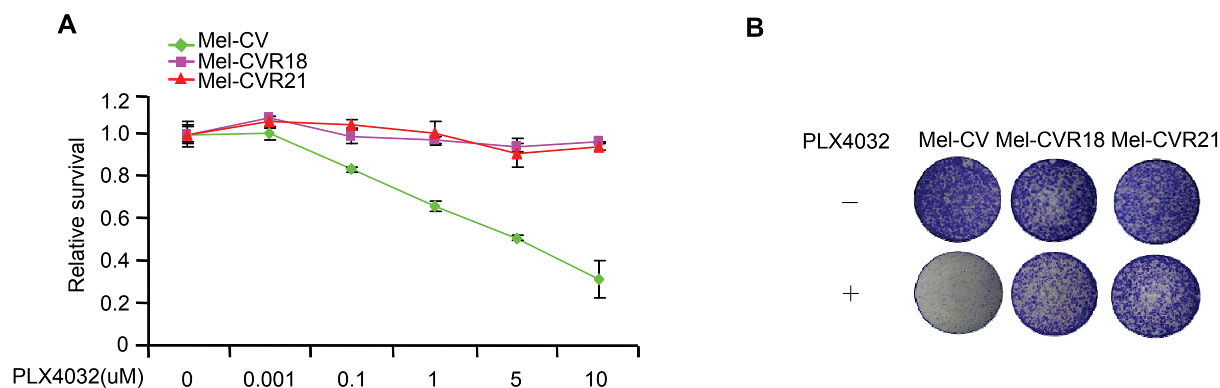

Figure S1
(A) Mel-CV, Mel-CVR18, or Mel-CVR21 cells were treated with indicated concentrations of PLX4032 for 36 h. Viability of cells was determined using MTT assays by measuring the absorbance at 490 nm in a micro plate reader.
(B) Long-term colony formation assay of Mel-CV, Mel-CVR18, and Mel-CVR21 cells. Cells were grown in the absence or presence of 3 μM PLX4032 for 3 weeks. All dishes were fixed at the same time, stained and photographed.

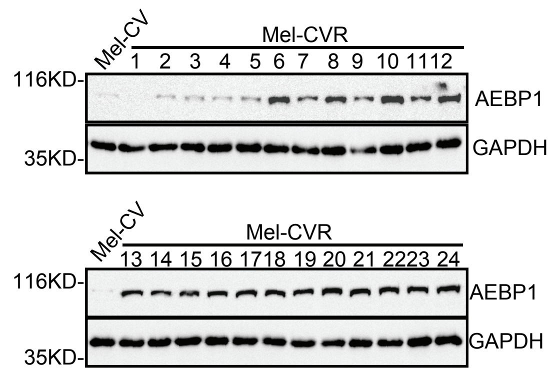

Figure S2
Western blot analysis of AEBP1 protein expression in Mel-CV parental cell line and 24 isolated PLX4032-resistant clones (Mel-CVR1-Mel-CVR24) without PLX4032 treatment.

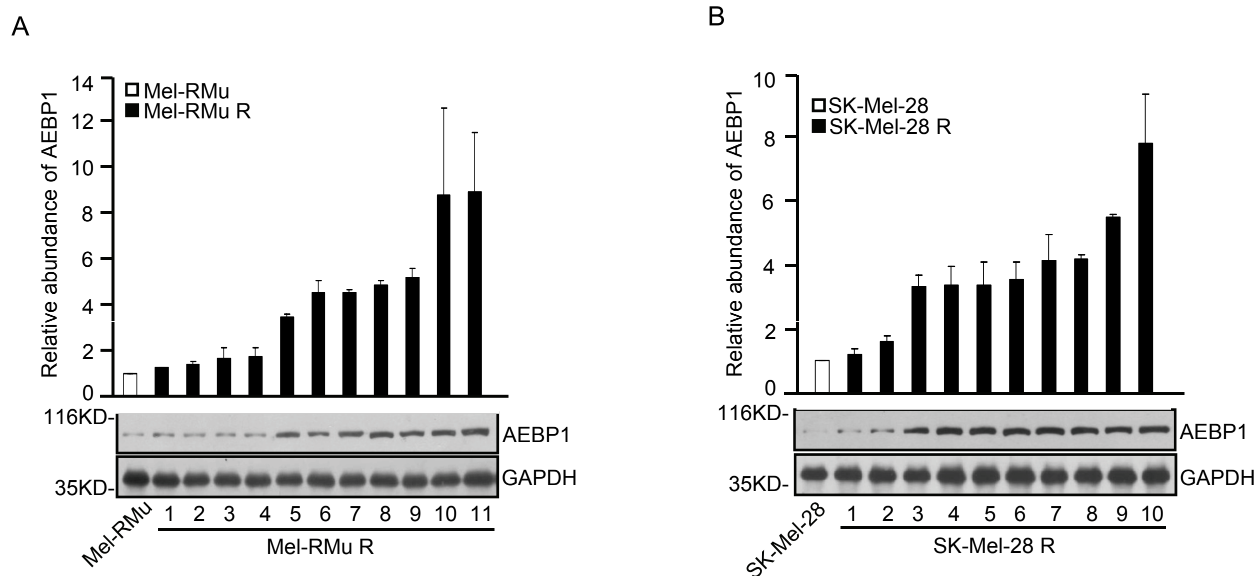

Figure S3

(A) Real time RT-PCR and Western blot analyses of AEBP1 expression in Mel-RMu parental cell line and 11 isolated PLX4032-resistant clones (Mel-RMuR1-Mel-RMuR11) without PLX4032 treatment. Mel-RMuR9 was used for the subsequent functional study and referred to as Mel-RMuR.

(B) Real time RT-PCR and Western blot analyses of AEBP1 expression in SK-Mel-28 parental cell line and 10 isolated PLX4032-resistant clones (SK-Mel-28R1-SK-Mel-28R10) without PLX4032 treatment. SK-Mel-28R9 was used for the subsequent functional study and referred to as SK-Mel-28R.

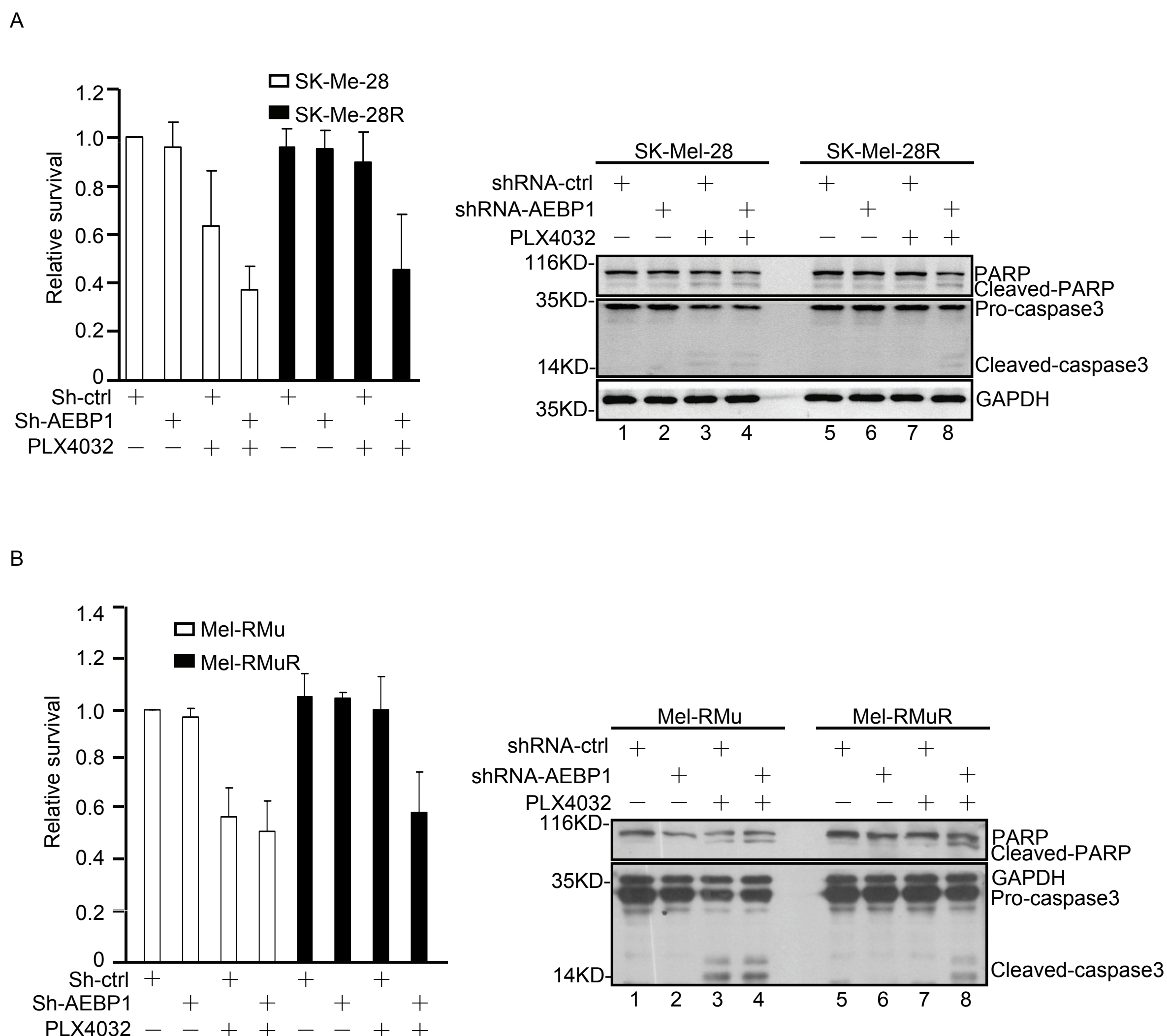

Figure S4
(A) SK-Mel-28 and SK-Mel-28R cells with and without stable knockdown of AEBP1 were treated with 5 μM PLX4032 for 36 h. Viability of cells was determined using MTT assays by measuring the absorbance at 490 nm in a micro plate reader. Cell lysates were also subjected to Western blot analysis with the indicated antibodies.
(B) Mel-RMu and Mel-RMuR cells with and without stable knockdown of AEBP1 were treated with 5 μM PLX4032 for 36 h. Viability of cells was determined using MTT assays by measuring the absorbance at 490 nm in a micro plate reader. Cell lysates were also subjected to Western blot analysis with the indicated antibodies.

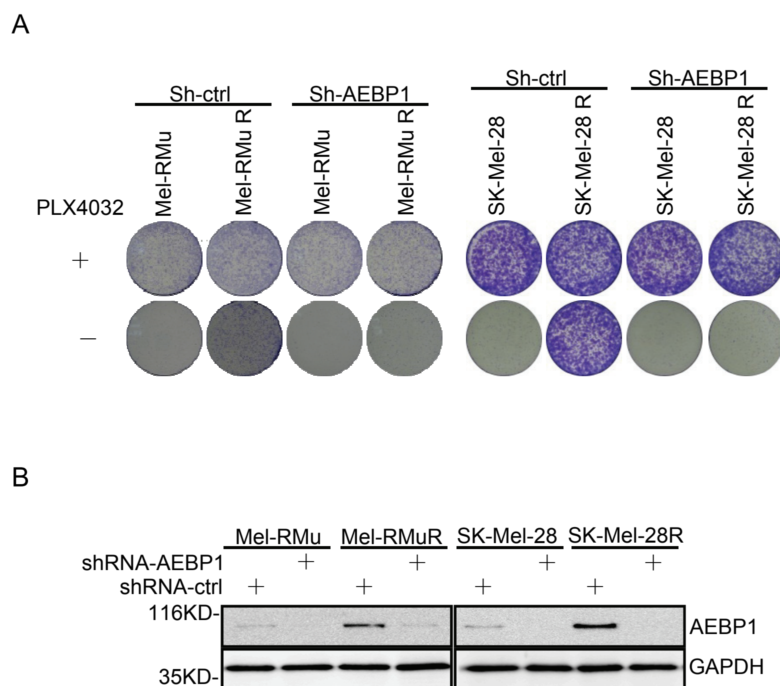

Figure S5

(A) Long-term colony formation assay of Mel-RMu, Mel-RMuR, SK-Mel-28 and SK-Mel-28R cells with and without stable knockdown of AEBP1. Cells were grown in the absence or presence of 3 μM PLX4032 for 3 weeks. All dishes were fixed, stained and photographed at the same time.

(B) AEBP1 knockdown efficiency in these cells were evaluated by Western blot analysis with the anti-AEBP1 antibody.

Figure S6

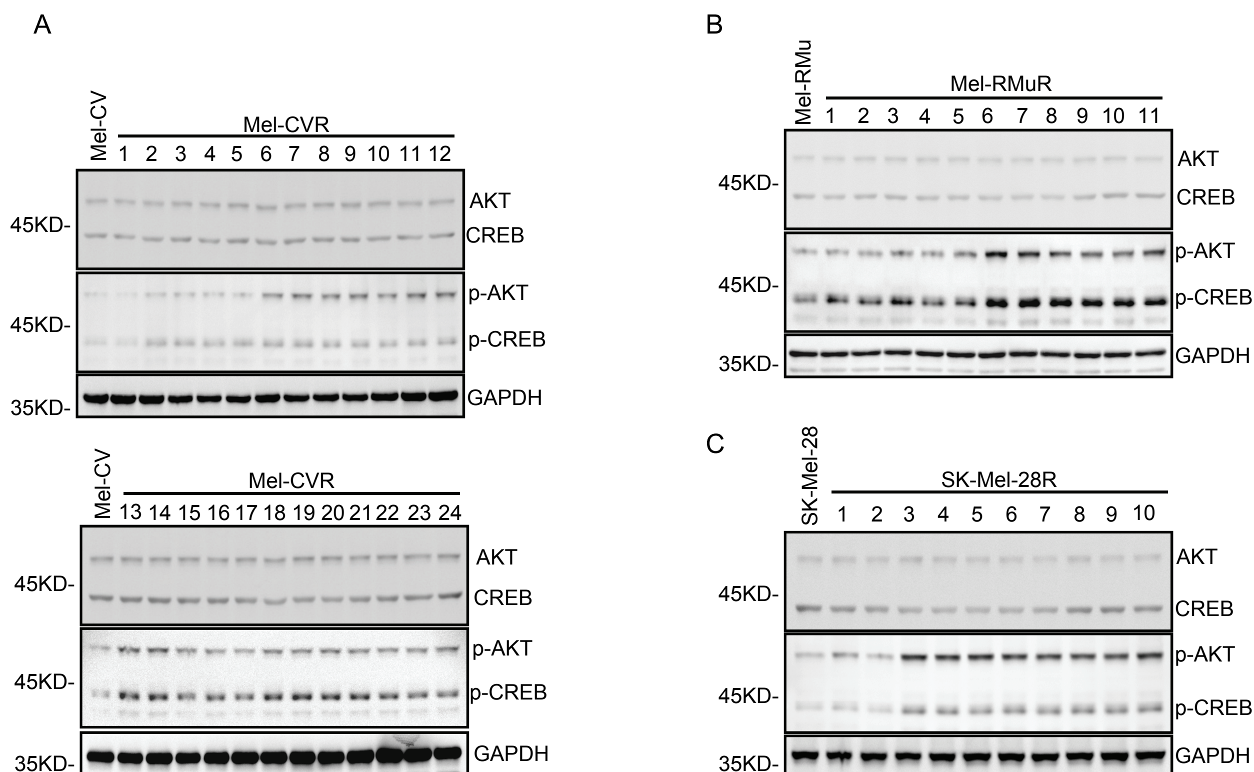

Figure S6

(A) Western blot analyses of AKT, CREB, p-AKT and p-CREB expression in Mel-CV parental cell line and 24 isolated PLX4032-resistant clones (Mel-CVR1-Mel-CVR24) without PLX4032 treatment.

(B) Western blot analyses of AKT, CREB, p-AKT and p-CREB expression in Mel-RMu parental cell line and 11 isolated PLX4032-resistant clones (Mel-RMuR1-Mel-RMuR11) without PLX4032 treatment.

(C) Western blot analyses of AKT, CREB, p-AKT and p-CREB expression in SK-Mel-28 parental cell line and 10 isolated PLX4032-resistant clones (SK-Mel-28R1-SK-Mel-28R10) without PLX4032 treatment.

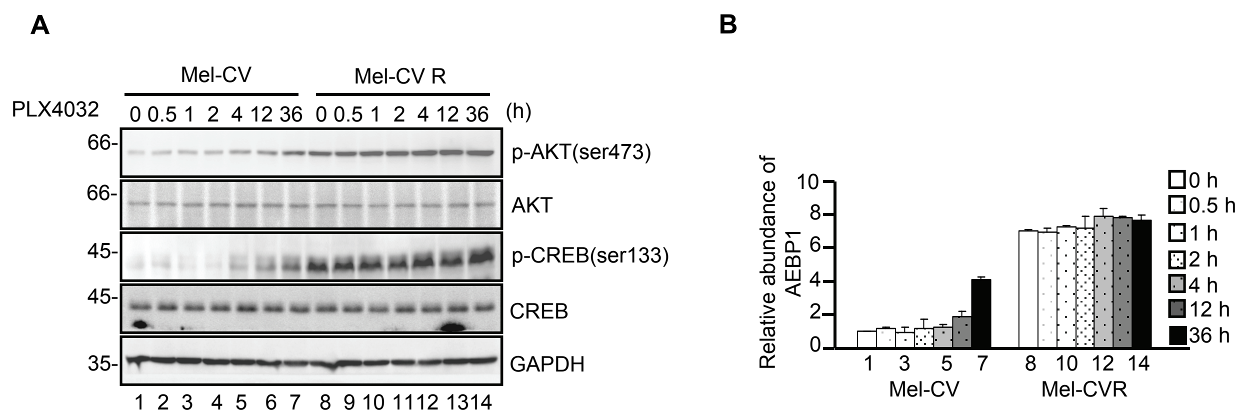

Figure S7
(A) Mel-CV and Mel-CVR cells were treated with 5 μM PLX4032 for the indicated periods of time. The remaining live cells were then collected and subjected to Western blot analysis with the indicated antibodies.
(B) Mel-CV and Mel-CVR cells were treated with 5 μM PLX4032 for the indicated periods of time. The remaining live cells were then collected and subjected to real time RT-PCR analysis.

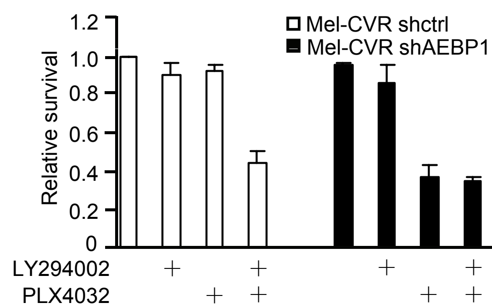

Figure S8
Mel-CVR cells with or without stable knockdown of AEBP1 were pre-treated with either LY294002 or DMSO for 4 h, followed by the treatment with 5 μM PLX4032 for an additional 36 h. Viability of cells was determined using MTT assays by measuring the absorbance at 490 nm in a micro plate reader.

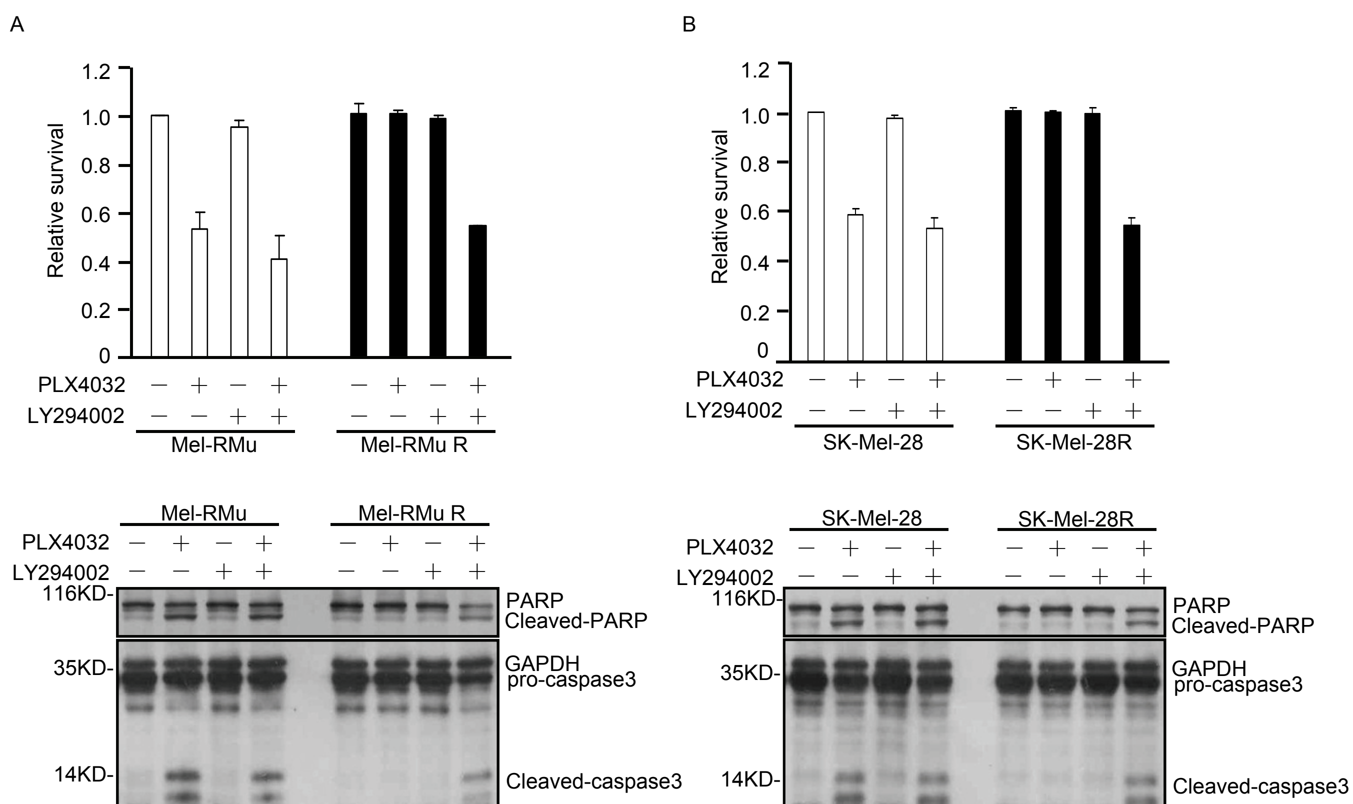

Figure S9

(A) Mel-RMu and Mel-RMuR cells were pre-treated with LY294002 or DMSO for 4 h. The cells were then incubated with 5 μM PLX4032 or mock control for another 36 h. Viability of cells was determined using MTT assays by measuring the absorbance at 490 nm in a micro plate reader. Cell lysates were also subjected to Western blot analysis with the indicated antibodies.

(B) SK-Mel-28 and SK-Mel-28R cells were pre-treated with LY294002 or DMSO for 4 h. The cells were then treated with 5 μM PLX4032 or mock control for another 36 h. Viability of cells was determined using MTT assays by measuring the absorbance at 490 nm in a micro plate reader. Cell lysates were also analyzed by Western blot with the indicated antibodies.

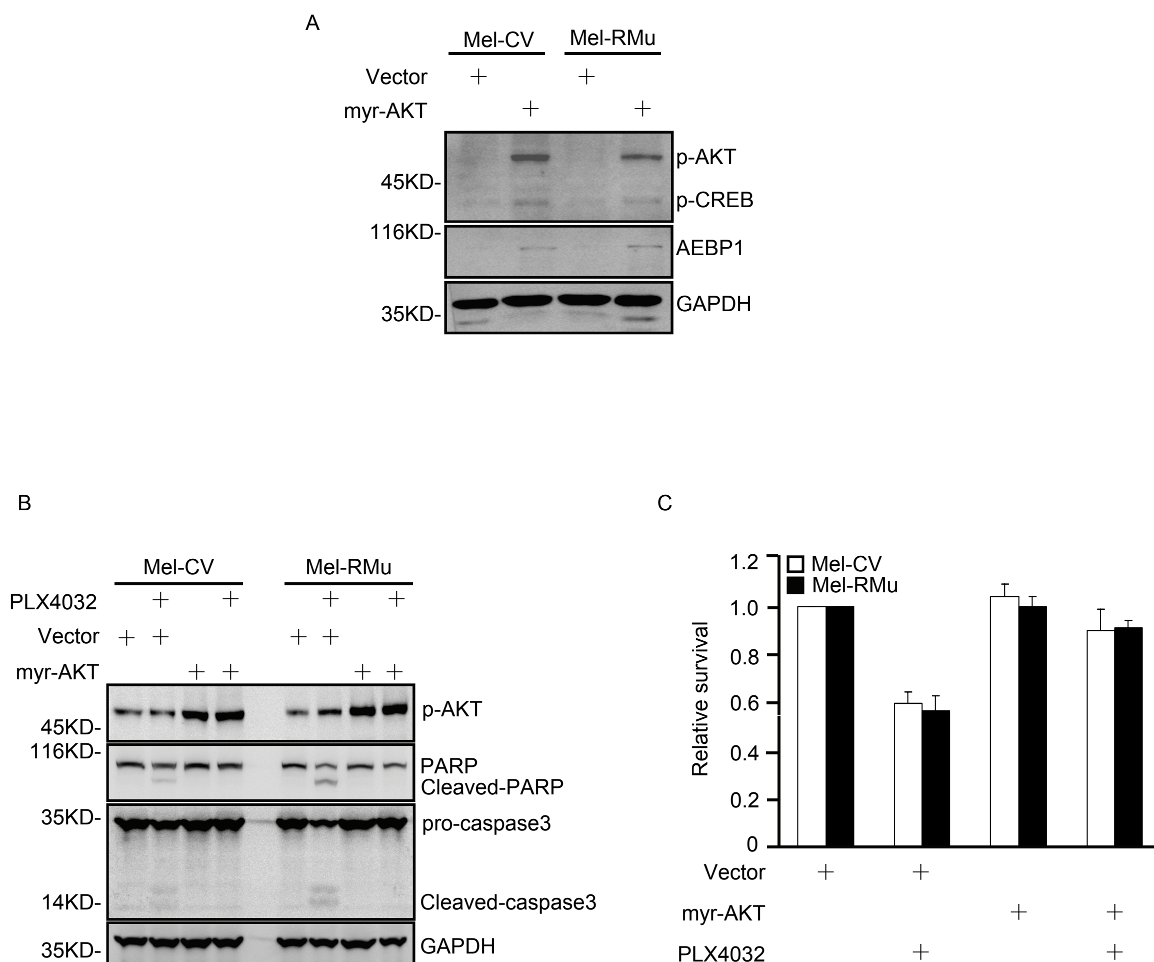

Figure S10

(A)Lysates from Mel-CV and Mel-RMu cells expressing myr-AKT or control proteins were analyzed by Western blot with the indicated antibodies.

(B) Mel-CV and Mel-RMu cells expressing myr-AKT or control proteins were treated with 5 μM PLX4032 for 36 h. Cell lysates were subjected to Western blot analysis with the indicated antibodies.

(C) Mel-CV and Mel-RMu cells expressing myr-AKT or control proteins were treated with 5 μM PLX4032 for 36 h. Viability of cells was determined using MTT assays.

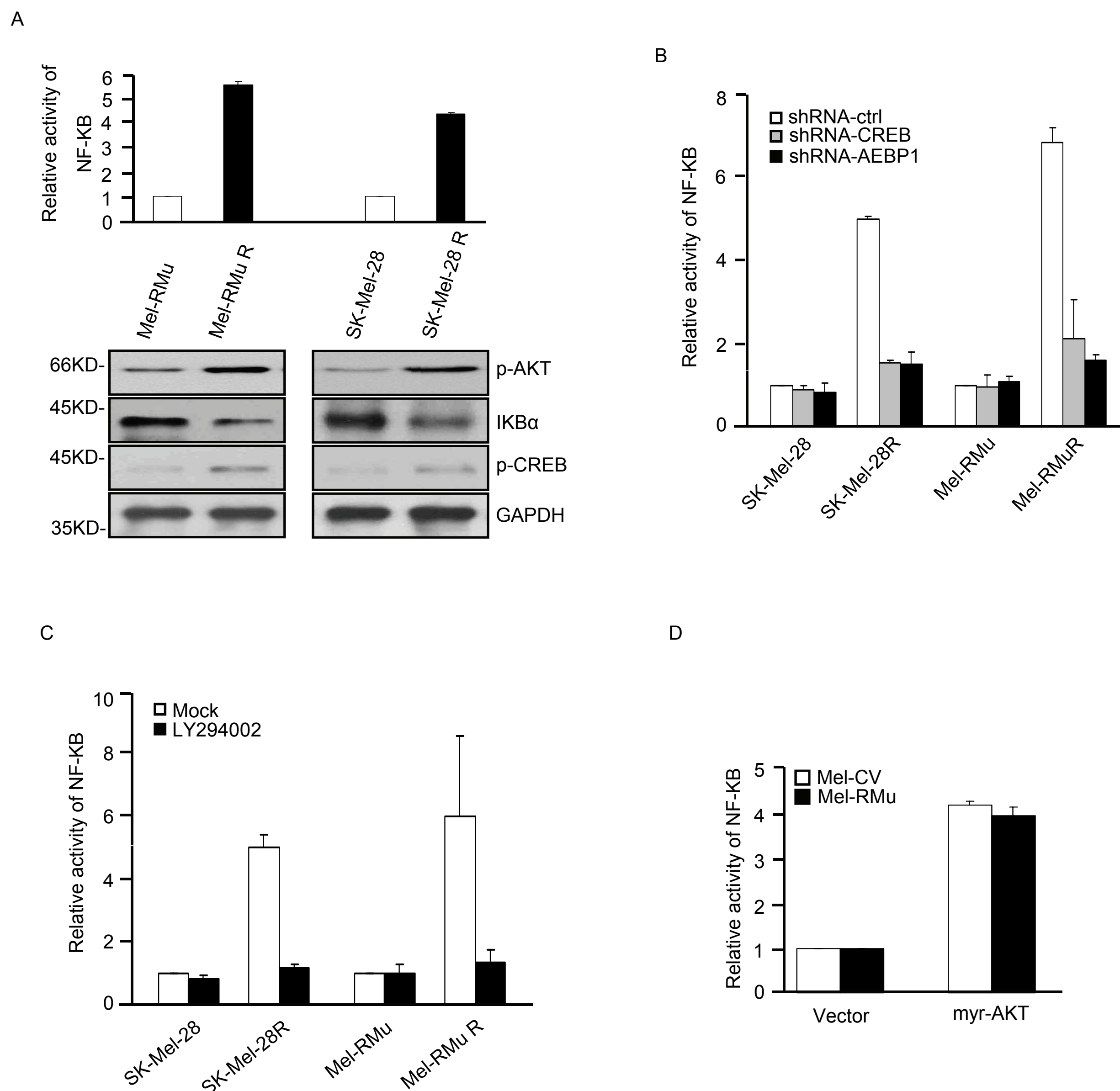

Figure S11

(A) Mel-RMu, Mel-RMuR, SK-Mel-28 and SK-Mel-28R cells were individually transfected with pGL3-3×NF-kB-BR construct together with Renilla luciferase plasmid. 24 h after transfection, the activity of NF-kB was determined by luciferase assays. Cell lysates were also analyzed by Western blot with the indicated antibodies.

(B) SK-Mel-28, SK-Mel-28R, Mel-RMu and Mel-RMuR cells individually expressing control shRNA, CREB shRNA, or AEBP1 shRNA were transfected with pGL3-3×NF-kB-BR construct together with Renilla luciferase plasmid. 24 h after transfection, the activity of NF-kB was determined by luciferase assays.

(C) SK-Mel-28, SK-Mel-28R, Mel-RMu and Mel-RMuR cells were co-transfected with pGL3-3×NF-kB-BR construct and Renilla luciferase plasmid. 24 h after trasfection, cells were treated with LY294002 or DMSO for another 4 h. The activity of NF-kB was then analyzed by luciferase assays.

(D) Mel-CV and Mel-RMu cells were co-transfected with pGL3×NF-kB-BR construct and myr-AKT as indicated. 24 h after transfection, NF-kB activity was measured by luciferase assays.

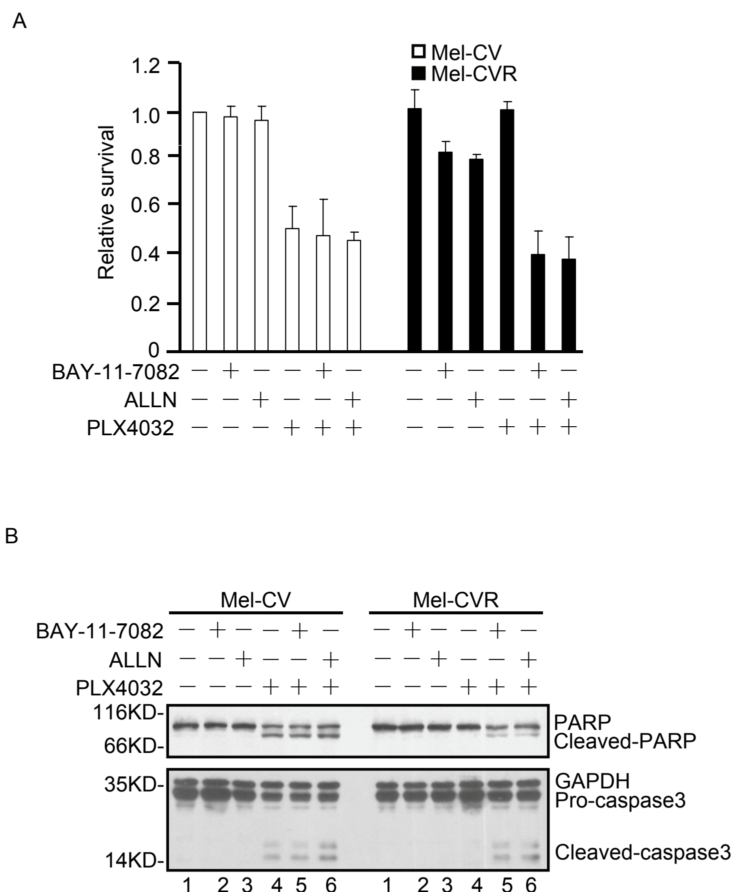

Figure S12
(A) Mel-CV and Mel-CVR cells were pre-treated with BAY-11-7082 or ALLN for 4h, followed by the treatment with 5 μM PLX4032 for another 36 h. Cell viability was determined by using MTT assays.
(B) Mel-CV and Mel-CVR cells were pre-treated with BAY-11-7082 or ALLN for 4h, followed by the treatment with 5 μM PLX4032 for another 36 h. Cell lysates were then analyzed by Western blot.

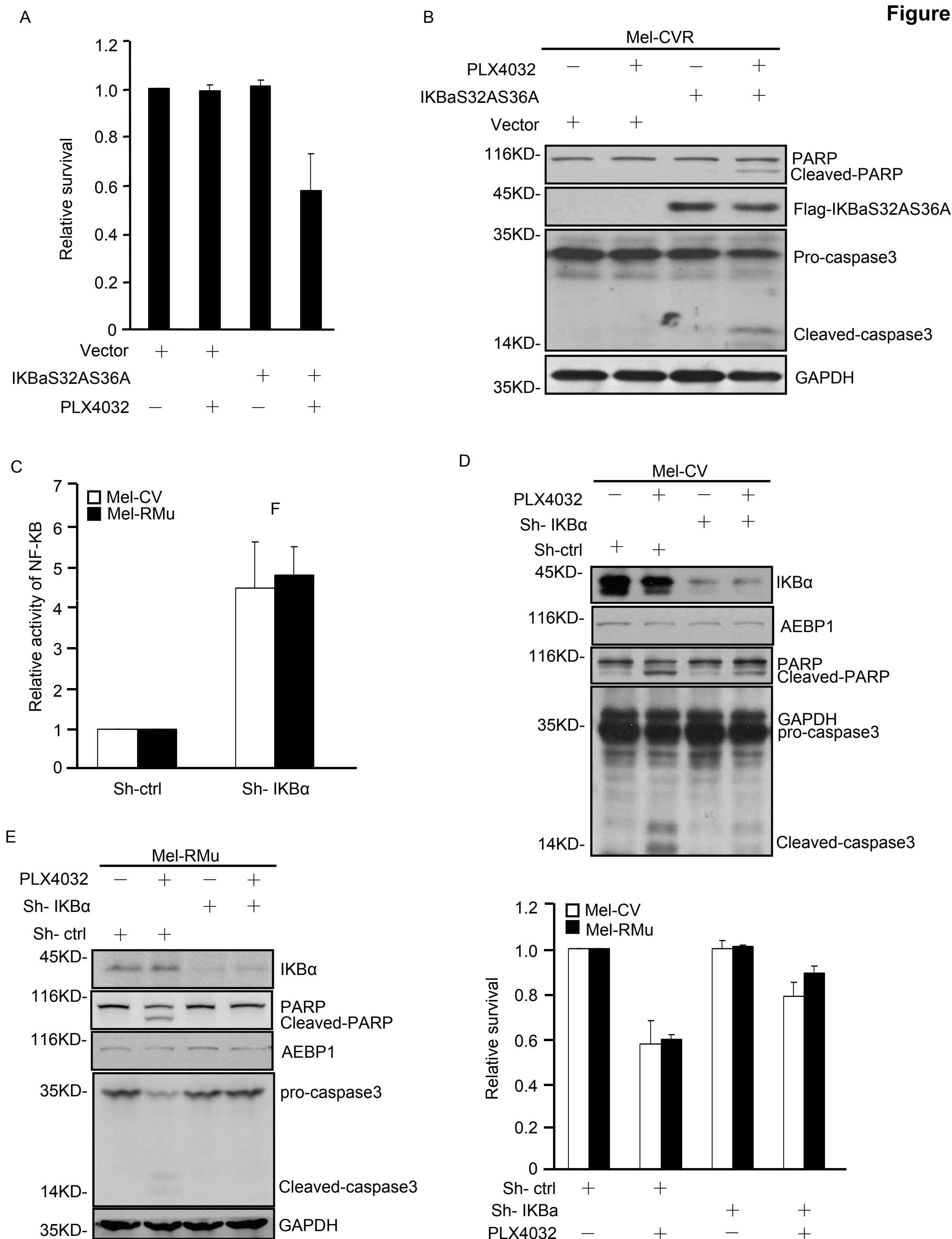

Figure S13

(A and B) Mel-CVR cells were tranfected with either vector or Flag-IKBaS32AS36A. 24 h after transfection, cells were treated with DMSO or 5 μM PLX4032 for an additional 36 h. (A) Viability of cells was determined using MTT assays. (B) Cell lysates were also subjected to Western blot analysis with the indicated antibodies.

(C) Mel-CV and Mel-RMu cells expressing control shRNA or IKBα shRNA were transfected with pGL3-3×NF-kB-BR construct. 24 h after transfection, NF-kB activity was determined by luciferase assays.

(D and E) Mel-CV cells (D) and Mel-RMu cells (E) with or without stable knockdown of IKBa were treated with 5 μM PLX4032 for 36 h. Cell lysates were subjected to Western blot analysis with the indicated antibodies.

(F) Mel-CV and Mel-RMu cells with or without stable knockdown of IKBa were treated with 5 μM PLX4032 for 36 h. Viability of cells was then examined by MTT assays.

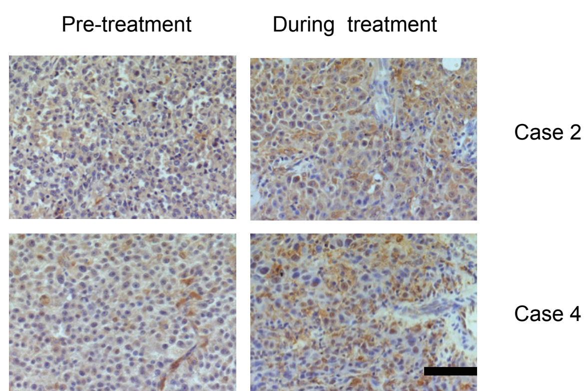

Figure S14
Representative microphotographs of immunohistochmistry staining of melanoma tissue sections showing that the expression levels of AEBP1 (brown) were increased in two of five melanomas progression samples (case 2 and case 4) from patients post-treatment with dabrafenib compared to the corresponding pre-treatment samples. Scale bar: 100μm.

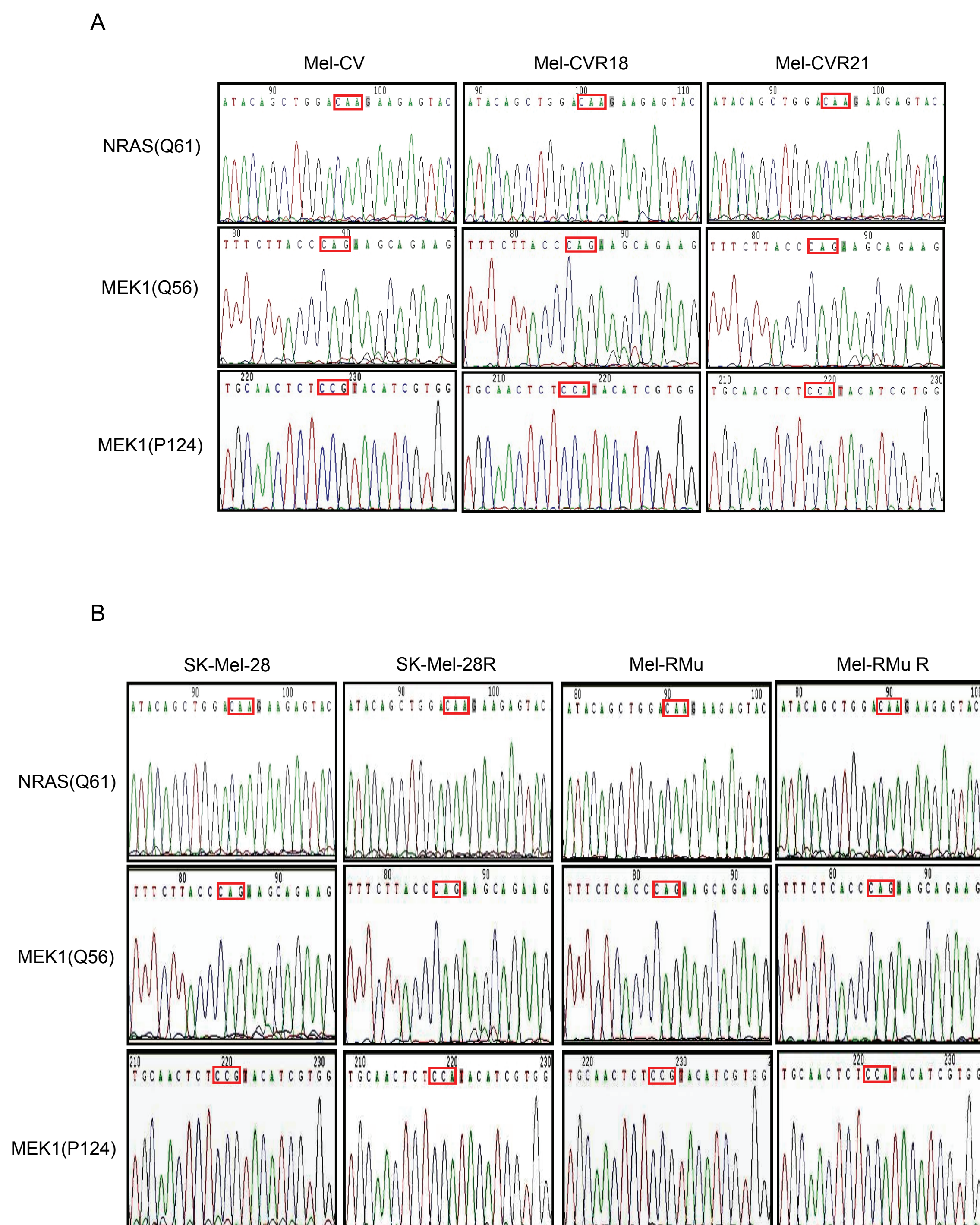

Figure S15

(A) The status of NRAS (Q61), MEK1 (Q56) and MEK (P124) in Mel-CV parental cell line and 2 isolated PLX4032-resistant clones (Mel-CVR18 and Mel-CVR21) were evaluated by exon sequencing.

(B) The status of NRAS (Q61), MEK1 (Q56) and MEK (P124) in SK-Mel-28, SK-Mel-28R, Mel-RMu and Mel-RMuR cells were evaluated by exon sequencing.

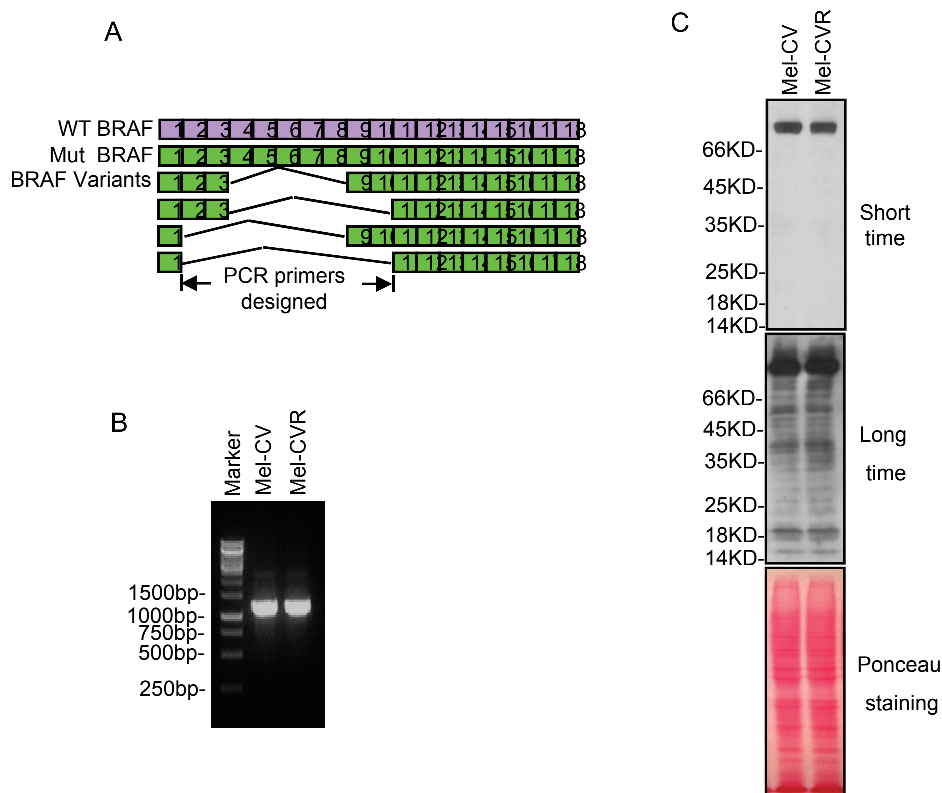

Figure S16

(A) A schematic illustration of the exon organization of the previously reported BRAF splicing variants found in relapsed melanomas in patients post-treament with BRAF inhibitor. The PCR primers were designed between the exon 1 and 11 to detect the BRAF splicing variants.

(B) PCR analysis of cDNA derived from Mel-CV and Mel-CVR using the primer pair shown in (A).

(C) Lysates from Mel-CV and Mel-CVR cells were analyzed by Western blot with the antibody against the C-terminal of BRAF (top and middle panels) and by Ponceau S staining (bottom panel).