**SUPPLEMENTARY INFORMATION**

## Material and methods

### Cell culture

Human hepatocellular carcinoma (HepG2) cells and human embryonic kidney (HEK)293 cells were grown in Dulbecco's modified Eagle's medium (Invitrogen AG, Basel, Switzerland) supplemented with 10% fetal calf serum (FCS; Invitrogen) at 37°C in a humidified atmosphere of 5% $CO_2$. Human primary hepatocytes (n=2) were purchased from Lonza (Lonza, Verviers, Belgium) and cultured according to the manufacturer's instructions. One donor was found to have metastatic liver disease with altered cellular response and was excluded from analysis; cells were kindly replaced with a novel batch of hepatocytes by Lonza. Prior to IL-6 stimulation, HepG2 cells were serum starved for period of 24h (0.5% FCS). IL-6 (R&D Systems, Abingdon, United Kingdom) was used as indicated.

### Quantitative real time-PCR (qPCR) analysis

Total RNA was extracted using the RNeasy kit (Qiagen AG, Hombrechtikon, Switzerland). RNA was reverse transcribed by using Random Hexamers and MultiScribe Reverse Transcriptase (both from Applied Biosystems, Rotkreuz, Switzerland). Quantification of specific RNA transcripts was performed by SYBR Green qPCR, using the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Sequences of primers used for amplification of human fibrinogen-γ-chain (FGG), human haptoglobin (Hp), human primary transcript of the miR-17/92 cluster (C13orf25), human signal transducer and activator of transcription (STAT)3, human protein inhibitor of activated STAT (PIAS)3 are shown in Suppl. Table S1. To confirm specific amplification, a dissociation curve analysis was performed. The amounts of loaded RNA were normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or hypoxanthine phosphoribosyltransferase 1 (HPRT1). Differential gene expression was calculated with the threshold cycle ($C_t$) method (1).

### Quantification of mature miRNAs

Total RNA was extracted using the mirVana (Applied Biosystems) or the miRNeasy miRNA isolation kit (Qiagen). Mature microRNA sequences were detected by specific stem-loop primers, reverse transcribed using MultiScribe Reverse Transcriptase and quantified by performing SYBR Green qPCR (2). Stem-loop primers and amplification primers were designed for miR-18a, miR-19a, miR-20a, and miR-92a (Suppl. Table S1). Obtained signals were normalized to the expression of GAPDH or RNU48 and specific amplification was confirmed by performing dissociation curve analysis.

### Western blot

For protein extraction cells were lysed with sample loading buffer. Whole-cell lysates were separated by 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and the proteins were transferred to nitrocellulose membrane. Membranes were blocked with dry milk and incubated with the following primary antibodies: anti – PIAS3, anti – Hp (both from Abcam, Cambridge, UK), anti - STAT3 (R&D Systems), anti – Phospho-STAT3 (Tyr705, Cell Signaling Technology, Danvers MA, USA), and anti – α-tubulin (Sigma, St. Louis, MO, USA). Bands were detected with species-specific secondary antibodies coupled to horseradish peroxidase (Jackson ImmunoResearch Europe Ltd, Suffolk, UK). Evaluation of the expression of proteins was performed by the Alpha Imager software (Alpha Innotech, San Leandro, CA, USA) via pixel quantification of the electronic image.

### Plasmid construction

For promoter studies, a fragment (878 bp) of the Hp promoter containing two STAT3 binding sites was amplified from human genomic DNA (Promega AG, Dübendorf, Switzerland) using PCR. The PCR product was digested with *Bgl*II and *Hind*III and cloned into the firefly luciferase-based pGL3basic vector (Promega). The same cloning strategy was applied for the cloning of the promoter of FGG (578 bp, containing one STAT3 binding site) into the pGL3basic vector. For normalization of the luciferase

activity, a modified vector encoding for *Renilla* luciferase (pRL-SV40, Promega) was used. The SV40 promoter of pRL-SV40 was removed by digestion with *Bgl*II and *Hind*III and replaced by the promoter of GAPDH (1063 bp). Primer sequences used for cloning are shown in Suppl. Table S1. The correct sequence of each insert was confirmed by sequencing.
For confirmation of direct miRNA - mRNA interactions, the 3'untranslated region (UTR) of PIAS3 (924 bp) was amplified from human genomic DNA using PCR. The obtained PCR product was digested with *Nhe*I and cloned into the pGL3control vector (Promega). As a negative control, the anti-sense construct was used (3). In addition, the corresponding miRNA seed match located in the 3' UTR of PIAS3 was specifically mutated by using mutagenesis PCR. The correct sequences and orientation of the inserts were confirmed by sequencing. The primer sequences are provided in Suppl. Table S1.

**Reporter gene assay**
For promoter activity studies, HepG2 cells were transfected either with pGL3basic-Hp or with pGL3basic-FGG (600ng/well) using Lipofectamine 2000 (Invitrogen). In addition, a vector for normalization (pRL-GAPDH, 300ng/well) was co-transfected. After 24h, the cells were stimulated with IL-6 (20ng/ml) for 4h, harvested, and luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega). The values obtained were normalized to the activity of *Renilla* luciferase (pRL-GAPDH).
For miRNA target validation, HEK293 cells were transfected with the pGL3control 3'UTR constructs of PIAS3 (150ng/well) using Lipofectamine 2000. Moreover, a vector for normalization (pRL-SV40, 80ng/well) and precursor molecules of miRNAs (negative control#1 or pre-miR-18a, both from Applied Biosystems/Ambion; 25nM) were added. After 24h, the cells were lysed, and luciferase activity was measured using the Dual-Luciferase Reporter Assay System. The values obtained were normalized to the activity of *Renilla* luciferase (pRL-SV40).

**Transfection of miRNAs**
For specific over expression of miRNAs in HepG2 cells precursor molecules of miRNAs were purchased from Applied Biosystems/Ambion (pre-miR-18a, -19a, -20a, -92a). Scrambled negative control #1 served as scrambled control. For over expression of the entire cluster (cocktail), 25nM of each miRNA was transfected. In addition, 100nM of each miRNA was used in single transfection studies. Nuclear transfection of HepG2 (100nM of RNA) was achieved by using the cell line nucleofector kit V (Amaxa GmbH, Cologne, Germany). Alternatively, HepG2 cells and primary hepatocytes were transfected with the same amount of pre-miRNAs using Lipofectamine 2000. For specific silencing of miR-18a HepG2 cells were transfected with anti-miR-18a (100nM, Applied Biosystems/Ambion) using cell line nucleofector kit V or Lipofectamine 2000. Following an incubation period of 72h, cells were stimulated with IL-6 (20ng/ml) for 4h, harvested, and gene expression analysis was performed. In addition, promoter activity studies in miRNA over expressing HepG2 cells were performed.

**Gene silencing**
HepG2 cells were transfected with 200nM of small interfering RNA molecules (siRNAs, all provided by Qiagen) using cell line nucleofector kit V (AMAXA). AllStars negative control siRNA (Qiagen) served as scrambled control. After an incubation period of 48h, cells were stimulated with IL-6 (20ng/ml) for 4h, harvested, and gene expression was analyzed. Furthermore, promoter studies in siRNA-transfected HepG2 cells were performed.

**Enzyme-linked immunosorbent assay (ELISA)**
HepG2 cells and primary hepatocytes were transfected with pre-miRNAs or anti-miRNAs (100nM) using Lipofectamine 2000. Following an incubation period of 72h, cells were stimulated with IL-6 (20ng/ml) for 8h or 24h and supernatants were collected. Quantitative determination of human Hp and FGG was achieved by using commercially available ELISA kits (GenWay Biotec Inc, San Diego, CA, USA) according to the manufacturer's instructions.

**FIGURE LEGENDS**

Fig. S1. **Over expression of miR-17/92 in transfected HepG2 cells.** HepG2 cells were transfected with miRNA precursor molecules (pre-miR-18a, -19a, -20a, -92a, and a cocktail of all) or scrambled negative control. (A) Stem-loop qPCR was used to quantify levels of miR-18a showing a significant up regulation of miR-18a in pre-miR-18a and cocktail transfected HepG2 cells (n=4). The same method was applied for quantification of miR-19a (B), miR-20a (C), and miR-92a (D) in pre-miRNA transfected HepG2 cells confirming a significant increase of miRNA expression, respectively (n=4). The respective scrambled transfection was set to 1. Statistical analysis by paired student's t-test.

Fig. S2. **Silencing of miR-18a decreases acute-phase response in HepG2 cells.** Antagonizing miR-18a expression was achieved by transfection of HepG2 cells with anti-miR-18a molecules. (A) Promoter activity of FGG and Hp was assessed by co-transfection of a luciferase-based vector system comprising promoter sequence of either FGG or Hp. IL-6 stimulation (20ng/ml) for 4h activated the promoter of FGG and Hp in all samples with the silencing of miR-18a reducing the activity of luciferase as compared to scrambled transfection (n=6). (B) Stem-loop qPCR was used to quantify levels of miR-18a showing a significant down regulation of miR-18a in anti-miR-18a transfected HepG2 cells (n=6). mRNA levels of FGG and Hp after 4h of IL-6 stimulation (20ng/ml) were found to be reduced in anti-miR-18a treated cells compared to scrambled transfection ($n \geq 5$). (C) Protein levels of fibrinogen and Hp in the supernatants of anti-miR-18a transfected HepG2 cells were quantified by ELISA. Release of fibrinogen after 8h of IL-6 stimulation (20ng/ml) was decreased in cells transfected with anti-miR-18a as compared to scrambled transfection. The same supernatants were assessed for quantification of Hp protein levels and showed significantly decreased secretion of Hp when miR-18a was blocked (n=6). (D) Western blot experiments showed increased expression of PIAS3 protein levels in anti-miR-18a transfected HepG2 cells confirming the regulation of PIAS3 by miR-18a (n=5). Statistical analysis was performed by using the student's t-test (unpaired for A, B, D and paired for C).

Fig. S3. **Expression of PIAS3 is reduced after IL-6 stimulation.** RNA levels of FGG, Hp, PIAS3, and C13orf25 were measured in HepG2 cells stimulated with IL-6 (20ng/ml). (A) mRNA levels of FGG and (B) Hp were found to be enhanced in a time-dependent manner by IL-6 stimulation, whereas the expression of (C) PIAS3 was significantly down regulated at 24h of IL-6 treatment ($0.89 \pm 0.09$ fold, $p=0.036$) ($n \geq 5$). (D) The expression of the primary transcript of miR-17/92 (C13orf25) was significantly induced at 1h of IL-6 treatment ($1.22 \pm 0.17$ fold, $p=0.001$) ($n \geq 5$). The correlation of PIAS3 and C13orf25 is deferred probably due to the miRNA maturation process (4). The dashed line indicates unstimulated control and was set as 1. Statistical analysis by paired student's t-test.

**Tab. S1. Primer sequences**

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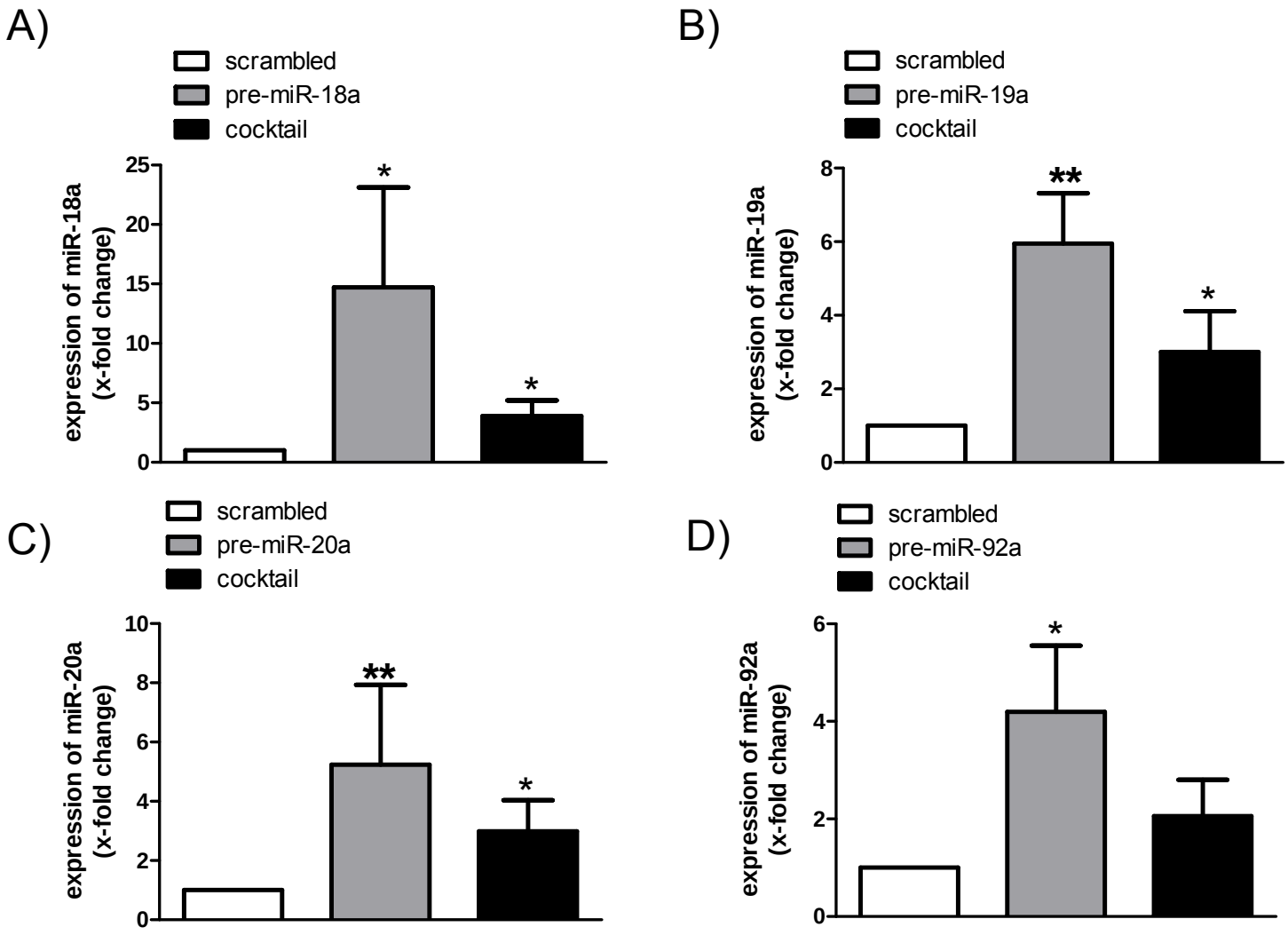
| **gene expression analysis** | |
| GAPDH for | 5’ – GGG AAG CTT GTC ATC AAT GGA – 3’ |
| GAPDH rev | 5’ – TCT CGC TCC TGG AAG ATG GT – 3’ |
| HPRT1 for | 5’ – ATG GAC AGG ACT GAA CGT CTT G – 3’ |
| HPRT1 rev | 5’ – GGC TAC AAT GTG ATG GCC TC – 3’ |
| FGG for | 5’ – TAT TAC CAA GGT GGC ACT TAC TC – 3’ |
| FGG rev | 5’ – CAT TAT CAT AAC CAT TAG GAG TAG ATG – 3’ |
| Hp for | 5’ – TCA CGG ATA TCG CAG ATG ACG – 3’ |
| Hp rev | 5’ – CAC ATA GCC ATG TGC AAT CTC G – 3’ |
| C13orf25 for | 5' – TTG CTA AGT GGA AGC CAG AAG – 3' |
| C13orf25 rev | 5' – CAT CCA CGT GGC AAA ACA T – 3' |
| PIAS3 for | 5' – GAT TCT TAC ATC CAG AGA GGT TC – 3' |
| PIAS3 rev | 5' – ACC TGT ATG GTA TAA TCA CAT TTG G – 3' |
| STAT3 for | 5' – TTC ACT TGG GTG GAG AAG GAC A – 3' |
| STAT3 rev | 5' – CGG ACT GGA TCT GGG TCT TAC C – 3' |
| **miRNA expression analysis** | |
| miR-18a RT | 5’ – GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC TAT CT – 3’ |
| miR-18a fwd | 5’ – GGC GGT AAG GTG CAT CTA GT – 3’ |
| miR-18a rev | 5’ – GTG CAG GGT CCG AGG T – 3’ |
| miR-19a RT | 5’ – GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACT CAG TT – 3’ |
| miR-19a fwd | 5’ – CGG CGG TGT GCA AAT CTA TGC – 3’ |
| miR-19a rev | 5’ – GTG CAG GGT CCG AGG T – 3’ |
| miR-20a RT | 5’ – GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACT ACC TG – 3’ |
| miR-20a fwd | 5’ – GCG GCG GTA AAG TGC TTA TAG TG – 3’ |
| miR-20a rev | 5’ – TGC AGG GTC CGA GGT AT – 3’ |
| miR-92a RT | 5’ – GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACA CAG GC – 3’ |
| miR-92a fwd | 5’ – CGG CGG TAT TGC ACT TGT CCC – 3’ |
| miR-92a rev | 5’ – GTG CAG GGT CCG AGG T – 3’ |
| RNU48 RT | 5’ – GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACG GTC AG – 3’ |
| RNU48 fwd | 5’ – CCA TGA GTG TGT CGC TGA TG – 3’ |
| RNU48 rev | 5’ – GTG CAG GGT CCG AGG T – 3’ |
| **plasmid construction** | |
| FGG promoter for | 5’ – GGG CTC GAG ATC TTG AGA AGT GAG AGC CTA TG – 3’ |
| FGG promoter rev | 5’ – AAT GCC AAG CTT TGT AAG CTC CTG GGA TAG – 3’ |
| Hp promoter for | 5’ – GGG CTC GAG ATC TGT TGA TGG GCA TTT GTC TTG – 3’ |
| Hp promoter rev | 5’ – AAT GCC AAG CTT GTT GGT CTT GCC TCT GG – 3’ |
| GAPDH promoter for | 5’ – GGG CTC GAG ATC TAC CTG TTC CCA CCG TGT G – 3’ |

| | |
|---|---|
| GAPDH promoter rev | 5’ – AAT GCC AAG CTT AGG CGG TGA CTC GGA CC – 3’ |
| PIAS3 3'UTR for | 5’ – TAA TGC TAG CGT TCC CTG GAT TAT GGA AAC – 3’ |
| PIAS3 3'UTR rev | 5’ – CGA CGC TAG CGA ACA TTC ACA ACC TTT ATT ATG – 3’ |
| PIAS3 3'UTR mut for | 5’ – CTG CAT TGG TCC AGG GAG GTG GAA G – 3’ |
| PIAS3 3'UTR mut rev | 5’ – CTG GAC CAA TGC AGA ATG AGC CAG G – 3’ |

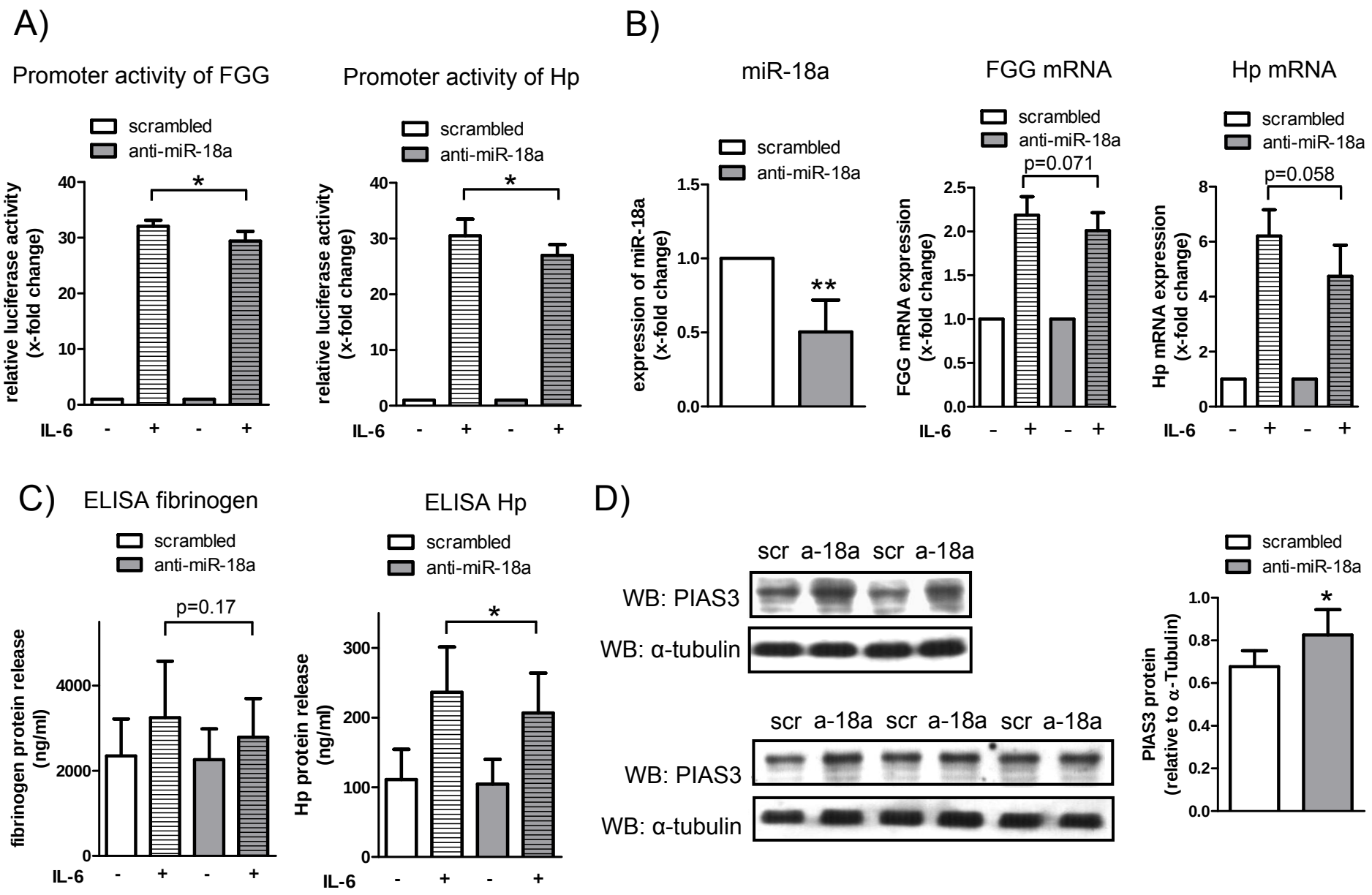
**References**


1. Schmittgen, T. D., and Livak, K. J. (2008) *Nat Protoc* **3**, 1101-1108
2. Chen, C., Ridzon, D. A., Broomer, A. J., Zhou, Z., Lee, D. H., Nguyen, J. T., Barbisin, M., Xu, N. L., Mahuvakar, V. R., Andersen, M. R., Lao, K. Q., Livak, K. J., and Guegler, K. J. (2005) *Nucleic Acids Res* **33**, e179
3. Kuhn, D. E., Martin, M. M., Feldman, D. S., Terry, A. V., Jr., Nuovo, G. J., and Elton, T. S. (2008) *Methods* **44**, 47-54
4. Bartel, D. P. (2004) *Cell* **116**, 281-297

Supplementary Figure S1



# Supplementary Figure S2



Supplementary Figure S3