

1 **Immune-responsive biodegradable scaffolds for enhancing neutrophil**
2 **regeneration**

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22 **Abstract**

23 Neutrophils are essential effector cells for mediating rapid host defense and their
24 insufficiency arising from therapy-induced side-effects, termed neutropenia, can lead to
25 immunodeficiency-associated complications. In autologous hematopoietic stem cell
26 transplantation (HSCT), neutropenia is a complication that limits therapeutic efficacy.
27 Here, we report the development and in vivo evaluation of an injectable, biodegradable
28 hyaluronic acid (HA)-based scaffold, termed HA cryogel, with myeloid responsive
29 degradation behavior. In mouse models of immune deficiency, we show that the
30 infiltration of functional myeloid-lineage cells, specifically neutrophils, is essential to
31 mediate HA cryogel degradation. Post-HSCT neutropenia in recipient mice delayed
32 degradation of HA cryogels by up to 3 weeks. We harnessed the neutrophil-responsive
33 degradation to sustain the release of granulocyte colony stimulating factor (G-CSF) from
34 HA cryogels. Sustained release of G-CSF from HA cryogels enhanced post-HSCT
35 neutrophil recovery, comparable to pegylated G-CSF, which, in turn, accelerated cryogel
36 degradation. HA cryogels are a potential approach for enhancing neutrophils and
37 concurrently assessing immune recovery in neutropenic hosts.

38 **Introduction**

39 Neutrophils mediate essential host defense against pathogens and are among the earliest
40 responders in tissue injury¹⁻³. Neutrophil deficiency, termed neutropenia, contributes to
41 opportunistic infections and could impair tissue regeneration in affected individuals⁴⁻⁷. In
42 autologous hematopoietic stem cell transplantation (HSCT) pre-conditioning
43 myelosuppressive regimens can contribute to a marked transient post-therapy
44 impairment of neutrophils and render recipients susceptible to immune deficiency-
45 associated complications for up to several weeks^{6, 8-11}.

46 Post-HSCT neutrophil regeneration follows successful bone marrow engraftment of
47 transplanted hematopoietic cells^{12, 13}, facilitated by granulocyte colony stimulating factor
48 (G-CSF)-mediated granulopoiesis of hematopoietic cells¹⁴⁻¹⁶. Neutropenia is typically
49 treated as an emergency and, in a subset of patients, the risk of neutropenia may be
50 prophylactically addressed with post-HSCT subcutaneous injection of recombinant
51 human G-CSF (filgrastim) to facilitate recovery^{6, 14, 17, 18}. Daily injections are used as G-
52 CSF has a half-life of a 3 – 4 hours, which can be extended by conjugating G-CSF with
53 polyethylene glycol (PEGylation)^{19, 20}. However, immune responses against PEG have
54 been demonstrated to enhance clearance of PEG-G-CSF in an antibody-dependent
55 manner²¹. As multiple cycles of PEG-G-CSF treatment are common, long-term treatment
56 could be rendered ineffective. Therefore, the development of a sustained release method
57 to deliver G-CSF while avoiding immune responses against PEG, and concurrently
58 assess neutrophil function could greatly improve the current standard-of-care.

59 Seeking to improve post-HSCT recovery of neutrophils and simultaneously assess
60 recovery, we developed a biodegradable depot to prophylactically deliver G-CSF in post-
61 HSCT recipients. The depot comprised a porous injectable scaffold made by low-
62 temperature crosslinking, termed cryogelation, of hyaluronic acid (HA), an easily sourced
63 and readily derivatized anionic glycosaminoglycan, termed 'HA cryogel.' As a component
64 of the extracellular matrix, endogenous HA is a substrate for degradation by myeloid cells
65 through enzymatic action and by neutrophil-mediated oxidation²²⁻²⁴. Harnessing the
66 immune-responsiveness of HA, we characterized in vivo degradation of HA cryogels in
67 immune deficient and post-HSCT mice and identified myeloid cell infiltration in HA

68 cryogels to be key mediators in facilitating degradation, which was significantly reduced
69 or altogether eliminated in mice with severely deficient neutrophil function. Transient but
70 profound post-HSCT myeloid depletion significantly delayed degradation of HA cryogels
71 until recovery of neutrophils²⁵. As the degradation profile of HA cryogels was responsive
72 to neutrophil recovery, we harnessed encapsulated G-CSF to facilitate the sustained
73 release, which was mediated by HA cryogel degradation. Neutrophil reconstitution was
74 enhanced in post-HSCT mice injected with G-CSF-encapsulated HA cryogels,
75 comparable to a single dose of PEGylated G-CSF, which accelerated HA cryogel
76 degradation.

77 **Results**

78 **Synthesis and characterization of HA cryogels**

79 Click-functionalized HA was prepared by conjugating either tetrazine (Tz) amine or
80 norbornene (Nb) methylamine to HA using carbodiimide chemistry. Nb- functionalized HA
81 (HA-Nb) was reacted with Tz-Cy5 to form Cy5-labeled HA-Nb (Cy5-HA-Nb) (**Fig. 1a**). Tz
82 amine-functionalized HA (HA-Tz) was prepared at 7% degree of substitution (termed
83 high-DOS). 0.8% DOS HA-Tz (termed low DOS) was also prepared for comparison.
84 Endotoxin levels of HA-Tz and Cy5-HA-Nb were quantified to be less than 5 endotoxin
85 units/kg, the threshold pyrogenic dose for preclinical species (**Supplementary Table 1**)
86²⁶. To maximize polymer concentration while maintaining proper viscosity to achieve
87 mixing, 0.6% w/v aqueous solutions of HA-Tz and Cy5-HA-Nb, pre-cooled to 4°C, were
88 well mixed in a 1:1 (v/v) ratio by vortexing (**Fig. 1a**). The solution was then pipetted onto
89 individual pre-cooled (-20°C) cryomolds (30 µL/mold) and immediately transferred to a -
90 20°C freezer and allowed to freeze (**Supplementary Note 1**), to generate Cy5-HA
91 cryogels (**Fig. 1b, c**).

92 To characterize Cy5-HA cryogels, we estimated the swelling ratio by comparing the
93 hydrated vs. cast volume and the aqueous mass composition from the wicked mass and
94 fully hydrated mass. The swelling ratio was 1.5 ± 0.1 in both low- and high-DOS Cy5-HA
95 cryogels (**Supplementary Fig. 1a**). The aqueous mass composition was $76.3 \pm 4.0\%$ and
96 $71.9 \pm 2.6\%$ in low- and high-DOS Cy5-HA cryogels respectively (**Supplementary Fig.**
97 **1b**).

98 To measure surface porosity of lyophilized Cy5-HA cryogels, we used scanning electron
99 microscopy (SEM) (**Fig. 1d, Supplementary Fig. 1c**). The surface pore structure images
100 were used to measure the average pore diameter using FIJI, which were between 80-
101 180 μ m and 40-90 μ m for low- and high-DOS Cy5-HA cryogels respectively
102 (**Supplementary Fig. 1d**). To characterize interconnectedness of the Cy5-HA cryogel
103 pore structure, we incubated fully hydrated low- and high-DOS Cy5-HA cryogels with
104 Fluorescein isothiocyanate (FITC)-labeled 10 μ m diameter melamine resin particles and
105 imaged using a confocal microscope (**Fig. 1e, Supplementary Fig. 1e**). Since the route
106 of administration of the Cy5-HA cryogels is through a needle, we repeated this experiment
107 with Cy5-HA cryogels after injection and observed similar penetration of the FITC-labeled
108 10 μ m particles (**Fig. 1e, Supplementary Fig. 1e**). Image analysis of z-stacked images
109 showed co-localization of the FITC-labeled 10 μ m particles with Cy5-HA up to a depth of
110 100 μ m below the surface, which was the limit of detection (**Supplementary Fig. 1f**). Both
111 low- and high-DOS Cy5-HA cryogels maintained pore morphology and relative surface
112 pore size distribution following lyophilization and rehydration (**Supplementary Fig. 1g,**
113 **1h**). Cy5-HA cryogels also maintained shape and structure post-injection
114 (**Supplementary Movie 1**).

115 To confirm susceptibility of Cy5-HA cryogels to enzymatic degradation, we used a
116 hyaluronidase-2 (HYAL2)-based in vitro assay (**Fig. 1f**). In native HA, HYAL2 cleaves
117 internal beta-N-acetyl-D-glucosaminidic linkages resulting in fragmentation of HA ²⁷.
118 Here, HYAL2 degraded HA cryogels and high DOS Cy5-HA cryogels degraded at a
119 slower rate compared to the low DOS Cy5-HA cryogels in vitro (**Fig. 1g, Supplementary**
120 **Fig. 2a**). To confirm in vivo degradation, low- and high-DOS Cy5-HA cryogels were
121 injected in subcutaneously in the hind flank of C57Bl/6J (B6) mice and degradation was
122 measured using in vivo imaging system (IVIS) fluorescence spectroscopy (**Fig. 1h**). In
123 contrast to in vitro degradation, both low- and high-DOS Cy5-HA cryogels degraded at a
124 similar rate (**Fig. 1i, Supplementary Fig. 2b**). This observation, together with the finding
125 of a similar pore size distribution in hydrated low- and high-DOS HA cryogels
126 (**Supplementary Fig. S1h**), supported the selection of one of the types of HA cryogels
127 for subsequent experiments, and we selected high-DOS HA cryogels. To characterize if
128 HA cryogels made from different batches of derivatized HA affected in vivo degradation,

129 we compared degradation of Cy5-HA cryogels made from three distinct batches of Cy5-
130 HA-Nb and HA-Tz and confirmed that all Cy5-HA-cryogels degraded at a similar rate
131 (**Supplementary Fig. 2c**).

132 **Depletion of immune cell subsets affects cellular infiltration into HA cryogels**

133 As the HSCT pre-conditioning regimen depletes all immune cell lineages, we first sought
134 to measure the effect of immune depletion on HA cryogel degradation. Cy5-HA cryogels
135 were subcutaneously injected into the hind flank of untreated B6 mice (**Fig. 2a**) and the
136 degradation profile was compared to that in B6 mice receiving (i) anti-Ly6G antibodies
137 and anti-rat κ immunoglobulin light chain to deplete neutrophils (**Fig. 2b**), (ii) clodronate
138 liposomes to deplete macrophages (**Fig. 2c**), (iii) anti-CD4 and anti-CD8 antibodies to
139 deplete T cells (**Supplementary Fig. 3a**), (iv) anti-B220 to deplete B-cells
140 (**Supplementary Fig. 3b**) and immune deficient NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG)
141 mice (**Fig. 2d**). The durability of depletion was assessed by measuring peripheral blood
142 cellularity throughout the duration of the degradation study (**Supplementary Fig. 3c-l**,
143 **Supplementary Table 2**). In untreated immune competent mice, the average half-life of
144 Cy5-HA cryogels, quantified as the time to achieve a 50% reduction in fluorescence
145 intensity, was about 9.5 days (**Supplementary Fig. 3m**). The average half-life in the
146 macrophage, neutrophil, T cell, and B cell depleted mice was similar at about 11.8 days,
147 11.3 days, 9.6, and 10.2 respectively (**Supplementary Fig. 3m**). In contrast, only a 35%
148 reduction in Cy5 signal intensity was measured after 3 months in the NSG mice (**Fig. 2d**).
149 Retrieval of Cy5-HA cryogels from sacrificed mice at the endpoint confirmed that the gels
150 had minimally degraded (**Supplementary Fig. 3n**).

151 To assess cellular infiltration and the foreign body response, Cy5-HA cryogels were
152 explanted from the above groups at 1-, 5-, and 10-days post-injection and stained using
153 haematoxylin and eosin (H&E). In Cy5-HA cryogels retrieved from all groups except NSG
154 mice, the total cellularity increased from day 1 to 10 and formed a distinct capsule
155 encapsulating the HA cryogel, indicative of a foreign body response (**Fig. 2e**,
156 **Supplementary Fig. 4a**). In NSG mice, some infiltrates were quantified on day 1,
157 however there was no appreciable increase in cellularity at the later timepoints or a
158 capsule by day 10 (**Fig. 2e**). H&E slides were further analyzed to quantify the cell density

159 in the different groups. Differences in infiltrates between the untreated and all
160 immunodepleted B6 mice were significant at the earlier timepoints, and either increased
161 or remained constant in all immunodepleted B6 (**Fig 2f, Supplementary Fig. 4b**). In
162 contrast, cell infiltrates in HA cryogels retrieved from NSG mice reduced steadily and were
163 80% lower than untreated B6 mice by day 10 (**Fig. 2f**).

164 To identify the immune cells contributing to HA cryogel degradation, we quantified cell
165 infiltrates in the Cy5-HA cryogels 1- and 10- days post-injection using flow cytometry in
166 untreated and immune depleted B6 and NSG mice (**Fig. 3a, b, Supplementary Fig. 5a,**
167 **b**). Viability of infiltrating cells, quantified by negative AnnexinV staining, was consistently
168 greater than 95% in all groups (**Supplementary Fig. 5c**).

169 Infiltration of total CD45⁺CD11b⁺ (myeloid) cells into HA cryogels of untreated B6 mice
170 and T cell depleted B6 mice were similar after 1- and 10-days post-injection
171 (**Supplementary Fig. 5d**). While there were comparable myeloid cells in HA cryogels
172 retrieved 1-day post-injection from B cell depleted B6 mice, by day 10 the number was
173 about 66% lower than in untreated B6 mice (**Supplementary Fig. 5e**). Similarly, myeloid
174 cell infiltration in Cy5-HA cryogels retrieved 1-day post-injection from macrophage
175 depleted mice was unaffected, however by day ten the number of infiltrating myeloid cells
176 was about 58% lower than untreated B6 mice (**Fig. 3c**). Neutrophil depletion in B6 mice
177 reduced the total number of myeloid cells in Cy5-HA cryogels compared with the
178 untreated B6 mice by about 77% 1-day and 68% 10-days post-injection respectively (**Fig.**
179 **3c**). In NSG mice myeloid cell infiltration in HA cryogels was 51% lower 1-day and 91%
180 lower 10-days post-injection as compared to untreated B6 mice (**Fig. 3c**).

181 CD45⁺CD11b⁺F4/80⁺ (macrophage) infiltration in Cy5-HA cryogels retrieved from all
182 groups except NSG mice reduced from 1- to 10-days (**Fig. 3d, Supplementary Fig. 5e**).
183 Intraperitoneal (i.p.) administration of clodronate liposomes minimally affected
184 macrophage infiltration in Cy5-HA cryogels even though it was effective in depleting
185 peripheral blood monocytes (**Fig. 3d, Supplementary Fig. 3g, h**). In NSG mice,
186 macrophage infiltration was 74% lower than in the untreated B6 mice on day 1 and
187 remained unchanged 10-days post-injection (**Fig. 3d**).

188 In CD45⁺CD11b⁺F4/80⁻Ly6G⁺ (neutrophil) depleted B6 mice, an additional intracellular
189 Ly6G staining step was included, as the method of neutrophil depletion is known to induce
190 internalization of the Ly6G receptor (**Supplementary Fig. 5f**)²⁸. Neutrophil infiltration in
191 Cy5-HA cryogels retrieved from untreated B6 and T cell depleted B6 mice was
192 comparable between 1- to 10-days post-injection (**Fig. 3e, Supplementary Fig. 5g**). B
193 cell depletion did not affect the initial neutrophil infiltration 1-day post-injection compared
194 with untreated controls but reduced the number of infiltrating neutrophils by 10-days post-
195 injection (**Supplementary Fig. 5g**). As expected, neutrophil depletion significantly
196 reduced initial neutrophil infiltration, by about 97%, compared to the untreated control. In
197 this group, neutrophils constituted less than 50% of infiltrating myeloid cells at all
198 timepoints assessed. Despite an increase by day 10, attributable to the internalization of
199 the Ly6G receptor which led to an approximate 4-fold increase in the infiltrating neutrophil
200 fraction (**Supplementary Fig. 5f**), the number of infiltrating neutrophils were still 84%
201 lower compared with untreated B6 mice (**Fig. 3e**). Macrophage depletion did not affect
202 the initial neutrophil infiltration 1-day post-injection compared with untreated B6 mice but
203 reduced the number of infiltrating neutrophils by 65% compared with untreated B6 mice
204 by day 10. HA cryogels retrieved from NSG mice had 50% fewer neutrophils than those
205 from untreated B6 mice on day 1 and very few to none were found by day 10 post-injection
206 (**Fig. 3e**). In NSG mice neutrophils constituted over 90% of total myeloid cells on day 1
207 but decreased to about 8% by day 10 (**Fig. 3e**). This observation along with minimal Cy5-
208 HA cryogel degradation in NSG mice (**Fig. 2d**), supported a key role of functional
209 neutrophils in mediating degradation. In all groups, the infiltration of CD45⁺CD11b⁺F4/80⁻
210 Ly6G⁻CD115⁺ (monocyte) cells were minimal and constituted a negligible portion of total
211 infiltrating myeloid cells (**Supplementary Fig. 5h, i**).

212 To provide additional confirmation of infiltrating neutrophils and macrophages, we used
213 immunohistochemical (IHC) staining to assess for Ly6G⁺ and F4/80⁺ cells respectively in
214 untreated B6 mice and NSG mice at 1-, 5-, and 10-days post-injection. Staining on day 1
215 corroborated the flow cytometry data in that there were more neutrophils than
216 macrophages within the Cy5-HA cryogels (**Supplementary Fig. 5i, Supplementary Fig.**
217 **6a**). On subsequent days, non-specific debris precluded accurate assessment in Cy5-HA
218 cryogels retrieved from B6 mice (**Supplementary Fig. 6a**). As a result of non-specific

219 staining of debris at later timepoints, IHC was only conducted on Cy5-HA cryogels
220 excised 1-day after injection in macrophage depleted, neutrophil depleted, T cell
221 depleted, and B cell depleted mice. Staining of these samples confirmed the presence of
222 both Ly6G and F4/80 in Cy5-HA cryogels confirming flow cytometry data
223 (**Supplementary Fig. 5i, Supplementary Fig. 6b**). In NSG mice, IHC staining of Ly6G⁺
224 and F4/80⁺ cells followed the results from flow cytometry analysis. Significantly more
225 neutrophils than macrophages in Cy5-HA cryogels were observed 1-day after injection
226 (**Supplementary Fig. 6c**). On day 5, there were significant macrophage and neutrophil
227 infiltrates (**Supplementary Fig. 6c**) and by day 10, the neutrophil infiltration reduced
228 significantly as expected from flow cytometry analysis (**Supplementary Fig. 5h,**
229 **Supplementary Fig. 6c**).

230 To further characterize the role of functional neutrophils, we compared the degradation
231 of Cy5-HA cryogels in B6 and B6.129S-Cybb^{tm1Din} (gp91^{phox-}) mice. Affected hemizygous
232 male gp91^{phox-} mice have a defect in the NADPH oxidase enzyme, which renders mice
233 deficient in neutrophil function through the production of reactive oxygen species^{29, 30}.
234 Cy5-HA cryogels were injected in gp91^{phox-} mice and B6 mice and degradation was
235 quantified using IVIS (**Fig. 3f**). Cy5-HA cryogels did not degrade appreciably in the
236 gp91^{phox-} over the course of the two-month study whereas the Cy5-HA cryogels in B6
237 mice degraded within 4 weeks, as expected (**Fig. 3g**).

238 Taken together, these results suggest that inducing immune deficiency by depletion
239 affects cell infiltration in Cy5-HA cryogels but does not affect degradation. However,
240 deficiencies which functionally impair neutrophils, modeled by NSG and gp91^{phox-} mice
241 are sufficient to significantly affect Cy5-HA cryogel degradation.

242 **HA cryogels are neutrophil responsive in post-HSCT mice**

243 We next quantified Cy5-HA cryogel degradation in post-HSCT mice. B6 recipients were
244 irradiated 48 hours prior to i.v. injection of lineage depleted hematopoietic stem cells (2 x
245 10⁵ cells, ~87% depleted) isolated from bone marrow of syngeneic B6 donor mice
246 (**Supplementary Fig. 7a**). Concurrently, B6 recipients and control mice (B6, non-
247 irradiated that do not receive a transplant) were injected subcutaneously with Cy5-HA
248 cryogels, and the degradation rate was compared (**Fig. 4a**). In contrast to non-irradiated

249 mice, a steady fluorescence signal was quantified for about 20 days in post-HSCT mice
250 after which it decreased, corresponding to HA cryogel degradation, at a rate comparable
251 to that in non-irradiated mice. The time interval to 50% of the initial fluorescence intensity
252 was approximately 30 days in post-HSCT mice whereas in non-irradiated mice, a
253 comparable decrease was achieved by day 13 (**Fig. 4b, Supplementary Fig. 7b**).

254 To quantify infiltrating myeloid subsets, Cy5-HA cryogels were excised on days 5 and 16
255 post-injection in non-irradiated mice and excised on days 5, 16, 21, and 26 in post-HSCT
256 mice (**Fig. 4c**). Viability of infiltrating cells, quantified by negative AnnexinV staining, was
257 initially lower 5- and 16-days post-injection, and increased by day 21 (**Supplementary**
258 **Fig. 7c**). In non-irradiated B6 mice, the number of infiltrating myeloid cells decreased by
259 96% from days 5 to 16 post-injection (**Fig. 4d**), mirroring near-complete Cy5-HA cryogel
260 degradation (**Fig. 4b, Supplementary Fig. 7b**). In contrast, myeloid cell infiltration in Cy5-
261 HA cryogels was significantly delayed and 97% lower than that of the non-irradiated
262 group, 5 days post-injection. In post-HSCT mice, appreciable myeloid infiltration was not
263 quantified until about day 21 post-HSCT, which was still 67% lower when compared with
264 HA cryogels from non-irradiated mice on day 5 (**Fig. 4d**).

265 Macrophage infiltration in Cy5-HA cryogels in non-irradiated mice decreased 87% from
266 days 5 to 16 (**Fig. 4e**). On day 5 in post-HSCT mice, macrophage infiltration in Cy5-HA
267 cryogels was reduced by about 92% compared to non-irradiated mice. By day 26,
268 infiltrating macrophages in some post-HSCT mice were quantified but remained
269 significantly lower than macrophage infiltration on day 5 in non-irradiated mice (**Fig. 4e**).

270 Neutrophils constituted a majority of the myeloid cells in Cy5-HA cryogels in non-
271 irradiated mice on day 5, but not by day 16 (**Fig. 4f**) when the majority of myeloid cells
272 were macrophages (**Supplementary Fig. 7d**). In contrast, very few cells were in HA
273 cryogels retrieved on day 5 in post-HSCT mice with a near absence of neutrophils, in
274 contrast with non-irradiated mice at the same timepoint. Neutrophil infiltration in Cy5-HA
275 cryogels was quantified 21 days post-injection but was still 62% lower than on day 5 in
276 non-irradiated mice (**Fig. 4f**). In post-HSCT mice, macrophages constituted most of the
277 cell infiltrates 5- and 16-days after injection, whereas a majority of myeloid cells were
278 neutrophils on days 21 and 26 (**Supplementary Fig. 7d**). This data supports that

279 irradiation reduces myeloid infiltration in Cy5-HA cryogels, delays cryogel degradation,
280 and degradation coincides with neutrophil recovery.

281 To assess whether the uniqueness of the results could be attributed to the HA cryogels,
282 we compared the results with hydrolytically degradable oxidized alginate (OxAlg), also
283 functionalized with Tz and Nb (**Supplementary Fig. 7e**). Unlike HA, OxAlg is not a
284 substrate for endogenous enzymes^{31, 32}. Tz-functionalized OxAlg was functionalized with
285 Cy5 and Cy5-OxAlg cryogels were formed in the same manner as high-DOS Cy5-HA
286 cryogels. In vitro, Cy5-OxAlg cryogels fully degraded in 1x PBS over 9-days
287 (**Supplementary Fig. 7f**). In contrast to Cy5-HA cryogels, Cy5-OxAlg cryogels injected
288 in B6 and post-HSCT B6 mice degraded rapidly at a comparable rate, with approximately
289 70% reduction in fluorescence signal within 24 hours post-injection (**Supplementary Fig.**
290 **7g**).

291 **HA cryogels sustain G-CSF delivery and enhance post-HSCT reconstitution of** 292 **neutrophils**

293 We sought to leverage the delay in post-HSCT degradation of HA cryogels to mediate G-
294 CSF release and enhance neutrophil recovery. As frequent bleeding to measure serum
295 G-CSF concentrations is challenging in post-HSCT mice, we assessed G-CSF release
296 from HA cryogels by labeling G-CSF with Cy5 and measuring the signal at the site of HA
297 cryogel injection using IVIS microscopy. 1 μ g of Cy5-labeled G-CSF (Cy5 G-CSF) was
298 encapsulated in HA cryogels and one cryogel was injected either in 1-day post-HSCT or
299 in non-irradiated B6 mice. Encapsulated Cy5 G-CSF was quantified using IVIS and
300 normalized to the initial 8-hour timepoint fluorescence signal (**Fig. 5a**). Cy5 G-CSF
301 release, assessed by fluorescence attenuation, from non-irradiated mice proceeded in a
302 sustained manner immediately post-injection with over 80% released after approximately
303 12-days post-injection. In post-HSCT mice, 20% Cy5 G-CSF released after approximately
304 12-days post-injection and subsequently released in a sustained manner (**Fig. 5b**). The
305 time to 50% fluorescence intensity in non-irradiated mice was 5.9 ± 3.0 days compared
306 to 15.5 ± 5.9 days in post-HSCT mice (**Fig. 5c**). To approximate G-CSF pharmacokinetics
307 (PK) in the blood, the release profile of G-CSF from HA cryogels in post-HSCT mice was
308 modeled as a piecewise function (**Supplementary Note 2**). We then sought to assess

309 the effect of G-CSF delivery on peripheral blood neutrophil recovery and acceleration of
310 Cy5-HA cryogel degradation. We compared mice receiving either two blank Cy5-HA
311 cryogels or two G-CSF-encapsulated HA-cryogels and, as a positive control, we included
312 mice with blank Cy5-HA cryogels injected systemically with 2 μ g pegylated (PEG) G-CSF
313 (**Fig. 5d**), corresponding to the clinical-equivalent dose for mice ^{33, 34}. Mice were bled at
314 pre-determined timepoints, and peripheral blood neutrophil concentration, quantified by
315 flow cytometry, was consistently higher when G-CSF from Cy5-HA cryogels was
316 delivered, and comparable with PEG G-CSF treatment than in mice which received blank
317 Cy5-HA cryogels (**Fig. 5e**). Moreover, Cy5-HA cryogel degradation was accelerated with
318 G-CSF or PEG G-CSF treatment (**Fig. 5f**). These results support that G-CSF release from
319 HA cryogels can improve neutrophil recovery in lethally irradiated mice and Cy5-HA
320 cryogel degradation may simultaneously be used as an indicator of functional neutrophil
321 recovery.

322 **Discussion**

323 Here we demonstrate that an immune responsive biodegradable HA cryogel scaffold
324 provides sustained G-CSF release and accelerates post-HSCT neutrophil recovery in
325 mice which, in turn, accelerates HA cryogel degradation in vivo. Harnessing post-HSCT
326 immune deficiency to sustain G-CSF release is distinct conceptually from other methods
327 of drug delivery. It is well established that immune cells sense implanted materials as
328 non-self and mount a well-characterized sequential response to isolate the implant in a
329 fibrous capsule ³⁵⁻³⁷. In this work, we observed neutrophil infiltration during the acute
330 stages of inflammation and show them to be key mediators in HA cryogel degradation.
331 Our finding is consistent with prior reports that have supported neutrophils as key
332 mediators of shaping the early implant microenvironment and for in vivo destruction of
333 implanted polymeric materials by neutrophil-derived oxidants ³⁸⁻⁴⁰. The finding of primarily
334 myeloid-lineage immune cell populations within the HA cryogel is consistent with previous
335 observations of cell infiltration occurring within scaffolds of a similar composition ^{41, 42}. We
336 demonstrate that the encapsulation and release of G-CSF from the polymer scaffold
337 mediated recovery of neutrophils in the peripheral blood, significantly faster than control

338 mice receiving blank HA cryogels and comparable to pegylated GCSF, which accelerated
339 HA cryogel degradation.

340 HA was selected as the primary constituent polymer as it is ubiquitous in the extracellular
341 matrix and has a long history of clinical use as a biodegradable material in a range of
342 biomedical applications⁴³⁻⁴⁸. In this work, commercially purchased HA was derivatized
343 with bioorthogonal Tz and Nb groups to facilitate crosslinking without the need for external
344 energy input or addition of external agents such as stabilizers and catalysts^{49, 50}, which
345 can make it challenging to purify the final product. The use of HA-Tz and HA-Nb also
346 facilitated cryogelation at a slower rate, compared to free-radical polymerization methods,
347 and consequently provided enhanced control over the crosslinking process^{51, 52}.
348 Moreover, other common cross-linking strategies that directly target the carboxylic acid
349 or hydroxyl side chains groups and unreacted agents may inadvertently react with
350 encapsulated proteins⁵³⁻⁵⁶. Further, Tz can be quantified spectroscopically and the DOS
351 was readily assessed^{57, 58}. Consistent with prior work, our results show that DOS affected
352 the rate of HA cryogel degradation by enzymatic cleavage^{22, 62}. On the other hand, the
353 paradoxical observation that DOS did not affect in vivo degradation is also consistent with
354 prior work that has demonstrated that partial degradation of HA by non-enzymatic means
355 in vivo overcomes steric factors which might otherwise hinder enzymatic access to HA
356 and, in our work, facilitated equalization of the in vivo degradation rate of low- and high-
357 DOS HA cryogels⁵⁹.

358 Our results support that activated neutrophils mediate degradation of HA cryogels in vivo,
359 consistent with past reports of the role of reactive oxygen species from activated
360 neutrophils in mediating HA degradation⁶⁰⁻⁶³ and of neutrophils in the acute phase of the
361 foreign body response^{35, 36}, which further clarifies how immune deficiency impacts the
362 rate of degradation^{9, 64}. We found that while despite successfully depleting neutrophils in
363 the peripheral blood, antibody-based depletion did not achieve a similar depletion of
364 infiltrating neutrophils in HA cryogels and degradation was unaffected in B6 mice. In NSG
365 mice, which have defective adaptive and innate immune cells, Cy5-HA cryogels degraded
366 minimally over 3 months and neutrophil infiltration into the HA cryogel was not sustained.
367 The observation is consistent with the well-documented lack of adaptive immune cells,
368 impaired innate immune cell subsets (e.g. macrophages) and a lack of a functional

369 complement system which affects the activation of neutrophils in these mice ⁶⁵⁻⁶⁸. We
370 expanded upon these results by quantifying Cy5-HA cryogel degradation in gp91^{phox-}
371 mice, which are on the B6 background, but gp91^{phox-} neutrophils in affected hemizygous
372 male mice lack superoxide production ^{29, 30}. The functional deficiency of neutrophils in
373 gp91^{phox-} is similar to the clinical observations of defective respiratory burst and
374 phagocytosis affecting neutrophils in chronic granulomatous disease, in which there are
375 normal neutrophil counts but impaired oxidative killing ²⁹. In these mice, the absence of
376 appreciable degradation of Cy5-HA cryogels provides additional support for the key role
377 of functional neutrophils in facilitating degradation.

378 The key role of functional neutrophils in HA cryogel degradation was further validated in
379 post-HSCT mice, modeling transient innate immune deficiency. Unlike antibody-based
380 depletion, irradiating mice achieves elimination of all immune cells and neutrophils
381 predominate the earliest immune cells that reconstitute post-HSCT ⁶⁹. Similar to gp91^{phox-}
382 mice, post-HSCT respiratory burst and phagocytosis of neutrophils are generally
383 decreased in humans for up to 3 months, underscoring the importance of qualitatively
384 assessing functionality of neutrophils ⁷⁰. We found that Cy5-HA cryogel degradation was
385 delayed until neutrophil infiltration into Cy5-HA cryogels recovered, further supporting the
386 role of neutrophils in mediating HA degradation and the immune responsive behavior of
387 HA cryogels. These results are consistent with past reports in which rapid neutrophil
388 infiltration and activation have been identified as one of the earliest cellular events of the
389 foreign body response ^{2, 71}. In contrast, we show that OxAlg cryogels, which have
390 hydrolytically labile groups but are not a substrate for endogenous enzymes, degrade
391 rapidly in vivo at similar rates in immune competent and post-HSCT mice ^{31, 32, 72}. These
392 observations characterizing the importance of neutrophils in HA cryogel degradation
393 support the unique immune responsiveness of HA cryogels.

394 Similar to PEG-G-CSF, we demonstrate the effect of G-CSF release from HA cryogels is
395 neutrophil-dependent ⁷³, and therefore might be characterized as self-regulating.
396 However, in contrast to PEG-G-CSF, G-CSF delivery from HA cryogels avoids the
397 potential of pre-existing or induced anti-PEG antibody (APA)-mediated rapid clearance ⁷⁴,

398 ⁷⁵. In immune competent mice, it has been demonstrated that the administration of PEG-
399 G-CSF at a clinically-relevant single dose elicits anti-PEG IgM antibodies in a dose-
400 dependent manner which subsequently accelerates clearance of a second PEG-G-CSF
401 dose via an anti-PEG IgM-mediated complement activation ²¹. De novo anti-PEG antibody
402 induction may not require T cell activation ⁷⁶ and therefore could also be induced in post-
403 HSCT immunodeficient hosts. PEG G-CSF may therefore be less effective with pre-
404 existing or induced APA ⁷⁷.

405 Therapy-induced neutropenia substantially limits the applicability of therapies that could
406 be life-saving. HA cryogels not only deliver G-CSF in a sustained manner to enhance
407 neutrophil regeneration, while avoiding the potential of APA-mediated enhanced
408 clearance, but also show a responsive degradation behavior. Collectively, our findings
409 support that the HA cryogels might be leveraged to enhance and functionally assess
410 neutrophil functionality and aid in treatment-related decisions for recipients of
411 myelosuppressive therapy.

412 **Figure Captions**

413 **Fig. 1| Production and characterization of Cy5-HA cryogel**

414 (a) Schematic for tetrazine (Tz) and norbornene (Nb) functionalization of HA, Cy5
415 functionalization of Nb functionalized HA (Cy5-HA-Nb) and crosslinking of Tz
416 functionalized HA with Cy5-HA-Nb. (b) Schematic for producing Cy5-HA cryogels. (c)
417 Representative photograph of lyophilized Cy5-HA cryogel. Scale bar = 1mm. (d)
418 Representative SEM image depicting Cy5-HA cryogels. Top scale bar = 1mm, middle
419 scale bar = 500 μ m, bottom scale bar = 100 μ m. (e) Confocal microscopy image, overhead
420 and side views, depicting hydrated Cy5-HA cryogels pre- and post-injection incubated
421 with 10 μ m FITC-labeled microparticles. Scale bar = 100 μ m. (f) Schematic depicting
422 workflow for in vitro Cy5-HA cryogel degradation study. (g) Measuring Cy5-HA cryogel
423 degradation in vitro by quantifying the Cy5-signal in supernatant at pre-determined
424 timepoints normalized to total Cy5-signal in supernatant across all timepoints. (h)
425 Schematic depicting workflow for in vivo Cy5-HA cryogel degradation study. (i)
426 Representative in vivo imaging system (IVIS) fluorescence images of gel degradation in
427 mice and measuring Cy5-HA cryogel degradation in vivo by quantification of total radiant
428 efficiency normalized to initial day 3 timepoint. IVIS Images are on the same scale and
429 analyzed using Living Image Software. Data in g represents mean \pm s.d. of n=4 HA
430 cryogels. Data in i represents mean \pm s.e.m. of n=4 HA cryogels. Part of figure 1b, f and
431 h were created with BioRender.com.

432

433 **Fig. 2| Cy5-HA cryogel degradation in immunodeficient mice**

434 Representative IVIS fluorescence images of Cy5-HA cryogel degradation and
435 quantification by measuring total radiant efficiency normalized to initial day 3 timepoint of
436 (a) untreated B6 mice, (b) neutrophil depleted B6 mice, (c) macrophage depleted B6
437 mice, and (d) NSG mice. IVIS Images are on the same scale and analyzed using Living
438 Image Software. (e) Hematoxylin and eosin (H&E) stained histological sections of
439 explanted Cy5-HA cryogels from the above groups, at days 1, 5, and 10 post-injection.
440 Full view scale bar = 800 μ m, magnified scale bar = 100 μ m. (f) Quantification of cellular
441 density in the sections from e. Data in a-d represent mean \pm s.e.m. of n=4-9 and are

442 representative of at least two separate experiments. Data in **f** represents mean \pm s.d. of
443 $n=7-12$ and were compared using student's t-test.

444

445 **Fig. 3| Assessment of innate immune cell infiltration into Cy5-HA cryogels**

446 **(a)** Schematic for the quantification of innate immune cell content in Cy5-HA cryogels. **(b)**
447 Representative flow cytometry plots depicting gating strategy to determine cellular identity
448 of CD45⁺ CD11b⁺ F4/80⁺ (macrophage) cells, CD45⁺ CD11b⁺ F4/80⁻ Ly6G⁺ (neutrophil)
449 cells, and CD45⁺ CD11b⁺ F4/80⁻ Ly6G⁻ CD115⁺ (monocyte) cells in untreated B6 mice,
450 anti-Ly6G and anti-rat κ immunoglobulin light chain antibody treated B6 mice, clodronate
451 liposome treated B6 mice, and NSG mice. **c-e** Quantification of total number of **(c)** CD45⁺
452 CD11b⁺ (myeloid) cells, **(d)** macrophages, and **(e)** neutrophils infiltrating HA cryogels in
453 untreated B6 mice, anti-Ly6G and anti-rat κ immunoglobulin light chain antibody treated
454 B6 mice, clodronate liposome treated B6 mice, and NSG mice. **(f)** Schematic depicting
455 workflow for in vivo Cy5-HA cryogel degradation study with gp91^{phox-} mice. **(g)**
456 Representative IVIS fluorescence images of gel degradation and quantification by
457 measuring total radiant efficiency normalized to initial day 3 timepoint of gp91^{phox-} mice
458 and B6 mice. IVIS Images are on the same scale and analyzed using Living Image
459 Software. Data in **c**, **d**, **e** represents mean \pm s.d. of $n=7-10$ Cy5-HA cryogels, are
460 representative of at least two separate experiments and compared using student's t-test.
461 Data in **g** represents mean \pm s.e.m. of $n=4-5$ and were compared using two-way ANOVA
462 with Bonferroni's multiple comparison test. Parts of figures **3a** and **f** were created with
463 BioRender.com.

464

465 **Fig. 4| Degradation kinetics of HA cryogels is impaired during transient** 466 **immunodeficiency following HSCT**

467 **(a)** Schematic depicting workflow for quantification of Cy5-HA cryogel degradation and
468 innate immune cell infiltration in control (non-irradiated mice that do not receive a
469 transplant) and post-HSCT B6 mice. **(b)** Representative IVIS fluorescence images of gel
470 degradation in non-irradiated and post-HSCT mice. Tracking gel degradation by
471 quantification of total radiant efficiency normalized to initial day 3 timepoint. **(c)**
472 Photograph of Cy5-HA cryogels in non-irradiated mice 5- and 16-days post-injection and

473 post-HSCT mice on days 5, 16, 21, and 26. **d-f** Cell infiltration of **(d)** CD45⁺CD11b⁺
474 (myeloid) cells, **(e)** CD45⁺CD11b⁺ F4/80⁺ (macrophage) cells, and **(f)** CD45⁺ CD11b⁺
475 F4/80⁻ Ly6G⁺ (neutrophil) cells into HA cryogels in non-irradiated mice 5- and 16-days
476 post-injection and 5-, 16-, 21-, and 26- days post-HSCT. Data in **b** represents mean \pm
477 s.e.m. of n = 7-9 mice and is representative of at least two separate experiments. Data in
478 **d, e, f** represents mean \pm s.d. of n = 6-10 HA cryogels and are representative of at least
479 two separate experiments. Data in **b** were compared using two-way ANOVA with
480 Bonferroni's multiple comparison test. Data in **d, e, f** were compared using student's t-
481 test. Part of figure **4a** was created with BioRender.com.

482

483 **Fig. 5| Enhanced reconstitution of peripheral blood neutrophil cells**

484 **(a)** Schematic depicting outline of study to quantify Cy5 G-CSF release from HA cryogels
485 in non-irradiated, non-transplanted B6 mice and post-HSCT B6 mice. **(b)** Representative
486 IVIS fluorescence images of Cy5 G-CSF release from HA cryogels and quantification by
487 measuring total radiant efficiency normalized to initial 8-hour timepoint. IVIS Images are
488 on the same scale and analyzed using Living Image Software. **(c)** Time to 50%
489 fluorescence intensity for Cy5 G-CSF encapsulated within HA cryogels in non-irradiated
490 and post-HSCT mice. **(d)** Schematic depicting outline of study to quantify neutrophil
491 reconstitution rate and Cy5-HA cryogel degradation rate in post-HSCT mice using G-CSF
492 encapsulated Cy5-HA cryogels. **(e)** Peripheral blood reconstitution of neutrophils in post-
493 HSCT mice, normalized to pre-irradiation neutrophil counts from a random subset of mice.
494 **(f)** Representative in vivo imaging system (IVIS) fluorescence images of gel degradation
495 in mice and measuring Cy5-HA cryogel degradation in vivo by quantification of total
496 radiant efficiency normalized to initial day 3 timepoint. IVIS Images are on the same scale
497 and analyzed using Living Image Software. Data in **b** represents mean \pm s.e.m. of n = 6-
498 9 mice. Data in **c** represents mean \pm s.d. of n = 6 – 9 mice. Data in **e** represents mean \pm
499 s.d. of n = 11-15 mice and is representative of at least two separate experiments. Data in
500 **f** represents \pm s.e.m. of n = 11-14 Cy5-HA cryogels and is representative of at least two
501 separate experiments. Data in **b, f** were compared using two-way ANOVA with
502 Bonferroni's multiple comparison test. Data in **c** were compared using student's t-test.

- 503 Data in **e** were compared using mixed-effect regression model with random intercepts.
- 504 Parts of figures **5a and d** were created with BioRender.com.

505 **Supplementary Figure Captions**

506 **Fig. S1| Supplementary HA cryogel materials characterization data**

507 (a) Volumetric swelling ratios for low- and high-DOS Cy5-HA cryogels. (b) Aqueous
508 weight percentage of low- and high-DOS Cy5-HA cryogels. (c) Representative SEM
509 image depicting low-DOS Cy5-HA cryogels. Top scale bar = 500 μ m, bottom scale bar =
510 100 μ m. (d) Average pore diameters of HA cryogels made from low- and high-DOS Cy5-
511 HA cryogels measured from SEM images (20 measurements/cryogel, n = 3 each for low-
512 and high-DOS HA cryogels). (e) Confocal microscopy images, overhead and side views,
513 depicting low-DOS Cy5-HA cryogels both pre-injection and post-injection incubated with
514 10 μ m FITC-labeled microparticles. Scale bar = 100 μ m. (f) Quantification of confocal
515 images showing penetration of 10 μ m FITC-labeled microparticles into both low- and high-
516 DOS Cy5-HA cryogels pre- and post-injection. (g) Representative confocal microscopy
517 images of low- and high-DOS Cy5-HA cryogels after thawing, after lyophilization, and
518 after lyophilization and rehydration. (h) Average surface pore diameter of Cy5-HA
519 cryogels measured from confocal images. Data in **a** and **b** represents mean \pm s.d. of n=10
520 Cy5-HA cryogels. Data in **d** represents mean \pm s.d. of n=3 Cy5-HA cryogels and was
521 compared using student's t-test. Data in **f** represents mean \pm s.d. of n=5 Cy5-HA cryogels.
522 Data in **h** represents mean \pm s.d. of n=3 HA cryogels and was compared using student's
523 t-test.

524

525 **Fig. S2| Supplementary Cy5-HA cryogel degradation characterization data**

526 (a) Measuring low-DOS Cy5-HA cryogel degradation in vitro by quantification of Cy5-
527 signal in supernatant at pre-determined timepoints normalized to total Cy5-signal in
528 supernatant across all timepoints. (b) Representative IVIS fluorescence images of gel
529 degradation in mice and measuring low-DOS Cy5-HA cryogel degradation in vivo by
530 quantification of total radiant efficiency normalized to the initial day 3 timepoint. (c)
531 Measuring Cy5-HA cryogel degradation by quantification of total radiant efficiency
532 normalized to initial day 3 timepoint. Data in **a** represents mean \pm s.d. of n=4 HA cryogels.
533 Data in **b** represents mean \pm s.e.m. of n=4 HA cryogels. Data in **c** represents mean \pm s.d.
534 of n=4-5 HA cryogels and were compared using two-way ANOVA with Bonferroni's
535 multiple comparison test.

536 **Fig. S3| Supplementary Cy5-HA cryogel degradation in immunodeficient mice**
537 **characterization data**

538 Representative IVIS fluorescence images of gel degradation and quantification by
539 measuring total radiant efficiency normalized to initial day 3 timepoint of (a) T cell depleted
540 B6 mice and (b) B cell depleted B6 mice. c-d Representative gating strategy to determine
541 identity of (c) innate immune cells and (d) adaptive immune cells in peripheral blood. (e)
542 Representative flow cytometry plot of peripheral blood neutrophils pre- and post-
543 administration of neutrophil depleted mice and (f) peripheral blood neutrophil
544 concentration. (g) Representative flow cytometry plot of peripheral blood monocytes pre-
545 and post- administration clodronate liposomes to mice and (h) peripheral blood monocyte
546 concentration. (i) Representative flow cytometry plot of peripheral blood T cells blood pre-
547 and post- administration of anti-CD4 and anti-CD8 antibody treatment to mice and (j)
548 peripheral blood T cell concentration. (k) Representative flow cytometry plot of peripheral
549 blood B cells blood pre- and post- administration of anti-B220 antibody treatment to B6
550 mice and (l) peripheral blood B cell concentration. (m) Overlay of normalized total radiant
551 efficiency curves and time to 50% fluorescence intensity of untreated B6, neutrophil
552 depleted, macrophage depleted, T cell depleted, and B cell depleted mice. (n)
553 Photograph of a Cy5-HA cryogel retrieved from NSG mice 3 months post-injection. Data
554 in a, b represents mean \pm s.e.m. of n=5 and are representative of at least two separate
555 experiments. Data in f, h, j, l represents mean \pm s.d. of n=4-5. Data in m represents mean
556 \pm s.d. of n=4-9 and were compared using student's t-test.

557
558 **Fig. S4| Supplementary histomorphometric analysis of Cy5-HA cryogels retrieved**
559 **from T- and B- cell depleted mice**

560 (a) Hematoxylin and eosin (H&E) stain of explanted Cy5-HA cryogels from T cell depleted
561 and B cell depleted mice at days 1, 5, and 10. Scale bar left = 800 μ m, scale bar right =
562 100 μ m. (b) Analysis of H&E stains to quantify cellular density in Cy5-HA cryogel. Data in
563 b represents mean \pm s.d. of n = 7-12 histological sections and was compared using
564 student's t-test.

565

566 **Fig. S5| Supplementary analysis of myeloid cell infiltration of Cy5-HA cryogels**
567 **retrieved from immunodeficient mice**

568 (a) Representative gating strategy to determine identity of innate immune cell infiltrates
569 of HA cryogel. (b) Representative flow cytometry plots gated to determine cellular identity
570 of CD45⁺ CD11b⁺ F4/80⁺ (macrophage) cells, CD45⁺ CD11b⁺ F4/80⁻ Ly6G⁺ (neutrophil)
571 cells, and CD45⁺ CD11b⁺ F4/80⁻ Ly6G⁻ CD115⁺ (monocyte) cells T cell depleted and B
572 cell depleted mice. (c) Percent of AnnexinV⁻ (live) cells within Cy5-HA cryogels one and
573 ten days after implant from flow cytometry analysis. d-e Quantification of total number of
574 (d) myeloid cells and (e) macrophages infiltrating Cy5-HA cryogels in untreated B6 mice,
575 T cell depleted mice, and B cell depleted mice. (f) Representative flow cytometry plots
576 from neutrophil depleted mice with and without intracellular Ly6G staining. Plotted data
577 assessing neutrophils as a percentage of total myeloid cells (CD45⁺CD11b⁺) with and
578 without intracellular Ly6G staining. (g) Quantification of total number of neutrophils
579 infiltrating Cy5-HA cryogels in untreated B6 mice, T cell depleted mice, and B cell
580 depleted mice. h,i Quantification of total number of (h) monocytes and (i) infiltrating
581 immune cell lineages plotted as a percentage of myeloid cells in untreated, neutrophil
582 depleted, macrophage depleted, T cell depleted, B cell depleted, and NSG mice. Data in
583 e represents mean \pm s.d. of n = 10. Data in c, d, e, g, h, i represents mean \pm s.d. of n =
584 7-10 and are representative of at least two separate experiments. Data in d, e, f, g were
585 compared using student's t-test.

586

587 **Fig. S6| Supplementary Immunohistochemical staining of Cy5-HA cryogels**
588 **retrieved from untreated B6 and NSG mice**

589 (a) Immunohistochemistry (IHC) staining for Ly6G (neutrophils, top, scale bar = 1mm)
590 and F4/80 (macrophages, bottom, scale bar = 60 μ m) of Cy5-HA cryogels excised from
591 untreated B6 mice 1-, 5-, and 10-days after injection. IHC was conducted on the same
592 Cy5-HA cryogels as in Fig. 2e. (b) IHC staining for Ly6G (top, scale bar = 1mm) and
593 F4/80 (bottom, scale bar = 60 μ m) of Cy5-HA cryogels excised from macrophage
594 depleted, neutrophil depleted, T cell depleted, and B cell depleted B6 mice 1-day after
595 injection. IHC was conducted on the same Cy5-HA cryogels as in Fig. 2e. (c) IHC staining
596 for Ly6G (top, scale bar = 1mm) and F4/80 (bottom, scale bar = 60 μ m) of Cy5-HA cryogels

597 excised from NSG mice 1-, 5-, and 10-days after injection. IHC was conducted on the
598 same Cy5-HA cryogels as in Fig. 2e.

599

600 **Fig. S7| Supplementary data for degradation kinetics of HA cryogels post-HSCT**

601 (a) Representative flow cytometry plots of bone marrow before and after lineage
602 depletion. (b) Time to 50% fluorescence intensity of Cy5-HA cryogels in non-irradiated
603 and post-HSCT mice. (c) Percent of AnnexinV- (live) cells within Cy5-HA cryogels 5- and
604 16-days post injection in non-irradiated mice and 5-, 16-, 21-, and 26-days post-injection
605 in post-HSCT mice. (d) Infiltrating immune cell lineages plotted as a percentage of
606 myeloid cells in non-irradiated and post-HSCT mice. (e) Schematic for tetrazine (Tz) and
607 norbornene (Nb) functionalization of oxidized alginate (OxAlg), Cy5 functionalization of
608 Nb functionalized OxAlg, and crosslinking of Tz functionalized HA with Cy5 functionalized
609 OxAlg. (f) Measuring Cy5-OxAlg cryogel degradation in vitro by quantifying the Cy5-signal
610 in supernatant at pre-determined timepoints normalized to total Cy5-signal in supernatant
611 across all timepoints. (g) Representative in vivo imaging system (IVIS) fluorescence
612 images of gel degradation in mice and measuring Cy5-tagged 40% oxidized alginate
613 cryogel degradation in vivo by quantification of total radiant efficiency normalized to initial
614 2-hour timepoint. IVIS Images are on the same scale and analyzed using Living Image
615 Software. Data in **b** represents n=7-9 Cy5-HA cryogels, is representative of at least two
616 separate experiments and were compared using student's t-test. Data in **c, d** represents
617 mean \pm s.d. of n=6-10 Cy5-HA cryogels and is representative of at least two separate
618 experiments. Data in **f** represents mean \pm s.d. of n=5 Cy5-OxAlg cryogels. Data in **g**
619 represents mean \pm s.e.m. of n=5 Cy5-OxAlg cryogels and were compared using two-way
620 ANOVA with Bonferroni's multiple comparison test.

621 **Supplementary Note 1**

622

623 The second order rate constant (k) for Tz-Nb reaction has been previously estimated to
624 be $1.3 - 1.7 \text{ M}^{-1}\text{s}^{-1}$ at 21°C (room temperature).^{1,2} In our system, for high-DOS Tz-HA
625 and Nb-HA the concentration is 0.55mM at the start of the reaction. The reaction rate
626 can be calculated as:

627

$$628 \quad \text{rate} = k[\text{Nb}][\text{Tz}] \quad (1)$$

629

630 We calculated the rate of reaction to be about $40\mu\text{M/s}$ from equation 1 and the time to
631 completion to be about 46.3 minutes at 21°C . In our system, the initial temperature is 4°C
632 and therefore the actual time for completion of the reaction would be significantly longer.

633

634 As the solution cools and freezes during the crosslinking process, we estimated the
635 freezing time. First, we determined the energy required to freeze $30\mu\text{L}$ of HA solution
636 starting from 4°C and ending at 0°C using:

637

$$638 \quad Q = \frac{\Delta H_i - \Delta H_f}{\rho V} \quad (2)$$

639

640 The energy required to freeze $30\mu\text{L}$ HA solution from 4°C to 0°C is 10.5J^3 . Since the HA
641 solution is very dilute ($0.6 \text{ wt}\%$), we have approximated the enthalpy of formation and
642 density to that of water.

643

644 To calculate the freezing time, we need to estimate the rate of energy extraction from
645 the HA solution. Since the teflon cryomold is pre-cooled to -20°C and rests on a metal
646 shelf in the freezer, we can estimate the rate of freezing using the thermal conductivity
647 of teflon, thickness of teflon (25mm), and conductive heat transfer area (5.75mm) using
648 equation 3. To simplify the analysis, we assume that convective heat loss at air-cryogel
649 interface is negligible.

650

651
$$\text{rate of heat transfer} = \frac{kA\Delta T}{L} \quad (3)$$

652
653 The rate of heat transfer is calculated to be 0.010J/s and therefore the time to reach 0°C
654 is ~16.8 minutes. Since we are ignoring conductive heat loss through the edge of the
655 cryogels and convective heat loss through the top, the calculated time represents an
656 overestimation for the freezing time but is still significantly below that of the time to
657 reaction completion. We have also experimentally verified that a 30uL drop of Tz-HA/Nb-
658 HA solution freezes in about 10 minutes.

659

660 References:

- 661 1. Vrabel M, Kölle P, Brunner KM, Gattner MJ, López-Carrillo V, de Vivie-Riedle R,
662 Carell T. Norbornenes in inverse electron-demand Diels-Alder reactions.
663 Chemistry. 2013;19(40):13309-12.
- 664 2. Devaraj, N. K., Weissleder, R., & Hilderbrand, S. A. (2008). Tetrazine-based
665 cycloadditions: application to pretargeted live cell imaging. Bioconjugate
666 chemistry, 19(12), 2297–2299. <https://doi.org/10.1021/bc8004446>
- 667 3. https://www.engineeringtoolbox.com/saturated-ice-steam-d_970.html

668 Supplementary Note 2

669

670 To model G-CSF pharmacokinetics (PK) in the blood, the release profile of G-CSF from
671 HA cryogels in post-HSCT mice was modeled as a piecewise function (**Fig. SN1**). Phase
672 1 was used to estimate G-CSF release from days 0-12 and phase 2 was used for days
673 12-40. We adopted parameters from previous reports^{1,2}.

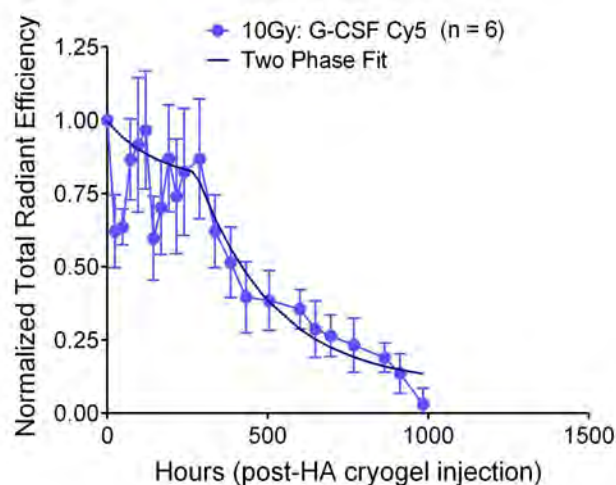
674

675

$$676 \quad \text{Phase 1: } G_{\text{Release}} = 0.7848 + 0.2112 \exp(-0.006138 t)$$

$$677 \quad \text{Phase 2: } G_{\text{Release}} = 0.09537 + 0.6989 \exp(-0.004115 [t - 286])$$

678



679

680 **Figure SN1.** Two-phase curve fit of G-CSF release from HA cryogel post-HSCT (as
681 depicted in **Fig. 5b**).

682

683 The model takes into account endogenous production of G-CSF and assumes two HA
684 cryogels loaded with 1 μ g of G-CSF each as sources. Renal clearance and internalization
685 by neutrophil progenitors are consumption terms (**Equations 1,2** and **Fig. SN2a**).

686

$$687 \quad \text{Equation 1: } \frac{dG_{\text{SubQ}}}{dt} = \frac{dG_{\text{Release}}}{dt} \frac{1}{V_s} - k_a G_{\text{SubQ}}$$

688

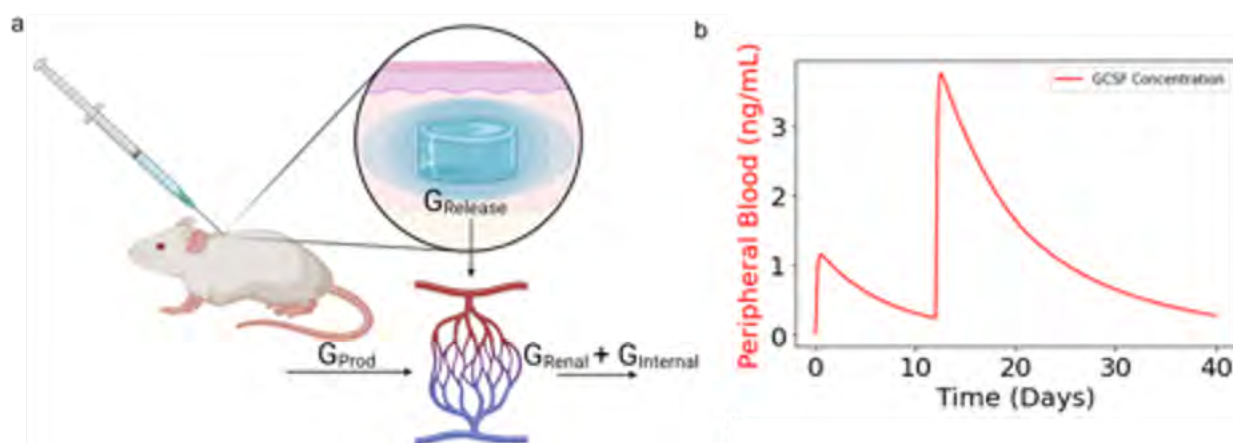
689 Equation 2:
$$\frac{dG_{Blood}}{dt} = \underbrace{zk_a G_{SubQ} + G_{Prod}}_{G_{Renal}} - \underbrace{k_{ren} G_{Blood} - NXk_{int} \frac{G_{Blood}^2}{G_{Blood}^2 + k_D}}_{G_{Internal}}$$

690

691

Parameter	k_a	k_{ren}	k_{int}	G_{prod}	z	V_S	N	X	k_D
Value	0.56 h ⁻¹	0.43 h ⁻¹	4.8 h ⁻¹	0.01 ng/mL/hr	30/2000	0.1 mL	Function of Time via reconstitution data	0.000246	1.44 ng/mL

692



698

699 **Figure SN2.** (a) Schematic depicting sources of G-CSF production and clearance in
 700 peripheral blood. (b) Predicted G-CSF concentration in peripheral blood from two
 701 subcutaneously administered G-CSF encapsulated HA cryogels based on experimentally
 702 determined G-CSF release from HA cryogels.

703 The resulting G-CSF concentration in the peripheral blood of mice is estimated in **Fig.**
 704 **SN2b.** The code underlying the model can be found here: <https://github.com/Shah-Lab-UCSD/GCSF-Release-Mod>

705

706

707

708

709

710

References:

1. Craig M, Humphries AR, Nekka F, Bélair J, Li J, Mackey MC. Neutrophil dynamics during concurrent chemotherapy and G-CSF administration: Mathematical modelling guides dose optimisation to minimise neutropenia. *Journal of Theoretical Biology.* 2015; 385: 77-89.
2. Krzyzanski W, Wiczling P, Lowe P, Pigeolet E, Fink M, et al. Population Modeling of Filgrastim PK-PD in Healthy Adults Following Intravenous and Subcutaneous Administrations. *The Journal of Clinical Pharmacology.* 2010;50(59): 101S-112S.

711 **Methods**

712 **General methods and statistics.** Sample sizes for animal studies were based on prior
713 work without use of additional statistical estimations. Results were analyzed where
714 indicated using student's t-test and two-way ANOVA with Bonferroni's test using
715 Graphpad Prism software. Mixed-model linear regression was conducted using IBM
716 SPSS statistical package. Alphanumeric coding was used in blinding for pathology
717 samples and cell counting.

718
719 **Chemicals.** Sodium hyaluronate (MW 1.5-2.2 MDa, Pharma Grade 150, lot: 18011K) and
720 sodium alginate (MW ~250 kDa, Pronova UP MVG) were purchased from NovaMatrix.
721 (2-morpholinoethanesulfonic acid (MES), sodium chloride (NaCl), sodium hydroxide
722 (NaOH), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
723 hydrochloride (EDC), sodium periodate (311448) and ammonia borane (AB) complex
724 (682098) were purchased from Sigma-Aldrich. (4-(1,2,4,5-tetrazin-3-
725 yl)phenyl)methanamine (tetrazine amine) was purchased from Kerfast (FCC659, lot:
726 2014). 1-bicyclo[2.2.1]hept-5-en-2-ylmethanamine (norbornene amine) was purchased
727 from Matrix Scientific (# 038023, lot: M15S). Cy5-tetrazine amine was purchased from
728 Lumiprobe (lot: 9D2FH). 1kDa molecular weight cutoff (MWCO) mPES membrane was
729 purchased from Spectrum (S02-E001-05-N).

730
731 **Derivatization of HA.** Tetrazine functionalized HA (HA-Tz) or norbornene functionalized
732 HA (HA-Nb) were prepared by reacting tetrazine amine or norbornene amine to HA using
733 EDC/NHS carbodiimide chemistry. Sodium hyaluronate was dissolved in a buffer solution
734 (0.75% wt/vol, pH ~ 6.5) of 100mM MES buffer. NHS and EDC were added to the mixture
735 to activate the carboxylic acid groups on the HA backbone followed by either tetrazine
736 amine or norbornene amine. HA was assumed to be 1.8 MDa for purposes of conjugation
737 reactions. To synthesize 7% DOS HA-Tz (high-DOS), the molar ratios of
738 HA:EDC:NHS:tetrazine are 1:25000:25000:2500. To synthesize 0.8% DOS HA-Tz (low-
739 DOS), the molar ratios of HA:EDC:NHS:tetrazine are 1:2860:2860:286. To synthesize
740 HA-Nb, the molar ratios of HA:EDC:NHS:norbornene are 1:25000:25000:2500. Each
741 reaction was stirred at room temperature for 24 hours and transferred to a 12,000Da MW

742 cutoff dialysis sack (Sigma Aldrich) and dialyzed in 4L of NaCl solutions of decreasing
743 molarity (0.125M, 0.100M, 0.075M, 0.050M, 0.025M, 0M, 0M, 0M, 0M) for 8 hours per
744 solution. After dialysis, solutions containing HA-Tz or HA-Nb were frozen overnight and
745 lyophilized (Labconco Freezone 4.5) for 48 hours. Cy5 conjugated HA-Nb (Cy5-HA-Nb)
746 was synthesized following a previously described technique with some modifications⁷⁸.
747 0.8mg of Cy5-Tz was reacted with 100mg of HA-Nb at 0.2 wt/vol in DI water for 24 hours
748 at 37 °C and purified by dialysis in DI water using a 12,000Da MW cutoff dialysis sack for
749 48 hours. Dialysis water bath was changed once every 8 hours. Cy5-HA-Nb was then
750 frozen overnight and lyophilized for 48 hours.

751
752 **Preparation of oxidized alginate.** Alginate was oxidized by mixing a 1% wt/vol solution
753 of sodium alginate in DI water with an aqueous solution of 23 mM sodium periodate
754 (Sigma Aldrich) to achieve a 1:586 molar ratio of alginate: periodate. The reaction was
755 stirred in the dark at room temperature overnight. Sodium chloride (1.8 grams/gram of
756 alginate) was added to solution to achieve a 0.3 M solution, followed by purification via
757 tangential flow filtration (TFF) using a mPES 1kDa molecular weight cutoff (MWCO)
758 membrane (Spectrum) and sequential solvent exchanges with 0.15 M – 0.10 M – 0.05 M
759 and 0.0 M sodium chloride in DI water. The resulting solution was treated with ammonia
760 borane (AB) complex (Sigma Aldrich) at 1:4 alginate:AB molar ratio and stirred at room
761 temperature overnight. Sodium chloride (1.8 grams/gram of alginate) was added to
762 solution to achieve a 0.3 M solution, followed by purification via TFF using a 1kDa MWCO
763 mPES membrane and sequential solvent exchanges with 0.15 M – 0.10 M – 0.05 M and
764 0.0 M sodium chloride in DI water. The resulting solution was lyophilized to dryness.

765
766 **Derivatization of oxidized alginate.** To synthesize tetrazine and norbornene
767 functionalized oxidized alginate (OxAlg-Tz, OxAlg-Nb respectively), oxidized alginate,
768 prepared as described above, was solubilized in 0.1 M MES buffer, 0.3 M sodium chloride,
769 pH 6.5 at 1%wt/vol. NHS and EDC were added to the mixture followed by either tetrazine
770 or norbornene. The molar ratio of oxidized alginate:NHS:EDC:tetrazine or norbornene
771 was 1:5000:5000:1000. The reaction is stirred in the dark at room temperature overnight.
772 The resulting solution is centrifuged at 4700 rpm for 15 minutes and filtered through a 0.2-

773 micron filter. The solution is purified via TFF using a mPES 1kDa molecular weight cutoff
774 (MWCO) membrane and sequential solvent exchanges with 0.15 M – 0.10 M – 0.05 M
775 and 0.0 M sodium chloride in DI water. The purified solution is treated with activated
776 charcoal (1 gram / gram of alginate) for 20 minutes at room temperature. The slurry is
777 filtered through 0.2-micron filter and the filtrate is lyophilized to dryness.

778

779 **Endotoxin Testing.** Endotoxin testing of high-DOS HA-Tz and Cy5-HA-Nb were
780 conducted using a commercially available endotoxin testing kit (88282, Thermo Fisher
781 Scientific, lot: VH310729) and following manufacturer's instructions. High-DOS HA-Tz
782 and Cy5-HA-Nb were solubilized at 0.6 wt% in endotoxin free water and samples were
783 tested in technical triplicates. To calculate endotoxin content of a single HA cryogel, the
784 EU/mL concentration for high-DOS HA-Tz and Cy5-HA-Nb were divided by 2 (relative
785 concentrations of HA-Tz and HA-Nb in HA cryogels are 0.3 wt%) and multiplied by 0.03
786 (30uL of volume per HA cryogel). EU/kg was calculated based on 2 HA cryogels
787 administered into a mouse with an average weight of 20 grams.

788

789 **Cryogel development.** We followed a previously described cryogelation method⁷⁸⁻⁸⁰. To
790 form cryogels, aqueous solutions of 0.6% wt/vol HA-Tz and HA-Nb or OxAlg- Tz and
791 OxAlg-Nb were prepared by dissolving lyophilized polymers into deionized water and left
792 on a rocker at room temperature for a minimum of 8 hours to allow for dissolution. The
793 aqueous solutions were then pre-cooled to 4°C before cross-linking to slow reaction
794 kinetics. HA-Tz and HA-Nb or OxAlg- Tz and OxAlg-Nb solutions were mixed at a 1:1
795 volume ratio, pipetted into 30µL Teflon molds which were pre-cooled to -20 °C, and
796 quickly transferred to a -20 °C freezer to allow for overnight cryogelation. Synthesis of
797 Cy5-HA or Cy5-OxAlg cryogels follows the same protocol as above, substituting Cy5-HA-
798 Nb for HA-Nb or Cy5-OxAlg-Nb for OxAlg-Nb.

799

800 **Pore size analysis of HA cryogels.** For scanning electron microscopy (SEM), frozen HA
801 cryogels were lyophilized for 24 hours and in a petri dish. Lyophilized HA cryogels were
802 adhered onto sample stubs using carbon tape and coated with iridium in a sputter coater.
803 Samples were imaged using secondary electron detection on a FEI Quanta 250 field

804 emission SEM in the Nano3 user facility at UC San Diego. Fluorescence images of Cy5-
805 HA cryogels were acquired using a Leica SP8 All experiments were performed at the UC
806 San Diego School of Medicine Microscopy Core. Pore size quantification of SEM images
807 and relative distribution of pore sizes of confocal images was done using FIJI image
808 processing package ⁸¹.

809

810 **HA cryogel pore-interconnectedness analysis.** Cy5-HA cryogels were synthesized
811 with low- and high-DOS HA-Tz and incubated in 1mL of FITC-labeled 10 μ M diameter
812 melamine resin micro particles (Sigma Aldrich) at 0.29mg/mL concentration on a rocker
813 at room temperature overnight. Fluorescence images of Cy5-HA cryogels with FITC-
814 labeled microparticles were acquired using a Leica SP8 confocal. Interconnectedness of
815 the HA cryogels was determined by generating 3D renderings of confocal z-stacks using
816 FIJI imaging processing package and assessing fluorescence intensity of both the Cy5
817 and FITC channels with depth starting from the top of the HA cryogel. To determine the
818 effect of injection on pore interconnectedness, HA cryogels were injected through a 16G
819 needle prior to incubation in FITC-labeled microparticle solution. All experiments were
820 performed at the UC San Diego School of Medicine Microscopy Core.

821

822 **In vitro degradation of Cy5-HA cryogels.** Cy5-HA cryogels synthesized with low- and
823 high-DOS HA-Tz and placed into individual 1.5mL microcentrifuge tubes (Thermo
824 Scientific) with 1mL of 100U/mL Hyaluronidase from sheep testes Type II (HYAL2,
825 H2126, Sigma Aldrich, lot: SLBZ9984) in 1x PBS. Degradation studies were conducted
826 in tissue culture incubators at 37 °C. Supernatant from samples were collected every 24
827 – 72 hours by centrifuging the samples at room temperature at 2,000G for 5 minutes and
828 removing 0.9mL of supernatant. Cy5-HA cryogels were resuspended by adding 0.9mL of
829 freshly made 100U/mL HYAL2 in 1x PBS. Fluorescence measurements were conducted
830 using a Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific) and these values
831 were normalized to sum of the fluorescence values over the course of the experiment. All
832 experiments were performed at UC San Diego.

833 **In vitro degradation of Cy5-OxAlg Cryogels.** Cy5-OxAlg cryogels were placed into
834 individual 1.5mL microcentrifuge tube with 1mL of 1x PBS. Degradation studies were

835 conducted in tissue culture incubators at 37°C. Supernatant from samples were collected
836 every 24 – 72 hours by removing visible Cy5-OxAlg cryogel material with tweezers and
837 transferring to new 1.5mL microcentrifuge tube with 1mL of 1x PBS. Fluorescence
838 measurements were conducted using a Nanodrop 2000 Spectrophotometer and these
839 values were normalized to sum of the fluorescence values over the course of the
840 experiment. All experiments were performed at UC San Diego.

841 **In vivo mouse experiments.** All animal work was conducted at the Moores Cancer
842 Center vivarium at UC San Diego, except NSG mouse IVIS imaging experiments, which
843 were conducted at the Harvard Biological Research Infrastructure vivarium at Harvard
844 University and approved by the respective Institutional Animal Care and Use Committee
845 (IACUC). All animal experiments followed the National Institutes of Health guidelines and
846 relevant AALAC-approved procedures. Female C57BL/6J (B6, Jax # 000664) and
847 NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG, Jax # 005557) mice were 6-8 weeks at the start
848 of the experiments. Male B6.129S-Cybb^{tm1Din} (gp91^{phox-}, Jax # 002365) mice were 6-8
849 weeks old at the start of experiments. All mice in each experiment were age matched and
850 no randomization was performed. The pre-established criteria for animal omission were
851 failure to inject the desired cell dose in transplanted mice and death due to transplant
852 failure. Health concerns unrelated to the study (e.g. malocclusion) and known mouse-
853 strain specific conditions that affected measurements (e.g. severe dermatitis and skin
854 hyperpigmentation in B6 mice) were criteria for omission.

855
856 **Immune depletion in mice.** Neutrophil depletion in B6 mice was achieved by following
857 the previously established protocol ^{28, 82}. Briefly, 25µL of anti-mouse Ly6G antibody (1A8,
858 Bio X Cell, lot: 737719A2)) was administered i.p. every day for the first week.
859 Concurrently, 50µL of anti-rat κ immunoglobulin light chain antibody (MAR 18.5, Bio X
860 Cell, lot: 752020J2) was administered every other day starting on the second day of
861 depletion. After one week, the dose of the anti-mouse Ly6G antibody was increased to
862 50µL. Macrophage depletion in B6 mice was induced by i.p. administration of 100uL of
863 clodronate liposomes (Liposoma, lot: C44J0920) every 3-days. B cell lineage depletion in
864 B6 mice was induced by i.p. administration of 400µg of anti-mouse B220/CD45R antibody
865 (RA3.31/6.1, Bio X Cell, lot: 754420N1) once every 3-days. T cell lineage depletion in B6

866 mice was induced by i.p. administration of 400 μ g dose of anti-mouse CD4 antibody
867 (GK1.5, Bio X Cell, lot: 728319M2) and 400 μ g dose of anti-mouse CD8 α antibody (2.43,
868 Bio X Cell, lot:732020F1) once every 3-days. For all lineage depletion models, mice
869 received intraperitoneal injections of 0.1mL (400 μ g) of antibodies or 0.1mL of clodronate
870 liposome solution 3 days before subcutaneous HA cryogel or Cy5-HA cryogel injection.
871 Depletion started 3-days prior to Cy5-HA cryogel administration to mice and continued
872 until complete cryogel degradation or until mice were euthanized and cryogels retrieved
873 for analysis. All experiments were performed at the Moores Cancer Center vivarium at
874 UC San Diego.

875 **Transplant models.** Irradiations were performed with a Cesium-137 gamma-radiation
876 source irradiator (J.L. Shepherd & Co.). Syngeneic HSCT (B6 recipients) consisted of 1
877 dose of 1,000 cGy + 1 x 10⁵ lineage-depleted bone marrow cells from syngeneic B6
878 donors. Bone marrow cells for transplantation (from donors) or analysis were harvested
879 by crushing all limbs with a mortar and pestle, diluted in 1x PBS, filtering the tissue
880 homogenate through a 70 μ m mesh and preparing a single-cell suspension by passing
881 the cells in the flowthrough once through a 20-gauge needle. Total cellularity was
882 determined by counting cells using a hemacytometer. Bone marrow cells were depleted
883 of immune cells (expressing CD3 ϵ , CD45R/B220, Ter-119, CD11b, or Gr-1) by magnetic
884 selection using a Mouse Hematopoietic Progenitor Cell Enrichment Set (BD Biosciences
885 # 558451, lot: 0114777). To confirm depletion, we incubated cells with a mix of Pacific
886 Blue-conjugated lineage specific antibodies (antibodies to CD3, NK1.1, Gr-1, CD11b,
887 CD19, CD4 and CD8) and with Sca-1 and cKit-specific antibodies for surface staining and
888 quantification of Lineage⁻ fraction of cells, which were \geq 87% lineage depleted.
889 Subsequently, cells were suspended in 100 μ L of sterile 1x PBS and administered to
890 anesthetized mice via a single retroorbital injection. All experiments were performed at
891 the Moores Cancer Animal Facility at UC San Diego Health. All flow cytometry
892 experiments were performed using an Attune[®] NxT Acoustic Focusing cytometer analyzer
893 (A24858) at UC San Diego.

894 **Subcutaneous cryogel administration:** While mice were anesthetized, a subset
895 received a subcutaneous injection of HA cryogel or OxAlg cryogel, which was suspended

896 in 200 μ L of sterile 1x PBS, into the dorsal flank by means of a 16G needle positioned
897 approximately midway between the hind- and forelimbs. The site of injection was shaved
898 and wiped with a sterile alcohol pad prior to gel injection.

899 **In vivo degradation.** In vivo Cy5-HA cryogel degradation was performed with Cy5-HA
900 cryogels synthesized with low- and high-DOS Tz-HA in untreated B6 mice, immune
901 deficient B6 mice, NSG mice, and gp91^{phox-} mice. In vivo Cy5 OxAlg cryogel degradation
902 was performed in non-irradiated, non-transplanted B6 mice and B6 mice post-HSCT. In
903 all cases, cryogels were administered into the dorsal flank of an anesthetized mouse and
904 the fluorescent intensity of the Cy5-HA cryogel was quantified using an IVIS spectrometer
905 (PerkinElmer) at predetermined timepoints and analyzed using LivingImage software
906 (PerkinElmer). At each timepoint, mice were anesthetized and the area around the
907 subcutaneous cryogel was shaved to reduce fluorescence signal attenuation.
908 Fluorescence radiant efficiency, the ratio of fluorescence emission to excitation, was
909 measured longitudinally as a metric to quantify fluorescence from subcutaneous cryogels.
910 These values were normalized to the measured signal on day 3. All experiments were
911 performed at the Moores Cancer Microscopy Core Facility at UC San Diego Health, with
912 the exception of NSG mouse in vivo degradation experiments, which were performed at
913 the Harvard Biology Research Infrastructure vivarium using an IVIS spectrometer
914 (PerkinElmer).

915 **Flow cytometry analysis.** Anti-mouse antibodies to CD45 (30-F11, lot: B280746),
916 CD11b (M1/70, lot: B322056), CD4 (RM4-5, lot: B240051), CD8 α (53-6.7, lot: B266721),
917 B220 (RA3-6B2, lot: B298555), Ly6-G/Gr-1 (1A8, lot: B259670), lineage cocktail
918 (17A2/RB6-8C5/RA3-6B2/Ter-119/M1/70, lot: B266946), Ly-6A/E/Sca-1 (D7, lot:
919 B249343), and CD117/cKit (2B8, lot: B272462) were purchased from Biolegend. Anti-
920 mouse F4/80 (BM8, lot: 2229150) and was purchased from eBioscience. All cells were
921 gated based on forward and side scatter characteristics to limit debris, including dead
922 cells. AnnexinV (Biolegend, lot: B300974) stain was used to separate live and dead cells.
923 Antibodies were diluted according to the manufacturer's suggestions. Cells were gated
924 based on fluorescence-minus-one controls, and the frequencies of cells staining positive
925 for each marker were recorded. To quantify T cells, B cells, monocytes, and neutrophils

926 in peripheral blood, blood was first collected from the tail vein of mice into EDTA coated
927 tubes (BD). Samples then underwent lysis of red blood cells and were stained with
928 appropriate antibodies corresponding to cell populations of interest. To quantify infiltrating
929 immune cells within Cy5-HA cryogels, mice were sacrificed, cryogels removed, and HA
930 cryogels crushed against a 70-micron filter screen before antibody staining. Absolute
931 numbers of cells were calculated using flow cytometry frequency. Flow cytometry was
932 analyzed using FlowJo (BD) software. All flow cytometry experiments were performed
933 using a Attune[®] NxT Acoustic Focusing cytometer analyzer (A24858) at UC San Diego.

934 **Histology.** After euthanasia, HA cryogels were explanted and fixed in 4%
935 paraformaldehyde (PFA) for 24 hours. The fixed HA cryogels were then transferred to
936 70% ethanol solution. Samples were routinely processed and sections (5 μ m) were
937 stained and digitized using an Aperio AT2 Automated Digital Whole Slide Scanner by the
938 Tissue Technology Shared Resource at the Moores Cancer Center at UC San Diego
939 Health. Digital slides were rendered in QuPath and positive cell detection was used to
940 quantify the total number of mononuclear cells within each image. Quantification of
941 mononuclear cell density was determined for each histological section.

942 **Immunohistochemistry (IHC).** Paraffin embedded HA cryogel sections were baked at
943 60 °C for 1 hr. Tissues were then rehydrated through successive washes (3x xylene, 2x
944 100% ethanol, 2x 95% ethanol, 2x 70% ethanol, di-water). After rehydration, antigen
945 retrieval was conducted using Unmasking solution (Citrate based, pH 6) (Vector
946 Laboratories, H-3300) at 95 °C for 30 minutes. Staining was performed using Intellipath
947 Automated IHC Stainer (Biocare). A peroxidase block, Bloxall (Vector Laboratories, SP-
948 6000) was performed for 10 minutes, followed by 2x washes in 1x tris-buffered saline with
949 0.1% Tween 20 (TBST, Sigma Aldrich), and a blocking step using 3% Donkey Serum for
950 10 minutes. Samples were stained using anti-Ly6G primary antibody (Rabbit, Cell
951 Signaling Technology, 87048S) at 1:100 concentration for 1 hr. Samples were washed
952 twice in TBST and anti-rabbit HRP polymer (Cell ID, 2HR-100) was added for 30
953 minutes. Samples were washed twice in TBST and DAB (brown) Chromogen (VWR,
954 95041-478) was added for 5 minutes. This was followed by 2x washes in di-water, 5-
955 minute incubation with Mayer's Hematoxylin (Sigma, 51275), 2x washes in TBST, and 1x

956 wash in di-water. Mounting was performed using a xylene-based mountant. IHC was
957 performed by the Tissue Technology Shared Resource at the Moores Cancer Center at
958 UC San Diego Health.

959 **G-CSF encapsulation.** To quantify G-CSF release from HA cryogels, recombinant
960 human G-CSF (300-23, Peprotech, lots: 041777 and 041877) was reacted with sulfo-Cy5
961 NHS ester (13320, Lumiprobe, lot 7FM7C) at a 1:250:25 molar ratio of G-CSF:EDC:sulfo-
962 Cy5 NHS ester in MES buffer to form Cy5 G-CSF. Unreacted EDC and sulfo-Cy5 NHS
963 ester was removed by overnight dialysis on a 10kDa dialysis membrane. 1 μ g of Cy5 G-
964 CSF was added to 0.6% wt/vol HA-Tz solution before mixing with HA-Nb and cryogelation
965 as described above. To track Cy5-HA cryogel degradation in mice which received G-CSF
966 loaded Cy5-HA cryogels, the same protocol is followed substituting G-CSF for Cy5 G-
967 CSF and Cy5-HA-Nb for HA-Nb.

968 **Neutrophil reconstitution models.** Mice were irradiated and administered an
969 autologous HSCT as described above. PEG G-CSF (MBS355608, MyBioSource, lot:
970 R15/2020J) or G-CSF was injected i.p. Cy5-HA cryogel encapsulating G-CSF was
971 injected subcutaneously as described above, 24 hours post-HSCT. Mice were bled at
972 predetermined timepoints and relevant immune subsets were stained for flow cytometry.

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974 analysis, investigation, resources, data curation, writing – original draft, writing – review
975 & editing and visualization. **David A. McBride:** Methodology, investigation, writing –
976 review and editing. **Wade T. Johnson:** Investigation and writing – review and editing.
977 **Arun K. Chumber:** Investigation and writing – review and editing. **Alexander J. Najibi:**
978 Investigation and writing – review and editing. **Bo Ri Seo.** Investigation and writing –
979 review and editing. **Alexander G. Stafford:** Resources. **David T. Scadden:** Funding
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1000

1001 **Data availability.** The data that support the findings of this study are available from the
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1003 **Competing interests.** M.D.K., D.J.M. and N.J.S. are named inventors on U.S.
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1005 **References**

- 1006 1. Mócsai, A. Diverse novel functions of neutrophils in immunity, inflammation, and
1007 beyond. *Journal of Experimental Medicine* **210**, 1283-1299 (2013).
- 1008 2. Anderson, J.M., Rodriguez, A. & Chang, D.T. in *Seminars in immunology*, Vol. 20
1009 86-100 (Elsevier, 2008).
- 1010 3. Wang, J. et al. Visualizing the function and fate of neutrophils in sterile injury and
1011 repair. *Science* **358**, 111-116 (2017).
- 1012 4. Lakshman, R. & Finn, A. Neutrophil disorders and their management. *J Clin*
1013 *Patho* **54**, 7-19 (2001).
- 1014 5. Crawford, J., Dale, D.C. & Lyman, G.H. Chemotherapy-induced neutropenia:
1015 risks, consequences, and new directions for its management. *Cancer* **100**, 228-
1016 237 (2004).
- 1017 6. Gil, L. et al. Neutropenic enterocolitis after high-dose chemotherapy and
1018 autologous stem cell transplantation: incidence, risk factors, and outcome.
1019 *Transplant Infectious Disease* **15**, 1-7 (2013).
- 1020 7. Ley, K. et al. Neutrophils: New insights and open questions. *Science immunology*
1021 **3** (2018).
- 1022 8. Celebi, H., Akan, H., Akcaglayan, E., Ustun, C. & Arat, M. Febrile neutropenia in
1023 allogeneic and autologous peripheral blood stem cell transplantation and
1024 conventional chemotherapy for malignancies. *Bone marrow transplantation* **26**,
1025 211-214 (2000).
- 1026 9. Sadtler, K. et al. The Scaffold Immune Microenvironment: Biomaterial-Mediated
1027 Immune Polarization in Traumatic and Nontraumatic Applications<sup/>. *Tissue*
1028 *engineering. Part A* **23**, 1044-1053 (2017).
- 1029 10. Balletto, E. & Mikulska, M. Bacterial Infections in Hematopoietic Stem Cell
1030 Transplant Recipients. *Mediterranean journal of hematology and infectious*
1031 *diseases* **7**, e2015045 (2015).
- 1032 11. Stoma, I., Karpov, I., Milanovich, N., Uss, A. & Iskrov, I. Risk factors for mortality
1033 in patients with bloodstream infections during the pre-engraftment period after
1034 hematopoietic stem cell transplantation. *Blood research* **51**, 102-106 (2016).
- 1035 12. Matsushima, H. et al. Neutrophil differentiation into a unique hybrid population
1036 exhibiting dual phenotype and functionality of neutrophils and dendritic cells.
1037 *Blood* **121**, 1677-1689 (2013).
- 1038 13. Weiskopf, K. et al. Myeloid Cell Origins, Differentiation, and Clinical Implications.
1039 *Microbiology spectrum* **4** (2016).
- 1040 14. Trivedi, M., Martinez, S., Corringham, S., Medley, K. & Ball, E.D. Optimal use of
1041 G-CSF administration after hematopoietic SCT. *Bone marrow transplantation* **43**,
1042 895-908 (2009).
- 1043 15. Mehta, H.M., Malandra, M. & Corey, S.J. G-CSF and GM-CSF in Neutropenia.
1044 *Journal of immunology (Baltimore, Md. : 1950)* **195**, 1341-1349 (2015).
- 1045 16. Bajrami, B. et al. G-CSF maintains controlled neutrophil mobilization during acute
1046 inflammation by negatively regulating CXCR2 signaling. *The Journal of*
1047 *experimental medicine* **213**, 1999-2018 (2016).

- 1048 17. Pinto, L. et al. Comparison of pegfilgrastim with filgrastim on febrile neutropenia,
1049 grade IV neutropenia and bone pain: a meta-analysis of randomized controlled
1050 trials. *Current medical research and opinion* **23**, 2283-2295 (2007).
- 1051 18. Singh, A.D. et al. Granulocyte Colony-Stimulating Factor Use after Autologous
1052 Peripheral Blood Stem Cell Transplantation: Comparison of Two Practices.
1053 *Biology of blood and marrow transplantation : journal of the American Society for*
1054 *Blood and Marrow Transplantation* **24**, 288-293 (2018).
- 1055 19. Ashrafi, F. & Salmasi, M. Comparison of the effects of pegylated granulocyte-
1056 colony stimulating factor and granulocyte-colony stimulating factor on cytopenia
1057 induced by dose-dense chemotherapy in breast cancer patients. *Journal of*
1058 *research in medical sciences : the official journal of Isfahan University of Medical*
1059 *Sciences* **23**, 73 (2018).
- 1060 20. Molineux, G. The design and development of pegfilgrastim (PEG-rmetHuG-CSF,
1061 Neulasta). *Current pharmaceutical design* **10**, 1235-1244 (2004).
- 1062 21. Elsadek, N.E. et al. Pegfilgrastim (PEG-G-CSF) induces anti-PEG IgM in a dose
1063 dependent manner and causes the accelerated blood clearance (ABC)
1064 phenomenon upon repeated administration in mice. *European Journal of*
1065 *Pharmaceutics and Biopharmaceutics* **152**, 56-62 (2020).
- 1066 22. Girard, N. et al. Human monocytes synthesize hyaluronidase. *British Journal of*
1067 *Haematology* **119**, 199-203 (2002).
- 1068 23. Soltes, L. et al. Degradative action of reactive oxygen species on hyaluronan.
1069 *Biomacromolecules* **7**, 659-668 (2006).
- 1070 24. Jiang, D., Liang, J. & Noble, P.W. Hyaluronan as an immune regulator in human
1071 diseases. *Physiological reviews* **91**, 221-264 (2011).
- 1072 25. Ito, M. et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse
1073 model for engraftment of human cells. *Blood* **100**, 3175-3182 (2002).
- 1074 26. Malyala, P. & Singh, M. Endotoxin limits in formulations for preclinical research.
1075 *Journal of pharmaceutical sciences* **97**, 2041-2044 (2008).
- 1076 27. Burdick, J.A., Chung, C., Jia, X., Randolph, M.A. & Langer, R. Controlled
1077 degradation and mechanical behavior of photopolymerized hyaluronic acid
1078 networks. *Biomacromolecules* **6**, 386-391 (2005).
- 1079 28. Boivin, G. et al. Durable and controlled depletion of neutrophils in mice. *Nature*
1080 *Communications* **11**, 2762 (2020).
- 1081 29. Pollock, J.D. et al. Mouse model of X-linked chronic granulomatous disease, an
1082 inherited defect in phagocyte superoxide production. *Nat Genet* **9**, 202-209
1083 (1995).
- 1084 30. Banerjee, E.R. & Henderson, W.R., Jr. Role of T cells in a gp91phox knockout
1085 murine model of acute allergic asthma. *Allergy Asthma Clin Immunol* **9**, 6 (2013).
- 1086 31. Bouhadir, K.H. et al. Degradation of Partially Oxidized Alginate and Its Potential
1087 Application for Tissue Engineering. *Biotechnology Progress* **17**, 945-950 (2001).
- 1088 32. Gao, C., Liu, M., Chen, J. & Zhang, X. Preparation and controlled degradation of
1089 oxidized sodium alginate hydrogel. *Polymer Degradation and Stability* **94**, 1405-
1090 1410 (2009).
- 1091 33. Jagasia, M.H. et al. Pegfilgrastim after high-dose chemotherapy and autologous
1092 peripheral blood stem cell transplant: phase II study. *Bone Marrow*
1093 *Transplantation* **35**, 1165-1169 (2005).

- 1094 34. Martino, M. et al. Pegfilgrastim compared with filgrastim after high-dose
1095 melphalan and autologous hematopoietic peripheral blood stem cell
1096 transplantation in multiple myeloma patients. *European journal of haematology*
1097 **77**, 410-415 (2006).
- 1098 35. Barr, S., Hill, E.W. & Bayat, A. Functional biocompatibility testing of silicone
1099 breast implants and a novel classification system based on surface roughness.
1100 *Journal of the Mechanical Behavior of Biomedical Materials* **75**, 75-81 (2017).
- 1101 36. Carnicer-Lombarte, A., Chen, S.-T., Malliaras, G.G. & Barone, D.G. Foreign
1102 Body Reaction to Implanted Biomaterials and Its Impact in Nerve
1103 Neuroprosthetics. *Frontiers in Bioengineering and Biotechnology* **9** (2021).
- 1104 37. Whitaker, R., Hernaez-Estrada, B., Hernandez, R.M., Santos-Vizcaino, E. &
1105 Spiller, K.L. Immunomodulatory Biomaterials for Tissue Repair. *Chemical*
1106 *Reviews* (2021).
- 1107 38. Sutherland, K., Mahoney, J.R., Coury, A.J. & Eaton, J.W. Degradation of
1108 biomaterials by phagocyte-derived oxidants. *The Journal of clinical investigation*
1109 **92**, 2360-2367 (1993).
- 1110 39. Ye, Q., Harmsen, M.C., van Luyn, M.J. & Bank, R.A. The relationship between
1111 collagen scaffold cross-linking agents and neutrophils in the foreign body
1112 reaction. *Biomaterials* **31**, 9192-9201 (2010).
- 1113 40. Labow, R.S., Meek, E. & Santerre, J.P. Neutrophil - mediated biodegradation of
1114 medical implant materials. *Journal of cellular physiology* **186**, 95-103 (2001).
- 1115 41. Shah, N.J. et al. A biomaterial-based vaccine eliciting durable tumour-specific
1116 responses against acute myeloid leukaemia. *Nature Biomedical Engineering* **4**,
1117 40-51 (2020).
- 1118 42. Verbeke, C.S. et al. Multicomponent Injectable Hydrogels for Antigen-Specific
1119 Tolerogenic Immune Modulation. *Adv Healthc Mater* **6**,
1120 10.1002/adhm.201600773 (2017).
- 1121 43. Frantz, C., Stewart, K.M. & Weaver, V.M. The extracellular matrix at a glance.
1122 *Journal of Cell Science* **123**, 4195-4200 (2010).
- 1123 44. Kaderli, S. et al. A novel biocompatible hyaluronic acid-chitosan hybrid hydrogel
1124 for osteoarthritis therapy. *International journal of pharmaceutics* **483**, 158-168
1125 (2015).
- 1126 45. Kaderli, S. et al. Efficacy study of two novel hyaluronic acid-based formulations
1127 for viscosupplementation therapy in an early osteoarthrotic rabbit model.
1128 *European journal of pharmaceutics and biopharmaceutics : official journal of*
1129 *Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* **96**, 388-395
1130 (2015).
- 1131 46. Buffa, R. et al. Conjugates of modified hyaluronic acid with amino compounds for
1132 biomedical applications. *Carbohydrate polymers* **189**, 273-279 (2018).
- 1133 47. Ma, X. et al. Injectable hydrogels based on the hyaluronic acid and poly (gamma-
1134 glutamic acid) for controlled protein delivery. *Carbohydrate polymers* **179**, 100-
1135 109 (2018).
- 1136 48. Purcell, B.P. et al. Injectable and bioresponsive hydrogels for on-demand matrix
1137 metalloproteinase inhibition. *Nature materials* **13**, 653-661 (2014).

- 1138 49. Barker, I.A. et al. Tetrazine-norbornene click reactions to functionalize
1139 degradable polymers derived from lactide. *Macromolecular rapid*
1140 *communications* **32**, 1362-1366 (2011).
- 1141 50. Hansell, C.F. et al. Additive-free clicking for polymer functionalization and
1142 coupling by tetrazine-norbornene chemistry. *Journal of the American Chemical*
1143 *Society* **133**, 13828-13831 (2011).
- 1144 51. Kennedy, S. et al. Rapid and extensive collapse from electrically responsive
1145 macroporous hydrogels. *Advanced healthcare materials* **3**, 500-507 (2014).
- 1146 52. Koshy, S.T., Zhang, D.K.Y., Grolman, J.M., Stafford, A.G. & Mooney, D.J.
1147 Injectable nanocomposite cryogels for versatile protein drug delivery. *Acta*
1148 *Biomater* **65**, 36-43 (2018).
- 1149 53. Qin, X.-H. et al. Enzymatic synthesis of hyaluronic acid vinyl esters for two-
1150 photon microfabrication of biocompatible and biodegradable hydrogel constructs.
1151 *Polym. Chem.* **5**, 6523-6533 (2014).
- 1152 54. Chen, F. et al. Self-crosslinking and injectable hyaluronic acid/RGD-
1153 functionalized pectin hydrogel for cartilage tissue engineering. *Carbohydrate*
1154 *polymers* **166**, 31-44 (2017).
- 1155 55. Tavsanlı, B. & Okay, O. Mechanically strong hyaluronic acid hydrogels with an
1156 interpenetrating network structure. *European Polymer Journal* **94**, 185-195
1157 (2017).
- 1158 56. Pedron, S. et al. Patterning Three-Dimensional Hydrogel Microenvironments
1159 Using Hyperbranched Polyglycerols for Independent Control of Mesh Size and
1160 Stiffness. *Biomacromolecules* **18**, 1393-1400 (2017).
- 1161 57. Šečkutė, J., Yang, J. & Devaraj, N.K. Rapid oligonucleotide-templated
1162 fluorogenic tetrazine ligations. *Nucleic Acids Research* **41**, e148-e148 (2013).
- 1163 58. Johann, K. et al. Tetrazine- and trans-cyclooctene-functionalised polypept(o)ides
1164 for fast bioorthogonal tetrazine ligation. *Polymer Chemistry* **11**, 4396-4407
1165 (2020).
- 1166 59. Greenwald, R.A. & Moy, W.W. Effect of oxygen - derived free radicals on
1167 hyaluronic acid. *Arthritis & Rheumatism: Official Journal of the American College*
1168 *of Rheumatology* **23**, 455-463 (1980).
- 1169 60. McNeil, J., Wiebkin, O., Betts, W. & Cleland, L. Depolymerisation products of
1170 hyaluronic acid after exposure to oxygen-derived free radicals. *Annals of the*
1171 *Rheumatic Diseases* **44**, 780-789 (1985).
- 1172 61. Šoltés, L. et al. Degradative action of reactive oxygen species on hyaluronan.
1173 *Biomacromolecules* **7**, 659-668 (2006).
- 1174 62. Duan, J. & Kasper, D.L. Oxidative depolymerization of polysaccharides by
1175 reactive oxygen/nitrogen species. *Glycobiology* **21**, 401-409 (2011).
- 1176 63. Greenwald, R.A. & Moak, S.A. Degradation of hyaluronic acid by
1177 polymorphonuclear leukocytes. *Inflammation* **10**, 15-30 (1986).
- 1178 64. Doloff, J.C. et al. Colony stimulating factor-1 receptor is a central component of
1179 the foreign body response to biomaterial implants in rodents and non-human
1180 primates. *Nature materials* **16**, 671-680 (2017).
- 1181 65. Shultz, L.D. et al. Multiple defects in innate and adaptive immunologic function in
1182 NOD/LtSz-scid mice. *Journal of immunology (Baltimore, Md. : 1950)* **154**, 180-
1183 191 (1995).

- 1184 66. Shultz, L.D. et al. Human lymphoid and myeloid cell development in NOD/LtSz-
1185 scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem
1186 cells. *Journal of immunology (Baltimore, Md. : 1950)* **174**, 6477-6489 (2005).
- 1187 67. Foreman, O., Kavirayani, A.M., Griffey, S.M., Reader, R. & Shultz, L.D.
1188 Opportunistic bacterial infections in breeding colonies of the NSG mouse strain.
1189 *Veterinary pathology* **48**, 495-499 (2011).
- 1190 68. Patton, J.B. et al. Methylprednisolone acetate induces, and Δ 7-dafachronic acid
1191 suppresses, *Strongyloides stercoralis* hyperinfection in NSG mice. *Proceedings*
1192 *of the National Academy of Sciences of the United States of America* **115**, 204-
1193 209 (2018).
- 1194 69. Pavlů, J. et al. Analysis of hematopoietic recovery after autologous
1195 transplantation as method of quality control for long-term progenitor cell
1196 cryopreservation. *Bone Marrow Transplant* **52**, 1599-1601 (2017).
- 1197 70. Ramaprasad, C., Pouch, S. & Pitrak, D.L. Neutrophil function after bone marrow
1198 and hematopoietic stem cell transplant. *Leukemia & Lymphoma* **51**, 756-767
1199 (2010).
- 1200 71. Grainger, D.W. All charged up about implanted biomaterials. *Nature*
1201 *biotechnology* **31**, 507-509 (2013).
- 1202 72. Freedman, B.R. et al. Degradable and Removable Tough Adhesive Hydrogels.
1203 *Advanced Materials* **33**, 2008553 (2021).
- 1204 73. Zamboni, W.C. Pharmacokinetics of pegfilgrastim. *Pharmacotherapy* **23**, 9S-14S
1205 (2003).
- 1206 74. Zhang, P., Sun, F., Liu, S. & Jiang, S. Anti-PEG antibodies in the clinic: Current
1207 issues and beyond PEGylation. *Journal of Controlled Release* **244**, 184-193
1208 (2016).
- 1209 75. McSweeney, M.D. et al. A minimal physiologically based pharmacokinetic model
1210 that predicts anti-PEG IgG-mediated clearance of PEGylated drugs in human
1211 and mouse. *Journal of Controlled Release* **284**, 171-178 (2018).
- 1212 76. Ishida, T., Wang, X., Shimizu, T., Nawata, K. & Kiwada, H. PEGylated liposomes
1213 elicit an anti-PEG IgM response in a T cell-independent manner. *Journal of*
1214 *Controlled Release* **122**, 349-355 (2007).
- 1215 77. Yang, Q. et al. Analysis of Pre-existing IgG and IgM Antibodies against
1216 Polyethylene Glycol (PEG) in the General Population. *Anal Chem* **88**, 11804-
1217 11812 (2016).
- 1218 78. Koshy, S.T. et al. Click-Crosslinked Injectable Gelatin Hydrogels. *Advanced*
1219 *healthcare materials* **5**, 541-547 (2016).
- 1220 79. Shah, N.J. et al. An injectable bone marrow-like scaffold enhances T cell
1221 immunity after hematopoietic stem cell transplantation. *Nat Biotechnol* **37**, 293-
1222 302 (2019).
- 1223 80. Desai, R.M., Koshy, S.T., Hilderbrand, S.A., Mooney, D.J. & Joshi, N.S. Versatile
1224 click alginate hydrogels crosslinked via tetrazine-norbornene chemistry.
1225 *Biomaterials* **50**, 30-37 (2015).
- 1226 81. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis.
1227 *Nature Methods* **9**, 676-682 (2012).
- 1228 82. Stackowicz, J., Jönsson, F. & Reber, L.L. Mouse models and tools for the in vivo
1229 study of neutrophils. *Frontiers in immunology* **10**, 3130 (2020).

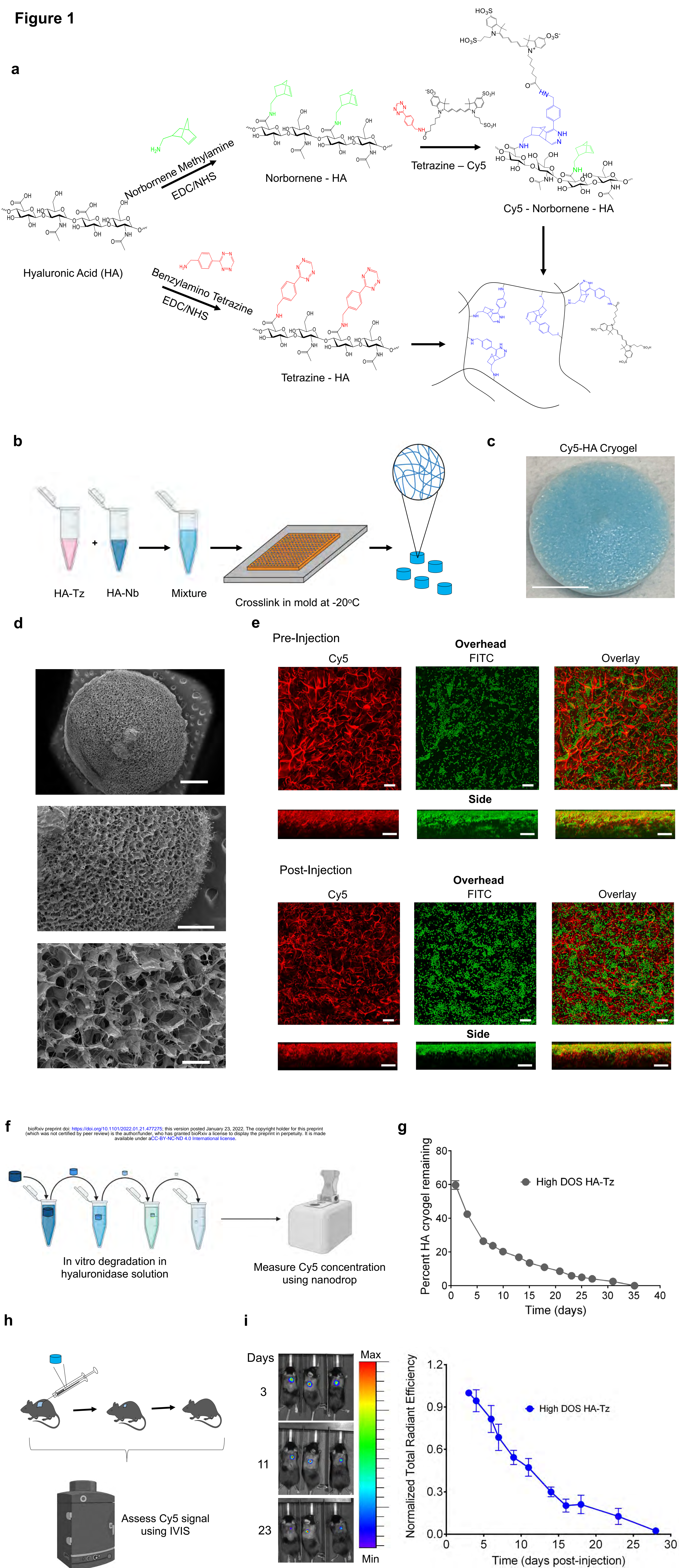
Figure 1

Figure 2

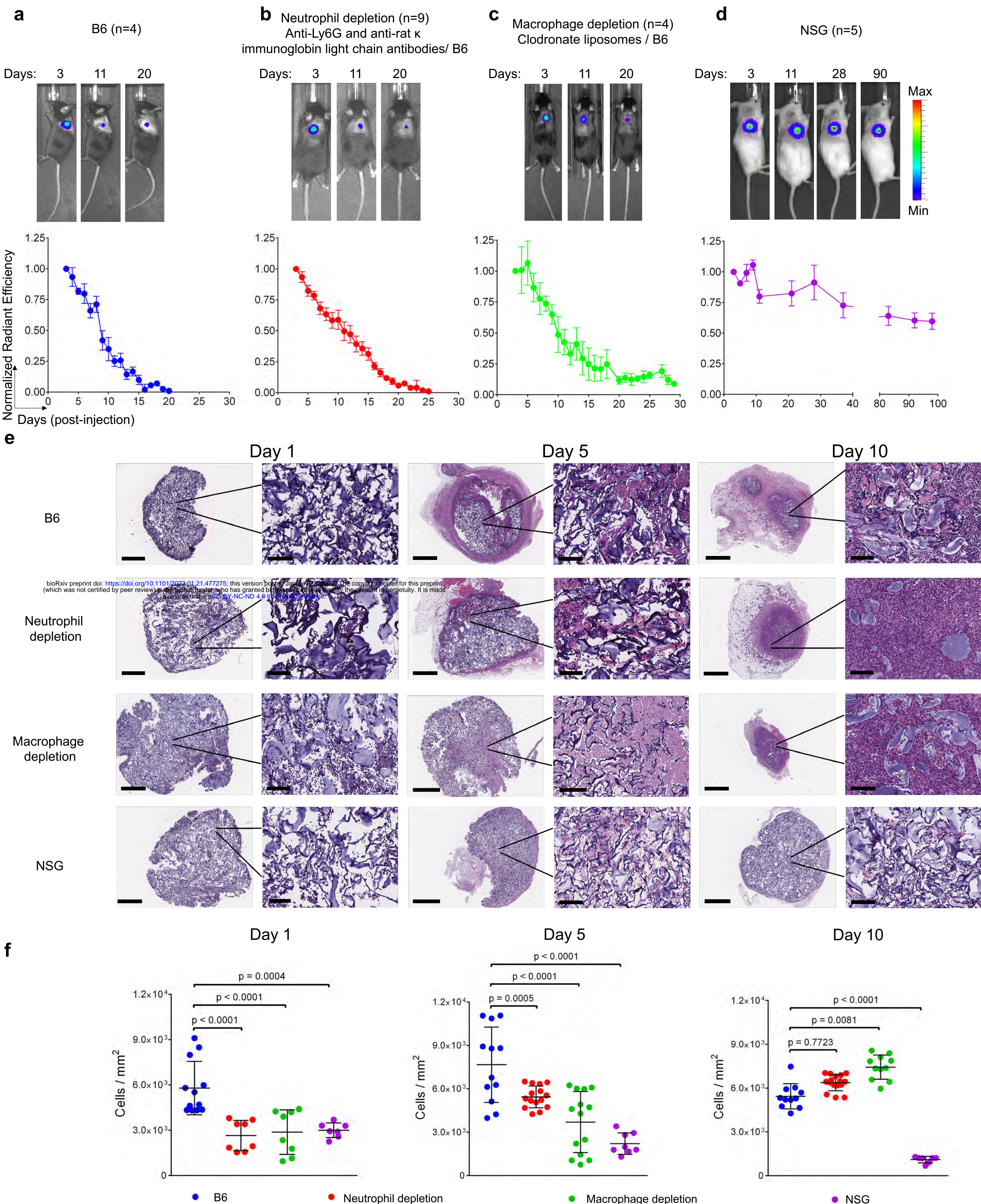
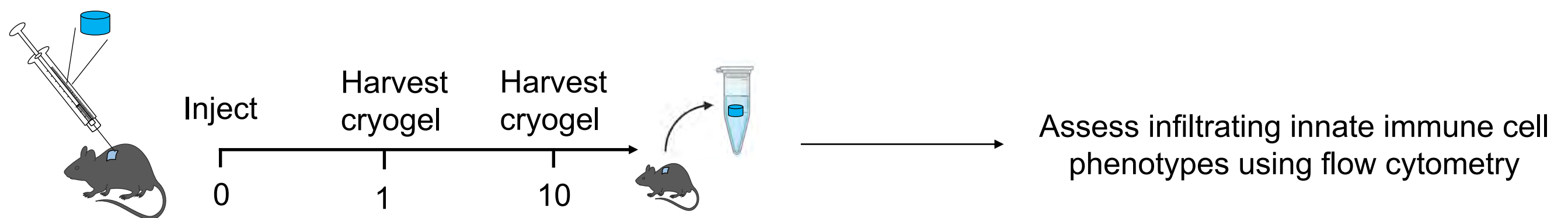
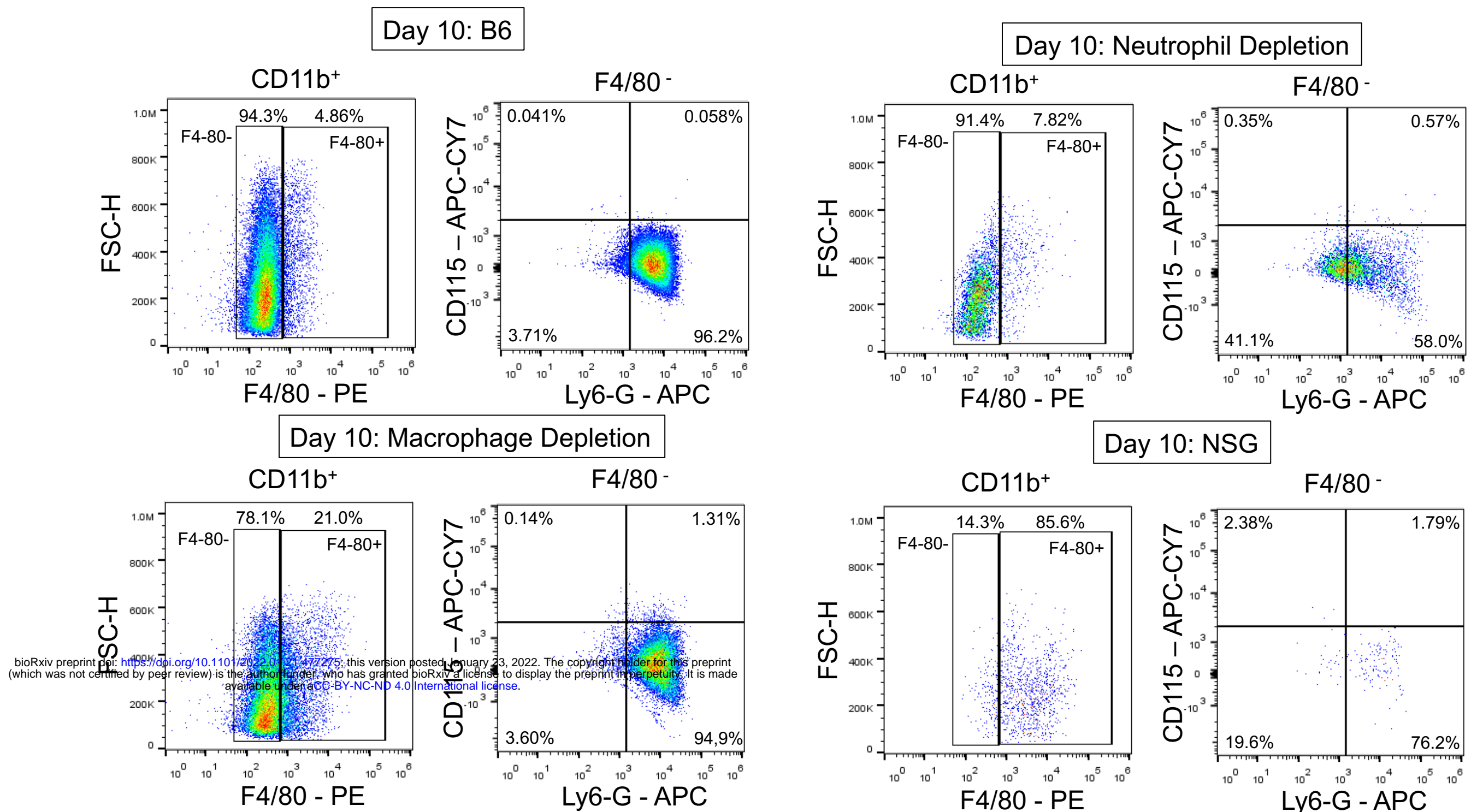
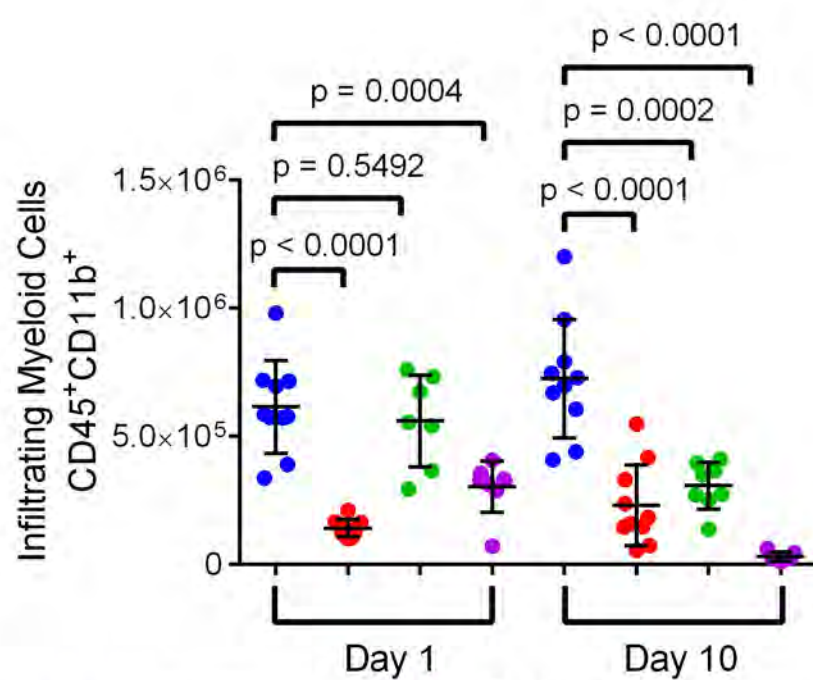


Figure 3**a****b****c**

● B6 (Day 1: n=10, Day 10: n=10)

● Neutrophil Depletion
(Day 1: n=10, Day 10: n=10)● Macrophage Depletion
(Day 1: n=7, Day 10: n=10)

● NSG (Day 1: n=8, Day 10: n=7)

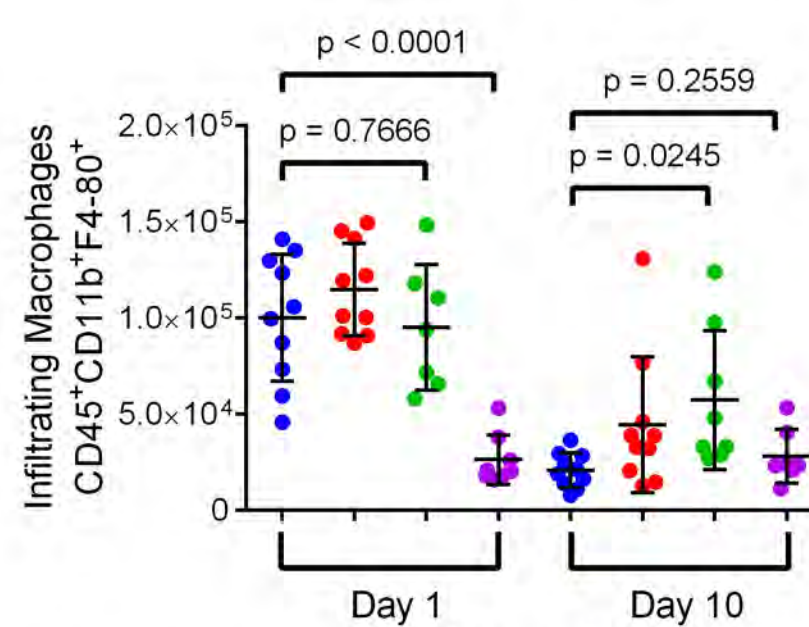
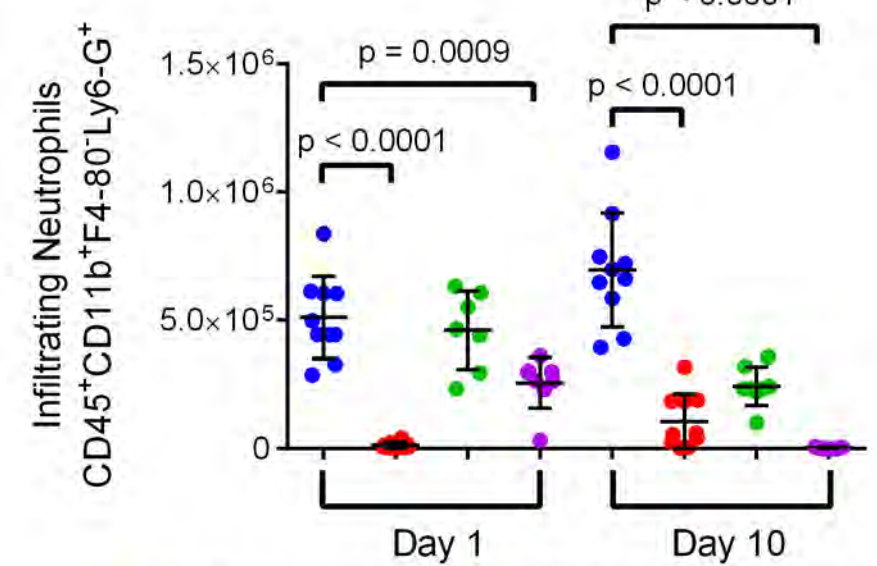
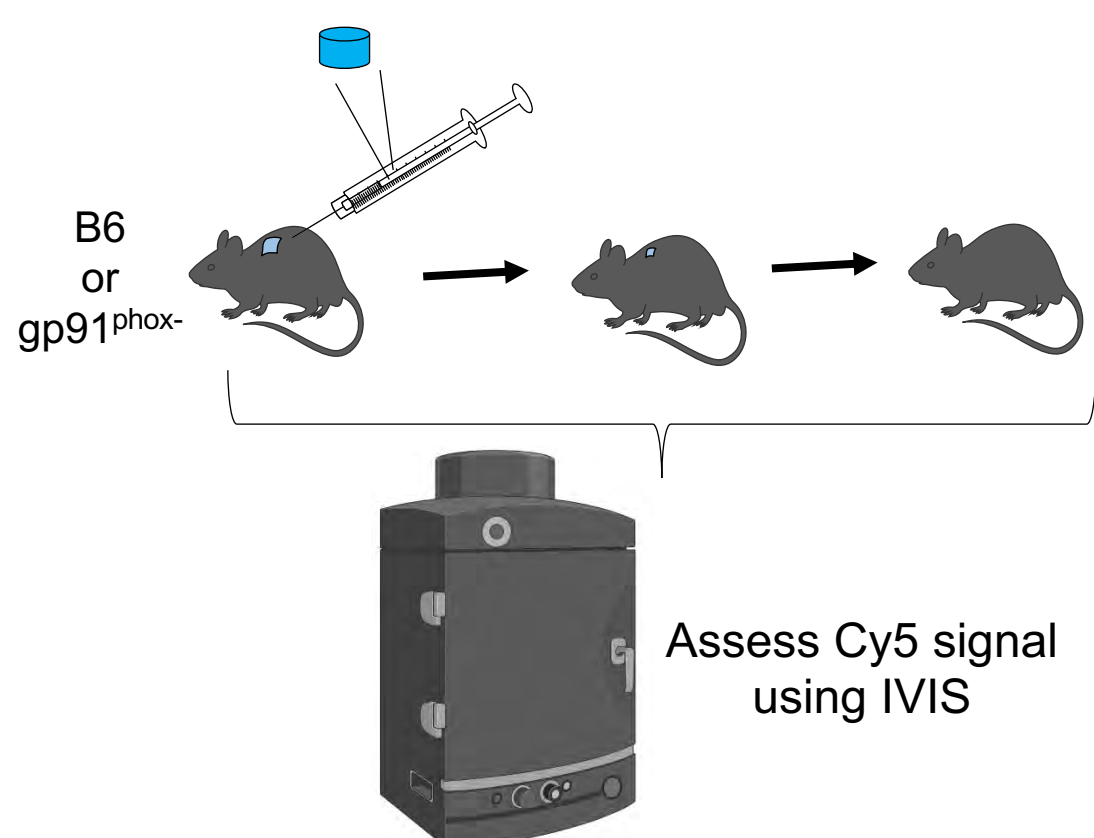
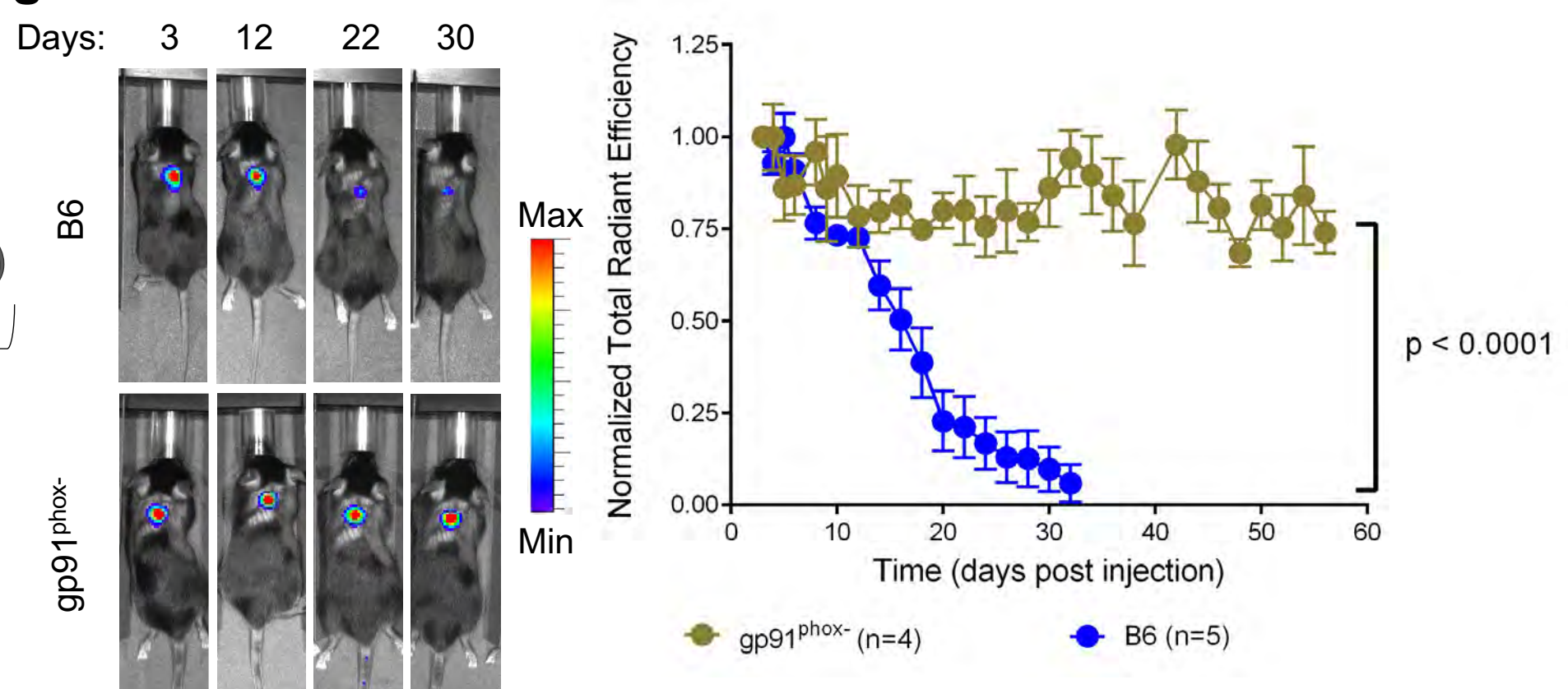
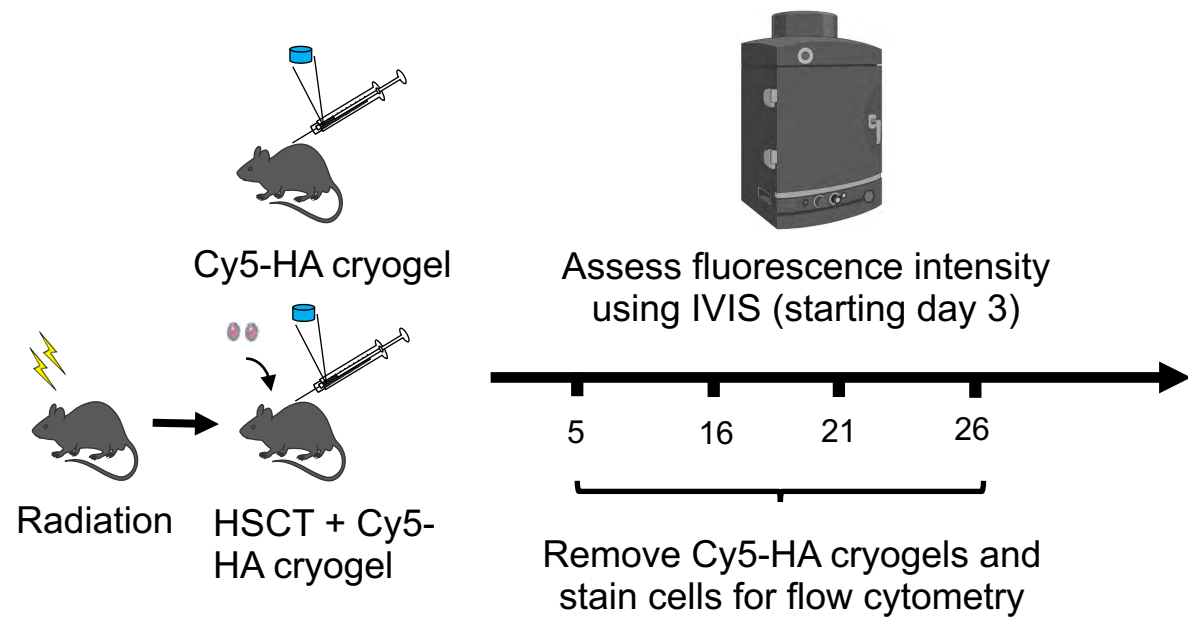
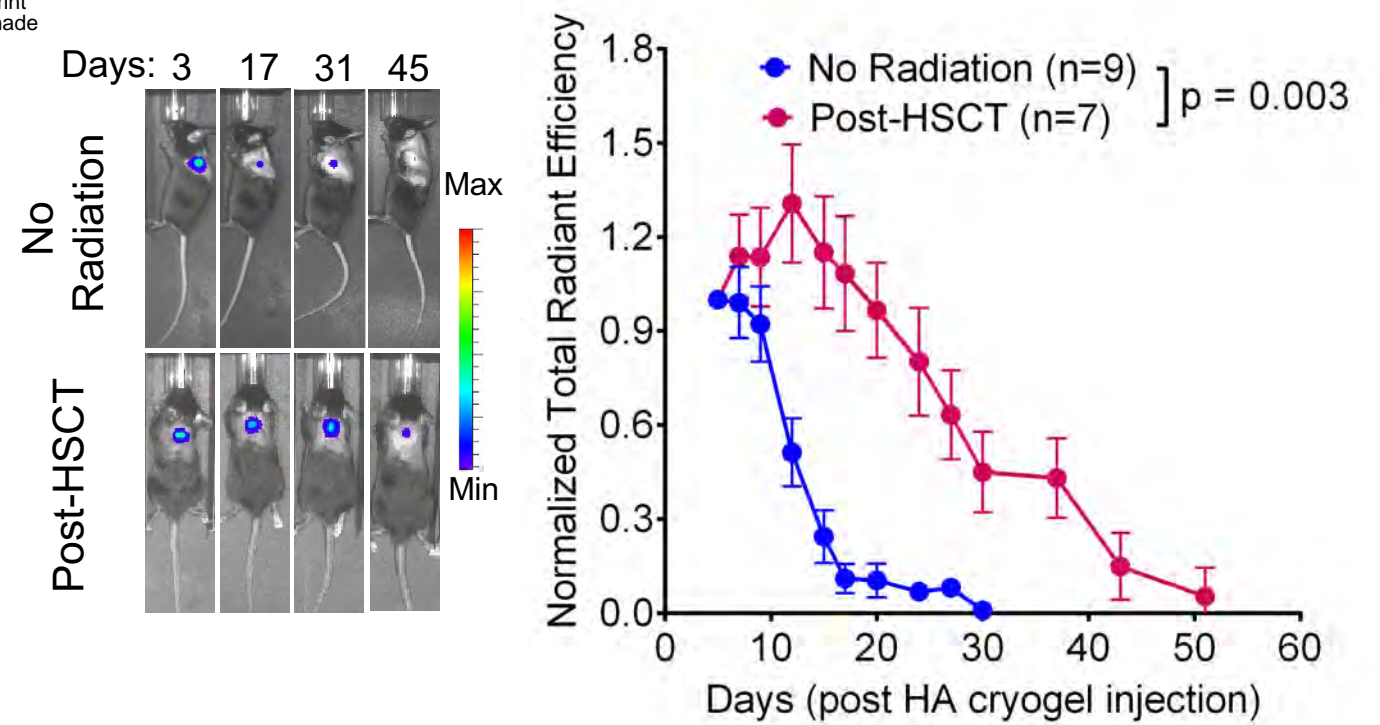
d**e****f****g**

Figure 4

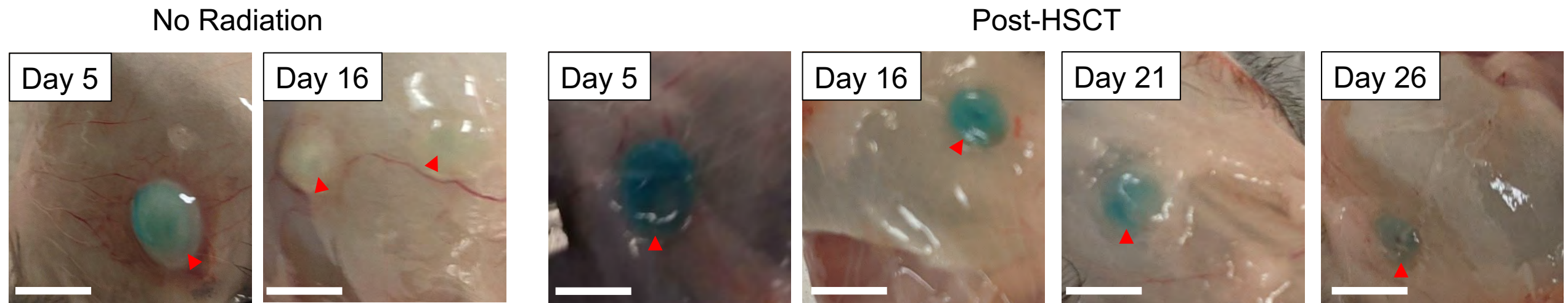
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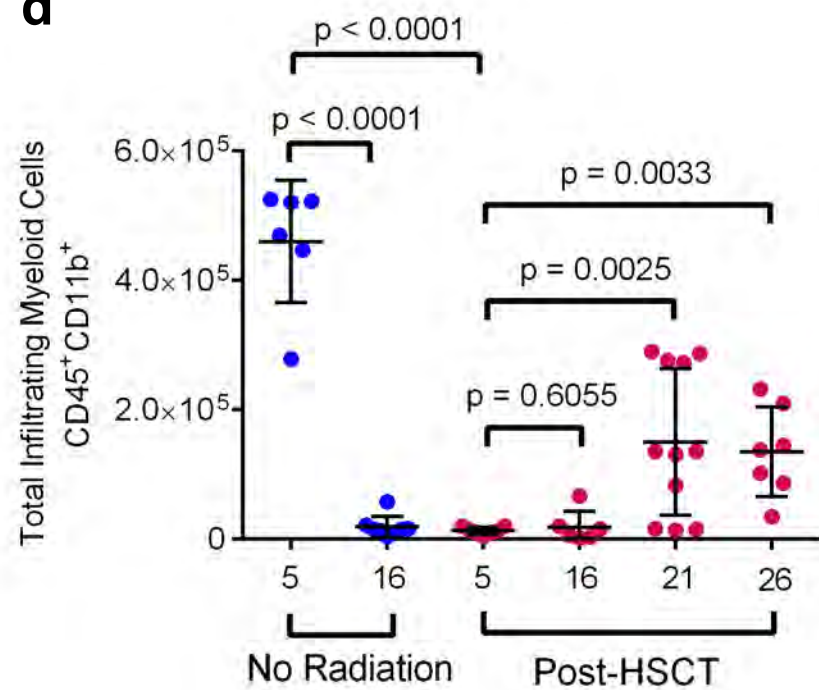
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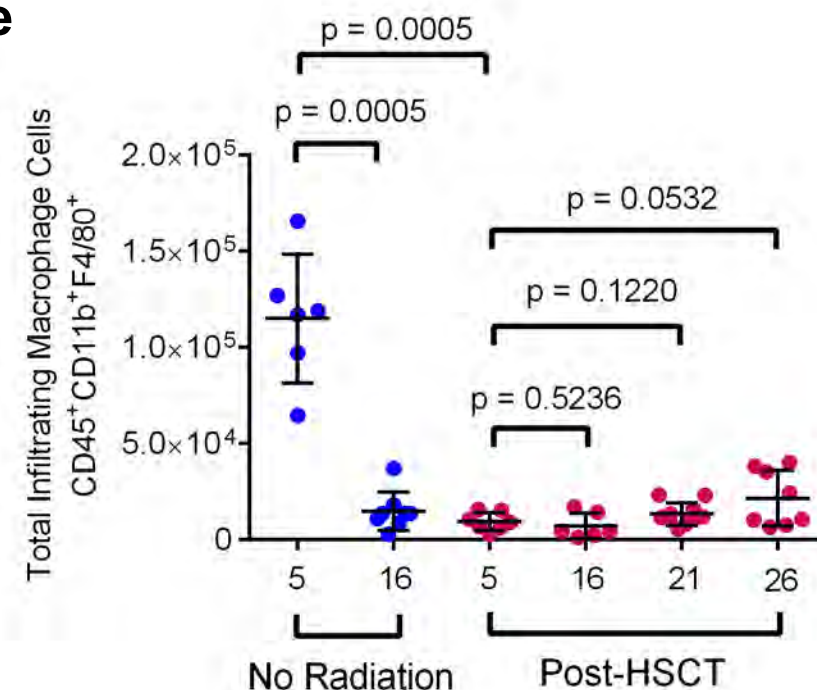
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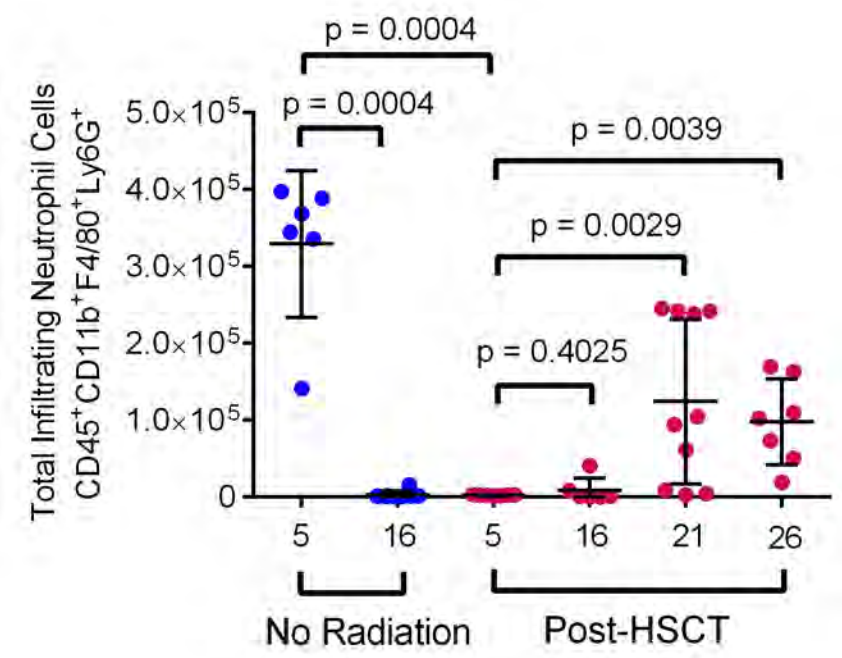
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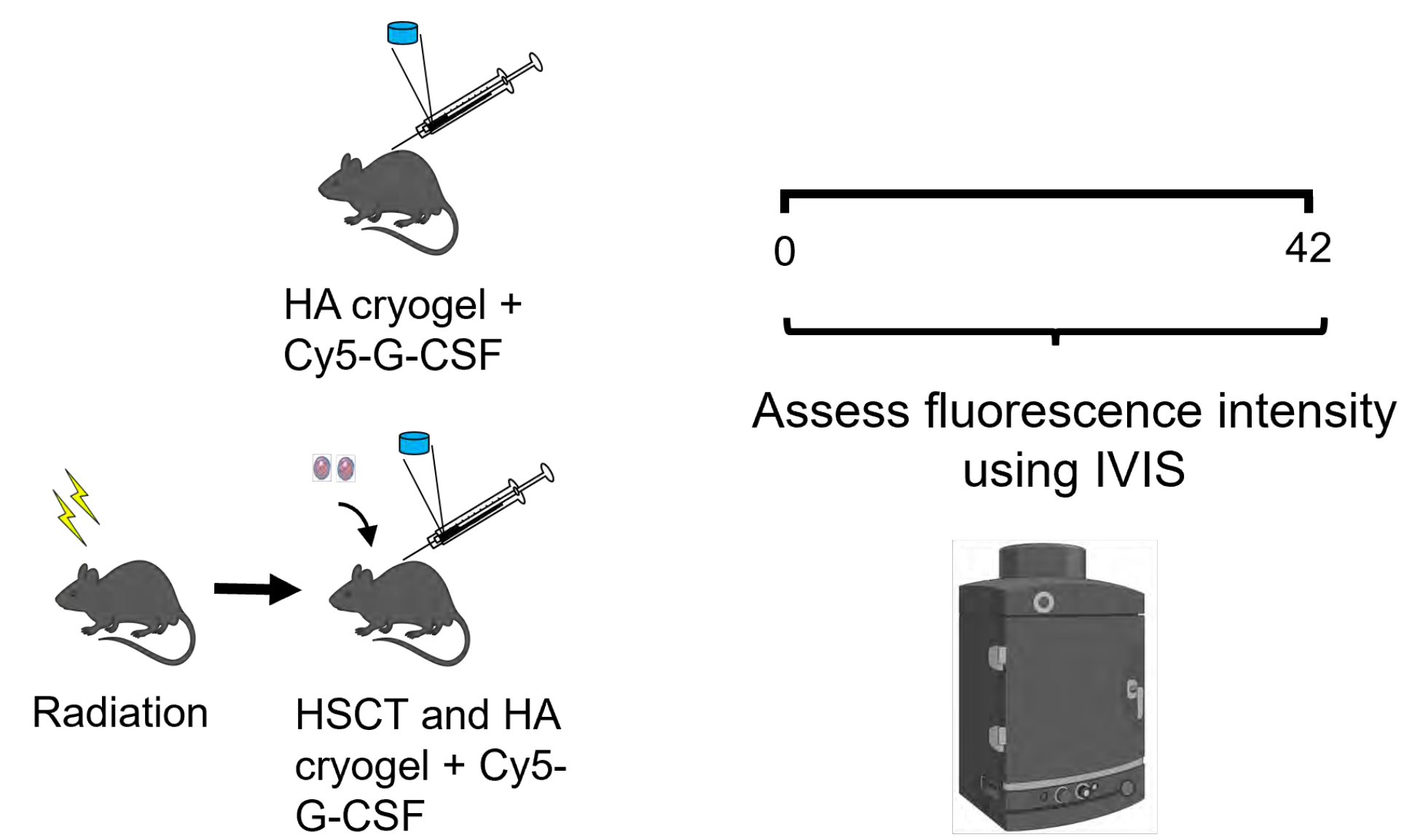
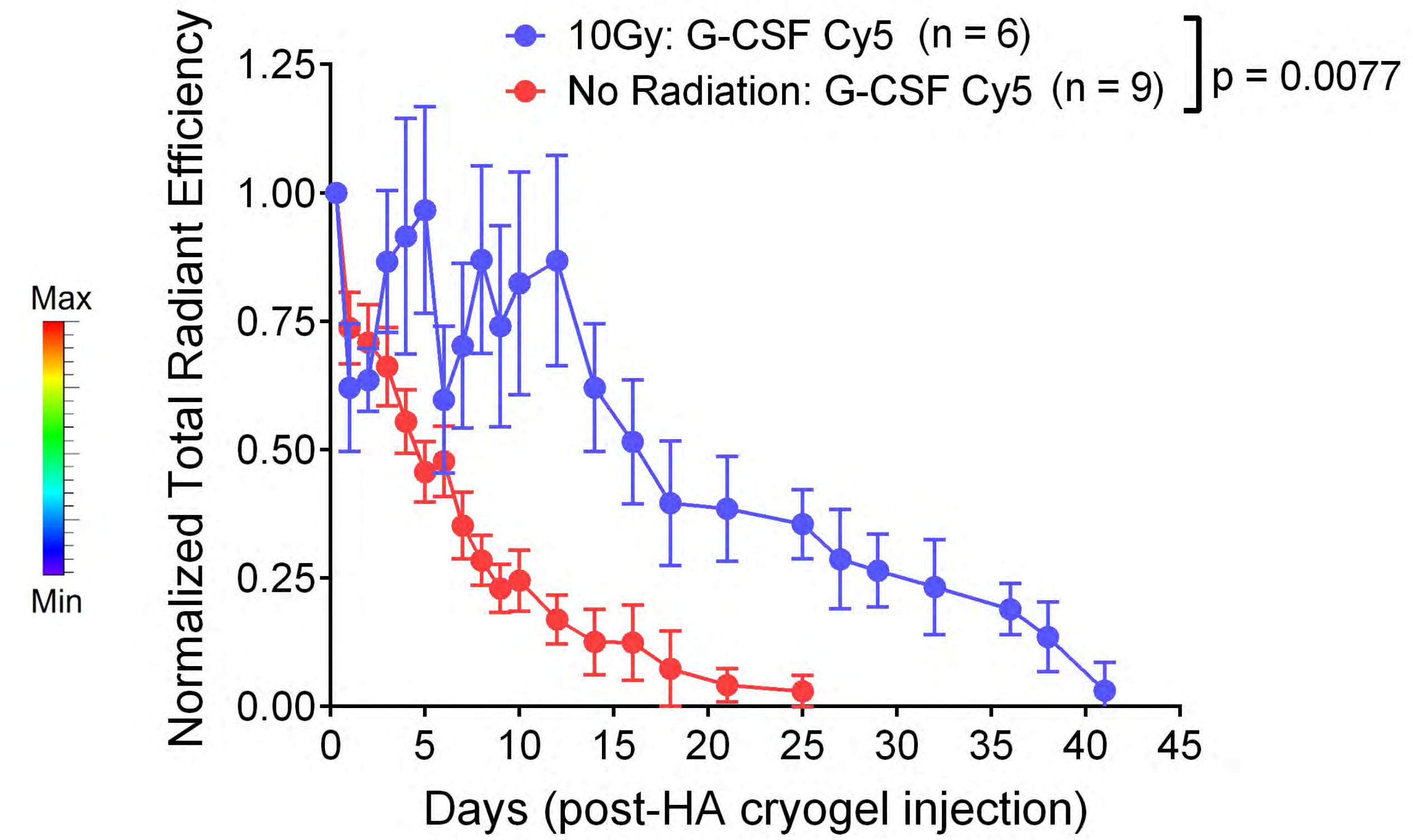
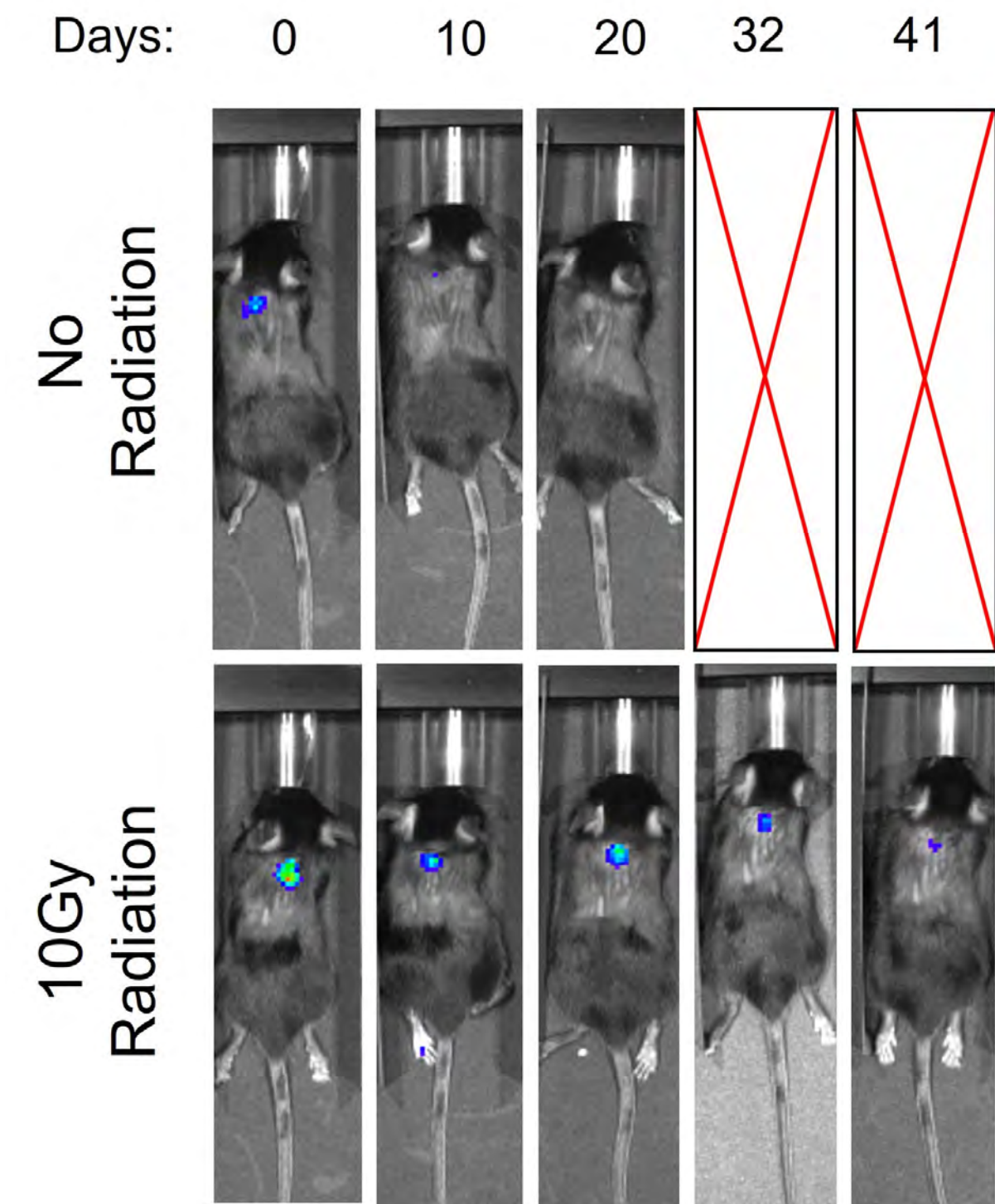
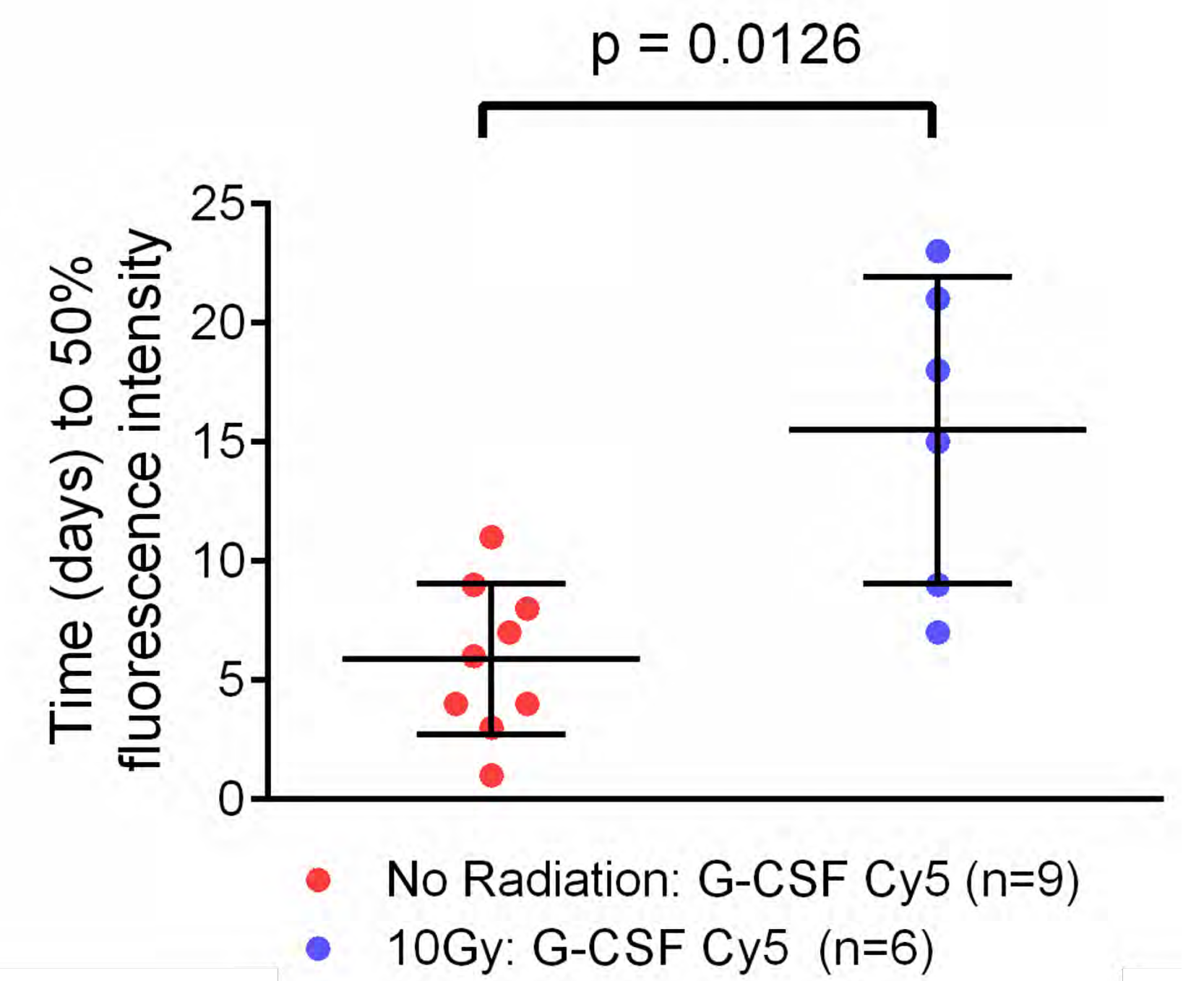


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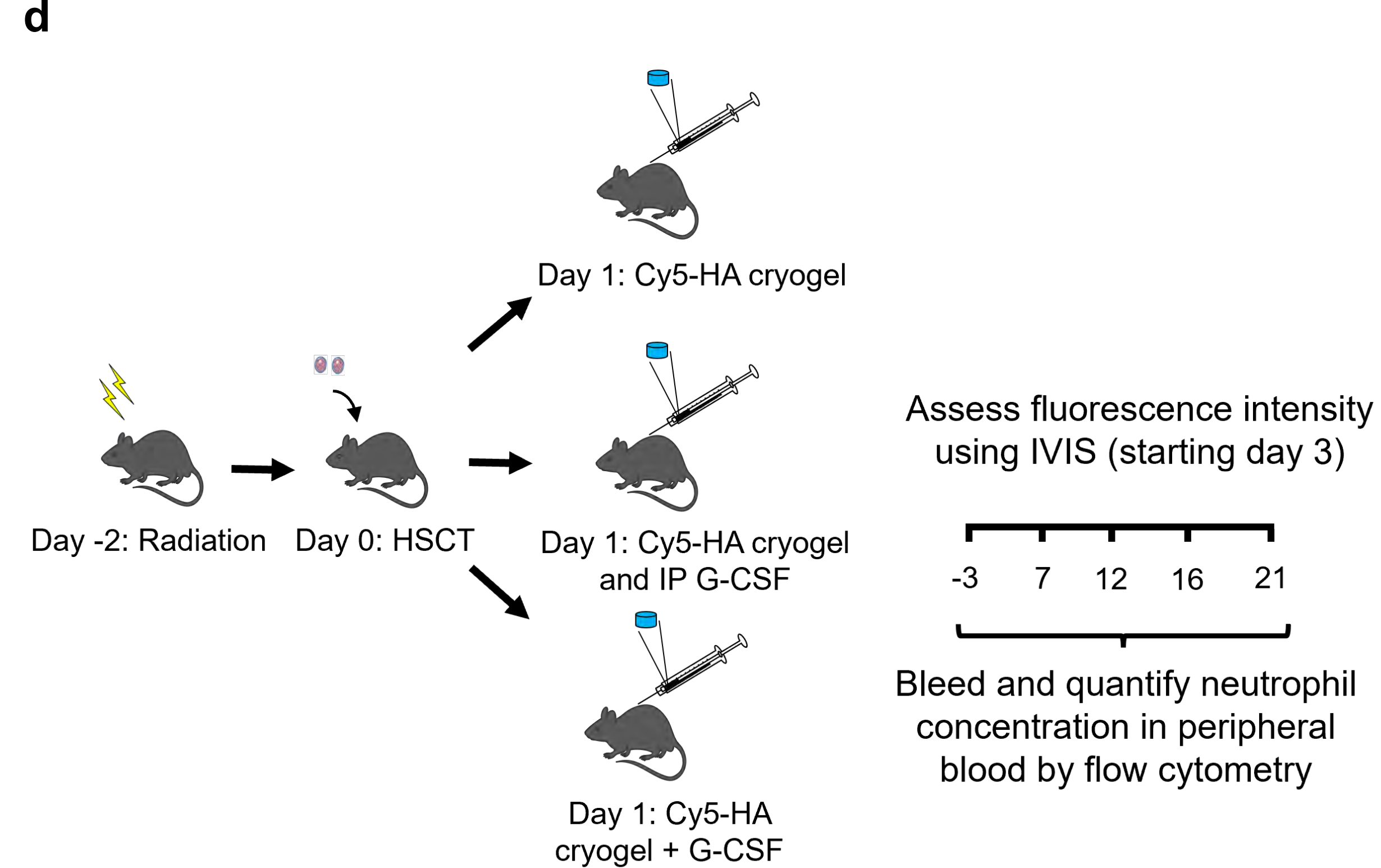
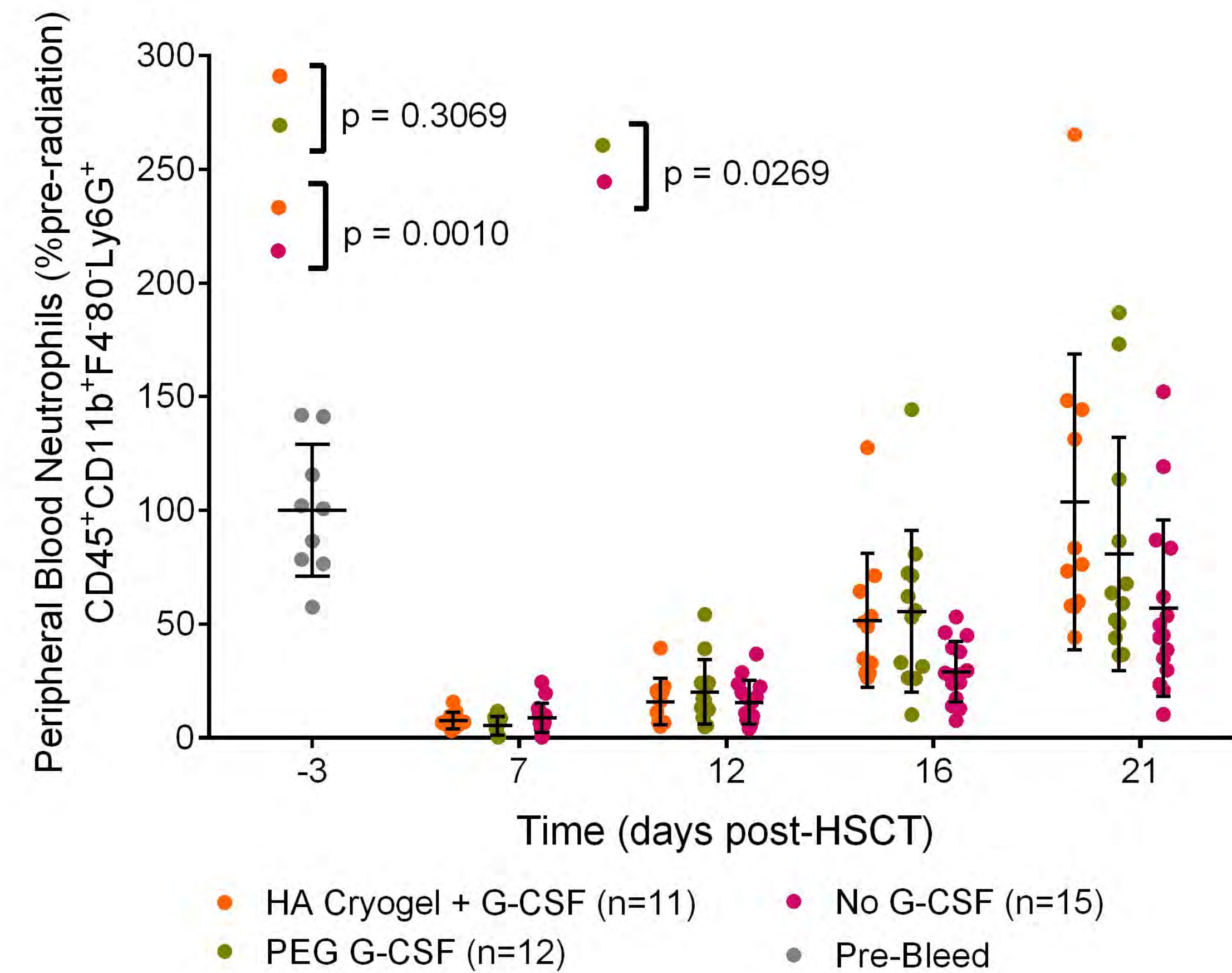
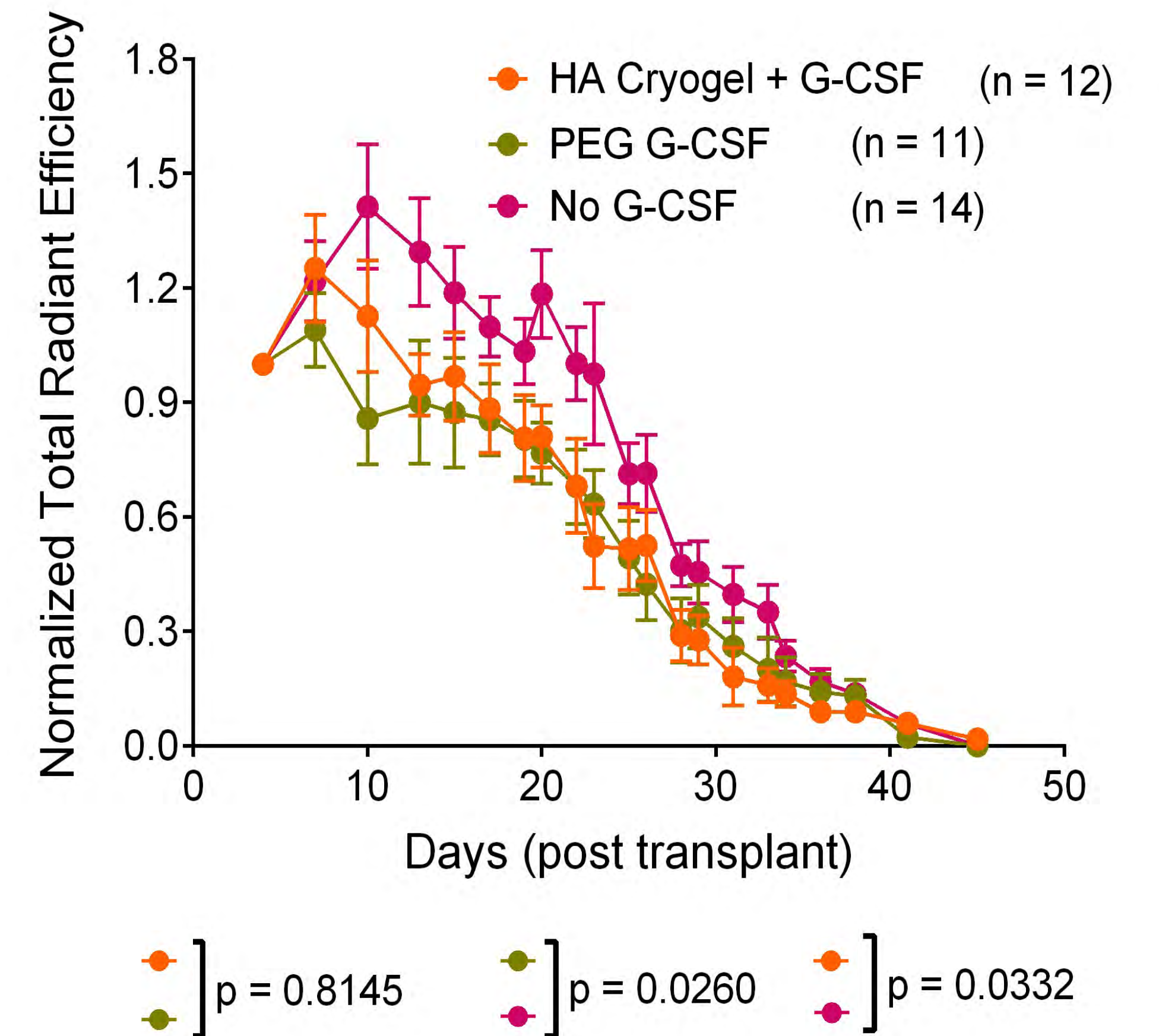
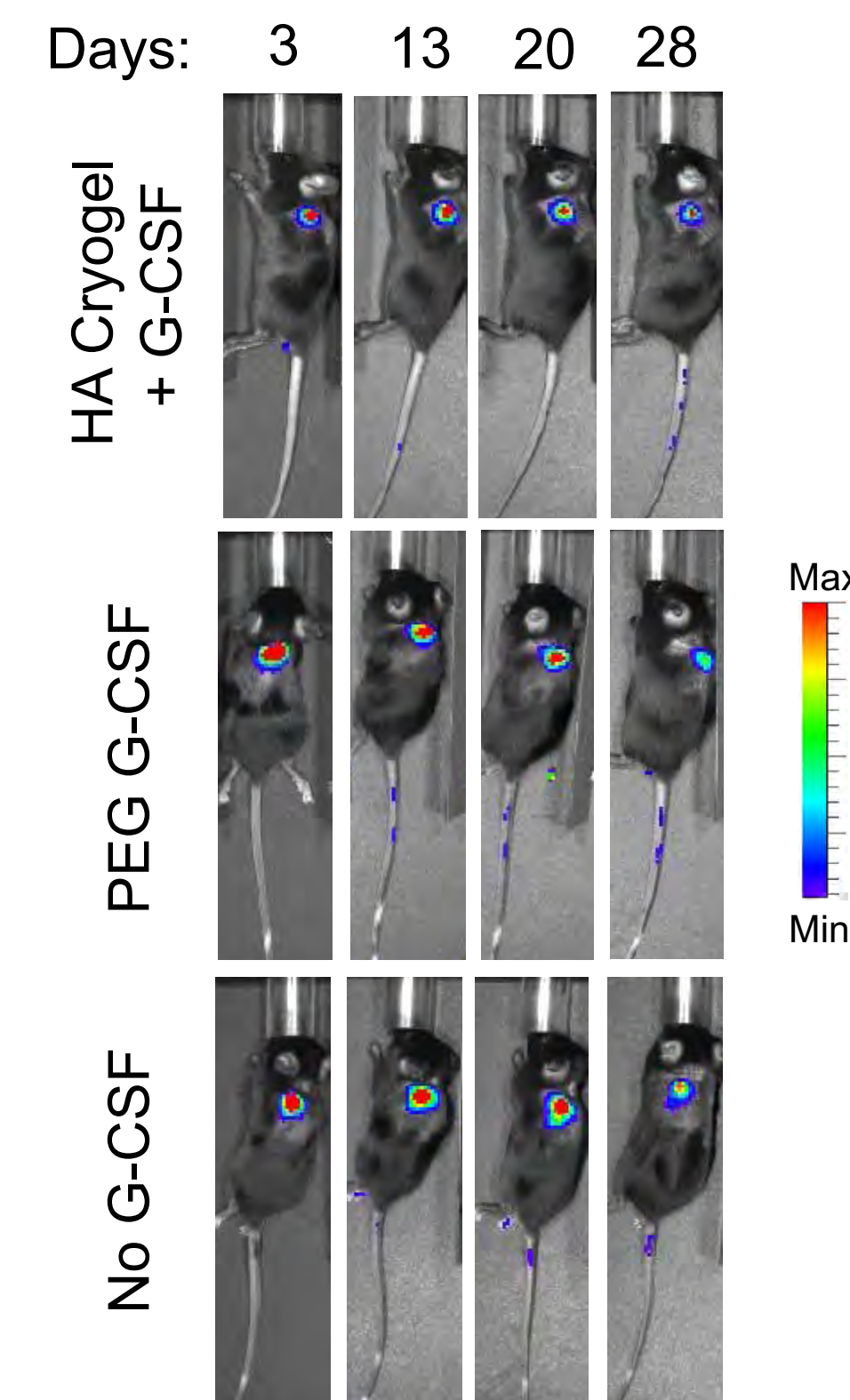


● No Radiation (Day 5: n=6, Day 16: n=8)

● Post-HSCT (Day 5: n=7, Day 16: n=6, Day 21: n=10, Day 26: n=7)

Figure 5**a****b****c****d**

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**e****f**

Supplementary Table 1

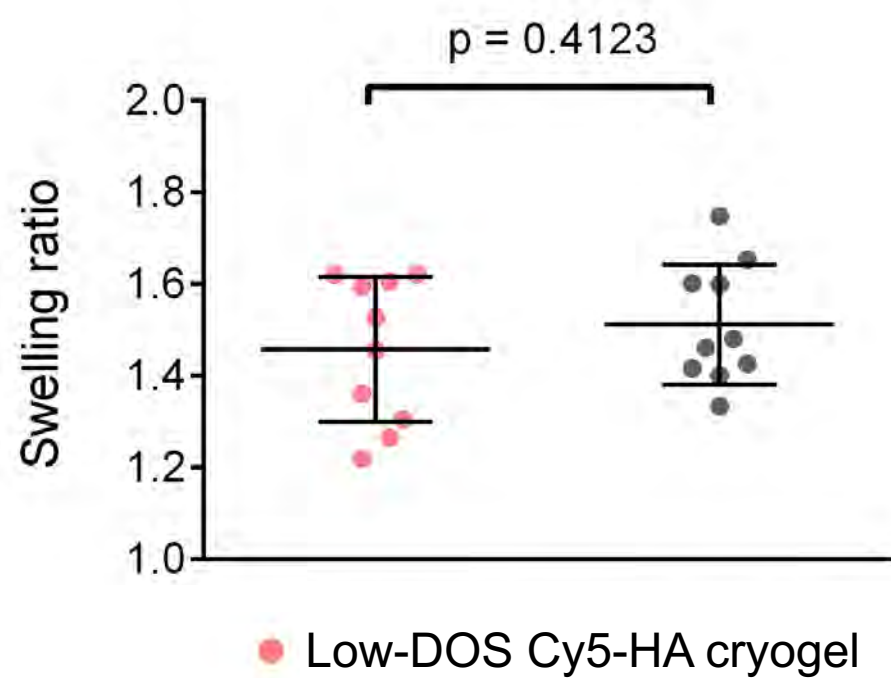
Material	Sample 1 (EU/mL)	Sample 2 (EU/mL)	Sample 3 (EU/mL)
HA-Tz	0.332	0.367	0.362
Cy5-HA-Nb	0.227	0.23	0.229
HA Cryogel Average Endotoxin Content	0.00874 EU		
EU/kg (2 HA cryogels/mouse)	0.874 EU/kg		

Supplementary Table 2

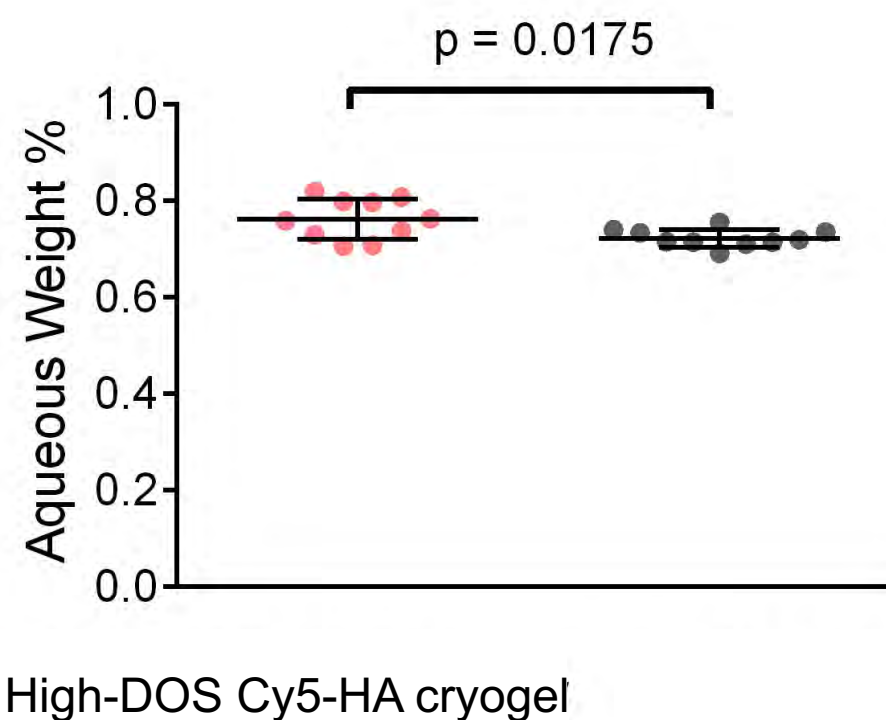
Depletion Type	Depletion Agent	Dose, Administration Route, Frequency	Depletion Efficiency
Neutrophil Depletion	Anti-mouse Ly6G antibody (1A8, Bio X Cell) Anti-rat kappa immunoglobulin light chain antibody (MAR 18.5, Bio X Cell)	25µL anti-mouse Ly6G - IP administration - Everyday for 1 week 50µL anti-mouse Ly6G - IP administration - Everyday after first week 50µL anti-rat kappa immunoglobulin light chain - IP administration - Every other day	Consistent - 98% Depletion of neutrophils in peripheral blood (Supplemental 5b,5c)
Macrophage/Monocyte Depletion	Clodronate Liposomes (Liposoma)	100µL - IP administration - 2x/week	Consistent - 80-95% Depletion of monocytes in peripheral blood (Supplemental 5d, 5e)
T-cell Depletion	Anti-mouse CD4 antibody (GK1.5, Bio X Cell) Anti-mouse CD8α antibody (2.43, Bio X Cell)	400µg anti-mouse CD4 - IP administration - 2x/week 400µg anti-mouse CD8α - IP administration - 2x/week	Consistent - 99% Depletion of T-cells in peripheral blood (Supplemental 5f, 5g)
B-cell Depletion	Anti-mouse B220/CD45R antibody (RA3.31, Bio X Cell)	400µg anti-mouse B220/CD45R - IP administration - 2x/week	Transient - 99% Depletion of B-cells in peripheral blood 4 days after start of depletion. Full reconstitution of B-cells by 3 weeks. (Supplemental 5h, 5i)

Supplementary Figure 1

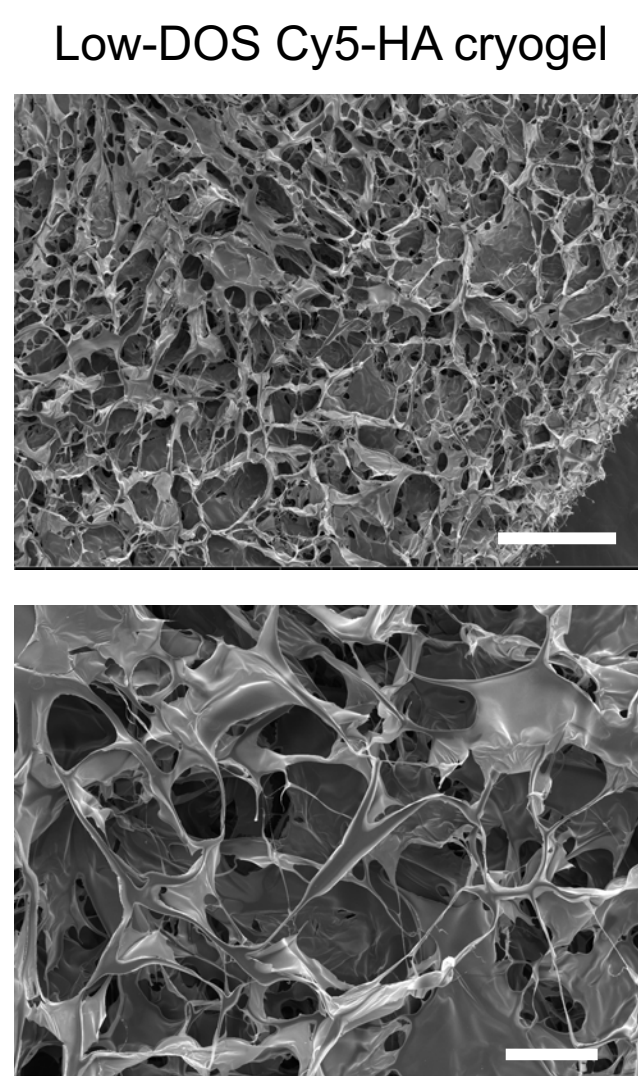
S1a



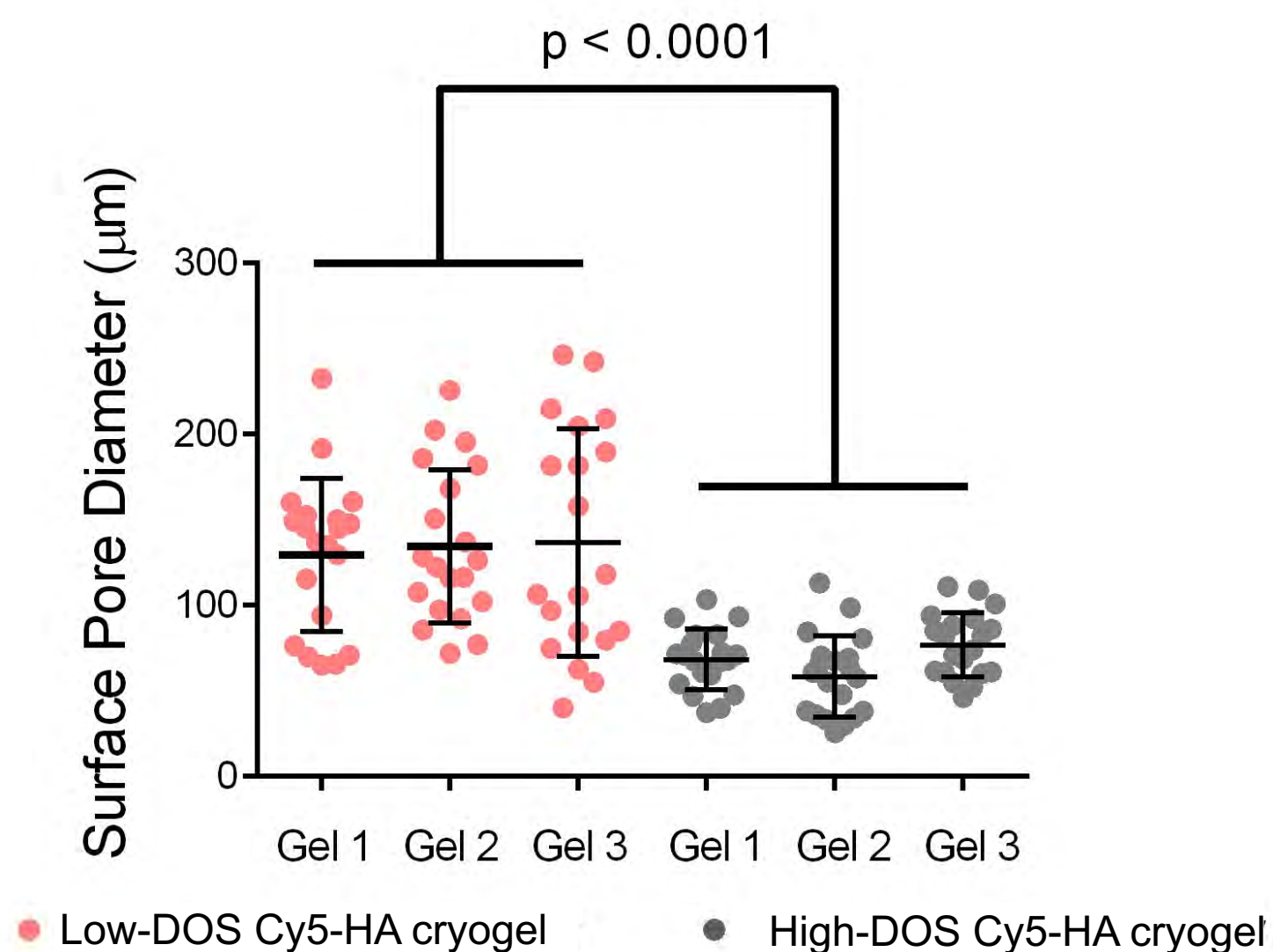
S1b



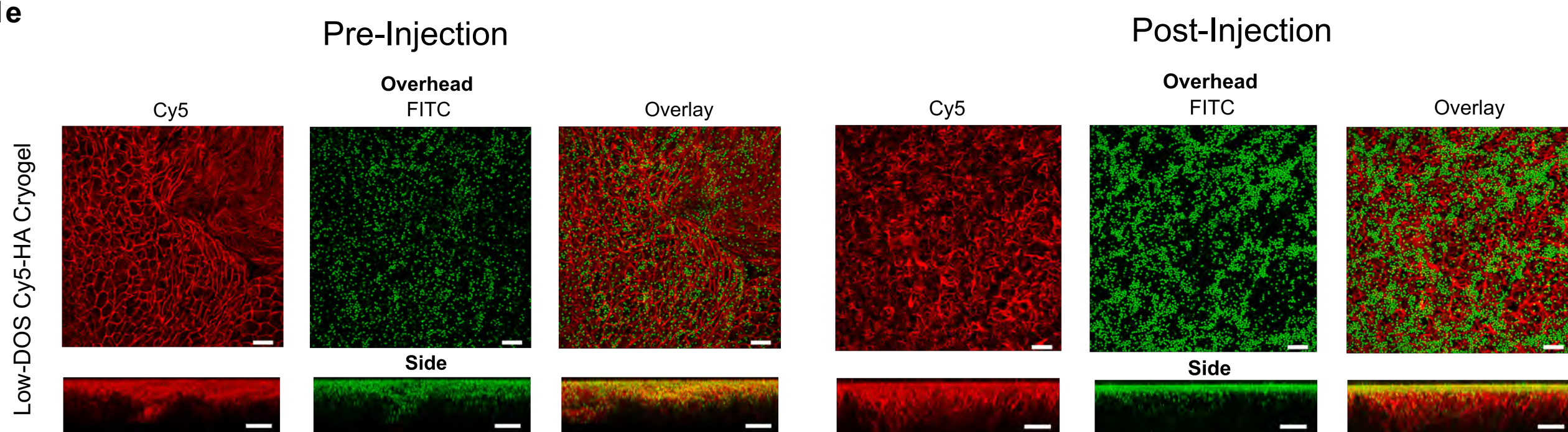
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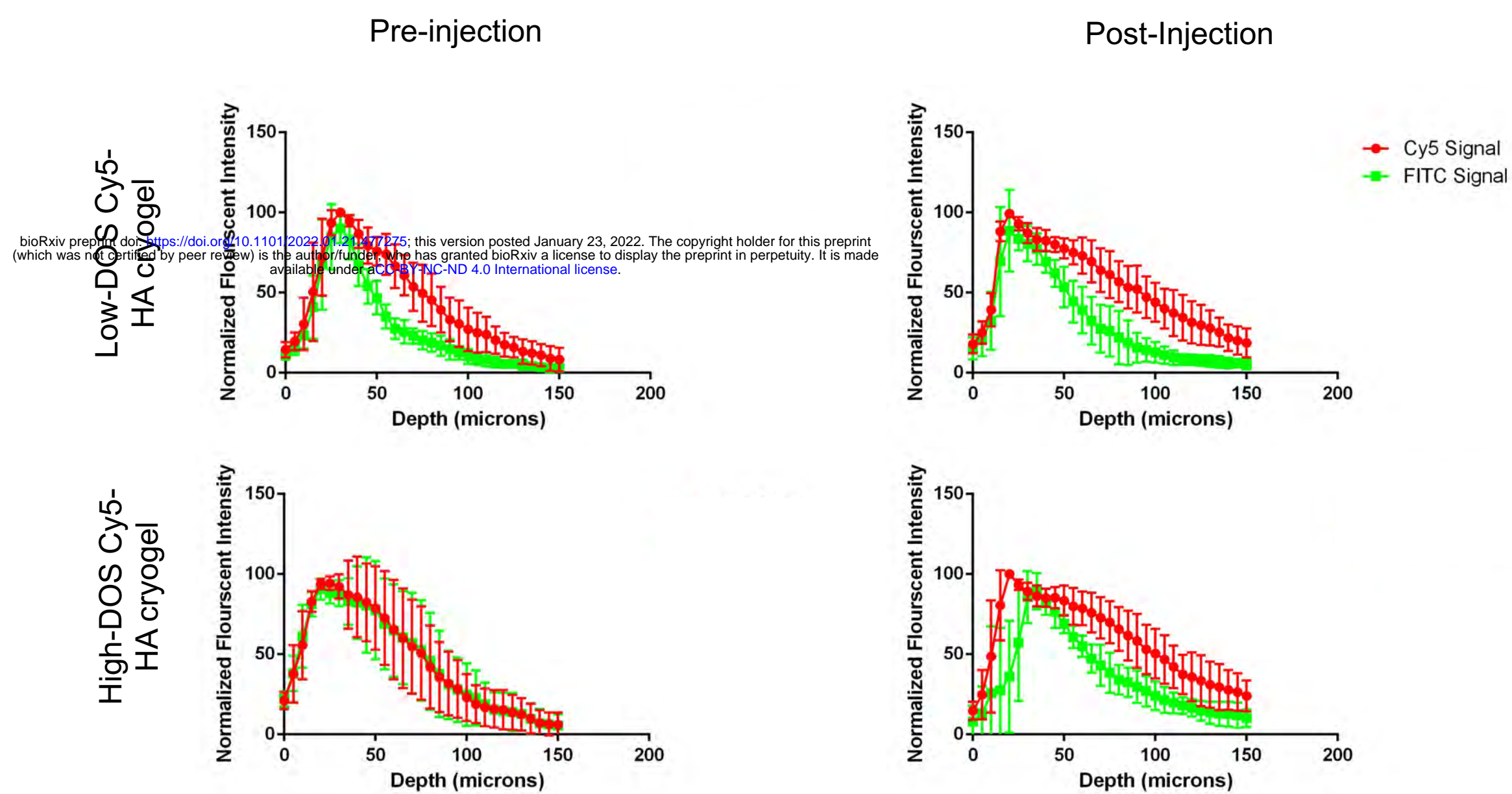
S1d



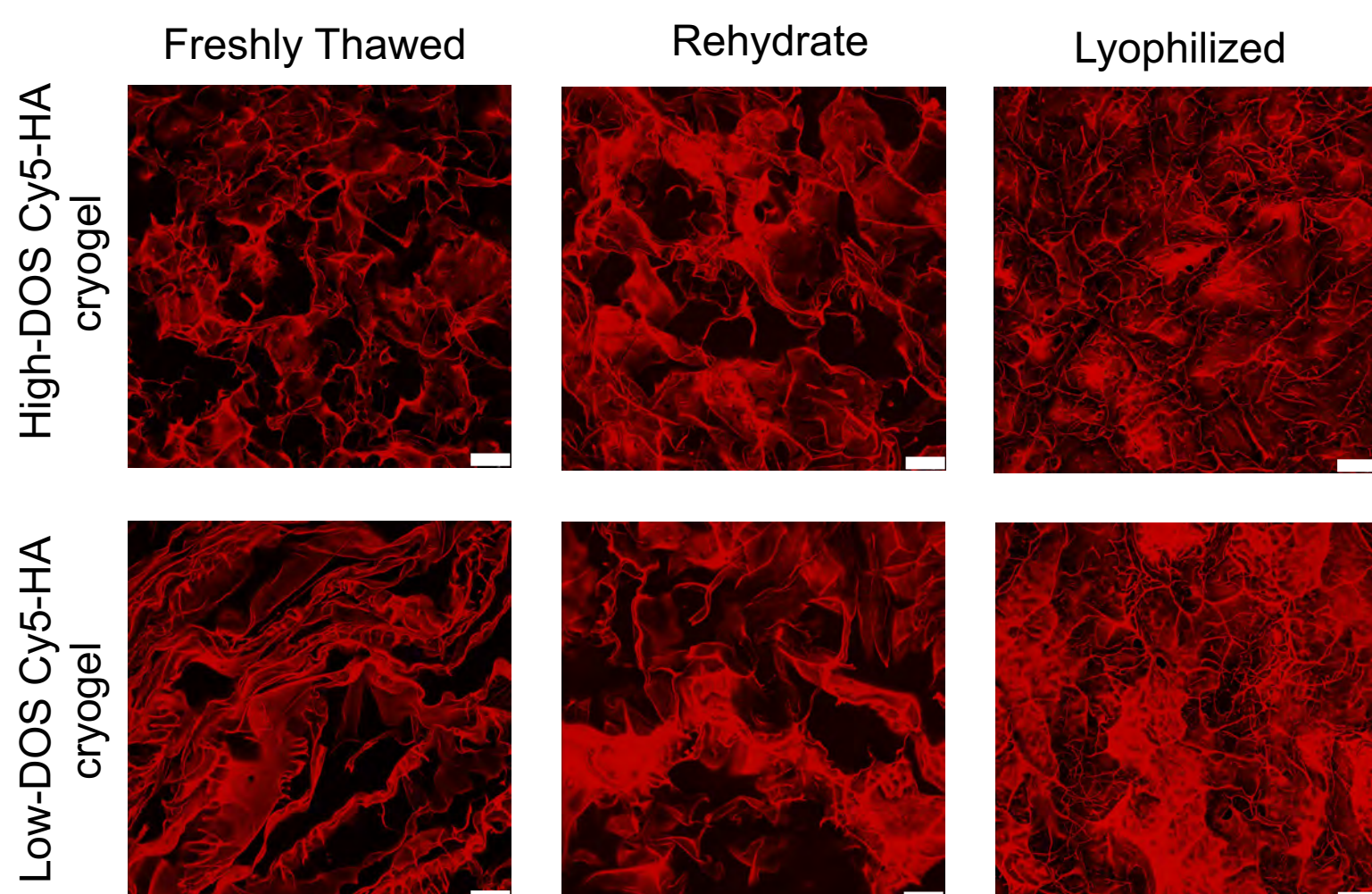
S1e



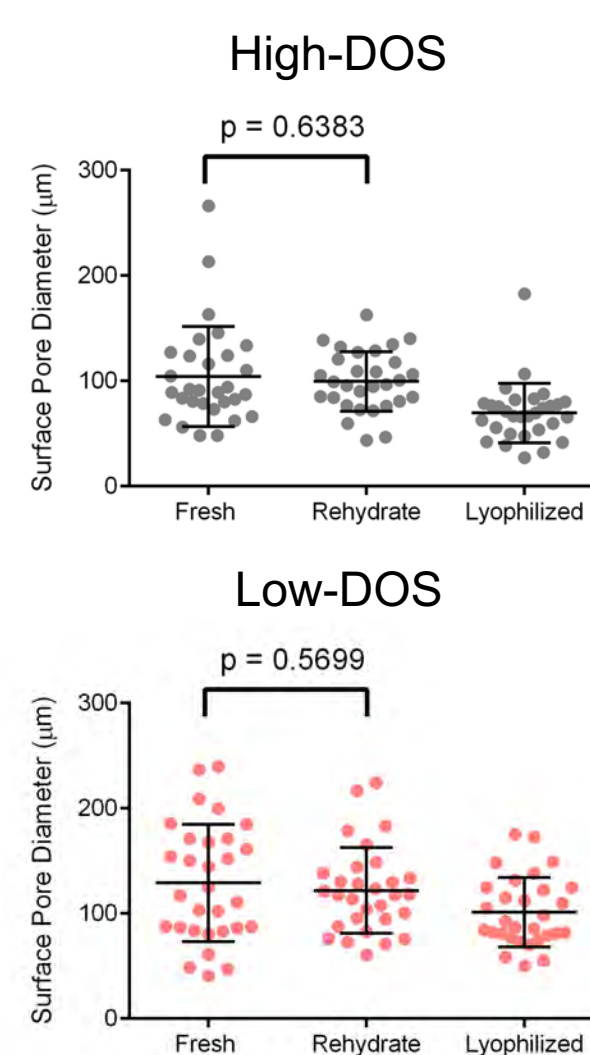
S1f



S1g



S1h



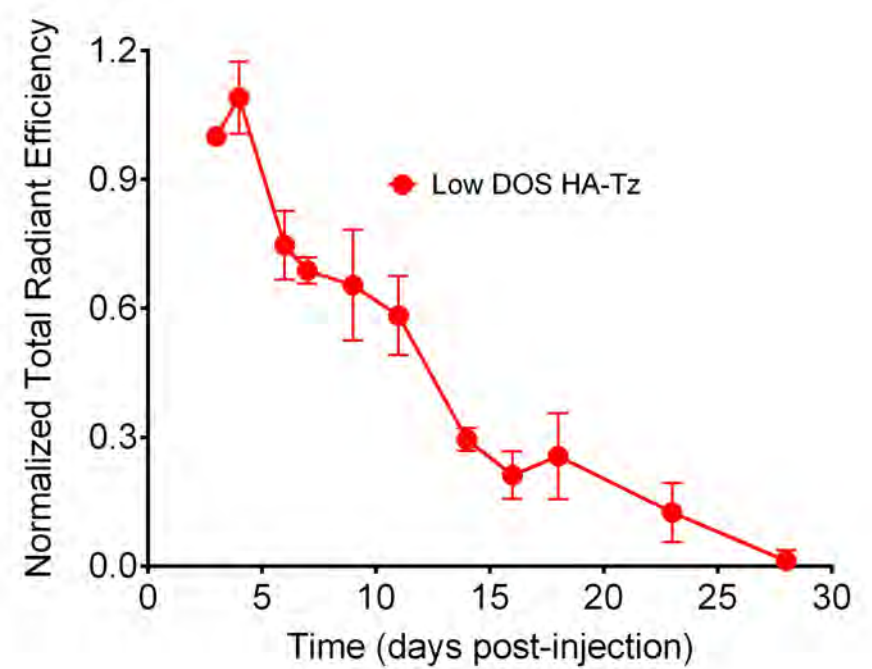
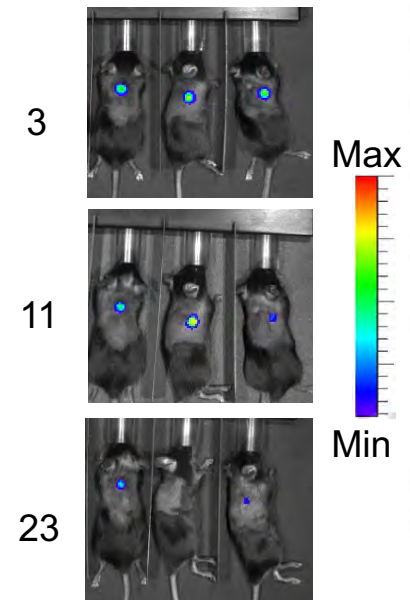
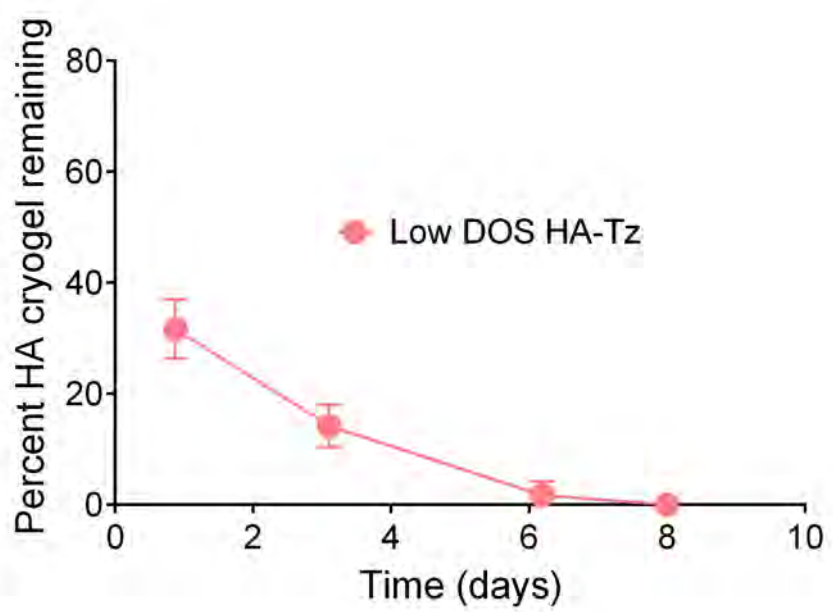
Supplementary Figure 2

S2a **S2b** **Low DOS**

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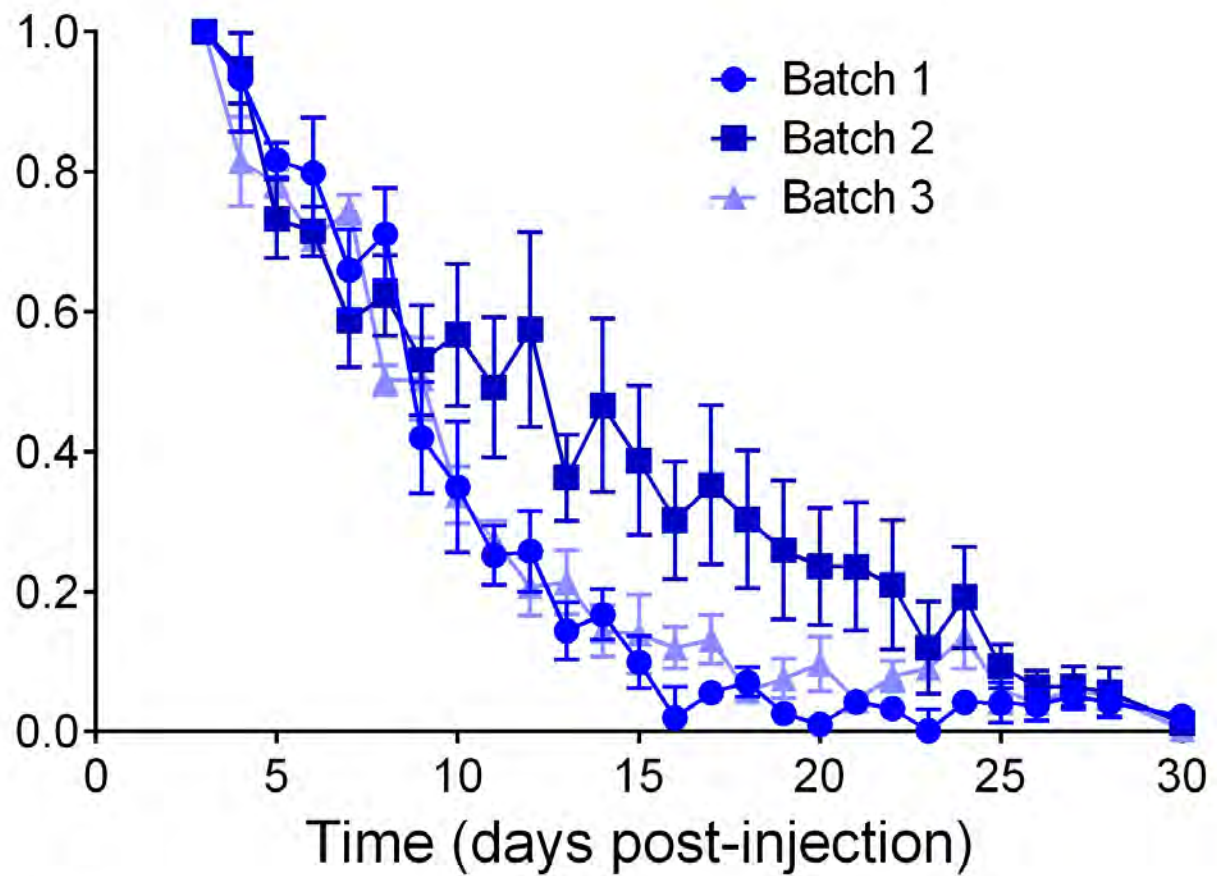
In vitro Cy5-HA cryogel degradation

Days: HA Cryogel



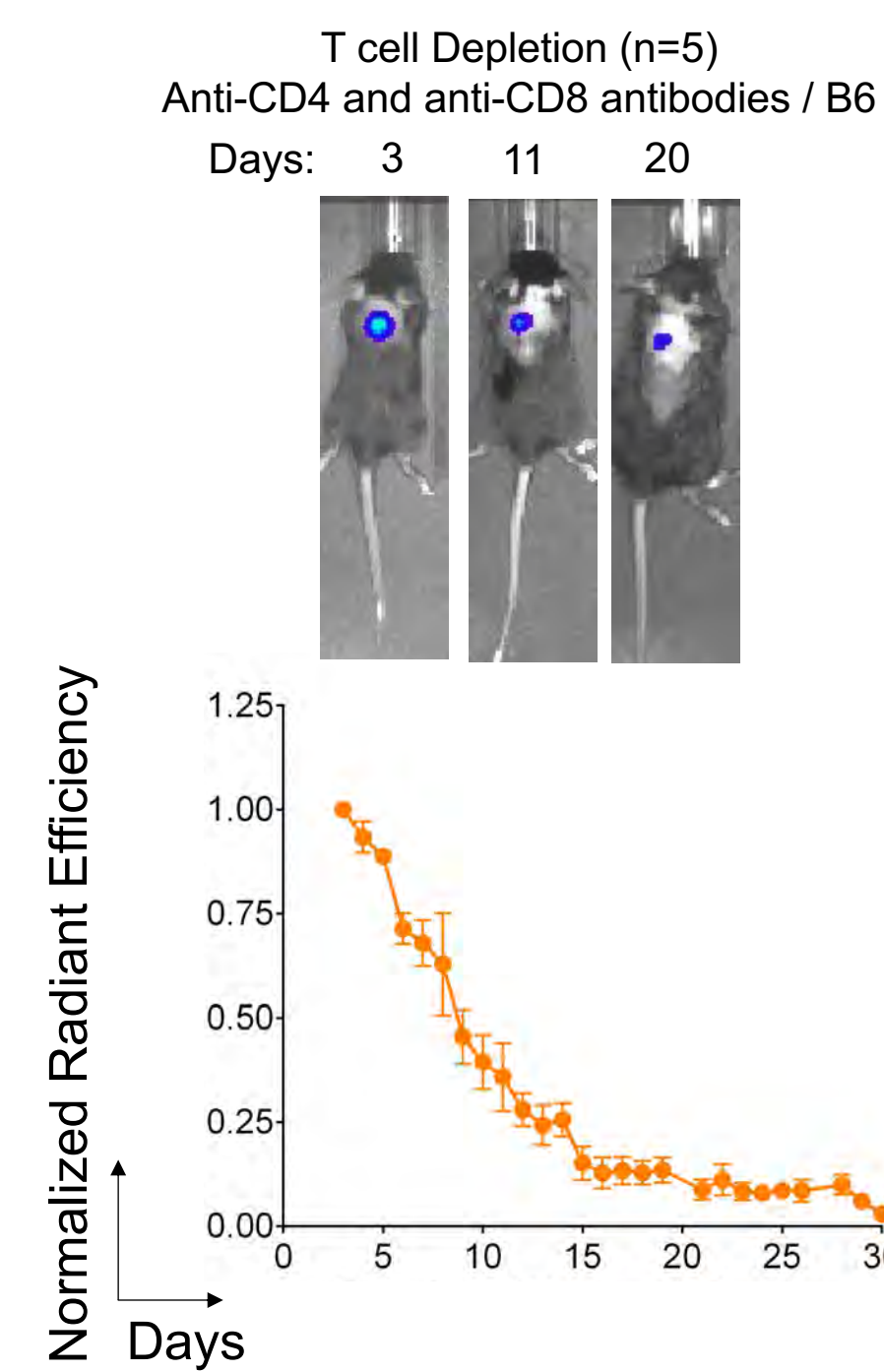
S2c

Normalized Total Radiant Efficiency

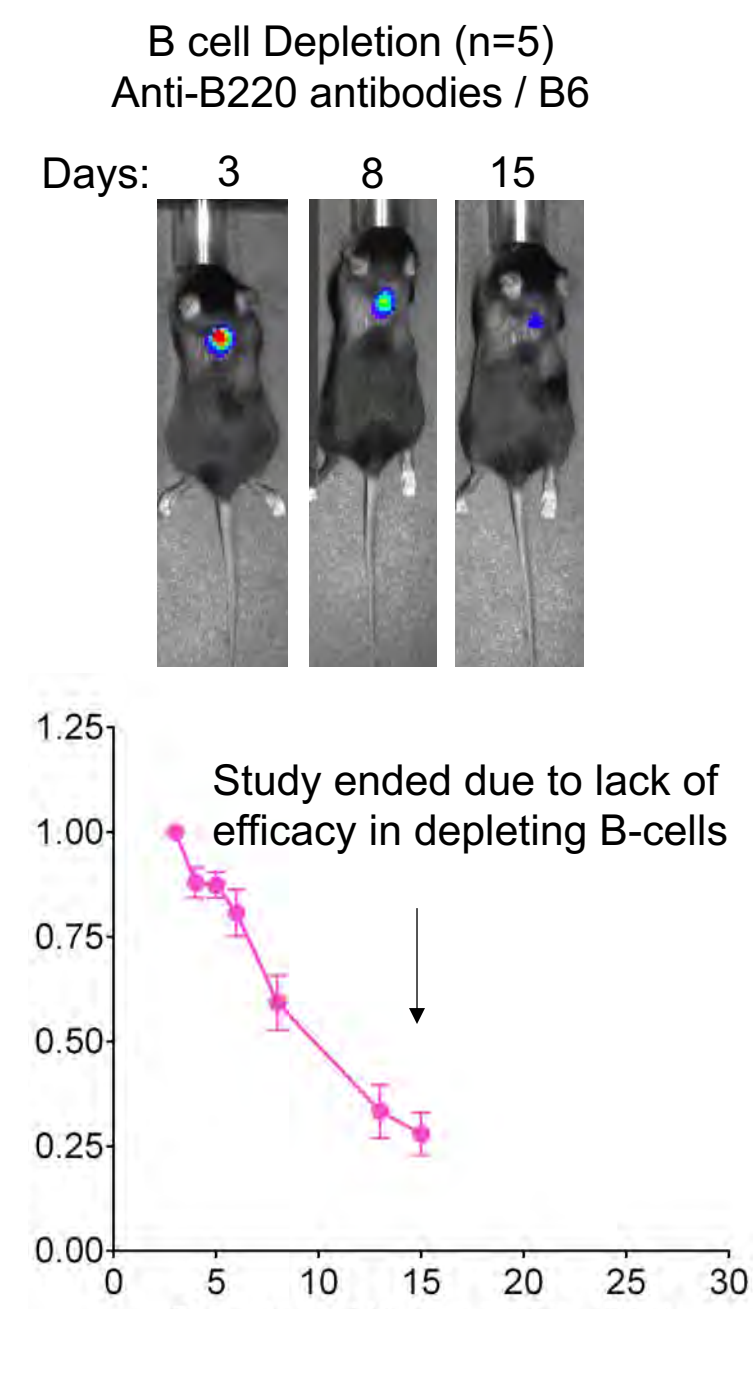


] $p = 0.0976$
] $p = 0.6742$
] $p = 0.1166$

S3a

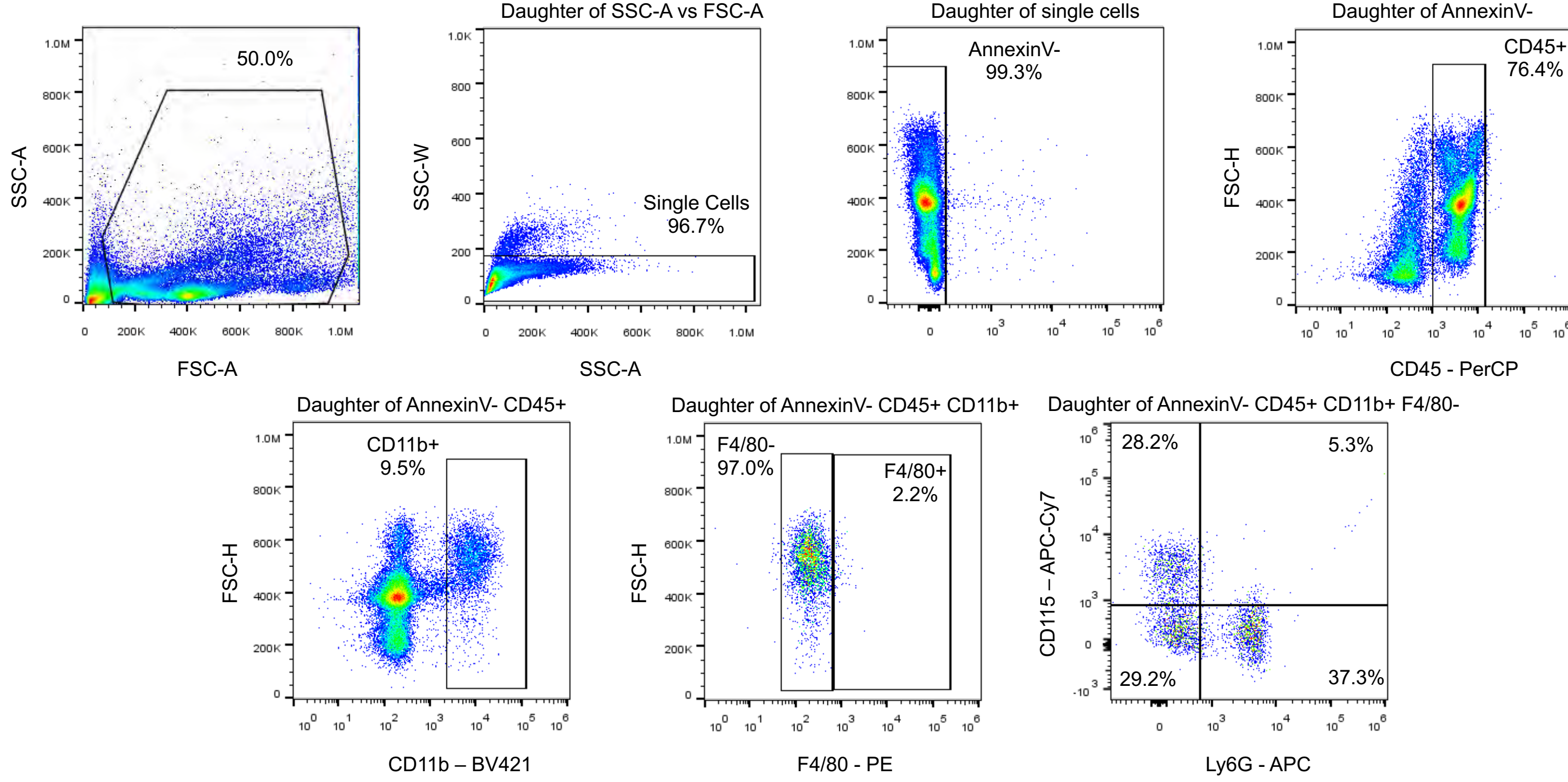


S3b



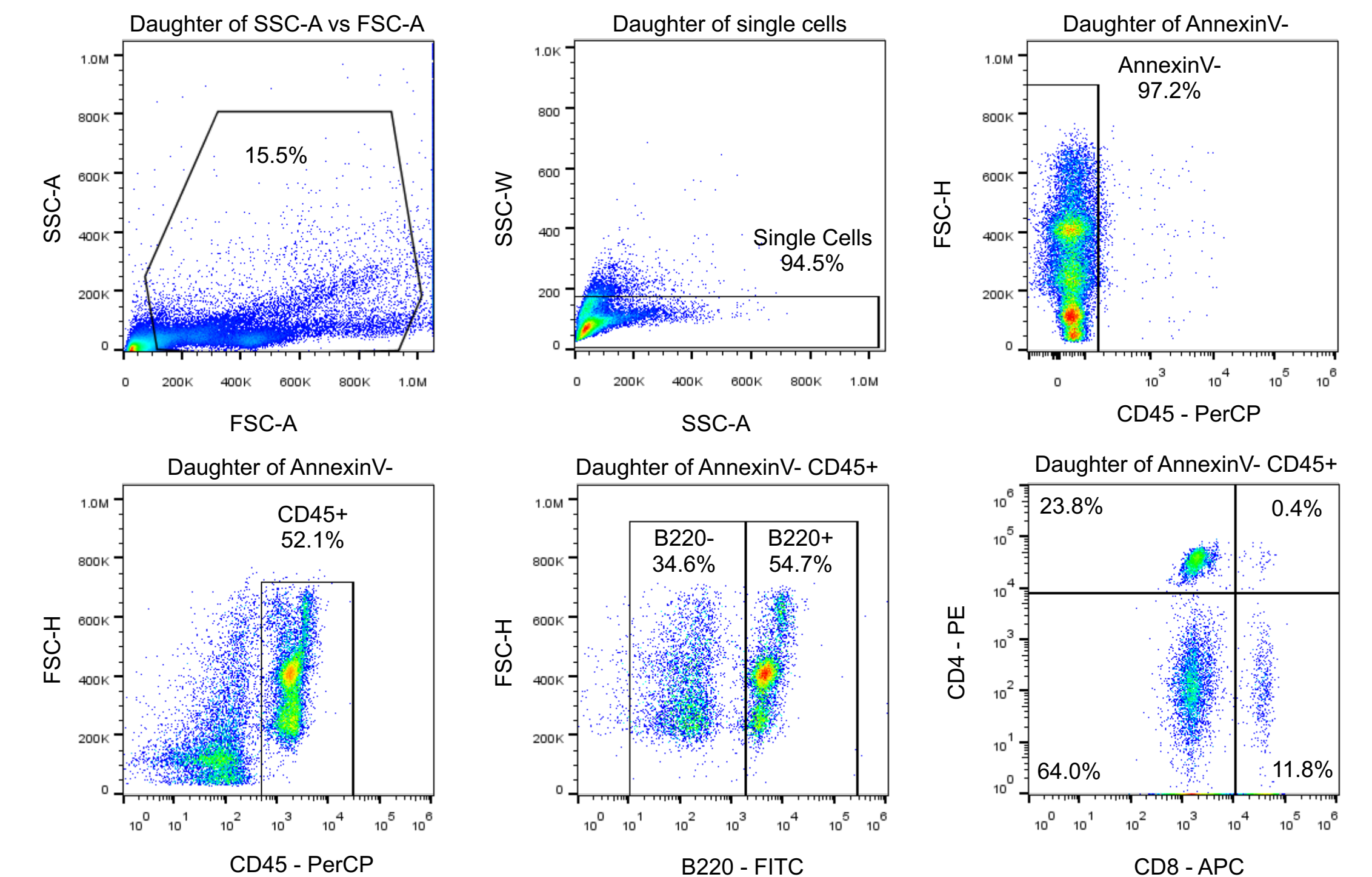
S3c

Innate immune cell stain – Representative flow plots (gating strategy) Peripheral Blood



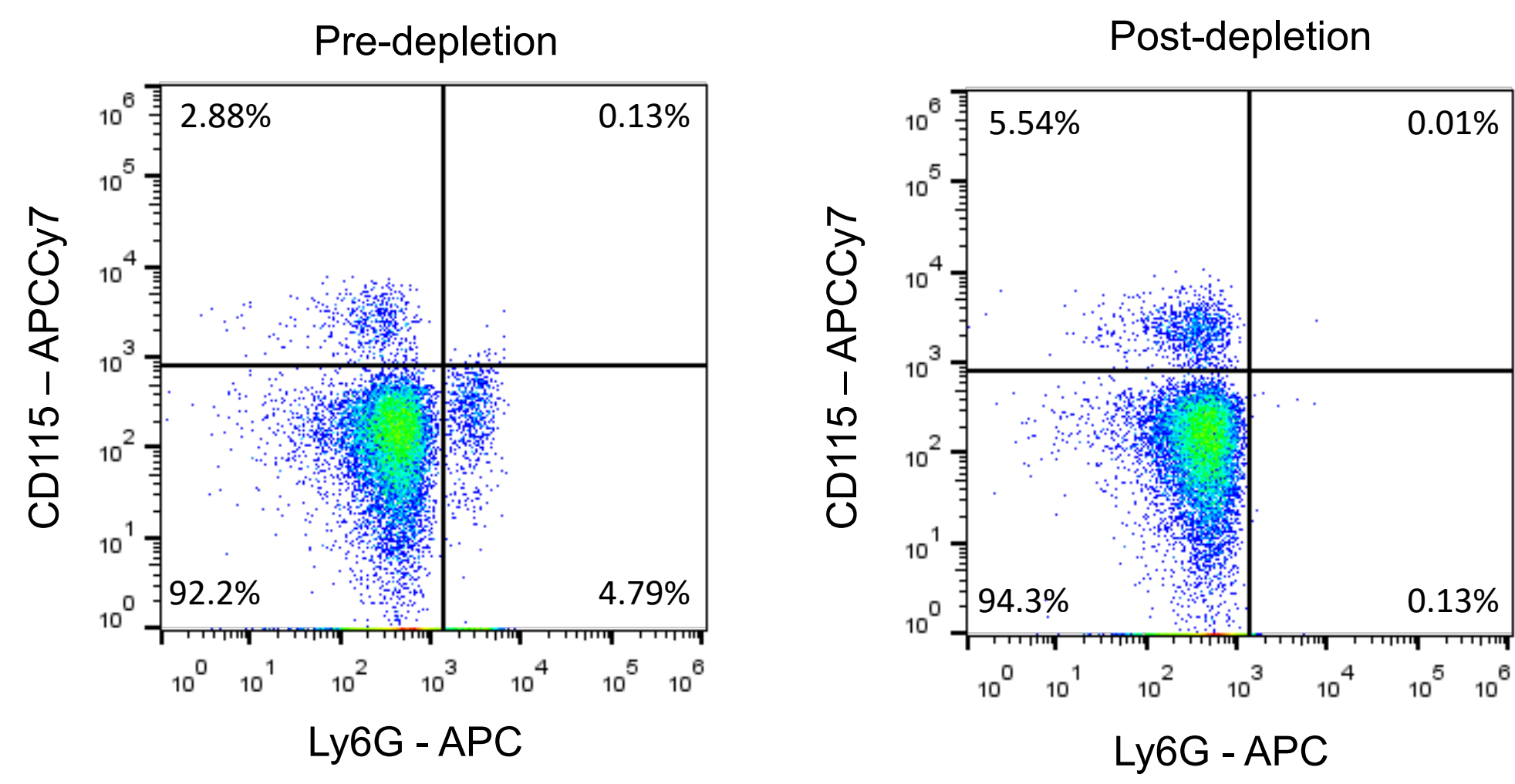
S3d

Adaptive immune cell stain – Representative flow plots (gating strategy) Peripheral Blood

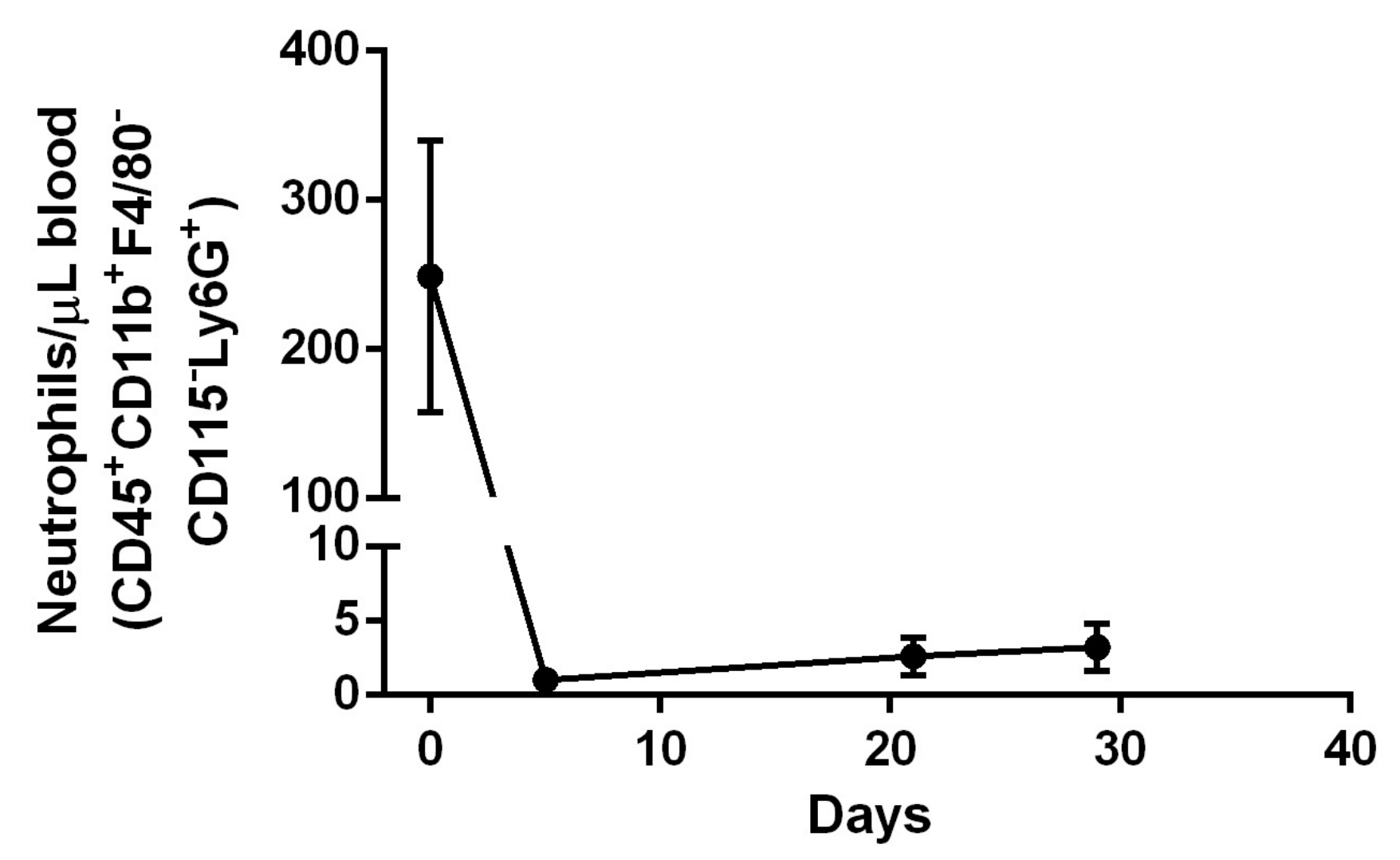


S3e

Neutrophil depletion
Anti-Ly6G and anti-rat k immunoglobulin light chain antibodies / B6

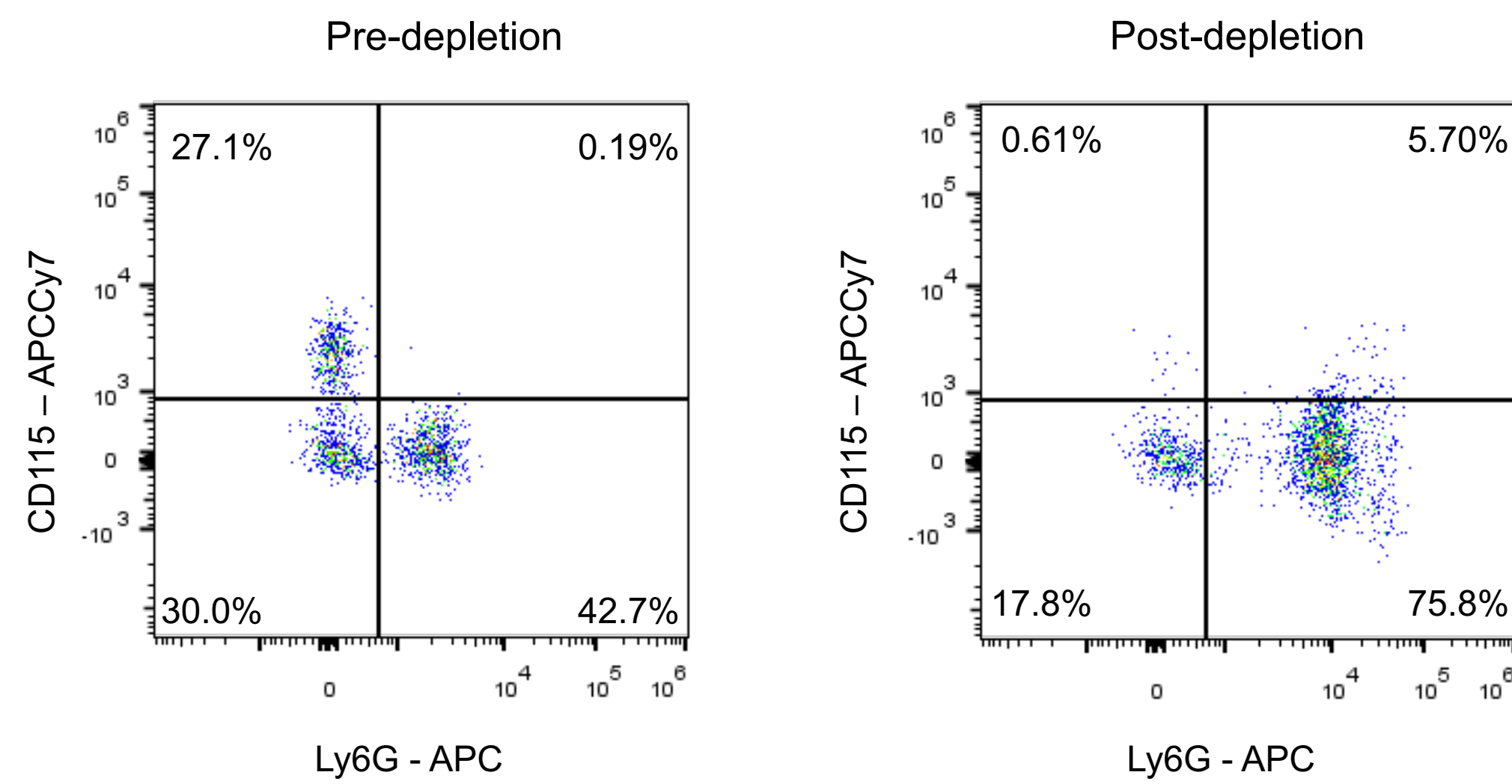


S3f

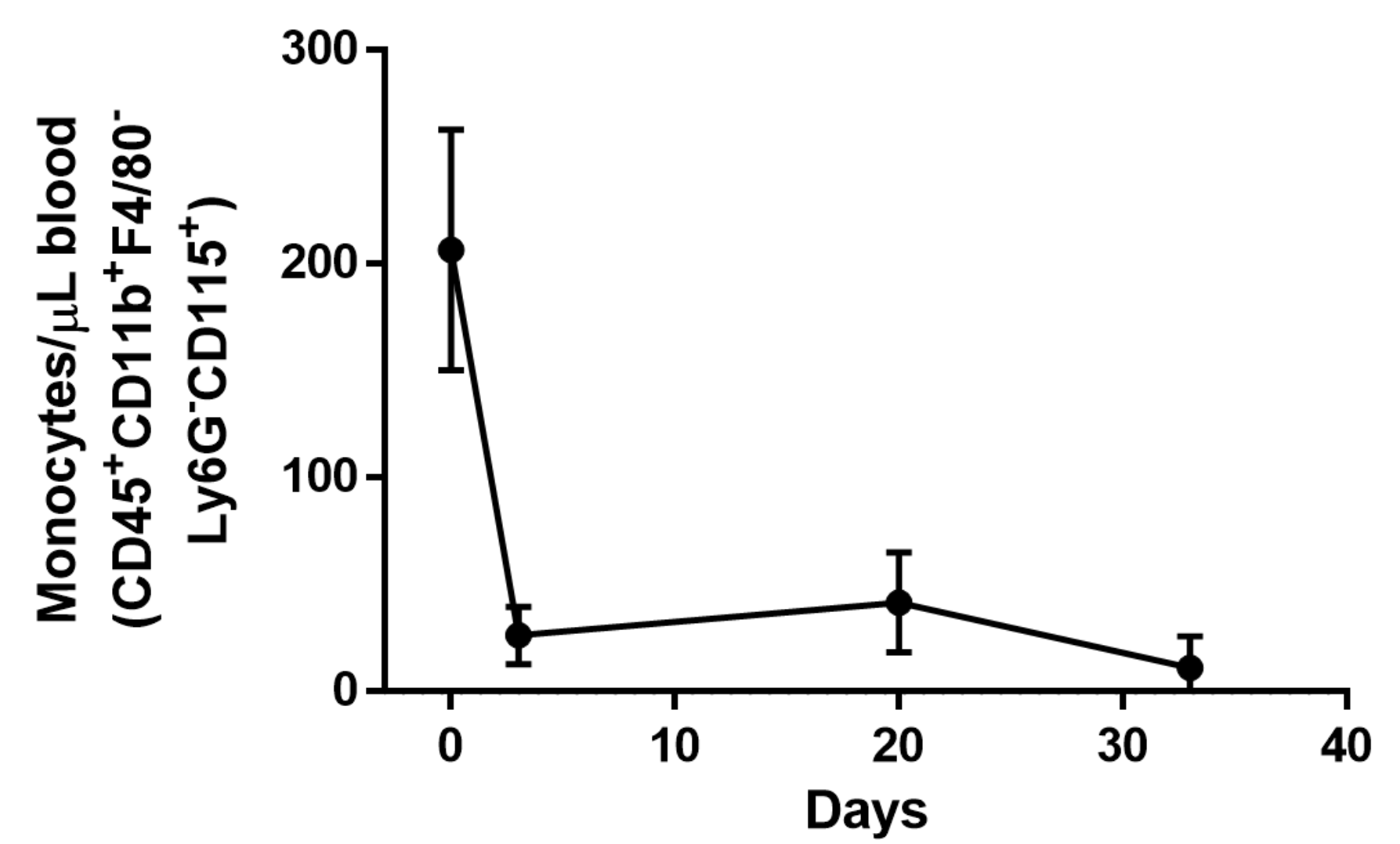


S3g

Macrophage depletion
Clodronate Liposomes / B6

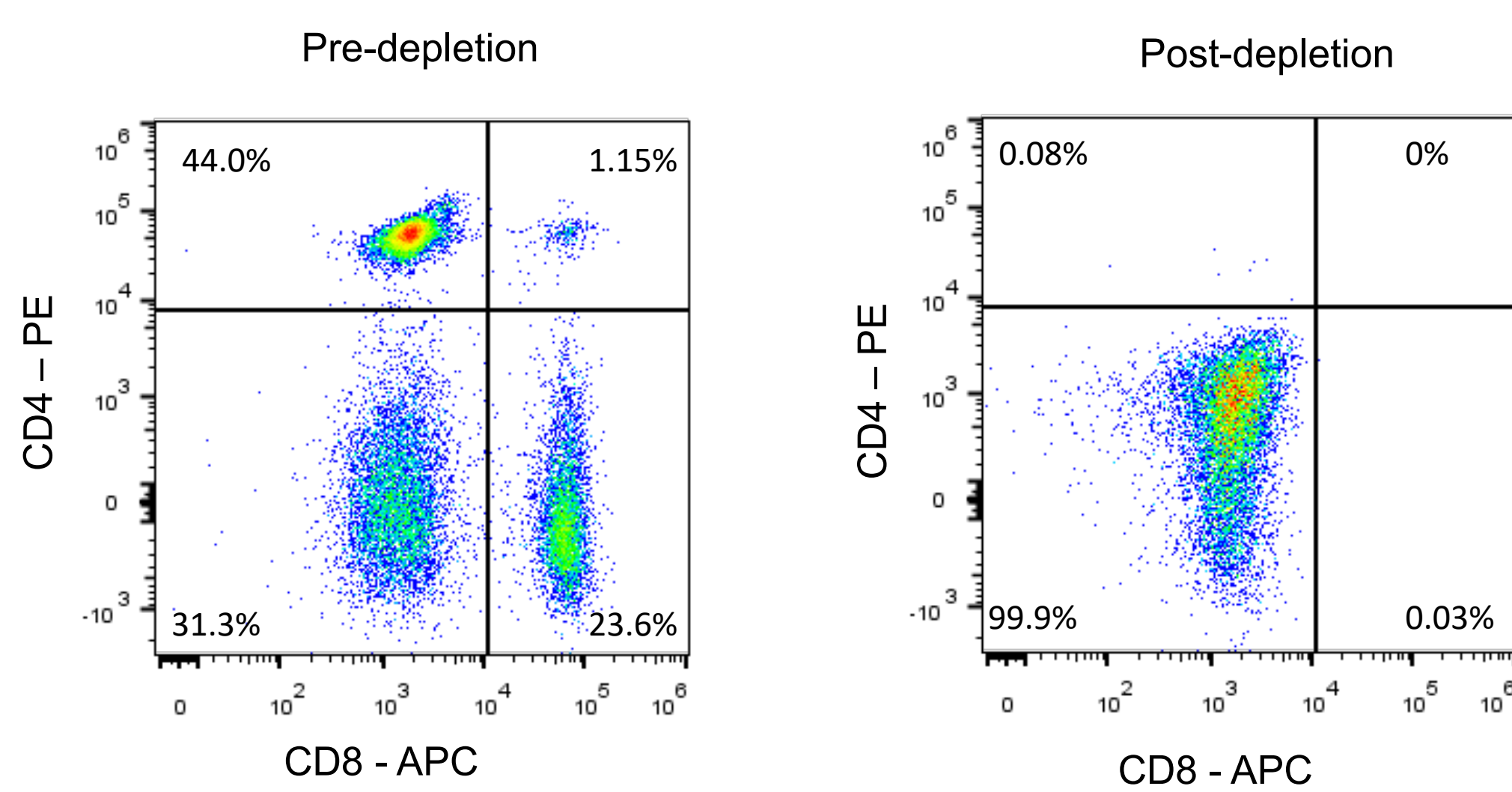


S3h

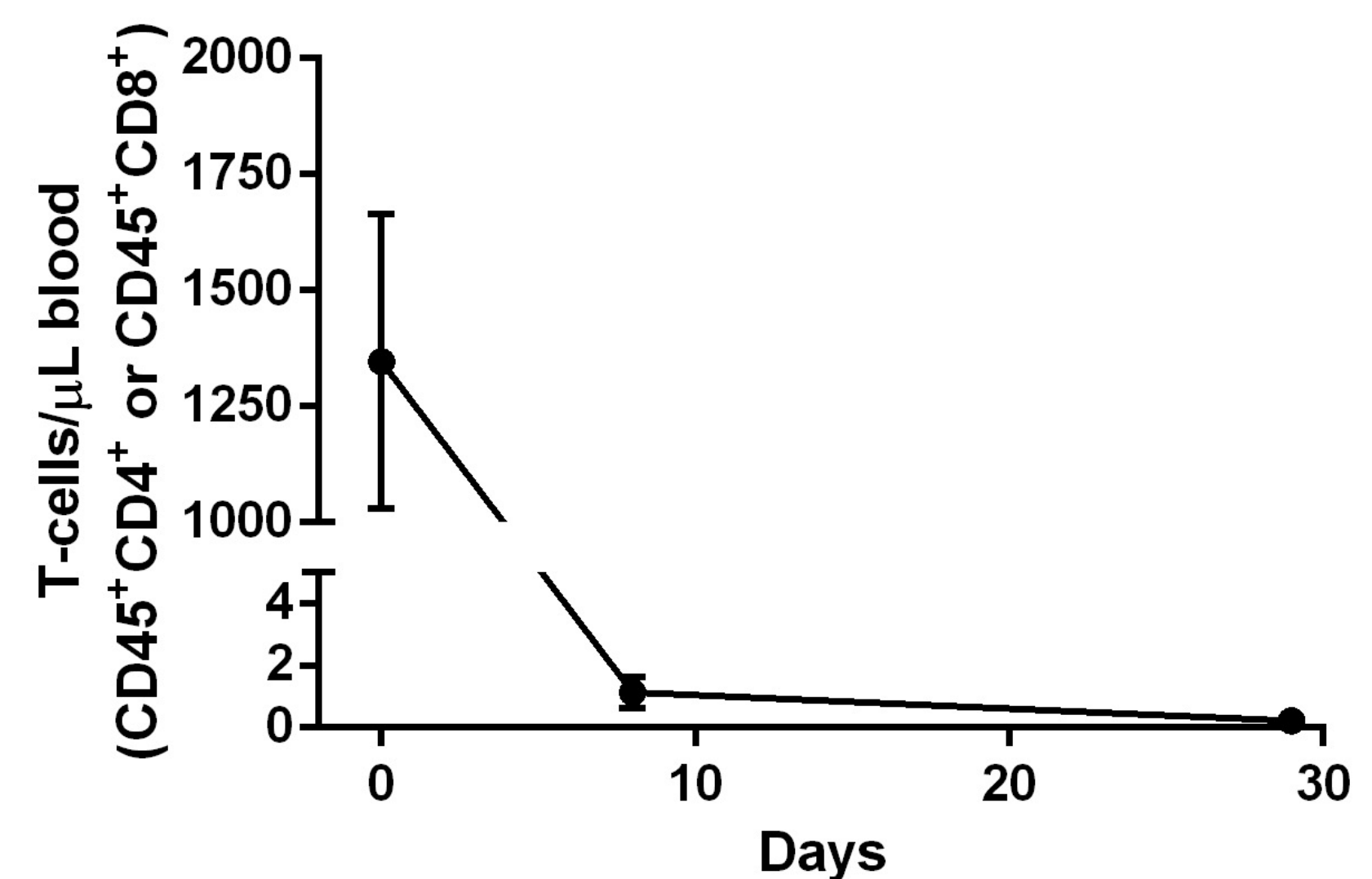


S3i

T cell depletion
Anti-CD4 and anti-CD8 antibodies / B6

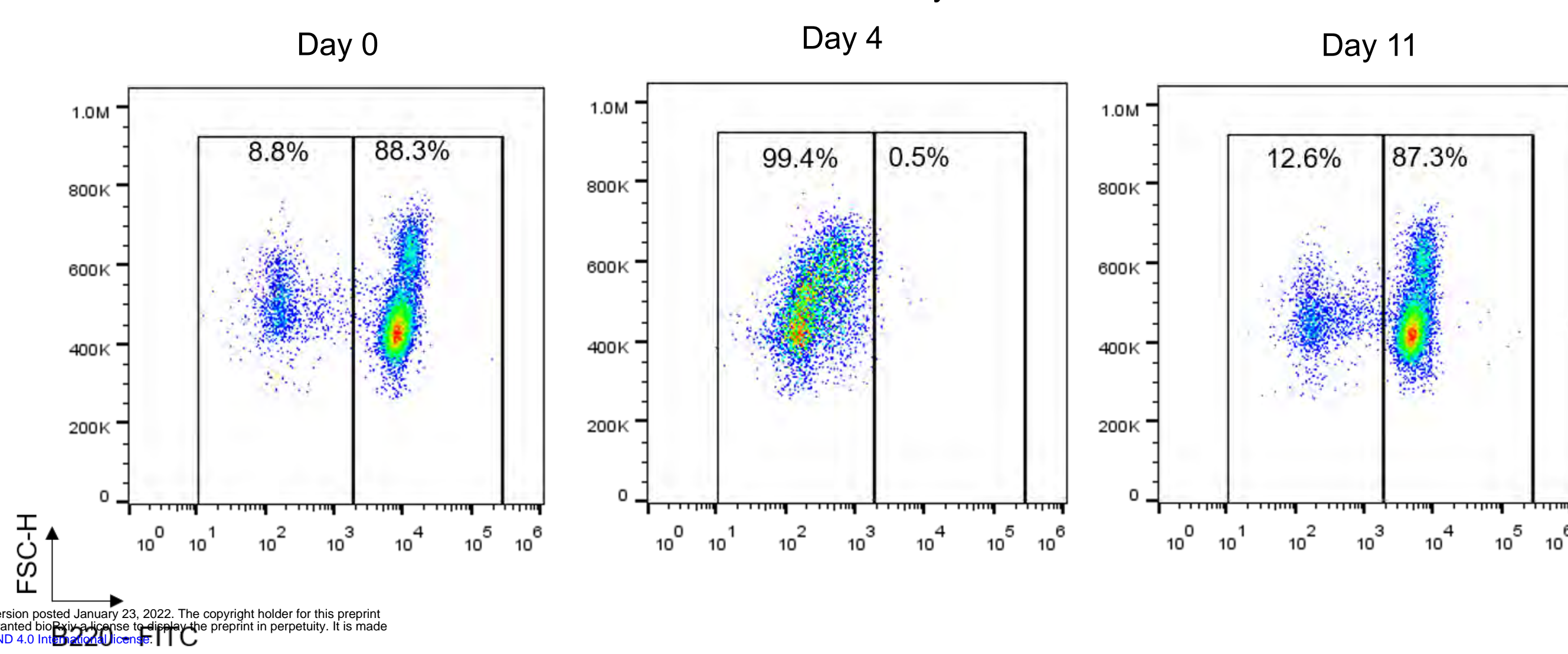


S3j

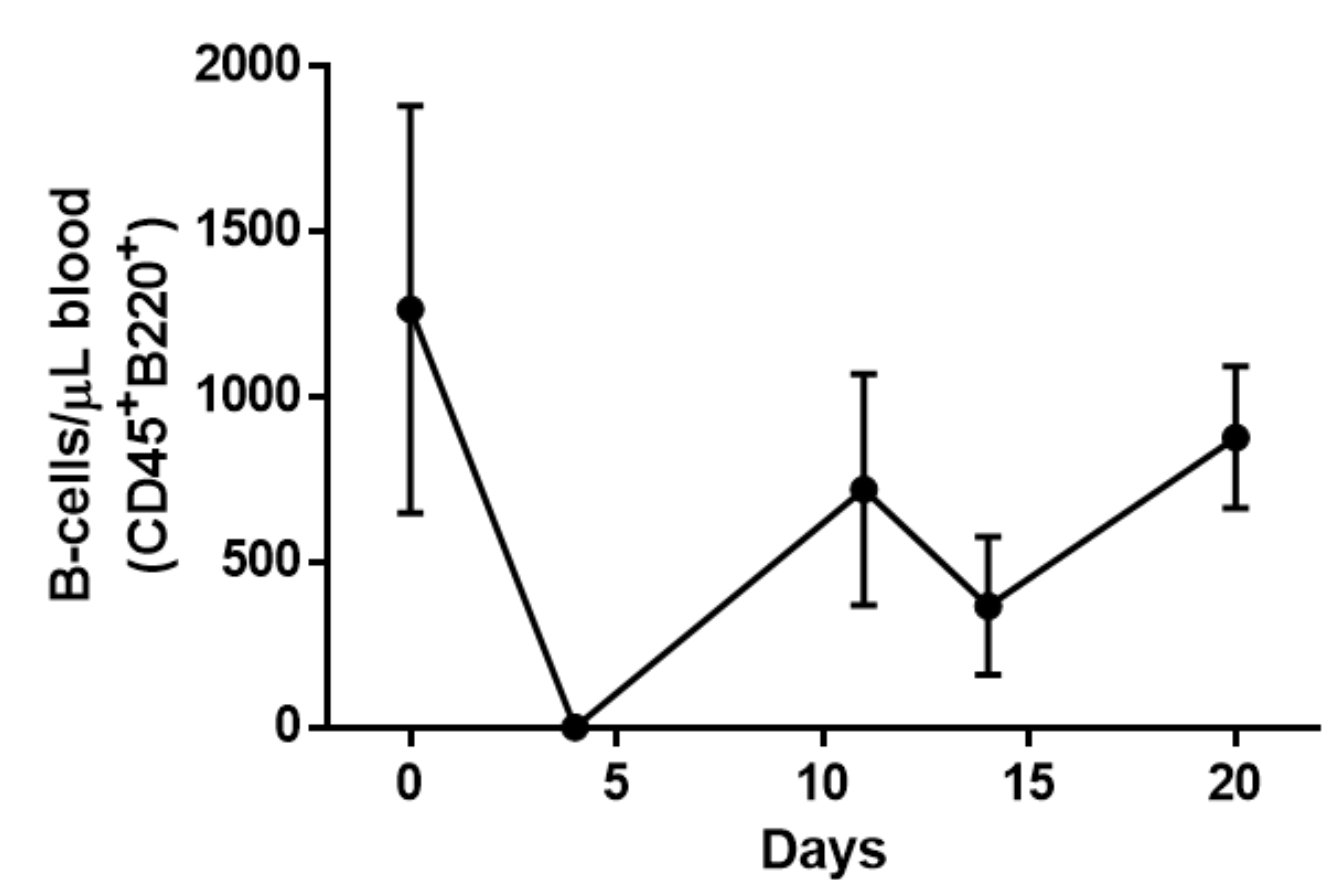


S3k

B cell depletion
Anti-B220 antibody / B6

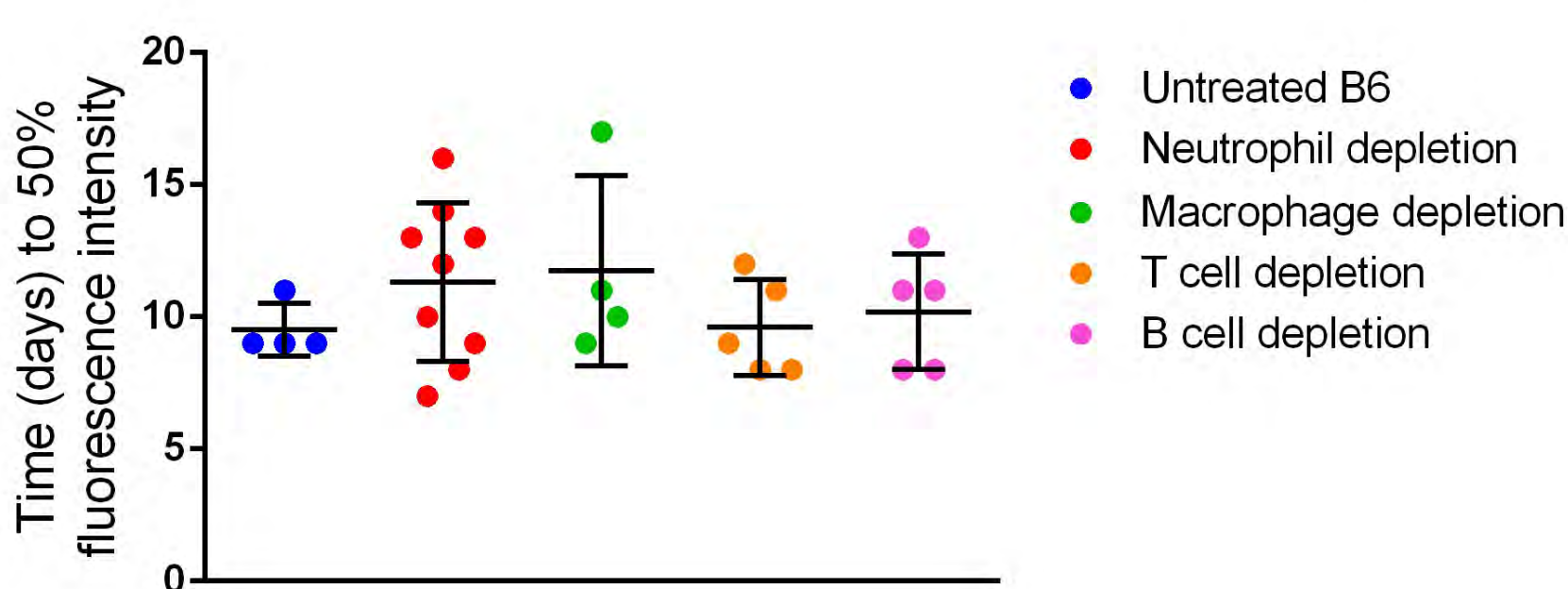
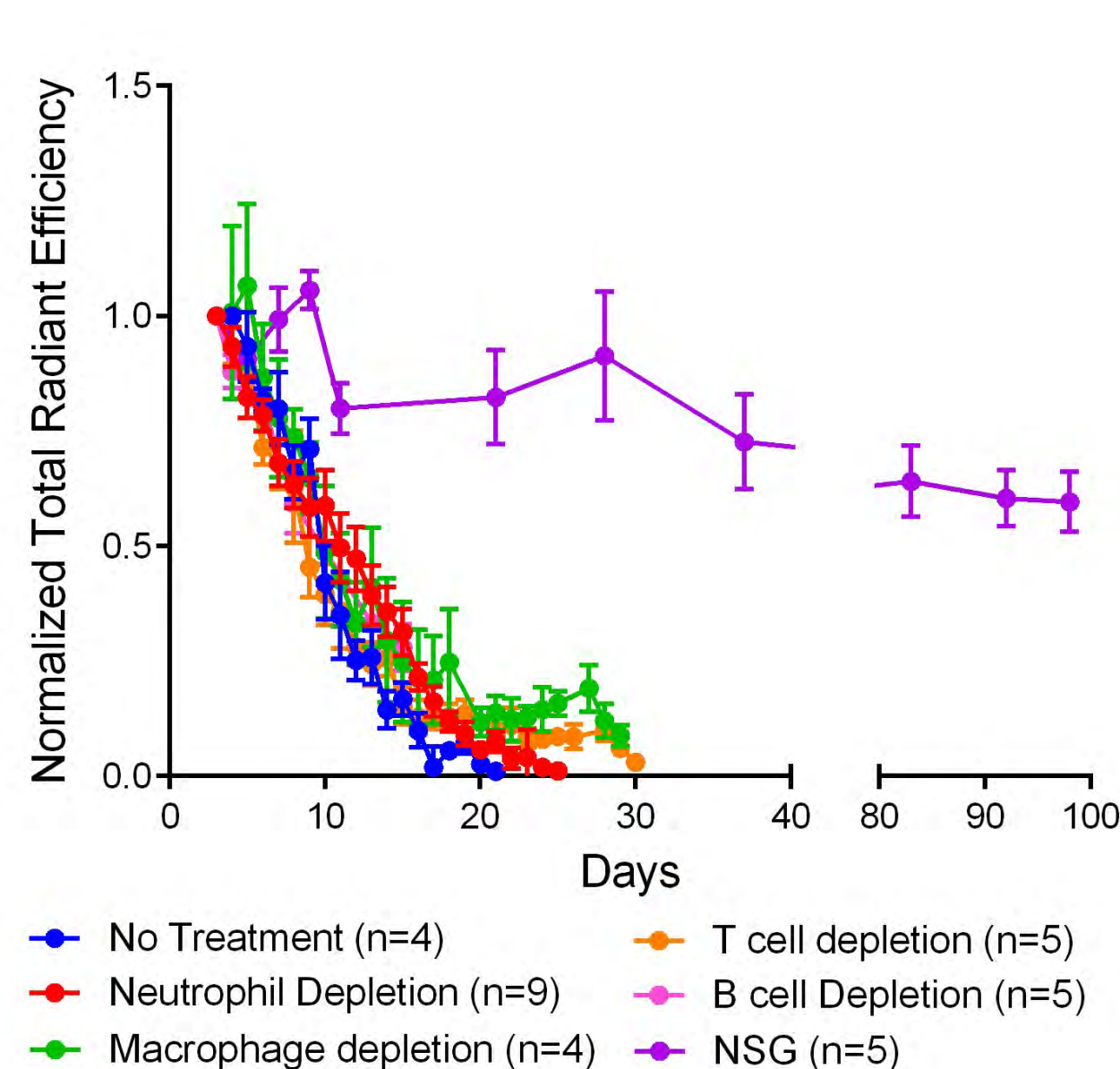


S3l



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S3m



Comparison	F	Df (Degrees of freedom)	P value
Untreated B6 vs neutrophil depletion	9	F _{8,3}	0.1300
Untreated B6 vs macrophage depletion	12.92	F _{3,3}	0.3036
Untreated B6 vs T cell depletion	3.3	F _{4,3}	0.9197
Untreated B6 vs B cell depletion	4.7	F _{4,3}	0.5453

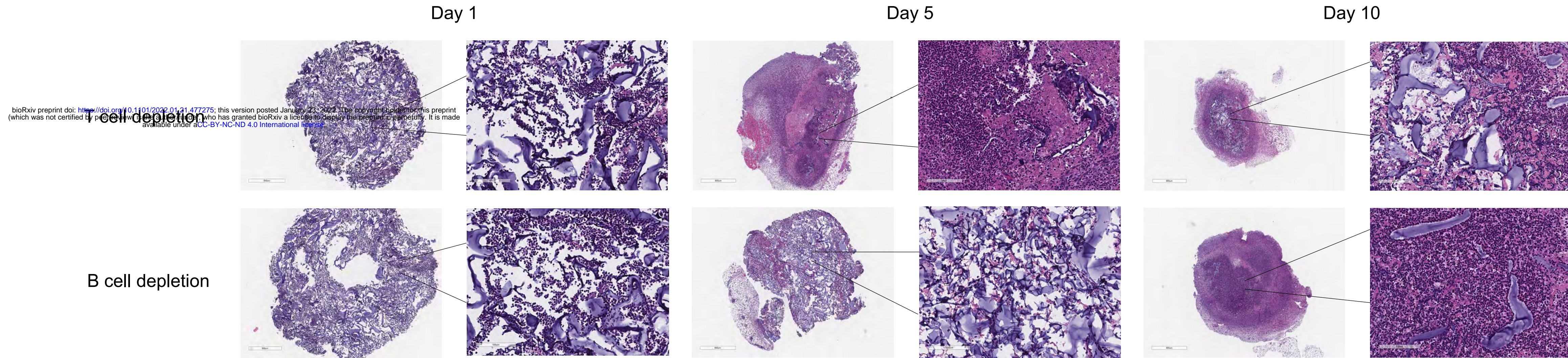
S3n

NSG (3 months)

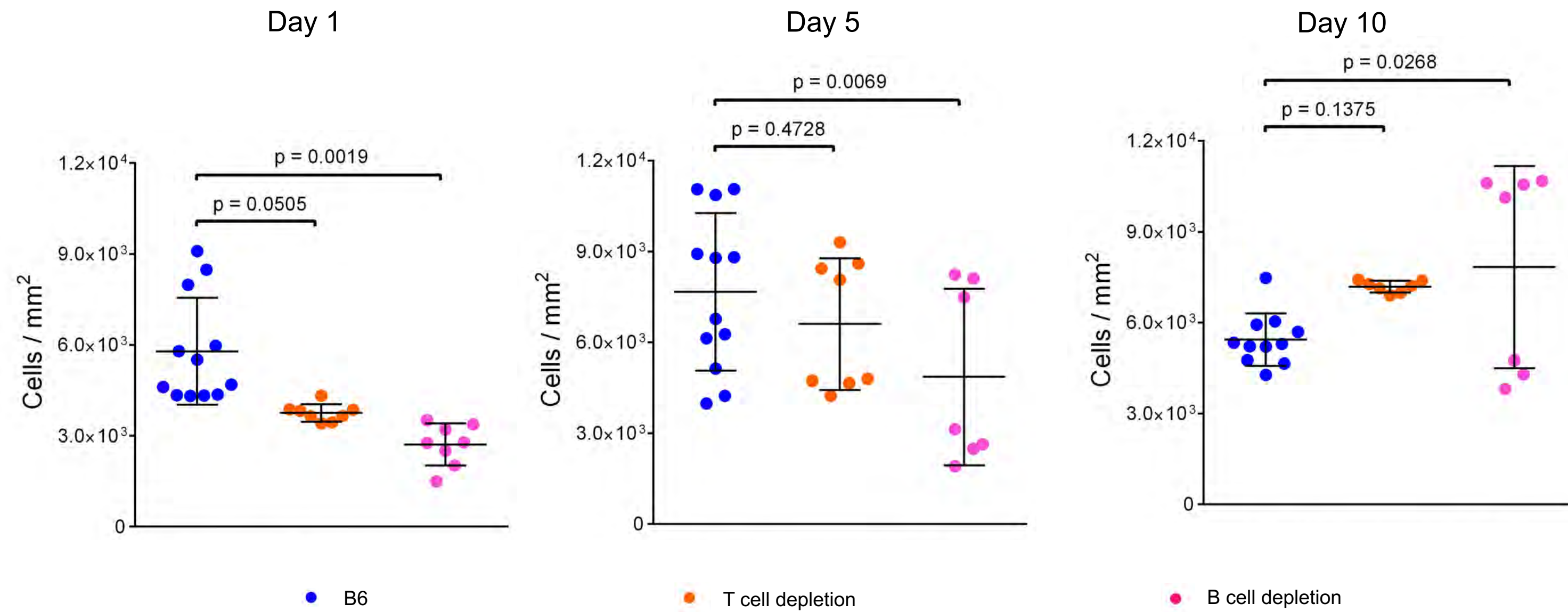


Supplementary Figure 4

S4a



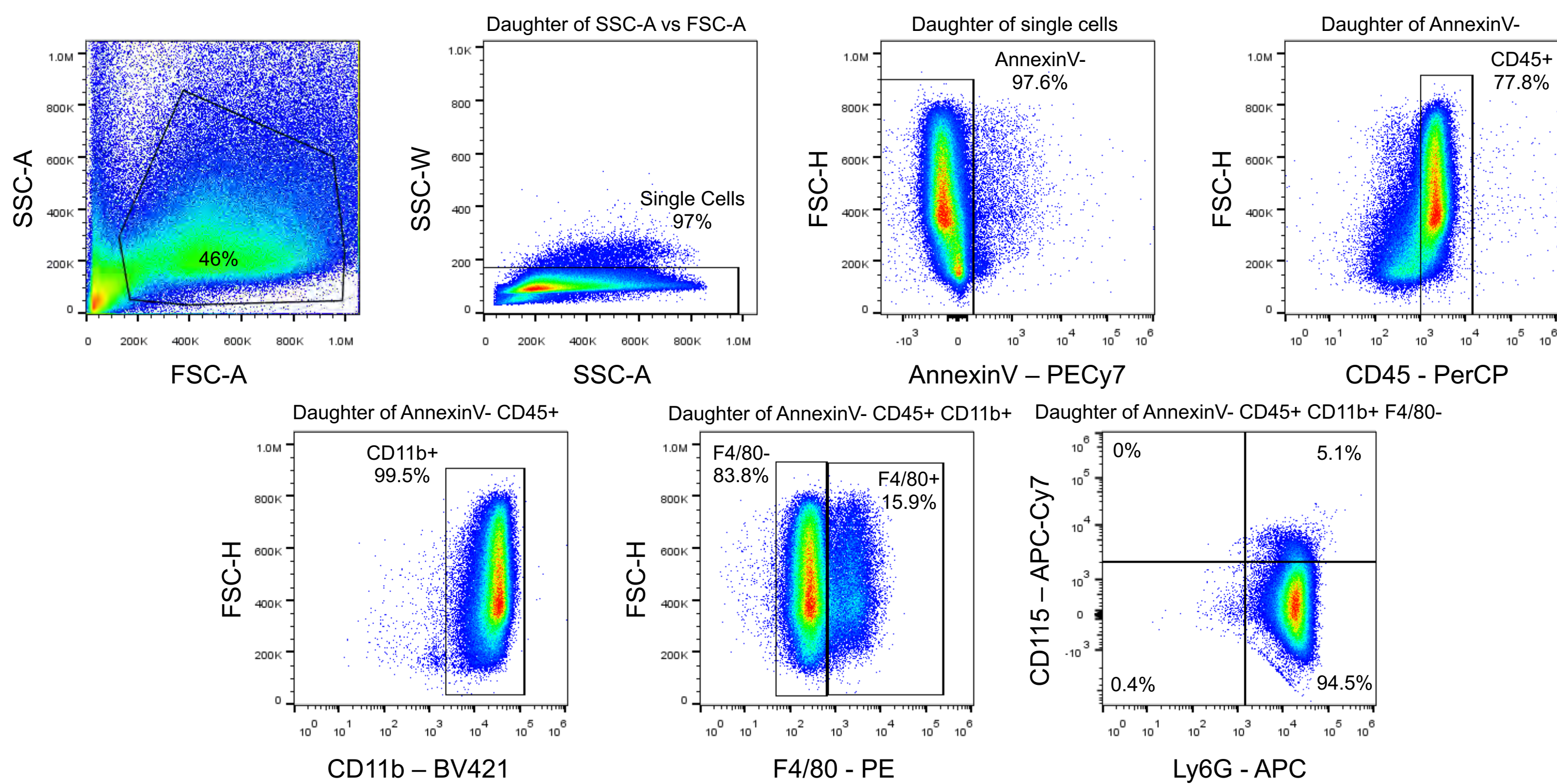
S4b



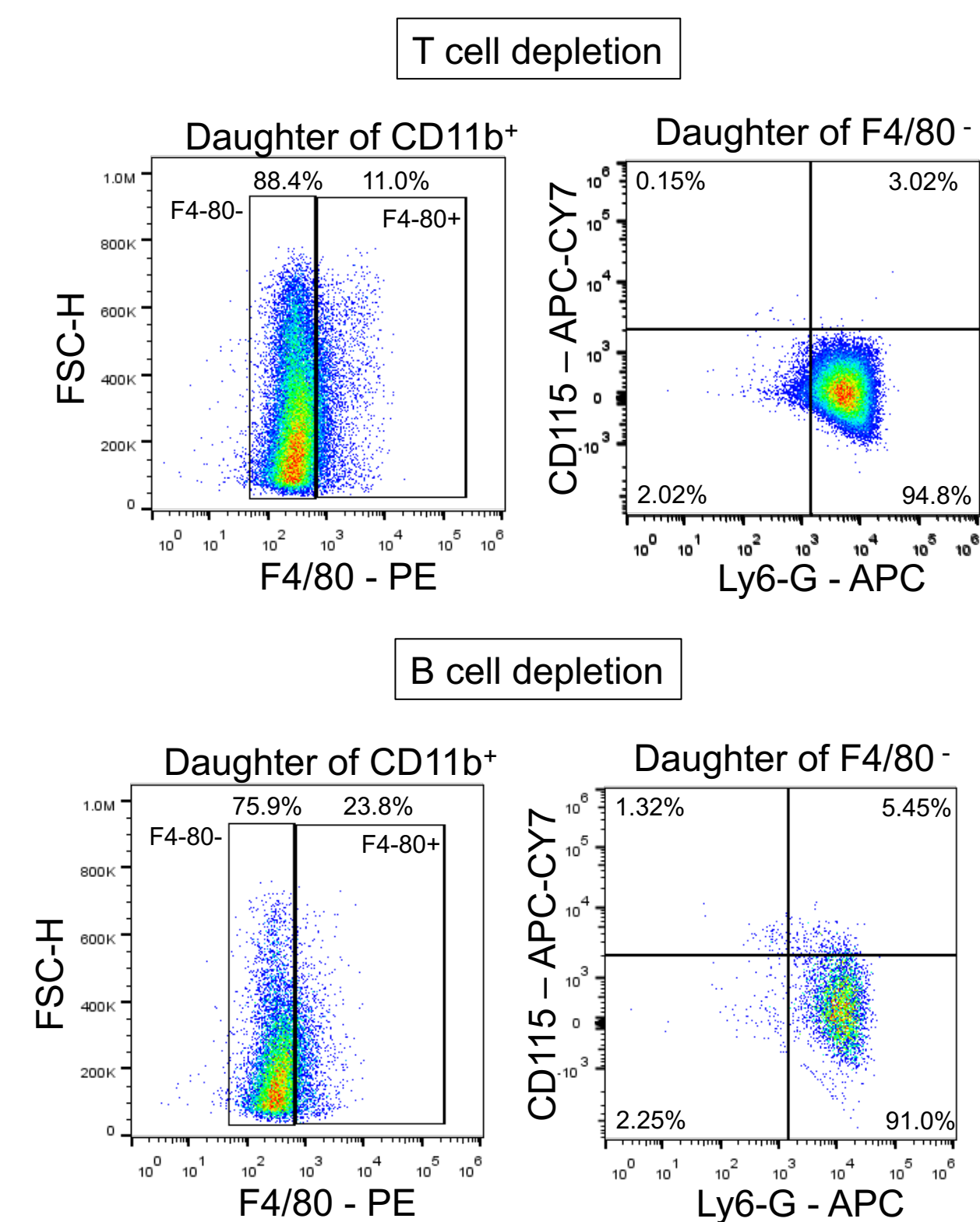
Supplementary Figure 5

S5a

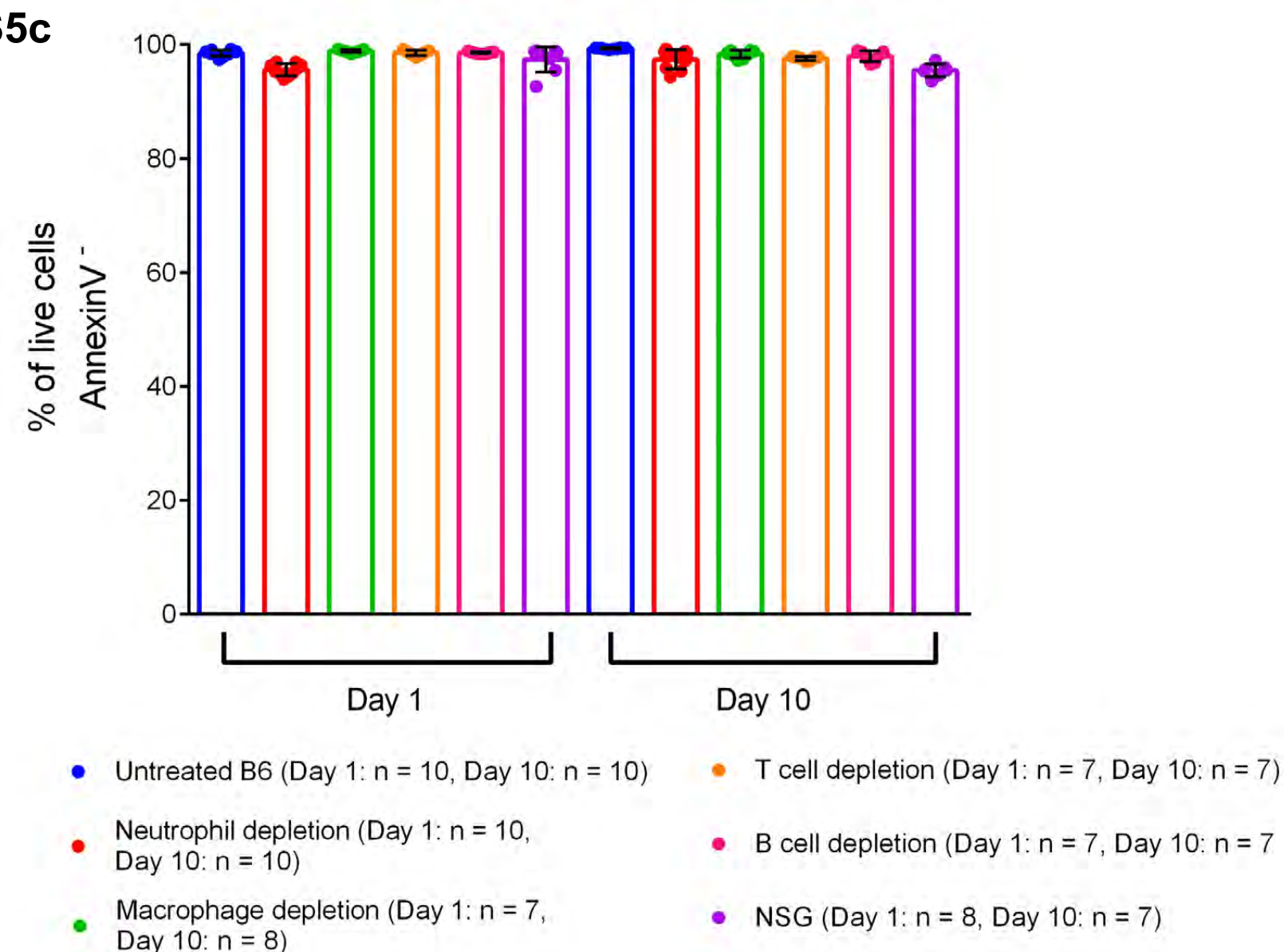
Innate immune cell infiltration – Representative flow plots (gating strategy)
Day 1: Untreated B6 mice



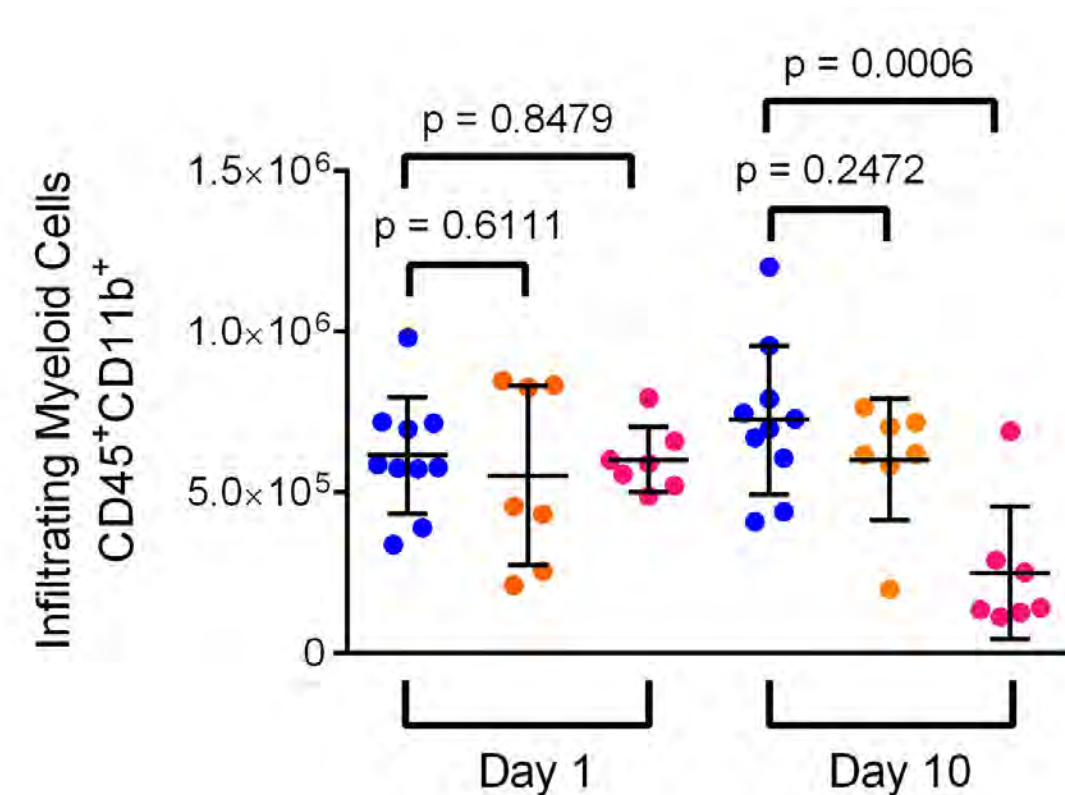
S5b



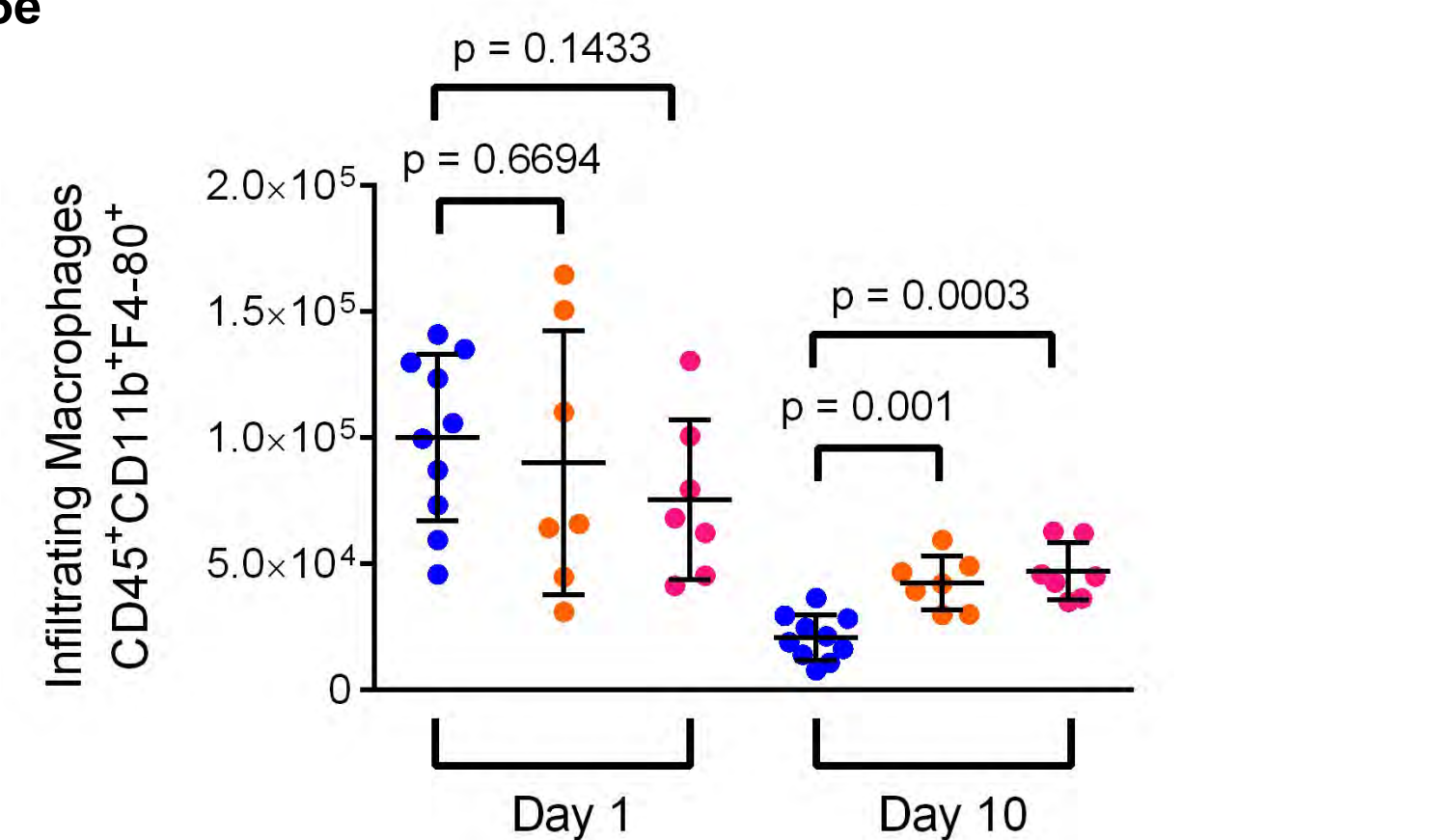
S5c



S5d

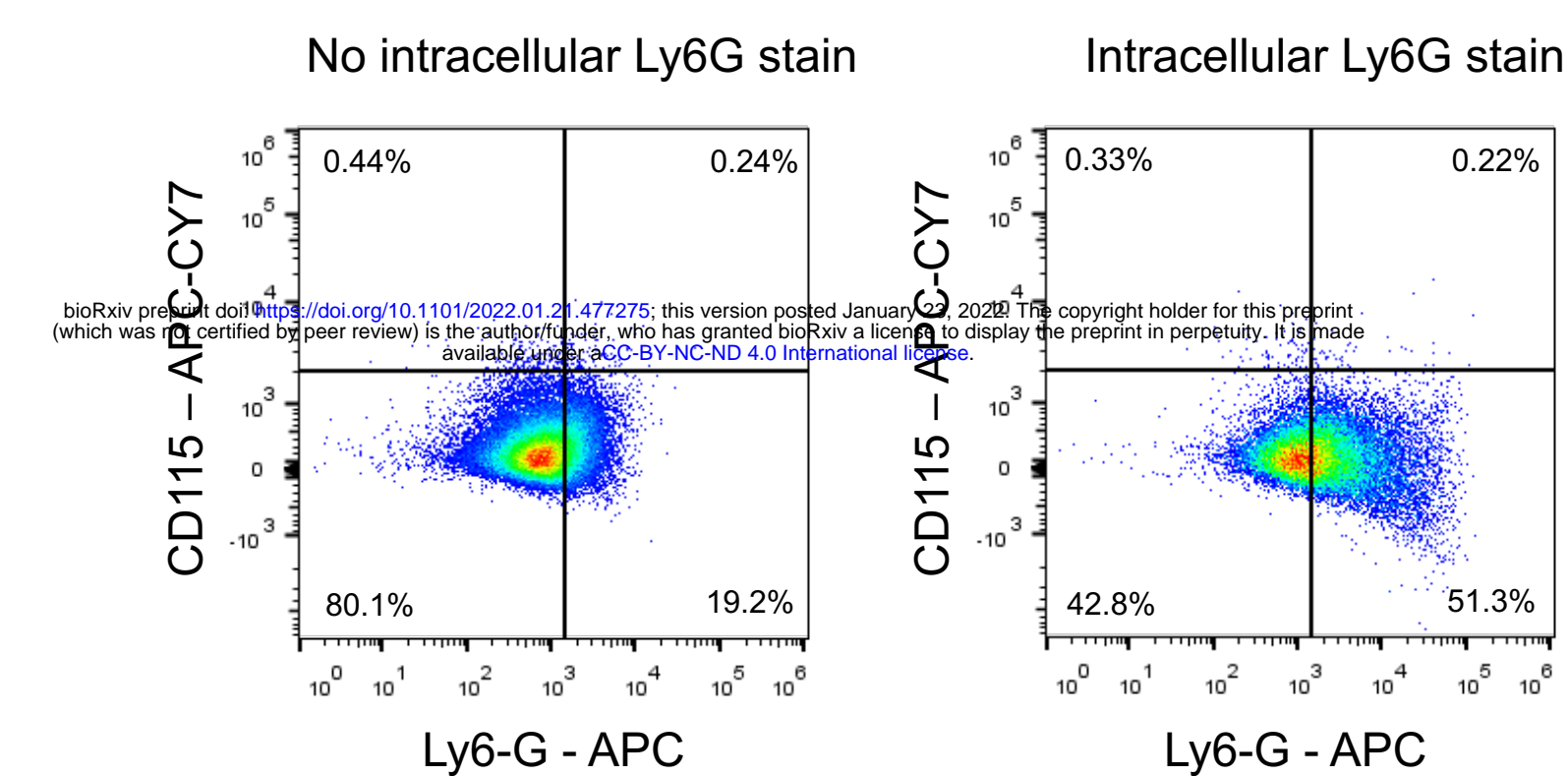


S5e

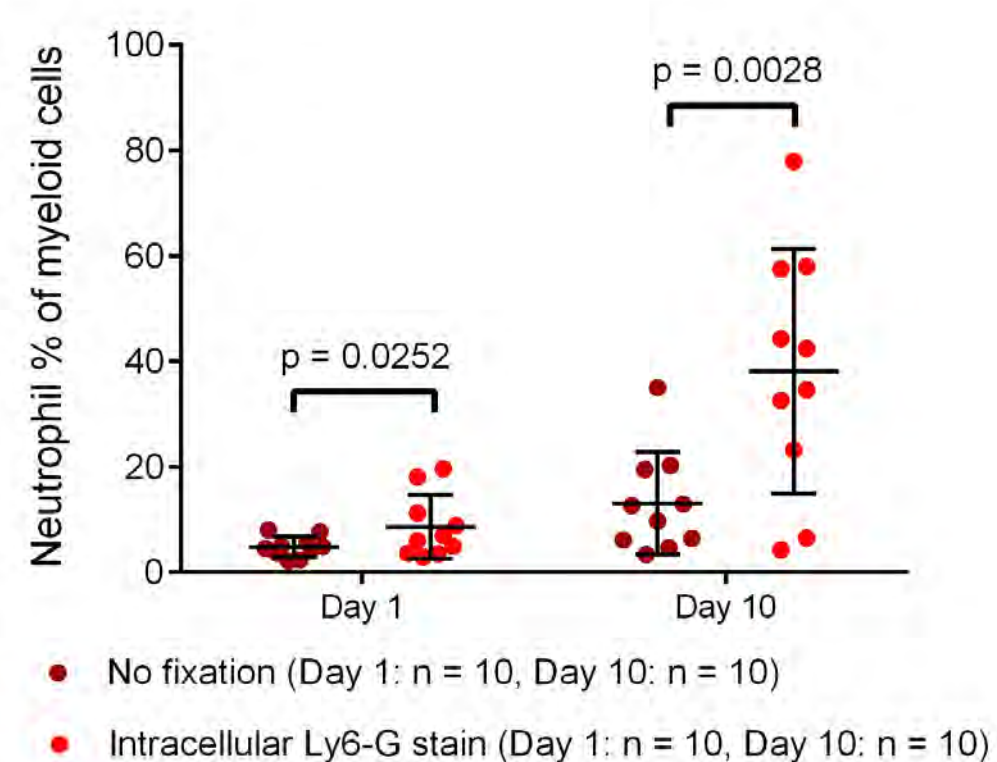


S5f

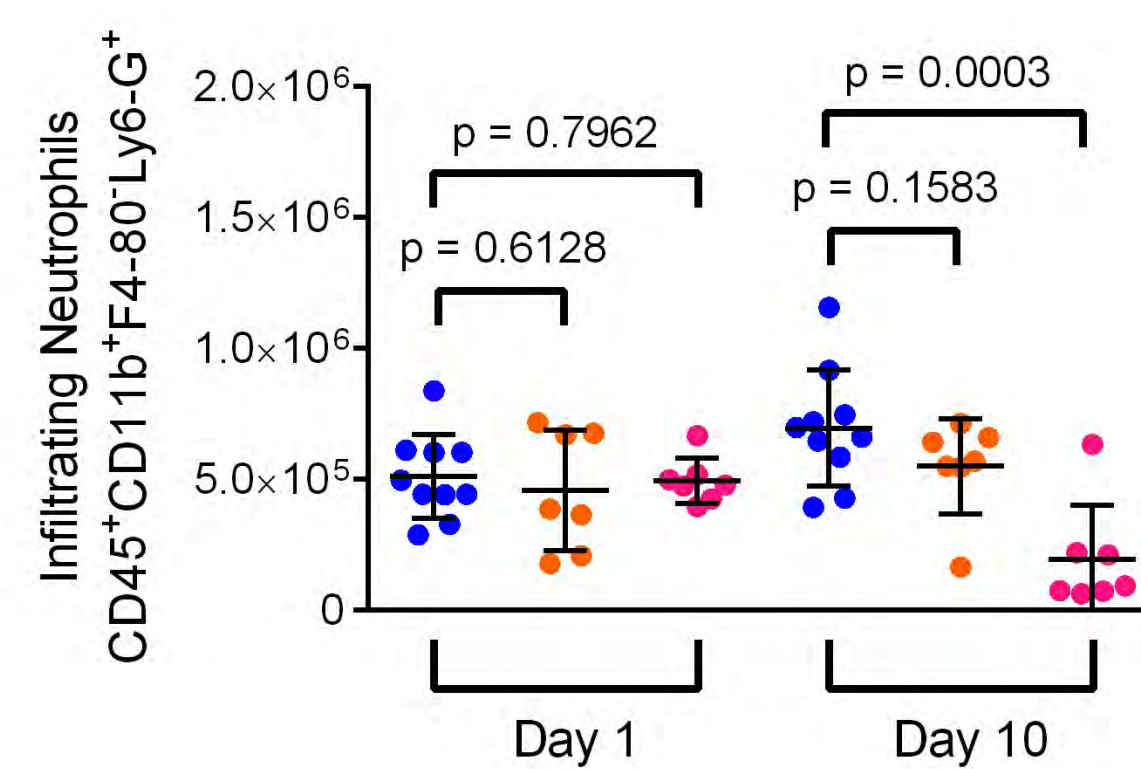
Neutrophil depletion
Day 10: Daughter of AnnexinV- CD45+ CD11b+ F4-80-



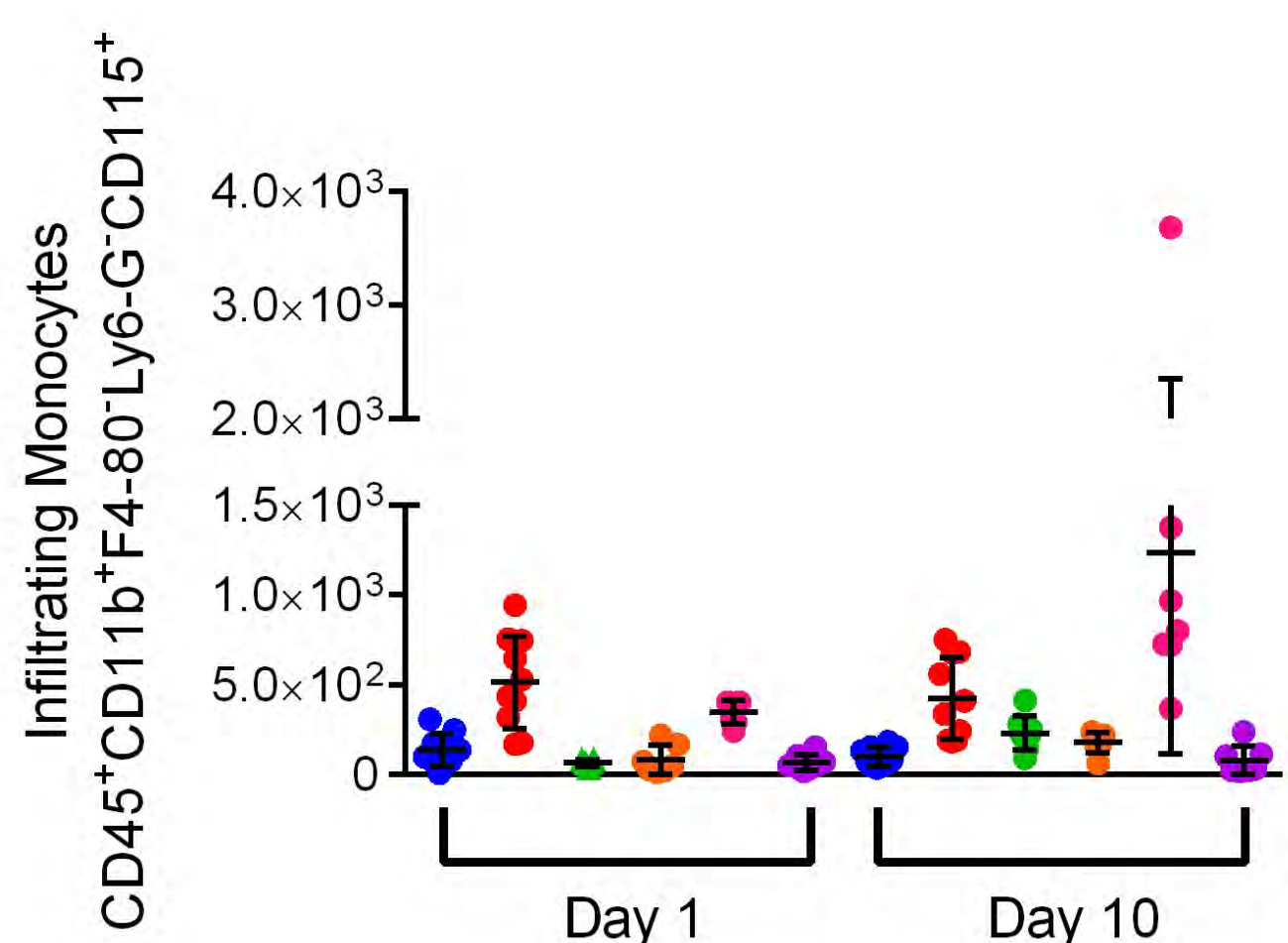
Neutrophil depletion



S5g

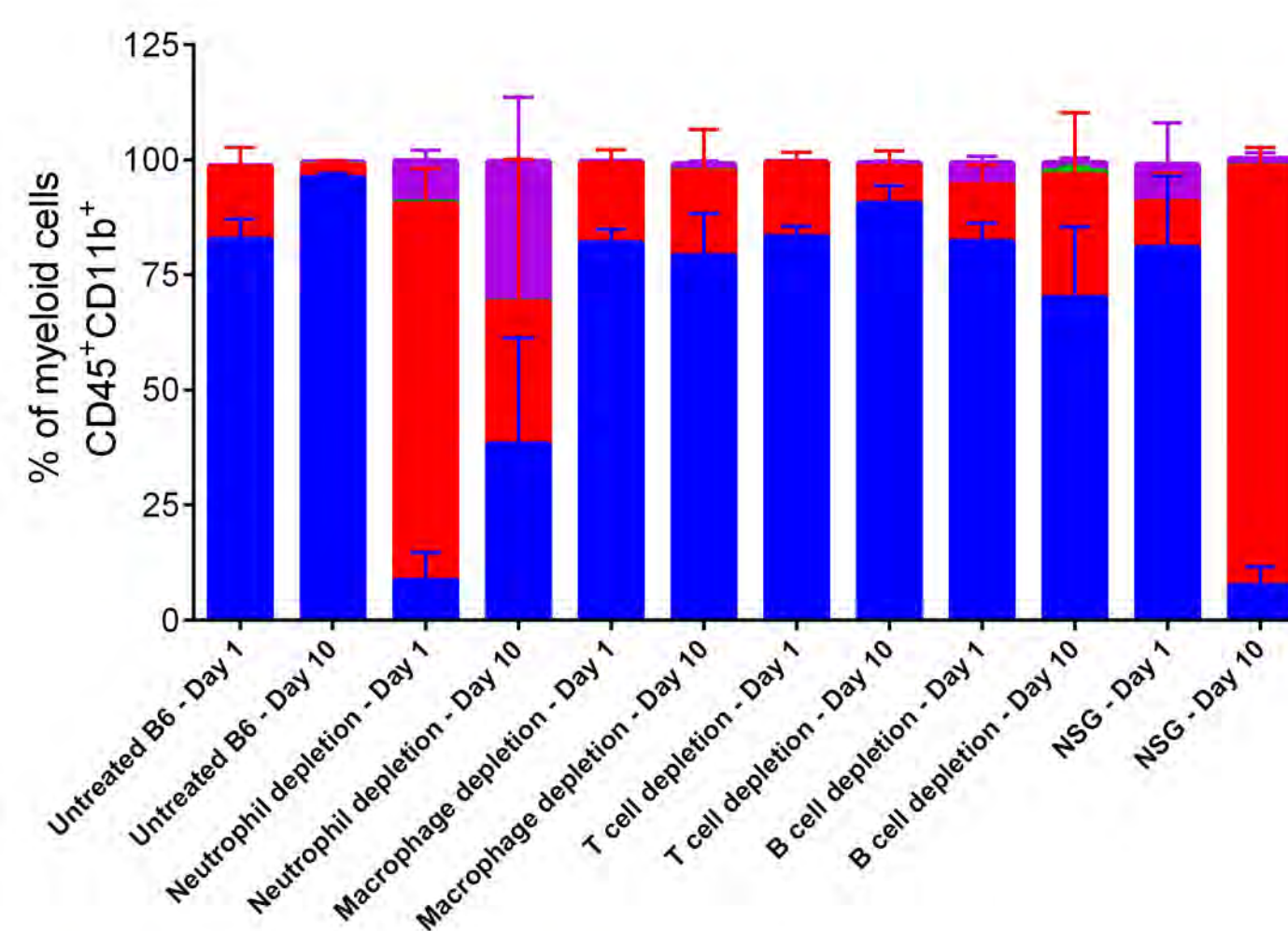


S5h



- Untreated B6 (Day 1: n = 10, Day 10: n = 10)
- Neutrophil depletion (Day 1: n = 10, Day 10: n = 10)
- Macrophage depletion (Day 1: n = 7, Day 10: n = 8)
- T cell depletion (Day 1: n = 7, Day 10: n = 7)
- B cell depletion (Day 1: n = 7, Day 10: n = 7)
- NSG (Day 1: n = 8, Day 10: n = 7)

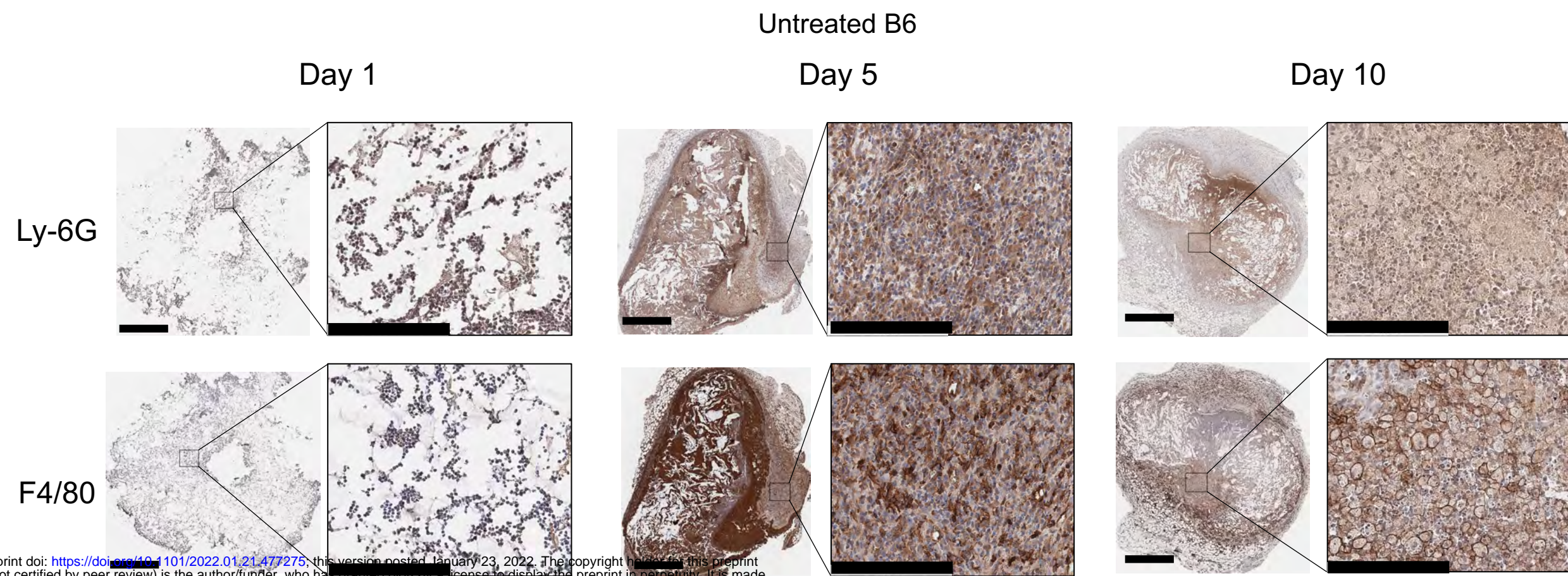
S5i



- Neutrophil %
- Macrophage %
- Monocyte %
- Other myeloid cell immunophenotypes %
CD45+CD11b+F4/80-CD115-Ly6G-

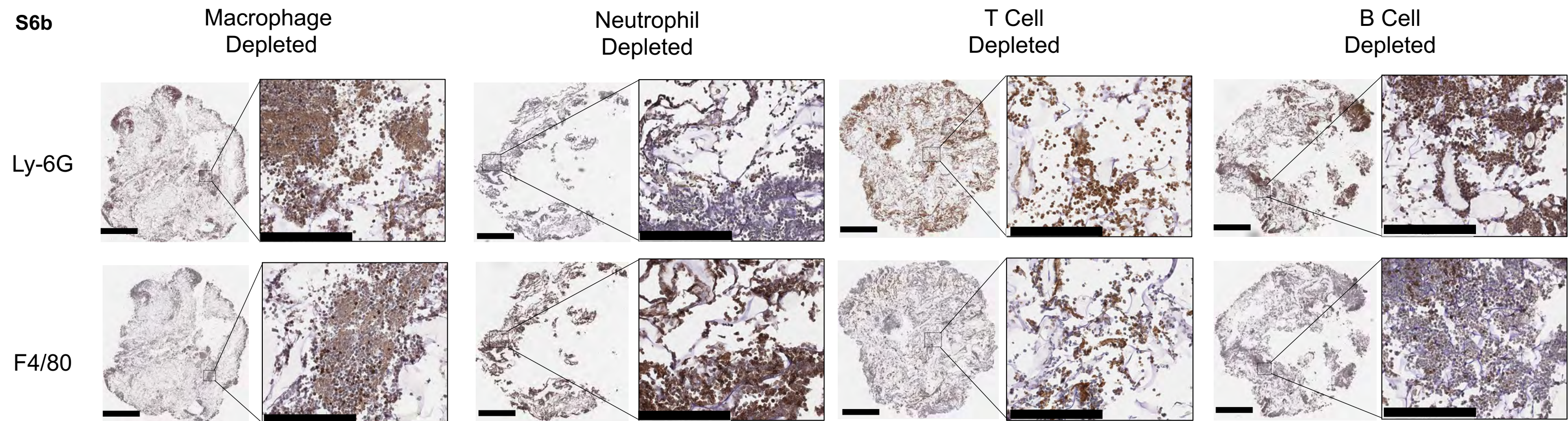
Supplementary Figure 6

S6a

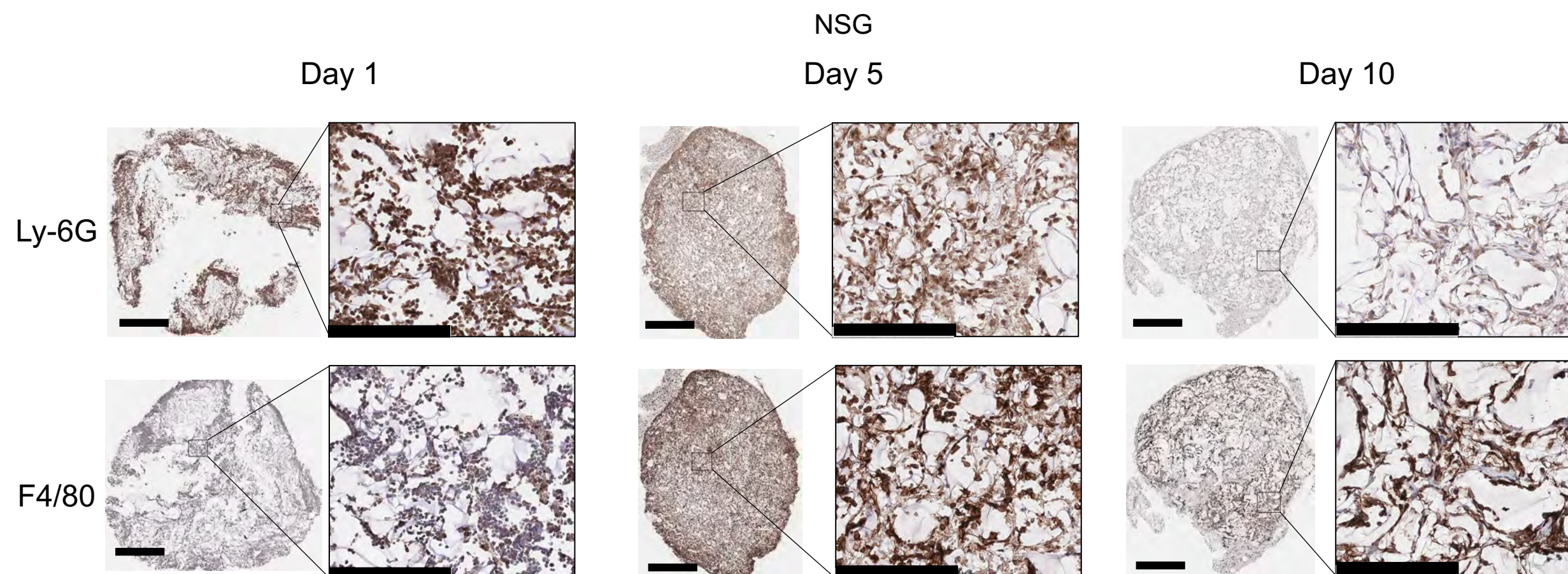


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S6b

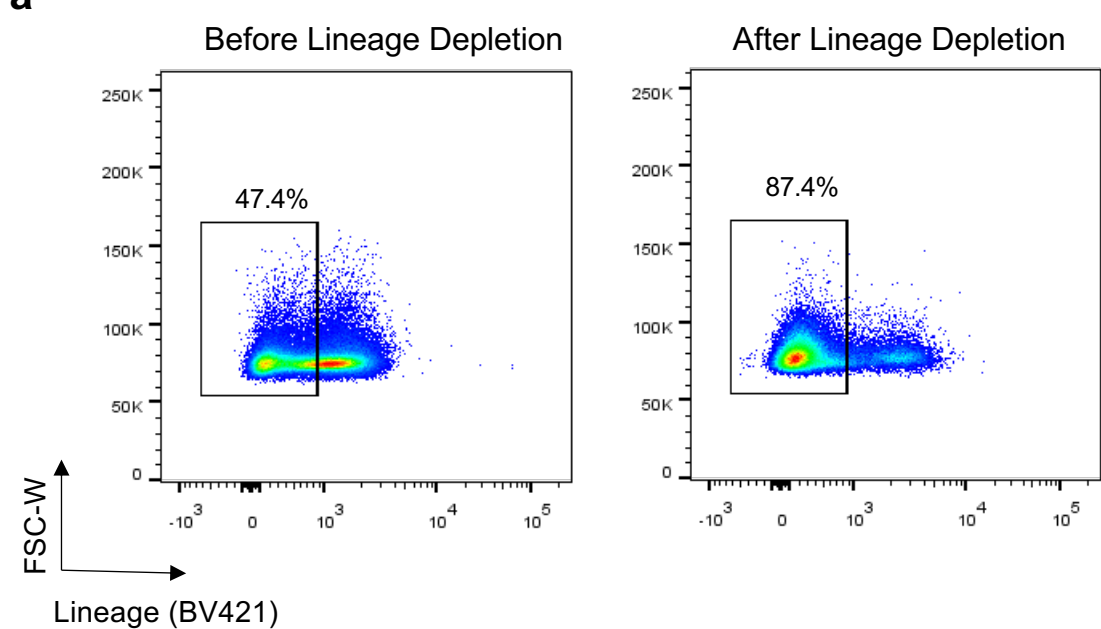


S6c

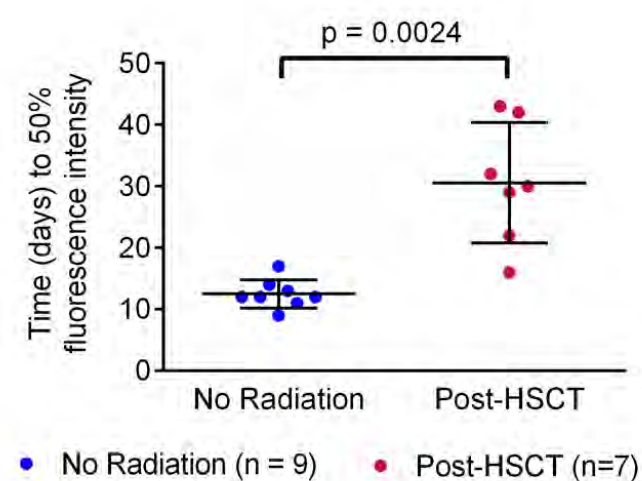


Supplementary Figure 7

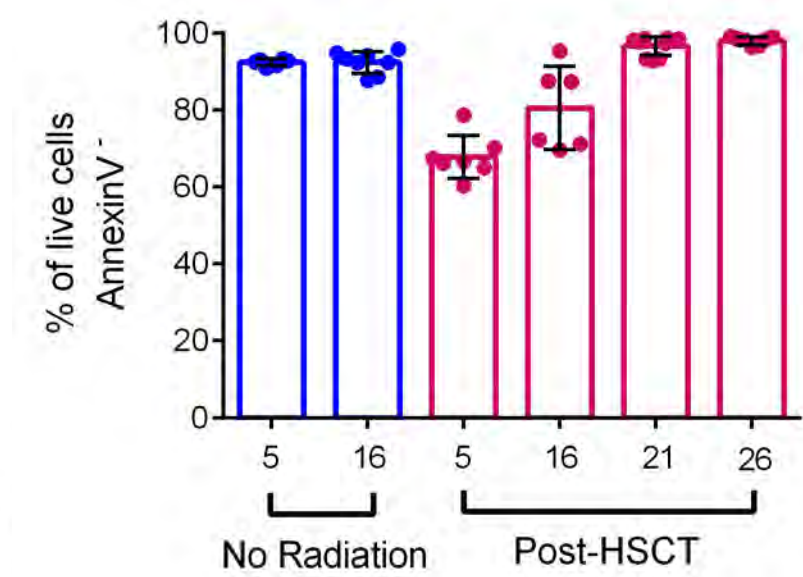
S7a



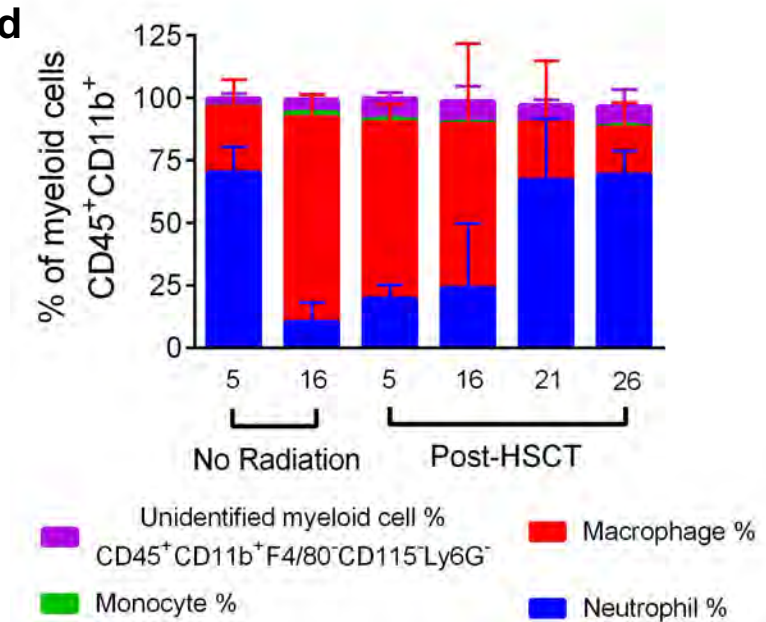
S7b



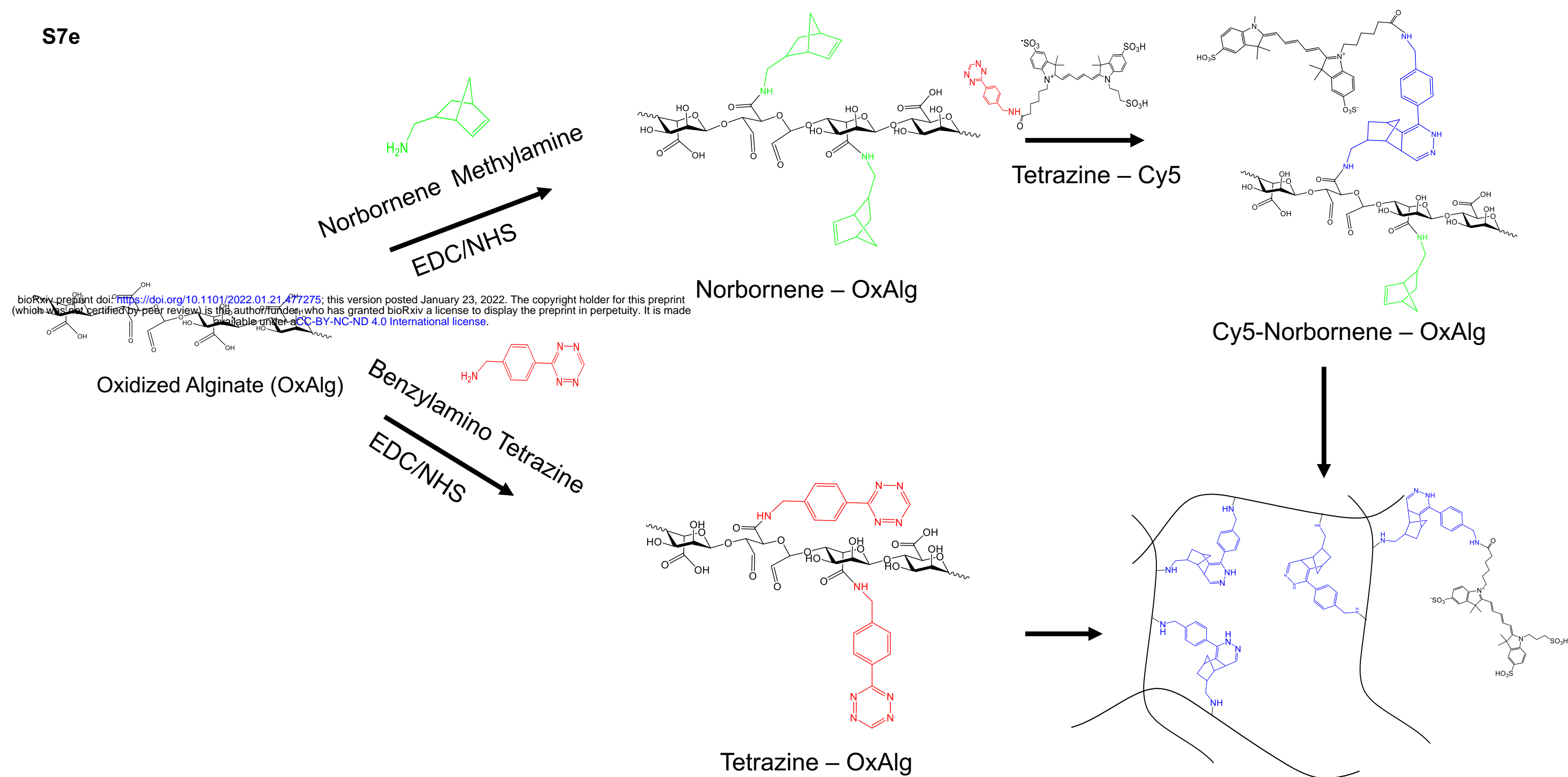
S7c



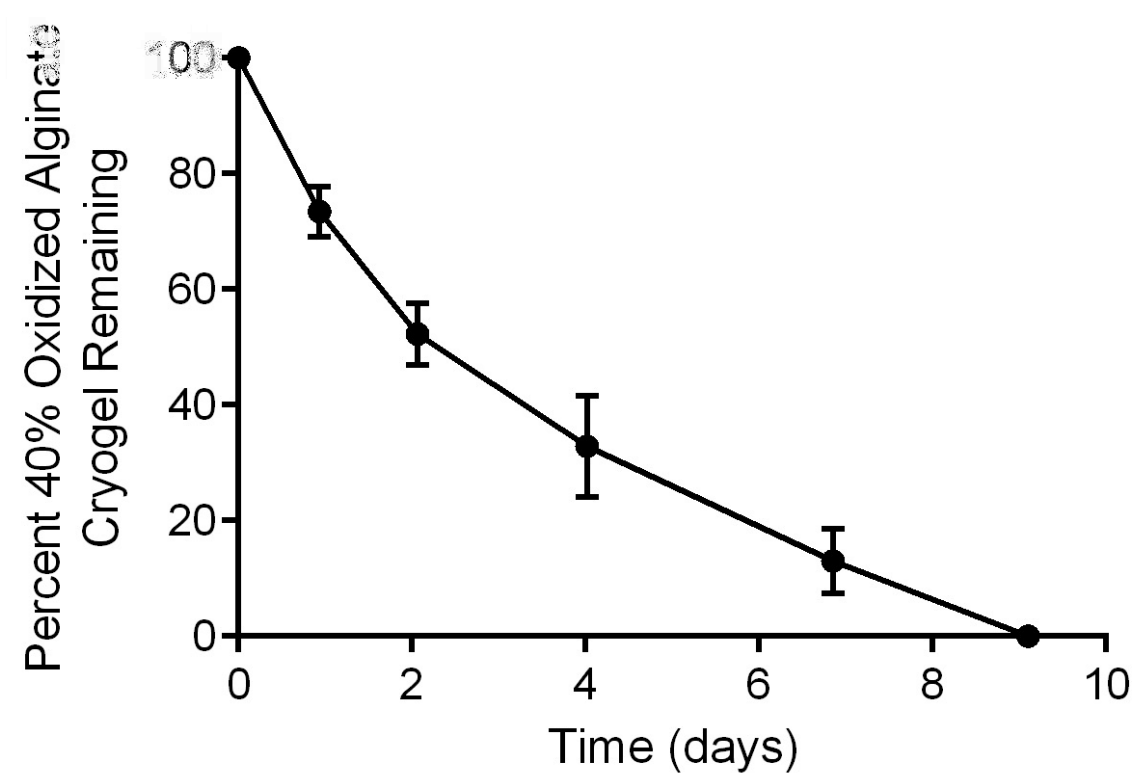
S7d



S7e



S7f



S7g

