P16^{INK4a} Deletion Ameliorated Renal Tubulointerstitial Injury in a Stress-induced Premature Senescence Model of *Bmi-1* Deficiency

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(a)

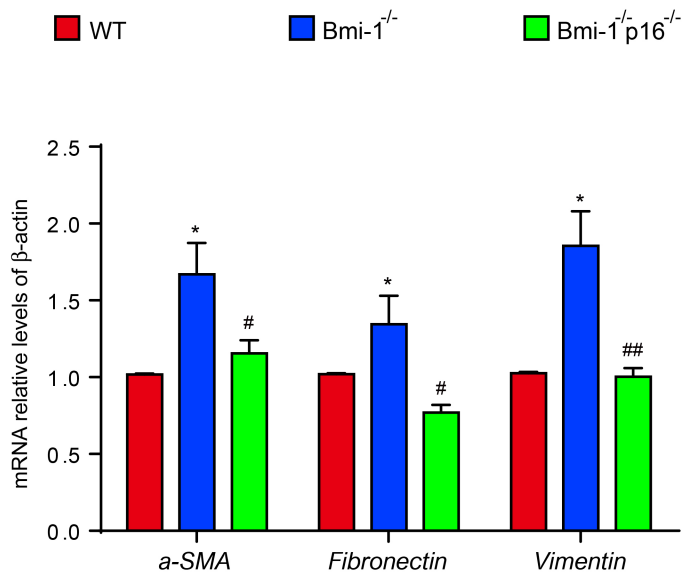

WT
Bmi-1-/-
Bmi-1-/-p16-/-
mRNA relative levels of β-actin
2.5
2.0
1.5
1.0
0.5
0.0
*
#
*
#
*
##
a-SMA
Fibronectin
Vimentin

Figure S1 | The mesenchymal characterization of renal interstitial fibroblasts. (a) Second-passage renal interstitial fibroblasts detected for *α-SMA, fibronectin* and *vimentin* mRNA levels by real-time RT-PCR, calculated as ratio to *β-actin* mRNA. Six biological replicates were used for these studies per experiment. Values are mean ± SEM of six determinations per group. *: $P < 0.05$ compared with WT group; #: $P < 0.05$, ##: $P < 0.01$ compared with *Bmi-1*$^{-/-}$ group.

Table S1 | Synthesized shDNAs encoding human *Bmi-1*-specific shRNA or Negative control

Name	5’	STEM	Loop	STEM	3’
human *Bmi-1*-RNAi	ccgg	AATGGACATACCTAATACT	CTCGAG	AGTATTAGGTATGTCCATT	TTTTTg
	aattcaaaaa	AATGGACATACCTAATACT	CTCGAG	AGTATTAGGTATGTCCATT	
Negative control	ccgg	CTACCGTTGTTAAGGTGT	CTCGAG	GACACCTATAACAACGGTAG	TTTTTg
	aattcaaaaa	CTACCGTTGTTAAGGTGT	CTCGAG	GACACCTATAACAACGGTAG	

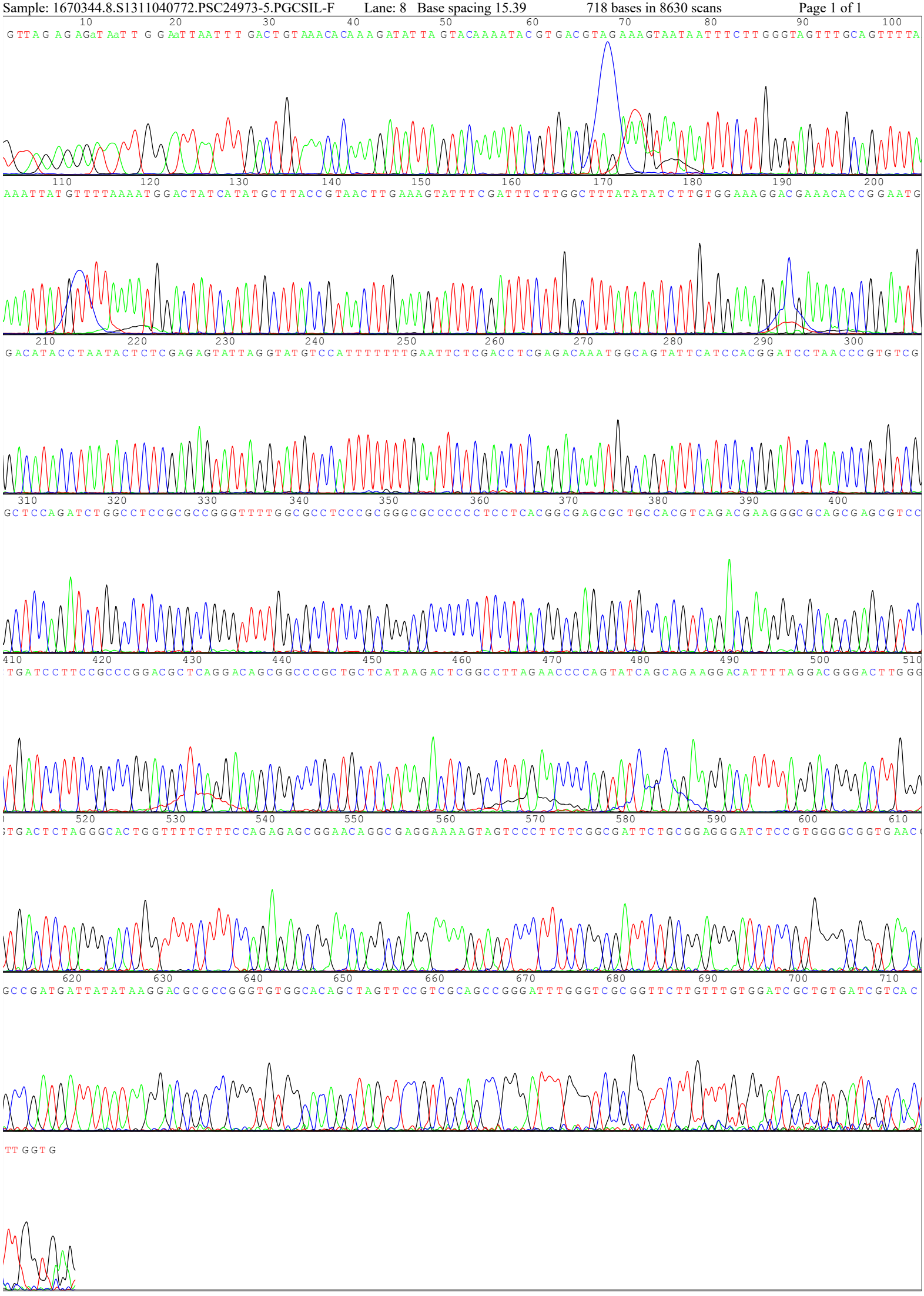
Table S2 | Primers for Real Time RT-PCR

Name	S/AS	Sequence	Species	Tm(°C)	Length (bp)
IL-1β	S	5'-GCAACTGTTCCTGAACTCAACT-3'	mouse	60	89
	AS	5'-ATCTTTTGGGGTCCGTCAACT-3'			
IL-6	S	5'-GTTGCCTTCTTGGGACTGATG-3'	mouse	58	192
	AS	5'-ATCAGAATTGCCATTGCACAA-3'			
TNF-α	S	5'-TCCCTCTCATCAGTTCTATGG-3'	mouse	55	100
	AS	5'-ACTTGGTGGTTTGCTACGAC-3'			
NF-κB	S	5'-CTTGCCAGACACAGATGATCG-3'	mouse	58	163
	AS	5'-GGGGACAGAAGTTGAGTTTC-3'			
1α(OH)ase	S	5'-CTTTGGGACACTTCGCACAG-3'	mouse	56	105
(*Cyp27b1*)	AS	5'-GCCCATGATGAGAAACTACAG-3'			
EPO	S	5'-GGAGAGGTACATCTTAGAGGC-3'	mouse	53	105
	AS	5'-TTGACTTTGGTATCTGGGACT-3'			
GAPDH	S	5'-CATTTCACTCAAGGTTGTCAGC-3'	mouse	55	346
	AS	5'-ATCATACTTGGCAGGTTTCTCC-3'			
α-SMA	S	5'- GCTGTGCTGTCCCTCTATGCC-3'	mouse	57	131
	AS	5'- GCCAAGTCCAGACGCATGATG-3'			
fibronectin	S	5'- TTTGTGGTCTCATGGGTCTCA-3'	mouse	57	136
	AS	5'- GCAGGTCAGGAATGTTCACG-3'			
vimentin	S	5'- GAGGTGGAGCGGGACAAC-3'	mouse	57	94
	AS	5'- GGGTGCTTTCGGCTTCCTCTC-3'			
β-actin	S	5'- GGCTGTATTCCCCTCCATCG-3'	mouse	58	326
	AS	5'- CCAGTTGGTAACAATGCCATGT-3'			
Bmi-1	S	5'-AAGGAGGAGGTGAATGATAA-3'	human	55	114
	AS	5'-ATCAATCTGGAAAGTATTAGG-3'			
GAPDH	S	5'-AGCGACACCCACTCCTCC-3'	human	55	108
	AS	5'-CCTGTTGCTGTAGCCAAATTC-3'			

S, sense; AS, antisense, sequence; Tm, annealing temperature; length, amplicon

DNA Sequencing

File: 1670344.8.S1311040772.PSC24973-5.PGCSIL-F.ab1 Run Ended: Nov 5, 2013, 23:40:44 Signal G:459 A:360 T:412 C:272 Comment:
Sample: 1670344.8.S1311040772.PSC24973-5.PGCSIL-F Lane: 8 Base spacing 15.39 718 bases in 8630 scans Page 1 of 1



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CTTGGTG

PSC24973-1 ccggAATGGACATACCTAATACTctcgagAGTATTAGGTATGTCCATTtttttg