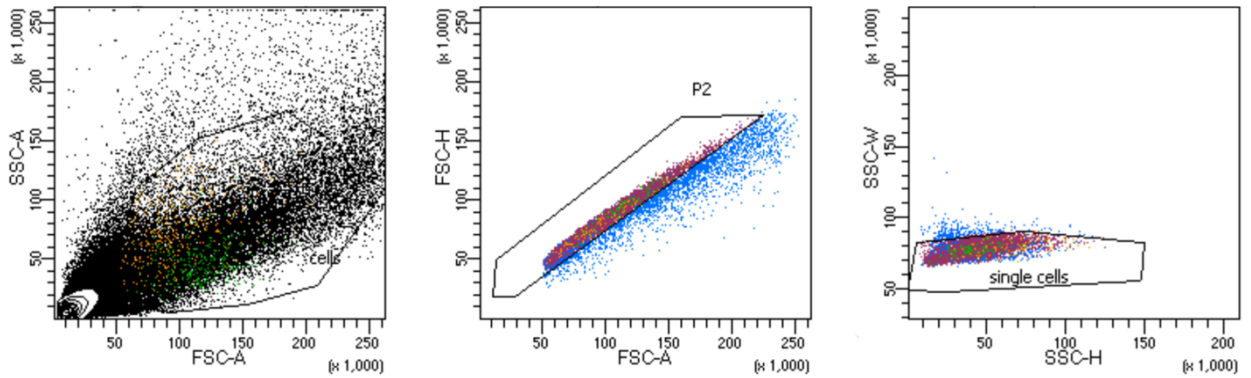
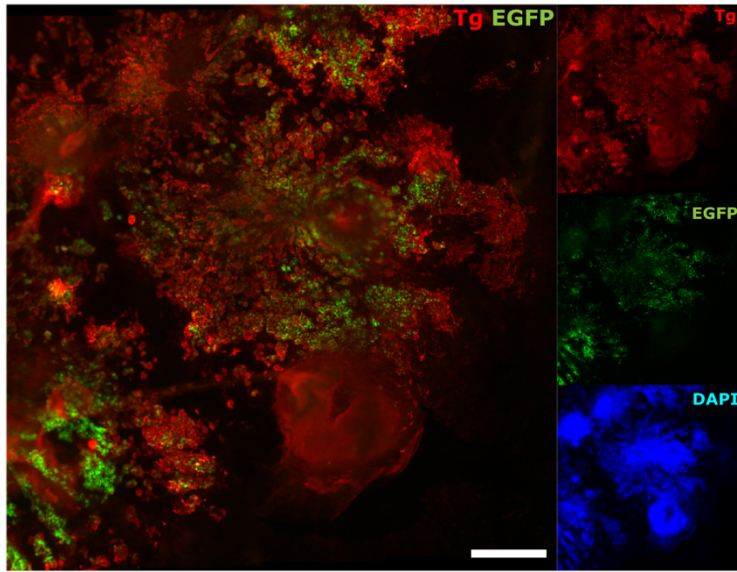


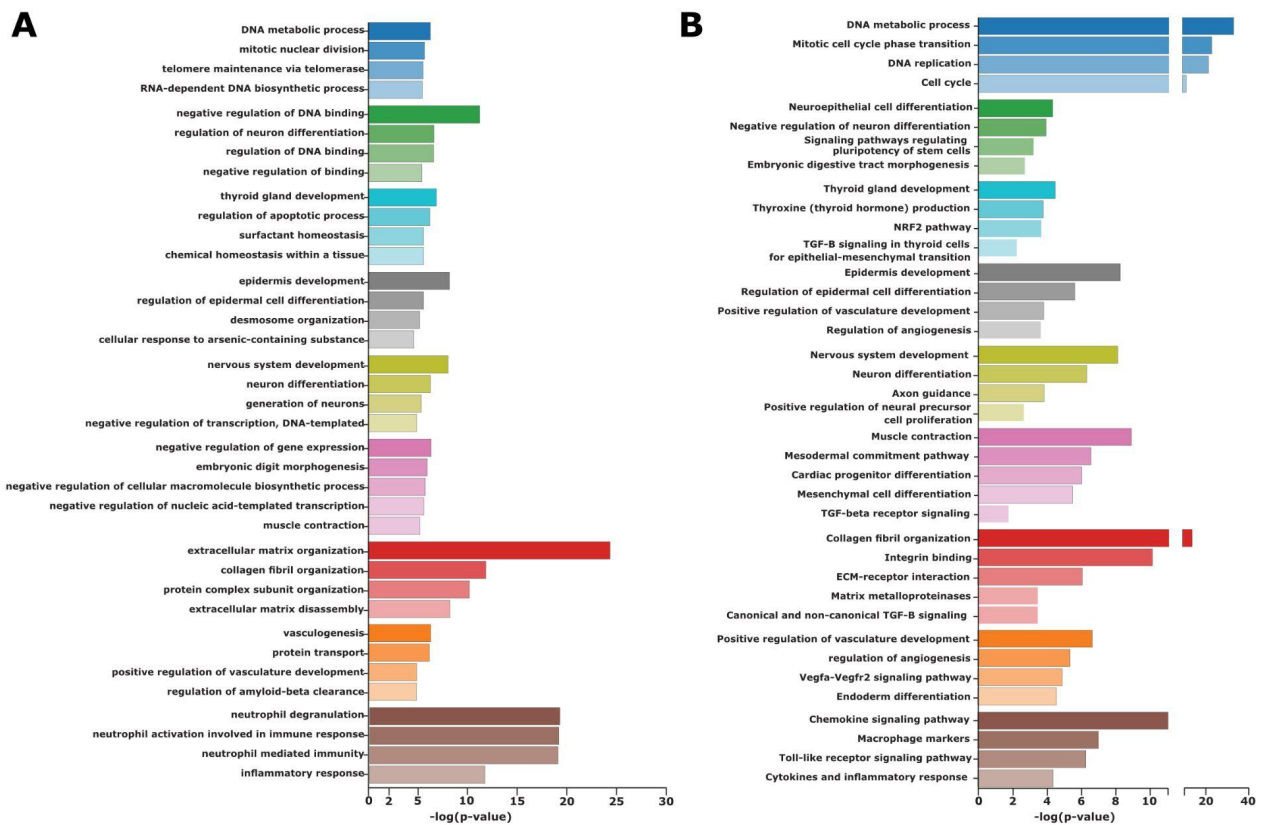
Supplementary Figures



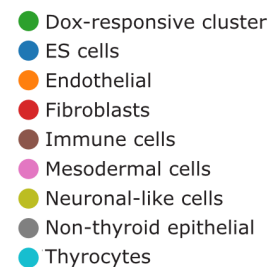
**Supplementary Figure 1.** Flow cytometry plots showing the gating strategy used for cell sorting. Cells were identified using a FSC-A/SSC-A plot and doublets were excluded based on gates in FSC-A/FSC-H and SSC-H/SSC-W plots. After, single cells were chosen according to calcein violet (viability) and EGFP+ fluorescence, as shown in Figure 1.



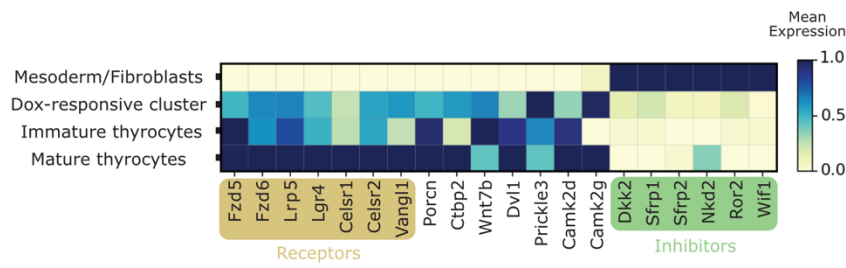
**Supplementary Figure 2.** Large view of thyroid organoids culture at differentiation day 22 shows several follicles expressing Tg (red) while a smaller proportion expresses the bTg\_EGFP reporter (green) (Scale bar: 500  $\mu$ m).



**Supplementary Figure 3.** GO term analysis for differentially expressed genes among the identified cell clusters. **(A)** The first more significant GO terms for each cluster according to biological processes classification. Color of the bar plots correspond to the color of the respective cell cluster. **(B)** GO terms selected according to the literature and relevance inside each cell type cluster.



## Supplementary Material



**Supplementary Figure 4.** Gene-expression matrix plot demonstrates increase in gene expression of Wnt/PCP receptors and effectors in mature thyroid cells compared to immature and Dox-responsive clusters. Mesoderm/fibroblast is used as a non-thyroid cell control.