Glucose and lactate isotope labeling

For glucose or lactate labeling (PMID: 29657327)(PMID: 35387341), glucose-free RPMI medium was supplemented with 10% dialyzed FBS and U^{13}C-glucose (2 g/liter; Cambridge Isotope Laboratories) or U^{13}C-lactate (2 g/liter; Cambridge Isotope Laboratories). All the organoids (50 organoids per condition) were washed with blank SILAC before being reconstituted in ^{13}C medium at a concentration of 10 eye organoids/ml The incubation was performed from 15 minutes to 2 hours for time-course analysis of the glucose flux. For the analysis in Fig. 4G, LDH inhibitor (GNE-140) treatment was done half an hour prior to the glucose or lactate labeling experiments (^{13}C labeling time was 30 minutes). The eye organoids were collected and washed twice with PBS before pellets were flash-frozen and stored at −80°C until metabolite extraction. Metabolite analysis was performed as described previously (PMID: 33931446). Briefly, for metabolite extraction, 80% methanol was used followed by the rapid freeze-thaw method to break the tissues. The supernatant underwent speedvac drying. The samples were prepared in 80% acetonitrile and were analyzed by High-Resolution Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). To control for the same concentration of protein/metabolites used as input for LC-MS/MS, we performed a series of controls as follows: 1. the same number of organoids are used for metabolite extraction; 2. when metabolite levels were analyzed, all data were normalized to total ion count/chromatogram (TIC)(PMID: 27718276); 3. When labeling data was analyzed, all isotopic enrichment for each metabolite were calculated against the total pool of unlabeled for each metabolite. For analyzing ^{13}C-glucose labeling, the standard % enrichment (fractional enrichment displayed as percentages) was calculated using the normalized peak area of each metabolite's isotopologue over its total pool as described in (PMID: 25731751). All data including TIC and unlabeled pools for each metabolite is available upon request.