

**Figure S1. Heatmap of DNA methylation expression levels of the NEK gene family in breast cancer by MethSurv platform.** cg02998883, cg05110629, cg26722769 of NEK1; cg15831905, cg17931972 of NEK2; cg19524009, cg22056112 of NEK3; cg02636488 of NEK4; cg15721359, cg18615369 of NEK5; cg14536906, cg13866149, cg13974765, cg14289738 of NEK6; cg04223956, cg09372617 of NEK7; cg17742559 of NEK8; cg04246305 of NEK9; cg09642369, cg17918906 of NEK10; cg01378599, cg06239593 of NEK11 displays the highest level of DNA methylation in breast cancer.

**Table S1: Prognostic Value of Single CpG of the NEK gene family in breast cancer by MethSurv platform.** The threshold of significance was LR Test p-value <0.05. A significant expression pattern was found in NEK2/6 between low and high risk groups for breast cancer.

Gene-CpG	HR	LR Test p-value
NEK1-Body-Open_Sea-cg02998883	1.216	0.33
NEK1-Body-Open_Sea-cg05110629	0.816	0.3
NEK1-Body-Open_Sea-cg26722769	0.751	0.16
NEK2-Body-N_Shelf-cg15831905	0.971	0.88
NEK2-TSS1500-S_Shore-cg17931972	0.558	0.0036*
NEK3-TSS1500-S_Shore-cg19524009	1.219	0.32
NEK3-Body-S_Shelf-cg22056112	0.77	0.19
NEK4-Body-N_Shore-cg02636488	1.073	0.72
NEK5-Body-N_Shelf-cg15721359	0.847	0.4
NEK5-Body-N_Shore-cg18615369	0.845	0.4
NEK6-5'UTR;1stExon;Body-Open_Sea-cg14536906	0.879	0.51
NEK6-5'UTR;Body-Open_Sea-cg13866149	0.963	0.85
NEK6-5'UTR;Body-Open_Sea-cg13974765	0.695	0.068
NEK6-Body-Open_Sea-cg14289738	0.56	0.0042*
NEK7-5'UTR-Open_Sea-cg04223956	0.976	0.9
NEK7-5'UTR-Open_Sea-cg09372617	1.15	0.48
NEK8-Body-N_Shelf-cg17742559	0.858	0.44
NEK9-3'UTR-Open_Sea-cg04246305	0.699	0.07
NEK10-TSS1500-S_Shore-cg09642369	0.749	0.14
NEK10-TSS1500-S_Shore-cg17918906	0.971	0.88
NEK11-Body-Open_Sea-cg01378599	1.056	0.78
NEK11-Body-S_Shelf-cg06239593	0.799	0.26

Table S2: Hallmark signaling pathway analysis of NEK2 in BRCA (GSEA Analysis, Supplementary table S2, Figure 7).

Pathway	pval	padj	log2err	ES	NES
HALLMARK_E2F_TARGETS	1.00E-10	8.33E-10		0.71994	3.11495
HALLMARK_G2M_CHECKPOINT	1.00E-10	8.33E-10		0.683988	2.959398
HALLMARK_MYC_TARGETS_V1	1.00E-10	8.33E-10		0.585011	2.531697
HALLMARK_MYC_TARGETS_V2	1.90E-10	1.35E-09	0.826657	0.664633	2.415173
HALLMARK_MTORC1_SIGNALING	1.00E-10	8.33E-10		0.481279	2.082339
HALLMARK_DNA_REPAIR	2.19E-07	1.09E-06	0.690132	0.46777	1.943888
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	2.40E-06	9.22E-06	0.627257	0.473169	1.898675
HALLMARK_GLYCOLYSIS	1.22E-06	5.09E-06	0.643552	0.416807	1.803391
HALLMARK_INTERFERON_ALPHA_RESPONSE	0.000154	0.000427	0.518848	0.455404	1.783309
HALLMARK_MITOTIC_SPINDLE	2.20E-05	6.87E-05	0.57561	0.397629	1.720994
HALLMARK_SPERMATOGENESIS	0.000511	0.001253	0.477271	0.398886	1.639428
HALLMARK_ESTROGEN_RESPONSE_LATE	0.000275	0.000725	0.498493	0.367052	1.588115
HALLMARK_PI3K_AKT_MTOR_SIGNALING	0.021276	0.036682	0.352488	0.358774	1.427884
HALLMARK_INTERFERON_GAMMA_RESPONSE	0.008478	0.016956	0.38073	0.324046	1.402341
HALLMARK_ESTROGEN_RESPONSE_EARLY	0.016549	0.030646	0.352488	0.320301	1.386305
HALLMARK_UV_RESPONSE_UP	0.020007	0.035726	0.352488	0.327529	1.369654
HALLMARK_ALLOGRAFT_REJECTION	0.110682	0.172941	0.149208	0.275883	1.193914
HALLMARK_OXIDATIVE_PHOSPHORYLATION	0.240669	0.325229	0.095288	0.256042	1.10805
HALLMARK_PANCREAS_BETA_CELLS	0.50234	0.558156	0.066284	0.290154	0.976768
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	0.490798	0.557724	0.066589	0.279271	0.97531
HALLMARK_PROTEIN_SECRETION	0.678273	0.721567	0.046882	0.228996	0.895558
HALLMARK_APICAL_SURFACE	0.806854	0.82332	0.044064	0.235104	0.801411
HALLMARK_KRAS_SIGNALING_DN	0.962773	0.962773	0.026395	0.177094	0.766229
HALLMARK_NOTCH_SIGNALING	0.634921	0.690131	0.081303	-0.254	-0.89576
HALLMARK_INFLAMMATORY_RESPONSE	0.748879	0.780082	0.10208	-0.18367	-0.90667
HALLMARK_ANGIOGENESIS	0.43454	0.50528	0.106323	-0.27918	-1.00469
HALLMARK_HEDGEHOG_SIGNALING	0.383152	0.456134	0.112843	-0.28342	-1.01703
HALLMARK_P53_PATHWAY	0.327273	0.409091	0.164406	-0.21337	-1.05046

HALLMARK_IL6_JAK_STAT3_SIGNALING	0.347079	0.423267	0.13649	-0.24641	-1.06307
HALLMARK_PEROXISOME	0.279851	0.358783	0.160801	-0.24548	-1.09361
HALLMARK_CHOLESTEROL_HOMEOSTASIS	0.270548	0.355984	0.156312	-0.26434	-1.10124
HALLMARK_ANDROGEN_RESPONSE	0.197183	0.273865	0.188204	-0.25668	-1.14047
HALLMARK_COMPLEMENT	0.15521	0.221729	0.249247	-0.23571	-1.16046
HALLMARK_APOPTOSIS	0.142276	0.20923	0.249247	-0.24794	-1.1832
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.061505	0.099202	0.321776	-0.24459	-1.21122
HALLMARK_COAGULATION	0.040394	0.067324	0.321776	-0.2757	-1.28536
HALLMARK_WNT_BETA_CATENIN_SIGNALING	0.119241	0.180668	0.213928	-0.34332	-1.2893
HALLMARK_IL2_STAT5_SIGNALING	0.008083	0.016839	0.38073	-0.2864	-1.41003
HALLMARK_APICAL_JUNCTION	0.002407	0.005233	0.431708	-0.29583	-1.45268
HALLMARK_HEME_METABOLISM	0.001156	0.002627	0.45506	-0.30426	-1.4941
HALLMARK_TGF_BETA_SIGNALING	0.008964	0.017238	0.38073	-0.39871	-1.5794
HALLMARK_XENOBIOTIC_METABOLISM	5.68E-05	0.000167	0.557332	-0.33553	-1.64767
HALLMARK_BILE_ACID_METABOLISM	0.000526	0.001253	0.477271	-0.37067	-1.65469
HALLMARK_KRAS_SIGNALING_UP	4.30E-06	1.43E-05	0.610527	-0.357	-1.76789
HALLMARK_HYPOXIA	2.81E-06	1.00E-05	0.627257	-0.36633	-1.80838
HALLMARK_FATTY_ACID_METABOLISM	9.78E-07	4.45E-06	0.643552	-0.41076	-1.96252
HALLMARK_TNFA_SIGNALING_VIA_NFKB	5.89E-09	3.27E-08	0.761461	-0.4113	-2.03038
HALLMARK_MYOGENESIS	3.39E-10	2.12E-09	0.814036	-0.43359	-2.13463
HALLMARK_UV_RESPONSE_DN	1.00E-10	8.33E-10		-0.51101	-2.39865
HALLMARK_ADIPOGENESIS	1.00E-10	8.33E-10		-0.53743	-2.66138

**Table S3: Pathway analysis of genes coexpressed NEK2 from public breast cancer databases using the MetaCore database (with p – value <0.05 set as the cutoff value) (Supplementary Table S3, Figure 8).**

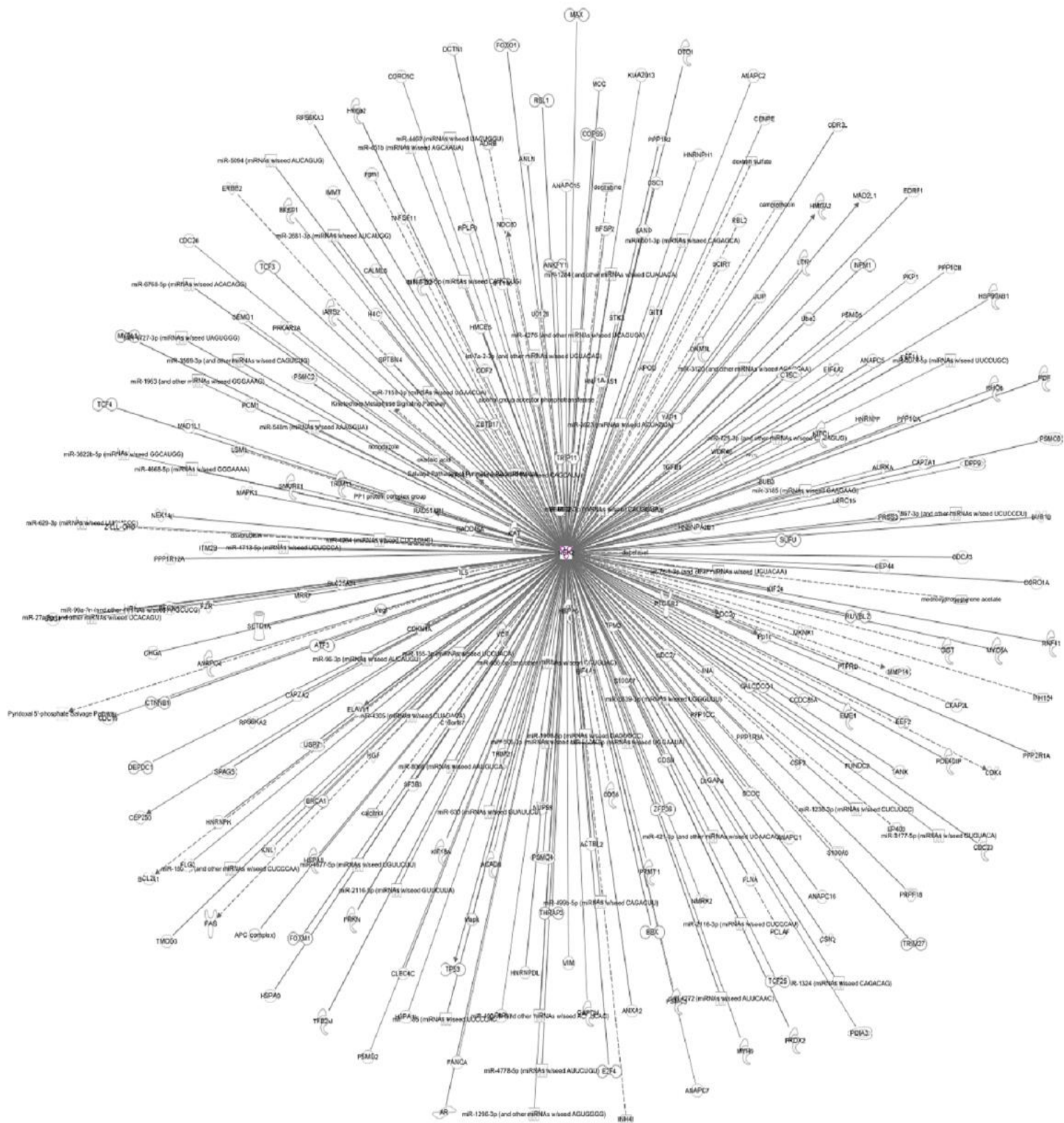
#	Maps	pValue	Network Objects from Active Data
1	Cell cycle_The metaphase checkpoint	6.790E-34	INCENP, Aurora-B, BUB1, HEC, SPBC25, CDCA1, CENP-A, CDC20, HZWint-1, Rod, CENP-F, SPBC24, DSN1, Zwilch, MAD2a, Survivin, CENP-H, CENP-E, AF15q14, Aurora-A, PLK1, BUBR1
2	Cell cycle_Role of APC in cell cycle regulation	3.599E-31	Aurora-B, BUB1, CDC25A, CDC18L (CDC6), CDC20, SKP2, Cyclin B, Tome-1, Geminin, MAD2a, Emi1, Securin, ORC1L, Cyclin A, CDK1 (p34), Aurora-A, PLK1, CDK2, CKS1, BUBR1
3	Cell cycle_Chromosome condensation in prometaphase	2.439E-27	INCENP, Aurora-B, BRRN1, CAP-H/H2, CAP-G, CAP-E, Cyclin B, Condensin, CAP-C, TOP2, Cyclin A, CNAP1, CAP-G/G2, CDK1 (p34), Aurora-A, CAP-D2/D3
4	Cell cycle_Start of DNA replication in early S phase	6.421E-23	Cdt1, CDC18L (CDC6), MCM10, ORC6L, Geminin, ASK (Dbf4), MCM4/6/7 complex, MCM4, CDC7, MCM3, MCM2, ORC1L, Cyclin E, CDK2, E2F1, CDC45L
5	Cell cycle_Spindle assembly and chromosome separation	1.233E-22	KNSL1, Aurora-B, HEC, Importin (karyopherin)-alpha, CDC20, TPX2, Tubulin alpha, Cyclin B, CSE1L, MAD2a, Separase, Securin, Ran, CDK1 (p34), Aurora-A, Tubulin (in microtubules)
6	DNA damage_ATM/ATR regulation of G2/M checkpoint: nuclear signaling	2.856E-18	CDC25C, GTSE1, WDHD1, CDC18L (CDC6), Cyclin B1, Cyclin B, Chk2, Cyclin B2, TTK, Cyclin A, CDK1 (p34), Claspin, Chk1, PLK1, CDK2
7	Cell cycle_Role of SCF complex in cell cycle regulation	3.422E-16	Cdt1, CDC25A, SKP2, Emi1, Cyclin E, CDK1 (p34), Skp2/TrCP/FBXW, Chk1, PLK1, CDK2, CKS1, E2F1
8	Abnormalities in cell cycle in SCLC	3.422E-16	Aurora-B, PCNA, Cyclin B1, SKP2, E2F2, Cyclin A, Cyclin E, CDK1 (p34), Cyclin E2, CDK2, CKS1, E2F1
9	Cell cycle_Cell cycle (generic schema)	1.878E-14	CDC25C, CDC25A, Cyclin B, E2F2, p107, Cyclin A, Cyclin E, CDK1 (p34), CDK2, E2F1
10	DNA damage_G2 checkpoint in response to DNA mismatches	3.081E-14	CDC25C, EXO1, TOPBP1, PCNA, MutSalp complex, MSH2, Chk2, MSH6, CDK1 (p34), Claspin, Chk1
11	Cell cycle_Role of Nek in cell cycle regulation	3.060E-12	HEC, Tubulin beta, Cyclin B1, TPX2, Tubulin alpha, MAD2a, Ran, CDK1 (p34), Aurora-A, Tubulin (in microtubules)

12	Cell cycle_Initiation of mitosis	1.347E-11	KNSL1, CDC25C, Lamin B, Cyclin B1, Cyclin B2, FOXM1, Kinase MYT1, CDK1 (p34), PLK1
13	DNA damage_ATM/ATR regulation of G2/M checkpoint: cytoplasmic signaling	2.153E-11	CDC25C, Aurora-B, UBE2C, CDC25A, Cyclin B1, BORA, Chk2, CDK1 (p34), Chk1, Aurora-A, PLK1
14	Cell cycle_ESR1 regulation of G1/S transition	2.770E-10	Cyclin A2, CDC25A, SKP2, Cyclin A, Cyclin E, Skp2/TrCP/FBXW, CDK2, CKS1, E2F1
15	Cell cycle_Transition and termination of DNA replication	5.710E-10	TOP2 alpha, PCNA, MCM2, TOP2, FEN1, Cyclin A, CDK1 (p34), CDK2
16	Cell cycle_Sister chromatid cohesion	7.790E-09	PCNA, Cyclin B, DCC1, RFC3, Separase, Securin, CDK1 (p34)
17	Cell cycle_Nucleocytoplasmic transport of CDK/Cyclins	8.763E-09	Importin (karyopherin)-alpha, Cyclin B1, Cyclin A, Cyclin E, CDK1 (p34), CDK2
18	Reproduction_Progesterone-mediated oocyte maturation	2.429E-08	CDC25C, BUB1, Cyclin B1, CDC20, Kinase MYT1, CDK1 (p34), Aurora-A, PLK1
19	DNA damage_Role of Brca1 and Brca2 in DNA repair	6.018E-08	PCNA, FANCD2, BRIP1, MSH2, Chk2, Rad51, MSH6
20	Nicotine / nAChR alpha-3/nAChR beta-2 signaling in NSCLC	5.946E-07	CDC25A, CDC18L (CDC6), E2F2, TYSY, Survivin, E2F1
21	Apoptosis and survival_DNA-damage-induced apoptosis	7.219E-07	BLM, FANCD2, Chk2, Chk1, E2F1
22	Cell cycle_Regulation of G1/S transition (part 2)	9.534E-07	Cyclin A2, p107, Cyclin A, Cyclin E, CDK2, E2F1
23	Mitogenic action of Estradiol / ESR1 (nuclear) in breast cancer	9.534E-07	SGOL2, CDC25A, Cyclin E, Cyclin E2, CDK2, E2F1
24	DNA damage_ATM/ATR regulation of G1/S checkpoint	9.805E-07	PCNA, CDC25A, Chk2, Cyclin A, Cyclin E, Chk1, CDK2
25	DNA damage_Mismatch repair	2.202E-06	EXO1, WDHD1, PCNA, MutSalpha complex, MSH2, MSH6
26	Immune response_IFN-alpha/beta signaling via PI3K and NF-kB pathways	2.249E-06	PCNA, b-Myb, CDC25A, p107, Cyclin A, Cyclin E, CDK1 (p34), CDK2, E2F1

27	Higher ESR1 / ESR2 ratio in breast cancer	4.544E-06	Cyclin A2, CDC25A, Cyclin B1, SKP2, FOXM1, Cyclin E
28	HCV-dependent transcription regulation leading to HCC	4.608E-06	SKP2, HSP70, Cyclin E, CDK2, E2F1
29	IL-6 signaling in colorectal cancer	5.369E-06	Cyclin B1, HSP70, Cyclin B, Survivin, Cyclin E, CDK1 (p34)
30	Cell cycle_Role of 14-3-3 proteins in cell cycle regulation	5.904E-06	CDC25C, CDC25A, Chk2, CDK1 (p34), Chk1
31	Microsatellite instability in gastric cancer	5.904E-06	EXO1, PCNA, MutSalph complex, MSH2, MSH6
32	Cell cycle_Regulation of G1/S transition (part 1)	6.311E-06	CDC25A, Chk2, Cyclin A, Cyclin E, Skp2/TrCP/FBXW, CDK2
33	Inhibition of remyelination in multiple sclerosis: regulation of cytoskeleton proteins	1.518E-05	chTOG, Tubulin beta, Tubulin alpha, Stathmin, Tubulin beta 4, Tubulin (in microtubules)
34	DNA damage_Brca1 as a transcription regulator	2.951E-05	PCNA, Cyclin B1, MSH2, Chk2, E2F1
35	Transcription_Ligand-dependent activation of the ESR1/SP pathway	2.951E-05	CDC25A, TYSY, Cyclin E, Cyclin E2, E2F1
36	dCTP/dUTP metabolism	3.381E-05	Small RR subunit, RRM1, Ribonucleotide reductase, RRM2, DNA polymerase theta, POLE2, '...
37	Microsatellite instability in colorectal cancer	5.538E-05	EXO1, PCNA, MutSalph complex, MSH2, MSH6
38	Rb proteins signaling in multiple myeloma	5.919E-05	p107, Cyclin E, CDK2, E2F1
39	Mechanisms of CAM-DR in multiple myeloma	6.397E-05	TOP2 alpha, HSP70, Cyclin A, Cyclin E, CDK2
40	Epigenetic alterations in ovarian cancer	6.580E-05	DNMT3B, Aurora-B, CDC20, SKP2, PARP-1, Aurora-A, EZH2

41	Brca1 and Brca2 in breast cancer	7.426E-05	BRIP1, MSH2, Rad51, Chk1
42	Cell cycle progression in Prostate Cancer	1.090E-04	CDC25A, Cyclin B, CDK1 (p34), CDK2, E2F1
43	Immune response_IL-4-induced regulators of cell growth, survival, differentiation and metabolism	1.102E-04	CDC25A, MCM6, MCM4, Cyclin A, Cyclin E, CDK2
44	Transcription_Role of heterochromatin protein 1 (HP1) family in transcriptional silencing	1.234E-04	Cyclin A2, CDC25A, Cyclin E, CDK1 (p34), E2F1
45	Proteolysis_Role of Parkin in the Ubiquitin-Proteasomal Pathway	1.940E-04	Tubulin beta, HSP70, Tubulin alpha, Cyclin E
46	DNA damage_Nucleotide excision repair	2.168E-04	Histone H2A, DNA polymerase kappa, PCNA, PARP-1, DTL (hCdt2), EZH2
47	The role of aberrations in CDKN2 locus and CDK4 in familial melanoma	3.599E-04	b-Myb, E2F2, p107, E2F1
48	Notch signaling in breast cancer	4.732E-04	Cyclin B1, HURP, Survivin, Cyclin A, CDK2
49	Cell cycle_Influence of Ras and Rho proteins on G1/S Transition	4.732E-04	Cyclin A2, SKP2, Cyclin E, CDK2, E2F1
50	IL-2 as a growth factor for T cells in multiple sclerosis	6.856E-04	PCNA, Cyclin E, CDK2, E2F1

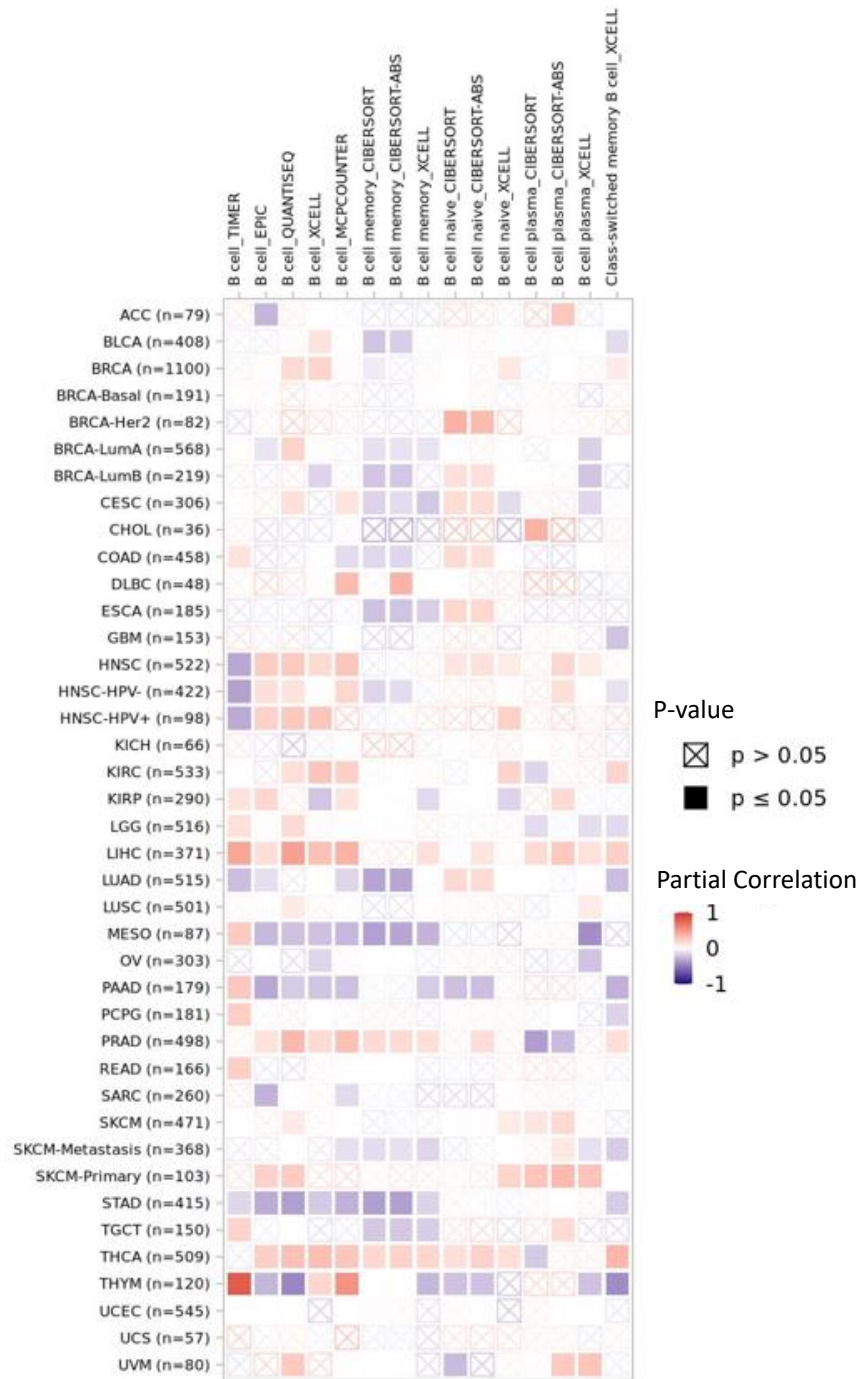




**Figure S2. Analysis of micro (mi)RNA networks with NEK2 in breast cancer.** We used the miRWalk database to identify associations with NEK2, and then network regulation was analyzed by Ingenuity Pathway Analysis (IPA). hsa-miR-1236-3p, hsa-miR-4264, hsa-miR-486-5p, hsa-miR-155-3p, and hsa-miR-6839-3p are co-expressed for breast cancer development.

**Table S4. Correlation of NEK2 expression with the level of immune infiltration of B cells in BRCA (Analysis of TIMER database).** rho shows the partial correlation value, and the level of statistical significance is shown with p-value and adjusted p-value. The threshold of significance was p-value <0.05.

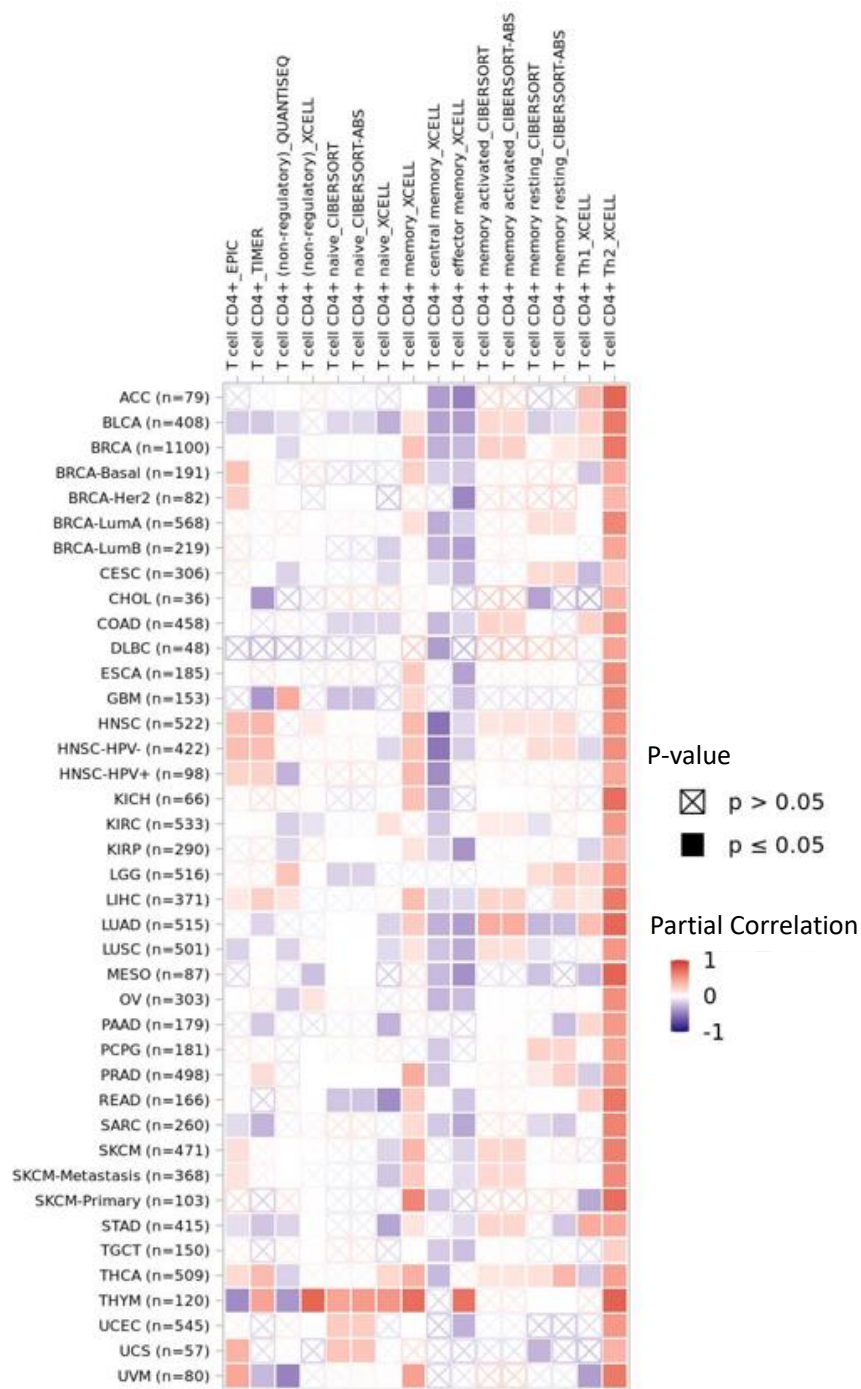
Cancer	Infiltrates	rho	p-value	Adjusted p-value
BRCA (n=1100)	B cell memory_CIBERSORT	-0.072	0.023	0.084
BRCA (n=1100)	B cell memory_CIBERSORT-ABS	-0.062	0.051	0.148
BRCA (n=1100)	B cell memory_XCELL	0.045	0.158	0.333
BRCA (n=1100)	B cell naive_CIBERSORT	0.000	0.992	0.997
BRCA (n=1100)	B cell naive_CIBERSORT-ABS	0.049	0.126	0.288
BRCA (n=1100)	B cell naive_XCELL	0.109	0.001	0.005
BRCA (n=1100)	B cell plasma_CIBERSORT	-0.036	0.259	0.478
BRCA (n=1100)	B cell plasma_CIBERSORT-ABS	0.007	0.814	0.909
BRCA (n=1100)	B cell plasma_XCELL	0.052	0.100	0.246
BRCA (n=1100)	B cell_EPIC	0.019	0.550	0.750
BRCA (n=1100)	B cell_MCPCOUNTER	0.029	0.355	0.593
BRCA (n=1100)	B cell_QUANTISEQ	0.180	0.000	0.000
BRCA (n=1100)	B cell_TIMER	0.061	0.053	0.150
BRCA (n=1100)	B cell_XCELL	0.202	0.000	0.000
BRCA (n=1100)	Class-switched memory B cell_XCELL	0.101	0.001	0.011



**Figure S3. Heatmap of NEK2 expression with the level of immune infiltration of B cells in different types of cancer (Analysis of TIMER database).** The partial correlation coefficient shows the size of the correlation, and the p-value indicates the level of statistical significance. The threshold of significance was p-value <0.05.

**Table S5. Correlation of NEK2 expression with the level of immune infiltration of CD4+ T Cells in BRCA (Analysis of TIMER database).** rho shows the partial correlation value, and the level of statistical significance is shown with p-value and adjusted p-value. The threshold of significance was p-value <0.05.

<b>Cancer</b>	<b>Infiltrates</b>	<b>rho</b>	<b>p-value</b>	<b>Adjusted p-value</b>
<b>BRCA (n=1100)</b>	T cell CD4+ (non-regulatory)_QUANTISEQ	-0.1357	0.0000	0.0001
<b>BRCA (n=1100)</b>	T cell CD4+ (non-regulatory)_XCELL	0.0401	0.2063	0.3350
<b>BRCA (n=1100)</b>	T cell CD4+ central memory_XCELL	-0.2947	0.0000	0.0000
<b>BRCA (n=1100)</b>	T cell CD4+ effector memory_XCELL	-0.2598	0.0000	0.0000
<b>BRCA (n=1100)</b>	T cell CD4+ memory activated_CIBERSORT	0.2223	0.0000	0.0000
<b>BRCA (n=1100)</b>	T cell CD4+ memory activated_CIBERSORT-ABS	0.2224	0.0000	0.0000
<b>BRCA (n=1100)</b>	T cell CD4+ memory resting_CIBERSORT	0.0617	0.0518	0.1188
<b>BRCA (n=1100)</b>	T cell CD4+ memory resting_CIBERSORT-ABS	0.1065	0.0008	0.0031
<b>BRCA (n=1100)</b>	T cell CD4+ memory_XCELL	0.2963	0.0000	0.0000
<b>BRCA (n=1100)</b>	T cell CD4+ naive_CIBERSORT	-0.0194	0.5406	0.6575
<b>BRCA (n=1100)</b>	T cell CD4+ naive_CIBERSORT-ABS	-0.0195	0.5399	0.6575
<b>BRCA (n=1100)</b>	T cell CD4+ naive_XCELL	-0.0297	0.3491	0.4943
<b>BRCA (n=1100)</b>	T cell CD4+ Th1_XCELL	0.1474	0.0000	0.0000
<b>BRCA (n=1100)</b>	T cell CD4+ Th2_XCELL	0.6766	0.0000	0.0000
<b>BRCA (n=1100)</b>	T cell CD4+_EPIC	0.0365	0.2496	0.3903
<b>BRCA (n=1100)</b>	T cell CD4+_TIMER	0.0045	0.8886	0.9314



**Figure S4. Heatmap of NEK2 expression with the level of immune infiltration of CD4+ T Cells in different types of cancer (Analysis of TIMER database).** The partial correlation coefficient shows the size of the correlation, and the p-value indicates the level of statistical significance. The threshold of significance was p-value <0.05.