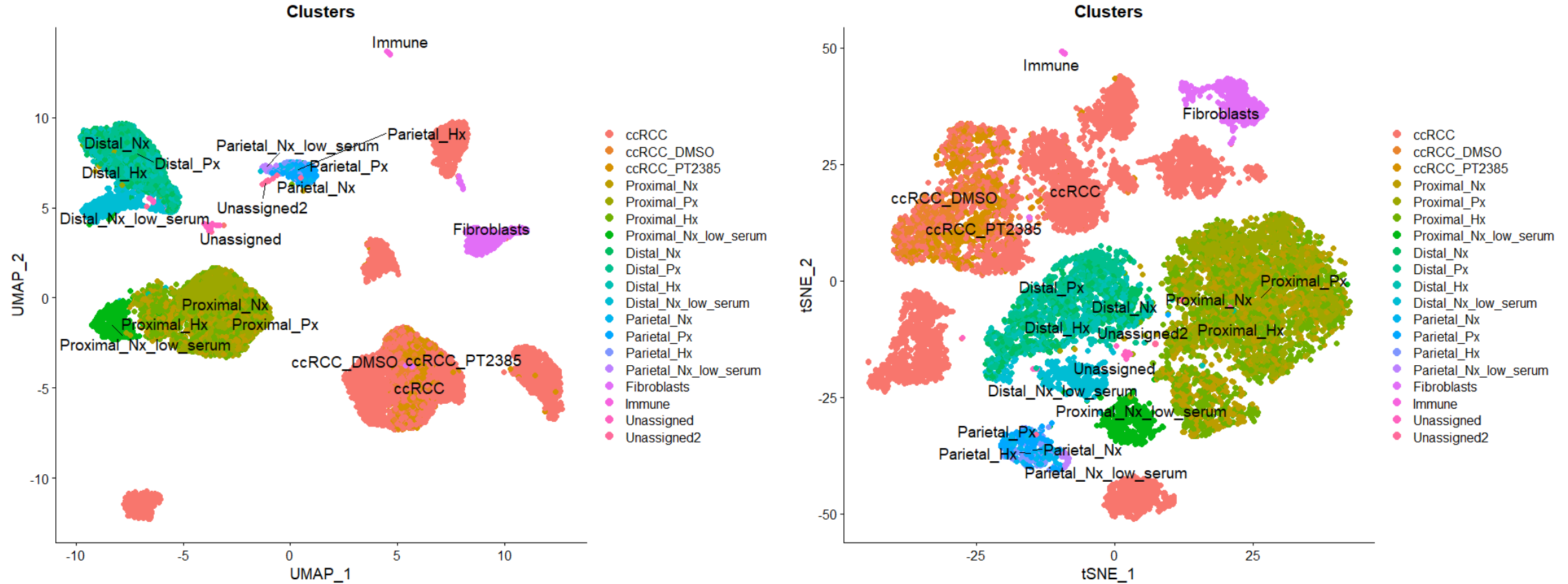
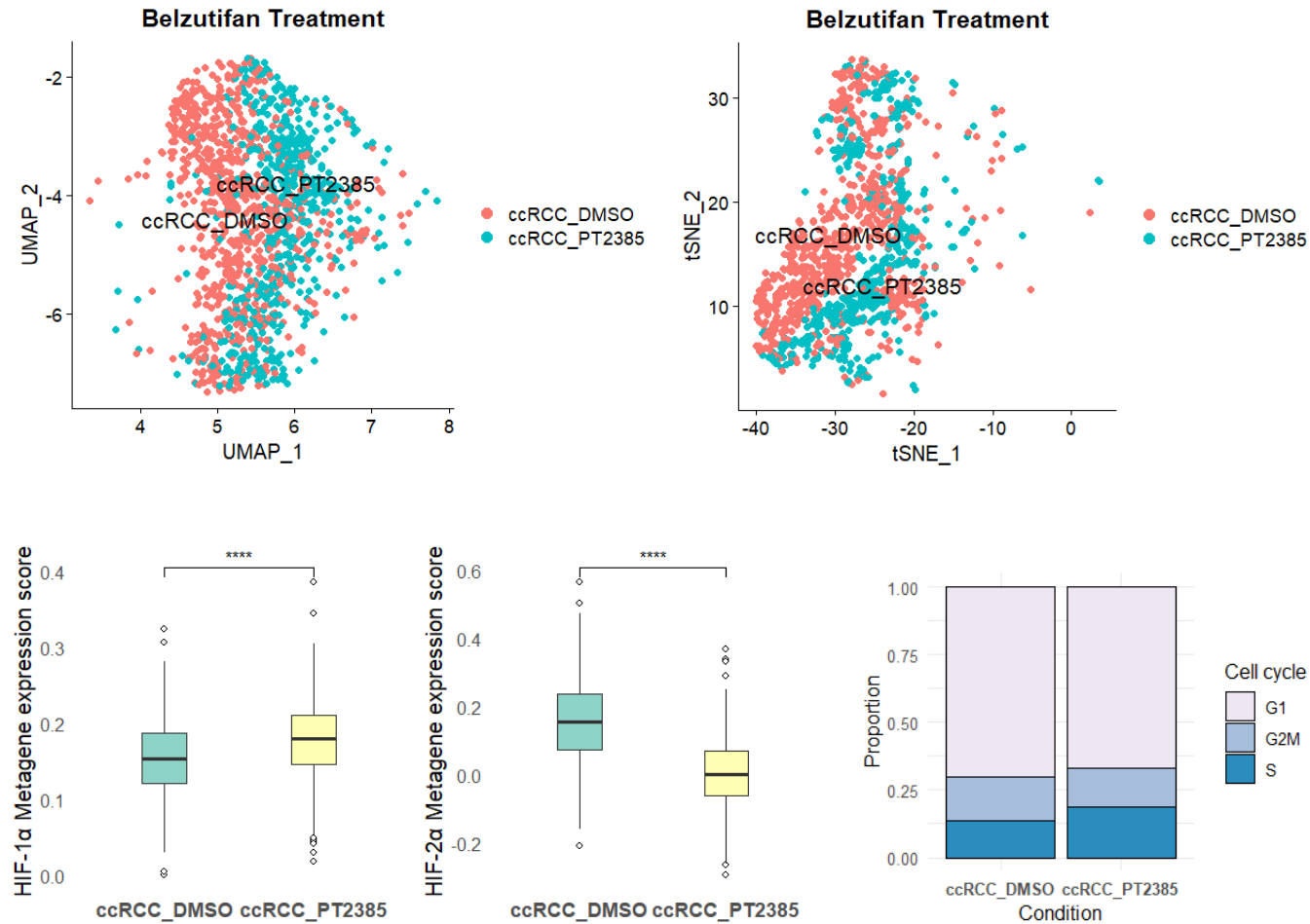


Single-cell RNA-seq using GSE269826

Non-linear dimensionality reduction for all sample



Belzutifan treatment ccRCC vs. ccRCC



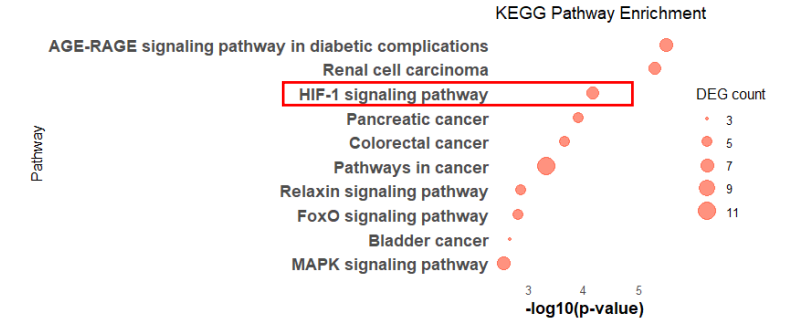
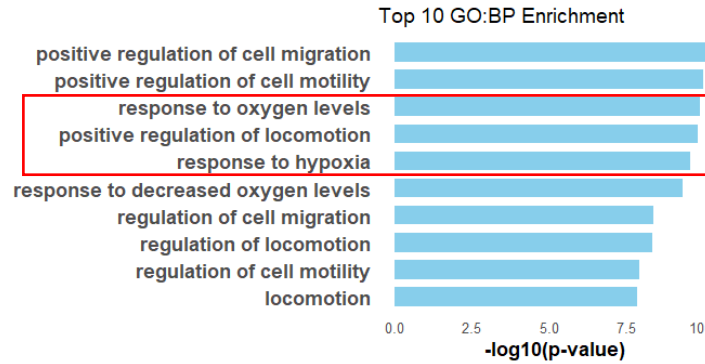
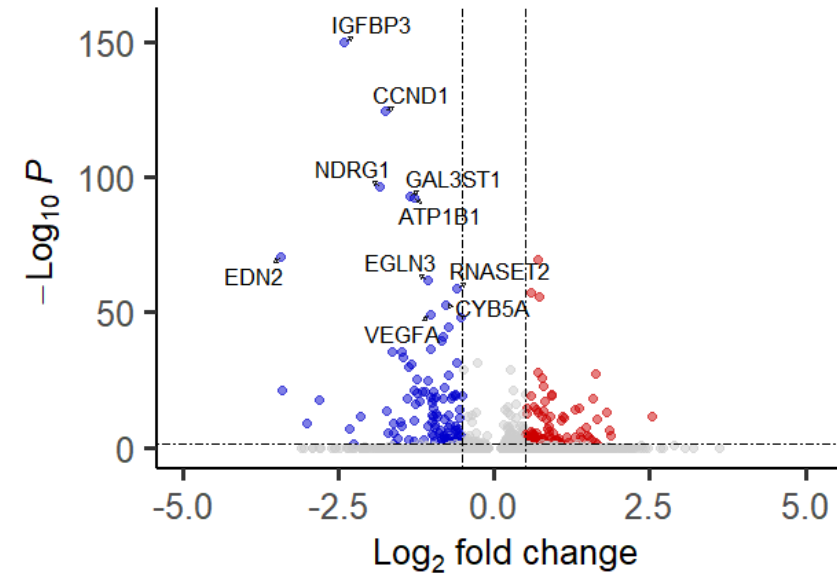
Each target gene expression of HIF-1α and HIF-2α is up-regulated and down-regulated after Belzutifan treatment

ccRCC_PT2385 = belzutifan 16 hours treat group

HIF-1 and HIF-2 target genes defined the HIF-1 specific and HIF-2 specific metagenes (excluding HIF1A and HIF2A/EPAS1 themselves)

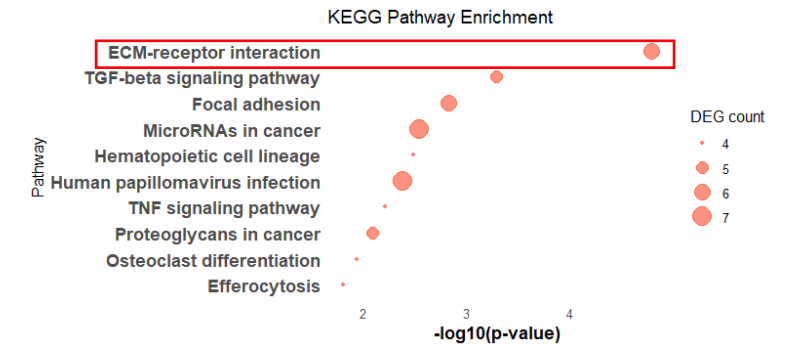
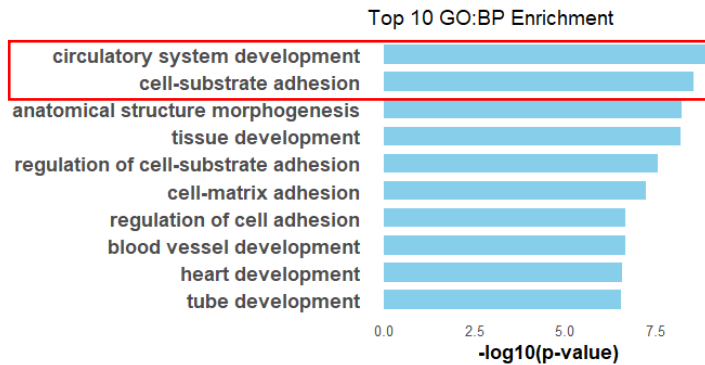
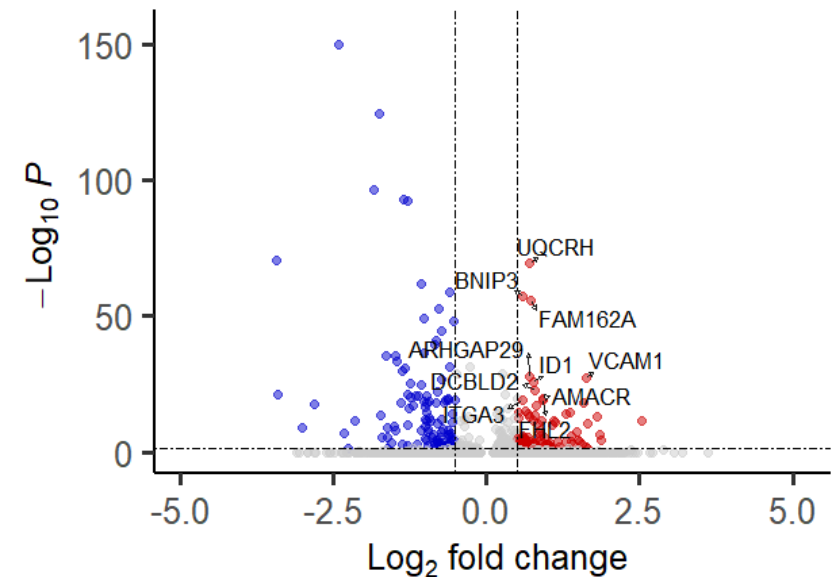
HIF-2 α target down-regulated & ECM adhesion, inflammation up-regulated

Down-regulated GO & KEGG analysis



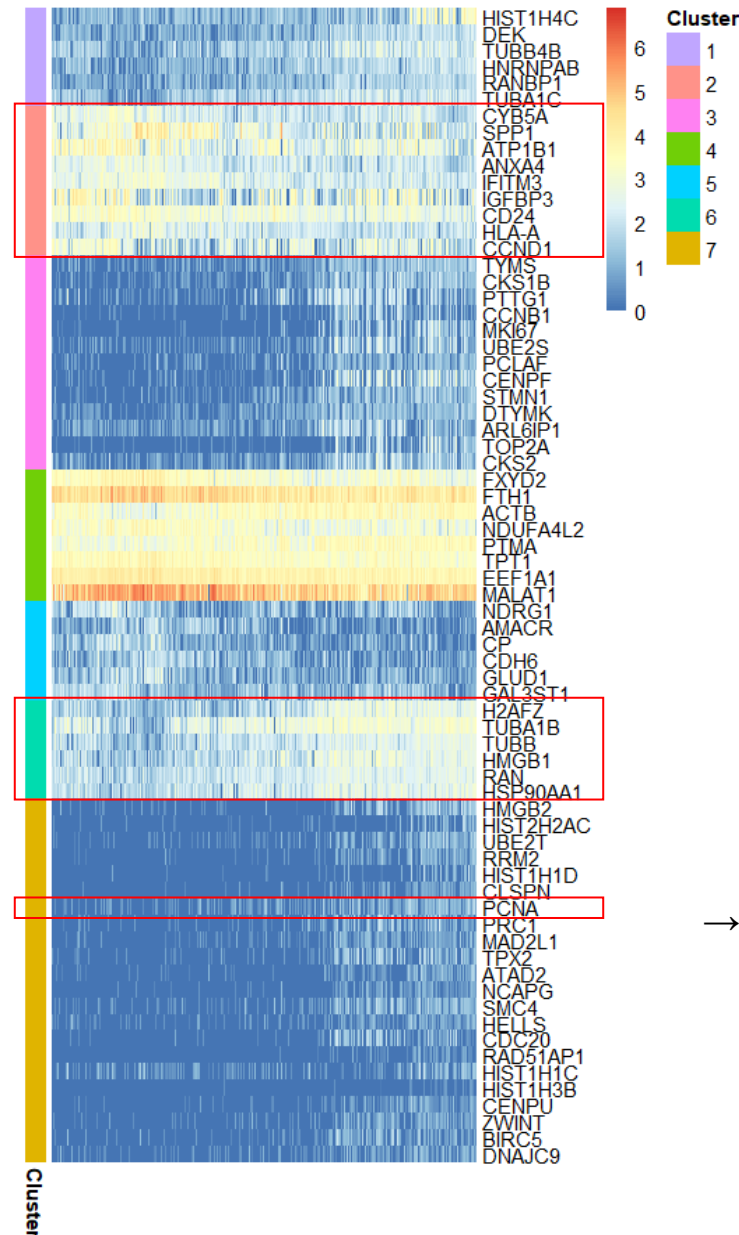
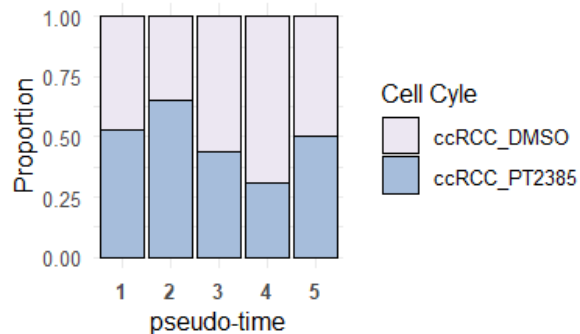
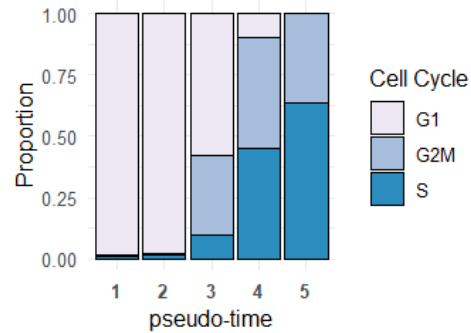
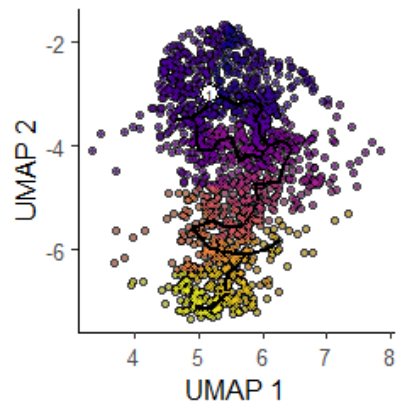
Cell migration down-regulated after Belzutifan treatment

Up-regulated GO & KEGG analysis



Cell-to-cell adhesion up-regulated after Belzutifan treatment

Pseudo-time trajectory analysis using Monocle3



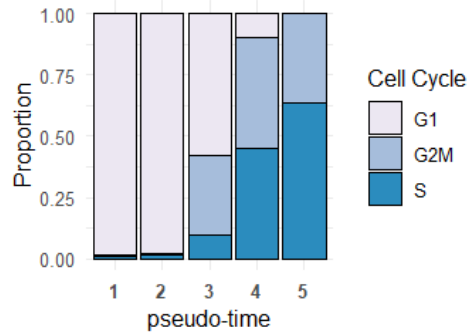
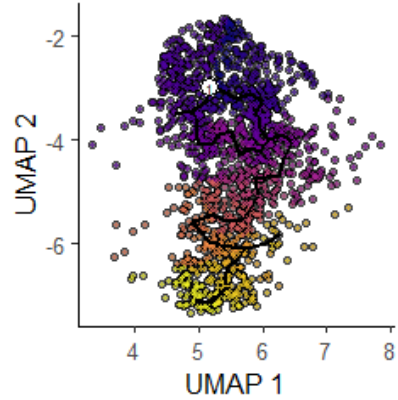
[Down-regulated gene following pseudo-time]

IGFBP3 (insulin-like growth factor binding protein)
CCND1 (cyclin D1)
→ Cells are transitioning into S phase

[Up-regulated gene following pseudo-time]

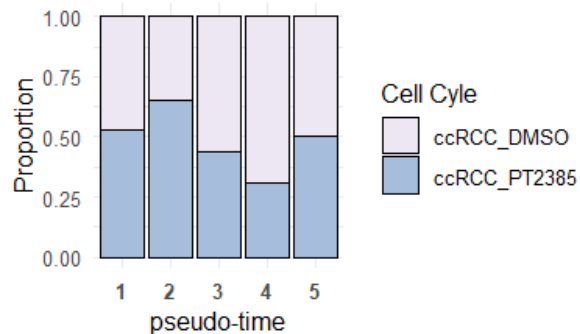
RAN (ras-related nuclear protein)
H2AFZ (H2A.Z Variant Histone 1)
TUBA1B (Tubulin Alpha 1a)
HMGB1 (High mobility group box 1)
PCNA (Proliferating Cell Nuclear Antigen)
→ Cells are transitioning in the direction of the S to G2/M phase

Pseudo-time trajectory analysis using Monocle3

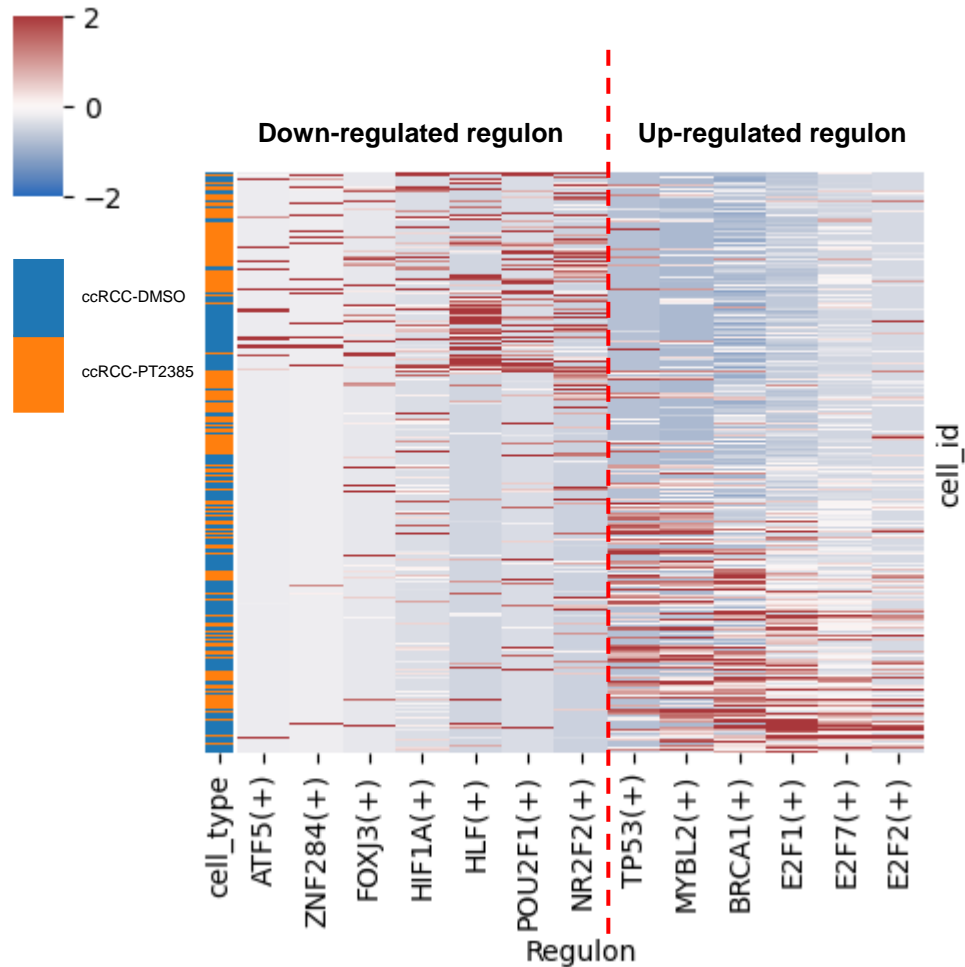


✓ Due to HIF-2 α inhibition, ccRCC cells may **bypass the G1 checkpoint** or **enter the S phase abnormally**, choosing a path of forced cell cycle (S \rightarrow G2 \rightarrow M phase) progression

✓ ccRCC cells may follow the same differentiation trajectory, but their transcriptional programs could be altered



Alterations of a regulon induced after Belzutifan treatment



- ✓ HIF-2 α inhibition suppresses a hypoxia-adaptive transcriptional network
- ✓ HIF-2 α inhibition leads to increase in the regulon activity of TP53, MYBL2, BRCA1, and members of the E2F family
- ✓ This suggests that cells undergo a state transition from stress adaptation toward **DNA repair and cell cycle reprogramming**