

Supplemental Discussion

Related to Fig.2 and Extended Data Fig.5: Hypoxia is associated with a form of metabolism that involves reductive carboxylation to transfer carbon from glutamine into fatty acids¹. A similar pathway is observed in cells with genetic defects in the electron transport chain or TCA cycle². In its entirety, this pathway involves reductive carboxylation to produce isocitrate/citrate, followed by citrate cleavage to generate acetyl-CoA and oxaloacetate, with the oxaloacetate then supplying pools of other TCA cycle intermediates (malate, fumarate, succinate). In cells cultured with [U-¹³C]glutamine, this produces citrate m+5, acetyl-CoA m+2, and oxaloacetate/malate/fumarate/succinate m+3 (see the opaque branch of the pathway in **Extended Data Fig.1b**). Acetyl-CoA m+2, if used in *de novo* fatty acid synthesis, generates even-numbered labeling of palmitate (m+2, m+4, m+6, etc). In the H460 cells studied here, hypoxia induced all of these changes, both in monolayer culture and spheroids (see labeling in citrate, malate and lipogenic acetyl-CoA in **Extended Data Fig.5**). However, normoxic spheroids displayed a more than doubling of the citrate m+5 fraction (**Fig.1d**), with only small changes in lipogenic acetyl-CoA labeling or in the m+3 fraction of malate (**Extended Data Fig.5**). Thus, while H460 cells can induce a typical hypoxia-associated pathway of reductive metabolism extending to labeling of fatty acids and 4-carbon TCA cycle intermediates, normoxic spheroids primarily display enhanced reductive labeling of citrate, without large changes in labeling of the other pools.

References

- 1, Metallo, C. M. *et al.* Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* **481**, 380-384, doi:10.1038/nature10602 (2012).
- 2, Mullen, A. R. *et al.* Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature* **481**, 385-388, doi:10.1038/nature10642 (2012).