Exome-sequencing of vitiligo lesions indicate lower burden of somatic variations: implications in risk for non-melanoma skin cancers

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Brief Summary

Given the protective nature of skin pigmentation, lower incidence of non-melanoma skin cancer among subjects with acquired depigmenting skin disorder vitiligo is enigmatic and a matter of longstanding debate. To address this, we performed high-coverage exome sequencing of matched non-lesional and lesional vitiligo skin along with whole blood to account for germline variations. Our analysis suggests lower somatic cancer-associated variations in exposed depigmented lesional skin compared to the cognate non-lesional pigmented skin. A detailed investigation of vitiligo skin transcriptome reveals elevation of DNA repair and cell-proliferation pathways, that could be attributed to possible expansion of holoclones in vitiligo. Our data supports the earlier demographic observation on lower risk of non-melanoma skin cancer in vitiligo subjects, providing an opportunity to learn strategies for cancer prevention from vitiligo.

To the Editor,

Genetic depigmentary conditions such as albinism with complete loss of epidermal pigmentation pose a higher risk for cutaneous malignancies (Lekalakala et al. 2015). By analogy, clinical management for photoprotection of the acquired depigmented skin in vitiligo is of serious concern. It is believed that vitiligo would pose a similar, elevated risk (Bae et al. 2020; Hexsel et al. 2009; Kim et al. 2020; Paradisi et al. 2014; Rodrigues 2017; Schallreuter et al. 2002; Teulings et al. 2013; Weng et al. 2021). Systematic evaluation of a large cohort of Caucasian vitiligo subjects indicated a decreased risk for both melanoma and non-melanoma skin cancers (Paradisi et al. 2014). Extrapolating from demographic studies, it is tempting to speculate that vitiligo could negatively influence either initiation or progression of cutaneous malignancies (Rodrigues 2017). Given the autoimmune etiology that targets melanocyte destruction, protection against melanoma could be rationalized, however a similar protection from non-melanoma skin cancer is perplexing. Therefore, these observations need to be substantiated with evidence at the tissue level. Recent advancements in genomics enables to map the somatic variations which would act as molecular correlate for cancer. In normal, seemingly healthy skin deep-sequencing of selected panel of cancer associated genes suggests pervasive positive selection of somatic variations that provides valuable insights into the origin of mutations and map their progression to skin cancers (Martincorena et al. 2015; Zheng et al. 2021).

At the tissue level, mutation burden in oncogenes would prove prognostic to objectively assess the “cancer-risk” and explain observations on demography-based incidence of non-melanoma skin cancers among vitiligo subjects. We based our study on 18 exomes derived from a set of 6 subjects, from whom matched sun-exposed lesional vitiligo skin from arms, unexposed non-lesional skin from the gluteal area and whole blood were procured for genome sequence comparisons **(Figure S1 & Figure S2; Table S1 & Table S2)**. Though we

had a relatively small sample size of six individuals, these were fairly homogenous in terms of body site, and sun exposure including the disuse of sunscreens. The paired nature of comparing lesion with cognate non-lesion provides sufficient statistical power for the study. The burden of somatic variations in cancer associated genes that were unique to skin was estimated after removing the variations observed in the whole blood **(Figure S2)**. Despite being diseased and sun-exposed, pair-wise comparison of vitiligo skin tissues reveal that lesional skin indeed have a lower mutation burden in 5 out of 6 subjects (**Figure 1b; Figure S3 & Figure S4; Table S3**). The difference was observed to be statistically significant based on paired t-test analysis **(Figure 1b)**. We created a circlepack plot depicting the clonal size of the variations in cancer driver genes **(Figure 1c).** We observed smaller clones in lesional tissue, and the clonal representation per gene was also observed to be low **(Table S4).** Lesional skin being depigmented and sun exposed, has UV as an external source that could cause somatic variations, whereas the matched non-lesional skin is pigmented and sun protected. Despite this the anticipated trend of more somatic variations was not observed in vitiligo lesions. Though vitiligo lesions account for only around 20-30% of body surface area on an average, the observed decrease in cancer incidence is seemingly more pronounced from the demography-based incidence data. Since autoimmunity plays a central role in vitiligo pathogenesis, it is likely that autoimmune alterations could have pervasive effects beyond localized lesions. Therefore, it is tempting to speculate that the non-lesional skin may be different from the normal skin of a healthy individual and could contribute towards cancer protection and needs to be systematically investigated. Thereby, our systematic pairwise comparison of the somatic mutation burden strongly supports the demographic observation on lower risk for non-melanoma skin cancers in vitiligo.

Insights into the possible mechanisms that result in this reduced mutation burden would add immense value to our understanding of the initiation and progression of cutaneous

malignancies. Towards this, we performed whole transcriptome sequencing of paired lesion and non-lesional skin tissue from 4 vitiligo subjects to possibly explain the differences in the burden of somatic variations (**Figure S5**). We observed an upregulation of nuclear division, as well as DNA repair response among the top upregulated pathways that could explain these observations **(Figure 2a and Table S5)**. Higher proliferation of skin cells could be attributed to thickened stratum corneum as observed earlier (Gniadecka et al. 1996; Singh et al. 2017). This could facilitate faster turnover of mutant clones and possibly explain the observed lower burden. A concomitant increase in DNA repair pathways strongly suggests a replication coupled repair response. This is discernable in a volcano plot of replication coupled-DNA repair pathway, wherein most of the genes were significantly upregulated in the lesional vitiligo skin **(Figure 2b, Figure S6).**

A recent study provides a foot-print of the three clonogenic pools of epidermal keratinocytes that are responsible for maintenance of the human epidermis. Holoclone-forming cell has hallmarks of stem cells and are bestowed with higher self-renewal capacity as well as their ability to repair DNA damage (Enzo et al. 2021). The set of holoclone signature was majorly found to be elevated in vitiligo lesions compared to non-lesional skin **(Figure 2c)**. All the RNA-sequencing observations could be recapitulated in the previously published microarray data (Singh et al. 2017) (**Figure S7**). Thereby, it is likely that despite the lack of melanin in vitiligo lesions, DNA damage could be efficiently repaired in the expanded holoclone population and account for lower mutation burden due to the augmented DNA repair machinery.

It is intriguing that rewiring of gene expression triggered by the disease, renders a state that offers protection from a more detrimental skin condition. In this regard the lesional vitiligo skin appears to be an enantiostatic state wherein the skin cells rewire and adapt to an alternate steady state (Singh et al. 2017). In future, detailed large-scale comparison that account for age,

skin type, gender, sun-exposure as well as matched samples across subjects including healthy volunteers free of the vitiligo is warranted. Thereby, our initial exploration does indicate towards an altered state in vitiligo, and raises further questions. The underlying mechanisms such as possible role of autoimmunity, as well as alterations in the non-lesional skin compared to the skin from healthy subjects would need to be systematically investigated in greater detail. Elaborate comparisons would identify key actionable players that could be translated not only for cancer prevention, but also to promote desirable mutations in skin cells to revert genodermatoses.

Data availability:

The transcriptomic data generated in this study have been submitted to the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number for RNA sequencing PRJNA554241 & for microarray GSE75819.

Conflict of interest:

RSG is the co-founder of Vyome Biosciences Pvt Ltd., a biopharmaceutical company working in the dermatology area.

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Figure Legends:

Figure 1: Spectrum of somatic variations across vitiligo skin tissues in cancer driver genes (A) Study design for sample collection from vitiligo subjects and identification of somatic variations. (B) Dumbbell plot (with an inset Boxplot) depicting the mutation burden in lesional skin compared to non-lesional across 6 vitiligo patients. (C) Circlepack plot depicting the clonal/subclonal variations across the lesional and non-lesional vitiligo skin. The outer coloured circle (Red & green) represents the tissue type; The white circles depict the cancer driver genes; innermost grey circle depicts variations. The size of the grey circle is proportional to the variant allele fraction (VAF) value in their respective tissue type.

Figure 2: Transcriptomic foot-print of the matched vitiligo skin implicates replication coupled DNA repair (A) Bubble plot depicting enriched processes from upregulated set of genes, based on the whole transcriptome sequencing of lesional wrt non-lesional vitiligo skin. (B) Volcano plot for genes pertaining to replication coupled repair pathway. Here red dot represents a gene to be significantly up in Lesional sample whereas, green dot represents significant downregulation, based a p-value cut off of 0.05 and a Log_2 fold change cut off of 0.5. (C) Bar plot showing the holoclone score contribution in the lesional and non-lesional tissues. Holoclone score is the summation of normalized expression values for the holoclone signature genes across each sample.

Supplementary figure legends

Figure S1: Workflow followed for the whole exome sequencing data analysis: From Fastq file till final VCF generation.

Figure S2: Post VCF filtering steps to obtain high confidence somatic variations.

Figure S3: Distribution of total variations across lesional and non-lesional tissues showing despite non-lesional tissue being sun protected the number of variations were higher compared to the sun exposed and diseased lesional tissue (in 4 out of 6 subjects). (A) Cumulative

distribution of variations in the 2 tissues. (B) Sample-wise distribution of variations across 6 vitiligo subjects.

Figure S4: Rank order plot depicting the trend of variation burden across the three cutaneous malignancies, healthy skin and vitiligo lesion and non-lesional skin samples. The mutation burden was estimated in the skin cancers across the entire genome sequenced whereas for the healthy skin and vitiligo sample the mutation burden was calculated for the cancer driver genes only.

Figure S5: Workflow followed for the transcriptome sequencing data analysis: From Fastq file till final normalized gene expression values.

Figure S6: Increased DNA damage & repair in lesional tissues via replication coupled repair. **(A)** Heatmap showing the expression pattern of DNA damage genes in vitiligo samples based on RNA sequencing. The green-red color represents the normalized expression values and the yellow-blue annotation bar is the lesional vs non-lesional fold change for that gene. **(B)** Volcano plot for genes pertaining to replication coupled repair pathways in vitiligo RNA sequencing dataset. Here red dot represents a gene to be significantly up in lesional sample whereas, green dot represents significant downregulation. **(C) & (D)** in vitiligo microarray samples.

Figure S7: Mapping of holoclone signature genes in vitiligo datasets. **(A) & (B)** Volcano plot showing the status of holoclone signatures in vitiligo RNA-sequencing and microarray datasets. **(C & D)** Heatmap showing that holoclone signatures are sufficient to segregate the lesional and non-lesional tissues in RNA-sequencing and microarray datasets.

Supplementary_Table_1: Meta-data for exome and transcriptome vitiligo samples.

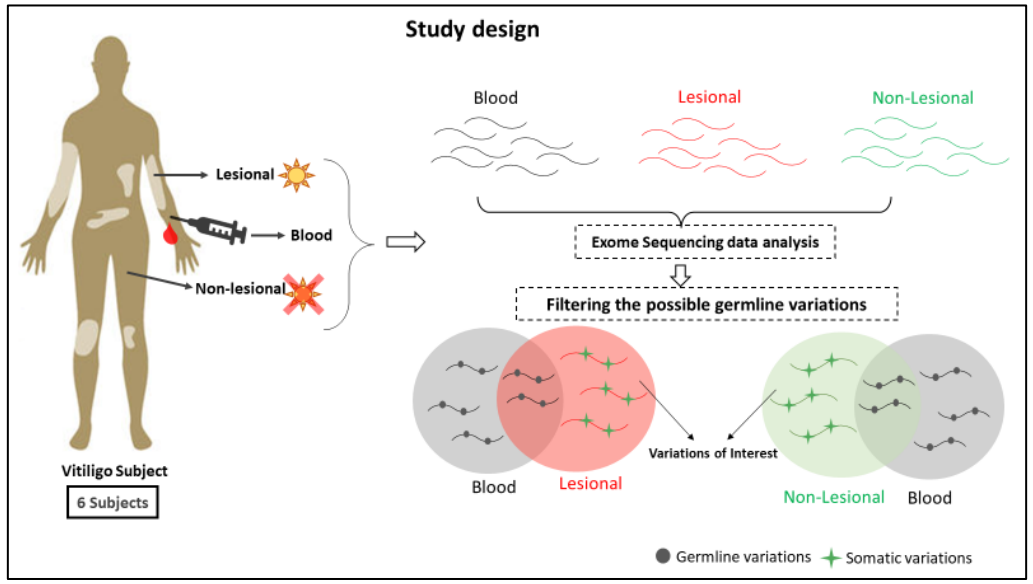
Supplementary_Table_2: Per tissue wise read count information for vitiligo exome samples.

Supplementary_Table_3: Mutation burden data for all cutaneous malignancies and healthy skin.

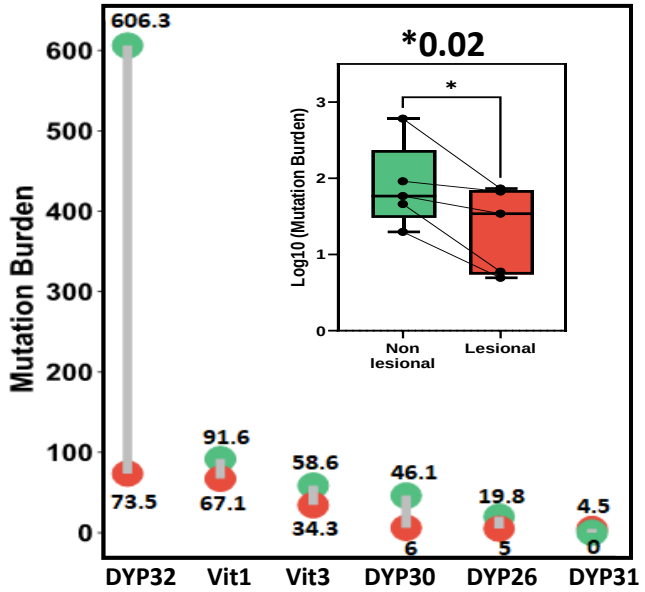
Supplementary_Table_4: Variations mapping to cancer driver genes for vitiligo samples.

Supplementary_Table_5: Up/down regulated pathways based on ToppGene suite from vitiligo transcriptomic dataset.

a


Study design
Lesional
Blood
Non-lesional
Vitiligo Subject
6 Subjects
Blood
Lesional
Non-Lesional
Exome Sequencing data analysis
Filtering the possible germline variations
Variations of Interest
Blood
Lesional
Non-Lesional
Blood
Germline variations
Somatic variations

b


*0.02
Mutation Burden
606.3
73.5
91.6
67.1
58.6
34.3
46.1
6
19.8
5
4.5
0
DYP32
Vit1
Vit3
DYP30
DYP26
DYP31
Log10 (Mutation Burden)
Non lesional
Lesional

c

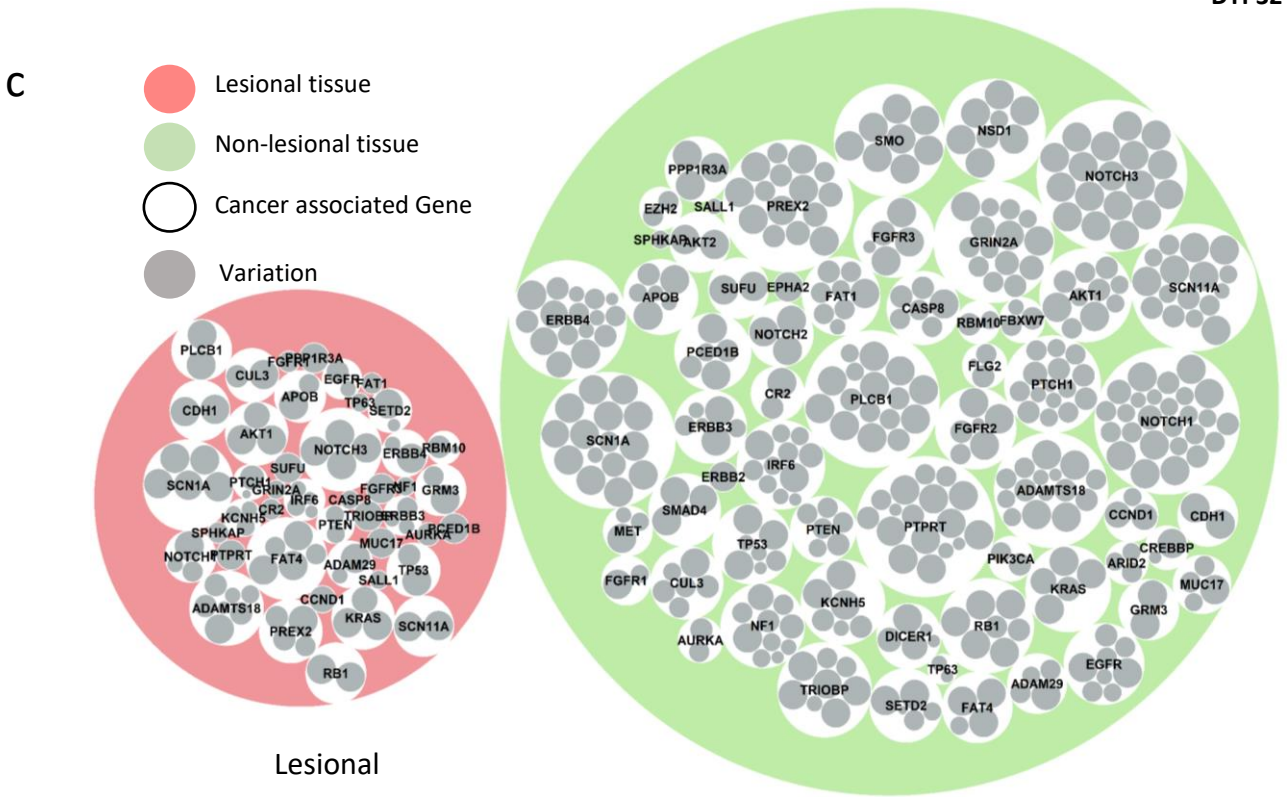

Lesional tissue
Non-lesional tissue
Cancer associated Gene
Variation
Lesional
Non-Lesional

Fig 1

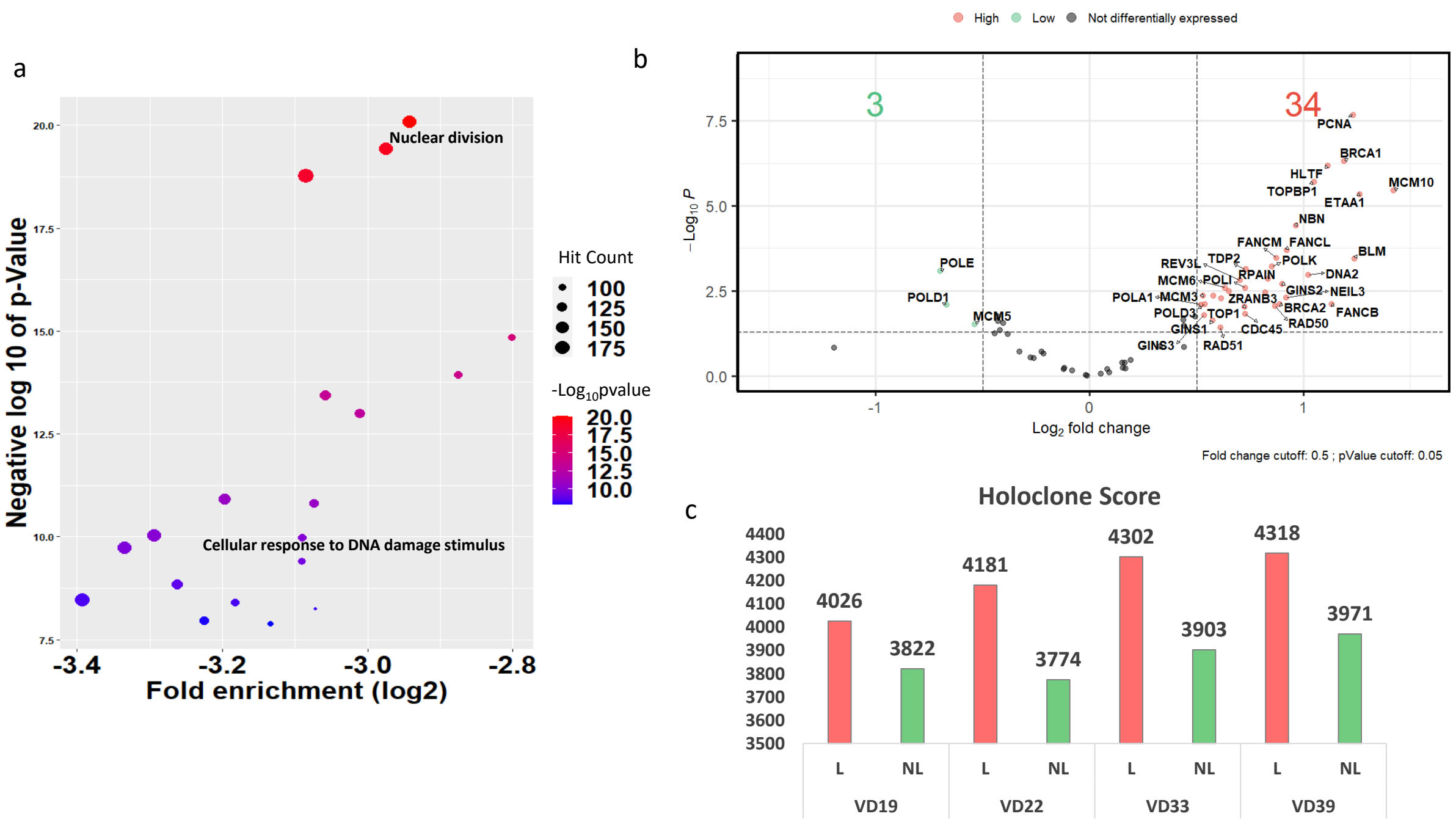

a
Nuclear division
Cellular response to DNA damage stimulus
Negative log 10 of p-Value
Fold enrichment (log2)
Hit Count
100
125
150
175
-Log10pvalue
20.0
17.5
15.0
12.5
10.0
b
High
Low
Not differentially expressed
3
34
PCNA
BRCA1
HLTF
TOPBP1
MCM10
ETAA1
NBN
FANCM
FANCL
BLM
POLK
TDP2
REV3L
RPAIN
DNA2
MCM6
POLI
GINS2
NEIL3
POLA1
MCM3
ZRANB3
BRCA2
FANCB
POLD3
TOP1
RAD50
GINS1
CDC45
GINS3
RAD51
POLE
POLD1
MCM5
−Log10 P
Log2 fold change
Fold change cutoff: 0.5 ; pValue cutoff: 0.05
c
Holoclone Score
4026
3822
4181
3774
4302
3903
4318
3971
L
NL
VD19
VD22
VD33
VD39

Fig 2