

Supporting Information for

Original article

Identification of a JAK–STAT–miR155HG positive feedback loop in regulating natural killer (NK) cells proliferation and effector functions

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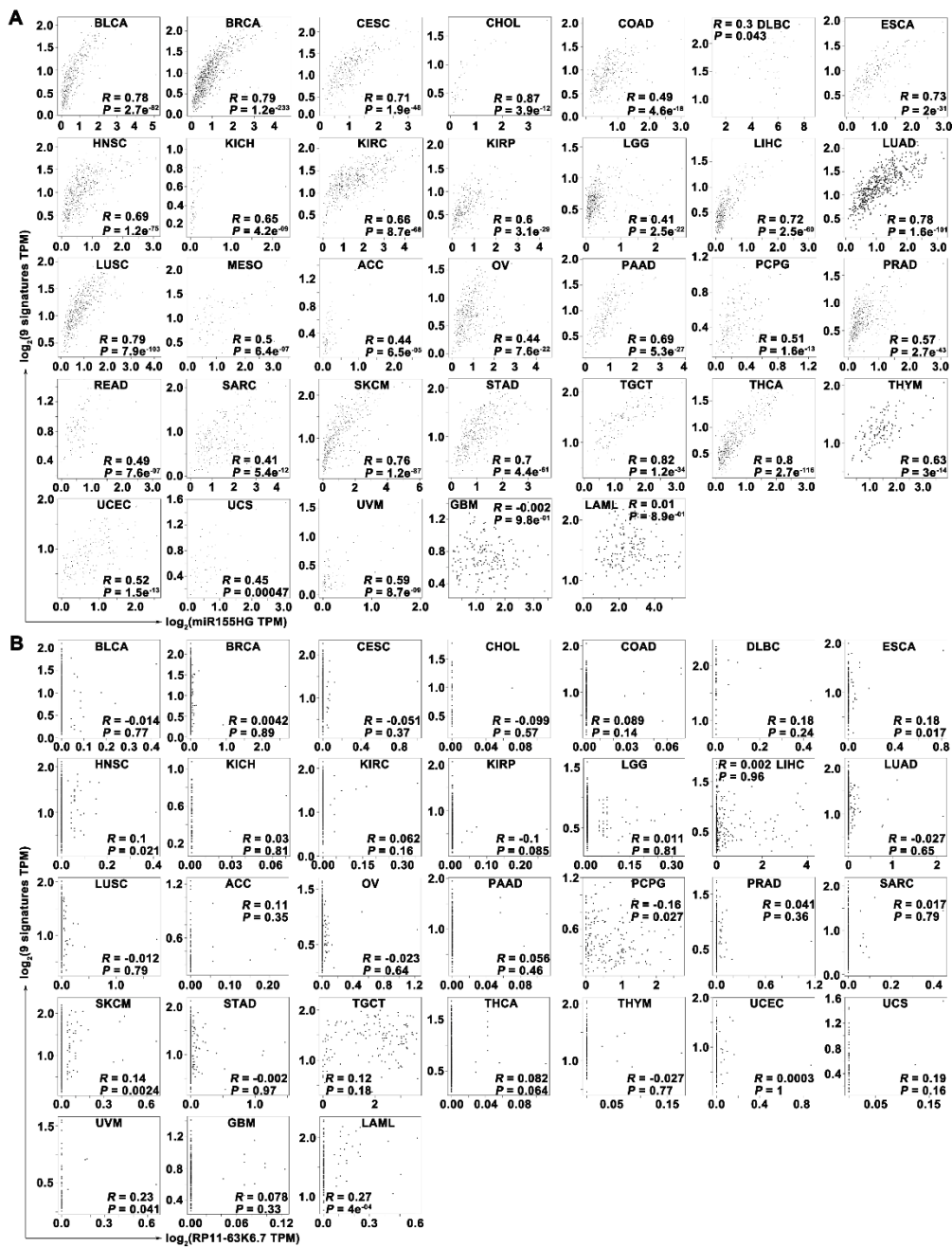


Figure S1 Correlation between miR155HG (A) or RP11-63K6.7 (B) TPM and module TPM of NK effector function related genes in 33 different cancer types. NK effector function related genes: *NCR1*, *EOMES*, *TBX21*, *CD69*, *DNAM1*, *KLRK1*, *PERF*, *IFNG*, and *GZMB*. Abbreviations: ACC, adrenocortical carcinoma; BLCA, adenocortical carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangio carcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, Brain lower grade glioma; LIHC, LIHC

liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; TPM, transcripts per kilobase of exon model per million mapped reads; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma. Spearman's correlation coefficient (R) and P values are shown.

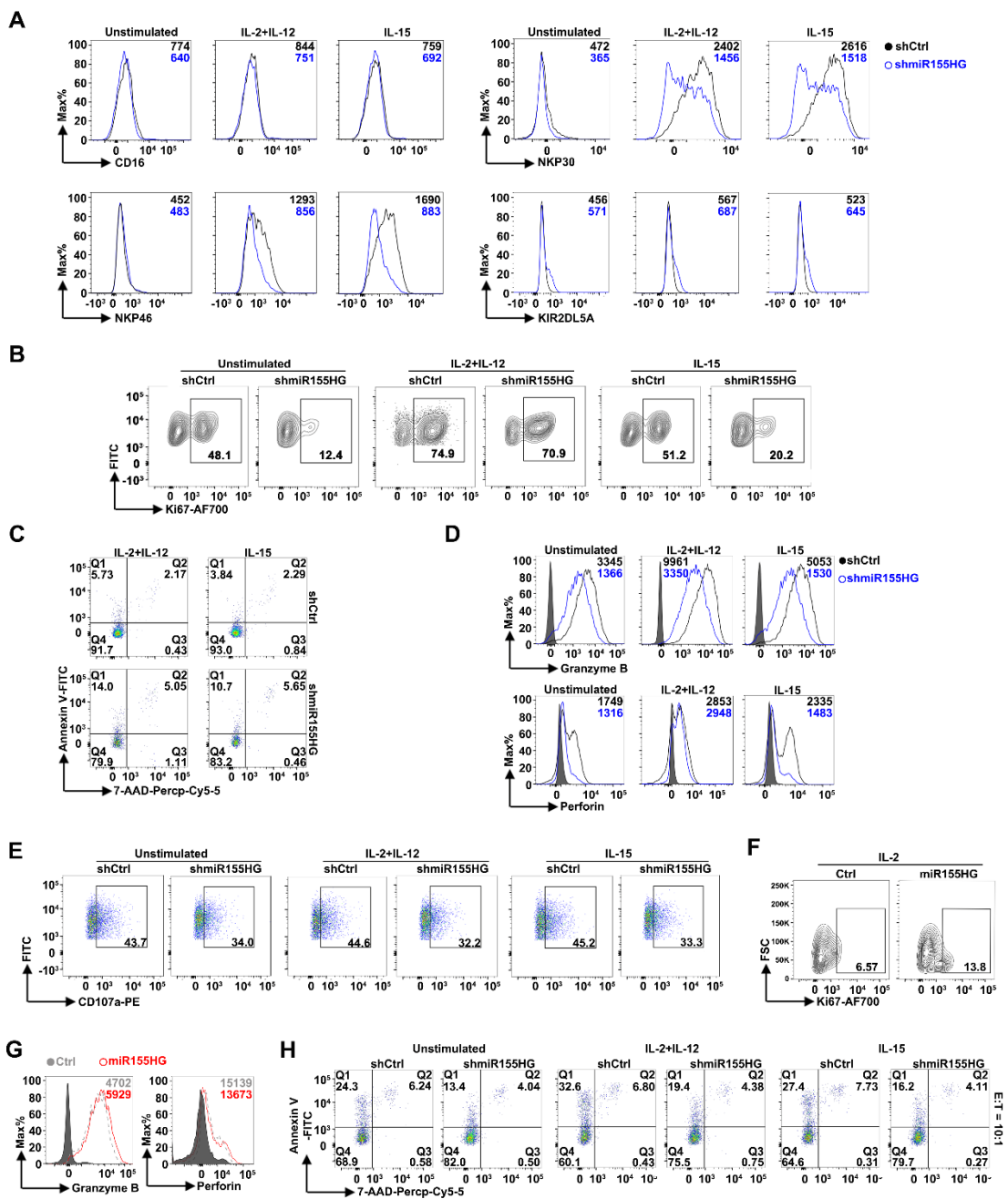


Figure S2 MiR155HG promotes NK cell growth and effector function. (A) Flowcytometry analysis of CD16, NKP30, NKP46 and KIR2DL5A expression on shmiR155HG and Ctrl NK92 cells after stimulated with or without cytokines for 24 h. gMFI: genomic mean fluorescence intensity. (B) Flowcytometry analysis of Ki67 expression on shmiR155HG and Ctrl NK92 cells after stimulated with or without cytokines for 24 h. (C) Flowcytometry analysis of the ratio of early apoptosis (Annexin V⁺7-AAD⁻) and late apoptosis (Annexin V⁺7-AAD⁺) of shmiR155HG and Ctrl NK92 cells after stimulated with cytokines for 48 h. (D) Flowcytometry analysis of the percentages of granzyme B and perforin in shmiR155HG and Ctrl NK92 cells after stimulated with or without cytokines for 24 h. (E) Flowcytometry analysis of the percentages of CD107a on shmiR155HG and Ctrl NK92 cells after co-cultured with K562 cells in the presence or absence of indicated cytokines for 5 h. (F) Flowcytometry analysis of Ki67 expression on miR155HG and Ctrl NK92 cells after stimulated with IL-2 (800 U/mL) for 24 h. (G) Flowcytometry analysis of the percentages of granzyme B and perforin in miR155HG and Ctrl NK92 cells after stimulated with IL-2 (800 U/mL) for 24 h. (H) Flowcytometry analysis of the percentages of live (Annexin V⁻7-AAD⁻) and early apoptosis (Annexin V⁺7-AAD⁻) of K562 cells after co-cultured with shmiR155HG or Ctrl NK92 cells with or without cytokines for 5 h.

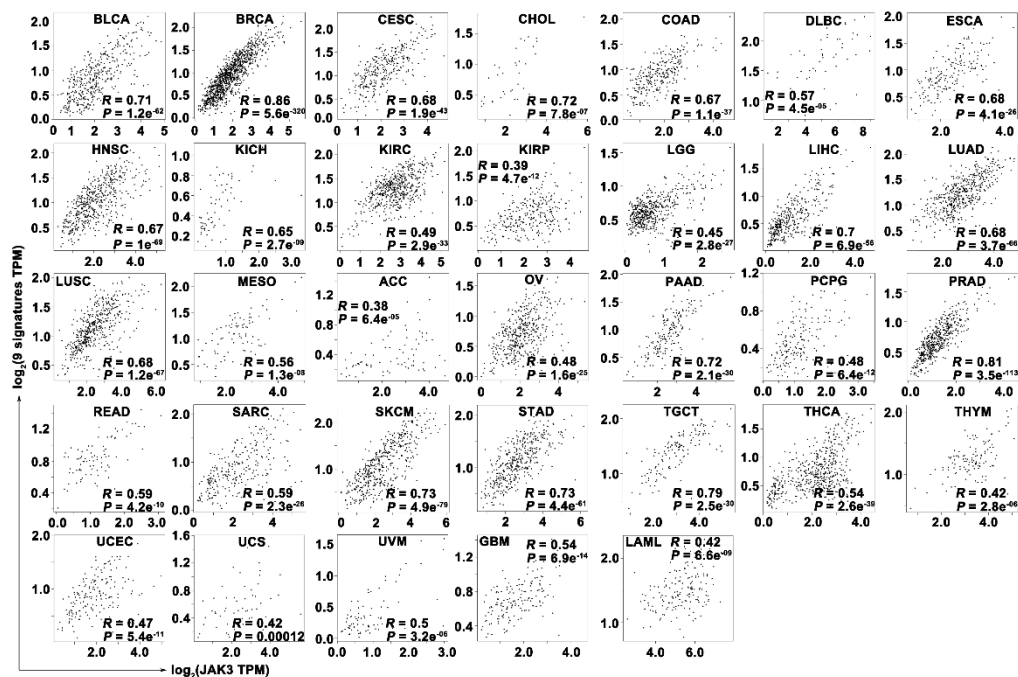


Figure S3 Correlation between JAK3 TPM and module TPM of NK effector function related genes in 33 different cancer types. The extensions of tumor abbreviations are listed in the legend of Fig. S1. Spearman's correlation coefficient (R) and P values are shown.

Table S1 Sequences of RNA and DNA oligonucleotides.

Name	Sense strand/Sense primer (5'–3')	Antisense strand/Antisense primer (5'–3')
Primers for qPCR		
miR155HG	TACCTGTCACCTTGGCTCTC	ACGGCAGCAATTTGTTCCAT
GAPDH	AGAAGGCTGGGGCTCATTTG	AGGGGCCATCCACAGTCTTC
JAK3	TTGGTTTGGGCTGGAGAAGT	TGACAGTCTTCAGCAGCTCT
AK096685	TGTCTCATCTGGTGCTCTTTCT	AACTGAACTGCCATAGAGCTT
RP11-63K6.7	CTCACGCATGTAATCCCTGC	TGGGTGTCTGTAGAGGGTGT
NR_158157.1	AACTGTACACCCACCTCCT	AAATCCGGCTTGTCCCTCTG
AF086143	CTTGTGTGTCTGTGTGCGTG	GTTACAGACACACACGCAC
ENSG00000290922	AGAGATGTGCCATGTCAGCC	ACAGTGAGCAGTTTCAGCCA
TCONS_00025394	TTGGAGAAAACGGTGGGGTT	CGGAAGCTGAAGTGGGAGAA
AK125615.1	AATGCTGCAGAGACCATGGG	ACTTCTGGCACACTGTTGGT
IFNG	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
GZMB	CCCTGGGAAAACACTCACACA	GCACAACCTCAATGGTACTGTCCG
MALAT1	GAAAGCGAGTGGTTGGTAAA	CCCTCAAAAGCTTCAGACAA
IL12RB2	CCAGAGTTGATTGTTGATGGCA	CCTTGTCTGGGCTTCAAAGAG
IL10	CCGTGGAGCAGGTGAAGAAT	AGCCCCAGATCCGATTTTGG
IL4R	TGCGAGTGGAAAGATGAATGGT	AGTTATCCGCACTGACCACG
CCND3	TGCACATGATTTCTGGCCT	TTCAGTGCCAGTGATCCCTG
IL18R1	CTGATATCCCAGGCCACGTC	TGGGCAAATCTCCACAGCA
IL18RAP	ATGCACAAAGTCCAGCGGTA	GCTCTGACTGTCCACGAACT
TNFRSF13C	GAGTGCTTCGACCTGCTG	CTCCAGCTCACCAGACCCA
XCR1	CCCAGAGAGCACCACCTTTT	CTGAGAGGCACAGGTTGAGG
CXCR6	TCAATGACAGCAGCCAGGAG	CCGTCAGGCTCTGCAACTTA
CCR1	CCAGAAGGTGAACGAGAGGG	CCAGGTTCAGGAGGTAGATGC
CCR6	TTTTCTGCCACAATGAGCG	CTGGAGAACTGCCTGACCTC
TNFRSF10A	ACGAGATTCTGAGCAACGCA	CAGCAGCCTCCTCCTCTGA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGA
hsa-miR-3614-3p		GCTAGCCTTCAGATCTTGGTGT
hsa-miR-4767		ATACGCGGGCGCTCCTGGCCGCCGCC
hsa-miR-5586-5p		GCCTATCCAGCTTGTACTATATGC
hsa-miR-6511b-5p		AGGCAGAAGTGGGGCTGACA
hsa-miR-6724-5p		ATACTGGGCCCCGCGCGGGCGTGGGG
hsa-miR-6756-5p		TATAGGGTGGGGCTGGAGGT
hsa-miR-6773-5p		GGCCCAGGAGTAAACAGGAT
hsa-miR-6778-5p		TAGTGGGAGGACAGGAGGCA
hsa-miR-6884-5p		AGAGGCTGAGAAGGTGATGTTG
hsa-miR-1233-5p		ATTGGGAGGCCAGGGCA
miRNA mimics		
miR-155	UAA AUGCUAAUCGUGAUAGGGGUU	CCCCUAUCACGAUUAGCAUUAUUU
miR-6756	AGGGUGGGGCGUGGAGGUGGGGCU	CCCACCUCAGCCCCACCCUUU
negative control (NC)	UGAAUUAGAUGGCGAUGUUdTdT	AACAUCGCCAUCUAAUUCAdTdT
miRNA inhibitors		
control inhibitor (anti-NC)	GUGGAUAUUGUUGCCAUCA	
anti-miR-6756	AGCCCCACCUCAGCCCCACCCU	

Primers for ChIP

p (-1982 to -1972)	GAGACATCATTATTGTCATT	TAGGAGTCAAATACACCTG
p (-1548 to -1411)	ATGGGAAATTCAGAAAGGC	TGATCATATGAGGGAGGAGC
GAPDH	TACTAGCGGTTTTACGGGCG	TCGAACAGGAGGAGCAGAGAGCGA

Probes for RAP

miR155HG-1	CCTATCACGATTAGCATTAACAGCATAACAGCCTACAG
miR155HG-3	CAGACAATCCATGATATAACTGGAGGTTAGTAGTCCTTC
miR155HG-4	ACCGTATTATGTGGCTAAGCTATTGAACTTGAACCTA
miR155HG-5	ACTGCATAGAAATCCAAACCTTAAATATTCTGCAAATCAA
Lac Z-1	GGGTTTTCCCAGTCACGACGTTGAAAACGACGGCCA
Lac Z-2	CGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGT
Lac Z-3	GGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGC
Lac Z-4	TACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCA
Lac Z-5	ACCATATGCGGTGTGAAATACCGCACAGATGCGTAAG

Table S2 The information of antibodies used.

Antibody	Clone	Catalog number	Source
P-JAK3 (Tyr980/981)	D44E3	5031T	CST
JAK3	EP909Y	ab45141	Abcam
P-JAK1(Tyr1022/1023)	EPR1899	ab138005	Abcam
JAK1	EPR349	ab133666	Abcam
P-STAT3 (Tyr705)	D3A7	9145T	CST
STAT3	79D7	4904S	CST
P-STAT4 (Tyr693)	D2E4	4134S	CST
STAT4	C46B10	2653S	CST
P-STAT5 (Tyr694)	C11C5	9359S	CST
STAT5	D2O6Y	94205S	CST
GAPDH	D16H11	8884S	CST
CD45	2D1	560178	BD Biosciences
CD45	HI30	304037	BioLegend
CD56	NCAM16.2	562780	BD Biosciences
CD56	QA17A16	392406	BioLegend
CD56	N901	IM2474	Beckman Coulter
CD3	HIT3a	300316	BioLegend
CD16	3G8	302012/302018	BioLegend
NKP46	9E2	331914	BioLegend
CD34	581	343516	BioLegend
CD43	CD43-10G7	343206	BioLegend
Perforin	dG9	308122	BioLegend
Granzyme B	GB11	515406	BioLegend
IFN- γ	4S.B3	502530	BioLegend
Ki67	Ki-67	350530	BioLegend
Ki67	11F6	151222	BioLegend
Granzyme B	GB11	561142	BD Biosciences
Perforin	B-D48	353314	BioLegend

CD107a	H4A3	555801/328616	BD Biosciences
CD337 (NKP30)	REA823	130-112-430	Miltenyi
KIR2DL5A	UP-R1	566330	BD Biosciences
