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Supplemental Information

The MicroRNA-92a/Sp1/MyoD Axis Regulates Hypoxic Stimulation of Myogenic Lineage Differentiation in Mouse Embryonic Stem Cells

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Supplementary Figure legends

Supplementary Figure S1. Effect of HIF1α or HIF2α overexpression on HIF-responsive genes in C57 ESCs under normoxia. Quantitative real-time PCR for bFGF and VEGF suggests that HIF plasmids were functional (n = 3; **p < 0.01, ***p < 0.001). ESCs were transfected with 1 μg pEGFP-HIF1α or pEGFP-HIF2α for 24 h, allowed to form EBs for 3 days under normoxic conditions, and real-time PCR were performed.

Supplementary Figure S2. Real-time PCR for *Sp1* and *MyoD*. (A) *Sp1* mRNA was significantly decreased by the transient transfection of shSp1 compared to that with the non-target shRNA control (shMock) (n = 4). **(B)** Sp1 knockdown suppressed the increased expression of *MyoD* mRNA even under hypoxia (n = 4). ***p < 0.001 versus Nor-EBs ###p < 0.001 versus Hyp-shMock-EBs. ESCs were transfected with 1 μg shMock or shSp1 for 24 h, formed EBs for 3 days under normoxia, and further incubated for 16 h under normoxic or hypoxic conditions.

Supplementary Figure S3. **Generation of Sp1-knockdown stable cells.** *Sp1* mRNA was remarkably reduced in all four Sp1 knockdown C57 EB clones. C57 ESCs were transfected with 1 μg shMock or shSp1 for 24 h, and further selected by puromycin treatment.

Supplementary Figure S4. Quantification of western blotting for Sp1. C57 ESCs were transfected with miRNA mimic-oligomer for 2 days under normoxic conditions, and western blotting was performed. miR-NC: miRNA-negative control; n=3, **p<0.01, ***p<0.001).

Supplementary Table 1. Primers used for real-time PCR and ChIP assay

Primer		Sequence	Size (bp)
Sp1	Forward	5′-CACCCTAACACCCATTGCCT-3′	187
	Reverse	5′-TCCATGATCACCTGGGGTGT-3′	
MyoD	Forward	5′-TGGAGATCCTGCGCAACGCC-3′	138
	Reverse	5′-TGTAGTGCTCGCTGCCACGG-3′	
Myf5	Forward	5′-CCACCATGCGCGAGCGTAGA-3′	194
	Reverse	5′-GCTCTGTCCCGGCAGGCTGTA-3′	
MyoG	Forward	5′-CTGCGCAGCGCCATCCAGTA-3′	222
	Reverse	5′-GGCGTCTGTAGGGTCAGCCG-3′	
AP2α	Forward	5′-CTCCAGAAGGGGTTGTGCAT-3′	171
	Reverse	5′-CGGGCCTGAAGAGGTTACTC-3′	
HIF1α	Forward	5′-TCCTGGAAACGAGTGAAAGG-3′	176
	Reverse	5′-CTGCCTTGTATGGGAGCATT-3′	
HIF2α	Forward	5′-CTTGGAGGGTTTCATTGCTG-3′	246
	Reverse	5′-ACCGTGCACTTCATCCTCAT-3′	
18s rRNA	Forward	5′-GTAACCCGTTGAACCTT-3′	151
	Reverse	5′-CCATCCAATCGGTAGTAGCG-3′	
OCT4	Forward	5′-GAAGCCCTCCCTACAGCAGA-3′	437
	Reverse	5′-CAGAGCAGTGACGGGAACAG-3′	
VEGF	Forward	5′-CGGATCAAACCTCACCAAAG-3′	131
	Reverse	5′-TTTCTCCGCTCTGAACAAGG-3′	
bFGF	Forward	5′-TCAAGGACCCCAAGCGGCTC-3′	170
	Reverse	5′-GTACCGGTTGGCACACACTC-3′	
ChIP-MyoD	Forward	5′-GTCTCTCTGCCCTCCTTCCTA-3′	185
	Reverse	5′-TATCCAGGGTAGCCTAAAAGCC-3′	
ChIP-NC	Forward	5′-GCCCACCCAACCCCATCTT-3′	226
	Reverse	5′-CCTCTTTCCTGAACTTGCCCT-3′	

Supplementary Table 2. Primers for cloning

pcDNA3.1-Sp1 For: 5′-GGTACCCACCATGAGCGACCAAGATCACTCCAT-3′ Rev: 5′-CTCGAGCCTTCTAATCTTAGAAACCATTGCCAC-3′
pGL4-MyoD promoter region For: 5′- GGTACCTTTTAATGATGATTCCCACTA-3′ Rev: 5′- CTCGAGCGTGAGAGTCGTCTTAAC TTT -3′
pGL4-MyoD promoter region- Δ1mt For: 5′- GGTACCTACACTCCTATTGGC -3′ Rev: 5′- CTCGAG CGTGAGAGTCGTCTTAAC TTT -3′
pGL4-MyoD promoter region- Δ2mt For: 5′- GGTACCTTTTAATGATGATTCCCACTA -3′ Rev: 5′- CTCGAGGCCTCAAGCCAATAG -3′
psiCHECK-2-Sp1-3′UTR-WT-Fragment A For: 5′-GGTACCGATTAGACACCCAGTGCCAGAGACA-3′ Rev: 5′-CTCGAGTGAGCGGCTCACAGACAGGGAG-3′
psiCHECK-2-Sp1-3′UTR-WT-Fragment B For: 5′-GGATCCAGTTACAAGCCGGCTTCGAGATGC-3′ Rev: 5′-AAGCTTACAAAGGAGCTACAGACTACATTG-3′
psiCHECK-2-Sp1-3′UTR-Fragment B – mt1 (3737–3765) For: 5′-GACGCTGCAGATCTTTGTA<u>AATTAACC</u>TA-3′ Rev: 5′-TA<u>GGTTAATT</u>TACAAAGATCTGCAGCGTC-3′ (Underline indicates mutations introduced at the miR-92a seed region of the *Sp1* 3′UTR)

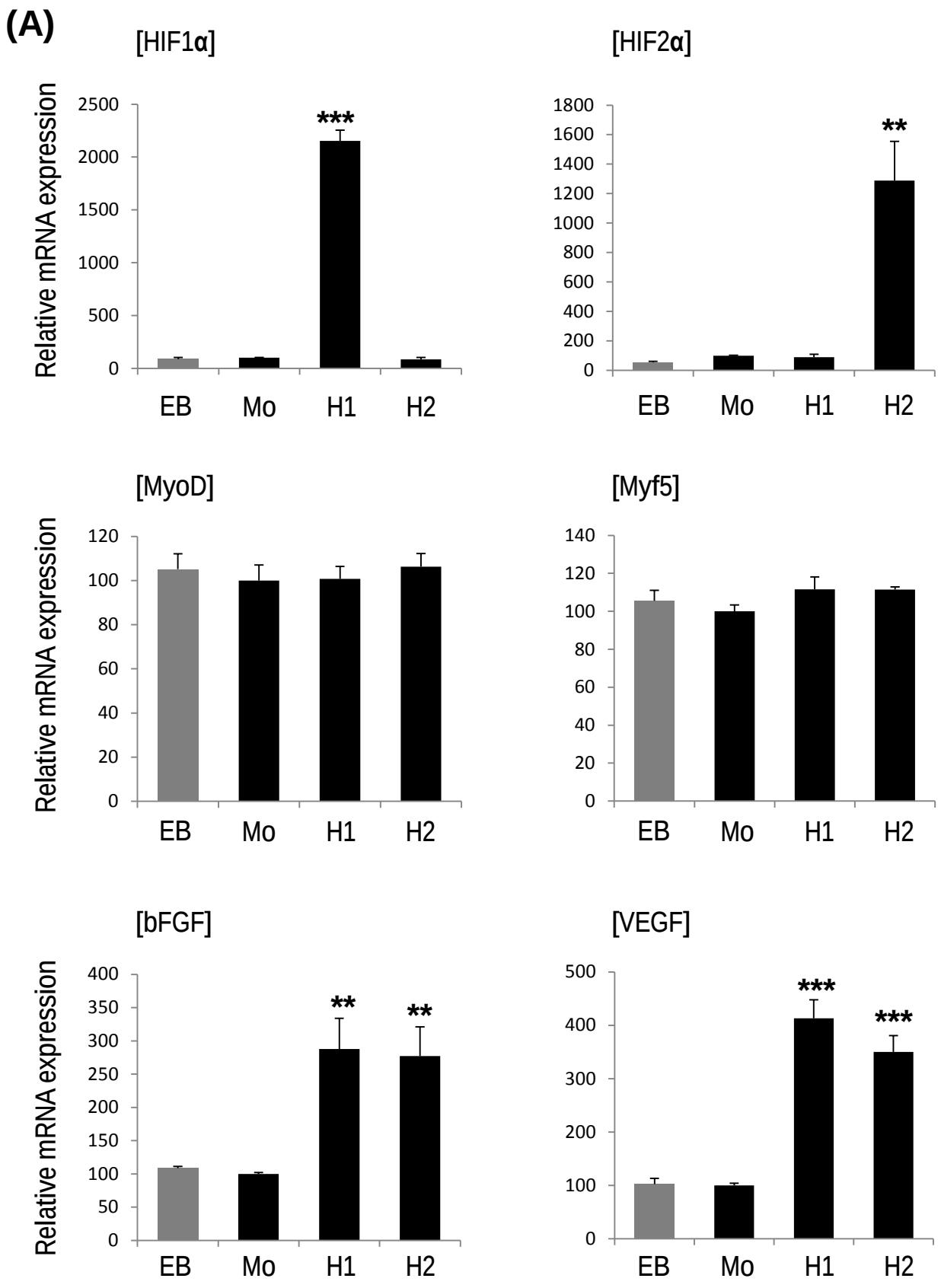
psiCHECK-2-Sp1-3’UTR-Fragment B – mt2 (4983–5011)

For: 5′-CCAGTTTATCTTTCTTAATACTAATCCGGACC-3′

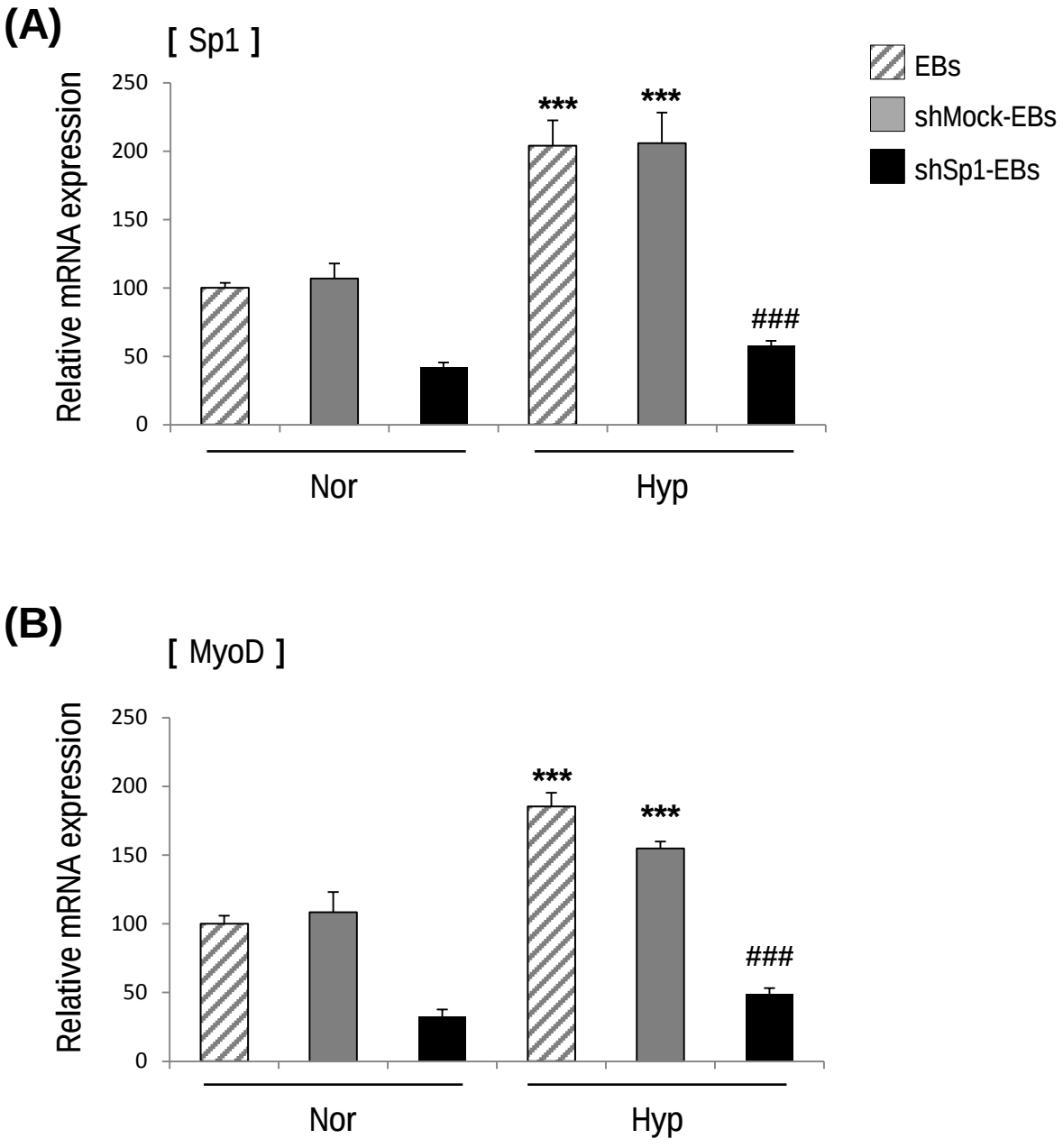
Rev: 5′-GGTCCGGATTAGTATTAAGAAAGATAAACTGG-3′

(Underline indicates mutations introduced at the miR-92a seed region of the *Sp1* 3′UTR)

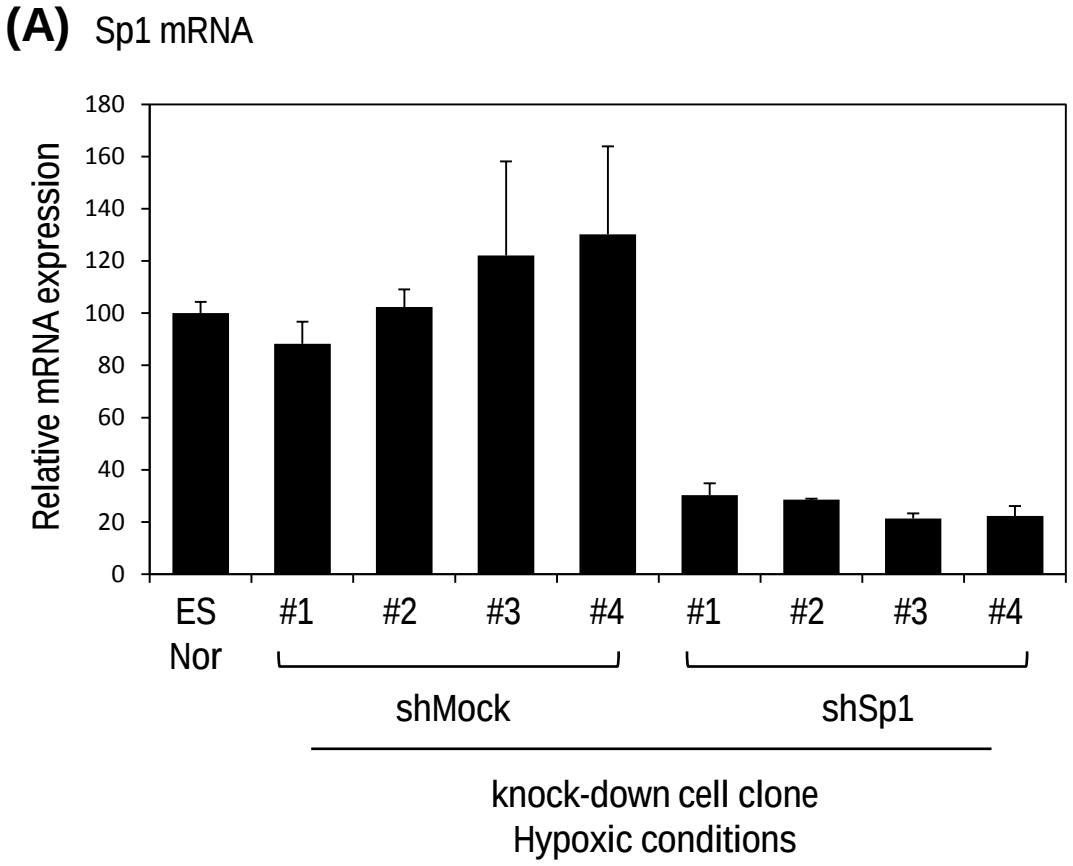
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4

(A) Sp1 protein