Background: Adipocyte hypertrophy is associated with glucose and lipid dysmetabolism, ectopic fat accumulation, and metabolic complications independently of the level of obesity [1]. In this study, we aimed to explore potential mechanisms driving the obesity-independent, hypertrophy-associated dysmetabolism at a single-cell resolution and to assess the applicability of single nuclei RNA-sequencing of flash-frozen human subcutaneous adipose tissue (AT) to probe for these metabolic differences.

Methods: In 40 healthy participants, peri-umbilical subcutaneous adipose tissue biopsies were collected by needle aspiration to determine adipocyte size. Tissue uptake of dietary fat was quantified via oral 18-fluoro-thiaheptadecanoic acid administration with positron emission tomography (PET) [2]. Rates of fatty acid oxidation and esterification were measured using intravenous [11C]-palmitate with PET and multi-compartmental modeling. PET/CT segmentation using deep convolutional neural networks was done with DeepImageTranslator [3,4]. Single nuclei RNA-sequencing was performed in two individuals with AT hypertrophy vs. hyperplasia but matched for the same sex, ethnicity, glucose-tolerance status, BMI, total and percent body fat, and waist circumference. Nuclei were extracted from frozen AT and purified by flow cytometry following a protocol that we have developed and validated. Capture of single nuclei and preparation of RNA libraries were done with 10X Genomics technology. Results: Unsupervised clustering identified a number of cell-type markers identical to those previously reported in murine epididymal AT and human AT. We found reduced quantities of fibroadipogenic cells per volume of AT with fat cell hypertrophy. Furthermore, the fraction of DPP4+ fibroblasts is increased with AT hypertrophy. Analyses of RNA velocity shows decreased velocity of adipocyte transformation from a transcriptionally active (TADA) phenotype to mature adipocytes in AT hypertrophy. Furthermore, in AT hypertrophy, adipocytes from the early TADA stage are destined to become insulin-resistant adipocytes, whereas those in AT hyperplasia are developing into an insulin-sensitive phenotype. Metabolic imaging results show that hypertrophied subcutaneous AT is associated with lower subcutaneous and higher visceral AT volume and dietary fatty acid storage capacity.