

## **SUBJECT AREAS: CANCER**

### **NQO1-induced activation of AMPK contributes to cancer cell death by oxygen-glucose deprivation**

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Figure S1.

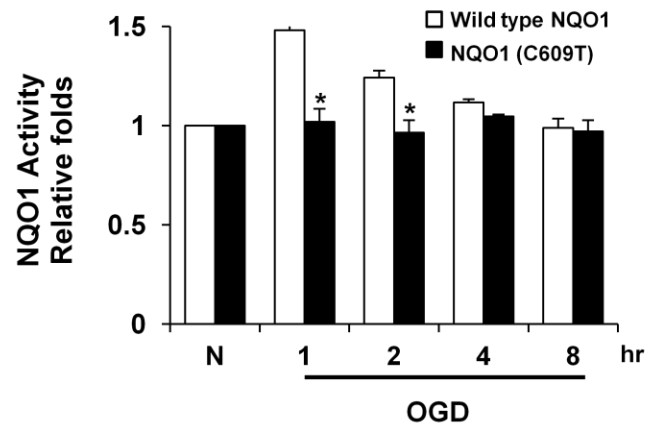
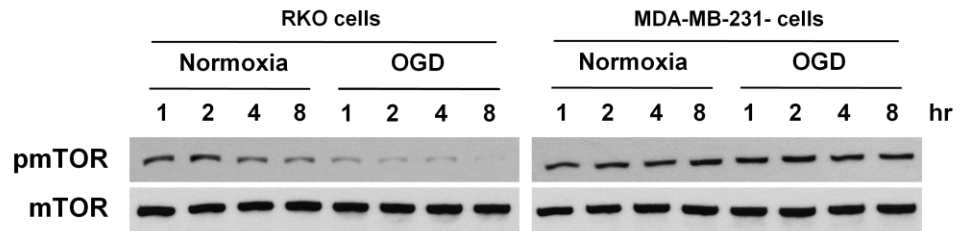


Figure S2.

A



B

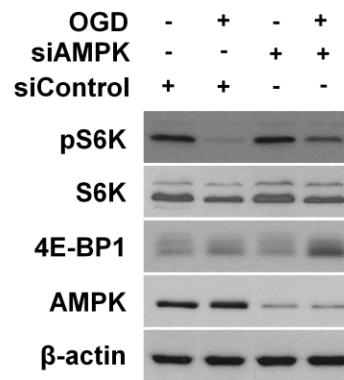


Figure S3.

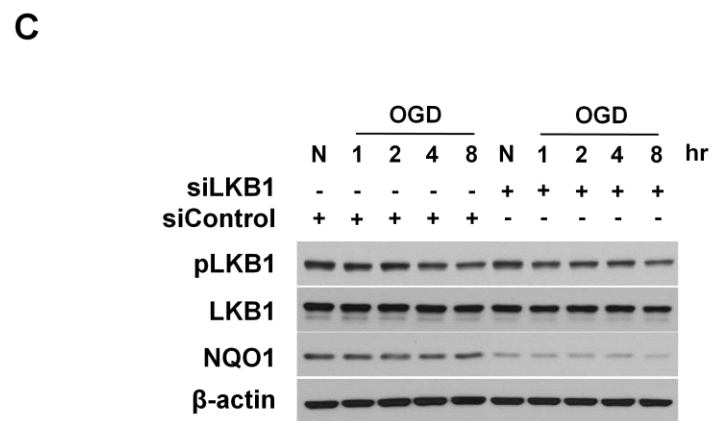
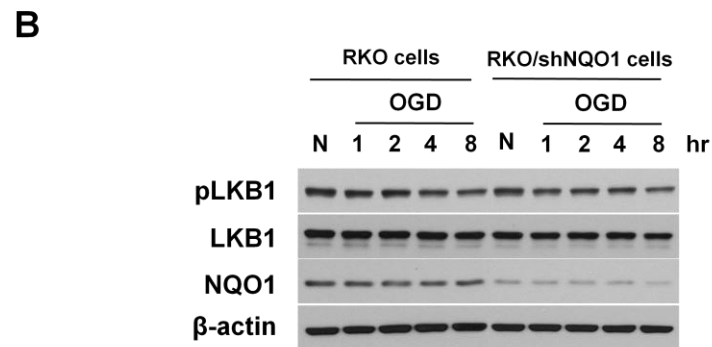
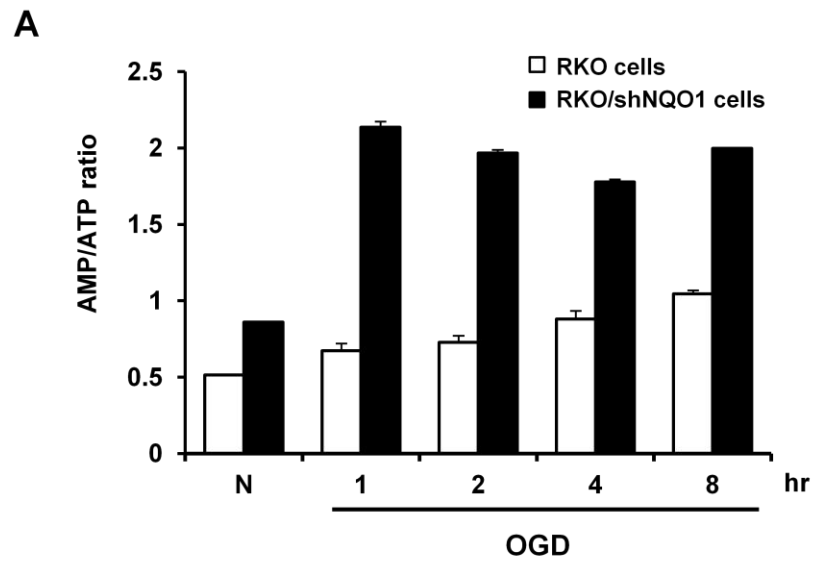
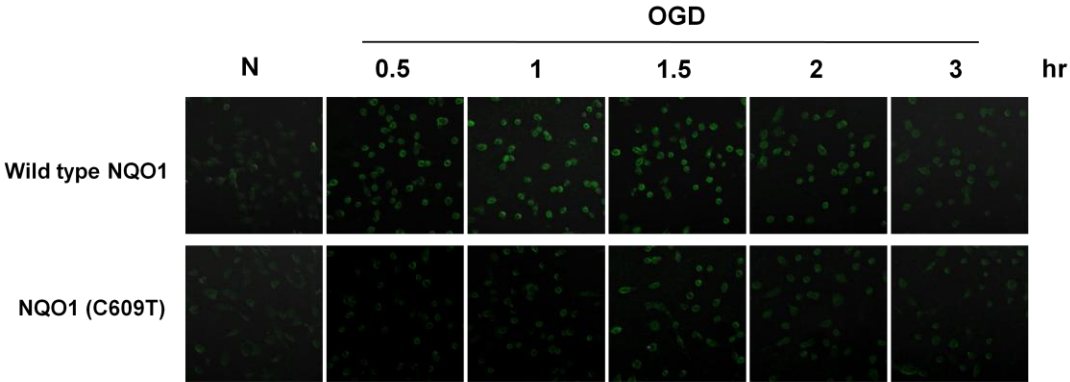


Figure S4.

A



B

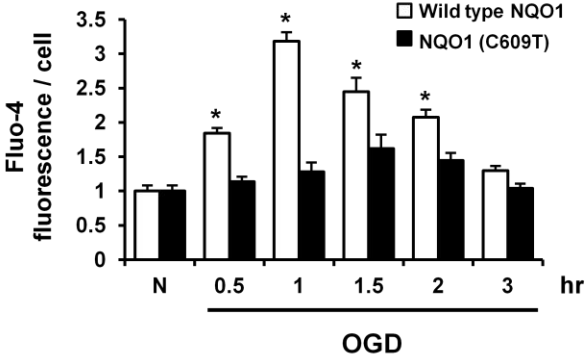


Figure S5.

A

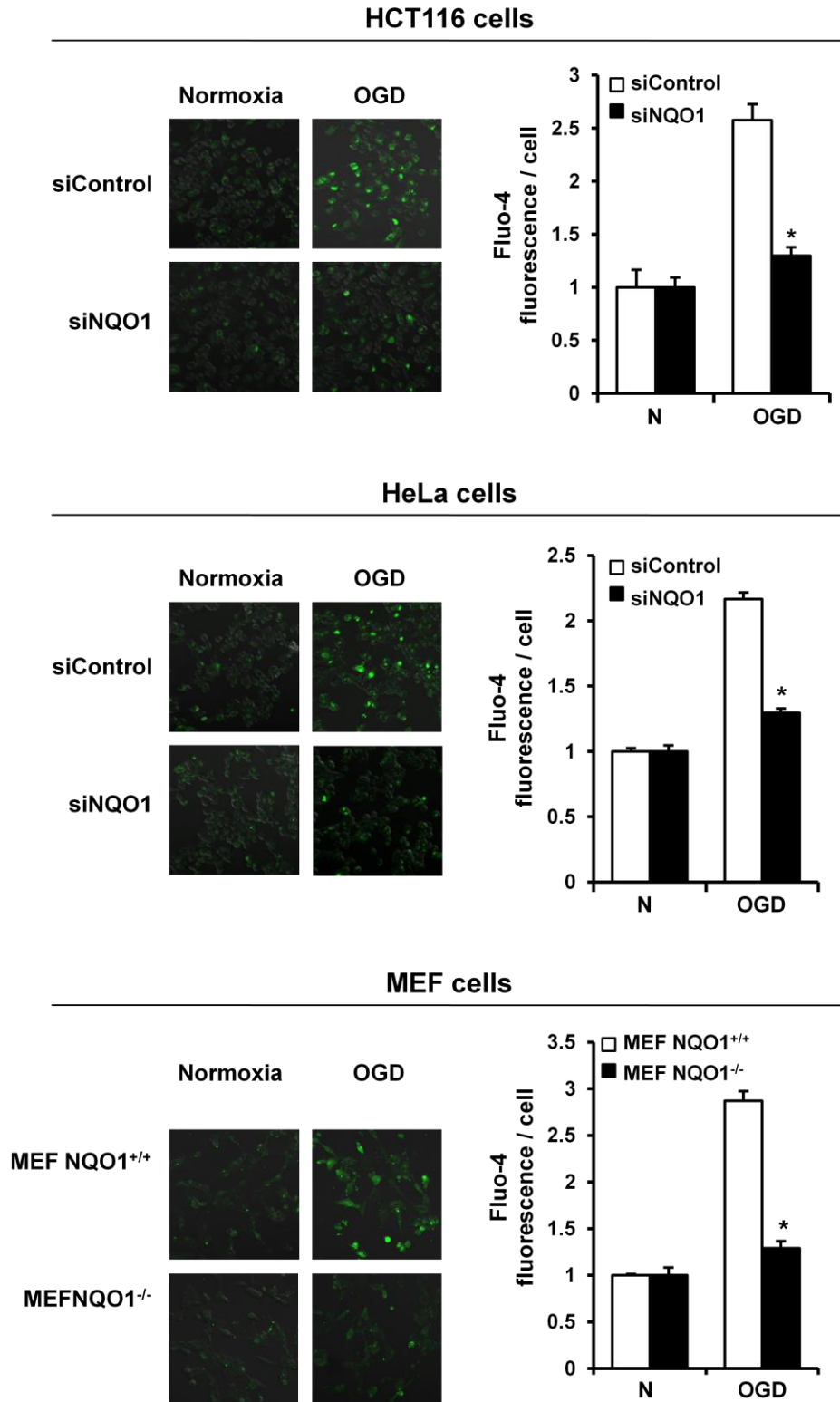
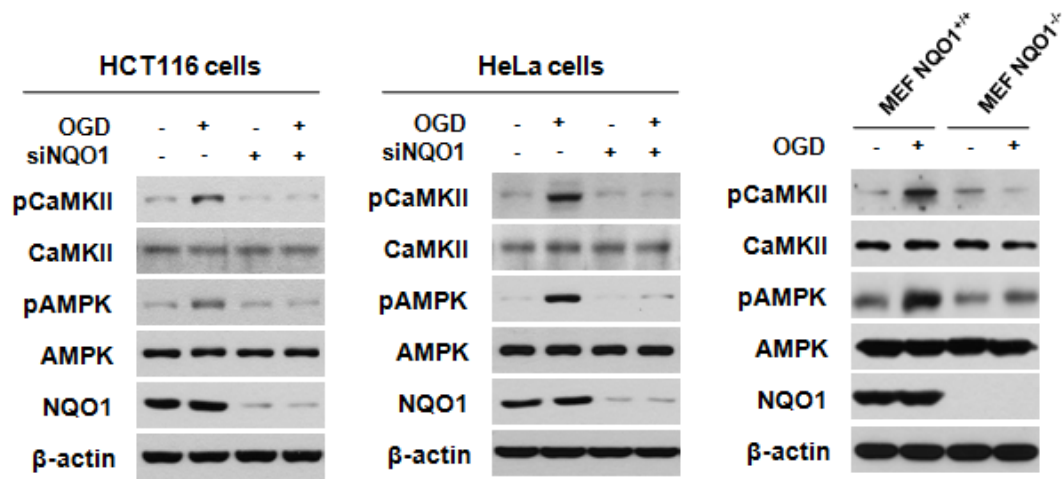
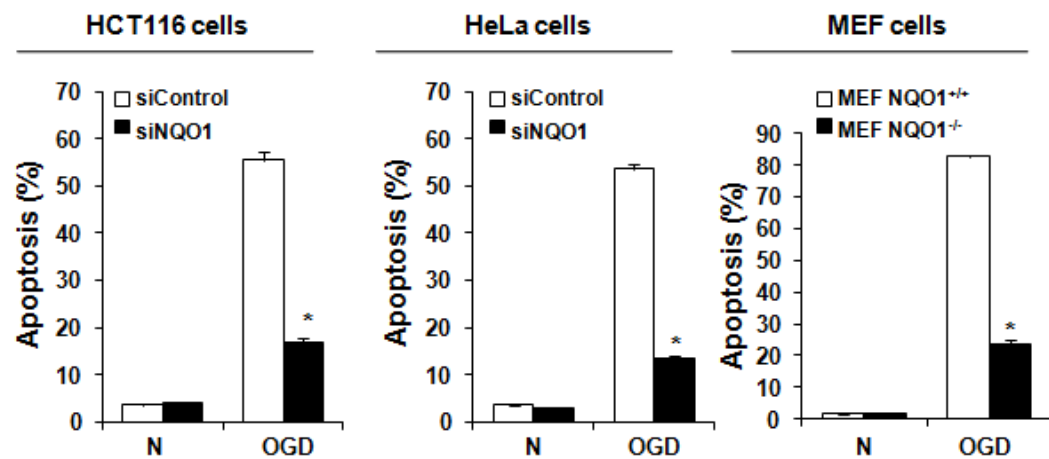


Figure S5. continued

**B**



**C**



## Supplementary figure caption

### Figure S1

Activities of wild-type and mutant (C609T) NQO1 under OGD. MDA-MB231-/pNQO1 and MDA-MB231-/pNQO1 (C609T) cells were exposed to OGD for the indicated times, and NQO1 activity was assessed. The results from three independent experiments are expressed as means  $\pm$  SEM (\*,  $P < 0.05$ ).

### Figure S2

Involvement of NQO1 in OGD-induced AMPK/mTOR signaling. (A) Expression levels of pmTOR and mTOR in parental RKO and MDA-MB-231- cells exposed to OGD for the indicated times. (B) RKO cells transfected with siRNAs against AMPK were exposed to OGD for 8 h, and the phosphorylation levels of S6K, S6K, 4E-BP1 and AMPK in whole-cell lysates were determined by immunoblotting.

### Figure S3

OGD does not act through LKB1 to activate AMPK. (A) Intracellular [AMP]/[ATP] ratios in RKO and RKO/shNQO1 cells exposed to OGD for the indicated times. (B) Expression of pLKB, LKB, and NQO1 in RKO and RKO/shNQO1 cells exposed to OGD for the indicated times. (C) Effect of LKB1 knockdown on the expression levels of pAMPK and pACC in RKO cells. Abbreviation: N, normoxic control.

### Figure S4

Effect of OGD on  $[Ca^{2+}]_i$  in MDA-MB231-/pNQO1 and MDAMB231-/pNQO1 (C609T) cells (A)-(B) Fluo-4-AM-loaded cells were exposed to OGD for the indicated times. Representative fluorescence microscopic images (A) and quantification of  $[Ca^{2+}]_i$  (B) are shown. The results from three independent experiments are expressed as means  $\pm$  SEM (\*,  $P < 0.05$ ). Abbreviation: N, normoxic control.

### Figure S5

Effect of NQO1 on OGD-induced activation of CaMKII/AMPK signaling pathway via increase of  $[Ca^{2+}]_i$  and cancer cell death in various cell lines. (A) Fluo-4-AM-loaded cells were exposed to OGD for 1 h. Representative fluorescence microscopic images and quantification of  $[Ca^{2+}]_i$  are shown. The results from three independent experiments are expressed as means  $\pm$  SEM (\*,  $P < 0.05$ ). (B) The cells were exposed to OGD for 1 h, and whole-cell lysates were analyzed by immunoblotting using antibodies against pCaMKII, pAMPK, CaMKII and AMPK. (C) Role of NQO1 in OGD-induced apoptosis. The cells were exposed to OGD for 48 h, and the apoptotic cells were analyzed using flow cytometry. The results from three independent experiments are expressed as means  $\pm$  SEM (\*,  $P < 0.05$ ). Abbreviation: N, normoxic control.