

Supplementary Information

Interleukin 32 exacerbates vitiligo by promoting inflammation and oxidative stress-induced injury in melanocytes

Authors: Tianqi Wei¹, Xin Huang¹, Jing Zhu¹, Guanying Liu¹, Baiyi Zuo¹, Zhaokang Zhou¹, Beilei Xu¹, Lingling Luo¹, and Chengrang Li^{1,2*}

Affiliation:

1 Hospital for Skin Diseases, Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College, Nanjing, China

2 Jiangsu Provincial Key Laboratory of Dermatology, Nanjing, China

* **Correspondence:** Chengrang Li, Hospital for Skin Diseases, Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College; Jiangsu Provincial Key Laboratory of Dermatology

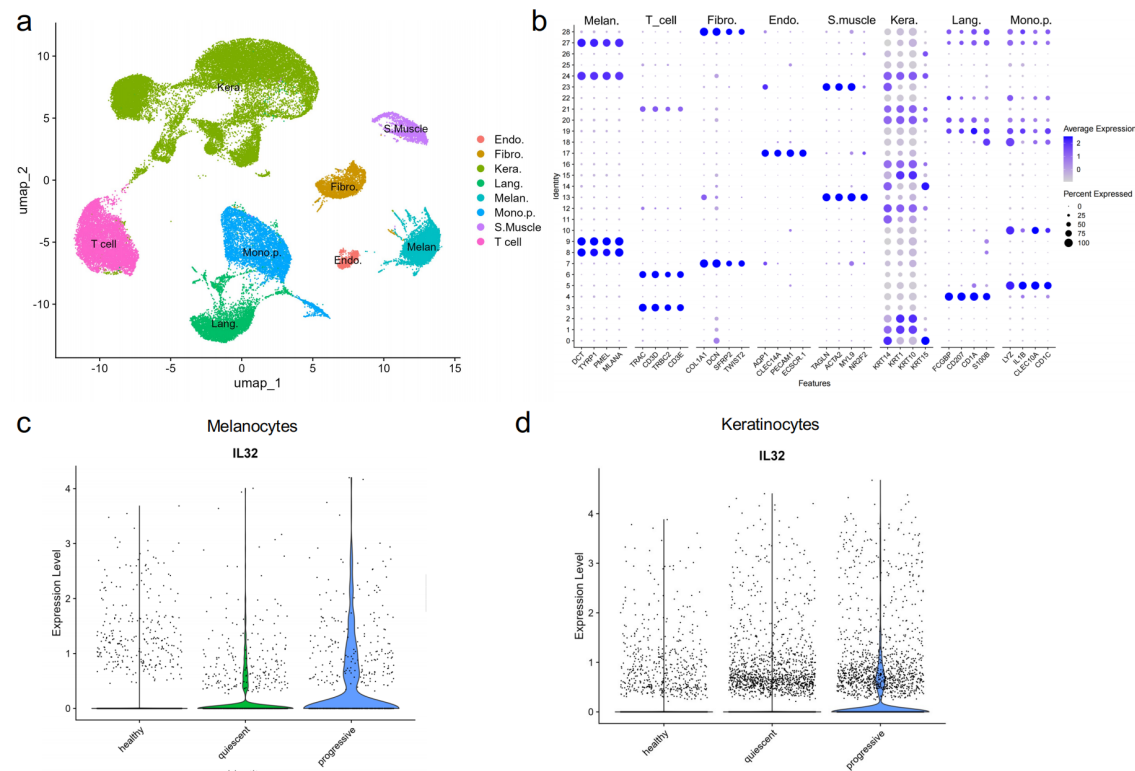
Email: dr_lcr72@163.com

Address: 12 Jiangwangmiao Street, Nanjing, Jiangsu, 210042, China

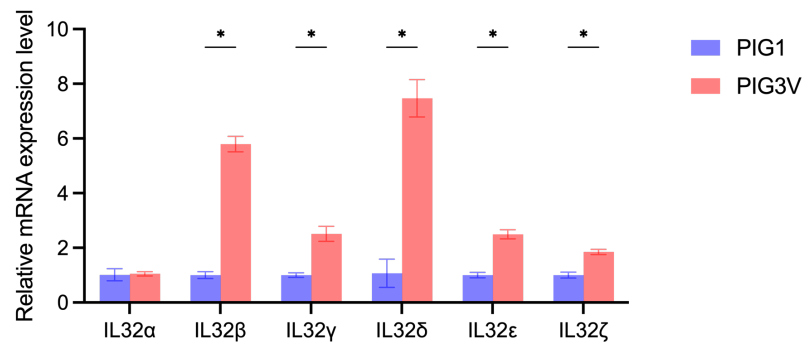
ORCID

Tianqi Wei <https://orcid.org/0000-0003-3405-9580>

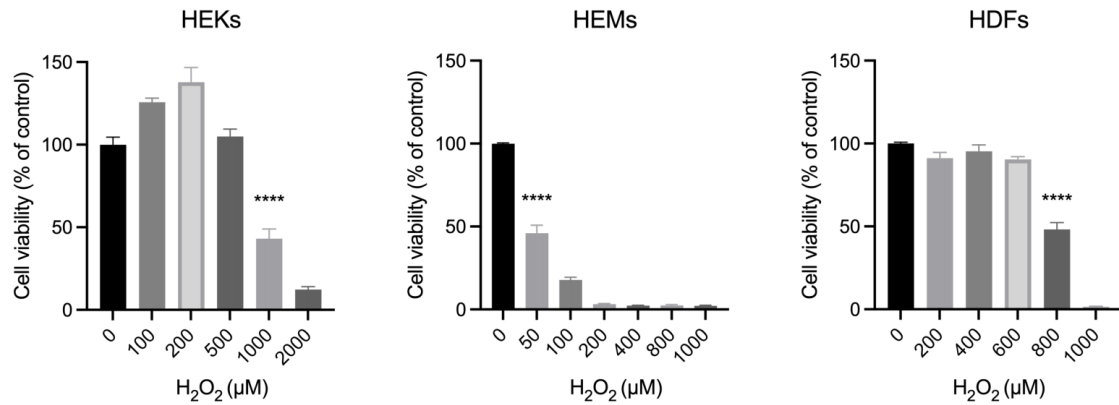
Supplementary Figure S1 Single-cell RNA-seq profiling of skin cells from vitiligo and healthy control samples. (a) UMAP plot of all cells after quality control and unsupervised clustering. Each dot represents a single cell, colored according to the assigned cell type. A total of eight major cell populations were identified: keratinocytes (Kera.), dermal fibroblasts (Fibro.), endothelial cells (Endo.), melanocytes (Melan.), smooth muscle cells (S. Muscle), monocyte/macrophages (Mono. p.), T cells, and Langerhans cells (Lang.). (b) Feature plots showing marker genes used for annotation in each cell type. (c-d) Violin plot of IL32 expression in the melanocytes (Melan.) and keratinocytes (Kera.) of healthy controls and different stages of vitiligo patients. Green represents stable vitiligo and blue represents progressive vitiligo.



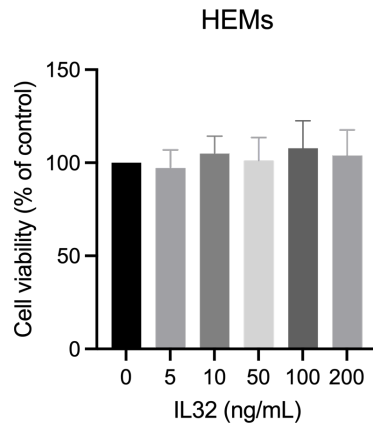
Supplementary Figure S2 Expression level of different *IL32* isoforms in PIG1 and PIG3V cells. Blue bars represent PIG1 cells and red bars represent PIG3V cells. Data were presented as mean $\pm$ SD. * $P < 0.05$. (two-way ANOVA).



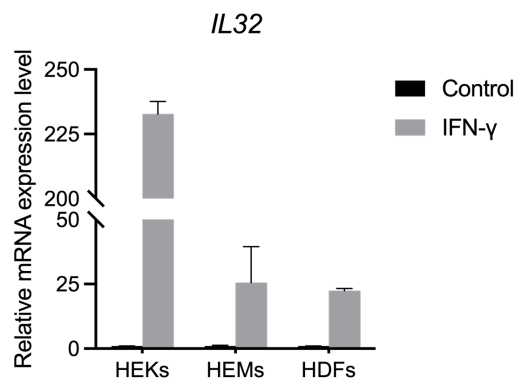
Supplementary Figure S3 Cell viability of different cells treated with H₂O₂ for 24h. Bar plots with different shades of gray represent cell viability of HEKs, HEMs, and HDFs after being treated with different concentrations of H₂O₂. Data were presented as mean $\pm$ SD. **** P < 0.0001. (one-way ANOVA).



Supplementary Figure S4 Cell viability of HEMs treated with rhIL32 β for 24h. Different shades of gray represent cell viabilities of HEMs under different rhIL32 β concentrations. There was no significant change (P > 0.05). Data were presented as mean $\pm$ SD. (one-way ANOVA).



Supplementary Figure S5 RT-qPCR analysis of IL32 in HEKs, HEMs, and HDFs treated with 20 ng/mL of IFN- γ for 24h compared to the control group.



Supplementary Table S1 Knockdown sequences for *IL32*.

small interfering RNA		
siIL32-1	sense	GAAUGCACCAGGCCAUAGATT
	antisense	UCUAUGGCCUGGUGCAUUCTT
siIL32-2	sense	GCUCACUCCUCUACUUGAATT
	antisense	UUCAAGUAGAGGAGUGAGCTT

Supplementary Table S2 Primer sequences used for qPCR.

Gene name		
<i>β-actin</i>	F	CCATCGTCCACCGCAAAT
	R	GCTGTCACCTTCACCGTTCC
<i>GAPDH</i>	F	TGCACCACCAACTGCTTA
	R	GGCATGGACTGTGGTCAT
<i>CXCL9</i>	F	CCAGTAGTGAGAAAGGGTCGC
	R	AGGGCTTGGGGCAAATTGTT
<i>CXCL10</i>	F	GTGGCATTCAAGGAGTACCTC
	R	TGATGGCCTTCGATTCTGGATT
<i>IL-32</i>	F	GACAGTGGCGGCTTATTATGAG
	R	CCTCGGCACCGTAATCCAT
<i>IL-32α</i>	F	GACAGTGGCGGCTTATTATGAG
	R	GCTCCGTAGGACTTGTCAAAA
<i>IL-32β</i>	F	GAAGACTGCGTGCAGAAGGT
	R	CTTTCTATGGCCTGGTGCAT
<i>IL-32γ</i>	F	AGGCCCGAATGGTAATGCT
	R	CCACAGTGTCTCAGTGTCAACA
<i>IL-32ζ</i>	F	GACGTGGACAGGACGACTTCA
	R	CCTCGGCACCGTAATCCAT
<i>IL-32ϵ</i>	F	AGGCCCGAATGGTGATGT
	R	GGCACCGTAATCCATCTCTT
<i>IL-32δ</i>	F	TCTGTCTCTCTCGGGTCCTCTCT
	R	TGTCTCCAGGTAGCCCTCTTTG