Metabolic trajectory of cellular differentiation in small intestine by Phasor Fluorescence Lifetime Microscopy of NADH

Chiara Stringari [1], Robert Edwards[2], Kira Pate[3], Marian Waterman[3], Peter J. Donovan [4,5] and Enrico Gratton[1]

Supplementary material

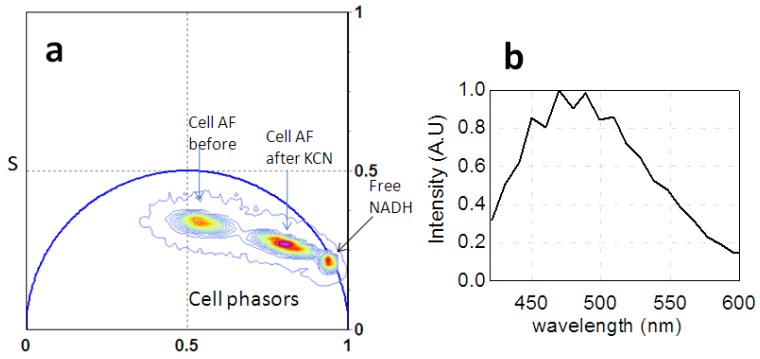


Figure SM1. NADH is the major intrinsic source in epithelial cells.

(a) FLIM phasor plot of DLD-1 colon cancer epithelial cells, excited at 740nm, before and after treatment with potassium cyanide (KCN). By blocking the respiratory chain the FLIM phasor distribution of cells shifts toward the location of the free reduced NADH **(b)** Emission spectrum measured in the epithelial cells within the round crypt in the small intestine excited with 740nm.

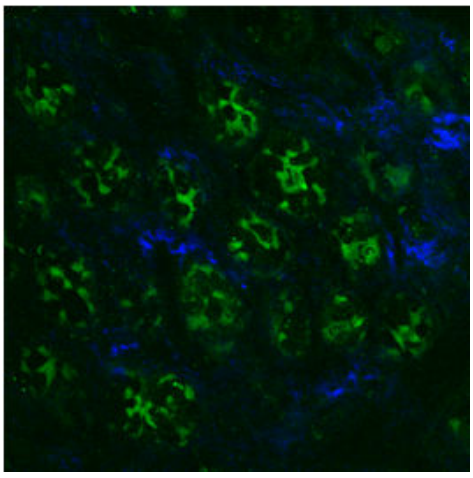


Fig.SM2 Two-photon emission (Green) and Second Harmonic Generation (Blue) images excited at 880 nm of the same field of view of the FLIM image of Fig. 1a and Fig 1c.

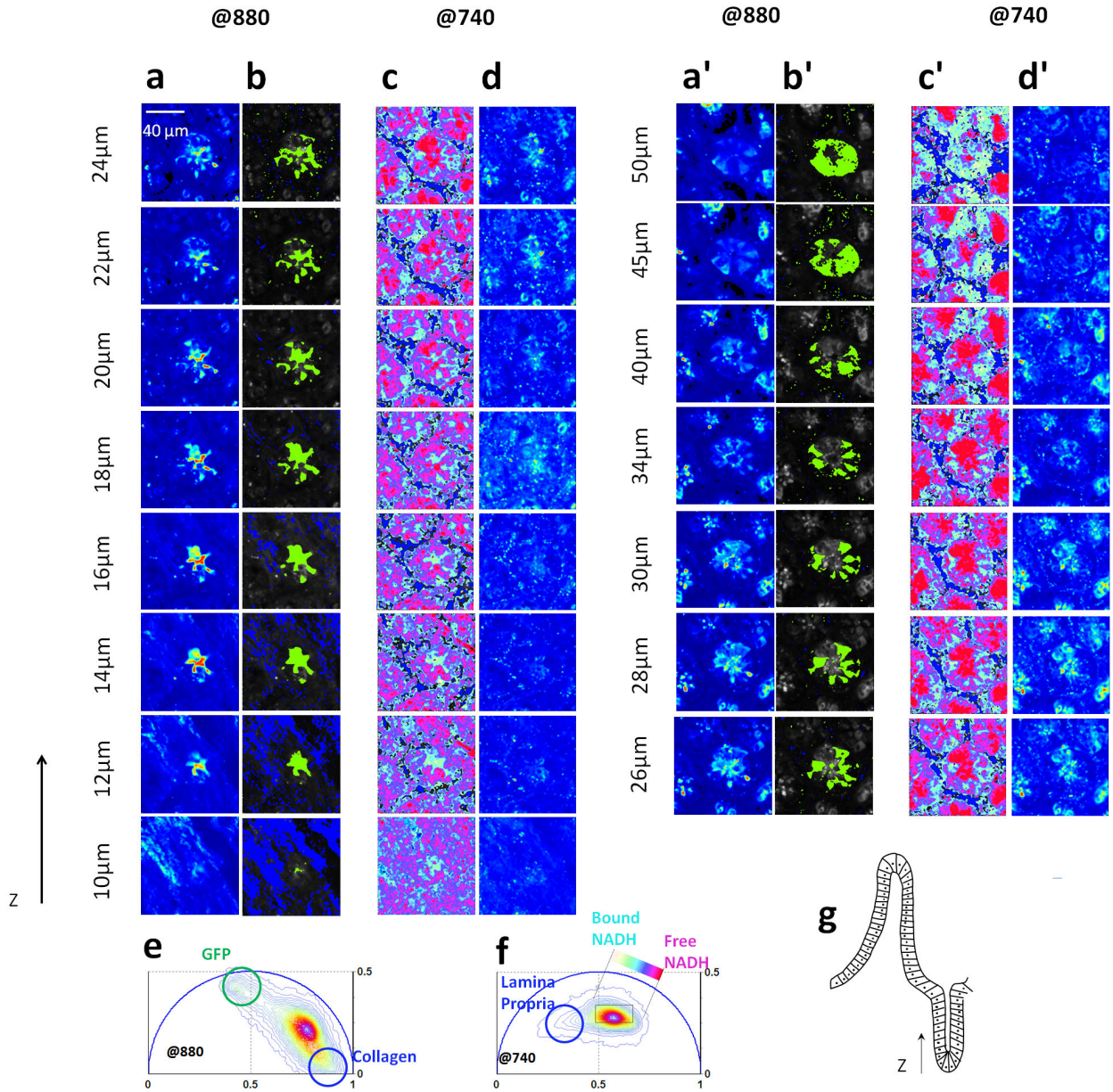
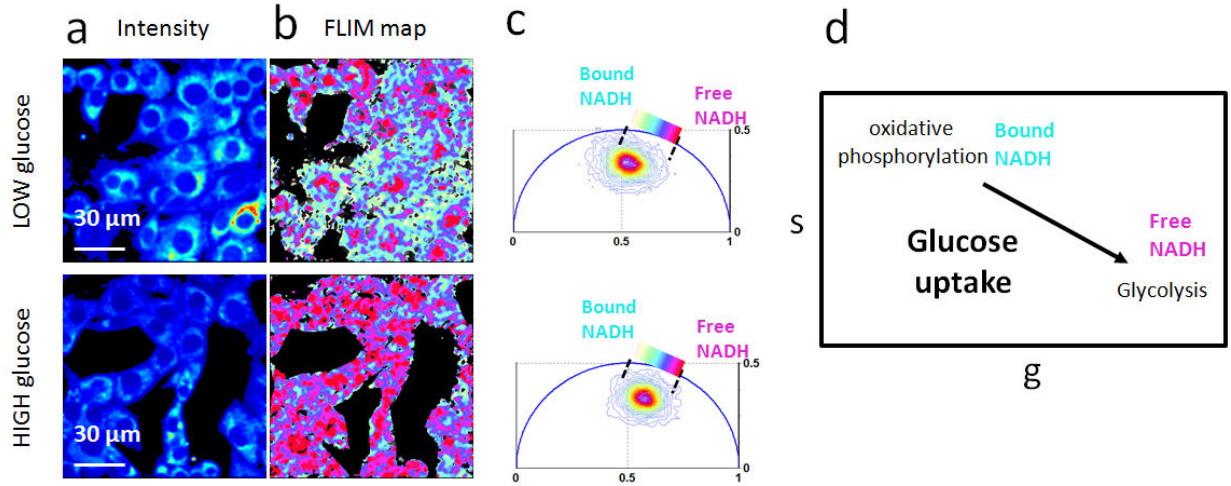


Fig SM3 Three-dimensional reconstruction of the small intestine crypt

Two-photon fluorescence intensity images excited at 880nm **(a-a`)** and 740nm **(d-d`)** of the small intestine from a Lgr5-GFP mouse that expresses GFP in the stem cells. **(b-b`)** Phasor color map at 880nm highlights GFP (green) stem cells and collagen (blue). **(c-c`)** Phasor color maps at 740nm of the relative concentrations of free NADH and bound NADH and porphyrin (blue) in the lamina propria. Purple color indicates a high free/bound NADH ratio, while violet, cyan and white indicate linearly and progressively decreasing ratios free/bound NADH ratio, as shown in f **(e)** Phasor histogram of the FLIM image excited at 880nm, with a circular cluster that highlights the GFP (green) and the collagen (blue). **(f)** Phasor

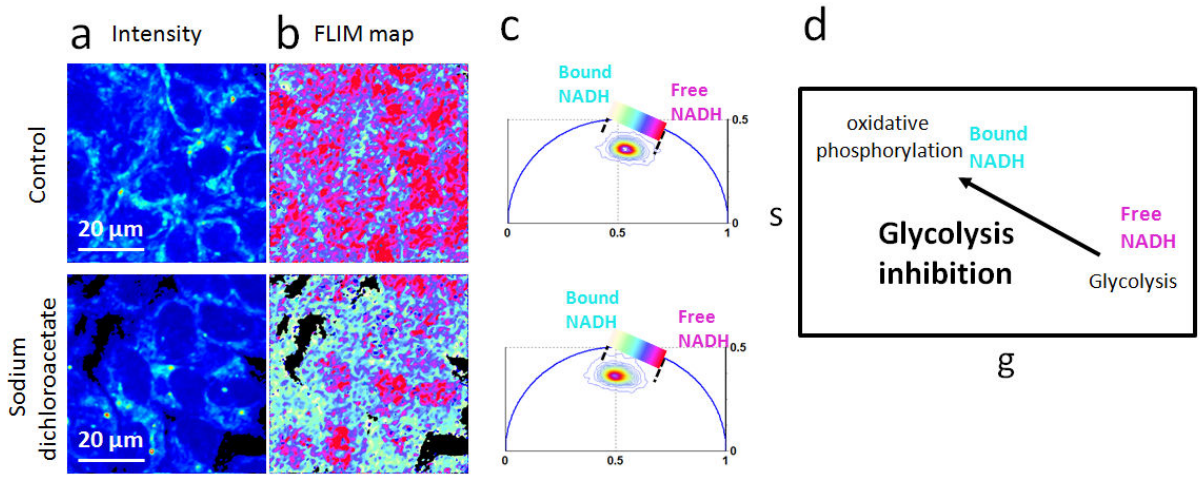
histogram of the FLIM image excited at 740nm. Phasor plot selection using a linear cluster that represents all possible relative concentrations of free NADH (purple) and bound NADH (white).



FigSM4

Glucose uptake by NIH3T3 fibroblast shifts the metabolic signature toward a glycolytic phenotype with high free/ bound NADH ratios

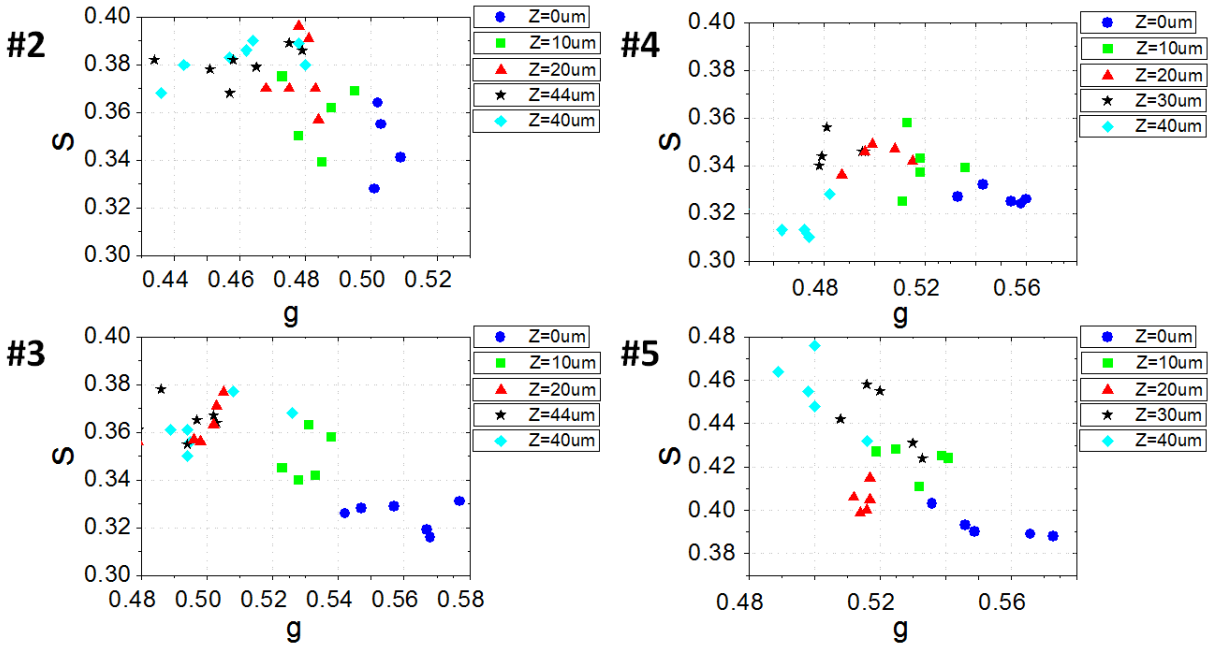
(a) Two-photon fluorescence intensity images excited at 740nm of cells in low glucose (4.5mM) and high glucose (22mM). **(b)** Phasor FLIM color maps at 740nm of the relative concentrations of free NADH (purple) and bound NADH (white) NADH. Cells with high glucose are characterized by a higher concentration of free NADH with respect to low glucose cells. **(c)** FLIM phasor plot of NIH3T3 cells in low and high glucose. Linear cluster represents all possible relative concentrations of free NADH (purple) and bound NADH (white). Phasor FLIM distribution shifts toward free NADH with an increasing concentration of glucose. **(d)** Schematic diagram indicates that glucose uptake shifts cellular metabolic signature toward a glycolytic phenotype with high ratios of free/bound NADH.



FigSM5

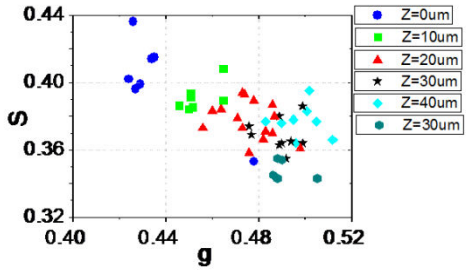
Inhibition of glycolysis in DLD-1 colon cancer epithelial cells shifts the metabolic signature toward an oxidative phosphorylation phenotype with low free/ bound NADH ratios

(a) Two-photon fluorescence intensity images excited at 740nm of control DLD-1 colon cancer cells and DLD-1 colon cancer cells treated with sodium dichloroacetate (DCA). Dichloroacetate ion inhibits pyruvate dehydrogenase kinase, resulting in the inhibition of glycolysis and a decrease in lactate production(b) Phasor color maps at 740nm of the relative concentrations of free NADH (purple) and bound NADH (white) NADH. Cells treated with DCA are characterized by a lower concentration of free NADH with respect to control cells. (c) FLIM phasor plot of control DLD-1 colon cancer cells and DLD-1 colon cancer cells treated with DCA. Linear cluster represents all possible relative concentrations of free NADH (purple) and bound NADH (white). Phasor FLIM distribution shifts toward bound NADH with DCA treatment. (d) Schematic diagram indicates that the inhibition of glycolysis through DCA treatment shifts cellular metabolic signature toward an oxidative phosphorylation phenotype with low ratios of free/bound NADH.



FigSM6

Scatter plots of the cell phasor of stem cells and differentiated epithelial cells at different depths from the collagen fibers of the basal membrane excited at 740nm, measured in 4 animals. Cyan diamond for Z=40µm, black stars for Z=30µm, red triangles for Z=20µm, green squares for Z=10 µm and blue circles for Z=0 µm). Along the Z axis the cell phasor shifts toward the longer lifetime indicating an increase of bound NADH with respect to free NADH. i.e. an decrease in NADH/NAD+ ratio.



FigSM7

Scatter plot of the cell phasor of stem cells and differentiated epithelial cells at different depths from the tip of the villi at 740nm, measured in one animal. Cyan diamond for Z=40µm, black stars for Z=30µm, red triangles for Z=20µm, green squares for Z=10 µm and blue circles for Z=0 µm). Along the Z axis the cell phasor shifts toward the shorter lifetime indicating an increase of free/bound NADH ratio.