Background The functional state of tumor infiltrating lymphocytes (TIL) is a critical determinant of antitumor immunity and response to immunotherapy. One of the key factors responsible for T cell dysfunction are metabolic barriers such as nutrient competition, low oxygen tension, and damaging byproducts in the tumor microenvironment. One of these metabolic barriers is the accumulation of oxidative stress, a critical contributor of T cell dysfunction observed during aging as well as in the tumor microenvironment. We and others have shown that accumulation of reactive oxygen species (ROS) accumulation drives T cell exhaustion. ROS can affect many cellular functions, but notably can induce DNA damage. We asked whether TIL accumulate DNA damage at telomeres as a consequence of terminal differentiation, and used a chemo-optogenetic approach to specifically induce telomeric DNA damage to explore its role in T cell biology, especially within the context of cancer.

Methods In this study we perform telomeric and centromeric FISH assays to analyze TIL for DNA damage accumulation. We used a chemo-optogenetic FAPS-TAPS to generate singlet oxygen and consequent 8-oxo-guanine lesions specifically at telomeres.1 We tethered GPX1 to TRF1 to generate a telomere-guided antioxidant protein.

Results Telo-FISH analysis demonstrated an accumulation of telomeric DNA damage, but not telomere shortening, in exhausted TIL from B16 mouse tumors, coordinate with presence of 53BP1 and gammaH2AX. Our data show that specific induction of mitochondrial and telomeric ROS cause the accumulation of DNA damage at telomeres, and consequent development of telomere fragility. These cells ultimately become dysfunctional showing a diminished capability for cytokine production. Importantly, targeting the ROS scavenger GPX1 directly to telomeres reduced telomere fragility and improved the function of therapeutic T cells in the B16 melanoma model.

Conclusions Our data suggest that dysfunctional T cells in cancer are not classically senescent, bearing short telomeres, but rather harbor damaged telomeres due to exposure to oxidative stress. Telomeric damage is sufficient to drive a dysfunctional state in newly activated T cells. Protecting telomeres through expression of a telomere-targeted antioxidant protein may preserve T cell function in the tumor microenvironment and drive superior responses to immunotherapy.

REFERENCE