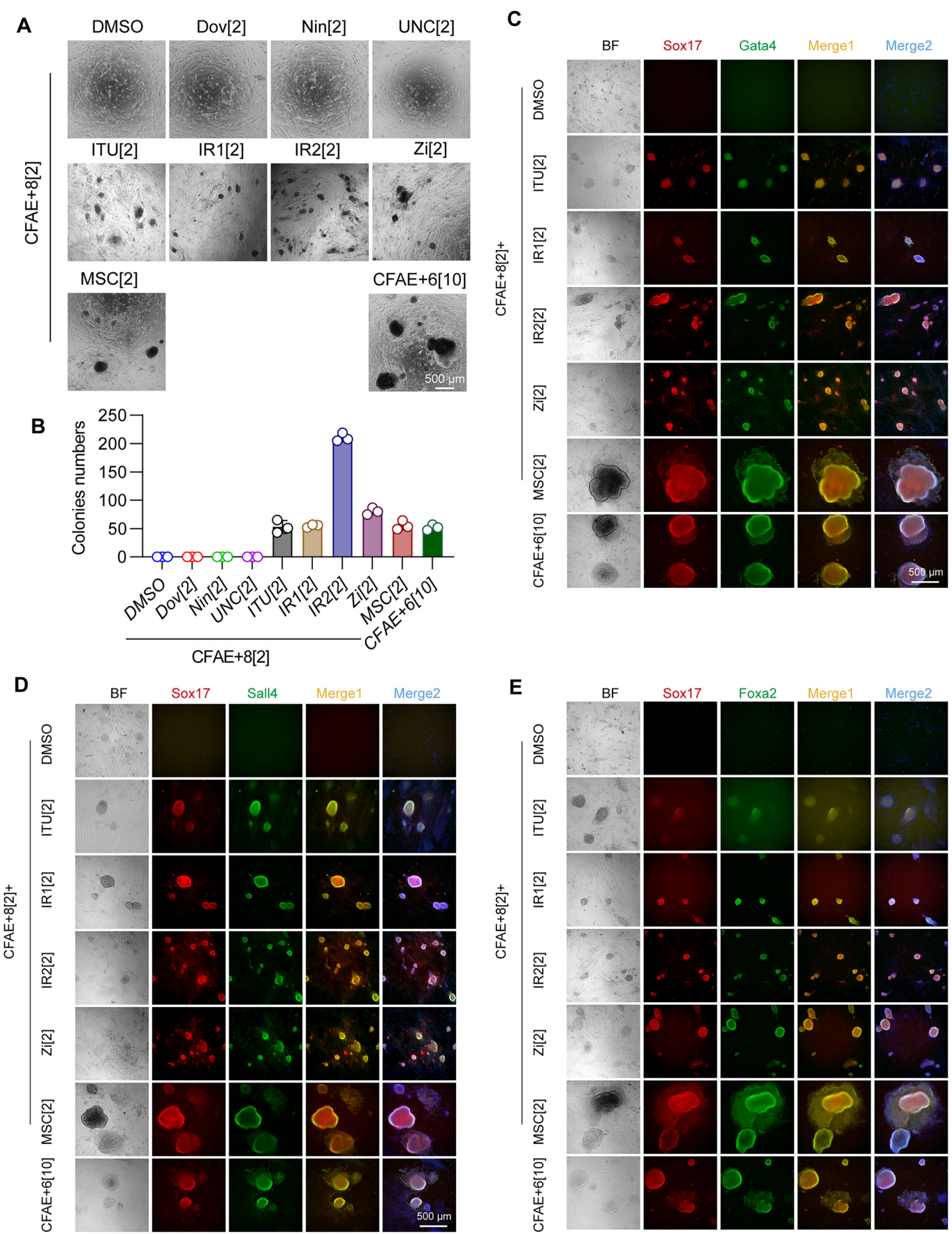


Expanded View Figures

Figure EV1. Identifying the candidate chemicals from the DRUG-seq2 screen that initiate MEF-XEN reprogramming.

(A) The morphologies of colonies in CFAE + 8 plus candidate chemicals from the DRUG-seq2 screen or in CFAE + 6 on day 12. (C CHIR99021, F Forskolin, A AM580, E EPZ04777, 6 E-616452, 8 A-83-01, Dov dovitinib lactate, Nin nintedanib esylate, UNC UNC2025, ITU 5-iodotubercidin, IR1 IRAK inhibitor 1, IR2 IRAK4-IN-1, Zi zimlovisertib, MSC MSC2530818). (B) Quantifying colony numbers in (A). Data were represented as mean $\pm$ SD. $n = 3$ biological replicates, respectively. (C-E) Individual raw images and merged panel related to Fig. 1A. Immunofluorescence staining of XEN master regulators Sox17, Gata4, Sall4, and Foxa2 in CFAE + 8 plus ITU/IR1/IR2/Zi/ MSC or CFAE + 6, respectively. Scale bar: 500 μ m. Source data are available online for this figure.



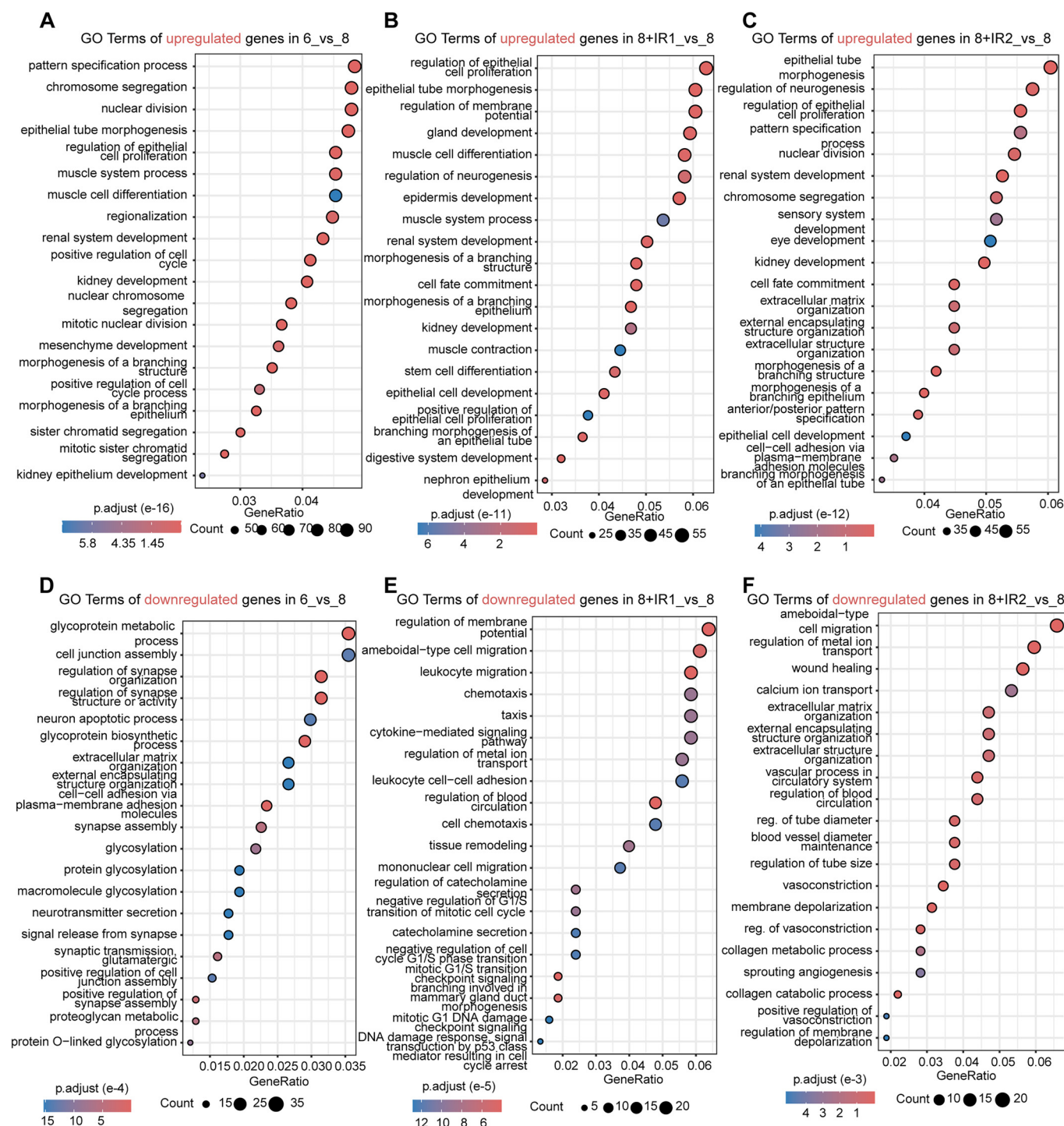


Figure EV2. Gene ontology analysis of DEGs in 6, 8 + IR1, or 8 + IR2 versus 8.

Biological processes (BP) of gene ontology (GO) enrichment in upregulated (A-C) or downregulated (D-F) genes of 6, 8 + IR1, or 8 + IR2 versus 8. The P value was calculated using the Hypergeometric test. Basal medium: CFAE. Optimal concentration determination of IR2 during MEF-XEN reprogramming. Related to Figs. 3 and 4.

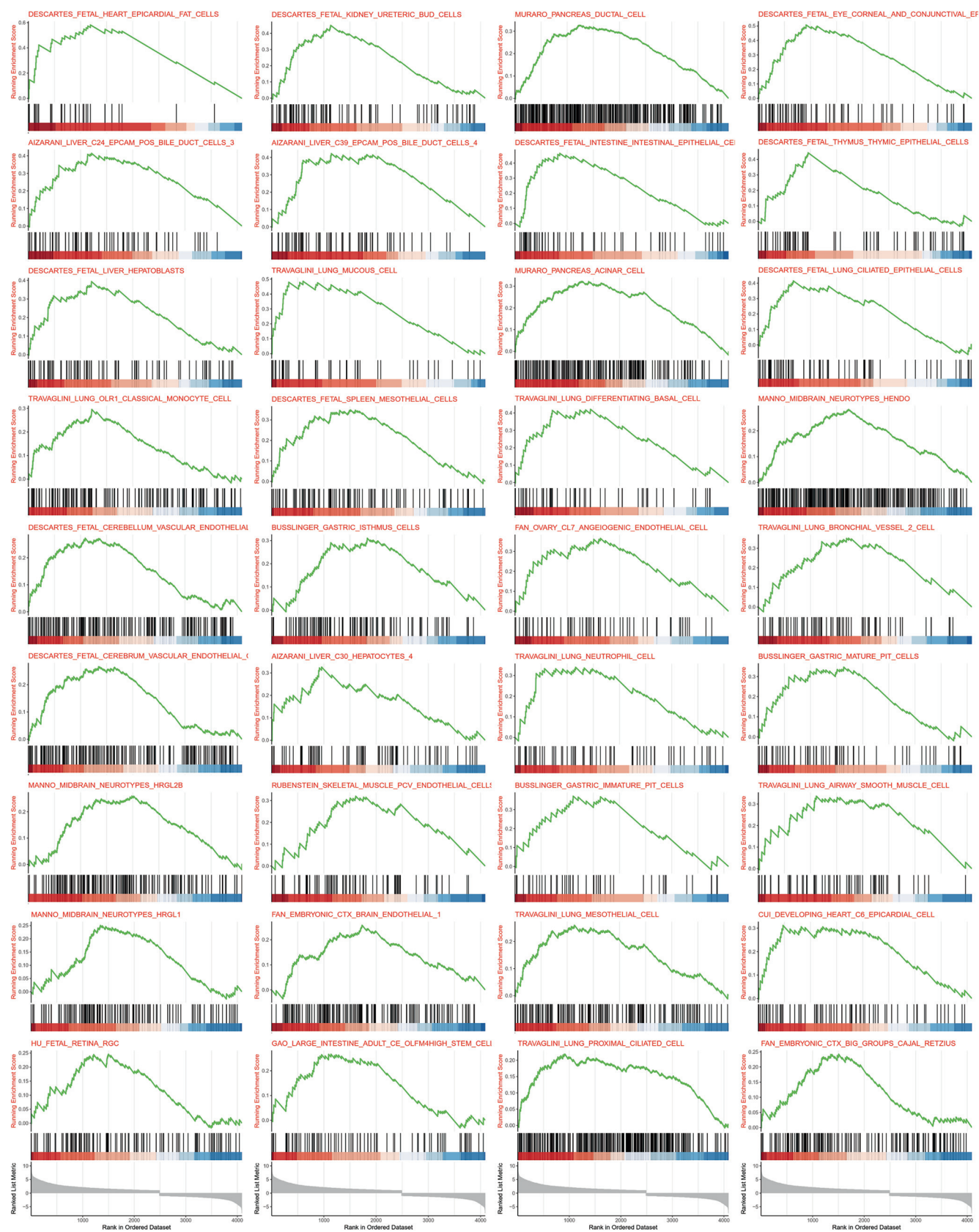
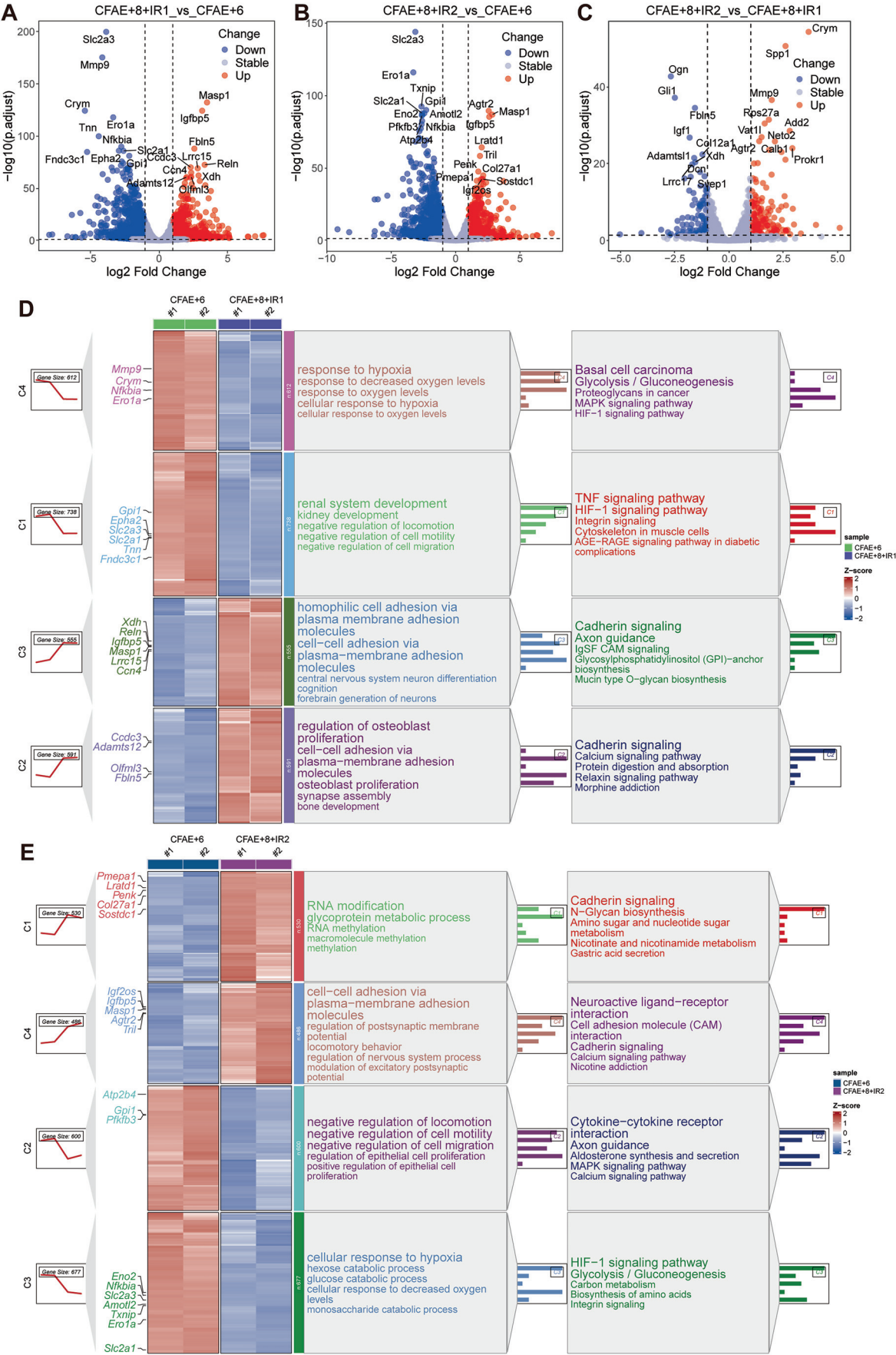


Figure EV3. GSEA of multi-lineage fate pathways in both CFAE + 6 and CFAE + 8 + IR1/IR2 versus the CFAE + 8 control.



◀ Figure EV4. Differential gene expression and comparative transcriptomic analysis between indicated conditions.

(A–C) Volcano plot of CFAE + 8 + IR1, CFAE + 8 + IR2 versus CFAE + 6, or CFAE + 8 + IR2 versus CFAE + 8 + IR1 (A–C: $n = 2$ biological replicates, respectively). The P value was calculated using the Wald test. Significantly up- and down-regulated genes are defined by $|\log_2FC| > 1$ and $p.adjust < 0.05$. (D, E) Heatmap, GO, and KEGG enrichments of differentially expressed transcripts in CFAE + 8 + IR1 or CFAE + 8 + IR2 versus CFAE + 6. Enriched GO-BP and KEGG pathways are displayed with font size scaled to significance.

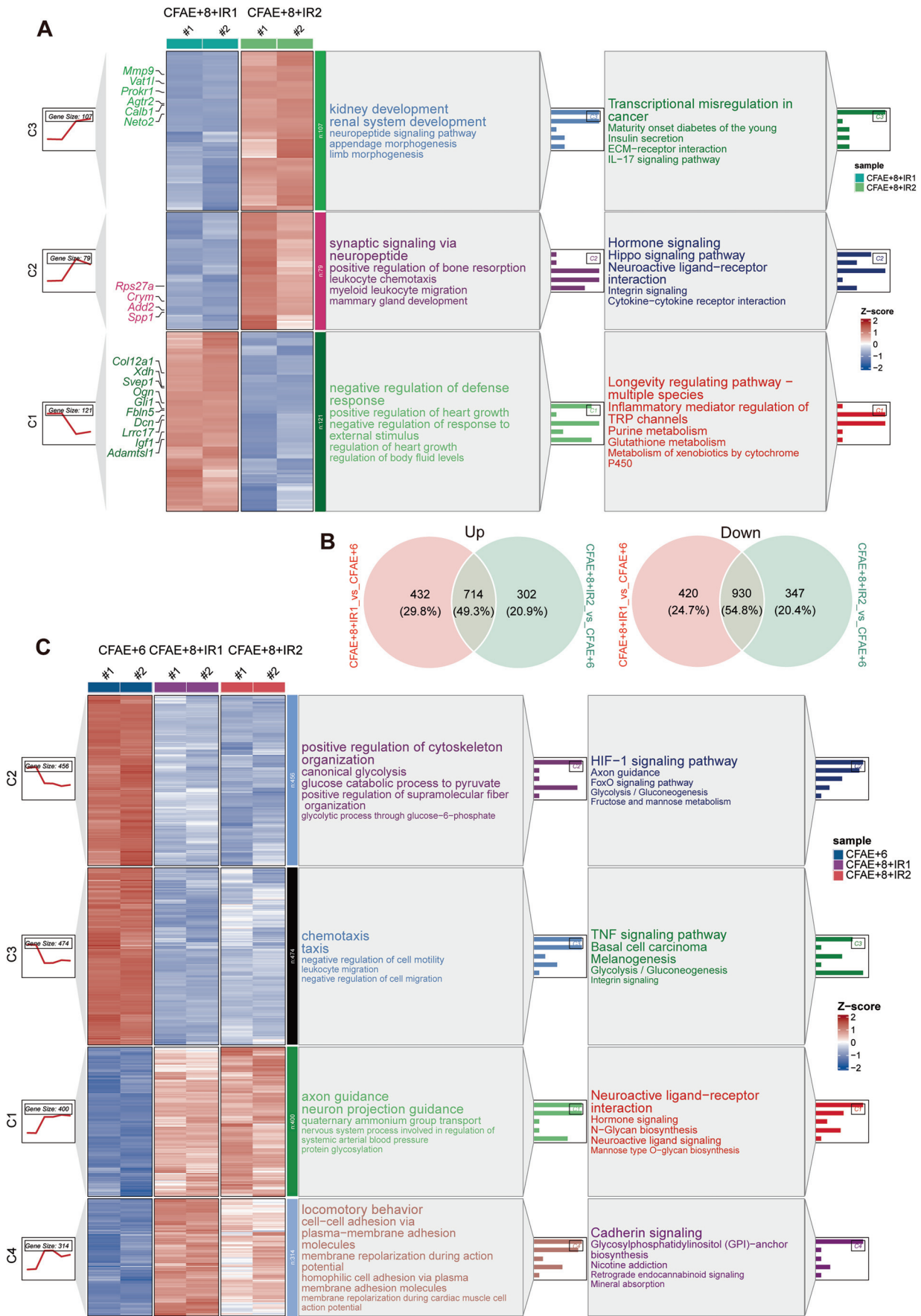


**Figure EV5. Comparative transcriptomic analysis between indicated conditions.**

(A) Heatmap, GO, and KEGG enrichments of differentially expressed transcripts in CFAE + 8 + IR2 versus CFAE + 8 + IR1. Enriched GO-BP and KEGG pathways are displayed with font size scaled to significance. (B) Venn diagrams show the common down- or up-regulated genes between CFAE + 8 + IR1 versus C6FAE and CFAE + 8 + IR2 versus C6FAE. (C) Heatmap, GO, and KEGG enrichments of transcripts in the intersection of comparisons. Enriched GO-BP terms and KEGG pathways for each cluster are displayed, with font size scaled to enrichment significance.