

Supplementary Information

Imaging immunomodulatory treatment responses in a multiple sclerosis mouse model using hyperpolarized ^{13}C metabolic MRI

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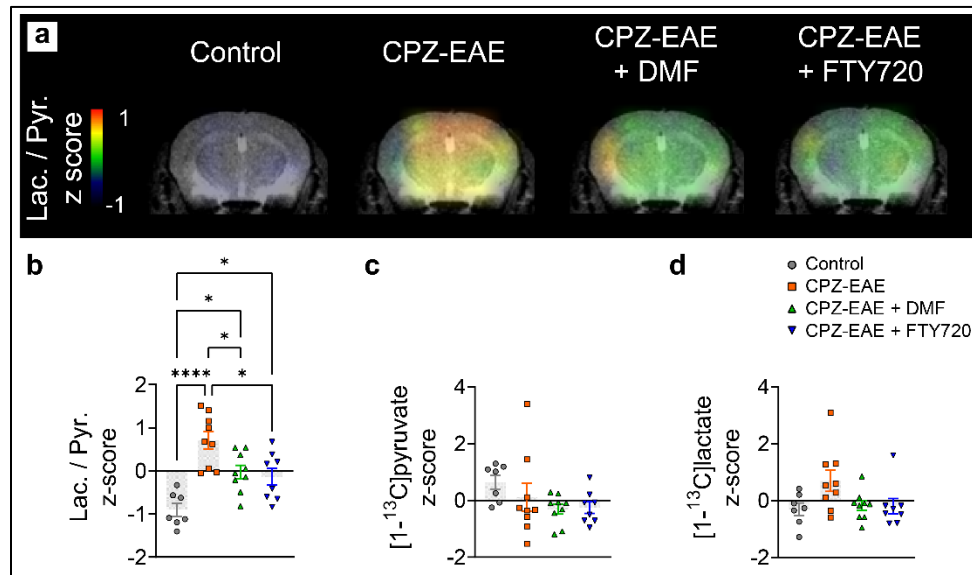
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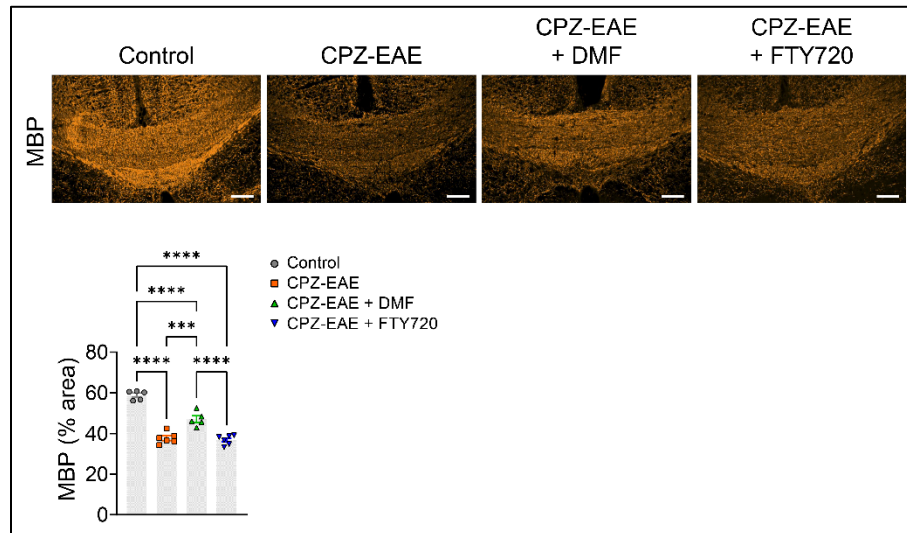
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Supplementary Figure 1. ^{13}C lactate/pyruvate mean z-score detects therapy responses.



(a) ^{13}C lactate/pyruvate mean z-score color maps overlaid on a representative T_2 -weighted image for the control, CPZ-EAE, CPZ-EAE + DMF and CPZ-EAE + FTY720 groups. **(b)** Quantitative analyses revealed significant increase of the ^{13}C lactate/pyruvate z-score in CPZ-EAE mice compared to Control mice. Both DMF and FTY720 showed lower ^{13}C lactate/pyruvate z-score compared to untreated CPZ-EAE mice. No significant differences were observed between groups for **(c)** the hyperpolarized $[1-^{13}\text{C}]$ pyruvate and **(d)** $[1-^{13}\text{C}]$ lactate z-scores. We noted a trend for an increase hyperpolarized $[1-^{13}\text{C}]$ lactate z-score in CPZ-EAE mice compared to control mice ($p = 0.079$). Abbreviations: Cuprizone and experimental autoimmune encephalomyelitis (CPZ-EAE), Dimethyl fumarate (DMF), Fingolimod (FTY720), lactate-to-pyruvate ratio (Lac. / Pyr.). Data is shown as mean $\pm$ standard error. Control are indicated by grey circles ($n = 7$ mice), CPZ-EAE by orange rectangles ($n = 9$ mice), CPZ-EAE + DMF by green triangles ($n = 9$ mice), and CPZ-EAE + FTY720 by blue inverted triangles ($n = 8$ mice). (* $p \leq 0.05$, **** $p \leq 0.0001$). Data to reproduce this figure are included in Supplementary Data 4.

Supplementary Figure 2. Evaluation of myelin basic protein levels following immunomodulatory therapies in the CPZ-EAE model.



Representative immunofluorescence staining of myelin basic protein (MBP, orange) in the corpus callosum from control (grey circles), CPZ-EAE (orange rectangles), CPZ-EAE + DMF (green triangles), and CPZ-EAE + FTY720 (blue inverted triangles) mice. Demyelination was confirmed by a 1.6 fold decrease MBP in untreated CPZ-EAE mice compared to control mice ($p < 0.0001$). DMF treated mice displayed an increased MBP content (+1.25 fold, $p = 0.0002$) compared to untreated CPZ-EAE mice, while no change was observed following FTY720. Abbreviations: Cuprizone and experimental autoimmune encephalomyelitis (CPZ-EAE), Dimethyl fumarate (DMF), Fingolimod (FTY720), myelin basic protein (MBP). Data is shown as mean $\pm$ standard error. Control are indicated by grey circles ($n = 5$ mice), CPZ-EAE by orange rectangles ($n = 6$ mice), CPZ-EAE + DMF by green triangles ($n = 5$ mice), and CPZ-EAE + FTY720 by blue inverted triangles ($n = 6$ mice). (** $p \leq 0.001$, **** $p \leq 0.0001$). Scale bar is 100 μ m. Data to reproduce this figure are included in Supplementary Data 5.