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A data integration workflow to identify drug combinations targeting synthetic lethal interactions

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TITLE:

A Data Integration Workflow to Identify Drug Combinations Targeting Synthetic Lethal Interactions

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KEYWORDS:

synthetic lethality, genetic screens, data integration, homology mapping, drug combinations, breast cancer, treatment, cell viability assay

SUMMARY:

Large genetic screens in model organisms have led to the identification of negative genetic interactions. Here, we describe a data integration workflow using data from genetic screens in model organisms to delineate drug combinations targeting synthetic lethal interactions in cancer.

ABSTRACT:

A synthetic lethal interaction between two genes is given when knock-out of either one of the two genes does not affect cell viability but knock-out of both synthetic lethal interactors leads to loss of cell viability or cell death. The best studied synthetic lethal interaction is between BRCA1/2 and PARP1, with PARP1 inhibitors being used in clinical practice to treat patients with BRCA1/2 mutated tumors. Large genetic screens in model organisms but also in haploid human cell lines have led to the identification of numerous additional synthetic lethal interaction pairs,

all being potential targets of interest in the development of novel tumor therapies. One approach is to therapeutically target genes with a synthetic lethal interactor that is mutated or significantly downregulated in the tumor of interest. A second approach is to formulate drug combinations addressing synthetic lethal interactions. In this article, we outline a data integration workflow to evaluate and identify drug combinations targeting synthetic lethal interactions. We make use of available datasets on synthetic lethal interaction pairs, homology mapping resources, drug-target links from dedicated databases, as well as information on drugs being investigated in clinical trials in the disease area of interest. We further highlight key findings of two recent studies of our group on drug combination assessment in the context of ovarian and breast cancer.

INTRODUCTION:

Synthetic lethality defines an association of two genes, where loss of one gene does not affect viability, but loss of both genes leads to cell death. It was first described in 1946 by Dobzhansky while analyzing various phenotypes of drosophila by breeding homozygous mutants[1]. Mutants that did not produce viable offspring, although viable themselves, exhibited lethal phenotypes when crossed with certain other mutants, setting ground for the establishment of the theory of synthetic lethality. Hartwell and colleagues suggested that this concept might be applicable for cancer therapy in humans[2]. Pharmacologically provoked synthetic lethality could rely on just one mutation, given that the mutated gene's synthetic lethal partner is targetable by a pharmacological compound. The first gene pair to enable pharmacological induction of synthetic lethality was BRCA(1/2) and PARP1. PARP1 functions as a sensor for DNA damage, and is tied to sites of double and single DNA strand-breaks, supercoils and crossovers[3]. BRCA1 and 2 play major roles in repair of DNA double-strand breaks through homologous recombination[4]. Farmer and colleagues published findings that cells deficient for BRCA1/2 were susceptible to PARP inhibition, while no cytotoxicity was observed in BRCA wild-type cells[5]. Ultimately, PARP inhibitors were approved for the treatment of BRCA deficient breast and ovarian cancer[6,7]. Further, synthetic lethality gene pairs leading to clinical approval of pharmacological compounds are much anticipated and a major area of recent cancer research efforts[8].

Synthetic lethal gene interactions were modelled in multiple organisms including fruit flies, C. elegans and yeast[2]. Using various approaches including RNA-interference- and CRISPR/CAS-library knockouts, novel synthetic lethal gene pairs were discovered in recent years[9–11]. A protocol on the experimental procedures of RNAi in combination with CRISPR/CAS was recently published by Housden and colleagues[12]. Meanwhile, researchers also conducted large screens in haploid human cells to identify synthetic lethal interactions[13,14]. In silico methods like biological network analysis and machine learning have also shown promise in the discovery of synthetic lethal interactions[15,16].

Conceptionally, one approach to make use of synthetic lethal interactions in the context of anti-tumor therapy is to identify mutated or non-functional proteins in tumor cells, making their synthetic lethal interaction partners promising drug targets for therapeutic intervention. Due to the heterogeneity of most tumor types, researchers have started the search for so-called synthetic lethal hub proteins. These synthetic lethal hubs have a number of synthetic lethal interaction partners that are either mutated and therefore non-functional or significantly downregulated in tumor samples. Addressing such synthetic lethal hubs holds promise in increasing drug efficacy or overcoming drug resistance as could be shown for instance in the context of vincristine resistant neuroblastoma[17]. A second approach to enhance drug treatment making use of the concept of synthetic lethal interactions is to identify drug combinations targeting synthetic lethal interactions. This could lead to new combinations of already approved single anti-tumor therapies and to the repositioning of drugs from other disease areas to the field of oncology.

In this article, we present a step-by-step procedure to yield a list of drug combinations that target synthetic lethal interaction pairs. In this workflow, we (i) use data on synthetic lethal interactions from BioGRID and (ii) information on homologous genes from Ensembl, (iii) retrieve drug-target pairs from DrugBank, (iv) build disease-drug associations from ClinicalTrials.gov, and (v) hence generate a set of drug combinations addressing synthetic lethal interactions. Lastly, we provide drug combinations in the context of ovarian and breast cancer in the representative results section.

PROTOCOL:

1. Retrieving synthetic lethal gene pairs

1.1. Data retrieval from BioGrid.

1.1.1. Download the latest BioGRID interaction file in tab2 format from https://downloads.thebiogrid.org/Download/BioGRID/Latest-Release/BIOGRID-ALL-LATEST.tab2.zip either using a web browser or directly from the Linux command line using curl or wget[18].

```
##download and unpack the latest BioGRID interaction file
#download latest BioGRID interaction file using curl
curl -o biogrid_latest.zip https://downloads.thebiogrid.org/Download/BioGRID/Latest-Release/BIOGRID-ALL-LATEST.tab2.zip
```

```
#unpack the downloaded data file
unzip biogrid_latest.zip
BG="BIOGRID-ALL-3.5.171.tab2.txt"
```

1.1.2. After the zip archive has been downloaded, unpack archive must and note the name of the actual dataset file (BIOGRID-ALL-X.X.X.tab2.txt) for subsequent steps. The BioGRID datafile holds interactions of different types that will be filtered in the next step.

NOTE: Other sources (e.g. DRYGIN, SynlethDB) holding synthetic lethal interactions exist, as outlined in the discussion.

1.2. Filter for synthetic lethality and negative genetic interactions (Experimental System).

1.2.1. Use information in the column “Experimental System” (column number 12) that indicates the nature of supporting evidence for an interaction to identify synthetic lethal interactions.

1.2.2. Restrict the dataset to entries with a value of either Negative Genetic or Synthetic Lethality. In the same step, filter columns and only retain columns relevant for subsequent analysis steps as listed in table 1 below.

```
##restrict the BioGRID interaction file to relevant columns and only retain interactions classified as negative genetic and synthetic lethality
cut -d "^I" -f 1,8,9,12,16,17 "${BG}" \
 | awk -F "\t" 'BEGIN{
 OFS="\t"
 }
 {
 if(NR == 1){
 print $0
 }else if($4 == "Negative Genetic" || $4 == "Synthetic Lethality"){
 print $0
 }
 }' > bg_synlet.txt
```

NOTE: In the code snippets ^I is used to represent horizontal tabs. Additional BioGRID categories such as synthetic growth defect may be included. Other columns of relevance for this workflow are listed in **Table 1**. BioGRID also retains the scores for individual interactions. Cutoffs may be used to identify strong/high confidence interactions.

[insert Table 1 here]

1.3. Identify species for which synthetic lethal interactions were reported.

1.3.1. Determine the number of synthetic lethal interaction partner tax-IDs to get an estimate on the number of synthetic lethal interactions being available per organism.

```
##count the number of appearances of each tax id in the previously extracted synthetic lethal interactions
cut -d "^I" -f5,6 bg_synlet.txt | tail -n +2 | tr "\t" "\n" \
 | sort | uniq -c | sort -r -g
```

NOTE: As a result of step 1, a list of synthetic lethal interactions with gene symbols from organisms in which the interactions were determined. The majority of synthetic lethal interactions have been determined in model organisms. When loading files into a spreadsheet program (e.g., Excel) avoid ruining Gene Symbols[19,20].

2. Translating synthetic lethal gene pairs to human orthologs

2.1. Retrieve human orthologs for relevant model organisms identified in step 1.3.

2.1.1. Retrieve human orthologs from Ensembl BioMart[21] by linking the respective model organism gene dataset with the human gene dataset. Use the gene symbols denoting the gene in the model organism and orthologous human genes for this task. Use the Ensembl BioMart webservice to automatize the retrieval process and send the query directly to BioMart RESTful access for retrieving the orthologous gene pairs (see example below and Ensembl BioMart Help & Documentation for further details).

```
##retrieve human orthologous for Saccharomyces Cerevisiae from Ensembl BioMart by using curl to send the BioMart query directly to the BioMart RESTful access service
curl -o s_cerevisiae.txt --data-urlencode 'query=<?xml version="1.0" encoding="UTF-8"?>
<!DOCTYPE Query>
<Query virtualSchemaName = "default" formatter = "TSV" header = "0" uniqueRows = "1" count = "" datasetConfigVersion = "0.6" >

 <Dataset name = "scerevisiae_gene_ensembl" interface = "default" >
 <Attribute name = "external_gene_name" />
 </Dataset>

 <Dataset name = "hsapiens_gene_ensembl" interface = "default" >
 <Attribute name = "external_gene_name" />
 </Dataset>
</Query>
' "http://www.ensembl.org/biomart/martservice"
```

In order to retrieve the orthologous human genes for other model organisms, replace the value of the name attribute of the first Dataset element with the name of the respective Ensembl dataset and re-execute the query.

NOTE: The process of ortholog mapping is well-documented in Ensembl BioMart Help & Documentation (http://www.ensembl.org/info/data/biomart/biomart_combining_species_datasets.html).

2.1.2. Access an example BioMart query for human orthologs for Saccharomyces cerevisiae, the top species identified in step 1.3, via the URL http://www.ensembl.org/biomart/martview/9b71da1415aba480a52b8dc7dd554d63?VIRTUALSCHEMANAME=default&ATTRIBUTES=scerevisiae_gene_ensembl.default.feature_page.external_gene_name|hsapiens_gene_ensembl.default.feature_page.external_gene_name&FILTERS=&VISIBLEPANEL=linkattributepanel.

NOTE: Other sources (e.g. roundup, oma browser, HomoloGene, inparanoid) for homology mapping exist, as outlined in the discussion section of this manuscript.

2.2. Add human orthologs to extracted synthetic lethal interactions.

2.2.1. Join synthetic lethal interactions based on organism tax-ID and gene symbol with the orthologous pairs retrieved in step 2.1. For human synthetic lethal interaction pairs either create artificial orthologous pairs for each human gene present in the dataset or make sure that human synthetic lethal interactions are not discarded while joining and transfer the human gene symbols into the newly added columns.

```
##collect ortholog mappings in a single file and join with synthetic lethal interaction file
#create a target file with headers for collecting ortholog mappings
echo "tax_id/gene_symbol^Ihuman_gene_symbol" > mapping.txt

#repeat this step for each model organism, take care to adapt input file name and tax-ID
#adds for each ortholog pair in s_cerevisiae.txt a new entry in mapping.txt: The Gene Symbol is prefixed with the tax id to ease subsequent joining with the synthetic lethal interactions file
awk -F "\t" 'BEGIN{
 OFS="\t"
 org_tax_id="559292"
 }
```

```
 {
 if($1 != "" && $2 != ""){
 print org_tax_id"/"$1, $2
 }
 }' s_cerevisiae.txt >> mapping.txt

#create artificial mapping entries for human genes
awk -F "\t" 'BEGIN{
 OFS="\t"
 human_tax_id="9606"
 }
 {
 if($5 == human_tax_id){
 print $5"/"$2, $2
 }
 if($6 == human_tax_id){
 print $6"/"$3, $3
 }
 }' bg_synlet.txt | sort -u >> mapping.txt

#add required join keys (tax id/Gene Symbol) to synthetic lethal interactions
awk -F "\t" 'BEGIN{
 OFS="\t"
 }
 {
 if(NR == 1){
 print $0, "Key Interactor A", "Key Interactor B"
 }else{
 print $0, $5"/"$2, $6"/"$3
 }
 }' bg_synlet.txt > tmp_bg_synlet_w_keys.txt

#join synthetical lethal interactions with orthologous pairs
merge tmp_bg_synlet_w_keys.txt mapping.txt 7 1 > tmp.txt
merge tmp.txt mapping.txt 8 1 > bg_synlet_mapped.txt
```

NOTE: The merge command used in this example is not a standard Unix command. However, its implementation with the help of the GNU Core Utilities sort and join is straightforward. The command has been introduced to hide the complexity of sorting the files before they can be joined with the command join. An implementation of merge can be found at https://github.com/aheinzel/merge-sh.

2.2.2. Use of any gene identifier uniquely identifying the gene in a certain namespace for best possible results.

NOTE: Step 2 results in a list of synthetic lethal interactions from multiple organisms mapped to human genes.

3. Mapping synthetic lethal interaction partners to drugs

3.1. Retrieve drug-target pairs from DrugBank.

3.1.1. Download DrugBank data from the downloads section of DrugBank and create an account first if not already created[22]. Use the CSV file with drug target identifiers (protein identifiers section: https://www.drugbank.ca/releases/latest#protein-identifiers) and the DrugBank vocabulary (open data section: https://www.drugbank.ca/releases/latest#open-data) with DrugBank identifiers and names. Alternatively, extract the required information from the XML database dump.

```
##restrict the DrugBank drug target file to relevant columns and only retain entries
for human molecular entities
DB_TARGETS="all.csv"
DB_NAMES="drugbank vocabulary.csv"

#extract relevant columns and reformat to use tab as column seperator
csvtool col 3,12,13 -u TAB "${DB_TARGETS}" > target_to_drugs_agg.txt

awk -F "\t" 'BEGIN{
 OFS="\t"
 }
 {
 if(NR == 1 || $2 == "Humans"){
 print $1, $3
 }
 }' target_to_drugs_agg.txt > human_target_to_drugs_agg.txt
```

NOTE: DrugBank data is provided in two main formats. The complete database is available as XML file. In addition, the majority of data is made available in a series of comma-separated value (CSV) files.

3.1.2. Be aware that DrugBank also records non-human drug-targets. The species column (column number 12) can be used to extract human drug-targets.

NOTE: For better readability names of the extracted columns are provided in **Table 2**. Other sources (e.g. the Therapeutic Target Database or Chembl) holding drug-target links exist, as outlined in the discussion section.

[Insert Table 2 here]

3.2. Add drug names to drug-targets.

3.2.1. Since drug name and drug-target information is provided in two separate CSV files, merge the information from the two files to subsequently add names of drugs targeting a synthetic lethal interaction partner to synthetic lethal interactions. Join the two datasets using the common DrugBank-drug-ID column. Normalize the drug-target dataset first that it only contains a single DrugBank-drug-ID per row, as the initial file may hold multiple DrugBank drug IDs in a row if a protein is targeted by multiple drugs.

```
##generate a single file holding drug target gene symbol, DrugBank drug ID and drug
name
#normalize drug-target dataset
awk -F "\t" 'BEGIN{
 OFS="\t"
 }
 {
 if(NR == 1){
 print $0
 }else if($1 != "" && $2 != ""){
 split($2, drug_targets, ";")
 for(i in drug_targets){
 drug_target = drug_targets[i]
 gsub(/ /, "", drug_target)
 print $1, drug_target | "sort -u"
 }
 }
 }' human_target_to_drugs_agg.txt > human_target_to_drug.txt

#extract relevant columns and reformat to use tab as column separator
csvtool col 1,3 -u TAB "${DB_NAMES}" > drugbank_id_to_name.txt

merge human_target_to_drug.txt \
 drugbank_id_to_name.txt 2 1 > db_human_drug_targets.txt
```

NOTE: Column one and three in the drugbank vocabulary.csv file hold the DrugBank drug ID and the respective name.

3.3. Add drugs targeting synthetic lethal interaction partners to synthetic lethal interaction dataset.

3.3.1. Join the synthetic lethal interaction dataset with the drug-target drug name file generated in the previous step using the gene symbol columns to add drugs to synthetic lethal interactions. Take care to add drug names for both partners of each synthetic lethal interaction.

```
##enhance the synthetic lethal interaction file by adding drugs targeting the
partners of each synthetic lethal interaction
merge bg_synlet_mapped.txt db_human_drug_targets.txt 9 1 > tmp.txt
merge tmp.txt db_human_drug_targets.txt 10 1 > bg_synlet_mapped_drugs.txt
```

NOTE: Step 3 results in synthetic lethal interaction from multiple organism with their orthologous human genes and drugs targeting these genes.

4. Establishing the set of currently tested drug combinations in clinical trials

4.1. Get access to ClinicalTrials.gov data.

4.1.1. Retrieve information on clinical trials in XML format from ClinicalTrials.gov on either (i) individual trials, (ii) trials resulting from a search query, or (iii) all trials in the database. Alternatively use the resources provided by the clinical trials transformation initiative which also hosts all data from ClinicalTrials.gov in a relational database. See step 4.4 for further details.

NOTE: A free account is required to access the cloud-hosted database instance hosted by the clinical trials transformation initiative. In addition, a plsql client is required.

4.2. Focus on interventional trials.

4.3. Filter for trials specific for the indication of interest.

NOTE: ClinicalTrials.gov provides disease names from the NCBI Medical Subject Headings (MeSH) controlled vocabulary. Contrary to submitter provided disease names, the controlled vocabulary allows to efficiently identify trials for the indication of interest. Nevertheless, one must keep in mind that the NCBI MeSH controlled vocabulary is a thesaurus. Therefore, check the MeSH Browser (https://meshb.nlm.nih.gov) if the general indication of interest has any child/narrower terms and include them if appropriate.

4.4. Retrieve the identified trials together with the drugs tested in these trials. A query for trials in the general indication of ovarian cancer is provided below.

```
##retrieve interventional trials for the general indication ovarian cancer from the
clinical trials transformation initiative hosted relational database containing
ClinicalTrials.gov data
cat <<EOF |
\pset footer off
SELECT DISTINCT s.nct_id, s.brief_title, i.intervention_type, i.name
FROM studies s
 INNER JOIN browse_conditions c ON(s.nct_id = c.nct_id)
 INNER JOIN interventions i ON(s.nct_id = i.nct_id)
WHERE s.study_type = 'Interventional'
 AND c.mesh_term IN (
 'Ovarian Neoplasms',
 'Carcinoma, Ovarian Epithelial',
 'Granulosa Cell Tumor',
 'Hereditary Breast and Ovarian Cancer Syndrome',
 'Luteoma',
 'Meigs Syndrome',
 'Sertoli-Leydig Cell Tumor',
 'Thecoma'
 )
ORDER BY s.nct_id, i.intervention_type;
EOF
psql --host="aact-db.ctti-clinicaltrials.org" --username="XXX" --password --no-align
--field-separator="^I" --output="clinical_trials.txt" aact
```

4.5. Extract drug names and map to DrugBank names.

NOTE: While it is tempting to directly use the drug names retrieved from clinical trials of interest one must be aware that intervention names in ClinicalTrials.gov are entered by the submitter as free text. As a consequence, the names are not standardized, brand names may be used instead of the common compound name and there is no guarantee for proper data normalization (e.g. multiple drug names in one entry). In addition, it is common that drugs are submitted with a different intervention type, differing from drug. Therefore, mapping of the retrieved intervention names to DrugBank drug names is best carried out manually.

```
##Obtain a list of interventions used in the previously retrieved set of clinical
trials.
cut -d "^I" -f3,4 clinical_trials.txt | tail -n +2 | sort -u
```

NOTE: Columns three and four hold type of intervention and intervention name, respectively.

4.6. Complement with drugs already in clinical use from guidelines

NOTE: Step 4 results in a list of drugs under evaluation/in use for the indication of interest.

5. Identification of drug combinations targeting synthetic lethal interactions

5.1. Search for synthetic lethal interactions being targeted by two drugs of interest. Restrict the dataset from step 3 to drugs of interest by filtering out lines in the file holding both drug A and drug B.

```
##only retain entries for synthetic lethal interactions and drugs triggering them
where both partners are targeted by the two drugs of interest (drug_a and drug_b)
awk -F "\t" '{
 if( ($12 == drug_a && $14 == drug_b) || ($12 == drug_b && $14 == drug_a) ){
 print $0
 }
 }' drug_a="XXX" drug_b="YYY" bg_synlet_mapped_drugs.txt
```

5.2. Ensure that neither of the two drugs alone is targeting both synthetic lethal interaction partners. Check the drug targets of each identified drug in the dataset from step 3.2 and evaluate whether both identified synthetic lethal partners are targets of the specific drug.

```
##find all drug target entries for a given drug name
awk -F "\t" '{
 if($3 == drug){
 print $0
 }
 }' drug="XXX" db_human_drug_targets.txt
```

NOTE: A drug that would target both synthetic lethal interaction pathways would be toxic to any cell, so theoretically it is not a valuable multi- target agent. That is the reason why this possibility is excluded in this step of the algorithm.

6. Testing selected new drug combinations in vitro

6.1. Treat human breast cancer cell lines and human benign mammary epithelial cells cultured in standard in vitro culturing methods in a humidified a 37 °C atmosphere with 5% CO_2 with various drug combinations.

6.2. Use media supplemented with fetal bovine serum and penicillin as well as streptomycin sulfate to hinder bacterial infection.

6.3. Dilute drugs in solvents such as DMSO or phosphate-buffered saline in at least four different concentrations based on their previously established IC50 (inhibitory concentration)

and use them in combination or alone for treatment of cells.

6.4. Perform cell viability assays and apoptosis assays such AnnexinV/7-AAD stainings to determine cytotoxic effects caused by treatments.

6.5. Monitor pharmacological inhibition of suspected molecular targets using western blots.

6.6. Distinguish synthetic lethality from purely additive effects calculating the combinatory index (CI) as described by Chou and others[23].

REPRESENTATIVE RESULTS:

Our group has recently published two studies applying the workflow depicted in this manuscript to identify drug combinations targeting synthetic lethal interactions in the context of ovarian and breast cancer[24,25]. In the first study, we evaluated drug combinations that are currently tested in late stage clinical trials (phase III and IV) or already being used in clinical practice to treat ovarian cancer patients regarding their impact on synthetic lethal interactions. In addition, we identified drug combinations that are currently not being tested in clinical trials but provide a rationale from the perspective of targeting synthetic lethal interactions. We therefore evaluated all possible drug combinations choosing drugs from the pool of all compounds in late stage ovarian cancer trials. We identified a unique set of 61 drug combinations that had been investigated in 68 late stage ovarian cancer trials. Twelve out of these 61 drug combinations addressed at least one synthetic lethal interaction. 84 additional drug combinations were proposed to address synthetic lethal interactions without being investigated in clinical trials to this date. 21 unique drugs contributed to the 84 identified drug combinations targeting a set of 39 synthetic lethal interactors as given in **Figure 1**.

[insert Figure 1 here]

Using the same workflow in a second study, we identified 243 promising drug combinations targeting 166 synthetic lethal gene pairs in the context of breast cancer. We experimentally tested selected drug combinations regarding their impact on cell viability and apoptosis in two breast cancer cell lines. In particular, the proposed low-toxicity drug combination of celecoxib and zoledronic acid showed cytotoxicity beyond additive effects in breast cancer cell lines as determined by their combinatorial index. Results of viability and apoptosis assays for this drug combination are displayed in **Figure 2**.

[insert figure 2 here]

FIGURES:

Figure 1: Network of proposed novel drug combinations in the context of ovarian cancer. Figure 1 displays synthetic lethal interactions where interactors are addressed by two drugs currently not being tested in clinical trials. Synlet interactions are displayed in red, whereas drug-target links are indicated by grey edges. Dotted lines represent synthetic lethal interactions being addressed by other drug combinations in late stage ovarian cancer clinical trials. These investigated drug combinations are indicated with an asterisk (*****), each in combination with paclitaxel with the additional investigated combination of cediranib and olaparib being indicated by a circle (**o**) [adapted from [25]].

Figure 2: Impact of celecoxib and zoledronic acid on viability and apoptosis in SKBR-3 cells. (A) Viability assay results for celecoxib (CEL), zoledronic acid (ZOL) and the combination of zoledronic acid and celecoxib (ZOL + CEL) in SKBR-3 breast cancer cell lines. Low and high CEL concentrations used were 50µM and 75µM. Low and high ZOL concentrations used were 500µM and 750µM. The drug combination had a significant synergistic effect on cell viability (** $p < 0.001$). **(B, C)** Annexin V (ANXA5) and 7-AAD stainings of SKBR-3 cells treated with CEL, ZOL, and the drug combination ZOL + CEL. The percentage of 7-AADpos/ANXA5pos cells was increased after treatment with the drug combination ZOL + CEL [adapted from [24]].

Table 1: Relevant columns of the BioGRID datafile.

DISCUSSION:

We have outlined a workflow to identify drug combinations impacting synthetic lethal interactions. This workflow makes use of (i) data on synthetic lethal interactions from model organisms, (ii) information of human orthologs, (iii) information on drug-target associations, (iv) drug information on clinical trials in the context of cancer, as well as (v) on information of drug-disease and gene-disease associations extracted from scientific literature. The consolidated information can be used to evaluate the impact of a given drug combination under investigation on synthetic lethal gene pairs. In addition, consolidated data can be used to evaluate a set of drugs currently being investigated or tested in clinical trials in the context of cancer to find combinations targeting the most relevant synthetic lethal interactions, therefore having a higher chance of impacting tumor cell survival. Lastly, the data generated can be used to screen for drug combinations consisting of drugs not initially developed for tumor treatment, thus providing a way for a computationally driven drug repositioning case.

For each step in the data integration workflow we present key data sources to complete the full data workflow but point out that the workflow can be further enhanced at various stages by making use of additional data sources. In our workflow we extracted synthetic lethal interaction pairs from the BioGRID database[18]. We specifically focused on interactions of experiment types “synthetic lethality” and “negative genetic”. Information in BioGRID on synthetic lethal interactions contains datasets from large genetic screen as for example a dataset published by Costanzo and colleagues[26], which is also available in the DRYGIN database[27], as well as data on single synthetic lethal interactions as described in individual experiments in scientific literature. There are additional data sources collecting and storing synthetic lethal interactions, as for example SynLethDB[28]. Further, on the level of orthology mapping, a large number of different tools and databases exist. We present a way to make use of Ensembl biomart to map synthetic lethal interaction partners identified in model organisms to their corresponding human orthologs. Other orthology databases and services include NCBI’s HomoloGene database[29], the

OMA orthology database from the Swiss Institute of Bioinformatics[30], or the InParanoid ortholog groups database maintained by the Stockholm Bioinformatics Center[31]. In our workflow, we focused on synthetic lethal interactions from multiple model organisms, with the largest number of synthetic lethal interactions coming from yeast. One might consider restricting the input set for the orthology mapping to data from mouse and rat only, which are evolutionary closer to humans. An additional way of defining the input set of synthetic lethal interactions is to only focus on synthetic lethal interactions being conserved in multiple species, thereby increasing the chances that the synthetic lethal interaction is truly positive. This on the other hand might reduce the set of synthetic lethal interactions dramatically, as there is already a large difference in the identified synthetic lethal interactions between S. cerevisiae and S. pombe. Another approach is to be not too stringent at the beginning and to even extend the set of experimental synthetic lethal interactions by machine learning algorithms as we did in the two studies listed in the representative results section. In brief, a random forest model was used to predict synthetic lethal interactions for human genes for which no orthologous genes existed in yeast. The random forest model was trained on the set of synthetic lethal interaction pairs from yeast and their orthologous human genes using data on pathway associations, Gene Ontology assignment as well as disease and drug associations as described previously[24,25]. This allowed us to consider human genes for which no ortholog mapping information was available in our integration workflow. A widely used database storing information on drug-target associations is DrugBank, which is also the primary source of interactions in the workflow. Other databases holding to some extent complementary information on drug targets are the Therapeutic Target Database (TTD)[32] or ChEMBL[33]. Major components of the workflow are also incorporated in the e.valuation platform from emergentec and SynLethDB, which has been developed by researchers from Nanyang Technological University. The last update of SynLethDB in 2015, however, was based on the datasets stored in the download section on their respective webpage[28].

A way to rank identified drug combinations and targeted synthetic lethal interaction pairs is using the association of synthetic lethal partners and/or drugs with the disease of interest via literature mining methods. In our work on the evaluation of drug combinations in the context of ovarian cancer, we ranked novel proposed drug combinations based on the number of publications on ovarian cancer mentioning either one of the two synthetic lethal interactors of a respective drug combination. MeSH annotation in Pubmed can be used to identify publications for a specific disease using the exact disease terms as given in the major MeSH branch C. Information on genes in the identified publications can be extracted using NCBI's gene2pubmed mapping file as described elsewhere[34]. Further, there are dedicated databases holding gene-disease and/or drug-disease links such as the Comparative Toxicogenomics Database[35], DisGeNET[36], or the e.valuation software platform. Ranking of drug combinations

based on disease associations is one way of supporting the final selection of drug combinations for experimental testing. Additional aspects need to be considered when selecting drug combinations for further testing, like for example individual toxicity profiles of the drugs or expression status of synthetic lethal interactors in the respective target organ.

In the representative results section, we present data for the drug combination of celecoxib and zoledronic acid, which was identified following the workflow to identify drug combinations in the context of breast cancer. This particular drug combination was selected for experimental testing due to the low toxicity profiles of both compounds. We used various concentrations in in-vitro experiments to evaluate the impact of the drug combination on cell viability and apoptosis. Ideally, drug concentrations could be significantly lowered for individual drugs to minimize side effects while at the same time maximizing efficacy by combining two drugs. Seeing impact on viability at lower doses is even more meaningful, as drug concentrations used for in vitro testing could be criticized to be supratherapeutical, that are not reached in in vivo models. However, the concentrations were chosen based on cell culture experiments with these given drugs in the literature. Drug dosing may further influence what targets are primarily affected, as most compounds have more than one drug target, potentially impacting a large set of known and unknown downstream molecules as well. Drug combinations showing synergistic effects on cell viability in in-vitro cell culture systems should therefore be further investigated in 3D or in-vivo models.

Summarizing, we present a workflow that integrates information from different data sources to evaluate and propose drug combinations targeting synthetic lethal interactions. To date, the largest information on synthetic lethal interactions is still coming from model organisms, requiring a mandatory orthology mapping step to the human genome. First screens in human haploid cells have led to the identification of synthetic lethal interactions in human cells. Additionally, the CRISPR/CAS technology has opened new ways of studying synthetic lethal interactions on a cellular level. With more high quality biological synthetic lethal interaction data becoming available, we propose that data integration efforts such as ours will transform clinical cancer treatment in the future, by discovering novel and clinically meaningful synthetic lethal gene pairs aside from BRCA(1/2)/PARP1.

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DISCLOSURES:

AH and PP were employees of emergentec biodevelopment GmbH at the time of performing the analyses leading to the results presented in the representative results section. MM and MK have nothing to disclose.

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Figure 1

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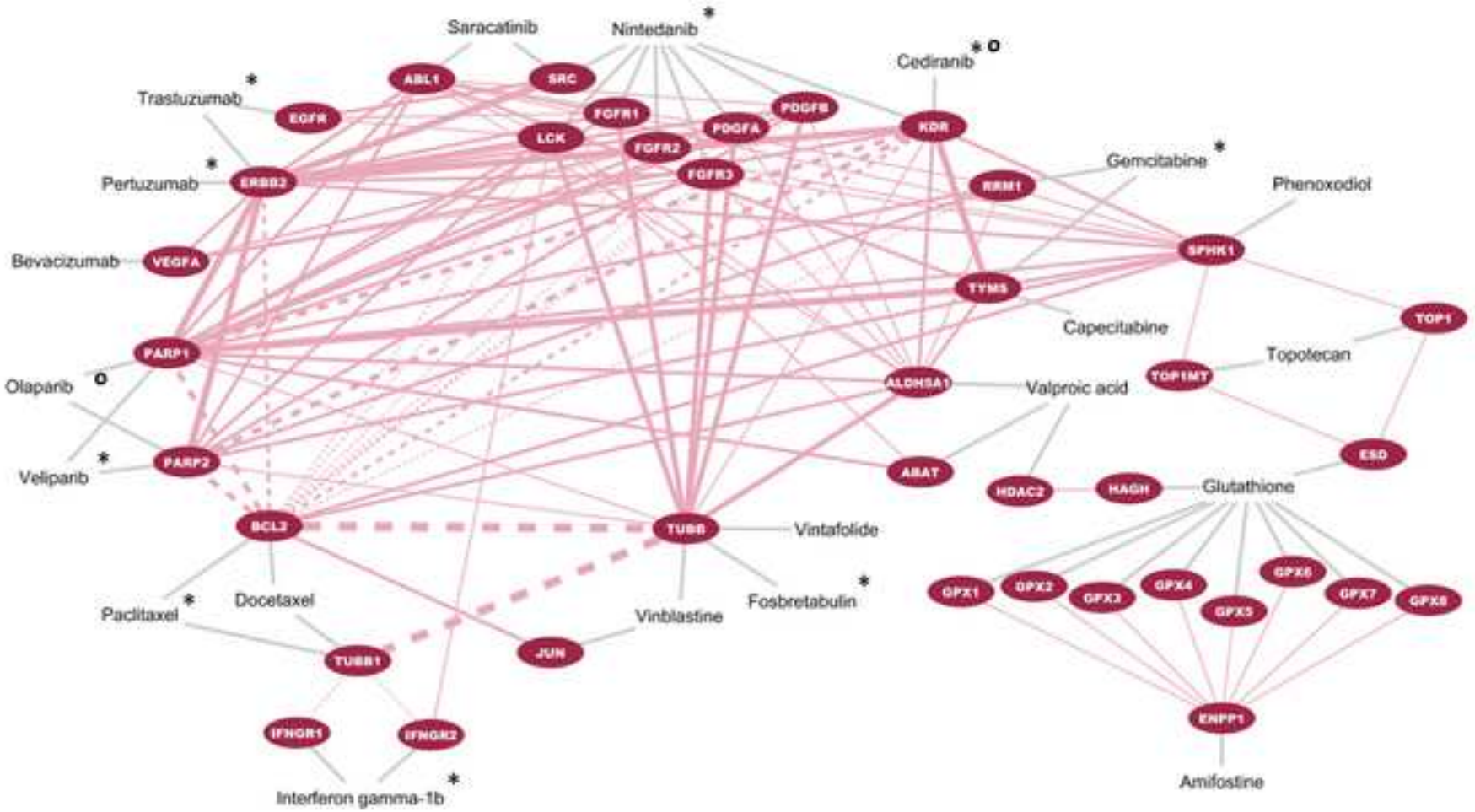

Figure 2

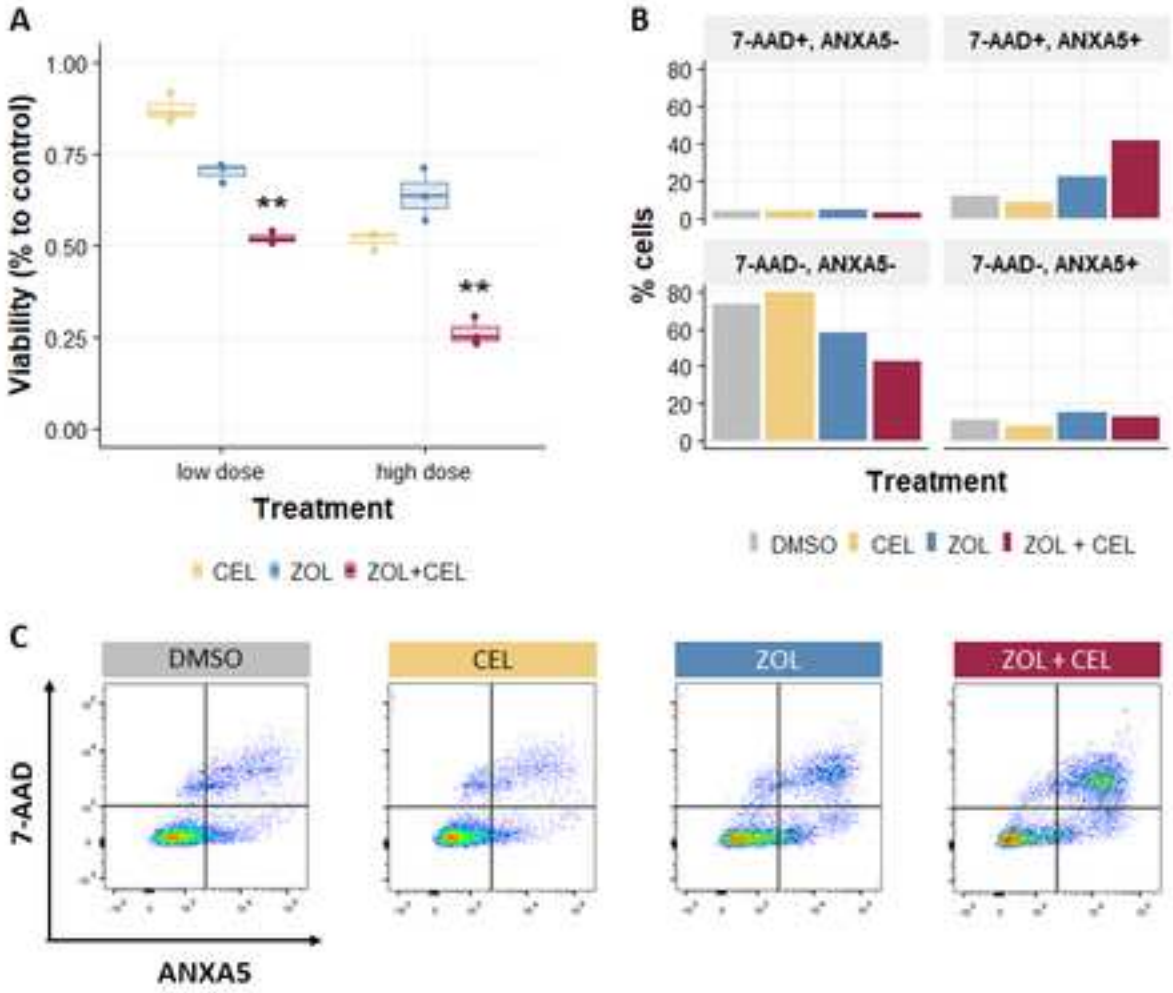

Table 1

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Column number	Column header name
1	#BioGRID Interaction ID
8	Official Symbol Interactor A
9	Official Symbol Interactor B
12	Experimental System
16	Organism Interactor A
17	Organism Interactor B

Table 2

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Column number	Column header name
3	Gene Name
12	Species
13	Drug IDs

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DrugBank	n/a	n/a	drugbank.ca
Ensembl BioMart	n/a	n/a	ensembl.org
for alternative computational databases please refer to the manuscript			
7-AAD	ebioscience	00-6993-50	
AnnexinV-APC	BD Bioscience	550474	
celecoxib	Sigma-Aldrich	PZ0008-25MG	
CellTiter-Blue Viability Assay	Promega	G8080	
FACS Canto II	BD Bioscience	n/a	
fetal bovine serum	Fisher Scientific/Gibco	16000044	
FloJo Software	FloJo LLC	V10	
McCoy's 5a Medium Modified	Fisher Scientific/Gibco	16600082	
penicillin G/streptomycin sulfate	Fisher Scientific/Gibco	15140122	
SKBR-3 cells	American Type Culture Collection (ATCC)	ATCC HTB-30	
zoledronic acid	Sigma-Aldrich	SML0223-50MG	
further materials or equipment will be made available upon request			

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October 4, 2019

RE: Resubmission of the manuscript titled "*A data integration workflow to identify drug combinations targeting synthetic lethal interactions*" JoVE60328 - [EMID: a39e91c34e87e70e]

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Dear Editors and Reviewers,

Thank you for the opportunity to revise our manuscript titled "*A data integration workflow to identify drug combinations targeting synthetic lethal interactions*". This letter includes a line-by-line response to each of your valuable and constructive comment; highlighted in bold to improve readability. We believe that our manuscript has substantially improved after making the suggested changes.

Sincerely,

Univ.-Prof. Dr. Michael Krainer

Editorial Comments

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.
We have proofread the manuscript and corrected all the typographical errors to the best of our knowledge.

• Protocol Language: The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief “Note” at the end of the step (please limit notes). Please re-write your ENTIRE protocol section accordingly. Descriptive sections of the protocol can be moved to Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study. Any reporting should be moved into the representative results.
1) Examples NOT in imperative voice: step 1.1, 2.1, 3.1, etc
We rewrote the Protocol section in imperative voice (with the exception of Notes).

2) For code snippets, please add a step before each code block describing the actions.
We have added a comment line before each code block summarizing the actions.

3) Please remove the enclosing boxes around the code blocks.
The enclosing boxes around the code blocks have been removed

4) Split up long steps (e.g., 2.1) into 2 or more steps.
We have tried our best to split long sentences for purposes of readability.In 2.1, we have omitted the example query and displayed only show the final data retrieval step.

• Protocol Detail: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
All commands as well as links for downloading data in order to execute the workflow have been provided in the form of code snippets.

• Protocol Highlight: Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.
1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.
We have highlighted the relevant steps in yellow as suggested.

• Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.
We currently have 5 paragraphs in the Discussion section elucidating the overall aim of the workflow, modifications and extensions along with notes on troubleshooting, limitations and potential modifications and filtering steps to overcome the limitations, as well as critical steps. Thus, we believe that the Discussion section sufficiently follows the guidelines of the journal.

• Tables: Please remove the embedded Table from the manuscript. All tables should be uploaded to the Editorial Manager site in the form of Excel files. A description of the table should be included with the Figure legends.

We have removed the embedded Table and added the Table legend to the Figure legends section. The Tables are now provided as separate Excel files.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."
Please see attached E-mail permissions to re-use the figures from previous publishers.

<u>Reviewers' Comments</u>

Reviewer #1
Manuscript Summary:
The manuscript proposes a workflow that integrates information from different sources to reveal potential drug combinations targeting synthetic lethal interactions, It provides a couple of previously reported examples of its applications. It is well-written; the protocol is crystal clear. Just minor comments from my side.

Minor Concerns:
* Could you please elaborate on the following statement "Relevance of organisms for the subsequent

translation step to human genes can be identified by counting the number of occurrences of organism tax-IDs". Please provide references.
The statement has been rewritten as follows: “Determine the number of synthetic lethal interaction partner tax-IDs to get an estimate on the number of synthetic lethal interactions being available per organism”.

* The authors say that, when testing drug combinations, it must be ensured that neither of the two drugs alone is targeting both synthetic lethal interaction partners. This is reasonable for the sole purpose of a combined therapy approach but if one of the retrieved drugs targets both targets simultaneously, it could be a valuable multi-target agent. Maybe a note on this would be appreciated.

The following Note has been added for purposes of clarity: “A drug that would target both synthetic lethal interaction pathways would be toxic to any cell, so theoretically it is not a valuable multi- target agent. That is the reason why this possibility is excluded in this step of the algorithm.”

* Please specify which folds may be advised in "Dilute drugs in solvents such as DMSO or phosphate-buffered saline and us them in combination or alone for treatment of cells at folds of their previously established IC50 (inhibitory concentration)."
We have edited the above sentence for purposes of clarity: "Dilute drugs in solvents such as DMSO or phosphate-buffered saline in at least four different concentrations based on their previously established IC50 inhibitory concentration and use them in combination or alone for treatment of cells.”

* Why have the authors tested such high levels of celecoxib and zoledronic acid in their combination experiments? As the authors themselves note, such levels would be hardly met in a therapeutic setting.
We have added the following sentence in the Discussion section for purposes of clarity: “The concentrations were chosen based on cell culture experiments with these given drugs in the literature.”

* By the way, I think "supratherapeutical" is better than "supraphysiological", since, as xenobiotics, the idea of a physiological concentration does not make sense.
We have substituted “supraphysiological” with “supratherapeuticel” as suggested by the reviewer.

There are minor typos.
We have proofread the manuscript and corrected all the typographical errors to the best of our knowledge.

Reviewer #2
Manuscript Summary:
The manuscript describes a protocol for extracting synthetically lethal pairs of genes from BioGrid, mapping the pairs to their human orthologues, and then integrating data from DrugBank and clinicaltrials.gov to identify druggable protein pairs.

These protocols are straightforward and could be successfully implemented by novice bioinformaticians -which would be helpful to the community.

Major Concerns:
i) My major issue is the underlying concept.
While is is true that if you simultaneously drug 2 synthetically lethal proteins you have the ability to kill a cell, this would be of little practical use as this would affect healthy cells as well as cancer cells.
The therapeutic attractiveness of synthetic lethality is when one protein is genetically inactivated in the tumour, ie is an inactivated tumour suppressor - BRCA1/2 in the clinic, and the other is inactivated pharmacologically - PARP1.
In healthy cells, BRAC1/2 work, so even though PARP1 in inhibited the cells can function.
This gives the therapeutic rationale (ie kills cancer cells but leaves healthy cells viable). This was not explained in the paper.
The example that they give is where they attack 2 proteins in a SSL pair; this would also be toxic in healthy cells.
The experimental confirmation although valid- they need a control. i.e it needs to be tested in a healthy cell as well as a cancer cell line. Most likely it will kill the healthy cell so is of no use as a drug treatment.
The concept does not directly result in ideal therapeutic combinations useful for cancer therapy. However, it can predict lethal interactions of non-toxic drugs based on synthetic lethality in cell culture. Consequently, in further experiments non-toxic drugs can be applied to cancer cells with defined genetic deficiencies in the affected pathway and a therapeutic index can be expected.

ii) The prediction method. Although there is certainly an enrichment of pairs of proteins synthetically lethal in humans that are synthetically lethal in model organisms, the actual prediction accuracy is very low. The authors should have produced a ROC curve to show the performance of their algorithm.
We agree with the reviewer that the prediction performance is of interest to the reader. Unfortunately, we feel that this would be out of the scope of this manuscript; however, reference 6 in reference 25 of this manuscript provides further details on the prediction performance.

iii) Finally, the genetic interaction data itself can be quite inaccurate. For model organisms where a lot of data is available, multiple observations improve the reliability of the data.
We are in full agreement with the reviewer and have added the following text in the Discussion section: "One might consider restricting the input set for the orthology mapping to data from mouse and rat only, which are evolutionary closer to humans. An additional way of defining the input set of synthetic lethal interactions is to only focus on synthetic lethal interactions being conserved in multiple species, thereby increasing the chances that the synthetic lethal interaction is truly positive."

Reviewer #3
Manuscript Summary:
The manuscript describes a work on identifying drug combinations that can target synthetic lethal proteins, so that in cancers, for instance, where the absence or mutation of one protein exists, the other protein can be a good candidate drug target to stop the proliferation. A protocol to identify such drug combinations is proposed. It is well written and easy to follow. I think it will be of great interest to researchers in this field.

Major Concerns:
None.

Minor Concerns:
My only minor concern is the quality of the figures. It is not of high quality in the current manuscript. It will increase the readability if the authors provide higher quality figures.
We have uploaded figures in high-quality for production.

Reviewer #4
Manuscript Summary:
This manuscript provides a protocol on how to generate a list of drugs through synthetic lethality using bioinformatics. The authors provided a workflow and the codes on the protocol, and showed the results in their studies. Overall, the manuscript is well written, and the steps are clear. However, the authors need to tidy up their codes, specifically providing more annotations/comments in their codes to show users what those values mean (e.g. column numbers, taxa etc).

Major Concerns:
1. In the codes, please make annotations for ease of understanding. For example, in pg9, code starting from line 198: what is the "559292" means in the "print 559292" ? is it the number of interactions? Or what? If this is a fix value, it should be defined properly. For example, the same code, the "9606" in the line if($5 == 9606), this reviewer understands that this is the taxa code for human. This should be defined as a variable "$TAXA = 9606"; and in the line (if $5 == $TAXA). Then user will know how to change the value "$TAXA" to other species. Similar cases exist in other places of various codes.
We thank the reviewer for this comment. As suggested, we have used variables for tax ids to avoid magic numbers in the code. We also agree with the reviewer that further constant values (only string constants e.g. Negative Genetic or Humans remaining) could be factored out into variables; however, we think that this would only complicate the code as the constant value is equally meaning full as any variable name that could be used instead. Therefore, we prefer to keep them directly in the code.

2. The authors assumed the users/readers will know the columns name. For example: in page 11, the authors are extracting some columns from DrugBank csvtool col 3,12,13 -u TAB "${DB_TARGETS}. The authors should do the annotation for these columns similar to Table 1 for BioGrid. This will inform the users, and potentially DrugBank might change their columns in the future, and the users still know which column to extract from.
Thank you for this valuable comment. The suggested Table 2 has been included.

Reviewer #5
Manuscript Summary:
The authors presented an integrated workflow for identification of drug targets for synthetic lethal interacting pairs against ovarian and breast cancer exploiting the information available in already existing databases. Their workflow provides a comprehensive step by step procedure which can easily be replicated. The authors have systematically applied the current protocol to two already published studies for analyzing drug combinations currently in clinical use or investigated in late stage phase III or IV clinical trials against ovarian and breast cancer. Additionally, they have proposed other drug combinations which are not being tested in clinical trials but exhibit a synthetic lethal mechanism of action for cancer. Overall, the paper is reporting valuable information on possibility of selectively targeting cancer cells, with the potential to reduce drug resistance and side effects. Even though only

one synthetically lethal interaction - between PARP and breast-cancer BRCA1 and BRCA2 is clinically approved, nevertheless such interactions have potential for clinical applications.

The article may be accepted in its current form.

Figure 1

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Figure1_synlets_novel-combos_2019-04-18_submitted_PLoS-One.pptx

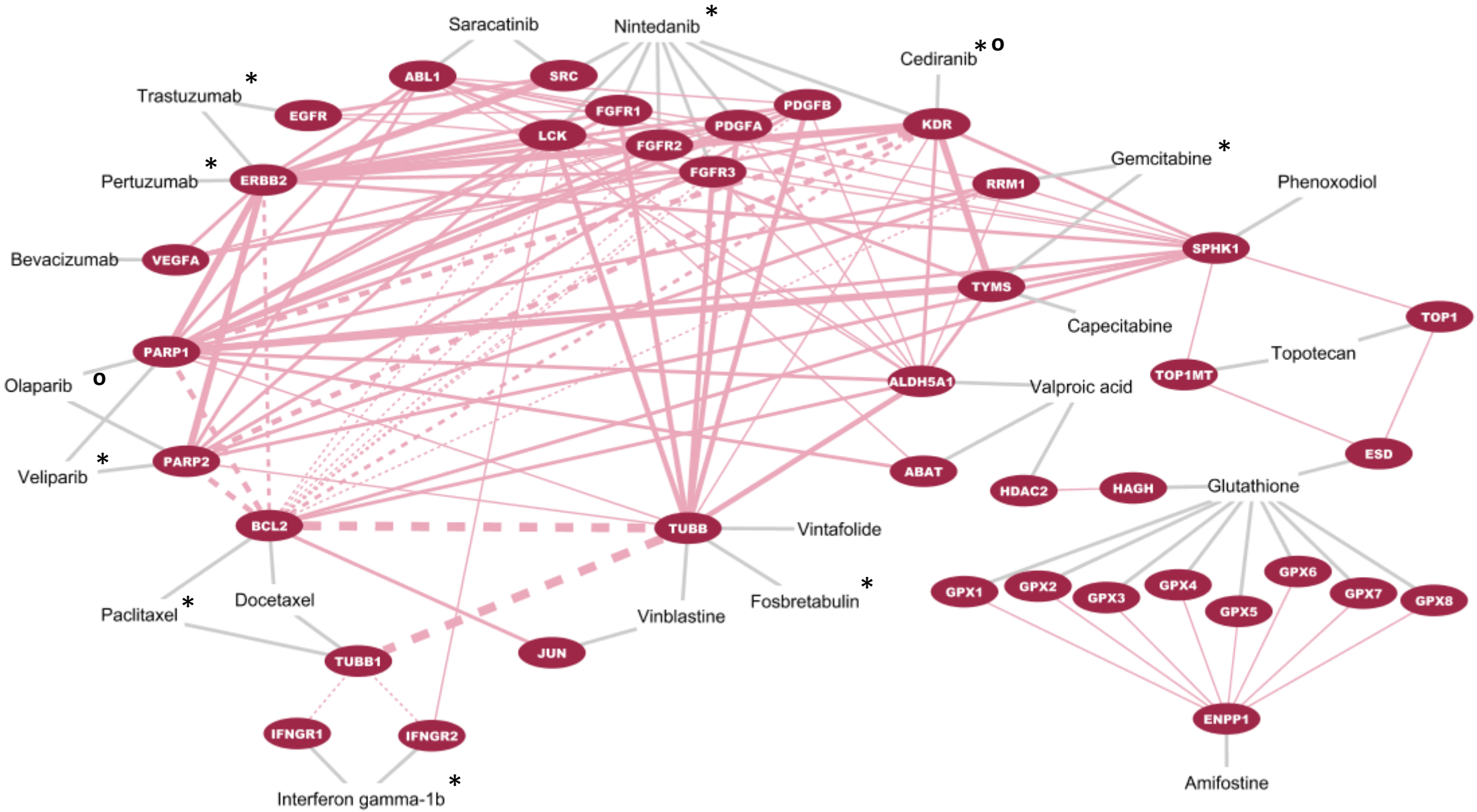
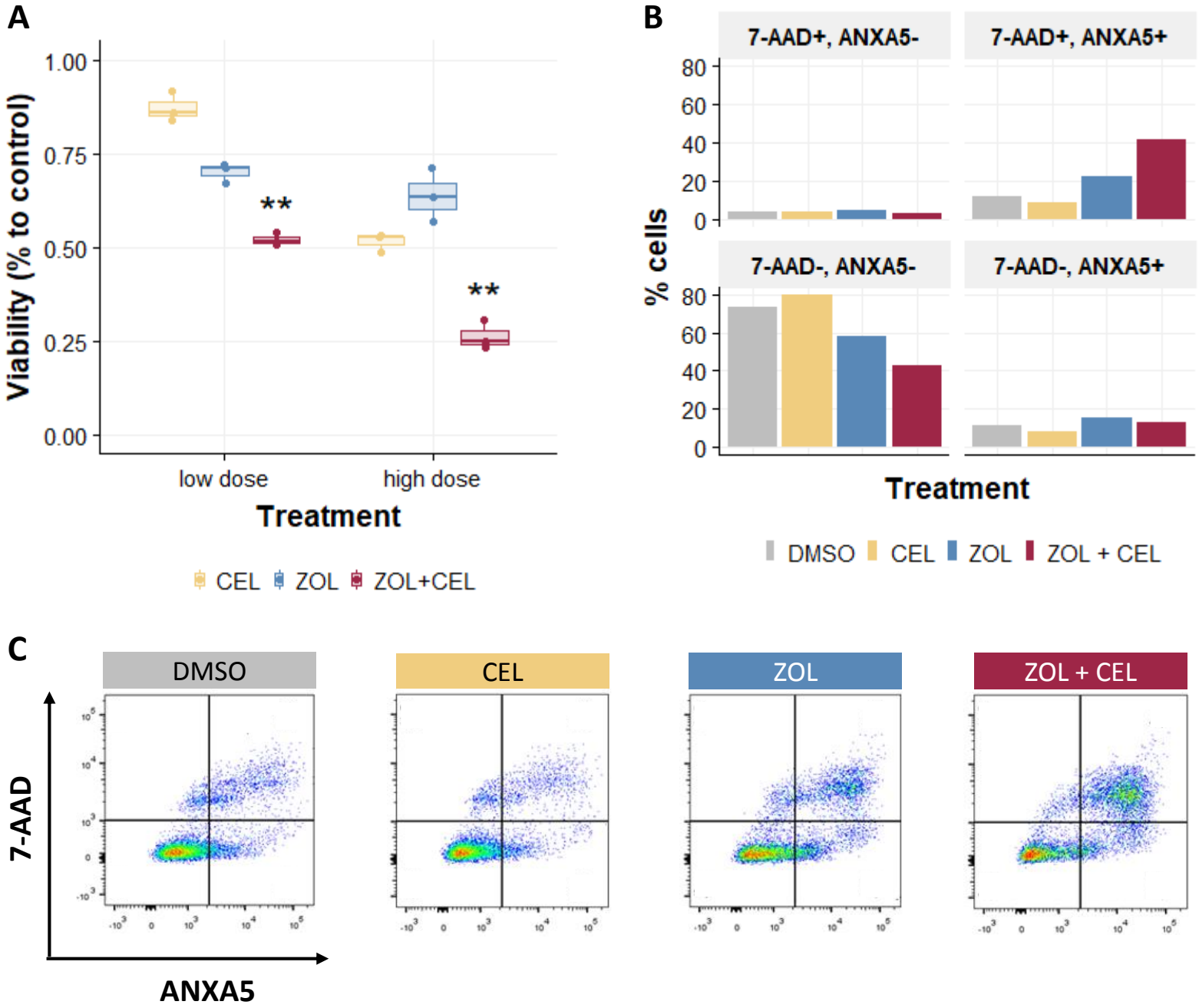


Figure 2



Mailverkehr bezüglich der 2. Figure für die JOVE Arbeit unten.

LG Paul

Paul Perco, PhD, PD

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Department of Internal Medicine IV
Anichstrasse 35
A-6020 Innsbruck, Austria

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w: www.paulperco.at

-----Original Message-----
From: Dr. med. univ. Maximilian Marhold, PhD <maximilian.marhold@meduniwien.ac.at>
Sent: Thursday, September 26, 2019 4:57 PM
To: paul.perco@i-med.ac.at
Subject: Fwd: RE: PONE-D-18-06274R2 Request to re-use figure 4

-------- Originalnachricht --------
Betreff: RE: PONE-D-18-06274R2 Request to re-use figure 4
Datum: 2019-04-10 21:03
Von: plosone <plosone@plos.org>
An: "maximilian.marhold@meduniwien.ac.at"
<maximilian.marhold@meduniwien.ac.at>
Kopie: "michael.krainer@prof-krainer.at"
<michael.krainer@prof-krainer.at>

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Please feel free to contact me if you have any other questions or concerns.

Kind regards,
Shayan Khan

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1160 Battery Street, Suite 100, San Francisco, CA 94111 plosone@plos.org

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---------- Original Message ----------

From: Dr. med. univ. Maximilian Marhold, PhD [maximilian.marhold@meduniwien.ac.at]
Sent: 4/6/2019 2:47 AM
To: plosone@plos.org
Cc: michael.krainer@prof-krainer.at
Subject: PONE-D-18-06274R2 Request to re-use figure 4

Dear editorial team,

after publishing our article in your prestigious journal, we were invited to publish a protocol paper about the methods presented in our PLOS One manuscript in JOVE, a peer-reviewed and pubmed-listed video journal. As JOVE calls for representative results, we kindly ask for permission to re-use Figure 4 in our JOVE contribution.

Our PLOS One article will be cited correctly and accordingly and a sentence in the acknowledgement section will read:

"Re-use of figures within this publication was kindly approved by Public Library of Sciences (PLOS) Publications."

We hope for your help in spreading knowledge and increasing citations of our publication in Plos One.

Best,

Maximilian Marhold, MDPhD

Am 2019-01-11 17:44, schrieb PLOS ONE:
> PONE-D-18-06274R2
> Synthetic lethality guiding selection of drug combinations in ovarian
> cancer
>
> Dear Dr. Marhold:
>
> Thank you for returning your manuscript and for your continued
> cooperation with our requests. There are still some outstanding issues
> that must be resolved before your manuscript can be formally accepted
> - please see below.
>
> To access your manuscript and complete these changes, please follow
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>
> Your task is due Jan 14 2019 11:59PM.
>
> We appreciate your patience during this process. Please contact
> plosone@plos.org with any questions or concerns.
>

> With kind regards,
> PLOS ONE staff
>
> JOURNAL REQUIREMENTS:
>
> 1. Please note we cannot accept changes to corresponding authorship,
> and therefore we have ensured Dr. Krainer will continue to be listed
> as corresponding author. If you have any questions, do not hesitate to
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>
> If this is acceptable, please submit your task and we will proceed to
> production
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Dr. med. univ. Maximilian Marhold, PhD

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Comprehensive Cancer Center (CCC) Medical University of Vienna
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Department of Internal Medicine I - Oncology Chair for Medical Breast Cancer Research
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Liebe Almas,

weitergeleitet die Nachricht von Max bezüglich der Verwendung der Figure aus dem Oncotarget paper.
Dies bitte bei der Resubmission des JOVE Manuscrtipts berücksichtigen und in die Reviewer Comments einbauen.

Ein zweites Mail bezüglich der anderen PLOS One Figure kommt auch gleich weitergeleitet.

Beste Grüße,

Paul

Paul Perco, PhD, PD

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m: paul.perco@i-med.ac.at
w: www.paulperco.at

-----Original Message-----
From: Dr. med. univ. Maximilian Marhold, PhD <maximilian.marhold@meduniwien.ac.at>
Sent: Thursday, September 26, 2019 4:57 PM
To: paul.perco@i-med.ac.at
Subject: Fwd: Re: 027244R4 - Request to re-use Figure 5B

-------- Originalnachricht --------
Betreff: Re: 027244R4 - Request to re-use Figure 5B
Datum: 2019-04-09 04:26
Von: Editorial Office <editorialoffice@oncotarget.com>
An: "Dr. med. univ. Maximilian Marhold, PhD" <maximilian.marhold@meduniwien.ac.at>
Kopie: "Dr. Michael Krainer" <michael.krainer@prof-krainer.at>

On Sat, Apr 6, 2019 at 4:44 AM Dr. med. univ. Maximilian Marhold, PhD <maximilian.marhold@meduniwien.ac.at> wrote:

> Dear editorial team, dear Dr. Blagosklonny,
>
> after publishing our article in your prestigious journal, we were

> invited to publish a protocol paper about the methods presented in our
>
> Oncotarget manuscript in JOVE, a peer-reviewed and pubmed-listed video
>
> journal. As JOVE calls for representative results, we kindly ask for
> permission to re-use Figure 5B and Figure S3 in our JOVE contribution.
>
> Our Oncotarget article will be cited correctly and accordingly and a
> sentence in the acknowledgement section will read:
>
> "Re-use of figures within this publication was kindly approved by
> Oncotarget (Impact Journals, LLC)."
>
> We hope for your help in spreading knowledge and increasing citations
> of our publication in Oncotarget.
>
> Best,
>
> Maximilian Marhold, MDPhD
>
> Am 2018-10-22 05:46, schrieb editorialoffice@oncotarget.com:
>> October 20, 2018
>>
>> Michael Krainer
>> Vienna General Hospital (AKH)
>> Oncology
>> Allgemeines Krankenhaus der Stadt Wien Waehringer Guertel 18-20
>> Vienna, Vienna 1090 Austria
>>
>> RE: Synthetic lethal combinations of low-toxicity drugs for breast
>> cancer identified in silico by genetic screens in yeast
>>
>> Dear Dr. Michael Krainer:
>>
>> I am pleased to inform you that your manuscript "Synthetic lethal
>> combinations of low-toxicity drugs for breast cancer identified in
>> silico by genetic screens in yeast" has been accepted for
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>> Sincerely,
>>
>> Mikhail
>> Prof. Mikhail V. Blagosklonny, MD, PhD Co-Editor-in-Chief Oncotarget
>>
>> ---
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> --
> Dr. med. univ. Maximilian Marhold, PhD
>
> Department of Internal Medicine I - Oncology Chair for Medical Breast
> Cancer Research Comprehensive Cancer Center (CCC) Medical University
> of Vienna
> Address: Währinger Gürtel 18-20, A-1090 Wien
> Mail: maximilian.marhold@meduniwien.ac.at
> Phone: +43 1 40400 73792

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Dr. med. univ. Maximilian Marhold, PhD

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