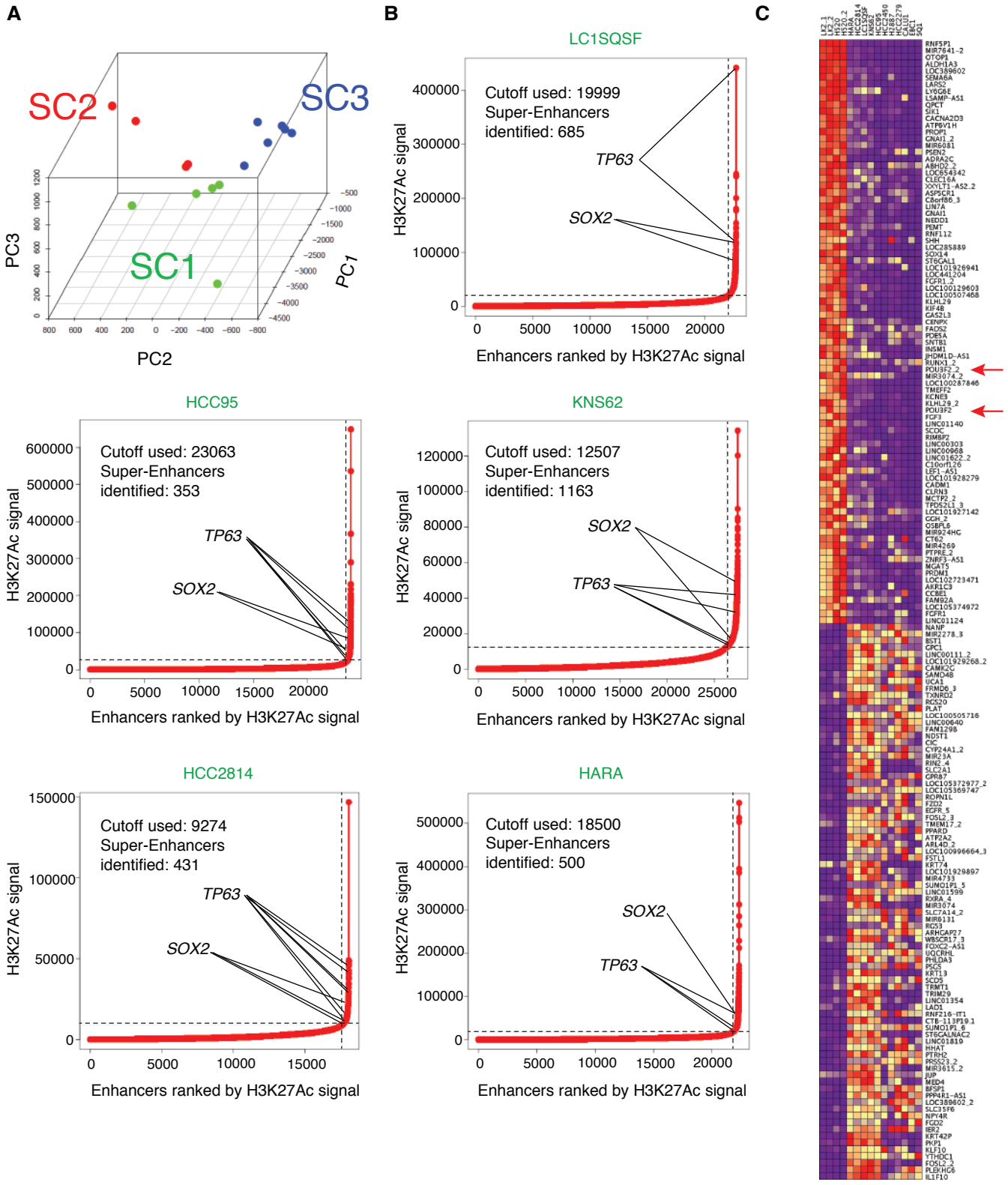
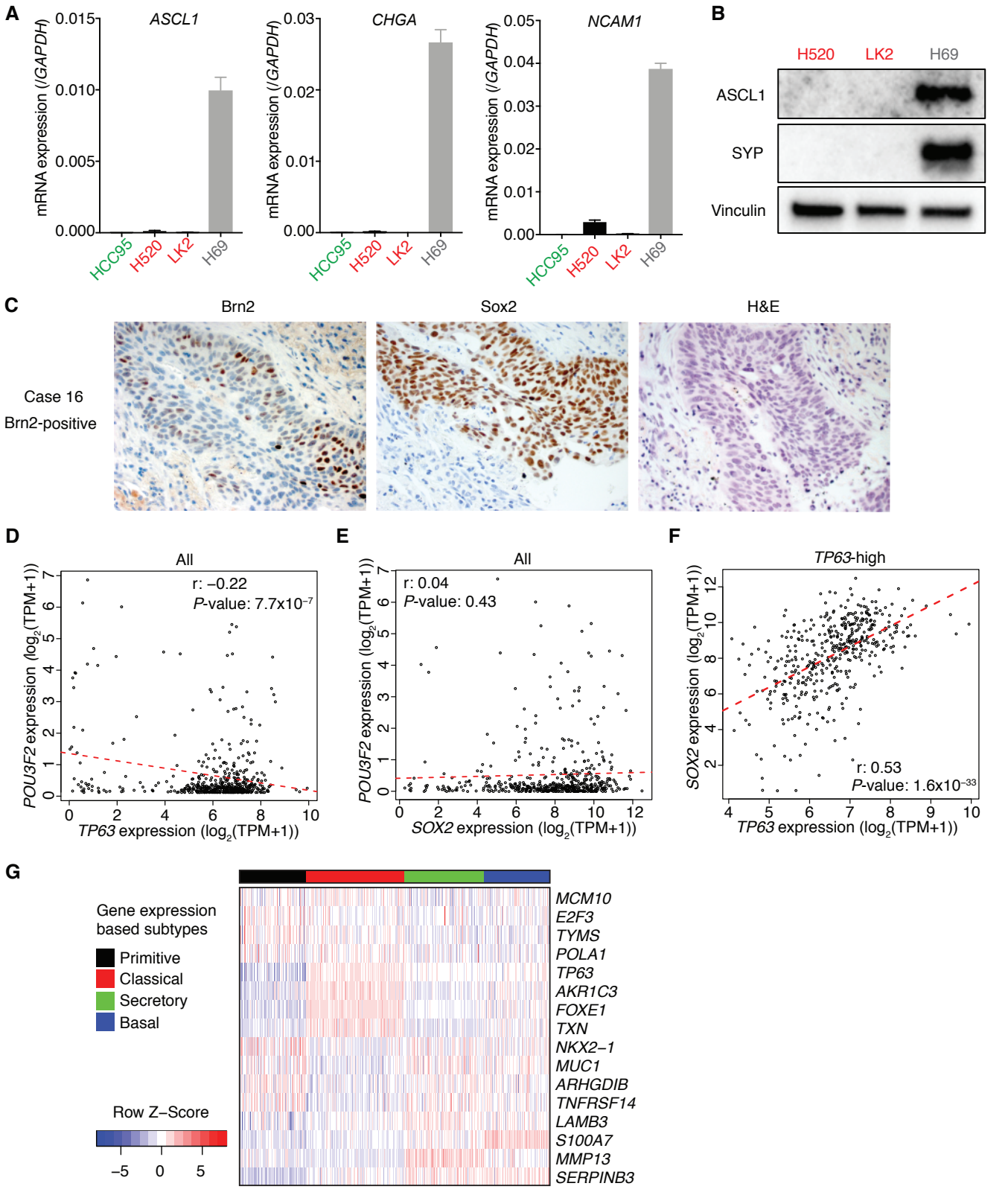
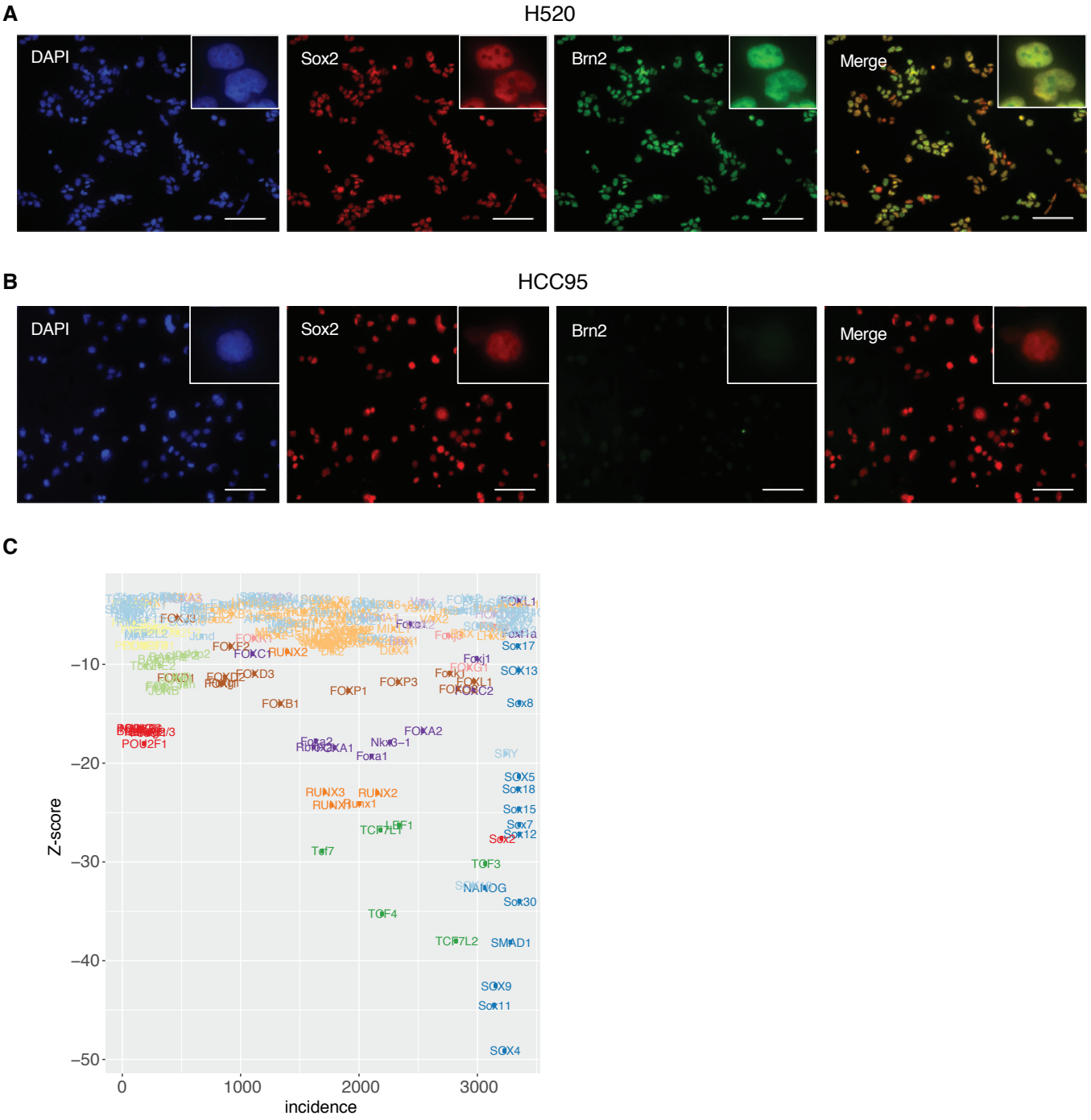


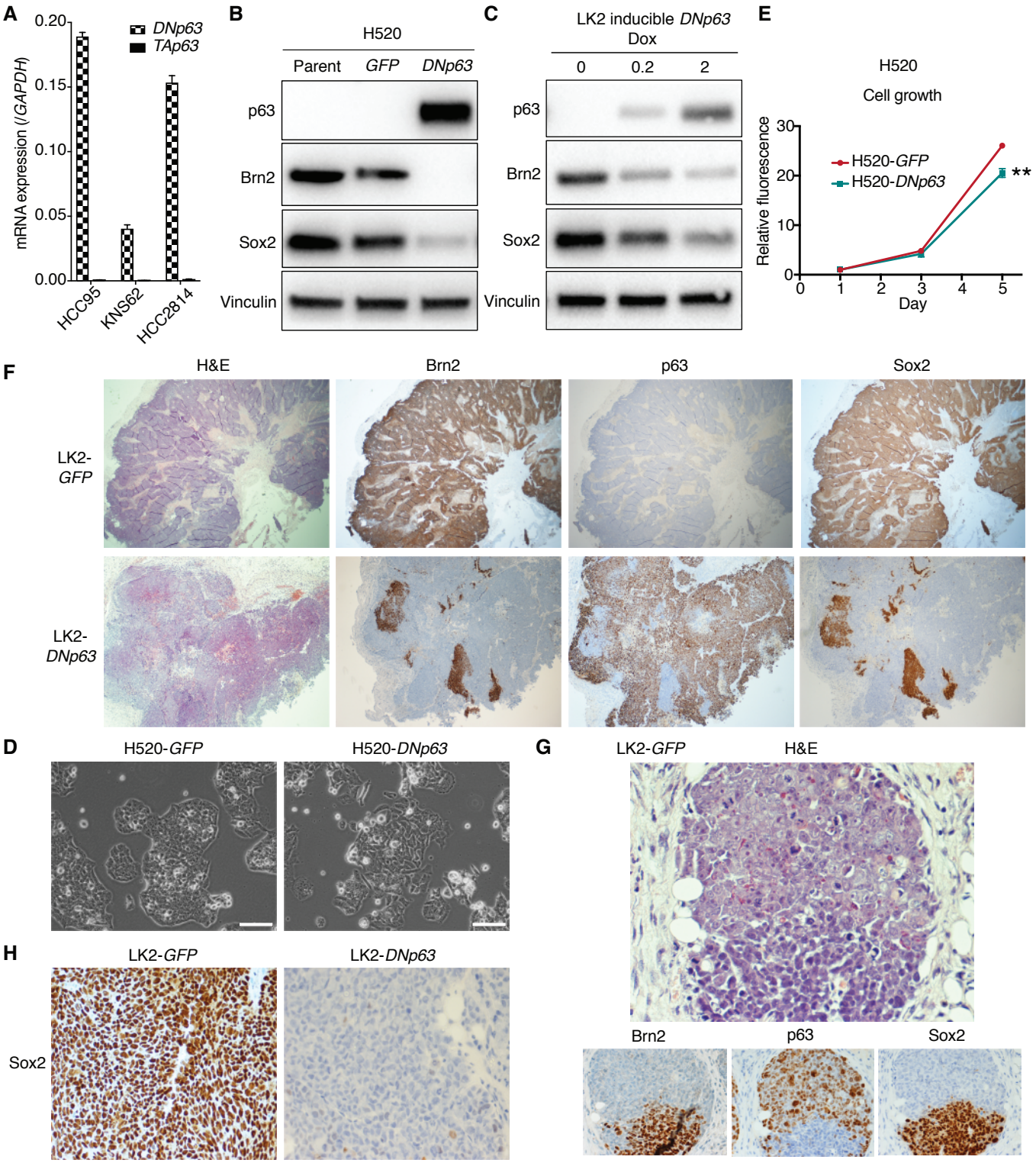
Supplementary Figure S1. Super-enhancer profiling identifies three subtypes of LUSC. **A,** Principal component analysis of super-enhancer profiles near transcriptional regulator genes in 13 LUSC cell lines. Each point represents a LUSC cell line that is colored based on unsupervised hierarchical clustering using super-enhancer scores near transcriptional regulator genes (Fig. 1A). **B,** Super-enhancer plots using H3k27ac scores in the classical subgroup of LUSC cell lines. **C,** Super-enhancer loci with differential super-enhancer scores near transcriptional regulator genes in the small subset of LUSC cell lines compared to the classical subgroup of LUSC cell lines.



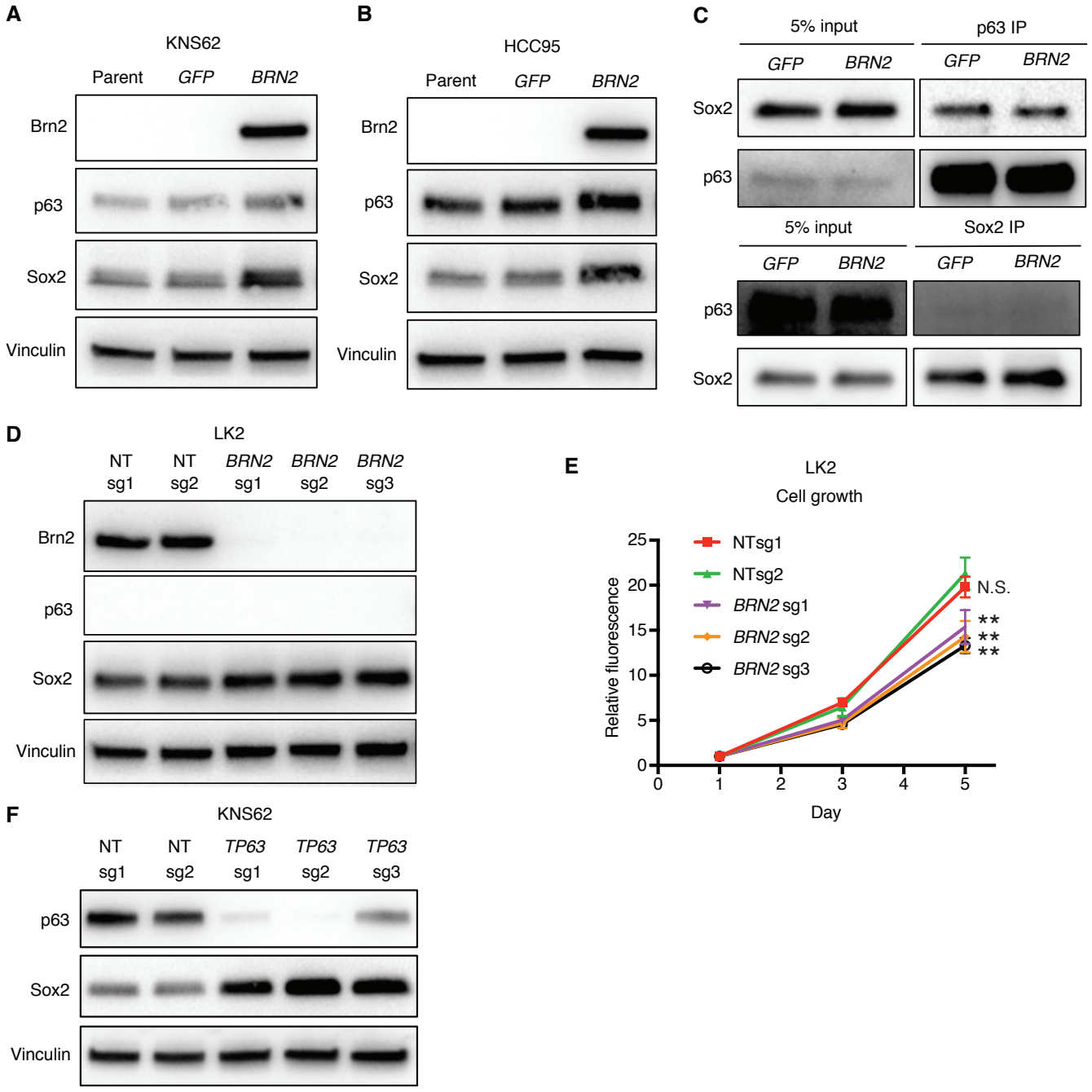
Supplementary Figure S2. Brn2 is expressed in a subset of human primary LUSC tumors. **A,** mRNA expression levels of neuroendocrine markers in the 'neural' LUSC cell lines compared with those in the classical LUSC cell line HCC95 and a SCLC line H69. **B,** Protein expression of ASCL1 and SYP in the 'neural' LUSC cell lines and H69. **C,** Immunohistochemical staining of Brn2 and Sox2 and H&E staining in Brn2-positive and negative human LUSC tumors. Representative images are shown (original Images, ×200). **D,** Scatter plots of expression of TP63 and POU3F2 among the entire dataset of TCGA-LUSC tumors. **E,** Scatter plots of expression of SOX2 and POU3F2 among the entire dataset of TCGA-LUSC tumors. **F,** Scatter plots of expression of TP63 and SOX2 in TP63-high LUSC tumors from TCGA. **G,** k-mean clustering (k=4) of 497 TCGA-LUSC samples for determining the gene expression subtype based on the subtype exemplar genes.



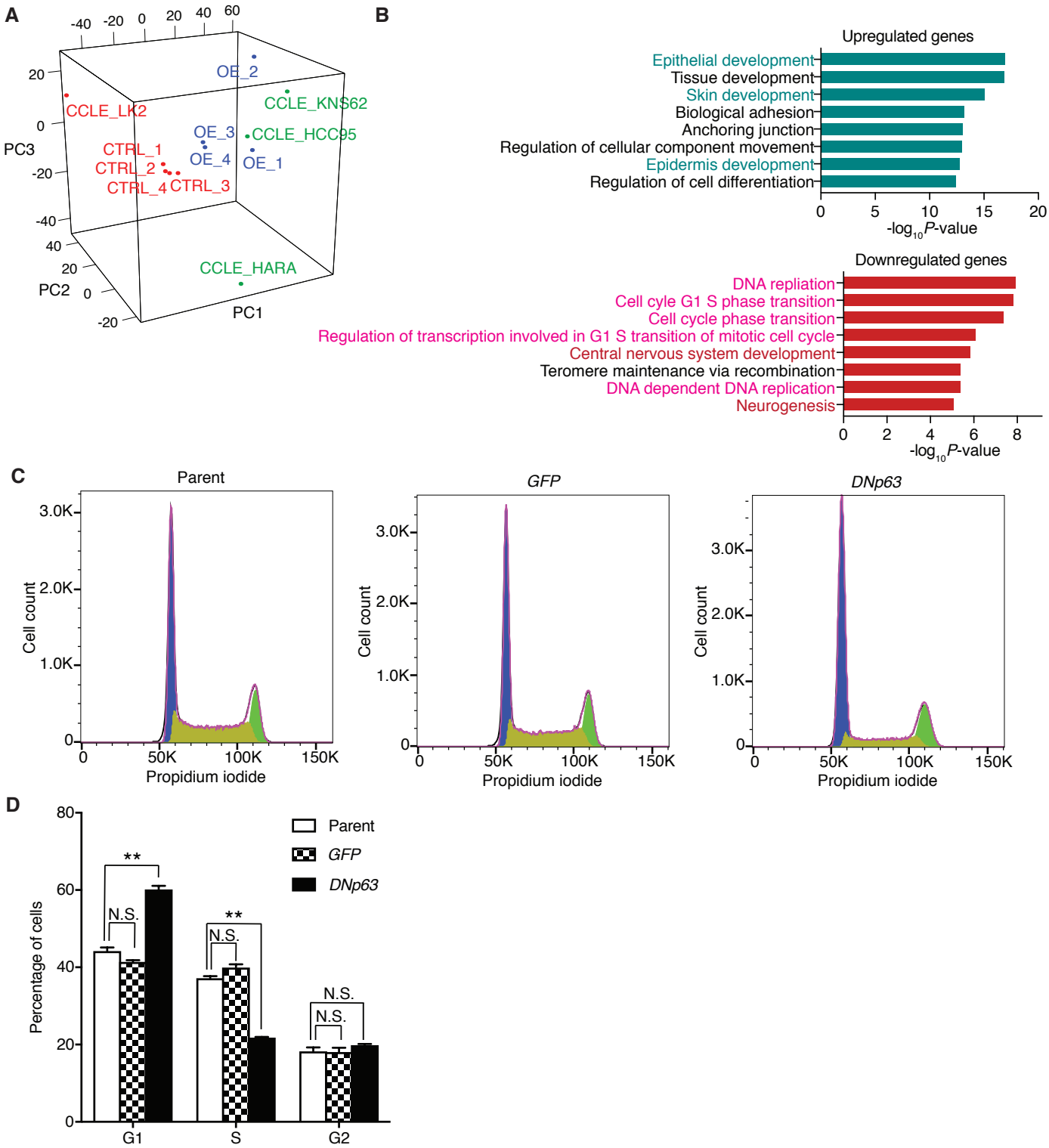
Supplementary Figure S3. Brn2 and Sox2 co-localize and collaborate in the 'neural' LUSC cells. **A** and **B,** Expression of endogenous Brn2 and Sox2 in NCI-H520 cells (A) and HCC95 cells (B), determined by immunofluorescence with anti-Sox2 (*green*) and anti-Brn2 (*red*) antibodies, respectively. DAPI staining (nuclei; *blue*) and merged images are also shown. Original magnification, ×200. Scale bar, 100 μm. **C,** Motif analysis for the Sox2/Brn2 peak-enriched cluster in Fig. 4C.



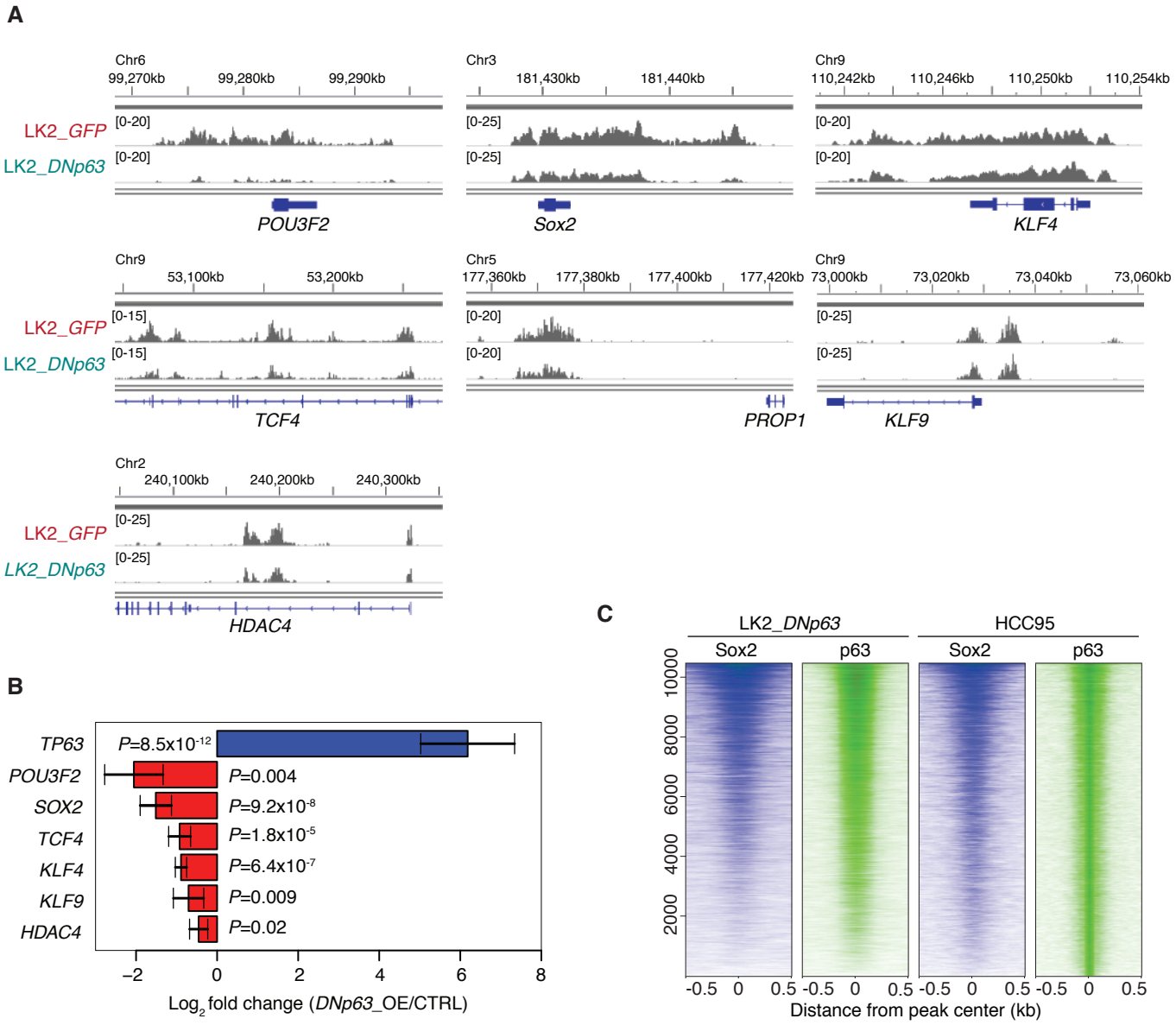
Supplementary Figure S4. DNp63 overexpression in the 'neural' LUSC cells suppresses Brn2 expression and induces phenotypic changes. **A,** mRNA expression levels of the two major isoforms of p63 in the 'classical' LUSC lines. **B,** Protein expression of p63, Brn2, Sox2 and vinculin as a loading control in parental, GFP-overexpressed and DNp63-overexpressed NCI-H520 cells. **C,** Protein expression of p63, Brn2, Sox2 and vinculin as a loading control in doxycycline-inducible DNp63-overexpressing LK2 cells. Cells were treated with doxycycline (Dox) for 11 days at the indicated concentrations. **D,** Phase-contrast microphotographs of GFP-overexpressed and DNp63-overexpressed NCI-H520 cells. Bar = 100 μm. **E,** Cell growth of GFP-overexpressed and DNp63-overexpressed NCI-H520 cells. Mean ± SD of sextuplicates are shown. **, $P<0.001$ vs. GFP-overexpressed NCI-H520 cells, *t*-test. **F,** H&E staining and immunohistochemical staining of Brn2, p63 and Sox2 in GFP-overexpressed or DNp63-overexpressed LK2 xenograft. Original Images, ×40. **G,** H&E staining and immunohistochemical staining of Brn2, p63 and Sox2 in the areas where both p63-positive and negative tumor cells were found in the DNp63-overexpressed LK2 xenograft. Original Images, ×400. **H,** Immunohistochemical staining of Sox2 in the DNp63-overexpressed LK2 xenograft. Original Images, ×400.



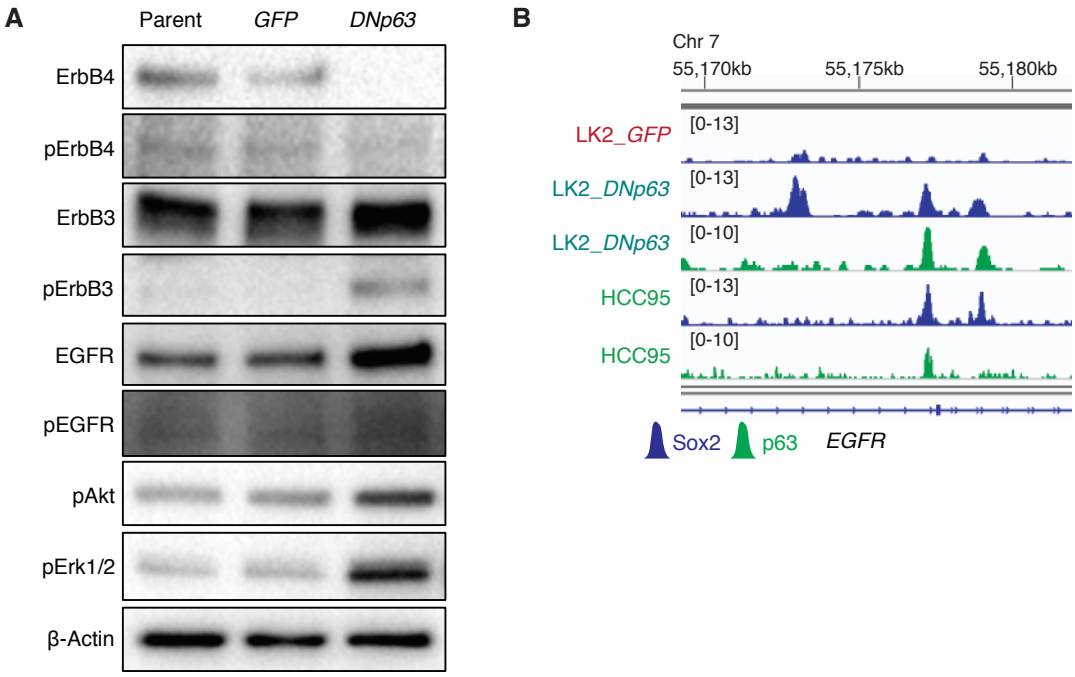
Supplementary Figure S5. Brn2 overexpression and p63 ablation in the 'classical' LUSC cells and Brn2 ablation in the 'neural' LUSC cells. **A** and **B,** Protein expression of Brn2, p63, Sox2 and vinculin as a loading control in parental, GFP-overexpressed and DNp63-overexpressed KNS62 cells (A) or HCC95 cells (B). **C,** Sox2-p63 interaction, shown by co-immunoprecipitation of Sox2 using an antibody against endogenous p63 (*top*) and that of p63 using an antibody against endogenous Sox2 (*bottom*) in control and Brn2-overexpressed HCC95 cells. **D,** Protein expression of Brn2, p63, Sox2 and vinculin in LK2 cells infected with 2 independent non-target sgRNAs (NT sg1 or 2) or 3 independent *BRN2* sgRNAs (BRN2 sg1, 2 or 3). **E,** Cell growth of LK2 cells infected with each non-target sgRNA or *BRN2* sgRNA. Mean ± SD of sextuplicates are shown. **, $P<0.001$ and N.S., $P>0.05$ vs. LK2 cells with non-target sgRNA2, *t*-test with Bonferroni correction. **F,** Protein expression of p63, Sox2 and vinculin in KNS62 cells infected with 2 independent non-target sgRNAs (NT sg1 or 2) or 3 independent *TP63* sgRNAs (*TP63* sg1, 2 or 3).



Supplementary Figure S6. DNp63 induces a classical squamous-cell transcriptional program and G1 phase arrest in the 'neural' LK2 cells. **A,** Principal component analysis of the transcriptomic profiles of the control (CTRL) and the DNp63-overexpressed (OE) LK2 cells, and the LUSC cell lines from the CCLE dataset. **B,** Gene ontology analyses for the differentially up-regulated (*top*) and down-regulated (*bottom*) genes upon DNp63 overexpression in LK2 cells. Likelihood ratio test model was used to identify genes significantly associated with conditions (control -> inducible overexpression -> stable overexpression) based on cutoffs of fold change>2 and FDR<0.01. Enriched functions for these genes are identified based on Fisher's exact test against GO terms curated in MSigDB. **C,** Propidium iodide staining followed by flow cytometry was used to analyze cell cycle distribution in parental (*left*), GFP-overexpressed (*middle*) and DNp63-overexpressed (*right*) LK2 cells. **D,** The percentages of cells in G1, S, and G2 phase of cell cycle were measured using flow cytometry after propidium iodide staining in triplicates of either parental, GFP-overexpressed or DNp63-overexpressed LK2 cells. Each bar represents the mean ± SD of triplicate measurements. **, $P<0.001$ and N.S., not significant ($P>0.05$), *t*-test with Bonferroni correction.



Supplementary Figure S7. DNp63 suppresses the 'neural' lineage program and induces the classical squamous-cell transcriptional program. **A,** Genome view tracks of H3K27ac ChIP-seq signals in control and DNp63-overexpressed LK2 cells at the super-enhancer regions specific to the 'neural' LUSC cells. **B,** Fold changes of mRNA expression levels of the genes at the super-enhancer regions specific to the 'neural' LUSC cells beteween control (CTRL) and DNp63-overexpressed (*DNp63*_OE) LK2 cells. **C,** Heatmap depicting analysis of ChIP-seq signals for Sox2 and p63 in DNp63-overexpressed LK2 cells and HCC95 cells at all p63 peak loci. ChIP-seq signal intensity is shown by color shading.



Supplementary Figure S8. DNp63 alters ErbB family signaling in the 'neural' LK2 cells. **A,** Protein expression of ErbB4, phospho-ErbB4, ErbB3, phospho-rbB3, EGFR, phospho-EGFR, phospho-Akt and phospho-Erk1/2 and β-Actin as a loading control in Parental, GFP-overexpressed and DNp63-overexpressed LK2 cells. Cells were cultured with 10% FBS. **B,** Genome view tracks of ChIP-seq signals for Sox2 and p63 at *EGFR* locus in GFP-overexpressed and DNp63-overexpressed LK2 cells and HCC95 cells.