protocols used to expand T cells often exhibit suboptimal tumor control. Failure in these therapies has been attributed to premature differentiation and impaired metabolism of the infused T cells. Previous work done in our lab showed that reduced PI3Kα signaling improved ACT. Because PI3Kγ and PI3Kδ have critical regulatory roles in T cell differentiation and function, we tested whether inhibiting PI3Kγ could recapitulate or synergize PI3Kδ blockade.

**Methods** To test this, we primed melanoma specific CD8+ pmel-1 T cells, which are specific to the glycoprotein 100 epitope, in the presence of PI3Kγ (PI-459), PI3Kδ (CAL101 or TGR-1202) or PI3Kγδ (PI-455) inhibitors following antigen stimulation with hgp100, and then infused them into 5Gy total body irradiated B16F10 tumor bearing mice. We characterized the phenotype of the transferred product by flow cytometry and then assessed their tumor control by measuring the tumor area every other day with clippers. For metabolic assays we utilized the 2-NBDG glucose uptake dye and the real time energy flux analysis by Seahorse.

**Results** Sole inhibition of PI3Kδ or PI3Kγ in vitro promoted greater tumor immunity and survival compared to dual inhibition. To understand how PI3Kδ or PI3Kγ blockade improved T cell therapy, we assessed their phenotype. CAL101 treatment produced more CD62LhiCD44lo T cells compared to IPI-459, while TGR-1202 enriched mostly CD62LhiCD44hi T cells. Because decreased T cell differentiation is associated with mitochondrial metabolism, we focused on CAL101 treated T cells to study their metabolism. We found that CAL101 decreased glucose uptake and increased mitochondrial respiration in vitro, indicating augmented mitochondrial function.

**Conclusions** These findings indicate that blocking PI3Kδ or PI3Kγ is sufficient to mediate lasting tumor immunity of adoptively transferred T cells by preventing premature differentiation and improving mitochondrial fitness. Our data suggest that addition of CAL101 to ACT expansion protocols could greatly improve T cell therapies for solid tumors by preventing T cell differentiation and improving mitochondrial function.