ABCB1 protects bat cells from DNA damage induced by genotoxic compounds

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Bats are unusual mammals, with the ability to fly, and long lifespans. In addition, bats have a low incidence of cancer, but the mechanisms underlying this phenomenon remain elusive. Here we discovered that bat cells are more resistant than human and mouse cells to DNA damage induced by genotoxic drugs. We found that bat cells accumulate less chemical than human and mouse cells, and efficient drug efflux mediated by the ABC transporter ABCB1 underlies this improved response to genotoxic reagents. Inhibition of ABCB1 triggers an accumulation of doxorubicin, DNA damage, and cell death. ABCB1 is expressed at higher levels in several cell lines and tissues derived from bats compared to humans. Furthermore, increased drug efflux and high expression of ABCB1 are conserved across multiple bat species. Our findings suggest that enhanced efflux protects bat cells from DNA damage induced by genotoxic compounds, which may contribute to their low cancer incidence.
Cancer is a leading cause of death among aging populations. Current cancer treatments are inefficient and have unacceptable side-effects, due in part to the biological complexity and incomplete understanding of the disease\(^1\). Therefore, there is an urgent need to develop alternative therapeutics. In-depth understanding of tumour suppressive mechanisms can provide effective strategies to treat and prevent cancer.

Most cancer research utilises cancer-prone, short-lived animals such as mouse and rat, which are amenable to genetic manipulation. Although these model organisms share some tumour suppressor mechanisms with humans, emerging evidence suggests that long-lived mammals have evolved unique and robust anti-cancer strategies\(^2\). For example, genome and transcriptome analyses of the bowhead whale, a large and long-lived mammal with a low rate of cancer, revealed a positive evolutionary selection of the excision repair cross-complementation group 1 (ERCC1) gene and duplication of the proliferating cell nuclear antigen (PCNA) gene, both DNA repair genes\(^3\). These data imply that an enhanced DNA repair pathway might protect whales from cancer by lowering the mutation frequency. Genomic analysis of elephants, which also have a low cancer incidence, uncovered a high number of TP53 pseudogenes, and elephant cells displayed an enhanced TP53-dependent DNA damage response compared to human cells\(^4,5\).

Some small mammals also show remarkable cancer resistance. Early contact inhibition is a unique mechanism of tumour suppression in the naked mole rat, mediated by the secretion of high-molecular-mass hyaluronic acid\(^6,7\). Blind mole rats also exhibit remarkable cancer resistance by inducing concerted necrotic cell death in response to hyperplasia\(^8\) and by having a stronger extracellular matrix to restrict tumour growth and metastasis\(^9\). Unravelling the mechanisms underlying low cancer rates provides important perspectives and insights into cancer biology and potential treatment strategies for humans.

Bats are small, long-lived mammals with an extremely low incidence of cancer\(^2,10\). They are the second largest order of mammals in the world\(^11\), the only mammal capable of powered-wing flight, and an asymptomatic reservoir for many deadly viruses\(^10\). Their longevity data predominately come from field-based studies, and therefore, their true longevities may be underestimated, and they may live longer than these reported records\(^12-14\). In general, longevity is positively correlated with the body size\(^12,13\). Austad and Fischer\(^13\) defined the longevity quotient (LQ) which takes the consideration of body mass in the estimated maximum lifespan of individual mammalian species. Bats possess one of the highest LQ value among the mammal order\(^12,13\), indicating that bats live much longer than other mammals of equivalent size. Their higher LQ makes bats interesting species to study since they may have unique tumour suppressive mechanisms compared to humans. Only a handful of cases of tumours have been recorded to date for bats in captivity\(^15-17\). However, the underlying mechanisms of tumour suppression in bats are still not fully understood. To understand such mechanisms, we previously performed genomic analyses of Pteropus alecto (P. alecto) and Myotis davidii, and discovered that the DNA damage response (DDR) pathway of bats had undergone positive evolutionary selection\(^18\). The DDR pathway is the major mechanism that detects and repairs damaged DNA and highly conserved in a diverse range of organisms\(^19\). DNA damage can induce cancer via multiple mechanisms, including genomic instability, inactivation of tumour suppressors or activation of oncogenes\(^20\). Living organisms are constantly exposed to environmental xenobiotics and internal metabolic by-products, some of which can possess genotoxic properties that can lead to DNA damage\(^21,22\). Elucidating whether and how bats efficiently resist DNA damage will advance our understanding of cancer biology.

Here, we describe a role for the ATP-binding cassette (ABC) transporter ABCB1 in DNA damage resistance in bats. We propose genotoxic efflux as a potential tumour suppressive mechanism that contributes to the low incidence of cancer in bats.

**Results**

**Bat-derived cells respond distinctively to chemical exposure.** To explore DNA damage resistant mechanisms in bat, we first examined the DNA damage response in bat, human and mouse cells exposed to different DNA damaging treatments.

First, we exposed PaLung (normal lung fibroblast from P. alecto), WI-38 (human normal lung fibroblast) and MEF (mouse embryonic fibroblast) cells to γ-irradiation, which induces double-strand breaks (DSBs) in DNA via free radical formation\(^23\). We exposed cells to 10 Gy of γ-irradiation and monitored the well-established DNA DSB marker phosphorylated histone 2A variant X (γH2AX)\(^24\). We found that γH2AX accumulated and diminished with similar kinetics in PaLung, WI-38 and MEF cells exposed to γ-irradiation (Fig. 1a). P. alecto PaKit03 cells (kidney cells transformed with SV40 large T antigen) and human HEK293T cells (embryonic kidney cell transformed with SV40 large T antigen) also showed similar changes in γH2AX levels in response to γ-irradiation (Supplementary Fig. 1A).

To verify that elevated γH2AX represented a DSB response rather than a preapoptotic signal associated with high levels of pan-nuclear γH2AX\(^25\), we monitored γH2AX in cells by immunofluorescence. We found that cells exposed to γ-irradiation displayed γH2AX foci rather than pan-nuclear accumulation (Supplementary Fig. 1B), consistent with DSBs.

To further confirm these results, we detected the DNA repair protein p53-binding protein 1 (53BP1) by immunofluorescence. We found that 53BP1 foci in PaLung and WI-38 cells exposed to γ-irradiation and monitored the γH2AX levels returned to basal levels within 3 h of etoposide removal in PaLung cells, whereas it remained high at least 12 h after irradiation (Fig. 1b). On the other hand, the number of 53BP1 foci increased slowly in γ-irradiated MEFs and remained high at least 12 h after irradiation (Fig. 1b), consistent with a previous report about DNA repair\(^28\). Together, these results suggest that lung fibroblasts from P. alecto and human are similarly sensitive and responsive to DNA damage induced by ionising radiation, whereas MEFs display a slightly slower response to the same treatment.

Next, we treated the same set of cell lines with the chemotherapeutic drug etoposide (50 μM). Etoposide inhibits topoisoasemerase II\(^25\) and thus induces DNA DSBs. We treated cells for 3 h, washed away the drug, and monitored the levels of γH2AX over time after drug removal. γH2AX was similarly induced by etoposide in all three cell lines (Fig. 1c, at 0 h time point after treatment). Unexpectedly, γH2AX levels returned to almost basal levels within 1–3 h of etoposide removal in PaLung cells, whereas it remained elevated for at least 12 h in WI-38 and MEF cells (Fig. 1c). Similarly, γH2AX levels returned to basal levels within 3 h of etoposide removal in P. alecto PaKit03 cells, but remained high in human HEK293T cells (Supplementary Fig. 1C). These results suggest that P. alecto cells are more resistant than human and mouse cells to etoposide-induced DNA damage.

**Bat cells accumulate less doxorubicin than human and mouse cells.** To extend our observations, we evaluated the response of these cells to the chemotherapeutic reagent doxorubicin. Like etoposide, doxorubicin inhibits topoisoasemerase II and induces DNA DSBs that lead to γH2AX induction. However, high
concentrations of doxorubicin can cause histone eviction that leads to reduced levels of γH2AX in human cells\(^3\). Indeed, WI-38 cells treated for 3 h with increasing concentrations of doxorubicin showed a dose-dependent decrease in γH2AX levels (Fig. 2a), consistent with histone eviction\(^3\). MEFs displayed a similar dose-dependent decrease in γH2AX levels (Fig. 2a). On the other hand, PaLung cells displayed a dose-dependent increase in γH2AX levels (Fig. 2a), consistent with increased DNA damage but without histone eviction.

As we did with etoposide, we treated cells with 5 µM doxorubicin for 3 h, washed away the drug, and monitored γH2AX levels over time for up to 12 h. We found that γH2AX in PaLung cells started to decrease immediately after washout and returned to almost basal levels within 6 h (Fig. 2b). In contrast, γH2AX levels in WI-38 and MEF cells accumulated after washout and were sustained at high levels until the last time point we monitored (Fig. 2b). The accumulation of γH2AX after doxorubicin washout was similarly observed in various human cell lines, but not in various \(P. alecto\) cell lines (Supplementary Fig. 2A).

To monitor DSB formation, we again detected γH2AX by immunofluorescence. We found that doxorubicin-treated PaLung, WI-38, and MEF cells displayed γH2AX foci but not pan-nuclear staining, consistent with DSBs (Supplementary Fig. 2B). Similar to γH2AX levels, 53BP1 foci in PaLung cells diminished soon after removal of 5 µM of doxorubicin (Fig. 2c). In contrast, 53BP1 foci accumulated in WI-38 and MEF cells after removal of doxorubicin (Fig. 2c).

Together, these findings suggest that treatment with 5 µM doxorubicin does not lead to histone eviction in \(P. alecto\) cells. In contrast, similarly treated human and mouse cells display transient histone eviction; the accumulation of γH2AX in human and mouse cells over time might reflect a decrease in intracellular doxorubicin levels, such that there is DNA damage without histone eviction.

We hypothesised that \(P. alecto\) cells accumulate less doxorubicin than similarly treated human and mouse cells, which would protect them from histone eviction since the only high dose of doxorubicin is known to cause histone eviction\(^3\) (Fig. 2a). To test this hypothesis, we treated the cells with increasing doses of doxorubicin and measured intracellular doxorubicin accumulation by flow cytometry\(^3\). The amount of intracellular doxorubicin was normalised to the cellular volume since there was some variation of the cell size among the cell lines (Fig. 2d).
We found that the amount of doxorubicin within PaLung cells was significantly lower than that within WI-38 and MEF cells, at all the treatment concentrations (Fig. 2e) and times (Fig. 2f). Similar trends were observed in P. alecto PaKIT03 cells in comparison with human HEK293T cells (Supplementary Fig. 2C–E). Together, these results suggest that P. alecto cells are more resistant than human and mouse cells to doxorubicin-induced DNA damage at least in part due to reduced accumulation of doxorubicin.

**Bat cells exhibit efficient drug efflux via ABC transporters.** Intracellular drug concentrations are influenced by the balance of drug influx and efflux. ABC transporters are the primary transporters that efflux genotoxic substances, thereby reducing intracellular xenobiotic concentrations. To determine if ABC transporters contribute to the difference in doxorubicin accumulation between bat, human, and mouse cells, we used verapamil (Vera) to inhibit efflux via ABC transporters, and assessed doxorubicin accumulation by fluorescence microscopy and flow cytometry. Specifically, we pre-treated cells with or without verapamil for 30 min, added doxorubicin for 3 h and measured intracellular doxorubicin levels. No notable differences were observed in the doxorubicin fluorescence intensities between control and verapamil-pre-treated WI-38 cells (Fig. 3a, b), suggesting that WI-38 cells exhibit no endogenous drug efflux capability via ABC transporters. Similarly, no obvious differences were detected in doxorubicin accumulation between control and verapamil-pre-treated MEF cells (Fig. 3a, b) and HEK293T cells (Fig. 3b and Supplementary Fig. 3). In contrast, verapamil-pre-treatment increased doxorubicin fluorescence in PaLung and PaKIT03 cells (Fig. 3a, b and Supplementary Fig. 3). Likewise, pre-treatment with cyclosporin A (CSA), another ABC transporter inhibitor, also led to an increase in doxorubicin...
**Fig. 3** Analysis of drug efflux capability via ABC transporters. 

**a** Doxorubicin (Dox) fluorescence or phase contrast images of PaLung, WI-38, and MEF cells. Cells were pre-treated without or with 5 μM verapamil (Vera) for 30 min before the treatment with 10 μM doxorubicin alone or together with Vera for an additional 3 h. Images were acquired with 20× objective lens, and scale bar represents 50 μm.

**b** Flow cytometry analyses of Dox accumulation in PaLung, PaKIT03, WI-38, HEK293T, and MEF cells. Cells were pre-treated without or with 5 μM verapamil (Vera, top row) or 5 μM cyclosporin A (CSA, bottom row) for 30 min before the treatment with 10 μM doxorubicin alone or together with Vera or CSA for an additional 3 h.

**c** Flow cytometry analyses of Dox accumulation in cells derived from different tissues of *P. alecto* in the presence or absence of verapamil. Cells were treated and analysed as in (b). All results shown are representative of at least three experimental repeats.
fluorescence in PaLung and PaKiT03 cells, but not in WI-38, HEK293T, and MEF cells (Fig. 3b). These results suggest that the drug efflux activity of ABC transporters is required to reduce doxorubicin accumulation in PaLung and PaKiT03 cells. Furthermore, primary P. alecto cell lines derived from kidney (PaKidney), spleen (PaSpleen), brain (PaBrain), and bone marrow (PaMarrow) also displayed a similar increase in doxorubicin accumulation with verapamil pre-treatment (Fig. 3c). Together, these results suggest that cells derived from multiple tissues of P. alecto possess drug efflux activity via ABC transporters.

**Drug efflux in bat cells reduces the exposure to doxorubicin genotoxicity.** We hypothesised that ABC transporter activity underlies the increased resistance of bat cells to chemotherapeutic drugs. This hypothesis predicts that pre-treatment with inhibitors of ABC transporters will increase the genotoxic effects of doxorubicin in P. alecto cells but not in WI-38, HEK293T and MEF cells, which lack substantial ABC transporter activity. Indeed, verapamil pre-treatment enhanced the induction of γH2AX levels by low dose of doxorubicin (1 µM) in PaLung and PaKiT03 cells, but not in WI-38, HEK293T and MEF cells (Fig. 4a, lanes 1, 2, 5 and 6), confirming that the ABC transporter activity helps to prevent DNA damage in PaLung and PaKiT03 cells. Treating PaLung and PaKiT03 cells with higher doses (5 and 10 µM) of doxorubicin in the presence of verapamil led to a doxorubicin dose-dependent decrease in γH2AX levels (Fig. 4a, lanes 6–8), as observed in WI-38, HEK293T and MEF cells with or without verapamil pre-treatment, and consistent with histone eviction30 (Fig. 4a, lanes 2–4 and 6–8). Similar results were obtained with the ABC transporter inhibitor CSA (Fig. 4b) and with cells derived from different tissues of P. alecto (Fig. 4c). Together, these results demonstrate that broad and efficient drug efflux in P. alecto cells contributes to reducing doxorubicin-mediated genotoxicity.

**ABC1 is responsible for increased drug efflux in bat cells.** To determine the transporter(s) responsible for the efflux of doxorubicin in bat cells, we re-analysed our previously published genome-wide transcriptome data set of P. alecto kidney cell line PaKiT0333. We identified three highly expressed ABC transporters (ABCB1, ABCC1 and ABCG2) that are well-characterised for doxorubicin efflux (Fig. 5a)32,34. To determine if one of these transporters is required for doxorubicin efflux in P. alecto cells, we reduced the expression of each gene in PaKiT03 cells via small-interfering RNA (siRNA)-mediated knockdown (Fig. 5b) and examined the effects on intracellular doxorubicin accumulation by flow cytometry (Fig. 5c). Verapamil was used as a positive control for efflux inhibition. We found that only ABCB1 knockdown increased doxorubicin accumulation to a similar extent as verapamil pre-treatment, suggesting that ABCB1 is the transporter responsible for doxorubicin efflux in P. alecto-derived cells.

To further verify our observation, we treated WI-38 and PaLung cells with cisplatin, which is not an efflux substrate of
ABC1 is highly and broadly expressed in *P. alecto*. To determine if *ABC1* is differentially expressed in *P. alecto* compared to human cells, we first performed RT-qPCR on a panel of cell lines. Consistent with our RNA-seq results from PaK103 and HEK293T cells (Fig. 5a), *ABC1* transcript levels were significantly higher in various *P. alecto* cell lines than in various human cell lines (Fig. 7a). Further, *ABC1* protein levels were higher in PaLung and PaK103 cells compared to human WI-38 cells from DNA damage, consequently ABC1 helps to prevent the DNA damage induced by doxorubicin in *P. alecto* cells.

In addition, we evaluated the effect of ABC1 inhibition on the viability of *P. alecto* cells treated with doxorubicin. We cultured PaK103 cells in media (+ or − verapamil) with doxorubicin for 3 h, followed by doxorubicin-free media for an additional 72 h. We then assessed cell viability by measuring ATP levels. We found that ABC1 inhibition significantly reduced the viability of PaK103 cells exposed to doxorubicin, further suggesting that ABC1 transport activity protects cells from genotoxic stress (Fig. 6f).
Fig. 6 Effect of ABCB1 knockdown on doxorubicin-induced DNA damage in bat cells. 

**a** The efficiency of siRNA knockdown. PaKiT03 cells were transfected with the indicated siRNA, and the expression of ABCB1 was analysed by Western blotting. C; control siRNA. Tubulin was used as a loading control.

**b** Comet assay of PaKiT03 cells. siRNA knockdown cells were treated without or with 10 μM doxorubicin (Dox) for 3 h, and DNA damage was visualised by comet assay.

**c** Quantification of the amount of DNA damage calculated by olive tail moments (OTM) for the cells in **b**.

**d** Comet assay of PaKiT03 cells. Cells were pre-treated without or with 5 μM verapamil (Vera) for 30 min before the treatment with 10 μM doxorubicin alone or together with Vera for an additional 3 h. DNA damage was visualised by comet assay.

**e** Quantification of the amount of DNA damage calculated by OTM for the cells in **d**.

**f** Quantification of cell viability. Cells were pre-treated without or with 5 μM verapamil for 30 min before the treatment with the indicated amount of doxorubicin alone or together with verapamil for an additional 3 h. Doxorubicin was then removed and replaced with the doxorubicin-free medium in the absence or presence of verapamil for 72 h before measuring cell viability. Cell viability (%) is presented relative to the control cells (the first grey bar). All data shown have at least three experimental repeats and the images are representative. Scale bar represents 50 μm. Bars represent the means ± SDs of three independent experiments. Statistical significances were calculated using unpaired student’s two-sided t test. p < 0.05 is represented with *, p < 0.01 with ** and p < 0.001 with ***.
ABC1 expression and efflux capability in human and multiple bat species. a qRT-PCR for ABCB1 mRNA expression in cell lines derived from human and P. alecto. ABCB1 mRNA expression is normalised to GAPDH and presented relative to the expression in WI-38 cells (mean ± SDs of three independent experiments). b Western blot analysis of ABCB1 in the indicated cell lines. Tubulin was used as a loading control. c qRT-PCR for ABCB1 mRNA expression in tissues derived from human (N = 1) and P. alecto (N = 3). ABCB1 mRNA expression is normalised to GAPDH and presented relative to the respective human tissues (mean ± SDs). d Western blot analysis of ABCB1 protein in the indicated tissues. Ponceau S staining of the membrane is shown as a loading control. Arrow indicates the size of ABCB1 protein. H and P. a indicate human and P. alecto respectively. Lysates from HEK293T transfected with control (−) or ABCB1 expressing plasmid (+) serve as negative and positive control, respectively. e Western blot analysis of ABCB1 in cells derived from the different bat species. Actin was used as a loading control. f Flow cytometry analysis of doxorubicin accumulation in cells derived from different bat species. Cells were pre-treated without or with 5 μM verapamil (Vera) for 30 min before the treatment with 10 μM doxorubicin alone or together with Vera for an additional 3 h. The fold change by Vera treatment is presented as the mean fluorescence intensity of cells treated with both verapamil and doxorubicin relative to the mean fluorescence intensity of cells treated with doxorubicin alone. Bars represent mean fold change ± SD of at least three experiments. Data shown in Figs. 3b, c and 7f are also included in this histogram. Statistical significances were calculated using unpaired student’s two-sided t-test. **p < 0.01, ***p < 0.001.
and HEK293T cells (Fig. 7b). Next, we examined ABCB1 transcript and protein levels in various normal tissues from *P. alecto* and human. We found that ABCB1 levels are higher in *P. alecto* than in human across multiple tissues (Fig. 7c, d), including tissues known to express ABCB1 in human (adrenal gland, kidney, small intestine and large intestine) as well as those that express no or very little ABCB1 in human (lung and spleen). These data suggest that ABCB1 is expressed at higher levels and more broadly in *P. alecto* compared to human.

A comparison of *P. alecto* ABCB1 amino acid sequence with the human sequence demonstrated similarity of 89.5% (Supplementary Table 1). In addition, Walker A-, Walker B-, and active transport signature-motifs, which are characteristic of ABC transporters, were 100% conserved across multiple phylogenetically distant mammals (Supplementary Fig. 4), indicating that the ABCB1 amino acid sequence is well-conserved among mammals. Therefore, the increased drug efflux via ABCB1 in *P. alecto* cells compared to human cells is likely due to the higher expression of ABCB1 rather than the amino acid sequence differences.

**ABCB1-mediated efflux capability is conserved in multiple bat species.** To determine if other families of bats also possess ABCB1-dependent drug efflux, we investigated cells derived from *Cynopterus brachyotis* (*Pteropodidae* family), *Rhinolophus lepidus* (*Rhinolophidae* family) and *Myotis muricola* and *Myotis davidii* (*Vespertilionidae* family), which have distinct maximum lifespans and body masses (Supplementary Table 2). Cells from these bat species showed high expression of ABCB1 protein, similar to *P. alecto* (*Pteropodidae* family) (Fig. 7e), as well as efficient doxorubicin efflux that depended on ABC transporter activity (Fig. 7f). Further, these cells displayed the induction of γH2AX by doxorubicin, but pre-treatment with verapamil led to a doxorubicin dose-dependent decrease in γH2AX levels (Fig. 7g), consistent with elevated levels of intracellular doxorubicin and histone eviction upon inhibition of ABCB1.

To further confirm that ABCB1-mediated drug efflux is unique to bats, we extended our analysis to a diverse range of cell lines from bats, mice, and humans. We examined doxorubicin accumulation in the presence or absence of verapamil by flow cytometry in 11 human cell lines, 2 mouse cell lines, 5 *P. alecto* cell lines, and 4 cell lines from other bat species (Supplementary Table 3). Verapamil pre-treatment increased doxorubicin accumulation in all of the bat cell lines, but not in any of the human and mouse cell lines (Fig. 7h). Together, these results suggest that high ABCB1 expression and enhanced drug efflux capacity are uniquely conserved in multiple bat species and may contribute to reducing DNA damage and perhaps, in turn, cancer incidence in bats.

**Discussion**

Emerging evidence suggests that several long-lived mammals possess unusually low rates of cancer occurrence2. Technological advances in genomic sequencing have facilitated analysis of these organisms and revealed unique mechanisms of cancer resistance that are not observed in human2. Bats are long-lived mammals with an extremely low incidence of cancer2,10. However, the mechanisms underlying their cancer resistance are not fully understood. We have access to the bat species *P. alecto*, a large, non-hibernating fruit bat of the *Pteropodidae* family. Using cell lines established from *P. alecto*, we discovered that *P. alecto* cells uniquely displayed increased resistance to DNA damage induced by specific chemicals. This resistance is due at least in part to increased efflux of the chemicals mediated by high and broad expression of the ABCB1 transporter.

ABC transporter family proteins regulate cellular drug efflux32. ABCB1, also known as the transmembrane protein P-glycoprotein (P-gp) and multidrug resistance protein 1 (MDR1), is one of the best-characterised efflux transporters and is highly conserved across mammals37. In mammals, ABCB1 is specifically expressed in regions of detoxification, excretion, and protective barriers such as the intestinal epithelium, lumens of the liver, proximal tubule of the kidney as well as blood-tissue barriers of the brain. In rodents, the ABCB1 gene encodes two isoforms, *Abcb1a* (*Mdr1a*) and *Abcb1b* (*Mdr1b*). Mice deleted for *Abcb1a* alone or both *Abcb1a* and *Abcb1b* are viable and not cancer-prone in laboratory conditions, which are free from genotoxic substances. However, they are more sensitive to toxins than wild-type mice38,39. We found that *P. alecto* expresses ABCB1 relatively highly and in additional tissues compared to human, such as lung and spleen. The broad expression of ABCB1 may contribute to more efficient protection from genotoxic substances in their environment.

ABCB1 was originally discovered in cancer cells where its high and functional expression promotes resistance to a variety of chemotherapeutic drugs, such as paclitaxel, vinblastine, etoposide and doxorubicin32,34,40. This broad chemoresistance is achieved by the promiscuous substrate specificity of ABCB1. Cancer cells acquire ABCB1 overexpression after prolonged chemotherapy, which is one of the major causes of cancer relapse since ABCB1-mediated drug efflux can reduce drug intake32,34. Likewise, generating cancer cell lines with high ABCB1 expression to study the biological implications of ABCB1 often requires prolonged exposure of cells to drugs for months41,42. It is, therefore, rather intriguing that various bat cell lines from many different tissues intrinsically possess and maintain high ABCB1 expression and drug efflux in the absence of known selective pressure. Notably, in addition to xenobiotics, ABCB1 is reported to transport endogenous compounds such as cytokines, short peptide and lipids43,44. It is possible that bat cells may possess endogenous substances that are ABCB1 efflux substrates, and thus express high levels of ABCB1 to maintain homeostasis.

ABCB1 expression is regulated by multiple signalling pathways and physiological factors. Previous studies have reported that c-Myc, c-Jun, HIF-1 and C/EBPβ can positively modulate ABCB1 expression in cancers, leading to the development of drug resistance45. Recent work on microRNA biology (miRNA) such as miR-27a46,47, miR-2348 and miR-45146, have also illustrated their potential roles in regulating ABCB1 expression. Furthermore, physiological substances including steroid hormones, environmental stresses such as temperature, osmotic pressure and pH, and xenobiotics such as insecticides, heavy metal and DNA damage reagents have been shown to significantly modulate ABCB1 expression in vitro45. Interestingly, human pathogenic virus and virus proteins are also reported to induce ABCB1 expression49,50. Since bats are renowned for being a reservoir of high mortality viruses like Ebola51 and SARS52, some viral proteins may induce ABCB1 expression. It remains to be elucidated how bat cells induce and retain the high expression of ABCB1.

High doses of γ-irradiation, such as 10 Gy used in this study, mainly cause DNA DSB53. DSBs are the most detrimental form of DNA breaks for cells, and most DSBs are repaired via non-homologous end-joining (NHEJ)26,54. γH2AX and 53BP1 protein are core components of the NHEJ pathway. Once phosphorylation of H2AX occurs in response to DSBs, 53BP1 rapidly forms foci and disappears upon repair. We observed that 53BP1 foci formation and clearance after 10 Gy of γ-irradiation were fairly comparable between the cells from *P. alecto* and human, whereas mouse cell displayed a slower 53BP1 response. On the other hand, γH2AX signals induced by the same treatment were similar between bat, human and mouse cells. Why there is a delay...
between γH2AX phosphorylation and 53BP1 foci formation in mouse cells compared to human and bat cells remains to be deciphered.

Nucleotide excision repair (NER) and base excision repair (BER) are utilised to repair single-strand breaks such as removing bulky DNA adducts caused by UV irradiation. Podlutsky et al. reported that human and bat cells have better BER than mouse cells, whereas human cells have better NER than bat and mouse cells. Our data suggest that human and bat cells have a better response to DSBs compared to mouse cells. It is likely that each species has evolved different strategies to cope with the different types of DNA damage.

Doxorubicin was originally isolated from a soil bacterium and can induce DSBs and single-stranded breaks via multiple mechanisms, including DNA intercalating to inhibit topoisomerase II activity, histone eviction, and induction of oxidative stress. The mechanisms of cell death induced by doxorubicin are cell context-dependent and could be mediated by DNA damage or be independent of DNA damage such as replication stress or oxidative stress to proteins and other organelles. Importantly, our data show that bat cells accumulate less doxorubicin compared to human and mouse cells, leading to reduced DSBs, and that ABCB1 is responsible for the reduced doxorubicin accumulation in bat cells.

Etoposide was originally isolated from plants and forms a ternary complex with DNA and topoisomerase II, thereby causing DNA DSBs. In our experiments, we found that γH2AX resolved faster in P. alecto cells than in human and mouse cells when transiently treated with etoposide. Etoposide is a well-known ABCB1 substrate, therefore human and mouse cells would be expected to have higher residual intracellular etoposide than P. alecto cells even after drug removal from the media, leading to more DNA damage and γH2AX accumulation than P. alecto cells. Thus, the prolonged γH2AX in human and mouse cells could reflect not only DNA repair but also DNA damage caused by residual etoposide. Similar to doxorubicin, the assessment of DNA repair caused by etoposide between the P. alecto, human and mouse cells is challenging since the amounts of intracellular etoposide are not equal among these animals.

Other mechanisms of cancer resistance in bats have been proposed previously. For example, genome studies of the longest-lived bat Myotis brandtii showed that cancer resistance might be attributed to growth suppression by specific mutations in the growth hormone receptor that may reduce the GH-insulin-like growth factor 1 signalling pathway. In addition, miRNA analysis revealed that three tumour suppressive miRNAs (miR-101-3p, miR-16-5p and miR-143-3p) were upregulated and a single tumour promoting miRNA (miR-221-5p) was downregulated in Myotis myotis. These data suggest that bats may have multiple strategies to prevent tumour formation.

Unlike conventional mouse models, in vivo validation of our proposed mechanism in bats is hindered by their low reproductive capacity and technological limitations for genetic manipulation. Since the function of ABCB1 in mice was reported to be limited to specific tissues, it would be of interest to determine whether transgenic mice with high and broad expression of ABCB1 are less tumour prone, particularly in response to ABCB1 substrates.

Laboratory-bred animals and their wild-caught counterparts likely respond differently to xenobiotic and environmental challenges. ABCB1 knockout mice are not cancer-prone in the laboratory conditions where is free from genotoxic substances but are more sensitive to toxins than wild-type mice. Therefore, whether ABCB1 knockout mice are cancer-prone in the wild remains to be investigated. Harper et al. have shown that cells derived from wild-trapped mice exhibited different sensitivity to various genotoxic insults than cells derived from laboratory-bred mice. Hence, we do not exclude the possibility that the environmental background could contribute to the differences we observed between bats and mice. It would be interesting to compare the ABCB1-mediated efflux capacity of bat cells to cells derived from wild mice.

In summary, using P. alecto as a model, we demonstrated that bats have a high and broad expression of ABCB1, which uniquely promotes resistance to DNA damage induced by specific chemicals. This phenotype is conserved in multiple bat species, possibly as a pan-bat biological feature, and might partly explain their low incidence of cancer. Future research into this potential tumour suppressor mechanism in bats could inform innovative strategies to prevent or treat human cancer, as well as chemoresistance.

**Methods**

**Cell culture.** P. alecto and M. davidi-derived cell lines listed in Supplementary Table 3 and established as previously described. Briefly, the tissues were first cut finely using a scalpel, washed with cold media and further processed by enzymatic reactions for primary cell culture. B. brachyotis, M. muricola and R. lepida were captured in Singapore under the National Parks Board permit NP/ RRI1-011 and NP/RRI1-109. Lung tissues were harvested to produce primary cell cultures. Lung tissues were sliced into 1 mm sized pieces and treated enzymatically (1X HBSS, 0.2% bovine serum albumin, 0.4 mg/ml collagenase A, 0.22 mm filtered) and incubated at 37 °C with rotation (60 rpm) for approximately 1 h to dissociate cells. Dissociated tissues were centrifuged at 430 g for 5 min, resuspended with complete RPMI medium 1640 (10% foetal bovine serum (Thermo Scientific), 2% penicillin/streptomycin (Thermo Scientific), 2.5% HEPEI) and plated in T-25 tissue culture flasks. Primary lung cells were maintained at 37 °C with 5% CO2 until confluence. After the second passaging, all cell lines were grown in Dulbecco’s modified Eagle’s medium (DMEM) with 10% foetal bovine serum and penicillin/streptomycin in a 37 °C incubator with 5% CO2, with exception of K562 and KCL22 cells that were maintained in RPMI medium 1640 with 10% foetal bovine serum and penicillin/streptomycin. Primary cell lines from the tissues of other bat species were established using identical method for lung tissues. Origin of cell lines and immortalisation type are listed in Supplementary Table 3. Tissue samples of the adrenal gland, lung, spleen, kidney, small and large intestines were obtained from P. alecto and were stored in Buffer RLT (Qiagen) at −80 °C for protein extraction. WI-38, IMR-90 and AG01518 cell lines were purchased from Coriell Institute, HEK293T, HepG2, MDA-MB-231, HT-29, WiDr, U251MG, K562 and NIH3T3 cell lines were purchased from American Type Culture Collection (ATCC). KCL-22 cell line was purchased from Leibniz Institute DSMZ-German. MEF cells were kindly provided by Dr Yanping Zhang (University of North Carolina at Chapel Hill).

**Reagents.** Doxorubicin (D4035), etoposide (E1383), cisplatin (P4394), verapamil hydrochloride (V4629) and cyclosporin A (C3642) were obtained from Sigma-Aldrich.

**Western blotting and antibodies.** Protein extract from cultured cells was conducted in sodium dodecyl sulfate (SDS) lysis buffer (50 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol). Protein extract from P. alecto tissues was performed in RIPA lysis buffer (10 mM Tris–HCl pH 7.6, 150 mM NaCl, 1% TritonX-100, 1 mM EDTA) supplemented with EDTA-free complete protease inhibitor cocktail (0469312001, Roche), phosphatase inhibitor cocktail 2 (P5726, Sigma-Aldrich), and phosphatase inhibitor cocktail 3 (P0944, Sigma–Aldrich) using cell pellet homogeniser (VWR) on ice. Protein lysates extracted from the human adrenal gland (sc-363761), lung (sc-363767), spleen (sc-363779), kidney (sc-363764), small intestine (sc-364225) and large intestine (sc-365757) were purchased from Santa Cruz biotechnology, Inc. Lysates were then quantified, and equal amounts of protein were loaded for Western blotting analyses. Antibodies used are as follow: γH2AX (05-636, Millipore, 1:3000), tubulin (ab49928, Abcam, 1:5000), actin (ab8226, Abcam, 1:10,000), γH2AX (05-636, Millipore, 1:3000), tubulin (ab49928, Abcam, 1:5000), actin (MAB1501, Millipore, 1:10,000), ABCB1 (sc-8313 H241, Santa Cruz, 1:1000), horseradish peroxidase (HRP)-conjugated secondary antibody (Thermo Scientific, 1:10,000), and fluorescence labelled secondary antibodies (Thermo Scientific, 1:5000). Detection of γH2AX, tubulin and actin were performed using LI-COR Odyssey (LI-COR Biosciences) or HRP substrate (Thermo Scientific). ABCB1 was detected using HRP substrate (Thermo Scientific). Uncropped Western blotting images are provided in the Supplementary Information.

**Measurement of 53BP1 foci formation.** Detection and quantification of 53BP1 foci formation were conducted using immunofluorescence and fluorescence microscopy. For immunofluorescence, WI-38 and MEF cells were treated with either 10 Gy of γ-irradiation or 5 μM doxorubicin for 3 h before subjected for analysis at the respective time points. In brief, cells were fixed with 4%
formaldehyde (Sigma-Aldrich) and permeabilized with 0.2% Triton X-100 (Bio-Rad) followed by staining with anti-33BP1 antibody (sc-22760, Santa Cruz, 1:250), Alexa Fluor 488-conjugated anti-rabbit IgG (Jackson ImmunoResearch, 1:100), and DAPI (Sigma-Aldrich). Imaging of cells was performed using Olympus IX83 fluorescence microscope at 405 nm for DAPI and 488 nm for 33BP1. As for quantification of 33BP1 foci, analysis of cells was carried out using cell image analysis software CellProfiler23 with the speckle counting pipeline24. A minimum of 100 cells was quantified per time point for each treatment condition. Average number of 33BP1 foci per cell was calculated using the equation: average number of 33BP1 foci per cell = (total number of foci)/(total number of cells quantified).

Measurement of intracellular doxorubicin accumulation. Accumulation of intracellular doxorubicin was monitored or measured by intrinsic fluorescence of doxorubicin using either fluorescence microscopy or flow cytometry. For fluorescence microscopy, PaLuG, PaKiT03, WI-38, HEK293T and MEF cells were pre-treated with or without 5 µM verapamil for 30 min, followed by incubation with doxorubicin for 3 h. Detection of doxorubicin in live cells was performed using Olympus IX71S13 fluorescence microscope. As for flow cytometry analysis, cells were pre-treated with or without 5 µM verapamil or cyclosporin A for 30 min, followed by incubation with doxorubicin for 3 h. Cells were then trypsinized, washed in PBS once and resuspended in PBS for analysis by MACSQuant Analyser 10 (Miltenyi Biotec). Laser excitation was set at 488 nm and filter with wavelengths 614/50 nm was used for detection. A total number of 30,000 live cells were analysed per sample. For Fig. 2c, e, and Supplementary Fig. 7d and E, the calculation was done using the equation: normalised doxorubicin fluorescence value = (mean fluorescence value)/(average cell volume). For Fig. 7h, the calculation was done using the equation: mean fluorescence doxorubicin fold change = (mean fluorescence with efflux inhibition)/(mean fluorescence without efflux inhibition).

Cell volume determination. Mean volume (µL) of WI-38, PaLuG, MEF, HEK293T and PaKiT03 cells were determined by Millipore Sceptre cell counter. Trypsinized cells were washed with PBS, before being measured using 60 µm sceptor tips (Millipore).

Comet assay (DNA damage measurements in cells). DNA damage in PaKiT03 cells was assessed by neutral comet assay as described48. Comets were acquired with propidium iodide for 30 min before subjected for analysis. Images were acquired with a 20× objective lens. A minimum of 30 comets was counted per treatment condition. Analysis of the comets and DNA damage calculated by olive tail moments were performed using CASPlab software67. Olive tail moments were performed using CASPlab software67. Olive tail moments were calculated using the formula: olive tail moments = (the percentage of DNA in comet tail) × (distance between comet head and tail centres of gravity).

Cell viability assay. PaKiT03 cells were seeded at 10,000 cells per well in a 96-well plate and kept overnight to allow attachment. Cells were pre-treated with or without 5 µM verapamil for 30 min, followed by co-treatment of increasing dose of doxorubicin (0.5, 1, 2.5, 5 and 10 µM doxorubicin) and verapamil for 3 h. Doxorubicin was then removed, and cells were replaced in doxorubicin-free medium with or without verapamil for 72 h before measuring cell viability using CellTiter-Glo® luminescent cell viability assay purchased from Promega.

sRNA knockdown. Transient transfection of sRNAs was performed using ScreenFect A (Wako) according to manufacturer’s instructions. Concentrations of sRNA for control, ABCB1, ABCG2 and ABCG2 were set at 20 nM. Control sRNA was purchased from Integrated DNA Technologies (NCl). Sense strand sequence of the sRNAs for ABCB1, ABCG2 and ABCG2 are as follows: ABCB1 #1 (5′-GAGGUCUGAGGAAGUAACACAAATTTG-3′), #2 (5′-GAUGAAGCUACUGCUACGCUAGTDA-3′); ABCG2 #1 (5′-CCCAACAGGACACCAAAAGGCGC-3′), #2 (5′-CUGGAGGAAGAAGUGCAAAUCAC-3′); ABCG2 #1 (5′-CGAUAGAUAUAUACUGCGUAUACDQA-3′), #2 (5′-GGAGAAGAACUUUCUGUAAGAACG-3′).

Quantitative RT-PCR analysis. Total RNA from cell lines and tissue samples was extracted using TRIzol (Life Technologies) and RNeasy Mini Kit (Qiagen). A single panel of human tissue total RNA was purchased from Clontech (human total RNA Master Panel II #636643) and cat tissue total RNA was harvested from three individual P. alecto. All cDNA was synthesised using iScript cDNA synthesis kit (Bio-Rad). Quantitative RT-PCR was performed with SYBR Green (KAPA Bio-systems) using the CFX96 System (BioRad). Relative expression of each gene was calculated using GAPDH as an internal control by BioRad CFX manager software. The primers used are as follows: GAPDH forward (5′-GGTACAGGAGGGTGAAGGTG-3′) and reverse (5′-AGACTCTGTTAGGTCAAATGAGGTC-3′); ABCB1 forward (5′-TGGGTTGTTGGTGATCGACAGAAGCT-3′) and reverse (5′-ACAGCTTCTTCACTCTCTACTGATG-3′); ABCG2 forward (5′-CCATGAGGGCTGATCGGCTG-3′) and reverse (5′-CTCCTCGGAAAGGTGAGATGC-3′).

RNAsq data analysis. Whole transcriptome shotgun sequencing data of PaKiT03 and HEK293T cells from our previous study was obtained from the NCBI Sequence Read Archive under the accessions [SRX661189] and [SRX665117]39. RNA-Seq reads were mapped using Tophat (version 1.3.2) and Bowtie (version 0.12.7). Fragments per kilobase million (FPKM) values were calculated using Cufflinks (version 1.2.1). Differential expression was calculated using DESeq2. FPKM values were normalised per gene to obtain relative expression values.

Protein sequence alignment and conservation comparison. Alignment of different mammalian ABCB1 protein sequences was generated with BioEdit programme. Percentages of identity with other mammalian ABCB1 were analysed with reference to human ABCB1 protein sequence using Vector NTI Advance version 11.5. In this study, amino acid sequences for ABCB1 were obtained for Bos taurus—cow (XP_010802430.1), Canis lupus familiaris—dog (NP_001032215.2), Felis catus—cat (NP_001163531.1), Heterocephalus glaber—naked mole rat (XP_012931457.1), Homo sapiens—human (NP_0009183.2), Macaca mulatta—Rhesus monkey (NP_001028059.1), Mus musculus—mouse ABCB1A (NP_035206.2) and mouse ABCB1B (NP_035205.1). M. davidii—David’s myotis (XP_006779228.1), P. alecto—black flying fox bat (XP_006925783) and Sus scrofa—pig (NP_00126175.1).

Data availability for different species of bats. Maximum lifespan and body mass of P. alecto, C. brachyotis were acquired from AnAge database of Animal Ageing and Longevity [http://genomics.senescence.info/species/]. For M. davidii, M. muricola and R. lepidus, maximum lifespan and body mass were determined by averaging Myotis genus and Rhinolophus genus, respectively using available information derived from the same database.

Statistical analysis. All statistical analyses in this study were conducted using the unpaired student’s two-sided t test.

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References

1. Cleeland, C. S. et al. Reducing the toxicity of cancer therapy: recognizing needs, taking action. Nat. Rev. Clin. Oncol. 9, 471–478 (2012).
2. Seluanov, A., Gladyshev, V. N., Vijg, J., Gorbonovna V. Mechanisms of cancer resistance in long-lived mammals. Nat. Rev. Cancer 18, 433–441 (2018).
3. Keane, M. et al. Insights into the evolution of longevity from the bowhead whale genome. Cell Rep. 10, 112–122 (2015).
4. Sulak M. et al. TP53 copy number expansion is associated with the evolution of increased body size and an enhanced DNA damage response in elephants. Proc. Natl Acad. Sci. USA 113, 6338–6343 (2016).
5. Abeggen, L. M. et al. Potential mechanisms for cancer resistance in elephants and comparative cellular response to DNA damage in humans. J. Am. Med. Assoc. 314, 1850–1860 (2015).
6. Seluanov, A. et al. Hypersensitivity to contact inhibition provides a clue to cancer resistance of naked mole-rat. Proc. Natl Acad. Sci. USA 106, 19352–19357 (2009).
7. Tian, X. et al. High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. Nature 499, 346–349 (2013).
8. Gorbonovna, V. et al. Cancer resistance in the blind mole rat is mediated by concerted necrotic cell death mechanism. Proc. Natl Acad. Sci. USA 109, 13992–13996 (2012).
9. Nasser, N. J. et al. Alternatively spliced Spalax heparanase inhibits extracellular matrix degradation, tumor growth, and metastasis. Proc. Natl Acad. Sci. USA 106, 2523–2528 (2009).

10. Wang, L. F., Walker, P. J. & Poon, L. L. M. Mass extinctions, biodiversity and mitochondrial function: are bats ‘special’as reservoirs for emerging viruses? Curr. Opin. Virol. 1, 649–657 (2011).

11. In praise of bats. Nat. Ecol. Evol. 1, 0071 (2017) https://doi.org/10.1038/s41559-017-0071.

12. Austad, S. N. Methusaleh: evidence from the bats and marsupials. Exp. Gerontol. 46, B47–B53 (1991).

13. Wilkinson, G. S. & South, J. M. Life history, ecology and longevity in bats. Aging Cell 1, 124–131 (2002).

14. Bradford, C., Jennings, R. & Ramos-Vara, J. Gastrointestinal leiomysarcoma in an Egyptian fruit bat (Rousettus aegyptiacus). J. Vet. Diagn. Invest. 22, 462–465 (2010).

15. McClelland, D. J., Dutton, C. J. & Barker, I. K. Sarcomatoid carcinoma in the lung of an Egyptian fruit bat (Rousettus aegyptiacus). J. Vet. Diagn. Invest. 21, 161–163 (2009).

16. Siegel-Willott, J., Heard, D., Siess, N., Naydan, D. & Roberts, J. Microchip-associated leiomyosarcoma in an Egyptian fruit bat (Rousettus aegyptiacus). J. Zoo. Wildl. Med. 38, 352–356 (2007).

17. Zhang, G. et al. Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. Science 339, 456–460 (2013).

18. Polo, S. E. & Jackson, S. P. Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. Genes Dev. 25, 409–433 (2011).

19. Tubbs, A. & Nussenzwang, A. Endogenous DNA damage as a source of genomic instability in cancer. Cell 168, 644–656 (2017).

20. Poirier, M. C. Linking DNA adduct formation and human cancer risk in chemical carcinogenesis. Environ. Mol. Mutagen. 57, 499–507 (2016).

21. Basu A. K. DNA Damage, Mutagenesis and cancer. Int. J. Mol. Sci. 19, E970 (2018).

22. Reisz, J. A., Bansal, N., Qian, J., Zhao, W. & Furdui, C. M. Effects of ionizing radiation on biological molecules—mechanisms of damage and emerging methods of detection. Antioxid. Redox Signal. 21, 260–292 (2014).

23. Fernandez-Capetillo, O., Lee, A., Nussenzwang, M. & Nussenzwang, A. HAX2: the histone guardian of the genome. DNA Repair 3, 959–967 (2004).

24. de Feraudy, S., Revet, I., Bezrookove, V., Feeney, L. & Cleaver, J. E. A minority of foci or pan-nuclear apoptotic staining of γH2AX in the S phase after UV damage contain DNA double-strand breaks. Proc. Natl Acad. Sci. USA 107, 6870–6875 (2010).

25. Panier, S. & Boulton, S. J. Double-strand break repair: 53BP1 comes into focus. Nat. Rev. Mol. Cell Biol. 15, 7–18 (2014).

26. Croco, E. et al. DNA damage detection by 53BP1: relationship to species longevity. J. Gerontol. A Biol. Sci. Med. Sci. 72, 763–770 (2017).

27. Cortopassi, G. A. & Wang, E. There is substantial agreement among interspecies estimates of DNA repair activity. Mech. Ageing Dev. 91, 211–218 (1996).

28. Montecucco, A., Zanetta, F. & Biamonti, G. Molecular mechanisms of etoposide. EXCLI J. 14, 95–108 (2015).

29. Pang, B. et al. Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin. Nat. Commun. 4, 1908 (2013).

30. de Lange, J. H. M. et al. Quantification by laser scan microscopy of intracellular doxorubicin distribution. Cytometry A 13, 571–576 (1992).

31. Robey, R. W., Puchino, K. M., Hall, M. D. & Fojo, A. T. Revisiting the role of ABC transporters in multidrug-resistant cancer. Nat. Rev. Cancer 18, 452–464 (2018).

32. Wyne, J. W. et al. Proteomics informed by transcriptomics reveals Hendra virus sensitizes bat cells to TRAIL-mediated apoptosis. Genome Biol. 15, 332 (2014).

33. Kathawala, R. J., Gupta, P., Ashby, C. R. & Chen, Z.-S. The modulation of ABC transporter-mediated multidrug cancer in review: a cancer of the past decade. Drug Resist. Updat. 18, 1–17 (2015).

34. Shi, Z. et al. Sildenafil reverses ABCB1- and ABCG2-mediated chemotherapeutic drug resistance. Cancer Res. 71, 3029–3041 (2011).

35. Loschmann, N. et al. ABCB1 as predominant resistance mechanism in cells with acquired SNS-032 resistance. Oncotarget 7, 58051–58064 (2016).

36. Wolf, S. J. et al. An update on ABCB1 pharmacogenetics: insights from a 3D model into the localization and evolutionary conservation of residues corresponding to SNPs associated with drug pharmacokinetics. Pharmacogenomics J. 11, 315–325 (2011).

37. Schinkel, A. H. et al. Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc. Natl Acad. Sci. USA 94, 4028–4033 (1997).
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Author contributions
J.K., E.X.Y.S. and A.K.G. performed the experiments. I.H.M., D.L. and Y.T.C. conducted the bat capturing and tissue sample collections. J.K. and Y.T.C. established the primary cell lines and immortalised one of the cell lines. J.K., Y.I., L.F.W. and K.I. designed the experiments, analysed the results, and wrote the paper.

Additional information
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