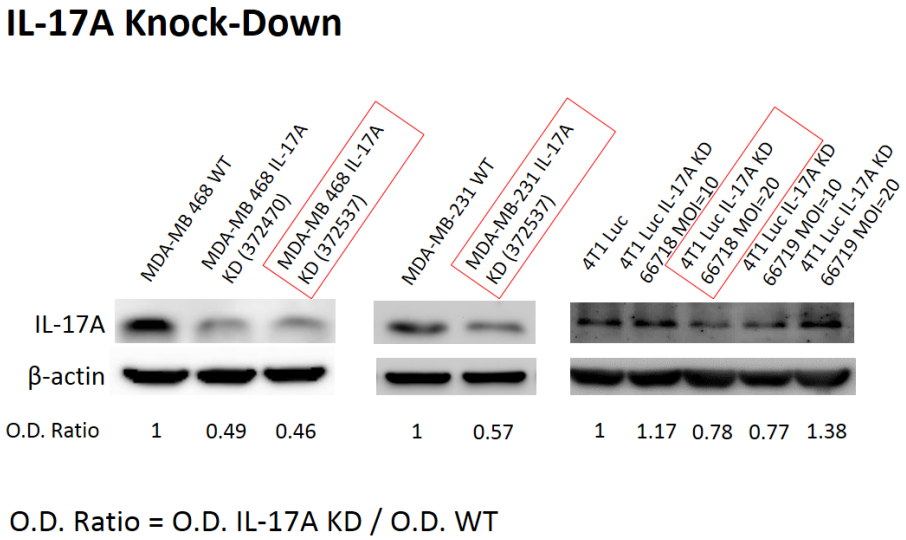
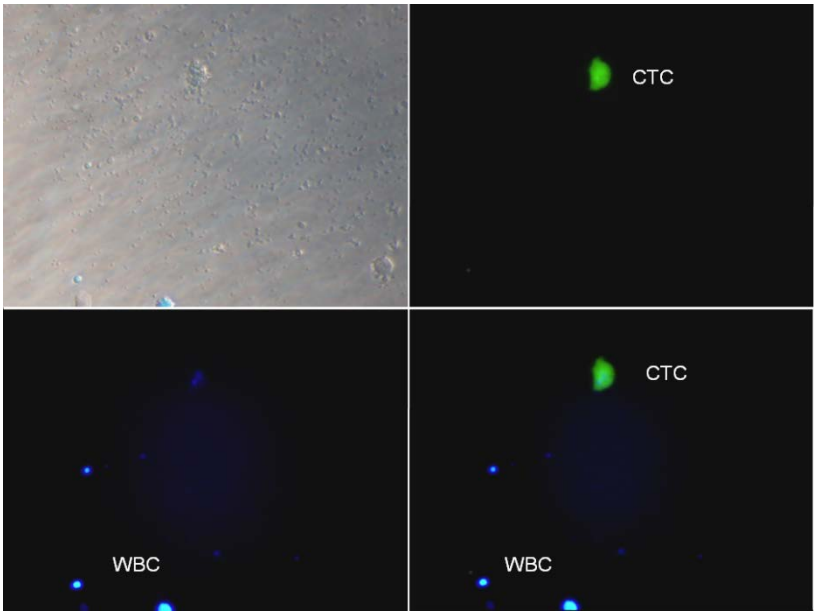
Supplementary references

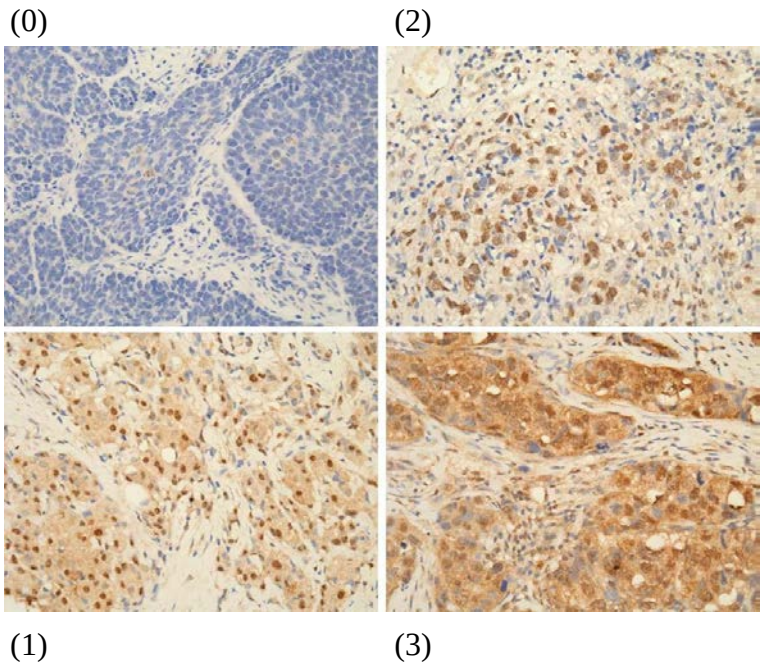
1. The knockdown efficiency of IL-17A in human and mouse cell lines.
   The lentiviral infection efficiency on human TNBC cell lines, MDA-MB-468, MDA-MB-231, and mouse 4T1 cell line was validated by Western blot analysis. Accordingly, we chose shRNA (372537) and shRNA (66718, MOI=20) to silent IL-17A gene in MDA-MD-231, MDA-MB-468, and mouse 4T1 cells, respectively.



2. The evidence confirming 4T1 circulating tumor cells.
   Since 4T1 is a mouse TNBC cell line with ER (-), PR (-), and HER2 (-), but has been characterized as a line resistant to 6-thioguanine (reference 21), we isolated CTCs from peripheral blood of 4T1-bearing mice. In short, after the anaesthetized 4T1-bearing mice had been sacrificed, blood cells were obtained and centrifuged (400 g) in Ficoll-Paque PREMIUM gradient medium. The peripheral mononuclear cells were then subjected to primary culture. After several passages, circulating 4T1 cells were selected using 6-thioguanine, which was followed by quantification using a 2-hydroxyethyl agarose colony assay. In other experiment, we inoculated 4T1-GFP cells on BALB/c mice and isolated CTCs from peripheral blood of tumor-bearing mice by 6-thioguanine selection. The results showed that GFP (+)-4T1 cells (green color) were isolated in the culture system, confirming the presence of 4T1 circulating tumor cells in mice blood.

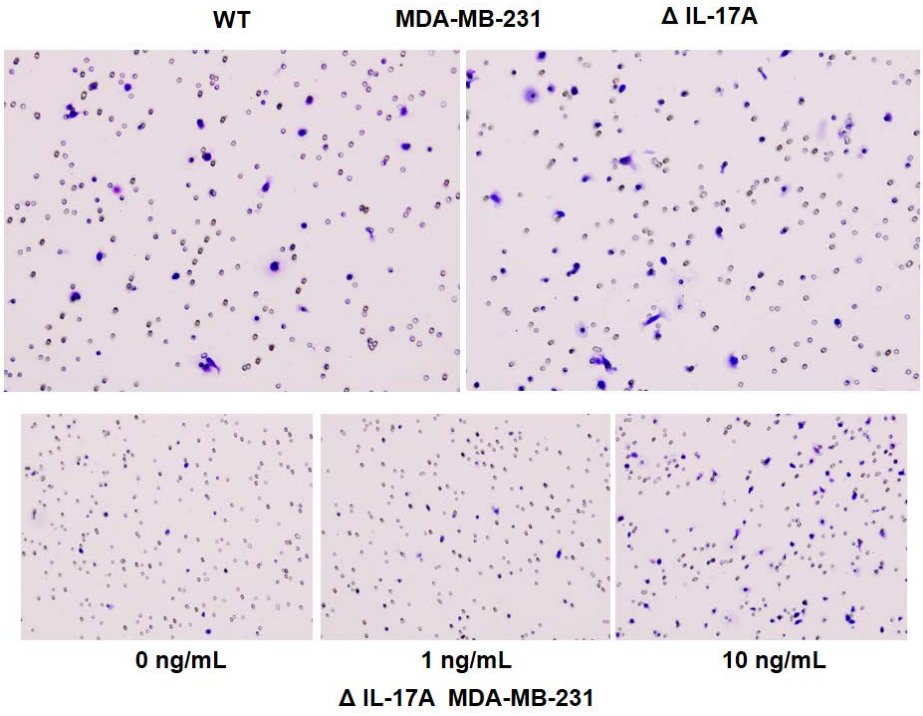


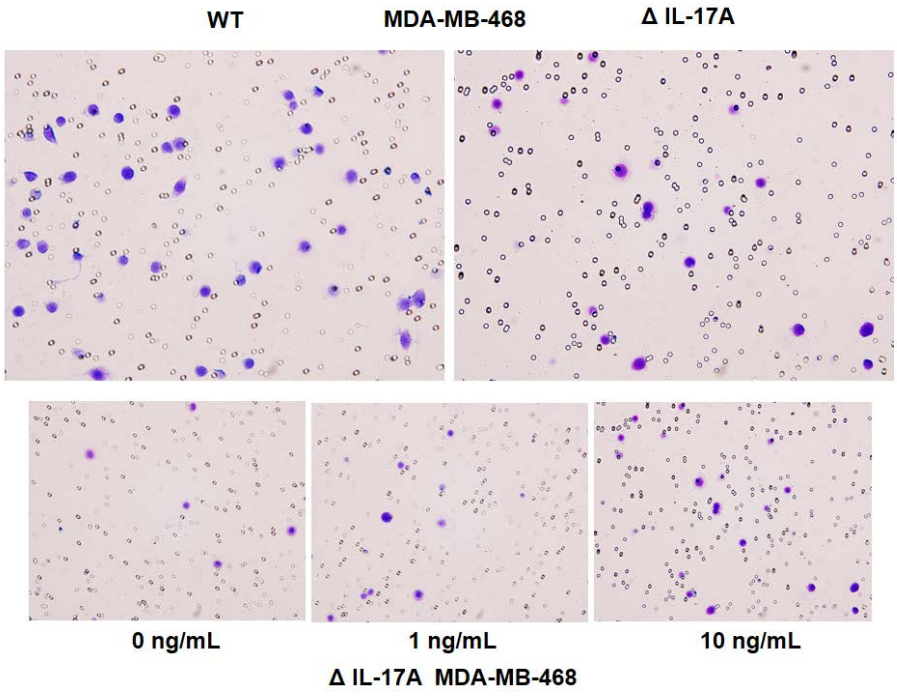
3. Protein expression of IL-17A expression in human triple negative breast cancer. The protein expression levels of IL-17A in a tissue array were assessed by immunohistochemical staining for IL-17A expression. The protein expression of IL-17A was semi-quantified and expressed as (0, left upper), <10%, (1, left lower), 11-25%, (2, right upper), 26-50%, and (3, right lower) >50% of the tumour cells examined.

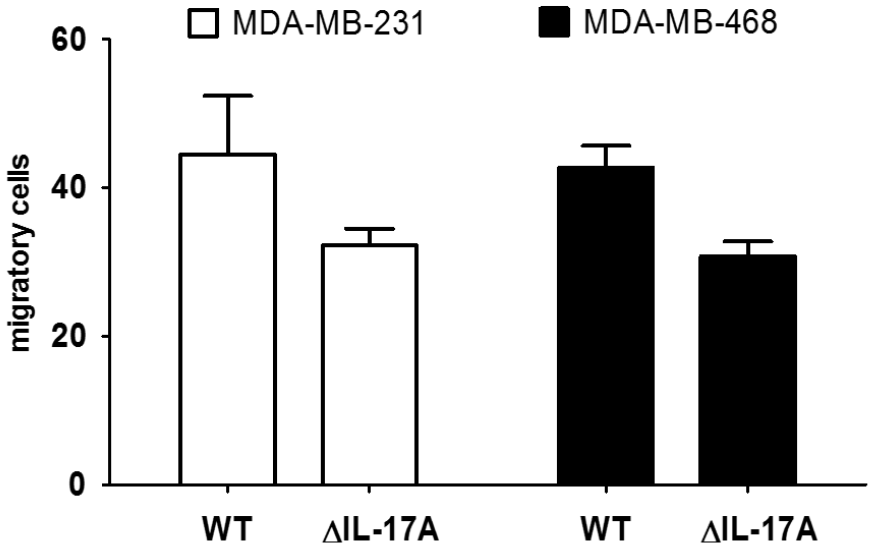


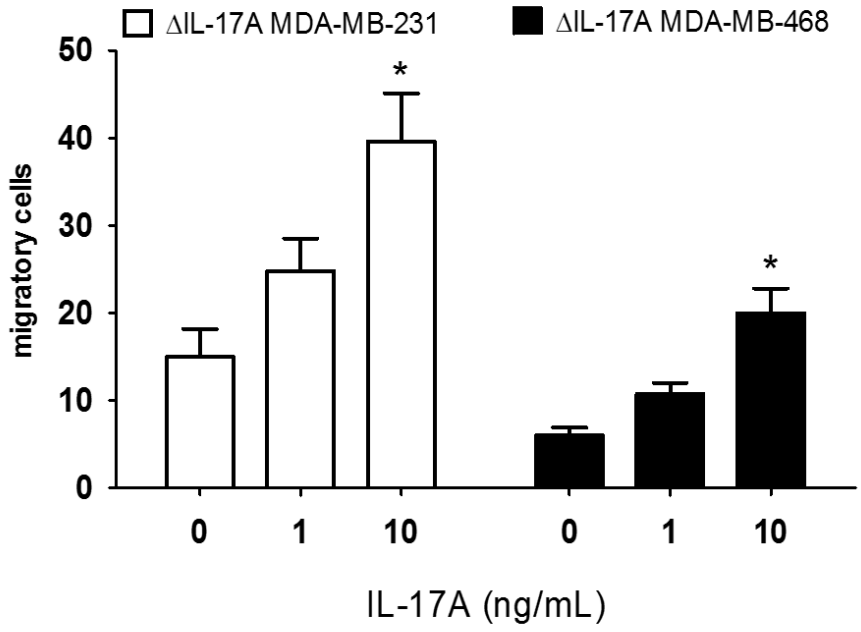
4. The role of IL-17A on migratory activity in human TNBC cell lines. After IL-17A gene was knocked down in MDA-MB-231/468 lines, the migratory activity was assessed using a trans-well system. Our results showed that there was a trend, but

no statistical significance ($p > 0.05$, Mann-Whitney U test), of decreased migratory activity in ΔIL-17A TNBC cells compared to the WT types. Besides, exogenous administration of IL-17 also increased the migratory activity in ΔIL-17A TNBC lines ($p < 0.05$, one way ANOVA with Dunnet's posthoc test). In our study, we did not knock down IL-17A receptor (IL-17AR) on TNBC lines. We attribute the fact thatΔIL-17A TNBC cells respond to IL-17A to the presence of IL-17A receptor in such cell lines.









When pretreatment of anti-IL-17A neutralizing antibody (3 μg/mL) in the culture medium of wild type MDA-MB-231 or MDA-MB-468, there is no significant difference on cell growth rate (two-way ANOVA) or migratory activity (Mann-Whitney U test) between WT and WT+Ab groups. Since there are many factors in 5% serum that might affect cell growth and migration activity of TNBC cell lines, it is reasonable to speculate that administration of anti-IL-17A antibody could

not completely blocks the effect of IL-17A (in the serum) on cell growth and migration.

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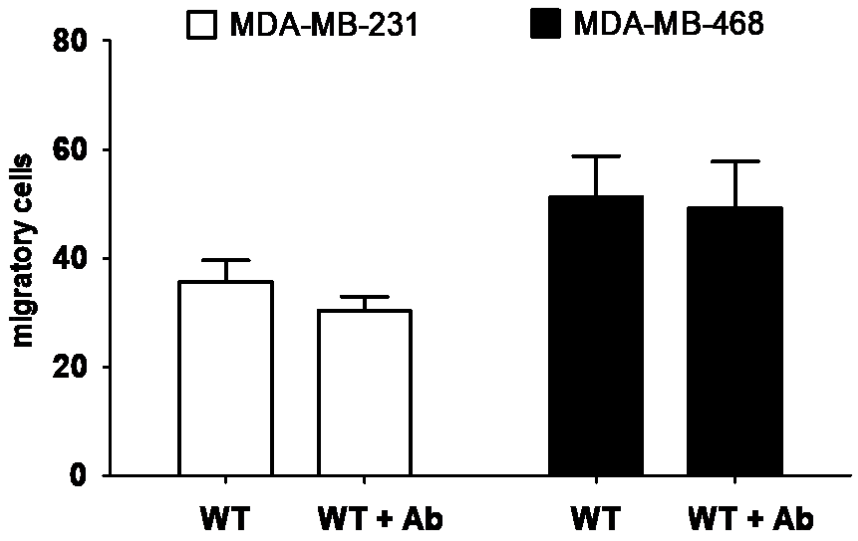
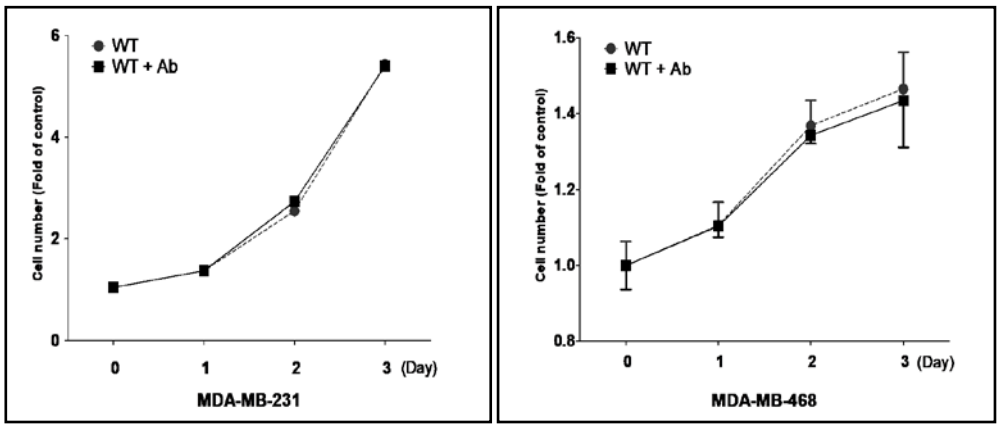
Cell migration by trans-well assay



Cell growth curve by MTT assay



5. The role of IL-17A on in vivo implanted 4T1 cells in BALB/c mice. Three groups of 4T1 –bearing BALB/c mice were designed, namely, wild type (WT), □IL-17A, and WT + neutralizing IL-17 antibody (WT+Ab). After tumor cells were implanted. Neutralizing IL-17A antibody (250 μg/100μL) was injected, twice a week, intraperitoneally in WT+Ab group. After 3 weeks after tumor cells implantation, animals were sacrificed under adequate anesthetized. Tumor weight was measured. Asterisk indicates a p value < 0.05 (Mann-Whitney U test), compared to the WT group.