Identification of DDX6 as a cellular modulator of VEGF expression under hypoxia

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**Supplementary Material**

- Supplementary Table 1: Primers, FISH probes and siRNAs
- Supplementary Materials and Methods
- Supplementary References
- Figure legend for Supplementary Figures S1
- Supplementary Figure S1
### Primers for cloning

<table>
<thead>
<tr>
<th>Description</th>
<th>sense</th>
<th>sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF 5’UTR</td>
<td>forward</td>
<td>CGGGTTACCAGCGCAGAGGCCATTGGGGC</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td>CGGCTCGAGGCTTTCGAGGGCCACCAGGG</td>
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<tr>
<td>VEGF 3’UTR</td>
<td>forward</td>
<td>AAAACTGCCAGCATGGTGAACAGCGAG</td>
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<tr>
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<td>TTTTCTGAGACCAAGAGGGAGGCTTGAGAT</td>
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<tr>
<td>Renilla reniformis luciferase ORF</td>
<td>forward</td>
<td>CGGCTGCAATGCGCTTGAAGTTTATGA</td>
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<tr>
<td></td>
<td>reverse</td>
<td>CGGAAGCTTCATAGATCATCTTGGTCTTTTCATTTT</td>
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</tbody>
</table>

### Primers for (quantitative) RT-PCR

<table>
<thead>
<tr>
<th>Description</th>
<th>sense</th>
<th>sequence</th>
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<tbody>
<tr>
<td>in vitro transcripts</td>
<td>forward</td>
<td>CTTCCGCATAGAATGCCT</td>
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<td>GTTTGTAATTAGCAGATGCAAG</td>
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<td>Firefly luciferase ORF</td>
<td>forward</td>
<td>GGAATTCGATTTGCAATGA</td>
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<td></td>
<td>reverse</td>
<td>GATTTGCATGAGCAGAAAA</td>
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<tr>
<td>CAT</td>
<td>forward</td>
<td>GGTGCTGAGCAGAAAAC</td>
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<td>GGGCAATGGCAATGAATG</td>
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### Endogenous mRNAs

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<tr>
<td>VEGF_{total} (all isoforms)</td>
<td>forward</td>
<td>CGAGGGCTGGAGTGTTG</td>
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<td></td>
<td>reverse</td>
<td>GGCCTTGGTGAAGGGTTTATGC</td>
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<tr>
<td>β-Actin (2)</td>
<td>forward</td>
<td>TCCCTGGAGAAGAGCTACG</td>
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<tr>
<td></td>
<td>reverse</td>
<td>GTAGTTTCGATGGAGCCACA</td>
</tr>
<tr>
<td>rpLP0 (3)</td>
<td>forward</td>
<td>GGGCAGTGGAGTCCAAC</td>
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<td>CATCAGCACCAGGCTTC</td>
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### Probes for FISH:

<table>
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<th>mRNA</th>
<th>sequence</th>
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<tbody>
<tr>
<td>VEGF</td>
<td>TGTAGGAAGCTCATCTCTCTATGTGCTG</td>
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<tr>
<td>β-Actin</td>
<td>TTTCTCCATGTCTCCAGTGGGCTGAG</td>
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### siRNAs:

<table>
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<tr>
<td>DDX6 #1 (4)</td>
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<td>ctrl. (5)</td>
<td>AGGUAGUUAUCGCUUGGdTdT</td>
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</tbody>
</table>
Supplementary Materials and Methods

Plasmid construction

pET16b-hnRNP K (6) and pET16b-DDX6 (4) were described previously. Primers used for cloning are summarized in Supplementary Table 1. VEGF 5’UTR was PCR amplified from HEK 293 cell DNA and cloned KpnI/XhoI in pBSIJKS(+). VEGF 3’UTR was amplified using Image clone BC065522 and inserted into PstI of pBSIJKS(+). To generate monocistronic VEGF 5’UTR-FLuc reporter mRNA (VF) for in vitro translation, the 5’UTR was inserted KpnI/XhoI in pT3-Luc (7). For the reporter mRNA containing the inverted VEGF 5’UTR (ViF) sites were blunt ended before ligation. For bi-cistronic reporter mRNAs the upstream Renilla luciferase ORF was inserted into the newly introduced PstI/HindIII in the KpnI site of VEGF 5’UTR-FLuc (RVF). 5’stem loop-RVF (SL-RVF) was generated by inserting a stem-loop (8) into PstI upstream of the Renilla ORF. For RF and SL-RF the KpnI fragment of RVF or SL-RVF was inserted into pT3-Luc-p(A), respectively. Tob-VEGF 5’UTR-sORF-VEGF 3’UTR (Tob-VEGF) was cloned employing the sORF (4). VEGF 5’UTR was cloned in KpnI/XhoI and VEGF 3’UTR was inserted in PstI. J6f1 aptamer (Tob) (9,10) was inserted in KpnI. For experiments shown in Figure 4A the 5’ and 3’UTR was cloned into pBSIJKS(+) containing the Tob aptamer in the KpnI site (pKS-Tob), VEGF 5’UTR, VEGF 3’UTR or β-globin 5’ leader (11) were inserted into XhoI /SpeI.
Supplementary References


Supplementary Figure legends

Supplementary Figure S1

A) Enlargement of the bottom panel in Figure 1D: Cy3 and FITC fluorescence monitored along the dashed lines is shown as relative signal intensity.

B) Enlargement of the bottom panels in Figure 3C to E: Cy3 and FITC fluorescence monitored along the dashed lines is shown as relative signal intensity.

C) Enlargement of the bottom panel in Figure 5C: Cy3 and FITC fluorescence monitored along the dashed lines is shown as relative signal intensity.
A) intensity profiles Figure 1D

![Intensity profiles for VEGF and β-Actin under normoxia and hypoxia conditions. The graphs show relative intensity versus distance in pixels (pxl).]
Supplementary Figure S1

B) intensity profiles Figure 3C

![Intensity profiles for VEGF and \(\beta\)-Actin under Normoxia and Hypoxia conditions](image1)

- **VEGF**
  - FISH-FITC
  - DDX6-Cy3

![Intensity profiles for VEGF and \(\beta\)-Actin under Normoxia and Hypoxia conditions](image2)

- **\(\beta\)-Actin**
  - FISH-FITC
  - G3BP1-Cy3

intensity profiles Figure 3D

![Intensity profiles for VEGF and Dcp1A under Normoxia and Hypoxia conditions](image3)

- **Dcp1A**
  - Dcp1A-FITC
  - DDX6-Cy3

![Intensity profiles for VEGF and Dcp1A under Normoxia and Hypoxia conditions](image4)

- **G3BP1**
  - G3BP1-FITC
  - DDX6-Cy3
C) intensity profiles Figure 5C

<table>
<thead>
<tr>
<th>siRNA</th>
<th>ctrl. DDX6 (#2)</th>
<th>ctrl. DDX6 (#2)</th>
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<tr>
<td>Normoxia</td>
<td>rel. intensity</td>
<td>rel. intensity</td>
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<tr>
<td>Hypoxia</td>
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- VEGF
- β-Actin

Legend:
- FISH-FITC
- rpL19-Cy3