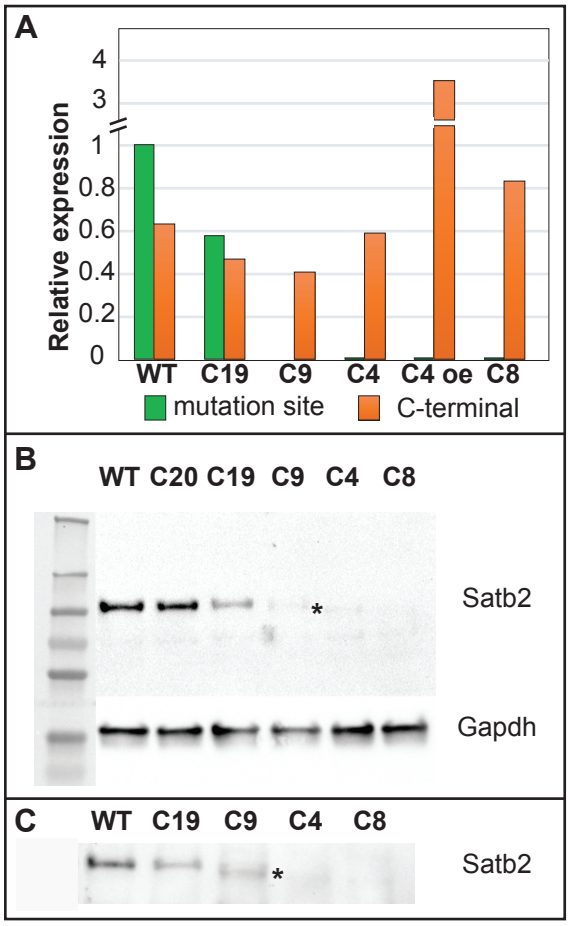
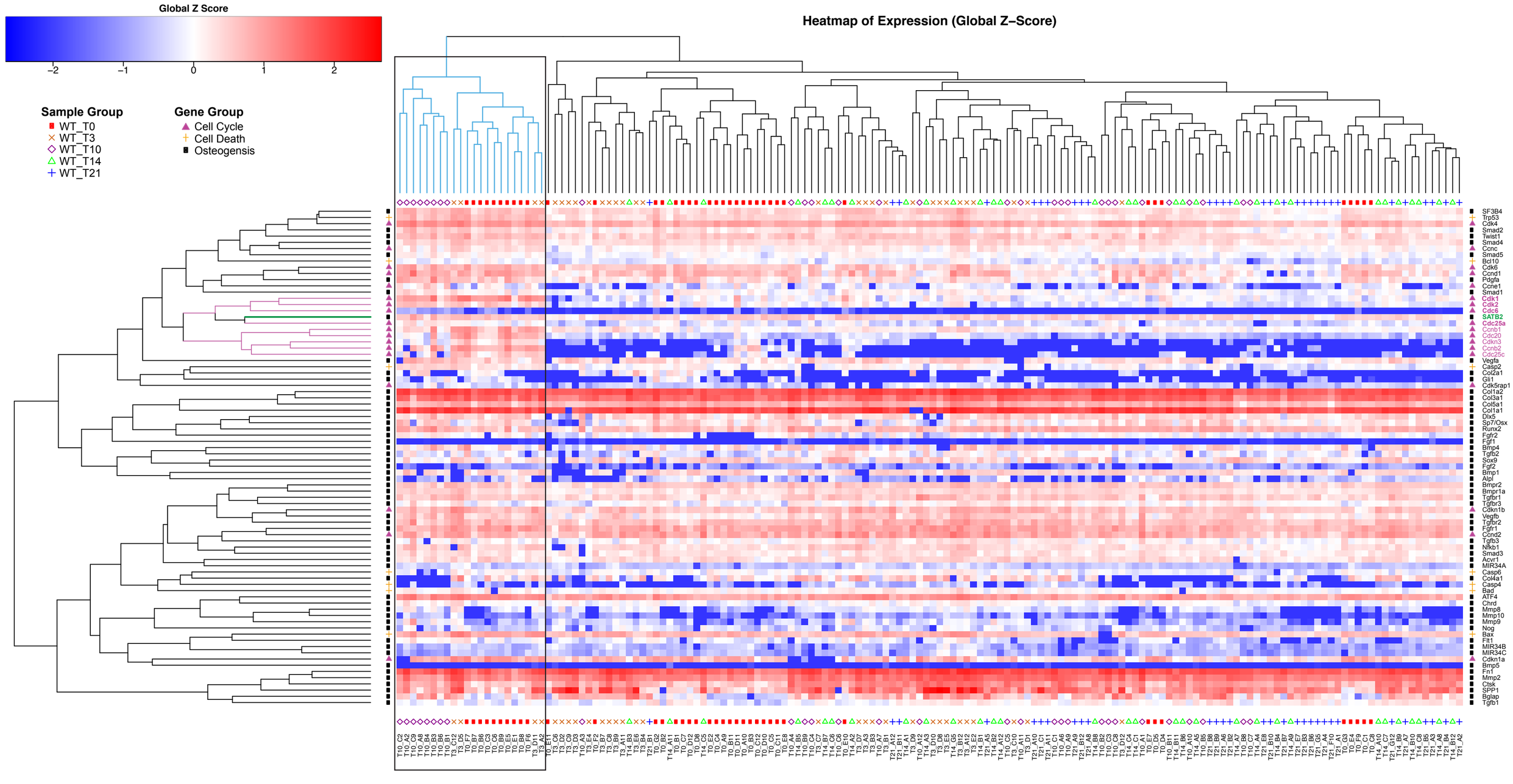
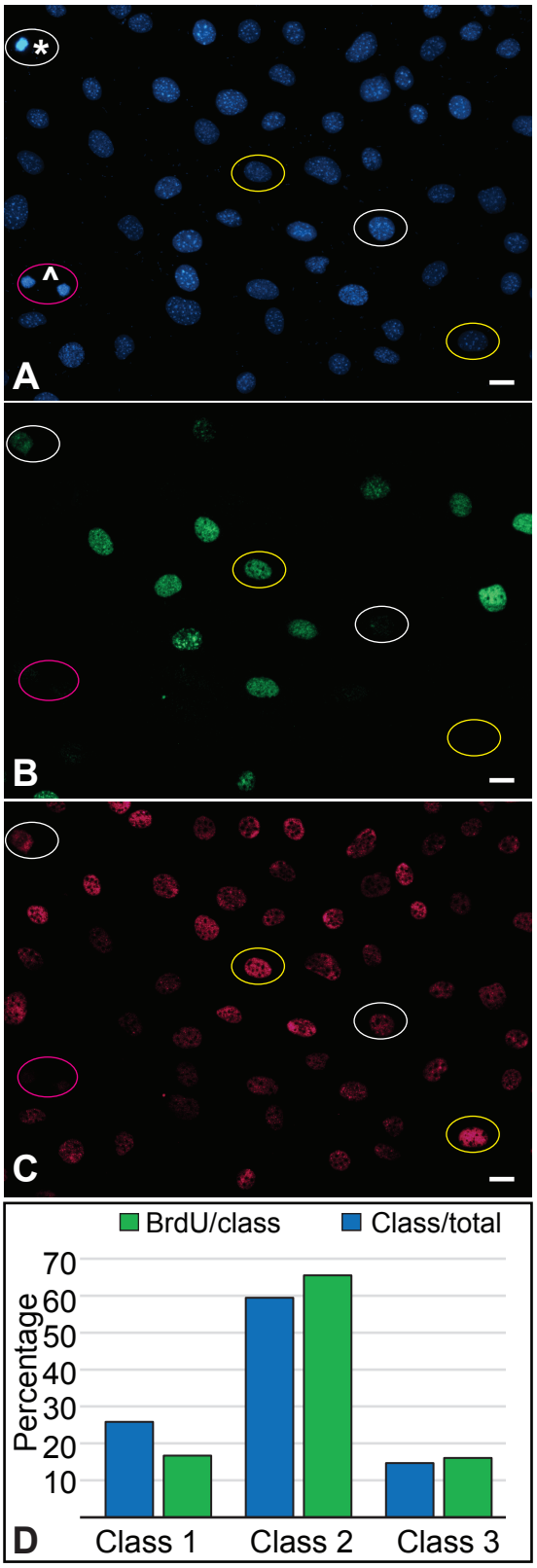
Supplemental Figure 1



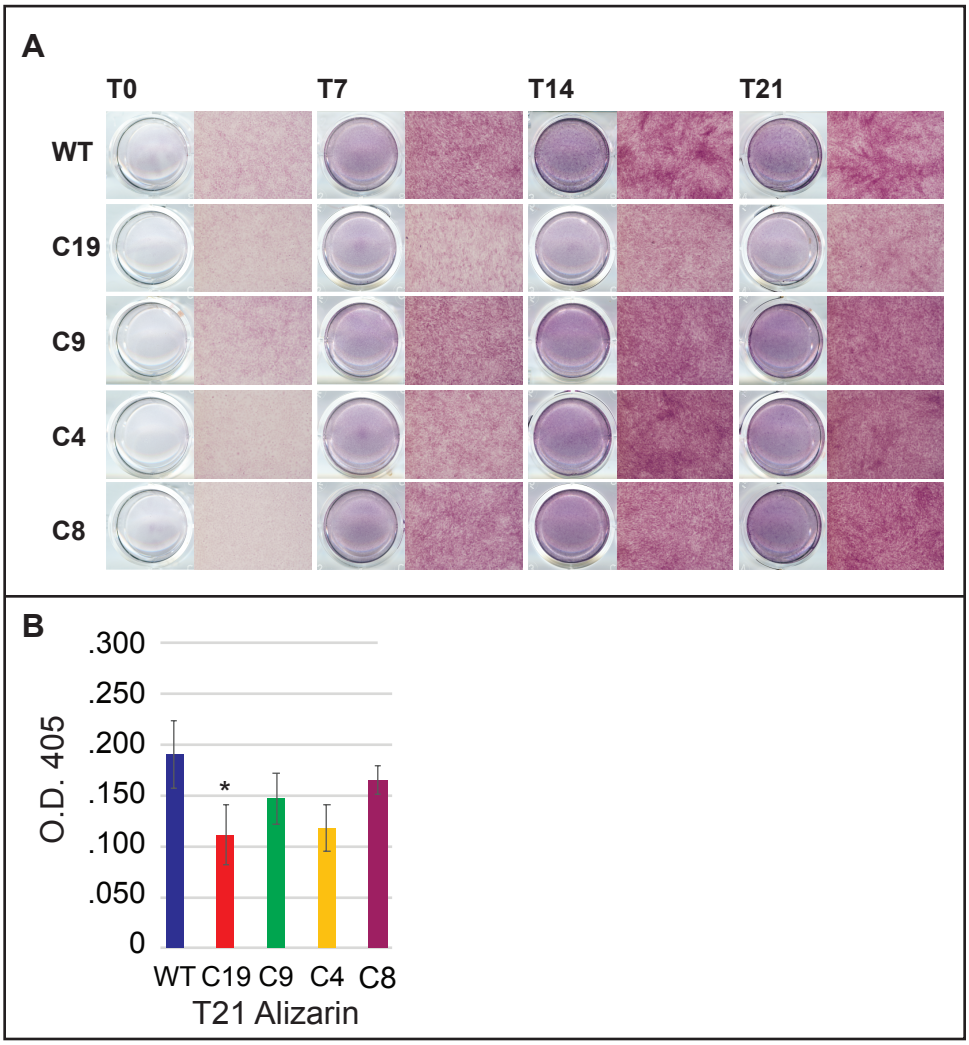
Supplemental Figure 2



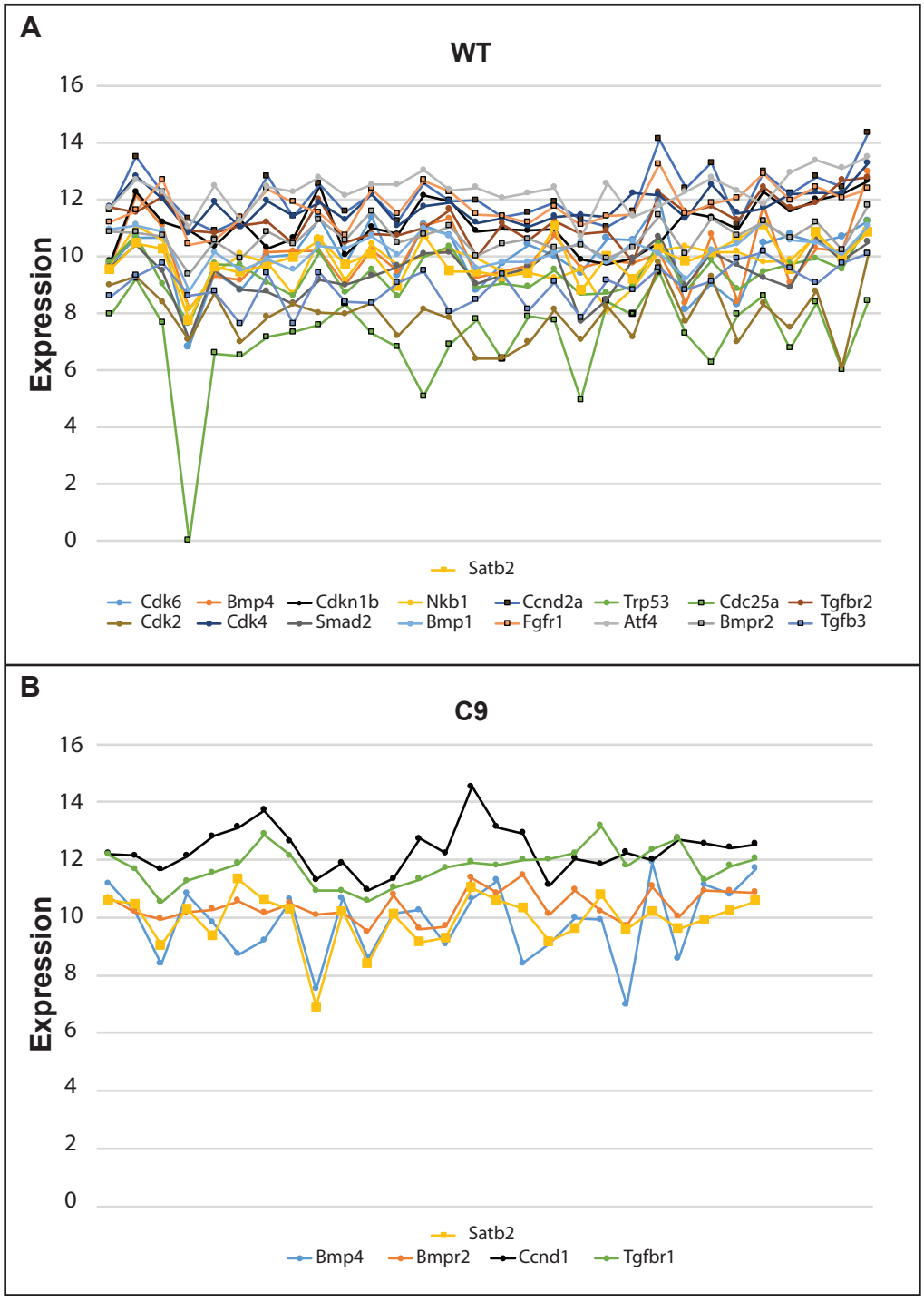
Supplemental Figure 3



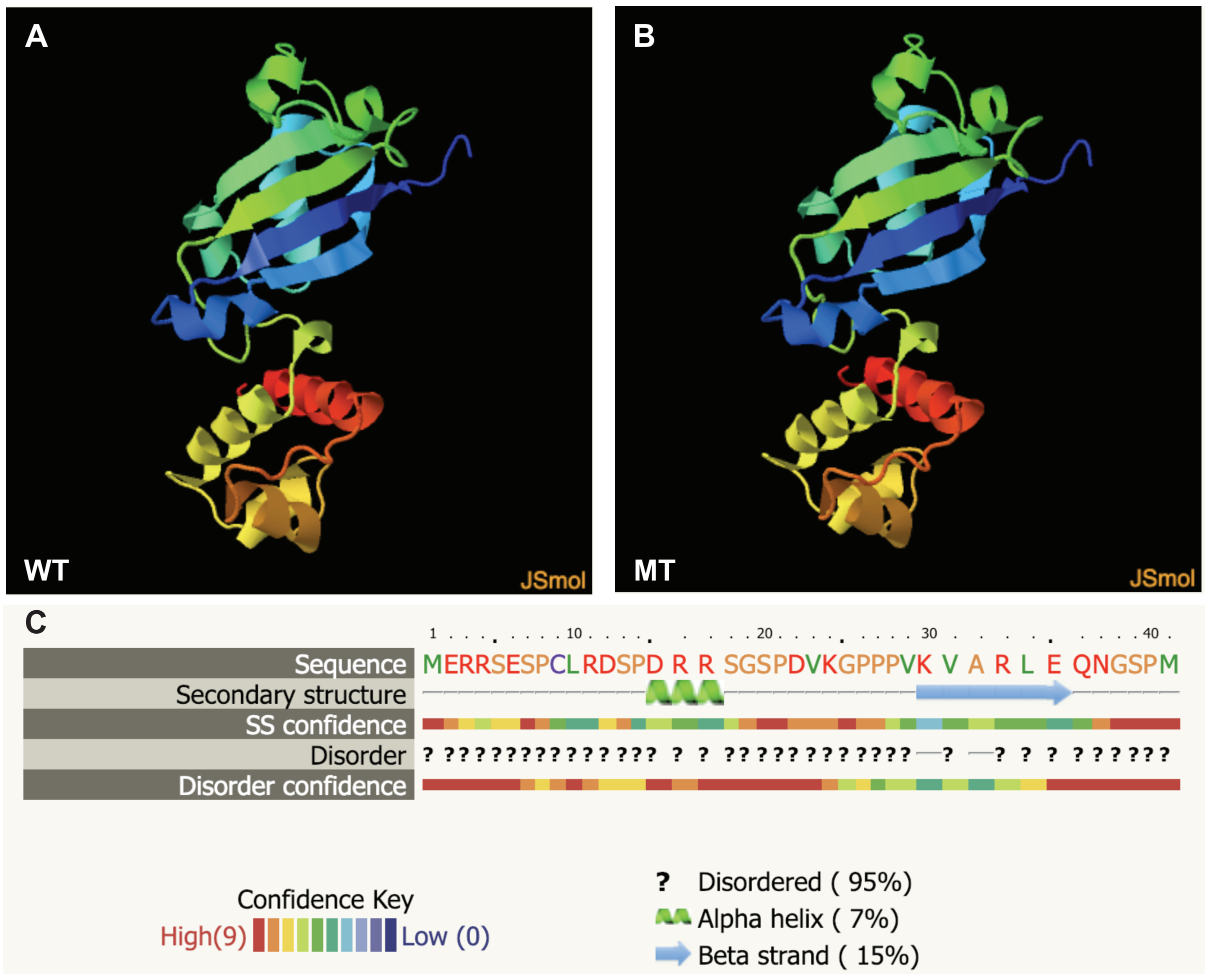
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Target	Forward Primer	Reverse Primer	Design RefSeq	Gene Symbol
Actb	ACAGCTTCTTTGCAGCTCCT	AGCGCAGCGATATCGTCAT	NM_007393.5	Actb
Acvr1	TGATGATGGCTTTCCCTTCC	CCCTCACACACACACATGTA	NM_007394.3	Acvr1
Alpl	GCAATGAGGTCACATCCATCC	GTTCACCCGAGTGGTAGTCA	NM_007431.2	Alpl
ATF4	GCCAAGCACTTGAAACCTCA	CATCCAATCTGTCCCGGAAAA	NM_009716.2	Atf4
Bad	GCAGCCACCAACAGTCATCA	GTACGAACTGTGGCGACTCC	NM_007522.2	Bad
Bax	GCGTGGTTGCCCTCTTCTA	CTGATCAGCTCGGGCACTTTA	NM_007527.3	Bax
Bcl10	CTCACGGAGGAGGATTTGAC	TTTCTCACACAGGTAAACACGTA	NM_009740.1	Bcl10
Bglap	GAACAGACAAGTCCCACACA	GTCAGCAGAGTGAGCAGAAA	NM_007541.2	Bglap
Bmp1	CCGTTTGTGATTGGAGGGAA	ATGCTTCTCCCAGTGTCTCA	NM_009755.3	Bmp1
Bmp2	GTGCGCAGCTTCCATCAC	CGTCACTGGGGACAGAACTTAA	NM_007553.2	Bmp2
Bmp3	CGGCTGGAGCGAATGGATTA	GTGGCGTGATTTGATGGTTTCA	NM_173404.3	Bmp3
Bmp4	GAACCGGGCTTGAGTACCC	GGTCCCTGGGATGTTCTCC	NM_007554.2	Bmp4
Bmp5	CTGCAAGAAGCACGAACTCTA	AGCATACCCTTCTGGTGCTA	NM_007555.3	Bmp5
Bmp6	CCAAGTCTTGCAGGAGCATCA	CCTTCTTCTGAGGCCCACAC	NM_007556.2	Bmp6
Bmp7	TGTACGTCAGCTTCCGAGAC	GTAGTAGGCAGCATAGCCTTCA	NM_007557.2	Bmp7
Bmpr1a	GGAAATGGCTCGTCGTTGTA	CACTGGGCACCATGTTGTAA	NM_009758.4	Bmpr1a
Bmpr1b	GAAGTTACGGCCTTCATTCCC	GCACTCTGTCATAAGCTTCCC	NM_007560.3	Bmpr1b
Bmpr2	GGGGAAGAAGATAATGCGGCTA	GGTTCACAGCTCCTTCTAGCA	NM_007561.3	Bmpr2
Casp2	TCCAAGTCTACAGAACAAGCCAAA	TCCATCTTGCTGGTCGACAC	NM_007610.1	Casp2
Casp4	CCACGTGGAGAAGGACTTCA	TCCTGTTTTGTCTCGGTAGGAC	NM_007609.3	Casp4
Casp6	ACAGAAACCGATGGCTTCTACA	TCCTCTTGTGGTCCATCTTGTAC	NM_009811.4	Casp6
Ccnb1	GCTGCTTCAGGAGACCATGTA	TAGCATCTTCTTGGGCACACA	NM_172301.3	Ccnb1
Ccnb2	GCCTCTTGCCTGTCTCAGAA	CACTCTCCATGTAGCCTGTGTA	NM_007630.2	Ccnb2
Ccnc	ACGGATCTCTGTCTGCTGTA	TGTTGTACGACACAGGCTAC	NM_016746.3	Ccnc
Ccnd1	TGCCGAGAAGTTGTGCATCTA	TGTTCACCAGAAGCAGTTCCA	NM_007631.2	Ccnd1
Ccnd2	TGCGGAAAAGCTGTGCATTTA	ACCCAACACTACCAGTTCCC	NM_009829.3	Ccnd2
Ccne1	CTGTGAAAAGCGAGGATAGCA	TGTTAGGGGTGGGGATGAAA	NM_007633.2	Ccne1
Cdc20	TCCCCTGCAAACATTCACTCA	ATATTGGACTGCCAGGGACAC	NM_023223.2	Cdc20
Cdc25a	TTGGACAGTGACCCAAGAGAC	AATCCTGATGCTTCCCAGAGAC	NM_007658.3	Cdc25a
Cdc25c	GATGTCTGCCTCTCCGCTTA	CTATCCAGAGGTCCAGATGAATCC	NM_009860.2	Cdc25c
Cdc6	TCTGCTGTTTCAGGAGACATCC	CCTGACATCCGACTCCACAA	NM_001025779.1	Cdc6
Cdk1	AAGTACCTGGACTCCATCCC	TCCCTGGAGGATTTGGTGTAA	NM_007659.3	Cdk1
Cdk2	GGAGAAGTTGTGGCGCTTAA	GAGATCTCTCGGATGGCAGTA	NM_183417.3	Cdk2
Cdk4	ATGTGGAGCGTTGGCTGTA	TGGTCGGCTTCAGAGTTTCC	NM_009870.3	Cdk4
Cdk5rap1	TCCTCACCCCAAGGATTTTCC	CTGGCAGGTGGATCTGCTTA	NM_025876.2	Cdk5rap1
Cdk6	TGGAAGTTCAGACGTGGATCA	GTCCCTAGGCCAGTCTTCC	NM_009873.2	Cdk6
Cdkn1a	GAACATCTCAGGGCCGAAAAC	TCTGCGCTTGGAGTGATAGAA	NM_007669.4	Cdkn1a

Cdkn1b	CAGTGTCCAGGGATGAGGAA	TTCGGGGAACCGTCTGAAA	NM_009875.4	Cdkn1b
Cdkn3	CAGACGAAGAACCTGTTGATGAA	ATTCACTCGCGACAGAGGTA	NM_028222.1	Cdkn3
Chrd	CCTTTGGGGAGATGAGCTGTA	GAACAATCGTCCCGCTCAC	NM_009893.2	Chrd
Col10a1	TTGCTAGCCCCAAGACACAA	GCCTTGTTCTCCTCTTACTGGAA	NM_009925.4	Col10a1
Col14a1	TGGTCAGATCAAGAGGACCAA	GGCCCATGATGTAGAGCAAA	NM_181277.3	Col14a1
Col1a1	TTCAGGGAATGCCTGGTGAA	ACCTTTGGGACCAGCATCA	NM_007742.3	Col1a1
Col1a2	GAAAAGGGTCCCTCTGGAGAA	AATACCGGGAGCACCAAGAA	NM_007743.2	Col1a2
Col2a1	GCACTTGCCAAGACCTGAAA	CCTGGTTGGGATCAATCCAGTA	NM_031163.3	Col2a1
Col3a1	TGCTGGAAAGAATGGGGAGAC	GGTCCAGAATCTCCCTTGTCAC	NM_009930.2	Col3a1
Col4a1	TCTGGCTGTGGAAAATGTGAC	TCCAATGACACCTTGCAACC	NM_009931.2	Col4a1
Col5a1	GGTCCTTTGGGGAAACCA	CTGGAGGACCTTCTTTTCCA	NM_015734.2	Col5a1
Ctsk	AGGGAAGCAAGCACTGGATA	TTCCGAGCCAAGAGAGCATA	NM_007802.3	Ctsk
Dlx5	TCTCTAGGACTGACGCAAAC	TGACTGTGGCGAGTTACAC	NM_010056.2	Dlx5
Fgf1	TGGACACCGAAGGGCTTTTA	GCATGCTTCTTGGAGGTGTAA	NM_010197.3	Fgf1
Fgf2	TCTTCCTGCGCATCCATCC	GCACACACTCCCTTGATAGACA	NM_008006.2	Fgf2
Fgfr1	GAGTAAGATCGGGCCAGACA	TCCATTTCCTTGTCGGTGGTA	NM_001079908.1	Fgfr1
Fgfr2	TCAAGTGGATGGCTCCTGAA	CACATTAACACCCCGAAGGAC	NM_010207.2	Fgfr2
Flt1	TTGCACGGGAGAGACTGAAA	GCCAAATGCAGAGGCTTGAA	NM_010228.3	Flt1
Fn1	GGAACCAGCAGAGTCCCAAA	CCTCGGTGTTGTAAGGTGGAA	NM_001276413.1	Fn1
Gapdh	CAAGGTCATCCCAGAGCTGAA	CAGATCCACGACGGACACA	NM_008084.2	Gapdh
Gdf10	CCCAAATCCTTTGACGCCTAC	CAATGCCCACAGCTCTGAC	NM_145741.2	Gdf10
Gli1	CAGAATCGGACCCACTCCAA	GCGAGCTGGGATCTGTGTA	NM_010296.2	Gli1
HOXA2	GAAGAAGGCGGCCAAGAAA	GCTGCCATCAGCTATTTCCA	NM_010451.1	Hoxa2
Ihh	TCTTCAAGGACGAGGAGAACAC	AGATGGCCAGTGAGTTCAGAC	NM_010544.2	Ihh
MIR34A	TGTCTTAGCTGGTTGTTGTGAGTA	GCAGCACTTCTAGGGCAGTA	NR_029751.1	Mir34a
MIR34B	CTCGGTTTGTAGGCAGTGTAA	TGTTTTGATGGCAGTGGAGTTA	NR_029655.1	Mir34b
MIR34C	AGTTACTAGGCAGTGTAGTTAGC	TTACCTGGCTGTGTGGTTAG	NR_029654.1	Mir34c
Mmp10	ACGTACTTCTTTGTAGGGGACAA	TGTCTTGGGAAGCCTTTATCCA	NM_019471.2	Mmp10
Mmp2	CGAGGACTATGACCGGGATA	GGGCACCTTCTGAATTTCCA	NM_008610.2	Mmp2
Mmp8	ACGGTCTTCAGGCTGCTTA	AGCCACTTAGAGCCCAGTAC	NM_008611.4	Mmp8
Mmp9	TCCCCAAAGACCTGAAAACC	GGGTGTAACCATAGCGGTAC	NM_013599.2	Mmp9
Nfkb1	ACCGTATGAGCCTGTGTTCA	GTAGCCTCGTGTCTTCTGTCA	NM_008689.2	Nfkb1
Nog	AGCAAGAAGCTGAGGAGGAA	TAGGTCATTCCACGCGTACA	NM_008711.2	Nog
Pdgfa	TGTAACACCAGCAGCGTCAA	GGCTTCTTCCTGACATACTCCA	NM_008808.3	Pdgfa
Runx2	TCTGGCCTTCCTCTCTCAGTAA	AACTGCCTGGGGTCTGAAAA	NM_009820.4	Runx2
SATB2	CCAGGAGTTTGGGAGATGGTA	TGAAAGGTTCTCTCGCTCCA	NM_139146.2	Satb2
SF3B4	ATGCCCAAGGACAGAGTCAC	TGGCATAGTCGGCATCTTCC	NM_153053.4	Sf3b4
Smad1	CTCAGCCCATGGACACGAA	CTCGTAAGCAACTGCCTGAAC	NM_008539.3	Smad1

Smad2	ACAAGTGACCAACAGTTGAACC	CAGGAGAGAGAGTAGTAGGAGACA	NM_001252481.1	Smad2
Smad3	TGCAGCCGTGGAACTTACAA	AAAGACCTCCCCTCCGATGTA	NM_016769.4	Smad3
Smad4	CCAACATTCCTGTGGCTTCC	GCTATCTGCAACAGTCCTTCAC	NM_008540.2	Smad4
Smad5	CCCAGCCTATGGATACAAGCA	CTCATAGGCGACAGGCTGAA	NM_008541.3	Smad5
Sost	CTCCCCACCATCCCTATGAC	CTGTCAGGAAGCGGGTGTA	NM_024449.5	Sost
Sox9	AGTACCCGCATCTGCACAA	GTCTCTTCTCGCTCTCGTTCA	NM_011448.4	Sox9
Sp7	AGGATGGCGTCCTCTCTG	AGAGCCGCCAAATTTGCT	NM_130458.3	Sp7
SPP1	TGCCTGACCCATCTCAGAA	AAGTCATCCTTTTCTTCAGAGGAC	NM_009263.3	Spp1
Tgfb1	GCTGCGCTTGCAGAGATTAA	GTAACGCCAGGAATTGTTGCTA	NM_011577.1	Tgfb1
Tgfb2	GCCCATATCTATGGAGTTCAGACA	AGCGGAAGCTTCGGGATTTA	NM_009367.3	Tgfb2
Tgfb3	TCAGGCCCTTGCCCATAC	CTCTGGGTTCAGGGTGTTGTA	NM_009368.3	Tgfb3
Tgfbr1	AATTGCTCGACGCTGTTCTA	ACCGATGGATCAGAAGGTACA	NM_009370.2	Tgfbr1
Tgfbr2	TCTGTGAGAAGCCGCATGAA	GGCAAACCGTCTCCAGAGTAA	NM_009371.3	Tgfbr2
Tgfbr3	TGGTGTGGCATGTGAAGACA	GATGAAAACTGGACCACAGAACC	NM_011578.3	Tgfbr3
Trp53	CACAGCGTGGTGGTACCTTA	CCCATGCAGGAGCTATTACACA	NM_011640.3	Trp53
Twist1	CATGTCCGCGTCCCACTA	TGTCCATTTTCTCCTTCTCTGGAA	NM_011658.2	Twist1
Vegfa	CCAGCACATAGGAGAGATGAG	CTGGCTTTGTTCTGTCTTTCTT	NM_001025250.3	Vegfa
Vegfb	GCCCCAGCCACCAGAA	GCTGGGCACTAGTTGTTTGAC	NM_001185164.N	Vegfb

Supplemental Data

Sup Fig. 1: Mutations in *Satb2* reduce protein levels

A) Satb2 expression determined by qPCR. Green bars reflect expression detected with primers binding at the mutation site. Note the absence of expression for these primers in C9, C4, and C8, all colonies lacking the N-terminal region of Satb2. C19 has one WT allele and produces roughly 60% of WT levels of *Satb2* full-length mRNA. Orange bars reflect expression detected with primers binding in the C-terminal region of Satb2. Note that all colonies produce mRNA, although not all of this RNA is expected to make protein. C4 cells over-expressing *Satb2* produce roughly 3.5 times as much *Satb2* mRNA. No RNA is detected at the cut site because the forward primer does not bind in our over-expression construct. **B)** Western Blot using antibodies directed against Satb2 (C-terminal region) and Gapdh. Note that C19 produces roughly half the amount of Satb2 protein as WT, as predicted by mRNA levels. C9 produces two separate proteins, including a shorter protein predicted to lack 40 N-terminal amino acids (asterisk). C4 also produces the short protein although at much lower levels, and C8 produces no protein. **C)** Second blot showing Satb2 bands at higher intensity, where the shorter protein can be seen more clearly.

SupFig2: *Satb2* expression is more closely related to cell cycle genes than osteogenic genes

Hierarchical clustering analysis of single cell gene expression. Individual cells are in columns. Genes are in rows. A cluster of highly expressing *Satb2* cells (black rectangle) is associated with high levels of cell cycle regulators associated with the S and G2 phases of the cell cycle (bold magenta). *Satb2* is listed in bold green.

Sup Fig. 3: Satb2 levels exhibit population-level variation

Undifferentiated (T0) WT cells were stained with **A)** Hoechst to identify DNA (blue) and immuno-stained for **B)** BrdU (green) and **C)** Satb2 (pink). Three Satb2 expression classes were defined. Class 1 (pink ovals) represents low Satb2 levels, class 2 (white ovals) represents medium Satb2 levels, and class 3 (yellow ovals) represents high Satb2 levels. The asterisk indicates a metaphase cell, and the caret indicates recently divided, early G1 phase cells. **D)** Bar graph showing the percentage of total cells per Satb2 expression class (blue bars) and the percentage of each Satb2 class that was BrdU positive (green bars).

Sup Fig 4: Mutations in Satb2 reduce osteogenic differentiation.

A) Overview scans of wells (left) and 4x magnification of Alizarin Red staining on WT, C19, C9, C4, and C8 cells after 0 (T0), 7 (T7), 14 (T14), and 21 days (T21) of differentiation. Data are shown for seeding densities of 100,000 cells. B) Quantification of Alizarin red staining at T21 after extraction. Data represent the means of 3 replicates with standard deviation. Asterisk indicates significant difference from WT (p=0.037).

Sup Fig 5: Mutations in *Satb2* dysregulate gene expression during differentiation

Genes whose expression is positively correlated with Satb2 with a correlation coefficient 0.5 and above are shown for **A)** WT and **B)** C9. Each point on the x-axis shows expression levels in a single cell (30 cells for WT; 26 cells for C9).

Sup Fig 6: Satb2 N-terminal disorder domain and protein folding

Predicted structure of **A)** wild-type (WT) and **B)** 40 amino acid N-terminal deletion (MT) proteins produced by the Phyre[2] software (Kelley *et al.*, 2015). **C)** Sequence, secondary structure, and disorder confidence predictions for the 40 amino acid N-terminal region of Satb2.

Supplementary Table 1: List of Fluidigm Delta Gene Assays

Genes and primer sequences used for single-cell qPCR Biomark analysis are listed.