Supplementary Materials: Evaluating the Role of p38 MAPK in the Accelerated Cell Senescence of Werner Syndrome Fibroblasts

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Table S1. Fibroblasts strains used in this study.

Strain [a]	Age of Donor	Replicative History [b]
Normal (N)		
N(GM05399A)	1	16 PDs
N(GM05400A)	6	15 PDs
N(AG06234)	17	15 PDs
N(AG13156)	44	9 PDs
N(AG04552)	65	10 PDs
N(AG11081)	78	13 PDs
N(AG13152)	79	10 PDs
N(AG11020)	79	10 PDs
Werner Syndrome (WS)		
WS(AG05229)	25	7 PDs
WS(AG03141B) [c]	30	12 PDs
WS(AG03141D) [c]	30	14 PDs
WS(AG03141F) [c]	30	6 PDs
WS(AG12800)	25	Passage 1

[a] To avoid confusion when referring to cell strains in this paper a prefix has been added to the strain code; [b] Number of population doublings (PDs) achieved at the Coriell Cell Banks prior to the receipt of the cells and the start of these growth experiments (where a passage number is given the PD value used has been set as zero); [c] Different batches of AG03141 WS cells.

Table S2. Comparison of CPDL [a] for inhibitor treated normal and WS fibroblasts.

Strain	PD at Start	PDs Achieved								
		Cont [b]	VX	UR	SB100	SB500	SB2500	B100	B500	B2500
Normal (N) young individuals (<25 years)										
N(AG06234)	15	34.1	nd [c]	nd	nd	nd	40.7	nd	nd	42.8
N(GM05399A)	15	41.6	nd	nd	nd	nd	55.5	nd	nd	68.6
N(GM05400B)	16	43.4	nd	nd	nd	nd	54.7	nd	nd	66.0
Mean ± SD [d]		39.7 ± 4.9					50.3 ± 8.3			59.1 ± 14.2
Normal (N) old individuals (>64 years)										
N(AG04552)	10	20.3	24.7	25.1	24.3	25	27.1	23.5	27.3	32.6
N(AG11020)	10	26.7	nd	nd	nd	nd	30.8	nd	nd	40.8
N(AG11081)	13	26.7	29.2	29.7	nd	nd	34.1	nd	nd	40.3
N(AG13152)	10	26.5	35.0	35.6	28.9	34.2	36.5	38.8	39	40.6
Mean ± SD[d]		25.1 ± 3.17					32.1 ± 4.1			38.6 ± 3.98
Normal (N) 44 years old individual										
N(AG13156)	9	29.9	nd	nd	nd	nd	41.6	nd	nd	48.6
Mean ± SD [e]		31.14 ± 8.0					40.1 ± 10.4			47.5 ± 13.0
Werner syndrome (WS)										
WS(AG05229)	7	20.9	29.2	32.6	28.3	33.2	36.7	32.9	42.9	53.7
WS(AG03141B)	12	16.9	nd	nd	nd	nd	21.5	nd	nd	36.1
WS(AG03141D)	14	20.7	nd	nd	nd	nd	nd	nd	nd	32.3
WS(AG03141F)	6	13.4	17.7	17.1	17.1	19.4	22.9	17.3	20.2	25.8
Mean ± SD [d]		18.0 ± 3.6					27.1 ± 8.4			36.9 ± 11.9
p (t-test) WS v N (all)		<0.012					>0.085			>0.203
WS(AG12800)	0	2.0	nd	nd	nd	nd	11.8	nd	nd	19.3

[a] CPDL = cumulative population doubling level (see methods for explanation of term); [b] Cont = DMSO treated cells, VX = 500 nM VX-745, UR = 1000 nM UR13756, SB = SB203580, B = BIRB 796. The numbers refer to nM concentrations, e.g., SB100 = SB203580 at 100 nM; [c] nd = not done; [d] Mean ± SD. Result only given for DMSO, SB2500 and B2500; [e] refers to all eight NDFs.

Table S3. Percentage increases in replicative capacity for primary fibroblasts using p38 inhibitors [a].

Strain	Inhibitors[b]							
	VX	**UR**	**SB100**	**SB500**	**SB2500**	**B100**	**B500**	**B2500**
N (Normal)								
N(AG04552)	42.7	45.6	38.5	45.6	66	31.5	68.5	119
N(AG11081)	18.2	22.0	nd [c]	nd	54	nd	nd	99.3
N(AG13152)	51.5	**55.1**	14.5	46.7	60.6	**74.5**	75.7	85.4
Mean ± SD	37.5	40.9	26.5	46.1	60.2	53	72.1	101.2
	17.2	17.1	17.0	0.78	6.0	30.4	5.1	16.9
WS (Werner Syndrome)								
WS(AG05229)	59.7	84.2	53	88.5	114	86.3	158.3	236
WS(AG03141F)	58.1	50	50	81.1	128.4	52.8	91.9	168
Mean ± SD	*58.9*	*67.1*	*51.5*	*84.8*	*121.2*	*69.6*	*125.1*	*201.8*
	1.13	24.2	2.1	5.23	10.2	23.7	46.9	48.4
p (t-test)	>0.19	>0.24	>0.17	<0.01	<0.004	>0.6	>0.25	<0.039

[a] The % increase is determined with reference to starting PD as far as possible: e.g., for N(AG04552) using SB203580 at 2500 nM the replicative capacity increase is (27.1 PDs–10 PDs)/(20.3 PDs–10 PDs) = 1.66% or a 66% increase in experimental replicative capacity. See Table S1 for starting PDs; [b] For definitions see Table S2; [c] nd = not done.

Table S4. Percentage increases in replicative capacity using SB203580 and BIRB796 [a].

Strain [b]	Inhibitors [c]	SB2500	B2500
Ny (Normal) < 25 years			
N(AG06234)		34.5	45.6
N(GM05399A)		52.2	101.5
N(GM05400B)		41.3	82.5
Mean ± SD		42.7 ± 8.9	76.5 ± 28.4
N (Normal) 44 years			
N(AG13156)		56.0	89.5
No (Normal) > 64 years			
N(AG04552)		66	119
N(AG11020)		24.6	84.4
N(AG11081)		54	99.3
N(AG13152)		60.6	85.4
Mean ± SD		51.3 ± 18.5	97.1 ± 16.1
p (t-test) Ny v No		>0.49	>0.27
WS (Werner Syndrome)			
WS(AG05229)		114	236
WS(AG03141F)		128.4	168
WS(AG03141B)		93.9	392
WS(AG03141D)		nd [d]	173
Mean ± SD		112 ± 17.3	242.1 ± 104.6
p (t-test) WS v N (all)		<0.00014	<0.0019
p (t-test) WS v Ny		<0.0036	<0.048
p (t-test) WS v No		<0.007	<0.034
WS(AG12800)		490	865

[a] For details see Table S2 notes; [b] Ny = young individuals, No = old individuals; [c] Inhibitors at 2.5 μM; [d] nd = not done.

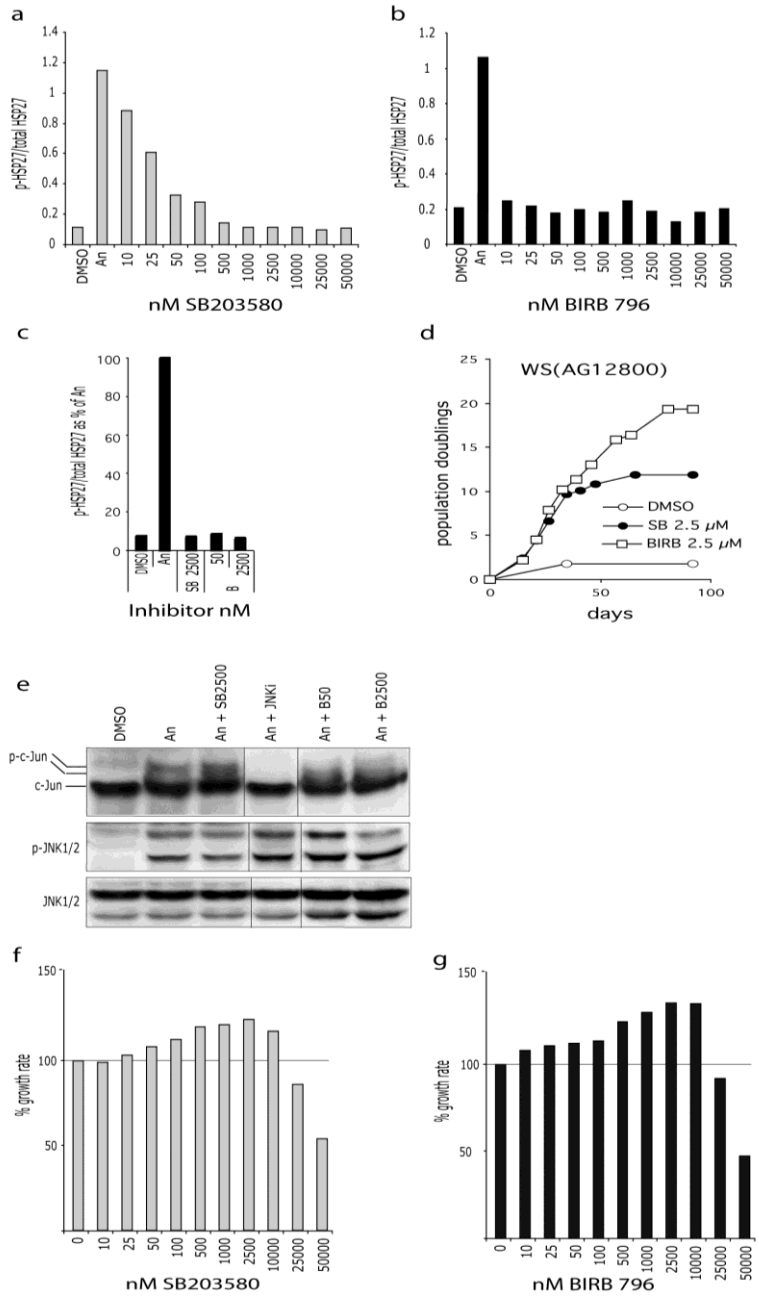

Figure S1. Supplementary Figures. (**a**, **b**) p38 inhibition profiles for SB203580 and BIRB 796 in HCA2^{tert} cells, assays as described in Materials and Methods. The graphs show ratio of pHSP27/HSP27. DMSO is control, An is cells stimulated with 30 μM anisomycin for 45 mins, 10 to 50,000 are cells pre-incubated with inhibitors for2 h prior to anisomycin stimulation. (**c**) Inhibition profiles for SB203580 (SB) and BIRB 796 (B) added to cells 24 h prior to anisomycin stimulation, concentrations given. (**d**) Growth curves for WS(AG12800) cells. (**e**) Immunoblot showing that neither SB203580 nor BIRB 796 significantly inhibit JNK1/2 or prevent their activation at 2.5 μM indicated by the presence of the p-c-Jun doublet in lanes 2,3,5 and 6 in the top panel and the doublet in lanes 2,3,5 and 6 in the middle panel (symbols as for c). Lane 4 shows the effect of a JNK1/2 inhibitor (see reference 37). Note that the sections of each panel are from the same immunoblot and each section has not been handled differently. (**f**, **g**) Effects of SB203580 and BIRB 796 on the growth rates of WS^{tert} cells (repeated experiment): see Figure 1 for details.

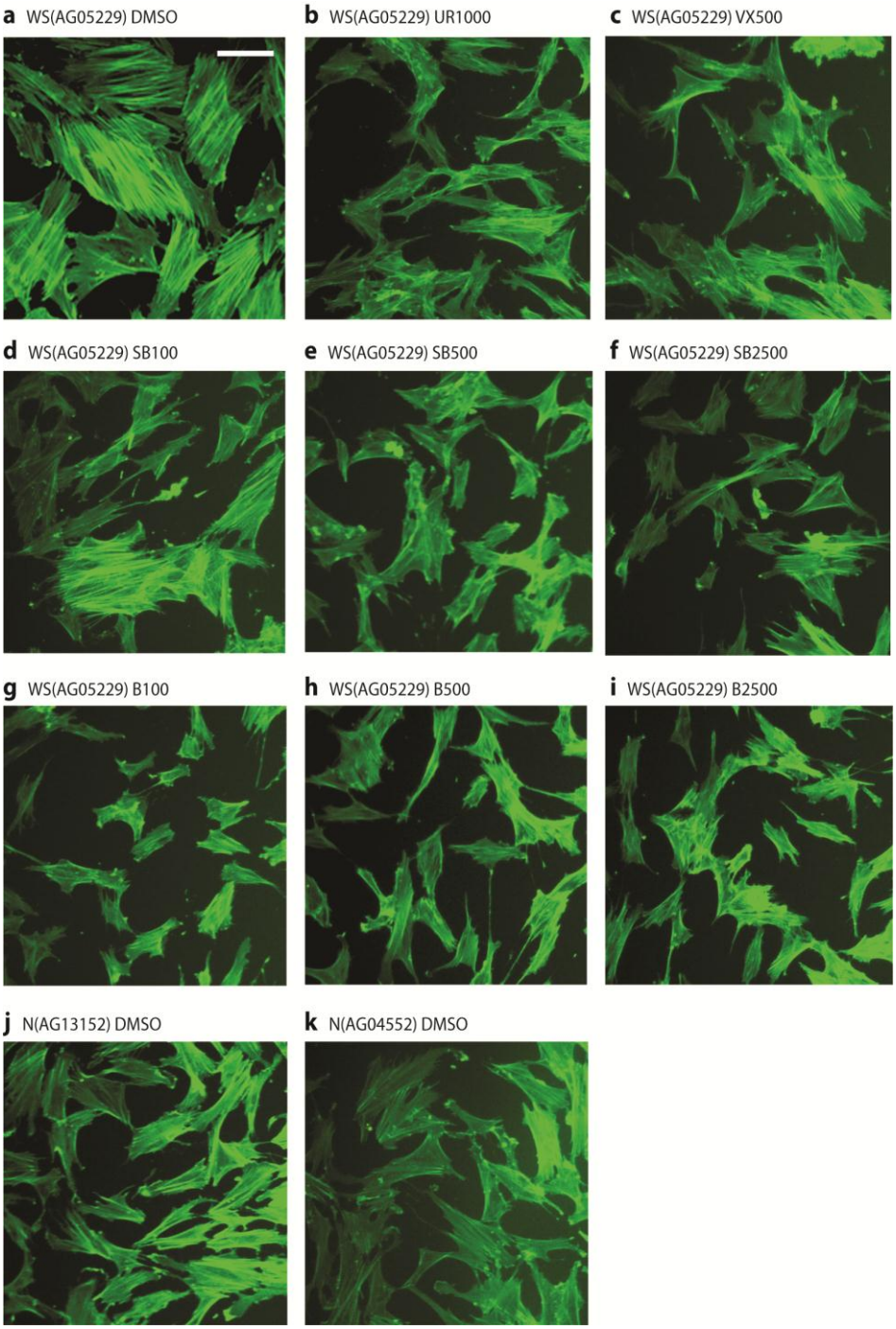

Figure S2. Figure 4. Stress fibre phenotypes of WS(AG05229) cells. Phalloidin stains for WS(AG05229) cells (**a**–**i**) and NDFs (**j**, **k**). Each panel labeled with strain and inhibitor used (symbols for inhibitors as in legend to Figure 3). These are representative figures from many experiments. Bar = 100 μm for each panel.