Legends to Supplemental Figures

Sup. Figure 1: Subcellular distribution of human CCR5 receptor in CHO-K1 cells. (A) CHO-K1 cells were transiently transfected with a plasmid encoding CCR5. 48 hours after transfection, cells were stained for surface (left panel) and total (after cell permeabilization, right panel) receptor with the 1/85a Alexa-Fluor^{R} 647-conjugated anti-human CCR5 antibody and analyzed by flow cytometry. Green histogram: specific labeling, gray histogram: isotypic control. (B) CHO-K1 cells, transfected as described above, were stained for surface (non permeabilized, NP) or total (permeabilized, P) CCR5 with the mouse 2D7 antibody, followed by an Alexa- Fluor^{R} 488-conjugated anti-mouse secondary antibody. Cells were subsequently examined by confocal microscopy. Scale bar: 10µm.

Sup. Figure 2: Subcellular localization of the human CCR5 in CHO-K1 cells. (A) CHO-K1 cells were transiently transfected with a construct coding for CCR5-YFP and, where indicated, with plasmid coding for ER-DsRed, a fluorescent protein retained in the ER. 48 hours after transfection, cells were permeabilized and stained for ER (Bip, Calnexin) or Golgi (Giantin, TGN46) markers and analyzed by confocal microscopy. Colocalization of CCR5-YFP (green) with Bip, calnexin, ER-DsRed, Giantin and TGN46 (all in red) is shown in orange (Merge). Scale bar: 10µm. (B) CHO-K1 cells were transfected with a construct coding for non-tagged CCR5 and, where indicated, with a plasmid coding for ER-DsRed. Cells were stained for CCR5 using the 2D7 anti-human CCR5, then an Alexa- Fluor^{R} 488-conjugated anti-mouse secondary antibody, and appropriate primary anti-markers and corresponding secondary antibodies (see methods). Images were examined by confocal microscopy. Colocalization of CCR5 (green) with calnexin, ER-DsRed, Giantin and TGN46 (all in red) is shown in orange (Merge). Scale bar: 10µm.

Sup. Figure 3: CCR5-CD4 association in intact cells revealed by co-IP experiments. CHO-K1 cells were transfected with plasmids coding for CD4 (lane 1), HA-CCR5-YFP (lane 2) or both (lane 4), as indicated. After cell lysis CCR5 was precipitated with a monoclonal anti-GFP antibody. In lane 3, lysates expressing either CD4 or HA-CCR5-YFP were mixed before the additon of the anti-GFP antibody. Co-precipitated CD4 was revealed by immunoblot using the 1F6 anti-CD4 antibody.

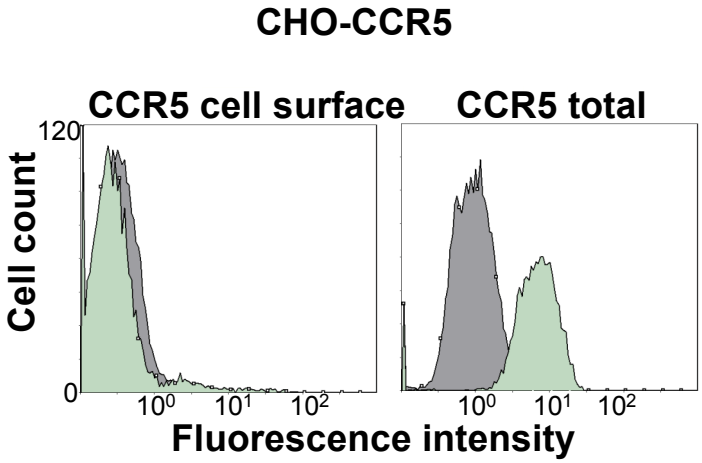
Sup. Figure 4: CCR5 and CD4 concentration in transfected CHO-K1 cells: comparison with values in THP-1 monocytes expressing endogenous co-receptors. (A) Cell samples from the indicated experiments (designated by asterisks in the corresponding figures) were processed for quantification by FACS analysis as described in the methods section. The expression level of CCR5 and CD4 was quantified using the 1/85a anti-human CCR5 and the monoclonal OKT4 antibody, respectively. Data represent the % of the signal measured in THP-1cells. (B) Surface CD4 in CHO-K1 cells as a function of increasing concentrations of total CD4. Aliquots of cells expressing increasing amounts of CD4-YFP, for the experiment in figure 4B, were analyzed by FACS after labeling with anti-CD4 OKT4 mouse monoclonal antibody, followed by an anti-mouse IgG Cy5-conjugated secondary antibody.

Sup. Figure 5: Effect of CD4 inhibition on total CCR5 expression. THP-1 cells nucleofected with siRNAs directed against CD4 (S1, S2 and S3) or scrambled control siRNA, were permeabilized and analyzed by flow cytometry for total CCR5 expression, using the 1/85a anti-human CCR5. Values were represented as the percentage (±SEM of 3 independents experiments in triplicate) of cells nucleofected with the control siRNA.

Sup. Figure 6: **Vpu-induced CD4 degradation negatively regulates cell surface expression of CCR5.** Top panel: Western blot analysis of CD4 from HeLa P4R5 cells transfected with

plasmids coding for Vpu-GFP, the inactive phosphorylation mutant $Vpu_{2/6}$-GFP or free GFP. CD4 was detected by the 1F6 anti-CD4 antibody. Lower panel : Quantitation of cell surface CCR5 and total CD4 total in cells transfected with the indicated plasmids by FACS analysis. Surface CCR5 and total CD4 total were measured in GFP-expressing cells using the 1/85a Alexa-Fluor 647 conjugated anti-human CCR5 and the monoclonal OKT4 anti-human CD4, followed by a cy5 conjugated anti-mouse antibody, respectively. Asteriks indicate significant difference from control free GFP-expressing cells ($p<0,01$).

A



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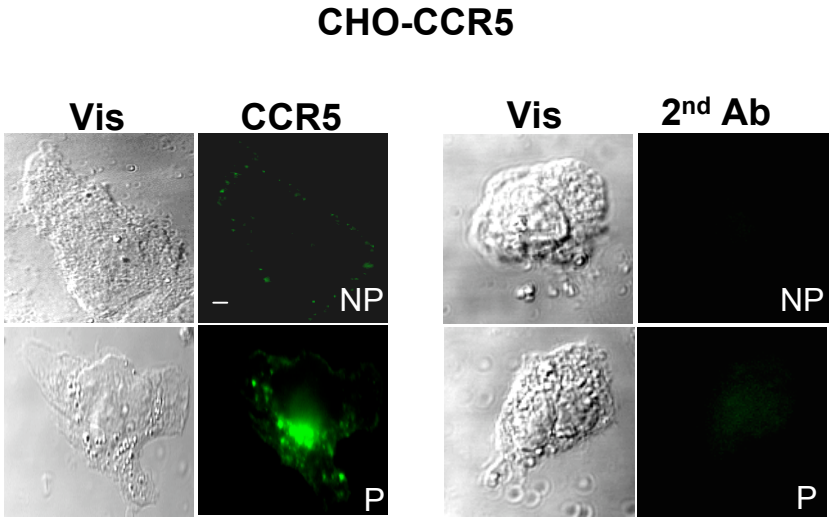
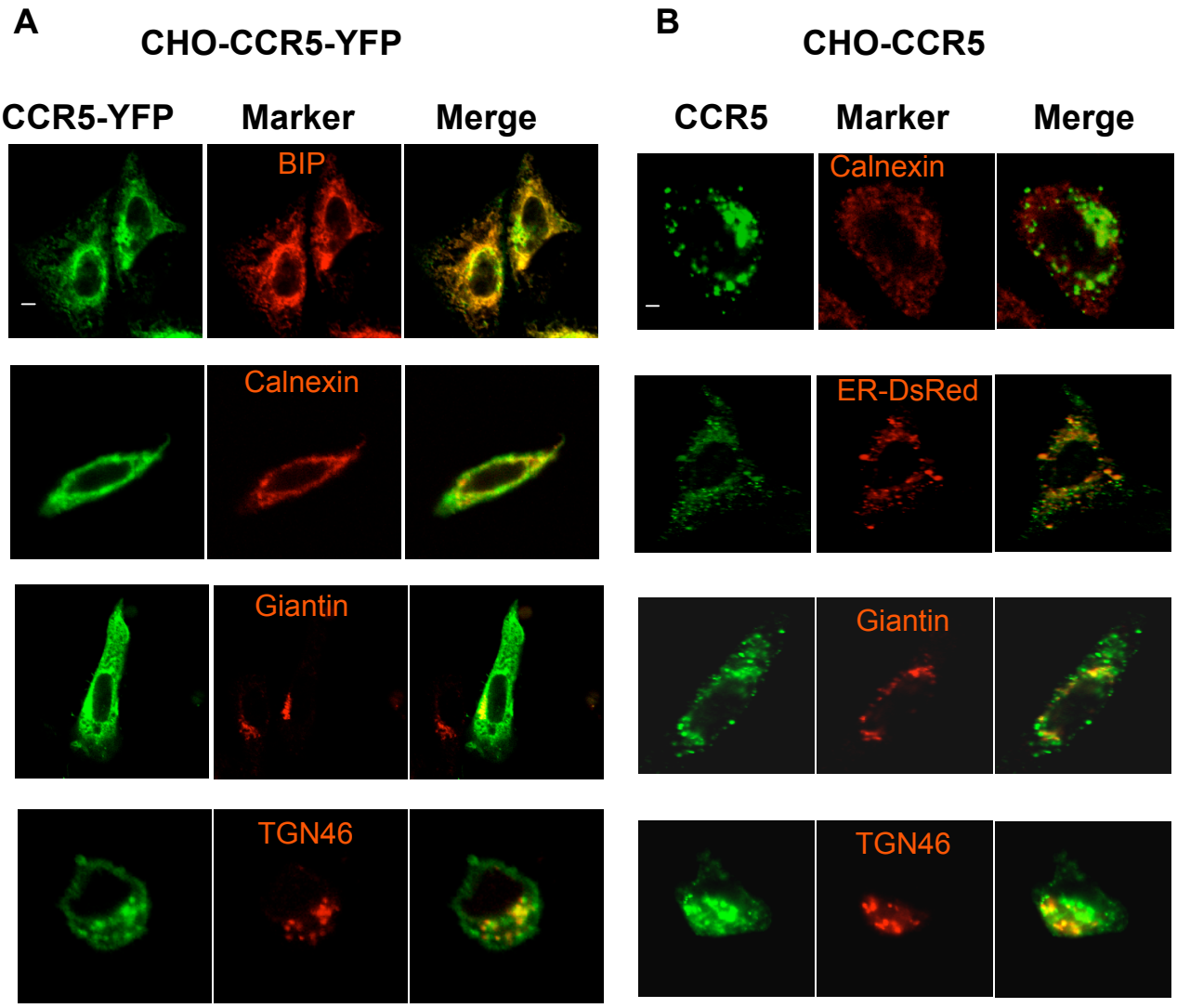
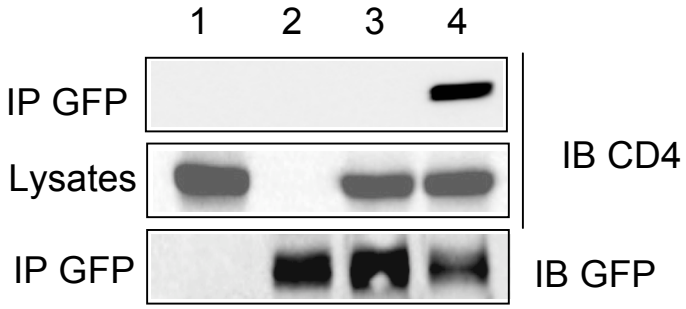


Supplemental Figure 1

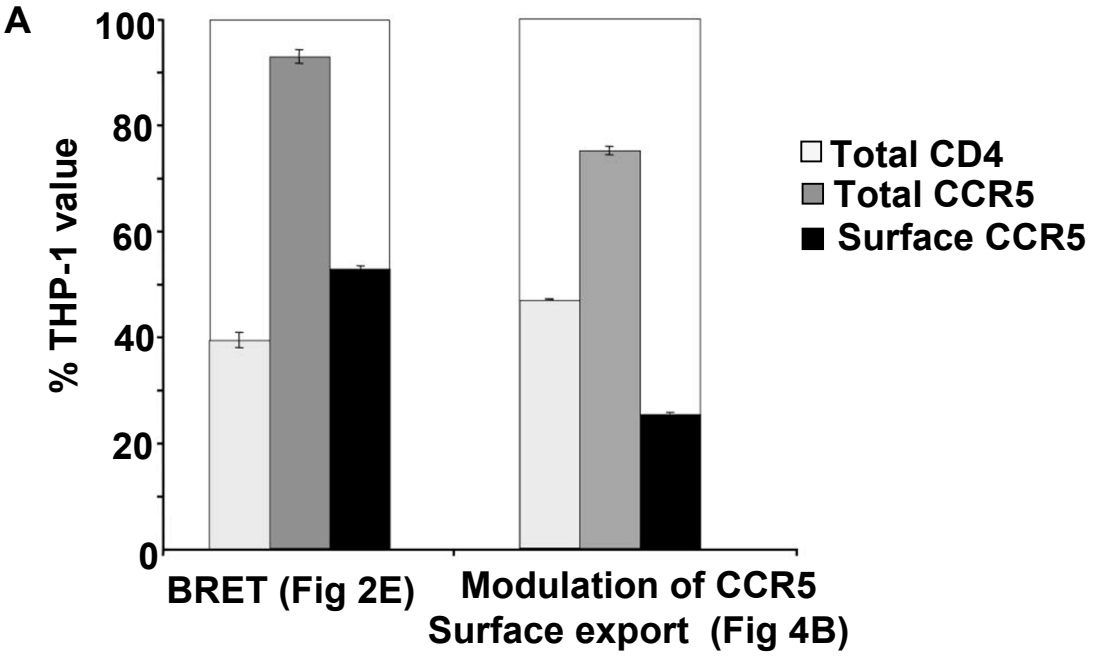


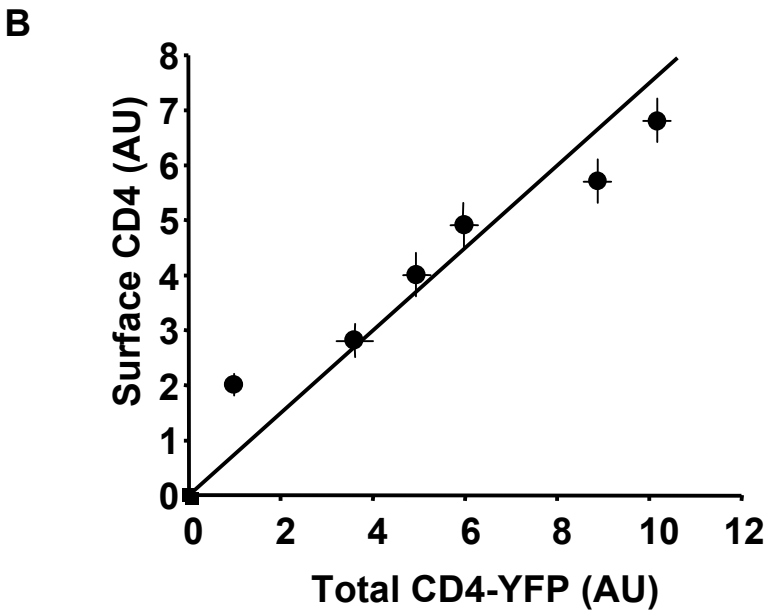
Supplemental Figure 2



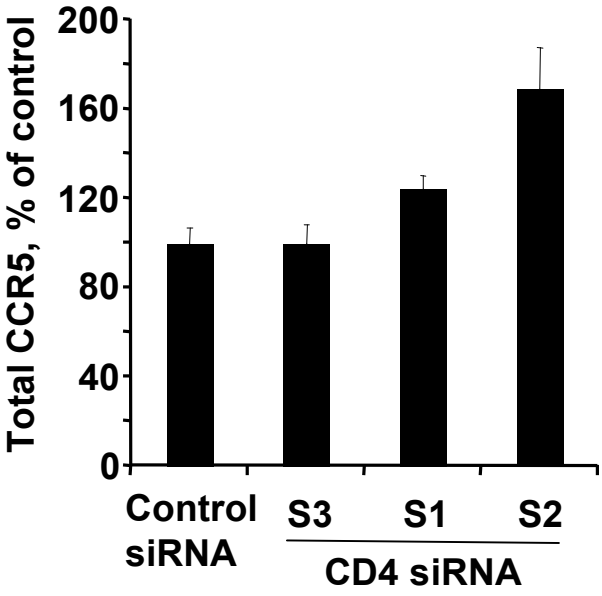
1. Transfection: CD4
2. Transfection: CCR5-YFP
3. 1 and 2 mixed before co-IP
4. Transfection CD4 and CCR5-YFP

Supplemental Figure 3

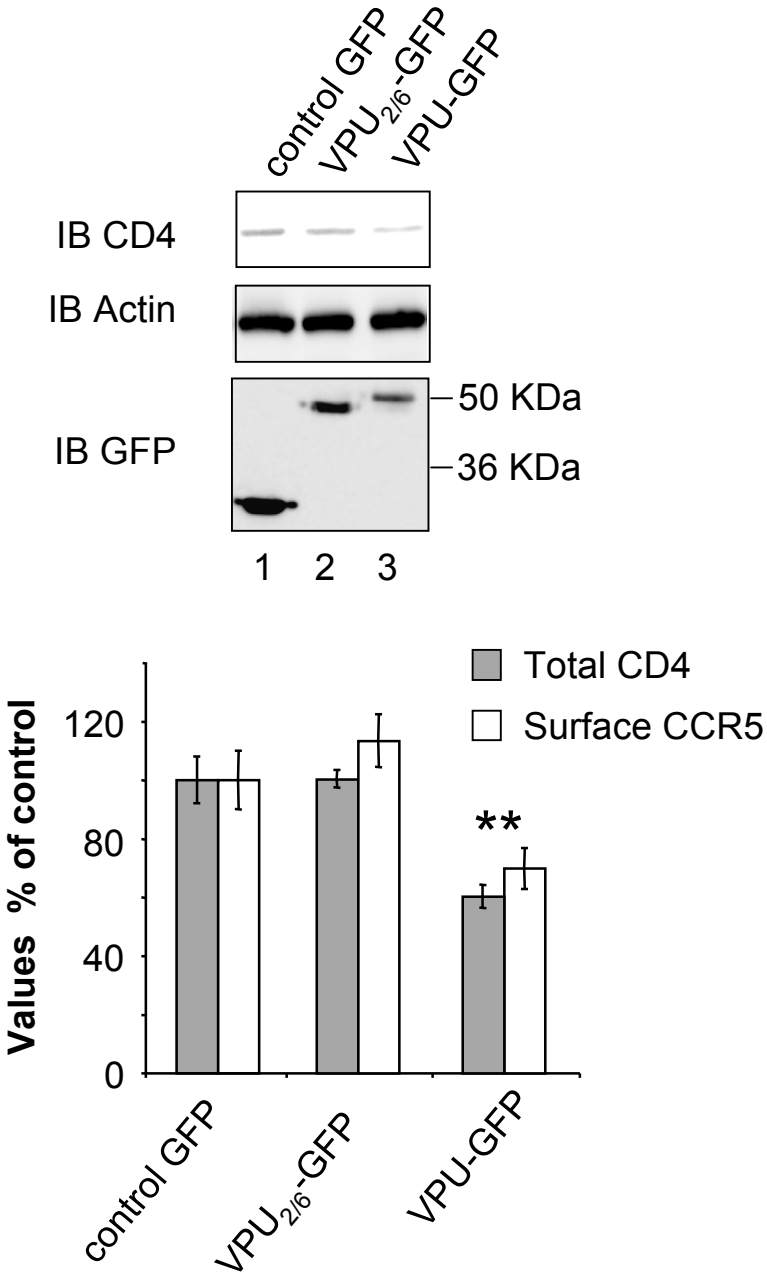




Supplemental figure 4



Supplemental Figure 5



Supplemental Figure 6