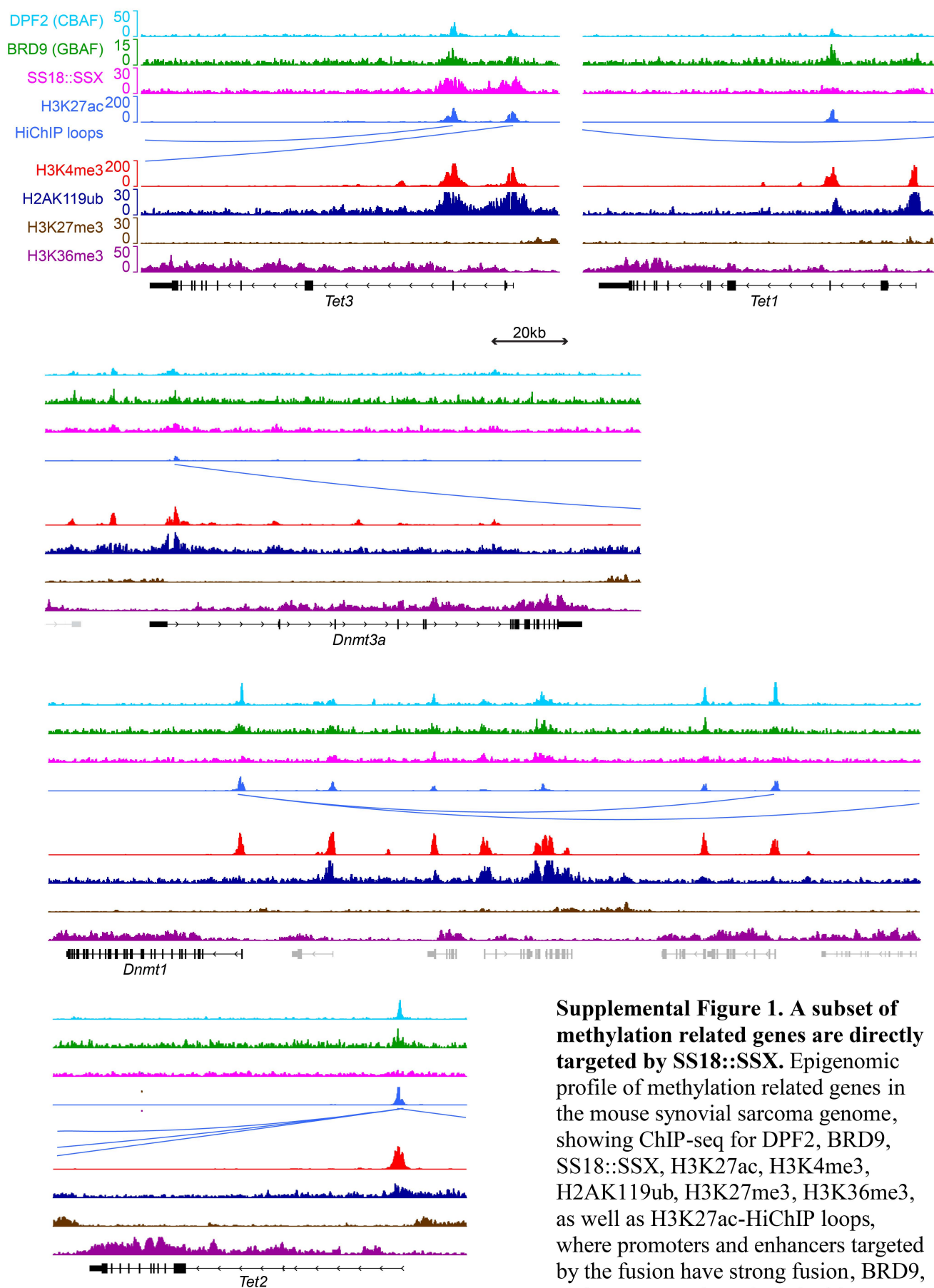
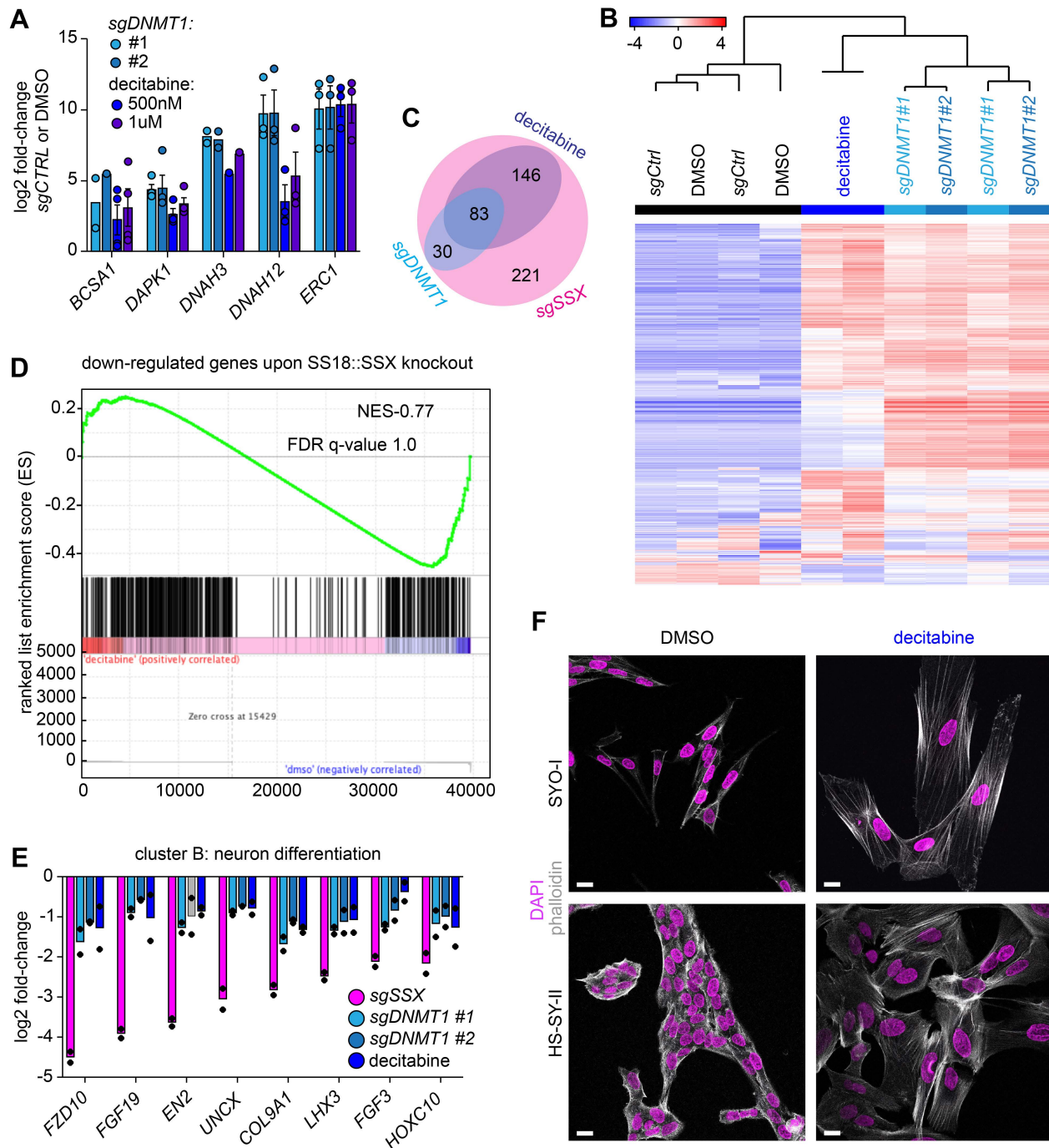
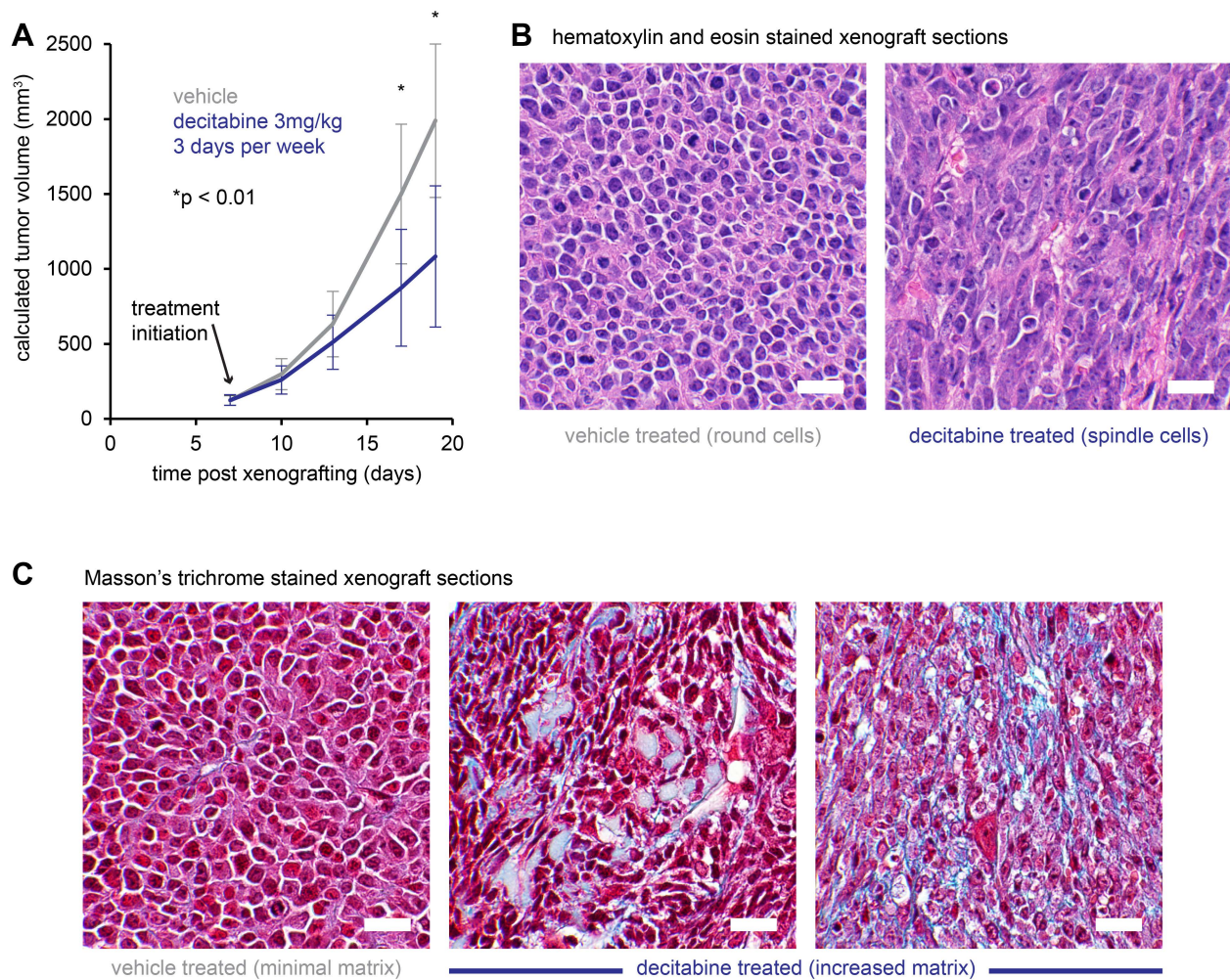




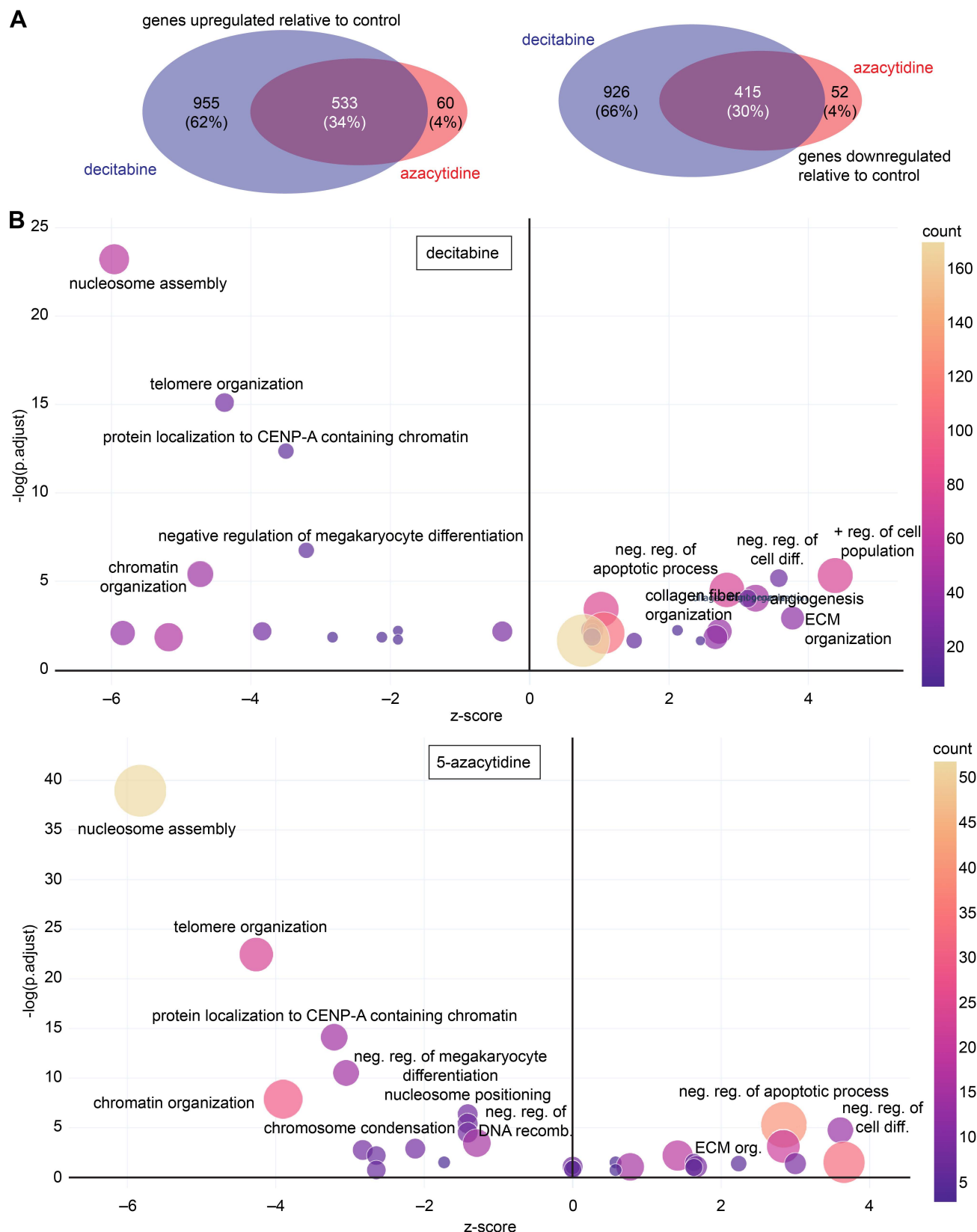
Supplemental Figure 1. A subset of methylation related genes are directly targeted by SS18::SSX. Epigenomic profile of methylation related genes in the mouse synovial sarcoma genome, showing ChIP-seq for DPF2, BRD9, SS18::SSX, H3K27ac, H3K4me3, H2AK119ub, H3K27me3, H3K36me3, as well as H3K27ac-HiChIP loops, where promoters and enhancers targeted by the fusion have strong fusion, BRD9, and H2AK119ub peaks coincident.



Supplemental Figure 2. DNMT1 inhibition or knock-out impacts transcription. (A) qRT-PCR of methylation sensitive promoters 12 days after *sgDNMT1* or decitabine treatment. Data represents the mean of $n = 1-4$ biological replicates. (B) RNA-seq heatmap showing 5000 most variable genes with a cut off z score of 4. (C) Venn diagram of genes for which log2 fold-changes of FPKM values were greater than 0.5, demonstrating the overlap of three groups from cluster C in Figure 2C. (D) Gene set enrichment analysis (GSEA) comparing the expression of the 411 genes present in cluster B in Figure 2C, neuron differentiation, with the top 500 genes downregulated upon SS18::SSX knockout. (E) Log2 fold-change of FPKM values from genes present in cluster B. Data represent the mean of two biological replicates. (F) Immunofluorescence of human SS HS-SY-II and SYO-1 cells stained with DAPI (magenta) and phalloidin (grey). Scale bars are 20 μ m in length.



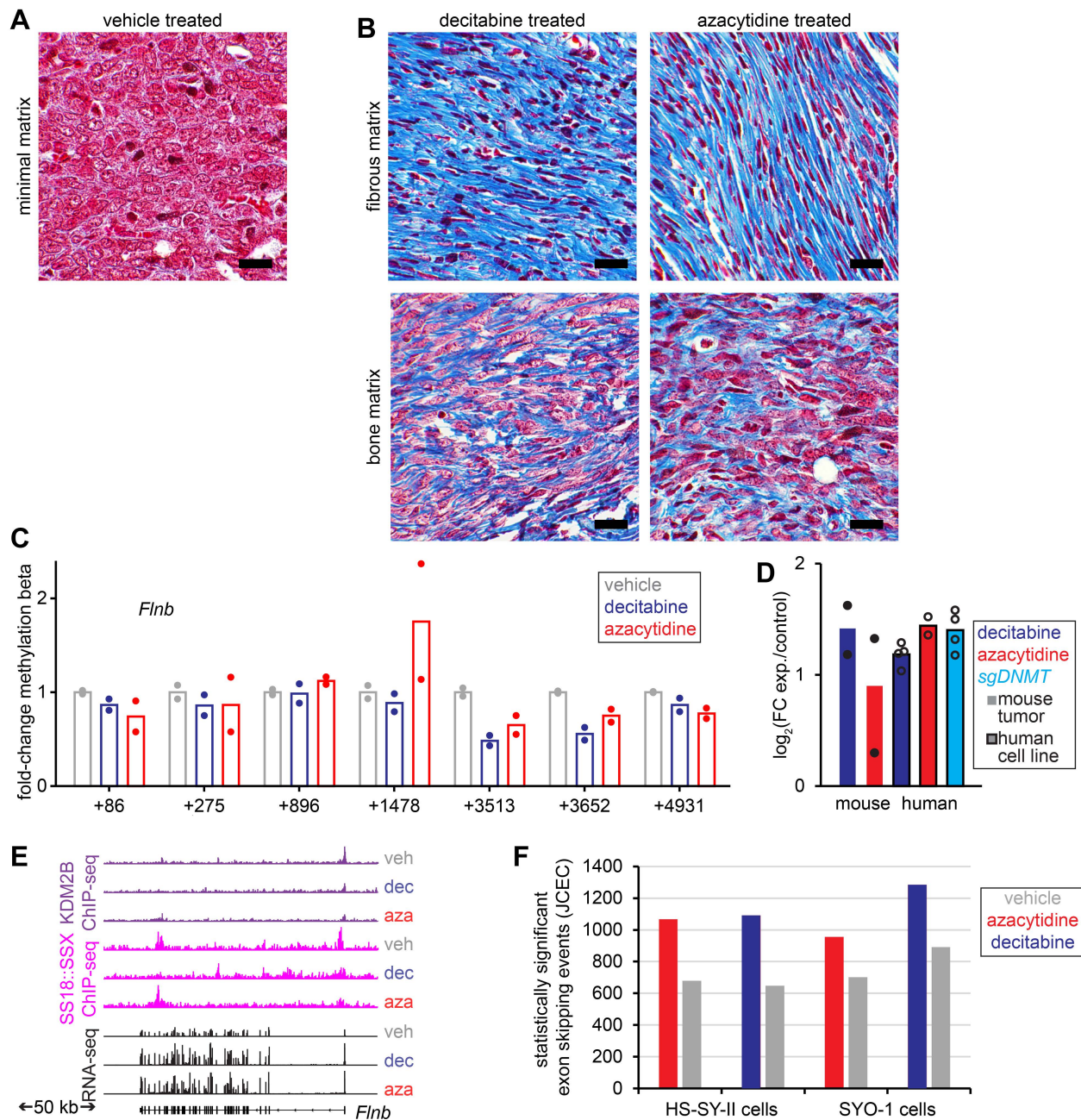
Supplemental Figure 3. Decitabine slows growth and increases mesenchymal differentiation in SYO-1 cell line xenografts. (A) Tumor growth curves for each group. Calculated tumor volumes are presented as mean $\pm$ SD. ($n = 10$ for both groups, but $n = 8$ for vehicle group on last two measurements, as tumors reached maximum size; p -values from two-tailed homoscedastic t-tests, with Bonferroni correction to $p < 0.01$.) (B) Photomicrographs of hematoxylin and eosin stained sections from the xenograft tumors after treatment, showing spindled histomorphology after decitabine. (C) Photomicrographs of Masson's trichrome stained sections of the SYO-1 xenografts, showing increased blue-staining collagen of the extracellular matrix after decitabine treatment. (All magnification bars are 20 μ m in length.)



Supplemental Figure 4. Demethylating agents impact transcription. (A) Genes with log₂ fold-change expression ($<-1/>1$) and adjusted $p < 0.05$ compared to control by decitabine and azacytidine in SYO-1 cells. (B) Bubble plots from GO analysis of up- and down-regulated genes in SYO-1 cells following decitabine or azacytidine treatment compared to control.



Supplemental Figure 5. GO analysis of the biological processes in genes upregulated in mouse tumors. (A) Analysis of 461 AZA group-only upregulated genes. (B) Analysis of 192 DAC-A-only upregulated genes. (C) Analysis of the 29 upregulated genes shared by the AZA and DAC-A groups. (D) Analysis of 320 AZA group-only genes. (E) Analysis of the 170 genes upregulated in common in both the AZA and DAC-B groups. (F) Analysis of 841 DAC-B group-only genes.



Supplemental Figure 6. Pharmacological hypomethylation increases extra-cellular matrix production in SS. (A) Photomicrographs of Masson's trichrome stained mouse SS tumor sections treated with vehicle or (B) azacytidine or decitabine, the latter showing blue stained collagen extracellular matrix and even some red stained matrix from partially calcified osteoid matrix. (C) Promoter methylation fold-changes for the *Flnb/FLNB* gene following vehicle, decitabine, or azacytidine treatments. (D) RNA-seq-determined $\log_2$ -transformed fold-changes of expression of *Flnb/FLNB* over controls for decitabine, azacytidine, or *sgDNMT1*. (E) ChIP-seq tracks of KDM2B (0-1.76) and SS18::SSX (0-0.88), with RNA-seq (reads per million 0-4.36) for mouse SSs treated with vehicle, decitabine or azacytidine. (F) RNA-seq-determined exon skipping alternative splicing events in two human cell lines following azacytidine or decitabine, each compared to vehicle, calculated by the junction counts and exon counts (JCEC) method.