SUPPLEMENTAL INFORMATION for

The nuclear receptor NR2E1/TLX controls senescence

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Including:

Supplemental Table 1. Primary antibodies used in this study.

Target	**Reference**
NR2E1	H6506, R&D systems
CBX6	Ref. 44
CBX7	Ab21873, Abcam
CBX8	A300-882A, Bethyl Laboratories
FLAG	M2, Sigma
BrdU	A21303, Invitrogen
$p16^{INK4a}$	JC-8, CRUK
$p21^{CIP1}$	CP74, Sigma
β-Actin	sc-47778, Santa Cruz Biotechnology
α-Tubulin	T6074, Sigma

Supplemental Table 2. Primers for qRT-PCR and Taqman probes used in this study.

Primers for qPCR

Human

Target	Forward primer	Reverse primer
p16	CGGTCGGAGGCCGATCCAG	GCGCCGTGGAGCAGCAGCAGCT
p21	CCTGTCACTGTCTTGTACCCT	GCGTTTGGAGTGGTAGAAATCT
RPS14	TCACCGCCCTACACATCAAACT	CTGCGAGTGCTGTCAGAGG

Mouse

Target	Forward primer	Reverse primer
p16	GTGTGCATGACGTGCGGG	GCAGTTCGAATCTGCACCGTAG
p21	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
Rps14	GACCAAGACCCCTGGACCT	CCCCTTTTCTTCGAGTGCTA

TaqMAN probes

Human

Target	Applied Biosystems Reference
CBX2	Hs01034268_m1
CBX4	Hs01106873_m1
CBX6	Hs00982441_g1
CBX7	Hs00545603_m1
CBX8	Hs00221034_m1
NR2E1	Hs01128417_m1
TBP	4333769F
RNU6B	1093

Mouse

Target	Applied Biosystems Reference
Cbx7	Mm00520005_m1
Nr2e1	Mm00455855_m1
Tbp	Mm00446971_m1

Supplemental Table 3. Sequence of siRNA and shRNA targeting NR2E1 used in this study.

siRNA

	Reference	Target sequence
Human		
siNR2E1.2	SI00050106	TCCCGTTAACATAGTGCTGAA
Mouse		
siNr2e1.4	SI00239400	TTGGGTATGAATCTATACTTA
siNr2e1.5	SI02698094	TACCAGCTTTACGGTCAATTA

shRNA

Human

shNR2E1.2
GATCCCCCAGAACTGAGTTAATAAGTGATTCAAGAGATCACTTATTAACTCAGTTCTGTTTTTA
shNR2E1.3
GATCCCCTCCCGTTAACATAGTGCTGAATTCAAGAGATTCAGCACTATGTTAACGGGATTTTTA

Mouse

shNr2e1.4
CCGGCGTGGACACAAGGAAGACAATCTCGAGATTGTCTTCCTTGTGTCCACGTTTTT
shNr2e1.5
CCGGCCGGTTGATGCTAACACTCTACTCGAGTAGAGTGTTAGCATCAACCGGTTTTT

Supplemental Table 4. Primers sets used for ChIP analysis.

Primer	Forward primer	Reverse primer
CBX7		
PS2	GAGGGAGAGGGAGAGGAAGA	GAGCCATCGGATTCCATCTA
PS3	CCTGTCCTCCTCTTGACTGC	AACGGATCCAGTGAGGTGAG
CDKN1A		
PS	TAGGGGAATGGTGAAAGGTG	TGAAAGCTGACTGCCCCTAT
NR2E1		
PS2	GCAGGATTTTTCCCCCTTTA	CCACACAGAGGGACTGCTCT
PS3	CCTTCTTTCCTTGGGAGACC	CTTTTTCCCCATTCCTGTCA
PS4	GCAGAGAGGGTCGTCTTGTC	CCTCTGAGGTCCATGAAAGC

Supplemental Table 5. Primers sets used for DPA analysis.

Probe	Forward oligonucleotide
0	GGGCGGATGGAGGGGTGGGTGGACGGACGTGGAGACACTGGCCA
1	AACTGCGCCGCGGAGAAGGCCGGATTAGGGAGACCTCGGCCCTG
2	CGACGTCCGGACACGGTGGGAAGCCTTTTGGGTCCCGGCTCTCC
3	TGCAACTCGAGGGGCTGCGCCTCGGGCCCAGTCTCCTCCGCTGC
4	GGGAGGTGAGCAAATGGCCTCCCCGCTCTGAGCCTTGACTTTGT
4.mut	GGGAGGTGAGCAAATGGCCTCCCCGCTCTGAGCCTCAGTCCTGT
5	CGTCGGCTCCACTGGGCCCTGCCACTTCGGCCCCTGGCAGTCAC
6	CGGGAGGCTATGAGGCGCCCGCCTGGGTATTAAGGATCACGTCC
7	CGCCCCGGCGTCACAGCAAGACGGGGCGCGCGAGCCTCCGCCCC
8	GCCCCAGCCGGTGACCGAGCCAAATAAGTCCCACGGCAGCGCTC
9	GCGGCTCGCGGCCCGCGGCCCAATCGCAACCCGCGGGGGCGGGC
10	CCCGGGGGCGGGGTTCCGATGGGGGCGGGGCTCGGGGGCGGAGC
11	TGACCCTCAGGGCGCGAGCCGAGCCCGCGGCCGTTCCGCGCGCT
12	CCCGCCCCGCCCCCTCCTTGCGCGCGCTCGCTCGCTGGCGCCGA
13	GGAAAACGTTGCGCAGGTTCAAAAATGGAACGTCGGCGGCGTGA
14	GGGAGCGCGAGGGGGTGTGCGCGCGTGCGCGTGCGCGTGCGCGC
15	CCGGACGAGGGTGACGGGGACCCCGCCAGCCCCAGCATCGCGCG
16	CCGCAGCCGCGGCCCCGCAGCTCCGCCCCCGGCCCGGCCCGGCC
17	CCGGGCCCGCTCGCCCGCCGCCCCGCATGGAGCTGTCAGCCATC
18	GGCGAGCAGGTGTTCGCCGTGGAGAGCATCCGGAAGAAGCGCGT
19	GCGGAAGGTGAGGCTGCCCGGGGGCGGCTCCCAGGACCCCAGTG
20	GGGTCCCTCCCGTCCCCAGCACCGCTCCCTCCACGCTGGGGCTG

Probe	Reverse oligonucleotide
0	TGGCCAGTGTCTCCACGTCCGTCCACCCACCCCTCCATCCGCCC
1	CAGGGCCGAGGTCTCCCTAATCCGGCCTTCTCCGCGGCGCAGTT
2	GGAGAGCCGGGACCCAAAAGGCTTCCCACCGTGTCCGGACGTCG
3	GCAGCGGAGGAGACTGGGCCCGAGGCGCAGCCCCTCGAGTTGCA
4	ACA**AAGTCA**AGGCTCAGAGCGGGGAGGCCATTTGCTCACCTCCC
4.mut	ACAGGACTGAGGCTCAGAGCGGGGAGGCCATTTGCTCACCTCCC
5	GTGACTGCCAGGGGCCGAAGTGGCAGGGCCCAGTGGAGCCGACG
6	GGACGTGATCCTTAATACCCAGGCGGGCGCCTCATAGCCTCCCG
7	GGGGCGGAGGCTCGCGCGCCCCGTCTTGCTGTGACGCCGGGGCG
8	GAGCGCTGCCGTGGGACTTATTTGGCTCGGTCACCGGCTGGGGC
9	GCCCGCCCCCGCGGGTTGCGATTGGGCCGCGGGCCGCGAGCCGC
10	GCTCCGCCCCCGAGCCCCGCCCCCATCGGAACCCCGCCCCCGGG
11	AGCGCGCGGAACGGCCGCGGGCTCGGCTCGCGCCCTGAGGGTCA
12	TCGGCGCCAGCGAGCGAGCGCGCGCAAGGAGGGGGCGGGGCGGG
13	TCACGCCGCCGACGTTCCATTTTTGAACCTGCGCAACGTTTTCC
14	GCGCGCACGCGCACGCGCACGCGCGCACACCCCCTCGCGCTCCC
15	CGCGCGATGCTGGGGCTGGCGGGGTCCCCGTCACCCTCGTCCGG
16	GGCCGGGCCGGGCCGGGGGCGGAGCTGCGGGGCCGCGGCTGCGG
17	GATGGCTGACAGCTCCATGCGGGGCGGCGGGCGAGCGGGCCCGG
18	ACGCGCTTCTTCCGGATGCTCTCCACGGCGAACACCTGCTCGCC
19	CACTGGGGTCCTGGGAGCCGCCCCCGGGCAGCCTCACCTTCCGC
20	CAGCCCCAGCGTGGAGGGAGCGGTGCTGGGGACGGGAGGGACCC

Bold: consensus NR2E1 binding motif
Underlined: mutated sequences

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. A reporter-based screen identifies NR2E1 as a regulator of CBX7 transcription.

(a) Re-testing of candidate cDNAs regulating CBX7 transcription using a human CBX7 promoter reporter in HEK283T cells. (b) NR2E1 wt and NR2E1 Δ40 are expressed at a similar level. HEK293T cells were transfected with expression vectors for NR2E1 wt or the DNA binding domain mutant NR2E1 Δ40 and NR2E1 protein level was checked by immunoblot. (c) Luciferase assay with a human CBX7 promoter reporter showing that NR2E1 activates the human CBX7 promoter through its DNA binding domain.

Figure S2. Regulation of the CBX7 promoter by NR2E1.

(a) DNA pulldown assay was performed using the probes described in Sup Table 5 that span the CBX7 human promoter. A preferential binding of NR2E1 to probe 4, that contains a consensus NR2E1 site is shown. A lower signal is detected with probes 8, 9 and 12 suggesting alternative binding sites. The arrow marks the size of the NR2E1 wt band. (b) Luciferase reporter assay using two mouse Cbx7 reporter constructs. -836 was used for the screen, while -536 lacks the homologous region shown to bind NR2E1 in the DPA assay. Luciferase activity is similar, suggesting that alternative binding sites o indirect mechanisms could contribute to Cbx7 activation by NR2E1.

Figure S3. NR2E1 overexpression inhibits senescence and extends cellular lifespan.

(a) NR2E1 wt but not NR2E1 DNA binding mutant delays senescence in IMR90 cells. (b) SA-β-Galactosidase staining of IMR90 cells infected with the indicated vectors is shown. (c) Mutation in the NR2E1 DNA binding domain impairs its ability to regulate INK4a and CDNK1a. qRT-PCR was performed using RNA from IMR90 cells infected with the indicated constructs. (d) NR2E1 overexpression extends the lifespan of WI-38 cells. WI-38 cells were infected with the indicated vectors. At passage 19, cells were seeded at low density, grown for 2 weeks and crystal violet, SA-

β-Galactosidase and BrdU staining were performed.

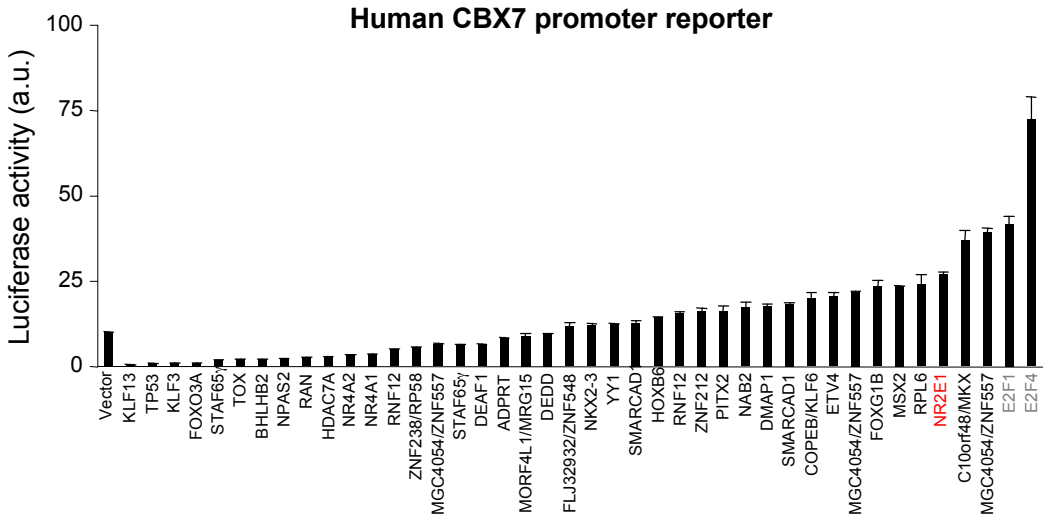
Figure S4. Knockdown of NR2E1 inhibits the proliferation of human fibroblasts and prostate epithelial cells (HPrEC).

(a) Cartoon locating the sequences of human NR2E1 targeted by the different shRNA and siRNA used in this study. (b) NR2E1 knockdown by shRNA inhibits cell proliferation in IMR90. IMR90 cells were infected with two different shRNA vectors targeting NR2E1 or with a control vector, knockdown efficiency was checked by qRT-PCR (left) and growth curves were performed (right). (c) NR2E1 knockdown in HPrEC causes a decrease in CBX7 mRNA level. HPrEC were transfected with siNR2E1.2 or a scrambled sequence and the mRNA levels of NR2E1 and CBX7 were assessed by qRT-PCR 5 days later. (d) Knocking down NR2E1 in HPrEC results in premature senescence. HPrEC were transfected with siNR2E1.2 or a scrambled sequence. 5 days after transfection cells were fixed and subjected to IF to count relative cell numbers (left) and the percentage of $p16^{INK4a}$ (middle) and $p21^{CIP1}$ (right) positive cells. (e) Knockdown efficiency of shRNA vectors targeting $p16^{INK4a}$ (shp16) or $p21^{CIP1a}$ (shp21) used in Figure 5e, as assessed by qRT-PCR for $p16^{INK4a}$ (left) and $p21^{CIP1}$ IF (right).

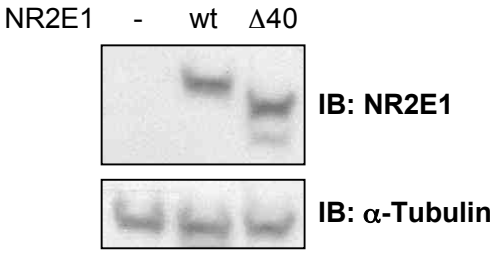
Figure S5. Effect of NR2E1 in neural cells.

(a) Cartoon locating the sequences of mouse Nr2e1 targeted by the different shRNA and siRNA used in this study. (b) Nr2e1 knockdown by shRNA in mouse neural stem cells (NSC) downregulates *Cbx7* and upregulates *Ink4a* and *Cdkn1a*. NSC were infected with shRNA vectors targeting Nr2e1 or with a control empty vector, selected and qRT-PCR were performed 2 days later.

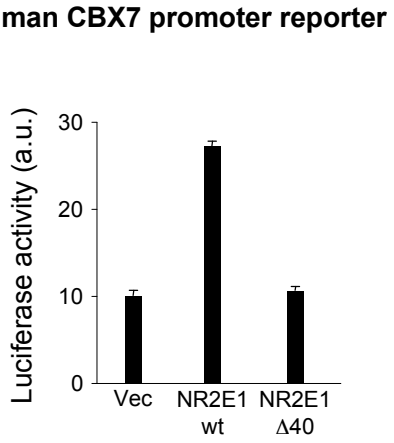
a


Human CBX7 promoter reporter
Luciferase activity (a.u.)
100
75
50
25
0
Vector
KLF13
TP53
KLF3
FOXO3A
STAF65γ
TOX
BHLHB2
NPAS2
RAN
HDAC7A
NR4A2
NR4A1
RNF12
ZNF238/RP58
MGC4054/ZNF557
STAF65γ
DEAF1
ADPRT
MORF4L1/MRG15
DEDD
FLJ32932/ZNF548
NKX2-3
YY1
SMARCAD
HOXB6
RNF12
ZNF212
PITX2
NAB2
DMAP1
SMARCAD1
COPEB/KLF6
ETV4
MGC4054/ZNF557
FOXG1B
MSX2
RPL6
NR2E1
C10orf48/MKX
MGC4054/ZNF557
E2F1
E2F4

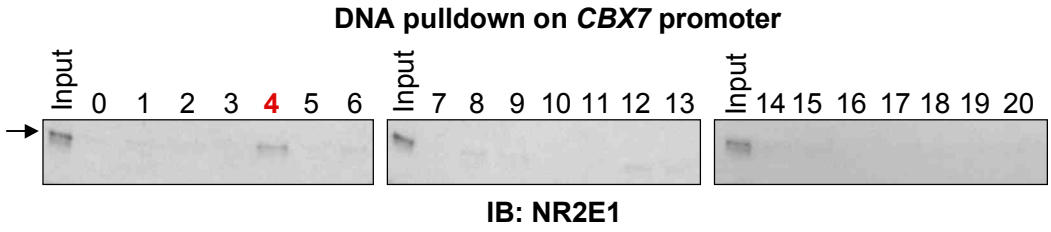
b


NR2E1 - wt Δ40
IB: NR2E1
IB: α-Tubulin

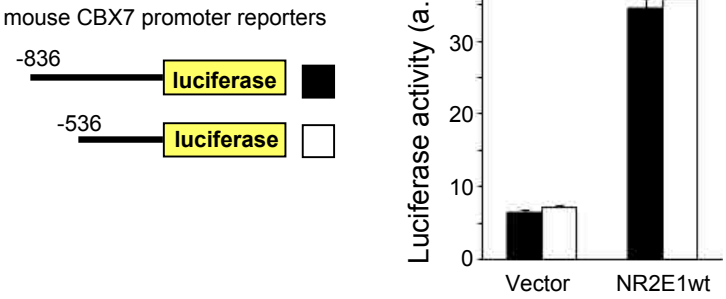
c

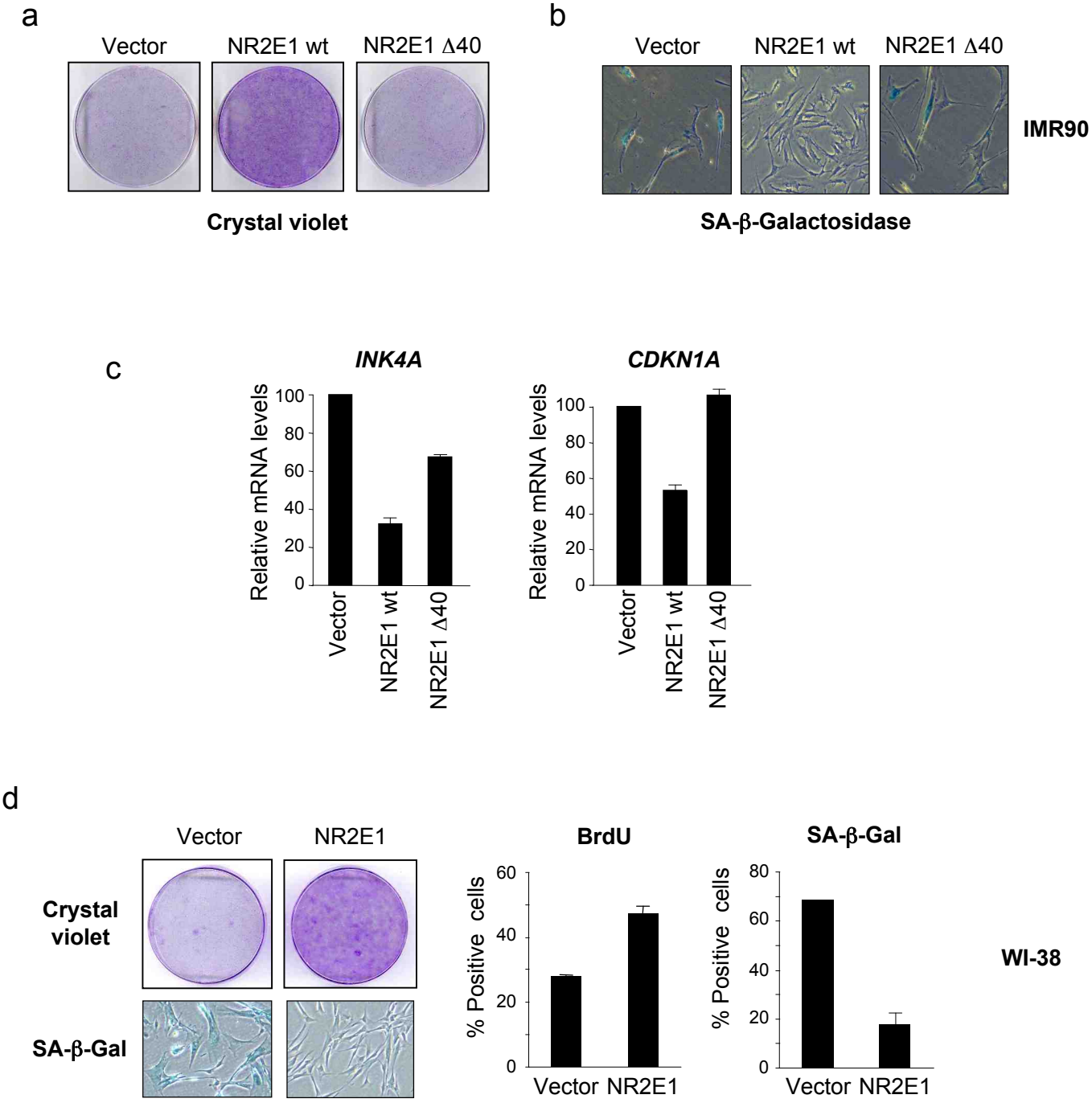

Human CBX7 promoter reporter
Luciferase activity (a.u.)
30
20
10
0
Vec
NR2E1 wt
NR2E1 Δ40

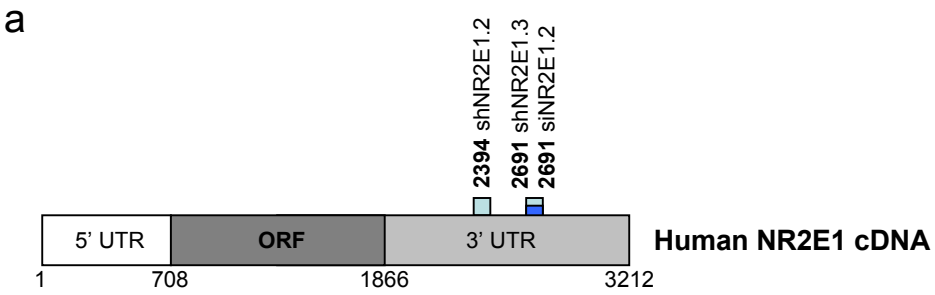
a

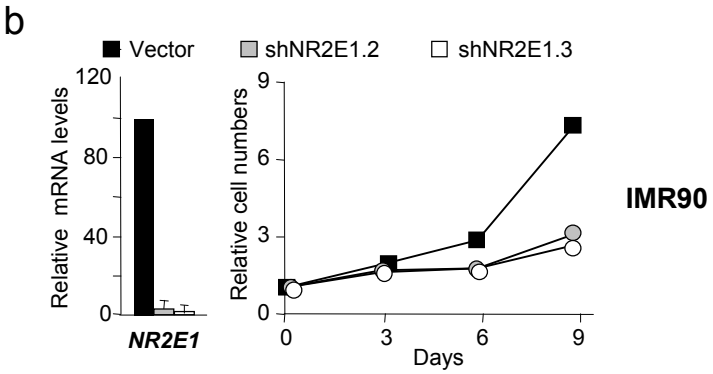

DNA pulldown on *CBX7* promoter
Input
0 1 2 3 4 5 6
Input
7 8 9 10 11 12 13
Input
14 15 16 17 18 19 20
IB: NR2E1

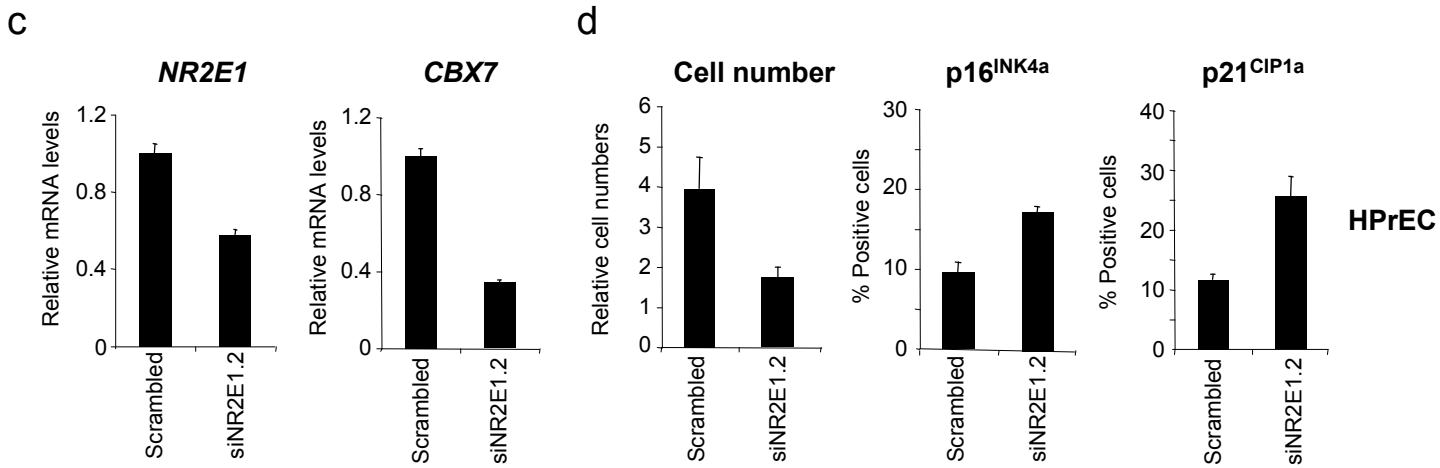
b

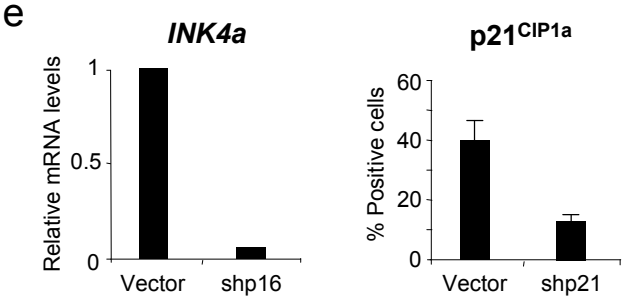

mouse CBX7 promoter reporters
-836
luciferase
-536
luciferase
Luciferase activity (a.u.)
40
30
20
10
0
Vector
NR2E1wt


a
Vector
NR2E1 wt
NR2E1 Δ40
Crystal violet
b
Vector
NR2E1 wt
NR2E1 Δ40
IMR90
SA-β-Galactosidase
c
INK4A
CDKN1A
Relative mRNA levels
100
80
60
40
20
0
Vector
NR2E1 wt
NR2E1 Δ40
d
Vector
NR2E1
Crystal violet
SA-β-Gal
BrdU
SA-β-Gal
% Positive cells
60
40
20
0
80
Vector NR2E1
WI-38

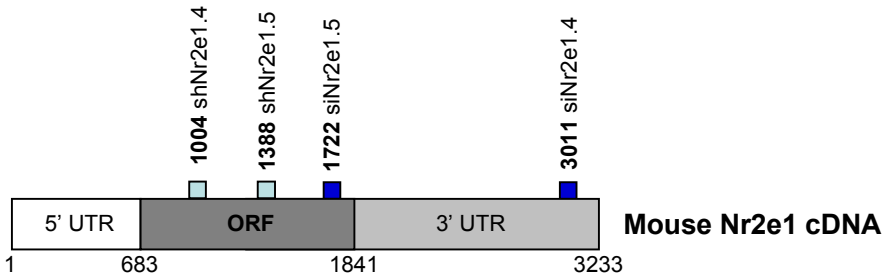

a
2394 shNR2E1.2
2691 shNR2E1.3
2691 siNR2E1.2
5' UTR
ORF
3' UTR
Human NR2E1 cDNA
1
708
1866
3212


b
Vector
shNR2E1.2
shNR2E1.3
Relative mRNA levels
NR2E1
Relative cell numbers
Days
IMR90

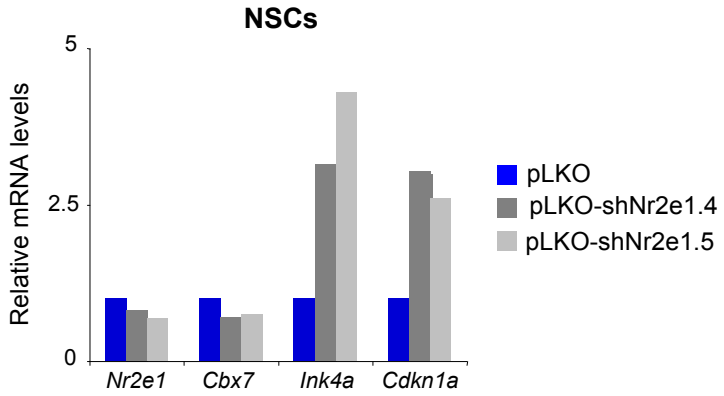

c
NR2E1
CBX7
Relative mRNA levels
Scrambled
siNR2E1.2
d
Cell number
p16INK4a
p21CIP1a
Relative cell numbers
% Positive cells
HPrEC


e
INK4a
p21CIP1a
Relative mRNA levels
% Positive cells
Vector
shp16
shp21

a


1004 shNr2e1.4
1388 shNr2e1.5
1722 siNr2e1.5
3011 siNr2e1.4
5' UTR
ORF
3' UTR
Mouse Nr2e1 cDNA
1
683
1841
3233

b


NSCs
Relative mRNA levels
5
2.5
0
Nr2e1
Cbx7
Ink4a
Cdkn1a
pLKO
pLKO-shNr2e1.4
pLKO-shNr2e1.5