1

Identification of highly penetrant Rb-related synthetic lethal interactions in triple negative breast cancer

Rachel Brough[1,2], Aditi Gulati[1,2], Syed Haider[1], Rahul Kumar[1,2], James Campbell[1,2], Erik Knudsen[3], Stephen J. Pettitt[1,2], Colm J. Ryan*[4,5], Christopher J. Lord[1,2]*

[1]The Breast Cancer Now Toby Robins Breast Cancer Research Centre and [2]CRUK Gene Function Laboratory, The Institute of Cancer Research, London, SW3 6JB, UK.

[3]Department of Medicine, University of Arizona, Tucson, AZ 85721, USA.

[4]Systems Biology Ireland and [5]School of Computer Science, University College Dublin, Dublin, Ireland.

*Corresponding authors: Dr. Colm J. Ryan Email Colm.Ryan Ryan@ucd.ie, Prof. Christopher Lord, Email Chris.Lord@icr.ac.uk

Conflict of Interest statement:

The authors have no conflict of interests to declare

Abstract

Although defects in the *RB1* tumour suppressor are one of the more common driver alterations found in triple negative breast cancer (TNBC), therapeutic approaches that exploit this have not been identified. By integrating molecular profiling data with data from multiple genetic perturbation screens, we identified candidate synthetic lethal (SL) interactions associated with *RB1* defects in TNBC. We refined this analysis by identifying the highly penetrant effects, reasoning that these would be more robust in the face of molecular heterogeneity and would represent more promising therapeutic targets. A significant proportion of the highly penetrant *RB1* SL effects involved proteins closely associated with RB1 function, suggesting that this might be a defining characteristic. These included nuclear pore complex components associated with the MAD2 spindle checkpoint protein, the kinase and bromodomain containing transcription factor TAF1 and multiple components of the SCF^{SKP} Cullin F box containing complex. Small molecule inhibition of SCF^{SKP} elicited an increase in p27^{Kip} levels, providing a mechanistic rationale for RB1 SL. Transcript expression of SKP2, a SCF^{SKP} component, was elevated in *RB1* defective TNBCs, suggesting that in these tumours, SKP2 activity might buffer the effects of *RB1* dysfunction.

Keywords: Rb, synthetic lethality, triple negative breast cancer, SKP2, TAF1, penetrance

Introduction

58

59 Patients who develop triple negative breast cancer (TNBC), i.e. those breast

60 cancers that lack amplification of the *ERBB2* gene as well as expression of

61 both the estrogen and progesterone receptors, tend to have a relatively poor

62 prognosis and represent a significant area of unmet clinical need, where novel

63 therapeutic approaches are acutely needed (recently reviewed in [5]). Although

64 some targeted approaches have been proposed for molecularly defined

65 subsets of TNBC patients, for most, classical chemotherapy regimens still

66 represent the mainstay of treatment, making the requirement to identify novel

67 targets in this disease critical. One approach to this problem has been to

68 define the molecular composition of TNBCs and then to use this information to

69 help identify therapeutic vulnerabilities that might operate in the disease.

70 Already, the delineation of genomic, transcriptomic and proteomic profiles of

71 tumours has identified a series of distinct molecular subtypes of TNBC, as

72 well as identifying likely cancer driver gene mutations that operate in the

73 disease[51].

74

75 One of the more frequent driver alterations in TNBCs involves deleterious

76 mutations (e.g. truncating mutation, gene deletions etc.) in the

77 *Retinoblastoma* tumour suppressor gene (*RB1* aka *Rb*). In non-tumour cells,

78 Rb's canonical role is in cell cycle progression, a function mediated in part by

79 the repressive effect Rb has on the E2F family of transcription factors[29]. A

80 somewhat reductionist model of Rb's role in tumour suppression, suggests

81 that loss of Rb's E2F repressive function allows cells to prematurely transition

82 through the G_1 cell cycle checkpoint; it also seems likely that loss of Rb

83 function in breast cancer also influences additional processes that contribute

84 to the development of the disease including the differentiation of stem and

85 progenitor cells and the transition of cells from an epithelial to a mesenchymal

86 phenotype[29].

87

88 Commensurate with its key role in cell cycle control, genomic alterations in the

89 *RB1* gene are relatively common in TNBCs[31, 32, 49] as well as in a series of

90 other malignancies[17, 27, 60]. In TNBC, loss of Rb protein expression is found in

91 > 40 % of cases[58, 61] (and reviewed in[30]). Although patients with Rb defective

92 tumours (as defined by immunohistochemistry and/or gene expression) tend

93 to have a relatively favorable response to classical chemotherapy[20, 26, 64]

94 many either fail to respond to therapy or later relapse with therapy resistant

95 disease, suggesting that novel therapeutic approaches are required to target

96 this patient subset.

97

98 One approach to identifying novel therapeutic targets that could be exploited

99 in patients with specific tumour suppressor gene defects has been to identify

100 synthetic lethal interactions associated with these genes. For example, the

101 identification of synthetic lethal interactions between *BRCA1* or *BRCA2*

102 tumour suppressor genes and inhibition of the PARP1 DNA repair protein, has

103 driven the clinical development and approval for use of PARP inhibitors for the

104 treatment of cancer[39]. One notable feature of the BRCA/PARP1 synthetic

105 lethal effect, that contributes to its translational value, is that it is highly

106 penetrant[2]; i.e. in otherwise molecularly diverse pre-clinical models, and

107 cancer patients, the presence of the predictive biomarker, in this case *BRCA1*

108 or *BRCA2* mutation, more often than not predicts a profound anti-tumour cell

109 response to a PARP inhibitor. This highly penetrant nature suggests that this

110 particular synthetic lethal effect is robust in the face of the molecular

111 heterogeneity that exists between different human cancers. Here, we describe

112 efforts to identify highly penetrant synthetic lethal effects associated with

113 deleterious Rb alterations in TNBC; we reasoned that those with greatest

114 penetrance will be more likely to operate in the diverse molecular contexts

115 within the TNBC subtype and thus represent more promising therapeutic

116 targets. Although genes such as *TSC2*[54] and elements of the Dicer pathway[44]

117 have been shown to be synthetic lethal with Rb defects, as far as we are

118 aware, the penetrance of these effects, or whether these operate in models of

119 TNBC, has not as yet been assessed. The availability of several, large-scale,

120 shRNA and siRNA screens[8, 9, 42, 62], conducted in multiple tumour cell lines,

121 some of which are derived from TNBCs, now make a detailed identification of

122 highly penetrant RB1-related synthetic lethal effects now possible. For this

123 reason, we describe here a detailed molecular analysis of Rb status in tumour

124 cell lines derived from TNBC. We then designed a straightforward data

125   analysis pipeline that allowed us to use this Rb annotation to interrogate both

126   in-house and publically available large-scale, shRNA and siRNA screens to

127   identify candidate Rb-related synthetic lethal effects. Included within this data

128   analysis pipeline, we included an estimate of penetrance. In triaging the

129   candidate Rb-synthetic lethal effects that operated in TNBC tumour cells to

130   identify those with greatest penetrance, we identified a series of

131   pharmacologically tractable effects, one of which, SKP2, we validated using

132   both genetic and pharmacological methods. We also noted that a significant

133   proportion of the highly penetrant Rb SL effects in TNBC involved proteins

134   closely associated with Rb function, suggesting that this might be a defining

135   characteristic.

136

137   ## Results

138   **Annotation of Rb status in TNBC tumour cell lines**

139   To identify highly robust synthetic lethal effects associated with Rb defects in

140   TNBC, we classified a molecularly diverse panel of TNBC tumour cell lines

141   (TCLs) according to Rb status and then used this Rb classification to

142   interrogate publically available genetic screen data using a data analysis

143   pipeline (described later) that identified highly penetrant synthetic lethal

144   effects. To do this, we first classified TNBC tumour cell lines using a

145   combination of genomic, transcriptomic and proteomic data to identify those

146   with Rb genetic or epigenetic defects. We used western blotting to assess Rb

147   protein expression in 30 breast TCLs, including 16 TNBC (Figure 1A,B). In

148   this analysis, we found that TCLs with deleterious mutations in the *RB1* gene

149   (BT549, *RB1* c.265_607del343, MDAMB468, *RB1* c.265_2787del2523,

150   DU4475, *RB1* c.1_2787del2787, MDAMB436, *RB1* c.607_608ins107) lacked

151   Rb protein expression, suggesting that the mutational status of *RB1*

152   correlated with protein expression (Figure 1A,B). We also found that SUM149

153   cells exhibited low Rb protein expression, an observation we found to be

154   consistent with reduced *RB1* mRNA levels (Supplementary Data 1). To

155   assess Rb protein status by orthogonal means, we compared our western blot

156   data with publically available mass spectrometry data describing the

157   proteomic profiles of 16 TNBC tumour cell lines[33]. Using average intensity-

158 based absolute protein abundance (iBAQ) data for Rb from mass

159 spectrometry profiling[33] (Supplementary Data 1), we found that TNBC TCLs

160 classified by western blotting as being Rb defective exhibited no Rb peptides

161 (MDAMB468, MDAMB436, HCC1937, DU4475, BT549) when compared to

162 those tumour cell lines we had classified as Rb proficient (Figure 1C,

163 p=0.0002), giving us some confidence in our classification. Examination of

164 transcriptomic profiles of TNBC TCLs[3] revealed that TCLs with reduced levels

165 of *RB1* mRNA exhibited low Rb protein expression (Figure 1D, p=0.0075),

166 suggesting that *RB1* mRNA expression levels could be used as a reasonable

167 proxy for protein expression. Taking proteomic, genomic and transcriptomic

168 data into consideration (Figure 1E), we then classified a total of 42 TNBC

169 TCLs according to Rb status, identifying 12 with one or more defects in Rb

170 ("Rb defective" e.g. low protein expression, truncating mutation/gene deletion,

171 reduced mRNA levels: BT549, HCC1937, DU4475, MDAMB436, MDAMB468,

172 CAL148, HDQP1, MB157, SUM149, HCC1187, HCC3153 and CAL851) and

173 30 TNBC TCLs as not exhibiting such defects ("Rb not altered" TCLs).

174

175 To further assess the validity of our Rb classification, we assessed the

176 transcriptomic profiles of Rb defective TNBC TCLs to assess whether these

177 reflected loss of Rb function (Figure 1F). Using the Rb defective and Rb not

178 altered classification described above for 42 TNBC TCLs, we identified 839

179 differentially expressed genes (452 with reduced expression in Rb defective

180 TCLs, 387 with elevated expression ($p<0.05$, Limma analysis, Supplementary

181 Data 2)). As expected, we found *RB1* itself to be the fifth most down-regulated

182 gene in the Rb defective TCLs compared to Rb not altered TCLs (log fold

183 change of -2.4, *p* value = 1.6×10^{-6} (Limma analysis); Supplementary Data 2

184 and Figure 1F). We also found that Rb defective TCLs exhibited elevated

185 expression of cyclins associated with G_1 checkpoint control and S phase

186 progression (Cyclin E1 (*p*=0.03), E2 (*p*=0.02), CDKN2A (p16; *p*=0.002)), as

187 well as elevated expression of the Rb regulated E2F3 transcription factor and

188 its binding partner TFDP1 (Figure 1F (*p*=0.005 and *p*=0.0001, respectively)).

189 Using the ENRICHR annotation tool[12] to identify pathways rather than

190 individual genes that were differentially expressed in the Rb defective group,

191 we found that pathways related to Rb and G_1 to S cell cycle control to be

192 among the most significantly dysregulated in Rb defective TNBC TCLs,
193 including "Retinoblastoma (RB) in Cancer WP2446" p=1.7 x 10^{-11} and "G$_1$ to S
194 cell cycle control WP45" p=1.4 x 10^{-9}, both of which included genes such as
195 *MCM4,6* and *7, TFDP1, CCNE1, CCNE2, CHEK1, E2F3,* and *RBP1*. Using
196 the same annotation tool we also searched for the key transcription factors
197 that regulated genes that were differentially expressed in Rb defective TNBC
198 TCLs. We found that a significant proportion of the differentially expressed
199 genes in Rb defective TNBC TCLs were targets of Rb-related E2F-family
200 transcription factors including E2F7, E2F4 and E2F1 (e.g. p =0.001; 0.005;
201 9.3 x 10^{-7} for E2F7; E2F4 and E2F1 respectively).

202

203 To compare the observations made in TCLs with TNBC tumours, we used the
204 same approach of exploiting *RB1* gene mutation/copy number status and RB1
205 mRNA expression profiles to classify 140 TCGA triple negative breast
206 tumors[10] according to Rb status; this approach identified 48 Rb defective
207 TNBC tumours and 92 where Rb was not altered. Assessing the
208 transcriptomic profiles of these TNBCs we again found that genes associated
209 with Rb and Rb-related G$_1$ to S cell cycle control were frequently dysregulated
210 in Rb defective TNBCs including *CDKN2A, TFDP1, CCNE1, CCNE2, E2F3,*
211 *CHEK1* and *DYRK1A*, a recently identified *RB1* synthetic lethal gene[9] (Figure
212 1G, Supplementary Data 3), consistent with the observations made in TNBC
213 TCLs. We also applied the same approach to classify 182 Metabric TNBC
214 tumours[46] according to Rb status; this approach identified 55 Rb defective
215 and 132 Rb not altered TNBC samples. Assessing the transcriptomic profiles
216 of these TNBCs we again found that genes associated with Rb and Rb-
217 related G$_1$ to S cell cycle control were frequently dysregulated in Rb defective
218 TNBCs (Figure 1H, Supplementary Data 4). These global transcriptional
219 patterns in Rb defective TNBC TCLs and human tumours suggested that our
220 Rb classification approach was somewhat valid.

221

222

223 **A pipeline for the identification of highly-penetrant Rb synthetic lethal**
224 **effects in TNBC**

225 Using the Rb classification of TNBC TCLs described above, we re-analysed

226 publically available genetic screen data (e.g. genome-wide shRNA screen

227 data[42, 62], gene subset shRNA screen data[43] or gene subset siRNA data[8, 9])

228 from TNBC TCLs, using a series of iterative data processing steps designed

229 to identify highly-penetrant synthetic lethal effects (Figure 2A). In summary,

230 these steps involved: (i) **step one – identification of candidate synthetic**

231 **lethal effects:** using shRNA screen data[42] for 12 Rb defective TNBC cell

232 lines and 30 Rb not altered cell lines we used the siMEM algorithm[42] to

233 identify those genes whose inhibition appeared to target the Rb defective

234 models to a greater extent than Rb proficient TNBC TCLs ($p < 0.05$). (ii) **steps**

235 **two and three – apply Z score thresholds to identify profound cell**

236 **inhibitory effects:** although step one allowed us to identify genes whose

237 inhibition selectively targeted Rb defective TCLs to a greater extent than Rb

238 not altered TCLs, we reasoned that the scale of cell inhibition in these two

239 TCL cohorts might also be critical in identifying the most suitable therapeutic

240 targets. For example, for the purposes of identifying novel therapeutic targets

241 we were less interested in genes whose inhibition profoundly inhibited both

242 Rb defective and Rb not altered groups, even if the Rb defective models

243 exhibited significantly greater sensitivity; we assumed that inhibition of these

244 targets would likely cause significant normal cell toxicity. Similarly, we also

245 assumed that candidate synthetic lethal effects that did not elicit profound cell

246 inhibitory effects in Rb defective TCLs would be less likely to elicit profound

247 anti-cancer therapeutic effects when exploited clinically. For these reasons,

248 we triaged the list of candidate synthetic lethal effects identified in step 1 to

249 remove from further analysis: (a) those genes that appeared to be synthetic

250 lethal with Rb but whose targeting elicited profound cell inhibition in Rb not

251 altered TCLs; and (b) those genes that appeared to be synthetic lethal with

252 Rb but whose targeting did not elicit profound cell inhibitory effects in Rb

253 defective TCLs. To do this we used shRNA/siRNA Z score thresholds to

254 estimate the effect of each RNAi reagent; (iii) **steps four and five – identify**

255 **effects with greatest penetrance:** We reasoned that the most clinically

256 effective synthetic lethal targets are likely to be those that have complete or

257   highly penetrant effects, i.e. the presence of the predictive biomarker (in this

258   case an Rb defect) is more often than not associated with profound sensitivity

259   to target inhibition. For this reason, we finally triaged synthetic lethal effects by

260   calculating synthetic lethal penetrance scores; in this case, synthetic lethal

261   penetrance (SLP) for each synthetic lethal effect was calculated as the

262   percentage of Rb defective TCLs that exhibited a Z score of less than -1 when

263   targeted with shRNA. We also calculated synthetic lethal coverage (SLC)

264   scores, i.e. the percentage of TNBC TCLs that were sensitive to shRNA that

265   were Rb defective, to estimate the specificity of synthetic lethal effects for Rb

266   defects.

267

268   To illustrate the concepts of penetrance and coverage as applied to functional

269   genomic screens in tumour cell lines, we examined a well-characterised and

270   therapeutically actionable oncogene addiction effect that operates in breast

271   cancer, namely that associated with amplification of the epidermal growth

272   factor receptor oncogene, *ERBB2*. Amplification and overexpression of

273   *ERBB2* is used clinically to stratify breast cancer patients for treatment with

274   ERBB2-targeting agents such as the monoclonal antibody trastuzumab[59]. In

275   examining the Colt2/Marcotte *et al* shRNA dataset (78,432 shRNAs targeting

276   16,056 genes in 77 breast TCLs representing TNBC and non-TNBC

277   subtypes[42], we found that, as shown before[42], that shRNA targeting of *ERBB2*

278   selectively targeted *ERBB2* amplified breast TCLs (siMEM, p<0.0001), elicited

279   minimal inhibition in *ERBB2* non-amplified TCLs (median Z -1), profound

280   inhibition in *ERBB2* amplified TCLs (median Z -3) and had a SLP

281   (penetrance) score of 80% and a SLC (coverage) score of 28% (Figure 2B);

282   the high penetrance score in this case, taken together with ERBB2 fulfilling

283   the other selection criteria, reconfirms *ERBB2* as a suitable therapeutic target

284   in appropriately stratified breast cancer patients.

285

286 **Identification of Rb synthetic lethal effects from shRNA screens**

287 To identify Rb synthetic lethal effects, we first used genome-wide shRNA data

288 from 42 TNBC TCLs described in the Colt2/Marcotte *et al* study[42]. In this

289 dataset, the combined effects of multiple shRNAs targeting a single gene are

290 described as Z normalised Gene Activity Ranking Profile (zGARP) scores[41].

291 We re-analysed this shRNA screen data using the siMEM mixed effect linear

292 model method (see **step one**, above), which identifies synthetic lethal effects

293 by estimating the depletion or "drop-out" rate of individual shRNAs in cohorts

294 of cell lines classified according to a molecular feature[42], in this case Rb

295 deficiency. Using the siMEM approach with our Rb classification of 42 TNBC

296 TCLs, we identified 1065 Rb-specific dependencies ($p < 0.05$, siMEM, Figure

297 2C): 437 genes where shRNA preferentially inhibited Rb defective TNBC (i.e.

298 Rb synthetic lethal effects) and 628 genes where shRNAs preferentially

299 targeted Rb proficient TNBC TCLs (Supplementary Data 5). Amongst these,

300 we noted that shRNAs targeting *CDK4, CDK6* or the CDK4,6 cyclin partner

301 gene *Cyclin D1* (*CCND1*) preferentially inhibited Rb proficient TNBC TCLs

302 (Figure 2C), consistent with the hypothesis that inhibition of CDK4,6 activity

303 restores cell cycle control in Rb proficient TNBC tumour cells and elicits cell

304 inhibition[1]. In terms of identifying Rb dependencies, we noted that the siMEM

305 analysis of the Colt2 dataset identified shRNA targeting the E2F family

306 transcription factor, E2F3, as being one of the most significant Rb synthetic

307 lethal effects (Figure 2C). These Rb-related observations thus gave us some

308 confidence in the approach. We also carried out similar analyses in

309 siRNA/shRNA datasets that included TNBC TCLs, from other sources: the

310 DRIVE dataset[43], the Achilles dataset[62] and the ICR-Intercell dataset[8, 9] and

311 provide the lists of Rb dependencies identified in these datasets in

312 Supplementary Data 6, 7, 8, respectively.

313

314

315 **Application of Z score thresholds identifies profound cell inhibitory**

316 **effects**

317 As described above, we were interested in identifying Rb synthetic lethal

318 effects that had profound effects in Rb defective TNBCs but had minimal

319 effects in cells without Rb defects. Although approaches such as siMEM are

320 useful in identifying putative vulnerabilities, they often do not, when used in

321 isolation, identify synthetic lethal effects with these favoured characteristics.

322 For example, Figure 2D and 2E illustrate a pair of p<0.05 Rb dependencies

323 identified by siMEM analysis (Step One), that had either profound cell

324 inhibitory effects in both Rb not altered as well as Rb defective TNBC TCLs

325 e.g. *PSMD1* (Figure 2D, siMEM p = 3 x 10^{-5}, median Z in Rb defective of -6,

326 median Z in Rb not altered of -4) or relative paucity of profound cell inhibition

327 effects in the Rb defective TCL cohort, e.g. *KLF16* (Figure 2E, siMEM p =

328 0.0002, median Z in Rb defective of -0.8). To eliminate such effects from

329 further study, we applied a pragmatic approach that removed from further

330 assessment p<0.05 synthetic lethal effects where the median zGARP score in

331 the Rb defective TCLs was >-1 (i.e. effects where profound cell inhibition in

332 Rb TCLs not observed) and those effects where median zGARP score in Rb

333 proficient effects was <-2 (i.e. dependencies which still elicited profound cell

334 inhibition in Rb proficient TCLs); three examples that fulfilled these criteria,

335 *GPS1, SNRPF and SNW1*, are shown in Figure 2F,G,H. This triage step

336 identified 122 Rb synthetic lethal effects in the Colt2 dataset that fulfilled these

337 criteria (Supplementary Data 9). Similarly, triaged dependencies were

338 identified in the DRIVE[43], Achilles[62] and ICR-Intercell datasets[8, 9]

339 (Supplementary Data 10, 11 and 12, respectively).

340

341 **Highly penetrant Rb synthetic lethal effects in TNBC include TAF1, TAF1**

342 **target genes, nuclear pore complex proteins and the SCF^{SKP}-COP9**

343 **signalosome complexes**

344 We next calculated synthetic lethal penetrance (SLP) and coverage (SLC)

345 scores for each Rb synthetic lethal effect triaged in steps two and three. In our

346 analysis of the *Colt2* dataset, we calculated SLP and SLC scores for 122 Rb

347 synthetic lethal effects, identifying 54 effects that had greater than 80 %

348 penetrance (Figure 3A (SLP>90% shown), Supplementary Data 9) and also

349   identified highly penetrant effects from our analysis of the DRIVE and Achilles

350   and ICR-Intercell datasets (Supplementary Data 10, 11 and 12, respectively).

351   Amongst the highly penetrant Rb SL candidates (>80% penetrant), a

352   significant number are known to be involved in RNA splicing (Supplementary

353   Figure 1, *p*=5.14×10^{-12}, GO Biological Processes 2017, "*mRNA splicing, via*

354   *spliceosome*", Enrichr[12], Supplementary Data 13), including: ISY1 (SLP =

355   92%); SON (SLP = 100%); CWC22 (SLP = 100%); DDX23 (SLP = 100%);

356   POLR2E (SLP = 100%); GEMIN5 (SLP = 92%); POLR2F (SLP = 100%);

357   TFIP11 (SLP = 92%); SRRM2 (SLP = 83%); LSM2 (SLP = 83%); SNW1 (SLP

358   = 83%); SART3 (SLP = 83%); FIP1L1 (SLP = 83%) and SNRPD3 (SLP = 92).

359   Interestingly, a RNAi screen in *C.elegans* also identified a synthetic lethal

360   interaction between Rb loss and components of the splicing machinery[11].

361   Similarly, a number of proteins involved in the regulation of translation were

362   also identified as candidate Rb SL targets (*p*=1.7×10^{-7}, GO Biological

363   Processes 2017, "*regulation of translation by machinery localisation*",

364   Enrichr[12], Supplementary Figure 1, Supplementary Data 13). These included:

365   RPS24 (SLP = 83%); RPS27 (SLP = 100%); RPS28 (SLP = 100%); RPL18A

366   (SLP = 92%); RPL13A (SLP = 92%); RPL10 (SLP = 92%); TCOF1 (SLP =

367   92%); GEMIN5 (SLP = 92%) and RPL38 (SLP = 92%).

368

369   We also identified two nuclear pore complex (NPC) components[4, 22], NUP88

370   (SLP = 100%) and NUP214 (SLP = 83%) as highly penetrant Rb synthetic

371   lethal partners (Supplementary Figure 1, Supplementary Data 13). NPCs are

372   responsible for trafficking proteins between the nucleus and the cytoplasm. In

373   particular, NPCs control spindle assembly, faithful chromosome segregation

374   and mitotic progression by controlling the temporal and spatial localisation of

375   proteins[6, 45, 66] including the E2F transcriptional target and spindle assembly

376   checkpoint (SAC) protein, MAD2, whose elevated expression is required for

377   chromosomal instability in Rb defective cells[50, 52]. It seems possible that the

378   highly penetrant synthetic lethalities between Rb and NUP88 or NUP214

379   could be related to the temporal and spatial localisation of MAD2, perhaps by

380   causing a level of excessive genomic instability that is incompatible with cell

381   viability.

382

383   When taking all of the highly penetrant Rb synthetic lethal effects identified in

384   the analysis of the *Colt2* dataset into account, a significant number of these

385   included genes/proteins known to be associated with Rb function including the

386   Rb interacting protein TAF1 (siMEM *p*=0.016, SLP 92% and SLC 38%, Figure

387   4A)[56]. TAF1 encodes the major 250 kDa subunit of the transcription initiation

388   factor, TFIID[57] which binds core promoter regions, including promoter start

389   sites in genes implicated in cell cycle control[36]. We found that over half (n =

390   33) of the highly penetrant Rb synthetic lethal genes encompassed putative

391   TAF1 binding sites, as defined by TAF1 Chip-Seq data[12, 16] a significant

392   enrichment over chance alone (p = 2.72 x10^{-13}, Figure 4B). One mechanistic

393   explanation for these observations might be that many of the highly penetrant

394   Rb synthetic lethal interactions that operate in TNBC are caused by aberrant

395   TAF1 activity, a downstream effect of Rb dysfunction. TAF1 encompasses

396   two protein domains, a kinase and bromodomain, which in principle are

397   pharmacologically tractable. When taken together with the highly penetrant

398   Rb synthetic lethal interaction, this might make TAF1 an attractive target for

399   cancer drug discovery[7, 18, 53].

400

401   SKP1 and SKP2 were also identified as highly penetrant Rb synthetic lethal

402   effects (Figure 4C,D, SLP 100% and 92% for SKP1 and SKP2, respectively).

403   Both SKP1 and SKP2 are part of an E3 ubiquitin ligase complex, SCF^{SKP2}

404   (Skp, Cullin F-box containing complex), whose activity is greatest during late

405   G_1/early S phase of the cell cycle. Using ubiquitin ligation, the SCF^{SKP2}

406   complex targets proteins for proteasomal degradation including the cyclin

407   dependent kinase inhibitors, p21 and p27[67]. Three other highly penetrant Rb

408   synthetic lethal effects identified were also associated with the SCF complex

409   (Figure 4E-G); *COPS1* (aka *GPS1,* SLP 83%, Figure 4E), *COPS3* (SLP 83%,

410   Figure 4F) and *COPS4* (SLP 92%, Figure 4G) encode components of the

411   COP9 (Constitutive photomorphogenesis 9) signalosome complex (CSN[35, 37]).

412   CSN regulates the ubiquitin ligase activity of SCF complexes via the

413   deneddylation of the ring finger subunits (e.g. Rbx1) within SCF[15] (Figure 4I).

414   We also noted that *CKS1B* (CDC28 protein kinase regulatory subunit 1B) also

415   represented a penetrant Rb synthetic lethal partner (SLP 58%, Figure 4H);

416   together with its co-factor SKP2, CKS1B provides the substrate specific

417   targeting of p27 by SCF[SKP2][47]. When we compared transcriptomic data from

418   RB1 defective TNBCs to those from TNBC with no apparent RB1 defect

419   (Supplementary Data 3 and 4), we noted that RB1 defective TNBCs

420   expressed significantly elevated levels of *SKP2* and *COPS1 (GPS1)* mRNA

421   (Figure 4J,K,L, $p<0.05$ for both TCGA[10] and Metabric[46] data, Wilcox rank sum

422   test), suggesting that in these particular tumours, elevated SKP2 activity might

423   buffer the effects of RB1 dysfunction.

424

425   SKP2 directly interacts with Rb[28] and has previously been shown to be

426   required for the hyper-proliferative phenotype of *Rb*-depleted human

427   retinoblastoma cells, via its regulatory control over p27 levels[67]. In cells with

428   normal G_1/S cell cycle control, Rb binds SKP2, impairing its activity within the

429   SCF[SKP2] complex; loss of Rb however, results in elevated SKP2 activity, a

430   resultant reduction in p27 and p21 protein levels, loss of Cyclin E-CDK2 and

431   Cyclin D-CDK4,6 inhibition and thus progression of cells through the G_1

432   restriction point and into S phase[40]. To directly test whether Rb loss in a

433   breast epithelial cell could cause synthetic lethality with SKP2 inhibition, we

434   silenced SKP2 using two different siRNAs in non-tumour breast epithelial

435   MCF10A cells expressing either a shRNA silencing *RB1* or a non-silencing

436   (NS) control shRNA (Figure 5A). Silencing of *SKP2* (Figure 5B) elicited

437   synthetic lethality in Rb defective cells but not Rb proficient cells (Figure 5C,

438   Student's t test $p<0.0001$, Supplementary Figure 2). We also found that the

439   toolbox SKP2 inhibitor SKPinC1, which impairs the binding of phosphorylated

440   p27 to the substrate recognition pocket formed by SKP2 and CKS1B[65], had a

441   profound synthetic lethal effect on Rb defective MCF10A cells, but minimal

442   effects in Rb wild type cells ($p<0.001$ two-way ANOVA, Figure 5D) and

443   induced apoptosis in Rb defective cells (Supplementary Figure 3). We also

444   assessed SKPinC1 sensitivity in 13 TNBC TCLs, and found that Rb defective

445   TCLs were more sensitive than TCLs with no apparent Rb defect (Figure 5E

446   and $p<0.0022$, Student's t test). In addition, we confirmed that exposure of

447   MCF10A cells with SKPinC1 inhibitor led to a stabilization of p27 protein

448   levels (Figure 5F). Targeting of p27 by siRNA also partially reversed the

449   inhibitory effect of SKPinC1 (Supplementary Figure 4), suggesting that the

450   effect of SKPinC1 was p27 dependent. Taken together, these observations

451 suggested that SKP2 small molecule inhibition, could in principle, elicit the

452 highly penetrant Rb synthetic lethal effect seen in TNBC tumour cells with

453 RNA interference reagents.

454

455 We also assessed whether other highly penetrant synthetic lethal effects

456 operated in the RB1 isogenic MCF10A system. Using an arrayed siRNA

457 screen we tested all 54 genes that we identified as highly penetrant (> 80%

458 penetrance) RB synthetic lethal effects in TNBC cell lines in the Colt2 study

459 along with an additional four controls (e.g. E2F3) for a total of 58 genes. We

460 found that over half of the identified dependencies (55%) were observed in

461 the isogenic system (Supplementary Figure 5), including profound synthetic

462 lethal effects associated with *TIMELESS, PCDH1, PITRM1, E2F3, SMN2* and

463 *TCOF1.* This suggests that these effects can be specifically associated with

464 RB loss, and that they are not an artefact of either the shRNA library used in

465 Colt2 or the pooled screening approach. It seemed possible that differences in

466 the proliferative rate of RB1 defective vs. wild type MCF10A cells could

467 account for the synthetic lethal effects observed. However, we found that the

468 proliferative rate of RB1 defective and wild type MCF10A cells was similar

469 (Supplementary Figure 6), suggesting this might not have a significant bearing

470 on the synthetic lethal effects identified. Moreover, analysis of previously

471 published doubling times for 17 TNBC cell lines identified no significant

472 difference between RB1 defective and RB1 proficient models (Mann-Whitney

473 U-test p=0.4)[25].

474

475 We also assessed which of the highly penetrant Rb synthetic lethal effects

476 identified in our analysis of the *Colt2* data were also identified as highly

477 penetrant effects in the TNBC TCLs in two other shRNA screens: Achilles[62]

478 and DRIVE[43]. Comparing the p<0.05 Rb penetrant synthetic lethal effects

479 between the three datasets, we noted that SKP2 was one of two synthetic

480 lethal effects identified in all three screens, the other effect being associated

481 with SART3, the RNA splicing factor (Figure 6A-C, Supplementary Data 9, 10

482 and 11). In each screen, SKP2/Rb synthetic lethality was highly penetrant with

483 SLP scores of 92% (Colt2), 75% (Achilles) and 100% (DRIVE dataset, Figure

484 6D-F). Whilst the TNBC TCLs described in these three datasets are partially

485   overlapping, the shRNA libraries used were independently designed and

486   synthesised and the screens independently carried out. As such, identifying

487   SKP2 as a highly penetrant Rb synthetic lethal effect in all three datasets

488   suggested that this effect was somewhat independent of the shRNA reagents

489   used.

490

491   **Rb/SKP2 synthetic lethality operates in tumour cells from lung and other**

492   **cancer histologies**

493   Rb defects are relatively prevalent in TNBC, but are not unique to this cancer

494   subtype and are also evident in in many tumour types including, for example,

495   small-cell lung cancers, bladder carcinomas, osteosarcomas, glioblastomas,

496   endometrial carcinomas and retinoblastomas[14, 17, 23, 27, 55]. To estimate

497   whether the highly penetrant Rb/SKP2 synthetic lethality in TNBC extended to

498   other cancer histologies, we re-analysed the results of two recent large-scale

499   shRNA screens that encompass TCLs derived from non-breast cancer

500   histologies. Project DRIVE includes 373 non-breast tumour cell lines with

501   available Rb mutation and copy number status that were derived from 18

502   different cancer histologies[43]. In an analysis of the DRIVE dataset that

503   excluded the breast tumour cell lines, we found a significant association

504   between *RB1* mutation/deletion and sensitivity to *SKP2* shRNA (Figure 7A,B;

505   MP-test $p<0.0001$, SLP=75%). Of over 6,000 genes tested, only *E2F3* (MP-

506   test $p<0.0001$) showed a stronger association with Rb status (Figure 7A).

507   Similarly, project Achilles includes 467 non-breast tumour cell lines with

508   available Rb mutation and copy number status, derived from 20 different

509   cancer histologies[62]. In an analysis of the Achilles dataset that excluded the

510   breast tumour cell lines, we also found a significant association between *RB1*

511   mutation/deletion and sensitivity to *SKP2* shRNA (Figure 7C,D; MP-test

512    $p<0.0001$, SLP=74%). Of over 17,000 genes, SKP2 synthetic lethality was the

513   second most significant effect associated with Rb loss, after *CDK2* (MP-test

514    $p<0.0001$) with *E2F3* being the third most significant effect (Figure 7C). The

515   elevated penetrance of the Rb/SKP2 synthetic lethal effect in tumour cell

516   models other than those derived from breast cancer suggested that this

517   genetic dependency might operate in multiple histologies. When we

518   considered the specific cancer histology of tumour cell lines, rather than

519   analysing these *en mass,* we found in both the Achilles and DRIVE datasets
520   the Rb/SKP2 synthetic lethality was detectable in tumour cell lines derived
521   from lung cancers (p=0.026/SLP=71% and p=0.0008/SLP=73% for DRIVE
522   and Achilles, respectively; Figure 7E,F). However, we do note that the
523   relatively small number of Rb defective tumour cell lines from non-lung cancer
524   histologies in these datasets might impair the ability to detect the Rb/SKP2
525   synthetic lethality. For example, in both DRIVE and Achilles datsets, we noted
526   that Rb defective tumour cell lines derived from prostate cancer,
527   osteosarcoma, liver cancer and oesophageal cancer, exhibited sensitivity to
528   SKP2 shRNA (Figure 7G).

529

530   **Discussion**
531   There is now a relatively long-standing history of using genetic concepts such
532   as synthetic lethality to identify novel therapeutic targets for the treatment of
533   cancer[24]. In part these efforts have been successful, with synthetic lethal
534   treatments[39] and drugs that exploit oncogene addiction effects now being
535   approved for the treatment of the clinical disease[38]. However, despite these
536   advances, one challenge to this approach has been in identifying highly
537   penetrant synthetic lethal effects that associate with the presence of a
538   molecular biomarker. Here we describe efforts to identify highly-penetrant
539   synthetic lethal effects associated with loss of the tumour suppressor *Rb* in
540   TNBC. Following the classification of 42 TNBC TCLs according to Rb status,
541   we interrogated genome-scale and focussed gene set shRNA screening
542   datasets, identified candidate synthetic lethal effects and then used stringent
543   filters to triage from the list of candidate synthetic lethal effects those most
544   likely to represent highly penetrant effects. This approach allowed us to
545   identify a number of highly penetrant synthetic lethal effects, many of which
546   are associated with known functions of Rb and/or associated with Rb
547   interacting proteins. These included TAF1 and TAF1 target genes as well as
548   members of the SCF^{SKP2} complex.

549

550   We note that there are considerable caveats associated with the approach we
551   have taken to identifying highly penetrant Rb-related synthetic lethal effects in
552   TNBC and the interpretation of the data; understanding these caveats is

553   critical to the use of the information we provide in this work. Primary amongst

554   these is that we have exclusively used data from *in vitro* genetic screens to

555   identify synthetic lethal effects and assess their penetrance. It seems likely

556   that some of the effects identified in our analysis only operate in the context of

557   two-dimensional *in vitro* culture and are abrogated, and therefore appear less

558   penetrant, in three dimensional and/or *in vivo* settings. Such a possibility thus

559   provides the rationale for also assessing synthetic lethal effects, and

560   assessing their penetrance, in *in vivo* models of cancer. Secondly, there is

561   little way of effectively estimating the true false negative rate of our approach;

562   it is entirely possible that we have not identified real, highly penetrant, Rb

563   related synthetic lethal effects either because the RNA interference reagents

564   used in genetic screens ineffectively inactive the genes they are designed to

565   target or because some other form of gene/protein inactivation (e.g. catalytic

566   inhibition of the target protein rather than gene silencing) is required to elicit a

567   synthetic lethal effect. Nevertheless, the identification in multiple,

568   independently-conducted, genetic screens of the highly penetrant Rb/SKP2

569   synthetic lethal effect, and its recapitulation with a small molecule inhibitor,

570   suggests that this highly penetrant synthetic lethality effect is unlikely to be a

571   false positive.

572

573   As well as targeting Rb defective TNBC, the potential for using SKP2

574   inhibition in other cancer histologies associated with Rb defects is

575   considerable. For example, the hyper-proliferative phenotype of *Rb*-depleted

576   human retinoblastoma cells and mouse melanotrophs is dependent upon the

577   SKP2[63, 67] and the SKP2/CKS1 pocket inhibitor, SKPinC1, inhibits *Rb/p53*

578   defective mouse prostate tumour cell organoids[68]. Finally, large-scale shRNA

579   screens in tumour cell lines from a variety of cancer histologies (and our

580   analysis described in Figure 6) suggest that the dependency of Rb defective

581   TCLs upon SKP2 might extend beyond models of TNBC. Whether the high

582   penetrance of the Rb/SKP2 synthetic lethality seen in models of TNBC

583   extends to each of these histologies remains to be seen, but our initial

584   analysis in Figure 6 suggests that highly penetrant effects might also be

585   apparent in lung cancer.

586

587   It might be interesting to speculate what characteristics might differentiate a

588   highly penetrant synthetic lethal effect that operates in cancer from less

589   penetrant effects; being able to understand the factors that distinguish one

590   from the other might eventually allow highly penetrant synthetic lethal effects

591   in cancer to be predicted from first principles, thus reducing the reliance upon

592   large-scale genetic screens in multiple tumour cell lines to empirically

593   establish penetrance. From our relatively unbiased analysis of genome-scale

594   shRNA screens, it is perhaps striking that many of the highly penetrant Rb

595   synthetic lethal effects we identified (e.g. SKP1, SKP2, CKS1B, COPS1,

596   COPS2, COPS3) have two characteristics: (i) they are closely, rather than

597   distally, involved in controlling an essential process in highly proliferating

598   tumour cells, namely G_1 cell cycle progression by Cyclin/CDK activity; and (ii)

599   this process is also closely, rather than distally, controlled by the synthetic

600   lethal partner, Rb. It seems reasonable to think that synthetic lethal

601   interactions that control essential processes in cancer cells via small-world

602   networks (i.e. those than contain a relatively small number of nodes between

603   synthetic lethal partners and proteins involved in essential processes) might

604   be less likely to be reversed, and therefore more likely highly penetrant, than

605   synthetic lethal effects that control essential processes via distal molecular

606   mechanisms that involve larger molecular networks with multiple intervening

607   nodes. Whether this turns out to be a general principle or not remains to be

608   seen but already others have started to establish that many synthetic lethal

609   effects associated with tumour suppressor genes other than Rb can be

610   classified into a defined, and relatively small number of classes, including

611   those between paralogs and those between genes in the same molecular

612   pathway[62]. This suggests that some of the principles that govern how

613   synthetic lethal effects operate in tumour cells can indeed be established.

614

615   **Materials and methods**

616

617   **Cell lines, compounds and siRNA**

618   All cell lines were obtained from the American Type Culture Collection (ATCC)

619   and maintained according to the supplier's instructions. Cell lines were

routinely STR typed and mycoplasma tested. Cell lines were grown and transfected with individual and SMARTpool siGENOME siRNA (Dharmacon) and transfected using RNAiMAX (Invitrogen) reagent as described in[9]. Transfection efficiency was monitored using positive control (siPLK1) and two different negative control siRNAs (siCON1 and siCON2; Dharmacon, catalogue numbers D-001210-01-20 and D-001206-14-20). SKPinC1 inhibitor was purchased from Tocris (no. 4817) and was solubilised as a 10 mM stock solution in DMSO.

Western blot analysis

Whole cell protein lysates were extracted from cells by lysis with NP250 buffer (20mM Tris pH 7.6, 1mM EDTA, 0.5% NP40, 235mM NaCl). In gene silencing experiments, cell were cultured for 48 hours after siRNA transfection, at which point lysates were generated. Following a 20 minute incubation at 4^0C, lysates were centrifuged and supernatents collected. Electrophoresis was performed using Novex precast Bis-Tris gels (Invitrogen) and gels were blotted onto nitrocellulose filters as described previously[21]. Blots were immunoblotted in 5% (w/v) milk at 4°C overnight using the following primary antibodies: anti-Rb1 (1/1000 (v/v) dilution in 5 % (v/v) milk, New England Biolab, 9309), anti-p16 (1/1000, abcam), anti-SKP2 (1/1000, New England Biolab, 4358), anti-p27 (1/1000, New Engand Biolab, 2552), anti-tubulin (1/1000, abcam) and anti-actin (1/1000, Santa Cruz, sc-1616). After washing, blots were incubated 1 hour at room temperature with secondary antibodies (Li-COR) diluted 1/10,000 (v/v) in 5% (w/v) milk. Protein bands were visualised and quantified using the Odyssey FC imaging system and ImageStudio software (Li-COR).

Tumour and cell line expression analysis

Limma[48] was performed to identify differentially expressed genes RB1 defective *vs.* RB1 not altered tumour cell lines, using data from [42] and [3]. A design matrix with the cell line RB1 classifications was created and a linear model was fitted to expression values of each gene. Next, an empirical Bayes method was used to obtain more precise gene-wise variability estimates

653 between the two groups. Differential expression between groups was
654 represented as log fold change scores with associated p-values and adjusted
655 p-values. Publically available cell line mRNA expression datasets used in this
656 study include Marcotte[42] and CCLE[3], as indicated.

657

658 For the analysis of TNBC tumours, mRNA expression, DNA copy number and
659 somatic mutation profiles for TCGA tumours were downloaded from GDAC
660 (https://gdac.broadinstitute.org/), release 2016_01_28. GISTIC v2 level 4 data
661 were used for copy number analysis and log_2 ratios were converted to
662 genomic gains/amplifications, neutral and loss/deletion states using threshold
663 of $\pm log_2(3/2)$. Raw Metabric data files were downloaded from the European
664 Genome-phenome Archive (EGA; study ID EGAS00000000083). The
665 Metabric breast cancer dataset was pre-processed, summarised and quantile-
666 normalised from the raw expression files generated by Illumina BeadStudio (R
667 packages: beadarray v2.4.2 and illuminaHuman v3.db_1.12.2). Probe to
668 gene-level mapping was performed by keeping the most variable (standard
669 deviation) probe. Metabric copy number calls were used as published in the
670 original study[19].

671

672 For TCGA breast cancer cohort, previously published[34] TNBC6 and TNBC4
673 calls were used resulting in 140 patients with matched mRNA, copy number
674 and mutation profiles. For the Metabric cohort, TNBC6 calls were successfully
675 derived from TNBC subtyping portal[13] (http://cbc.mc.vanderbilt.edu/tnbc) for
676 187 patients with matched mRNA and copy number profiles. In the TCGA
677 cohort, TNBC samples with RB1 mRNA z-score < -1 were regarded as Rb-
678 low, RB1 copy number log_2 ratio < -0.585 were regarded as Rb loss and
679 samples with RB1 truncating mutations were considered as Rb inactivated.
680 This resulted in 48 Rb defective and 92 Rb not altered TNBC samples. In the
681 Metabric cohort, samples with RB1 mRNA z-score < -1 were regarded as Rb
682 low, whilst samples with RB1 loss/deletions were regarded as Rb loss. This
683 resulted in 55 Rb defective and 132 Rb not altered TNBC samples.

684

685 Differential gene expression analysis on TCGA TNBC samples was
686 performed using Limma voom[48].

687

Association Testing

689 The si/shRNA Mixed Effect Model (siMEM) was used to measure the
690 essentiality of genes in the Colt2 genome-wide shRNA dropout screen[42].
691 siMEM uses hierarchical linear regression and considers level of each shRNA
692 to be a regression function of its initial abundance over time, baseline trend in
693 abundance over time and difference in abundance trend between samples
694 sharing a common feature. siMEM results are represented as the difference
695 between the dropout rate of hairpins in the two cell line groups being
696 compared.

697 For analysis of the DRIVE and Achilles shRNA screen datasets, where only a
698 single screen time-point was collected, a one-sided median permutation (MP)
699 test was used to identify associations between *RB1* status of cell lines and
700 their sensitivities to shRNA targeting of genes. For each gene, the observed
701 difference between the median of scores of RB1 defective and RB1 not
702 altered groups of tumour cell lines was compared. A total of one million
703 random samples with the same sample size as in the two groups were
704 created. The differences in the medians of the groups were calculated for
705 each random sample and the statistical significance of the difference was
706 determined.

Cell viability assays

708 500 cells per well were seeded into 384-well plates. After 24 hours cells were
709 exposed to increasing concentrations of SKPinC1 inhibitor diluted in DMSO
710 using an Echo 550 liquid handler (Labcyte). Cells were incubated with the
711 inhibitor for five days after which cell viability was estimated with CellTitre-Glo
712 reagent (Promega). For gene silencing experiments cells were incubated for
713 five days following siRNA transfection prior to viability assessment.
714 Luminescence readings from drug exposed cells were normalised to those
715 from DMSO exposed cells to calculate Surviving Fractions (SF). SFs were
716 used to derive four parameter logistic regression dose/response curves using

717 Graphpad Prism software, as per the manufacturer's instructions. Apoptosis
718 was measure using Caspase-Glo 3/7 reagent (Promega).
719

720 **Statistical analysis**
721 Unless otherwise stated all data is represented here as mean $\pm$ standard error
722 using Graphpad Prism Software (La Jolla, CA). All conditions were performed
723 in triplicate in at least triplicate experiments. Statistical significance was
724 measured using either Student's t-test, pearson coefficient correlation, Mann-
725 Whitney U test or two-way ANOVA. *p*<0.05 was considered significant.
726

727

728 **Conflict of interest statement**
729 There are no conflicts of interest to declare.
730

745

746

References

1   Asghar US, Barr AR, Cutts R, Beaney M, Babina I, Sampath D *et al*. Single-Cell Dynamics Determines Response to CDK4/6 Inhibition in Triple-Negative Breast Cancer. Clin Cancer Res 2017; 23: 5561-5572.

2   Ashworth A, Lord CJ, Reis-Filho JS. Genetic interactions in cancer progression and treatment. Cell 2011; 145: 30-38.

3   Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S *et al*. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012; 483: 603-607.

4   Bernad R, Engelsma D, Sanderson H, Pickersgill H, Fornerod M. Nup214-Nup88 nucleoporin subcomplex is required for CRM1-mediated 60 S preribosomal nuclear export. J Biol Chem 2006; 281: 19378-19386.

5   Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol 2016; 13: 674-690.

6   Blower MD, Nachury M, Heald R, Weis K. A Rae1-containing ribonucleoprotein complex is required for mitotic spindle assembly. Cell 2005; 121: 223-234.

7   Bouche L, Christ CD, Siegel S, Fernandez-Montalvan AE, Holton SJ, Fedorov O *et al*. Benzoisoquinolinediones as Potent and Selective Inhibitors of BRPF2 and TAF1/TAF1L Bromodomains. J Med Chem 2017; 60: 4002-4022.

8   Brough R, Frankum JR, Sims D, Mackay A, Mendes-Pereira AM, Bajrami I *et al*. Functional viability profiles of breast cancer. Cancer Discov 2011; 1: 260-273.

9   Campbell J, Ryan CJ, Brough R, Bajrami I, Pemberton HN, Chong IY *et al*. Large-Scale Profiling of Kinase Dependencies in Cancer Cell Lines. Cell Rep 2016; 14: 2490-2501.

10   Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature 2012; 490: 61-70.

11   Ceron J, Rual JF, Chandra A, Dupuy D, Vidal M, van den Heuvel S. Large-scale RNAi screens identify novel genes that interact with the C. elegans retinoblastoma pathway as well as splicing-related components with synMuv B activity. BMC Dev Biol 2007; 7: 30.

12   Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV *et al*. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics 2013; 14: 128.

795
796 13   Chen X, Li J, Gray WH, Lehmann BD, Bauer JA, Shyr Y *et al*. TNBCtype: A
797   Subtyping Tool for Triple-Negative Breast Cancer. Cancer Inform 2012; 11:
798   147-156.
799
800 14   Choi W, Ochoa A, McConkey DJ, Aine M, Hoglund M, Kim WY *et al*.
801   Genetic Alterations in the Molecular Subtypes of Bladder Cancer: Illustration
802   in the Cancer Genome Atlas Dataset. Eur Urol 2017; 72: 354-365.
803
804 15   Chung D, Dellaire G. The Role of the COP9 Signalosome and Neddylation in
805   DNA Damage Signaling and Repair. Biomolecules 2015; 5: 2388-2416.
806
807 16   Consortium EP. The ENCODE (ENCyclopedia Of DNA Elements) Project.
808   Science 2004; 306: 636-640.
809
810 17   Corney DC, Flesken-Nikitin A, Choi J, Nikitin AY. Role of p53 and Rb in
811   ovarian cancer. Adv Exp Med Biol 2008; 622: 99-117.
812
813 18   Crawford TD, Tsui V, Flynn EM, Wang S, Taylor AM, Cote A *et al*. Diving
814   into the Water: Inducible Binding Conformations for BRD4, TAF1(2), BRD9,
815   and CECR2 Bromodomains. J Med Chem 2016; 59: 5391-5402.
816
817 19   Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ *et al*. The
818   genomic and transcriptomic architecture of 2,000 breast tumours reveals novel
819   subgroups. Nature 2012; 486: 346-352.
820
821 20   Ertel A, Dean JL, Rui H, Liu C, Witkiewicz AK, Knudsen KE *et al*. RB-
822   pathway disruption in breast cancer: differential association with disease
823   subtypes, disease-specific prognosis and therapeutic response. Cell Cycle
824   2010; 9: 4153-4163.
825
826 21   Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB *et al*.
827   Targeting the DNA repair defect in BRCA mutant cells as a therapeutic
828   strategy. Nature 2005; 434: 917-921.
829
830 22   Fornerod M, van Deursen J, van Baal S, Reynolds A, Davis D, Murti KG *et*
831   *al*. The human homologue of yeast CRM1 is in a dynamic subcomplex with
832   CAN/Nup214 and a novel nuclear pore component Nup88. EMBO J 1997; 16:
833   807-816.
834
835 23   Goldhoff P, Clarke J, Smirnov I, Berger MS, Prados MD, James CD *et al*.
836   Clinical stratification of glioblastoma based on alterations in retinoblastoma
837   tumor suppressor protein (RB1) and association with the proneural subtype. J
838   Neuropathol Exp Neurol 2012; 71: 83-89.
839
840 24   Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH. Integrating
841   genetic approaches into the discovery of anticancer drugs. Science 1997; 278:
842   1064-1068.
843

844   25   Haverty PM, Lin E, Tan J, Yu Y, Lam B, Lianoglou S *et al*. Reproducible
845   pharmacogenomic profiling of cancer cell line panels. Nature 2016; 533: 333-
846   337.
847

848   26   Herschkowitz JI, He X, Fan C, Perou CM. The functional loss of the
849   retinoblastoma tumour suppressor is a common event in basal-like and luminal
850   B breast carcinomas. Breast Cancer Res 2008; 10: R75.
851

852   27   Higashiyama M, Doi O, Kodama K, Yokouchi H, Tateishi R. Retinoblastoma
853   protein expression in lung cancer: an immunohistochemical analysis.
854   Oncology 1994; 51: 544-551.
855

856   28   Ji P, Jiang H, Rekhtman K, Bloom J, Ichetovkin M, Pagano M *et al*. An Rb-
857   Skp2-p27 pathway mediates acute cell cycle inhibition by Rb and is retained
858   in a partial-penetrance Rb mutant. Mol Cell 2004; 16: 47-58.
859

860   29   Jiang Z, Jones R, Liu JC, Deng T, Robinson T, Chung PE *et al*. RB1 and p53
861   at the crossroad of EMT and triple-negative breast cancer. Cell Cycle 2011;
862   10: 1563-1570.
863

864   30   Johnson J, Thijssen B, McDermott U, Garnett M, Wessels LF, Bernards R.
865   Targeting the RB-E2F pathway in breast cancer. Oncogene 2016; 35: 4829-
866   4835.
867

868   31   Jones RA, Robinson TJ, Liu JC, Shrestha M, Voisin V, Ju Y *et al*. RB1
869   deficiency in triple-negative breast cancer induces mitochondrial protein
870   translation. J Clin Invest 2016; 126: 3739-3757.
871

872   32   Knudsen ES, McClendon AK, Franco J, Ertel A, Fortina P, Witkiewicz AK.
873   RB loss contributes to aggressive tumor phenotypes in MYC-driven triple
874   negative breast cancer. Cell Cycle 2015; 14: 109-122.
875

876   33   Lawrence RT, Perez EM, Hernandez D, Miller CP, Haas KM, Irie HY *et al*.
877   The proteomic landscape of triple-negative breast cancer. Cell Rep 2015; 11:
878   630-644.
879

880   34   Lehmann BD, Jovanovic B, Chen X, Estrada MV, Johnson KN, Shyr Y *et al*.
881   Refinement of Triple-Negative Breast Cancer Molecular Subtypes:
882   Implications for Neoadjuvant Chemotherapy Selection. PLoS One 2016; 11:
883   e0157368.
884

885   35   Li P, Xie L, Gu Y, Li J, Xie J. Roles of Multifunctional COP9 Signalosome
886   Complex in Cell Fate and Implications for Drug Discovery. J Cell Physiol
887   2017; 232: 1246-1253.
888

889   36   Lin CY, Tuan J, Scalia P, Bui T, Comai L. The cell cycle regulatory factor
890   TAF1 stimulates ribosomal DNA transcription by binding to the activator
891   UBF. Curr Biol 2002; 12: 2142-2146.
892

37 Lingaraju GM, Bunker RD, Cavadini S, Hess D, Hassiepen U, Renatus M *et al*. Crystal structure of the human COP9 signalosome. Nature 2014; 512: 161-165.

38 Lord CJ, Ashworth A. Biology-driven cancer drug development: back to the future. BMC Biol 2010; 8: 38.

39 Lord CJ, Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. Science 2017; 355: 1152-1158.

40 Lu Z, Bauzon F, Fu H, Cui J, Zhao H, Nakayama K *et al*. Skp2 suppresses apoptosis in Rb1-deficient tumours by limiting E2F1 activity. Nat Commun 2014; 5: 3463.

41 Marcotte R, Brown KR, Suarez F, Sayad A, Karamboulas K, Krzyzanowski PM *et al*. Essential gene profiles in breast, pancreatic, and ovarian cancer cells. Cancer Discov 2012; 2: 172-189.

42 Marcotte R, Sayad A, Brown KR, Sanchez-Garcia F, Reimand J, Haider M *et al*. Functional Genomic Landscape of Human Breast Cancer Drivers, Vulnerabilities, and Resistance. Cell 2016; 164: 293-309.

43 McDonald ER, 3rd, de Weck A, Schlabach MR, Billy E, Mavrakis KJ, Hoffman GR *et al*. Project DRIVE: A Compendium of Cancer Dependencies and Synthetic Lethal Relationships Uncovered by Large-Scale, Deep RNAi Screening. Cell 2017; 170: 577-592 e510.

44 Nittner D, Lambertz I, Clermont F, Mestdagh P, Kohler C, Nielsen SJ *et al*. Synthetic lethality between Rb, p53 and Dicer or miR-17-92 in retinal progenitors suppresses retinoblastoma formation. Nat Cell Biol 2012; 14: 958-965.

45 Orjalo AV, Arnaoutov A, Shen Z, Boyarchuk Y, Zeitlin SG, Fontoura B *et al*. The Nup107-160 nucleoporin complex is required for correct bipolar spindle assembly. Mol Biol Cell 2006; 17: 3806-3818.

46 Pereira B, Chin SF, Rueda OM, Vollan HK, Provenzano E, Bardwell HA *et al*. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat Commun 2016; 7: 11479.

47 Reed SI. Ratchets and clocks: the cell cycle, ubiquitylation and protein turnover. Nat Rev Mol Cell Biol 2003; 4: 855-864.

48 Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W *et al*. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.

49 Robinson TJ, Liu JC, Vizeacoumar F, Sun T, Maclean N, Egan SE *et al*. RB1 status in triple negative breast cancer cells dictates response to radiation treatment and selective therapeutic drugs. PLoS One 2013; 8: e78641.

943

944 50 Rodriguez-Bravo V, Maciejowski J, Corona J, Buch HK, Collin P, Kanemaki
945 MT *et al*. Nuclear pores protect genome integrity by assembling a premitotic
946 and Mad1-dependent anaphase inhibitor. Cell 2014; 156: 1017-1031.
947

948 51 Russnes HG, Lingjaerde OC, Borresen-Dale AL, Caldas C. Breast Cancer
949 Molecular Stratification: From Intrinsic Subtypes to Integrative Clusters. Am J
950 Pathol 2017; 187: 2152-2162.
951

952 52 Schvartzman JM, Duijf PH, Sotillo R, Coker C, Benezra R. Mad2 is a critical
953 mediator of the chromosome instability observed upon Rb and p53 pathway
954 inhibition. Cancer Cell 2011; 19: 701-714.
955

956 53 Sdelci S, Lardeau CH, Tallant C, Klepsch F, Klaiber B, Bennett J *et al*.
957 Mapping the chemical chromatin reactivation landscape identifies BRD4-
958 TAF1 cross-talk. Nat Chem Biol 2016; 12: 504-510.
959

960 54 Searle JS, Li B, Du W. Targeting Rb mutant cancers by inactivating TSC2.
961 Oncotarget 2010; 1: 228-232.
962

963 55 Semczuk A, Schneider-Stock R, Miturski R, Skomra D, Tomaszewski J,
964 Roessner A *et al*. RB protein expression in human endometrial carcinomas--an
965 immunohistochemical study. Pathol Res Pract 2000; 196: 41-46.
966

967 56 Shao Z, Ruppert S, Robbins PD. The retinoblastoma-susceptibility gene
968 product binds directly to the human TATA-binding protein-associated factor
969 TAFII250. Proc Natl Acad Sci U S A 1995; 92: 3115-3119.
970

971 57 Shao Z, Siegert JL, Ruppert S, Robbins PD. Rb interacts with
972 TAF(II)250/TFIID through multiple domains. Oncogene 1997; 15: 385-392.
973

974 58 Stefansson OA, Jonasson JG, Olafsdottir K, Hilmarsdottir H, Olafsdottir G,
975 Esteller M *et al*. CpG island hypermethylation of BRCA1 and loss of pRb as
976 co-occurring events in basal/triple-negative breast cancer. Epigenetics 2011; 6:
977 638-649.
978

979 59 Takada M, Ishiguro H, Nagai S, Ohtani S, Kawabata H, Yanagita Y *et al*.
980 Survival of HER2-positive primary breast cancer patients treated by
981 neoadjuvant chemotherapy plus trastuzumab: a multicenter retrospective
982 observational study (JBCRG-C03 study). Breast Cancer Res Treat 2014; 145:
983 143-153.
984

985 60 Thomas DM, Carty SA, Piscopo DM, Lee JS, Wang WF, Forrester WC *et al*.
986 The retinoblastoma protein acts as a transcriptional coactivator required for
987 osteogenic differentiation. Mol Cell 2001; 8: 303-316.
988

989 61 Trere D, Brighenti E, Donati G, Ceccarelli C, Santini D, Taffurelli M *et al*.
990 High prevalence of retinoblastoma protein loss in triple-negative breast
991 cancers and its association with a good prognosis in patients treated with
992 adjuvant chemotherapy. Ann Oncol 2009; 20: 1818-1823.

993

994 62 Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS
995 *et al*. Defining a Cancer Dependency Map. Cell 2017; 170: 564-576 e516.
996

997 63 Wang H, Bauzon F, Ji P, Xu X, Sun D, Locker J *et al*. Skp2 is required for
998 survival of aberrantly proliferating Rb1-deficient cells and for tumorigenesis
999 in Rb1+/- mice. Nat Genet 2010; 42: 83-88.
1000

1001 64 Witkiewicz AK, Ertel A, McFalls J, Valsecchi ME, Schwartz G, Knudsen ES.
1002 RB-pathway disruption is associated with improved response to neoadjuvant
1003 chemotherapy in breast cancer. Clin Cancer Res 2012; 18: 5110-5122.
1004

1005 65 Wu L, Grigoryan AV, Li Y, Hao B, Pagano M, Cardozo TJ. Specific small
1006 molecule inhibitors of Skp2-mediated p27 degradation. Chem Biol 2012; 19:
1007 1515-1524.
1008

1009 66 Wu Z, Jin Z, Zhang X, Shen N, Wang J, Zhao Y *et al*. Nup62, associated with
1010 spindle microtubule rather than spindle matrix, is involved in chromosome
1011 alignment and spindle assembly during mitosis. Cell Biol Int 2016; 40: 968-
1012 975.
1013

1014 67 Xu XL, Singh HP, Wang L, Qi DL, Poulos BK, Abramson DH *et al*. Rb
1015 suppresses human cone-precursor-derived retinoblastoma tumours. Nature
1016 2014; 514: 385-388.
1017

1018 68 Zhao H, Lu Z, Bauzon F, Fu H, Cui J, Locker J *et al*. p27T187A knockin
1019 identifies Skp2/Cks1 pocket inhibitors for advanced prostate cancer.
1020 Oncogene 2017; 36: 60-70.
1021

1022

1023

1024 **Figure and Table legends**

1025 **Figure 1. Rb status in TNBC tumour cell lines**. **A.** Western blot illustrating

1026 Rb and p16 protein expression in 30 breast tumour cell lines (TCLs). BT549,

1027 MDAMB436, MDAMB468 and DU4475 each possess loss of function

1028 mutations in the *RB1* gene. SUM149 cells express reduced *RB1* mRNA. **B.**

1029 Scatter plot illustrating quantification of Rb protein levels delineated from (A).

1030 Protein expression in Rb defective *vs.* not altered, p=0.0484, Student's t test.

1031 **C**. Scatter plot illustrating Rb protein expression in defective and proficient

1032 TNBC TCLs estimated by mass spectrometry data from[33]. TNBC TCLs were

1033 classified into "Rb defective" and "not altered" groups according to western

1034 blot data from (A); using this classification, normalised iBAQ Rb peptide

1035 scores were compared and are shown. p=0.0002, Fishers exact test. **D**.

1036 Scatter plot illustrating the correlation between Rb protein and mRNA

1037 transcript levels in TNBC TCLs. Rb protein levels from (A,B) are compared to

1038 Rb mRNA transcript levels obtained from the CCLE database[3]. Correlation

1039 r=0.7, p=0.0075, Pearson's correlation. **E**. Oncoprint illustrating Rb and breast

1040 cancer subtype status amongst 42 TNBC TCLs. **F**. Volcano plot illustrating

1041 mRNAs that are differentially expressed (limma analysis p<0.05) in Rb

1042 defective (*vs.* Rb not altered) TNBC TCLs. Genes functionally related to Rb

1043 are highlighted, as is Rb itself. **G**. Volcano plot of mRNAs that are differentially

1044 expressed (limma analysis p<0.05) in 48 Rb defective (*vs.* Rb not altered)

1045 triple negative breast tumours from the TCGA study[10]. Genes functionally

1046 related to Rb are highlighted, as is Rb itself. **H**. Volcano plot of mRNAs that

1047 are differentially expressed (limma analysis p<0.05) in 55 Rb defective (*vs.* Rb

1048 not altered) triple negative breast tumours from the Metabric study[46]. Genes

1049 functionally related to Rb are highlighted, as is Rb itself.

1050

1051

1052

1053 **Figure 2. Identifying highly penetrant Rb synthetic lethal effects that**

1054 **operate in TNBC**. **A.** Schematic illustrating the data analysis workflow used.

1055 In the first instance, 16,056 gene zGARP scores from shRNA screens in 42

1056 TNBC cell lines described in the Colt2 dataset were analysed; parallel

1057 analyses were carried out using data from the DRIVE and Achilles datasets

1058 (see main text). **B.** Scatter plot illustrating *ERBB2* Z GARP scores in 77 breast

1059 tumour cell lines partitioned according to effects in ERBB2 amplified and non-

1060 amplified TCLs. *ERBB2* shRNA elicits a p<0.0001 oncogene addiction effect

1061 (siMEM) with 80% penetrance in *ERBB2* amplified tumour cell lines (red).

1062 Coverage is also shown. **C.** Scatter plot illustrating 1065 p<0.05 significant

1063 siMEM Rb synthetic lethal effects identified from the siMEM analysis of TNBC

1064 TCLs in the *Colt2* study (step one in (A)). p<0.05 effects are ranked ordered

1065 by siMEM *p* value. E2F3 (synthetic lethal in Rb defective), CDK6, CDK4 and

1066 the CDK4,6 cyclin partner, Cyclin D1 (*CCND1*) (dependencies in Rb not

1067 altered) are highlighted. **D,E.** Scatter plots illustrating Z scores in 42 TNBC

1068 TCLs for two siMEM p<0.05 candidate Rb synthetic lethal effects, PSMD1 (D)

1069 and KLF16 (E), removed from further analysis by the use of Z score filters

1070 (step two and three in (A)). zGARP scores for *PSMD1* (preferentially target Rb

1071 defective, siMEM *p* value 3 x 10^{-5}) indicate all but two Rb not altered tumour

1072 cell lines exhibit Z score of <-2 (median Z in not altered group < -4). zGARP

1073 scores for *KLF16* (preferentially target Rb defective, siMEM *p* value 0.0002)

1074 indicate that the majority of Rb defective TCLs exhibit Z score of >-1 (median

1075 Z in defective group = -0.8), despite median Z scores being significantly

1076 different in Rb not altered *vs.* deficient TCLs. **F-H.** Scatter plots illustrating Z

1077 scores in 42 TNBC TCLs for three siMEM p<0.05 candidate Rb synthetic

1078 lethal effects, GPS1, SNRPF and SNW1 where median Z in defective group

1079 <-1 and median Z in proficient group >-2 (step two and three in (A)).

1080

1081

1082 **Figure 3. Highly penetrant Rb synthetic lethal effects.** Scatter plots
1083 illustrating Z scores in 42 TNBC TCLs for 30 candidate Rb synthetic lethal
1084 effects which pass Z score threshold assessment and demonstrate a
1085 penetrance score of greater than 90%, as summarised in steps 1-5 of Figure
1086 2A.

1087

1088 **Figure 4. TAF1 and SKP2 as central nodes in highly-penetrant Rb**
1089 **synthetic lethal networks. A.** Scatter plot illustrating Z scores in 42 TNBC
1090 TCLs for TAF1 from the data analysis illustrated in Figure 2A. **B.** Cytoscape
1091 network plot illustrating 33 highly penetrant (>80% penetrance) Rb synthetic
1092 lethal effects identified as TAF1 transcription factor target genes, as
1093 annotated by ENCODE data[12, 16]. **C-H.** Scatter plots illustrating Z scores in 42
1094 TNBC TCLs for SKP1, SKP2, COPS1,3,4 and CKS1B from the data analysis
1095 in Figure 2A. **I.** Pathway diagram highlighting the role of multiple highly
1096 penetrant Rb synthetic lethal effects in the control of p27 activity. **J.** Volcano
1097 plot illustrating mRNAs from highly penetrant Rb SL genes that are
1098 differentially expressed (limma analysis p<0.05) in 48 Rb defective *vs.* 92 Rb
1099 not altered triple negative breast tumours from the TCGA study[10]. Four highly
1100 SCF^{SKP}/COP9 complex genes, highlighted in red, demonstrate significantly
1101 higher mRNA expression levels in the Rb defective cell lines. **K.** As per panel
1102 (J), using data from the Metabric study[46] **L.** Box plots illustrating elevated
1103 SKP2 or GPS1 (COPS1) mRNA expression in Rb defective TNBC from both
1104 the TCGA [10] and Metabric studies[46]. *p* values shown calculated with Wilcox
1105 rank sum test.

1106

1107

1108 **Figure 5. Small molecule inhibition of SKP2 in Rb defective breast cell**
1109 **lines is synthetic lethal. A.** Western blot illustrating loss of Rb expression in
1110 isogenic MCF10A non-tumour breast epithelial cells with constitutive
1111 expression of either a control non-targeting shRNA (shCONTROL) or an Rb-
1112 targeting shRNA (shRB1). **B.** Western blot illustrating loss of SKP2 protein
1113 expression in MCF10A cells 48 hours after transfection with SKP2 or control,
1114 non-targeting, siRNAs (siCON1 or siCON2). **C.** Bar chart illustrating cell
1115 inhibitory effects in isogenic MCF10A cells with/without stable expression of
1116 Rb shRNA transfected with SKP2 siRNA. Cells were reverse transfected with
1117 siRNAs as shown and cultured for five continuous days, at which point cell
1118 viability was assessed by use of CellTitre Glo reagent. SKP2 siRNA caused
1119 significant cell inhibition (p<0.001, Student's t test) in cells with stable Rb
1120 silencing, but not in cells with wild type Rb expression. **D.** Dose response
1121 survival curves illustrating the cell inhibitory effects of the SKP2 small
1122 molecule inhibitor, SKPinC1, in isogenic MCF10A cells with/without stable
1123 expression of Rb shRNA. Cells were exposed to SKPinC1 for five continuous
1124 days at which point cell viability was assessed by use of CellTitre Glo reagent.
1125 Rb defective cells demonstrated a profound sensitivity, compared to Rb wild
1126 type cells ($p<0.0001$, two-way ANOVA, $SF_{50} = 1$ μM and >10 μM in Rb
1127 defective and wild type cells, respectively). **E.** Tumour cell inhibitory effect of
1128 SKPinC1 in 13 TNBC TCLs classified according to Rb status. Cells were
1129 exposed to 1 μM SKPinC1 as in (H). Surviving fractions are shown ($p=0.0022$,
1130 Student's t test). **F.** Western blot illustrating p27 protein levels in Rb defective
1131 cells exposed to 0.5 μM and 1 μM SKPinC1 for 24 hours.

1132

1133

1134 **Figure 6. SKP2 identified as a highly-penetrant Rb synthetic lethal effect**
1135 **in multiple, independently derived, datasets. A-C.** Scatter plots comparing
1136 p values (-$\log_{10}$) for Rb synthetic lethal effects identified in Colt2[42], Achilles[62]
1137 and DRIVE[43] datasets. $p<0.05$ effects in any single screen are shown. $p<0.05$
1138 effects in two screens are shown the top right hand quadrant of each plot.
1139 SKP2 and SART3, which were identified in all three screens as synthetically
1140 lethal with Rb defects, are highlighted in red. $p<0.05$ effects in Colt2 data
1141 were identified by siMEM, followed by Z score and penetrance filtering (see
1142 Figure 2A); $p<0.05$ effects in DRIVE and Achilles data were identified by
1143 median permutation t test, followed by Z score and penetrance filtering (see
1144 Figure 2A) **D.** Scatter plots illustrating Z scores in 42 TNBC TCLs for SKP2
1145 from the Colt2 data analysis. **E.** Scatter plots illustrating Z scores in 16 TNBC
1146 TCLs for SKP2 from the Achilles data analysis. **F.** Scatter plots illustrating Z
1147 scores in 12 TNBC TCLs for SKP2 from the Drive data analysis.

1148

1149 **Figure 7. SKP2 identified as a highly-penetrant Rb synthetic lethal effect**
1150 **in other histologies in two independently derived datasets. A.** Scatter plot
1151 of 775 p<0.05 significant Rb synthetic lethal effects identified from the MP-test
1152 analysis of 373 non-breast cancer TCLs in the Drive study (step one in Figure
1153 1A). All 775 p<0.05 effects are ranked ordered by MP test *p* value. SKP2 and
1154 E2F3 are highlighted. **B.** Scatter plot illustrating RSA scores in 373 non-breast
1155 TCLs with Rb annotation for SKP2 sensitivity from the Drive data analysis. **C.**
1156 Scatter plot of 1467 p<0.05 significant Rb synthetic lethal effects identified
1157 from the MP-test analysis of 467 non-breast TCLs in the Achilles study (step
1158 one in Figure 1A). All 1467 p<0.05 effects are ranked ordered by MP test *p*
1159 value. SKP2 and E2F3 are highlighted. **D.** Scatter plots illustrating Demeter
1160 scores in 1467 non-breast TCLs with Rb annotation for SKP2 sensitivity from
1161 the Achilles data analysis. **E,F.** Scatter plots illustrating RSA and Demeter
1162 scores in 63 and 115 lung TCLs with Rb annotation for SKP2 sensitivity from
1163 the Drive and Achilles studies, respectively. **G.** Scatter plot of intersect of cell
1164 line between the two datasets showing SKP2 sensitivity in Drive RSA scores
1165 (x axis) and Achilles Demeter scores (y axis) for selected histologies with only
1166 a single Rb defective line. This graph illustrates a trend between Rb defects
1167 and sensitivity to SKP2 shRNA across seven different histotypes.