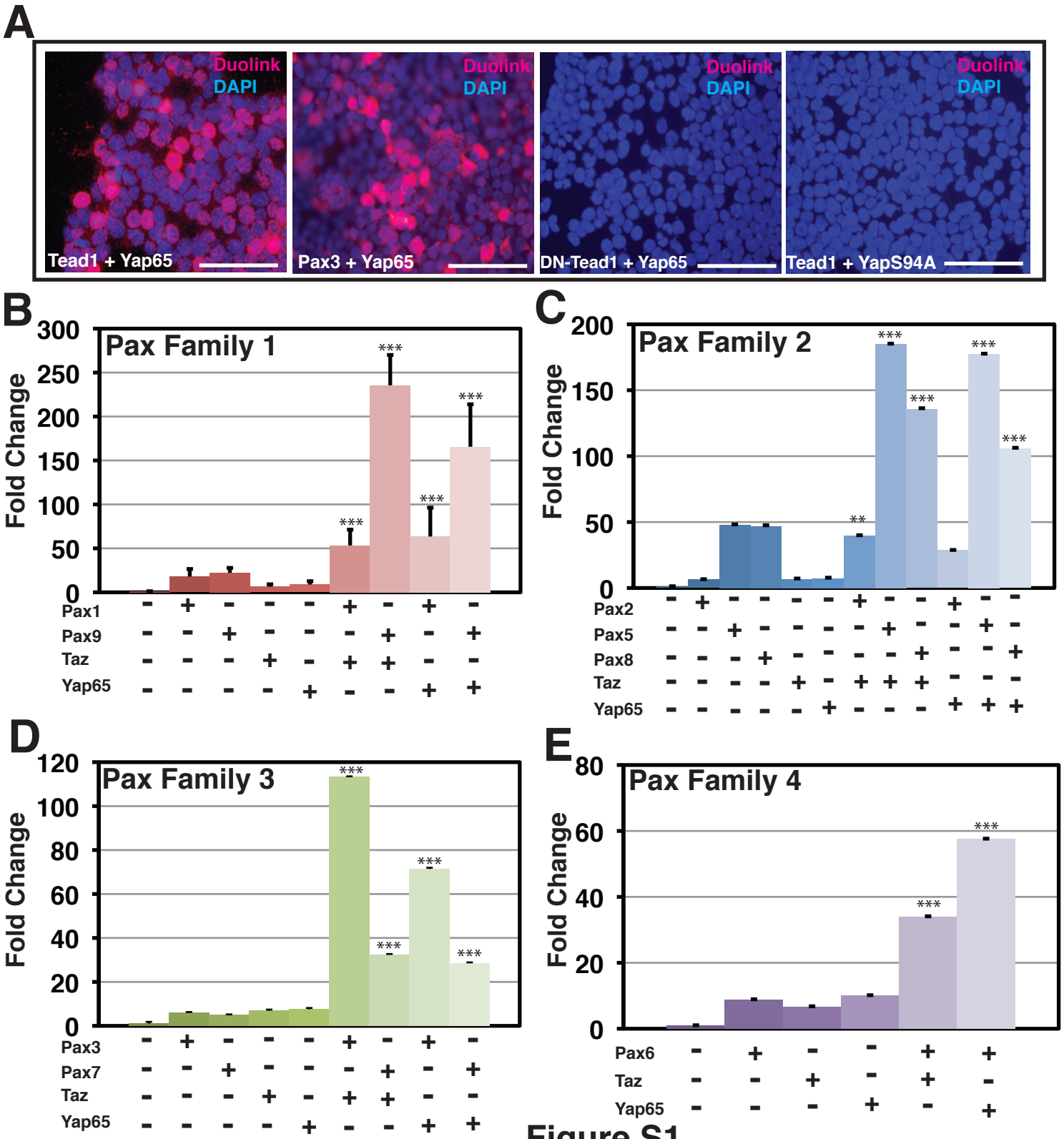
A
Duolink
DAPI
Tead1 + Yap65
Pax3 + Yap65
DN-Tead1 + Yap65
Tead1 + YapS94A
B
Pax Family 1
Fold Change
300
250
200
150
100
50
0
***
Pax1
Pax9
Taz
Yap65
C
Pax Family 2
200
150
100
50
0
**
Pax2
Pax5
Pax8
Taz
Yap65
D
Pax Family 3
120
100
80
60
40
20
0
Pax3
Pax7
Taz
Yap65
E
Pax Family 4
80
60
40
20
0
Pax6
Taz
Yap65

Figure S1

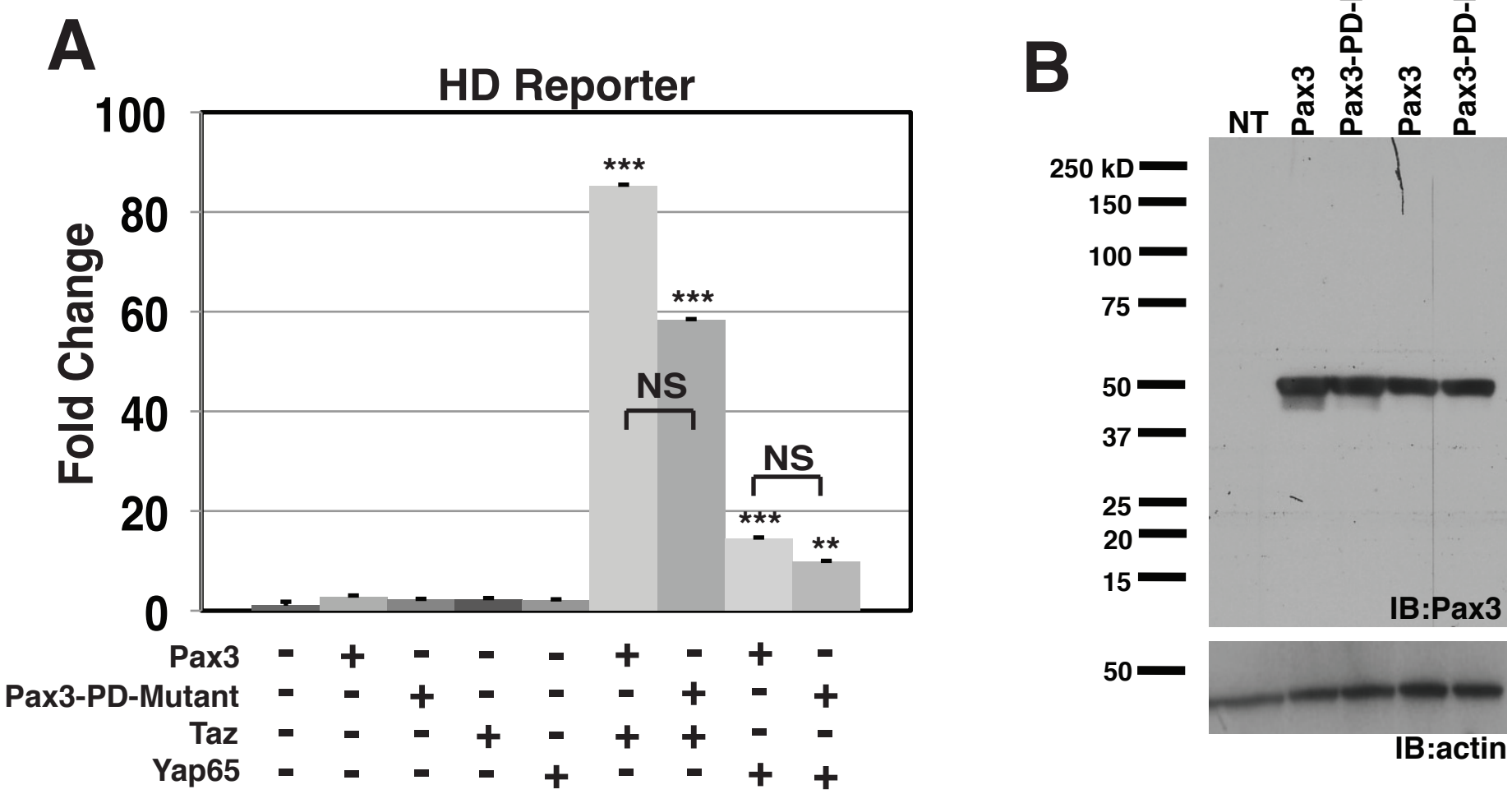
A
HD Reporter
Fold Change
100
80
60
40
20
0
***
***
NS
NS
***
**
Pax3 - + - - - + - + -
Pax3-PD-Mutant - - + - - - + - +
Taz - - - + - + + - -
Yap65 - - - - + - - + +
B
NT
Pax3
Pax3-PD-Mutant
Pax3
Pax3-PD-Mutant
250 kD
150
100
75
50
37
25
20
15
IB:Pax3
50
IB:actin

Figure S2

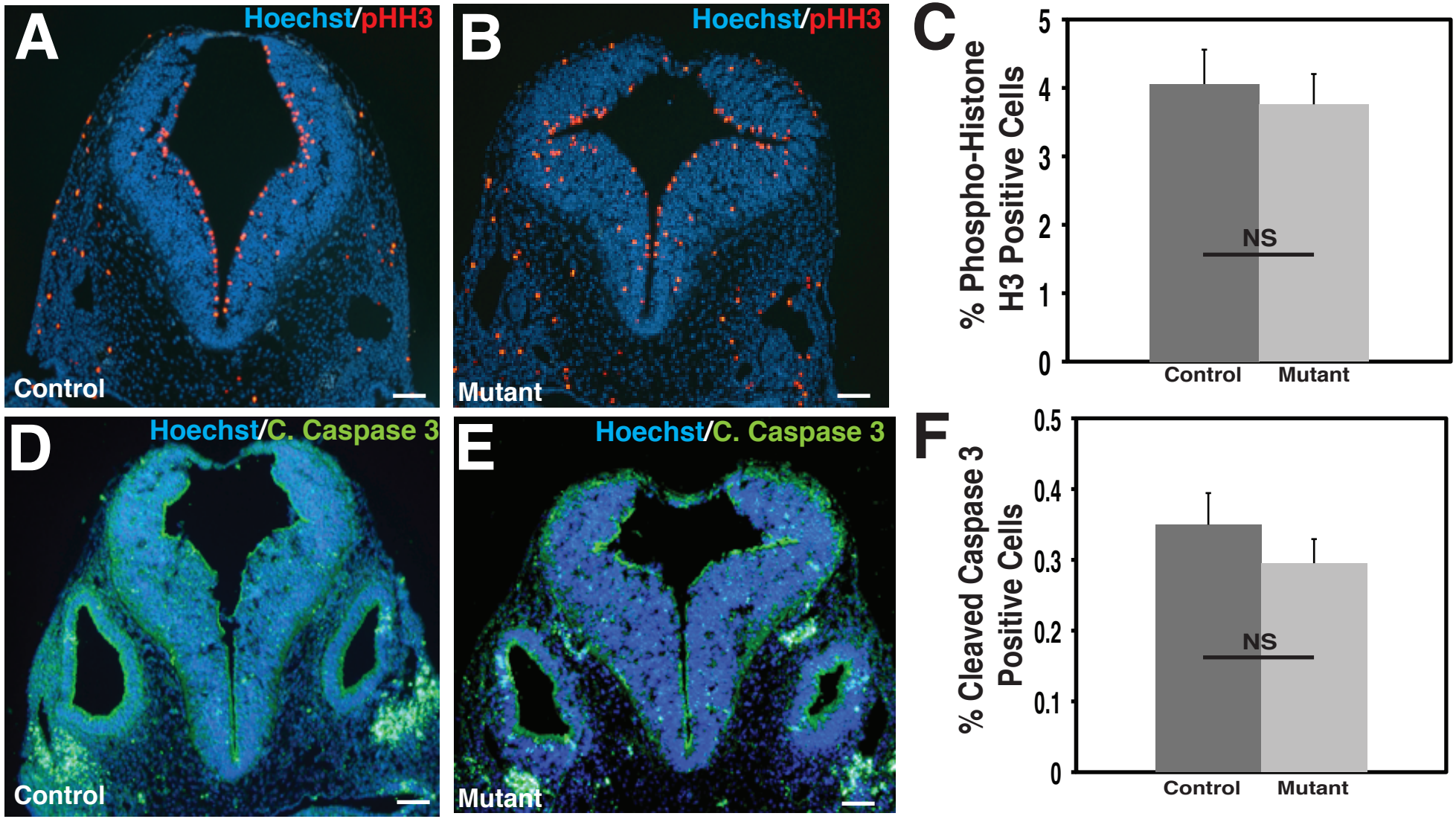
A
Hoechst/pHH3
Control
B
Hoechst/pHH3
Mutant
C
% Phospho-Histone H3 Positive Cells
5
4
3
2
1
0
NS
Control
Mutant
D
Hoechst/C. Caspase 3
Control
E
Hoechst/C. Caspase 3
Mutant
F
% Cleaved Caspase 3 Positive Cells
0.5
0.4
0.3
0.2
0.1
0
NS
Control
Mutant

Figure S3

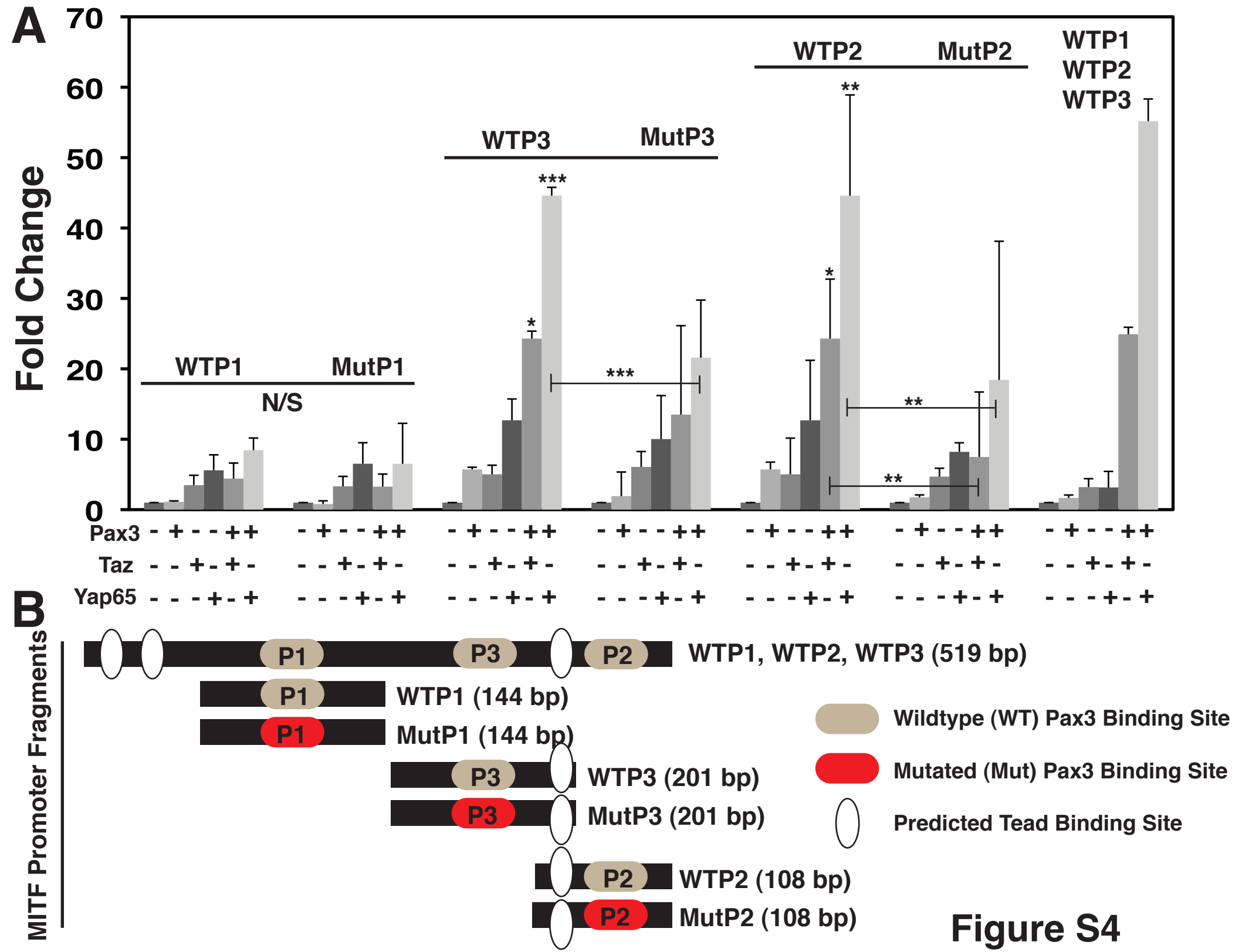
A
Fold Change
70
60
50
40
30
20
10
0
WTP1
MutP1
N/S
WTP3
MutP3
WTP2
MutP2
WTP1
WTP2
WTP3
*
**
***
Pax3 - + - - ++ - + - - ++ - + - - ++ - + - - ++ - + - - ++ - + - - ++ - + - - ++
Taz - - +- + - - - +- + - - - +- + - - - +- + - - - +- + - - - +- + - - - +- + -
Yap65 - - - +- + - - - +- + - - - +- + - - - +- + - - - +- + - - - +- + - - - +- +
B
MITF Promoter Fragments
P1
P3
P2
WTP1, WTP2, WTP3 (519 bp)
WTP1 (144 bp)
MutP1 (144 bp)
WTP3 (201 bp)
MutP3 (201 bp)
WTP2 (108 bp)
MutP2 (108 bp)
Wildtype (WT) Pax3 Binding Site
Mutated (Mut) Pax3 Binding Site
Predicted Tead Binding Site

Figure S4

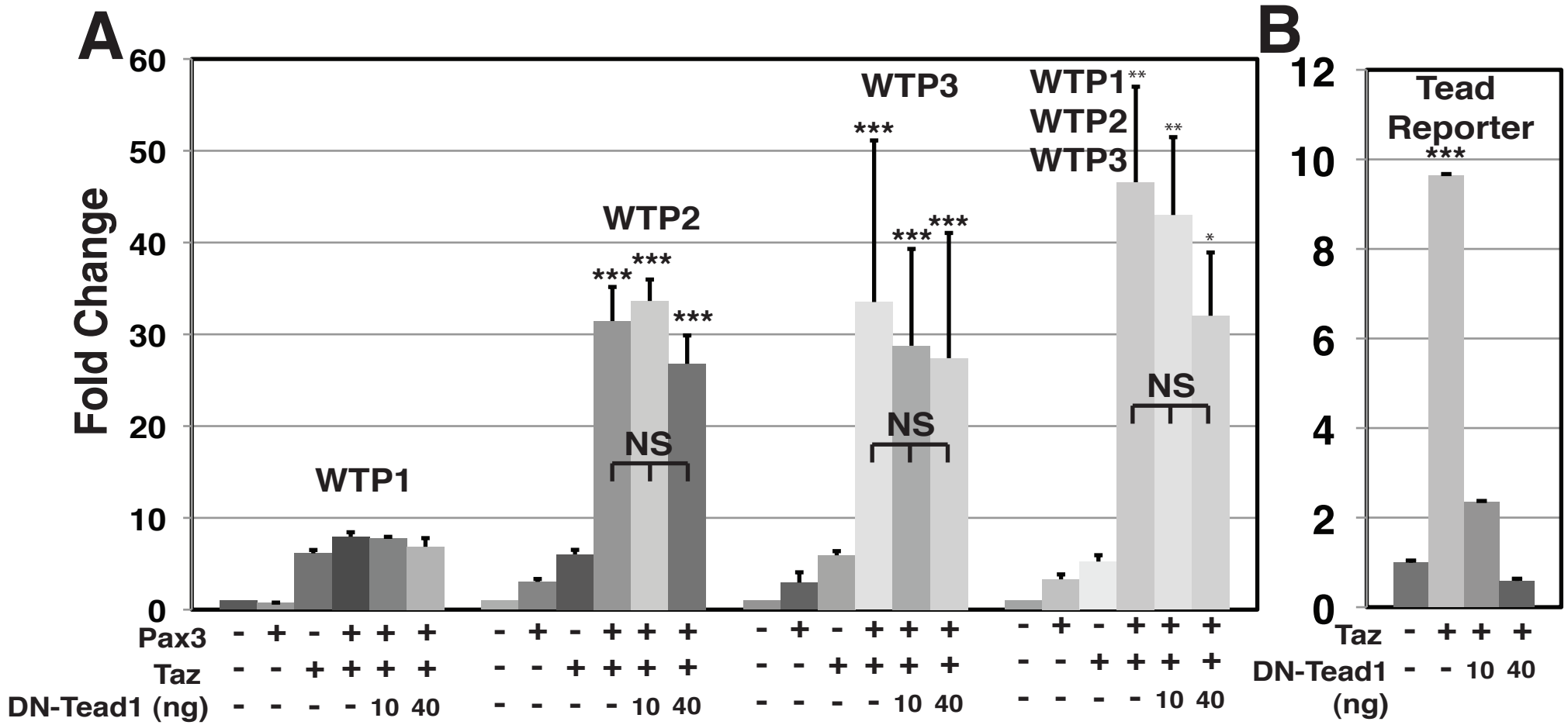
A
B
Fold Change
60
50
40
30
20
10
0
WTP1
WTP2
WTP3
WTP1
WTP2
WTP3
***
***
***
***
***
**
**
*
NS
NS
NS
Pax3
Taz
DN-Tead1 (ng)
- + - + + +
- - + + + +
- - - - 10 40
12
10
8
6
4
2
0
Tead
Reporter
***
Taz
DN-Tead1
(ng)
- + + +
- - 10 40

Figure S5

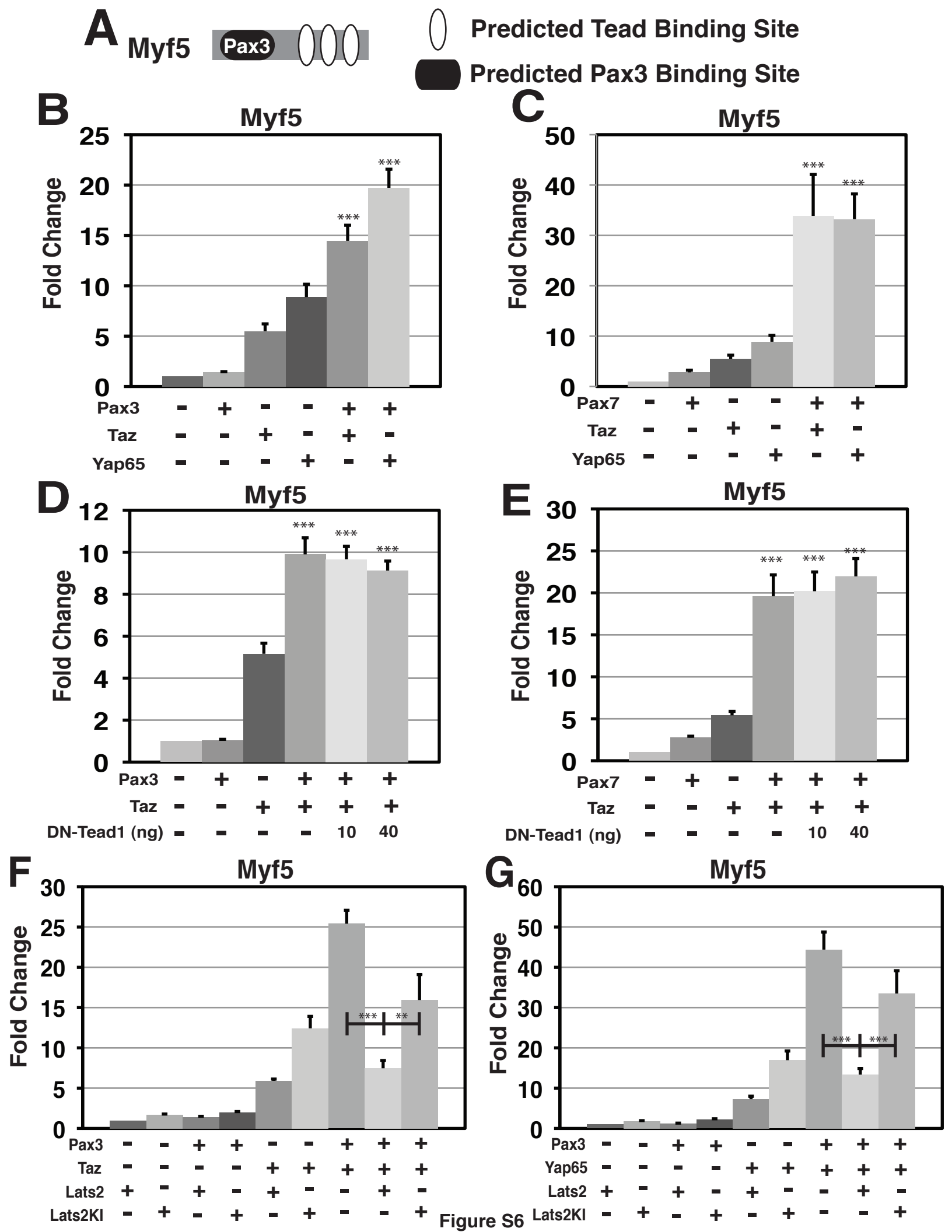
A
Myf5
Pax3
Predicted Tead Binding Site
Predicted Pax3 Binding Site
B
Myf5
Fold Change
Pax3
Taz
Yap65
C
Myf5
Fold Change
Pax7
Taz
Yap65
D
Myf5
Fold Change
Pax3
Taz
DN-Tead1 (ng)
E
Myf5
Fold Change
Pax7
Taz
DN-Tead1 (ng)
F
Myf5
Fold Change
Pax3
Taz
Lats2
Lats2KI
G
Myf5
Fold Change
Pax3
Yap65
Lats2
Lats2KI

Figure S6

Supplemental Figure Legends

Figure S1 (Related to Figure 1). **Pax3 and Yap65 can interact and the transcriptional activity of all Pax gene families are significantly increased in the presence of Taz or Yap65.** (A) Demonstration of Pax3-Yap65 interaction using Duolink® reagents. Interaction is documented as red fluorescence. Samples were co-transfected with the specified plasmids. Tead1+Yap65 served as the positive control. DN-Tead1 is truncated protein that retains DNA-binding capacity but cannot recruit Yap (Zhao et al., 2008). YapS94A is a point mutant shown previously to prevent interaction with Tead1 (Zhao et al., 2008). Scale bars: 100μm. (B) Results of dual luciferase reporter assays in HEK293T cells with Pax3-luciferase reporter in the presence (+) or absence (-) of Pax1, Pax9, Taz or Yap65. All activities were normalized to that observed in the absence of Pax1, Pax9, Taz or Yap65 (lane 1). (C) Results of dual luciferase reporter assays in HEK293T cells with Pax3-luciferase reporter in the presence (+) or absence (-) of Pax2, Pax5, Pax8, Taz or Yap65. All activities were normalized to that observed in the absence of Pax2, Pax5, Pax8, Taz or Yap65 (lane 1). (D) Results of dual luciferase reporter assays in HEK293T cells with Pax3-luciferase reporter in the presence (+) or absence (-) of Pax3, Pax7, Taz or Yap65. All activities were normalized to that observed in the absence of Pax3, Pax7, Taz or Yap65 (lane 1). (E) Results of dual luciferase reporter assays in HEK293T cells with Pax6-luciferase reporter in the presence (+) or absence (-) of Pax6, Taz or Yap65. All activities were normalized to that observed in the absence of Pax6, Taz or Yap65 (lane 1). In all experiments, luciferase activity was first normalized

to the activity of a co-transfected renilla luciferase construct. All experiments were performed in duplicate a minimum of three individual occasions. Data depicted are the mean + standard error of the mean (SEM). Statistics were completed using an ANOVA with a Tukey-Kramer post-hoc comparison test. *** $p<0.001$, ** $p<0.01$

Figure S2 (Related to Figure 1). Pax3 Paired-Domain mutant can activate a Homeodomain reporter. (A) Results of dual luciferase reporter assays in HEK293T cells with Pax3-HomeoDomain (HD)-luciferase reporter in the presence (+) or absence (-) of Pax3, Pax3-Paired Domain (PD)-Mutant, Taz or Yap65. Data depicted are the mean + standard error of the mean (SEM). Statistics were completed using an ANOVA with a Tukey-Kramer post-hoc comparison test. *** $p<0.001$, ** $p<0.01$ (B) Anti-Pax3 western blot from HEK293T cells either non-transfected (NT), transfected with Pax3 or transfected the Pax3-Paired Domain (PD)-Mutant. Western blots of the same cellular protein lysates were also probed with anti-actin to demonstrate equivalent protein loading among samples.

Figure S3 (Related to Figure 3). Taz/Yap65 neural crest null embryos display no alterations in proliferation or apoptosis. (A) Cross section of a Wnt1-Cre; $Taz^{flox/+}$;$Yap^{flox/+}$ E10.5 embryo (Control). (B) Cross section of a Wnt1-Cre; $Taz^{flox/flox}$;$Yap^{flox/flox}$ E10.5 embryo (Mutant). A and B are both stained for phospho-Histone H3 (pHH3) as a marker of proliferation and Hoechst for a nuclear counterstain. (C) Quantitation of percent pHH3 positive cells in neural tubes from four control and five mutant embryos. (D) Cross section of a Wnt1-

Cre; $Taz^{flox/+}$;$Yap^{flox/+}$ E10.5 embryo (Control). (E) Cross section of a Wnt1-Cre; $Taz^{flox/flox}$;$Yap^{flox/flox}$ E10.5 embryo (Mutant). D and E are both stained for Cleaved Caspase 3 (C. Caspase 3) as a marker of apoptosis and Hoechst for a nuclear counterstain. (F) Quantitation of percent C. Caspase 3 positive cells in neural tubes from four control and three mutant embryos. Data depicted in panels C and F are the mean + standard error of the mean (SEM). Statistics were completed using a Student's t-test. NS=Not Significant, ***$p<0.001$. Panels A-B, D-E, scale bars: 100μm.

Figure S4 (Related to Figure 5). Pax3 P2 and P3 binding sites within Mitf promoter fragments display Taz/Yap65 synergistic activation. (A) Results of dual luciferase reporter assays in HEK293T cells with Mitf-promoter fragment luciferase reporters in the presence (+) or absence (-) of Pax3, Taz or Yap65. Fragments containing wildtype (WT) Pax3 binding sequences were compared to the same fragment differing only by the presence of a mutated (Mut) Pax3 binding sequences for each site (P1, P2 and P3). (B) Schematic representation of the Mitf-promoter fragment luciferase reporter plasmids. The Pax3 binding sites are denoted within oblong circles, labeled P1, P2 and P3, representing the consensus sequences AGTTCCAGTAGTATTAATA, ATTAGCTTAGGTTAT, and AAGCATGACGTCAAGCCA, respectively. Mutated Pax3 binding sites are represented in the red oblong circles. Mutant P1 represents the sequence A<u>C</u>TTC<u>G</u>AGTAGTAT<u>CGC</u>TA. Mutant P2 represents the sequence <u>GCG</u>AGCTTAG<u>C</u>TT<u>C</u>T. Mutant P3 represents the sequence AA<u>A</u>CA<u>C</u>GAG<u>C</u>TC<u>G</u>AG<u>T</u>CA Underlined nucleotides indicate altered positions.

Predicted TEAD binding sites are denoted with white ovals, representing the consensus sequence XDGHATXT. The size of all fragments are included following the construct designation. In all experiments, luciferase activity was first normalized to the activity of a co-transfected renilla luciferase construct, then to the activity observed in the absence of specificed cDNAs. All experiments were performed in duplicate a minimum of three individual occasions. Data depicted are the mean + standard error of the mean (SEM). Statistics were completed using an ANOVA with a Tukey-Kramer post-hoc comparison test. *** $p<0.001$, **$p<0.01$, * $p<0.05$, N/S=Not significant

Figure S5 (Related to Figure 5). The Pax3-Taz/Yap65 co-activation of Mitf promoter fragments P2 and P3 is Tead independent. (A) Results of dual luciferase reporter assays in HEK293T cells with Mitf-promoter fragment luciferase reporters in the presence (+) or absence (-) of Pax3, Taz or or the specified nanogram (ng) amount of DN-Tead1. In all experiments, luciferase activity was first normalized to the activity of a co-transfected renilla luciferase construct, then to the activity observed in the absence of specificed cDNAs. All experiments were performed a minimum of three individual occasions. Data depicted are the mean + standard error of the mean (SEM). Statistics were completed using an ANOVA with a Tukey-Kramer post-hoc comparison test. *** $p<0.001$, **$p<0.01$, * $p<0.05$, N/S=Not significant

Figure S6 (Related to Figure 6). The Pax3-Taz/Yap65 co-activation of endogenous Pax3 target gene Myf5 is Tead independent and can be inhibited by Lats2. (A) Schematic representation of the Myf5 luciferase reporter

plasmid. The Pax3 binding site is denoted within the black oblong circles, representing the consensus sequence CGTCACGCTT. Predicted TEAD binding sites are denoted with white ovals, representing the consensus sequence XDGHATXT. (B) Results of dual luciferase reporter assays in HEK293T cells with Myf5-luciferase reporter in the presence (+) or absence (-) of Pax3, Taz or Yap65. (C) Results of dual luciferase reporter assays in HEK293T cells with Myf5-luciferase reporter in the presence (+) or absence (-) of Pax7, Taz or Yap65. (D) Results of dual luciferase reporter assays in HEK293T cells with Myf5-luciferase reporter in the presence (+) or absence (-) of Pax3, Taz or the specified nanogram (ng) amount of DN-Tead1. (E) Results of dual luciferase reporter assays in HEK293T cells with Myf5-luciferase reporter in the presence (+) or absence (-) of Pax7, Taz or the specified nanogram (ng) amount of DN-Tead1. (F) Results of dual luciferase reporter assays in HEK293T cells with Myf5-luciferase reporter in the presence (+) or absence (-) of Pax3, Taz, Lats2 or Lats2KI. (G) Results of dual luciferase reporter assays in HEK293T cells with Myf5-luciferase reporter in the presence (+) or absence (-) of Pax3, Yap65, Lats2 or Lats2KI. In all experiments, luciferase activity was first normalized to the activity of a co-transfected renilla luciferase construct, then to the activity observed in the absence of specificed cDNAs (lane 1). All experiments were performed in duplicate a minimum of three individual occasions. Data depicted are the mean + standard error of the mean (SEM). Statistics were completed using an ANOVA with a Tukey-Kramer post-hoc comparison test. *** $p<0.001$, **$p<0.01$

Supplemental Tables

	Gene Symbols	Accession Numbers	Fold Change
1	**TAZ, WWTR1**, C78399,2310058J06RIK,2610021I22RIK	AW907926,AW907926.1,AW909855,AW909855.1,BC004640,BC004640.1	24.18429
2	SDF5, SFRP2, A1851596	BC014722,BC014722.1,BG246137,BG246137.1	14.57312
3	TBP	BC110341,BQ072304	10.74361
4	ESX1, ESX1L, ESXR1	BC042633,BI831835	6.21598
5	**YAP, YAP1, YAP2, YAP65**	BC038235,BC038235.1,BM918433,BM918433.1	3.872002

Table S1. (Related to Figure 1) Pax3 High Throughput Screen Top Co-Activator Molecules

Supplemental Experimental Procedures

Plasmids

To generate a Pax6 reporter construct, a previously indentified Pax6 DNA-binding motif (Epstein et al., 1994a), TTTTTCACGCTTGAGTT, was synthetically generated and re-iterated six times. This sequence was then cloned upstream of pGL4.27 for use in luciferase assays (Promega). The *Myf5* reporter sequence, a 145-bp element located -57.5 kb upstream of *Myf5* (chr10:107,543,161-107,543,305 (mm10)), was subcloned into pGL4.27 (Promega, (Bajard et al., 2006)). Human *TEAD1* was provided by Kun-Liang Guan. The human *YAP-S94A* expression vector was described previously (Zhao et al, 2008, Addgene plasmid 33102). The murine *Pax1* expression construct was previously characterized (Brent et al., 2003, Addgene #13898) and was cloned into pCS3-6myc-DEST for expression. The murine *Pax2* expression construct was previously described (Lang et al., 2000 JCI). The human *PAX5* expression construct was a gift of M. Busslinger. The human *PAX6* expression construct was previously described (Epstein et al., 1994b) but was subcloned into pcDNA3 (Invitrogen). Murine *Pax8* was amplified from an expression library and cloned into pCMV-Sport6 (Invitrogen). The murine *Pax9* expression construct was previously described (Lang et al., 2000) and was subcloned into pcDNA3 (Invitrogen).

Human *MITF* promoter fragments were PCR-amplified from a human *MITF* reporter described previously (Lang et al., 2005). Pax3-binding site mutants were PCR-generated with primers containing the mutagenic nucleotides. The fragment primer sequences are as follows and genomic positions are referenced

using the UCSC Genome Browser on human Dec. 2013 (GRCh38/hg38) assembly:

MITF Fragment containing Pax3 P1, P3, and P2 binding sites (519 bp)
chr3:69936067-69936585
Forward: GAATTCAAGCTTAGGTCAATAGGCAGGTTACAC
Reverse: GTGTCTAAGCTTTTTATCACAG

MITF Fragment Pax3 P1 (144 bp)
chr3:69,936,263-69,936,406
Forward: GAATTCAAGCTTTGATGTCTCCTCCAAAGGGG
Reverse: GAATTCAAGCTTAAGTGACGAGCTATCAAAGTCAAAC

MITF Fragment Pax3 P2 (108 bp)
chr3:69,936,478-69,936,585
Forward: GAATTCAAGCTTAAGACCAAACTCGTAGGGC
Reverse: GAATTCAAGCTTTTTATCACAGAAGCCCTGC

MITF Fragment Pax3 P3 (201 bp)
chr3:69,936,346-69,936,546
Forward: GAATTCAAGCTTAAGATGAATAGTGAATTGGCCTTG
Reverse: GAATTCAAGCTTTAGTTATGCATGTAAAAAAAAACGTTCAC

Mutations in the respective Pax3 P1, P2 or P3 binding sites were introduced by recombinant circle PCR using pairs of reactions (Reaction 1 and Reaction 2) using the following primers:

MITF Fragment Mutated Pax3 P1 BS
Reaction 1
Forward: CTTTACTTCGAGTAGTATCGCTAGACAATGGTATTTCTC
Reverse: ATGAATAGTGAATTGGCCTTGATC
Reaction 2
Forward: TTTCTCTTTCAGCAATAGGTTAATAG
Reverse: TACCATTGTCTAGCGATACTACTCGAAGTAAAGATG

MITF Fragment Mutated Pax3 P2 BS
Reaction 1
Forward: TGCATAACTAGCGAGCTTAGCTTCTTATAAGCAGGGCTTCTGTG
Reverse: TGTAAAAAAAAACGTTCACATAAGGGCC
Reaction 2
Forward: TGTGATAAAAAGCTTATCGATACCGTCGACCTCG
Reverse:
GAAGCCCTGCTTATAAGAAGCTAAGCTCGCTAGTTATGCATG

MITF Fragment Mutated Pax3 P3 BS
Reaction 1
Forward: ATGAAAAAAAACACGAGCTCGAGTCAGGGGGAAAAA
Reverse: AAATATAAAAGAACCTTTTTAAGTGACGAGCTATC
Reaction 2
Forward: TTGATATCAACATTTAAGACCAAACTCGTAGGGCTTCC
Reverse: TTTTTCCCCCTGACTCGAGCTCGTGTTTTTTTTCAT

All *MITF* promoter fragments were sequence verified to exclude polymerase errors after insertion into the pGL4.27 vector for use in luciferase assays.

Histology and immunohistochemistry

Samples were harvested, fixed overnight in 4% paraformaldehyde and dehydrated through an ethanol series. All samples were paraffin embedded and sectioned. The antibody used for phospho-histone H3 (pHH3) immunofluorescence was anti-pHH3 (Ser 10, 6G3) mouse monoclonal (9706L, Cell Signaling). The antibody used for Cleaved Caspase 3 (C. Caspase 3) immunofluorescence was anti-Caspase 3 (Cleaved) rabbit polyclonal (CP229A, Biocare). Nuclei were stained blue with Hoechst dye. Quantitation of neural tube cell numbers was performed on Hoechst-stained sections using ImageJ software (National Institutes of Health, Bethesda, MD).

Proximity Ligation Assay

The proximity ligation assay was performed on transfected HEK293T cells. All transfections were completed using FuGene6 (Roche). Experiments utilized 40ng of each specified plasmid. Primary antibodies were incubated for 1 hour at room temperature and included anti-Pax3 mouse monoclonal (developed by C.P. Ordahl and obtained from the Developmental Studies Hybridoma Bank, created

by the NICHD of the NIH), anti-Yap rabbit polyclonal (Cell Signaling #4912S) and anti-myc mouse monoclonal (Cell Signaling #2276) as Tead1 and DN-Tead1 contain an N-terminal myc epitope tag. Staining was performed using the Duolink® In Situ Detection Reagents- Red kit (Sigma-Aldrich) following manufacturer's instructions. The DAPI nuclear stain was included in the Duolink® mounting media (Sigma-Aldrich).

Western Blot

Anti-Pax3 membranes were probed with primary antibody overnight (Pax3 mouse monoclonal antibody, developed by C.P. Ordahl and obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH).

Supplemental References

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