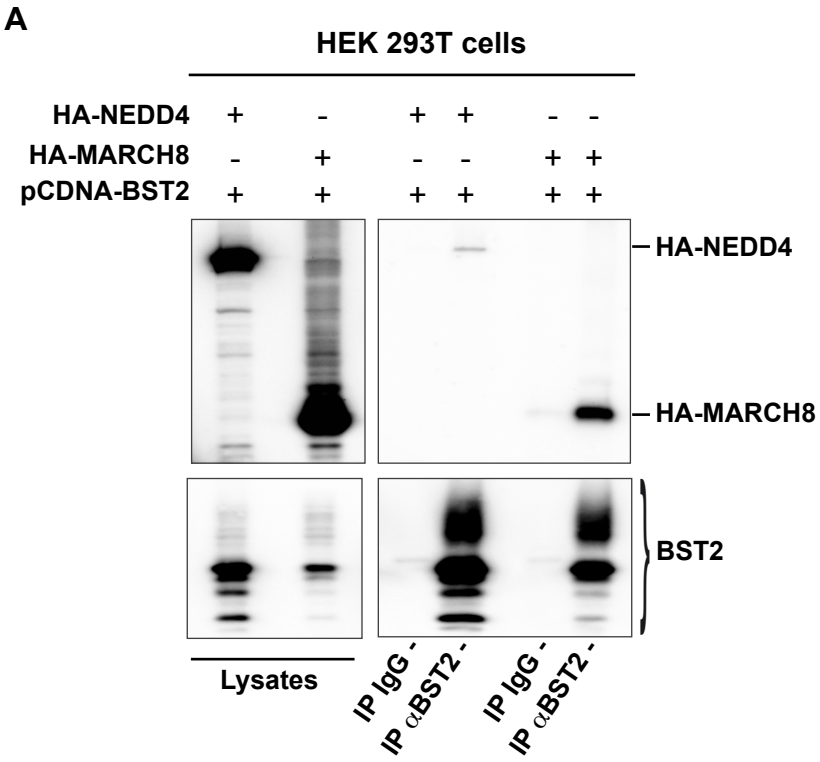
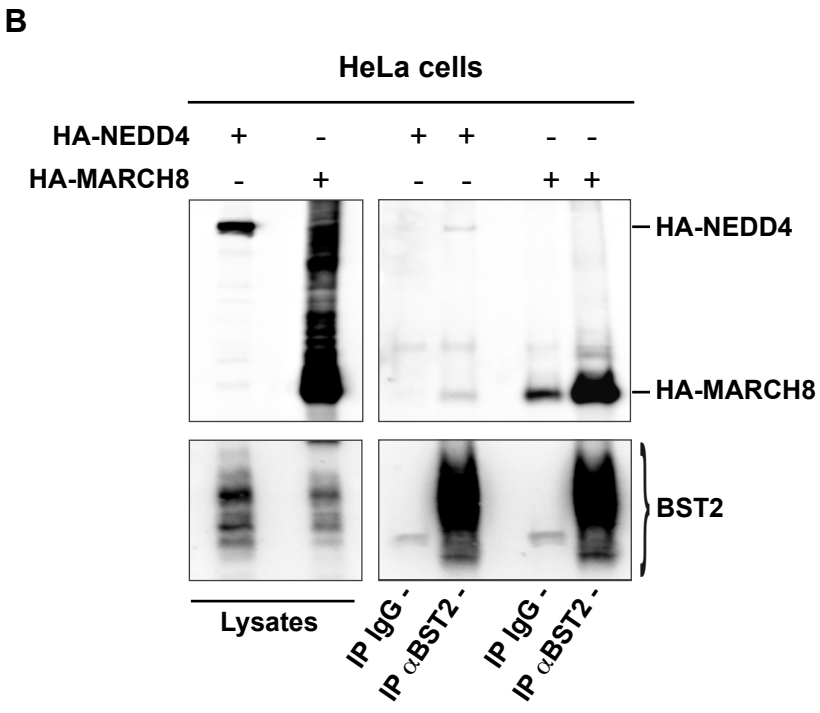
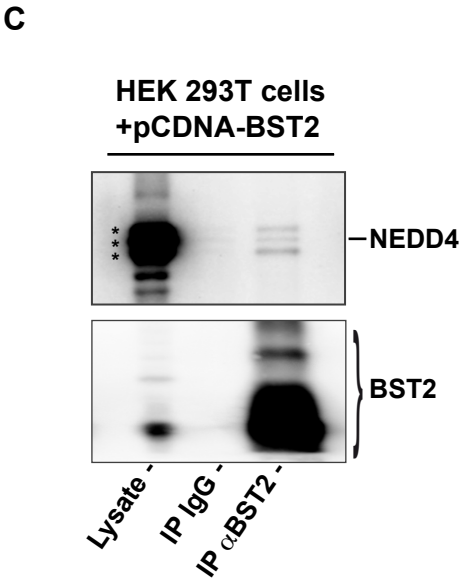
## Supplementary Figures



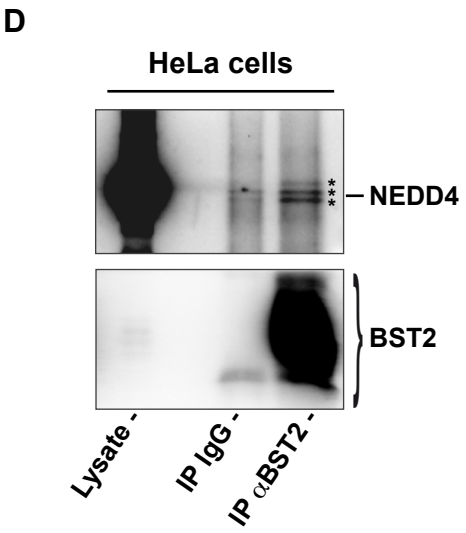



**Fig. S1 (related to Fig. 3). (A-B) Analysis of BST2 interaction with HA-NEDD4 and HA-MARCH8.** **(A)** HEK293T cells were transfected with plasmids encoding BST2 (pCDNA-BST2) along with expression vectors for HA-NEDD4 or HA-MARCH8. Binding of HA-E3 ubiquitin ligases and BST2 was assessed by immunoprecipitation using monoclonal anti-BST2 antibody or IgG1 isotype as a negative control, followed by western-blot analyses using antibodies against the HA tag and BST2. Left panels represent the input and right panels represent bound proteins. Data representative of 3 independent experiments. **(B)** HeLa cells were transfected with expression vectors for HA-NEDD4 or HA-MARCH8. Binding of the HA-ligase with endogenous BST2 was assessed as described in (A). Data representative of 3 independent experiments. **(C-D) Analysis of BST2 interaction with endogenous NEDD4.** **(C)** HEK293T cells were transfected with plasmid encoding untagged BST2. Interaction of BST2 with endogenous NEDD4 was addressed by immunoprecipitation using monoclonal anti-BST2 antibody or IgG1 isotype as a negative control. Samples were resolved on a 4-12% gradient acrylamide gel followed by western blotting using antibodies against BST2 and NEDD4. Data representative of 3 independent experiments. **(D)** Binding of endogenous BST2 with NEDD4 was assessed in Hela cells by co-immunoprecipitation assay as described in (C)**.** Data representative of 2 independent experiments. **(C-D)** Western-blotting of samples resolved on gradient acrylamide gel with anti-NEDD4 antibody, directed against the WW2 domain of NEDD4, enables detection of 3 major species (*) that might correspond to isoforms of NEDD4.

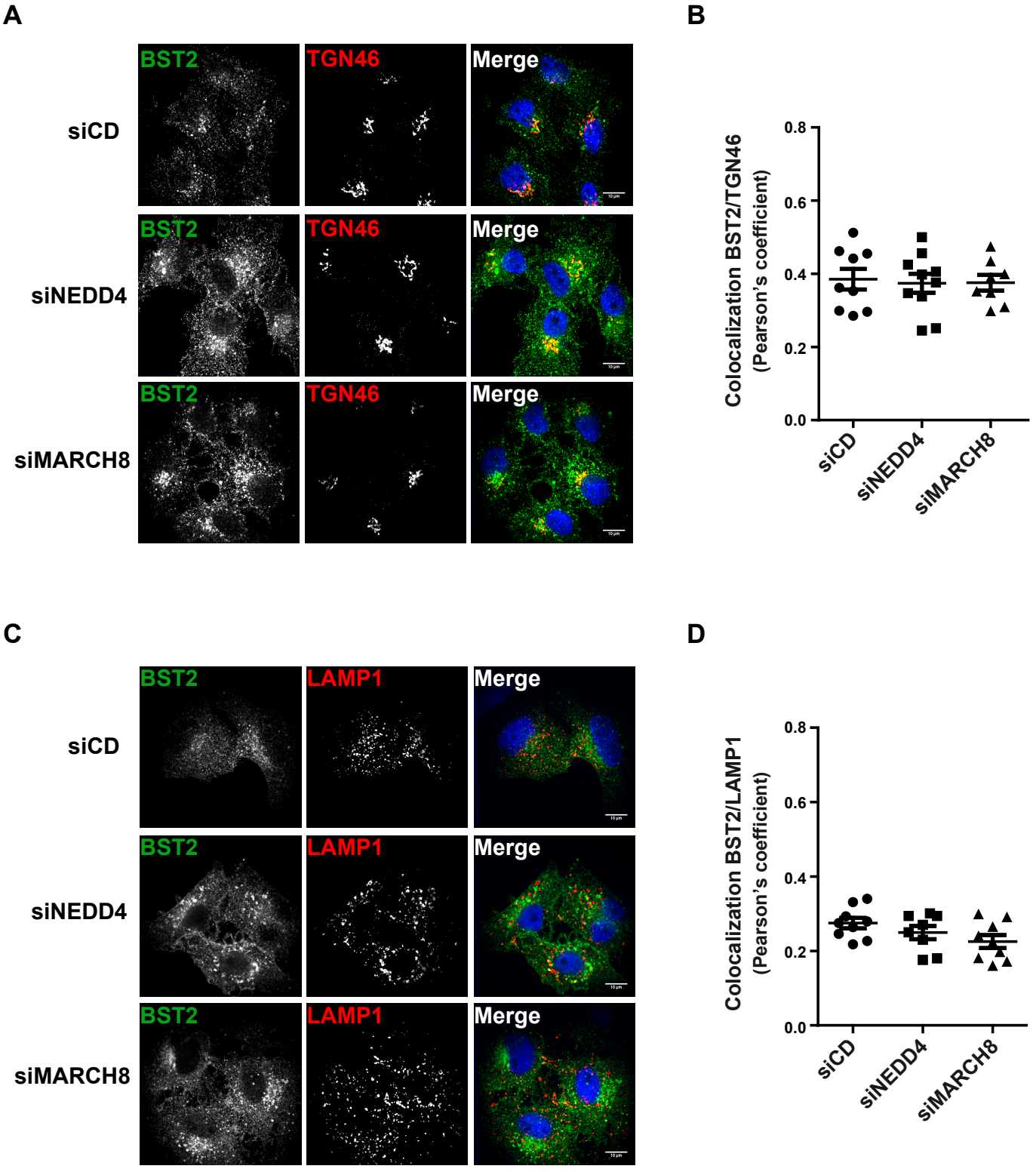


**Fig. S2 (related to Fig. 5). Effects of NEDD4, or MARCH8 silencing on BST2 subcellular localization. (A and C)** HeLa cells transfected with siRNA targeting NEDD4, MARCH8 or control siRNA, were permeabilized before fixation and immuno-stained for BST2 (green) along with the TGN marker TGN46 **(A)** or the lysosomal membrane protein LAMP1 (lysosomes) **(C)** (red) along with Dapi (blue) to visualize the nucleus of the cells, followed by analysis by confocal microscopy. Scale bars = 10μm. **(B, D) BST2-TGN46 and BST2-LAMP1 colocalizations.** Colocalization was assessed by calculating the Pearson's correlation coefficient on 8 images per conditions, using the JACoP plugin on ImageJ. Each dot represents the Pearson's correlation coefficient of one image featuring at least 3 cells. Bars represent the mean -/+ SEM from each image. The data is representative of 3 independent experiments.

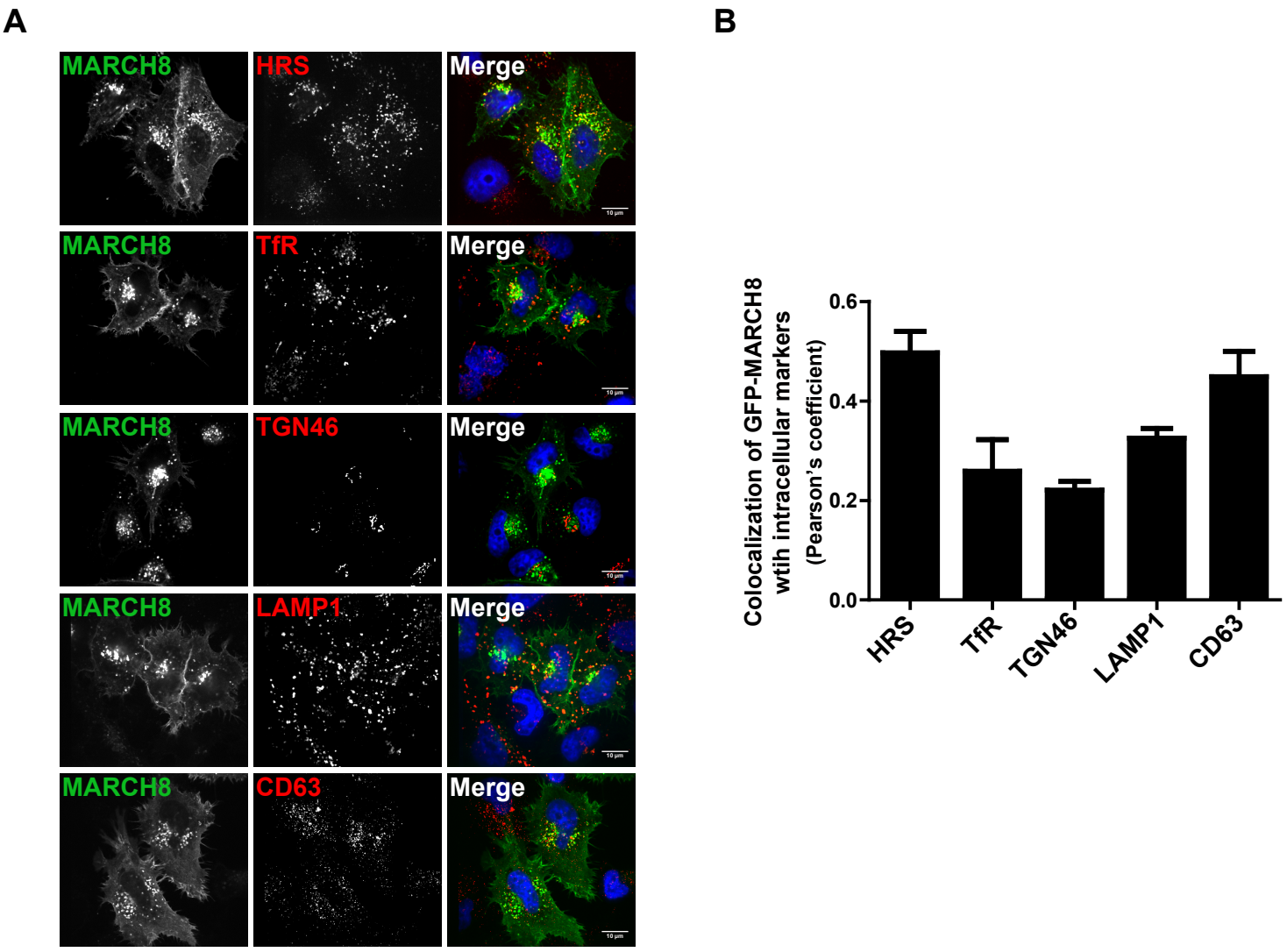



**Fig. S3 (related to Fig. 6). MARCH8 is localized at the plasma membrane and in endosomal compartments. (A)** HeLa cells were transfected with plasmid encoding GFP-tagged MARCH8 (GFP-MARCH8) (green). Twenty-four hours later, cells were permeabilized before fixation and staining with antibodies directed against specific markers of cellular compartments such as HRS (endosomes/MVBs), TfR (early/recycling endosomes), TGN46 (TGN), LAMP1 (lysosomes) or CD63 (late endosomes) (red). Cells were then analyzed by confocal microscopy. Scale bars = 10µm. Colocalizations between GFP-MARCH8 and subcellular compartment markers were assessed by calculating the Pearson's correlation coefficient on 7 images featuring at least 3 cells per conditions. Bars represent the mean -/+ SEM from each image. The data is representative of 2 independent experiments.

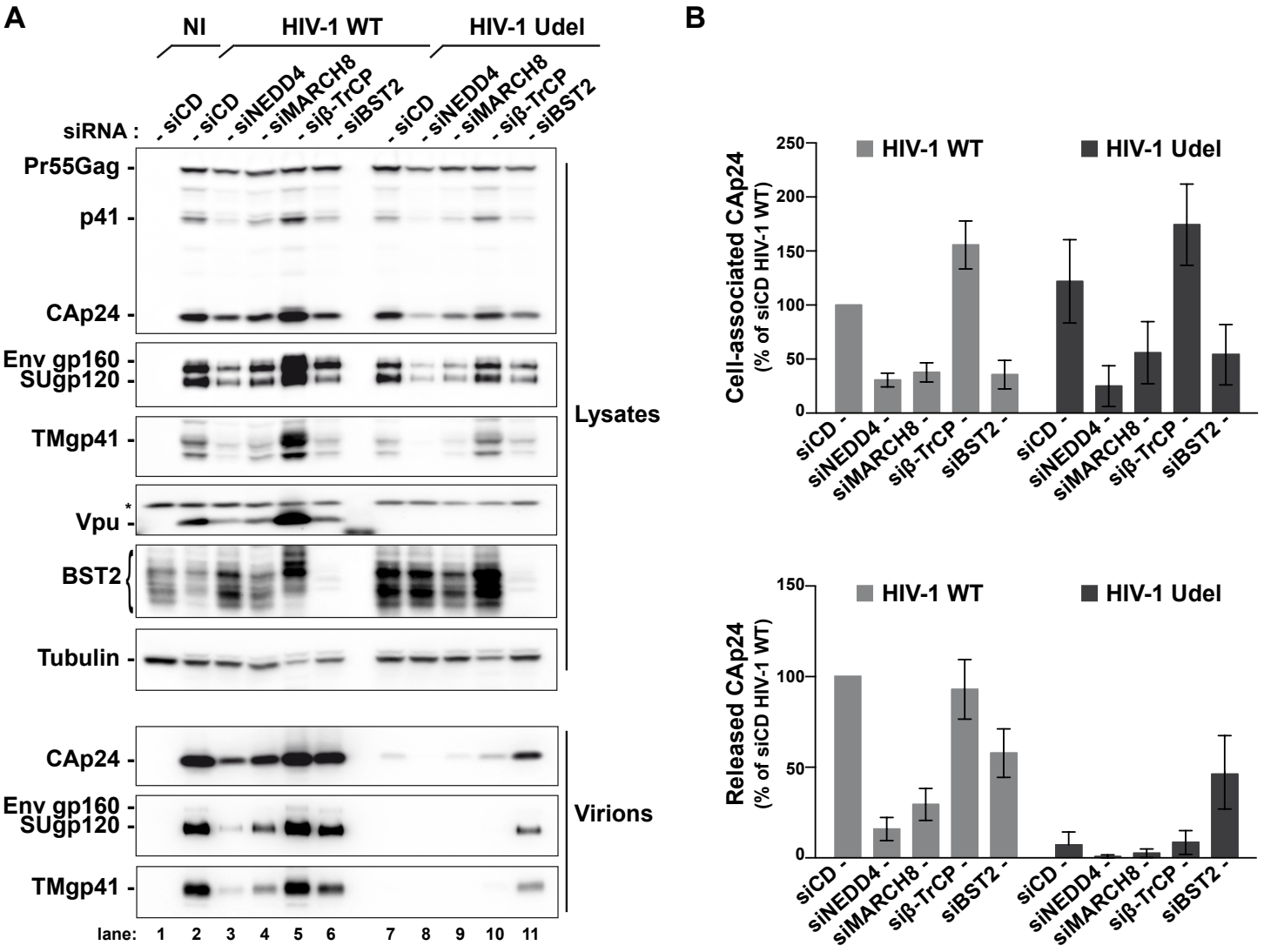



**Fig. S4 (related to Fig. 7). Consequences of NEDD4, MARCH8 and βTrCP depletion on HIV-1 production.** HeLa cells transfected with either control siRNA (siCD) or siRNA targeting BST2, NEDD4, MARCH8 or β-TrCP were infected with VSV-G pseudotyped NL4-3 HIV-1 (NL4-3 WT) or VSV-G pseudotyped Vpu-defective NL4-3 (NL4-3 Udel) at a MOI of ~0,5. **(A)** Western blot analyses of infected siRNA treated cells (upper panels) and pelleted virus (lower panels) with antibodies against BST2, Gag; Env, Vpu and Tubulin as a loading control. Asterisks (*) indicate non-specific bands. **(B)** CAp24 present within the cells (Cell-associated CAp24) and released in the supernatant of the infected cells (Released CAp24) was measured by ELISA. The values were normalized to those obtained for control cells (siCD) infected with WT viruses set to 100%. Bars represent the mean -/+ SD from 5 independent experiments.

**Table S1**: Primary and secondary antibodies used for western blot analyses

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**Table S2:** Primary and secondary antibodies used for Immunofluorescence staining followed by Microscopy or Flow cytometry analyses

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