SUPPLEMENTAL MATERIAL

Disruption of NSD1 in head and neck cancer promotes favorable chemotherapeutic responses linked to hypomethylation

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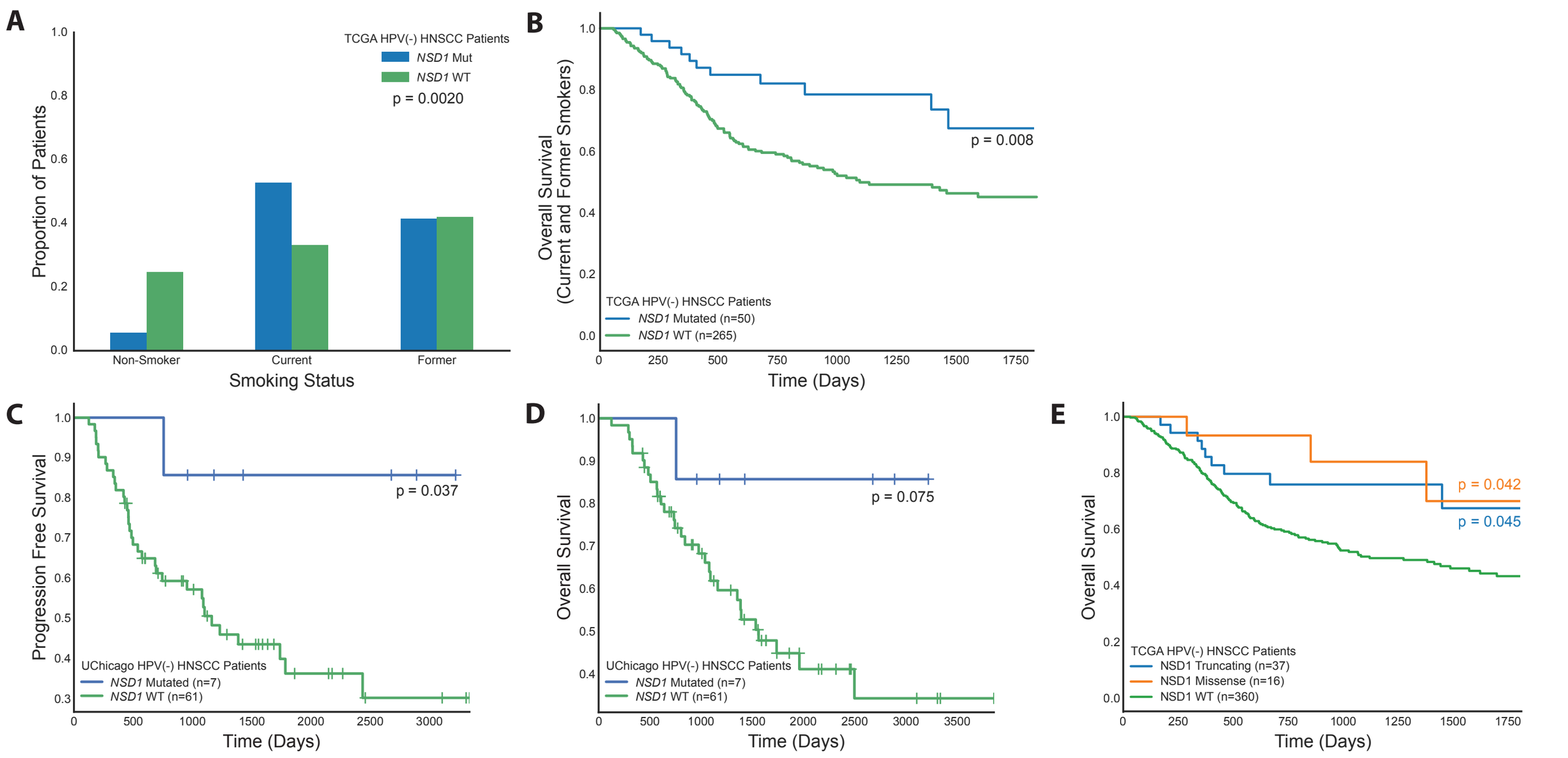
Supplemental Table 1. Descriptions of the CpG-associated genes reveal a predominance of genes that function in MHC class or skin/connective tissue structure.

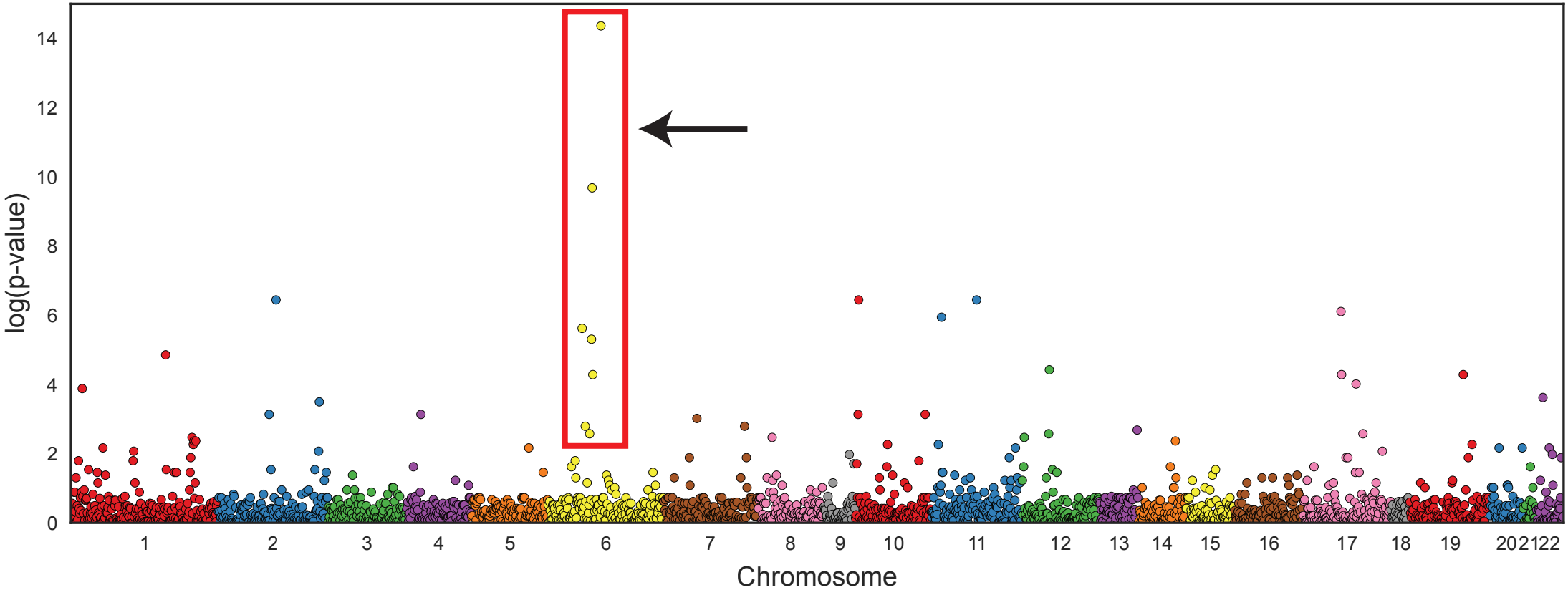
HUGO Symbol	Location	Function
TNXB	MHC Class III	An tenascin (extracellular matrix glycoprotein) with anti-adhesive effects involved in wound healing. This has been implicated as a biomarker for malignant mesothelioma (1).
COL11A2		Alpha 2 chain of type XI collagen, an minor fibrillar collagen.
PSORS1C1	MHC Class I	Confers susceptibility to psoriasis and systemic sclerosis
MUC21		Large membrane-bound O-glycosylated protein involved in forming mucous barriers and intracellular signalling. MUC21 expression has been associated with colorectal cancers among others (2).
HCG22		HLA Complex Group 22
C6ORF15		Protein coding gene that is related to heparin binding and fibronectin binding in GO.
CYP21A2		Cytochrome P450 family member which are monooxygenases involved in drug metabolism and synthesis of cholesterols, steroids, and other lipids.
STK19	MHC Class III	A serine/theronine kinase with unknown function. STK19 mutations have been associated with melanoma (3).
CDSN	MHC Class I	A protein involved in desquamination and a variety of skin related diseases.
TNXA		Tenascin XA is a pseudogene. Expression of this lncRNA was associated with bladder cancer (4).
C4A/B	MHC Class III	Complement factor involved in the complement pathways, allowing for the immune system to defend against foreign pathogens.

Supplemental Table 2. Consistently hypomethylated genes in *NSD1* disrupted cell lines.

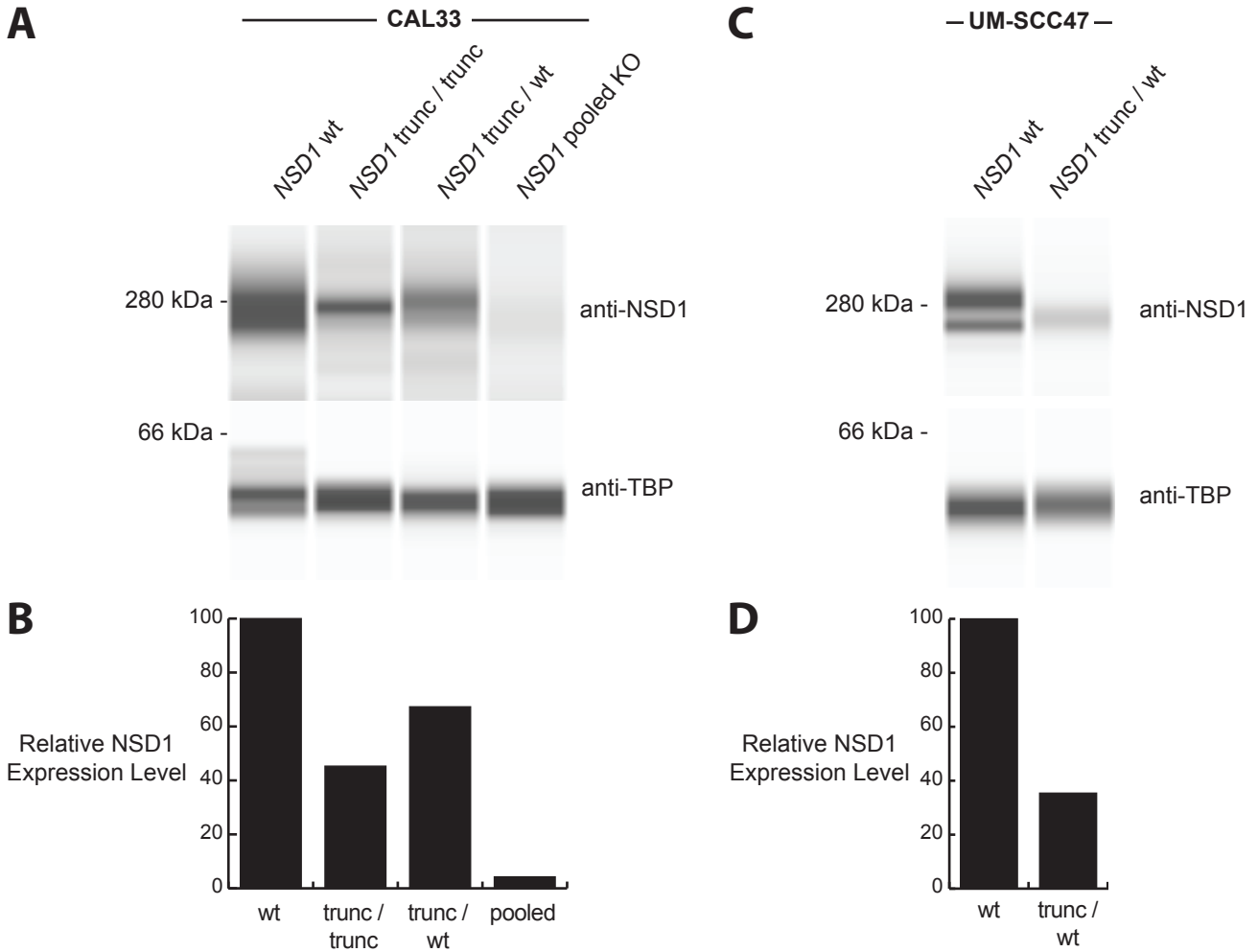
Gene	Function
COL13A1	Alpha chain of a nonfibrillar collagen. Involved in cell-matrix and cell-cell adhesion. Differential gene expression of this COL13A1 may be associated with chemotherapy response in breast cancer (5).
PRSS16	A serine protease typically expressed in the thymus that may play a role in T-cell development. PRSS16 is associated with Type I Diabetes and is located on chromosome 6 near the major histocompatibility complex class I region, similar to genes in supplemental Figure 2b. Has shown differential gene expression in chemoresistant tumors in ovarian cancer (6).
ARRDC5	A gene that contains the arrestin domain. Not much else is known about this protein.
NTM	A gene from a family of cell adhesion molecules that contain an immunoglobulin domain involved in promoting the growth of neurites.
PDE1A	Calcium/Calmodulin-dependent cyclic nucleotide phosphodiesterase. This gene is involved in many cell signalling processes and has been implicated in ALL (7) and melanoma (8).
LMX1A	Transcription factor that promotes insulin production and may also play a role in neuron production in embryogenesis. LMX1A has been suggested as a tumor suppressor in gastric and cervical cancers (9,10).
F11	Encodes the Factor XI blood coagulation factor. Like PRSS16, Factor XI is a serine protease. Factor XI deficiency is a cause of Hemophilia C.
NR1H4	A ligand-activated transcription factor primarily regulating genes involved in bile acid synthesis and transport. NR1H4 also has many other isoforms involved in the regulation of many different genes. Also known as FXR, NR1H4 has been implicated as a tumor suppressor in colon cancer (11).



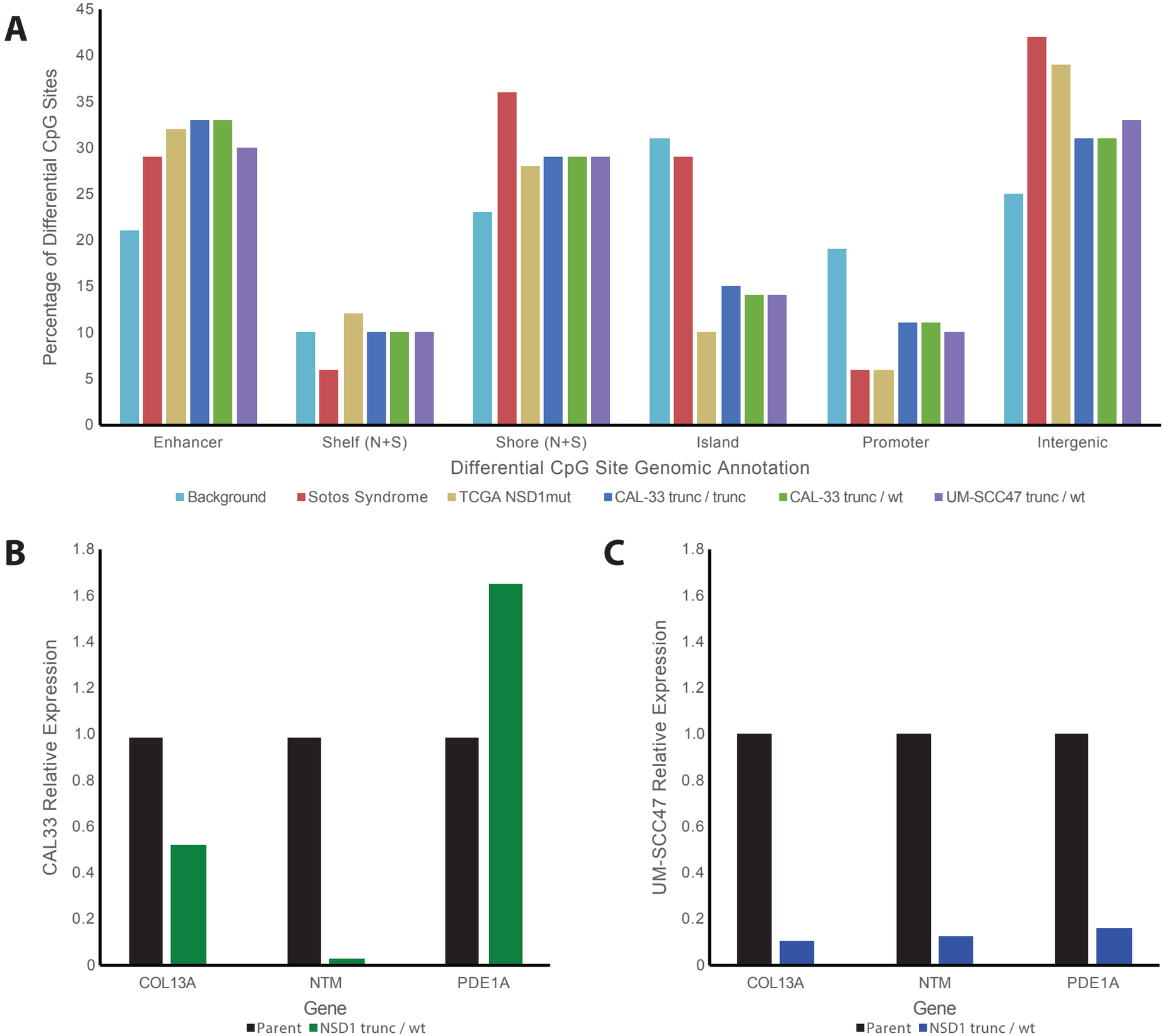
Supplemental Figure 1. Effect of various factors on NSD1 mutation survival effect in TCGA and validation of NSD1 survival effect in a second independent cohort from the University of Chicago (12). **A,** Distribution of smokers and non-smokers and **B,** Kaplan-Meier curve for overall survival of current and former smokers from the HPV(–) HNSCC cohort in TCGA (13). **C,** Kaplan-Meier curve for progression free survival and **D,** for overall survival for the HPV(–) HNSCC cohort from the University of Chicago. **E,** Kaplan-Meier curve for overall survival of patients with truncating (blue) or missense (orange) NSD1 mutations versus those with wild type NSD1 (green). Analysis is for the TCGA HPV(–) HNSCC cohort. All p-values for Kaplan-Meier curves in this figure were derived from the Log-Rank Test.



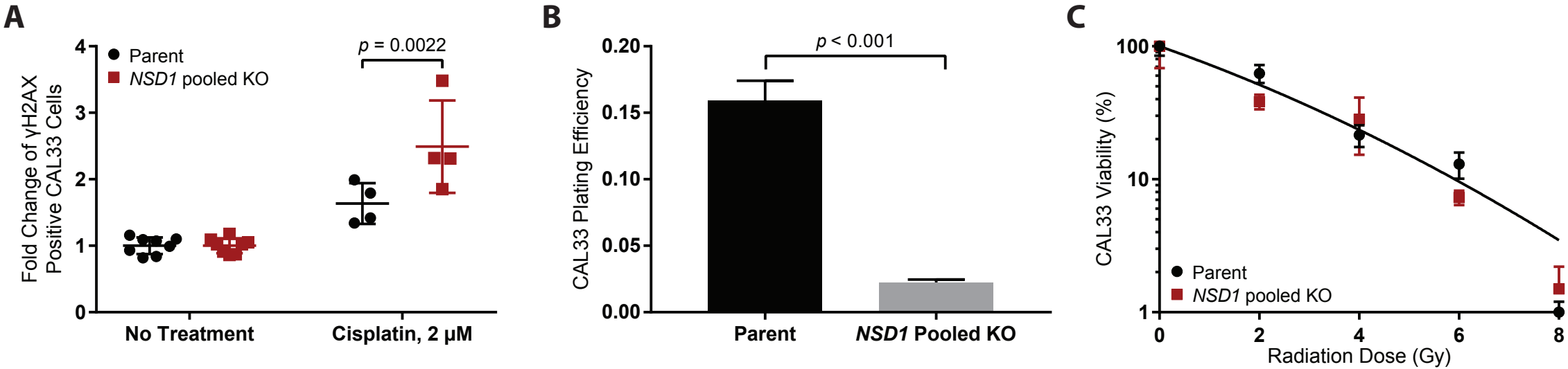
Supplemental Figure 2. Regional analysis of differentially methylated CpG sites in TCGA patients. A strong cluster of differentially methylated CpG sites appear around chromosome 6 from 31 MB – 33 MB.



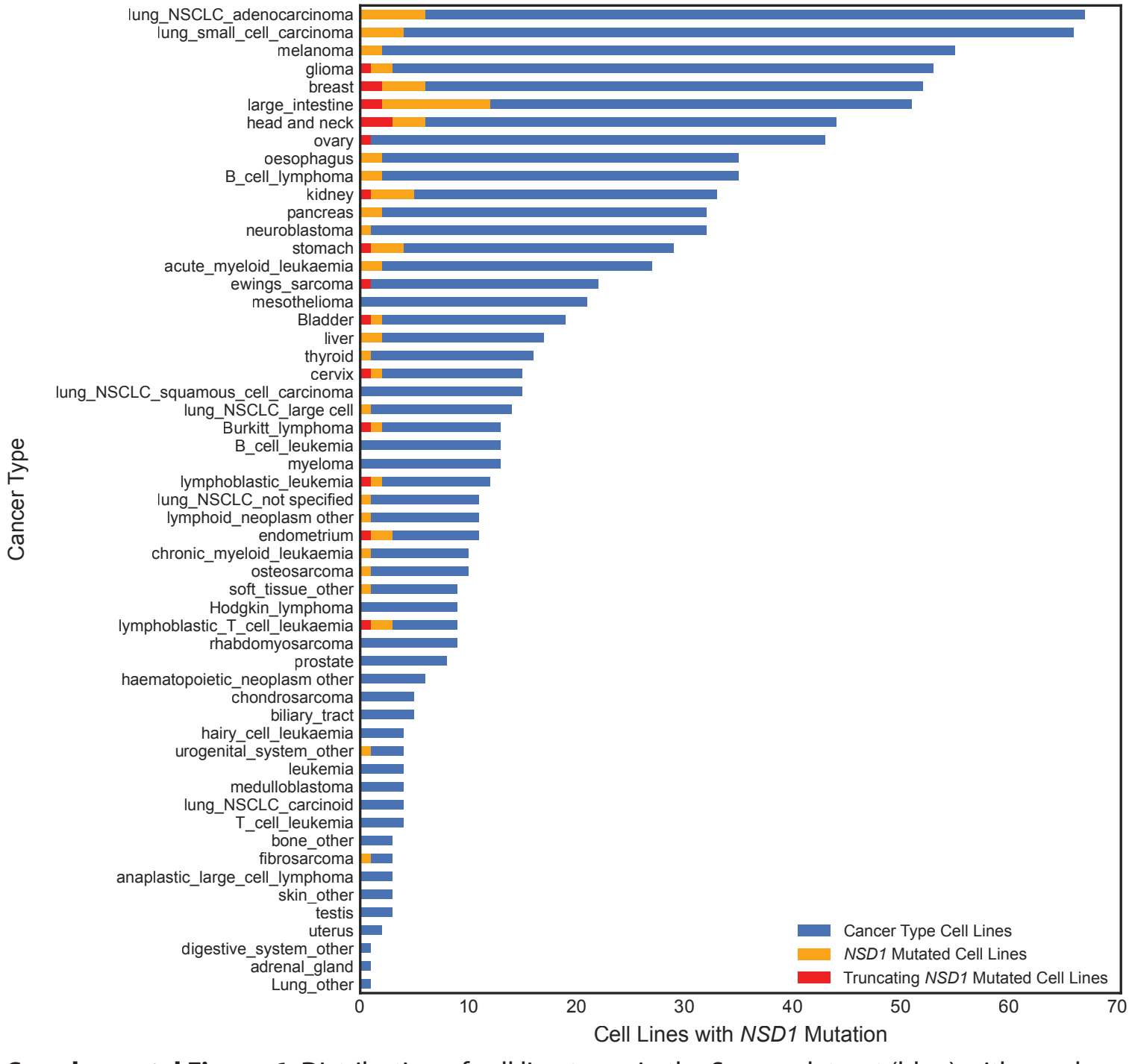
Supplemental Figure 3. NSD1 protein expression levels in parental and monoclonal cell lines with NSD1 disrupted by CRISPR-Cas9. **A,** Immunoblots of NSD1 and TBP (TATA Binding Protein) for NSD1 wild type CAL33, two monoclonal and one polyclonal NSD1 disrupted cell lines. **B,** Relative NSD1 expression levels were calculated by taking the ratio of intensity of the NSD1 band relative to the TBP band and setting the wild type expression level to 100%. **C,** Immunoblots of NSD1 and TBP (TATA Binding Protein) for NSD1 wild type UM-SCC47 and one monoclonal NSD1 disrupted cell line. **D,** Same as B except with the NSD1 expression levels from the UM-SCC47 cell lines. NSD1 alleles from monoclonal populations are characterized as follows: wt, wild type; trunc, contains a truncating mutation.



Supplemental Figure 4. Effect of NSD1 mutations on CpG site methylation and gene expression in HNSCC cell lines. **A,** Clustered bar chart describing the distribution of all differentially methylated CpG sites (DMRs) for Sotos Syndrome patients (14) (red), TCGA HNSCC NSD1 mutant tumors (gold), and the top 10,000 most differentially methylated probes from CAL33 (blue, green) and UM-SCC47 (purple) NSD1 knockout cell lines (Methods) compared to the standard distribution of CpG sites in the Illumina 450k chip for each site feature (light blue). **B and C,** Differential mRNA expression by RT-qPCR of selected genes in CAL33 (B) and UM-SCC47 (C) cell lines, with and without NSD1 disruption.



Supplemental Figure 5. Effect of NSD1 mutations on cell line sensitivity to radiation and DNA damage repair. **A,** Relative gain in percentage of γ H2AX positive CAL33 cells when treated with cisplatin compared to cells without cisplatin treatment for CAL33 wild-type and pooled NSD1-knock-out cells. γH2AX gain is significantly increased at 2μM cisplatin (Holm-Sidak corrected p-value < 0.005, Student's T-Test), consistent with the signifi-cant difference in cisplatin IC50 between the two groups. **B,** Clonogenic plating efficiency differences between CAL33 wild-type and pooled NSD1-knockout cells. **C,** Clonogenic survival assay of CAL33 wild-type and pooled NSD1-knockout cells with increasing doses of radiation. Two independent curves do not fit the data significantly better than a single curve (extra-sum-of-squares F-Test).



Supplemental Figure 6. Distribution of cell line types in the Sanger dataset (blue) with number of NSD1 mutations (red) and truncating NSD1 mutations (orange).

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