

Figure S1

Overview of the study

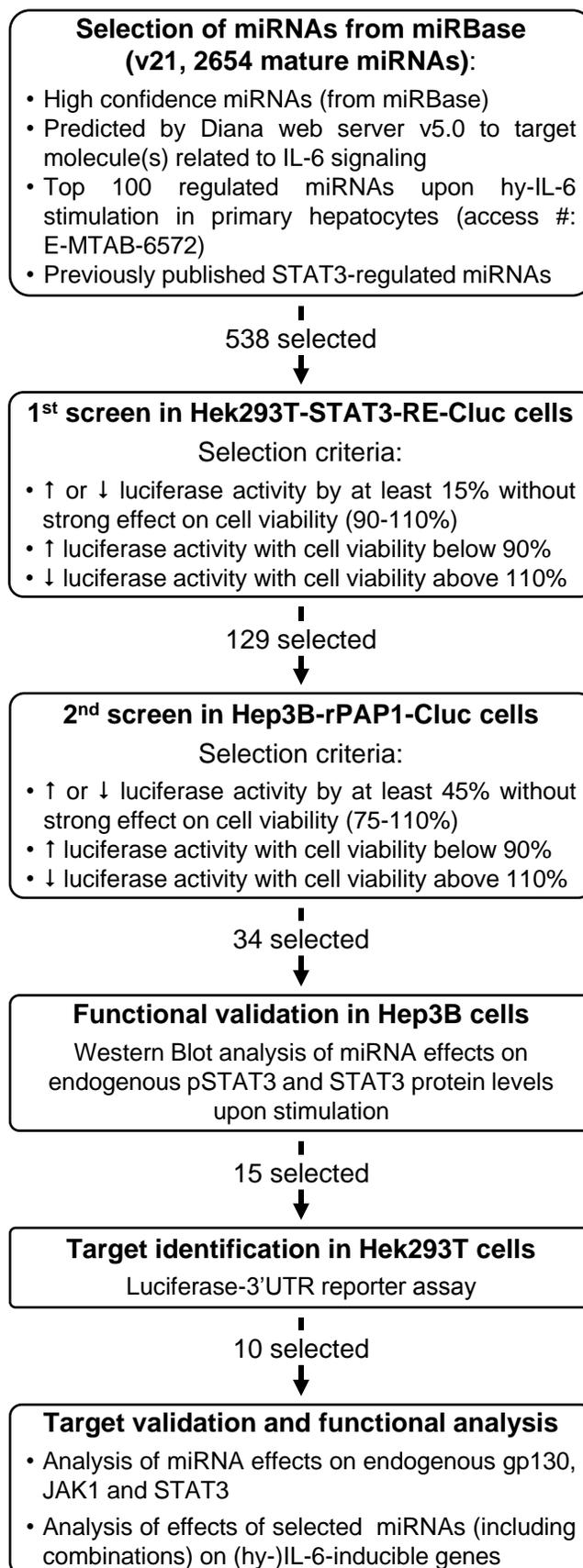
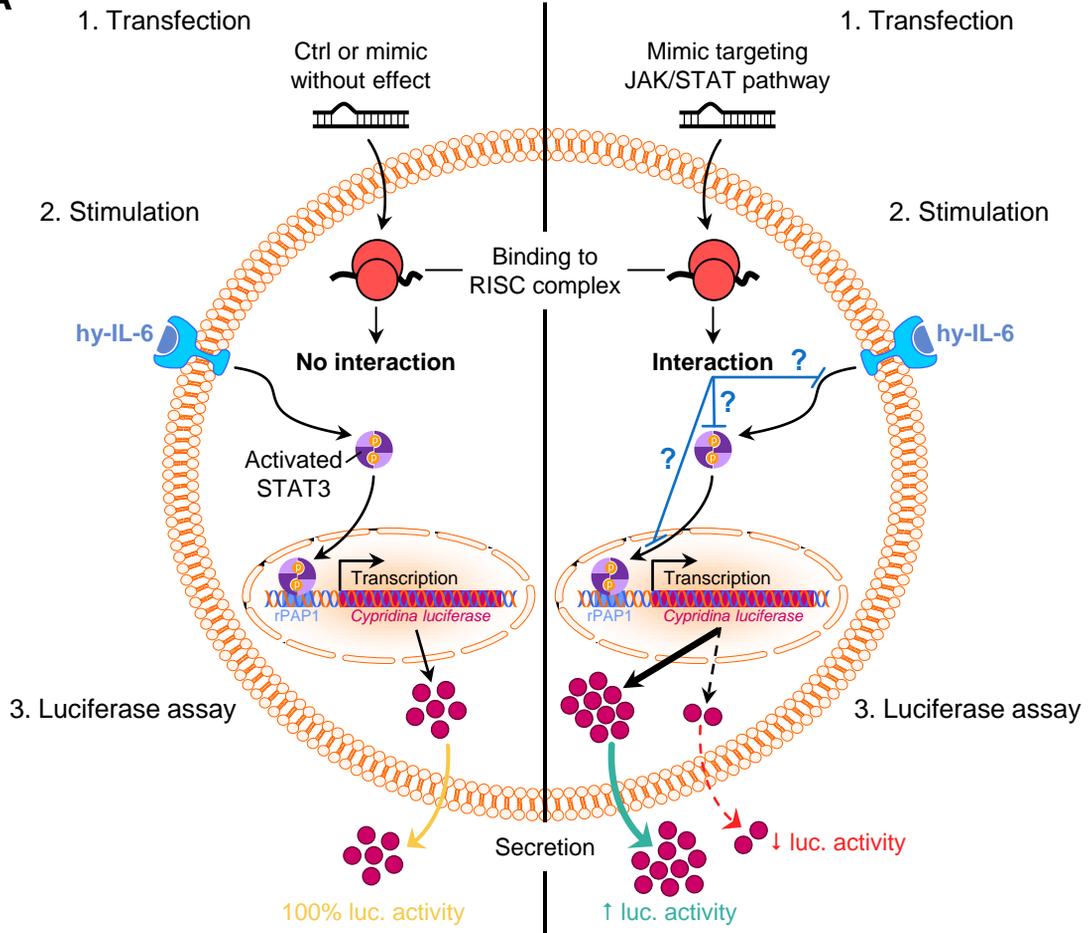
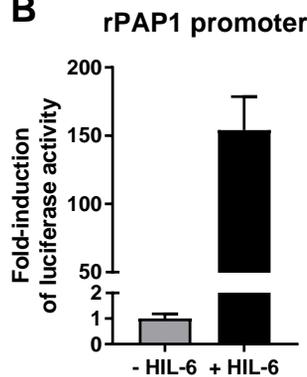


Figure S2

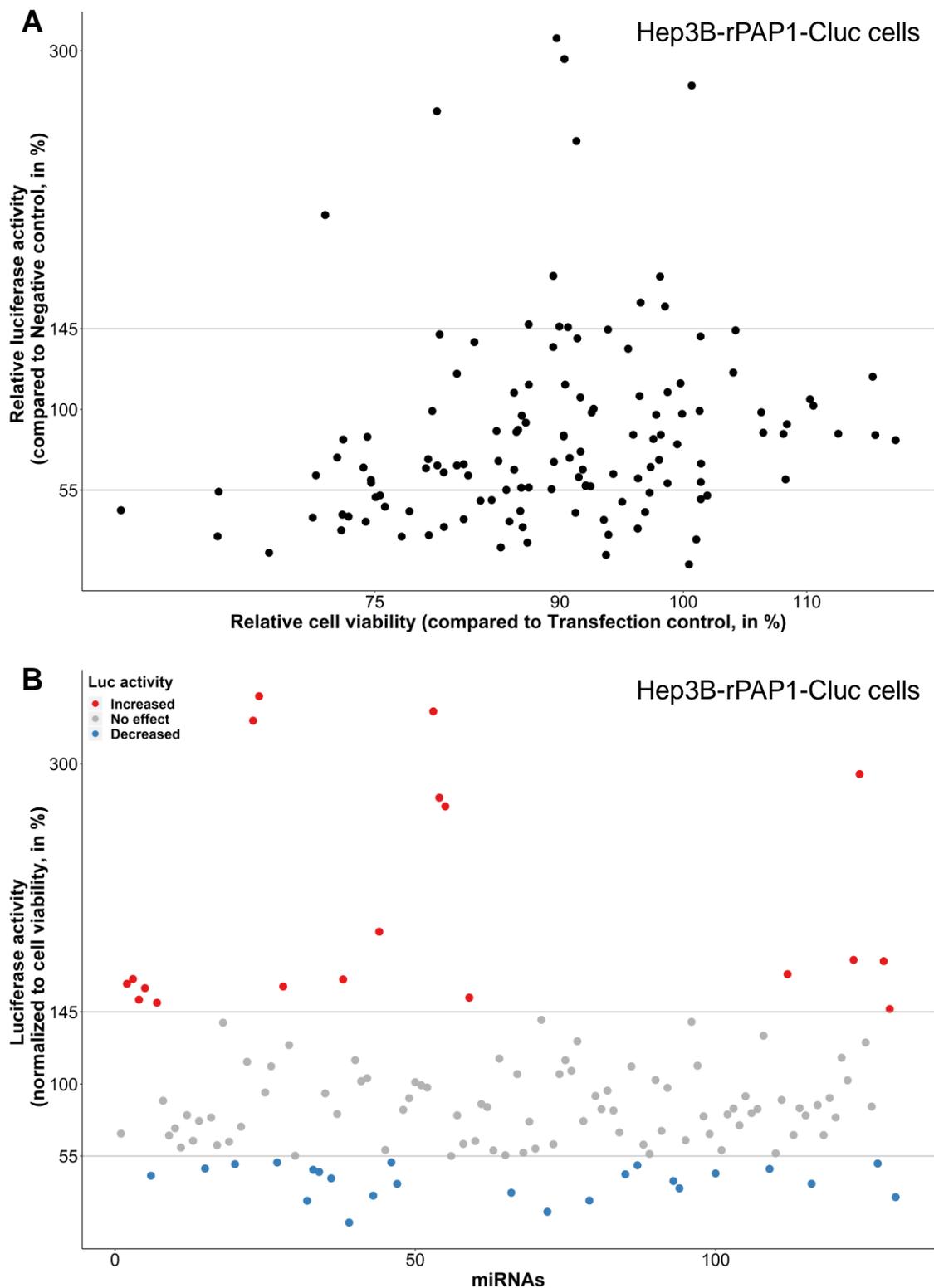
A



B

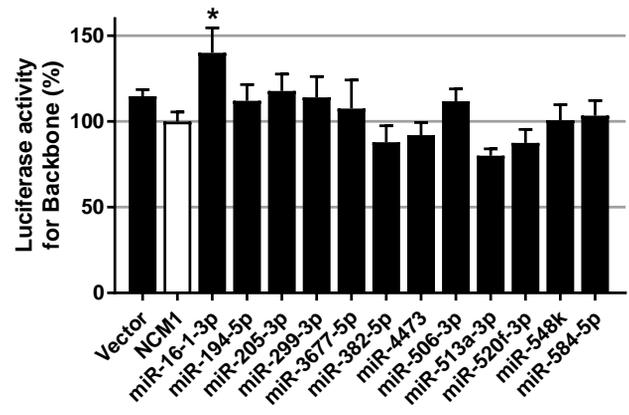


(A) Principle of the luciferase reporter assay. At day 1, cells were reverse transfected with a library of selected miRNA mimics (20 nM) and stimulated with 20 ng/mL hy-IL-6 at day 3. Upon stimulation, the secreted *Cypridina luciferase* gene is expressed in a STAT3-dependent manner. After 24 h, cell 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

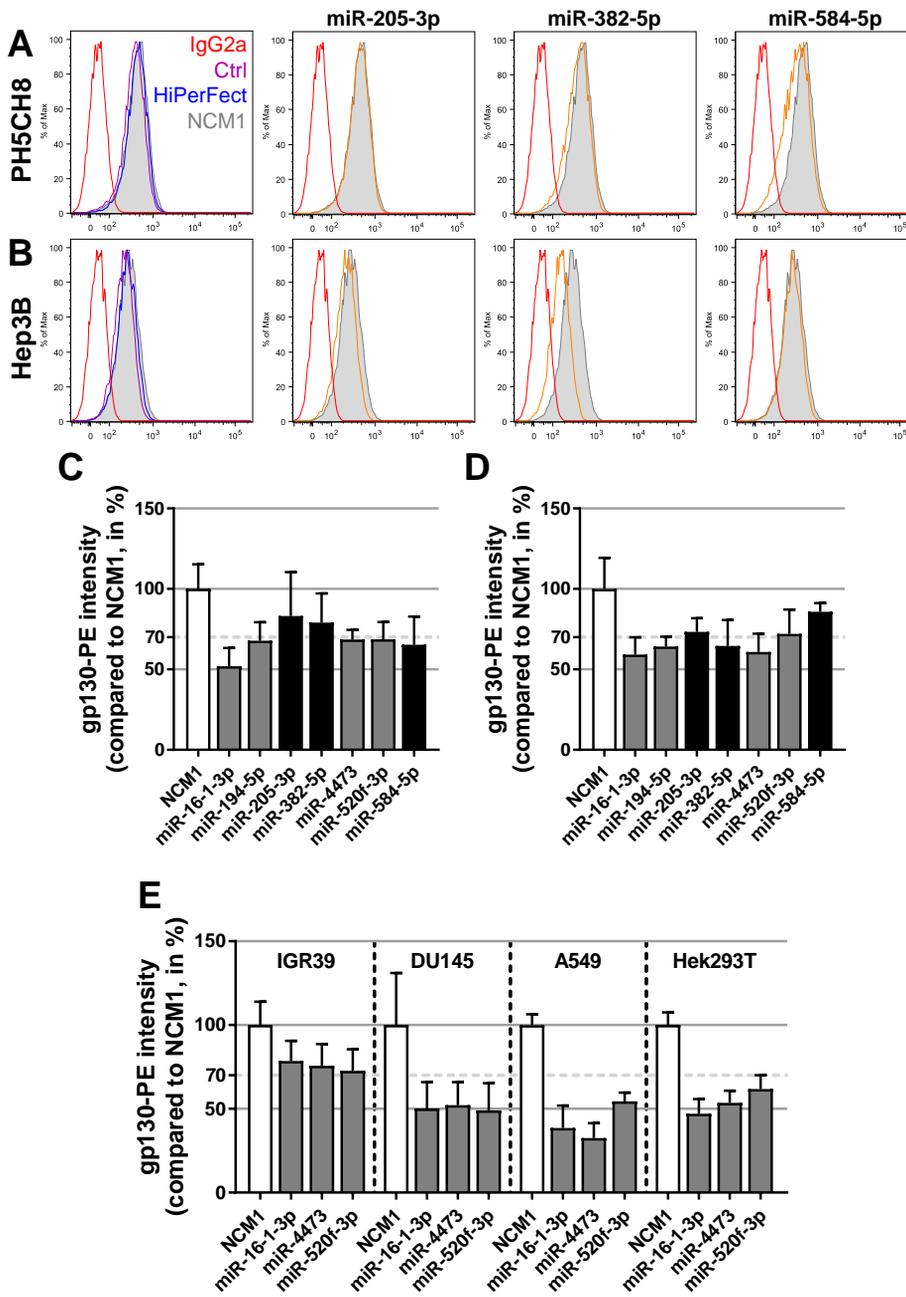
Screening results for the 129 selected candidate miRNAs in Hep3B-reporter cells (stably expressing the *Cypridina luciferase* gene under the control of the IL-6-inducible rPAP1 promoter). Cells were transfected with 20 nM mimics or Negative Control (NCM1) and stimulated with 20 ng/mL hy-IL-6 for 24 h. **(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



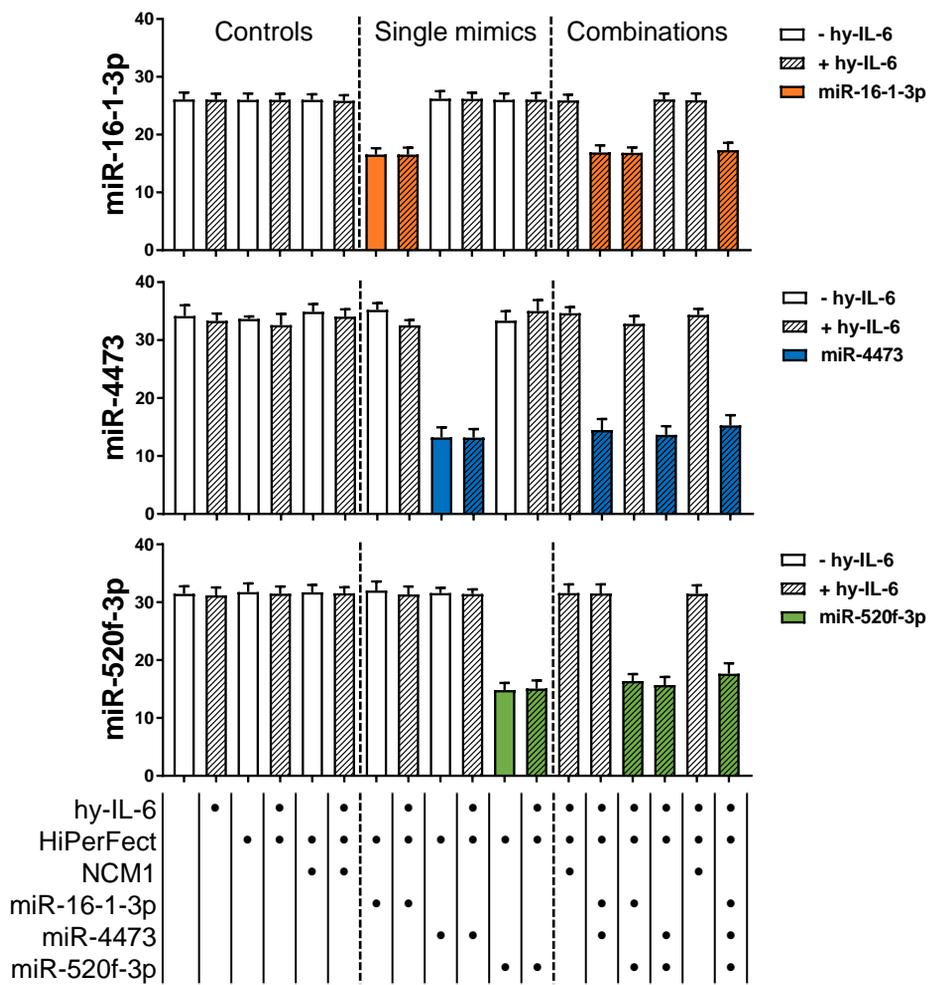
Hek293T cells were co-transfected with the selected mimics and the backbone vector harboring the *Gaussia luciferase* cDNA without 3'UTR inserted. Negative control mimic 1 (NCM1) is shown in white. Error bars represent standard deviation of 3 biological replicates. Kruskal-Wallis followed by Dunn's Post-Hoc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

Figure S5



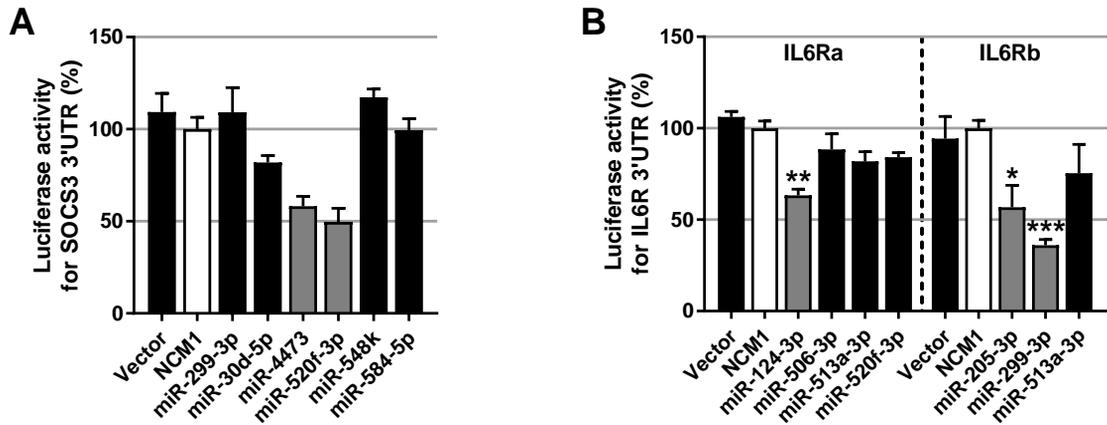
(A, C) Non-neoplastic PH5CH8 liver cells and (B, D) Hep3B hepatoma cells were either left untreated (Ctrl, in purple) or transfected with a negative control (NCM1, in gray) or one of the selected miRNA mimics and analyzed for gp130 expression level by flow cytometry. (A-B) Overlap. (C-E) Relative median 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



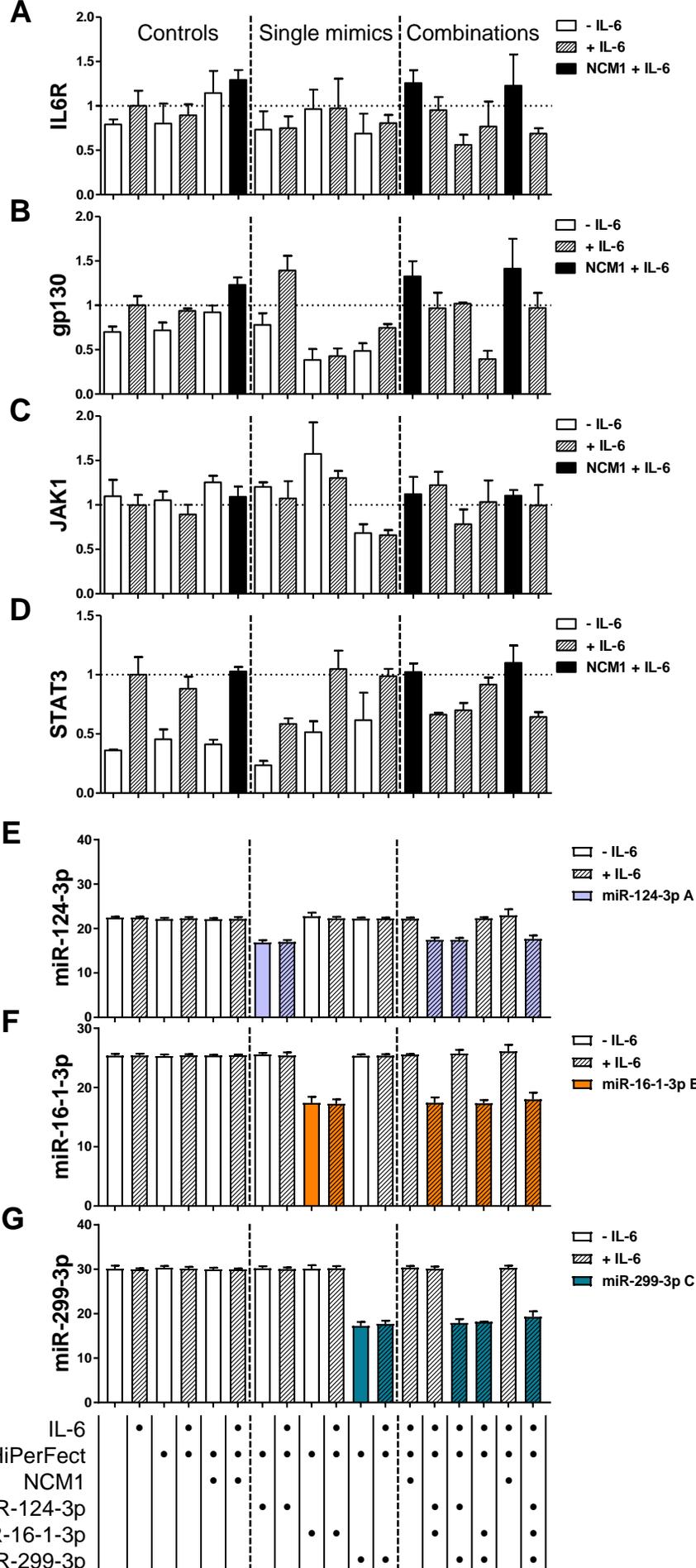
Expression levels of miR-16-1-3p, miR-4473 and/or miR-520f-3p upon reverse transfection of PH5CH8 cells with the corresponding mimics (represented by the Ct values obtained by qPCR; thus, successful transfection is reflected by reduced Ct values). Panel at the bottom indicates the applied treatments 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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Table S1, continued

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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						
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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