Supplemental Materials

Molecular Biology of the Cell

Chigaev et al.

FRET detection of lymphocyte function-associated antigen-1 conformational extension

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Supplementary information

Supplementary Figure 1

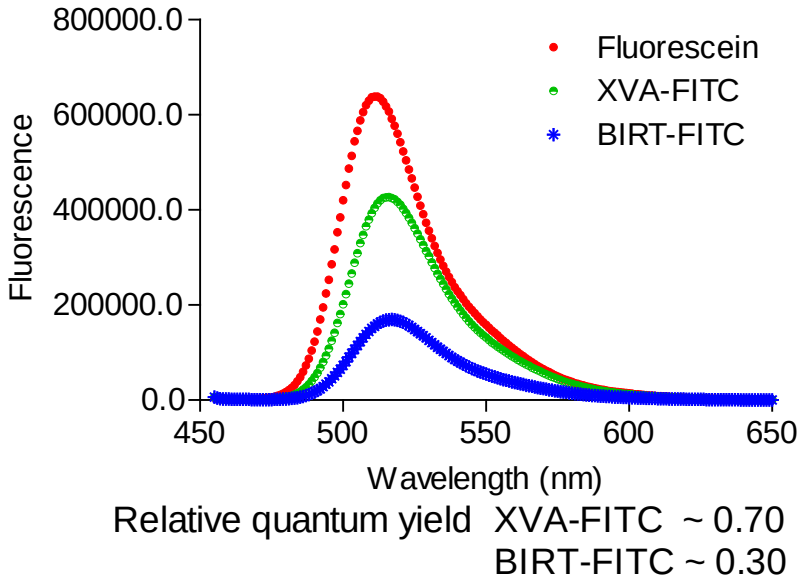
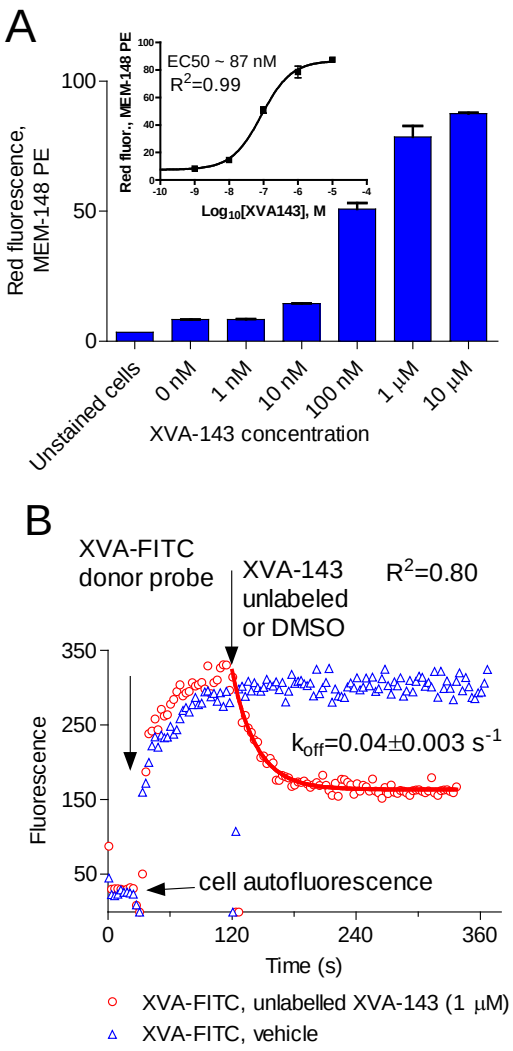


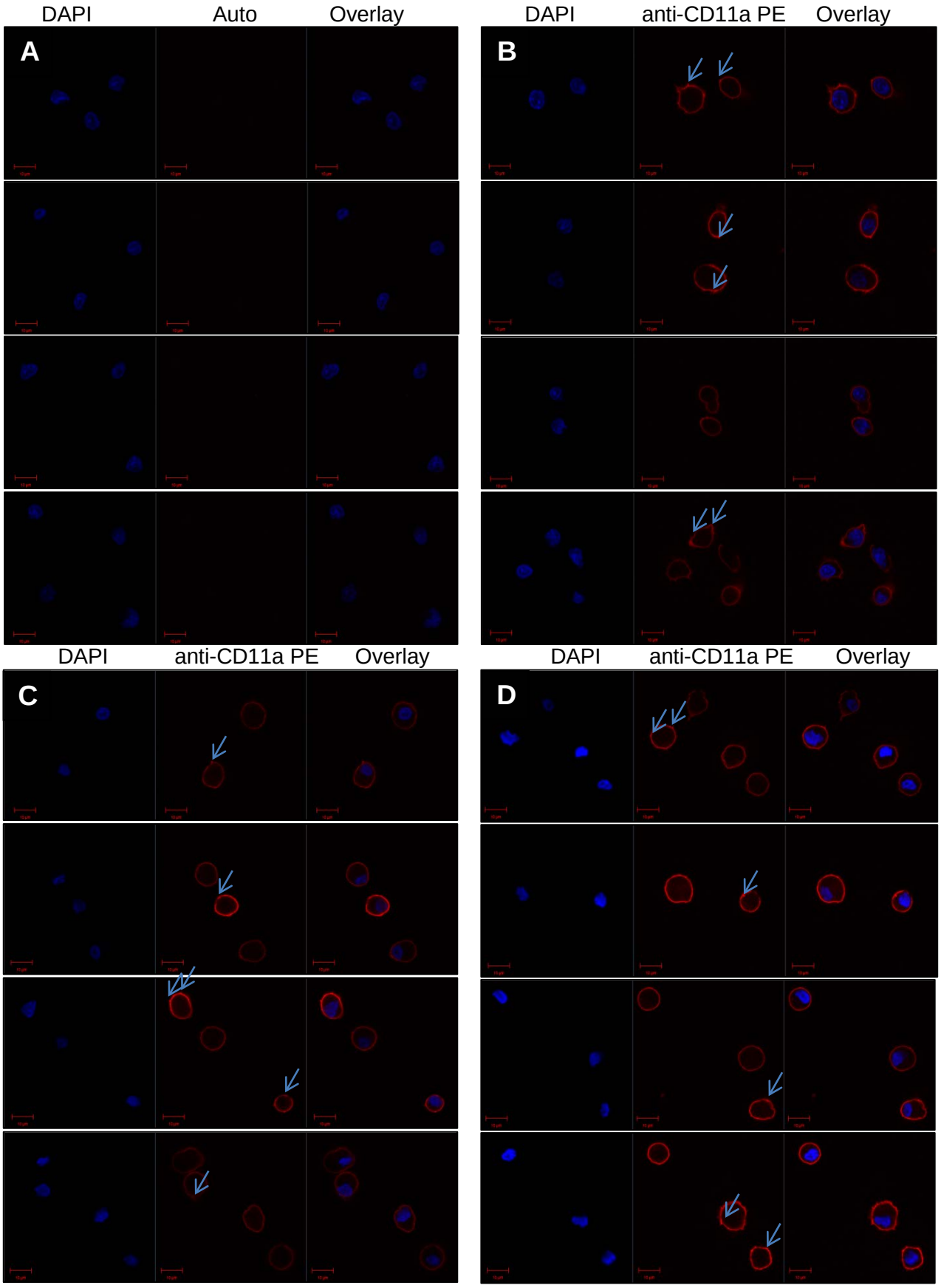
Figure 1. Emission spectra of XVA-FITC, BIRT-FITC donor probes and fluorescein standard in phosphate buffered saline Absorption-matched (at 490 nm) samples of the LFA-1 probes (XVA-FITC and BIRT-FITC) and a fluorescein standard solution in phosphate buffered saline were used to obtain emission spectra (excitation at 450 nm). The relative amount of light emitted by the probes was calculated as the ratio of the area under the emission peak for the LFA-1 probes and the fluorescein standard. Each line represents the mean of three independent determinations.

Supplementary Figure 2



Supplementary Figure 2. Binding affinity of XVA-143. (A) Binding of the integrin-specific ligand determined using ligand induced binding sites (LIBS) antibodies. Cells were incubated with phycoerythrin labeled anti-CD18 mAbs MEM-148 (Abcam, Cambridge, MA) in the presence of increasing concentrations of unlabeled XVA-143. LIBS exposure was fitted to a sigmoidal dose-response equation (inset). Because exposure of LIBS epitope is triggered through conformational change induced by the ligand binding, the EC50 for LIBS antibody binding is reporting the ligand Kd (Chigaev *et al.*, 2009). Each line represents the mean±SEM out of two determinations (n=2). (B) The dissociation of the XVA-FITC probe. Cells were sequentially treated with 100 nM XVA-FITC, and 1 μM unlabelled XVA-143 or vehicle (DMSO). One representative experiment of four experiments is shown. Ligand dissociation rate constant (k_{off}) was determined by fitting the signal decay to a single exponential equation (Chigaev *et al.*, 2001).

Supplementary Figure 3



Supplementary Figure 3. Imaging of LFA-1 before and after cell activation. Four representative fields for each experimental condition are shown. Cells were treated in a manner analogous to flow cytometry experiments, fixed, and stained with primary labeled (phycoerythrin) fluorescent mAbs (mouse anti-human CD11a/LFA-1α, clone HI111 (PE) as described in details in Materials and Methods. Nuclear stain (DAPI) was included on the microscope slide mounting reagent (SlowFade Gold Antifade Mountant with DAPI). (A) Cell autofluorescence. (B) Cells were fixed prior to TPA/TG activation (resting cells). (C) Cells were fixed 3 min after TPA/TG activation. (D) Cells were fixed 6 min after TPA/TG activation. Notice that complete unquenching of the probe signal was observed within 3 min after TPA/TG addition (Figure 6).

Reference List

Chigaev,A., Blenc,A.M., Braaten,J.V., Kumaraswamy,N., Kepley,C.L., Andrews,R.P., Oliver,J.M., Edwards,B.S., Prossnitz,E.R., Larson,R.S., Sklar,L.A. (2001). Real time analysis of the affinity regulation of alpha 4-integrin. The physiologically activated receptor is intermediate in affinity between resting and Mn(2+) or antibody activation. Journal of Biological Chemistry *276*, 48670-48678.

Chigaev,A., Waller,A., Amit,O., Halip,L., Bologa,C.G., Sklar,L.A. (2009). Real-time Analysis of Conformation-sensitive Antibody Binding Provides New Insights into Integrin Conformational Regulation. Journal of Biological Chemistry *284*, 14337-14346.