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

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ABSTRACT

Prior genome-wide association studies have identified a melanoma-associated locus on chr1q42.1 that encompasses a ~100 kb region spanning the PARP1 gene. eQTL analysis in multiple cell types of melanocytic lineage consistently demonstrated that the 1q42.1 melanoma risk allele (rs3219090, G) is correlated with higher PARP1 levels. In silico fine-mapping and functional validation identified a common intronic indel, rs144361550 (-/GGGCCC, r^2 =0.947 with rs3219090) as displaying allele-specific transcriptional activity. A proteomic screen identified RECQL as binding to rs144361550 in an allele-preferential manner. In human primary melanocytes, PARP1 promotes cell proliferation and rescues $BRAF^{V600E}$-induced senescence

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

To date, genome-wide association studies (GWAS) have identified twenty common, genome-wide significant melanoma susceptibility loci[1-9], most of which do not appear to be explained by protein-coding variants. A subset of these loci harbor known pigmentation genes that mediate melanoma-associated phenotypes such as eye, hair, and skin color. While several loci harbor genes implicated in cancer, evidence directly linking common risk variants within most of these loci to altered function of specific genes is lacking.

MacGregor and colleagues initially identified a melanoma risk locus tagged by rs3219090 on chromosome band 1q42.1 in an Australian case-control study at a near genome-wide level of significance ($P = 9.3 \times 10^{-8}$, OR = 0.87, protective allele A)[8]. The association has since been replicated by multiple other studies[3,10], including most recently by a meta-analysis of 12,874 melanoma cases (rs1858550, $P = 1.7 \times 10^{-13}$)[7]. Notably, the locus at 1q42.1 has also been associated with melanoma survival[11], where the melanoma risk allele correlates with increased survival, an association that has since been replicated[12]. The region of association spans from 226.52 Mb to 226.63 Mb (hg19) of chromosome 1, encompassing the entirety of the poly(ADP-ribose) (PAR) polymerase-1 (PARP1) (OMIM: 173870) gene, and fine-mapping suggests that the association is best explained by a single-SNP model[3].

While a number of other genes are located in the vicinity of the association peak, PARP1 has the most well-established role in cancer. PARP1 is best known for its role as a DNA repair enzyme and genotoxic sensor that functions in base excision repair (BER), single-strand break repair, and double-strand break repair[13]. Once PARP1 binds to damaged DNA, its enzymatic

function is activated, and it covalently attaches PAR polymers to acceptor proteins, including histones and PARP1 itself[14]. PARP1 amplifies DNA damage signals, modifies chromatin structures to accommodate DNA damage response proteins, and further recruits DNA repair proteins[13,15,16]. While PARP1 is not directly involved in repair of UV signature mutations via nucleotide excision repair, its role in the repair of DNA lesions induced by reactive oxygen species (ROS) is well-established[17]. ROS are generated by UVA exposure[18], are a byproduct of melanin production[19], and appear to play a role in oncogene-induced senescence (OIS)[20,21]. Aside from DNA repair, PARP1 functions in regulating gene expression by modifying chromatin structure, associating with promoters and enhancers, and acting as a transcriptional co-regulator[22,23]. While many of these roles rely on PARP1 catalytic activity, some are also PARylation-independent, as in the transcriptional co-regulator function for NF-kB and B-MYB[24,25].

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

RESULT

The rs3219090 risk allele is correlated with high PARP1

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

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

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

RNAseq (data not shown). Subsequent PARP1 ASE analysis in TCGA and GTEx RNAseq datasets demonstrated that a higher allelic proportion of mapped reads was also observed for the risk allele across TCGA tumor samples (P = 0.011, two-tailed Wilcoxon signed rank test, n = 48, copy-neutral and heterozygous; **Fig. 1c**), as well as in sun-exposed and non-sun-exposed skin tissues (GTEx, P = 1.16 $x10^{-5}$, n = 139; P = 8.9 x 10^{-5}, n = 69; respectively, two-tailed Wilcoxon signed rank test; **Supplementary Fig. 1e-f**). These data demonstrate that the melanoma risk allele of rs3219090 is significantly associated with increased PARP1 expression in tissues of melanocytic origin and skin with striking consistency across multiple datasets.

Fine-mapping and functional annotation of candidate SNPs

Given that high PARP1 levels are correlated with the melanoma risk allele of rs3219090, we next sought to identify functional risk variant(s) that may influence PARP1 expression. Previously, fine-mapping of this locus in a large European population provided support for a model in which a single variant accounts for the association signal in this region[3], a finding confirmed as part of the meta-analysis conducted by Law and colleagues[7]. We prioritized 65 variants that are highly correlated with the lead SNP as candidate functional variants (r^2>0.6 with lead SNPs from the discovery or meta-analysis lead SNPs[3,7], rs3219090 and rs1858550, respectively; LD based on 1KG phase3, EUR and CEU). Given the absence of amino acid-changing PARP1 variants within this set of candidates, an absence of evidence for alternative splicing as a likely mechanism (**Supplementary Note**), and considerable evidence for allelic differences in PARP1 expression levels, we focused on those located within annotated melanocyte- or melanoma-specific cis-regulatory elements using data from the ENCODE[26] and Roadmap projects[27] (**Supplementary Note**, **Supplementary Table 5-6, Supplementary Fig. 2-3**). The four of the most strongly supported variants are situated at the center of melanocyte DHS peaks as well as within regions harboring promoter or enhancer histone marks (H3K4me1, H3K4me3, or H3K27ac) in the majority of melanocyte/melanoma cultures assayed

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

An intronic indel displays allelic transcriptional activity

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

To identify proteins that bind rs144361550 in an allele-preferential manner, we utilized quantitative mass-spectrometry employing dimethyl label swapping[28,29 30]. Mass-spectrometry

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

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

PARP1 facilitates melanocyte growth and transformation

Given that the risk allele of rs3219090 is associated with increased levels of PARP1 expression, we tested whether increased PARP1 levels could lead to altered cellular phenotypes relevant to melanomagenesis. As $BRAF^{V600E}$ -induced senescence is well accepted as a barrier to malignant transformation in early events of melanomagenesis [31-33] and ROS seems to present a potential functional link between PARP1 and OIS [21], we examined whether elevated PARP1 modulates OIS by overexpressing PARP1 in human primary melanocytes expressing oncogenic BRAF ($BRAF^{V600E}$). Consistent with published results, ectopic expression of $BRAF^{V600E}$ in human melanocytes induced a robust arrest of cell growth and proliferation, accompanied by heterochromatic H3K9Me3 focus formation (**Fig. 5a-c, Supplementary Fig.14**), and a moderate increase in β-galactosidase activity (**Supplementary Fig. 15**), both hallmarks of OIS. Overexpression of PARP1 prior to induction of $BRAF^{V600E}$, however, prevented the cell cycle arrest and H3K9Me3 focus formation as well as β-galactosidase activity observed in melanocytes expressing $BRAF^{V600E}$ alone (**Fig. 5a-c, Supplementary Fig. 14-15**), demonstrating a rescue from $BRAF^{V600E}$-induced senescence. Crystal violet staining of cells three weeks following PARP1 expression indicated that PARP1 by itself can also increase cell proliferation in the absence of $BRAF^{V600E}$, suggesting an effect on cell proliferation by PARP1 (**Fig. 5a**). PARP1 consistently exerted weak but significant effects on cell proliferation and OIS reversal even at a moderate induction level (~1.5 fold, **Supplementary Fig. 16**) recapitulating the subtle allelic expression differences observed in melanomas carrying risk or protective alleles. Several oncogenes, including MYC [34] and PIK3CA [33], have been reported to stimulate malignant transformation in melanoma cells by abrogating OIS and restarting cell proliferation. To further evaluate if PARP1 can stimulate malignant transformation by affecting melanocyte proliferation, we examined the effect of PARP1 on anchorage-independent growth of TERT-immortalized human melanocytes (p'mel[35]) by soft-agar assay. PARP1 cooperated with $BRAF^{V600E}$ to enhance colony formation of p'mel cells in soft agar, similar to the previously reported effect of MITF[35], albeit to a lesser degree (**Fig. 5d-e**). Thus, like deregulated MITF

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

Most of the well-characterized functions of PARP1, including those involving DNA repair, are closely linked to its poly-ADP ribosylation activity. We therefore tested whether PARP1-mediated rescue of melanocytes from $BRAF^{V600E}$-induced OIS is dependent on PARP1 catalytic activity. Melanocytes overexpressing both PARP1 and $BRAF^{V600E}$ were treated with the PARP1 inhibitor BYK204165[36], and the inhibition of PAR-activity was confirmed by Western blotting with an antibody recognizing PAR (**Fig. 6a**). BYK204165 treatment did not block the cell proliferation effect of PARP1 in $BRAF^{V600E}$-expressing cells as shown by similarly increased cell growth in crystal violet staining (**Supplementary Fig. 17a**)**,** an increased percentage of BrdU-positive cells and reduced G0/G1 population in PARP1-expressing cells (**Fig. 6b**), nor did it affect H3K9Me3 focus formation (**Supplementary Fig. 17b**). We also tested a catalytically inactive mutant version of PARP1[37-39] for effect on melanocyte growth and proliferation (**Supplementary Fig. 18a-d**), resulting in similar phenotypes to those observed using BYK204165.

PARP1 transcriptionally activates MITF

To begin to understand the mechanism of PARP1-mediated senescence rescue we first examined ROS content changes in $BRAF^{V600E}$ and/or PARP1 over-expressing melanocytes. While we observed a minor increase of ROS content by $BRAF^{V600E}$ expression, we did not detect meaningful changes in ROS content by PARP1 in our melanocyte system (data not shown). We then turned our attention to melanocyte lineage survival oncogene, MITF, which is frequently amplified in malignant melanomas and can transform melanocytes in the context of $BRAF^{V600E}$ [35]. Previous studies have observed that MITF expression is inhibited by oncogenic $BRAF^{V600E}$ in melanomas[40]. Consistent with this finding, we demonstrate that persistent activation of MAPK pathway by $BRAF^{V600E}$ also suppressed MITF expression in primary melanocytes (**Fig. 6a**). Significantly, PARP1 induction, at either strong (**Fig. 6a**, **Supplementary**

Fig. 19) or more modest levels (**Supplementary Fig. 16e-f**), increases MITF at both mRNA and protein levels, in a PARylation-independent manner (**Supplementary Fig. 18d-f**, **Fig. 6a**). PARP1 also partially restores MITF expression in $BRAF^{V600E}$-expressing melanocytes, suggesting a link between OIS rescue and MITF restoration (**Fig. 6a, Supplementary Fig. 16e,f, Supplementary Fig. 18d-f, Supplementary Fig. 19**). Consistent with this finding, we observed a weak but significant positive correlation between PARP1 and MITF transcript levels (**Supplementary Fig. 20**) in 409 melanoma samples from TCGA (Pearson's r = 0.198, P = 5.7 x 10^{-5}) as well as in the smaller subset of 189 tumors that are copy-neutral at the MITF locus (Pearson's r = 0.252, P = 4.8 x 10^{-4}). To address potential issues with tumor heterogeneity and random factors driving this correlation in melanomas, we also looked at the correlation in our early-passage melanoma cell lines. While we did not observe a significant correlation in the full set of 59 cell lines (Pearson's r = 0.15, P = 0.26; **Supplementary Fig. 21a**), when we further subdivide them into MITF-high and MITF-low groups to account for distinct cellular states signified by MITF levels[41], a strong correlation of PARP1 and MITF levels was observed only in MITF-high group (Pearson's r = 0.560, P = 5.5 x 10^{-3}, n = 23; P = 6.6 x 10^{-3} when MITF copy number is adjusted; **Supplementary Fig. 21b**). A trend of association with PARP1 levels were also observed in a subset of MITF target genes (**Supplementary Fig. 21c-d, Supplementary Table 12**).

MITF is an essential melanocyte survival gene, and therefore we could not directly test if MITF mediates PARP1 senescence rescue by knocking down MITF in our system. While MITF depletion by shRNA dramatically reduces melanocyte growth approximately two weeks following knockdown (**Fig. 6c,d**), at an earlier time point (D9 after infection), we noticed that MITF depletion itself introduced a weak but distinct senescence-associated H3K9Me3 phenotype (**Fig. 6e**) as previously reported [42]. Interestingly, in the context of MITF knockdown, PARP1 senescence rescue is not observed in terms of partial reversal of H3K9Me3 focus

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

Given that PARP1 activates MITF at the mRNA level, we performed ChIP to determine whether PARP1 directly localizes to the melanocyte-specific MITF-M [43,44] promoter in human primary melanocytes. ChIP experiments were performed both via overexpression of PARP1 in melanocytes, as well as in melanocytes expressing endogenous levels. Among seven primer sets spanning the MITF-M promoter region (–1263 to +172bp of the transcription start site; TSS), the two that are more proximal to the TSS displayed the most significant enrichment, indicating PARP1 binding (~8-19 fold above IgG background at endogenous level, Primer4 and Primer5, from -587 to -136 bp; **Fig. 7**). Localization of PARP1 to the MITF-M promoter was observed both with PARP1 over-expression as well as endogenous levels, while no detectable enrichment was observed when PARP1 was knocked down using an shRNA (data not shown). Notably, the region where PARP1 localizes overlaps with consensus binding sites of previously known transcriptional regulators of the MITF-M promoter: SOX10, TCF/LEF, and CRE (**Fig. 7**); and a putative PARP1 binding motif[45] was also predicted in this core region (see Methods section).

To assess whether PARP1 directly regulates MITF transcription, we performed luciferase reporter assays using constructs containing the MITF-M promoter regions[46]. Altered PARP1 levels via over-expression or shRNA-mediated knockdown failed to modulate reporter activity (**Supplementary Fig. 22**), suggesting a potential epigenetic role for PARP1 in regulation of the MITF-M locus that is not recapitulated via ectopic expression of a reporter gene. Bisulfite sequencing of two assayable CpGs near the MITF-M TSS[47] indicated that introduction of $BRAF^{V600E}$ does not alter their methylation status (**Supplementary Fig. 23**), speaking against DNA methylation-mediated regulation in this context. Examination of a promoter histone mark, H3K4Me3, by ChIP revealed that H3K4Me3 is enriched in the MITF-M proximal promoter when PARP1 is expressed either endogenously or ectopically (**Supplementary Fig. 24a**), but is

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

DISCUSSION

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

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

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

Our unbiased approach using quantitative mass-spectrometry identified RECQL as a protein binding allele-preferentially to rs144361550. Importantly, RECQL binding to rs144361550 does not appear to be driven by sequence specificity but rather by DNA secondary structure. While genomic sequence encompassing rs144361550 suggested G4-forming potential (**Supplementary Table 11**), which might explain a regulatory role[48], our in vitro assays failed to provide definitive evidence for G4 structure either by insertion or deletion allele. However, formation of another differential structural motif, such as a transient hairpin structure (formed by single-stranded sequences**, Supplementary Table 13**) or a locally perturbed double-helix structure at the hexanucleotide repeat domain[49], inducing DNA bending and serving as a recognition motif for allele-specific protein binding[50], cannot be excluded.

Although PARP1 is most well-known for its role in DNA repair, to date GWAS have identified this region only for melanoma susceptibility (GWAS catalog; see URL section), suggesting a potential melanoma-specific role of PARP1. Indeed, ectopic expression of PARP1 led to increased melanocyte proliferation both alone, as well as in the presence of oncogenic mutated version of BRAF ($BRAF^{V600E}$). $BRAF^{V600E}$ is found in most melanocytic nevi[51], and as

reported earlier[31] its over-expression in primary melanocytes led to markedly decreased cell proliferation as well as H3K9Me3 focus formation, a hallmark of cellular senescence[52]. Our data show that these senescence-like phenotypes are partially reversed by elevated PARP1 levels. Further, ectopic expression of PARP1 in TERT-immortalized (p'mel) melanocytes expressing $BRAF^{V600E}$ [35] led to a tumorigenic phenotype exemplified by anchorage-independent growth. These data suggest a role for increased PARP1 in the early stages of melanomagenesis promoting increased melanocyte growth and/or escape from $BRAF^{V600E}$-induced senescence. In this context, PARP1 seems to be functionally linked to melanocyte lineage survival oncogene, MITF, which itself is an established melanoma susceptibility gene[53,54]. While we did not exhaustively explore all the major functions of PARP1 including DNA repair and inflammation in the context of melanomagenesis, our findings highlight a novel role for PARP1 in a melanocyte-specific context, providing a potential link between a susceptibility gene and a lineage-specific oncogene.

Most well-established functions of PARP1 are dependent on the poly-ADP ribosylation activity including its role in DNA damage response and repair. While some transcriptional co-regulator functions of PARP1 do not require its enzymatic activity[24,25], the role of PARP1 in chromatin structure modification mainly relies on NAD+-dependent association with nucleosomes[55] or PARylation of histones and chromatin modulators such as histone demethylase KDM5B[56,57]. Notably, the effects of PARP1 expression on melanocyte proliferation, $BRAF^{V600E}$-induced senescence, and restoring MITF-M expression do not appear to depend on the catalytic activity of PARP1. Interestingly, PARP1-mediated MITF-M up-regulation seems to involve chromatin modulation, as the promoter histone mark H3K4Me3 is markedly enriched near the TSS of MITF-M upon PARP1 expression relative to its depleted state in $BRAF^{V600E}$-expressing melanocytes. While this is an important observation of potential PARylation-independent chromatin modulation function, further investigation is required to establish a causal relationship and molecular link.

URLs.

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AUTHOR CONTRIBUTIONS

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

was analyzed by T.Z., M.H.L., H.P., and M.M.I. Fine mapping of GWAS data was performed by M.M.I., D.T.B., J.A.N-B., S.M., and M.H.L. Melanoma cell line eQTL and ASE experiments were 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., and K.M.B.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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

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

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

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

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

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

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

Figure 6 MITF expression is restored in primary human melanocytes co-expressing PARP1 and $BRAF^{V600E}$ in a PARylation-independent manner, concurrent with partial reversal of senescence phenotypes. (**a**) Western blot detection of MITF (long and short exposures), BRAF, PARP1, and PAR. Primary human melanocytes were co-infected with PARP1, $BRAF^{V600E}$ followed by treatment with the PARP inhibitor, BYK204165. Cell extract was generated at day 13 after initial infection with PARP1 construct. Blot images were cropped. (**b**) BrdU flow cytometry of primary human melanocytes co-infected with PARP1, $BRAF^{V600E}$, and/or MITF-shRNA followed by treatment with the PARP inhibitor, BYK204165. (**c**) Crystal Violet staining of melanocytes co-infected with PARP1, $BRAF^{V600E}$, and/or MITF-shRNA. Primary melanocytes were first infected with pIN20-PARP1 or empty pIN20 vector concurrently with pLKO-shRNA-MITF-M or pLKO-empty vector followed by G418 treatment and doxycycline induction. Cells were then infected with HIV-CSCG-$BRAF^{V600E}$ or empty HIV-CSCG vector at day 6 after PARP1 infection followed by Blasticidine selection. A representative crystal violet (CV) staining (n=3) image is displayed at day 17 of infection. (**d**) Quantification of solubilized CV staining by measuring absorbance at 540nm at day 17 of infection is plotted. Median with range (n=3, t-test assuming unequal variance, a representative set from three experiments is shown) (**e**) PARP1-mediated partial reversal of $BRAF^{V600E}$-induced H3K9Me3 focus formation in melanocytes is blocked by knockdown of MITF. H3K9Me3 staining was performed at day 13 after initial infection. Scale bars are shown for 10µm.

Figure 7 PARP1 binds to MITF-M promoter. Human melanocytes were transduced with lentiviral constructs of empty pIN20 (Empty = endogenous PARP1 levels) or PARP1 cDNA (PARP1 = over-expression of PARP1) and harvested five days after infection. Chromatin immunoprecipitation was performed using antibody against PARP1 (anti-PARP1) or rabbit normal IgG (IgG) and pulled-down DNA was amplified using seven sets of primers spanning -1263bp through to +172bp relative to MITF-M TSS

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

ONLINE METHODS

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

672 genotyped on the array) was imputed using IMPUTE2.2.2[58] 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 SNPTEST 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 colleagues[7]; 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 database[26] 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 MACS[59](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

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

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

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

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

Mass-spectrometry. Quantitative AP-MS/MS following SNP DNA pulldown and in-solution dimethyl chemical labeling was performed based on procedures described previously[29,30]. Nuclear extract from the melanoma cell line UACC2331 was collected as described previously using the Dignam lysis protocol[60]. For DNA pulldowns, 500 pmol of annealed, forward strand 5'-biotinylated oligo probes were coupled to streptavidin sepharose beads (GE Healthcare). Insertion and deletion allele probe sequences are listed in **Supplementary Table 14**. Beads were incubated with 450 µg of nuclear extract for 90 minutes plus 10 µg of non-specific competitor DNA (either 10 µg of poly-dAdT or 5 µg of poly-dAdT plus 5 µg poly-dIdC). After washes, beads were resuspended in 100mM TEAB buffer, proteins were reduced with 5mM TCEP, alkylated with 10 mM MMTS, and digested overnight with 0.25 µg trypsin. Digested peptides were labelled using in-solution dimethyl chemical labelling as described previously[28].

All experiments were performed in duplicate, and labels were swapped between replicate pairs to prevent labeling bias. Heavy and light labelled peptides were mixed and prepared using C18-StageTips. Peptides were loaded on a column packed with 1.8 µm Reprosil-Pur C18-AQ beads (gift from Dr. Maisch) and eluted using a 120 minute gradient from 7%-32% buffer B (80% acetonitrile, 0.1% formic acid) at a flow rate of 250nL/min. Peptides were sprayed directly onto a Thermo QExactive mass spectrometer. Data was collected in top10 data-dependent acquisition mode. Thermo RAW files were analyzed with MaxQuant (version 1.3.0.5) by searching against the Uniprot curated human proteome. Methionine oxidation and N-terminal acetylation were considered as variable modifications and cysteine-dithiomethane was set as a fixed modification. Protein ratios normalized by median ratio shifting as described previously[61] were used for outlier calling. An outlier cutoff of 1.5 IQRs (inter-quartile ranges) in two out of two biological replicates was used.

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

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

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

Lentiviral vectors and lentiviral infection of melanocytes. HIV-CSCG-BRAF^{V600E} was used to express BRAF^{V600E} in normal human melanocytes (provided by Dr. Daniel Peeper, Netherlands Cancer Institute). The PARP1 cDNA clone (BC037545) was purchased from Thermo Scientific and sub-cloned and expressed in three different lentiviral vectors: a. under a tetracycline-inducible promoter in the pInducer 20 vector backbone (Addgene) as pIN20-PARP1, which expresses high level of PARP1; b. under a tetracycline-inducible promoter in the pLU-TCMV-FMCS-pPURO vector (a generous gift from Dr. Meenhard Herlyn's laboratory at the Wistar Institute) as pLU-TCMV-PARP1 which expresses low level of PARP1; c. with a CMV promoter in the pLX304 backbone (Addgene) as pLX-PARP1. The PARP1 catalytic mutant p.Glu988Lys[39] was generated using the QuickChange II XL Site-Directed Mutagenesis kit (Agilent). The MITF-M cDNA clone (NM_000248) was purchased from Origene and was sub-cloned and expressed in pLX304 backone as pLX-MITF. pLKO_MITF shRNAs (a pool of 4 shRNAs with clone IDs from TRC0000019119 to TRC0000019123) were purchased from Sigma in lenitiviral-based vectors. Primary human melanocytes were obtained from Invitrogen and/or

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

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

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

Cruz Biotechnology). For immunofluorescence staining, cells were fixed and permeabilized followed by staining with anti-H3K9Me3 antibody (07-442 from Millipore, Billerica, MA) or anti-HP1γ antibody (ab56978 from Abcam). Cells were then labeled with Alexa Fluor488 donkey anti-Rabbit secondary Ab or Alexa Fluor568 goat anti-Rabbit secondary antibody (Invitrogen, Carlsbad, CA) before treated with anti-fade gold mounting medium with DAPI (Invitrogen, Carlsbad, CA). Images were examined and processed by a Zeiss LSM 700 confocal microscope with total 630X magnification. Representative images are shown. To measure H3K9Me3 level by western blotting, total histone was extracted using HCL following a standard protocol (Abcam) and detected with rabbit anti-Histone H3 (61277, Active Motif) or rabbit anti-Histone H3K9Me3 (ab8898, Abcam) antibody; ratios of H3K9Me3 to total H3 did not correlate with H3K9Me3 focus formation (**Supplementary Fig. 26**).

Colony formation assays. Anchorage-independent growth was assayed with p'mel/BRAFV600E cells (provided by Dr. Hans Widlund, Dana-Farber Cancer Institute). Briefly, 2,500 cells were mixed in HAMF12 plus 10% FBS medium containing 0.4% SeaKem LE agarose from Lonza, and plated on top of the bottom layer with 0.65% agarose prepared in same HAMF12 medium in 6-well plate. After the agarose is solidified, each well was covered with 0.5 mL feeding medium which was refreshed twice a week. Colonies were counted and imaged between 3-4 weeks after seeding under a regular microscope with total magnification of 40X. Colonies were counted from three wells of each condition, and significant difference from the control was assessed by t-test assuming unequal variance.

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

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

Statistical analyses

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

Accession codes

dbGAP Accession: phs000178.v9.p8

dbGaP Accession: phs000424.v6.p1

GEO Accession: GSE60666

DATA AVAILABILITY STATEMENT

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

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