Ⅲ．学会等発表実績

様式第１９

学　会　等　発　表　実　績

委託業務題目「ゲノム不安定性を示す難治性遺伝性疾患群の症例収集とゲノム・分子機能解析による病態解明研究」

機関名　長崎大学

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
放射線感受性および各種発達異常を示す遺伝性疾患の新規責任遺伝子の同定と分子機能解析(口頭発表・ポスター発表)	中沢由華、荻朋男	第37回日本分子生物学会年会	2014年11月	国内
Molecular and functional study on the initiation of transcription coupled nucleotide excision repair（ポスター発表）	Guo C, Ogi T	第37回日本分子生物学会年会	2014年11月	国内
転写と共役したヌクレオチド除去修復のin vitro反応系の構築　（ポスター発表）	唐田清伸、郭朝万、荻朋男	第37回日本分子生物学会年会	2014年11月	国内
コケイン症候群様の臨床症状を示す遺伝性疾患の責任遺伝子探索　（ポスター発表）	宮崎仁美、荻朋男	第37回日本分子生物学会年会	2014年11月	国内
エキソーム解析を用いたDNA修復機構欠損性疾患の新規責任遺伝子の探索（ポスター発表）	嶋田繭子、荻朋男	第37回日本分子生物学会年会	2014年11月	国内
Molecular cloning and characterisation of new human DNA repair genes (口頭発表)	Ogi T	3R Symposium Program	2014年11月	国内
Molecular characterization and functional analysis of XRCC4, a novel pathological gene for radiation sensitivity and developmental abnormalities (ポスター発表)	Guo C, Ogi T	3R Symposium Program	2014年11月	国内
ERCC1/XPF deficiency causes three NER-deficient disorders: a patient with various symptoms of xeroderma pigmentosum, Cockayne syndrome, and Fanconi	Nakazawa Y, Kashiyama K, Pilz DT, Guo C, Shimada M, Sasaki K, Fawcett H, Wing JF,	3R Symposium Program	2014年11月	国内

anemia（ポスター発表）	Lewin SO, Carr L, Li TS, Yoshiura K, Utani A, Hirano A, Greenblatt D, Nardo T, Stefanini M, McGibbon D, Sarkany R, Fassihi H, Mitsutake N, Lehmann AR, Ogi T			
ゲノム不安定性疾患群の新規責任遺伝子の同定と分子機能解析（招待講演・口頭発表）	荻朋男	第57回日本甲状腺学会学術集会	2014年11月	国内
ヒストンH3K9メチル化酵素類のDNA二重鎖切断修復反応への関与（シンポジウム・口頭発表）	荻朋男	第87回日本生化学会大会	2014年10月	国内
転写共役ヌクレオチド除去修復の開始反応の分子機構（ワークショップ・口頭発表）	荻朋男	日本放射線影響学会第57回大会	2014年10月	国内
DNA修復機構の異常により発症する先天性疾患とゲノム不安定性/発がん（口頭発表）	荻朋男	第20回日本家族性腫瘍学会学術集会	2014年6月	国内
Transcription, DNA damage and Repair（招待講演・口頭発表）	Ogi T	International Symposium on Xeroderma Pigmentosum and Related Diseases	2014年3月	国内
光線過敏症の最近の動向（口頭）	錦織千佳子	第113回日本皮膚科学会総会・学術大会	2014年5月	国内
小児色素性乾皮症C群の1例（口頭）	錦織千佳子	第38回日本小児皮膚科学会学術大会	2014年7月	国内
特異な臨床症状を示した小児色素性乾皮症Ｃ群(XP-C)の１例（口頭）	正木太朗、中野 英司、錦織千佳子、鈴木民夫	第36回日本光医学・光生物学会	2014年7月	国内
細胞の紫外線損傷DNA修復能の蛍光検出（口頭）	栂達也、倉岡功、渡邉駿、中野英司、竹内聖二、錦織千佳子、菅澤薫、岩井成憲	第36回日本光医学・光生物学会	2014年7月	国内
UV and Melanoma: Insights from Clinical View Points.（口頭）	Nishigori C	XXII International Pigment Cell Conference	2014年9月	国外

Photocarcinogenesis is a complex process caused by DNA damage, inflammation and immunesuppression.（口頭）	Nishigori C	16th International Congress on Photobiology	2014年9月	国外
Genotype -Phenotype Correlation Among Xeroderma Pigmentosum Complementation Group D.（口頭）	Nakano E, Ono R, Masaki T, Takeuchi S, Takaoka Y, Sugasawa K, Nishigori C	3rd Eastern Asia Dermatology Congress	2014年9月	国外
ナローバンド、ブロードバンドＵＶＢ照射後のケラチノサイトとメラノサイトにおけるマイクロアレイ解析（ポスター）	正木太朗、竹内聖二、松田外志朗、錦織千佳子	第73回日本癌学会学術総会	2014年9月	国内
角化細胞と色素細胞における、UVB照射時のマイクロアレイを用いた遺伝子変動解析（ポスター）	竹内聖二、正木太朗、松田外志朗、錦織千佳子	第73回日本癌学会学術総会	2014年9月	国内
A群色素性乾皮症患者細胞における低線量紫外線照射時の網羅的遺伝子発現解析（口頭）	竹内聖二、松田外志朗、小野竜輔、錦織千佳子	第57回日本放射線影響学会	2014年10月	国内
低線量紫外線照射が遺伝子発現プロファイルに与える影響（口頭）	松田外志朗、竹内聖二、小野竜輔、錦織千佳子	第57回日本放射線影響学会	2014年10月	国内
光線過敏症　最近の話題（口頭）	錦織千佳子	第368回日本皮膚科学会山形地方会	2014年12月	国内
Microarray analysis in the keratinocyte and melanocyte exposed to Narrow-band UVB and Broad-band UVB.（ポスター）	Masaki T, Takeuchi S, Matsuda T, Nishigori C	日本研究皮膚科学会第39回年次学術大会・総会	2014年12月	国内
診療科横断的な色素性乾皮症の診療経験（口頭）	錦織千佳子、正木太朗、中野英司、竹内聖二、山下大介、苅田典生、酒井良忠	第438回日本皮膚科学会京滋地方会	2014年12月	国内
Genetic alterations in thyroid cancer and their clinical implications	Mitsutake N	11st Asia Oceania Thyroid Association Congress	2014年11月	国外
Association of polymorphism in the FOXE1 gene locus with papillary thyroid carcinoma in Japanese patients	Saenko V, Rogounovitch T, Bychkov A, Mitsutake N, Matsuse M, Nishihara E, Hirokawa M, Nikitsky A,	第87回日本内分泌学会学術総会	2014年4月	国内

	Minami S, Yamanouchi K, Yoshiura K, Miyauchi A, Yamashita S			
Rare T allele of rs944289 associates not only with risk of malignant but also of benign thyroid tumors	ログノビッチタチアナ、サエンコ ウラジミール、ビチコブ アンドレイ、ニキツキー アリャクサンドル、高橋めい子、中島正洋、林徳眞吉、廣川満良、宮内昭、石垣克、重松和人、松瀬美智子、光武範吏、西原永潤、南恵樹、山内孝彰、伊東正博、吉浦孝一郎、松田文彦、山下俊一	第87回日本内分泌学会学術総会	2014年4月	国内
放射線発がんプロセスに関与する甲状腺生物学	鈴木啓司、光武範吏、山下俊一	第72回日本癌学会学術集会	2014年10月	国内
三次元培養法を用いた新たな甲状腺初代培養法の開発	松瀬美智子、鈴木啓司、山之内孝彰、光武範吏、山下俊一	第57回日本甲状腺学会学術集会	2014年11月	国内
Effect of targeted overexpression of Foxe1 on a mouse thyroid	Nikitski A, Mitsutake N, Saenko V, Rogounovitch T, Yamashita S	第57回日本甲状腺学会学術集会	2014年11月	国内
新規小頭症/放射線感受性症責任遺伝子の同定と機能解析（口頭発表）	中沢由華、郭朝万、嶋田繭子、賈楠、唐田清信、永山雄二、荻朋男	第10回広島大学・長崎大学連携研究事業カンファランス	2014年5月	国内
Four cases of xeroderma pigmentosum diagnosed by non-radioactive system using ethynyluracil derivatives	Tomimura S, Nakazawa Y, Kuwatsuka S, Kuwatsuka Y, Ogi T, Utani A	The 39th Annual Meeting of the Japanese Society for Investigative Dermatology	2014年12月	国内
Chk2 and Rad18 contribute	Tanoue Y,	29th RBC-NIRS	2013年11月	国内

cooperatively to maintain genomic stability, leading to prevention of tumorigenesus and germ cells maintenance	Tateishi S	INTERNATIONAL SYMPOSIUM		
Chk2 and Rad18 concertedly maintain genomic integrity to prevent tumor formation	Tateishi S	International Conference Replication, repair and transcription; coupling mechanisms and chromatin dynamics for genome integrity,	2014年2月	国内
遺伝性髄様癌になぜ乳頭癌が合併するのか	内野眞也	第26回日本内分泌外科学会	2014年5月	国内
甲状腺髄様癌の遺伝子診断	内野眞也	第73回日本癌学会総会、シンポジウム	2014年9月	国内
IgGサブクラス欠損を示したI型高IgE症候群の1例	金子英雄、大西秀典、川本典生、加藤善一郎、舩戸道徳、小田紘嗣、小原収、深尾敏幸	日本免疫不全症研究会	2014年1月	国内
Multiple Ubiquitination pathways suppress RAD51 recruitment through facilitating RPA S4/S8 phosphorylation	Nakajima K, Nakada S	Keystone Symposium	2015年3月	国外

2．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
A rapid comprehensive assay system for DNA repair activity and cytotoxic effects of DNA damaging reagents by measuring unscheduled DNA synthesis and recovery of RNA synthesis after DNA damage.	Jia N, Nakazawa Y, Guo C, Shimada M, Sethi M, Takahashi Y, Ueda H, Nagayama Y, Ogi T.*	Nature Protocols	2015年	国外
Hypomorphic PCNA mutation underlies a human DNA repair disorder.	Baple EL, Chambers H, Cross HE, Fawcett H, Nakazawa Y, Chioza BA, Harlalka GV, Mansour S, Sreekantan-Nair A, Patton	Journal of Clinical Investigation	2014年	国外

	MA, Muggenthaler M, Rich P, Wagner K, Coblentz R, Stein CK, Last JI, Taylor AM, Jackson AP, Ogi T, Lehmann AR, Green CM, Crosby AH.			
Differences in clinical phenotype among patients with XP complementation group D: 3D structure and ATP-docking of XPD *in silico*.	Nakano E, Ono R, Masaki T, Takeuchi S, Takaoka Y, Maeda E, Nishigori C	Journal of Investigative Dermatology	2014年	国外
Fluorescence detection of cellular nucleotide excision repair of damaged DNA.	Toga T, Kuraoka I, Watanabe S, Nakano E, Takeuchi S, Nishigori C, Sugasawa K, Iwai S	Scientific Reports	2014年	国外
Photocarcinogenesis and inflammation.	Nishigori C	Cancer and Inflammation Mechanisms: Chemical, Biological, and Clinical Aspects. Edited by Yusuke Hiraku, Shosuke Kawanishi, Hiroshi Ohshima, John Wiley & Sons, Inc,	2014年	国外
Inhibitory effects of dietary *Spirulina platensis* on UVB-induced skin inflammatory responses and carcinogenesis.	Yogianti F, Kunisada M, Nakano E, Ono R, Sakumi K, Oka S, Nakabeppu Y, Nishigori C	Journal of Investigative Dermatology	2014年	国外
色素性乾皮症（XP）バリアント型	錦織千佳子	皮膚病診療	2014年	国内
Heparin inhibits melanosome uptake and inflammatory response coupled with phagocytosis through blocking PI3k/Akt	Makino-Okamura C, Niki Y, Takeuchi S, Nishigori C, Declercq L,	Pigment Cell & Melanoma Research	2014年	国外

and MEK/ERK signaling pathways in human epidermal keratinocytes.	Yarosh DB, Saito N			
光老化のモデルとしての色素性乾皮症	中野英司、錦織千佳子	医学の歩み	2014年	国内
Case of Hermansky-Pudlak syndrome 1 patient with milder symptoms in Japanese.	Takeuchi S, Abe Y, Yamada T, Kawano S, Hozumi Y, Ito S, Suzuki T, Nishigori C	Journal of Dermatology	2014年	国外
The common genetic variant rs944289 at chromosome 14q13.3 associates with risk of both malignant and benign thyroid tumors in Japanese population.	Rogounovitch T, Bychkov A, Takahashi M, Mitsutake N, Nakashima M, Nikitski A, Hayashi T, Hirokawa M, Ishigaki K, Shigematsu K, Bogdanova TI, Matsuse M, Nishihara E, Minami S, Yamanouchi K, Ito M, Kawaguchi T, Kondo H, Takamura N, Ito Y, Miyauchi A, Matsuda F, Yamashita S, Saenko V.	Thyroid	2015年	国外
Radiation signatures in childhood thyroid cancers after the Chernobyl accident: Possible roles of radiation in carcinogenesis.	Suzuki K, Mitsutake N, Saenko V, Yamashita S.	Cancer Science	2014年	国外
Studies on Expression of Aldehyde Dehydrogenase in Normal and Cancerous Tissues of Thyroids.	Kurashige T, Shimamura M, Yasui K, Mitsutake N, Matsuse M, Nakashima M, Minami S, Eguchi S, Nagayama Y.	Hormone and Metabolic Research	2014年	国外
Morphological difference in adult thyroid papillary	Ito M, Bogdanova T,	Endocrine Journal	2014年	国外

carcinoma between Japan and Ukraine.	Saenko V, Rogounovitch T, Mitsutake N, Kondo H, Maeda S, Nakashima M, Yamashita S.			
A Bead-based Normalization for Uniform Sequencing depth (BeNUS) protocol for multi-samples sequencing exemplified by HLA-B.	Hosomichi K, Mitsunaga S, Nagasaki H, Inoue I*	BMC Genomics	2014年	国外
Molecular interplays involved in the cellular uptake of octaarginine on cell surfaces and the importance of syndecan-4 cytoplasmic V domain for the activation of protein kinase Calpha.	Nakase I, Osaki K, Tanaka G, Utani A, Futaki S.	Biochemical and Biophysical Research Communications	2014年	国外
Pseudoxanthoma elasticum-like skin lesions with congenital erythropoietic porphyria.	Mine Y, Iwanaga A, Ikehara S, Koike Y, Takamura N, Utani A.	European Journal of Dermatology	2014年	国外
Cold agglutinin disease-associated digital gangrene treated with plasmapheresis.	Koike Y, Akiyama Y, Utani A.	Indian Journal of Dermatology, Venereology and Leprology	2014年	国外
Letter: Sacral pressure ulcer successfully treated with traction, resulting in a reduction of wound deformity.	Mizokami F, Furuta K, Matsumoto H, Utani A, Isogai Z.	International Wound Journal	2014年	国外
Angiosarcoma of the scalp successfully treated with pazopanib.	Tomita H, Koike Y, Asai M, Ogawa F, Abe K, Tanioka M, Utani A.	Journal of the American Academr Y of Dermatology	2014年	国外
Calcification of the placenta in a woman with pseudoxanthoma elasticum with a mutation of the ABCC6 gene.	Tanioka M, Utani A, Tamura H, Yoshimura N, Kashiwagi N, Kondo E, Konishi I, Miyachi Y.	Journal of Dermatology	2014年	国外
Mycetoma caused by Nocardia transvalensis	Ichinomiya A, Nishimura	Journal of Dermatology	2014年	国外

with repeated local recurrences for 25 years without dissemination to viscera.	K, Takenaka M, Utani A, Nishimoto K.			
Proposal of the new name "eruptive papular collagenoelastopathy" to unify two indistinguishable entities: eruptive collagenoma and papular elastorrhexis.	Kuwatsuka S, Kuwatsuka Y, Tomimura S, Utani A.	Journal of Dermatology	in press	国外
Yusho patients show increased serum IL-17, IL-23, IL-1beta, and TNFalpha levels more than 40 years after accidental polychlorinated biphenyl poisoning.	Kuwatsuka Y, Shimizu K, Akiyama Y, Koike Y, Ogawa F, Furue M, Utani A.	Journal of Immunotoxicology	2014年	国外
A survey of 165 sporotrichosis cases examined in Nagasaki prefecture from 1951 to 2012.	Takenaka M, Yoshizaki A, Utani A, Nishimoto K.	Mycoses	2014年	国外
Nationwide survey of Cockayne syndrome in Japan: its incidence, clinical course and prognosis	Kubota M, Ohta S, Ando A, Koyama A, Terashima H, Kashii H, Hoshino H, Sugita K. Hayashi M.	Pediatrics International	in press	国内
コケイン症候群	久保田雅也	日本臨床別冊神経症候群Ⅲ	2014	国内
Seckel症候群	久保田雅也	日本臨床別冊神経症候群Ⅳ	2014	国内
Canavan disease: Clinical features and recent advances in research.	Hoshino H, Kubota M	Pediatrics International	2014	国外
RAD18 activates the G2/M checkpoint through DNA damage signaling to maintain genome integrity after ionizing radiation exposure.	Sasatani M, Xu Y, Kawai H, Cao L, Tateishi S, Shimura T, Li J, Iizuka D, Noda A, Hamasaki K, Kusunoki Y, Kamiya K*.	PLos One	in press	国外
Regulation of Y-Family Translesion Synthesis (TLS) DNA polymerases by	Vaziri C*, Tateishi S, Yang Y,	Editors: Domenico Maiorano & Dr. Jean-Sébastian	2014 年	国外

RAD18. In 'Translesion DNA polymerases: from DNA repair and beyond'	Greenwalt A.	Hoffmann,		
Two replication fork maintenance pathways fuse inverted repeats to rearrange chromosomes.	Hu L, Kim TM, Son MY, Kim SA, Holland CL, Tateishi S, Kim DH, Yew PR, Montagna C, Dumitrache LC, Hasty P*.	Nature	2013年	国外
A non-catalytic role of DNA polymerase η in recruiting Rad18 and promoting PCNA monoubiquitination at stalled replication forks.	Durando M, Tateishi S, Vaziri C*.	Nucleic Acids Research	2013年	国外
Mutations in UVSSA cause UV-sensitive syndrome and impair RNA polymerase IIo processing in transcription-coupled nucleotide-excision repair.	Nakazawa Y, Sasaki K, Mitsutake N, Matsuse M, Shimada M, Ohyama K, Ito K, Masuyama R, Kudo T, Utani A, Takenaka K, Miki Y, Nardo T, Stefanini M, Takahashi Y, Yamashita S, Tateishi S, Lehmann A, Yoshiura K, Ogi T*.	Nature Genetics	2012年	国外
An extended family with familial medullary thyroid carcinoma and Hirschsprung's disease.	Igarashi T, Okamura R, Jikuzono T, Uchino S*, Sugitani I, Shimizu K	Journal of Nippon Medical School	2014年	国内
甲状腺癌の遺伝子異常	内野眞也	Medical Practice	2014年	国内
多発性内分泌腫瘍症2型の診断と治療.	内野眞也	日本甲状腺学会雑誌	2014年	国内
甲状腺癌の治療戦略 家族性甲状腺癌.	内野眞也	ENTONI	2014年	国内
家族性腫瘍における遺伝子診断の実際.	内野眞也	日本外科学会雑誌	2014年	国内
A complement factor B mutation in a large	Funato M, Uemura O,	Journal of Clinical Immunology	2014年	国外

kindred with atypical hemolytic uremic syndrome.	Ushijima K, Ohnishi H, Orii K, Kato Z, Yamakawa S, Nagai T, Ohara O, Kaneko H, Kondo N.			
添加物・仮性薬理活性物質（仮性アレルゲン）	金子英雄	小児科	2014年	国内
食物アレルギーによっておこる症状はどんなものがありますか。食物アレルギーハンドブック 2014 —子供の食に関わる方々へ—	金子英雄	日本小児アレルギー学会、協和企画	2014年	国内
ブルーム症候群	金子英雄	別冊日本臨床No28神経症候群（第2版）	2014年	国内
Baller-Gerold症候群	金子英雄	別冊日本臨床No29神経症候群（第2版）	2014年	国内
FBH1 influences DNA replication fork stability and homologous recombination through ubiquitylation of RAD51.	Chu WK, Payne MJ, Beli P, Hanada K, Choudhary C, Hickson ID.	Nature Communications	2015年	国外
Molecular analysis of the BCR-ABL1 kinase domain in chronic-phase chronic myelogenous leukemia treated with tyrosine kinase inhibitors in practice: Study by the Nagasaki CML Study Group.	Itonaga H, Tsushima H, Imanishi D, Hata T, Doi Y, Mori S, Sasaki D, Hasegawa H, Matsuo E, Nakashima J, Kato T, Horai M, Taguchi M, Matsuo M, Taniguchi H, Makiyama J, Sato S, Horio K, Ando K, Moriwaki Y, Sawayama Y, Ogawa D, Yamasaki R, Takasaki Y, Imaizumi Y, Taguchi J, Kawaguchi Y, Yoshida S, Joh T, Moriuchi Y, Nonaka H,	Leukemia Research	2014年	国外

	Soda H, Fukushima T, Nagai K, Kamihira S, Tomonaga M, Yanagihara K, Miyazaki Y.			
Expression of myeloperoxidase in acute myeloid leukemia blasts mirrors the distinct DNA methylation pattern involving the downregulation of DNA methyltransferase DNMT3B.	Itonaga H, Imanishi D, Wong YF, Sato S, Ando K, Sawayama Y, Sasaki D, Tsuruda K, Hasegawa H, Imaizumi Y, Taguchi J, Tsushima H, Yoshida S, Fukushima T, Hata T, Moriuchi Y, Yanagihara K, Miyazaki Y.	Leukemia	2014 年	国外
Comprehensive analysis of genetic alterations and their prognostic impacts in adult acute myeloid leukemia patients.	Kihara R, Nagata Y, Kiyoi H, Kato T, Yamamoto E, Suzuki K, Chen F, Asou N, Ohtake S, Miyawaki S, Miyazaki Y, Sakura T, Ozawa Y, Usui N, Kanamori H, Kiguchi T, Imai K, Uike N, Kimura F, Kitamura K, Nakaseko C, Onizuka M, Takeshita A, Ishida F, Suzushima H, Kato Y, Miwa H, Shiraishi Y, Chiba K, Tanaka H, Miyano S,	Leukemia	2014 年	国外

	Ogawa S, Naoe T.			
Longitudinal Analysis of DNA Methylation in CD34+ Hematopoietic Progenitors in Myelodysplastic Syndrome.	Wong YF, Micklem CN, Taguchi M, Itonaga H, Sawayama Y, Imanishi D, Nishikawa S, Miyazaki Y, Jakt LM.	Stem Cells Translational Medicine	2014年	国外
Rad18 and Rnf8 facilitate homologous recombination by two distinct mechanisms, promoting Rad51 focus formation and suppressing the toxic effect of nonhomologous end joining.	Kobayashi S, Kasaishi Y, Nakada S, Takagi T, Era S, Motegi A, Chiu RK, Takeda S, Hirota K.	Oncogene	in press	国外

Ⅳ．研究成果の刊行物・別刷

PROTOCOL

A rapid, comprehensive system for assaying DNA repair activity and cytotoxic effects of DNA-damaging reagents

Nan Jia[1–3,8], Yuka Nakazawa[2,3,8], Chaowan Guo[2,3], Mayuko Shimada[2,3], Mieran Sethi[4], Yoshito Takahashi[5,7], Hiroshi Ueda[1], Yuji Nagayama[3] & Tomoo Ogi[2,3,6]

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DNA repair systems protect cells from genomic instability and carcinogenesis. Therefore, assays for measuring DNA repair activity are valuable, not only for clinical diagnoses of DNA repair deficiency disorders but also for basic research and anticancer drug development. Two commonly used assays are UDS (unscheduled DNA synthesis, requiring a precise measurement of an extremely small amount of repair DNA synthesis) and RRS (recovery of RNA synthesis after DNA damage). Both UDS and RRS are major endpoints for assessing the activity of nucleotide excision repair (NER), the most versatile DNA repair process. Conventional UDS and RRS assays are laborious and time-consuming, as they measure the incorporation of radiolabeled nucleosides associated with NER. Here we describe a comprehensive protocol for monitoring nonradioactive UDS and RRS by studying the incorporation of alkyne-conjugated nucleoside analogs followed by a fluorescent azide-coupling click-chemistry reaction. The system is also suitable for quick measurement of cell sensitivity to DNA-damaging reagents and for lentivirus-based complementation assays, which can be used to systematically determine the pathogenic genes associated with DNA repair deficiency disorders. A typical UDS or RRS assay using primary fibroblasts, including a virus complementation test, takes 1 week to complete.

INTRODUCTION

The DNA repair system is important for maintaining genome integrity[1,2]; therefore, failures in the system are implicated in various pathological conditions including carcinogenesis and aging. Furthermore, congenital defects in the DNA repair system give rise to genetic disorders associated with genome instability[1–5]. NER is one of the most versatile DNA repair processes operating in mammals[6]. It deals with a diversity of DNA lesions, including UV-induced photo-lesions, chemical DNA adducts and some forms of oxidative damage. There are two subpathways in NER: global genome repair (GGR), which removes damage from both expressed and silent genomic regions[7], and transcription-coupled repair (TCR), which removes damage specifically from actively transcribed genes[8,9]. Xeroderma pigmentosum (XP) and Cockayne syndrome (CS) are representative genetic disorders associated with defects in NER[10,11]. XP and CS patients are characterized by photosensitivity, but there is variation in their clinical features. For example, XP patients show greater pigmentary changes and have a very high incidence of skin cancer. The most severe XP cases suffer from neurological degeneration and mental retardation as a consequence of defective NER. CS patients have several developmental abnormalities, including physical and mental retardation, microcephaly and premature aging, but they have no susceptibility to skin cancer[3,4].

XP results from defects in one of the seven NER-related genes, *XPA* to *XPG* (complementation groups XP-A to XP-G), and in *POLH* (encoding DNA polymerase-η; referred to hereafter as *XPV* for 'XP variant')[12], whereas two genes, *ERCC8* (referred to hereafter as *CSA*) and *ERCC6* (referred to hereafter as *CSB*) are mutated in CS[1,7,9,13]. The proteins, XPA to XPG, and CSA and CSB, have distinct roles in NER. The lesion recognition in GGR is mediated by XPC and XPE. XPB and XPD are the helicase subunits of the transcription factor TFIIH, which stimulates unwinding of damaged DNA after damage recognition[14]. XPA is responsible for the lesion-verification process before damaged DNA is incised by the ERCC1-XPF endonuclease complex from the 5′ side and by another endonuclease, XPG, from the 3′ side. Unlike GGR, TCR is initiated by the recognition of stalled RNA polymerase IIo (RNA polIIo) at sites of DNA damage. The recently identified factor UV-stimulated scaffold protein A (UVSSA)[15–17], which has been associated with UV-sensitive syndrome (UV^SS) in its mutated form, and CSA, CSB and other partner proteins are recruited to facilitate the removal of RNA polIIo enzymes stalled at the DNA damage site. Processing the stalled RNA polIIo allows TFIIH to bind at the DNA damage site, before damage incision by ERCC1-XPF and XPG endonucleases. As summarized in **Table 1**, patients with XP, CS or UV^SS have defects in one of the NER genes described above; however, different mutations in the same NER gene can result in different disorders. *XPB*, *XPD* and *XPG* are required for the proper function and stability of TFIIH; therefore, mutations in these genes may compromise global transcription as well as NER activity, and may result in a wide range of clinical features associated with XP, CS or related diseases[18–21].

Aside from the clinical features, definitive diagnoses of NER-deficiency disorders requires measurement of DNA repair activity, which includes UDS and RRS[22,23]. UDS refers to damage-induced, non-S-phase DNA repair synthesis and is used

TABLE 1 | Summary of UV-induced DNA damage–repair pathway genes.

Assay			Deficiency in DNA repair pathway	Related genes	Related diseases	Assay method used in virus complementation
UDS	RRS	Cell sensitivity				
+	–	ND	TCR	*CSA, CSB* *UVSSA*	CS or UV^SS UV^SS	RRS
–	+	ND	GGR	*XPC, XPE*	XP	UDS
–	–	ND	GGR and TCR	*XPA* *XPB, XPD, XPF, XPG, ERCC1*	XP XP, CS or others	UDS/RRS[a]
+	+	–	Translesion synthesis	*POLH (XPV)*	XP-V	Cell sensitivity

+, normal; –, deficiency; ND, not necessary to do.
[a]Either UDS or RRS can be performed in this condition.

to determine GGR activity. RRS is used as a measurement of TCR activity, as unrepaired DNA damage elicits transient inhibition of mRNA transcription.

Assays to measure DNA damage repair

Generally, a 254-nm UVC germicidal lamp is used to induce DNA damage in cells; UDS or RRS activity is then measured by evaluating the incorporation of radiolabeled nucleoside or nucleoside analog. Conventionally, UDS and RRS are measured by autoradiography of incorporated ^{3}H-thymidine (^{3}H-deoxythymidine, ^{3}H-thy) or ^{3}H-uridine, respectively[22–25]. Nuclear silver grain counting provides a measurement of UDS and RRS, as each individual event of nucleotide incorporation can be detected. Although the procedure is laborious and time-consuming, this autoradiographic technique is still commonly used in many diagnostic and basic research laboratories, as it is a sensitive and accurate method.

Direct scintillation counting of an incorporated radionucleotide is another option for measuring UDS and RRS; however, although this process is much quicker than nuclear silver grain counting, it is less accurate. BrdU and bromouridine (BrU) can be used in place of ^{3}H-thymidine and ^{3}H-uridine in UDS and RRS, respectively, for immunofluorescence-based detection methods[26]. Although these assays avoid the use of radioactive materials and save time, they are not in widespread use owing to their low sensitivity and low resolution. The host cell reactivation assay[27] is used for XP and CS clinical diagnoses in several laboratories, but the technique is not suitable for the direct measurement of DNA repair activity.

Alkyne-conjugated nucleoside analogs 5-ethynyl-2′-deoxyuridine (EdU) and 5-ethynyluridine (EU) were recently developed for labeling of S-phase DNA and nascent RNA synthesis, respectively[28,29]. The incorporated nucleotides can be detected after conjugation with fluorescent azide dye via a copper-catalyzed alkyne-azyde–coupling click-chemistry reaction[30]. We established a method for the precise measurement of an extremely small amount of DNA synthesis associated with UDS[31], which was then used to develop a semiautomated assay system for UDS and RRS measurement, using EdU and EU as alternatives to conventionally used radiolabeled nucleosides or BrdU-labeled nucleosides[32]. As EdU and EU can be directly and specifically conjugated to a fluorescent dye, the sensitivity and background signal associated with our approach are markedly improved compared with conventional approaches, equaling the more recently developed autoradiographic methods[31,32]. These procedures use plastic 96-well tissue culture plates and a high-content screening (HCS) imaging system—an automated plate scanner equipped with a fluorescence microscope and a CCD camera, which saves time and greatly improves the accuracy of the procedure and thus the reproducibility of these assays.

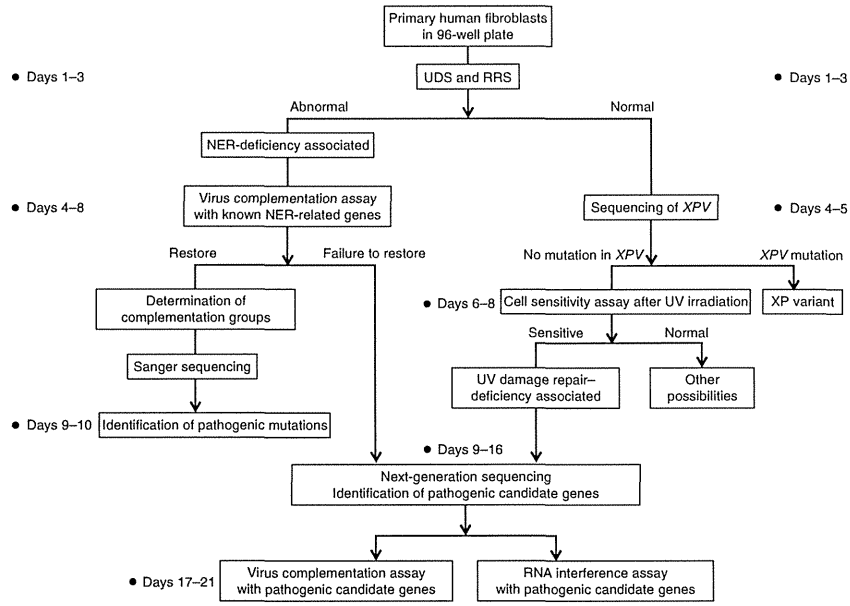

Figure 1 | A flowchart diagram showing standard procedures for diagnosing UV-sensitive DNA repair–deficiency disorders using the assay system.

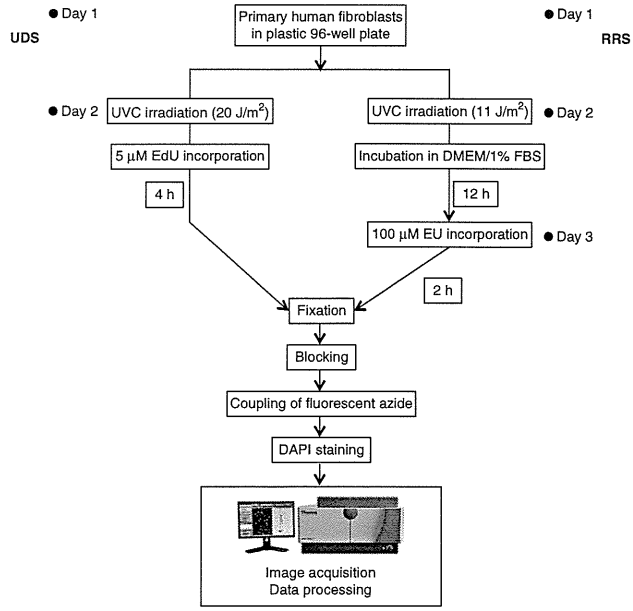

Figure 2 | Overview of the experimental procedure for the UDS and RRS assays.

Our system can also be used for EdU-based measurement of cell sensitivity after DNA-damaging treatment. This cell sensitivity assay protocol is based on a measurement of cell proliferation potency by incorporation of EdU during S-phase. The protocol is compatible with most standard cell biology techniques such as immunofluorescence staining[31], ectopic gene expression and RNA interference–based gene-knockdown assays[33]. We have also used the system to develop a lentivirus-based complementation assay[15], which can be used to systematically determine the pathogenic genes linked to DNA repair deficiency disorders[15,34]. In this assay, primary fibroblasts are infected with a set of lentiviruses carrying DNA repair genes and evaluated for complementation of UDS activity, RRS activity or cell viability. The protocol is also applicable to siRNA screening studies; DNA repair–related genes are knocked down by a set of siRNA oligonucleotides in normal cells, and the automated assay system is used to measure UDS and RRS levels[32].

EdU-based UDS and EU-based RRS, together with cell sensitivity and virus complementation assays, could feasibly become gold-standard techniques for the diagnosis of NER-deficient disorders. **Figure 1** summarizes the standard diagnostic procedures with the assay system for photosensitive DNA repair–deficiency disorders such as XP, CS and UV-sensitive syndrome. After the patient's primary fibroblast cells are received, we first perform UDS and RRS assays to confirm or refute an NER deficiency and to determine which subpathway of NER, GGR or TCR is affected (**Figs. 2–4** and **Table 1**). In confirmed NER-deficiency cases, we perform the lentivirus complementation assay to determine responsible genes (**Figs. 3, 5** and **6**), followed by identification of mutations in the genes by Sanger DNA sequencing. In refuted cases with normal UDS and RRS activity, we perform cell sensitivity assay to evaluate whether the patient's symptoms are associated with UV-induced DNA damage repair deficiency (**Figs. 3, 5** and **7**). In cases of suspected XP-V, direct genomic DNA sequencing of the *XPV* gene is performed[12]. In both NER-related and non-NER-related cases, unidentified DNA repair genes may be involved. In these cases, potential pathogenic mutations that relate to the DNA repair system can be further investigated by next-generation sequencing[15]. Lentivirus complementation and RNA interference

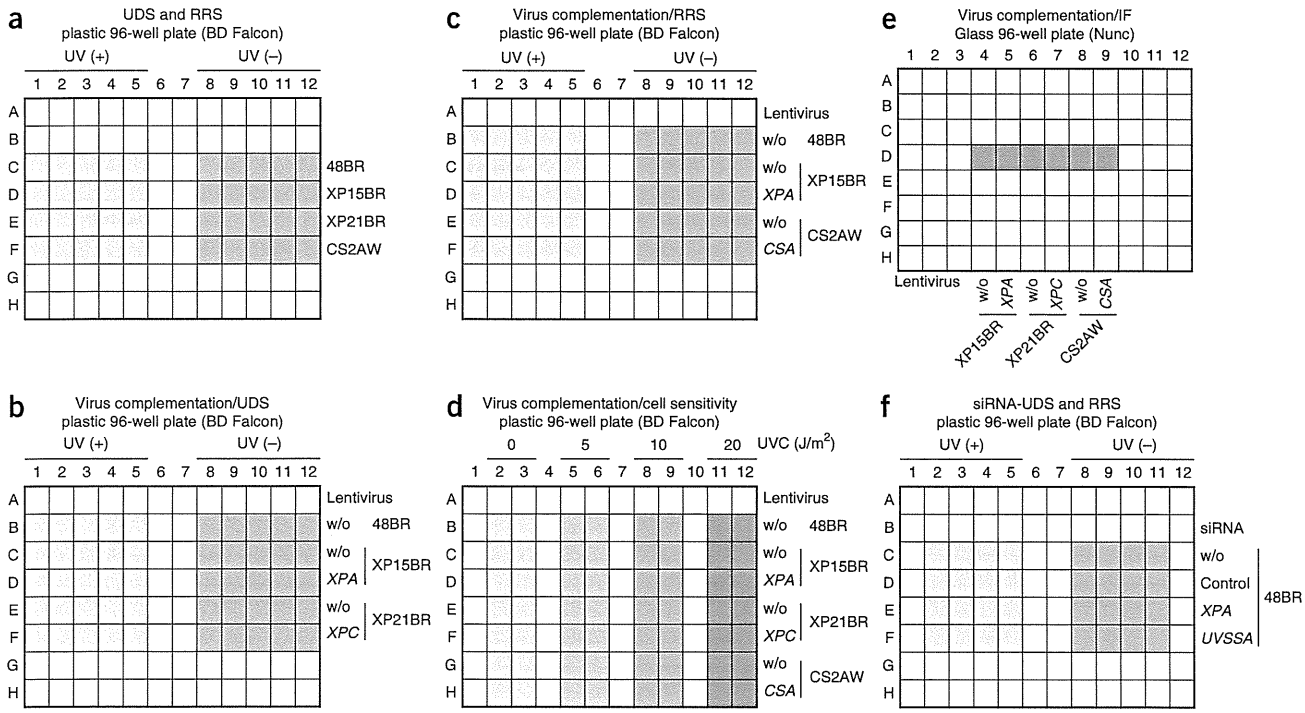

Figure 3 | Typical 96-well plate formats. (**a–f**) UDS and RRS assays (**a**); virus complementation experiments for UDS (**b**), RRS (**c**) and cell sensitivity assays (**d**); immunofluorescence (IF) staining for measurement of viral infection efficiency (**e**); RNA interference assay (**f**). w/o, without virus infection or siRNA transfection.

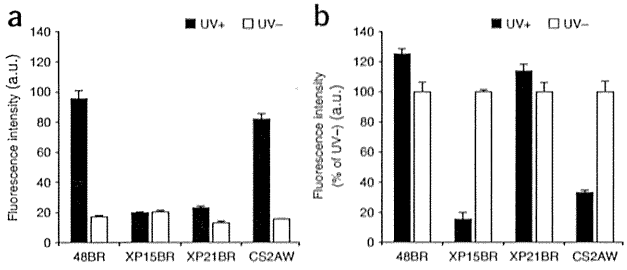

Figure 4 | Measurement of NER activity by UDS and RRS assays. Normal 48BR, XP patient–derived XP15BR (XPA) and XP21BR (XPC), and CS patient–derived CS2AW (CSA) cells were used. (**a**) EdU-based UDS assay: indicated cells were seeded on a plastic 96-well plate. UV irradiation was performed on half of the assay plate (black bars, 20 J/m^2 UVC irradiation; white bars, no UV irradiation), followed by 4 h of incubation with 5 μM EdU. Incorporated EdU was detected as described in the main PROCEDURE (Steps 7–16). (**b**) EU-based RRS assay: indicated cells were seeded on a plastic 96-well plate. UV irradiation was performed on half of the assay plate (black bars, 11 J/m^2 UVC irradiation; white bars, no UV irradiation), followed by 12 h incubation with DMEM supplemented with 1% (vol/vol) FBS. EU incorporation and detection were performed as described in the main PROCEDURE (Steps 7–16). Bars and error bars represent average fluorescence intensity of quintuplicates and s.d., respectively. In **b**, RRS was normalized to activity measurement in nonirradiated cells. a.u., arbitrary units.

assays (**Figs. 3, 5** and **8**) are then performed to confirm involvement of newly identified DNA repair candidate genes[34].

Applications of the protocol

In the comprehensive protocol presented here, we provide step-by-step instruction on how to perform UDS, RRS, cell sensitivity, virus complementation and RNA interference assays in primary human fibroblasts. In our studies, we have focused on the use of human primary fibroblasts derived from patients with DNA repair deficiencies; however, in principle, the protocol could be used with any adherent cells, such as MRC5, HEK293 and HeLa cells. We have shown that the protocol can be applied to immortalized lymphoblastoid cell lines (LBLs) by performing a few preceding steps[32] (**Supplementary Methods 1–5**); however, the assays are not as sensitive and specific with LBLs as they are with primary fibroblasts (**Supplementary Fig. 1**).

A key advantage of this protocol over other methods is that it can be used as a high-throughput screening system. The protocol is not restricted to the study of UV-induced DNA damage repair–deficiency disorders in human cells. On the basis of the cell sensitivity assay, we are also able to investigate DNA repair activity for DNA damage, such as γ-ray–induced double-strand breaks (DSBs)[35] (**Supplementary Method 4** and **Supplementary Fig. 2**) or mitomycin C–induced DNA interstrand cross-links (ICLs)[34] (**Supplementary Method 4** and **Supplementary Fig. 3**). The UDS and RRS assays are also potentially applicable to drug screening of small chemical compounds.

Overview of the protocol

EdU-based UDS and EU-based RRS assays. The experimental schemes for the UDS and RRS assays are summarized in **Figure 2**. Normal and DNA repair–deficient patient-derived fibroblasts are seeded in quintuplicate on a plastic 96-well plate (**Fig. 3a–c**) and cultured for 16 h in FBS-supplemented DMEM. Half of the plate is irradiated with UVC (UV+), whereas the other half is wrapped in aluminum foil (UV−). In the UDS assay, cells are incubated in serum-free EdU-supplemented DMEM immediately after irradiation. For the RRS assay, cells are irradiated at a lower dose

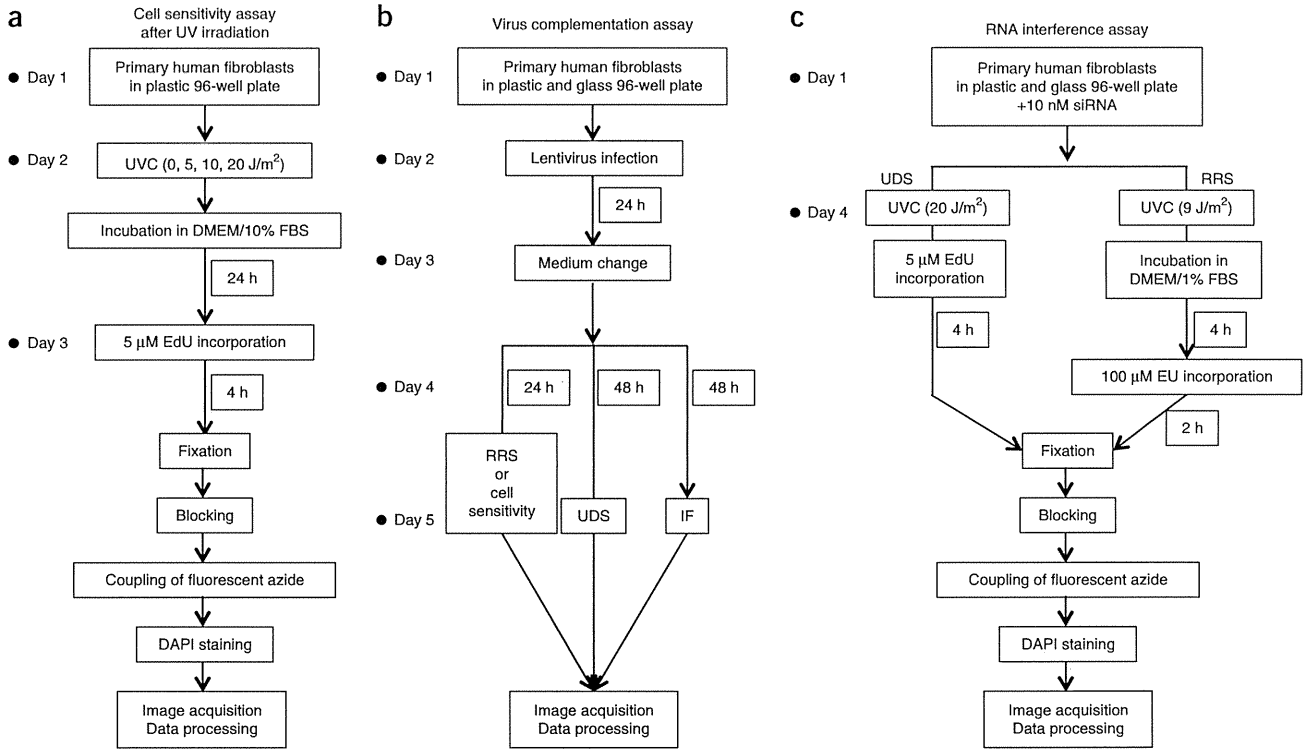

Figure 5 | Experimental schemes. (**a–c**) Shown are schemes for cell sensitivity assay after UV irradiation (**a**), virus complementation assay (**b**) and RNA interference assay (**c**).

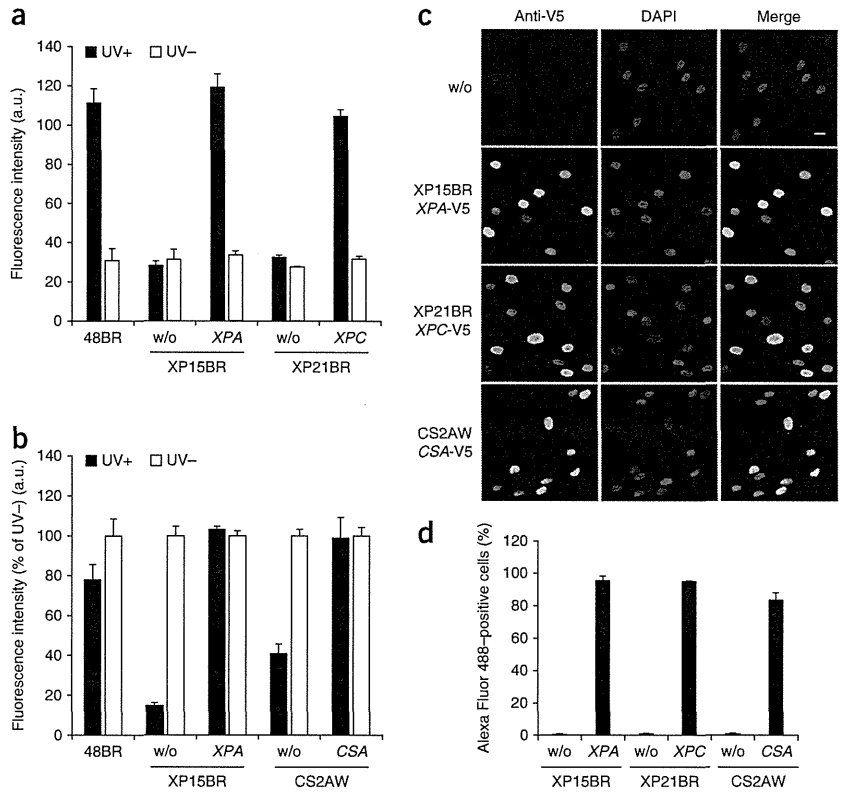

Figure 6 | Virus complementation experiments for UDS and RRS assays. (**a**) UDS assay for normal 48BR, and XP patient–derived XP15BR and XP21BR cells. Cells were seeded on a plastic 96-well plate, followed by infection with a set of lentiviruses expressing *XPA* or *XPC* cDNA. At 72 h after virus infection, cells were UV irradiated (black bars, 20 J/m^2 UVC irradiation; white bars, no UV irradiation), followed by EdU incorporation and detection as described in the main PROCEDURE (Steps 6–16). (**b**) RRS assay for normal 48BR, XP patient–derived XP15BR and CS patient–derived CS2AW cells. Cells were seeded on a plastic 96-well plate, followed by infection with a set of lentiviruses expressing *XPA* or *CSA* cDNA. At 60 h after virus infection, cells were UV irradiated (black bars, 11 J/m^2 UVC irradiation; white bars, no UV irradiation), followed by 12 h of incubation with DMEM supplemented with 1% (vol/vol) FBS. EU incorporation and detection were performed as described in the main PROCEDURE (Steps 6–16). Bars and error bars represent average fluorescence intensity of quintuplicates and s.d. in **a** and **b**, respectively. In **b**, RRS was normalized to activity measurement in nonirradiated cells. (**c**,**d**) Viral infection efficiency was confirmed by immunofluorescence staining with anti-V5-tag antibody. Cells were seeded on a glass-bottomed 96-well plate, followed by infection with a set of lentiviruses expressing *XPA*, *XPC* or *CSA* cDNA. At 72 h after infection, immunofluorescence staining was performed as described in step 5B of **Box 2**. Infection efficiency was calculated as a number of Alexa 488–positive cells using the VTI system. Scale bar, 20 μm. a.u., arbitrary units; w/o, without virus infection.

and incubated in FBS-supplemented DMEM to allow RRS before incubation in serum-free EU-supplemented DMEM. After incorporation of EdU or EU, cells are fixed, exposed to Alexa Fluor 488–azide coupling solution and then stained with DAPI. Image acquisition and data processing are automated using an HCS system, the ARRAY^{SCAN} VTI (Thermo Scientific). Plates are scanned and photographed with a CCD camera–equipped fluorescence microscope. Captured images are directly processed using the integrated analysis software, Cellomics Scan (Cellomics), which is programmed for the detection of nuclei (DAPI staining) and measurement of the fluorescence intensity of Alexa Fluor 488. In the UDS assay, all non-S-phase cells in a single captured field are evaluated; the average fluorescence intensity of 25 different fields is calculated for each well. In the RRS assay, all cells in a single

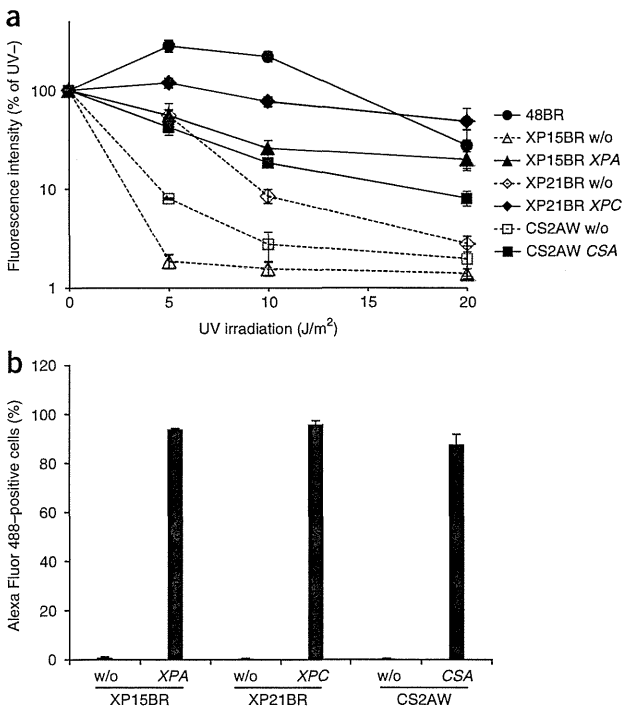

Figure 7 | Virus complementation experiment for cell sensitivity assay. (**a**) Cell sensitivity assay for normal 48BR, XP patient–derived XP15BR, XP21BR and CS patient–derived CS2AW cells. Cells were seeded on a plastic 96-well plate, followed by infection with a set of lentiviruses expressing *XPA*, *XPC* or *CSA* cDNA. At 48 h after virus infection, UV irradiation was performed with indicated doses (0, 5, 10 and 20 J/m^2), followed by 24 h of incubation with DMEM supplemented with 10% (vol/vol) FBS. EdU incorporation and detection were performed as described in the cell sensitivity assay (steps 9–11 of **Box 1**). Data were normalized to the EdU incorporation level measured in nonirradiated cells, and data points represent average fluorescence intensity (in arbitrary units) of duplicate wells. Error bars represent s.d. of three experiments. (**b**) Viral infection efficiency was confirmed by immunofluorescence staining. Cells were seeded on a glass-bottomed 96-well plate, followed by infection with a set of lentiviruses expressing *XPA*, *XPC* or *CSA* cDNA. At 72 h after infection, immunofluorescence staining was performed as described in step 5B of **Box 2**. Infection efficiency was calculated as a number of Alexa Fluor 488–positive cells using the VTI system. w/o, without virus infection.