Qing G supplementary Table

Summary of HIF-α staining in 15 primary neuroblastoma tumors.

sample	S00-4578	S02-3700	S08-6611	S08-2426	S03-2888	S08-7220	S92-4105	S00-4481	S01-3408	S95-268	S96-1558	892-4105	903-2568	S95-3787	900-1545
MYCN status	amp	amp	amp	amp	amp	amp	amp	amp	amp	amp	amp	amp	amp	no	no
HIF-1α	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HIF-2α	–	+	+	+	–	–	–	–	–	–	–	–	–	+	+

("+": positive HIF-α staining; "–": negative HIF-α staining)

Figure legends for supplemental figures

Figure S1. Immunoblot analysis of HIF-1α and HIF-2α expression in neuroblastoma cells at 5% O_2. (A-F) Neuroblastoma cells were cultured at 21% and 5% O_2 for 48 hr, and respective HIF-α levels were detected using specific antibodies. Actin was used as a loading control.

Figure S2. Immunoblot analysis of HIF-1α and HIF-2α expression in neuroblastoma cells at 1.5% O_2. (A-I) Neuroblastoma cells cultured at 21% and 1.5% O_2 were harvested at different time points, and respective HIF-α levels were detected using specific antibodies. Actin was used as a loading control.

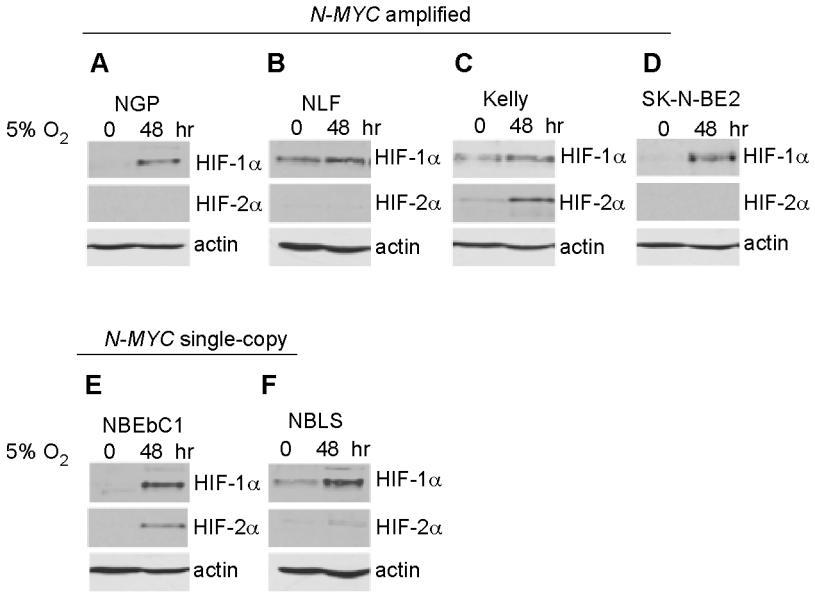
Figure S3. Relative levels of HIF-1α and HIF-2α mRNA in primary neuroblastoma samples with different tumor risks. (A) Relative levels of HIF-1α. (B) Relative levels of HIF-2α. The levels of both HIF-α isoforms within each tumor risk group were normalized based on that of actin. Numbers of tumors used in the assay: low risk group (28), intermediate (21), *MYCN* single-copy, high risk (32) and *MYCN* amplified, high risk (20).

Figure S4. Proliferation of *MYCN* amplified cell lines under normoxia and hypoxia. (A-C) NPG, IMR32, and SMS-SAN cells were cultured at 21% and 1.5% O_2 and cell counting was performed at different time points shown in the figure. (D) LAN5 cells were cultured at 21% and 0.5% O_2 and cell counting was performed at different time points shown in the figure. Data are shown as an average of triplicates.

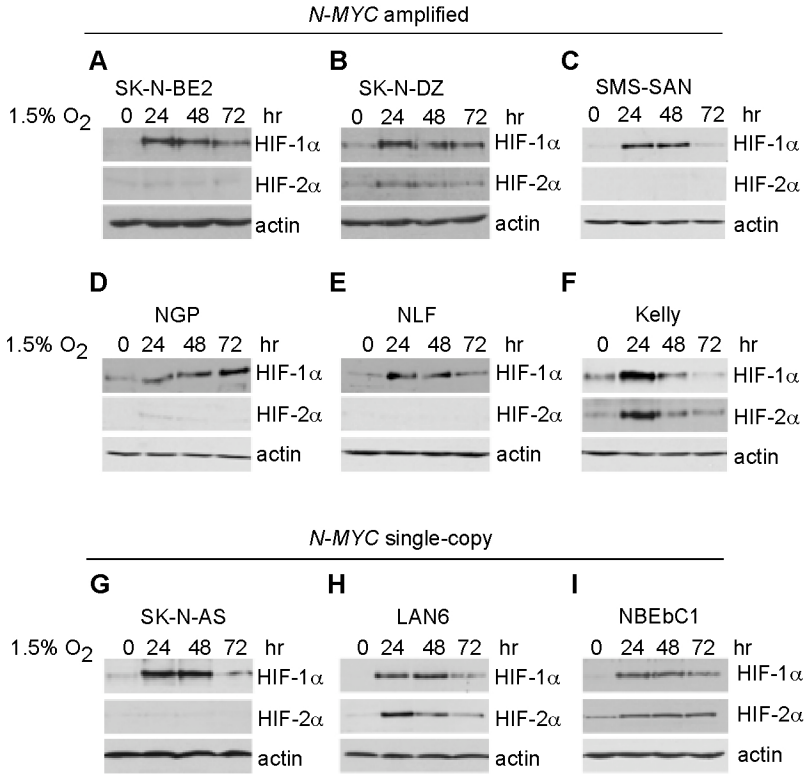
Figure S5. Western analysis of HIF-1α levels in control and knockdown cells. HIF-1α was inhibited by control or specific shRNAs in LAN5 cells, and the protein levels at either normoxia or hypoxia were determined by immunoblot assay. β-actin was used as a loading control.

Figure S6. HIF-2α expression in SK-N-BE2 cells and possible effects on glucose consumption, cell cycle progression and tumorigenesis. (A) Detection of HIF-2α by immunoblot assay. (B) Expression of HIF-α target genes; $p<0.01$. (C) Glucose consumption as analyzed for 1 million cells. Data are shown as an average of triplicates. (D) Representative FACS plots from control and HIF-2α transfected cells grown at 1.5% O_2 for 48 hr. (E) Tumorigenesis analyzed in a xenograft animal model. 5 million control or HIF-2α transfected SK-N-BE2 cells were injected into nude mice, with each group consisting of 5 tumors. $p= 0.886$.

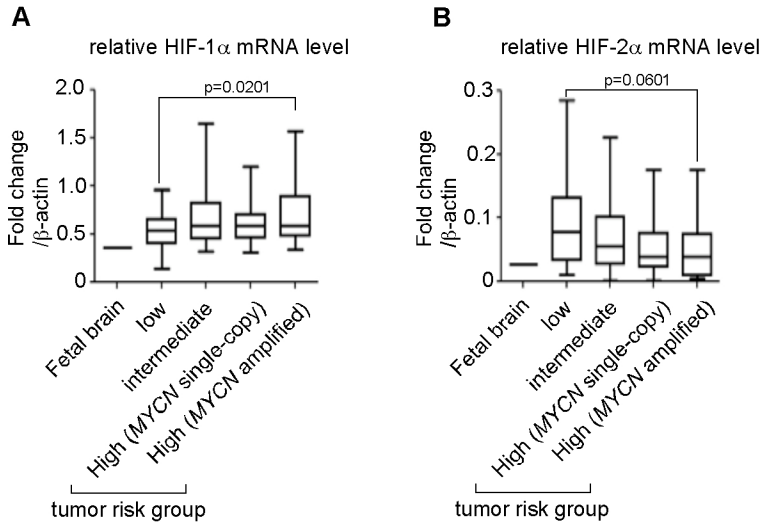
Qing G supplementary Figure 1



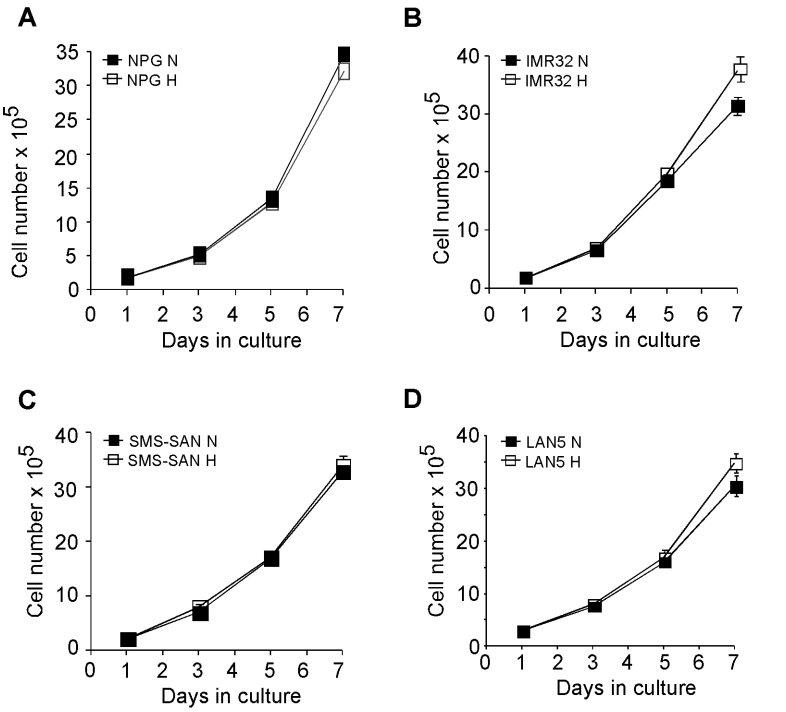
Qing G supplementary Figure 2



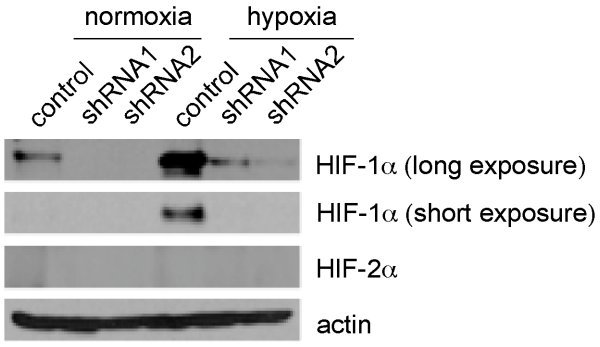
Qing G supplementary Figure 3

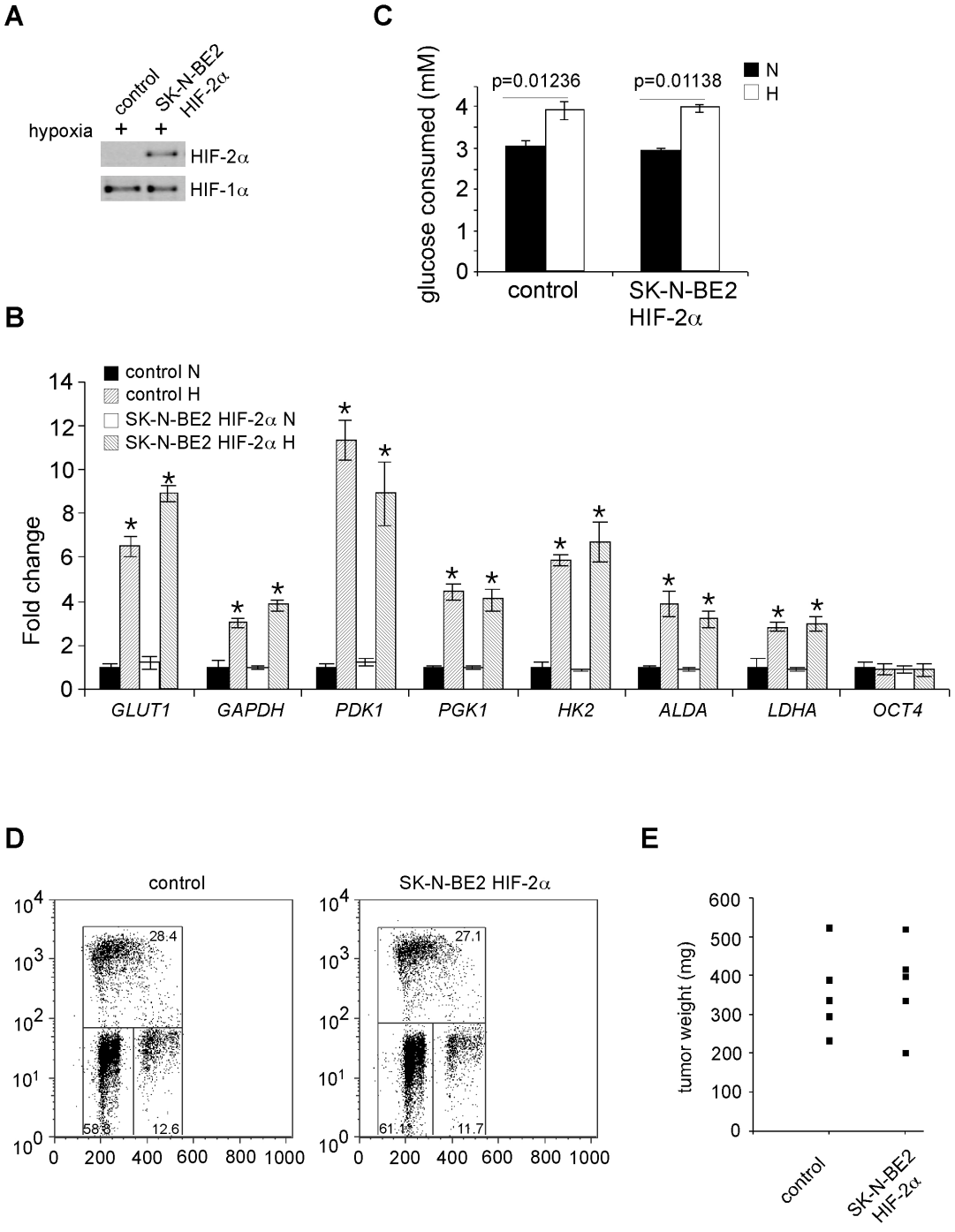


Qing G supplementary Figure 4



Qing G supplementary Figure 5




A
control
SK-N-BE2 HIF-2α
hypoxia + +
HIF-2α
HIF-1α
C
p=0.01236
p=0.01138
N
H
glucose consumed (mM)
control
SK-N-BE2 HIF-2α
B
control N
control H
SK-N-BE2 HIF-2α N
SK-N-BE2 HIF-2α H
Fold change
GLUT1
GAPDH
PDK1
PGK1
HK2
ALDA
LDHA
OCT4
D
control
SK-N-BE2 HIF-2α
28.4
58.8
12.6
27.1
61.1
11.7
E
tumor weight (mg)
control
SK-N-BE2 HIF-2α