61392-RG-2 (supplementary data)

# RhoGDI2 suppresses metastasis via reduction of versican expression and macrophage infiltration in murine models

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**Running Title:** RhoGDI2 Suppresses Bladder Cancer Metastasis

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## SUPPLEMENTARY FIGURES

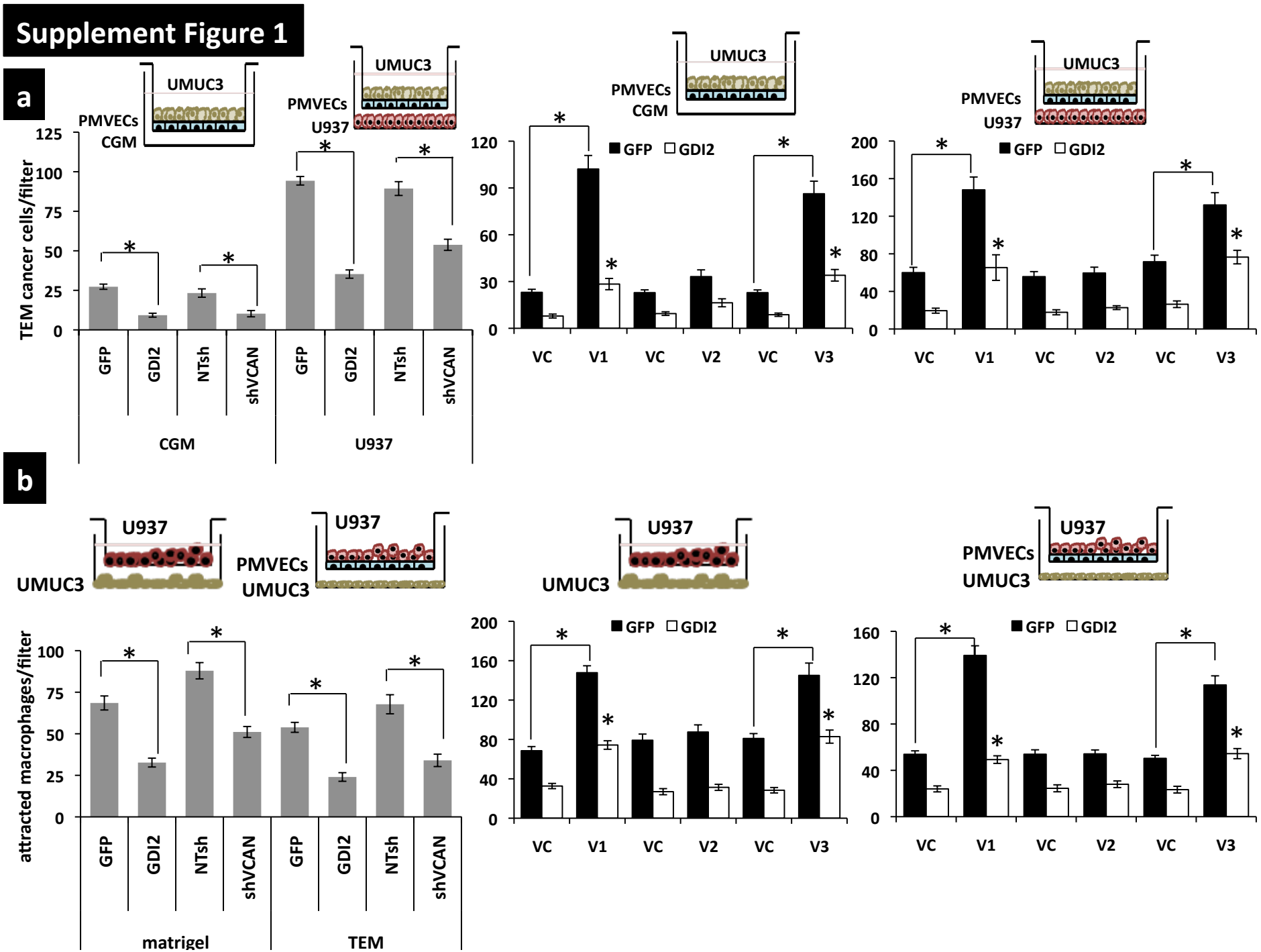
**Supplement Figure 1. a.** Engineered UMUC3 cells in **Figure 3** were allowed to invade through 8-μm pore polycarbonate inserts coated with either matrigel, or confluent monolayers of primary human pulmonary microvascular endothelial cell (PMVECs) towards complete growth medium (CGM) or U937 in the bottom chamber. **b.** Monolayers of transfected/transduced cancer cells allowed to attract U937 cells through 3-μm pore inserts coated with either matrigel or confluent monolayers of PMVECs. Invading/attracted cells on the undersurface of the filters were counted. Bars represent mean ± SEM of three independent experiments performed in triplicates.*$P$<0.05, Students'$t$-test.

**Supplement Figure 2. RhoGDI2 and versican expression in T24/T24 cell lines. a.** Western blots (WB) showing the expression of endogenous Rho-GDI2 protein in the two isogenic cell lines T24 and T24T. **b.** WB showing the expression of V1 and V3 versican in T24T and T24 cells were transduced with lentiviruses shRNA targeting all VCAN isoforms (shVCAN), an irrelevant target (non-target, NTsh) or **c.** retroviruses overexpressing V1, and V3 and their empty vector controls (VC), respectively. Equal protein loading was confirmed by tubulin (tub).

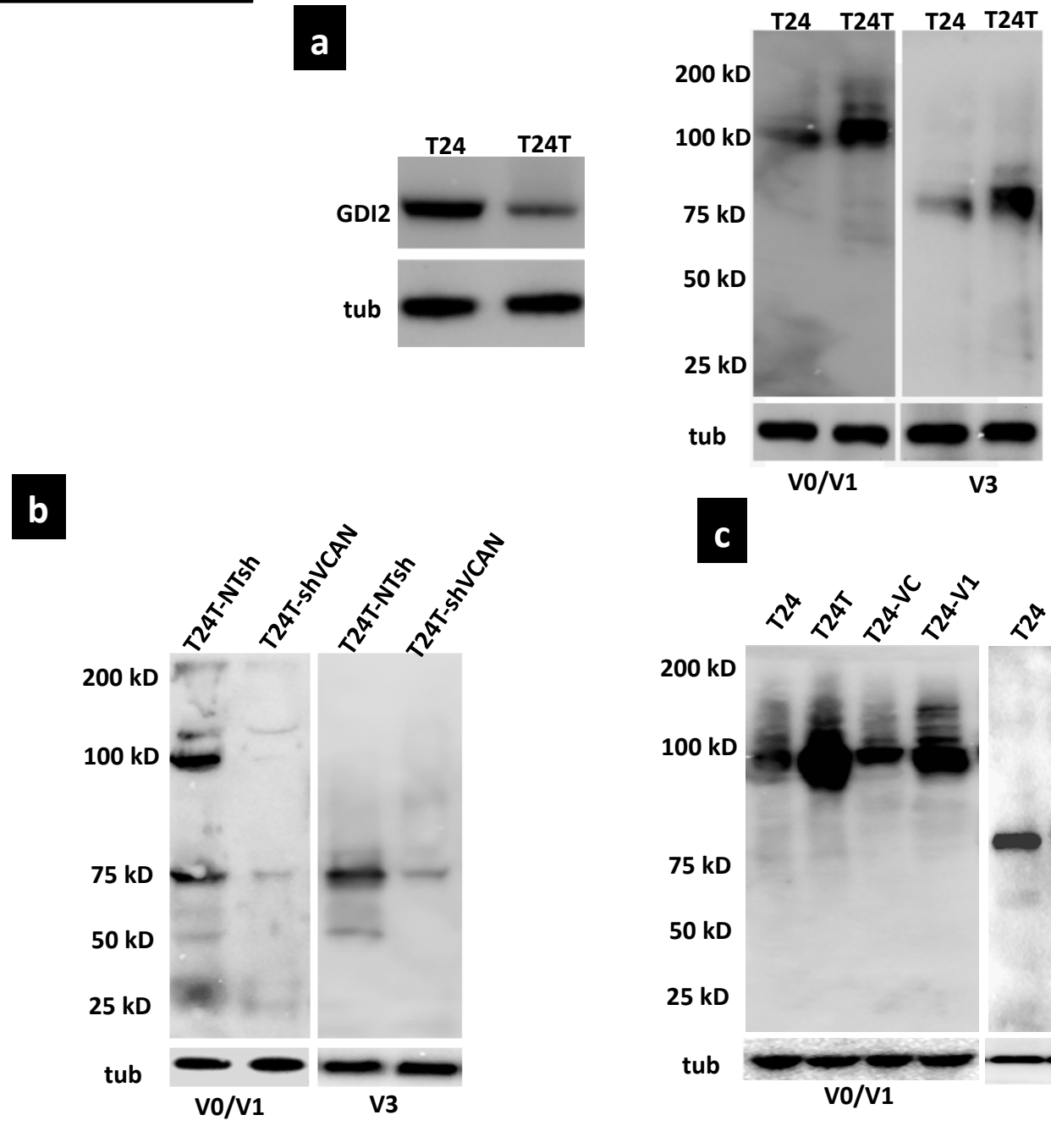
**Supplement Figure 3. GDI2 modulates cancer cell-macrophage interactions through VCAN in T24/T24T isogenic cell lines.** effect of depletion of versican in T24T (upper) and overexpression of V1 and V3 in T24 cells (lower) on their ability to: **a.** invade through 8-μm pore polycarbonate inserts coated with matrigel towards complete growth medium (CGM, lower left) or U937 lower right) in the bottom chamber. **b.** invade through 8-μm pore polycarbonate inserts coated with PMVECs towards complete growth medium (CGM, lower left) or U937 (lower right) in the bottom chamber. **c.** monolayers of T24T and T24 depleted of (upper) or overexpressing (lower) VCAN were allowed to attract U937 cells through 3-μm pore inserts coated with either matrigel or confluent monolayers of PMVECs. Invading/attracted cells on the undersurface of the filters were counted. Bars represent mean ± SEM of three independent experiments performed in triplicates.*$P$<0.05, Students'$t$-test.

**Supplement Figure 4. Depletion of VCAN in T24T cells abrogates their metastatic capability. a.** Photos of lung metastasis (circled in yellow) and scatter plot of the incidence and number of visible metastases (mets) 6W post-injection of T24T-NTsh and T24T-shVCAN cells developing after tail vein injection of $1x10^6$ cancer cells in nude mice. *$P$<0.05, $\chi^2$ test; comparing the incidence, and **$P$<0.01, Student's $t$ test, comparing the number of mets. **b.** WB of the expression of VCAN isoforms in representative lung lysates (n=2) 6W post-injection of cancer cells **c.** Macrophages infiltration in metastatic foci (circled in yellow) and surrounding lung parenchyma was determined by mac2 immunostaining. Bars represent the mean±SEM of macrophage number, counted in 6 random high power fields (HPF); **$P$<0.01, Student's $t$-test. **d-e.** Human IL-6, and CCL2 (hIL-6**,** hCCL2), murine IL-6, and CCL2 (mIL-6, mCCL2), and Cox2 activity were determined in lungs' lysates. Bars represent mean±SEM, (n=3, performed in duplicates). *$P$<0.05, **$P$<0.01 Student's $t$-test.

# Supplement Figure 1

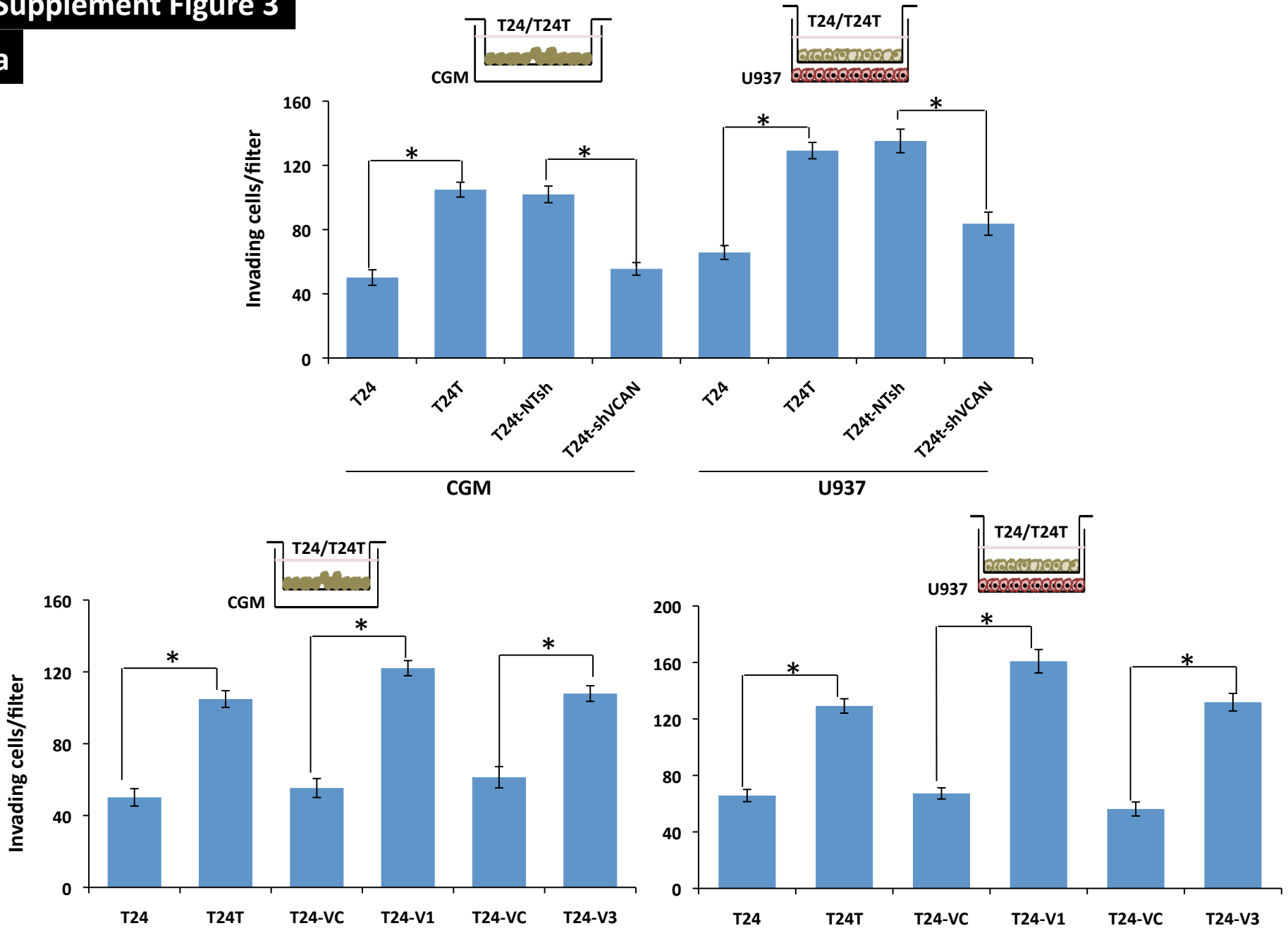


**Supplement Figure 2**



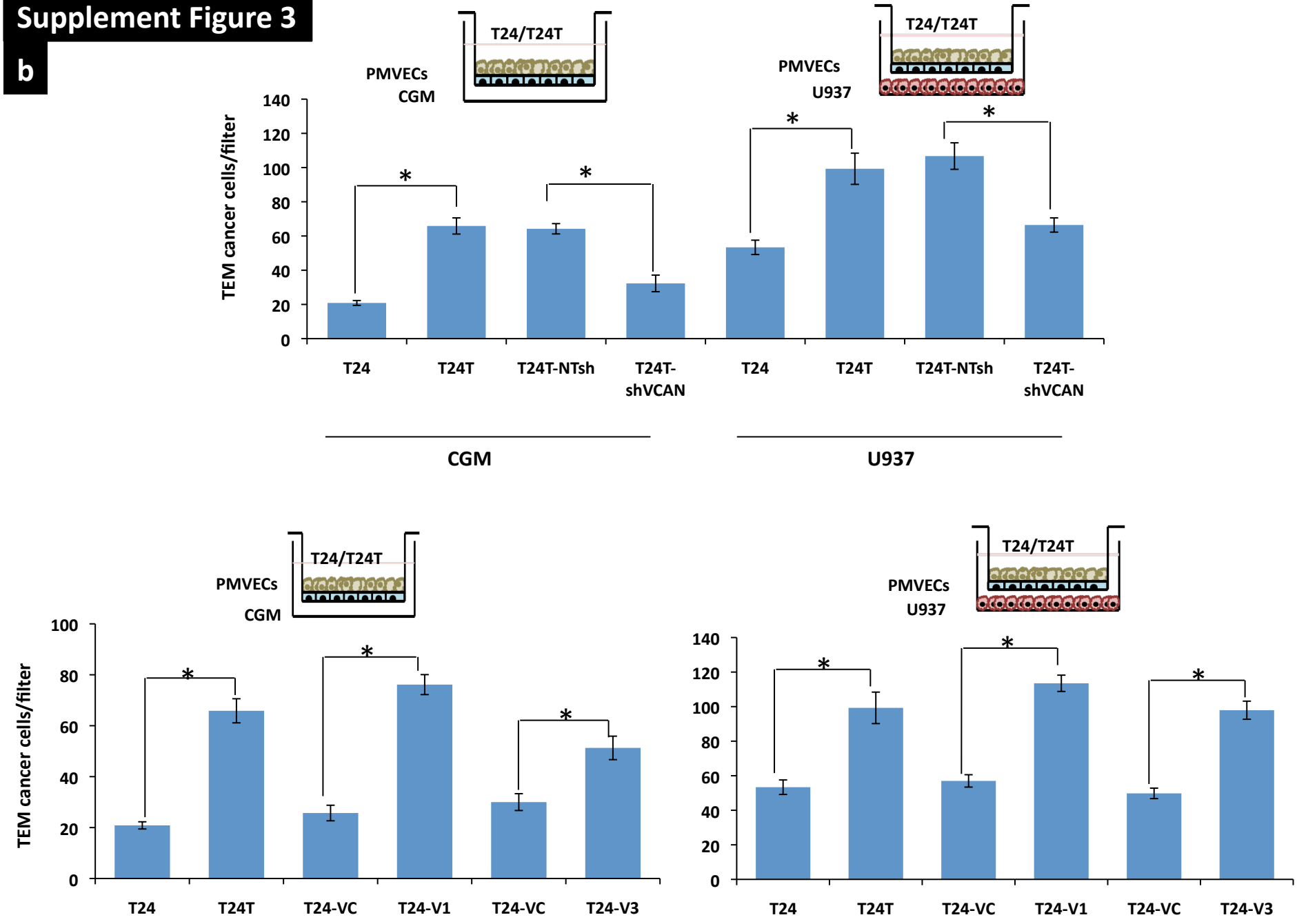
**Supplement Figure 3**

**a**



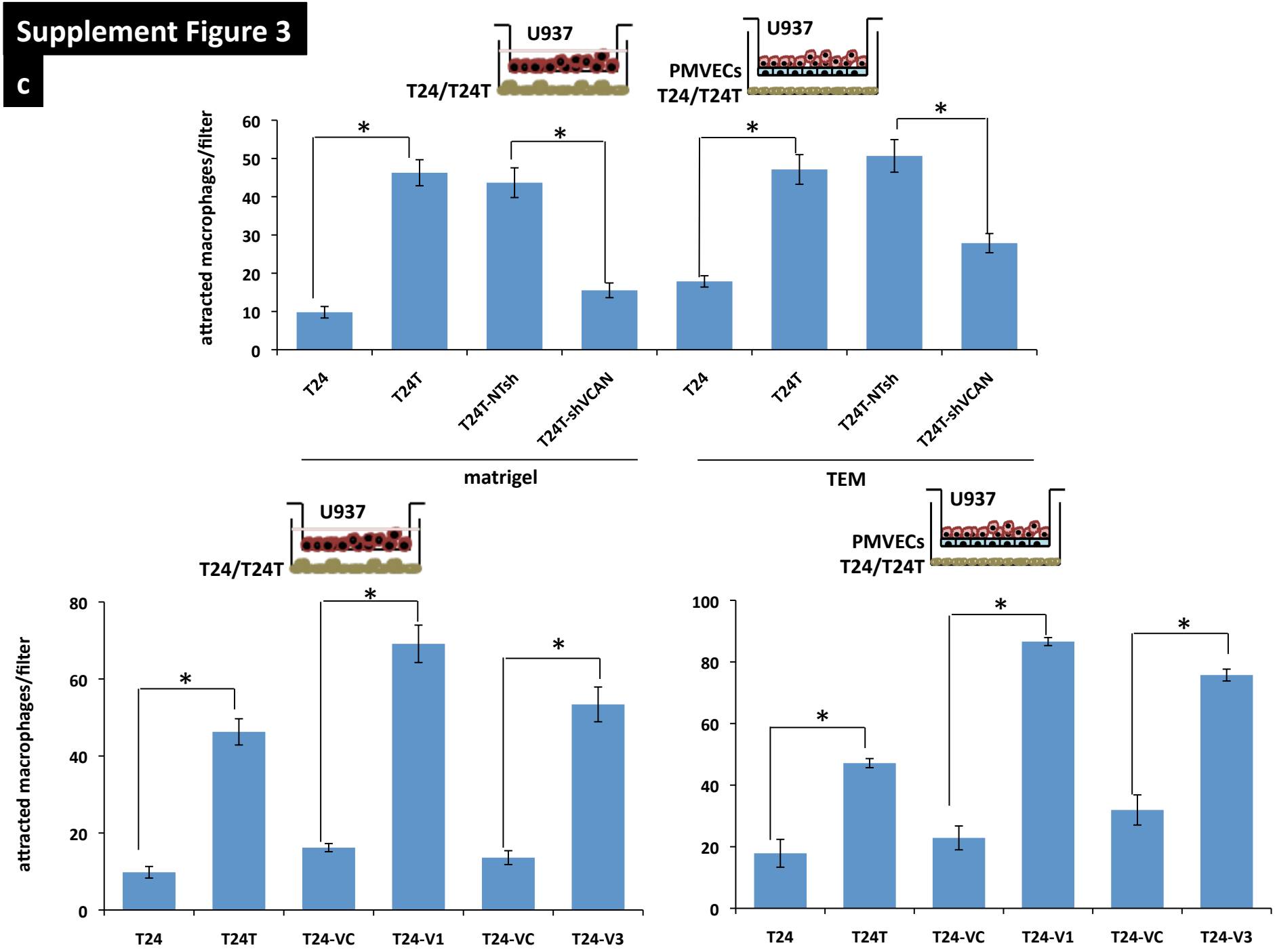
**Supplement Figure 3**

**b**



**Supplement Figure 3**

**c**



# Supplement Figure 4