Article Title: Ca-TAT mediated gene silencing of CCL2 enhances autophagy and necrosis of luminal B breast cancer cells

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Supplemental Figure legends

Supplemental Fig. 1 Carcinoma cells comprise the bulk of MMTV-PyVmT mammary tumors**.** MMTV-PyVmT mammary tumors were digested into single cell suspensions, immunostained for CD24 (epithelial marker), Fsp1 (fibroblast marker), or Cd11b (macrophage marker), and analyzed by flow cytometry. N=4**.** Values are shown as Mean±SEM

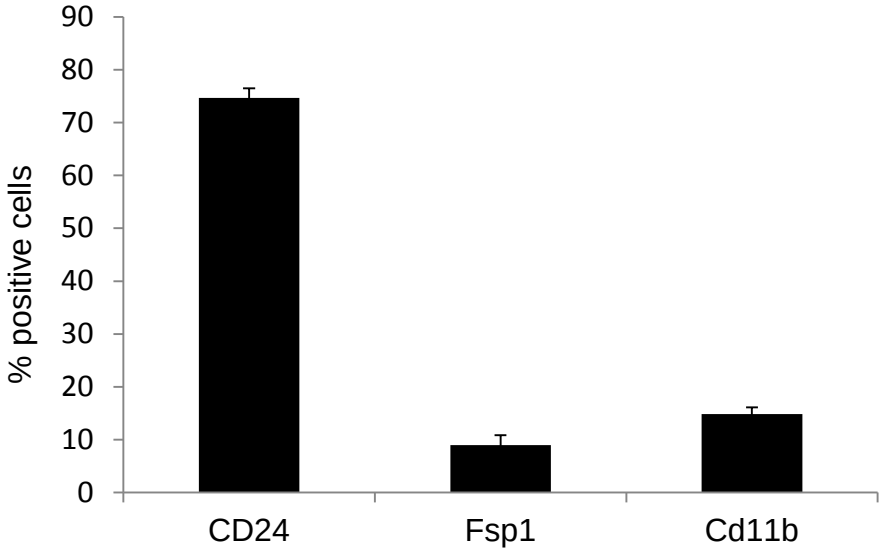
Supplemental Fig. 2 CCL2 knockdown inhibits growth of PyVmT mammary carcinoma cells. PyVmT mammary carcinoma cells were transfected with Ca-TAT complexed to control siRNAs (Con) or murine CCL2 siRNAs (mCCL2si) for up to 48 hours. Parental cells (Par) or transfected cells were counted at 24 and 48 hours post-transfection. Statistical analysis was performed by One way ANOVA followed by Bonferonni post-hoc comparisons. Statistical significance was determined by p- value < 0.05. *p<0.001. Values are shown as Mean±SEM

Supplemental Fig. 3 Ca-TAT delivery of CCL2 siRNAs does not affect growth and viability of fibroblasts and macrophages. Raw 264.7 macrophages (Mϕ) or mammary fibroblasts (Fib) were transfected with Ca-TAT complexed to control siRNAs (Con) or murine CCL2 siRNAs (mCCL2si). Parental (Par) or transfected cells were analyzed for the following: **a** Cell proliferation by immunocytochemistry staining for Ki67 expression. **b** Cell viability by trypan blue staining. Statistical analysis was performed by One way ANOVA followed by Bonferonni post-hoc comparisons. Statistical significance was determined by p- value < 0.05. ****p>0.05. Values are shown as Mean±SEM

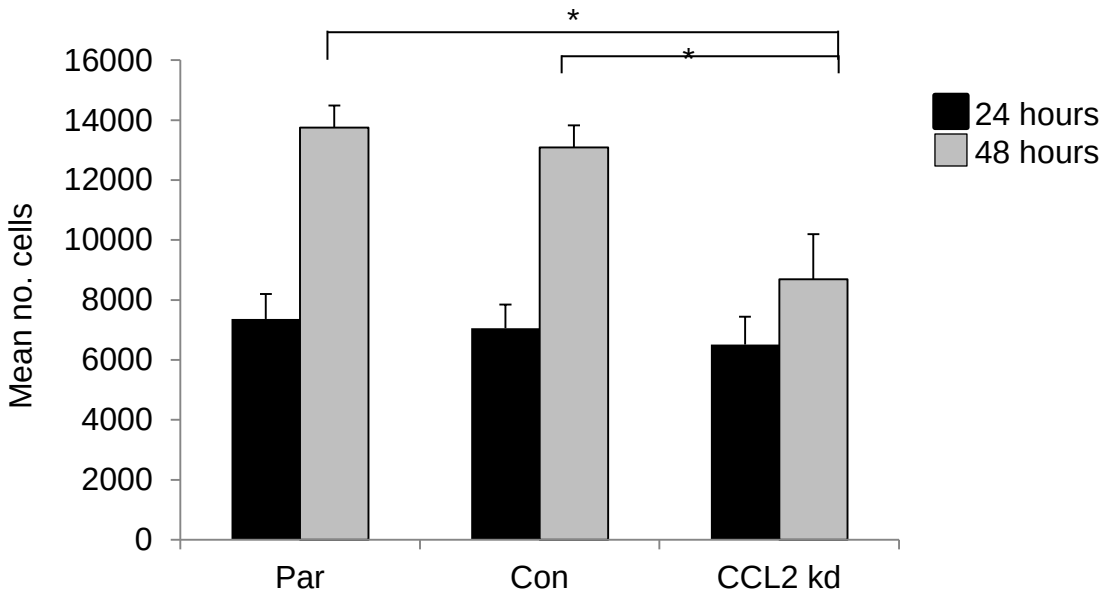
Supplemental Fig. 4 Design of siRNAs to target human CCL2 gene expression. Two siRNA sequences (huCCL2si1, huCCL2si2) target non-overlapping sequences of human CCL2 RNA

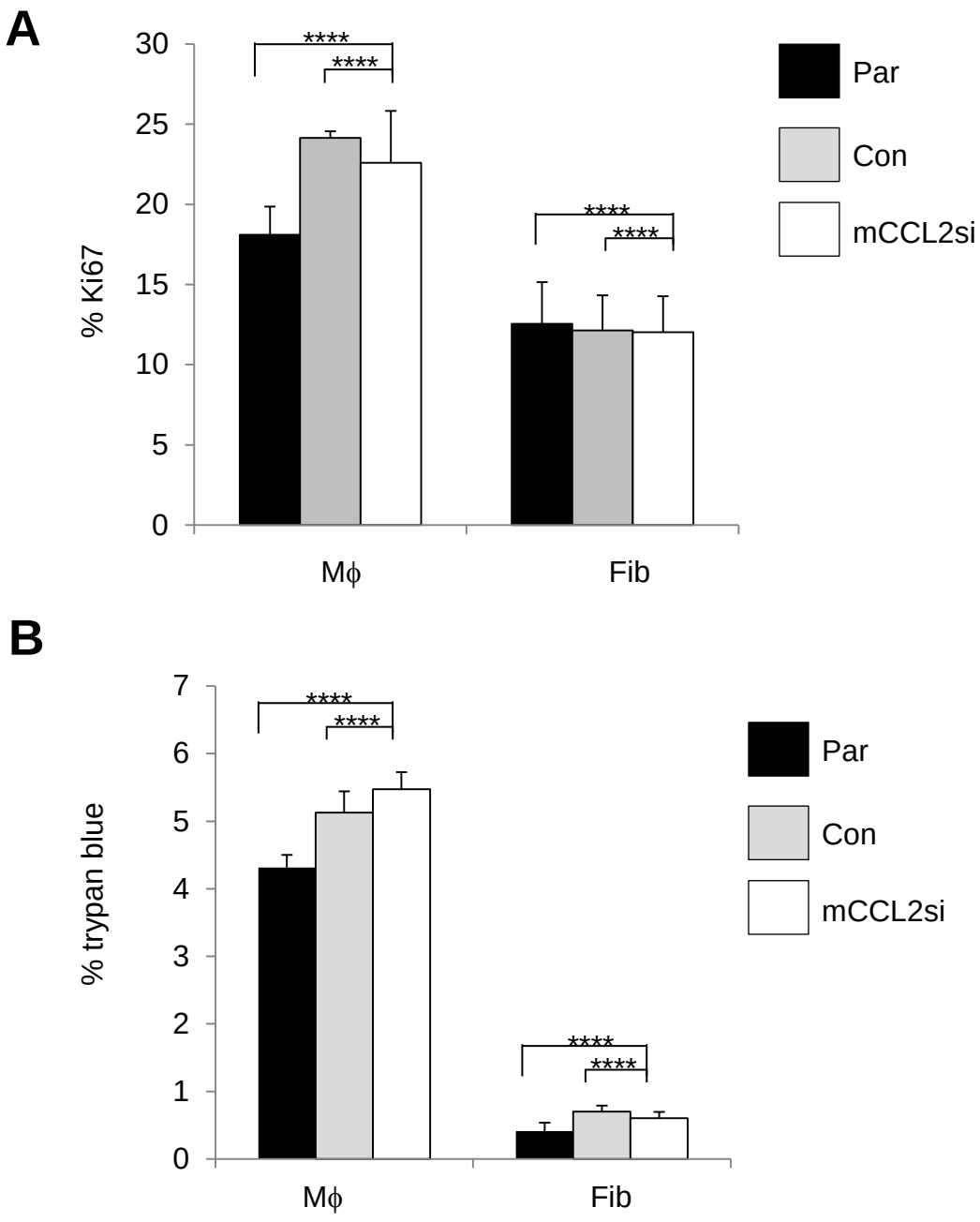
Supplemental Fig. 5 CCL2 knockdown inhibits growth of BT474 breast cancer cells. BT474 breast cancer cells were transfected with Ca-TAT complexed to control siRNAs (Con) or CCL2 siRNAs (huCCL2si1 or huCCL2si2) for up to 48 hours. Parental cells (Par) or transfected cells were counted at 24 and 48 hours post-transfection. Statistical analysis was performed by One way ANOVA followed by Bonferonni post-hoc comparisons. Statistical significance was determined by p- value < 0.05. *p<0.001. Values are shown as Mean$\pm$SEM

Supplemental Fig. 1



Supplemental Fig. 2

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Par
Con
mCCL2si
Mϕ
Fib

Position 5’ **Position 3’**

90- CCCCAGUCA**CCUGCUGUUAUAACUUCA**CCAAU **-120**

huCCL2si1

160- GAAUCACCAG**CAGCAAGUGUCCCAAAGAA**GCU **-190**

huCCL2si2

Supplemental Fig. 5