Background The accumulation of depolarized mitochondria in tumor-infiltrating T lymphocytes (TILs) has been shown to orchestrate transcriptomic and epigenetic reprogramming for exhaustion. However, the underlying mechanisms by which the accumulation of dysfunctional mitochondria orchestrate T cell exhaustion remain unclear.

Methods First, a degron-mCherry reporter and a regulatory heme (RH) detection assay were developed to examine protein degradation. Second, the correlation between RH and CD8+ T cell differentiation was investigated by detecting RH concentration and BACH2 and BLIMP1 expression in exhausted CD8+ T cells. Third, hemin, a heme mimic, was used to assess the effect on CD8+ T cell exhaustion. Furthermore, the heme-binding site mutated BACH2 (BACH2MUT) was utilized to explore the mechanism of heme-induced exhaustion. In addition, PGRMC2, responsible for heme import into the nucleus, was knocked out to verify the impact of heme cellular distribution on driving TILs' exhaustion.

Results We found that terminally exhausted T cells elevated gene signature involved in proteasome degradation. In support of this, our results revealed that T cells accumulating depolarized mitochondria displayed an elevated mitochondrial protein degradation, which result in increased abundance of RH due to the degradation of hemoproteins. Interestingly, since RH concentration is positively correlated with the exhaustion severity and the decline of stemness in CD8+ T cells within the tumor microenvironment, we then speculated the elevation of RH can orchestrate commitment of T cell exhaustion. Indeed, exposure of exogenous heme during the in vitro repetitive stimulation facilitated commitment of T cell exhaustion and induced alterations in transcriptomic networks linking to T cell exhaustion. Mechanistically, RH destabilized BACH2 protein and abolished BACH2 DNA-binding ability, which resulted in increased BLIMP1 expression for driving T cell exhaustion. By overexpressing BACH2MUT rather than wild type BACH2, we found that CD8+ T cells sustained the differentiation of stem-like CD8+ T cells and maintained functional capacity on producing effector molecules. In addition, knocking out PGRMC2 ameliorated CD8+ T cell exhaustion and enhanced mitochondrial fitness of tumor-infiltrating CD8+ T cells.

Conclusions Our work unveils the unexplored mechanism, mitochondrial protein degradation and heme-mediated transcriptional network, on orchestrating T cell exhaustion in response to the accumulation of depolarized mitochondria in CD8+ T cells. We further highlight that manipulating heme signaling axis can be exploited to restore mitochondrial fitness and tailor T cell-based cellular therapies.

Ethics Approval Mouse strains were maintained in the SPF animal facility of the University of Lausanne. Health status was checked every 3 months following FELASA guidelines. Animal experiments were conducted in accordance with protocols approved by the veterinary authorities of the Canton de Vaud (VD3765).