SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Retroviral vectors express miR-9 and miR-181.

IMR90 cells were infected with the indicated vectors and the expression of miR-9 or miR-181 assessed by qRT-PCR.

Supplementary Figure 2. Controls for CBX7 overexpression and knockdown.

(A) Overexpression of mouse Cbx7 results in $p16^{INK4a}$ repression. qRT-PCR was carried out in cells used in the experiment described in Fig 2C, to assess the levels of *INK4a* mRNA (encoding for $p16^{INK4a}$ and the exogenously expressed mouse Cbx7 (*Cbx7*). (B) Knockdown of CBX7 results in $p16^{INK4a}$ induction. qRT-PCR was carried out in cells used in the experiment described in Fig 2D-E, to assess *INK4a* and CBX7 mRNA levels.

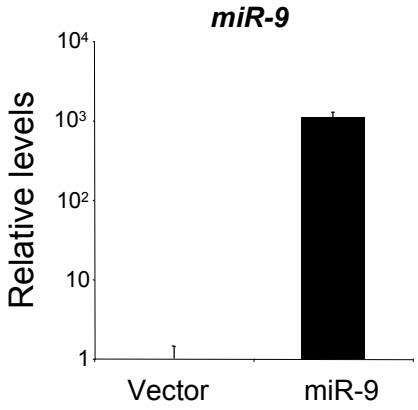
Supplementary Figure 3. Characterization of the miR-9 induced arrest. (A) IMR90 cells were infected with control or miR-9 expressing vectors and the expression of the indicated genes was assessed by qRT-PCR. (B-C) The expression of (B) $p21^{CIP}$ and (C) p53 was assessed by IF, and the percentage of positive cells were quantified. (D) miR-9 induces $p16^{INK4A}$ expression in IMR90 cells. $p16^{INK4a}$ expression was assessed by IF in IMR90 cells transfected with miR-9 or miR-181 mimics or with anti-miR-9.

Supplementary Figure 4. Analysis of $p16^{INK4a}$-deficient Leiden fibroblasts suggests that miR-9-induced senescence is $p16^{INK4a}$-dependent.

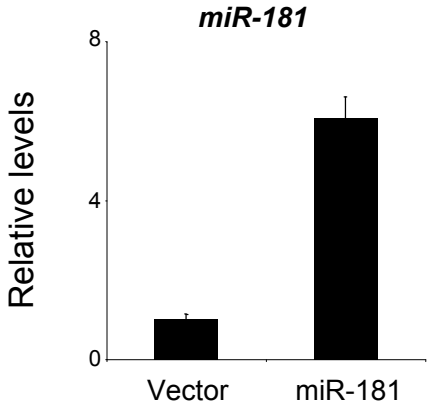
IMR90 and $p16^{INK4a}$-defective Leiden fibroblasts (Brookes *et al.* 2002) were infected with pMSCV-miR-9 or empty vector. (A) Immunofluorescence for $p16^{INK4a}$ was performed in both cells types. Quantification (left) and representative images (right) are shown. (B) Cells were seeded at

low density and stained with crystal violet after 2 weeks. Quantification (left) and representative images (right) are shown. (C) BrdU incorporation was assessed in the indicated cells.

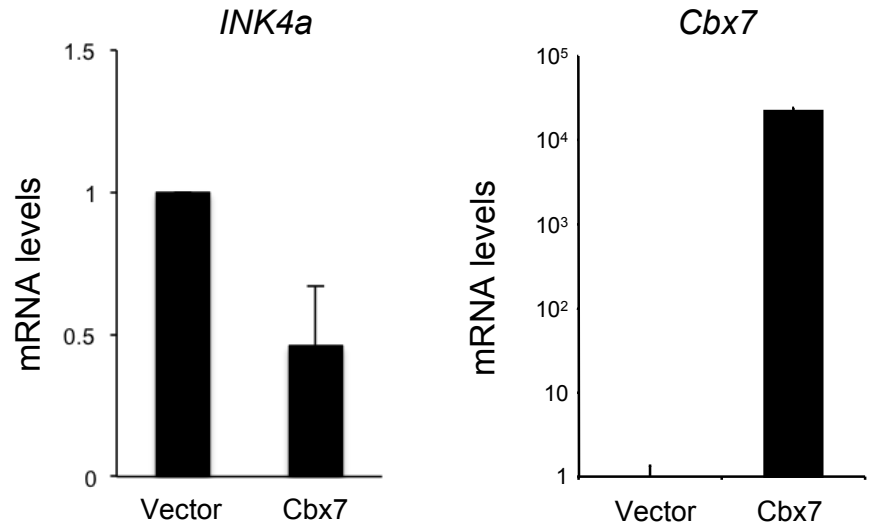
(A)


miR-9
Relative levels
10^4
10^3
10^2
10
1
Vector
miR-9

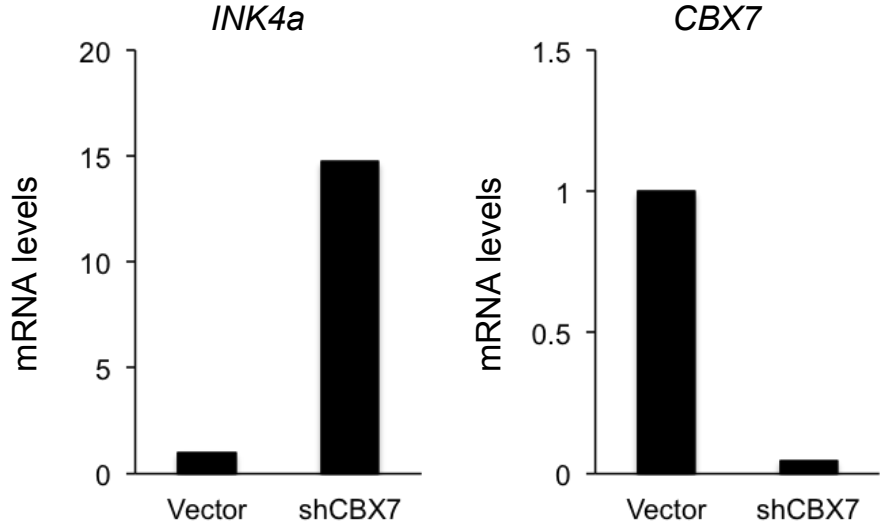
(B)

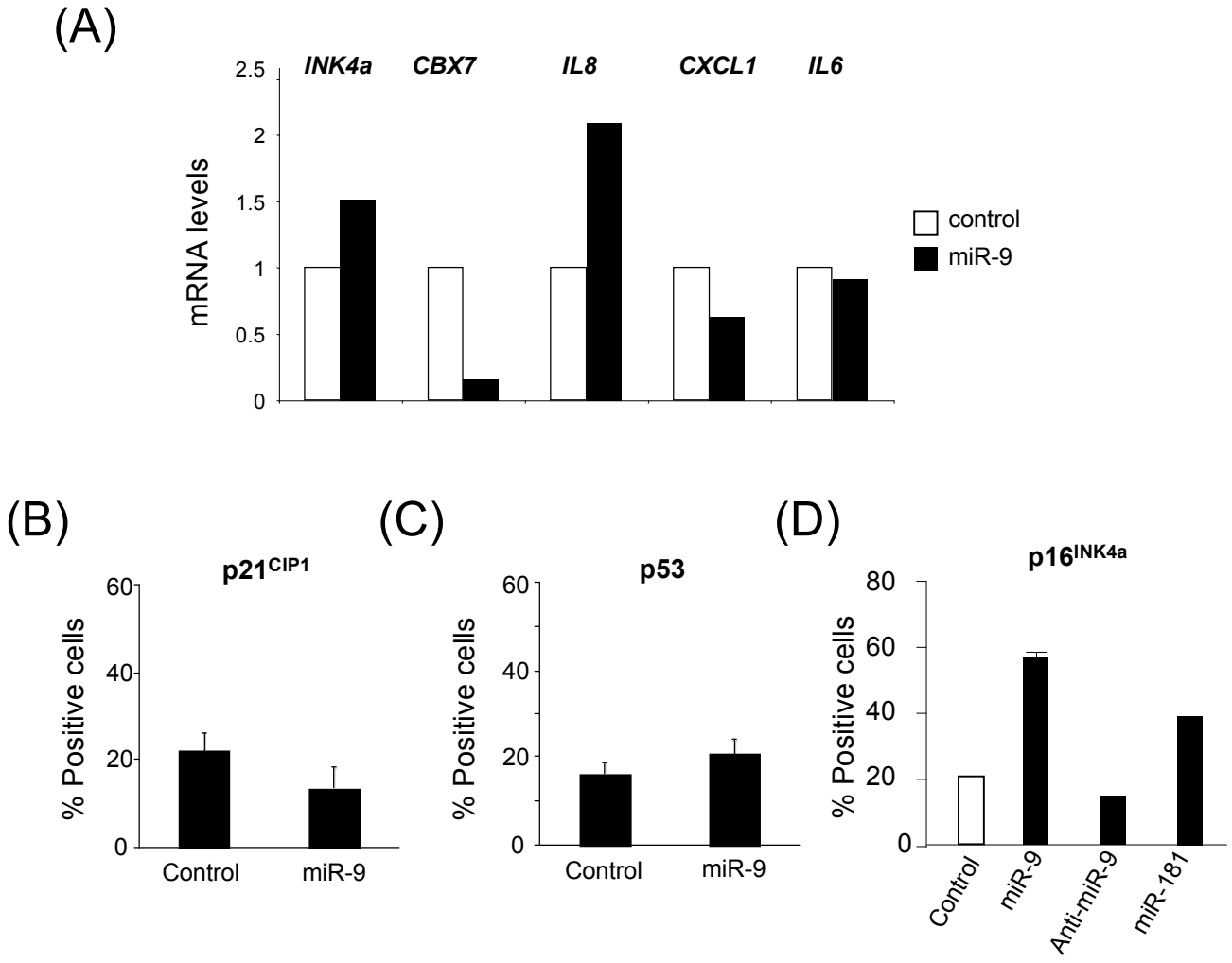

miR-181
Relative levels
8
4
0
Vector
miR-181

(A)

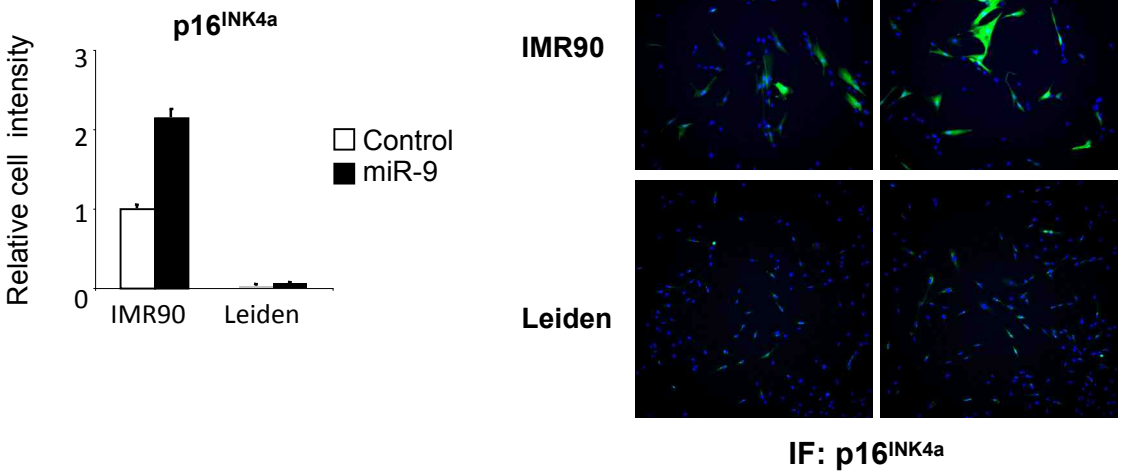

INK4a
mRNA levels
1.5
1
0.5
0
Vector
Cbx7
Cbx7
mRNA levels
10⁵
10⁴
10³
10²
10
1
Vector
Cbx7

(B)

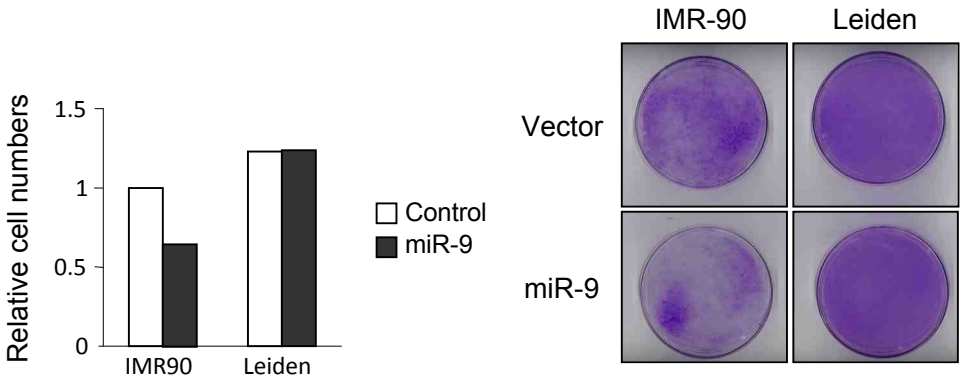

INK4a
mRNA levels
20
15
10
5
0
Vector
shCBX7
CBX7
mRNA levels
1.5
1
0.5
0
Vector
shCBX7


(A)
INK4a
CBX7
IL8
CXCL1
IL6
mRNA levels
2.5
2
1.5
1
0.5
0
control
miR-9
(B)
p21CIP1
% Positive cells
60
40
20
0
Control
miR-9
(C)
p53
% Positive cells
60
40
20
0
Control
miR-9
(D)
p16INK4a
% Positive cells
80
60
40
20
0
Control
miR-9
Anti-miR-9
miR-181

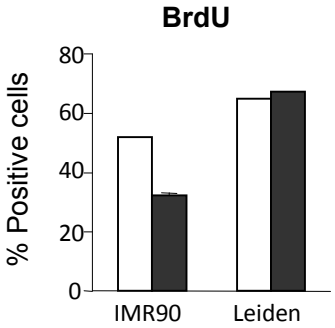
(A)


p16INK4a
Relative cell intensity
3
2
1
0
IMR90
Leiden
Control
miR-9
Control
miR9
IMR90
Leiden
IF: p16INK4a

(B)


Relative cell numbers
1.5
1
0.5
0
IMR90
Leiden
Control
miR-9
IMR-90
Leiden
Vector
miR-9

(C)


BrdU
% Positive cells
80
60
40
20
0
IMR90
Leiden