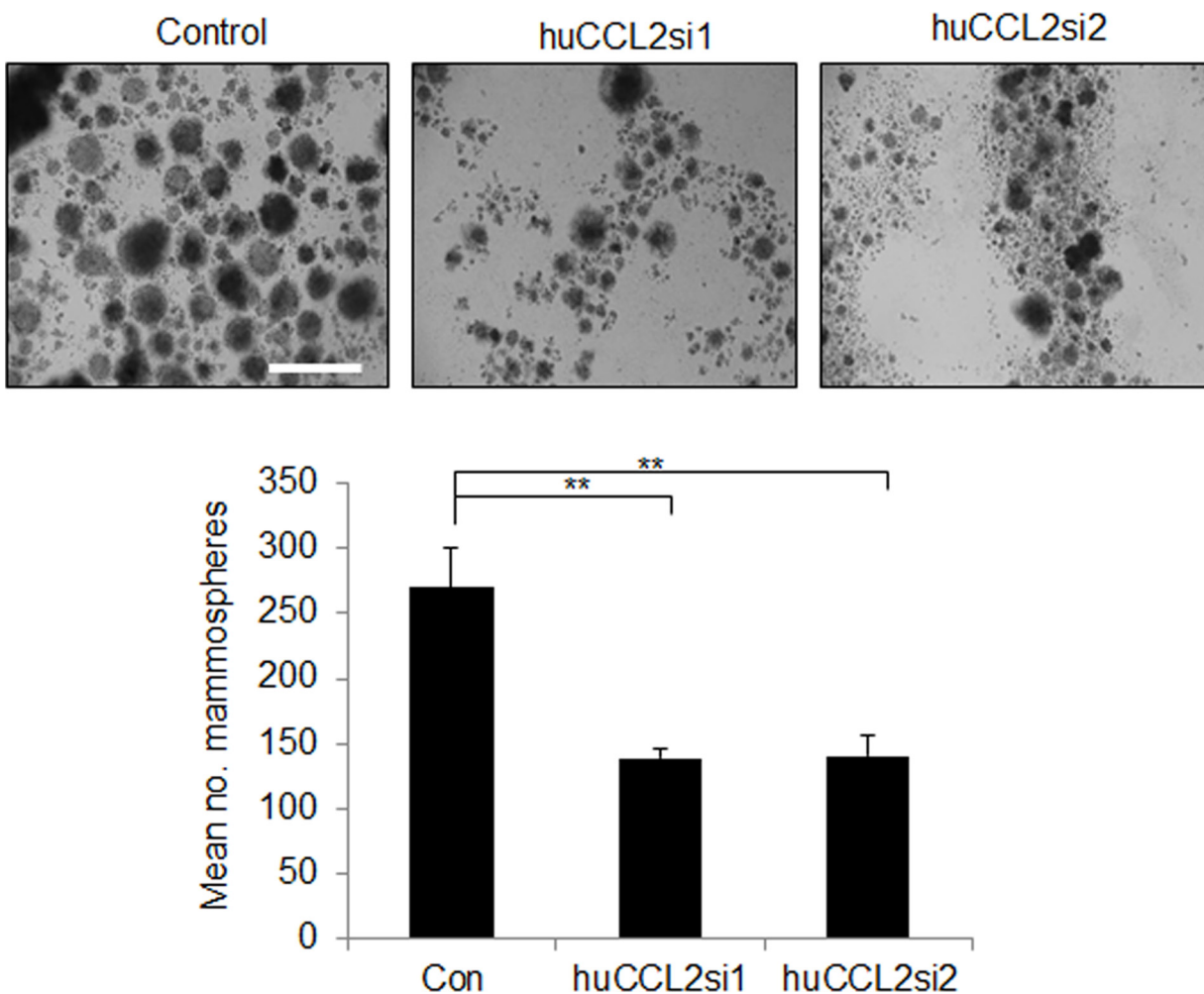
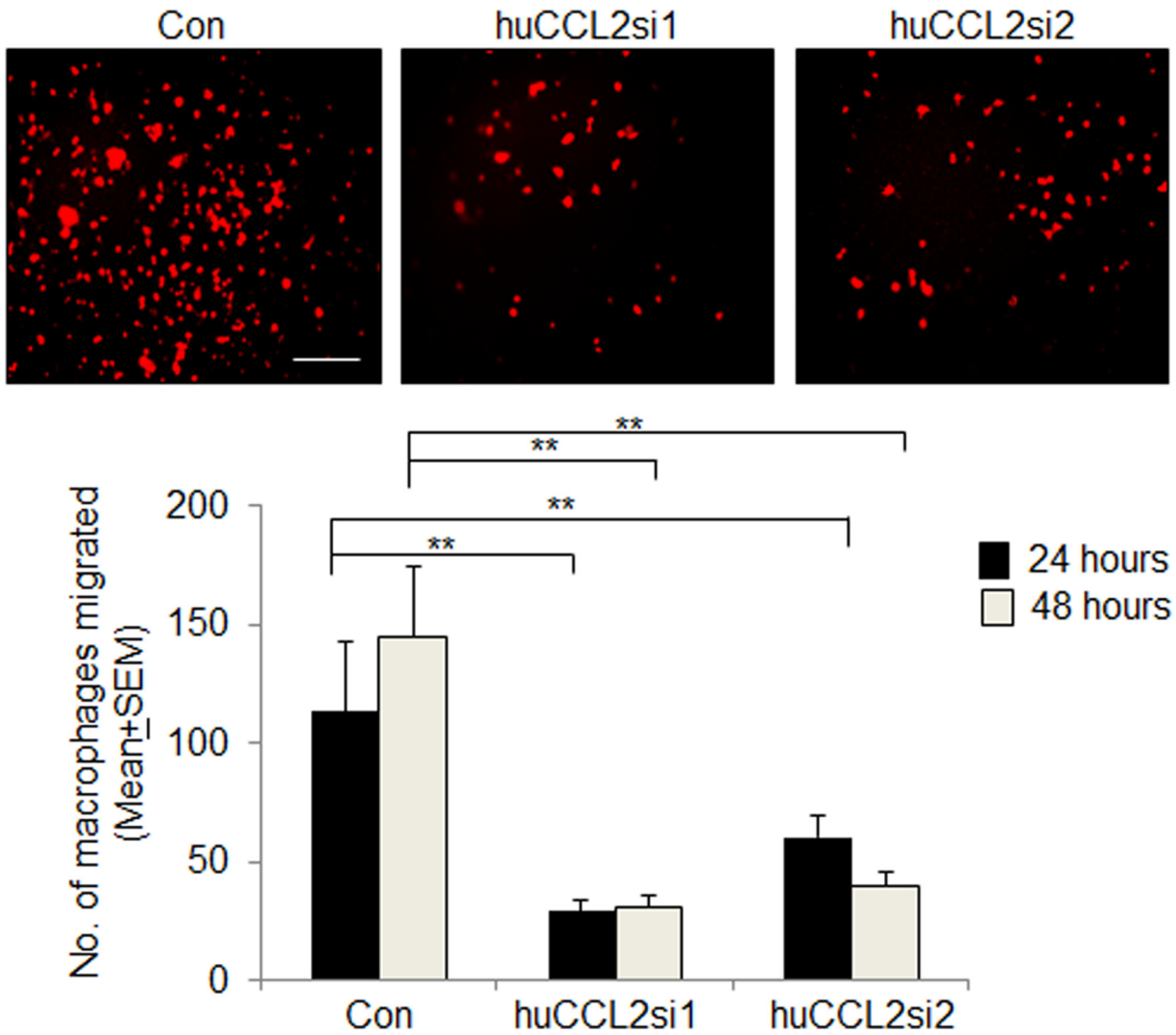


## Targeted gene silencing of CCL2 inhibits triple negative breast cancer progression by blocking cancer stem cell renewal and M2 macrophage recruitment

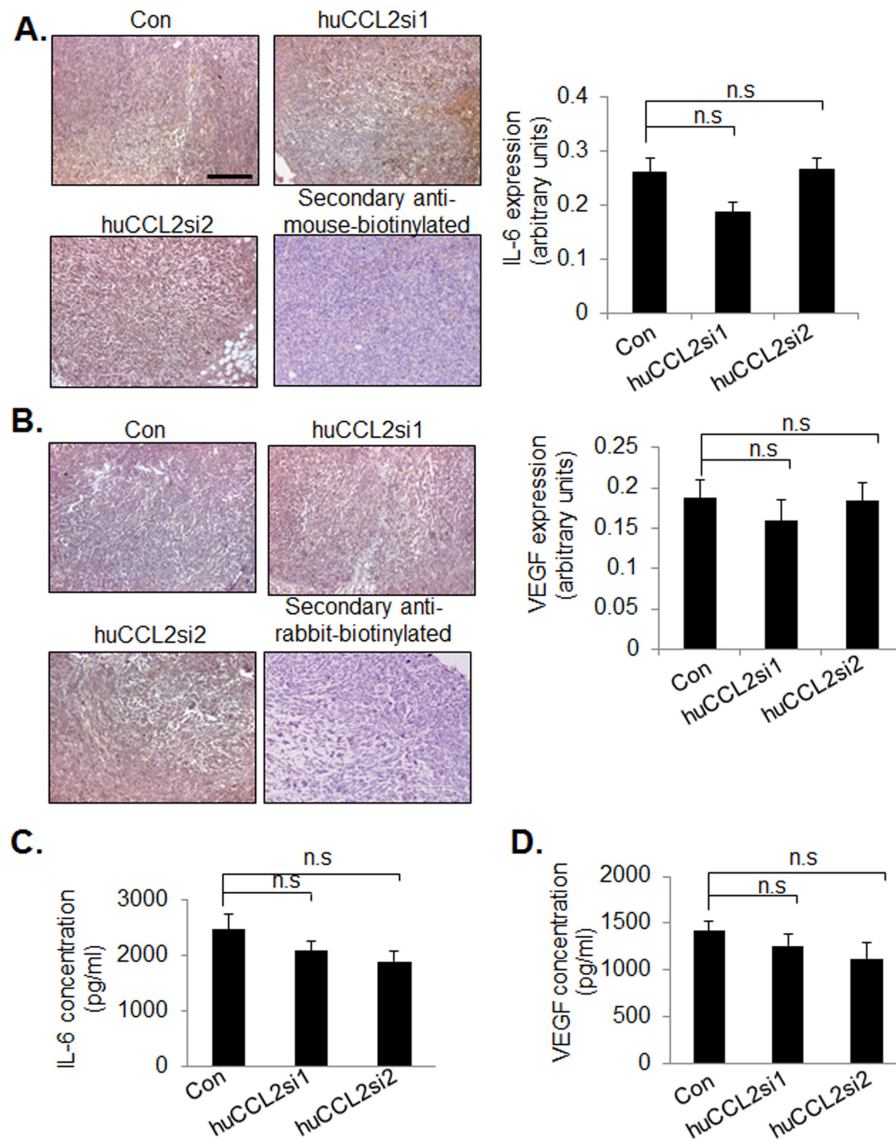
### SUPPLEMENTARY FIGURES



**Supplementary Figure S1: Ca-TAT mediated delivery of CCL2 siRNAs decreases the numbers of DCIS.com mammospheres.** DCIS.com cells were transfected with Ca-TAT/siRNA complexes and analyzed for mammosphere formation for 4 passages. Representative images of mammospheres at passage 4 are shown. Scale bar=200 microns. Mammospheres were quantified by Image J analysis. Mean number of mammospheres at passage 4 is shown. Statistical analysis was performed using One Way ANOVA with Bonferonni post-hoc analysis. Statistical significance was determined by p-value less than 0.05. \*\*p<0.01. Mean±SEM is shown.



**Supplementary Figure S2: CCL2 gene silencing in DCIS.com breast cancer cells inhibits macrophage infiltration into the MMD.** DCIS.com cells were cultured in 3D collagen in the MMDs, transfected with Ca-TAT/siRNA complexes, and analyzed for macrophage infiltration into the MMD. Scale bar=100 microns. Macrophage infiltration was quantified by Image J; arbitrary units shown. Statistical analysis was performed by One way ANOVA followed by Bonferonni post-hoc analysis. Statistical significance was determined by p-value less than 0.05. \*\*p<0.01. Mean+SEM is shown.



**Supplementary Figure S3: Ca-TAT delivery of CCL2 siRNAs does not significantly affect VEGF or IL-6 expression in MDA-MB-231 breast tumor xenografts or cells.** A-B. MDA-MB-231 breast tumor xenografts were immunostained for protein expression of IL-6 (A) or VEGF (B). Scale bar= 200 microns. Protein expression was quantified using Image J software. C-D. MDA-MB-231 breast cancer cells were embedded in 3D collagen, transfected with Ca-TAT peptides complexed to siRNAs, and analyzed for expression of IL-6 (C) or VEGF (D) in conditioned media by ELISA. Statistical analysis was performed by One way ANOVA followed by Bonferonni post-hoc analysis. Statistical significance was determined by p-value less than 0.05. n.s.=not significant. Mean±SEM is shown.