**Supplementary Figure 1.** a-e Luciferase assays of HEK-293 cells transfected with the indicated luciferase reporter plasmids and pcDNA3.1 expression plasmids for the indicated genes. f, The role of TRIM5 in signaling. Horizontal double line, cytoplasmic membrane; vertical dashed line separates the two waves of innate signaling.
Supplementary Figure 2. a, Schematic of the lentiviral vector pAPM where both puromycin N-acetyltransferase and microRNA-based shRNA expression are driven by the spleen focus forming virus (SFFV) pol II promoter. b, TE671 cells, transduced with pAPM stably expressing the indicated shRNAs, were assayed for TRIM5 knockdown by qRT-PCR. c, cycling THP-1 monocytes, stably expressing pAPM shRNA targeting TRIM5 or control shRNA, were challenged with VSV-G-pseudotyped N- or B-MLV GFP reporter vectors and assayed by FACS 72 h post-transduction. d, qRT-PCR for the indicated mRNAs harvested 2 to 8 hrs after LPS-treatment of THP-1 macrophages, depending on the peak values for that gene. e, qRT-PCR for the indicated mRNAs harvested 2 to 8 hrs after LPS-treatment of MDM, depending on the peak values for that gene.
Supplementary Figure 3. MDDC (a) or MDM (b) were treated with the indicated compounds for 24 h, challenged with a VSV-G-pseudotyped HIV-1 GFP reporter minimal vector, and assayed by FACS 72 h post-transduction. TPA-differentiated THP-1 cells (c) were treated with ultrapure *E. coli* K12 LPS for 24 h and challenged with a VSV-G-pseudotyped HIV-1 luciferase reporter virus, and assayed by luciferase assay 72 h post-transduction. MDM (d) or TPA-differentiated THP-1 cells (e) were treated with the indicated compounds for 16 h, and qRT-PCR was performed for TRIM5. TPA-differentiated THP-1 cells stably expressing human TRIM5α, or empty vector control cells, were challenged with an HIV-1 luciferase reporter virus (f) or Vesicular Stomatitis Virus bearing a GFP reporter (g). HIV-1 was assayed by luciferase assay 72 h after challenge and VSV was assayed by FACS 20 h after challenge.
Supplementary Figure 4. a, CD4/CCR5-THP-1 macrophages transduced with lentiviral vectors expressing pol II-driven microRNA-based shRNAs targeting TRIM5 or control, were treated with LPS or vehicle, and challenged with CCR5-tropic HIV-1 luciferase reporter virus. b, Correlation between TRIM5 KD efficiency, as measured by qRT-PCR, and magnitude rescue of HIV-1 infectivity from LPS.
from LPS. Immunoblot of whole-cell lysates from TPA-differentiated THP-1 cells transduced with pAPM expressing shRNA targeting IRF3 (c), STAT2 (d) or control shRNA. STAT2 KD and control KD cells were either treated with recombinant IFN-β for 16 h, or left untreated, prior to lysis. e, THP-1 macrophages were treated with LPS or vehicle and challenged with a VSV G-pseudotyped SIVMAC239GFP reporter virus. MDM (f) or MDDCs (g-i), transduced with lentiviral KD vectors targeting TRIM5 (f and h) or IRF3 (g and i), were treated for 20 hrs with LPS (f) or as indicated (h and i), and then challenged with an SIVMAC239GFP reporter virus. Results are reported as percent infected (i) or as fold-change compared to control (h and i). Lysates were probed with the indicated antibodies in (g).
**Supplementary Figure 5.** a, Immunoblot of the indicated FLAG-tagged proteins immunoprecipitated from transfected 293T cells. b, Immunoblot of TAK1 knockdown in HEK-293 cells. c, Luciferase assay of TAK1 or control KD HEK-293 cells transfected with an AP-1 luciferase reporter plasmid, and either empty pcDNA or a c-Jun expression plasmid. d, Immunoblot of TAK1 KD TPA-differentiated THP-1 cells. e, THP-1 cells expressing either wild type AtTRIM5Cyp or the H436Q mutant (or an empty vector control line) were challenged with a VSV-G-pseudotyped HIV-1 GFP reporter virus and analyzed by FACS 72 after transduction. f, PCR for full-length N-tropic MLV viral cDNA in TRIM5, TAK1, or control KD THP-1 cells.
Supplementary Figure 6. a, Immunoblot of UBC13 KD in HEK-293 cells. b, Luciferase assay of UBC13 or control KD HEK-293 cells transfected with an AP-1 luciferase reporter plasmid, and either empty pcDNA or a c-Jun expression plasmid. c, Luciferase assay of control KD, UBC13 KD, or UEV1A KD HEK-293 cells transfected with an AP-1 luciferase reporter plasmid, and a human TRIM5α expression plasmid. d, Immunoblot of UBC13 KD in TPA-differentiated THP-1 cells. e, HEK-293 cells were transfected with the indicated plasmids, along with an NF-κB luciferase reporter. f, Immunoblot of whole cell lysates from HEK-293 cells transfected with the indicated plasmids.
Supplementary Figure 7. a, Coomassie-stained SDS-PAGE gels showing insoluble (Pellets) or soluble (Elution) protein fractions. N-terminal tag fusions to Owl monkey TRIM5Cyp and human TRIM5α were screened for soluble expression in transfected Sf9 cells. The fusion constructs all carried an N-terminal His-tag. To detect soluble expressed constructs, fusion constructs were batch purified on Ni-NTA beads and analyzed by SDS-PAGE after elution with 250 mmol/L imidazole (Elution). Insoluble fractions were obtained by centrifugation of lysed cells (Pellets). b, Gel filtration chromatogram showing the elution profile of recombinant TRIM5Cyp protein on a Superdex 200 column, and a coomassie-stained SDS-PAGE gel of the indicated fractions (insert). The apparent molecular mass was estimated by comparison to standard molecular weight markers depicted in the chromatogram. c, d, SEC-MALS results. The elution profiles are depicted in refractive index detection and the calculated
molecular masses obtained from the light scattering data are shown as
distributions across the peaks. Comparable molecular masses were obtained
for TRIM5Cyp WT (c) and TRIM5Cyp H436Q (d) showing that both proteins
behave the same in solution.
Supplementary Figure 8. a, Products of in vitro reactions with ATP, purified Ubiquitin, UBE1, the UBC13/UEV1A E2 complex, and increasing amounts of the E3 Ubiquitin Ligase AtTRIM5Cyp, revealed by Coomassie. Gel pieces in the higher molecular weight (> 170 kDa) and lower molecular weight (40 – 70 kDa) regions were cut out separately (black boxes) and analyzed by MALDI-MS/MS after in-gel tryptic digestion. b, Immunoblot of products from in vitro reactions with ATP, purified WT Ubiquitin, or the indicated Ubiquitin mutants, UBE1, the UBC13/UEV1A E2 complex, and AtTRIM5Cyp. c, Immunoblot of the indicated FLAG-tagged proteins immunoprecipitated from lysates of transfected HEK-293 cells. d, Immunoblot of products of in vitro reactions with ATP, purified Ubiquitin, UBE1, the UBC13/UEV1A E2 complex, and the indicated transfected FLAG-tagged proteins immunoprecipitated from HEK-293 lysates.
**Supplementary Figure 9.** Identification of K63-linked polyubiquitin chains formed by the E3-ligase activity of AtTRIM5Cyp: Gel pieces containing products from an *in vitro* ubiquitinylation assay are analyzed by MS after in-gel tryptic digestion. (a) MALDI spectrum of tryptically digested fragments: Mass over charge ratios (m/z) of signals matching theoretical peptide fragments of Ubiquitin are labeled in the spectrum. Numbers below the indicated m/z ratios correspond to residues in the Ubiquitin sequence (shown on top of the spectrum). Detected signals cover 94% of the Ubiquitin sequence. Fragments with detected masses matching Ubiquitin peptides carrying a modified lysine are marked as red numbers in the spectrum and were further analyzed by MS/MS. MS/MS spectra of the peptides 2244.2 m/z (b) and 2513.4 mZ (c).
show signals for fragment ions (a-, b-, and y-ions labelled in different colours) with mass differences corresponding to single amino acids in the peptide sequence (shown on top of the spectrum). The mass differences $K^*$ match a lysine modified with amino acids GG or LRGG. (b) MS/MS spectrum identifying fragment 2244.2 m/z as Ubiquitin peptide 55-72 with additional amino acids GG linked to K63 (see inset). (c) MS/MS spectrum identifying fragment 2513.4 m/z as Ubiquitin peptide 55-72 with additional amino acids LRGG linked to K63 (see inset).
**Supplementary Figure 10.** Figure S10: Purification of recombinant TAK1 complex components. In panel a, FS-TAK1, FS-TAB1, and FS-TAB2 were produced in 293T and purified by Streptactin Sepharose chromatography as described under Experimental Procedures. Aliquots (1 µL) of the soluble lysate (lane L), the Streptactin Sepharose flow-through (lane FT), and wash 1 (lane W1) fractions, and aliquots (10 µl) of the wash 5 (lane W5), and the 2.5 mM Desthiobiotin eluate fractions were analyzed by SDS–PAGE. The polypeptides were visualized by staining the gel with Coomassie Blue dye. The positions and sizes (kDa) of marker polypeptides are indicated. In panel b, c, d, and e, aliquots (10 µl) were analyzed by immunoblotting with the indicated antibodies.
Supplementary Figure 11. qRT-PCR for the indicated mRNAs from MDM (a) or MDDC (b). mRNA was harvested 6 h after challenge with VSV-G-pseudotyped N-tropic or B-tropic MLV. Data are expressed as fold change versus media control.
**Supplementary Figure 12.**

a, Electron micrograph of HIV-1 capsid tubes made with oxidized A14C/E45C mutant in the absence of salt (magnification, 33,000x).
b, Coomassie-stained SDS-PAGE gels of samples from the *in vitro* ubiquitylation time-course reactions using recombinant TRIM5Cyp WT or the H436Q mutant carried out in the presence of UBE1 and UBC13/UEV1A, with or without the addition of assembled HIV-1 capsid (CA A14C E45C).
c, Immunoblot of samples from an *in vitro* ubiquitylation time-course reaction using recombinant TRIM5Cyp WT or the H436Q mutant, carried out in the presence of UBE1 and UBC13/UEV1A.
d, Immunoblot of samples from an *in vitro* ubiquitylation time-course reaction using the recombinant TRIM5Cyp H436Q mutant, carried out in the presence of UBE1 and UBC13/UEV1A, with or without the addition of assembled HIV-1 capsid (CA A14C E45C).