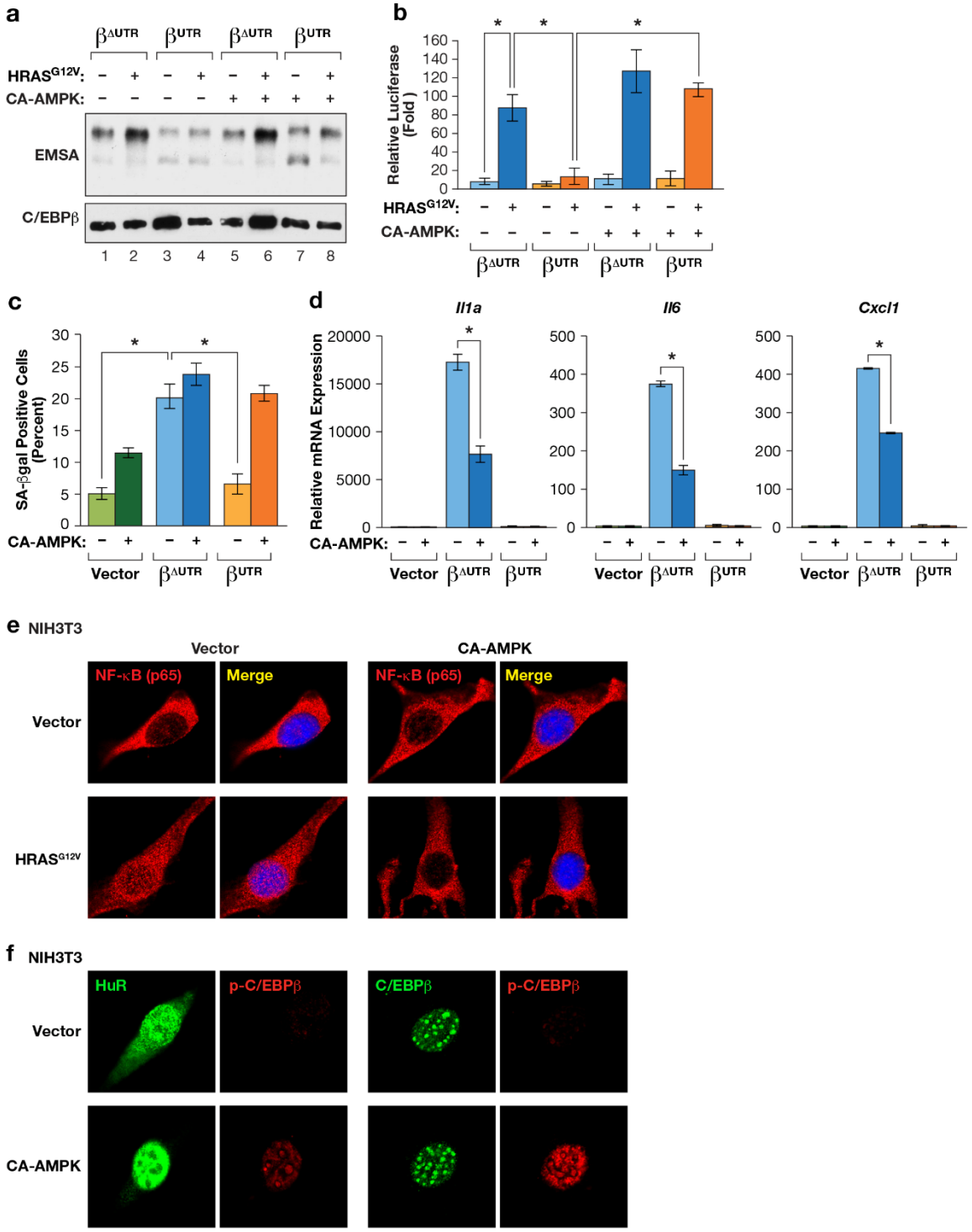
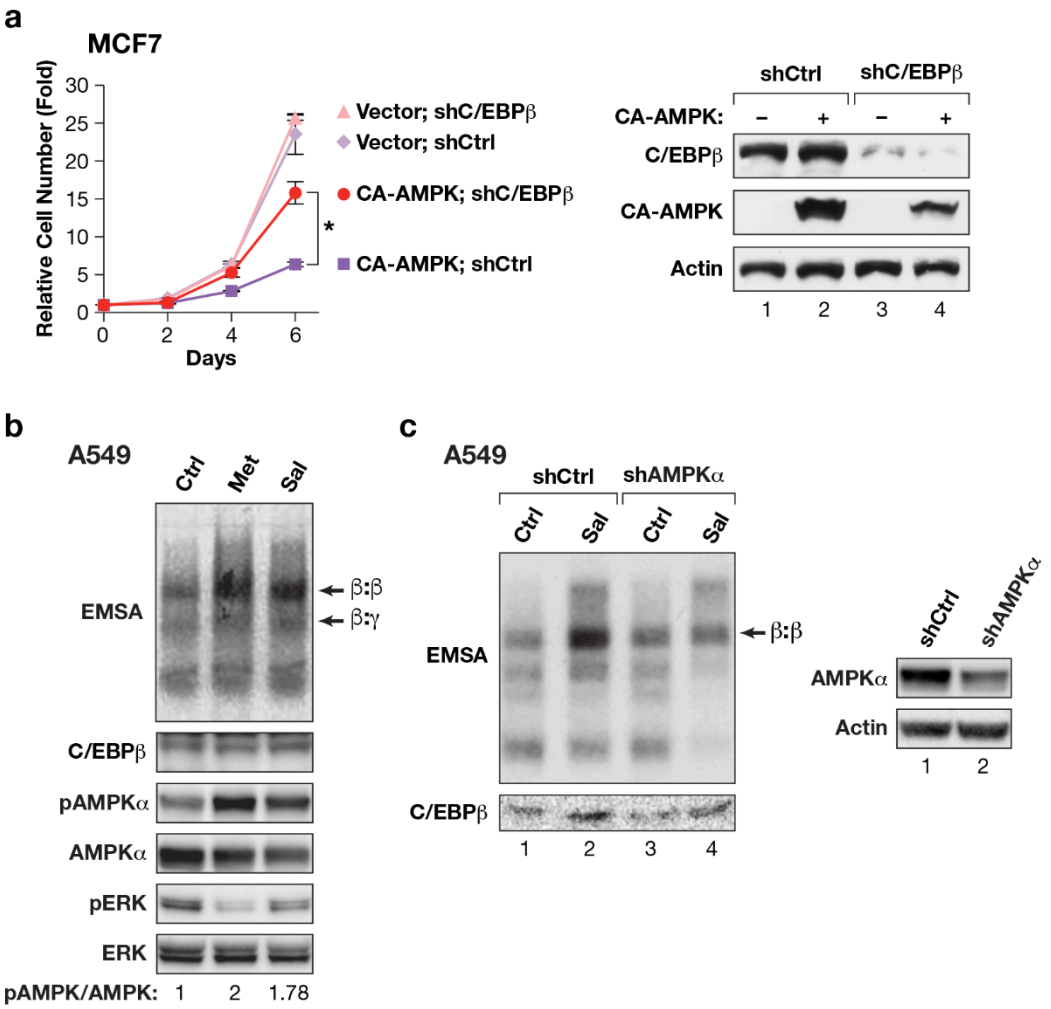
SUPPLEMENTARY INFORMATION

Supplementary Figure 1



Supplementary Figure 1 AMPK signaling overrides 3'UTR inhibition of C/EBPβ activity (UPA). **(a)** EMSA showing the effect of CA-AMPK on 3'UTR inhibition of oncogenic RAS-induced C/EBPβ DNA-binding activity. 293T cells were transfected with the indicated constructs and nuclear extracts analyzed by EMSA using a radiolabeled oligonucleotide probe containing a canonical C/EBP site. C/EBPβ levels in the lysates were determined by immunoblotting (bottom panel). **(b)** Transactivation assays showing the effect of CA-AMPK on C/EBPβ UPA. 293T cells were transfected with the 2XC/EBP-Luc reporter construct and the indicated expression plasmids. 24 hr later the cells were serum starved for 16 hr, lysates were prepared and assayed for luciferase activity. Reporter activity was normalized to total protein in the lysate. n=3 biological replicates; values represent the mean ± S.E.M. Statistics were determined using Student's two tailed t test; *p<0.05. **(c)** Quantitation of senescent (SA-β-Gal^{+}) cells in the experiment of Figure 1f. n=3 biological replicates; values represent the mean ± S.E.M. Statistics were determined using Student's two tailed t test; *p<0.05. **(d)** Effect of CA-AMPK on C/EBPβ-induced expression of SASP genes in NIH3T3^{RAS} cells. Levels of representative SASP mRNAs (*Il1a*, *Il6,* and *Cxcl1*) in the cells described in Fig. 1d-f were analyzed by qRT-PCR. n=3 biological replicates; values represent the mean ± S.E.M. Statistics were determined using Student's two tailed t test; *p<0.05. **(e)** NF-κB activation by oncogenic RAS is suppressed by CA-AMPK. Control and HRAS^{G12V}-expressing NIH3T3 cells, without or with expression of CA-AMPK, were immunostained for NF-κB p65 (RelA). Nuclei are visualized in the DAPI stained (merge) images. **(f)** Analysis of HuR localization, p-C/EBPβ and total C/EBPβ in NIH3T3 cells, without or with CA-AMPK. NIH3T3 cells were infected with control or CA-AMPK retroviruses and then co-immunostained for HuR and phospho-C/EBPβ (Thr188) (left panels), or total C/EBPβ and phospho-C/EBPβ (right panels).

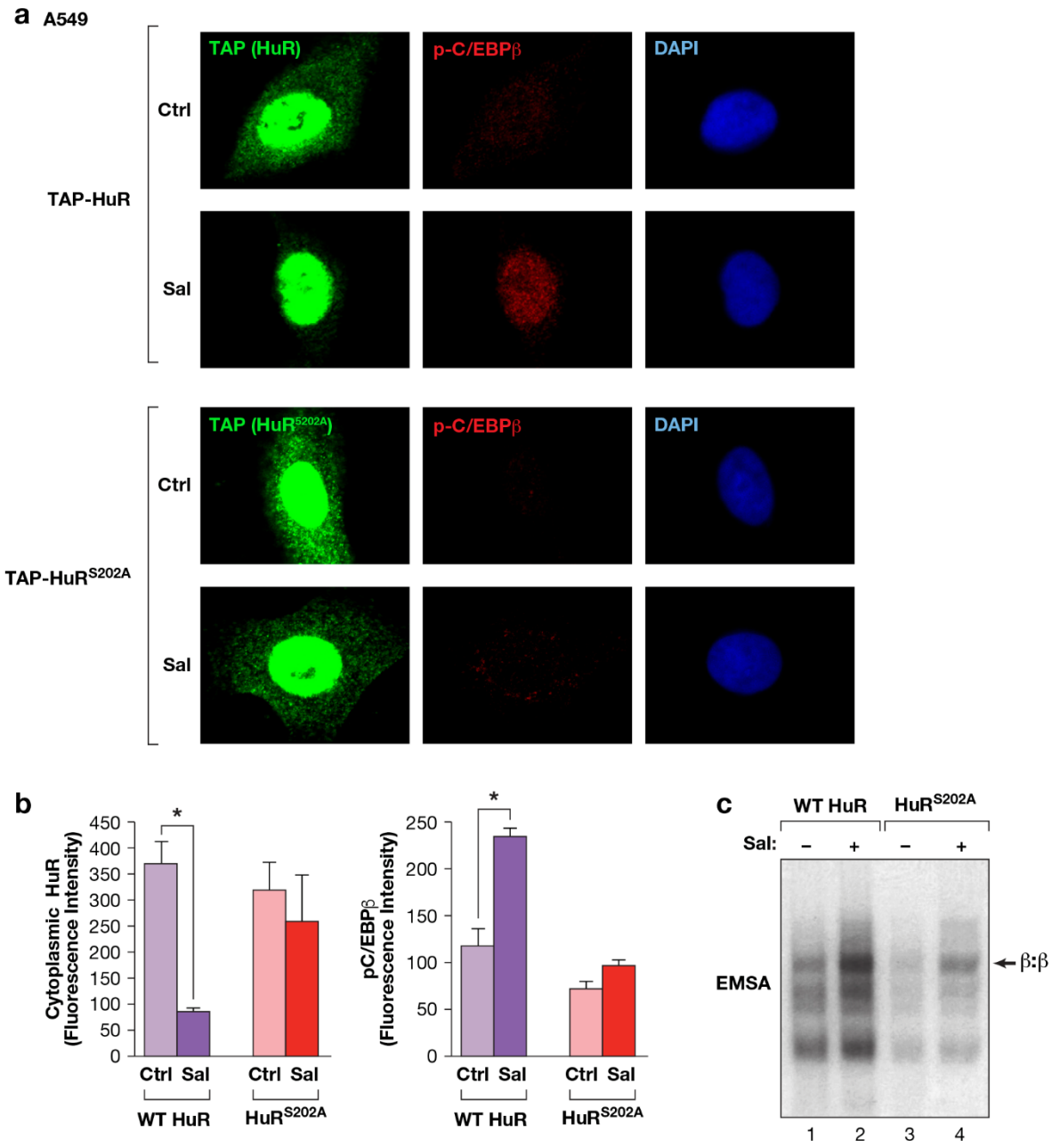
Supplementary Figure 2



Supplementary Figure 2 AMPK signaling activates C/EBPβ and causes C/EBPβ-dependent growth arrest in human tumor cells. **(a)** Proliferation of control and CA-AMPK-expressing MCF7 breast cancer cells, without or with C/EBPβ depletion. Transduced cells were analyzed over a six-day time course. n=2 independent biological replicates, each time point assayed in triplicate; error bars represent S.E.M. Statistics were determined for day 6 using Student's two tailed t test.; *p<0.05. Right panel: Western blots showing levels of C/EBPβ and CA-AMPK. **(b)** Augmentation of C/EBPβ DNA binding by AMPK activators in A549 cells. Cells were treated with vehicle, 1 mM metformin or 3 mM salicylate for 24 hr. Nuclear extracts were prepared, normalized for C/EBPβ levels and analyzed by EMSA using a C/EBP site probe. Levels of p-AMPKα (Thr172), total AMPKα, p-ERK and total ERK were analyzed by immunoblotting. p-AMPKα:AMPKα ratios were determined by quantitating the

chemoluminescence scans. **(c)** Salicylate-induced stimulation of C/EBPβ DNA binding requires AMPK. A549 cells were infected with retroviruses containing non-specific (shCtrl) or pan-AMPKα-specific shRNAs[1], and then treated with vehicle or 3 mM salicylate for 24 hr. Nuclear extracts normalized for C/EBPβ levels were analyzed by EMSA. Levels of total AMPKα were assessed by immunoblotting using actin as an internal standard.

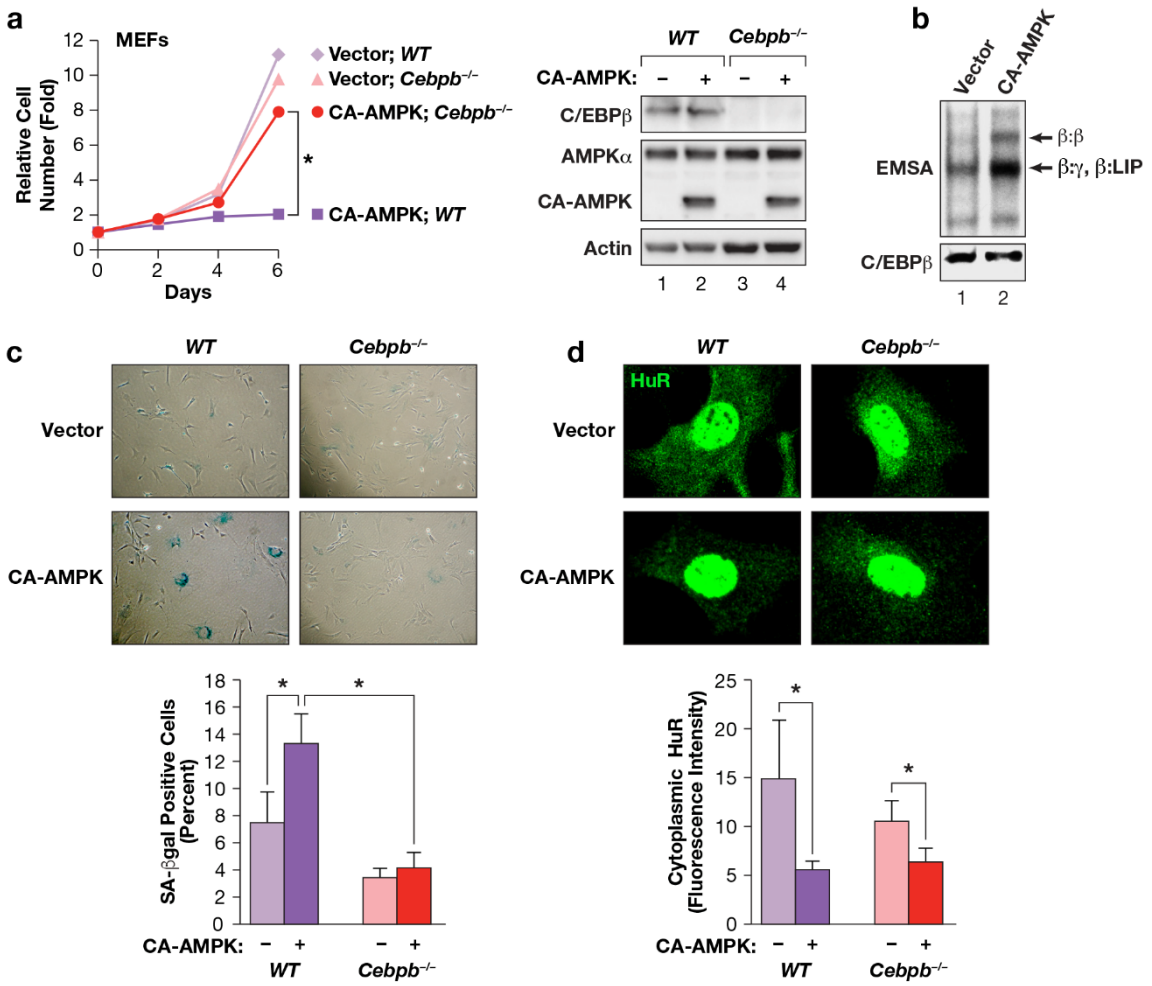
Supplementary Figure 3



Supplementary Figure 3 A constitutively cytoplasmic HuR mutant (S202A)[2] prevents AMPK-induced activation of C/EBPβ. **(a)** TAP-tagged WT HuR or HuR^{S202A} proteins were expressed in A549 cells by transient transfection. The cells were treated for 24 hr with vehicle or 3 mM salicylate and the over-expressed HuR proteins were visualized by immunostaining using a TAP tag antibody. Cells were also co-stained for p-C/EBPβ Thr235 (the ERK site in human C/EBPβ). **(b)** Quantitation of cytoplasmic HuR and p-C/EBPβ levels in the experiment

of panel **(a)**. n=5 cells; error bars represent S.E.M. Statistical differences were determined by Student's t test; *$p<0.05$. **(c)** HuR^{S202A} blocks salicylate-induced stimulation of C/EBPβ DNA binding. A549 cells expressing WT HuR or HuR^{S202A} were treated with either vehicle or 3 mM salicylate for 24 hr. Nuclear extracts were prepared and analyzed by EMSA using a canonical C/EBP site probe.

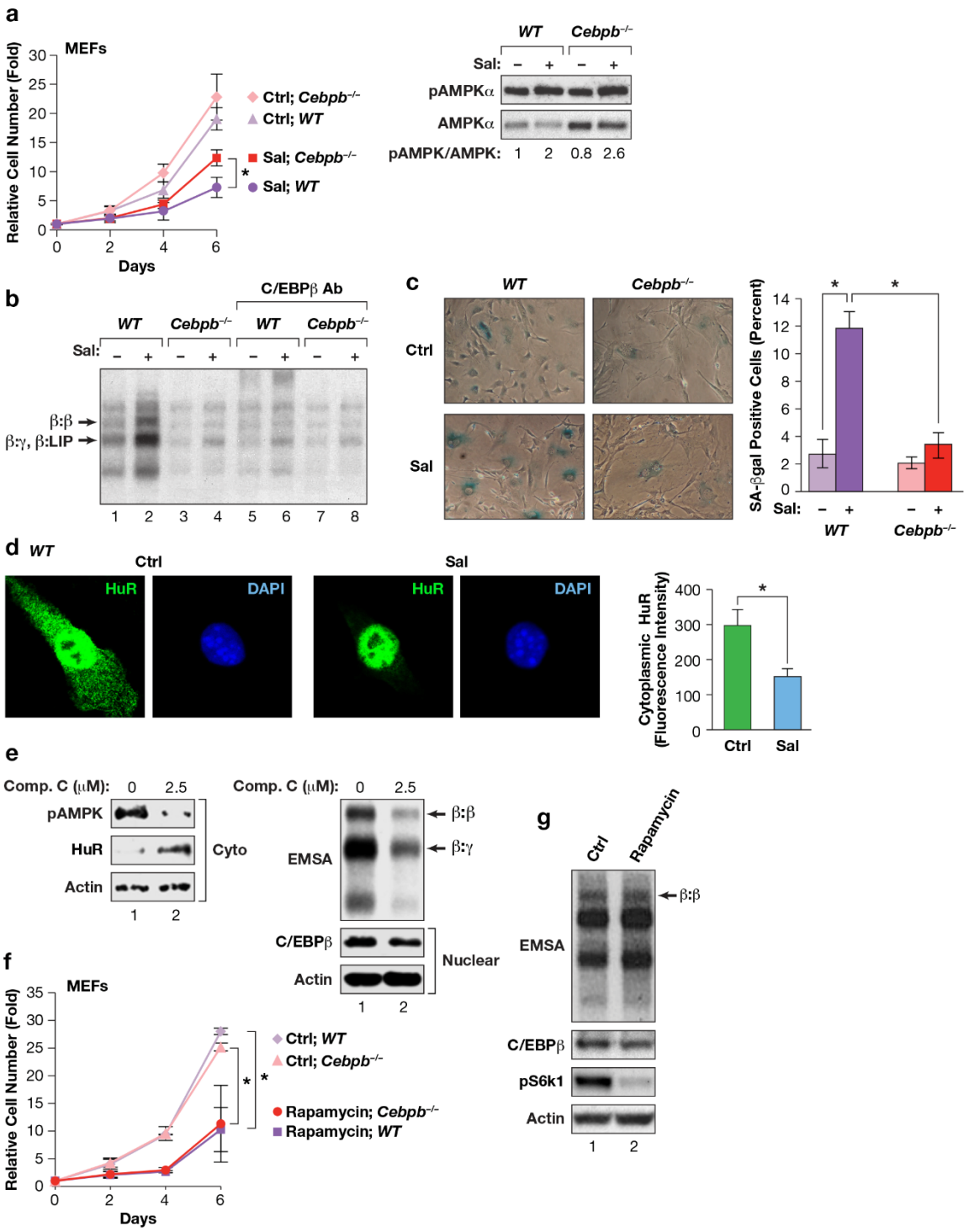
Supplementary Figure 4



Supplementary Figure 4 AMPK-induced senescence in primary MEFs requires C/EBPβ. **(a)** CA-AMPK was expressed in *WT* and *Cebpb*$^{-/-}$ MEFs and cell proliferation was analyzed over a six-day time course. n=2 independent biological replicates, each time point assayed in triplicate; error bars represent S.E.M. Statistics were determined for day 6 using Student's two tailed t test.; *p<0.05. The immunoblot (right panel) shows C/EBPβ and CA-AMPK levels in these cells. **(b)** AMPK signaling stimulates C/EBPβ DNA binding in MEFs. EMSA was performed using nuclear extracts from *WT* MEFs lacking or expressing CA-AMPK and a canonical C/EBP site probe. C/EBPβ levels were equalized in the EMSA assay. **(c)** CA-AMPK induces senescence in a C/EBPβ-dependent manner. Senescence was evaluated by SA-βGal staining. The percentage of SA-β-Gal^{+} cells in *WT* and *Cebpb*$^{-/-}$ MEFs, without or with CA-AMPK, was determined and is plotted in the lower panel. Data are from 3 independent experiments (n>200 cells scored in total); values represent the mean ± S.E.M. Statistics were

determined using Student's two tailed t test; *p<0.05. **(d)** HuR undergoes nuclear translocation in MEFs expressing CA-AMPK. IF staining of HuR was performed in *WT* and *Cebpb*$^{-/-}$ cells, without and with expression of CA-AMPK. Lower panel shows the average HuR cytoplasmic staining intensity in each cell population. n=5; values represent the mean ± S.E.M. Statistics were determined using Student's two tailed t test; *p<0.05.

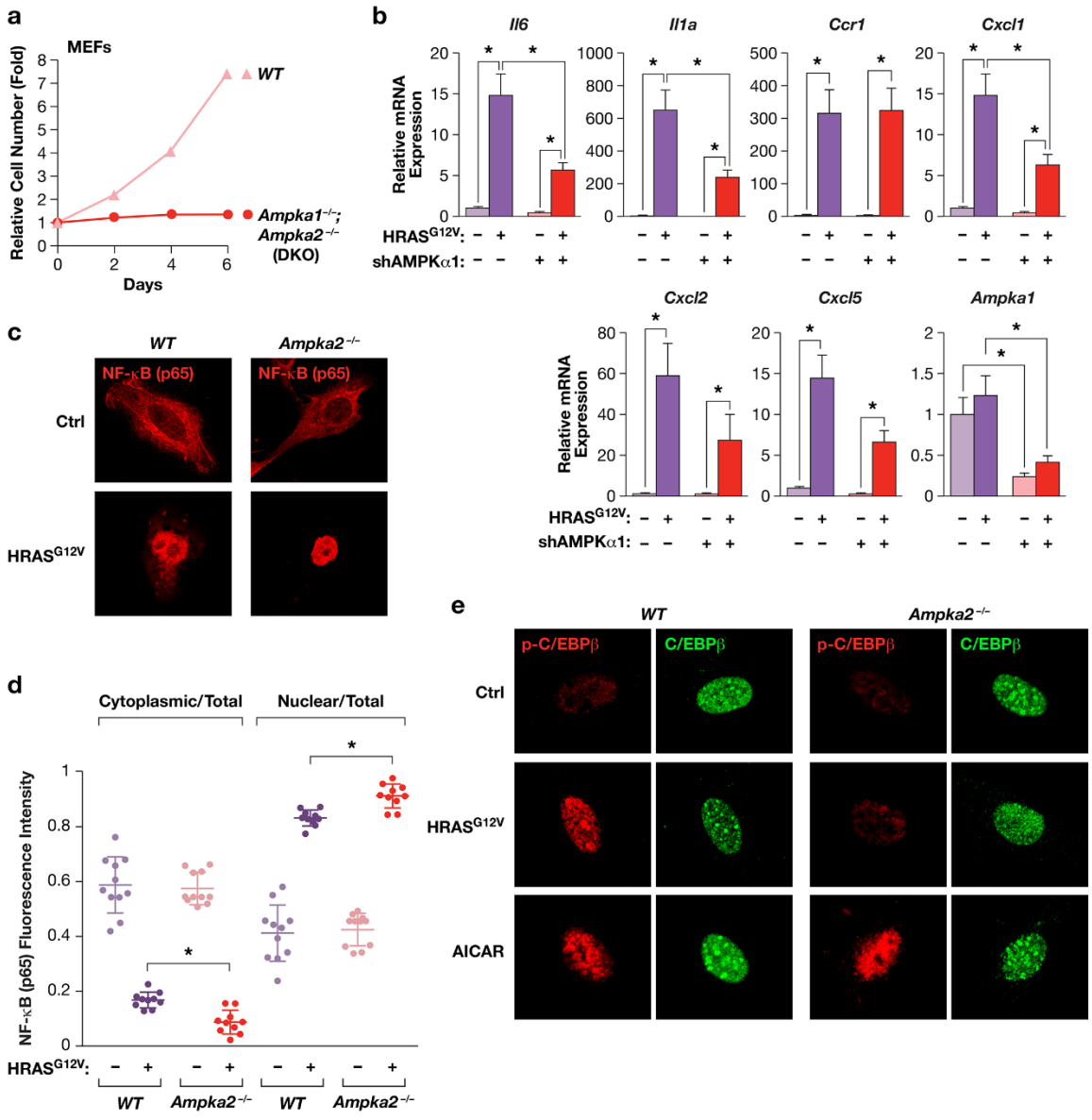
Supplementary Figure 5



Supplementary Figure 5 Salicylate activates C/EBPβ and induces C/EBPβ-dependent senescence in MEFs. **(a)** Proliferation assays of *WT* and *Cebpb*$^{-/-}$ MEFs, without and with salicylate treatment (0.3 mM). n=3 independent biological replicates, each time point assayed in triplicate; error bars represent S.E.M. Statistics were determined for day 6 using Student's

two tailed t test.; *p<0.05. Right panel: immunoblot showing levels of p-AMPKα (Thr172) and total AMPKα, with the ratios calculated. **(b)** EMSA showing C/EBPβ DNA-binding activity in *WT* and *Cebpb*$^{-/-}$ MEFs, without and with salicylate treatment. In lanes 5-8, the same samples were analyzed by supershift with a C/EBPβ antibody. **(c)** Proportion of senescent (SA-β-Gal^{+}) cells in *WT* and *Cebpb*$^{-/-}$ MEFs, without and with salicylate treatment. Data are from 3 independent experiments (n>200 cells scored in total); values represent the mean ± S.E.M. Statistics were determined using Student's two tailed t test; *p<0.05. **(d)** IF staining of HuR in control and salicylate-treated MEFs. Right panel: quantitation of cytoplasmic HuR fluorescence intensity. n=5 cells; error bars represent S.EM. Statistical differences were determined by Student's t test; *p<0.05. **(e)** The AMPK inhibitor, compound C, decreases C/EBPβ DNA binding in MEFs. Cells were treated with vehicle or 2.5 μM compound C for 24 hr prior to harvest. Cytoplasmic extracts were analyzed for p-AMPKα (Thr172) and HuR levels by immunoblotting (left panel). Nuclear extracts were analyzed by EMSA using a C/EBP site probe (right panel). **(f)** Proliferation of *WT* and *Cebpb*$^{-/-}$ MEFs in the absence and presence of rapamycin (100 pM). n=2 independent experiments, assayed in triplicate; error bars represent S.EM. Statistical differences were determined by Student's t test; *p<0.05. **(g)** EMSA showing C/EBPβ DNA binding in *WT* MEFs without or with rapamycin treatment (1 nM). Long exposures of this gel and the one in panel **(e)** were used to visualize C/EBPβ DNA binding in these cells, which do not express oncogenic RAS. Lower panels show immunoblots for the mTOR target, phospho-S6k1, and C/EBPβ. β-actin was used as a loading control.

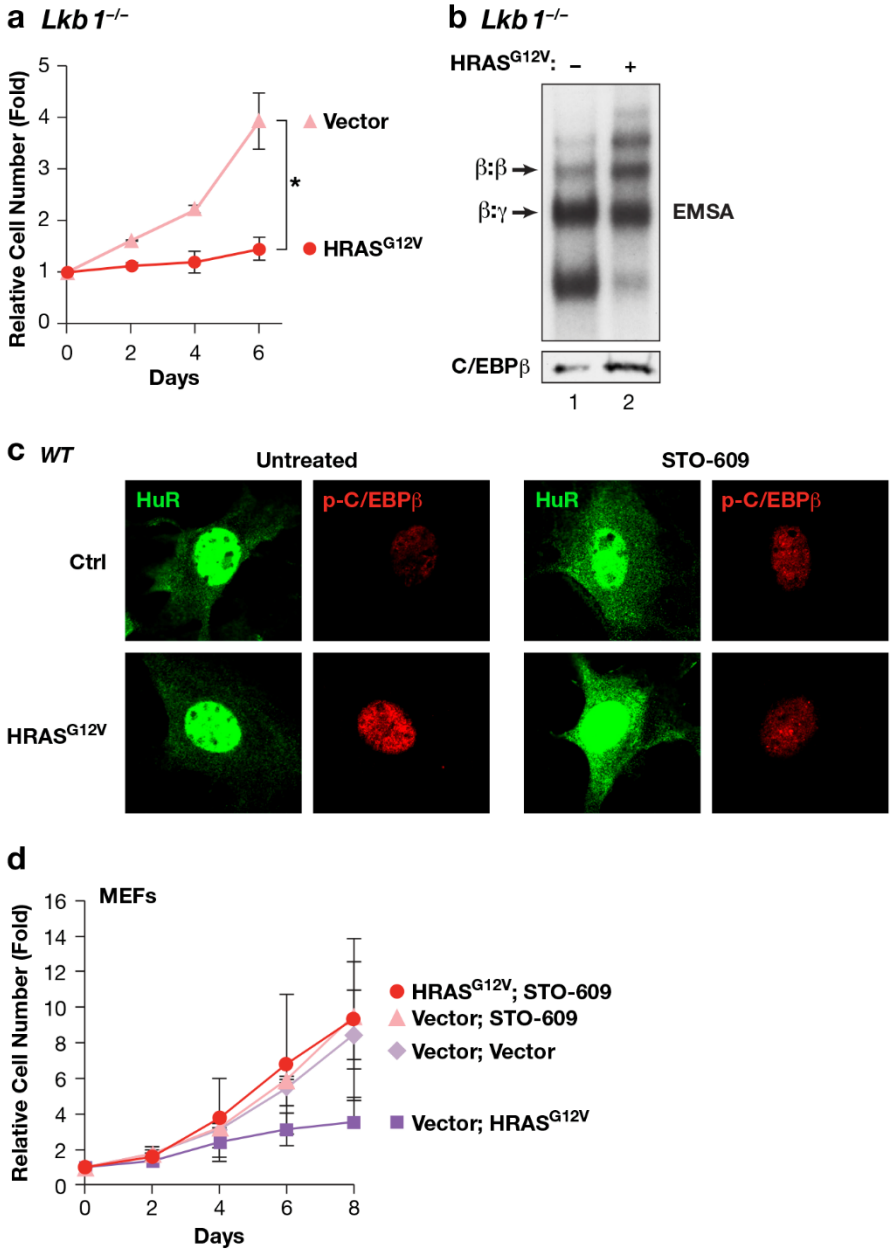
Supplementary Figure 6



Supplementary Figure 6 Effect of AMPKα deficiency on cell proliferation, SASP induction and activation of NF-κB and C/EBPβ. **(a)** Impaired growth of primary *Ampka1*$^{-/-}$*;Ampka2*$^{-/-}$ (DKO) MEFs. Low passage *WT* and mutant MEFs were analyzed for proliferation over a 6-day time course. n=1, each time point analyzed in triplicate. **(b)** Effect of AMPKα1 depletion on the SASP gene expression. *WT* MEFs infected with lentiviral vectors expressing non-specific and AMPKα1-specific shRNAs without and with HRAS^{G12V} were analyzed for SASP gene expression by qRT-PCR. n=2 biological replicates, each sample assayed in triplicate. Values are averages and error bars represent S.D. Statistical significance was calculated using Student's

two-tailed t test; *p<0.05. **(c)** Enhanced NF-κB activation in *Ampka2*$^{-/-}$ MEFs. Control and HRAS^{G12V}-expressing *WT* and *Ampka2*$^{-/-}$ MEFs were immunostained for NF-κB p65 (RelA). **(d)** Quantitation of NF-κB p65 nucleocytoplasmic distribution in *WT* and *Ampka2*$^{-/-}$ MEFs. Nuclear and cytoplasmic p65 signals were quantitated using ImageJ from two independent experiments (n=10 cells scored in total), and cytoplasmic/total and nuclear/total ratios were calculated. Error bars represent S.E.M.; statistical differences were determined by Student's t test; *p<0.05. **(e)** Analysis of C/EBPβ phosphorylation in *WT* and *Ampka2*$^{-/-}$ MEFs, without and with HRAS^{G12V} expression or AICAR treatment. Cells were co-immunostained using anti-p-C/EBPβ (Thr188) and total C/EBPβ antibodies.

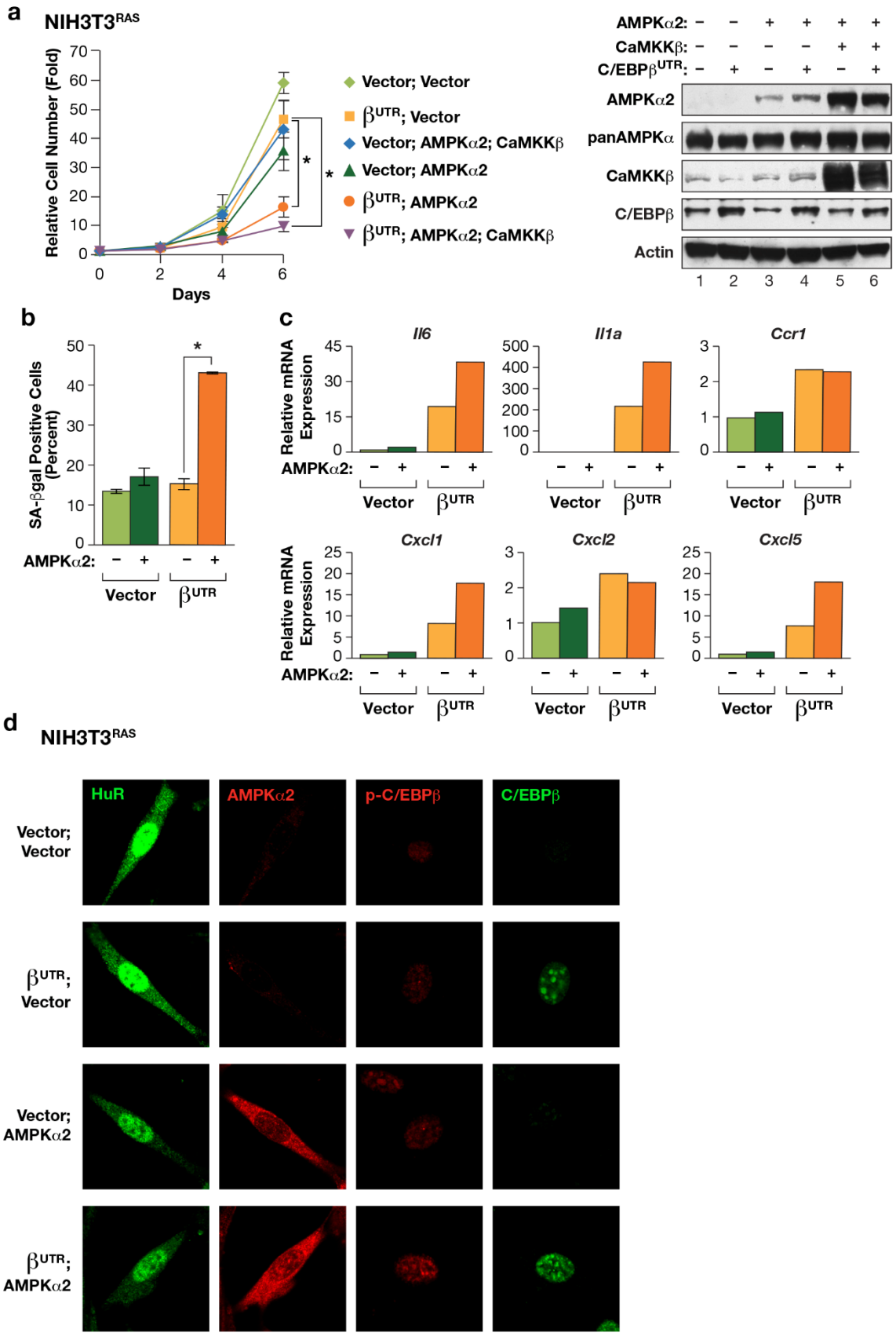
Supplementary Figure 7



Supplementary Figure 7 HRAS^{G12V}-induced growth arrest, activation of C/EBPβ and HuR translocation is independent of the AMPK kinase, LKB1. **(a)** Proliferation assays of *Lkb1*$^{-/-}$ MEFs, without and with HRAS^{G12V}. n=2; each time point analyzed in triplicate. Error bars represent S.EM. Statistical differences were determined by Student's t test; *p<0.05. **(b)** C/EBPβ DNA-binding activity in *Lkb1*$^{-/-}$ MEFs in the absence or presence of HRAS^{G12V}. Nuclear extracts were normalized for C/EBPβ levels and analyzed by EMSA. **(c)** The CaMKK inhibitor, STO-609, blocks HRAS^{G12V}-induced HuR nuclear translocation and C/EBPβ

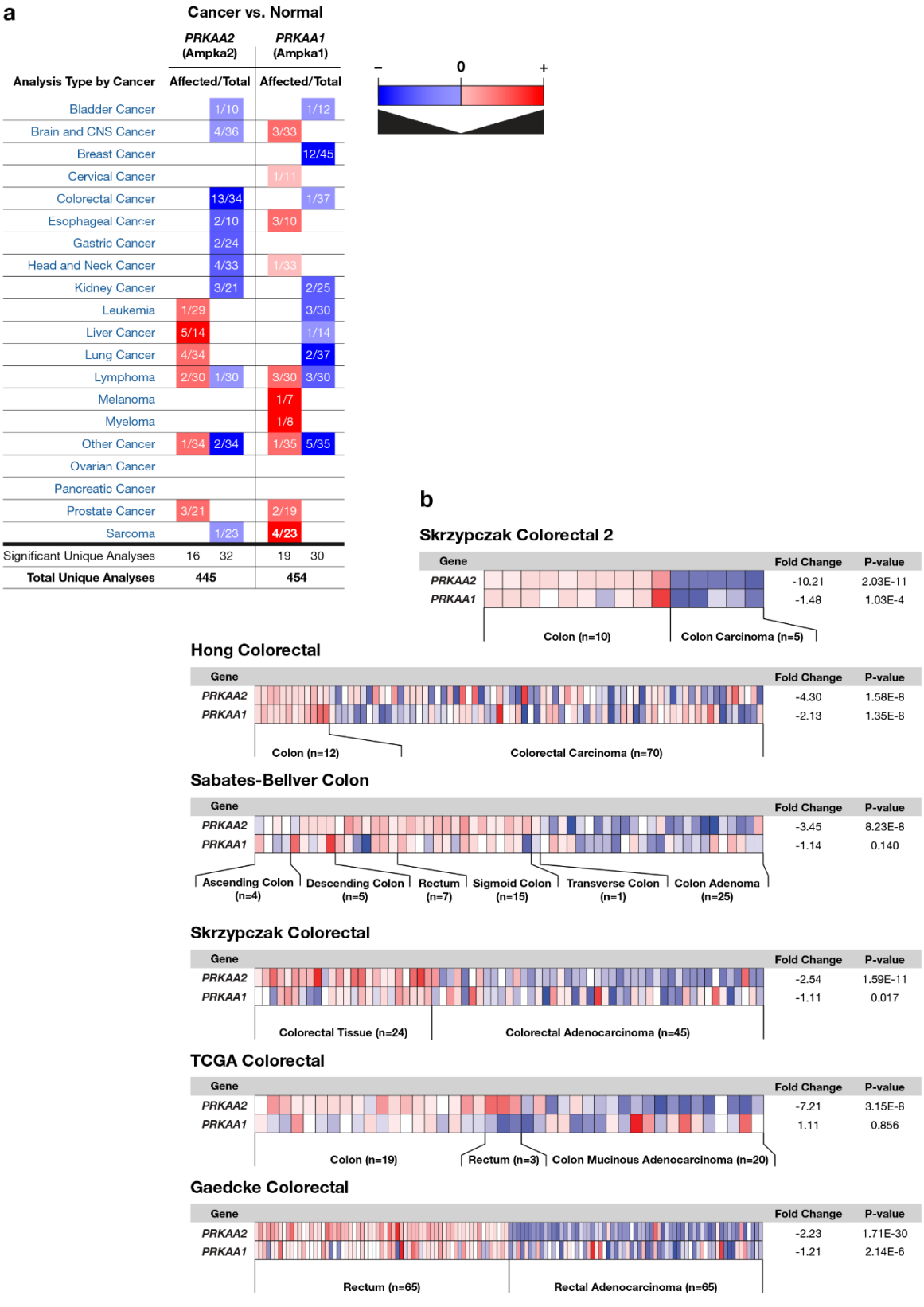
phosphorylation. Control and $HRAS^{G12V}$-expressing MEFs were treated with vehicle or 1 μg/ml STO-609 for 16 hr prior to fixation. The cells were immunostained for HuR and p-C/EBPβ (Thr188). **(d)** STO-609 abrogates $HRAS^{G12V}$-induced growth arrest in MEFs. Cells were treated with vehicle or 0.1 μg/ml STO-609; the drug was administered immediately following retroviral infection and maintained throughout the experiment. n=2 independent biological replicates, assayed in triplicate; values represent means ± S.E.M.

Supplementary Figure 8



Supplementary Figure 8 (a) Expression of *Cebpb*^{UTR} together with AMPKα2 and CaMKKβ inhibits proliferation and induces senescence in NIH3T3^{RAS} cells. Proliferation was analyzed over a 6-day time course in cells expressing the indicated proteins. n=3 independent biological replicates, each time point assayed in triplicate. Error bars represent S.EM. Statistical differences were determined by Student's t test; *p<0.05. Immunoblots for AMPKα2, pan-AMPKα, CaMKKβ, and total C/EBPβ levels are shown on the right. **(b)** Quantitation of senescent (SA-β-Gal^{+}) cells in *Cebpb*^{UTR} and AMPKα2 expressing NIH3T3^{RAS} cells. Data are from two independent experiments (n>300 cells scored in total); values represent the mean ± S.E.M. Statistics were determined using Student's two tailed t test; *p<0.05. **(c)** *Cebpb*^{UTR} and AMPKα2 increase expression of SASP genes in NIH3T3^{RAS} cells. n=1, samples assayed in triplicate. **(d)** Over-expression of AMPKα2 in NIH3T3^{RAS} cells elicits HuR nuclear translocation and C/EBPβ phosphorylation. AMPKα2 was expressed ± *Cebpb*^{UTR} and the cells were immunostained for HuR, AMPKα2, p-C/EBPβ (Thr188) and total C/EBPβ.

Supplementary Figure 9



Supplementary Figure 9 *AMPKA1* and *AMPKA2* mRNA expression in human tumors. **(a)** Summary of human cancer studies showing tumor-associated changes in *AMPKA1* (*PRKAA1*) or *AMPKA2* (*PRKAA2*) mRNA expression. Meta-analysis of microarray data is from Oncomine[3]. Thresholds of 1.5-fold change and $p<0.05$ were used for the analysis. Tables show fraction of studies with significant differences in mRNA levels in tumors vs. normal tissue, listed by tumor type. **(b)** Heat map representations of fold changes in *PRKAA1* or *PRKAA2* mRNA expression in several independent colorectal tumor studies. Tumor samples were compared to their respective normal tissue. Thresholds of 1.5-fold change and $p<0.05$ were used for the analysis. P-values were calculated using Student's t test.

SUPPLEMENTARY REFERENCES

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3 Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D *et al*. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 2004; 6: 1-6.