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1 **A common intronic variant of *PARP1* confers melanoma risk and mediates melanocyte**
2 **growth via regulation of *MITF***

3 Jiyeon Choi^{1,7}, Mai Xu^{1,7}, Matthew M. Makowski², Tongwu Zhang¹, Matthew H. Law³, Michael A.
4 Kovacs¹, Anton Granzhan⁴, Wendy J. Kim¹, Hemang Parikh¹, Michael Gartside³, Jeffrey M.
5 Trent⁵, Marie-Paule Teulade-Fichou⁴, Mark M. Iles⁶, Julia A. Newton-Bishop⁶, D. Timothy
6 Bishop⁶, Stuart MacGregor³, Nicholas K. Hayward³, Michiel Vermeulen², Kevin M. Brown^{1*}

7 ¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD;

8 ²Department of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life

9 Sciences, Radboud University Nijmegen, the Netherlands; ³QIMR Berghofer Medical Research

10 Institute, Brisbane, QLD, Australia; ⁴CNRS UMR9187, INSERM U1196, Institut Curie, PSL

11 Research University, and Université Paris Sud, Université Paris Saclay, Orsay, France;

12 ⁵Translational Genomics Research Institute, Phoenix, AZ; ⁶Section of Epidemiology and

13 Biostatistics, Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, UK; ⁷These

14 authors contributed equally to this work. *Correspondence should be addressed to K.M.B at:

15 Email: kevin.brown3@nih.gov

16 **ABSTRACT**

17 Prior genome-wide association studies have identified a melanoma-associated locus on

18 chr1q42.1 that encompasses a ~100 kb region spanning the *PARP1* gene. eQTL analysis in

19 multiple cell types of melanocytic lineage consistently demonstrated that the 1q42.1 melanoma

20 risk allele (rs3219090, G) is correlated with higher *PARP1* levels. *In silico* fine-mapping and

21 functional validation identified a common intronic indel, rs144361550 (-/GGGCCC, $r^2 = 0.947$

22 with rs3219090) as displaying allele-specific transcriptional activity. A proteomic screen

23 identified RECQL as binding to rs144361550 in an allele-preferential manner. In human primary

24 melanocytes, *PARP1* promotes cell proliferation and rescues *BRAF*^{V600E}-induced senescence

25 phenotypes in a PARylation-independent manner. PARP1 also transforms *TERT*-immortalized
26 melanocytes expressing *BRAF*^{V600E}. PARP1-mediated senescence rescue is accompanied by
27 transcriptional activation of melanocyte lineage survival oncogene, *MITF*, highlighting a new role
28 of PARP1 in melanomagenesis.

29

30 To date, genome-wide association studies (GWAS) have identified twenty common,
31 genome-wide significant melanoma susceptibility loci¹⁻⁹, most of which do not appear to be
32 explained by protein-coding variants. A subset of these loci harbor known pigmentation genes
33 that mediate melanoma-associated phenotypes such as eye, hair, and skin color. While several
34 loci harbor genes implicated in cancer, evidence directly linking common risk variants within
35 most of these loci to altered function of specific genes is lacking.

36 MacGregor and colleagues initially identified a melanoma risk locus tagged by
37 rs3219090 on chromosome band 1q42.1 in an Australian case-control study at a near genome-
38 wide level of significance ($P = 9.3 \times 10^{-8}$, OR = 0.87, protective allele A)⁸. The association has
39 since been replicated by multiple other studies^{3,10}, including most recently by a meta-analysis of
40 12,874 melanoma cases (rs1858550, $P = 1.7 \times 10^{-13}$)⁷. Notably, the locus at 1q42.1 has also
41 been associated with melanoma survival¹¹, where the melanoma risk allele correlates with
42 increased survival, an association that has since been replicated¹². The region of association
43 spans from 226.52 Mb to 226.63 Mb (hg19) of chromosome 1, encompassing the entirety of the
44 poly(ADP-ribose) (PAR) polymerase-1 (*PARP1*) (OMIM: 173870) gene, and fine-mapping
45 suggests that the association is best explained by a single-SNP model³.

46 While a number of other genes are located in the vicinity of the association peak, *PARP1*
47 has the most well-established role in cancer. PARP1 is best known for its role as a DNA repair
48 enzyme and genotoxic sensor that functions in base excision repair (BER), single-strand break
49 repair, and double-strand break repair¹³. Once PARP1 binds to damaged DNA, its enzymatic

50 function is activated, and it covalently attaches PAR polymers to acceptor proteins, including
51 histones and PARP1 itself¹⁴. PARP1 amplifies DNA damage signals, modifies chromatin
52 structures to accommodate DNA damage response proteins, and further recruits DNA repair
53 proteins^{13,15,16}. While PARP1 is not directly involved in repair of UV signature mutations via
54 nucleotide excision repair, its role in the repair of DNA lesions induced by reactive oxygen
55 species (ROS) is well-established¹⁷. ROS are generated by UVA exposure¹⁸, are a byproduct of
56 melanin production¹⁹, and appear to play a role in oncogene-induced senescence (OIS)^{20,21}.
57 Aside from DNA repair, PARP1 functions in regulating gene expression by modifying chromatin
58 structure, associating with promoters and enhancers, and acting as a transcriptional co-
59 regulator^{22,23}. While many of these roles rely on PARP1 catalytic activity, some are also
60 PARylation-independent, as in the transcriptional co-regulator function for NF- κ B and B-
61 MYB^{24,25}.

62 In this study, we functionally characterized the 1q41.2 melanoma risk locus,
63 demonstrating a consistent correlation of the risk genotype with levels of *PARP1* gene
64 expression in tissues of melanocytic origin, identifying a gene regulatory variant within the first
65 intron of *PARP1*, and elucidating a role for PARP1 in melanocyte OIS via regulation of the
66 melanocyte master regulatory transcription factor, *MITF*.

67

68 **RESULT**

69 **The rs3219090 risk allele is correlated with high *PARP1***

70 We performed expression quantitative trait locus (eQTL) analysis in order to identify
71 genes for which expression levels are correlated with 1q42.1 risk genotype in tissues of
72 melanocytic lineage. Initially we evaluated the correlation of rs3219090 with expression of
73 genes within +/-1Mb in 59 early-passage melanoma cell lines using expression microarray data.
74 The results indicated that the rs3219090 risk allele is associated with higher levels of *PARP1*
75 expression ($P = 1.4 \times 10^{-3}$, linear regression; **Fig. 1a**). Notably, *PARP1* is the only gene in the

76 region that passed a Bonferroni-corrected P -value threshold (corrected for 14 genes, $P < 3.6 \times$
77 10^{-3} ; **Supplementary Table 1**), and this eQTL subsequently validated via qPCR assay ($P =$
78 0.031 , linear regression; **Supplementary Fig. 1a**). We then sought independent replication of
79 *PARP1* and other nominally significant eQTL genes ($P < 0.05$) in publicly available RNA-
80 sequencing datasets for melanoma-relevant tissues. When 409 melanoma tumors from The
81 Cancer Genome Atlas (TCGA) project (dbGAP Accession: phs000178.v9.p8) were tested, the
82 melanoma risk allele of rs3219090 was again significantly correlated with higher *PARP1*
83 expression levels ($P = 3.9 \times 10^{-3}$, linear regression using copy number as a covariate;
84 **Supplementary Fig. 1b**) while no other genes were significantly correlated (**Supplementary**
85 **Table 2**). Similarly, the *PARP1* eQTL was replicated in normal skin samples collected through
86 the Genotype-Tissue Expression (GTEx) Project (dbGAP Accession: phs000424.v6.p1),
87 including those derived from both sun-exposed skin ($P = 2 \times 10^{-4}$, linear regression, $n = 302$)
88 and non-sun-exposed skin ($P = 0.011$, linear regression, $n = 196$) (**Supplementary Fig 1c-d**,
89 **Supplementary Table 3-4**). Together, these data identified *PARP1* as the strongest eQTL gene
90 in the chr1q42.1 locus whose expression displayed the most consistent correlation with
91 genotypes of the lead SNP in sample panels of melanocytic lineage as well as human skin.

92 To complement eQTL data and rule out the possibility of any sample-specific
93 confounding factors masking genotype effect, we performed allele-specific expression (ASE)
94 analysis for *PARP1* in samples carrying both risk and protective alleles. Fourteen melanoma cell
95 lines that are heterozygous for rs3219090 and harbor normal regional copy number were
96 assayed using a quantitative allelic TaqMan assay for a synonymous coding surrogate SNP
97 (rs1805414; $r^2 = 0.98$ with rs3219090 in 1KG phase3 EUR), where allelic ratio was inferred from
98 known ratios of allelic standards. The results demonstrated a significant allelic imbalance
99 towards a higher proportion of *PARP1* expressed from the risk allele in the majority of
100 heterozygous cell lines ($P = 1.2 \times 10^{-4}$, two-tailed Wilcoxon signed rank test; **Fig. 1b**). Significant
101 allelic imbalance was also observed when a subset of these cell lines were analyzed by

102 RNAseq (data not shown). Subsequent *PARP1* ASE analysis in TCGA and GTEx RNAseq
103 datasets demonstrated that a higher allelic proportion of mapped reads was also observed for
104 the risk allele across TCGA tumor samples ($P = 0.011$, two-tailed Wilcoxon signed rank test, $n =$
105 48, copy-neutral and heterozygous; **Fig. 1c**), as well as in sun-exposed and non-sun-exposed
106 skin tissues (GTEx, $P = 1.16 \times 10^{-5}$, $n = 139$; $P = 8.9 \times 10^{-5}$, $n = 69$; respectively, two-tailed
107 Wilcoxon signed rank test; **Supplementary Fig. 1e-f**). These data demonstrate that the
108 melanoma risk allele of rs3219090 is significantly associated with increased *PARP1* expression
109 in tissues of melanocytic origin and skin with striking consistency across multiple datasets.

110

111 **Fine-mapping and functional annotation of candidate SNPs**

112 Given that high *PARP1* levels are correlated with the melanoma risk allele of rs3219090,
113 we next sought to identify functional risk variant(s) that may influence *PARP1* expression.
114 Previously, fine-mapping of this locus in a large European population provided support for a
115 model in which a single variant accounts for the association signal in this region³, a finding
116 confirmed as part of the meta-analysis conducted by Law and colleagues⁷. We prioritized 65
117 variants that are highly correlated with the lead SNP as candidate functional variants ($r^2 > 0.6$
118 with lead SNPs from the discovery or meta-analysis lead SNPs^{3,7}, rs3219090 and rs1858550,
119 respectively; LD based on 1KG phase3, EUR and CEU). Given the absence of amino acid-
120 changing *PARP1* variants within this set of candidates, an absence of evidence for alternative
121 splicing as a likely mechanism (**Supplementary Note**), and considerable evidence for allelic
122 differences in *PARP1* expression levels, we focused on those located within annotated
123 melanocyte- or melanoma-specific *cis*-regulatory elements using data from the ENCODE²⁶ and
124 Roadmap projects²⁷ (**Supplementary Note, Supplementary Table 5-6, Supplementary Fig.**
125 **2-3**). The four of the most strongly supported variants are situated at the center of melanocyte
126 DHS peaks as well as within regions harboring promoter or enhancer histone marks (H3K4me1,
127 H3K4me3, or H3K27ac) in the majority of melanocyte/melanoma cultures assayed

128 **(Supplementary Table 6)**. Based on these data, we proceeded with functional characterization
129 of these four candidates **(Supplementary Table 6, Supplementary Fig. 2)**.

130

131 **An intronic indel displays allelic transcriptional activity**

132 We assessed all four candidate functional variants for gene regulatory potential using
133 luciferase reporter assays, as well as for allelic patterns of protein binding via electrophoretic
134 mobility shift assay (EMSA). For these assays, we sought to identify variants that display 1)
135 transcriptional activation consistent with ENCODE annotation, 2) higher activity for the risk allele
136 consistent with the eQTL data, and 3) allele-specific protein binding. Among four candidate
137 variants, only rs144361550, a GGGCCC indel variant, met all these criteria **(Fig. 2-3,**
138 **Supplementary Figs. 4-6;** summarized in **Supplementary Table 7)**. Namely, luciferase assays
139 conducted in a melanoma cell line demonstrated that the genomic region around rs144361550
140 exhibits strong transcriptional activity in both long (905bp covering the larger DHS region, ~17-
141 20 fold higher than control levels) and short cloned fragments (22 or 28bp covering the
142 GGGCCC repeats, ~1.7-2.5 fold higher than control levels; **Fig. 3a**), where the risk-associated
143 deletion allele exhibited higher reporter activity than the insertion allele (30-45% higher). In
144 primary melanocytes, where transfection efficiency is considerably lower, allelic activity was not
145 observed, but the long deletion and insertion fragments displayed weak but significant
146 transcriptional activity ($P = 1.2 \times 10^{-3}$ and 5.9×10^{-4} , respectively, two-tailed, paired t-test; **Fig.**
147 **3c**). EMSAs using nuclear extract from melanoma cell lines or cultured primary human
148 melanocytes displayed preferential binding of nuclear proteins to the insertion allele **(Fig. 3b,d)**.
149 Given the potential for miscalling genotype of this functional indel, we directly genotyped
150 rs144361550 in a large reference set to confirm LD with the lead SNP **(Supplementary Note,**
151 **Supplementary Table 8-9, Supplementary Fig. 7-8)**.

152 To identify proteins that bind rs144361550 in an allele-preferential manner, we utilized
153 quantitative mass-spectrometry employing dimethyl label swapping^{28,29 30}. Mass-spectrometry

154 using melanoma cell line extract identified exclusively insertion allele-preferential interactors, the
155 majority of which are not conventional transcription factors, including the RECQL helicase (**Fig.**
156 **4a**). While two transcription factors previously found by the ENCODE Project to localize to the
157 region overlapping rs144361550 via chromatin immunoprecipitation (ChIP) were found to bind
158 rs144361550 probes (TFAP2A, ZBTB7A), neither did so in an allele-preferential manner (data
159 not shown), in line with the observation that rs144361550 creates no new sequence motifs but
160 rather extends a poly-G repeat stretch. We then performed a series of antibody supershifts and
161 EMSAs using purified recombinant proteins for multiple candidates and validated that RECQL is
162 an unequivocal allele-preferential binder to rs144361550 (**Fig. 4b, Supplementary Figs. 9, and**
163 **Supplementary Table 10**). ChIP assays indicated that RECQL indeed binds to the *PARP1*
164 indel region in melanoma cells and primary human melanocytes carrying an insertion allele (**Fig.**
165 **4c, Supplementary Fig. 10**). We also performed a series of *in silico* and *in vivo* assays testing
166 for alternative DNA secondary structure formation (G-quadruplex or G4), with the results
167 suggesting RECQL-specific allelic binding mechanism rather than the one through G4
168 (**Supplementary Note, Supplementary Table 10-11, Supplementary Fig. 11-13**).

169 Ectopic expression of RECQL in three melanoma cell lines carrying insertion or deletion
170 alleles at a moderate level using lentiviral transduction resulted in a mild increase in *PARP1*
171 transcription (**Fig. 4d**). We then performed luciferase assays for rs144361550 with or without
172 RECQL over-expression in cells with low baseline levels of RECQL relative to melanomas
173 (HEK293FT cells). At a basal level, insertion and deletion alleles did not display differential
174 luciferase activity, but upon RECQL over-expression, significant allele-specific transcriptional
175 activity we previously observed in melanoma cell lines was recapitulated (**Fig. 4e**). Together,
176 these data suggest that RECQL may play a role in *PARP1* allelic expression in cells of
177 melanocytic lineage through the melanoma risk-associated indel, rs144361550.

178

179 ***PARP1* facilitates melanocyte growth and transformation**

180 Given that the risk allele of rs3219090 is associated with increased levels of *PARP1*
181 expression, we tested whether increased *PARP1* levels could lead to altered cellular
182 phenotypes relevant to melanomagenesis. As *BRAF*^{V600E}-induced senescence is well accepted
183 as a barrier to malignant transformation in early events of melanomagenesis³¹⁻³³ and ROS
184 seems to present a potential functional link between *PARP1* and OIS²¹, we examined whether
185 elevated *PARP1* modulates OIS by overexpressing *PARP1* in human primary melanocytes
186 expressing oncogenic *BRAF* (*BRAF*^{V600E}). Consistent with published results, ectopic expression
187 of *BRAF*^{V600E} in human melanocytes induced a robust arrest of cell growth and proliferation,
188 accompanied by heterochromatic H3K9Me3 focus formation (**Fig. 5a-c, Supplementary**
189 **Fig. 14**), and a moderate increase in β-galactosidase activity (**Supplementary Fig. 15**), both
190 hallmarks of OIS. Overexpression of *PARP1* prior to induction of *BRAF*^{V600E}, however,
191 prevented the cell cycle arrest and H3K9Me3 focus formation as well as β-galactosidase activity
192 observed in melanocytes expressing *BRAF*^{V600E} alone (**Fig. 5a-c, Supplementary Fig. 14-15**),
193 demonstrating a rescue from *BRAF*^{V600E}-induced senescence. Crystal violet staining of cells
194 three weeks following *PARP1* expression indicated that *PARP1* by itself can also increase cell
195 proliferation in the absence of *BRAF*^{V600E}, suggesting an effect on cell proliferation by *PARP1*
196 (**Fig. 5a**). *PARP1* consistently exerted weak but significant effects on cell proliferation and OIS
197 reversal even at a moderate induction level (~1.5 fold, **Supplementary Fig. 16**) recapitulating
198 the subtle allelic expression differences observed in melanomas carrying risk or protective
199 alleles. Several oncogenes, including *MYC*³⁴ and *PIK3CA*³³, have been reported to stimulate
200 malignant transformation in melanoma cells by abrogating OIS and restarting cell proliferation.
201 To further evaluate if *PARP1* can stimulate malignant transformation by affecting melanocyte
202 proliferation, we examined the effect of *PARP1* on anchorage-independent growth of *TERT*-
203 immortalized human melanocytes (p^{mel}³⁵) by soft-agar assay. *PARP1* cooperated with
204 *BRAF*^{V600E} to enhance colony formation of p^{mel} cells in soft agar, similar to the previously
205 reported effect of *MITF*³⁵, albeit to a lesser degree (**Fig. 5d-e**). Thus, like deregulated *MITF*

206 expression, increased expression of *PARP1* can both rescue and further transform human
207 melanocytes from *BRAF*^{V600E}-induced senescence.

208 Most of the well-characterized functions of PARP1, including those involving DNA repair,
209 are closely linked to its poly-ADP ribosylation activity. We therefore tested whether PARP1-
210 mediated rescue of melanocytes from *BRAF*^{V600E}-induced OIS is dependent on PARP1 catalytic
211 activity. Melanocytes overexpressing both *PARP1* and *BRAF*^{V600E} were treated with the PARP1
212 inhibitor BYK204165³⁶, and the inhibition of PAR-activity was confirmed by Western blotting with
213 an antibody recognizing PAR (**Fig. 6a**). BYK204165 treatment did not block the cell proliferation
214 effect of PARP1 in *BRAF*^{V600E}-expressing cells as shown by similarly increased cell growth in
215 crystal violet staining (**Supplementary Fig. 17a**), an increased percentage of BrdU-positive
216 cells and reduced G0/G1 population in PARP1-expressing cells (**Fig. 6b**), nor did it affect
217 H3K9Me3 focus formation (**Supplementary Fig. 17b**). We also tested a catalytically inactive
218 mutant version of *PARP1*³⁷⁻³⁹ for effect on melanocyte growth and proliferation (**Supplementary**
219 **Fig. 18a-d**), resulting in similar phenotypes to those observed using BYK204165.

220

221 ***PARP1* transcriptionally activates *MITF***

222 To begin to understand the mechanism of PARP1-mediated senescence rescue we first
223 examined ROS content changes in *BRAF*^{V600E} and/or *PARP1* over-expressing melanocytes.
224 While we observed a minor increase of ROS content by *BRAF*^{V600E} expression, we did not
225 detect meaningful changes in ROS content by PARP1 in our melanocyte system (data not
226 shown). We then turned our attention to melanocyte lineage survival oncogene, *MITF*, which is
227 frequently amplified in malignant melanomas and can transform melanocytes in the context of
228 *BRAF*^{V600E}³⁵. Previous studies have observed that *MITF* expression is inhibited by oncogenic
229 *BRAF*^{V600E} in melanomas⁴⁰. Consistent with this finding, we demonstrate that persistent
230 activation of MAPK pathway by *BRAF*^{V600E} also suppressed *MITF* expression in primary
231 melanocytes (**Fig. 6a**). Significantly, PARP1 induction, at either strong (**Fig. 6a, Supplementary**

232 **Fig. 19)** or more modest levels (**Supplementary Fig. 16e-f**), increases *MITF* at both mRNA and
233 protein levels, in a PARylation-independent manner (**Supplementary Fig. 18d-f, Fig. 6a**).
234 PARP1 also partially restores *MITF* expression in *BRAF*^{V600E}-expressing melanocytes,
235 suggesting a link between OIS rescue and *MITF* restoration (**Fig. 6a, Supplementary Fig.**
236 **16e,f, Supplementary Fig. 18d-f, Supplementary Fig. 19**). Consistent with this finding, we
237 observed a weak but significant positive correlation between *PARP1* and *MITF* transcript levels
238 (**Supplementary Fig. 20**) in 409 melanoma samples from TCGA (Pearson's $r = 0.198$, $P = 5.7 \times$
239 10^{-5}) as well as in the smaller subset of 189 tumors that are copy-neutral at the *MITF* locus
240 (Pearson's $r = 0.252$, $P = 4.8 \times 10^{-4}$). To address potential issues with tumor heterogeneity and
241 random factors driving this correlation in melanomas, we also looked at the correlation in our
242 early-passage melanoma cell lines. While we did not observe a significant correlation in the full
243 set of 59 cell lines (Pearson's $r = 0.15$, $P = 0.26$; **Supplementary Fig. 21a**), when we further
244 subdivide them into *MITF*-high and *MITF*-low groups to account for distinct cellular states
245 signified by *MITF* levels⁴¹, a strong correlation of *PARP1* and *MITF* levels was observed only in
246 *MITF*-high group (Pearson's $r = 0.560$, $P = 5.5 \times 10^{-3}$, $n = 23$; $P = 6.6 \times 10^{-3}$ when *MITF* copy
247 number is adjusted; **Supplementary Fig. 21b**). A trend of association with *PARP1* levels were
248 also observed in a subset of *MITF* target genes (**Supplementary Fig. 21c-d, Supplementary**
249 **Table 12**).

250 *MITF* is an essential melanocyte survival gene, and therefore we could not directly test if
251 *MITF* mediates PARP1 senescence rescue by knocking down *MITF* in our system. While *MITF*
252 depletion by shRNA dramatically reduces melanocyte growth approximately two weeks
253 following knockdown (**Fig. 6c,d**), at an earlier time point (D9 after infection), we noticed that
254 *MITF* depletion itself introduced a weak but distinct senescence-associated H3K9Me3
255 phenotype (**Fig. 6e**) as previously reported⁴². Interestingly, in the context of *MITF* knockdown,
256 PARP1 senescence rescue is not observed in terms of partial reversal of H3K9Me3 focus

257 formation (**Fig. 6e**). These results suggest that PARP1 function in senescence rescue is likely
258 upstream of *MITF* expression.

259 Given that PARP1 activates *MITF* at the mRNA level, we performed ChIP to determine
260 whether PARP1 directly localizes to the melanocyte-specific *MITF-M*^{43,44} promoter in human
261 primary melanocytes. ChIP experiments were performed both via overexpression of PARP1 in
262 melanocytes, as well as in melanocytes expressing endogenous levels. Among seven primer
263 sets spanning the *MITF-M* promoter region (-1263 to +172bp of the transcription start site;
264 TSS), the two that are more proximal to the TSS displayed the most significant enrichment,
265 indicating PARP1 binding (~8-19 fold above IgG background at endogenous level, Primer4 and
266 Primer5, from -587 to -136 bp; **Fig. 7**). Localization of PARP1 to the *MITF-M* promoter was
267 observed both with PARP1 over-expression as well as endogenous levels, while no detectable
268 enrichment was observed when *PARP1* was knocked down using an shRNA (data not shown).
269 Notably, the region where PARP1 localizes overlaps with consensus binding sites of previously
270 known transcriptional regulators of the *MITF-M* promoter: SOX10, TCF/LEF, and CRE (**Fig. 7**);
271 and a putative PARP1 binding motif⁴⁵ was also predicted in this core region (see Methods
272 section).

273 To assess whether *PARP1* directly regulates *MITF* transcription, we performed
274 luciferase reporter assays using constructs containing the *MITF-M* promoter regions⁴⁶. Altered
275 *PARP1* levels via over-expression or shRNA-mediated knockdown failed to modulate reporter
276 activity (**Supplementary Fig. 22**), suggesting a potential epigenetic role for PARP1 in regulation
277 of the *MITF-M* locus that is not recapitulated via ectopic expression of a reporter gene. Bisulfite
278 sequencing of two assayable CpGs near the *MITF-M* TSS⁴⁷ indicated that introduction of
279 *BRAF*^{V600E} does not alter their methylation status (**Supplementary Fig. 23**), speaking against
280 DNA methylation-mediated regulation in this context. Examination of a promoter histone mark,
281 H3K4Me3, by ChIP revealed that H3K4Me3 is enriched in the *MITF-M* proximal promoter when
282 *PARP1* is expressed either endogenously or ectopically (**Supplementary Fig. 24a**), but is

283 diminished upon knocking down *PARP1* expression (**Supplementary Fig. 24b**). Furthermore,
284 expression of *BRAF*^{V600E} resulted in diminished H3K4Me3 signal in the *MITF-M* promoter, but
285 was restored to the usual high levels upon co-expression with *PARP1* (**Supplementary Fig.**
286 **24c**). CHIP analyses using antibodies recognizing the C-terminal domain (CTD) of RNA
287 polymerase II (RNA Pol II) or CTD with phosphorylated Serine 5 demonstrated that RNA Pol II
288 and its initiation-signature, Serine 5 phosphorylation, are both enriched near *M-MITF* TSS at
289 endogenous *PARP1* levels, but diminished upon *PARP1* knock-down (**Supplementary Fig. 25**).
290 These results are consistent with a model in which *PARP1* influences *MITF-M* promoter activity
291 and influences *MITF* levels via transcriptional regulation.

292

293 **DISCUSSION**

294 In this study, eQTL and ASE analyses suggest *PARP1* as the susceptibility gene
295 underlying the melanoma risk locus on chromosome band 1q42.1. When we evaluated the set
296 of genes in +/- 1Mb of the lead melanoma risk SNP (rs3219090) to account for potential long-
297 range regulation, we observed a highly-reproducible eQTL with *PARP1*, but not with other
298 nearby genes. The correlation between the risk allele and higher levels of *PARP1* expression
299 was highly reproducible across multiple melanoma-relevant tissues, including early-passage
300 melanoma cell lines, melanoma tumors, and human skin biopsies in both eQTL and ASE
301 analyses. While eQTL and ASE analyses cannot completely rule out a potential role for other
302 genes within the larger genomic region surrounding the GWAS peak, these data strongly
303 implicate *PARP1* as functionally mediating melanoma risk at this locus.

304 While this region is relatively small in size, 65 variants are nonetheless strongly
305 correlated ($r^2 > 0.6$) with the lead GWAS SNP. To efficiently prioritize functional candidates we
306 took advantage of potential gene regulatory regions annotated in human melanocyte and
307 melanoma samples by the ENCODE and Roadmap Projects. We chose to focus on variants

308 located in most consistently annotated regulatory elements across different individuals and
309 cellular conditions because of the strikingly consistent eQTL and ASE data observed in both
310 melanocytes and melanomas. Subsequent characterization of these candidate variants
311 highlighted a single variant, rs144361550, as a strong functional candidate. Of the variants
312 tested, only rs144361550 demonstrated both allele-specific transcriptional activity and protein
313 binding pattern in a manner consistent with the observed pattern of genotype/expression
314 correlation. While these data provide support for rs144361550 as a functional melanoma risk
315 variant influencing levels of *PARP1* expression, they nonetheless cannot rule out other variants
316 in this region as also contributing to the observed correlation between *PARP1* levels and
317 genotype.

318 Our unbiased approach using quantitative mass-spectrometry identified RECQL as a
319 protein binding allele-preferentially to rs144361550. Importantly, RECQL binding to
320 rs144361550 does not appear to be driven by sequence specificity but rather by DNA
321 secondary structure. While genomic sequence encompassing rs144361550 suggested G4-
322 forming potential (**Supplementary Table 11**), which might explain a regulatory role⁴⁸, our *in*
323 *vitro* assays failed to provide definitive evidence for G4 structure either by insertion or deletion
324 allele. However, formation of another differential structural motif, such as a transient hairpin
325 structure (formed by single-stranded sequences, **Supplementary Table 13**) or a locally
326 perturbed double-helix structure at the hexanucleotide repeat domain⁴⁹, inducing DNA bending
327 and serving as a recognition motif for allele-specific protein binding⁵⁰, cannot be excluded.

328 Although PARP1 is most well-known for its role in DNA repair, to date GWAS have
329 identified this region only for melanoma susceptibility (GWAS catalog; see URL section),
330 suggesting a potential melanoma-specific role of *PARP1*. Indeed, ectopic expression of *PARP1*
331 led to increased melanocyte proliferation both alone, as well as in the presence of oncogenic
332 mutated version of *BRAF* (*BRAF*^{V600E}). *BRAF*^{V600E} is found in most melanocytic nevi⁵¹, and as

333 reported earlier³¹ its over-expression in primary melanocytes led to markedly decreased cell
334 proliferation as well as H3K9Me3 focus formation, a hallmark of cellular senescence⁵². Our data
335 show that these senescence-like phenotypes are partially reversed by elevated PARP1 levels.
336 Further, ectopic expression of *PARP1* in *TERT*-immortalized (p'mel) melanocytes expressing
337 *BRAF*^{V600E}³⁵ led to a tumorigenic phenotype exemplified by anchorage-independent growth.
338 These data suggest a role for increased PARP1 in the early stages of melanomagenesis
339 promoting increased melanocyte growth and/or escape from *BRAF*^{V600E}-induced senescence. In
340 this context, PARP1 seems to be functionally linked to melanocyte lineage survival oncogene,
341 *MITF*, which itself is an established melanoma susceptibility gene^{53,54}. While we did not
342 exhaustively explore all the major functions of PARP1 including DNA repair and inflammation in
343 the context of melanomagenesis, our findings highlight a novel role for PARP1 in a melanocyte-
344 specific context, providing a potential link between a susceptibility gene and a lineage-specific
345 oncogene.

346 Most well-established functions of PARP1 are dependent on the poly-ADP ribosylation
347 activity including its role in DNA damage response and repair. While some transcriptional co-
348 regulator functions of PARP1 do not require its enzymatic activity^{24,25}, the role of PARP1 in
349 chromatin structure modification mainly relies on NAD⁺-dependent association with
350 nucleosomes⁵⁵ or PARylation of histones and chromatin modulators such as histone
351 demethylase KDM5B^{56,57}. Notably, the effects of *PARP1* expression on melanocyte
352 proliferation, *BRAF*^{V600E}-induced senescence, and restoring *MITF-M* expression do not appear
353 to depend on the catalytic activity of PARP1. Interestingly, PARP1-mediated *MITF-M* up-
354 regulation seems to involve chromatin modulation, as the promoter histone mark H3K4Me3 is
355 markedly enriched near the TSS of *MITF-M* upon PARP1 expression relative to its depleted
356 state in *BRAF*^{V600E}-expressing melanocytes. While this is an important observation of potential
357 PARylation-independent chromatin modulation function, further investigation is required to
358 establish a causal relationship and molecular link.

359

360 **URLs.**

361 https://mathgen.stats.ox.ac.uk/impute/impute_v2.html

362 https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html

363 <http://firebrowse.org/>

364 <https://tcga-data.nci.nih.gov/tcga/>

365 http://www.bios.unc.edu/research/genomic_software/Matrix_eQTL/

366 <http://www.gtexportal.org/home/testyourown>

367 www.broadinstitute.org/cancer/cga/gistic

368 <http://www.cbioportal.org/>

369 <https://www.encodeproject.org/>

370 <http://www.roadmapepigenomics.org/>

371 <http://genome.ucsc.edu/>

372 <http://liulab.dfci.harvard.edu/MACS/>

373 <http://splice.uwo.ca/>

374 <http://bioinformatics.ramapo.edu/QGRS/analyze.php>

375 <http://unafold.rna.albany.edu/?q=mfold>

376 <https://www.ebi.ac.uk/gwas/>

377

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404

405 **AUTHOR CONTRIBUTIONS**

406 J.C., M.X., and K.M.B. designed the study. J.C., M.M.M., M.A.K., and W.J.K. conducted
407 experiments for molecular characterization of PARP1 risk variants. M.X. performed phenotypic
408 analyses of PARP1 in primary and immortalized melanocytes. Proteomics analysis was
409 conducted by M.M.M. and M.V. CD and TDS analysis was performed by A.G. and M.T. Data

410 was analyzed by T.Z., M.H.L., H.P., and M.M.I. Fine mapping of GWAS data was performed by
411 M.M.I., D.T.B., J.A.N-B., S.M., and M.H.L. Melanoma cell line eQTL and ASE experiments were
412 performed by K.M.B., N.K.H., J.M.T., M.G., and J.C. The manuscript was written by J.C., M.X.,
413 and K.M.B.

414

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416 The authors declare no competing financial interests.

417

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538 **Figure 1** The melanoma risk-associated G allele of rs3219090 is correlated with increased *PARP1*
539 expression. (a) eQTL analysis was performed for rs3219090 using expression microarray and SNP array
540 genotypes derived from a panel of 59 early-passage melanoma cell lines. A significant eQTL was
541 observed for *PARP1*, and the result is plotted for rs3219090 genotype ($P = 1.4 \times 10^{-3}$; linear regression).
542 G is the risk allele and A the protective allele of rs3219090. A.U.; arbitrary unit. (b) The allelic ratios of
543 *PARP1* transcripts were measured in 14 copy-neutral melanoma cell lines that were heterozygous for
544 both rs3219090 and a synonymous mRNA-coding surrogate SNP (rs1805414, $r^2=0.98$ with rs3219090)
545 using Taqman genotyping assays. Allelic ratios were inferred from a known amount of allelic standards
546 and plotted as a ratio of *PARP1* expression from the risk over protective allele ($P = 1.2 \times 10^{-4}$, two-tailed
547 Wilcoxon signed rank test, average value of PCR triplicates were considered as a single data point). (c)
548 Allelic ratios of *PARP1* transcripts were measured using RNA sequencing data from 48 copy-neutral
549 TCGA skin melanoma samples that were heterozygous for both rs3219090 and rs1805414. The mapped
550 numbers of RNAseq reads encompassing each allele of rs1805414 were used for calculating allelic ratios
551 ($P = 0.011$, two-tailed Wilcoxon signed rank test). Solid line marks 1:1 ratio, and dashed line represents
552 median ratio.

553

554 **Figure 2** Functional annotation of a 3kb region encompassing rs144361550 in primary melanocytes.
555 Histone modifications (H3K4Me1, H3K4Me3, and H3K27Ac) and DNaseI hypersensitivity sites (DHS) in
556 primary melanocytes are shown for a 3kb region encompassing rs144361550. The red dashed vertical
557 line indicates the position of rs144361550, overlapping histone marks, DHS, and transcription factor
558 binding sites. Genomic positions are based on hg19. Transcription factor binding sites are from UCSC
559 genome browser track “Transcription Factor ChIP-seq (161 factors) from ENCODE with Factorbook
560 Motifs” representing multiple ENCODE cell types. “Chromatin Primary Core Marks Segmentation by HMM
561 from Roadmap Project” track is also shown for three melanocyte samples. TssA: Active_TSS, TssF:
562 Flanking_Active_TSS, Enh: Enhancers. For DHS, traces from two experimental replicates of Melanocyte
563 1 and 2 are displayed. The scale of each track is uniformly set throughout the region of the *PARP1* gene
564 to cover the highest peaks, with 0 as the baseline (see online methods for details of each track).

565

566 **Figure 3** The melanoma-associated indel, rs144361550, drives allelic transcriptional activity and protein
567 binding. (a,c) Luciferase assays for rs144361550 were conducted using the melanoma cell line

568 UACC2331 (a) or primary melanocytes (c). 905bp or 22/28bp encompassing rs144361550, respectively,
569 were cloned 5' of minimal promoter in pGL4.23 vector and transfected into the cells. Luciferase activity
570 was measured 24hrs after transfection and was normalized against Renilla luciferase activity. Relative
571 luciferase levels were plotted as percent of the minimal promoter control. *P*-values are shown or * *P* <
572 0.05 against Ctrl (two-tailed, paired *t*-test). Two (a) and three (c) independent cell cultures and
573 transfections of *n* = 3 were combined to present the median with range, 75 & 25 percentiles, and each
574 data point. Ctrl: minimal promoter control, Del: deletion/risk allele construct, Ins: insertion/protective allele
575 construct. (b,d) EMSA was performed using biotin-labeled double stranded oligos for the deletion/risk
576 (Del, D) or insertion/protective (Ins, I) alleles of rs144361550 and nuclear extracts from melanoma cell
577 lines UACC2331 and UACC457 (b) or primary melanocytes (d). Sequences shown in panel b for the 22
578 bp deletion and 28 bp insertion probes were used for both b and d (bold and italic bases highlight
579 potential G4 structure forming nucleotides). 50X, 200X or 500X molar excess of unlabeled competitors
580 were added in specified lanes.

581 **Figure 4** RECQL binds to the insertion allele and mediates allelic expression. (a) Insertion allele-specific
582 binding proteins were identified by mass-spectrometry using melanoma cell nuclear extract and
583 biotinylated double-stranded oligos. The ratio of proteins bound to heavy/light-dimethyl labeled probes is
584 plotted on x and y-axis for labeling swapping. Red circles: enriched above the background in both
585 directions. Circle sizes represent relative abundance. (b) RECQL EMSA/supershift. D: deletion, I:
586 insertion (c) ChIP was performed using anti-RECQL antibody or IgG and melanoma cell chromatin
587 followed by qPCR. DNA quantity was normalized to input DNA for each IP (*n*=3). Neg: gene desert, Pos:
588 a known RECQL binding locus, Indel: rs144361550 region. A representative set from four independent
589 experiments is shown. (d) RECQL under tetracycline-inducible promoter was expressed in three
590 melanoma cell lines. *PARP1* levels (top) and *RECQL* RNA (middle) and protein (bottom) levels were
591 measured at 48hrs of doxycycline induction (blot images were cropped). Transcript levels are shown as
592 fold over Empty vector after normalizing to *B2M* control (*n* = 6, 5, and 6 for each cell line). (e) Luciferase
593 assays were performed using 905bp deletion (Del) or insertion allele (Ins) constructs with RECQL or
594 Empty vector co-transfection in HEK293FT cells. Renilla-normalized relative luciferase activities were
595 plotted as percent of the minimal promoter control (Ctrl) (*n* = 6). (c-e) Each graph shows median with
596 range, 75 & 25 percentiles, and each data point. Two-tailed, *t*-test assuming unequal variance for all *P*-
597 values shown. * *P* < 0.05 against Ctrl (e) or Empty (d).

598

599 **Figure 5** Cell growth and H3K9Me3 focus formation in primary human melanocytes expressing *PARP1*
600 and *BRAF*^{V600E}. (a) Crystal violet (CV) staining of melanocytes expressing *PARP1*, *BRAF*^{V600E}, or both.
601 Cells were first infected with pIN20-*PARP1* followed by G418 treatment and doxycycline induction. Cells
602 were then infected with HIV-CSCG-*BRAF*^{V600E} vector at day 6 after *PARP1* infection followed by
603 blasticidine selection. Equal cell numbers were seeded at day 11 after pIN20-*PARP1* infection and

604 stained with CV 9 days after seeding. **(b)** BrdU staining of primary human melanocytes expressing either
605 *PARP1*, *BRAF^{V600E}*, or both. BrdU staining was done at day 13 after initial infection of pIn20-*PARP1* (n =
606 4, median with range and 25 & 75 percentiles, *t*-test assuming unequal variance, representative set from
607 three experiments). **(c)** H3K9Me3 staining of primary human melanocytes at day 13 after initial infection.
608 Scale bars are shown for 10µm. **(d)** Colony formation in soft-agar of p'mel/*BRAF^{V600E}* cells expressing
609 either *PARP1* or *MITF-M*. Immortalized melanocytes (p'mel cells) expressing active *BRAF^{V600E}* were
610 infected with pLX304 empty, pLX304-*PARP1* or pLX304-*MITF* and seeded in soft-agar plates after
611 blasticidine selection. Images were taken at day 22 and colonies were counted from two sets of
612 triplicates at day 26 after plating (n=6, from two experiments, median with range and 25 & 75 percentiles).
613 *t*-test assuming unequal variance **(e)** Representative images of colonies are shown. The same
614 magnification (40X) was applied for all the images.

615

616 **Figure 6** *MITF* expression is restored in primary human melanocytes co-expressing *PARP1* and
617 *BRAF^{V600E}* in a PARylation-independent manner, concurrent with partial reversal of senescence
618 phenotypes. **(a)** Western blot detection of MITF (long and short exposures), BRAF, PARP1, and PAR.
619 Primary human melanocytes were co-infected with *PARP1*, *BRAF^{V600E}* followed by treatment with the
620 PARP inhibitor, BYK204165. Cell extract was generated at day 13 after initial infection with *PARP1*
621 construct. Blot images were cropped. **(b)** BrdU flow cytometry of primary human melanocytes co-infected
622 with *PARP1*, *BRAF^{V600E}*, and/or *MITF*-shRNA followed by treatment with the PARP inhibitor, BYK204165.
623 **(c)** Crystal Violet staining of melanocytes co-infected with *PARP1*, *BRAF^{V600E}*, and/or *MITF*-shRNA.
624 Primary melanocytes were first infected with pIn20-*PARP1* or empty pIn20 vector concurrently with
625 pLKO-shRNA-*MITF-M* or pLKO-empty vector followed by G418 treatment and doxycycline induction.
626 Cells were then infected with HIV-CSCG-*BRAF^{V600E}* or empty HIV-CSCG vector at day 6 after *PARP1*
627 infection followed by Blasticidine selection. A representative crystal violet (CV) staining (n=3) image is
628 displayed at day 17 of infection. **(d)** Quantification of solubilized CV staining by measuring absorbance at
629 540nm at day 17 of infection is plotted. Median with range (n=3, *t*-test assuming unequal variance, a
630 representative set from three experiments is shown) **(e)** *PARP1*-mediated partial reversal of *BRAF^{V600E}*-
631 induced H3K9Me3 focus formation in melanocytes is blocked by knockdown of *MITF*. H3K9Me3 staining
632 was performed at day 13 after initial infection. Scale bars are shown for 10µm.

633

634 **Figure 7** PARP1 binds to *MITF-M* promoter. Human melanocytes were transduced with lentiviral
635 constructs of empty pIn20 (Empty = endogenous *PARP1* levels) or *PARP1* cDNA (*PARP1* = over-
636 expression of *PARP1*) and harvested five days after infection. Chromatin immunoprecipitation was
637 performed using antibody against PARP1 (anti-PARP1) or rabbit normal IgG (IgG) and pulled-down DNA
638 was amplified using seven sets of primers spanning -1263bp through to +172bp relative to *MITF-M* TSS

639 (black arrow). Gray arrows depict locations of qPCR primers relative to the genomic region of *MITF*-M
640 promoter shown below. Relative quantities were calculated by normalizing each sample amount to
641 matched input DNA. Average quantity with range, and individual data points for qPCR triplicates are
642 shown. Experiments were repeated 3 times yielding similar results, and the results from a representative
643 set are displayed. Known (closed symbols) or predicted (open symbols) binding sites for transcription
644 factors are presented to their relative genomic positions on *MITF*-M promoter. The genomic sequence of -
645 325bp through to -150bp relative to the TSS are shown in the box, with known or predicted transcription
646 factor binding sites underlined.

647 **ONLINE METHODS**

648 **Early passage melanoma cell line eQTL analysis.** Early passage melanoma cell lines were
649 obtained from the University of Arizona Cancer Center (UACC), and eQTL analysis was
650 performed by combining gene expression profiling and SNP genotyping data. The use of cell
651 lines was approved by National Institutes of Health Office of Human Subject Research. Early
652 passage melanoma cell lines were grown in the medium containing RPMI1640, 10% FBS, 20
653 mM HEPES, and penicillin/streptomycin until ~70% confluent. All cell lines were tested negative
654 for mycoplasma contamination. For RNA isolation, cells were washed twice with cold PBS on
655 ice and lysed with Trizol. Trizol was heated to 65°C for 5 min to maximize melanin removal.
656 Following heating, 1 mL chloroform was added per 5 mL of Trizol, vortexed, cooled on ice for 5
657 min, and centrifuged. The aqueous phase was removed, and equal volume of 70% EtOH was
658 added dropwise while vortexing at low speed. Ethanol /supernatant mixtures were added to
659 Qiagen RNeasy midi columns, with the flow-through reapplied once. Samples were then
660 processed per manufacturer's protocol. RNA quantity and integrity were assessed using
661 Bioanalyzer, which yielded RIN>7 for all samples. Total RNA were expression profiled on
662 Affymetrix U133Plus2 expression microarrays, with labeling, hybridization, washing, and
663 scanning performed according to manufacturer's protocol. Background correction and quantile
664 normalization of gene expression data were performed using Robust Multi-array Average (RMA)
665 algorithm with the default settings (Affymetrix). For genomic DNA isolation, Qiagen DNeasy
666 Blood and Tissue kit was used. DNA quantity was measured using NanoDrop and PicoGreen
667 fluorescent assay. All samples were profiled using Applied Biosystems Identifiler STR panel
668 prior to genotyping on Illumina OmniExpress arrays. After quality assessment of genotypes
669 samples with >0.1 missing rate were excluded from the analysis. Loci with > 0.1 missing rate,
670 MAF < 0.01, or Hardy-Weinberg Equilibrium *P*- value < 5E-5 were also excluded. The genomic
671 region encompassing 6Mb around the GWAS lead SNP rs3219090 (which was directly

672 genotyped on the array) was imputed using IMPUTE2.2.2⁵⁸ and 1KG phase1 v3 April 2012
673 (build 37) as a reference data. After assigning imputed genotypes for 2 samples that were
674 missing direct genotype for rs3219090 (recoded as 0.333 probability of each genotype), 59 total
675 samples were qualified for eQTL analysis with gene expression and genotype data available.
676 Affymetrix U133Plus2 annotates 17 genes and 11 other transcripts in the 2 Mb region centering
677 at rs3219090. Among these, probes for 12 genes and 2 other transcripts passed QC, including
678 PARP1. eQTL analysis was then performed for these samples and gene/transcripts using
679 SNPTTEST v2.5 (see URL section) considering an additive model for genotypes.

680 **Allele discrimination qPCR.** cDNA from early passage melanoma cell lines heterozygous for
681 both rs3219090 and coding surrogate SNP rs1805414 as well as of normal genomic copy were
682 assayed using custom-designed Taqman genotyping probe sets that do not recognize genomic
683 DNA. To act as a standard, the same amplicon was PCR-amplified for each allele from cDNA
684 and subsequently cloned into the pCR2.1 TOPO vector (Invitrogen) and sequence verified. A
685 standard curve was then generated using known amounts of cloned amplicons by plotting 11
686 different points of allelic ratio against VIC/FAM signal ratio. Allele discrimination qPCR was
687 performed in triplicate, and allelic ratio was calculated from the average ratio of VIC/FAM signal
688 using the standard curve. Departure from expected allelic ratio (major/minor allele) of 1 was
689 assessed using two-tailed Wilcoxon signed rank test.

690 **Nomination of candidate functional variants.** All LD r^2 values used for candidate variant
691 nomination were from 1KG phase 3 data. r^2 values based on both the EUR and CEU
692 populations were considered to extract the maximum r^2 of each variant with the lead SNPs,
693 rs3219090 and rs1858550. Meta-analysis P -values were obtained from the previously published
694 work of Law and colleagues⁷; all samples used in the meta-analysis were collected with
695 informed consent and ethics committee approvals as previously described. DHS peaks for the
696 primary human melanocyte culture “melano” (ENCODE/Duke) were obtained from ENCODE

697 database²⁶ through UCSC Genome browser (see URL section). DNase-seq data for penis
698 foreskin melanocyte primary cell cultures “skin 01” and “skin 02” (shown as Melanocyte_1 and
699 Melanocyte_2 in **Fig 2** and **Supplementary Fig 2**) were obtained from Roadmap database
700 (03/09/2015) and DHS peaks were called using MACS⁵⁹(see URL section) using the default
701 settings and FDR 1% cutoff. DHS peak intervals were overlaid with the genomic position of
702 each candidate variant to determine whether each candidate localizes within a DHS peak.
703 Experimental duplicates for skin 01 (DS18590, DS18601) and skin 02 (DS19662, DS18668),
704 and analytical duplicates for melano were used for our analysis. To call a variant to be within
705 DHS in one sample, DHS overlapping the variant in either of the duplicates were counted. DHS
706 peaks from DNase-seq data for two melanoma cell lines Mel2183 (ENCODE/Duke) and RPMI-
707 7951 (ENCODE/UW) were obtained from the ENCODE database. Peaks from FAIREseq data
708 for 11 melanoma culture samples were obtained from GEO (accession number: GSE60666).
709 Histone mark annotation was performed in the same way. Primary melanocyte histone marks
710 were taken from subsets of three individuals through Roadmap database (skin 01, skin 02, and
711 skin 03; shown as Melanocyte_1, Melanocyte_2, and Melanocyte_3 in **Fig 2** and
712 **Supplementary Fig 2**). Source of each track visualized on UCSC genome browser is as
713 follows: Melanocyte track names used for Fig 2 (Track 1: UCSF-UBC-USC Penis Foreskin
714 Melanocyte primary Cells Histone H3K27ac Donor skin03 Library A15584 EA Release 9, Track
715 2: UCSF-UBC-USC Penis Foreskin Melanocyte primary Cells Histone H3K4me1 Donor skin03
716 Library A15579 EA Release 8, Track 3: UCSF-UBC-USC Penis Foreskin Melanocyte primary
717 Cells Histone H3K4me3 Donor skin03 Library A15580 EA Release 8, Track 4: Melano DNaseI
718 HS Density Signal from ENCODE/Duke, Track 5: Penis Foreskin Melanocyte Primary Cells
719 Donor skin 01 DNase Uniformly Signal from Roadmap, Track 6: UW Penis Foreskin Melanocyte
720 Primary Cells DNase Hypersensitivity Donor skin02 Library DNase DS19662 EA Release 9.),
721 Melanoma track names used for Supplementary Fig 2 (Track 1 through 11: H3K27ac ChIP-seq
722 signal from F-seq, Track 12 through 22: FAIRE-seq signal from F-seq, Track 23: Mel2183

723 DNaseI HS Density Signal from ENCODE/Duke, Track 24: RPMI-7951 DNaseI HS Raw Signal
724 Rep 1 from ENCODE/UW.).

725 **Luciferase reporter assays.** Luciferase constructs were generated to include the DHS region
726 encompassing each SNP. Sequences encompassing each variant were amplified from
727 genomic DNA of HapMap CEU panel samples using the primers listed in **Supplementary Table**
728 **14**, cloned into pCR2.1TOPO vector, and subsequently cloned into pGL4.23 vector using
729 EcoRV and HindIII enzymes or directly into pGL4.23 vector using primers with HindIII and XhoI
730 sequence overhangs. Sequence-verified pGL4.23 constructs were then co-transfected with
731 pGL4.74 (Renilla luciferase) into a melanoma cell lines UACC2331, UACC457, or UACC1308
732 using Lipofectamine 2000 reagent (Thermo Fisher) or electroporation with Lonza Amaxa SE kit
733 and DS-150 protocol on 4D-Nucleofector system. Electroporation of primary human melanocytes
734 (HEMn-LP, Invitrogen) was performed using the Lonza Amaxa P2 kit and protocol CA-137
735 (Lonza). When luciferase assays were combined with *RECQL* over-expression, empty pCMV6-
736 Entry vector or human *RECQL* cDNA clone (Origene, RC200427) were co-transfected with
737 luciferase constructs into HEK293FT cells using Lipofectamine 2000 reagent. Cells were
738 collected 24hr following transfection and luciferase activity was measured using Dual-Luciferase
739 reporter system (Promega) on GLOMAX multi detection system (Promega). All cell lines and
740 primary cultures used here and onward were tested negative for mycoplasma contamination.

741 **EMSA and antibody supershift.** Nuclear extracts were prepared from actively growing normal
742 human melanocytes (HEMn-LP, Invitrogen) or melanoma cell lines (UACC) using NE-PER
743 nuclear and cytoplasmic extraction kit (Thermo Scientific). DNA oligos for each variant were
744 synthesized with 5' biotin labeling, and HPLC-purified (Life Technologies; probe sequences are
745 listed in **Supplementary Table 14**). Forward and reverse strands were then annealed to make
746 double stranded DNA probes. Probes were bound to 0.5-4 μ g nuclear extracts pre-incubated
747 with 1 μ g poly d(I-C) in binding buffer containing 10mM Tris (pH 7.5), 50 mM KCl, 1 mM DTT, 10

748 mM MgCl₂, with or without 5% glycerol at 4°C for 30min. Unlabeled competitor oligos were
749 added to the reaction mixture 5min prior to the addition of probes. Completed reactions were
750 run on 5% or 4-20% native acrylamide gel and transferred blots were developed using LightShift
751 Chemiluminescent EMSA kit (Thermo Scientific) and exposed on film. Supershift antibodies
752 (RecQL, sc-25547, Santa Cruz) or rabbit normal IgG (sc-2027, Santa Cruz) were bound to
753 nuclear extract prior to poly d(I-C) incubation at 4 °C for 1hr. EMSAs with purified recombinant
754 protein were performed using RECQL (TP300427, Origene), where purified recombinant
755 proteins were used in place of nuclear extract and poly d(I-C). Additional antibodies and
756 recombinant proteins for validations are as follows. Antibodies are from Santa Cruz unless
757 otherwise specified: anti-NCL (sc-8031), anti-SRSF3 (sc-13510), anti-CIRP (sc-161012), anti-
758 BLM (sc-7790), anti-hnRNPd (sc-22368), anti-RBM3 (sc-162080), anti-TOP3A (sc-11257), anti-
759 RPA1 (sc-14696), anti-DHX36 (A300-525A, Bethyl), anti-RPA3 (ab167593, Abcam).
760 Recombinant proteins are from Origene: NCL (TP319082), CIRP (TP301639),
761 RPA1(TP302066), hnRNPd (TP300660), RBM3 (TP760298).

762 **Mass-spectrometry.** Quantitative AP-MS/MS following SNP DNA pulldown and in-solution
763 dimethyl chemical labeling was performed based on procedures described previously^{29,30}.
764 Nuclear extract from the melanoma cell line UACC2331 was collected as described previously
765 using the Dignam lysis protocol⁶⁰. For DNA pulldowns, 500 pmol of annealed, forward strand 5'-
766 biotinylated oligo probes were coupled to streptavidin sepharose beads (GE Healthcare).
767 Insertion and deletion allele probe sequences are listed in **Supplementary Table 14**. Beads
768 were incubated with 450 µg of nuclear extract for 90 minutes plus 10 µg of non-specific
769 competitor DNA (either 10 µg of poly-dAdT or 5 µg of poly-dAdT plus 5 µg poly-dIdC). After
770 washes, beads were resuspended in 100mM TEAB buffer, proteins were reduced with 5mM
771 TCEP, alkylated with 10 mM MMTS, and digested overnight with 0.25 µg trypsin. Digested
772 peptides were labelled using in-solution dimethyl chemical labelling as described previously²⁸.

773 All experiments were performed in duplicate, and labels were swapped between replicate pairs
774 to prevent labeling bias. Heavy and light labelled peptides were mixed and prepared using C18-
775 StageTips. Peptides were loaded on a column packed with 1.8 μm Reprosil-Pur C18-AQ beads
776 (gift from Dr. Maisch) and eluted using a 120 minute gradient from 7%-32% buffer B (80%
777 acetonitrile, 0.1% formic acid) at a flow rate of 250nL/min. Peptides were sprayed directly onto a
778 Thermo QExactive mass spectrometer. Data was collected in top10 data-dependent acquisition
779 mode. Thermo RAW files were analyzed with MaxQuant (version 1.3.0.5) by searching against
780 the Uniprot curated human proteome. Methionine oxidation and N-terminal acetylation were
781 considered as variable modifications and cysteine-dithiomethane was set as a fixed
782 modification. Protein ratios normalized by median ratio shifting as described previously⁶¹ were
783 used for outlier calling. An outlier cutoff of 1.5 IQRs (inter-quartile ranges) in two out of two
784 biological replicates was used.

785 **Chromatin immunoprecipitation of RECQL.** UACC2331 melanoma cells or primary human
786 melanocytes (HEMn-LP, Invitrogen) were fixed with 1% formaldehyde when 80-90% confluent,
787 following the instructions of Active Motif ChIP-ITexpress kit or ChIP-IT high sensitivity kit. $7.5 \times$
788 10^6 cells were then sheared by sonication using a Bioruptor (Diagenode) at high setting for
789 15min, with 30 sec on and 30 sec off cycles. Sheared chromatin from 1 to 4×10^6 cells were
790 used for each immunoprecipitation with antibodies against RECQL, H110 (sd-25547; Santa
791 Cruz), and A300 (A300-450A; Bethyl), or normal rabbit IgGs (sc-2027; Santa Cruz) following the
792 manufacturer's instructions. Purified pulled-down DNA was assayed by SYBR Green qPCR for
793 enrichment of target sites using primers listed in **Supplementary Table 14**. A commercial
794 primer set (71001, Active Motif) recognizing a gene desert on chromosome 12 was used for a
795 negative control (Neg).

796 **Overexpression of RECQL in melanoma cell lines.** RECQL was cloned from a cDNA
797 construct (RC200427, purchased from Origene) into lentiviral pLU-TCMV-FMCS-pPURO vector

798 (a generous gift from Dr. Meenhard's lab at Wistar) containing tetracycline-inducible promoter.
799 Lentiviral vectors were co-transfected into 293 cells with packaging vectors psPAX2, pMD2-G,
800 and pCAG4-RTR2. Virus was collected two days after transfection and concentrated by
801 Vivaspin. Cells were incubated with virus for 24 hr, followed by puromycin (1-2 µg/ml) selection
802 for 2-3 days. After drug selection cells were seeded and grown in the media containing varying
803 amount of doxycycline (0,0.5, and 1 µg/ml). Cells were harvested after 48 hrs of doxycycline
804 induction for RNA and protein isolation. cDNA was generated from total RNA and transcript
805 levels were measured using Taqman qPCR. *RECQL* and *PARP1* transcript levels normalized to
806 the levels of *B2M*, and PCR triplicates were averaged and considered as one data point.
807 Western blotting of RECQL and GAPDH was performed using the following antibodies:
808 RecQL1, sc-25547, Santa Cruz, GAPDH, sc-51907, Santa Cruz.

809 **Lentiviral vectors and lentiviral infection of melanocytes.** HIV-CSCG-*BRAF*^{V600E} was used
810 to express *BRAF*^{V600E} in normal human melanocytes (provided by Dr. Daniel Peeper,
811 Netherlands Cancer Institute). The *PARP1* cDNA clone (BC037545) was purchased from
812 Thermo Scientific and sub-cloned and expressed in three different lentiviral vectors: a. under a
813 tetracycline-inducible promoter in the pInducer 20 vector backbone (Addgene) as pIN20-
814 *PARP1*, which expresses high level of PARP1; b. under a tetracycline-inducible promoter in the
815 pLU-TCMV-FMCS-pPURO vector (a generous gift from Dr. Meenhard Herlyn's laboratory at the
816 Wistar Institute) as pLU-TCMV-PARP1 which expresses low level of PARP1; c. with a CMV
817 promoter in the pLX304 backbone (Addgene) as pLX-*PARP1*. The PARP1 catalytic mutant
818 p.Glu988Lys³⁹ was generated using the QuickChange II XL Site-Directed Mutagenesis kit
819 (Agilent). The *MITF-M* cDNA clone (NM_000248) was purchased from Origene and was sub-
820 cloned and expressed in pLX304 backbone as pLX-*MITF*. pLKO-*MITF* shRNAs (a pool of 4
821 shRNAs with clone IDs from TRC0000019119 to TRC0000019123) were purchased from Sigma
822 in lentiviral-based vectors. Primary human melanocytes were obtained from Invitrogen and/or

823 the Yale SPORE in Skin Cancer Specimen Resource Core and grown under standard culture
824 conditions using Medium M254 with supplements (Invitrogen). For lentivirus production,
825 lentiviral vectors were co-transfected into 293 cells with packaging vectors psPAX2, pMD2-G,
826 and pCAG4-RTR2. Virus was collected two days after transfection and concentrated by
827 Vivaspin. Cells were incubated with virus for 24 hr, followed by drug selection, before being
828 subjected to various experimental treatments and assays. Dose of BYK204165 was chosen by
829 performing a titration assay. Briefly, melanocytes were treated with 1, 5, 20, and 40 μM
830 BYK204165 for 1, 7, and 24 hrs, and PARylation was assessed by western blotting. PARylation
831 was mostly diminished with 5 μM BYK204165, and completely undetectable with 20 μM after 1
832 hour treatment. Based on these data, we chose to use 10 μM BYK204165 for our assays.

833 **Cell proliferation assays.** Cell proliferation was assayed by a bromodeoxyuridine (BrdU) flow
834 kit (BD Pharmingen, San Jose, CA) according to the manufacturer's protocol. Briefly, cells were
835 labeled with 10 μM BrdU for three hours before they were fixed, permeabilized and subjected to
836 DNaseI treatment. Cells were then stained with FITC-conjugated anti-BrdU antibody and 7-
837 AAD, followed by flow cytometry analysis using a FACSCalibur (BD Pharmingen, San Jose,
838 CA). For most cases, BrdU assay was performed on cells two weeks after initial infection of
839 *PARP1* vector. For crystal violet (CV) staining, cells were seeded at equal numbers after
840 infection and drug selection, and stained with CV between 2-3 weeks after initial *PARP1*
841 infection.

842 **Western and immunofluorescence staining.** For western blot analysis, total cell lysates were
843 generated with RIPA buffer (Thermo Scientific, Pittsburgh, PA) and subjected to water bath
844 sonication. Samples were resolved by 4-12% Bis-Tris ready gel (Invitrogen, Carlsbad, CA)
845 electrophoresis. The primary antibodies used were rabbit anti-PAR (551813, BD Pharmingen,
846 CA), rabbit anti-PARP (9542, Cell Signaling Technology), mouse anti-MITF (MS-771-P1,
847 Thermo Scientific), mouse anti- β -Actin (A5316, Sigma), and mouse anti-Raf-B (sc-5284, Santa

848 Cruz Biotechnology). For immunofluorescence staining, cells were fixed and permeabilized
849 followed by staining with anti-H3K9Me3 antibody (07-442 from Millipore, Billerica, MA) or anti-
850 HP1 γ antibody (ab56978 from Abcam). Cells were then labeled with Alexa Fluor488 donkey
851 anti-Rabbit secondary Ab or Alexa Fluor568 goat anti-Rabbit secondary antibody (Invitrogen,
852 Carlsbad, CA) before treated with anti-fade gold mounting medium with DAPI (Invitrogen,
853 Carlsbad, CA). Images were examined and processed by a Zeiss LSM 700 confocal
854 microscope with total 630X magnification. Representative images are shown. To measure
855 H3K9Me3 level by western blotting, total histone was extracted using HCL following a standard
856 protocol (Abcam) and detected with rabbit anti-Histone H3 (61277, Active Motif) or rabbit anti-
857 Histone H3K9Me3 (ab8898, Abcam) antibody; ratios of H3K9Me3 to total H3 did not correlate
858 with H3K9Me3 focus formation (**Supplementary Fig. 26**).

859 **Colony formation assays.** Anchorage-independent growth was assayed with
860 p^umel/BRAFV600E cells (provided by Dr. Hans Widlund, Dana-Farber Cancer Institute). Briefly,
861 2,500 cells were mixed in HAMF12 plus 10% FBS medium containing 0.4% SeaKem LE
862 agarose from Lonza, and plated on top of the bottom layer with 0.65% agarose prepared in
863 same HAMF12 medium in 6-well plate. After the agarose is solidified, each well was covered
864 with 0.5 mL feeding medium which was refreshed twice a week. Colonies were counted and
865 imaged between 3-4 weeks after seeding under a regular microscope with total magnification of
866 40X. Colonies were counted from three wells of each condition, and significant difference from
867 the control was assessed by t-test assuming unequal variance.

868 **Chromatin immunoprecipitation of PARP1, H3K4me3, and RNA PolIII.** Primary human
869 melanocytes (HEMn-LP, Invitrogen) were fixed with 0.1% formaldehyde when 80-90%
870 confluent. About 1×10^7 cells were then sheared by sonication using a Bioruptor (Diagenode) at
871 high setting for 10 cycles with 20 sec on and 30 sec off. Chromatin were then purified and
872 immunoprecipitated following the instructions of Active Motif CHIP-IT high sensitivity kit. 5-10 μ g

873 sheared chromatin were used for each immunoprecipitation with antibodies against
874 PARP1,46D11 (#9532, Cell Signaling Technology), H3K4me3 (ab8580, Abcam), RNA PolII
875 CTD (17-672, Millipore), RNA PolII CTD Phospho-Ser5 (ab5408, abcam) or normal rabbit IgGs
876 (ab46540, Abcam) and normal mouse IgGs (17-672, Millipore). Purified pulled-down DNA was
877 assayed by SYBR Green qPCR for enrichment of target sites across *MITF*-M promoter using
878 primers listed in **Supplementary Table 14**.

879 **Statistical analyses**

880 All cell-based experiments were repeated at least three times with separate cell cultures, except
881 for Fig3a (repeated twice), Fig4d-e (six technical replicates), and Fig5d-e (repeated twice).
882 When a representative set was shown, replicate experiments displayed similar patterns. For all
883 the plots, individual data points are shown with median or mean, range (maximum and
884 minimum), and 25 & 75 percentile (where applicable). Statistical method, number of data points,
885 and number and type of replicates are indicated in each figure legend.

886 **Accession codes**

887 dbGAP Accession: phs000178.v9.p8

888 dbGaP Accession: phs000424.v6.p1

889 GEO Accession: GSE60666

890

891 **DATA AVAILABILITY STATEMENT**

892 The data generated during the current study have been deposited in NCBI's Gene Expression
893 Omnibus (Edgar et al., 2002) with the accession code GSE99221 (super series) which includes
894 GSE99193 (genotype data) and GSE78995 (expression data).

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