# Supplementary materials

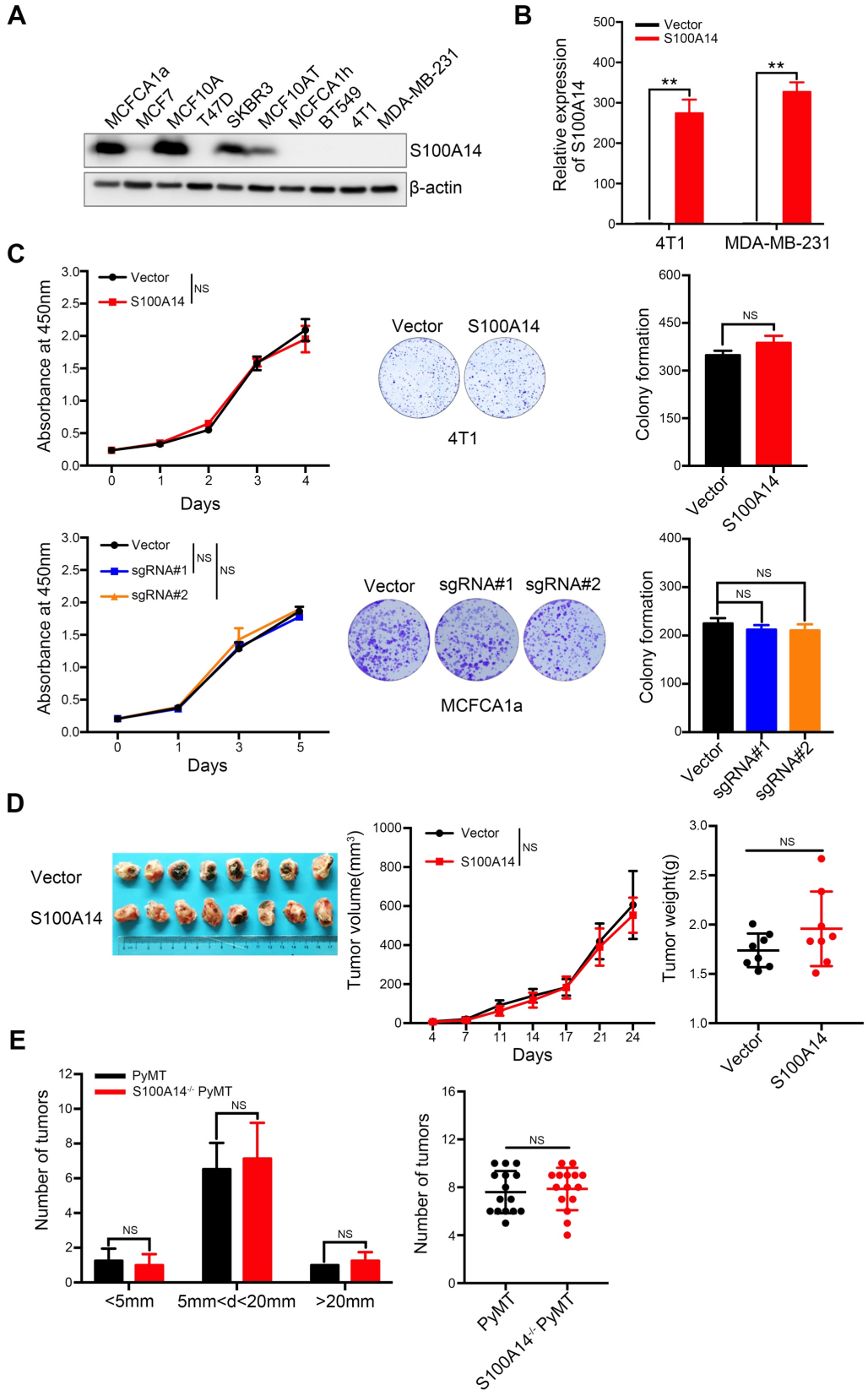

**Figure S1.** S100A14 has no effect on breast cancer cell growth *in vitro* and *in vivo*. (**A**) S100A14 expression was examined in nine breast cancer cell lines and one normal breast epithelial cell line by Western blot. β-actin was used as a loading control. (**B**) The efficiency of S100A14 overexpression was detected by qRT-PCR. (**C**) Left, cell proliferation was detected by CCK-8 assay after the ectopic overexpression of S100A14 in 4T1 cells (upper) and knockout of S100A14 in MCFCA1a cells (lower). Right, colony formation assays were performed after the ectopic overexpression of S100A14 in 4T1 cells (upper) and knockout of S100A14 in MCFCA1a cells (lower). Representative images and statistical analyses of number of colonies are shown. (**D**) The tumor pictures, tumor volumes and tumor weights of mice injected with S100A14-overexpressing 4T1 and control cells (n=8). (**E**) Statistical analyses of the number and diameter of tumors from S100A14$^{-/-}$ PyMT and PyMT mice. The birth date of PyMT mice was recorded. After 22 weeks, PyMT mice were killed, and the number and diameter of tumors were analyzed (n=15). Data in **C** and **D** are presented as the mean of biological replicates in a representative experiment ± SD. Data in **B** and **E** are presented as the mean ± SD, and *P* values are based on Student's *t*-test (2-sided). $^{**}P<0.01$. NS means no significant difference.

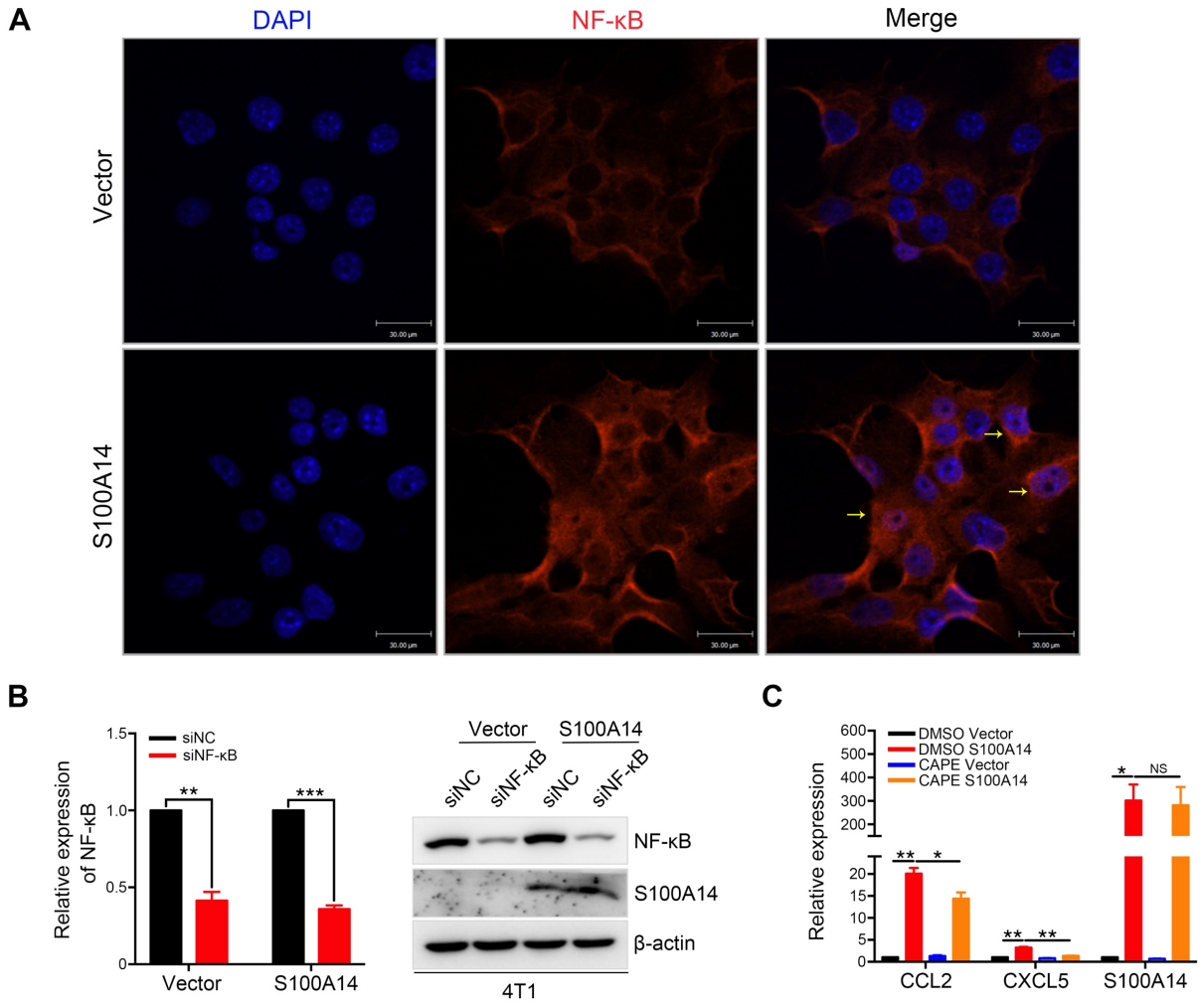


**Figure S2.** The expression of CCL2 and CXCL5 induced by S100A14 depends on NF-κB. **(A)** Immunofluorescence for detecting the distribution of NF-κB. Scale bars=30 μm. **(B)** S100A14-overexpressing 4T1 and control cells were transfected with siRNA (60 nM) for 24 h. The expression of NF-κB was detected by qRT-PCR (**Left**) and western blot (**Right**). (**C**) S100A14-overexpressing 4T1 and control cells were treated with CAPE (2 μM) and vehicle. After 48 h, the expression of S100A14, CCL2 and CXCL5 was detected by qRT-PCR. Data in **B** and **C** are presented as the mean ± SD; 2-sided t-test; **P*<0.05, ***P*<0.01, ****P*<0.001. NS means no significant difference.

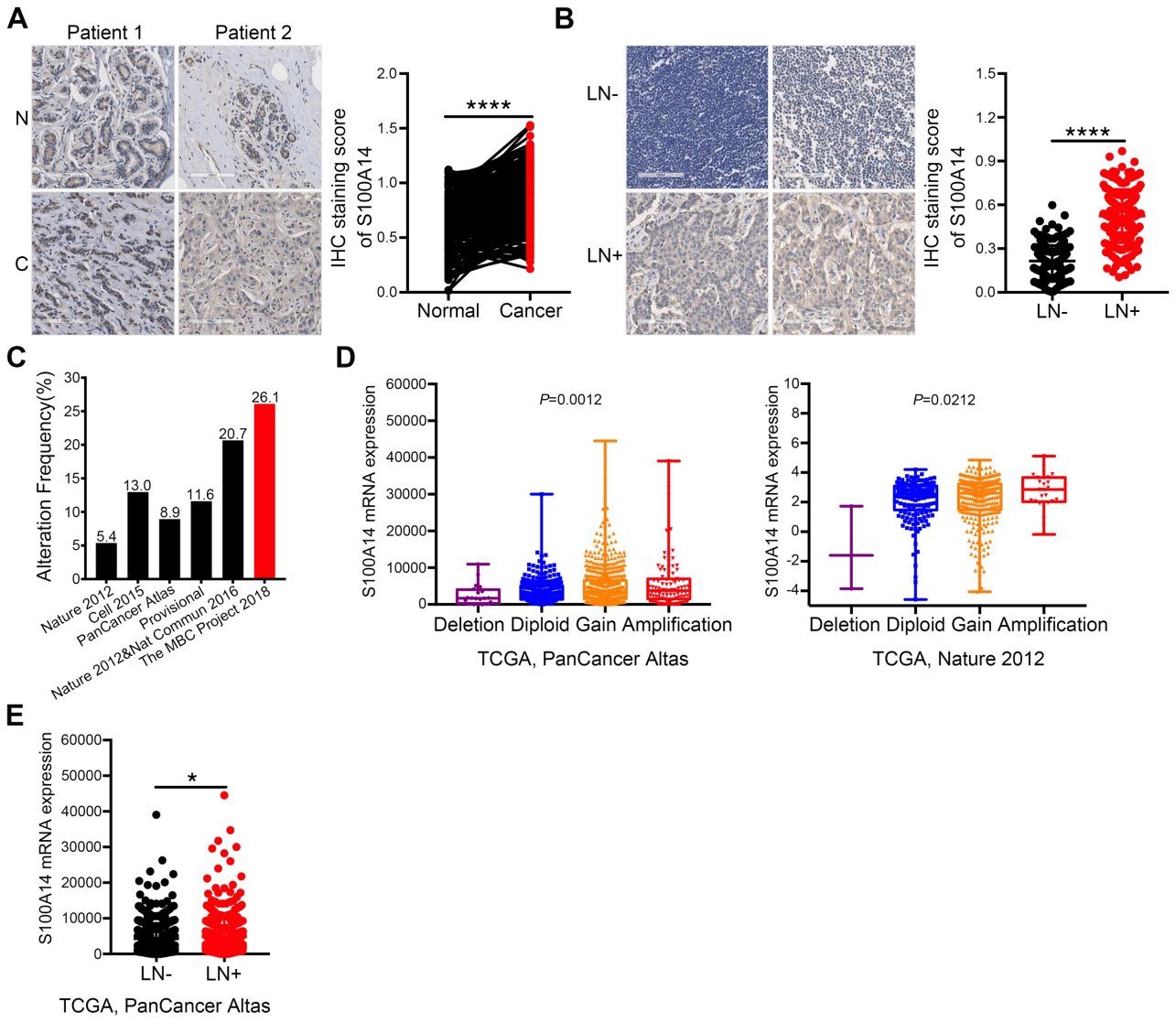


**Figure S3.** S100A14 displays copy number amplification and is overexpressed in breast cancer. **(A)** Histological analysis of S100A14 expression in the paired primary breast cancer tissues and adjacent normal tissues. Representative photographs (**left**) and statistical analyses (**right**) are shown. (**B**) Histological analysis of S100A14 expression in lymph node tissues with or without metastasis. Representative photographs (**left**) and statistical analyses (**right**) are shown. **(C)** The copy number amplification of S100A14 in breast cancer was analyzed using the TCGA dataset including METABRIC (Nature 2012), Breast Invasive Carcinoma (Cell 2015), PanCancer Atlas, Provisional, Nature 2012 & Nat Commun 2016 and The Metastatic Breast Cancer Project (Provisional, October 2018). From left to right, n = 778, 816, 2173, 1070, 1080, and 237. **(D)** The correlation between S100A14 amplification and its mRNA expression in the TCGA breast cancer dataset was analyzed

with the Kruskal-Wallis test. The mRNA expression values of S100A14 and the copy number variations were downloaded from the TCGA PanCancer Atlas and Nature 2012 dataset. **(E)** The correlation between S100A14 mRNA expression and lymph node metastasis was analyzed using the TCGA PanCancer Atlas dataset. Data in **A**, **B**, **D** and **E** are presented as the mean ± SD; paired or 2-sided t-test; **P*<0.05, *****P*<0.0001.

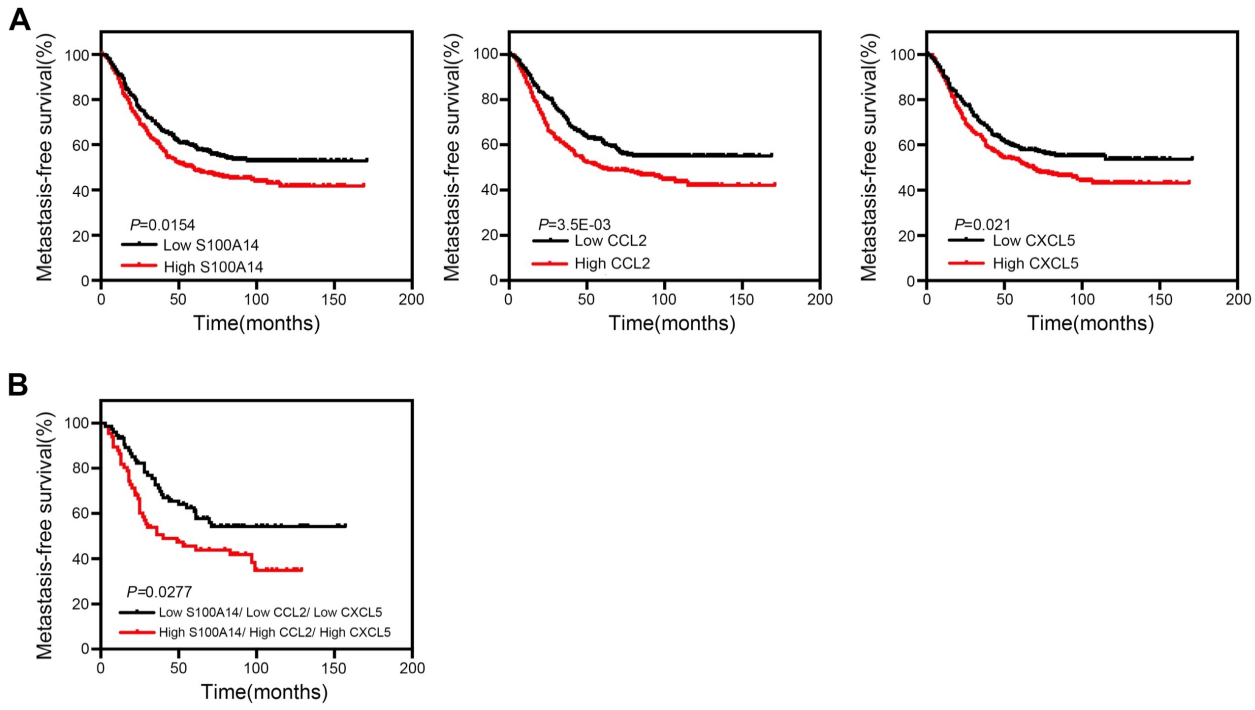


**Figure S4.** S100A14, CCL2 and CXCL5 expression can predict metastasis-free survival in breast cancer patients. Kaplan-Meier analysis for metastasis-free survival of breast cancer patients in the GEO database (GSE2034, GSE2603, GSE5327 and GSE12276). Patients were divided into two groups based on the median value for S100A14, CCL2 and CXCL5 (**A**). Patients were divided into two groups based on the median value for high S100A14 / high CCL2 / high CXCL5 and low S100A14/ low CCL2 / low CXCL5 (**B**). The log-rank test *P* values are shown.

**Table S1. Primers, sgRNAs used in this study.**

| Name | Species | Forward Primer Sequence (5' to 3') | Reverse Primer Sequence (5' to 3') | Used for |
|---|---|---|---|---|
| S100A14 | Human | TGCTCTAGAATGGGACAGTGTCGGTCAGCC | CGCGGATCCTCAGTGCCCCCGGACAGGCCT | Cloning Primer |
| S100A14 | Mouse | GGAAGATCTAGTGTACCAGGAACGATGGGA | CCGCTCGAGTTCCAAGTAGAAGTCCTCAGC | Cloning Primer |
| S100A14 -sgRNA#1 | Human | CACCGGGCTGACCGACACTGTCCCA | AAACTGGGACAGTGTCGGTCAGCCC | sgRNA |
| S100A14 -sgRNA#2 | Human | CACCGTCCCACCCTCCACGGAGTAC | AAACGTACTCCGTGGAGGGTGGGAC | sgRNA |
| S100A14 | Human | CTGACCCCTTCTGAGCTACG | TTCTCTTCCAGGCCACAGTT | RT-PCR Primer |
| β-actin | Human | AGGCACCAGGGCGTGAT | GCCCACATAGGAATCCTTCTGAC | RT-PCR Primer |
| S100A14 | Mouse | ATGGGACAGTGTCGGTCAG | CCGCCACAGAGTATTTATGGAAG | RT-PCR Primer |
| β-actin | Mouse | CATTGCTGACAGGATGCAGAAGG | TGCTGGAAGGTGGACAGTGAGG | RT-PCR Primer |
| IL1A | Mouse | CGAAGACTACAGTTCTGCCATT | GACGTTTCAGAGGTTCTCAGAG | RT-PCR Primer |
| CXCL1 | Mouse | TCCAGAGCTTGAAGGTGTTGCC | AACCAAGGGAGCTTCAGGGTCA | RT-PCR Primer |
| CXCL3 | Mouse | CCATCCAGAGCTTGACGGTGAC | GGCTCAGCTGGACTTGCCGCTC | RT-PCR Primer |
| CXCL5 | Mouse | TGCGTTGTGTTTGCTTAACCG | CTTCCACCGTAGGGCACTG | RT-PCR Primer |
| Cav1 | Mouse | GCGACCCCAAGCATCTCAA | ATGCCGTCGAAACTGTGTGT | RT-PCR Primer |
| Rab25 | Mouse | ATGGGGAATCGAACAGATGAAGA | GAGAACTCAACCCCGATGGT | RT-PCR Primer |
| Csf3 | Mouse | ATCCCGAAGGCTTCCCTGAGTG | AGGAGACCTTGGTAGAGGCAGA | RT-PCR Primer |
| PSMB8 | Mouse | GTGCAGGTTGTATTATCTTCGGA | CGAGTCCCATTGTCATCTACG | RT-PCR Primer |
| NTN1 | Mouse | GGTCGGAGGAAACCTTGTTATC | ATTCCGTAGTTGTTGGATGCC | RT-PCR Primer |
| CCL2 | Mouse | GTTGGC TCAGCCAGATGCA | AGCCTACTCATTGGGATCATCTTG | RT-PCR Primer |
| DAPK2 | Mouse | CCCAGAAGGAGTCGTTAAGTGA | GGCTTGAGATCAAAGTGAGCAAT | RT-PCR Primer |
| Mtus1 | Mouse | GCTGGGAAAACAAGCCACGAATG | AGTCTGGCTTGGAACTGCTCCT | RT-PCR Primer |
| MMP13 | Mouse | CTTCTTCTTGTTGAGCTGGACTC | CTGTGGAGGTCACTGTAGACT | RT-PCR Primer |
| Hspb1 | Mouse | GCTCACAGTGAAGACCAAGGAAG | TGAAGCACCGAGAGATGTAGCC | RT-PCR Primer |

| | | | | |
|---|---|---|---|---|
| CCL2 | Mouse | CCGATGGAGCTGCATGTATATCA | CCCTCTTTATTGGACCGAAGAGT | RT-PCR Primer for ChIP |
| CXCL5 | Mouse | GCAAAGAGCCACTCCTCCTAGCC | ACCCACTGCACCCCTTTTATCTG | RT-PCR Primer for ChIP |
| Arg1 | Mouse | CATTGGCTTGCGAGACGTAGAC | GCTGAAGGTCTCTTCCATCACC | RT-PCR Primer |
| iNOS | Mouse | GAGACAGGGAAGTCTGAAGCAC | CCAGCAGTAGTTGCTCCTCTTC | RT-PCR Primer |
| IL12 | Mouse | CAATCACGCTACCTCCTCTTTT | CAGCAGTGCAGGAATAATGTTTC | RT-PCR Primer |
| CD206 | Mouse | CTCTGTTCAGCTATTGGACGC | TGGCACTCCCAAACATAATTTGA | RT-PCR Primer |
| MRC1 | Mouse | GTTCACCTGGAGTGATGGTTCTC | AGGACATGCCAGGGTCACCTTT | RT-PCR Primer |
| MRC2 | Mouse | TACAGCTCCACGCTATGGATT | CACTCTCCCAGTTGAGGTACT | RT-PCR Primer |
| Fizz1 | Mouse | CTTGTGGCTTTGCCTGTGGA | GCAGTGGTCCAGTCAACGAG | RT-PCR Primer |
| Ym1 | Mouse | AGAAGGGAGTTTCAAACCTGGT | GTCTTGCTCATGTGTGTAAGTGA | RT-PCR Primer |
| MGL1 | Mouse | TGAGAAAGGCTTTAAGAACTGGG | GACCACCTGTAGTGATGTGGG | RT-PCR Primer |
| MGL2 | Mouse | TTAGCCAATGTGCTTAGCTGG | GGCCTCCAATTCTTGAAACCT | RT-PCR Primer |
| TNFα | Mouse | GGTGCCTATGTCTCAGCCTCTT | GCCATAGAACTGATGAGAGGGAG | RT-PCR Primer |
| TNFβ | Mouse | TGATACGCCTGAGTGGCTGTCT | CACAAGAGCAGTGAGCGCTGAA | RT-PCR Primer |
| IL6 | Mouse | TACCACTTCACAAGTCGGAGGC | CTGCAAGTGCATCATCGTTGTTC | RT-PCR Primer |
| Loxp1 | Mouse | CTTCTGTTATGAGGGTTTGCGCC | GAAGACACTCTAGAGGCCAGAATCCTG | Genotype identification |
| Loxp2 | Mouse | TCTGTAACAGGTGATGCTGAA | CTAGACTTCTCCAACGAGGC | Genotype identification |
| CMV-Cre | Mouse | ATTTGCCTGCATTACCGGTC | ATCAACGTTTTCTTTTCGG | Genotype identification |
| KO#1 | Mouse | CCTCATGTGGGTTGTATCTCCTTCAG | CCAAGTAGAAGTCCTCAGCTCCGAGT | Genotype identification |
| KO#2 | Mouse | CTTCTGTTATGAGGGTTTGCGCC | CTAGACTTCTCCAACGAGGC | Genotype identification |
| MMTV | Mouse | GGAAGCAAGTACTTCACAAGGG | GGAAAGTCACTAGGAGCAGGG | Genotype identification |
| MMTV | Mouse | CAAATGTTGCTTGTCTGGTG | GTCAGTCGAGTGCACAGTTT | Genotype identification |

**Table S2. siRNAs used in this study.**

| Name | Species | Sequence (5' to 3') | Used for |
|---|---|---|---|
| ON-TARGETplus SMARTpool | Mouse | GCUCAAGAUCUGCCGAGUA | knock-down NF-κB |
| | | GGCAUGCGAUUCCGCUAUA | |
| | | GGGAUGAGAUCUUCUUGCU | |
| | | CCAGACCGCAGUAUCCAUA | |
| ON-TARGETplus Control siRNA | Mouse | UGGUUUACAUGUCGACUAA | Negative control |
| | | UGGUUUACAUGUUGUGUGA | |
| | | UGGUUUACAUGUUUUCUGA | |
| | | UGGUUUACAUGUUUUCCUA | |

**Table S3. Antibodies used in this study.**

| Antibody | Catalogue Number | Purpose |
|---|---|---|
| S100A14 | NBP1-90000, Novus | WB IHC |
| NF-κB p65 | 8242, CST | ChIP IF WB |
| Hsp70 | EXOAB-Hsp70A-1, SBI | WB |
| CD9 | ab92726, Abcam | WB |
| CD81 | sc-166029, Santa Cruz | WB |
| TSG101 | 14497-1-AP, Proteintech | IHC |
| Lamin B1 | 66095-1-Ig, Proteintech | WB |
| β-tubulin | 66240-Ig, Proteintech | WB |
| β-actin | A5316, Sigma-Aldrich | WB |
| CCL2 | GTX60582, GeneTex | IHC |
| CXCL5 | AF254, Novus | WB |
| F4/80 | GB11027, Servicebio | IHC |