Table S1 Primers used for RT-PCR

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
CD68	TGGGGCAGAGCTTCAGTTG	TGGGGCAGGAGAAACTTTGC
IFNγ	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
CD163	AAAAAGCCACAACAGGTCGC	CTTGAGGAAACTGCAAGCCG
CD206	TGAATTGTACTGGTCTGTCCT	CTGTGGTGCTGTGCATTTATCT
TGFβ	CCTGAGTGGCTGTCTTTTGA	CGTGGAGTTTGTTATCTTTGCTG
IL4	CGGCAACTTTGTCCACGGA	TCTGTTACGGTCAACTCGGTG
IL-10	TTAAGGGTTACCTGGGTTGC	CTGGGTCTTGGTTCTCAGCTT
VEGF	AGGGCAGAATCATCACGAAGT	AGGGTCTCGATTGGATGGCA
NEK2	CATTGGCACAGGCTCCTAC	GAGCCATAGTCAAGTTCTTTCCA
GAPDH	TGGAAGGACTCATGACCACA	TTCAGCTCAGGGATGACCTT

Table S2 siRNA sequence

	sense（5'-3'）	antisence（5'-3'）
NC	UUCUCCGAACGUGUCACGUTT	UAUCUGACAGGGCUUGAGCTT
siRNA-1	GCUCAAGCCCUGUCAGAUATT	AAAGCCAAAGGGAAUAUGCTT
siRNA-2	GCAUUAAUGCCUCCAUUUATT	UAAAUGGAGGCAUUAAUGCTT
siRNA-3	GCUUGUUUCUGAAGUGAAUTT	AUUCACUUCAGAAACAAGCTT

Table S3 Antibodies used for Western Blot.

| Target	Manufacturer	Catalog number	Dilution factor
NEK2	Affinity	DF7296	1:1000
GAPDH	Proteintech	10494-1-AP	1:1000
P21	Proteintech	10355-1-AP	1:1000
P27	Proteintech	25614-1-AP	1:1000
CDK2	Proteintech	10122-1-AP	1:1000
CDK4	Proteintech	11026-1-AP	1:1000
CDK6	Proteintech	14052-1-AP	1:1000
CyclinD1	Proteintech	26939-1-AP	1:1000
E-Cadherin	Abclonal	A3044	1:1000
N-Cadherin	Abclonal	A19083	1:1000
Vimentin	Abclonal	A19607	1:1000
MMP9	Abclonal	A2095	1:1000
c-Myc	Proteintech	10828-1-AP	1:1000
β-catenin	Proteintech	51067-2-AP	1:1000
p-GSK3β	Cell Signaling Technology	#5558	1:1000
t-GSK3β	Cell Signaling Technology	#12456	1:1000
IL-10	Affinity	DF6894	1:1000
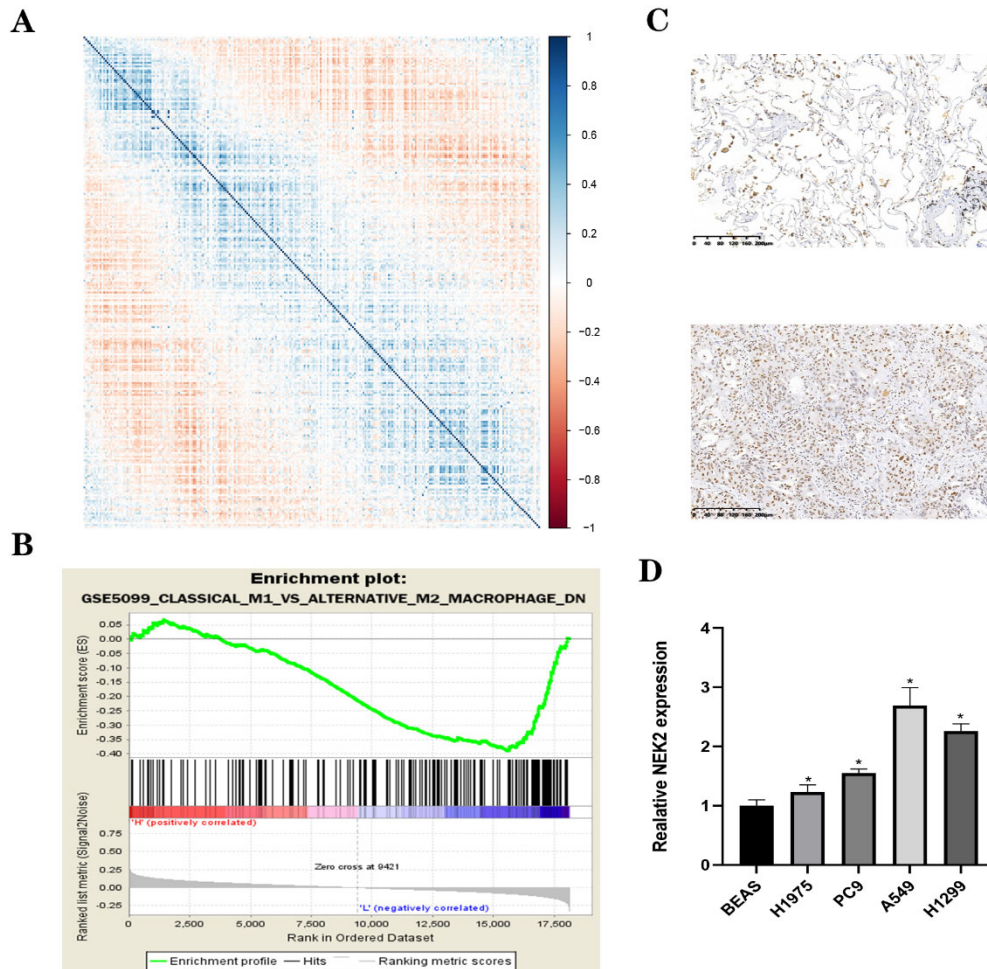
Figure S1. Expression and immune functions of NEK2. (A) Expression correlation heatmap for all DEGs based on TCGA. (B) NEK2 single-gene GSEA analysis showed that the NEK2 gene participated in the process of macrophage polarization. (C) The expression of the NEK2 proteins in paired NSCLC tissues was examined by immunohistochemistry (up: normal; below: tumor). Scale bar: 200 μm. (D) mRNA levels of NEK2 in a normal lung cell line and NSCLC cell lines were examined by RT-qPCR. Data are present as mean ± SD of 3 independent experiments. *$P < 0.05$.
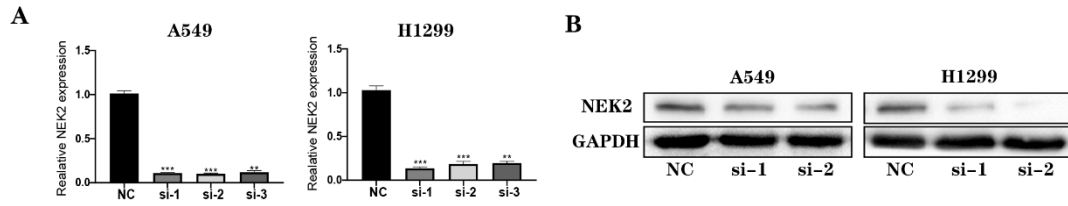
Figure S2. Knockdown efficiency of NEK2. (A-B) NEK2 expression in A549 and H1299 cells transfected with NEK2-specifific siRNAs (si-1 and si-2) or non-specific control siRNA (NC) was analyzed by RT-qPCR and immunoblotting. Data are presented as mean ± SD of 3 independent experiments. *P < 0.05.
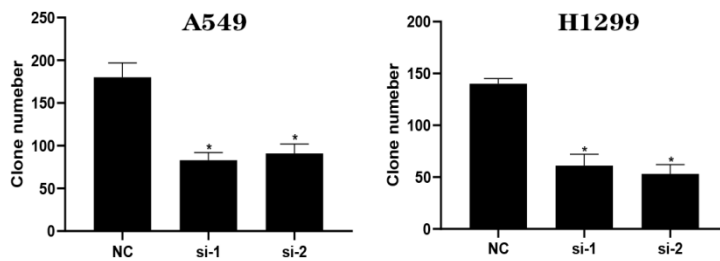

Figure S3. Quantifications of clone formation. Clone numbers of A549 and H1299 cells transfected with NEK2-specifific siRNAs (si-1 and si-2) or non-specific control siRNA (NC) in colony formation assay. Data are presented as mean ± SD of 3 independent experiments. *P < 0.05.
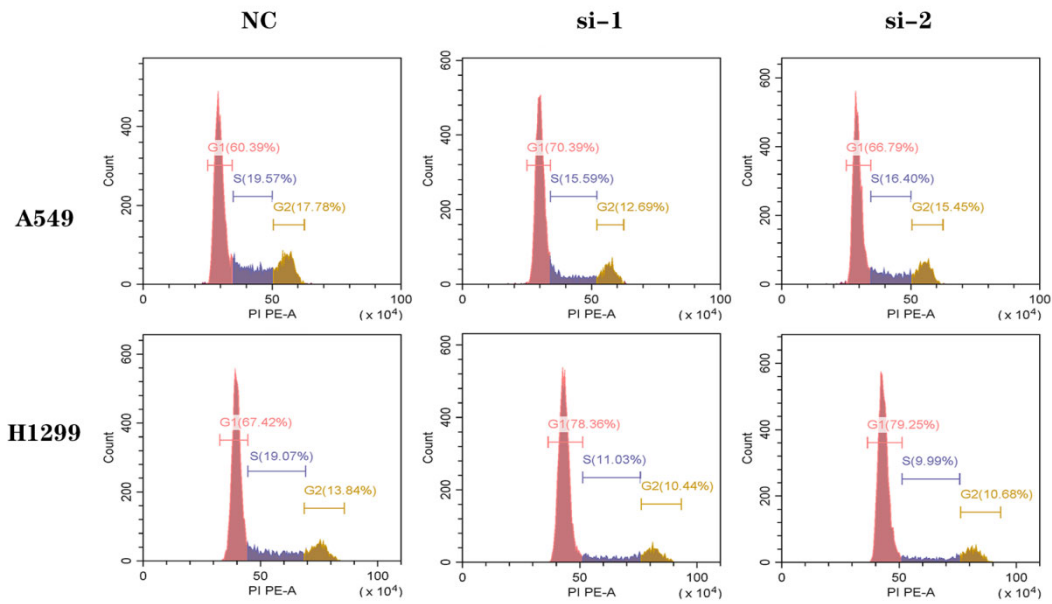
Figure S4. Representative photographs of flow cytometry. Cell distribution at different cell cycle phases 48 h after transfection with NEK2 siRNAs or non-specific control siRNA. Data are presented as mean ± SD of 3 independent experiments. *P < 0.05.
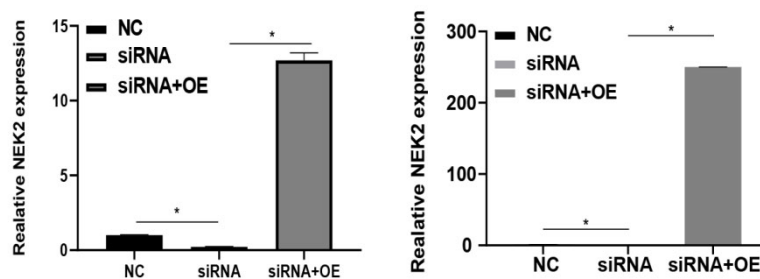

Figure S5. Rescue efficiency of NEK2. NEK2 expression in A549 and H1299 cells transfected with non-specific control siRNA (NC), NEK2-specific siRNA (siRNA) and NEK2-specific siRNA with NEK2 overexpression plasmid (siRNA+OE) was analyzed by RT-qPCR. Data are presented as mean ± SD of 3 independent experiments. *P < 0.05.
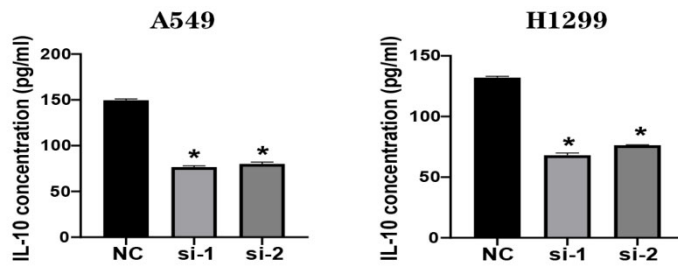
Figure S6. Concentration of IL-10 in conditioned media. ELISA was used

to detect the production of IL-10 in the conditioned media of A549 and H1299.

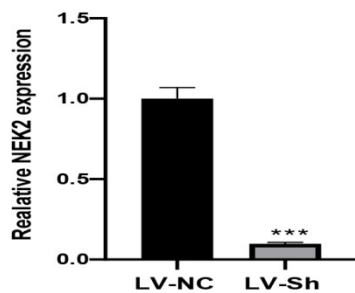Data are presented as mean ± SD of 3 independent experiments. *P < 0.05.

Figure S7. Knockdown efficiency of NEK2 with lentivirus. We used

RT-qPCR to detect the knockdown efficiency of lentiviruses in H1299 cells.

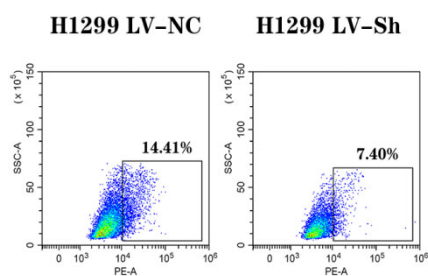Data are presented as mean ± SD of 3 independent experiments. *P < 0.05.

Figure S8. Representative photographs of flow cytometry. Flow cytometry detected the proportion of 163+ M2 cells. Data are presented as mean ± SD of 3 independent experiments. *P < 0.05.
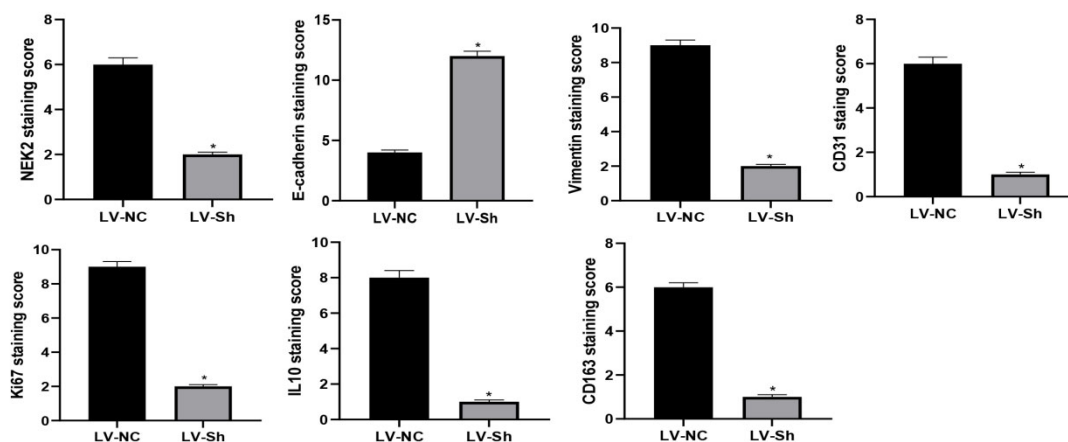

Figure S9. Quantification of immunohistochemistry. Expression of NEK2, CD163, IL-10, CD31, E-cadherin, Vimentin, and Ki67 in nude mouse tumor tissues. Data are presented as mean ± SD of 3 independent experiments. *P < 0.05.