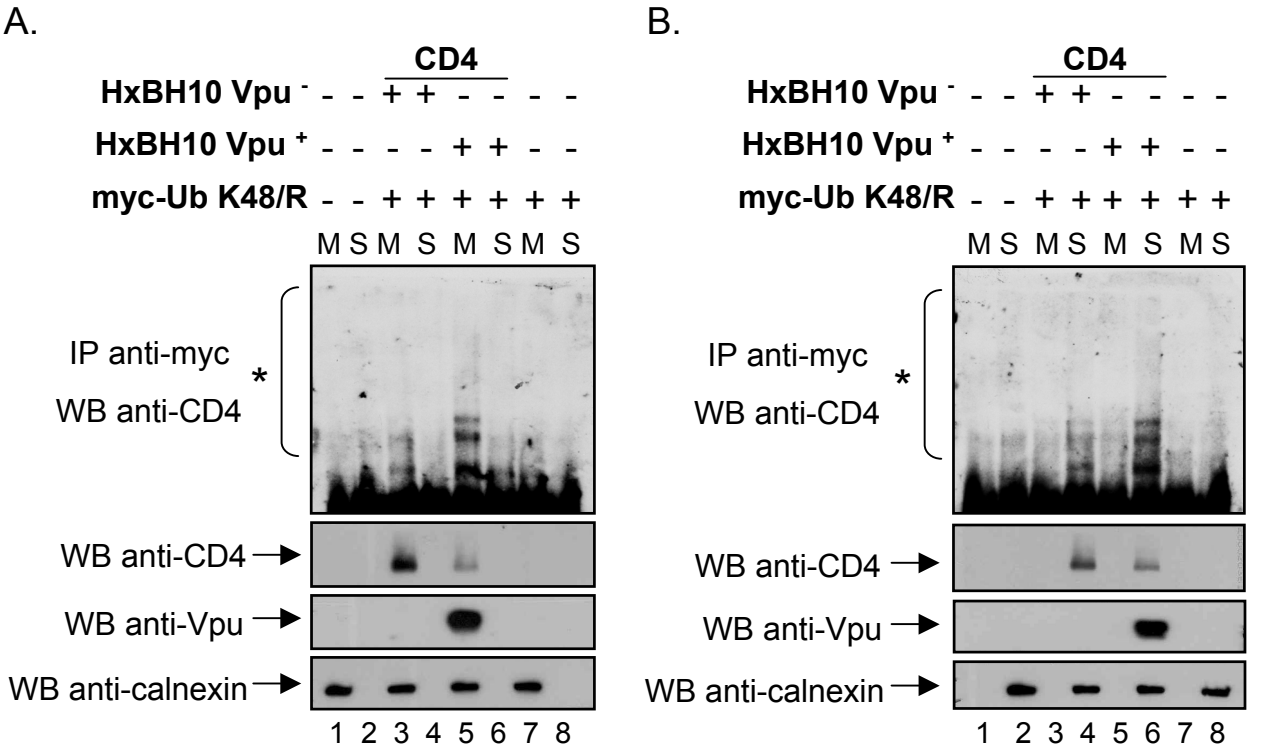

Additional file 2. HEK 293T cells were mock-transfected or co-transfected with 1 μg of pHIV CD4 wt, 10μg of envelope-defective provirus (HxBc2-pr$^-$, vpu$^-$, env$^-$ or HxBH10-pr$^-$, vpu$^+$, env$^-$) and 15 μg of his(6)/c-myc-Ub K48/R expression plasmid where indicated. Cells were treated with BFA for 2 h prior to mechanical lysis. Membrane (M) were treated with either NaCl (pH 7.0) (panel A.) or RIPA-DOC (panel B.) as described in materials and methods. The treated membranes (M) and supernatants (S) were subsequently isolated by centrifugation. CD4-Ub conjugates were immunoprecipitated with anti-myc monoclonal antibodies prior to western-blot using anti-CD4 polyclonal antibodies whereas control proteins in each fraction were directly revealed by western-blot. Calnexin was used as a membrane-associated protein control. (asterisk) represents the area of the autoradiogram that we considered as poly-ubiquitinated CD4 molecules.