

# Viral microRNAs Target a Gene Network, Inhibit STAT Activation, and Suppress Interferon Responses

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## **Supplementary Information**

**Figure S1.** Phosphorylated and total STAT information.

**Figure S2.** Full-length blots for Figure 3.

**Figure S3.** Full-length blots for Figures 4, 5, and 7.

**Table S1.** Luciferase data is shown (mean and standard deviation, n = 3) for all tested combinations of 3'UTR luciferase reporters and KSHV miRNAs reported in Figure 1.

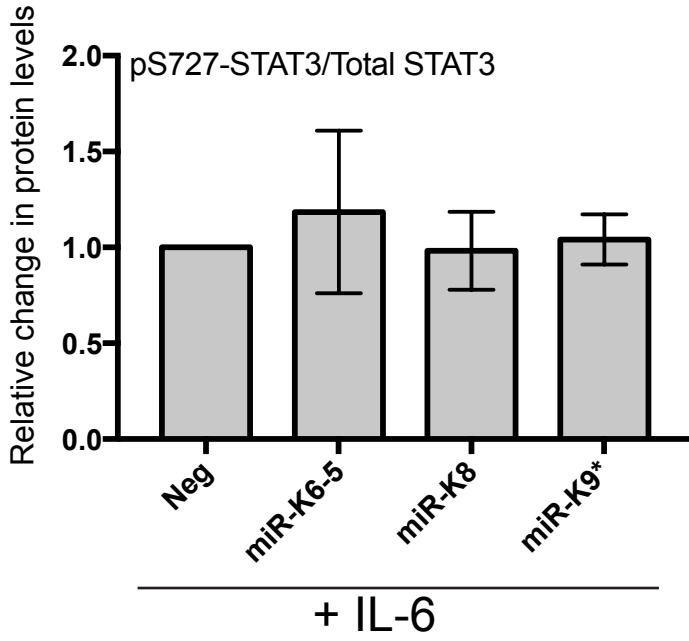
**Table S2.** Sequences of the oligonucleotide primers used for the identification of non-canonical binding sites in the 3' untranslated regions of KSHV miRNA target genes reported in Figure 1.

**Table S3.** Interactions report from MetaCore analysis lists the publications that each interaction is based upon in Figure 2.

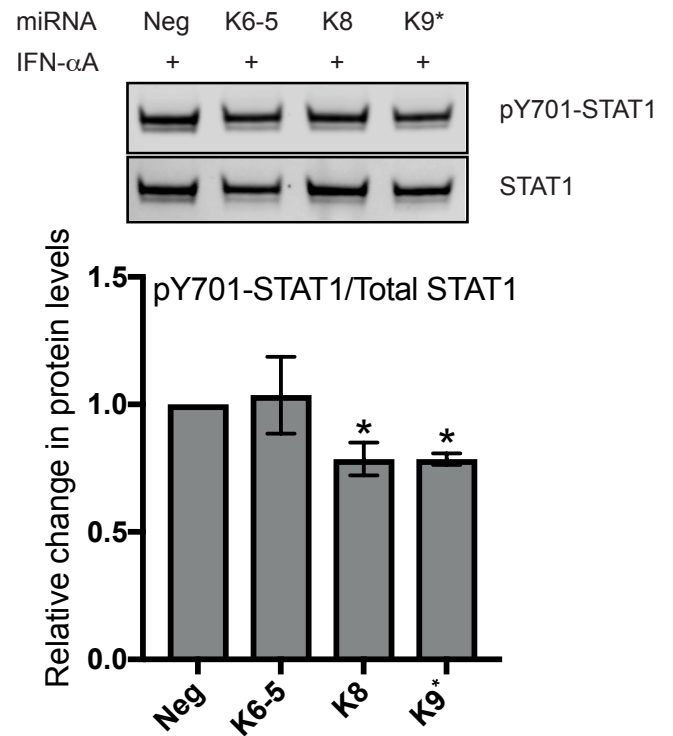
**Table S4.** BIRC5 3'UTR luciferase data.

Figure S1

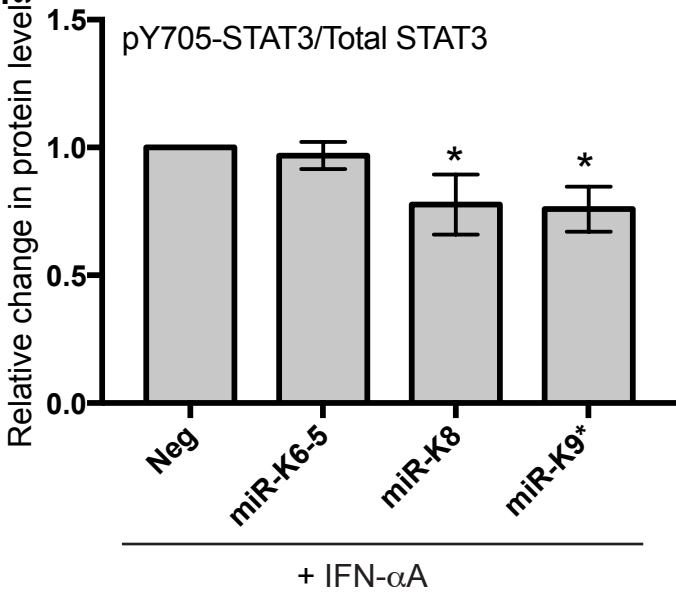
A.



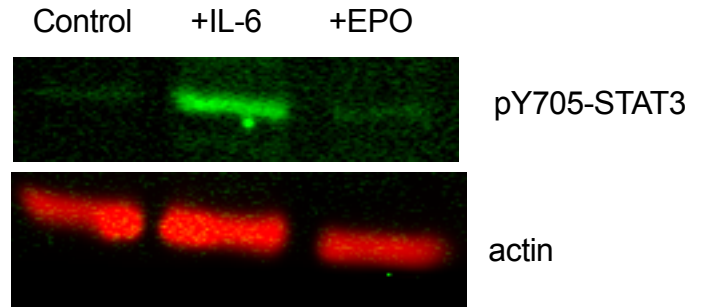
B.



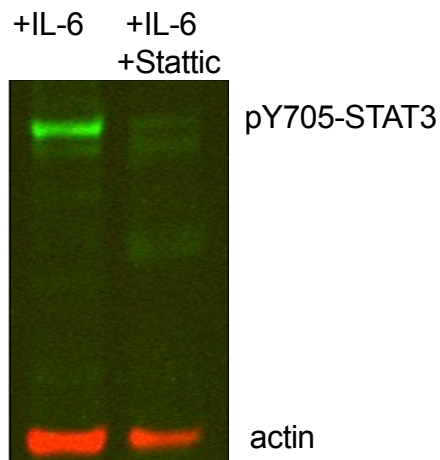
C.



D.



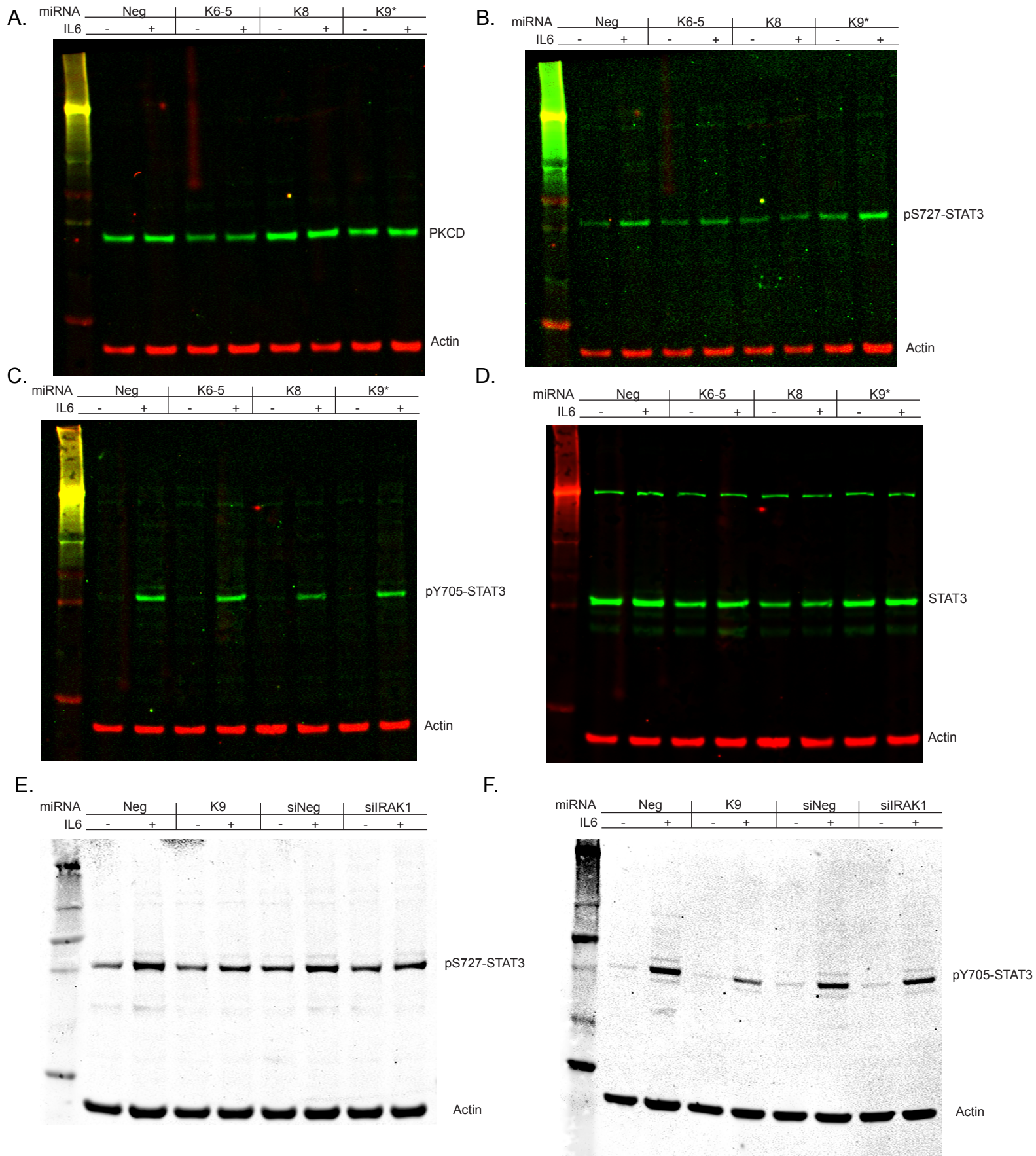
E.



Supplementary Figure S1. Analysis of phosphorylated STAT proteins.

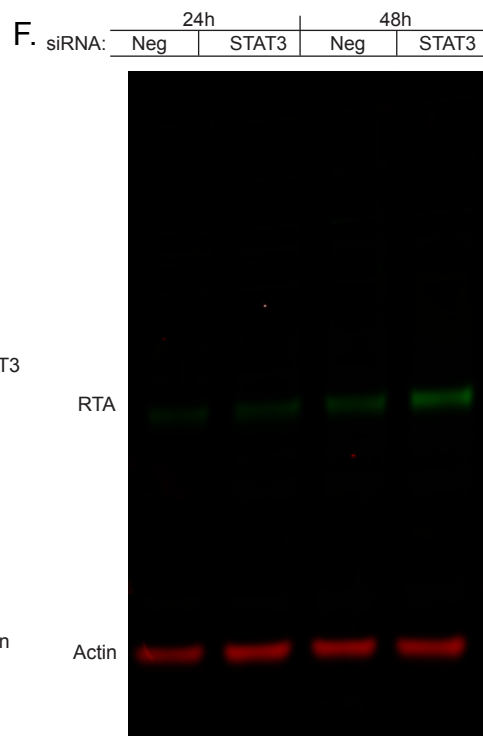
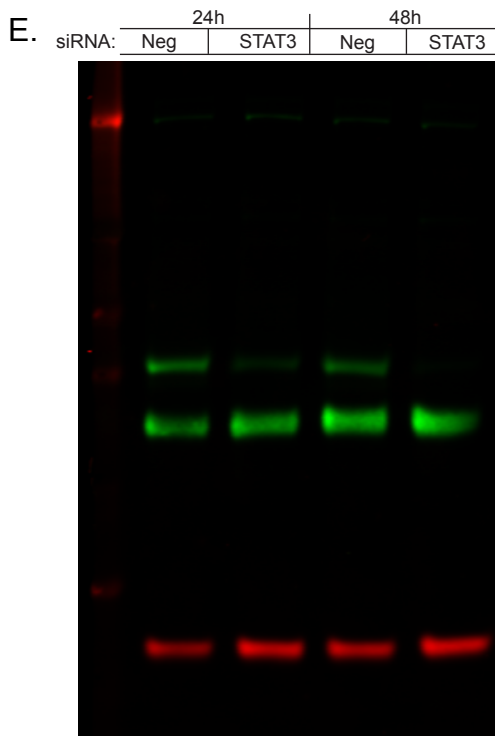
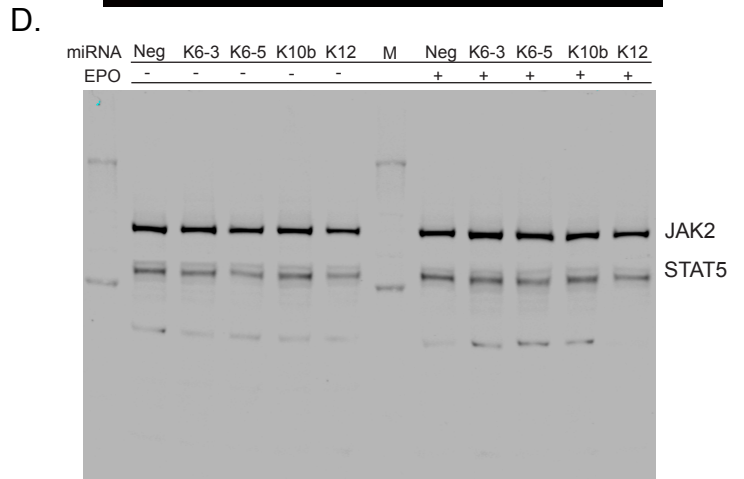
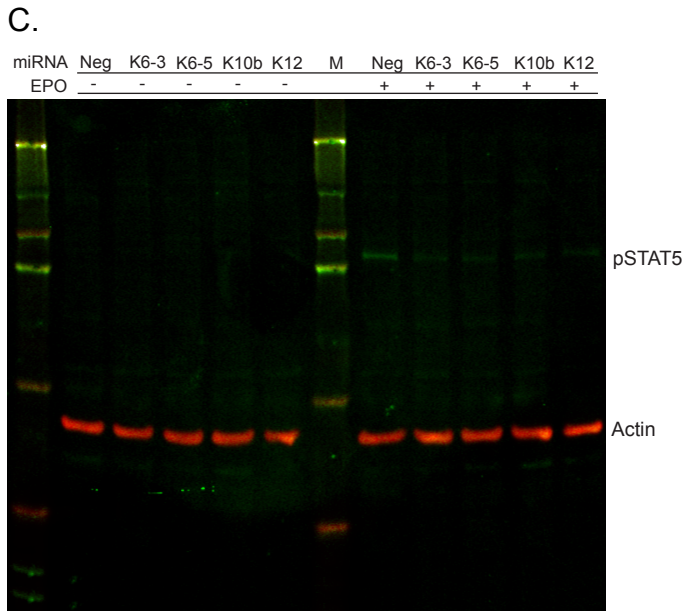
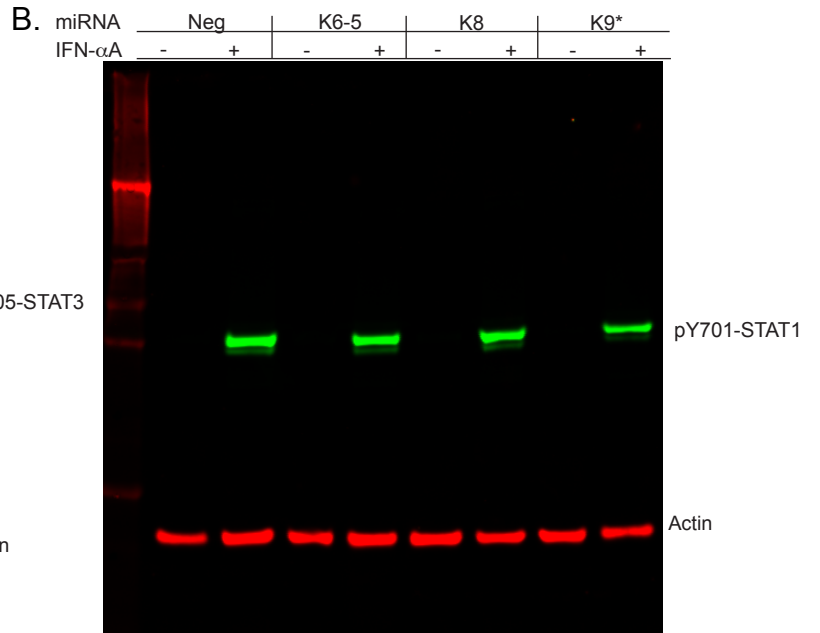
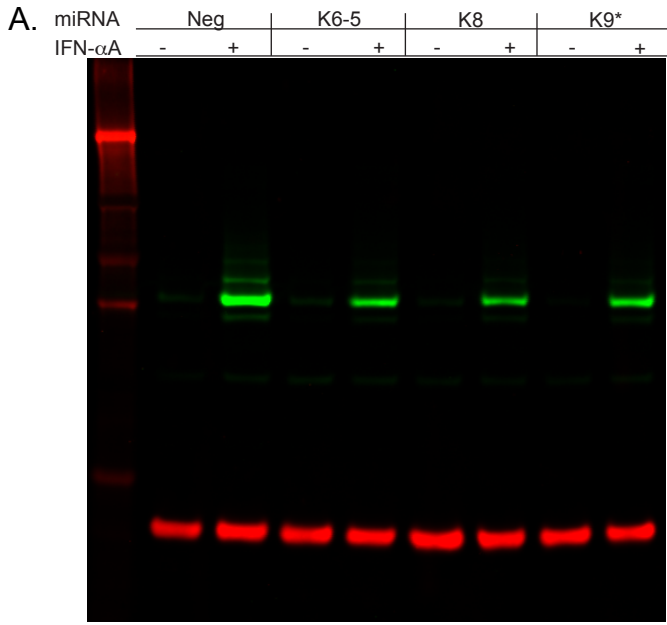
A. Ratios of pS727-STAT3 to total STAT3 are shown for IL-6 treated HUVECs. B. Western blot and ratios of pY701-STAT1 to total STAT1 are shown for IFN- $\alpha$ A treated HUVECs. C. Ratios of pY705-STAT3 to total STAT3 are shown for IFN- $\alpha$ A treated HUVECs. D. Western blot for IL-6 and EPO treated HUVECs are shown. E. Western blot for BCBL-1 cells treated with IL-6 and Stattic are shown.

Figure S2



Supplementary Figure S2. Immunoblots in main figures.  
A-D. Full blots for Figure 3A. E-F. Full blots for Figure 3B.

Figure S3



Supplementary Figure S3.  
Immunoblots in main figures.  
A-B. Full blots for Figure 4.  
C-D. Full blots for Figure 5.  
E-F. Full blots for Figure 7.

# Supplementary Table 1

3'UTR/miRNA	1	2	3	3star	4_3	4_5	5	6_3	6_5	7	8	9	9star	10a	10b	11	12-5
CTSZ				1.142										0.929			
IRAK1	0.548	0.944	0.738	0.621	1.056	0.894	0.841	0.691	0.953	0.817	0.865	0.576	0.829	1.025	0.932	0.920	
TNFRSF12A	1.123	1.251	1.188	0.956	1.337	1.244	1.142	0.913	1.130	1.114	1.253	1.166	1.112	0.480	1.077	1.078	
VCL		0.955	0.973					0.967	1.153		1.280						
EPOR												1.439	0.823		1.176		
TAGLN		1.165		1.009							1.188						
FOXK2					0.975						1.161						
JARID1B																0.395	
MET	0.974					1.756	1.514								0.882	1.481	
CORT												1.123	0.856	0.930			
DCBLD2										1.173	1.387	1.158					
CUL5										0.930	1.496				1.098	0.844	
ABI2	1.090	0.740	1.048	1.457											1.111	1.749	
PPM1E			0.638												0.620	0.916	
HMG3							1.485		1.196						1.256	1.491	
PHF17										1.096							
LN2	0.668		0.608												0.459		
OCRL			1.435	2.087											1.143	2.038	
TMEM97					1.102	1.193			0.660						0.757		
SEN7				1.214											0.775		
ESCO2	0.584	0.652						1.059		0.804							
CBX1		0.619					1.071		1.038						0.826	1.560	
ARID1A													0.851	0.813			
CDR2						1.365								1.148			
UPF3B				0.715					0.898					0.778			
CENPA				0.798				0.943				1.044			0.758		
CENPM	1.170							0.996		0.876	1.131			0.991			
GADD45B								1.204	1.031			0.785	1.001		1.351		0.755
TPM1 Ex13		1.254		1.169		1.141	0.664	1.099	0.998				0.848	0.886	0.820		
TPM1 Ex14		1.730		1.041		1.379	1.058	1.023	1.091				0.999	0.859	0.877		
TPM1 Ex15		0.866		0.877		1.279	1.016	0.533	0.854				0.783	0.587	0.484		
NISCH	1.086	0.835	0.395	0.882	0.443	1.161			0.425						1.315		
IL10RB										0.691		0.899	0.615				
PARP12		1.692										2.117	2.089	1.884	2.146		
DVL2			0.757	0.839	0.968	0.870	0.496				1.346		1.113	0.998	0.612		
COQ3								0.705		1.238		2.166		1.337	1.675		
INTS3					1.445					1.237							1.062
PRKCD				1.341	1.427				0.646								
PDAP1								1.159	1.618		1.349				1.178		
UBA3									1.829					1.702	0.917	0.722	
VBP1					2.220									2.215	0.919		
N4BP2	0.760					1.177				0.642	0.732						
FANCC								1.021		0.823		0.869					
E2F2								0.817	1.079	1.329							0.445
TNPO3		1.670			1.522	1.778		1.381								1.895	
UPF3B				0.885				0.654	0.905		0.975				1.359		
S100PBP			0.880			1.531								1.187	1.582		
NUP188											1.435	1.396	0.957				
SLC7A5	0.802										1.240	0.873					
NUSAP1										1.055				1.065	1.624		
RNF115					1.116		1.129			1.512					1.065		
BIRC5	0.792	1.323	0.917	1.400	1.344	1.116	0.998			0.907	0.907	0.789	0.793			0.995	0.697

3'UTR/miRNA	1	2	3	3star	4_3	4_5	5	6_3	6_5	7	8	9	9star	10a	10b	11	12-5
CTSZ				0.224										0.117			
IRAK1	0.053	0.060	0.035	0.075	0.088	0.051	0.016	0.099	0.091	0.126	0.152	0.082	0.110	0.194	0.167	0.187	
TNFRSF12A	0.062	0.043	0.080	0.277	0.046	0.195	0.225	0.192	0.118	0.032	0.131	0.200	0.112	0.030	0.065	0.146	
VCL		0.033	0.021					0.047	0.007		0.189						
EPOR												0.111	0.096		0.069		
TAGLN		0.237		0.205							0.379						
FOXK2					0.302						0.309						
JARID1B																	
MET	0.148					0.389	0.505								0.332	0.571	0.113
CORT												0.316	0.095	0.137			
DCBLD2										0.528	0.543			0.414			
CUL5										0.082	0.637			0.116	0.172		
ABI2	0.238	0.167	0.170	0.397										0.286	0.425		
PPM1E			0.086											0.077	0.577		
HMG3							0.134		0.330					0.241	0.046		
PHF17										0.167				0.018			
LN2	0.067		0.036											0.133			
OCRL			0.059	0.412										0.219	0.516		
TMEM97					0.203	0.216			0.088					0.112			
SEN7				0.464										0.044			
ESCO2	0.068	0.057						0.253		0.133							
CBX1		0.118					0.383		0.338					0.258	1.068		
ARID1A													0.215	0.169			
CDR2						0.238								0.212			
UPF3B				0.247					0.200					0.134			
CENPA				0.060				0.204				0.377			0.210		
CENPM	0.288							0.621		0.146	0.092			0.228			
GADD45B								0.151	0.119			0.047	0.189		0.283		0.063
TPM1 Ex13		0.180		0.062		0.212	0.048	0.069	0.165				0.166	0.093	0.140		
TPM1 Ex14		0.319		0.161		0.147	0.123	0.068	0.273				0.061	0.143	0.239		
TPM1 Ex15		0.172		0.155		0.227	0.178	0.032	0.155				0.121	0.084	0.084		
NISCH	0.046	0.107	0.046	0.066	0.052	0.076			0.087						0.133		
IL10RB										0.054		0.387	0.145				
PARP12		0.331										0.646	0.231	0.743	0.852		
DVL2			0.166	0.166	0.033	0.227	0.111				0.299		0.132	0.306	0.214		
COQ3								0.383		0.258		0.986		0.199	0.247		
INTS3					0.195				0.090		0.188				0.088		
PRKCD				0.330	0.122												
PDAP1								0.491	0.019		0.449					0.417	
UBA3									0.623					0.051	0.205	0.119	
VBP1					0.356									0.378	0.165		
N4BP2	0.115					0.107				0.119	0.070						
FANCC								0.150		0.182		0.123					
E2F2								0.130	0.126	0.048							0.082
TNPO3		0.116			0.023	0.303		0.311								0.113	
UPF3B				0.270				0.065	0.155		0.305				0.284		
S100PBP			0.090			0.582								0.142	0.366		
NUP188											0.388	0.116	0.012				
SLC7A5	0.069										0.449	0.109					
NUSAP1										0.110				0.213	0.278		
RNF115					0.195		0.265				1.045				0.207		
BIRC5	0.030	0.067	0.092	0.394	0.051	0.062	0.196			0.372	0.074	0.066	0.070			0.135	0.098

**Oligo name    Oligo sequence (5' to 3')**

LNX2-a-f    AACTGATTTTCTGGTTTATTTCTACTGTAATCTCTCAATAA  
LNX2-a-r    TATTGAGAGATTACAGTAGAAATAAACCAGAAAATCAGTTA  
LNX2-b-f    AGATAAAACCATATTATTAAATACCTATATATCACAGCTAA  
LNX2-b-r    TAGCTGTGATATATAGGTATTTAATAATATGGTTTTATCTA  
LNX2-c-f    TGTCAGCAAAGCCAGTAACAACAGCGTGTACTGCCACTGTA  
LNX2-c-r    ACAGTGGCAGTACACGCTGTTGTTACTGGCTTTGCTGACAA  
UPF3B-b-f    GAATTTGGGTGGAGCAGAGTCGCTTTGAAGCCTTGTTCCG  
UPF3B-b-r    CGGAACAAGGCTTCAAAGCGACTCTGCTCCACCCAAATTC  
UBA3-a-f    AGTGCCATAGAGGCCAATATGCACAATATTAATAATGCCA  
UBA3-a-r    GGCATTAGTTAATATTGTGCATATTGGCCTCTATGGCACTA  
NISCH-a-f    TTGCTGTTGCTGTTGGCATCTTGCTGCTAATCCTGAGGCTA  
NISCH-a-r    AGCCTCAGGATTAGCAGCAAGATGCCAACAGCAACAGCAAA  
SLC7A5-a-f    GTGTGGCTGGGACCTTCTTTATTCTGTGTTAATGGCTAACA  
SLC7A5-a-r    GTTAGCCATTAACACAGAATAAAGAAGGTCCCAGCCACACA

LNX2-a-m1f    AACTGATTTTCTGGTTTATTTCTAGGCTAATCTCTCAATA  
LNX2-a-m1r    TATTGAGAGATTAGCCTAGAAATAAACCAGAAAATCAGTT  
LNX2-b-m1f    AGATAAAACCATATTATTAAATATAACATATATCACAGCTA  
LNX2-b-m1r    TAGCTGTGATATATGTATATTTAATAATATGGTTTTATCT  
LNX2-c-m1f    TGTCAGCAAAGCCAGTAACAACAGCGTAGCCTGCCACTGT  
LNX2-c-m1f    ACAGTGGCAGGCTACGCTGTTGTTACTGGCTTTGCTGACA  
NISCH-a-m1f    TTGCTGTTGCTGTTGGCATCTTGATGTAATCCTGAGGCT  
NISCH-a-m1r    AGCCTCAGGATTACATGCAAGATGCCAACAGCAACAGCAA

# Supplementary Table 5

## Interactions Report

#	From Network Object "FROM"	Object Type	To Network Object "TO"	Object Type	Effect	Mechanism	Homo sapiens	Link Info	References
1	STAT3	Transcription factor	GADD45 beta	Generic binding protein	Activation	Transcription regulation	x	putative Stat3 binding site on GADD45 beta was validated by measuring in vitro binding and in vivo occupancy.	19282476;2046778
2	BIRC5	Generic binding protein	STAT3	Transcription factor	Inhibition	Binding	x	Acetylated BIRC5 binds to the N-terminal transcriptional activation domain of the STAT3 dimer and represses STAT3 transactivation of target gene promoters.	20928784
3	STAT3	Transcription factor	NABP2	Generic kinase	Unspecified	Transcription regulation	x	Stat3 binds to gene NABP2 promoter.	18005416
4	PKC-delta	Protein kinase	HGF receptor (Met)	Receptor with enzyme activity	Inhibition	Phosphorylation	x	PKC-delta phosphorylates HGF receptor (Met) and decreases its activity.	15075332
5	RAK1	Protein kinase	STAT3	Transcription factor	Activation	Phosphorylation	x	RAK1 phosphorylates STAT3 and increases its activity.	15465816
6	STAT3	Transcription factor	BIRC5	Generic binding protein	Activation	Transcription regulation	x	Activated Stat3 signaling contributes to breast cancer progression and resistance to chemotherapy by, at least in part, inducing expression of the antiapoptotic protein, BIRC5.	136778425;16397018;16529541;17533050;17934481;18075512;18242580;18744341;19880237;19937972;21521803
7	STAT3	Transcription factor	STAT3	Transcription factor	Activation	Binding	x	Dimeric and tetrameric STAT3 complexes formation is required for transcriptional activity of STAT3.	7701321;8609222;8762827;10458605;11438543;18849387;19053881
8	HGF receptor (Met)	Receptor with enzyme activity	GRB2	Generic binding protein	Activation	Binding	x	HGF receptor (Met) directly binds to GRB2 and triggers RAS pathway.	1571210;8628899;91828210;9550480;100599803;11430831;11439336;11900489;12766170;15546961;16724056;17065554;17440121;8900286;9224282
9	Epo receptor	Generic receptor	Epo receptor	Generic receptor	Activation	Binding	x	Our results indicate that Epo exerts its antiapoptotic effects on differentiated SH-SY5Y and PC-12 cells through the standard stoichiometry of one molecule of Epo binding to two Epok subunits, comprising the HGF stimulates recruitment of Stat-3 to the receptor, tyrosine phosphorylation, nuclear translocation and binding to the specific promoter element SIE.	9793257;10912517;1287756;17045762
10	HGF receptor (Met)	Receptor with enzyme activity	STAT3	Transcription factor	Activation	Binding	x	Stat3 tyrosine phosphorylation is required for Stat3 association with PKC-delta.	9440892;10068003;15254691;15468908;16724056
11	PKC-delta	Protein kinase	STAT3	Transcription factor	Activation	Phosphorylation	x	Stat3 tyrosine phosphorylation is required for Stat3 association with PKC-delta.	934341;13872331;10448219;10521505;16770494;1133571142;361954;12576423;12763138;1451213;15380937;16418226;16064308;17065554
12	EPF2	Transcription factor	BIRC5	Generic binding protein	Unspecified	Transcription regulation	x		15271987;17916908
13	HGF receptor (Met)	Receptor with enzyme activity	HGF receptor (Met)	Receptor with enzyme activity	Activation	Phosphorylation	x	HGF receptor (Met) phosphorylates HGF receptor (Met) and increases its activity.	11779195
14	Epo receptor	Generic receptor	STAT3	Transcription factor	Activation	Binding	x	Epo receptor interacts with STAT3 and activates it.	11756198;15752978

Pubmed IDs

<b>BIRC5 3'UTR data</b>	<b>24h</b>	<b>SD</b>	<b>N</b>	<b>48h</b>	<b>SD</b>	<b>N</b>
Neg1	1.00	0.00	3	1.00	0.00	3
miR-K1	0.99	0.06	3	0.79	0.03	3
miR-K2	0.97	0.25	3	1.32	0.07	3
miR-K3	0.87	0.12	3	0.92	0.09	3
miR-K3*	1.13	0.35	3	1.40	0.39	3
miR-K4-3	0.98	0.23	3	1.34	0.05	3
miR-K8	0.98	0.17	3	0.91	0.07	3
mir-K9	0.94	0.17	3	0.79	0.07	3
miR-K11	0.80	0.21	3	0.99	0.13	3
miR-K12-5	0.63	0.16	3	0.70	0.10	3

**miRNAs that didn't show repression**

Neg1	1.00	0.00	3	1.00	0.00	3
miR-K6-3	1.19	0.04	3	1.17	0.28	3
miR-K6-5	1.69	0.13	3	2.54	1.07	3
miR-K10b	1.46	0.05	3	1.61	0.53	3
miR-K12	1.44	0.11	3	1.90	0.67	3

Red if  $p < 0.05$