Supplementary information

HIF1 driven transcriptional activity regulates steroidogenesis and proliferation of bovine granulosa cells

Authors: Vijay Simha Baddela[1], Arpna Sharma[1], Marten Michaelis[2], Jens Vanselow[1]*

Affiliations: [1] Experimental Reproductive Biology Unit, Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), 18196, Dummerstorf, Germany; [2] Reproductive Biochemistry Unit, Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany

Correspondence: Jens Vanselow, Experimental Reproductive Biology Unit, Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany; Tel: +49 38208 68750; Fax: +49 38208 68752;
e-mail: vanselow@fbn-dummerstorf.de

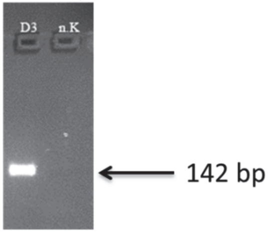
S1: PCR primers:

Gene		Sequence	Accession No.	Product Length
RPLP0	For	TGGTTACCCAACCGTCGCATCTGTA	NM_001012682	142 bp
	Rev	CACAAAGGCAGATGGATCAGCCAAG		
TBP	For	GCCTTGTGCTTACCCACCAACAGTTC	NM_001034494	200 bp
	Rev	TGTCTTCCTGAAACCCTTCAGAATAGGG		
VEGFA	For	AGGAGCCTCCCTCAGGGTTTCGGG	NM_001075742	183 bp
	Rev	CGCTCCGGACCCAAAGTGCTCTGC		
HIF1A	For	GCATCGCGGGCACCGATTCACCAT	NM_174339.3	165 bp
	Rev	TGGGGGAGTGGCAACTGATGAGCA		
STAR	For	TTGTGAGCGTACGCTGTACCAAG	NM_174189.2	237 bp
	Rev	CTGCGAGAGGACCTGGTTGATG		
CYP19A1	For	GCTTTTGGAAGTGCTGAACCCAAGG	NM_174305	172 bp
	Rev	GGGCCCAATTCCCAGAAAGTAGCTG		
CYP19A1	For	CCACCTCTGGAATGAGCTGC	NM_174305	272 bp
(ChIP)	Rev	GTACCGTAGAGATTGGCGCTT		
HSD3B	For	TGTTGGTGGAGGAGAAGGATCTG	NM_174343	208 bp
	Rev	GCATTCCTGACGTCAATGACAGAG		
CCND2	For	CGCAGGGCCGTGCCGGACGCCAAC	NM_001076372	279 bp
	Rev	CACGGCCCCCAGCAGCTGCAGATGG		
PCNA	For	GTGAACCTGCAGAGCATGGACTCGT	NM_001034494	192 bp
	Rev	CGTGTCCGCGTTATCTTCAGCTCTT		
VNN2	For	TCCCACAGCTTGGATGAACGTTTTG	NM_001163920	267 bp
	Rev	TAGGCACTCCAATTCATGGCTGGTG		

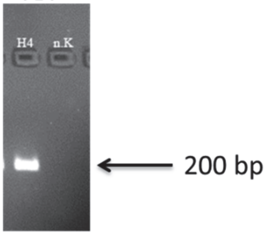
S2: PCR product specificity of different genes under the study:

All the PCR primers used in the study were verified for single product amplification using 3% agarose gel electrophoresis after each run. The gel pictures below indicate a positive control (standard) and negative control (n.k) samples. The negative control well contains nuclease pure water instead of DNA.

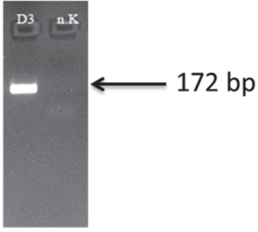
RPLP0



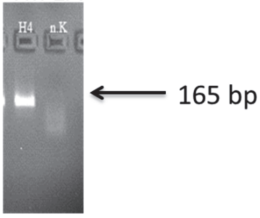
TBP



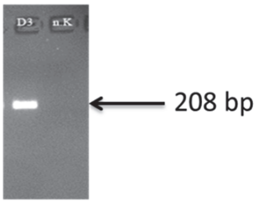
CYP19A1



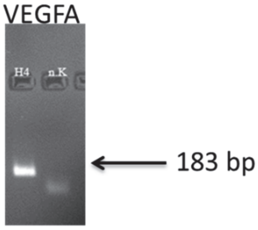
HIF1A



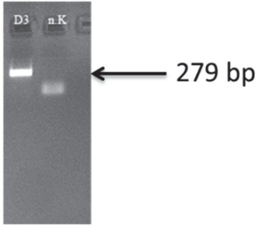
HSD3B



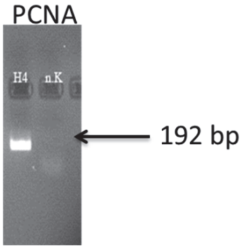
VEGFA



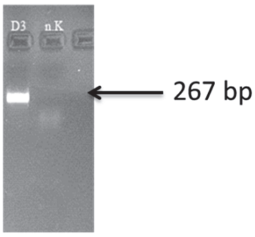
CCND2



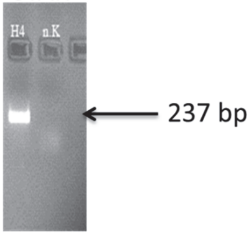
PCNA



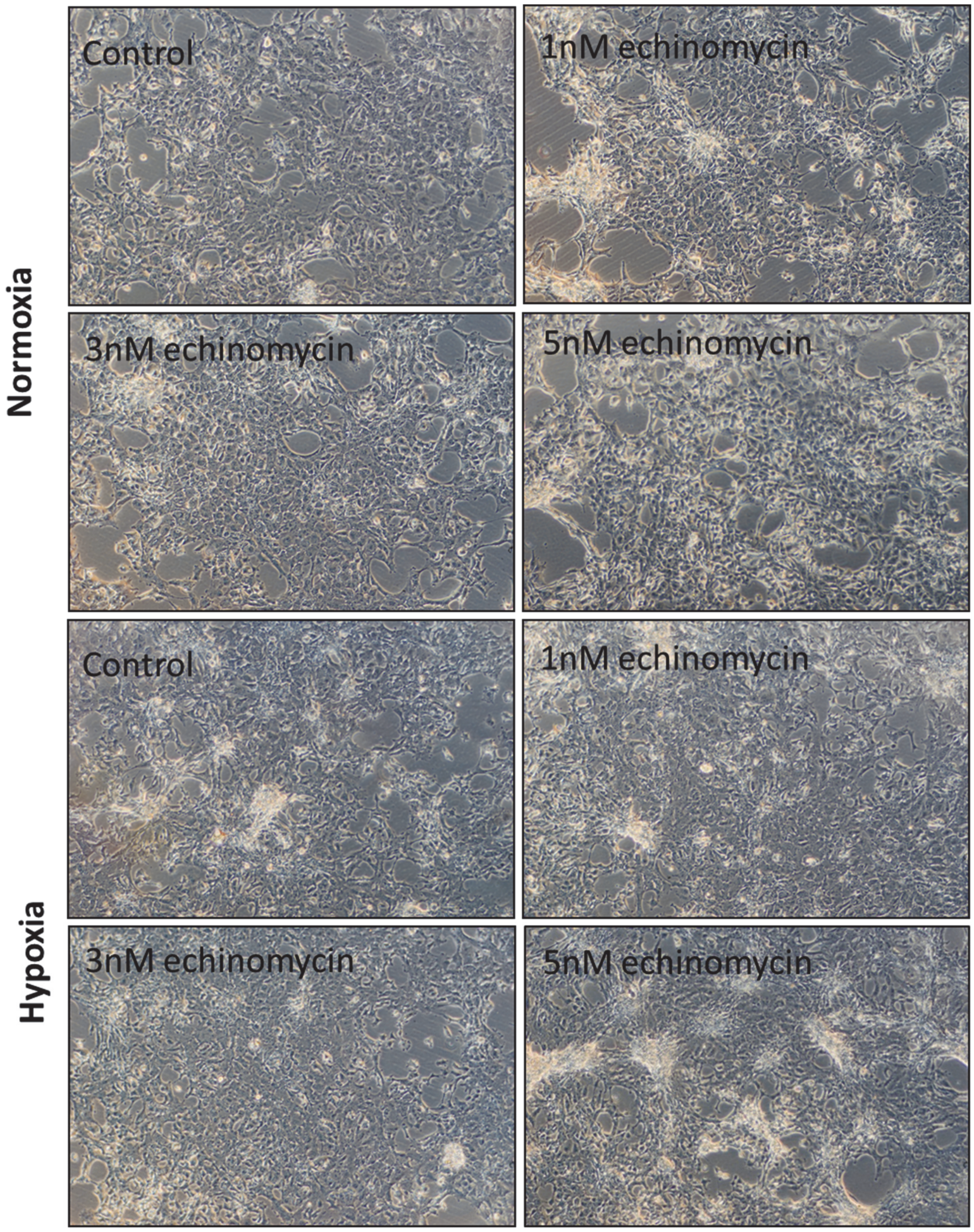
VNN2



STAR



S3: Morphology of Granulosa cells after treating with echinomycin under normoxic and hypoxic conditions:

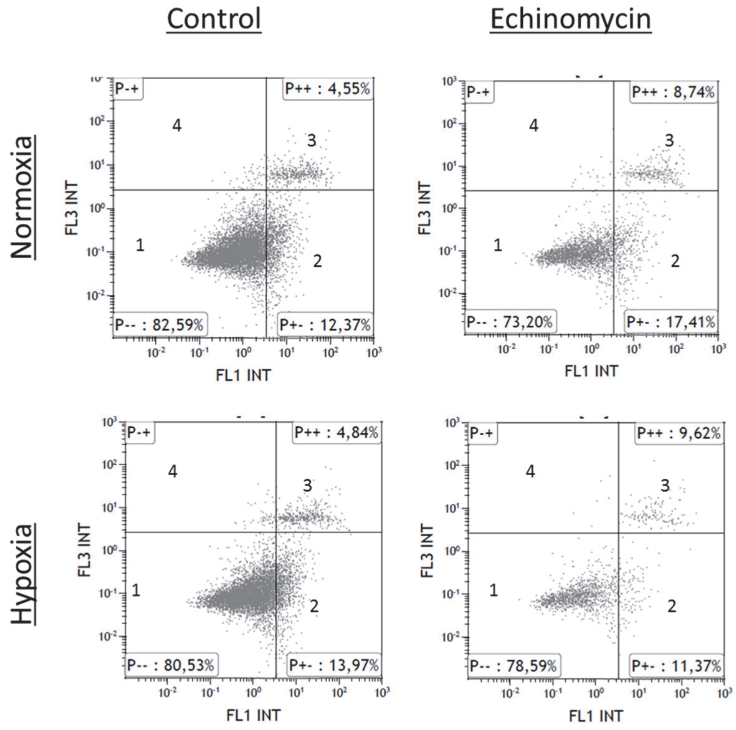


Photomicrographs of GC cultured under serum-free culture system for 8 days. On 6th day GC were treated with different concentrations of echinomycin. Photomicrographs were obtained from Nikon TMS-F inverted microscope with 200X magnification.

S4: Cell viability status in granulosa cells after treatment with echinomycin:

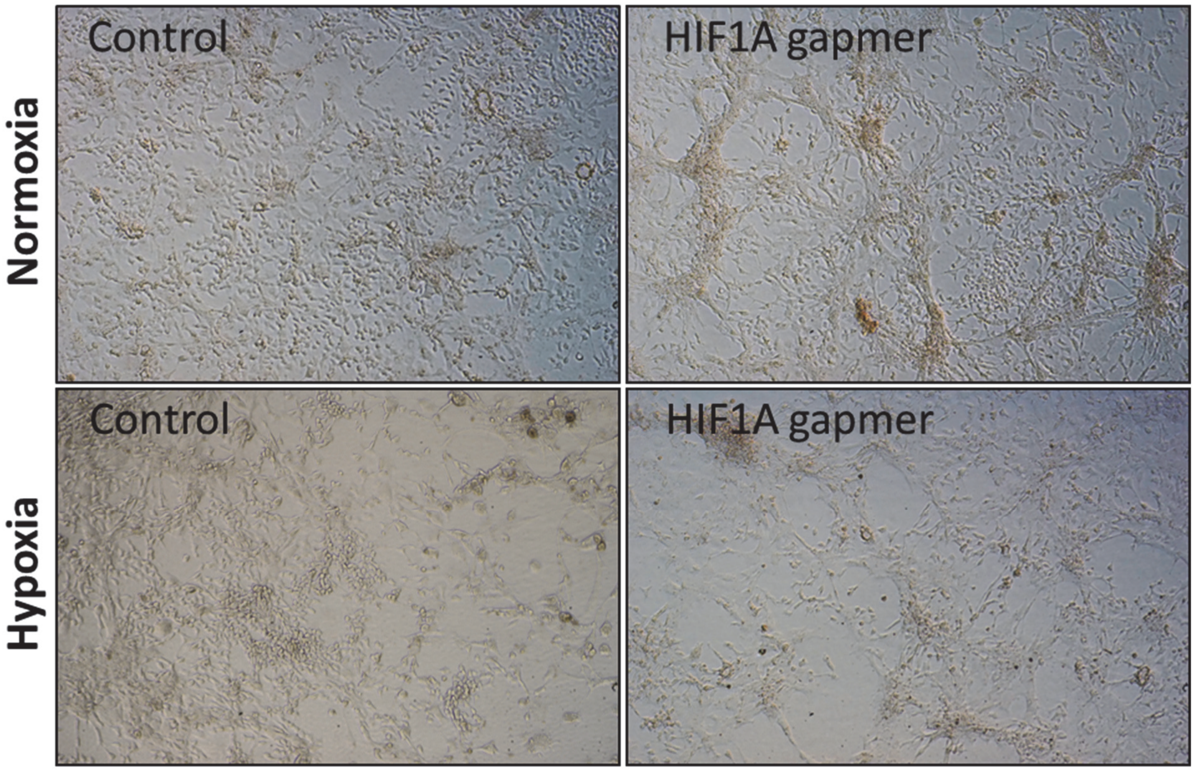
	21% Oxygen			1% Oxygen		
(%)	**Viable cells**	**Apoptotic cells**	**Dead cells**	**Viable cells**	**Apoptotic cells (%)**	**Dead cells (%)**
Control	**78.167±2.64**	**15.37±1.83**	**5.62±0.67**	**78.65±4.67**	**15.76±3.79**	**5.137±0.96**
5nM Echinomycin	**79.160±5.43**	**9.49±1.46**	**10.49±4.27**	**81.70± 4.25**	**11.72±2.84**	**6.013±11.36**

- Representative histograms of FACS analysis used for identifying the cell viability and apoptotic status is below.



1: viable cells, 2: apoptotic, 3: dead, 4: necrotic cell proportions in the FACS analysis.

<u>S5: Morphology of Granulosa cells after treating with control and HIF1A gapmers:</u>

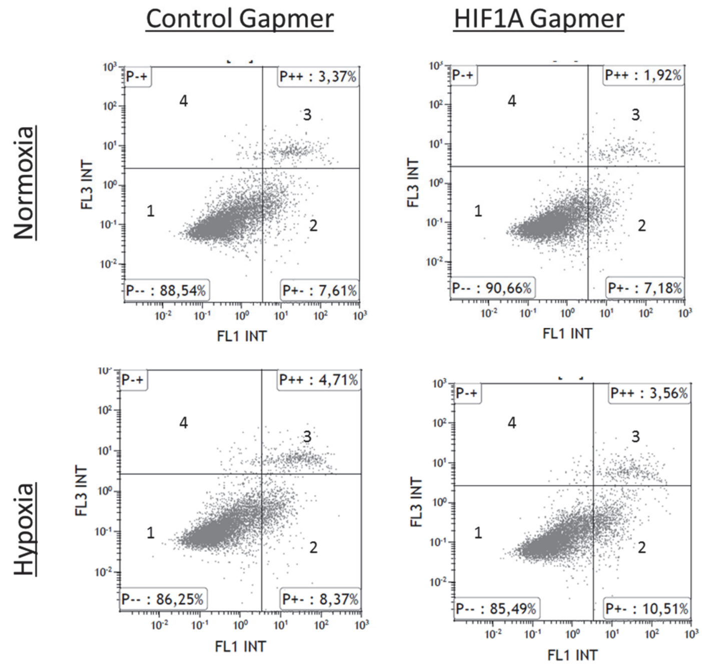


Photomicrographs of GC cultured under serum-free culture system for 8 days. On day 2, GC were incubated with 50nM of HIF1A gapmer for 6 days. Photomicrographs were obtained from Nikon TMS-F inverted microscope with 200X magnification.

S6: Cell viability status in granulosa cells after treating with control and HIF1A gapmers:

	21% Oxygen			1% Oxygen		
(%)	**Viable cells**	**Apoptotic cells**	**Dead cells**	**Viable cells**	**Apoptotic cells (%)**	**Dead cells (%)**
Control Gapmer	**91.5±1.5**	**5.6±1.0**	**2.4±0.4**	**90.1±1.9**	**5.3±1.5**	**3.9±0.3**
HIF1A Gapmer	**92.0±0.8**	**5.8±0.7**	**1.9±0.1**	**89.5±2.0**	**7.1±1.7**	**2.8±0.3**

- Representative histograms of FACS analysis used for identifying the cell viability and apoptotic status is below



1: viable cells, 2: apoptotic, 3: dead, 4: necrotic cell proportions in the FACS analysis.