Supporting Information

HIV-1 Nef dimers short-circuit immune receptor signaling by activating Tec-family kinases at the cell membrane

Contents

Figure S1. HIV and SIV Nef proteins induce Itk activation loop autophosphorylation at the cell membrane. A) Itk was expressed either alone (Control) or together with HIV-1 Nef (SF2 isolate) or SIV Nef (mac239) as BiFC pairs in the absence or presence of the Btk/Itk inhibitor ibrutinib (1 µM) in 293T cells. Cells were fixed and stained for confocal microscopy with phosphospecific antibodies to the Itk activation loop phosphotyrosine (pY511; red) and to the Itk protein (V5 epitope; blue). Nef interaction with Itk is observed as fluorescence complementation of the YFP variant, Venus (BiFC; green). B) Single-cell image analysis. Mean fluorescence intensities for the pY511 and Itk signals were determined for ≥ 100 cells from each condition using Image J. The fluorescence intensity ratio (pY511:Itk expression) for each cell is shown as a horizontal bar, with the median value indicated by the red bar. Student's t-tests demonstrated significant increases in Itk activation loop phosphorylation in the presence of both HIV-1 and SIV Nef ($P < 0.0001$ in each case).

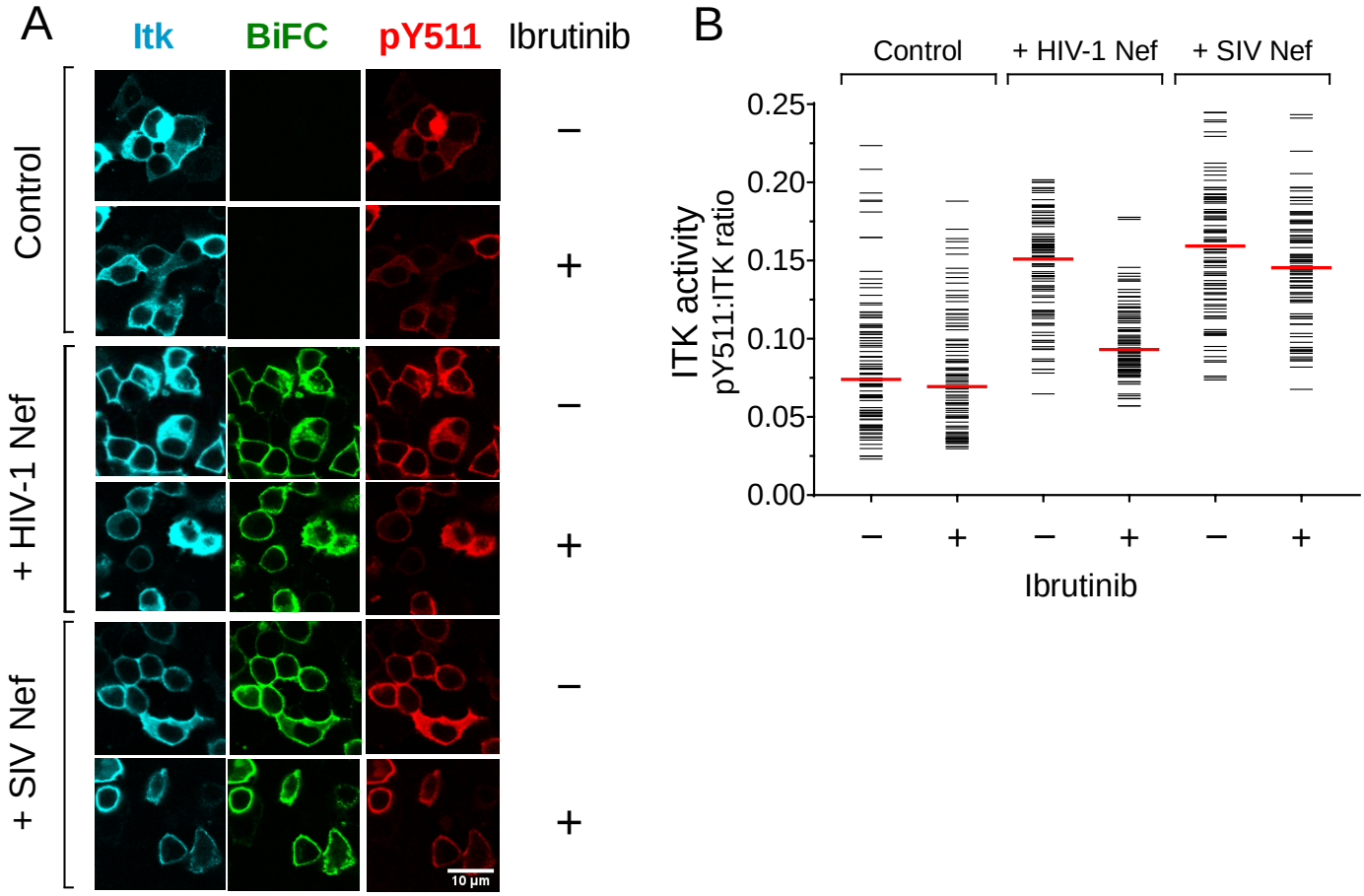

Figure S2. HIV and SIV Nef proteins induce constitutive Btk activation loop autophosphorylation at the cell membrane. A) Btk was expressed either alone (Control) or together with HIV-1 Nef (SF2 isolate) or SIV Nef (mac239) as BiFC pairs in the absence or presence of the Btk/Itk inhibitor ibrutinib (1 µM) in 293T cells. Cells were fixed and stained for confocal microscopy with phosphospecific antibodies to the Btk activation loop phosphotyrosine (pY551; red) and to the Btk protein (V5 epitope; blue). Nef interaction with Btk is observed as fluorescence complementation of the YFP variant, Venus (BiFC; green). B) Single-cell image analysis. Mean fluorescence intensities for the pY551 and Btk signals were determined for ≥ 100 cells from each condition using Image J. The fluorescence intensity ratio (pY551:Btk expression) for each cell is shown as a horizontal bar, with the median value indicated by the red bar. Student's t-tests demonstrated significant increases in Btk activation loop phosphorylation in the presence of both HIV-1 and SIV Nef ($P < 0.0001$ in each case).

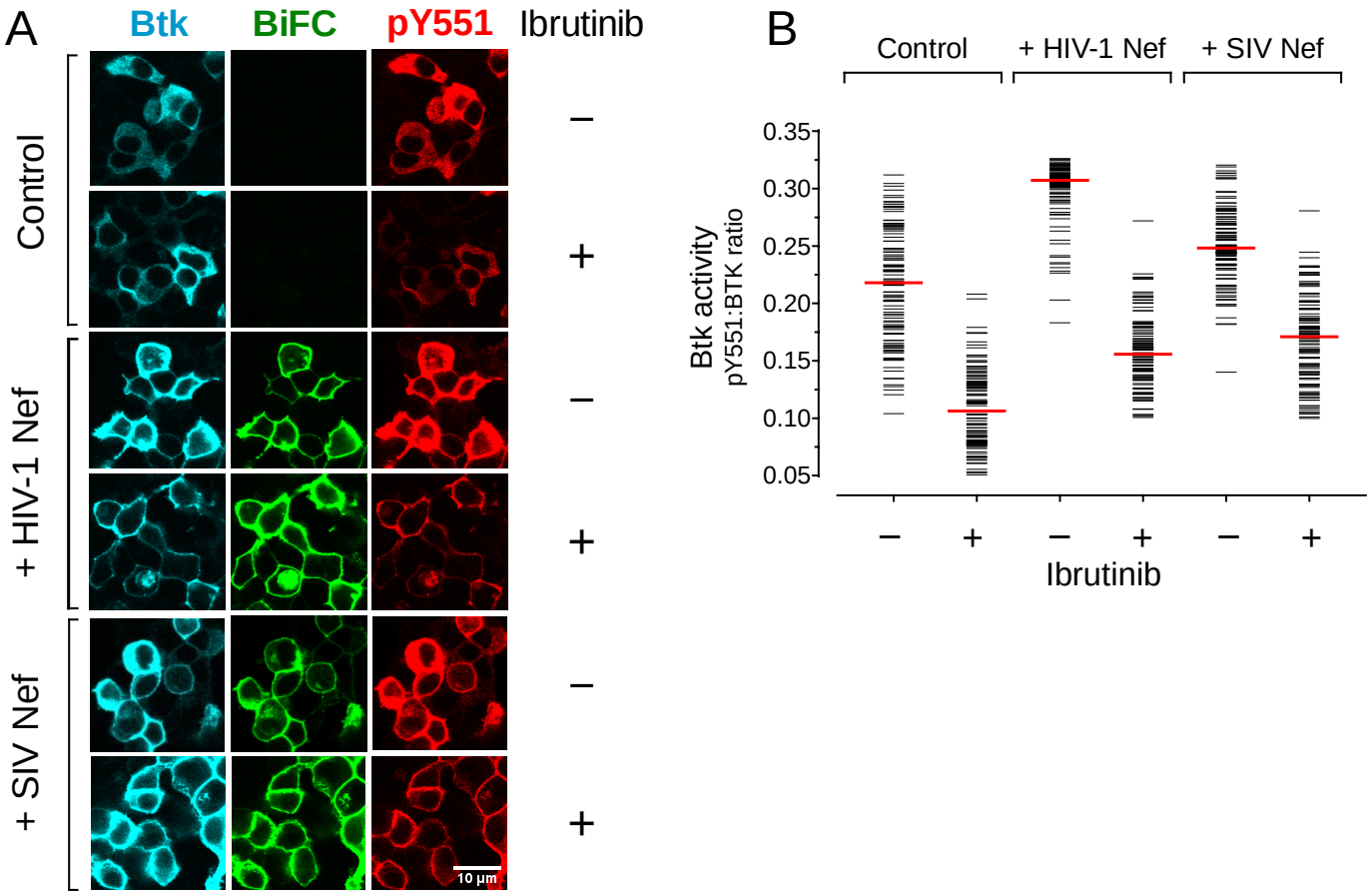

Figure S3. Dimerization-defective Nef mutants bind Itk but fail to induce kinase autophosphorylation. A) Itk was expressed either alone or together with wild-type Nef (WT) or homodimerization-defective mutants as BiFC pairs in 293T cells. Cells were fixed and stained for confocal microscopy with phosphospecific antibodies to the Itk activation loop phosphotyrosine (pY511; red) and to the Itk protein (V5 epitope; blue) to verify expression. Nef interaction with Itk is observed as Venus fluorescence (BiFC; green). B) Single-cell image analysis was performed as per the legend to Figure S1. The fluorescence intensity ratio (pY511:Itk expression) for each cell is shown as a horizontal bar, with the median value indicated by the red bar. Student's t-tests were performed on the groups indicated by horizontal lines above the plot; $P < 0.0001$ in each case. In addition, ratios obtained with the F121A and LY/DD mutants were significantly lower than the baseline value observed for Itk without Nef (Control), suggesting a dominant negative effect ($P < 0.0001$).

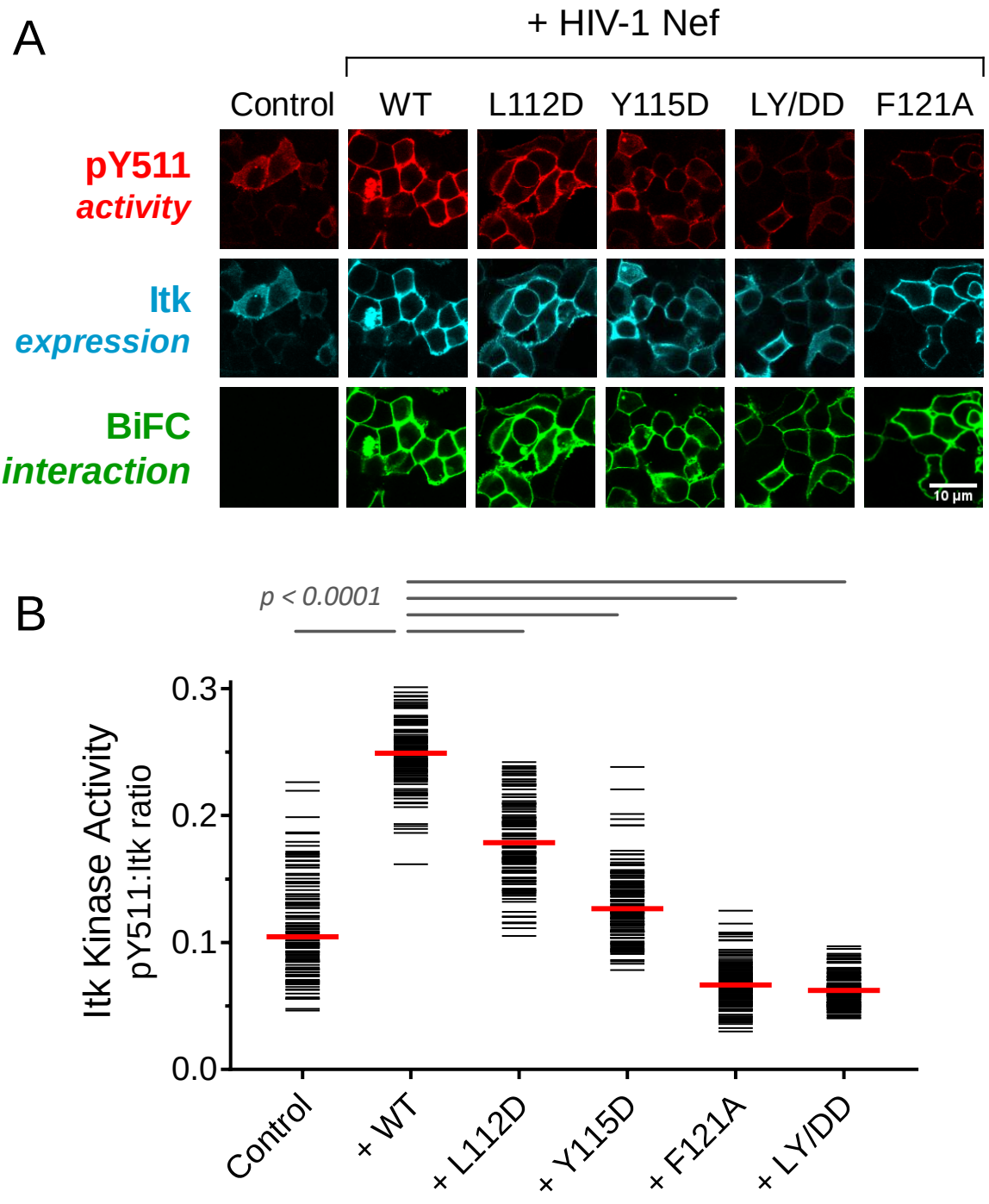

Figure S4. Dimerization-defective Nef mutants bind Btk but fail to induce kinase autophosphorylation. A) Btk was expressed either alone or together with wild-type Nef (WT) or homodimerization-defective mutants as BiFC pairs in 293T cells. Cells were fixed and stained for confocal microscopy with phosphospecific antibodies to the Btk activation loop phosphotyrosine (pY551; red) and to the Btk protein (V5 epitope; blue) to verify expression. Nef interaction with Btk is observed as Venus fluorescence (BiFC; green). B) Single-cell image analysis was performed as per the legend to Figure S1. The fluorescence intensity ratio (pY551:Btk expression) for each cell is shown as a horizontal bar, with the median value indicated by the red bar. Student's t-tests were performed on the groups indicated by horizontal lines above the plot; $P < 0.0001$ in each case. In addition, ratios obtained with the F121A and LY/DD mutants were significantly lower than the baseline value observed for Btk in the absence of Nef (Control), suggesting a dominant negative effect ($P < 0.0001$).