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Supplemental Information

miR-146b-5p Enhances the Sensitivity of NSCLC to EGFR Tyrosine Kinase Inhibitors by Regulating the IRAK1/NF-κB Pathway

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Supporting Information:

Supplementary Table S1. miRNAs showing differential expression in PC9 versus PC9/gef lung cancer cells, as evaluated with TaqMan Array Human MicroRNA A+B Cards Set (submitted as a separate file)

Supplementary Table S2. miRNAs showing differential expression in HCC827 versus HCC827/gef lung cancer cells, as evaluated with TaqMan Array Human MicroRNA A+B Cards Set (submitted as a separate file)

Supplementary Table S3: Clinical characteristics of the 30 lung adenocarcinoma patients with malignant pleural effusions

	Pleural effusion			
	Patient No.	**Before treatment**	**Acquired resistance to TKI**	***P****
Total No.	30	15	15	
Age, median years (range)	66.1 (29.5-88.0)	68.9 (42.6-88.0)	63.8 (29.5-85.3)	0.567[#]
Sex				0.710
Female	18	10	8	
Male	12	5	7	
Smoking				1.000
Nonsmokers	24	12	12	
Smokers	6	3	3	
ECOG PS[#]				1.000
			2	
EGFR				0.311
Del-19	12	4	8	
L858R	16	10	6	
other	2	1[◎]	1[※]	

* by Fisher's exact test; [#]by Mann-Whitney Test

[◎] G719A; [※]L858R+R776H

[#] Eastern Cooperative Oncology Group, performance status

Supplementary Table S4: EGFR mutations and IC_{50} values for osimertinib of lung cancer cells which were isolated from patients' pleural effusions

Cancer cell	Treatment duration (months)	EGFR mutation	IC_{50} of osimertinib (μM)
PE2988	5	del E746-A750+T790M	4.53
PE3479	14	L858R+T790M+C797S	1.31

Supplementary Table S5: Potential targets of miR-146b-5p using TargetScan software

Target gene	Conserved sites total	Conserved 8mer sites	Conserved 7mer-m8 sites	Conserved 7mer-A1 sites
TRAF6	3	3	0	0
ST5	3	0	2	1
IRAK1	2	2	0	0
ACKR2	2	2	0	0
CDKN2AIP	2	1	1	0
NOVA1	2	1	1	0
KLF7	2	1	1	0
MED1	2	1	1	0
VPS52	2	0	2	0
ZNRF3	2	0	1	1
LRRC15	2	0	2	0
PRX	2	0	2	0
MYO5A	2	0	1	1
ATG7	2	0	0	2
Target gene	**Poorly conserved sites total**	**Poorly conserved 8mer sites**	**Poorly conserved 7mer-m8 sites**	**Poorly conserved 7mer-A1 sites**
EGFR	3	1	1	1

Supplementary Table S6: Clinical characteristics of the 74 lung adenocarcinoma patients with malignant pleural effusions

		Pleural effusion			
		Patient No.	**Before treatment**	**Acquired resistance to TKI**	***P***
Total No.		74	40	34	
Age, median years (range)		65.7 (29.5-89.4)	66.8 (32.7-89.4)	64.7 (29.5-89.4)	0.389
Sex					0.326
	Female	50	29	21	
	Male	24	11	13	
Smoking					0.359
	Nonsmokers	61	31	30	
	Smokers	13	9	4	
ECOG PS					0.496
	0-1	47	24	23	
	2-4	27	16	11	
EGFR mutation					0.585
	Del-19	41	21	20	
	L858R	33	19	14	

Supplementary Table S7: List of primers

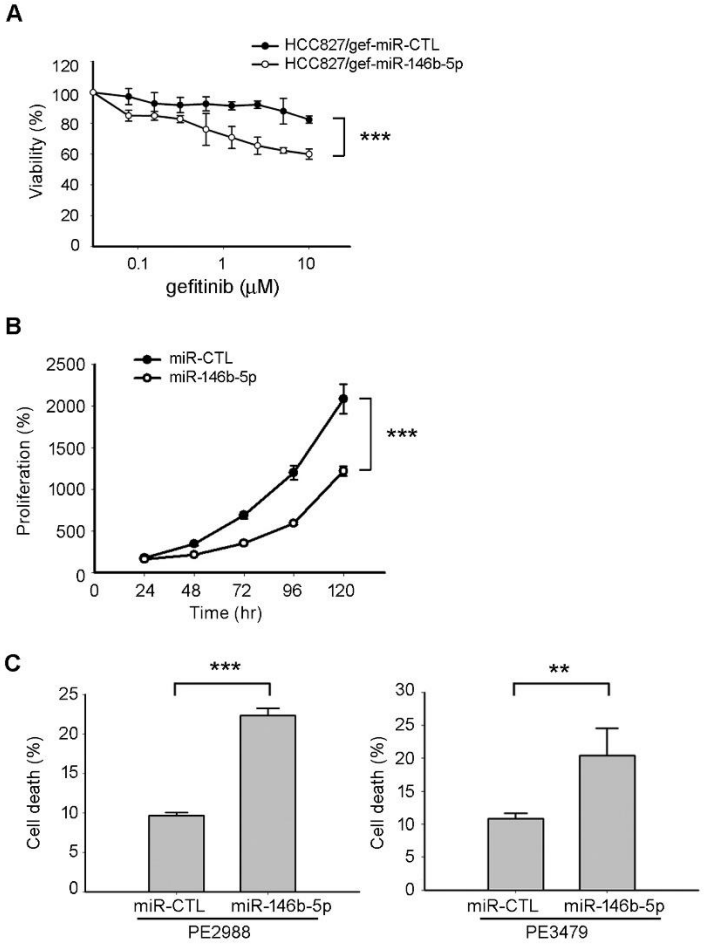
Gene	Primer
EGFR-F	5'- CGCAAAGGGCATGAACTACTT-3'
EGFR-R	5'- CTTGACATGCTGCGGTGTTT-3'
TRAF6-F	5'- TTTTGGTTGCCATGAAAAGA-3'
TRAF6-R	5'-TTCTCATGTGTGACTGGGTGT-3'
IRAK1-F	5'-GAGACCTTGGCTGGTCAGAG-3'
IRAK1-R	5'-GTGCTTCTCAAAGCCACTCC-3'
IL6-F	5'-TCAGCCCTGAGAAAGGAGACAT-3'
IL6-R	5'-CATCCATCTTTTTCAGCCATCTT-3'
IL8-F	5'-ACTCCAAACCTTTCCACCC-3'
IL8-R	5'-AAACTTCTCCACAACCTCTG-3'
TBP-F	5'-CACGAACCACGGCACTGATT-3'
TBP-R	5'-TTTTCTTGCTGCCAGTCTGGAC-3'

Supplementary Table S8: Antibodies and plasmids used in the study

Name	Company	Catalog number
PARP	Cell Signaling Technology	#9542
Caspase-3	Cell Signaling Technology	#9662
TRAF6	Cell Signaling Technology	#8028s
IRAK1	Cell Signaling Technology	#4504s
Phospho-EGFR (Tyr1045)	Cell Signaling Technology	#2237
EGFR	Santa Cruz Biotechnology	#sc-03
β-actin	Millipore	#MAB1501
α-tubulin	Millipore	#04-1117
NF-κB p65	Cell Signaling Technology	#8242
NF-κB1 p105/p50	Cell Signaling Technology	#12540
Lamin B	Santa Cruz	#sc-6210
IRAK1-3'UTR-WT reporter	Addgene	#15095
IRAK1-3'UTR-Mut reporter	Addgene	#15096

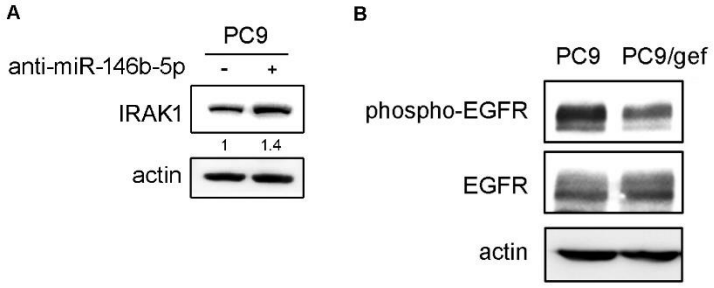
Supplementary Figure

Figure S1



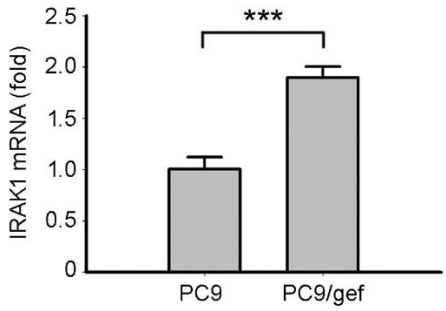
Supplementary Fig. S1. The effect of miR-146b-5p on gefitinib-induced cell death and proliferation. (A) HCC827/gef cells were transfected with miR-Ctl (scrambled control) or miR-146b-5p mimic and incubated for 24 h, followed by treatment with the indicated concentrations of gefitinib. Cell viability was assessed using the MTT assay as described in "Materials and Methods". (B) PC9/gef cells were plated overnight, and then transiently transfected with miR-Ctl or miR-146b-5p. The day of transfection was set as "0" and the growth of cells was determined at the indicated time points by MTT assay. (C) After transfection with miR-Ctl (scrambled control) or miR-146b-5p mimic, the ratio of apoptotic cells was analyzing by annexinV assays.

Figure S2



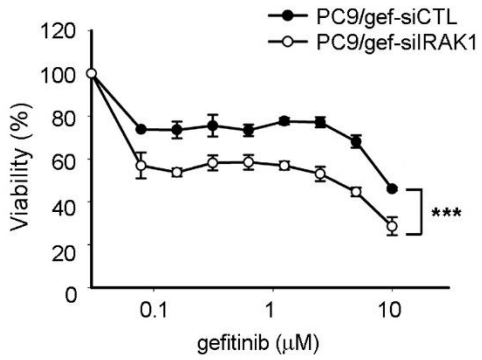
Supplementary Fig. S2. Expression of downstream targets of miR-146b-5p. (A) The expression levels of the IRAK1 protein after transfection of anti-miR-Ctl or anti-miR-146b-5p inhibitor in PC9 cells. (B) The phospho-EGFR and EGFR expressions were detected using and immunoblotting.

Figure S3



Supplementary Fig. S3. IRAK1 expression. The levels of *IRAK1* mRNA were detected using RT-qPCR.

Figure S4

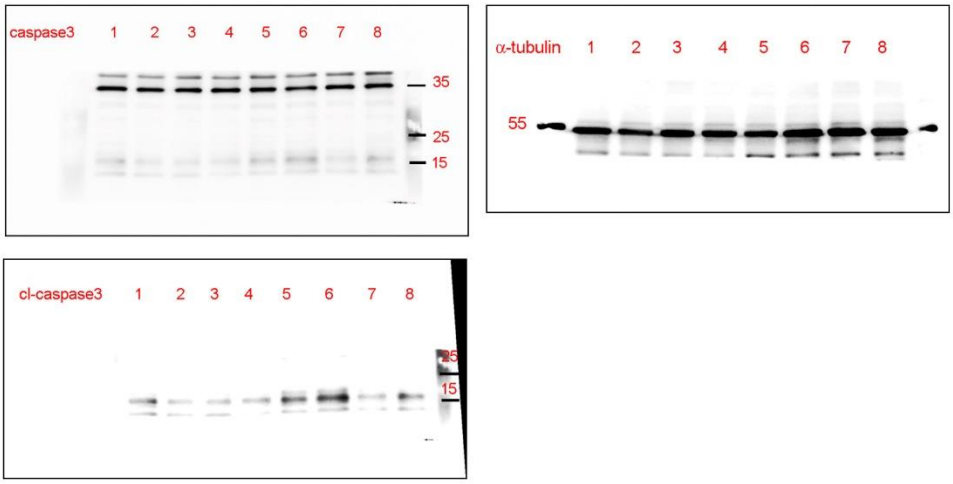


Supplementary Fig. S4. *IRAK1* knockdown enhanced gefitinib-mediated cell death.

PC9/gef cells were transfected with siCTL (scrambled control) or si*IRAK1* for 24 h, and then treated with the indicated concentrations of gefitinib for 72 h. Cell viability was assessed using the MTT assay as described in the "Materials and Methods".

Figure S5 (refer to Figure 2)

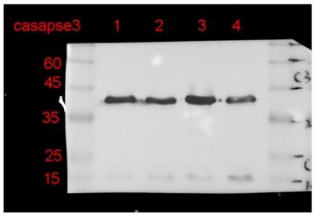
(refer to Fig. 2C)



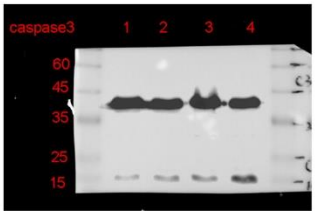
(refer to Fig. 2E)

(Fig. 2E, left)

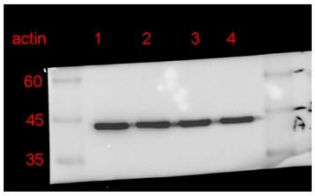
caspase3 (short exposure)



caspase3 (long exposure)



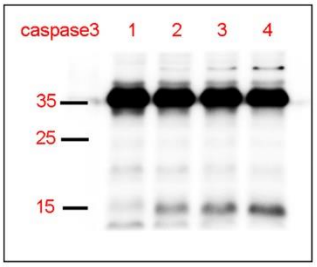
actin



(Fig. 2E, right)

caspase3 (short exposure)

caspase3 (long exposure)

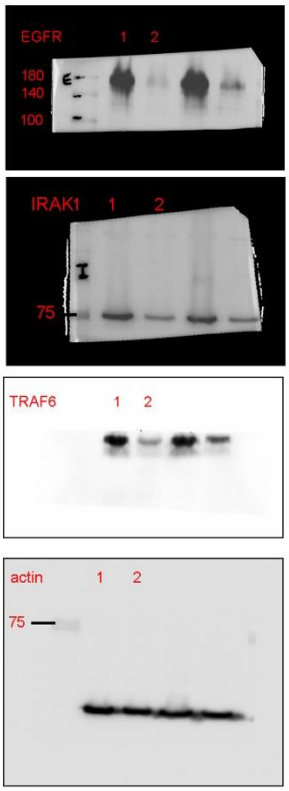


actin

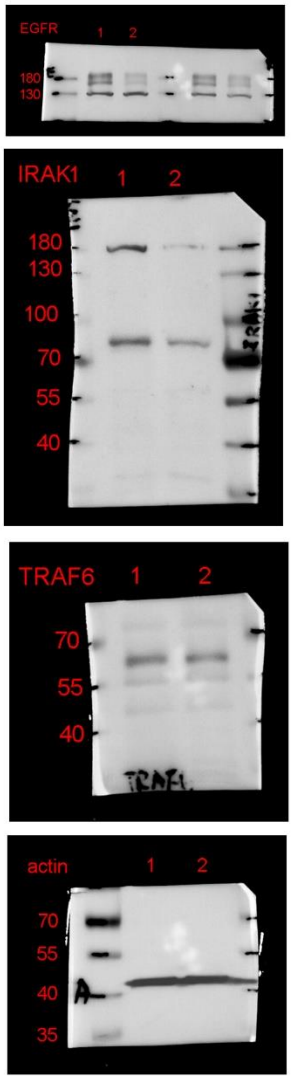
Supplementary Fig. S5. Original films refer to figure 2C and 2E.

Figure S6 (refer to Figure 3)

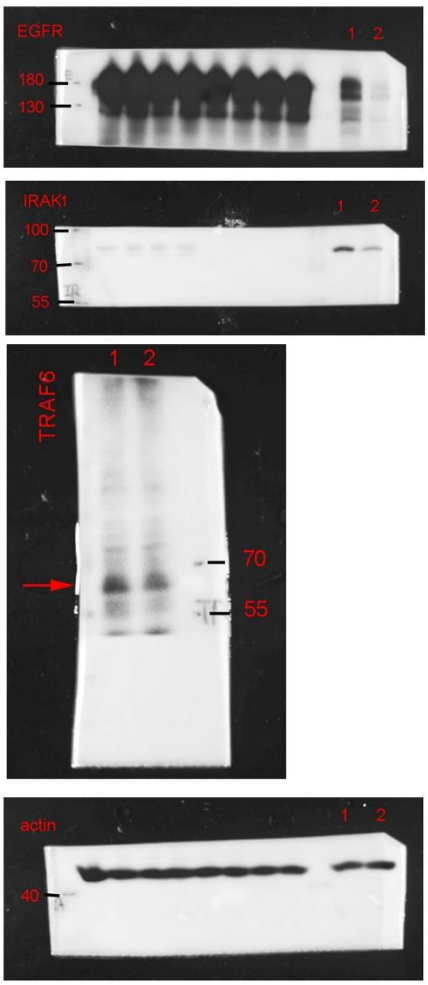
(refer to Fig.3B, left)



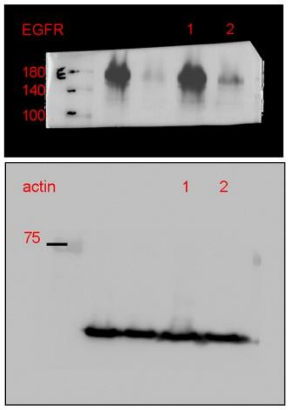
(refer to Fig.3B, middle)



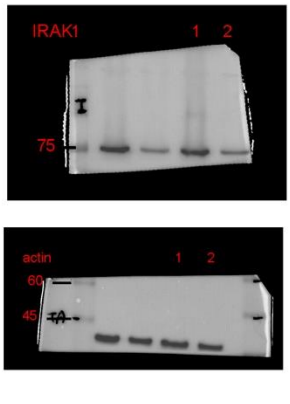
(refer to Fig.3B, right)



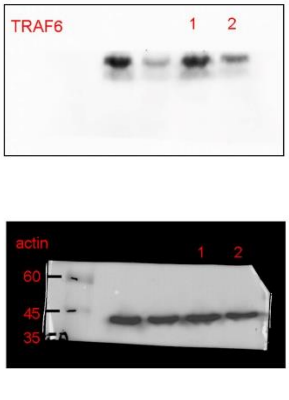
(refer to Fig.3C, left)



(refer to Fig.3C, middle)

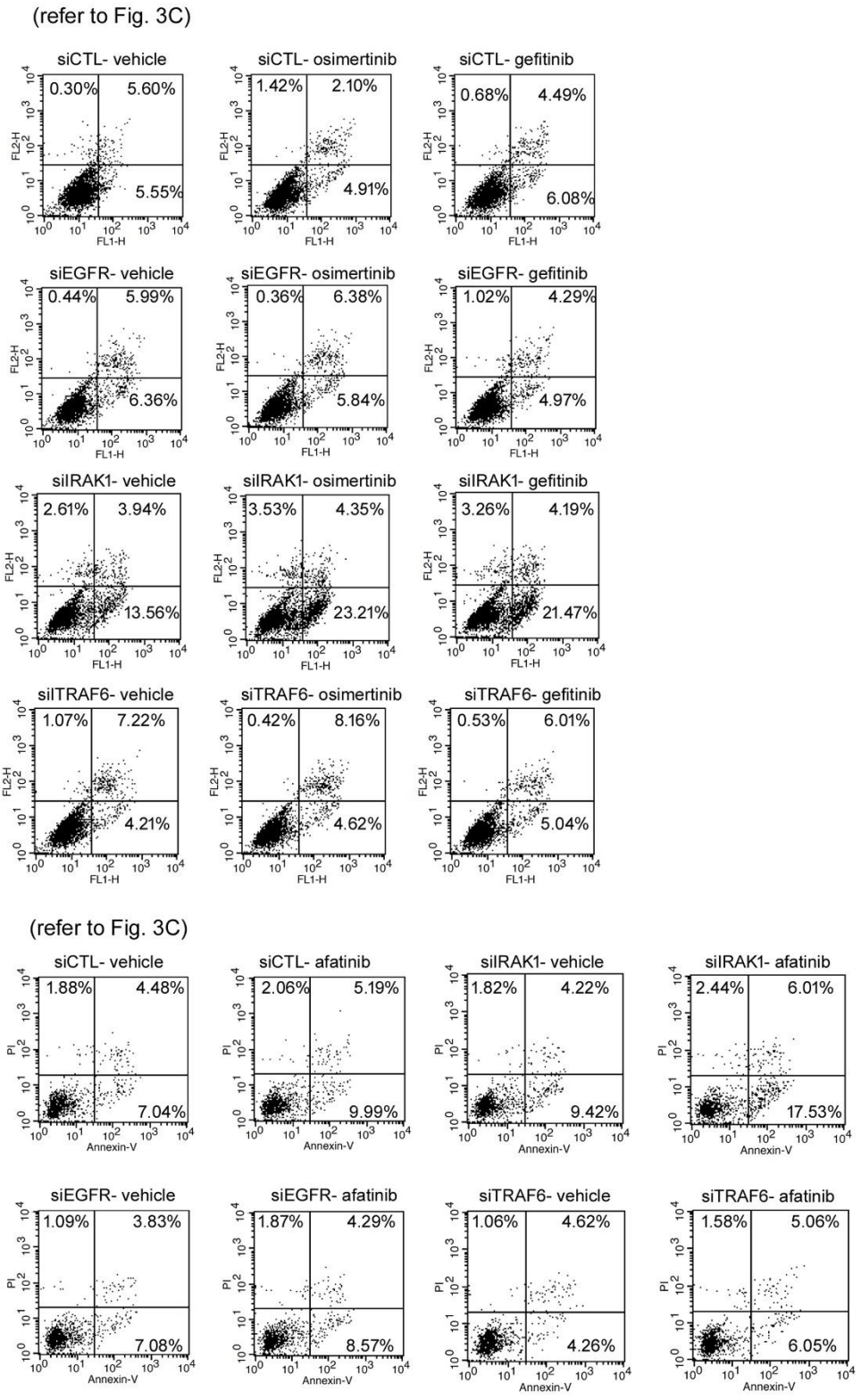


(refer to Fig.3C, right)



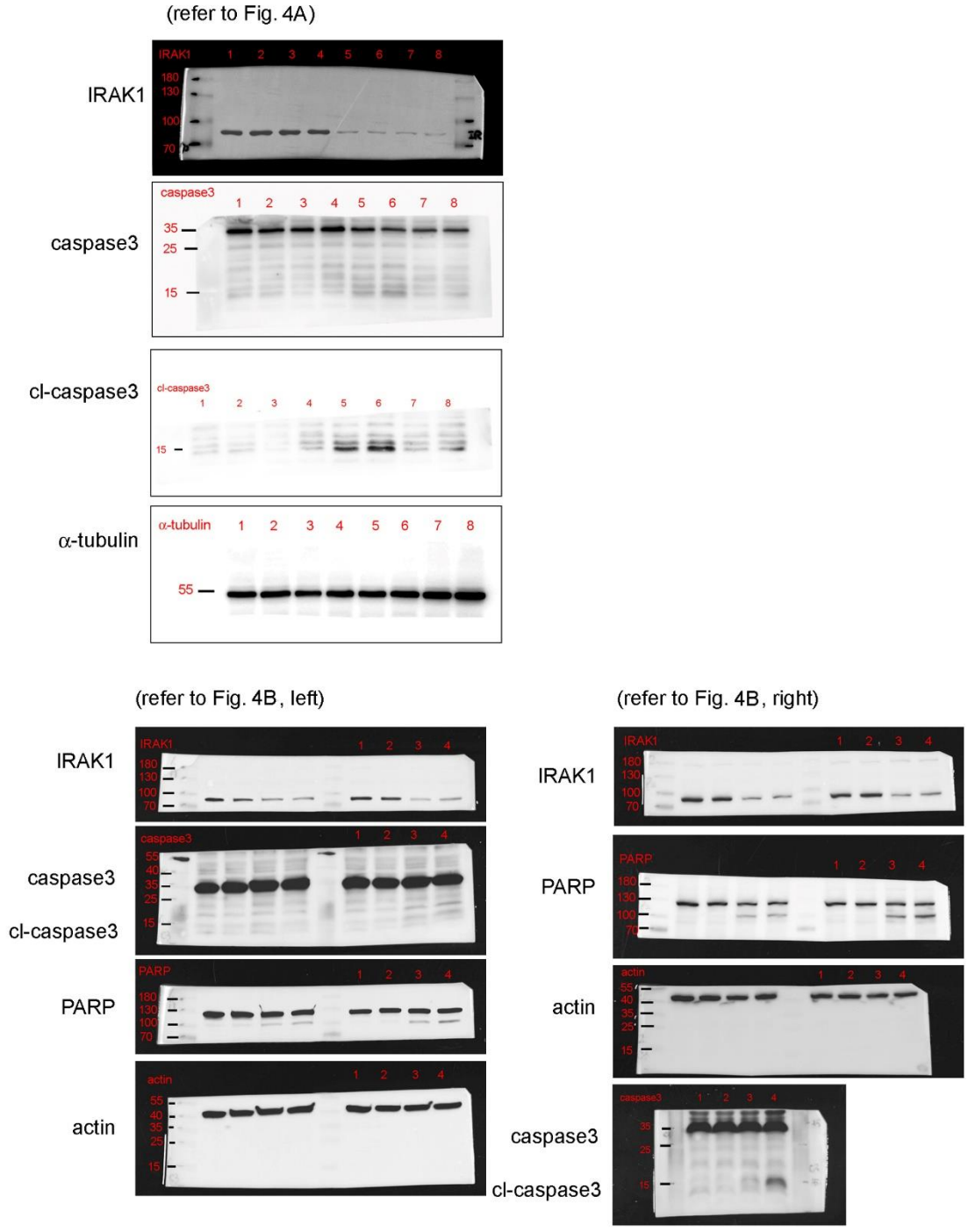
Supplementary Fig. S6. Original films refer to figure 3B and 3C.

Figure S7 (refer to Figure 3)



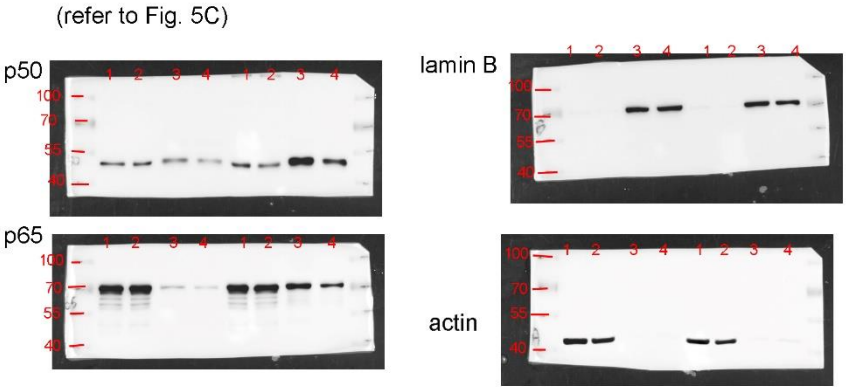
Supplementary Fig. S7. Original dot plots refer to figure 3C.

Figure S8 (refer to Figure 4)



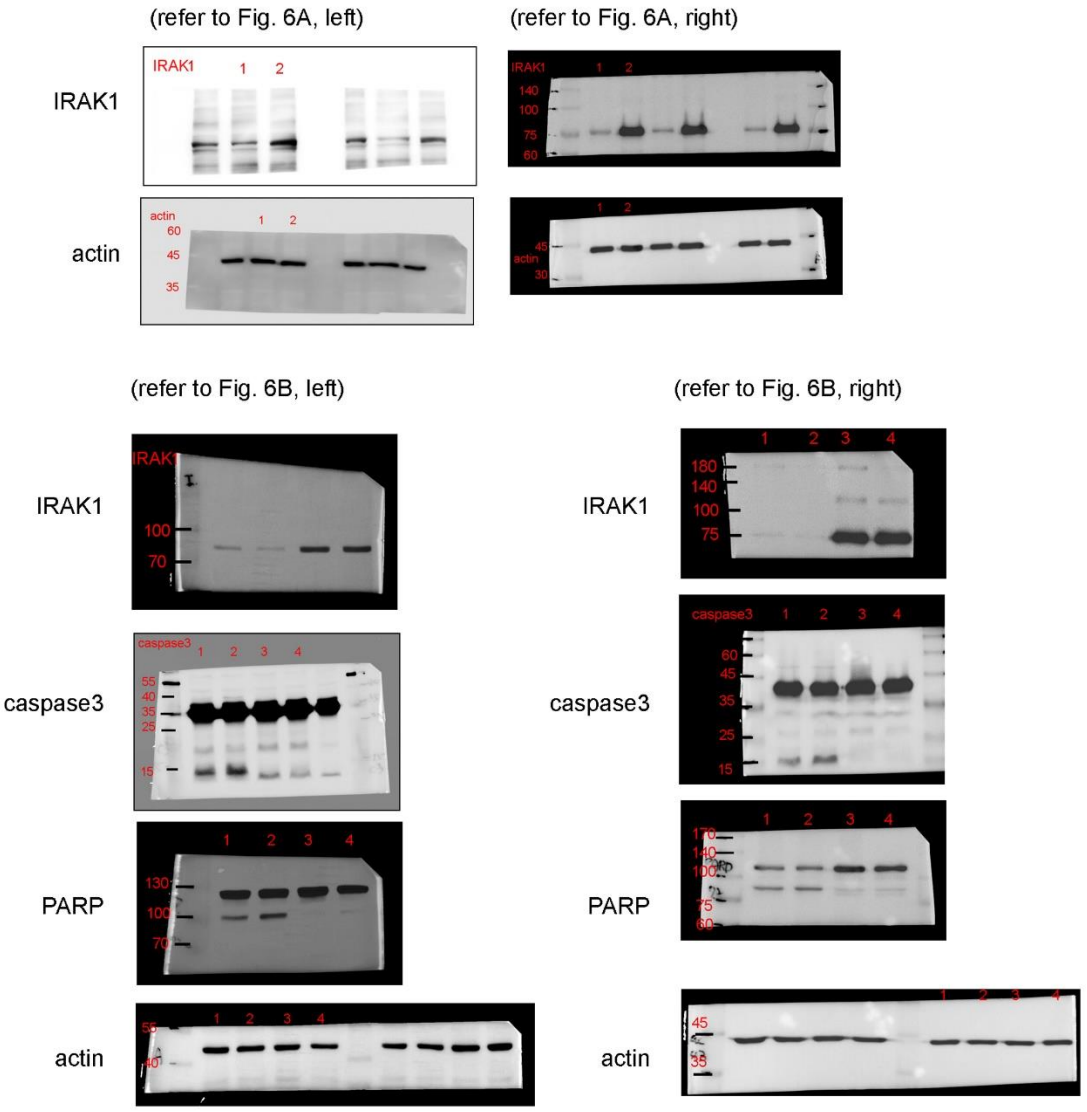
Supplementary Fig. S8. Original films refer to figure 4A and 4B.

Figure S9 (refer to Figure 5)



Supplementary Fig. S9. Original films refer to figure 5C.

Figure S10 (refer to Figure 6)



Supplementary Fig. S10. Original films refer to figure 6A and 6B.