Background
Pancreatic ductal adenocarcinoma (PDAC) features a dense, fibrotic stroma that obstructs infiltration of immune cells. Our group investigates methods to improve immunotherapy efficacy in PDAC by targeting and manipulating the PDAC stroma. We hypothesize that dysregulated CD26/DPP4 enzymatic activity in PDAC contributes to constrained efficacy of immunotherapy. In addition, we postulate that in vivo pharmacological targeting of CD26 enzyme activity in PDAC, via inhibitors that are FDA-approved for adult patients with Type 2 Diabetes Mellitus, can influence immune cell infiltration and enhance responses to immune checkpoint blockade in murine models of PDAC.

Methods
CD26 protein expression was evaluated in immortalized CAFs and PDAC cells by immunoblot and flow cytometry, while soluble CD26 was measured in supernatants by ELISA. In vivo studies used immune-competent C57BL/6 mice orthotopically implanted with syngeneic luciferase-expressing KPC-tumor cells into the pancreas. Tumor establishment was verified by bioluminescence imaging (BLI). Mice were randomized to the following treatment groups: sitagliptin (75mg/kg in drinking water, CD26/DPP4 inhibitor), anti-PD-L1 Ab (200 ug 3x/week), or both combined for 9 days. Controls received vehicle and/or isotype control Ab. BLI monitored tumor growth throughout the study. Tumors were harvested and weighed at study endpoint (day 9 of treatment) and immune cell infiltration was evaluated by flow cytometry. An additional in vivo study was performed to assess differences in efficacy of concurrent versus consecutive administration (2 weeks) of sitagliptin (75mg/kg) and anti-PD-L1 Ab by measuring BLI and pancreas weight at study endpoint.

Results
CD26 protein expression was observed in both immortalized human CAFs (HT137 and h-iPSC-PDAC-1) and PDAC cells (HPAC and PANC1) through immunoblot and flow cytometry, however soluble CD26 was only detected in CAF supernatants. Concurrent administration of sitagliptin and anti-PD-L1 Ab limited tumor progression and increased tumor infiltrating CD4+ T cells (p=.0302, p=.0208 vs isotype control and anti-PD-L1, respectively), CD8+ T cells (p=.0336 vs anti-PD-L1), and macrophages compared to vehicle, sitagliptin, and anti-PD-L1 Ab alone. Further studies showed no significant difference in efficacy between concurrent and consecutive administration of sitagliptin together with anti-PD-L1 Ab.

Conclusions
Our results reveal that CD26 enzyme inhibition (sitagliptin), augments anti-tumor activity of anti-PD-L1 Ab in PDAC tumor-bearing mice. This work identifies immune cell populations, including T cells and macrophages, that provide insight into mechanisms of efficacy in orthotopic murine models of PDAC. Continued investigations will evaluate the versatility of CD26 inhibition and its capacity to modulate immune checkpoint molecules, thus, broadening efficacy studies across other targets with relevance to PDAC.