

1 **Non-canonical IL-22 receptor signaling remodels the mucosal barrier during fungal
2 immunosurveillance**

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18 Th22, STAT3

19 **Abstract**

20 Mucosal barrier integrity is vital for homeostasis with commensal organisms while preventing
21 pathogen invasion. We unexpectedly found that fungal-induced immunosurveillance enhances
22 resistance to fungal outgrowth and tissue invasion by remodeling the oral mucosal epithelial
23 barrier in mouse models of adult and neonatal *Candida albicans* colonization. Epithelial subset
24 expansion and tissue remodeling were dependent on interleukin-22 (IL-22) and signal transducer
25 and activator of transcription 3 (STAT3) signaling, through a non-canonical receptor complex
26 composed of glycoprotein 130 (gp130) coupled with IL-22RA1 and IL-10RB. Immunosurveillance-
27 induced epithelial remodeling was restricted to the oral mucosa, whereas barrier architecture was
28 reset once fungal-specific immunity developed. Collectively, these findings identify fungal-induced
29 transient mucosal remodeling as a critical determinant of resistance to mucosal fungal infection
30 during early stages of microbial colonization.

31 **Main**

32 Oral mucosal tissues are continuously exposed to food, airborne antigens, and commensal
33 microbes, including fungi (1). The finely tuned implementation of innate and adaptive immune
34 responses enables the host to maintain homeostasis with commensal species and neutralize
35 invading organisms (2-4). As a central constituent of the mycobiome, the fungus *Candida albicans*
36 colonizes the oral mucosa of up to 75% of healthy individuals (5). Settings of local or systemic
37 immunosuppression result in oropharyngeal candidiasis (OPC) (6). Since, in healthy individuals
38 *C. albicans* causes no harm, fungal colonization appears to be evolutionarily selected for
39 appropriate metabolic function and immune priming (7-10). While *C. albicans* has been
40 extensively studied as a pathogen (11-14), the primary lifestyle of this fungus in the oral cavity is
41 as a commensal (15-17). The natural diversity in *C. albicans* influences the outcome of the
42 interaction between the fungus and the host (16) implying that the involvement of specific immune
43 pathways to the host defense against *C. albicans* is modulated in a strain-dependent manner. In
44 mouse models of OPC, *C. albicans* strains can be grouped into two categories: pathogenic-like
45 (PL) and commensal-like (CL) (16). While PL *C. albicans* strains induce a strong pro-inflammatory
46 response that leads to rapid clearance in the oral mucosa (acute OPC), CL *C. albicans* strains
47 instead trigger a tempered inflammatory response that permits long-term fungal colonization, thus
48 mimicking commensal colonization in humans. Still, the regulation of mucosal homeostasis during
49 commensal fungal colonization remains poorly understood.

50 The epithelial architecture of mucosal surfaces such as the oral cavity is crucial for its host
51 defensive function (18-20), providing structural immunity by initiating and coordinating immune
52 responses (21). However, relatively little is known about how this is coordinated in the oral
53 mucosa. Furthermore, the epithelium balances a multiplicity of roles in early life (22), while the
54 acquired microbiome contributes to the development of immunity in newborns. Following
55 exposure, the mucosal immune system of neonates undergoes successive, non-redundant
56 phases that support the developmental needs of the infant to establish immune homeostasis (23).
57 While tissue remodeling has been associated with pathological features post-injury or disease
58 (24, 25), commensal organisms may induce changes in barrier structures to promote homeostasis
59 (26).

60 Here, we studied mucosal immune responses and tissue homeostasis in mouse models of
61 persistent *Candida* oral colonization at distinct ranges of age. We show that immunosurveillance-
62 induced epithelial expansion and remodeling mediates resistance against fungal outgrowth during
63 the onset of fungal colonization. IL-22, a critical cytokine in epithelial homeostasis and host
64 defense at mucosal surfaces, has long been associated with signaling through a well-
65 characterized receptor complex (27). Traditionally, IL-22 exerts its protective effects through
66 binding to the IL22RA1-IL10RB receptor complex. However, our study reveals that IL-22
67 recognition and signaling extend beyond this canonical pathway, expanding the current
68 understanding of its biological roles. Oral mucosal remodeling required IL-22-mediated gp130
69 activation in non-canonical cytokine receptor complexes with IL-22RA1 and IL-10RB. IL-22
70 mediated oral epithelial remodeling was transitory and a subsequent mucosal remodeling event
71 in later stages of colonization required *Candida*-specific immunity. Finally, fungal-induced
72 mucosal remodeling prevents tissue invasion in a mouse model of neonatal colonization. These
73 findings provide novel insights into the molecular mechanisms of IL-22-mediated signaling,
74 constituting a new pathway for antifungal immunity, and expand our understanding of microbe-
75 induced epithelial remodeling and homeostasis through a non-canonical receptor complex.

76 **Persistent *C. albicans* colonization induces epithelial remodeling**

77 In a mouse model of OPC (28), acute infection with a prototypic PL *C. albicans* strain (SC5314)
78 leads to its rapid clearance from the oral cavity, whereas a CL *Candida* strain (CA101) persists in

79 the oral mucosa (Fig. S1)(15). Several host cell types respond to *C. albicans* encounter in the oral
80 cavity. To develop a single cell transcriptome profile of the oral mucosa, we evaluated gene
81 expression in tissues of CL colonized mice and from mice after pathogenic *Candida* clearance
82 (Fig. 1A). Our analysis identified 18 distinct cell subpopulations, which were present in both, CL
83 colonized mucosa and mucosal tissue after PL clearance (Fig. 1B). The identified subpopulations
84 expressed cell-type specific marker genes (Fig. S2) consistent with classical, well-established
85 markers for each respective cell population. The scRNA-sequencing analysis revealed that the
86 epithelial proportions increased during persistent fungal colonization (Figure 1C). Accordingly,
87 epithelial cells from colonized mice had higher expression of proliferation marker genes (*Mki67*,
88 *Cenpf*, *Cenpa*) (Fig. 1D). Tissue histology during the onset of CL *Candida* colonization revealed
89 that the epithelial layer expanded (Fig. 1E) and basal epithelial cell proliferation increased
90 indicated by Ki67 staining (Fig. 1F, G). Next, we performed RNA sequencing of epithelial-enriched
91 mucosal tissue (Fig. 1H). Using Gene Set Enrichment Analysis (GSEA), we found that genes
92 involved in keratinization and keratinocyte differentiation were significantly enriched in fungal
93 colonized epithelial tissues (Fig. 1I). Similarly, genes for keratinization were enriched in epithelial
94 cells from colonized mice in the scRNA-sequencing data set (Fig. S3). Keratins influence the
95 epithelial architecture by determining cell compartmentalization and differentiation (29). Several
96 keratins, including keratin 14 (K14), were highly expressed in the mucosa of colonized mice (Fig.
97 1J). Basal epithelial cells express K14 in all regions, while the suprabasal epithelial layer (SEL)
98 expresses K13 (30). Consistent with these findings the K14 epithelial layer expanded during
99 commensal colonization, while the epithelium had similar architecture after PL encounter and
100 clearance compared to Sham infection (Fig. 1K, L). Notably, the K13 layer distribution and
101 thickness remained unchanged during CL colonization (Fig. S4). Time course studies revealed
102 that K14 epithelial expansion occurs after 8 days of fungal colonization (Fig. 1M, S5). Collectively,
103 these data show that persistent fungal colonization induces distinct epithelial subset expansion;
104 thus, indicating that *Candida* commensalism influences the oral mucosal architecture.

105 **CD4 T cells are a major source of IL-22 during commensal colonization**

106 T cells play vital roles in the mucosal antifungal immunity (31, 32), while some CD4⁺ T cells reside in
107 the oral mucosa of healthy individuals (20). In fact, T cells can promote stromal cell proliferation
108 through secretion of cytokines (33-35). Thus, we compared protein expression kinetics of various T
109 helper (Th) cell-associated cytokines and chemokines during persistent *C. albicans* colonization and
110 acute OPC. Acute infection with the PL *C. albicans* strain led to early TNF α and IL-1 β induction,
111 whereas the CL *Candida* strain colonized the oral mucosa without inducing inflammation (Fig.
112 2A). In acute OPC, IL-17 and IL-22 are expressed by Type 17 cells with similar kinetics (11, 36-38).
113 In contrast, our data show that IL-22 and IL-17 are differentially expressed during persistent
114 colonization (Fig. 2A, B). Mice infected with a PL strain induced IL-17A and IL-22 similarly followed by
115 rapid decline after fungal clearance. However, in CL colonized mice, mucosal IL-22 levels remained
116 high, while IL-17A levels were low at the onset of colonization (Fig. 2B). IL-22 neutralization after CL
117 *C. albicans* colonization (Fig. 2C) resulted in fungal proliferation (Fig. 2D), while antibody
118 neutralization of IL-17A did not alter the fungal burden in colonized mice. This suggested that IL-22
119 may have a prominent role in controlling fungal outgrowth after mucosal colonization. Next, we
120 determined the cellular origin of IL-22. Our scRNA-sequencing dataset suggested that CD4 T cells
121 highly express *Il22* during persistent fungal colonization (Fig. 2E). Using *IL22TdTTomato* reporter mice,
122 we confirmed that CD4 T cells are the major source of IL-22 during colonization with CL *Candida* (Fig.
123 2F). Next, we evaluated localization of CD4 T cells during commensal colonization within the oral
124 mucosa. CD4 T cells were exclusively found in the epithelial and submucosal layers of commensal
125 colonized mice, which differed markedly from PL- or Sham-infected mice (Fig. 2G). Within the helper
126 T cells, IL-22 is mainly produced by Th17 and Th22 subsets (39). *Ex vivo* stimulation of mucosal
127 resident CD4 T cells from commensal colonized and pathogenic infected mice showed that during

128 commensal colonization Th17 and Th22 cells are the major source of IL-22, while Th22 cell
129 frequencies increased (Fig. 2H, I).

130 **Epithelial expansion depends on IL-22 signaling via non-canonical gp130 receptor
131 complexes**

132 The IL-17- and the IL-22 receptors, which mediate anti-*C. albicans* immunity during acute OPC,
133 play distinct and restricted roles in distinct sublayers of the stratified oral epithelium (36). During
134 CL-*Candida* colonization, IL-22RA1 protein expression extended into the suprabasal layer (Fig.
135 S6) consistent with K14 expression (Fig. 1K). IL-22 has been implicated in multiple aspects of
136 epithelial barrier function and wound repair, including regulation of cell growth (40). Accordingly,
137 IL-22 induced proliferation of human oral epithelial cells in a dose-dependent manner, while high
138 cytokine concentrations inhibited oral epithelial cell growth (Fig. 3A, S7). Similarly, IL-22 deficient
139 mice and IL-22 depletion during CL colonization reduced K14 epithelial layer expansion and
140 proliferation (Fig. 3B-D, Fig. S8), as well as resistance against fungal outgrowth (Fig. 3E) in the
141 setting of intact IL-17 signaling (Fig. S9). IL-22-mediated proliferation required JAK-TYK2-STAT3
142 signaling in human oral epithelial cells (Fig. S10). Classical IL-22 signal transduction is mediated
143 by binding of the cytokine to a receptor complex consisting of IL-22RA1 and IL-10RB (27).
144 However, while mice deficient for either receptor, IL-22RA1 and IL-10RB respectively, were more
145 susceptible to CL *Candida* outgrowth (Fig. 3F), the K14 layer surprisingly still underwent
146 expansion in the absence of either receptor chain (Fig. 3G, S11). Notably, IL-10 signals through
147 a heterotetrameric complex comprising of IL10Ra and IL10RB but was dispensable for fungal
148 control and epithelial remodeling (Fig. S12). Recently, studies in synthetic cytokine biology have
149 challenged the classical view of IL-22 signaling via a heterodimeric IL-22RA1 and IL-10RB
150 complex. Synthetic cytokine receptor chains of IL-22RA1 and IL-10RB form functional
151 heterodimeric receptor signaling complexes with a IL-6 receptor chain of gp130 to induce STAT3
152 signal transduction (41). Proximity ligation assays and co-immunoprecipitation identified the
153 classical heterodimeric IL-22RA1 and IL-10RB complex, while revealing that IL-10RB, as well as
154 IL-22RA1, form receptor complexes with gp130 in human oral epithelial cells (Fig. 3H, S13). We
155 tested whether antibody blockade of either receptor chain, IL22RA1 and IL-10RB, would abolish
156 STAT3 signal transduction in human oral epithelial cells. While antibodies blocking IL-22RA1 or
157 IL-10RB induced similar STAT3 activation following IL-22 exposure, treatment with both
158 antibodies reduced epithelial activation and IL-22 mediated proliferation (Fig. 3I, J, S14). Next,
159 we determined the contribution of gp130 signaling to IL-22-mediated oral epithelial proliferation.
160 Pharmacological inhibition of gp130 signaling reduced IL-22-mediated oral epithelial proliferation
161 *in vitro* (Fig. 3K), while reducing STAT3 activation (Fig. 3L, S14). IL-6 binds to membrane-bound
162 IL-6R to induce signaling via gp130 (42). However, IL-22-mediated epithelial proliferation was
163 independent of IL-6-gp130 signaling (Fig. S15). Of note, STAT3 activity remained unchanged
164 during inhibition of gp130 in intestinal epithelial cells (Fig. S16), suggestive of organ-specific
165 receptor complex signaling in response to IL-22. Inhibition of gp130 signaling during oral CL
166 persistence increased the fungal burden and inhibited K14 expansion and remodeling (Fig. 3M-
167 P). Thus, IL-22 signals via various receptor chain combinations to provide oral mucosal antifungal
168 immunity during fungal immunosurveillance, while oral epithelial proliferation and expansion
169 depends on non-canonical gp130 signaling receptor complexes.

170 **Transient epithelial expansion depends on development of *Candida*-specific immunity**

171 While oral CL infection leads to epithelial expansion at the onset of colonization, at later time
172 points of fungal persistence the K14 expansion is reset to levels comparable to the baseline cell
173 turnover seen in naïve mice (Fig. 4A-C), suggesting that mucosal remodeling is transitory,
174 regulated, and depends on an auxiliary mechanism. A unifying theme of susceptibility to
175 mucocutaneous candidiasis is seen in both humans and mice with a variety of genetic defects

176 within the IL-17 pathway (3, 31, 43). The generation of organism-specific adaptive immune
177 responses takes time to generate sufficient cells, due to the inherent demands for extensive
178 proliferation and differentiation of naive cells into effector cells (44). Thus, we tested if *Candida*-
179 specific immunity is required to remodel the oral mucosal barrier in subsequent phases of
180 colonization. During late stages of fungal persistence, increased numbers of *Candida*-specific IL-
181 17 secreting cells in cervical lymph nodes (Fig. 4D) inversely correlated with reversion of the K14
182 barrier thickness (Fig. 4E) suggesting the requirement of *Candida*-specific immunity to reset
183 barrier architecture. *Rag1*^{-/-} were more susceptible to fungal outgrowth at late stages during
184 colonization (Fig. 4F), while K14 epithelial expansion remained high (Fig. 4G, H). Collectively, we
185 show that IL-22-mediated oral epithelial remodeling is transitory and requires *Candida*-specific
186 immunity for a subsequent mucosal remodeling event in later stages of colonization.

187 **Commensal-induced epithelial expansion prevents fungal tissue invasion in neonates**

188 Neonates exhibit differentially adapted immune responses when compared to adults (45), with
189 important implications for immunity to OPC; indeed, oral thrush is commonly seen in infants (46),
190 though the underlying basis for this is not well defined. In this regard, neonatal umbilical cord
191 blood-derived CD4 T cells are intrinsically less able to differentiate into Th17 cells, but rather tend
192 to skew towards an IL-22 phenotype (47). Here, we established a mouse model of neonatal fungal
193 colonization to study early-life interactions between the oral epithelium and *Candida*, which occurs
194 in humans (Fig. 5A, B). Consistent with our findings in adult animals, oral CL colonization in
195 neonates induced K14 epithelial expansion (Fig. 5C, D) and CD4 T cell infiltration at the onset of
196 colonization (Fig. S17). Neonatal mice deficient in IL-22 had increased oral fungal burden,
197 abrogated K14 expansion, and decreased fungal-induced epithelial proliferation (Fig. 5F-H).
198 Furthermore, IL-22 deficiency in neonates increased the depth of fungal tissue invasion (Fig. 5I,
199 J) which was not observed in adult *Il22*^{-/-} mice (Fig. 5J) suggesting that IL-22-mediated immunity
200 and associated tissue remodeling plays a critical role preventing fungal invasion during early life,
201 highlighting the importance of IL-22 in neonatal immune defense and epithelial protection.

202 **Discussion**

203 Resistance to mucosal fungal infection is tightly linked to maintenance of barrier integrity (20, 48).
204 The communication between epithelial and immune cells enables coordinated responses that
205 maintain homeostasis and elicit host defenses (21, 49). Our findings support a paradigm by which
206 fungal-induced immunosurveillance in the oral mucosa leads to transitory mucosal tissue
207 remodeling and enhanced barrier resistance to fungal outgrowth.

208 The microbiome is a crucial factor for shaping and modulating immune system responses through
209 cytokine signatures (50). As such, our data unveil an IL-22-mediated pathway that critically
210 regulates mucosal antifungal host defense in mice following continued exposure to fungi. We
211 demonstrate that oral epithelial IL-22 signaling relies on various receptor chain combinations to
212 mediate antifungal immunity, while gp130 receptor complexes drive epithelial remodeling and
213 proliferation, which expands on reported tissue-specific protective roles of IL-22 receptor signaling
214 (51). Our findings of a non-canonical IL-22 receptor complex to orchestrate epithelial remodeling
215 represents a novel mechanism of mucosal antifungal defense, challenging the classical
216 understanding of IL-22 signaling. The IL-22 recognizing receptor complexes contribute to different
217 degrees to oral antifungal immunity indicated by diverse level of fungal dysbiosis. These findings
218 align with clinical phenotypes of inborn errors of immunity in IL-10RB, in which patients are
219 resistant to oral candidiasis (52) while a small fraction of patients with gp130-dependent Hyper-
220 IgE syndrome develop chronic mucocutaneous candidiasis (53, 54). Future studies will be
221 required to determine additive and compensatory mechanisms of the IL-22RA1, IL-10RB, and
222 gp130 epithelial receptor complexes during fungal colonization and infection in the oral mucosa.
223 Inhibition of gp130, as well as dual receptor blockage of IL-22RA1 and IL-10RB, impairs IL-22-

224 mediated STAT3 phosphorylation and epithelial proliferation, while some transcription factor
225 activity remains. T cell differentiation requires optimal STAT3 phosphorylation (55) suggesting a
226 similar mechanism requiring fine-tuned STAT3 activity exist in epithelial cells to induce
227 proliferation. On the other hand, IL-22 signaling via IL-22RA1, IL-10RB, and gp130 complexes is
228 organ- and context dependent since IL-22-mediated STAT3 activation in intestinal epithelial cells
229 occurs independent of gp130.

230 *Candida*-responsive CD4⁺ T cells are primed in all healthy individuals as a consequence of
231 exposure to the commensal fungus (4). Predominantly characterized by a Th17 profile,
232 *C. albicans*-specific T cells are detected in epithelial tissues at the onset of colonization in
233 experimental models of mucosal infection (56, 57). During the initial phases of acute mucosal
234 infection in a naïve host, anti-*Candida* immunity is driven by epithelial pattern recognition and
235 damage, and subsequent innate and bystander T cell responses (13, 58). Within the oral mucosa,
236 tissue-resident Flt3L-dependent dendritic cells (DCs) and CCR2-dependent monocyte-derived
237 DCs collaborate in fungal antigen presentation and T cell priming (59). The generation of antigen-
238 specific immunity depends on its nature and the site of exposure. Transitory epithelial proliferation
239 and oral mucosal remodeling enhances resistance to fungal outgrowth, while reversal of the
240 epithelial expansion occurs once *Candida*-specific immunity is developed (Fig. S18). The
241 sustained epithelial expansion and increased fungal burden in mice lacking fungal-specific
242 immunity underscore the importance of functional adaptive immunity in controlling both, epithelial
243 remodeling and fungal persistence.

244 During early life, newborns encounter an abundance of antigenic challenges derived from
245 commensal and pathogenic organism. Although 5–7% of infants develop oral candidiasis (46),
246 aberrant cellular innate immune responses and an inexperienced adaptive immune system do
247 not elaborate high resistance against fungal outgrowth. Thus, neonates may exhibit a degree of
248 immunological tolerance to fungal colonization to prevent harmful immune reactions in mucosal
249 tissues. After birth, the neonatal epithelium is permeable, while lacking pattern recognition
250 receptors (60, 61) suggesting that neonatal oral epithelial cells have reduced sensitivity to
251 microbial exposure relative to the adult to prevent excessive immune reactivity (61). However, the
252 microbiota induces a temporary protective mechanism in the neonatal oral epithelium by
253 increasing salivary antimicrobial components that shape the oral bacterial composition and
254 burden (60, 61). Here we show that neonatal oral *Candida* colonization and consequently IL-22-
255 mediated mucosal remodeling prevents fungal epithelial invasion. Given that neonates mount a
256 vigorous T cell response to microbial exposure rather than developing immunological memory
257 (62), oral mucosal remodeling may play a key role to control colonizing fungi during early life.
258 Understanding such early mechanisms are important for human health, because a deficiency of
259 this remodeling process could translate into oral and systemic pathologies in adult life. Future
260 studies will be required to determine epithelial remodeling and turnover to age-related differences
261 in bystander and antigen-specific T cell activation to maintain mucosal homeostasis.

262 Collectively, our findings identify oral mucosal remodeling through non-canonical IL-22 receptor
263 signaling as a critical determinant of resistance to mucosal fungal infection and highlight the
264 importance of tissue-specific immune responses in the control of infectious disease.

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275 **Author contributions**

276 Conceptualization: MS
277 Methodology: NM, JS, NS, AM, FA, AW, MSL, SLG, NJ, SGF, MS
278 Investigation: NM, JS, NS, AM, FA, AW, MS
279 Visualization: NM, JS, FA, AW, MS
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281 Project administration: MS
282 Supervision: NJ, MS
283 Writing – original draft: MS
284 Writing – review & editing: NM, JS, NS, AM, FA, AW, MSL, SLG, NJ, SGF, MS

285 **Competing interests**

286 The authors declare no competing interests.

287 **Data and materials availability**

288 The authors declare that the data supporting the findings of this study are available within the
289 paper and the accompanying supplementary information files. The high-throughput sequencing
290 data from this study have been deposited with links to BioProject accession numbers xxx and xxx
291 in the NCBI BioProject database (accession numbers will be provided once paper is accepted for
292 publication).

293 **Figure 1 Oral *C. albicans* colonization induces epithelial remodeling.** **A** Experimental setup
294 for single cell collection and sequencing. **B** Cell types identified in the oral mucosa after 11 days
295 of CL and PL infection with UMAP projections of scRNA-seq data. **C** Proportions of identified cell
296 types separated by PL and CL infection. **D** Expression of *Miki67*, *Cenpf*, and *Cenpa* in epithelial
297 cells of PL and CL infected mice. **E** Representative pictures of PAS staining of CL-infected mice
298 over time. **F** Representative pictures of Ki67 staining of CL-infected mice over time. Scale bar
299 50 μ m. **G** Quantification of Ki67 $^{+}$ DAPI $^{+}$ cells CL-infected mice over time. $N=3$; Ordinary one-way
300 ANOVA. **H** Experimental setup for sequencing of epithelial-enriched tissues. **I** GSEA of
301 keratinocyte differentiation and keratinization sequencing data of epithelial-enriched tissues.
302 $N=3-4$. **J** Z-scores of keratin genes in epithelial-enriched tissues after PL and CL infection (Day
303 11). $N=3-4$ **K** Representative pictures of K14 staining of PL- and CL-infected mice after 11 days.
304 **L** Quantification of K14 thickness in Sham-, PL, and CL-infected mice after 11 days. $N=6$;
305 combined data of two independent experiments. Ordinary one-way ANOVA. **M** Quantification of
306 K14 thickness in CL-infected mice over time. $N=5$; combined data of two independent
307 experiments. Ordinary one-way ANOVA. PL, pathogen-like; CL, commensal-like.

308 **Figure 2 Th17/Th22 cells produce IL-22 during *C. albicans* colonization.** **A** Heat map
309 presented in fold change of various cytokines during PL, CL, and Sham infection over time $N=10$;
310 combined data of two independent experiments. Two-tailed Mann–Whitney Test. **B** Levels of IL-
311 17A and IL-22 in PL-, CL-, and Sham-infected mice; $N=10$. Two-tailed Mann–Whitney Test.
312 **C** Scheme of IL-17 and IL-22 depletion during CL-colonization. **D** Oral fungal burden of mice
313 colonized with CL after treatment on day 11 and 13 with anti-IL-17A or anti-IL-22 antibodies. $N=6$;
314 combined data of two independent experiments. Two-tailed Mann–Whitney Test. The y-axis is
315 set at the limit of detection (20 CFU/g tissue). **E** Expression of *Il22* in CD4 T cells identified in the
316 single cell RNA-sequencing data set. **F** Quantification of IL-22 expressing cells during CL
317 colonization using *IL22TdTTomato* reporter mice. $N=4$. Two-tailed Mann–Whitney Test. **G**
318 Representative immunofluorescence pictures of localization of CD4 T cells in tissues after 11
319 days of infection. Scale bar 50 μ m. **H** Representative flow cytometry plots for intracellular IL-17A
320 and IL-22 levels in CD4+ cells. **I** Levels of Th17 and Th22 cells after 11 days of infection. $N=6$;
321 combined data of two independent experiments. Two-tailed Mann–Whitney Test. PL, pathogen-
322 like; CL, commensal-like.

323 **Figure 3 Epithelial expansion requires non-classical IL-22 signaling** **A** Area under the curve
324 (AUC) of oral epithelial cells in response to different concentrations of IL-22. Growth was
325 determined by confluence over time. $N=8$. Ordinary one-way ANOVA. **B** Representative pictures
326 of K14 staining of Sham- and CL-infected mice after 11 days. Scale bar 50 μ m. **C** Quantification
327 of K14 thickness in Sham- and CL-infected WT and *Il22* $^{-/-}$ mice at day 11. $N=6$; combined data
328 of two independent experiments. Two-tailed Mann–Whitney Test. **D** Quantification of Ki67 $^{+}$ DAPI $^{+}$
329 cells CL-infected mice over time. $N=6$; combined data of two independent experiments. Two-
330 tailed Mann–Whitney Test. **E** Oral fungal burden of WT and *Il22* $^{-/-}$ mice colonized with CL after
331 11 days. $N=8-9$; combined data of two independent experiments. Two-tailed Mann–Whitney
332 Test. The y-axis is set at the limit of detection (20 CFU/g tissue). **F** Oral fungal burden of indicated
333 mice colonized with CL after 11 days. $N=7$; combined data of two independent experiments.
334 Brown-Forsythe and Welch ANOVA. The y-axis is set at the limit of detection (20 CFU/g tissue).
335 **G** Quantification of K14 thickness in indicated mice at day 11. $N=6$; combined data of two
336 independent experiments. Ordinary one-way ANOVA. **H** (Left) Representative Images for
337 proximity ligation assay (PLA) for indicated receptor complexes. Scale bar 50 μ m. Red dots
338 indicate receptor complexes. (Right) Quantification of average number of complexes per cell. N
339 = 5. **I** Representative immunoblot of STAT3 activation during IL-22 incubation in the presence of
340 IL-22RA1, IL-10RB, or combination of IL-22RA1/IL-10RB blocking antibodies. **J** Area under the

341 curve (AUC) of oral epithelial cells in response to IL-22 treatment in the presence of IL-22RA1,
342 IL-10RB, or combination of IL-22RA1/IL-10RB blocking antibodies. Growth was determined by
343 confluence over time. $N = 8$. Ordinary one-way ANOVA. **K** Area under the curve (AUC) of oral
344 epithelial cells in response to IL-22 treatment in the presence of the gp130 inhibitor SC144
345 (igp130). Growth was determined by confluence over time. $N = 8$. Ordinary one-way ANOVA. **L**
346 Representative immunoblot of STAT3 activation during IL-22 incubation in the presence of gp130
347 inhibitor, IL-22RA1, IL-10RB, or combination. **M** Oral fungal burden of mice treated with gp130
348 inhibitor (igp130) or vehicle control (Veh) colonized with CL after 11 days. $N = 6$; combined data
349 of two independent experiments. Two-tailed Mann–Whitney Test. The y-axis is set at the limit of
350 detection (20 CFU/g tissue). **N** Representative pictures of K14 staining of Veh- and igp130 treated
351 mice after 11 days of CL infection. Scale bar 50 μ m **O** Quantification of K14 thickness in Veh- and
352 igp130 treated mice after 11 days of CL infection. $N = 6$; combined data of two independent
353 experiments. Two-tailed Mann–Whitney Test. **P** Quantification of Ki67⁺ DAPI⁺ cells CL-infected
354 mice in the presence and absence of gp130 signaling. $N = 6$; Two-tailed Mann–Whitney Test. PL,
355 pathogen-like; CL, commensal-like.

356 **Figure 4 Transitory epithelial K14 expansion depends on *Candida*-specific immunity. A**
357 Oral fungal burden of mice colonized with CL after indicated time points. $N = 6$; combined data of
358 two independent experiments. Two-tailed Mann–Whitney Test. The y-axis is set at the limit of
359 detection (20 CFU/g tissue). **B** Representative pictures of K14 staining of CL-infected mice after
360 11 and 31 days. Scale bar 50 μ m. **C** Quantification of K14 thickness in CL-infected mice at
361 indicated time points. $N = 6$; combined data of two independent experiments. Two-tailed Mann–
362 Whitney Test. **D** Determination of IL-17 secreting cells isolated from cervical lymph nodes mice
363 and stimulated with *Candida* antigen pools for 24 hours using ELISpot. $N = 6$. Ordinary one-way
364 ANOVA. **E** Correlation of K14 thickness and *Candida* (Ca)- specific IL-17 secreting cells. $N = 6$.
365 Correlation was determined by Pearson. **F** Oral fungal burden of WT and *Rag1*^{−/−} mice colonized
366 with CL after 31 days. $N = 5$; combined data of two independent experiments. Two-tailed Mann–
367 Whitney Test. The y-axis is set at the limit of detection (20 CFU/g tissue). **G** Representative
368 pictures of K14 staining of CL-infected mice after 31 days. Scale bar 50 μ m. **H** Quantification of
369 K14 thickness in CL-infected mice after 31 days. $N = 5$; combined data of two independent
370 experiments. Two-tailed Mann–Whitney Test. PL, pathogen-like; CL, commensal-like.

371 **Figure 5 Epithelial remodeling prevents fungal invasion during neonatal colonization. A**
372 Experimental setup for neonatal colonization of wild type mice. **B** Oral fungal burden of neonatal
373 mice Sham-infected or colonized with CL after indicated time points. $N = 4-6$; combined data of
374 two independent experiments. Two-tailed Mann–Whitney Test. The y-axis is set at the limit of
375 detection (20 CFU/g tissue). **C** Representative pictures of K14 staining of Sham-infected or CL-
376 infected mice at day 14 of life. Scale bar 50 μ m. **D** Quantification of K14 thickness in Sham-infected
377 or CL-infected mice at day 14 of life. $N = 5$; combined data of two independent experiments. Two-
378 tailed Mann–Whitney Test. **E** Oral fungal burden of neonatal WT and *Il22*^{−/−} mice colonized with
379 CL at day 14 of life. $N = 6$; combined data of two independent experiments. Two-tailed Mann–
380 Whitney Test. The y-axis is set at the limit of detection (20 CFU/g tissue). **F** Representative
381 pictures of K14 staining of WT and *Il22*^{−/−} mice colonized with CL at day 14 of life. Scale bar 50 μ m.
382 **G** Quantification of K14 thickness in neonatal WT and *Il22*^{−/−} mice colonized with CL at day 14 of
383 life. $N = 5$; combined data of two independent experiments. Two-tailed Mann–Whitney Test. **H**
384 Quantification of Ki67⁺ DAPI⁺ cells in neonatal WT and *Il22*^{−/−} mice colonized with CL at day 14 of
385 life. $N = 6$; combined data of two independent experiments. Two-tailed Mann–Whitney Test. **I**
386 Representative PAS staining of tongue tissues of neonatal (day 14 of life/day 12 of CL
387 colonization) and adult (day 11 of CL colonization) WT and *Il22*^{−/−} mice. Scale bar 50 μ m. **J**
388 Quantification of depth of fungal invasion in neonatal WT and *Il22*^{−/−} mice colonized with CL at
389 day 14 of life and adult mice after 11 days of CL colonization. $N = 8-12$; combined data of four

390 independent experiments. Two-tailed Mann–Whitney Test. PL, pathogen-like; CL, commensal-like.

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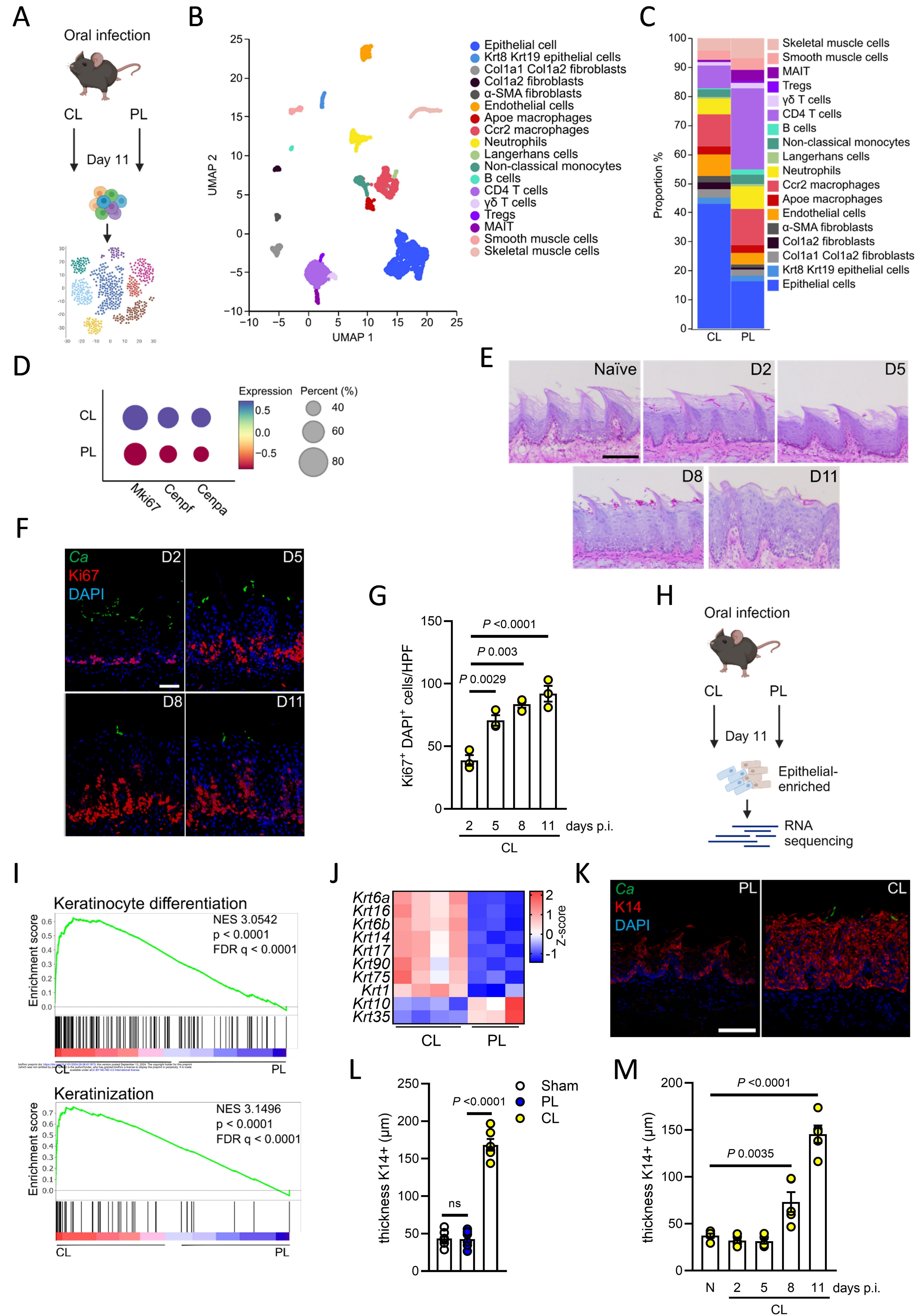
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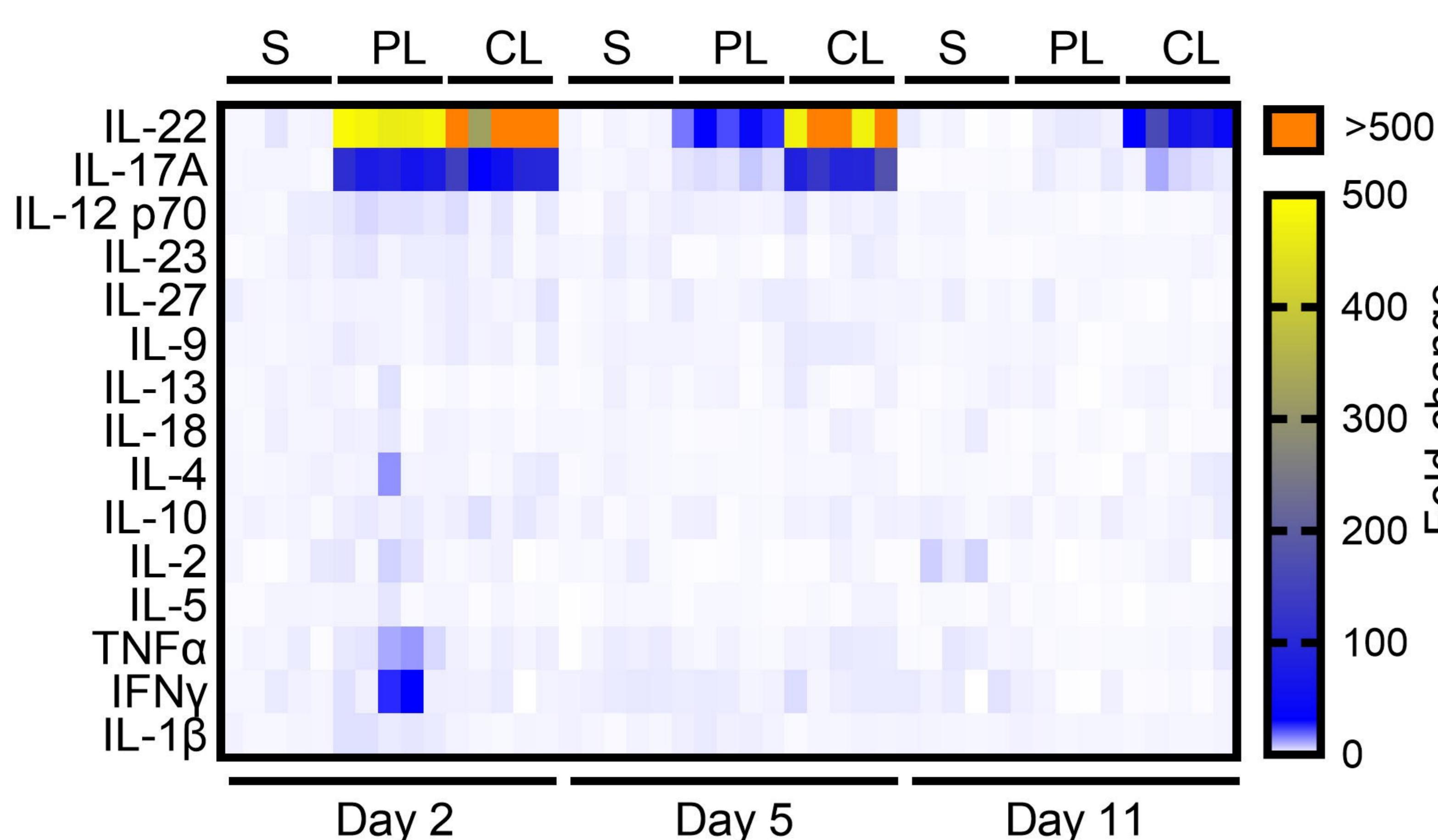
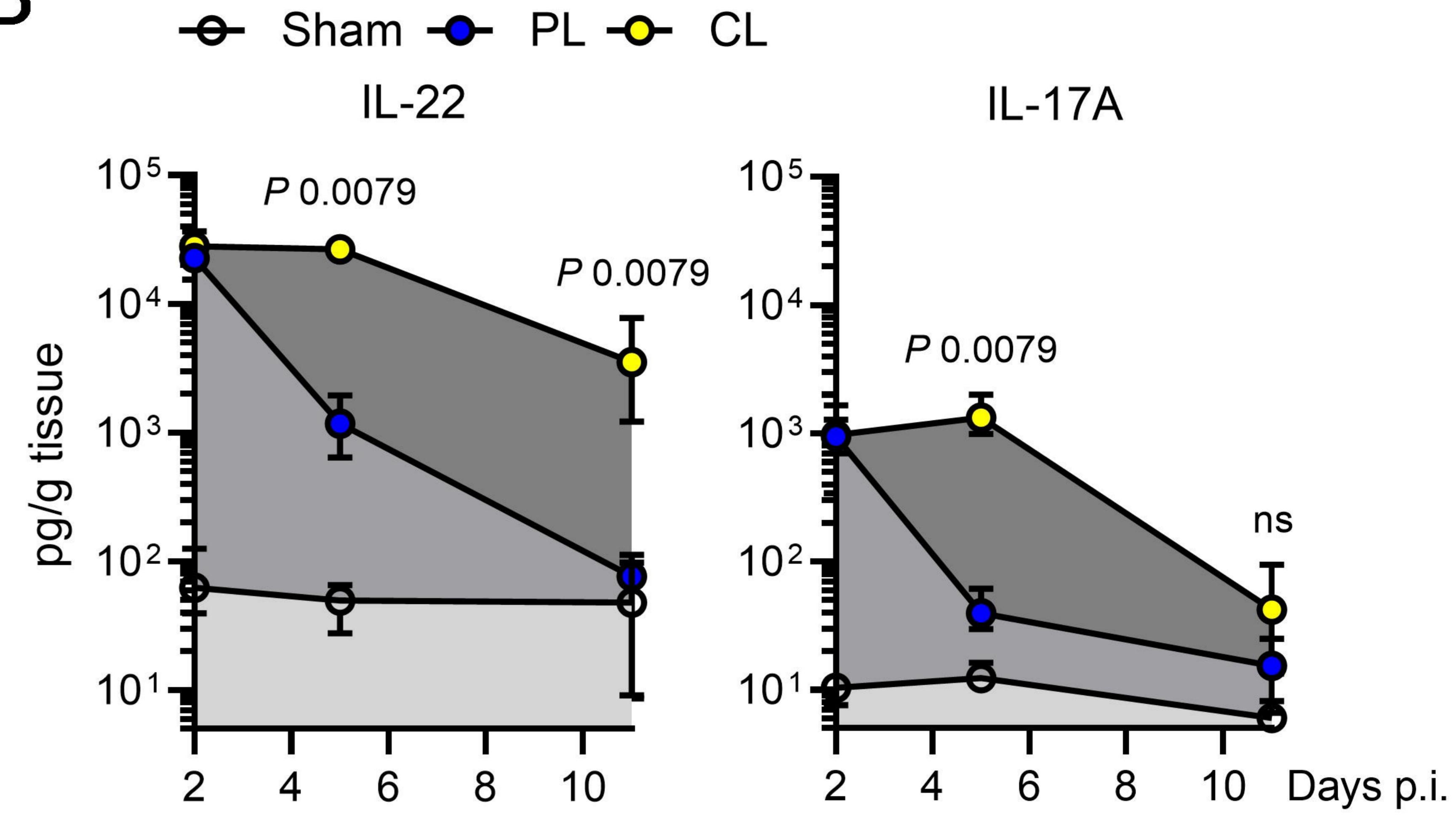
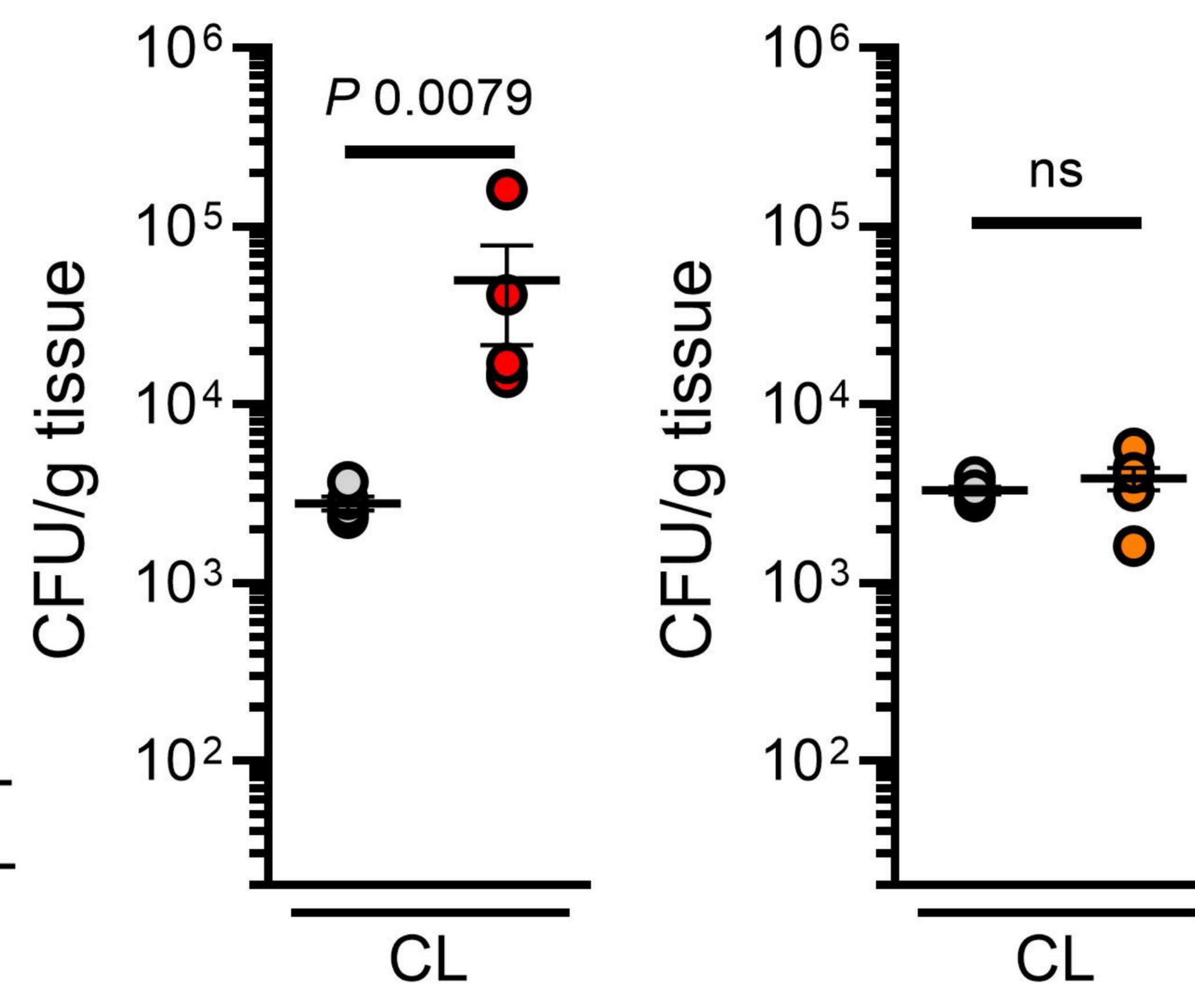
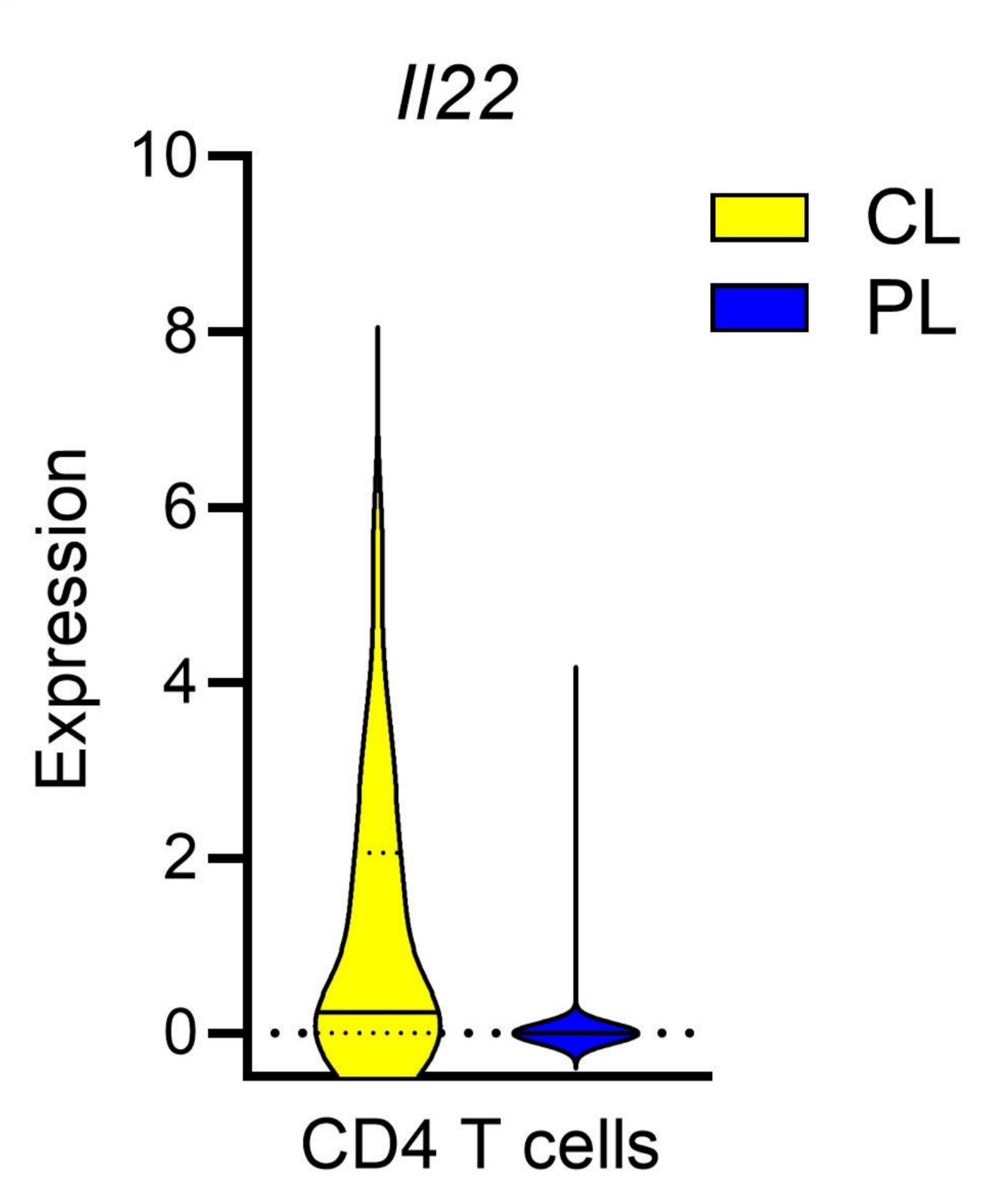
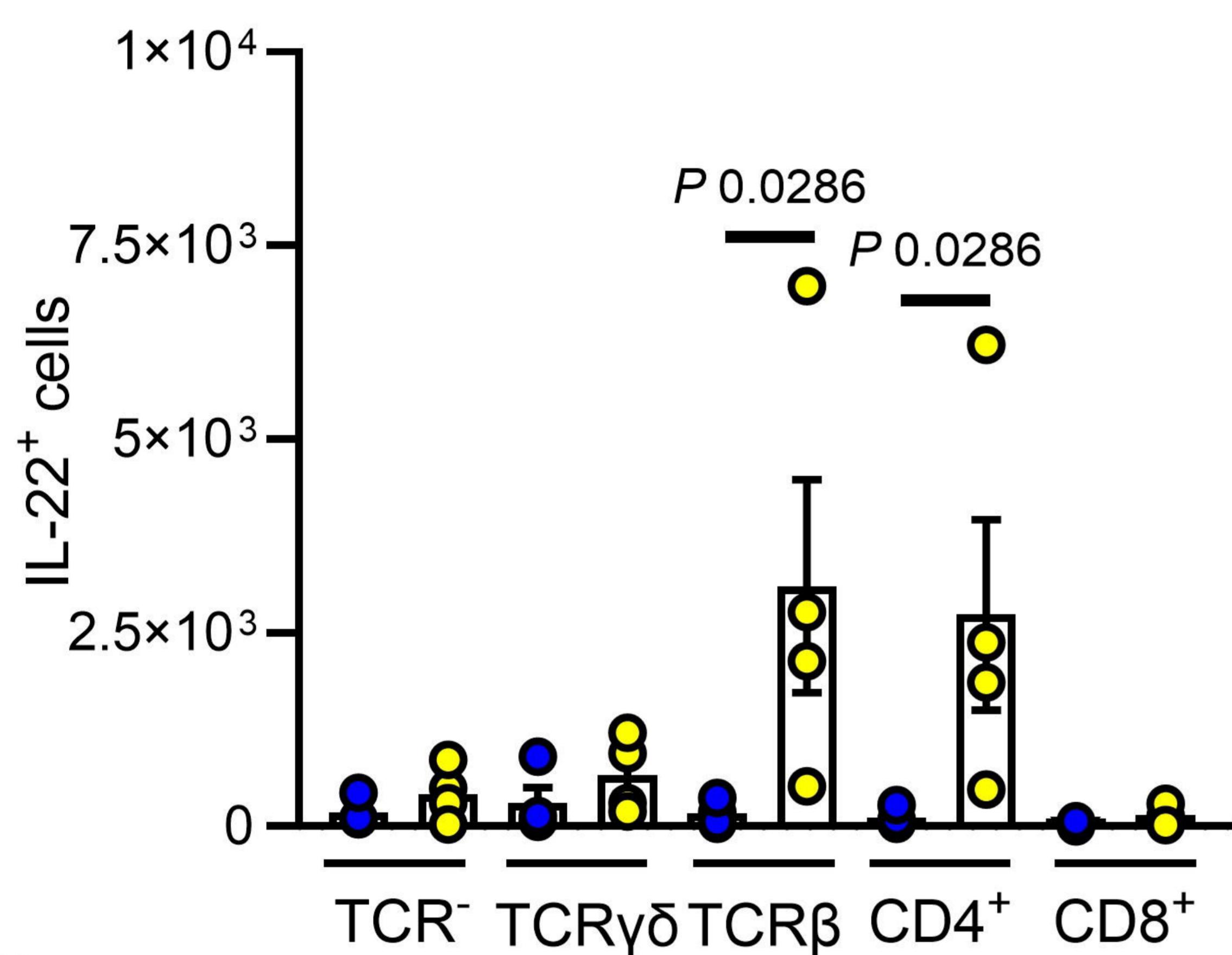
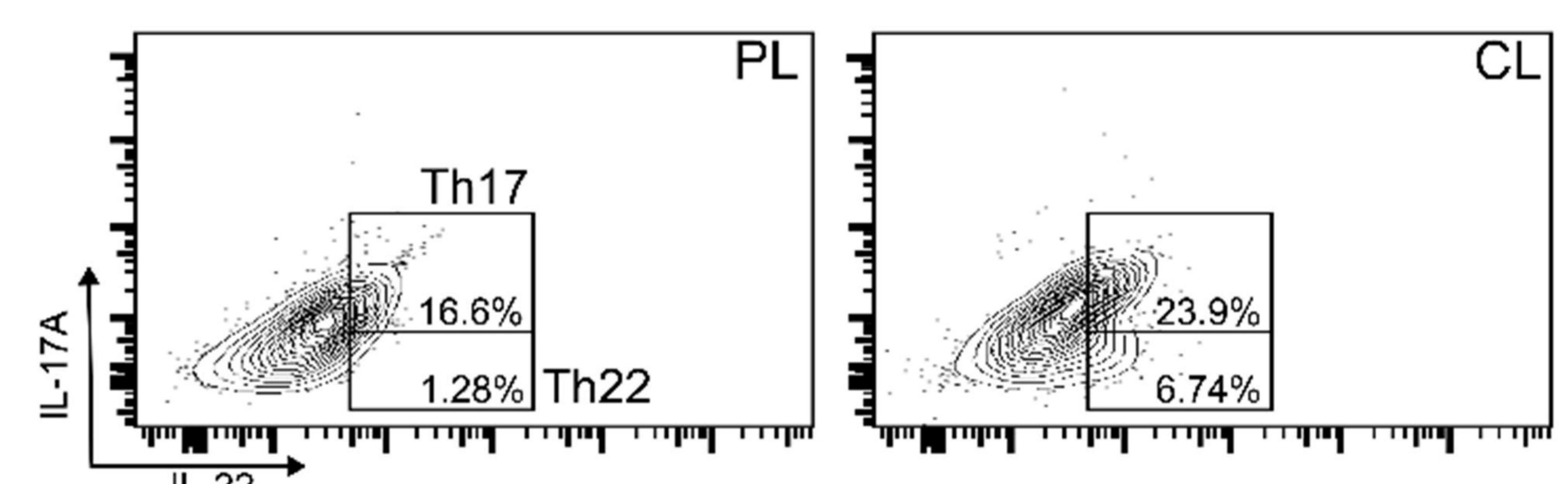
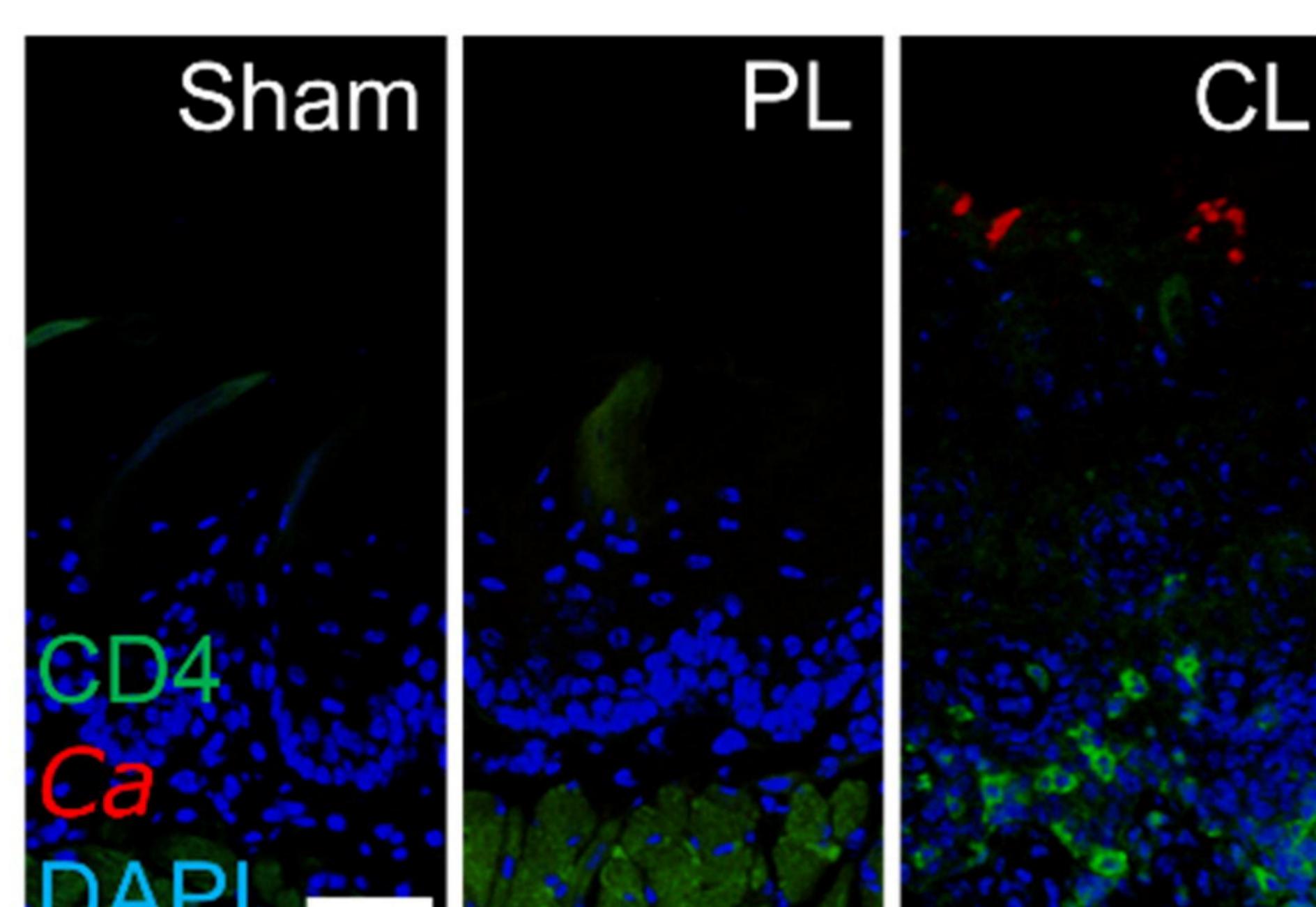
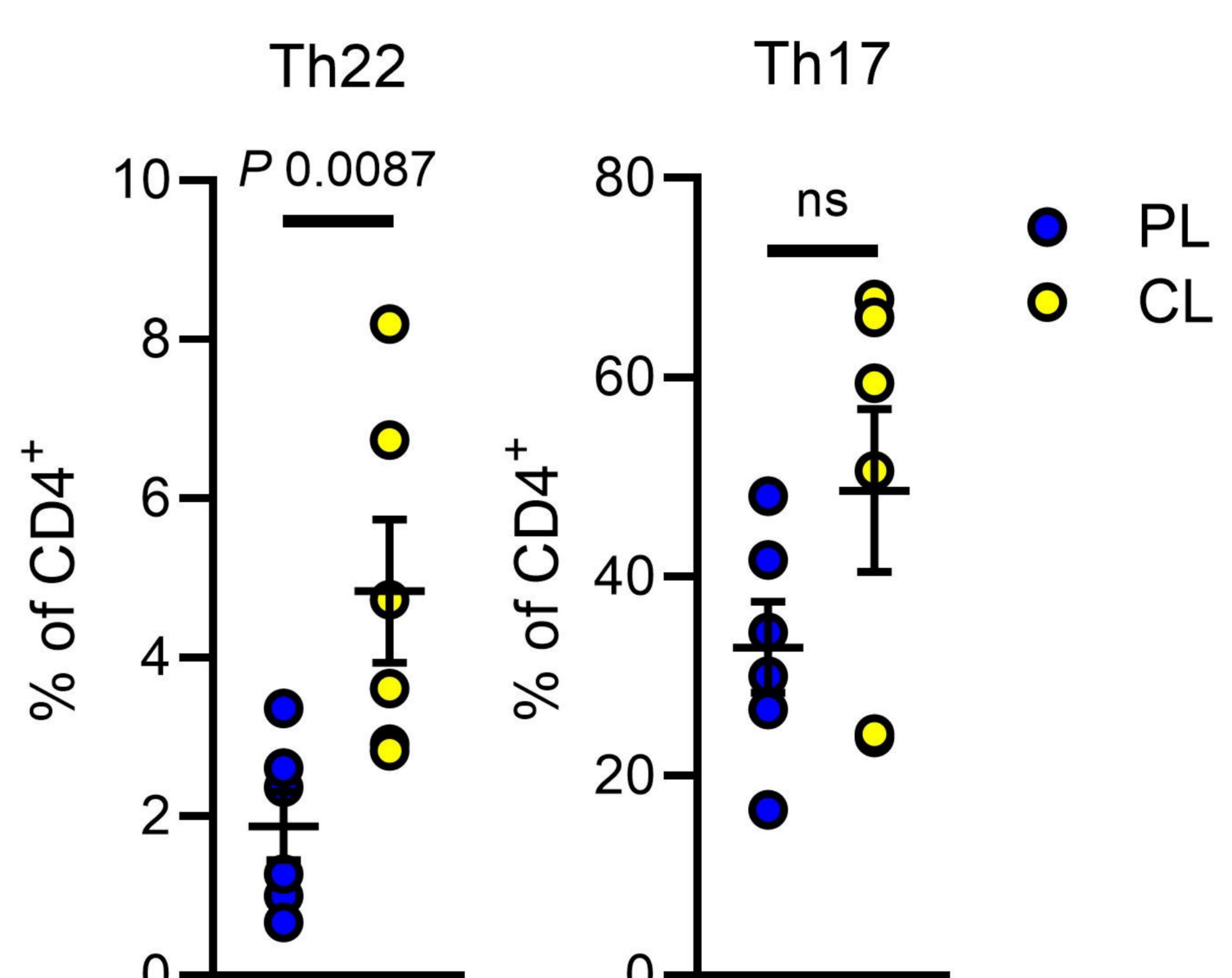
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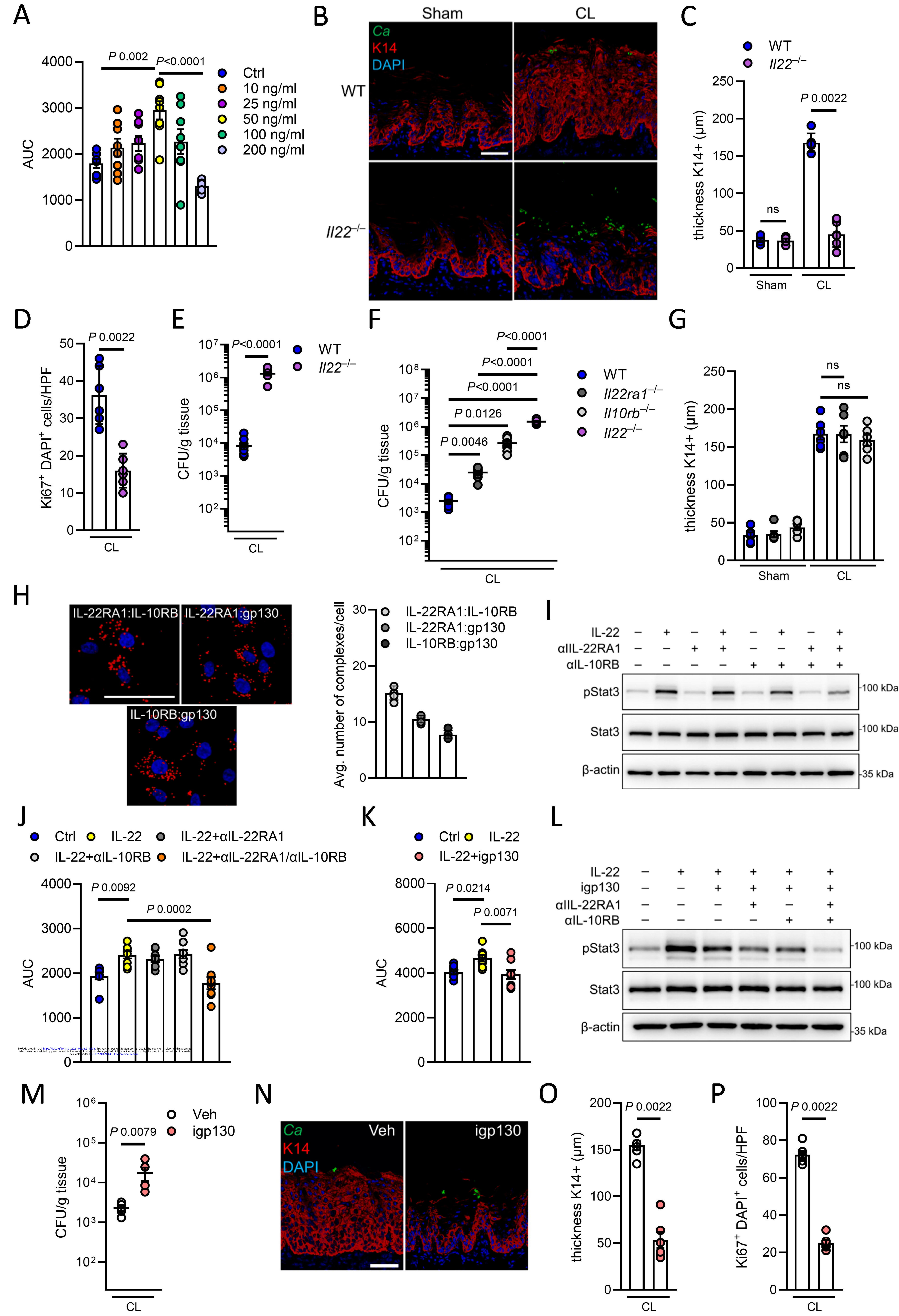
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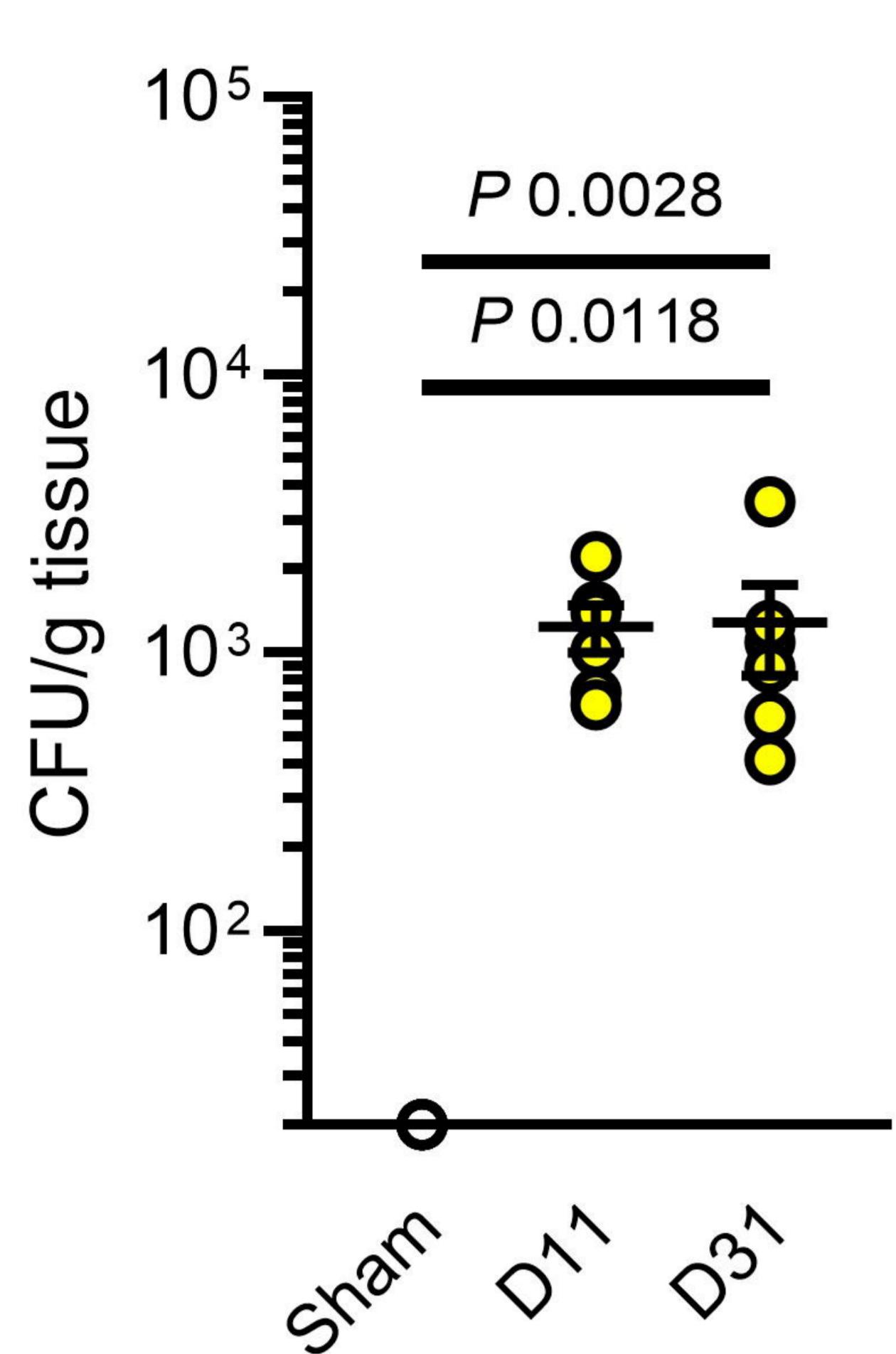
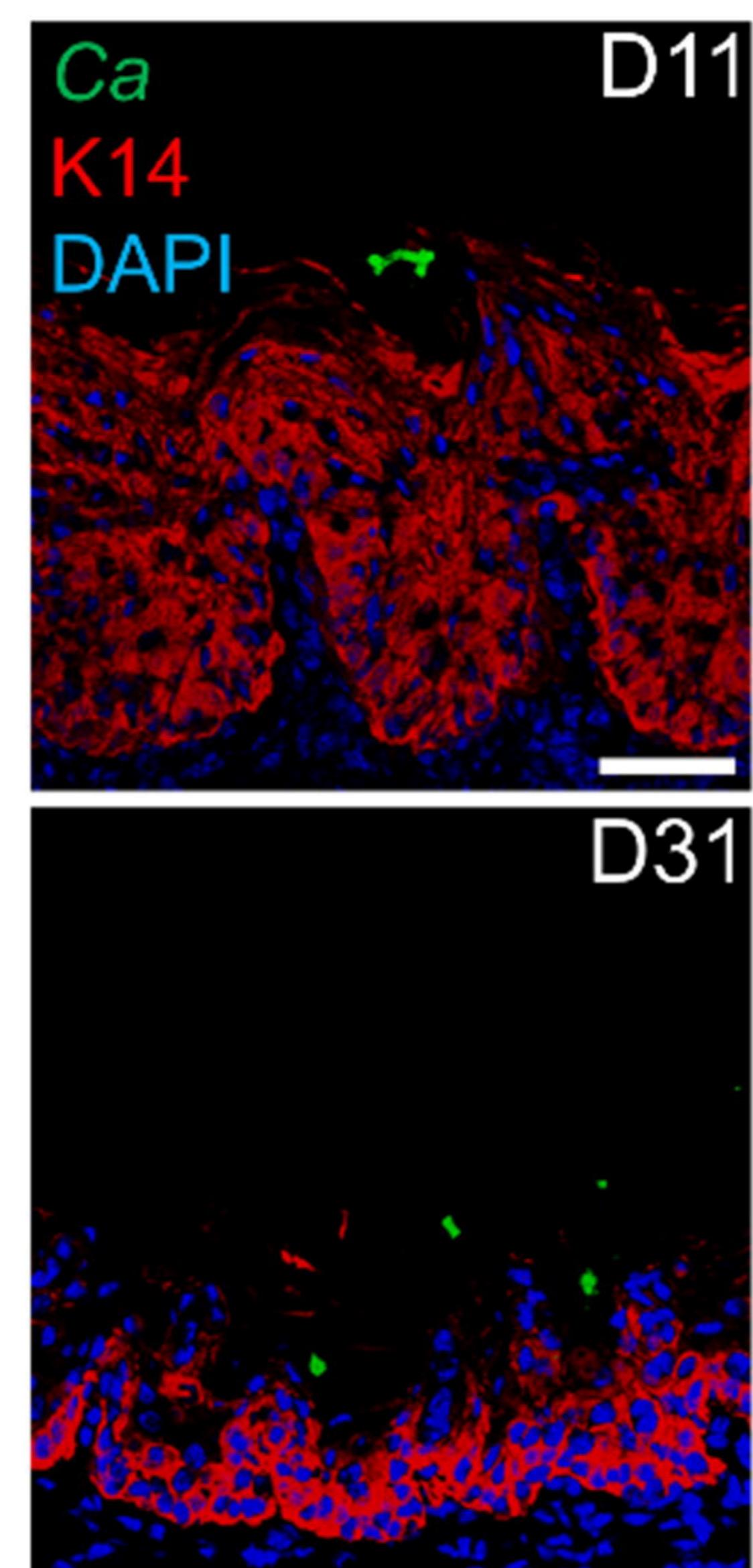
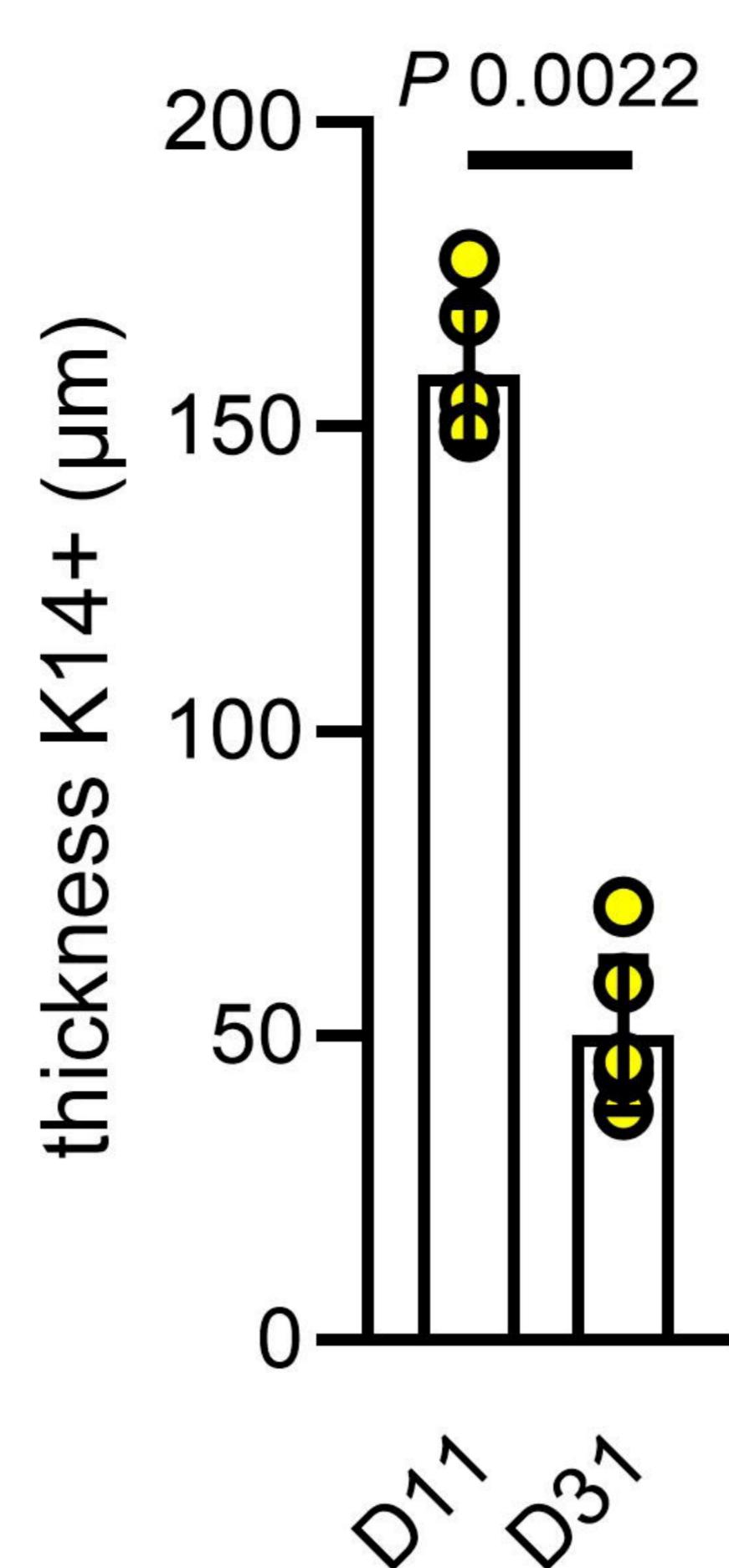
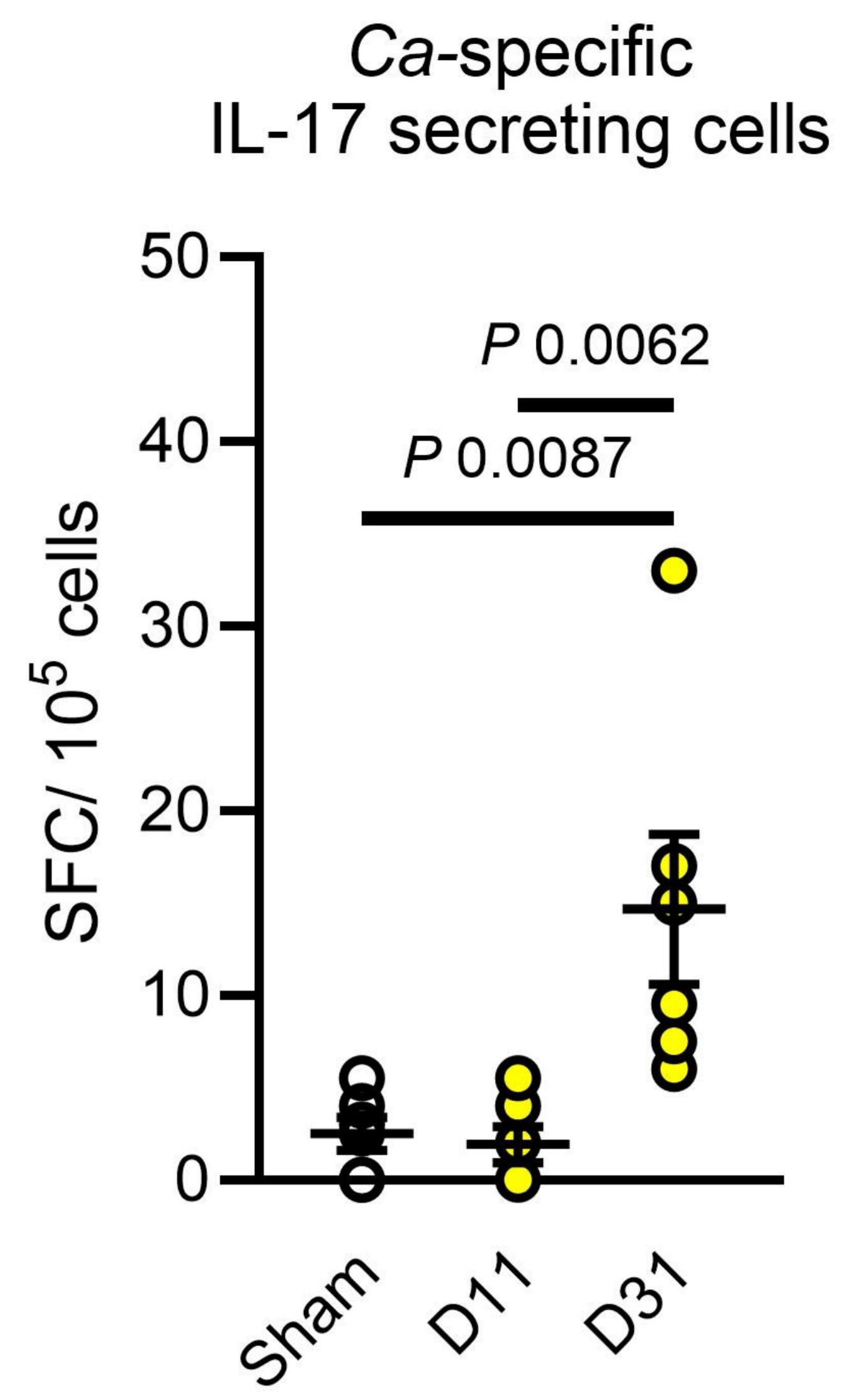
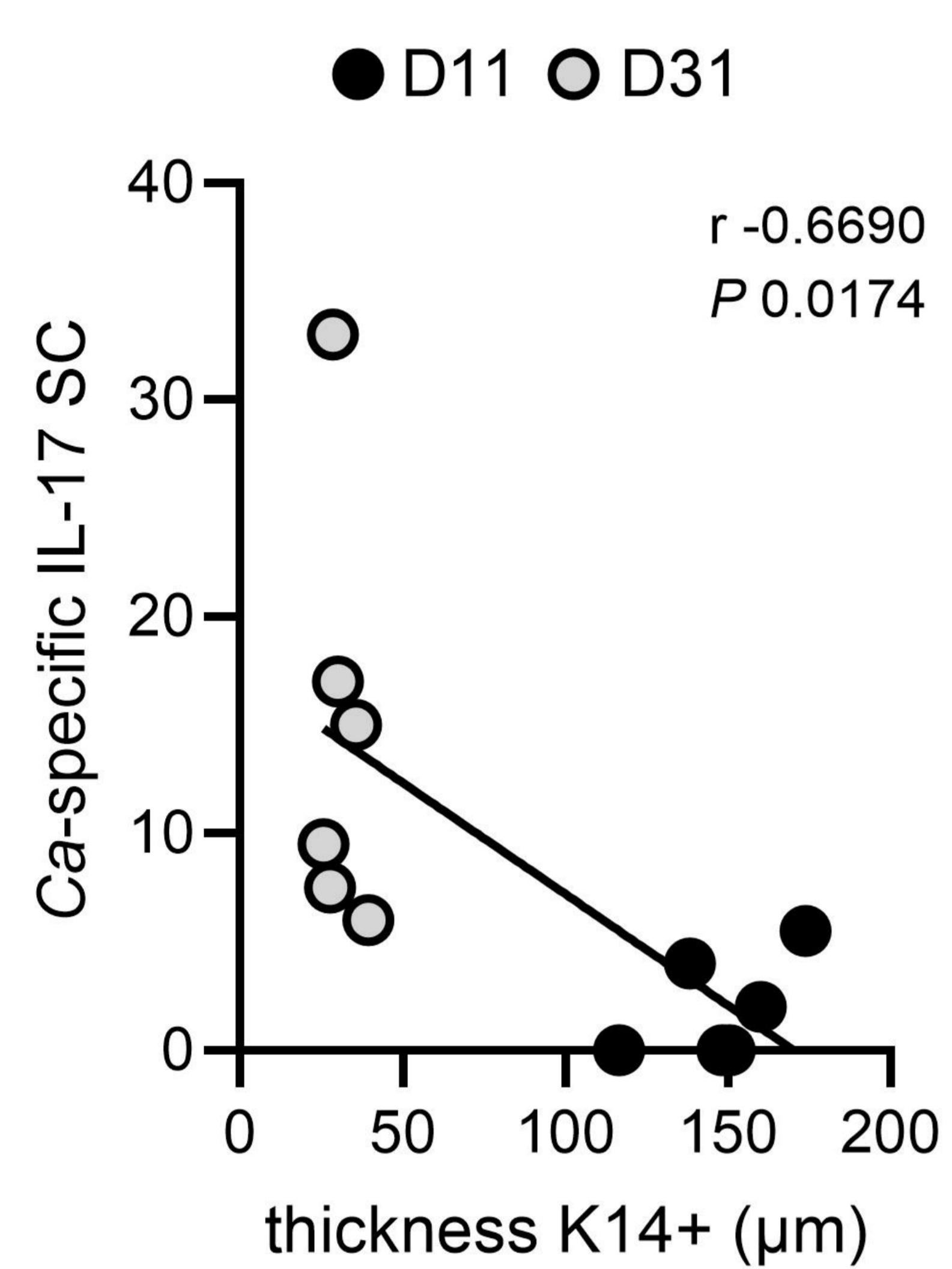
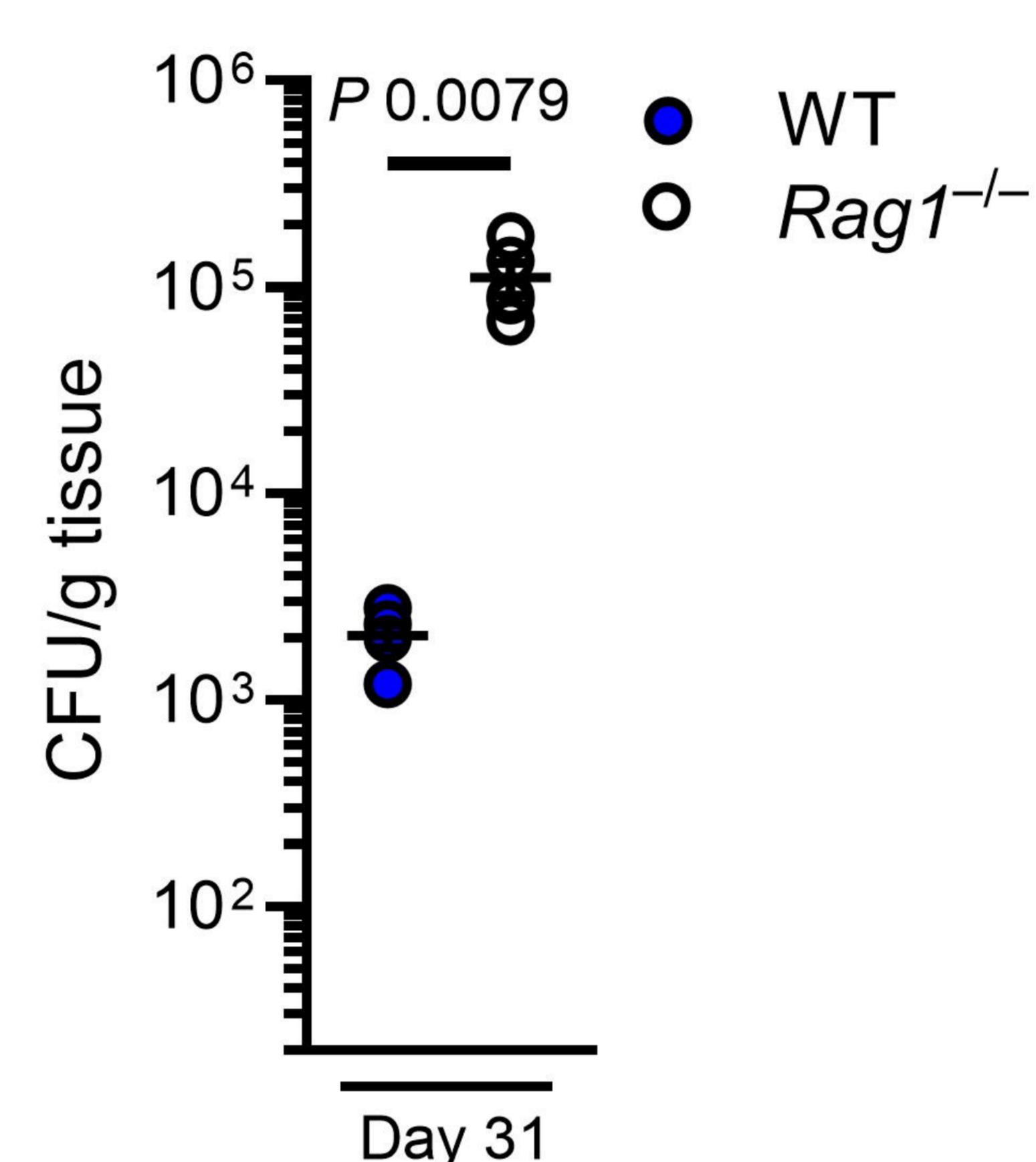
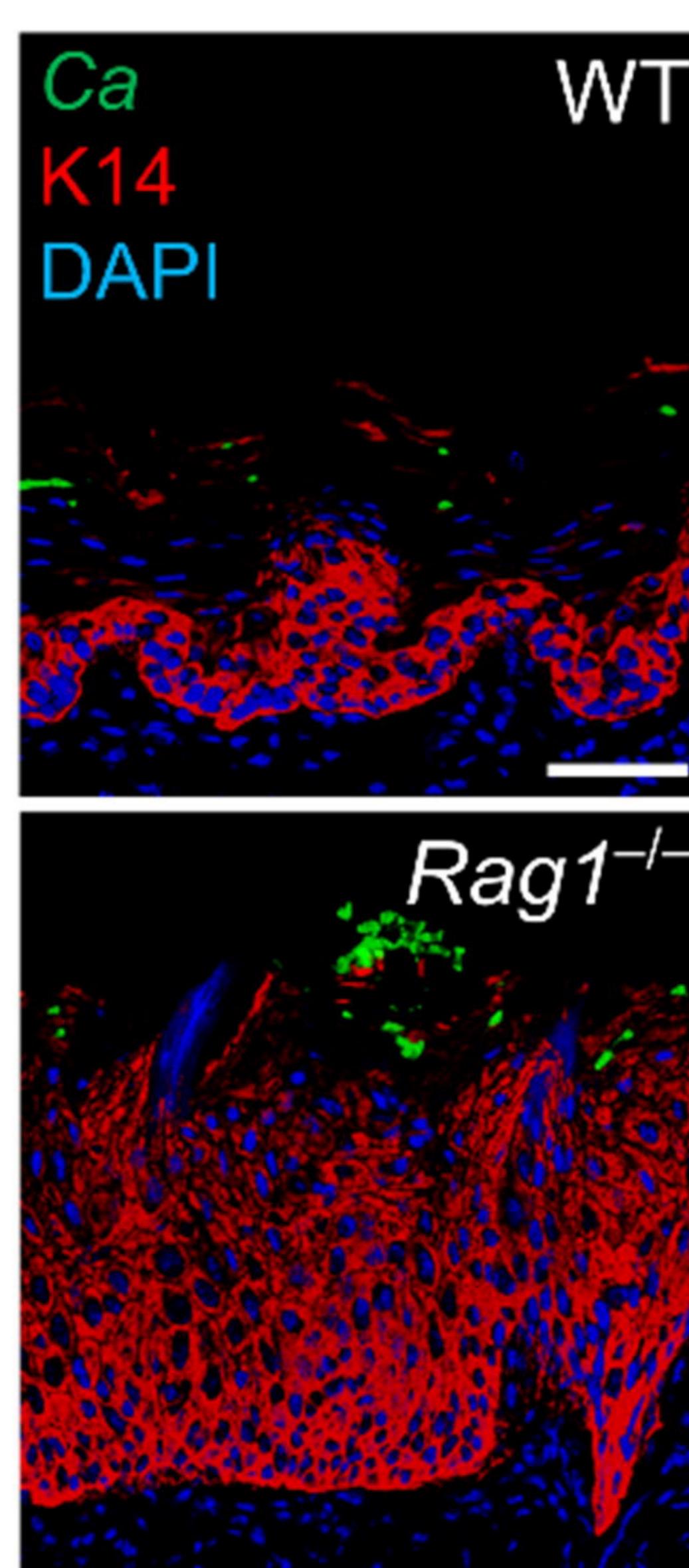
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