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Neurogenesis is disrupted in human hippocampal progenitor cells upon exposure to serum samples from hospitalized COVID-19 patients with neurological symptoms

Alessandra Borsini, PhD[1*], Blair Merrick, MD[2], Jonathan Edgeworth, PhD, MD[3], Gargi Mandal, BSc[1], Deepak Srivastava, PhD[4,5], Anthony C. Vernon, PhD[4,5], Gaia Nebbia, PhD, MD[3], Sandrine Thuret, PhD[4], Carmine M. Pariante, PhD, MD[1]

[1] Stress, Psychiatry and Immunology Laboratory, Institute of Psychiatry, Psychology and Neuroscience, Department of Psychological Medicine, King's College London, UK

[2] Centre for Clinical Infection and Diagnostics Research, Guy's and St Thomas' NHS Foundation Trust, London, UK

[3] School of Immunology and Microbial Sciences, Guy's and St Thomas' NHS Foundation Trust, London, UK

[4] Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK

[5] MRC Centre for Neurodevelopmental Disorders, King's College London, UK

* Corresponding Authors

Alessandra Borsini, PhD

Stress, Psychiatry and Immunology Lab & Perinatal Psychiatry

Institute of Psychiatry, Psychology and Neuroscience, King's College London

G.32.01, The Maurice Wohl Clinical Neuroscience Institute

Cutcombe Road, London, SE5 9RT

Tel: 020 7848 0726

Email: alessandra.borsini@kcl.ac.uk

32 **Abstract**

33 Coronavirus disease 2019 (COVID-19), represents an enormous new threat to our healthcare
34 system and particularly to the health of older adults. Although the respiratory symptoms of
35 COVID-19 are well recognized, the neurological manifestations, and their underlying cellular
36 and molecular mechanisms, have not been extensively studied yet. Our study is the first one to
37 test the direct effect of serum from hospitalised COVID-19 patients on human hippocampal
38 neurogenesis using a unique *in vitro* experimental assay with human hippocampal progenitor
39 cells (HPC0A07/03C). We identify the different molecular pathways activated by serum from
40 COVID-19 patients with and without neurological symptoms (i.e., delirium), and their effects
41 on neuronal proliferation, neurogenesis, and apoptosis. We collected serum sample twice, at
42 time of hospital admission and approximately 5 days after hospitalization. We found that
43 treatment with serum samples from COVID-19 patients with delirium (n=18) decreased cell
44 proliferation and neurogenesis, and increases apoptosis, when compared with serum samples
45 of sex- and age-matched COVID-19 patients without delirium (n=18). This effect was due to
46 a higher concentration of interleukin 6 (IL6) in serum samples of patients with delirium
47 (mean±SD: 229.9±79.1pg/ml, vs. 32.5±9.5pg/ml in patients without delirium). Indeed,
48 treatment of cells with an antibody against IL6 prevented the decreased cell proliferation and
49 neurogenesis and the increased apoptosis. Moreover, increased concentration of IL6 in serum
50 samples from delirium patients stimulated the hippocampal cells to produce IL12 and IL13,
51 and treatment with an antibody against IL12 or IL13 also prevented the decreased cell
52 proliferation and neurogenesis, and the increased apoptosis. Interestingly, treatment with the
53 compounds commonly administered to acute COVID-19 patients (the Janus kinase inhibitors,
54 baricitinib, ruxolitinib and tofacitinib) were able to restore normal cell viability, proliferation
55 and neurogenesis by targeting the effects of IL12 and IL13. Overall, our results show that serum
56 from COVID-19 patients with delirium can negatively affect hippocampal-dependent

neurogenic processes, and that this effect is mediated by IL6-induced production of the downstream inflammatory cytokines IL12 and IL13, which are ultimately responsible for the detrimental cellular outcomes.

Keywords: delirium; COVID-19; hippocampal neurogenesis; apoptosis; interleukin 6 (IL6); IL12; IL13

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the novel coronavirus that causes coronavirus disease 2019 (COVID-19), represents an enormous new threat to our healthcare system and particularly to the health of older adults[1, 2]. Although the respiratory symptoms of COVID-19 are well recognized[3], the neurological manifestations and their underlying mechanisms have not been extensively studied yet. So far, evidence has shown that COVID-19 patients can develop several neurological symptoms during the acute disease, including headaches, dizziness, loss of smell, cognitive problems, and in some cases even delirium symptoms[4, 5]. In older people, delirium, with the associated severe cognitive disturbances, including confusion and altered level of attention and consciousness, is associated with adverse outcomes, including prolonged hospitalization and death, even in the absence of COVID-19 infection[6].

So far, studies indicate that 20–30% of COVID-19 patients will present with or develop delirium or mental status changes during the course of the disease, with rates of 60–70% in cases of severe illness, at all ages[7-9]. While admission in intensive therapy units (ITU) is itself associated with delirium symptoms[10], a sub-group of COVID-19 patients start to show these symptoms early on before hospitalisation[7, 8], suggesting that the infection itself, rather than the hospital or ITU admission, plays a role in the development and/or exacerbation of an already compromised neurological scenario, especially in older patients. However, the exact mechanisms underlying the association between COVID-19 and delirium (or other neurological symptoms) are still not well understood.

It is likely that a major contributing mechanism to the development of neurological symptoms in COVID-19 patients is a hyper-activated immune system. Indeed, the virus infection is now well-known for its ability to induce an over-reacting immune response (defined as “cytokine storm” syndrome) characterized by the production of multiple inflammatory cytokines and chemokines measurable in the periphery, including interleukin 2 (IL2), IL6, IL8, IL12, IL13, interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α)[11-13]. Once produced, these peripheral inflammatory cytokines can then penetrate the blood brain barrier (BBB)[14-16] and directly affect brain mechanisms and induce neurological symptoms affecting cognition, memory, alertness and emotional state, and leading to delirium[17]. Among these brain mechanisms, hippocampal neurogenesis is regarded as one of the most important cellular processes involved in the regulation of cognition[18-20]. However, which of the cytokines that are elevated by COVID-infection are also directly relevant to the development of neurological symptoms is currently unknown.

Using an *in vitro* model of human hippocampal progenitor cells, we have already demonstrated that treatment with high concentrations of IL6, similar to those found in peripheral blood of COVID-19 patients (~50-500pg/ml)[12, 21], can reduce neurogenesis and increase apoptosis[22, 23]. Other proinflammatory cytokines, like IL1β and interferon-alpha (IFN-α), have similar effects in this cellular model[24-27]. Moreover, we have shown that treatment of the same cellular model with serum samples from patients receiving IFN-α-treatment for Hepatitis C also increases apoptosis and reduces neurogenesis[28], and that such changes are predictive of the development of IFN-α-induced depression. Of note, similarly to COVID-19 patients, patients receiving IFN-α treatment also can present with cognitive impairments, inattention, memory loss and confusion[29]. This therefore suggests that reduction of hippocampal neurogenesis and/or increased apoptosis is one of the mechanisms underpinning the inflammation-induced

139 neurological symptoms in COVID-19 infected patients, and that using serum samples from
140 affected patients in this cellular model might be a mechanistically valid way to understand
141 these effects.
142
143 Here we use this unique, well-established[22-25, 30-36], *in vitro* model of human hippocampal
144 progenitor cells to assess the direct effect of treatment with serum samples from COVID-19
145 patients on hippocampal neurogenesis, and to investigate the molecular pathways activated by
146 serum from patients with or without delirium. In particular, we tested the hypothesis that
147 treatment of human hippocampal progenitor cells with serum from delirium patients would
148 decrease cell proliferation and neuronal differentiation, and would increase apoptosis, when
149 compared with serum from patients without delirium. We also identified the candidate
150 inflammatory mechanisms that are involved in these effects of serum from patients.
151
152 **METHODS AND MATERIALS**
153 **Patients' cohort:** The study comprises serum samples collected from a total of 36 patients who
154 were admitted to Guy's and St Thomas' NHS Foundation Trust during the first wave of the
155 COVID-19 pandemic in UK (March-June 2020). Samples used in this study were collected at
156 time of hospital admission (*Time point 1*) and during admission (~5 days after hospitalization;
157 *Time point 2*). All patients (with and without clinical diagnosis of delirium) started to present
158 COVID-19 symptoms ~5 days before hospitalisation. N=18 patients presenting with delirium
159 symptoms at the time of hospital admission were age- and sex-matched with N=18 patients
160 who did not present any symptoms of delirium, both at time of hospitalization and across
161 disease progression (see Table 1). None of these patients have been admitted to ITU prior or at
162 either time of samples collection. Delirium symptoms were documented in clinical notes by
163 the treating physician around the time of blood collection (during admission, Time point 1; and

164 ~5 days (range: 2-7 days) after hospitalization, Time point 2). In both groups, the majority of
165 patients presented with two or more medical comorbidities, with hypertension and type 2
166 diabetes being the most frequently observed; there were no differences in respiratory symptoms
167 severity, and survival rate, between the two groups (see Table 1). Levels of C reactive protein
168 (CRP) in serum also did not significantly differ between the two groups, at both time points
169 (group effect: $F=1.3$, $p=0.2$; time effect: $F=0.4$, $p=0.8$, see Table 1). The study was approved
170 by the King's College Hospital Research Ethics Committee (Ref: 20/SC/0310).
171
172 **Cell culture:** Multipotent human hippocampal progenitor cell line HPC0A07/03C (provided by
173 ReNeuron, Surrey, UK) was used[22-25, 30-34, 36]. This model has been previously validated using
174 a hippocampal newborn neuron specific marker, Prospero homeobox protein 1 (Prox1) [31]. Cells
175 were left to proliferate in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12
176 (DMEM/F-12) media to which we added the growth factors epidermal growth factor (EGF),
177 basic fibroblast growth factor (bFGF) and 4-hydroxytamoxifen (4-OHT). Differentiation was
178 initiated by removal of the growth factors and 4-OHT. Detailed information on this cell line
179 can be found in our previous publication[31].
180
181 **In vitro treatment with serum samples from COVID-19 patients with and without**
182 **delirium:** Cells were plated on 96 well plates (Nunclon) at a density of 1.2×10^4 cells per
183 well. Following our established model[28], after 1 day of proliferation, cells were treated with
184 media containing 1% serum (from each COVID-19 patient, delirium and non-delirium) and
185 0.5mg/ml penicillin for 2 days. At this stage cells were either fixed with 4% PFA for 20 min
186 at room temperature and immunostained, or left to differentiate for additional 4 days, again
187 in presence of 1% serum (from the same COVID-19 patient previously used in the
188 proliferation phase) and 0.5mg/ml penicillin, and then again fixed and immunostained. In

189 addition, at the end of 1 day of serum treatment during proliferation, and of 1 day of serum
190 treatment during differentiation, cell supernatant was collected for subsequent cytokines
191 measurement (Figure 1a, b, c and d). For the experiment with the antibodies against
192 cytokines, anti-IL6 antibody (0.1ug/ml) was added during both proliferation and
193 differentiation, anti-IL12 antibody (0.3ug/ml) during proliferation, and anti-IL13 antibody
194 (0.1ug/ml) during differentiation (Figure 1a-e), based on the results of supernatant cytokines
195 measurement. Finally, for the experiments with the Janus kinase (JAK) inhibitors, baricitinib,
196 ruxolitinib or tofacitinib (all 1nM) were added during proliferation or differentiation together
197 with recombinant IL12 or IL13 at concentrations previously found in supernatant of cells
198 exposed to serum from patients with delirium (IL12: 20pg/ml, IL13: 25pg/ml) or without
199 delirium (IL12: 3pg/ml, IL13: 4pg/ml) (Figure 1f, g, Supplementary Figure 5a-e).
200
201 **Immunocytochemistry and quantification of immunofluorescence:** During the proliferation
202 stage, fixed cells were stained for markers of stemness (sex determining region Y-box 2 (Sox2),
203 Alexa 488 donkey anti-rabbit; 1:1000, Invitrogen; Nestin, Alexa 555 donkey anti-mouse, 1:1000,
204 Invitrogen), proliferation (Ki67, Alexa 488 donkey anti-rabbit; 1:1000, Invitrogen) and apoptosis
205 (CC3, Alexa 555 donkey anti-mouse, 1:1000, Invitrogen). During the differentiation stage, cells
206 were stained for markers of neuroblasts and mature neurons (respectively, doublecortin (DCX),
207 Alexa 555 donkey anti-rabbit; 1:1000, Invitrogen; microtubule-associated protein 2 (Map2),
208 Alexa 488 donkey anti-mouse, 1:1000, Invitrogen), astrocytes (S100 calcium-binding protein β
209 (S100β), Alexa 488 donkey anti-rabbit; 1:1000, Invitrogen), and again apoptosis (CC3; Alexa
210 555 donkey anti-rabbit, 1:1000, Invitrogen). All cells were labelled using DAPI dye, as in
211 previous publications[22, 25, 28, 37-39]. The percentage of Sox2, Nestin, Ki67, DCX, Map2, S100β
212 and CC3 positive cells over total DAPI positive cells was counted using an insight automated
213 imaging platform (CellInsight) (Supplementary Figure 1a-h for representative images). At least

214 six independent experiments were conducted on independent biological cultures, and each
215 sample was tested in quadruplicate.
216
217 **Multiplex cytokine measurement in cell supernatant and serum samples:** Cell supernatants
218 and serum samples from COVID-19 patients with and without delirium (collected both at *Time*
219 *point 1* and *2*) were used for cytokines measurement (IL1β, IL2, IL4, IL6, IL8, IL10, IL12,
220 IL13, TNF-α, IFN-γ), using the Human ProInflammatory Multiplex Very-Sensitive Kit from
221 Meso Scale Discovery (MSD) (Gaithersburg, MD) and the SECTOR Imager MSD device,
222 according to the manufacturers' instructions. Detailed information on the cytokines analyses
223 procedure can be found in our previous publication[22, 23, 40]. At least six independent experiments
224 were conducted on independent biological cultures, and each sample was tested in duplicates.
225
226 **Statistical Analysis:** Statistical analyses were performed with IBM SPSS statistical software
227 version 25, StataCorp STATA version 16 and GraphPad Prism version 8 and consisted of two-
228 way mixed analysis of variance (ANOVA), Chi-square $\chi 2$ test, Mann-Whitney U-test, followed
229 by Bonferroni's post hoc analyses where appropriate. Variance was tested using the Shapiro–
230 Wilk test. Data are presented as mean±SD, and p-values≤0.05 were considered significant.
231

232 **RESULTS**

233 **Treatment with serum samples from COVID-19 patients with delirium decreased cell**
234 **proliferation, neurogenesis, and increased apoptosis, when compared with serum from**
235 **non-delirium patients.**

236 As in our established protocol[28], we exposed cells to 2 days treatment during proliferation,
237 with 1% serum sample from each COVID-19 patient with or without delirium, collected both
238 at time of hospital admission (*Time point 1*) and during admission (*Time point 2*). We

239 measured markers of cell proliferation (Ki67), apoptosis (CC3) and stemness (Sox2, Nestin)
240 (Figure 1a). Interestingly, we found that treatment of cells with serum samples from delirium
241 patients decreased cell proliferation (group effect: $p<0.0001$; time effect: $p=0.6$, Figure 2a,
242 Supplementary Figure 1i) and increased apoptosis (group effect: $p<0.0001$; time effect: $p=0.5$,
243 Figure 2b, Supplementary Figure 1j), with no differences between cell treated with serum
244 samples collected at *Time point 1* or *Time point 2*.
245

246 We did not observe any difference in the marker of stemness (Sox2 or Nestin), when
247 comparing the two groups (delirium vs non-delirium) and the two time points (Supplementary
248 Figure 2a, b).
249

250 In the differentiation experiments, after the 2 days of treatment during proliferation, we left
251 cells to differentiate for additional 4 days, again in presence of 1% serum. We measured
252 markers of neurogenesis (DCX, Map2), astrogliogenesis (S100β) and apoptosis (CC3)
253 (Figure 1b). We found that treatment of cells with serum samples from delirium patients
254 decreased neurogenesis (DCX: group effect: $p<0.0001$; time effect: $p=0.9$, Figure 2c,
255 Supplementary Figure 1k; Map2: group effect: $p<0.0001$; time effect: $p=0.9$, Figure 2d,
256 Supplementary Figure 1l) and increased apoptosis (group effect: $p<0.0001$; time effect: $p=0.9$,
257 Figure 2e, Supplementary Figure 1m), again with no differences between the two time points.
258

259 Also, we did not observe any differences in the marker of astrogliogenesis (S100β), when
260 comparing the two groups (delirium vs non-delirium) and the two time points (Supplementary
261 Figure 2c).
262

263 **Higher concentration of IL6 in serum samples from COVID-19 patients with delirium**
264 **induces cells to produce IL12 and IL13, respectively during the proliferation and**
265 **differentiation stage.**
266 In order to investigate the molecular mechanisms through which cell treatment with serum
267 samples from delirium patients detrimentally affected cell proliferation, neurogenesis and
268 apoptosis, we first measured levels of candidate cytokines (IL1β, IL2, IL4, IL6, IL8, IL10,
269 IL12, IL13, TNF-α, IFN-γ) known to be modulated by the SARS-CoV-2 virus[11-13], in serum
270 samples of both patients with delirium and without delirium.
271
272 Interestingly, we found a significantly higher concentration of IL6 in serum samples of
273 patients with delirium (229.9±79.1pg/ml, Figure 3a-b), when compared with serum samples
274 from patients without delirium (32.5±9.5pg/ml, Figure 3a-b), again with no differences
275 between serum samples collected at *Time point 1* or *Time point 2* (group effect: $p<0.0001$;
276 time effect: $p=0.4$, Figure 3b). None of the other cytokines were differentially expressed
277 between the two groups or the two time points (Supplementary Figure 3a-i).
278
279 We then measured the same panel of cytokines in supernatant of cells exposed to treatment
280 with serum samples from patients with or without delirium (collected both at *Time point 1*
281 and *2*). In particular, cell supernatant was collected after 1 day of serum treatment during
282 proliferation, and 1 day of serum treatment during differentiation (Figure 1a and b).
283
284 Results showed significantly higher levels of IL12 during the proliferation, and IL13 during
285 the differentiation, in the supernatant of cells treated with serum from delirium patients vs
286 non-delirium patients (IL12: 20.6±2.7pg/ml vs 2.8±2pg/ml, Figure 3c-d; IL13: 24.6±3pg/ml
287 vs 3.9±3.1pg/ml, Figure 3e-f), with no differences between supernatant of cells treated with

288 serum samples collected at *Time point 1* or *Time point 2* (IL12: group effect: $p<0.0001$; time
289 effect: $p=0.8$, Figure 3d; IL13: group effect: $p<0.0001$; time effect: $p=0.9$, Figure 3f).
290
291 Of note, concentrations of the same cytokines (IL12 and IL13) in the original serum samples
292 were much lower, and did not differ between delirium and non-delirium patients (IL12:
293 2.1±1pg/ml vs 2.05±1pg/ml, Supplementary Figure 3f; IL13: 2.7±0.7pg/ml vs 2.8±1pg/ml,
294 Supplementary Figure 3g), indicating that these differences in the supernatant levels are due
295 to new production of these cytokines by the cells.
296
297 Finally, none of the other cytokines were differentially expressed in the supernatant of cells
298 treated with serum samples from delirium or non-delirium patients, or from *Time point 1* or
299 *Time point 2* (Supplementary Figure 3j-zz).
300
301 **During proliferation, treatment with an antibody against IL6 prevents the detrimental**
302 **effect of serum from COVID-19 patients with delirium on both cell proliferation and**
303 **apoptosis, and decreases IL12 production.**
304 In order to confirm that the detrimental effect of treatment with serum from delirium patients
305 on cell proliferation and apoptosis was due to the higher levels of IL6 in the serum samples
306 of the same patients, we exposed cells to treatment with serum from COVID-19 patients
307 together with an antibody against IL6 (Figure 1c).
308
309 Interestingly, treatment with IL6 antibody prevented the decrease in proliferation (Ki67) and
310 increase in apoptosis (CC3) caused by treatment with serum from delirium patients, when
311 compared with treatment with serum from non-delirium patients (*Time point 1*, delirium vs
312 delirium + IL6 antibody; for Ki67, 40.2% vs 61.3%, $p<0.0001$; for CC3, 10.2% vs. 3.4%,

p<0.0001), with no differences between cells treated with serum samples collected at *Time point 2* (Figure 4a-d).

Moreover, treatment of cells with serum samples from delirium patients and IL6 antibody prevented the production of IL12 in cell supernatant (*Time point 1*, delirium vs delirium + IL6 antibody; IL12: 20.6±2.7pg/ml vs 4.1±2.1pg/ml, p<0.0001,), again with no differences between *Time point 1* and *Time point 2* (Figure 1c, Figure 4e, f).

This finding seems to suggest that, during proliferation, the detrimental effect on cell proliferation and apoptosis caused by IL6, which is produced in higher concentrations in serum from delirium patients, may be mediated by the production of IL12 in the brain environment. Indeed, treatment of cells with an antibody against IL12 prevented the decrease in cell proliferation (Ki67) and increase in apoptosis (CC3) caused by treatment with serum from delirium patients (*Time point 1*, delirium vs delirium + IL12 antibody; for Ki67, 40.2% vs 62.2%, p<0.0001; for CC3, 10.2% vs 3.2%, p<0.0001). Again, there were no differences between cells treated with serum samples collected at *Time point 1* or *Time point 2* (Figure 1c, Figure 4g-j).

During differentiation, treatment with an antibody against IL6 prevents the detrimental effect of serum from COVID-19 patients with delirium on both neurogenesis and apoptosis, and decreases IL13 production.

In order to investigate whether the detrimental effect of treatment with serum from delirium patients on neurogenesis and apoptosis during *differentiation* was also mediated by a higher concentration of IL6 in the serum samples of the same patients, we exposed cells to treatment

337 with serum and an antibody against IL6, first during proliferation, then during differentiation,
338 and finally during both proliferation and differentiation (Figure 1d, e).
339
340 Exposing cells to IL6 antibody during the proliferation stage did not prevent the effect of
341 serum on the aforementioned cellular outcomes (Supplementary Figure 4a-f). However,
342 treatment of cells with IL6 antibody during the differentiation stage prevented the reduction
343 in neurogenesis (DCX, Map2) and increase in apoptosis (CC3) previously observed upon
344 treatment with serum from delirium patients, when compared with serum from non-delirium
345 patients (*Time point 1*, delirium vs delirium + IL6 antibody; for DCX, 19.1% vs 34.8%,
346 $p<0.0001$; for Map2, 25.1% vs 40.7%, $p<0.0001$; for CC3, 17.3% vs 3.4%, $p<0.0001$). Again,
347 there were no differences between cells *Time point 1* and *Time point 2* (Figure 5a-f). Results
348 were confirmed when exposing cells to IL6 antibody during both the proliferation and
349 differentiation stage (Supplementary Figure 4g-l).
350
351 Moreover, similar to the effects of IL6 antibody during the proliferation phase on the
352 production of IL12, treatment of cells with serum samples from delirium patients and IL6
353 antibody during the differentiation stage prevented the production of IL13 in cell supernatant,
354 (*Time point 1*, delirium vs delirium + IL6 antibody; IL13: 24.6±3pg/ml vs 5.1±2.7pg/ml,
355 $p<0.0001$,), again with no differences between *Time point 1* and *Time point 2* (Figure 1e,
356 Figure 5g, h).
357
358 In this case, finding seems to suggest that, during differentiation, the detrimental effect on
359 neurogenesis and apoptosis caused by the high IL6 concentrations in serum from delirium
360 patients is mediated by production of IL13. Indeed, treatment of cells with an antibody against
361 IL13 prevented the decrease in neurogenesis (DCX, Map2) and increase in apoptosis (CC3)

caused by treatment with serum from delirium patients (*Time point 1*, delirium vs delirium + IL13 antibody; for DCX, 19.1% vs 33.8%, $p<0.0001$; for Map2, 25.1% vs 41.4%, $p<0.0001$; for CC3, 17.3% vs 3.8%, $p<0.0001$). Again, there were no differences between *Time point 1* and *Time point 2* (Figure 1c, Figure 5i-n).

Treatment with recombinant IL12 or IL13, at concentrations previously found in supernatant of cells exposed to serum from patients with delirium, decreases cell proliferation, neurogenesis and increase apoptosis, whereas co-treatment with the JAK inhibitors, baricitinib, ruxolitinib and tofacitinib, prevents these detrimental effects.

Having identified the downstream production of IL12 and IL13 in supernatant of cells exposed to serum from patients with delirium as the mechanism responsible for the reduced cell proliferation and neurogenesis and increased apoptosis, we decided to test whether 1) these same effects could be replicated by treating cells directly with IL12 and IL13 at the same concentrations measured in the supernatant following treatment with serum; and 2) if treatment with effective and commonly administered therapeutic compounds for patients with acute COVID-19, the JAK inhibitors baricitinib, ruxolitinib and tofacitinib (all 1nM), could prevent these detrimental effects by IL12 and IL13.

For this purpose, we treated cells directly with recombinant IL12 and IL13, used at concentrations previously found in supernatant of cells exposed to serum from patients with delirium (IL12 delirium: 20pg/ml and IL13 delirium: 25pg/ml) or without delirium (IL12 without delirium: 3pg/ml, IL13 without delirium: 4pg/ml, Figure 1f, g), with or without the JAK inhibitors.

386 As hypothesised, during proliferation, treatment with IL12 (20pg/ml) was able to cause a
387 decrease in cell proliferation (Ki67) and increase in apoptosis (CC3), when compared with
388 IL12 (3pg/ml) (for Ki67, 42.8% vs 71.6%, p<0.0001; for CC3, 11.5% vs 5.6%, p<0.0001,
389 Supplementary Figure 5a, b). This is similar to what previously shown upon exposure of cells
390 to serum from patients. Interestingly, co-treatment with baricitinib, ruxolitinib or tofacitinib
391 were able to prevent these detrimental effects (IL12 20pg/ml vs IL12 3pg/ml +
392 vehicle/baricitinab/ruxolitinab/tofacitinab; for Ki67, 42.8% vs 68.4%, p<0.001, vs 69%,
393 p<0.001, vs 71.4%, p<0.001; for CC3, 11.5% vs 5.7%, p<0.0001, vs 5%, p<0.0001, vs 5.4%,
394 p<0.0001, Supplementary Figure 5a, b).
395
396 Similarly, during differentiation, treatment with IL13 (25pg/ml) was able to cause a decrease
397 in neurogenesis (DCX and Map2) and increase in apoptosis (CC3), when compared with IL13
398 (4pg/ml) (for DCX, 20.8% vs 31%, p<0.0001; for Map2, 27.2% vs 41%, p<0.0001; for CC3,
399 15.8% vs 7.6%, p<0.0001, Supplementary Figure 5c-e). This is, again, similar to what
400 previously shown upon exposure of cells to serum from patients. Again, co-treatment with
401 baricitinib, ruxolitinib or tofacitinib were able to prevent these detrimental effects (IL13
402 25pg/ml vs IL13 4pg/ml + vehicle/baricitinab/ruxolitinab/tofacitinab; for DCX, 20.8% vs
403 30.4%, p<0.001, vs 29%, p<0.001, vs 30%, p<0.001; for Map2, 27.2% vs 39.1%, p<0.001,
404 vs 39.7%, p<0.001, vs 39.7%, p<0.001; for CC3, 15.8% vs 7.4%, p<0.0001, vs 8.3%,
405 p<0.0001, vs 8.1%, p<0.0001, Supplementary Figure 5c-e).
406

407 DISCUSSION

408 To our knowledge, this is the first study to identify the high IL6 levels in the serum of COVID-
409 19 hospitalised patients with neurological symptoms (delirium) as the molecular mechanism
410 leading to disruption in neurogenic processes, measured *in vitro* using a unique human model

411 of hippocampal precursors. Interestingly, IL-6-induced increased production by these
412 hippocampal cells of IL12 (during proliferation) and IL13 (during differentiation) is the
413 molecular mechanisms downstream to IL6, suggesting that brain production of cytokines in
414 response to peripheral inflammation is important in neurological symptoms induced by
415 COVID-19 infection. Indeed, treatment with an antibody against IL12 or IL13 prevented the
416 effect of serum from delirium patients on cell proliferation, cell differentiation and apoptosis.
417 Of note, treatment with the therapeutic compounds commonly administered to acute COVID-
418 19 patients (the JAK inhibitors baricitinib, ruxolitinib and tofacitinib) were able to restore
419 normal cell viability, proliferation and neurogenesis by targeting the effects of IL12 and IL13.
420 The novelty of our study resides in the use of a well-established but, at the same time, unique
421 model of human hippocampal neurogenesis[25, 30-34], and a validated "blood-brain-axis"
422 experimental assay[28] using serum samples from COVID-19 patients.
423

424 While there is evidence showing that patients with COVID-19 present with neurological
425 symptoms both during and after the disease (the latter phenomenon is the so-called "long
426 COVID")[7, 8, 41], the brain mechanisms through which this occur have not been identified yet.
427 So far there is some indication that the SARS-CoV-2 can affect transcriptionally brain cells
428 from the parenchymal and choroid plexus, and is able to reduce hippocampal neurogenesis in
429 animal and human models of COVID-19[42-44]. Similarly, there is evidence for an association
430 between neuroinflammation and neurological symptoms during the acute and mild phase of
431 COVID-19[45-47]. Our study identifies IL6 and IL6-induced IL12 and IL13 as the putative
432 mechanisms mediating these effects. Specifically, we found a 6-fold increase in the
433 concentrations of IL6 in serum samples of patients with delirium (229.9±105.2pg/ml), when
434 compared with patients without delirium (32.5±9.5pg/ml). In line with our findings, higher
435 levels of IL6 have been reported also by other studies in the periphery and in the cerebrospinal

436 fluid (CSF) of COVID-19 patients[12, 13, 21, 48, 49], as well as in children with acute encephalitis-
437 like syndrome during infection with coronavirus-OC43[50]. Overall, these findings identify IL6
438 an important therapeutic target not only for COVID-19 symptoms in general, but also for those
439 neurological manifestations which are commonly observed as a consequence of the disease.
440 Interestingly, this result is consistent with our recent study showing that exposing the same
441 cells to concentrations of IL6 ranging from 50 to 5000pg/ml dramatically decrease
442 neurogenesis and increase apoptosis[22]. Likewise, previous findings generated in other
443 laboratories also demonstrated that exposure to concentrations of IL6 similar to those detected
444 in the present study is associated with a significant reduction in neurogenesis and increase in
445 apoptosis, both *in vitro* and *in vivo*[51-53].
446
447 Of note, treatment with serum from delirium patients induced our cells to produce two well-
448 known inflammatory cytokines, IL12 and IL13, in the supernatant; these cytokines were in
449 very low quantities in serum, indicating a direct production by cells. The causal role of IL6
450 and IL6-induced IL12 and IL13 in mediating the effect of serum from delirium patients on
451 the neurogenic outcomes is confirmed by the use of an antibody against these cytokines in
452 our experiments. Interestingly, IL12 was produced only during the proliferation stage,
453 whereas IL13 only during the differentiation stage, but always as a consequence of IL6
454 stimulation. Interestingly, while both IL12 and IL13 are expressed in the brain[54, 55], IL12 is
455 primarily known to regulate cell proliferation[54], whereas IL13 is mainly involved in
456 differentiation-related processes[55]. Indeed, IL12 reduces cell proliferation and migration in
457 several neuronal cultures via activation of distinct pro-inflammatory signalling pathways,
458 including janus-activated kinase and signal transducers and activators of transcription (JAK-
459 STAT) pathway[56, 57], which we have also previously identified to be involved in the regulation
460 of neuronal apoptosis upon exposure of hippocampal cell to IFN-α treatment[24]. Accordingly,

461 previous evidence has reported an increase in the production of IL12 and IL13 in the CSF of
462 COVID-19 patients with neurological manifestations[58, 59]. Similarly, in mice, studies have
463 shown that increased cerebral expression of IL12 is able to induce neurological symptoms,
464 and that these symptoms are worsen by an infectious disease[54]. Likewise, in humans, IL13
465 expression has shown to be associated with the development of neurological disorders, and
466 with the experience of more severe neuropsychiatric symptoms. In particular, individuals with
467 high IL13 expression are more likely to develop neurological diseases, including
468 Parkinson's[60], whereas in those individuals who already suffer from a major depressive
469 disorder, higher IL13 levels are associated with more severe depression and a higher number
470 of suicide attempts[61].
471
472 Our study identifies multiple pharmacological approaches that could be beneficial for
473 treatment of neurological symptoms in the. context of COVID-19 infection, and thus
474 potentially in other conditions associated with high levels of IL6. First, IL6 itself can be
475 considered a valuable therapeutic target in treating COVID-19 patients. Indeed, treatment
476 with IL6 receptor antagonists, such as tocilizumab or sarilumab, have so far shown a relatively
477 good level of efficacy in reducing COVID-19 mortality rates, although these compounds have
478 a high molecular weight, making them unable to act centrally[62]. Second, there are several
479 IL12 inhibitors available, such as ustekinumab and briakinumab, which have already been
480 tested for their efficacy and tolerability in clinical context[63]. However, these compounds are
481 not exclusively targeting IL12, but rather the p40 subunit of both IL12 and IL23, and have a
482 relatively high molecular weight, making them less likely to exert their properties centrally[64].
483 The situation is similar for IL-13: although a selective inhibitor, lebrikizumab, has already
484 been developed and tested in a phase 2b trial, this compound also has a very high molecular
485 weight, making it unsuitable for treatment of brain inflammatory neuropathologies[65]. Finally,

486 we have shown that the JAK inhibitors, baricitinib, ruxolitinib and tofacitinib, prevent the
487 reduction in cell proliferation and neurogenesis, and the increase in apoptosis, caused by
488 treatment with recombinant IL12 or IL13. Recently randomized, placebo-controlled trials of
489 these have shown these compounds to be among the most effective treatment interventions
490 for acute COVID-19 patients, especially for those with a high level of inflammation[66-68].
491 Moreover, these JAK inhibitors have low molecular weight (< 400Da) making them suitable
492 to act centrally, and potentially on mechanisms linked to the neuronal production of IL12 and
493 IL13 that we describe. All together, we propose that baseline stratification of patients based
494 on their level of inflammatory biomarkers, including IL6, IL12 and IL13, or the presence of
495 neurological symptoms, may represent the best therapeutic approach for those individuals
496 experiencing severe COVID-19 infection.
497
498 We acknowledge several limitations associated with our study, including the use of an *in vitro*
499 system of immortalized cell line. However, while theoretically this system may differ from
500 the scenario of an adult in vivo environment and the adult neurogenic niche, in particular
501 because of the absence of microglia cells and the fetal status of the immortalized cells, we
502 have extensive confirmation from previous studies that our results are relevant to the human
503 and animal brains. Indeed, over the years we have been able to replicate all our results
504 obtained in this *in vitro* model in both the animal brain and the human blood, including
505 changes in neurogenesis by cortisol, antidepressants, IL1β, IFN-α, and serum samples from
506 depressed patients, and changes in stress-, antidepressants- and inflammation-regulated
507 mRNA gene expression in both the hippocampal mRNA of animal models of depression and
508 the whole blood mRNA of depressed patients[22, 25, 28, 30-33, 40, 69, 70]. Nevertheless, in future
509 studies, we aim to expand this cellular model using both neurons and microglia co-cultures,
510 in order to create a more comprehensive and realistic experimental scenario. Also, our study

does not investigate the direct effects of COVID-19 on these cells. However, considering that there is evidence showing that the virus has difficulty crossing the BBB in humans, and that its central effect is instead mediated by the activation of peripheral mechanisms[71-73], we believe that our experimental approach, exposing brain cells to serum from COVID-19 infected patients rather than to the virus itself, provides mechanistic understanding on how COVID-19 indirectly regulates brain function. Finally, the number of samples used in this study, although matched for age and sex, was also relatively small, 18 patients per group. Therefore, the results presented here should be replicated in a bigger cohort of COVID-19 patients with and without delirium, including patients with long COVID, who are also known to experience persistent neurological manifestations[41]. However, while the sample size is small, this is the first study demonstrating for the first time that it is the elevated peripheral concentration of IL-6 (rather than of other pro-inflammatory cytokines) that clinically affects cognition and mechanistically affects neurogenesis in the same COVID-19 patients with delirium. In addition, the identification of the IL12 and IL13 as downstream mediators is a completely novel mechanism for the IL-6-induced reduction in neurogenesis, and the novel experiments using JAK inhibitors performed in the present study offer additional mechanistic insights into these pathways.

In summary, our *in vitro* study demonstrates that that serum from COVID-19 patients with delirium can detrimentally affect human hippocampal neurogenesis, and that this effect is mediated by IL6-induced production of the downstream inflammatory cytokines IL12 and IL13, which are ultimately responsible for the negative cellular outcomes. Understanding the mechanisms through which neurogenesis is altered in COVID-19 patients with neurological symptoms will ultimately contribute to the development of novel interventional and

535 preventative strategies for this particular sub-group of patients, and potentially for patients with

536 cognitive and neurological symptoms in the context of inflammation.

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Author Contributions

AB, BM, JE and CMP designed research; AB performed research and analysed data; AB, BM, JE, GM, DS, ACV, GN, ST and CMP wrote the paper.

Statement of Interest

Dr Alessandra Borsini, Professor Carmine M. Pariante and Professor Sandrine Thuret have received research funding from Johnson & Johnson for research on depression and inflammation which included cellular work (2012-2018), but this work is unrelated to that funding; moreover, less than 10% of Professor Pariante's support in the last 10 years derives from commercial collaborations, including: a strategic award from the Wellcome Trust (Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA) Consortium, grant 104025), in partnership with Janssen, GlaxoSmithKline, Lundbeck and Pfizer; and consultation and speakers fees from Boehringer Ingelheim, Eli Lilly, Compass, Eleusis, GH Research, Lundbeck, and Värde Partners. Professor Thuret received funding for a project on neural stem cell aging co-funded by the UK Medical Research Council and Merck Sharp &

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References:

1. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020.

2. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A *et al.* Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. *JAMA* 2020.

3. Acter T, Uddin N, Das J, Akhter A, Choudhury TR, Kim S. Evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as coronavirus disease 2019 (COVID-19) pandemic: A global health emergency. *The Science of the total environment* 2020; **730:** 138996.

4. Gklinos P. Neurological manifestations of COVID-19: a review of what we know so far. *J Neurol* 2020.

5. Taquet M, Geddes JR, Husain M, Luciano S, Harrison PJ. 6-month neurological and psychiatric outcomes in 236 379 survivors of COVID-19: a retrospective cohort study using electronic health records. *Lancet Psychiatry* 2021; **8**(5)**:** 416-427.

6. Oh ES, Fong TG, Hshieh TT, Inouye SK. Delirium in Older Persons: Advances in Diagnosis and Treatment. *JAMA* 2017; **318**(12)**:** 1161-1174.

7. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q *et al.* Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol* 2020.

8. Kennedy M, Helfand BKI, Gou RY, Gartaganis SL, Webb M, Moccia JM *et al.* Delirium in Older Patients With COVID-19 Presenting to the Emergency Department. *JAMA Netw Open* 2020; **3**(11)**:** e2029540.

9. Pun BT, Badenes R, Heras La Calle G, Orun OM, Chen W, Raman R *et al.* Prevalence and risk factors for delirium in critically ill patients with COVID-19 (COVID-D): a multicentre cohort study. *Lancet Respir Med* 2021; **9**(3)**:** 239-250.

10. O'Hanlon S, Inouye SK. Delirium: a missing piece in the COVID-19 pandemic puzzle. *Age Ageing* 2020.

652 11. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ *et al.* COVID-
653 19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;
654 **395**(10229)**:** 1033-1034.
655
656 12. Zhao Y, Qin L, Zhang P, Li K, Liang L, Sun J *et al.* Longitudinal COVID-19 profiling
657 associates IL-1RA and IL-10 with disease severity and RANTES with mild disease.
658 *JCI Insight* 2020; **5**(13).
659
660 13. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y *et al.* Clinical features of patients infected
661 with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**(10223)**:** 497-506.
662
663 14. Yarlagadda A, Alfson E, Clayton AH. The blood brain barrier and the role of cytokines
664 in neuropsychiatry. *Psychiatry (Edgmont)* 2009; **6**(11)**:** 18-22.
665
666 15. Gampierakis IA, Koutmani Y, Semitekolou M, Morianos I, Polissidis A, Katsouda A
667 *et al.* Hippocampal neural stem cells and microglia response to experimental
668 inflammatory bowel disease (IBD). *Molecular psychiatry* 2021; **26**(4)**:** 1248-1263.
669
670 16. Marx W, Lane M, Hockey M, Aslam H, Berk M, Walder K *et al.* Diet and depression:
671 exploring the biological mechanisms of action. *Molecular psychiatry* 2021; **26**(1)**:** 134-
672 150.
673
674 17. Simone MJ, Tan ZS. The role of inflammation in the pathogenesis of delirium and
675 dementia in older adults: a review. *CNS neuroscience & therapeutics* 2011; **17**(5)**:** 506-
676 513.
677
678 18. Anacker C, Hen R. Adult hippocampal neurogenesis and cognitive flexibility - linking
679 memory and mood. *Nat Rev Neurosci* 2017; **18**(6)**:** 335-346.
680
681 19. Alam A, Hana Z, Jin Z, Suen KC, Ma D. Surgery, neuroinflammation and cognitive
682 impairment. *EBioMedicine* 2018; **37:** 547-556.
683
684 20. Boldrini M. The Many Avenues Leading to Adult Neurogenesis Adaptations.
685 *Biological psychiatry* 2020; **88**(8)**:** 588-590.
686
687 21. McElvaney OJ, McEvoy NL, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM *et*
688 *al.* Characterization of the Inflammatory Response to Severe COVID-19 Illness.
689 *American journal of respiratory and critical care medicine* 2020; **202**(6)**:** 812-821.
690
691 22. Borsini A, Di Benedetto MG, Giacobbe J, Pariante CM. Pro- and anti-inflammatory
692 properties of interleukin (IL6) in vitro: relevance for major depression and for human
693 hippocampal neurogenesis. *Int J Neuropsychopharmacol* 2020.
694
695 23. Borsini A, Nicolaou A, Camacho-Munoz D, Kendall AC, Di Benedetto MG, Giacobbe
696 J *et al.* Omega-3 polyunsaturated fatty acids protect against inflammation through
697 production of LOX and CYP450 lipid mediators: relevance for major depression and
698 for human hippocampal neurogenesis. *Molecular psychiatry* 2021; **26**(11)**:** 6773-6788.
699
700 24. Borsini A, Cattaneo A, Malpighi C, Thuret S, Harrison NA, Consortium MRCI *et al.*
701 Interferon-Alpha Reduces Human Hippocampal Neurogenesis and Increases Apoptosis

702 via Activation of Distinct STAT1-Dependent Mechanisms. *Int J*
703 *Neuropsychopharmacol* 2018; **21**(2)**:** 187-200.
704
705 25. Borsini A, Alboni S, Horowitz MA, Tojo LM, Cannazza G, Su KP *et al.* Rescue of IL-
706 1beta-induced reduction of human neurogenesis by omega-3 fatty acids and
707 antidepressants. *Brain, behavior, and immunity* 2017; **65:** 230-238.
708
709 26. Crupi R, Cambiaghi M, Spatz L, Hen R, Thorn M, Friedman E *et al.* Reduced adult
710 neurogenesis and altered emotional behaviors in autoimmune-prone B-cell activating
711 factor transgenic mice. *Biological psychiatry* 2010; **67**(6)**:** 558-566.
712
713 27. Koo JW, Wohleb ES. How Stress Shapes Neuroimmune Function: Implications for the
714 Neurobiology of Psychiatric Disorders. *Biological psychiatry* 2021; **90**(2)**:** 74-84.
715
716 28. Borsini A, Pariante CM, Zunszain PA, Hepgul N, Russell A, Zajkowska Z *et al.* The
717 role of circulatory systemic environment in predicting interferon-alpha-induced
718 depression: The neurogenic process as a potential mechanism. *Brain, behavior, and*
719 *immunity* 2019; **81:** 220-227.
720
721 29. Perini G, Cotta Ramusino M, Sinforiani E, Bernini S, Petrachi R, Costa A. Cognitive
722 impairment in depression: recent advances and novel treatments. *Neuropsychiatr Dis*
723 *Treat* 2019; **15:** 1249-1258.
724
725 30. Zunszain PA, Anacker C, Cattaneo A, Choudhury S, Musaelyan K, Myint AM *et al.*
726 Interleukin-1beta: a new regulator of the kynurenine pathway affecting human
727 hippocampal neurogenesis. *Neuropsychopharmacology : official publication of the*
728 *American College of Neuropsychopharmacology* 2012; **37**(4)**:** 939-949.
729
730 31. Anacker C, Cattaneo A, Luoni A, Musaelyan K, Zunszain PA, Milanesi E *et al.*
731 Glucocorticoid-related molecular signaling pathways regulating hippocampal
732 neurogenesis. *Neuropsychopharmacology : official publication of the American*
733 *College of Neuropsychopharmacology* 2013; **38**(5)**:** 872-883.
734
735 32. Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S *et al.*
736 Antidepressants increase human hippocampal neurogenesis by activating the
737 glucocorticoid receptor. *Molecular psychiatry* 2011; **16**(7)**:** 738-750.
738
739 33. Anacker C, Cattaneo A, Musaelyan K, Zunszain PA, Horowitz M, Molteni R *et al.* Role
740 for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal
741 neurogenesis. *Proceedings of the National Academy of Sciences of the United States of*
742 *America* 2013; **110**(21)**:** 8708-8713.
743
744 34. Horowitz MA, Wertz J, Zhu DH, Cattaneo A, Musaelyan K, Nikkheslat N *et al.*
745 Antidepressant Compounds Can Be Both Pro- and Anti-Inflammatory in Human
746 Hippocampal Cells. *International Journal of Neuropsychopharmacology* 2015; **18**(3).
747
748 35. de Lucia C, Murphy T, Maruszak A, Wright P, Powell TR, Hartopp N *et al.* Serum
749 from Older Adults Increases Apoptosis and Molecular Aging Markers in Human
750 Hippocampal Progenitor Cells. *Aging Dis* 2021; **12**(8)**:** 2151-2172.
751

752 36. Borsini A, Stangl D, Jeffries AR, Pariante CM, Thuret S. The role of omega-3 fatty
753 acids in preventing glucocorticoid-induced reduction in human hippocampal
754 neurogenesis and increase in apoptosis. *Translational psychiatry* 2020; **10**(1)**:** 219.
755
756 37. Borsini A, Cattaneo A, Malpighi C, Thuret S, Harrison NA, Zunszain PA *et al.*
757 Interferon-Alpha Reduces Human Hippocampal Neurogenesis and Increases Apoptosis
758 via Activation of Distinct STAT1-Dependent Mechanisms. *Int J Neuropsychoph* 2018;
759 **21**(2)**:** 187-200.
760
761 38. Smeeth DM, Kourouzidou I, Duarte RRR, Powell TR, Thuret S. Prolactin, Estradiol
762 and Testosterone Differentially Impact Human Hippocampal Neurogenesis in an In
763 Vitro Model. *Neuroscience* 2021; **454:** 15-39.
764
765 39. Houghton V, Du Preez A, Lefevre-Arbogast S, de Lucia C, Low DY, Urpi-Sarda M *et*
766 *al.* Caffeine Compromises Proliferation of Human Hippocampal Progenitor Cells.
767 *Front Cell Dev Biol* 2020; **8:** 806.
768
769 40. Hepgul N, Cattaneo A, Agarwal K, Baraldi S, Borsini A, Bufalino C *et al.*
770 Transcriptomics in Interferon-alpha-Treated Patients Identifies Inflammation-,
771 Neuroplasticity- and Oxidative Stress-Related Signatures as Predictors and Correlates
772 of Depression. *Neuropsychopharmacology : official publication of the American*
773 *College of Neuropsychopharmacology* 2016; **41**(10)**:** 2502-2511.
774
775 41. The Lancet N. Long COVID: understanding the neurological effects. *Lancet Neurol*
776 2021; **20**(4)**:** 247.
777
778 42. Boldrini M, Canoll PD, Klein RS. How COVID-19 Affects the Brain. *JAMA Psychiatry*
779 2021; **78**(6)**:** 682-683.
780
781 43. Klein R, Soung A, Sissoko C, Nordvig A, Canoll P, Mariani M *et al.* COVID-19
782 induces neuroinflammation and loss of hippocampal neurogenesis. *Res Sq* 2021.
783
784 44. Fernández-Castañeda A, Lu P, Geraghty AC, Song E, Lee M-H, Wood J *et al.* Mild
785 respiratory SARS-CoV-2 infection can cause multi-lineage cellular dysregulation and
786 myelin loss in the brain. *bioRxiv* 2022**:** 2022.2001.2007.475453.
787
788 45. Mazza MG, De Lorenzo R, Conte C, Poletti S, Vai B, Bollettini I *et al.* Anxiety and
789 depression in COVID-19 survivors: Role of inflammatory and clinical predictors.
790 *Brain, behavior, and immunity* 2020; **89:** 594-600.
791
792 46. Mazza MG, Palladini M, De Lorenzo R, Magnaghi C, Poletti S, Furlan R *et al.*
793 Persistent psychopathology and neurocognitive impairment in COVID-19 survivors:
794 Effect of inflammatory biomarkers at three-month follow-up. *Brain, behavior, and*
795 *immunity* 2021; **94:** 138-147.
796
797 47. Fernández-Castañeda A, Lu P, Geraghty AC, Song E, Lee MH, Wood J *et al.* Mild
798 respiratory SARS-CoV-2 infection can cause multi-lineage cellular dysregulation and
799 myelin loss in the brain. *bioRxiv* 2022.
800

801 48. Bodro M, Compta Y, Llanso L, Esteller D, Doncel-Moriano A, Mesa A *et al.* Increased
802 CSF levels of IL-1beta, IL-6, and ACE in SARS-CoV-2-associated encephalitis. *Neurol*
803 *Neuroimmunol Neuroinflamm* 2020; **7**(5).
804
805 49. Brodin P. Immune determinants of COVID-19 disease presentation and severity.
806 *Nature medicine* 2021; **27**(1)**:** 28-33.
807
808 50. Kiernan EA, Smith SM, Mitchell GS, Watters JJ. Mechanisms of microglial activation
809 in models of inflammation and hypoxia: Implications for chronic intermittent hypoxia.
810 *The Journal of physiology* 2016; **594**(6)**:** 1563-1577.
811
812 51. Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal
813 neurogenesis. *Science* 2003; **302**(5651)**:** 1760-1765.
814
815 52. Zonis S, Ljubimov VA, Mahgerefteh M, Pechnick RN, Wawrowsky K, Chesnokova V.
816 p21Cip restrains hippocampal neurogenesis and protects neuronal progenitors from
817 apoptosis during acute systemic inflammation. *Hippocampus* 2013; **23**(12)**:** 1383-1394.
818
819 53. Vallieres L, Campbell IL, Gage FH, Sawchenko PE. Reduced hippocampal
820 neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-
821 6. *The Journal of neuroscience : the official journal of the Society for Neuroscience*
822 2002; **22**(2)**:** 486-492.
823
824 54. Hofer M, Hausmann J, Staeheli P, Pagenstecher A. Cerebral expression of interleukin-
825 12 induces neurological disease via differential pathways and recruits antigen-specific
826 T cells in virus-infected mice. *The American journal of pathology* 2004; **165**(3)**:** 949-
827 958.
828
829 55. Yu JT, Lee CH, Yoo KY, Choi JH, Li H, Park OK *et al.* Maintenance of anti-
830 inflammatory cytokines and reduction of glial activation in the ischemic hippocampal
831 CA1 region preconditioned with lipopolysaccharide. *Journal of the neurological*
832 *sciences* 2010; **296**(1-2)**:** 69-78.
833
834 56. Ireland DD, Reiss CS. Expression of IL-12 receptor by neurons. *Viral immunology*
835 2004; **17**(3)**:** 411-422.
836
837 57. Turka LA, Goodman RE, Rutkowski JL, Sima AA, Merry A, Mitra RS *et al.* Interleukin
838 12: a potential link between nerve cells and the immune response in inflammatory
839 disorders. *Mol Med* 1995; **1**(6)**:** 690-699.
840
841 58. Espindola OM, Gomes YCP, Brandao CO, Torres RC, Siqueira M, Soares CN *et al.*
842 Inflammatory Cytokine Patterns Associated with Neurological Diseases in Coronavirus
843 Disease 2019. *Ann Neurol* 2021; **89**(5)**:** 1041-1045.
844
845 59. Bernard-Valnet R, Perriot S, Canales M, Pizzarotti B, Caranzano L, Castro-Jimenez M
846 *et al.* Encephalopathies Associated With Severe COVID-19 Present Neurovascular
847 Unit Alterations Without Evidence for Strong Neuroinflammation. *Neurol*
848 *Neuroimmunol Neuroinflamm* 2021; **8**(5).
849

60. Mori S, Maher P, Conti B. Neuroimmunology of the Interleukins 13 and 4. *Brain Sci* 2016; **6**(2).

61. Vai B, Mazza MG, Cazzetta S, Calesella F, Aggio V, Lorenzi C *et al.* Higher Interleukin 13 differentiates patients with a positive history of suicide attempts in major depressive disorder. *Journal of Affective Disorders Reports* 2021; **6:** 100254.

62. Group TWREAfC-TW. Association Between Administration of IL-6 Antagonists and Mortality Among Patients Hospitalized for COVID-19: A Meta-analysis. *JAMA* 2021; **326**(6)**:** 499-518.

63. Almradi A, Hanzel J, Sedano R, Parker CE, Feagan BG, Ma C *et al.* Clinical Trials of IL-12/IL-23 Inhibitors in Inflammatory Bowel Disease. *BioDrugs* 2020; **34**(6)**:** 713-721.

64. Banks WA. Characteristics of compounds that cross the blood-brain barrier. *BMC Neurol* 2009; **9 Suppl 1:** S3.

65. Bilusic M, Heery CR, Collins JM, Donahue RN, Palena C, Madan RA *et al.* Phase I trial of HuMax-IL8 (BMS-986253), an anti-IL-8 monoclonal antibody, in patients with metastatic or unresectable solid tumors. *J Immunother Cancer* 2019; **7**(1)**:** 240.

66. Guimaraes PO, Quirk D, Furtado RH, Maia LN, Saraiva JF, Antunes MO *et al.* Tofacitinib in Patients Hospitalized with Covid-19 Pneumonia. *The New England journal of medicine* 2021; **385**(5)**:** 406-415.

67. Marconi VC, Ramanan AV, de Bono S, Kartman CE, Krishnan V, Liao R *et al.* Efficacy and safety of baricitinib for the treatment of hospitalised adults with COVID-19 (COV-BARRIER): a randomised, double-blind, parallel-group, placebo-controlled phase 3 trial. *Lancet Respir Med* 2021; **9**(12)**:** 1407-1418.

68. Han MK, Antila M, Ficker JH, Gordeev I, Guerreros A, Bernus AL *et al.* Ruxolitinib in addition to standard of care for the treatment of patients admitted to hospital with COVID-19 (RUXCOVID): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Rheumatol* 2022; **4**(5)**:** e351-e361.

69. Horowitz MA, Wertz J, Zhu D, Cattaneo A, Musaelyan K, Nikkheslat N *et al.* Antidepressant compounds can be both pro- and anti-inflammatory in human hippocampal cells. *Int J Neuropsychopharmacol* 2014; **18**(3).

70. Cattaneo A, Ferrari C, Turner L, Mariani N, Enache D, Hastings C *et al.* Whole-blood expression of inflammasome- and glucocorticoid-related mRNAs correctly separates treatment-resistant depressed patients from drug-free and responsive patients in the BIODEP study. *Translational psychiatry* 2020; **10**(1)**:** 232.

71. Fernandez-Castaneda A, Lu P, Geraghty AC, Song E, Lee MH, Wood J *et al.* Mild respiratory SARS-CoV-2 infection can cause multi-lineage cellular dysregulation and myelin loss in the brain. *bioRxiv* 2022.

899 72. Khan M, Yoo SJ, Clijsters M, Backaert W, Vanstapel A, Speleman K *et al.* Visualizing
900 in deceased COVID-19 patients how SARS-CoV-2 attacks the respiratory and olfactory
901 mucosae but spares the olfactory bulb. *Cell* 2021; **184**(24)**:** 5932-5949 e5915.
902
903 73. Serrano GE, Walker JE, Arce R, Glass MJ, Vargas D, Sue LI *et al.* Mapping of SARS-
904 CoV-2 Brain Invasion and Histopathology in COVID-19 Disease. *medRxiv* 2021.
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911 **Figures Caption**

912 **Table 1. Characteristics of COVID-19 patients with and without delirium.** Demographics of the
913 COVID-19 patients' cohort. Legend: * Mann-Whitney U-test; ** Chi squared test; # Severity Score:
914 0=Asymptomatic or no requirement for supplemental oxygen, 1= Requirement for supplemental
915 oxygen (fraction of inspired oxygen (FiO2) < 0.4) for at least 12 h, 2= Requirement for supplemental
916 oxygen (FiO2 ≥ 0.4) for at least 12 h, 3= Requirement for supplemental oxygen (FiO2 > 0.6) for at least
917 12 h, and not a candidate for escalation above level one (ward-based) care, 4= Requirement for
918 supplemental oxygen (FiO2 > 0.8) and peripheral oxygen saturations <90% (with no history of type 2
919 respiratory failure (T2RF)) or <85% (with known T2RF) for at least 12 h, 5= Requirement for ECMO;
920 ## Comorbidities: hypertension, type 2 diabetes mellitus, (paroxysmal) atrial fibrillation aortic stenosis,
921 benign prostatic hypertrophy/ hyperplasia, benign paroxysmal positional vertigo, cancer, congestive
922 cardiac failure, chronic kidney disease, chronic obstructive pulmonary disease, gastroesophageal reflux
923 disease, ischaemic heart disease, monoclonal gammopathy of undetermined significance, osteoarthritis,
924 osteoporosis, peripheral vascular disease, rheumatoid arthritis, transient ischaemic attack, venous
925 thromboembolism.
926
927 **Figure 1. Timeline of *in vitro* experiments with the same serum samples. (a-e)** Cells were treated
928 with media containing 1% serum (from each COVID-19 patient, delirium and non-delirium) with or
929 without IL6 Antibody (A) (0.1ug/ml) or IL12A (0.3ug/ml) for additional 2 days. At this stage cells were
930 either fixed or left to differentiate for additional 4 days, again in presence of 1% serum (from the same
931 COVID-19 patient previously used in the proliferation phase), with or without IL6A or IL13A
932 (0.1ug/ml). At the end of day 2 of proliferation and day 4 of differentiation, cell supernatant was
933 collected for subsequent cytokines measurement. Legend: Sox2, sex determining region Y-box 2; DCX,
934 doublecortin; Map2, microtubule-associated protein 2; S100β, S100 calcium-binding protein β; CC3,
935 caspase 3; interleukin, IL.
936
937 **Figure 2. Cell treatment with serum samples from COVID-19 patients with delirium decreased**
938 **cell proliferation, neurogenesis, and increased apoptosis, when compared with serum from non-**
939 **delirium patients. (a-b)** Cell treatment with serum samples from delirium patients decreased
940 proliferation (KI67+cells) and increased apoptosis (CC3+ cells), when compared with serum from non-
941 delirium patients. There were no differences across Time point 1 and 2. **(c-e)** Treatment with serum
942 samples from delirium patients decreased neurogenesis (DCX+ and Map2+cells) and increased
943 apoptosis (CC3+ cells), when compared with serum from non-delirium patients. Again, there were no
944 differences across Time point 1 and 2. Two-way mixed ANOVA was performed.
945
946 **Figure 3. Higher concentration of IL6 in serum samples from COVID-19 patients with delirium**
947 **induced cells to produce IL12 and IL13, respectively during the proliferation and differentiation**
948 **stage. (a-b)** Serum samples from delirium patients showed increased levels of IL6, when compared
949 with non-delirium patients, both at Time point 1 and 2. **(c-d)** During proliferation, exposure of cells
950 with serum samples from delirium patients (collected both at Time point 1 and 2) induced the production
951 of IL12 in the supernatant, when compared with treatment with serum samples from non-delirium

952 patients. **(e-f)** During differentiation, exposure of cells with serum samples from delirium patients
953 (collected both at Time point 1 and 2) induced the production of IL13 in the supernatant, when
954 compared with treatment with serum samples from non-delirium patients. Mann-Whitney U-test and
955 two-way mixed ANOVA were performed. Data are shown as mean; ****p<0.0001 comparisons as
956 indicated.
957
958 **Figure 4. During proliferation, treatment with an antibody against IL6 prevented the detrimental**
959 **effect of serum from COVID-19 patients with delirium on both cell proliferation and apoptosis**
960 **and decreased IL12 production. (a-d)** Cell treatment with IL6A (0.1ug/ml) prevented the decrease in
961 proliferation (Ki67+cells) and increase in apoptosis (CC3+cells) previously observed upon treatment
962 with serum samples from patients with delirium. This is confirmed across treatment with serum samples
963 collected at Time point 1 and 2. **(e, f)** Treatment with IL6A also prevented production of IL12 in cell
964 supernatant. **(g-j)** Cell treatment with IL12A (0.3ug/ml) prevented the decrease in proliferation
965 (Ki67+cells) and increase in apoptosis (CC3+cells) previously observed upon treatment with serum
966 samples from patients with delirium. Again, this is confirmed across treatment with serum samples
967 collected at Time point 1 and 2. Two-way mixed ANOVA with Bonferroni's post hoc test was
968 performed. Data are shown as mean; ****p<0.0001 comparisons as indicated.
969
970 **Figure 5. During differentiation, treatment with an antibody against IL6 prevented the**
971 **detrimental effect of serum from COVID-19 patients with delirium on both neurogenesis and**
972 **apoptosis and decreased IL13 production. (a-f)** Cell treatment with IL6A (0.1ug/ml) prevented the
973 decrease in neurogenesis (DCX+ and Map2+cells) and increase in apoptosis (CC3+cells) previously
974 observed upon treatment with serum samples from patients with delirium. This is confirmed across
975 treatment with serum samples collected at Time point 1 and 2. **(g, h)** Treatment with IL6A also
976 prevented production of IL13 in cell supernatant. **(i-n)** Cell treatment with IL13A (0.1ug/ml) prevented
977 the decrease in neurogenesis (DCX+ and Map2+cells) and increase in apoptosis (CC3+cells) previously
978 observed upon treatment with serum samples from patients with delirium. Again, this is confirmed
979 across treatment with serum samples collected at Time point 1 and 2. Two-way mixed ANOVA with
980 Bonferroni's post hoc test was performed. Data are shown as mean; ****p<0.0001 comparisons as
981 indicated.
982
983

	Delirium *(n = 18)*	**Non Delirium** *(n = 18)*	***p* value**
Age (years)			
Mean±SD	76.2±14.7	75.4±14.2	*p*=0.9*
Gender			
Male	12 (66.7%)	11 (61.1%)	*p*=0.7**
Severity Score#			
Mean±SD	1.2±1.2	1.3±1.2	*p*=0.9*
Survival			
Yes	13 (72.2%)	16 (88.9%)	*p=0.2***
Two or more comorbidities##			
Baseline	15 (83.3%)	14 (77.8%)	*p=0.3***
Hypertension			
Baseline	13 (72.2%)	12 (66.7%)	*p=0.7***
Type 2 Diabetes			
Baseline	5 (27.8%)	8 (44.4%)	*p=0.3***
CRP *Time point 1*			
Mean±SD	72±53.9 mg/L	115.5±94.4 mg/L	*p=0.2**
CRP *Time point 2*			
Mean±SD	83.7±88.4 mg/L	97.5±102.7 mg/L	*p=0.9**

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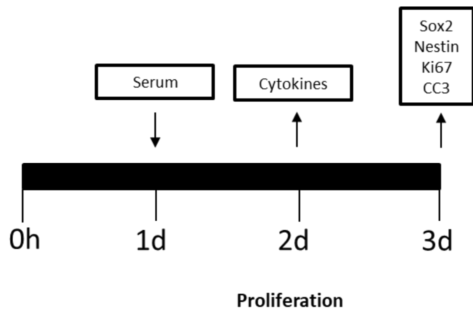

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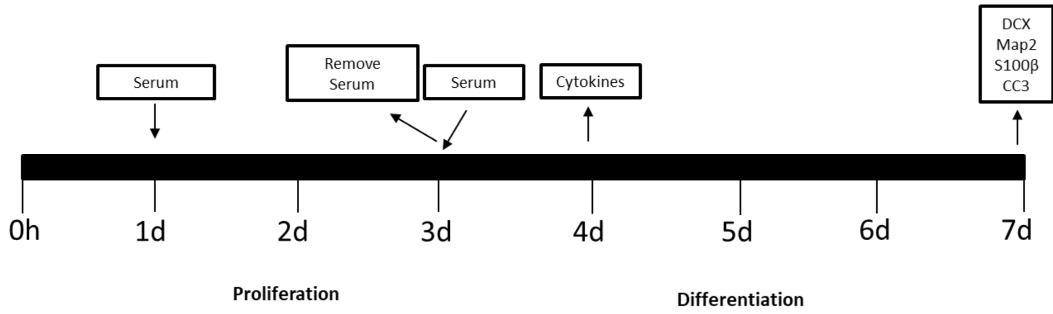

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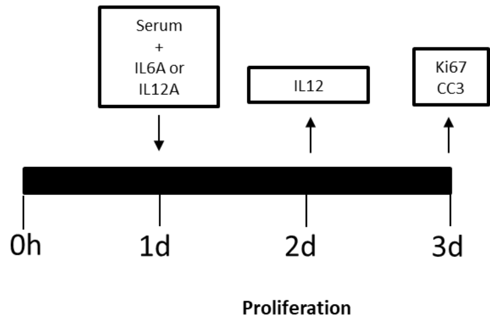

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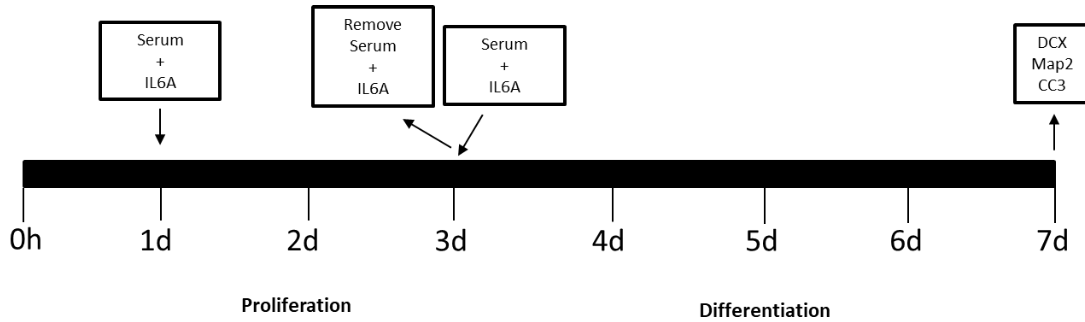

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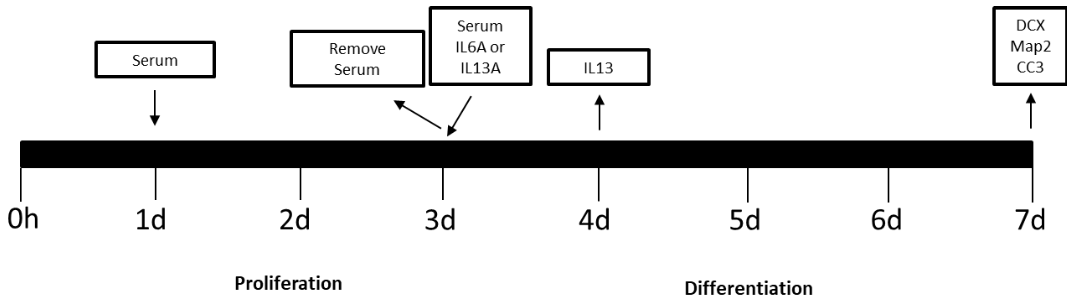

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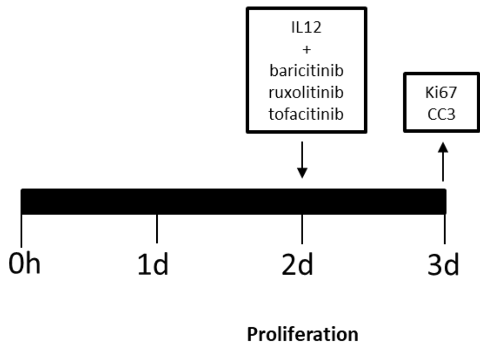

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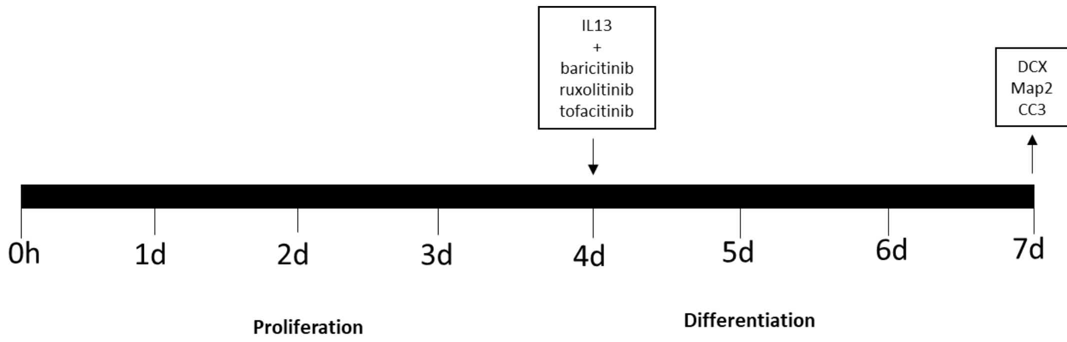

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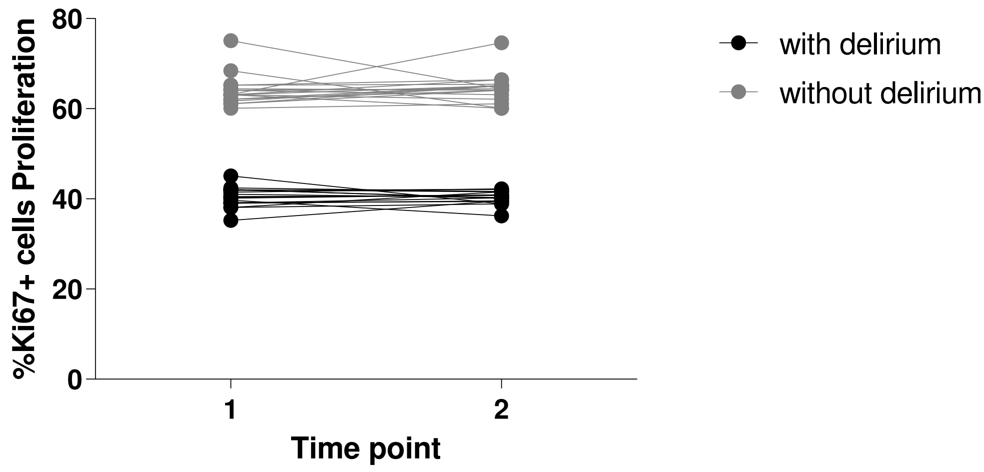

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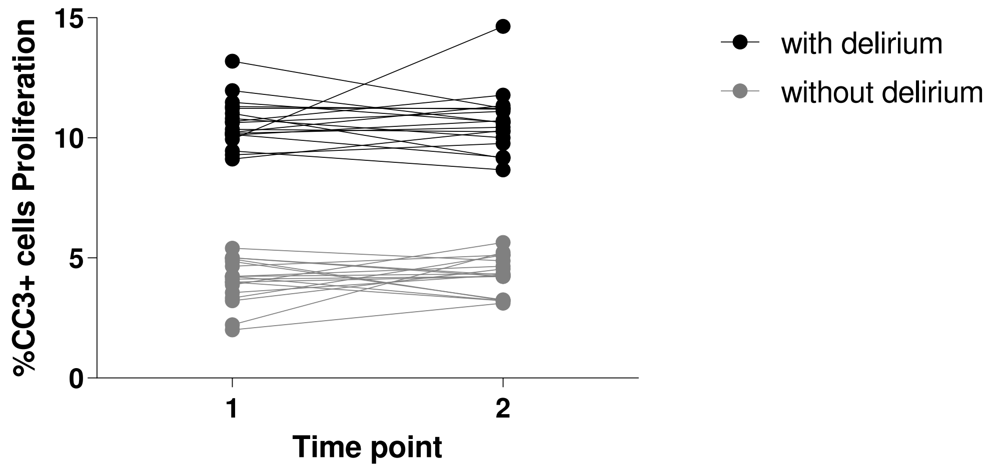

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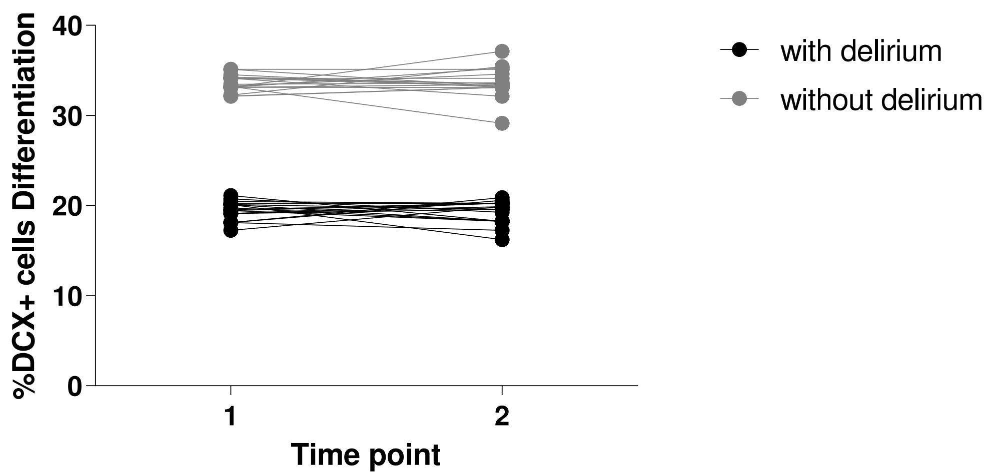

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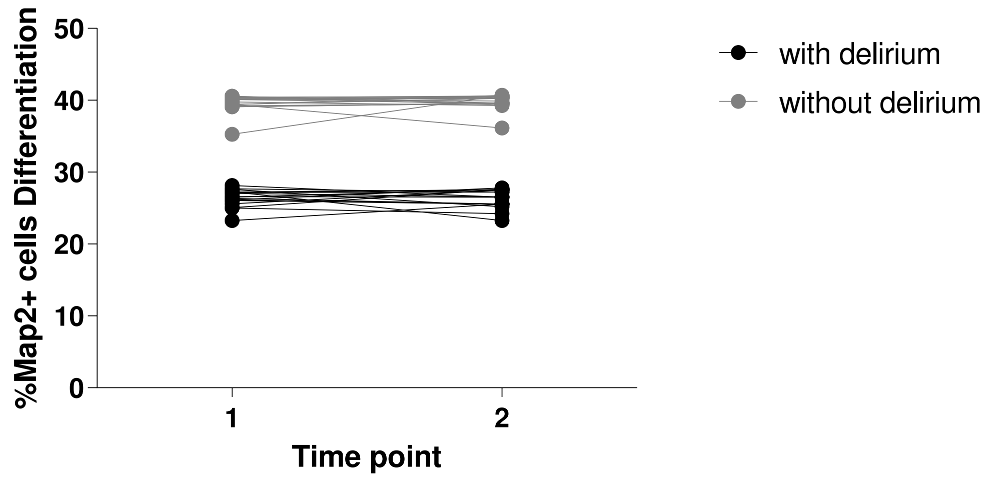

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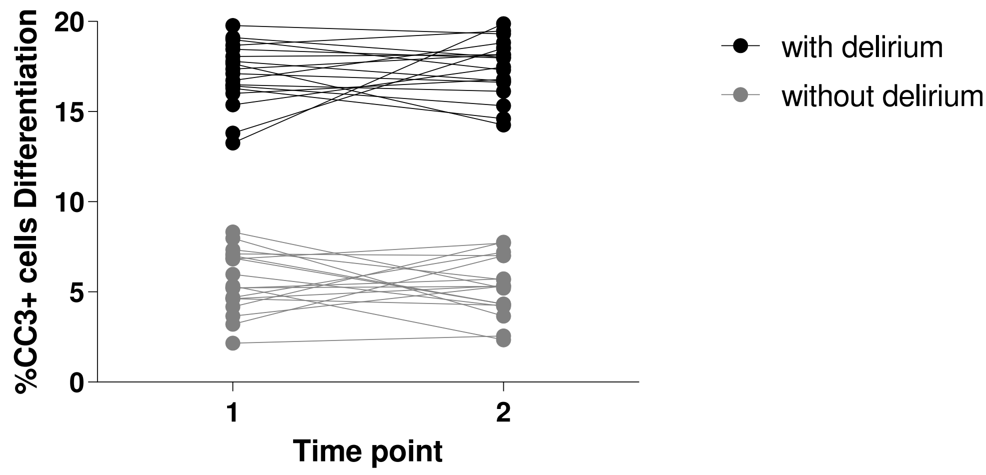

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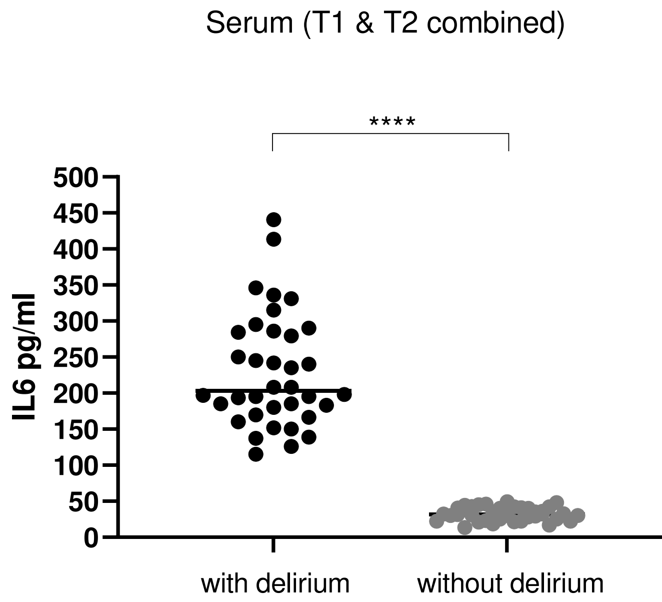

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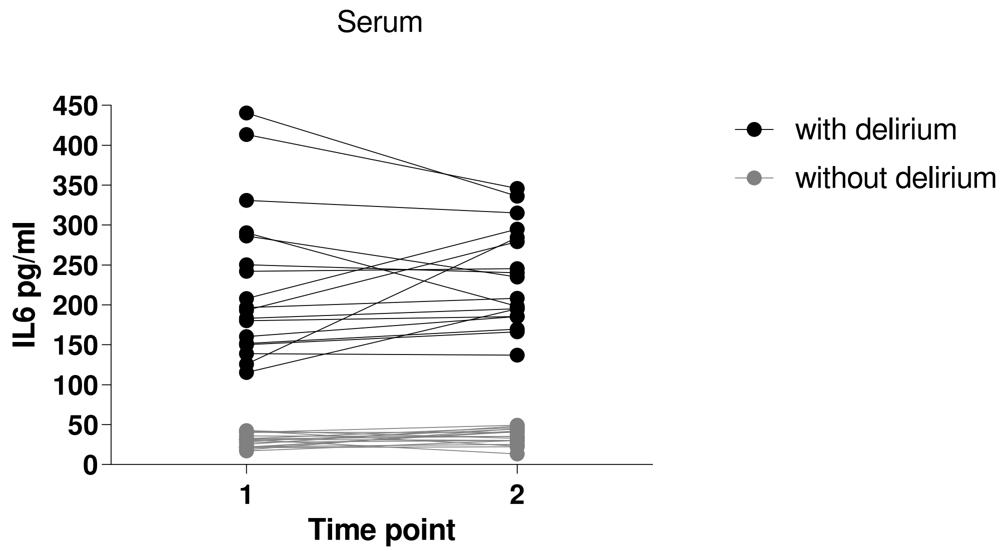

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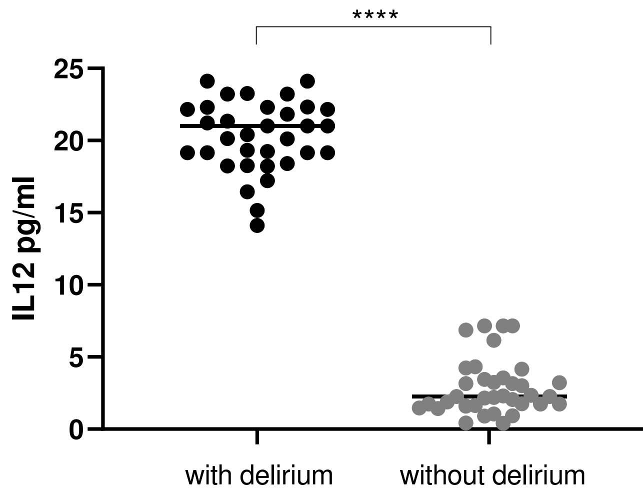

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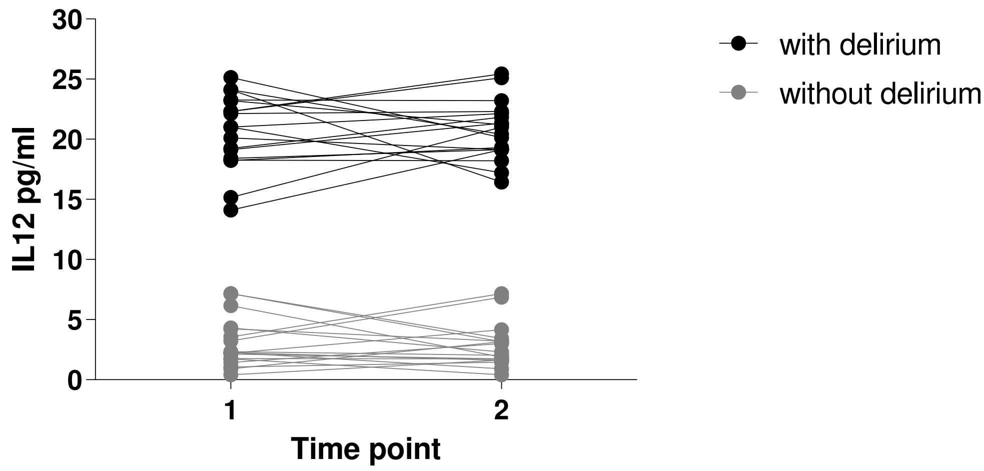

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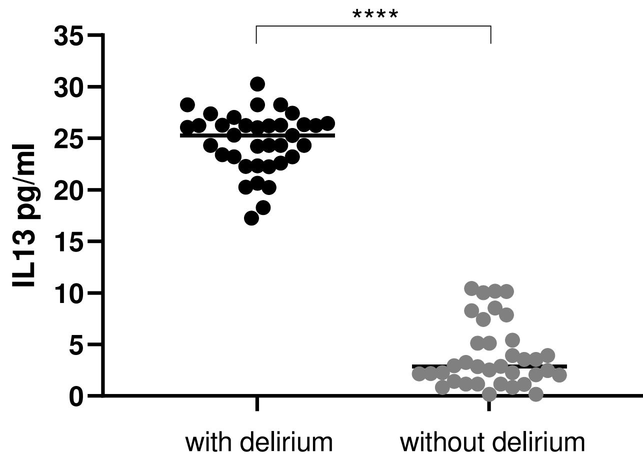

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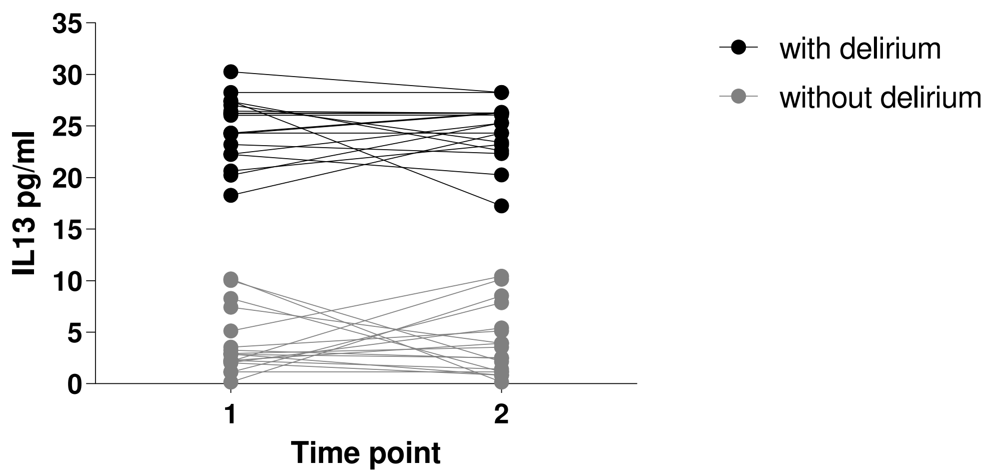

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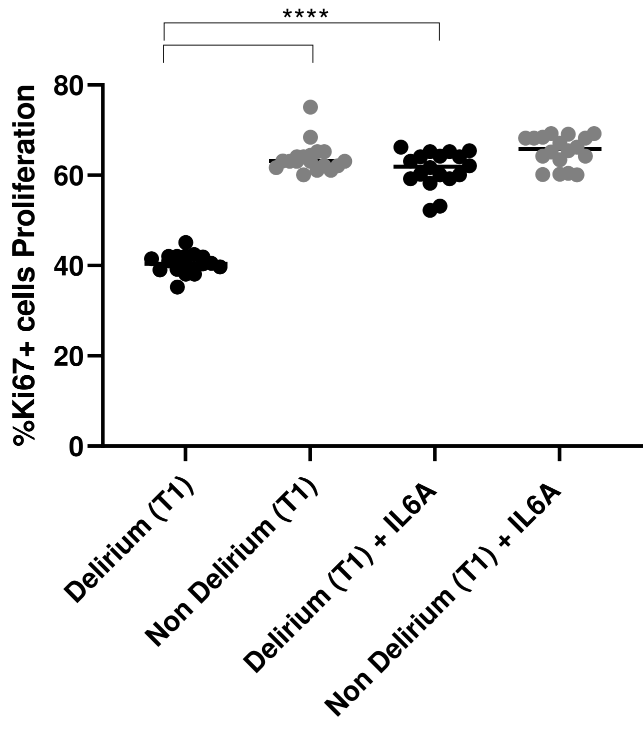

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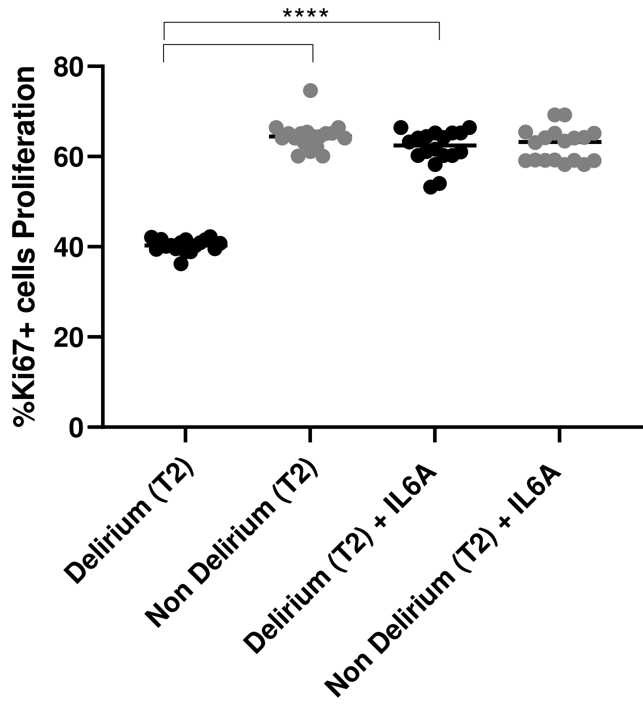


c)

****

%CC3+ cells Proliferation

15
10
5
0

Delirium (T1)
Non Delirium (T1)
Delirium (T1) + IL6A
Non Delirium (T1) + IL6A

d)

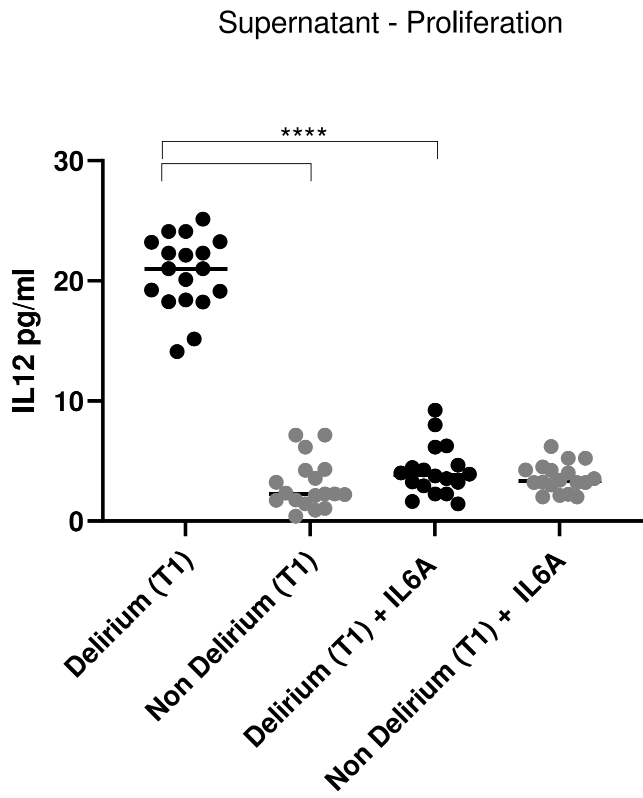
****

%CC3+ cells Proliferation

15
10
5
0

Delirium (T2)
Non Delirium (T2)
Delirium (T2) + IL6A
Non Delirium (T2) + IL6A

e)



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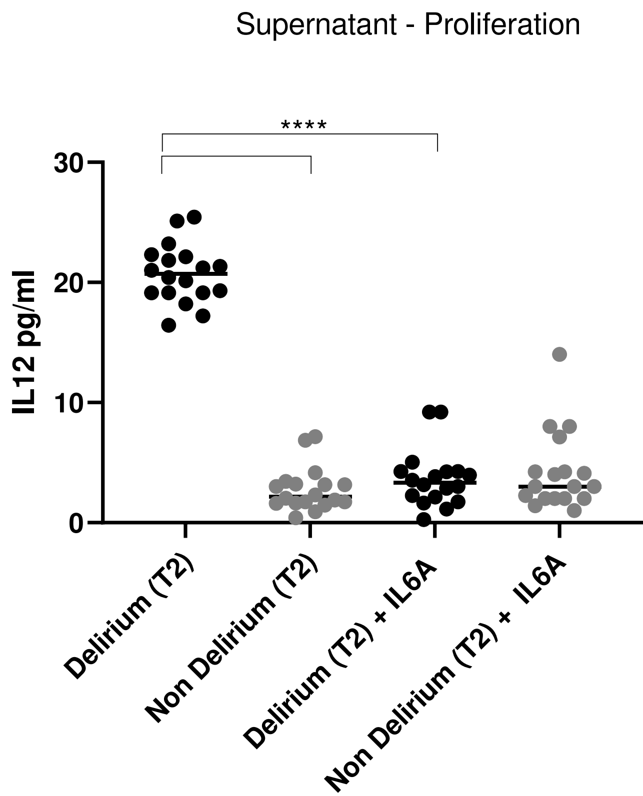

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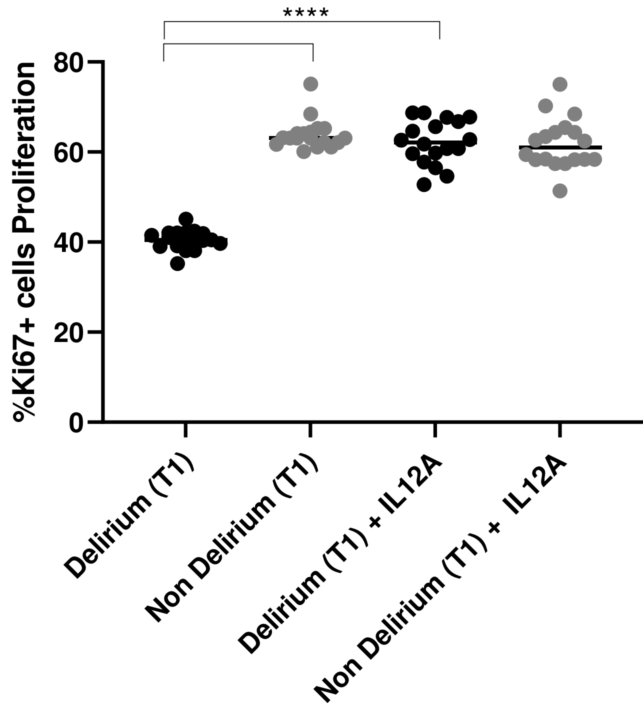

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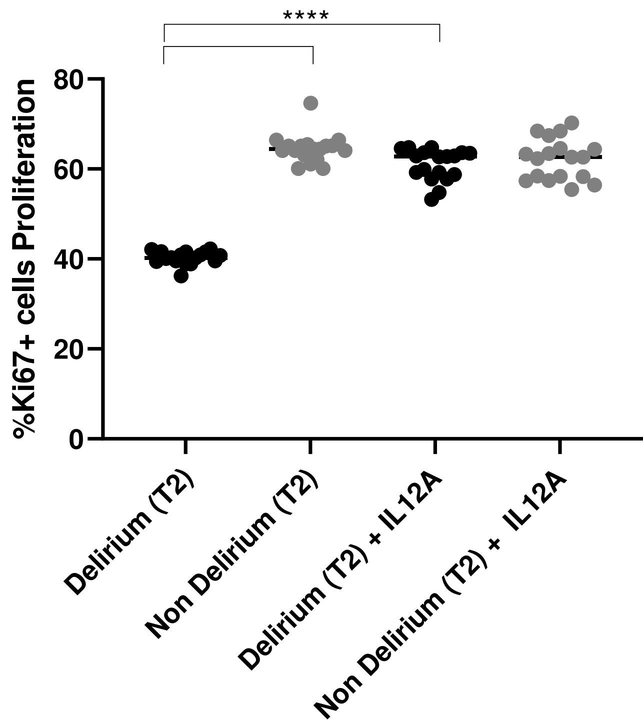

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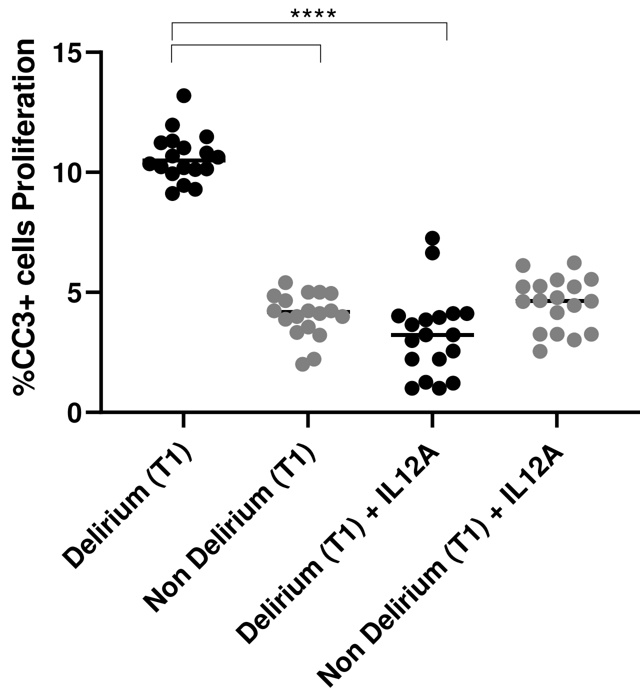

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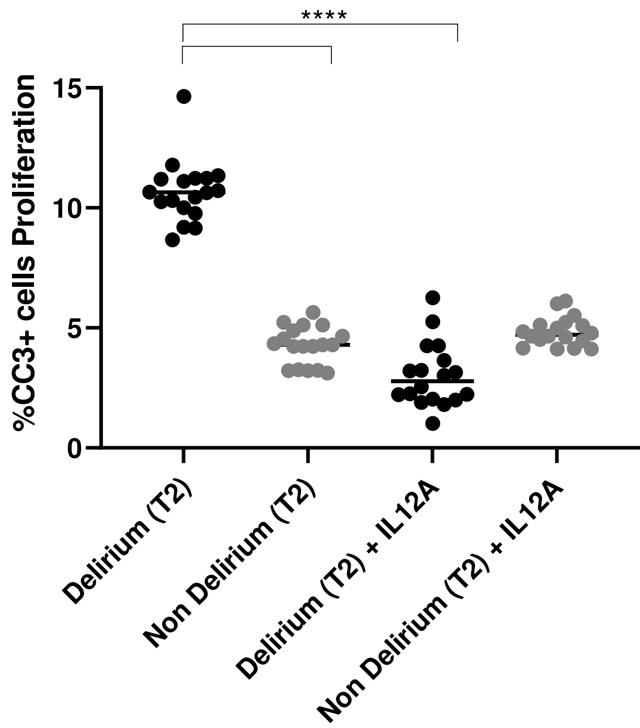

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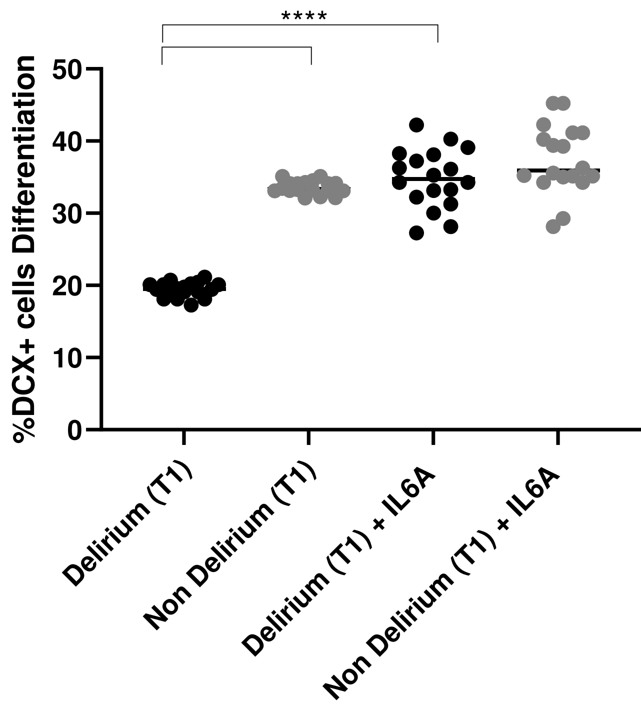

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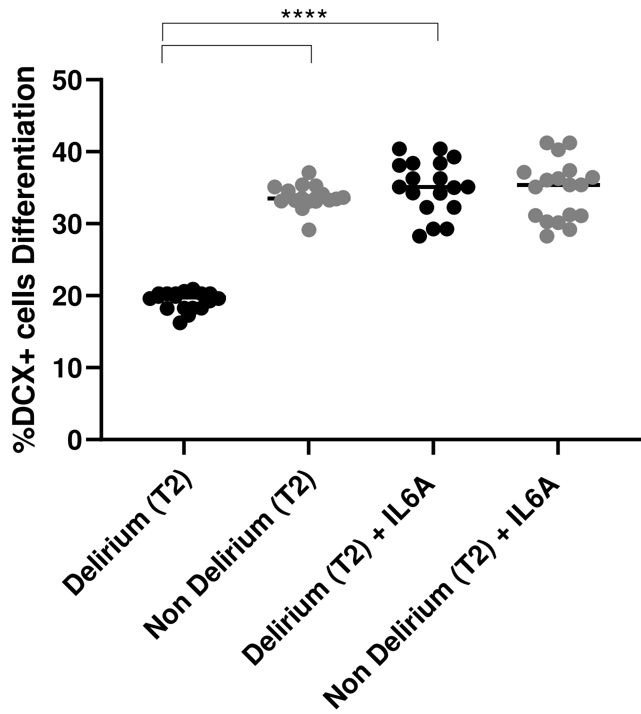

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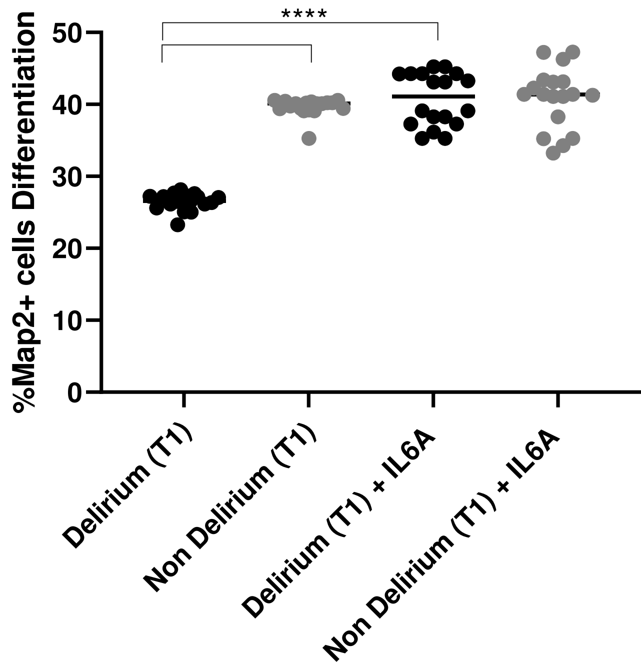

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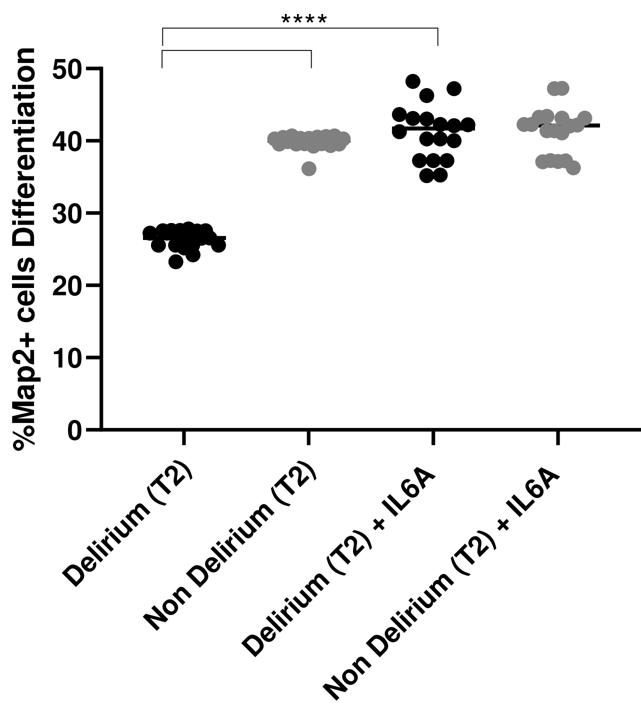

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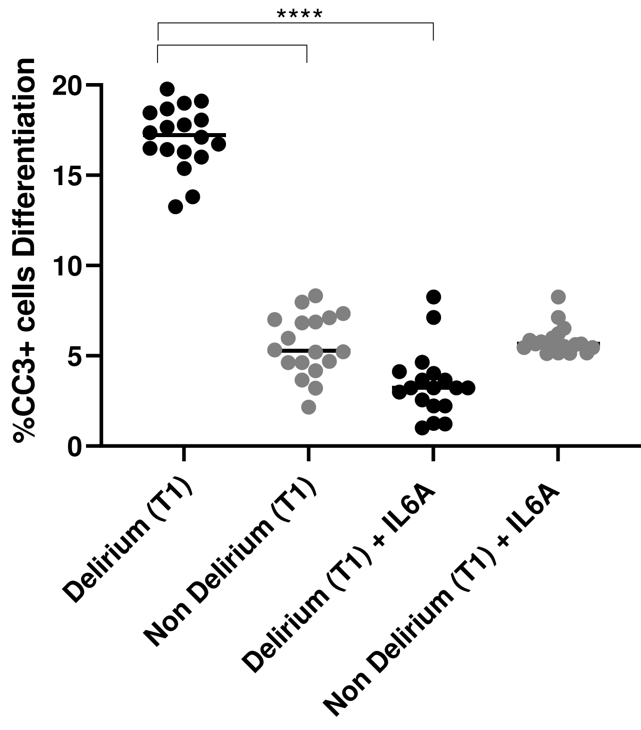

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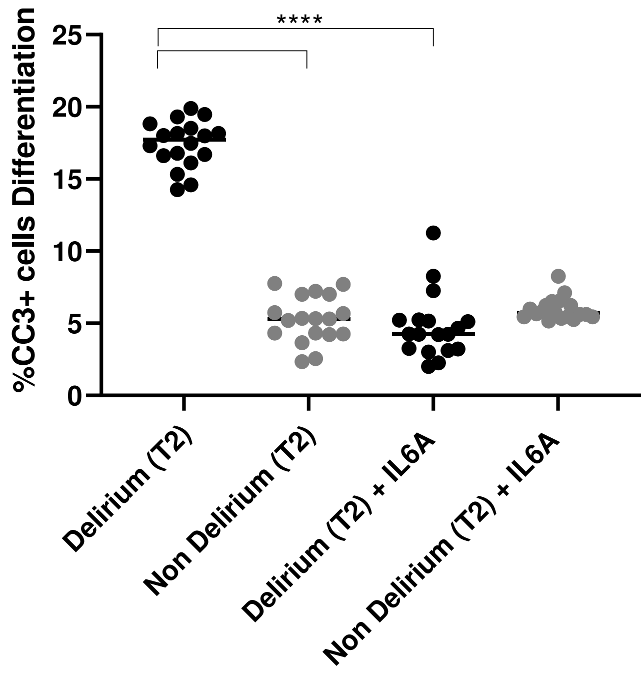

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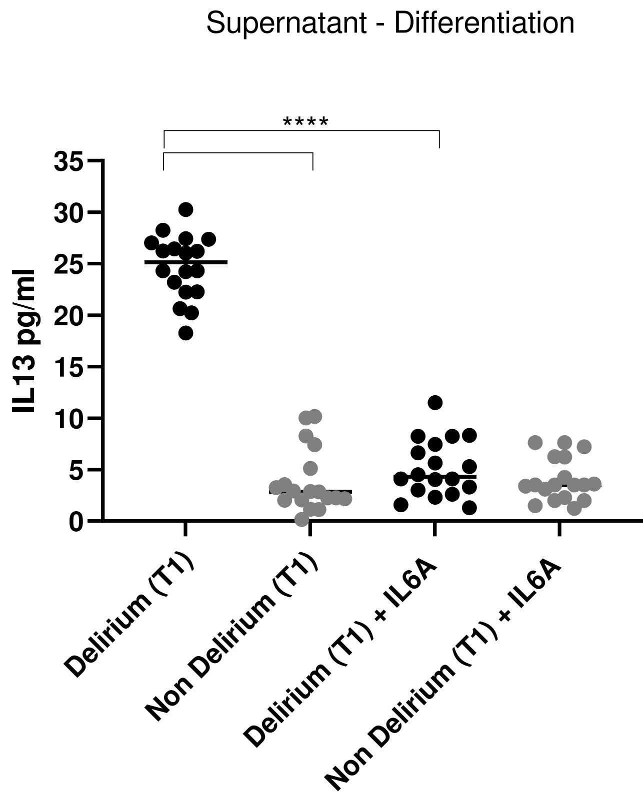

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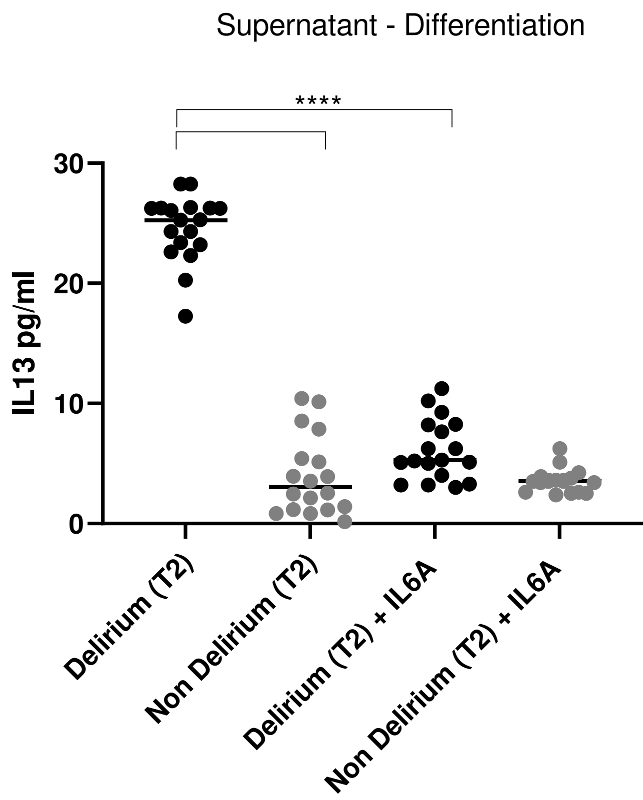

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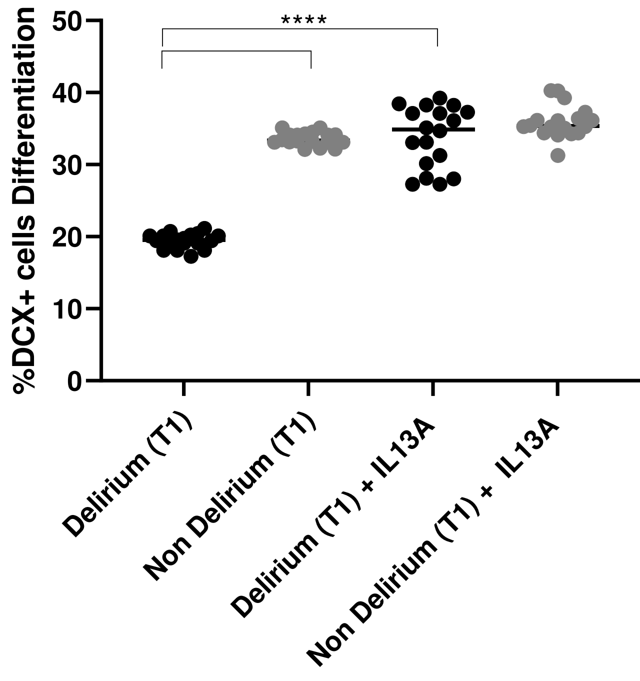

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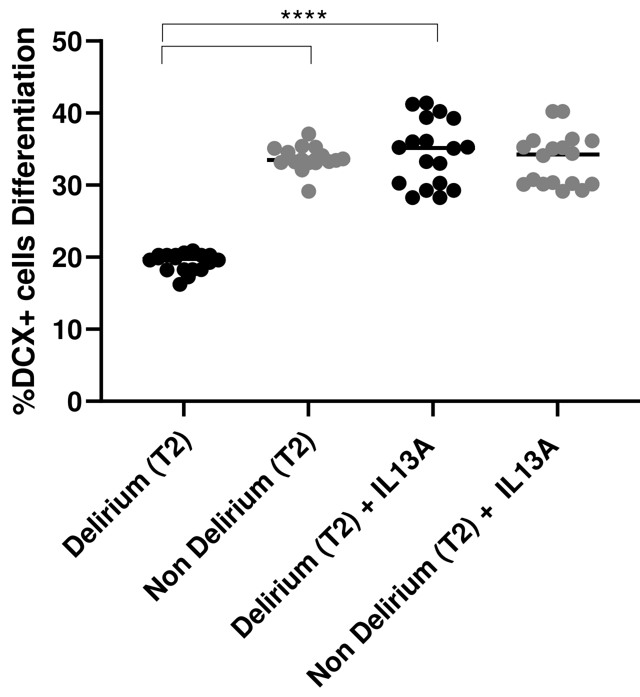

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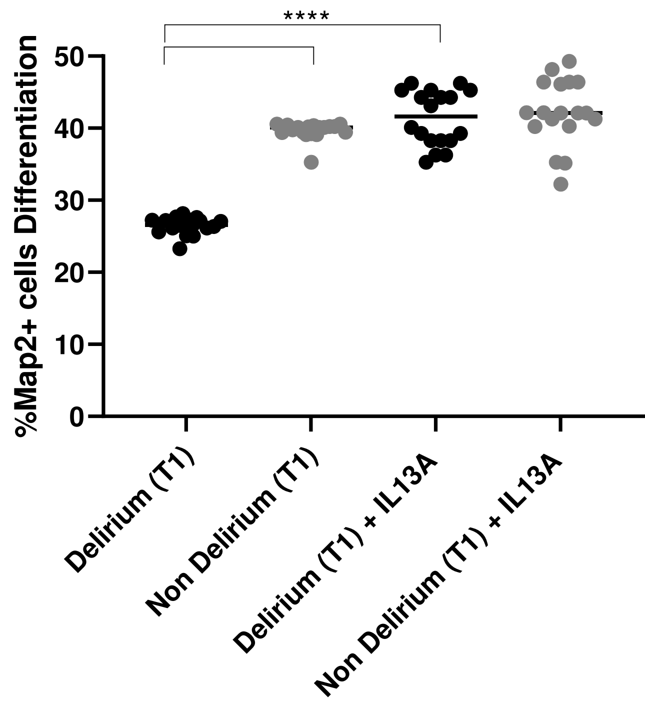

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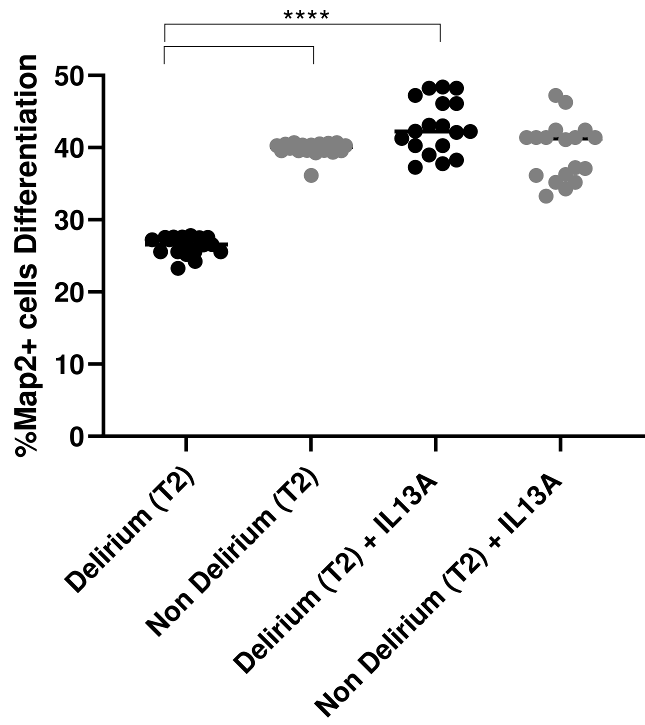

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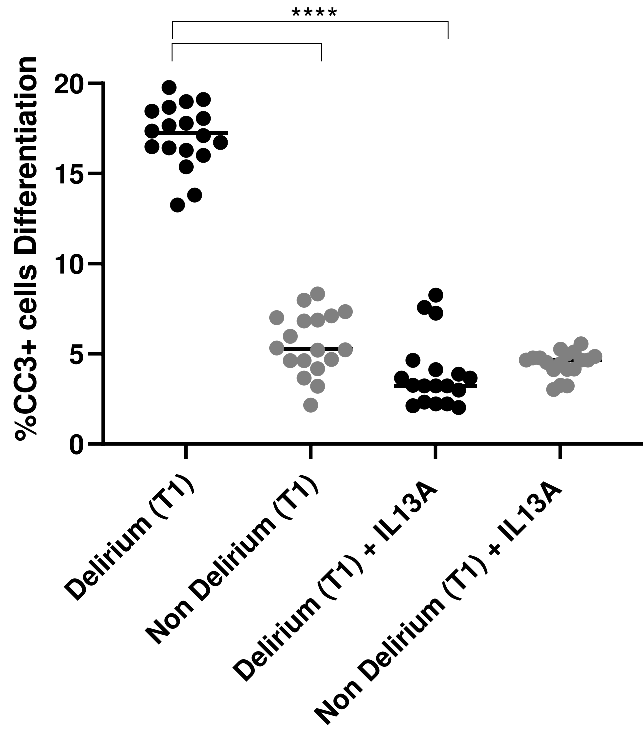

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