 430 and scanning on a GeneChip Scanner 3000 7G, commanded by the Affymetrix GeneChip Command Console software. Probe-level analysis including background subtraction and quantile normalization took place with the Robust Multi Array Average Algorithm using the Affymetrix Expression Console Software 1.3. DEGs (p < 0.05 and fold change >1) from the GeneChip® Gene 2.0 ST array and p < 0.01 and fold change > 1.5 for the GeneChip® PrimeView™ were determined using the Transcriptome Analysis Console v3.0. Raw and processed Affymetrix data have been deposited in the Gene Expression Omnibus repository under accession GSE120127.

**Ingenuity Pathway analysis.** DEGs were used as input for IPA to infer canonical pathways and upstream regulators that could explain gene expression changes caused by loss of BAP1 for z-scores >2 and a p-value <0.01. The positive or negative sign of z-score indicates activation or repression, respectively, of a particular canonical pathway or upstream regulator in the context of BAP1 loss. A Fisher’s exact test was used to determine the statistical significance.

**Chromatin immunoprecipitation.** Cells (1 x 10⁷) were cross-linked in 1% formaldehyde for 20 min, quenched with 125 mM glycine for 5 min, and lysed in ice-cold 1% SDS, 1% Triton X-100 in PBS (pH 7.4) and spun down at 4 °C, 16,000 x g for 10 min on ice. Nuclei were pelleted, digested with 1 μl micrococcal nuclease for 3 min at 37 °C to fragment chromatin, resuspended in SDS lysis buffer (50 mM Tris-HCl pH 8.0, 1% SDS, 150 mM NaCl, and 5 mM EDTA), and briefly sonicated. Cells were centrifuged 16,000 x g for 10 min at 4 °C, and ten volumes of dilution buffer (16.7 mM Tris pH 8.0, 167 mM NaCl, 0.1% SDS, 1% Triton X-100, and 1.2 mM EDTA) was added to the sample fraction. Chromatin immunoprecipitation (ChIP-Seq) took place on protein A agarose beads with antibodies against H3K4me1, H3K4me3, H3K27me3, H3K27ac, H3K219ub9, H2BK210ub, and BAP1 (Cell Signaling, Supplementary Table 2) overnight at 4 °C. Beads were washed twice with 100 mM HEPES, pH 7.4, 1 mg/mL MgCl₂, 0.5% Nonidet P40, and low-salt buffer with: low-salt buffer (50 mM HEPES, pH 8.0, 10 mM MgCl₂, 0.1% SDS, 1% Triton X-100, and 2 mM EDTA), high-salt buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1% SDS, 1% Triton X-100, and 2 mM EDTA), LiCl buffer (10 mM Tris-HCl pH 8.0, 0.25 M LiCl, 1% Igepal CA-630, 1% Na-Deoxycholate, and 1 mM EDTA), and TE (10 mM Tris pH 7.5, 1 mM EDTA). DNA–protein complexes were eluted with 2× cross-link reversal buffer at 95 °C for 5 min, and analyzed by western blotting. Supplementary Table 2 provides the catalog numbers and dilutions of antibodies.
1 h at 42 °C and cleaning of the eluates took place with the QIAquick PCR Purification Kit (Qiagen). Libraries were prepared with the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs) and sequenced on an Illumina NextSeq 2000 instrument.

**ChiP-seq data analysis.** The FastQC (Version 0.10.1) software was used to examine the read quality. Reads were aligned to the mouse (mm9) or human (GRCh37/hg19) genome using Bowtie (Version 1.1.1 [25]) with default parameters and keeping only uniquely aligned reads. SICER (Version 1.1 [26]) was used for peak calling to identify chromatin domains enriched for BAP1 binding and histone modifications. Several read counts, which are expected to be the result of PCR artifacts, were filtered before peak calling. Window size = 200 bp and gap size = 200 bp was used for H3K27ac, H3K4me3, and BAP1, and window size = 600 bp and gap size = 600 bp for H3K27me3, H3K4me1, H2A.K191Ub, H2B.K120Ub. False discovery rate threshold was set to 1e − 8 for all histone modifications and 1e − 5 for BAP1. For peak annotation, we employed the RegSeq transcripts from the UCSC genome browser with a ±2 kb window spanning the TSS. Genome-wide comparison and K-means clustering of signal intensity as well as plotting the read density profile over the TSS and SE took place with EaSeq [27] software. Island-filtered reads from SICER were used as input. For calling super-enhancers, we employed ROSE (Rank Ordering of Super-Enhancers) [28] software using H3K27ac ChIP-Seq signal as input and default parameters. The GREAT (version 3.0.0) online tool was used to identify genes linked to SE based on the association rule: Basis+extension: 1 kb upstream, 1 kb downstream, 1000 bp max extension. Raw and processed data from ChiP-seq experiments have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE120460 [29]. Raw and processed data from human ChiP-seq data sets for BAP1 and H2A.K191Ub have been deposited in the Gene Expression Omnibus repository under accession number GSE121709 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121709). Raw and processed data from human ChiP-seq data sets for BAP1 and H2A.K191Ub have been deposited in the Gene Expression Omnibus repository under accession number GSE120460 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120460). Raw ChiP-seq data for H3K4me3 (ENCFF46XSSZ), POLR2Aser5 (ENCFF06FNP2), H3K36me3 (ENCFF356QYS), and H3K27me3 (ENCFF072KNY) for PANC1 were downloaded from ENCODE (https://www.encodeproject.org/).

**Induction of DNA damage and drug sensitivity.** Cells were exposed to 10 Gy of IR (157 Cs source), camptothecin (topoisomerase I inhibitor), cisplatin (DNA crosslinking agent), gemcitabine, and PARP inhibitors (olaparib, 3-ABA, and PJ-34) as indicated in the figure legends. The FastQC (Version 0.10.1) software was used to align the reads, which are expected to be the result of PCR artifacts, were filtered before peak calling. Window size = 200 bp and gap size = 200 bp was used for H3K27ac, H3K4me3, and BAP1, and window size = 600 bp and gap size = 600 bp for H3K27me3, H3K4me1, H2A.K191Ub, H2B.K120Ub. False discovery rate threshold was set to 1e − 8 for all histone modifications and 1e − 5 for BAP1. For peak annotation, we employed the RegSeq transcripts from the UCSC genome browser with a ±2 kb window spanning the TSS. Genome-wide comparison and K-means clustering of signal intensity as well as plotting the read density profile over the TSS and SE took place with EaSeq [27] software. Island-filtered reads from SICER were used as input. For calling super-enhancers, we employed ROSE (Rank Ordering of Super-Enhancers) [28] software using H3K27ac ChiP-Seq signal as input and default parameters. The GREAT (version 3.0.0) online tool was used to identify genes linked to SE based on the association rule: Basis+extension: 1 kb upstream, 1 kb downstream, 1000 bp max extension. Raw and processed data from ChiP-seq experiments have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE120460 [29]. Raw and processed data from human ChiP-seq data sets for BAP1 and H2A.K191Ub have been deposited in the Gene Expression Omnibus repository under accession number GSE121709 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121709). Raw and processed data from human ChiP-seq data sets for BAP1 and H2A.K191Ub have been deposited in the Gene Expression Omnibus repository under accession number GSE120460 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120460). Raw ChiP-seq data for H3K4me3 (ENCFF46XSSZ), POLR2Aser5 (ENCFF06FNP2), H3K36me3 (ENCFF356QYS), and H3K27me3 (ENCFF072KNY) for PANC1 were downloaded from ENCODE (https://www.encodeproject.org/).
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Acknowledgements

We thank Dr. H Pei (GWU) for providing the BARD1 expression constructs, the Welcome Trust Sanger Institute Mouse Genetics Project and its funders for providing the Bap1 mutant mouse line, and the European Mouse Mutant Archive (www.emmanet.org) partner EUCOMM from which the mouse line was received. S.P. was supported by the Institute for Biomedical Sciences (GWU). This work was supported by a seed grant from the Hirshberg Foundation for Pancreatic Cancer Research, the GW Cancer Center, the Elaine Snyder Cancer Research Award, and National Cancer Institute grants R01CA158582, R21CA182662, R03CA212068, R03CA219523-01A1, and R01CA222930-01A1 to AT.
Author contributions
S.P. and A.T. designed and performed the experiments. J.A. performed the Affymetrix gene expression studies and contributed in data analysis. S.P., Y.K., and A.T. performed data mining and bioinformatics analyses. A.T. supervised the project. All authors discussed the results and commented on the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-16589-8.

Correspondence and requests for materials should be addressed to A.T.

Peer review information Nature Communications thanks Marina Pasca di Magliano and the other, anonymous reviewer(s) for their contribution to the peer review of this work.

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