Figures and figure supplements

Long non-coding RNA *Neat1* and paraspeckle components are translational regulators in hypoxia

Anne-Claire Godet and Emilie Roussel *et al*.

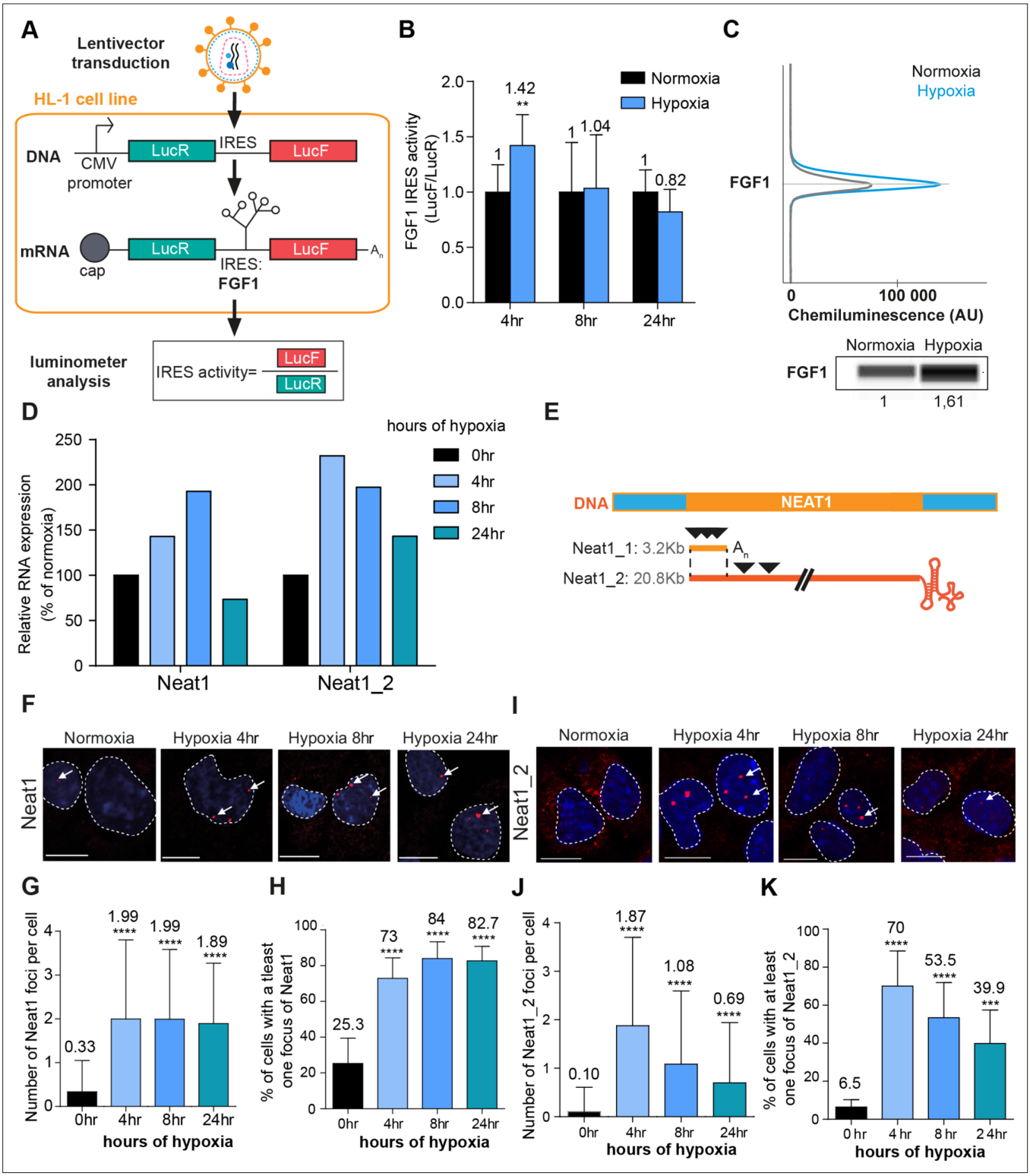

Figure 1. *FGF1* IRES activation during hypoxia correlates with *Neat1* induction and paraspeckle formation. (**A**) Schema depicting the Lucky Luke bicistronic construct and HL-1 cells transduced by a lentivector carrying the transgene. The LucF/LucR ratio indicates the IRES activity. (**B**) Activity of the human *FGF1* IRES in HL-1 cardiomyocytes at 4 hr, 8 hr, or 24 hr of hypoxia normalized to normoxia. The corresponding luciferase values are presented in ***Figure 1—figure supplement 1***, ***Supplementary file 1***. (**C**) Detection of endogenous mouse FGF1 by capillary Simple Western in normoxic and hypoxic (2 hr) cardiomyocytes. The curve corresponds to the chemiluminescence signal detected with FGF1 antibody. A numerical blot is represented. Below the blot is shown the quantification of FGF1 normalized to total proteins and to control gapmer. Total proteins are detected by a dedicated

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Figure 1 continued

channel in capillary Simple Western. The full raw unedited gel is provided in ***Figure 1—figure supplement 1*** (***Figure 1—figure supplement 1—source data 1***). (**D**) HL-1 cells were subjected to normoxia (0 hr) or to hypoxia during 4 hr, 8 hr, and 24 hr. *Neat1* and *Neat1_2* expression was analyzed by droplet digital PCR (Primer sequences in ***Supplementary file 2***). RNA expression is normalized to the normoxia time point. (**E**) Schema depicting the *Neat1* mouse gene and the *Neat1_1* and *Neat1_2* RNA isoform carrying a poly(A) tail or a triple helix, respectively. Black arrowheads represent FISH probes against *Neat1* and *Neat1_2* (sequences in ***Supplementary file 2***). (**F–K**) *Neat1* (**F**) or *Neat1_2* (**I**) FISH labeling in HL-1 cardiomyocytes in normoxia or at 4 hr, 8 hr, and 24 hr of 1% O_2. DAPI staining is represented in blue and *Neat1* or *Neat1_2* cy3 labeling in red. Nuclei are delimited by dotted lines. Scale bar = 10 µm. Larger fields are presented in ***Figure 1—figure supplement 2***. (**G and J**) Quantification of *Neat1* (**G**) or *Neat1_2* (**J**) foci per cell by automated counting (ImageJ). (**H and K**) Percentage of cell harboring at least one focus of *Neat1* (**H**) or *Neat1_2* (**K**); Histograms correspond to means ± standard deviation, with Mann-Whitney (n=12) (**B**) or one-way ANOVA (**G-H**, n=269–453) and (**J-K**, n=342–499); **p<0.01, ***<0.001, ****p<0.0001.

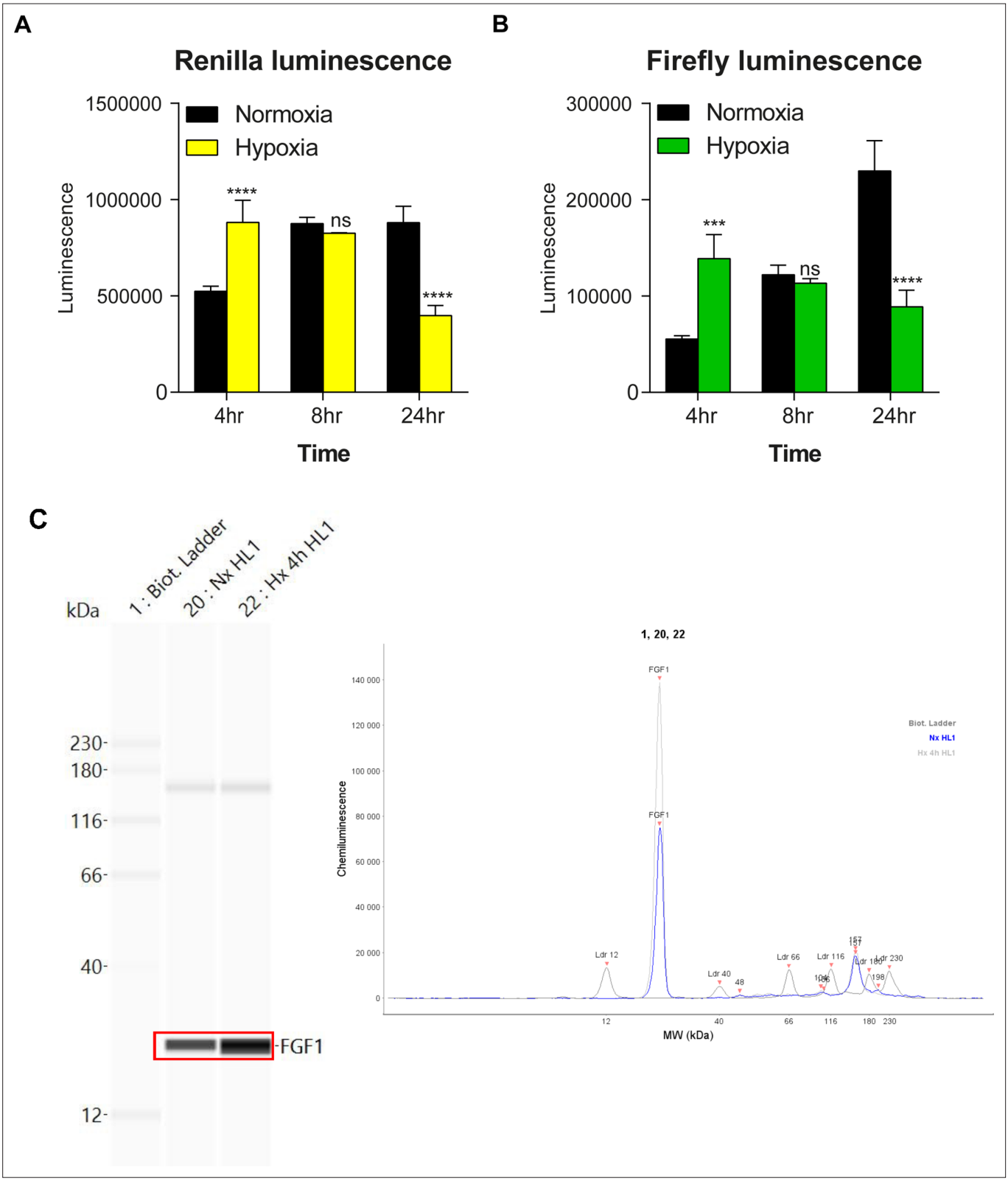

Figure 1—figure supplement 1. Bicistronic vector LucR and LucF expression and endogenous FGF1 protein expression in hypoxic HL-1 cells. Renilla (**A**) and firefly (**B**) luciferase activities were measured in HL-1 cardiomyocytes transduced by the bicistronic vector with the *FGF1* IRES, after 4 hr, 8 hr, or 24 hr of hypoxia or normoxia. The experiments were performed with triplicates from four distinct transduced cell samples (***Supplementary file 1***). The graphs show a representative triplicate experiment. (**C**) Raw gel and chemiluminescence curve of endogenous FGF1 expression analyzed by capillary Simple Western. Source data of the capillary Simple Western are provided (***Figure 1—figure supplement 1—source data 1***).

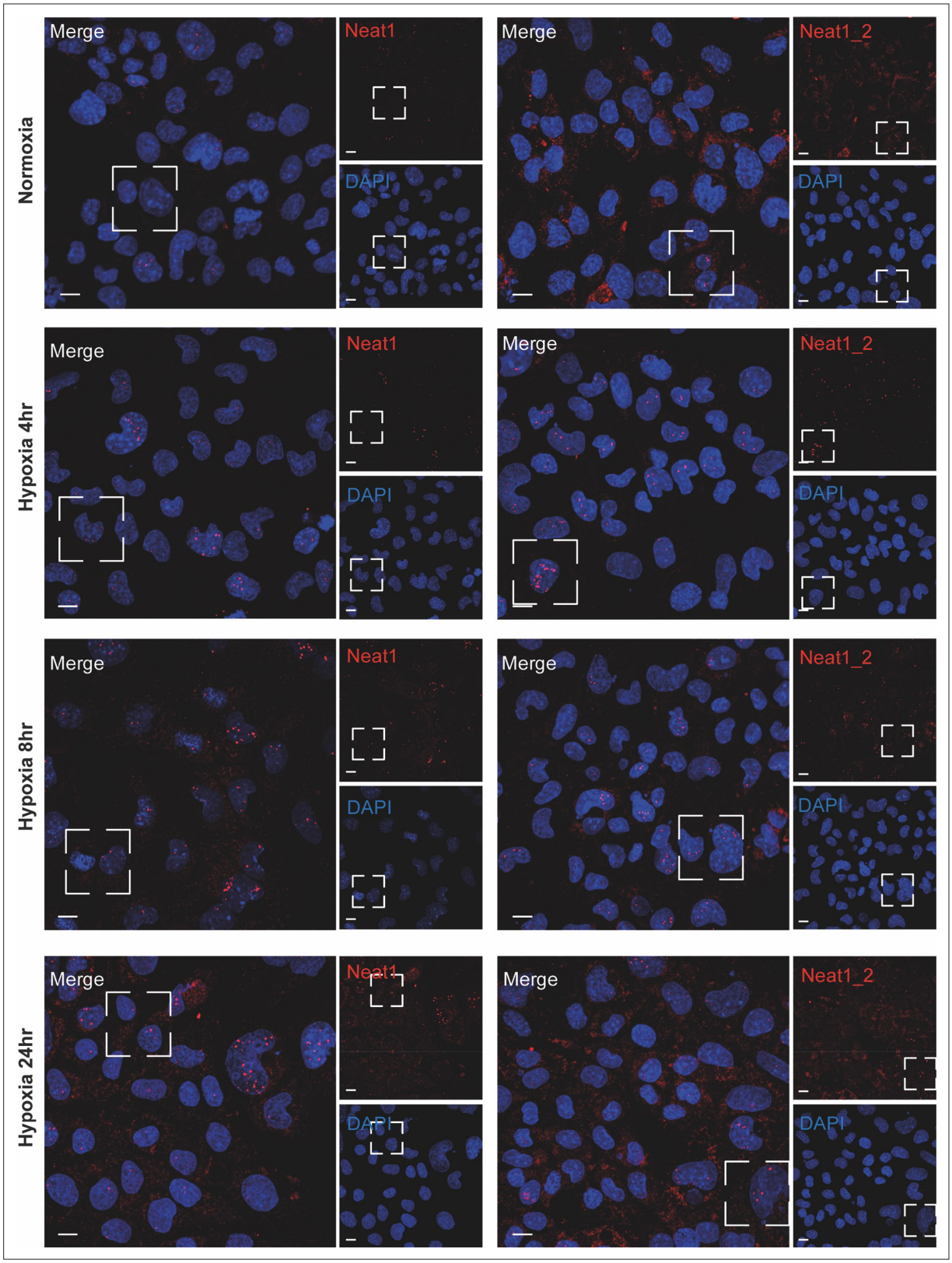

Figure 1—figure supplement 2. Detection of *Neat1* and *Neat1_2* in hypoxic HL-1 by FISH. FISH experiment with representative images of *Neat1* (both isoforms)(left panels) or *Neat1_2* isoform (right panels) in normoxia and hypoxia at 4 hr, 8 hr, 24 hr in HL-1 cardiomyocytes. DAPI, *Neat1* cy3 or *Neat1_2* cy3 labelling and merge. The open square represents magnified zones presented in ***Figure 1F and I***. Scale bar: 10μm.

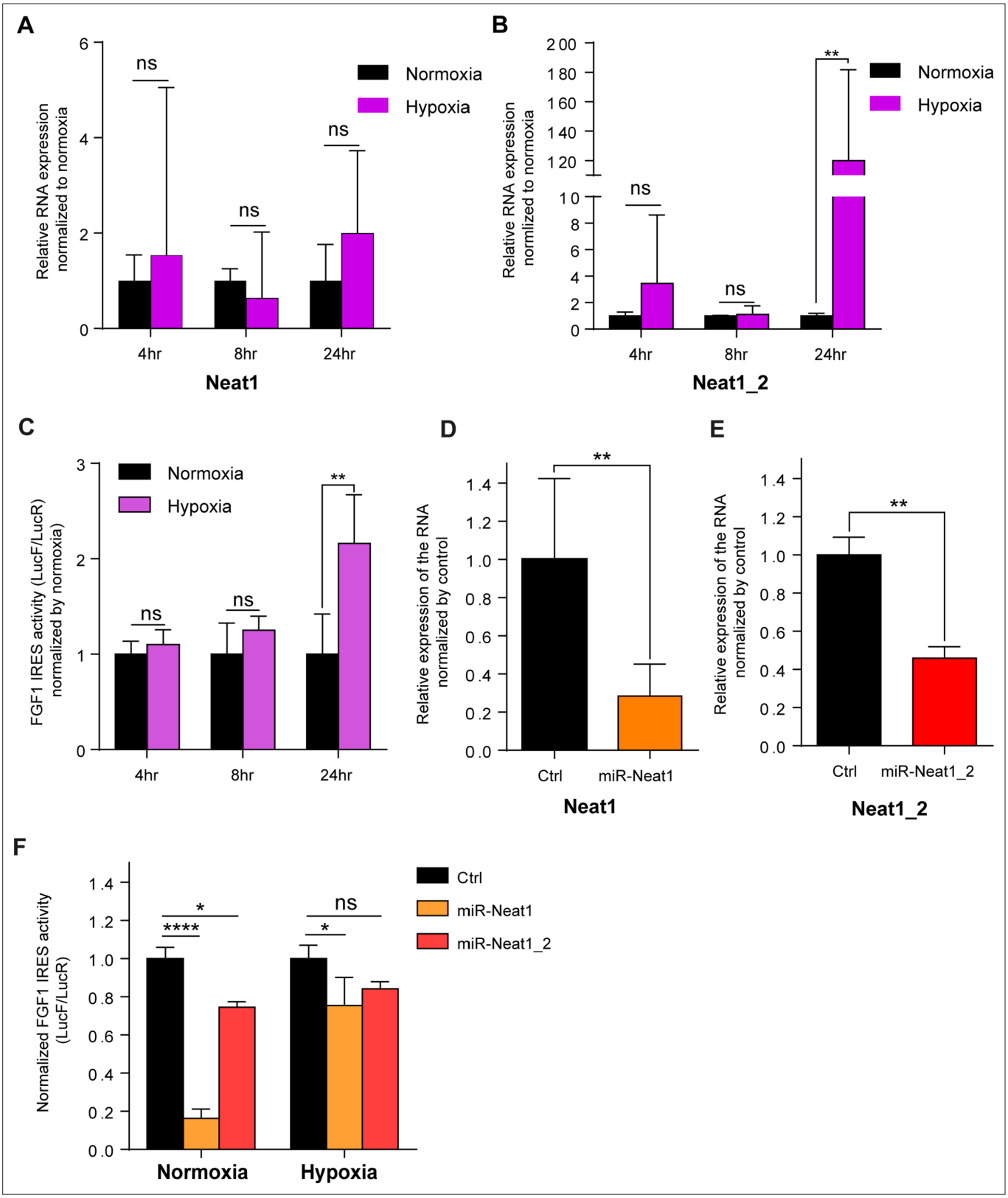

Figure 1—figure supplement 3. *FGF1* IRES is activated by hypoxia in correlation with *Neat1* induction in 67NR cells and inactivated after *Neat1* knock-down. (**A–B**) 67NR cells (mouse breast cancer) were subjected to normoxia (0 hr) or to hypoxia (1% 0_2) during 4 hr, 8 hr and 24 hr. *Neat1* (**A**) or *Neat1_2* (**B**) expression was analyzed by RT qPCR (Primer sequences in ***Supplementary file 2***). RNA expression is normalized to normoxia time point. (**C**) Activity of the human *FGF1* IRES in 67NR cells at 4 hr, 8 hr, or 24 hr of hypoxia normalized to normoxia. (**D–E**) *Neat1* or *Neat1_2* knock-down obtained by

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transduction of 67NR cells with lentivectors expressing an artificial miRNA targeting *Neat1* (miR-Neat1, **D**) or *Neat1_2* (miR-Neat1_2, **E**). To knock down *Neat1_2*, a pool of two lentivectors coding two different miRNAs was used (sequences in ***Supplementary file 2***). (**C**) Activity of the human *FGF1* IRES in 67NR cells transduced with lentivector miR-Neat1 or miR-Neat1_2 and submitted to normoxia or 24 hr of hypoxia 1% O_2. Statistics were performed by two-way ANOVA with multiple comparison Dunnet test. Normoxia versus hypoxia for each time. *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001.

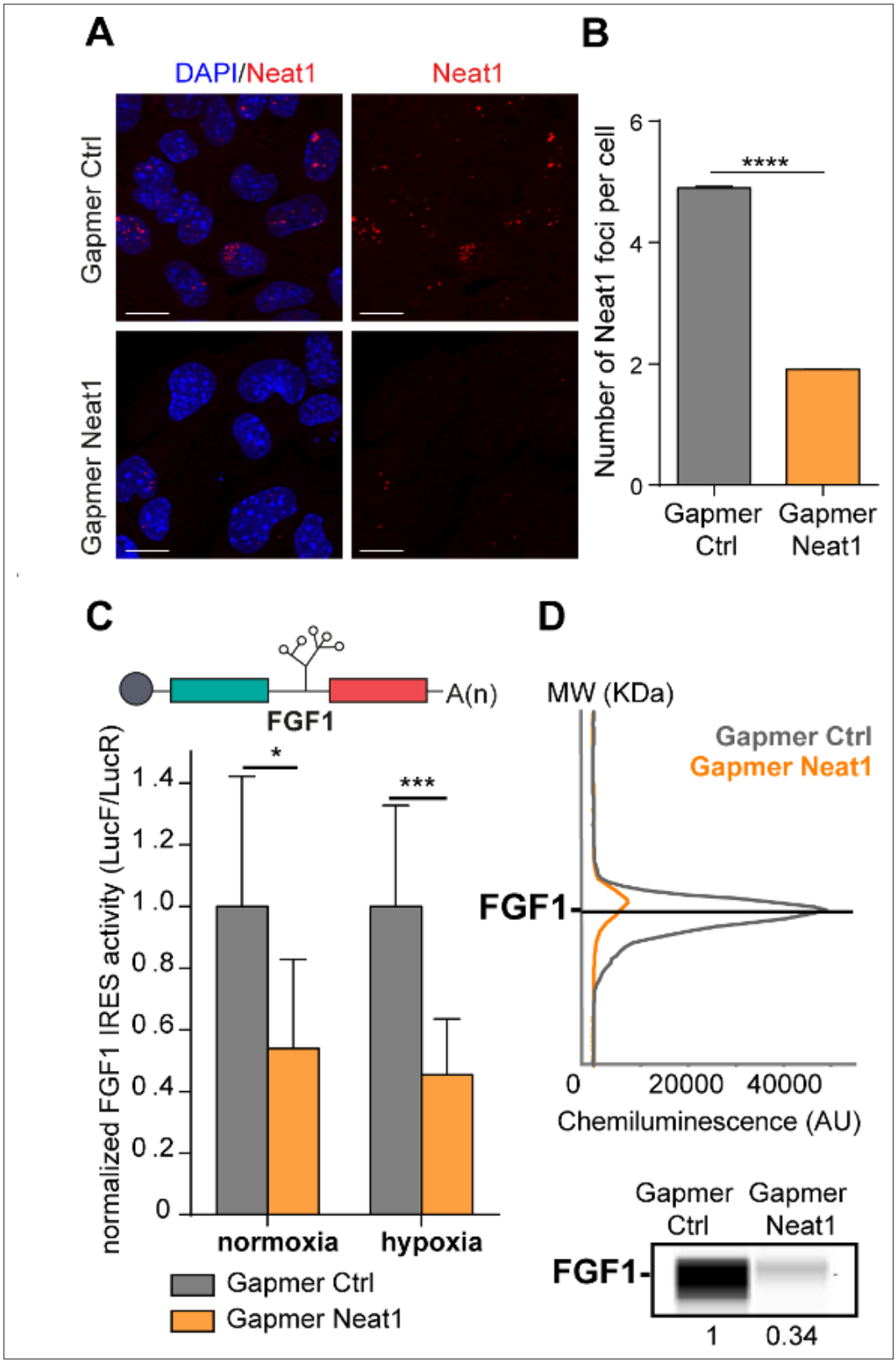

Figure 2. LncRNA *Neat1* knock-down drastically affects the *FGF1* IRES activity and endogenous FGF1 expression. (**A**) SmiFISH imaging of *Neat1* knock-down by a pool of LNA gapmers targeting both isoforms (Sequences in ***Supplementary file 2C***). Cells were treated during 48 hr with the gapmers. Scale bar = 10 µm. (**B**) *Neat1* foci counting per cell for the control gapmer and *Neat1* LNA gapmer pool, using unpaired two-tailed student t-test

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with n=249 for control and 187 for Neat1 LNA gapmer. (**C**) *FGF1* IRES activities in HL-1 cells transduced with Lucky Luke bicistronic reporter and treated with gapmer *Neat1* or control during normoxia or hypoxia (1% O_2). Histograms correspond to means ± standard deviation of the mean. Non-parametric Mann-Whitney test was performed with n=9. *p<0.05, ***<0.001, ****p<0.0001. The mean has been calculated with nine cell culture biological replicates, each of them being already the mean of three technical replicates (27 technical replicates in total). Detailed values of biological replicates are presented in ***Supplementary file 3***. (**D**) Detection of endogenous mouse FGF1 by capillary Simple Western. The curve corresponds to the chemiluminescence signal detected with FGF1 antibody. A numerical blot is represented. Below the blot is shown the quantification of FGF1 normalized to total proteins and to control gapmer. The source data of the capillary Simple Western are provided in ***Figure 2—figure supplement 2***. Total proteins are detected by a dedicated channel in capillary Simple Western.

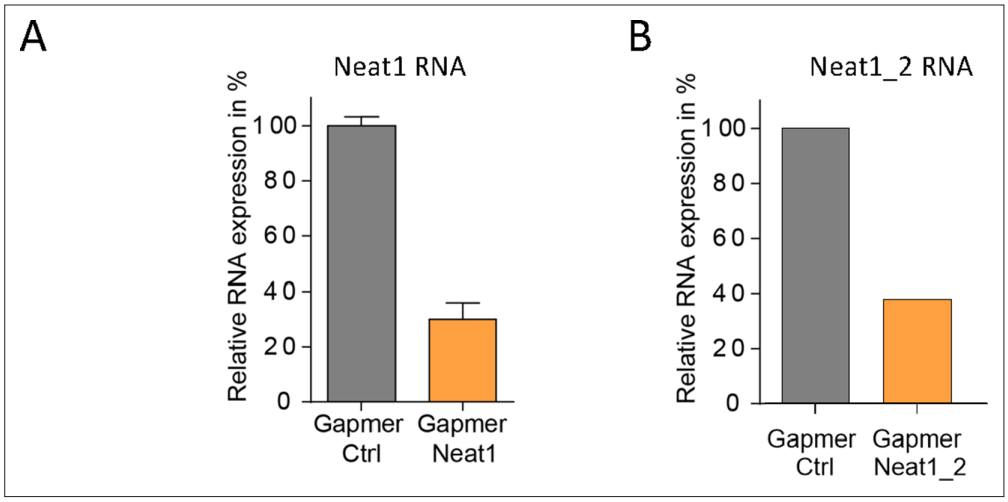


Figure 2—figure supplement 1. Knock-down of *Neat1* and *Neat1_2* in HL-1 cardiomyocytes. *Neat1* knock-down was performed in HL-1 cells using pooled LNA gapmers against *Neat1* (48 hr)(**A**) or *Neat1_2* (72 hr)(**B**). *Neat1* and *Neat1_2* RNA expression was measured by droplet digital PCR and normalized to gapmer control (Ctrl) at 100% expression.

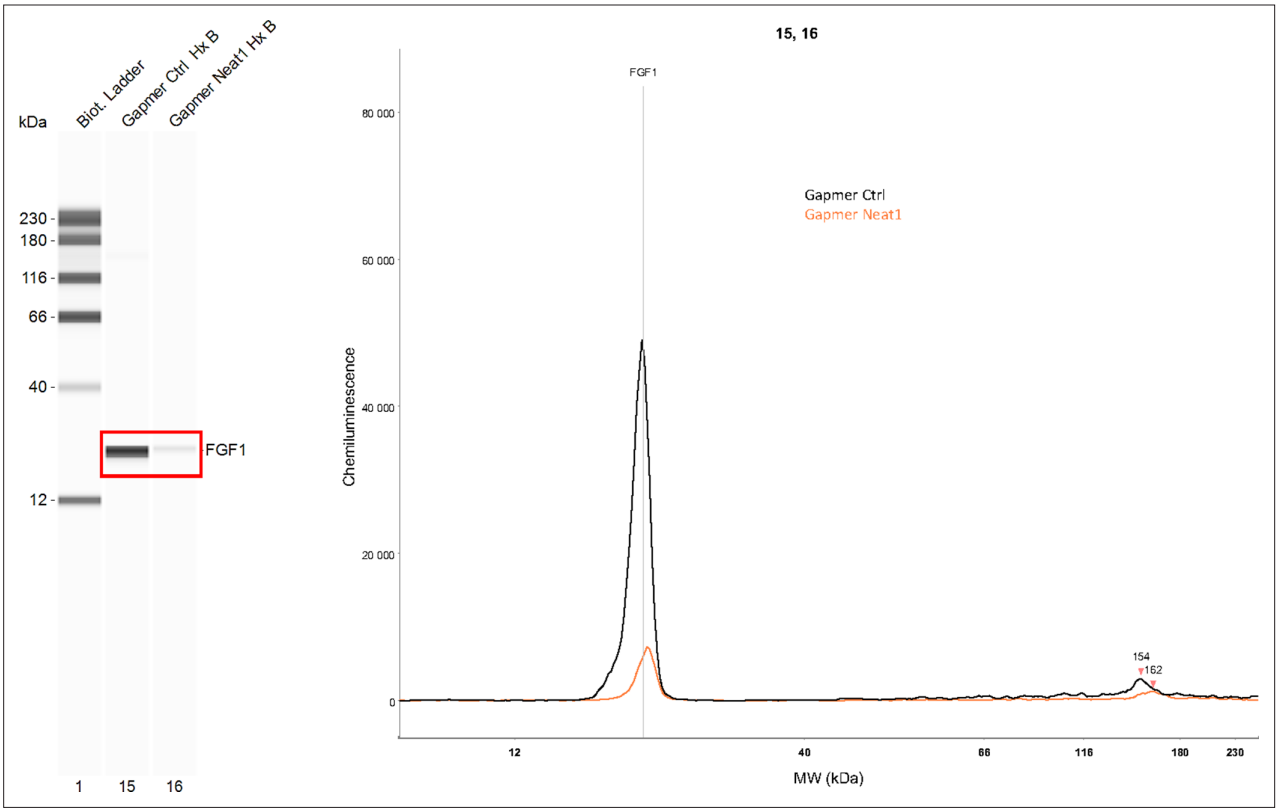


Figure 2—figure supplement 2. Effect of *Neat1* knock-down on endogenous FGF1 protein expression. Endogenous mouse FGF1 was detected using anti-FGF1 antibody in hypoxic HL-1 cells treated either by control gapmer (ctrl) or *Neat1_2* gapmer by capillary Simple Western. The source data of the capillary Simple Western are presented (***Figure 2—figure supplement 2—source data 1***). The curve corresponds to the chemiluminescence signal detected with FGF1 antibody.

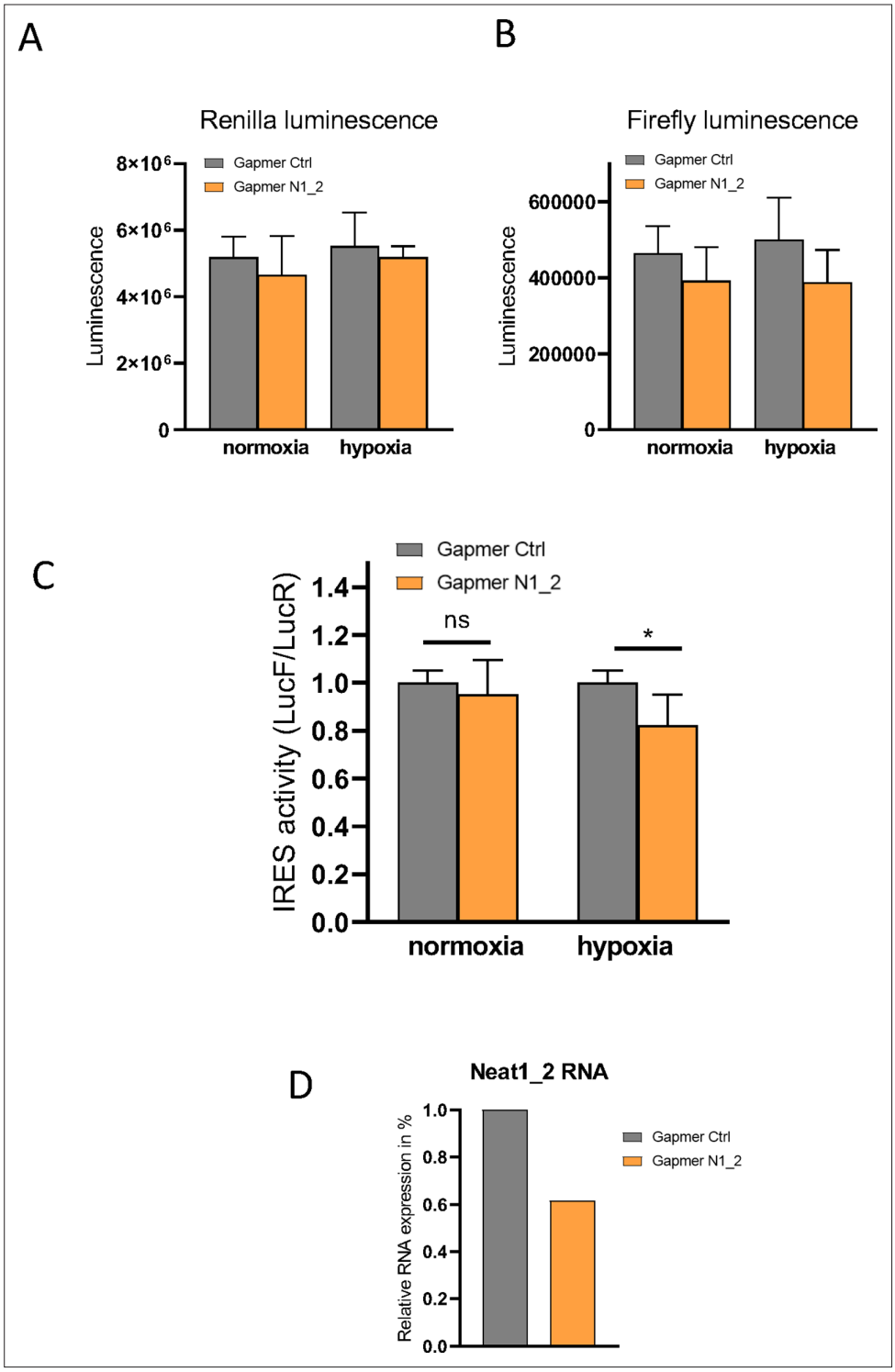
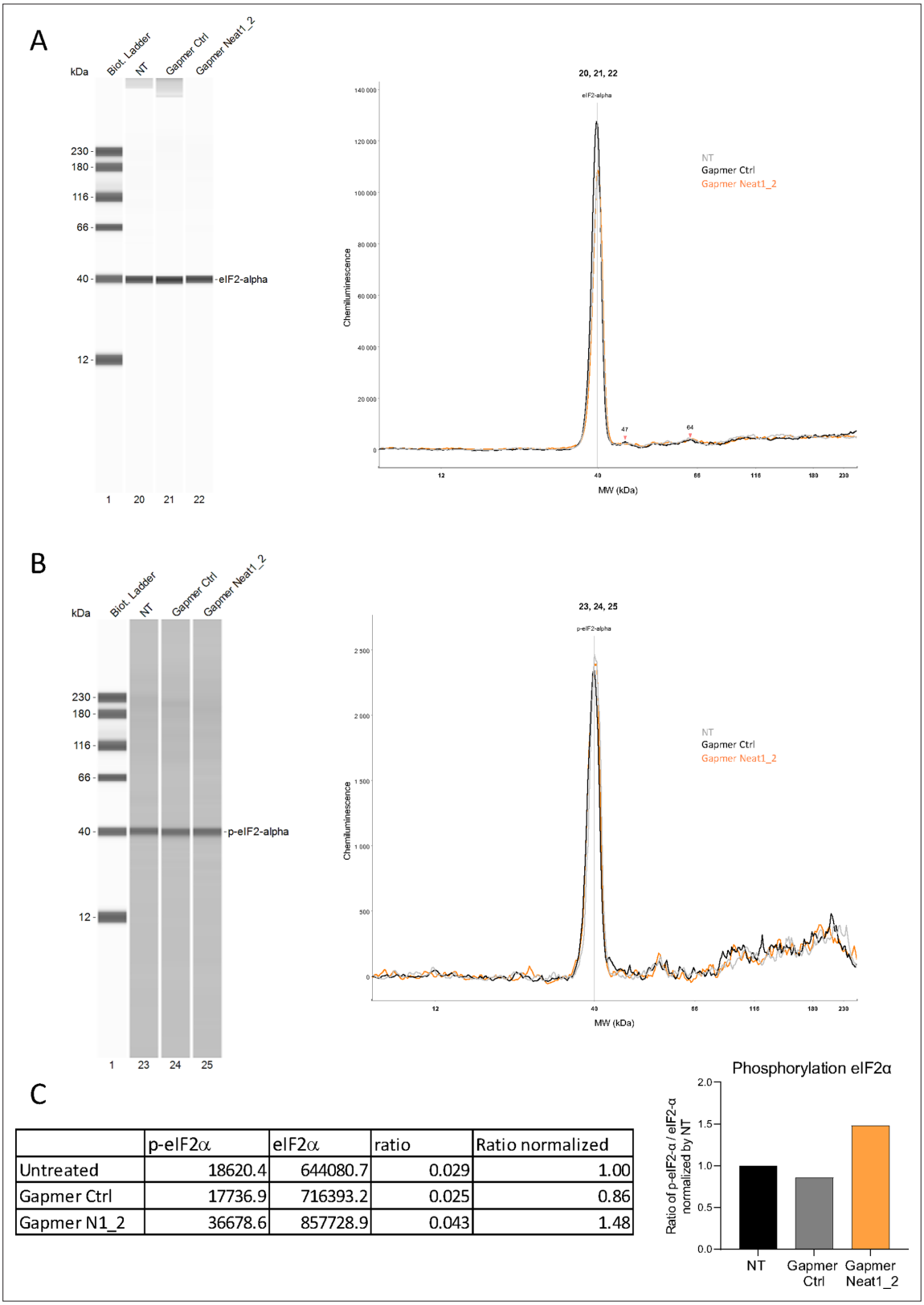
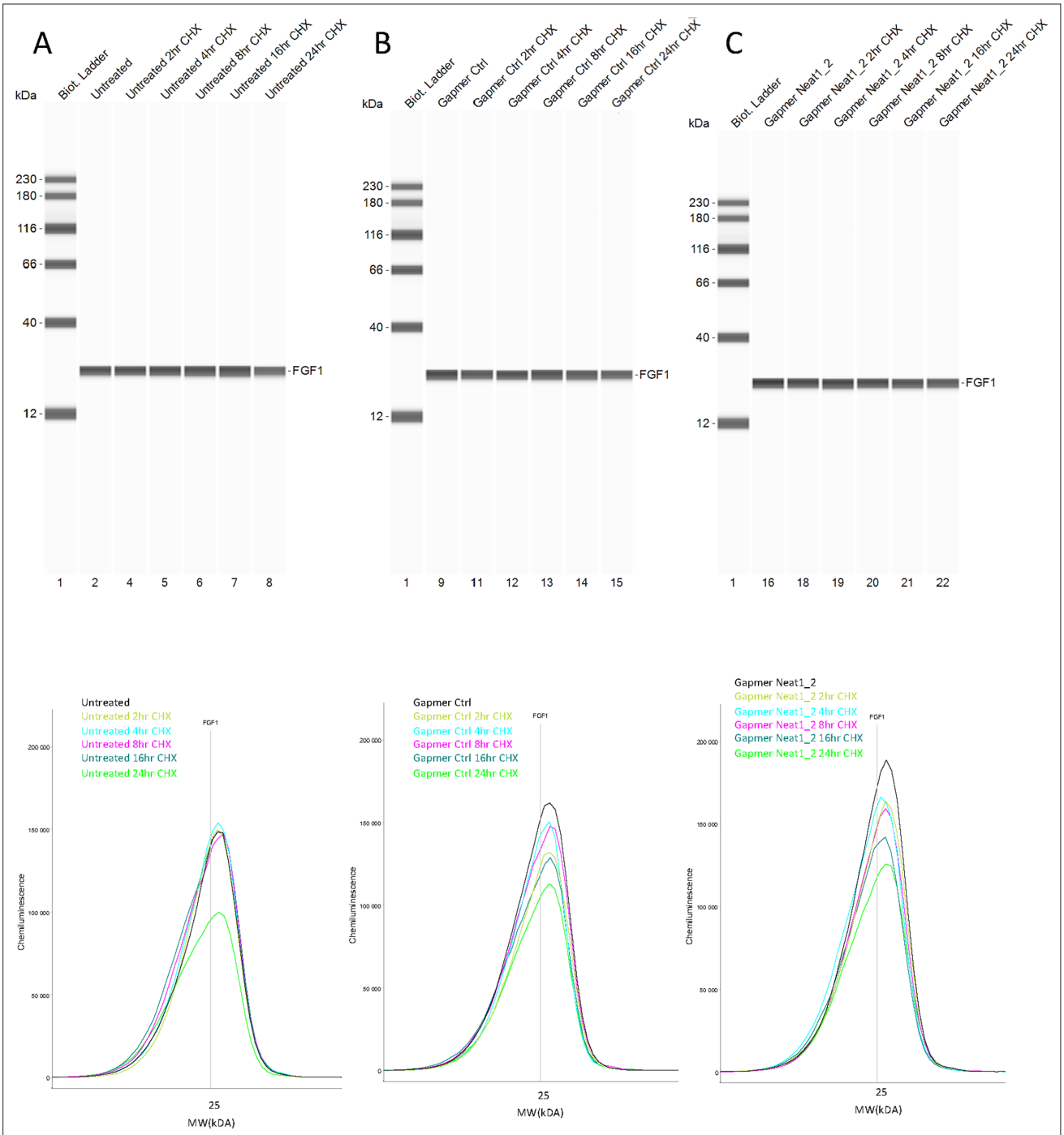


Figure 2—figure supplement 3. Effect of *Neat 1_2* knock-down on *FGF1* IRES activity. *Neat1-2* knock-down was performed in HL-1 cells transduced by the bicistronic lentivector with the *FGF1* IRES during normoxia or hypoxia (1% O_2). Luciferase activities as well as the LucF/LucR ratios (defined as IRES activities) are presented. (**A–B**) LucR and LucF activities. (**C**) *FGF1* IRES activities. (**D**) *Neat1_2* RNA expression was measured by RT-qPCR and normalized to control gapmer.



	p-eIF2α	eIF2α	ratio	Ratio normalized
Untreated	18620.4	644080.7	0.029	1.00
Gapmer Ctrl	17736.9	716393.2	0.025	0.86
Gapmer N1_2	36678.6	857728.9	0.043	1.48

Figure 2—figure supplement 4. Effect of *Neat1_2* knock-down on eIF2α phosphorylation. Expression and phosphorylation of eIF2α in HL-1 cells either untreated (NT) or treated by control gapmer (ctrl) or *Neat1_2* gapmer were measured using anti-eIF2α (**A**) and anti-phospho-eIF2α antibodies (**B**), respectively, by Jess capillary Simple Western, normalized to the Jess quantification of total proteins. The ratio p-eIF2α/ eIF2α was calculated and normalized by untreated cells (**C**) The source data of the capillary Simple Western are provided (***Figure 2—figure supplement 4—source data 1***).



Percentage of FGF1

CHX time	0 hr	2 hr	4 hr	8 hr	16 hr	24 hr
Untreated	100%	94%	93%	92%	86%	79%
Gapmer Ctrl	100%	92%	83%	89%	85%	87%
Gapmer Neat1_2	100%	92%	85%	77%	77%	87%

Figure 2—figure supplement 5. FGF1 half-life after *Neat1_2* gapmer treatment. (**A–E**) FGF1 half-life was determined in HL-1 cells treated with control gapmer, *Neat1_2* gapmer or untreated. The half-life determination was performed by blocking protein synthesis with cycloheximide at 10 µg/mL, with time-course points at 0 hr, 2 hr, 4 hr, 8 hr, 16 hr, and 24 hr. FGF1 protein stability was measured bu capillary western. P21 was used as a control for its short half life (***Figure 2—figure supplement 6***). Capillary western are presented, showing FGF1 protein during the cycloheximide time course in HL-1

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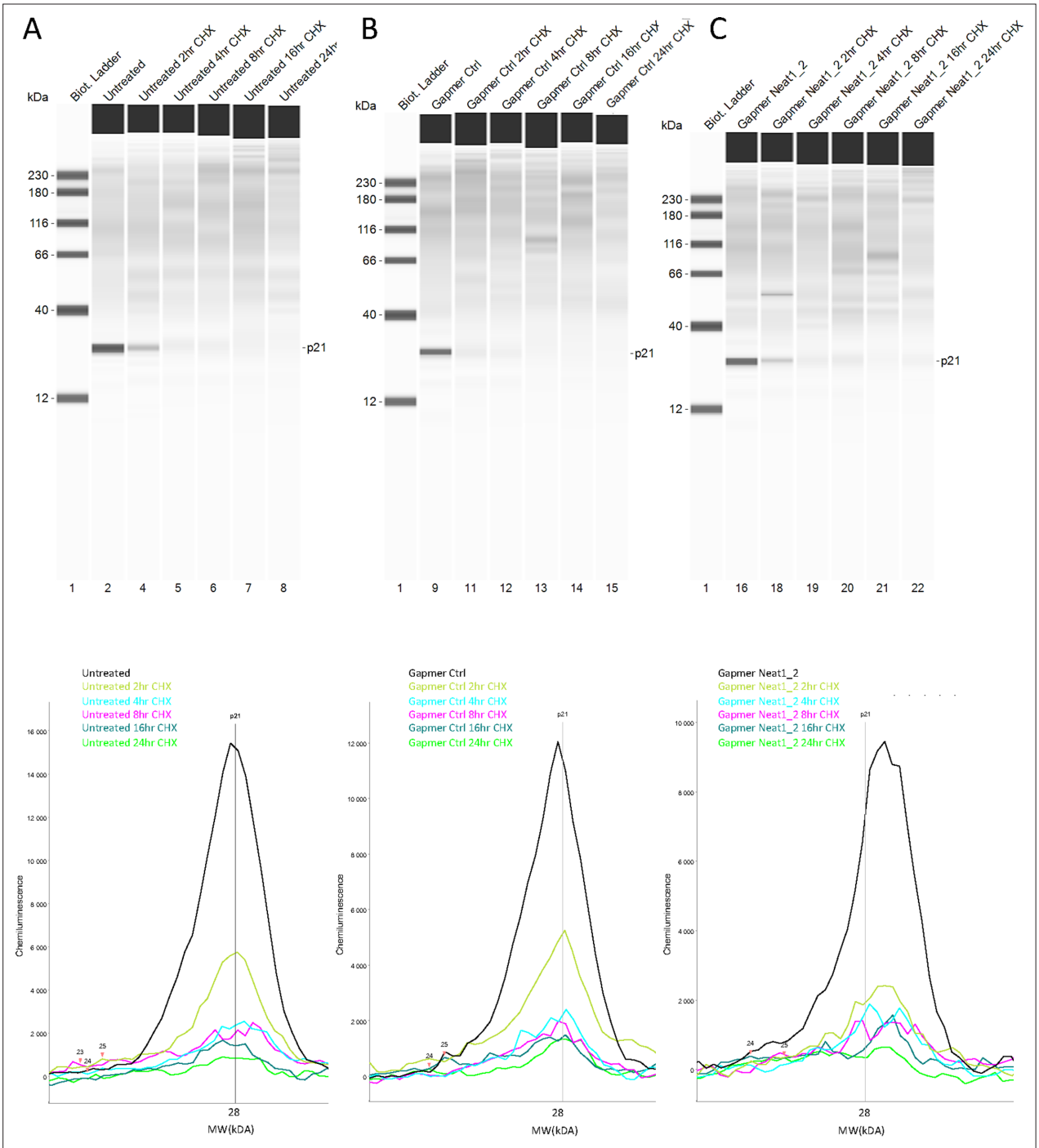
cells untreated (**A**), treated with gapmer control (**B**) or with gapmer *Neat1_2*. (**C**) Percentage of FGF1 is normalized to the 0 hr time course point. Source data of capillary Simple Western are provided (***Figure 2—figure supplement 5—source data 1*** and ***Figure 2—figure supplement 6—source data 1***)



Percentage of p21

CHX time	0 hr	2 hr	4 hr	8 hr	16 hr	24 hr
Untreated	100%	35%	17%	17%	9%	6%
Gapmer Ctrl	100%	34%	23%	24%	11%	10%
Gapmer Neat1_2	100%	50%	17%	13%	ND	10%

Figure 2—figure supplement 6. p21 half-life after *Neat1_2* gapmer treatment. (**A–E**) p21 half-life half-life was determined as a control in HL-1 cells treated with control gapmer, *Neat1_2* gapmer or untreated, in the experiment shown in ***Figure 2—figure supplement 5***. p21 protein stability was measured by capillary western, showing p21 protein level during the cycloheximide time course in HL-1 cells untreated (**A**) treated with gapmer control (**B**) or with gapmer *Neat1_2*. (**C**) Percentage of p21 is normalized to the 0 hr time course point. Source data of capillary Simple Western are provided (***Figure 2—figure supplement 5—source data 1*** and ***Figure 2—figure supplement 6—source data 1***).

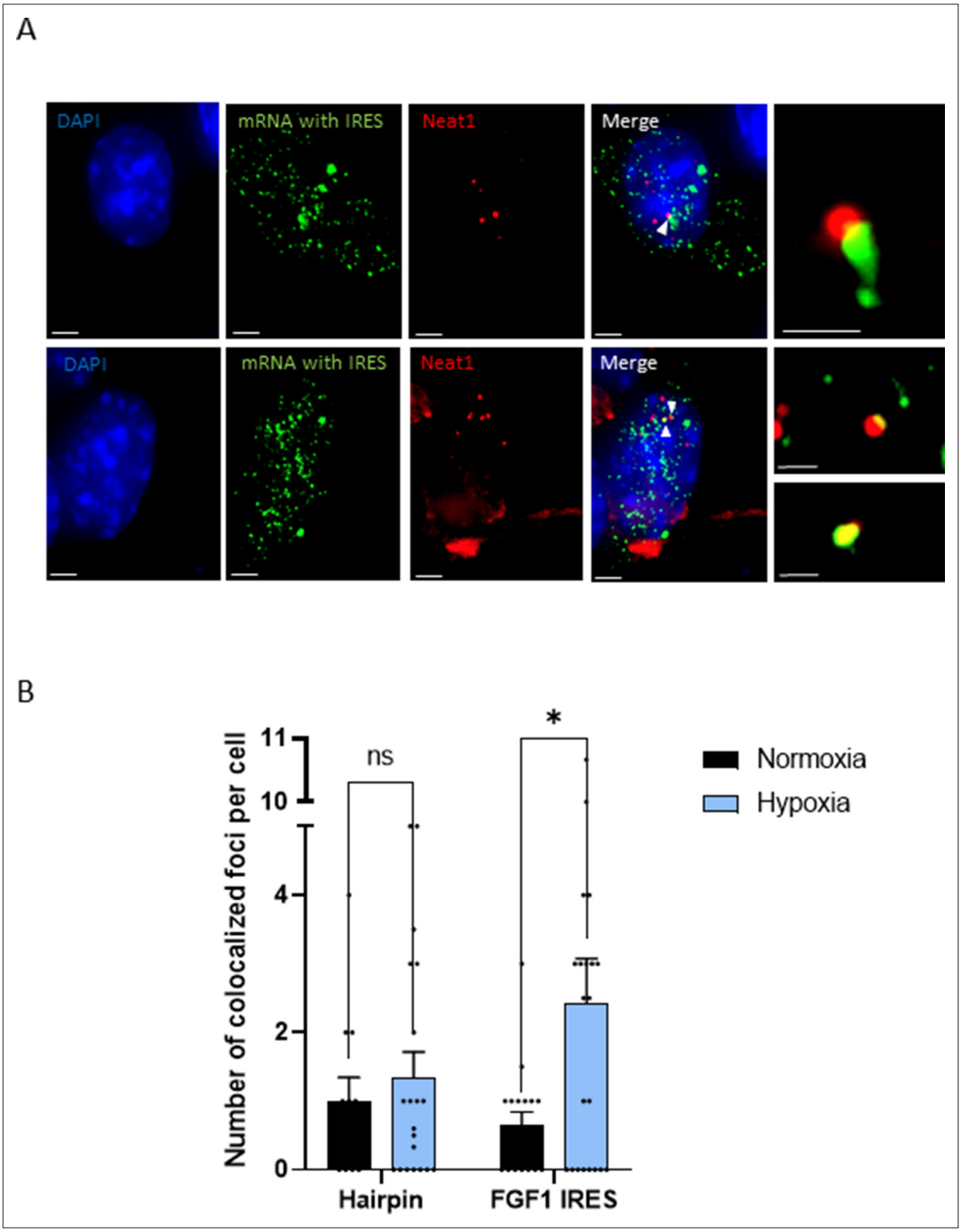


Figure 3. IRES-containing mRNA is colocalized with *Neat1* in hypoxic HL-1 cells. Cells were transduced with lentivectors carrying bicistronic Lucky Luke constructs with the *FGF1* IRES or a hairpin (control), subjected or not to 4 hr hypoxia. SmiFISH experiments were performed. (**A**) SmiFISH images showing the bicistronic mRNA carrying the *FGF1* IRES (green) colocalized with *Neat1* RNA (red) in hypoxia condition. Two representative cells are presented. Scale bars are 3 µm for higher panels, 4 µm for lower panesl and 1 µm for zoomed images of colocalized spots. (**B**) Quantification of colocalized spots per cell (n=30). Unpaired two-tailed Student T-test was performed.

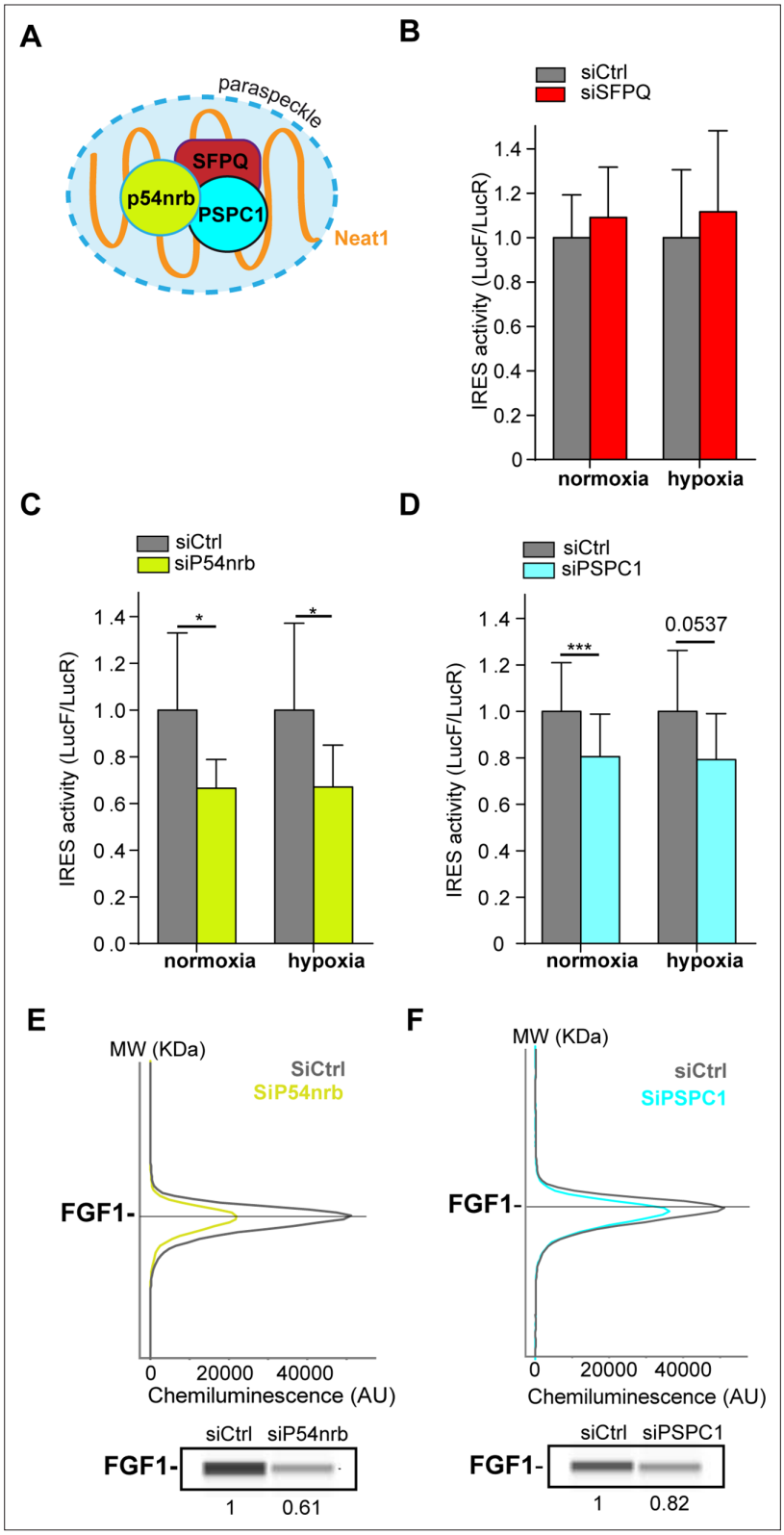


Figure 4. Paraspeckle proteins p54^{nrb} and PSCP1, but not SFPQ, are ITAFs of the *FGF1* IRES. (**A**) Schema of paraspeckle and DBHS proteins. (**B–D**) *FGF1* IRES activity upon knock-down of SFPQ (**B**), P54^{nrb} (**C**) or PSPC1 (**D**) in HL-1 cell (***Figure 4—figure supplement 1—source data 1***) transduced with Lucky Luke bicistronic reporter during normoxia or hypoxia was measured as in ***Figure 2***. Cells were harvested 72 hr after siRNA treatment. The IRES activity values have been normalized to the control siRNA. Histograms correspond to means ± standard

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deviation of the mean, with a non-parametric Mann-Whitney test with n=9; *p<0.05, ***<0.001. The mean has been calculated with nine cell culture biological replicates, each of them being already the mean of three technical replicates (27 technical replicates in total). Detailed values of biological replicates are presented in ***Supplementary file 3***, ***Supplementary file 4***, ***Supplementary file 5***. (**E and F**) Capillary Simple Western detection of endogenous FGF1 protein with $P54^{nrb}$ (**E**) or PSPC1 (**F**) knock-down. Source data of capillary Simple Western are presented in ***Figure 4—figure supplement 2*** (***Figure 4—figure supplement 2—source data 1***).

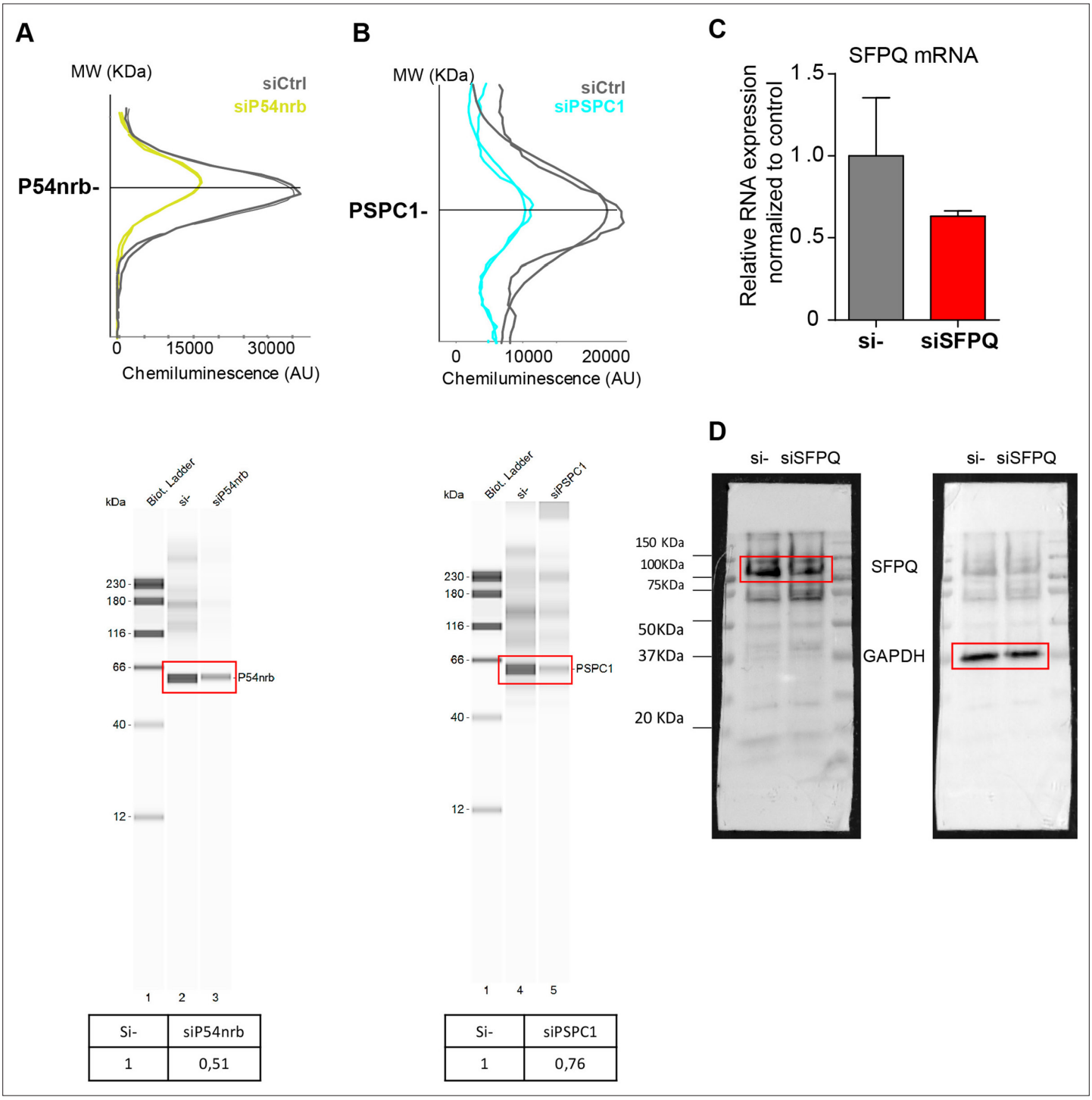


Figure 4—figure supplement 1. Knock-down of p54^{nrb}, PCPC1 and SFPQ in HL-1 cardiomyocytes. (**A–B**) Capillary Simple Western detection (as described in ***Figure 2***) of p54^{nrb} (**A**) and PSPC1 (**B**) proteins using anti-p54nrb and anti-PSPC1 antibodies, respectively, following p54^{nrb} and PSPC1 knock-down using siRNAs. Western detection was performed 72 hr after siRNA treatment. (**C–D**) SFPQ knock-down was performed in HL-1 cells using siRNA against SFPQ. SFPQ RNA expression was measured by RT-qPCR and normalized to control siRNA (**C**). One representative experiment is shown with n=2 biological replicates. SFPQ protein expression was visualized by Western Blot using an anti-SFPQ antibody (**D**). Histograms correspond to means ± standard deviation. Source data of capillary Simple Western are provided (***Figure 4—figure supplement 1—source data 1***).

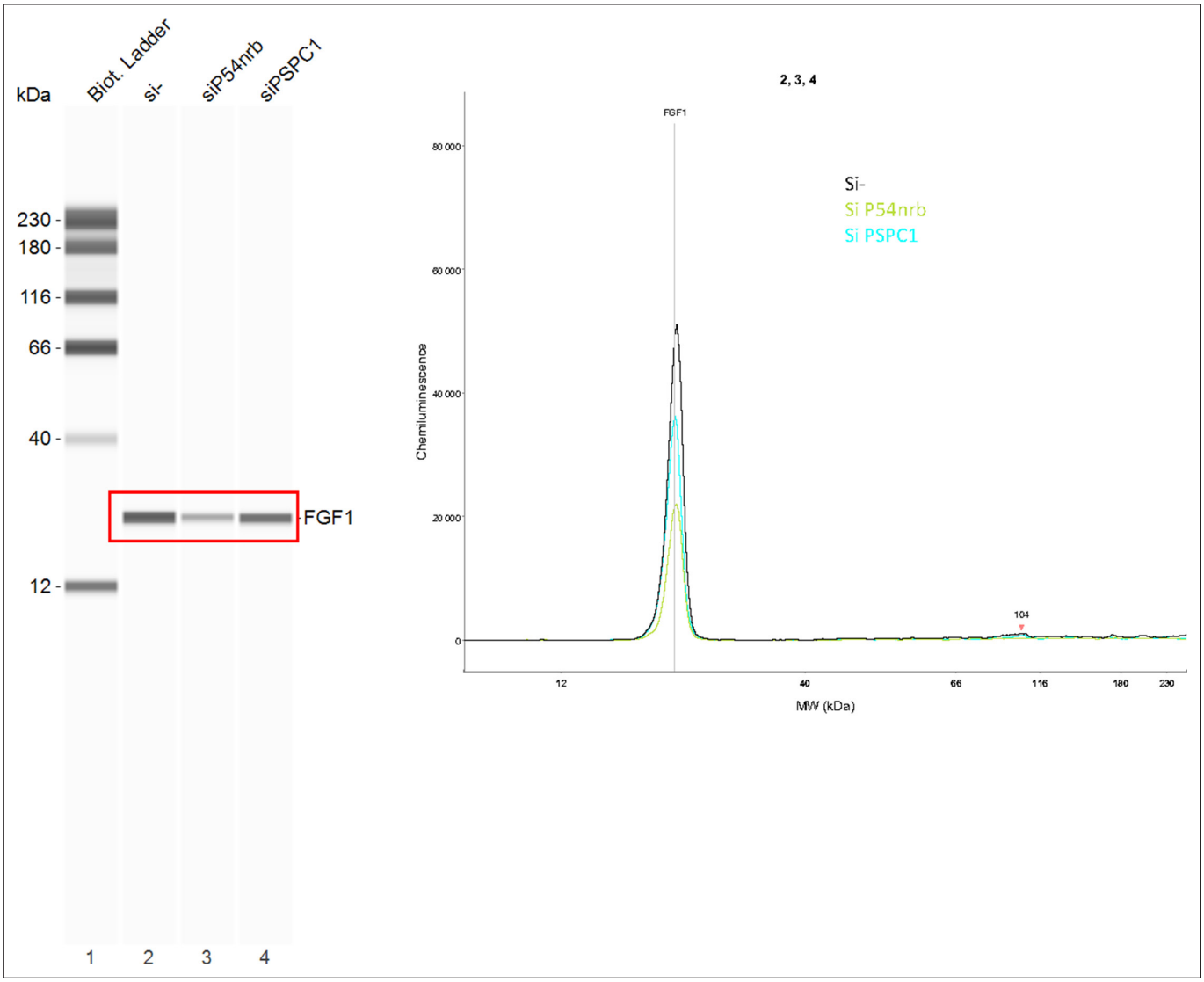
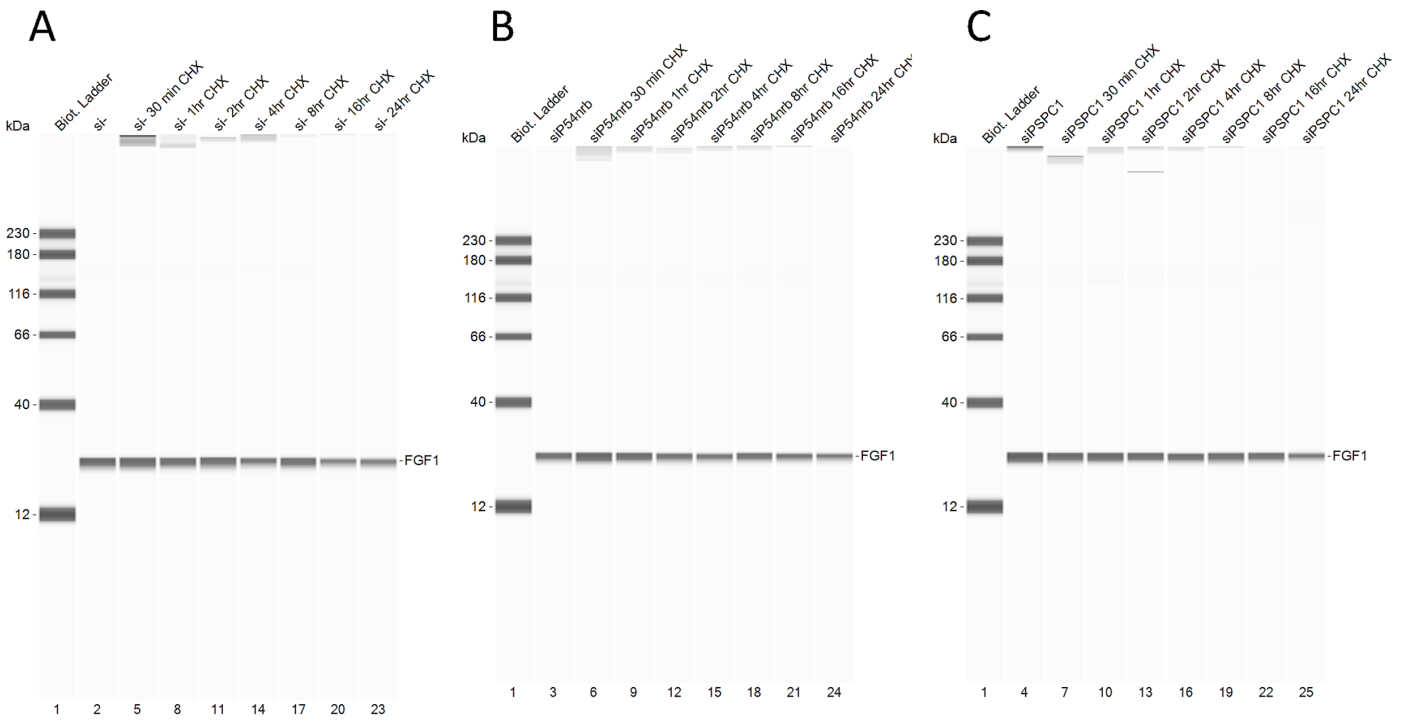


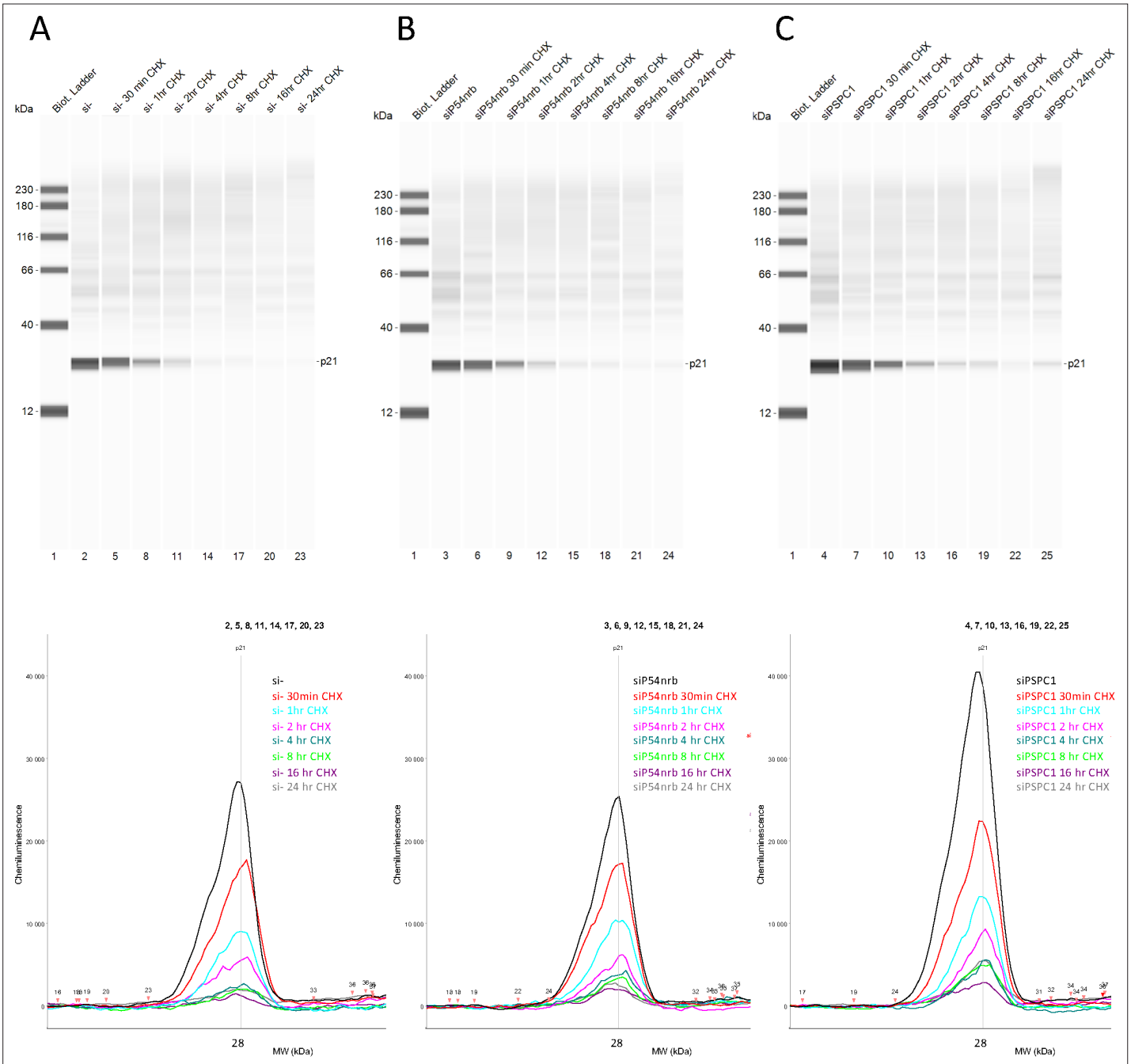
Figure 4—figure supplement 2. FGF1 protein expression in response to p54^{nrb} or PSPC1 knock-down. Endogenous FGF1 protein was detected by Capillary Simple Western in conditions of P54^{nrb} or PSPC1 knock-down mentioned in ***Figure 4—figure supplement 1***. The raw data presented correspond to the experiment shown in ***Figure 4E–F*** . Source data of capillary Simple Western are provided (***Figure 4—figure supplement 2—source data 1***).



Percentage of FGF1

CHX time	0 hr	30min	1 hr	2 hr	4 hr	8 hr	16 hr	24 hr
Untreated	100%	97%	87%	98%	85%	127%	64%	63%
siRNA p54^{nrb}	100%	105%	112%	104%	101%	146%	76%	84%
siRNA PSPC1	100%	71%	81%	75%	75%	82%	62%	68%

Figure 4—figure supplement 3. FGF1 half-life in response to p54^{nrb} or PSPC1 knock-down. FGF1 half-life was determined on HL-1 cells treated with p54^{nrb} siRNA, PSPC1 siRNA or control siRNA, by blocking protein synthesis with cycloheximide at 10 µg/mL, with time-course points at 0 hr, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 16 hr, and 24 hr. FGF1 protein stability was measured by capillary Western, with normalization to 0 hr time-course point. Source data of capillary Simple Western are provided (***Figure 4—figure supplement 3—source data 1***).



Percentage of p21

CHX time	0 hr	30min	1 hr	2 hr	4 hr	8 hr	16 hr	24 hr
Untreated	100%	65%	33%	25%	11%	8%	4%	9%
siRNA p54^{nrb}	100%	79%	59%	28%	24%	17%	10%	12%
siRNA PSPC1	100%	59%	37%	24%	13%	13%	7%	15%

Figure 4—figure supplement 4. p21 half-life in response to p54^{nrb} or PSPC1 knock-down. p21 half-life was determined on HL-1 cells treated with p54^{nrb} siRNA, PSPC1 siRNA or control siRNA in the same experiment as in that presented in ***Figure 4—figure supplement 3***. p21 protein stability was measured by capillary western, with normalization to 0 hr time-course point. Source data of capillary Simple Western are provided (***Figure 4—figure supplement 4—source data 1***).

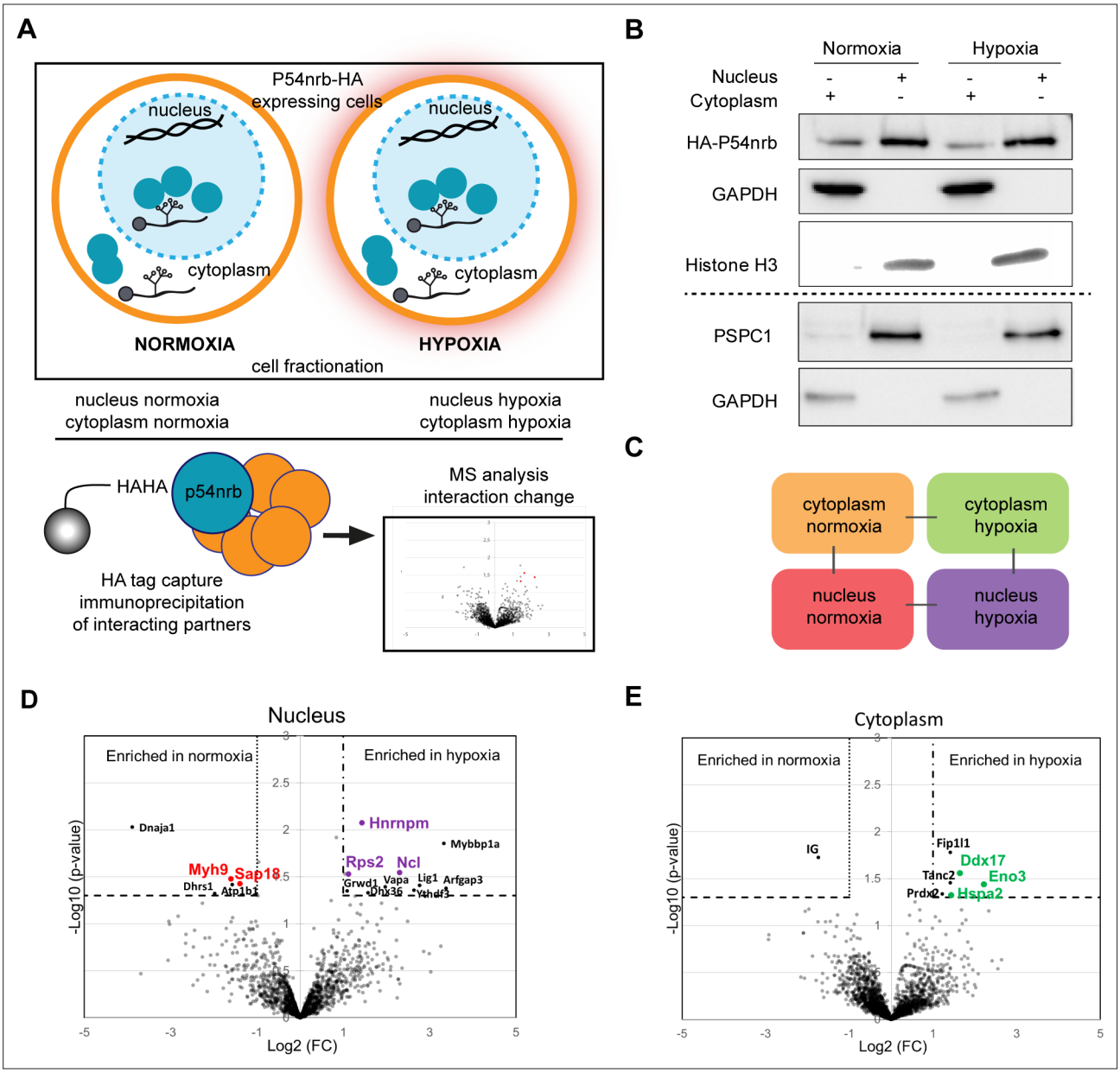


Figure 5. P54^{nrb} interactome in normoxic and hypoxic cardiomyocytes. (**A**) Experimental workflow: p54^{nrb}-HA transduced HL-1 cells were subjected to normoxia or hypoxia, then nucleus and cytoplasm fractionation was performed and extracts were immunoprecipitated using anti-HA antibody. Enriched interacting proteins were identified by using a label-free quantitative mass spectrometry approach. (**B**) Western blot of fractionation experiment of HL-1 cells in normoxia and hypoxia. Histone H3 was used as a nuclear control and GAPDH as a cytoplasm control. The dotted line delineates two different blots of the same fractionation experiment. (**C**) Schema of the four pairwise comparisons submitted to statistical analysis. (**D and E**) Volcano plots showing proteins enriched (bold black) and significantly enriched (after elimination of false-positive hits from quantitation of low-intensity signals) in the nucleus for hypoxia (purple) versus normoxia (red) (**D**) or in the cytoplasm for hypoxia (green) versus normoxia (**E**). An unpaired bilateral student t-test with equal variance was used. Enrichment significance thresholds are represented by an absolute log2-transformed fold-change (FC) greater than 1 and a -log10-transformed (p-value) greater than 1.3. Details are provided in ***Supplementary file 7***.

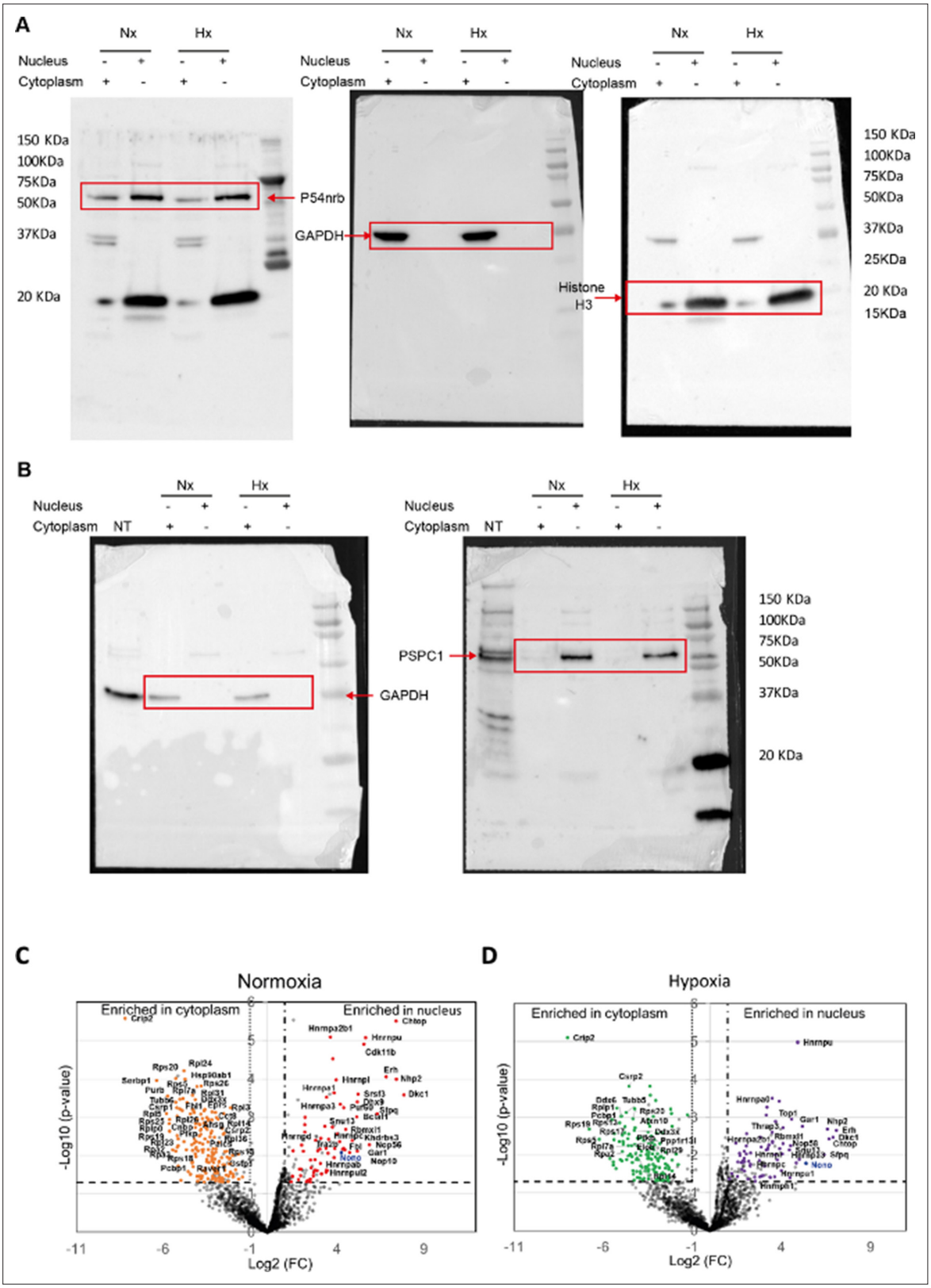


Figure 5—figure supplement 1. Western blot of fractionation experiment of HL-1 cells and label-free quantitative analysis of HA-P54^{nrb}-bound proteins identified by mass spectrometry in different conditions. (A-B) Raw data of the Western blots using nuclear or cytoplasmic extracts of HL-1 cells in normoxia and hypoxia, presented in Figure 5B. (A) Western blot successively blotted with anti-GAPDH, anti-histone H3, and anti-p54nrb antibodies. (B) Western blot successively blotted with anti-GAPDH and anti-PSPC1 antibodies. Size markers are indicated. NT corresponds to total extracts of non-

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transduced cells. (C-D) Volcano plots showing proteins significantly enriched in the normoxia condition for nucleus (dots in red) versus cytoplasm (dots in orange) (C) or in the hypoxia condition (4 hr) for nucleus (dots in purple) versus cytoplasm (dots in green) (D). The $p54^{nrb}$ bait (endogenous mouse *Nono*) is indicated in blue. An unpaired bilateral student t-test with equal variance was performed. Enrichment significance thresholds are represented by an absolute log2-transformed fold-change (FC) greater than 1 and a -log10-transformed (p-value) greater than 1.3.

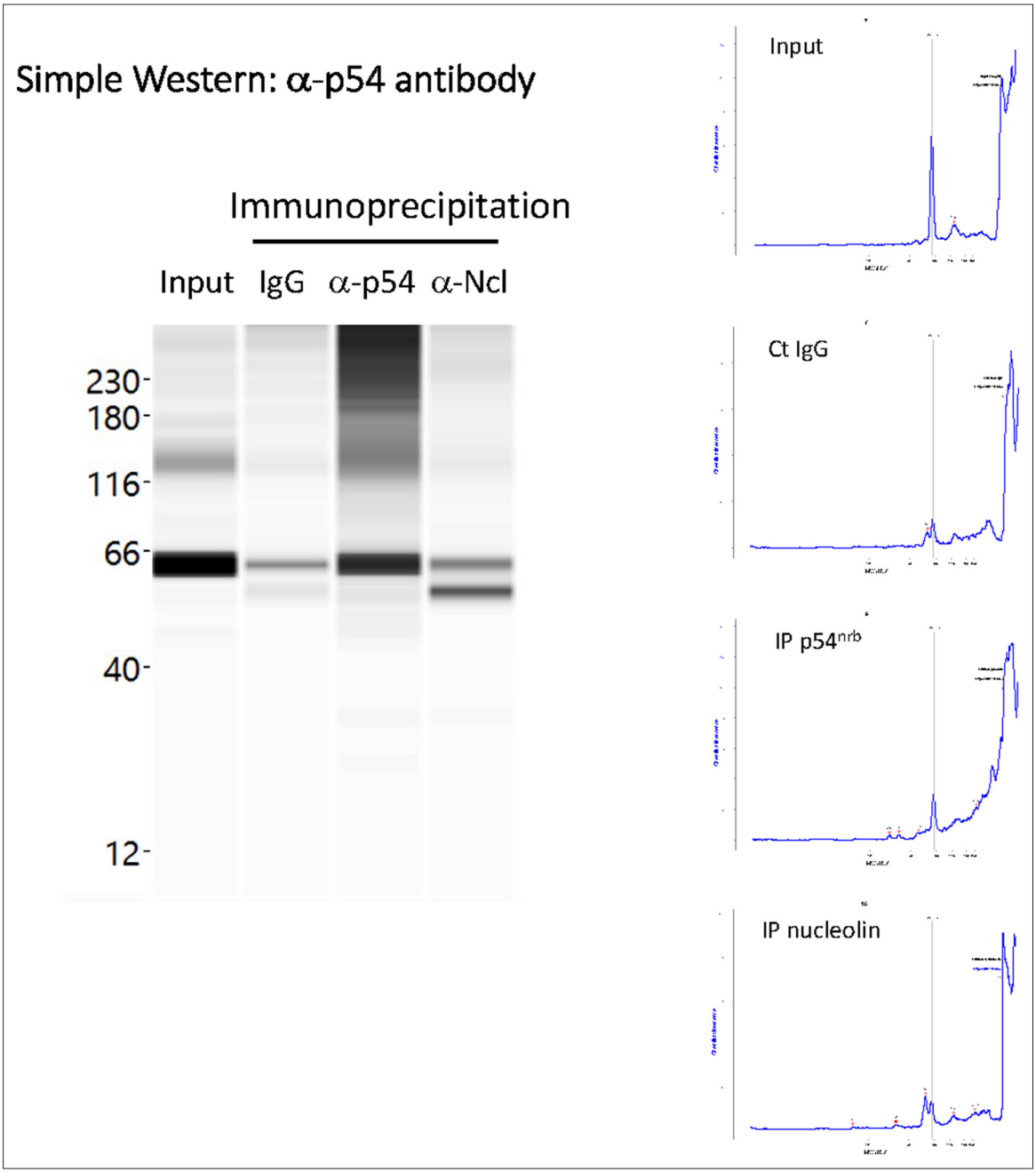

Figure 5—figure supplement 2. p54^{nrb} is co-immunoprecipitated by anti-nucleolin antibody. Immunoprecipitation was performed from HL-1 cell nuclear extracts, using either IgG (negative control), or antibody against p54^{nrb}, or antibody against nucleolin. Capillary Simple Western (Jess) was then performed using a-p54^{nrb} antibody. Data show an enrichment of p54 using anti-nucleolin antibody. Interestingly, a smaller isoform of p54 is efficiently co-immunoprecipitated, described in a previous report. Source data of capillary Simple Western are provided (***Pavao et al., 2001***; ***Figure 5—figure supplement 2—source data 1***).

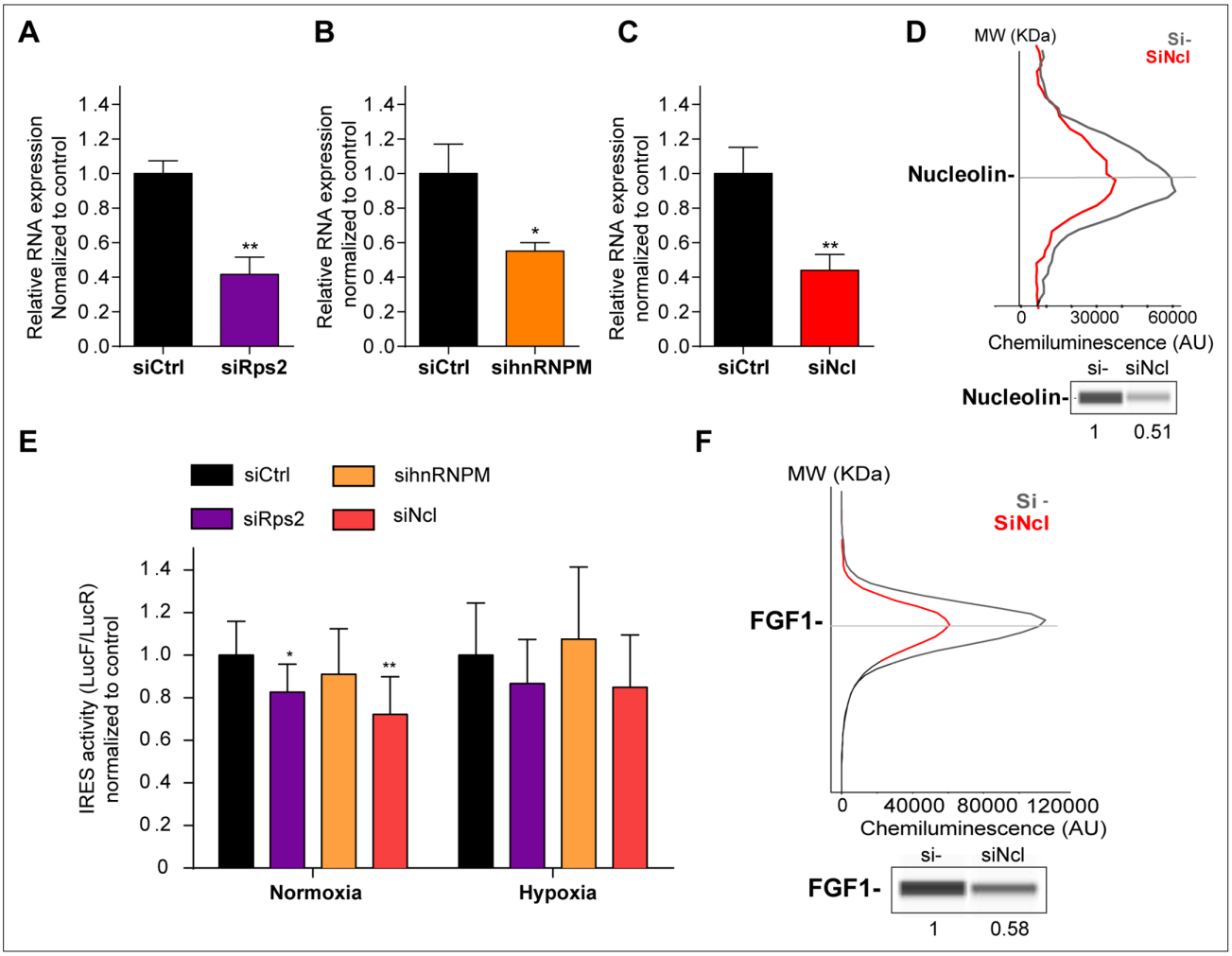

Figure 6. p54^{nrb}-interacting proteins, nucleolin and RPS2, control the *FGF1* IRES activity. (**A–C**) Quantification of RPS2 (**A**), hnRNPM (**B**) and nucleolin (**C**) RNA expression in HL-1 cells transfected with siRNAs against *Rps2*, hnRNPM or nucleolin mRNA, respectively. RNA expression was measured by RT-qPCR and normalized to control siRNA. One representative experiment is shown with n=3 biological replicates. Student two-tailed t-test was performed with n=3 or Mann-Whitney test with n=9; *p<0.05, **p<0.01, ***<0.001, ****p<0.0001. (**D**) Capillary Simple Western of nucleolin following nucleolin knock-down. The full raw unedited gel is provided in ***Figure 6—figure supplement 1A*** (***Figure 6—figure supplement 1—source data 1***). (**E**) *FGF1* IRES activity with knock-down by siRNA interference of candidate ITAF nucleolin in HL-1 in normoxia or hypoxia 1% O_2 was performed as in ***Figure 2***. The IRES activity values have been normalized to the control siRNA. Histograms correspond to means ± standard deviation of the mean, with a non-parametric Mann-Whitney test *p<0.05, **p<0.01. The mean has been calculated with nine cell culture biological replicates, each of them being already the mean of three technical replicates (27 technical replicates in total but the M-W test was performed with n=9). Detailed values of biological replicates are presented in ***Supplementary file 6***. (**F**) Capillary Simple Western of endogenous FGF1 following nucleolin knock-down. Histograms correspond to means ± standard deviation. The source data or capillary Simple Western are provided in ***Figure 1—figure supplement 1B*** (***Figure 6—figure supplement 1—source data 1***).

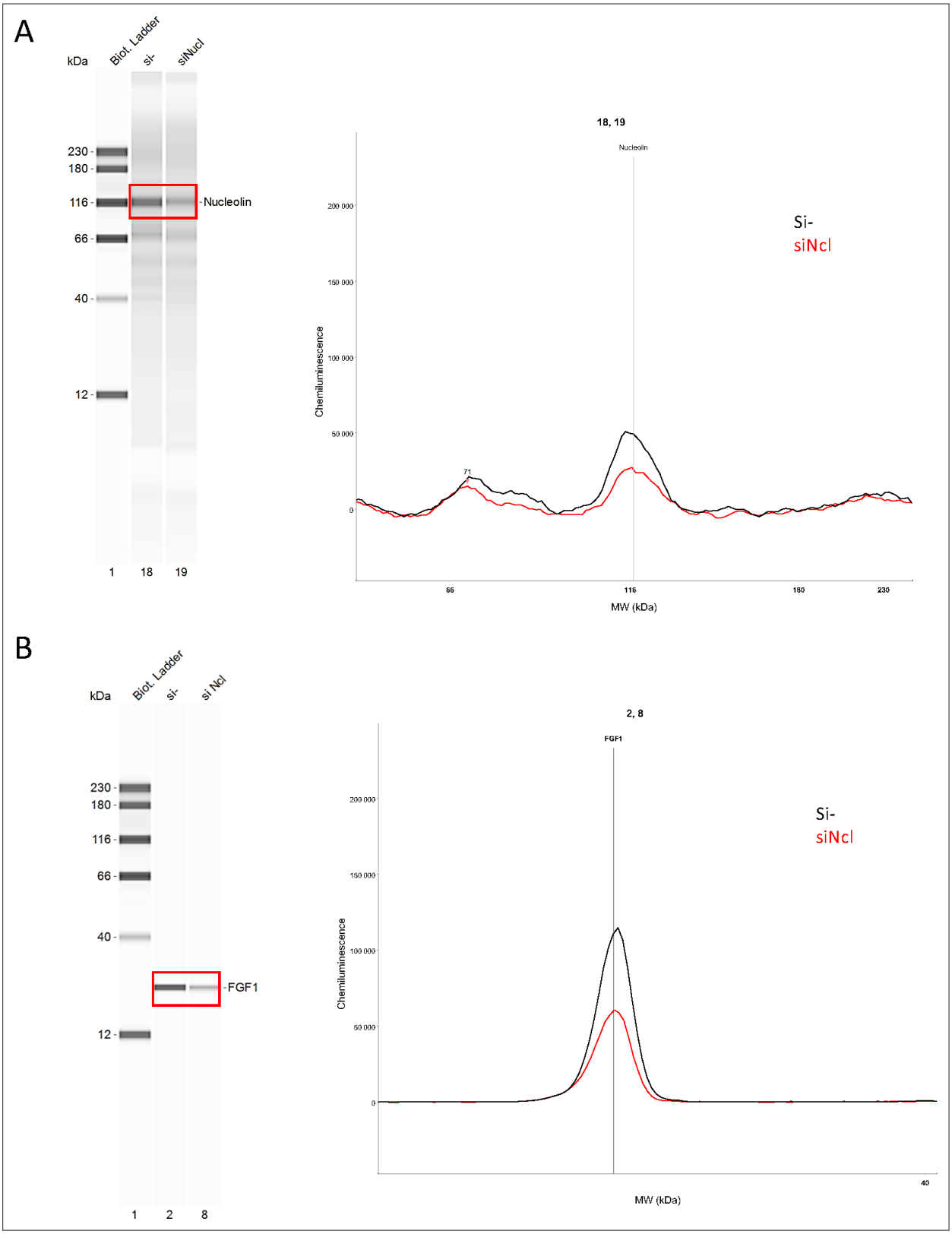

Figure 6—figure supplement 1. Endogenous FGF1 protein expression is down-regulated following nucleolin knock-down. Knock-down of nucleolin was achieved in HL-1 cells using siRNA against nucleolin mRNA. Capillary Simple Western were performed using anti-nucleolin (**A**) of anti-FGF1 antibody (**B**). The source data of the capillary Simple Western are provided (***Figure 6—figure supplement 1—source data 1***).

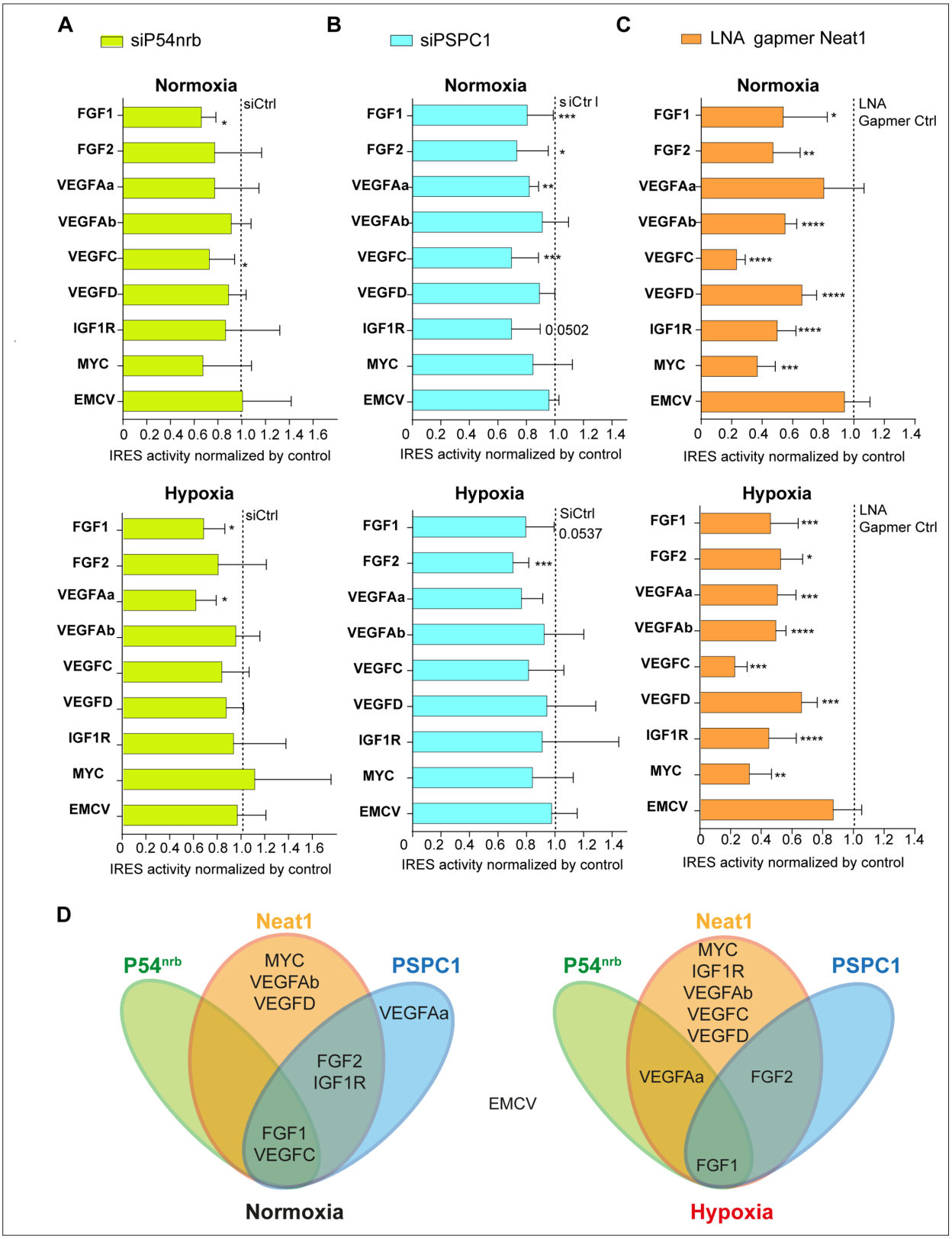

Figure 7. *Neat1* is the key activator of (lymph)angiogenic and cardioprotective factor mRNA IRESs. (**A–C**) HL-1 subjected to normoxia or 1% O_2 hypoxia were transduced by Lucky Luke bicistronic lentivectors with *FGF1*, *FGF2*, *VEGFAa*, *VEGFAb*, *VEGFC*, *VEGFD*, *IGF1R*, *MYC*, or EMCV IRES, then the knock-down of p54^{nrb}(A) PCPC1 (**B**) and *Neat1* (**C**) was performed as in ***Figure 2*** and ***Figure 4***. IRES activities were measured and normalized to activities in normoxia. IRES activity in normoxia is represented by a dotted line at 1. Histograms correspond to means ± standard deviation, and Mann-Whitney

Figure 7 continued on next page

Figure 7 continued

test with n=9 or n=12 for *FGF1* IRES; *p<0.05, **p<0.01, ***<0.001, ****p<0.0001. For each IRES the mean has been calculated with nine cell culture biological replicates, each of them being already the mean of three technical replicates (27 technical replicates in total). Detailed values of biological replicates are presented in ***Supplementary file 3***, ***Supplementary file 5***, ***Supplementary file 6***. (**D**) Schema depicting groups of IRESs regulated by *Neat1*, PSPC1, or P54^{nrb} in normoxia or hypoxia.

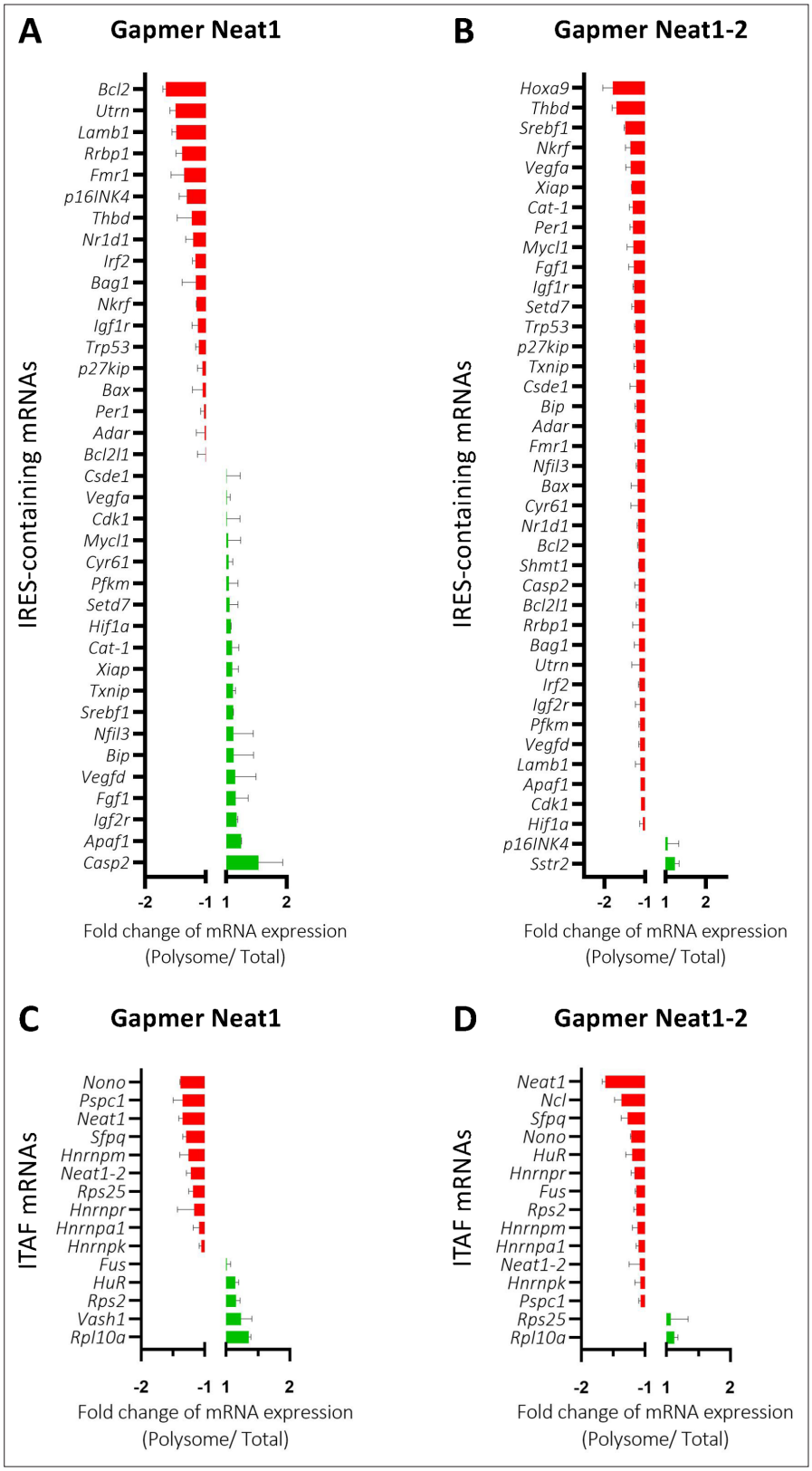

Figure 8. *Neat1_2* knock-down down-regulates translation of most IRES-containing RNAs as well as mRNAs coding ITAFs. HL-1 cardiomyocytes were transfected with gapmer *Neat1*, *Neat1_2*, or control. Polysomes were purified on sucrose gradient as described in Star Methods. The polysome profile is presented in ***Figure 8—figure supplement 1***. RNAs were purified from cytoplasmic extracts and from pooled polysomal fractions and analyzed

Figure 8 continued on next page

Figure 8 continued

on a Fluidigm deltagene PCR array from two biologicals replicates (cell culture dishes and cDNAs), each of them measured in three technical replicates (PCR reactions) (***Supplementary file 8***). IRES-containing mRNAs (**A–B**) and ITAF mRNA levels in polysomes (**C–D**) polysomal RNA/ total RNA were analyzed. Relative quantification (RQ) of mRNA level was calculated using the $2^{-\Delta\Delta CT}$ method with normalization to GAPDH mRNA and to HL-1 tranfected by gapmer control, and is shown as fold change of repression (red) or induction (blue).

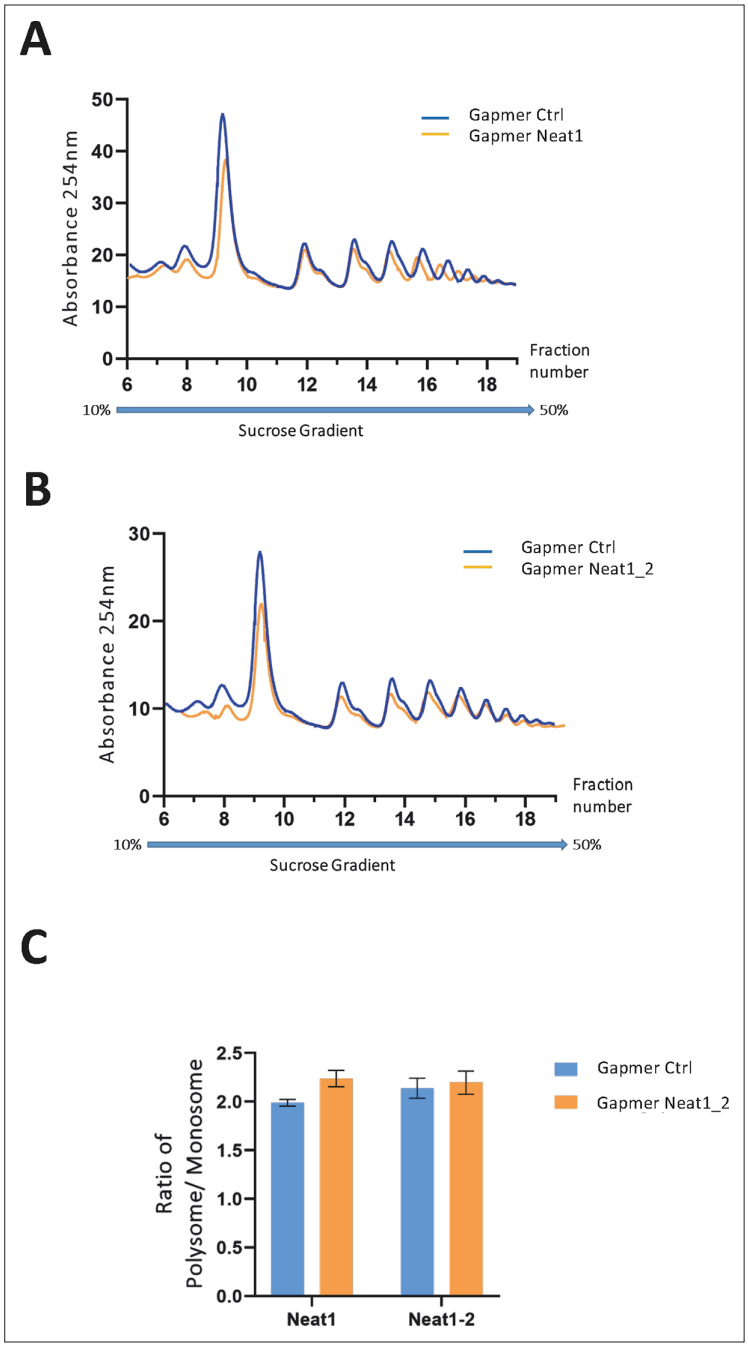

Figure 8—figure supplement 1. Polysome profiles of HL-1 cardiomyocytes treated by gapmer *Neat1* or *Neat1_2*, compared to gapmer control. In order to isolate translated mRNAs, polysomes from transfected HL-1 cardiomyocytes were purified on a sucrose gradient, as described in Star Methods. Polysomal profiles of HL-1 cardiomyocytes transfected by gapmer *Neat1* (**A**) or *Neat1_2* (**B**) was analyzed and compared to gapmer control. The P/M ratio (polysome/monosome) (**C**) was determined by delimiting the 80 S and polysome peaks, taking the lowest plateau values between each peak and calculating the area under the curve (AUC). Then the sum of area values of the nine polysome peaks was divided by the area of the 80 S peak.

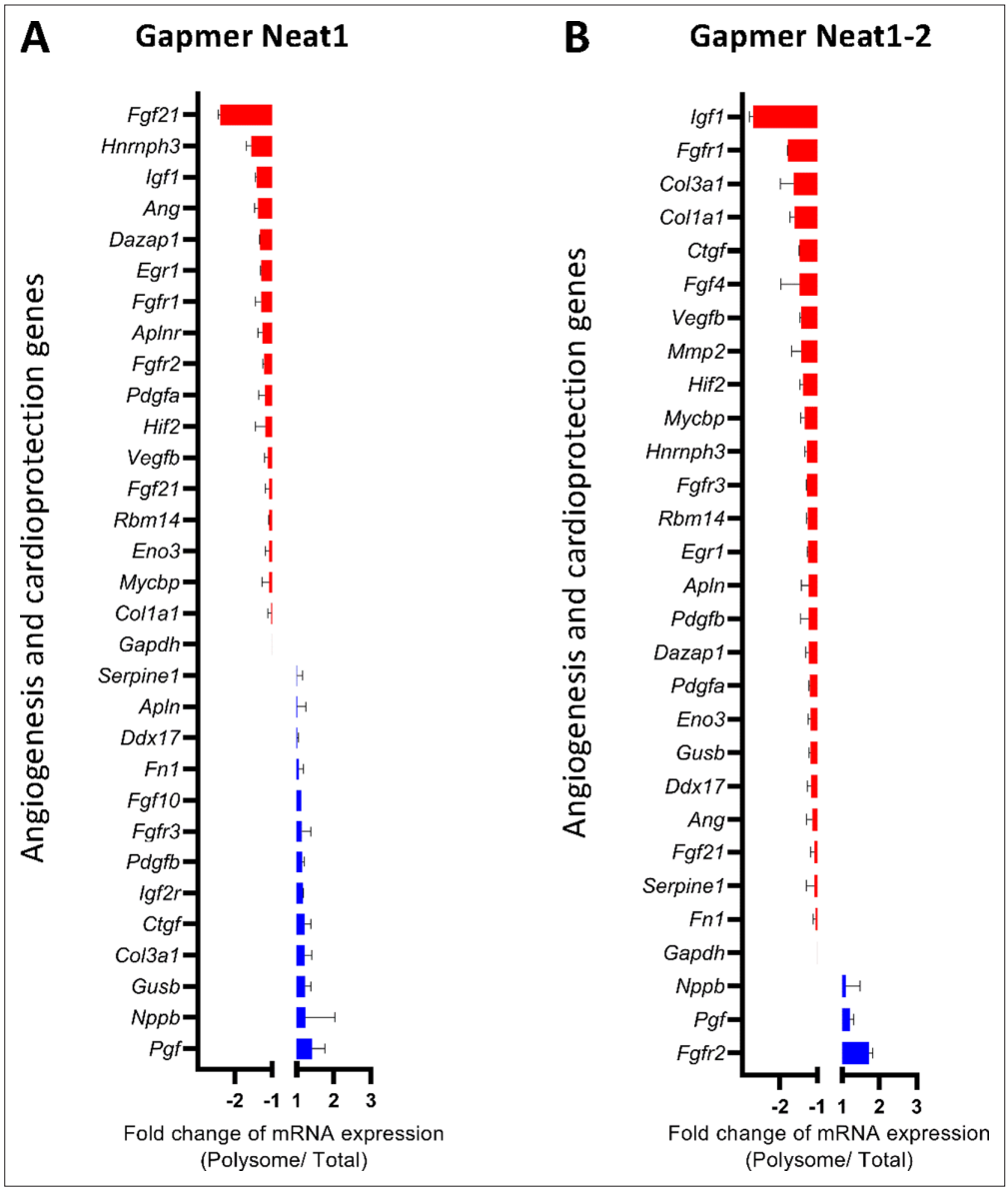

Figure 8—figure supplement 2. Effect of *Neat1* and *Neat1_2* knock-down on translation of mRNAs coding angiogenic and cardioprotective factors. HL-1 cardiomyocytes were transfected with gapmer *Neat1*, *Neat1-2*, or control. Polysomes were purified on sucrose gradient as described in Star Methods. Polysome profile is presented in ***Figure 8—figure supplement 1***. RNAs were purified from cytoplasmic extracts and from pooled polysomal fractions and analyzed on a Fluidigm deltagene PCR array from two biologicals replicates (cell culture dishes and cDNAs), each of them measured in three technical replicates (PCR reactions) (***Supplementary file 8***). Angiogenesis and cardioprotection gene mRNA levels in polysomes (polysomal RNA/ total RNA) were analyzed after knock-down of *Neat1* (**A**) or *Neat1_2* (**B**). Relative quantification (RQ) of mRNA levels was calculated using the 2–ΔΔCT method with normalization to GAPDH mRNA and to HL-1 tranfected by gapmer control, and is shown as fold change of repression (red) or induction (blue).

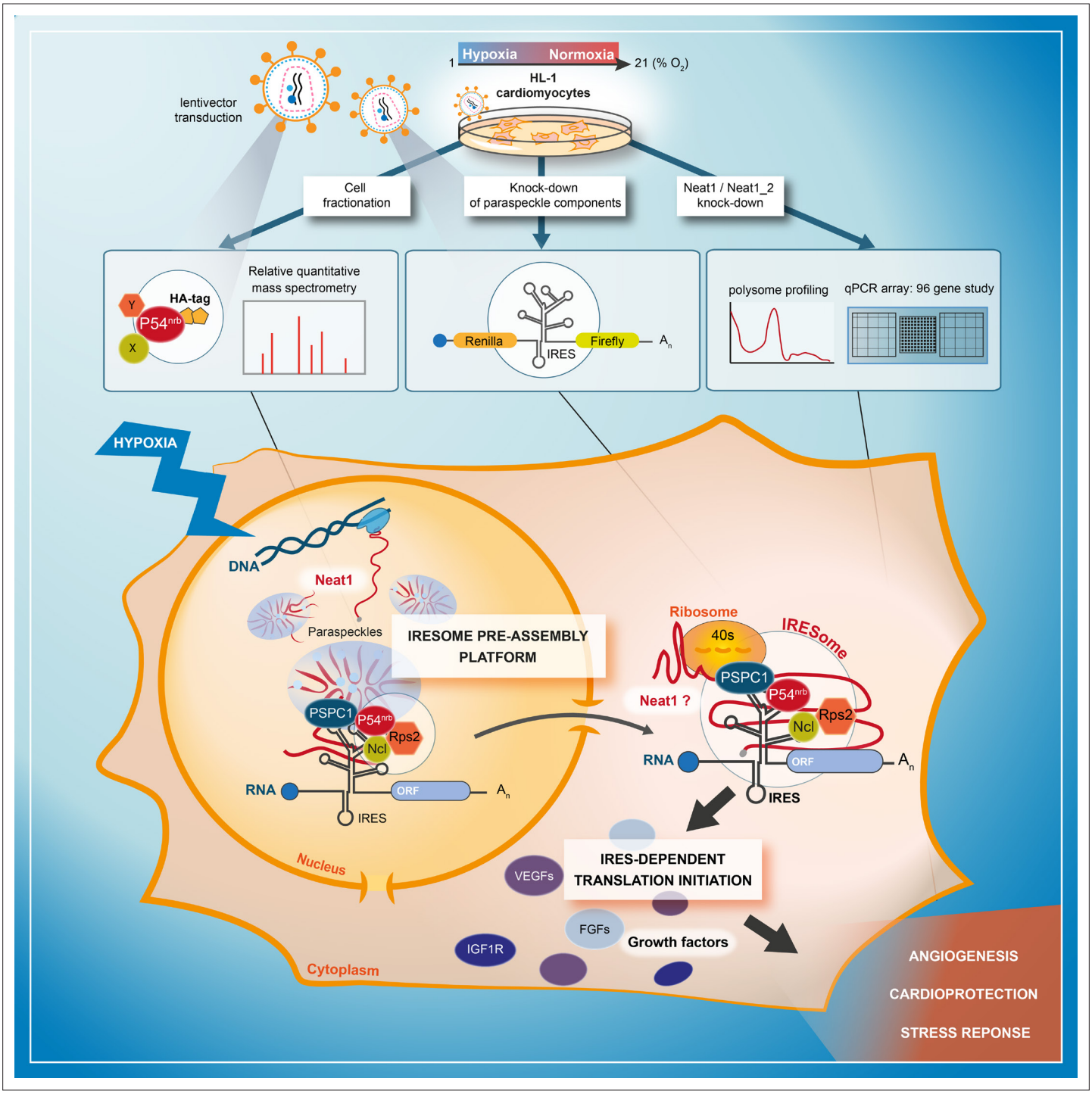

Figure 9. Model of IRESome formation in the paraspeckle. According to the present data, we propose that the paraspeckle may be a recruitment platform for IRES-containing mRNAs in hypoxic cardiomyocytes. *Neat1* and proteins present in the paraspeckle (among them major paraspeckle components such as p54^{nrb} and PSPC1) would assemble the IRESome, then mRNA would be exported from the nucleus and translated in the cytosol. Identification of Neat1 in the cytoplasm suggests that it might be part of the IRESome and have a direct role in translation. However this latter hypothesis remains to be elucidated.