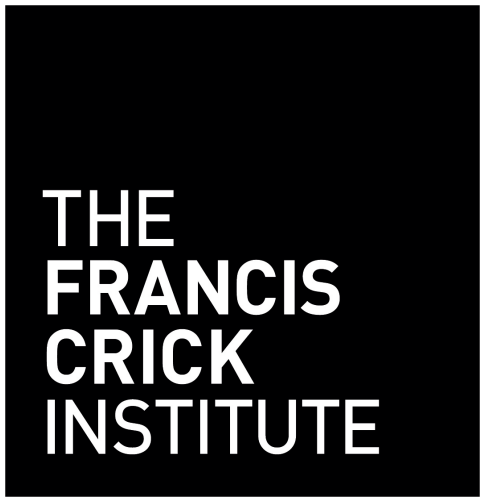


TET2-Mutant Hematopoietic Clones Shape the Lung Immune Microenvironment in NSCLC Initiation and Progression

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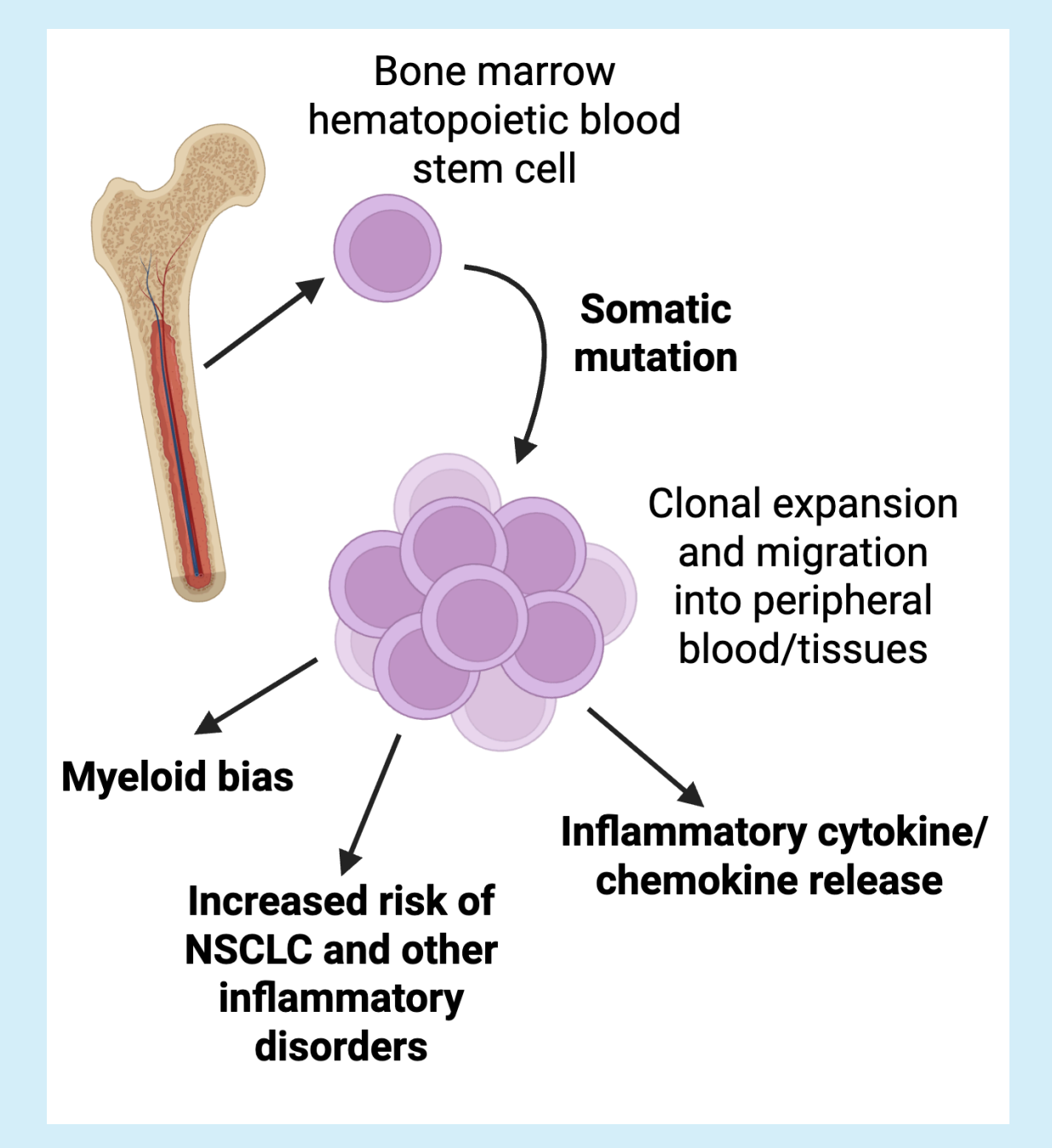


INTRODUCTION

Clonal Hematopoiesis of Indeterminate Potential (CHIP), an aging-related clonal expansion of mutant hematopoietic blood stem cells in the absence of hematologic malignancy, increases the risk of lung cancer and other diseases^{1,2}. **TET2** is one of the most frequently mutated genes in CHIP³, and TET2-mutant hematopoietic cells differentiate with **myeloid bias** and migrate to distal organs, such as the lungs. They have also been shown to promote a **pro-inflammatory tumor microenvironment (TME)**³; for instance, TET2-mutant macrophages produce elevated levels of **IL-1 β** , an inflammatory cytokine⁴. Exposure to other inflammatory agents, such as particulate matter (PM) air pollution, may exaggerate these effects.

Here, we investigated how CHIP alters the lung immune microenvironment to drive non-small cell lung cancer (NSCLC) initiation and progression via:

- 1) In-vitro culture of primary alveolar macrophages (AMs) and interstitial macrophages (IMs)—in either the absence or presence of exogenous PM—assesses proliferation of TET2-mutant immune cells.
- 2) In-vitro AT2 organoid assays examine the effects of inflammatory cytokines on organoid growth.
- 3) In-vivo EGFR-mutant NSCLC mouse model probes how TET2-mutant hematopoietic cells affect EGFR+ architecture and modulate the composition and compartmentalization of the lung immune TME.



CHIP PROMOTES MACROPHAGE CLONAL EXPANSION, PROLIFERATION

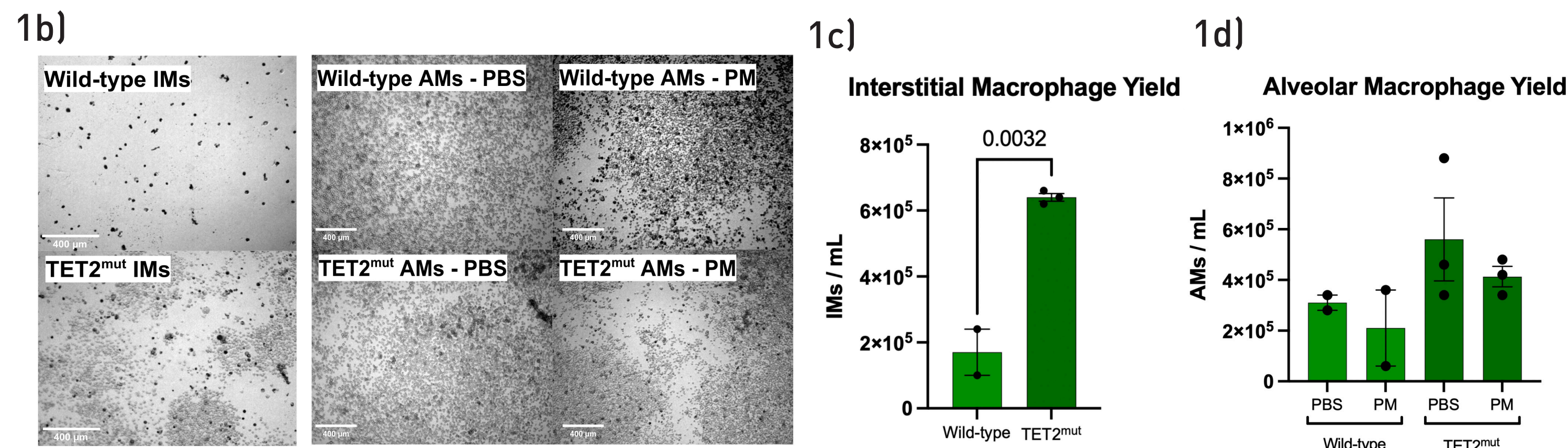
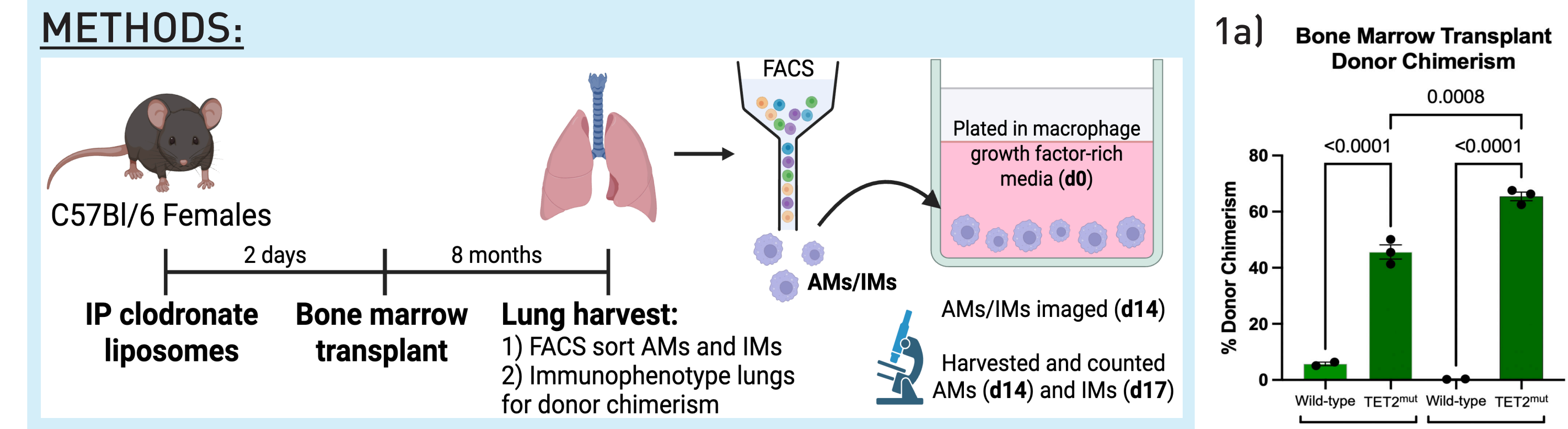


Figure 1: a) TET2-mutant bone marrow (BM) transplants promoted higher donor chimerism and expansion of donor BM cells. **b)** Representative brightfield images of plated PM-exposed IMs and PBS- or PM-exposed AMs (d14). **c)** PM-exposed TET2-mutant IMs were more proliferative than wild-type counterparts. **d)** Greater TET2-mutant AM expansion in both PBS and PM conditions as compared to wild-type, with PM exposure resulting in a lower yield across both genotypes.

IL-1 β DRIVES IFN- γ -SENSITIVE ORGANOID GROWTH

Since TET2-mutant macrophages upregulate IL-1 β release⁴ (potentially increased if stimulated with PM), we asked how tumor growth correlates with IL-1 β concentration. We also examined whether IFN- γ , a cytokine produced by T cells adjacent in the TME, affects growth since IFN- γ is known to have both macrophage-activating and cytostatic/cytotoxic effects⁵.

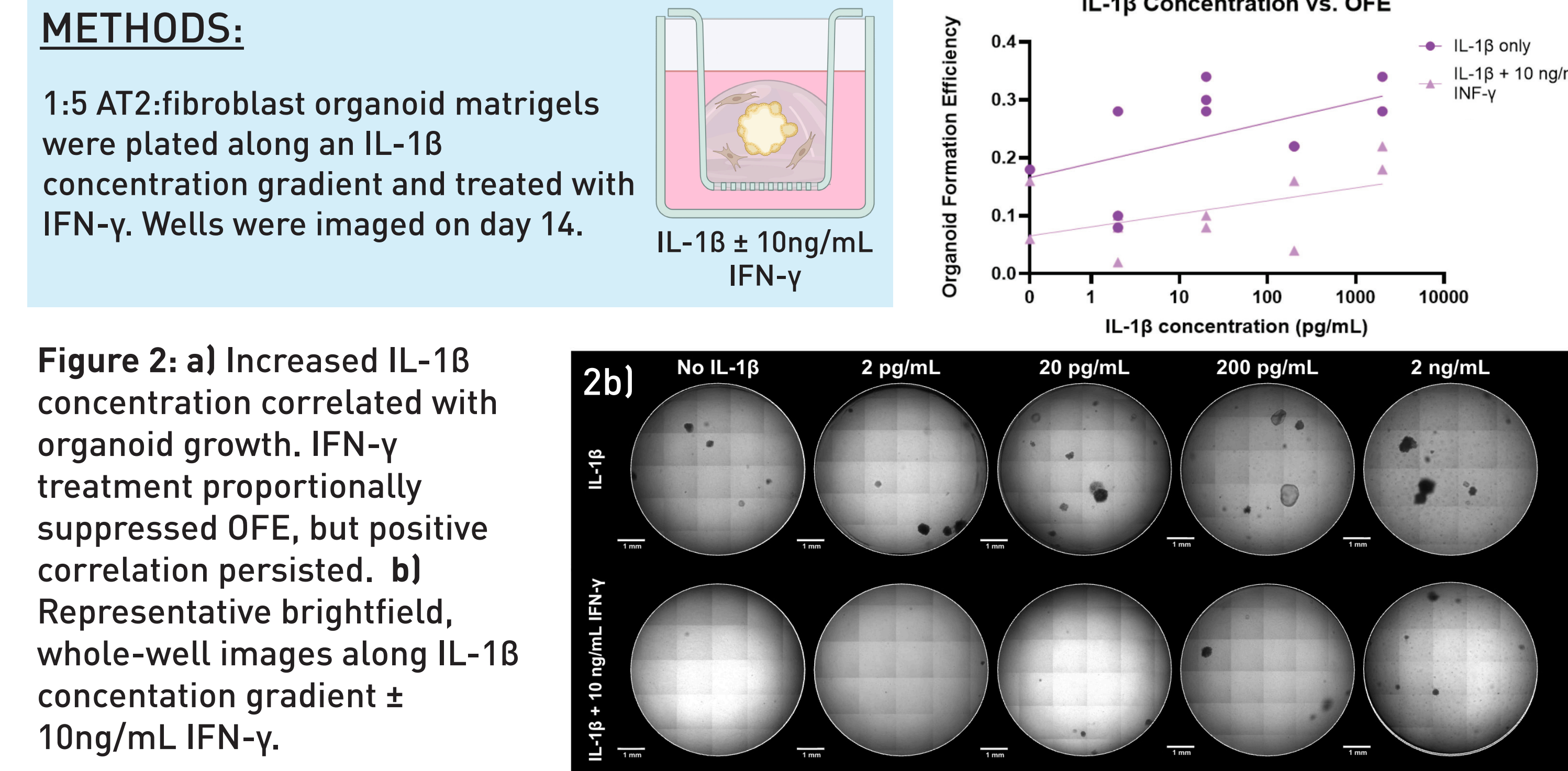


Figure 2: a) Increased IL-1 β concentration correlated with organoid growth. IFN- γ treatment proportionally suppressed OFE, but positive correlation persisted. **b)** Representative brightfield, whole-well images along IL-1 β concentration gradient $\pm$ 10ng/mL IFN- γ .

ACKNOWLEDGEMENTS

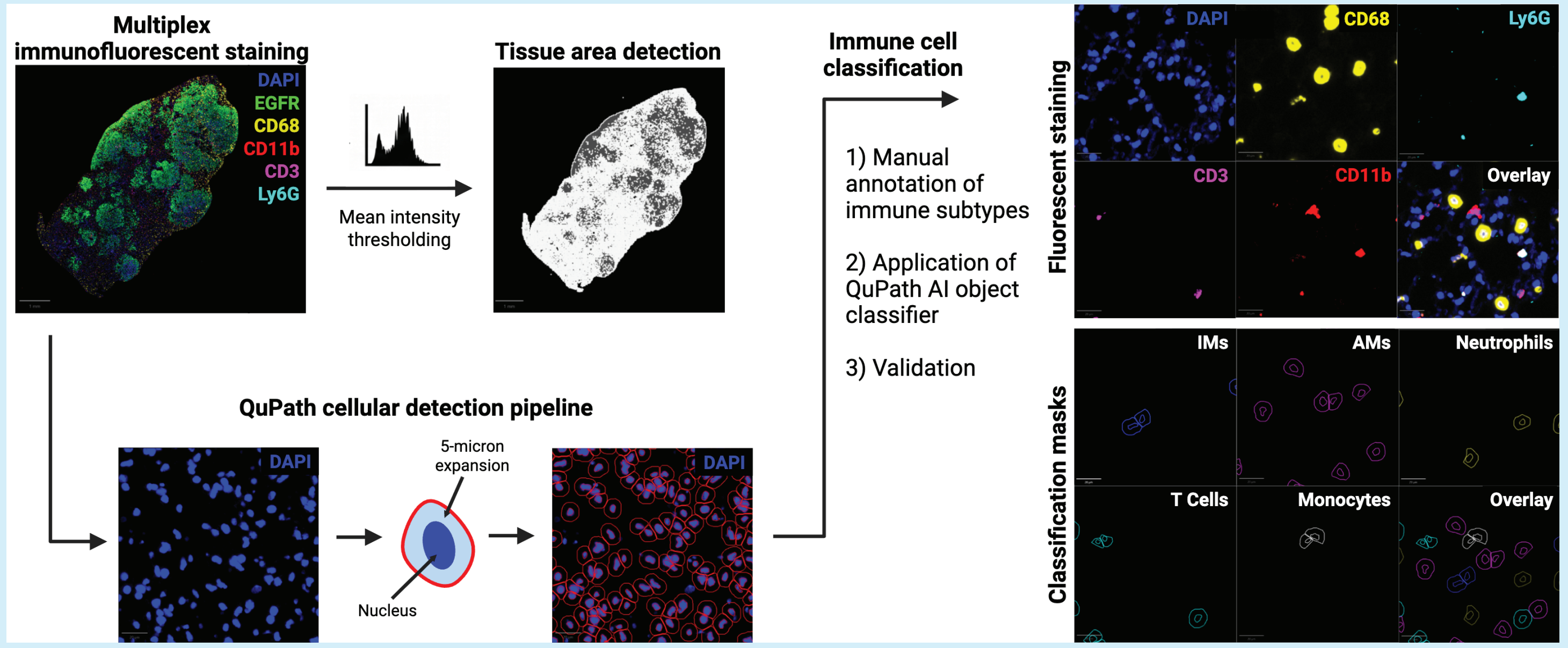
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NSCLC PROGRESSION

METHODS: EGFR-mutant, tumor-bearing mice (Rosa²⁶LSL-tTa/LSL-tTomato;TetO-EGFR^{L858R}) were treated with PEG-clodronate to deplete the immune compartment and then transplanted with WT or 1:2 WT:TET2-knockout BM cells. 13 weeks later, lungs were harvested, immunostained, and analyzed via a **QuPath image analysis pipeline**:



EGFR+ ARCHITECTURE:

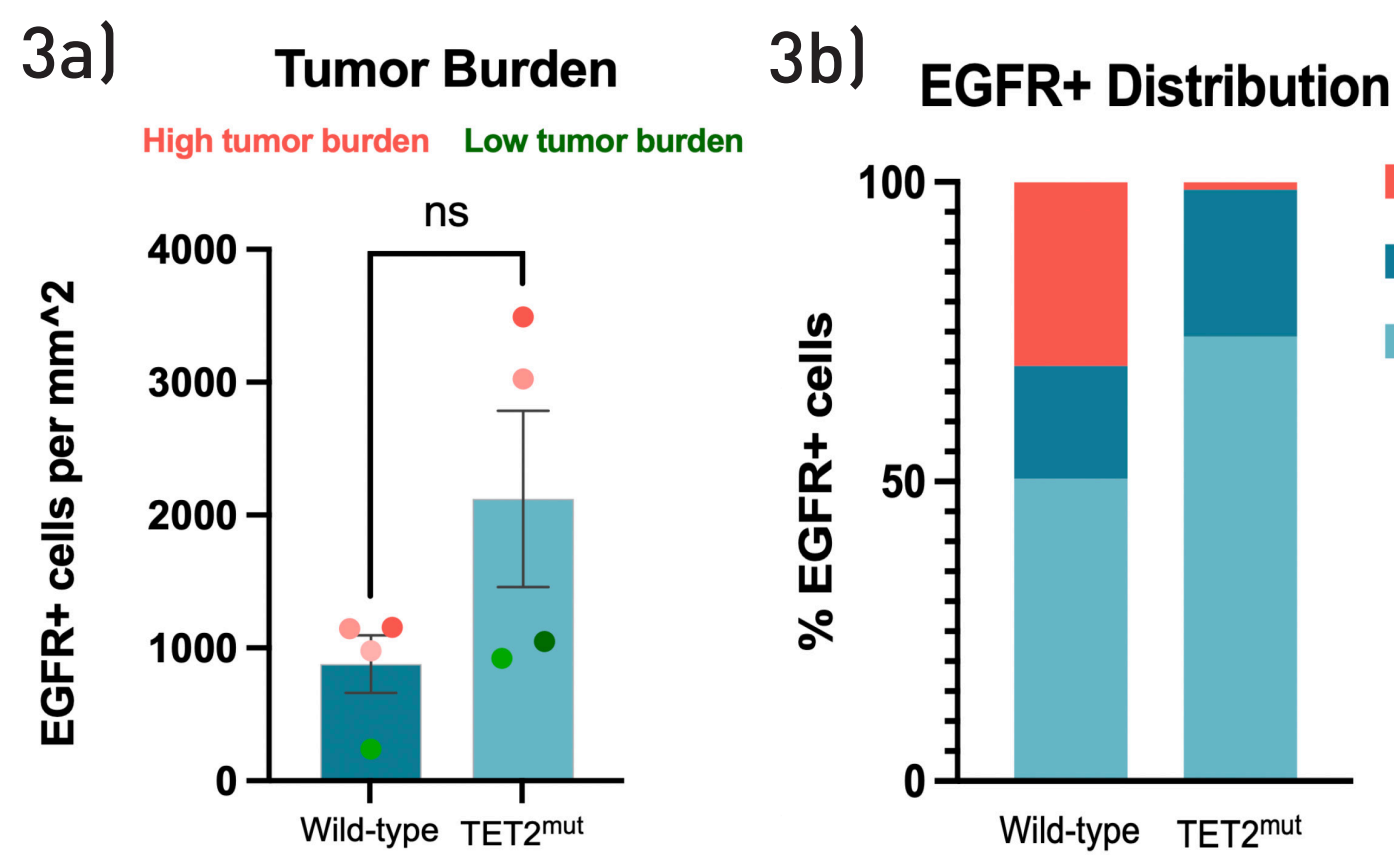
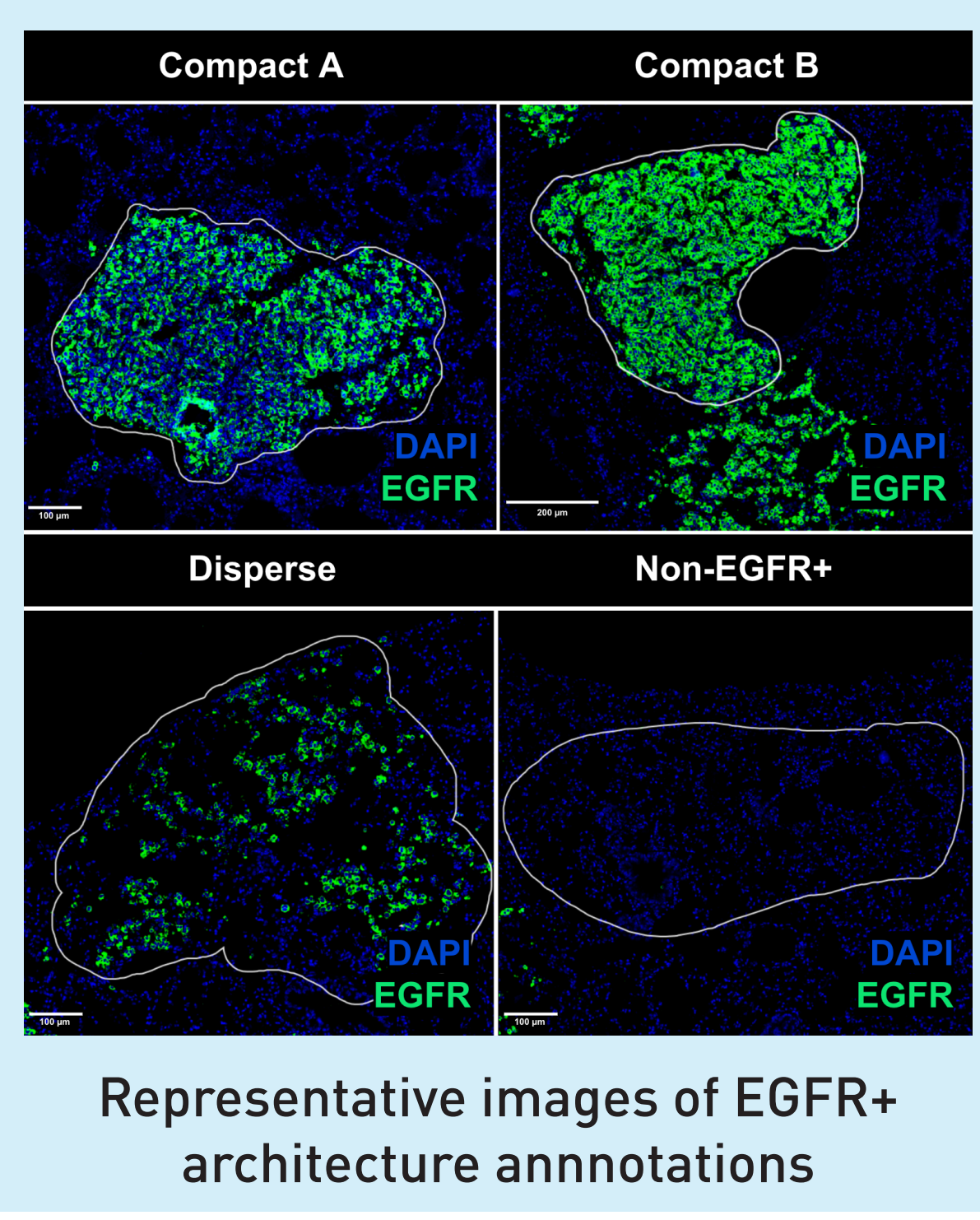
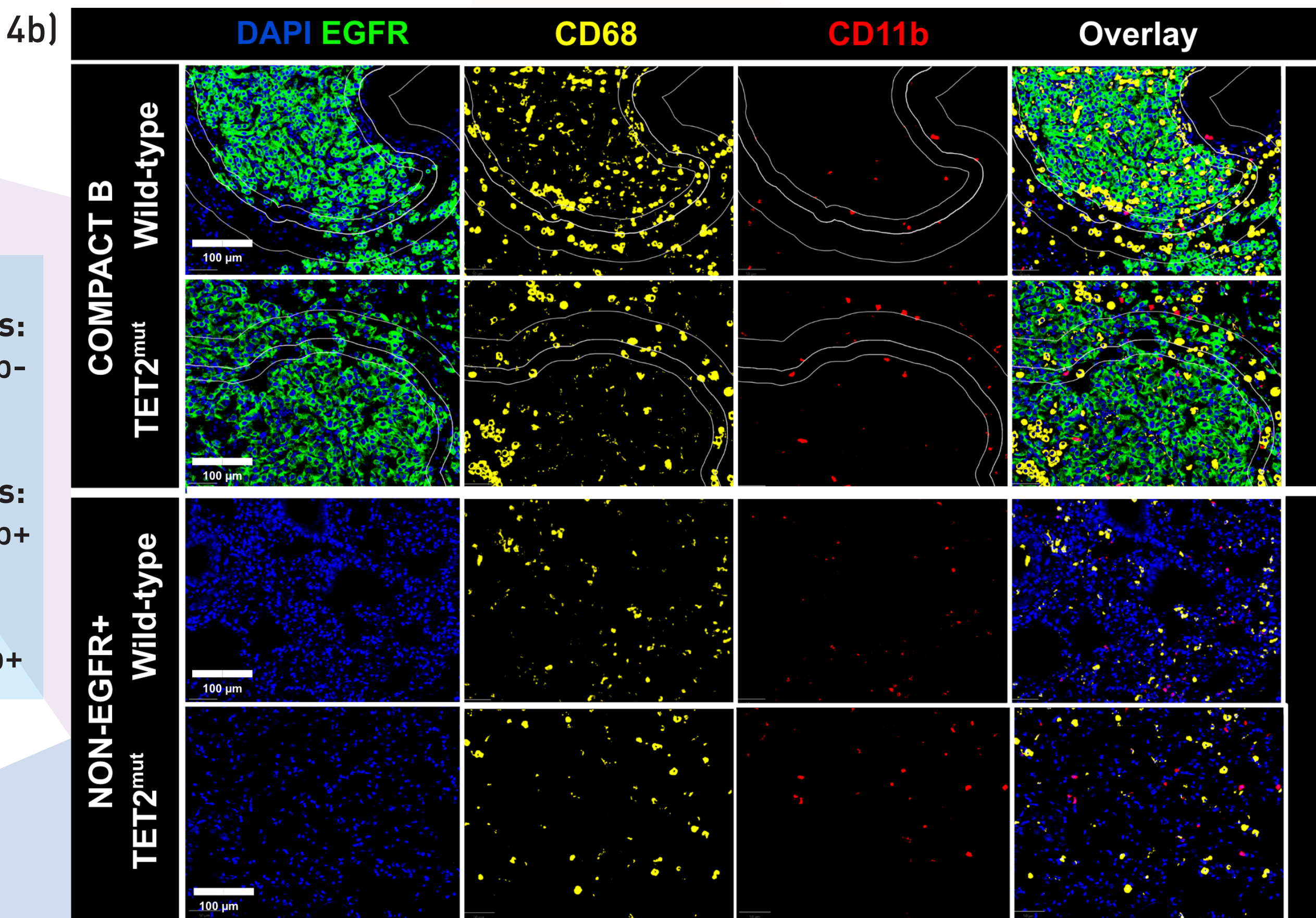
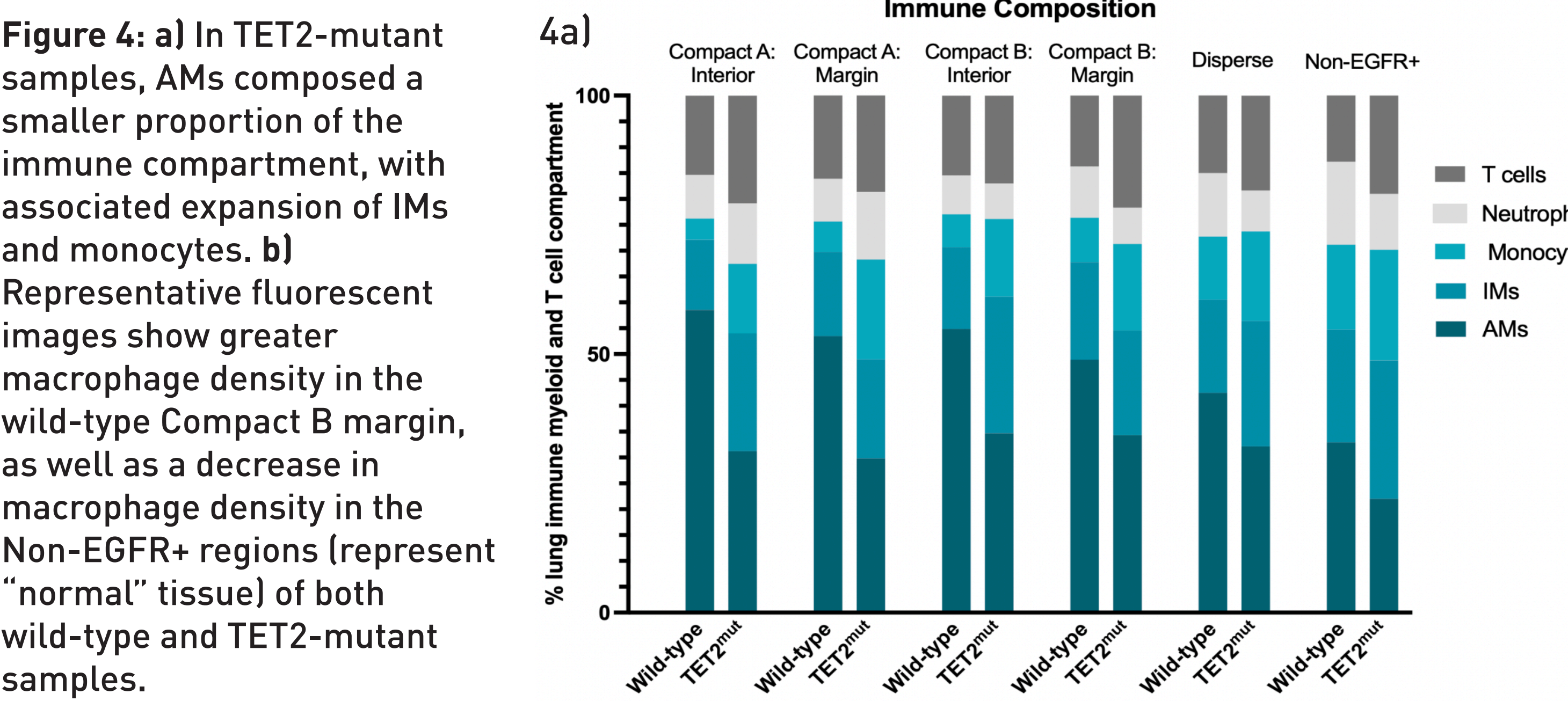


Figure 3: a) TET2-mutant samples trended toward higher tumor burden; note intra-cohort variability. **b)** TET2-mutant mice exhibited a greater proportion of disperse and Compact B lesions, likened to a more advanced, aggressive NSCLC state.



IMMUNE COMPARTMENTALIZATION:



FUTURE DIRECTIONS & CONCLUSION

Future work will compare IM proliferation when exposed to either PBS or PM and expand immunofluorescent staining panels to include B cells and T cell subtypes. Furthermore, direct organoid cultures with primary IMs and AMs will help validate the reported cytokine-driven organoid growth results.

Here, we found that (1) TET2 mutations in hematopoietic stem cells lead to more proliferative IMs and AMs, (2) IL-1 β has a concentration-dependent, IFN- γ -sensitive effect on organoid formation, and (3) in NSCLC tumor-bearing animals, TET2-mutant bone marrow transplant drives a more aggressive, spatially disperse EGFR+ architecture, as well as a decrease in AMs and an expansion of other bone marrow-derived myeloid populations. These results provide further evidence for the impact of pro-inflammatory CHIP on NSCLC initiation and progression.