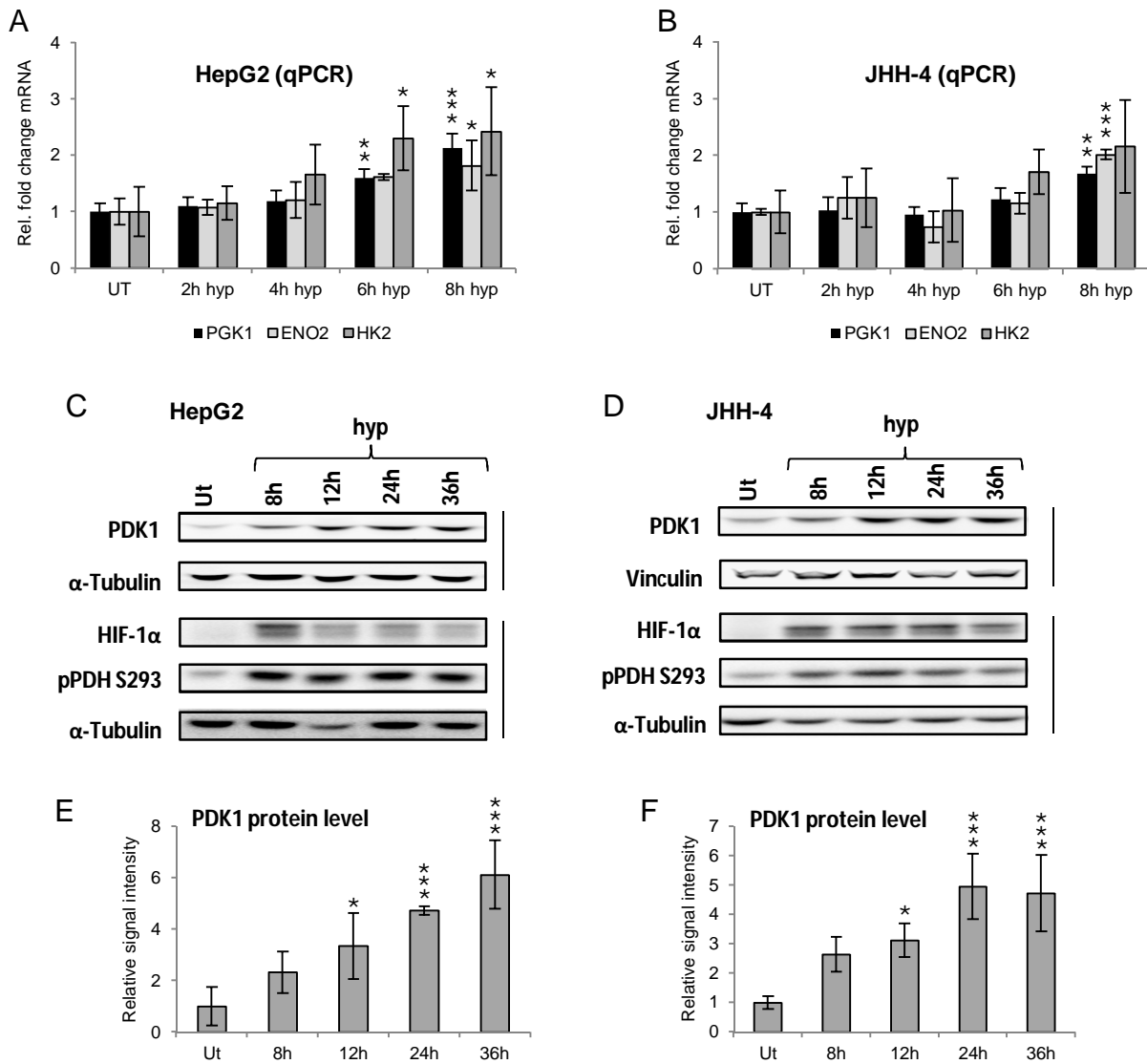


## Supplemental Figure 1:

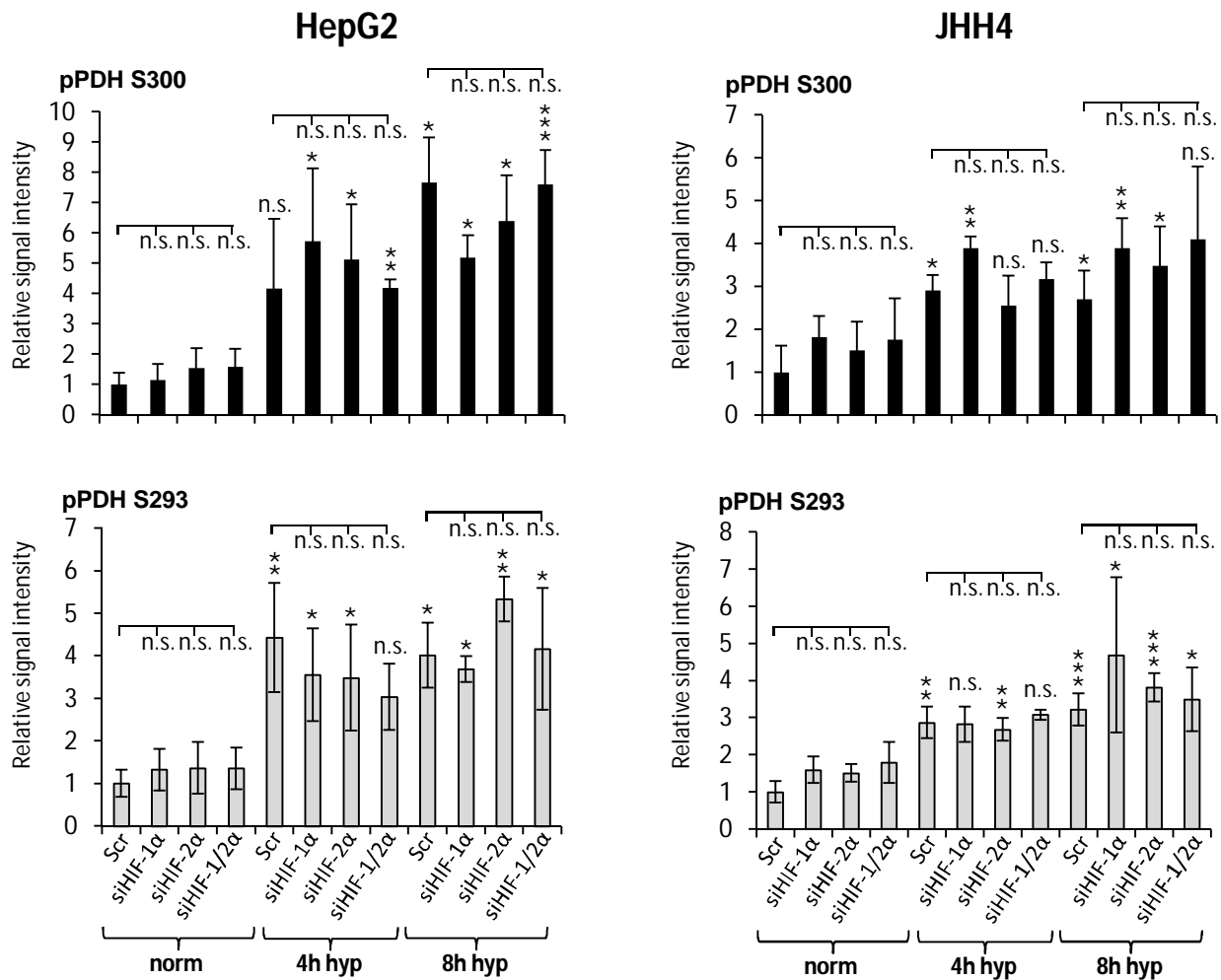


### Supplemental Figure 1: HIF target gene upregulation following hypoxia.

HepG2 and JHH-4 cells were cultivated under hypoxia (hyp; 1% O<sub>2</sub>) for the indicated time periods. For the untreated control (Ut), cells were left without any treatment and harvested together with the other samples. (A & B) qPCR validation of canonical HIF-1 target genes. The target gene expression was normalized to the untreated normoxia control (Ut). Standard deviations are shown for three biological replicates, each time performed in triplicate. Abbreviations: PGK1: phosphoglycerate kinase 1; ENO2: enolase 2; HK2: hexokinase 2. (C & D) Western blot analysis of PDK1 expression and the phosphorylation status of PDH E1 $\alpha$  S293. Detections from one membrane are indicated by vertical lines. (E & F) Quantification of PDK1 protein level at different time points, normalized to the untreated control. Vinculin or  $\alpha$ -tubulin served as loading control. Standard deviations are shown for three biological replicates.

\*\*\*: P < 0.001, \*\*: P < 0.01, \*: P < 0.05.

## Supplemental Figure 2:



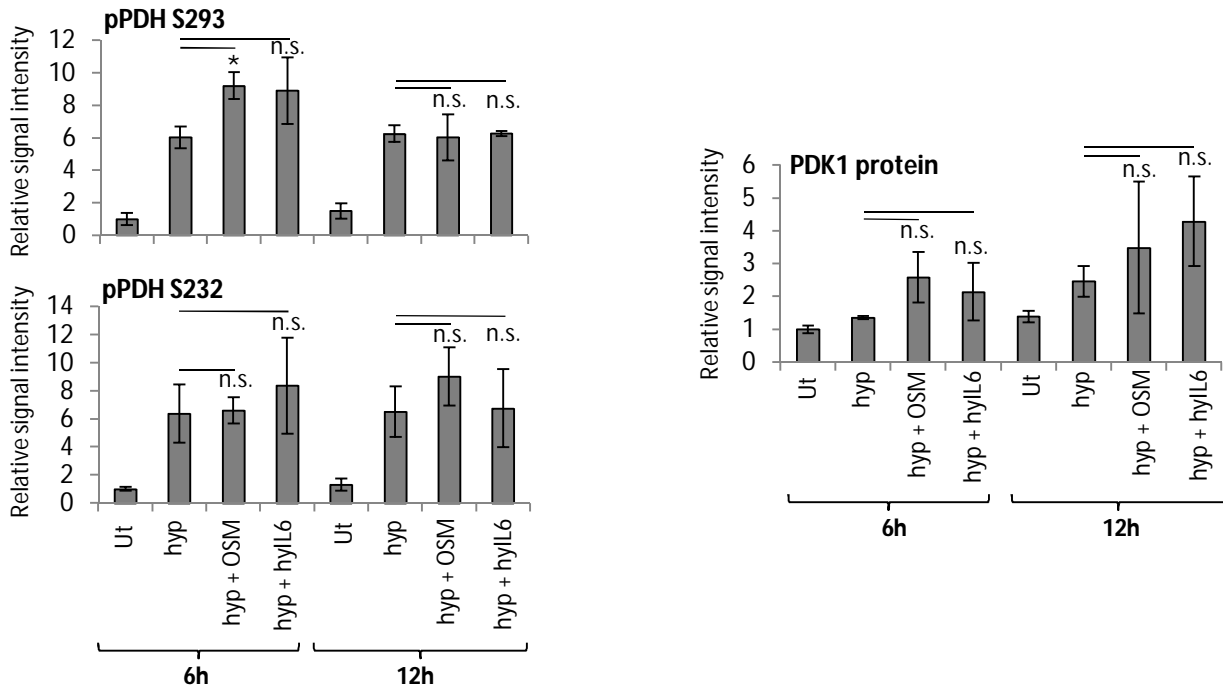
### Supplemental Figure 2: Hypoxia-induced PDH E1 $\alpha$ phosphorylation on S293 and S300 occurs independently of HIF-1 $\alpha$ .

The quantitation of phosphorylated residues S300 and S292 from Western blots presented in Figure 2 are shown. All error bars represent the standard deviation of three biological replicates. Symbols directly located over the bars represent the significance of upregulation with respect to the respective normoxia control. Horizontal bars with corresponding symbols represent the significance of the difference between the Scr- and the siRNA-treated samples at normoxia or at the different time points of hypoxic treatment.

\*\*\*:  $P < 0.001$ , \*\*:  $P < 0.01$ , \*:  $P < 0.05$ , n.s.: not significant.

## Supplemental Figure 3

### HepG2

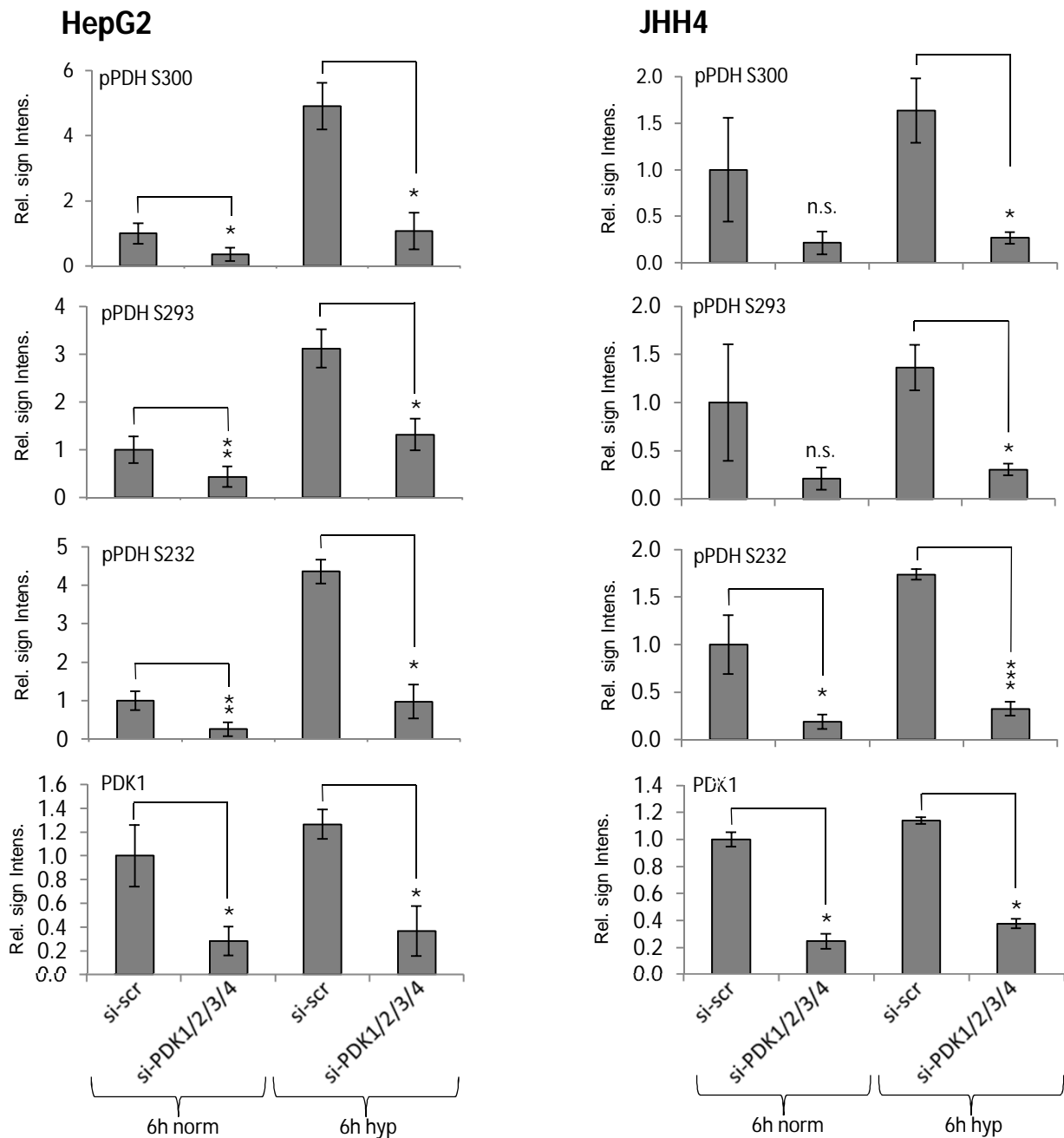


### Supplemental Figure 3: PDH E1 $\alpha$ phosphorylation does not correlate with the amount of induced HIF-1 $\alpha$ protein.

HepG2 cells were cultivated under hypoxia (hyp; 1% O<sub>2</sub>) and left untreated or treated with oncostatin M (OSM; 20 ng/ml) or hyper-IL6 (hIL6; 50 ng/ml) for 6 and 12 hours. Untreated cells under normoxia (Ut) were used as a control. The PDH phosphorylation level on serine 293 and 232 as well as the PDK1 protein expression were quantitated and normalized to the corresponding Western blot signal of the “hypoxia only” treated control. Standard deviations are shown for three biological replicates.

\*: P < 0.05, n.s.: not significant.

## Supplemental Figure 4

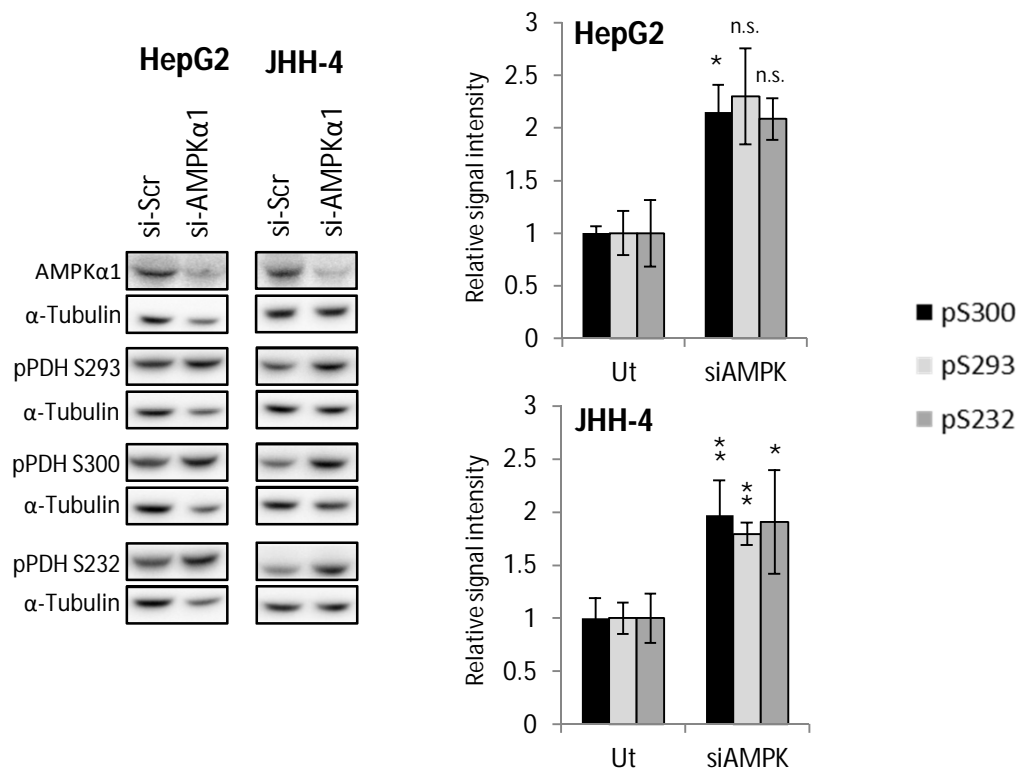


### Supplemental Figure 4: PDKs are crucially involved in the phosphorylation of PDH E1 $\alpha$ .

HepG2 and JHH-4 cells were transfected with siRNA against all four PDK isoforms (PDK1-4; 40 nM siRNA for each isoform) or a scrambled control and cultivated under normoxic (norm) or hypoxic (1% O<sub>2</sub>) conditions for 6h. Western blots of the lysates were prepared and analysed by immune-detection with the indicated antibodies. A quantitation of the PDH phosphorylation level on serine 232, 293 and 300 is shown. PDK1 protein levels are also presented.

\*\*\*: P < 0.001, \*\*: P < 0.01, \*: P < 0.05, n.s.: not significant.

## Supplemental Figure 5



### Supplemental Figure 5: Knock-down of AMPK mediates an upregulation of PDH E1α phosphorylation.

HepG2 and JHH-4 cells were transfected with siRNA against AMPK or a scrambled control. After 48 hours the cells were subjected for 6 hours to hypoxia (1% O<sub>2</sub>). Western blots of the lysates were prepared and analysed by immune-detection with the indicated antibodies. A quantitation of the PDH phosphorylation level on serines 232, 293 and 300 is shown.

\*\* : P < 0.01, \* : P < 0.05, n.s.: not significant.