

Supplementary Information for

Cells exhibiting strong $p16^{INK4a}$ promoter activation in vivo display features of senescence

Jie-Yu Liu^{a,b}, George P. Souroullas^c, Brian O. Diekman^{b,d,e}, Janakiraman Krishnamurthy^b, Brandon M. Hall^f, Jessica A. Sorrentino^b, Joel S. Parker^{b,g}, Garrett A. Sessions^d, Andrei V. Gudkov^{f,h}, and Norman E. Sharpless^{a,b,i,j,1}

Norman E. Sharpless

Email: Norman.Sharpless@NIH.gov

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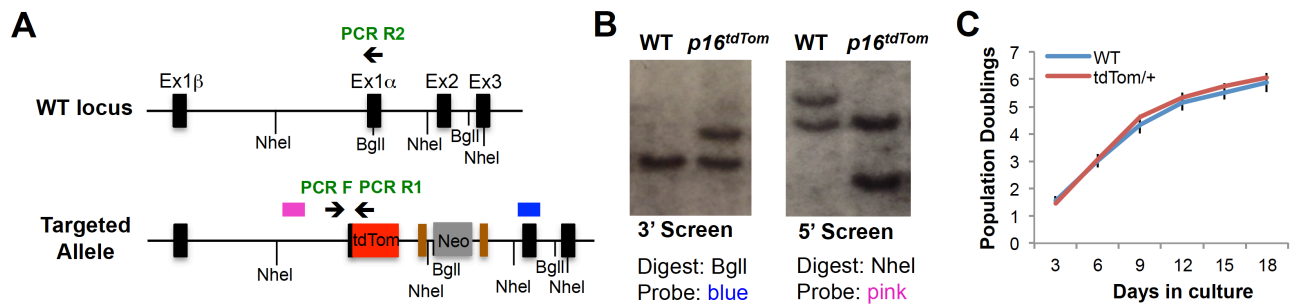


Fig. S1. Validation of the *p16*^{tdTom} allele design (A) Schematic of Southern blotting and PCR genotyping strategy. tdTom, tandem-dimer Tomato. Neo, neomycin selection cassette. Brown bars denote flippase (FLP) recognition sites. The 5' and 3' Southern probes are shown as pink and blue boxes. PCR primers are shown in arrowheads. (B) Southern blot analysis demonstrating correct 3' and 5' insertion of the tdTom construct in targeted embryonic stem cells. (C) Growth curves generated in littermate MEFs cultured on 3T3 schedule. Data shown correspond to four biological replicates for each genotype. Error bars represent SEM.

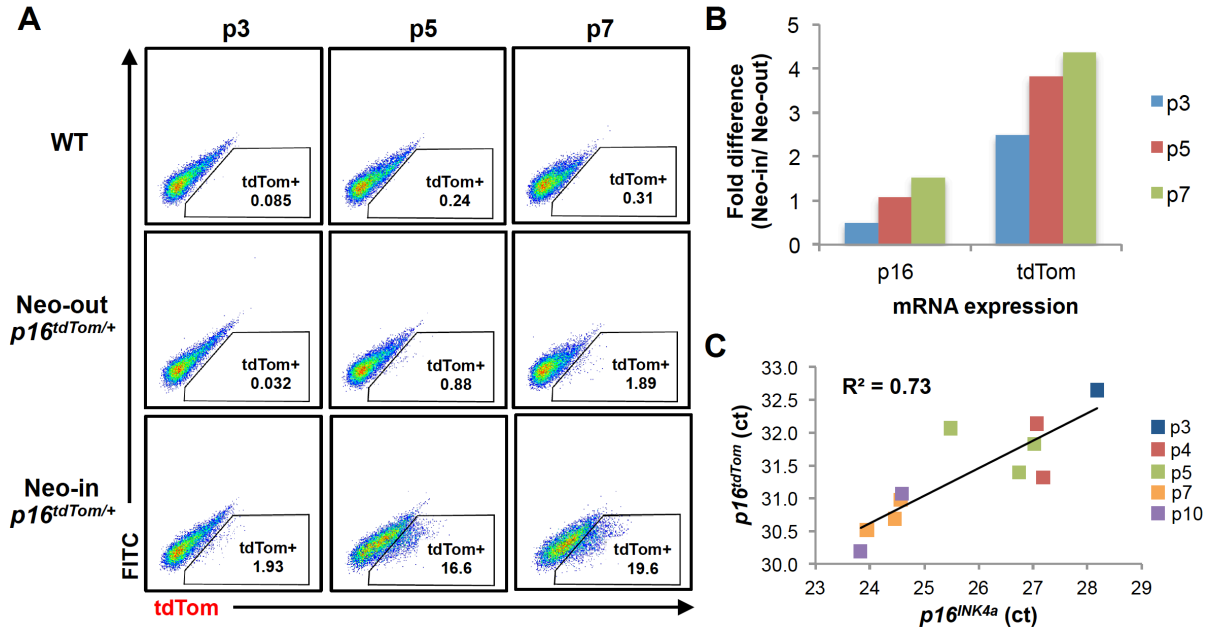


Fig. S2. Influence of a retained Neomycin selection cassette on $p16^{tdTom}$ expression. (A) Induction of tdTomato (tdTom) expression in $p16^{tdTom/+}$ MEFs with (Neo-in) or without (Neo-out) the Neo cassette with serial passage. p3 = passage 3; p5 = passage 5; p7= passage 7. Representative FACS analysis of MEFs at indicated genotypes and passage number are shown. (B) Expression of $p16^{INK4a}$ and $tdTom$ by qRT-PCR. Fold difference was calculated by dividing the Neo-in value by the Neo-out value. (C) Correlation of $p16^{INK4a}$ and $tdTom$ mRNA expression in Neo-out $p16^{tdTom/+}$ MEFs. Passage number is presented by different colors. Linear regression was used to calculate the coefficient of determination (R^2).

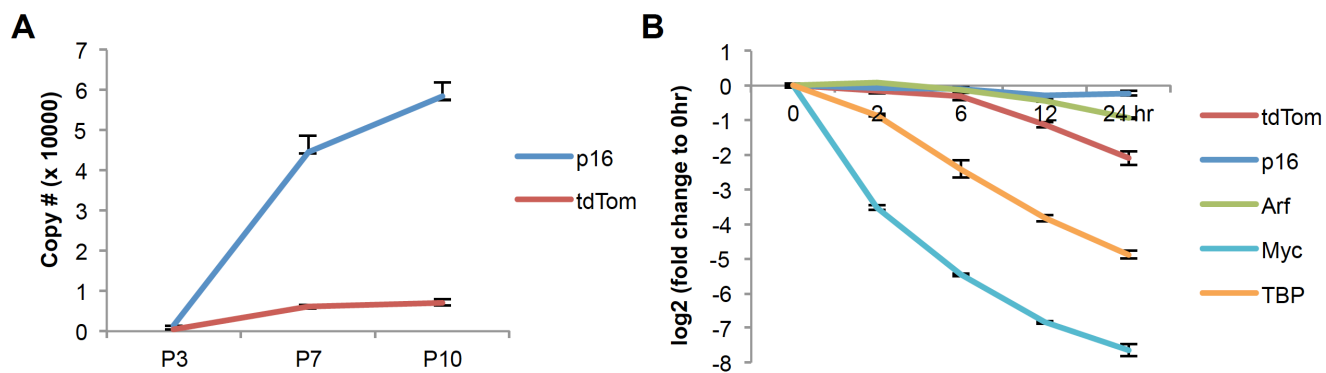


Fig. S3. Transcript stability of *tdTom* and *p16^{INK4a}*. (A) Absolute copy number of the *p16^{INK4a}* and *tdTom* transcripts as determined by qRT-PCR in *p16^{tdTom/+}* MEFs shown at the indicated passage numbers. (B) The stability of the indicated transcripts in *p16^{tdTom/+}* MEFs was determined by inhibition of mRNA synthesis using actinomycin D treatment followed by relative quantification of the mRNA levels of the indicated genes by qRT-PCR at the indicated time points. Fold change was calculated versus the time zero mRNA level with normalization to highly stable ribosomal RNA (18S). Throughout, error bars represent SEM.

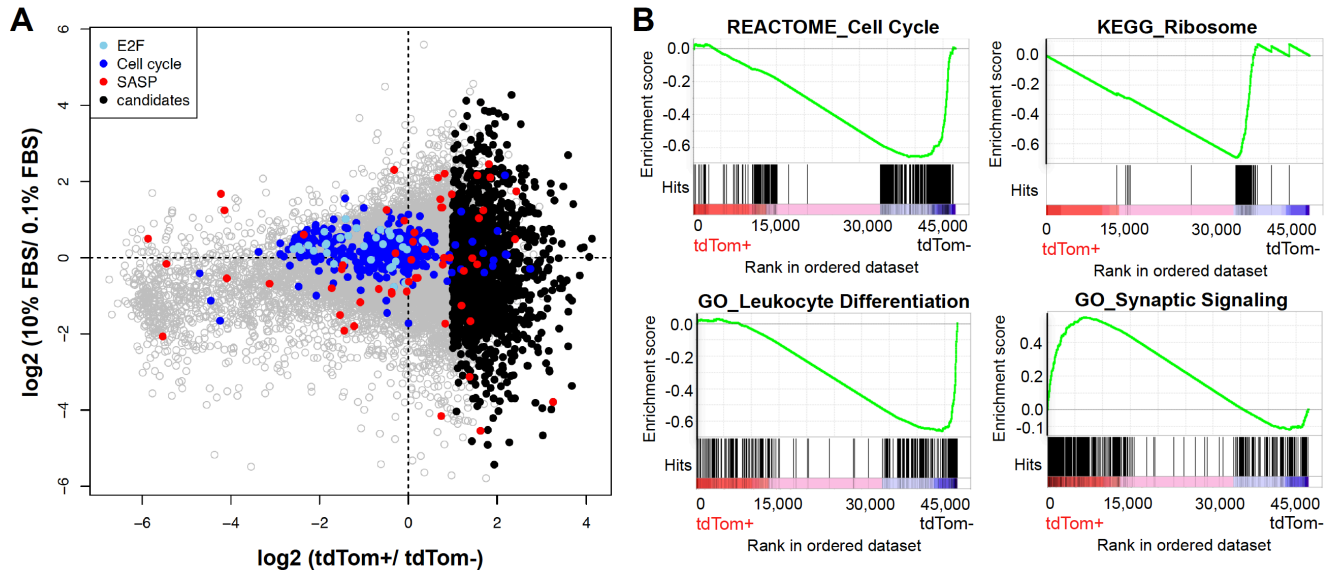


Fig. S4. Gene expression profile of $p16^{\text{INK4a}}$ -activated MEFs. (A) Plot of differentially expressed genes (DEGs) in tdTom^- vs. tdTom^+ populations from MEFs at passage 7 as determined by whole transcriptome RNA-seq. \log_2 transformed ratios of mRNA expression in tdTom^+ vs. tdTom^- is shown on the x-axis and normal serum (10%FBS) versus serum starved cells (0.1%FBS) is shown on the y-axis. Each dot represents a unique DEG and is colored as indicated groups (E2F, Cell cycle, SASP and candidates). Candidates are DEGs upregulated more than 2-fold in tdTom^+ populations. (B) GSEA of tdTom^- vs. tdTom^+ MEFs. Representative plots for significantly enriched gene sets at false-discovery rate < 0.01 are shown.

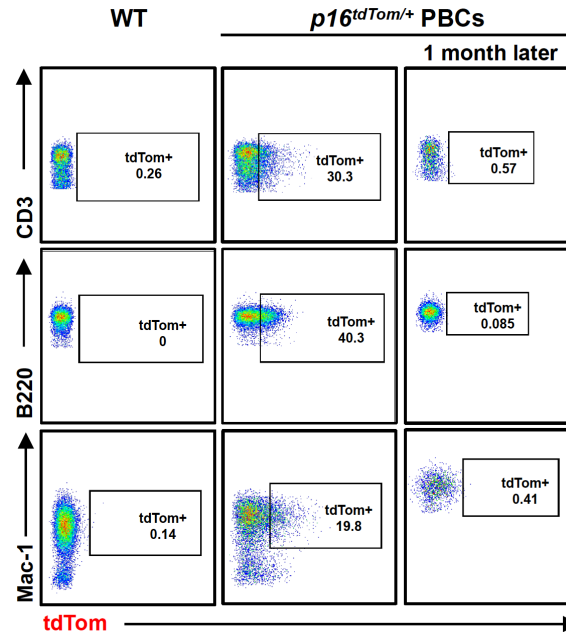


Fig. S5. Transient *p16^{INK4a}* activation in peripheral blood cells (PBCs). Representative FACS analysis of CD3⁺ (T cells), B220⁺ and Mac-1⁺ (myeloid cells) populations in the peripheral blood harvested from a 18-wk-old wild-type (WT) mouse as well as a *p16^{tdTom/+}* littermate mouse. For the *p16^{tdTom/+}* mouse, results are also shown one month after the initial observation.

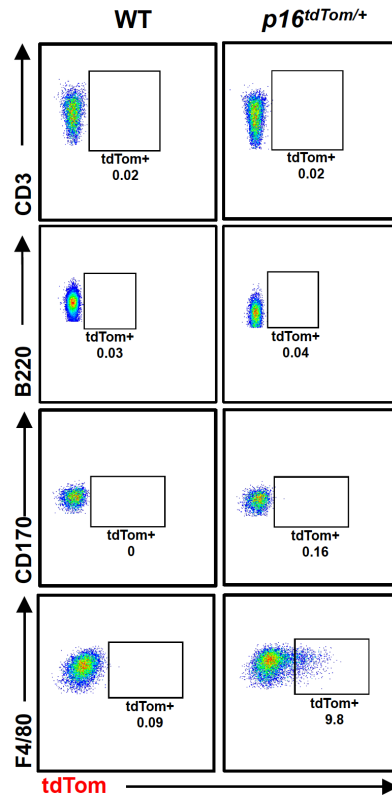


Fig. S6: Expression of tdTom in peritoneal lavage cells. Representative FACS analysis of CD3⁺ (T cells), B220⁺, CD170⁺ (eosinophils) and F4/80⁺ (macrophages) in the peritoneal lavage harvested from wild-type (WT) and *p16*^{tdTom/+} mice 21 days after NDF-bead injection.

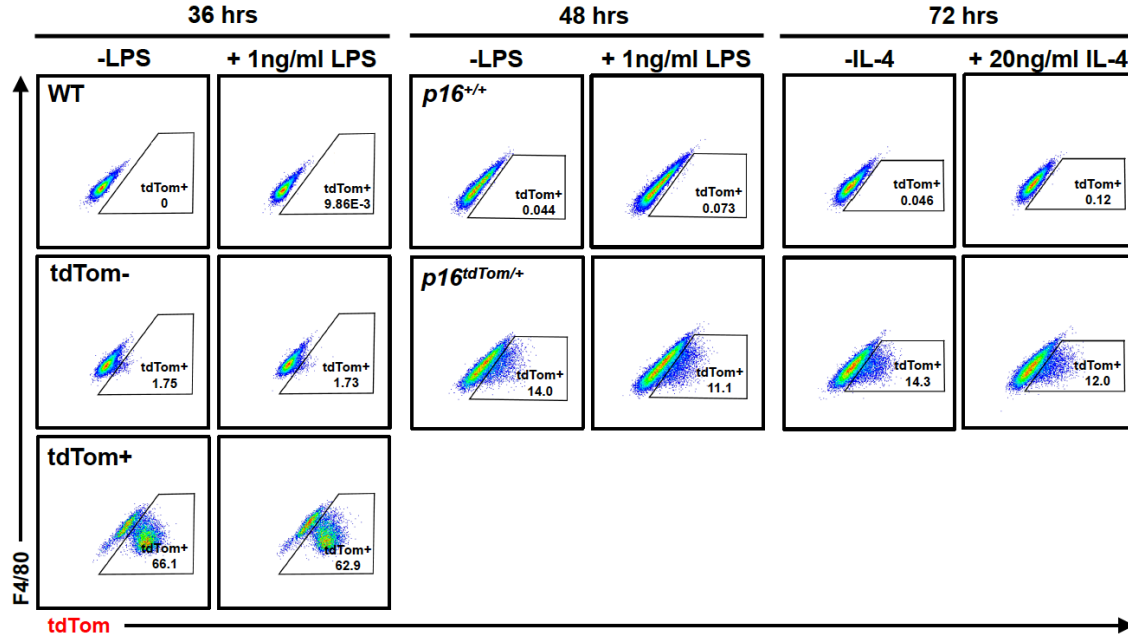


Fig. S7: Effect of M1/M2-polarizing agents on the $p16^{INK4a}$ promoter activity in beads-elicited macrophages. (A) Representative FACS analysis of sorted tdTom⁻ and tdTom⁺ populations after treated with LPS (1 ng/mL) for 36 hours. (B-C) Representative FACS analysis of peritoneal lavage cells after treated with either LPS (1 ng/mL) for 48 hours (B) or IL-4 (20 ng/mL) for 72 hours (C).

Table S1. Expression of SASPs in tdTom⁺ peritoneal macrophages (MACS) and tdTom⁺ MEFs

	MACS baseMean	MACS log2FoldChange	MACS padj	MEF baseMean	MEF log2FoldChange	MEF padj
Ang	131.501	0.430	0.182	744.207	-1.727	0.000
Areg	151.283	0.156	0.759	93.364	-0.492	0.325
Axl	2911.053	-0.098	0.922	64334.084	-0.091	0.760
Ccl1	0.242	0.003	NA	1.265	2.407	0.024
Ccl2	285.096	0.315	0.615	5715.144	-0.291	0.398
Ccl25	137.336	-0.181	0.643	335.850	0.375	0.029
Ccl3	118.996	0.517	0.235	2132.738	-5.538	0.000
Ccl5	12.386	0.342	0.464	80.699	-1.479	0.002
Ccl8	24.778	0.543	NA	155.004	3.251	0.000
Csf2	0.518	0.002	NA	1.411	0.667	0.585
Csf3	77.000	0.071	0.873	20.866	0.738	0.319
Ctsb	137262.390	0.441	0.000	229706.379	-1.084	0.012
Cxcl1	3946.179	0.301	0.544	638.640	-0.373	0.563
Cxcl12	32.169	1.751	0.000	24769.308	0.212	0.746
Cxcl13	391.744	1.212	0.000	3.097	-1.444	0.196
Cxcl16	1235.565	-0.154	0.792	7636.033	-4.093	0.000
Cxcl2	20384.673	0.224	0.740	3860.968	-5.866	0.000
Cxcl3	47.158	0.030	0.966	401.548	-4.144	0.000
Egf	65.384	0.153	0.789	70.988	-0.376	0.102
Egfr	10.187	0.788	0.011	8599.624	0.807	0.020
Ereg	27.593	0.148	0.653	1348.615	0.719	0.086
Fas	796.679	0.177	0.772	2057.387	1.398	0.000
Fgf2	120.586	0.124	0.871	8781.698	1.552	0.000
Fgf7	1.747	0.031	NA	2157.231	0.137	0.823
Glb1	15766.051	0.828	0.001	6237.621	0.168	0.620
Hgf	42.499	0.653	0.089	1289.884	0.831	0.001
Icam1	1848.381	0.571	NA	2863.047	-1.538	0.000
Igfbp4	115.900	-0.332	0.532	20754.398	-0.667	0.013
Igfbp6	95.692	-0.548	0.195	1120.102	1.550	0.000
Igfbp7	1.432	0.102	NA	39901.597	1.590	0.000
Il11	3.313	-0.190	0.766	427.149	1.848	0.000
Il15	406.580	0.215	0.649	314.598	-1.222	0.000
Il1a	7302.602	0.305	0.580	73.566	-4.224	0.000
Il1b	2007.471	0.083	0.903	568.525	-5.454	0.000
Il6	21207.990	0.059	0.952	152.437	0.820	0.003
Il7	104.459	0.944	0.002	270.007	0.939	0.000
Lmnb1	726.760	-0.441	0.297	6238.705	-2.356	0.000
Mif	2236.540	-0.306	0.049	10969.887	0.105	0.692
Mmp12	145.194	0.922	0.003	1267.262	-3.128	0.000

Mmp13	19.772	0.395	0.426	1059.124	-1.501	0.024
Mmp14	613.516	-0.241	0.738	38723.161	0.775	0.000
Ngf	2.013	-0.012	NA	2415.422	0.979	0.006
Nrg1	126.298	-0.342	0.573	2882.071	1.252	0.000
Pgf	5.673	0.076	0.938	435.082	1.688	0.000
Pigf	218.036	-0.205	0.673	1144.901	0.069	0.679
Plaur	16656.705	0.257	0.653	4729.421	-0.317	0.487
Timp2	83558.985	0.476	0.002	96689.802	1.442	0.000
Tnfrsf1a	10795.358	-0.017	0.957	10041.863	0.010	0.961
Ccl11	NA					
Ccl13	NA					
Ccl16	NA					
Ccl20	NA					
Ccl26	NA					
Cxcl4	NA					
Cxcl5	NA					
Cxcl8	NA					
Icam3	NA					
Ifng	NA					
Igfbp1	NA					
Igfbp2	NA					
Igfbp3	NA					
Il13	NA					
Il8	NA					
kitlg	NA					
Lep	NA					
Mmp1	NA					
Mmp10	NA					
Mmp3	NA					
Sgp130	NA					
Tnfrsf10c	NA					
Tnfrsf11b	NA					
Vefga	NA					
Vegf	NA					