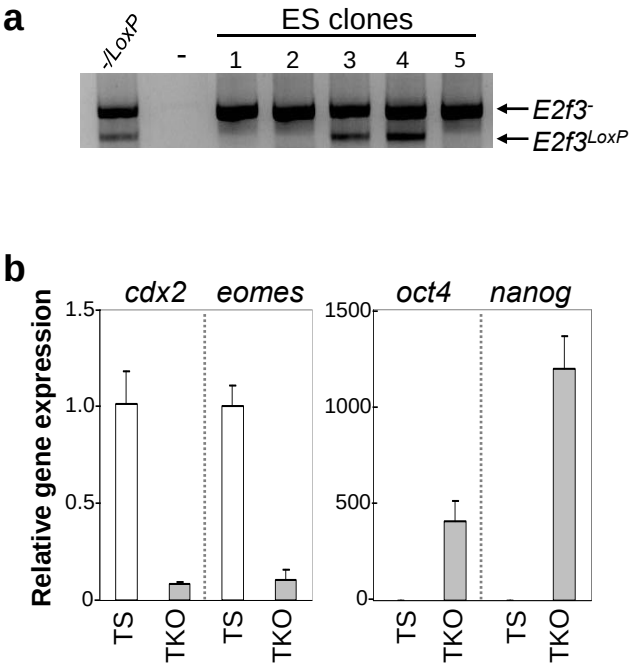
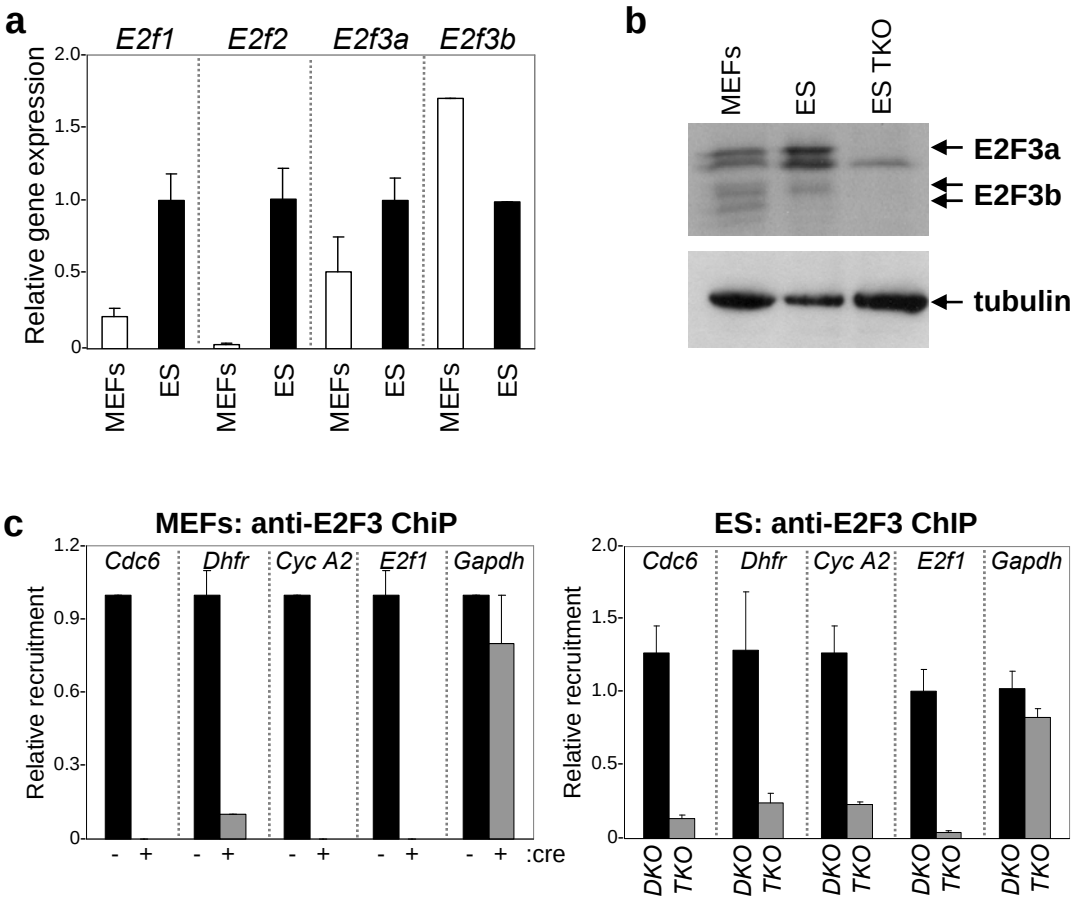
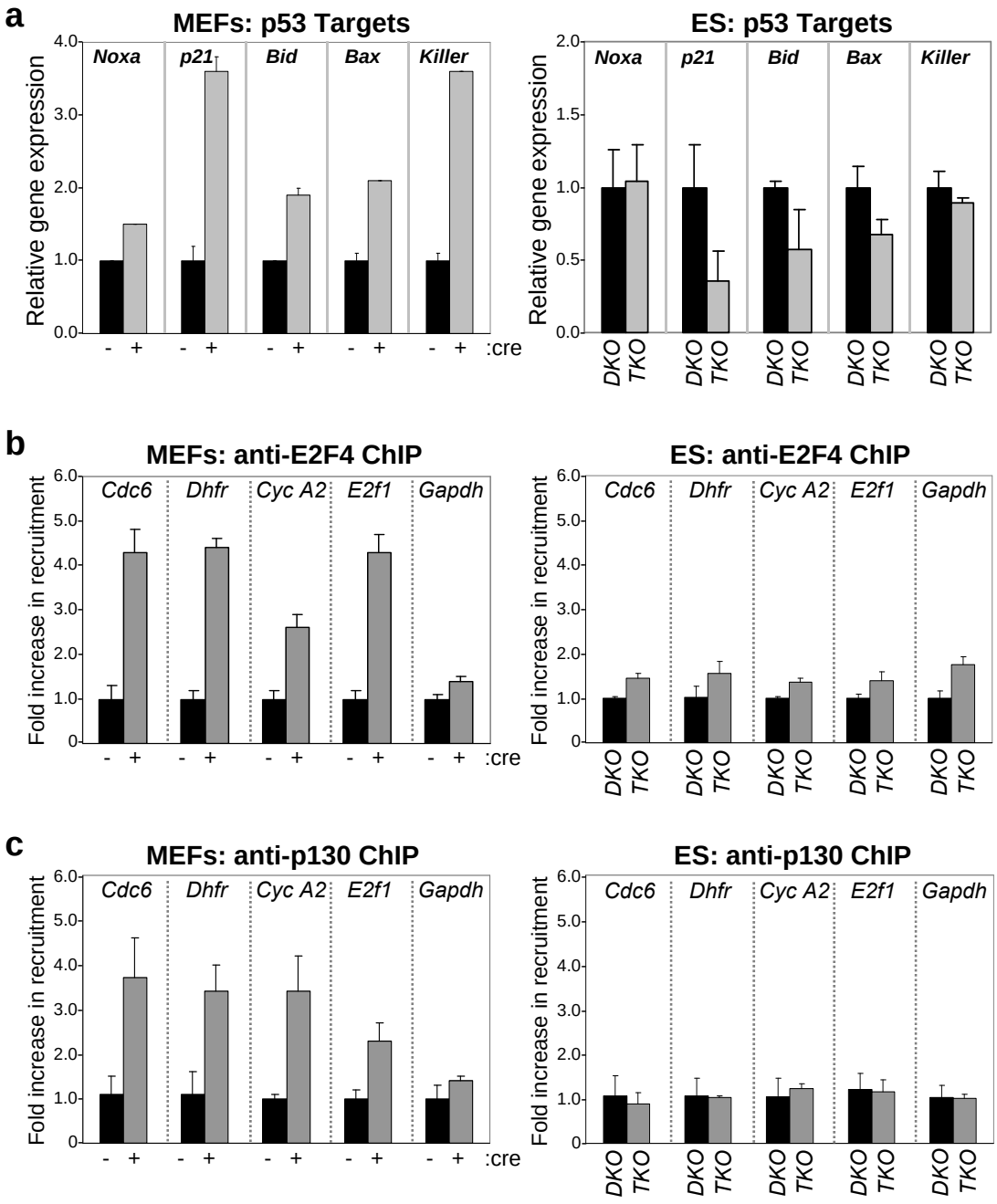

a
-/LoxP
-
ES clones
1
2
3
4
5
E2f3-
E2f3LoxP
b
Relative gene expression
cdx2
eomes
oct4
nanog
1.5
1.0
0.5
0
1500
1000
500
0
TS
TKO
TS
TKO
TS
TKO
TS
TKO


a
E2f1
E2f2
E2f3a
E2f3b
Relative gene expression
2.0
1.5
1.0
0.5
0
MEFs
ES
b
MEFs
ES
ES TKO
E2F3a
E2F3b
tubulin
c
MEFs: anti-E2F3 ChiP
Cdc6
Dhfr
Cyc A2
E2f1
Gapdh
Relative recruitment
1.2
0.9
0.6
0.3
0
- + :cre
ES: anti-E2F3 ChIP
2.0
1.5
1.0
0.5
DKO
TKO

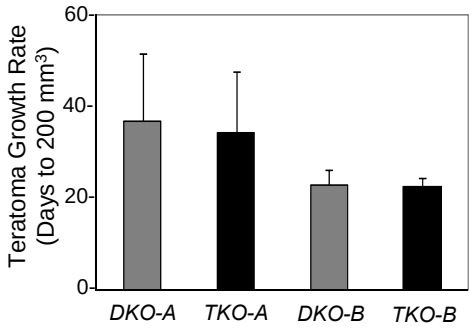

a
MEFs: p53 Targets
ES: p53 Targets
Relative gene expression
Noxa
p21
Bid
Bax
Killer
- + - + - + - + - + :cre
DKO TKO
b
MEFs: anti-E2F4 ChIP
ES: anti-E2F4 ChIP
Fold increase in recruitment
Cdc6
Dhfr
Cyc A2
E2f1
Gapdh
c
MEFs: anti-p130 ChIP
ES: anti-p130 ChIP

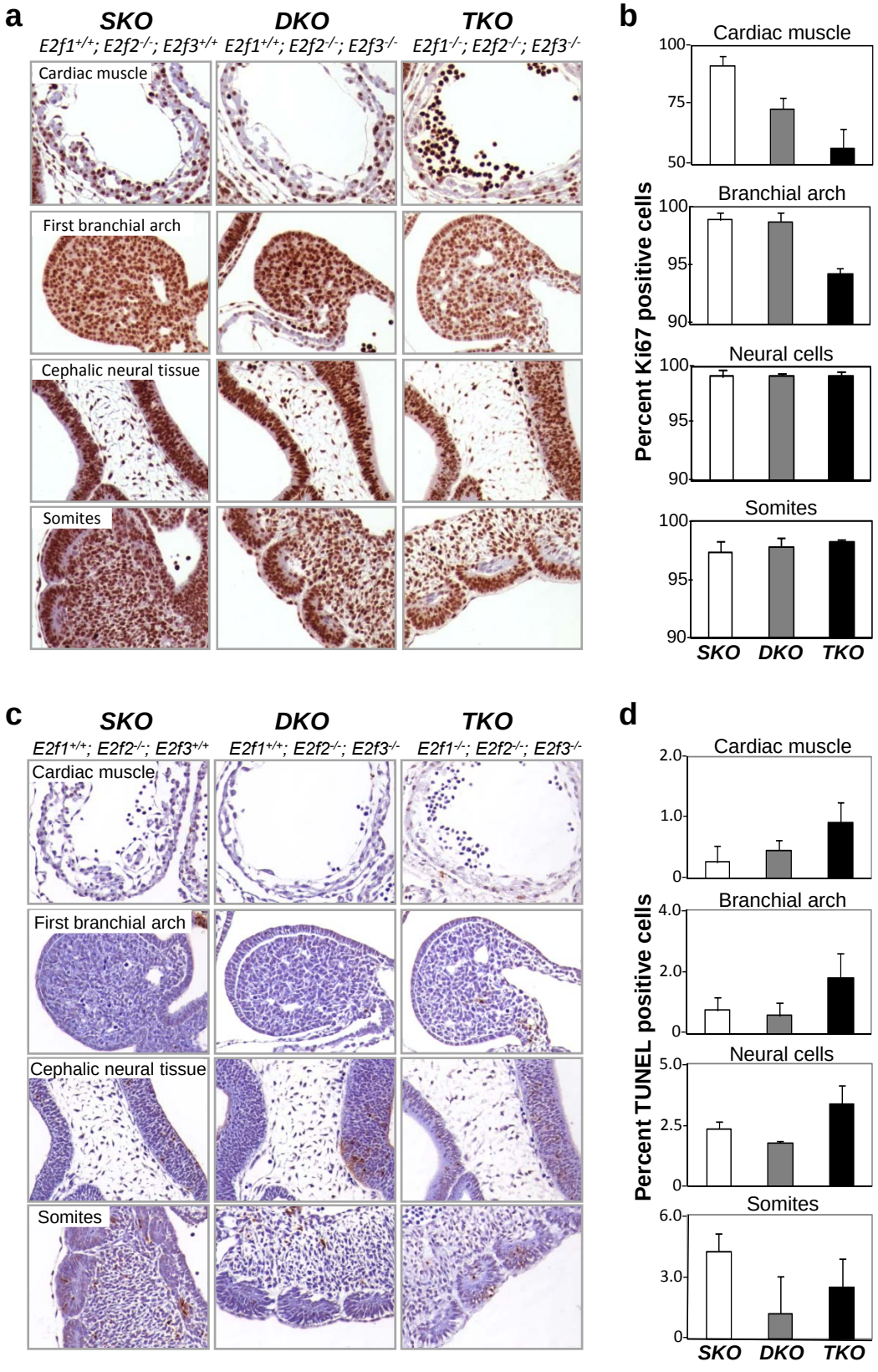
a

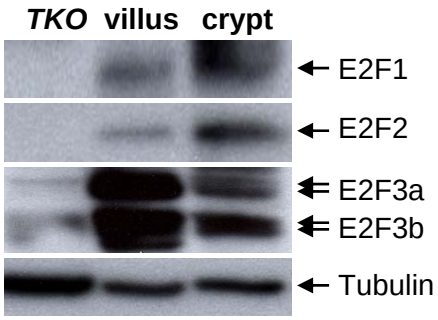
Lineages present in teratomas derived from various ES cell lines

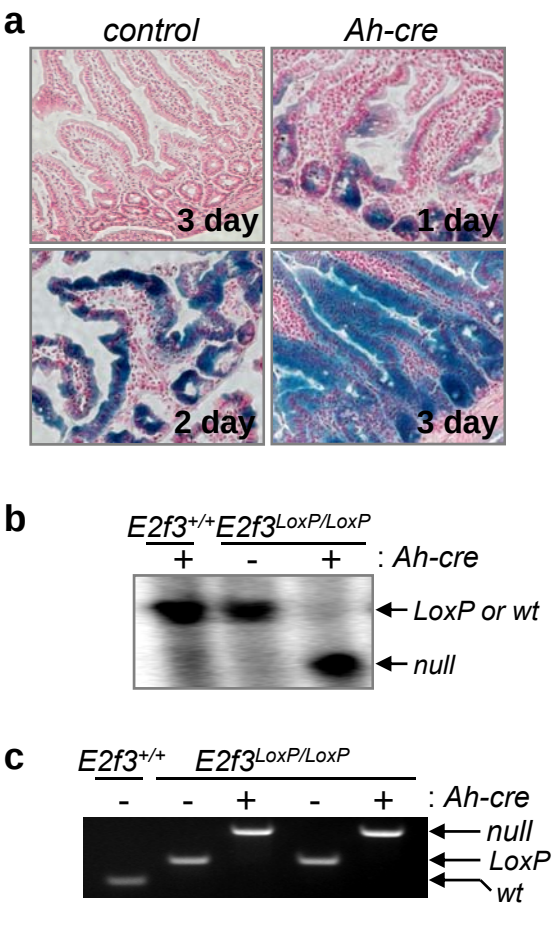
	TC-1	DKO	TKO
Total # of teratomas	7	12	17
Neural	7	10	15
Muscle	7	11	17
Epithelial	7	12	13
Skin	1	7	3
Inflammatory	7	12	10

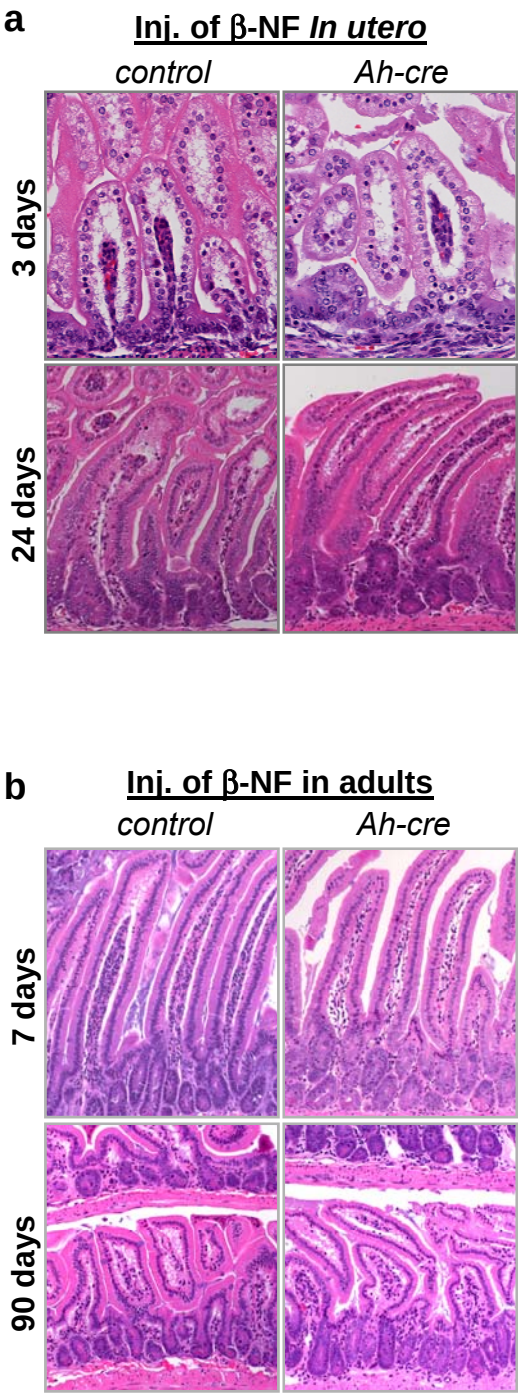
b

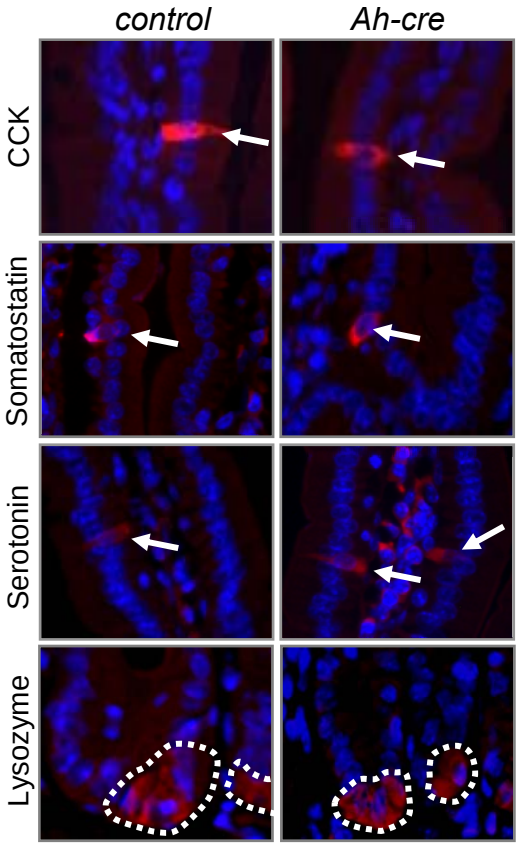


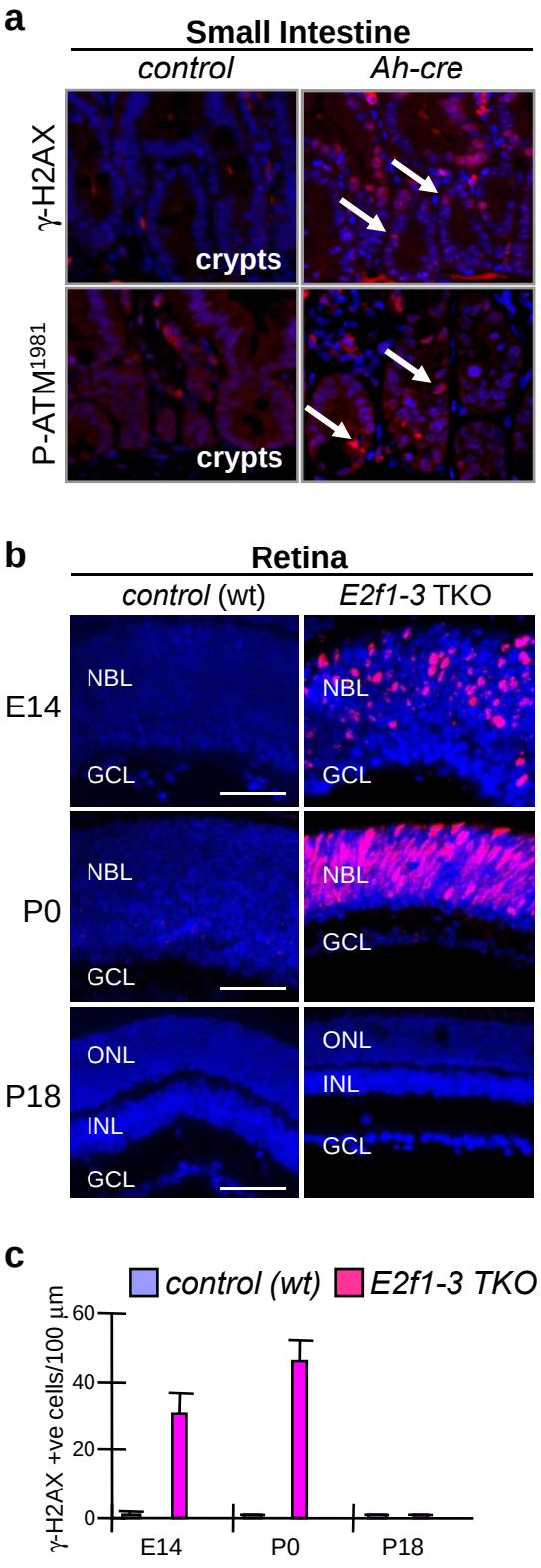

a
SKO
DKO
TKO
E2f1+/+; E2f2-/-; E2f3+/+
E2f1+/+; E2f2-/-; E2f3-/-
E2f1-/-; E2f2-/-; E2f3-/-
Cardiac muscle
First branchial arch
Cephalic neural tissue
Somites
b
Cardiac muscle
Branchial arch
Neural cells
Somites
Percent Ki67 positive cells
100
95
90
75
50
SKO
DKO
TKO
c
SKO
DKO
TKO
E2f1+/+; E2f2-/-; E2f3+/+
E2f1+/+; E2f2-/-; E2f3-/-
E2f1-/-; E2f2-/-; E2f3-/-
Cardiac muscle
First branchial arch
Cephalic neural tissue
Somites
d
Cardiac muscle
Branchial arch
Neural cells
Somites
Percent TUNEL positive cells
2.0
1.0
0
4.0
2.0
5.0
2.5
6.0
3.0
SKO
DKO
TKO

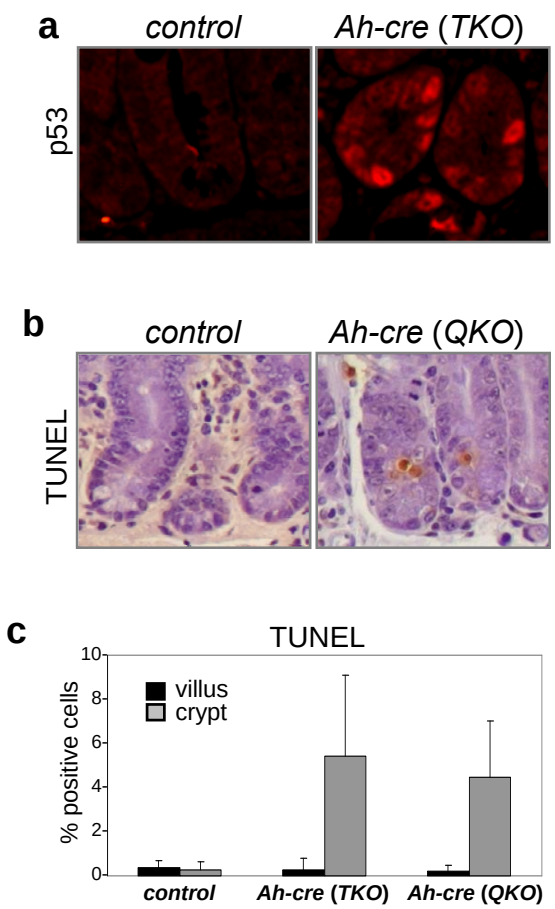

TKO
villus
crypt
E2F1
E2F2
E2F3a
E2F3b
Tubulin


a
control
Ah-cre
3 day
1 day
2 day
3 day
b
E2f3+/+ E2f3LoxP/LoxP
+ - + : Ah-cre
LoxP or wt
null
c
E2f3+/+ E2f3LoxP/LoxP
- - + - + : Ah-cre
null
LoxP
wt
d
Villus
Crypt
Relative gene expression
E2f4
E2f5
E2f6
E2f7
E2f8
con
Ah-cre
con
Ah-cre

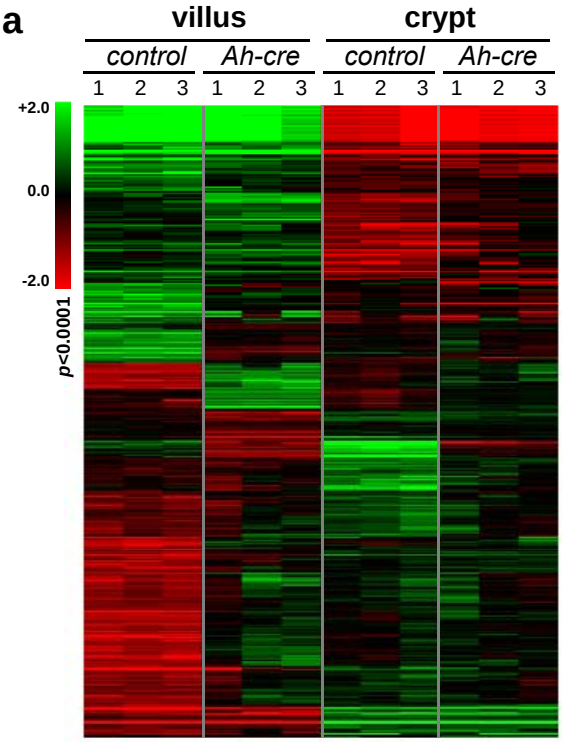

a
Inj. of β-NF *In utero*
control
Ah-cre
3 days
24 days
b
Inj. of β-NF in adults
control
Ah-cre
7 days
90 days


control
Ah-cre
CCK
Somatostatin
Serotonin
Lysozyme


a
Small Intestine
control
Ah-cre
γ-H2AX
P-ATM1981
crypts
crypts
b
Retina
control (wt)
E2f1-3 TKO
E14
P0
P18
NBL
GCL
ONL
INL
c
control (wt)
E2f1-3 TKO
γ-H2AX +ve cells/100 µm
0
20
40
60
E14
P0
P18

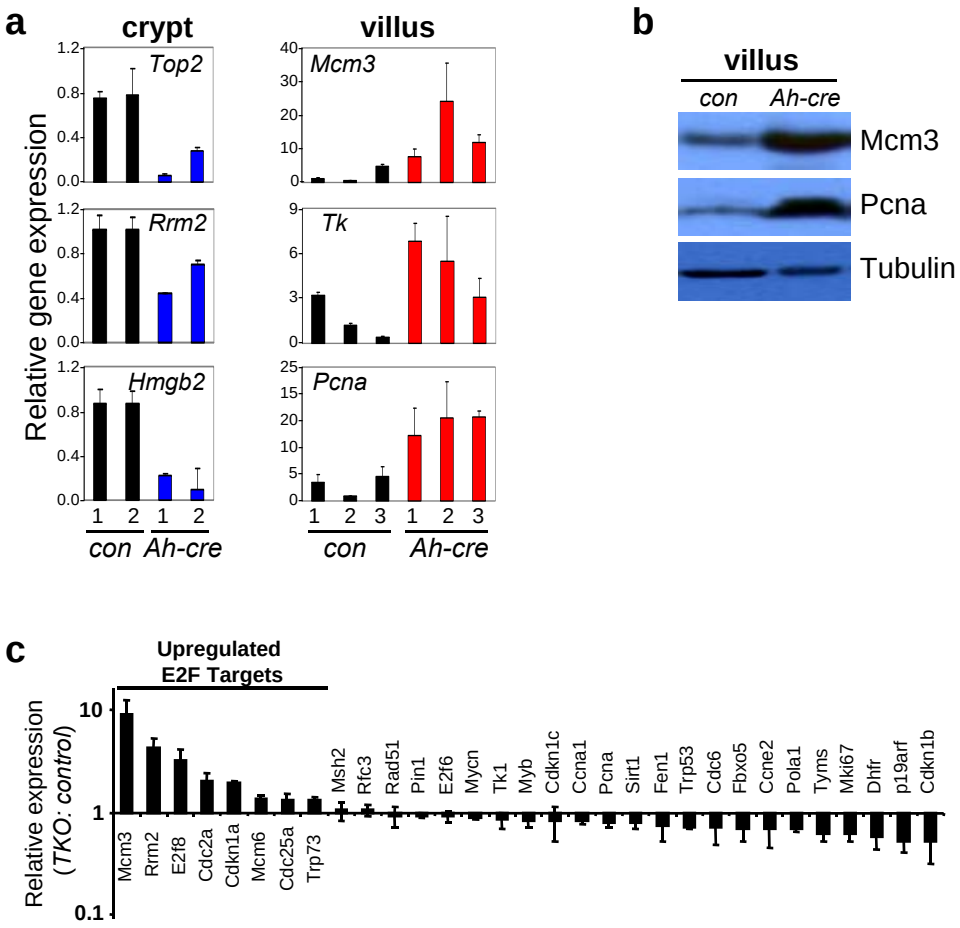

a
control
Ah-cre (TKO)
p53
b
control
Ah-cre (QKO)
TUNEL
c
TUNEL
% positive cells
villus
crypt
0
2
4
6
8
10
control
Ah-cre (TKO)
Ah-cre (QKO)

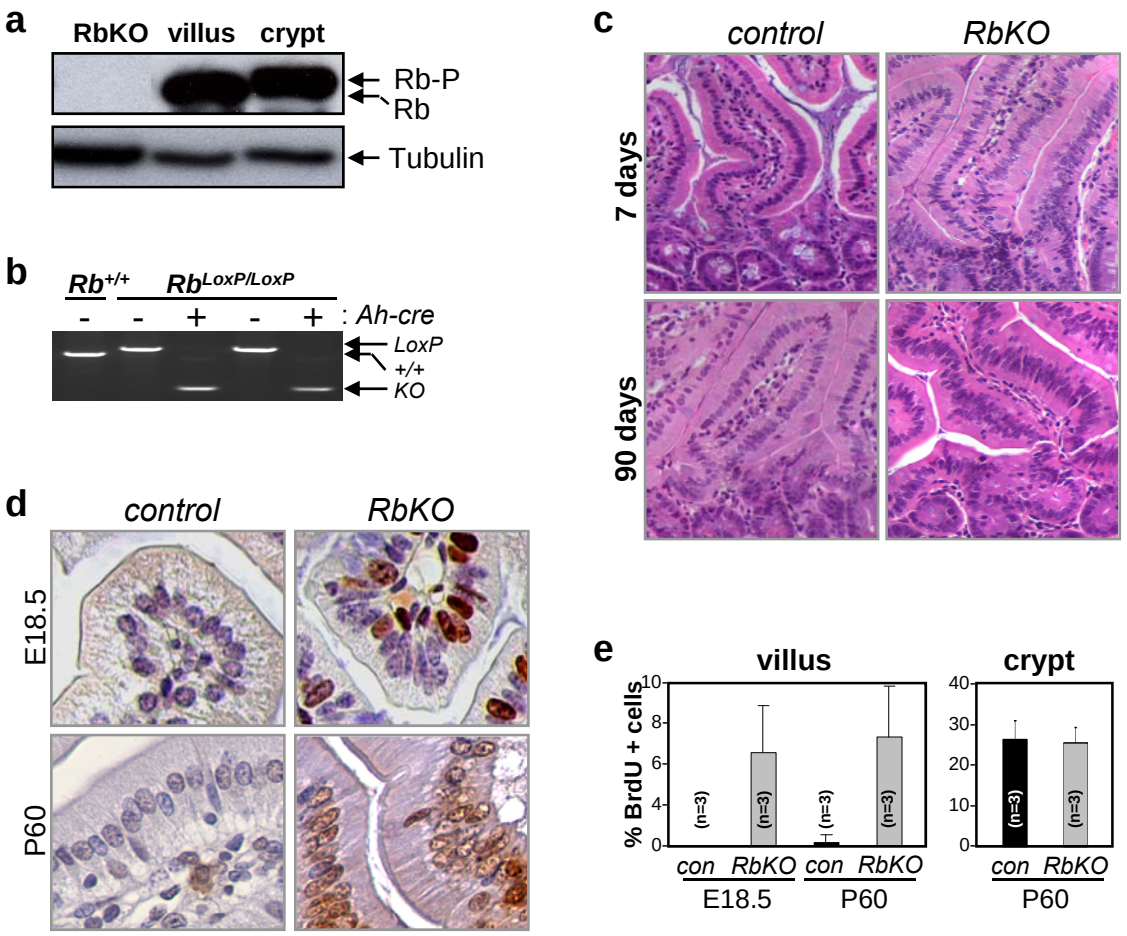
a

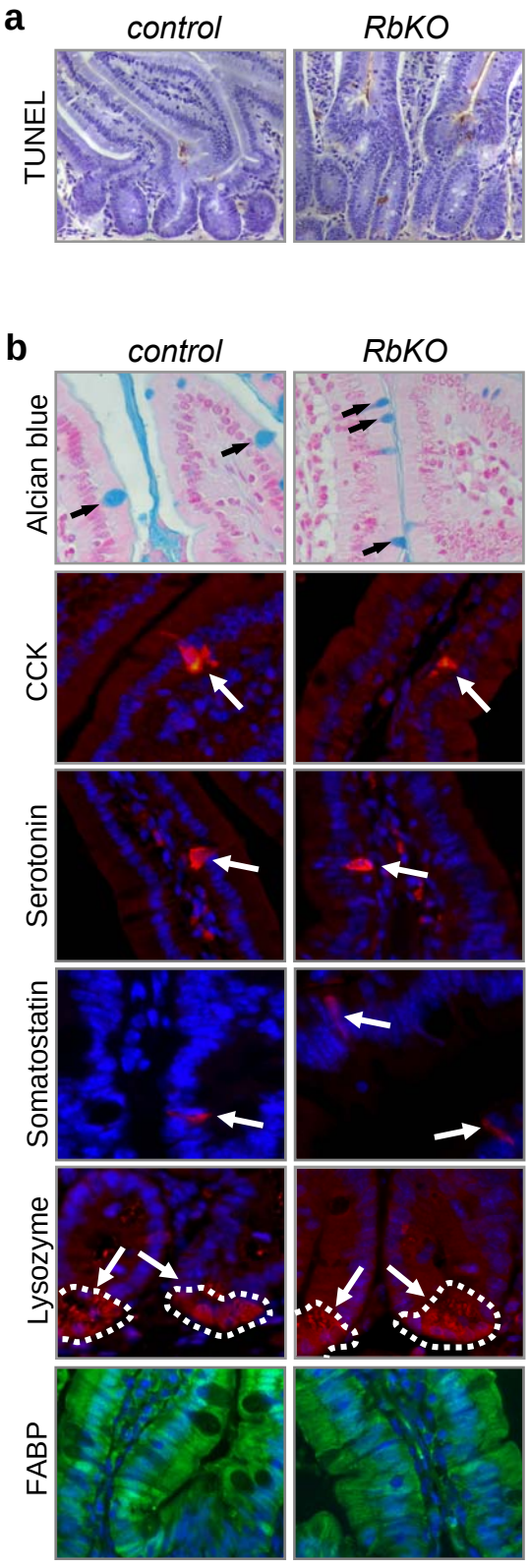


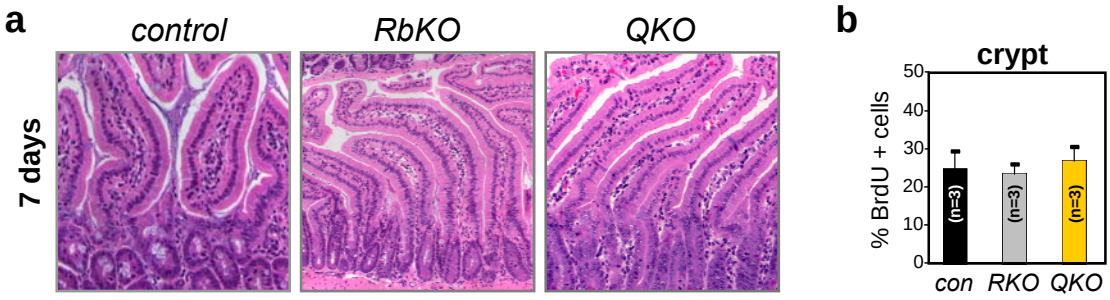
b

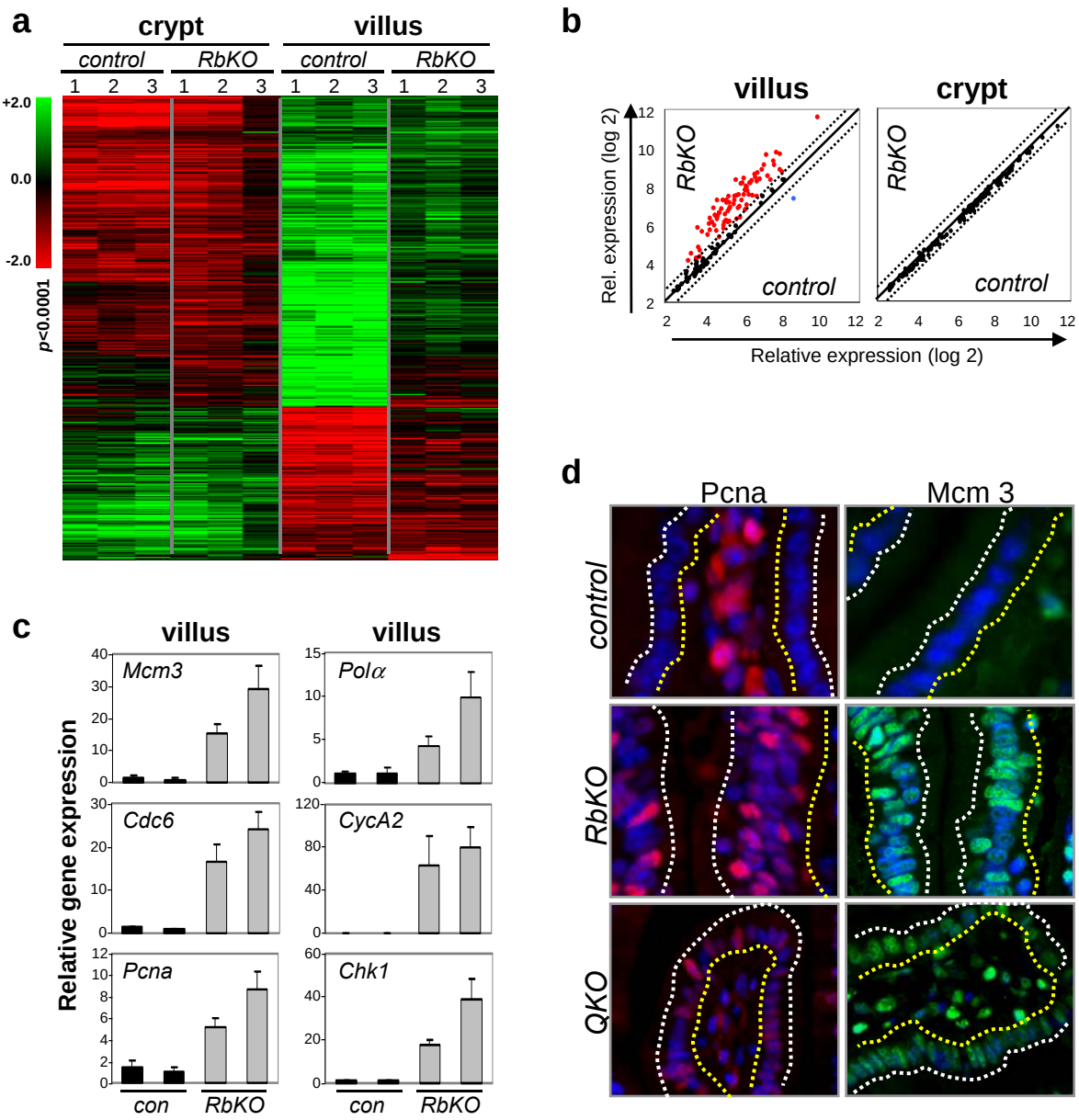
Validated E2F target genes (regulated and bound by E2F transcription factors)					
ASF1B	CDC25A	E2F3	LMNB1	PLK1	RRM1
BARD1	CDC6	FANCL	MAP3K5	POLD3	SERPINE1
BLM	CDC7	FEN1	MCM3	PRC1	SMC2
BNIP3	CDCA3	H2AFX	MKI67	RACGAP1	SSX2IP
BOK	CDCA5	HIST1H2AD	NASP	RAD51	TK1
BRCA1	CENPF	HMGB2	NRP1	RAD51AP1	TMPO
BUB1	CENPK	HMMR	NUF2	RAD54L	TOP2A
BUB1B	DLG7	IQGAP3	PBK	RBL1	TPX2
CCDC5	DNA2L	KIF20A	PBX3	RFC3	TYMS
CCNA2	E2F1	KIF2C	PCNA	RFC4	UBE2C

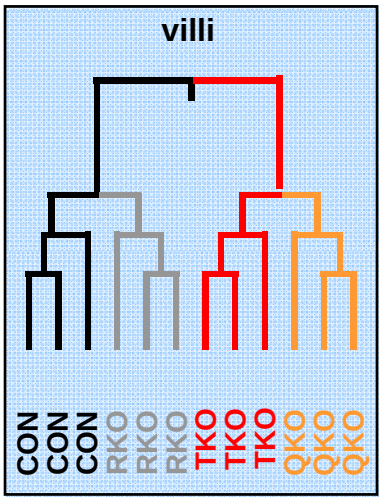

a
crypt
villus
Relative gene expression
Top2
Mcm3
Rrm2
Tk
Hmgb2
Pcna
con
Ah-cre
b
villus
con
Ah-cre
Mcm3
Pcna
Tubulin
c
Upregulated
E2F Targets
Relative expression
(TKO: control)
Mcm3
Rrm2
E2f8
Cdc2a
Cdkn1a
Mcm6
Cdc25a
Trp73
Msh2
Rfc3
Rad51
Pin1
E2f6
Mycn
Tk1
Myb
Cdkn1c
Ccna1
Pcna
Sirt1
Fen1
Trp53
Cdc6
Fbxo5
Ccne2
Pola1
Tyms
Mki67
Dhfr
p19arf
Cdkn1b


a
RbKO villus crypt
Rb-P
Rb
Tubulin
b
Rb+/+
RbLoxP/LoxP
- - + - + : Ah-cre
LoxP
+/+
KO
c
control
RbKO
7 days
90 days
d
control
RbKO
E18.5
P60
e
villus
crypt
% BrdU + cells
(n=3)
con RbKO con RbKO
E18.5 P60
con RbKO
P60


a
control
RbKO
TUNEL
b
control
RbKO
Alcian blue
CCK
Serotonin
Somatostatin
Lysozyme
FABP


a
control
RbKO
QKO
7 days
b
crypt
% BrdU + cells
50
40
30
20
10
0
(n=3)
(n=3)
(n=3)
con
RKO
QKO


a
crypt
villus
control
RbKO
control
RbKO
1 2 3 1 2 3 1 2 3 1 2 3
+2.0
0.0
-2.0
p<0.0001
b
villus
crypt
Rel. expression (log 2)
RbKO
control
RbKO
control
2 4 6 8 10 12
Relative expression (log 2)
c
villus
villus
Relative gene expression
Mcm3
Polα
Cdc6
CycA2
Pcna
Chk1
con
RbKO
con
RbKO
d
Pcna
Mcm 3
control
RbKO
QKO


villi
CON
CON
CON
RKO
RKO
RKO
TKO
TKO
TKO
QKO
QKO
QKO

a

Primers used for genotyping

Gene	Sense primer(s)	Antisense primer(s)
Ah-cre	CCTGACTAGCATGGCGATAC	ATTGCCCCTGTTTCA CTATC
Rb	GGCGTGTGCCATCAA TG	CTCAAGAGCTCAGACTCATGG
ROSA26 Rep	AAAGTCGCTCTGAGTTGTTAT	GGAGCGGGAGAAATGGATAT
		GCGAAGAGTTTGTCCTCAACC
E2f1	AGAAGTCACGCTATGAAACCTCAC	AGCCACTGGATATGATTCTTGGAC
	AGTGCCAGCGGGGCTGCTAAAG	
E2f2	CCTGAGCGAGTCGGAGGATGG	GCCCCTAACACATGCACCCATTGG
	ACCAAAGAACGGAGCCGGTTGGCG	
E2f3	TGTGAATAATTTTTGGCATGTTTT	AAGGGAAGGGAAAATTAAATCTGA
		CTTATTCTGAGTGTGGACATACCG

b

Primers used for chromatin immunoprecipitation

Gene	Sense primer(s)	Antisense primer(s)
Cdc6	TGATGAGTGACAACTAATCAG	GAGCTTTGCACTCTTCAGG
CycA2	TGTAAGATTCCCGTCGGGCCTTC	AGGCGGGAGGAGCGTAGAGCC
CycE1	AAGAACACGCCCCCCGGGAGGCCAC	AAGCTGTGTCCGCCGCAGGCAGGCG
Mcm3	GTCTCTGCTCCATGCTTCCTCCAC	GCTTCCCGCAGCTCCACATCATC

c

Antibodies used in this study

name	company	Cat #
Anti-Mcm3	Santa Crus	sc-9850
Anti-Pcna	Santa Crus	sc-56
Anti-Atm-ps1981	NOVUS	NB600-622
Anti-Phospho-H2AX (ser139)	Upstate	05-636
Anti-E2F3	Santa Crus	sc-878
Anti-Rb	BDPharmingen	554136
Anti-Cholecystokinin	Immunostar	20078
Anti-L-Fabp	Santa Crus	sc-16064
Anti-lysozyme	Dako	A0099
Anti-serotonin	Dako	M0758
Anti-somatostatin	Dako	A0566
anti-cleaved caspase-3	Cell Signaling	9661-L
anti-phosphorylated histone H3	Millipore	06-570

Primers used for real-time RT-PCR

Gene	Sense primer(s)	Antisense primer(s)
Mcm3	CGCAGGAAGAATGAAAAGAGGG	CTGAGGAAGCAGGAAGTGAGAGTC
Cdc6	AGTTCTGTGCCCGCAAAGTG	AGCAGCAAAGAGCAAACCAGG
CycE1	GAGCAGGAGACAGAATGACCAAAC	ACAGCAACCTACAACACCCGAG
Dhfr	GTAGAGAACTCAAAGAACCACCACG	TTTTCCTCCTGGACCTCAGAGAG
Polα	TCTTCCCCTTCATTTCTCCCG	TCTTCCCCTTCATTTCTCCCG
Pcna	AGGCTCTCAAAGACCTCATCAATG	CCTGTTCTGGGATTCCAAGTTG
CycA2	CCCCCAGAAGTAGCAGACTTTGTG	TGTGACTGTGTAGAGAGCCAAGTGG
p19^{ARF}	CACCGGAATCCTGGACCAG	GCAGTTCGAATCTGCACCGT
p21	CTTGTCGCTGTCTTGCACTC	CTCCTGACCCACAGCAGAAG
Rrm2	AGAGGATGGGAGTCATGTCG	CTTTTAATGGCAAGGGGACA
Hmgb2	CGCGGAGAACTCTGCAAAACAAG	CGAAGAAGGCGTACGAGGACATT
top 2	CACCGCTGCAGCCTGTAAATGA	CACATTTGCTGGGTCACTAACTCCAC
GAPDH	CGGTGTGAACGGATTTGGC	TTTGATGTTAGTGGGGTCTCGC
E2f4	CTTCTACCTCCTTTGAGCCCATC	TCACAGACACCTTCACTCTCGTCC
E2f5	ACCTGATGACCTCACACAGCCTTC	GGGGTAGGAGAAAGCCGTAAAAG
E2f6	CATCCACAATGCCCAGAGAAAG	CAATAGAGCACAAGAGCACTCCG
E2f7	GCCAAGCAGGAAACAGAAGA	ACCGTGCCAACCATACTGAT
E2f8	GAGAAATCCCAGCCGAGTC	CATAAATCCGCCGACGTT
Cdc2a	ATGATCCTGCCAAACGAATC	TCCATCCAGAGGGCTACATC
Cdkn1a	GTGGCCTTGTCGCTGTCTT	GCGCTTGGAGTGATAGAAATCTG
Mcm6	CTGAAAGGCTCCAGTGAAGG	GTCCCTGGCCATCAAGTTAC
Trp73	GCCCATCAAAGAGGAGTTCA	TCCCACTTCCAAGAGCAGTT
Cdc25a	AGTCGCCTGAAGAAGCTCTG	CCCCTTTGCTTACTGCTCTG
Msh2	TCAAGCAGGTGCCCTTTACT	GTTGTTCTTTGCGACCACCT
Rfc3	GTGGCGAAGTTTATGGCACT	CATCTGTTGTGCTTGGAGGA
Rad51	TGGGGCAGTCTTTTGTCTTC	ACCACCCATTCCCACTTACA
Pin1	AAAGCAGACGCTCCATACCTG	CCAGATTTAATGGAAGGTGCG
Myc	CAGATCAGCAACAACCGCAA	GACGTTGTGTGTCCGCCTCT
Myb	GCGTTCTCAGCTCGAACTCT	GAGCCATGTGTCGAAAGTCA
Sirt1	GGAGTCAGCACCGTGTCTGG	GGGCCTGTTTGGACATTACCA
Fen1	TCCTGATTGCTGTTCGTCAG	ATGCGGATGGTACGGTAGAA
Fbox5	CAAACGGGAAAGCTGTCAAT	GGCAAAGGACCCACTTTACA
Ccne2	TGTTCACAGGAGGTTTGGCA	GGTTCCAGGTCAGAATGCAGA
Tyms	CGTGCAGGATAGGGTGAAGT	TGGAGCCTTCCTCCCTAGAT
Ki67	GAGTGAGAGGGCAGTTCTGG	GCTGCAGTAACTGTGGGTCA
Cdkn1b	TCTCTTCGGCCCGGTCAAT	GGGGCTTATGATTCTGAAAGTCG