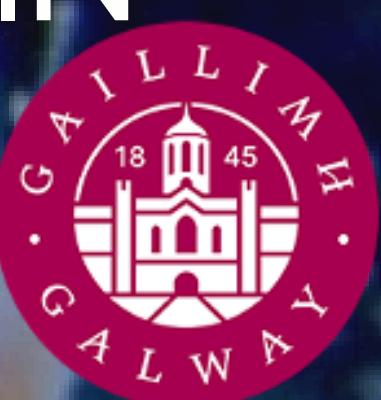


IN VIVO ANALYSIS OF THE EPIGENETIC LANDSCAPE IN *HYDRACTINIA SYMBIOLONGICARPU*S

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ABSTRACT

Epigenetic modifications like DNA methylation and histone modifications play an essential role in inducing and maintaining cell identity through regulation of gene expression. Much of our understanding of these processes comes from *in vitro* studies. This study was carried out on the cnidarian *Hydractinia* *in vivo*. We aimed to identify a key epigenetic regulator and validate its expression using SABER-FISH. We also carried out immunofluorescence analysis of H3K9me3 and H4K16ac to investigate chromatin organisation in distinct cell types.

1. DNMT3 HOMOLOG IDENTIFICATION

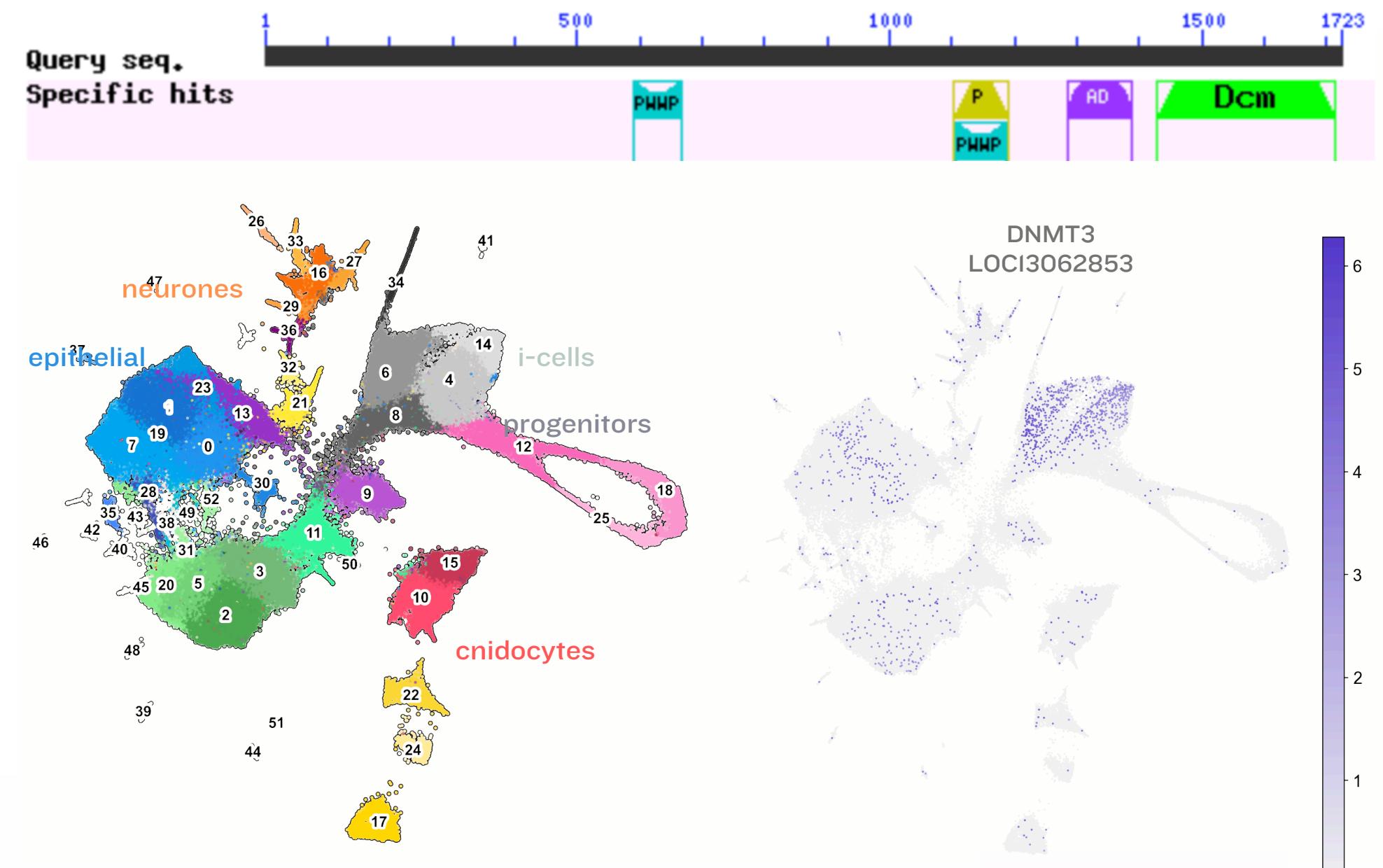


Fig 1. (A) UMAP projection of *Hydractinia* cell atlas where clusters are coloured by their cell-identity. (B) UMAP showing presence of LOC13062853 RNA in the dataset. (C) Specific domain hits identified by the CDD.

Our identification of a DNMT3 homolog provides key insights into the conservation of DNA methylation mechanisms in *Hydractinia*. Identified functional domains are responsible for de novo methylation, suggesting that this gene may function similarly to vertebrates. While our attempts to confirm DNMT3 expression using FISH were inconclusive, its enrichment in i-cells and progenitors suggests that it may play a role in maintaining epigenetic plasticity.

2. H3K9ME3 REVEALS CHROMATIN REPRESSION

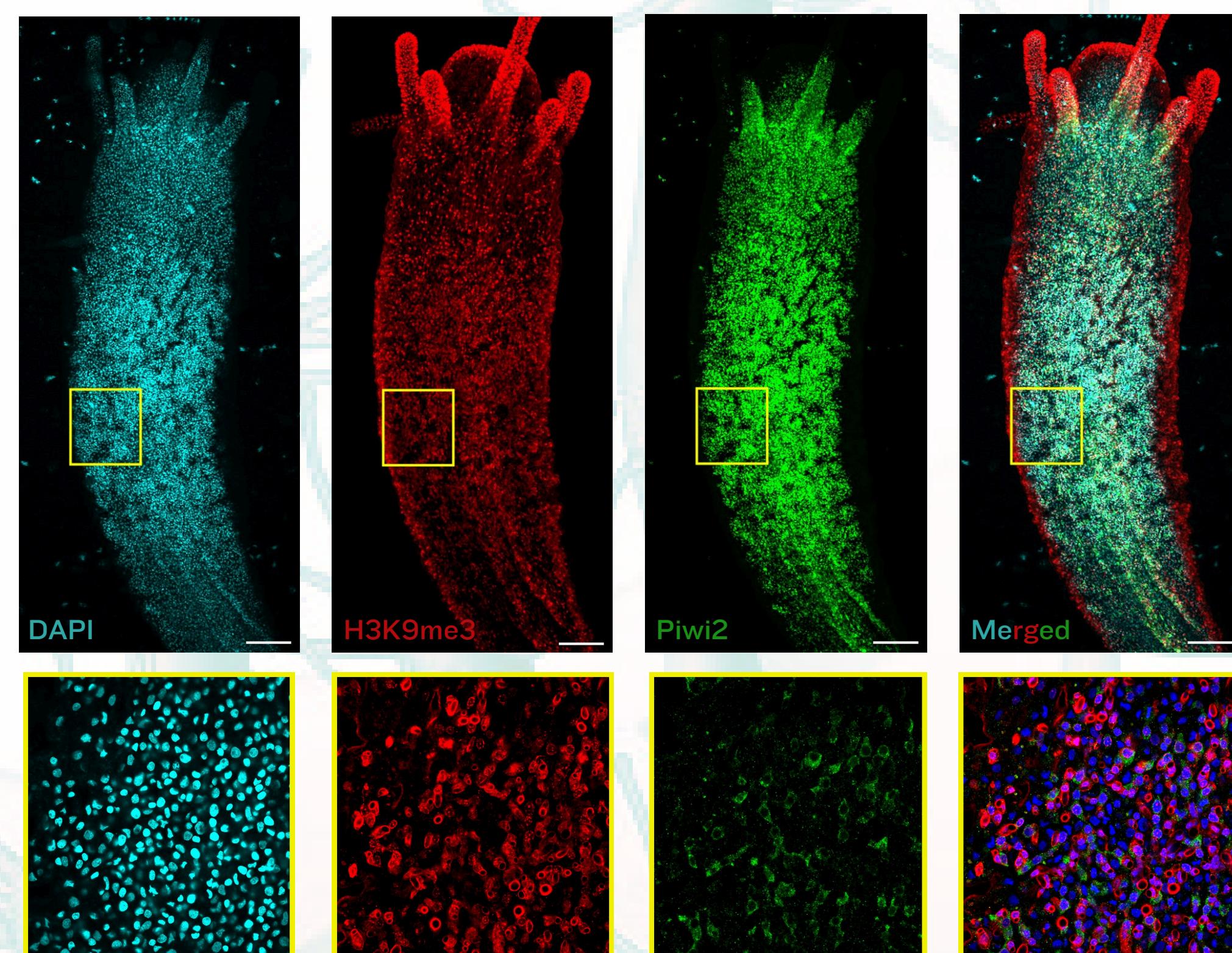


Fig 2. H3K9me3 Immunofluorescence Procedure. Maximum projection of DAPI, H3K9me3 and Piwi2 in a feeding polyp.

The histone modification H3K9me3 is an established marker of heterochromatin. In cnidocytes, H3K9me3 clustered around the nuclear periphery. In contrast, it appeared as speckled pattern in i-cells suggesting the presence of localised heterochromatic regions within an otherwise euchromatic nucleus. Quantification of H3K9me3 staining was challenging due to the presence of non-specific large red ring stains (Fig. 2).

3. H4K16AC HIGHLIGHTS PLURIPOTENT IDENTITY

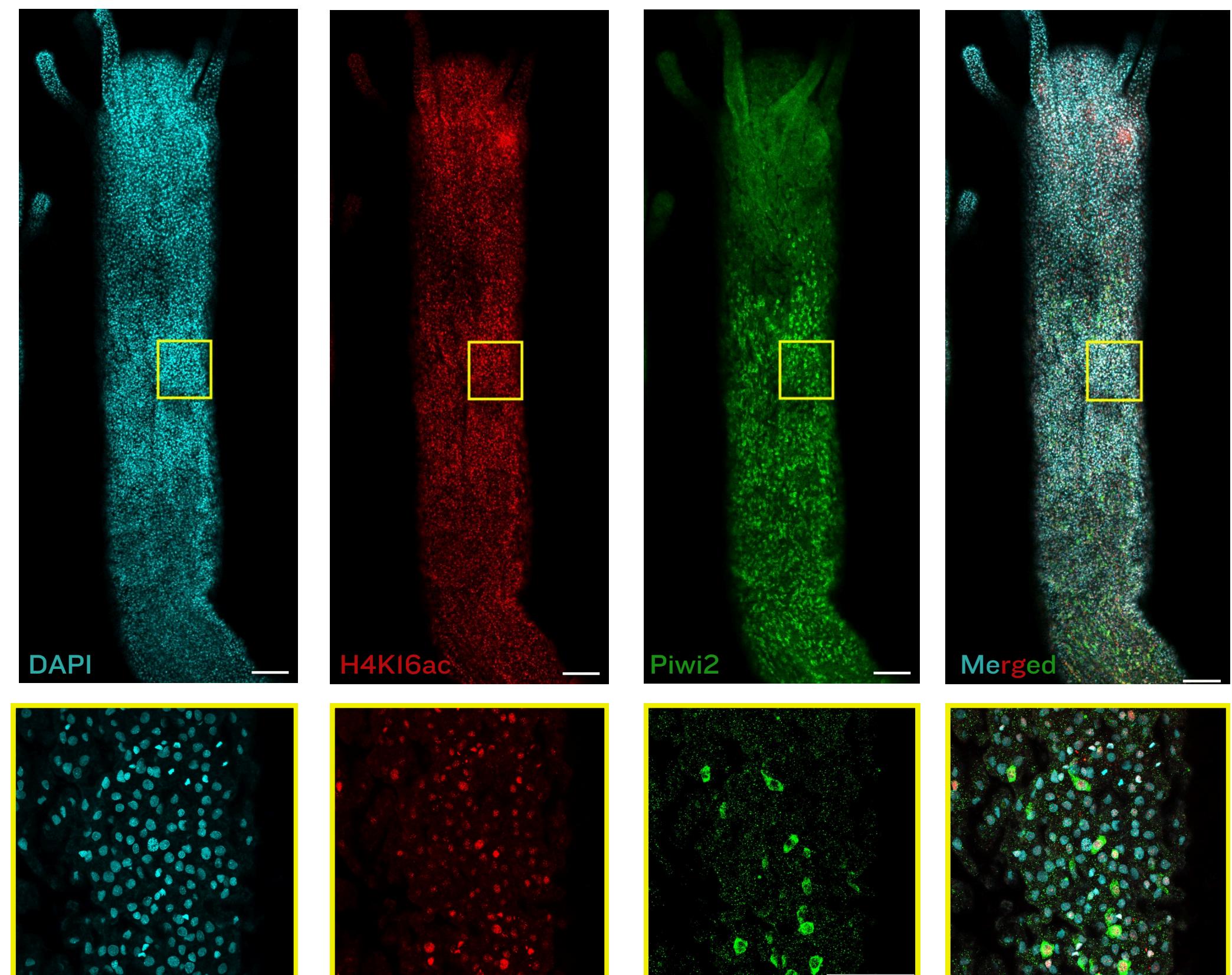


Fig 3. H4K16ac Immunofluorescence Procedure. Maximum projection of DAPI, H4K16ac and Piwi2 in a feeding polyp.

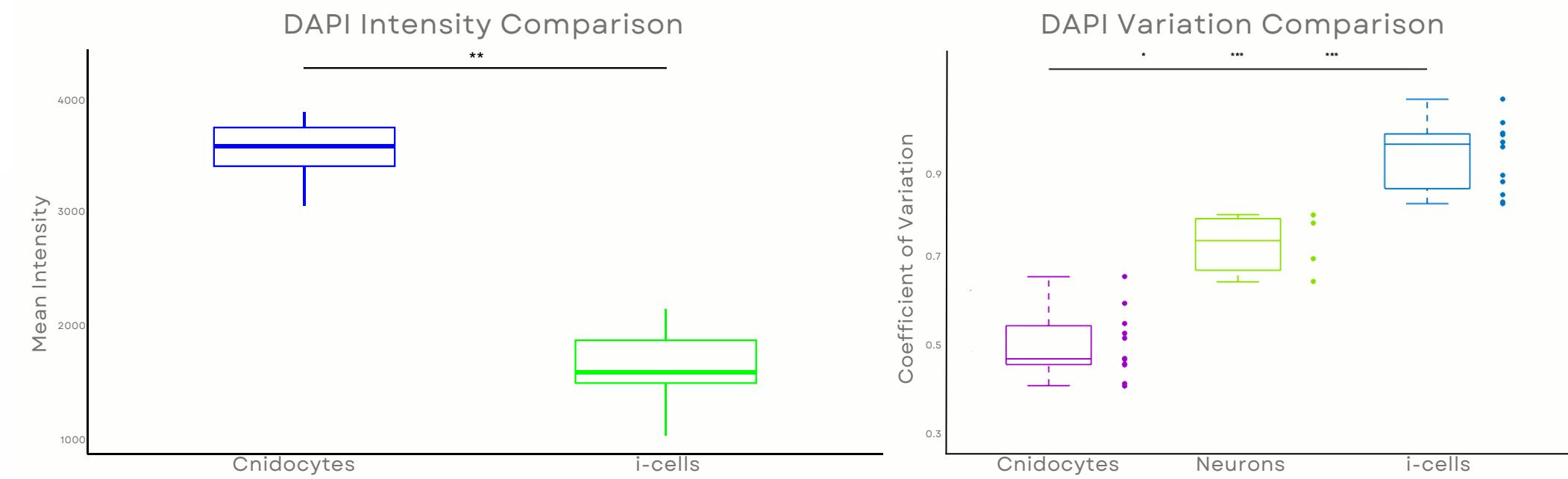


Fig 4. (A) Mean DAPI fluorescence intensity per nucleus comparison between i-cells and cnidocytes quantified from confocal microscopy images. (B) CV fluorescence intensity per nucleus comparison between cnidocytes, neurons and i-cells quantified from confocal microscopy images.

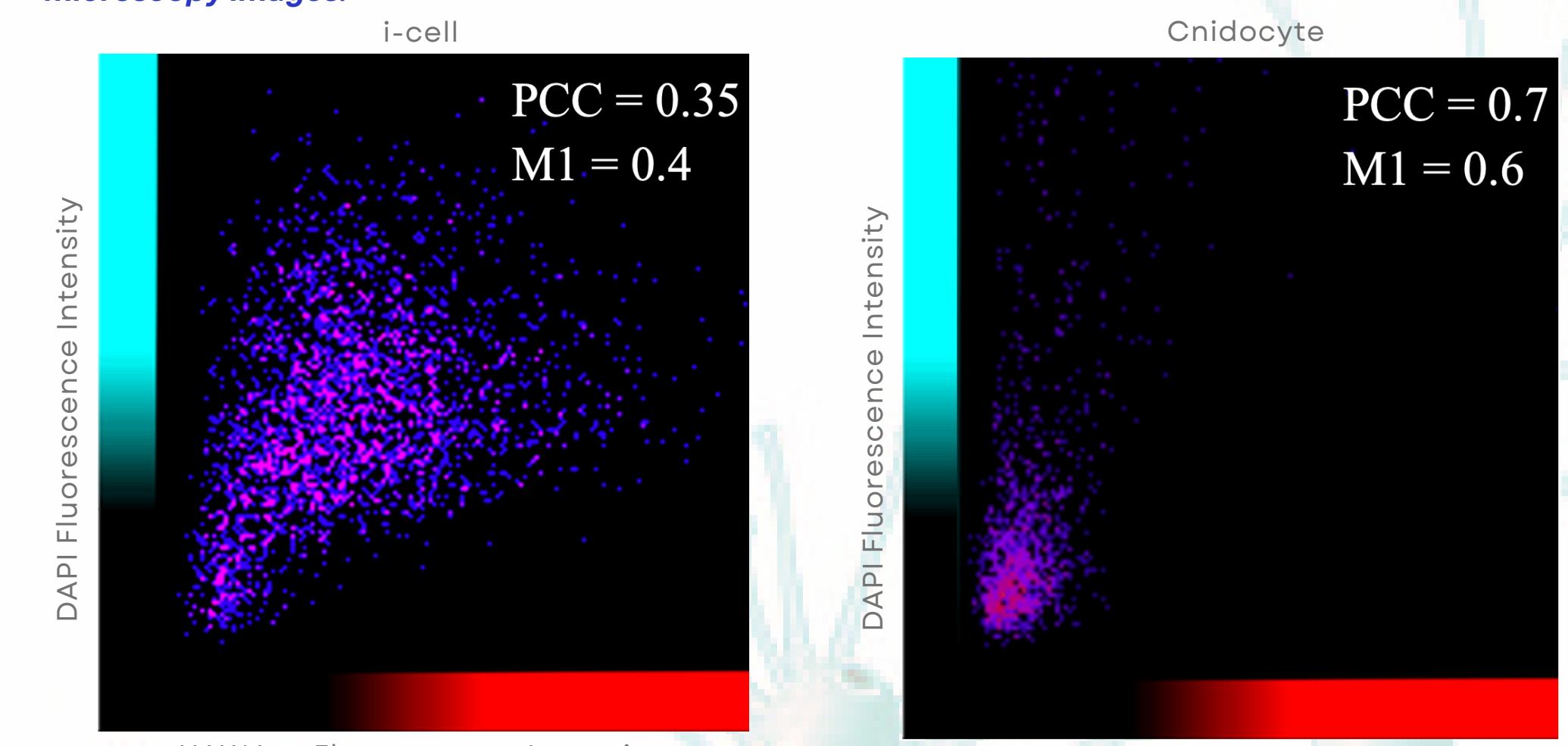


Fig 5. Fluorogram displaying the distribution of fluorescence intensities for DAPI and H4K16ac.

H4K16ac is a hallmark of open chromatin. It was observed as discrete nuclear foci in i-cells (Fig.3). Its low Pearson's Correlation (PCC) suggests that regions with high H4K16ac intensity correspond to areas with low DAPI intensity. In contrast, H4K16ac staining in cnidocytes appeared more diffuse. Its high PCC of 0.7 suggests it does not drive transcription as despite H4K16ac presence, DAPI intensity remained high suggesting a more compact state (Fig. 5).

CONCLUSIONS

These findings set the stage for further investigating the precise mechanisms governing stem cell pluripotency and differentiation in *Hydractinia*. While SABER-FISH repetition is a clear next step, future research could explore the role of DNMT3 in detail. The contrasting chromatin states revealed by the immunofluorescence procedures emphasises the importance of epigenetic modifications in establishing and maintaining cell identity.

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