SUPPLEMENTAL INFORMATION

SUPPLEMENTAL METHODS

Preparation of MDCK cell line stably expressing CXCR4-GFP. T293 cells were transfected with pHR'-CMV-CXCR4-GFP vector in a conditional packaging system to produce a sufficient amount of lentivirus. Subsequently, MDCK cells were infected with this lentivirus, and clones expressing CXCR4-GFP were selected by single-cell cloning method and screening by Nikon TE-2000S inverted fluorescence microscope (Nikon, Melville, NY).

Chemokine Receptor 4 (CXCR4)-GFP localization

The MDCK cells expressing CXCR4-GFP were grown in Corning transwells (Corning Glassworks, Corning, NY) for 3 days, in MEM-EAGLE containing 5% FCS, 1% penicillin-streptomycin solution (media and related reagents were purchased from Biological Industries, Beit Haemek, Israel). The expression of CXCR4-GFP was upregulated by stimulating the CMV promoter through the addition of 0.5 mM Na-butyrate from a stock solution of 0.5 M (Sigma-Aldrich Chemical Co., St. Louis, MO) for 18 h prior to AMB or AMB-AGC treatment. These cells were then incubated for 1 h at 37ºC with AMB (40 µg/ml, Alpharma, Copenhagen, Denmark) or AMB-AGC (400 µg/ml AMB equivalent). Cells in medium without drug were used as a control. Cells were fixed with 4% PFA as previously described (1). Images were captured using a Nikon TE-2000S inverted fluorescence microscope with a plan Apo 60X objective,

equipped with a Z stepper and a Hamamatsu CCD ORCAII camera. Images were processed by Image-Pro Plus v. 4.5 software (Media Cybernetics Inc., Bethesda, MD).

REFERENCES

1. **Altschuler, Y., S. Liu, L. Katz, K. Tang, S. Hardy, F. Brodsky, G. Apodaca, and K. Mostov.** 1999. ADP-ribosylation factor 6 and endocytosis at the apical surface of Madin-Darby canine kidney cells. J Cell Biol **147:**7-12.

Figure S1

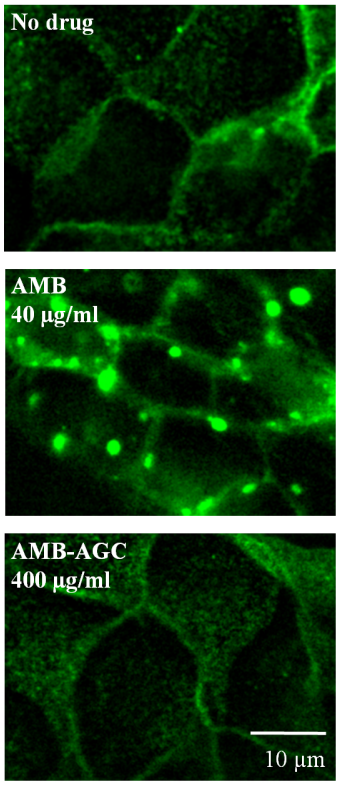

Fig. S1 - AMB but not AMB-AGC modulates the distribution of CXCR4 in MDCK cells. MDCK cells stably expressing CXCR4-GFP were treated as follows: no-drug control, AMB (40 µg/ml), AMB-AGC (400 µg/ml, AMB equivalent). The cells were visualized by fluorescence microscopy after fixation.

Figure S2A

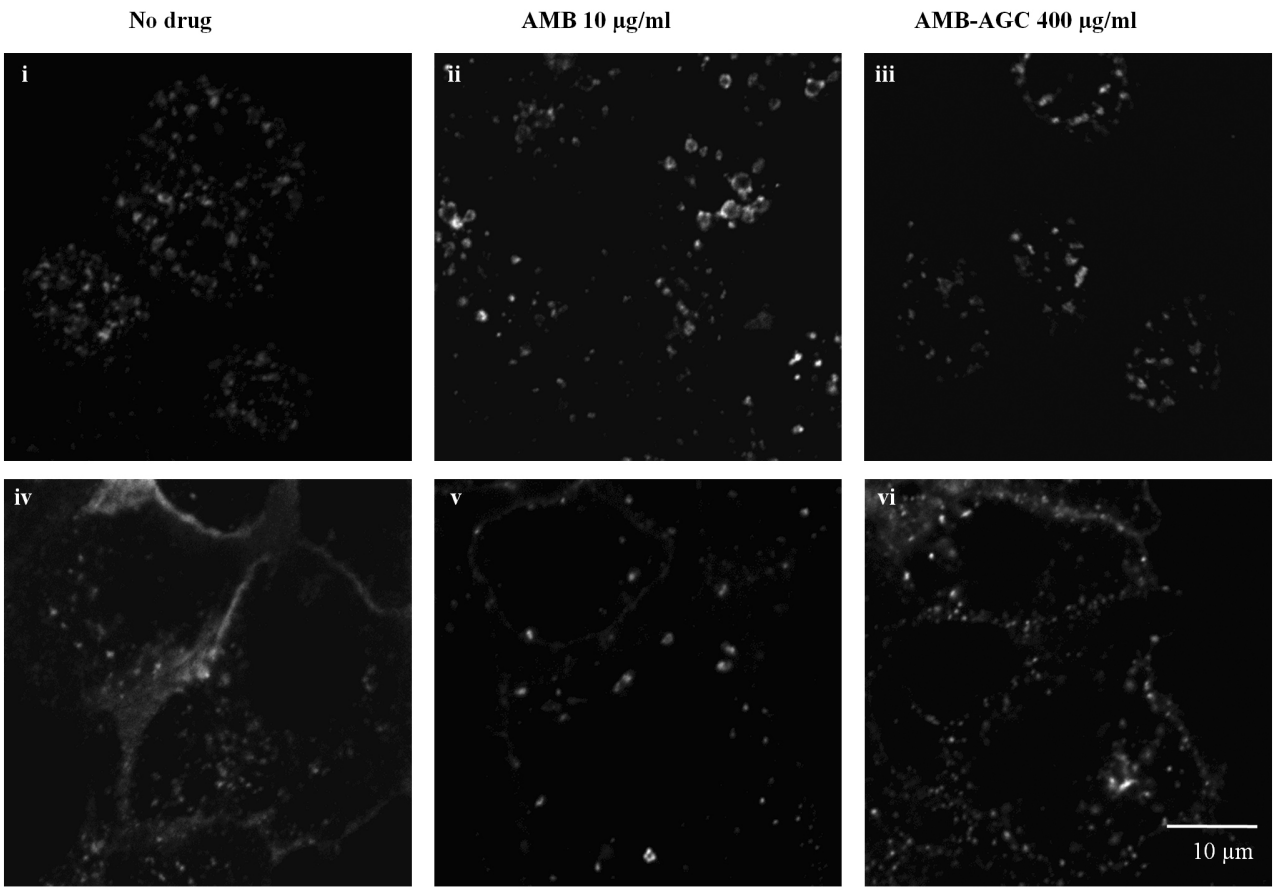

Figure S2B

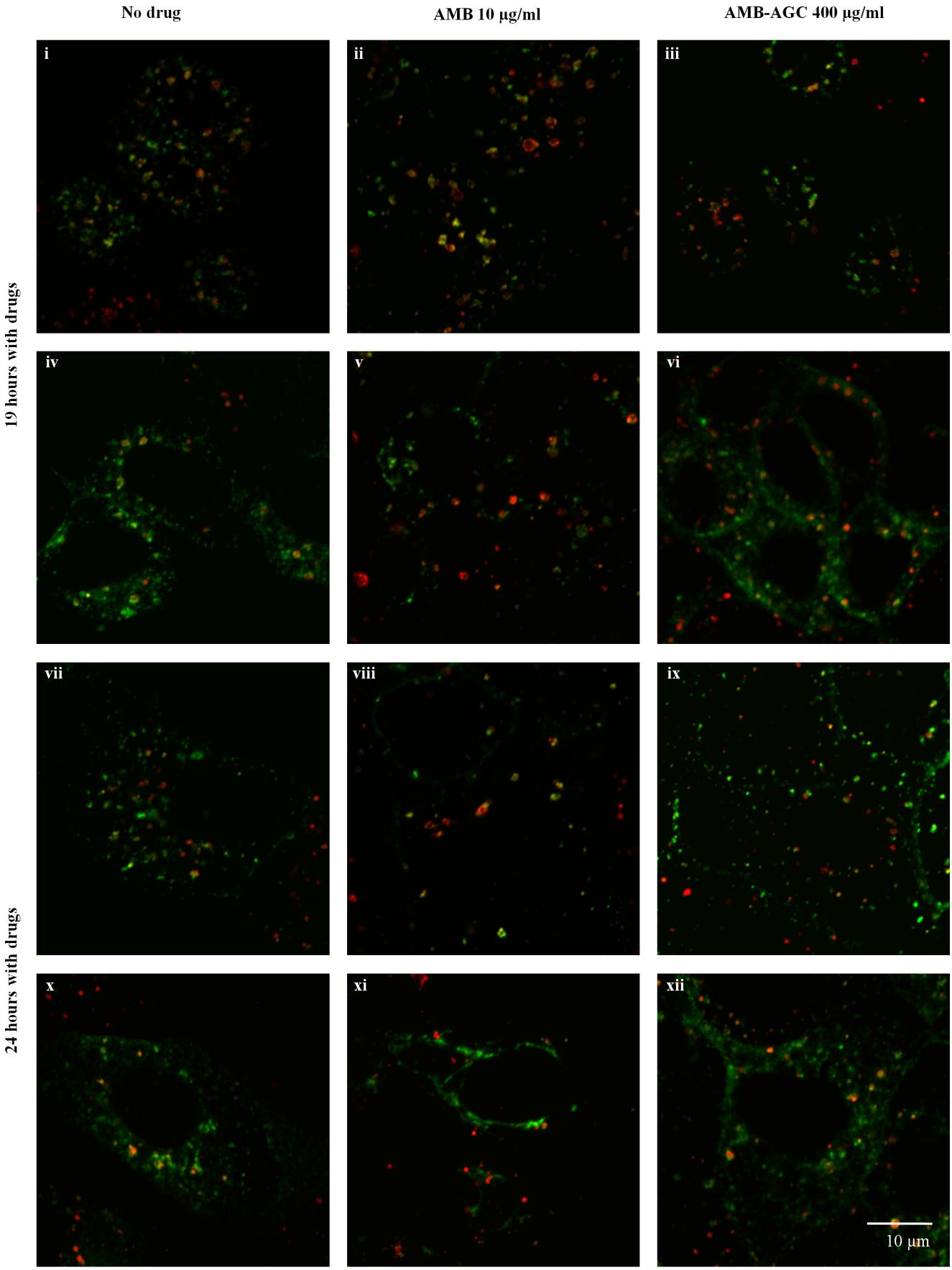
No drug
AMB 10 µg/ml
AMB-AGC 400 µg/ml
19 hours with drugs
24 hours with drugs
i
ii
iii
iv
v
vi
vii
viii
ix
x
xi
xii
10 µm

Figure S2C

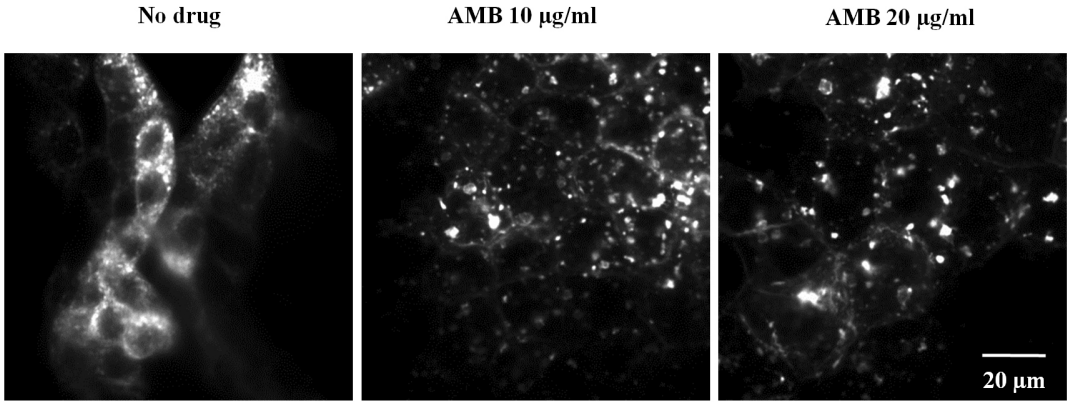

Fig. S2 AMB but not AMB-AGC modulates the morphology of early endosomes (EEs) and recycling endosomes (REs) in MDCK cells.

(A) MDCK-PTR9 cells grown on glass coverslips in MEM/BSA media were treated for 19 h (i–iii) or 24 h (iv–vi) with AMB (10 µg/ml) or AMB-AGC (400 µg/ml, AMB equivalent) and then incubated with FITC- hTfn (60 µg/ml) for 60 min on ice followed by 5 min at 37°C to induce endocytosis. Cells were then washed with acidic buffer (pH 5) to strip membrane-attached FITC-hTfn from plasma membrane, fixed and visualized by confocal microscopy.

(B) Cells were treated as described in Fig. 4B and Fig. S2A. (i–vi) 19 h treatment with drugs. (vii–xii) 24 h treatment with drugs. (i–iii, vii–ix): 5 min FITC-hTfn internalization. (iv–vi, x–xii): 30 min FITC-hTfn internalization. After fixation, cells were labeled with mouse anti-EEA1, and then with goat anti-mouse Cy5. Samples were analyzed by confocal microscopy. FITC-hTfn: green, EEA1: red. The sections shown in iv–vi are more basolateral compared to those shown in Fig. 4Bi–iii.

(C) MDCK-PTR9 cells were grown on glass coverslips and incubated for 20 h in MEM/BSA media without drugs and an additional 2 h with AMB (10 or 20 µg/ml) or no drug. Cells were then incubated for 60 min on ice with 60 µg/ml of hTfn-Alexa594, washed and transferred for 20 min to 37°C to induce endocytosis. Cells were fixed, and visualized by fluorescence microscopy.