



Single vs. Double Genetic Manipulations of Intestinal Stem Cells in *Drosophila melanogaster*



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Introduction

Intestinal stem cells (ISCs) maintain gut homeostasis by dividing asymmetrically. Through mitosis, they make a new copy of themselves and either of two sister cell types: an intermediate progenitor known as the enteroblast (EB), which differentiates into an absorptive enterocyte (EC), or an enteroendocrine precursor cell (EEP) that turns into a hormone-secreting enteroendocrine cell (EE) - Fig. 1.

Past studies showed that manipulating “master regulators genes” versus their downstream targets often yields contradictory outcomes, suggesting that these genes do not follow simple linear pathways, but instead interact in complex ways to determine cell fate. To test these interactions more directly, our project uses a strategy called double manipulation, where two genes are altered at the same time. We focused on the genes *klaroid* (Koi) and CAP. These genes have not been studied in the context of ISC's and are not predicted to interact based on their known cellular roles.

If these genes work separately, then the double manipulations will produce the additive effect of each single-gene manipulation.

However, if the outcome differed from the expected sum of effects, this would indicate that these genes interact with one another to influence ISC fate.

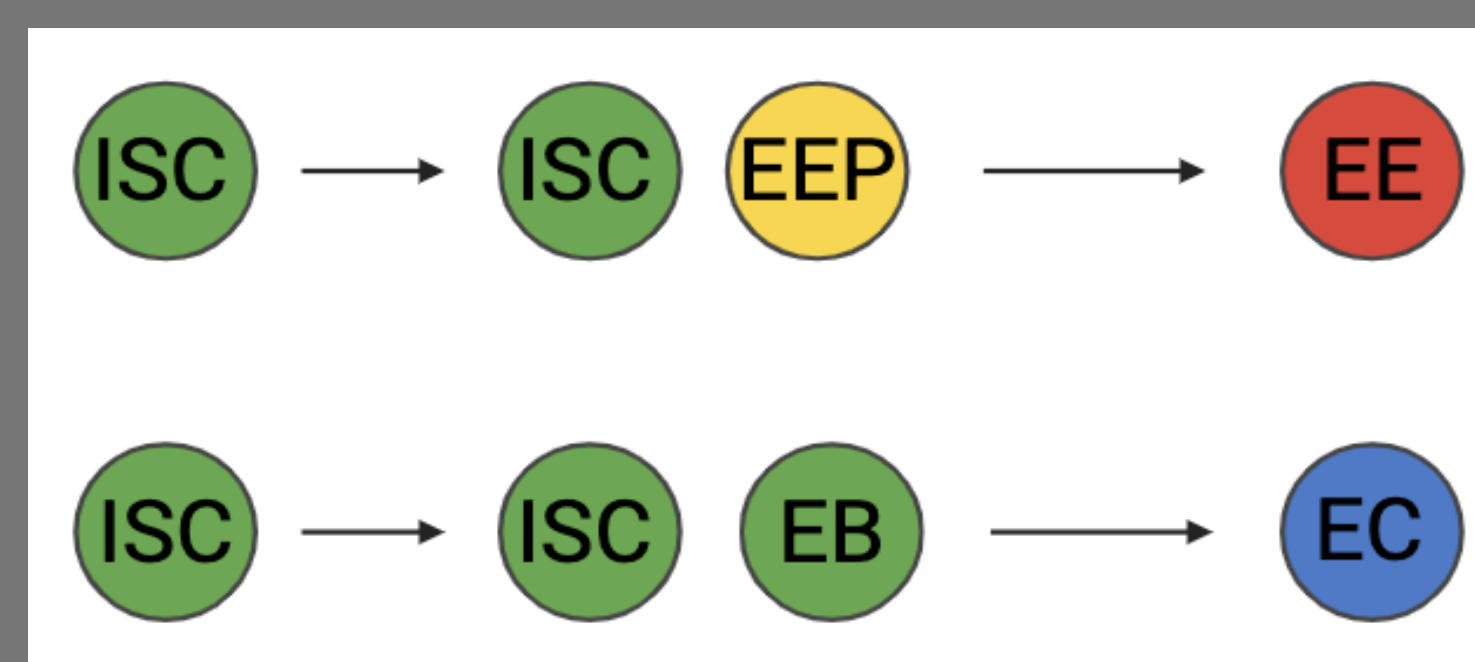
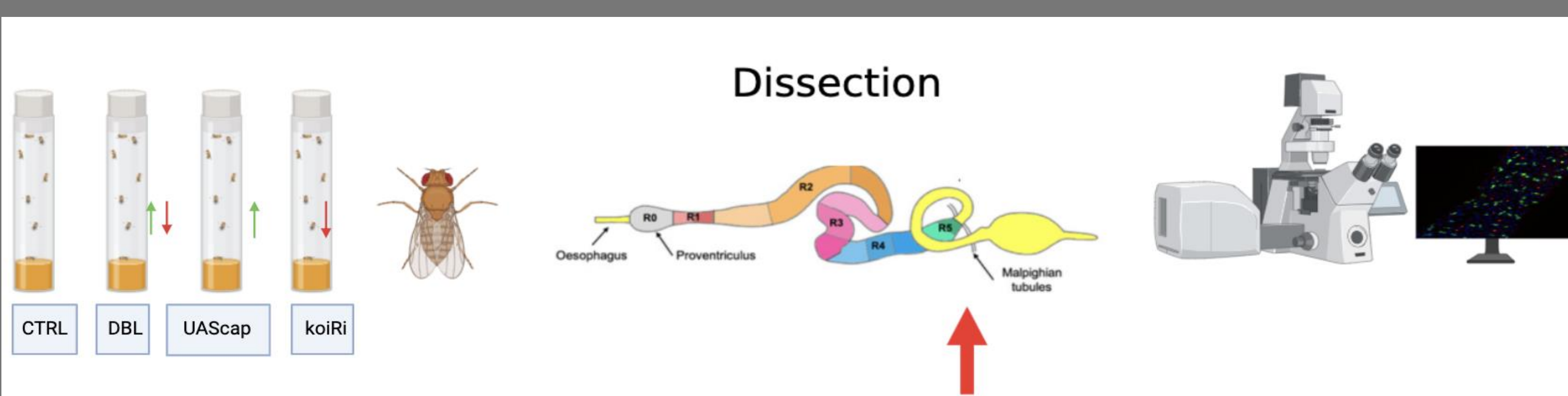


Figure 1. The possible pathways of *Drosophila* intestinal lineage

Methodology

We collected flies from established genetic crosses designed to manipulate one or both of our genes of interest. To overexpress CAP, we used the Gal4-upstream activating sequence (Gal4-UAS) system. To knock-down Koi, we used Gal4-UAS to overexpress a Koi RNAi transgene. The flies were then incubated for 7 days to allow for sufficient transgene activity, before dissecting the gut and preparing it for imaging. We used the following fluorescent stains: DAPI (blue) to stain all nuclei, *esg*-GFP (green) to stain progenitors, and Pros (red), to stain EEs. These stains allowed us to clearly visualize the cells under the Zeiss microscope.



We focused our imaging on the posterior midgut, the best-studied region of the intestine, which provides a reliable reference for normal cell patterns. Our microscopy images (Fig. 2a) were processed with CellProfiler to automatically detect and measure various parameters per cell type (Fig. 2b-d) and the data were analyzed statistically using JASP (Fig. 3).

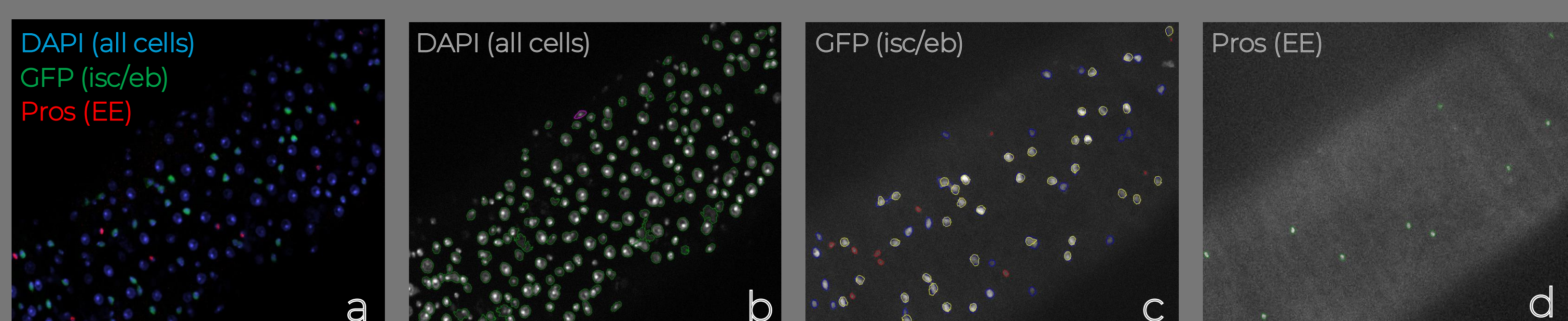


Figure 2. (a) Example of a fluorescence microscopy image from the posterior *Drosophila* midgut. Cell types were identified based on fluorescence staining as indicated. (b-d) CellProfiler identified different cell types in each fluorescence channel.

Results

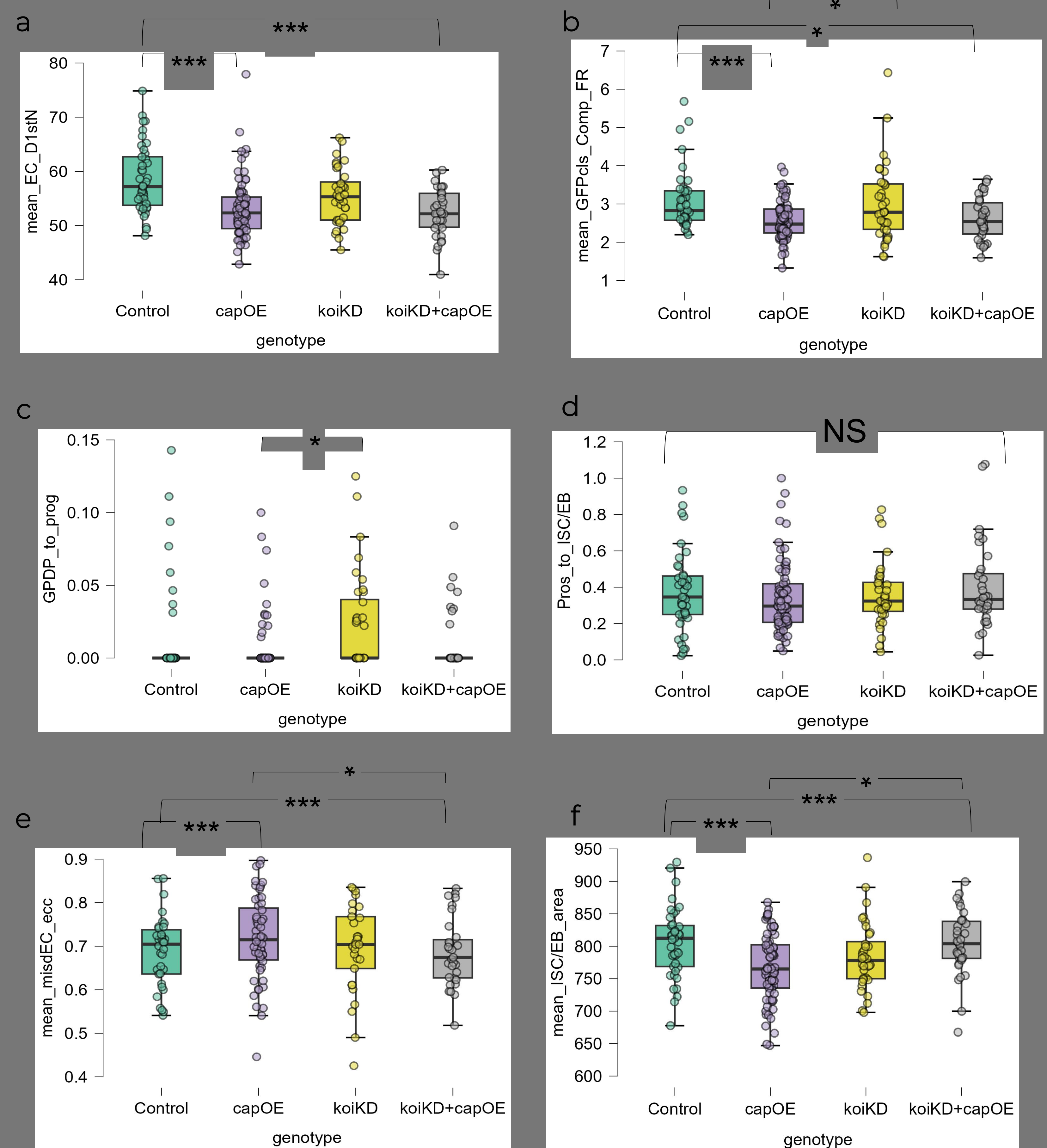


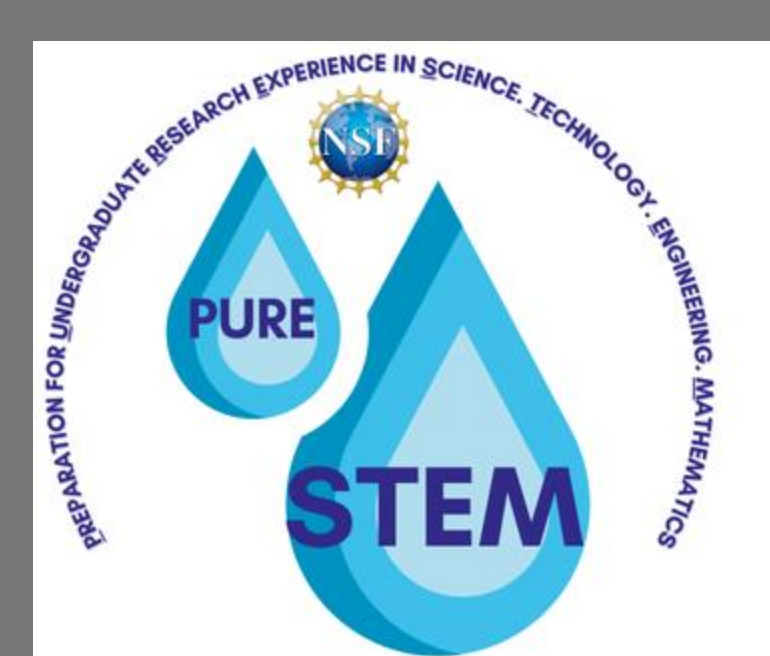
Figure 3. Effect of single vs. double-genetic manipulations across different morphological features. (a) EC_D1stN measures the distance of ECs to their closest neighbor. (b) GFPcls_Comp_FR denotes the compactness/Feret Ratio, used to characterize the shape of GFP+ clusters (a higher ratio corresponds to rounder shapes). (c) GFPD/prog stands for the ratio of GFP/Pros Double-positive cells (EEPs) to ISC/EBs. (d) Pros_to_ISC/EBs is used to show the relative proportion of EEs to ISC/EBs. (e) misdEC_ecc refers to the eccentricity of mis-differentiated, GFP+ ECs. (f) ISC/EB_area refers to the surface area of ISC/EB nuclei. (* = $p < 0.05$) (** = $p < 0.01$) (***) = $p < 0.001$) (NS = Not significant)

Conclusion

We observed that the double-manipulation of Koi and CAP produced additive effects for some variables (e.g. Fig. 3a,b), and non-linear outcomes for others (e.g. Fig. 3c,e,f). This supports the hypothesis that MR genes, their targets and their phenotypic outcomes are not connected via simple linear pathways but are likely controlled by more complex genetic interactions.

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