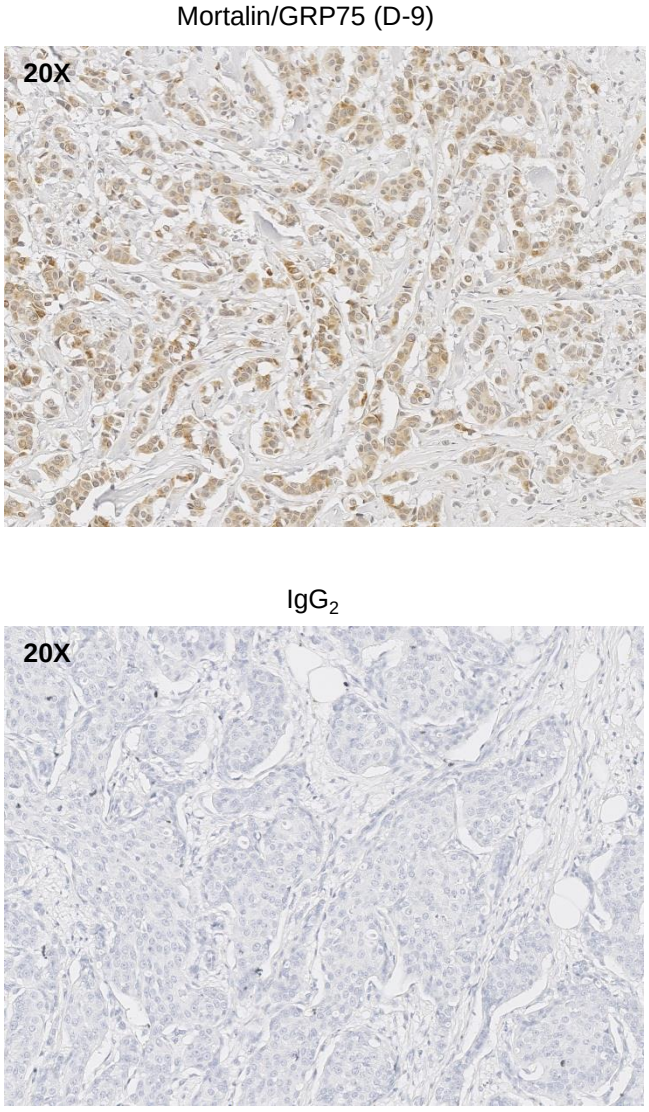

Figure S1. Validation of mortalin antibody (D-9) for immunohistochemistry. Mortalin antibody (D-9) and its control IgG type 2 were tested for specific recognition of mortalin in human breast cancer tissues (antibody dilution, 1:200).

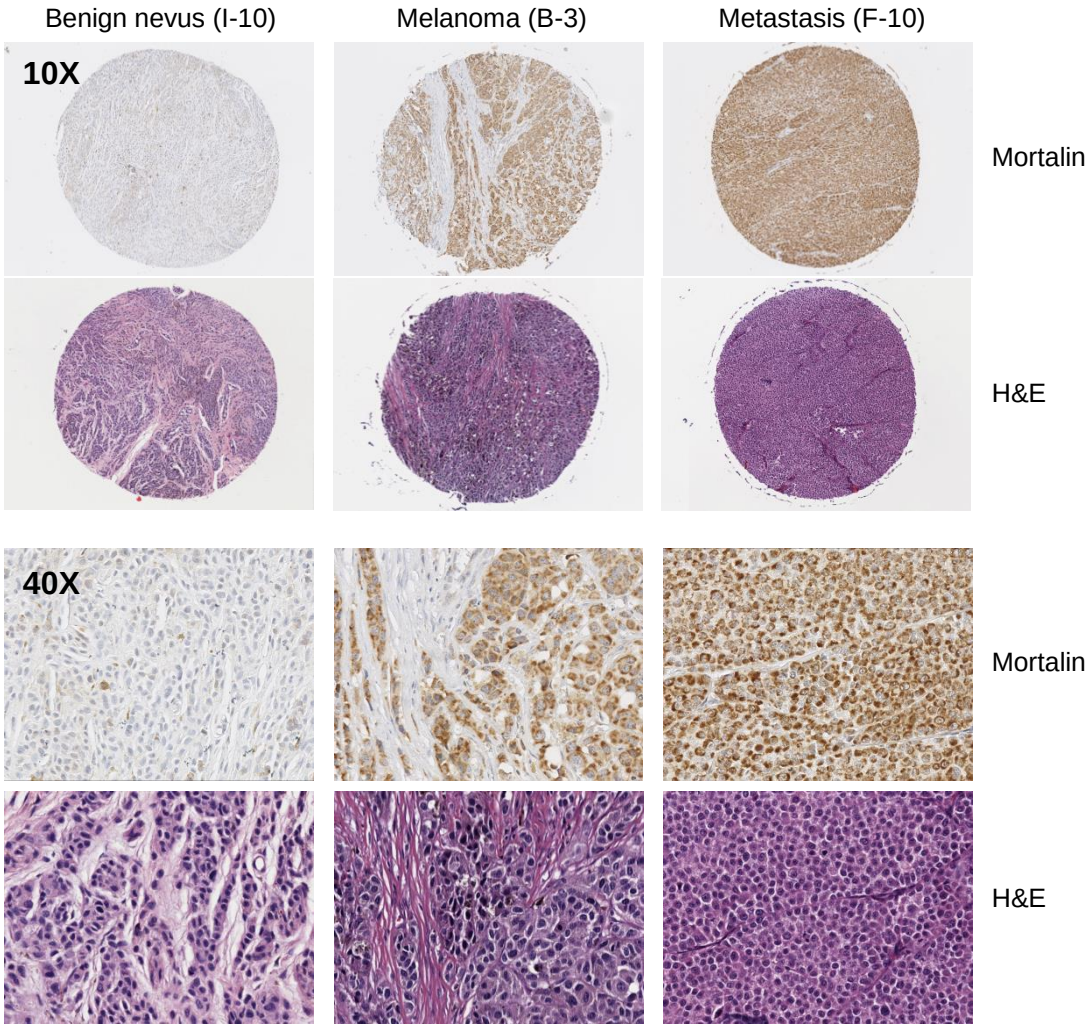

Figure S2. H&E staining images corresponding to Fig. 2C. Formalin-fixed, paraffin-embedded 5 µm sections of melanoma tissue microarray (ME1004a), containing 100 cases (melanoma, 56; metastatic melanoma, 20; and nevus, 24) was purchased from US Biomax (Rockville, MD). The matching images of H&E staining were downloaded from the supplier's website (http://www.biomax.us/tissue-arrays/Melanoma/ME1004a). Indicated are the core numbers in the tissue array.

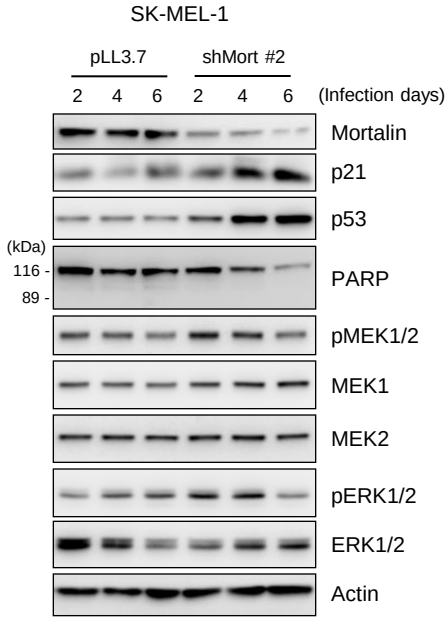

Figure S3. Effects of shMort #2 on SK-MEL-1. SK-MEL-1 cells were infected with the control pLL3.7 virus and shMort #2 for 5 days before Western blot analysis.

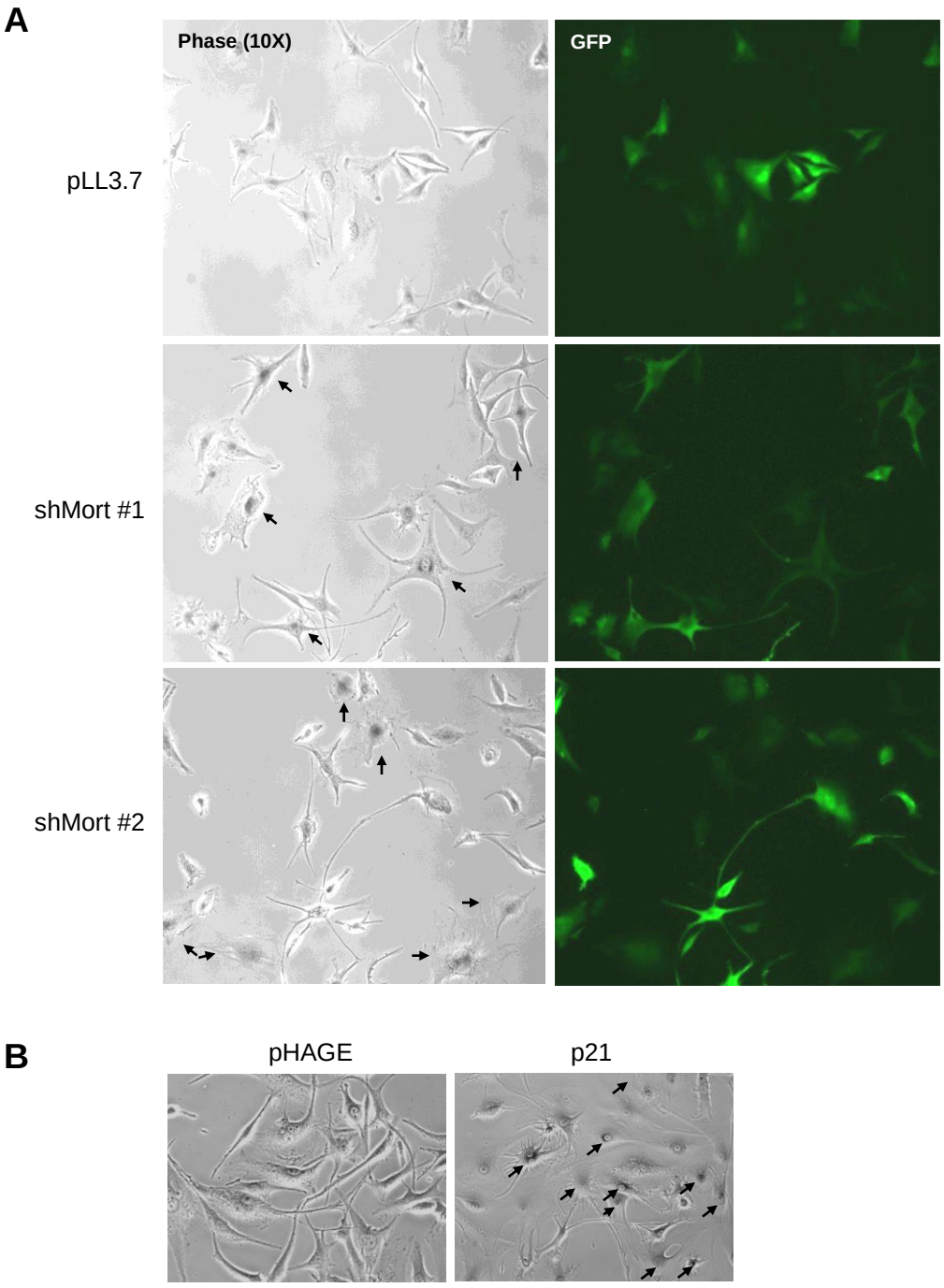

Figure S4. Mortalin knockdown or ectopic p21^{CIP1} expression increases SA β-gal staining in SK-MEL-28 culture. **(A)** Cells infected with viruses expressing two different shRNAs that target mortalin mRNA (shMort #1 and shMort #2) for 10 days were examined for SA β-gal staining (indicated by arrows). Similar infection ratio was verified by GFP expression (right panels). The empty pLL3.7 virus-infected cells were used for comparison. **(B)** Cells infected with lentiviral pHAGE expressing p21^{CIP1} or empty pHAGE were examined for SA β-gal staining at day 5.

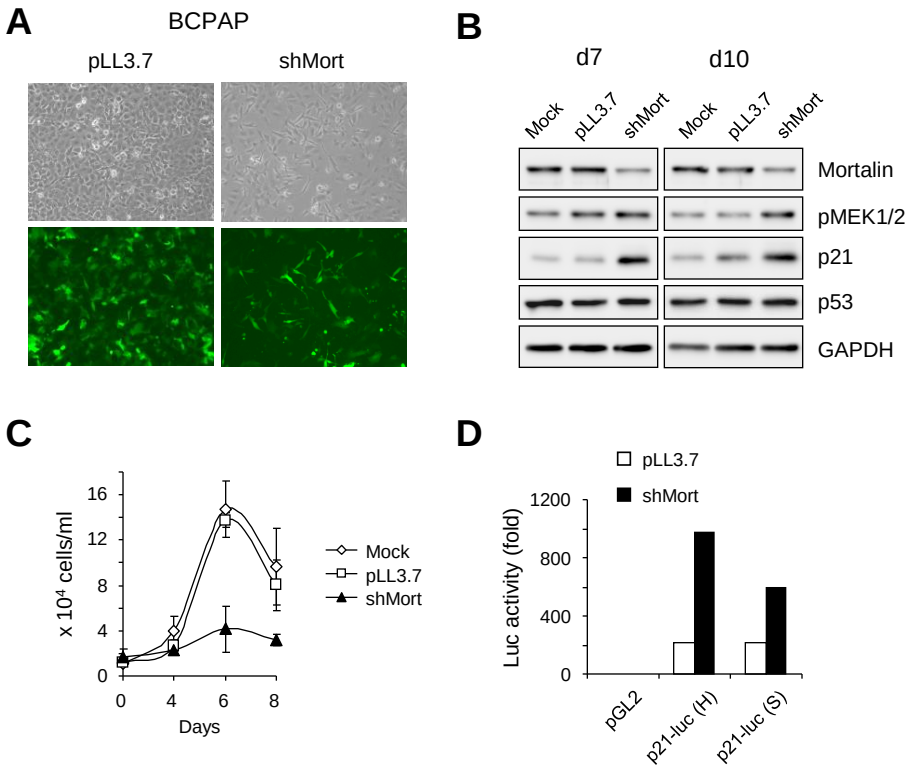

Figure S5. Mortalin knockdown induces growth inhibition in B-Raf^{V600E}-transformed thyroid cancer line, BCPAP. **(A)** BCPAP cells were infected with shMort#1 and its control empty pLL3.7 virus. GFP expression indicates infection efficiency (lower panels). **(B)** Cells infected for 7 and 10 days were examined for expression of the indicated proteins by Western blot analysis. **(C)** Proliferation rates were monitored by cell counting. Data (mean ± SEM) are from a representative experiment performed in triplicate. **(D)** Cells were co-transfected for 3 days with shMort#1 and the p21-luciferase reporters, H2320 and S2260, before determining luciferase activity. pGL2 is the control luciferase reporter vector. Data (mean ± SEM) from a representative experiment performed in triplicate are expressed as fold changes relative to the cells co-transfected with pGL2 and pLL3.7.

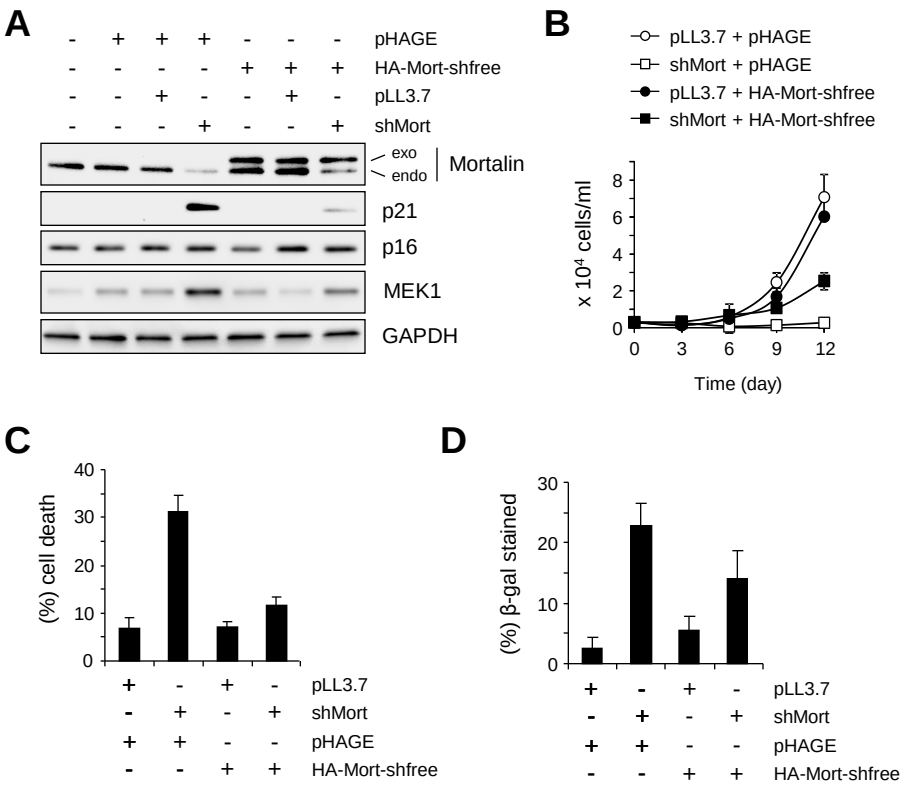

Figure S6. Expression of non-shRNA-targetable mortalin abrogates mortalin knockdown effects. SK-MEL-28 cells were co-infected with shMort#1 virus and the lentivirus expressing non-shMort-targetable mortalin tagged with HA at the N-terminus (HA-Mort-shfree). The empty viruses, pHAGE and pLL3.7, are the controls for HA-Mort-shfree and shMort viruses, respectively. Cells were examined for expression of the indicated proteins by Western blotting at post-infection day 5 **(A)**, proliferation rates by cell counting **(B)**, cell death by trypan blue staining at post-infection day 5 **(C)**, and SA β-gal staining at post-infection day 10 **(D)**. *P* value is < 0.05 for the shMort/Mort-shfree pair compared to the control shMort/pHAGE pair in (B, day 12), (C), and (D), respectively (Student's *t* test). Data (mean ± SEM) are from a representative experiment performed in triplicate.

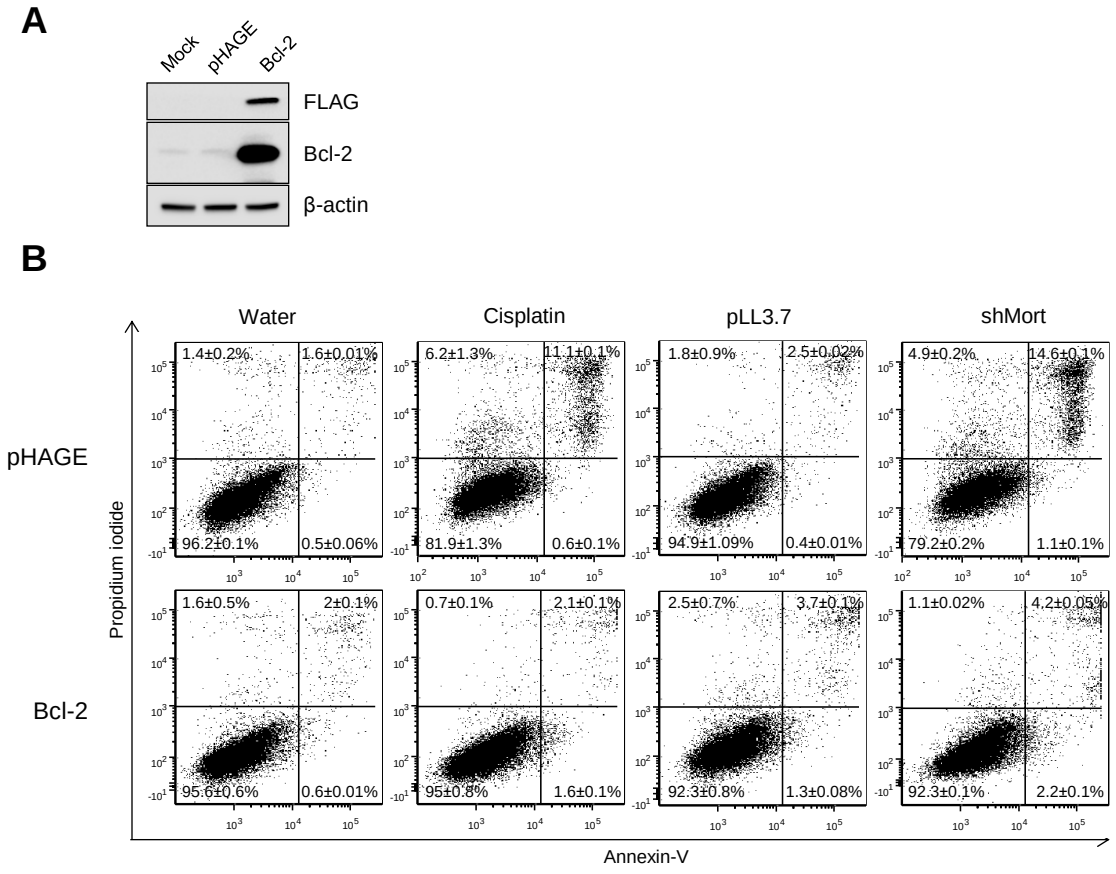

Figure S7. Bcl-2 overexpression renders HCT116 p21$^{-/-}$ cells resistant to cell death induced by cisplatin or mortalin knockdown. (A) HCT116 p21$^{-/-}$ cells were infected with lentiviral pHAGE-puro-FLAG-Bcl-2. Western blotting confirms expression of the exogenous Bcl-2. **(B)** Cells in (A) were treated with 50 μM cisplatin for 24h or were infected with lentiviral shMort for 4 days before the annexin V assay. Cisplatin was dissolved in water. (%) population of annexin V stained cells are scored in Fig. 4E.

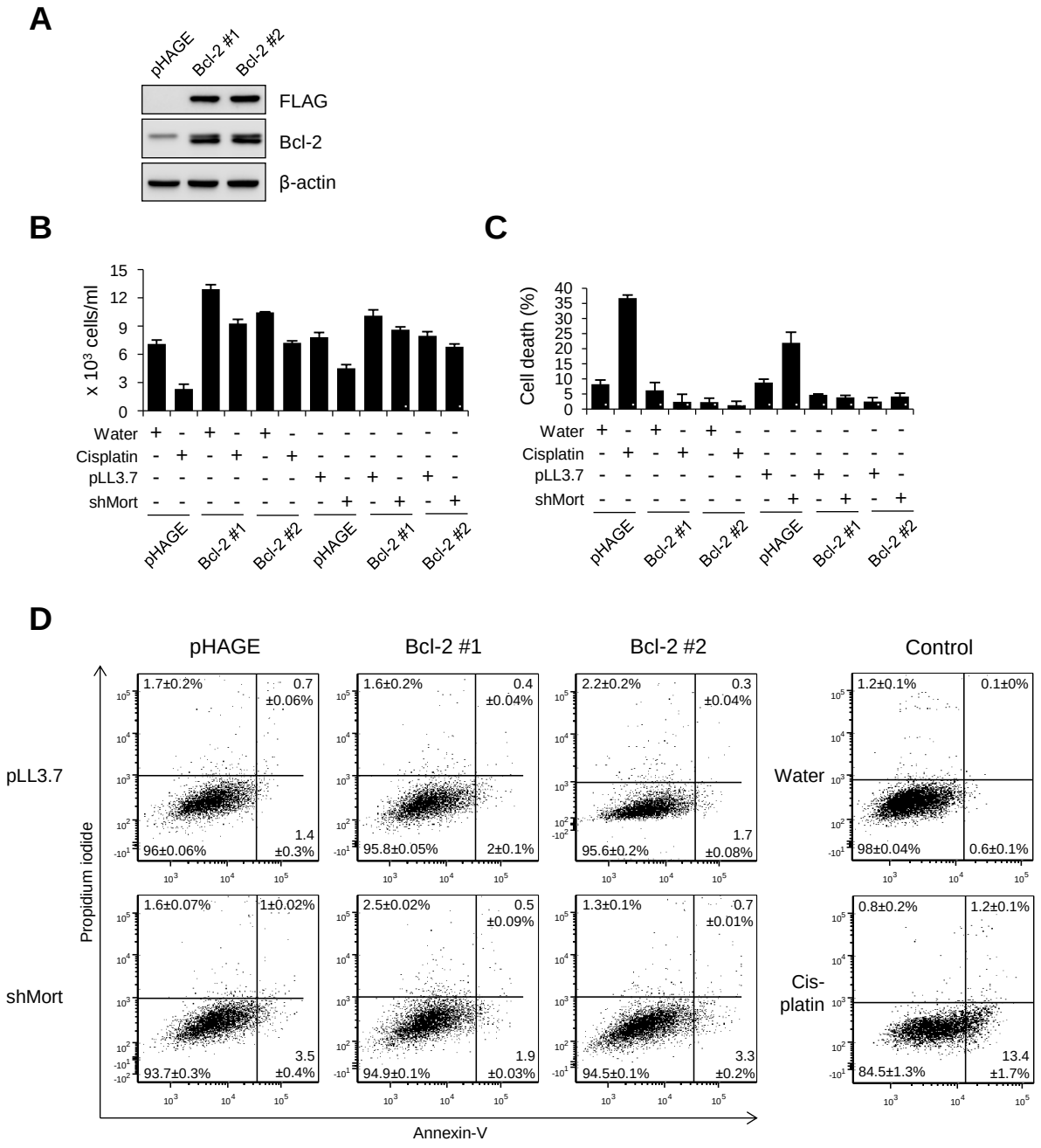

Figure S8. Bcl-2 overexpression renders SK-MEL-28 cells resistant to cell death induced by cisplatin or mortalin knockdown. (A) SK-MEL-28 cells were infected with lentiviral pHAGE-puro-FLAG-Bcl-2. Two independent SK-MEL-28 cells stably expressing FLAG-Bcl-2 were derived by puromycin selection. Western blotting confirms expression of the exogenous Bcl-2. **(B and C)** Cells in (A) were treated with 50 μM cisplatin for 24h (control for apoptosis induction) or were infected with lentiviral shMort for 4 days before counting live (B) and dead (C) cells using trypan blue. Cisplatin was dissolved in water. **(D)** shMort-infected cells in (B and C) were examined by the annexin V assay. Note that mortalin knockdown does not efficiently increase annexin V staining.

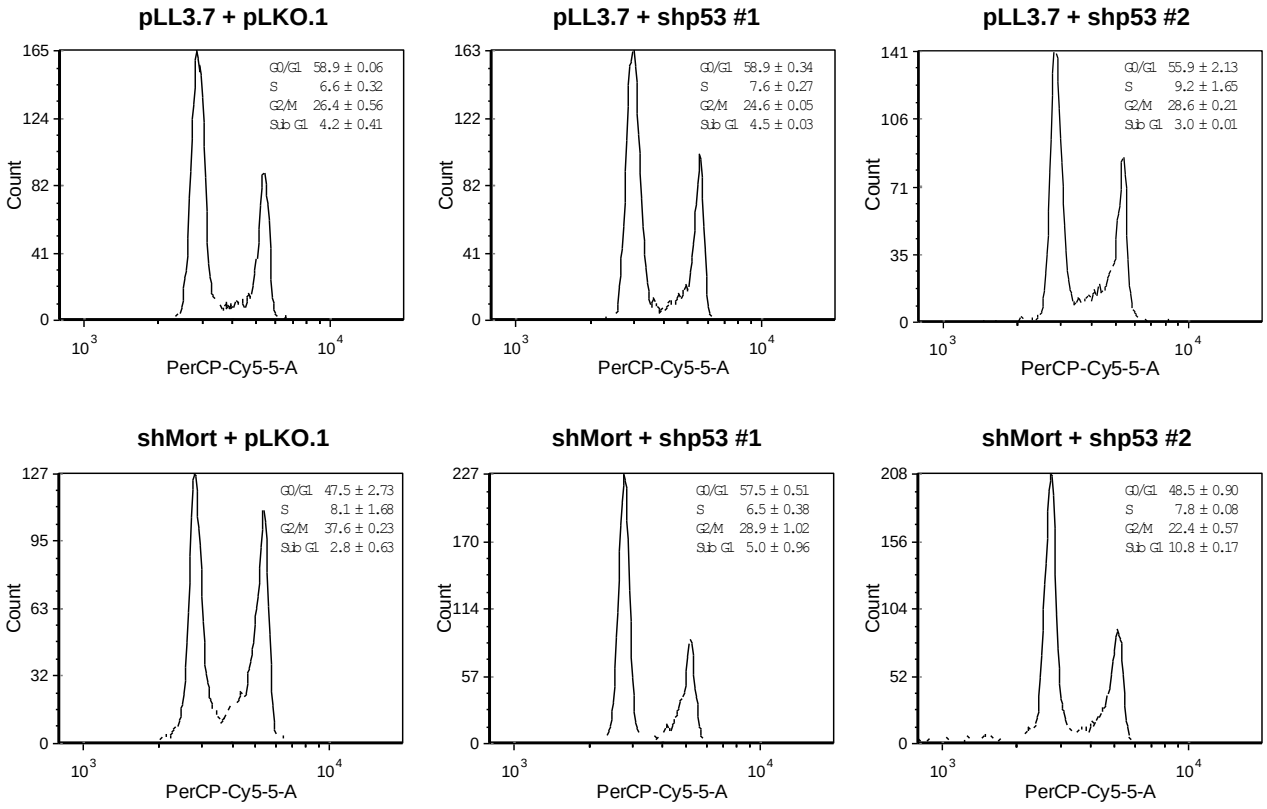

Figure S9. p53 knockdown abrogates G2/M arrest induced by mortalin knockdown in SK-MEL-1 cells. SK-MEL-1 cells were co-infected for 5 days with lentiviral shMort #1 and pLKO.1 expressing two different p53-targeting shRNAs (shp53 #1 and shp53 #2). pLKO.1 and pLL3.7 are controls for shp53 and shMort, respectively. Data (mean ± SEM) are from a representative experiment performed in triplicate.

Table S1. MAPK pathway and *TP53* status in test lines[1]

Cell line (tumor type)	MAPK pathway alteration	*TP53*
SK-MEL-1 (mel)	B-RafV600E (hetero)	WT
SK-MEL-28 (mel)	B-RafV600E (homo)	L145R (homo)
A375 (mel)	B-RafV600E (homo)	WT
RPMI-7951 (mel)	B-RafV600E (hetero)	S166* (homo)
SK-MEL-2 (mel)	N-RasQ61R (homo)	G245S (hetero)
MeWo (mel)	NF1Q1336* (homo)	E258K (hetero), Q317* (hetero)
8505C (PTC)	B-RafV600E (hetero)	R248G (homo)
BCPAP (PTC)	B-RafV600E (homo)	D259Y (homo)
HT29 (colon)	B-RafV600E (hetero)	R273H (homo)
HCT116 (colon)	K-RasG13D (homo)	WT
HCT116 $p21^{-/-}$	K-RasG13D (homo)	WT

[1]Information collected from the Cancer Genome Project at Sanger Institute (http://www.sanger.ac.uk/)

*non-sense mutation

Abbreviations: mel, melanoma; PTC, papillary thyroid cancer; het, heterozygous mutation; homo, homozygous mutation

Table S2. Primers used for generation of the gene expression constructs

Constructs		Sequences
pHAGE-HA-Mort	Foward	TTATCCGCTAGCATAAGTGCCAGCCGAGCTGCAGCAGCCCG
	Reverse	TTTCTGGGATCCTTACTGTTTTTCCTCCTTTTGATCTTCC
pHAGE-HA-Mort-shfree	Foward	CATTGGCCGGCGATACGACGACCCAGAAGTACAGAAAGAC
	Reverse	GTCTTTCTGTACTTCTGGGTCGTCGTATCGCCGGCCAATG
pHAGE-HA-HSC70	Foward	CCAGCAGCTAGCTCCAAGGGACCTGCAGTTGGTATTGATCTTG
	Reverse	CACTTGGGATCCTTAATCAACCTCTTCAATGGTGGGCCCTGAG
pHAGE-HA-BIP	Foward	GCTGGCGCTAGCAAGCTCTCCCTGGTGGCCGCGATGCTGCTG
	Reverse	GCAGATGGATCCCTACAACTCATCTTTTTCTGCTGTATCCTC
pHAGE-HA-HSP72	Foward	GGAACCGCTAGCGCCAAAGCCGCGGCGATCGGCATCGACCTG
	Reverse	TTGGAAGGATCCCTAATCTACCTCCTCAATGGTGGGGCCTGA
pHAGE-ERK1-K71R/T202A/Y204F	Foward	CACACCGGCTTCCTGGCGGAGTTTGTGGCTACGCGCTGG
	Reverse	GTGTGGCCGAAGGACCGCCTCAAACACCGATGCGCGACC

Table S3. Primers used for qPCR

Targets		Sequences
$p21^{CIP1}$ (526–844)	Foward	CTGGAGACTCTCAGGGTCGAA
	Reverse	CCAGCACTCTTAGGAACCTCTCA
MEK1 (574-885)	Foward	CAGAAGAAGCTGGAGGAGCTAG
	Reverse	CCATCGCTGTAGAACGCACCAT
MEK2 (343-658)	Foward	CGAGGCAAACCTGGTGGACCT
	Reverse	CCGTAGAAGCCCACGATGTAC
Actin (1,543-1,731)	Foward	GTCCTCTCCCAAGTCCACAC
	Reverse	GGGAGACCAAAAGCCTTCAT
GAPDH (191-498)	Foward	CGGAGTCAACGGATTTGGTCGTAT
	Reverse	AGCCTTCTCCATGGTGGTGAAGAC