Cellular Fitness Phenotype of Cancer Target Genes in Repurposing Cancer Therapeutics

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ABSTRACT

Currently approved drugs in oncology have been mostly developed using candidate target-driven approaches for a given cancer. To define the global significance of cellular targets oncology drug in cancer, we examined the fitness-dependency ï the loss of cancer cellôs viability in the absence of a test gene, of cancer drug targets across human cancer cells in a CRISPR-Cas9 fitness screening dataset, wherein genes were selectively knocked-down before assaying for the fitness-dependency of 7470 genes in 324 cancer cell lines representing 19 cancer-types. We observed that depletion of 35 out of 47 fitness targets of oncology drugs did not result in an otherwise expected loss of cell fitness in appropriate cancer-types for which drugs targeting these molecules were approved. This raises the possibility of undesirable drug-associated off-target effects in such cancers. In addition, our analysis allowed recognition of 41 drug targets as fitness genes in several cancer-types ascandidate targets for repurposing approved oncology drugs for cancer-types in which these drugs were not approved. For example, we found widespread upregulation and associated reduction in the duration of overall survival of cancer patients, and fitness-dependency of the components of mevalonate and purine biosynthesis pathways (currently targeted by bisphosphonates, statins and pemetrexed in certain cancers) in breast and other hard-to-treat cancers for which these drugs are not approved. In brief, the present analysis raises caution about the likelihood of off-target and undesirable drug-associated effects of certain oncology drugs in a subset of cancers where intended drug targets are not fitness genes. This also offers a rationale for repurposing a set of approved oncology drugs for cancer-types that have significant fitness-dependencyon a set of cellular targets of such approved drugs.

INTRODUCTION

Over the past few decades, cancer treatment has witnessedtremendous progressin enhancing the duration of disease-free survival, and delaying or preventing cancer recurrence in patients. The first generation of cancer chemotherapeutic and cytotoxic drugs actedthrough targetingnucleic acids,protein synthesis and cell metabolism ï all fundamental to the growth of both cancer and normal cells. Owing to this non-specificity, such drugs exhibit both anti-cancer as well as toxic side effects[1,2,3,4]. In contrast, targeted cancer therapy targets specific cellular biomolecules and pathway(s) that are differentially overexpressed and/or hyperactivated in cancer cells as compared to normal cells, and has emerged as a preferred option of cancer treatment[5,6,7]. In this context, the Food and Drug Administration (FDA) of the United States has approved about 235 oncology drugs until May 2019 which targetabout 232 cellular genes. The core of targeted cancer therapy is the intended cellular target against which an inhibitory moleculewasdeveloped. However, beneficial clinicalas well as toxiceffects of targeted cancer therapeutics generally could result from both on- and/or off-target effects of the drug[2,8,9]. The mere presence of an upregulated cellular cancer target does not ensure that a given cancer drug will exhibit a homogenous therapeutic response across the patient population. This might be due to inherent genomic and cellular heterogeneity and acquired compensatory rewiring of proliferative pathways upon treatment with such drugs, leading to acquired therapeutic resistance. For example, in-spite of HER2overexpression of all breast cancer patients who receive Trastuzumabas a single agent, only about 26% show beneficial clinical response[10]; and only about 34% of EGFR-positive metastatic colorectal cancer patients show stable disease upon receiving cetuximab as a single agent, and the majority of patients shows progressive disease in monotherapy settings[11]. This is because of a whole range of reasons beyond the target.Similarly, HER2-directed therapies such as Trastuzumab in breast cancer results in the median survival of over 3 years[12], whereas there is a modest increase in survival of about 4 months in patients with gastric cancer[13]. It is not clear whether differential effectiveness of targeted therapy in these settings was due to lack of the

intended target or ineffectiveness of targeted therapy in inhibit the target, in addition of other reasons.

Historically, currently available FDA-approved oncology drugshave beendeveloped by intended molecule-driven empirical approaches[14].This has also been very fruitful and was essential to reach the current stage of targeted cancer therapy. However, this approach had not always taken advantage of post-genomic data in selecting the target for developing a drug[15]. Further, the fact that cancer is a polygenic disease[16] was not always factored during development of FDA-approved oncology drugs - although the notion of polygenic nature was considered in developing combination regimens targeting distinct pathways. However, post-genomic data and high-throughput screening platforms are actively utilized for molecular classification and diagnosis of tumors, assessing the therapeutic sensitivity, and patient-stratification for improving the effectiveness of existing oncology drugs.

Targeted cancer therapy still remains far from achieving the promise of targeted cancer therapy to inhibit the growth of tumor cells in all patients if indeed such patients are selected on basis of the intended target for a given oncology drug. It is possible that a new approach is required for additional significant gains and benefitsfor cancer patients. In this context, Behan *et al.*developed a most comprehensive portrait of gene-dependency of human cancer[17], wherein the team utilized the CRISPR-Cas9 approach to selectively knock-down about 7460 genes in 324 genomically characterized cell lines[18], representing 19 cancer tissues and assayed the requirementof each gene for the cellular fitness (i.e. viability) of cancer cells which was then presented the outcome as a fitness gene (i.e. the loss of cell viability in the absence of a test gene, depicted as negative fitness effect) or not a fitness gene (i.e. no loss of cell viability in the absence of a test gene, depicted as positive fitness effect) of each gene for each cell line[17,19]. The work identified 628 priority-genes distributed across 19 cancer-types for cancer therapeutic out of 7,470 fitness genes with significant fitness-dependency in multiple cancer-types[17]. Approved oncology drugs act in a given cancer types by impairing the functionality of specific cellular

targets. However, it remains unknown whether cellular targets of approved oncology drugs are also fitness genes in cancer types for which drugs targeting these targets are not approved, and is being investigated here.

RESULTS and DISCUSSION

To define the global significance of oncology drugsô cellular targets in cancer cell growth, here we examined the fitness-dependency(i.e. required for the cell viability or growth) of cancer drug targets in cancer cell lines. We examined the presence of 232 cellular targets that are targeted by 235 FDA-approved oncology drugs (Supplementary Table 1) in the CRISPR-Cas9 fitness screen datasets[17]. We found the presence of 100 cellular targets in the fitness screen. Forty seven out of these 100cancer drug targets of FDA-approved drugs are fitness genes across 19 cancer-types in the cancer-dependency screen (Supplementary Fig. 1a,b), while 53 cancer drug targets are without any loss of fitness upon knocking down a specific target(Supplementary Table 1).

We first focussed on the 47 cellular cancer targets of FDA-approved drugs in the subsequent studies presented here. When compared with the recently identified priority-genes for cancer therapeutics[17], we observed that 15 of the 47oncology drug targets overlap with 628priority therapeutic targets (Fig. 1a, Supplementary Table 1). Both 47 cellular targets of FDA approved drugs and its subset of 15 shared drug targetswere distributed across cancer-types for which drugs targeting these cellular targets were approved (Supplementary Fig. 1c) or not approved (Fig. 1b). Three and ten of 15 targets shared between priority therapeutic targets[17] and targets of oncology drugs were also targeted by therapeutic antibodies and small molecules, respectively (Supplementary Table 1). These observations not only confirmed the recent findings of detecting cellular targets of approved cancer drugs as priority therapeutic targets[17], but also recognized41 cellular targets with excellent fitness effect in cancer-types for which drugs targeting these targets are not approvedand that 53 cellular targets of oncology drugs are without

any cellular fitness effect upon their depletion (Fig. 1a). In addition, a small number of 47 cellular targets could be fitness gene not fitness genein cancer type context manner. For example, Phosphoribosylglycinamide Formyltransferase (GART) is an excellent fitness gene in Ovarian cancer for which pemetrexed which targets GART was approvedas well as it is not a fitness gene for Lung and Kidney cancer cancers.

[Figure1]

To determine the requirement of 47 cellular drug targets in cancer-types fitness, we examined the fitness-dependency of these genes in the CRISPR-Cas9 derived cancer-dependency map[17]. We observed that depletion these targets in appropriate cancer-types for which drugs targeting these cellular molecules were approved, resulted in a significant loss of cell fitness, implying that there is a role of these cellular targetsin the growth of these cancer cells as expected (Fig. 1c, dark green boxes; Supplementary Fig. 1d). For example, depletion of 13 targets (i.e. RRM1, TOP2A, TYMS etc.) in breast cancer cells, 8 targets (i.e. RRM1, TOP1, MTOR etc.) inglioblastoma and 6 targets (i.e TYMS, RRM1, TOP1 etc.) in pancreatic cancer cells result in a significant loss of cellular fitness as depicted by negative fitness effect. Like-wise, we found that depletion of 41 targets in cancer-types for which drugs targeting these cellular molecules were not approved, also accompanied by the loss of cell fitness (Fig. 1c, light green boxes). Surprisingly, we also noticed that the deletion of 88cellular targets of approved drugs, inclusive of 35 out of 47 fitness genes in certain cancersdid not result in a significant loss of cell fitness in multiple cancer-types for which drugs targeting these molecules were approved (Fig. 2a and Fig. 2b). For example, knocking down of FCGR1A, FCGR2B and FCGR3a ï all targets of Bevacizuab in ovarian, intestinal and kidney cancer (Fig. 2c), and of CDK4 and CDK6 (target of Palbociclib/Ibrancein breast cancer), ERBB2 (target of Trastuzumab/Herceptin in breast cancer), BTK (target of Ibrutinib/Imbruvica in haematopoietic cancer), CRBN (target of lenalidomide/Revlimid in Skin) and CYP17A1 (target of Abiraterone Acetate/Zytiga in prostate

cancer (Supplementary Fig. 2b) did not influence the fitness of cancer cell-types.In contrast, we noticed that depletion of such target genes without fitness-loss was often accompanied by significantly improved fitness in cancer-types (Fig. 2c; Supplementary Fig. 2b**)**, implying an improved cell growth if such genes are attempted to be targetedin the cancer-type which could be point of some concerns.In this context, a number of recent reports have demonstrated growth-promoting activities of cancer treatment drugs in physiologicallyrelevant whole animal models[20,21,22,22,23,24]. Theseobservations raisedtwo important possibilities for targeted cancer therapy, abeneficial antitumor and therapy-associated toxic effects may result from off-targeteffects of certain oncology drugs if theintended target of such drugs is not a fitness-gene for the cell growth/viability in certain cancer-types, second in the absence of loss of fitness-dependency, attempt to inhibitsuch cellular target genes could lead to increased proliferation of certain cancer-types, perhaps, via indirect pathways. The latter possibility implies that if indeedintended cellular drug targetsare not affected by the drugs, this could lead to undesirable effects in some cancer-types.

[Figure2]

To reveal a broader significance of 47 cellular targets which exhibited a significant fitness-dependency, we next determined whether these targetsare required for the fitness/growth of cancer-types for which drugstargeting these molecules were not approved. Interestingly, we found that depletion of 41 of 47 cellular targets in multiple cancer-types associates with a substantial loss of cell fitness (Fig. 1c, Fig. 2d, Supplementary Fig. 3).Further a direct comparison of the status of cellular targets which are either approved or not approved for a given cancer revealed that the majority of targets with significant fitness-dependency are in cancer-types for which drugs targeting these molecules are not approved for that cancer. Results in Fig. 2e illustrate the distribution of fitness-dependency value of molecules which are targets of approved (dark green) or not approved (light green) drugs for breast cancer, pancreatic cancer and

glioblastoma. Fitness-dependency of 41 targets of approved oncology drugs across the remaining 15 cancers is shown in Supplementary Figs. 3 and 4. In general, targets of cancer drugs exhibited a widespread fitness-dependency in cancer-types for which drugs targeting these cellular targets are not approved (light green) as compared to cancer-types for which drugs targeting such molecules are approved (dark green).For example,cellularfitness of breast, ovarian and endometrial cancer cell lines was significantly compromised by depletion of 14 (i.e. GGPS1, FDPS, GART etc.), 24 (i.e. GGPS1, FCGR1A, TUBD1 etc.), and 25 (i.e. GGPS1, FDPS, BRAF etc.) molecules targeted by approved oncology drugs, respectively (Fig. 2e, Supplementary Fig. 5a). Interestingly, multi-variant analysis of tumors with high overexpression versus low expression of these fitness genes was associated with a highly significant, reduction in the overall survival of respective cancers patients (Supplementary Figs. 5b and 5c). Similarly, fitness of esophageal, pancreatic and stomach cancercell lines was significantly compromised by depletion of 31, 27 and 29 genes, respectively (Supplementary Fig. 6a). Multi-variant analysis of high overexpression versus low expression of these fitness genes was also associated with a highly significant, overall reduction in the survival of respective cancers patients (Supplementary Figs. 6b and 6c). Interestingly, all of these 41 fitness-genes are targets of FDA-approved drugs in cancer-types for which drugs targeting these are not approved (Supplementary Table 1). These results revealed the significance of cellular targets (of approved oncology drugs) in cellular fitness for cell viability in cancer-types, and raising the possibility of repurposing cancer drugs for cancer-types for which these drugs are not approved, but cellular targets of such drugs in such cancer-types show a significant fitness-dependency.

Because of our interest in womenôs cancer, we next evaluated the expression of 14 cancer drug targets, of which 11 are shared among breast, ovarian and endometrial cancers, with significant fitness-dependencyin breast cancer (Supplementary Fig. 5a and Fig. 2e).Among these cell fitness targets, we observed a widespread mRNA overexpressionand/or copy number amplification of Geranylgeranyl pyrophosphate synthase(GGPS1), Farnesyldiphosphate synthase

(FDPS).andPhosphoribosylglycinamideFormyltransferase (GART, also known as glycinamideribonucleotideformyltransferase - GARFT)in breast tumors(Fig. 3a and Supplementary Fig. 7). GGPS1 and FDPS enzymes are components of the mevalonate pathway which plays a pivotal role in cholesterol biosynthesis and pathobiology of bone metastasis of breast cancer, prostate cancer and multiple myeloma[25,26,27,28,29,30], while GARTôs protein product[31] is a mandatory trifunctional enzyme with an essential role in purine biosynthesis (Supplementary Fig. 8a).We found that the levels of GGPS1, FDPS and GART weresignificantly elevated in breast tumors (Fig. 3b) as compared to matching adjacent normal[32], in breast cancer cell lines (Fig. 3c), in breast cancer sub-types (Fig. 3d), upregulatedin triple negative breast cancer (TNBC) as compared to matched normal or non-TNBC tumors (Fig. 3e)[33]. The noticedoverexpression of GGPS1, FDPS and GART mRNAs in breast tumors was also accompanied by their respective proteins (Fig. 3f) in breast tumors[34]. Interestingly, we also noticed coexpression of GGPS1, FDPS, or GART proteins in several of the same breast tumors (shown by empty blocks).

[Figure3]

GGPS1 and FDPS are targets of nitrogen-containing bisphosphonates, such as zoledronic acid derivatives which are widely to prevent the risk of skeleton related events related to breast cancer relapse and reduce mortality and in postmenopausal women by inhibiting bone metastasis by suppressing osteoclast-mediated bone resorption[25,26,27,28]. They accomplish this by inhibiting osteoclast activity, decreasing the bone turnover as supported by a reduction in the levels of bone resorption markers N-telopeptide and c-telopeptide[25,26,27,28,29,30]. In general, bisphosphonates are considered supportive therapy and not anti-cancer therapy for solid tumors due to a modest modifying effect on the overall survival of patients with solid tumors in clinical trials undertaken by two of the authors of this study[28,29,30], while it increases the overall survival in multiple myeloma[35]. However, the nature and context of bisphosphonateôs targets in breast cancer, i.e.

GGPS1 and FDPS,are expected to be different from its targets in bone. In this context, a recently completed clinical trial provides clues about the beneficial anti-tumor activity of bisphosphonates against breast cancer in adjuvant setting in a subset of postmenopausal women[36, 37] which were negative for MAF transcription factor ï previously implicated in regulating genes important in breast-to-bone metastasis[38], and other cancers[39,40]. It remains an open question whether the responders in this study were GGPS1 and/or FDPS positive, in addition to MAF-negative solid tumor patients treated in bisphosphonates clinical studies were positive for GGPS1 and/or FDPS or not as emerging data implicate both GGPS1 and FDPS in oncogenesis[41,42,43]. Zoledronic acid acts by inhibiting these enzymes due to its analogue nature with naturally occurring pyrophosphate, and suppressing geranylgeranylation and farnesylation of the small GTPases (Fig. S8a). The GART is one of three targets of antifolates such as pemetrexed which are approved for ovarian and kidney cancer, the two other targets of pemetrexed include, being dihydrofolatereductase andthymidylate synthase[31]. Overexpression of GGPS1, FDPS and GART in breast cancer was also associated with a highly significant, overall reduction in the duration of overall survival of breast cancer patients as compared to patients without overexpression(Fig. 3g, left).As most of the relapses occur in the first five years in, it would be interesting to learn whether fitness genes are responsible for carcinogenesis, progression, or recurrence of cancer in future studies.Significance of overexpression of GGPS1, FDPS and GART in the pathophysiology of breast cancer is also evident by the fitness-dependency of breast cancer cells on these genes (Fig. 3h).

In addition to breast cancer including TNBC (Fig. 3, Supplementary Fig. 9), GGPS1, FDPS, and GART are also upregulated in multiple cancers, including hard-to-treat cancers such as esophageal, pancreatic, glioblastoma, lung and oral cancer (Fig. 2, Supplementary Figs. 4, 8-11).Significance of overexpression of GGPS1, FDPS and GART in the pathophysiology of other cancer-types is also evident by the fitness-dependency of multiple cancer cell-types, including hard-to-treat, such as esophageal, CNS, Head & Neck and ovarian cancers, on the presence of

GGPS1, FDPS and GART in additional to other genes (Fig. 4a, b).Interestingly, overexpression GGPS1, FDPS, GART or HMGCS1 was also associated with a highly significant, overall reduction in the duration of overall survival of patients with esophgeal and pancreatic, and GGPS1, GART and HMGCS1 in glioblastoma, and in ovarian and endometrial cancers but not in prostate cancer (Supplementary Fig. 10). In addition, we found that the levels of GGPS1, FDPS and GART are not or albeit upregulated in prostate cancer (Supplementary Fig. 11d) as well as did not exhibit any fitness-dependency of prostate cancer cells onGGPS1 while other two, FDPS and GART are without fitness value(Supplementary Fig. 10f). As Zoledronic acid is also used for prostate cancer bone metastases, this suggests that there might be some degree of cell-type specificity of fitness-dependency of the same set of genes between the breast and prostate cancer cells for reasons which remain poorly understood at present.

[Figure2]

FDPS and GGPS1 are downstream components of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (Supplementary Fig. 8a) - a rate-limiting enzyme and target of statins[44]. Interestingly, statin treatment of cancer cells lead to a compensatory upregulation of 3-hydroxy-3-methylglutaryl-CoA synthase 1(HMGCS1, placed just upstream of HMGCS1) which itself is widely upregulated in breast[41,42,43] as well as other cancer-types (Fig. 3;Supplementary Fig. 7 and Fig. 10). Although HMGSC1 is not a target of any FDA-approved oncology drug, itsô knockdown as well as of HMGCR in fitness screen wasaccompanied by a significant fitness-dependency of breast cancer cells and other cancer cell-types (Fig. 4a and 4b). We also observed that overexpression of HMGCS1 along with GGPS1, FDPS and GART in breast (Fig. 3g, right), and ovarian, endometrial, pancreatic and CNS cancers (Supplementary Fig. 10, 11) correlated with a significant reduction in overall survival of patients as compared to patients without overexpression.

Because a large body of prior data suggests that use of statins may be associated with a reduced incidence of breast as well as esophageal cancer etc.[45,46,47,48,49] and the fact that all four enzymes, i.e. GGPS1, FDPS, HGMCS1 and HGMCR, belong to the mevalonate pathway, these observations provide scientific reasoning for potentially combining bisphosphonates with statins (along with strategies to target HMGCS1) for cancer-types for which these drugs are not approved (Fig. 4c). However completed clinical trials does not appear to not involve pre-screening of the status and/or activity of targets of bisphosphonates (i.e., FDPS and GGPS1) or statins (HMGCR and HMGCS1) which is the premise of targeted therapy. It is possible that the combination of bisphosphonates and statins (and perhaps, with pemetrexed) will yield to a superior therapy response in a sub-set of cancers such as TNBC, ovarian cancer, pancreatic cancer and CNS cancer if such patients are stratified on the basis of expression of FDPS, GGPS1, HMGCS1, and GART in future clinical trials (Fig. 4c). Interestingly, Zoledronic acid has been shown to exhibit synergistic growth inhibitory activity with other aniticancer agents in cellular models[50], a recently completed breast cancer clinical trial aimed to repurpose zoledronic acid in a neoadjuvant setting suggests that zoledronic acid promotes anti-cancer activity of chemotherapy and anti-HER2 therapy[51]. Regarding potential toxicity, the scientific community has an immense experience about the safe use of bisphosphonates or statins over an extended period of time. In this context, we found that the relative expression of GGPS1, FDPS, GART and HMGCS1 are albeit in the body map atlas, different lineages, normal cells as compared to respective cancer-types and immune cell-types(Supplementary Fig. 12), and in human blood cells (Supplementary Fig. 13).

Outlook: In brief, in addition to repurposing bisphosphonates and statins, with or without pemetrexed for breast cancer (and other cancer-types), the present analysis provides a rationale for repurposing a range of approved oncology drugs in cancer-types for which such drugs are not approved but targets of such drugs are excellent fitness genes in these cancer-types. Data in

Supplementary Fig. 14 illustrates that there is a substantial increase in the number of fitness gene targets in cancer-types for which oncology drugs targeting these targets are not approved as compared to cancer-types for which such drugs are approved as shown by the number of lines connecting the target and cancer-types. This also opens an avenue for utilizing post-genomic data for the benefit of cancer patients by repurposing approved cancer therapeutics by integrating another layer of matrix involving fitness-dependency of the intended target, its cellular overexpression, and role in overall survival of patients with high versus low expression of fitness gene or genes in multi-variant analysis versus. As with any new finding, the present study provides new hypotheses to be tested in future using appropriate preclinical model systems, such as Cell Model Passports models used in the original CRISPER-fitness screen[17], and subsequently, novel cancer-specific clinical trial. For example, as certain targets of oncology drugs are also detected in extracellular fluids as secretory proteins (i.e. FDPS, GART and HMGCS1 etc.[52]), in addition to being fitness genes as well as overexpressed in tumors, it will be important to evaluate the potential relationship between the levels of such cancer therapeutic targets in serum/plasma and tumors in future studies, as such secretory fitness gene products could be potentially developed as surrogate biomarkers of assess the diseaseôs status as well as therapeutic responsiveness.

Materials and Methods

Datasets

U.S. Food and Drug Administration approved oncology drugs during the period of 1952-September 2019 were collected from the FDA site (https://www.accessdata.fda.gov). All drug targets of FDA approved oncology drugs were collected from DrugBank databases ((https://www.drugbank.ca/; version 5.1.4, accessed on 09/13/02019). Fitness score for the gene targets were collected from the Cancer Dependency Map dataset (https://score.depmap.sanger.ac.uk/gene).

U.S. Food and Drug Administration Approved Drugs

The Drugbank database mined for the targets of 235 oncology drugs included 185 small molecules, 5 enzymes and 45 biotechnology drugs. Among these targets, 230 are approved/re-approved after January 2000. Drug accession number, type of molecule, and weight of the molecule are collected and documented for each drug (Supplementary Table1).

Drug-Target Data

Drug associated with 232 targets were extracted from the Drug bank database with one to one and one to many elationships. Drug targets included DNA, enzymes, protein complexes and genes. Among the target 109 genes of oncology drugs, 100 genes were found to be also present in the quality-control passed list of 7460 genes in the Cancer Dependency Map dataset[19].

Cell-Fitness Data

One hundred genes targeted by oncology drugs were analyzed for the fitness dependency using Cancer Dependency Map database comprising of CRISPR-Cas9-mediated knockdown of 7460 genes in 324 cancer cell lines representing 19 cancer-types. Fitness effect for each of 47 targets of FDA approved drugs was collected in various cell lines, and categorized for cancer types and subtypes based on the cell line model information available with it.

Analysis and Plots

Genome alterations and gene expression analysis for selected genes in corresponding cancer datasets were performed using the cBioPortal[53,54] andXena Browser[55]. Alteration graphs and heatmap representations are directly exported from online analysis tools from above portals. Survival analyses were performed using the SurvExpress program[56]. Boxplots and heatmaprepresentations for fitness score data were created using the R program.

Genome Alterations and Gene Expression Analysis

Genome level alterations and gene expression changes for selected genes were analysed in cancer samples using the cBioPortal [53,54] and Xena Browser[55].

Survival Analysis

Survival plots for selected genes for corresponding datasets were performed using the SurvExpress tool[56].

Drug-Target-Cancer Relationship Diagram

Drug-Target-Cancer relationship was represented as Sankey chart diagram using SankeyMATICtool (http://sankeymatic.com/).

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Figures

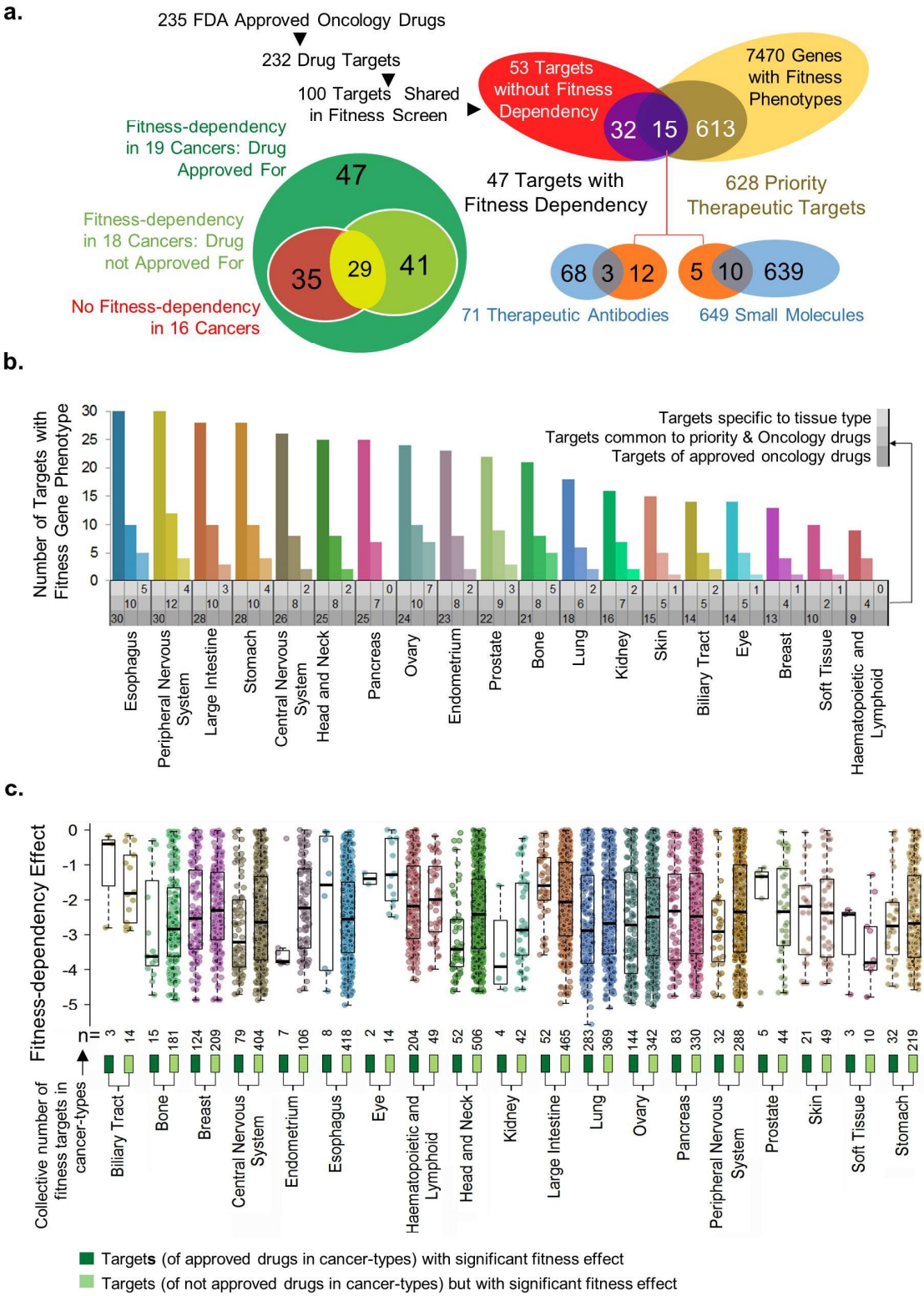

Figure 1

Figure 1: Oncology drug targets as good or poor cellular fitness genes. **a**, Strategy to examine fitness-dependency of cancer types for which oncology drugs targeting these targets were approved or not approved. **b**, Distribution of 41 cancer targets of FDA-approved drugs, a subset of its 14 targets shared with 628 priority therapeutic targets, and common targets between these two groups across cancer-types for which drugs targeting these cellular targets are not approved. Color distribution of bars for the cancer types are as per reference number 15. **c**, Distribution of significant fitness-dependency of 47 targets across 19 cancer types, for which drugs targeting these molecules are either approved (dark green boxes) or not approved (light green boxes). n, collective number of target fitness values among cancer cell lines in a given cancer-type; one dot per target per cell line.

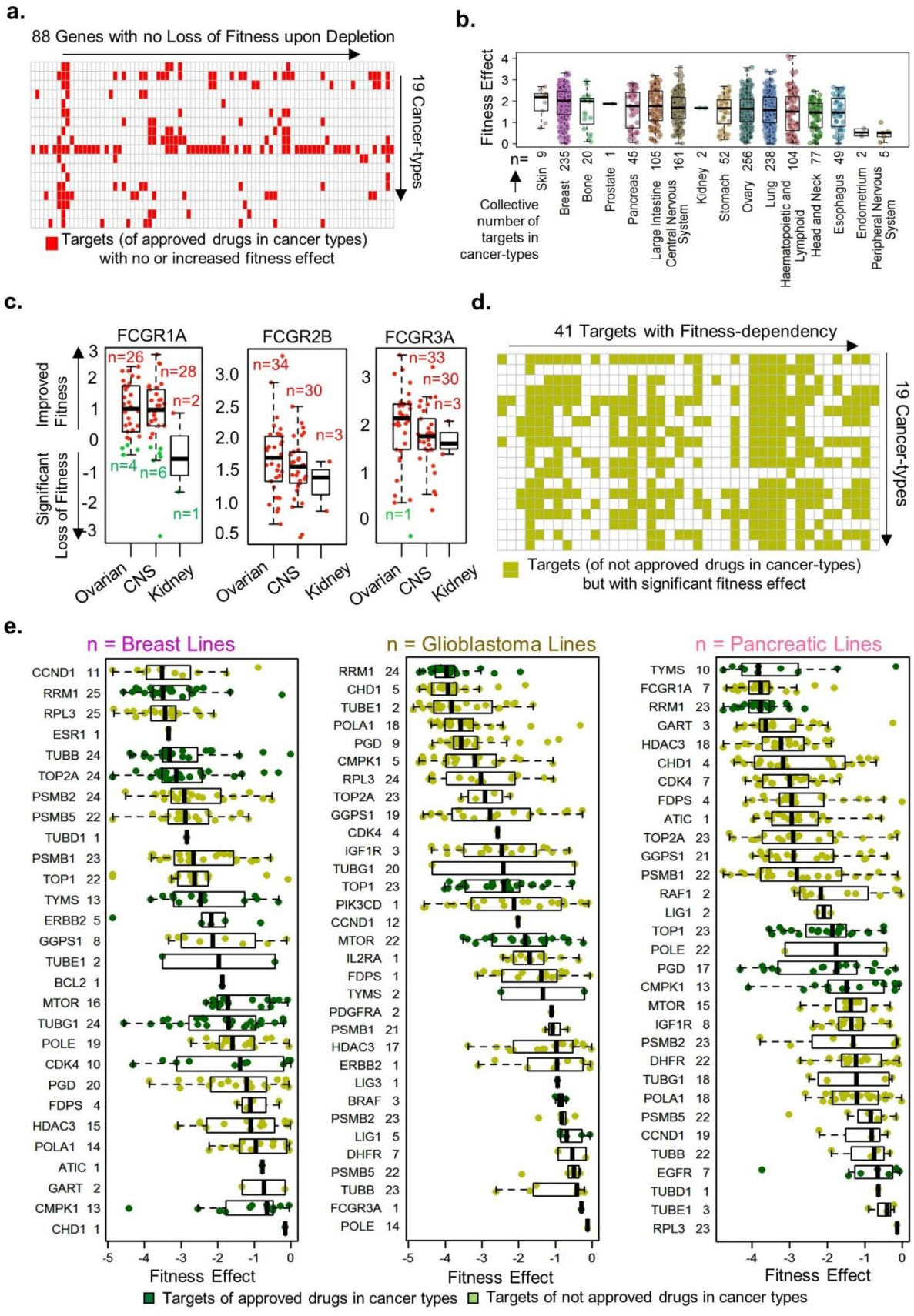

Figure 2

Figure 2: Revelation of fitness targets with differential effects on cellular fitness. a, Overall distribution of ~~47~~ 88 cancer targets with no loss of cellular fitness upon depletion across 19 cancer-types. **b**, Distribution of a positive fitness effect of depleting 47 targets across 19 cancer types, for which drugs targeting these molecules are either approved or not approved. n, collective number of target fitness values among cancer cell lines in a given cancer-type; one dot per target per cell line. **c**, Representative examples of three above fitness genes targeted by Bevacizumab in referred three cancer-types. **d**, Distribution of 41 cancer cell fitness targets with a significant loss of cellular fitness upon depletion across 19 cancer-types. **e**, Distribution of the loss of cellular fitness upon depletion of targets of approved (dark green) or not approved (light green) oncology drugs in breast cancer, pancreatic cancer, or glioblastoma.

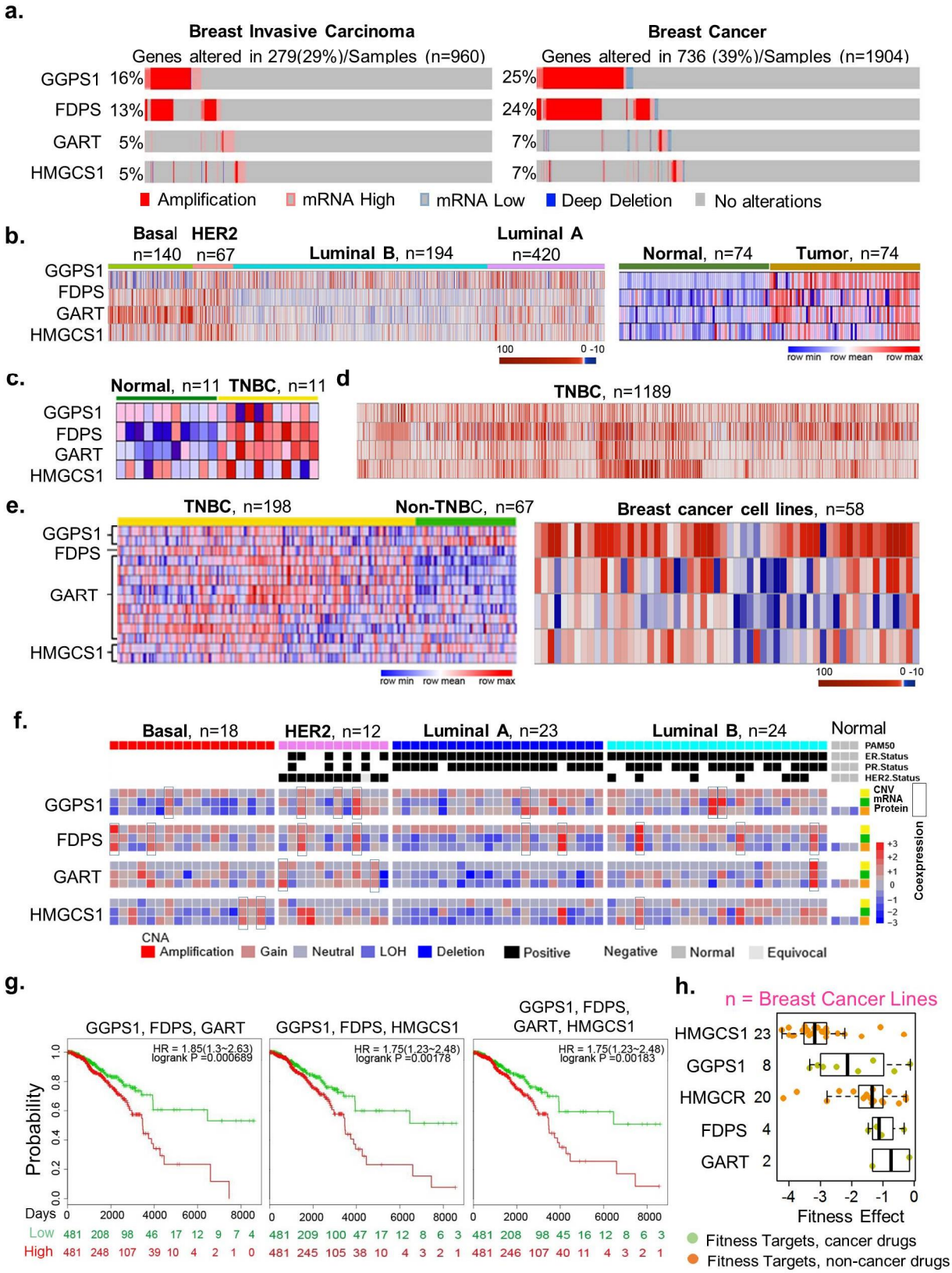

Figure 3

Figure 3: GGPS1, FDPS, HMGCS1 and GART are fitness-dependent targets in breast cancer. a, Amplification and expression of indicated molecules in breast tumors in TCGA (left) and Metaberic data [right, ref. 42] using CNV and gene alteration rate data from cBioPortal[53,54]. **b**, Expression of GGPS1, FDPS, GART and HMGCS1 mRNAs in breast cancer sub-types, in breast tumors and adjacent matched normal tissues using the data from cBioPortal [right panel] [53,54] and from Xena Browser [right panel][55]. **c-e**, Expression of indicated four mRNAs in TNBC and matched normal tissues fromXena Browser [right panel][55], in TNBC samples[57], in TNBC and non-TNBC breast tumors, and breast cancer cell lines[58] [Left lane;] Gene expression representation using heatmap in breast cancer cell lines - AU565, BT20, BT474, BT483, BT549, CAL120, CAL148, CAL51, CAL851, CAMA1, DU4475, EFM192A, EFM19, HCC1143, HCC1187, HCC1395, HCC1419, HCC1428, HCC1500, HCC1569, HCC1599, HCC1806, HCC1937, HCC1954, HCC202, HCC2157, HCC2218, HCC38, HCC70, HDQP1, HS274T, HS281T, HS343T, HS578T, HS606T, HS739T, HS742T, JIMT1, KPL1, MCF7, MDAMB134VI, MDAMB157, MDAMB175VII, MDAMB231, MDAMB361, MDAMB415, MDAMB436, MDAMB453, MDAMB468, SKBR3, T47D, UACC812, UACC893, YMB1, ZR751, ZR7530, EVSAT and HMC18 cells [right panel] using data from cBioPortal[53,54]. **f**, Proteogenomics expression status of indicated four targets in breast tumors. Yellow, CNV; Green, RNAseq; and Orange, protein[34]. **g**, SurvExpress[45] survival analysis of GGPS1, FDPS and GART, and GGPS1, FDPS, GART and HMGCS1 in patients with breast tumors. **h**, Status of cellular fitness of target cells upon knocking down GGPS1, FDPS, HMGCS1, or GART in breast cancer-types for which drugs targeting these molecules are not approved (light green), or by non-oncology drugs (orange).

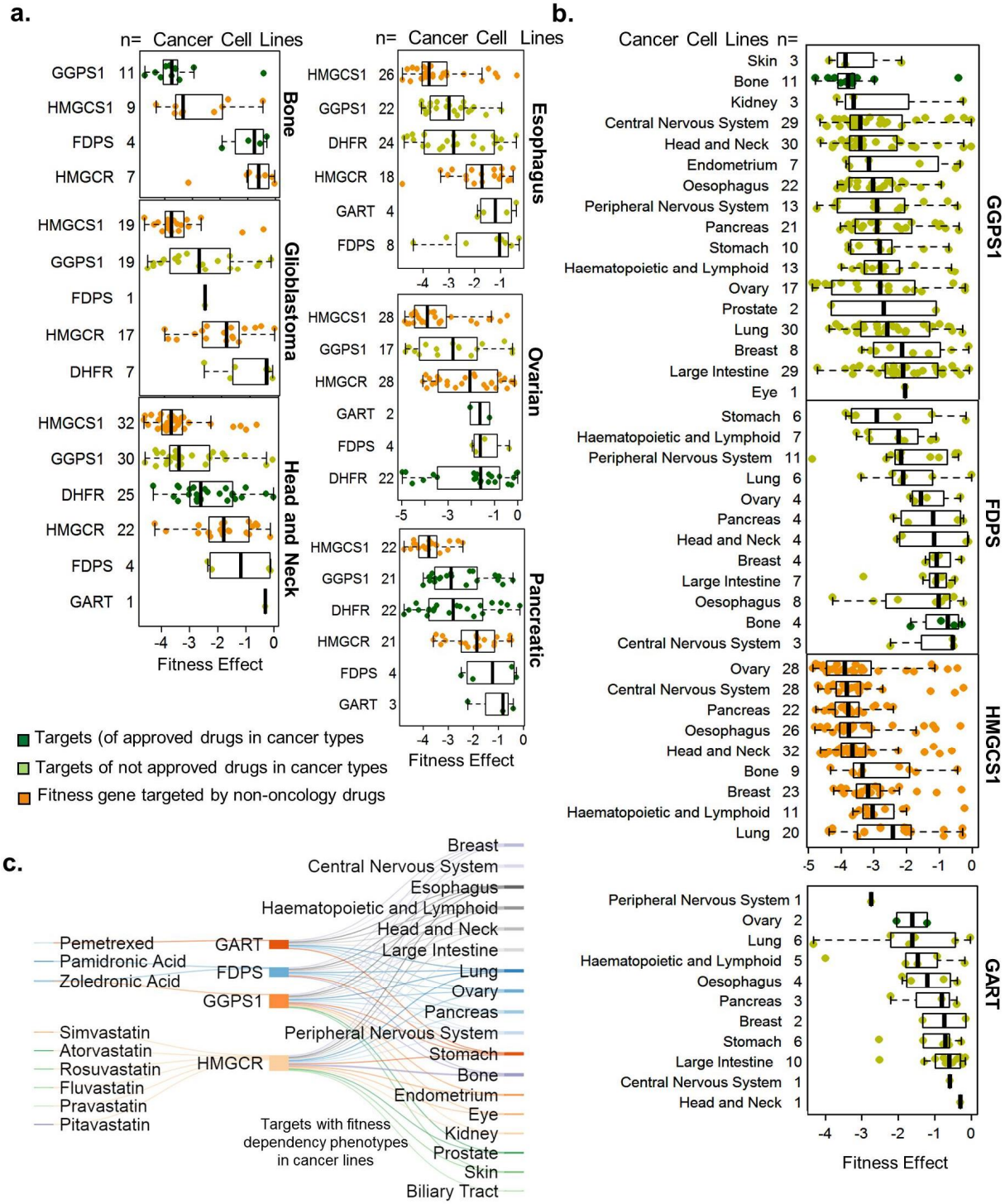

Figure 4

Figure 4: Fitness-dependency of four enzymes of the mevalonate/cholesterol pathway in cancer. **a**, Status of cellular fitness of representative cancer cell lines upon knocking down the indicated molecules, such as GGPS1, HMGCS1, FDPS, HMGCR or so on in cancer-types for which drugs targeting these molecules are approved (dark green dots), not approved (light green), or by non-oncology drugs (orange). **b**, Status of cellular fitness of cancer-types upon knocking down GGPS1, FDPS, HMGCS1, or GART in cancer-types for which drugs targeting these molecules are approved (dark green dots), not approved (light green), or by non-oncology drugs (orange). **c**, Relationship between four cellular targets and fitness-dependency of cancer-types for which indicated drugs targeting these molecules are not approved.