

INTRODUCTION

Vitronectin (VTN) is a multifunctional glycoprotein regulating lung repair and immune homeostasis¹. Our previous studies established VTN as a key biomarker for radiation pneumonitis (RP) and confirmed that the rs704 (T400M) polymorphism increases RP susceptibility². This study further explored the molecular mechanism by which this VTN mutant exacerbates radiation-induced lung inflammation and fibrosis.

METHODS

To uncover the mechanistic basis for T400M-mediated radiation pneumonitis exacerbation, we established stable VTN-WT and VTN-T400M overexpressing human lung fibroblast lines via lentiviral transduction. Following 8 Gy irradiation, dynamic expression of fibrotic markers and inflammatory cytokine IL-6 and TGF- β was validated by qPCR, Western blot and ELISA. IP-MS was further performed to identify differential interacting proteins, revealing key molecular regulators driving mutant-specific pro-fibrotic and pro-inflammatory responses.

RESULTS

The VTN T400M mutation significantly increases the expression of radiation damage biomarkers.

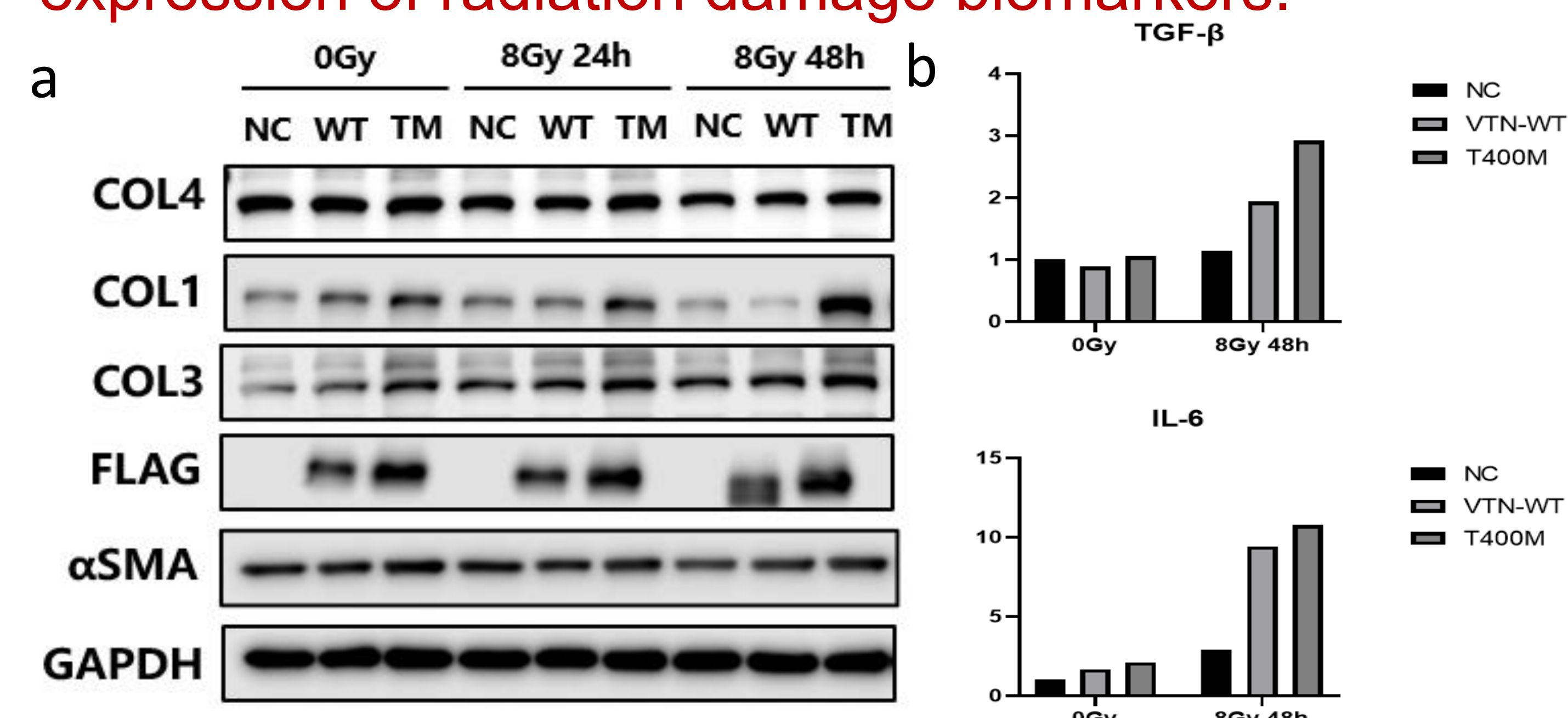


Fig.1 (a) WB analysis of expression of fibrotic markers in irradiated HFL1 cells across groups. (b) RT-qPCR analysis of IL-6 and TGF- β expression following irradiation

Co-IP analyses revealed robust specific interaction between NDUFA9 and wild-type VTN, with nearly abolished binding to mutant VTN.

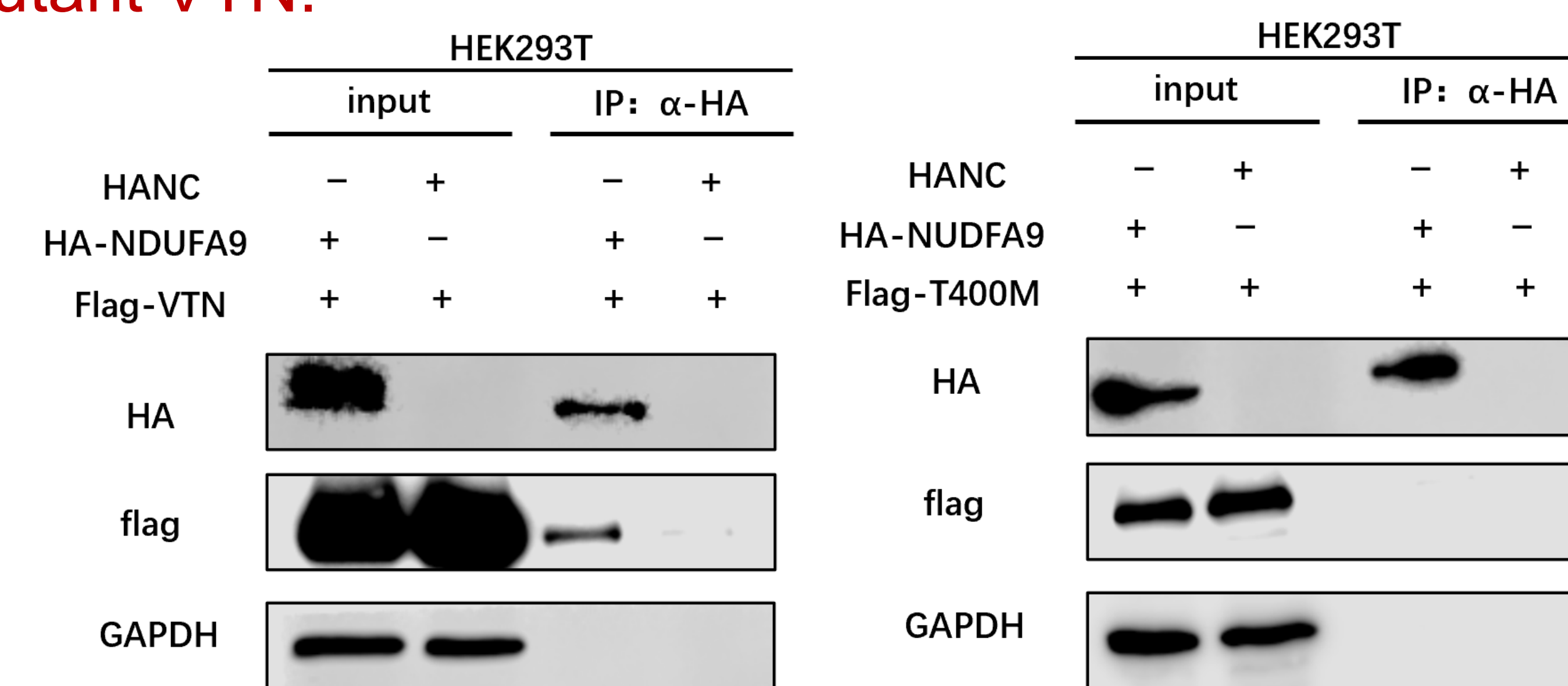


Fig.3 Co-IP validation of interactions between wild-type/mutant VTN and NDUFA9

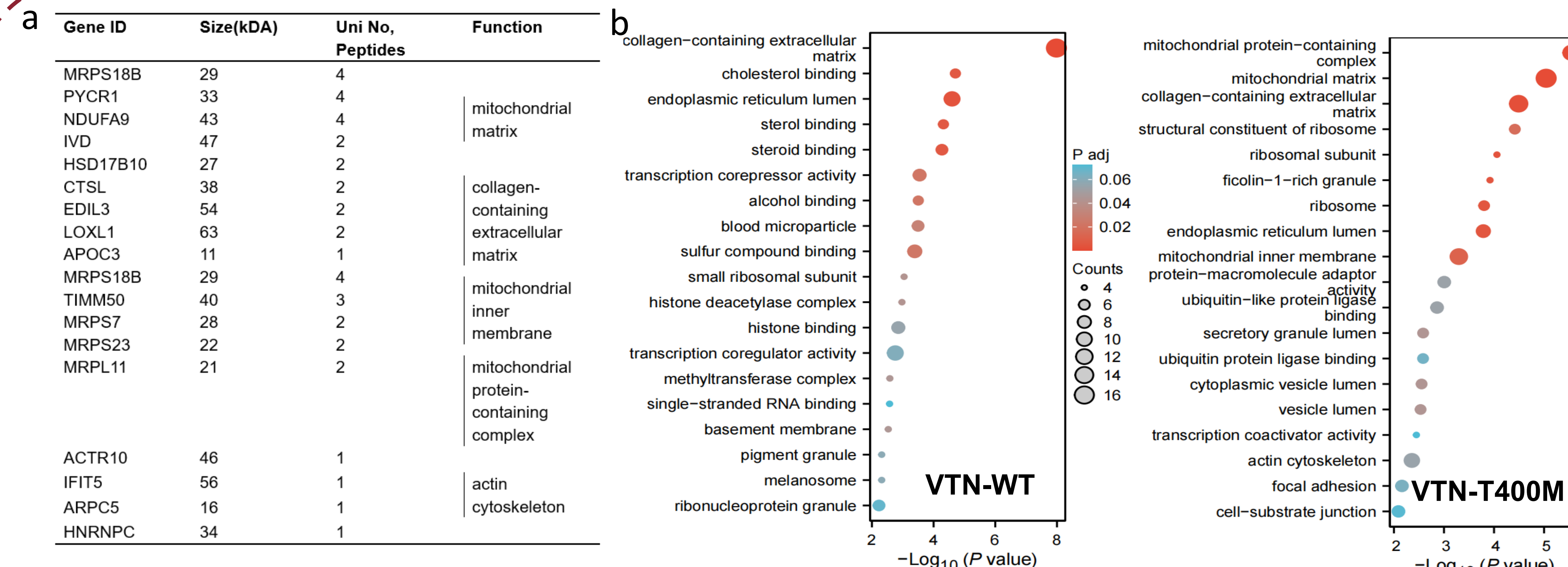


Fig.2 (a) IP-MS analysis of major enriched proteins. (b) KEGG pathway enrichment analysis based on IP-MS data

IP-MS revealed divergent interactome profiles for wild-type and mutant VTN in pulmonary fibroblasts. Mutant VTN interactors showed marked enrichment in extracellular matrix organization, fibrosis, and integrin signaling pathways.

CONCLUSION

The VTN T400M polymorphism drives pro-fibrotic and pro-inflammatory phenotypes in irradiated lung fibroblasts. Mechanistically, this variant disrupts VTN-NDUFA9 binding, triggering aberrant mitochondrial localization of NDUFA9, ROS accumulation, and downstream activation of NF- κ B/TGF- β 1 signaling.

REFERENCES & ACKNOWLEDGEMENTS

REFERENCES

- Preissner KT, Reuning U. Vitronectin in vascular context: facets of a multitasking matricellular protein. *Semin Thromb Hemost*. 2011 Jun;37(4):408-24
- Jiahua Yu; Qingya Zhao; Yuan Liu; Xueru Zhu; Zhangru Yang; Xiaolong Fu; Xuwei Cai; The Plasma Levels and Polymorphisms of Vitronectin Predict Radiation Pneumonitis in Patients With Lung Cancer Receiving Thoracic Radiation Therapy. *Int J Radiat Oncol Biol Phys*. 2021, 110(3): 757-765

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No.82273575)