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Accessibility
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Germ-line mutations in PALB2 lead to a familial predisposition to breast and pancreatic cancer or to Fanconi Anemia subtype N. PALB2 performs its tumor suppressor role, at least in part, by supporting homologous recombination-type double strand break repair (HR-DSBR) through physical interactions with BRCA1, BRCA2, and RAD51. To further understand the mechanisms underlying PALB2-mediated DNA repair and tumor suppression functions, we targeted Palb2 in the mouse. Palb2-deficient murine ES cells recapitulated DNA damage defects caused by PALB2 depletion in human cells, and germ-line deletion of Palb2 driven by K14-Cre led to mammary tumor formation with long latency. Codelletion of both Palb2 and Tumor protein 53 (Trp53) accelerated mammary tumor formation. Like BRCA1 and BRCA2 mutant breast cancers, these tumors were defective in RAD51 focus formation, reflecting a defect in Palb2 HR-DSBR function, a strongly suspected contributor to BRca1, BRca2, and Palb2 mammary tumor development. However, unlike the case of Brca1-mutant cells, Trp53bp1 deletion failed to rescue the genomic instability of Palb2- or Brca2-mutant primary lymphocytes. Therefore, Palb2-driven DNA damage control is, in part, distinct from that executed by Brca1 and more similar to that of Brca2. The mechanisms underlying Palb2 mammary tumor suppression functions can now be explored genetically in vivo.

Results and Discussion

Targeting the Mouse Palb2 Gene and Generation of Palb2-Deficient ES Cells. To generate a Palb2 allele that could be conditionally inactivated upon Cre recombinase expression, we insertedloxP sites flanking exons 2 and 3 of the Palb2 gene (Fig. S1 A and B). These exons encode a putative nuclear localization signal sequence and the PALB2 coiled-coil domain (Fig. 1A, Fig. S1 A). The latter mediates the PALB2 interaction with BRCA1 (5, 6). Deletion of these exons would result in out-of-frame reading of exon 4 and premature termination of the PALB2 translation before the BRCA2-interacting, seven-bladed WD40-type β-propeller domain (Fig. 1A, Fig. S1 A). Due to premature truncation...
the Palb2 ORF, the resulting transcript is also a candidate for degradation via nonsense-mediated decay.

Targeting of the Palb2 locus and integration of bothloxP recombinase sites was confirmed by Southern blot analysis (Fig. S1C). Heterozygous ES cells (Palb2loxfl) were injected into blastocysts, and the resulting chimeras from two individual clones were bred to either Fip-deleter mice (to eliminate the Frt-flanked neomycin resistance cassette and generate a conditional allele) or Cre-deleter mice (to generate a conventional Palb2 KO allele). Germ-line transmission of the Palb2lox allele occurred from nearly all chimeras, and mice were successfully genotyped for the Flp- and the Cre-recombined alleles (Palb2fl or Palb2+, respectively).

We attempted to derive ES cells from the Palb2fl allele. Three, independent Palb2fl ES cell lines were derived from a single heterozygous Palb2fl cross. Expression analysis of Palb2 mRNA by quantitative real-time RT-PCR (qRT-PCR) confirmed that one of these ES lines was Palb2flfl and the other two were Palb2fl− (Fig. S1D). The loss of full-length Palb2 expression from these lines was further confirmed by Western blotting, using a polyclonal antimalouse PALB2 antibody raised against the N-terminal 200 residues of the mouse PALB2 protein (Fig. 1B). Therefore, Palb2 loss did not prevent ES cell derivation and subsequent survival. The three ES cell lines we derived were morphologically comparable, proliferated at the same rate as wild-type (WT) ES cells and were capable of differentiation into embryoid bodies.

ES cells that are deficient for Brca1 or Brca2 have been notoriously difficult to isolate and are severely compromised in their proliferation (17, 18). In keeping with these findings, Palb2−/− ES cells could not be derived from embryos carrying a conventional PALB2 gene-trap allele (19). Because PALB2 operates immediately upstream of BRCA2 and is required for BRCA2 localization at DNA double strand breaks, it is possible that the viability and robustness of our Palb2−/− ES cells were due to residual expression of a truncated PALB2 species. Such a polypeptide could, in theory, result from translation initiation downstream of the engineered Palb2 genomic deletion. No such truncated protein was detected with our polyclonal antibody (Fig. 1B).

To test whether the conditional gene targeting approach that was used had generated an allele that would be rendered null after Cre action, the response of Palb2flfl and Palb2fl−/− ES cells to DNA damaging agents that cause double strand breaks was analyzed. Normally, exposure of PALB2-proficient cells to ionizing radiation (IR) leads to the formation of phosphorylated histone H2AX (γH2AX) nuclear foci and subsequent recruitment of BRCA1, BRCA2, and RAD51 to these structures. As expected, after exposure to IR, γH2AX and BRCA1 IRIF formation was unaffected in Palb2−/− cells (Fig. 1C, Fig. S2A). However, the recruitment of RAD51 was severely compromised (Fig. 1C). This defect was also evident at the biochemical level because no increase in chromatin loading of RAD51 after IR could be detected in nuclear extracts of IR-treated Palb2−/− cells (Fig. 1D).

Because biallelic PALB2 mutations in humans cause Fanconi anemia, a hallmark of which is increased sensitivity to DNA cross-linking agents such as mitomycin C (MMC), the sensitivity of Palb2−/− ES cells to MMC as well other DNA damaging agents was assayed. Both Palb2−/− ES lines displayed increased sensitivity to MMC, IR, and the radiomimetic drug, neocarzinostatin (Fig. 1E, Fig. S2 B and C). These findings further imply that these Palb2fl−/− cells are functional KOs for Palb2 because they are compromised in multiple, known Palb2-associated functions. Thus, upon Cre-mediated recombination in vivo, the aforementioned conditional Palb2 allele appears to be converted to a Palb2-null allele.

Loss of Palb2 in the Germ Line Results in Early Embryonic Lethality. Germ-line deletion of Brca1 or Brca2 results in early embryonic lethality (17, 20, 21). Although Palb2fl−/− ES cells displayed no apparent growth defects compared with Palb2flfl controls, Palb2fl−/− loss could still be deleterious in differentiated progeny cells, and thereby negatively affect mouse development. Indeed, we were unable to obtain Palb2−− mice from heterozygous crosses (Fig. S3A), thereby consistent with previous reports (19, 22). Dissection of embryos from timed pregnancies revealed that Palb2-null embryos could be recovered only up to E12.5, but even then at sub-Mendelian ratios. These embryos repeatedly exhibited severe malformations. At earlier time points, morphological aberrations of Palb2−− embryos were less obvious. However, these mutant embryos were clearly smaller than WT or heterozygous littermate embryos (Fig. S3F), and some displayed exencephaly as well as malformations of the placental labyrinth and yolk sac-associated blood islets (Fig. S3 C–F). The fact that Palb2 nullizygosity resulted in embryonic lethality detectable at E8.5–E10.5 is consistent with earlier reports showing that homozygous Palb2-deficient mice also die during embryogenesis at ~E8.5 (19, 22). Embryonic lethality due to loss of Brca1 or Brca2 can be delayed by concomitant loss of P53 (encoded by Trp53) or the CDK inhibitor p21 (encoded by Cdkn1a gene) (23, 24). Trp53 loss also delayed the lethality of Palb2 KO embryos, which otherwise exhibited increased p21 abundance (22). We therefore tested whether loss of p21 expression affects Palb2−− embryonic lethality by generating Palb2−/− Cdkn1a−/− embryos. As expected, loss of p21 expression did delay embryonic lethality of Palb2 KO embryos by 2–3 d (Fig. S3A). However, all Palb2−/−Cdkn1a double KO embryos still displayed multiple malformations and impaired growth compared with Palb2 heterozygous or WT littermates.
and were eventually resorbed. Therefore, these \textit{Palb2}\textsuperscript{+/−} embryonic rescue effects were incomplete and failed to suppress embryonic lethality.

Because the establishment of the placenta and onset of embryonic hematopoiesis are critical steps in development that take place around the time of lethality of \textit{Palb2} embryos, we asked whether the lethality of \textit{Palb2} KO embryos could be bypassed by a WT placenta. To this end, we used the \textit{Palb2}\textsuperscript{−/−} and \textit{Palb2}\textsuperscript{+/−} ES cells we had generated to perform tetraploid complementation assays. In this assay, diploid KO ES cells are aggregated to tetraploid WT blastocysts to generate KO embryos that are supported by a WT placenta because the tetraploid WT blastomeres are still capable of forming a placenta but cannot contribute to the embryo.

We found that embryos derived from the \textit{Palb2}\textsuperscript{+/−} ES cells were already underdeveloped and malformed at E9.5 compared with their \textit{Palb2}\textsuperscript{+/+} counterparts (Fig. S3 I and J). At E12.5, embryos derived from \textit{Palb2}\textsuperscript{−/−} ES cells appeared normal whereas embryos from \textit{Palb2}\textsuperscript{+/−} ES cells had been resorbed (Fig. S3 K and L). Likewise, breeding of the \textit{Palb2}\textsuperscript{−/−} conditional allele to \textit{Meox2-Cre} knock-in (KI) mice (in which Cre is expressed from the endogenous \textit{Meox2} locus only in the embryo proper and not in the placenta or extraembryonic tissues) (25) only yielded viable mice in which the \textit{Palb2} deletion was incomplete. Collectively, these findings indicate that \textit{Palb2} is an essential gene during development, and its deficiency in the embryo proper is incompatible with life. These findings are analogous to previous results showing that the lethality of \textit{Brea1}\textsuperscript{−/−} embryos could not be rescued by tetraploid complementation assay (20).

\textbf{\textit{Palb2} is a Breast Tumor Suppressor in Mice.} To assess the effect of \textit{Palb2} loss-of-function on mammary tumorigenesis, we crossed \textit{Palb2}\textsuperscript{−/−} mice with keratin 14 promoter-driven \textit{Cre} (K14-Cre) transgenic mice (26). K14-Cre transgenic animals preferentially express Cre recombinase in the basal epithelium of the mammary ducts, as well as in skin and oral mucosa. K14-Cre has previously been used to model murine \textit{Brea1} and \textit{Brea2} mammary tumorigenesis (27).

PALB2, like BRCA1 and -2, appears to be a breast cancer suppressor in humans (9, 10, 28). Therefore, in an effort to develop a tractable system for studying how \textit{Palb2} operates in this regard, we set out to develop a \textit{Palb2} mouse breast cancer model. Mammary tumor formation initiated by BRCA1 or BRCA2 loss requires concomitant loss of functional p53 (encoded by \textit{Trp53} in mice) (27, 29). This observation was considered in efforts to establish a \textit{Palb2} model.

We first generated \textit{Palb2}\textsuperscript{−/−}/\textit{Trp53} conditional double conditional mice by crossing \textit{Palb2}\textsuperscript{−/−}, K14-Cre transgenic mice with \textit{Trp53} conditional normal mice. All mice that harbored the conditional alleles for \textit{Palb2} and/or \textit{Trp53} in the absence of K14-Cre were phenotypically normal, fertile, and capable of nursing their litters. During the period of tumor monitoring (up to 600 d after birth), \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} female mice developed spontaneous mammary tumors with a frequency of ~80% and a mean tumor-free interval (T\textsubscript{1/2}) of 320 d. By contrast, \textit{Palb2}\textsuperscript{−/−}, \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} double conditional mice developed tumors much faster (T\textsubscript{1/2} = 192 d, P = 2.4 × 10^{-5}), indicating that \textit{Palb2} loss accelerates tumor formation on a \textit{Trp53}-null background (Fig. 2A). These latencies are comparable with \textit{Brea2}\textsuperscript{−/−}, \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} and \textit{Brea1}\textsuperscript{−/−}, \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} mice (T\textsubscript{1/2} = 181 and 213 d, respectively) (27, 30).

Somatic loss of one \textit{Trp53} allele displayed, as expected, a haploinsufficient tumor suppressor phenotype (27, 30), given that \textit{Palb2}\textsuperscript{−/−}, \textit{Trp53}\textsuperscript{−/+}/\textit{K14-Cre} mice developed tumors significantly faster than \textit{Palb2}\textsuperscript{−/−}, \textit{Trp53}\textsuperscript{−/+}/\textit{K14-Cre} mice (T\textsubscript{1/2} = 225 d vs. 420 d, respectively, P = 2.5 × 10^{-12}, Fig. S4C). \textit{Palb2} loss of function also accelerated tumor formation on a \textit{Trp53} heterozygous (\Omega+/−) background, again reflecting the genetic interaction of these two genes (Fig. 2B).

\textbf{K14-Cre-mediated loss of \textit{Palb2} and \textit{Trp53} led, predominantly, to tumor formation in breast, skin, and oral mucosa (Fig. 2E), as previously reported for K14-Cre-driven \textit{Brea1} and \textit{Brea2} cancers (27, 30–32). All of the mammary cancers were estrogen and progesterone receptor (ER/PR)-negative, basal-like (Fig. S4 D–F), much like the BREA1 and -2 tumors generated by K14-Cre (30, 32). Although most of the tumors found in \textit{Palb2}−/+;\textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} mice were breast carcinomas, \textit{Palb2}/\textit{Trp53} compound KO mice displayed an expanded spectrum of tissues affected by tumors (Fig. 2E), suggesting that combined loss of \textit{Palb2} and \textit{P53}, possibly due to some expression of Cre in other tissues, also results in tumor formation that is not restricted to the mammary gland.

Mice harboring conditional alleles for \textit{Palb2} and \textit{Trp53}, but no K14-Cre transgene, and \textit{Palb2}\textsuperscript{−/+}, \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} mice did not display overt tumor formation during the observation period (Fig. 2C), despite the intrinsic mutagenic activity of Cre in mammalian cells (31, 33). Therefore, tumor formation in the above-noted experiments is a product of targeted gene deletion.

All tumors from \textit{Palb2}\textsuperscript{−/−}, \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} mice (n = 12) had lost both copies of \textit{Palb2} and \textit{Trp53} (Fig. 2D). Similarly, in all tumors from \textit{Palb2}\textsuperscript{−/−}, \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} mice (n = 15), the conditional \textit{Palb2} and \textit{Trp53} alleles were recombined. The WT copy of \textit{Trp53} was also lost in most tumors, probably through LOH.

Early reports describing a lack of \textit{PALB2} LOH in clinical tumors samples from heterogeneous patients suggested that \textit{PALB2} could be a haploinsufficient tumor suppressor in humans whereas other reports showed that multiple \textit{PALB2} tumors revealed \textit{PALB2} LOH, implying that the \textit{PALB2} tumor formation process is not uniform (9, 34, 35). In our experimental setting, no haploinsufficiency for tumor suppression was observed for \textit{Palb2}, as indicated by the comparable latency in \textit{Palb2}\textsuperscript{−/−}, \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} and \textit{Palb2}\textsuperscript{−/+}/\textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} tumor development (P = 0.46, Fig. S4A). Similarly, when compared on a \textit{Trp53}\textsuperscript{−/−}/\textit{K14-Cre} background, cohorts of \textit{Palb2}\textsuperscript{−/+} and \textit{Palb2}\textsuperscript{+/−} mice developed tumors with similar latency and frequency (P =
which encodes mouse 53BP1) expression is reduced in Trp53 dose breast tumor samples. The tumors displayed apparently greater genomic instability by LOH. The majority of these tumors were small lesions in the head and neck, and a few were mammary tumors. All of these mammary tumors were Palb2−/−, and all displayed either mutations in or loss of Trp53 by LOH.

The finding that loss of Palb2 alone is sufficient to induce long latency tumor formation contrasts with most Brca1 and Brca2 mouse models in which significant numbers of these tumors could not be detected, unless Trp53 was codelleted (27, 30, 36, 37). One explanation for this finding is that somatic Palb2 loss might be better tolerated than somatic Brca1 loss on a Trp53 WT background. This hypothesis fits with the finding that Palb2 nulligosity gives rise to a less severe phenotype in ES cells than biallelic Brca1 or Brca2 loss (17, 18, 27, 38).

Genomic instability is a hallmark of human cancer, and it promotes tumor initiation and progression. Experimental mouse tumor models have recapitulated this aspect of human tumorigenesis (39). To gain insight into the genomic structures of the tumors that arose due to the loss of Palb2, we performed high-resolution comparative genomic hybridization (CGH) (40) analysis of Palb2/Trp53, Brca1/Trp53, Brca2/Trp53, and Trp53 only-deficient mammary tumors that arose in K14-Cre mice (Fig. 3 A–C).

Segmentation analysis of the CGH data was performed for each tumor to assess the number of genomic segments with deviating copy number changes (aka genomic segmentation), as a readout of genomic instability (41). Palb2/Trp53 and Brca1/Trp53 tumors displayed apparently greater genomic instability (genomic segmentation) compared with Brca2/Trp53 and Trp53-only tumors, but the difference was not statistically significant. However, when the relative dose of amplified segments (log2 dose ≥ 0.5) was analyzed, Palb2/Trp53 mammary tumors (n = 8) displayed a significantly higher average dose of amplified segments than either Brca1/Trp53 (n = 4; P < 0.0001, Fig. 3C) or Brca2/Trp53 tumors (n = 5; P = 0.0014, Fig. 3C). The low numbers of deletions (log2 dose ≤ -0.5) detected in Brca2/Trp53 and Trp53-only tumors precluded further analysis of this aspect of genomic instability (Fig. 3B).

No significant difference in focal genome amplification appeared when Palb2/Trp53 tumors were compared with Trp53-only tumors, indicating that Palb2/Trp53 tumors share a similar amplification-prone genomic profile with Trp53-only tumors, despite their marked difference in tumor formation kinetics. The inability of Palb2 loss to suppress focal genomic amplifications (unlike what was observed in Brca1/Trp53 and Brca2/Trp53 tumors) could be accounted for by three, alternative explanations.

First, these differences could be due to residual activity of the conditional Palb2 allele we generated, and other Palb2 loss of function mutations might trigger the formation of true Brca2 tumor phenocopies. Second, our allele is a functional null, as our studies suggest, but complete loss of Palb2 is similar to a BRCA2 hypomorphic phenotype rather than a complete loss of BRCA2 function. Alternatively, there are PALB2 functions that are, at least in part, nonoverlapping with the tumor suppressing functions of its BRCA2 partner protein. New experiments with additional Palb2 and Brca2 mutant mouse strains would be required to distinguish between these possibilities.

Finally, tumor heterogeneity likely affected the CGH profiles (Fig. S5) in ways that make it difficult to identify regions of chromosomal imbalances that were unique to Palb2/Trp53 tumors. Conceivably, a more comprehensive analysis with a much larger collection of tumor samples would reveal such regions.

Loss of 53BP1 Fails to Rescue the HR Defect Caused by PALB2 Deficiency. Loss of 53BP1 can rescue the HR defect and lethality observed in either Brca1Δ1/Δ1 or Brca1-null cells and mice (42–45). Similarly, decreased expression of the P53 binding protein 1 (53BP1) was detected in triple negative breast cancers as well as human BRCA1 tumors (45). Therefore, we asked whether Tip53bp1 (which encodes mouse 53BP1) expression is reduced in Palb2/Trp53 KO tumors, and whether its absence rescues the HR defect associated with Palb2 loss. Quantitative RT-PCR analysis of Tip53bp1 mRNA in freshly isolated Palb2 breast tumor samples.
showed that levels of $\text{Trp53bp1}$ messenger varied considerably among the tumors that were analyzed. However, overall $\text{Trp53bp1}$ mRNA levels were not significantly different in $\text{Palb2}$-deficient and $\text{Palb2}$-proficient tumors (Fig. 4A).

To determine whether HR deficiency due to $\text{Palb2}$ loss is complemented by $\text{Trp53bp1}$ loss in primary cells (cultured primary splenic B cells), we generated $\text{Palb2}^{+/+}$, $\text{CD19-Cre}$ mice that were or were not deficient in $\text{Trp53bp1}$. Cultured primary splenocytes from these mice were then assayed for HR competence upon treatment with PARPi inhibitors (PARPi), which selectively induces DNA damage and chromosome aberrations in HR-deficient cells (46). Treatment with $\text{KU0058948}$ (PARPi) led to an accumulation of chromosomal and chromatid breaks, and radial structures were evident in chromosomal spreads from cultured $\text{Palb2}^{+/+}$; $\text{Trp53bp1}^{+/+}$; $\text{CD19-Cre}$ primary splenocytes (Fig. 4B). The number of chromosome aberrations observed was not reduced in $\text{Palb2}^{+/+}$; $\text{Trp53bp1}^{+/+}$; $\text{CD19-Cre}$ splenocytes, implying that $\text{Trp53bp1}$ deletion did not complement the HR defect caused by $\text{Palb2}$ deficiency (Fig. 4B). $\text{Trp53bp1}$ deletion also failed to rescue the chromosomal aberrations found in spreads from PARPi-treated $\text{Brcal}^{+/+}$; $\text{Trp53bp1}^{-/-}$; $\text{CD19-Cre}$ splenocytes (Fig. S6A), which appeared to be even more extensive than those observed in PARPi-treated $\text{Palb2}^{+/+}$; $\text{Trp53bp1}^{-/-}$; $\text{CD19-Cre}$ cells (Fig. 4B). As has been previously described (43, 45), complete rescue of the DNA repair deficiency in $\text{Brcal}^{+/+}$; $\text{Trp53bp1}^{-/-}$; $\text{CD19-Cre}$ splenocytes was observed (Fig. S6B).

Of note, both $\text{Palb2}/\text{Trp53bp1}$ and $\text{Brcal}/\text{Trp53bp1}$ compound KO cells displayed more chromosomal aberrations after PARPi exposure than $\text{Palb2}$ or $\text{Brcal}$ single mutants (Fig. 4B, Fig. S6A). Thus, whereas $\text{Brcal}$, $\text{Palb2}$, and $\text{Brcal}$2 manifest closely related, even overlapping functions, loss of $\text{Palb2}$ or $\text{Brcal}$2 also resulted in a different DNA damage response after $\text{Trp53bp1}$ elimination from that manifested by $\text{Brcal}$ KO cells, in which $\text{Trp53bp1}$ codetection rescued the genomic instability observed after PARP inhibition.

These observations suggest that the contributions of $\text{PALB2}$ and $\text{BRCA2}$ to HR-based DSB repair are distinct from those of $\text{BRCA1}$ and cannot be complemented by $\text{53BP1}$ loss. In keeping with existing evidence, $\text{PALB2}$ and $\text{BRCA2}$ may be de facto HR effectors that cannot be replaced or bypassed, except by artificially forcing the loading of RAD51 onto chromatin at/near DSB, which $\text{53BP1}$ loss has not yet been shown to promote (45, 47–49). These observations, along with earlier results (4), also suggest that PARP inhibition might be a potential therapeutic regimen in $\text{PALB2}$-deficient tumors, as it is in $\text{BRCA1}$- and $\text{BRCA2}$-associated tumors (46).

Conclusions

In summary, we have shown that $\text{Palb2}$ is a breast tumor suppressor gene in mice as it is in humans and that it synergizes with $\text{Trp53bp1}$ to suppress tumor formation. The outcome of dual $\text{Palb2}$/$\text{Trp53bp1}$ nullizygosity in the mouse mammary gland is highly penetrant breast cancer. In keeping with the fact that $\text{Palb2}$ is also a breast tumor suppressor in humans, $\text{Palb2}$ might be viewed as a $\text{BRCA3}$-like allele. Moreover, tumorigenesis driven by $\text{Palb2}$ loss in the mouse is not entirely suppressed on a $\text{Trp53}$ WT germ-line background, unlike most $\text{Brcal}$ and $\text{Brcal2}$ mouse models of breast tumorigenesis (50).

Despite many similarities to $\text{Brcal}/\text{Trp53}$ and $\text{Brcal}/\text{Trp53}$ breast tumors, $\text{Palb2}$ tumors displayed certain divergent genomic features that might be viewed as separating them from $\text{BRCA1}$ and -2 cancers. Specifically, we observed patterns of genomic aberrations that were different in $\text{Palb2}/\text{Trp53}$-derived tumors from those detected in $\text{Brcal}/\text{Trp53}$- or $\text{Brcal2}/\text{Trp53}$-derived tumors. These data are consistent with the hypothesis that $\text{PALB2}$ possesses biological functions that extend beyond those of its major interactors, $\text{BRCA1}$ and $\text{BRCA2}$. Alternatively, the effect of $\text{Palb2}$ deletion may mimic a phenotype akin to partial loss of $\text{BRCA2}$, resulting in a less dramatic genomic instability profile in the relevant tumor cells.

Although the genomic instability of $\text{Brcal}$-deficient cells can be rescued by loss of $\text{Trp53bp1}$, deletion of the latter had, if anything, an adverse effect in $\text{Palb2}$ KO cells. In that context, $\text{Palb2}$ is more similar to $\text{Brcal}$2, the absence of which leads to an HR defect that also cannot be rescued by $\text{Trp53bp1}$ deletion.

Haploinsufficiency for $\text{Palb2}$ tumor suppression was not detected in this model although one cannot rule out the possibility that it would be manifest in a different model system and/or with enlarged cohorts of experimental mice. For example, the tumors in this mouse model driven by $\text{K14-Cre}$ were uniformly of the triple negative phenotype. This characteristic might well contribute to the absence of haploinsufficiency in our system, in the same way that $\text{BRCA1}$ mammary tumors derived from distinct cell populations display preferential patterns of consecutive LOH events along the tumorigenesis pathway (32, 51).

We believe that this mouse model will be useful in understanding how $\text{Palb2}$ serves its breast cancer suppression function.
Mice harboring tumors were humanely killed when the tumor diameter reached 2.0 cm in its greatest dimension. Mice that were otherwise severely diseased/dystresed were also killed according to institutional guidelines. ManteX-Col logrank test was applied for comparison of tumor-free survival of mouse cohorts.

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