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Stromal cells regulate malignant B-cell spatial organization, survival, and drug response in a new 3D model mimicking lymphoma tumor niche

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1 **Stromal cells regulate malignant B-cell spatial organization, survival, and drug**
2 **response in a new 3D model mimicking lymphoma tumor niche**

3

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35 ABSTRACT

36

37 Non-Hodgkin B-cell lymphomas (B-NHL) mainly develop within lymph nodes
38 as densely packed aggregates of tumor cells and their surrounding
39 microenvironment, creating a tumor niche specific to each lymphoma subtypes. Until
40 now, *in vitro* preclinical models mimicking biomechanical forces, cellular
41 microenvironment, and 3D organization of B lymphomas remain scarce while all
42 these parameters constitute key determinants of lymphomagenesis and drug
43 resistance. Using a microfluidic method based on the encapsulation of cells inside
44 permeable, elastic, and hollow alginate microspheres, we developed a new tunable
45 3D-model incorporating extracellular matrix and/or stromal cells. Lymphoma B cells
46 and stromal cells dynamically formed self-organized 3D spheroids, thus initiating a
47 coevolution of these two cell types, reflecting their bidirectional crosstalk, and
48 recapitulating the heterogeneity of B-NHL subtypes. In addition, this approach makes
49 it suitable to assess in a relevant *in vitro* model the activity of new therapeutic agents
50 in B-NHL.

51

52

53 INTRODUCTION

54

55 Non-Hodgkin lymphoma (NHL) is a group of common hematological
56 malignancies, with the majority of them originating from B cells. Follicular lymphoma
57 (FL) and diffuse large B-cell lymphomas (DLBCL), the two most frequent B-cell NHL
58 (B-NHL) (Smith et al., 2011; Teras et al., 2016), result from the malignant
59 transformation of germinal center (GC) or post-GC B cells. FL are indolent
60 lymphomas characterized by the occurrence of the t(14;18)(q32;q21) translocation
61 combined with additional recurrent somatic alterations (Huet et al., 2018). Two major
62 subtypes of aggressive DLBCL, GC B-cell (GCB) and activated B-cell (ABC)-DLBCL,
63 have been identified using gene expression profiling, reflecting their putative cell of
64 origin and molecular alterations (Alizadeh et al., 2000). Despite a better
65 understanding of the pathophysiology of these tumors, frontline therapy remains
66 based on a combination of conventional chemotherapies such as CHOP
67 (Cyclophosphamide, Hydroxydaunorubicin, Vincristine and Prednisone) and a
68 monoclonal antibody against CD20. Nonetheless, 30–40% of DLBCL patients
69 develop relapse or have refractory disease that cannot be cured with the standard
70 immunochemotherapy (Sehn and Gascoyne, 2015). In addition, FL remains
71 essentially an incurable disease and about 20% of patients progress or relapse in the
72 first 2 years following treatment initiation, with a dismal prognosis (Huet et al., 2018).

73 It is now widely accepted that tumors constitute a complex ecosystem
74 composed of many cell types regulated by biological, structural, chemical, and
75 mechanical cues, that altogether participate in the effectiveness of therapeutic
76 molecules (Bissell and Radisky, 2001). The tumor microenvironment of B-cell
77 lymphomas contains highly variable numbers of immune cells, stromal cells, blood

78 vessels, and extracellular matrix (ECM) and the interplay between these elements
79 produces a tumor niche specific to each lymphoma subtypes (Scott and Gascoyne,
80 2014; Verdière et al., 2018). FL exhibits a high dependence on a GC-like
81 microenvironment where immune and stromal cells support survival, proliferation,
82 and migration of malignant B cells. In turn, FL cells modulate the phenotype and
83 function of their surrounding microenvironment. In particular, FL-infiltrating stromal
84 cells are engaged in a bidirectional crosstalk with malignant B cells within infiltrated
85 lymph nodes and bone marrow (Guilloton et al., 2012; Pandey et al., 2017).
86 Conversely DLBCL have been proposed as less dependent on lymph node
87 microenvironment but specific stromal signatures have been shown to impact DLBCL
88 prognosis (Fowler et al., 2016; Scott and Gascoyne, 2014). Importantly, studies
89 exploring functional interactions between lymphoma B cells and stromal cells have
90 been essentially performed in 2D *in vitro* culture models whereas biomechanical
91 forces and 3D organization emerged as key determinants of lymphoma pathogenesis
92 and drug resistance.

93 *In vitro*, 2D culture of cell lines is the model mainly used to screen new
94 molecules. However, this constitutes an “ideal” model where nutrients, oxygen, and
95 drugs reach freely all cells. In addition, these cell cultures do not consider the spatial
96 architecture of tissues, and lack microenvironment and ECM identified as tumor
97 safety guards from chemotherapies. In this context, multicellular spheroids or tumor
98 organoids represent promising models allowing high-throughput screening of anti-
99 cancer drugs in versatile systems mixing several cell types and ECM components.
100 While 3D culture models are increasingly developed for solid cancer (Clevers, 2016),
101 their transfer to lymphoma modeling is still limited and includes: i) multicellular
102 aggregates of lymphoma cells obtained using hanging drop method, which are useful

103 for testing drug efficacy but do not account for the effect of cell-cell and cell-ECM
104 interactions (Decaup et al., 2019; Gravelle et al., 2014), ii) 3D lymphoma organoid
105 models integrating lymphoid-like stromal cells or integrin-specific binding peptides to
106 recapitulate more accurately lymphoma microenvironment, but difficult to handle and
107 low throughput (Tian et al., 2015).

108 In the present study, we aimed at providing a 3D lymphoma model that both
109 integrates interactions between stromal cells and lymphoma B cells and is amenable
110 to high throughput screening. Using a microfluidic method based on the
111 encapsulation of cells inside permeable, elastic, and hollow alginate microspheres
112 (Alessandri et al., 2013), we developed a new tunable 3D model incorporating ECM
113 and/or stromal cells. We showed that lymphoma B cells and stromal cells form 3D
114 spheroids whose internal architecture is driven by self-organization, thus initiating a
115 bidirectional crosstalk and a co-evolution of these two cell types, recapitulating the
116 heterogeneity of B-NHL subtype. In addition, this approach makes it possible to
117 assess drug efficacy in a relevant *in vitro* B-NHL model.

118 RESULTS

119 **1) B-NHL cell lines form cohesive spheroids in scaffold-free alginate** 120 **capsules**

121 To produce spheroids from lymphoma B-cell lines, we used the cellular capsule
122 technology, initially developed to generate spheroids from solid cancer tumor cells
123 (Alessandri et al., 2013, 2016). Three B-NHL cell lines have been selected for
124 encapsulation as representative models for GCB-DLBCL (SU-DHL-4), ABC-DLBCL
125 (HLY1) and FL (DOHH2). From image-based analysis (N=656), the mean of the
126 capsule diameter distribution was $215.9 \pm 1.3 \mu\text{m}$ and the capsules contained an
127 average of 18.2 ± 0.8 cells/ capsule (N=48) (Figure S1). During the first 11 days, SU-
128 DHL-4 and HLY1 proliferated and aggregated to form spheroids. Alike the
129 terminology used for 2D cultures, we called '3D confluence', the stage when cells
130 filled up the capsule and reached the internal wall of the alginate shell. Contrastingly,
131 DOHH2 cells were not able to grow and form spheroids even after 17 days (Figure
132 1A) suggesting their requirement of a supportive microenvironment. Cell survival in
133 SU-DHL-4 and HLY1 spheroids was evaluated at various culture time points using
134 propidium iodide labelling. Upon spheroid dissociation and flow cytometry
135 quantification, we found that the percentage of dead cells remained as low as 20-
136 30% during the first week in culture and then increased to about 60% after 8 to 12
137 days (Figure 1B). As a corollary, EdU staining revealed that the early stages of
138 spheroid growth were characterized by a high proliferation rate while post-confluent
139 stages exhibited a significant decrease in cell proliferation concomitant with cell death
140 increase (Figure S2). To determine whether this survival decrease was associated
141 with the formation and growth of a dead cellular core, as observed in solid tumor
142 spheroids (Däster et al., 2016; Sutherland, 1988), we performed immunostaining for

143 cell proliferation (Ki67) and cell death (cleaved caspase 3). Besides an increased cell
144 death after confluence (Figure 1B and Figure S2), neither Ki67 nor cleaved-caspase-
145 3 regionalization was detected at prior or after 3D confluence (Figure 1C).

146 To check that the capsule content was not only a confined cell suspension but
147 formed truly cohesive spheroids, the alginate shell was dissolved in PBS containing 1
148 mM EGTA. As shown in figure 2A (and movie 1), the integrity of the cellular
149 aggregates observed after confluence was maintained upon capsule dissolution,
150 suggesting that 3D culture may induce the secretion of cohesive ECM. We thus
151 compared by immunofluorescence the expression of laminin, fibronectin, and
152 collagen I, three ECM components expressed in secondary lymphoid organs (Chen
153 et al., 2012; Sobocinski et al., 2010), between cells cultured in suspension (2D) or in
154 capsules (3D). SU-DHL-4 already expressed collagen 1 in 2D culture and
155 upregulated laminin and fibronectin in 3D spheroids. Conversely, HLY1 displayed
156 similar laminin expression in 2D and 3D cultures but increased fibronectin and
157 collagen 1 in 3D spheroids (Figure 2B). Altogether these data argue for a promotion
158 of ECM expression in 3D cultures of lymphoma cells.

159 Altogether, these data show that the cellular capsule technology is a valuable
160 approach to produce cohesive lymphoma spheroids from DLBCL.

161

162 **2) Tumor B-cell and stromal cell co-encapsulation and 3D self-organization** 163 ***promote spheroid growth***

164 Since B-NHL are organized as mixed aggregates of tumor cells and
165 lymphoma-supportive non-malignant components (Verdière et al., 2018), we decided
166 to aim for a more physiopathological model by introducing lymphoid stromal cells
167 isolated from human tonsils (Tonsil Stromal Cells, TSC) (Ame-Thomas et al., 2007)

168 and ECM components (Matrigel). We observed that lymphoma cell growth, evaluated
169 by cell counting, was differentially regulated by the presence of ECM and stromal
170 cells. Indeed, while the presence of stromal cells had no significant effect on HLY1
171 spheroid growth, they significantly increased the growth kinetic of SU-DHL-4
172 spheroids, and they even promoted DOHH2-derived spheroid formation and growth,
173 which were not achieved in the absence of TSC (Figure 3A). These results suggest
174 that our 3D model recapitulates the high TSC-dependence of FL B cells whereas
175 DLBCL exhibit a more variable dependence (Ciavarella et al., 2018).

176 We next sought to decipher how stromal cells play a supportive role on DOHH2
177 spheroid formation. DOHH2 cells were co-encapsulated with TSC in the presence or
178 absence of a layer of Matrigel anchored to the inner wall of the capsules (Alessandri
179 et al., 2016). In the absence of Matrigel, the capsule wall remained cell-repellent and
180 stromal cells formed clusters inside the capsules but were unable to induce DOHH2
181 spheroid growth (Figure 3B and C). In contrast, in the presence of Matrigel, TSC
182 spread onto ECM, leading to the formation of a ramified 3D network of stromal cells,
183 that subsequently supported B cell proliferation (Figure 3D and movies 2-3).

184 We then monitored the stromal cell network dynamic by imaging capsules after 2,
185 24, 48, 72, and 96h of culture using confocal microscopy (Figure 4A). By solely
186 focusing on stromal cells, we performed 3D+time image analysis and measured
187 sphericity and branchness that both characterized the evolution of the stromal cell
188 network complexity. High sphericity index indicated a compact morphology of the
189 stromal network, thus offering a limited surface of interaction with B cells. Conversely,
190 high branchness index was associated to a ramified network that increased the
191 probability of B cell-stromal cell physical contacts. Stromal cell morphology was
192 significantly modified as early as 24h after encapsulation, as revealed by the

193 opposite variations of the sphericity and branchness indexes (Figure 4B and C). In
194 addition, after a rapid initial change, both parameters underwent a slower but
195 continuous evolution in time in the same correlated manner, indicating that the
196 increased branching architecture of the stromal network was not a disorganized and
197 randomly observed feature but was sustained by a self-organization process (Figure
198 4D).

199

200 3) ***Microencapsulated 3D co-cultures recapitulate phenotypic features of B*** 201 ***lymphomas***

202 We decided to focus on the 3D TSC-DOHH2 co-culture to investigate the
203 stromal/B-cell bidirectional crosstalk. Previously, we have demonstrated that FL B
204 cells could contribute to the commitment of mesenchymal precursors into lymphoid-
205 like differentiation (at least partly through the secretion of TNF α (TNF)) (Ame-
206 Thomas et al., 2007; Guilloton et al., 2012). Interestingly, we revealed that 3D cell co-
207 cultures induced a stromal cell phenotype similar to that observed *in situ* in FL lymph
208 nodes (Figure 5). More specifically DOHH2 induced podoplanin (PDPN) expression
209 in stromal cells while this was not the case in 2D co-cultures in which only the
210 treatment of TSC cells with a combination of TNF and lymphotoxin $\alpha 1\beta 2$ (LT), the
211 two factors involved in lymphoid stroma differentiation, was able to trigger stromal
212 PDPN expression (Figure 5 and Figure S3). Within spheroids, stromal cells thus
213 acquired characteristics mimicking those observed *in situ*, suggesting that 3D spatial
214 organization is an added value to recapitulate the pathophysiology of lymphoma
215 tissues.

216 We next studied the gene expression profile (GEP) of DOHH2 cells cultured for
217 10 days either in monoculture in 2D (noted 2D), or in co-culture with TSC in 2D or 3D

218 (respectively noted 2D + TSC and 3D + TSC). Data were then compared with the
219 GEP of purified FL B cells and normal tonsil centrocytes (CC) obtained by Affymetrix
220 microarrays. Expression of 37 genes involved in several transduction pathways
221 including NF- κ B cascade, migration signaling, cell cycle/quiescence, and apoptosis,
222 was studied by RT-qPCR (Table 1, Figure S4). Analyses revealed a shift in GEP of B
223 cells cultured in 3D compared to cells cultured in 2D with or without TSC (Figure 6 A).
224 In particular, we observed that the expression of *CDKN1A* and *CDKN1B*, two
225 regulators of cell quiescence (Heldt et al., 2018; Sgambato et al., 2000; Zhang et al.,
226 2013), was similarly up regulated in 3D vs 2D co-cultures and in FL-B cells vs GC B-
227 cells (Figure 6B). Moreover, *BCL2* was upregulated in DOHH2 after the 3D co-culture
228 and was highly expressed in FL B cells (Figure 6C).

229 The hypoxic tumor environment has been often reproduced in 3D tumor *in vitro*
230 models (Benien and Swami, 2014). We confirmed this observation in our lymphoma
231 3D model, since both the upstream HIF1A transduction pathway regulator (*EGLN1*)
232 and some HIF1A target genes (*VEGF*, *CXCR4*) were upregulated compared with 2D
233 co-culture condition, reflecting, at least in part, the expression pattern obtained in FL
234 B cells compared with normal CC (Figure 6D and E).

235 FL is a disseminated disease and we thus sought to determine the impact of 3D
236 architecture on the expression of genes involved in cell confinement or cell migration.
237 Interestingly, *RGS13*, known to limit GC size and GC B-cell number by interfering
238 with *CXCL12* and *CXCL13* signals (Hwang et al., 2013), was specifically
239 downregulated in 3D co-cultures and FL B cells. Conversely, *CXCR4* expression was
240 similarly upregulated in FL-spheroids and FL B cells (Figure 6E).

241 Activation of the NF- κ B pathway has been extensively described in B-NHL
242 (Kennedy and Klein, 2018), leading us to investigate the expression of this pathway

243 in our 3D model. Our analyses revealed an upregulation of the noncanonical (*RELB*)
244 NF- κ B pathway in spheroids compared to 2D co-cultures. Concomitantly, the
245 expression of *TNFAIP3*, a negative regulator of the canonical NF- κ B pathway, as
246 well as that of *BCL21A* and *CFLAR*, two target genes of NF- κ B, were increased in 3D
247 compared to 2D co-cultures (Figure 6F). These results suggest that a functional non-
248 canonical NF- κ B pathway is activated in FL-spheroids.

249 Collectively, these results suggest that the co-culture of DOHH2 with TSC in 3D
250 exhibits high degree of similarity with FL biopsies and partly mimics the lymphoma
251 niche.

252 **4) B-NHL sensitivity to chemotherapy in 3D vs 2D cell culture**

253 We wondered whether the 3D architecture could alter the drug response of
254 lymphoma B cells. For that purpose, we evaluated the effect of doxorubicin, one of
255 the components of the classical polychemotherapy used in B-NHL (CHOP), on the
256 cell death of B-NHL cell lines cultured in 2D or 3D in the absence or presence of
257 stromal cells. As shown in Figure 7A, we observed that the induction of apoptosis by
258 doxorubicin tested at a concentration corresponding to its EC50 for the different cell
259 lines (Figure S5), was abolished or significantly decreased in cells cultured in 3D
260 compared to 2D. Based on our results showing that *BCL2* expression was
261 upregulated in FL spheroids, we then tested the effect of a BCL2 inhibitor. ABT-199
262 induced massive cell death in DOHH2 co-cultured in 2D with or without stromal cells,
263 as well as in 3D co-cultures, suggesting that malignant B cells retained their
264 dependence on BCL2 expression for survival (Figure 7B).

265 Altogether, these results reveal that our 3D lymphoma model may be useful to
266 perform precision medicine or drug screening.

267 **DISCUSSION**

268 Lymphomas develop as complex cell structures including tumor cells and their
269 microenvironment within mechanically constraint lymph nodes (LN). Previous
270 lymphoma spheroid models incorporating only malignant B cells (Decaup et al., 2013;
271 Gravelle et al., 2014) have already suggested that the 3D cell architecture is a key
272 feature for the regulation of lymphoma growth and therapeutic response. LN are
273 highly dynamic structures expanding and becoming mechanically stiff under immune
274 cell recruitment and proliferation, whereas immune response resolution is associated
275 with LN contraction and return to a baseline of mechanical softness (Fletcher et al.,
276 2015). LN mechanical properties rely on several determinants including the external
277 constraint of the capsule and the internal tension created by the TSC network of
278 specialized myofibroblasts able to produce and contract ECM. Moreover,
279 mechanosensing has been recently demonstrated to control T-cell activation and
280 metabolism (Meng et al., 2020) together with mechanical forces are involved in TCR
281 (Hu and Butte, 2016) and BCR (Apoorva et al., 2018) signaling. Mouse models of FL
282 and DLBCL cannot entirely recapitulate the biology of these tumors (Ramezani-Rad
283 and Rickert, 2017) and, due to their inherent complexity and cost, they are not
284 suitable to study specific cellular interactions contributing to the organization of
285 lymphoma cell niche or to perform drug screening. The development of 3D lymphoma
286 models including mechanical constraints is therefore of particular relevance to
287 advance the understanding and treatment of these neoplasia. In the present work,
288 we adapted a microfluidic approach to recapitulate the lymphoma microenvironment
289 in hollow, permeable alginate shells, forming high-throughput (~5000/s), size-
290 controlled, and easy to handle spheroids (Alessandri et al., 2013). Such a strategy is
291 ideally suited for drug testing and the versatility of the technique allows generating

292 3D monocultures (i.e. from tumor B cells only) or co-cultures with human stromal
293 cells, and to evaluate the impact of ECM.

294 First, the proliferation of malignant B cells alone within spheroids results in
295 cohesive multicellular aggregates promoted by the over-secretion of ECM in 3D
296 compared to 2D cultures. Although expression and secretion of ECM have been
297 mainly described in stromal cells, we demonstrated here that lymphoma B cells
298 display a similar ability, as already proposed for normal plasmablasts (Della-Torre et
299 al., 2020) and plasma cells (Tancred et al., 2009), or leukemic B cells (Mikaelsson et
300 al., 2005). How ECM expression is regulated remains unclear. We could however
301 hypothesize that it may originate from the activation of a mechanotransduction
302 pathway as already described in other cell types (Coplen et al., 2003; He et al.,
303 2004). Remarkably, alginate capsules are elastic, meaning that cell growth past
304 confluence dilates the capsule, which conversely applies a compressive force onto
305 the spheroid (Alessandri et al., 2013). Although not investigated in depth, we
306 qualitatively observed that the cohesion of spheroids was stronger for post-confluent
307 capsules, which would support this mechanotransduction hypothesis.

308 Second, as previously reported using other 3D models, some B-cell lines,
309 including DOHH2, were unable to grow and form spheroids when maintained alone in
310 capsules, reproducing the strong dependency of FL on their niche for survival and
311 growth. Guided by our previous studies showing that lymphoid-like stromal cells have
312 a supportive effect on FL B cells (Ame-Thomas et al., 2007; Gallouet et al., 2014;
313 Guilloton et al., 2012), we added TSC to generate hybrid stroma-tumor 3D lymphoma
314 models. Interestingly, our 3D model reproduced the different levels of NHL
315 microenvironment dependency, mimicking the biology of aggressive versus indolent
316 lymphomas (Scott and Gascoyne, 2014). By contrast with other 3D culture

317 approaches that consist in embedding cells in an ECM scaffold (Gravelle et al., 2014;
318 Purwada et al., 2015; Sabhachandani et al., 2019; Tian et al., 2015), our alginate
319 spheroid model allows cell motions and interactions, leading to an early self-
320 organization of a stroma-tumor B cell network that could organize localized 3D niches
321 promoting cell interactions within the spheroids. Besides favoring or allowing
322 malignant B-cell growth, the 3D co-culture was also associated with coevolution of
323 stromal cells. In particular, TSC displayed increased podoplanin expression in 3D,
324 mimicking the phenotype of FL-infiltrating stromal cells (Pandey et al., 2017),
325 whereas such induction was not observed in 2D co-culture. Podoplanin is a
326 transmembrane glycoprotein known to mediate the lymphoid stromal cell contractile
327 function within LN (Astarita et al., 2015). It is thus tempting to speculate that
328 podoplanin upregulation could contribute to the regulation of spheroid mechanical
329 constraints. Production of TNF by malignant B cells has been proposed as a key
330 mechanism of their dynamic interaction with stromal cells, leading to the upregulation
331 of podoplanin, CCL2, and IL-8 in 2D culture conditions *in vitro* (Guilloton et al., 2012).
332 However, neither TNF nor LT were found upregulated in DOHH2 maintained in 3D
333 culture (data not shown), confirming a recent work performed on composite hydrogel-
334 based lymphoma spheroids (Sabhachandani et al., 2019), suggesting that other
335 soluble factors, but also 3D architecture and spatial constraints could contribute to
336 the reprogramming of stromal cells.

337 Overall, the analysis of B-cell gene expression profile within spheroids
338 revealed a profound shift associated with the transition from a 2D to a 3D context,
339 mimicking the phenotype of primary FL B cells. The drop of B-cell proliferation that
340 took place when cells filled the capsule was associated with an upregulation of
341 quiescence genes also strongly expressed by FL B cells in agreement with their

342 indolent phenotype. Even in the absence of a necrotic core within spheroids, the
343 increase of *EGLN1* and *VEGF* expression could reflect a response to hypoxia, a
344 process associated with a limitation of energy consumption through reduction of cell
345 proliferation and DNA repair (Goda et al., 2003; Koshiji et al., 2004). Such
346 association of response to hypoxia and quiescence has been previously described in
347 a 3D model where spheroids were generated by the hanging drop method with
348 lymphoma B cells alone (Gravelle et al., 2014). Interestingly, *BCL2* was found
349 upregulated in 3D spheroids and has been previously shown to display anti-
350 proliferative effects in normal and malignant B cells (Tellier et al., 2014; Zinkel et al.,
351 2006), suggesting that it could contribute to the decrease of cell proliferation. Even if
352 the t(14;18) is the main driver of *BCL2* deregulation in FL, *BCL2* expression shows a
353 high level of inter- and intra-tumoral heterogeneity and is correlated with IgH
354 expression and NF- κ B activation (Barreca et al., 2014; Heckman et al., 2002).
355 Accordingly, we highlighted an upregulation of *RELB* and NF- κ B target genes in 3D
356 co-cultures, indicating that the NF- κ B pathway was activated in lymphoma spheroids.
357 Of note, a recent study revealed that fluid shear stress upregulates BCR expression
358 and signaling in ABC-DLBCL (Apoorva et al., 2018), suggesting that engagement of
359 BCR/NF- κ B/*BCL2* loop by biophysical forces could be a general feature of B-cell
360 lymphomas. Finally, we described in our 3D model an upregulation of *CXCR4*,
361 previously associated with FL B-cell migration, adhesion, and survival. Together with
362 the reduction of *RGS13* involved in the organization of GC into dark and light zones
363 and the limitation of GC size (Hwang et al., 2013; Shi et al., 2002), this could
364 reproduce some of the factors sustaining FL dissemination and will require further
365 investigations.

366 BCL2 expression is a key determinant of response to therapy in FL patients
367 (Barreca et al., 2014). Moreover, the 3D-induced drug resistance has been already
368 reported in solid cancers and hematological malignancies (Desoize, 2000; Gravelle et
369 al., 2014; Tian et al., 2015). We confirmed here that the sensitivity to doxorubicin
370 dramatically dropped when cells were cultured in 3D compared to 2D in all
371 investigated B NHL cell lines. By contrast, the pro-apoptotic activity of ABT-199,
372 mediating the dissociation of BCL2 with the proapoptotic BH3 only proteins, was
373 maintained in 3D co-cultures despite BCL2 upregulation suggesting that ABT-199
374 might be considered as a good alternative for FL patient treatment. More generally,
375 the cellular capsule technology adapted for lymphoma 3D culture seems a useful tool
376 to screen new therapeutic approaches.

377 In summary, we report the development of a new lymphoma 3D model,
378 including stromal cells and ECM, and recapitulating the dynamic crosstalk between
379 tumor cells and their supportive microenvironment. The versatility of this high-
380 throughput platform makes it possible to evaluate the role of various ECM
381 components, stromal cell subtypes, and soluble factors on the spatial organization
382 and function of the lymphoma niche. Moreover, this approach is particularly suitable
383 for the testing of new therapeutic agents in B-NHL.

384

385 **MATERIALS AND METHODS**

386 **Reagents and antibodies**

387 Alginic acid sodium salt from brown algae, D-Sorbitol and CaCl₂ hexahydrate,
388 EGTA, paraformaldehyde were supplied by Merck (Fontenay sous bois, France).
389 Matrigel was provided by Corning (UGAP, France). Propidium iodide and Phalloidin-
390 Alexa 594 were from Thermo Fisher Scientific (Courtaboeuf, France). Hoechst 33258
391 and ABT-199 were purchased from Tocris Bioscience (Lille, France). The
392 comprehensive cancer center pharmacy department of the Institut Bergonié kindly
393 provided doxorubicin. APC Annexin V was from BD Biosciences (Le pont de Claix,
394 France).

395 Rabbit polyclonal anti-laminin, rabbit polyclonal anti-fibronectin, rabbit
396 monoclonal anti-human collagen I and chicken polyclonal anti-GFP antibodies were
397 supplied by Abcam (Paris, France). Mouse anti-human CD20 APC-H7 and mouse
398 anti-human CD73 PE were from e-Bioscience (Thermo Fisher Scientific). Monoclonal
399 mouse anti-human Ki67 was from Dako Agilent Technologies (Les Ulis, France) and
400 rabbit polyclonal anti-cleaved caspase 3 antibody was provided by Cell Signaling
401 Technology (Ozyme, Saint Cyr l'école, France). All Alexa-conjugated secondary
402 antibodies were purchased from Thermo Fisher Scientific.

403

404 **Encapsulation procedure**

405 Cell encapsulation was performed by adapting the protocols from (Alessandri
406 et al., 2016; Doméjean et al., 2016; Trushko et al., 2020). Briefly, it includes the
407 fabrication of the device, the preparation of the solutions and the operation itself for
408 cellular capsules formation.

409 First, the microfluidic device was printed with EnvisionTEC Micro Hi-Res Plus
410 3D printer (Arkety3D, Le Raincy, France) using the resin HTM140V2 (EnvisionTEC),
411 with the following printing parameters: burn-in range thickness 400 μ m, base plate of
412 300 μ m, and exposure time 3000ms. The printed device was washed using propanol
413 and air-dried. In parallel, a glass capillary was pulled using a micropipette puller (P30,
414 Sutter Instrument, Micro Mecanique SAS, Saint Germain en Laye, France), then cut
415 and polished with micro-abrasive films (1 μ m grain, 3M) to reach the desired tip
416 diameter (typically between 130 and 180 μ m). This glass tip, which serves as a
417 nozzle for the encapsulation device, was hydrophobized (1H,1H,2H,2H-
418 Perfluorooctyltrimethoxysillane, ABCR) following standard protocols (Perret et al.,
419 2002) and glued to the exit of the microfluidic plastic device with epoxyglue EA M-
420 31CL (Loctite).

421 To run an encapsulation, the microfluidic device is connected to three syringes
422 (10MDR-LL-GT SGE, Analytical Science, Milton Keynes, UK) mounted on pumps
423 (Nemesys, Cetoni GmbH, Korbussen, Germany) for flow rate control. Three different
424 solutions are injected into the three coaxial cones of the device. The outermost cone
425 contains the alginate solution, which is prepared by dissolving 2% wt/vol sodium
426 alginate (Protanal LF200S) in water and by adding 0.5mM SDS surfactant (VWR
427 International, Fontenay sous bois, France). The solution was filtered at 1 μ m (glass
428 filters, Pall Life Science) and stored at 4 °C. The intermediate cone contains a 300
429 mM sorbitol (Merck) solution. The innermost cone contains cells suspended in a
430 sorbitol solution or cells/Matrigel/sorbitol solution (when ECM is needed) in a ratio of
431 2:1:2 (v/v). Lymphoma B cells lines were taken from cell suspension routinely
432 cultured while adherent stromal cells were detached from the culture flask with
433 trypsin-EDTA (Thermo Fisher Scientific). After rinse, cells were centrifuged and

434 counted. For encapsulation of lymphoma cells or stromal cells only (mono-culture), a
435 suspension of 2×10^6 cells were resuspended in culture DMEM (Thermo Fisher
436 Scientific). When adherent and lymphoma B cells were co-encapsulated, a
437 suspension of 2×10^6 cells of each cell type were used. 200 μ L of the cell suspension
438 is injected into a cooling part to maintain Matrigel liquid (Alessandri et al., 2016). The
439 flow rates are set to 45mL/h, 45mL/h and 30mL/h for the alginate, sorbitol and cell
440 suspension, respectively. The device is positioned 40-50 cm above a petri dish
441 containing 100mM CaCl₂ solution for capsule gelling and collection. To improve
442 capsule shape and monodispersity an alginate charging part and copper ring (21mm
443 OD, RadioSpares), both connected to a high voltage (2 kV) generator (Stanford
444 Research PS350, Acal BFI, France) are introduced, as described in (Trushko et al.,
445 2020). The charged formed droplets passing through the copper ring under electrical
446 tension get deflected as they cross the ring, creating a splay-like jet that prevents
447 capsule coalescence. Once formed inside the calcium bath, capsules are washed
448 with DMEM and transferred to cell culture medium.

449

450 **Cell lines, suspension, monolayer and encapsulated cell culture**

451 Stromal cells (TSC) were isolated as previously described (Ame-Thomas et
452 al., 2007; Guilloton et al., 2012) from human tonsils collected from children
453 undergoing routine tonsillectomy, considered as surgical wastes. GFP-expressing
454 TSC cells were established after transduction with lentivirus carrying the pUltra (gift
455 from Malcolm Moore (Addgene plasmid # 24129; <http://n2t.net/addgene:24129>;
456 RRID: Addgene_24129), which contained the enhanced GFP gene under the control
457 of Ubc promoter and produced as previously described (WU and LU, 2007). TSC
458 were transduced in RPMI supplemented with 2% FBS containing pUltra concentrated

459 lentiviral supernatant with MOI of 4. On the next day, the medium was replaced with
460 standard culture medium. After one week, eGFP^{high} TSC were isolated using a cell
461 sorter (FACS Aria, BD Biosciences). For all the experiments reported in the present
462 work, TSC were used from P9 to P13. DLBCL SU-DHL-4 and FL DOHH2 cell lines
463 were obtained from the DSMZ cell collection (Braunschweig, Germany). HLY-1 cell
464 line was kindly provided by Dr F. Meggetto (CRCT, Toulouse, France). Cells were
465 routinely cultured in RPMI medium (Thermo Fisher Scientific), supplemented with
466 10% FBS (Dutscher, Brumath, France) under humid atmosphere containing 5% CO₂,
467 with medium changed 3 times a week. One day prior to encapsulation, cells were
468 cultured in DMEM supplemented with 10% FBS. Once cellular capsules were formed
469 following the protocol described above, they were cultured in 75 cm² flasks in DMEM
470 supplemented with 10% FBS under humid atmosphere containing 5% CO₂, with
471 medium changed 2 times a week. Depending on the type of experiments, capsules
472 were sampled and transferred to dedicated culture supports, e.g. for imaging, or
473 treated in bulk conditions and subsequently analyzed, e.g. for FACS.

474

475 **Spheroid growth measurement**

476 At various post-encapsulation culture times, we selected about 200 capsules.
477 Capsules were dissolved by incubation in PBS containing 1 mM EGTA and the
478 spheroids were mechanically dissociated by pipetting. When spheroids were too
479 cohesive, trypsin-EDTA was added to improve cell dissociation. The cell suspension
480 was centrifuged and re-suspended in 200 µL of PBS and 50 µL of Precision Count
481 Beads (BioLegend, Ozyme). The number of cells was then determined in a given cell
482 suspension volume of 100 µL using flow cytometry (FACS Calibur, BD biosciences)
483 according to the manufacturer's instructions. To evaluate the cell death in spheroids,

484 propidium iodide (PI) (10 µg/mL) was added to the cell suspension and PI
485 fluorescence was analyzed by flow cytometry using Cell Quest or FlowJo (BD
486 biosciences).

487

488 **Immunofluorescence staining of spheroids**

489 *In capsule staining*

490 Capsules were fixed in DMEM w/o phenol red (Pan Biotech, Dutscher),
491 containing 4% paraformaldehyde for 20 min at room temperature (RT). After rinsing,
492 capsules were incubated for 30 min in DMEM w/o phenol red/ 0.1% triton X-100.
493 Following a step of saturation with a solution containing DMEM w/o phenol red/ BSA
494 1% /FBS 2% for 1h at RT, capsules were incubated with primary antibody overnight
495 at 4°C under shaking. Primary antibody was revealed by incubation of capsules with
496 secondary Alexa-coupled antibody for 2h at RT. Nuclei were stained by incubation of
497 capsules with Hoechst 33258 for 30 min at RT. To determine the role of Matrigel in
498 the stromal network establishment, immunofluorescence using a rabbit anti-human
499 pan laminin antibody revealed by goat anti rabbit Alexa 633 secondary antibody, was
500 performed to visualize Matrigel, stromal cells were GFP positive and nuclei was
501 stained with Hoechst 33258. Z-stacks were acquired using a Zeiss LSM 510 meta
502 confocal microscope with a 25x objective (N.A 0.7). 3D rendering was obtained using
503 UCSF Chimera software (RBVI, San Francisco, USA).

504

505 *FFPE staining*

506 To analyze protein expression inside spheroids, immunofluorescence on
507 FFPE (Formalin-Fixed Paraffin-Embedded) spheroids sections (3-5 µm thick) were
508 performed. Alginate shells were dissolved and spheroids were fixed as described

509 above. Spheroids were then pre-embedded in 2% agarose and further embedded in
510 a paraffin block. Immunofluorescence staining was then performed as previously
511 described (Andrique et al., 2019). Images were acquired using a Zeiss LSM 510
512 meta confocal microscope (Zeiss, Göttingen, Germany) with a 25x objective (N.A
513 0.7). Then images obtained were processed using Fiji.

514

515 **3D cell morphology quantification**

516 Capsules were fixed at various post-encapsulation culture times and each
517 capsule was placed in an agarose well of the Universlide microscopy chamber
518 (Alessandri et al., 2017), which allowed immobilization of the capsules as required for
519 stable z-stack acquisition and medium throughput 3D imaging. Cell nuclei were
520 stained with Hoechst 33258 and stromal cells were visualized by the GFP
521 expression. Images were acquired using a Leica SP8 confocal microscope with a
522 20X dry objective (NA 0.7). To measure the evolution of TSC complexity over time

523 two parameters were measured: the sphericity defined as $\sqrt[3]{\frac{36 \times \pi \times \text{Volume of particle}^2}{\text{Surface of particle}^3}}$ and

524 the branchness defined as $\frac{\text{Volume of particle convex hull}}{\text{Volume of particle}}$. To obtain these values, we

525 applied a locally adjusted threshold to the green signal, performed 3D segmentation
526 (3D suite plugin, Fiji (Ollion et al., 2013)). For the two parameters, the values of all
527 “particles” contained in one capsule were averaged. Thus, each capsule was
528 characterized by one mean value for sphericity and branchness. 10-18 capsules
529 were analyzed per time point

530

531 **Multiplex fluorescence microscopy**

532 Patients were recruited under institutional review board approval following
533 informed consent process according to the declaration of Helsinki and the French
534 National Cancer Institute ethic committee recommendations. Tissue samples
535 included tonsils collected from routine tonsillectomy, and lymph nodes obtained from
536 grade 1-2 FL and from DLBCL patients.

537 Four-micrometer-thick whole-slide sections, obtained with a microtome
538 (Histocore multicut Leicabiosystems, Nanterre, France) from FFPE tissue or
539 spheroids blocks, were transferred onto plus-charged slides (VWR international),
540 followed by multiplex immunofluorescence staining with a U DISCOVERY 5 plex
541 immunofluorescence (Roche Diagnostics, Meylan, France). Three sequential rounds
542 of staining were performed each including heat deactivation step, followed by
543 incubation with primary antibody and corresponding HRP secondary antibody.
544 Hence, rat anti-podoplanin (Thermo Fisher Scientific NZ-1.3), mouse Anti-PAX5
545 antibody (Abcam ab224660), and sheep anti-transglutaminase (R&D AF4376)
546 expressions were visualized on the same section using HRP enzyme mediated
547 deposition of the tyramide coupled to rhodamine, DCC and FAM fluorophores (kits
548 Ventana Medical Systems, Tucson, Ariz), respectively, that covalently binds to the
549 tissue at the site of the reaction. After 3 sequential reactions, sections were
550 counterstained with DAPI and coverslipped using fluoromount (Enzo Life Sciences,
551 Farmingdale, NY, USA). Visualization was performed with the Nanozoomer
552 (Hamamatsu Photonics, Massy, France) equipped with the multicolor fluorescence
553 module.

554

555 **Cell co-culture and cell sorting:**

556 2D co-cultures were performed over 3 days. 3×10^5 DOHH2 (1.5×10^5 /mL) were plated
557 on 5×10^4 TSC /cm² in 6 well plates. For cell sorting, cells were trypsinized (Thermo
558 Fisher Scientific) for 12 min at 37 °C and washed with medium DMEM 10% FBS. 3D
559 co-cultures were performed over 10 days. For cell sorting, after dissolution of the
560 alginate shells, cells were rinsed with DMEM 10% FBS and centrifuged for 7 min at
561 1700 rpm, incubated with trypsin at 37 °C for 15 min, and rinsed with medium DMEM
562 10% FBS. Cells were stained for 20 min on ice with mouse anti-human CD20 APC-
563 H7 and mouse anti-human CD73 PE. After DAPI staining, cell sorting was processed
564 by the FACS Aria II (BD Biosciences).

565

566 **RT q-PCR arrays**

567 After cell sorting, RNA extraction from DOHH2 co-cultured in 2D or 3D with
568 TSC cells was done using Nucleospin RNA XS Micro kit (PN 740990) from Macherey
569 Nagel (Hoerd, France). cDNA were synthesized using reverse transcription master
570 mix (Fluidigm, USA, PN 100-6297), followed by gene expression preamplification
571 with Fluidigm Preamp Master mix (PN 100-5744) and Taq Man assays and followed
572 by RT-QPCR. Assay-on-demand primers, probes, and TaqMan gene expression
573 Master Mix were obtained from Thermo Fisher Scientific. Gene expression was
574 measured using 96.96 Dynamic Array™ IFCs and the BioMark™ HD System
575 from Fluidigm, based on Δ Ct calculation method. A tonsil pool was used as
576 appropriate DOHH2 expression external control. For each sample, the Ct value for
577 the gene of interest was determined and normalized to its respective mean value of
578 housekeeping genes (*ABL1* and *GAPDH*). Data were represented through a
579 heatmap using the pheatmap R package. In addition, differentially expressed genes

580 were analyzed in Affymetrix data sets comparing purified FL B cells and purified
581 centrocytes (GSE85233 and GSE136249) (Pangault et al., 2020).

582

583 **Chemotherapeutic toxicity measurement**

584 To compare the drug toxicities between lymphoma cells cultured in
585 suspension and in spheroids, 0.5×10^6 cells/well co-cultured or not with 5×10^4 TSC
586 /cm² or 100 confluent spheroids were distributed in 6-well plates and treated or not
587 with doxorubicin for 24h. Then, cell death was assessed by flow cytometry after
588 Annexin V-APC labelling according to the manufacturer's instructions.

589

590 **Statistical analyses**

591 All data were expressed as means \pm SEM. The significance of differences was
592 calculated using the parametric ANOVA or the non-parametric U-Mann and Whitney
593 or Wilcoxon tests, as appropriate. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).
594 Statistical analyses were performed using GraphPad Prism 8 software.

595

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616

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845

846 **FIGURE LEGENDS**

847 **Figure 1: B NHL spheroids obtained by using the cellular capsule technology.**

848 **A:** Representative images of cell growth in alginate capsule shells over time for the
849 various cell lines. Scale bar: 100 μ m. **B:** Percentage of SU-DHL-4 and HLY1 cell
850 death measured after shell dissolution and cell dissociation at various culture time
851 points. To evaluate the cell death propidium iodide (PI) (10 μ g/mL) was added to the
852 cell suspension and PI fluorescence was analyzed by flow cytometry (n=3). **C:**
853 Immunostaining showing the repartition of proliferating and apoptotic cells in SU-
854 DHL-4 and HLY1-derived spheroids at day 8 or 12 post-encapsulation. Five
855 micrometers thick sections were stained with anti-Ki67 (Ki67, in green), and anti-
856 cleaved caspase 3 (CC3, in red), to identify proliferating and dead cells, respectively.
857 Nuclei are depicted in blue. Scale bar: 100 μ m.

858

859 **Figure 2: B NHL spheroids are cohesive and express extracellular matrix.**

860 **A:** Pictures showing the maintenance of SU-DHL-4 spheroids after the capsule
861 dissolution by incubation in PBS containing 1 mM EGTA. Scale bar: 100 μ m. **B:**
862 Immunostaining showing the expression of extracellular matrix in cells cultured in
863 suspension (2D) or in spheroids (3D). After fixation, cell suspension was centrifuged
864 and pellet was embedded in 2% agarose and then in paraffin, as performed for
865 spheroids. Extracellular matrix components were visualized by staining with anti-pan
866 laminin, anti- fibronectin and anti- collagen I antibodies on 5 μ m thick sections. Nuclei
867 are depicted in blue. Scale bar: 50 μ m.

868

869 **Figure 3: The establishment of a stromal network is necessary to reproduce**
870 **stromal-dependent B NHL cell growth.**

871 **A:** Effect of Matrigel (Mg) and TSC on spheroid growth. Left panel, representative
872 images of spheroids obtained from B cells encapsulated with or without Mg and TSC
873 at D7 (HLY1), D6 (SU-DHL-4) or D10 (DOHH2) of culture. Scale bar: 200 μ m. Right
874 panel, the cell number was evaluated at various culture time points in the different
875 conditions (n=3). **B:** TSC network needs Mg to be established. *Left*, example of TSC
876 encapsulated without Mg. *Right*, example of TSC encapsulated with Mg. TSC were
877 visualized in green by stable expression of GFP and nuclei were stained in blue with
878 Hoechst 33252. Capsules were imaged 3 days after encapsulation. Images were
879 maximum intensity projection from z-stacks. Scale bar: 100 μ m. **C:** TSC network is
880 necessary to induce DOHH2 spheroid growth. DOHH2 were co-encapsulated with
881 TSC with or without Mg for 10 days. TSC were visualized in green by stable
882 expression of GFP. **D:** TSC/Mg interaction. TSC were encapsulated in the presence
883 of Mg. After 3 days in culture, capsules were fixed and immunofluorescence was
884 performed. Mg and TSC were visualized by staining with anti-human pan laminin (in
885 red) and anti-GFP (in green), respectively. Nuclei were counterstained in blue with
886 Hoechst 33258. Images were maximum intensity projection from z-stacks. Scale bar:
887 50 μ m. Yellow square is a crop showing the hanging of TSC on Mg coating. Scale
888 bar: 10 μ m.

889

890 **Figure 4: Self-organization of the stromal network.**

891 **A:** Representatives images of capsules containing DOHH2 with Mg and TSC at
892 different culture time point. After fixation, 10 to 18 spheroids for each time point were
893 placed in an Universlide (Alessandri et al 2017). Images shown are Z-projection. TSC
894 are shown in green and all the analyses were performed on green signal exclusively.
895 Scale bar: 50 μ m **(B)** and **(C)** Sphericity and branchness indexes of green particles

896 contained in spheroids at different time after encapsulation, respectively. To
897 determine significant differences, Anova was performed (* $P < 0.05$, ** $P < 0.01$,
898 *** $P < 0.001$, **** $P < 0.0001$). (D) Correlation of sphericity and branchness. For each
899 time point, small dots represent individual spheroids whereas big dots represent the
900 average values of all spheroids. Pearson correlation coefficient (R) was calculated
901 using R software. The black line represents the regression line and the grey area
902 represents the 95% confidence interval of the linear regression.

903

904 **Figure 5: Spheroids recapitulate the FL stromal cell phenotype.**

905 **A:** Tonsil B cells (revealed with anti-PAX5 antibody in blue) interact mainly with
906 stromal cells expressing podoplanin (red). In FL lymph node, tumor B cells interact
907 with stromal cells expressing podoplanin (red) and transglutaminase (green) markers.
908 Scale bar: 250 μ m. In the 3D model TSCs adopt a particular phenotype in contact
909 with DOHH2 by expressing transglutaminase (in green) and podoplanin (in red)
910 markers. Scale bar: 100 μ m. **B:** 3D model at a higher magnification represents
911 DOHH2 (in blue) in close contact with the podoplanin (shown by arrows in red) and
912 transglutaminase (in green) expressing TSC network. Scale bars: 50 μ m. All images
913 were stained with multiplex immunofluorescence microscopy method and visualized
914 by Nanozoomer software.

915

916 **Figure 6: Comparison of DOHH2 and FL B cell gene transcriptional expression**
917 **profile.**

918 **A:** Heatmap of DOHH2 gene expression levels evaluated by Real Time quantitative
919 PCR (RT-qPCR) in cells cultured in 2D without (n=5) or with TSC (n=3), and in 3D
920 with TSC (n=5). Analysis of DOHH2 transcriptional gene expression profiles, by RT-

921 qPCR, for **B**: *CDKN1A* and *CDKN1B*, **C**: *BCL2*, **D**: *EGLN1* and *VEGFA*, **F**: *RGS13*
922 and *CXCR4*, **G**: *RELB*, *TNFAIP3*, *BCL2A1* and *CFLAR*. DOHH2 were cultured in 2D
923 without (n=5) or with TSC (n=3) and in 3D with TSC (n=5) for 10 days. Variation gene
924 expression study between tonsil centrocytes (n=6) and FL lymph nodes tumoral B
925 cells (n=10) was studied by using Affymetrics micro-array analysis. A Kruskal Wallis
926 test was performed for *in vitro* experiments and a two tailed Mann Whitney test was
927 performed for the variation gene expression study between tonsil centrocytes (CC)
928 and LF lymph nodes tumoral B cells (FL C). ($p < 0.05^*$, $p < 0.01^{**}$). Bar represent the
929 geometric mean of independent experiments.

930

931 **Figure 7: Impact of 3D culture on drug response in B-NHL.**

932 **A-C**: Comparison of cell death induced by doxorubicin in SU-DHL-4 (**A**), HLY1 (**B**)
933 and DOHH2 (**C**) cultured in 2D or in 3D alone (*left panel*) or with TSC (2D+TSC or
934 3D+TSC) (*right panel*). For 2D culture, B cells were seeded alone or in presence of
935 TSC . For 3D culture, 100 confluent capsules, containing B cells alone or B cells with
936 TSC in presence of Matrigel, were seeded. 2D and 3D cultures were exposed to
937 doxorubicin at a concentration corresponding to its EC50 and cell death was
938 evaluated after 24h.. **D**: Comparison of cell death induced by ABT-199 (1 μ M) in
939 DOHH2 cells cultured in 2D (*left panel*) or in 3D with TSC and Matrigel (*right panel*),
940 for 24h. The data represent mean \pm SE of, at least, three independent experiments
941 (Wilcoxon test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$).

942

943 **Movie 1: Dissolution of alginate shell.** Alginate shell of capsule containing SU-
944 DHL-4 spheroid obtained after 10 days in culture is dissolved in a bath of PBS/EGTA.
945 The images were acquired every 15 seconds until complete dissolution of capsule
946 using an epifluorescence microscope (Leica DMI8).

947

948 **Movie 2: Establishment of the stromal cell network.** TSC expressing GFP and
949 DOHH2 expressing dTomato were co-encapsulated in presence of Matrigel and
950 placed in an Universlide in DMEM containing 10% FCS. The images were acquired
951 immediately after encapsulation every 30 minutes for 12 hours using an
952 epifluorescence microscope (Leica DMI8) equipped with a thermostatic and CO₂
953 chamber (Live on Stage, Leica). Scale bar: 100µm.

954

955 **Movie 3: Spatial organization of lymphoma spheroid.** 3D reconstruction from
956 optical sections using confocal microscopy of capsule containing DOHH2 with Mg
957 and TSC cultured for 6 days.

958

Pathway	Gene
Cell survival	<i>CFLAR</i> <i>BCL2</i> <i>BRCA1</i> <i>BCL2A1</i>
Hypoxia	<i>VEGFA</i> <i>EGLN1</i>
B cell and BCR activation	<i>EBI3</i> <i>FYN</i> <i>CD79b</i> <i>CD79a</i> <i>RRM2</i>
Proliferation and differentiation	<i>CD24</i> <i>MET</i> <i>CD72</i>
Cell cycle	<i>CDC20</i> <i>CDK1</i> <i>CDK4</i>
Mechanosensitive	<i>PIEZO 1</i>
NFκB	<i>TNFAIP3</i> <i>BCL10</i> <i>RELB</i>
Quiescence	<i>CDKN1B</i> <i>CDKN1A</i>
Confinement and motility	<i>CXCR4</i> <i>RGS13</i> <i>P2RY8</i> <i>S1PR1</i> <i>CCR7</i> <i>GPR183</i> <i>CXCR5</i>
Apoptosis	<i>CASP8</i>
Cell and matrix interactions	<i>ADAM8</i>
Adhesion	<i>CD52</i>
Others	<i>RGS10</i> <i>KLF4</i> <i>LMO2</i> <i>IL6R</i>
Housekeeping genes	<i>GAPDH</i> <i>ABL1</i>

959 **Table 1: List of genes studied by RT-qPCR arrays.**

960

Figure 1

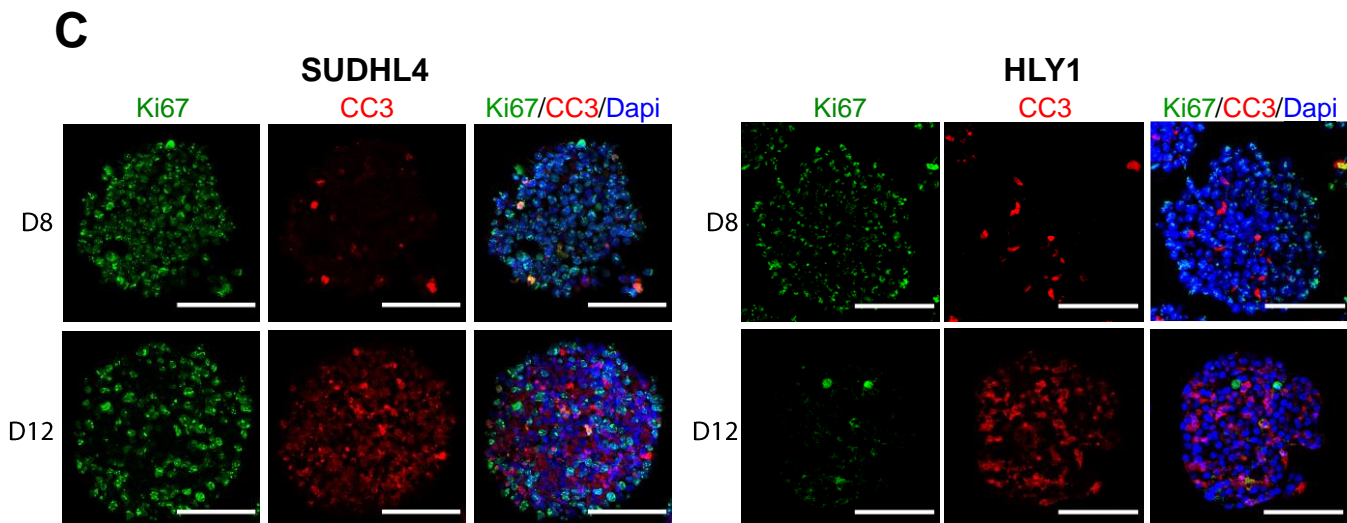
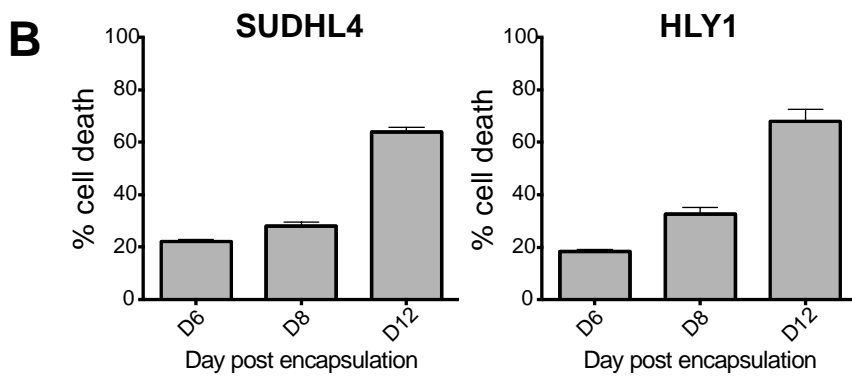
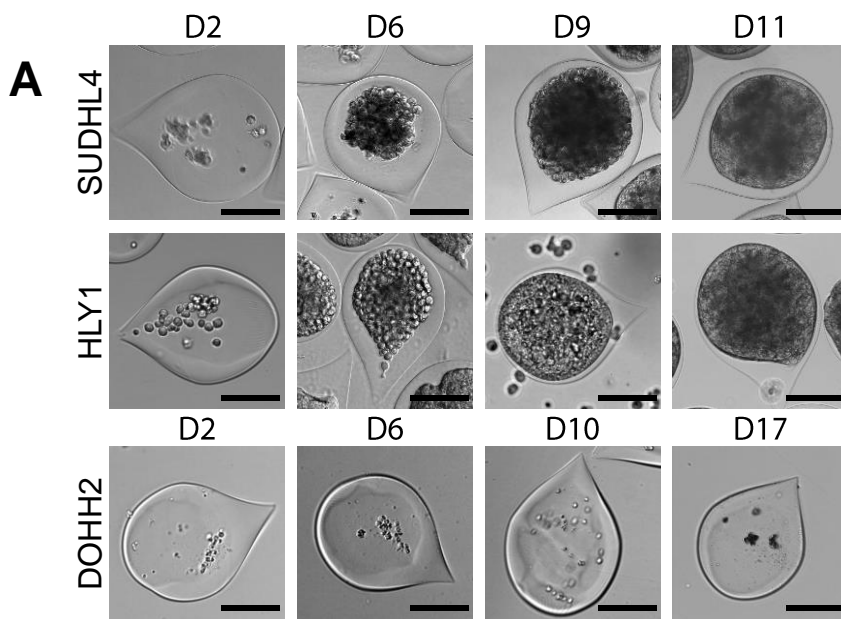


Figure 2

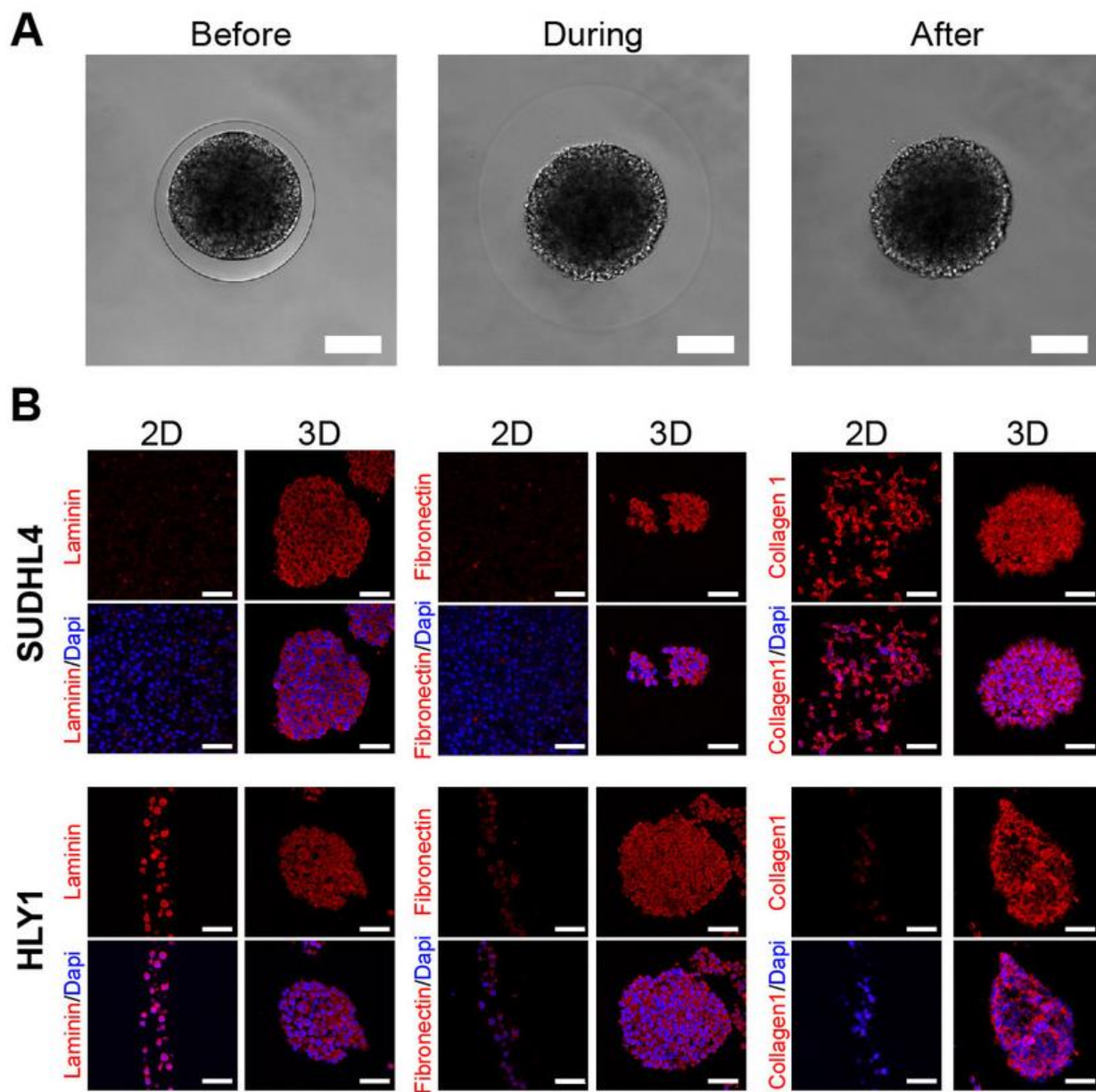


Figure 3

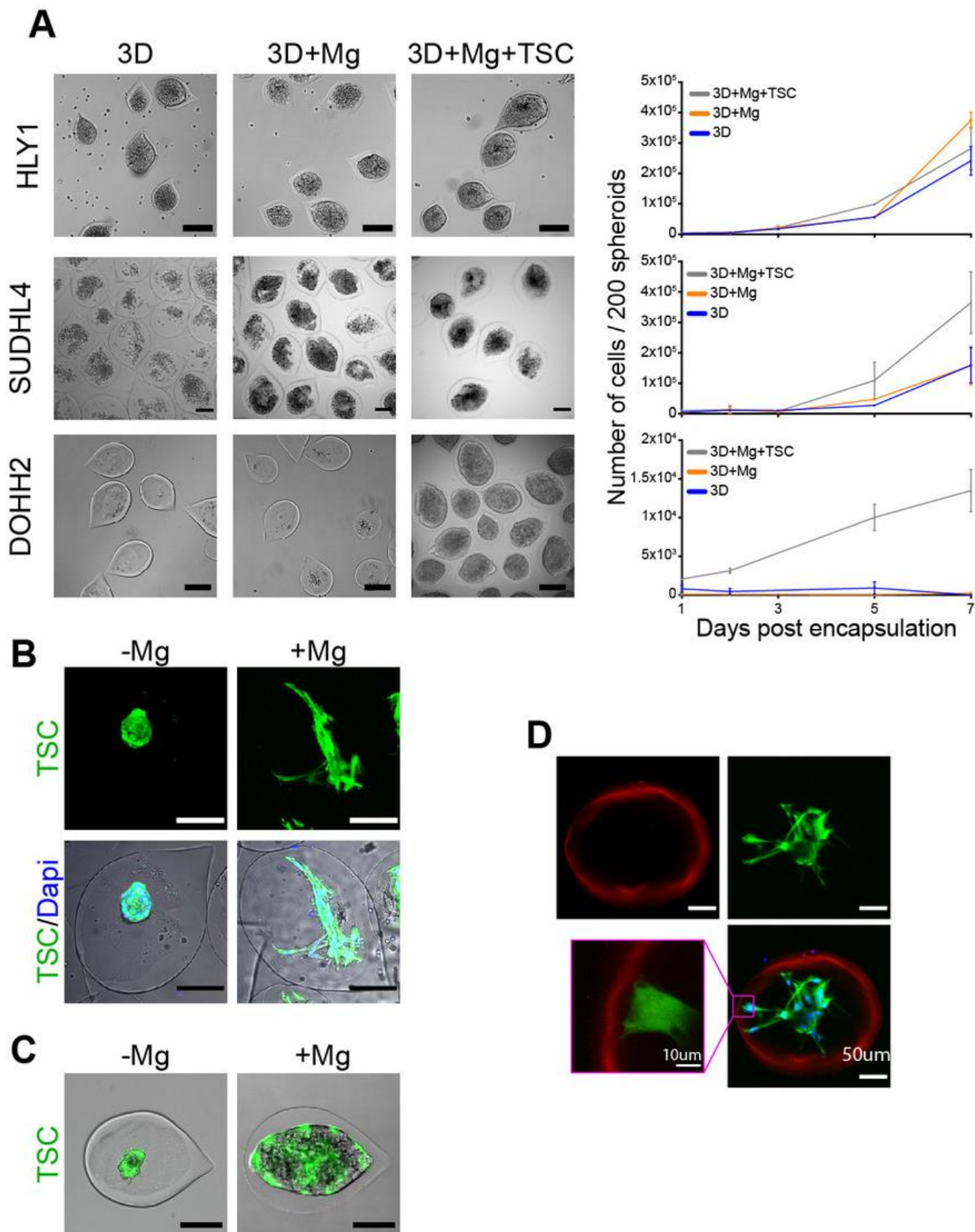


Figure 4

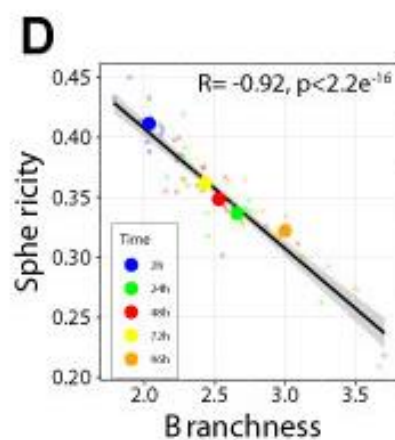
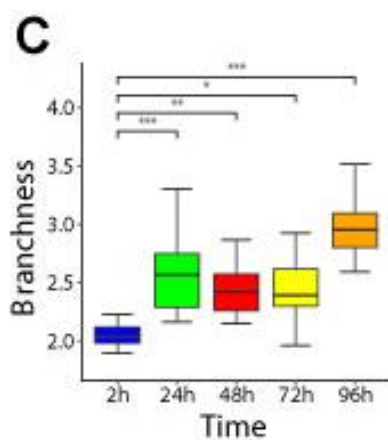
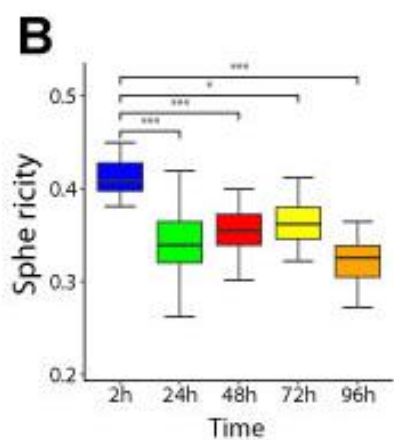
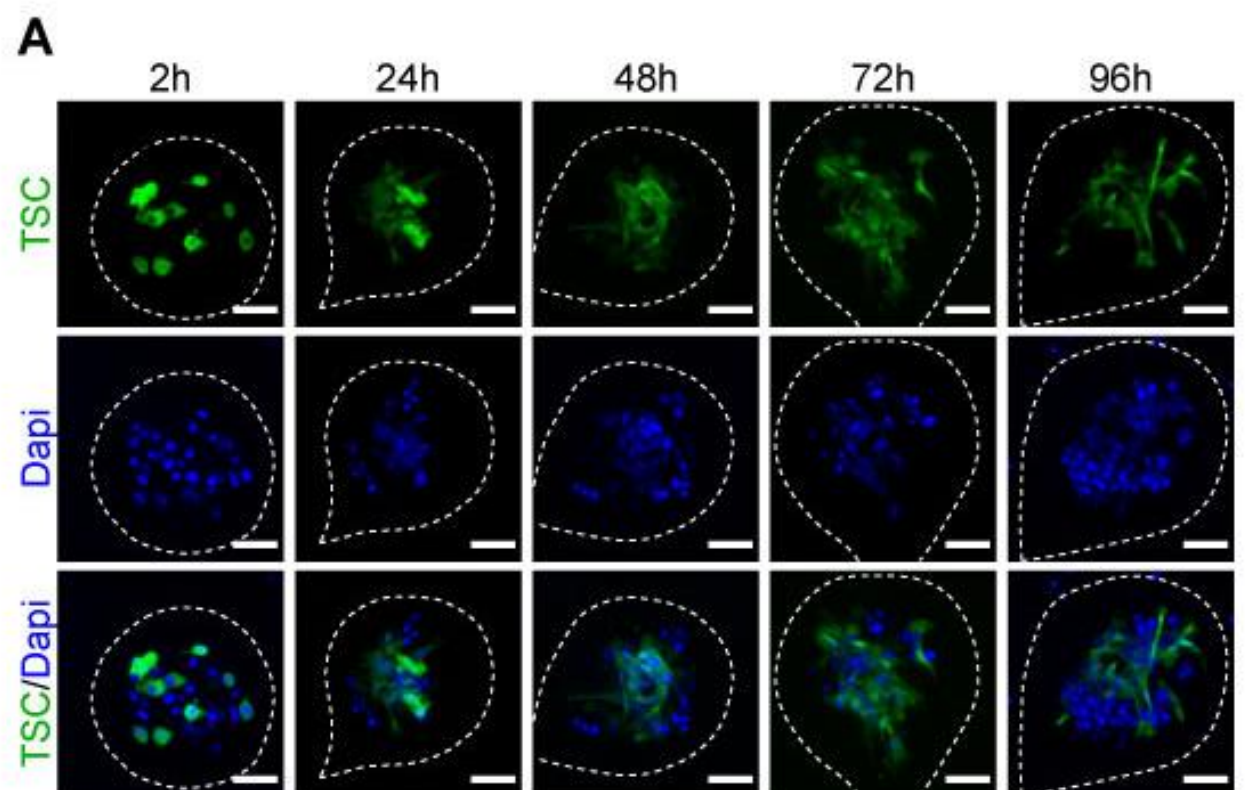


Figure 5

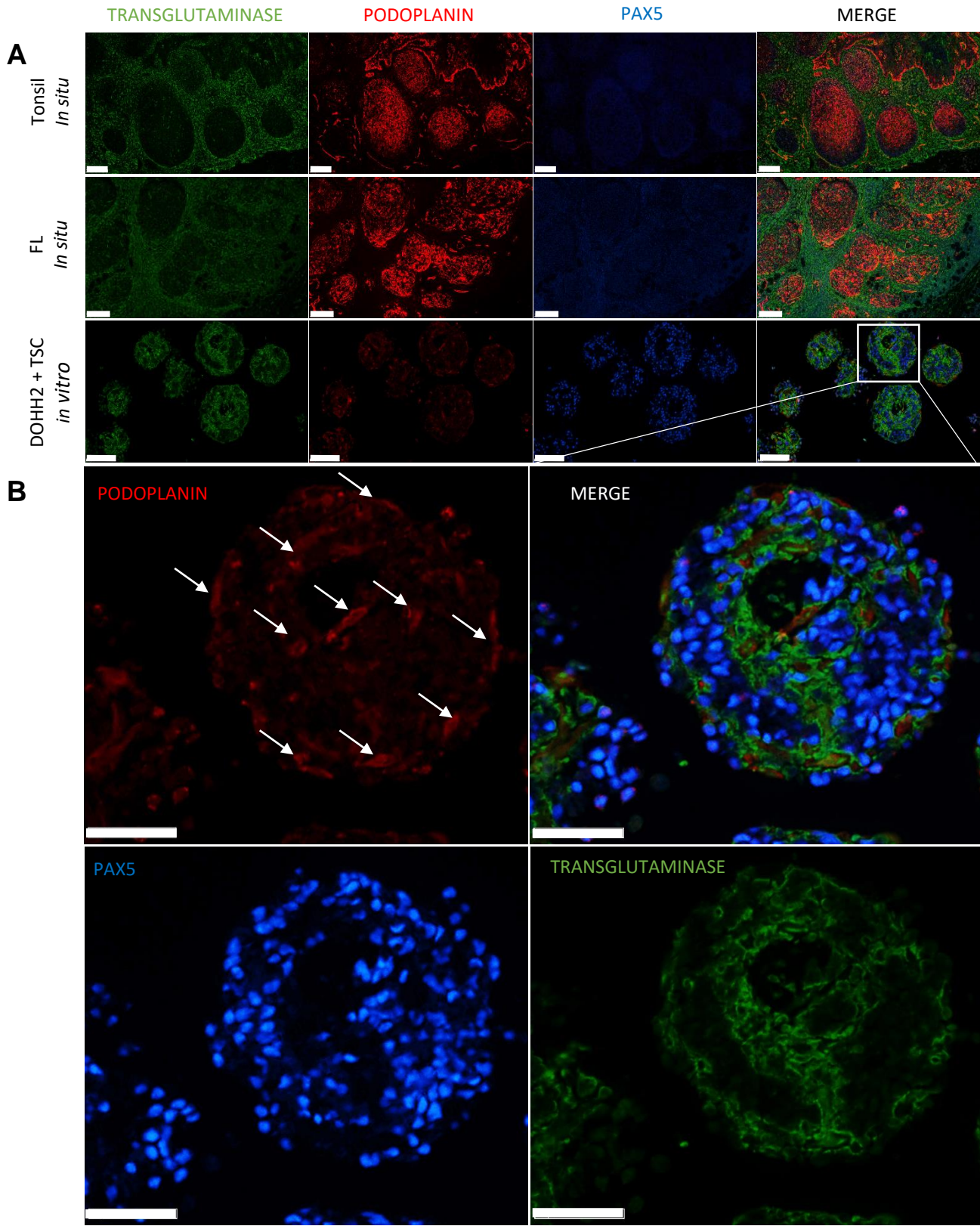


Figure 6

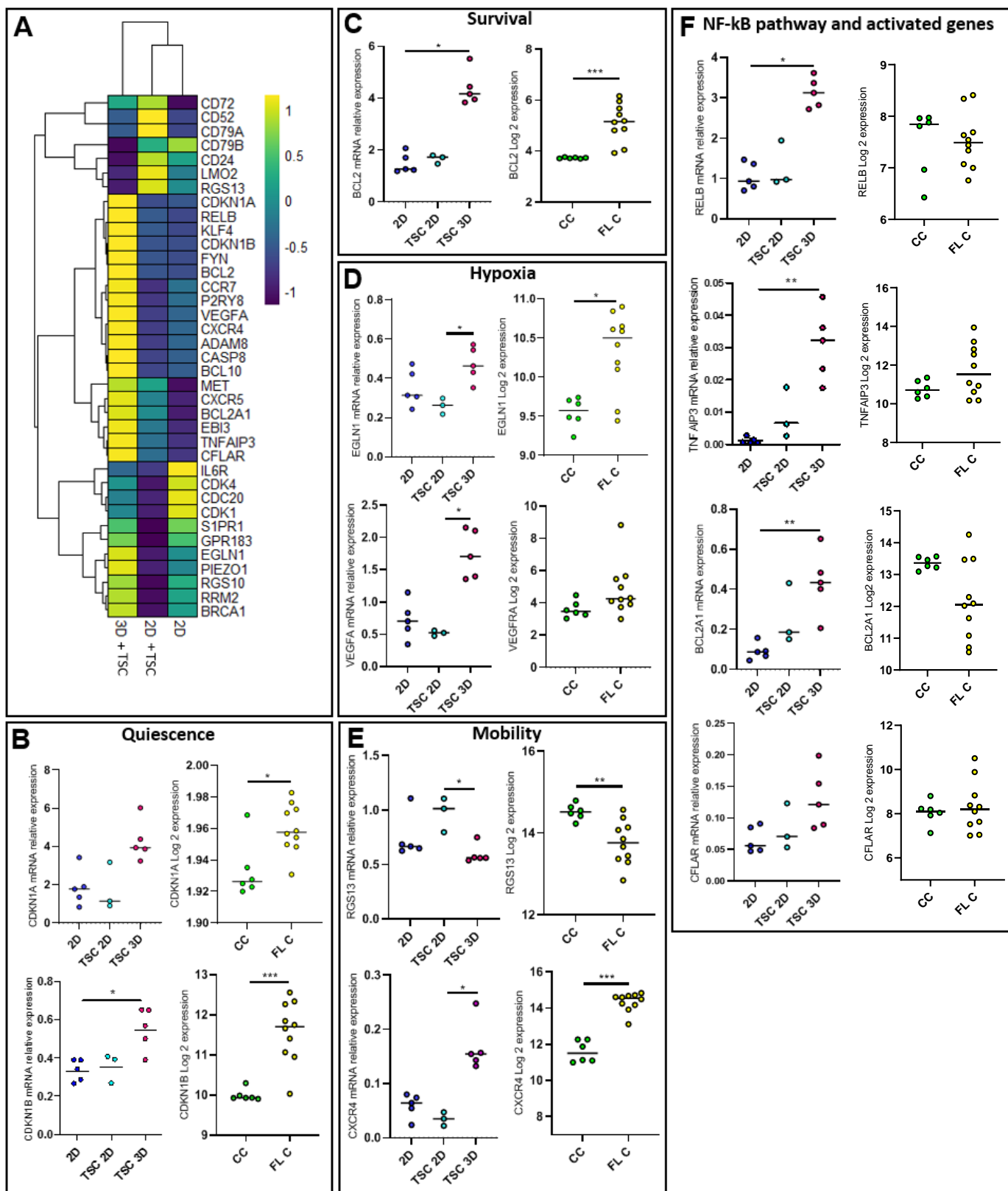


Figure 7

