Groehler and Lannigan, http://www.jcb.org/cgi/content/full/jcb.201002124/DC1

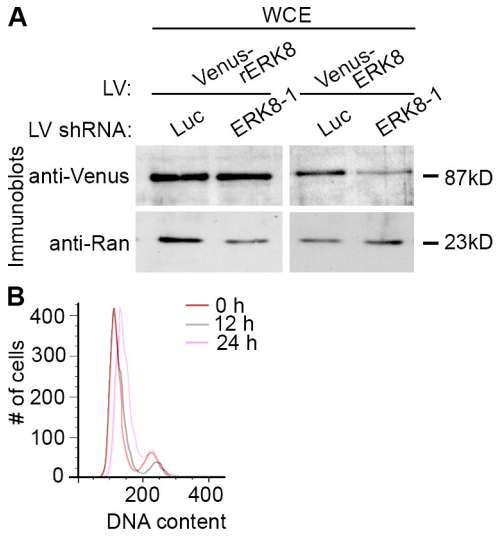

Figure S1. **ERK8 regulates the cell cycle.** (A) Triple silent mutations in ERK8 prevent silencing by wild-type ERK8-specific shRNA. MCF-10A cells transduced with wild-type or resistant (rERK8) ERK8 followed by a second transduction with shRNA to luciferase (Luc) or an ERK8-specific shRNA. At 5 d after transduction, lysates were normalized for Ran expression and immunoblotted for Venus. LV, lentivirus; WCE, whole cell extract. (B) Flow cytometric analysis of MCF-10A cells synchronized by arresting the cells by serum and growth factor depletion, followed by release into the cell cycle by the addition of complete media. The times indicate the sampling times after release into the cell cycle.

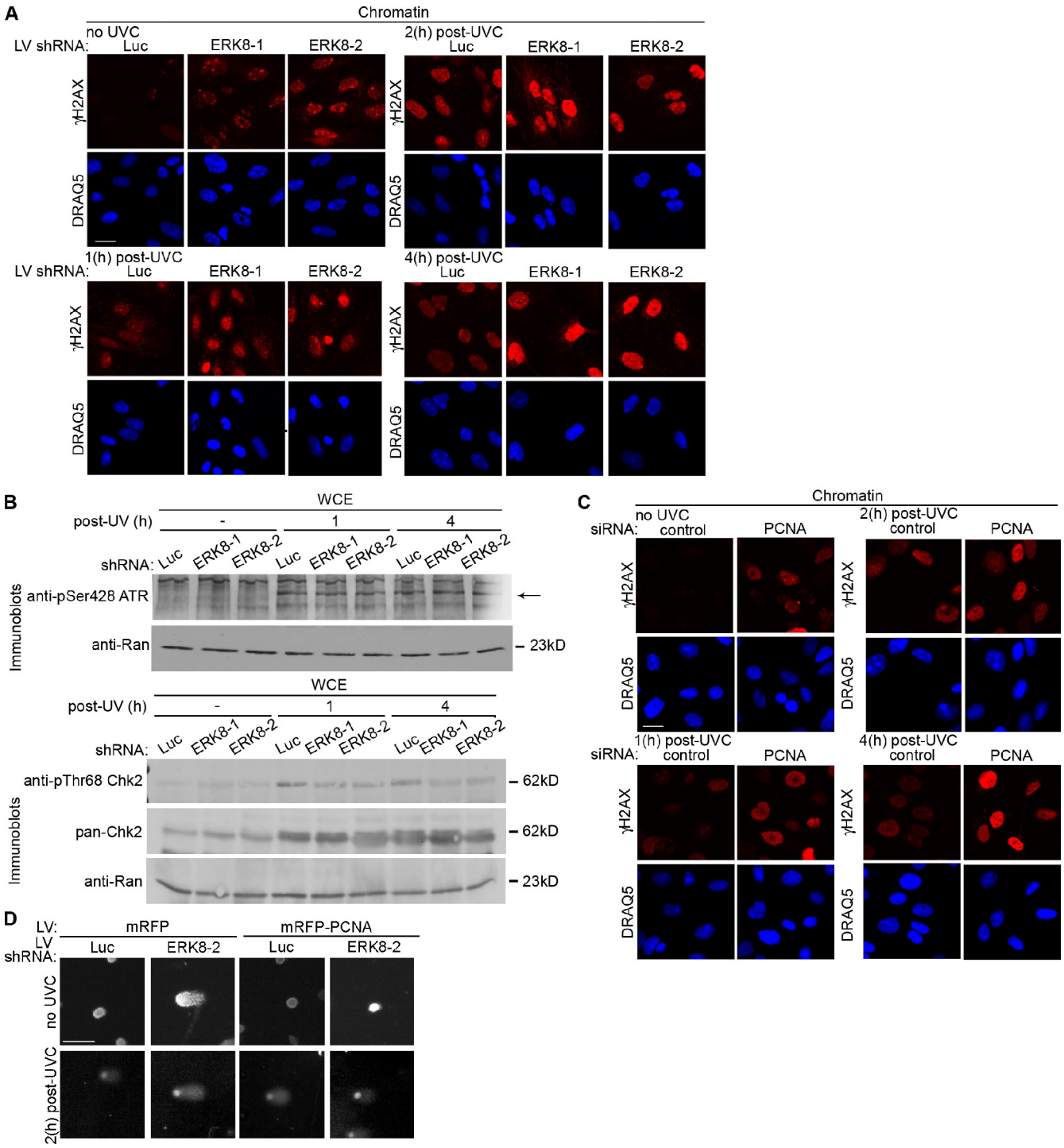

Figure S2. **Loss of ERK8 increases DNA damage.** (A) Representative images of indirect detection of γ-H2AX in ERK8 knockdown cells. MCF-10A cells transduced for 5 d were treated with or without (−) 20 J/m^2 UVC. The times indicated refer to the length of time after irradiation. At the indicated time, the cells were treated with detergent, fixed, and immunostained with an anti–γ-H2AX antibody and an anti–mouse fluorescent secondary antibody. Nuclei were stained with DRAQ5. (B) Analysis of activation of DNA damage regulators in response to UVC. MCF-10A cells were transduced and treated as in A. Lysates were normalized for Ran and immunoblotted. The arrow indicates phospho-Ser428 ATR. (C) Representative images of indirect detection of γ-H2AX in PCNA knockout cells. MCF-10A cells were transfected with control or PCNA-specific siRNA. After 4 d, the transfected cells were treated as in A. (D) Representative images of the comet assay performed on MCF-10A cells transduced with control or mRFP-PCNA followed by a second transduction with control or ERK8-specific shRNA. After 5 d of transduction, the comet assay was performed on cells treated as in A. Luc, luciferase; LV, lentivirus. Bars: (A and C) 10 µm; (D) 50 µm.

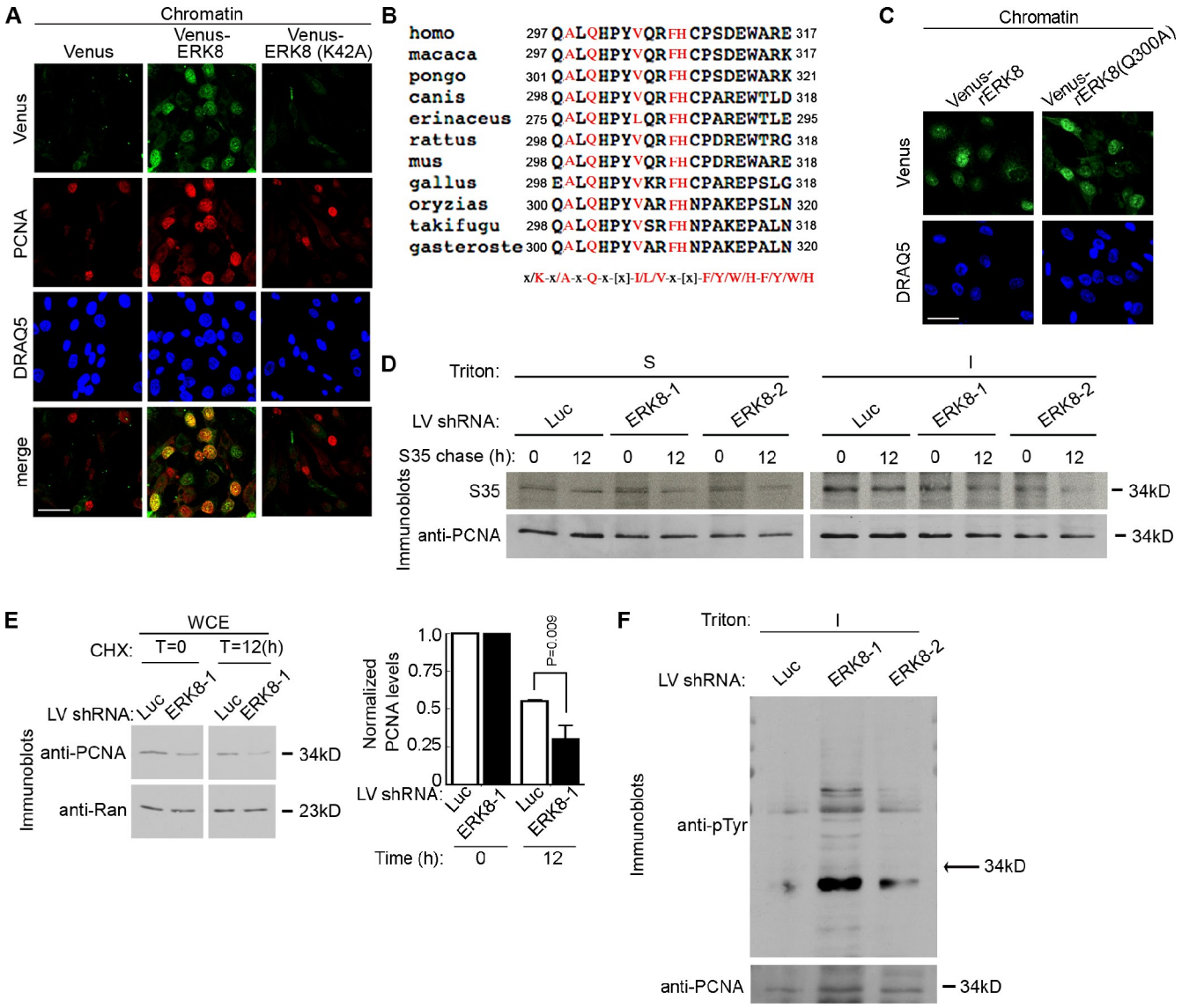

Figure S3. **ERK8 regulates PCNA turnover.** (A) Representative images of wild-type and mutant ERK8^{CHROMATIN} and PCNA^{CHROMATIN}. Transduced cells were treated with detergent and a high salt wash, fixed, and immunostained with anti-PCNA antibody and an anti–mouse secondary antibody. Venus-ERK8 proteins were detected by direct fluorescence. (B) The conserved PIP box present in ERK8 and the consensus PIP box (Moldovan et al., 2007) are shown in red. x is any amino acid. [x] indicates variable spacing. (C) Representative images of wild-type and mutant ERK8^{CHROMATIN}. MCF-10A cells were transduced with resistant forms of wild-type (rERK8) or PIP box mutant of ERK8 (rERK8(Q300A)). Transduced cells were treated with detergent and a high salt wash and fixed. Venus-ERK8 proteins were detected by direct fluorescence. (D) Representative images of the data obtained for the time course of PCNA degradation by [^{35}S]Met pulse-chase labeling. MCF-10A cells transduced for 5 d were depleted of intracellular Met, labeled with [^{35}S]Met for 4 h, washed, and incubated in excess, cold Met for 0 or 12 h. Lysates were separated into insoluble (I) and soluble (S) fractions, and each fraction was immunoprecipitated with an anti-PCNA antibody. (E) Analysis of the time course of PCNA destruction using the protein synthesis inhibitor cycloheximide (CHX). Transduced MCF-10A cells were treated without or with 50 μM cycloheximide for 12 h. Lysates were normalized for Ran expression and immunoblotted for PCNA. The right panel shows the quantitation of PCNA levels normalized for Ran relative to cycloheximide for 0 h. Each transduction was separately normalized. Mean is shown (n = 2, in duplicate), and error bars indicate SEM. (F) Analysis of phospho-Tyr in PCNA. PCNA was immunoprecipitated from MCF-10A cells that were transduced for 5 d. The amount of PCNA was normalized between samples, and the immunoprecipitates were immunoblotted with an anti–phospho-Tyr (anti-pTyr) antibody. The immunoblot was reprobed with anti-PCNA antibody. The arrow indicates the location of PCNA. Luc, luciferase; LV, lentivirus; WCE, whole cell extract. Bars, 50 μm.

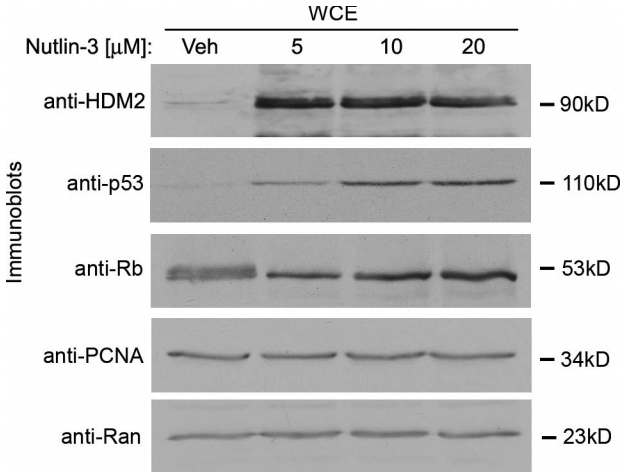

Figure S4. **Inhibition of p53 degradation does not alter PCNA protein levels.** Analysis of inhibition of p53 degradation in MCF-10A cells. MCF-10A cells were treated with varying concentrations of nutlin-3 for 24 h. Lysates of the treated cells were normalized for Ran levels and immunoblotted. Veh, vehicle; WCE, whole cell extract.

Reference

Moldovan, G.L., B. Pfander, and S. Jentsch. 2007. PCNA, the maestro of the replication fork. *Cell*. 129:665–679. doi:10.1016/j.cell.2007.05.003

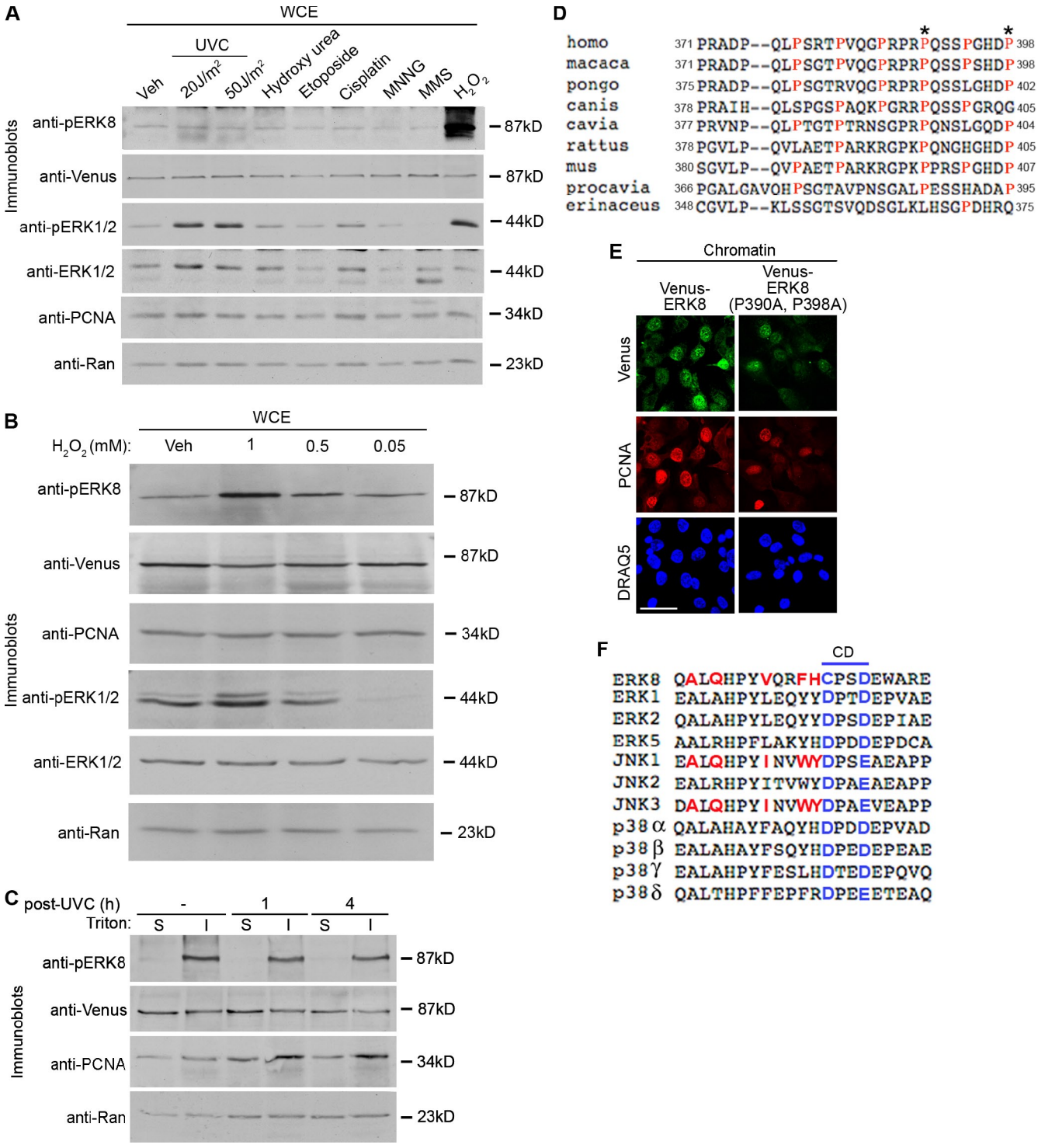

Figure S5. **ERK8 activation.** (A) Analysis of ERK8 activation in response to DNA-damaging agents. MCF-10A cells were treated with vehicle (Veh), 2 mM hydroxyurea for 2 h, 10 μM etoposide for 2 h, 10 μM cisplatin for 12 h, 2 μM MNNG for 12 h, 2 mM MMS for 2 h, and 1 mM H_2O_2 for 1 h. Additionally, some cells were treated with 20 J/m^2 or 50 J/m^2 UVC, and 2 h after irradiation, lysates were generated. Lysates of the treated cells were normalized for Ran levels and immunoblotted. (B) Analysis of ERK8 activation in response to varying doses of H_2O_2. MCF-10A cells were treated with vehicle or differing doses of H_2O_2 for 1 h. Lysates of the treated cells were normalized for Ran expression and immunoblotted. (C) Analysis of the subcellular distribution of ERK8 and PCNA in response to UVC. MCF-10A cells were transduced with Venus-ERK8 for 2 d and treated with or without (−) UVC. At the indicated time, lysates were separated into insoluble (I) and soluble (S) fractions, and the fractions were normalized for Ran levels. (D) Repeating PXXXP motif identified in the C-terminal extension of ERK8 in various members of the class Mammalia, subclass placental are shown in red. Asterisks indicate mutated residues in human ERK8. (E) Representative images of wild-type and mutant ERK8^{CHROMATIN}. MCF-10A cells were transduced with wild-type or a mutant of ERK8 (ERK8 (P390A, P398A)) and fixed and immunostained with anti-PCNA antibody and an anti–mouse secondary antibody. Venus-ERK8 constructs were detected by direct fluorescence. Bar, 50 μm. (F) The PIP box in ERK8 is adjacent to the CD domain. Alignment of members of the MAPK superfamily, in which the residues in blue are part of the CD and residues in red are homologous to the consensus PIP box. The human sequences for each are shown.