RSC Chemical Biology

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Chem. Biol.*, 2026, 7, 136

Application of HIV-1 viral protein R-derived-peptides as new E3 ligase-binding components of BRD4 degraders†

Kohei Tsuji, ‡*[a] Xueyuan Huang,‡[a] Maho Miyamoto,[bc] Sayaka Sukegawa,[d] Hidetomo Yokoo,[b] Hiroaki Takeuchi,[e] Yosuke Demizu [bcf] and Hirokazu Tamamura [a]

Proteolysis targeting chimeras (PROTACs) have become a new modality for drug development of particular importance for cancer chemotherapy. PROTACs are composed of a ligand that binds to the protein of interest (POI) tethered by a linker to a ubiquitin E3 ligase-binding motif. These molecules can degrade the POI by ubiquitination and subsequent digestion using the ubiquitin-proteasome system (UPS). Although more than six hundred E3 ligases are encoded in human genome, only a small number are currently utilized by PROTACs. Because the expression levels and activities of E3 ligases vary among the cell lines, it can be advantageous to develop PROTACs that utilize new E3 ligase-binding components. In our current work we report new E3 ligase-binding ligands that employ viral protein R (Vpr), an accessory protein of the human immunodeficiency virus type-1 (HIV-1). Vpr can bind to both the E3 ligase complex, Cul4A-DDB1-DCAF1 and host proteins, such as UNG2 and facilitate host protein degradation *via* the UPS. We envisioned that Vpr fragments may function in PROTACs as new E3 ligase-binding ligands. Herein, we designed, synthesized and evaluated bromodomain 4 (BRD4)-targeting PROTACs (BRD4-PROTACs) that employ a well-known BRD4 inhibitor (JQ1) as a warhead and Vpr-derived peptides as the E3 ligase-binding ligands. We successfully demonstrate that the Vpr-derived peptides can function as E3 ligase-targeting ligands for PROTAC development. We also evaluated PROTACs based on the HIV-1 latency-reversing activity of JQ-1. The chemical degraders are less effective than the parent inhibitor as a latency-reversing agent (LRA). However, the low cytotoxicity of the new peptidic PROTACs allowed the compounds to be tolerated at high does, leading to potent LRA activity.

Received 16th May 2025,
Accepted 20th October 2025

DOI: 10.1039/d5cb00125k

rsc.li/rsc-chembio

Introduction

The ubiquitin-proteasome system (UPS) is a crucial cellular pathway for protein degradation. It regulates a variety of cellular processes by selectively degrading misfolded, damaged, or regulatory proteins.[1] Ubiquitin, E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligases are involved in the UPS and cooperatively transfer ubiquitin to target proteins. The E3 facilitates ubiquitin transfer from the E2 enzyme to lysine residues on the target protein.[2] Subsequent polyubiquitination results in recognition by the 26S proteasome followed by proteolytic degradation.[3]

Proteolysis targeting chimeras (PROTACs) were initially reported by Crews and colleagues in 2001.[4] PROTACs function to induce degradation of a protein of interest (POI) *via* the UPS. PROTACs offer significant therapeutic potential for treatment of a variety of diseases.[5–9] PROTACs are generally composed of three integral components: a ligand that binds to a POI, a component that binds to an E3 ubiquitin ligase and a linker

[a] *Laboratory for Biomaterials and Bioengineering, Institute of Integrated Research, Institute of Science Tokyo, 2-3-10 Kandasurugadai, Chiyoda-ku, Tokyo 101-0062, Japan. E-mail: ktsuji.mr@tmd.ac.jp; Fax: +81-3-5280-8039; Tel: +81-3-5280-8038*
[b] *National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki 210-9501, Japan*
[c] *Graduate School of Medical Life Science, Yokohama City University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan*
[d] *Department of Molecular Virology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan*
[e] *Department of High-risk Infectious Disease Control, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan*
[f] *Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1, Tsushimanaka, Kita, Okayama, Okayama 700-8530, Japan*

† Dedicated to Professor Akira Otaka on the occasion of his retirement from Tokushima University.

‡ Equally contributed.

that joins the two together. PROTACs simultaneously bind to the POI and the E3 ligase to facilitate the ubiquitination of the POI and subsequent proteolytic degradation.[10] This leverages the endogenous protein degradation machinery to provide a strategy for the selective elimination of pathogenic proteins.[11]

Despite the identification of more than six hundred E3 ligases, only a limited number of ligases have been utilized in the development of PROTACs. These include VHL, CRBN, cIAP, and keap1.[12,13] However, E3 ligases can exhibit significant variation among cell lines in terms of enzymatic activity, target specificity, expression level, and tissue distribution.[14] In order to expand the utility of PROTACs it would be beneficial to employ new E3 ligases and their ligands.

The E3 ligase complex DCAF-1-DDB1-cullin4A (Cul4) has recently been reported as being suitable for use in PROTACs. Several small molecules have been reported as DCAF1-binding components within PROTACs.[15,16] This E3 ligase is also known as a target protein of viral protein R (Vpr), a human immunodeficiency virus type-1 (HIV-1) accessory protein. Vpr interacts with the DCAF1-DDB1-Cul4 E3 ligase to induce degradation of target proteins *via* the host cell UPS. Targets include uracil-DNA glycosylases UNG2 and SMUG1 to aid viral replication and immune evasion.[17–19] We envisioned that Vpr fragment peptides might serve as E3 ligase-binding ligands for use in PROTACs.

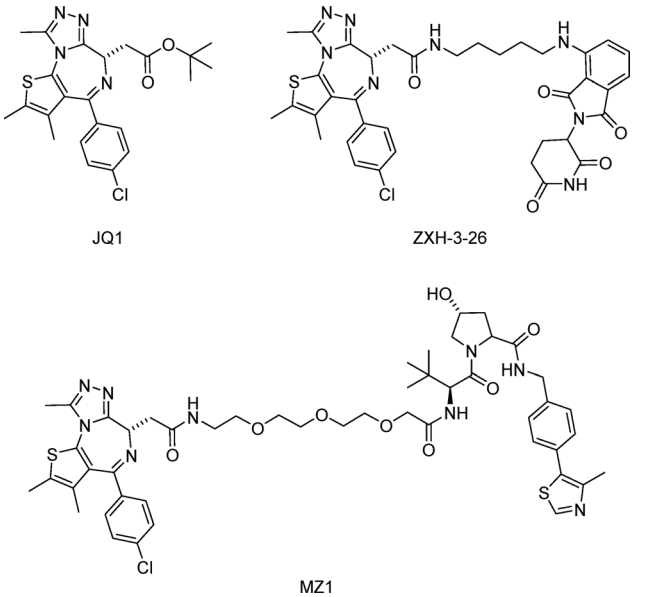

Fig. 1 Structures of the BRD4 inhibitor JQ1 and the BRD4-PROTACs ZXH-3-26 and MZ1.

Results and discussion

Design of BRD4-PROTACs possessing Vpr-derived peptides

Bromodomain-containing protein 4 (BRD4) is a member of the bromodomain and extraterminal domain (BET) family. This protein binds to acetylated histones to promote gene transcription. In cancer cells, BRD4 is correlated with the expression of several oncoproteins, including Myc.[20] Therefore, BRD4 represents a potentially attractive target for anticancer drug development. JQ1 is a well-established BRD4 inhibitor that also targets other BET family members, including BRD2, BRD3, and BRDT (Fig. 1).[21]

JQ1 binds to bromodomains and prevents interaction with acetylated histones. JQ1 inhibits the roles of bromodomains in epigenetic regulation of gene expression and shows anti-cancer activity.[21] JQ1 is used as a warhead in BRD4-targeting PROTACs (BRD4-PROTACs). ZXH-3-26 is a PROTAC in which JQ1 serves as a BET binding moiety that is tethered to pomalidomide, a cereblon (CRBN) E3 ligase-binding ligand. ZXH-3-26 selectively degrades BRD4 relative to BRD2 and BRD3 (Fig. 1).[22] MZ1 is a PROTAC that JQ1 is tethered to VHL-1 as a VHL E3 ligase-binding ligand. MZ1 degrades BRD4 as well as BRD2 and BRD3 (Fig. 1).[23] A crystal structure of a UNG2-Vpr-DCAF1-DDB1-Cul4 complex shows how the N-terminal region of Vpr (Vpr (1–14)) and the C-terminal region (Vpr (60–74)) engage with DCAF1 (Fig. 2).

We took these Vpr regions as potential DCAF1-binding peptides, and we designed the BRD4-PROTACs **1–6**. These are composed of JQ1, a polyethylene glycol (PEG) linker, Vpr-derived peptides as the DCAF1 ligands, and octa-arginine as a cell penetrating peptide (Fig. 3).[24]

Compounds **1** and **2** consist of JQ1, a PEG3 linker and either Vpr (1–14) or Vpr (60–74), respectively. Compounds **3** and **4** have JQ1, a PEG3 linker and either Vpr (1–14) or Vpr (60–74), and octa-arginine with a two-glycine spacer at the C-terminus of the Vpr peptides. Compounds **5** and **6** have JQ1, a PEG3 linker, an octa-arginine on the lysine ε-amino group and either Vpr (1–14) or Vpr (60–74), and octa-arginine at the C-terminus of the Vpr peptides.

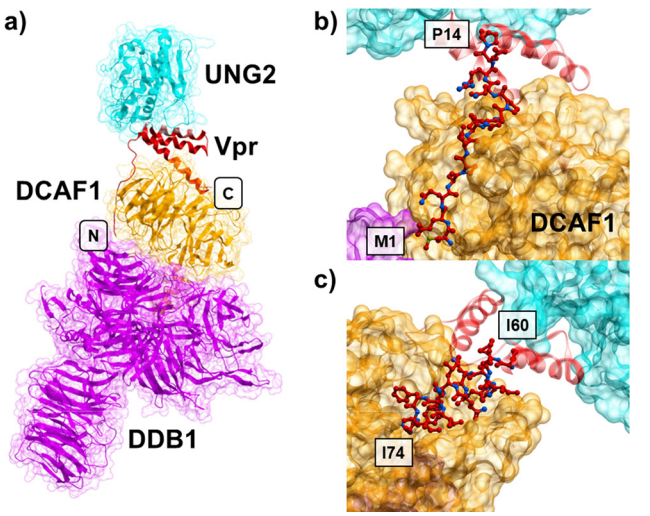

Fig. 2 Structure of a UNG2-Vpr-DCAF-1-DDB1 complex (PDB: 5JK7). (a) A whole complex structure; (b) and (c) regions enlarged on Vpr-DCAF-1 interaction sites, Vpr (1–14) and Vpr (60–74), respectively. UNG2: cyan ribbon and surface, Vpr: red stick and ribbon, DCAF1: orange ribbon and surface, DDB1: purple ribbon and surface.

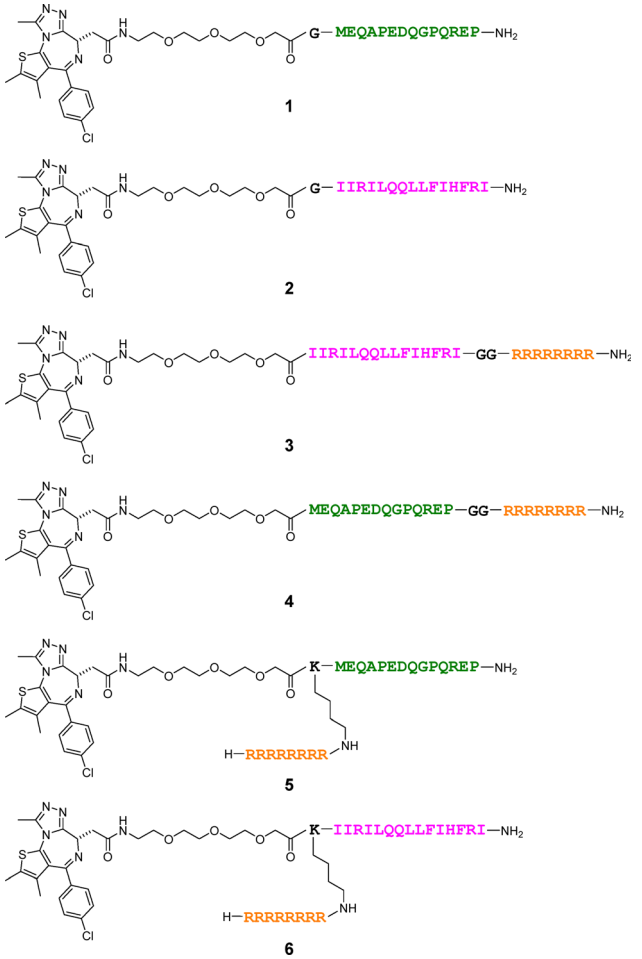

Fig. 3 Structures of synthetic JQ1-based BRD4-PROTACs **1–6**, in which JQ1 is tethered at the N-termini of Vpr-derived peptides (Vpr (1–14, green) or Vpr (60–74, magenta)). The cell penetrating peptide octa-arginine (orange), is attached to the N- or C-terminus of the Vpr peptides.

Initial evaluation of BRD4 degrading ability

Compounds **1–6** were evaluated by western blots for their ability to degrade BRD4 using the human breast cancer cell line, MCF-7 (Fig. 4 and Fig. S1a).[25–27] The results of the initial assays (Fig. 4a and b) showed that compounds **1**, **2**, and **4** did not exhibit significant BRD4 degradation activity. Compounds **3** and **6** showed cytotoxicity in high concentration range possibly arisen from their hydrophobic nature of the peptide moiety. However, compound **5** did demonstrate weak but dose-dependent BRD4 degradation. Compound **5** was subjected to western blot analysis at high concentrations that ranged up to 100 μM (Fig. 4c). The results clearly showed that BRD4 degradation occurred at 50 and 100 μM concentrations.

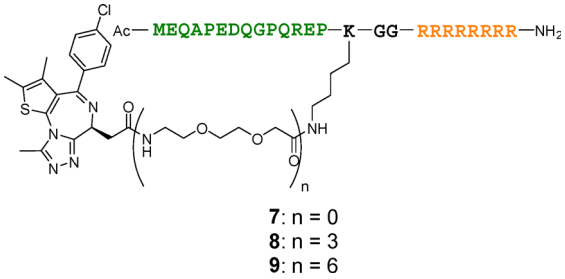

Fig. 5 Structures of synthesized JQ1-based BRD4-PROTACs **7–9**, in which JQ1 and octa-arginine (orange) were tethered with Vpr (1–14, green) at the C-terminus.

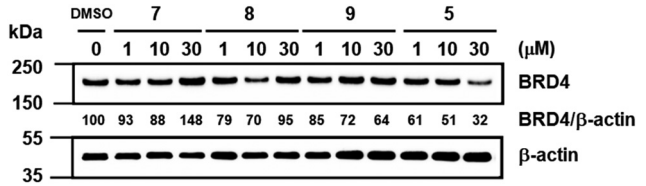

Fig. 6 Results from western blot assays using MCF-7 cells to evaluate the BRD4 degradation ability of compounds **7–9** from 1 to 30 μM.

BRD4-PROTACs bearing C-terminal JQ1 conjugation

Next, we designed and synthesized BRD4-PROTACs possessing JQ1 appended to the C-terminus of Vpr (1–14) using lysine for **7**, lysine with three 8-amino-3,6-dioxaoctanoic acid (miniPEG) units for **8**, and lysine with six miniPEG units for **9**. An octa-arginine was appended to the C-terminus of the Vpr (1–14) using a two-glycine spacer (Fig. 5).

Evaluation of the BRD4 degrading ability of compounds 7–9

Compounds **7** and **8** did not show BRD4 degradation, whereas compound **9** showed slight degrading activity, but less than compound **5** (Fig. 6 and Fig. S1b). These results suggest that conjugation of JQ1 at the N-terminus of Vpr (1–14) is preferred to conjugation at the C-terminus.

BRD4-PROTACs with different PEG linkers

Next, we designed and synthesized the compound **5** derivatives **10–13** that possess different PEG linkers (Fig. 7). Compound **10** has a miniPEG unit between the octa-arginine and the lysine ε-

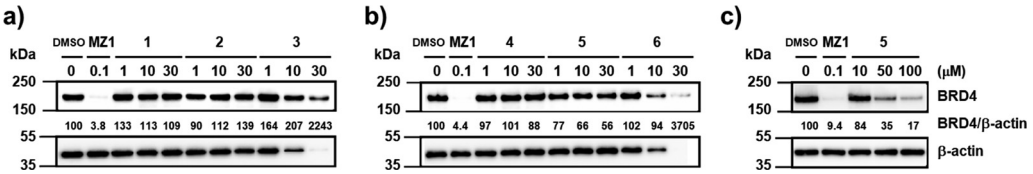

Fig. 4 Results from western blot assays using MCF-7 cells to evaluate BRD4 degradation by compounds **1–6**. (a) and (b) Initial evaluation of compounds **1–6** from 1 to 30 μM; (c) evaluation of compound **5** at higher concentration ranges (10 to 100 μM).

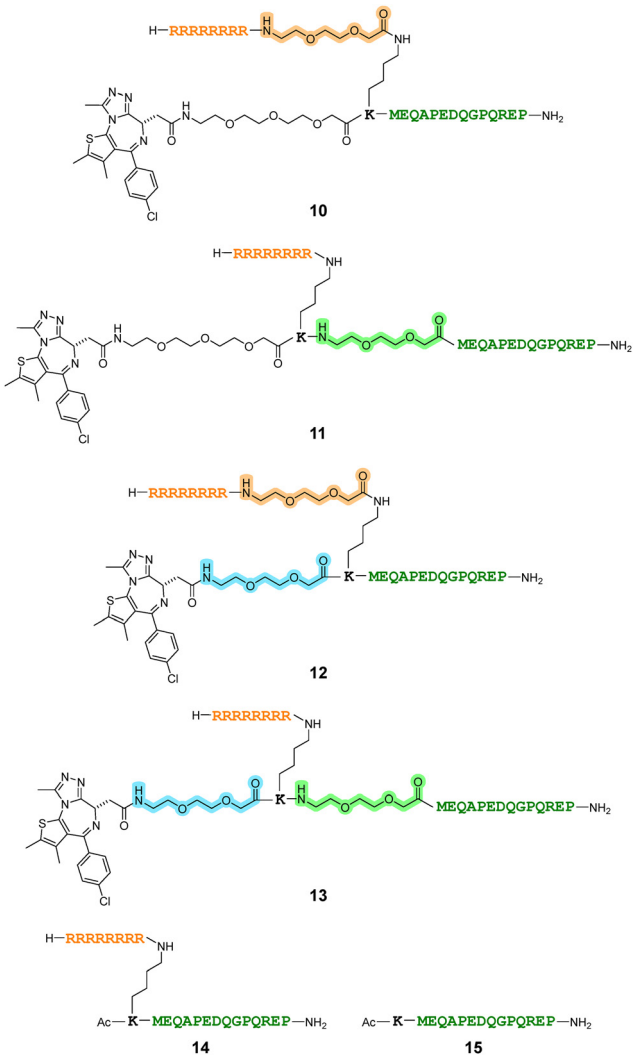

Fig. 7 Structures of compound **5** derivatives **10–13** possessing varied PEG linkers and Vpr peptides **14** and **15**.

amino group. Compound **11** has a miniPEG unit between lysine and Vpr (1–14). Compound **12** replaces the PEG3 linker with a miniPEG unit. Compound **13** replaces the PEG3 linker with a miniPEG unit while having a miniPEG unit between lysine and Vpr (1–14).

Evaluation of BRD4 degrading ability of compounds 10–13

All compound **5** derivatives **10–13** showed improved BRD4 degradation activity. Derivatives **11** and **13** having PEG linkers incorporated at N- and C-terminal lysine residues showed

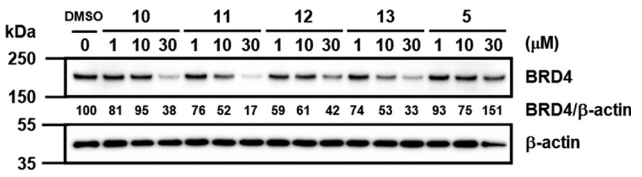

Fig. 8 Results from western blot assays using MCF-7 cells to evaluate BRD4 degradation ability of compounds **10–13** from 1 to 30 μM.

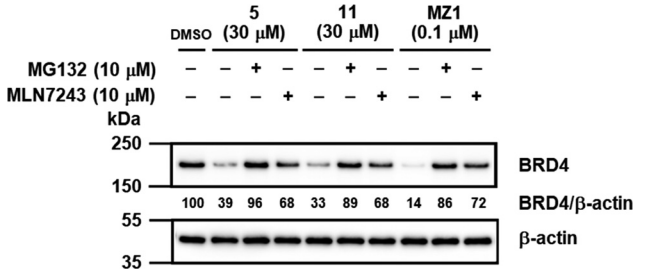

Fig. 9 Results from western blot assays using MCF-7 cells to evaluate the BRD4 degrading ability of compounds **5** and **11** at 30 μM in the presence of 10 μM of MG-132 or 10 μM of MLN7243.

significantly greater potent activity as compared to compound **5** (Fig. 8 and Fig. S1c). Compound **11** was selected for further evaluation.

Evaluation of BRD4 degrading ability of compounds 5 and 11 in the presence of the proteasome inhibitor MG-132, a ubiquitin activating enzyme inhibitor MLN7243, or Vpr (1–14) peptides

In order to determine whether PROTACs bearing Vpr-derived peptides as E3 ligase-binding ligands degrade BRD4 *via* the UPS, we performed co-treatment of compound **5** or **11** with the proteasome inhibitor MG-132[23,28] or with a ubiquitin activating enzyme (UAE, E1) inhibitor MLN7243 (Fig. 9).[29] We found that BRD4 degradation by compounds **5** and **11** was suppressed by these inhibitors. The partial degradation of BRD4 in the presence of MLN7243 might be because of co-treatment of the compounds and MLN7243. MLN7243 is a mechanism-based inhibitor, and the active species is the ubiquitin covalently bound form.[29] Therefore, inhibitory activity of MLN7243 was partially attenuated.[30,31] We also conducted the western blot assays of compounds **5** and **11** with the pre-treatment of a neural precursor cell-expressed developmentally downregulated protein 8 (NEDD8)-activating enzyme (NAE) inhibitor, MLN4924, which inhibits DCAF1-mediated degradation pathway (Fig. S2),[15,32] and with the co-treatment of JQ1, Vpr (1–14) peptides **14** or **15** to test whether the each moiety is required for their degrading ability (Fig. 7 and Fig. S2, S3). The results demonstrated that the treatment of MLN4924 and competitive inhibition with excess JQ1 and Vpr (1–14) peptides decreased the BRD4 degrading activity of compounds **5** and **11**. These results suggest that the Vpr-derived peptides function as E3 ligase-binding ligands and that compounds **5** and **11** degrade their target protein BRD4 *via* UPS.

Evaluation of cytotoxicity of compounds 5, 11, and 14

Next, we performed MTT assays to evaluate the cytotoxicities of compounds **5**, **11**, and **14** against MCF-7 cells and two HIV-1 latently infected model cell lines; HIV-1 gene integrated human T lymphocyte cells (Jurkat cells) in which a GFP gene replaces with *Nef* gene (J-Lat 10.6 cells)[33] and HIV-1 NL4–3 strain[34] -based viral vector, pNLn-NanoLuc-Kp, integrated human monocytic leukemia cells (THP-1 cells) in which a NanoLuc gene is incorporated as a reporter in the *Nef* region (THP-1

Table 1 Results from MTT assays using MCF-7, J-Lat 10.6, and THP-1 NLuc#225 cells

Compound	CC_{50}[a] (μM)		
	MCF-7	J-Lat 10.6	THP-1 NLuc#225
JQ1	2.4 ± 0.18	9.5 ± 1.8	22 ± 0.48
MZ1	0.065 ± 0.010	2.8 ± 0.090	0.44 ± 0.040
5	52 ± 5.4	>50	>50
11	45 ± 3.6	>50	>50
14	>200	>50	>50

[a] CC_{50} values were determined from three independent experiments and shown as the average IC_{50} value ± standard error of the mean (SEM, μM).

NLuc#225 cells, Table 1 and Fig. S4).[35–37] Compounds **5** and **11** showed lower cytotoxicities than JQ1 and MZ-1, and compound **14** did not show significant cytotoxicity in MCF-7 cells. This could imply low cell membrane permeability of compounds **5** and **11** due to their peptidic features and it supports further optimization using additional CPPs. J-Lat 10.6 and THP-1 NLuc#225 cells were less sensitive to BRD4 inhibitors and degraders in terms of cytotoxicity even though MZ1 showed improved activity compared to JQ1. Compounds **5**, **11**, and peptide **14** did not show significant cytotoxicity against either cell line up to 50 μM.

Evaluation of HIV latency-reversing activity of the synthesized compounds 5 and 11

The BRD4 inhibitor, JQ1 is known as a highly potent HIV-1 latency-reversing agent (LRA).[38] In the forty years since the discovery of HIV-1,[39] acquired immune deficiency syndrome (AIDS) and HIV-1 infection-related diseases have change from fatal infections to controllable chronic infections because of the development of efficient drugs and their use in combination antiretroviral therapy (cART).[40,41] Despite this, medications have yet to be developed that can completely eliminate HIV-1 from infected individuals. One barrier to the achieving a HIV-1 cure is latent infection.[42] Latently infected cells are not responsive to anti-HIV drugs and the virus rebounds rapidly when cART is stopped. A ''shock and kill'' approach has been studied as a means of eradicating HIV-1 from latently infected cells.[43] The shock process involves HIV-1 latency reversal by LRAs followed by viral clearance *via* the host immune response and apoptotic cell death. In theory, cART combined with the shock and kill approach can eliminate HIV-1 entirely from the

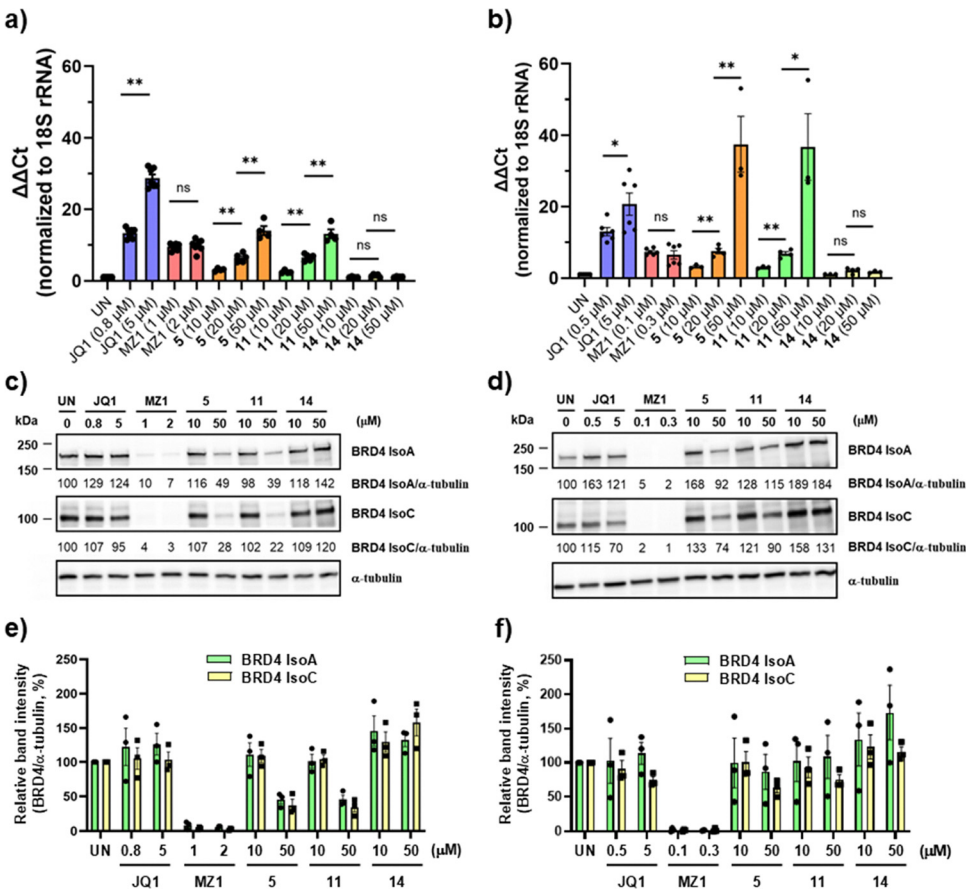

Fig. 10 LRA activity of JQ1, MZ1, **5**, **11**, and the Vpr-derived peptide **14** using J-Lat 10.6 cells (a) and THP-1 NLuc#225 cells (b). Results from western blot assays using J-Lat 10.6 cells (c) and THP-1 NLuc#225 cells (d) to evaluate BRD4 degrading abilities of JQ1, MZ1, **5**, **11**, and **14**. Average numbers of BRD4/α-tubulin ratio from three independent experiments using J-Lat 10.6 cells (e) and THP-1 NLuc#225 cells (f). UN: untreated (PBS control); ns: not significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$.

host body. However, effective LRAs in *in vivo* have not yet been reported. Although JQ1 has shown potent LRA activity,[23] the JQ1-based PROTACs, ZXH-3-26 and MZ1 show much less LRA activity than JQ1 in Jurkat cell-derived latency model cell lines.[44–46] Therefore, we evaluated the LRA activities of the BRD4-PROTACs MZ1, **5**, **11** with the Vpr-derived peptide **14** as a negative control. These studies were conducted using J-Lat 10.6 and THP-1 NLuc#225 cells as latently infected cell models (Fig. 10).[35–37] In comparison to JQ1, MZ-1 showed lower LRA activity in both cell lines, which was consistent with previous reports (Fig. 10a and b).[45,46] The reason why chemical degraders show lower LRA activity than the parent compound remains unclear. However, Turner and co-workers have recently proposed a mechanism related to BRD4-related latency reversal.[46] These authors showed that the BRD4 inhibitor JQ1 releases BRD4 from chromatin, and the released BRD4 then interacts with the positive transcription elongation factor b (P-TEFb) in the 7SK small nuclear ribonucleoprotein (snRNP) complex. This interaction facilitates dissociation of P-TEFb-BRD4 from the 7SK complex and increases free active P-TEFb levels, resulting in HIV transcriptional initiation. This rationale may explain why BRD4 degraders do not show potent LRA activity. Additionally, it is difficult to use high concentrations of MZ1 due to cytotoxicity. However, the BRD4-PROTACs **5** and **11** are less toxic than MZ1 and JQ1 (Table 1), and therefore these PROTACs can be used at much higher concentrations than JQ1 and MZ1. Indeed, compounds **5** and **11** demonstrated potent LRA activities at high dosages (Fig. 10a and b). Results from western blot assays showed that MZ1 effectively degraded BRD4 in both cell lines, whereas BRD4 degradation by **5** and **11** in J-Lat 10.6 cells was observed rather than that in THP-1 NLuc#225 cells (Fig. 10c–f and Fig. S5). The difference between these cell lines might result in the more potent LRA activity of **5** and **11** in the THP-1 NLuc#225 cells than that in J-Lat 10.6 cells due to increase of the free BRD4 in the cells. Since peptide **14** composed of Vpr (1–14) and octa-arginine showed no LRA activity in both cell lines (Fig. 10a and b), the LRA activities of **5** and **11** were thought to be from JQ1 moiety. These results are consistent with Turner's proposed mechanism[46] and suggest that chemical knockdown of BRD4 may not result in effective LRA activity. This is similar to knockdown by siRNA.[46,47]

Conclusions

PROTACs represent a new modality in drug development, and researchers are actively engaged in developing clinically usable agents using this approach. Numerous PROTACs have been developed that are based on a limited set of E3 ligase-binding ligands that include VHL, CRBN, cIAP, and keap1. However, certain cell lines do not express VHL[48] or exhibit CRBN resistance.[49] Yet, DCAF-1-mediated PROTACs are often able to work in these cell lines.[16] Therefore, utilizing DCAF-1 as an E3 ligase may expand the application of PROTACs. Our current work focusses on using the HIV-1 accessory protein Vpr in the construction of BRD4-PROTACs. We demonstrated BRD4 degradation by compound **5** that possesses the Vpr-derived peptide Vpr (1–14) as a E3 ligase-binding ligand (Fig. 3 and 4). Structural optimization provided the more potent compound **11** (Fig. 7 and 8). Mechanistic analysis using proteasome and UAE inhibitors and Vpr (1–14) peptides showed that these compounds function as PROTACs to degrade BRD4 *via* UPS (Fig. 9 and Fig. S2, S3). The LRA activities of MZ1, **5**, and **11** were also evaluated (Fig. 10). Results demonstrated that MZ1 is not suitable for use as LRAs. However, the BRD4-PROTACs **5** and **11** have low cytotoxicities and BRD4 degradation abilities. Although these features are not advantageous to the development of BRD4-targeting anti-cancer agents, by increasing dosages PROTACs **5** and **11** showed more potent HIV-1 LRA activities in both J-Lat 10.6 and THP-1 NLuc#225 cells as compared to MZ1. Several reports demonstrate a relationship between BRD4 isoforms and HIV-1 latency.[50–52] There is a long BRD4 isoform, isoform A (isoA, BRD4 (1–1362)), which contains the P-TEFb-interacting domain (PID) at the C-terminus and a short BRD4 isoform, isoform C (isoC, BRD4 (1–722)) that does not have the extended C-terminal region and PID. As mentioned in the Turner's proposed mechanism,[46] releasing BRD4 isoA from chromatin into cytoplasm by BRD4 inhibitors facilitates BRD4-P-TEFb interactions resulting in LRA activity. In contrast, isoC lacking a PID binds to BRM/SWI2-related gene 1 (BRG1, SMARCA4) in SWI/SNF chromatin-remodeling complexes (also known as BRG1-associated factors, BAF) on the latent HIV-1 promoter and represses its transcription.[50] These reports and our findings suggest that there is room for fine-tuning of BRD4 inhibitors and degraders to reduce cytotoxicity and increase isoform selectivity especially for isoC-selective degradation. This may lead to the development of potent and safe LRAs.

Overall, our results identified the HIV-1 accessory protein-derived Vpr (1–14) as a new E3 ligase-binding ligand, that is suitable for PROTAC development. Further investigation is in progress to apply these findings for developing PROTACs targeting other proteins and more potent LRAs.

Author contributions

KT: conceptualization, funding acquisition, investigation, project administration, supervision, writing – original draft, writing – review & editing; XH: investigation, writing – original draft; MM: investigation; SS: funding acquisition, investigation; HY: funding acquisition, investigation, writing – review & editing; HTakeuchi: funding acquisition, supervision, writing – review & editing; YD: funding acquisition, supervision, writing – review & editing; HTamamura: funding acquisition, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information: synthesis and characterization data of compounds including HPLC charts, and experimental procedures and results of biological evaluations. See DOI: **https://doi.org/10.1039/d5cb00125k**.

Acknowledgements

This work was supported in part by JSPS KAKENHI Grant Numbers 22K15243 and 25K08852 (K. T.), 24K18260 (H.Y.), 23H04926 (Y. D.), and 24K02144 (H.Tamamura); Japan Agency for Medical Research and Development (AMED) JP25ama121043 and JP24ama121043 (Research Support Project for Life Science and Drug Discovery, BINDS) (H.Tamamura), 23fk0410052h0002 and 24fk0410052h0003 (H.Takeuchi); Takeda Science Foundation and Pharmacodynamics research foundation (S. S.); TMDU Blue-Bird Contest (BBC) grant (S. S. and K. T.); JST SPRING, Grant Number JPMJSP2180 (X. H.). This work was also supported in part by MEXT Promotion of Development of a Joint Usage/Research System Project: Coalition of Universities for Research Excellence Program (CURE) Grant Number JPMXP1324134721. This research was conducted under the auspices of the Cooperative Research Project of the Research Center for Biomedical Engineering. The authors thank Dr. Terrence R. Burke, Jr., NCI/NIH, for editing this manuscript and Dr. Shinya Fujii, Institute of Science Tokyo (currently Osaka Medical and Pharmaceutical University), for providing MCF-7 cells.

References

1 A. Varshavsky, *Annu. Rev. Biochem.*, 2012, **81**, 167–176.
2 A. Ciechanover, *Nat. Rev. Mol. Cell Biol.*, 2005, **6**, 79–87.
3 D. Nandi, P. Tahiliani, A. Kumar and D. Chandu, *J. Biosci.*, 2006, **31**, 137–155.
4 K. M. Sakamoto, K. B. Kim, A. Kumagai, F. Mercurio, C. M. Crews and R. J. Deshaies, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 8554–8559.
5 X. Zheng, N. Ji, V. Campbell, A. Slavin, X. Zhu, D. Chen, H. Rong, B. Enerson, M. Mayo, K. Sharma, C. M. Browne, C. R. Klaus, H. Li, G. Massa, A. A. McDonald, Y. Shi, M. Sintchak, S. Skouras, D. M. Walther, K. Yuan, Y. Zhang, J. Kelleher, G. Liu, X. Luo, N. Mainolfi and M. M. Weiss, *J. Med. Chem.*, 2024, **67**, 18022–18037.
6 J. A. Livingston, J. Y. Blay, J. Trent, C. Valverde, M. Agulnik, M. Gounder, A. Le Cesne, M. McKean, M. J. Wagner, S. Stacchiotti, S. Agresta, A. Quintás-Cardama, S. A. Reilly, K. Healy, D. Hickman, T. Zhao, A. Ballesteros-Perez, A. Khalil, M. P. Collins, J. Piel, K. Horrigan, A. Lefkovith, S. Innis, A. J. Lazar, G. M. Cote and A. J. Wagner, *Clin. Cancer Res.*, 2025, **31**, 628–638.
7 D. P. Petrylak, M. McKean, J. M. Lang, X. Gao, R. Dreicer, D. M. Geynisman, T. F. Stewart, M. Gandhi, L. J. Appleman, T. B. Dorff, G. S. Chatta, R. Tutrone, J. De La Cerda, E. Berghorn, J. Gong, T. Yu, E. Dominy, E. Chan and N. D. Shore, *J. Clin. Oncol.*, 2024, **42**, 5011.
8 J. Li, W. Xu, P. Yan, Y. Cao, M. Hu and W. Daley, *Cancer Res.*, 2023, **83**, CT128.
9 D. W. Robbins, M. A. Noviski, Y. S. Tan, Z. A. Konst, A. Kelly, P. Auger, N. Brathaban, R. Cass, M. L. Chan, G. Cherala, M. C. Clifton, S. Gajewski, T. G. Ingallinera, D. Karr, D. Kato, J. Ma, J. McKinnell, J. McIntosh, J. Mihalic, B. Murphy, J. R. Panga, G. Peng, J. Powers, L. Perez, R. Rountree, A. Tenn-McClellan, A. T. Sands, D. R. Weiss, J. Wu, J. Ye, C. Guiducci, G. Hansen and F. Cohen, *J. Med. Chem.*, 2024, **67**, 2321–2336.
10 M. Pettersson and C. M. Crews, *Drug Discovery Today:Technol.*, 2019, **31**, 15–27.
11 I. Churcher, *J. Med. Chem.*, 2018, **61**, 444–452.
12 P. P. Chamberlain and L. G. Hamann, *Nat. Chem. Biol.*, 2019, **15**, 937–944.
13 T. Ishida and A. Ciulli, *SLAS Discovery*, 2021, **26**, 484–502.
14 G. M. Burslem and C. M. Crews, *Cell*, 2020, **181**, 102–114.
15 Y. Tao, D. Remillard, E. V. Vinogradova, M. Yokoyama, S. Banchenko, D. Schwefel, B. Melillo, S. L. Schreiber, X. Zhang and B. F. Cravatt, *J. Am. Chem. Soc.*, 2022, **144**, 18688–18699.
16 M. Schröder, M. Renatus, X. Liang, F. Meili, T. Zoller, S. Ferrand, F. Gauter, X. Li, F. Sigoillot, S. Gleim, T. M. Stachyra, J. R. Thomas, D. Begue, M. Khoshouei, P. Lefeuvre, R. Andraos-Rey, B. Chung, R. Ma, B. Pinch, A. Hofmann, M. Schirle, N. Schmiedeberg, P. Imbach, D. Gorses, K. Calkins, B. Bauer-Probst, M. Maschlej, M. Niederst, R. Maher, M. Henault, J. Alford, E. Ahrne, L. Tordella, G. Hollingworth, N. H. Thomä, A. Vulpetti, T. Radimerski, P. Holzer, S. Carbonneau and C. R. Thoma, *Nat. Commun.*, 2024, **15**, 275.
17 F. Forouzanfar, S. Ali, C. Wallet, M. De Rovere, C. Ducloy, H. El Mekdad, M. El Maassarani, A. Aït-Ammar, J. Van Assche, E. Boutant, F. Daouad, F. Margottin-Goguet, C. Moog, C. Van Lint, C. Schwartz and O. Rohr, *Sci. Rep.*, 2019, **9**, 13154.
18 Y. Wu, X. Zhou, C. O. Barnes, M. DeLucia, A. E. Cohen, A. M. Gronenborn, J. Ahn and G. Calero, *Nat. Struct. Mol. Biol.*, 2016, **23**, 933–940.
19 H. J. Sharifi, A. K. Furuya, R. M. Jellinger, M. D. Nekorchuk and C. M. de Noronha, *J. Virol.*, 2014, **88**, 6944–6958.
20 C. Otto, S. Schmidt, C. Kastner, S. Denk, J. Kettler, N. Müller, C. T. Germer, E. Wolf, P. Gallant and A. Wiegering, *Neoplasia*, 2019, **21**, 1110–1120.
21 P. Filippakopoulos, J. Qi, S. Picaud, Y. Shen, W. B. Smith, O. Fedorov, E. M. Morse, T. Keates, T. T. Hickman, I. Felletar, M. Philpott, S. Munro, M. R. McKeown, Y. Wang, A. L. Christie, N. West, M. J. Cameron, B. Schwartz, T. D. Heightman, N. La Thangue, C. A. French, O. Wiest, A. L. Kung, S. Knapp and J. E. Bradner, *Nature*, 2010, **468**, 1067–1073.
22 R. P. Nowak, S. L. DeAngelo, D. Buckley, Z. He, K. A. Donovan, J. An, N. Safaee, M. P. Jedrychowski, C. M. Ponthier, M. Ishoey, T. Zhang, J. D. Mancias,

N. S. Gray, J. E. Bradner and E. S. Fischer, *Nat. Chem. Biol.*, 2018, **14**, 706–714.
23 M. Zengerle, K. H. Chan and A. Ciulli, *ACS Chem. Biol.*, 2015, **10**, 1770–1777.
24 S. Futaki, T. Suzuki, W. Ohashi, T. Yagami, S. Tanaka, K. Ueda and Y. Sugiura, *J. Biol. Chem.*, 2001, **276**, 5836–5840.
25 N. Ohoka, Y. Morita, K. Nagai, K. Shimokawa, O. Ujikawa, I. Fujimori, M. Ito, Y. Hayase, K. Okuhira, N. Shibata, T. Hattori, T. Sameshima, O. Sano, R. Koyama, Y. Imaeda, H. Nara, N. Cho and M. Naito, *J. Biol. Chem.*, 2018, **293**, 6776.
26 H. Xu, T. Kurohara, R. Takano, H. Yokoo, N. Shibata, N. Ohoka, T. Inoue, M. Naito and Y. Demizu, *Chemistry-Open*, 2022, **11**, e202200131.
27 H. Xu, T. Kurohara, N. Ohoka, G. Tsuji, T. Inoue, M. Naito and Y. Demizu, *Bioorg. Med. Chem.*, 2023, **86**, 117293.
28 K. L. Rock, C. Gramm, L. Rothstein, K. Clark, R. Stein, L. Dick, D. Hwang and A. L. Goldberg, *Cell*, 1994, **78**, 761–771.
29 M. L. Hyer, M. A. Milhollen, J. Ciavarri, P. Fleming, T. Traore, D. Sappal, J. Huck, J. Shi, J. Gavin, J. Brownell, Y. Yang, B. Stringer, R. Griffin, F. Bruzzese, T. Soucy, J. Duffy, C. Rabino, J. Riceberg, K. Hoar, A. Lublinsky, S. Menon, M. Sintchak, N. Bump, S. M. Pulukuri, S. Langston, S. Tirrell, M. Kuranda, P. Veiby, J. Newcomb, P. Li, J. T. Wu, J. Powe, L. R. Dick, P. Greenspan, K. Galvin, M. Manfredi, C. Claiborne, B. S. Amidon and N. F. Bence, *Nat. Med.*, 2018, **24**, 186–193.
30 I. G. Cowell, E. M. Ling, R. L. Swan, M. L. W. Brooks and C. A. Austin, *Mol. Pharmacol.*, 2019, **96**, 562–572.
31 N. C. Payne, S. Maksoud, B. A. Tannous and R. Mazitschek, *Cell Chem. Biol.*, 2022, **29**, 1333–1340.
32 S. Kumar, Y. Tomooka and M. Noda, *Biochem. Biophys. Res. Commun.*, 1992, **185**, 1155–1161.
33 A. Jordan, D. Bisgrove and E. Verdin, *EMBO J.*, 2003, **22**, 1868–1877.
34 A. Adachi, H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson and M. A. Martin, *J. Virol.*, 1986, **59**, 284–291.
35 J. Gohda, K. Suzuki, K. Liu, X. Xie, H. Takeuchi, J.-I. Inoue, Y. Kawaguchi and T. Ishida, *Sci. Rep.*, 2018, **8**, 3521.
36 S. C. Iwase, P. Miyazato, H. Katsuya, S. Islam, B. T. J. Yang, J. Ito, M. Matsuo, H. Takeuchi, T. Ishida, K. Matsuda, K. Maeda and Y. Satou, *Sci. Rep.*, 2019, **9**, 12326.
37 H. Kitamura, S. Sukegawa, K. Matsuda, K. Tanimoto, T. Kobayakawa, K. Takahashi, H. Tamamura, K. Tsuchiya, H. Gatanaga, K. Maeda and H. Takeuchi, *Biochem. Biophys. Res. Commun.*, 2023, **641**, 139–147.
38 Z. Li, J. Guo, Y. Wu and Q. Zhou, *Nucleic Acids Res.*, 2012, **41**, 277–287.
39 F. Barré-Sinoussi, J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum and L. Montagnier, *Science*, 1983, **220**, 868–871.
40 K. A. Freedberg, E. Losina, M. C. Weinstein, A. D. Paltiel, C. J. Cohen, G. R. Seage, D. E. Craven, H. Zhang, A. D. Kimmel and S. J. Goldie, *New Eng. J. Med.*, 2001, **344**, 824–831.
41 T. W. Mahungu, A. J. Rodger and M. A. Johnson, *Clin. Med.*, 2009, **9**, 125–128.
42 A. L. Hill, D. I. S. Rosenbloom, F. Fu, M. A. Nowak and R. F. Siliciano, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 13475–13480.
43 S. Klinnert, C. D. Schenkel, P. C. Freitag, H. F. Günthard, A. Plückthun and K. J. Metzner, *Gene Ther.*, 2024, **31**, 74–84.
44 R. P. Nowak, S. L. DeAngelo, D. Buckley, Z. He, K. A. Donovan, J. An, N. Safaee, M. P. Jedrychowski, C. M. Ponthier, M. Ishoey, T. Zhang, J. D. Mancias, N. S. Gray, J. E. Bradner and E. S. Fischer, *Nat. Chem. Biol.*, 2018, **14**, 706–714.
45 S. D. Falcinelli, J. J. Peterson, A. W. Turner, D. Irlbeck, J. Read, S. L. Raines, K. S. James, C. Sutton, A. Sanchez, A. Emery, G. Sampey, R. Ferris, B. Allard, S. Ghofrani, J. L. Kirchherr, C. Baker, J. D. Kuruc, C. L. Gay, L. I. James, G. Wu, P. Zuck, I. Rioja, R. C. Furze, R. K. Prinjha, B. J. Howell, R. Swanstrom, E. P. Browne, B. D. Strahl, R. M. Dunham, N. M. Archin and D. M. Margolis, *J. Clin. Invest.*, 2022, **132**, e157281.
46 A.-M. W. Turner, F. M. Bashore, S. D. Falcinelli, J. A. Fox, A. L. Keller, A. D. Fenton, R. F. Geyer, B. Allard, J. L. Kirchherr, N. M. Archin, J. I. Lindsey and D. M. Margolis, *J. Virol.*, 2025, **99**, e01777-24.
47 J. K. Kisaka, D. Rauch, M. Griffith and G. B. Kyei, *Virol. J.*, 2024, **21**, 80.
48 M. Baba, S. Hirai, H. Yamada-Okabe, K. Hamada, H. Tabuchi, K. Kobayashi, K. Kondo, M. Yoshida, A. Yamashita, T. Kishida, N. Nakaigawa, Y. Nagashima, Y. Kubota, M. Yao and S. Ohno, *Oncogene*, 2003, **22**, 2728–2738.
49 A. K. Gandhi, D. Mendy, M. Waldman, G. Chen, E. Rychak, K. Miller, S. Gaidarova, Y. Ren, M. Wang, M. Breider, G. Carmel, A. Mahmoudi, P. Jackson, M. Abbasian, B. E. Cathers, P. H. Schafer, T. O. Daniel, A. Lopez-Girona, A. Thakurta and R. Chopra, *Br. J. Haematol.*, 2013, **164**, 233–244.
50 R. J. Conrad, P. Fozouni, S. Thomas, H. Sy, Q. Zhang, M.-M. Zhou and M. Ott, *Mol. Cell*, 2017, **67**, 1001–1012.e6.
51 Z. Li, U. Mbonye, Z. Feng, X. Wang, X. Gao, J. Karn and Q. Zhou, *PLoS Pathog.*, 2018, **14**, e1007012.
52 E. Hsieh, D. H. Janssens, P. J. Paddison, E. P. Browne, S. Henikoff, M. OhAinle and M. Emerman, *PLoS Pathog.*, 2023, **19**, e1011101.