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Supplemental Material to:

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Integrated regulation of autophagy and apoptosis by EEF2K controls cellular fate and modulates the efficacy of curcumin and velcade against tumor cells

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Fig. 1

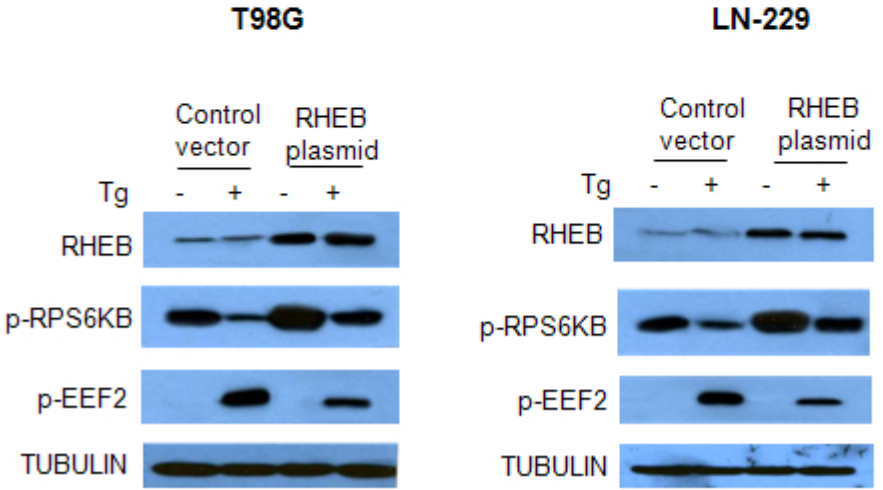

T98G
Control vector
RHEB plasmid
Tg
- + - +
RHEB
p-RPS6KB
p-EEF2
TUBULIN
LN-229
Control vector
RHEB plasmid
Tg
- + - +
RHEB
p-RPS6KB
p-EEF2
TUBULIN

Fig. 2

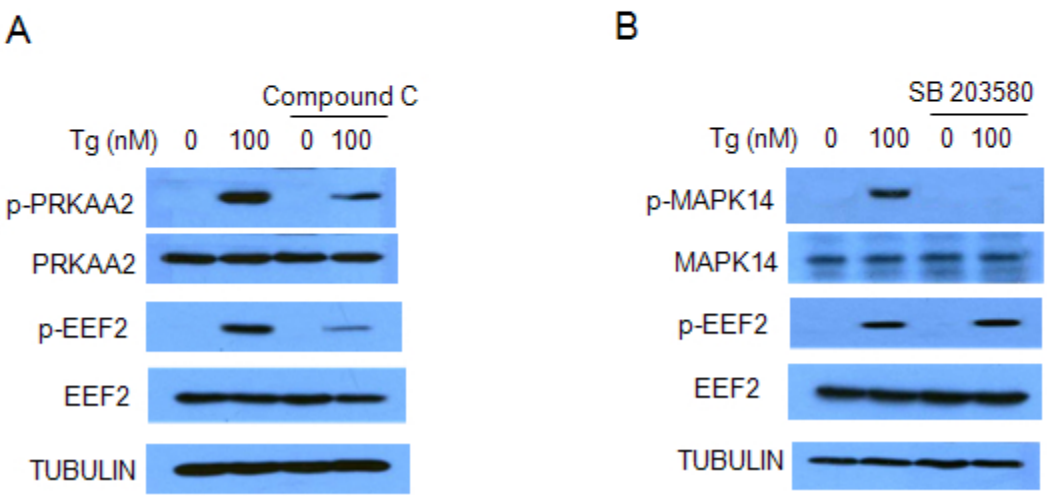
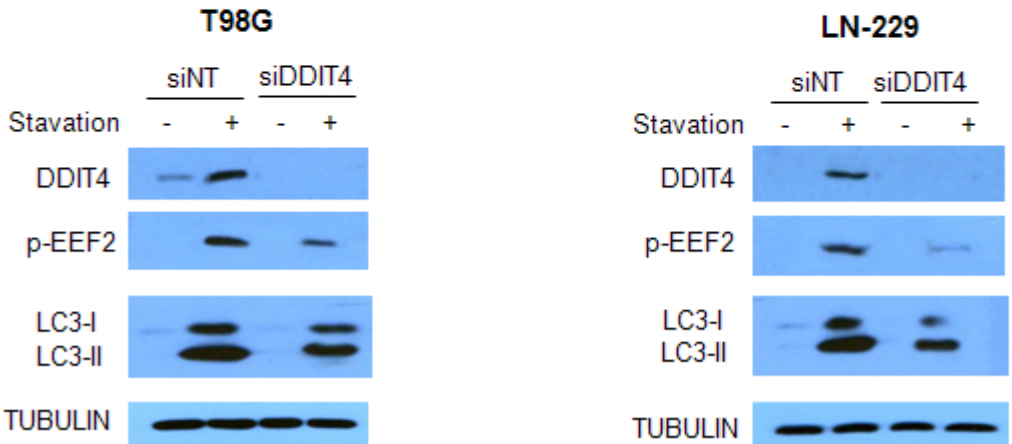

A
Compound C
Tg (nM) 0 100 0 100
p-PRKAA2
PRKAA2
p-EEF2
EEF2
TUBULIN
B
SB 203580
Tg (nM) 0 100 0 100
p-MAPK14
MAPK14
p-EEF2
EEF2
TUBULIN

Fig. 3


T98G
siNT
siDDIT4
Stavation
-
+
-
+
DDIT4
p-EEF2
LC3-I
LC3-II
TUBULIN
LN-229
siNT
siDDIT4
Stavation
-
+
-
+
DDIT4
p-EEF2
LC3-I
LC3-II
TUBULIN

Legends to Supplementary Figures

Figure S1. Enforced expression of RHEB decreases the activity of EEF2K in tumor cells subjected to ER stress. LN-229 or T98G cells were transfected with a control plasmid or a RHEB plasmid, followed by treatment with Tg or vehicle for 24 h. At the end of treatment, the levels of RHEB, p-RPS6KB, and p-EEF2 were examined by western blot. TUBULIN was used as a loading control.

Figure S2. Effects of the inhibitors of PRKAA2 and MAPK14 on the ER stress-induced activation of EEF2K. (A) LN-229 cells were treated with Tg for 24 h in the presence or absence of the PRKAA2 inhibitor, Compound C. The levels of p-PRKAA2, PRKAA2, p-EEF2 and EEF2 were examined by western blot. TUBULIN was used as a loading control. **(B)** LN-229 cells were treated with Tg for 24 h in the presence or absence of the MAPK14 inhibitor, SB203580. The levels of p-MAPK14, MAPK14, p-EEF2 and EEF2 were examined by western blot. TUBULIN was used as a loading control.

Figure S3. Silencing of DDIT4 expression blocks activation of EEF2K and suppressed autophagy in tumor cells subjected to nutrient starvation. LN-229 or T98G cells were transfected with a non-targeting RNA or a siRNA targeting *DDIT4*, followed by treatment with PBS for 2 h. The levels of DDIT4, p-EEF2 and LC3 were examined by western blot. TUBULIN was used as a loading control.