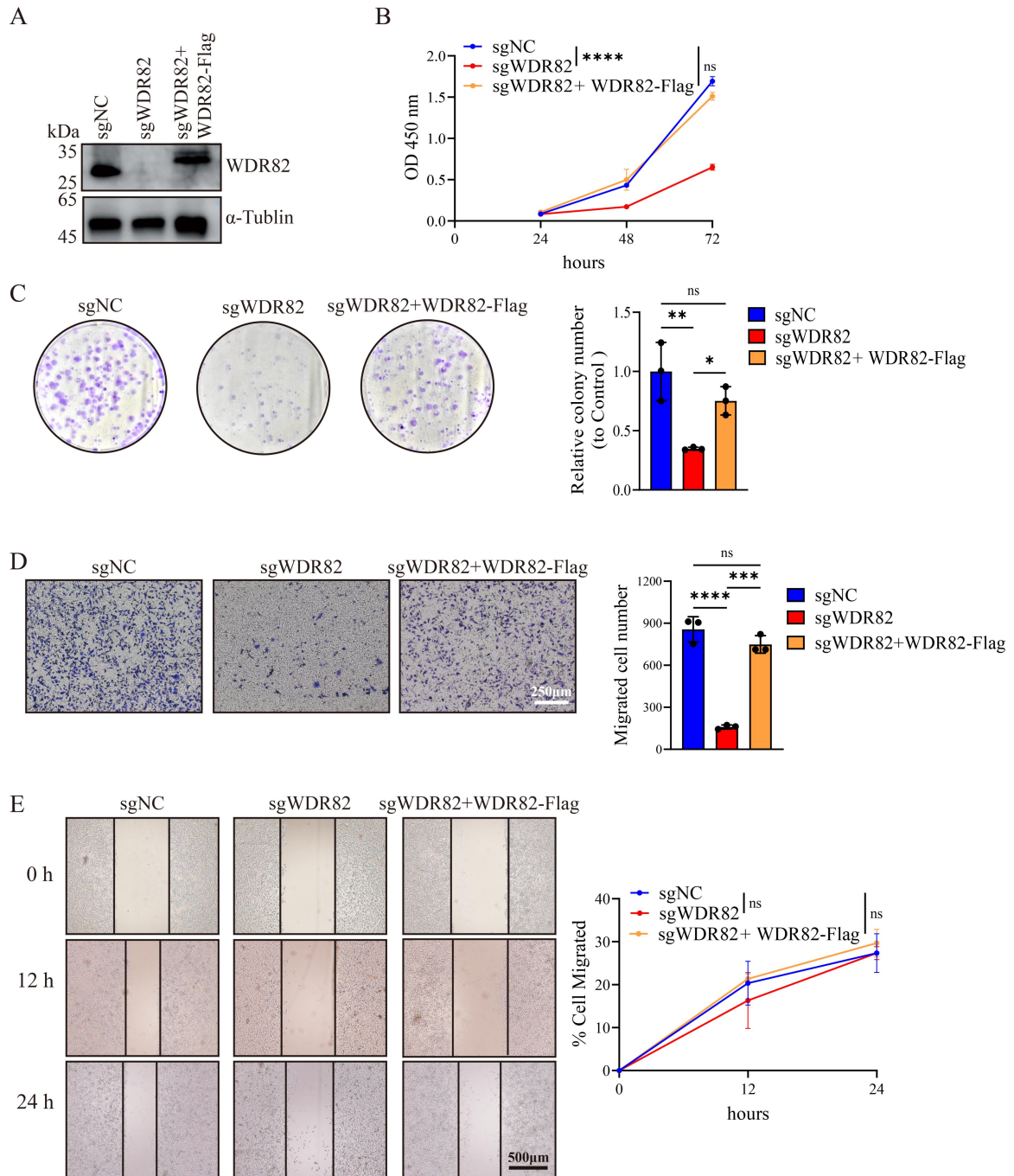


**WDR82 suppresses breast cancer progression by inhibiting ERK-driven chemokine expression and neutrophil infiltration**

Qinyi Yu, Jiang Yang, Fengjiao Lu, Wenxiu Liu, Zeyao Han, Yuanchao Bao, Xiaorui Xu, Jialei Xu, Wanfeng Gao, Boyi Cong, Yangyang Chai, Xuetao Cao\*

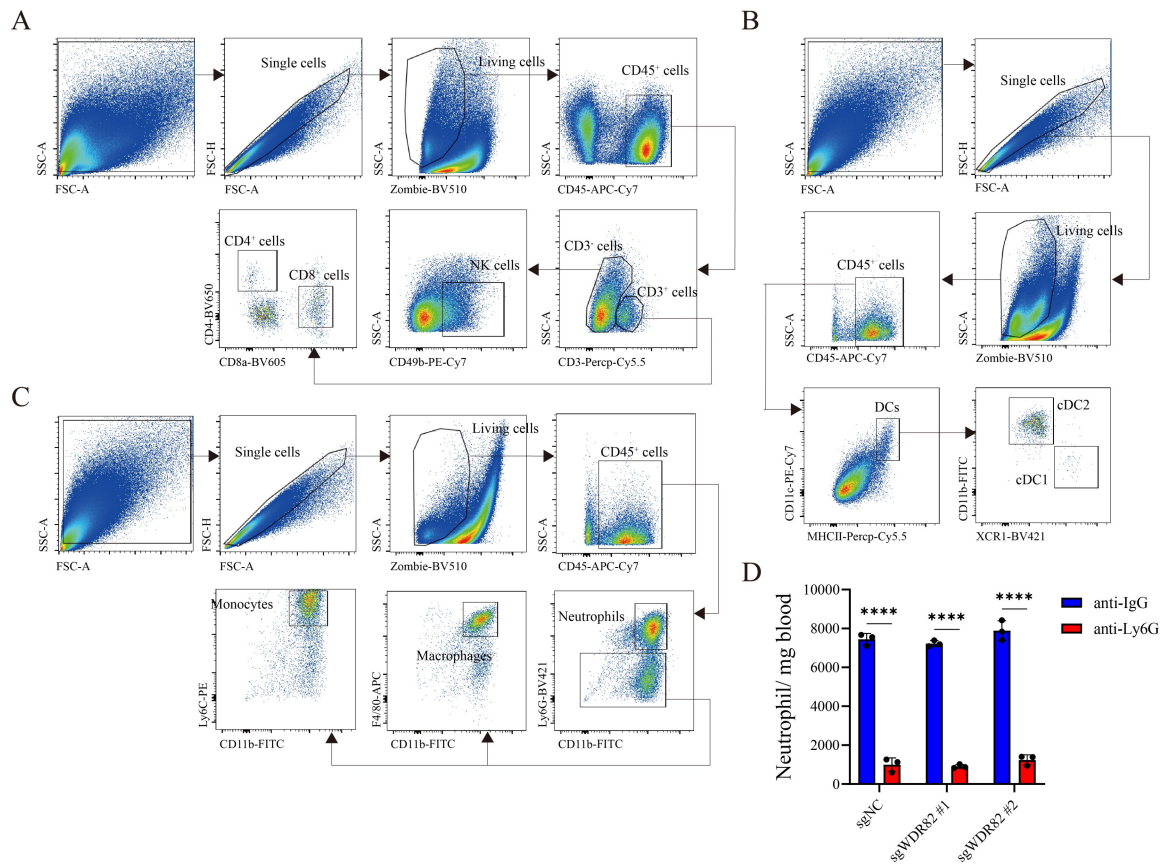
## Supplementary Figure 1-3



Supplementary Figure 1

***Wdr82* ablation in 4T1 cell inhibits cell proliferation and invasion *in vitro*.** (A) WB analysis of WDR82 expression in *Wdr82*<sup>-/-</sup> (sgWDR82), *Wdr82*<sup>-/-</sup> transfected with pcDNA3.1-WDR82-flag (sgWDR82+WDR82-Flag) or control (sgNC) 4T1 cells. The 4T1 cells transfected with empty vector were used as control. (B) CCK8 analysis of indicated cells (n=3). (C) Colony formation of indicated cells (n=3). (D) Transwell

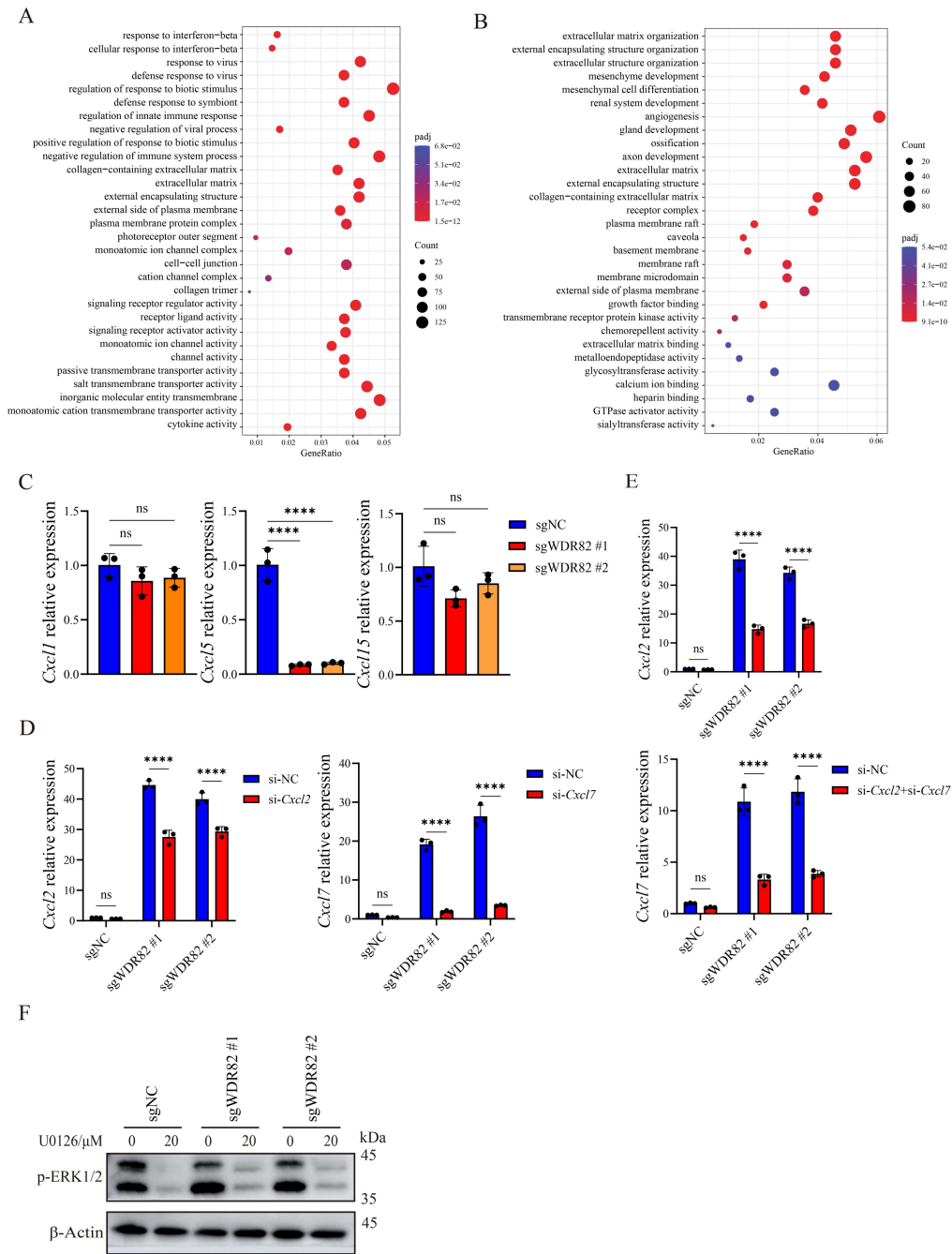
assay of indicated cells (n=3, scale bar, 250 $\mu$ m). (E) Wound healing assay of indicated cells (n=3, scale bar, 500 $\mu$ m). Data show the mean  $\pm$  SD of biological replicates or are representative graphs of three (A, C-E) independent experiments. Two-way ANOVA (B, E) or one-way ANOVA with Tukey's multiple comparison (C-D). \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001, ns, not significant.



Supplementary Figure 2

***Wdr82* loss promotes tumor progression through elevating neutrophil infiltration.**

(A-C) The gating strategy for analyzing tumor infiltrating lymphoid cells (A), DCs (B) and myeloid cells (C). (D) Number of neutrophils in peripheral blood from mice with indicated treatment (n=3). Data show the mean  $\pm$  SD of biological replicates. Two-way ANOVA (D). \*\*\*\*p<0.0001.



Supplementary Figure 3

***Wdr82* knockout promotes CXCL2/CXCL7 expression through increasing ERK activation.** (A) GO analysis showing enrichment of up-regulated genes in *Wdr82*<sup>-/-</sup> 4T1 cells from bulk RNA-seq analysis (n=3). (B) GO analysis showing enrichment of down-regulated genes in *Wdr82*<sup>-/-</sup> 4T1 cells from bulk RNA-seq analysis (n=3). (C) qRT-PCR analysis of *Cxcl1* (left), *Cxcl5* (middle) or *Cxcl15* (right) expression in *Wdr82*<sup>-/-</sup> or Control 4T1 cells (n=3). (D) qRT-PCR analysis of *Cxcl2* (left) or *Cxcl7*

(right) expression in *Wdr82*<sup>-/-</sup> or control 4T1 cells transfected with si-NC, si-*Cxcl2* or si-*Cxcl7* (n=3). (E) qRT-PCR analysis of *Cxcl2* (up) or *Cxcl7* (down) expression in *Wdr82*<sup>-/-</sup> or control 4T1 cells transfected with si-NC or si-*Cxcl2* + si-*Cxcl7* (n=3). (F) WB analysis of ERK phosphorylation in *Wdr82*<sup>-/-</sup> or control 4T1 cells pre-treated with U0126 or DMSO for 1 hour followed by EGF treatment. Data show the mean ± SD of biological replicates or are representative graphs of three (F) independent experiments. One-way ANOVA with Tukey's multiple comparison (C) or two-way ANOVA (D-E). \*\*\*\*p<0.0001, ns, not significant.

## Supplementary Table 1-2

Supplementary Table 1 sgRNAs used for *Wdr82* knockout

Gene name	Oligonucleotides
<i>Wdr82-sgRNA-1</i>	Fw: 5'-CACCAACTGCCAGGTAATGGTACC-3' Rv: 5'-AAACGGTACCATTACCTGGCAGTT-3'
<i>Wdr82-sgRNA-2</i>	Fw: 5'-CACCTAGGGCCTCATGCATCTACA-3' Rv: 5'-AAACTGTAGATGCATGAGGCCCTA-3'

Supplementary Table 2 Primers used for qRT-PCR assays

Gene name	Oligonucleotides
<i>Actb</i>	Fw: 5'-GGCTGTATTCCCCTCCATCG-3' Rv: 5'-CCAGTTGGTAACAATGCCATGT-3'
<i>Cxcl1</i>	Fw: 5'-CTGGGATTACCTCAAGAACATC-3' Rv: 5'-CAGGGTCAAGGCAAGCCTC-3'
<i>Cxcl2</i>	Fw: 5'-CCAACCACCAGGCTACAGG-3' Rv: 5'-GCGTCACACTCAAGCTCTG-3'
<i>Cxcl5</i>	Fw: 5'-TCCAGCTCGCCATTCATGC-3' Rv: 5'-TTGCGGCTATGACTGAGGAAG-3'
<i>Cxcl7</i>	Fw: 5'-CTCAGACCTACATCGTCCTGC-3' Rv: 5'-GTGGCTATCACTTCCACATCAG-3'
<i>Cxcl15</i>	Fw: 5'-CAAGGCTGGTCCATGCTCC-3' Rv: 5'-TGCTATCACTTCCTTTCTGTTGC-3'
<i>Wdr82</i>	Fw: 5'-GTGTTCCGCGAGAACTCAGAC-3' Rv: 5'-TCATAGAGCACGATGGAGTCA-3'