Imaging a hallmark of cancer: hyperpolarized ^{13}C-magnetic resonance spectroscopy can non-invasively monitor TERT expression in low-grade gliomas *in vivo*

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Highlights:

- Telomerase reverse transcriptase (*TERT*) expression is a hallmark of cancer, including in low-grade oligodendrogliomas.
- Metabolic imaging of TERT status can inform on tumor burden and response to therapy.
- Hyperpolarized [U-^{13}C,U-^{2}H]-glucose and hyperpolarized [1-^{13}C]-alanine can non-invasively monitor TERT status in low-grade oligodendrogliomas *in vivo*

Abstract (496/500)

Gliomas constitute the majority of malignant primary brain tumors and are a significant clinical problem with long-lasting physical and cognitive effects. Recent advances in cancer genomics have uncovered a variety of clinically relevant mutations in gliomas. There is an urgent need to identify methods of non-invasively imaging these oncogenic events, thereby integrating this genomic information into clinical patient management. Mutations in the telomerase reverse transcriptase (*TERT*) promoter are defining molecular features of high-grade primary glioblastomas and low-grade oligodendrogliomas. TERT is responsible for the synthesis of telomeres, which are chromosomal structures essential for cell proliferation. TERT is silenced in normal somatic cells and, therefore, inhibiting TERT expression is an attractive therapeutic strategy for gliomas. ***The goal of this study was to identify translational, hyperpolarized ^{13}C-magnetic resonance spectroscopy (MRS)-detectable biomarkers of TERT status that can be used to non-invasively monitor tumor burden in low-grade oligodendrogliomas.***

We examined immortalized normal human astrocytes without ($NHA_{control}$) and with TERT (NHA_{tert}) expression. Unbiased principal component analysis of ^{1}H-MRS profiles indicated that TERT induced unique metabolic reprogramming (Fig. 1A). Notably, levels of reduced glutathione (GSH), NAD(P)H, aspartate and AXP (AMP/ADP/ATP) were elevated in NHA_{tert} cells relative to $NHA_{control}$ cells (Fig. 1B). Since ^{1}H-MRS cannot distinguish between NADPH and NADH, we used spectrophotometric assays to confirm that both NADH and NADPH were elevated in NHA_{tert} cells.

Glucose flux through the pentose phosphate pathway (PPP) is a major producer of NADPH, which, in turn maintains GSH in the reduced state. Using thermally-polarized ^{13}C-MRS following administration of [2-^{13}C]-glucose, we established that fractional flux through the PPP was elevated in NHA_{tert} cells relative to $NHA_{control}$ cells. In order to leverage this TERT-induced increase in PPP flux for non-invasive hyperpolarized ^{13}C-MRS, we examined the metabolism of hyperpolarized [U-^{13}C,U-^{2}H]-glucose in our models (Fig. 2C). The flux of hyperpolarized [U-^{13}C,U-^{2}H]-glucose to the PPP metabolite 6-phosphogluconate (6-PG) was significantly elevated in NHA_{tert} cells relative to $NHA_{control}$ (Fig. 2D). Importantly, 2D EPSI imaging studies indicated that

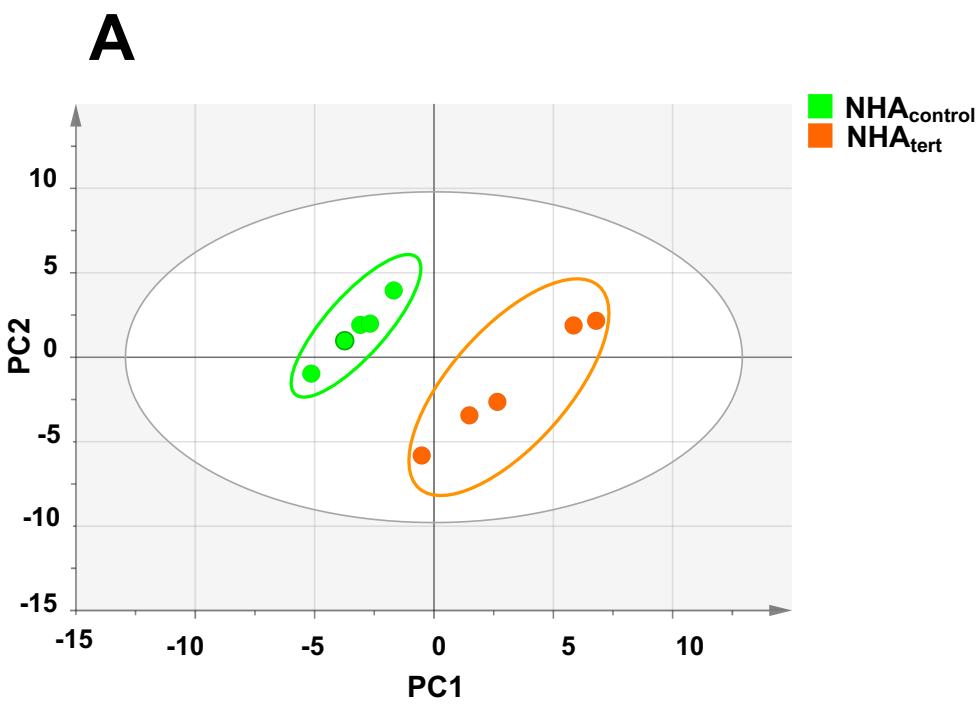
6-PG production clearly differentiated tumor from normal brain in orthotopic NHA_{tert} tumor xenografts *in vivo* (Fig. 2E).

Next, we exploited the observation that TERT expression increased NADH, which is essential for the metabolism of hyperpolarized [1-^{13}C]-alanine to [1-^{13}C]-lactate. Lactate production from hyperpolarized [1-^{13}C]-alanine was higher in NHA_{tert} cells relative to $NHA_{control}$ (Fig. 2F-2G). Importantly, 2D EPSI imaging of hyperpolarized [1-^{13}C]-alanine metabolism in orthotopic NHA_{tert} tumor xenografts revealed pronounced differences in lactate production between tumor tissue and normal brain (Fig. 2H).

In summary, we demonstrate, for the first time, non-invasive *in vivo* imaging of TERT status in low-grade oligodendrogliomas. Since TERT is inextricably linked to tumor proliferation and is not expressed in normal, non-cancerous tissue, imaging TERT has the potential to distinguish between "true" tumor burden and treatment-related effects like pseudoprogression and pseudoresponse. Imaging TERT can also provide a readout of response to standard radio- and chemotherapy as well as novel inhibitors of TERT expression currently under development. Therefore, our imaging biomarkers, once translated to the clinic have the potential to significantly impact outcome for glioma patients.

Figure Legends: Hyperpolarized ^{13}C-MRS can non-invasively monitor TERT expression in low-grade gliomas. **(A)** Scores plot of principal component analysis for $NHA_{control}$ and NHA_{tert} cells. **(B)** Univariate quantification of metabolite concentrations by ^{1}H-MRS in $NHA_{control}$ and NHA_{tert} cells. **(C)** ^{13}C spectral array showing metabolism of hyperpolarized [U-^{13}C,U-^{2}H]-glucose to 6-phosphogluconolactone and 6-PG in NHA_{tert} cells. **(D)** Quantification of 6-PG production in $NHA_{control}$ and NHA_{tert} cells. **(E)** 2D EPSI imaging of hyperpolarized [U-^{13}C,U-^{2}H]-glucose metabolism to 6-PG in the tumor region in orthotopic NHA_{tert} tumor xenografts. **(F)** ^{13}C spectral array showing metabolism of hyperpolarized [1-^{13}C]-alanine to [1-^{13}C]-lactate in NHA_{tert} cells. **(G)** Quantification of lactate production in $NHA_{control}$ and NHA_{tert} cells. **(H)** 2D EPSI imaging of hyperpolarized [1-^{13}C]-alanine metabolism to lactate in the tumor region in orthotopic NHA_{tert} tumor xenografts.

Figure 1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NHAtert
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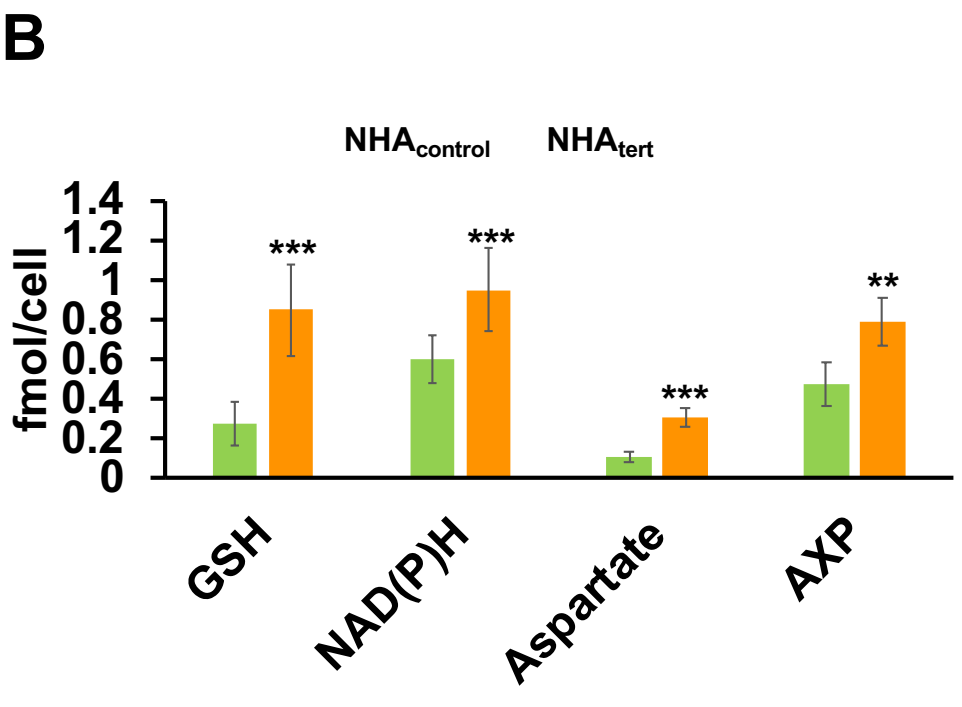
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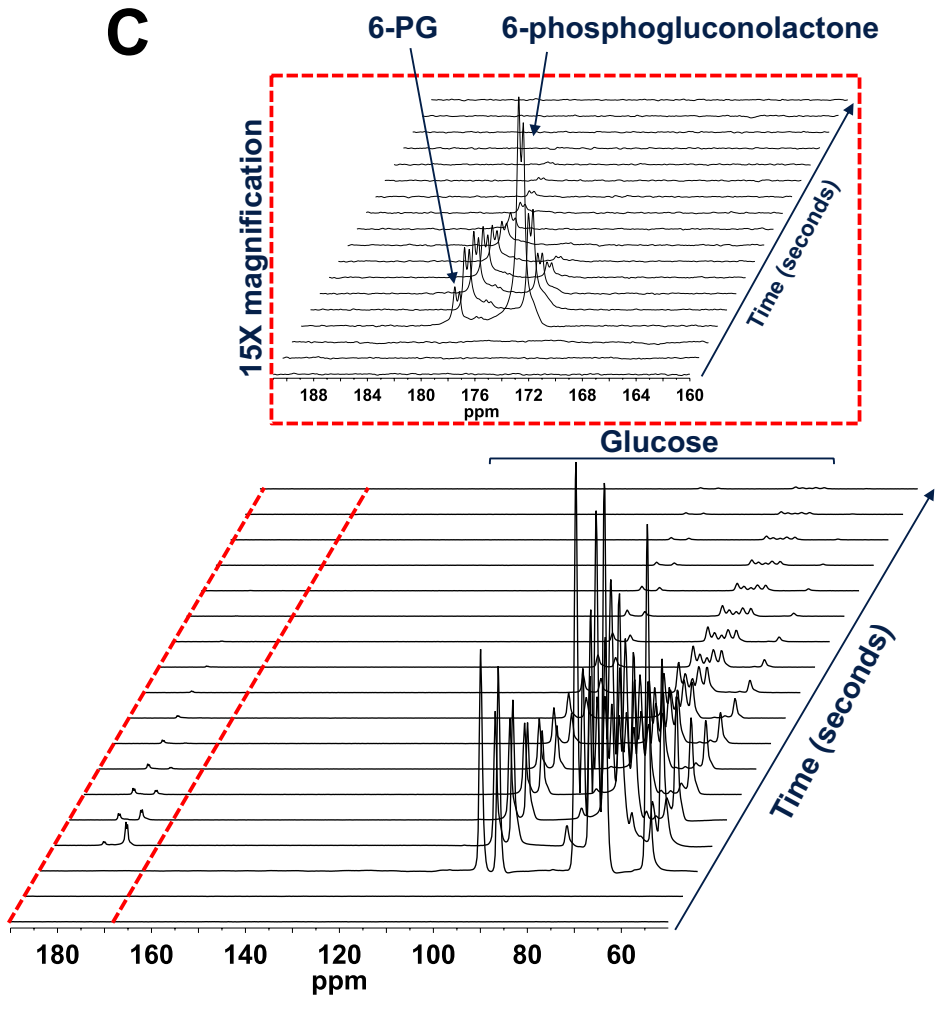
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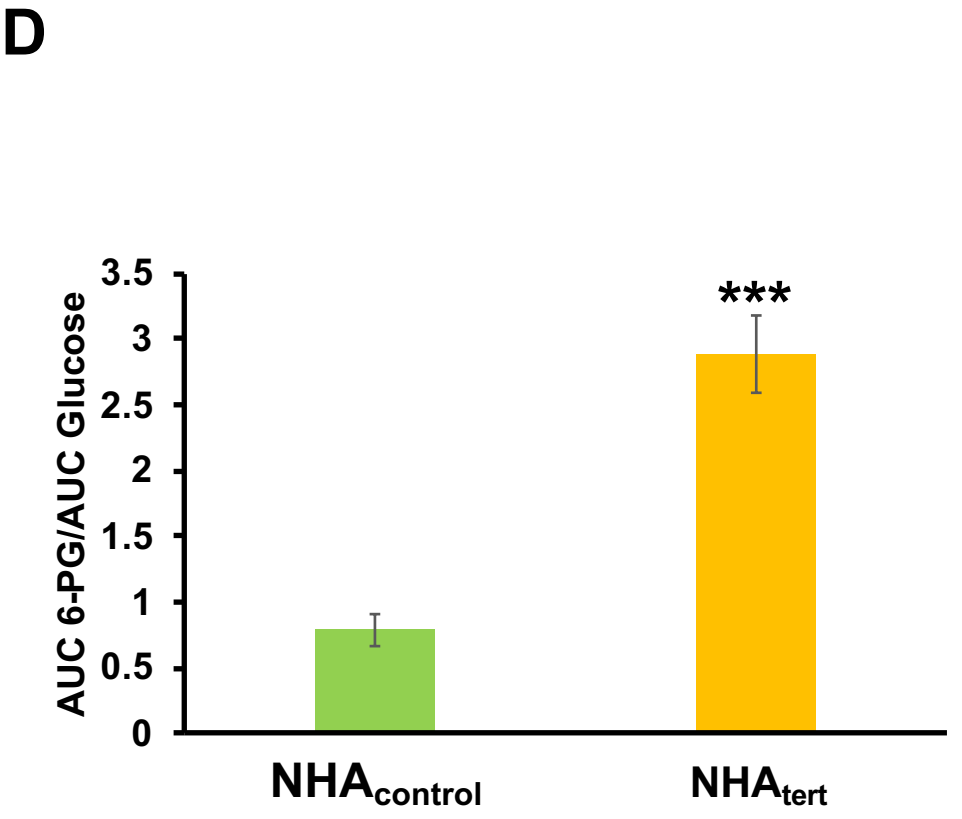
ppm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2.5
2
1.5
1
0.5
0
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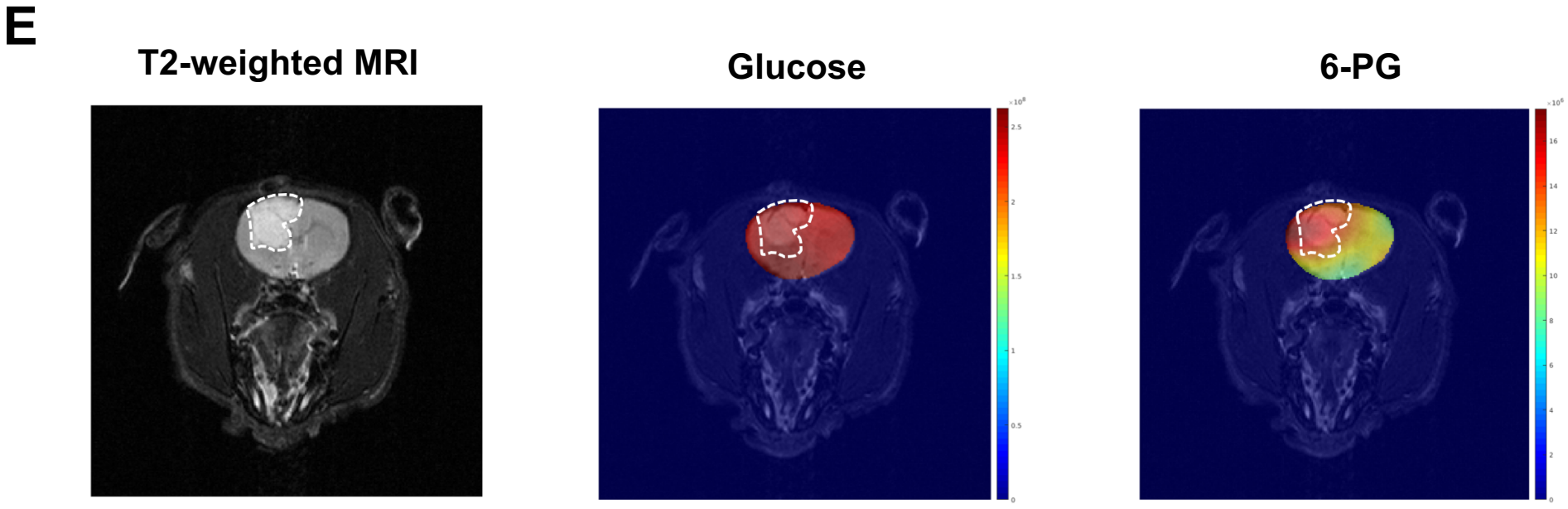
NHAcontrol
NHAtert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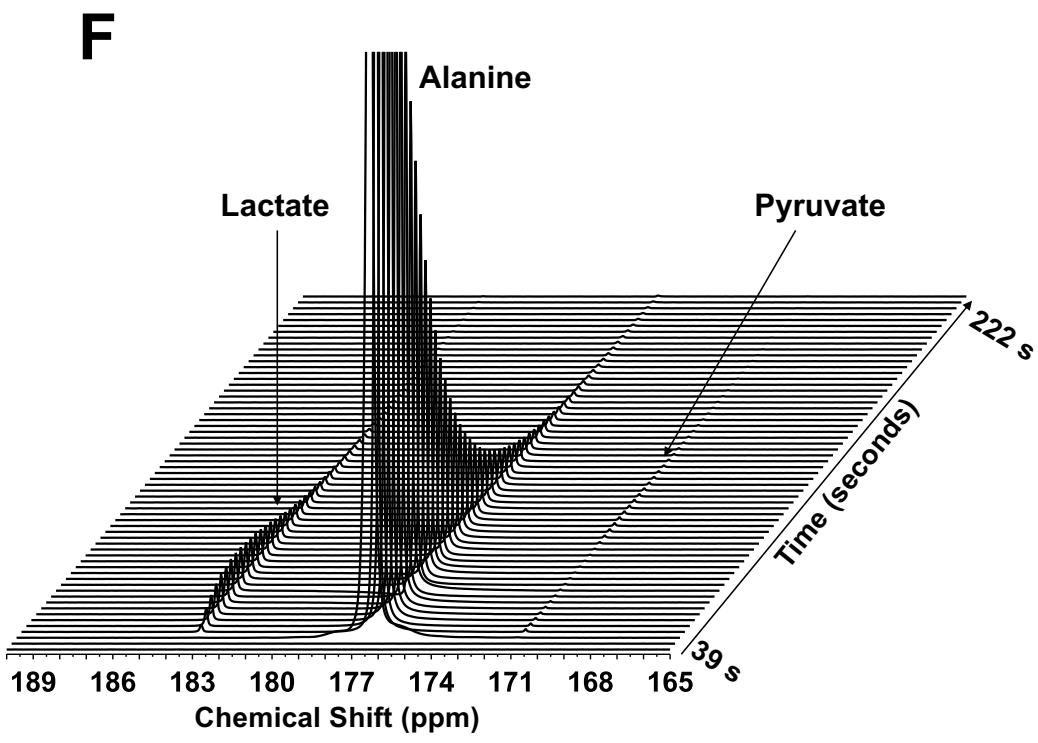
Glucose
6-PG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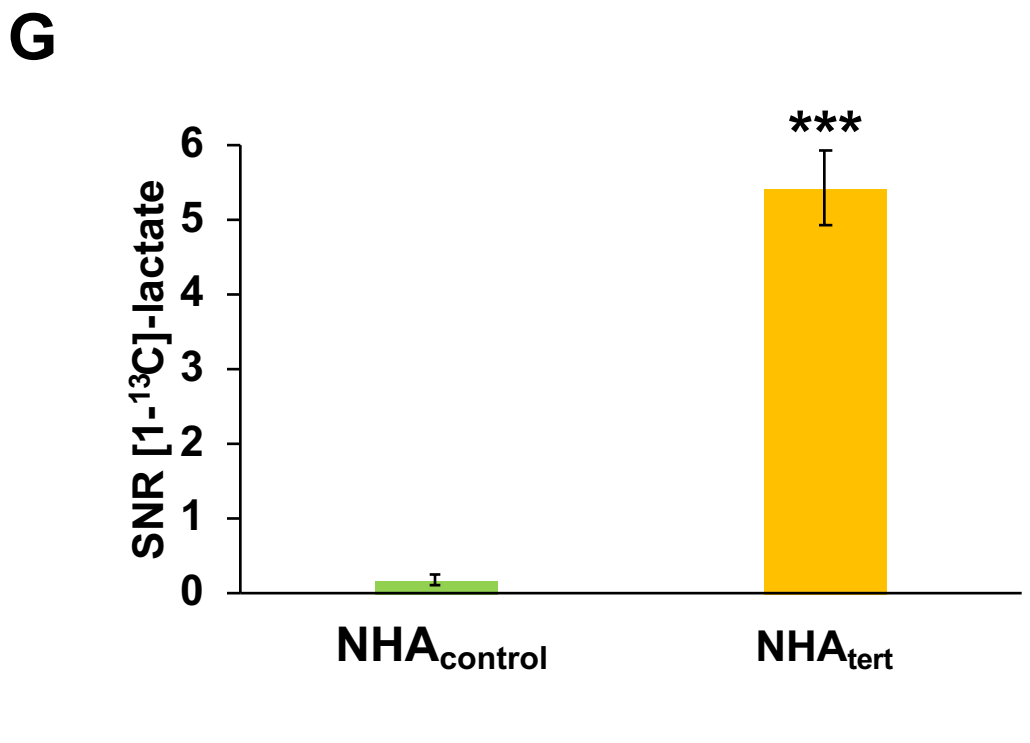
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0
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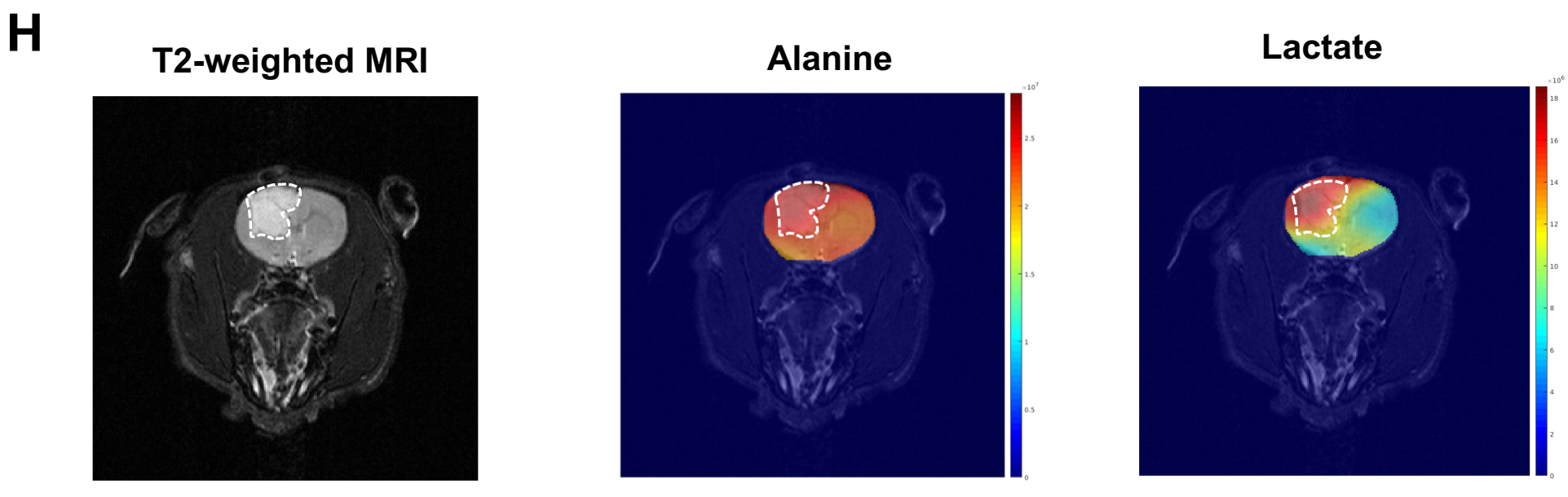
NHAcontrol
NHAtert


H
T2-weighted MRI
Alanine
Lactate