Figure S1

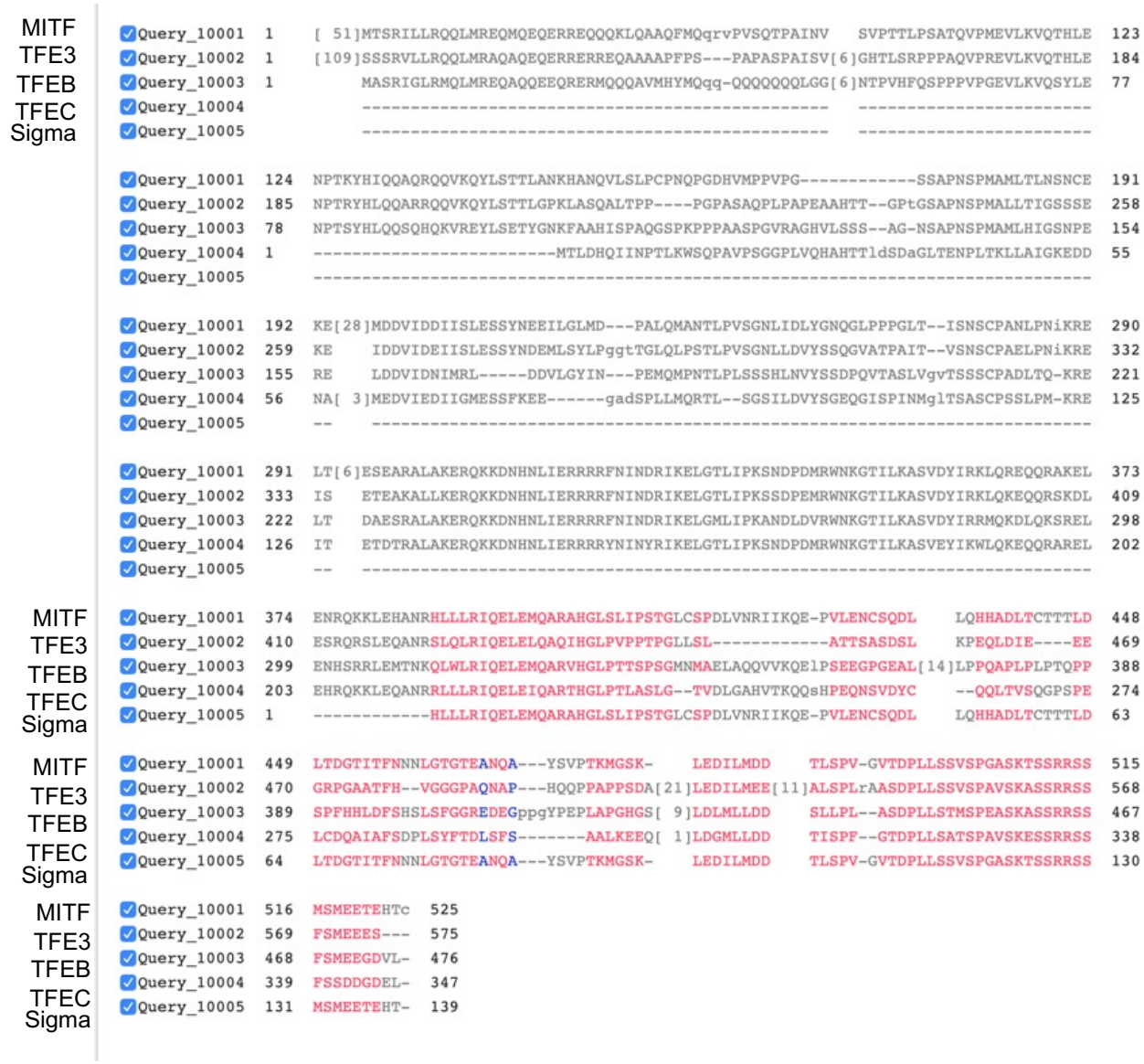
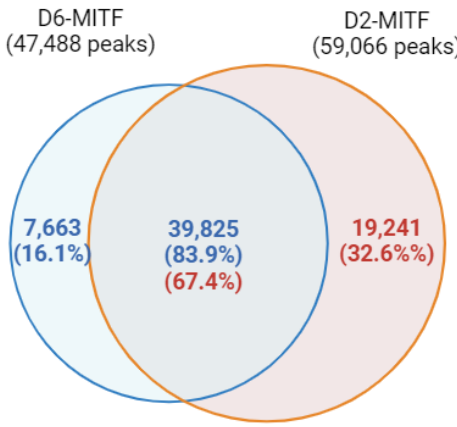
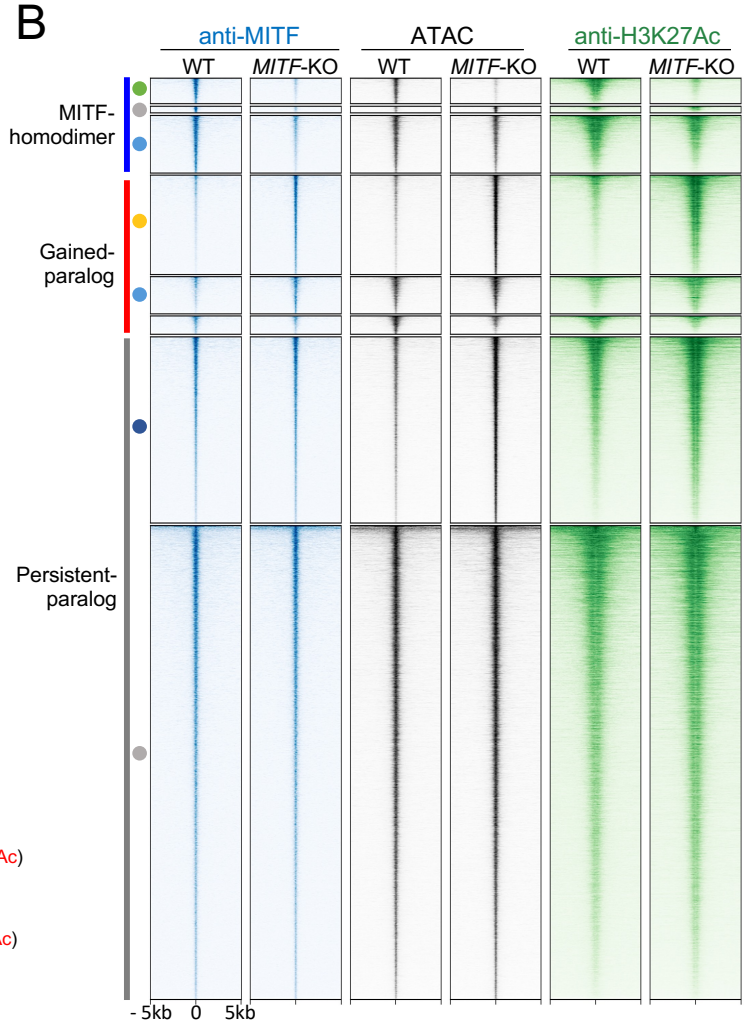

MITF
TFE3
TFEB
TFEC
Sigma
Query_10001 1 [51]MTSRILLRQQLMREQMQEQERREQQQKLQAAQFMQqrvPVSQTPAINV SVPTTLPSATQVPMEVLKVQTHLE 123
Query_10002 1 [109]SSSRVLLRQQLMRAQAQEQERRERREQAAAAPFPS---PAPASPAISV[6]GHTLSRPPPAQVPREVLKVQTHLE 184
Query_10003 1 MASRIGLRMQLMREQAQQEEQRERMQQQAVMHYMQqq-QQQQQQQLGG[6]NTPVHFQSPPPVPGEVLKVQSYLE 77
Query_10004 ---------- ----------
Query_10005 ---------- ----------
Query_10001 124 NPTKYHIQQAQRQQVKQYLSTTLANKHANQVLSLPCPNQPGDHVMPPVPG----------SSAPNSPMAMLTLNSNCE 191
Query_10002 185 NPTRYHLQQARRQQVKQYLSTTLGPKLASQALTPP----PGPASAQPLPAPEAAHTT--GPtGSAPNSPMALLTIGSSSE 258
Query_10003 78 NPTSYHLQQSQHQKVREYLSETYGNKFAAHISPAQGSPKPPPAASPGVRAGHVLSSS--AG-NSAPNSPMAMLHIGSNPE 154
Query_10004 1 ----------MTLDHQIINPTLKWSQPAVPSGGPLVQHAHTTldSDaGLTENPLTKLLAIGKEDD 55
Query_10005 ----------
Query_10001 192 KE[28]MDDVIDDIISLESSYNEEILGLMD---PALQMANTLPVSGNLIDLYGNQGLPPPGLT--ISNSCPANLPNiKRE 290
Query_10002 259 KE IDDVIDEIISLESSYNDEMLSYLPggtTGLQLPSTLPVSGNLLDVYSSQGVATPAIT--VSNSCPAELPNiKRE 332
Query_10003 155 RE LDDVIDNIMRL-----DDVLGYIN---PEMQMPNTLPLSSSHLNVYSSDPQVTASLVgvTSSSCPADLTQ-KRE 221
Query_10004 56 NA[3]MEDVIEDIIGMESSFKEE------gadSPLLMQRTL--SGSILDVYSGEQGISPINMglTSASCPSSLPM-KRE 125
Query_10005 -- ----------
Query_10001 291 LT[6]ESEARALAKERQKKDNHNLIERRRRFNINDRIKELGTLIPKSNDPDMRWNKGTILKASVDYIRKLQREQQRAKEL 373
Query_10002 333 IS ETEAKALLKERQKKDNHNLIERRRRFNINDRIKELGTLIPKSSDPEMRWNKGTILKASVDYIRKLQKEQQRSKDL 409
Query_10003 222 LT DAESRALAKERQKKDNHNLIERRRRFNINDRIKELGMLIPKANDLDVRWNKGTILKASVDYIRRMQKDLQKSREL 298
Query_10004 126 IT ETDTRALAKERQKKDNHNLIERRRRYNINYRIKELGTLIPKSNDPDMRWNKGTILKASVEYIKWLQKEQQRAREL 202
Query_10005 -- ----------
MITF
TFE3
TFEB
TFEC
Sigma
Query_10001 374 ENRQKKLEHANRHLLLRIQELEMQARAHGLSLIPSTGLCSPDLVNRIIKQE-PVLENCSQDL LQHHADLTCTTTLD 448
Query_10002 410 ESRQRSLEQANRSLQLRIQELELQAQIHGLPVPPTPGLLSL----------ATTSASDSL KPEQLDIE----EE 469
Query_10003 299 ENHSRRLEMTNKQLWLRIQELEMQARVHGLPTTSPSGMNMAELAQQVVKQElPSEEGPGEAL[14]LPPQAPLPLPTQPP 388
Query_10004 203 EHRQKKLEQANRRLLLRIQELEIQARTHGLPTLASLG--TVDLGAHVTKQQsHPEQNSVDYC --QQLTVSQGPSPE 274
Query_10005 1 ----------HLLLRIQELEMQARAHGLSLIPSTGLCSPDLVNRIIKQE-PVLENCSQDL LQHHADLTCTTTLD 63
MITF
TFE3
TFEB
TFEC
Sigma
Query_10001 449 LTDGTITFNNNLGTGTEANQA---YSVPTKMGSK- LEDILMDD TLSPV-GVTDPLLSSVSPGASKTSSRRSS 515
Query_10002 470 GRPGAATFH--VGGGPAQNAP---HQQPPAPPSDA[21]LEDILMEE[11]ALSPLrAASDPLLSSVSPAVSKASSRRSS 568
Query_10003 389 SPFHHLDFSHSLSFGGREDEGppgYPEPLAPGHGS[9]LDLMLLDD SLLPL--ASDPLLSTMSPEASKASSRRSS 467
Query_10004 275 LCDQAIAFSDPLSYFTDLSFS-------AALKEEQ[1]LDGMLLDD TISPF--GTDPLLSATSPAVSKESSRRSS 338
Query_10005 64 LTDGTITFNNNLGTGTEANQA---YSVPTKMGSK- LEDILMDD TLSPV-GVTDPLLSSVSPGASKTSSRRSS 130
MITF
TFE3
TFEB
TFEC
Sigma
Query_10001 516 MSMEETEHTc 525
Query_10002 569 FSMEEES--- 575
Query_10003 468 FSMEEGDVL- 476
Query_10004 339 FSSDDGDEL- 347
Query_10005 131 MSMEETEHT- 139

Figure S2

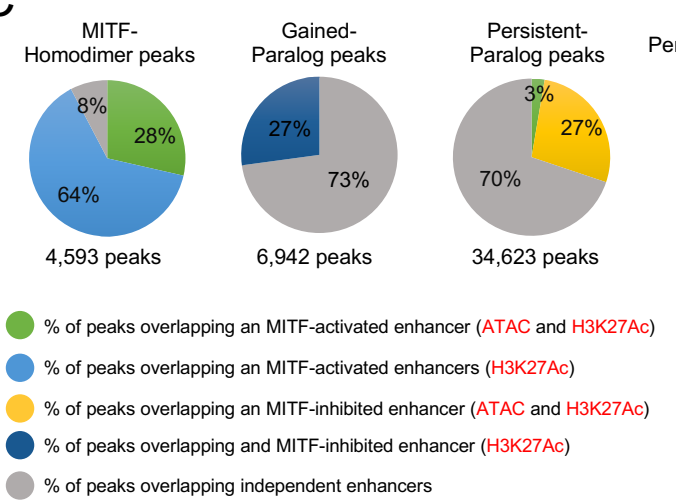
A



B



C



D

MITF-Homodimer peaks
Gene Ontology analysis (Panther)

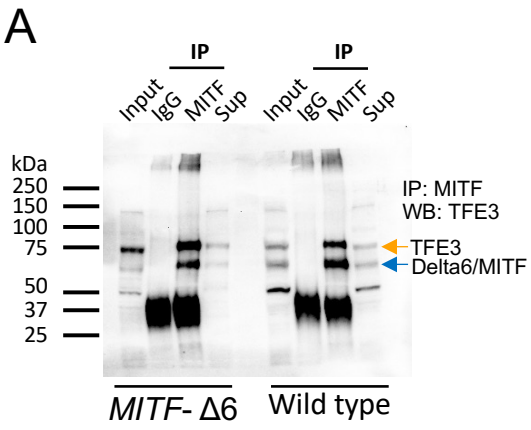
GO	No. of genes	FDR
Developmental processes	1103	2.6×10^{-15}
Cell differentiation	710	1.7×10^{-09}
Developmental pigmentation	21	3.7×10^{-05}
Regulation of cytokine production	173	8.9×10^{-05}
Melanocyte differentiation	10	2.6×10^{-03}
Organelle localization	119	5.0×10^{-03}
Pigment cell differentiation	14	8.4×10^{-03}
Melanin biosynthetic process from tyrosine	4	1.9×10^{-02}

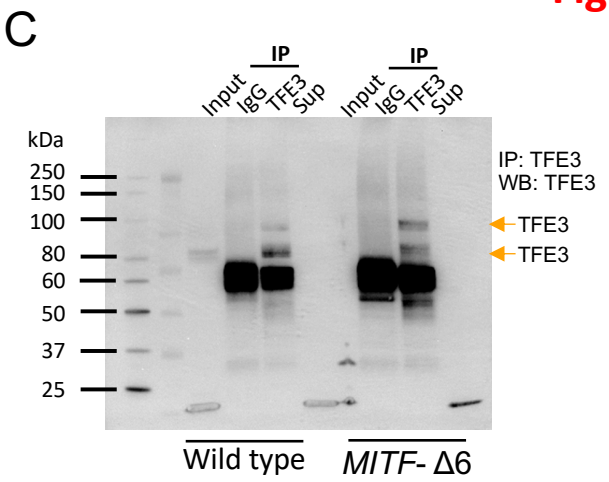
E

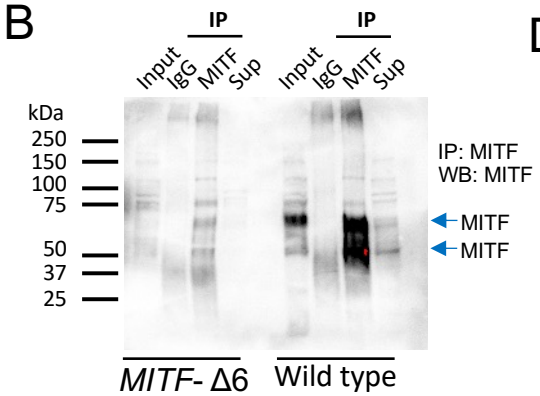
Gained-paralog peaks
Gene Ontology analysis (Panther)

GO	No. of genes	FDR
Regulation of cell migration	266	1.5×10^{-17}
Extracellular matrix organization	87	1.0×10^{-13}
Regulation of metabolic process	1287	2.4×10^{-13}
Regulation of metabolic process	1287	2.4×10^{-13}
Kidney development	97	8.9×10^{-09}
Regulation of MAPK cascade	170	4.5×10^{-08}
Regulation of epithelial to mesenchymal transition	35	3.8×10^{-4}
Wound healing	78	4.0×10^{-03}

Figure S3


A
IP
IP
Input
IgG
MITF
Sup
Input
IgG
MITF
Sup
kDa
250
150
100
75
50
37
25
IP: MITF
WB: TFE3
TFE3
Delta6/MITF
MITF- Δ6
Wild type


C
IP
IP
Input
IgG
TFE3
Sup
Input
IgG
TFE3
Sup
kDa
250
150
100
80
60
50
37
25
IP: TFE3
WB: TFE3
TFE3
TFE3
Wild type
MITF- Δ6


B
IP
IP
Input
IgG
MITF
Sup
Input
IgG
MITF
Sup
kDa
250
150
100
75
50
37
25
IP: MITF
WB: MITF
MITF
MITF
MITF- Δ6
Wild type

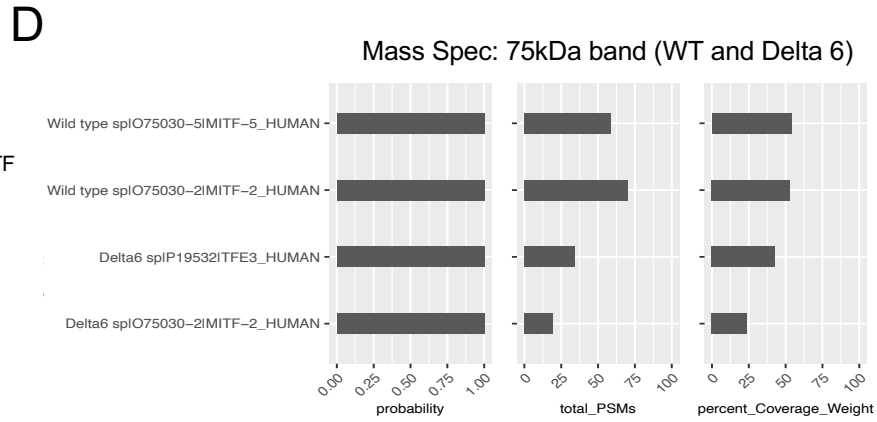
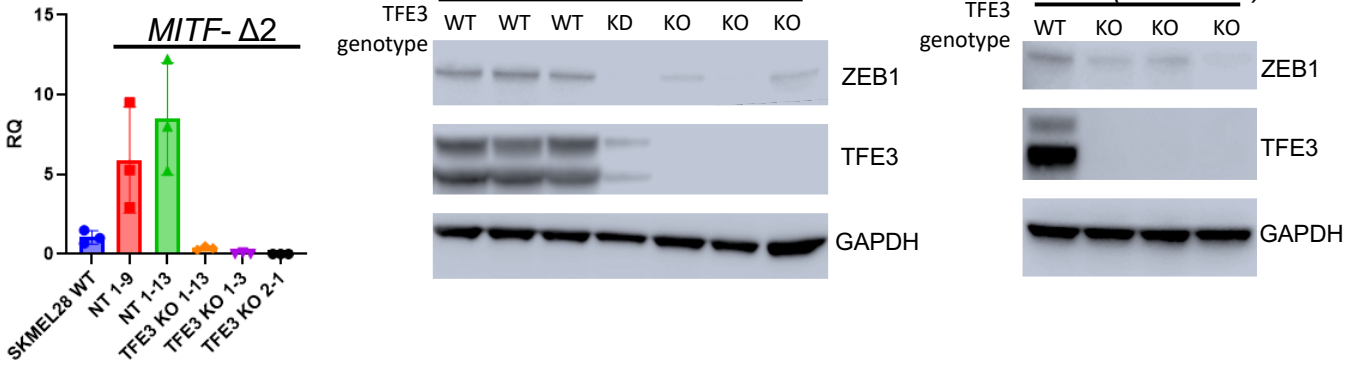

D
Mass Spec: 75kDa band (WT and Delta 6)
Wild type sp|O75030-5|MITF-5_HUMAN
Wild type sp|O75030-2|MITF-2_HUMAN
Delta6 sp|P19532|TFE3_HUMAN
Delta6 sp|O75030-2|MITF-2_HUMAN
0.00
0.25
0.50
0.75
1.00
probability
0
25
50
75
100
total_PSMs
0
25
50
75
100
percent_Coverage_Weight

Figure S4

A



B

Gene Ontology (GO) analysis: TFE3-dependent

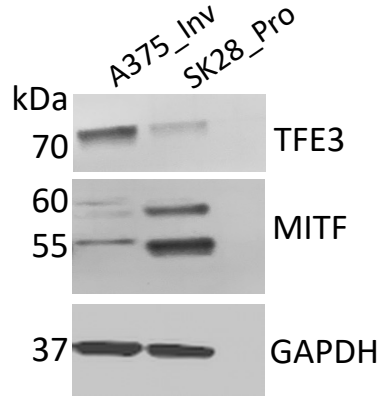
GO	# of genes	FDR
Cell differentiation	129	$1.5x10^{-07}$
Angiogenesis	32	$9.5x10^{-05}$
Cell motility	46	$1.8x10^{-04}$
Cell migration	33	$2.8x10^{-04}$

C

Gene Ontology (GO) analysis: TFE3-independent

GO	# of genes	FDR
Multicellular organismal process	229	$8.1x10^{-11}$
Basement membrane assembly	34	$1.9x10^{-04}$
Regulation of type B pancreatic cell proliferation	33	$2.8x10^{-04}$
Lymphocyte mediated immunity	20	$3.8x10^{-02}$

E



Inv – Invasive cell lines
Pro - Proliferative cell lines

F

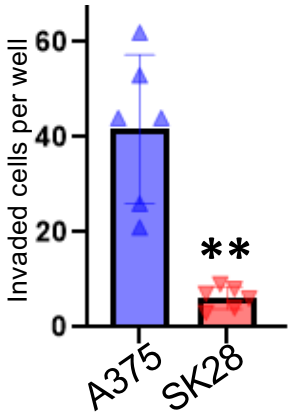
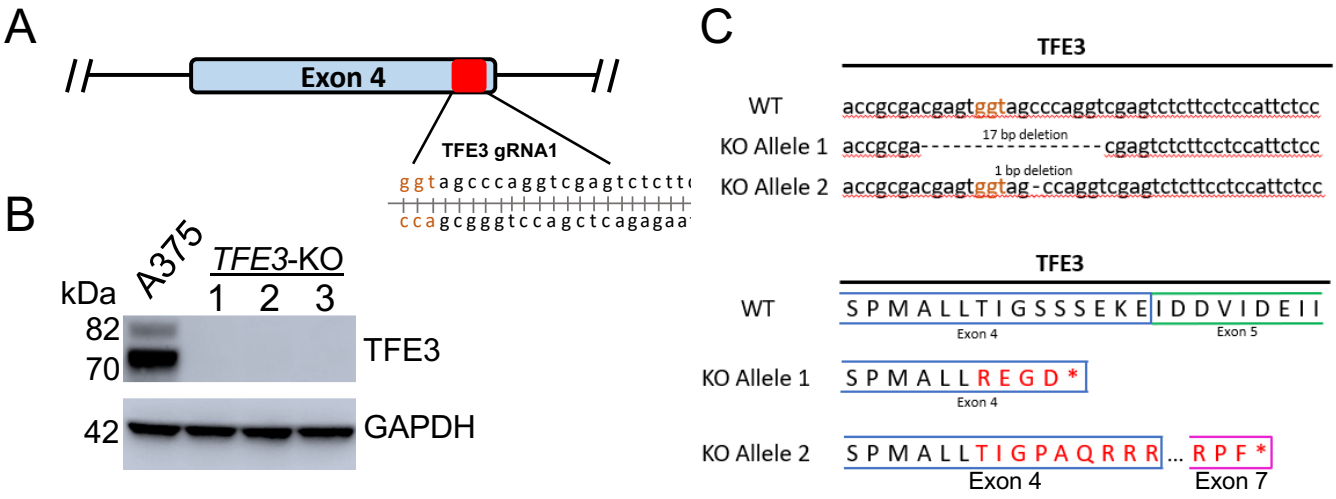


Figure S5


A
Exon 4
TFE3 gRNA1
ggtagcccaggtcgagtctctt
ccagcgggtccagctcagagaa
B
A375
TFE3-KO
1 2 3
kDa
82
70
TFE3
42
GAPDH
C
TFE3
WT
accgcgacgagtggtagcccaggtcgagtctcttcctccattctcc
KO Allele 1
accgcga----------cgagtctcttcctccattctcc
17 bp deletion
KO Allele 2
accgcgacgagtggtag-ccaggtcgagtctcttcctccattctcc
1 bp deletion
TFE3
WT
S P M A L L T I G S S S E K E I D D V I D E I I
Exon 4
Exon 5
KO Allele 1
S P M A L L R E G D *
Exon 4
KO Allele 2
S P M A L L T I G P A Q R R R ... R P F *
Exon 4
Exon 7

D

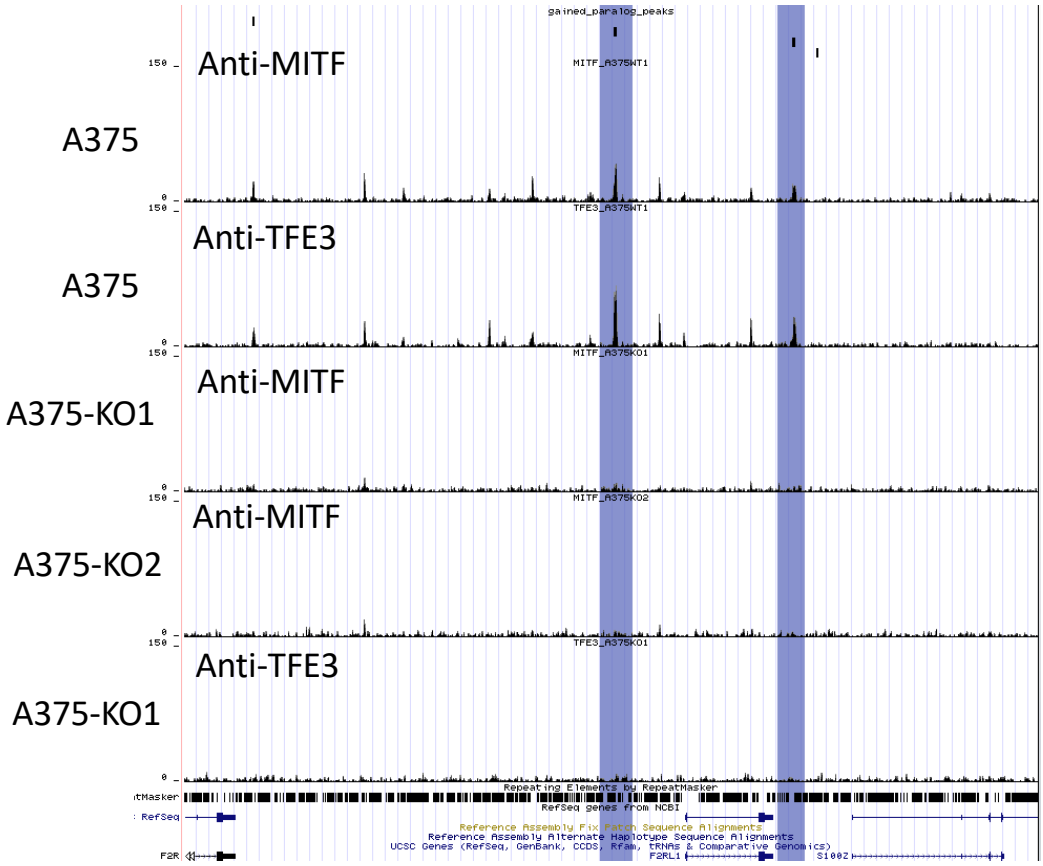

Anti-MITF
A375
Anti-TFE3
A375
Anti-MITF
A375-KO1
Anti-MITF
A375-KO2
Anti-TFE3
A375-KO1
gained_paralog_peaks
MITF_A375WT1
TFE3_A375WT1
MITF_A375KO1
MITF_A375KO2
TFE3_A375KO1
Repeating Elements by RepeatMasker
RefSeq genes from NCBI
Reference Assembly Fix Patch Sequence Alignments
Reference Assembly Alternate Haplotype Sequence Alignments
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)

Figure S6

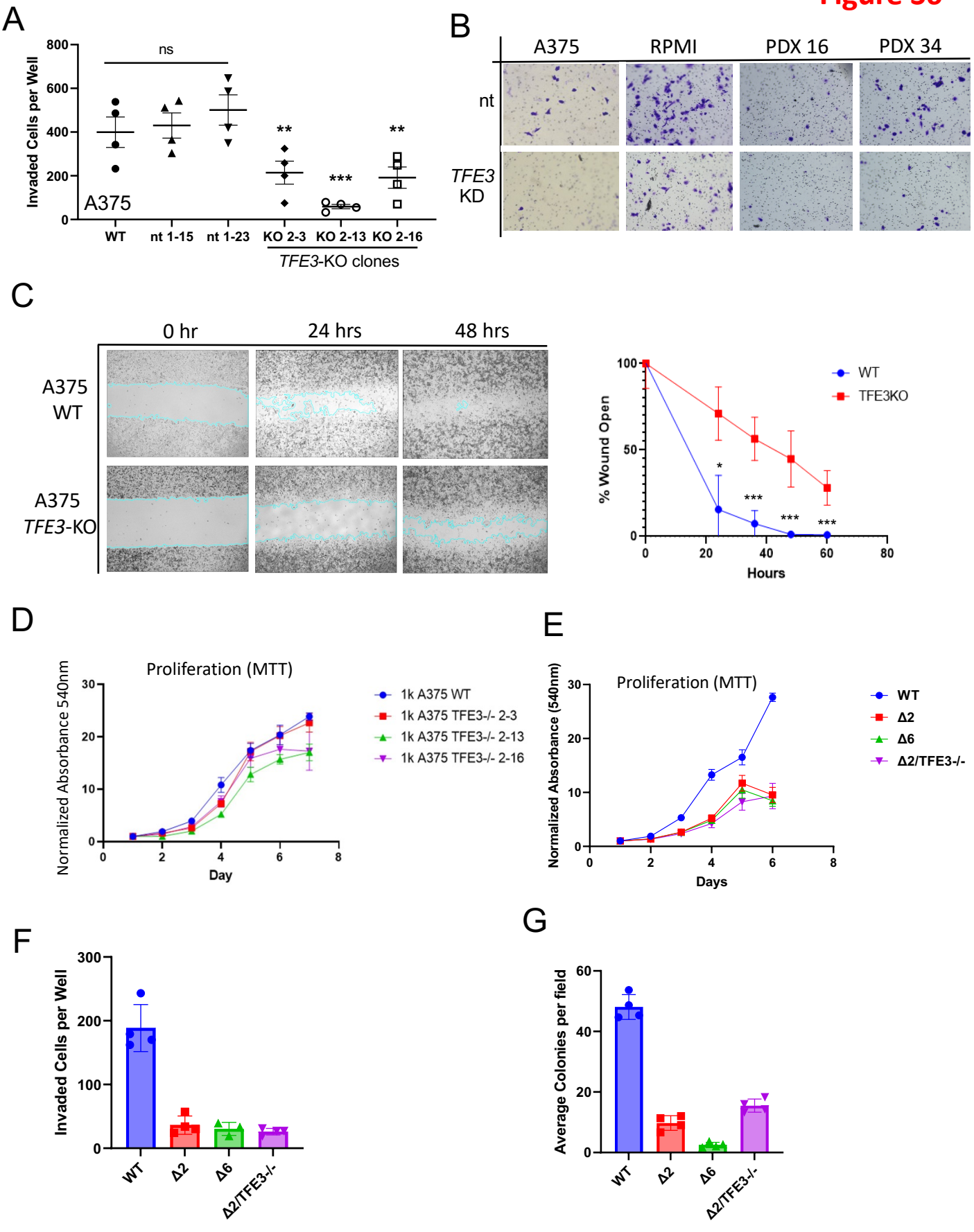
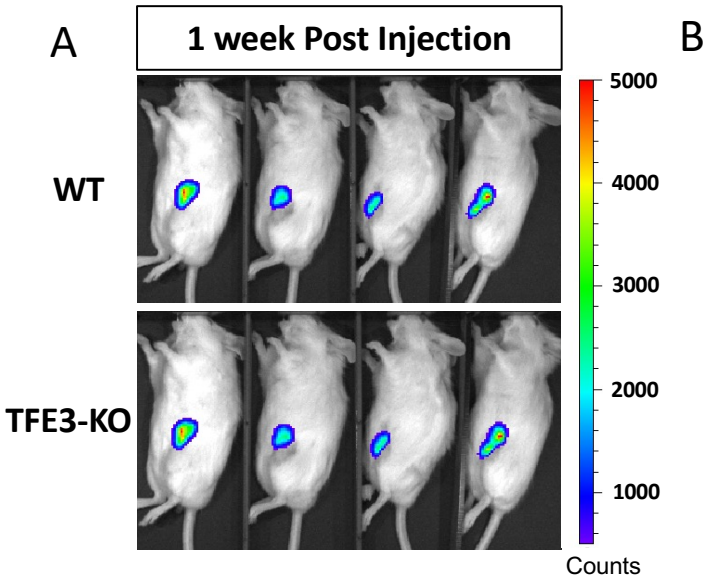

A
Invaded Cells per Well
800
600
400
200
0
ns
**
***
**
A375
WT
nt 1-15
nt 1-23
KO 2-3
KO 2-13
KO 2-16
TFE3-KO clones
B
A375
RPMI
PDX 16
PDX 34
nt
TFE3 KD
C
0 hr
24 hrs
48 hrs
A375 WT
A375 TFE3-KO
% Wound Open
100
50
0
WT
TFE3KO
*
***
***
***
0
20
40
60
80
Hours
D
Proliferation (MTT)
Normalized Absorbance 540nm
30
20
10
0
0
2
4
6
8
Day
1k A375 WT
1k A375 TFE3-/- 2-3
1k A375 TFE3-/- 2-13
1k A375 TFE3-/- 2-16
E
Proliferation (MTT)
Normalized Absorbance (540nm)
30
20
10
0
0
2
4
6
8
Days
WT
Δ2
Δ6
Δ2/TFE3-/-
F
Invaded Cells per Well
300
200
100
0
WT
Δ2
Δ6
Δ2/TFE3-/-
G
Average Colonies per field
60
40
20
0
WT
Δ2
Δ6
Δ2/TFE3-/-

Figure S7


A
1 week Post Injection
WT
TFE3-KO
5000
4000
3000
2000
1000
Counts

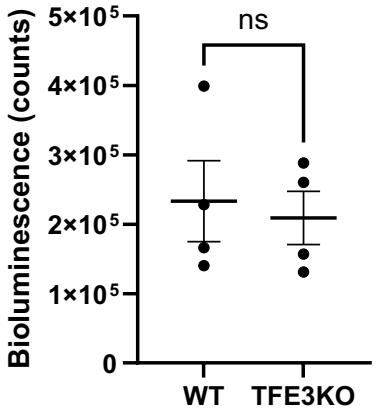
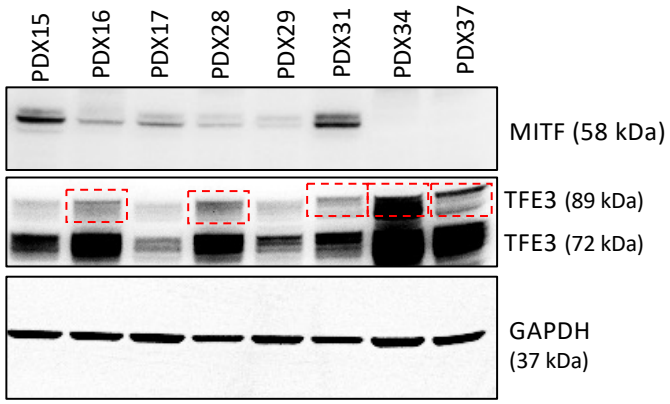
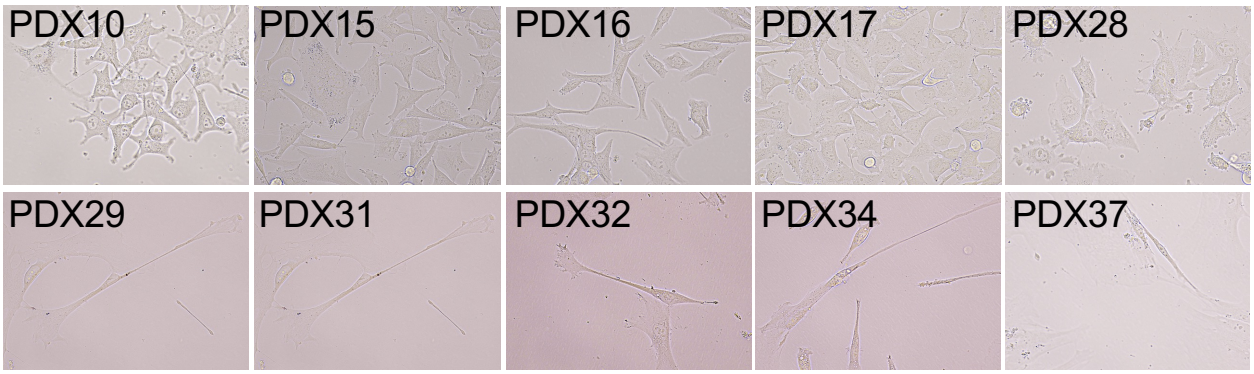

B
Bioluminescence (counts)
5×10⁵
4×10⁵
3×10⁵
2×10⁵
1×10⁵
0
ns
WT
TFE3KO

Figure S8

A


PDX15
PDX16
PDX17
PDX28
PDX29
PDX31
PDX34
PDX37
MITF (58 kDa)
TFE3 (89 kDa)
TFE3 (72 kDa)
GAPDH
(37 kDa)

B


PDX10
PDX15
PDX16
PDX17
PDX28
PDX29
PDX31
PDX32
PDX34
PDX37