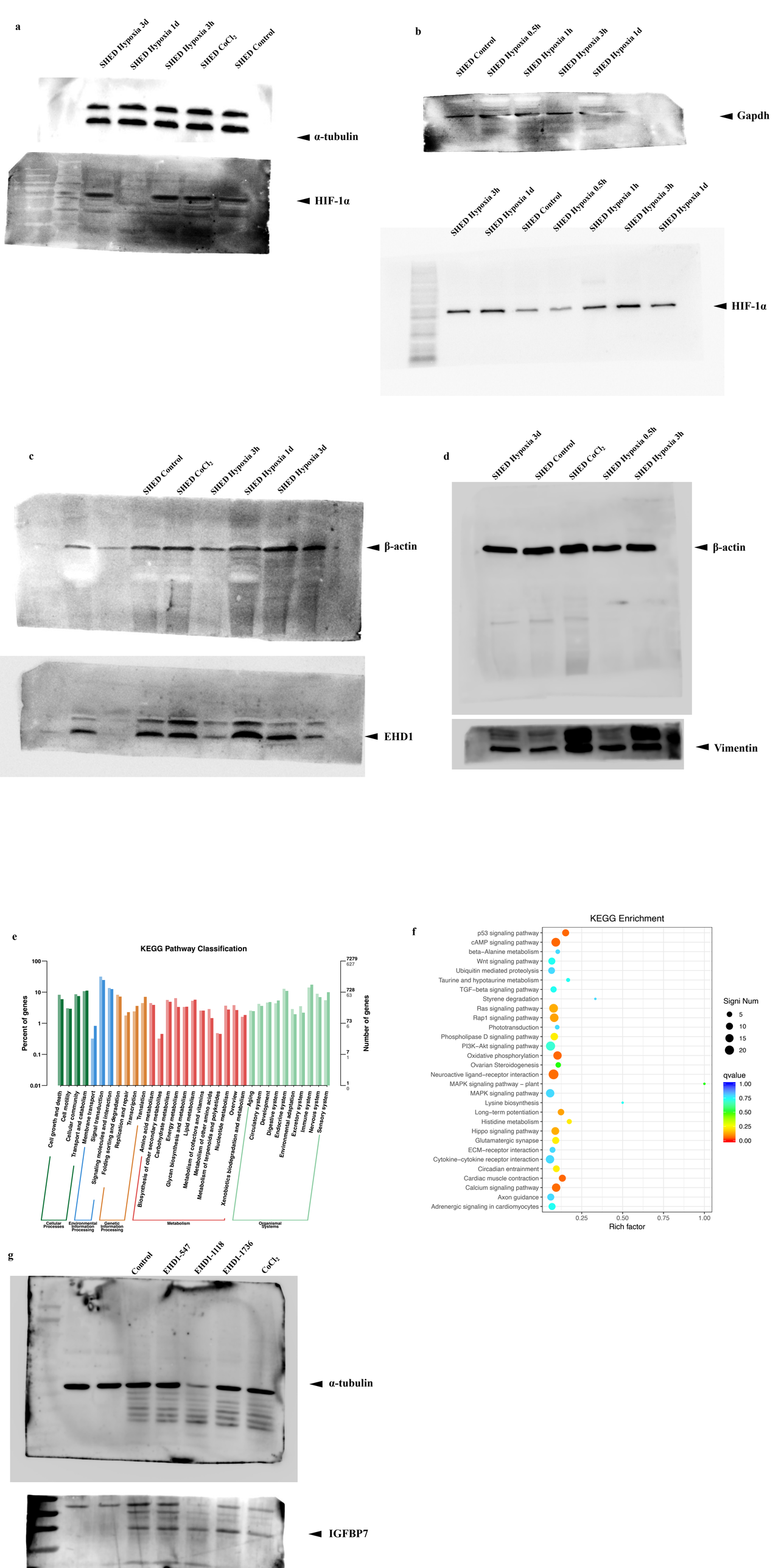
a
SHED Hypoxia 3d
SHED Hypoxia 1d
SHED Hypoxia 3h
SHED CoCl2
SHED Control
α-tubulin
HIF-1α
b
SHED Control
SHED Hypoxia 0.5h
SHED Hypoxia 1h
SHED Hypoxia 3h
SHED Hypoxia 1d
Gapdh
SHED Hypoxia 3h
SHED Hypoxia 1d
SHED Control
SHED Hypoxia 0.5h
SHED Hypoxia 1h
SHED Hypoxia 3h
SHED Hypoxia 1d
HIF-1α
c
SHED Control
SHED CoCl2
SHED Hypoxia 3h
SHED Hypoxia 1d
SHED Hypoxia 3d
β-actin
EHD1
d
SHED Hypoxia 3d
SHED Control
SHED CoCl2
SHED Hypoxia 0.5h
SHED Hypoxia 3h
β-actin
Vimentin
e
KEGG Pathway Classification
Percent of genes
Number of genes
Cellular Processes
Environmental Information Processing
Genetic Information Processing
Metabolism
Organismal Systems
f
KEGG Enrichment
p53 signaling pathway
cAMP signaling pathway
beta−Alanine metabolism
Wnt signaling pathway
Ubiquitin mediated proteolysis
Taurine and hypotaurine metabolism
TGF−beta signaling pathway
Styrene degradation
Ras signaling pathway
Rap1 signaling pathway
Phototransduction
Phospholipase D signaling pathway
PI3K−Akt signaling pathway
Oxidative phosphorylation
Ovarian Steroidogenesis
Neuroactive ligand−receptor interaction
MAPK signaling pathway − plant
MAPK signaling pathway
Lysine biosynthesis
Long−term potentiation
Histidine metabolism
Hippo signaling pathway
Glutamatergic synapse
ECM−receptor interaction
Cytokine−cytokine receptor interaction
Circadian entrainment
Cardiac muscle contraction
Calcium signaling pathway
Axon guidance
Adrenergic signaling in cardiomyocytes
Rich factor
Signi Num
qvalue
g
Control
EHD1-547
EHD1-1118
EHD1-1736
CoCl2
α-tubulin
IGFBP7

Supplemental information. (a, b) Original western blot results of SHED with varying durations of hypoxic preconditioning (using antibodies from different brands). Based on the results, a 3-hour period was selected as the preconditioning time point, along with the appropriate choice of antibody brand. (c) Western blot results with varying durations of hypoxic preconditioning show that, following 3 hours of preconditioning, the expression of EHD1 was upregulated, and after 24 hours of treatment, the expression of EHD1 reached its maximum. An increase in EHD1 expression was also observed after 3 days of preconditioning, which may be attributed to chronic hypoxic stimulation enhancing cellular tolerance, resulting in the upregulation of numerous factors. This finding aligned with the reported dualistic effects of hypoxic stimulation in the literature. The present study concentrates on the short-term stimulation to augment the cells' differentiation capabilities, and thus, the effects of long-term stimulation are not the subject of extensive investigation. (d) Western blot results of vimentin in SHEDs with different durations of hypoxic preconditioning. (e, f) KEGG results map the differentially expressed genes onto their respective pathways, reflecting the impact of gene expression differences on metabolic pathways. (g) In previous studies, IGFBP7 was identified as one of the regulatory factors for SHED differentiation. After EHD1 was knocked down using three types of siRNA, a western blot assay was conducted to examine the expression of IGFBP7, with no significant association found.