LETTER

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TRIM5 is an innate immune sensor for the retrovirus capsid lattice

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TRIM5 is a RING domain-E3 ubiquitin ligase that restricts infection by human immunodeficiency virus (HIV)-1 and other retroviruses immediately following virus invasion of the target cell cytoplasm[1,2]. Antiviral potency correlates with TRIM5 avidity for the retrovirion capsid lattice[3,4] and several reports indicate that TRIM5 has a role in signal transduction[5–7], but the precise mechanism of restriction is unknown[8]. Here we demonstrate that TRIM5 promotes innate immune signalling and that this activity is amplified by retroviral infection and interaction with the capsid lattice. Acting with the heterodimeric, ubiquitin-conjugating enzyme UBC13–UEV1A (also known as UBE2N–UBE2V1), TRIM5 catalyses the synthesis of unattached K63-linked ubiquitin chains that activate the TAK1 (also known as MAP3K7) kinase complex and stimulate AP-1 and NFκB signalling. Interaction with the HIV-1 capsid lattice greatly enhances the UBC13–UEV1A-dependent E3 activity of TRIM5 and challenge with retroviruses induces the transcription of AP-1 and NF-κB-dependent factors with a magnitude that tracks with TRIM5 avidity for the invading capsid. Finally, TAK1 and UBC13–UEV1A contribute to capsid-specific restriction by TRIM5. Thus, the retroviral restriction factor TRIM5 has two additional activities that are linked to restriction: it constitutively promotes innate immune signalling and it acts as a pattern recognition receptor specific for the retrovirus capsid lattice.

To determine if TRIM5 contributes to signal transduction, the effect of ectopic human TRIM5α expression on transcriptional reporters in HEK-293 cells was examined. TRIM5 stimulated either of two luciferase reporters for AP-1 with a magnitude comparable to that of MAVS or the AP-1 transcription factor c-Jun (Fig. 1a and Supplementary Fig. 1a). TRIM5 also stimulated NF-κB (Fig. 1b) but minimally activated *IFNB1*-, or IRF3-dependent, luciferase reporters (Fig. 1c and Supplementary Fig. 1b and c). The TRIM5–cyclophilin A fusion protein from owl monkey[1] activated AP-1 and NF-κB to similar levels as human TRIM5α (Supplementary Fig. 1d, e). Although TRIM5 was not sufficient to activate *IFNB1*, induction of *IFNB1* by IRF3 was greatly enhanced by TRIM5 (Fig. 1c), consistent with the fact that *IFNB1* transcription requires NF-κB and AP-1, as well as IRF3 (Supplementary Fig. 1f)[9].

To determine if endogenous TRIM5 regulates AP-1 and NF-κB signalling pathways, the effect of *TRIM5* knockdown was assessed in myeloid cells. THP-1 cells were transduced with lentiviral vectors engineered to confer puromycin-resistance and to express RNA polymerase II (Pol II)-driven, microRNA-based short hairpin RNAs (shRNAs) targeting either *TRIM5* or control RNAs (Supplementary Fig. 2a–c). Pools of puromycin-resistant cells were generated with each knockdown vector and global expression profiles were assessed. The effect of *TRIM5* knockdown was extraordinarily specific in that, of 25,000 genes probed, only 33 were significantly decreased (Fig. 1d). The majority of these were NF-κB- and AP-1-responsive inflammatory mediators, 70% being inflammatory chemokines and cytokines (Supplementary Table 1).

Lipopolysaccharide (LPS), a pathogen-associated molecular pattern (PAMP) recognized by the pattern recognition receptor (PRR) TLR4-MD-2, activates AP-1 and NF-κB-signalling and this culminates in the expression of inflammatory genes like those perturbed by *TRIM5* knockdown[10,11]. Monocyte-derived dendritic cells (MDDC), macrophages (MDM) and THP-1 cells were challenged with LPS and induction of the AP-1- and NF-κB-dependent genes *CXCL9*, *CXCL10*, *CCL8*, *IL6*, *IL8* and *PTGS2* (also known as *COX2*), was found to be attenuated by *TRIM5* knockdown (Fig. 1e and f and Supplementary Fig. 2d and e). These results demonstrate that TRIM5 activates MAPK- and NF-κB-dependent genes and makes a major contribution to LPS signalling and gene induction (Supplementary Fig. 1f).

Given the contribution of TRIM5 to the production of inflammatory mediators by LPS, the effect of TRIM5 on the previously reported anti-HIV-1 activity of LPS[12] was examined. Transduction of MDDC, MDM or THP-1 macrophages by vesicular stomatitis virus (VSV) G-pseudotyped HIV-1 was blocked by LPS, by other PAMPs, and by type 1 IFN (Supplementary Fig. 3a–c). *TRIM5* mRNA increased tenfold in response to these factors (Supplementary Fig. 3d, e), but this increase was not sufficient for the anti-HIV-1 state (Supplementary Fig. 3f, g). Nonetheless, *TRIM5* knockdown rescued HIV-1 from LPS, although not from type 1 IFN, and the magnitude rescue correlated with the efficiency of *TRIM5* knockdown (Fig. 1g and Supplementary Fig. 4a and b). These phenotypes were indistinguishable from those observed with knockdown of *IRF3*, a critical transcription factor that acts proximal to *IFNB1* (ref. 10; Fig. 1h and Supplementary Fig. 4c). In contrast, knockdown of *STAT2*, a factor that acts downstream of the type I IFN receptor, blocked the anti-HIV-1 activity of either LPS or type 1 IFN (Fig. 1i and Supplementary Fig. 4d).

Rescue from LPS seems to be independent of capsid-recognition by TRIM5 in that *TRIM5* knockdown rescued a molecular clone of simian immunodeficiency virus (SIV_{MAC}), a retrovirus that differs greatly from HIV-1 in terms of its sensitivity to TRIM5-mediated restriction[1,2], as well as two non-retroviruses, the rhabdovirus vesicular stomatitis virus and the paramyxovirus Newcastle disease virus (Fig. 1j and Supplementary Fig. 4e–i). Although TRIM5 is not sufficient to activate IFNB1 (Fig. 1c), it promotes the first wave of innate immune signalling upstream of IFNB1 and thereby contributes to the antiviral state established by LPS (Supplementary Fig. 1f).

To understand how TRIM5 activates AP-1 and NF-κB, 20 candidate proteins, selected on the basis of signalling activity above the MAPK/NF-κB bifurcation in the LPS signalling pathway, were tested for the ability to immunoprecipitate with TRIM5. Strong signal was observed with TAK1, TAB2, and TAB3 (Fig. 2a and Supplementary Fig. 5a), all components of the TAK1 kinase complex that phosphorylates proximal MAPK and NF-κB kinases in response to LPS[11]. Like TRIM5, TAK1 potently activated AP-1 and modestly activated NF-κB (Fig. 2b). 5Z-7-oxozeaenol, a TAK1-inhibitor, blocked AP-1 induction by TRIM5 or

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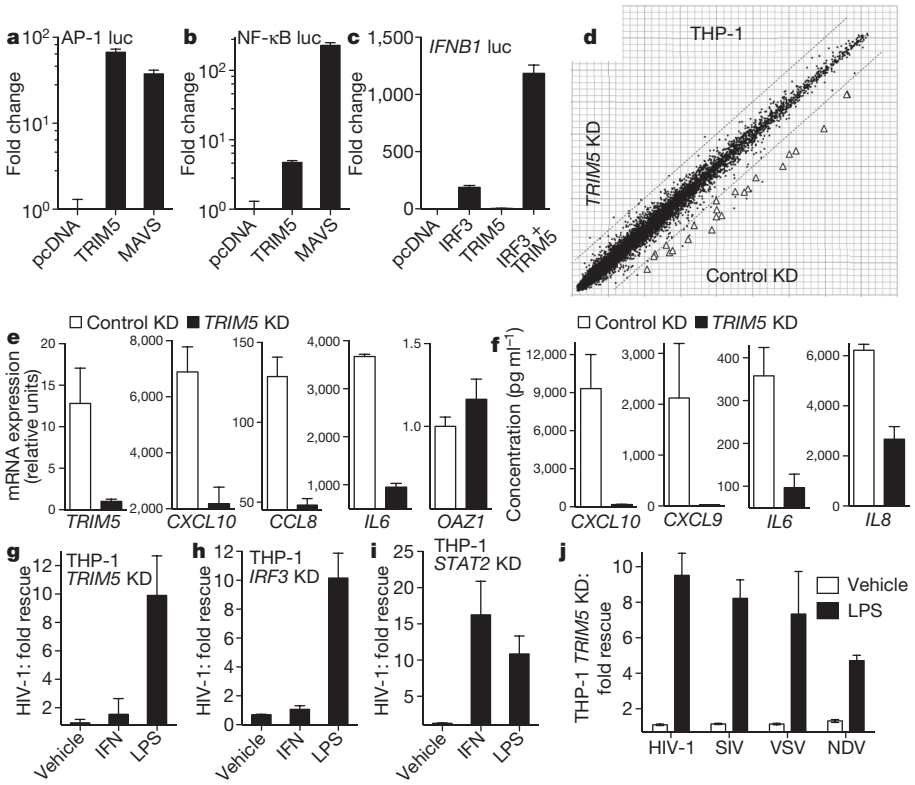

Figure 1 | TRIM5 promotes innate immune signalling. **a**–**c**, HEK-293 cells transfected with the indicated pcDNA-based expression plasmids and luciferase reporters for AP-1 (**a**), NF-κB (**b**) or *IFNB1* (**c**). Bars show mean luciferase activity ± s.d. ($n = 6$). **d**, Global expression profile comparing *TRIM5* knockdown (KD) to control KD THP-1 macrophages. Triangles indicate inflammatory genes significantly downregulated in *TRIM5* KD. **e**, qRT–PCR for the indicated mRNAs collected from MDDCs 2 to 8 h after LPS treatment, depending on the peak values for that gene. Shown are the means ± s.e.m ($n = 3$) relative to untreated cells. **f**, Concentration of the indicated proteins in the culture supernatant, 24 h after LPS treatment (mean ± s.d., $n = 3$). RNA and protein data are representative of at least three separate donors. **g**–**j**, THP-1 macrophages transduced with miR30-based lentivirus KD vectors targeting either *TRIM5* (**g** and **j**), *IRF3* (**h**), or *STAT2* (**i**), were treated for 24 h with the indicated compounds and challenged with VSV-G pseudotyped HIV-1 luciferase reporter virus (**g**–**i**) or with the indicated green fluorescent protein (GFP) reporter viruses (**j**). Data are expressed as fold-change compared to control KD cells, with s.e.m ($n = 4$). All data are representative of at least three independent experiments.

TAK-1 without effect on AP-1 induction by the downstream effector c-Jun (Fig. 2b). *TAK1* knockdown blocked AP-1 activation by TRIM5 (Fig. 2c), but not by c-Jun (Fig. 2c and Supplementary Fig. 5c). *TRIM5* knockdown blocked LPS-induced TAK1 autophosphorylation on threonine 187 (Fig. 2d), a post-translational modification required for TAK1 activation[11]. Like *TRIM5* knockdown, *TAK1* knockdown rescued HIV-1 from the LPS-induced antiviral state (Fig. 2e and Supplementary Fig. 5d), and either TAB2 or TAB3 acted synergistically with TRIM5 to activate AP-1 (Fig. 2f). These results indicate that TRIM5 and the TAK1 kinase complex cooperate to promote signal transduction, and given that TAK1 phosphorylates both IκB kinases (IKKs) and mitogen-activated protein kinase kinases (MKKs)[11], explains how TRIM5 activates both MAPK and NF-κB signalling pathways.

The well-characterized restriction of HIV-1 by owl monkey TRIMCyp[1,13] (a TRIM5–CypA fusion protein) was exploited to determine if TAK1 contributes to TRIM5-mediated, capsid-specific restriction. Pools of THP-1 cells were selected for puromycin-resistance after transduction with a bicistronic lentiviral vector encoding owl monkey TRIM5Cyp[13]. As shown previously, these cells were resistant to infection with wild-type HIV-1, but not to the HIV-1 G89V capsid mutant, and the infectivity of wild-type HIV-1 was rescued by cyclosporine[13] (Supplementary Fig. 5e). Control cells transduced with a vector bearing TRIM5Cyp(H436Q), a mutant that does not bind HIV-1 capsid and does not restrict HIV-1 (ref. 13), were infected with efficiency equal to that of cells transduced with the empty vector. THP-1 cells transduced with either wild-type or H436Q mutant TRIM5Cyp were then subjected to a second round of selection after transduction with miR30-based knockdown vectors targeting *TAK1* or luciferase control and expressing hygromycin-resistance. The pools of puromycin/hygromycin double-resistant THP-1 cells were then challenged with HIV-1. *TAK1* knockdown rescued HIV-1 transduction and nascent HIV-1 cDNA synthesis (Fig. 2g, h). This effect was specific to the cells with TRIM5Cyp-mediated restriction activity because *TAK1* knockdown had no effect on HIV-1 transduction in the non-restrictive, H436Q control cells (Fig. 2g).

The contribution of TAK1 to restriction of N-tropic murine leukemia virus (MLV) by human TRIM5α was examined using miR30-based knockdown vectors in THP-1, HeLa and HT1080 cells. Inhibition of both N-tropic and B-tropic MLV infection by the *TAK1* knockdown was observed, perhaps because, unlike HIV-1, infection with MLV is cell-cycle dependent[14], and these viruses were sensitive to growth inhibitory effects of the knockdown. This precluded assessment of capsid-specific effects on reporter gene transduction, although nascent viral cDNA synthesized after infection of THP-1 cells was rescued by the *TAK1* knockdown in an N-tropic MLV-specific manner (Supplementary Fig. 5g). Similar non-specific effects on MLV were observed after transfection of double stranded RNA (dsRNA) oligonucleotides targeting *TAK1*. Like HIV-1, equine infectious anaemia virus (EIAV) is a lentivirus that infects non-dividing cells, but it differs from HIV-1 in that it is relatively sensitive to human TRIM5α-mediated restriction[15]. Transfection of dsRNAs targeting *TAK1* rescued EIAV transduction almost to the same level as the *TRIM5* knockdown (Fig. 2i). These results indicate that TAK1 contributes to capsid-specific restriction mediated by TRIM5.

AP-1 induction by TRIM5 was impaired by mutants of the RING E3 ubiquitin (Ub)-ligase domain (Fig. 3a). This raised the question which

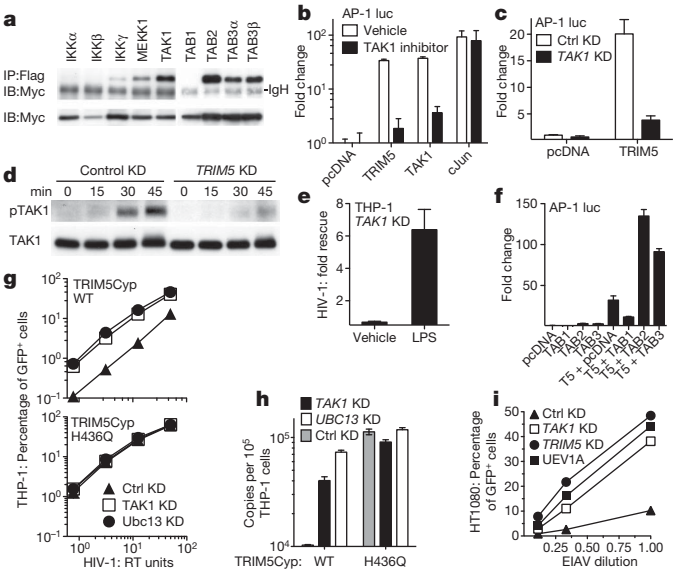

Figure 2 | The TAK1 kinase complex interacts biochemically and functionally with TRIM5. **a**, HEK-293T cells were co-transfected with Myc-tagged human TRIM5α and the indicated Flag-tagged constructs. Shown are immunoblots (IB) with anti-Myc antibody after immunoprecipitation with anti-Flag (upper panel), or of total cell lysate (bottom panel). **b**, **c**, and **f**, HEK-293 cells were transfected with the indicated pcDNA-based expression plasmids and an AP-1 luciferase reporter and show the effect of TAK1 inhibitor 5Z-7-oxozeaenol (**b**) or *TAK1* KD (**c**). *TAK1* KD and control KD THP-1 macrophages were treated with LPS for the indicated times and immunoblotted with anti-TAK1 antibody (lower panel) or anti-phospho-TAK1 antibody (upper panel) (**d**), or, cells were treated 24 h with LPS or vehicle and challenged with an HIV-1 luciferase reporter virus (**e**). The results in (**e**) are reported as fold rescue due to *TAK1* KD, with respect to control KD. **g** and **h**, THP-1 cells were transduced with lentiviral vectors encoding owl monkey TRIM5Cyp, either wild-type (WT) or the H436Q mutant. Pools of each were then transduced with lentiviral KD vectors targeting either *TAK1*, *UBC13* or control, and challenged with an HIV-1-GFP reporter vector. Infectivity was monitored by FACS (**g**) or by PCR for synthesis of full-length viral cDNA (**h**). **i**, HT1080 cells were transfected with dsRNA oligonucleotides targeting *TRIM5*, *TAK1* or *UEV1A* and challenged with EIAV-GFP reporter vector.

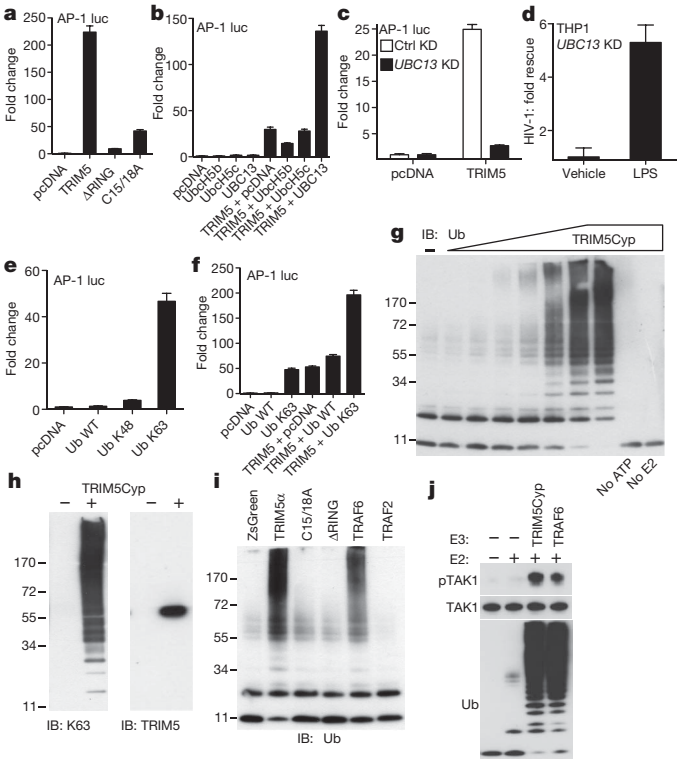

Figure 3 | TRIM5 acts with UBC13–UEV1A to synthesize free K63-linked Ub chains that activate TAK1. **a**–**c**, **e** and **f**, HEK-293 cells were transfected with an AP-1 luciferase reporter and the indicated pcDNA-based expression plasmids. Bars show mean ± s.d. ($n = 6$). In **c**, HEK-293 cells had stable *UBC13* KD or control KD. **d**, *UBC13* KD or control KD THP-1 macrophages were treated for 24 h with LPS or vehicle and challenged with an HIV-1 luciferase reporter virus. Shown is the fold rescue due to *UBC13* KD, with respect to the control KD. **g**-**j**, Products of *in vitro* reactions with ATP, Ub, UBE1, UBC13–UEV1A, and the indicated E3 Ub ligases were revealed by immunoblot for total Ub (**g**, **i**, and **j**), K63-linked Ub chains (left panel of **h**), or TRIM5 (right panel of **h**). E3 ubiquitin ligases included purified owl monkey TRIM5Cyp (**g**, **h**, and **j**), or the indicated Flag-tagged proteins immunoprecipitated from HEK-293T cells (**i**). **j**, *In vitro* Ub reactions like those in (**i**) were incubated with purified TAK1 kinase complex. Products were probed in immunoblot with the indicated antibodies.

of the many E2 Ub-conjugating enzymes might be relevant for TRIM5-mediated effects on signal transduction. Among candidate E2s, UBC13 synergized with TRIM5 to activate AP-1 (Fig. 3b). Interestingly, the TAK1 kinase complex is activated by the heterodimeric E2 UBC13–UEV1A[11]. Knockdown of *UBC13* or *UEV1A* severely blocked AP-1 activation by TRIM5 (Fig. 3c and Supplementary Fig. 6a–c), rescued HIV-1 from the LPS-induced antiviral state in THP-1 macrophages (Fig. 3d and Supplementary Fig. 6d), and rescued HIV-1 and EIAV from TRIM5-mediated restriction (Fig. 2g–i).

The UBC13–UEV1A E2 heterodimer is notable in that it generates K63-linked Ub chains that are unlinked to substrates; these free Ub chains multimerize and activate the TAK1 kinase complex via the Ub binding components, TAB2 and TAB3 (ref. 11). Ub in which all lysines except K63 are mutated to arginine (Ub K63) activated AP-1 and NF-κB (Fig. 3e and Supplementary Fig. 6e, f), and enhanced the ability of TRIM5 to activate AP-1 (Fig. 3f). K48-only Ub did not have these activities (Fig. 3e, f), nor did wild-type Ub, perhaps because of the dominance of competing Ub metabolic pathways and the tight regulation of K63 chains within cells[16]. These experiments indicate that the heterodimeric E2 UBC13–UEV1A and the K63-linked Ub chains that it produces have a role in TRIM5-mediated signalling.

Because TRIM5 interacted biochemically and functionally with TAK1, TAB2, TAB3, UBC13, UEV1A and K63-Ub, the ability of TRIM5 to synthesize K63-linked Ub chains was assessed. A purification protocol was established that yielded 0.5 mg of soluble, full-length, owl monkey TRIM5Cyp from 1 l of Sf9 cell culture (Supplementary Fig. 7). No procedure has been reported to date for the production of purified, full-length, recombinant TRIM5 protein[17]. Purified TRIM5Cyp was incubated with purified Ub, E1 and the E2 Ub-conjugases UBC13 and UEV1A, and reaction products were separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). With increasing TRIM5Cyp concentration, monomeric Ub was progressively depleted and the yield of Ub chains increased (Fig. 3g and Supplementary Fig. 8a). Synthesis of Ub chains was ATP-dependent and required both UBC13 and UEV1A.

The Ub chains generated by TRIM5Cyp were detected with antibody specific for K63-linked Ub and immunoblot showed TRIM5Cyp to be a monomer with no detectable incorporation into the Ub chains (Fig. 3h). To obtain an independent assessment of their identity, reaction products were isolated by PAGE and analysed by matrix-assisted laser desorption/ionization and tandem mass spectrometry (Supplementary Figs 8a and 9a–c). These methods identified peptides corresponding to K63-linked Ub and failed to detect conjugates with other Ub lysines or peptides corresponding to TRIM5Cyp, confirming that reaction products were free, unattached K63 Ub chains. Additionally, synthesis of Ub chains was undetectable with a Ub mutant in which K63 was mutated to arginine (Supplementary Fig. 8b). Conversely, Ub was efficiently incorporated into chains when all lysines except K63 were mutated to arginine (Supplementary Fig. 8b), indicating that K63 was necessary and sufficient to form the Ub chains.

Human TRIM5α, produced by transfection of 293T cells and enriched by immunoprecipitation, catalysed the synthesis of free K63 Ub chains like those of TRIM5Cyp, in a RING domain-dependent manner (Fig. 3i and Supplementary Fig. 8c, d). It had at least as much activity as TRAF6 (Fig. 3i), an E3 Ub ligase previously reported to synthesize unattached K63 chains that activate TAK1 (ref. 11). TRAF2, a close paralogue of TRAF6 that does not interact with UBC13 (ref. 18), lacked activity (Fig. 3i).

Free K63-linked Ub chains generated by TRAF6 result in TAK1 autophosphorylation on threonine 187 (ref. 11), a modification required for TAK1 activation. To test the effect of K63-linked Ub chains generated by TRIM5 on TAK1 activation, the essential components of a TAK1 kinase complex, TAK1, TAB1 and TAB2 (ref. 11), were purified and combined (Supplementary Fig. 10). This complex was then incubated with Ub, UBC13–UEV1A, and either TRAF6 or purified owl monkey TRIM5Cyp. TAK1 phosphorylation was observed in response to the K63-linked Ub chains synthesized by either TRAF6 or by TRIM5Cyp (Fig. 3j). Kinase activity required the TAK1-associated TAB1, the Ub receptor TAB2, and UBC13–UEV1A (Fig. 3j). These experiments show that, like TRAF6 (ref. 11), TRIM5 synthesizes free K63-linked Ub chains that activate TAK1 autophosphorylation.

If TRIM5 were a PRR specific for the retroviral capsid lattice, infection with retroviruses would activate signalling, the magnitude of which would correlate with TRIM5 avidity for the capsid of the challenge virus. To determine if this is the case, myeloid cells were challenged with pairs of retroviruses that differ with respect to TRIM5 avidity for the capsid[3,4] and the subsequent induction of NF-κB- and MAPK-dependent genes was assessed. VSV G-pseudotyped N-tropic and B-tropic MLV vectors, normalized for exogenous reverse transcriptase activity and for titre on non-restrictive MDTF cells[19], were used to challenge THP-1 macrophages. The multiplicity of infection of the non-restricted B-tropic MLV on cycling THP-1 cells was 0.1. mRNA was harvested from the THP-1 cells and processed by reverse transcription and quantitative PCR (qRT–PCR). Greater induction of *PTGS2*, *CXCL10*, *CCL8* and *IL6* mRNA was observed after challenge with N-MLV than with B-MLV (Fig. 4a, b), in correlation with the higher avidity of human TRIM5α for the capsid of N-tropic MLV than for the capsid of B-tropic MLV[3]. *TRIM5* knockdown suppressed the higher inflammatory gene induction by N-MLV, indicating its dependence upon endogenous TRIM5 (Fig. 4b). Similar differential induction of *PTGS2*, *CXCL10*, *CCL8* and *IL6* mRNAs by N-tropic and B-tropic MLV was observed after challenge of MDDCs or MDMs (Supplementary Fig. 11).

Retroviral cDNA activates innate immune signalling under some conditions[20]. Restriction by human TRIM5α results in N-MLV cDNA levels that are an order of magnitude lower than for B-MLV cDNA[21] so the experiments described above might underestimate the effect of N-MLV capsid on TRIM5-mediated signalling. Therefore, MDDCs were challenged with matched pairs of virus-like particles (VLPs) devoid of the viral genome that serves as the reverse transcription template. VLPs bearing N-MLV capsid activated *CXCL9*, *CXCL10*, *IFIT1* and *IFIT2* mRNAs from 5- to 55-fold over the levels in untreated MDDCs (Fig. 4c). Inflammatory gene induction was not detected with VLPs bearing the unrestricted NB-MLV capsid[22] (Fig. 4c). As with the mRNA, soluble IL8, CCL5, CXCL9 and CXCL10 protein was differentially induced by N-MLV (Fig. 4d).

To determine if differential gene induction after retrovirus challenge was peculiar to N-tropic MLV, a similar experiment was performed with OMK, a kidney cell line from the owl monkey, *Aotus trivirgatus*. The TRIM5 orthologue in this species restricts HIV-1 but not SIV[1]. VSV G-pseudotyped HIV-1 and SIV vectors, normalized for exogenous reverse transcriptase activity and for titre on HeLa cells, were used to challenge OMK cells. The multiplicity of infection of the unrestricted SIV on OMK cells was 0.3. Among the MAPK- and NF-κB-dependent gene products that were detectable in this species using human probes, transcriptional activation of *PTGS2*, *IFIT1* and *IFIT2* mRNAs, and

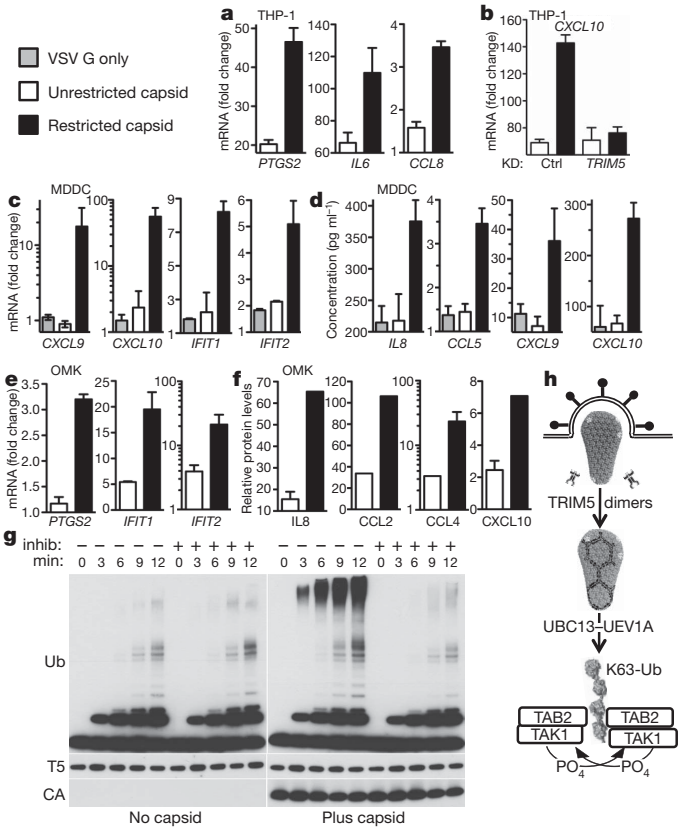

Figure 4 | Retrovirus capsid sensing by TRIM5. THP-1 cells (**a** and **b**), MDDCs (**c** and **d**), or owl monkey kidney cells (OMK; **e** and **f**), were challenged with matched pairs of VSV G-pseudotyped particles bearing retrovirion capsids that are restricted by the TRIM5 orthologue endogenous to that cell type (black bars), or unrestricted (white bars), or VSV G-derived particles that are devoid of capsid (grey bars). Restricted capsids were from N-tropic MLV (**a**–**d**) or HIV-1 (**e** and **f**). Unrestricted capsids were B-tropic MLV (**a** and **b**), N/B-tropic MLV (**c** and **d**) or SIV_{MAC239} (**e** and **f**). Particles bore viral genomes in **a**, **b**, **e** and **f**, but not in **c** and **d**. mRNA was harvested for qRT–PCR (**a**–**c** and **e**) and reported as fold change versus media control. Protein in the supernatant was quantified (**d** and **f**). Bars show means ± s.d. ($n = 3$), and are representative of at least three independent experiments. **g**, Immunoblots with the indicated antibodies of products from *in vitro* time-course with ATP, Ub, UBE1 (E1), UBC13–UEV1A (E2) and purified owl monkey TRIM5Cyp, with or without assembled HIV-1 capsid-A14C/E45C, and with or without competitive inhibitor MeIle4CsA. (**h**) Schematic showing entry of an HIV-1 virion core[27] (courtesy of Pornillos and Yeager) into the target cell cytoplasm where it induces dimeric TRIM5 to form a hexameric lattice[25] with increased E3 Ub ligase activity. With UBC13–UEV1A, TRIM5 synthesizes free K63 Ub chains that are recognized by TAB2, which multimerizes and activates the TAK1 kinase complex.

secretion of IL8, CCL2, CCL4 and CXCL10 proteins, was higher after challenge with the restricted virus (Fig. 4e, f).

TRIM5 senses retrovirus capsids in the target cell cytoplasm (Fig. 4a–f) and activates MAPK- and NF-κB-dependent transcription via the synthesis of TAK1-activating, K63-linked Ub chains (Fig. 3g–j). If these observations were linked functionally, interaction with capsid would be expected to stimulate the synthesis of Ub chains by TRIM5Cyp. Soluble, recombinant HIV-1 capsid or capsid hexamers generated by the oxidation of recombinant capsid bearing strategically-placed cysteine substitutions (A14C/E45C/W184A/M185A)[23] had no effect on the synthesis of K63-linked Ub chains (data not shown). Current models of the HIV-1 capsid lattice are based on cylinders generated under high salt with either capsid or capsid-nucleocapsid fusion protein[17,24]; both preparations were generated but the high salt necessary to maintain capsid cylinders blocked E3 Ub ligase activity. Capsid cylinders were then assembled with A14C/E45C-substituted

capsid protein in 1 M NaCl and the cysteines were oxidized. These oxidized cylinders were stable in the absence of salt (Supplementary Fig. 12a) and greatly stimulated the production of K63-linked Ub chains by TRIM5Cyp (Fig. 4g). No Ub-linked products were detected with anti-capsid (p24) or anti-TRIM5 antibodies, indicating that the reaction products were unattached Ub chains (Fig. 4g).

Finally, two factors that disrupt the HIV-1 capsid-TRIM5Cyp interaction and block restriction activity—a non-immunosuppressive cyclosporine analogue[1] or the TRIM5Cyp(H436Q) mutant protein[13]—each eliminated the enhancement of E3 Ub ligase activity by the A14C/E45C capsid cylinders, without effect on the baseline activity in the absence of capsid (Fig. 4g and Supplementary Figs 7d and 12b–d).

The experiments presented here demonstrate that TRIM5 is a multifunctional component of the innate immune system. In addition to functioning as a retroviral capsid-specific restriction factor, TRIM5 synthesizes K63 Ub chains that activate TAK1 and inflammatory transcription, most probably via multimerization of the TAK1-associated Ub-binding protein TAB2 (ref. 11; Fig. 4h). This activity was greatly increased by the hexameric capsid lattice, a molecular signature of HIV-1 and other retroviruses. TRIM5, then, satisfies criteria for a bona fide PRR[10]. Interestingly, TRIM5 spontaneously forms an hexagonal lattice that is complementary to the capsid lattice[25], but the efficiency of TRIM5 lattice formation is greatly stimulated by the capsid hexameric lattice[25] (Fig. 4h). Little is known about how the innate immune system detects retroviruses[26] and the discovery that TRIM5 acts as a PRR is an important step towards filling this critical gap. The cellular factors required for TRIM5 E3 activity and inflammatory gene induction, UBC13, UEV1A and TAK1, also promoted capsid-specific restriction activity, indicating that the multiple functions of TRIM5 are mechanistically linked. Identification of relevant TAK1 substrates will inform future attempts to pinpoint the mechanism of restriction.

METHODS SUMMARY

Plasmids, cells and viruses. These methods were described previously[1,13,19] or are detailed in the Supplementary Information.

Recombinant protein. Production of full-length, soluble, TRIM5Cyp is described in the supplement. CA A14C E45C was produced and assembled into tubes as described[23,24].

Microarray. Illumina HumanHT-12 V3.0 expression bead chips were probed with RNA from *TRIM5* knockdown THP-1 cells. Data set and methods are available at the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) under accession number GSE25041.

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