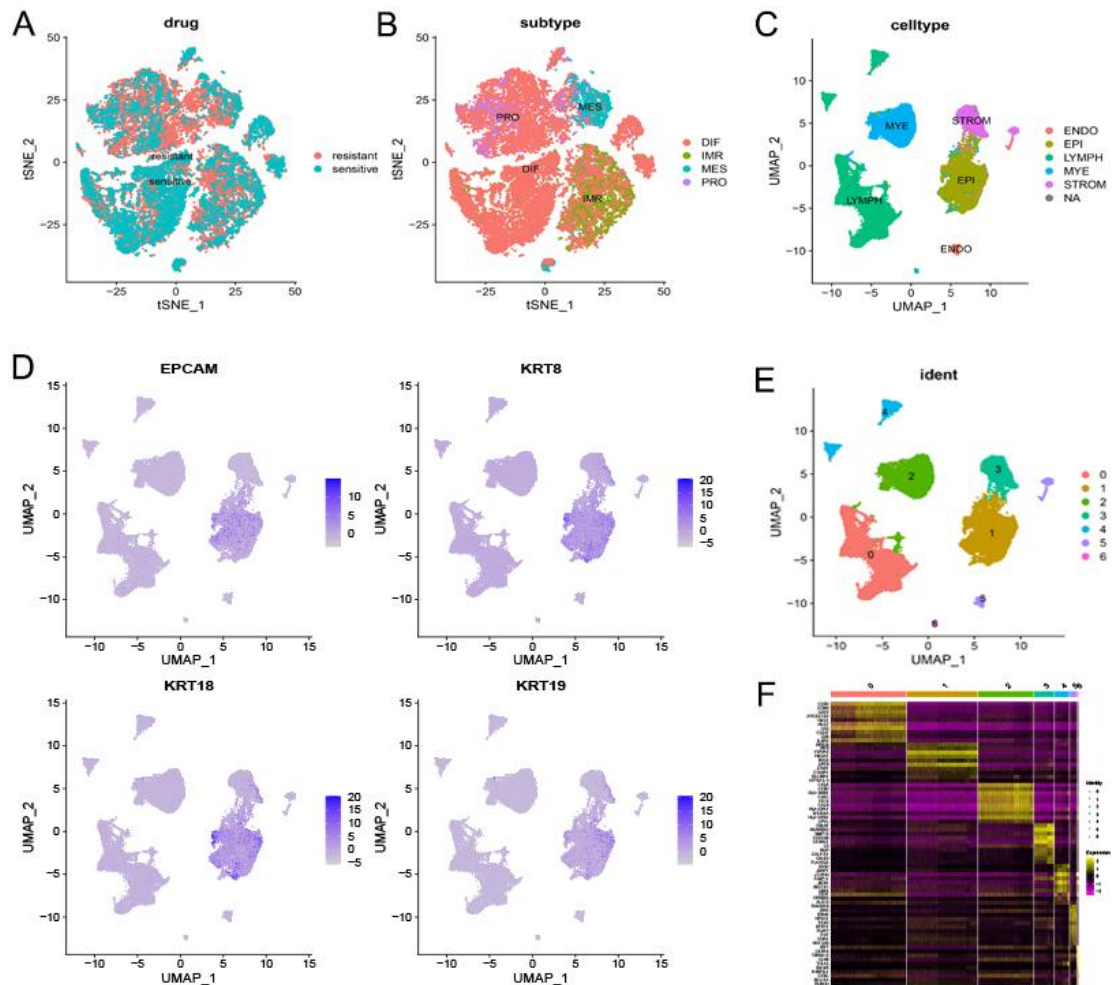


## Supplementary information

### **CSTF3 contributes to platinum resistance in ovarian cancer through alternative polyadenylation of lncRNA NEAT1 and generating the short isoform NEAT1\_1**

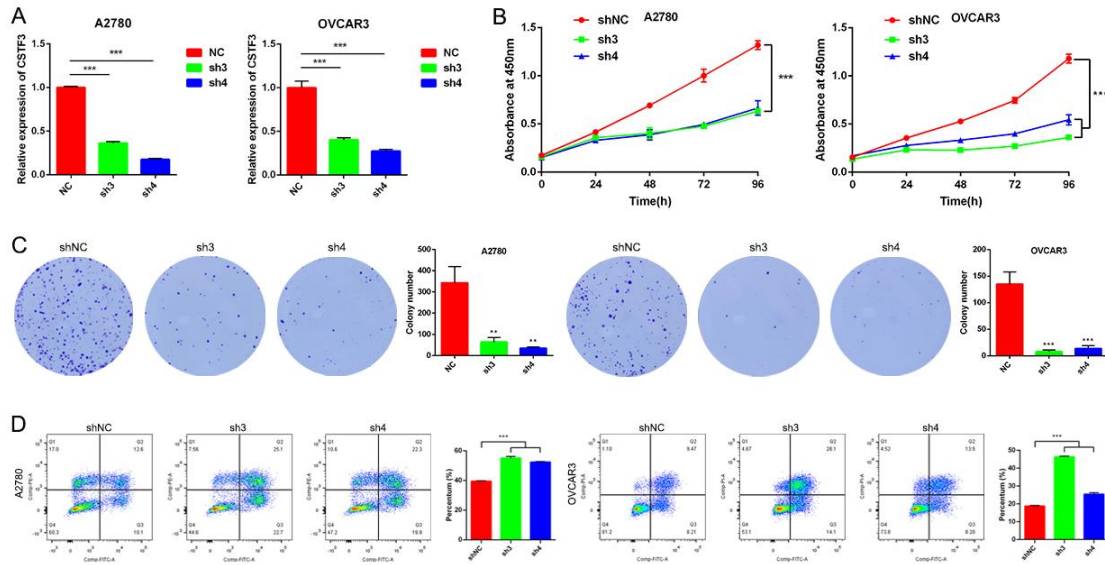
Xin Luo<sup>1,4</sup>, Qinglv Wei<sup>2,4</sup>, Xiaoyan Jiang<sup>1,4</sup>, Ningxuan Chen<sup>1</sup>, Xinzhao Zuo<sup>1</sup>, Hongyan Zhao<sup>3</sup>, Yujiao Liu<sup>1</sup>, Xiaoyi Liu<sup>1</sup>, Lingcui Xie<sup>1</sup>, Yu Yang<sup>1</sup>, Tao Liu<sup>1</sup>✉, Ping Yi<sup>1</sup>✉, Jing Xu<sup>1</sup>✉

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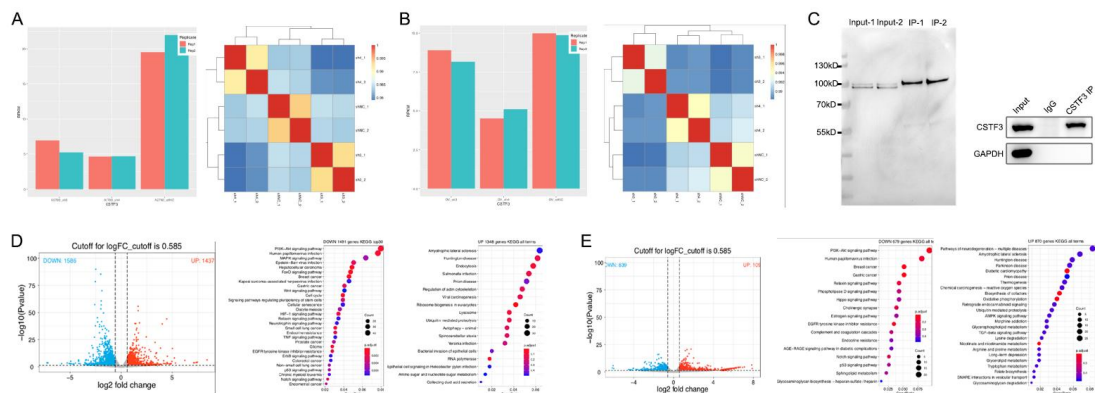


**Figure S1. scRNA-seq analysis with the platinum resistance and sensitivity of OC datasets.**

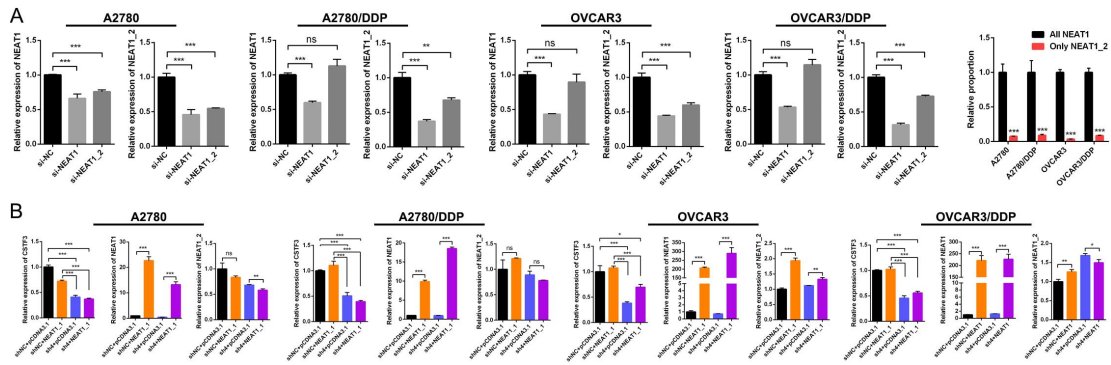
Integrating all scRNA-seq data, dimension reduction by tSNE or UMAP aimed at drug treatment (A), subtype (B) and cell type (C). (D) The epithelial marker genes EPCAM, KRT8, KRT18, and KRT19 annotated epithelial cell (EPI) using UMAP. (E) Cells were clustered based on the expression of highly variable genes and visualized using UMAP, and heatmap of average ten specific genes show the features of each cluster (F).



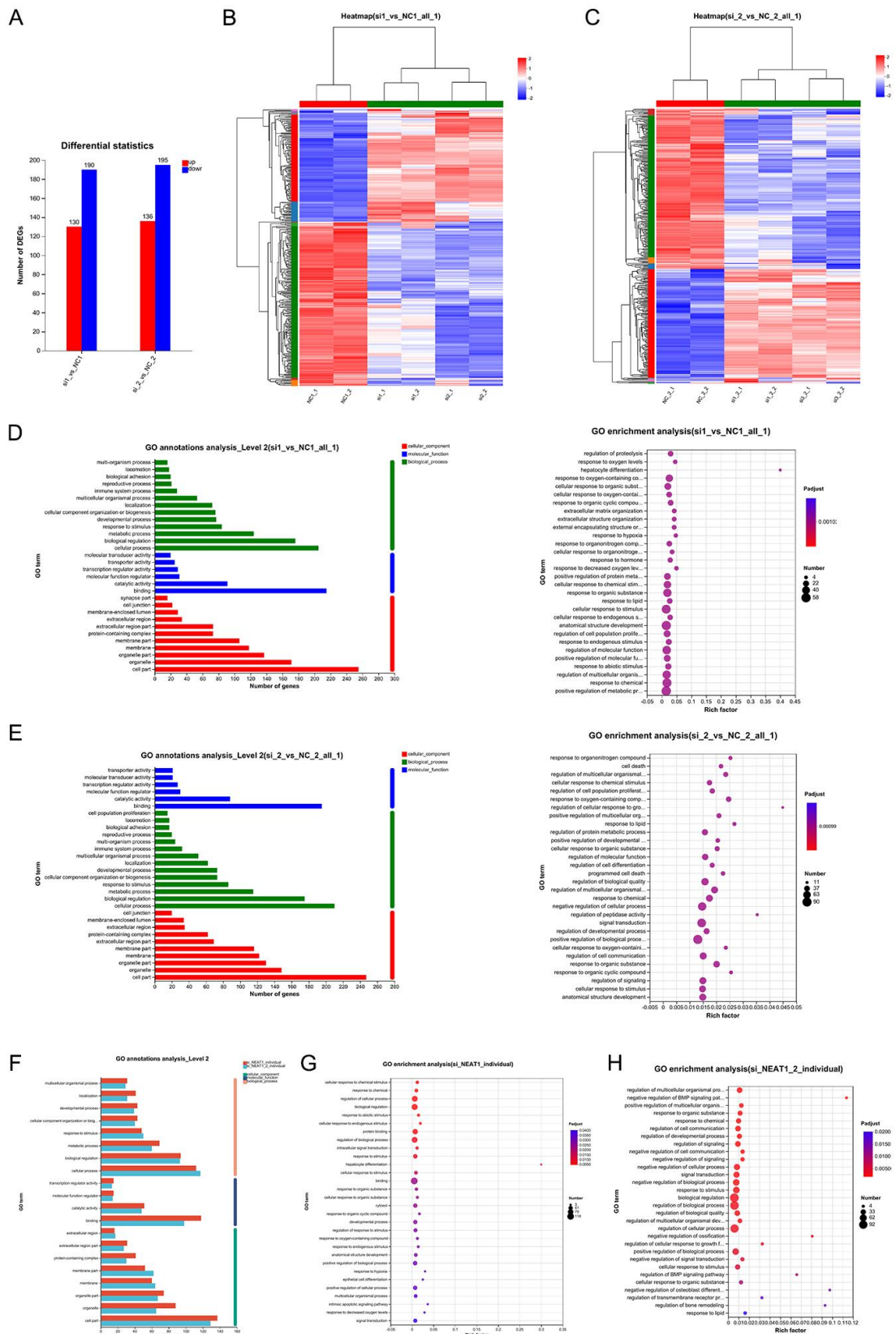
**Figure S2. CSTF3 knockdown suppressed OC cell proliferation and induced apoptosis.** A. CSTF3 mRNA was measured to confirm the knockdown efficiency in A2780 and OVCAR3 cells. CCK8 (B) and colony formation (C) assays were performed to determine the proliferation of OC cells after downregulation of CSTF3 expression. (D) Apoptosis was measured by flow cytometry. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. shNC. ns not significant.



**Figure S3. Analysis of PAS-seq with CSTF3 knockdown and quality control of CSTF3 eCLIP-seq and eCLIP-qPCR.** (A) The knockdown efficiency of the CSTF3 gene and the correlation of knockdown and control samples in PAS-seq of A2780 cells. (B) The image of knockdown efficiency and heatmap of the samples' correlation in OVCAR3 cells. (C) Immunoprecipitation of CSTF3 eCLIP-seq and eCLIP-qPCR. (D, E) Analysis of differentially expressed genes as well as KEGG pathway enrichment with upregulated and downregulated genes for PAS-seq results of A2780 and OVCAR3 cells.



**Figure S4. Silencing NEAT1 and NEAT1\_2 in A2780, OVCAR3 and corresponding platinum-resistant cells as well as overexpressing NEAT1\_1 in A2780, OVCAR3 and platinum-resistant cells with CSTF3 knockdown. (A) The silencing verification and expression examination of NEAT1 and NEAT1\_2 in A2780, OVCAR3 and corresponding platinum-resistant cells by RT-qPCR. (B) The expression of CSTF3, NEAT1 and NEAT1\_2 were detected when NEAT1\_1 was overexpressed in A2780, OVCAR3 and platinum-resistant cells with CSTF3 knockdown. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .**



**Figure S5. Enrichment analysis of DEGs after silencing NEAT1 or NEAT1\_2 in OVCAR3 cells.** (A) Histogram of DEGs after silencing NEAT1 and NEAT1\_2 in OVCAR3 cells. (B, C) Heatmap of DEGs from OVCAR3 cells enriched transfected with NEAT1 siRNA, NEAT1\_2 siRNA and

control siRNA. (D) GO and KEGG enrichment analysis of DEGs for silencing NEAT1 in OVCAR3 cells. (E) GO and KEGG enrichment analysis of DEGs for silencing NEAT1\_2 in OVCAR3 cells. (F) GO annotation analysis of independent DEGs with NEAT1 and NEAT1\_2 silenced. (G) GO enrichment analysis of the individual DEGs list with NEAT1 silenced. (H) GO enrichment analysis of the individual DEGs list with NEAT1\_2 silenced.

## Supplementary tables

**Table S1. Sequences of oligos by interfering CSTF3, NEAT1 and NEAT1\_2**

Target names		Sequences (5'–3')
CSTF3 shRNA-3	Forward	CCGGGTGATGAAGCTGCTAATATATCTCGAGATATATT AGCAGCTTCATCACTTTTTG
	Reverse	AATTCAAAAAGTGATGAAGCTGCTAATATATCTCGAG ATATATTAGCAGCTTCATCAC
CSTF3 shRNA-4	Forward	CCGGGCCCGATTTCTAGCATTTGAACTCGAGTTCAAAT GCTAGAAATCGGGCTTTTTG
	Reverse	AATTCAAAAAGCCCgATTTCTAGCATTTGAACTCGAGT TCAAATGCTAGAAATCGGGC
si-NC	Forward	UUCUCCGAACGUGUCACGUTT
	Reverse	ACGUGACACGUUCGGAGAATT
NEAT1 siRNA-1	Forward	CAGGAGGCUACCAUUUAAATT
	Reverse	UUUAAAUGGUAGCCUCCUGTT
NEAT1 siRNA-2	Forward	GCAGGUUGAAGGAAUUCUTT
	Reverse	AGAAUCCCUUCAACCUGCTT
NEAT1_2 siRNA-1	Forward	CCAGGUCUGUCAUAUUAATT
	Reverse	UUAUAUUGACAGACCUGGTT
NEAT1_2 siRNA-2	Forward	GGCCUCAUAUAAGUGUAAUTT
	Reverse	AUUACACUUAUAUGAGGCCTT

**Table S2. Sequences of primers used in this study**

Gene	Primer	Sequence (5'–3')
Primers for RT-qPCR		
CSTF3	Forward	CTGAGTATGTCCCAGAGAAGGT
	Reverse	TGCTCCAAGCATCAAGGTCAT
NEAT1 Distal	Forward	GGTGGGACTGTTCTGTCCTTG
	Reverse	AACAGGCCCAGGTGAGTAGA
NEAT1 Proximal	Forward	TGGGCGAGGTGCCTTTACTA
	Reverse	CCCAGAAGACAGAAAGATCCCA
GAPDH	Forward	GTCAAGGCTGAGAACGGGAA
	Reverse	AAATGAGCCCCAGCCTTCTC
Primers for eCLIP-qPCR		
NEAT1-1	Forward	TCGACCCCTATCACATTGCC
	Reverse	ACTTGGAGCTAGCAAATCTAGACC
NEAT1-2	Forward	GGGATCTTTCTGTCTTCTGGGTT
	Reverse	ATACCCGAGACTACTTCCCCA
NEAT1-3	Forward	GGTGGGGAGTACTTTGCCATA
	Reverse	CAAATCCCAGGCACATTCCAG