A comparative genomic approach for identifying synthetic lethal interactions in human cancer

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Abstract

Synthetic lethal interactions enable a novel approach for discovering specific genetic vulnerabilities in cancer cells that can be exploited for the development of therapeutics. Despite successes in model organisms such as yeast, discovering synthetic lethal interactions on a large scale in human cells remains a significant challenge. We describe a comparative genomic strategy for identifying cancer relevant synthetic lethal interactions whereby candidate interactions are prioritized based on genetic interaction data available in yeast, followed by targeted testing of candidate interactions in human cell lines. As proof of principle, we describe two novel synthetic lethal interactions in human cells discovered by this approach, one between the tumor suppressor gene *SMARCB1* and *PSMA4*, and another between alveolar soft-part sarcoma-associated *ASPSCR1* and *PSMC2*. These results suggest therapeutic targets for cancers harboring mutations in *SMARCB1* or *ASPSCR1*, and highlight the potential of a targeted, cross-species strategy for identifying synthetic lethal interactions relevant to human cancer.

Introduction

Synthetic lethality is an exciting new avenue to disrupt cancer cells for targeted treatment. Two genes are said to be synthetic lethal if mutations in both genes cause cell death but a mutation in either of them alone is not lethal. In applying synthetic lethality to the discovery of cancer drugs, the goal would be to identify a target gene that when mutated or chemically inhibited, kills cells that harbor a specific cancer-related alteration, but spares otherwise identical cells lacking the cancer-related alteration (1). This concept has recently been

exploited in the development of *PARP* inhibitors as novel chemotherapeutics for breast cancer. While *PARP* is not an essential gene in normal cells, *BRCA* mutant cells are dependent on *PARP* for their survival. The described efficacy of an oral *PARP* inhibitor, olaparib (AZD2281), in early phase clinical trials for treating *BRCA* mutant tumors, is a remarkable success story for translational cancer therapeutics (2). Importantly, strategies based on synthetic lethal interactions enable drug targeting of cancer-specific alterations in tumor suppressors which might otherwise be undruggable. Several recent studies have reported large-scale assays based on RNA-interference (RNAi) technology to discover synthetic lethal interactions with common cancer mutations, including *BRCA1/2* and *RAS* genes (3–9). These studies typically target cells with a well-defined genetic background using a library of short hairpin RNAs (shRNAs) to identify combinations that result in cell death or growth inhibition. While such approaches have the potential to rapidly discover genetic interactions at a full genome scale, a number of technological challenges remain to be solved, and the number of independently validated interactions produced by these efforts has been relatively limited to date (10).

One complementary strategy to whole-genome screens in cancer cell lines is motivated by the wealth of potentially relevant interaction data in model organisms. Publication of the first eukaryotic genome-scale genetic interaction map in yeast (*Saccharomyces cerevisiae*) (11), where approximately 30% of all possible gene pairs were tested for interactions, provides a unique opportunity for discovering potentially therapeutic synthetic lethal interactions. For example, putative synthetic lethal interactions in human could be inferred based on yeast synthetic lethal interactions between conserved genes in yeast and human. These predicted pairs of human genes provide a rich database of possible candidates for further study in the context of human disease. In fact, several interactions related to chromosome stability have already been mapped from yeast to worm to human (12), suggesting that such a strategy has the potential to yield promising new drug targets. In combination with the exponentially accumulating volume of data regarding the landscape of genomic alterations in human cancer, such an approach has the potential to become increasingly powerful going forward.

We describe a combined computational and experimental approach whereby yeast interactions between human orthologs are filtered by cancer association and interaction strength in yeast, and candidates from the prioritized list are then validated in human cell lines. Using this approach, we discovered two previously unknown synthetic sick interactions, one between *SMARCB1* (yeast *SNF5*) and *PSMA4* (yeast *PRE9*), and another between *ASPSCR1* (yeast *UBX4*) and *PSMC2* (yeast *RPT1*). The predicted synthetic sick/ lethal interactions between these genes were validated with shRNA double knock-down in multiple cell lines and single knock-down of *PSMA4* in two cancer cell lines containing endogenous *SMARCB1* mutations. These interactions suggest potentially new therapeutic targets for *SMARCB1* and *ASPSCR1* mutated cancers, and more broadly, illustrate the potential of this cross-species approach.

Materials and Methods

Cell culture and shRNAs

IMR90,293TN, A-204, G-401 and 293 cell lines were obtained from American Type Culture Collection (ATCC). IMR90, 293TN and 293 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), Penicillin, and Streptomycin. A-204 and G-401 cells were cultured in McCoy's 5A media containing 10% Fetal Bovine Serum (FBS), Penicillin, and Streptomycin. Bacterial stocks of control and validated gene-specific shRNA expressing vectors including *PSMA4* and *SMARCB1*

shRNAs were selected from the RNAi consortium database and purchased from Sigma-Aldrich (St Louis, MO).

Preparation of viral particles

Bacterial stocks of validated shRNAs clones were amplified and DNA extracted using the HiSpeed Plasmid purification kit (Qiagen, Valencia, CA). 293TN cells were then transfected with shRNA vector clones mixed with viral package vectors pMD2 and psPAX2 using Lipofectamine 2000 transfection reagent (Invitrogen). After 48 hours, culture media containing viral particles were mixed with polybrene and centrifuged at 10,000 rpm to precipitate and concentrate the viral particles.

RNAi mediated gene knockdown

IMR90 cells were seeded into 96-well plates and transduced with pre-determined pairs of shRNAs to generate four conditions with 6 replicates each: control shRNAs, control shRNA/*PSMA4*, control shRNA/*SMARCB1* and *PSMA4*/*SMARCB1*. A similar set-up of four conditions and replicates were used for *PSMC2* and *ASPSCR1* interaction. Cells from each treatment were cultured for 8 and 10 days, and the number of viable cells determined by the CellTiter-Glo Luminescence Cell Viability Assay (Promega, Madison, WI). This assay determines the number of viable cells in culture based on the amount of ATP produced by the living cells and is designed for use with multiwell plate formats and high-throughput screening (HTS) for cell proliferation and cytotoxicity assays. The addition of the assay reagent results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present, which is directly proportional to the number of living cells present in each well. The intensity of the luminescent signal was measured in relative luminescence units (RLU) using the Beckman Coulter DTX 880 multimode plate reader.

Immunoblotting

Cell lysates from 293, A-204, G-401 and IMR90 control cells, and from shRNA infected cells were extracted after 5 days incubation and quantified using Bio-Rad Protein Assay reagent. An equal amount of protein (50ug) was subjected to SDS-PAGE, transferred onto a PVDF membrane and blocked with non-fat milk. The membranes were then incubated in primary antibody overnight at 4°C and then with anti-mouse (1:6000) or anti-rabbit (1:1000) secondary antibody at room temperature for 1 hr. Primary antibodies rabbit anti-SNF5 (SMARCB1) and rabbit anti-TUG (ASPSCR1) were purchased from Cell Signaling whereas rabbit anti-PSMC2, 20S Proteosome -4 (PSMA4) and anti- -actin-HRP were obtained from Santa Cruz biotechnology. Protein expression was detected using enhanced chemoluminiscence (ECL) substrate (Pierce).

Estimation of the significance of genetic interactions in human shRNA experiments

Growth rate of a single or double shRNA knockdown relative to the empty shRNA vector control are calculated using

$$f_A = \frac{RLUCount_A}{RLUCount_{Control}}$$

Where f_A is relative growth rate for a single or double knock down experiment (A, B or AB), and $RLUCount_A$ is the intensity of the luminescent signal measured in relative luminescence units (RLU). Since f_A is a ratio of two quantities that has error associated with it, error for f_A is given by

$$\sigma_A = \sqrt{\left(\frac{\Delta RLUCount_A}{RLUCount_A}\right)^2 + \left(\frac{\Delta RLUCount_{Control}}{RLUCount_{Control}}\right)^2} * f_A$$

where $RLUCount_A$ is the standard deviation in the n (= 6 for our experiment) observations of $RLUCount_A$.

Expected double mutant fitness and error associated with it is given by

$$f'_{AB} = f_A * f_B \quad \sigma'_{AB} = \sqrt{\left(\frac{\sigma_A}{f_A}\right)^2 + \left(\frac{\sigma_B}{f_B}\right)^2} * f'_{AB}$$

In order to assess the significance of the interaction, we assumed normal distribution for f'_{AB} and f_{AB}, and compared f'_{AB} with f_{AB} using Welch t-test(13). p-value for f_{AB} is less than f'_{AB} can be calculated using one tailed t-test, which requires the t-test score t (13) and degree of freedom given by the Welch-Satterthwaite equation(14), as follows:

$$t = \frac{f'_{AB} - f_{AB}}{\sqrt{\frac{\sigma'_{AB}^2}{n'_{AB}} + \frac{\sigma_{AB}^2}{n_{AB}}}} \quad \nu = \frac{\left(\frac{\sigma'_{AB}^2}{n'_{AB}} + \frac{\sigma_{AB}^2}{n_{AB}}\right)^2}{\frac{\sigma'_{AB}^4}{n'_{AB}^2(n'_{AB}-1)} + \frac{\sigma_{AB}^4}{n_{AB}^2(n_{AB}-1)}}$$

Here $n_{AB} = n'_{AB} = 6$ because we have 6 replicate observations for control, single and double knock-down experiments.

Orthology mapping

InParanoid7 (15) was used to map yeast genes to human genes. Only 1:1 orthologs were used for our study (Supplementary Table S1).

Collection and processing of yeast genetic interaction data

Yeast genetic interaction data was taken from Costanzo *et al.* 2010 (11), which reported data for interactions between 1711 query genes and 3885 array genes. We applied a p-value cutoff < 0.05 on all interactions. Furthermore, we applied an interaction cutoff in two ways: first, we considered stringent negative genetic interactions (< −0.2), and second, we allowed intermediate interactions (< −0.08), which were reported in reciprocal screens. Specifically, in the Costanzo *et al.* network, query genes were screened against the entire non-essential deletion array, and in some cases, genes present on the array were also screened as queries. For these cases, an interaction between genes A and B was tested in both screens: A (query)×B (array) and B (query)× A (array). In such cases, we applied an intermediate cutoff because an interaction appearing in both of these screens is of high confidence.

11 new SGA screens were also used to generate candidate gene pairs, including screens for the following queries (human/yeast orthologs): *XPC/RAD4, VTI1A/VTI1, NOP56/NOP56, POLD2/POL31, MLH1/MLH1, XPO1/CRM1, UBA3/UBA3, ERCC4/RAD1, XPA/RAD14, PSMC2/RPT1, PSMB1/PRE7*. A screen involving a temperature sensitive (TS) allele of yeast RPT1 (human PSMC2) was the basis for testing the human interaction *PSMC2-ASPSCR1*, so the yeast interaction data supporting that inference are included here

(Supplementary Table 2). A genome-wide screen for the *RPT1* TS allele's genetic interactions was conducted as described in Baryshnikova et al, 2010 (16). Briefly, a rpt1-1 mutant strain marked with a nourseothricin (NatMX4) resistance cassette and harboring the SGA haploid specific markers and reporter (16) was mated to an array of ~4000 viable *S. cerevisiae* deletion mutants. Nourseothricin- and geneticin-resistant heterozygous diploid mutants were selected and sporulated and MATa rpt1-1 double mutants were subsequently selected (16). To confirm the SGA results, all gene deletions were constructed in a SSL204 MATa strain and crossed with an isogenic rpt1-1 MAT   strain. Diploid cells were sporulated at 25°C and dissected. Plates were incubated for 3–5 days at either 25°C or 30°C.

Yeast tetrad dissection

Confirmations by tetrad analyses were performed as described in Amberg *et al* 2006 (17).

Results

To discover cancer-associated genetic interactions in human cells, we first selected a set of highly significant interactions between yeast genes from the large network of synthetic genetic interactions that has recently been mapped in yeast. A recent study reported testing genetic interactions for 5.4 million yeast gene pairs, consisting of instances where two non-essential genes were deleted in combination, or a temperature-sensitive mutation of an essential gene was used along with a deletion of a non-essential gene (11). In total, approximately 116,000 pairs were reported as having a detectable synthetic sick or lethal interaction, of which around 24,000 interactions connect two genes that both have human orthologs (Fig. 1). More than 500 of these latter interactions involve at least one gene that has been previously associated with mutations in cancer (Sanger Institute Cancer Gene Census (18); Fig. 1B), suggesting a large number of candidate pairs can be generated by this approach (Supplementary Table S3).

To narrow the candidate list for testing in human cells, we first applied a very stringent cutoff on interactions in yeast, either requiring a high-magnitude effect, high-confidence interaction to be reported (< −0.2, p < 0.05) or selecting gene-pairs for which interactions were reproduced in two reciprocal screens (see Methods for details). Furthermore, we restricted our search to genes with one-to-one orthologs in human to increase the likelihood of functional conservation between yeast and human, and to avoid potentially buffering effects of paralog functional redundancy (19) (Supplementary Table S1). Applying these relatively stringent criteria, we obtained 1522 putative synthetic sick/lethal interactions between human orthologs of yeast genes, of which 70 interactions involved a gene that has been previously implicated in some form of human cancer (Fig. 1B and Supplementary Table S4). In addition to these published interactions, we applied the same criteria to 11 previously unpublished yeast screens involving human orthologs (see Methods and Supplementary Table S2). Candidate interaction pairs involving cancer-associated mutations (Sanger Institute Cancer Gene Census) were ranked based on the strength of the yeast interactions and were selected in order up to a maximum of 3 interactions per gene. In total, 21 pairs of genes representing mutations associated with a diverse set of cancers were selected for further experiments in human cell lines (Fig. 2).

The candidate synthetic sick or lethal pairs derived from the yeast genetic interaction network were screened in normal human IMR90 fibroblast cells using an RNAi approach. IMR90 cells were chosen because the cell line was established from the lungs of a 16-week female fetus and have the advantage of early passage and a low likelihood of accumulated genetic alterations. This stable genetic background allowed us to assess the validity of candidate interactions with the lowest possibility of unknown, confounding genetic alterations. We screened the selected 21 pairs of potential interactions using a CellTiter-Glo

luminescence viability assay. We found evidence for significant synthetic sick or lethal interactions for 6 of the 21 tested pairs (see Supplementary Fig. 1 for data, Fig. 2 for significant interactions). We focused further validation efforts on the strongest 2 of the 6 significant interactions: *SMARCB1*/*PSMA4* and *ASPSCR1*/*PSMC2* Panels 1 and 2 for Fig. 2B).

To further validate these two interactions, we first retested them in yeast cells by dissecting tetrads (Fig. 3, which indeed confirmed a strong synthetic sick effect between the pairs of yeast orthologs, *SNF5* / *PRE9* human *SMARCB1*/*PSMA4* and *UBX4* / *RPT1* (human *ASPSCR1*/*PSMC2*) (Fig. 3). In human cells, we repeated the same viability assay and additionally performed knock-downs with independent targeting shRNAs for both pairs of genes. After simultaneous depletion of the targeted gene pairs, the number of cells that survived was significantly reduced in all cases (Fig. 4 B, C, E, F). Importantly, the extent of survival was significantly lower than the expected survival of double knock-downs estimated from the single shRNA effects (Fig. 4B of *PSMA4*/*SMARCB1*; Welch t-test (13) score = 8.11 at Day 8, 8.90 at Day 10; Fig. 4E for ASPSCR1-PSMC2; Welch t-test score = 14.86 at Day 7 and 20.95 at Day 10; pval < 0.0001 in all cases). Expected double knock-down effects were calculated assuming a multiplicative null model, which has been widely used in the genetic interaction community (20) (see Methods for details). Similar results were observed when different shRNA clones for *PSMA4*/*SMARCB1* and *ASPSCR1*/ *PSMC2* knock-down were used (Fig. 4 C, F). We also confirmed the effectiveness of shRNA silencing of the targeted genes by conducting protein expression analyses using Western blots (Fig. 4A, D), which showed greatly reduced protein levels in the shRNA-infected cells.

The discovery of cancer related synthetic lethal interactions can directly impact therapeutic potential, as the synthetic lethal interactor of a cancer related gene can be targeted selectively to kill cancer cells. To test the clinical relevance of the *PSMA4* and *SMARCB1* interactions, we identified an epithelial muscle rhabdosarcoma cell line (A-204) and a renal rhabdoid sarcoma cell line (G-401), each harboring *SMARCB1* mutations, and used embryonic kidney HEK-293 cells expressing wild type *SMARCB1* as a control (Fig. 5). We observe that *PSMA4* knock-down almost completely kills the cell lines harboring *SMARCB1* mutations, and that this observation is exaggerated versus controls when following the cells to later time points (Day 7, Fig. 5A-D). We also demonstrated the complete absence of SMARCB1 protein in cell lines A-204 and G-401 by Western blotting (Fig. 5E). A-204 carries a TC deletion of codons 181 and 182 in exon 5 whereas G-401 harbors a homozygous deletion of exons 1–9 (21) In both cell lines harboring *SMARCB1* mutation, the decrease in growth is greater than expected by the multiplicative combination of the individual *SMARCB1* mutation and *PSMA4* knock-down effects as estimated from the control cell line (Fig. 5; p value < $2.5*10^{-6}$ for all days, all replicate and both cell lines).

Discussion

We describe an experimental pipeline where we prioritized synthetic genetic interactions from the global map of yeast interactions to test candidate synthetic sick/lethal pairs involving cancer-associated mutations in human cells. We propose this general approach, involving computational prioritization followed by experimental validation, as a complementary strategy to large-scale RNAi screens that are in progress by several other groups.

Based on the synthetic sick/lethal interaction we discovered between *SMARCB1* and *PSMA4*, we hypothesize that targeting *PSMA4* in therapeutic approaches could selectively inhibit the growth of cancer cells harboring *SMARCB1* mutations. Human *PSMA4* is a

proteasome subunit component expressed across numerous tissues. *PSMA4* mRNA levels are increased in lung tumors compared with normal lung tissues, and down-regulation of *PSMA4* expression in lung cancer cell lines decreases proteasome activity and induces apoptosis (22). Human *SMARCB1* is a core component of the BAF ATP-dependent chromatin-remodeling complex, known to play important roles in cell proliferation and differentiation, and inhibition of tumor formation. Deletions in *SMARCB1* are associated with epitheliod sarcomas (23), and are a known cause of rhabdoid tumor predisposition syndrome (RTPS), a highly malignant group of neoplasms that usually occur in early childhood (24, 25). No described direct protein interaction exists between *PSMA4* and *SMARCB1*. Although the clinical implications of this synthetic lethal interaction await further study, one potential application could be in the use of existing proteasome inhibitors such as bortezomib for the treatment of tumors harboring *SMARCB1* mutations. Interestingly, interactions between other SWI/SNF subunits and the proteasome were also observed in yeast (11), suggesting the possibility that perturbations in multiple combinations of subunits across these complexes could have the same effect. Whether interactions exist in human between other genes encoding the SWI/SNF complex and the proteasome remains to be determined, but this merits further study since mutations in other subunits of SWI/SNF have been observed in many other types of cancer (26, 27).

The direct clinical implications of the *ASPSCR1-PSMC2* synthetic sick interaction are less clear, but this case also merits further study. *ASPSCR1* is a relatively uncharacterized gene that has been associated with alveolar soft-part sarcoma (ASPS), a rare class of tumors that typically occur in younger patients. (28). Most cases of this cancer are associated with an unbalanced translocation der(17)t(X;17) (p11;q25) that results in an ASPSCR1-TFE3 fusion protein. The fusion protein appears to act as an aberrant transcription factor, inducing unregulated transcription of TFE3-regulated genes (28, 29). This fusion truncates one *ASPSCR1* allele, leaving the other allele intact in most cases (28, 29). How this genetic interaction could be leveraged for therapeutic purposes awaits further investigation, but one possibility is the potential combined effect of reduced expression of *ASPSCR1* in conjunction with proteasome inhibition.

Interestingly, the two strongest synthetic sick/lethal interactions we observed involved components of the proteasome, even though we tested a variety of genes from multiple pathways that were produced by our approach. These data suggest the proteasome may be a rich target for synthetic lethal approaches in human cancer therapy, and indeed, successful cancer treatment involving proteasome inhibitors has been reported recently in a number of different contexts (30). The availability of several approved proteasome inhibitors may make such interactions between cancer-associated mutations and the proteasome immediately translatable to several clinical settings. These results also highlight the potential for discovering interactions within core biologic pathways with strong yeast/human homology. Importantly however, one of the limitations of our approach is its dependence on genes and proteins with such homology, and an inability to reflect many known oncogenic pathways where yeast/human homology does not exist. We also note that a recent study identified both *PSMA4* and *PSMC2* as 2 of a set of 56 genes (and the only proteasomal components) for which gene knock-down inhibited the growth of cells with partial copy number loss in the same gene (31). Our independent finding of synthetic sick/lethal interactions for these same proteasomal subunits is intriguing and suggests that perturbations of these subunits have a relatively unique effect on proteasome function that may not be replicated by manipulation of its other components.

The appeal of a targeted approach for identifying synthetic sick/lethal interaction candidates is strengthened by the fact that there are currently large numbers of tumor genome sequencing efforts in progress which will produce new, potentially lengthy, lists of

mutations associated with various types of cancers. As we gain richer knowledge of the spectrum of mutations present in cancer, we can continue to directly screen the most promising candidate synthetic sick/lethal interactions involving these genes. The identification of specific cancer subtypes harboring specific mutations may provide therapeutic opportunities for synthetic lethal approaches that are not currently appreciated. Furthermore, in future studies, we intend to leverage data beyond sequence-similarity and literature-derived functional information to prioritize interactions for testing across species. For example, the large collections of functional genomic data in both yeast and human could allow for a more robust and unbiased assessment of the likelihood of functional conservation of genes and conserved synthetic lethal interactions between them. Our initial results highlight the feasibility of this comparative genomic approach, and suggest its potential utility for rapid translation of novel sequence variants into new therapeutic targets. We believe this approach has the potential to provide a dramatic increase in the number of therapeutic targets beyond those currently available for drug development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH. Integrating genetic approaches into the discovery of anticancer drugs. Science. 1997; 278(5340):1064–1068. Epub 1997/11/14. [PubMed: 9353181]

2. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet. 2010; 376(9737):235–244. Epub 2010/07/09. [PubMed: 20609467]

3. Scholl C, Frohling S, Dunn IF, Schinzel AC, Barbie DA, Kim SY, et al. Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. Cell. 2009; 137(5):821–834. Epub 2009/06/06. [PubMed: 19490892]

4. Luo J, Emanuele MJ, Li D, Creighton CJ, Schlabach MR, Westbrook TF, et al. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell. 2009; 137(5):835–848. Epub 2009/06/06. [PubMed: 19490893]

5. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature. 2009; 462(7269):108–112. Epub 2009/10/23. [PubMed: 19847166]

6. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005; 434(7035):913–917. Epub 2005/04/15. [PubMed: 15829966]

7. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. The New England journal of medicine. 2009; 361(2):123–134. Epub 2009/06/26. [PubMed: 19553641]

8. Marcotte R, Brown KR, Suarez F, Sayad A, Karamboulas K, Krzyzanowski PM, et al. Essential gene profiles in breast, pancreatic, and ovarian cancer cells. Cancer discovery. 2012; 2(2):172–189. Epub 2012/05/16. [PubMed: 22585861]

9. Cheung HW, Cowley GS, Weir BA, Boehm JS, Rusin S, Scott JA, et al. Systematic investigation of genetic vulnerabilities across cancer cell lines reveals lineage-specific dependencies in ovarian cancer. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(30):12372–12377. Epub 2011/07/13. [PubMed: 21746896]

10. Iorns E, Lord CJ, Turner N, Ashworth A. Utilizing RNA interference to enhance cancer drug discovery. Nature reviews Drug discovery. 2007; 6(7):556–568. Epub 2007/06/30.

11. Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, et al. The genetic landscape of a cell. Science. 2010; 327(5964):425–431. Epub 2010/01/23. [PubMed: 20093466]

12. McManus KJ, Barrett IJ, Nouhi Y, Hieter P. Specific synthetic lethal killing of RAD54B-deficient human colorectal cancer cells by FEN1 silencing. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106(9):3276–3281. Epub 2009/02/17. [PubMed: 19218431]

13. Welch BL. The generalisation of student's problems when several different population variances are involved. Biometrika. 1947; 34(1–2):28–35. Epub 1947/01/01. [PubMed: 20287819]

14. Satterthwaite FE. An approximate distribution of estimates of variance components. Biometrics. 1946; 2(6):110–114. Epub 1946/12/01. [PubMed: 20287815]

15. Ostlund G, Schmitt T, Forslund K, Kostler T, Messina DN, Roopra S, et al. InParanoid 7: new algorithms and tools for eukaryotic orthology analysis. Nucleic acids research. 2010; 38((Database issue)):D196–D203. Epub 2009/11/07. [PubMed: 19892828]

16. Baryshnikova A, Costanzo M, Dixon S, Vizeacoumar FJ, Myers CL, Andrews B, et al. Synthetic genetic array (SGA) analysis in Saccharomyces cerevisiae and Schizosaccharomyces pombe. Methods in enzymology. 2010; 470:145–179. Epub 2010/10/16. [PubMed: 20946810]

17. Amberg DC, Burke DJ, Strathern JN. Tetrad dissection. CSH protocols. 2006; 2006(1) Epub 2006/01/01.

18. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, et al. A census of human cancer genes. Nature reviews Cancer. 2004; 4(3):177–183. Epub 2004/03/03.

19. VanderSluis B, Bellay J, Musso G, Costanzo M, Papp B, Vizeacoumar FJ, et al. Genetic interactions reveal the evolutionary trajectories of duplicate genes. Mol Syst Biol. 2010; 6:429. Epub 2010/11/18. [PubMed: 21081923]

20. Mani R, St Onge RP, Hartman JLt, Giaever G, Roth FP. Defining genetic interaction. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(9):3461–3466. Epub 2008/02/29. [PubMed: 18305163]

21. DeCristofaro MF, Betz BL, Wang W, Weissman BE. Alteration of hSNF5/INI1/BAF47 detected in rhabdoid cell lines and primary rhabdomyosarcomas but not Wilms' tumors. Oncogene. 1999; 18(52):7559–7565. Epub 1999/12/22. [PubMed: 10602515]

22. Liu Y, Liu P, Wen W, James MA, Wang Y, Bailey-Wilson JE, et al. Haplotype and cell proliferation analyses of candidate lung cancer susceptibility genes on chromosome 15q24-25.1. Cancer research. 2009; 69(19):7844–7850. Epub 2009/10/01. [PubMed: 19789337]

23. Modena P, Lualdi E, Facchinetti F, Galli L, Teixeira MR, Pilotti S, et al. SMARCB1/INI1 tumor suppressor gene is frequently inactivated in epithelioid sarcomas. Cancer research. 2005; 65(10):4012–4019. Epub 2005/05/19. [PubMed: 15899790]

24. Schneppenheim R, Fruhwald MC, Gesk S, Hasselblatt M, Jeibmann A, Kordes U, et al. Germline nonsense mutation and somatic inactivation of SMARCA4/BRG1 in a family with rhabdoid tumor predisposition syndrome. American journal of human genetics. 2010; 86(2):279–284. Epub 2010/02/09. [PubMed: 20137775]

25. Lee RS, Stewart C, Carter SL, Ambrogio L, Cibulskis K, Sougnez C, et al. A remarkably simple genome underlies highly malignant pediatric rhabdoid cancers. The Journal of clinical investigation. 2012; 122(8):2983–2988. Epub 2012/07/17. [PubMed: 22797305]

26. Weissman B, Knudsen KE. Hijacking the chromatin remodeling machinery: impact of SWI/SNF perturbations in cancer. Cancer research. 2009; 69(21):8223–8230. Epub 2009/10/22. [PubMed: 19843852]

27. Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. Oncogene. 2009; 28(14): 1653–1668. Epub 2009/02/24. [PubMed: 19234488]

28. Folpe AL, Deyrup AT. Alveolar soft-part sarcoma: a review and update. Journal of clinical pathology. 2006; 59(11):1127–1132. Epub 2006/10/31. [PubMed: 17071801]

29. Ladanyi M, Lui MY, Antonescu CR, Krause-Boehm A, Meindl A, Argani P, et al. The deR17)t(X; 17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. Oncogene. 2001; 20(1):48–57. Epub 2001/03/13. [PubMed: 11244503]

30. Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer research. 2001; 61(7):3071–3076. Epub 2001/04/18. [PubMed: 11306489]

31. Nijhawan D, Zack TI, Ren Y, Strickland MR, Lamothe R, Schumacher SE, et al. Cancer vulnerabilities unveiled by genomic loss. Cell. 2012; 150(4):842–854. Epub 2012/08/21. [PubMed: 22901813]
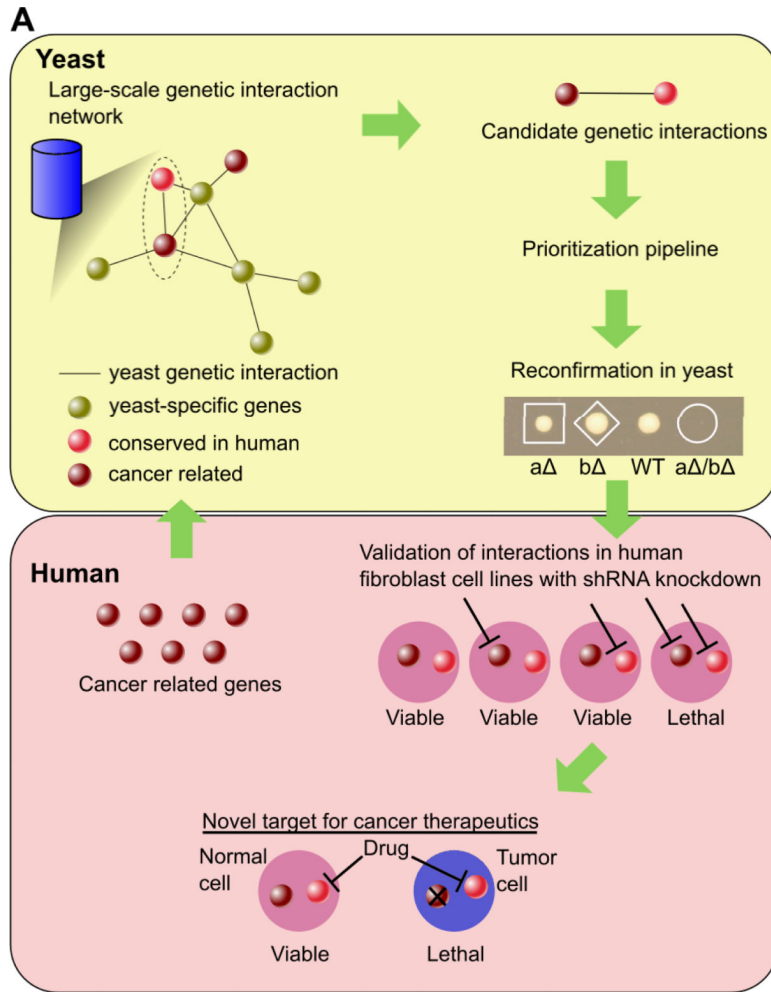
B

	Complete set		Filtered set	
	Interactions	Genes	Interactions	Genes
Total synthetic sick/lethals in *S. cerevisiae*	116,339	4,391	21,948	3,385
Synthetic sick/lethals *H. sapiens* orthologs	24,205	1666	1,522	633
Cancer-related synthetic sick/lethals	599	431	70	70

Figure 1.
Comparative genomic approach for discovering cancer related synthetic sick/lethal interactions in human. (A) Flowchart describing steps to use the wealth of synthetic sick/lethal interactions available in yeast and knowledge of genes commonly mutated in cancer (Sanger Institute Cancer Gene Census) for discovery of novel cancer drug targets in human. (B) Summary of yeast synthetic sick/lethal interaction network statistics and mapping of interactions between human orthologs. The "Complete set" contains all significant synthetic sick or lethal interaction pairs at an intermediate confidence cutoff as described in (11) (< −0.08; p-value < 0.05), and human totals include any genes with human orthologs. The "Filtered set" contains only high confidence interactions (< −0.2; p-value < 0.05) or

interactions replicated in two independent experiments, and human totals include only gene pairs with one-to-one orthologs (see Methods – Processing yeast genetic interaction data).
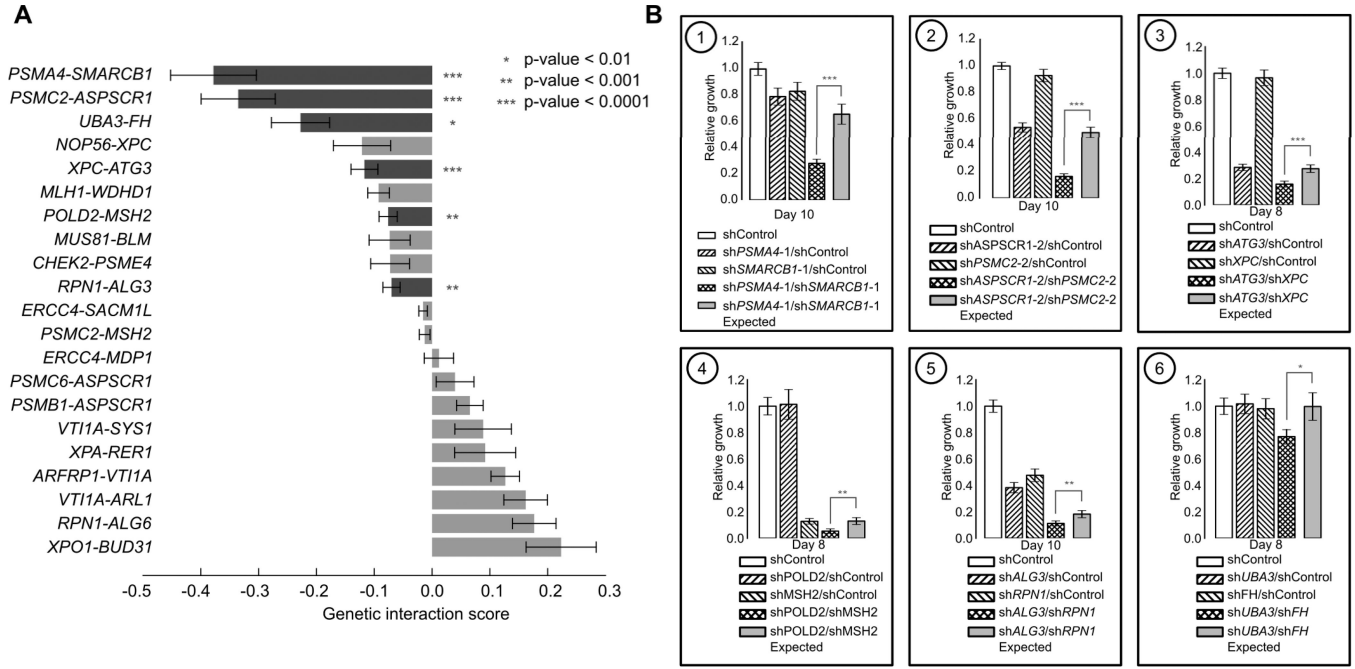
Figure 2.
Interaction testing of 21 selected candidate synthetic sick/lethal interactions in human fibroblast cell lines. (A) The interaction scores for all human interactions tested. The interaction score is the difference between observed and expected growth rate based on a multiplicative model. The significant negative genetic interactions are colored red and strength of the significance is denoted by the number of asterisks, according to the legend shown. (B) Results for each significant interaction tested. The number of days the fibroblast cells were grown in the presence of shRNAs is indicated in each plot. The error bars for both (A) and (B) represent twice the width of the standard error in the interaction scores and growth rates.
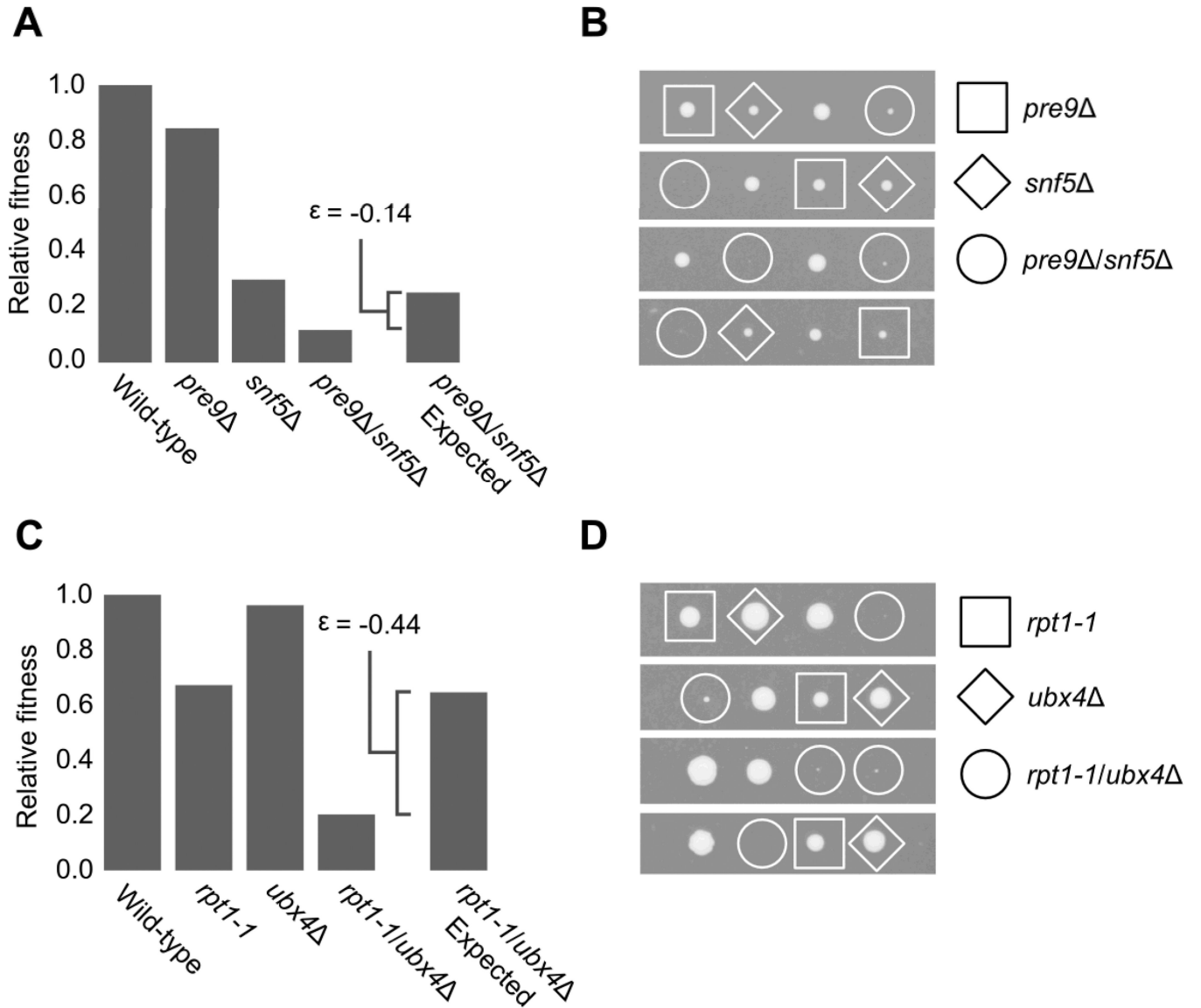
Figure 3.
Yeast data for the candidate synthetic sick/lethal interaction between *SNF5-PRE9* (human *SMARCB1- PSMA4*) and *UBX4-RPT1* (human *ASPSCR1-PSMC2*). (A) Fitnesses of the single and double mutants relative to wild-type for the *SNF5-PRE9* interaction. The interaction score ( ) was estimated by comparing the observed double mutant fitness with the fitness expected based on the single mutant fitnesses. (B) Confirmation of the synthetic sick/lethal interaction using tetrad dissection analysis for the *SNF5-PRE9* double mutant. Each tetrad is oriented horizontally and represents four meiotic progeny of a heterozygous double mutant between *pre9* ::natMX4/*PRE9* and *snf5* ::kanMX4/*SNF5*. Four representative tetrads are shown. The genes knocked-out are identified by the presence of the natMX and kanMX markers, respectively. The identified double knock-out spore colonies are enclosed in circles while single gene knock-out strains are enclosed in squares or diamonds, and wild type strains are not enclosed. (C) and (D) present similar data for query mutant *rpt1-1*, a temperature-sensitive conditional mutant of *RPT1*, and yeast *ubx4*
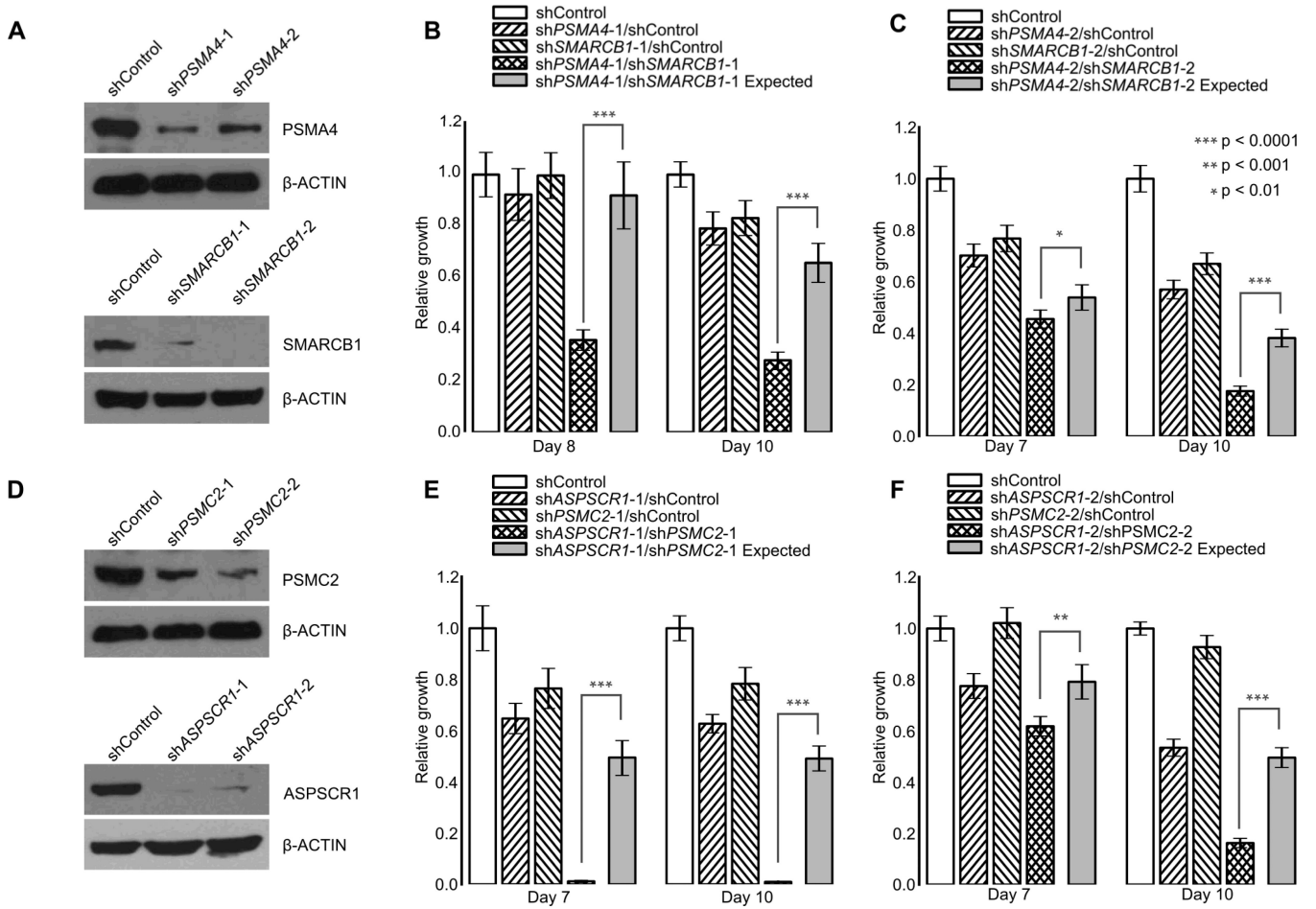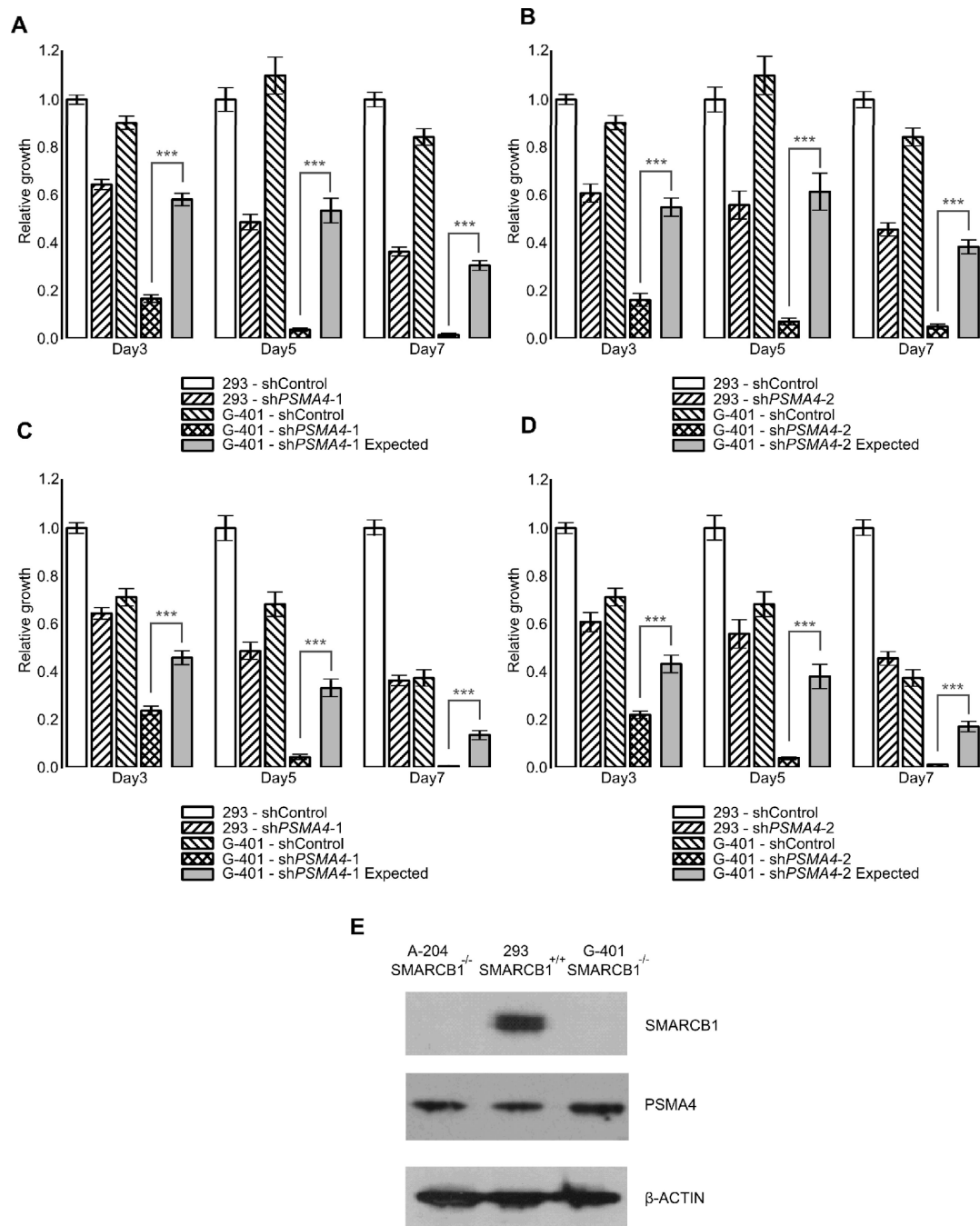
Figure 4.
Validation of two candidate synthetic sick/lethal interactions in human fibroblast cell lines. (A) A Western blot of IMR90 cells transduced with *PSMA4* and *SMARCB1* shRNA virus showing down-regulation of respective protein expression. (B and C) Cell viability analyses of *PSMA4* and *SMARCB1* interaction using two different clones which showed decreased cell survival in cells depleted of both *PSMA4* and *SMARCB1* compared to cells expressing shRNAs of individual genes and compared to the expected effect from depletion of both genes. (D) Immunoblotting showing knockdown of PSMC2 and ASPSCR1 expression in IMR90 cells treated with viral particles encoding *PSMC2* or *ASPSCR1* shRNA. (E,F) Synthetic lethal interaction effect of *PSMC2* and *ASPSCR1* in IMR90 cells as shown by significantly decreased survival in cells expressing shRNAs of both genes compared to the expected effect from depletion of both genes.

Figure 5.
Validation of the PSMA4-SMARCB1 synthetic lethal interaction in cancer cell lines harboring SMARCB1 loss-of-function mutations. (A) Cell viability analyses of cell lines with (A-204) or without (293) endogenous SMARCB1 mutation, grown with or without *PSMA4* shRNA knock-down (shPSMA4-1), demonstrate the therapeutic potential for this cancer associated synthetic lethal interaction. (B) The experiment in (A) is repeated with a different *PSMA* shRNA construct (shPSMA4-2). (C,D) The experiment in A,B is repeated with a different *SMARCB1* deficient cell line, G-401. (E) A Western blot showing the complete absence of SMARCB1 protein in cell lines, A-204 and G-401, which have endogenous null *SMARCB1* mutations, and normal expression of SMARCB1 in the control

293 cell line. The endogenous PSMA4 and -actin protein levels detected serve as loading controls.