Expanded View Figures

Figure EV1. UPLC derivatization methods and heterologous expression in *Xenopus* oocytes.

A, B BCKAs (KIV, KIC, KMV) are detected by ultra performance liquid chromatography (UPLC) coupled to fluorescence detection in cell extracts using the derivatization with DMB (A) or in cell culture supernatants using the OPD derivatization reagent (B).

C, D Co-expression of BCAT1 and either MCT1 or MCT4 facilitates the excretion of BCKAs from *Xenopus* oocytes. BCKAs (KIV, KIC, KMV) levels detected by ultra performance liquid chromatography (UPLC) coupled to fluorescence detection in native oocytes or oocytes expressing BCAT1, MCT1, MCT4, NBCe1, or co-expressing MCT1 or MCT4 and BCAT1 stimulated with BCAAs (1 nmol L-valine, 1 nmol L-leucine, 1 nmol L-isoleucine) and α-ketoglutarate (3 nmol) for 2 h at RT (C) and in the oocytes culture medium (D). Heterologous expression of the human or rat proteins in the oocytes was performed by injection of the respective cRNA into the oocyte. The cRNA concentrations used are 5 ng rat MCT1, 5 ng rat MCT4, 12 ng human BCAT1, 7 ng human NBCe1 or 5 ng MCT1 or MCT4, and 12 ng BCAT1. Batches of 10 oocytes are used per condition. Values are mean ± SD of three independent biological replicates. KIV: α-ketoisovalerate. KIC: α-ketoisocaproate. KMV: α-keto-β-methylvalerate. NBCe1: sodium/bicarbonate cotransporter. Unpaired Student's *t*-test *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

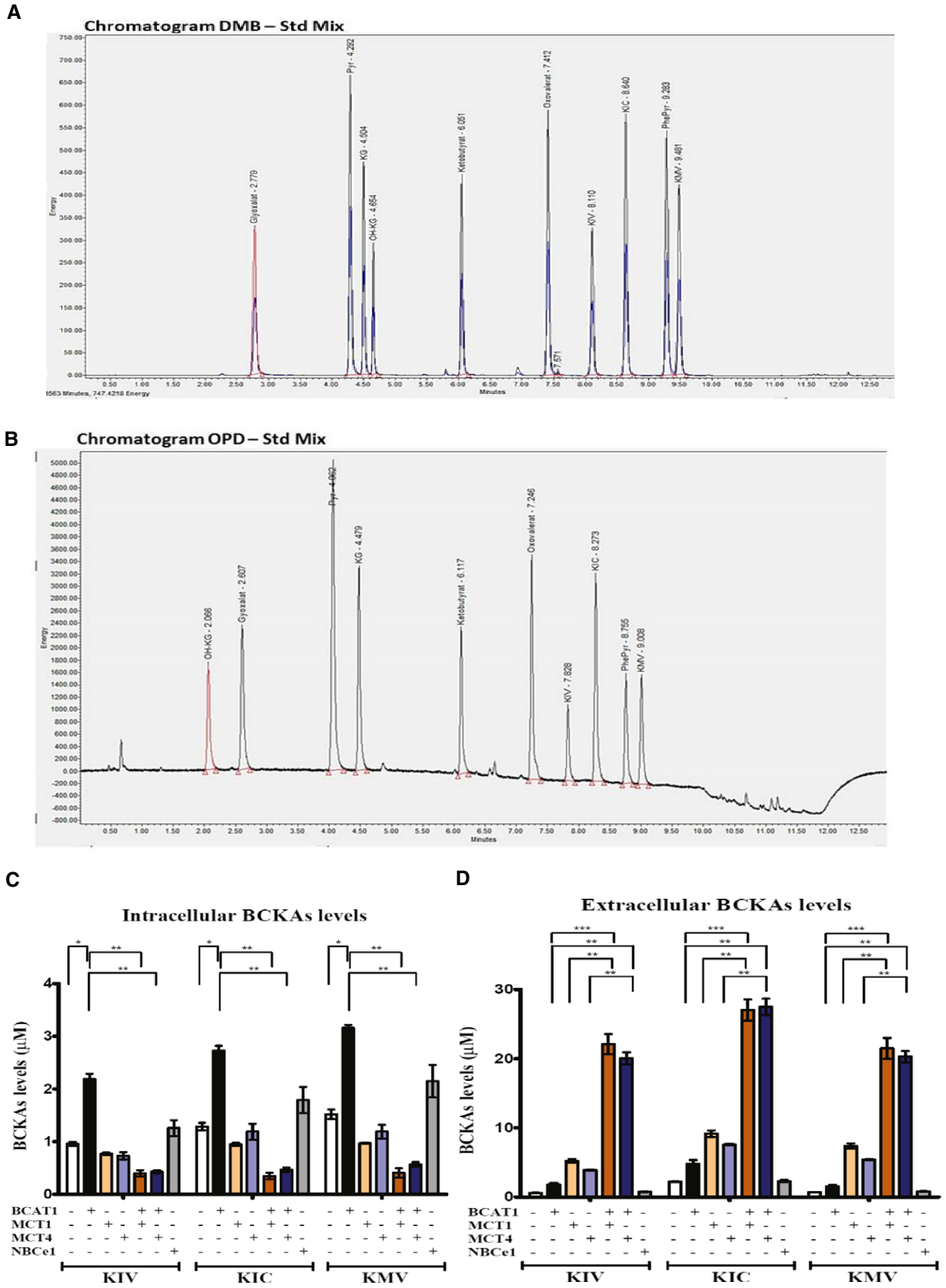

Figure EV1.

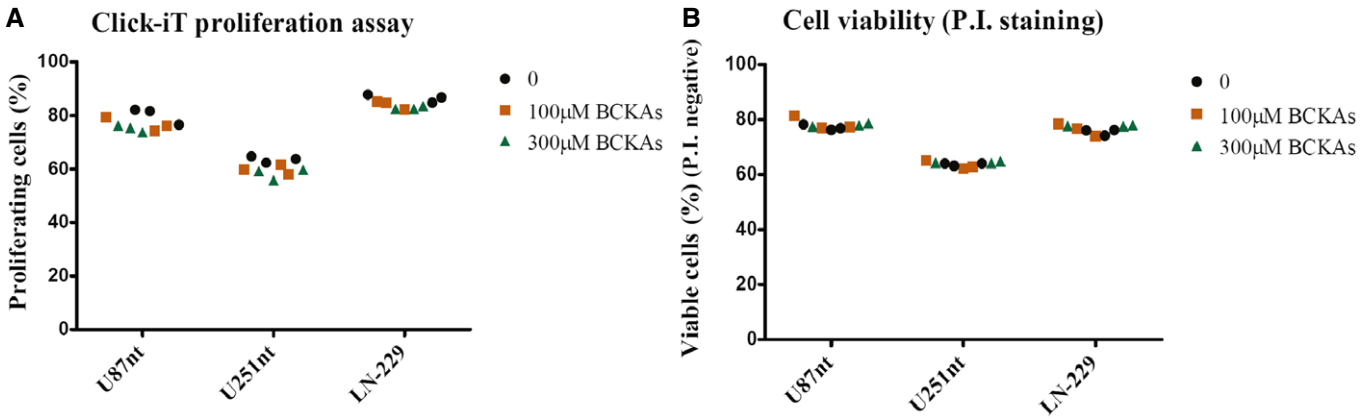

Figure EV2. BCKAs do not impact on glioblastoma cell phenotype.

A Effect of 100 or 300 μM BCKAs on cell proliferation of U87nt, U251nt, and LN-229 cells determined using the Click-iT proliferation assay. n = 3 technical replicates.

B Effect of 100 or 300 μM BCKAs on cell proliferation of U87nt, U251nt, and LN-229 cells on cell viability determined by propidium iodide (P.I) staining, and analysis of P.I. negative (viable) cells was done using flow cytometry (FACS Canto II, BD Biosciences). n = 3 technical replicates.

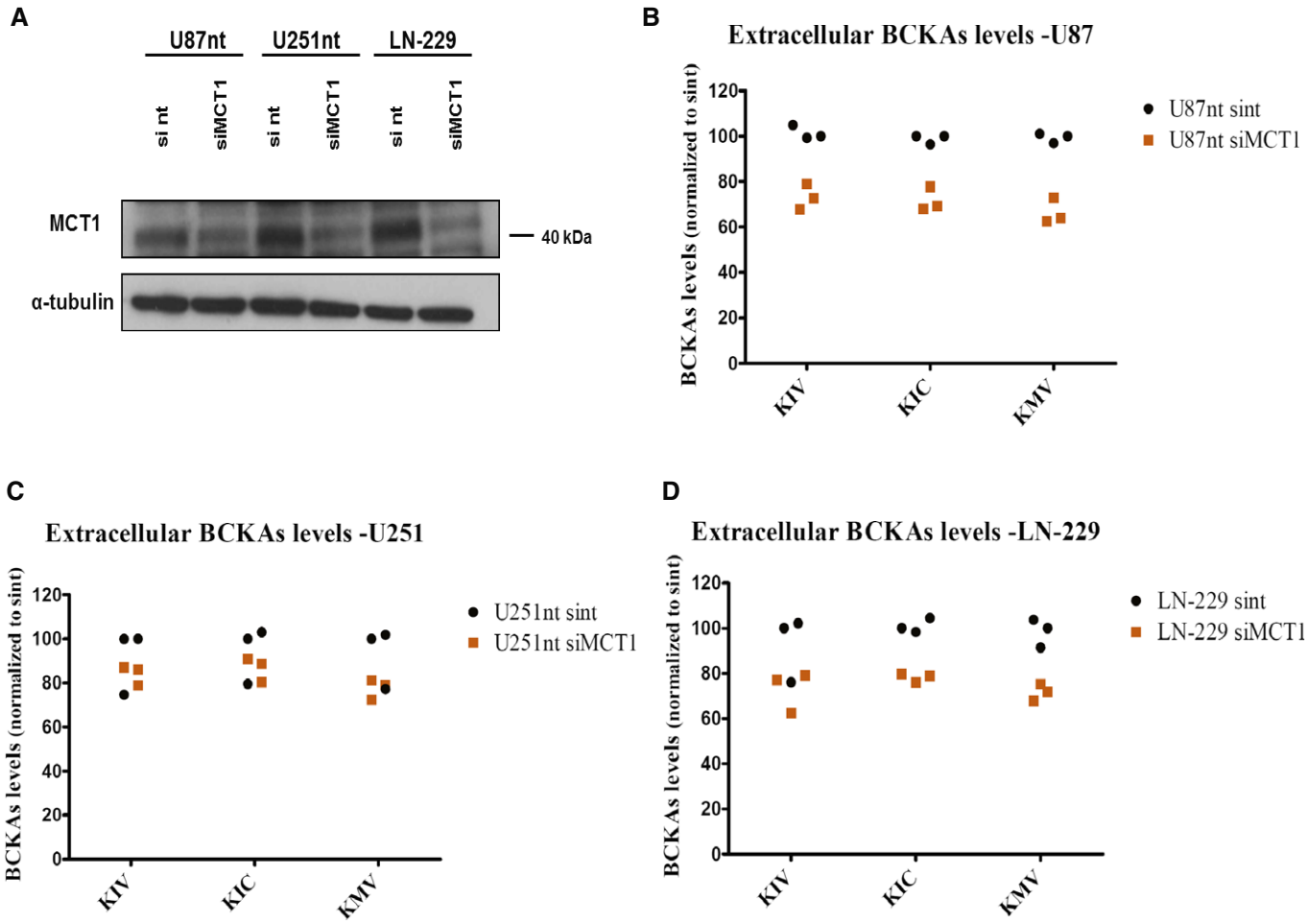

Figure EV3. MCT1 knockdown decreases BCKA excretion.

A Western blot analysis of MCT1 levels in U87-MG cells, U251-MG expressing normal levels of BCAT1 (U87nt and U251nt, respectively) and LN-229 cells 48 h post-transfection using DharmaFECT 1 transfection reagent (Dharmacon) with 25 nmol of MCT1 siRNA smart pool (Dharmacon), or non-target (nt) #2 siRNA pool (Dharmacon). Anti-MCT1 antibody (AB3538P, Millipore) was used at 1:5,000 dilution. α-tubulin was used as loading control.

B–D BCKAs (KIV, KIC, KMV) levels are determined by ultra performance liquid chromatography (UPLC) coupled to fluorescence detection in supernatants from U87nt (B), U251nt (C), and LN-229 (D) cells 48 h after transfection. BCKAs levels are normalized to total protein content and to the levels detected in U87nt, U251nt, or LN-229 cells transfected with non-target #2 siRNA (U87nt sint, U251nt sint, or LN-229 sint, respectively). n = 3 technical replicates. KIV: α-ketoisovalerate. KIC: α-ketoisocaproate. KMV: α-keto-β-methylvalerate.

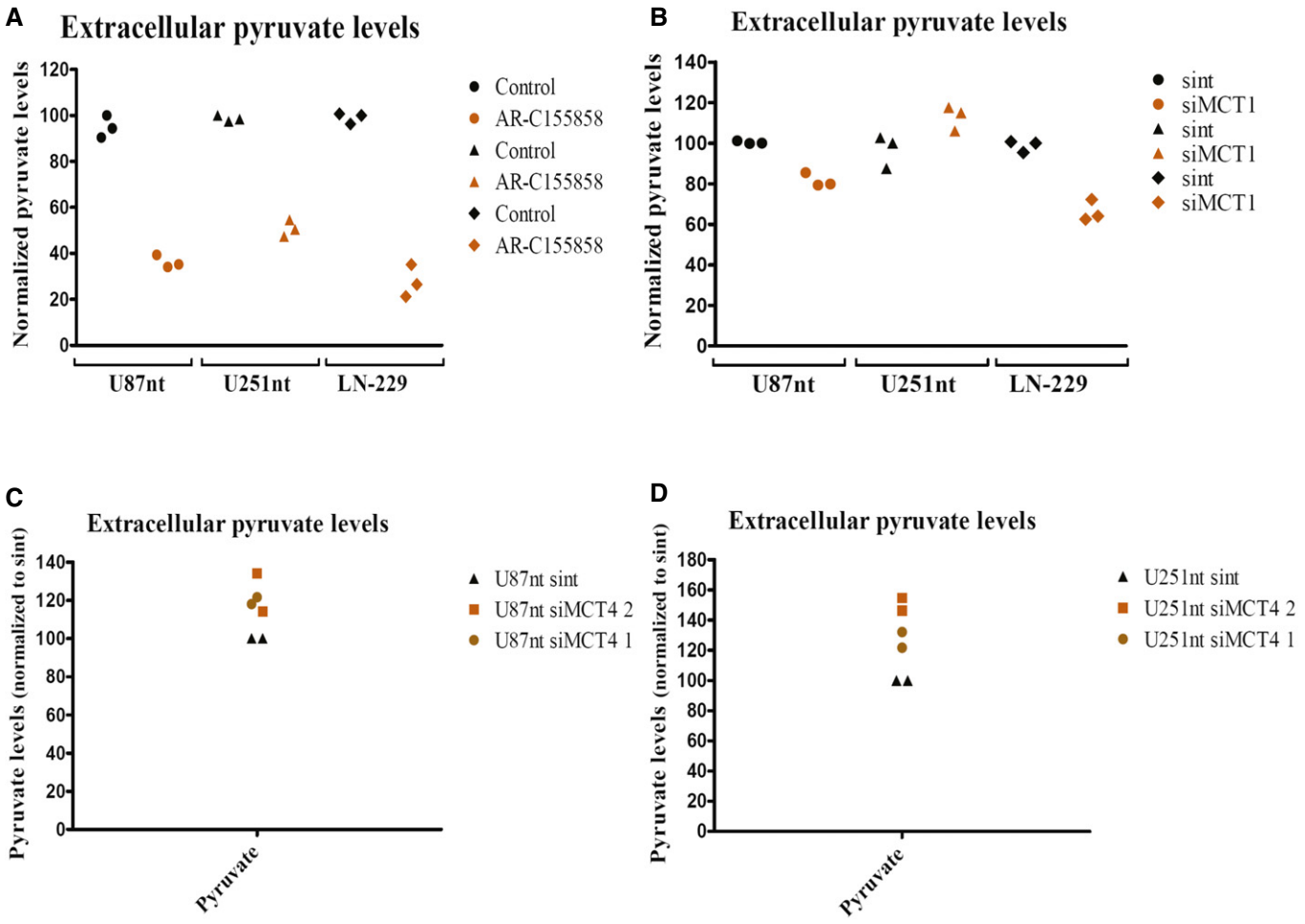

Figure EV4. MCT1 inhibition or knockdown decreased pyruvate excretion in glioblastoma cells.

A, B Pyruvate levels are determined by UPLC coupled to fluorescence detection in supernatants from U87nt, U251nt, and LN-229 cells treated with AR-C155858 for 24 h at 37°C 10% CO_2 (A) and cells expressing normal MCT1 levels (sint) or low MCT1 levels (siMCT1) (B). n = 3 technical replicates.

C, D Pyruvate levels are determined from U87nt (C) or U251nt (D) cells expressing normal MCT4 levels (sint) or low MCT4 levels (siMCT4). Pyruvate levels are normalized to total protein content and to the detected levels in the respective cells treated with DMSO (Control) or to the detected levels in cells expressing normal MCT1 or MCT4 levels (sint). n = 2 independent biological replicates.

Figure EV5. Effects of BCKAs on macrophage viability and nutrient consumption.

A The effect of BCKAs on macrophage viability was assessed using propidium iodide (P.I.) staining, and analysis of P.I. negative (viable) was done using flow cytometry (FACS Canto II, BD Biosciences). M-CSF differentiated macrophages were incubated in the absence (medium control) or presence of 300 μM BCKAs (KIV, KIC, KMV) at 37°C for 24 h. Cells were isolated from two independent donors, run in triplicates.

B, C Consumption of BCAAs (B), pyruvic acid, lactic acid, succinic acid, and hexoses (C) by monocyte-derived macrophages incubated in the absence (medium control) or presence of 100 or 300 μM BCKAs (KIV, KIC, KMV) at 37°C for 24 h was determined by GC-MS in the supernatants collected from macrophage cultures. Nutrient levels are normalized to protein content and to the levels detected in the supernatant of macrophages incubated in the absence of BCKAs (Medium control: DMEM 6046 10% FCS 1%P/S). Val: valine. Ile: isoleucine. Leu: leucine.

Data information: Data show $n = 3$ technical replicates.

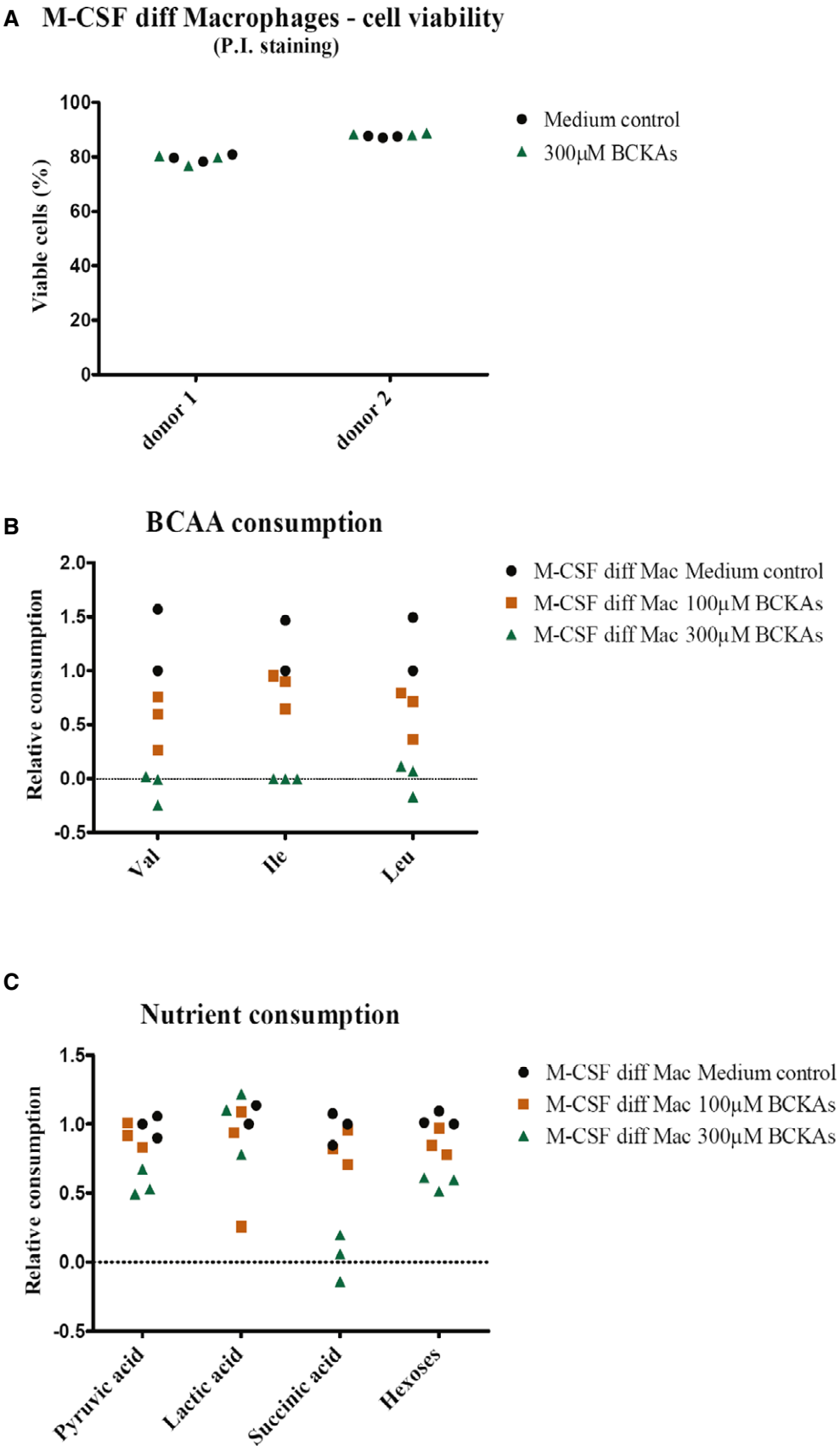

Figure EV5.