

1 Prevalence, causes and impact of *TP53*-loss 2 phenocopying events in human tumors

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9

10 Abstract

11 *TP53* is a master tumor suppressor gene, mutated in approximately half of all human
12 cancers. Given the many regulatory roles of the corresponding p53 protein, it is
13 possible to infer loss of p53 activity -- which may occur from trans-acting alterations --
14 from gene expression patterns. We apply this approach to transcriptomes of ~8,000
15 tumors and ~1,000 cell lines, estimating that 12% and 8% of tumors and cancer cell
16 lines phenocopy *TP53* loss: they are likely deficient in the activity of the p53 pathway,
17 while not bearing obvious *TP53* inactivating mutations. While some of these are
18 explained by amplifications in the known phenocopying genes *MDM2*, *MDM4* and
19 *PPM1D*, others are not. An analysis of cancer genomic scores jointly with
20 CRISPR/RNAi genetic screening data identified an additional *TP53*-loss phenocopying
21 gene, *USP28*. Deletions in *USP28* are associated with a *TP53* functional impairment in
22 2.9-7.6% of breast, bladder, lung, liver and stomach tumors, and are comparable to
23 *MDM4* amplifications in terms of effect size. Additionally, in the known CNA segments
24 harboring *MDM2*, we identify an additional co-amplified gene (*CNOT2*) that may
25 cooperatively boost the *TP53* functional inactivation effect. An analysis using the
26 phenocopy scores suggests that *TP53* (in)activity commonly modulates associations
27 between anticancer drug effects and relevant genetic markers, such as *PIK3CA* and
28 *PTEN* mutations, and should thus be considered as a relevant interacting factor in
29 personalized medicine studies. As a resource, we provide the drug-marker
30 associations that differ depending on *TP53* functional status.

31

32 Introduction

33 Mutations in the *TP53* tumor suppressor gene are a very common feature across
34 almost all types of human cancer. These mutations abrogate or reduce *TP53* activity
35 via various mechanisms: dominant-negative acting missense mutations, loss-of-
36 function missense, nonsense, frameshift indel, splice site, or synonymous mutations, or
37 copy number losses that frequently delete one *TP53* allele while the other allele is
38 inactivated by a mutation. That such *TP53* genetic alterations occur at high frequency
39 in many cancer types implies that they have very strong selective advantages for the
40 expanding cancer cell clones (1, 2); indeed this is borne out in experimental data on
41 cell lines and animal models of cancer (3, 4).

42

43 The large selective advantage of *TP53* losses are consistent with its roles in arresting
44 the cell cycle or triggering apoptosis upon threats to genome integrity. *TP53*-null cells
45 better tolerate genomic instability, which can result from endogenous causes, most
46 prominently oncogene-overexpressing and thus replication-stress inducing cancerous
47 genetic backgrounds. Consistently, *TP53*-mutant tumors have higher frequencies of
48 segmental copy number alterations (CNA), whole-genome duplications, and overall
49 mutation rates (5, 6). Moreover, *TP53*-null cells better tolerate DNA damaging
50 conditions that would normally trigger cell cycle checkpoints, such as those resulting
51 from DNA-acting drugs or radiation (7, 8). Consistently, *TP53*-mutation bearing tumors
52 tend to be more resistant to various cancer chemotherapies (4, 9-11) and radiotherapy
53 (10–12), and more aggressive i.e. *TP53* R273 and R248 mutants are associated with
54 accelerated cancer progression in colorectal tumors (13).

55

56 The frequency of *TP53* mutations --highest of all cancer genes, standing at 37% in the
57 TCGA cohort-- indicates that most cancers benefit from the loss of *TP53*. However,
58 there are nonetheless many tumors which do not bear a mutation in *TP53*. A part of
59 those is explained by genetic events that phenocopy *TP53* loss i.e. that have similar
60 downstream phenotypic consequences as *TP53* loss itself. There are three
61 established examples of *TP53* loss phenocopying events occurring in tumors. Most
62 prominently, this is the amplification of the *MDM2* and *MDM4* oncogenes and
63 overexpression of the corresponding proteins. These negatively regulate *TP53* protein
64 levels by promoting its proteasomal degradation, and that otherwise inhibit *TP53*
65 activity by binding to its transactivation domain(14–16). The third implicated gene is
66 *PPM1D*, whose amplification overexpresses a serine/threonine phosphatase acting
67 upon various targets including *TP53*, reducing its activity. (We note that *PPM1D* can
68 also be affected by point mutations that result in gain-of-function(17–19))

69

70 Given the strong selective advantages of the *TP53* activity loss in cancer evolution, we
71 hypothesized that *TP53* loss phenocopying in human cancers extends beyond these
72 known examples of *MDM2*, *MDM4* and *PPM1D* alterations. If indeed other common
73 mechanisms of *TP53* phenocopying exist, this would be relevant to predicting tumor
74 cell response to various drugs, and to predicting tumor aggressiveness, thus having
75 implications to personalized medicine. Because *TP53* loss has clear consequences on
76 the mRNA expression levels of various downstream targets (4, 21), the *TP53*-null-like
77 phenotype can be inferred from large scale transcriptomic data (20-23). Here, we
78 apply a statistical framework to jointly analyse ~966 cancer cell line and ~8000 tumor
79 genomes and transcriptomes, to identify additional *TP53* phenocopying genetic events
80 and impact on drug sensitivity. We find that *TP53* loss phenocopies are remarkably
81 common across tumors and cancer cell lines, and we identify *USP28* deletions as one
82 cause of *TP53* loss phenocopying, and reveal many links between drugs and their
83 targets that are modulated by *TP53* activity.

84

85

86

87 Results

88 Inferring the functional *TP53* status of tumors from 89 transcriptomes

90
91 We developed a machine learning method to detect *TP53* phenocopies in tumors and
92 cell lines, integrating RNA-seq data with *TP53* mutation data in a logistic regression,
93 regularized with an Elastic Net penalty (very similar cross-validation accuracy was
94 obtained with Ridge or Lasso penalties; see Methods). Regression models were
95 trained using cross-validation on mRNA levels of ~8000 tumor samples from the TCGA
96 project, across 20 different cancer types, controlling for cancer type. In addition to
97 using this global analysis mRNA expression levels to infer the functional *TP53* status
98 state of each tumor, we also identified the expression patterns of which genes are
99 associated with *TP53* status. Tumors with *TP53* putatively causal mutations were
100 included as a positive set (*TP53* status was categorized according to GDSC
101 methodology; see Methods). Previously known phenocopying events (*MDM2*, *MDM4*
102 and *PPM1D* amplifications), as well as samples with *TP53* deletions were excluded
103 from the training set (these known phenocopying events will be used to calibrate
104 decision thresholds; see below). Our classifier learned a combination of relevant gene
105 weights that differentiate samples with an aberrant *TP53* activity. Tumor samples that
106 are not *TP53* mutated (by GDSC criteria), but are classified as mutated by the machine
107 learning model are considered to be *TP53* phenocopies.

108
109 Our classifier showed a high performance with an area under the receiver operating
110 characteristic (AUROC) curve of 96% in cross-validation on TCGA tumors (out-of-
111 sample accuracy), and 95% on the testing set (consisting of 10% of the samples held
112 out from training set, Fig.1A). Thus, we were able to often correctly detect *TP53* status
113 in unseen tumor samples the classifier was not exposed to, with an area under
114 precision-recall curve=0.9654. The *TP53* loss phenocopy scores for each TCGA tumor
115 sample and the cancer cell lines are provided in Supplementary Data 1.

116
117 Out of the ~12000 genes available to the classifier, 217 genes were deemed relevant
118 for *TP53* status classification (non-zero coefficients; gene score provided in
119 Supplementary Data 2). These represent a sparse (but not necessarily exhaustive) set
120 of genes that are, considered together, highly informative for predicting *TP53* status.

121
122 Expectedly, many of the classifier's most relevant genes are known to be related to
123 *TP53* functionality. For instance, *apoptosis-enhancing nuclease (AEN)* was the gene
124 with the highest absolute importance score. This exonuclease is a direct *TP53* target
125 whose expression is regulated by the phosphorylation of *TP53* and its tumor
126 suppressor role has been reported (25). Tumors with a high expression of *AEN* are
127 expected to be p53 functional, and indeed highly expressed *AEN* was associated with
128 *TP53* WT status in our classifier's coefficients. On the other extreme, *COP1*, a ubiquitin
129 ligase that acts as an important p53 negative regulator, was the strongest coefficient
130 associated with *TP53* mutated status in the classifier (26). We further performed a GO
131 enrichment analysis, revealing that top functional enriched sets were related to
132 apoptotic signals, supporting the biological rationale underlying this set (Supp Fig. 1A).

133 Most enriched pathways were: *Intrinsic apoptotic signalling pathway in response to*
134 *DNA damage by p53 class mediator* (8.1-fold enrichment, FDR=4.2%), *Pyrimidine*
135 *deoxyribonucleoside monophosphate biosynthetic process* (47.4 fold enrichment,
136 FDR=1.9%) and *Response to UV-B* (17.2 fold enrichment, FDR=3.7%) (ShinyGO, see
137 Methods).

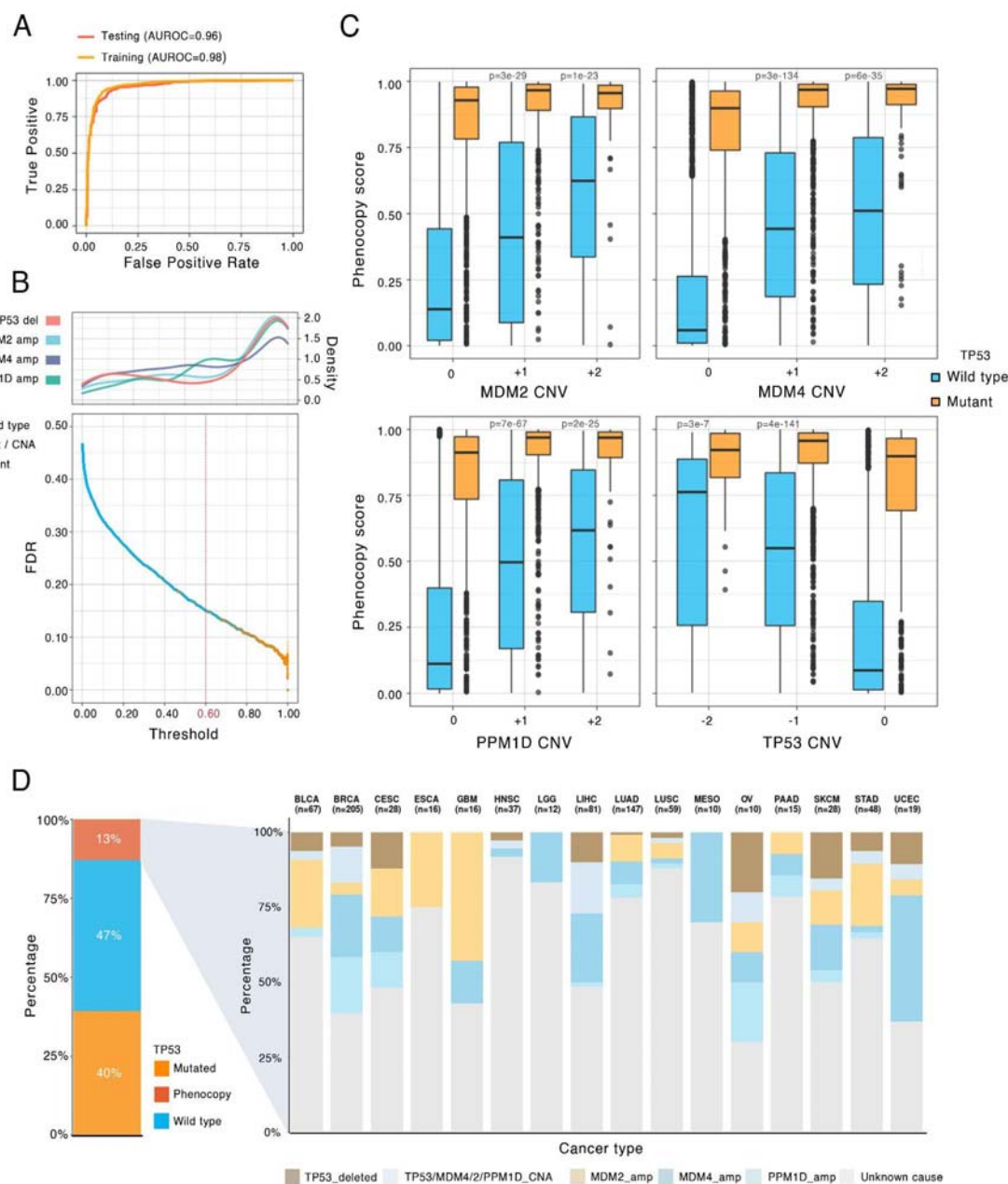
138
139 Our classifier extends recent gene expression-based models for TP53 functionality (20-
140 23) by being able to generalize across both tumor and cancer cell lines (important for
141 identifying drug sensitivity associations, see below), and moreover it can provide
142 calibrated FDR estimates for TP53 status of each tumor or cell line. In particular, to
143 assess the reliability of the individual predictions from the model, FDR for each tumor
144 was computed via the analysis of cross-validation precision-recall curves (Fig.1B). The
145 previously known phenocopies (*MDM2*, *MDM4* and *PPM1D* amplifications) and *TP53*
146 deep deletions, which were held out from the training set, were largely scored as *TP53*
147 mutated. Tumors harbouring a known phenocopying amplification were assigned
148 higher scores than the rest of *TP53* wild-type tumors (means=0.56 and 0.27
149 respectively, $p=1e-65$ by t-test). Cells harbouring a *TP53* deep deletion also had higher
150 scores (mean *TP53* deleted=0.47, mean *TP53* not deleted=0.27, $p=1e-08$). Our choice
151 of threshold to detect *TP53* phenocopied tumors was set based on these known
152 phenocopies, conservatively, corresponding to score >0.6, Methods; Fig.1B).

153
154 This resulted in an empirical FDR estimated at 15% (i.e. precision of 85%), based on
155 the known *TP53* mutations. Importantly this 15% is a conservative upper-bound
156 estimate of FDR, since it is based on the assumption that there do not exist any
157 unknown *TP53* phenocopying events: it classifies all high-scoring *TP53* *wild-type*
158 tumors as false positives. Conversely, using the known phenocopying events we
159 estimate a lower-bound recall (sensitivity) of this classifier at 63% (Fig. 1B). Again, this
160 estimate is conservatively biased, since it is not a priori known whether every copy
161 number gain in *MDM2/MDM4/PPM1D* causes a phenocopy; some low-level gains may
162 not have effects and thus would appear as false-negatives.

163
164 To additionally validate the classifier, we inspected the relationship between known
165 phenocopy genes' allele copy-number (see Methods), and the *TP53* phenocopy score.
166 There were significant positive correlations between three known phenocopying genes
167 copy-number, and the *TP53* phenocopy score in *TP53* wild-type tumors (Fig.1C).

168
169 The prevalence of phenocopying events was substantial: overall 12 % of all tumor
170 samples were redefined into a *TP53* mutated-like category (Fig.1D) by our criteria.
171 Different cancer types display different phenocopy frequencies (Fig.1D), overall
172 frequency ranging from 19% for breast cancer (BRCA cancer type) to 3% for B-cell
173 lymphoma (DLBC cancer type, overall phenocopy frequencies are shown in Supp Fig.
174 1B). For instance, most breast cancer *TP53*-phenocopied tumors derive from
175 previously known events i.e. the *MDM4/MDM2/PPM1D* amplifications are the most
176 common event, while a remaining 27% of the phenocopies (5% of all breast cancer
177 samples) is not associated with a known phenocopying event (proportion shown for
178 every cancer type Fig.1D). We do note that it is still possible that individual examples of
179 tumor may be erroneously classified as TP53-deficient at this threshold. More
180 generally, 51% of *TP53*-loss phenocopied tumor samples across all cancer types were

181 not linked with one of the three known genes nor a CNA deletion in *TP53* itself,
 182 suggesting that additional *TP53* phenocopying mechanisms are commonly occurring in
 183 tumors.



184
 185 **Figure 1. Evaluation of the functional *TP53*-loss score classifier and prevalence**
 186 **of *TP53* loss phenocopying events in cancer.**
 187 A. Receiver operating characteristic (ROC) curve and area under the ROC (AUROC) curve for
 188 training and testing sets in TCGA tumor transcriptomes.
 189 B. Bottom: False discovery rate (FDR) for each tumor sample. X axis is the classification
 190 threshold for each tumor sample. The general threshold used for classification (0.6) is
 191 highlighted. Top: the histogram of frequency of CNV events (“density” refers to smoothed
 192 relative frequency) affecting *TP53* and the known phenocopying genes *MDM4*, *MDM2* and
 193 *PPM1D* at various phenocopy-score thresholds.

- 194 C. *TP53* loss phenocopying score stratified by 3 known phenocopying CNA events and by
195 *TP53* deletions. Data points are tumor samples coloured by *TP53* status; boxes show
196 median, Q1 and Q3, while whiskers show range (outlying examples shown as separate
197 dots). X axis represents the GISTIC thresholded CNV of each given gene. Tumor samples
198 with deletions in the corresponding genes (for *MDM2*, *MDM4* and *PPM1D*) and
199 amplifications (*TP53*) are omitted for simplicity. P values represent results from the t-test
200 comparison of the *TP53* phenocopy score between each CNV category to neutral CNV (0)
201 category in *TP53* wild-type samples.
- 202 D. *TP53* functional status classification across TCGA cancers. Left: pan-cancer; “*Phenocopy*”
203 refers to *TP53*-loss phenocopying tumors according to the classifier in panels A, B. Right:
204 showing only the *TP53* loss phenocopying tumor samples, stratified by cancer type and by
205 cause of the phenocopy. Tumor samples harbouring a known event that affects *TP53*
206 functionality are shown with colours, and the remaining *TP53*-loss phenocopy tumors are
207 labelled as “Unknown cause

210 ***USP28* deletion phenocopies a *TP53* mutated state in tumors**

211
212 Prompted by the abundance of tumor samples that are functionally *TP53* null but
213 lacking an obvious *TP53* loss or a known phenocopying event, we sought to identify
214 other phenocopying genes across all cancer types. We designed a custom association-
215 testing methodology that combines six different statistical tests across four different
216 genomic data types with this goal (see Methods).

217
218 In brief, our methodology is based on the rationale that genes that cause a phenocopy
219 via altered dosage at DNA and mRNA levels should exhibit a distinct copy number
220 variant (“CNV” tests) and also gene expression (“GE” tests) pattern. Each of these two
221 genomic data types is considered in two tests, one comparing *TP53* phenocopying
222 against *TP53* wild-type tumors, and other comparing *TP53* phenocopying against
223 *TP53*-mutant tumors, for a total of four tests. As two additional tests, we considered
224 external data from genetic screens across large panels of cancer cell lines (28,29). In
225 particular we test for significant codependency scores, explaining how a knockout
226 (“CRISPR”) or knock-down (“RNAi”) of a candidate phenocopying gene affects fitness
227 across a panel of cell lines, when compared with the fitness profile of a *TP53*
228 knockout/knock-down across the same panel(30, 31). An example supporting the use
229 of this methodology that combines cancer genomic analysis and genetic screening
230 data analysis, a CRISPR knockout of the known *TP53* negative regulator *MDM2*
231 decreases cell line fitness, in a manner anticorrelated to a *TP53* knockout across cell
232 lines. (Supp Fig. 3A)

233
234 In summary, we tested differences of tumor genomics CNV and GE patterns (two tests
235 each as above), additionally considering “CRISPR” and “RNAi” test scores from genetic
236 screens, for each gene, performing tests stratified by cancer type. Our final score
237 combines each of the 6 tests together providing a ranking of potential *TP53*
238 phenocopying genes.

239
240 As anticipated, top 3 prioritization scores correspond to *MDM2*, *MDM4* and *PPM1D*
241 genes (Fig. 2A). Following those known *TP53* phenocopies, the gene *USP28* was the
242 4th ranked gene in terms of overall statistical significance ($p=5.9e-07$, combined across

243 all six tests), and in particular scored highly on CRISPR codependency (pan-cancer
244 score for *USP28*=0.54, compared with -0.71 for *MDM2* and -0.53 for *MDM4*). A break-
245 down of our *custom prioritization* scores by different cancer types is provided in
246 Supplementary Figure 2. We note that, in contrast to *MDM2* and *MDM4*, it is the
247 deletions not amplifications of *USP28* that were associated with *TP53* phenocopies;
248 this is reflected in the mirrored direction of the codependency score. *USP28* encodes a
249 deubiquitinase enzyme with substantial evidence from previous biochemistry and cell
250 model studies that link it to p53 activity. In particular, *USP28* was linked to DNA
251 damage apoptotic response through the Chk2-p53-PUMA pathway (32). Recent
252 evidence suggests that the *TP53BP1-USP28* complex might positively regulate p53
253 and influence arrest after centrosome loss and prolonged mitosis (33). It has been
254 proposed that *TP53BP1-USP28* complexes could counteract *MDM2*-dependent p53
255 ubiquitination (34). Additional studies have linked *USP28* loss with a defective
256 apoptotic response (35). A 10% of the total of 437 tumors classified as *TP53* loss
257 phenocopied but with an undefined source (Supp Fig.1B) had a *USP28* deletion.

258

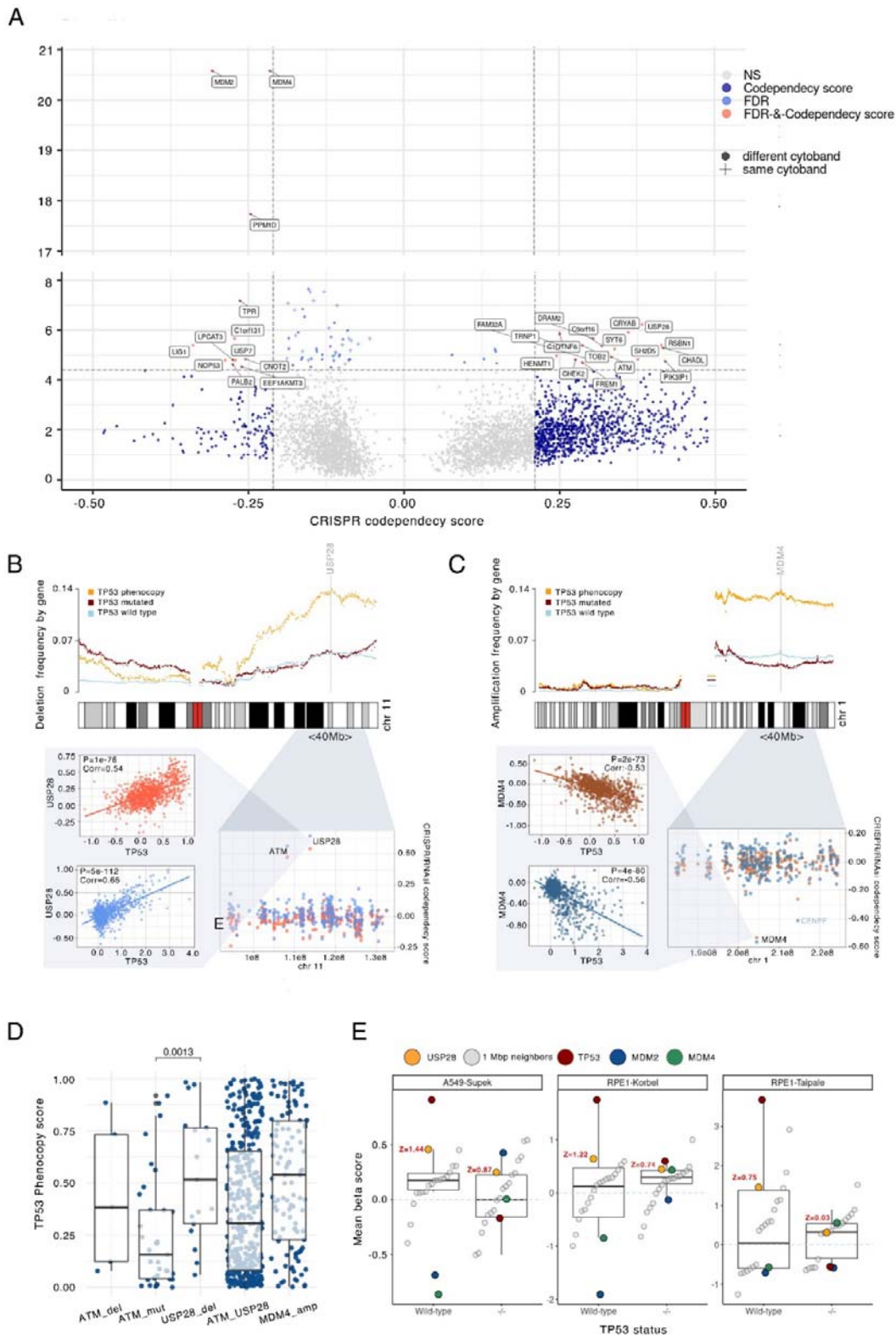
259 Overall, diverse experimental evidence from genetic screens strongly supports our
260 identification of *USP28* deletions as p53-loss phenocopying events, and our genomic
261 analysis suggests a widespread distribution of causal *USP28* deletions across human
262 tumors.

263

264 Additional hits from this association study might provide promising genes for follow-up.
265 For instance, *MSI2* was the 5th most highly prioritized gene, predicted to phenocopy
266 *TP53* loss by amplification. *MSI2* encodes a transcriptional regulator that has been
267 recently identified as an oncogene in hematologic and solid cancers (36–38). Similar
268 results to CRISPR analyses were observed using RNAi screening codependency
269 scores, further supporting the role of *USP28* loss in the *TP53* phenocopying, as well as
270 *MSI2* gains (Supp Fig. 3B). Other apoptosis-related genes such as *DRAM2*, *CHEK2*, or
271 *ATM* (39–41) were also in the prioritized genes in our analysis albeit at more modest
272 statistical significance. Of note, the *TPR* gene also had a highly significant
273 codependency score but was driven by a single cancer type (kidney) and thus with less
274 clear relevance to diverse tumor types.

275

276



277

278 **Figure 2: Transcriptomics scores predicting phenocopying events can pinpoint**
 279 **causal genes in CNA-affected chromosomal segments.**

280 **A.** Prioritization score of genes for *TP53* loss phenocopying effects. Y axis shows gene
 281 significance (FDR) when combining six statistical tests (two cancer genomic/transcriptomic,

282 and two based on CRISPR and RNAi screens), and further pooling p-values across cancer
283 types; see Methods for details. X axis represents the effect size from the CRISPR
284 codependency score of each gene. Crosses represent gene neighbours (same cytoband) to
285 a known phenocopying gene. Relevant hits in terms of FDR and codependency score are
286 labelled. Shown thresholds for effect size and significance were determined based on
287 scores of known phenocopy events (Methods).

288 **B.** Top: CNV frequency in tumors, and their associations with *TP53* phenocopy transcriptomic
289 scores, of the segment of chromosome 1 containing *MDM4*. Each dot represents one gene,
290 while colours represent groups of tumor samples by *TP53* status. Bottom: A zoomed-in view
291 of a commonly amplified region of the chromosome, showing the CRISPR (blue) and the
292 RNAi (red) *TP53*-codependency scores for each gene. The determination of the *TP53* co-
293 dependency score is shown for the top score of the region (left panels), showing the actual
294 CRISPR and RNAi fitness effects for the *MDM4* disruption (Y axis) across many cell lines
295 (dots), compared to *TP53* disruption fitness effects (X axis) across the same cell lines.

296 **C.** Same as (B), but for *USP28*, a gene we identified to be associated with a *TP53* loss
297 phenocopying via a deletion. Here, the y axis on the top plot shows frequency of gene
298 deletions in tumors, divided by *TP53* functional status, whereas panel B shows frequency of
299 amplification. Bottom plots are the same as in B.

300 **D.** Comparison of the *TP53* phenocopy score of *USP28* CNV deletions (by negative GISTIC
301 score), *ATM* deletions, *ATM* mutations and *MDM4* amplifications. Each dot represents a
302 tumor sample. Only *TP53 wild-type* samples were considered. P-values by Mann-Whitney
303 test.

304 **E.** Fitness effect of *USP28* knock-out in *TP53 wild-type* and mutant isogenic cell lines.
305 Comparison of the mean beta score (fitness effect upon CRISPR gene disruption, y-axis) of
306 *USP28*, with the mean beta scores of genes located within its 1Mbp immediate
307 surroundings as negative controls ("1 Mbp neighbours", see Methods). Genes *TP53*,
308 *MDM2*, and *MDM4* are also shown as a reference. x-axis bottom labels indicate the *TP53*
309 status of the cell line. *USP28* Z-scores, comparing to the distribution of neighbouring
310 genes, are plotted in red (see Methods)

311
312
313

314 **Phenocopy scores prioritize causal genes in CNA-affected** 315 **chromosomal segments**

316

317 Amplifications of certain chromosomal segments or whole arms in case of *MDM2*,
318 *MDM4* and *PPM1D* commonly underlie *TP53* phenocopies. Such CNA genetic events
319 in cancers however often affect multiple adjacent genes, where an open question in
320 cancer genomics is which of the gene or genes in the affected segment are causal
321 (42). We hypothesized that the known *TP53* phenocopying gene CNA segments might
322 in some cases harbor more than one causal gene. Our combination-test approach can
323 prioritize genes with enriched gene expression and CNA in our *TP53* phenocopying
324 group. Considered together with CRISPR and RNAi codependency, this method
325 provided a plausible ranking of possible *TP53* loss phenocopying genes. Applied
326 globally, this identified *USP28* as a novel phenocopying gene (see above). To more
327 formally study if the *USP28*-adjacent genes could contribute to this, we considered that
328 the same method could be applied on a local scale: examining profiles of CNVs and
329 our genomic prioritization scores would be able to single out the causal gene(s) in the
330 chromosomal segment of recurrent CNA.

331

332 As a control for this approach, we sought to confirm previously known phenocopies.
333 Indeed, *MDM4* amplification was found to be enriched in the *TP53*-phenocopying group
334 of tumor samples, but not in the rest of tumor groups --the *TP53* mutant and the non-
335 phenocopying *TP53* wild-type (Fig. 2B). The local profile of this enrichment for the
336 chromosome 1q segment 32.1 peaks at the *MDM4* gene and falls off towards its
337 flanking genes (Fig. 2B). Our CRISPR and RNAi data analysis, consistently, indicate
338 *MDM4* as the gene with the strongest effect in the region (Fig. 2B). As expected,
339 similar CNA and CRISPR/RNAi profiles were observed at *PPM1D* (Supp Fig. 3C).
340 Next, the *MDM2* CNA enrichment score segment peak was narrower, suggesting a
341 more focal gene amplification process (Supp Fig. 3C)

342

343 Next, we examined the shape of the local *USP28*-adjacent CNA profiles. *USP28*
344 deletions were found to be enriched in the *TP53* phenocopying group when compared
345 to the rest of tumor groups (2.3-fold in *TP53* w.t, 2.8-fold in *TP53* mutant). *USP28*
346 enrichment was comparable to *MDM4* region enrichments of 2.5-3.7-fold (*TP53* wt.,
347 *TP53* mutant) (Fig. 2B, C). *TP53* phenocopying tumor samples appear to have more
348 deletions in the *USP28* region than *TP53* wild-type (non-phenocopying) and *TP53*
349 mutant samples. The local profile of enrichments presents a plateau-like pattern rather
350 than a sharp peak, and *USP28* is within the top-ranked genes in the plateau however
351 some neighbouring genes appear similarly so. Therefore, we further used the CRISPR
352 and RNAi codependency scores to prioritize the causal genes in the segment; this
353 score clearly distinguishes *USP28* from immediate neighbours (Fig. 2C), suggesting
354 that *USP28* is indeed the main causal gene in the chromosomal segment.

355

356 Furthermore, this 'local scan' can be applied chromosome-wide, where we noted
357 another small region on chromosome 11q.12.1-q1.13.1 modestly enriched with
358 amplifications in *TP53*-phenocopying tumors (Supp Fig. 3D), thus raising our interest.
359 However, neither genes in this region nor other chromosome 11 regions showed a
360 positive CRISPR codependency score of even half of *USP28* score (Fig. 2C). We note
361 here that the *USP28* codependency score exceeds, in absolute magnitude, the score
362 of the known *MDM4* phenocopy (Fig. 2B, C).

363

364 In the broader neighborhood of *USP28*, the gene *ATM* seems to also be frequently
365 deleted in the *TP53*-phenocopying tumor group, meaning *ATM* is also a candidate for
366 the causal gene in this deletion segment at chr11 q22.3-q23.2. However, the statistical
367 support from genomic enrichment scores (using our custom methodology for
368 meta-analysis across 6 statistical tests) for *ATM* were less strong than for *USP28* ($p=1e-5$
369 versus $p=6e-7$, respectively). Consistently, comparing the RNAi and CRISPR *TP53*-
370 codependency scores of *ATM* versus *USP28* shows a stronger effect of the *USP28*
371 knockout (*USP28* RNAi codependency score $p=4.9e-112$ versus *ATM* $p=3e-80$, in a
372 pan-cancer analysis; Supp Fig. 3E). To further rule out that *ATM* has the causal role in
373 this deleted segment, we considered the cases of tumors where *ATM* is disrupted by a
374 point mutation; unlike CNA in the *ATM* gene, these cases are not commonly linked with
375 disruptions in *USP28*. The *ATM* mutated but *USP28* wild-type tumors had considerably
376 weaker *TP53* phenocopy transcriptomic scores (median=0.36) than the *USP28* deleted
377 but *ATM* non-mutated tumors (median=0.84; $p=0.0013$ by Mann-Whitney test; Fig. 2D).
378 The cases where both *USP28* and *ATM* were disrupted, by deletion or mutation, had

379 very similar phenocopy scores (median=0.73) as the *USP28* deleted but *ATM* non-
380 mutated cases. This analysis of *ATM* mutations supports that *USP28* deletion, rather
381 than *ATM* disruption, is the causal change in the deleted segment at chr11 q22-q23.

382

383 To validate the *USP28* finding, we analyzed an independent CRISPR data set,
384 consisting of 3 genome-wide screens performed on *TP53* wild-type and *TP53* -/
385 isogenic pairs of cell lines: one on the A549 cell line pair and two on the RPE1 cell line
386 pairs (see details in Methods). In the *TP53* wild-type background, the *TP53* k.o.
387 increases cell fitness (as expected for a high-effect tumor suppressor gene; Fig. 2E).
388 Thus, if the *USP28* loss were to phenocopy *TP53* loss, the *USP28* k.o. by CRISPR
389 should also increase fitness. Indeed, it does so: compared to the 10 neighboring
390 control genes residing within 1 Mb of *USP28*, the *USP28* k.o. has a stronger fitness
391 effect (beta score from MAGeCK tool, see Methods) for 10 out of 10 genes in 2 out of 3
392 screens, and 8 out of 10 neighboring genes in the remaining screen (Fig. 2E). For
393 *ATM*, this effect is less pronounced (Supp Fig. 3F). In 3 out of 3 cell screening
394 experiments, *USP28* fitness effect was stronger than *ATM* effect (1.4-fold, 2.4-fold and
395 2.6-fold increased beta score). To further support this finding, we asked if the fitness
396 gain resulting from *USP28* loss is because of downstream effects on *TP53* activity. We
397 thus considered the isogenic cells where *TP53*-/ was ablated, in which indeed the
398 fitness gain from *USP28* k.o. was attenuated or disappeared (Fig. 2E) compared to
399 *TP53* wild-type cells. In 2 out of 3 cell line screens, the fitness attenuation effect of
400 *TP53*-/ background cells was stronger in *USP28* than in the neighboring *ATM* gene,
401 supporting the causal role of *USP28* in that segment (Supplementary Data 3). Of note,
402 in this analysis the effect sizes of *USP28* k.o. were less than of full *TP53* k.o., however
403 they were still substantial: in 2 out of 3 screens considered, the fitness gain effect of
404 *USP28* disruption was comparable in size to the fitness loss effect of *MDM4* disruption
405 (Fig. 2E).

406

407 Overall, these analyses highlight *USP28* as the likely causal gene for *TP53* loss
408 phenocopying via deletion CNVs in the chr11 q22-q23 segment.

409

410 **Cancer type specificity of *TP53* phenocopying events**

411

412 As stated above, not every cancer type seems to be affected by the same types of
413 phenocopies. For instance, *MDM2* amplification phenocopy occurs often in BRCA,
414 CESC, BLCA, LUAD and STAD but it does not in HNSC, OV, MESO nor LIHC
415 (Fig.1D). To further elucidate the tissue-specificity of *USP28* phenocopying events, we
416 considered the prioritization scores separately for different cancer types (Supp Fig. 2).
417 We observed that BRCA, BLCA and LUAD were the cancer types which showed the
418 strongest signal in our prioritization score for *USP28* phenocopies, with a suggestive
419 signal in STAD.

420

421 To elucidate the cancer type spectrum of the *USP28* phenocopies, we considered the
422 *USP28* amplifications as a negative control (deletions are expected to phenocopy). In
423 particular, we determined in which tumor types *USP28* deletions had a higher *TP53*
424 phenocopy score than *USP28* copy number amplified samples. As expected, statistical
425 significance when comparing the *TP53* phenocopy score of *USP28* copy number-

426 neutral tumor samples versus those bearing deletions was higher than comparing
427 neutral to amplifications. This supports that the impact of *USP28* deletions on *TP53*
428 loss phenocopy score was stronger than for the amplification CNVs. The strongest
429 effect was found in BLCA, STAD, BRCA, LIHC and LUAD (Fig. 2E). In further support
430 of this tissue spectrum, when CRISPR *TP53* codependency scores were checked,
431 highest *USP28* scores were found in cancer cell lines originating from BLCA, STAD,
432 BRCA, LIHC, LIHC and LUAD (Fig. 2E). The leading codependency score was found in
433 BLCA (Effect size=0.73, $p=2.2e-08$) and BLCA also had the most significant value
434 when comparing deletions to neutral copy numbers *TP53* phenocopy score ($p=4.2e-06$,
435 Supp Fig. 3G). LUAD had the second most significant codependency p -value
436 ($p=3.78e-6$), and is also highly ranked in comparison of phenocopy score between
437 deletion *versus* neutral *USP28* CNV tumors (Fig. 3F). We found a positive association
438 between *USP28* CRISPR codependency score and the effect of *USP28* deletions in
439 *TP53* phenocopying score across cancer types (Supp Fig. 3G). Of note, that the
440 “oncogene-tumor suppressor” dichotomy of *USP28* was reported (43), which might
441 imply that *USP28* amplification could also result in a *TP53* phenocopy in certain
442 contexts. However, our analysis did not support this in the majority of cancer types: out
443 of 14 cancer types, only 3 of them had a stronger *TP53* phenocopy score in *USP28*-
444 amplified samples than in *USP28*-deleted samples (Fig. 2E); this was the case for none
445 of the primary cancer types for *USP28* phenocopying (BLCA, STAD, LIHC, BRCA and
446 LUAD).

447

448 Taken together, these results suggest that *USP28* deletion is a novel *TP53* phenocopy
449 that commonly affects major cancer types such as breast cancer (6.2% of total breast
450 tumors, not counting known phenocopying events and *TP53* deletions) and also
451 bladder, lung, liver and stomach cancer (7.6 %, 7.0%, 3.8% and 2.9% cases).

452

453

454 **Multiple neighboring genes in a CNA segment can contribute to** 455 **a *TP53* loss state**

456

457 Some of the top hits found in our combined testing approach were near to known *TP53*
458 loss phenocopying genes such as *MDM2*. We thus hypothesized that there exist cases
459 of ‘collaboration’ of neighboring genes, affected by a single copy-number alteration,
460 which may bear upon the *TP53* loss phenotype. This would represent a special case of
461 epistasis between two genes, caused by a single alteration that affects both genes. Our
462 data suggests that the *CNOT2* gene, residing in the *MDM2* segment in the
463 chromosome 12q15, is likely an example of this relationship.

464

465 In particular, in our data, *MDM2* was frequently co-amplified with *CNOT2*, in 72% of the
466 cases of *MDM2* amplification (Supp Fig. 4A, check by cancer type at Supp Fig. 4B).
467 Data from CRISPR and RNAi screening experiments can help resolve associations
468 from genomic analysis, where effects of neighboring genes are in genetic linkage (here
469 implying being jointly affected by CNA). No other gene in that neighborhood that was
470 amplified together with *MDM2* had as high CRISPR codependency scores as *CNOT2*
471 (effect size=-0.24, $p=4.1e-14$, Fig. 3A, B); next best gene in the 20Mb neighborhood is
472 *CDK4* with effect size=-0.16, $p=3e-7$. However, *CDK4* is co-amplified with *MDM2* in

473 only 20% of the cases (Fig. 3A). *CNOT2*-only amplifications (i.e. without concurrent
474 *MDM2* CNA) do not significantly associate with *TP53* phenocopy score (Pearson's
475 *TP53* phenocopy score vs *CNOT2* CNV $p=0.45$, effect size=-0.83, Supp Fig. 4C). More
476 interestingly, *MDM2* CNV was not found to be associated with our *TP53* phenocopy
477 score when *MDM2*-only amplified without *CNOT2* (Pearson's *TP53* phenocopy score
478 vs *MDM2* CNV $p=0.57$, effect size=0.09, Supp Fig. 4C). On the other hand, *MDM2*-
479 *CNOT2* co-amplifications were significantly associated with a *TP53* deficiency
480 transcriptomic score in tumors (Pearson's correlation *TP53* phenocopy score vs *MDM2*
481 CNV $p=2e-05$, effect size=0.41, Supp Fig. 4C).

482

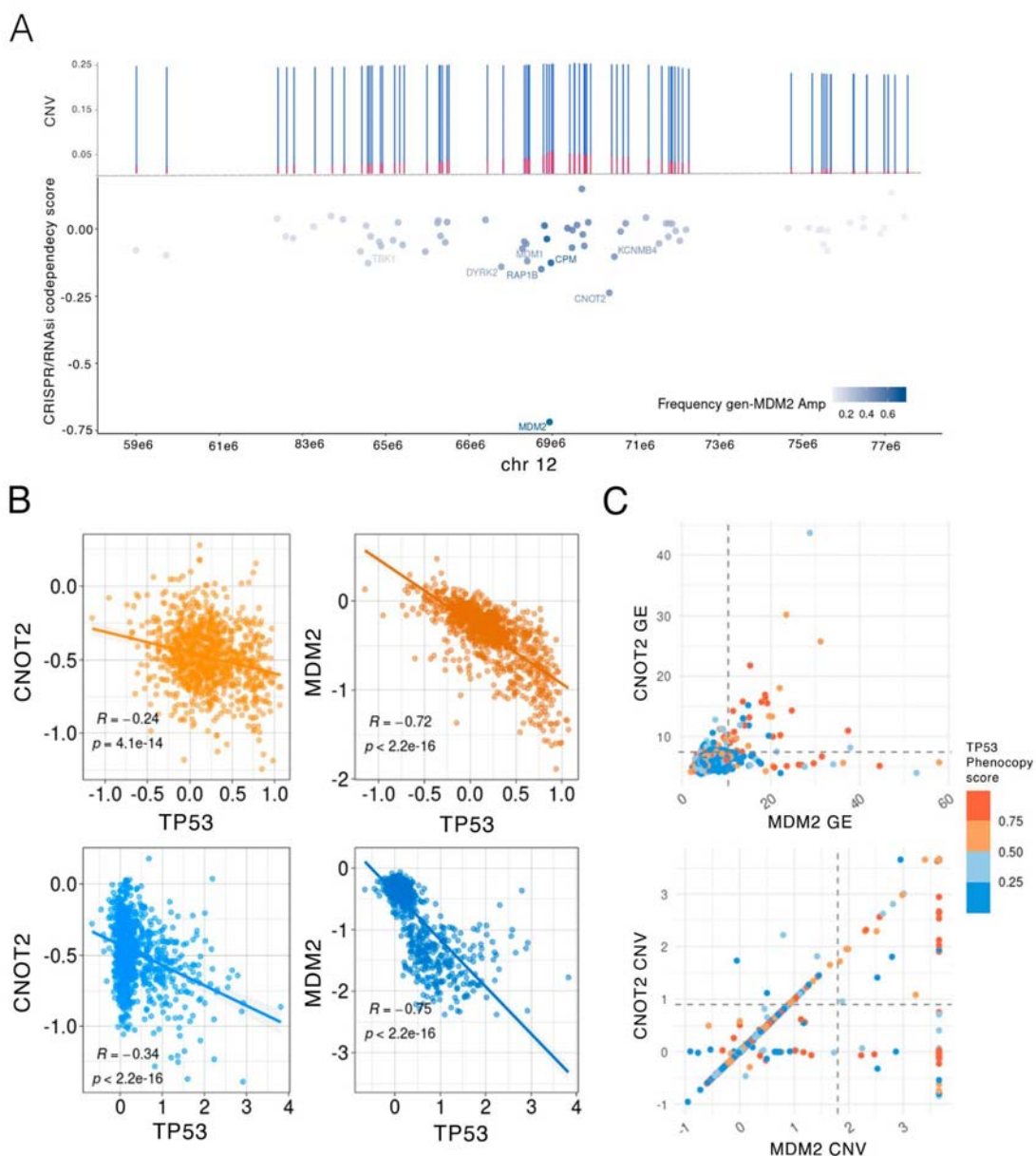
483 This genomic evidence we provide here is supported by recent experimental studies,
484 indicating a role for *CNOT2* in p53-dependent apoptosis, and suggesting therapeutic
485 potential of *CNOT2* suppression (see Supplementary Text S1 for a summary and
486 references). As supporting evidence, we considered fitness effects of *CNOT2* k.o. by
487 CRISPR in various subsets of cell lines. The *MDM2*-gain but *CNOT2*-neutral genetic
488 backgrounds had more modest fitness effects of *CNOT2* k.o. (median=-0.37) than the
489 *CNOT2*-gain but *MDM2*-neutral genetic backgrounds (median=-0.62; $p=0.072$ by
490 Mann-Whitney test, Supplementary Fig. 4D). Consistently, the *CNOT2* k.o. by CRISPR
491 had stronger fitness effects (median=-0.55) in the *TP53* *wild-type* backgrounds than in
492 *TP53*-mutant background cell lines (median=-0.45, $p=0.0091$ by Mann-Whitney test).
493 In other words, fitness effects of *CNOT2* disruption by CRISPR are conditional upon
494 *MDM2* alterations and *TP53* alterations, implicating *CNOT2* in a genetic interaction with
495 the two other genes.

496

497 We hypothesized that this role of *CNOT2* in boosting the *TP53*-phenocopying effect of
498 *MDM2* amplification may be variable across tissues. Our data suggests that in some
499 cancer types *TP53* functional loss seems to rely on amplifications of both genes
500 together, rather than solely *MDM2*, but not all (Supplementary Text 2). This suggests a
501 model where the *MDM2-CNOT2* coamplification enhances the *TP53* loss effect via a
502 genetic interaction, and of *MDM2* alone but not *CNOT2* alone able to generate a *TP53*
503 functional loss phenotype. Gene expression profiles match this observation seen in
504 CNA: having a *MDM2* and *CNOT2* co-overexpression (over the 97th percentile; $n=40$)
505 implies a high mean *TP53* phenocopy score (above the 84th percentile, mean
506 phenocopy score *MDM2_CNOT2*=0.65, Fig. 3C, Supp Fig. 4F), however less so for a
507 *MDM2*-only overexpression (76th percentile; mean *MDM2* only=0.46, Fig 3 C, Supp
508 Fig. 4F), and, expectedly, even less so for a *CNOT2*-only overexpression (73th
509 percentile; mean phenocopy score *CNOT2* only=0.41).

510

511 This principle might extend beyond the *MDM2-CNOT2* pair. For instance, the *MSI2*
512 gene, another highly prioritized hit in our combined test (Supp Fig. 4 G, H, I), resides
513 near the known phenocopying gene *PPM1D* and thus has the potential to boost the
514 effects of the linked amplification of the *PPM1D* gene to cause a *TP53* deficient state.
515 Considered jointly, these findings suggest the possibility of *TP53*-loss like phenotype
516 being a result of multiple phenocopying events generated by a single segmental CNA.



517 **Figure 3. *MDM2-CNOT2* co-amplifications are associated with *TP53*-loss**
 518 **phenocopy score.**

519 **A.** Top: CNV of *MDM2* gene neighborhoods (20Mb segment). Y axis represents the
 520 percentage of GISTIC CNV gain states +1 (blue) and +2 (red), compared to neutral CNV
 521 state (0). Bottom: CRISPR *TP53*-codependency scores (y axis) shown by gene on
 522 chromosome 12 (x axis). Genes labeled have a codependency score <math>< -0.1</math>, suggesting
 523 *TP53* phenocopying effects. Color shows the frequency of CNV amplification of each gene,
 524 together with *MDM2* amplifications.

525 **B.** Co-dependency source data. CRISPR and RNAi fitness effect scores for phenocopying
 526 gene *MDM2* and candidate gene *CNOT2* (y axis), and fitness effect scores for *TP53* in the
 527 genetic screens (x axis). Top plots represent RNAi screening data and bottom plots
 528 CRISPR screening data.

529 **C.** Association between *MDM2* and *CNOT2* gene expression (GE, top) and CNV status
 530 (bottom). Each dot represents a tumor sample, coloured based on the *TP53*-loss
 531 phenocopy score provided by the classifier. Dashed lines represent the 97th quantile
 532 across genes, for each data type.

533 **Detecting *TP53* loss phenocopies in cancer cell line panels**

534

535 It is well known that *TP53* mutations associate with overall poorer drug response in
536 tumors (44–46), consistent with a lower ability of *TP53* deficient cells to trigger cell
537 cycle arrest and/or apoptosis response(47–51). We hypothesized that, in addition to
538 conferring a generalized drug resistance, the *TP53* function loss may also modulate the
539 association between certain drugs and their target genes. In other words, we asked
540 whether in *TP53* *wild-type* cancer cells, for instance, amplification in a particular
541 oncogene predicts sensitivity to a particular drug, while in *TP53* mutant cells the same
542 amplification does not associate with sensitivity. Cancer cell line screening panels (52,
543 53) are a convenient system to test this hypothesis, because many drugs were tested
544 systematically across both *TP53* wild-type and mutant cells of multiple cancer types.
545 Considering *TP53* function loss via phenocopy should afford additional statistical power
546 and clarify the associations discovered; otherwise, some effectively *TP53* null cells
547 would be erroneously considered wild-types during association testing, making it more
548 difficult to identify associations.

549

550 First, we aimed to generalize our tumor *TP53* phenotype classifier to cancer cell lines.
551 Because cell lines exhibit strong global (i.e. affecting many genes) shifts in gene
552 expression patterns, compared to their tumor tissue of origin, we applied an adjustment
553 methodology as in our recent work (54), using the COMBAT tool (55).

554 Upon adjusting gene expression data from cell lines in the CCLE and GDSC panels to
555 make it comparable with TCGA tumor data (see Methods), we applied the *TP53*
556 classifier and obtained ranked scores. Reassuringly, the classifier assigned a
557 significantly higher *TP53* phenotype score to *TP53* mutated cell lines (mean
558 *TP53*_wt=0.43, *TP53*_mut=0.83, $p=1.1e-49$ t-test), therefore cell line data served as
559 an independent validation set for the classifier. Of the 610 cell lines labeled as *TP53*
560 mutant based on genomic sequence (see Methods), 87% were classified as *TP53*-loss
561 phenotype (Fig. 4A), suggesting a reasonable ability of the classifier trained on TCGA
562 tumors transcriptomes to generalize to cell line data.

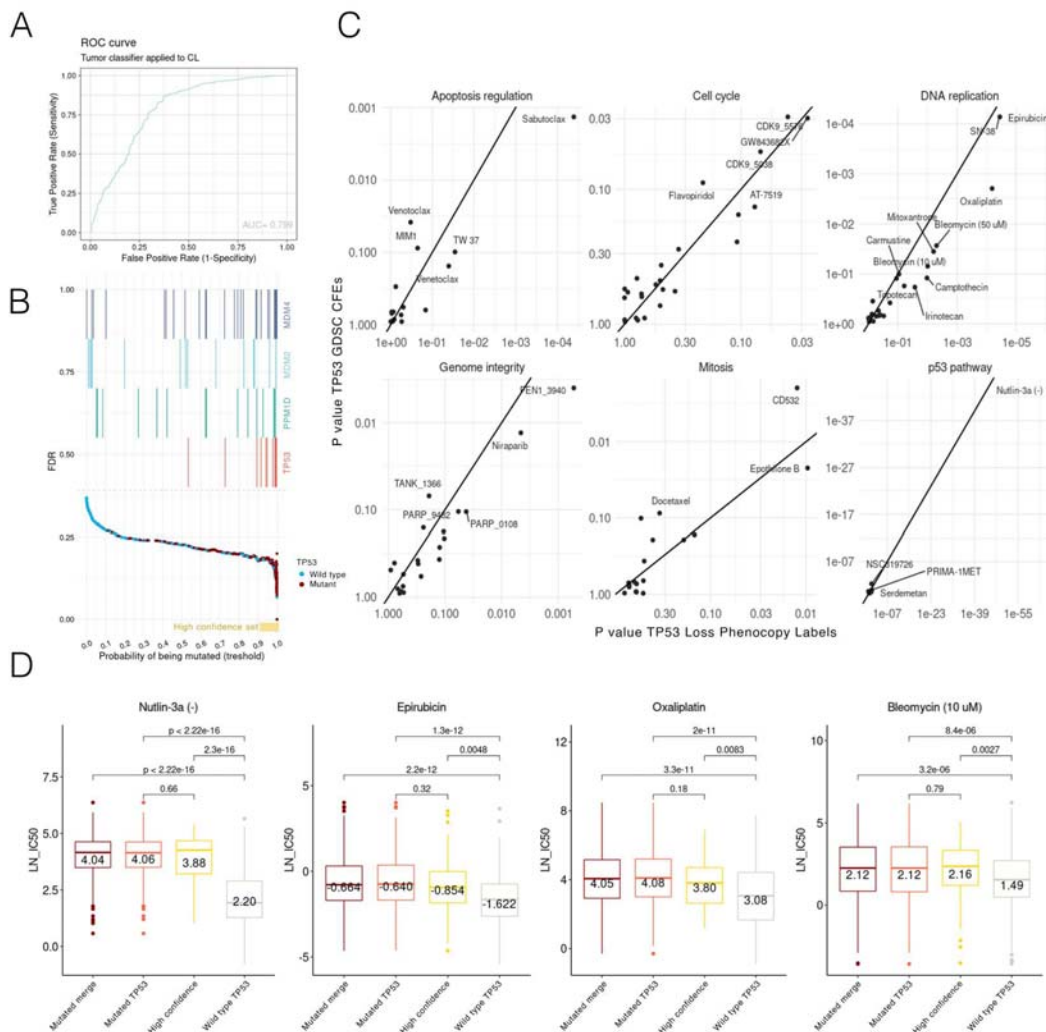
563

564 Similarly, as in tumors, a notable fraction of cell lines were apparent false positives,
565 labelled as *TP53* wild-type by the genomes, but classified as *TP53* deficient using the
566 phenocopy score. We stratified these apparent false positives into a high-confidence
567 set (“high-confidence set”); the *TP53* phenotype score of the *TP53* deleted tumor
568 samples was used as the threshold (see Methods). The high-confidence set was
569 composed of 76 cell lines (FDR=18%, see Methods, Fig 4 B). Only 79% of the total
570 number of cells labelled as *TP53* wild-type genetically were also classified as *TP53*
571 wild-type by the phenocopy score, suggesting that *TP53*-loss phenocopying events are
572 common among cancer cell lines. In comparison, this percentage was 77% in cancer
573 samples.

574

575

576 Some of the apparent false positive cell lines had a *MDM2*, *MDM4* or *PPM1D*
 577 amplification or a *USP28* deletion (43 out of 109, 39% of the high-confidence set).
 578 Samples harboring one of these CNA in known phenocopying genes were assigned
 579 higher scores than the rest of *TP53* wild-type cell lines (mean score=0.58 and 0.37,
 580 respectively; t-test $p=5.4e-5$, Supp Fig.5A). Cells harboring a *TP53* deep deletion (90th
 581 percentile of CNA scores) also had higher phenocopy scores than samples without
 582 deletion (mean score=0.78 and 0.33, respectively, t-test $p=5.4e-8$). 28% of the cell
 583 lines in the high-confidence harbor a *TP53* deep deletion (22 out of 76, 90th percentile
 584 of *TP53* deletion CNA). These data support that the apparent false positives are often
 585 *bona fide TP53* phenocopying events in cancer cell lines. All *TP53* phenocopy scores
 586 and cell line functional *TP53* status information is provided in Supplementary Data 4.



587 **Figure 4: *TP53* loss phenocopy as estimated by the transcriptome score impact**
 588 **drug sensitivity**

589 A. *TP53* functional status classifier, derived from tumors, is applied to cancer cell lines.
 590 Receiver operating characteristic (ROC) curve and area under curve (AUC) are shown.

591 B. The false discovery rate (FDR) for each cell line is shown as a dot. X axis represents the
 592 phenocopy score threshold at which each cell line would be classified as *TP53* functionally
 593 deficient. Yellow horizontal bar represents the range for the high-confidence set t of *TP53*

594 phenocopying cell lines (FDR=0.18, threshold=0.93). In the top part of the plot, cell lines
595 harboring deletions of *TP53*, and amplifications of known phenocopying genes *MDM4*,
596 *MDM2* and *PPM1D* are marked.

597 C. *TP53* status - drug sensitivity associations. Each panel represents drugs targeting genes in
598 a given pathway. Each dot represents an association of a drug with two possible *TP53*
599 functional status labels: X axis with the *TP53* phenocopy score and Y axis with the *TP53*
600 mutational status (“CFE” labels by the GDSC, see Methods). P-values are from a pan-
601 cancer regression of a given drug log IC50 versus the *TP53* status. The Y axis represents
602 the same but using *TP53* labels according to GDSC. Associations with FDR<0.25 are
603 labeled.

604 D. Distributions of log IC50 values for several example drugs where *TP53* status is known to
605 confer resistance. The X axis illustrates the different categories based on *TP53* mutated
606 status (“Mutated *TP53*”), wild type *TP53* (“Wild type *TP53*”) and a high *TP53* phenocopy
607 score (“High confidence”); the “Mutated merge” is a combination of the two. Statistical tests
608 results comparing the groups (Mann-Whitney test, two-sided) are plotted on top. Median
609 values are provided inside of each box.

610

611

612 **Effects of *TP53* on general drug resistance are clarified by *TP53*** 613 **phenocopy scores**

614

615 Next, we considered the GDSC drug response distributions for various drugs, in light of
616 the *TP53* functional status, as determined by the *TP53* mutations, or alternatively by
617 our *TP53* phenocopy scores. To identify drugs to which response is affected by *TP53*
618 mutation status, we predicted drug response (log IC50) values of 449 GDSC drugs
619 individually, using *TP53* status as an independent variable (see Methods).

620 For most of the tested drugs (105 out of 188 drugs that were significantly associated at
621 <25% FDR, pan-cancer), the associations with *TP53* had a lower FDR when testing
622 using *TP53* phenocopy score, over the *TP53* CFE labels (mutations which alter gene
623 function) (Fig. 4C, effect size at Supp Fig. 5B). For the drugs that affected pathways
624 related to *TP53* functionality, this effect of improved significance by using the
625 phenoscore was prominent (hits FDR *TP53* phenocopy score < *TP53* CFE labels: DNA
626 replication, 12/12 drugs, genome integrity, 8/10, p53 pathway, 3/5, Apoptosis
627 regulation, 4/6, Cell cycle, 4/7, Supp Fig. 5C). As a negative control, randomized *TP53*
628 labels were not significantly associated with any drug. As a positive control, the drugs
629 known to be affected by *TP53* status such as nutlin-3a (Effect size= 1.48 vs 1.01, p=
630 6.7e-68 vs 1.2e-44) or bleomycin (Effect size=0.25 vs 0.16, p= 0.009 vs 0.07), exhibit a
631 stronger association with the *TP53* phenotypic score than with *TP53* CFE mutation
632 (Fig. 4C).

633 We examined the IC50 drug sensitivity values of all drugs together, considering the
634 different groups of cell lines defined by our *TP53* functional status classifier (Supp Fig.
635 5D). Here, the mean IC50 values of our high-confidence cell lines is more similar to the
636 *TP53* mutated cell-lines than to the *TP53* wild-type cell lines. In drugs known to be
637 affected by *TP53* status, such as bleomycin, (Fig. 4D), IC50 values were not notably
638 different between *TP53* mutant and the *TP53* phenocopying high-confidence cell lines.
639 All drug associations effect size and p-value are plotted in Supplementary Figure 6 A,
640 B. Cancer type-specific associations are shown at Supplementary Figure 6 C.

641

642 Taken together, the above analyses support the utility of the phenocopy score in
643 identifying *TP53*-associated drug sensitivity, and also support that our tumor-derived
644 classifier is able to generalize to cancer cell line transcriptomes to detect functional
645 *TP53* loss phenotype.
646

647 **Associations between drug sensitivity and genetic markers is** 648 **modified by functional *TP53* status**

649
650 A central goal in personalized cancer medicine is to discover actionable mutations,
651 which are used as genetic markers to decide which therapy to apply. Based on the role
652 of *TP53* mutations in dysregulating various processes relevant to tumorigenesis, we
653 hypothesized that various druggable cancer vulnerabilities may be conditional on *TP53*
654 functional status. To investigate, a regression was fit to predict activity (log IC₅₀) for
655 each drug, from cancer type and each cancer gene mutated status (via the CFE
656 classification, see Methods) and additionally introducing *TP53* status (either via *TP53*
657 mutation (CFE), or via phenocopy status) as an interaction term. Comparing *TP53*
658 phenocopy FDRs against *TP53* mutation FDR suggested that use of phenocopy score
659 allowed to more confidently identify the drug-gene associations where *TP53* status
660 modulates the effect size; see the comparison of FDR values (Fig. 5A), broken down
661 by pathway that targets the drug. Out of the identified three-way associations (gene x
662 drug x *TP53* status), 34% were found only by using the *TP53* phenocopy score, but not
663 by the *TP53* mutation status (Fig. 5A), while for comparison only 15% are uniquely
664 identified by *TP53* mutation status. We provide a tally of all gene-drug associations that
665 were conditional upon *TP53* in Supp Fig. 7A and a by-gene comparison of associations
666 identified with *TP53* phenocopy score labels, versus those identified by *TP53*
667 mutational status, in Supp Fig. 7B.

668
669 Next, we aimed to select the more robust associations. To this end, we applied the
670 “two-way” testing approach to identify replicated drug-marker links (56). In this test, it is
671 enforced that the drug-gene association replicates across two or more drugs that share
672 the same target gene or pathway. These were tested separately for specific cancer
673 types, comparing *TP53*-deficient versus wild-type cells. Here, this “two-way” test (56)
674 was further modified to be able to detect interactions with a third factor, the *TP53*
675 functional status. As an additional criterion ensuring confidence of associations, only
676 the hits that appear in more than one cancer type were taken into consideration (as a
677 trade-off, this will cause highly tissue-specific associations to be missed). Stratifying by
678 *TP53* functional status, we identified a number of drug-gene CFE associations that
679 were not significant when ignoring the *TP53* status (60 % of total, <25% FDR, Supp
680 Fig. 7 C). This corresponds to a total of 2303 associations of a drug to specific gene
681 mutational status by cancer type (total number of tests ignoring *TP53*=486417 versus
682 n=402945 controlling for *TP53* status, Supp Fig. 7D). 133 associations were found in
683 both approaches, but revealed a lower FDR when considering *TP53* stratification
684 (mean FDR=15% versus =19% if not stratifying=5e-08); all associations from the “two-
685 way” replication test are listed in Supplementary Data 5.

686
687

688

689

690

691 **Sensitizing effects of driver mutations on HDAC and ATR** 692 **inhibitors are modulated by *TP53***

693

694 Several studies suggested a role of the drug AR-42 (a HDAC1 inhibitor) in prolonging
695 p53 life and triggering apoptosis (57, 58). We hypothesized that, if p53 activity is
696 impaired, this effect of HDAC inhibitors should be reduced. Interestingly, our testing
697 reveals that mutations in the *PIK3CA* oncogene are associated with sensitivity to
698 HDAC1 inhibition in a manner conditional upon *TP53* mutation. In other words, when
699 *TP53* is functional, the resistance to HDAC1 inhibitor AR-42 due to *PIK3CA* mutation is
700 higher than when *TP53* is mutant or otherwise inactivated as indicated by phenocopy
701 score (*TP53* wild-type A *PIK3CA*_mut regression coefficient test $p=0.005$, Cohen's
702 $d=1.3$, *TP53* mutant *PIK3CA* regression coefficient test $p=0.08$, Cohen $d=-0.38$, Fig.
703 5B). We would not retrieve this association ignoring *TP53* status (test on regression
704 coefficient only using *PIK3CA* mutation status $p=0.67$, Cohen $d=-0.08$). In particular, in
705 LUAD the difference in AR-42 sensitivity (median of normalized log IC50 across cell
706 lines) between *PIK3CA* mutant and wild-type is hardly evident: 0.26 versus 0.24
707 respectively, while in *TP53*-functional LUAD this difference is -0.43 (*PIK3CA* wild-type)
708 versus 0.35 (*PIK3CA* wild-mutant). This response is observed across three different
709 HDAC inhibitors and in three different cancer types. AR-42 and belinostat were found
710 significantly associated with *PIK3CA* mutation in HNSC+LUSC (here considered jointly
711 because of known molecular similarities of the cancer types), BRCA, and LUAD cancer
712 types (Fig 5 B). Similarly, the AR-42 association with *PIK3CA* mutation was supported
713 in the HDAC1-targeting drug CAY10603 (Supp Fig. 7E). Furthermore, when we
714 analyzed an independent drug screening dataset, the PRISM screen (53), we were
715 able to recover the same associations (Supp Fig. 7E). This example illustrates how
716 being aware of *TP53* functional inactivation status, allows to detect drug-gene
717 associations that may be specific to the *TP53* wild-type or to the *TP53* deficient
718 backgrounds.

719

720 We also noted that the HDAC1i-*PIK3CA* mutation association (conditional upon *TP53*
721 functional status) was only recovered when controlling for *TP53* phenocopy score, but
722 not when using simply the *TP53* mutation status (per CFE method, see Methods) as an
723 interaction term (Belinostat IC50-*PIK3CA* mutation Mann-Whitney test, in the *TP53*
724 mutation wild-type background $p=0.13$, while in the *TP53* w.t. phenocopy labels
725 background $p=0.01$, Fig. 5B). This example illustrates how the use of *TP53* phenocopy
726 scores provides additional power to identify drug-gene associations, as already
727 indicated by the comparison of FDR scores for many associations above (Fig. 5A).

728

729 Recent reports have pointed out the potential therapeutic benefit of ATR inhibitors such
730 as VE-821 or VE-822 in PTEN-defective breast, glioma and melanoma cells (59, 60).
731 ATR is a crucial kinase regulating DNA repair and safeguarding genome integrity. ATR
732 inhibition in PTEN-deficient cells was associated with accumulation of DSBs, cell cycle
733 arrest and induction of apoptosis (59, 60), thus based on these phenotypes we
734 hypothesized that the functional status may modulate this effect. Inspecting our data

735 supports that the ATR inhibitors VE-821, VE-822, and AZD6738 were associated with a
736 lower fitness in *PTEN*-mutant cells of the SKCM, OV, BRCA and DLBC cancers (Fig.
737 5C, Supp Fig. 7F). This effect was however revealed only when *TP53* status was taken
738 into consideration, since p53 defective cells had an increased survival that obscured
739 this association (Fig. 5C, Supp Fig. 7F). Significance of the *TP53* interaction term was
740 not reached in this particular example, probably as the number of cell lines with a
741 *PTEN* mutation (but *TP53* wild-type) was low. Nevertheless, association of ATRi IC50
742 values was found to be more significant in a *TP53* wild type context than in a *TP53*
743 deficient context. This means there was a more robust difference in cell fitness
744 comparing *PTEN*-mutated to *PTEN* wild-type cells in a *TP53*-proficient background
745 (*TP53* wild-type IC50-*PTEN* Cohen's $d=-0.41$ vs *TP53* deficient AZD6738 IC50-*PTEN*
746 Cohen's $d=-0.05$).

747

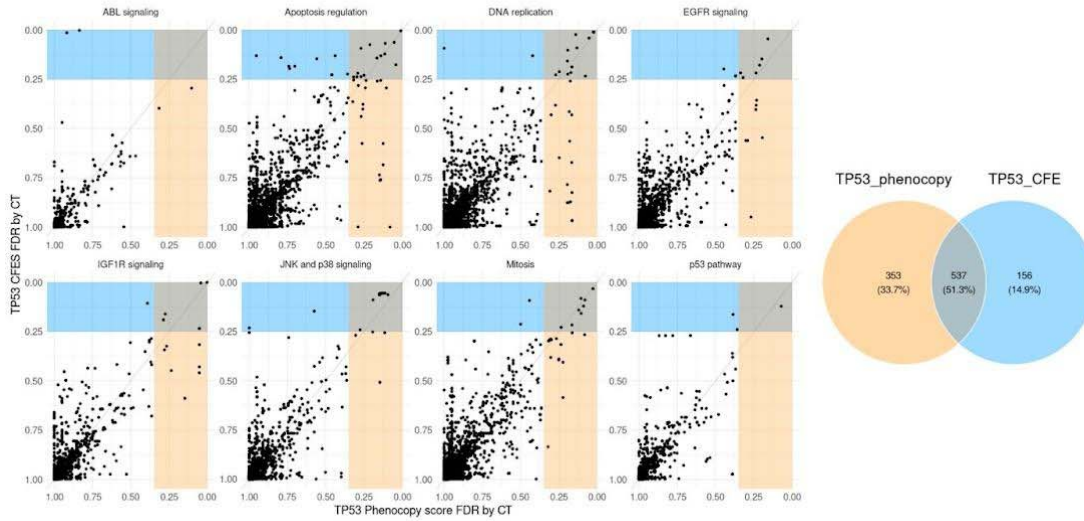
748 Overall, above we highlighted two examples where *TP53* functional status modulates
749 the association between HDAC1 inhibitors and *PIK3CA* mutations, and ATR inhibitors
750 and *PTEN* mutations. There were however many other significant three-way
751 associations involving *TP53* status, cancer driver gene mutations (CNA) and drugs
752 (listed in Supplementary Data 5), for example the association between *PIK3R1*
753 mutations and sensitivity to MET inhibitors (Supplementary Fig. 7 G).

754

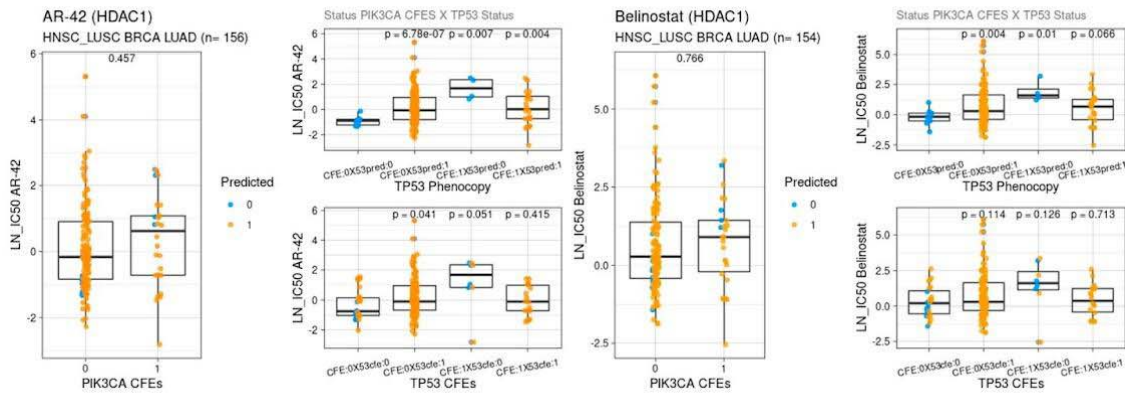
755 To estimate the importance in considering *TP53* in discovering drug associations, we
756 considered overlap in associations recovered when *TP53* status was accounted for
757 versus associations obtained when *TP53* status was ignored. Only 14% of significant
758 associations of a given molecular target to driver gene alteration status were shared
759 between two approaches (Supp Fig. 7 F), indicating that considering *TP53* status
760 strongly alters the drug-gene links recovered from statistical testing of drug screens.
761 The *TP53* status-aware testing recovered a higher number of associations ($n=12150$
762 versus 7853, both at $<25\%$ FDR). We also noted this effect depended on the particular
763 gene: Drug responses in genes such as *KRAS* or *TP53BP1* are well explained by gene
764 mutational status alone, not benefitting from *TP53* stratification (Supp Fig. 7 G).
765 Nevertheless, for most of the gene, their drug associations are often more confidently
766 retrieved when *TP53* status was accounted for (e.g. *BRAF*, *HRAS*, *ATM*, *APC*; $n=18$
767 genes total). Overall, the above data suggests that *TP53* should be considered when
768 matching drugs to cancer patients based on the driver mutations in their tumor, and
769 that this *TP53* functional status should ideally be estimated via the phenocopying score
770 rather than *TP53* gene mutations.

771

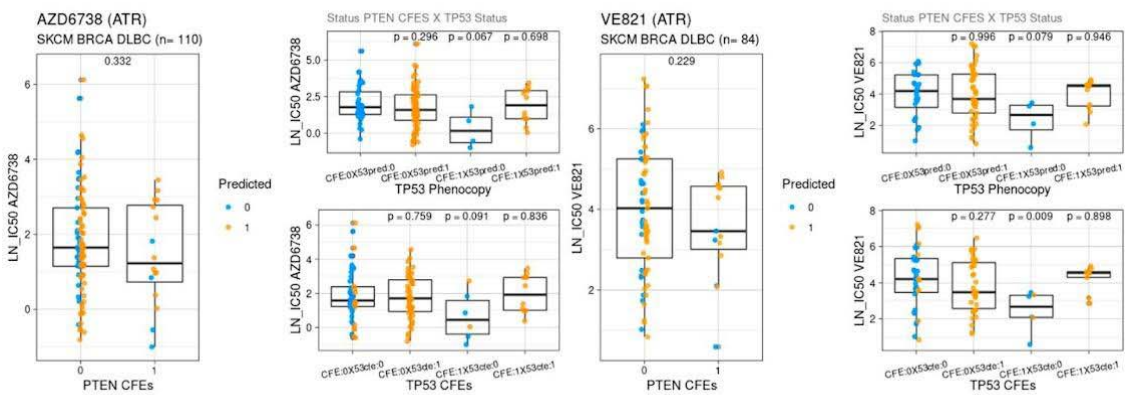
A



B



C



772

Figure 5. Associations between drug response and genetic markers are commonly affected by TP53 functional status

773

774 **A.** Associations of mutations in various genes with antitumour drug sensitivity, controlling for
 775 *TP53* status. Each panel represents a pathway targeted by drugs, and each dot represents
 776 a gene - drug - cancer type combination. Associations are conditioned on *TP53* status by
 777 including an interaction term in the regression, where the Y axis shows associations using
 778 *TP53* mutational status using GDSC labels (*TP53* CFEs), while the X axis represents the
 779 same using *TP53* phenocopy score-based labels. Yellow-shaded area contains

780 associations with $FDR < 0.25$ for *TP53* phenocopy labels, and blue-shaded area shows the
781 same for *TP53* CFEs. Total counts of associations in shaded areas are shown in the Venn
782 diagram.

783 **B.** Association of *PIK3CA* mutation status with HDAC1 targeting drugs (AR-42 and
784 CAY10603), after controlling for *TP53* status. Large plots show the association without
785 stratification by *TP53* labels. “CFE” denotes mutated (1) or wild-type (0) *PIK3CA* state. An
786 association p-value is shown on top of each box by Mann-Whitney u-test. Each dot is a
787 tumor sample belonging to one of the cancer types listed above the panel. Dots are colored
788 according to *TP53* phenocopy score labels. Small panels represent the same association
789 but upon stratification by *TP53* status. Top row, stratification using *TP53* phenocopy score
790 labels; bottom row, using *TP53* CFEs (“cancer functional events”, functional mutation
791 status, see Methods). The X axis represents tumor samples stratified by both the *PIK3CA*
792 and *TP53* status. *PIK3CA* CFEs groups refer to *PIK3CA* stratification (1=mut, 0=w.t)
793 ignoring *TP53* status. Labels are as follows: “CFE:(1/0)X53pred:(1/0)” refers to stratification
794 of *PIK3CA* (CFE i.e. driver mutation status) using *TP53* phenocopy labels (53pred). “Last
795 CFE:(1/0)X53cfe:(1/0)” refers to stratification of *PIK3CA* (CFE) using *TP53* mutation labels
796 (53cfe). “CFE:(1/0)X53pred:(1/0)” refers to stratification of *PIK3CA* (CFE) using *TP53*
797 phenocopy labels (53pred). Lastly, “CFE:(1/0)X53cfe:(1/0)” refers to stratification of *PIK3CA*
798 (CFE) using *TP53* mutation labels (53cfe)

799 **C.** Association of *PTEN* mutation status with ATR targeting drugs (AZD6738 and VE821), after
800 controlling for *TP53* status. Organization of the plots matches Fig. 5B, C.

801
802
803

804 Discussion

805

806 Disabling the master tumor suppressor gene *TP53* provides cancer cells with important
807 advantages such as avoiding cell cycle arrest or apoptosis upon replication stress or
808 DNA damage. Because *TP53* acts as a transcription factor controlling expression of
809 hundreds of genes, a functional read-out of *TP53* activity can be obtained using gene
810 expression data, both at the level of mRNA or ncRNA, or at the protein level (20-23).
811 These scores were reported to have potential clinical relevance in predicting cancer
812 aggressiveness/patient survival and therapy response(22, 23, 61, 62).

813 In this study, we developed a global transcriptome score of *TP53* deficiencies, and
814 applied it to ~8,000 tumors and ~1,000 cancer cell lines, to answer three questions.

815

816 Firstly, we asked how common are the *TP53*-mutation phenocopying events across
817 various human cancers. We estimated a 12% frequency of *TP53* loss phenocopies,
818 compared to a 58% prevalence of *TP53* mutant tumors. In some cancer types such as
819 BRCA and BLCA, the *TP53* phenocopies may constitute a high fraction of 19% and
820 16% tumor samples, respectively, suggesting that the *TP53* status of tumors should
821 preferentially be measured via functional readout (here, transcriptome-wide signature)
822 rather than considering only mutations. Supporting this notion, a recent study using a
823 four-gene expression signature of *TP53* activity demonstrated that this significantly
824 predicts patient survival across 11 cancer types, and that in the majority of those it
825 performs better than considering *TP53* mutations (22).

826

827 Secondly, given the high prevalence of *TP53* phenocopies we observed, we asked if
828 there exist additional genetic events that are associated with these phenocopies. We

829 developed a method considering CNA profiles and gene expression in tumors,
830 integrating external data from CRISPR and RNAi screens, which identified the *USP28*
831 gene deletion as a common *TP53*-loss phenocopying event. This is relevant for at least
832 five cancer types: BLCA, STAD, BRCA, LIHC and LUAD, and affects 2.9%-7.6% tumor
833 samples therein. The same statistical methodology also highlighted additional genes
834 neighbouring the known phenocopies *MDM2* and *PPM1D* -- *CNOT2* and *MSI2*
835 respectively -- which are often co-amplified with the 'primary' gene in the CNA gain
836 segment and may boost the resulting *TP53*-loss phenotype. This analysis provides an
837 example of how molecular phenotypes (here, a transcriptional signature and fitness
838 effects from a CRISPR screen) can be used to identify multiple causal genes in a CNA
839 segment. Analogous genomics methodologies could be applied in future work to
840 interrogate various recurrent CNA events observed in tumors, for which the causal
841 gene(s) are often not known with confidence.

842

843 Thirdly, we asked if a better measurement of the *TP53* functional inactivation status
844 may be impactful in terms of predicting response to antitumor drugs based on genetic
845 markers. Given that *TP53* deficiencies have myriad downstream consequences on the
846 cell, including e.g. suppression of cell cycle checkpoints, or inactivation of various DNA
847 repair pathways (4) it is conceivable that the *TP53* background may affect the ability of
848 various drugs to kill cancer cells, including drugs targeted towards a particular driver
849 mutation. We searched for three-way interactions involving *TP53* status, each drug,
850 and each mutated cancer driver gene, finding for instance that the *TP53* status
851 modulates the selective activity of HDAC1-inhibitors on *PIK3CA*-mutant cells. The
852 associations were filtered to retain those supported in multiple compounds targeting the
853 same protein or pathway; enforcing agreement across multiple measurements may
854 allay concerns of reproducibility in cell line screening databases (63–65). Recent work
855 by us and others (56, 66) has used statistical methods to integrate over various
856 screening datasets, considering drug and CRISPR genetic screens jointly, to improve
857 reliability of drug-target association discovery. Our robustly supported set of drug-
858 target gene links (Supplementary Data 5) that may be modulated by *TP53* status
859 provides a resource for follow-up work to validate the role of *TP53* functional status in
860 modulating particular gene-drug associations.

861

862 The statistical method we employed to identify *TP53* loss phenocopying events draws
863 on the expression levels of 217 genes. Given that the model's predictive accuracy is
864 high (demonstrated using cross-validation and application to an independent data set
865 of cancer cell line transcriptomes), the errors it makes are of interest. While the
866 apparent false-positives are often *TP53* loss phenocopies, as addressed extensively in
867 this study, it would also be interesting to look into the apparent false negatives in
868 future. These *TP53*-mutant tumors classified as *wild-type*-like by our transcriptome
869 score were not considered here, because of their relatively modest number, making
870 statistical analyses difficult. Going forward, analyses of genomes from larger cohorts of
871 cancer patients may provide enough such examples to reveal mechanisms of re-
872 establishing *TP53* activity in certain cancers. Conceivably, this may happen by
873 normalizing expression of the *TP53*-downstream genes which have been dysregulated
874 by the *TP53* mutation; understanding these events may inspire new avenues for
875 therapy of *TP53* mutant tumors.

876

877 The general approach presented here could be applied beyond *TP53* also to other
878 sorts of phenocopying events which may occur in tumors. For instance, RAS pathway
879 activation transcriptomic scores were proposed (20), and similarly homologous
880 recombination repair scores based on mutational signatures (86,87) Conceivably,
881 other important cancer pathways may be similarly addressed as well, analyzing their
882 distribution across tumors to identify possible phenocopying events, as well as their
883 implications to drug response prediction, as we have done here for *TP53* phenocopies.
884
885
886
887

888 **Materials and methods**

889 **Data collection and preparation**

890 **Gene expression and Copy Number Alteration (CNA) data**

891 We downloaded gene expression data (transcripts per million, TPM) from GDC Data
892 Portal (74) for human tumor samples (TCGA) and from GDSC (52) and CCLE (75) for
893 cell line samples (CL). We filtered out genes with missing values in more than 100
894 samples and selected the overlapping genes between cell lines and tumors. Cancer
895 types with less than 10 samples were filtered out. Low expressed genes were removed
896 (TPM < 1 in 90% of the samples) and applied a square-root transformation to TPM.
897 Cancer types. Tumors with less than 10 samples were filtered out. In total, we have
898 12,419 features for 966 CL samples and 9149 TCGA samples. We collected CNA from
899 GDC Data Portal (74) for TCGA samples and from DepMap (64) for CL samples.

900

901 **Data alignment between tumors and cell lines**

902 In order to later generalize the model to cell lines we proceed to align TCGA and CL
903 data. For this, we applied ComBat, a batch adjustment method, to account for intrinsic
904 differences between tumor signal and cell lines signal (55). For the alignment of TCGA
905 and CL data, we first applied quantile normalization (normalize.quantiles function,
906 preprocessCore R 1.48.0 package) using tumor data as reference and then applied
907 ComBat (ComBat function, R package sva 3.32.1). Each group (TCGA, GDSC or
908 CLLC) was treated as a different batch.

909

910 ***TP53* status label (according to GDSC)**

911 TCGA Pan-Cancer Atlas somatic mutation data were extracted from the MC3 Public
912 MAF (v0.2.8) data set (76). We followed the Iorio et al. methodology (24) to determine
913 bona fide *TP53* mutations (0:wild type, 1: mutated). We identify recurrent variants that
914 are likely to contribute to carcinogenesis. We considered mutated variants: non-
915 synonymous missense mutations, indels (in frame insertions and deletions and out of
916 frame insertions and deletions), nonsense mutations and specific splice-site mutations
917 (such as “p.X125_splice”). Samples without any of these mutations annotated were
918 considered *TP53* wild type. Just in 5% of the cases (179 out of 3416) our labels
919 differed from the ones provided by Iorio et al. In total, we obtained *TP53* labels for 7788
920 TCGA tumors.

921

922 ***TP53* score classifiers in human tumors**

923 We used the aligned human tumor data to train a supervised elastic (20-23) net
924 penalized logistic regression (using `cv.glmnet` function with $\alpha = 0.5$, R package
925 `glmnet` 4.0-2) classifier with cyclical coordinate descent optimization (77). The
926 choice of Elastic net penalization aims to deal with two concerns: the large number of
927 variables can lead to high complexity (overfitting) and the feature multicollinearity.
928 Elastic net regressions are seen as a good trade-off that benefit from the
929 dimensionality reduction provided by Lasso penalization while keeping as many
930 informative variables as possible (Ridge penalization). Of note, these three
931 regularization methods yielded similar cross-validation accuracy: Elastic net (i.e.
932 $\alpha=0.5$) AUC 0.960, Lasso (i.e. $\alpha=1$) AUC 0.965, and Ridge (i.e. $\alpha=0$) AUC
933 0.952, suggesting that the default $\alpha=0.5$ in Elastic net method is a reasonable
934 choice. The model is trained using RNAseq data (X matrix) to infer *TP53* status (Y
935 matrix). As a reference (Y) during training we used *TP53* mutation status labels.

936 For the training set, we excluded the tumor samples that have an amplification (not
937 neutral, >0 , according to GISTIC CNA thresholded calls downloaded using
938 `FirebrowseR` package, `Analyses.CopyNumber.Genes.Thresholded` function) in
939 previously known *TP53* phenocopying genes (*MDM2*, *MDM4*, *PPM1D*) or a deep
940 deletion of *TP53*, to prevent the model from relying too much on dosage effects of
941 these genes, instead of the downstream response.

942 In addition, to control for cancer type specific signals we included cancer type as a
943 dummy variable. To control for class imbalance, we included weights in the classifier.

944 The model learns a vector of gene-specific weights that better classifies *TP53* status.
945 The score from the models determines the probability of a given tumor of being *TP53*
946 deficient. Optimization of the penalized regression formula and further details of the
947 classifier can be consulted at (77)

948

949 **Assessment of the classifier and calculation of FDR score**

950 Using 90% of the training set and 5 balanced folds (balanced based on *TP53*
951 mutational state) we performed cross-validation. We measured the performance of the
952 training set (folds used for training) and the testing set (10% held out). Areas under the
953 Receiving Operating Curve (AUROC) and the Precision Recall curve (AUPRC) were
954 calculated for both training (cross-validation) and testing sets.

955

956 FDR was calculated by sample using each sample probability score from the classifier
957 as threshold for determining positive and negative samples $FDR = \text{false positive} / (\text{false}$
958 $\text{positive} + \text{true positive})$. Samples harboring an amplification (GISTIC thresholded
959 amplifications, `FirebrowseR` package, `Analyses.CopyNumber.Genes.Thresholded`
960 function) of known phenocopying genes (*MDM2*, *MDM4*, *PPM1D*) or *TP53* deletions
961 (GISTIC thresholded deep deletions, `FirebrowseR` package,
962 `Analyses.CopyNumber.Genes.Thresholded` function)) were considered as true
963 positives when calculating FDR.

964

965 In Figure 1B, density of known phenocopies was calculated using *MDM4*, *MDM2*,
966 *PPM1D* (amplifications) and *TP53* (deletions) CNA over/under the 95/0.05 th quantile.

967 All *TP53* Phenocopy scores (probabilities of being *TP53* dysfunctional) are provided at
968 Data S2.

969

970 The classifier coefficients were analyzed using the GO enrichment tool ShinyGO (78).
971 The 12419 genes from the gene expression matrix with a coefficient equal to zero were
972 used as background. Full classifiers relevant coefficients are provided at Data S1.

973 The coefficients of the *TP53* model should be interpreted with care, for several
974 reasons: some of these genes may change in expression levels via indirect association
975 meaning they may not be directly regulated by *TP53*; the gene set may omit genes that
976 are *bona fide* *TP53* targets if the information contained in them is redundant with other
977 genes; and finally these genes may individually be only weakly associated with *TP53*
978 status, since the method optimizes the expression markers' collective power.
979 Visualization was performed using Revigo (27).

980

981 ***TP53* status detection in cell lines**

982 Using the downloaded RNAseq from GDSC cell lines data we applied our trained tumor
983 classifier to cell lines. As stated above, RNAseq data was square rooted, normalized
984 and ComBat batch corrected. Cell line prediction performance was measured using as
985 reference *TP53* COSMIC labels (79) combined with Iorio et al methodology (24) as we
986 did in tumors. FDR was calculated again using samples harboring an amplification of
987 known phenocopying genes (*MDM2*, *MDM4*, *PPM1D*) or *TP53* deletions as true
988 positives.

989

990 Using the classifier scores we separate the cell lines high-confidence set (FDR ≤ 18%)
991 using as threshold reference GISTIC thresholded *TP53* deep deletions (-2)
992 (threshold = 0.93) (FirebrowseR package, Analyses.CopyNumber.Genes.Thresholded
993 function). Therefore, we determine 3 sets derived from our Phenocopy score: high-
994 confidence set (predicted *TP53* phenocopies, classified as mutant but originally labeled
995 as wild type), *TP53* mutant (classified and labeled as mutant) and *TP53* wild type
996 (classified and labeled as wild type). All cell line predictions are provided at Data S3.

997

998 Due to a lack of positive controls, samples that were classified as wild type being
999 originally labeled as *TP53* mutant were not considered further. However, in the future,
1000 analyses with a higher number of cancer genomes may reveal mechanisms of re-
1001 establishing *TP53* activity in some *TP53* mutant cancers (e.g. by normalizing
1002 expression of the *TP53*-downstream genes which have been dysregulated by the *TP53*
1003 mutation).

1004

1005 **Gene co-dependency with *TP53* knockout/knockdown**

1006 Following data of the 2021 Q4 release downloaded from the DepMap project website:
1007 CRISPR data from PROJECT Score (28) ("Achilles_gene_effect.csv"), combined RNAi
1008 from DEMETER2 project (29) ("D2_combined_gene_dep_scores.csv"), and the cell line
1009 metadata ("sample_info.csv"). In this data, negative scores imply cell growth inhibition
1010 and/or death following gene knockout.

1011 CRISPR data is normalized so non-essential genes scores are close to 0. We used
1012 Pearson's correlation to correlate the gene effect of CRISPR *TP53* knockout in every

1013 cell line to other genes' effect. We tested 990 cell lines for our 12419 genes. This score
1014 was calculated both by pan-cancer and by cancer type.
1015 Equally to CRISPR codependency data we correlated gene knockdown effect with
1016 *TP53* knockdown effect using Pearson's correlation test. We tested 700 cell lines for
1017 our 12419 genes. This score was calculated both for pan-cancer and by cancer type.
1018

1019 **Calculation of the prioritization score**

1020 We sought to rank possible *TP53* loss phenocopying genes testing different data: copy
1021 number variant data, gene expression data (RNAseq), RNAi codependency score and
1022 CRISPR codependency score. We used the downloaded tumor data (previously
1023 described) and our *TP53* Phenocopy score to test for differences across our 3 main
1024 *TP53* groups: *TP53* wild type (labeled and classified as wild type), *TP53* mutated
1025 (labeled and classified as mutated) and predicted *TP53* phenocopied (labeled as wild
1026 type but classified as mutated). We guessed that phenocopying genes should have a
1027 differential expression in the phenocopies group when comparing to wild type and
1028 mutated *TP53* groups individually. We tested 12419 genes (by cancer type) in the
1029 following manner (via Student's t-test):

- 1030
- 1031 CNV_gene(i)_*TP53*_wt versus CNV_gene(i)_*TP53*_phenocopies (CNV0 test),
- 1032 CNV_gene(i)_*TP53*_mut versus CNV_gene(i)_*TP53*_phenocopies (CNV1 test)
- 1033 GE_gene(i)_*TP53*_wt versus GE_gene(i)_*TP53*_phenocopies (GE0 test)
- 1034 GE_gene(i)_*TP53*_mut versus GE_gene(i)_*TP53*_phenocopies (GE1 test)
- 1035 RNAi_score_gene(i) versus RNAi_score_*TP53* (RNAi codependency score,
1036 methodology described above)
- 1037 CRISPR_score_gene(i) versus CRISPR_score_*TP53* (CRISPR codependency
1038 score, methodology described above).

1039

1040 3010 genes out of 12419 did not have gene expression data so GE1 and GE0 tests
1041 were omitted from the combination for those genes. We combined the p-values values
1042 from the tests by cancer type using Fisher's method for combining p-values. For each
1043 category (CNV and GE) we only use in the combination the worst p-value (max)
1044 between CNV0 and CNV1 and GE1 and GE0 as a way of controlling. Genes in which
1045 the test direction is not coherent in CNV, GE and codependency score were dropped.
1046 A gene with a negative codependency score, as negative regulators such as *MDM2*, is
1047 expected to cause a phenocopy of *TP53* by amplification and overexpression
1048 (therefore a higher expression in the phenocopies group than *TP53* wt or mut). P-values
1049 were FDR adjusted using Benjamini-Hochberg method (p.adjust function of the stats
1050 package). We further merged each cancer type combined score into one single FDR
1051 value using Fisher's approach. That way we obtained the final Prioritization score for
1052 each gene in a cancer-combined way. We set as reference the known phenocopies
1053 (*MDM2*, *MDM4*, *PPM1D*) FDR and CRISPR codependency score. To establish a
1054 stringent threshold for new possible phenocopying genes, we determine that the gene's
1055 prioritization score (combined by cancer type) should have an FDR as significant as the
1056 best ranked phenocopying gene (by cancer type). Same was applied for CRISPR
1057 codependency score. The known phenocopying genes with the best score by cancer
1058 type was *MDM4* in LUAD, with an FDR of 4e-05 and a CRISPR codependency score of
1059 -0.26.

1060

1061 ***TP53* wild-type and *TP53* -/- isogenic cell line screens**

1062 Mean beta scores were calculated using MAGeCK-MLE (80) for *TP53*-isogenic pair cell
1063 lines A549 (81) and two RPE1 cell lines (82, 83). Beta scores represent the effect that
1064 gene knock-out has on cell fitness.

1065 We calculated the Z-scores (distance from the mean expressed as number of standard
1066 deviations) of either *USP28* or ATM within the distribution of their respective neighbor
1067 genes, for each dataset and *TP53* status "1Mbp neighbor genes" are genes present in
1068 Brunello (84) and Gecko v2 (85) libraries and located within a 1Mbp window
1069 surrounding either *USP28* or ATM, obtained from genecards.weizmann.ac.il
1070

1071 **Drug response associations with *TP53* status**

1072 We collected GDSC (24) drug data for a total of 1000 cell lines. We used IC50 as a
1073 measure of activity of a compound against a specific cell line. If drug data was
1074 available in both GDSC1 and GDSC2 versions, GDSC1 data was selected.

1075 We also collected each drug putative target and target pathway information from the
1076 GDSC website (<https://www.cancerrxgene.org/>). We filtered out NA values and
1077 transformed IC50 to log scale. We downloaded GDSC mutational Cancer Functional
1078 Events (CFEs) (24) in order to: make comparisons between *TP53* Phenocopy score
1079 and GDSC *TP53* CFEs and to test other gen status drug responses controlling for
1080 *TP53* status. Mutational CFEs consist of a GDSC curated set of cancer genes (CGs)
1081 for which the mutation pattern in whole-exome sequencing (WES) data is consistent
1082 with positive selection.

1083 We first used drug response (IC50) values of 449 GDSC drugs to fit a pan-cancer
1084 regressions against *TP53* status using cancer type as control variable. We fit three
1085 different regressions per drug response: against *TP53* CFEs, against predicted *TP53*
1086 Phenocopy thresholded scores and against *TP53* random labels.

1087 $\log(\text{IC}_{50}) \sim TP53.status + cancer.type$

1088 For the *TP53* status we used the groups obtained from our Phenocopy score being the
1089 *TP53* high-confidence set (classified as mutant, labeled as *wild-type*) and *TP53* mutant
1090 set (classified as mutant, labeled as mutant) the *TP53* deficient set (*TP53*.status = 1)
1091 and *TP53* wild type (classified as *wild-type*, labeled as *wild-type*) as wild type set
1092 (*TP53*.status = 0). Due to uncertainty, we filtered out samples with a *TP53* mutation
1093 classified as *wild-type*. Cancer types with less than 3 cases for any category were
1094 filtered out. We used the *esc* R package to calculate effect size (*cohens_d* function). P-
1095 values of associations were FDR corrected using the Benjamini-Hochberg ("fdr")
1096 correction of the *p.adjust* function (*stats* package).
1097

1098 We separate the drugs into groups according to the pathway the gene they target
1099 belong to. By pathway, we calculated the slope resulting from the comparison of the
1100 FDR Phenocopy score regression versus the FDR *TP53* CFEs. For visualization we
1101 plotted raw IC50 values of different drugs and all drugs together across the different
1102 cell line defined sets. For further analysis, we merged the cancer types that were
1103 similar: HNSC with LUSC (jointly known as HNSC_LUSC), GBM with LGG
1104 (LGG_GBM) and OV with UCEC (OV_UCEC).

1105

1106

1107 **Drug response associations of gene status controlling for *TP53* status**

1108 We collected drug screening data from the PRISM project (53) and GDSC project (52).
1109 NA values were filtered out and IC50 values were transformed to logarithmic scale. We
1110 downloaded mutation features (GDSC mutational CFEs, see above) from (24).

1111

1112 First, we fit a regression for each drug and gene CFE including *TP53* loss Phenocopy
1113 score and the interaction term as it follows:

1114 $\log(IC50) \sim \text{genCFEs} + TP53\text{Phenocopy.status} + \text{genCFEs} * TP53\text{Phenocopy.status}$

1115 For comparison, we performed the same analysis using *TP53* random and *TP53* CFEs
1116 instead of *TP53* Phenocopy.status.

1117 We tested every gene mutational CFEs out of the 300 genes provided by GDSC. We
1118 filtered out cases with less than 3 samples in any category (mutated:1 or wildtype:0) for
1119 *TP53* status and gen CFEs. Regressions were fitted by cancer type using *glm* package
1120 (glmnet 4.0-2 R package). We selected genCFEs p.value and FDR correct using the
1121 Benjamini-Hochberg (“fdr”) correction of the p.adjust function (stats R package). The
1122 coefficient of the genCFEs variable informs us about the fold change of the different
1123 variable states (mutant:1-wildtype:0) when *TP53*Phenocopy.status is set to its
1124 reference levels (wildtype:0). We compared these scores when using *TP53* Phenocopy
1125 to *TP53* CFEs by plotting FDR values and calculating slope (Figure 5 A,
1126 Supplementary Figure 7 A).

1127

1128

1129 **Two-way association tests**

1130 To further analyze *TP53* interaction in a more stringent way we implemented a version
1131 of the “two-way association test” approach developed by Levatic et al (56). In this
1132 methodology we enforced that, for a given drug, an association between a gen feature
1133 (GDSC gen mutational CFEs) and GDSC drug response is reproduced in other drugs
1134 with the same molecular target (controlled by *TP53* status as an interaction).

1135

1136 For this, we curated 996 sets of two drugs with the same target (ie: Dabrafenib and
1137 AZ628, target=BRAF). For the two drugs separately, we fitted a regression comparing
1138 the GDSC drug response against gen status (GDSC mutational CFEs) controlling for
1139 *TP53* status (as stated above) by cancer type. We tested the different labels in the
1140 regression: *TP53* CFEs, *TP53* Random labels and *TP53* Phenocopy labels. We
1141 considered associations by cancer type. We calculated the two-way association score
1142 by averaging the estimates (effect size) obtained between drug 1 and drug 2. To
1143 calculate the p-value for each drug-drug combination, we shuffled the *TP53* labels and
1144 compared the obtained random estimates with the actual estimate as described in our
1145 previous work (56).

1146

1147 For an association to be selected, we require that it is observed in more than one
1148 cancer type (merged cancer types excluded), FDR<25% across all cancer types where
1149 the hit is observed and that the direction (value from gen CFEs variable estimate) is

1150 maintained across drugs. When selecting relevant hits we also required that each hit
1151 *TP53* interaction term variable in regression is significant (FDR<25%). This informs us
1152 of deviation from the behavior of the regression variables *gen_status=1* and
1153 *gen_status=0* when *TP53* is controlled as interaction. We filtered out cases with less
1154 than 3 samples in any category (mutated:1 or wildtype:0) for *TP53* status and *gen*
1155 CFEs in a cancer type manner. Supported hits by this methodology are reported at
1156 Figure 6 B C, Supplementary Figure 7 C, D and E and in Supplementary Data 5.
1157 In addition, as a validation for some hits we performed a “two-way” using PRISM data.
1158 In this case we enforced that, for a given drug, an association between a *gen* feature
1159 (GDSC *gen* mutational CFEs) and GDSC drug response is reproduced in the same
1160 drug using the PRISM dataset. The rest of the methodology was applied in the same
1161 manner (see GDSC “two-way test” above).

1162
1163 As control, we followed the same procedure of the two-way testing method but fitting
1164 regressions of IC50 ~ *gen* CFEs (without interaction term). FDR corrected p-values of
1165 *gen* CFEs coefficient in regressions with and without interaction term were compared.
1166 We made different types of comparisons: by gene associations (Supplementary Figure
1167 7 B), molecular target-*gen* CFEs associations (different 2-sets of drugs can target the
1168 same molecular feature) and all associations (Supplementary Figure 7 A)

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1482 **Supplementary material**

1483

1484 **Supplementary Text 1.**

1485 CCR4-NOT is a transcription complex (CNOT), composed of 11 subunits, that plays an
1486 important role in multiple functions in terms of regulating translation, mRNA stability,
1487 and RNA polymerase I and II transcriptions (67,68). *CNOT2*, one of the CCR4-NOT
1488 subunits, plays a critical role in deadenylase activity and the structural integrity of the
1489 complex (69) among other functions. An increasing number of studies have suggested
1490 *CNOT2*'s role in tumor progression, such as in metastasis, proliferation and
1491 angiogenesis (70, 71). *CNOT2* depletion and CCR4-NOT disruption have been linked
1492 to an apoptotic response via MID1IP1 and increased p53 activity (70, 72) . *CNOT2* has
1493 been reported to be among the top 5 amplified genes in chromosome 12, together with
1494 *MDM2* (73). Appealingly its overexpression has been demonstrated in several cancer
1495 types such as pancreas, prostate, liver, urinary, ovarian and breast (71). Experiments
1496 inducing *CNOT2* overexpression led to increased p21 and p53 expression, decreased
1497 apoptosis and decreased TNF-related apoptosis-inducing ligand (TRAIL) sensitivity
1498 (72, 73).

1499

1500 **Supplementary Text 2.**

1501

1502 In BLCA, co-amplifications are associated with a higher *TP53* phenocopy score, and
1503 are more frequent than *MDM2*-only amplifications (21 out of 32 are co-amplifications,
1504 Supp Fig. 4E). In BRCA, we found almost exclusively *MDM2-CNOT2* co-amplifications
1505 and no *MDM2* only amplifications. In STAD co-amplifications of *MDM2* and *CNOT2* are
1506 more frequent (10 out of 13) than *MDM2* solely. Just GBM was found to rely more on
1507 *MDM2* only amplifications (8 out of 14, Supp Fig. 4E).

1508 Only 3 tumor samples were *CNOT2* amplified but *MDM2*-non amplified (all 3 having a
1509 *TP53* phenocopy score lower than 0.5, Supp Fig. 4E). No cancer type relied on *CNOT2*
1510 only amplifications.

1511

1512

1513 **Supplementary Data are attached as separate files.**

1514 Supplementary Data 1 - TCGA TP53 Phenocopy scores

1515 Supplementary Data 2 - Gene coefficients

1516 Supplementary Data 3 - USP28/ATM fitness effect

1517 Supplementary Data 4 – Cell lines TP53 Phenocopy scores

1518 Supplementary Data 5 - Two-way associations

1519

1520 **Supplementary Figures 1-7 are given in a separate document.**

1521