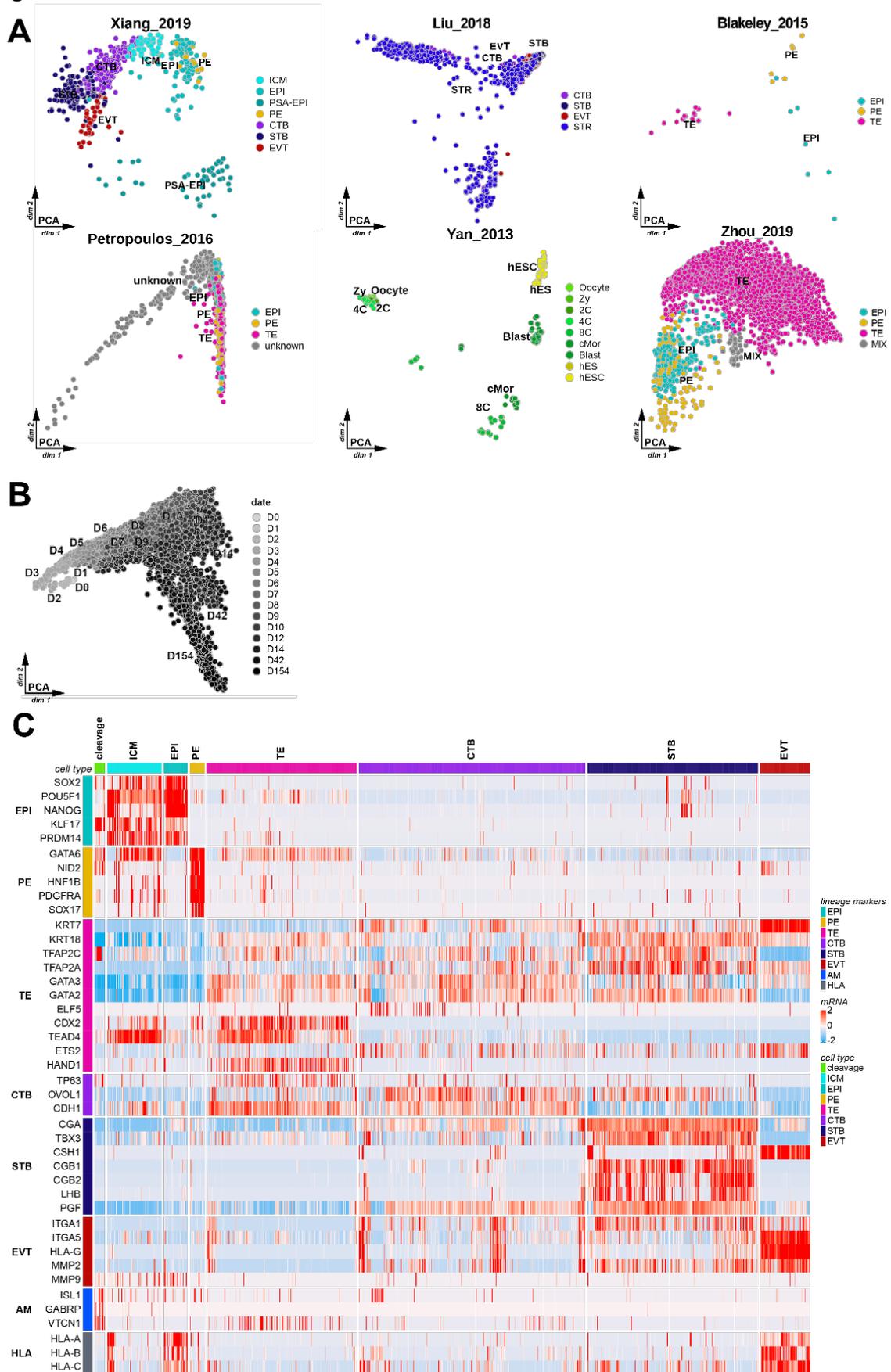


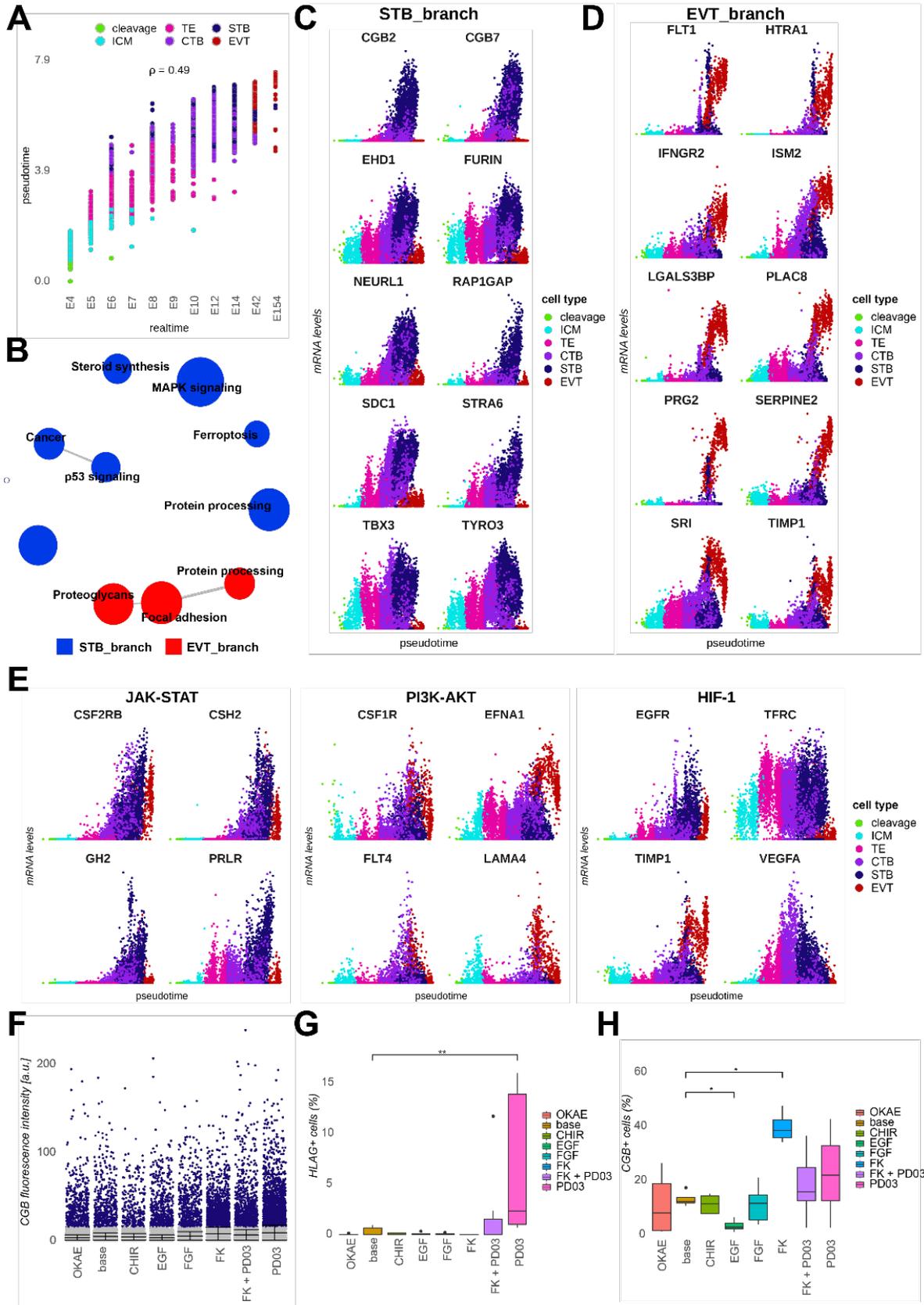
Figure S1



**Fig. S1. Integration of six scRNA-seq datasets into a trophoblast developmental trajectory.**

(A) Principal component analysis (PCA) for each of the six datasets used in this study.  
(B) PCA of the combined dataset coloured by developmental time (D). (C) Normalized expression counts of lineage markers for each annotated lineage.

Figure S2



**Fig. S2. Pseudotime trajectory identifies lineage specific signalling pathways.** (A)

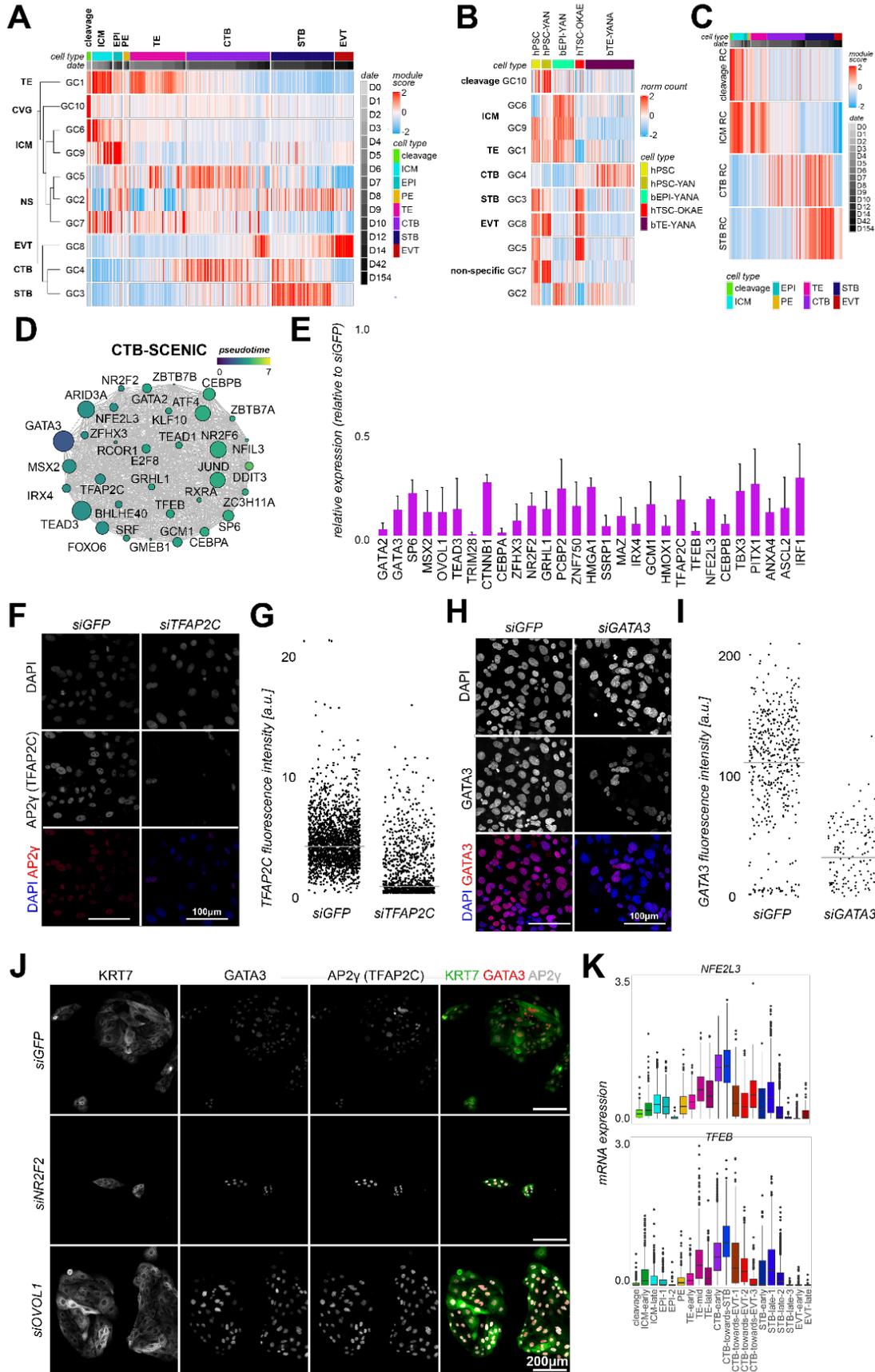
Pearson correlation between pseudotime with embryonic day. (B) Over-represented KEGG terms of significant differentially expressed genes along the STB and EVT branches. ECM: extracellular matrix. (C) Normalized expression counts of the most highly differentially expressed genes along the STB branch over the pseudotime. The counts are scaled to a mean of 0 and standard deviation of 1. (D) Normalized expression counts of the most highly differentially expressed genes along the EVT branch over the pseudotime. Counts are scaled to a mean of 0 and standard deviation of 1. (E) Scaled transcript counts of the most dynamically expressed genes in indicated KEGG terms. (F) Quantification of CGB fluorescence in indicated conditions (n=3). (G) Quantification of HLAG positive cells in indicated conditions (n=3). (H) Quantification of CGB positive cells in indicated conditions (n=3).



**Fig. S3. Staging trophoblast *in vitro* cells using pseudotime**

(A) PCA projection of hPSCs onto the trophoblast developmental trajectory. (B) PCA of different lineage subclusters. (C) Scaled correlation score of indicated *in vitro* cells with trophoblast development lineage subclusters. (D) Relative probability of matching the transcriptomic profile of *in vivo* lineages to the trophoblast trajectory. (E) Relative probability of matching the transcriptomic profile of *bTE-YU* and *hPSCs* to the trophoblast trajectory. (D) Relative probability of matching the transcriptomic profile of *bTSC-YU* and *hPSC* to the trophoblast trajectory. (E) Enriched KEGG terms for differentially expressed genes between *hTSC-OKAE*, *bTSC-YU*, and *bTE-YANA* and *in vivo* lineages. (F) Enriched KEGG terms for differentially expressed genes between *in vitro* lineages.

Figure S4



**Fig. S4. Weighted gene co-expression network analysis identified trophoblast cell type specific transcription factor network.**

(A) Module scores of transcription factor network in each cell. GC:(transcription factor) gene cluster, CVG: cleavage, NS: non-specific. (B) Module scores of each transcription factor network in each *in vitro* cell. (C) Heatmap of module scores of SCENIC regulon clusters in each cell. RC: transcription factor regulon cluster. (D) SCENIC transcription factor regulon network associated with each trophoblast cell type. Edge width: proportional to the Pearson correlation value; node size: mean gene expression; colour: mean pseudotime. The CTB network is from the CTB RC in Figure S4A. (E) Quantitative PCR of siRNA knockdown of all genes normalized to siGFP on day 3 (n=3). (F) Immunofluorescence of siGFP and siTFAP2C treated hTSCs on day 4. (G) Quantification of TFAP2C fluorescence in indicated conditions (n=3). (H) Immunofluorescence of siGFP and siGATA3 treated hTSCs on day 4. (I) Quantification of GATA3 fluorescence in indicated conditions (n=2). (J) Immunofluorescence of siGFP, siNR2F2, and siOVOL1 for trophoblast lineage markers. (K) mRNA expression of indicated genes from Cambridge trophoblast atlas.

**Table S1. Trophoblast atlas average gene expression**

Average gene expression of in vivo and in vitro lineages in trophoblast atlas.

[Click here to download Table S1](#)

**Table S2. Differential expression analysis between in vivo lineages**

Pairwise differential expressed analysis between in vivo lineages.

[Click here to download Table S2](#)

**Table S3. Differential expression analysis between in vitro lineages**

Pairwise differential expressed analysis between in vitro lineages.

[Click here to download Table S3](#)

**Table S4. Lineage specific transcription factor modules**

CTB, STB, and EVT specific transcription factor modules. kWithin: intra-modular connectivity; avgExpr: average expression.

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**Table S5. Transcriptional validation of siRNA knockdowns**

qPCR results of siRNA KO validation with primer sequences.

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**Table S6. siRNA sequences**

Target sequences of human siRNA screen.

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