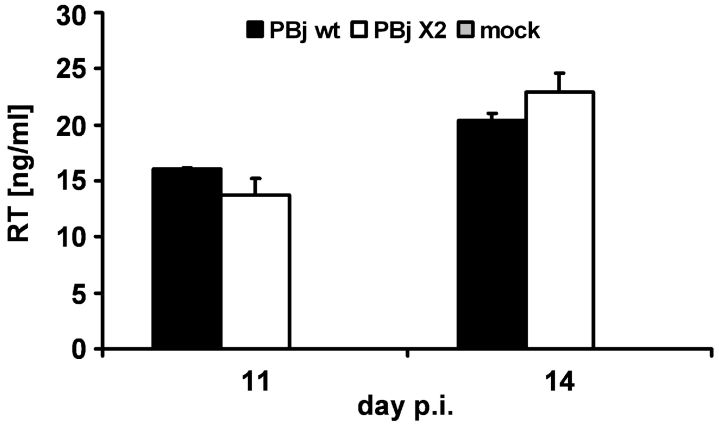
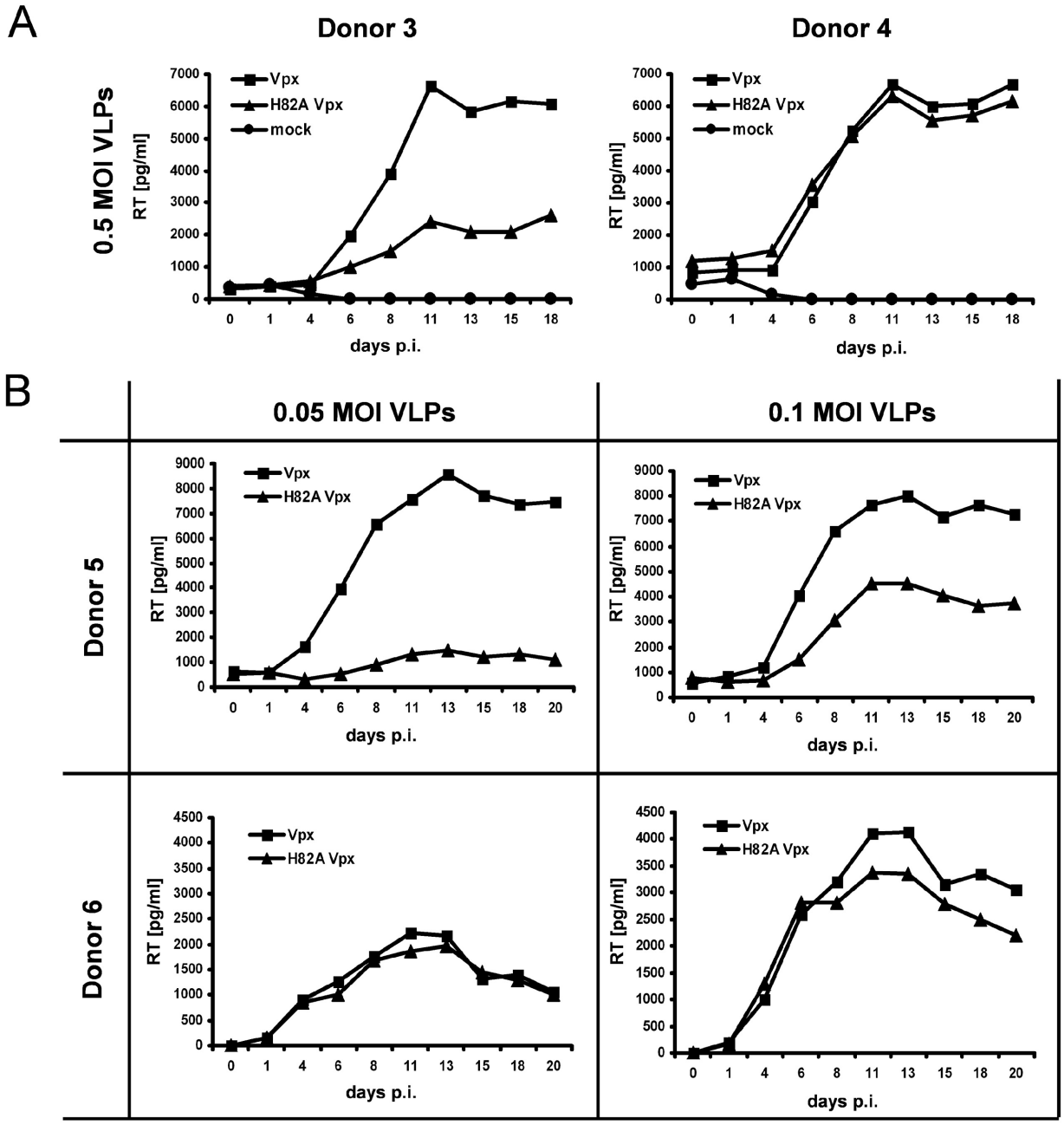
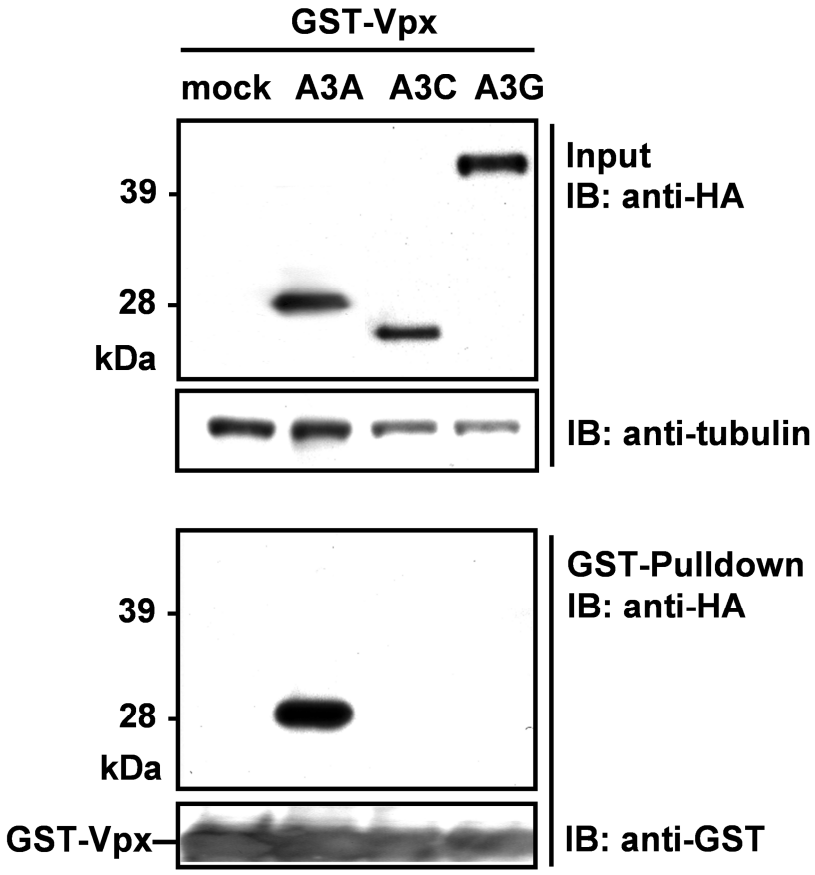


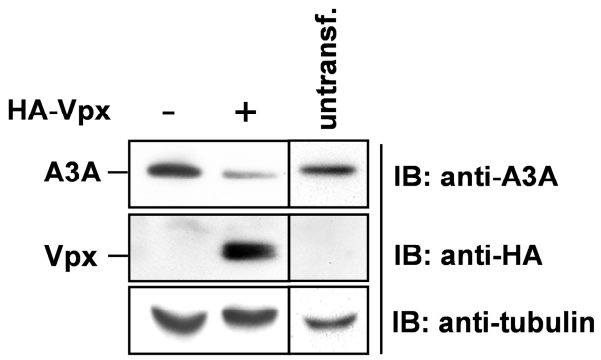
Suppl. Fig. 1. PBj wt and PBj X2 replicate in C8166 lymphocytes. C8166 cells were infected with 0.3 MOI of PBj wt, PBj X2 or left uninfected. The cells were passaged equally and at days 11 and 14, the supernatant was analyzed for RT concentration.



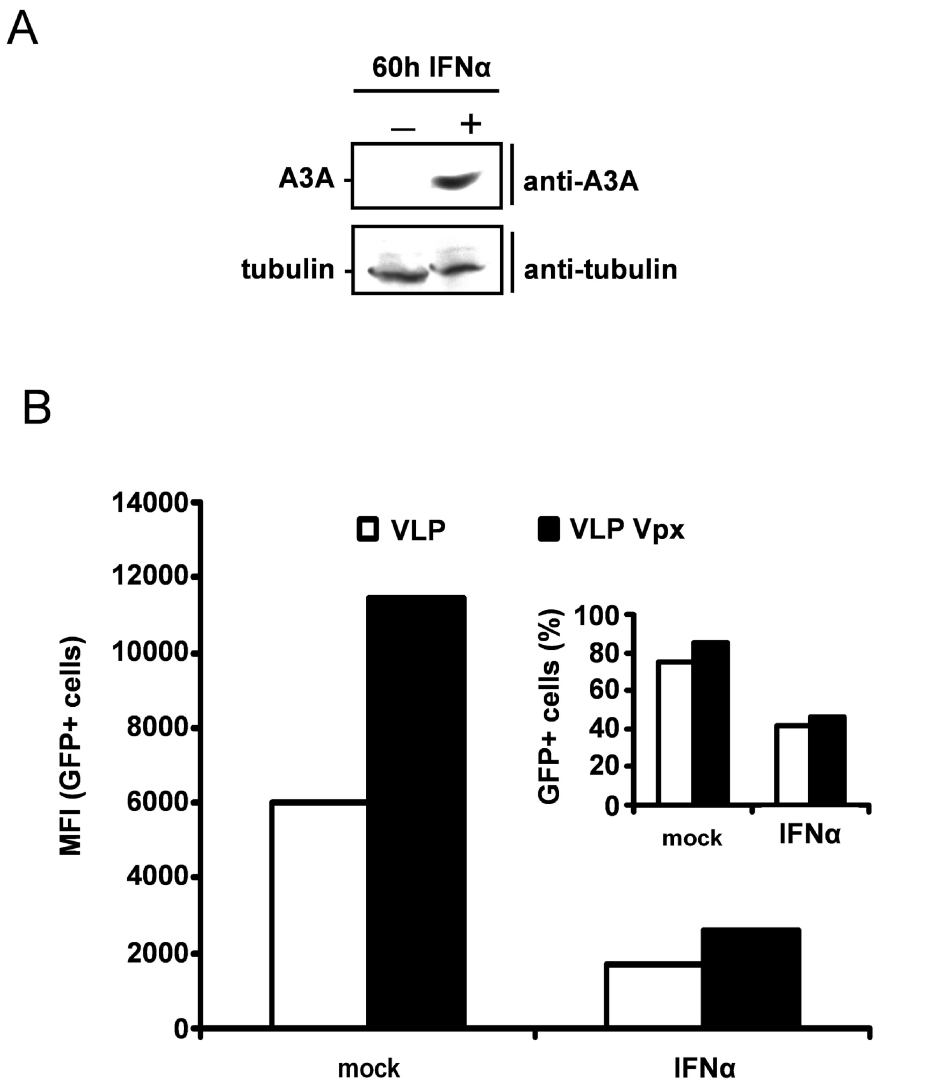
Suppl. Fig. 2. Vpx enhances HIV-1 replication in human monocytes and H82A Vpx is impaired in a dose- and donor-dependent manner. *A.* 5x10^5 human monocytes were freshly isolated from healthy donors with the Monocyte Isolation Kit II (Miltenyi) and incubated with 0.5 MOI of Vpx, H82A Vpx or empty VLPs and 1 MOI HIV-1 (SF162) After 5h, the cells were washed and new medium was applied. At indicated days post-infection, reverse transcriptase concentration in the supernatant was determined with the Lenti RT Activity Kit (Cavidi). *B*. Experiments were performed as described in A, but human monocytes were either incubated with 0.05 or 0.1 MOIeq of Vpx or H82A Vpx-containing VLPs prior HIV-1 infection.



Suppl. Fig. 3. GST-Vpx does interact with A3A, but not with A3C or A3G. 10µg purified recombinant GST-Vpx and 20µl glutathione sepharose 4B (50% resin) (GE Healthcare) were incubated with 600µg 293T cell lysates that transiently expressed HA-A3A, HA-A3C or HA-A3G (upper panel). After 1h gentle rotation at 4°C, the resin was washed 4 times with lysis buffer. GST-Vpx-associated APOBECs were analyzed via immunoblot using an anti-HA antibody. GST-Vpx was detected with an anti-GST antibody (lower panel).



Suppl. Fig. 4. Vpx reduces endogenous A3A protein levels in U937 monocytes. $1x10^6$ U937 cells were nucleofected with either 2µg empty vector or HA-Vpx encoding plasmid using the Cell Line Nucleofector Kit C (Amaxa) and the manufacturer's recommended protocol. After two days, protein levels of A3A and Vpx were analyzed with Western Blot.



Suppl. Fig. 5. Vpx enhances transgene expression but does not overcome the IFNα-induced restriction in cycling THP-1 monocytes. *A*. THP-1 cells were incubated with 20ng/ml IFNα or solvent for 60h and subjected to Western Blot analysis with an anti-A3A antibody. *B*. $5x10^5$ THP-1 cells were stimulated with 20ng/ml IFNα or solvent for 5h and subsequently incubated with 1 MOI non-replicative HIV-1-EGFP reporter virus in the presence of 1 MOIeq of Vpx VLPs or empty VLPs. After 5 days, cells were washed and the amount of GFP+ cells and their mean fluorescence intensity (MFI) was determined by FACS analysis.