Vasohibin1, a new IRES trans-acting factor for induction of (lymph)angiogenic factors in early hypoxia

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Running title: IRES regulation during hypoxia

ABSTRACT

Hypoxia, a major inducer of angiogenesis, is known to trigger major changes of gene expression at the transcriptional level. Furthermore, global protein synthesis is blocked while internal ribosome entry sites (IRES) allow specific mRNAs to be translated. Here we report the transcriptome and translatome signatures of (lymph)angiogenic genes in hypoxic HL-1 cardiomyocytes: most genes are not induced at the transcriptome-, but at the translatome level, including all IRES-containing mRNAs. Our data reveal activation of (lymph)angiogenic mRNA IRESs in early hypoxia. We identify vasohibin1 (VASH1) as an IRES trans-acting factor (ITAF) able to activate FGF1 and VEGFD IRESs in hypoxia while it inhibits several IRESs in normoxia. Thus this new ITAF may have opposite effects on IRES activities. These data suggest a generalized process of IRES-dependent translational induction of (lymph)angiogenic growth factors expression in early hypoxia, whose pathophysiological relevance is to trigger formation of new functional vessels in ischemic heart. VASH1 is not always required, indicating that the IRESome composition is variable, thus allowing subgroups of IRESs to be activated under the control of different ITAFs.

Key words: cardiomyocyte/ hypoxia / IRES/ translational control/ vasohibin

INTRODUCTION

Hypoxia constitutes a major stress in different pathologies including cancer, as well as ischemic pathologies where artery occlusion leads to hypoxic conditions. In all these pathologies, hypoxia induces a cell response that stimulates angiogenesis to re-feed starved cells with oxygen and nutrients (1). Recently it has been shown that lymphangiogenesis is also induced by hypoxia (2). Hypoxia-induced (lymph)angiogenesis is mediated by strong modification of gene expression at both transcriptional and post-transcriptional levels (1, 3). A major way of gene expression regulation is mediated at the transcriptional level by the hypoxia inducible factor 1 (HIF1), a transcription factor stabilized by oxygen deprivation, that activates transcription from promoters containing hypoxia responsive elements (HRE). One of the well-described HIF1 targets is vascular endothelial growth factor A (VEGFA), a major angiogenic factor (4, 5). However, two other major angiogenic or lymphangiogenic growth factors, fibroblast growth factor 2 (FGF2) and VEGFC, respectively, are induced by hypoxia in a HIF-independent manner by a translational mechanism, indicating the importance of the post-transcriptional regulation of gene expression in this process (2, 6).

Translational control of gene expression plays a crucial role in the stress response. In particular, translation of most mRNAs, occurring by the classical cap-dependent mechanism, is silenced whereas alternative translation mechanisms allow enhanced expression of a small group of messengers involved in the control of cell survival (3, 7, 8). One of the major alternative mechanisms able to overcome this global inhibition of translation by stress depends on internal ribosome entry sites (IRESs) that correspond to RNA structural elements allowing the direct recruitment of the ribosome on mRNA. As regards the molecular mechanisms of IRES activation by stress, several studies have reported the involvement of RNA binding proteins, called IRES trans-acting factors (ITAFs), able to stabilize the adequate RNA conformation allowing ribosome recruitment (9-13). Interestingly, subcellular relocalization of ITAFs plays a critical role in IRES-dependent translation (14). Indeed, many RNA-binding proteins are known to shuttle between nucleus and cytoplasm, and it has been reported that cytoplasmic relocalization of ITAFs such as PTB, PCBP1, RBM4 or nucleolin is critical to activate IRES-dependent translation (10, 13, 14). In contrast, other ITAFs such as hnRNPA1, may have a

negative impact on IRES activity when accumulating in the cytoplasm (15). However, how ITAFs participate in the regulation of the hypoxic response remains a challenging question to address.

IRESs are present in the mRNAs of several (lymph)angiogenic growth factors in the FGF and VEGF families, suggesting that the IRES-dependent mechanism might be a major way to activate angiogenesis and lymphangiogenesis during stress (2, 10, 13, 16-19). However, most studies of the role of hypoxia in gene expression regulation have been performed in tumoral hypoxia, while it has been reported that tumoral angiogenesis leads to formation of abnormal vessels that are non functional, which strongly differs from non tumoral angiogenesis that induces formation of functional vessels (20). This suggests that gene expression regulation in response to hypoxia may be different in cancer versus ischemic pathologies. In particular, the role of IRESs in the control of gene expression in ischemic heart, the most frequent ischemic pathology, remains to be elucidated.

Here we analyzed the transcriptome and the translatome of (lymph)angiogenic growth factors in hypoxic cardiomyocytes, and studied regulation of IRES activities in early and late hypoxia. Data show that in cardiomyocyte, (lymph)angiogenic growth factors are mostly regulated at the translational level. Interestingly, FGF and VEGF mRNA IRESs are sequentially activated at different times of early hypoxia in contrast to IRESs of non angiogenic messengers. We also looked for ITAFs governing IRES activation in hypoxia and identified vasohibin1 (VASH1) as a new ITAF specific of the earliest activated FGF1 IRES in cardiomyocytes. VASH1 knock-down strongly down-regulates the earliest-induced FGF1 IRES but not the other IRESs, revealing that this protein is a new IRES trans-acting factor (ITAF) in cardiomyocytes, specific of early hypoxia.

RESULTS

Most (lymph)angiogenic genes are not induced at the transcriptome level of hypoxic cardiomyocytes.

In order to analyze expression of angiogenic and lymphangiogenic growth factors in hypoxic cardiomyocytes, the HL-1 cell line was chosen: although immortalized, it keeps the beating phenotype specific to cardiomyocyte (21). HL-1 cells were submitted to increasing times of hypoxia, from 5 minutes to 24 hours and their trancriptome was analyzed on a Fluidigm Deltagene PCR array targeting 96 genes of angiogenesis, lymphangiogenesis and/or stress (Fig. 1, EV Fig. 1, EV Table 1). Data showed a significant increase of *Vegfa*, PAI-1 and apelin (*Apln*) mRNA levels, with a peak at 8h of hypoxia for *Vegfa* and PAI1 and 24h for *Apln*. These three genes are well described HIF1 targets (4, 22, 23). However, only 5-8% of the genes were induced, while the mRNA levels of several major angio- or lymphangiogenic factors, such as FGF2 and VEGFC, were strongly decreased after 4 h or 8 h of hypoxia. These data indicate that the transcriptional response to hypoxia in cardiomyocytes is not the major mechanism controlling expression of (lymph)angiogenic factors, suggesting that post-transcriptional mechanisms are involved.

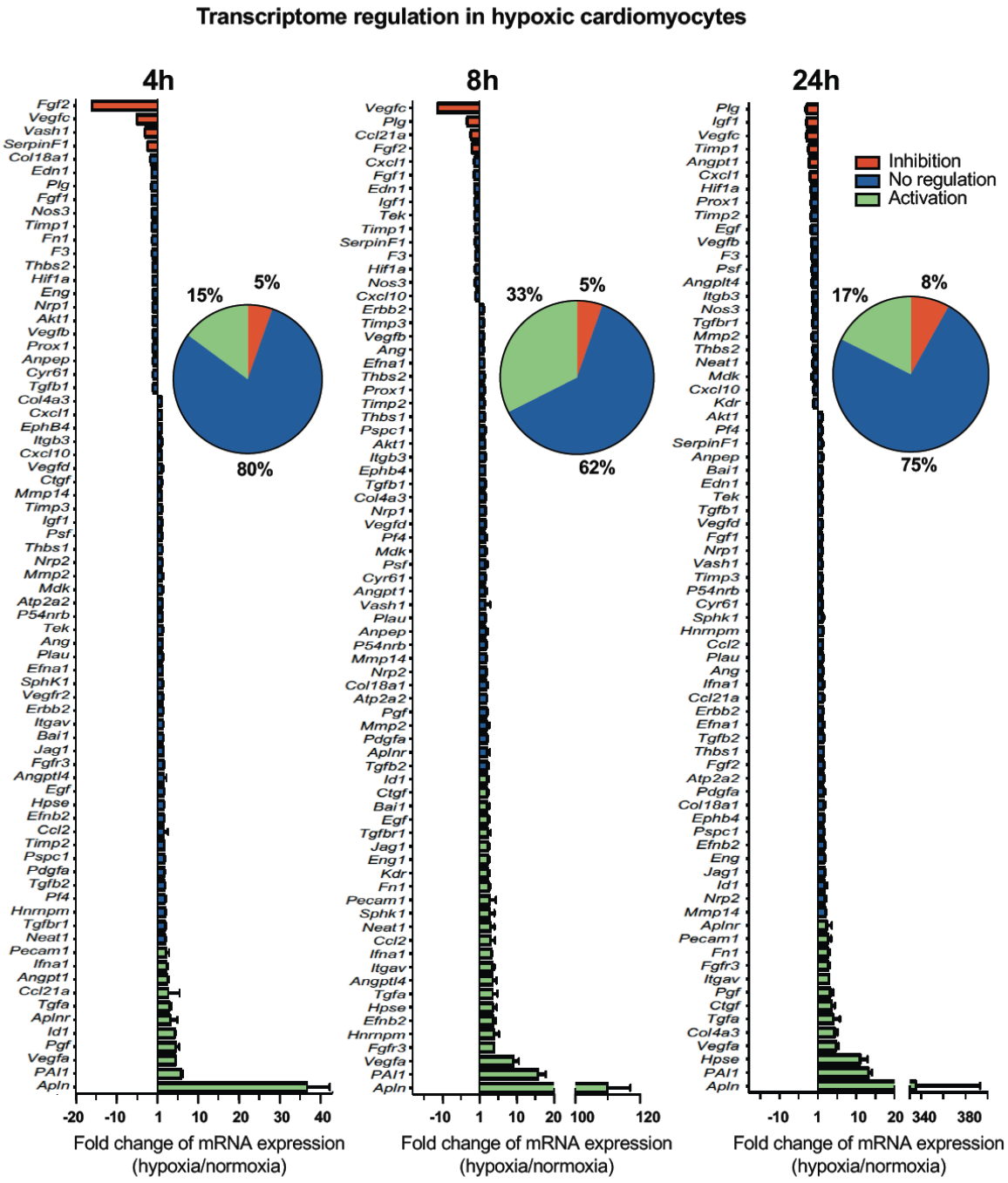
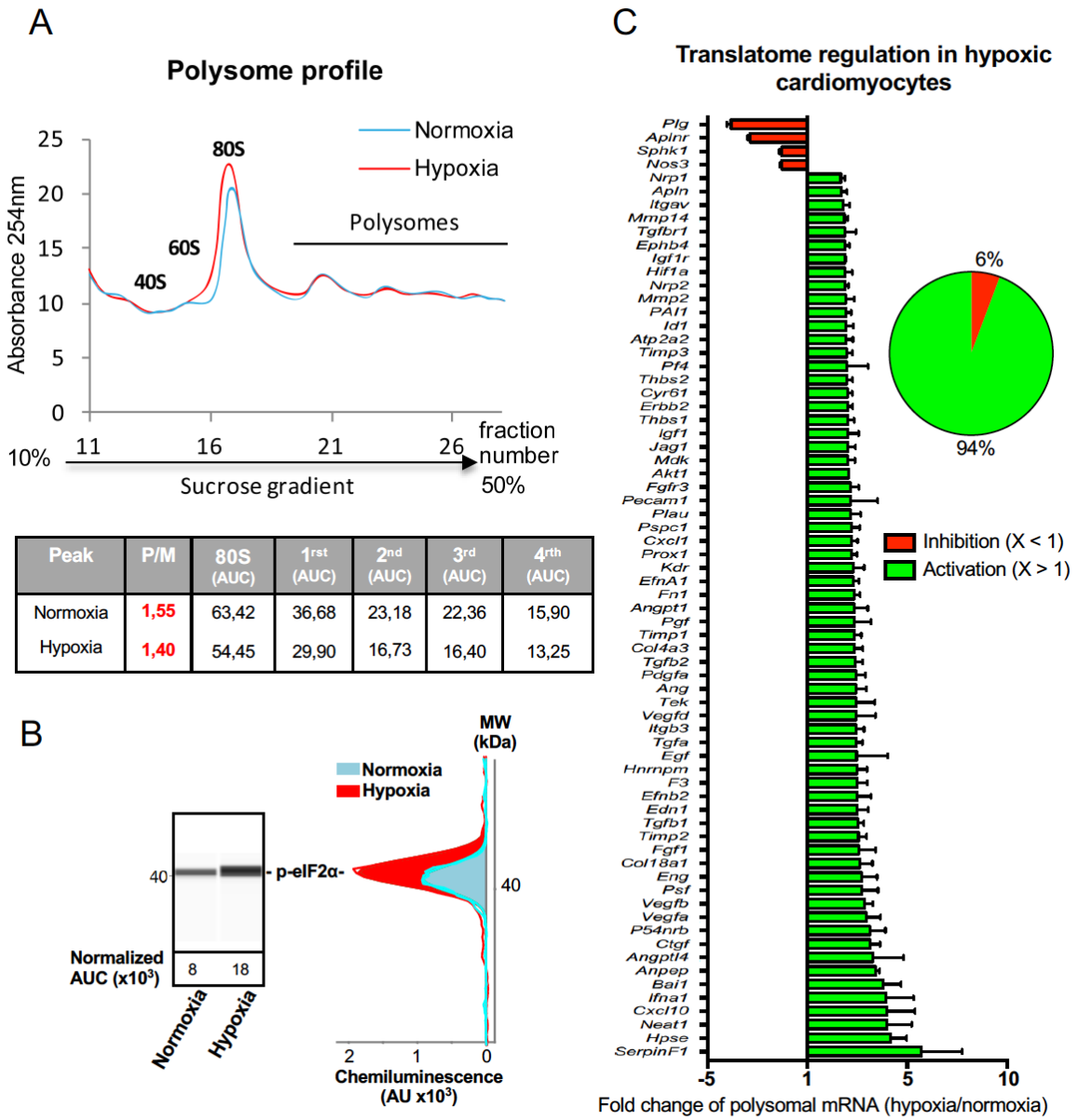


Figure 1. Most (lymph)angiogenic genes are not induced at the transcriptome level in hypoxic cardiomyocytes.

Total RNA was purified from HL-1 cardiomyocytes submitted to increasing times from 5 min to 24 h of hypoxia at 1% O_2, as well as from normoxic cardiomyocytes as a control. cDNAs were synthesized and used for a Fluidigm deltagene PCR array dedicated to genes related to (lymph)angiogenesis or stress (EV Table 6). Relative quantification (RQ) of gene expression during hypoxia was calculated using the $2^{-\Delta\Delta CT}$ method with normalization to 18S and to normoxia. mRNA levels are presented by histograms for the times of 4 h, 8 h and 24 h, as the fold change of repression (red) or induction (green) normalized to normoxia. Non-regulated mRNAs are represented in blue. When the RQ value is inferior to 1, the fold change is expressed as -1/RQ. The percentage of repressed, induced, and non-regulated mRNAs is indicated for each time. For earlier times of 5 min to 2 hr, the percentages are shown in EV Fig. 1. The detailed values for all the times of the kinetics are presented in EV Table 1.

mRNAs of most (lymph)angiogenic genes are recruited into polysomes in hypoxic cardiomyocytes.

Based on the fact that mRNA present in polysomes are actively translated, we tested the hypothesis of translational induction by analysing the recruitment of mRNAs into polysomes. This experiment was performed in early and late hypoxia. The polysome profile showed that translational activity in normoxic HL-1 cells was low but decreased after 4 h of hypoxia, with a shift of the polysome to monosome ratio from 1,55 to 1,40 (Fig. 2A). 4E-BP1 appeared as a single band and its phosphorylation profile did not change upon hypoxia, suggesting that it is already hypophosphorylated in normoxia in these cells (EV Fig. 2A and 2B). In contrast, translation blockade was confirmed by the strong phosphorylation of eIF2α (Fig. 2B, EV Fig.2C). 94% of the genes of the (lymph)angiogenic array showed a more sustained recruitment into polysomes under hypoxic conditions (Fig. 2C, EV Table 2). This translational induction not only targets major angiogenic factors and their receptors (*Vegfa, Fgf1, Pdgfa, Fgfr3, Vegfr2*...), but also genes involved in cardiomyocyte survival in ischemic heart (*Igf1, Igf1R*) or in inflammation (BAI1, *Tgfb*). These data suggest that in cardiomyocytes, the main response to early hypoxia of (lymph)angiogenic genes is not transcriptional, but translational.



Peak	P/M	80S (AUC)	1rst (AUC)	2nd (AUC)	3rd (AUC)	4rth (AUC)
Normoxia	1,55	63,42	36,68	23,18	22,36	15,90
Hypoxia	1,40	54,45	29,90	16,73	16,40	13,25

Figure 2. ***mRNAs of most (lymph)angiogenic genes are mobilized into polysomes in hypoxic cardiomyocytes.***
A-C In order to isolate translated mRNAs, polysomes were purified on sucrose gradient from HL-1 cardiomyocytes in normoxia or after 4 hr of hypoxia at 1% O_2, as described in Materials and Methods. P/M ratio (polysome/monosome) was determined by delimiting the 80s and polysome peaks by taking the lowest plateau values between each peak and by calculating the area under the curve (AUC). Then the sum of area values of the four polysome peaks was divided by the area of the 80s peak (A). Translation blockade was measured by eIF2α phosphorylation quantification by capillary Simple Western using normalization against total protein as described in Mat. & Meth. (B).
RNA was purified from polysome fractions and from cell lysate before loading. cDNA and PCR array were performed as in Figure 1. Polysomes profiles are presented for normoxic and hypoxic cardiomyocytes. Relative quantification (RQ) of gene expression during hypoxia was calculated using the $2^{-\Delta\Delta CT}$ method with normalization to 18S and to normoxia. mRNA levels (polysomal RNA/total RNA) are shown as fold change of repression (red) or induction (green) in hypoxia normalized to normoxia as in Figure 1 (C). When the RQ value is inferior to 1, the fold change is expressed as -1/RQ**.** The detailed values are available in EV Table 2.

IRES-containing mRNAs are more efficiently mobilized into polysomes under hypoxic conditions.

IRES-dependent translation has been reported to drive translation of several mRNAs in stress conditions (2, 3, 6, 24). Thus we focused onto the regulation of the different IRES-containing mRNAs present in the Fluidigm array (Fig. 3). Interestingly, the only IRES-containing mRNA to be significantly induced at the transcriptome level by hypoxia was *Vegfa* (Fig. 3A and EV Fig. 3). Expression of the apelin receptor (*Aplnr*), presumably devoid of IRES but transcriptionally induced during hypoxia, is also shown for comparison.
Polysome recruitment of these IRES-containing mRNAs is shown in figure 3B. Clearly, *Fgf1, Vegfa, Vegfd, Cyr61*, *Hif1a* and *Igf1r* mRNAs were recruited into polysomes under hypoxia 2 to 3 times more than in normoxia, suggesting an important induction in terms of translation. In contrast, *Aplnr* mRNA recruited into polysomes decreased about three times. The data are not available for *Fgf2* and *Vegfc* mRNAs, which were not detectable. These results indicate that hypoxia in cardiomyocytes, although blocking global cap-dependent translation, induces translation of all detectable IRES-containing angiogenic factor mRNAs. This mechanism occurs as soon as 4 hours after oxygen deprivation, thus corresponding to an early event in the hypoxic response.

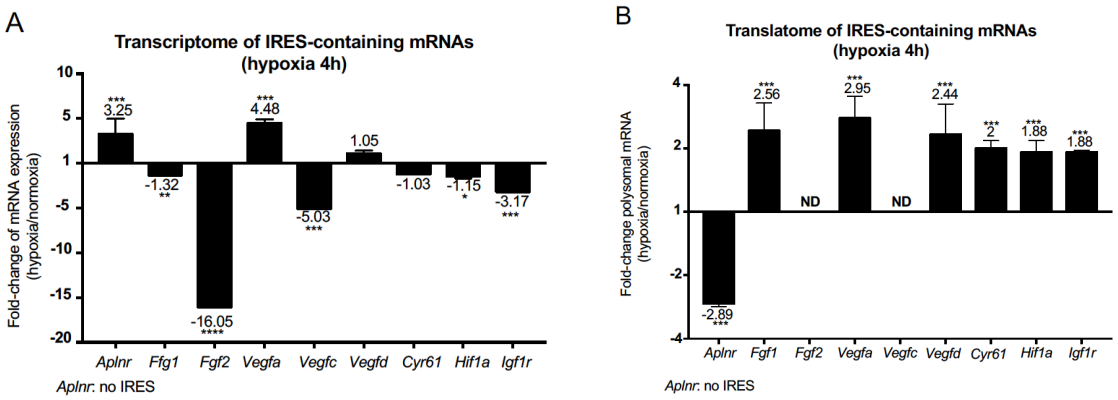
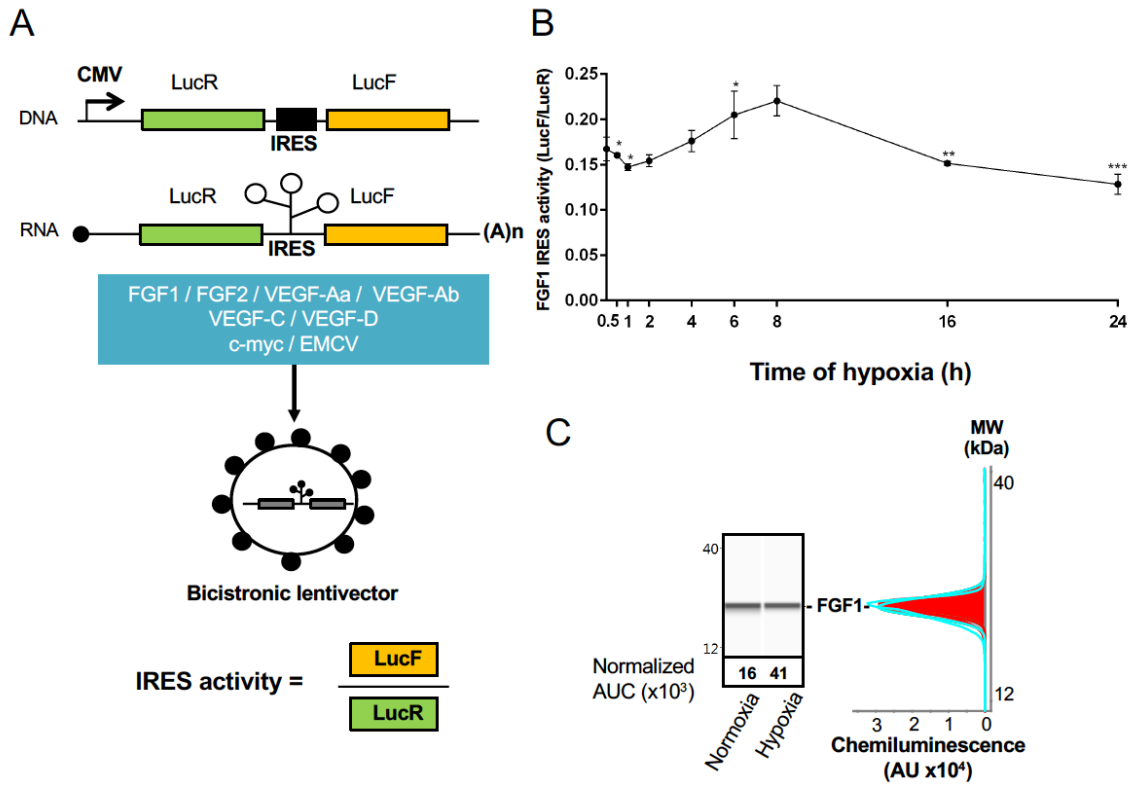


Figure 3. IRES-containing mRNAs are more efficiently associated to polysomes in hypoxic conditions.
A-B RQ values for IRES-containing mRNA transcriptome (A) and translatome (B) extracted from the PCR arrays shown in Figures 1 and 2. The gene *Aplnr* (apelin receptor) was chosen as a control without an IRES. *Vegfc* and *Fgf2* mRNAs, repressed in the transcriptome, were below the detection threshold in polysomes (ND).
Histograms correspond to means ± standard deviation, *p<0.05, **p<0.01, ***<0.001, ****p<0.0001 compared to normoxia.

IRESs of (lymph)angiogenic factor mRNAs are activated during early hypoxia.

To confirm that the polysome recruitment of IRES-containing mRNAs actually corresponds to a stimulation of IRES-dependent translation, IRESs from FGF and VEGF mRNAs were introduced into a bicistronic dual luciferase gene expression cassette (Fig. 4A). As controls, two IRESs from non angiogenic mRNAs, c-myc and EMCV IRESs, were used. A negative control without IRES was provided by an hairpin inserted between the two cistrons (25). The well established bicistronic vector strategy, previously validated by us and others, allows to measure IRES activity revealed by expression of the second cistron, LucF (2, 25). The bicistronic cassettes were subcloned into lentivectors, as HL-1 cells are not efficiently transfected by plasmids but can be easily transduced by lentivectors with an efficiency of more than 80% (not shown). HL-1 cardiomyocytes were first transduced with the lentivector containing the FGF1 IRES and a kinetics was performed from 1 to 24 hours of hypoxia. Luciferase activities were measured from cell extracts and IRES activities reported as the LucF/LucR luminescence ratio. Data showed an increase of IRES activity between 4 to 8 hours whereas it decreased from 16 h to 24 h (Fig. 4B). Expression of endogenous FGF1 was analyzed after 8 hours of hypoxia. FGF1 protein quantification normalized to total proteins showed that IRES induction correlates with an increased expression of FGF1 protein (Fig. 4C). This is also consistent with the increase of FGF1 mRNA recruitment into polysomes observed above (Fig. 3C, EV Table 2). To determine whether this transient induction could affect other IRESs, HL-1 cells were then transduced by the complete series of lentivectors described above (Fig. 4A) and submitted to 4, 8 or 24 hours of hypoxia. Results showed an increase of all FGF and VEGF IRES activities in early hypoxia (4 hours and/or 8 hours), while the c-myc IRES was activated only in late hypoxia after 24 hours (Fig. 4D). The viral EMCV IRES was activated in both early (4 hours) and late (24 hours) hypoxia. The hairpin control was not induced (EV Table 3J). These data revealed two waves of IRES activation in response to hypoxia: a first wave concerns IRESs from (lymph)angiogenic growth factor mRNAs that are activated during early hypoxia, while a second wave concerns “non-angiogenic” c-myc and EMCV IRESs, that are activated in late hypoxia.



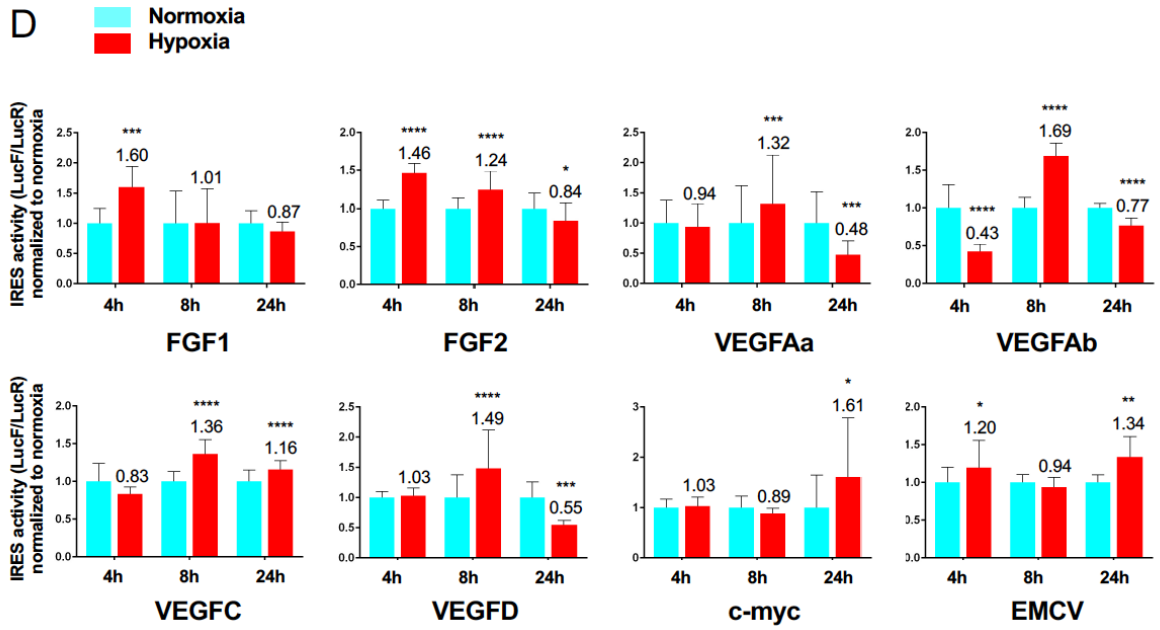


Figure 4. IRESs from (lymph)angiogenic factor mRNAs are activated in early hypoxia.
A-D To measure IRES-dependent translation during hypoxia, HL-1 cardiomyocytes were transduced with bicistronic dual luciferase lentivectors (termed "Lucky-Luke") containing different IRESs cloned between the genes of renilla (LucR) and firefly (LucF) luciferase (A). In bicistronic vectors, translation of the first cistron LucR is cap-dependent whereas translation of the second cistron LucF is IRES-dependent (25). Cardiomyocytes transduced by the CRF1AL+ lentivector Lucky-Luke reporter containing FGF1 IRES were submitted to an hypoxia time-course (0h, 1h, 2h, 4h, 6h, 8h, 16h and 24h) and each time point compared to 0h point with one-tailed t-test (B). Endogenous FGF1 protein expression was measured by capillary Simple Western, from extracts of cardiomyocytes in normoxia or submitted to 8h of hypoxia (C). HL-1 cardiomyocytes transduced by different Lucky-Luke constructs were submitted to 4h, 8h or 24h of hypoxia and luciferase activities measured. IRES activities during hypoxia, expressed as LucF/LucR ratio, are normalized to normoxia. Three groups of activation were identified: FGF IRESs, VEGF IRESs, non-angiogenic IRESs at 4h, 8h or 24h of hypoxia, respectively.
Histograms correspond to means ± standard deviation of the mean, with a one-tailed t-test *p<0.05, **p<0.01, ***<0.001, ****p<0.0001, compared to normoxia. For each IRES the mean has been calculated from three independent experiments with three biological replicates (n=9). All detailed values are presented in EV Table 3. A no-IRES control was also performed and values are presented in EV Table 3 J.

Identification of IRES-bound proteins in hypoxic cardiomyocytes reveals vasohibin1 as a new RNA-binding protein.

Early activation of angiogenic factor IRESs during hypoxia suggested that specific ITAFs may be involved between 4 and 8 hours. In an attempt to identify such ITAFs, we used the technology of biomolecular analysis coupled to mass spectrometry (BIA-MS), validated for ITAF identification in two previous studies (13, 26). Biotinylated RNAs corresponding to FGF1 (4 hours activation), VEGFAa (8 hours activation) and EMCV IRESs (24 hours activation) were used as probes for BIA-MS. Hooked proteins from normoxic and hypoxic HL-1 cells were then recovered and identified (Fig. 5A-B, EV Table 4). Surprisingly, except for nucleolin bound to VEGFAa and EMCV IRES in normoxia, no known ITAF was identified as bound to these IRESs in normoxia or in hypoxia. Interestingly, besides several proteins unrelated to (lymph)angiogenesis, we detected the presence of vasohibin1 (VASH1), a protein described as an endothelial cell-produced angiogenesis inhibitor, but also for its role in stress tolerance and cell survival (Fig. 5C) (27, 28). However, this secreted protein has never been reported for any RNA-binding activity. VASH1 bound to the FGF1 IRES under 4 hours or 8 hours of hypoxia , but not under normoxia (EV Table 4). This protein also bound to the EMCV IRES both in normoxia and hypoxia but not to the VEGFA IRES. In order to address the RNA binding

potential of VASH1, we performed an *in silico* analysis of VASH1 protein sequence that predicted two conserved RNA-binding domains (RBD) in the N- and C-terminal parts of the full length protein, respectively (Fig. 5C, EV Fig. 4A and 4B). The direct interaction of VASH1 with FGF1, VEGFAa and EMCV IRESs was assessed by surface plasmon resonance using the full length recombinant 44 kDa protein, resulting in the measurement of affinity constants of 6.5 nM, 8.0 nM and 9.6 nM, respectively (Fig. 5D-5F). These data indicate that VASH1 exhibits a significant RNA binding activity.

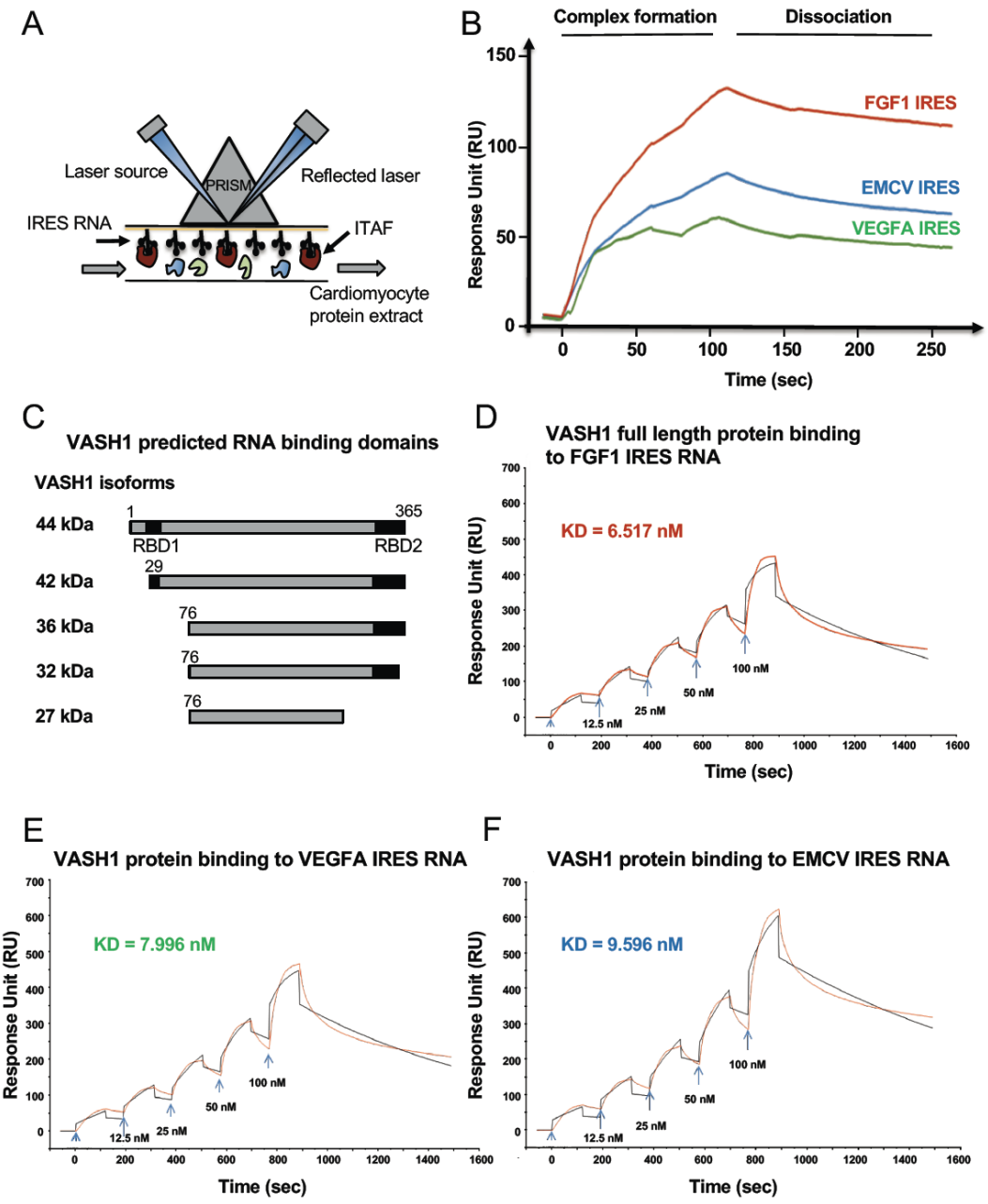


Figure 5. Identification of IRES-bound proteins in hypoxic cardiomyocytes reveals vasohibin1 as a new RNA-binding protein.

A-F Biotinylated IRES RNAs were transcribed *in vitro* and immobilized on the sensorchip of the BIAcore T200 optical biosensor device (A). Total cell extracts from normoxic or hypoxic HL-1 cardiomyocytes were injected in the device. Complex formation and dissociation were measured (see Mat. & Meth) (B). Bound proteins were recovered as described in Mat. & Meth. and identified by mass spectrometry (LC-MS/MS) after tryptic digestion. The list of proteins bound in normoxia and hypoxia to FGF1, VEGFAa and EMCV IRESs is shown in EV Table 4. VASH1 protein was identified bound to FGF1 (hypoxia) and EMCV IRESs (hypoxia and normoxia), but not to VEGFA IRES. A diagram of VASH1 RNA-binding properties is shown, with VASH1 isoforms described by Sonoda et al **(37)** (C). The predicted RNA binding domains (RBD1 and RBD2) shown in EV Figure

4, conserved in mouse and human, are indicated (C). Recombinant full-length 44 kDa VASH1 was injected into the Biacore T200 device containing immobilized FGF1 (D), VEGFAa (E) or EMCV (F) IRES as above. The affinity constants (KD) were calculated (D, E, F) with a Single Cycle Kinetics (SCK) strategy.

Vasohibin1 is translationally induced and nuclearized in early hypoxia.

VASH1 has been previously described for its expression in endothelial cells but never in cardiomyocytes (27). The present BIA-MS study provides evidence that it is expressed in HL-1 cardiomyocytes (EV Table 4). We analyzed the regulation of VASH1 expression during hypoxia: *Vash1* mRNA level strongly decreases after 4 hours of hypoxia whereas it is slightly upregulated after 8 hours (EV Table 1, Fig. 6A). In contrast, analysis of *Vash1* mRNA recruitment into polysomes showed a strong increase at 4 hours of hypoxia (about 7 times)(Fig. 6B), whereas it was not detectable in polysomes at 24 hours (EV Table 2). This indicates that *Vash1* mRNA translation is strongly induced in early hypoxia. VASH1 immunodetection confirmed a strong expression of VASH1 at 4 hours of hypoxia, despite the decrease of its mRNA. VASH1 appeared as foci in both cytoplasm and nucleus (Fig. 6C). The number of foci did not change, but their size significantly increased in hypoxia (Fig. 6D and 6E).

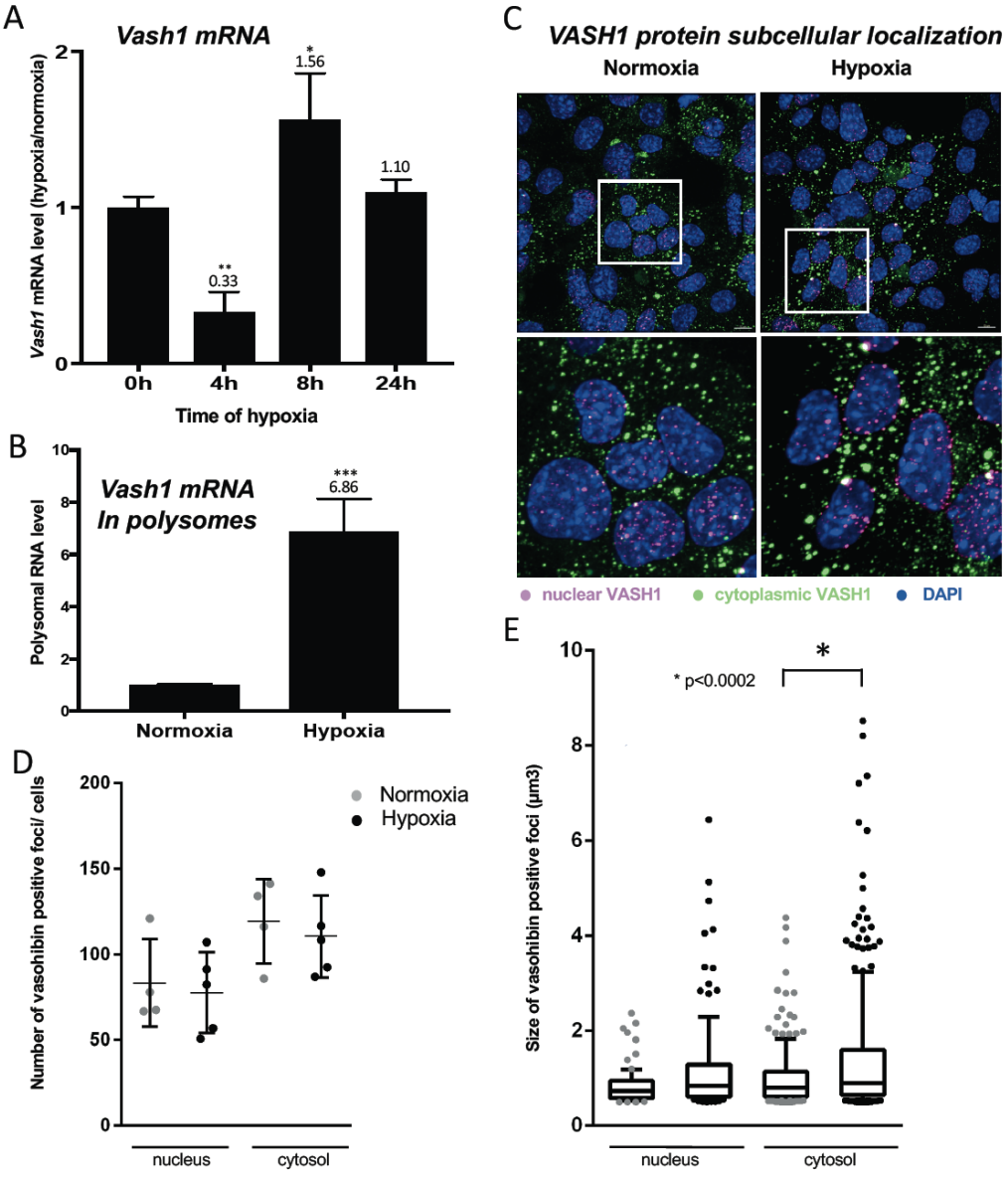
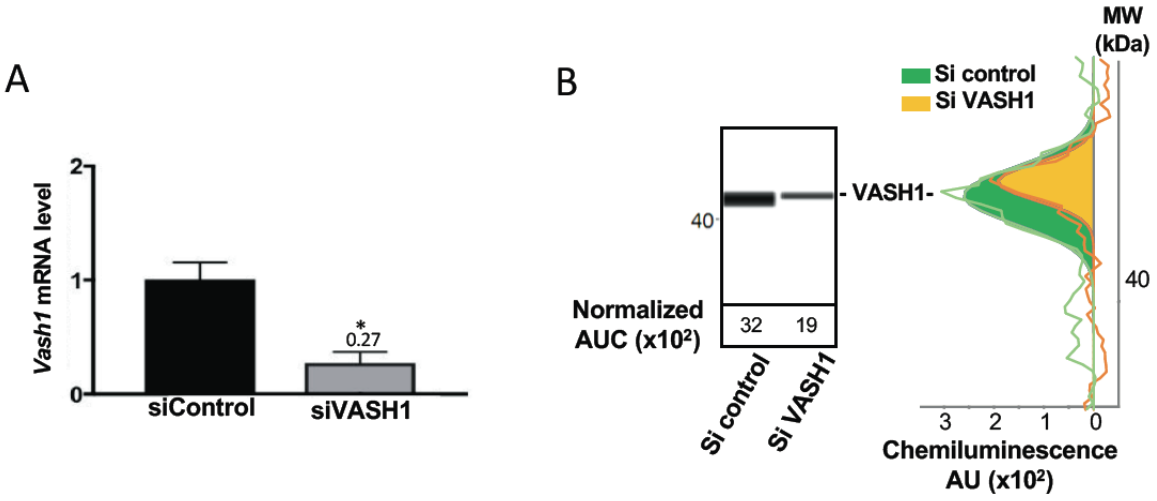


Figure 6. Vasohibin1 is translationally induced and nuclearized in early hypoxia.
A-D VASH1 expression was analyzed by RT-qPCR in HL-1 cardiomyocytes in response to hypoxia at the transcriptome and translatome levels. Total RNA was purified from cardiomyocytes in normoxia, or submitted to 4 h, 8 h or 24 h of hypoxia (A). Polysomal RNA was purified from cardiomyocytes in normoxia or after 4 h of hypoxia (B). Histograms correspond to mean ± standard deviation of the mean, with two-tailed t-test, *p<0.05, **p<0.01, ***<0.001, compared to normoxia. Experiments have been reproduced 3 times independently and a representative triplicate experiment is shown.
VASH1 was immunodetected in HL-1 cardiomyocytes in normoxia or after 4h of hypoxia (C). DAPI staining allows to detect VASH1 nuclear localization (MERGE). VASH1 foci in the nucleus are shown in purple and in the cytoplasm in green using Imaris software. The number of VASH1 foci was quantified in the nucleus and in the cytoplasm in normoxia and after 4 h of hypoxia (n = 4-5 images with a total cell number of 149 in normoxia and 178 in hypoxia) (D). Boxplots of volume of vasohibin foci in normoxia and hypoxia (E). All foci above 0,5 μm^3 were counted. Whiskers mark the 10 % and the 90% percentiles with the mean in the center. One-way Anova with Tukey's comparisons test was applied.

Vasohibin1 is a new ITAF selectively active in early hypoxia.

The putative ITAF function of VASH1 was assessed by a knock-down approach using an siRNA smartpool (siVASH1). Transfection of HL-1 cardiomyocytes with siVASH1 was able to knock-down VASH1 mRNA with an efficiency of 73% (Fig. 7A). The knock-down of VASH1 protein measured by capillary Western was only 59% (Fig. 7B). This moderate knock-down efficiency was probably due to the long half-life of VASH1, superior to 24h (EV Fig. 5). The effect of VASH1 knock-down was analyzed in HL-1 cells transduced with different IRES-containing bicistronic lentivectors in normoxia or after 8 h of hypoxia. In normoxia, VASH1 knock-down generated a moderate increase of activity for several IRESs (13-16%), significant for VEGFD and EMCV IRESs (Fig. 7C). In contrast, in hypoxia, VASH1 knock-down resulted in a significant decrease of FGF1, VEGFD and EMCV IRES activities, by 64%, 12% and 5%, respectively (Fig. 7D). These data showed that VASH1 behaves as an activator ITAF in hypoxia, limited to FGF1, VEGFD and EMCV IRESs, while it has an inhibitory role on the activities of these IRES in normoxia (Fig. 7C).



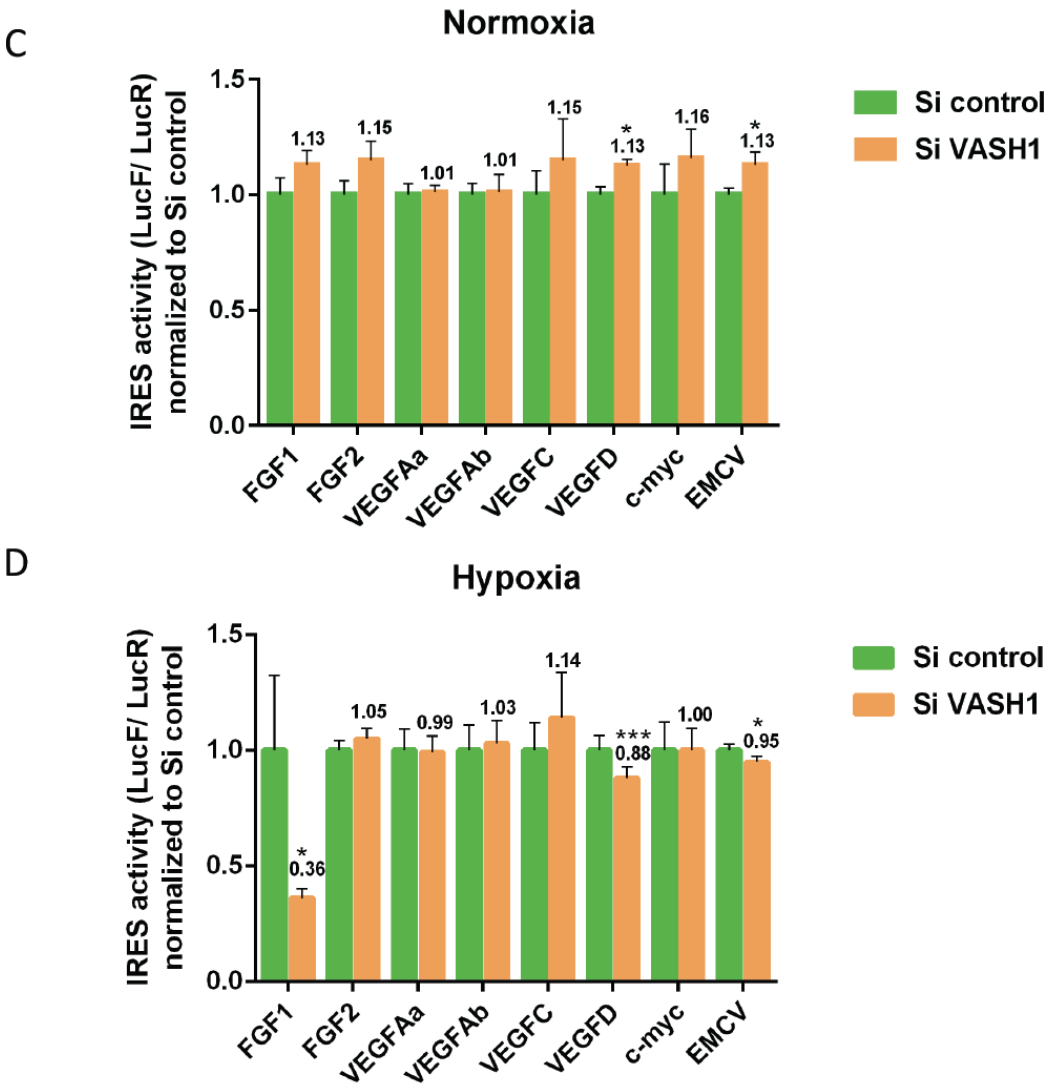


Figure 7. Vasohibin1 is a new ITAF selectively active in early hypoxia.
A-B VASH1 knock-down was performed in HL-1 cardiomyocytes using siRNA smartpools targeting VASH1 (siVASH1) or control (siControl). VASH1 mRNA level was measured by RT-qPCR (A) and VASH1 protein expression analyzed by capillary Simple Western method using an anti-VASH1 antibody and quantified by normalization to total proteins. The experiments have been reproduced three times and representative results are shown (B).
Knock-down experiment of VASH-1 performed on cardiomyocytes transduced by a set of IRES-containing lentivectors used in Fig. 4. After 8 h of hypoxia, IRES activities (LucF/LucR ratio) were measured in cell extracts. The IRES activity values have been normalized to the control siRNA. Histograms correspond to means ± standard error of the mean of the mean, with a one-tailed t-test *p<0.05, ***<0.001, compared to siControl. For each IRES the mean of three independent experiments with three biological replicates (n=9) is shown in normoxia (C) and hypoxia (D). All detailed values as well as standard deviations are presented in EV Table 5.

DISCUSSION

The present study highlights the crucial role of translational control in cardiomyocyte response to hypoxia. Up to now, although a few genes had been described for their translational regulation by hypoxia, it was thought that most genes are transcriptionally regulated. Here we show that translational control, revealed by mRNA recruitment in polysomes during hypoxia, concerns the majority of the genes involved in angiogenesis and lymphangiogenesis. IRES-dependent translation appears as a key mechanism in this process, as we show that all the

(lymph)angiogenic mRNAs known to contain an IRES are up-regulated. Furthermore, our data reveal that IRESs of angiogenic factor mRNAs are activated during early hypoxia, while non angiogenic mRNA IRESs are activated in late hypoxia. We have identified an angiogenesis- and stress-related protein, vasohibin1, as a new ITAF responsible for the activation of several, but not all IRESs, in early hypoxia.

Translational control in tumoral versus non tumoral hypoxia.

Most studies of gene expression in response to stress have been performed at the transcriptome level in tumoral cells of different origins, whereas the present study is focused on cardiomyocytes. HL-1 cardiomyocytes are immortalized but still exhibit the beating phenotype (21). Thus this cell model, although not perfectly mimicking the cardiomyocyte behavior *in vivo*, is still close to a physiological state. The strong translational response to hypoxia revealed by our data, that differs from the transcriptional response usually observed in tumor cells, may reflect mechanisms occurring in cells that are not engaged into the cell transformation process leading to cancer, or at least not too far. Indeed, HL-1 cells respond to hypoxia very early, whereas various murine or human tumor cell lines described in other reports require a longer time of hypoxia for IRES-dependent translation to be stimulated. In human breast cancer BT474 cells, VEGFA, HIF and EMCV IRESs are all activated after 24h of hypoxia (29). In murine 4T1 and LLC cells (breast and lung tumor, respectively) as well as in human CAPAN-1 pancreatic adenocarcinoma, the VEGFA and VEGFC IRESs are activated after 24 hours of hypoxia whereas the EMCV IRES is not activated (2). The same observation of late activation in 4T1 cells has been made for the FGF1 IRES, while this IRES is activated in early hypoxia in HL-1 cardiomyocytes (Godet AC & Prats AC, unpublished data)(Fig. 4). Also, the VEGFD IRES is differently regulated in HL-1 cardiomyocytes compared to 4T1 tumor cells: only heat shock, but not hypoxia, is able to activate this IRES in 4T1 cells, whereas it is activated by hypoxia in HL-1 cardiomyocytes (Fig. 4)(13). These observations suggest that many tumoral cell lines developing resistance to hypoxia are not able to govern subtle regulations of gene expression such as the waves of IRES regulation observed in HL-1 cells.

VASH1, an ITAF of early hypoxia.

We also consider the hypothesis that the important process of translational regulation observed in our study may be cardiomyocyte-specific. In such a case, IRES-dependent translation would depend on cell type specific ITAFs as well as the early response to hypoxia. These results are of great importance in regard to the acute stress response in ischemic heart that is necessary for recovery. In contrast, a delayed chronic response is known to be deleterious for heart healing (30). In agreement with this hypothesis, VASH1 expression is cell-type specific: described up to now as endothelial-specific, this protein is not expressed in tumoral cells (27). In the present study, we show that this cell-type specificity extends to cardiomyocytes. Consistent with our data, this protein has been described as a key actor of striated muscle angio-adaptation (31). VASH1 may thus have a role in the early hypoxic response in a limited number of cell types. The ITAF role of VASH1 identified here is physiologically relevant if one considers the VASH1 function in angiogenesis and stress tolerance (28). According to previous reports, VASH1 is induced during angiogenesis in endothelial cells and halts this process, while its overexpression also renders the same cells resistant to senescence and cell death induced by stress (28). Furthermore, it has been reported that VASH1 is induced after 3 hours of cell stress at the protein level but not at the transcriptional level in endothelial cells (32). This is in agreement with our observation in cardiomyocytes where VASH1, although downregulated in the transcriptome in early hypoxia, is more efficiently recruited in polysomes at the same time (Fig. 6).

It is noteworthy that VASH1 itself seems to be induced translationally by stress (Fig. 6) (32). In endothelial cells, Myashita et al report that the protein HuR upregulates VASH1 by binding to its mRNA. HuR may bind to an AU-rich element present in the 3' untranslated region of the

VASH1 mRNA. However, in other studies, HuR has also been described as an ITAF, thus it is possible that VASH1 itself may be induced by an IRES-dependent mechanism (10, 33, 34).
The anti-angiogenic function of VASH1 may appear inconsistent with its ability to activate the IRES of an angiogenic factor. However, our data also suggest that VASH1 might be an activator or an inhibitor for (lymph)angiogenic factor mRNA translation. Such a double role may explain the unique dual ability of VASH1 to inhibit angiogenesis and to promote endothelial cell survival (28, 32). This could result from the existence of different VASH1 isoforms of 44 kDa, 42 kDa, 36 kDa, 32 kDa and 27 kDa, resulting from alternative splicing and/protein processing (31, 35-37). Interestingly p42 and p27 are the main isoforms expressed in heart, while the p44 is undetectable (31, 37). One can expect that the ITAF function is carried by p42 which contains the two predicted RNA binding domains (Fig.5C, EV Fig. 4). VASH1 has been observed in both the nucleus and the cytoplasm, and no striking nucleocytoplasmic relocalization is visible in response to hypoxia, in contrast to other ITAFs such as hnRNPA1 or nucleolin which shuttle to cytoplasm upon stress (10, 13, 15, 38, 39). Interestingly, VASH1, appears as foci whose size increases in hypoxia, suggesting that it could be partly translocated to stress granules. This translocation has been reported for other ITAFs such as hnRNPA1 and polypyrimidine tract binding protein (PTB)(10, 40, 41).

VASH1 regulates several, but not all IRESs.
Although all IRESs of (lymph)angiogenic factor mRNAs are activated in early hypoxia, only FGF1 and VEGFD IRESs are regulated by VASH1. This suggests that other ITAFs are involved in activation of FGF2, VEGFA and VEGFC IRESs. Furthermore, the knock-down of VASH1 only partially silenced FGF1 and VEGFD IRES activities. This could result from the moderate efficiency of the knock-down, due to the strong stability of VASH1 protein, but it also suggests that VASH1 acts with other partners in the IRESsome. The double role of VASH1 as an activator or an inhibitor of IRES activity in hypoxia or in normoxia, respectively, also favors the hypothesis that VASH1 interacts with different partners in the IRESome. Thus, our study shows that IRESs of (lymph)angiogenic growth factor mRNAs, although they are all activated in early hypoxia, are regulated by different IRESome complexes whose composition is still to be discovered.

MATERIALS & METHODS

Lentivector construction and production

Bicistronic lentivectors coding for the *renilla* luciferase (LucR) and the stabilized firefly luciferase Luc+ (called LucF in the text) were constructed from the dual luciferase lentivectors described previously, which contained Luc2CP (2, 13). The LucR gene used here is a modified version of LucR where all the predicted splice donor sites have been mutated (sequence is available upon request). The cDNA sequences of the human FGF1, -2, VEGFA, -C, -D, c-myc and EMCV IRESs were introduced between the first (LucR) and the second cistron (LucF) (19, 42, 43). IRES sequences sizes are : 430 nt (FGF1), 480nt (FGF2), 302 nt (VEGFAa), 485 nt (VEGFAb), 419 nt (VEGFC), 507 nt (VEGFD), 363 nt (c-myc), 640 nt (EMCV)(2, 13, 16, 17, 19, 42). The two IRESs of the VEGFA have been used and are called VEGFAa and VEGFAb, respectively (16). The expression cassettes were inserted into the SIN lentivector pTRIP-DU3-CMV-MCS vector described previously (43). All cassettes are under the control of the cytomegalovirus (CMV) promoter.
Lentivector particles were produced using the $CaCl_2$ method-based by tri-transfection with the plasmids pLvPack and pLvVSVg, $CaCl_2$ and Hepes Buffered Saline (Sigma-Aldrich, Saint-Quentin-Fallavier, France), into HEK-293FT cells. Viral supernatants were harvested 48 hours after transfection, passed through 0.45 µm PVDF filters (Dominique Dutscher SAS, Brumath, France) and stored in aliquots at -80°C until use. Viral production titers were assessed on

HT1080 cells with serial dilutions and scored for GFP expression by flow cytometry analysis on a BD FACSVerse (BD Biosciences, Le Pont de Claix, France).

Cell culture, transfection and transduction

HEK-293FT cells and HT1080 cells were cultured in DMEM-GlutaMAX + Pyruvate (Life Technologies SAS, Saint-Aubin, France), supplemented with 10% fetal bovine serum (FBS), and MEM essential and non-essential amino acids (Sigma-Aldrich).

Mouse atrial HL-1 cardiomyocytes were a kind gift from Dr. William Claycomb (Department of Biochemistry & Molecular Biology, School of Medicine, New Orleans) (21). HL-1 cells were cultured in Claycomb medium containing 10% FBS, Penicillin/Streptomycin (100U/mL-100µg/mL), 0.1mM norepinephrine, and 2mM L-Glutamine. Cell culture flasks were pre-coated with a solution of 0.5% fibronectin and 0.02% gelatin 1h at 37°C (Sigma-Aldrich). To keep HL-1 phenotype, cell culture was maintained as previously described (21).

For hypoxia, cells were incubated at 37°C at 1%O_2.

HL-1 cardiomyocytes were transfected by siRNAs as follows: one day after being plated, cells were transfected with 10 nM of small interference RNAs from Dharmacon Acell SMARTpool targeting VASH1 (siVASH1) or non-targeting siRNA control (siControl), using Lipofectamine RNAiMax (Invitrogen) according to the manufacturer's recommendations, in a media without penicillin-streptomycin and norepinephrine. Cells were incubated 72h at 37°C with siRNA (siRNA sequences are provided in EV Table 6).

For lentivector transduction, 6.10^4 HL-1 cells were plated into each well of a 6-well plate and transduced overnight in 1 mL of transduction medium (OptiMEM-GlutaMAX, Life Technologies SAS) containing 5 µg/mL protamine sulfate in the presence of lentivectors (MOI 2). GFP-positive cells were quantified 48h later by flow cytometry analysis on a BD FACSVerse (BD Biosciences). HL-1 cells were transduced with an 80% efficiency. siRNA treatment on transduced cells was performed 72h after transduction (and after one cell passage).

To achieve protein half-life measurement, HL-1 cardiomyocytes were treated with cycloheximide (InSolution CalBioChem) diluted in PBS at a final concentration of 10 µg/mL in well plates. Time-course points were taken by stopping cell cultures after 0h, 4h, 6h 8h 16h or 24h of incubation and subsequent capillary Western analysis of cell extracts.

Reporter activity assay

For reporter lentivectors, luciferase activities *in vitro* and *in vivo* were performed using Dual-Luciferase Reporter Assay (Promega, Charbonnières-les-Bains, France). Briefly, proteins from HL-1 cells were extracted with Passive Lysis Buffer (Promega France). Quantification of bioluminescence was performed with a luminometer (Centro LB960, Berthold, Thoiry, France).

Capillary electrophoresis

Diluted protein lysate was mixed with fluorescent master mix and heated at 95°C for 5 minutes. 3 μL of protein mix containing Protein Normalization Reagent, blocking reagent, wash buffer, target primary antibody (mouse anti-VASH-1 Abcam EPR17420 diluted 1:100; mouse anti-FGF1 Abcam EPR19989 diluted 1:25; mouse anti-P21 Santa Cruz sc-6546 (F5) diluted 1:10; rabbit anti eIF2α Cell Signaling Technology 9721 diluted 1:50; mouse anti-phospho-eIF2α Cell Signaling Technology 2103 diluted 1:50; rabbit anti-4EBP-1 Cell Signaling Technology 9452 diluted 1:50; rabbit anti-phospho-4EBP-1 Cell Signaling Technology 9451 diluted 1:50), secondary-HRP (ready to use rabbit "detection module", DM-001), and chemiluminescent substrate were dispensed into designated wells in a manufacturer provided microplate. The plate was loaded into the instrument (Jess, Protein Simple) and proteins were drawn into individual capillaries on a 25 capillary cassette (12-230kDa)(SM-SW001). Data were analyzed on compass software provided by the manufacturer.

RNA purification and cDNA synthesis

Total RNA extraction from HL-1 cells was performed using TRIzol reagent according to the manufacturer's instructions (Gibco BRL, Life Technologies, NY, USA). RNA quality and quantification were assessed by a Xpose spectrophotometer (Trinean, Gentbrugge, Belgium). RNA integrity was verified with an automated electrophoresis system (Fragment Analyzer, Advanced Analytical Technologies, Paris, France).

500 ng RNA was used to synthesize cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Villebon-sur-Yvette, France). Appropriate no-reverse transcription and no-template controls were included in the PCR array plate to monitor potential reagent or genomic DNA contaminations, respectively. The resulting cDNA was diluted 10 times in nuclease-free water. All reactions for the PCR array were run in biological triplicates.

qPCR array

The DELTAgene Assay™ was designed by Fluidigm Corporation (San Francisco, USA). The qPCR-array was performed on BioMark with the Fluidigm 96.96 Dynamic Array following the manufacturer's protocol (Real-Time PCR Analysis User Guide PN 68000088). The list of primers is provided in EV Table 6. A total of 1.25 ng of cDNA was preamplified using PreAmp Master Mix (Fluidigm, PN 100-5580, 100-5581, San Francisco, USA) in the plate thermal cycler at 95°C for 2 min, 10 cycles at 95°C for 15sec and 60°C for 4 min. The preamplified cDNA was treated by endonuclease I (New England BioLabs, PN M0293L, Massachusetts, USA) to remove unincorporated primers.

The preamplified cDNA was mixed with 2x SsoFast EvaGreen Supermix (BioRad, PN 172-5211, California, USA), 50 µM of mixed forward and reverse primers and sample Loading Reagent (Fluidigm, San Francisco, USA). The sample was loaded into the Dynamic Array 96.96 chip (Fluidigm San Francisco, USA). The qPCR reactions were performed in the BioMark RT-qPCR system. Data was analyzed using the BioMark RT-qPCR Analysis Software Version 2.0.

18S rRNA was used as a reference gene and all data were normalized based on 18S rRNA level. Hprt was also assessed as a second reference gene but was not selected as its level was not stable during hypoxia. Relative quantification (RQ) of gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. When the RQ value was inferior to 1, the fold change was expressed as -1/RQ. The oligonucleotide primers used are detailed in EV Table 6.

Polysomal RNA preparation

HL-1 cells were cultured in 150-mm dishes. 15 min prior to harversting, cells were treated by cycloheximide at 100 µg/ml. Cells were washed three times in PBS cold containing 100 µg/mL cycloheximide and scraped in the PBS/cycloheximide. After centrifugation at 3,000 rpm for 2 min at 4°C, cells were lysed by 450µl hypotonic lysis buffer (5 mM Tris-HCL, pH7.5 ; 2.5 mM $MgCl_2$; 1.5 mM KCl). Cells were centrifuged at 13,000 rpm for 5 min at 4°C, the supernatants were collected and loaded onto a 10-50% sucrose gradient. The gradients were centrifuged in a Beckman SW40Ti rotor at 39,000 rpm for 2.5 h at 4°C without brake. Fractions were collected using a Foxy JR ISCO collector and UV optical unit type 11. RNA was purified from pooled heavy fractions containing polysomes (fractions 19-27), as well as from cell lysate before gradient loading.

Preparation of biotinylated RNA

The FGF1, VEGFA or EMCV IRESs was cloned in pSCB-A-amp/kan plasmid (Agilent) downstream from the T7 sequence. The plasmid were linearized and *in vitro* transcription was performed with MEGAscript T7 kit (Ambion), according to the manufacturer's protocol, in the

presence of Biotin-16-UTP at 1 mM (Roche), as previously described (26). The synthesized RNA was purified using RNeasy kit (Qiagen).

BIA-MS experiments

BIA-MS studies based on surface plasmonic resonance (SPR) technology were performed on BIAcore T200 optical biosensor instrument (GE Healthcare), as described previously **(13, 26)**. Immobilization of biotinylated IRES RNAs was performed on a streptavidin-coated (SA) sensorchip in HBS-EP buffer (10 mM Hepes pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20) (GE Healthcare). All immobilization steps were performed at a flow rate of 2 μl/min with a final concentration of 100 μg/ml.

Binding analyses were performed with normoxic or hypoxic cell protein extracts at 100 μg/ml over the immobilized IRES RNA surface for 120 sec at a flow rate of 30 μl/min. The channel (Fc1) was used as a reference surface for non-specific binding measurements. The recovery wizard was used to recover selected proteins from cell protein extracts. This step was carried out with 0.1% SDS. Five recovery procedures were performed to get enough amounts of proteins for MS identification.

Eluted protein samples from BIA experiment were digested *in gel* with 1 μg of trypsin (sequence grade, Promega) at 37°C OVN. Peptides were then subjected to LC-MS/MS analysis. The peptides mixtures were loaded on a YMC-Triart C18 150x300 μm capillary column (particle diameter 3 μm) connected to a RS3000 Dionex HPLC system. The run length gradient (acetonitrile and water) was 30 minutes. Then, on the AB Sciex 5600+ mass spectrometer, data were acquired with a data dependent analysis. Data were then loaded on Mascot software (Matrix Science) that attributes peptide interpretations to MS/MS recorded scans. The higher the score, the lower the probability of false positive (a score of 20 corresponds to a 5% probability of false positive).

Surface Plasmon Resonance assays.

For kinetic analysis, immobilization of biotinylated FGF1 IRES RNA was performed on a streptavidin-coated (SA) sensorchip in HBS-EP buffer (10 mM Hepes pH 7.4, 150 mM NaCl, 3mM EDTA, 0.005% surfactant P20) (GE Healthcare). Immobilization step was performed at a flow rate of 2 µl/min with a final concentration of 100 µg/ml. Total amount of immobilized FGF1 IRES RNA was 1500 RU.

Binding analyses were performed with recombinant protein VASH1 (Abnova H00022846-P01) at 100 µg/ml over the immobilized FGF1. This recombinant VASH1 contains the 27 kDa N-terminal part of the protein coupled to glutathione S-transferase. The channel (Fc1) was used as a reference surface for non-specific binding measurements.

A Single-Cycle Kinetics (SCK) analysis to determine association, dissociation and affinity constants (ka, kd, and KD respectively) was carried out by injecting different protein concentrations (16.25 nM-300 nM). Binding parameters were obtained by fitting the overlaid sensorgrams with the 1:1. Langmuir binding model of the BIAevaluation software version 3.0.

Immunocytology

Cells were plated on glass coverslip and incubated for 4 h of normoxia or hypoxia. They were fixed with cold methanol at -20°C during 5 min, washed 3 times with PBS and permeabilized 1 min with 0.1% Triton. Then, cells were incubated 5 min with blocking solution (1% FBS, 0.5% BSA) and 30 min with anti-VASH1 antibody (1/50; abcam ab176114) and Alexa 488 conjugated anti-mouse secondary antibody. Images were acquired with LSM780 Zeiss confocal microscope, camera lens x60 with Z acquisition of 0.36 µM. A single plan is shown Fig. 6C.

Imaris software was used to represent vasohibin staining in Figure 6C. To differentiate vasohibin in the nucleus and cytoplasm, nucleus was delimitated with Dapi staining and all Vasohibin foci in the nucleus are shown in purple and in the cytoplasm in green.

Using imaris software, the mean of vasohibin foci was counted and the volume of vasohibin foci was quantified, a threshold was applied and all particles above 0,5 μm^3 was selected and quantified.

Statistical analysis

All statistical analyses were performed using one-way Anova with Tukey's comparisons test or one-tailed Student's t-test and are expressed as mean +- standard deviation, *p<0.05, **p<0.01, ***<0.001, ****<0.0001.

ACKNOWLEDGMENTS

Our thanks go to J.J. Maoret and F. Martin from the Inserm UMR1048 GeT-TQ plateau of the GeT plateform Genotoul (Toulouse), F. Lopez and L. Tonini from the proteomic platform genotoul (Toulouse), J. Iacovoni from the Inserm UMR 1048 bioinformatics plateau, as well as L. van den Berghe and C. Segura from the Inserm UMR1037 vectorology plateau (Toulouse) and A. Lucas from the We-Met Functional Biochemistry Facility (Toulouse). We also thank V. Poinsot for helpful discussion and W. Claycomb for providing HL-1 cells.

This work was supported by Région Midi-Pyrénées, Association Française contre les Myopathies (AFM-Téléthon), Association pour la Recherche sur le Cancer (ARC), European funding (REFBIO), Fondation Toulouse Cancer Santé and Agence Nationale pour la Recherche AAPG2018-RIBOCARD. F.H. had fellowships from the Région Midi-Pyrénées and from the Ligue Nationale Contre le Cancer (LNCC). E.R.G. had a fellowship from AFM-Telethon. A.C. Godet had a fellowship from LNCC.

EXPANDED VIEW MATERIAL

EV Figure 1, 2 and 3, 4, 5, EV Tables 1, 2, 3, 4, 5, 6, 7

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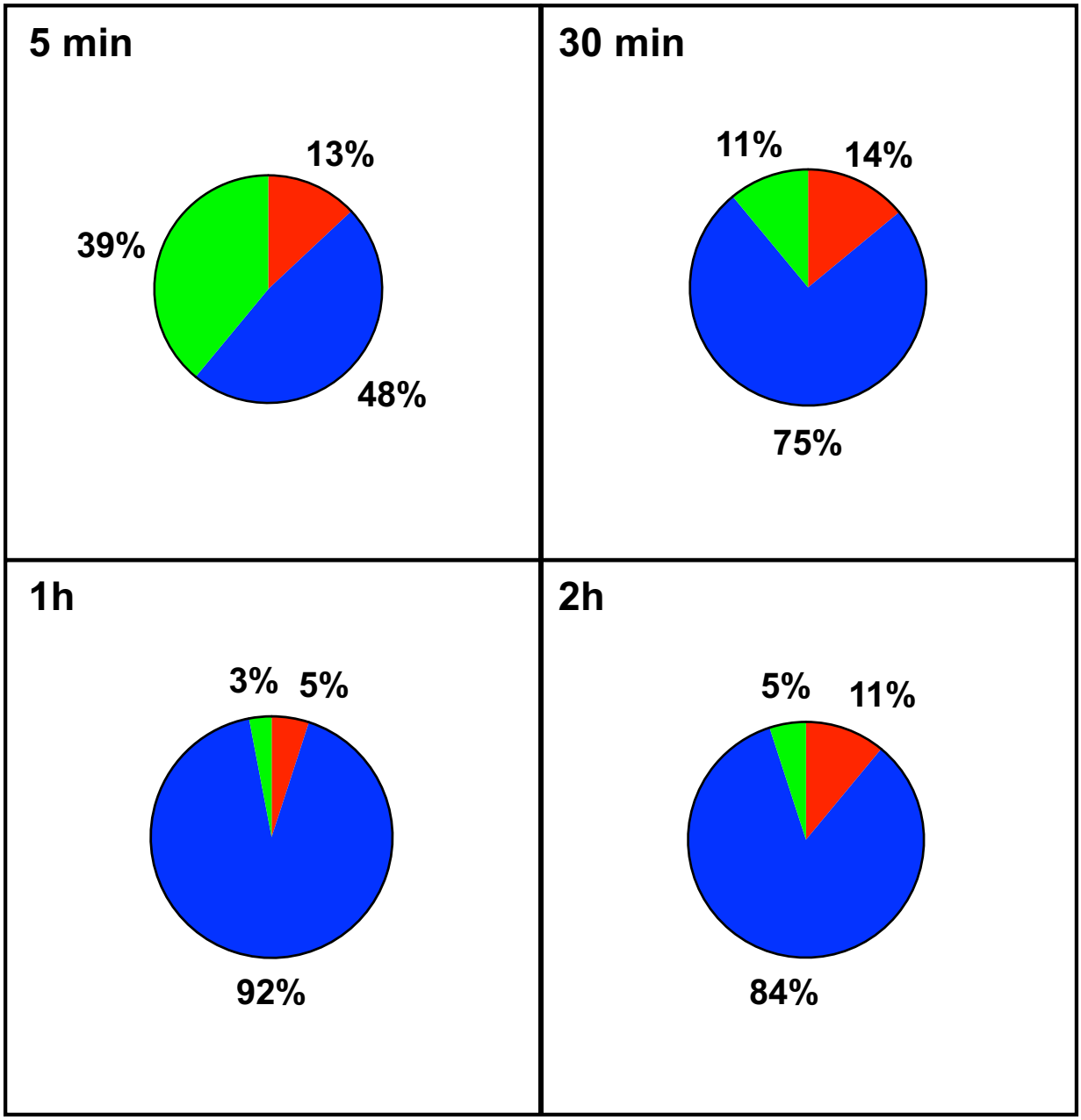
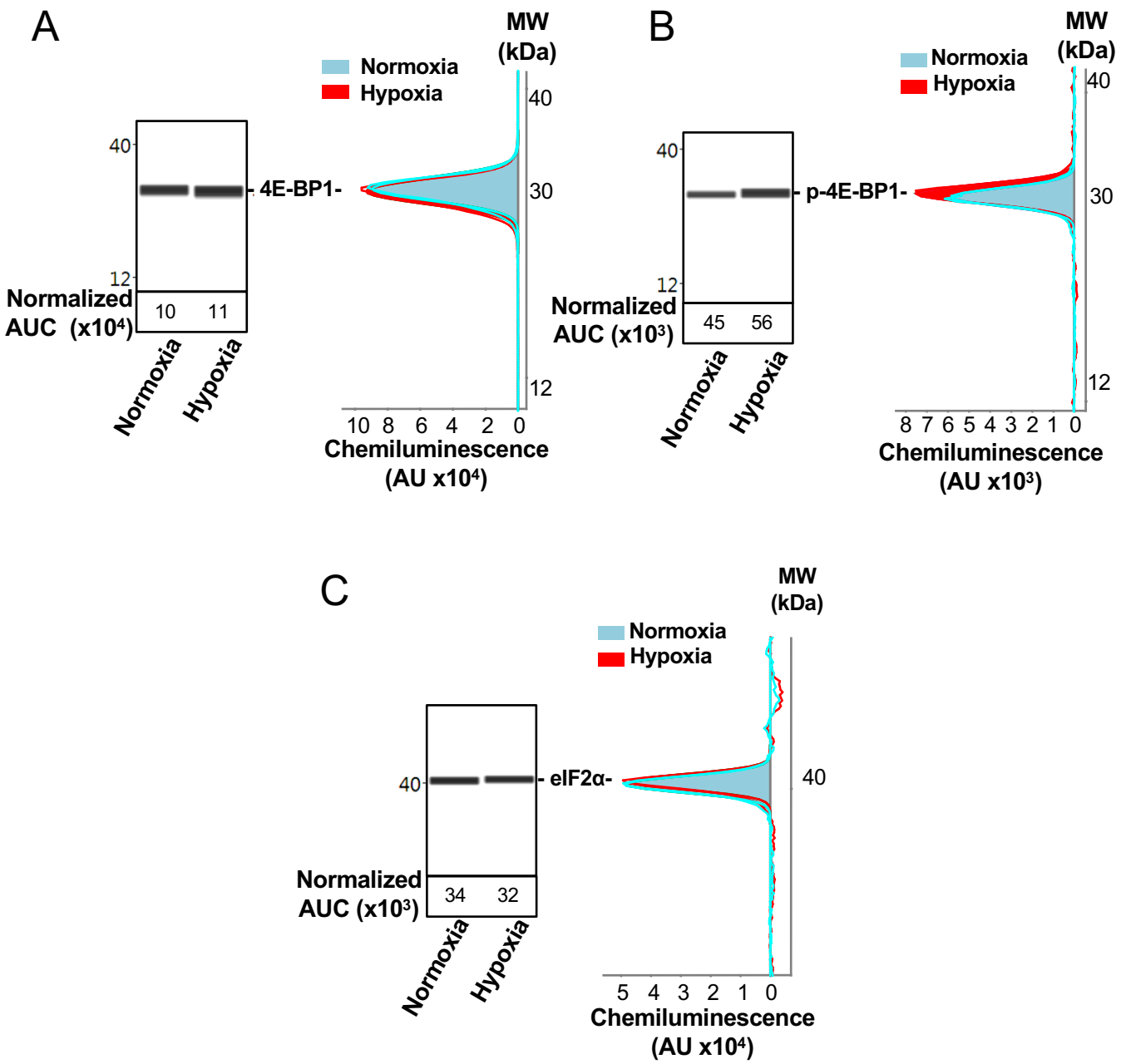
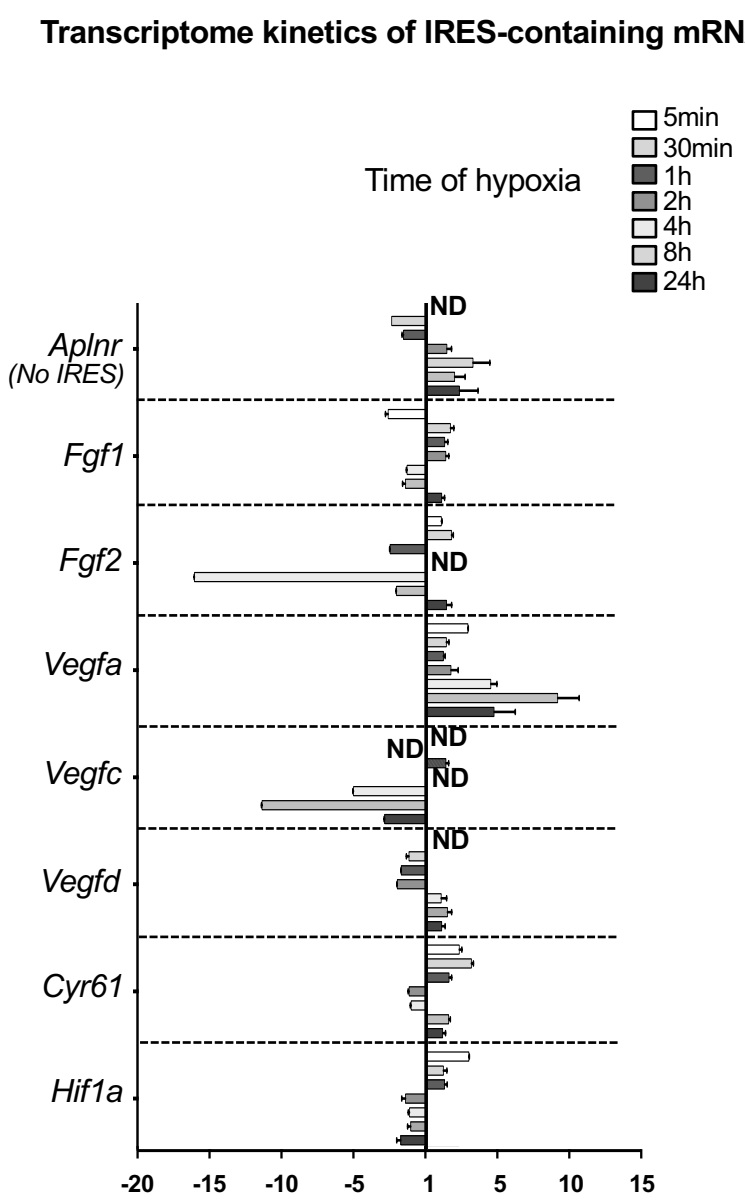


Expanded View Figure 1. Transcriptome of IRES-containing mRNAs in hypoxic cardiomyocytes.
Total RNA was purified from HL-1 cardiomyocytes submitted to increasing times from 5 min to 24 h of hypoxia at 1% O_2, as well as from normoxic cardiomyocytes as a control. cDNA was synthesized and used for a Fluidigm deltagene PCR array dedicated to genes related to (lymph)angiogenesis or stress (EV Table 6). Relative quantification (RQ) of gene expression during hypoxia was calculated using the $2^{-\Delta\Delta CT}$ method with normalization to 18S and to normoxia. The percentage of repressed (red), induced (green) and non-regulated (blue) mRNAs is shown for the earlier times of the kinetics. The later times are shown in Fig. 1. The detailed values for all the times of the kinetics are presented in EV Table 1.



Expanded View Figure 2. Capillary electrophoresis immunodetection of 4E-BP1 and eIF2α.

A-C 4E-BP1 expression (A) and phosphorylation (B), as well as eIF2α expression (C) in normoxia and hypoxia (8 h) were analysed and quantified by capillary Simple Western, as described in Mat. & Meth. The quantified values, expressed in arbitrary units of luminescence (AUC) are normalized to total proteins. Analysis of eIF2α phosphorylation is shown in Figure 2.



Expanded View Figure 3. Transcriptome of IRES-containing mRNAs in hypoxic cardiomyocytes.
RQ values for IRES-containing mRNA transcriptome kinetics extracted from the PCR arrays shown in EV Table 1. The gene *Aplnr* (apelin receptor) was chosen as a control without an IRES.

A

Mouse vasohibin-1 (NP_796328.2)

BindN prediction of RNA-binding residues

Summary

Input sequence length: 375 amino acids
Predicted binding sites: 125 residues
User-defined specificity: 80.00%
Estimated sensitivity: 53.95%

Overview

```
Sequence:    MPGGKKVVPSGSSSASPNAAATTTAAAAAAAAAPHSGTKRLETTEGASAQRDEEPEEEGE
Prediction:  ----++--++++++++++--+++---------+++++++-++++-++-++---------
Confidence:  43347732665888474752365644455543357878992566636826622344677 5

Sequence:    EDLRDGGVPFFINRGGLPVDEATWERMWKHVAKIHPDGEKVALRIRGATDLPKIPIPSVP
Prediction:  ---+---------+----------+------+------+---+-+------+----+--
Confidence:  66767748466945655275463455752587672336455267764523636736346 4

Sequence:    TFQPTTPVPERLEAVQRYIRELQYNHTGTQFFEIKKSRPLTGLMDLAKEMTKEALPIKCL
Prediction:  +----+---------+---+---++-+--------+++--+-------------------
Confidence:  53232535342855742497272452632446372668235777787266226564938 9

Sequence:    EAVILGIYLTNSMPTLERFPISFKTYFSGNYFRHIVLGVNFGGRYGALGMSRREDLMYKP
Prediction:  -------------+--+-----+--+---+----------+---++-+++-------
Confidence:  69999793922364476575737434524348669997933338242462675323434 4

Sequence:    PAFRTLSELVLDYEAAYGRCWHVLKKVKLGQCVSHDPHSVEQIEWKHSVLDVERLGREDF
Prediction:  ---+----------+-+--------+--+--+-------------------+--++--
Confidence:  34353745999986655568338722855265644453395385665588694744752 4

Sequence:    RKELERHARDMRLKIGKGTGPPSPTKDRKKDVSSPQRAQSSPHRRNSRSERRPSGEKKPA
Prediction:  ++---+--+--+-+--+-++++++++++++++++++++++++++++++++++++++++++-
Confidence:  76352736735775438296999999789874875795899989999999988688863

Sequence:    EPKAMPDLSGYQIRV
Prediction:  +-+-----+-+--+-
Confidence:  52465457554287 8
```

```
*** Prediction:  binding residues are labeled with '+' and in red;
                 non-binding residues labeled with '-' and in green.
*** Confidence:  from level 0 (lowest) to level 9 (highest).
```

B

Human vasohibin-1 (NP_055724.1)

BindN prediction of RNA-binding residues

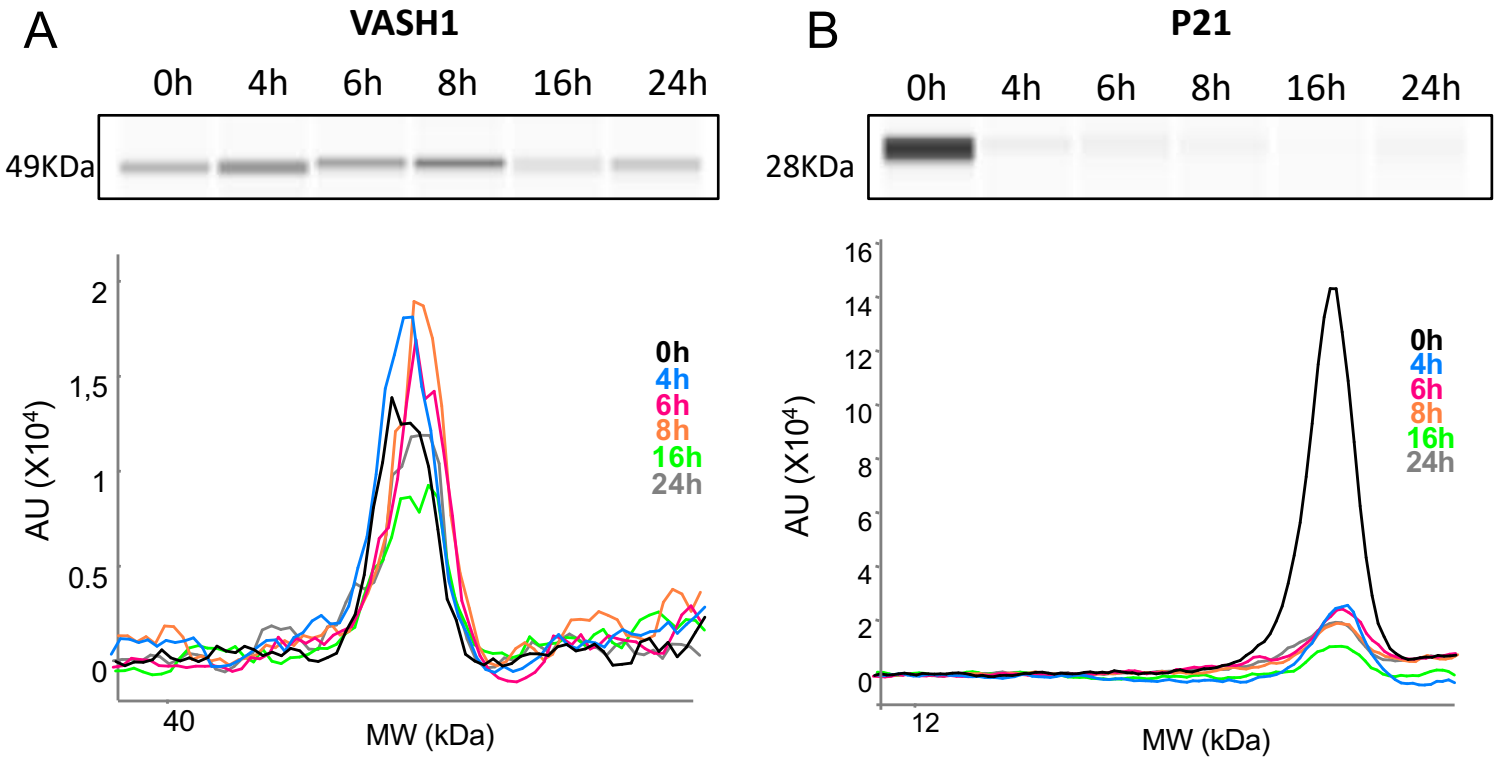
Summary

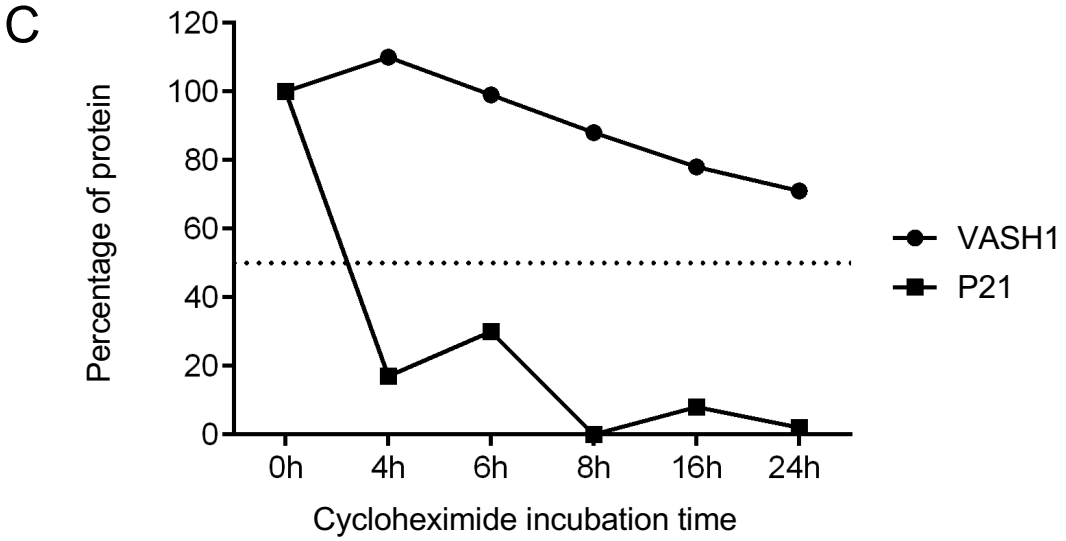
nput sequence length: 365 amino acids
Predicted binding sites: 125 residues
User-defined specificity: 80.00%
Estimated sensitivity: 53.95%

Overview

```
equence:     MPGGKKVAGGGSSGATPTSAAATAPSGVRRLETSEGTSAQRDEEPEEEGEEDLRDGGVPF
rediction:   ----++-++-+++++++++--++-+++-++--+++-++++++----------+------
onfidence:   43337735725998785782456468549832667288577423446775667677484 6

equence:     FVNRGGLPVDEATWERMWKHVAKIHPDGEKVAQRIRGATDLPKIPIPSVPTFQPSTPVPE
rediction:   ---+----------+------+------+---+-+------+----+-+----+----
onfidence:   69456552754634557525876723364443265743236367363464532326243 4

equence:     RLEAVQRYIRELQYNHTGTQFFEIKKSRPLTGLMDLAKEMTKEALPIKCLEAVILGIYLT
rediction:   -----+---+---++-+--------+++--+-----------------------------
onfidence:   28557424972724526324463726682357777872662265649389693999793 92

equence:     NSMPTLERFPISFKTYFSGNYFRHIVLGVNFAGRYGALGMSRREDLMYKPPAFRTLSELV
rediction:   ----+-+-----+-+-+----+----------+--++-+++-------+------+-----
onfidence:   23644765757374345243486699979335373524626753234344343537459 9

equence:     LDFEAAYGRCWHVLKKVKLGQSVSHDPHSVEQIEWKHSVLDVERLGRDDFRKELERHARD
rediction:   ------+-+--------+-+++-+-------------------+--++--++---+-+-
onfidence:   99976656683387228534744534334395385665588694744742476352736 73

equence:     MRLKIGKGTGPPSPTKDRKKDVSSPQRAQSSPHRRNSRSERRPSGDKKTSEPKAMPDLNG
rediction:   -+-+--+-++++++++++++++++++++++++++++++++++++++++++++++-------
onfidence:   57754382969999997898748757958999899999999888989876576554725

equence:     YQIRV
rediction:   ---+-
onfidence:   22778
```

Expanded View Figure 4. Conservation of predicted RNA binding domains in mouse and human vasohibin-1.
A-B RNA binding domains in mouse (A) and human (B) VASH1 proteins were predicted using BindN software (https://omictools.com/bindn-2-tool)





time	0 h	4 h	6 h	8 h	16 h	24 h
VASH-1	100%	110%	99%	88%	78%	71%
p21	100%	17%	30%	ND	8%	2%

Expanded View Figure 5. VASH1 half life is superior to 24h.
A-C VASH1 half-life determination experiment were performed by blocking protein synthesis with cycloheximide at 10 µg/mL, with time-course points at 0 h, 4 h, 6 h, 8 h, 16 h and 24 h. VASH1 (A) and P21 (B) protein stability was measured by capillary Simple Western with normalization to 0 h time-course point. P21 was used as a control for its short half-life (C).

Hantelys et al, EV Table 1

Hypoxia time	5min		30min		1h		2h		4h		8h		24h	
Gene name	**RQ**		**RQ**		**RQ**		**RQ**		**RQ**		**RQ**		**RQ**	
Akt1	1.60	±0.13	1.30	±0.06	1.20	±0.15	1.36	±0.25	-1.10	±0.09	1.40	±0.13	1.00	±0.32
Ang	2.65	±0.25	1.23	±0.36	-1.04	±0.12	1.24	±0.04	1.24	±0.12	1.09	±0.08	1.26	±0.28
Angpt1	5.51	±0.17	1.20	±0.30	1.19	±0.07	1.90	±0.49	2.43	±0.36	1.56	±0.43	-2.27	±0.06
Angptl4	1.03	±0.02	-3.14	±0.09	1.65	±0.54	1.60	±0.46	1.56	±0.67	3.50	±1.07	-1.37	±0.35
Anpep	ND		-3.26	±0.29	1.65	±0.01	-8.05	±0.06	-1.04	±0.14	1.62	±0.62	1.03	±0.43
Apln (Apelin)	2.11		-19,21	±0.01	-6.17	±0.05	-29.04	±0.01	36.77	±5.59	109.98	±6.98	335.72	±57.52
Aplnr	ND		-2.36	±0.03	-1.57	±0.10	1.44	±0.32	3.25	±1.70	1.98	±0.72	2.31	±1.29
Atp2a2 (SERCA2)	1.46	±0.08	1.21	±0.13	1.26	±0.14	1.50	±0.01	1.20	±0.06	1.84	±0.18	1.48	±0.32
Bai1	-1.52	±0.19	2.90	±0.91	1.17	±0.06	1.65	±0.52	1.36	±0.13	2.05	±0.60	1.04	±0.30
Ccl2	ND		-1.09	±0.49	1.76	±0.46	-2.76	±0.16	1.57	±1.01	2.99	±1.13	1.23	
Ccl21a	ND		-32.39	±0.01	2.97	±0.85	-1.90	±0.03	2.65	±2.78	-2.27	±0.21	1.32	±0.39
Col18a1	1.66	±0.71	1.58	±0.39	1.14	±0.14	1.45	±0.04	-1.69	±0.09	1.82	±0.43	1.57	±0.43
Col4a3	19.64	±0.07	1.52	±0.19	1.20	±0.21	1.31	±0.04	1.00	±0.03	1.45	±0.30	4.40	±0.80
Ctgf	1.29	±0.09	1.67	±0.26	1.16	±0.06	1.30	±0.13	1.06	±0.13	2.05	±0.30	3.66	±0.76
Cxcl1	1.32	±0.64	1.11	±0.27	1.67	±0.19	1.58	±0.12	1.01	±0.09	-1.43	±0.08	-2.04	±0.13
Cxcl10	1.37	±0.86	1.36	±0.29	1.11	±0.14	1.33	±0.25	1.05	±0.09	-1.02	±0.13	-1.08	±0.23
Cyr61	2.30	±0.18	3.14	±0.14	1.59	±0.18	-1.18	±0.07	-1.03	±0.06	1.56	±0.12	1.15	±0.21
Edn1	-2.43	±0.28	2.93	±0.31	1.72	±0.32	-1.4	±0.24	-1.39	±0.06	-1.33	±0.09	1.04	±0.26
Efna1	2.08	±0.15	1.23	±0.10	-1.52	±0.12	-1.16	±0.19	1.25	±0.09	1.17	±0.05	1.35	±0.33
Efnb2	1.74	±0.14	1.55	±0.25	1.14	±0.23	-1.03	±0.18	1.57	±0.18	3.71	±0.61	1.65	±0.42
Egf	ND		1.35	±0.14	1.32	±0.30	1.56	±0.44	1.56	±0.11	2.14	±0.53	-1.61	±0.35
Eng	2.96	±0.94	1.55	±0.39	1.18	±0.19	-1.02	±0.14	-1.11	±0.15	2.27	±0.33	1.65	±0.34
Ephb4	2.22	±0.13	1.67	±0.16	-1.03	±0.14	1.10	±0.13	1.01	±0.12	1.45	±0.09	1.57	±0.21
Erbb2 (Her2)	1.87	±0.19	1.85	±0.26	-1.01	±0.18	1.16	±0.18	1.32	±0.11	1.00	±0.18	1.34	±0.09
F3	1.12	±0.94	1.45	±0.18	1.28	±0.11	-1.57	±0.23	-1.23	±0.07	-1.09	±0.10	-1.56	±0.21
Fgf1 (aFGF)	-2.63	±0.18	1.69	±0.23	1.29	±0.22	1.37	±0.21	-1.32	±0.05	-1.43	±0.20	1.08	±0.20
Fgf2 (bFGF)	1.06	±0.04	1.76	±0.13	-2.5	±0.03	ND		-16.05	±0.02	-2.05	±0.07	1.43	±0.35
Fgfr3	1.41	±0.07	1.34	±0.27	-1.08	±0.12	1.38	±0.31	1.47	±0.27	3.93	±0.55	2.69	±0.39

Hypoxia time	5min		30min		1h		2h		4h		8h		24h	
Gene name	**RQ**		**RQ**		**RQ**		**RQ**		**RQ**		**RQ**		**RQ**	
Fn1	3.30	±0.07	1.70	±0.15	-1.02	±0.17	1.16	±0.01	-1.25	±0.06	2.45	±0.36	2.61	±0.49
Hif1a	2.97	±0.03	1.20	±0.24	1.28	±0.18	-1.42	±0.26	-1.15	±0.08	-1.06	±0.21	-1.75	±0.22
Hnrnpm	1.55	±0.18	1.17	±0.18	1.54	±0.15	2.26	±0.20	1.86	±0.25	3.86	±1.33	1.23	±0.26
Hpse	ND		3.23	±1.07	1.25	±0.34	1.64	±0.16	1.56	±0.14	3.52	±1.07	11.04	±1.95
Id1	-1.61	±1.12	1.58	±0.18	2.63	±0.50	3.01	±1.27	4.23	±0.19	2.01	±0.40	1.79	±0.64
Ifna1	ND		1.72	±0.17	-1.09	±0.06	1.73	±0.07	2.19	±0.33	3.11	±0.25	1.31	±0.49
Igf1	10.07	±0.59	1.54	±0.22	1.42	±0.15	1.06	±0.04	1.10	±0.22	-1.25	±0.14	-2.86	±0.14
Igf1r	ND		ND		ND		ND		-3.17	±0.08	2.37	±0.20	5.14	±0.32
Itgav	ND		1.19	±0.11	-1.36	±0.14	1.33	±0.29	1.33	±0.12	3.41	±0.56	2.82	±0.64
Itgb3	-4.33	±0.30	1.34	±0.19	-1.35	±0.02	1.04	±0.20	1.03	±0.20	1.42	±0.34	-1.37	±0.10
Jag1	2.13	±0.09	1.80	±0.24	1.03	±0.13	1.66	±0.46	1.46	±0.06	2.26	±0.37	1.76	±0.21
Mdk	1.14	±0.21	2.45	±0.17	-1.07	±0.17	-1.27	±0.00	1.16	±0.32	1.52	±0.38	-1.16	±0.46
Mmp14	-1.77	±0.03	1.30	±0.22	1.02	±0.06	-1.16	±0.17	1.06	±0.11	1.70	±0.24	1.95	±0.15
Mmp2	1.46	±0.18	1.96	±0.50	1.20	±0.05	1.68	±0.44	1.14	±0.33	1.94	±0.66	-1.23	±0.37
Neat1	4.03	±0.48	-1.17	±0.23	-1.59	±0.05	2.65	±0.00	1.89	±0.15	2.96	±1.02	-1.16	±0.29
Nos3	9.29		-4.01	±0.10	2.00	±0.54	-2.58	±0.26	-1.30	±0.14	-1.02	±0.40	-1.32	±0.19
Nrp1	2.46	±0.06	1.32	±0.17	1.04	±0.15	-1.25	±0.25	-1.10	±0.07	1.47	±0.25	1.10	±0.16
Nrp2	2.50	±0.07	1.55	±0.05	1.02	±0.12	1.02	±0.12	1.14	±0.19	1.72	±0.32	1.83	±0.39
P54nrb	2.96	±0.09	1.58	±0.42	1.21	±0.20	1.42	±0.29	1.22	±0.13	1.66	±0.30	1.11	±0.23
PAI1 (SerpinE1)	-1.73	±0.12	2.18	±0.54	1.46	±0.15	1.74	±0.65	5.75	±0.57	15.76	±2.01	13.18	±1.02
Pdgfa	3.65	±0.43	1.45	±0.18	1.29	±0.26	1.59	±0.36	1.71	±0.11	1.97	±0.35	1.54	±0.34
Pecam1	1.48		-11.55	±0.02	-1.09	±0.24	2.86	±1.08	2.09	±0.77	2.70	±1.72	2.60	±0.86
Pf4	ND		-39.36	±0.02	-1.79	±0.33	-2.20	±0.18	1.80	±0.40	1.48	±0.55	1.01	±0.28
Pgf	ND		2.17	±0.14	-1.36	±0.19	-7.89	±0.09	4.47	±0.89	1.85	±0.29	3.22	±0.85
Plau (upa)	1.13	±0.29	-1.04	±0.24	-1.36	±0.16	-1.31	±0.13	1.25	±0.30	1.58	±0.26	1.24	±0.24
Plg	ND		-2.71	±0.26	-1.05		2.45	±0.63	-1.35	±0.19	-3.33	±0.16	-2.94	±0.25
Prox1	7.13	±0.20	1.48	±0.23	1.26	±0.14	-1.13	±0.14	-1.08	±0.13	1.27	±0.29	-1.72	±0.07

Hypoxia time	5min		30min		1h		2h		4h		8h		24h	
Gene name	**RQ**		**RQ**		**RQ**		**RQ**		**RQ**		**RQ**		**RQ**	
Psf/Sfpq	1.45	±0.17	1.37	±0.09	1.30	±0.19	1.22	±0.08	1.10	±0.15	1.55	±0.59	-1.43	±0.16
Pspc1	1.00	±0.80	1.34	±0.11	1.50	±0.01	1.64	±0.58	1.67	±0.19	1.37	±0.31	1.59	±0.23
SerpinF1	ND		-31.89	±0.02	-9.09	±0.01	-1.91	±0.33	-2.38	±0.11	-1.14	±0.17	1.02	±0.37
Sphk1	ND		1.91	±0.80	1.70	±0.04	-1.51	±0.19	1.30	±0.11	2.78	±1.20	1.17	±0.43
Tek	1.97	±1.55	1.98	±0.23	1.55	±0.41	1.48	±0.20	1.24	±0.14	-1.25	±0.09	1.04	±0.13
Tgfa	ND		-2.01	±0.13	-7.94	±0.05	-6.9	±0.03	2.91	±0.63	3.50	±1.29	4.05	±1.71
Tgfb1	-5.26	±0.02	1.22	±0.23	-1.45	±0.14	1.03	±0.10	-1.02	±0.22	1.45	±0.24	1.05	±0.25
Tgfb2	-2.45	±0.08	1.50	±0.31	1.22	±0.07	1.37	±0.12	1.78	±0.24	2.00	±0.39	1.43	±0.40
Tgfbr1	-2.03	±0.57	1.33	±0.16	1.28	±0.25	1.48	±0.14	1.86	±0.34	2.23	±0.77	-1.27	±0.16
Thbs1	2.65	±0.09	1.88	±0.12	1.19	±0.09	1.47	±0.33	1.12	±0.07	1.30	±0.22	1.44	±0.08
Thbs2	2.15	±0.12	1.52	±0.10	1.03	±0.16	1.20	±0.18	-1.20	±0.01	1.21	±0.15	-1.22	±0.21
Timp1	-2.20	±0.04	1.13	±0.27	-1.32	±0.29	-1.96	±0.17	-1.28	±0.12	-1.22	±0.11	-2.33	±0.19
Timp2	2.21	±0.04	1.46	±0.22	1.14	±0.01	1.13	±0.09	1.67	±0.13	1.28	±0.12	-1.69	±0.15
Timp3	ND		2.70	±0.43	1.40	±0.23	1.98	±0.46	1.09	±0.16	1.04	±0.29	1.11	±0.17
Vash1	ND		ND		ND		ND		-3.01	±0.14	1.56	±0.30	1.10	±0.08
Vegfa	2.90	±0.02	1.40	±0.18	1.20	±0.12	1.72	±0.50	4.48	±0.43	9.12	±1.48	4.71	±0.69
Vegfb	1.38	±0.04	1.33	±0.10	-1.2	±0.11	1.02	±0.04	-1.09	±0.07	1.04	±0.22	-1.59	±0.10
Vegfc	ND		ND		1.38	±0.18	ND		-5.03	±0.05	-11.37	±0.03	-2.86	±0.16
Vegfd	ND		-1.19	±0.17	-1.73	±0.22	-1.99	±0.23	1.05	±0.37	1.48	±0.29	1.08	±0.24
Vegfr2 (Kdr)	-2.05	±0.05	1.25	±0.13	1.07	±0.04	1.43	±0.21	1.31	±0.10	2.29	±0.38	-1.01	±0.30

EV Table 1. Transcriptome of (lymph)angiogenic factor genes in hypoxic HL-1 cardiomyocytes.
Total RNA was purified from HL-1 cardiomyocytes submitted to increasing times from 5 min to 24 h of hypoxia at 1% O_2, as well as from normoxic cardiomyocytes as a control. cDNA was synthesized and used for a Fluidigm deltagene PCR array dedicated to genes related to (lymph)angiogenesis or stress (EV Table 6). Relative quantification (RQ) of gene expression in hypoxia was calculated using the $2^{-\Delta\Delta CT}$ method with normalization to 18S and to normoxia. Standard deviation is indicated. When the RQ value is inferior to 1, the fold change is expressed as -1/RQ. ND means “non detected”.

Hantelys et al. EV Table 2

Hypoxia: 4 h

	Total mRNA		Polysome bound mRNA		Fold change		Standard deviation	
Gene name	RQ 1	RQ 2	RQ 1	RQ 2	RQ(polysomes)/ RQ(total mRNA) 1	RQ(polysomes)/ RQ(total mRNA) 2	ST DEV 1	ST DEV 2
Akt1	0.54	0.63	1.12	1.45	2.07	2.29	0.07	0.05
Ang	0.22	0.59	0.54	0.44	2.42	-1.35	0.53	0.01
Angpt1	0.16	0.48	0.37	0.32	2.35	-1.48	0.67	0.01
Angptl4	0.21	0.39	0.67	0.48	3.26	1.23	1.56	0.15
Anpep	0.41	0.40	1.39	1.59	3.41	3.96	0.21	0.83
Apelin	35.38	2.85	60.39	4.47	1.71	1.57	0.27	0.10
Aplnnr	3.25	ND	1.12	ND	-2.86	ND	0.35	ND
Atp2a2	0.31	0.45	0.6	0.84	1.94	1.84	0.34	0.07
Bai1	0.16	0.39	0.61	1.97	3.8	5.06	0.89	0.30
Ccl2	1.57	1.96	ND	ND	ND	ND	ND	ND
Ccl21a	2.65	ND	ND	ND	ND	ND	ND	ND
Col18a1	0.45	0.57	1.18	0.88	2.64	1.53	0.61	0.19
Col4a3	0.37	0.51	0.87	1.15	2.36	2.24	0.41	0.13
Ctgf	0.29	0.48	0.9	0.13	3.15	-3.77	0.49	0.00
Cxcl1	0.1	0.56	0.22	0.29	2.21	-1.95	0.3	0.04
Cxcl10 (inp10)	0.19	0.43	0.74	0.13	3.96	-3.26	1.43	0.02
Cyr 61	0.45	0.72	0.91	0.62	2	-1.16	0.24	0.04
Edn1	0.27	0.48	0.68	0.68	2.49	1.42	0.55	0.03
Efna1	0.3	0.48	0.69	0.82	2.29	1.69	0.29	0.16
Efnb2	0.53	0.55	1.31	0.68	2.48	1.23	0.71	0.01
Egf	0.21	0.56	0.53	2.34	2.46	4.16	1.56	0.67
Eng	0.8	0.63	2.16	1.36	2.71	2.16	0.78	0.25
Ephb4	0.31	0.50	0.58	0.68	1.87	1.36	0.23	0.05
Erbb2(her2)	0.27	0.41	0.55	0.60	2.02	1.44	0.24	0.08
F3	0.44	0.78	1.1	0.51	2.48	-1.53	0.51	0.00
Fgf1	0.3	0.40	0.76	1.16	2.56	2.90	0.87	0.19
Fgf2	0.06	-	ND	-	ND	-	ND	-
Fgfr3	0.63	0.70	1.35	0.88	2.15	1.25	0.41	0.12
Fibrillarin	ND	0.61	ND	1.06	ND	1.73	ND	0.04
Fn1	0.48	0.46	1.13	0.57	2.34	1.23	0.3	0.04
Hif1a	0.52	0.55	0.97	0.90	1.88	1.63	0.36	0.18
Hif2a	ND	0.39	ND	0.64	ND	1.64	ND	0.14
Hnrnpm	0.74	0.68	1.84	1.42	2.48	2.08	0.5	0.01
Hpse	0.66	0.61	2.75	1.26	4.17	2.07	0.79	0.16
Id1	0.47	ND	0.9	0.87	1.93	ND	0.38	ND

Ifna1	0.34	ND	1.35	1.91	3.93	ND	1.38	ND
Igf1	0.22	0.40	0.44	0.79	2.03	1.97	0.54	0.13
Igf1r	1.05	0.79	1.97	1.49	1.88	1.88	0.06	0.06
Itgav	0.48	0.61	0.86	0.75	1.79	1.22	0.31	0.01
Itgb3	0.48	0.56	1.18	0.52	2.44	-1.07	0.4	0.09
Jag1	0.17	ND	0.35	ND	2.03	ND	0.38	ND
Mdk	0.47	ND	0.96	0.66	2.03	ND	0.35	ND
Mmp14	0.44	0.79	0.82	0.90	1.85	1.13	0.18	0.13
Mmp2	0.27	0.24	0.52	1.26	1.91	5.14	0.44	2.48
Neat-1	0.22	0.43	0.88	4.21	3.98	9.90	1.24	0.03
Nos3	1.08	0.20	0.84	2.35	-1.30	11.86	0.1	4.49
Nrp1	0.54	0.48	0.9	0.35	1.67	-1.37	0.22	0.00
Nrp2	0.51	0.60	0.96	0.78	1.88	1.29	0.18	0.09
P54nrb	0.57	0.73	1.77	1.32	3.12	1.81	0.78	0.09
Pai-1	4.77	2.31	9.15	5.12	1.92	2.22	0.27	0.27
Pdgfa	0.41	0.59	0.99	1.33	2.42	2.24	0.48	0.06
Pecam1	0.55	0.93	1.18	0.95	2.15	1.03	1.37	0.15
Pf4	0.44	0.54	0.86	0.47	1.97	-1.15	1.07	0.03
Pgf	0.65	0.20	1.53	3.70	2.36	18.38	0.83	2.53
PLAU(upa)	0.31	0.36	0.68	0.73	2.16	2.03	0.5	0.59
Plg	0.67	ND	0.17	ND	-3.85	ND	0.77	ND
Prox1	0.33	0.51	0.72	1.52	2.22	2.95	0.27	0.51
Psf/sfpq	0.44	0.65	1.21	0.75	2.74	1.15	0.8	0.02
Pspc1	0.36	0.47	0.8	0.79	2.19	1.68	0.43	0.03
Serpinf1	0.31	0.12	1.79	0.89	5.7	7.61	2.05	0.95
Sphk1	0.58	0.63	0.45	2.32	-1.30	3.65	0.12	1.09
Tek	0.22	0.44	0.54	1.15	2.43	2.62	0.95	0.24
Tgfa	0.85	0.43	2.09	1.03	2.45	2.40	0.31	1.00
Tgfb1	0.29	0.52	0.74	0.57	2.52	1.08	0.31	0.03
Tgfb2	0.3	0.63	0.72	0.52	2.39	-1.22	0.39	0.04
Tgfbr1	0.44	0.52	0.81	0.51	1.86	-1.02	0.57	0.02
Thbs1	0.31	0.48	0.62	0.38	2.03	-1.27	0.3	0.23
Thbs2	0.35	0.47	0.7	0.67	1.98	1.43	0.28	0.01
Timp1	0.27	0.46	0.63	0.43	2.36	-1.07	0.34	0.15
Timp2	0.27	0.52	0.69	0.90	2.55	1.73	0.42	0.04
Timp3	0.22	0.57	0.44	0.77	1.95	1.35	0.31	0.02
Vash1	-	0.33	-	2.28	-	6.86	-	4.27
Vegfa	1.82	2.09	5.38	2.28	2.95	1.09	0.7	0.04
Vegfb	2.94	0.59	8.39	1.40	2.85	2.37	0.43	0.07
Vegfc	1.05	-	ND	-	ND	-	ND	-
Vegfd	0.23	0.48	0.56	0.85	2.44	1.78	0.96	0.23
Vegfr2 (kdr)	0.25	0.37	0.58	0.57	2.29	1.55	0.55	0.15

Hypoxia: 24 h

Gene name	Total mRNA	Polysome bound mRNA	Fold change	Standard deviation
Akt1	1.80	5.69	3.17	0.22
Ang	1.88	2.38	1.26	0.15
Angpt1	1.17	2.62	2.24	0.08
Angptl4	ND	ND	ND	ND
Anpep	4.68	ND	ND	ND
Apelin	31.49	ND	ND	ND
Aplnnr	ND	ND	ND	ND
Atp2a2	3.34	2.73	-1.22	0.12
Bai1	1.39	ND	ND	ND
Ccl2	ND	ND	ND	ND
Ccl21a	ND	0.83	ND	ND
Col18a1	4.29	12.60	2.94	0.27
Col4a3	5.14	9.53	1.85	0.21
Ctgf	1.83	2.75	1.50	0.07
Cxcl1	1.09	1.04	-1.06	0.23
Cxcl10 (inp10)	10.42	ND	ND	ND
Cyr 61	4.57	5.52	1.21	0.12
Edn1	2.40	2.13	-1.12	0.04
Efna1	4.02	2.79	-1.44	0.04
Efnb2	2.86	3.03	1.06	0.07
Egf	2.33	ND	ND	ND
Eng	4.50	ND	D	ND
Ephb4	2.67	3.37	1.26	0.01
Erbb2(her2)	2.26	4.96	2.20	0.34
F3	3.72	5.21	1.40	0.06
Fgf1	1.00	ND	ND	ND
Fgf2	-	-	-	-
Fgfr3	5.01	9.20	1.84	0.35
Fibrillarin	2.13	2.49	1.17	0.22
Fn1	ND	ND	ND	ND
Hif1a	1.24	2.75	2.21	0.21
Hif2a	1.56	ND	ND	ND
Hnrnpm	1.25	7.91	6.34	0.76
Hpse	17.68	7.70	-2.30	0.07
Id1	1.75	ND	ND	ND
Ifna1	ND	ND	ND	ND
Igf1	1.73	1.42	-1.22	0.07
Igf1r	4.65	4.94	1.06	0.00
Itgav	5.01	7.29	1.46	0.17
Itgb3	1.99	15.07	7.56	0.49

Jag1	ND	ND	ND	ND
Mdk	2.08	ND	ND	ND
Mmp14	3.04	4.42	1.45	0.17
Mmp2	2.88	ND	ND	ND
Neat-1	3.20	3.44	1.08	0.17
Nos3	ND	ND	ND	ND
Nrp1	3.04	2.84	-1.07	0.12
Nrp2	4.42	5.27	1.19	0.07
P54nrb	2.55	7.61	2.99	0.08
Pai-1	18.19	ND	ND	ND
Pdgfa	2.90	10.05	3.46	0.34
Pecam1	11.37	ND	ND	ND
Pf4	1.16	ND	ND	ND
Pgf	6.82	ND	ND	ND
PLAU(upa)	4.42	2.28	-1.94	0.00
Plg	ND	ND	ND	ND
Prox1	1.10	3.35	3.04	0.28
Psf/sfpq	0.86	4.59	5.32	0.05
Pspc1	1.23	1.73	ND	ND
Serpinf1	15.49	ND	ND	ND
Sphk1	6.11	ND	ND	ND
Tek	4.07	5.49	1.35	0.16
Tgfa	4.75	ND	ND	ND
Tgfb1	3.41	5.67	1.66	0.14
Tgfb2	4.72	1.80	-2.63	0.06
Tgfbr1	2.08	2.37	1.14	0.23
Thbs1	5.35	4.11	-1.30	0.10
Thbs2	3.77	3.29	-1.15	0.01
Timp1	2.75	2.05	-1.34	0.12
Timp2	2.12	1.94	-1.09	0.14
Timp3	5.20	ND	ND	ND
Vash1	7.43	ND	ND	ND
Vegfa	8.15	14.73	1.81	0.19
Vegfb	1.94	6.41	3.31	0.34
Vegfc	-	-	-	-
Vegfd	2.77	ND	ND	ND
Vegfr2 (kdr)	3.04	2.28	-1.33	0.13

EV Table 2. Translatome of (lymph)angiogenic factor genes in hypoxic HL-1 cardiomyocytes. Polysomes were purified on sucrose gradient from HL-1 cardiomyocytes either in normoxia or after 4 h or after 24 h of hypoxia at 1% O_2, as described in Materials and Methods. RNA was purified from polysome-bound and from cell lysate (before gradient loading). cDNA and PCR array was performed as in Figure 1 and in EV Table 1. Relative quantification (RQ) of gene expression in hypoxia was calculated using the $2^{-\Delta\Delta CT}$ method

(polysomal RNA/total RNA normalized to normoxia). The 4 h time of hypoxia array was repeated in two independent arrays (RQ1 and RQ2). The values presented in Figures 2 and 3 correspond to RQ1 values. For each array, gene expression analysis was performed in three replicates. Standard deviation is indicated. When the RQ value is inferior to 1, the fold change is expressed as -1/RQ. ND means “non detected”. “-“ means that the gene was not included in the array.

Hantelys et al, EV Table 3

A/ Kinetics of FGF1 IRES activity in hypoxia (30 min to 24 h)

Normoxia

LucF	A	B	C	Mean	SD
30min/ 16h	1379893	1430614	1379161	1396556	29498
1h	1125637	1523078	1518366	1389027	228115
2h	1261000	1469939	1356217	1362385	104606
4h	1154339	1436444	1532732	1374505	196654
6h	1357302	1583219	1525817	1488779	117424
8h/ 24h	1772344	1668645	1416015	1619001	183278

LucR	A	B	C	Mean	SD
30min/ 16h	7946845	8739701	8113491	8266679	418038
1h	7848356	8700328	8205407	8251364	427841
2h	7763820	8766358	8196176	8242118	502846
4h	6957128	8160444	8944535	8020702	1001046
6h	8571896	9444154	9233090	9083047	455075
8h/ 24h	8896288	8402478	7130206	8142991	911187

Hypoxia

LucF	A	B	C	Mean	SD
30min	1758060	1725309	1674165	1719178	42282
1h	1475799	1562285	1591315	1543133	60092
2h	1728494	1889719	1927154	1848456	105562
4h	1891657	1744526	2255783	1963989	263192
6h	1815024	2151709	2541526	2169420	363575
8h	2143188	2354330	2311918	2269812	111691
16h	1860159	1762647	1940120	1854309	88881
24h	1733141	1936317	2026315	1898591	150184

LucR	A	B	C	Mean	SD
30min	11000223	10866380	10294397	10720333	374893
1h	10257343	10319905	10828619	10468622	313332
2h	11652705	11702822	12633961	11996496	552629
4h	10450445	10725351	12210832	11128876	947030
6h	10117963	10586170	10958961	10554365	421400
8h	10592865	10001462	10350135	10314821	297279
16h	12250023	11839180	12615589	12234930	388424
24h	14326454	13708075	16512683	14849071	1473534

LucF/ LucR

		Biological replicates					IRES activity: LucF/LucR *100				
Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
30min	Normoxia	0.1736	0.1637	0.1700	0.1691	0.0050	16.91	0.50			
	Hypoxia	0.1598	0.1588	0.1626	0.1604	0.0020	16.04	0.20	0.040990334	*	0.95
1h	Normoxia	0.1434	0.1751	0.1850	0.1678	0.0217	16.78	2.17			
	Hypoxia	0.1439	0.1514	0.1470	0.1474	0.0038	14.74	0.38	0.10537981		0.88
2h	Normoxia	0.1624	0.1677	0.1655	0.1652	0.0026	16.52	0.26			
	Hypoxia	0.1483	0.1615	0.1525	0.1541	0.0067	15.41	0.67	0.022944683	*	0.93
4h	Normoxia	0.1659	0.1760	0.1714	0.1711	0.0051	17.11	0.51			
	Hypoxia	0.1810	0.1627	0.1847	0.1761	0.0118	17.61	1.18	0.319890477		1.03
6h	Normoxia	0.1583	0.1676	0.1653	0.1637	0.0048	16.37	0.48			
	Hypoxia	0.1794	0.2033	0.2319	0.2049	0.0263	20.49	2.63	0.046232591	*	**1.25**
8h	Normoxia	0.1992	0.1986	0.1986	0.1988	0.0004	19.88	0.04			
	Hypoxia	0.2023	0.2354	0.2234	0.2204	0.0167	22.04	1.67	0.080176714		1.11
16h	Normoxia	0.1736	0.1637	0.1700	0.1691	0.0050	16.91	0.50			
	Hypoxia	0.1518	0.1489	0.1538	0.1515	0.0025	15.15	0.25	0.007194029	**	0.90
24h	Normoxia	0.1992	0.1986	0.1986	0.1988	0.0004	19.88	0.04			
	Hypoxia	0.1210	0.1413	0.1227	0.1283	0.0112	12.83	1.12	0.004341785	***	0.65
	Normoxia mean	0.1672	0.1748	0.1760	0.1726	0.0131	17.26	1.31			

*= p<0.05
**=p<0.01
***=p<0.005

B/ FGF1 IRES

LucF

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	77 310	91 175	63 445	77 310	13865
		Hypoxia	248 088	192 876	303 300	248 088	55212
	8h	Normoxia	3 721	5 041	2 401	3 721	1320
		Hypoxia	10 760	12 896	8 624	10 760	2136
	24h	Normoxia	231 071	198 199	263 943	231 071	32872
		Hypoxia	138 279	153 534	123 025	138 279	15255
2	4h	Normoxia	52 728	58 279	56 285	55 764	2812
		Hypoxia	138 626	171 997	152 394	154 339	16770
	8h	Normoxia	72 622	72 505	73 578	72 902	589
		Hypoxia	70 529	72 640	33 059	58 743	22268
	24h	Normoxia	208 454	220 996	210 670	213 373	6694
		Hypoxia	100 770	113 450	99 785	104 668	7621
3	4h	Normoxia	59 485	53 665	53 223	55 458	3495
		Hypoxia	110 552	157 006	149 316	138 958	24899
	8h	Normoxia	121 992	112 068	132 044	122 035	9988
		Hypoxia	118 486	109 078	112 198	113 254	4792
	24h	Normoxia	226 971	199 587	262 787	229 782	31694
		Hypoxia	105 268	70 597	90 603	88 823	17404

LucR

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	604 174	507 891	700 457	604 174	96283
		Hypoxia	996 143	899 215	1 093 070	996 143	96928
	8h	Normoxia	12 580	13 628	11 532	12 580	1048
		Hypoxia	35 746	46 304	25 188	35 746	10558
	24h	Normoxia	1 010 280	1 134 589	885 971	1 010 280	124309
		Hypoxia	662 414	716 741	608 087	662 414	54327
2	4h	Normoxia	452 118	345 763	472 356	423 412	68003
		Hypoxia	714 978	915 991	757 182	796 050	105993
	8h	Normoxia	700 579	710 733	712 400	707 904	6398
		Hypoxia	631 364	634 140	633 140	632 881	1406
	24h	Normoxia	1 021 135	1 036 550	1 020 270	1025985	9160
		Hypoxia	519 324	514 720	769 988	601 344	146068
3	4h	Normoxia	495 500	529 252	547 526	524 093	26394
		Hypoxia	754 720	974 680	917 680	882 360	114154
	8h	Normoxia	912 286	860 603	855 579	876 156	31390
		Hypoxia	826 664	827 015	822 262	825 314	2649
	24h	Normoxia	956 252	896 252	789 825	880 776	84286
		Hypoxia	455 239	386 245	351 256	397 580	52910

LucF/ LucR

			Biological replicates					**AU : LucF/LucR *100**				
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
1	4h	Normoxia	0.1280	0.1795	0.0906	0.1327	0.0447	13.27	4.47			
		Hypoxia	0.2490	0.2145	0.2775	0.2470	0.0315	24.70	3.15	0.061		**1.86**
	8h	Normoxia	0.2958	0.3699	0.2082	0.2913	0.0809	29.13	8.09			
		Hypoxia	0.3010	0.2785	0.3424	0.3073	0.0324	30.73	3.24	0.415		1.05
	24h	Normoxia	0.2287	0.1747	0.2979	0.2338	0.0618	23.38	6.18			
		Hypoxia	0.2088	0.2142	0.2023	0.2084	0.0060	20.84	0.60	0.292		0.89
2	4h	Normoxia	0.1166	0.1686	0.1192	0.1348	0.0293	13.48	2.93			
		Hypoxia	0.1939	0.1878	0.2013	0.1943	0.0068	19.43	0.68	0.049	*	**1.44**
	8h	Normoxia	0.1037	0.1020	0.1033	0.1030	0.0009	10.30	0.09			
		Hypoxia	0.1117	0.1145	0.0522	0.0928	0.0352	9.28	3.52	0.335		0.90
	24h	Normoxia	0.2041	0.2132	0.2065	0.2079	0.0047	20.79	0.47			
		Hypoxia	0.1940	0.2204	0.1296	0.1813	0.0467	18.13	4.67	0.204		0.87
3	4h	Normoxia	0.1201	0.1014	0.0972	0.1062	0.0122	10.62	1.22			
		Hypoxia	0.1465	0.1611	0.1627	0.1568	0.0089	15.68	0.89	0.027	*	**1.48**
	8h	Normoxia	0.1337	0.1302	0.1543	0.1394	0.0130	13.94	1.30			
		Hypoxia	0.1433	0.1319	0.1365	0.1372	0.0058	13.72	0.58	0.406		0.98
	24h	Normoxia	0.2374	0.2227	0.3327	0.2643	0.0597	26.43	5.97			
		Hypoxia	0.2312	0.1828	0.2579	0.2240	0.0381	22.40	3.81	0.090		0.85

Final values		**AU : LucF/LucR *100**				
Time	Condition	Total mean	SD	ratio	t-test	Significance
4h	Normoxia	12.46	3.07			
	Hypoxia	19.94	4.27	**1.60**	0.003	***
8h	Normoxia	17.79	9.57			
	Hypoxia	17.91	10.10	1.01	0.954	
24h	Normoxia	23.53	4.95			
	Hypoxia	20.46	3.56	0.87	0.072	

C/ FGF2 IRES

LucF

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	89 743	98 302	91 878	93 308	4455
		Hypoxia	109 876	111 252	98 034	106 387	7267
	8h	Normoxia	146 589	149 931	129 865	142 128	10751
		Hypoxia	124 357	130 350	152 345	135 684	14737
	24h	Normoxia	116 853	120 498	124 567	120 639	3859
		Hypoxia	182 431	173 530	165 431	173 797	8503
2	4h	Normoxia	104 059	102 906	102 632	103 199	757
		Hypoxia	109 548	111 252	120 034	113 611	5627
	8h	Normoxia	84 185	90 263	75623	83 357	7355
		Hypoxia	85 227	82 565	78 265	82 019	3513
	24h	Normoxia	226 539	198 256	161 526	195 440	32598
		Hypoxia	245 261	256 154	198 782	233 399	30470
3	4h	Normoxia	80 652	78 568	70 687	76 636	5256
		Hypoxia	85 698	90 565	84 568	86 944	3187
	8h	Normoxia	80 256	77 895	79 568	79 240	1214
		Hypoxia	80 268	82 568	79 635	80 824	1543
	24h	Normoxia	178 258	170 625	168 262	172 382	5224
		Hypoxia	258 684	298 365	224 265	260 438	37081

LucR

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	89 743	98 302	91 878	93 308	4455
		Hypoxia	109 876	111 252	98 034	106 387	7267
	8h	Normoxia	146 589	149 931	129 865	142 128	10751
		Hypoxia	124 357	130 350	152 345	135 684	14737
	24h	Normoxia	116 853	120 498	124 567	120 639	3859
		Hypoxia	182 431	173 530	165 431	173 797	8503
2	4h	Normoxia	104 059	102 906	102 632	103 199	757
		Hypoxia	109 548	111 252	120 034	113 611	5627
	8h	Normoxia	84 185	90 263	75623	83 357	7355
		Hypoxia	85 227	82 565	78 265	82 019	3513
	24h	Normoxia	226 539	198 256	161 526	195 440	32598
		Hypoxia	245 261	256 154	198 782	233 399	30470
3	4h	Normoxia	80 652	78 568	70 687	76 636	5256
		Hypoxia	85 698	90 565	84 568	86 944	3187
	8h	Normoxia	80 256	77 895	79 568	79 240	1214
		Hypoxia	80 268	82 568	79 635	80 824	1543
	24h	Normoxia	178 258	170 625	168 262	172 382	5224
		Hypoxia	258 684	298 365	224 265	260 438	37081

LucF/ LucR

			Biological replicates					AU : LucF/LucR *100				
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
1	4h	Normoxia	0.1427	0.1630	0.1808	0.1621	0.0190	16.21	1.90			
		Hypoxia	0.2402	0.2215	0.2604	0.2407	0.0194	24.07	1.94	0.010	**	**1.48**
	8h	Normoxia	0.1673	0.1480	0.1651	0.1602	0.0106	16.02	1.06			
		Hypoxia	0.2445	0.2029	0.2014	0.2163	0.0244	21.63	2.44	0.021	*	**1.35**
	24h	Normoxia	0.2026	0.1685	0.2508	0.2073	0.0414	20.73	4.14			
		Hypoxia	0.2338	0.1478	0.1675	0.1831	0.0450	18.31	4.50	0.270		0.88
2	4h	Normoxia	0.1517	0.1520	0.1636	0.1558	0.0067	15.58	0.67			
		Hypoxia	0.2226	0.2436	0.2525	0.2396	0.0154	23.96	1.54	0.003	*	**1.54**
	8h	Normoxia	0.1301	0.1271	0.1154	0.1242	0.0078	12.42	0.78			
		Hypoxia	0.1439	0.1483	0.1295	0.1406	0.0098	14.06	0.98	0.010	*	**1.13**
	24h	Normoxia	0.3299	0.2926	0.2571	0.2932	0.0364	29.32	3.64			
		Hypoxia	0.1974	0.2435	0.1885	0.2098	0.0295	20.98	2.95	0.040	*	0.72
3	4h	Normoxia	0.1760	0.1973	0.1744	0.1826	0.0128	18.26	1.28			
		Hypoxia	0.2216	0.2538	0.2832	0.2529	0.0308	25.29	3.08	0.035	*	**1.39**
	8h	Normoxia	0.1677	0.1591	0.1574	0.1614	0.0055	16.14	0.55			
		Hypoxia	0.1886	0.2072	0.1965	0.1974	0.0093	19.74	0.93	0.023	*	**1.22**
	24h	Normoxia	0.3081	0.3101	0.2992	0.3058	0.0058	30.58	0.58			
		Hypoxia	0.2643	0.3480	0.2504	0.2876	0.0528	28.76	5.28	0.292		0.94

Final values		**AU : LucF/LucR *100**				
Time	Condition	Total mean	SD	ratio	t-test	Significance
4h	Normoxia	16.68	1.70			
	Hypoxia	24.44	2.08	**1.46**	0.000	****
8h	Normoxia	14.86	1 .96			
	Hypoxia	18.48	3.69	1.24	0.0009	****
24h	Normoxia	26.88	5.41			
	Hypoxia	22.68	6.03	0.84	0.047	*

D/ VEGFA IRES a

LucF

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	106 987	7 470	80 384	64 947	51523
		Hypoxia	198 708	10 543	112 982	107 411	94206
	8h	Normoxia	140 987	13 381	229 805	128 058	108790
		Hypoxia	291 799	15 874	217 091	174 921	142714
	24h	Normoxia	95 467	11 501	66 548	57 839	42655
		Hypoxia	43 998	3 278	18 093	21 790	20610
2	4h	Normoxia	80 568	75 356	78 658	78 194	2637
		Hypoxia	187 525	175 862	168 568	177 318	9562
	8h	Normoxia	100 578	96 235	97268	98 027	2269
		Hypoxia	186 568	202 525	164 570	184 554	19057
	24h	Normoxia	102 540	110 402	98 758	103 900	5940
		Hypoxia	60 568	55 845	68 025	61 479	6141
3	4h	Normoxia	68 256	60 658	58 698	62 537	5049
		Hypoxia	105 364	121 231	135 682	120 759	15165
	8h	Normoxia	230 264	214 235	221 658	222 052	8022
		Hypoxia	402 562	380 256	351 214	378 011	25748
	24h	Normoxia	125 214	123 214	110 254	119 561	8122
		Hypoxia	70 658	65 252	64 154	66 688	3482

LucR

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	557 863	620 150	654 367	610 793	48928
		Hypoxia	1 387 607	1 255 594	987 656	1 210 286	203789
	8h	Normoxia	1 098 767	1 618 781	1 579 760	1 432 436	289624
		Hypoxia	1 465 480	1 285 249	1 354 320	1 368 350	90931
	24h	Normoxia	1 246 587	1 620 876	1 674 890	1 514 118	233257
		Hypoxia	1 054 689	879 823	987 698	974 070	88226
2	4h	Normoxia	512 362	458 685	398 568	456 538	56927
		Hypoxia	985 365	987 878	865 258	946 167	70081
	8h	Normoxia	758 652	684 594	705635	716 294	38162
		Hypoxia	1 025 268	985 698	1 014 254	1 008 407	20423
	24h	Normoxia	878 985	985 635	868 457	911 026	64828
		Hypoxia	1 121 245	1 002 568	987 685	1 037 166	73194
3	4h	Normoxia	369 568	398 658	405 235	391 154	18981
		Hypoxia	875 698	870 258	865 234	870 397	5233
	8h	Normoxia	656 265	652 485	698 584	669 111	25594
		Hypoxia	878 258	852 012	878 565	869 612	15243
	24h	Normoxia	758 587	765 625	720 258	748 157	24416
		Hypoxia	975 685	985 625	945 252	968 854	21035

LucF/ LucR

			Biological replicates					AU : LucF/LucR *100				
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
1	4h	Normoxia	0.1918	0.0120	0.1228	0.1089	0.0907	10.89	9.07			
		Hypoxia	0.1432	0.0084	0.1144	0.0887	0.0710	8.87	7.10	0.146		0.81
	8h	Normoxia	0.1283	0.0083	0.1455	0.0940	0.0748	9.40	7.48			
		Hypoxia	0.1991	0.0124	0.1603	0.1239	0.0986	12.39	9.86	0.143		**1.32**
	24h	Normoxia	0.0766	0.0071	0.0397	0.0411	0.0348	4.11	3.48			
		Hypoxia	0.0417	0.0037	0.0183	0.0213	0.0192	2.13	1.92	0.081		0.52
2	4h	Normoxia	0.1572	0.1643	0.1974	0.1730	0.0214	17.30	2.14			
		Hypoxia	0.1903	0.1780	0.1948	0.1877	0.0087	18.77	0.87	0.144		1.09
	8h	Normoxia	0.1326	0.1406	0.1378	0.1370	0.0041	13.70	0.41			
		Hypoxia	0.1820	0.2055	0.1623	0.1832	0.0216	18.32	2.16	0.030	*	**1.34**
	24h	Normoxia	0.1167	0.1120	0.1137	0.1141	0.0024	11.41	0.24			
		Hypoxia	0.0540	0.0557	0.0689	0.0595	0.0081	5.95	0.81	0.004	***	0.52
3	4h	Normoxia	0.1847	0.1522	0.1448	0.1606	0.0212	16.06	2.12			
		Hypoxia	0.1203	0.1393	0.1568	0.1388	0.0183	13.88	1.83	0.218		0.86
	8h	Normoxia	0.3509	0.3283	0.3173	0.3322	0.0171	33.22	1.71			
		Hypoxia	0.4584	0.4463	0.3998	0.4348	0.0309	43.48	3.09	0.005	**	**1.31**
	24h	Normoxia	0.1651	0.1609	0.1531	0.1597	0.0061	15.97	0.61			
		Hypoxia	0.0724	0.0662	0.0679	0.0688	0.0032	6.88	0.32	0.001	****	0.43

Final values		AU : LucF/LucR *100				
Time	Condition	Total mean	SD	ratio	t-test	Significance
4h	Normoxia	14.75	5.61			
	Hypoxia	13.84	5.66	0.94	0.718	
8h	Normoxia	18.77	11.64			
	Hypoxia	24.73	15.24	**1.32**	0.001	***
24h	Normoxia	10.50	5.47			
	Hypoxia	4.99	2.42	**0.48**	0.0002	****

E/ VEGFA IRES b

LucF

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	140 874	98 149	100 854	113 292	23925
		Hypoxia	100 976	91 883	93 743	95 534	4804
	8h	Normoxia	159 873	208 754	170 983	179 870	25624
		Hypoxia	378 983	513 871	389 750	427 535	74963
	24h	Normoxia	499 864	537 099	556 098	531 020	28606
		Hypoxia	350 980	439 788	345 230	378 666	53011
2	4h	Normoxia	102 368	112 327	106 258	106 984	5019
		Hypoxia	86 358	87 652	85 365	86 458	1147
	8h	Normoxia	130 254	112 584	112 547	118 462	10212
		Hypoxia	298 654	305 265	300 245	301 388	3451
	24h	Normoxia	498 567	465 856	475 554	479 992	16801
		Hypoxia	345 268	344 568	324 265	338 034	11929
3	4h	Normoxia	104 241	124 251	114 212	114 235	10005
		Hypoxia	80 242	78 658	81 542	80 147	1444
	8h	Normoxia	124 212	110 226	102 265	112 234	11110
		Hypoxia	310 245	302 142	298 265	303 551	6113
	24h	Normoxia	487 568	452 028	435 982	458 526	26400
		Hypoxia	285 475	284 658	235 268	268 467	28754

LucR

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	1 087 650	2 019 783	1 876 580	1 661 338	501961
		Hypoxia	2 098 750	2 993 873	3 542 712	2 878 445	728868
	8h	Normoxia	2 178 659	2 269 092	2 157 890	2 201 880	59126
		Hypoxia	2 765 909	3 127 386	2 987 652	2 960 316	182282
	24h	Normoxia	3 987 600	3 794 801	3 654 780	3 812 394	167106
		Hypoxia	3 265 890	3 614 913	2 764 579	3 215 127	427434
2	4h	Normoxia	986 256	968 586	987 584	980 809	10606
		Hypoxia	1 987 584	1 876 258	1 785 625	1 883 156	101156
	8h	Normoxia	1 587 568	1 658 258	1 785 547	1 677 124	100329
		Hypoxia	2 258 586	2 457 268	2 358 625	2 358 160	99342
	24h	Normoxia	3 685 658	3 545 658	3 365 245	3 532 187	160631
		Hypoxia	2 998 586	3 058 248	3 124 025	3 060 286	62744
3	4h	Normoxia	876 548	857 625	985 562	906 578	69053
		Hypoxia	1 685 265	1 457 235	1 471 457	1 537 986	127746
	8h	Normoxia	1 325 328	1 245 258	1 475 682	1 348 756	116985
		Hypoxia	2 425 241	2 574 625	2 145 258	2 381 708	217969
	24h	Normoxia	3 258 654	3 214 651	3 254 478	3 242 594	24290
		Hypoxia	3 021 214	2 875 625	2 874 246	2 923 695	84457

LucF/ LucR

			Biological replicates					AU : LucF/LucR *100				
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
1	4h	Normoxia	0.1295	0.0486	0.0537	0.0773	0.0453	**7.73**	4.53			
		Hypoxia	0.0481	0.0307	0.0265	0.0351	0.0115	**3.51**	1.15	0.083		**0.45**
	8h	Normoxia	0.0734	0.0920	0.0792	0.0815	0.0095	**8.15**	0.95			
		Hypoxia	0.1370	0.1643	0.1305	0.1439	0.0180	**14.39**	1.80	0.005	***	**1.77**
	24h	Normoxia	0.1254	0.1415	0.1522	0.1397	0.0135	**13.97**	1.35			
		Hypoxia	0.1075	0.1217	0.1249	0.1180	0.0093	**11.80**	0.93	0.008	**	**0.84**
2	4h	Normoxia	0.1038	0.1160	0.1076	0.1091	0.0062	**10.91**	0.62			
		Hypoxia	0.0434	0.0467	0.0478	0.0460	0.0023	**4.60**	0.23	0.001	***	**0.42**
	8h	Normoxia	0.0820	0.0679	0.0630	0.0710	0.0099	**7.10**	0.99			
		Hypoxia	0.1322	0.1242	0.1273	0.1279	0.0040	**12.79**	0.40	0.003	***	**1.80**
	24h	Normoxia	0.1353	0.1314	0.1413	0.1360	0.0050	**13.60**	0.50			
		Hypoxia	0.1151	0.1127	0.1038	0.1105	0.0060	**11.05**	0.60	0.026	*	**0.81**
3	4h	Normoxia	0.1189	0.1449	0.1159	0.1266	0.0159	**12.66**	1.59			
		Hypoxia	0.0476	0.0540	0.0554	0.0523	0.0042	**5.23**	0.42	0.007	**	**0.41**
	8h	Normoxia	0.0937	0.0885	0.0693	0.0838	0.0129	**8.38**	1.29			
		Hypoxia	0.1279	0.1174	0.1390	0.1281	0.0108	**12.81**	1.08	0.037	*	**1.53**
	24h	Normoxia	0.1496	0.1406	0.1340	0.1414	0.0079	14.14	0.79			
		Hypoxia	0.0945	0.0990	0.0819	0.0918	0.0089	9.18	0.89	0.003	***	**0.65**

Final values		**AU : LucF/LucR *100**				
Time	Condition	Total mean	SD	ratio	t-test	Significance
4h	Normoxia	10.43	3.25			
	Hypoxia	4.45	0.98	**0.43**	0.0000	****
8h	Normoxia	7.88	1.11			
	Hypoxia	13.33	1.33	**1.69**	0.0000	****
24h	Normoxia	13.90	0.85			
	Hypoxia	10.68	1.37	**0.77**	0.0002	****

F/ VEGFC IRES

LucF

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	17 645	16 237	15 672	16 518	1016
		Hypoxia	21 673	18 377	17 834	19 295	2078
	8h	Normoxia	21 673	18 377	17 834	19 295	2078
		Hypoxia	27 742	18 374	19 875	21 997	5032
	24h	Normoxia	23 451	21 422	22 765	22 546	1032
		Hypoxia	30 194	22 544	26 876	26 538	3836
2	4h	Normoxia	15 245	14 582	13 258	14 362	1012
		Hypoxia	19 875	18 625	17 485	18 662	1195
	8h	Normoxia	20 235	20 146	19 826	20 069	215
		Hypoxia	26 587	21 457	20 358	22 801	3325
	24h	Normoxia	21 568	20 148	18 759	20 158	1405
		Hypoxia	28 568	27 532	26 352	27 484	1109
3	4h	Normoxia	18 750	16 985	19 754	18 496	1402
		Hypoxia	17 258	16 238	14 587	16 028	1348
	8h	Normoxia	16 987	17 258	16 784	17 010	238
		Hypoxia	30 268	31 245	30 216	30 576	580
	24h	Normoxia	19 867	20 235	28 220	22 774	4720
		Hypoxia	19 287	24 568	22 586	22 147	2668

LucR

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	287 654	316 145	365 786	323 195	39540
		Hypoxia	302 234	354 485	345 290	334 003	27894
	8h	Normoxia	425 763	455 482	480 013	453 753	27166
		Hypoxia	403 294	341 909	375 634	373 612	30742
	24h	Normoxia	420 632	391 071	375 100	395 601	23102
		Hypoxia	452 093	342 612	410 973	401 893	55302
2	4h	Normoxia	195 685	185 247	182 457	187 796	6973
		Hypoxia	298 538	290 265	284 242	291 015	7177
	8h	Normoxia	390 584	382 546	367 856	380 329	11525
		Hypoxia	390 268	352 632	342 268	361 723	25258
	24h	Normoxia	410 214	402 158	402 387	404 920	4586
		Hypoxia	402 358	412 586	398 574	404 506	7249
3	4h	Normoxia	205 236	204 325	210 214	206 592	3170
		Hypoxia	280 142	270 268	262 874	271 095	8664
	8h	Normoxia	356 258	321 252	384 276	353 929	31577
		Hypoxia	410 226	401 236	410 256	407 239	5199
	24h	Normoxia	398 268	396 216	381 568	392 017	9107
		Hypoxia	398 265	396 246	362 142	385 551	20298

LucF/ LucR

			Biological replicates					AU : LucF/LucR *100				
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
1	4h	Normoxia	0.0613	0.0514	0.0428	0.0518	0.0093	**5.18**	0.93			
		Hypoxia	0.0717	0.0518	0.0516	0.0584	0.0115	**5.84**	1.15	0.083		1.13
	8h	Normoxia	0.0509	0.0403	0.0372	0.0428	0.0072	**4.28**	0.72			
		Hypoxia	0.0688	0.0537	0.0529	0.0585	0.0089	**5.85**	0.89	0.003	***	**1.37**
	24h	Normoxia	0.0558	0.0548	0.0607	0.0571	0.0032	**5.71**	0.32			
		Hypoxia	0.0668	0.0658	0.0654	0.0660	0.0007	**6.60**	0.07	0.026	*	1.16
2	4h	Normoxia	0.0779	0.0787	0.0727	0.0764	0.0033	**7.64**	0.33			
		Hypoxia	0.0666	0.0642	0.0615	0.0641	0.0025	**6.41**	0.25	0.004	***	0.84
	8h	Normoxia	0.0518	0.0527	0.0539	0.0528	0.0011	**5.28**	0.11			
		Hypoxia	0.0681	0.0608	0.0595	0.0628	0.0046	**6.28**	0.46	0.045	*	**1.19**
	24h	Normoxia	0.0526	0.0501	0.0466	0.0498	0.0030	**4.98**	0.30			
		Hypoxia	0.0710	0.0667	0.0661	0.0679	0.0027	**6.79**	0.27	0.001	***	1.37
3	4h	Normoxia	0.0914	0.0831	0.0940	0.0895	0.0057	**8.95**	0.57			
		Hypoxia	0.0616	0.0601	0.0555	0.0591	0.0032	**5.91**	0.32	0.010	*	0.66
	8h	Normoxia	0.0477	0.0537	0.0437	0.0484	0.0051	**4.84**	0.51			
		Hypoxia	0.0738	0.0779	0.0737	0.0751	0.0024	**7.51**	0.24	0.002	***	**1.55**
	24h	Normoxia	0.0499	0.0511	0.0740	0.0583	0.0136	**5.83**	1.36			
		Hypoxia	0.0484	0.0620	0.0624	0.0576	0.0079	**5.76**	0.79	0.462		0.99

Final values		**AU : LucF/LucR *100**				
Time	Condition	Total mean	SD	ratio	t-test	Significance
4h	Normoxia	7.26	1.75			
	Hypoxia	6.05	0.67	0.83	0.062	
8h	Normoxia	4.80	0.62			
	Hypoxia	6.55	0.91	**1.36**	0.0002	****
24h	Normoxia	5.50	0.82			
	Hypoxia	6.38	0.64	**1.16**	0.031	*

G/ VEGFD IRES

LucF

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	1 123 453	1 092 536	1 242 342	1 152 777	79091
		Hypoxia	1 087 648	1 129 996	1 250 434	1 156 026	84457
	8h	Normoxia	899 766	837 544	657 889	798 400	125600
		Hypoxia	1 141 352	977 778	855 973	991 701	143198
	24h	Normoxia	1 187 460	1 382 536	1 289 532	1 286 509	97573
		Hypoxia	521 596	706 269	506 468	578 111	111246
2	4h	Normoxia	125 682	123 122	120 268	123 024	2708
		Hypoxia	110 236	112 025	99 856	107 372	6571
	8h	Normoxia	89 526	75 862	78 588	81 325	7232
		Hypoxia	112 252	85 211	92 252	96 572	14028
	24h	Normoxia	147 214	132 250	145 258	141 574	8134
		Hypoxia	50 231	48 215	47 528	48 658	1405
3	4h	Normoxia	1 252 025	985 986	1 025 254	1 087 755	143610
		Hypoxia	1 452 638	1 258 326	1 425 325	1 378 763	105192
	8h	Normoxia	1 418 588	1 325 682	1 312 022	1 352 097	57986
		Hypoxia	1 912 212	1 858 562	1 757 552	1 842 775	78529
	24h	Normoxia	1 547 252	1 457 258	1 325 206	1 443 239	111685
		Hypoxia	485 625	465 258	475 528	475 470	10184

LucR

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	11 987 690	12 069 833	12 069 833	12 042 452	47425
		Hypoxia	10 657 845	12 059 630	11 656 849	11 458 108	721716
	8h	Normoxia	13 799 872	14 349 216	12 764 891	13 637 993	804472
		Hypoxia	11 678 964	10 969 410	12 345 654	11 664 676	688233
	24h	Normoxia	14 908 768	16 101 138	15 779 780	15 596 562	616939
		Hypoxia	8 765 789	9 428 743	9 076 890	9 090 474	331686
2	4h	Normoxia	1 265 226	1 125 268	1 125 363	1 171 952	80777
		Hypoxia	1 025 236	989 265	1 125 236	1 046 579	70453
	8h	Normoxia	1 325 662	1 685 236	1 256 364	1 422 421	230227
		Hypoxia	1 152 635	1 232 568	1 152 332	1 179 178	46237
	24h	Normoxia	1 658 235	1 751 820	1 256 250	1 555 435	263293
		Hypoxia	895 652	952 325	875 252	907 743	39934
3	4h	Normoxia	10 252 682	9 253 258	11 220 214	10 242 051	983521
		Hypoxia	11 212 241	13 258 233	12 258 225	12 242 900	1023082
	8h	Normoxia	12 452 120	11 572 582	11 457 582	11 827 428	544046
		Hypoxia	10 214 785	9 865 236	11 525 852	10 535 291	875474
	24h	Normoxia	10 475 685	11 452 232	10 254 362	10 727 426	637380
		Hypoxia	9 258 352	8 562 265	8 956 825	8 925 814	349078

LucF/ LucR

			Biological replicates					AU : LucF/LucR *100				
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
1	4h	Normoxia	0.0937	0.0905	0.1029	0.0957	0.0064	**9.57**	0.64			
		Hypoxia	0.1021	0.0937	0.1073	0.1010	0.0068	**10.10**	0.68	0.039	*	1.06
	8h	Normoxia	0.0652	0.0584	0.0515	0.0584	0.0068	**5.84**	0.68			
		Hypoxia	0.0977	0.0891	0.0693	0.0854	0.0146	**8.54**	1.46	0.014	*	**1.46**
	24h	Normoxia	0.0796	0.0859	0.0817	0.0824	0.0032	**8.24**	0.32			
		Hypoxia	0.0595	0.0749	0.0558	0.0634	0.0101	**6.34**	1.01	0.024	*	**0.77**
2	4h	Normoxia	0.0993	0.1094	0.1069	0.1052	0.0052	**10.52**	0.52			
		Hypoxia	0.1075	0.1132	0.0887	0.1032	0.0128	**10.32**	1.28	0.413		0.98
	8h	Normoxia	0.0675	0.0450	0.0626	0.0584	0.0118	**5.84**	1.18			
		Hypoxia	0.0974	0.0691	0.0801	0.0822	0.0142	**8.22**	1.42	0.011	*	**1.41**
	24h	Normoxia	0.0888	0.0755	0.1156	0.0933	0.0204	**9.33**	2.04			
		Hypoxia	0.0561	0.0506	0.0543	0.0537	0.0028	**5.37**	0.28	0.035	*	**0.58**
3	4h	Normoxia	0.1221	0.1066	0.0914	0.1067	0.0154	**10.67**	1.54			
		Hypoxia	0.1296	0.0949	0.1163	0.1136	0.0175	**11.36**	1.75	0.290		1.06
	8h	Normoxia	0.1139	0.1146	0.1145	0.1143	0.0004	**11.43**	0.04			
		Hypoxia	0.1872	0.1884	0.1525	0.1760	0.0204	**17.60**	2.04	0.018	*	**1.54**
	24h	Normoxia	0.1477	0.1272	0.1292	0.1347	0.0113	**13.47**	1.13			
		Hypoxia	0.0525	0.0543	0.0531	0.0533	0.0010	**5.33**	0.10	0.004	***	**0.40**

Final values		**AU : LucF/LucR *100**				
Time	Condition	Total mean	SD	ratio	t-test	Significance
4h	Normoxia	10.25	1.01			
	Hypoxia	10.59	1.28	**1.03**	0.435	
8h	Normoxia	7.70	2.88			
	Hypoxia	11.45	4.83	**1.49**	0.0008	****
24h	Normoxia	10.35	2.67			
	Hypoxia	5.68	0.72	**0.55**	0.002	***

H/ c-myc IRES

LucF

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	2 567 872	3 651 734	3 454 332	3 224 646	577283
		Hypoxia	2 029 809	3 147 437	3 256 210	2 811 152	678845
	8h	Normoxia	3 546 872	3 161 720	3 478 284	3 395 625	205450
		Hypoxia	3 508 730	2 749 546	2 876 921	3 045 066	406564
	24h	Normoxia	7 768 312	8 684 097	6 987 312	7 813 240	849284
		Hypoxia	7 513 771	5 782 962	7 981 903	7 092 879	1158317
2	4h	Normoxia	356 812	375 210	351 278	361 100	12529
		Hypoxia	275 895	265 826	245 628	262 450	15413
	8h	Normoxia	368 258	450 268	452 025	423 517	47864
		Hypoxia	325 120	301 245	298 547	308 304	14625
	24h	Normoxia	358 582	258 625	247 583	288 263	61148
		Hypoxia	486 250	460 258	410 258	452 255	38623
3	4h	Normoxia	360 212	410 251	420 215	396 893	32155
		Hypoxia	310 215	325 230	312 020	315 822	8198
	8h	Normoxia	415 231	398 652	350 652	388 178	33539
		Hypoxia	350 655	316 522	310 222	325 800	21755
	24h	Normoxia	362 025	352 014	342 062	352 034	9982
		Hypoxia	658 250	568 260	487 210	571 240	85559

LucR

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	29 784 420	27 508 124	26 098 324	27 796 956	1859945
		Hypoxia	20 988 822	19 862 926	21 341 228	20 730 992	772141
	8h	Normoxia	24 900 832	29 630 539	30 983 221	28 504 864	3193622
		Hypoxia	23 987 042	24 750 406	25 987 577	24 908 342	1009576
	24h	Normoxia	36 987 908	35 408 692	23 788 902	32 061 834	7207949
		Hypoxia	18 898 912	13 608 479	16 988 904	16 498 765	2679057
2	4h	Normoxia	3 025 325	2 758 632	2 658 985	2 814 314	189411
		Hypoxia	2 025 128	2 212 570	2 325 201	2 187 633	151583
	8h	Normoxia	2 856 985	2 140 236	2 562 120	2 519 780	360245
		Hypoxia	2 653 213	2 012 251	2 120 124	2 261 863	343184
	24h	Normoxia	3 856 658	4 021 522	4 010 698	3 962 959	92219
		Hypoxia	4 582 632	4 658 230	4 215 120	4 485 327	237040
3	4h	Normoxia	2 879 258	2 586 258	2 586 258	2 683 925	169164
		Hypoxia	2 145 251	2 014 214	2 147 542	2 102 336	76324
	8h	Normoxia	3 125 368	2 582 321	2 836 124	2 847 938	271716
		Hypoxia	2 785 624	2 658 325	2 625 258	2 689 736	84672
	24h	Normoxia	4 025 215	4 085 368	4 125 214	4 078 599	50342
		Hypoxia	4 658 284	4 075 250	4 875 251	4 536 262	413724

LucF/ LucR

			Biological replicates					AU : LucF/LucR *100				
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
1	4h	Normoxia	0.0862	0.1328	0.1324	0.1171	0.0268	**11.71**	2.68	0.026	*	1.16
		Hypoxia	0.0967	0.1585	0.1526	0.1359	0.0341	**13.59**	3.41			
	8h	Normoxia	0.1424	0.1067	0.1123	0.1205	0.0192	**12.05**	1.92	0.181		1.02
		Hypoxia	0.1463	0.1111	0.1107	0.1227	0.0204	**12.27**	2.04			
	24h	Normoxia	0.2100	0.2453	0.2937	0.2497	0.0420	**24.97**	4.20	0.0002	****	**1.73**
		Hypoxia	0.3976	0.4250	0.4698	0.4308	0.0365	**43.08**	3.65			
2	4h	Normoxia	0.1179	0.1360	0.1321	0.1287	0.0095	**12.87**	0.95	0.307		0.94
		Hypoxia	0.1362	0.1201	0.1056	0.1207	0.0153	**12.07**	1.53			
	8h	Normoxia	0.1289	0.2104	0.1764	0.1719	0.0409	**17.19**	4.09	0.081		0.80
		Hypoxia	0.1225	0.1497	0.1408	0.1377	0.0139	**13.77**	1.39			
	24h	Normoxia	0.0930	0.0643	0.0617	0.0730	0.0173	**7.30**	1.73	0.031	*	**1.38**
		Hypoxia	0.1061	0.0988	0.0973	0.1007	0.0047	**10.07**	0.47			
3	4h	Normoxia	0.1251	0.1586	0.1625	0.1487	0.0206	**14.87**	2.06	0.443		1.01
		Hypoxia	0.1446	0.1615	0.1453	0.1505	0.0095	**15.05**	0.95			
	8h	Normoxia	0.1329	0.1544	0.1236	0.1370	0.0158	**13.70**	1.58	0.121		0.88
		Hypoxia	0.1259	0.1191	0.1182	0.1210	0.0042	**12.10**	0.42			
	24h	Normoxia	0.0899	0.0862	0.0829	0.0863	0.0035	8.63	0.35	0.038	*	**1.47**
		Hypoxia	0.1413	0.1394	0.0999	0.1269	0.0234	12.69	2.34			

Final values		**AU : LucF/LucR *100**				
Time	Condition	Total mean	SD	ratio	t-test	Significance
4h	Normoxia	13.15	2.23			
	Hypoxia	13.57	2.32	1.03	0.536	
8h	Normoxia	14.31	3.30			
	Hypoxia	12.71	1.48	0.89	0.066	
24h	Normoxia	13.63	8.82			
	Hypoxia	21.95	16.04	**1.61**	0.010	*

I/ EMCV IRES

LucF

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	1 698 754	1 842 473	1 523 420	1 688 216	159787
		Hypoxia	1 768 791	1 848 197	1 212 320	1 609 769	346483
	8h	Normoxia	2 453 998	2 217 362	2 178 642	2 283 334	149062
		Hypoxia	1 098 422	1 388 910	1 053 523	1 180 285	182064
	24h	Normoxia	2 987 530	3 331 188	3 097 981	3 138 900	175445
		Hypoxia	3 208 903	3 551 010	3 254 552	3 338 155	185746
2	4h	Normoxia	1 852 625	1 798 265	1 658 985	1 769 958	99875
		Hypoxia	1 658 986	1 725 542	1 875 258	1 753 262	110769
	8h	Normoxia	2 547 240	2 580 264	1 985 520	2 371 008	334250
		Hypoxia	1 658 250	1 752 220	1 425 632	1 612 034	168128
	24h	Normoxia	2 658 568	2 784 240	2 645 280	2 696 029	76681
		Hypoxia	2 985 568	3 025 106	3 685 210	3 231 961	393022
3	4h	Normoxia	1 985 625	1 895 032	1 758 320	1 879 659	114430
		Hypoxia	1 254 214	1 158 220	1 258 210	1 223 548	56611
	8h	Normoxia	2 652 124	2 758 210	2 650 210	2 686 848	61809
		Hypoxia	1 452 025	1 252 620	1 158 203	1 287 616	150005
	24h	Normoxia	2 875 620	2 750 215	2 458 982	2 694 939	213748
		Hypoxia	3 025 240	2 920 213	3 857 920	3 267 791	513758

LucR

Experiment	Time	Condition	A	B	C	Mean	SD
1	4h	Normoxia	1 897 642	1 739 558	1 676 425	1 771 208	113954
		Hypoxia	1 497 245	1 416 380	1 123 235	1 345 620	196789
	8h	Normoxia	2 453 210	2 270 622	1 987 430	2 237 087	234694
		Hypoxia	1 345 232	1 423 859	1 125 412	1 298 168	154690
	24h	Normoxia	2 234 123	2 586 791	3 142 423	2 654 446	457914
		Hypoxia	2 076 764	1 593 918	2 090 845	1 920 509	282924
2	4h	Normoxia	1 325 225	1 250 265	1 325 052	1 300 181	43228
		Hypoxia	998 565	865 258	1 125 580	996 468	130174
	8h	Normoxia	2 025 872	2 125 672	2 110 253	2 087 266	53725
		Hypoxia	1 257 253	1 520 252	1 427 856	1 401 787	133423
	24h	Normoxia	1 987 562	2 358 268	2 145 214	2 163 681	186042
		Hypoxia	2 125 147	2 235 126	1 989 987	2 116 753	122785
3	4h	Normoxia	2 015 210	1 989 570	1 997 212	2 000 664	13164
		Hypoxia	1 328 620	1 285 210	1 275 487	1 296 439	28290
	8h	Normoxia	2 251 012	2 325 026	2 452 012	2 342 683	101657
		Hypoxia	1 352 210	1 245 253	1 124 210	1 240 558	114072
	24h	Normoxia	2 454 210	2 325 620	2 332 210	2 370 680	72414
		Hypoxia	2 452 012	2 145 217	2 052 982	2 216 737	208908

LucF/ LucR

			Biological replicates					AU : LucF/LucR *100				
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance	H/N
1	4h	Normoxia	0.8952	1.0592	0.9087	0.9544	0.0910	**95.44**	9.10			
		Hypoxia	1.1814	1.3049	1.0793	1.1885	0.1130	**118.85**	11.30	0.010	*	**1.25**
	8h	Normoxia	1.0003	0.9765	1.0962	1.0244	0.0634	**102.44**	6.34			
		Hypoxia	0.8165	0.9755	0.9361	0.9094	0.0828	**90.94**	8.28	0.091		0.89
	24h	Normoxia	1.3372	1.2878	0.9859	1.2036	0.1902	**120.36**	19.02			
		Hypoxia	1.5451	2.2278	1.5566	1.7765	0.3909	**177.65**	39.09	0.057	*	**1.48**
2	4h	Normoxia	1.3980	1.4383	1.2520	1.3628	0.0980	**136.28**	9.80			
		Hypoxia	1.6614	1.9943	1.6660	1.7739	0.1909	**177.39**	19.09	0.020	*	**1.30**
	8h	Normoxia	1.2574	1.2139	0.9409	1.1374	0.1715	**113.74**	17.15			
		Hypoxia	1.3189	1.1526	0.9984	1.1567	0.1603	**115.67**	16.03	0.340		1.02
	24h	Normoxia	1.3376	1.1806	1.2331	1.2504	0.0799	**125.04**	7.99			
		Hypoxia	1.4049	1.3534	1.8519	1.5367	0.2741	**153.67**	27.41	0.116		**1.23**
3	4h	Normoxia	0.9853	0.9525	0.8804	0.9394	0.0537	**93.94**	5.37			
		Hypoxia	0.9440	0.9012	0.9865	0.9439	0.0426	**94.39**	4.26	0.469		1.00
	8h	Normoxia	1.1782	1.1863	1.0808	1.1484	0.0587	**114.84**	5.87			
		Hypoxia	1.0738	1.0059	1.0302	1.0367	0.0344	**103.67**	3.44	0.049	*	0.90
	24h	Normoxia	1.1717	1.1826	1.0544	1.1362	0.0711	113.62	7.11			
		Hypoxia	1.2338	1.3613	1.8792	1.4914	0.3418	149.14	34.18	0.136		**1.31**

Final values		**AU : LucF/LucR *100**				
Time	Condition	Total mean	SD	ratio	t-test	Significance
4h	Normoxia	108.55	22.02			
	Hypoxia	130.21	38.62	**1.20**	0.011	*
8h	Normoxia	110.34	11.29			
	Hypoxia	103.42	14.11	0.94	0.062	
24h	Normoxia	119.68	11.99			
	Hypoxia	160.16	32.22	**1.34**	0.007	**

J/ Control without IRES (hairpin)

LucF

			Biological replicates				
Experiment	Time	Condition	A	B	C	Mean	SD
1	8h	Normoxia	2082	2490	1901	2158	302
		Hypoxia	1937	3592	2125	2552	906
	24h	Normoxia	2262	1232	1692	1729	516
		Hypoxia	2063	3082	4574	3240	1263
2	8h	Normoxia	4203	4273	3176	3884	614
		Hypoxia	5664	1600	4811	4025	2143
3	8h	Normoxia	5188	5900	6041	5710	457
		Hypoxia	3270	4499	ND	3884	2015

LucR

			Biological replicates				
Experiment	Time	Condition	A	B	C	Mean	SD
1	8h	Normoxia	91411	99067	79850	90109	9674
		Hypoxia	98850	86360	51747	78986	24402
	24h	Normoxia	95815	50314	72697	72942	22752
		Hypoxia	49263	68572	104606	74147	28090
2	8h	Normoxia	155803	142912	127454	142056	14194
		Hypoxia	100895	73120	120118	98044	23628
3	8h	Normoxia	163996	161011	198604	174537	20896
		Hypoxia	69871	75614	ND	72743	41668

LucF/ LucR

			Biological replicates					AU: LucF/LuR *100			
Experiment	Time	Condition	A	B	C	Mean	SD	Mean	SD	t-test	Significance
1	8h	Normoxia	0.0228	0.0251	0.0238	0.0239	0.0012	2.39	0.12		
		Hypoxia	0.0236	0.0245	0.0233	0.0238	0.0006	2.38	0.06	0.833	
	24h	Normoxia	0.0196	0.0416	0.0411	0.0341	0.0126	3.41	1.26		
		Hypoxia	0.0419	0.0449	0.0437	0.0435	0.0015	4.35	0.15	0.280	
2	8h	Normoxia	0.0270	0.0299	0.0249	0.0273	0.0025	2.73	0.25		
		Hypoxia	0.0561	0.0219	0.0400	0.0394	0.0171	3.94	1.71	0.381	
3	8h	Normoxia	0.0316	0.0366	0.0304	0.0329	0.0033	3.29	0.33		
		Hypoxia	0.0468	0.0595	ND	0.0531	0.0090	5.31	0.90	0.127	

EV Table 3. IRES activities at different times of hypoxia in HL-1 cells.

Luciferase activity values and IRES activities corresponding to the experiments presented Figure 4.

A/ Kinetics of FGF1 IRES activity from 30 min to 24 h

B-I/ Activities of the different IRES at 4 h, 8 h and 24 h of hypoxia

J/ Negative control with a lentivector containing a hairpin (no IRES) between the two luciferase cistrons.

Biological replicates are indicated as A, B and C, whereas independent experiments are indicated as 1, 2, 3. Means, standard deviation (SD) and t-test of IRES activities were calculated. The panels “final values” correspond to means of all experiments (nine values) which are reported in the histograms of Figure 4. P-value significance is indicated: *p<0.05, **p<0.01, ***<0.001, ****p<0.0001.

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A. Proteins bound to FGF1 IRES

Normoxia (0 h)

Symbol	Full name	Score	Spectres	Peptides
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	29	2	1
KCTD9_MOUSE	BTB/POZ domain-containing protein KCTD9 OS=Mus musculus GN=Kctd9 PE=2 SV=1	23	1	1
EMAL6_MOUSE	Echinoderm microtubule-associated protein-like 6 OS=Mus musculus GN=Eml6 PE=2 SV=1	35	1	1
EF1A1_MOUSE	Elongation factor 1-alpha 1 OS=Mus musculus GN=Eef1a1 PE=1 SV=3	53	1	1
H11_MOUSE	Histone H1.1 OS=Mus musculus GN=Hist1h1a PE=1 SV=2	28	1	1
GPR19_MOUSE	Probable G-protein coupled receptor 19 OS=Mus musculus GN=Gpr19 PE=2 SV=2	22	1	1
GUF1_MOUSE	Translation factor Guf1, mitochondrial OS=Mus musculus GN=Guf1 PE=1 SV=1	13	4	1

Hypoxia (4 h)

Symbol	Full name	Score	Spectres	Peptides
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	51	2	2
ATPA_MOUSE	ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1	52	2	2
C2C2L_MOUSE	C2 domain-containing protein 2-like OS=Mus musculus GN=C2cd2l PE=1 SV=3	25	1	1
PDE8B_MOUSE	High affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8B OS=Mus musculus GN=Pde8b PE=1 SV=1	23	1	1
GVIN1_MOUSE	Interferon-induced very large GTPase 1 OS=Mus musculus GN=Gvin1 PE=1 SV=1	29	1	1
ACOX1_MOUSE	Peroxisomal acyl-coenzyme A oxidase 1 OS=Mus musculus GN=Acox1 PE=1 SV=5	23	1	1
TAM41_MOUSE	Phosphatidate cytidylyltransferase, mitochondrial OS=Mus musculus GN=Tamm41 PE=1 SV=2	23	1	1
ALBU_MOUSE	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	35	1	1
TCPG_MOUSE	T-complex protein 1 subunit gamma OS=Mus musculus GN=Cct3 PE=1 SV=1	28	1	1
VASH1_MOUSE	**Vasohibin-1 OS=Mus musculus GN=Vash1 PE=2 SV=4**	26	2	1

Normoxia (4 h)

Symbol	Full name	Score	Spectres	Peptides
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	48	1	1
EMAL6_MOUSE	Echinoderm microtubule-associated protein-like 6 OS=Mus musculus GN=Eml6 PE=2 SV=1	43	2	1
FXL14_MOUSE	F-box/LRR-repeat protein 14 OS=Mus musculus GN=Fbxl14 PE=2 SV=1	15	1	1
BRCC3_MOUSE	Lys-63-specific deubiquitinase BRCC36 OS=Mus musculus GN=Brcc3 PE=1 SV=1	20	1	1
MYBA_MOUSE	Myb-related protein A OS=Mus musculus GN=Mybl1 PE=1 SV=2	26	1	1
NUDC2_MOUSE	NudC domain-containing protein 2 OS=Mus musculus GN=Nudcd2 PE=1 SV=1	17	1	1
GUF1_MOUSE	Translation factor Guf1, mitochondrial OS=Mus musculus GN=Guf1 PE=1 SV=1	14	25	1
TMPSD_MOUSE	Transmembrane protease serine 13 OS=Mus musculus GN=Tmprss13 PE=2 SV=2	15	1	1

Hypoxia (8 h)

Symbol	Full name	Score	Spectres	Peptides
ACTA_MOUSE	Actin, aortic smooth muscle OS=Mus musculus GN=Acta2 PE=1 SV=1	36	1	1
ADT1_MOUSE	ADP/ATP translocase 1 OS=Mus musculus GN=Slc25a4 PE=1 SV=4	32	1	1
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	51	2	2
C2C2L_MOUSE	C2 domain-containing protein 2-like OS=Mus musculus GN=C2cd2l PE=1 SV=3	22	1	1
CSPRS_MOUSE	Component of Sp100-rs OS=Mus musculus GN=Csprs PE=2 SV=1	23	1	1
MCM8_MOUSE	DNA helicase MCM8 OS=Mus musculus GN=Mcm8 PE=1 SV=3	25	1	1
EMAL6_MOUSE	Echinoderm microtubule-associated protein-like 6 OS=Mus musculus GN=Eml6 PE=2 SV=1	34	2	1
GKAP1_MOUSE	G kinase-anchoring protein 1 OS=Mus musculus GN=Gkap1 PE=1 SV=1	26	1	1
H1T_MOUSE	Histone H1t OS=Mus musculus GN=Hist1h1t PE=1 SV=4	41	1	1
ALBU_MOUSE	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	41	1	1
SPE39_MOUSE	Spermatogenesis-defective protein 39 homolog OS=Mus musculus GN=Vipas39 PE=1 SV=1	20	1	1
GUF1_MOUSE	Translation factor Guf1, mitochondrial OS=Mus musculus GN=Guf1 PE=1 SV=1	22	1	1
VASH1_MOUSE	**Vasohibin-1 OS=Mus musculus GN=Vash1 PE=2 SV=4**	28	1	1

Normoxia (8 h)

Symbol	Full name	Score	Spectres	Peptides
RM17_MOUSE	39S ribosomal protein L17, mitochondrial OS=Mus musculus GN=Mrpl17 PE=1 SV=1	26	1	1
RS10_MOUSE	40S ribosomal protein S10 OS=Mus musculus GN=Rps10 PE=1 SV=1	42	1	1
RS19_MOUSE	40S ribosomal protein S19 OS=Mus musculus GN=Rps19 PE=1 SV=3	41	1	1
RS4X_MOUSE	40S ribosomal protein S4, X isoform OS=Mus musculus GN=Rps4x PE=1 SV=2	28	1	1
RS7_MOUSE	40S ribosomal protein S7 OS=Mus musculus GN=Rps7 PE=2 SV=1	57	2	2
ADT1_MOUSE	ADP/ATP translocase 1 OS=Mus musculus GN=Slc25a4 PE=1 SV=4	604	9	5
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	83	5	1
ATPA_MOUSE	ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1	515	14	10
ATPB_MOUSE	ATP synthase subunit beta, mitochondrial OS=Mus musculus GN=Atp5b PE=1 SV=2	582	15	11
ATPG_MOUSE	ATP synthase subunit gamma, mitochondrial OS=Mus musculus GN=Atp5c1 PE=1 SV=1	67	3	2
ATPO_MOUSE	ATP synthase subunit O, mitochondrial OS=Mus musculus GN=Atp5o PE=1 SV=1	579	20	9
ATAD3_MOUSE	ATPase family AAA domain-containing protein 3 OS=Mus musculus GN=Atad3 PE=1 SV=1	19	1	1
CE295_MOUSE	Centrosomal protein of 295 kDa OS=Mus musculus GN=Cep295 PE=1 SV=3	32	4	1
CERU_MOUSE	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	106	5	3
CC178_MOUSE	Coiled-coil domain-containing protein 178 OS=Mus musculus GN=Ccdc178 PE=2 SV=2	21	1	1
TRI33_MOUSE	E3 ubiquitin-protein ligase TRIM33 OS=Mus musculus GN=Trim33 PE=1 SV=2	78	4	1
ECI1_MOUSE	Enoyl-CoA delta isomerase 1, mitochondrial OS=Mus musculus GN=Eci1 PE=1 SV=2	30	1	1
HNRPU_MOUSE	Heterogeneous nuclear ribonucleoprotein U OS=Mus musculus GN=Hnrnpu PE=1 SV=1	72	2	2
H12_MOUSE	Histone H1.2 OS=Mus musculus GN=Hist1h1c PE=1 SV=2	146	7	3
H2AV_MOUSE	Histone H2A.V OS=Mus musculus GN=H2afv PE=1 SV=3	60	3	2
H2B1B_MOUSE	Histone H2B type 1-B OS=Mus musculus GN=Hist1h2bb PE=1 SV=3	203	7	4
H4_MOUSE	Histone H4 OS=Mus musculus GN=Hist1h4a PE=1 SV=2	25	1	1
HXA1_MOUSE	Homeobox protein Hox-A1 OS=Mus musculus GN=Hoxa1 PE=1 SV=2	25	1	1
ITIH2_MOUSE	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	43	2	2
MYBA_MOUSE	Myb-related protein A OS=Mus musculus GN=Mybl1 PE=1 SV=2	26	1	1
MTA70_MOUSE	N6-adenosine-methyltransferase subunit METTL3 OS=Mus musculus GN=Mettl3 PE=1 SV=2	35	1	1
MPCP_MOUSE	Phosphate carrier protein, mitochondrial OS=Mus musculus GN=Slc25a3 PE=1 SV=1	51	1	1
PLXA3_MOUSE	Plexin-A3 OS=Mus musculus GN=Plxna3 PE=1 SV=2	25	1	1
PTRF_MOUSE	Polymerase I and transcript release factor OS=Mus musculus GN=Ptrf PE=1 SV=1	67	2	2
BSN_MOUSE	Protein bassoon OS=Mus musculus GN=Bsn PE=1 SV=4	25	1	1
RTKN2_MOUSE	Rhotekin-2 OS=Mus musculus GN=Rtkn2 PE=1 SV=2	29	1	1
RUNX1_MOUSE	Runt-related transcription factor 1 OS=Mus musculus GN=Runx1 PE=1 SV=1	25	1	1
AT2A2_MOUSE	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS=Mus musculus GN=Atp2a2 PE=1 SV=2	28	1	1
SPTC2_MOUSE	Serine palmitoyltransferase 2 OS=Mus musculus GN=Sptlc2 PE=1 SV=2	125	8	1
SDPR_MOUSE	Serum deprivation-response protein OS=Mus musculus GN=Sdpr PE=1 SV=3	56	2	2
SUCA_MOUSE	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial OS=Mus musculus GN=Suclg1 PE=1 SV=4	25	1	1
SAE1_MOUSE	SUMO-activating enzyme subunit 1 OS=Mus musculus GN=Sae1 PE=1 SV=1	21	1	1
GUF1_MOUSE	Translation factor Guf1, mitochondrial OS=Mus musculus GN=Guf1 PE=1 SV=1	17	1	1
ECHA_MOUSE	Trifunctional enzyme subunit alpha, mitochondrial OS=Mus musculus GN=Hadha PE=1 SV=1	119	3	3
ECHB_MOUSE	Trifunctional enzyme subunit beta, mitochondrial OS=Mus musculus GN=Hadhb PE=1 SV=1	81	3	3
TNNT2_MOUSE	Troponin T, cardiac muscle OS=Mus musculus GN=Tnnt2 PE=1 SV=2	23	1	1
RS27A_MOUSE	Ubiquitin-40S ribosomal protein S27a OS=Mus musculus GN=Rps27a PE=1 SV=2	35	1	1
CN159_MOUSE	UPF0317 protein C14orf159 homolog, mitochondrial OS=Mus musculus PE=1 SV=1	26	2	1
VDAC1_MOUSE	Voltage-dependent anion-selective channel protein 1 OS=Mus musculus GN=Vdac1 PE=1 SV=3	48	1	1
VDAC2_MOUSE	Voltage-dependent anion-selective channel protein 2 OS=Mus musculus GN=Vdac2 PE=1 SV=2	64	2	2

B. Proteins bound to VEGFA IRES

Normoxia (0 h)

Symbol	Full name	Score	Spectres	Peptides
NUCL_MOUSE	Nucleolin OS=Mus musculus GN=Ncl PE=1 SV=2	209	7	7
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	28	3	1
RN181_MOUSE	E3 ubiquitin-protein ligase RNF181 OS=Mus musculus GN=Rnf181 PE=1 SV=1	26	3	1
CSPRS_MOUSE	Component of Sp100-rs OS=Mus musculus GN=Csprs PE=2 SV=1	23	4	1
C2C2L_MOUSE	C2 domain-containing protein 2-like OS=Mus musculus GN=C2cd2l PE=1 SV=3	14	1	1
SAE1_MOUSE	SUMO-activating enzyme subunit 1 OS=Mus musculus GN=Sae1 PE=1 SV=1	14	1	1

Hypoxia (4 h)

Symbol	Full name	Score	Spectres	Peptides
ATPA_MOUSE	ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1	42	1	1
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	38	3	1
RN181_MOUSE	E3 ubiquitin-protein ligase RNF181 OS=Mus musculus GN=Rnf181 PE=1 SV=1	30	2	1
DOC10_MOUSE	Dedicator of cytokinesis protein 10 OS=Mus musculus GN=Dock10 PE=1 SV=3	26	2	1
NID2_MOUSE	Nidogen-2 OS=Mus musculus GN=Nid2 PE=1 SV=2	26	2	1
C2C2L_MOUSE	C2 domain-containing protein 2-like OS=Mus musculus GN=C2cd2l PE=1 SV=3	25	3	1
PLCL1_MOUSE	Inactive phospholipase C-like protein 1 OS=Mus musculus GN=Plcl1 PE=1 SV=3	21	1	1

Normoxia (4 h)

Symbol	Full name	Score	Spectres	Peptides
ATPA_MOUSE	ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1	912	25	16
ATPO_MOUSE	ATP synthase subunit O, mitochondrial OS=Mus musculus GN=Atp5o PE=1 SV=1	374	12	7
ATPB_MOUSE	ATP synthase subunit beta, mitochondrial OS=Mus musculus GN=Atp5b PE=1 SV=2	372	9	8
ADT2_MOUSE	ADP/ATP translocase 2 OS=Mus musculus GN=Slc25a5 PE=1 SV=3	364	8	5
HNRPU_MOUSE	Heterogeneous nuclear ribonucleoprotein U OS=Mus musculus GN=Hnrnpu PE=1 SV=1	348	12	9
ACTA_MOUSE	Actin, aortic smooth muscle OS=Mus musculus GN=Acta2 PE=1 SV=1	147	2	1
ECHA_MOUSE	Trifunctional enzyme subunit alpha, mitochondrial OS=Mus musculus GN=Hadha PE=1 SV=1	134	8	8
ACTB_MOUSE	Actin, cytoplasmic 1 OS=Mus musculus GN=Actb PE=1 SV=1	119	1	1
ALBU_MOUSE	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	100	1	1
SUCA_MOUSE	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial OS=Mus musculus GN=Suclg1 PE=1 SV=4	86	1	1
ATPG_MOUSE	ATP synthase subunit gamma, mitochondrial OS=Mus musculus GN=Atp5c1 PE=1 SV=1	78	1	1
THIM_MOUSE	3-ketoacyl-CoA thiolase, mitochondrial OS=Mus musculus GN=Acaa2 PE=1 SV=3	57	1	1
SERPH_MOUSE	Serpin H1 OS=Mus musculus GN=Serpinh1 PE=1 SV=3	54	1	1
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	52	3	1
PTRF_MOUSE	Polymerase I and transcript release factor OS=Mus musculus GN=Ptrf PE=1 SV=1	51	1	1
AT2A2_MOUSE	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS=Mus musculus GN=Atp2a2 PE=1 SV=2	46	1	1
ILF2_MOUSE	Interleukin enhancer-binding factor 2 OS=Mus musculus GN=Ilf2 PE=1 SV=1	43	2	2
MOCS3_MOUSE	Adenylyltransferase and sulfurtransferase MOCS3 OS=Mus musculus GN=Mocs3 PE=1 SV=1	39	1	1
MYOG_MOUSE	Myogenin OS=Mus musculus GN=Myog PE=1 SV=2	33	1	1
TAGAP_MOUSE	T-cell activation Rho GTPase-activating protein OS=Mus musculus GN=Tagap PE=2 SV=2	27	1	1
CH60_MOUSE	60 kDa heat shock protein, mitochondrial OS=Mus musculus GN=Hspd1 PE=1 SV=1	26	1	1
C2C2L_MOUSE	C2 domain-containing protein 2-like OS=Mus musculus GN=C2cd2l PE=1 SV=3	25	2	1
EFC14_MOUSE	EF-hand calcium-binding domain-containing protein 14 OS=Mus musculus GN=Efcab14 PE=2 SV=1	24	1	1
SPIR1_MOUSE	Protein spire homolog 1 OS=Mus musculus GN=Spire1 PE=1 SV=1	24	1	1
ATAD3_MOUSE	ATPase family AAA domain-containing protein 3 OS=Mus musculus GN=Atad3 PE=1 SV=1	20	1	1

Hypoxia (8 h)

Symbol	Full name	Score	Spectres	Peptides
SPTC2_MOUSE	Serine palmitoyltransferase 2 OS=Mus musculus GN=Sptlc2 PE=1 SV=2	20	1	1
CCD66_MOUSE	Coiled-coil domain-containing protein 66 OS=Mus musculus GN=Ccdc66 PE=1 SV=3	28	1	1
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	35	1	1

Normoxia (8 h)

Symbol	Full name	Score	Spectres	Peptides
SPTC2_MOUSE	Serine palmitoyltransferase 2 OS=Mus musculus GN=Sptlc2 PE=1 SV=2	20	1	1
CCD66_MOUSE	Coiled-coil domain-containing protein 66 OS=Mus musculus GN=Ccdc66 PE=1 SV=3	28	1	1
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	35	1	1
ATPA_MOUSE	ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1	60	1	1
PHRF1_MOUSE	PHD and RING finger domain-containing protein 1 OS=Mus musculus GN=Phrf1 PE=1 SV=2	41	7	1
OXR1_MOUSE	Oxidation resistance protein 1 OS=Mus musculus GN=Oxr1 PE=1 SV=3	26	1	1
KCTD9_MOUSE	BTB/POZ domain-containing protein KCTD9 OS=Mus musculus GN=Kctd9 PE=2 SV=1	24	2	1
BIK_MOUSE	Bcl-2-interacting killer OS=Mus musculus GN=Bik PE=1 SV=1	21	2	1

C. Proteins bound to EMCV IRES

Normoxia (0 h)

Symbol	Full name	Score	Spectres	Peptides
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	55	2	1
ATPA_MOUSE	ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1	70	2	2
KCTD9_MOUSE	BTB/POZ domain-containing protein KCTD9 OS=Mus musculus GN=Kctd9 PE=2 SV=1	23	1	1
BRCC3_MOUSE	Lys-63-specific deubiquitinase BRCC36 OS=Mus musculus GN=Brcc3 PE=1 SV=1	30	1	1
NDE1_MOUSE	Nuclear distribution protein nudE homolog 1 OS=Mus musculus GN=Nde1 PE=1 SV=1	21	1	1
VASH1_MOUSE	**Vasohibin-1 OS=Mus musculus GN=Vash1 PE=2 SV=4**	**26**	**1**	**1**

Hypoxia (4 h)

Symbol	Full name	Score	Spectres	Peptides
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	81	5	1
KCTD9_MOUSE	BTB/POZ domain-containing protein KCTD9 OS=Mus musculus GN=Kctd9 PE=2 SV=1	23	2	1
NUDC2_MOUSE	NudC domain-containing protein 2 OS=Mus musculus GN=Nudcd2 PE=1 SV=1	20	1	1
PLPL1_MOUSE	Patatin-like phospholipase domain-containing protein 1 OS=Mus musculus GN=Pnpla1 PE=2 SV=1	24	1	1
TAM41_MOUSE	Phosphatidate cytidylyltransferase, mitochondrial OS=Mus musculus GN=Tamm41 PE=1 SV=2	21	1	1
VASH1_MOUSE	**Vasohibin-1 OS=Mus musculus GN=Vash1 PE=2 SV=4**	**22**	**3**	**1**

Normoxia (4 h)

Symbol	Full name	Score	Spectres	Peptides
LOX15_MOUSE	Arachidonate 15-lipoxygenase OS=Mus musculus GN=Alox15 PE=1 SV=4	23	1	1
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	88	6	1
KCTD9_MOUSE	BTB/POZ domain-containing protein KCTD9 OS=Mus musculus GN=Kctd9 PE=2 SV=1	23	2	1
BRCC3_MOUSE	Lys-63-specific deubiquitinase BRCC36 OS=Mus musculus GN=Brcc3 PE=1 SV=1	27	1	1
PCLO_MOUSE	Protein piccolo OS=Mus musculus GN=Pclo PE=1 SV=4	24	1	1
RPAP1_MOUSE	RNA polymerase II-associated protein 1 OS=Mus musculus GN=Rpap1 PE=1 SV=2	23	1	1
ALBU_MOUSE	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	73	1	1
GUF1_MOUSE	Translation factor Guf1, mitochondrial OS=Mus musculus GN=Guf1 PE=1 SV=1	24	1	1

Hypoxia (8 h)

Symbol	Full name	Score	Spectres	Peptides
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	36	2	1
EMAL6_MOUSE	Echinoderm microtubule-associated protein-like 6 OS=Mus musculus GN=Eml6 PE=2 SV=1	30	1	1
BRCC3_MOUSE	Lys-63-specific deubiquitinase BRCC36 OS=Mus musculus GN=Brcc3 PE=1 SV=1	24	1	1
RPAP1_MOUSE	RNA polymerase II-associated protein 1 OS=Mus musculus GN=Rpap1 PE=1 SV=2	23	1	1
VASH1_MOUSE	**Vasohibin-1 OS=Mus musculus GN=Vash1 PE=2 SV=4**	**26**	**1**	**1**

Normoxia (8 h)				
Symbol	**Full name**	**Score**	**Spectres**	**Peptides**
ASAP2_MOUSE	Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2 OS=Mus musculus GN=Asap2 PE=1 SV=3	38	4	1
ATAD3_MOUSE	ATPase family AAA domain-containing protein 3 OS=Mus musculus GN=Atad3 PE=1 SV=1	26	2	1
DOC10_MOUSE	Dedicator of cytokinesis protein 10 OS=Mus musculus GN=Dock10 PE=1 SV=3	24	1	1
NUCL_MOUSE	**Nucleolin OS=Mus musculus GN=Ncl PE=1 SV=2**	**40**	**1**	**1**
RPAP1_MOUSE	RNA polymerase II-associated protein 1 OS=Mus musculus GN=Rpap1 PE=1 SV=2	29	1	1
VASH1_MOUSE	**Vasohibin-1 OS=Mus musculus GN=Vash1 PE=2 SV=4**	**28**	**3**	**1**

EV Table 4. BIA-MS analysis of IRES-bound proteins in hypoxic cardiomyocytes.
Total cell extracts from normoxic or hypoxic HL-1 cardiomyocytes were injected into the BIAcore T200 optical biosensor device where biotinylated IRES RNAs had been immobilized. The list of bound proteins identified by mass spectrometry (LC-MS/MS) after tryptic digestion is shown for FGF1 (A), VEGF-Aa (B) or EMCV (C) IRESs, respectively. The score and the number of spectra and peptides identified are indicated. For each time of hypoxia, cells were cultivated the same time in normoxia as a control (Normoxia 4h and 8h).

FGF1 IRES

LucF			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	6165	5235	4839	5413	681
		2	9056	8546	8165	3917	650
		3	24718	28513	71475	41569	25969
	Si VASH1	1	6061	4321	6130	5504	1025
		2	2819	4382	4771	3991	907
		3	25587	41809	41011	36136	9144
Hypoxia	Si Control	1	23268	23282		23275	10
		2	9056	7434	4327	6939	2134
		3	35934	27425	41617	34992	7143
	Si VASH1	1	3476	3798	3703	3659	165
		2	918	794	159	624	356
		3	35078	27053	26439	29523	4820

LucR			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	53438	31326	43193	42652	11066
		2	26638	18202	41546	28795	10241
		3	215819	361595	537143	371519	160892
	Si VASH1	1	33640	25524	46369	35178	10507
		2	20966	30708	37007	29560	7132
		3	169661	310210	232371	237414	70410
Hypoxia	Si Control	1	20104	28719	24302	24375	4308
		2	47957	40352	28489	38933	8713
		3	166883	126093	212489	168488	43220
	Si VASH1	1	17915	14606	17491	16671	1800
		2	6722	7810	5492	6675	1043
		3	202073	157173	139745	166330	32157

IRES activity

LucF/ LucR			Biological replicates					AU : LucF/LucR *100				
	SiRNA	Experiment	A	B	C	Mean	SD	Mean	SD	Ratio	t-test	Significance
Normoxia	Si Control	1	0.1154	0.1671	0.1120	0.1315	0.0309	13.15	3.09			
		2	0.1325	0.1124	0.1632	0.1360	0.0226	13.60	2.26			
		3	0.1145	0.0789	0.1331	0.1088	0.0276	10.88	2.76			
	Si VASH1	1	0.1235	0.1379	0.1419	0.1311	0.0154	13.11	1.54	1.00	0.440	
		2	0.0954	0.1482	0.1614	0.1350	0.0307	13.50	3.07	0.99	0.483	
		3	0.1508	0.1348	0.1765	0.1540	0.0210	15.40	2.10	1.42	0.008	**
Hypoxia	Si Control	1	1.1574	0.8107		0.9840	0.2451	98.40	24.51			
		2	0.1888	0.1840	0.1520	0.1750	0.0187	17.50	1.87			
		3	0.2153	0.2175	0.1959	0.2096	0.0119	20.96	1.19			
	Si VASH1	1	0.1729	0.1323	0.1524	0.1525	0.0203	15.25	2.03	0.15	0.004	***
		2	0.1364	0.1016	0.0289	0.0890	0.0476	8.90	4.76	0.51	0.026	*
		3	0.1736	0.1721	0.1892	0.1783	0.0095	17.83	0.95	0.85	0.063	

Final values		AU : LucF/LucR *100		Normalized to Si control			
Time	Condition	Total mean	SD	Ratio	SD	t-test	Significance
Normoxia	Si Control	12.54	2.74	1.00	0.22		
	Si VASH1	14.12	2.31	1.13	0.18	**0.094**	
Hypoxia	Si Control	39.02	37.86	1.00	0.97		
	Si VASH1	13.99	4.96	0.36	0.13	**0.047**	*

FGF2 IRES

LucF			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	22826	21218	12844	18963	5360
		2	1378436	1082836	1164807	1208693	152608
		3	83190	93828	99601	92206	8325
	Si VASH1	1	21094	24819	20555	22156	2322
		2	2209438	826675	1840332	1625481	715981
		3	67392	76305	73470	72389	4554
Hypoxia	Si Control	1	19965	12844	11697	14835	4480
		2	1017433	1179299	829663	1008798	174978
		3	67103	78868	71632	72534	5934
	Si VASH1	1	15522	15043	16528	15698	758
		2	893314	749103	966069	869495	110427
		3	59989	68906	68663	65852	5080

LucR			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	180511	135180	129077	148256	28100
		2	12202038	11842478	11986298	12010272	180975
		3	590388	751723	757328	699813	94806
	Si VASH1	1	125421	125058	135793	128757	6096
		2	14135403	8953356	10851103	11313287	2621757
		3	556354	613876	606021	592084	31191
Hypoxia	Si Control	1	96016	67708	75468	79731	14628
		2	6222023	7669135	5269169	6386776	1208436
		3	334572	394644	375540	368252	30692
	Si VASH1	1	68476	61198	64605	64760	3642
		2	4535768	4011896	5170671	4572778	580273
		3	392981	376414	347094	372163	23237

IRES activity

LucF/ LucR			Biological replicates					AU : LucF/LucR *100				
	SiRNA	Experiment	A	B	C	Mean	SD	Mean	SD	Ratio	t-test	Significance
Normoxia	Si Control	1	0.1265	0.1570	0.0995	0.1276	0.0287	12.76	2.87			
		2	0.1130	0.0914	0.0972	0.1005	0.0111	10.05	1.11			
		3	0.1409	0.1248	0.1315	0.1324	0.0081	13.24	0.81			
	Si VASH1	1	0.1169	0.1836	0.1592	0.1532	0.0338	15.32	3.38	1.20	0.165	
		2	0.1563	0.0923	0.1696	0.1394	0.0413	13.94	4.13	1.39	0.101	
		3	0.1211	0.1243	0.1212	0.1222	0.0018	12.22	0.18	0.92	0.104	
Hypoxia	Si Control	1	0.2079	0.1897	0.1550	0.1842	0.0269	18.42	2.69			
		2	0.1635	0.1538	0.1575	0.1582	0.0049	15.82	0.49			
		3	0.2006	0.1998	0.1907	0.1971	0.0055	19.71	0.55			
	Si VASH1	1	0.1617	0.2222	0.2190	0.2009	0.0341	20.09	3.41	1.09	0.330	
		2	0.1969	0.1867	0.1868	0.1902	0.0059	19.02	0.59	1.20	0.001	*
		3	0.1526	0.1831	0.1978	0.1778	0.0230	17.78	2.30	0.90	0.026	*

Final values		AU : LucF/LucR *100		Normalized to Si control			
Time	Condition	Total mean	SD	Ratio	SD	t-test	Significance
Normoxia	Si Control	12.02	2.18	1.00	0.18		
	Si VASH1	13.83	2.99	1.15	0.25	**0.073**	
Hypoxia	Si Control	17.98	2.21	1.00	0.12		
	Si VASH1	18.97	2.31	1.05	0.13	**0.235**	

VEGFA IRES a

LucF			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	1063	768	835	889	155
		2	10177	10903	9795	10292	563
		3	8927	10090	10353	9790	759
	Si VASH1	1	ND	ND	ND		
		2	12008	10355	13047	11803	1357
		3	7081	6763	7085	6976	185
Hypoxia	Si Control	1	944	938	958	947	10
		2	21334	25229	24602	23722	2091
		3	6919	8893	10550	8787	1818
	Si VASH1	1	985	1284	1040	1103	159
		2	18934	25104	26388	23475	3985
		3	6087	5714	5375	5725	356

LucR			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	110313	88187	88187	95563	12774
		2	858315	1006460	1131780	998852	136891
		3	800801	851785	976533	876373	90409
	Si VASH1	1	104087	104234	88328	98883	9141
		2	1122644	949786	1245834	1106088	148717
		3	781558	904997	1041730	909428	130143
Hypoxia	Si Control	1	110313	88187	88187	95563	12774
		2	1201671	1239986	1269291	1236983	33910
		3	397755	412761	462290	424269	33772
	Si VASH1	1	104087	104234	88328	98883	9141
		2	1178924	1314113	1411139	1301392	116629
		3	397766	338569	370795	369043	29638

IRES activity

LucF/ LucR			Biological replicates					AU : LucF/LucR *100				
	SiRNA	Experiment	A	B	C	Mean	SD	Mean	SD	Ratio	t-test	Significance
Normoxia	Si Control	1	0.0084	0.0083	0.0091	0.0086	0.0004	0.86	0.04			
		2	0.0119	0.0108	0.0087	0.0104	0.0016	1.04	0.16			
		3	0.0111	0.0118	0.0106	0.0112	0.0006	1.12	0.06			
	Si VASH1	1	ND	ND	ND							
		2	0.0107	0.0109	0.0105	0.0107	0.0002	1.07	0.02	1.02	0.403	
		3	0.0089	0.0098	0.0101	0.0096	0.0007	0.96	0.07	0.86	0.053	
Hypoxia	Si Control	1	0.0086	0.0106	0.0109	0.0100	0.0013	1.00	0.13			
		2	0.0178	0.0203	0.0194	0.0192	0.0013	1.92	0.13			
		3	0.0178	0.0164	0.0153	0.0165	0.0012	1.65	0.12			
	Si VASH1	1	0.0089	0.0146	0.0118	0.0118	0.0028	1.18	0.28	1.17	0.185	
		2	0.0161	0.0191	0.0187	0.0180	0.0017	1.80	0.17	0.94	0.027	*
		3	0.0153	0.0169	0.0145	0.0156	0.0012	1.56	0.12	0.94	0.194	

Final values		AU : LucF/LucR *100		Normalized to Si control			
Time	Condition	Total mean	SD	Ratio	SD	t-test	Significance
Normoxia	Si Control	1.01	0.15	1.00	0.14		
	Si VASH1	1.01	0.07	**1.01**	**0.07**	**0.162**	
Hypoxia	Si Control	1.52	0.42	1.00	0.28		
	Si VASH1	1.51	0.32	**0.99**	**0.21**	**0.417**	

VEGFA IRES b

LucF			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	24407	29293	28866	27522	2706
		2	636888	673362	711456	673902	37287
		3	336029	485230	456130	425796	79091
	Si VASH1	1	26057	23895	24286	24746	1152
		2	754530	811206	796490	787408	29409
		3	377720	490184	368048	411984	67896
Hypoxia	Si Control	1	25539	27250	27360	26716	1021
		2	1455967	1495875	1461388	1471077	21646
		3	252396	325862	292610	290290	36788
	Si VASH1	1	29633	27197	27734	28188	1280
		2	1442573	1496275	1406109	1448319	45357
		3	290051	301154	292703	294636	5799

LucR			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	204606	259243	265657	243169	33550
		2	3953413	4461082	4955118	4456538	500868
		3	2785870	3180830	2979085	2981929	197495
	Si VASH1	1	129783	131937	114093	125271	9740
		2	4588230	4469611	4706020	4587954	118204
		3	3035339	3373906	2706872	3038706	333530
Hypoxia	Si Control	1	207937	216079	211792	211936	4073
		2	5227968	5137726	5358864	5241519	111190
		3	1206699	1464757	1496579	1389345	158974
	Si VASH1	1	179775	171019	145280	165358	17930
		2	5346747	5752000	5164154	5420967	300869
		3	1221397	1296195	1237799	1251797	39315

IRES activity

LucF/ LucR			Biological replicates					AU : LucF/LucR *100				
	SiRNA	Experiment	A	B	C	Mean	SD	Mean	SD	Ratio	t-test	Significance
Normoxia	Si Control	1	0.1193	0.1130	0.1087	0.1136	0.0053	11.36	0.53			
		2	0.1611	0.1509	0.1436	0.1519	0.0088	15.19	0.88			
		3	0.1206	0.1525	0.1531	0.1421	0.0186	14.21	1.86			
	Si VASH1	1	0.1274	0.0922	0.0914	0.1036	0.0205	10.36	2.05	0.91	0.064	
		2	0.1644	0.1815	0.1692	0.1717	0.0088	17.17	0.88	1.13	0.071	
		3	0.1244	0.1453	0.1360	0.1352	0.0104	13.52	1.04	0.95	0.019	*
Hypoxia	Si Control	1	0.1228	0.1261	0.1292	0.1260	0.0032	12.60	0.32			
		2	0.2785	0.2912	0.2727	0.2808	0.0094	28.08	0.94			
		3	0.2092	0.2225	0.1955	0.2091	0.0135	20.91	1.35			
	Si VASH1	1	0.1425	0.1259	0.1309	0.1331	0.0085	13.31	0.85	1.06	0.190	
		2	0.2698	0.2601	0.2723	0.2674	0.0064	26.74	0.64	0.95	0.140	
		3	0.2375	0.2323	0.2365	0.2354	0.0027	23.54	0.27	1.13	0.050	

Final values		AU : LucF/LucR *100		Normalized to Si control			
Time	Condition	Total mean	SD	Ratio	SD	t-test	Significance
Normoxia	Si Control	13.59	2.02	1.00	0.15		
	Si VASH1	13.69	3.20	1.01	0.24	**0.438**	
Hypoxia	Si Control	20.53	6.76	1.00	0.33		
	Si VASH1	21.20	6.10	1.03	0.30	**0.186**	

VEGFC IRES

LucF			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	246375	242933	314902	268070	40594
		2	217056	137257	253247	202520	59346
		3	34901	43369	30196	36155	6676
	Si VASH1	1	487878	477149	492814	485947	8009
		2	229290	246978	182056	219441	33563
		3	38245	41693	27884	35941	7187
Hypoxia	Si Control	1	335045	434617	425637	398433	55079
		2	281808	313489	279125	291474	19113
		3	24456	25053	23295	24268	894
	Si VASH1	1	356286	400146	398669	385034	24907
		2	307224	258394	357857	307825	49734
		3	20099	20436	22661	21065	1392

LucR			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	3487442	3595724	2160847	3081337	799005
		2	1940200	1317594	1986534	1748109	373556
		3	517859	522417	438634	492970	47111
	Si VASH1	1	5663195	5510976	2230869	4468347	1939207
		2	1895114	2069968	1309904	1758329	398067
		3	562597	580376	487313	543428	49404
Hypoxia	Si Control	1	3743871	4296534	1914906	3318437	1246509
		2	2035704	2389202	2247759	2224222	177921
		3	288505	254400	261953	268286	17913
	Si VASH1	1	3382601	3988748	1245215	2872188	1441227
		2	2242942	2337124	2558068	2379378	161757
		3	190852	185250	218449	198184	17773

IRES activity

LucF/ LucR			Biological replicates					AU : LucF/LucR *100				
	SiRNA	Experiment	A	B	C	Mean	SD	Mean	SD	Ratio	t-test	Significance
Normoxia	Si Control	1	0.0706	0.0676	0.1457	0.0946	0.0443	9.46	4.43			
		2	0.1119	0.1042	0.1275	0.1145	0.0119	11.45	1.19			
		3	0.0674	0.0830	0.0688	0.0731	0.0086	7.31	0.86			
	Si VASH1	1	0.0861	0.0866	0.2209	0.1312	0.0777	13.12	7.77	1.39	0.100	
		2	0.1210	0.1193	0.1390	0.1264	0.0109	12.64	1.09	1.10	0.010	*
		3	0.0680	0.0718	0.0572	0.0657	0.0076	6.57	0.76	0.90	0.103	
Hypoxia	Si Control	1	0.0895	0.1012	0.2223	0.1376	0.0735	13.76	7.35			
		2	0.1384	0.1312	0.1242	0.1313	0.0071	13.13	0.71			
		3	0.0848	0.0985	0.0889	0.0907	0.0070	9.07	0.70			
	Si VASH1	1	0.1053	0.1003	0.3202	0.1753	0.1255	17.53	12.55	1.27	0.171	
		2	0.1370	0.1106	0.1399	0.1291	0.0162	12.91	1.62	0.98	0.429	
		3	0.1053	0.1103	0.1037	0.1065	0.0034	10.65	0.34	1.17	0.013	*

Final values		AU : LucF/LucR *100		Normalized to Si control			
Time	Condition	Total mean	SD	Ratio	SD	t-test	Significance
Normoxia	Si Control	9.41	2.94	1.00	0.31		
	Si VASH1	10.78	5.05	1.15	0.54	**0.074**	
Hypoxia	Si Control	11.99	4.32	1.00	0.36		
	Si VASH1	13.70	7.02	1.14	0.59	**0.079**	

VEGFD IRES

LucF			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	145323	155346	154191	151620	5484
		2	47796	48288	42436	46173	3246
		3	75842	68756	63160	69253	6355
	Si VASH1	1	194207	225219	224077	214501	17584
		2	41472	53177	48922	47857	5925
		3	50506	69895	67848	62750	10653
Hypoxia	Si Control	1	195382	241996	212936	216772	23542
		2	86899	103834	88966	93233	9239
		3	38323	41266	45431	41673	3571
	Si VASH1	1	179053	197152	112369	162858	44651
		2	79555	81056	77725	79445	1668
		3	29441	36166	41950	35852	6260

LucR			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	1907458	2093765	2160847	2054023	131286
		2	567674	597668	541654	568999	28030
		3	858060	837030	638997	778029	120864
	Si VASH1	1	1991236	2298870	2230869	2173658	161600
		2	517993	560985	512755	530578	26463
		3	593777	780959	789452	721396	110603
Hypoxia	Si Control	1	1619167	1897011	1914906	1810361	165821
		2	525002	615275	500416	546898	60479
		3	326465	350347	383692	353501	28743
	Si VASH1	1	1641814	1672889	1245215	1519972	238454
		2	536248	545353	584930	555510	25882
		3	278668	318382	374719	323923	48265

IRES activity

LucF/ LucR			Biological replicates					AU : LucF/LucR *100				
	SiRNA	Experiment	A	B	C	Mean	SD	Mean	SD	Ratio	t-test	Significance
Normoxia	Si Control	1	0.0762	0.0742	0.0714	0.0739	0.0024	7.39	0.24			
		2	0.0842	0.0808	0.0783	0.0811	0.0029	8.11	0.29			
		3	0.0884	0.0821	0.0988	0.0898	0.0084	8.98	0.84			
	Si VASH1	1	0.0975	0.0980	0.1004	0.0986	0.0016	9.86	0.16	1.33	0.004	***
		2	0.0801	0.0948	0.0954	0.0901	0.0087	9.01	0.87	1.11	0.154	
		3	0.0851	0.0895	0.0859	0.0868	0.0024	8.68	0.24	0.97	0.332	
Hypoxia	Si Control	1	0.1207	0.1276	0.1112	0.1198	0.0082	11.98	0.82			
		2	0.1655	0.1688	0.1778	0.1707	0.0064	17.07	0.64			
		3	0.1174	0.1178	0.1184	0.1179	0.0005	11.79	0.05			
	Si VASH1	1	0.1091	0.1179	0.0902	0.1057	0.0141	10.57	1.41	0.88	0.028	*
		2	0.1484	0.1486	0.1329	0.1433	0.0090	14.33	0.90	0.84	0.045	*
		3	0.1057	0.1136	0.1120	0.1104	0.0042	11.04	0.42	0.94	0.040	*

Final values		AU : LucF/LucR *100		Normalized to Si control			
Time	Condition	Total mean	SD	Ratio	SD	t-test	Significance
Normoxia	Si Control	8.16	0.83	1.00	0.10		
	Si VASH1	9.19	0.70	1.13	0.09	**0.033**	*
Hypoxia	Si Control	13.61	2.65	1.00	0.19		
	Si VASH1	11.98	1.97	0.88	0.14	**0.002**	***

c-myc IRES

LucF			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	252109	291920	918687	487572	373887
		2	325307	384619	326642	345523	33865
		3	249643	233046	183453	222047	34439
	Si VASH1	1	1424225	1180035	1295701	1299987	122151
		2	352401	300184	317795	323460	26565
		3	186267	182128	179026	182474	3633
Hypoxia	Si Control	1	816497	422387	80789	439891	368166
		2	575691	601917	534370	570659	34053
		3	183838	194270	220011	199373	18619
	Si VASH1	1	622268	530019	497331	549872	64791
		2	518194	406193	591820	505402	93472
		3	178636	177011	163963	173203	8043

LucR			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	2603194	2581366	4956962	3380507	1365294
		2	1216742	1371688	1207546	1265325	92227
		3	1738743	1532354	1438325	1569807	153671
	Si VASH1	1	6466612	5522384	5429788	5806261	573752
		2	1197183	1080280	1156255	1144572	59321
		3	1513541	1375704	1410980	1433408	71603
Hypoxia	Si Control	1	3585096	2670578	530645	2262106	1567659
		2	1405500	1480296	1467212	1451002	39946
		3	775271	746257	985294	835607	130442
	Si VASH1	1	2552808	2119514	2073981	2248768	264289
		2	1449064	1365072	1405441	1406526	42006
		3	868202	777132	863443	836259	51261

IRES activity

LucF/ LucR			Biological replicates					AU : LucF/LucR *100				
	SiRNA	Experiment	A	B	C	Mean	SD	Mean	SD	Ratio	t-test	Significance
Normoxia	Si Control	1	0.0968	0.1131	0.1853	0.1318	0.0471	13.18	4.71			
		2	0.2674	0.2804	0.2705	0.2728	0.0068	27.28	0.68			
		3	0.1436	0.1521	0.1275	0.1411	0.0125	14.11	1.25			
	Si VASH1	1	0.2202	0.2137	0.2386	0.2242	0.0129	22.42	1.29	1.70	0.023	*
		2	0.2944	0.2779	0.2748	0.2824	0.0105	28.24	1.05	1.04	0.197	
		3	0.1231	0.1324	0.1269	0.1274	0.0047	12.74	0.47	0.90	0.085	
Hypoxia	Si Control	1	0.2277	0.1582	0.1522	0.1794	0.0420	17.94	4.20			
		2	0.4096	0.4066	0.3642	0.3935	0.0254	39.35	2.54			
		3	0.2371	0.2603	0.2233	0.2402	0.0187	24.02	1.87			
	Si VASH1	1	0.2438	0.2501	0.2398	0.2445	0.0052	24.45	0.52	1.36	0.059	
		2	0.3576	0.2976	0.4211	0.3588	0.0618	35.88	6.18	0.91	0.275	
		3	0.2058	0.2278	0.1899	0.2078	0.0190	20.78	1.90	0.86	0.0002	****

Final values		AU : LucF/LucR *100		Normalized to Si control			
Time	Condition	Total mean	SD	Ratio	SD	t-test	Significance
Normoxia	Si Control	18.19	7.26	1.00	0.40		
	Si VASH1	21.13	6.83	1.16	0.38	**0.065**	
Hypoxia	Si Control	27.10	9.91	1.00	0.37		
	Si VASH1	27.04	7.55	1.00	0.28	**0.489**	

EMCV IRES

LucF			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	1702630	1760136	1302068	1588278	249527
		2	107301	144501	143440	131747	21177
		3	330170	293299	266000	296490	32204
	Si VASH1	1	5352755	7029602	3647215	5343191	1691214
		2	112778	121482	113111	115790	4932
		3	291868	245382	237669	258306	29320
Hypoxia	Si Control	1	2686430	2928766	2764654	2793283	123678
		2	223538	216316	233362	224405	8556
		3	244519	254123	209543	236062	23462
	Si VASH1	1		886021	2250249	1568135	964655
		2	221733	209587	204733	212018	8756
		3	216546	204516	186109	202390	15329

LucR			Biological replicates				
	SiRNA	Experiment	A	B	C	Mean	SD
Normoxia	Si Control	1	2208003	2016725	1796654	2007127	205843
		2	147772	179110	161146	162676	15725
		3	365365	327375	301181	331307	32272
	Si VASH1	1	5336122	6838676	2959448	5044749	1955959
		2	144691	146769	138309	143256	4409
		3	316197	262296	254808	277767	33491
Hypoxia	Si Control	1	2156164	2373157	1936666	2155329	218246
		2	177395	182885	198846	186375	11143
		3	173211	189437	175377	179341	8810
	Si VASH1	1	ND	730239	1752792	1241515	723054
		2	191909	168829	202128	187622	17058
		3	170625	166261	149249	162045	11294

IRES activity

LucF/ LucR			Biological replicates					AU : LucF/LucR *100				
	SiRNA	Experiment	A	B	C	Mean	SD	Mean	SD		t-test	Significance
Normoxia	Si Control	1	0.7711	0.8728	0.7247	0.7895	0.0757	78.95	7.57			
		2	0.7261	0.8068	0.8901	0.8077	0.0820	80.77	8.20			
		3	0.9037	0.8959	0.8832	0.8943	0.0103	89.43	1.03			
	Si VASH1	1	1.0031	1.0279	1.2324	1.0878	0.1258	108.78	12.58	1.38	0.054	
		2	0.7794	0.8277	0.8178	0.8083	0.0255	80.83	2.55	1.00	0.494	
		3	0.9231	0.9355	0.9327	0.9304	0.0065	93.04	0.65	1.04	0.028	*
Hypoxia	Si Control	1	1.2459	1.2341	1.4275	1.3025	0.1084	130.25	10.84			
		2	1.2601	1.1828	1.1736	1.2055	0.0475	120.55	4.75			
		3	1.4117	1.3415	1.1948	1.3160	0.1107	131.60	11.07			
	Si VASH1	1	ND	1.2133	1.2838	1.2486	0.0498	124.86	4.98	0.96	0.285	
		2	1.1554	1.2414	1.0129	1.1366	0.1154	113.66	11.54	0.94	0.202	
		3	1.2691	1.2301	1.2470	1.2487	0.0196	124.87	1.96	0.95	0.191	

Final values		AU : LucF/LucR *100		Normalized to Si control			
Time	Condition	Total mean	SD	Ratio	SD	t-test	Significance
Normoxia	Si Control	83.05	7.41	1.00	0.09		
	Si VASH1	94.22	13.73	1.13	0.17	**0.043**	*
Hypoxia	Si Control	127.47	9.64	1.00	0.08		
	Si VASH1	120.66	8.74	0.95	0.07	**0.029**	*

EV Table 5. Knock-down of VASH1 in HL-1 cells.

HL-1 cells transduced by the different IRES-containing lentivectors were transfected with siRNA siVASH of SiControl and submitted to 8 h of hypoxia. Luciferase activity and IRES activities (ratio LucF/LucR x 100) were measured. The values correspond to the experiments presented Figure 4.

Biological replicates are indicated as A, B and C, whereas independent experiments are indicated as 1, 2, 3. Means, standard deviation (SD) and t-test of IRES activities were calculated. The panels “final values” correspond to means of all experiments (nine values) which are reported in the histograms of Figure 7. P-value significance is indicated: *p<0.05, **p<0.01, ***<0.001, ****p<0.0001.

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Target	Forward primer 5' to 3'	Reverse primer 5' to 3'
Akt1	AGAACTCTAGGCATCCCTTCC	CGTTGGCATACTCCATGACA
Ang	TCCTGACTCAGCACCATGAC	ACATCTTTGCAGGGTGAGGTTA
Angpt1	ACAACACCGGGAAGATGGAA	TTCACCAGAGGGATTCCCAAAA
Angpt2	GAACCAGACAGCAGCACAAA	TCGAGTCTTGTCGTCTGGTTTA
Angptl4	CTTGGGACCAAGACCATGAC	TGGCTACAGGTACCAAACCA
Anpep	TGGGACTTTGTCCGAAGCA	TCCCTGGATGAGATTGGCAAA
Apln (Apelin)	GCAGGAGGAAATTTCGCAGAC	ACTTGGCGAGCCCTTCAA
Aplnr	TTGACTGGCCTTTTGGAACC	GCAAAAGACACTGGCGTACA
Atp2a2	CGGTCCAAGAGTCTCCTTCTA	GCACAATCCACTCCATCGAA
Bai1	GGTCCTGAGAAGCAAACCAA	GACCATTCGTTCCAGTTTCCA
Ccl11(Eotaxin)	CAACAACAGATGCACCCTGAA	CACAGATCTCTTTGCCCAACC
Ccl2 (mcp-1)	AGCAGCAGGTGTCCCAAA	TTCTTGGGGTCAGCACAGAC
Ccl21a	GTCAGGACTGCTGCCTTAAGTA	GCTTCCTATAGCCTCGGACAA
Cdh5	AACGAGGACAGCAACTTCAC	TGGCATGCTCCCGATTAAAC
Col18a1	CAGGACCAAAGGGTGACAAA	TTCCAGGTGGAAGAGGTCAA
Col4a3	GCTGGTACAAAGGGCAACAA	TAAGCCTGGCAATCCATCCA
Ctgf	AAGCTGACCTGGAGGAAAACA	TGCAGCCAGAAAGCTCAAAC
Cxcl1	CCTGAAGCTCCCTTGGTTCA	TTCTCCGTTACTTGGGGACAC
Cxcl10 (Inp10)	ATCCGGAATCTAAGACCATCAAGAA	GCTCTCTGCTGTCCATCCA
Cxcl5 (ena78/lix)	GGCATTTCTGTTGCTGTTCAC	TGCGGCTATGACTGAGGAA
Cxcl9	AGCCCCAATTGCAACAAAAC	TCTTCACATTTGCCGAGTCC
Cyr61	CCACACCAAGGGGTTGGAA	CACAGGGTCTGCCTTCTGAC
Edn1	CCTGGACATCATCTGGGTCAA	AACGCTTGGACCTGGAAGAA
Efna1	TGGGCAAGGAGTTCAAGGAA	GCACTGGGATTCCTGATGGTA
Efnb2	TGCCAGACAAGAGCCATGAA	GTCTTGTTGGACCGTGATTCC
Egf	GGAGAGACTGCTGAGTGTCA	AGCCAGCACACACTCATCTA
Eng	AGGCATCCAACACCATCGAA	TCTAGCTGGACTGTGACCTCA
Ephb4	CCTCACGGAATTCATGGAGAAC	ACCAGCTGGATGACTGTGAA
Erbb2 (Her2)	ATTCTCAGACGCCGGTTCA	TTGGCCCCAAAGGTCATCA
F3	ACCCAAACCCACCAACTATACC	GTGTCTGTGGTCGAGAAGCA
Fgf1 (aFGF)	TGGACACCGAAGGGCTTTTA	GCATGCTTCTTGGAGGTGTAA
Fgf2 (bFGF)	TCTTCCTGCGCATCCATCC	GCACACACTCCCTTGATAGACA
Fgfr3	AGGATTTAGACCGCATCCTCAC	CCTGGCGAGTACTGCTCAAA
Flt1	TTGCACGGGAGAGACTGAAA	GCCAAATGCAGAGGCTTGAA
Fn1	CGTCATTGCCCTGAAGAACA	AAGGGTAACCAGTTGGGGAA
Hgf	CATCAAATGCCAGCCTTGGAA	TCTTTACCGCGATAGCTCGAA
Hif1a	TCGACACAGCCTCGATATGAA	TTCCGGCTCATAACCCATCA
Hnrnpm	GATGCCAACCATCTGAGCAAA	CCAAATCCTATGCCTTCCATTCC
Hpse	GCCTCGAGGGAAGACAGTTAAA	TGCCATGTAAGAGAGTCGATCAC

Id1	ACCCTGAACGGCGAGATCA	GATCGTCGGCTGGAACACA
Ifna1	TCCACCAGCAGCTCAATGAC	TCTTCCTGGGTCAGGGGAAA
Ifng	GGCACAGTCATTGAAAGCCTA	GCCAGTTCCTCCAGATATCCA
Igf1	GAGCTGGTGGATGCTCTTCA	CTCCGAATGCTGGAGCCATA
Igf1r	ATGGAGCCTGAGAACATGGA	CCTTGTGTCCTGAGTGTCTT
Il1b	TGGCAACTGTTCCTGAACTCA	GGGTCCGTCAACTTCAAAGAAC
Il6	CCAGAAACCGCTATGAAGTTCC	GTTGTCACCAGCATCAGTCC
Il8	GGCTACTGTTGGCCCAATTAC	GCTTCATTGCCGGTGGAAA
Itgav	AAAGGCAGATGGCAAGGGAA	GGCTCCCTTCTGCTTGAGTTTA
Itgb3	CCCACCACAGGCAATCAAAA	GCGTCAGCACGTGTTTGTA
Jag1	TCCCAAGCATGGGTCTTGTA	GATGCACTTGTCGCAGTACA
Lect1	CCTGCCGATTTTCTGGCTTA	AGAGGGAGCACTGTTTCTCA
Lep	AGACCATTGTCACCAGGATCA	ATGAAGTCCAAGCCAGTGAC
Mdk	TTGCCCTCTTGGTGGTCAC	CCAGGTCCACTCCGAACAC
Mmp14	CAAGGCTGATTTGGCAACCA	GCCTTGATCTCAGTCCCAAAC
Mmp2	CGAGGACTATGACCGGGATA	GGGCACCTTCTGAATTTCCA
Mmp9	TCCCCAAAGACCTGAAAACC	GGGTGTAACCATAGCGGTAC
Neat1	GGGAAGCTGATTGCCAAGAA	ATGGTTTCAGAGCCCACAAC
P54nrb	TGGTACTCCAGCTCCTCCA	CAGCTTGGCCAAAACGTTCA
Nos3	GGGATTCTGGCAAGACAGACTA	GCAGCCAAACACCAAAGTCA
Notch4	ACCTGCTTGCAACCTTCCA	GGTGCACTCATTGACCTCCA
Nrp1	CCTGTATCCTGGGAAACTGGTA	GCCCAACATTCCAGAGCAA
Nrp2	GTGGATCAGCAGCGCTAAC	GCCATCACTCTGCAGTTTCAA
PAI1 (serpinE1)	CAGACAATGGAAGGGCAACA	GAGGTCCACTTCAGTCTCCA
Pdgfa	TGTAACACCAGCAGCGTCAA	GGCTTCTTCCTGACATACTCCA
Pecam1	GCACAGTGATGCTGAACAAC	GTCACCTTGGGCTTGGATAC
Pf4	CCAGCCTGGAGGTGATCAA	GGCAAATTTTCCTCCCATTCTTCA
Pgf	CCAATCGGGATCCACATTTCTA	GCCTTTGTCGTCTCCAGAATA
Plau (upa)	TAGCCTAGGCCTGGGGAAA	AGGCCAATCTGCACATAGCA
Plg	TGGAATTGCCCACAGTTTCC	CCGATAGTCTTTGCCATTCCC
Prok2	GGCTTGGCGTGTTTAAGGAC	GGGTCGCATTTCAAGTTCCTAC
Prox1	GCCCTCAACATGCACTACAAC	CGTGATCTGCGCAACTTCC
Psf/Sfpq	TGAAAAGCTGGCCCAGAAGAA	TGTGCCATGCTGAGCAAAAC
Pspc1	TCCCCGTGGAGCAATAAACA	ATACCCATCATTGGAGGAGGAC
Ptgs1	TATCACCTGCGGCTCTTCAA	GTTCCACGGAAGGTGGGTA
S1pr1	CGGTGTAGACCCAGAGTCC	GAGAGGCCTCCGAGAAACA
SerpinF1	AGAACCTCAAGAGTGCTTCCA	TTCTCCAGAGGGGCAACAAA
Sphk1	GGCAGCTTCTGTGAACCACTA	CAGCAGGTTCATGGGTGACA
Tek	GTTGGATGGCAATCGAATCAC	CCAGAGCAATACACCATAGGAC
Tgfa	CCCTGGCTGTCCTCATTATCA	CAGTGTTTGCGGAGCTGAC
Tgfb1	GCTGCGCTTGCAGAGATTAA	GTAACGCCAGGAATTGTTGCTA
Tgfb2	GCCCATATCTATGGAGTTCAGACA	AGCGGAAGCTTCGGGATTTA

Tgfbr1	AATTGCTCGACGCTGTTCTA	ACCGATGGATCAGAAGGTACA
Thbs1	CCCCAGAAGACATTCTCAGGAA	CGTTCACCACGTTGTTGTCA
Thbs2	GACTGCACGTCATGGTGAAC	CCCAATGAGCTCCAAAAGGAAC
Tie1	CCTTTGCTCAGATCGCACTA	CTCAAACAGCGACATGTTCAC
Timp1	TCCCCAGAAATCAACGAGACC	CATTTCCCACAGCCTTGAATCC
Timp2	GAAGAGCCTGAACCACAGGTA	TCATCCGGGGAGGAGATGTA
Timp3	CCCTTTGGCACTCTGGTCTA	ACGTGGGGCATCTTACTGAA
Tnf	CAAATGGCCTCCCTCTCATCA	TGGGCTACAGGCTTGTCAC
Tymp	GGCACACTGGATAAGCTGGAA	CAGCAGCCGACTTCCTCAA
Vash1	GGCTGCCAAGTTGGGGTGTGTT	AAACCAGGGCGTGGCTCCTGTA
Vegfa	CCAGCACATAGGAGAGATGAG	CTGGCTTTGTTCTGTCTTTCTT
Vegfb	GAGATGTCCCTGGAAGAACACA	TGGCTTCACAGCACTCTCC
Vegfc	AGACGTTCTCTGCCAGCAA	AGGCATCGGCACATGTAGTTA
Vegfd (figf)	TCCATTCAGACCCCAGAAGAA	GTGTTATCCCACAGCATGTCA
Vegfr2 (Kdr)	ATTTCACCTGGCACTCTCCA	TCCCAGGAAAGGGTTTCACA
Vegfr3 (Flt4)	CTCGCTCGGGACATCTACAAA	GGGCCATCCATTTCAGAGGAA
18S	CAACTAAGAACGGCCATGCA	AGCCTGCGGCTTAATTTGAC

EV Table 6. List of genes and primer couples used in the Fluidigm Deltagene PCR array.

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siControl (5’ -> 3’)	siVASH1 (5’->3’)
ACCAAAUGUACAGCUGAUU	GACACUAGGACCCUUAAAU
ACCAAAUGUACAAAAGACU	CGAAGUUCUGGAUAAAGAG
ACCAAAUGUACAAAAGGAU	CCCUCCUGGACUACAUGUU
ACCAAAUGUACAACACACU	CAUGUUAGUGUGUCCCUGU

EV Table 7. SiRNA sequences. The sequences of the four siRNAs present in the siControl and siVASH1 Smartpools are indicated.