Supporting Information

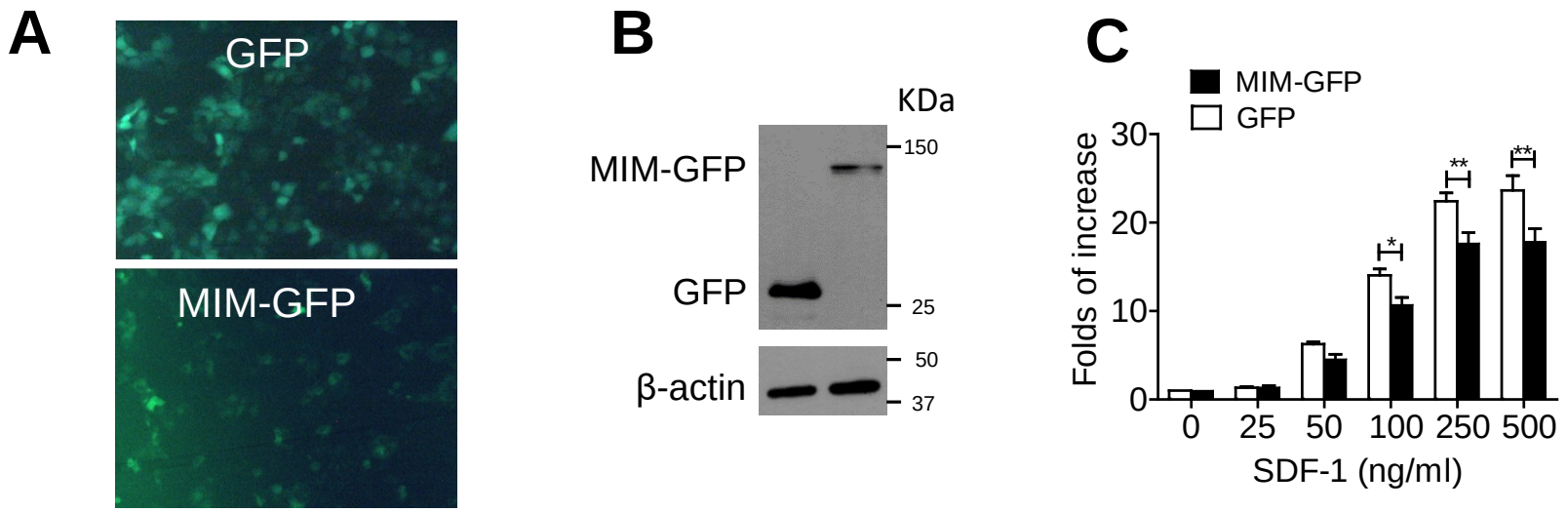


Fig S1. MIM downregulates chemotaxis of MDA-MB-231 cells toward SDF-1. (A) MDA-MB-231 cells were transfected with plasmids encoding MIM-GFP or GFP with FuGene Transfection Reagent. After 48h of transfection, cells were selected in DMEM containing 5% of bovine serum, 1% Pen-Strep and 0.5 mg/ml G418 for two weeks. Cell images were acquired by a fluorescent microscope under a10X objective lens. (**B**) Immunoblot analysis of protein levels of MIM-GFP and GFP in transfected cells. (**C**) MDA-MB-231 cells expressing MIM-GFP or GFP were treated with SDF-1 at different concentrations as indicated. The motility of the treated cells was analyzed by Transwell assay and quantified as described in the legend of Fig. 1D. The data represents mean ± s.e.m. (n=3). *, $P<0.05$; and **, $P<0.01$.

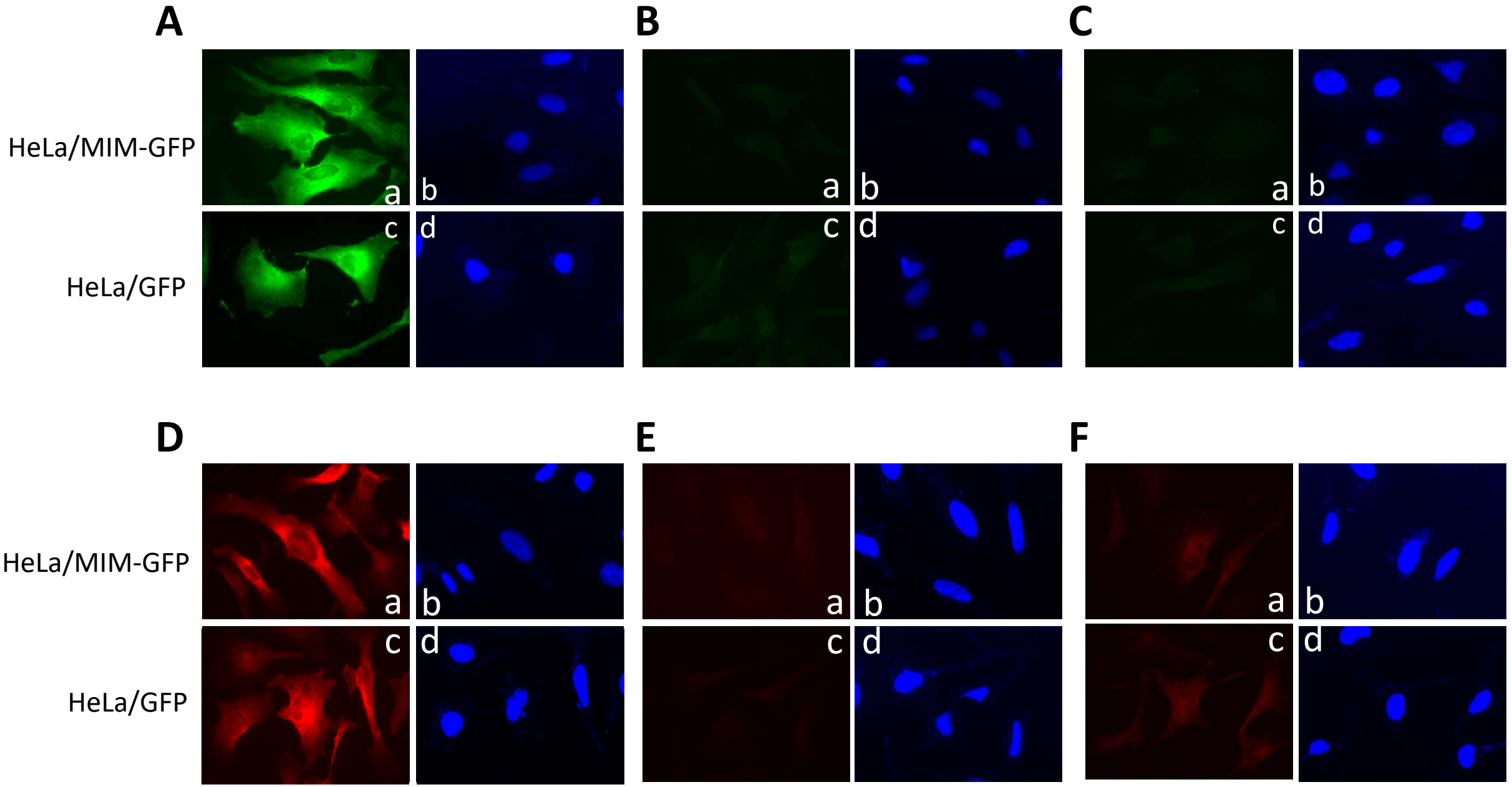

A
B
C
HeLa/MIM-GFP
HeLa/GFP
a
b
c
d
D
E
F
HeLa/MIM-GFP
HeLa/GFP

Fig. S2. Immunofluorescence analysis of GFP and MIM-GFP expression in cells. (**A**) HeLa/MIM-GFP (a and b) or HeLa/GFP cells (c and d) were stained with rabbit anti-GFP antibody followed by staining with Alexa-488-conjugated goat anti-rabbit IgG antibody plus DAPI. The stained cells were inspected by immunofluorescence microscopy under either green (a and c) or blue channel (b and d) using a 60 x oil objective lens. Digital images were captured at a fixed exposing time of 1s for green channel, and 5s for blue channel. (**B** and **C**) HeLa cells expressing GFP or MIM-GFP as A were stained with either normal rabbit (**B**) or normal mouse (**C**) IgG, then stained with Alexa-488-conjugated goat anti-rabbit (**B**) or mouse (**C**) antibody. The stained cells were inspected as A. (**D-F**) HeLa/MIM-GFP and HeLa/GFP cells were treated and examined exactly same as A-C except that Alexa 568-conjugated secondary antibodies were used.

Condition: Cells were plated on fibronectin coated coverslips in a 6-well plate at $3x10^5$ cells/well. After 16 h, the cells were fixed in 4% paraformaldehyde for 10 min followed by incubation in 0.05% saponin for 10 min and then blocked in 5% goat serum in PBS for 30 min. The blocked cells were incubated with either primary antibody or normal IgG at 4 µg/ml for 1 h at room temperature in dark and then with secondary antibody at 2 µg/ml plus DAPI for 1 h. All treated cells were mounted to a slide and inspected by a florescent microscope (Nikon TE2000-U) using a 60 x oil objective lens with aperture of 1.40. Digital images were captured by QImaging CCD camera driven by MetaMorph 7.8 software. The captured images were presented without any digital modifications except of DAPI images some of which were modified by Photoshop software to highlight all the nucleus.

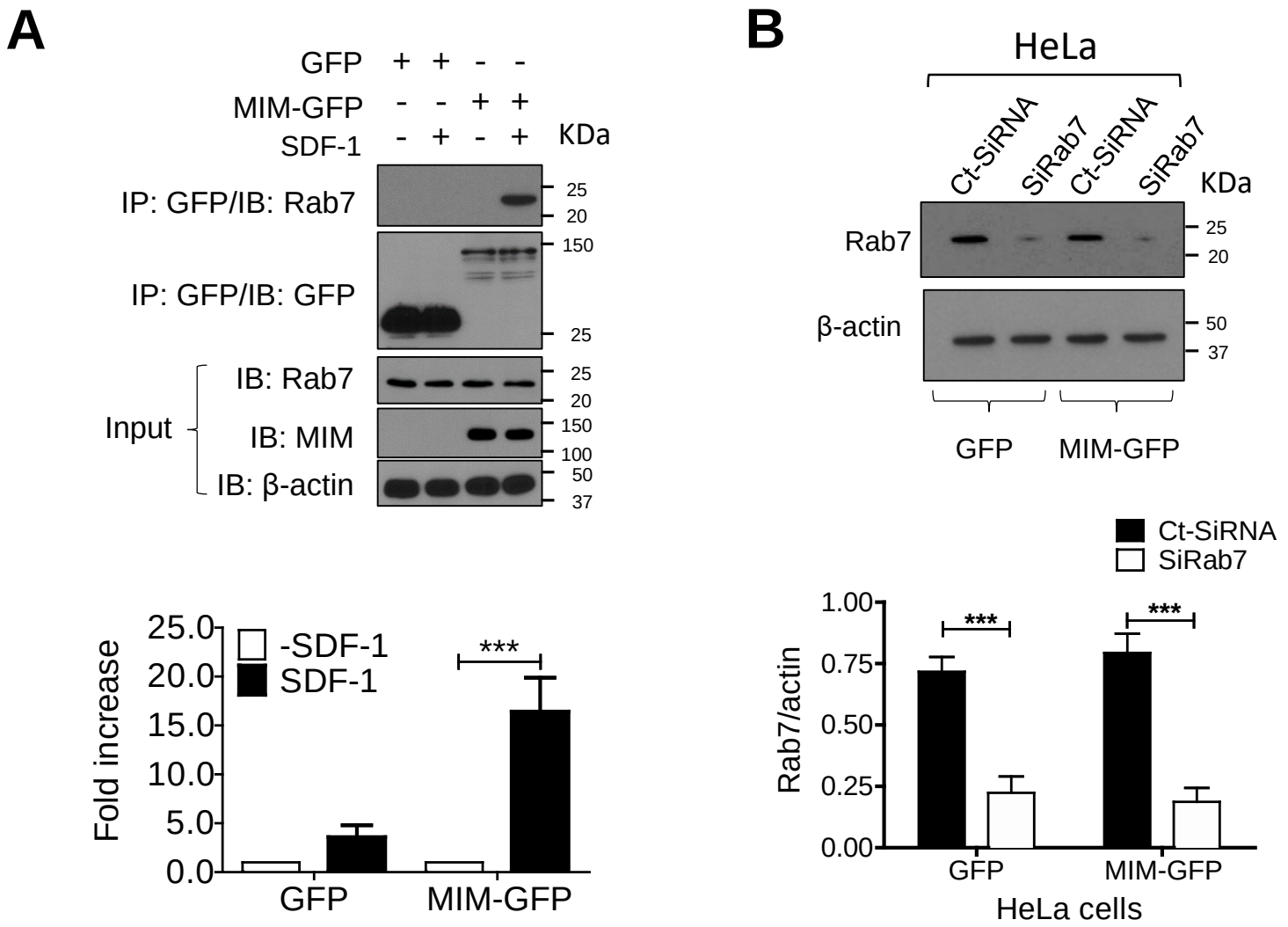
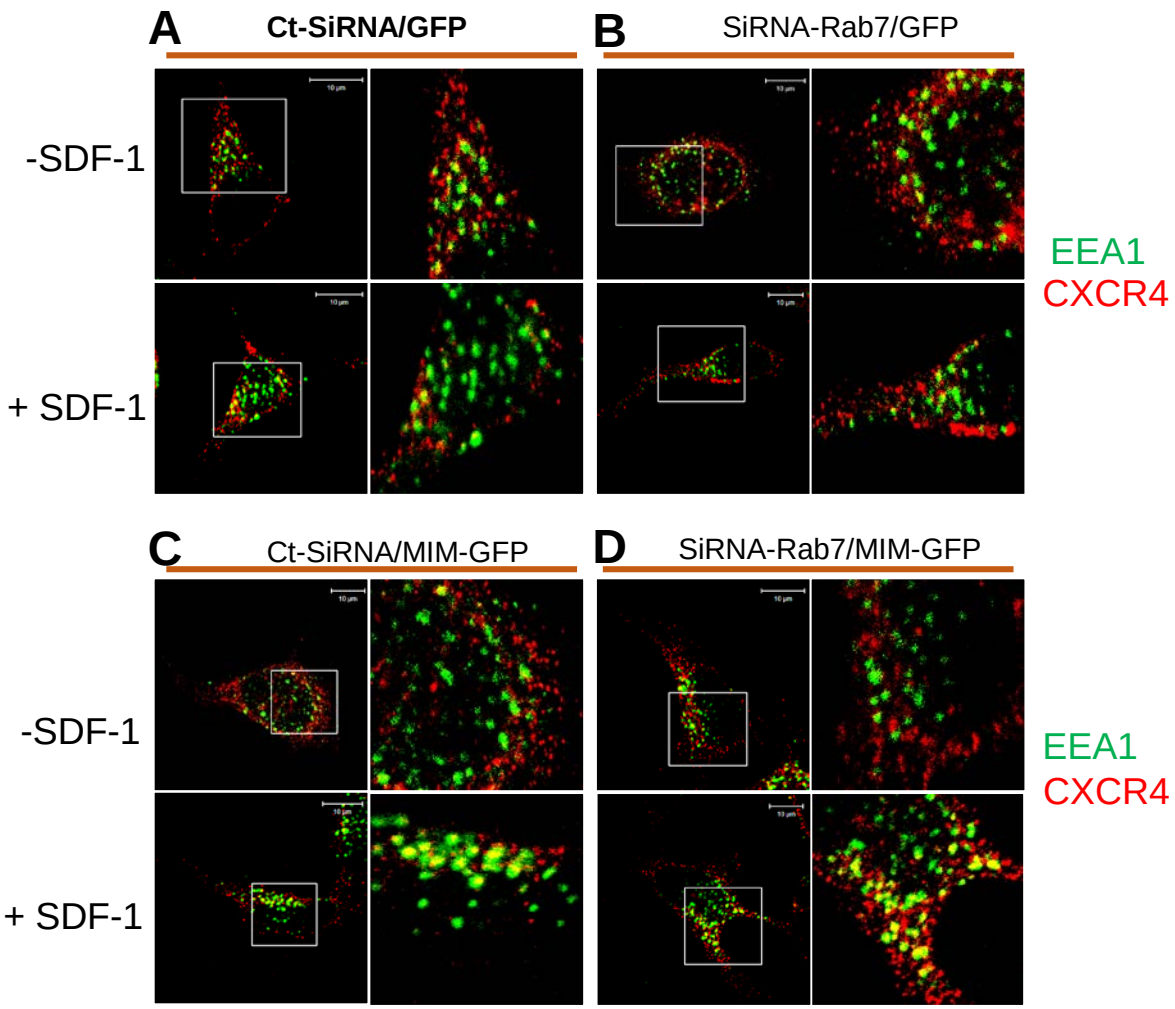


Fig. S3. Suppression of Rab7 expression in HeLa cells. (A) HeLa cells expressing MIM-GFP or GFP were stimulated with 150 ng/ml SDF-1 for 30 min. The lysates of stimulated cells were subjected to IP with GFP antibody followed by IB with antibodies as indicated. The level of precipitated Rab7 protein was normalized to that of cells expressing GFP without SDF-1 treatment. (**B**) HeLa cells expressing GFP or MIM-GFP were treated with siRNAs against Rab7a (siRab7) or a siRNA with scrambled sequence (Ct-siRNA). Protein levels of Rab7 were evaluated by immunoblot and normalized to that of β-actin. All the data represent mean ± s.e.m. (n=3). ***, P< 0.001.



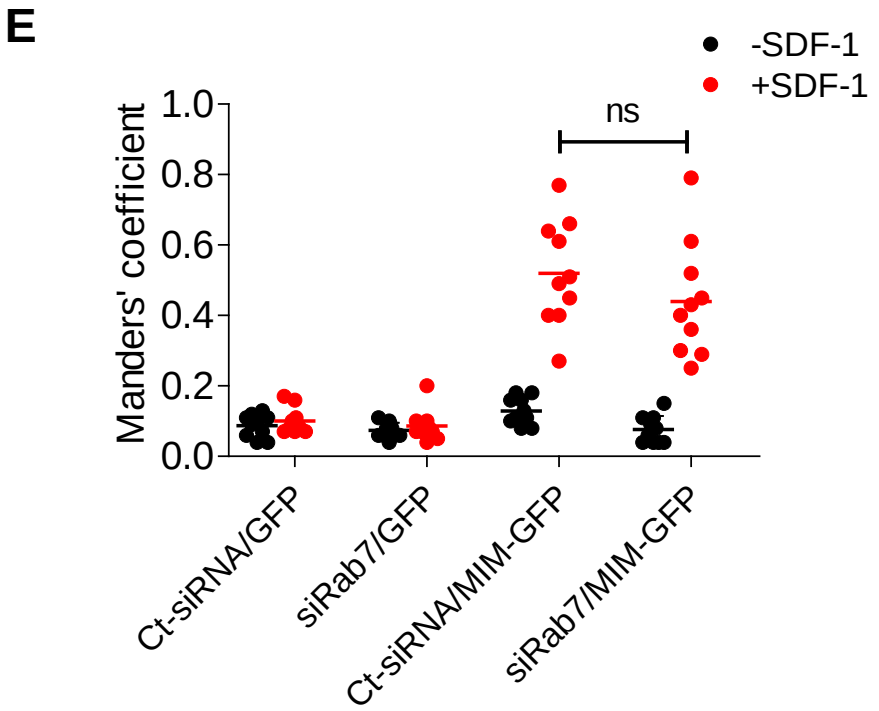
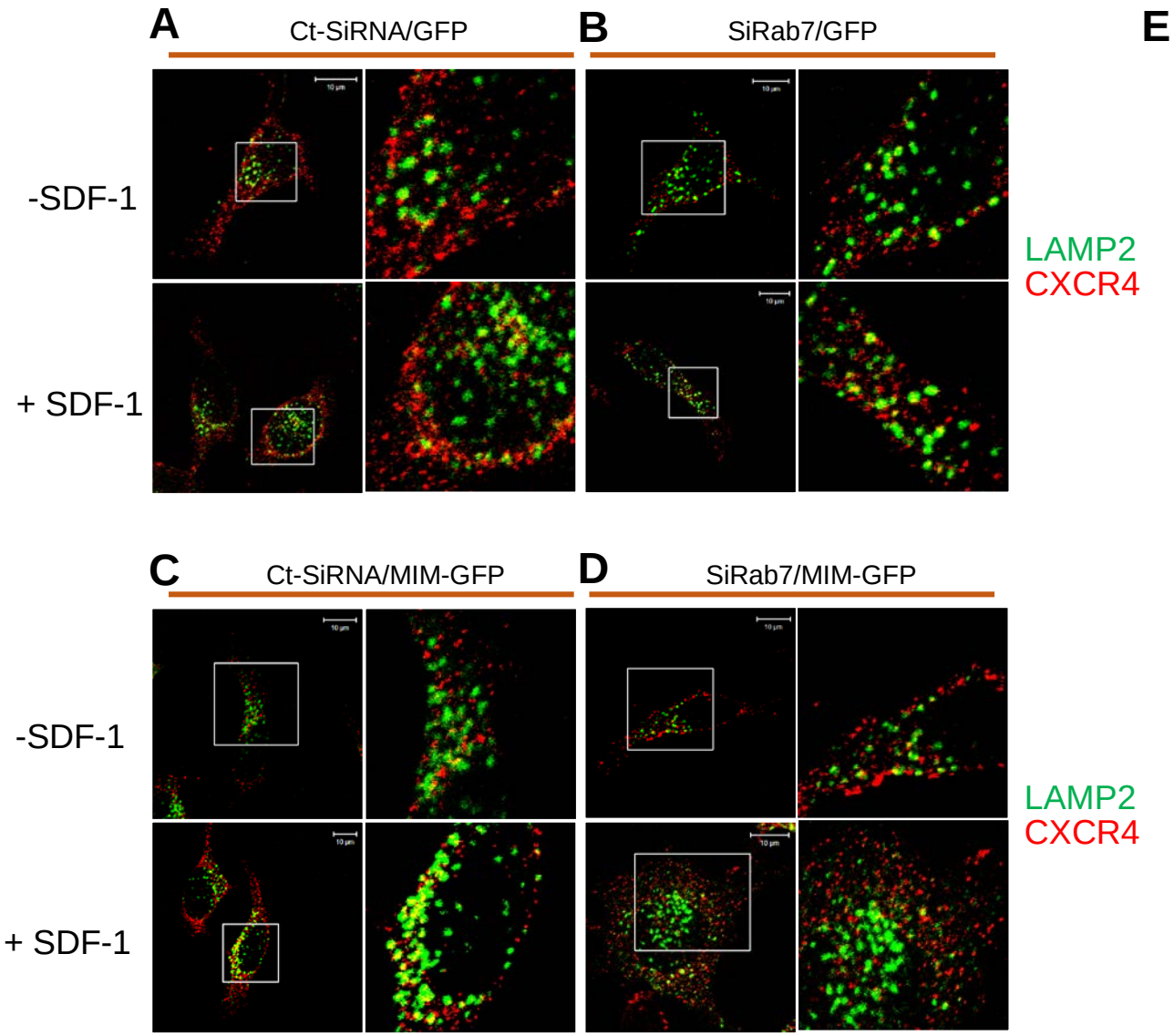


Fig. S4. Rab7 is dispensable for MIM-mediated recruitment of CXCR4 to early endosomes. (A-D) HeLa cells expressing GFP (A and B) or MIM-GFP (C and D) were plated in a 6-well plate and treated with siRab7 or Ct-siRNA for 48h prior to treatment with 500 ng/ml SDF-1 for 90 min. Treated cells were stained with anti-CXCR4 (Red) and EEA (Green) and inspected by confocal microscopy. The images shown were the representative of 10 images inquired by the microscope. (**E**) Quantification of co-localization of CXCR4 and EEA was conducted based on Manders' coefficient. Ns (n=10), not significant, referring to the difference between MIM-GFP cells treated with siRab7 and those treated with control siRNA.



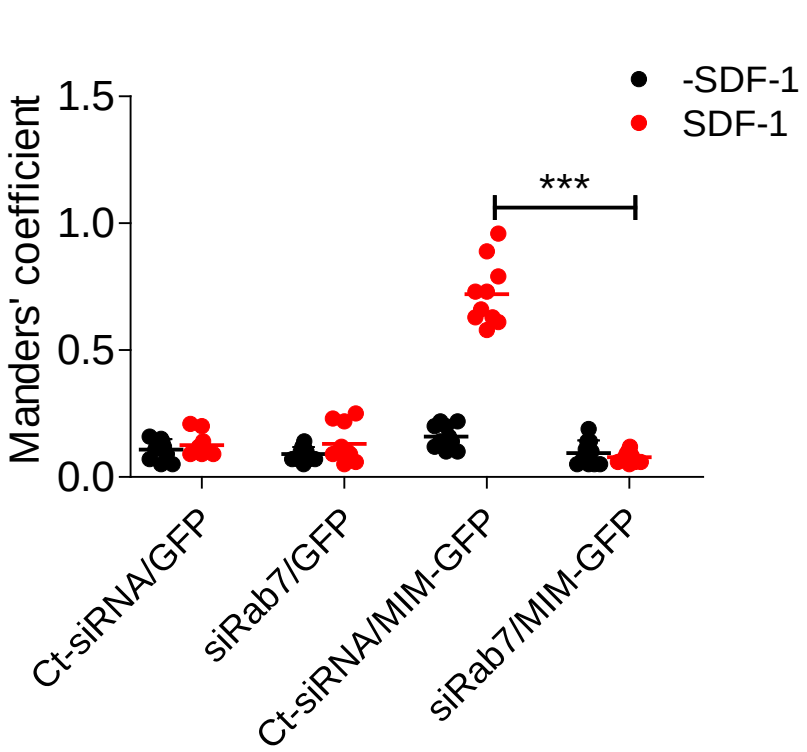


Fig. S5. Rab7 is indispensable for MIM-mediated recruitment of CXCR4 into lysosomes. (A-D) HeLa cells expressing GFP (A and B) or MIM-GFP (C and D) were plated in 6-well plates and treated with siRab7 or Ct-siRNA for 48h prior to treatment with 500 ng/ml SDF-1 for 90 min. Treated cells were stained with anti-CXCR4 (red) and LAMP2 (green) and inspected by confocal microscopy. The images shown were the representatives of 10 images inquired by the microscope. (**E**) Co-localization of CXCR4 with LAMP2 was quantified based on Manders' coefficient. ***, $P < 0.001$ (n=10), referring to the difference between MIM-GFP cells treated with siRab7 and those treated with control siRNA.

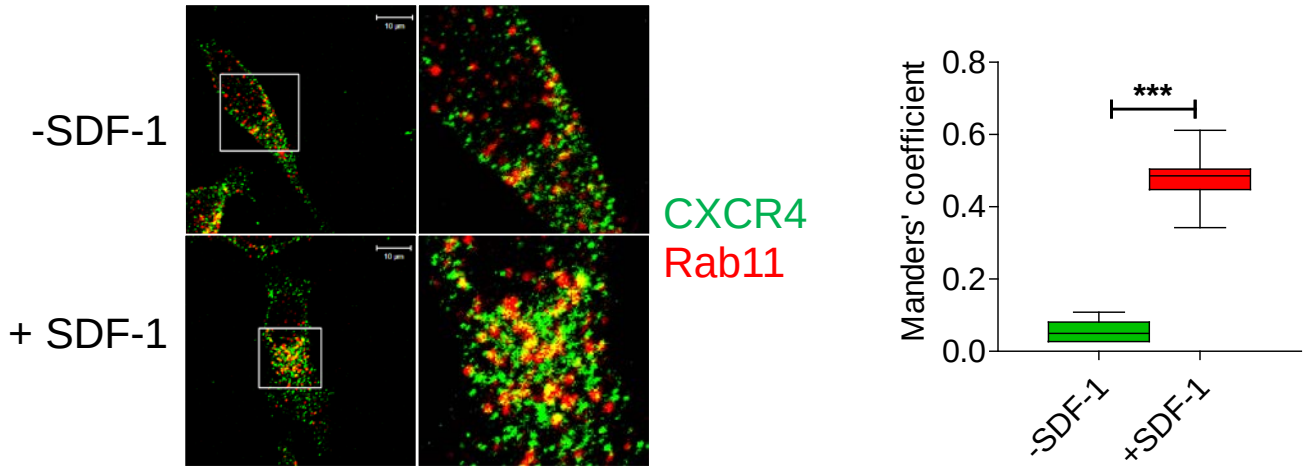


Fig. S6. Analysis of recruitment of CXCR4 to Rab11-associated endosomes in IRTKS expressing cells. HeLa cells expressing IRTKS-GFP were treated with with 500 ng/ml SDF-1 for 30 min prior to co-staining with anti-CXCR4 (green) and anti-Rab11 (red). The stained cells were inspected by confocal microscopy. Quantification of co-localization of CXCR4 and Rab11 was performed based on Manders' coefficient. Data represent mean ± s.e.m. (n=10). ***, $P < 0.001$.

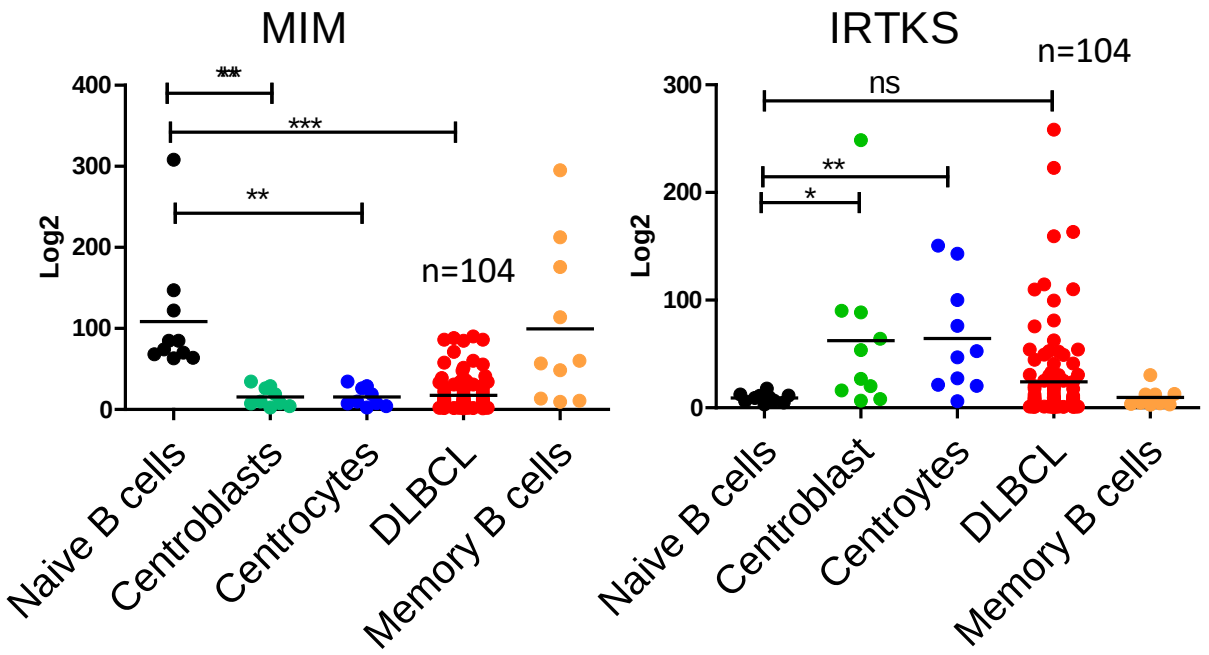


Fig. S7. MIM and IRTKS are differentially expressed in normal and malignant B cells. Expressions of MIM and IRTKS in several types of normal human B cells and diffused large B cell lymphoma (DLBCL) were plotted based on public microarray databases (Amazonia!). Note the opposite distribution of MIM and IRTKS expression in those cells. Except of DLBCL, which has a cohort size of 104, all others have a cohort size of 10. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0001$; and ns, not significant.