SUPPORTING INFORMATION

E3 ubiquitin ligase PARK2, an inhibitor of melanoma cell growth, is repressed by the oncogenic ERK1/2-ELK1 transcriptional axis

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Supplementary Figure Legends

Supplementary Figure 1. Kaplan-Meier progression free survival (left) and overall survival (right) curves in metastatic melanoma patients from the TCGA cohort (n=24). Patients were stratified into two groups based on high (> 4.587) and low (< 4.587) *PARK2* expression values.

Supplementary Figure 2. PARK2 is negatively modulated by the RAS-RAF-MEK1/2-ERK1/2 signaling pathway. Western blot analysis of PARK2 and pERK1/2 in 501-Mel melanoma cells treated with vemurafenib (**A**) or SCH-772984 (**B**) for the indicated time. HSP90 was used as loading control.

Supplementary Figure 3. PARK2 expression is negatively modulated by EGF. qPCR analysis of *Cyclin D1* (**A**)*, c-Fos* (**B**) and *PARK2* (**C**) in HEK-293T cells treated with EGF (20ng/ml) for the indicated time. The y axis represents expression ratio of gene/(*GAPDH* and *β-ACTIN* average), with control equated to 1. Data shown are mean ± s.e.m. of at least three biological replicates, each performed in triplicate.

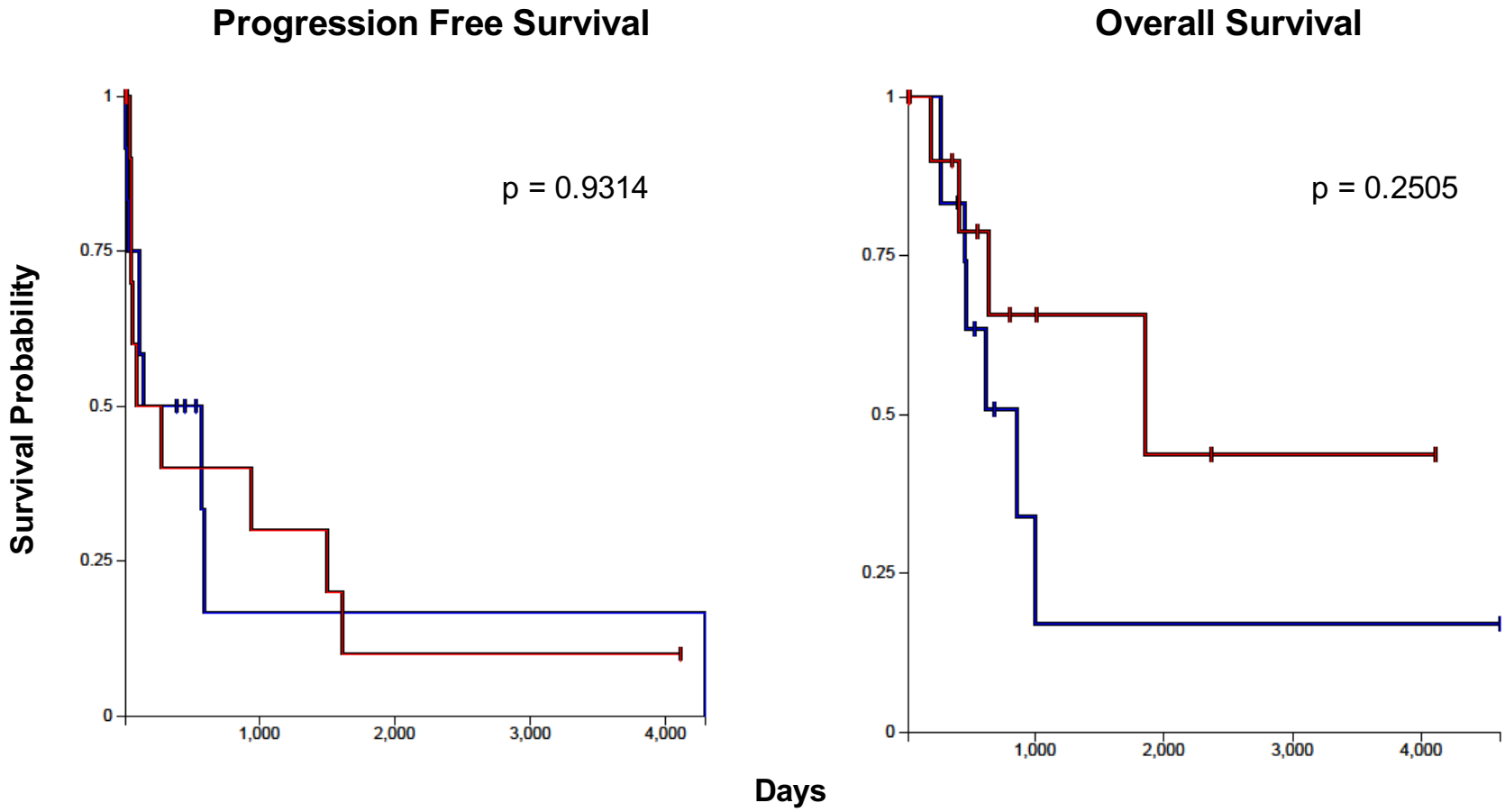
Supplementary Figure 4. Sequence of *PARK2* promoter. The position of ELK1 binding site is highlighted in green, sequences of primers for ChIP are in bold. Primers for mutagenesis of the ELK1 binding site are reported.

Supplementary Figure 5. ELK1 negatively modulates the expression of PARK2. **A.** Chromatin immunoprecipitation (ChIP) assay showing that exogenous ELK1 binds to *PARK2* promoter in HEK-293T cells transfected as indicated. Two different specific anti-ELK1 antibodies were used. **B.** ChIP assay showing that silencing of ELK1 reduces the binding to *PARK2* promoter in A375 cells transduced as indicated. The y-axis represents the relative promoter enrichment, normalized on the input material. IgG was used as negative control and set to 1. **C.** Expression of *ELK1* and *PARK2* mRNA by qPCR in A375 cells transduced as indicated. The y axis represents expression ratio of gene/(*GAPDH* and *β-ACTIN* average), with the level of control equated to 1. **D.** Western blot (WB) analysis of PARK2 and ELK1 in A375 cells transduced as indicated. HSP90 was used as loading control. **E.** Expression of *PARK2* and *ELK1* mRNA by qPCR in SK-Mel-5 cells transduced as indicated. The y axis represents expression ratio of gene/(*GAPDH* and *β-ACTIN* average), with control equated to 1. **F.** WB analysis of PARK2 and ELK1 in SK-Mel-5 cells transduced as indicated. HSP90 was used as loading control. **G.** qPCR analysis of *PARK2* and *ELK1* mRNA in HEK-293T cells overexpressing empty vector (pCAG) or ELK1. The y axis represents expression ratio of gene/(*GAPDH* and *β-ACTIN* average), with control equated to 1. **H.** WB analysis of PARK2 and ELK1 in HEK-293T cells transfected as indicated. HSP90 was used as loading control. Data shown are mean ± s.d. (A, B) or mean ± s.e.m. (C, E, G) of at least three biological replicates, each performed in triplicate. *, $p<0.05$; **, $p<0.01$.

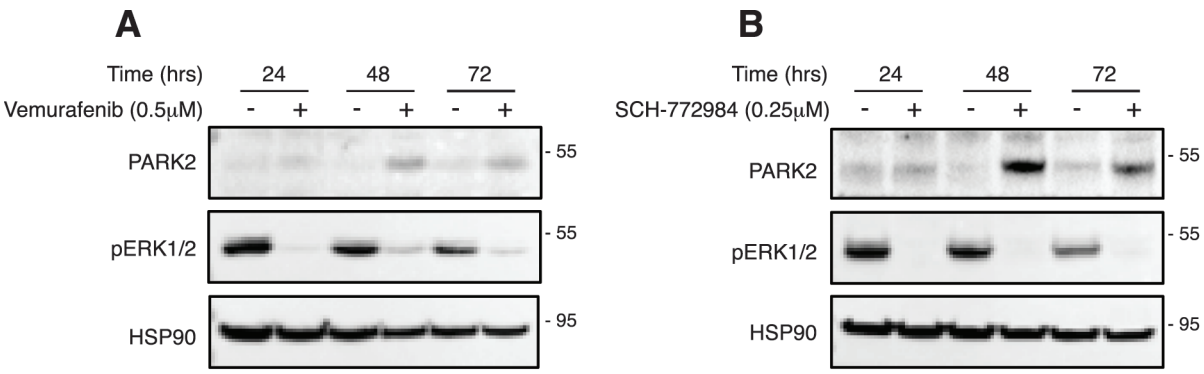
Supplementary Figure 6. ELK1 negatively modulates the expression of PARK2 in glioblastoma and breast cancer cells. **A-B.** Expression of *PARK2* and *ELK1* mRNA by qPCR in U87MG (HTB-14) glioblastoma and MCF7 (HTB-22) breast cancer cell lines transiently transfected with empty vector (pCAG) or pELK1. Both cell lines were grown in EMEM supplemented with 10% fetal bovine serum (FBS), 1% Penicillin-streptomycin, 2 mM L-Glutamine (Lonza, Basel, Switzerland). The y axis represents expression ratio of gene/(*GAPDH* and *β-ACTIN* average), with control equated to 1. **C.** Western blot analysis of endogenous PARK2 in U87MG and MCF7 cell lines transiently transfected as indicated. HSP90 was used as loading control. Data shown are mean ± s.e.m. of at least three biological replicates, each performed in triplicate. **, $p<0.01$.

Supplementary Figure 7. Overexpression of PARK2 reduces melanoma cell growth and increases apoptosis. **A.** Western blot (WB) analysis of PARK2 in 501-Mel and SK-Mel-28 melanoma cells transduced with pBABE or PARK2. HSP90 was used as loading control. **B-C.** Growth curves in 501-Mel and SK-Mel-28 transduced as indicated. **D-E.** Histogram of the quantification of colony assay in 501-Mel and SK-Mel-28 cells transduced as indicated. **F-G.** 501-Mel and SK-Mel-28 melanoma cells transduced as indicated, were subjected to cytometric analysis of apoptotic cells after staining with Annexin V/7-AAD (Annexin V+/7-AAD−: early apoptosis; Annexin V+/7-AAD+: late apoptosis). **H.** WB analysis of BCL-2 and BCL-XL in 501-Mel and SK-Mel-28 transduced as described. HSP90 was used as loading control. **I.** Western blot analysis of pAKT in melanoma cell lines transduced as indicated. HSP90 was used as loading control. Protein quantifications are indicated in italic. Data shown are mean ± s.d. of at least three biological replicates, each performed in triplicate. *, $p<0.05$; **, $p<0.01$.

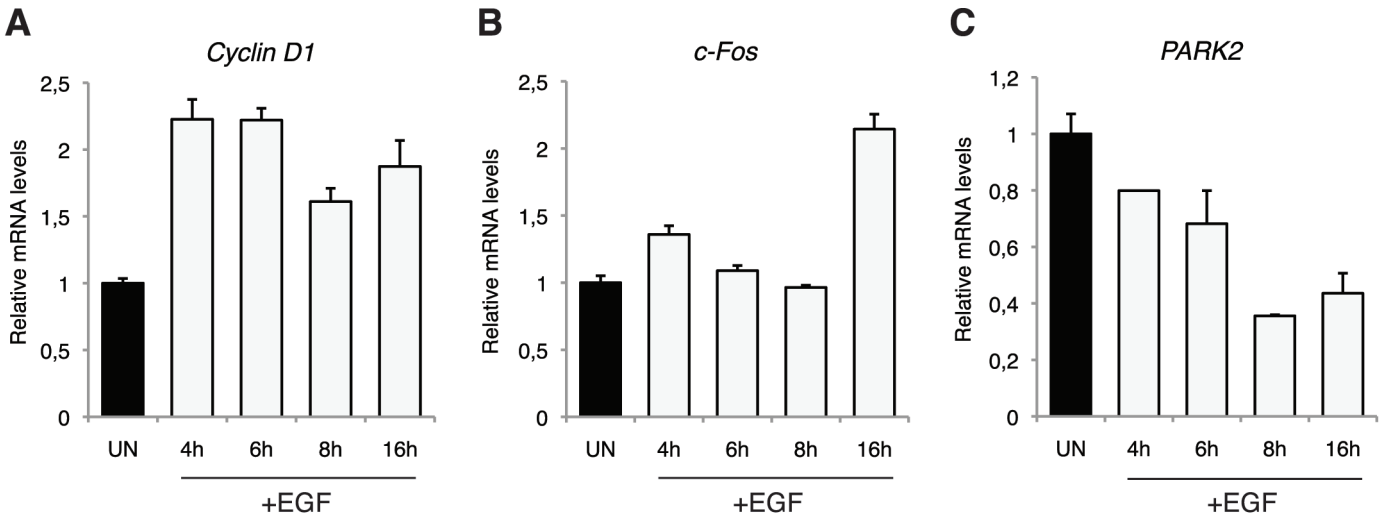
Supplementary Figure 8. PARK2 depletion increases melanoma cell growth reducing apoptosis. **A.** Western blot (WB) analysis of PARK2 in M51 melanoma cells transduced with LV-c, LV-shPARK2-1 or LV-shPARK2-2. HSP90 was used as loading control. **B.** Growth curves in M51 cells transduced as indicated. **C.** M51 melanoma cells transduced as indicated, were subjected to cytometric analysis of apoptotic cells after staining with Annexin V/7-AAD (Annexin V+/7-AAD−: early apoptosis; Annexin V+/7-AAD+: late apoptosis). **D.** WB analysis of Caspase-3 and BCL-2 in M51 cells transduced as indicated. HSP90 was used as loading control. **E.** WB analysis of pAKT in melanoma cells transduced as indicated. HSP90 was used as loading control. Protein quantifications are indicated in italic. Data shown are mean ± s.d. of at least three biological replicates, each performed in triplicate. *, $p<0.05$.



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

PARK2 promoter sequence

chr6:162727669-162728401 GRCh38/hg38 (-635 +98 from TSS)

GATCACTTACGACTGAGTTTTTCATCATGGCTTTGACTGTGAAACCCTCA
GAAACCAGAGAGTGAGGCTGGTGCACCGGGAGCGGCTGTTGTGCCAGCAG
CTTGGTCCTCTTCGGCATCTTGTCTGGGCATTTGTTTAAGCTCAGGGTCT
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AGGTTGATCCAGATGTTTGGCAGCTCCTAGGTGAAGGGAGCTGGACCCTA
GGGGCGGGGCGGGAAGAGGGCAGGACCTTGGCTAGAGCTGCAACAAGCTT
CCAAAGGTAAGCCTCCCGGTT**GCTAAGCGACTGGTCAACAC**GGCGGGCGC
ATAGCCCCGCCCCCCGGTGACGTAAGATTGCTGGGCCTGAAGCCGGAAAG
GGCGGCGGTGGGGGGCTGGGGGCAGGAGG**CGTGAGGAGAAACTACGCGTT**
AGAACTACGACTCCCAGCAGGCCCTGGGCCGCGCCCTCCGCGCGTGCGCA
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AGAGCCGCTGGTGGGAGGCGCGGCTGGCGCCGCTGCGCGCATGGGCCTGT
TCCTGGCCCGCAGCCGCCACCTACCCAGTGACC

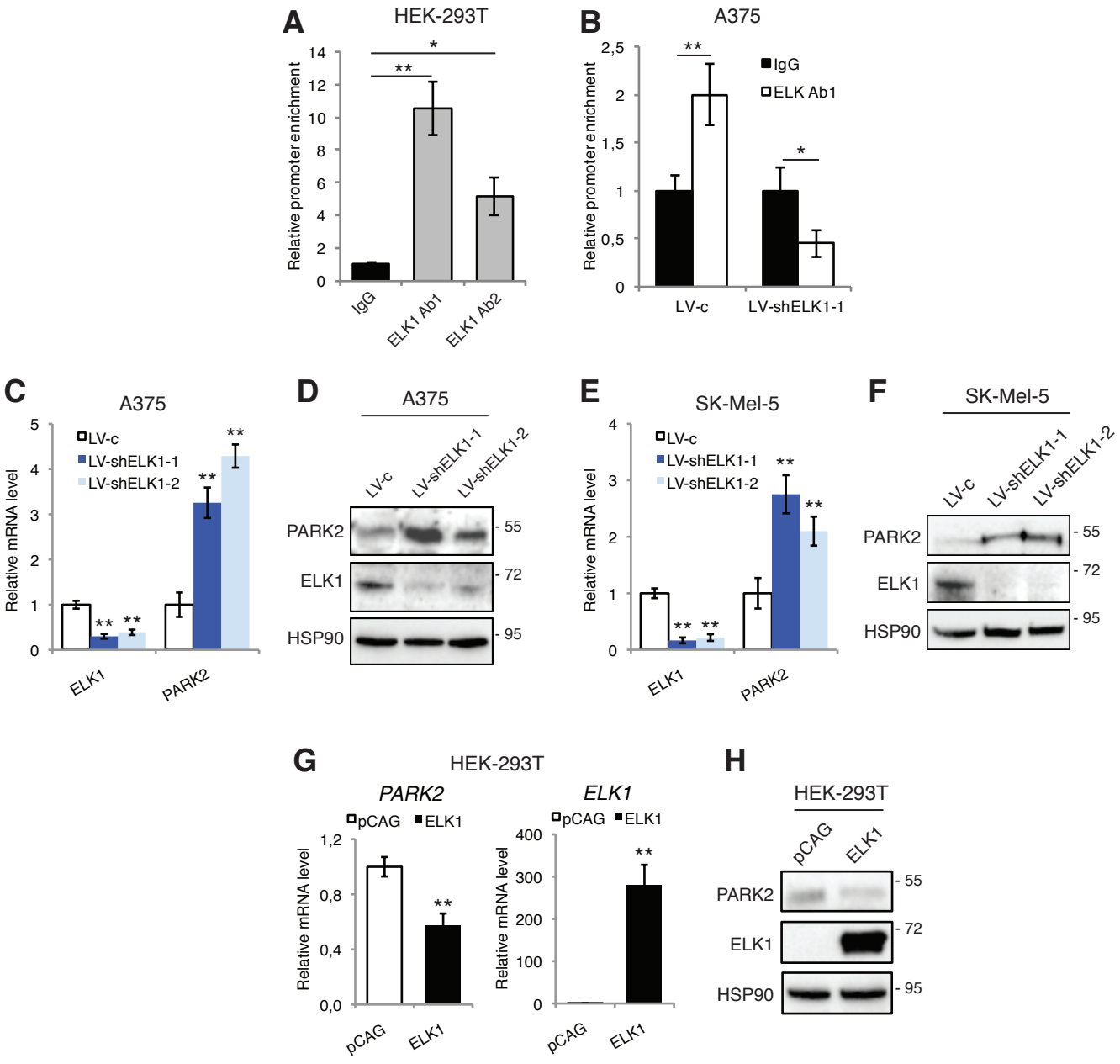
CCGGAAA ELK1 binding site (BS) -143 from TSS
AGG Start TSS
Bold: primers used for ChIP

Primers (ChIP)	**Sequence (5'->3')**
PARK2chip F2	GCTAAGCGACTGGTCAACAC
PARK2chip R2	AACGCGTAGTTTCTCCTCACG

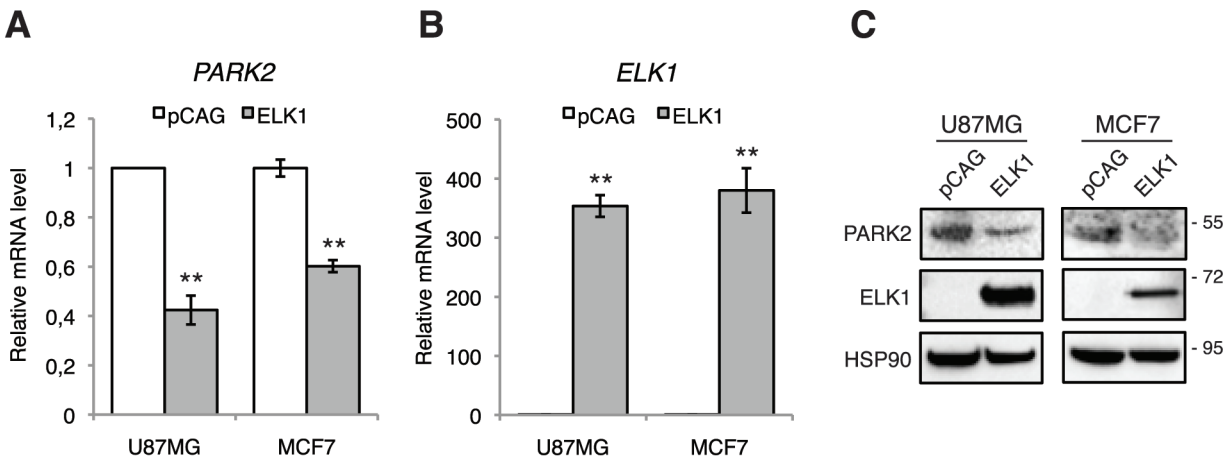
CC**CCC**AA mut ELK1 binding site (BS) -143 from TSS

Primers (Mutagenesis)	**Sequence (5'->3')**
PARK2prom FW mut1	CTGGGCCTGAAGCCCCCAAGGGCGGCGGTGG
PARK2prom RV mut1	CCACCGCCGCCCTTGGGGGCTTCAGGCCCAG

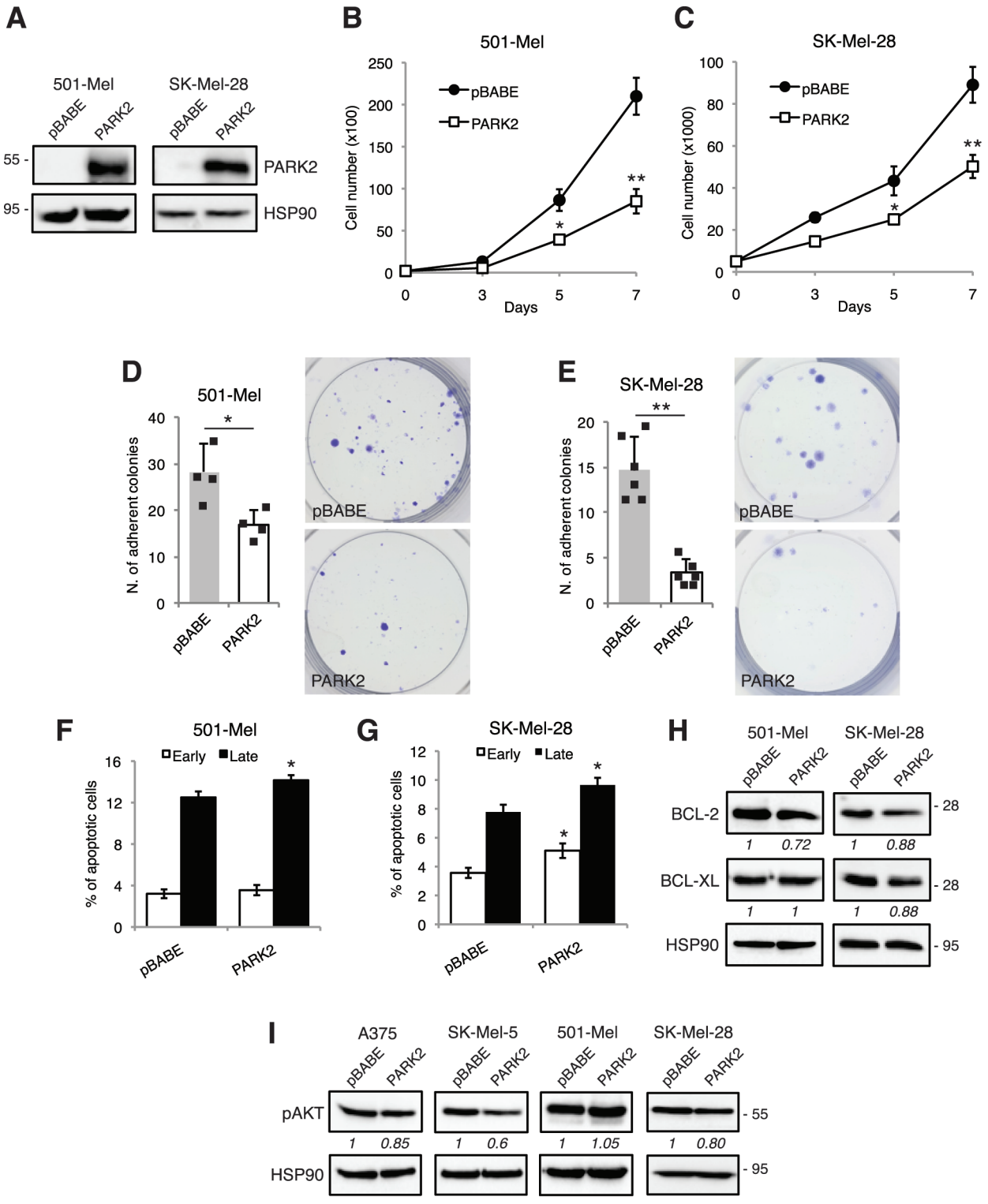
Supplementary Figure 4



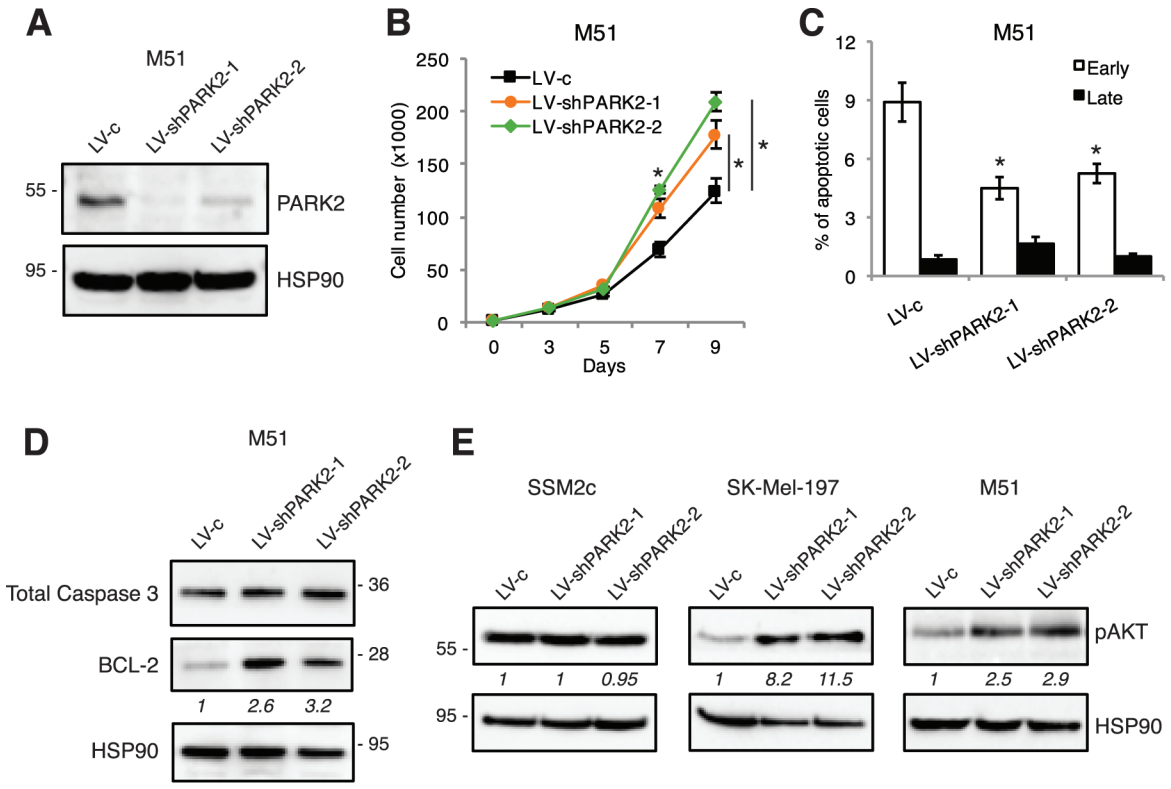
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8