

The Genetic Drivers and Therapeutic Vulnerabilities of Metastatic Breast Cancer

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The Genetic Drivers and Therapeutic Vulnerabilities of Metastatic Breast Cancer

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Abstract

The understanding of genetic basis and potential therapeutic targets for metastatic breast cancer (mBC) remains limited. Here we systematically identify the genetic and epigenetic causes underlying breast cancer metastasis from clinically defined alterations through multiplexed CRISPR knockout and base editing screens in various models. Multiple drivers converge on the down-regulation of SMARCA1, a core component of ISWI chromatin-remodeling complexes, to drive metastasis, largely through potentiating TGF β signaling. Using druggable gene CRISPR screens, we identify SOD1, a key antioxidant enzyme, as a prominent vulnerability in mBC. We further validate the *in vivo* efficacy of SOD1 pharmacological inhibitor LCS-1 in treating mBC in multiple preclinical models. The exacerbated SOD1 vulnerability in mBC is due to elevated TGF β signaling which impinges on mitochondrial homeostasis, thereby resulting in superoxide radical overload and subsequently triggering ferroptosis. Together, our work provides a comprehensive landscape on the genetic basis and informs therapeutic interventions for mBC.

Introduction

Breast cancer is the most commonly diagnosed malignancy and a leading cause of cancer-related mortality in women¹. Most of the deaths caused by breast cancer are due to metastasis – the spreading of cancer cells from original sites to distinct organs²⁻⁴. Lung, liver, brain and bone are the major colonization sites for metastatic breast cancer (mBC)⁵. As metastasis is a complicated process involving both the tumor cell-intrinsic properties and their interactions with many other cell types in surrounding tissues, advanced *in vivo* experimental systems are usually necessary to model and decipher metastasis⁶⁻⁸. This adds more complexity and difficulty compared to *in vitro* cell culture-based systems, thus significantly limiting the progress of metastasis research. Although multi-omics studies revealed specific genomic or epigenomic features of mBC⁹⁻²⁰, whether these alterations are causal to metastasis remains largely unknown. Only a handful of genes within the tumors or tumor microenvironment were proved so far to regulate breast cancer metastasis²¹⁻⁴⁷. Furthermore, despite the growing functional appreciation of cancer-associated single nucleotide variants (SNVs) or mutations, which and how these SNVs underlie metastasis in breast cancer are still poorly understood.

The application of CRISPR-based genetic perturbation tools (such as Cas9 nuclease-mediated gene knockout or Cas9 nickase-dependent base editors) combined with high throughput screening techniques offers a great opportunity to investigate causal genes or SNVs in varied biological contexts including cancer⁴⁸⁻⁵². Recent pioneer studies have tried to implement CRISPR knockout or activation screens for metastasis regulators in breast cancer⁵³⁻⁵⁹. However, due to dramatic variations in terms of cancer types, cell lines, metastasis models, screening libraries, etc., it remains difficult to obtain consensus or comparable conclusions across different studies. Furthermore, base editing screens have not been performed yet for uncovering metastasis-associated SNVs in breast cancer. In addition, CRISPR knockout screens with druggable gene library can also help to identify the genetic vulnerabilities or potential drug targets for specific cancer cell populations or states⁴⁸. Nevertheless, such efforts have not yet been systematically devoted into mBC yet.

Here, by leveraging multiple CRISPR knockout and base editing screens, we establish a comprehensive landscape of genetic causes in driving breast cancer metastasis, and delineate actionable drug targets in treating mBC. Mechanistically, a consensus SMARCA1-centric regulatory program is revealed to promote metastasis via TGF β signaling coordination. The mitochondria-involved reactive oxygen species (ROS) and ferroptosis underlie the vulnerabilities of genetic or pharmacological inhibition of SOD1 in mBC.

Results

Systematic CRISPR knockout screens for metastasis genes in breast cancer

To determine the genes whose loss-of-function due to genetic mutations or epigenetic down-regulation functionally contribute to human breast cancer metastasis, we designed a human CRISPR knockout library (hBRCA-KO library) with 3,961 sgRNAs targeting 611 genes that are either recurrently mutated or down-regulated in human mBC compared to primary tumor or normal tissue according to public datasets (Fig. 1a; Supplementary Table1; see Methods). We lentivirally transduced this library into human breast cancer MCF7 or MDA-MB-231 (MDA-231 for short) cells at various multiplicity of infection (MOI: 0.3, 3 or 6), and transplanted them into immunocompromised mice either subcutaneously (s.c.) or intravenously (i.v.) to allow for the metastasis formation (Fig. 1b). The metastatic tumors, along with matched primary tumors (via subcutaneous inoculation) and *in vitro* cultured parental cells, were harvested for sgRNA quantification and bioinformatic analysis to determine the functional metastasis genes (Fig. 1b). As a luminal breast cancer cell line, wild-type (WT) MCF7 cells barely undergo metastasis in mouse xenograft model, whereas triple-negative breast cancer cell (TNBC) MDA-MB-231 represents a common model for studying metastasis. Primary tumors inoculated subcutaneously would be more difficult to metastasize than those via i.v. injection, as an additional intravasation process is needed for s.c. tumors. After pooled CRISPR editing, the growth rate of s.c. primary tumors did not change much compared to control in 6-week duration (Extended Data Fig. 1a, b), and metastasis foci could be visible in multiple organs or sites (e.g., lung, liver, diaphragm, or abdomen) at screening endpoint (Fig. 1c, d). As expected, the metastasis rate for MDA-231 group (either s.c. or i.v.) was 100% (Extended Data Fig. 1e). In contrast, sgControl (vector) tumors of MCF7 background had no metastasis events at all (Fig. 1d). Interestingly, CRISPR-edited MCF7 tumors were indeed capable to metastasize, albeit at a low or moderate rate, with i.v. group (38% - 50%) higher than s.c. group (10% - 20%) (Fig. 1d). These results indicated the validity of our screening systems for functional metastasis genes.

The sgRNA distribution was highly distorted in metastatic samples compared to those of primary tumors or *in vitro* culture (Extended Data Fig. 1d), and individual sgRNAs were highly enriched in metastatic tumors (Fig. 1e), suggesting a strong selection pressure during metastasis progression. Therefore, functional metastasis genes were defined to meet the following basic criteria: (1) at least one sgRNA is among the top 20 enriched sgRNAs in each metastasis sample; and (2) the read count of sgRNA should account for more than 1% of the total reads in the corresponding metastasis sample. On top of that, if (1) the qualified sgRNA or (2) the corresponding gene occurs in ≥ 2 metastatic tumors, or (3) the gene can be supported by ≥ 2 qualified sgRNAs, then the corresponding gene would be considered as a hit (Fig. 1f). Using these criteria, a handful of functional metastasis genes were identified under different groups, and the top enriched genes were highly reproducible across conditions (Fig. 1f), including tumor suppressors (e.g., *PTEN* and *TP53*), epigenetic regulators (e.g., *SMARCA4* and *KMT2D*), signaling molecules (e.g., *LEP* and *PTPN13*), and extracellular matrix (ECM)

modulators (e.g., *ITIH5*, *ITIH2* and *ADAMTS7*). Several of them were known regulators for breast cancer outgrowth and metastasis^{2, 4, 25, 60}, suggesting the robustness of our screening systems. Among different metastasis groups, the same cell background (MB-231 s.c. vs. MB-231 i.v. or MCF7 s.c. vs. MCF7 i.v.) and s.c. inoculation manner (MCF7 s.c. vs. MB-231 s.c.) significantly shared metastasis genes (Fig. 1g). Indeed, the genetic background and primary tumor inoculation routes were more influential than organ/site tropism in defining the functional metastasis genes across conditions (Extended Data Fig. 1e). A total of 104 metastasis genes were identified as high-confidence hits that occurred in at least 2 conditions, with clear classification patterns regarding to cell specificity and inoculation routes (Fig. 1h). The metastasis genes were enriched in functional terms relating to “senescence”, “transcription”, “chromatin remodeling”, “stress response”, “growth” and “extracellular region” (Fig. 1i and Extended Data Fig. 1f).

To determine which metastasis gene acts through enhancing tumor growth or fitness, we looked into cell fitness screening data in matched primary tumors or *in vitro* cultured parental cells. MAGeCK-derived β -score was employed to identify cell fitness genes⁶¹, with a higher positive score indicating stronger positive selection and a lower negative score meaning stronger negative selection. As expected, core essential genes were negatively selected in both parental cells and primary tumors (Extended Data Fig. 1g, h), indicating the high quality of these screens. We found that only a few high-confidence metastasis genes were among the top 10% positively selected cell fitness hits (Extended Data Fig. 1i-l), including several typical tumors suppressors (e.g., *TSC2*, *PTEN*, *NF1*, *TP53* and *RB1*). These results were consistent with functional gene enrichment analysis (Fig. 1i and Extended Data Fig. 1f), suggesting that metastasis genes exert functions via multiple mechanisms.

Using METABRIC and TCGA-BRCA cohorts, we found that patients whose tumors had either low-expression or mutation of those high-confidence metastasis genes consistently displayed bad overall survival (Fig. 1j-m), further corroborating the functional impacts and clinical relevance of these metastasis genes.

Functional validation of metastasis genes

To validate the functions of metastasis genes identified from systematic screens, we employed the MCF7 s.c. condition as the test model in which WT MCF7-derived tumors cannot undergo distal metastasis at all. We firstly constructed a small sub-library containing 40 sgRNAs for knocking out the top 20 ranked metastasis genes in MCF7 s.c. group (Extended Data Fig. 2a; Supplementary Table1). Transwell experiments showed that MCF7 cells transduced with the sub-library exhibited significantly enhanced migration and invasion, compared to control cells (Extended Data Fig. 2b). More importantly, s.c. inoculation of the sub-library infected MCF7 tumors indeed led to the occurrence of lung metastasis, whereas control tumors could not (Extended Data

Fig. 2c, d), proving the *in vivo* functions of these metastasis genes. To further pinpoint the key genes, individual sgRNAs were used to target several candidate genes (Extended Data Fig. 2e). We surmise that the perturbation of a single gene might not be sufficient to drive MCF7 s.c. metastasis, and therefore, the gene combination was also considered according to their co-occurrence in metastatic tumors (Extended Data Fig. 2a). Using *in vitro* migration assay, we determined that each single gene perturbation generally produced moderate phenotype, while the multi-gene combination significantly enhanced the migration capacity of perturbed cells (Extended Data Fig. 2f). Next, we selected two representative double-gene knockout models (*ADAMTS7* + *FRMD1*, *AF*; *PTEN* + *TENM3*, *PT*), which displayed comparable effect size to multi-gene combination groups, for downstream analysis (Extended Data Fig. 2f). Single clonal cells were generated for double knockout (DKO) or single knockout (KO) of the above-mentioned genes (Fig. 1n). Transwell-based migration and invasion assay in MCF7 cells demonstrated significantly increased effects in gene-perturbed cells compared to control group, especially for DKO groups (Extended Data Fig. 2g). The mobility-promotive effects of DKO were also confirmed by the scratch or wound healing assay in MCF7 cells (Extended Data Fig. 2h). Similar *in vitro* results were also obtained using another luminal T47D cells (Extended Data Fig. 2i, j).

To determine the *in vivo* functions of these genes, we transplanted control, *AF*^{DKO}, or *PT*^{DKO} MCF7 cells into immunocompromised mice via either s.c. or i.v. routes, and examined metastasis status after six weeks. For the most difficult MCF7 s.c. metastasis model, unlike the top 20 gene perturbation (Extended Data Fig. 2c, d), neither *AF*^{DKO} nor *PT*^{DKO} could sufficiently form visible metastasis foci (Fig. 1o, p), suggesting that more genes were required to drive this complicated process. However, in MCF7 i.v. model, both DKO models successfully formed metastasis at a high penetration rate (78% for *AF*^{DKO}; 89% for *PT*^{DKO}) (Fig. 1o, p). Moreover, the metastasis-driving role of the four genes or *AF/TP* combo was also confirmed in MDA-231 cells using *in vitro* transwell and scratch assays (Extended Data Fig. 2k, l) as well as *in vivo* MDA-231 i.v. metastasis model (Extended Data Fig. 2m). These data clearly proved the functions of these genes in driving breast cancer metastasis, reinforcing the validity of our screening results.

Metastasis gene screening in immunocompetent mice

Transplantation of human tumors into immunocompromised mice fell short of simulating normal *in vivo* context or tumor microenvironment due to deficiency of immune system. To overcome this limitation and more comprehensively evaluate the tumor-intrinsic metastasis genes, we constructed a matched mouse CRISPR knockout library (mBRCA-KO library) with 3,817 sgRNAs targeting 585 mouse genes corresponding to those human genes in hBRCA-KO library (Fig. 1a; Supplementary Table2; see Methods). We lentivirally transduced this library into JC cells, a non-metastatic luminal breast cancer line of mouse origin, at different MOI (0.3 and 3). Next,

the CRISPR-edited cell pool was transplanted into syngeneic and immunocompetent BALB/c mice. In addition to s.c. and i.v. routes, orthotopic (ortho.) inoculation via mammary fat pad was also executed (Fig. 2a). Interestingly, ortho. transplanted tumors displayed faster growth rate than s.c. tumors (Extended Data Fig. 3a), highlighting the importance of tumor microenvironment. After 6 weeks, the metastasis status was determined. As expected, the non-edited control JC tumors could not metastasize at all, while the CRISPR-edited tumors did metastasize to the lung, liver, diaphragm, etc. despite at a low or moderate frequency (13%-50%) (Fig. 2b). The i.v. route was the easiest for developing metastasis followed by the ortho. inoculation, while the s.c. approach was the most difficult (Fig. 2b). Then, the metastatic, primary and *in vitro* cultured parental samples were collected for high-throughput sgRNA quantification and downstream analysis (Fig. 2a). Similarly, the sgRNAs were highly selected in metastatic tumors (Fig. 2c and Extended Data Fig. 3b). Using the same analytic pipeline as before, we catalogued a cohort of metastasis genes in immunocompetent context (Fig. 2d; Supplementary Table2). Among the top enriched genes, typical tumor suppressors such as *Nf1*, *Trp53* and *Pten* were apparently shared between different groups while others were implicated in immune response (e.g., *Il17b*), transcriptional control (e.g., *Men1* and *Id4*), cell motility (e.g., *Actg2* and *C1xn*), lipid metabolism (e.g., *Fabp4* and *Mlxip1*), etc. (Fig. 2d). Intriguingly, the ortho. metastasis genes more resembled to those in the i.v. group than in the s.c. condition (Fig. 2e and Extended Data Fig. 3c). A total of 29 genes were identified as high-confidence (in at least 2 conditions) metastasis genes in immunocompetent mice using JC cell derived tumors (Fig. 2f), with prominent functions relating to “cell migration”, “proliferation” and “angiogenesis” (Fig. 2g). When comparing with high-confidence metastasis genes of luminal tumors (MCF7-derived) in immunocompromised context, a significant overlap was observed (Extended Data Fig. 3d). When checking the cell fitness effects in primary tumor and *in vitro* cultured cells, only three high-confidence metastasis hits (*Nf1*, *Trp53* and *Nup98*) could act by enhancing tumor growth (Extended Data Fig. 3e-h), again suggesting that multiple mechanisms were involved in regulating metastasis. Consistently, the low-expression or mutation of these murine-derived immunocompetent metastasis genes also significantly correlated with bad patient survival (Extended Data Fig. 3i-l), further supporting their functions and clinical relevance.

One prominent hit *Il17b*, which was the 3rd-ranked high-confidence metastasis gene in immunocompetent context but not identified in immunocompromised condition, caught our attention (Fig. 2f, 1g). This gene in human encodes interleukin 17B, a member of IL-17 family cytokines which play multifaceted roles in inflammatory and malignant disorders^{62, 63}. To determine its role during breast cancer metastasis, we knocked out *Il17b* in JC cells with two independent sgRNAs (Fig. 2h). Interestingly, *in vitro* migration and invasion assay did not reveal apparent effects in *Il17b* KO cells (Extended Data Fig. 3m), however, *in vivo* transplantation of JC-derived *Il17b* KO

tumors into immunocompetent mice exhibited significantly increased metastasis via i.v. route compared to the control tumors (Fig. 2i, j). No metastasis was observed for ortho. groups (Fig. 2i, j), indicating that additional gene perturbations might be required for the complete metastasis process. These results suggest that tumor microenvironment is essentially required for *Il17b* KO tumors to develop metastasis. To explore the tumor microenvironment, we performed single cell RNA sequencing (scRNA-seq) in JC-derived *Il17b* KO primary and lung metastatic tumors (Fig. 2k and Extended Data Fig. 3n). A significant immune cell infiltration was observed in metastasis versus primary tumors (Extended Data Fig. 3o), and *Il17b* KO tumors caused aberrant changes of tumor immune microenvironment compared to control possibly via dysregulation of immune regulatory genes in cancer (Fig. 2l-n). These data underscore the importance of tumor microenvironment in decoding the gene functions during metastasis.

Base editing screens to identify driver mutations for breast cancer metastasis

SNV, a major form of cancer mutations, is a pivotal driving force for cancer development and progression. SNVs can act in either loss-of-function or gain-of-function manner. To gain a comprehensive view on how cancer SNVs affect metastasis, we constructed two base editing libraries with 12,676 sgRNAs for adenine or cytosine base editor (ABE or CBE) to install 2,253 highly frequent SNVs (A•T-to-G•C or C•G-to-T•A) in BC as well as other major cancer types (hPANCAN-BE libraries) (Fig. 3a, b; Supplementary Table3; see Methods). MCF7 or MDA-231 cells were firstly transduced with lentiviruses delivering highly efficient and PAM-less SpRY-ABE8e or SpRY-evoA-BE4max base editors that we generated previously⁶⁴. The hPANCAN-BE libraries were then lentivirally transduced at a low MOI (< 0.3) to generate the mutated cell pool followed by either i.v. transplantation into immunodeficient mice to allow for metastasis or parallelly *in vitro* cell culture, before harvesting for sgRNA quantification (Fig. 3c). Interestingly, we did observe apparent metastasis foci in the lung and liver for BE-edited tumors compared to vector control, even at 100% metastasis rate for both MCF7 and MDA-231 derived tumors (Fig. 3d, e). This strong phenotype penetration suggested that these SNVs may act in a gain-of-function manner, in contrast to the moderate penetration rate of loss-of-function metastasis genes in above knockout screens. Again, the sgRNAs were also highly selected in metastatic samples (Fig. 3f, Extended Data Fig. 4a, b). The sgRNAs targeting the essential genes were negative selected in cells as expected (Extended Data Fig. 4c), indicated a high quality of our BE screens.

To nominate a functional metastasis SNV, the following criteria were applied: (1) a qualified sgRNA targeting the SNV is defined when it is among the top 30 enriched sgRNAs in each metastasis sample and the read count of the sgRNA should account for more than 1% of the total reads in the corresponding metastasis sample; (2) the same qualified sgRNA is determined in at least two metastasis samples, or different qualified sgRNAs targeting the same SNV are identified in at least two metastasis

samples, or at least two qualified sgRNAs targeting the same SNV are found in one metastasis sample. Under such criteria, we identified a total of 127 A>G/T>C and 141 C>T/G>A metastasis-driving SNVs in MCF7 and MDA-231 derived tumors, respectively (Fig. 3g; Supplementary Table3), and a few of them displayed promotive functions of tumor growth as evidenced by the *in vitro* cell fitness screens (Extended Data Fig. 4d-g). These functional SNVs affected around 25% of breast cancer patients (Extended Data Fig. 4h), and patients bearing these SNVs tended to have a bad overall survival (Fig. 3h, i). Interestingly, the genes bearing these functional SNVs were significantly enriched in multiple oncogenic signaling pathways (Extended Data Fig. 4i), and these genes were largely different from functional metastasis genes identified from knockout screens (Extended Data Fig. 4j). The detailed metastasis SNVs in breast cancer were shown in Fig. 3j and 3k. Interestingly, among those top SNVs, we found that their parental genes were functionally important and highly mutated in breast cancer such as *ESR1* (encoding estrogen receptor α), *ERBB2* (encoding HER2), and *TP53* (Fig. 3j, k). Our results deepened the understanding of these genes at single nucleotide resolution in driving breast cancer metastasis. Using an individual sgRNA and SpRY-evoA-BE4max base editor, we installed a top representative but less characterized metastasis SNV *DUSP19_c.650C>T* in MDA-231 cells (Extended Data Fig. 4k). This SNV converts a serine to a leucine at site 217 of DUSP19 protein (a dual-specificity phosphatase). Both transwell-based *in vitro* assays and i.v. transplanted *in vivo* models could validate the metastasis-promotive role of this SNV (Extended Data Fig. 4l and Fig. 3l, m), confirming the accuracy of base editing screens. Altogether, our data presented a comprehensive mutation landscape of clinically-relevant causal variants at single nucleotide resolution that underlie breast cancer metastasis.

Repression of SMARCA1 as a core driver for metastasis

To further reveal the detailed mechanisms of breast cancer metastasis, we employed two independent cellular models with high metastasis capability that were validated in our above efforts - *AF^{DKO}* and *PT^{DKO}* along with their matched non-metastatic MCF7 parental control cells. RNA-seq based transcriptome profiling and mass spectrometry-based proteome characterization were performed to determine the metastasis associated gene expression signatures (Supplementary Table4). Interestingly, the two independent models (*AF^{DKO}* vs. *AF^{DKO}*) correlated well and identified significantly overlapped gene signatures at either transcriptional or protein levels especially for down-regulated genes in metastasis^{high} states (Fig. 4a, b and Extended Data Fig. 5a-d). The differentially expressed metastasis-associated genes were prominently enriched in the processes or cellular components involving “ECM”, “cell adhesion”, “epithelial mesenchymal transition”, “estrogen response” and “mitochondrion” (Fig. 4c, d and Extended Data Fig. 5e, f), indicating the diverse features and/or mechanisms underlying breast cancer metastasis.

By scrutinizing the individual hits in the signatures, we were able to pinpoint a single consensus gene *SMARCA1* that was down-regulated across all the four comparison groups (Fig. 4e and Extended Data Fig. 5g). *SMARCA1* is an ATPase and catalytic subunit of ISWI chromatin-remodeling complexes, which can regulate nucleosome sliding and chromatin accessibility in transcriptional control⁶⁵. We then knocked out *SMARCA1* by individual sgRNAs in MCF7 cells (Fig. 4f), and found that knockout of *SMARCA1* enhanced cell migration and invasion capabilities compared to control cells during *in vitro* assays (Fig. 4g). Such phenotypes were rescued by ectopic expression of *SMARCA1* (Fig. 4f, g), confirming the specificity of *SMARCA1*-targeting sgRNAs. More importantly, MCF7 derived *SMARCA1* KO tumors indeed developed *in vivo* lung metastasis in nude mice via i.v. injection while control tumors did not (Fig. 4h, i). Using public datasets, we observed low-expression of *SMARCA1* mRNA in advanced stages or highly metastatic subtypes of human breast cancers (Fig. 4j and Extended Data Fig. 5h). We further confirmed this observation by *SMARCA1* immunohistochemistry using a human breast cancer tissue microarray (Fig. 4k). Moreover, low-expression of *SMARCA1* correlated with poor survival of breast cancer patients (Fig. 4l, m). These results corroborated that down-regulation of *SMARCA1* is a *bona fide* driver for human breast cancer metastasis.

To determine the detailed mechanisms of *SMARCA1* in regulating metastasis, we firstly profiled *SMARCA1* target genes by RNA-seq in *SMARCA1* KO MCF7 cells (Supplementary Table4). Interestingly, a significant overlap of differentially expressed genes was observed between sg*SMARCA1*, *AF*^{DKO} and *PT*^{DKO} groups (Fig. 4n and Extended Data Fig. 5i, j), indicating that *SMARCA1* down-regulation might be a key mediator for the two cellular models to display high metastasis capability. Indeed, ectopic expression of *SMARCA1* in *AF*^{DKO} or *PT*^{DKO} MCF7 cells could significantly reverse their enhanced cell migration and invasion phenotypes (Extended Data Fig. 5k). This phenomenon was also recapitulated in T47D and MDA-231 cells (Extended Data Fig. 5l, m), further confirming the central role of *SMARCA1* in metastasis. When checking the enriched functional terms of *SMARCA1* target genes, several top terms were related to “transcription” or “gene expression” (Fig. 4o, n), consistent with the chromatin remodeling role of *SMARCA1*. Particularly, top terms also included “TGFβ signaling pathway” or “epithelial mesenchymal transition” (Fig. 4o, p and Extended Data Fig. 5n). Considering the pivotal role of TGFβ signaling in driving breast cancer metastasis⁶⁶, we surmised that repressed *SMARCA1* may act through augmenting the TGFβ signaling. Indeed, we observed increased levels of phosphorylated SMAD2/3 (p-SMAD2/3) in *SMARCA1* knockout or *AF*^{DKO} / *PT*^{DKO} metastasis^{high} MCF7 or MDA-231 cells (Fig. 4q, and Extended Data Fig. 5o). Concordantly, the DNA binding signals of SMAD2/3 were generally increased upon *SMARCA1* KO in MCF7 cells (Fig. 4r). These data suggested a reinforced TGFβ response and signaling cascades in *SMARCA1* repressed cells. Furthermore, a physical protein-protein interaction between SMAD2/3 and *SMARCA1* was observed (Extended Data Fig. 5p), implying

that *SMARCA1* might also directly orchestrate the SMAD2/3-dependent transcriptional program in the nucleus. Using ATAC-seq, we observed a significant increase of chromatin accessibility ($n = 2618$ increased peaks; $n = 381$ decreased peaks) upon *SMARCA1* KO in MCF7 cells, which was accompanied with enhanced SMAD2/3 binding among these regions (Fig. 4s). Motif and TFLink analysis also indicated a significant enrichment of SMAD2/3 onto these increased open regions (Fig. 4t, u). Moreover, these altered chromatin states were also relevant to the gene expression change upon *SMARCA1* KO (Fig. 4v, w). Altogether, these data demonstrate that down-regulation of *SMARCA1*, as a convergently regulatory node of clinical relevance, can potentiate TGF β signaling, thereby driving breast cancer metastasis.

Therapeutic vulnerabilities of metastatic breast cancer

We hypothesized that the gain of certain advantageous features (e.g., high metastasis capacity) of tumor cells might meanwhile bring some new weakness or at a negative cost (e.g., special vulnerability). If so, such weakness or vulnerability would open a door for the treatment of metastatic cancer. To test this, we employed a druggable gene CRISPR knockout library that we generated before with $\sim 12,000$ sgRNAs targeting $\sim 1,700$ druggable genes in the human genome⁴⁸. Cell fitness CRISPR screens were performed with this library in the two metastasis^{high} MCF7 cell models (single clonal *AF*^{DKO} and *PT*^{DKO} lines generated by SaCas9) as well as the parental control cells (see Methods) (Fig. 5a, and Extended Data Fig. 6a-e). Special vulnerabilities in metastasis^{high} cells were defined when a gene hit was more negatively selected in metastasis^{high} cells compared to control cells and determined with the following cutoff: $\text{score}^{\text{DKO}} < -1$ & $\text{score}^{\text{control}} < 0.5$ & $(\text{score}^{\text{DKO}} - \text{score}^{\text{control}} < -1)$ (Supplementary Table5). Among the special vulnerability hits shared between the two metastasis^{high} cell models (Extended Data Fig. 6f, g), we found that *SOD1*, *MAP3K3*, *SMARCA4* and *MVK* ranked among the top (Fig. 5b, c). Interestingly, *SMARCA4* was identified as a metastasis gene in MDA-231 cells whose knockout promoted tumor growth and metastasis (Fig. 1f, and Extended Data Fig. 1k, l). This function of *SMARCA4* in TNBC was also supported by another recent report⁶⁰. The divergent roles of the same gene between luminal and TNBC manifested the context importance and regulatory complexity of cancer subtypes. Functional gene enrichment analysis indicated that mBC vulnerability genes significantly converged on mitochondria related processes (Fig. 5d). Using independent sgRNAs to ablate three top hits *SOD1*, *SMARCA4* and *MVK* (Fig. 5e), we confirmed these exacerbated vulnerabilities in metastasis^{high} *AF*^{DKO} and *PT*^{DKO} MCF7 cells (Fig. 5f). Interestingly, ectopic expression of *SOD1* could rescue the cytostatic effects of other top gene knockout (Extended Data Fig. 6h-k), indicating that *SOD1* is epistatic to others to mediate mBC vulnerability.

As these druggable genes all have ready-to-use inhibitors or drugs, we next employed a specific *SOD1* inhibitor compound LCS-1 to evaluate its therapeutic efficacy. First, we achieved significant inhibition of cell viability in multiple metastasis^{high} models

(AF^{DKO} , PT^{DKO} or sgSMARCA1) of MCF7, T47D and MDA-231 origin, compared to their corresponding controls (Fig. 5g-i). Intriguingly, the highly metastatic MDA-231 cells were more sensitive to LCS-1 treatment compared to non- or low-metastatic MCF7 or T47D cells (Fig. 5g-i). Second, we employed two *in vivo* experimental metastasis models based on MDA-231 or 4T1 derived tumors via i.v. inoculation in immunocompromised or immunocompetent mice, respectively. As shown in Fig. 5j and 5k, significant reductions of metastasis signals or areas in the lung were observed upon LCS-1 treatment in both models. Third, we further tested LCS-1 in a spontaneous metastasis model with transgenic MMTV-PyMT mice, which spontaneously develop mammary tumors and metastasis with characteristics resembling human breast cancer⁸. Consistently, LCS-1 treatment could not only alleviate the metastasis burden but also significantly decrease the growth of primary tumors in this preclinical model (Fig. 5l, m). Together, these data strongly suggest that targeted inhibition of SOD1 might be an attractive approach to therapeutically treat mBC.

Targeting SOD1 to elicit ferroptosis in metastatic breast cancer

We further explored the underlying mechanisms of SOD1 vulnerability in mBC. As an enzyme to convert toxic superoxide radical $O_2^{\bullet-}$ into less harmful hydrogen peroxide H_2O_2 , SOD1 is the major intracellular superoxide dismutase and an important scavenger of ROS in the antioxidant defense system⁶⁷⁻⁶⁹. Indeed, either genetic ablation of *SOD1* or pharmacological inhibition using LCS-1 caused enhanced total ROS levels in both metastasis^{high} (AF^{DKO} , PT^{DKO} or sgSMARCA1) and parental control MCF7 cells (Fig. 6a, b). Interestingly, the levels of ROS were significantly higher in metastasis^{high} versus control cells (Fig. 6a, b). Particularly, the intracellular and mitochondrial superoxide $O_2^{\bullet-}$ levels were also increased upon SOD1 inhibition, especially in metastasis^{high} (AF^{DKO} , PT^{DKO} or sgSMARCA1) cells (Fig. 6c, d and Extended Data Fig. 7a). These data suggest that the exacerbated SOD1 vulnerability of metastasis^{high} cells might link to higher oxidative stress which causes ferroptosis. As a programmed cell death mechanism, ferroptosis is characterized by iron-dependent membrane lipid peroxidation^{70, 71}. Using a series of assays, we did find that the oxidized polyunsaturated fatty acids (PUFAs) (C11-BODIPY probe), phospholipid hydroperoxides (PLOOHs) (MDA assay) and the Fe^{2+} iron (FerroOrange probe) levels were significantly accumulated in metastasis^{high} (AF^{DKO} , PT^{DKO} or sgSMARCA1) cells upon SOD1 inhibition compared to those in control MCF7 cells (Fig. 6e-g). In addition, the expression levels of ACSL4 (ferroptosis driver) and GPX4 (ferroptosis suppressor) were apparently increased or decreased, respectively, upon SOD1 inhibition or genetic knockout especially in metastasis^{high} (AF^{DKO} , PT^{DKO} or sgSMARCA1) MCF7 cells (Fig. 6h and Extended Data Fig. 7b). More importantly, the cell death was reinforced with ferroptosis inducer Erastin for LCS-1 treated metastasis^{high} (AF^{DKO} , PT^{DKO} or sgSMARCA1) cells, while the ferroptosis inhibitor Ferrostatin-1 (Fer-1) could fully reverse the exacerbated cell death phenotype of metastasis^{high} cells over their parental control counterparts (Fig. 6i). These results corroborated that the pronounced

vulnerability of mBC to SOD1 inhibition is due to enhanced oxidative stress and resulting ferroptosis.

Mitochondria are the primary source of ROS production especially for the superoxide $O_2^{\bullet-}$ species during mitochondrial respiration⁶⁷⁻⁶⁹. We found that the mitochondrial membrane potential of metastasis^{high} (AF^{DKO} , PT^{DKO} or sgSMARCA1) cells was significantly lower (enhanced monomer/aggregates ratio of JC-1 probe) than that of control MCF7 cells (Fig. 6i), which suggested a more compromised mitochondrial function in metastasis^{high} cells. LCS-1 treatment further exacerbated such effects possibly via increased production of harmful superoxide $O_2^{\bullet-}$ (Fig. 6j). The mitochondrial function decline of metastasis^{high} cells was also evidenced by the significantly lower oxygen consumption rate (OCR) compared to control cells (Fig. 6k). Thus, the mitochondrial dysfunction likely underlined the elevated levels of ROS in metastasis^{high} cells, which echoed with significant enrichment of mitochondrial genes in metastasis-associated gene signatures and vulnerabilities (Extended Data Fig. 5e, and Fig. 5d). Previous studies in mammalian cells and *C. elegans* indicated that mitochondrial homeostasis can be affected by the ECM and TGF β signaling⁷². To determine whether enhanced TGF β response in metastasis^{high} tumors impinges on mitochondrial function, we knocked out *TGFBR1* or *TGFBR2* (Extended Data Fig. 7c), which encodes type I or II TGF β receptor, respectively, in multiple metastasis^{high} and control MCF7 cell models. Intriguingly, the decreased mitochondrial OCR in multiple metastasis^{high} cells were fully rescued when genetically ablating *TGFBR1* or *TGFBR2* (Fig. 6l). More importantly, the inhibition of cell viability by SOD1 blockade in metastasis^{high} cells was further reinforced upon exogenous TGF β -1 addition, while significantly relieved by the treatment of TGF β type I receptor inhibitor SB-431542 (Fig. 6m). Interestingly, we also found that ectopic expression of SMARCA1 in AF^{DKO} or PT^{DKO} metastasis^{high} MCF7 cells significantly rescued the ROS accumulation and reversed the cytotoxic effects of LCS-1 treatment (Extended Data Fig. 7d, e), consistent with the role of SMARCA1 in orchestrating TGF β signaling. Altogether, these data revealed a “TGF β -mitochondria-ROS-ferroptosis” regulatory axis as the centric mechanism to endow mBC with pronounced SOD1 vulnerability.

Discussion

Deciphering the genetic basis and vulnerabilities of mBC is a prerequisite for rationally developing effective therapeutics against this deadly disease. Through systematic CRISPR knockout and base editing screens along with mechanistic investigations, we presented a comprehensive view on the tumor-intrinsic metastasis driving forces from clinically-relevant mutational or expressional alterations. More importantly, we further proposed potential drug targets and therapeutic strategies to treat mBC.

Although different *in vivo* mouse models are usually employed for studying metastasis, there is no single experimental system that can perfectly model the whole profiles of

human cancer metastasis^{6-8, 73}. For instance, injection of human MDA-MB-231 cells into the tail vein of immunocompromised mice is one the most commonly used experimental metastasis system for modelling lung metastasis of breast cancer. Although this system can reflect the extravasation step that tumor cells leave the circulation routes to lodge into secondary organs, it fails to recapitulate the initial intravasation process by which tumors leave the primary sites to enter the circulation system. Another widely used spontaneous metastasis model using orthotopic implantation of murine breast cancer cells (e.g., 4T1) into syngeneic and immunocompetent mice overcomes the above drawbacks, but the tumors and tumor microenvironment are not of human origin^{7, 8}. To obtain the most relevant and comprehensive information underlying breast cancer metastasis, we performed genetic perturbation screens using multiple *in vivo* models covering different subtypes of tumor cells, varied tumor implantation routes and sites, and immune-competent or -compromised microenvironment. Particularly, we succeeded to construct a subcutaneous xenograft metastasis model based on luminal MCF7 cells via genetic perturbations. Such new model will be fairly useful to investigate the whole process of metastasis (both intravasation and extravasation) for lumina types of breast cancer of human origin.

Determining the functional genes underlying metastasis is one of the central tasks in this field. We employed CRISPR knockout screens to functionally perturb the genes *en masse* that are either mutated in mBC or transcriptionally down-regulated compared to primary tumors. We found that both genetic and epigenetic alterations on those functional genes can promote metastasis. The driving effects could be greatly enhanced via the combination of multiple alteration events. Moreover, we further determined the metastasis-driving SNVs in mBC via base editing screens, which have not been characterized before. The delineation of these functional alterations not only helps to decode the metastasis mechanisms, but also holds prognostic or predictive values to inform metastasis after clinical genetic tests. The findings of SMARCA1 down-regulation in multiple mBC models indicate that different driving events might converge on consensus downstream nodes or nuclear programs to execute metastasis. The deregulation of SMARCA1-involved chromatin remodeling might create a permissive chromatin state that favors TGF β /SMAD signaling-elicited transcriptional programs underlying breast cancer metastasis.

It is also interesting and important to observe that different mBC cells could share consensus vulnerabilities, suggesting that a common therapeutic regimen might be possible to treat a broad population of mBC patients. The top vulnerabilities all converged to SOD1, further strengthening the mBC dependency on this superoxide radical scavenger. As altered ECM or enhanced TGF β signaling in mBC impacts mitochondrial homeostasis to produce superfluous ROS, simultaneous blocking of SOD1 would cause aberrant accumulation of harmful superoxide radical O₂^{•-} within

the cell. $O_2^{\bullet-}$ can not only target iron–sulfur (Fe-S) clusters to release Fe^{2+} iron, but also forms peroxynitrite ($ONOO^-$) with nitric oxide (NO). In addition to causing oxidative damages to proteins and nucleic acids, $ONOO^-$ also induces lipid peroxidation⁶⁷⁻⁶⁹. Ferroptosis is then triggered via excessive lipid peroxidation along with Fe^{2+} iron supply^{70, 71}. In support of this model, the inter-connections between ECM, TGF β , mitochondria and ROS have been separately suggested in different scenarios rather than mBC by other studies^{72, 74, 75}. Furthermore, mBC and TNBC display pronounced sensitivity to varied ferroptosis inducers⁷⁶⁻⁷⁸. More importantly, our data showed that either genetic or pharmacological inhibition of SOD1 exhibited appreciable therapeutic effects against mBC in multiple models largely via ferroptosis. Altogether, targeting tumor-intrinsic SOD1 might be a promising avenue for developing therapeutics against mBC in clinics.

Our research is subject to a few limitations. First, our current study primarily focused on breast cancer metastasis to the lungs or livers but not to the brains or bones due to the choice of model systems. Whether the brain or bone metastasis of breast cancer obey similar rules uncovered here needs further verification. Second, this work mainly aims to investigate the human tumor-intrinsic alteration events during metastasis. Other driving effects from alterations in other cell types in the tumor microenvironment or along the metastatic routes have not been explored. Third, we did not investigate those genes that are transcriptionally up-regulated in mBC and possibly promote metastasis in a gain-of-function manner during perturbation screens. This might be resolved once highly efficient CRISPR activation tools or high-quality cDNA expression libraries compatible to high-throughput screening are readily available. Fourth, the clinical efficacy of SOD1-targeting strategies in mBC patients awaits future investigation.

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Methods

Cell lines

HEK293FT (#CRL-1573), MCF7 (#HTB-22), MDA-MB-231 (#HTB-26), T47D (#HTB-133), JC (#CRL-2116), and 4T1 (#CRL-2539) cell lines were obtained from the American Type Culture Collection (ATCC). MCF7, MDA-MB-231, and HEK293FT cells were maintained in DMEM medium (VivaCell BIOSCIENCES, #C3114-0500) while JC and 4T1 cells were cultured in RPMI-1640 medium (VivaCell BIOSCIENCES, #C3420-0500). All media were supplemented with 10% fetal bovine serum (FBS, ExCell, #FSP500) and 1% penicillin/streptomycin (VivaCell BIOSCIENCES, #C3420-0100).

Animals

Female BALB/c nude mice (BALB/cA-nu, Beijing HFK Bioscience, #13001A) and wild-type BALB/c mice (BALB/c, Beijing HFK Bioscience, #11002A) were purchased from Beijing HFK Bioscience Co., Ltd. FVB-MMTV-PyMT mice (Strain #T004993) were purchased from GemPharmatech Co., Ltd. (China). All mice were housed under specific pathogen-free (SPF) conditions in individually ventilated cages (IVCs) with a 12-hour light/dark cycle, an ambient temperature of 24–26 °C, and a relative humidity of 40%–60%. Following an acclimatization period of at least 5–7 days, the mice were utilized for experiments. Food and water were provided *ad libitum*.

Plasmid construction

The pLenti-EGFP-Luciferase-Blast plasmid was constructed by digesting the pLenti-Cas9-Blast vector (Addgene, #52962) with XbaI (Takara, #1634) and BamHI (Takara, #1605) restriction enzymes to substitute the Cas9-encoding sequence with the EGFP-Luciferase fusion gene. To construct pLenti-SaCas9-Blast vector, SaCas9 and gRNA scaffold were cloned from AAV-SaCas9 (Addgene, #61591) and inserted into pLentiCRISPRv2-Blast (Addgene, #83480) backbone to replace the original SpCas9 and its gRNA scaffold. The pHAGE-SOD1-Blast and pHAGE-FLAG-SMARCA1-Blast expression plasmids were constructed using the pHAGE-EF1 α -puro vector (Addgene, #118692) digested with AgeI (NEB, #R3552) and BamHI (Takara, #1605). The human *SOD1* coding sequence (NM_000454) and the FLAG-tagged human *SMARCA1* coding sequence (NM_003069) were amplified by PCR and subsequently ligated into the linearized vector using a T4 DNA ligase (Thermo Fisher Scientific, #EL0016). The lentiCRISPRv2-puro vector (Addgene, #52961) was employed to insert individual sgRNAs for SpCas9-mediated gene knockout. The sgRNA sequences are listed in Supplementary Table6.

Lentivirus production and infection

Lentiviruses were produced by co-transfecting HEK293FT cells with the lentivector plasmid and the packaging plasmids pCMVR8.74 (Addgene, #22036) and pMD2.G (Addgene, #12259) by the Neofect DNA transfection reagent (Neofect biotech., #TF20121201). The viral supernatant was harvested at 48 hours (h) and 72 h post-

transfection after centrifuging at $1580 \times g$ for 5 min and filtering through a $0.45 \mu\text{m}$ PVDF membrane. The clarified virus was aliquoted and stored at -80°C until use. Target cells for lentiviral infection were transduced by incubation with lentiviral supernatant in the presence of $6 \mu\text{g/mL}$ polybrene (Solarbio, #H8761). Transduced cells were usually subjected to antibiotic selection for 3-5 days to eliminate non-transduced cells. For blasticidin (Solarbio, #3513-03-9) selection, the following concentrations were used: MCF7 ($5 \mu\text{g/mL}$), MDA-MB-231 ($10 \mu\text{g/mL}$), T47D ($15 \mu\text{g/mL}$), 4T1 ($2 \mu\text{g/mL}$), and JC ($2 \mu\text{g/mL}$). For puromycin (Solarbio, #58-58-2) selection, the concentrations were: MDA-MB-231 ($2 \mu\text{g/mL}$), MCF7 ($2 \mu\text{g/mL}$), T47D ($3.5 \mu\text{g/mL}$), 4T1 ($3.5 \mu\text{g/mL}$), and JC ($5 \mu\text{g/mL}$).

Design and construction of BRCA CRISPR knockout library

A human breast cancer (BRCA) CRISPR knockout library (hBRCA-KO library) was designed to target 611 human genes that are potentially implicated in breast cancer metastasis. The gene was selected with the following criteria: (1) frequently mutated gene ($>1\%$) in BRCA cohorts according to COSMIC database; (2) highly mutated gene ($>5\%$) in BRCA mutation cohorts from individual literatures; and (3) significantly down-regulated gene in primary (versus normal, $\log_2$ fold-change < -2) or metastatic (versus primary, $\log_2$ fold-change < -4) tumors in BRCA cohorts according to The Cancer Genome Atlas (TCGA) database (data freeze, January 28, 2016). A total of 611 genes were selected and the targeting sgRNAs (3,645 sgRNAs) were chosen from the Human CRISPR Knockout Library H3 (Addgene #133914). The hBRCA-KO library also included 316 control sgRNAs, yielding a total of 3,961 sgRNAs (Supplementary Table1). A matched mouse CRISPR knockout library (mBRCA-KO library) was also constructed by converting the human genes to their murine homologs, resulting in 585 target genes. A total of 3531 targeting sgRNAs were designed along with 286 control sgRNAs to assemble the final mBRCA-KO library with 3,817 sgRNAs (Supplementary Table2). Libraries were synthesized as pooled oligonucleotides (Synbio Tech) and cloned into the Esp3I-digested lentiCRISPRv2-puro vector. The ligated products were transformed into Stable *E. coli* cells with over 100-fold library coverage, and plasmid libraries were prepared using Endo-Free Maxi-prep kits. For the focused small subLib-hMeta library that targets the top 20 ranked metastasis genes in MCF7 s.c. condition, 40 sgRNAs (two sgRNAs per gene) were retrieved from the hBRCA-KO library and individually cloned into the lentiCRISPRv2-puro vector. The resulting plasmids were pooled together at equimolar ratios as the final subLib-hMeta library.

Design and construction of PanCancer base editing library

A human PanCancer base editing sgRNA library (hPANCAN-BE library) was designed for base editors to install frequently mutated SNVs across several major cancer types: Breast Cancer (BRCA), Colorectal Cancer (CRC), Hepatocellular Carcinoma (HCC), Cholangiocarcinoma (CHC), Non-Small Cell Lung Cancer (NSCLC), and Small Cell Lung Cancer (SCLC). The target SNVs (A $\rightarrow$ G or T $\rightarrow$ C for ABE; C $\rightarrow$ T or G $\rightarrow$ A for CBE)

were selected from the COSMIC database with the following criteria: (1) mutational frequency $\geq 1\%$ in the corresponding patient cohort of ≥ 1000 individuals; or (2) recurrently occur in tumors with a minimum of two affected patients in smaller cohorts (<1000 individuals). A total of 552 SNVs and 1,701 SNVs were selected for ABE and CBE, respectively. The sgRNAs were designed in a saturated manner regardless of PAM restriction within the activity windows for most ABEs or CBEs. The final libraries also incorporated multiple control sgRNAs, including non-targeting controls, AAVS1-targeting guides, splicing-site controls, and stop-codon controls, yielding a final pool of 3,453 sgRNAs for ABE library and 9,223 sgRNAs for CBE library, respectively. The library synthesis, cloning (pLenti-CRISPRv2-Guide-Puro vector⁶⁴) and plasmid maxiprep followed similar procedures as above.

***In vivo* metastasis screens**

Human (MCF7, MDA-MB-231) and mouse (JC) breast cancer cell lines stably expressing an EGFP-luciferase fusion gene were lentivirally transduced with respective lentiviral libraries. For the knockout screens, cells were transduced with the hBRCA-KO library at MOI of 0.3, 3, and 6, or the mBRCA-KO library at MOI of 0.3 and 3. For the base editing screen, a two-step protocol was used. Cells were first transduced with lentiviruses expressing the base editor (pLenti-SpRY-ABE8e-Blast or pLenti-SpRY-evoA-BE4max-Blast) and selected with blasticidin to generate stable editor-expressing cell lines. These stable lines were then lentivirally transduced with the hPANCAN-BE sgRNA libraries at an MOI of 0.3. After two days post-transduction, all cell pools were selected with puromycin for 3 days, followed by a two-day recovery period in standard culture medium. Then, these edited cells were ready for inoculation into the mice. Meanwhile, a fraction of Day 0 sample as the screening start point were harvested. For the subcutaneous tumor model, 5×10^6 cells per tumor were resuspended in 100 μL of PBS containing 30% Matrigel (Corning, #356231) and bilaterally injected into the flanks of 6-week-old female BALB/cA-nu mice. For the orthotopic model, 5×10^6 cells in 100 μL of PBS/Matrigel were bilaterally injected into the inguinal mammary fat pads of immunocompetent BALB/c mice. For the experimental metastasis model, 3×10^6 cells in 100 μL of PBS were administered intravenously via tail vein injection into BALB/cA-nu mice (for human cell lines) or BALB/c mice (for murine cell lines). Tumor growth in subcutaneous and orthotopic models was monitored by regular caliper measurements. Metastatic burden was assessed by *in vivo* bioluminescence (luciferase) imaging, *ex vivo* fluorescence (EGFP) imaging (for the whole lung tissues), histological analysis by H&E staining, or visually morphological inspection. For bioluminescence imaging, mice received an intraperitoneal injection of D-luciferin (Yeason, #40901ES10, 150 mg/kg) and were imaged using the MS FXPRO *in vivo* imaging system (Carestream Health). Image acquisition and analysis were performed using the Bruker molecular imaging software. For EGFP imaging, freshly isolated lungs were rinsed in PBS and immediately imaged using the MS FXPRO imaging system (Carestream Molecular Imaging, USA).

Fluorescence images were captured and quantified using the Bruker molecular imaging software. In parallel with the *in vivo* metastasis modeling, another batch of cells from the same parental Day 0 sample fractions underwent *in vitro* cell fitness screens in Petri dishes under normal cell culture media. At the experimental endpoint (6 weeks post-inoculation), primary tumors, metastatic lesions, *in vitro* cultured cells and parental (Day 0) cells were collected for genomic DNA extraction and subsequent sgRNA sequencing.

To extract genomic DNA, tissues were mechanically dissociated on ice to obtain single-cell suspensions. After centrifugation, cell pellets containing $\sim 5 \times 10^6$ cells were lysed in 1.5 mL tubes with 500 μ L of lysis buffer (300 mM NaCl, 0.2% SDS, 2 mM EDTA, 10 mM Tris-HCl, pH 8.0) supplemented with 5 μ L RNase A (10 mg/mL), followed by incubation at 65°C for 1 h with agitation. Proteinase K (5 μ L, 10 mg/mL) was then added for an overnight incubation at 55°C. Lysates were extracted with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1), and genomic DNA was precipitated from the aqueous phase with isopropanol. The DNA pellet was washed twice with 75% ethanol, air-dried, and resuspended in nuclease-free water for quantification. To construct sgRNA sequencing libraries, a two-step PCR amplification was employed as described previously⁴⁸. The final libraries were gel-purified and proceed to high-throughput sequencing on an Illumina platform with PE150 mode.

CRISPR screens with druggable gene library

Two metastasis^{high} MCF7 cell models (*AF*^{DKO} and *PT*^{DKO}) alongside a parental control (sgAAVS1) cell line were lentivirally transduced with the pooled druggable gene CRISPR knockout library⁴⁸ at a low MOI (< 0.3). As the CRISPR libraries were constructed under lentiCRISPRv2-puro vector with SpCas9-sgRNA cassette, the *AF*^{DKO} and *PT*^{DKO} cells models used here were generated with lentivirally delivered and genomically integrated SaCas9-sgRNA cassette (different sgRNA scaffold sequence from SpCas9 system) to avoid the sgRNA sequencing contamination from *AF*- or *PT*-targeted sgRNAs. After two days post-infection, puromycin selection was performed for 3 days followed by a two-day recovery in normal media. After collecting the Day 0 sample, the rest of cells with enough coverage were continually cultured for cell fitness selection in normal media for 30 days before harvesting. Two independent replicate screens were performed. Genomic DNA was extracted to construct the sgRNA sequencing libraries as described above. The sgRNA abundance was determined by high-throughput sequencing on an Illumina platform with PE150 mode.

CRISPR screen data analysis

Sequencing data from all CRISPR screens were processed using the MAGeCK pipeline (v0.5.9.4). Raw FASTQ files were aligned to the corresponding sgRNA library references and read counts for each sgRNA were generated using the mageck count

function with default parameters, forming the count matrices for all subsequent analyses. Analytic details for each type of screens are described below.

In vivo CRISPR knockout screen

For metastatic tumors, the sgRNA distribution is highly distorted due to strong positive selection. A sgRNA was considered a qualified candidate in each metastatic sample if it ranked among the top 20 most enriched sgRNAs and its read count constituted >1% of the total reads in that sample. On top of that, a gene was then designated as a functional metastasis hit if it met the following criteria: (i) the same qualified sgRNA was present in ≥ 2 metastatic tumors, (ii) the gene was picked out (via any qualified sgRNA) in ≥ 2 metastatic tumors, or (iii) the gene was supported by ≥ 2 distinct qualified sgRNAs. For primary tumors and *in vitro* cultured parental cells, gene essentiality was quantitatively assessed using the β -score derived from the MAGeCK Maximum Likelihood Estimation (MLE) model. A higher positive score indicates stronger positive selection and a lower negative score means stronger negative selection.

In vivo base editing screen

For metastatic tumors, the base editing screen samples also underwent strong positive selection. A sgRNA was classified as a qualified candidate in a metastasis sample if it ranked among the top 30 enriched sgRNAs and its read count represented >1% of the total reads. A functional metastasis SNV was subsequently defined if it satisfied the following criteria: (a) the same qualified sgRNA was identified in at least two independent metastatic samples, or (b) at least two different qualified sgRNAs targeting the identical SNV were recovered in the screens.

In vitro druggable gene CRISPR screen

The druggable gene CRISPR screen data were analyzed using the MAGeCK Robust Rank Aggregation (RRA) algorithm. A $\log_{10}$ transformed RRA scores (tRRA) obtained using the ReadRRA function were assigned to each gene to inform the phenotypic effect during the screens. Similar to β -score, a higher positive tRRA score indicating stronger positive selection and a lower negative tRRA score meaning stronger negative selection. To identify the special vulnerabilities in metastasis^{high} cells, we particularly focused on those negative selection hits. Special genetic vulnerabilities were defined when the genes exhibited significantly stronger negative selection in metastasis^{high} cells compared to control cells. The tRRA score cutoff was as follows: $\text{score}^{\text{metastasis_high}} < -1$ & $\text{score}^{\text{control}} < 0.5$ & $(\text{score}^{\text{metastasis_high}} - \text{score}^{\text{control}}) < -1$.

RNA-seq analysis

For each RNA-seq sample, total RNA was extracted from at least 1×10^7 cells using TRIzol reagent. Sequencing libraries were prepared by Novogene Co., Ltd. and sequenced on an Illumina platform with PE150 mode. Three biological replicates were

performed for each experimental condition. Raw sequencing reads were processed using Trimmomatic (v0.39), and then aligned to the human reference genome (GRCh38.p13) using HISAT2 (v2.2.1) with the default parameters. Transcript abundance was quantified and assembled using StringTie (v2.1.4). A count matrix for genes across all samples was generated using the prepDE.py script. Differential expression analysis was performed with DESeq2 (v1.30.1) in R, and genes with base mean > 10, an adjusted *p*-value < 0.05 and |log2 fold-change| > 1 were considered as differentially expressed gene hits.

ATAC-seq assay

ATAC-seq libraries were prepared from 3×10^4 cells following the Omni-ATAC protocol⁷⁹. Cells were lysed in 50 μ L of ice-cold lysis buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.1% IGEPAL CA-630, 0.1% Tween-20, and 0.01% digitonin) for 3 min. After washing with 1 mL of wash buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM MgCl₂, and 0.1% Tween-20), the nuclei pellet was resuspended in 50 μ L of transposition mix from the TruePrep DNA Library Prep Kit V2 (Vazyme, #TD501) and incubated at 37°C with 1000 rpm shaking for 30 min. The DNA in the reaction was purified using VAHTS DNA Clean Beads (Vazyme, #N411), and then amplified by PCR using primers from the TruePrep Index Kit V2 (Vazyme, #TD202). Amplified libraries underwent double-sided size selection with VAHTS DNA Clean Beads to enrich fragments between 200-700 bp. Library concentration was measured using Qubit™, and sequencing was performed on an Illumina platform with PE150 mode.

CUT&Tag assay

CUT&Tag assays were performed using the Hyperactive Universal CUT&Tag Assay Kit for Illumina Pro (Vazyme, #TD904) in conjunction with the Hyperactive TF Enhancer Module (Vazyme, #TD904-C2) according to the manufacturer's instructions. In brief, approximately 2×10^5 cells per sample were harvested and subjected to TF Enhancer treatment to stabilize protein-DNA interactions. Cells were bound to Concanavalin A-coated magnetic beads, permeabilized, and incubated with the primary antibody against SMAD2/3 (Cell Signaling Technology, #8685) overnight at 4°C. After washing, samples were sequentially incubated with a species-matched secondary antibody and the Protein A/G-Tn5 Transposase (pA/G-Tnp Pro). Tagmentation was carried out at 37°C for 1 h to fragment the DNA. The reaction was terminated, and the DNA was purified using DNA Extract Beads Pro. Final libraries were amplified by PCR, purified with VAHTS DNA Clean Beads (Vazyme, #N411), and quantified by Qubit™. Libraries were sequenced on an Illumina platform with PE150 mode.

Epigenomic analysis

Adapter sequences and low-quality bases of the ATAC-seq and CUT&Tag sequencing reads were trimmed using Trim Galore (v0.6.10) with a quality threshold of 20 for quality control. The cleaned reads were aligned to the human reference genome

GRCh38 using Bowtie2 (v2.4.5). PCR duplicates were removed using sambamba markdup (v0.8.0). For ATAC-seq, mitochondrial reads were excluded, and only properly paired, uniquely mapped reads with a mapping quality (MAPQ) ≥ 30 were retained. Peak calling for ATAC-seq data was performed using MACS2 (v2.2.7.1) with the parameters --nomodel --shift -100 --extsize 200. For CUT&Tag data, peaks were called against matched IgG control samples using MACS2 with a q-value threshold of 0.05. Blacklisted regions from the ENCODE project were removed from all peak sets. Normalized bigWig files for visualization were generated with deepTools (v3.5.1) bamCompare (for CUT&Tag) and bamCoverage (for ATAC-seq). A consensus peak set was created for each condition by merging replicate peaks using the DiffBind (v3.18.0) in R. Read counts for these consensus regions were quantified directly within DiffBind using the summarizeOverlaps method via the dba.count function. Differential peak analysis was then conducted using the DESeq2 algorithm integrated within DiffBind, and regions with an adjusted p -value < 0.05 and $|\log_2 \text{fold-change}| > 1.5$ were deemed significant. Motif enrichment analysis on differential peaks was performed using HOMER (v4.11) findMotifsGenome.pl. Transcription factor-target gene linkages (TFLink) were derived by annotating peaks to genes with ChIPseeker R package followed by transcription factor enrichment analysis using DAVID (v2024q1). Profile plots and heatmaps were generated with deepTools, computeMatrix and plotHeatmap.

Functional enrichment analysis

Functional enrichment analyses were performed on specified gene lists. Gene Ontology (GO) enrichment analysis was conducted using DAVID. Gene Set Enrichment Analysis (GSEA) was specifically applied to the transcriptomic data using the clusterProfiler R package (v4.0). For GSEA, a pre-ranked gene list was generated based on the $\log_2$ fold-change from the differential gene expression analysis. Enrichment was tested against the MSigDB Hallmark gene sets, with a significance cutoff of adjusted p -value < 0.05 (Benjamini-Hochberg method).

Public database interrogation

Mutational profiles of genes of interest were queried from the COSMIC Cancer Gene Census (<https://cancer.sanger.ac.uk/cosmic/login>). Gene expression data across normal and tumor tissues were obtained from the GTEx portal (<https://www.gtexportal.org>) and the TCGA program (<https://portal.gdc.cancer.gov/>). Analysis of significantly mutated genes in TCGA cohorts was performed using the Broad Institute GDAC Firehose pipeline (stddata__2016_01_28 run).

Survival Analysis

Clinical and molecular data from TCGA (BRCA) and the METABRIC (<https://ega-archive.org/studies>, accession number: EGAS00000000083, EGAS00001001753) consortia were utilized. For gene expression-based survival analysis, patients were stratified into "High" and "Low" groups based on the median expression level of a

predefined gene list. For mutation-based survival analysis, patients were dichotomized into "Mutated" versus "Non-mutated" groups based on the alteration status of specific genes or SNVs. Overall survival was the primary endpoint. Kaplan-Meier survival curves were generated, and differences between groups were assessed using the log-rank test.

Cell migration and invasion assays

Cell migration and invasion abilities were assessed using the wound healing and transwell assays. For the wound healing or scratch assay, cells were seeded in 6-well plates and cultured to around 50% confluence. A uniform wound was created using a sterile 200 μ L pipette tip. After washing to remove detached cells, fresh medium containing serum was added. Wound closure was monitored at 0, 24 and 48 h, and the migration rate was quantified using Fiji software (NIH). For the transwell migration and invasion assays, 24-well transwell chambers (8- μ m pore size) were used. For invasion assays, chambers were pre-coated with a thin layer of Matrigel (Corning, #356234) and allowed to solidify at 37°C for 4 h prior to cell seeding. MCF7 and T47D cells (1×10^5) and MDA-MB-231 cells (5×10^4) were seeded in serum-free medium in the upper chambers, with medium containing 20% FBS as a chemoattractant in the lower chambers. MCF7 cells were starved for 12 h prior to the assay. After incubation for 24 h (MCF7, T47D) or 12 h (MDA-MB-231), non-migrated/invaded cells were removed, and cells on the lower membrane were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and quantified. MCF7 and T47D cells were counted across the entire membrane, while MDA-MB-231 cells were quantified from five randomly selected fields per chamber.

Cell viability assay

Cell viability was assessed by direct cell counting using a hemocytometer. For growth curve analysis, cells were plated in culture dishes and harvested at 3-day intervals over a 9-day period. At each time point, cells were trypsinized and counted, with total cell numbers calculated by applying appropriate dilution factors from routine passaging to track population expansion. For drug treatment experiments, cell viability was measured using the Cell Counting Kit-8 (CCK-8) (Beyotime, #C0037). Cells were seeded in 96-well plates at a density of $4\sim5 \times 10^3$ cells per well with 5-8 technical replicates per drug concentration. After treatment for specified durations, 10 μ L of CCK-8 solution was added to each well and incubated at 37°C for 4 h. Absorbance was measured at 450 nm using a microplate reader.

***In vivo* metastasis assays**

Female BALB/cA-nu mice (6-8 weeks old) were used. For the subcutaneous inoculation model, 5×10^6 cells in 100 μ L PBS containing 30% Matrigel were injected into the flanks of mice. For the experimental metastasis model, 3×10^6 cells in 100 μ L PBS were injected intravenously via the tail vein. Metastatic progression was

monitored over 3-6 weeks. For longitudinal monitoring of metastasis, mice were anesthetized and imaged using the Quantum GX2 microCT imaging system at specified time points. At the experimental endpoint, lungs were collected for *ex vivo* analysis. To detect EGFP-expressing metastatic lesions, freshly isolated lungs were rinsed in PBS and immediately imaged using the MS FXPRO imaging system (Carestream Molecular Imaging, USA). Fluorescence images were captured and quantified using the Bruker molecular imaging software.

***In vivo* therapeutic assays against mBC**

Experimental metastasis model

To evaluate the therapeutic efficacy of SOD1 inhibitor LCS-1 (MCE, #HY-115445), EGFP-expressing human MDA-MB-231 and murine 4T1 breast cancer cells were used. Female BALB/cA-nu mice (6–8 weeks old) were injected intravenously via the tail vein with 3×10^6 MDA-MB-231 cells, while immunocompetent BALB/c mice (6–8 weeks old) were injected with 5×10^5 4T1 cells, all suspended in PBS. One week after inoculation, mice were randomly assigned to control or treatment groups. The treatment group received intraperitoneal (i.p.) administration of LCS-1 at 50 mg/kg every two days for two weeks, whereas the control group received an equal volume of vehicle on the same schedule. At the experimental endpoint, mice were euthanized, and lungs were harvested. *Ex vivo* fluorescence imaging of lung tissues was performed using an MS FXPRO *in vivo* imaging system to quantify EGFP-positive metastatic burden.

Spontaneous metastasis model in MMTV-PyMT mice

Primary tumor growth inhibition: Female MMTV-PyMT transgenic mice, which develop spontaneous mammary tumors, were enrolled in the study at 10 weeks of age when primary tumors had reached a measurable volume. Mice were randomized into vehicle and LCS-1 treatment groups. The treatment group received LCS-1 at 50 mg/kg via i.p. injection every two days for two weeks. Primary tumor dimensions were measured every two to three days using vernier calipers, and tumor volume was calculated using the formula: Volume = (Length $\times$ Width²) / 2.

Post-surgical metastasis inhibition: To assess the efficacy of LCS-1 in reducing the metastatic burden, a separate cohort of MMTV-PyMT mice underwent surgical resection of the primary mammary tumors by removing the entire mammary fat pad and adjacent lymph nodes at 10 weeks of age. After a one-week postoperative recovery period, mice were randomized and treated as described above (LCS-1, 50 mg/kg, i.p., every two days for two weeks). At the experimental endpoint, mice were anesthetized and subjected to final microCT imaging using a Quantum GX2 system to assess metastatic burden, followed by lung collection for macroscopic nodule counting.

Determination of base editing efficiency

To determine the editing efficiency of base editors, targeted deep sequencing was performed on genomic DNA from both cultured cells and metastatic tissues. Genomic DNA from cellular and tissue samples was subjected to PCR amplification of target regions. The sequencing library was prepared as described above. Next-generation sequencing (Illumina PE150) was performed to quantify the base conversion rates according to allele frequencies. The PCR primers for target amplification are listed in Supplementary Table6.

Immunoblot and immunoprecipitation

Cells were washed twice with ice-cold PBS and lysed in RIPA buffer (Beyotime, #P0013C) containing protease and phosphatase inhibitor cocktails (Meilunbio, #MB26780, #MB12707) for 15 min at 4°C. Lysates were clarified by centrifugation at 14000 rpm for 15 min at 4°C, and supernatants were collected as whole-cell extracts. For co-immunoprecipitation, clarified lysates were incubated with anti-FLAG (Beyotime, #P2115) or anti-MYC magnetic beads (Beyotime, #P2118) overnight at 4°C with gentle agitation. Beads were washed three times with 1× TBS, and bound proteins were eluted in 5× SDS loading buffer by heating at 100°C for 5 min. Protein samples were resolved by 8-15% bis-tris polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Pall Corporation, #27574625). Membranes were blocked with 5% non-fat milk in TBST and probed with specific primary antibodies, followed by incubation with HRP-conjugated secondary antibodies. Signals were visualized using an enhanced chemiluminescence detection system (Tanon 5200). The following antibodies were used: ADAMTS7 (Bioss, #bs-0733R), FRMD1 (Immunoway, #YN3638), PTEN (Immunoway, #YT5752), TENM3 (Novus, #NBP2-13687), USP44 (Immunoway, #YT4841), TSC2 (Proteintech, #68380-1-Ig), I17b (Cohension, #CQA6921), SMARCA1 (Proteintech, #29461-1-AP), SMARCA1 (ABclonal, #A10248), SMAD2/3 (CST, #8685), Phospho-SMAD2 (Ser465/467) (CST, #9520), Phospho-SMAD3 (Ser423/425) (CST, #3108), TGFBR1 (Proteintech, #30117-1-AP), TGFBR2 (Proteintech, #66636-1-Ig), GAPDH (Santa, #sc-32233), α -Tubulin (Proteintech, #66031-1-Ig), SOD1 (Huana, #ET1702-36), BRG1 (Huaan, #ET1611-85), MVK (Huaan, #A9G12), GPX4 (Proteintech, #67763-1-Ig), ACSL4 (Proteintech, #22401-1-AP), Flag (Invitrogen, #MA1-91878), α -Tubulin (Proteintech, #66031-1-Ig), Goat anti-Rabbit IgG (Thermo Fisher Scientific, #31460), and Goat anti-Mouse IgG (Thermo Fisher Scientific, #A-11001).

Reactive oxygen species (ROS) detection

Total intracellular ROS detection

Total intracellular ROS levels were measured using the cell-permeant fluorescent probe H2DCFDA (MedChemExpress, #HY-D0940). Cells were incubated with 5 μ M H2DCFDA in PBS at 37°C for 30 min in the dark. After incubation, cells were washed twice with PBS. Fluorescence intensity of the oxidized product (DCF) was quantified using a Synergy HTX multi-mode microplate reader (BioTek) at excitation/emission of

488/525 nm. For confocal imaging, cells grown on glass-bottom dishes were processed identically and imaged immediately after washing using a STELLARIS 5 confocal microscope (Leica), with excitation at 488 nm and emission collection between 500-550 nm.

Superoxide anion ($O_2^{\cdot-}$) detection

Cellular superoxide anion ($O_2^{\cdot-}$) levels were quantified using the Superoxide Assay Kit (Beyotime, #S0060). This assay utilizes WST-1, which is reduced by superoxide to a water-soluble formazan product. Cells were incubated with the superoxide detection working buffer (containing WST-1 and catalase) at 37°C for 3 min, and absorbance was immediately measured at 450 nm using the Synergy HTX multi-mode microplate reader.

Mitochondrial superoxide detection

Mitochondrial superoxide was detected using the Mitochondrial Superoxide Assay Kit (MitoSOX Red) (Beyotime, #S0061). Cells were co-stained with 5 μ M MitoSOX Red and 100 nM MitoTracker Green (Beyotime, #C1048) in PBS at 37°C for 30 min. Following two washes with warm PBS, live cells were immediately imaged using a STELLARIS 5 confocal microscope (Leica). MitoSOX Red fluorescence (indicative of mitochondrial superoxide) was captured with excitation at 510 nm and emission at 580 nm. MitoTracker Green fluorescence (indicative of mitochondrial mass) was captured with excitation at 490 nm and emission at 516 nm.

Measurement of lipid peroxidation

Cellular lipid peroxidation was assessed using the fluorescent probe BODIPY 581/591 C11 (Thermo Fisher Scientific, #D-3861). Live cells grown on glass-bottom dishes were incubated with 2 μ M BODIPY 581/591 C11 in complete culture medium at 37°C for 30 min. After washing, cells were immediately imaged under live-cell conditions using a STELLARIS 5 confocal microscope (Leica). The fluorescence of the reduced form (ex/em: 581/591 nm) and oxidized form (ex/em: 488/510 nm) were captured to calculate the green-to-red fluorescence ratio using Fiji software, where a higher ratio indicates increased lipid peroxidation.

Measurement of MDA content

Malondialdehyde (MDA) content, a terminal product of lipid peroxidation, was quantified using the Lipid Peroxidation MDA Assay Kit (Beyotime, #S0131). Cells were lysed, and protein concentration was determined using a BCA assay for normalization. Lysates were reacted with thiobarbituric acid (TBA) at 100°C for 15 min to form the MDA-TBA adduct. After centrifugation, supernatant absorbance was measured at 532 nm using a Synergy HTX microplate reader (BioTek). MDA concentrations were calculated from a standard curve and normalized to total protein quantity.

Detection of labile iron pool (Fe²⁺)

The labile iron pool (Fe²⁺) was detected using FerroOrange (MedChemExpress, #HY-D1913). Cells were incubated with 1 µM FerroOrange in HBSS at 37°C for 30 min, washed, and imaged using a STELLARIS 5 confocal microscope (Leica) with excitation at 543 nm and emission at 570–620 nm.

Assessment of mitochondrial membrane potential (ΔΨ_m)

Mitochondrial membrane potential was measured using JC-1 fluorescent probe (MedChemExpress, #HY-15534). Cells were stained with 5 µM JC-1 in serum-free medium at 37°C for 30 min. After washing, fluorescence was captured using a STELLARIS 5 confocal microscope (Leica). J-aggregates (intact ΔΨ_m) and monomers (depolarized) were detected at ex/em 585/590 nm (red) and 514/529 nm (green), respectively. The green-to-red fluorescence ratio was calculated, with a higher ratio indicating a decreased membrane potential or mitochondrial depolarization.

Seahorse mitochondrial stress assay

Mitochondrial respiratory function was assessed using the Seahorse XF Cell Mito Stress Test Kit (Agilent, #103015-100). Cells were seeded in XF96-well cell culture microplates (Agilent, #103793-100) at a density of 4,000 cells/well in complete growth medium and incubated overnight for adherence. One hour prior to the assay, the medium was replaced with pre-warmed XF assay medium (Agilent, #103575-100) supplemented with 2 mM glutamine, 1 mM pyruvate, and 10 mM glucose (pH 7.4), and cells were incubated in a non-CO₂ incubator at 37°C. The sensor cartridge was hydrated in XF calibrant solution at 37°C in a non-CO₂ incubator overnight. Sequential injections of mitochondrial inhibitors were loaded into designated ports: port A, oligomycin (1.5 µM); port B, FCCP (1.0 µM); and port C, rotenone/antimycin A (0.5 µM). Oxygen consumption rate was measured using an XFe96 analyzer (Agilent).

Hematoxylin and Eosin (H&E) staining and immunohistochemistry (IHC)

The collected tissues were fixed in 4% paraformaldehyde, processed, and embedded in paraffin. Sections of 4-5 µm thickness were cut and mounted on slides. For histological analysis, the sections were deparaffinized in xylene, rehydrated through a graded ethanol series, and stained with Hematoxylin and Eosin (H&E) following standard procedures. The stained sections were imaged under a light microscope to visualize and quantify the metastatic foci.

Immunohistochemical staining was performed on human breast cancer tissue microarrays by a commercial service provider (Pantomics, #BRM962). In brief, following deparaffinization and antigen retrieval, tissue sections were incubated with a primary antibody against SMARCA1 (ABclonal, #A10248). Detection was carried out using the Pantomics Mini IHC Kit with a horseradish peroxidase (HRP)-conjugated secondary antibody and DAB chromogen, followed by hematoxylin counterstaining.

Quantification and statistical analysis

No statistical methods were used to predetermine the sample size, and no data were excluded from the analyses. Data are presented as mean $\pm$ SEM, as specified in the Fig. legends. Statistical significance between two groups was determined using an unpaired two-tailed Student's t-test. Associations between categorical variables were assessed using Fisher's exact test or Hypergeometric test. Correlation analysis was performed using Pearson's method, with the correlation coefficient denoted as "r". A p -value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 10 software. Significance levels are denoted as follows: ** $p < 0.01$; and *** $p < 0.001$.

Data availability

All data are available in the main text or the supplementary materials. The raw sequencing and proteomics data have been deposited and are publicly available in the Genome Sequence Archive and the OMIX in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences, under accession code (GSA-Human: HRA015015; GSA: CRA034450; OMIX: OMIX013421) at <https://ngdc.cncb.ac.cn/>.

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Author contributions

T.F. conceived the study and designed the research. Y.L. performed most of the experiments and bioinformatics analysis. T.F. and Y.L. wrote the manuscript with the input from all the other authors. All authors discussed the data and approved the manuscript. T.F. supervised the study.

Competing interests

Authors declare that they have no competing interests.

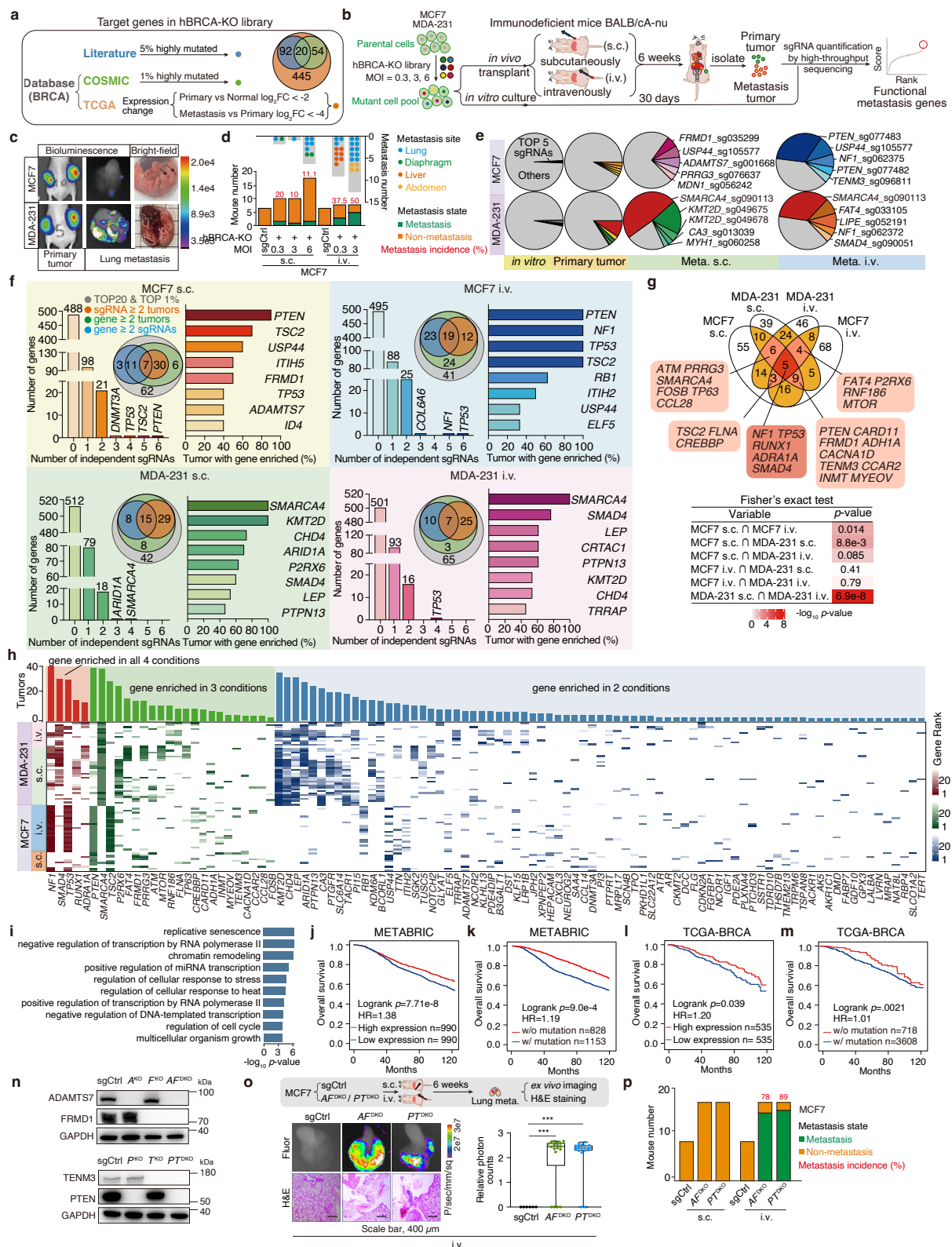


Fig. 1 | Systematic *in vivo* CRISPR screens in immunodeficient mice identify functional metastasis genes in mBC.

a, Design and composition of the human BRCA CRISPR knockout (hBRCA-KO) library for functional metastasis gene screening.

b, Workflow of the *in vivo* CRISPR knockout screens for functional metastasis genes in immunodeficient mice models.

c, Representative bioluminescence imaging (BLI) and bright-field images of primary tumors or metastatic foci at the 6-week screening endpoint. The metastases are indicated by black arrows or dashed circles.

d, Bar graphs showing the metastasis statistics for the MCF7 model according to indicated classifications. Each dot represents one metastasis event.

e, Pie charts depicting the count distribution of sgRNAs in a representative sample of each group. The top 5 most enriched sgRNAs are labeled.

f, Top functional metastasis genes identified in each group from these screens according to indicated cutoffs.

g, Venn diagram showing the overlap of functional metastasis gene hits across the four experimental conditions.

h, Heatmap showing the summary of 104 high-confidence functional metastasis genes identified from these screens. Genes in each metastatic tumor are color-coded according to the ranking of top 20 enriched sgRNAs and screening conditions.

i, Gene Ontology (GO) enrichment for the 104 high-confidence metastasis genes.

j-m, Kaplan-Meier curves for overall survival in the METABRIC (**j**, **k**) and TCGA-BRCA (**l**, **m**) cohorts. Breast cancer patients are stratified by the expression levels (**j**, **l**) or mutation status (**k**, **m**) of the 104 high-confidence metastasis genes.

n, Immunoblot analysis confirming the establishment of single clonal MCF7 cells with single knockout (KO) or double knockout (DKO) of indicated genes. *ADAMTS7* + *FRMD1* (*AF*), *PTEN* + *TENM3* (*PT*).

o,p, DKO of indicated metastasis gene pairs in MCF7 tumors causes metastatic dissemination *in vivo*. Representative *ex vivo* fluorescence imaging, corresponding H&E staining and quantification of metastatic nodules according to EGFP fluorescence of lung sections are shown in (**o**). Mean $\pm$ SEM (n=16 mice per group), *** $p < 0.001$ (unpaired t-test). Statistics of metastasis incidence is shown in (**p**).

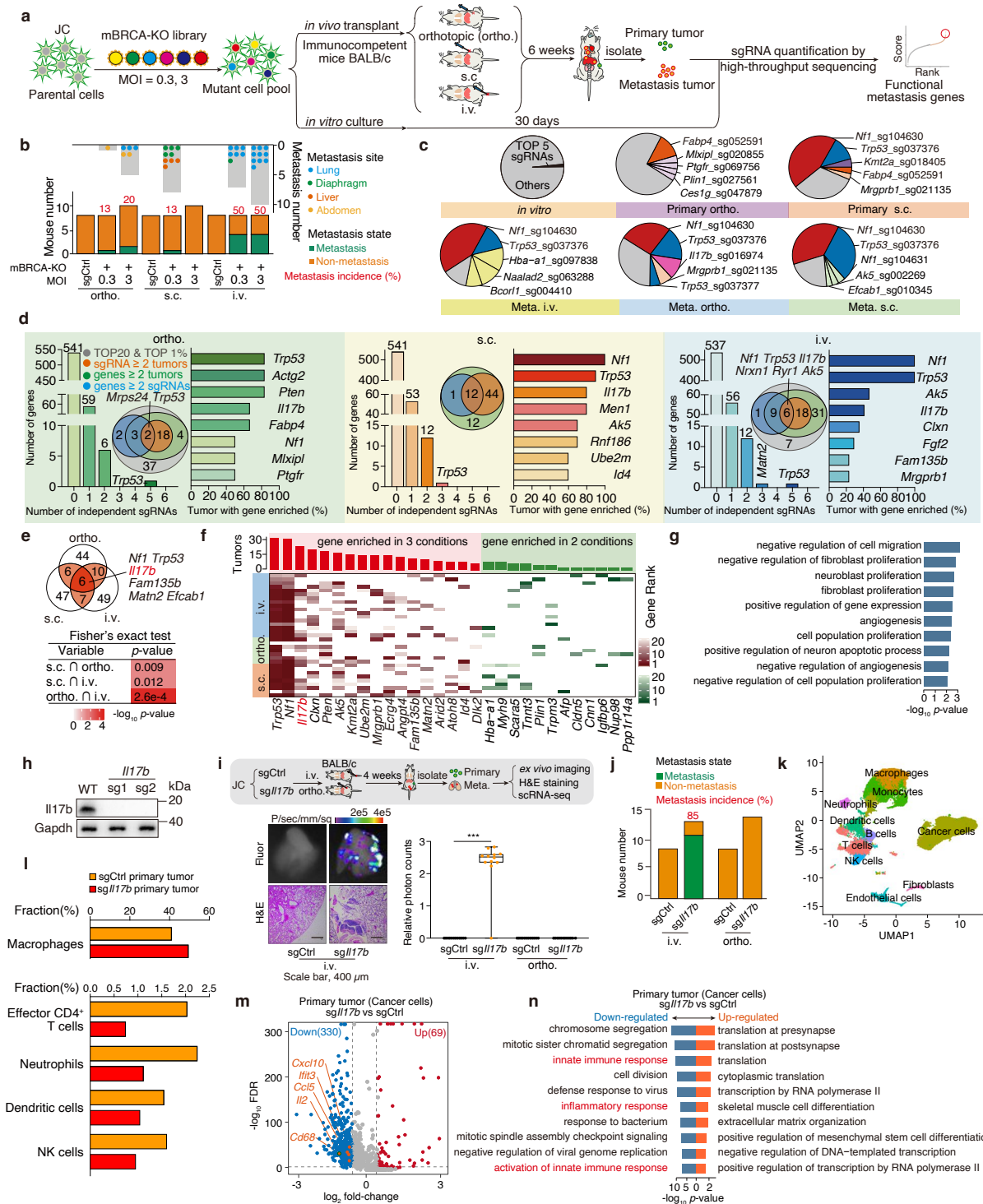


Fig. 2 | Systematic *in vivo* CRISPR screens in immunocompetent mice for functional metastasis genes.

a, Workflow of the *in vivo* CRISPR knockout screens for functional metastasis genes in immunocompetent mice models.

b, Bar graphs showing the metastasis statistics for the JC model according to indicated classifications. Each dot represents one metastasis event.

c, Pie charts depicting the sgRNA count distribution in a representative sample of each group. The top 5 most enriched sgRNAs are labeled.

1336 **d**, Top functional metastasis genes identified in each group from these screens
 1337 according to indicated cutoffs.

1338 **e**, Venn diagram showing the overlap of functional metastasis gene hits across the
 1339 three experimental conditions.

1340 **f**, Heatmap showing the summary of 29 high-confidence functional metastasis genes
 1341 identified from the current screens. Genes in each metastatic tumor are color-coded
 1342 according to the ranking of top 20 enriched sgRNAs and screening conditions.

1343 **g**, GO enrichment for the 29 high-confidence metastasis genes.

1344 **h**, Immunoblot analysis confirming successful knockout of *Il17b* with two independent
 1345 sgRNAs in JC cells.

1346 **i,j**, *Il17b* knockout promotes metastatic dissemination via i.v. inoculation in
 1347 immunocompetent mice. Representative *ex vivo* fluorescence imaging, H&E staining
 1348 and metastasis quantification of lung sections from mice are shown in (i). Mean $\pm$ SEM
 1349 ($n = 8-12$ mice per group), *** $p < 0.001$ (unpaired t-test). Statistics of metastasis
 1350 incidence is shown in (j).

1351 **k**, UMAP of single cell classification from scRNA-seq data of JC-derived *Il17b* KO
 1352 primary and lung metastasis tumors.

1353 **l**, Bar graph showing the proportional abundance of major immune cell types in
 1354 indicated groups according to scRNA-seq data.

1355 **m**, Volcano plot displaying differentially expressed genes in ortho. primary tumors
 1356 between sg*Il17b* and sgCtrl groups based on scRNA-seq analysis.

1357 **n**, GO enrichment terms from differentially expressed genes in (m).
 1358

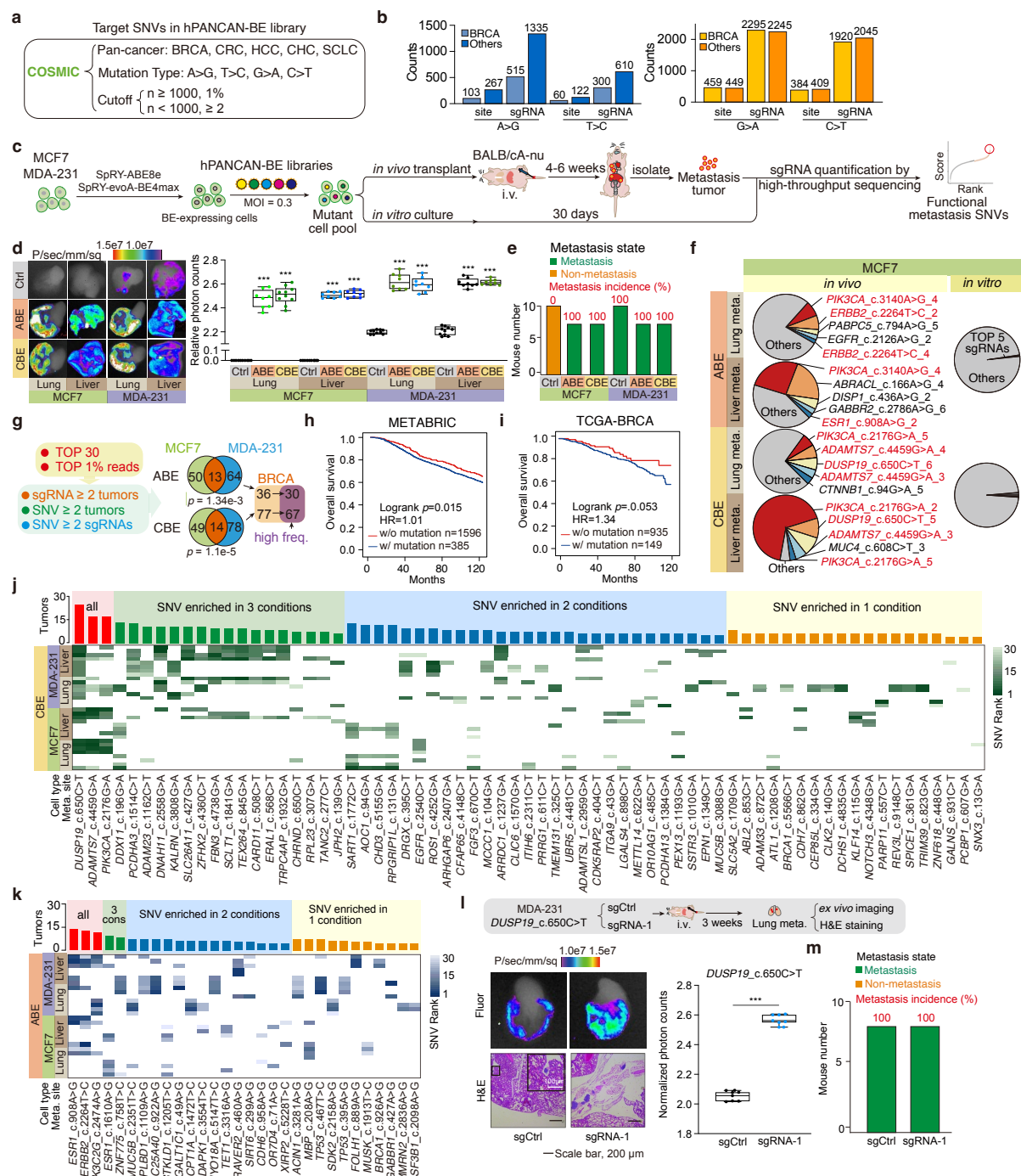


Fig. 3 | In vivo base editing screens identify driver SNVs for breast cancer metastasis.

a, Design and composition of the human Pan-cancer base editing (hPANCAN-BE) sgRNA libraries for ABE/CBE to install cancer-associated SNVs.

b, The statistics of SNVs (sites) or their targeting sgRNAs in hPANCAN-BE libraries. SNV or mutations are further categorized by their prevalence in BRCA versus other cancer types.

c, Workflow of the *in vivo* base editing screens for functional metastasis SNVs in breast cancer.

d, Representative *ex vivo* fluorescence imaging and metastasis quantification of lung and liver sections from mice inoculated with MCF7 or MDA-MB-231 derived tumors transduced with the hPANCAN-BE sgRNA libraries (ABE, CBE) or control (Ctrl, empty vector of ABE and CBE). Mean $\pm$ SEM (n=5-8 mice per group), *** $p < 0.001$ (unpaired t-test, vs Ctrl).

e, Bar graph showing the metastasis incidence for indicated groups.

f, Pie charts depicting the count distribution of sgRNAs for installing indicated SNVs in a representative sample of each group during the MCF7 screens. The top 5 most enriched sgRNAs are labeled.

g, Cross-condition analysis identifies high-confidence metastatic driver SNVs. Venn diagrams showing the overlap of functional metastasis SNVs across different groups. The union of ABE and CBE screens is in pink, with driver SNVs frequently mutated in BRCA cohorts are in dark pink (n = 97). *P*-value is calculated by Fisher's exact test.

h,i, Kaplan-Meier curves for overall survival in the METABRIC (**h**) or TCGA-BRCA (**i**) cohorts. Breast cancer patients are stratified by the mutation status of the 97 functional metastasis SNVs.

j,k, The landscape of functional metastasis SNVs identified from base editing screens via ABE (**j**) or CBE (**k**), respectively. BRCA SNVs in each metastatic tumor are color-coded according to the ranking of top 30 enriched sgRNAs in the screens.

l, The *DUSP19_c.650C>T* variant promotes metastatic dissemination *in vivo*. Representative *ex vivo* fluorescence imaging, H&E staining (bottom) and metastasis quantification of lung sections from mice of indicated groups. Mean $\pm$ SEM (n = 8-12 mice per group), *** $p < 0.001$ (unpaired t-test).

m, Bar graph showing the metastasis incidence for indicated groups.

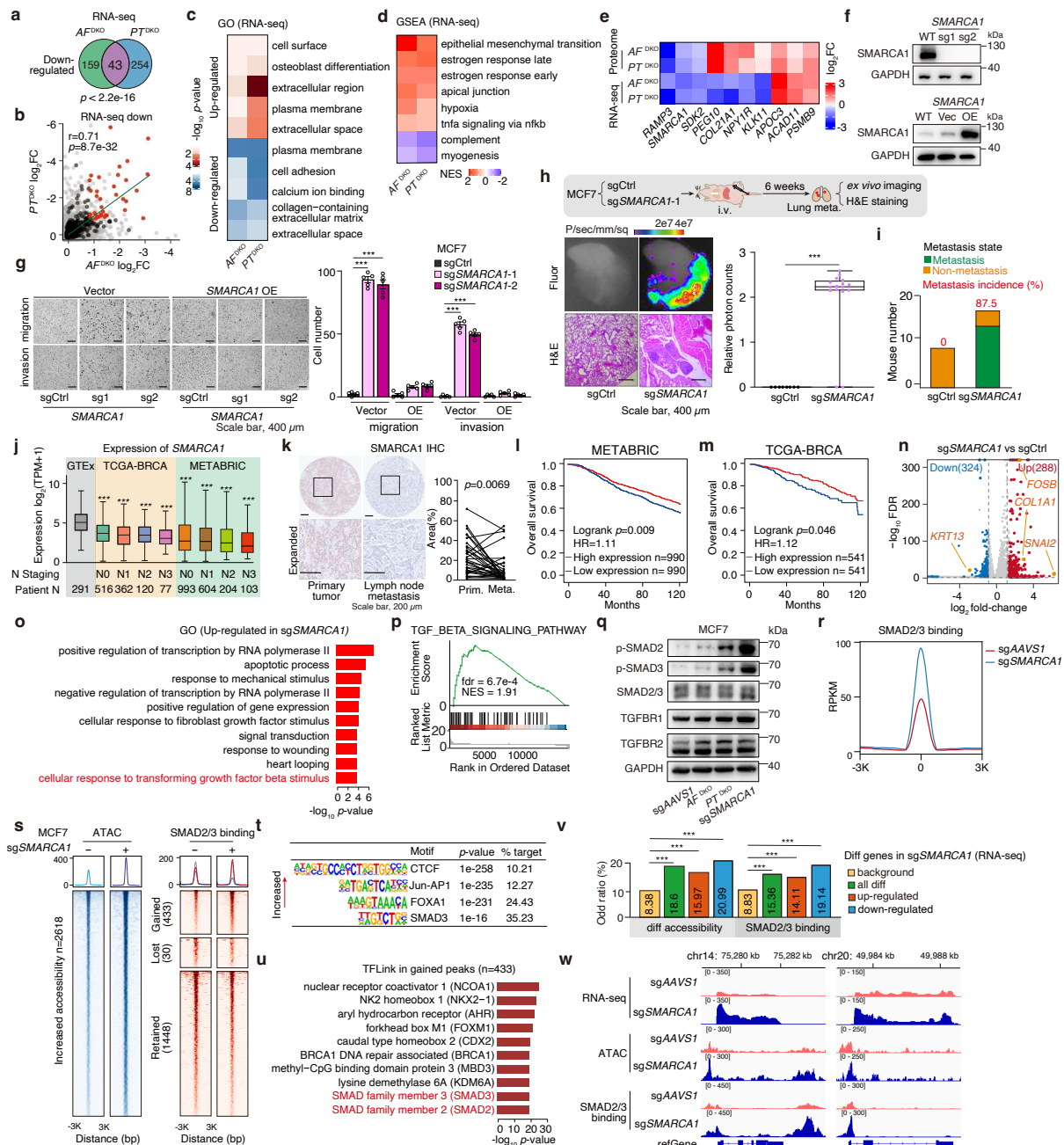


Fig. 4 | Mechanisms of SMARCA1 in mediating breast cancer metastasis.

a, Venn diagram showing the overlap of down-regulated genes between the two MCF7 metastasis^{high} cell models (AF^{DKO} or PT^{DKO}, versus parental control).

b, Pairwise correlation between the down-regulated genes between the two indicated MCF7 metastasis^{high} cell models. Black dots indicate $p < 0.05$; red dots indicate $\log_2$ fold-change > 1 & $p < 0.05$.

c-d, Heatmap showing the top enriched GO c or GSEA d terms for differentially expressed genes in the two indicated MCF7 metastasis^{high} cell models.

e, Heatmap showing the top consensus differential genes in both RNA-seq and proteomics data for the two indicated MCF7 metastasis^{high} cell models.

f, Immunoblot analysis confirming efficient knockout (using sgRNAs) or ectopic expression (cDNA overexpression) of *SMARCA1* in MCF7 cells.

g, Transwell-based migration and invasion assays for indicated MCF7 samples under sgRNA-mediated *SMARCA1* knockout and / or *SMARCA1* overexpression conditions. Representative images and result quantification are shown. Mean $\pm$ SEM, $n = 5$, *** $p < 0.001$ (unpaired t-test).

h,i, *SMARCA1* inactivation promotes metastatic dissemination *in vivo* using MCF7 i.v. models in BALB/c nude mice. Representative *ex vivo* fluorescence imaging, H&E staining and metastasis quantification of lung sections from mice are shown in (**h**). Mean $\pm$ SEM ($n = 8-16$ mice per group), *** $p < 0.001$ (unpaired t-test). Statistics of metastasis incidence is shown in (**i**).

j, Box plots showing *SMARCA1* mRNA expression in human breast tumors categorized by lymph node burden (N0, N1, N2-N3) compared to normal breast tissue. *** $p < 0.001$ (unpaired t-test).

k, Representative immunohistochemical (IHC) staining of *SMARCA1* and result quantification on a tissue microarray (TMA) containing human primary breast tumors ($n = 30$) and matched lymph node metastases ($n = 30$). Mean $\pm$ SEM, p -value (paired t-test).

l-m, Kaplan-Meier curves for overall survival in the METABRIC (**l**) or TCGA-BRCA (**m**) cohorts. Patients are stratified by the expression status of *SMARCA1*.

(n) Volcano plot displaying differentially expressed genes from RNA-seq in *SMARCA1* knockout (sg*SMARCA1*) versus control (sgCtrl) MCF7 cells.

o, The top 10 enriched GO terms for up-regulated genes upon *SMARCA1* knockout in MCF7 cells.

p, GSEA plot showing significant enrichment of the TGF- β signaling pathway hallmark gene set in *SMARCA1* knockout (sg*SMARCA1*) versus control (sgCtrl) MCF7 cells.

q, Immunoblot analysis of target proteins in indicated samples of MCF7 cells.

r, *SMARCA1* loss enhances global SMAD2/3 chromatin binding as evidenced by CUT&Tag signals for indicated MCF7 samples.

s, (Left) Heatmap of increased ATAC-seq signals upon *SMARCA1* knockout, and (Right) corresponding changes of SMAD2/3 CUT&Tag signals at the same regions.

t, Representative transcription factor binding motifs enriched in increased ATAC-seq peaks upon *SMARCA1* knockout.

u, Bar plot displaying the top enriched transcription factors from TFLink analysis of genes associated with genomic regions that gain both accessibility and SMAD2/3 binding upon *SMARCA1* knockout.

v, Chromatin alterations are linked to transcriptional changes upon *SMARCA1* loss. Bar plot showing the odds ratios of indicated gene expression change over altered chromatin accessibility or SMAD2/3 binding events upon *SMARCA1* loss. *** $p < 0.001$, Fisher's exact test.

w, Representative Integrative Genomics Viewer (IGV) tracks for indicated genes.

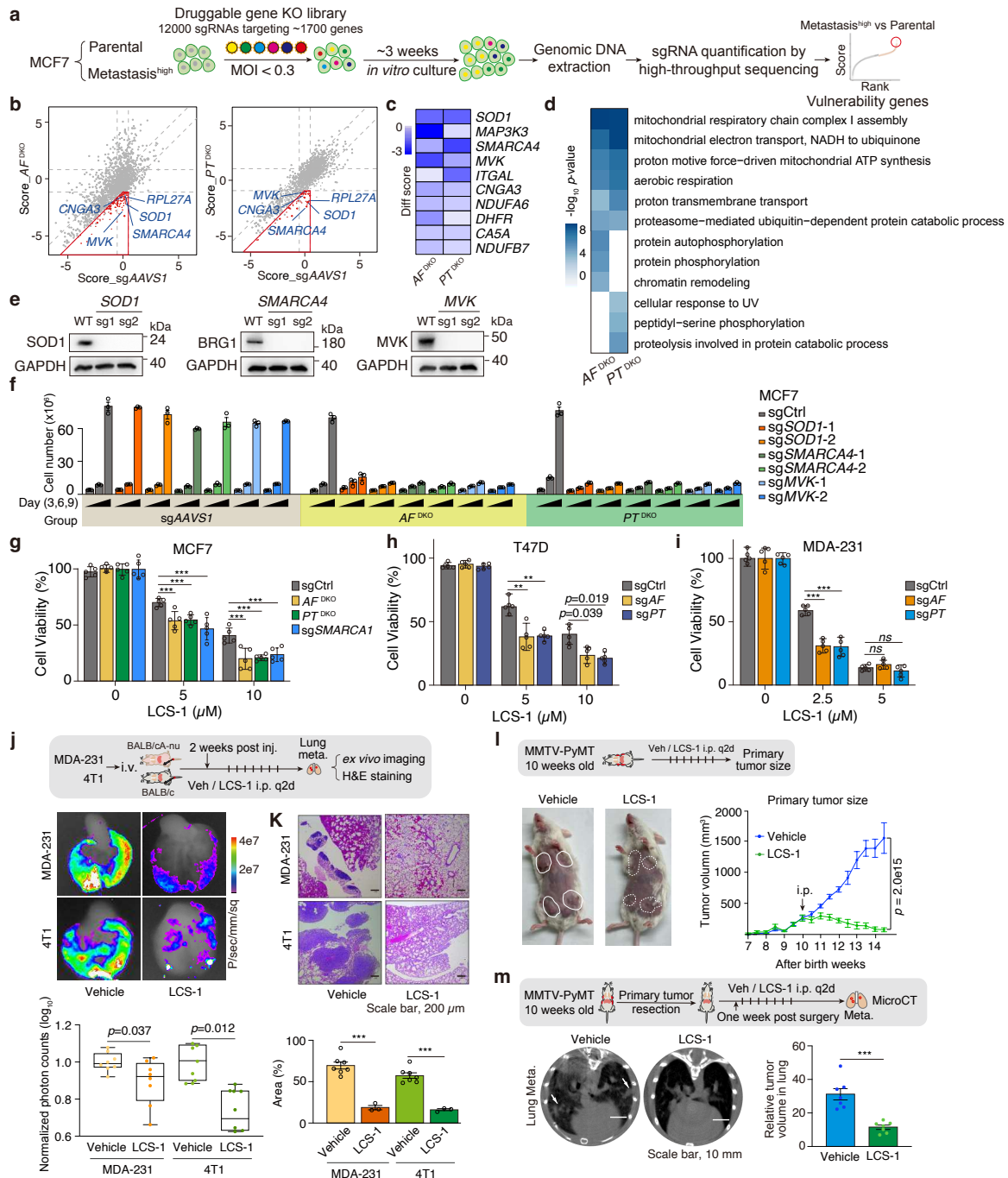


Fig. 5 | Druggable gene CRISPR screens identify therapeutic vulnerabilities in metastatic breast cancer.

a, Workflow of the druggable gene CRISPR knockout screens in mBC models.

b, Scatter plot of tRRA scores for all genes screened in the druggable gene library, comparing metastasis^{high} MCF7 models (*AF*^{DKO}, *PT*^{DKO}) with the parental control cells (sgAAVS1). Genes within the red box represent preferential vulnerabilities in metastasis^{high} cells.

c, Heatmap displaying the top 10 ranked vulnerability hits according to differential tRRA score ($\text{score}^{\text{metastasis_high}} - \text{score}^{\text{sgAAVS1_Control}}$) across the two metastasis^{high} models.

d, Heatmap displaying the top 10 significantly enriched GO terms for preferential vulnerabilities in metastasis^{high} cells.

e, Immunoblot analysis confirming efficient knockout of *SOD1*, *SMARCA4* (encoding BRG1), and *MVK* in MCF7 cells using two independent sgRNAs per gene.

f, Growth curves of MCF7 cells upon indicated gene knockout over a 9-day period.

g-i, Bar graphs showing the cell viability of indicated MCF7 cells (*AF*^{DKO}, *PT*^{DKO}, sg*SMARCA1*) (**g**) T47D cells (**h**), or MDA-231 cells (**i**) relative to sgCtrl control at multiple LCS-1 concentrations.

j-k, LCS-1 treatment reduces metastasis burden *in vivo* using both immunodeficient and immunocompetent mice models. Representative *ex vivo* fluorescence (**j**) and H&E staining (**k**) as well as corresponding result quantification of lungs from experimental metastasis models (MDA-231 or 4T1 tumors, n=8 mice per group) following LCS-1 or vehicle treatment. Mean ± SEM, ****p* < 0.001 (unpaired t-test). i.p., intraperitoneal injection; q2d, every two days.

l, LCS-1 inhibits primary tumor growth in MMTV-PyMT transgenic mice. Representative photographs of MMTV-PyMT mice with solid or dashed circles indicating the locations of primary mammary tumors. Primary tumor growth curves are plotted for indicated groups. Mean ± SEM, n = 7, *p*-value (unpaired t-test).

m, LCS-1 suppresses spontaneous metastasis in the MMTV-PyMT model. Representative microCT images of spontaneous lung metastases in MMTV-PyMT mice following LCS-1 or vehicle treatment. The metastases are indicated by white arrows. Quantification of metastatic burden according to microCT results are shown. Mean ± SEM, n = 7 mice per group, ****p* < 0.001 (unpaired t-test).

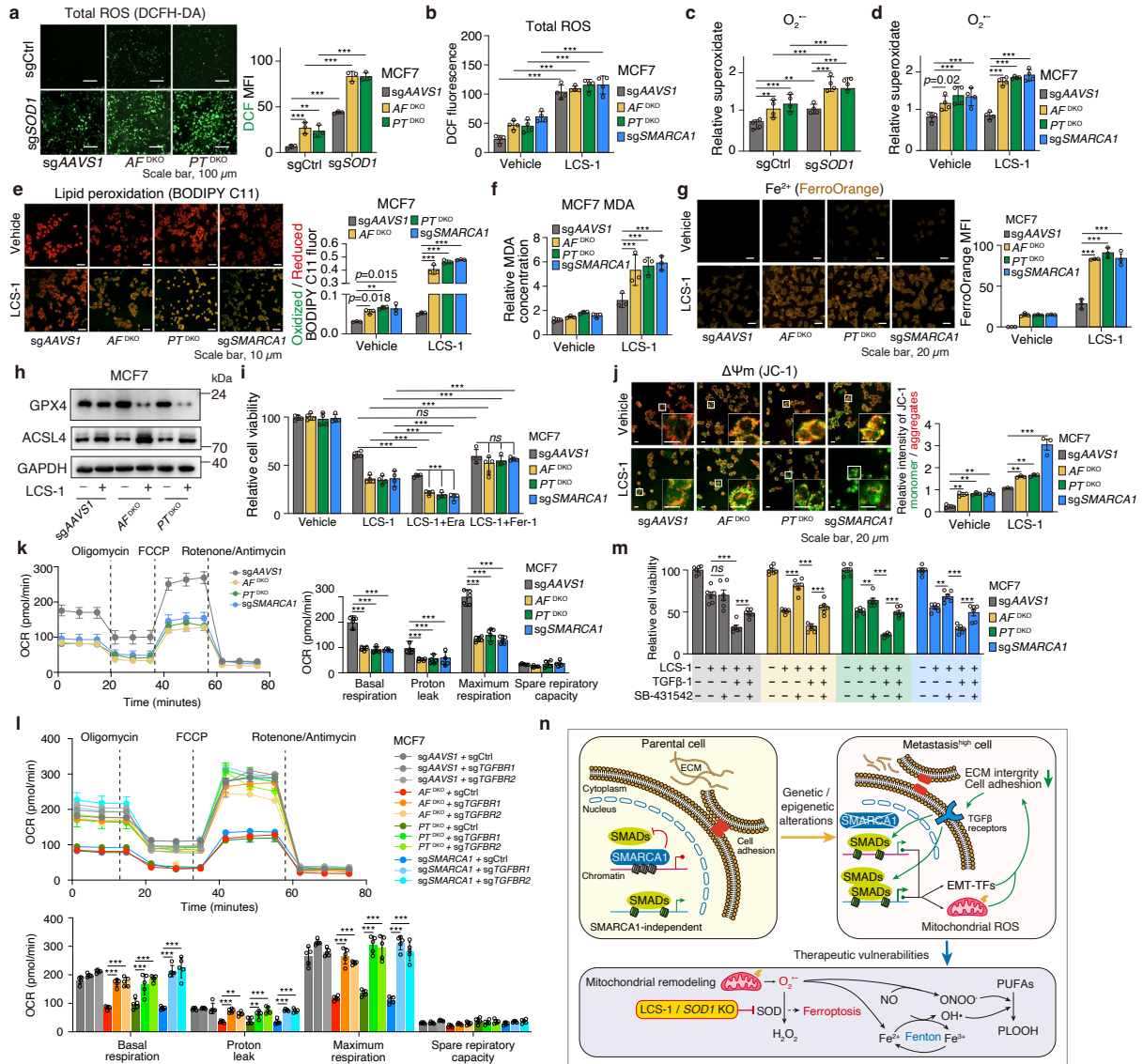


Fig. 6 | Targeting SOD1 induces ferroptosis in metastatic breast cancer.

a, Measurement of total ROS levels by DCFH assay for indicated samples upon genetic ablation of *SOD1*. Mean $\pm$ SEM, $n = 3$, $^{**}p < 0.01$, $^{***}p < 0.001$ (unpaired t-test).

b, DCFH assay measuring total ROS levels for indicated samples upon LCS-1 treatment. Mean $\pm$ SEM, $n = 4$, $^{***}p < 0.001$ (unpaired t-test).

c-d, Measurement of superoxide anion ($O_2^{\cdot -}$) levels using WST-1-based detection kit upon *SOD1* knockout c or LCS-1 treatment d for indicated MCF7 samples. Mean $\pm$ SEM, $n = 4$, $^{**}p < 0.01$, $^{***}p < 0.001$ (unpaired t-test).

e-g, Quantification of lipid peroxidation using BODIPY 581/591 C11 staining e, malondialdehyde (MDA) content (a key biomarker of lipid peroxidation and core driver of ferroptosis) f, and Fe^{2+} using FerroOrange staining g upon LCS-1 treatment (8 μ M, 24 hours) for indicated MCF7 samples. Mean $\pm$ SEM, $n = 3$, $^{**}p < 0.01$, $^{***}p < 0.001$ (unpaired t-test).

h, Immunoblot analysis showing the decrease of GPX4 and increase of ACSL4 upon LCS-1 treatment (6 μ M, 48 hours) for indicated MCF7 samples. GAPDH serves as a loading control.

i, Treatment with ferroptosis inhibitor Ferrostatin-1 (Fer-1, 5 μ M, 48 h) rescues LCS-1 (6 μ M) induced cell death in MCF7 metastatic models (*AF*^{DKO}, *PT*^{DKO}, and *sgSMARCA1*) (vs. *sgAAVS1*, vehicle), whereas treatment with ferroptosis inducer Erastin (Era, 3 μ M) enhances LCS-1 efficacy. Mean $\pm$ SEM, n = 3, ****p* < 0.001 (unpaired t-test), ns means not significant.

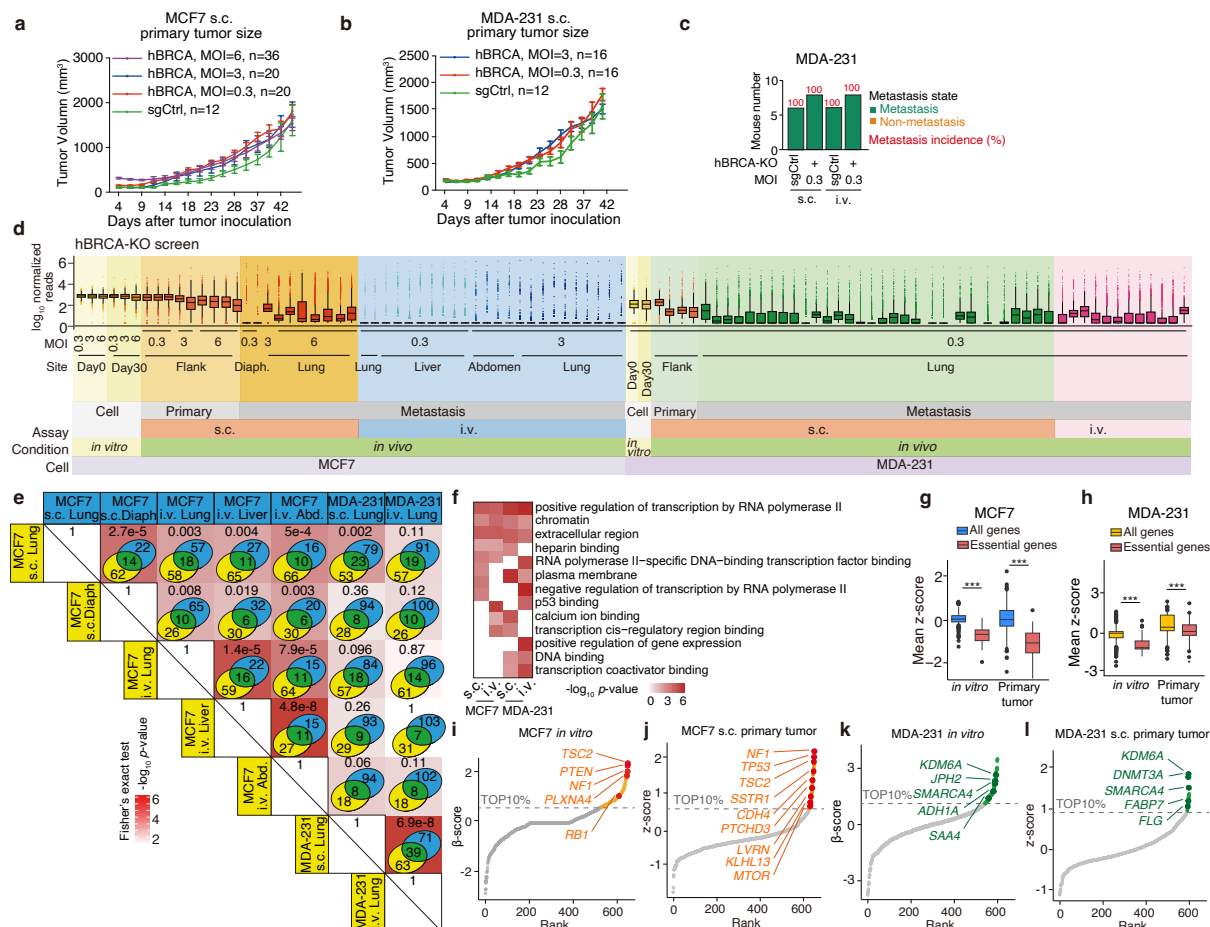
j, Measurement of mitochondrial membrane potential using the JC-1 probe for indicated MCF7 samples. Mean $\pm$ SEM, n = 3, ***p* < 0.01, ****p* < 0.001 (unpaired t-test). $\Delta\Psi_m$, mitochondrial membrane potential.

k, Seahorse mitochondrial stress test reveals compromised mitochondrial function in MCF7 metastasis^{high} models (*AF*^{DKO}, *PT*^{DKO}, and *sgSMARCA1*) compared to *sgAAVS1* control. Key parameters of mitochondrial respiration are determined. OCR, oxygen consumption rate. Mean $\pm$ SEM, ****P* < 0.001 (unpaired t-test).

l, Genetic ablation of *TGFBR1* or *TGFBR2* reverses the mitochondrial respiratory defects in MCF7 metastasis^{high} models (*AF*^{DKO}, *PT*^{DKO}, and *sgSMARCA1*) by the Seahorse mitochondrial stress test. Mean $\pm$ SEM, ***p* < 0.01, ****p* < 0.001 (unpaired t-test).

m, Treatment with TGF β 1 (6 ng/mL) enhances LCS-1 induced cell death in MCF7 metastasis^{high} models (*AF*^{DKO}, *PT*^{DKO}, and *sgSMARCA1*), while treatment with the TGF β receptor inhibitor SB-431542 (10 μ M) reverses this effect. Mean $\pm$ SEM, n = 6, ***p* < 0.01, ****p* < 0.001 (unpaired t-test), ns means not significant.

n, A schematic working model for the core drivers and vulnerabilities of mBC.



Extended Data Fig. 1 | CRISPR screens for functional metastasis genes in immunodeficient mice.

a-b, Growth curves of subcutaneously transplanted tumors in mice of MCF7 (**a**) or MDA-231 (**b**) cell origin during the screens. Mean $\pm$ SEM ($n = 12-36$ tumors per group).

c, Bar graphs summarizing the metastatic incidence for the MDA-231 model.

d, Box plots showing the $\log_{10}$ normalized read counts for sgRNA abundance of each sequenced sample in the screens.

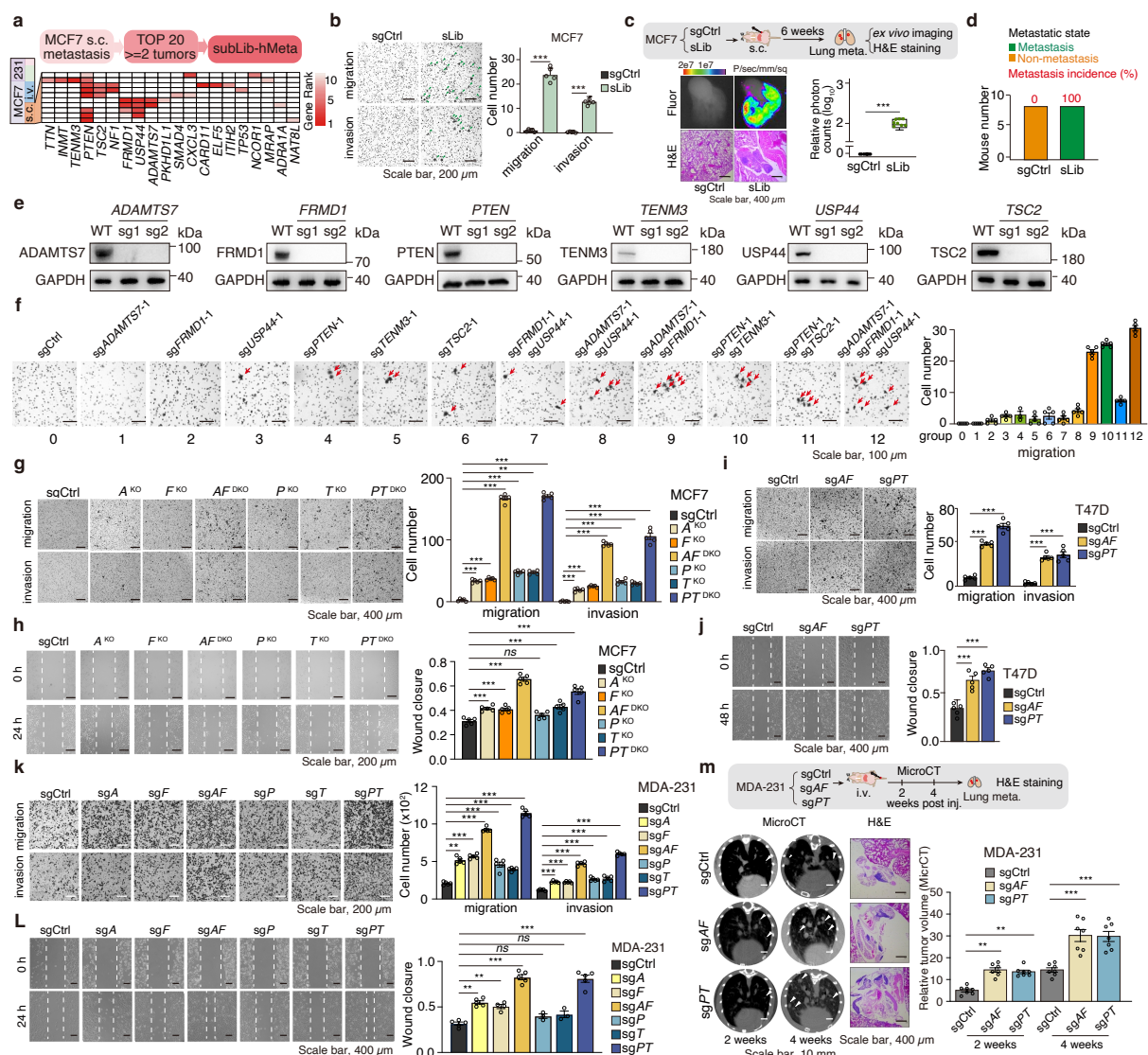
e, Statistical association of enriched sgRNAs across all pairwise conditions. Each Venn diagram compares the sets of significantly enriched sgRNAs between two conditions and is color-coded according to the $-\log_{10} p$ -value from Fisher's exact test. Diaph., Diaphragms; Abd., Abdomen.

f, Heatmap displaying the top 10 most significantly enriched GO biological process terms for each of the four experimental conditions.

g-h, Distribution of z-normalized β -scores for all genes and core essential genes in MCF7 (**g**) or MDA-231 (**h**) screening models for both the *in vitro* cell culture and primary tumors. Unpaired two-sided t-test; *** $p < 0.001$.

i-l, Genes are ranked based on their cell-fitness screening scores during *in vitro* culture (**i**, **k**) or in primary tumors (**j**, **l**) for MCF7 (**i**, **j**) or MDA-231 (**k**, **l**) models, respectively.

1545 The top 10% positively selected hits are colored, and those belonging to the high-
1546 confidence metastasis genes are labeled.
1547



Extended Data Fig. 2 | Validation of functional metastasis genes.

a, Heatmap displaying the top metastasis genes in the MCF7 s.c. group and candidate targets for the small sub-library.

b, MCF7 cells lentivirally transduced with the sub-library (sLib) exhibit increased capacity of migration and invasion compared to control (sgCtrl) cells in transwell-based assays. Representative images (Left) and quantification of results (Right) are shown. Green arrows indicate representative migrating or invading cells. Mean $\pm$ SEM, $n = 5$, *** $p < 0.001$ (unpaired t-test).

c, The sLib transduced MCF7 tumors display metastatic dissemination *in vivo*. (Left) Representative *ex vivo* fluorescence imaging (top) and corresponding H&E staining (bottom) of lung sections from mice are shown. Metastatic nodules are indicated by EGFP fluorescence. (Right) Quantification of the total EGFP signal area in lungs is shown. Mean $\pm$ SEM ($n=8$ mice per group), *** $p < 0.001$ (unpaired t-test).

d, Bar graph showing the metastasis incidence for indicated groups.

e, Immunoblot analysis confirming the successful knockout of indicated genes with two independent sgRNAs per gene in MCF7 cells.

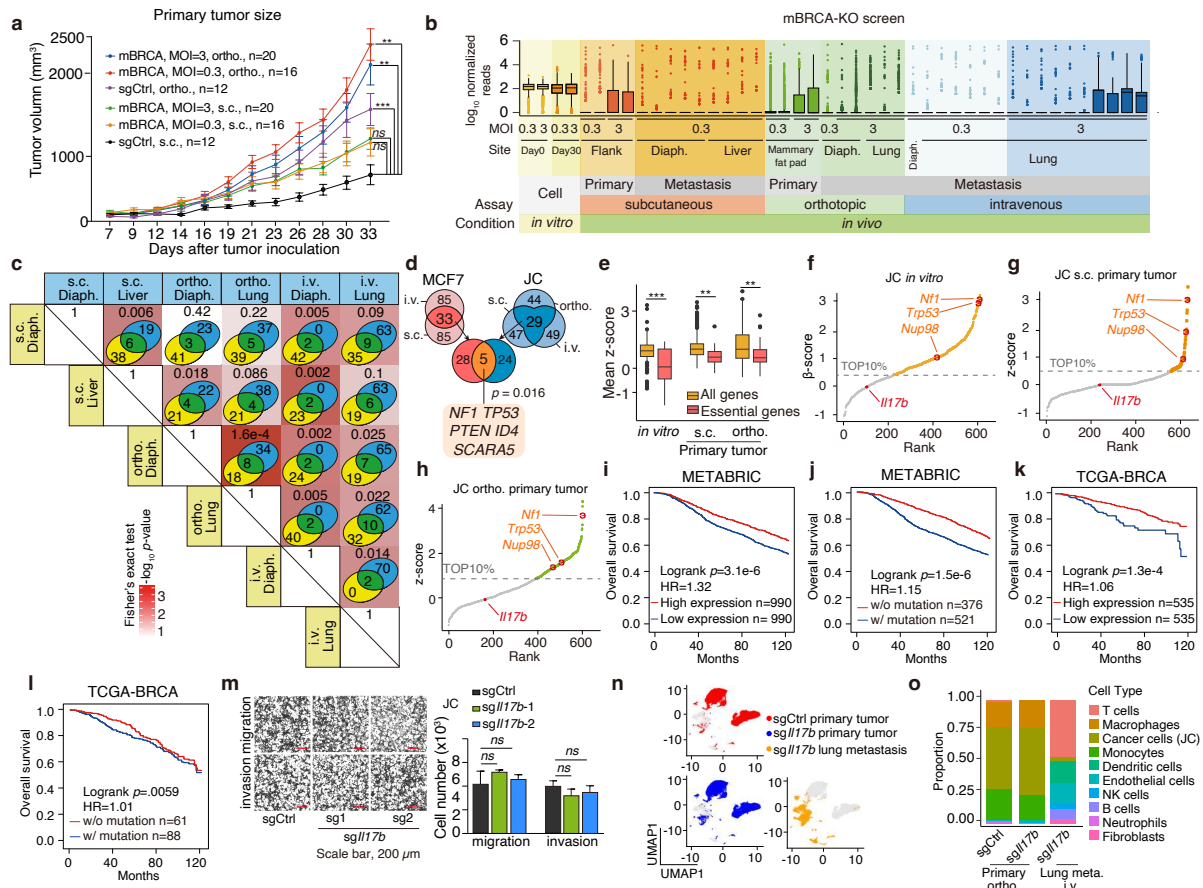
f, Transwell-based migration assays for indicated MCF7 cells. Red arrows indicate representative migrating cells. Scale bar, 100 μ m. Mean $\pm$ SEM, n =3.

g, Transwell-based migration and invasion assays for indicated MCF7 samples. Representative images (Left) and result quantification (Right) are shown. Mean $\pm$ SEM, n = 5, **p < 0.01, ***p < 0.001 (unpaired t-test).

h, Wound healing or scratch assays for indicated MCF7 samples. Representative images (Left) and result quantification (Right) are shown. Mean $\pm$ SEM, n = 5, ***p < 0.001 (unpaired t-test), ns means not significant.

i-l, Transwell-based migration and invasion assays (**i**, **k**) and scratch assays (**j**, **l**) for indicated samples in T47D (**i**, **j**) or MDA-231 (**k**, **l**) cells. Mean $\pm$ SEM, n =5 for (**i**, **j**), n = 5 for (**k**, **l**), *p < 0.05, **p < 0.01, ***p < 0.001 (unpaired t-test), ns means not significant.

m, DKO of indicated metastasis gene pairs in MDA-231 tumors promotes metastatic dissemination *in vivo*. Representative micro-CT images, H&E staining and metastasis quantification according to micro-CT imaging of lung sections are shown. The metastases are indicated by white arrows. Mean $\pm$ SEM (n=7 mice per group), **p < 0.01, ***p < 0.001 (unpaired t-test).



Extended Data Fig. 3 | CRISPR screens for functional metastasis genes in immunocompetent mice.

a, Growth curves of subcutaneous or orthotopic JC-derived tumors in immunocompetent mice during the screens. Mean $\pm$ SEM ($n = 12-20$ tumors per group), ** $p < 0.01$, *** $p < 0.001$ (unpaired t-test), ns means not significant.

b, Box plots showing the $\log_{10}$ normalized read counts for sgRNA abundance of each sequenced sample in the screens.

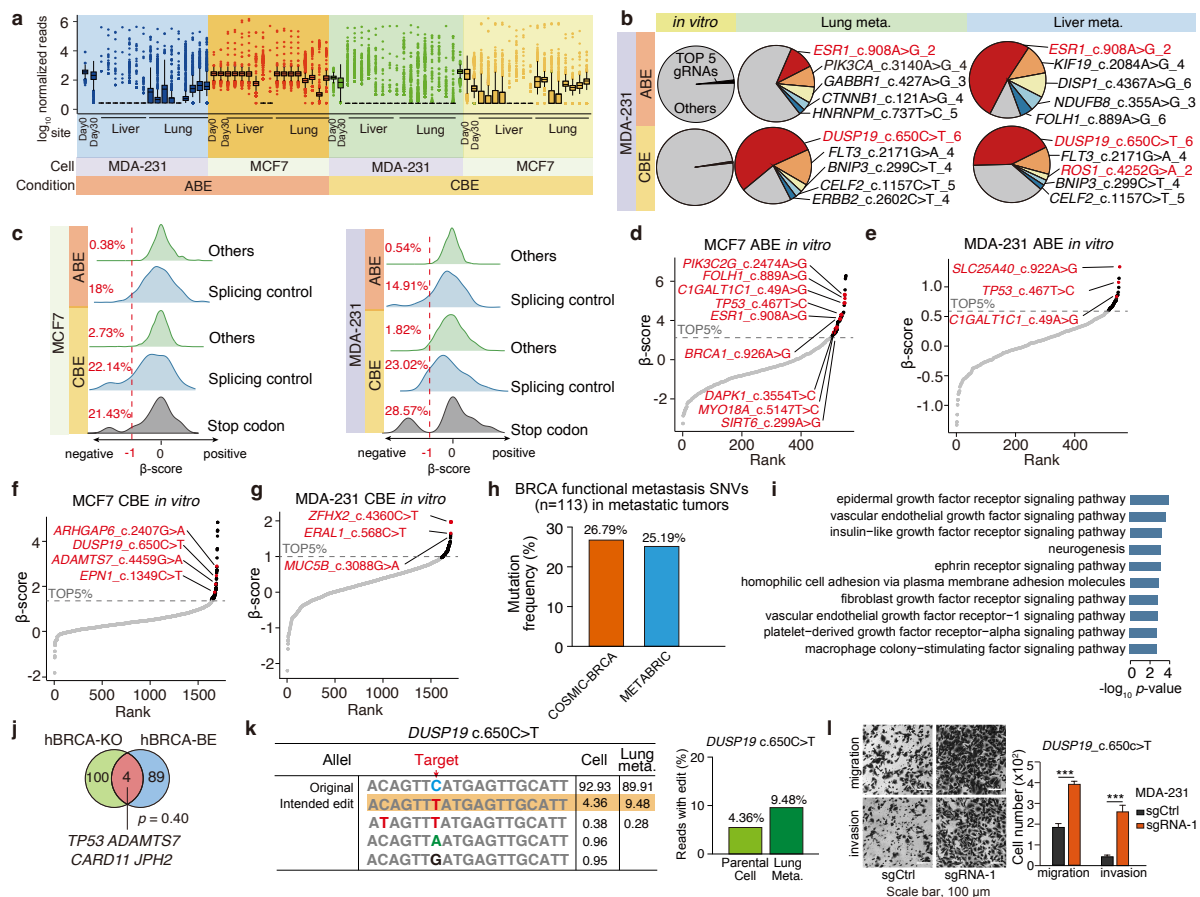
c, Statistical association of enriched sgRNAs across all pairwise conditions. Each Venn diagram compares the sets of significantly enriched sgRNAs between two conditions and is color-coded according to the $-\log_{10} p$ -value from Fisher's exact test. Diaph., Diaphragms.

d, Venn diagram comparing the sets of functional metastasis genes across indicated groups.

e, Distribution of z-normalized β -scores for all genes and core essential genes in JC-based screening models for both the *in vitro* cell culture and s.c. or ortho. inoculated primary tumors. Unpaired two-sided t-test; ** $p < 0.01$, *** $p < 0.001$.

f-h, Genes are ranked based on their cell-fitness screening scores during *in vitro* culture (**f**) or in primary tumors (**g, h**) for JC-based models. The top 10% positively selected hits are colored, and those belonging to the 29 high-confidence metastasis genes are labeled.

1606 **i-l**, Kaplan-Meier curves for overall survival in the METABRIC (**i, j**) and TCGA-BRCA
1607 (**k, l**) cohorts. Breast cancer patients are stratified by the expression levels (**i, k**) or
1608 mutation status (**j, l**) of the 29 high-confidence metastasis genes.
1609 **m**, Transwell-based migration and invasion assays for indicated samples in JC cells.
1610 **n**, UMAP visualization of single-cell transcriptomes from three JC samples: sgCtrl
1611 primary (red), *sg//17b* primary (blue), and *sg//17b* metastasis (yellow). Dimensionality
1612 reduction analysis shows substantial transcriptional overlap between primary tumors
1613 of both genotypes, while *sg//17b* metastases form a distinct cluster.
1614 **o**, Bar plot displaying the relative proportions of major cell types in indicated groups,
1615 as determined from single-cell RNA-seq data.
1616



Extended Data Fig. 4 | Base editing screens for functional metastasis SNVs.

a, Box plots showing the $\log_{10}$ normalized read counts for sgRNA abundance of each sequenced sample in the screens.

b, Pie charts depicting the count distribution of sgRNAs for installing indicated SNVs in a representative sample of each group during the MDA-231 screens. The top 5 most enriched sgRNAs are labeled.

c, Ridge plot displaying the β -score distribution of sgRNAs targeting splicing control sites, stop-codon mutations or other SNVs during the base editing screens. The proportion of sgRNAs with β -score < -1 (indicating negative selection) is labeled for each category.

d-g, SNVs are ranked based on their cell-fitness scores of base editing screens during *in vitro* culture for MCF7 ABE screen (**d**), MDA-231 ABE screen (**e**), MCF7 CBE screen (**f**), and MDA-231 CBE screen (**g**). The top 5% positively selected hits are colored, and those belonging to the 97 high-frequency metastasis SNVs are labeled.

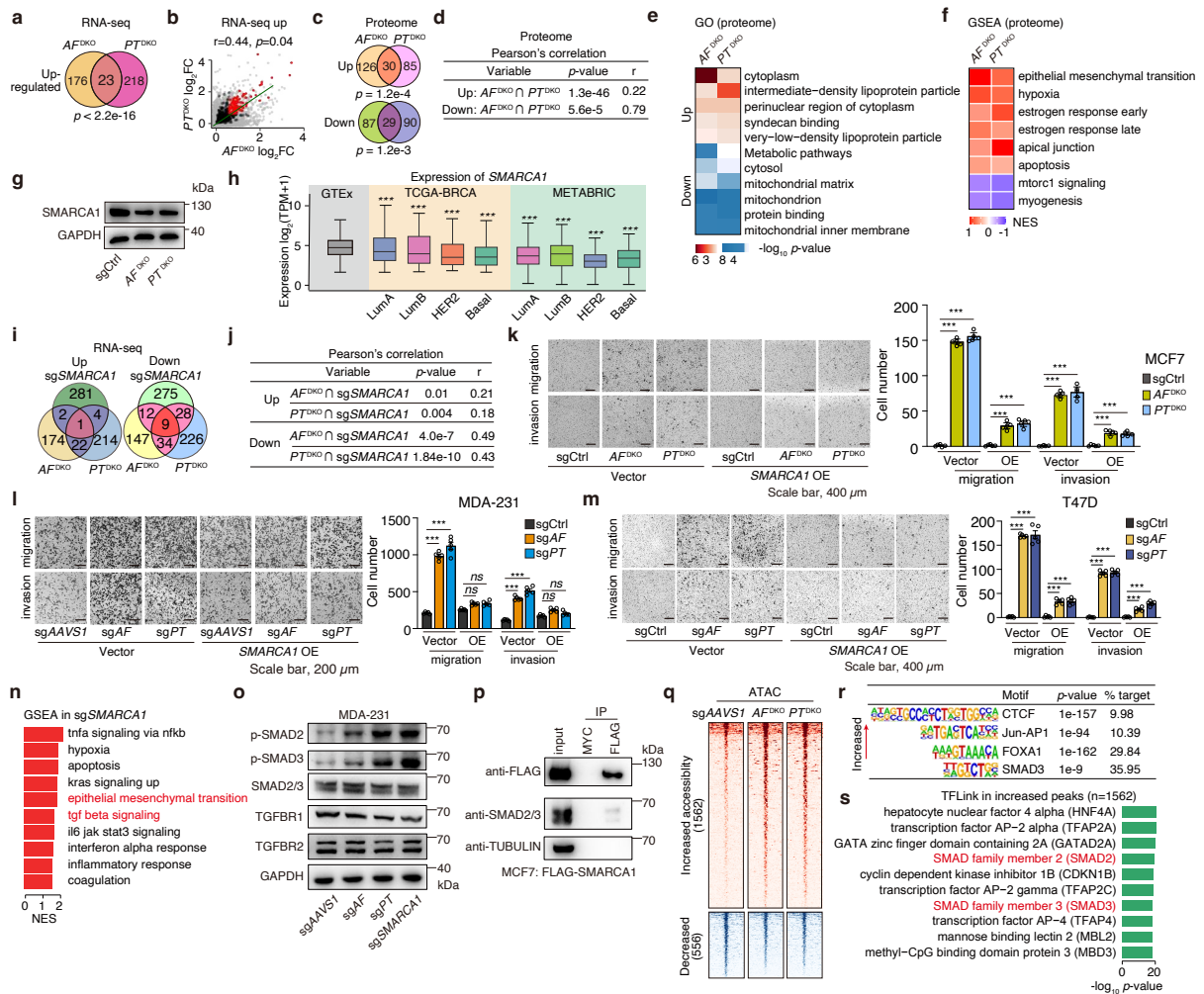
h, The mutational frequencies of functional metastasis SNVs identified from base editing screens in mBC of COSMIC or METABRIC clinical cohorts.

i, Enriched GO terms for genes associated with the 97 high-frequency metastasis SNVs.

j, Venn diagram showing the overlap between the 93 genes associated with the 97 high-frequency metastasis SNVs and the 104 high-confidence metastatic genes from the hBRCA-KO *in vivo* CRISPR screens. *P*-value is calculated by Hypergeometric test.

k, Base editing efficiency (allele frequency) of target site for *DUSP19_c.650C>T* SNV in samples from *in vivo* metastatic tumors and *in vitro* parental MDA-231 cells.

l, The *DUSP19_c.650C>T* variant enhances migratory and invasive capacity *in vitro*. Representative images (Left) and quantification (Right) of transwell-based migration and invasion assays performed with MDA-231 cells expressing the *DUSP19_c.650C>T* variant or sgCtrl control. Mean $\pm$ SEM, $n = 5$, *** $p < 0.001$ (unpaired t-test).



Extended Data Fig. 5 | Mechanisms of *SMARCA1* underlying breast cancer metastasis.

a, Venn diagram showing the overlap of up-regulated genes between the two MCF7 metastasis^{high} cell models (AF^{DKO} or PT^{DKO} , versus parental control).

b, Pairwise correlation between the up-regulated genes between the two indicated MCF7 metastasis^{high} cell models. Black dots indicate $p < 0.05$; red dots indicate $\log_2$ fold-change > 1 & $p < 0.05$.

c, Venn diagram showing the overlap of differential proteins between the two MCF7 metastasis^{high} cell models (AF^{DKO} or PT^{DKO} , versus parental control).

d, Pearson's correlation of up- or down-regulated proteins between the two indicated MCF7 metastasis^{high} cell models.

e,f, Heatmap showing the top enriched GO (**e**) or GSEA (**f**) terms for differential proteins for the two indicated MCF7 metastasis^{high} cell models.

g, Immunoblot analysis of SMARCA1 protein expression in wild-type (WT), AF^{DKO} , and PT^{DKO} MCF7 cells.

h, SMARCA1 is down-regulated in aggressive molecular subtypes of human breast cancer. SMARCA1 mRNA levels across indicated subtypes in the TCGA-BRCA and

1667 METABRIC cohorts, compared to normal breast tissue (GTEx). *** $p < 0.001$ (unpaired
1668 t-test).

1669 **i,j**, Venn diagram showing the overlap (**i**) and pairwise correlation (**j**) between indicated
1670 gene lists.

1671 **k-m**, Transwell-based migration and invasion assays for indicated samples in MCF7
1672 (**k**), T47D (**l**) and MDA-231(**m**) cells. OE, overexpression. Mean $\pm$ SEM, $n = 5$, *** $p <$
1673 0.001 (unpaired t-test).

1674 **n**, GSEA analysis showing the top 10 most significantly enriched hallmark gene sets
1675 by normalized enrichment score (NES) from differential genes between *SMARCA1* KO
1676 versus control MCF7 cells in RNA-seq.

1677 **o**, Immunoblot analysis of target proteins in indicated samples of MDA-231 cells.

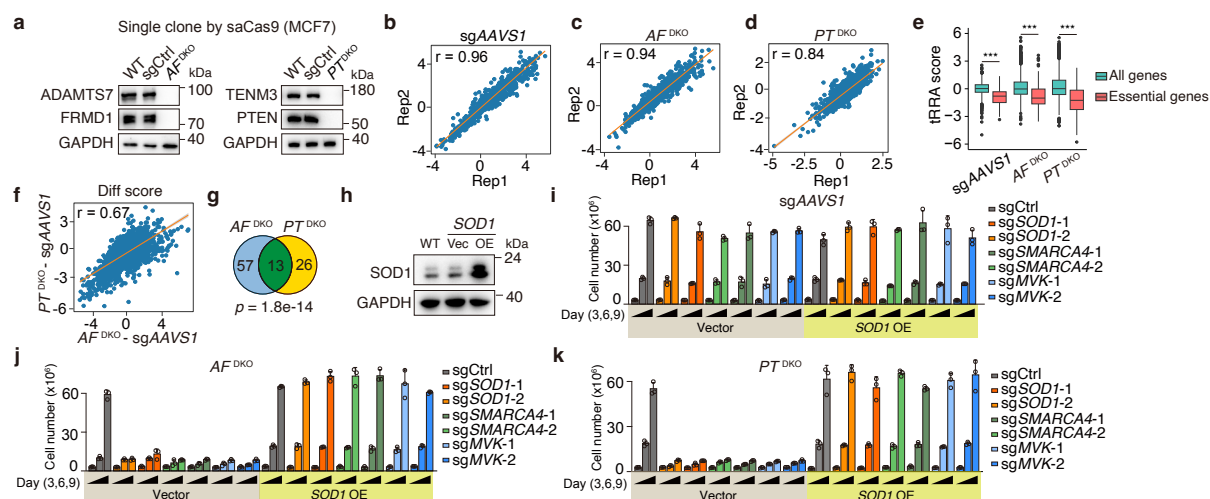
1678 **p**, Co-immunoprecipitation (Co-IP) of endogenous SMAD2/3 with FLAG-tagged
1679 SMARCA1 using an anti-FLAG antibody in MCF7 cells. MYC antibody serves as a
1680 control. Immunoblotting was performed with antibodies against SMAD2/3 and FLAG.

1681 **q**, *AF^{DKO}* and *PT^{DKO}* models share a common chromatin remodeling signature.
1682 Heatmap of ATAC-seq signals at genomic regions with concordantly increased (top,
1683 $n=1562$) or decreased (bottom, $n=556$) chromatin accessibility in both *AF^{DKO}* and
1684 *PT^{DKO}* MCF7 cells compared to sgAAVS1 control.

1685 **r**, Representative transcription factor binding motifs enriched in increased ATAC-seq
1686 peaks in both *AF^{DKO}* and *PT^{DKO}* MCF7 cells.

1687 **s**, Bar plot displaying the top enriched transcription factors from TFLink analysis of
1688 genes associated with genomic regions that gain accessibility in both *AF^{DKO}* and *PT^{DKO}*
1689 MCF7 cells.

1690



Extended Data Fig. 6 | Druggable gene CRISPR screens for therapeutic vulnerabilities of mBC.

a, Immunoblot analysis confirming the successful establishment of single clonal AF^{DKO} and PT^{DKO} MCF7 lines by SaCas9.

b-d, Scatter plots displaying the Pearson's correlation of tRRA scores between the two independent replicates (R1 vs. R2) for each screening model: parental control (**b**), AF^{DKO} (**c**), and PT^{DKO} (**d**).

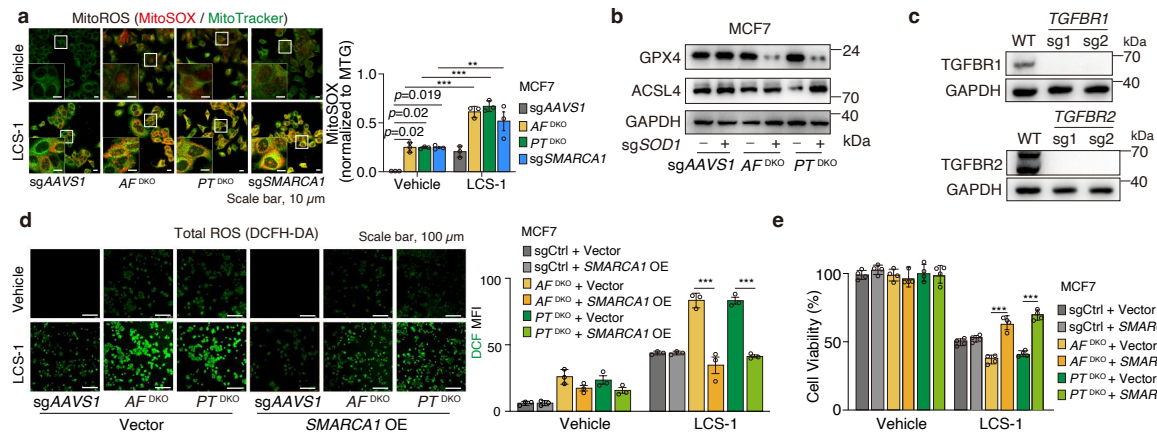
e, Box plot comparison of tRRA scores for core essential genes versus all genes in the druggable gene CRISPR screens for indicated samples. *** $p < 0.001$ (unpaired t-test).

f, Scatter plot showing the pairwise comparison of differential tRRA scores (AF^{DKO} or PT^{DKO} versus sgAAVS1) between the two metastasis^{high} models. r , Pearson's correlation coefficient.

g, Venn diagram showing the significant overlap of preferential vulnerability hits between AF^{DKO} and PT^{DKO} models. P -value is calculated by Fisher's exact test.

h, Immunoblot analysis confirming the ectopic expression of SOD1 in MCF7 cells.

i-k, Ectopic SOD1 expression rescues the cell viability defect elicited by *SOD1*, *SMARCA4* or *MVK* knockout in MCF7 cells. Growth curves of indicated samples in sgAAVS1 (**i**), AF^{DKO} (**j**), and PT^{DKO} (**k**) cell models are shown.



Extended Data Fig. 7 | SOD1 is a promising therapeutic target against metastatic breast cancer.

a, Measurement of mitochondrial superoxide levels using MitoSOX Red staining upon LCS-1 treatment (8 μ M, 24 h) in indicated MCF7 samples. Cells are co-stained with MitoTracker Green to normalize for mitochondrial mass. Mean $\pm$ SEM, n = 3, ** p < 0.01, *** p < 0.001 (unpaired t-test).

b, Immunoblot analysis showing the decrease of GPX4 and increase of ACSL4 after sgRNA-mediated SOD1 knockout in MCF7 metastasis^{high} models (AF^{DKO} or PT^{DKO}) compared to sgAAVS1 control group.

c, Immunoblot analysis confirming efficient knockout of TGFBR1 and TGFBR2 in MCF7 cells using two independent sgRNAs per target. GAPDH serves as a loading control.

d, DCFH assay showing the total ROS levels upon LCS-1 treatment (8 μ M, 24 h) for indicated MCF7 samples. Ectopic SMARCA1 expression partially reverses the elevated total ROS levels induced by LCS-1 treatment in metastasis^{high} models. Mean $\pm$ SEM, n = 3, *** p < 0.001 (unpaired t-test).

e, Cell viability assay showing that ectopic SMARCA1 expression partially reverses the cytotoxic effects of LCS-1 treatment (8 μ M, 24 h) in MCF7 metastasis^{high} models (AF^{DKO} or PT^{DKO}). Mean $\pm$ SEM, n = 4, *** p < 0.001 (unpaired t-test).

1734 **Supplementary Table 1** (attached dataset)
1735 hBRCA KO library and screening data.
1736
1737 **Supplementary Table 2** (attached dataset)
1738 mBRCA KO library and screening data.
1739
1740 **Supplementary Table 3** (attached dataset)
1741 BE library and screening data.
1742
1743 **Supplementary Table 4** (attached dataset)
1744 Omics data.
1745
1746 **Supplementary Table 5** (attached dataset)
1747 Druggable gene screening data.
1748
1749 **Supplementary Table 6** (attached dataset)
1750 Oligos and primers.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.hBRCAKOlibraryandscreeningdata.xlsx](#)
- [TableS2.mBRCAKOlibraryandscreeningdata.xlsx](#)
- [TableS3.BElibraryandscreeningdata.xlsx](#)
- [TableS4.Omicdata.xlsx](#)
- [TableS5.Druggablegenescreeningdata.xlsx](#)
- [TableS6.Oligosandprimers.xlsx](#)