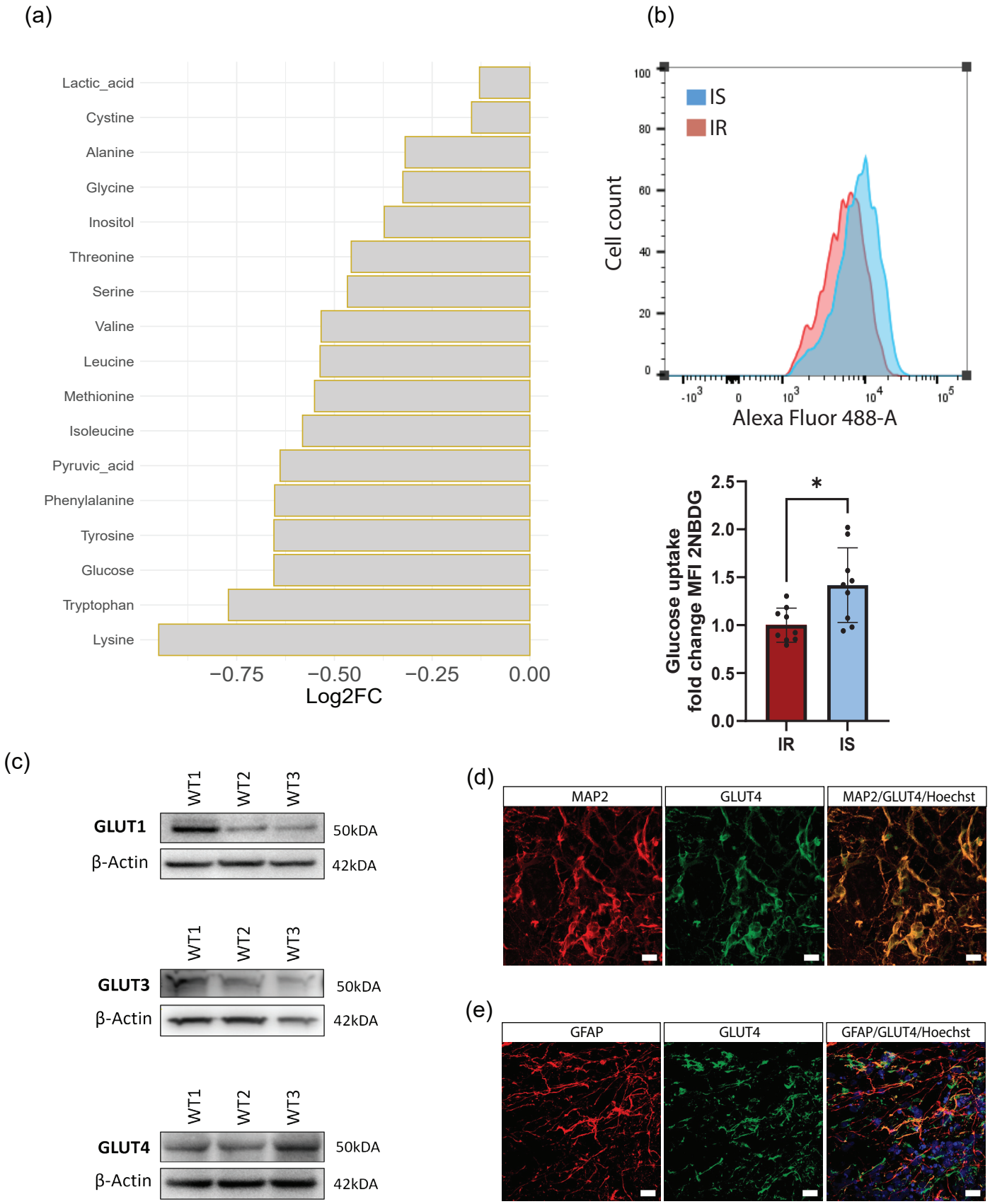

Figure S5. Insulin-dependent metabolic changes and glucose transporter presence in midbrain organoids. (a) GC-MS detected metabolite relative concentration in insulin sensitive (IS) organoid spent media demonstrated as log2 fold change (FC) against insulin resistant (IR) samples. Metabolite relative abundance normalised to the organoid area. (b) Representative histogram of cells positive to the Alexa-Fluor488 signal, demonstrating uptake of fluorescent glucose analog - 2NBDG. Measurement performed after 30 min incubation of high (standard media) or reduced (self-made N2 media) insulin concentration after organoid dissociation. Statistical significance tested with two-tailed, paired t-test. P value = 0.0157; n=9. (c) Representative images of Western Blots showing GLUT1, GLUT3 and GLUT4 presence in midbrain organoids. (d) Representative immunofluorescence staining images of MAP2 and GLUT4 at day 30 of organoid culture. Scale bars: 20µm. (e) Representative immunofluorescence staining images of GFAP and GLUT4 at day 60 of organoid culture. Scale bars: 20µm.