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Supplemental Information

Modulation of the IL-6-Signaling Pathway in Liver Cells by miRNAs Targeting gp130, JAK1, and/or STAT3

Florence A. Servais, Mélanie Kirchmeyer, Matthias Hamdorf, Nadège W.E. Minoungou, Stefan Rose-John, Stephanie Kreis, Claude Haan, and Iris Behrmann

Figure S1

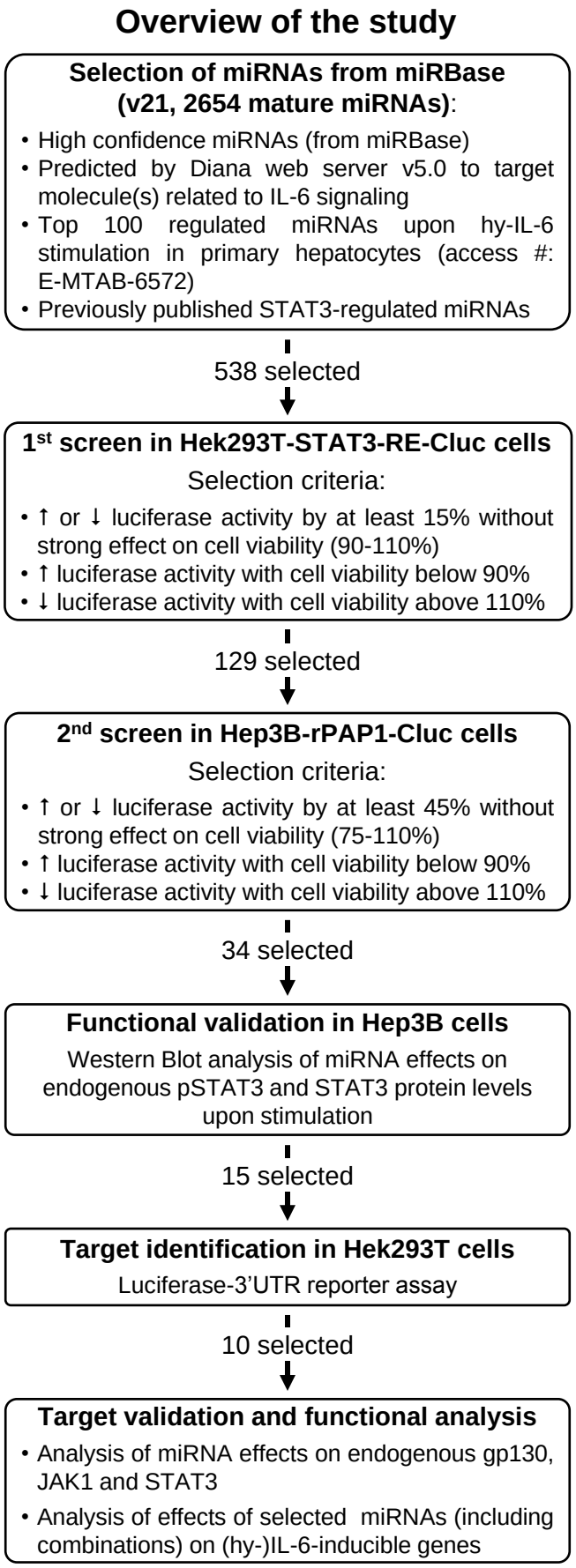
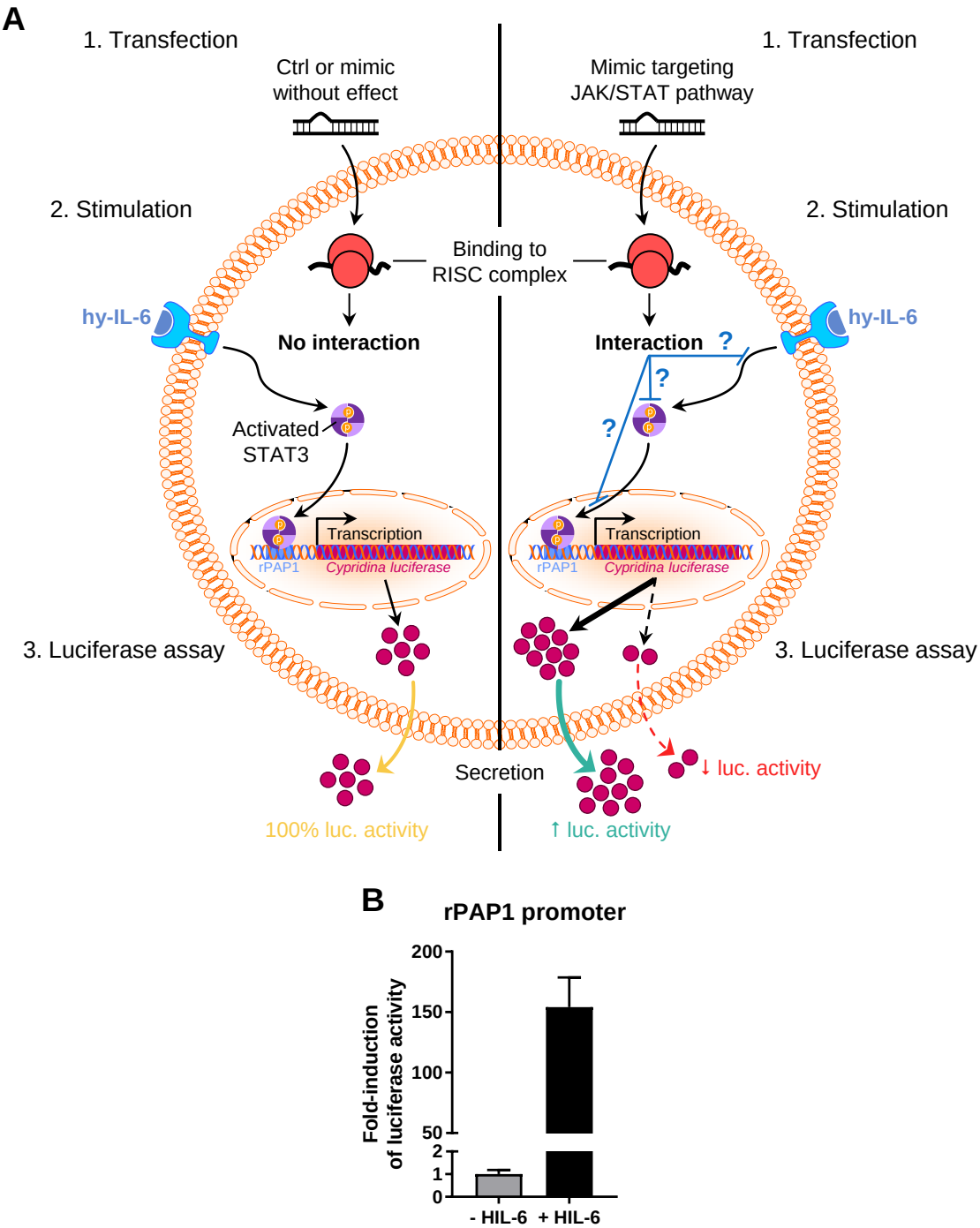
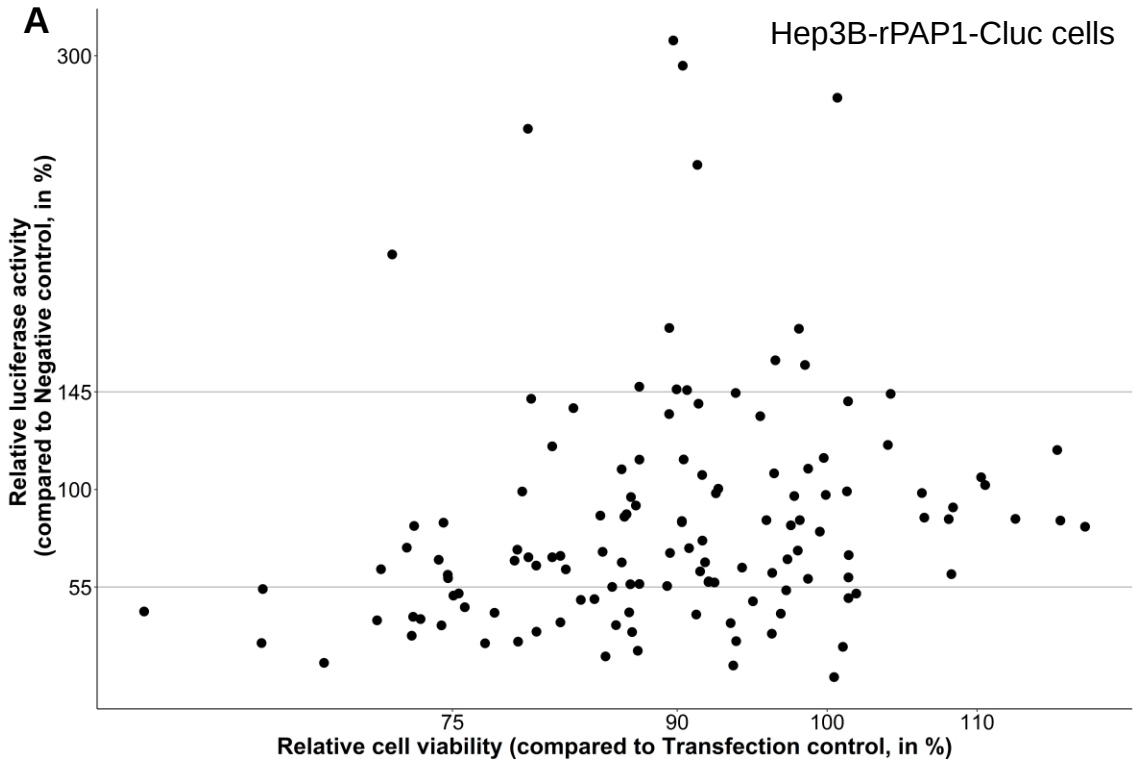

Overview of the study
Selection of miRNAs from miRBase (v21, 2654 mature miRNAs):
• High confidence miRNAs (from miRBase)
• Predicted by Diana web server v5.0 to target molecule(s) related to IL-6 signaling
• Top 100 regulated miRNAs upon hy-IL-6 stimulation in primary hepatocytes (access #: E-MTAB-6572)
• Previously published STAT3-regulated miRNAs
538 selected
1st screen in Hek293T-STAT3-RE-Cluc cells
Selection criteria:
• ↑ or ↓ luciferase activity by at least 15% without strong effect on cell viability (90-110%)
• ↑ luciferase activity with cell viability below 90%
• ↓ luciferase activity with cell viability above 110%
129 selected
2nd screen in Hep3B-rPAP1-Cluc cells
Selection criteria:
• ↑ or ↓ luciferase activity by at least 45% without strong effect on cell viability (75-110%)
• ↑ luciferase activity with cell viability below 90%
• ↓ luciferase activity with cell viability above 110%
34 selected
Functional validation in Hep3B cells
Western Blot analysis of miRNA effects on endogenous pSTAT3 and STAT3 protein levels upon stimulation
15 selected
Target identification in Hek293T cells
Luciferase-3'UTR reporter assay
10 selected
Target validation and functional analysis
• Analysis of miRNA effects on endogenous gp130, JAK1 and STAT3
• Analysis of effects of selected miRNAs (including combinations) on (hy-)IL-6-inducible genes

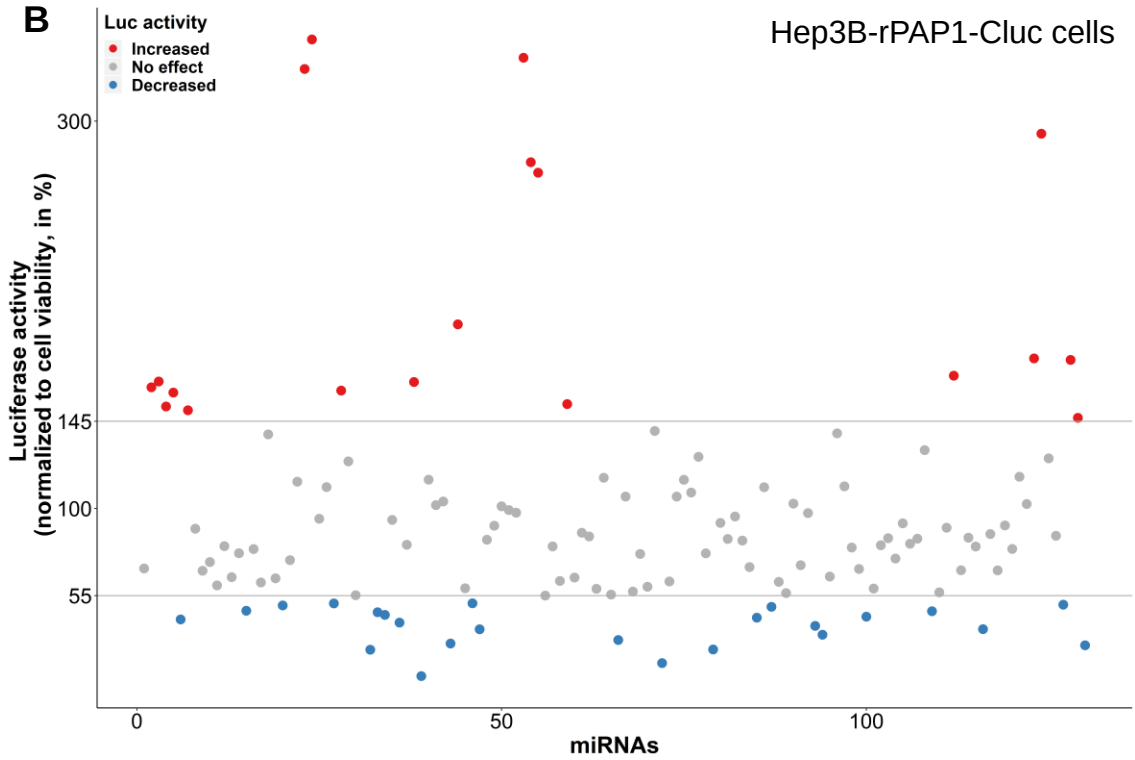
Figure S2



(**A**) Principle of the luciferase reporter assay. At day 1, cells were reverse transfected with a library of selected miRNA mimics (20 nM) and stimulated with 20 ng/mL hy-IL-6 at day 3. Upon stimulation, the secreted *Cypridina luciferase* gene is expressed in a STAT3-dependent manner. After 24 h, cell supernatants were collected and used for luciferase assay. **Left panel**: Some miRNA mimics as well as the negative control have no effect on the JAK/STAT3 signaling pathway, therefore a « normal » luciferase activity was measured in the supernatant of the cells. **Right panel**: Some mimics could have positive (e.g. by targeting negative regulators such as SOCS3) or negative effects (e.g. by targeting signaling molecules such as the receptor gp130) on the pathway, leading, respectively, to an increased or decreased luciferase activity measured. The STAT3-specific promoter rPAP1 was used for the screen performed in engineered Hep3B cells. (**B**) Fold induction of the luciferase activity measured in the supernatant of unstimulated and stimulated cells.

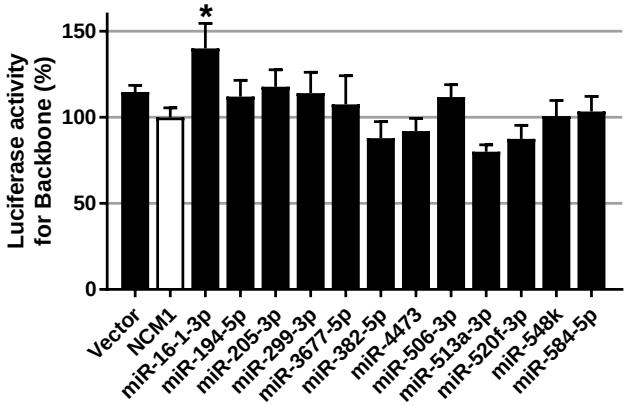
Figure S3





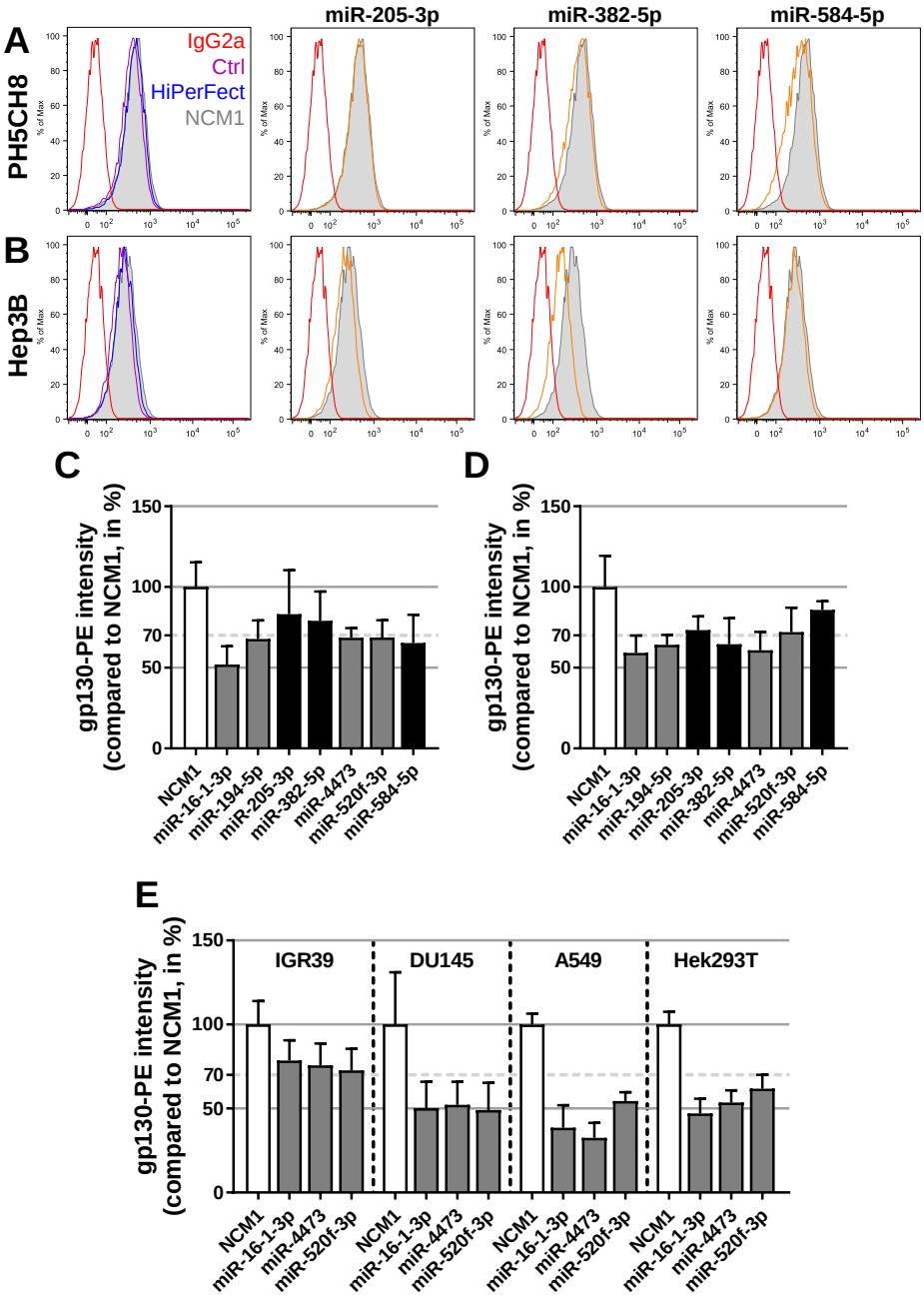
Screening results for the 129 selected candidate miRNAs in Hep3B-reporter cells (stably expressing the *Cypridina luciferase* gene under the control of the IL-6-inducible rPAP1 promoter). Cells were transfected with 20 nM mimics or Negative Control (NCM1) and stimulated with 20 ng/mL hy-IL-6 for 24 h. (**A**) (**Y axis**) Relative luciferase activity of transfected samples normalized to NCM1-transfected control samples. **(X axis)** Relative cell viability of transfected samples was determined with PrestoBlue, normalized to control samples with transfection reagent only. (**B**) The luciferase activity for each miRNA mimic was normalized to the associated viability. Dots represent the averaged luciferase activity obtained from 3 biological replicates. Some miRNA mimics reduced the level of pSTAT3 but were not further considered as they have been already associated with the JAK/STAT pathway (e.g. miR-17-5p targets STAT3 [He *et al.*, 2013]) or well-studied (e.g. miR-25-3p, reviewed in Sárközy *et* al., 2018, for its target genes and role in tumorigenesis).

Figure S4



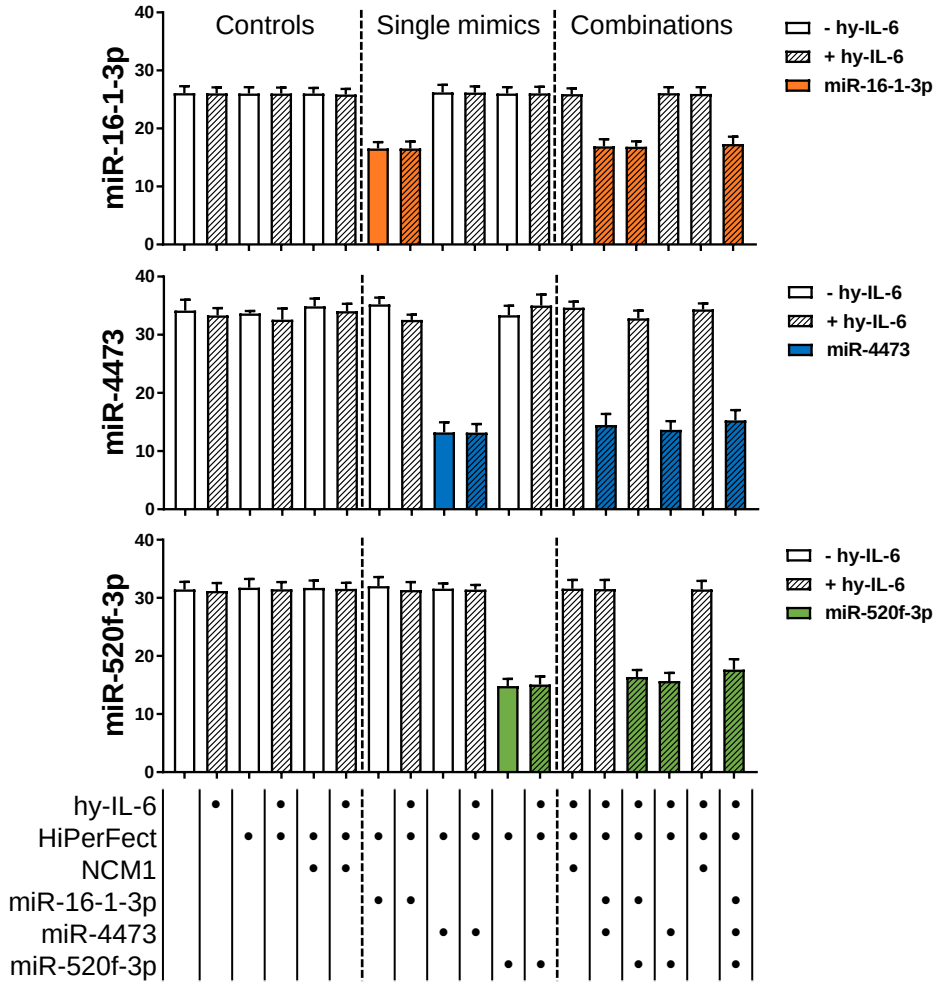
Hek293T cells were co-transfected with the selected mimics and the backbone vector harboring the *Gaussia luciferase* cDNA without 3'UTR inserted. Negative control mimic 1 (NCM1) is shown in white. Error bars represent standard deviation of 3 biological replicates. Kruskal-Wallis followed by Dunn's Post-Hoc test were performed to assess statistical significance represented with ⋆adjusted p-value <0.05. Refers to **Figures 2, 4** and **S7**.

Figure S5



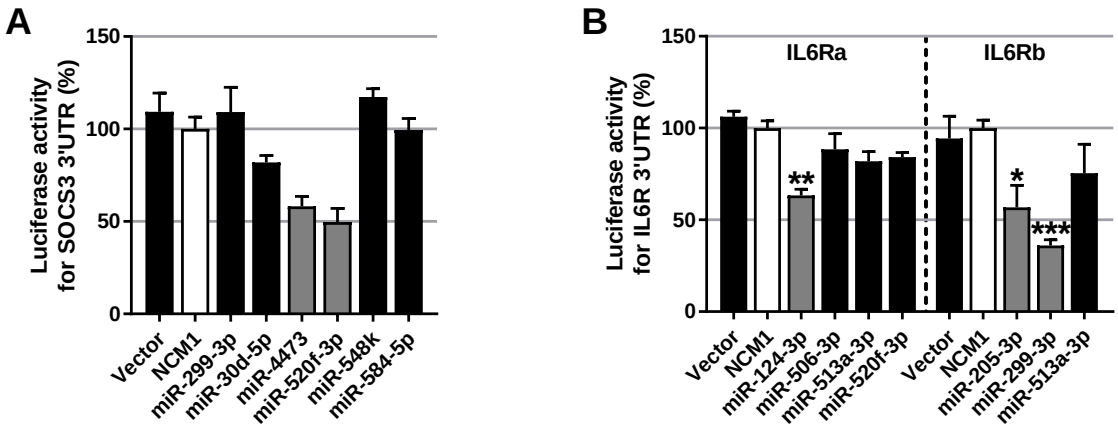
(**A**, **C**) Non-neoplastic PH5CH8 liver cells and (**B**, **D**) Hep3B hepatoma cells were either left untreated (Ctrl, in purple) or transfected with a negative control (NCM1, in gray) or one of the selected miRNA mimics and analyzed for gp130 expression level by flow cytometry. (**A-B**) Overlap. (**C-E**) Relative median of gp130-PE intensity in (**C**) PH5CH8, (**D**) Hep3B, and (**E**) in IGR39, DU145, A549, and HEK293T cells. Error bars represent the mean of 3 biological replicates. Refers to **Figures 2** and **6**.

Figure S6



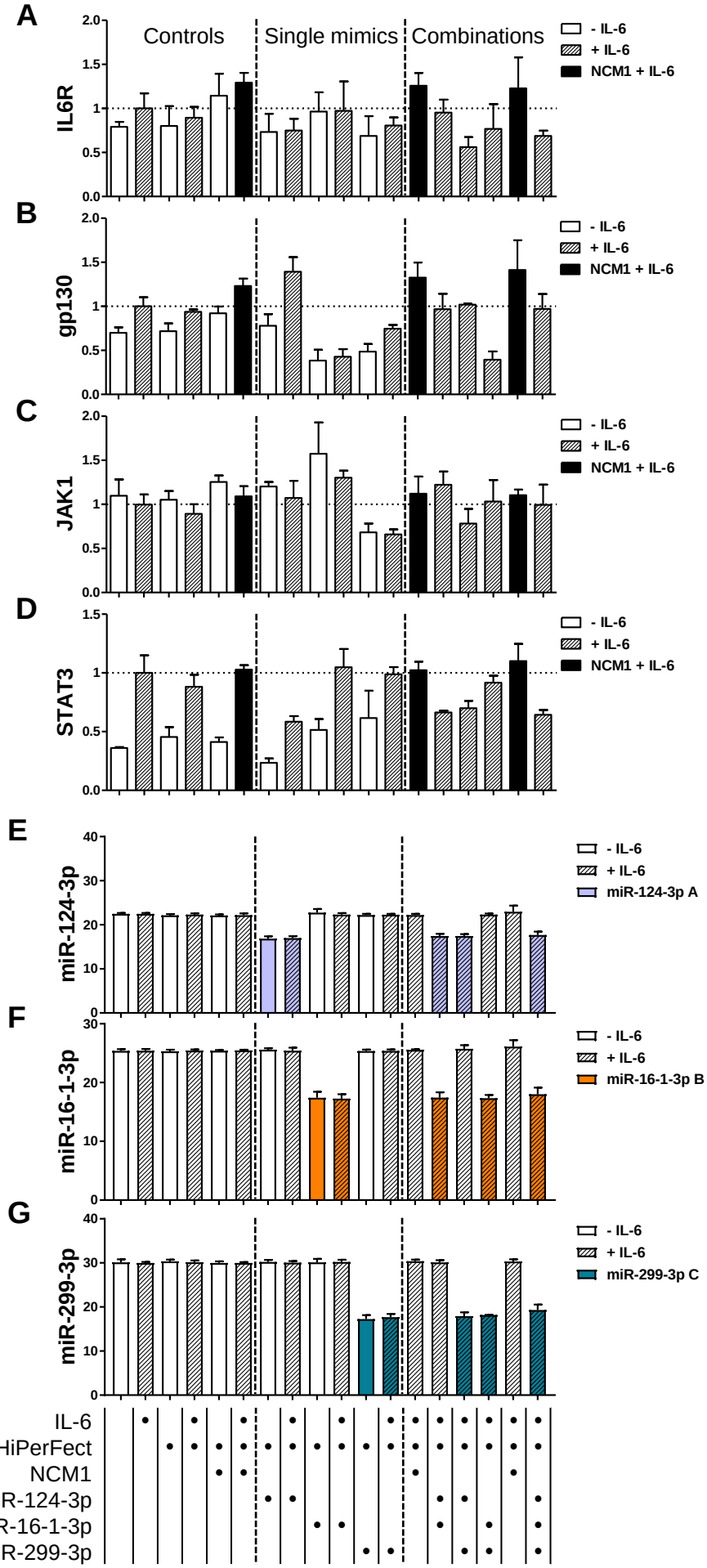
Expression levels of miR-16-1-3p, miR-4473 and/or miR-520f-3p upon reverse transfection of PH5CH8 cells with the corresponding mimics (represented by the Ct values obtained by qPCR; thus, successful transfection is reflected by reduced Ct values). Panel at the bottom indicates the applied treatments per lane. Refers to **Figure 3**.

Figure S7



Hek293T cells were co-transfected with the selected mimics and the vectors harboring the *Gaussia luciferase* cDNA fused to the 3'UTR of (**A**) SOCS3 or (**B**) IL6R. Negative control mimic 1 (NCM1) is shown in white. Error bars represent standard deviation of 3 biological replicates. Kruskal-Wallis followed by Dunn's Post-Hoc test were performed to assess statistical significance represented with ⋆ adjusted p-value (adj.p.)<0.05, ⋆⋆adj.p.<0.01, ⋆⋆⋆ajd.p.<0.001. Refers to **Figure 6**.

Figure S8



Expression levels of IL6R, gp130, JAK1, STAT3 (relative to IL-6 sample) as well as of miR-124-3p, miR-16-1-3p and /or miR-299-3p upon reverse transfection of Hep3B cells with the corresponding mimics (represented by the Ct values obtained by qPCR; thus, successful transfection is reflected by reduced Ct values). Panel at the bottom indicates the applied treatments per lane. Refers to **Figure 5**.

Table S1

List of all miRNAs present in the library.

538 human miRNA mimics were screened in Hek293T reporter cells.

miRNA	Accession #	miRNA	Accession #	miRNA	Accession #	miRNA	Accession #	miRNA	Accession #	miRNA	Accession #
let-7a-3p	MIMAT0004481	miR-1306-3p	MIMAT0005950	miR-181d-5p	MIMAT0002821	miR-219b-3p	MIMAT0019748	miR-3142	MIMAT0015011	miR-365	MIMAT0000710
let-7a-5p	MIMAT0000062	miR-1307-3p	MIMAT0005951	miR-182-5p	MIMAT0000259	miR-221-3p	MIMAT0000278	miR-3143	MIMAT0015012	miR-3652	MIMAT0018072
let-7b-5p	MIMAT0000063	miR-130a-3p	MIMAT0000425	miR-183-5p	MIMAT0000261	miR-221-5p	MIMAT0004568	miR-3144-5p	MIMAT0015014	miR-3656	MIMAT0018076
let-7d-5p	MIMAT0000065	miR-130a-5p	MIMAT0004593	miR-185-5p	MIMAT0000455	miR-222-3p	MIMAT0000279	miR-3148	MIMAT0015021	miR-3658	MIMAT0018078
let-7e-5p	MIMAT0000066	miR-130b-3p	MIMAT0000691	miR-186-5p	MIMAT0000456	miR-223-3p	MIMAT0000280	miR-3149	MIMAT0015022	miR-365b-5p	MIMAT0022833
let-7f-1-3p	MIMAT0004486	miR-1323	MIMAT0005795	miR-188-5p	MIMAT0000457	miR-22-3p	MIMAT0000077	miR-3150a-3p	MIMAT0015023	miR-3660	MIMAT0018081
let-7f-5p	MIMAT0000067	miR-132-3p	MIMAT0000426	miR-18a-5p	MIMAT0000072	miR-224-3p	MIMAT0009198	miR-3155a	MIMAT0015029	miR-3662	MIMAT0018083
let-7g-5p	MIMAT0000414	miR-133a-3p	MIMAT0000427	miR-18b-5p	MIMAT0001412	miR-224-5p	MIMAT0000281	miR-3158-3p	MIMAT0015032	miR-3671	MIMAT0018094
miR-100-5p	MIMAT0000098	miR-133b	MIMAT0000770	miR-1908-5p	MIMAT0007881	miR-23a-3p	MIMAT0000078	miR-31-5p	MIMAT0000089	miR-3677-5p	MIMAT0019221
miR-101-3p	MIMAT0000099	miR-1343-3p	MIMAT0019776	miR-190a-5p	MIMAT0000458	miR-23c	MIMAT0018000	miR-3163	MIMAT0015037	miR-3678-3p	MIMAT0018103
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miR-10a-5p	MIMAT0000253	miR-138-5p	MIMAT0000430	miR-193a-3p	MIMAT0000459	miR-26b-5p	MIMAT0000083	miR-320a	MIMAT0000510	miR-370-3p	MIMAT0000722
miR-10b-5p	MIMAT0000254	miR-139-5p	MIMAT0000250	miR-193b-3p	MIMAT0002819	miR-27a-3p	MIMAT0000084	miR-320b	MIMAT0005792	miR-371b-3p	MIMAT0019893
miR-1185-1-3p	MIMAT0022838	miR-1-3p	MIMAT0000416	miR-194-5p	MIMAT0000460	miR-27a-5p	MIMAT0004501	miR-320c	MIMAT0005793	miR-372-3p	MIMAT0000724
miR-1185-2-3p	MIMAT0022713	miR-141-3p	MIMAT0000432	miR-195-5p	MIMAT0000461	miR-27b-3p	MIMAT0000419	miR-320d	MIMAT0006764	miR-373-3p	MIMAT0000726
miR-1207-5p	MIMAT0005871	miR-141-5p	MIMAT0004598	miR-196a-5p	MIMAT0000226	miR-27b-5p	MIMAT0004588	miR-323a-3p	MIMAT0000755	miR-374a-3p	MIMAT0004688
miR-122-3p	MIMAT0004590	miR-142-3p	MIMAT0000434	miR-196b-5p	MIMAT0001080	miR-28-5p	MIMAT0000085	miR-32-3p	MIMAT0004505	miR-374a-5p	MIMAT0000727
miR-122-5p	MIMAT0000421	miR-142-5p	MIMAT0000433	miR-1972	MIMAT0009447	miR-2861	MIMAT0013802	miR-32-5p	MIMAT0000090	miR-374b-5p	MIMAT0004955
miR-1226-3p	MIMAT0005577	miR-143-3p	MIMAT0000435	miR-197-3p	MIMAT0000227	miR-296-5p	MIMAT0000690	miR-328-3p	MIMAT0000752	miR-376a-3p	MIMAT0000729
miR-124-3p	MIMAT0000422	miR-144-3p	MIMAT0000436	miR-199a-3p	MIMAT0000232	miR-299-3p	MIMAT0000687	miR-329-3p	MIMAT0001629	miR-376c-3p	MIMAT0000720
miR-124-5p	MIMAT0004591	miR-1469	MIMAT0007347	miR-199a-5p	MIMAT0000231	miR-29a-3p	MIMAT0000086	miR-330-3p	MIMAT0000751	miR-377-3p	MIMAT0000730
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miR-1252-3p	MIMAT0026744	miR-146a-5p	MIMAT0000449	miR-19a-3p	MIMAT0000073	miR-29c-3p	MIMAT0000681	miR-335-3p	MIMAT0004703	miR-378a-3p	MIMAT0000732
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Table S1, continued

miRNA	Accession #	miRNA	Accession #	miRNA	Accession #	miRNA	Accession #	miRNA	Accession #	miRNA	Accession #
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miR-421	MIMAT0003339	miR-4700-5p	MIMAT0019796	miR-513c-5p	MIMAT0005789	miR-548c-3p	MIMAT0003285	miR-577	MIMAT0003242	miR-4659b-5p	MIMAT0019733
miR-422a	MIMAT0001339	miR-4707-3p	MIMAT0019808	miR-514a-3p	MIMAT0002883	miR-548c-5p	MIMAT0004806	miR-581	MIMAT0003246	miR-4668-3p	MIMAT0019746
miR-423-3p	MIMAT0001340	miR-4707-5p	MIMAT0019807	miR-514b-5p	MIMAT0015087	miR-548d-3p	MIMAT0003323	miR-582-5p	MIMAT0003247	miR-4669	MIMAT0019749
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miR-4263	MIMAT0016898	miR-4734	MIMAT0019859	miR-5196-3p	MIMAT0021129	miR-548g-5p	MIMAT0022722	miR-589-5p	MIMAT0004799	miR-513b-5p	MIMAT0005788
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miR-4420	MIMAT0018933	miR-4793-3p	MIMAT0019966	miR-520d-3p	MIMAT0002856	miR-548p	MIMAT0005934	miR-627-5p	MIMAT0003296	miR-885-5p	MIMAT0004947
miR-4422	MIMAT0018935	miR-4793-5p	MIMAT0019965	miR-520e	MIMAT0002825	miR-548t-3p	MIMAT0022730	miR-628-5p	MIMAT0004809	miR-887-3p	MIMAT0004951
miR-4429	MIMAT0018944	miR-4796-3p	MIMAT0019971	miR-520f-3p	MIMAT0002830	miR-548t-5p	MIMAT0015009	miR-629-5p	MIMAT0004810	miR-92a-2-5p	MIMAT0004508
miR-4430	MIMAT0018945	miR-483-3p	MIMAT0002173	miR-522-3p	MIMAT0002868	miR-548v	MIMAT0015020	miR-638	MIMAT0003308	miR-92a-3p	MIMAT0000092
miR-4441	MIMAT0018959	miR-485-5p	MIMAT0002175	miR-526b-3p	MIMAT0002836	miR-548w	MIMAT0015060	miR-642a-5p	MIMAT0003312	miR-92b-3p	MIMAT0003218
miR-4451	MIMAT0018973	miR-487b-3p	MIMAT0003180	miR-532-3p	MIMAT0004780	miR-548x-3p	MIMAT0015081	miR-6503-5p	MIMAT0025462	miR-93-3p	MIMAT0004509
miR-4457	MIMAT0018979	miR-488-3p	MIMAT0004763	miR-539-5p	MIMAT0003163	miR-548x-5p	MIMAT0022733	miR-6511a-3p	MIMAT0025479	miR-9-3p	MIMAT0000442
miR-4459	MIMAT0018981	miR-490-3p	MIMAT0002806	miR-541-5p	MIMAT0004919	miR-548y	MIMAT0018354	miR-651-5p	MIMAT0003321	miR-941	MIMAT0004984
miR-4463	MIMAT0018987	miR-491-5p	MIMAT0002807	miR-542-3p	MIMAT0003389	miR-548z	MIMAT0018446	miR-652-3p	MIMAT0003322	miR-942-5p	MIMAT0004985
miR-4468	MIMAT0018995	miR-494-3p	MIMAT0002816	miR-543	MIMAT0004954	miR-550a-5p	MIMAT0004800	miR-654-5p	MIMAT0003330	miR-944	MIMAT0004987
miR-4473	MIMAT0019000	miR-495-3p	MIMAT0002817	miR-545-3p	MIMAT0003165	miR-550b-2-5p	MIMAT0022737	miR-660-5p	MIMAT0003338	miR-9-5p	MIMAT0000441
miR-4477a	MIMAT0019004	miR-497-3p	MIMAT0004768	miR-548a-3p	MIMAT0003251	miR-551b-3p	MIMAT0003233	miR-664b-3p	MIMAT0022272	miR-96-5p	MIMAT0000095
miR-4482-3p	MIMAT0020958	miR-497-5p	MIMAT0002820	miR-548a-5p	MIMAT0004803	miR-556-5p	MIMAT0003220	miR-664b-5p	MIMAT0022271	miR-98-5p	MIMAT0000096
miR-4492	MIMAT0019027	miR-499a-3p	MIMAT0004772	miR-548aa	MIMAT0018447	miR-5571-5p	MIMAT0022257	miR-671-5p	MIMAT0003880	miR-99a-5p	MIMAT0000097
miR-4508	MIMAT0019045	miR-499a-5p	MIMAT0002870	miR-548ab	MIMAT0018928	miR-5572	MIMAT0022260	miR-675-5p	MIMAT0004284	miR-99b-5p	MIMAT0000689
miR-450a-5p	MIMAT0001545	miR-5001-5p	MIMAT0021021	miR-548ac	MIMAT0018938	miR-5581-5p	MIMAT0022275	miR-708-5p	MIMAT0004926		
miR-450b-5p	MIMAT0004909	miR-500a-3p	MIMAT0002871	miR-548ae-3p	MIMAT0018954	miR-5582-3p	MIMAT0022280	miR-7-1-3p	MIMAT0004553		
miR-4514	MIMAT0019051	miR-5010-3p	MIMAT0021044	miR-548ah-3p	MIMAT0020957	miR-5583-3p	MIMAT0022282	miR-7-2-3p	MIMAT0004554		
miR-4516	MIMAT0019053	miR-5010-5p	MIMAT0021043	miR-548aj-3p	MIMAT0018990	miR-5584-3p	MIMAT0022284	miR-744-5p	MIMAT0004945		
miR-4517	MIMAT0019054	miR-502-5p	MIMAT0002873	miR-548aj-5p	MIMAT0022739	miR-559	MIMAT0003223	miR-758-3p	MIMAT0003879		
miR-451a	MIMAT0001631	miR-503-5p	MIMAT0002874	miR-548ak	MIMAT0019013	miR-5590-3p	MIMAT0022300	miR-7-5p	MIMAT0000252		
miR-452-3p	MIMAT0001636	miR-504-5p	MIMAT0002875	miR-548am-3p	MIMAT0019076	miR-561-3p	MIMAT0003225	miR-762	MIMAT0010313		
miR-4524a-3p	MIMAT0019063	miR-505-3p	MIMAT0002876	miR-548am-5p	MIMAT0022740	miR-5683	MIMAT0022472	miR-765	MIMAT0003945		
miR-4524a-5p	MIMAT0019062	miR-506-3p	MIMAT0002878	miR-548ap-5p	MIMAT0021037	miR-5688	MIMAT0022479	miR-766-3p	MIMAT0003888		
miR-4526	MIMAT0019065	miR-508-3p	MIMAT0002880	miR-548aq-3p	MIMAT0022264	miR-5691	MIMAT0022483	miR-767-3p	MIMAT0003883		
miR-4530	MIMAT0019069	miR-509-3-5p	MIMAT0004975	miR-548ar-3p	MIMAT0022266	miR-5692a	MIMAT0022484	miR-767-5p	MIMAT0003882		
miR-4536-5p	MIMAT0019078	miR-509-3p	MIMAT0002881	miR-548ar-5p	MIMAT0022265	miR-5692b	MIMAT0022497	miR-769-5p	MIMAT0003886		
miR-454-3p	MIMAT0003885	miR-509-5p	MIMAT0004779	miR-548as-3p	MIMAT0022268	miR-5692c	MIMAT0022476	miR-873-5p	MIMAT0004953		
miR-455-5p	MIMAT0003150	miR-510-5p	MIMAT0002882	miR-548as-5p	MIMAT0022267	miR-5693	MIMAT0022486	miR-874-3p	MIMAT0004911		

Table S2

Summary of the luciferase and PrestoBlue results obtained in the 1st and 2nd screens for each of the 34 miRNAs selected for functional analysis of their effects on STAT3 activation. Results represent the Luciferase or PrestoBlue signals measured, compared to the corresponding control and expressed in %. Errors (SD) represent standard deviation of 3 biological replicates.

miRNA	1st screen				2nd screen			
	Luciferase		PrestoBlue		Luciferase		PrestoBlue	
	% to Ctrl	SD	% to Ctrl	SD	% to Ctrl	SD	% to Ctrl	SD
let-7e-5p	126	18	107	13	157	8	99	1
let-7f-1-3p	66	30	103	9	34	9	81	10
miR-124-3p	**85**	**27**	**95**	**3**	**46**	**3**	**76**	**3**
miR-1277-5p	83	25	101	7	39	7	82	6
miR-130a-5p	80	9	100	1	43	7	87	6
miR-133a-3p	126	18	102	2	295	14	90	11
miR-133b	127	8	103	0	307	22	90	14
miR-142-3p	58	15	108	8	48	2	95	7
miR-155-5p	86	36	126	25	26	8	87	2
miR-16-1-3p	**72**	**33**	**104**	**9**	**23**	**5**	**85**	**12**
miR-17-5p	85	11	98	9	42	6	91	0
miR-188-5p	77	12	90	1	38	9	94	5
miR-193a-3p	117	15	100	2	160	24	97	5
miR-194-5p	**60**	**15**	**92**	**5**	**14**	**4**	**100**	**9**
miR-205-3p	**74**	**18**	**105**	**8**	**20**	**3**	**66**	**12**
miR-208b-3p	82	15	105	23	174	10	89	10
miR-25-3p	71	13	109	7	49	7	84	7
miR-299-3p	**67**	**15**	**103**	**8**	**30**	**6**	**79**	**12**
miR-30a-5p	141	7	105	6	266	43	80	4
miR-30c-5p	136	8	109	5	281	13	101	11
miR-30d-5p	**126**	**10**	**107**	**1**	**250**	**41**	**91**	**10**
miR-363-3p	72	20	104	7	49	12	84	6
miR-3677-5p	**136**	**20**	**105**	**21**	**43**	**15**	**78**	**7**
miR-382-5p	**84**	**11**	**90**	**13**	**30**	**5**	**94**	**15**
miR-4473	**74**	**35**	**117**	**4**	**19**	**7**	**94**	**17**
miR-494-3p	**77**	**17**	**97**	**1**	**27**	**6**	**101**	**25**
miR-506-3p	**74**	**8**	**99**	**1**	**37**	**6**	**86**	**11**
miR-513a-3p	**69**	**17**	**91**	**0**	**50**	**4**	**101**	**10**
miR-519e-5p	81	8	99	9	34	9	87	10
miR-520f-3p	**72**	**9**	**102**	**4**	**33**	**4**	**96**	**12**
miR-548k	**76**	**13**	**98**	**1**	**43**	**2**	**97**	**17**
miR-584-5p	**83**	**23**	**110**	**10**	**29**	**5**	**77**	**14**
miR-7-2-3p	81	13	92	14	174	12	98	6
miR-744-5p	117	15	92	10	208	95	71	12

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