Table S1. List of primers used in the study

Gene name	Gene reference number		Sequence	Product length (bp)
CELL CYCLE REGULATION				
ATM	NM000051	Forward	5'-TGTTCCAGGACACGAAGGGAGA-3'	138
		Reverse	5'-CAGGGTTCTCAGCACTATGGGA-3'	
ATR	NM001184	Forward	5'-GGAGATTTCCTGAGCATGTTCGG-3'	100
		Reverse	5'-GGCTTCTTTACTCCAGACCAATC-3'	
BRCA1	NM007294	Forward	5'-CTGAAGACTGCTCAGGGCTATC-3'	155
		Reverse	5'-AGGGTAGCTGTTAGAAGGCTGG-3'	
CHEK1	NM001274	Forward	5'-CTCATGGCAGGGGTGGTTTATC-3'	122
		Reverse	5'-ACTGTTGCCAAGCCAAAGTCTG-3'	
E2F4	NM001950	Forward	5'-GGAAGGTATCGGGCTAATCGAG-3'	126
		Reverse	5'-AGCTCCTCGATCTCTGCCTTGA-3'	
NFKB1	NM003998	Forward	5'-GCAGCACTACTTCTTGACCACC-3'	130
		Reverse	5'-TCTGCTCCTGAGCATTGACGTC-3'	
P16	NM012308	Forward	5'-CTCGTGCTGATGCTACTGAGGA-3'	134
		Reverse	5'-GGTCGGCGCAGTTGGGCTCC-3'	
P53	NM000546	Forward	5'-CCTCAGCATCTTATCCGAGTGG-3'	128
		Reverse	5'-TGGATGGTGGTACAGTCAGAGC-3'	
RB1	NM000321	Forward	5'-AGAGCTTGGTTAACTTGGGAGA-3'	120
		Reverse	5'-CTCATCTAGGTCAACTGCTGC-3'	
DNA REPAIR				
APEX1	NM001641	Forward	5'-TCTTACGGCATAGGCGATGA-3'	111
		Reverse	5'-CAGACCTCGGCCTGCATTAG-3'	
TERT	NM198253	Forward	5'-GCTGACGTGGAAGATGAGCG-3'	146
		Reverse	5'-GCTCGACGACGTACACACTC-3'	
XRCC4	NM003401	Forward	5'-TGATGGTCATTCAGCATGGACT-3'	109
		Reverse	5'-TGCTTTTCTCAGTTCACCAACA-3'	
XRCC6	NM001469	Forward	5'-TGCGTGGATTGTCGTCTTCT-3'	111
		Reverse	5'-CTTCTTCATCGCCCTCGGTT-3'	
APOPTOSIS				
BAX	NM138761	Forward	5'-TCAGGATGCGTCCACCAAGAAG-3'	103
		Reverse	5'-TGTGTCCACGGCGGCAATCATC-3'	
BCL2	NM000633	Forward	5'-ATCGCCCTGTGGATGACTGAGT-3'	127
		Reverse	5'-GCCAGGAGAAATCAAACAGAGGC-3'	
BIRC5	NM001012271	Forward	5'-CCACTGAGAACGAGCCAGACTT-3'	115
		Reverse	5'-GTATTACAGGCGTAAGCCACCG-3'	
CASP3	NM004346	Forward	5'-GGAAGCGAATCAATGGACTCTGG-3'	146
		Reverse	5'-GCATCGACATCTGTACCAGACC-3'	
CASP8	NM001228	Forward	5'-AACCTCGGGGATACTGTCTG-3'	297
		Reverse	5'-CCTGTCCATCAGTGCCATAG-3'	
CASP9	NM001229	Forward	5'-GTTTGAGGACCTTCGACCAGCT-3'	129
		Reverse	5'-CAACGTACCAGGAGCCACTCTT-3'	
ANTIOXIDATION				
GSTA5	NM153699	Forward	5'-CCAAGCTCCACTACTCCAATGC-3'	144
		Reverse	5'-GGAACAGCAAACTCCCATCATTTCT-3'	
PRDX2	NM005809	Forward	5'-CCTTCCAGTACACAGACGAGCA-3'	136
		Reverse	5'-CTCACTATCCGTTAGCCAGCCT-3'	
PRDX5	NM012094	Forward	5'-TGATGCCTTTGTGACTGGCGAG-3'	135
		Reverse	5'-CCAAAGATGGACACCAGCGAATC-3'	
SOD1	NM000454	Forward	5'-CTCACTCTCAGGAGACCATTGC-3'	129
		Reverse	5'-CCACAAGCCAAACGACTTCCAG-3'	
TXN	NM003329	Forward	5'-TTGGTGCTTTGGATCCATTTCCAT-3'	107
		Reverse	5'-CAAGGCTTCCTGAAAAGCAGTCT-3'	

Table S1. Continued.

Gene name	Gene reference number		Sequence	Product length (bp)
EPIGENETICS				
DNMT1	NM001379	Forward	5′-ACCGCTTCTACTTCCTCGAGGCCTA-3′	335
		Reverse	5′-GTTGCAGTCCTCTGTGAACACTGTGG-3′	
HOUSEKEEPING				
ACTB	NM001101	Forward	5′-GAGCTACGAGCTGCCTGAC-3′	110
		Reverse	5′-GGATGCCACAGGACTCCATG-3′	

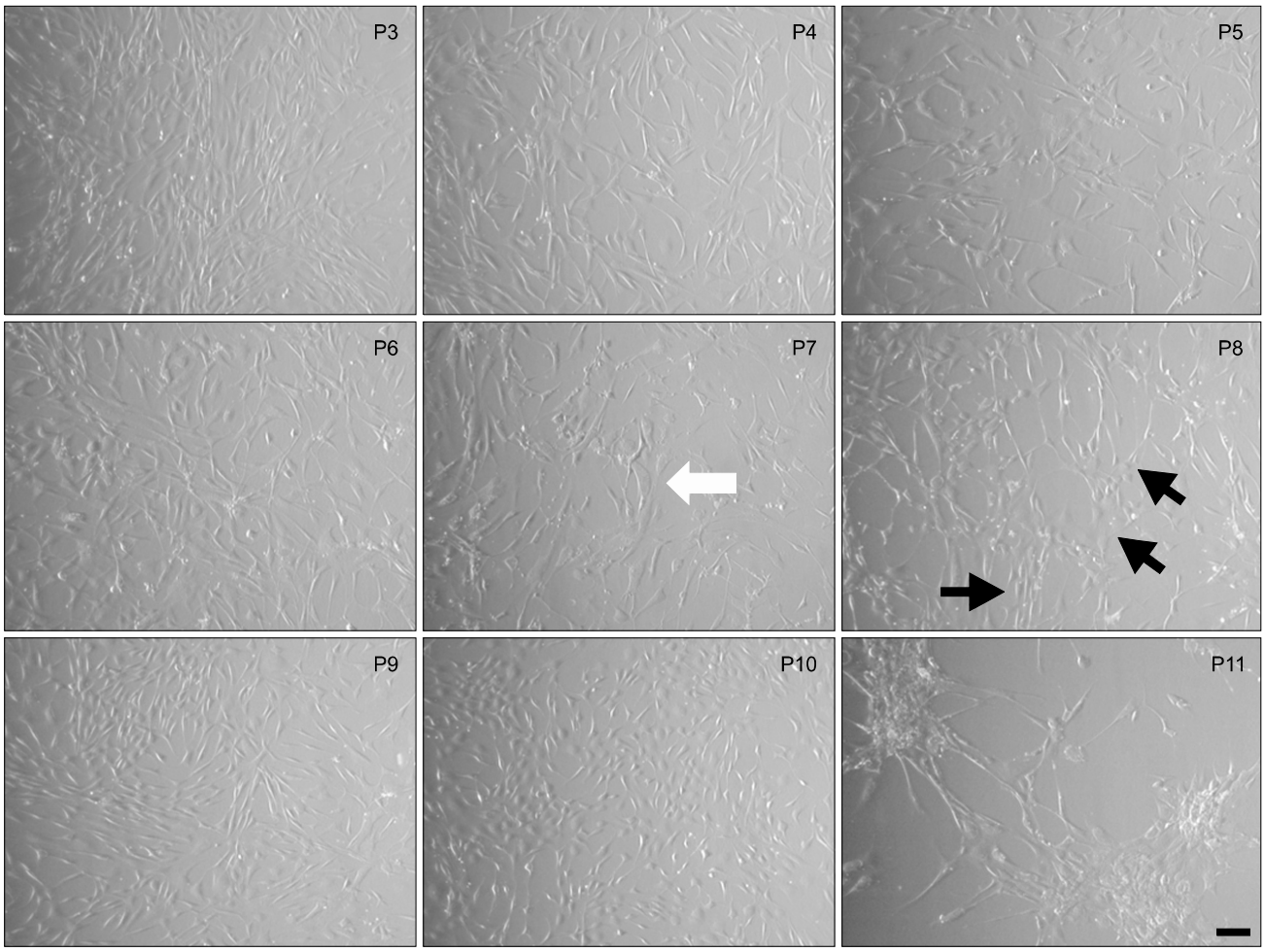

Fig. S1. Changes of adipose-derived mesenchymal stem cell morphology during long-term cultivation. Representative images of sample CS-1 are shown. Note the common senescence-associated enlargement and flattening of cells at passage 7 (P7) (white arrow). The appearance of spindle- and triangular-shaped cells in P8 is depicted by black arrows. These cells dominate during P9 and P10. Scale bar – 100 μm.

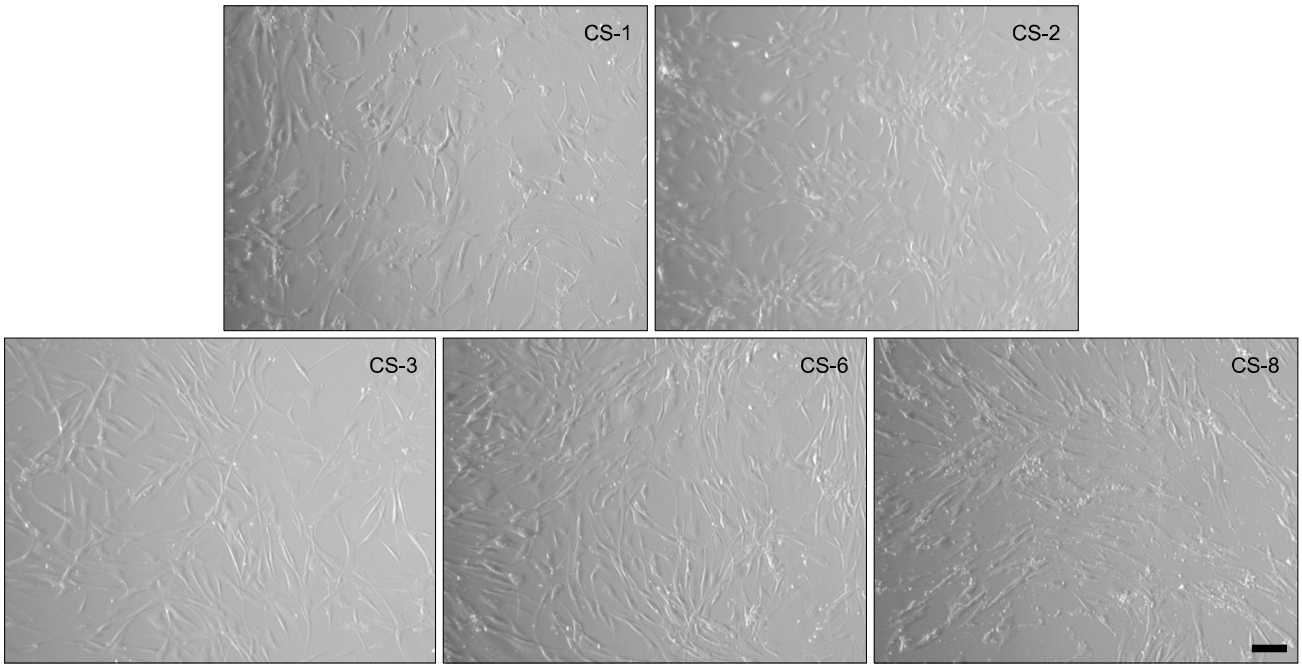

Fig. S2. Morphology of adipose-derived mesenchymal stem cells (ADSCs) obtained from different donors. Comparison of cells at passage 7 (P7) is shown. Cells from donor CS-1 are enlarged and irregular in shape which are typical signs of senescence. For CS-2, small triangular cells clustering in groups are seen. ADSCs of CS-3 appear spindle-shaped with minimal signs of senescence. Image of CS-6 presents a mixed population of cells where large, senescenct cells can be seen among spindle-shaped ones. For CS-8, ADSCs maintain a spindle-shaped appearance and possess a donor-specific pattern to grow in a parallel orientation to each other making the monolayer appear streaky. A lot of cell debris is present. Scale bar = 100 μm.

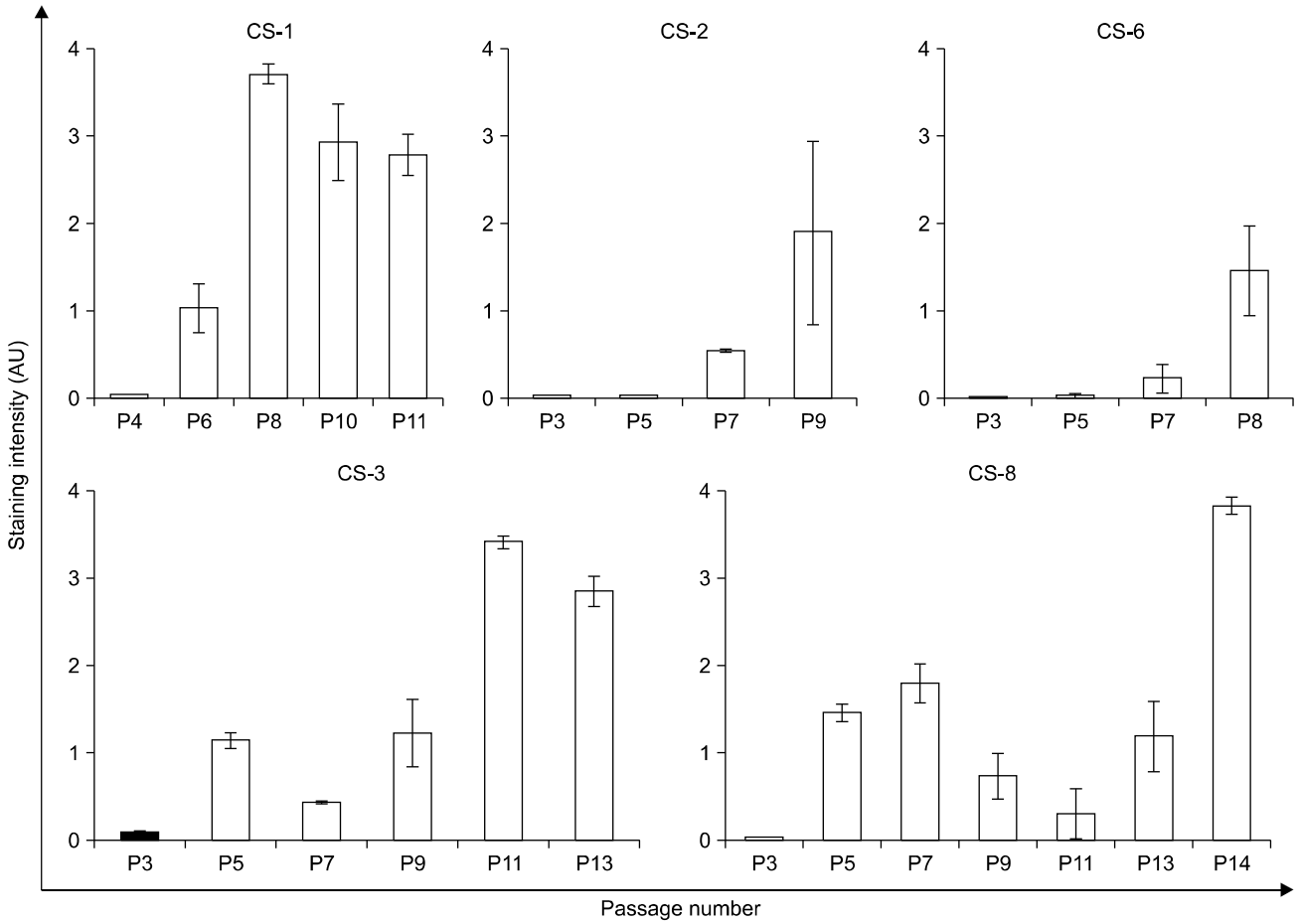

Fig. S3. Quantitative evaluation of senescence-associated β-galactosidase (SA-β-gal) expression. Adipose-derived mesenchymal stem cells from five donors (CS-1,2,3,6,8) were culture-expanded until entering senescence. SA-β-gal expression was tested histochemically. Images were subjected to visual evaluation by two observers with regard to color intensity and percentage of stained cells per field of view. Error bars show standard deviation from two measurements.

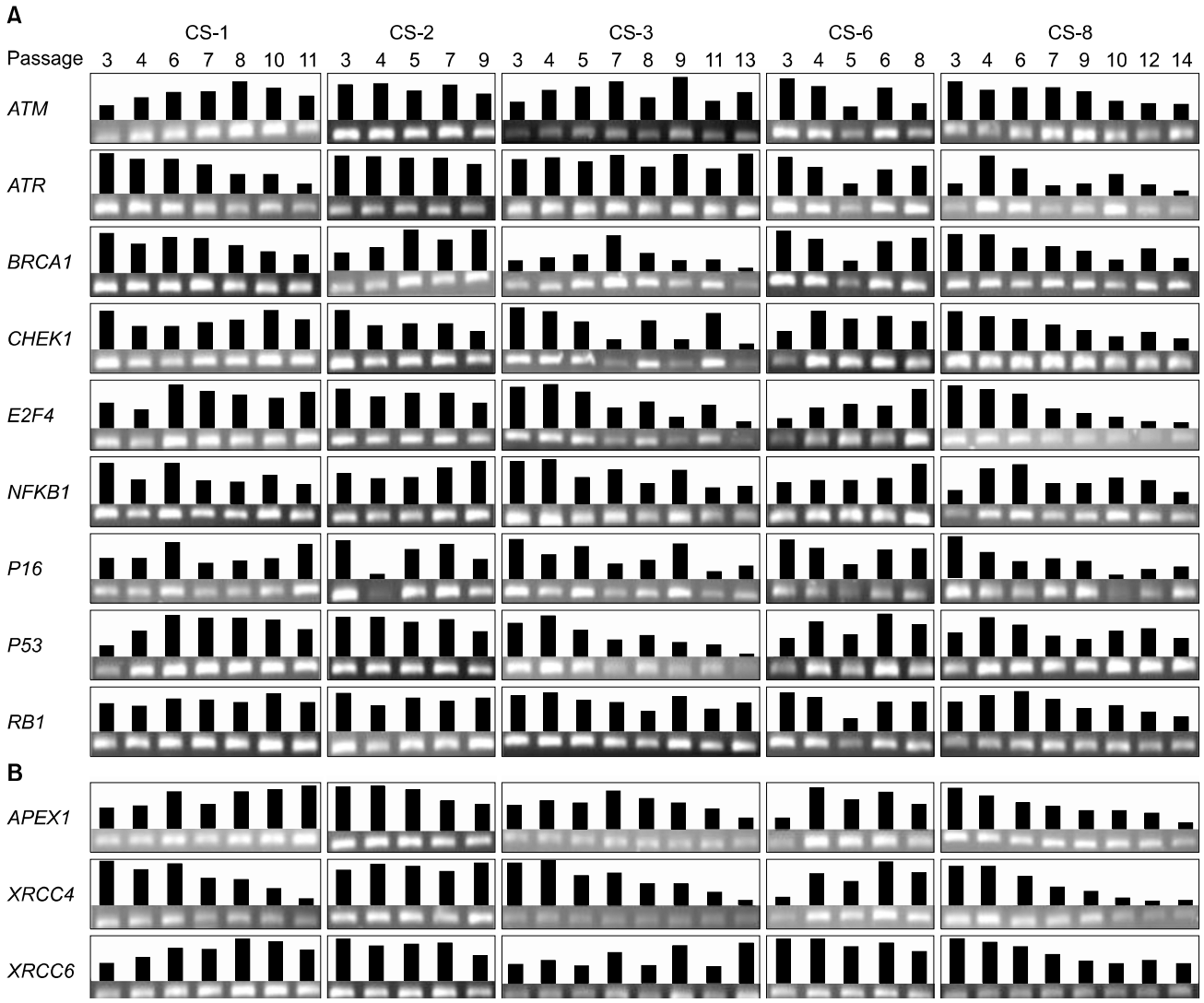

Fig. S4. Gene expression in adipose-derived mesenchymal stem cells (ADSCs) during long-term culture. Messenger RNA was detected in five ADSC cultures (CS-1,2,3,6,8) by reverse-transcriptase polymerase chain reaction. Amplification products were visualized in UV light by ethidium bromide staining. Signal intensity was quantified by pixel density analysis in ImageJ software (version 1.48) and normalized against housekeeping gene signal intensity. Expression of genes involved in (A) cell cycle regulation, (B) DNA repair, (C) apoptosis, (D) antioxidation, and (E) epigenetics was tested. β-actin was used as housekeeping gene (F). Quantification results are expressed in arbitrary intensity units (black bars). The respective gel bands are shown below.

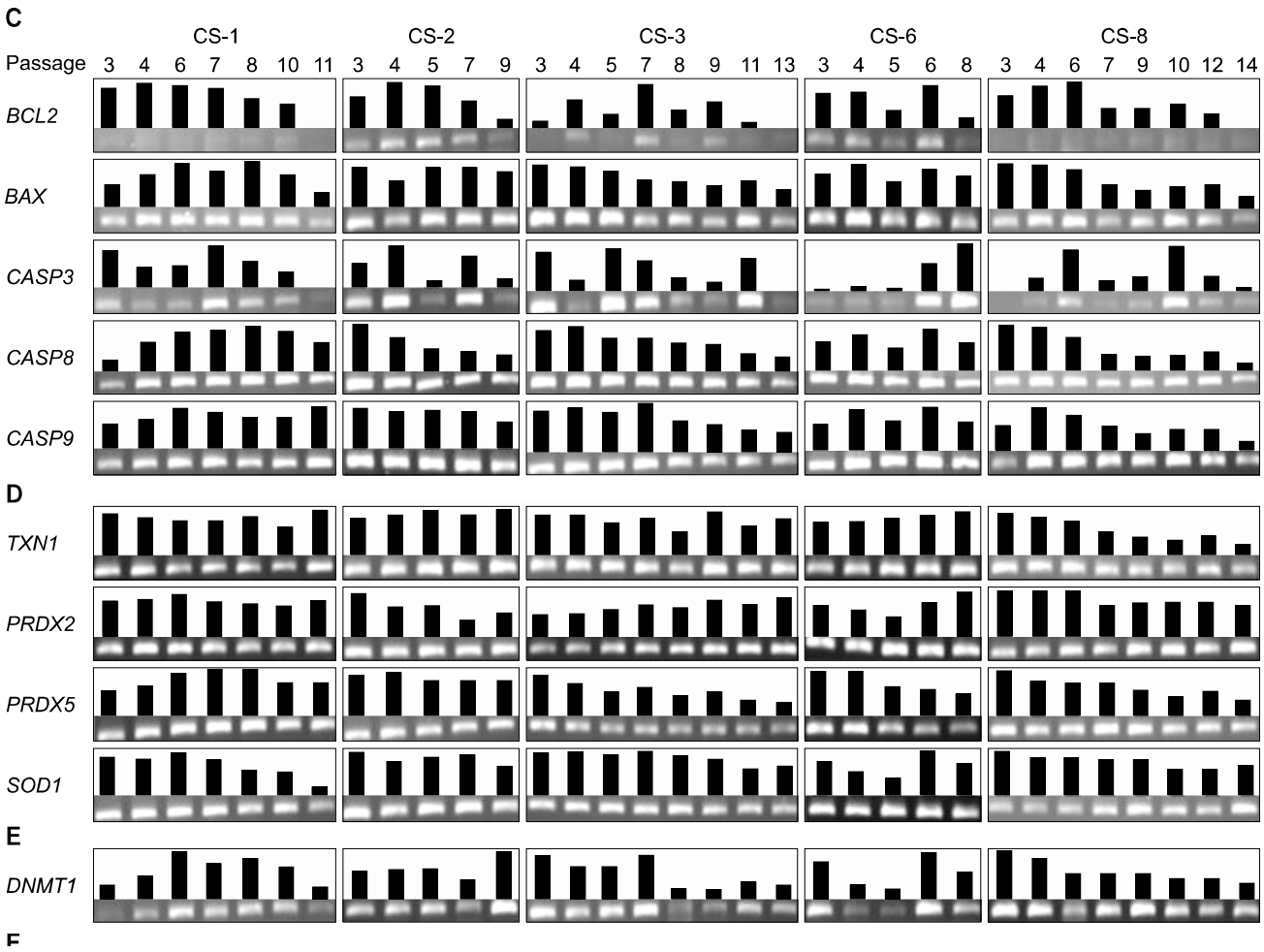

Fig. S4. Continued.