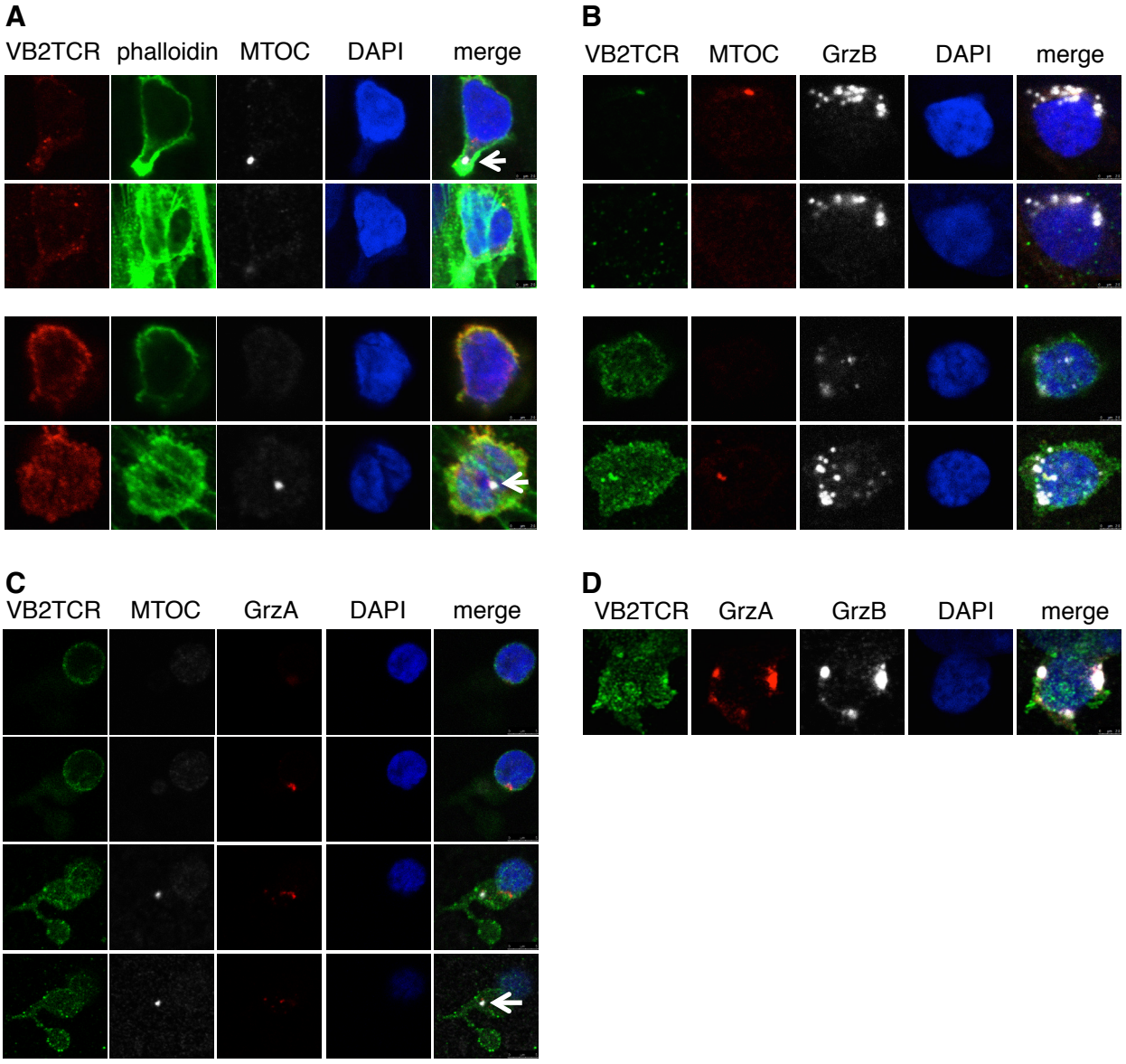
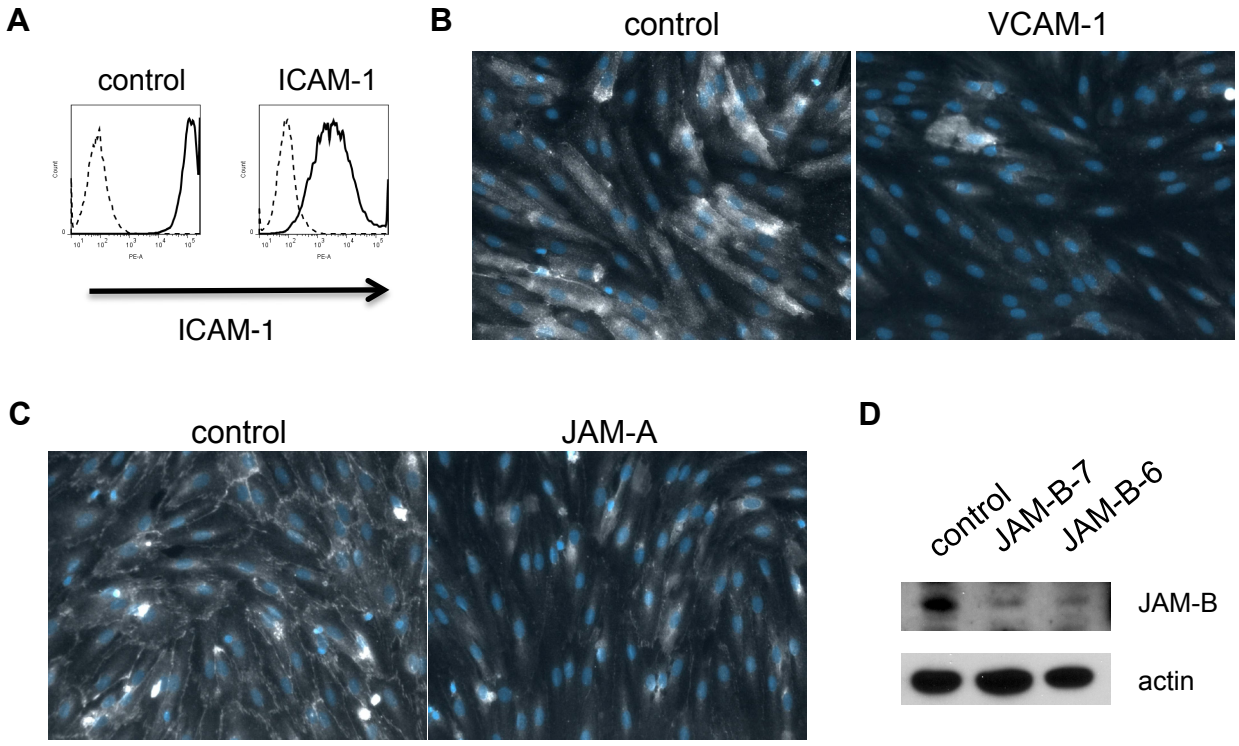


Supplemental Figure I

Supplemental Figure I. MTOC and granule localization during CR- and TCR-driven TEM of CD8 T_{EM} cells. **A**. MTOC localization in CR-driven TEM (upper two panels) and TCR-driven TEM (lower two panels) at 5 min flow. Representative T cells are stained for the MTOC marker γ-tubulin (white) in addition to F-actin (phalloidin, green), Vβ2TCR (VB2TCR, red) and nuclei (DAPI, blue) and analyzed by confocal microscopy. The confocal images are taken in a plane parallel to the EC monolayer. The lower panels of each set are at a plane at the apical surface of the EC, and the upper panels are 1.2 and 1.5 μm above in CR and TCR, respectively. The white dot in the γ-tubulin column identifies the MTOC, which is situated behind the T cell in CR-driven TEM but located between the T cell and the EC in TCR-driven TEM, as indicated by white arrows in the merged images. Note the T cell nuclei between the EC F-actin in CR-driven TEM. **B**. Lytic granule localization in CR-driven TEM (upper two panels) and TCR-driven TEM (lower two panels) at 5 min flow. Representative T cells are stained for granzyme B (GrzB, white), γ-tubulin (MTOC, red), Vβ2TCR (VB2TCR, green) and nuclei (DAPI, blue) and analyzed by confocal microscopy. The lower panels of each set are at the EC apical surface, and the upper panels of each set are 1.8 and 1.9 μm above for CR and TCR, respectively. Note that granzyme B is located on the same side of the cell as the MTOC, namely, in the back in CR-driven TEM, and towards the T cell-EC interface in TCR-driven TEM. **C**. The MTOC precedes the nucleus in TCR-driven TEM. Representative staining of TCR-driven TEM at later time points, showing the MTOC (white) localized beneath the EC monolayer in the TEP (green, lowest panels), as indicated by the white arrow in the bottom merged image, and lytic granules situated nearby (GrzA, red, middle panels) and the nuclei remaining on the apical surface (upper panels). The distance between each slice is 0.8 μm. **D**. Colocalization of granzymes A and B in CD8 T_{EM} cells. Representative sample stained for Vβ2TCR (VB2TCR, green), granzyme A (GrzA, red), granzyme B (GrzB, white) and nuclei (DAPI, blue). Note the identical staining pattern of granzymes A and B.



Supplemental Figure II. Analysis of siRNA knockdowns. A. ICAM-1 siRNA. Histograms show FACS analysis of EC transfected with control siRNA (left) and ICAM-1 siRNA (right) and stained with anti-ICAM-1 mAb. The corrected median fluorescence intensities are 115,923 and 3141 for control and ICAM-1, respectively. B. VCAM-1 siRNA. EC transfected with control siRNA (left) and VCAM-1 siRNA (right) and stained with anti-VCAM-1 mAb. C. JAM-A siRNA. EC transfected with control siRNA (left) and JAM-A siRNA (right) and stained with anti-JAM-A mAb. D. JAM-B siRNA. Western blots of EC transfected with control siRNA and JAM-B siRNAs and probed with anti-JAM-B Ab.

Supplemental Figure II