Supplementary material and methods

Structural modeling of VRK1-Y213H variant

The 3D structure of the human Vaccinia-Related Kinase 1 (VRK1) wild-type protein was obtained from the Protein Data Bank (PDB id: 2LAV)[1]. The first conformer (of the 20 NMR conformers included in the PDB file) was selected for further processing. Model for VRK1-Y213H variant was generated using the wild-type structure as template. Models were built using the SWISS-MODEL server (http://swissmodel.expasy.org) and their structural quality were within the range of those accepted for homology-based structure (Anolea/Gromos/QMEAN4) [2].

Molecular Dynamics simulation

Prior to molecular dynamics (MD) procedures, 3D structures were energy minimized using the GROMOS 43B1 force field implemented in DeepView (http://spdbv.vital-it.ch/), using 500 steps of steepest descent minimization followed by 500 steps of conjugate-gradient minimization.

Wild-type VRK1 structure and VRK1-Y213H model were subjected to 200 ns of MD simulation using the AMBER18 molecular dynamics package (http://ambermd.org/; University of California-San Francisco, CA). The 3D structures were solvated with a periodic octahedral pre-equilibrated solvent box using the LEaP module of AMBER, with 12 Å as the shortest distance between any atom in the protein subdomain and the periodic box boundaries. Free MD simulation were performed essentially as previously described [3], using the PMEMD program of AMBER18 and the ff14SB force field (http://ambermd.org/). The SHAKE algorithm was used, allowing a time step of 2 fs. Systems were initially relaxed over 15,000 steps of energy minimization with a cut-off of 12 Å. Simulations were then started with a 20 ps heating phase, raising the temperature from 0 to 300 K in 10 temperature change steps, after each of which velocities were reassigned. During minimization and heating, the Cα trace dihedrals were restrained with a force constant of 500 kcal mol^{-1} rad^{-2} and gradually released in an equilibration phase in which the force constant was progressively reduced to 0 over 200 ps. After the equilibration phase, 200 ns of unrestricted MD simulation were obtained. MD trajectories were analyzed using VMD software [4]. The trajectories were continuously monitored by the measurement of root-mean square deviation (RMSD).

Figures were generated using the Pymol Molecular Graphics System (https://pymol.org/; Schrödinger, LLC, Portland, OR).

Plasmids and mutagenesis

Human VRK1 was expressed from mammalian expression vector, pCEFL-HA-VRK1 [5], and bacterial expression pGEX-4T-VRK1 [5-8]. The following primers were used to generate the Y213H mutations in human and murine VRK1. Mutations in VRK1 were performed using the GeneArt Site-Directed Mutagenesis System (Invitrogen-ThermoFisher) with the following primers for human VR K1-Y213H forward (5'-AGGAGTTCATAAAGAACACAAAGAAGACCCCAAAA-3') and reverse (5'-TTTTGGGGTCTTCTTTGTGTTCTTTATGAACTCCT-3'); and for murine VRK1-Y213H forward (5'- TGGAGTTCATAAAGAGCACAAGGAAGATCCCAAA-3') and reverse (5'- TTTGGGATCTTCCTTGTACTCTTTATGAACTCCA-3). Sanger sequencing was used to confirm all variants generated.

Human VRK1 wild type and the VRK1-Y213H variant were expressed from constructs pGEX4T-GST-VRK1 plasmid expressed in *E.coli* BL21 strain competent cells. The following plasmids were used to express the substrates: pGST4T-53BP1 (1-346) [9, 10]; GST-p53(1-85)[11] [6, 12], and pGEX4T-GST-Coilin(160-214) [13]. All plasmids were expressed in BL21 *E.coli* to purify the fusion protein used as substrate in kinase assays as previously reported [8, 13]. Human H3 is a purified recombinant protein (Merck-Millipore).

Kinase assays

The kinase assays were performed as previously described [5, 8, 14]. Briefly, In vitro kinase assays with [^{32}- P]-γATP were performed with GST-VRK1 wild-type and variants [5, 13, 15]. Assays with the following substrates were previously published: p53 [12, 16], histone H3 [7, 17], 53BP1 [10], and GST-coilin [13].

The Serine-Threonine kinase activity of VRK1 was analysed by performing in vitro kinase assays using 2 µg of GST-VRK1 and variant recombinant proteins that were purified from BL21 cells. The following proteins (2 µg) were used as specific substrates, GST-53bp1(1-346) [10] , GST-Coilin(160-214) [13], GST-p53(1-84) [6, 18, 19], and human recombinant histones H3 [7, 17]. To perform the in vitro kinase assay [19], it was used a specific buffer (20mM Tris-HCl pH 7.5, 5mM $MgCl_2$, 0.5mM DTT

and 150mM KCl), 5µM ATP and 5µCi (0.1µM) radiolabelled [ɣ-^{32}P]ATP in a final volume of 40µl during 45 min at 30ºC [8]. H3T3ph was detected with a rabbit polyclonal antibody (Upstate-Millipore) [15, 17]. In radioactive assays, film exposure was in the lineal response range for all assays.

Electrophoresis, antibodies and immunoblots

Proteins were separated in SDS-PAGE gels in running buffer (25mM Tris-HCl, ph 8.0, 200mM glycine, 1.7mM SDS), and transferred to a PVDF membrane (Immobilon-FL, Millipore) in buffer (25mM Tris-HCl, pH 8.0, 19.2 mM glycine, 15% methanol) as previously described [13, 15, 20, 21]. The primary and secondary antibodies are listed in Supplementary Table S1. The secondary antibodies were incubated for an hour and the fluorescence was detected with LI-COR Odyssey Infrared Imaging System or with ECL Western Blotting Detection Reagent (Sigma-Aldrich) were used if the secondary antibodies were conjugated with peroxidase.

Cell lines, transfection and cell lysate

For the study of Cajal bodies formation, the validated HeLa (ATCC-CCL2) cell line was grown in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich) and transfected using Lipofectin [13, 15, 20]. Cell extracts were prepared by using a mild lysis buffer (50mM Tris-HCl, pH 8.0, 150mM NaCl, 1% Triton X-100 and 1mM EDTA) supplemented with protease inhibitors (1mM PMSF, 10 µg/mL aprotinin and 10 µg/mL leupeptin) and phosphatase inhibitors (1mM sodium orthovanadate, 1mM NaF) and incubated for 20 minutes [8, 13, 15].

Statistical analysis

Statistical analysis were performed using the IBM SPSS 28 statistics package. All assays were performed in the lineal response range and in identical conditions for all substrates [8]. Individual quantitative experiments were repeated three times, and statistical significance was analyzed using two-tailed T-test with Welch' correction [22].

Reagents

Recombinant human histones H3 and H2AX (Millipore, Merck), Cycloheximide (Sigma-Aldrich). All other chemical were from Sigma-Merck (Darmstadt, Germany).

Tissue culture media and reagents were from GIBCO-ThermoFisher Scientific (Waltham, MA).

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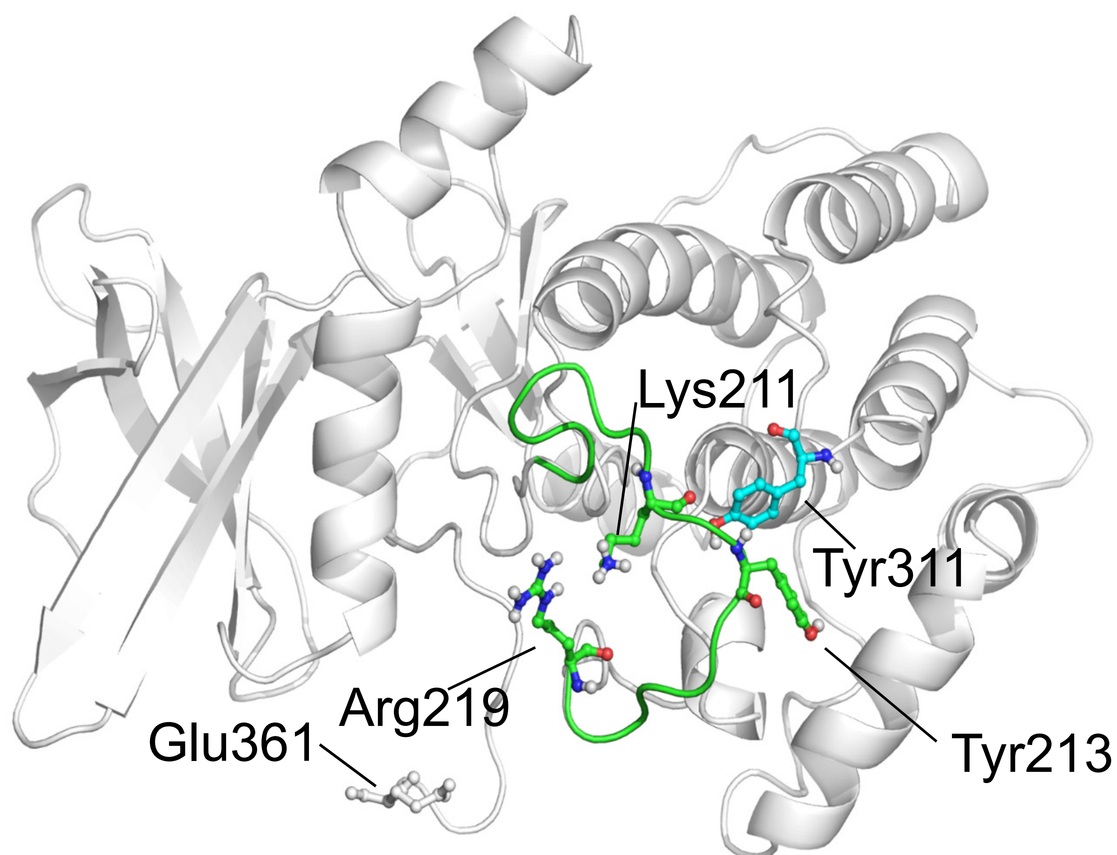
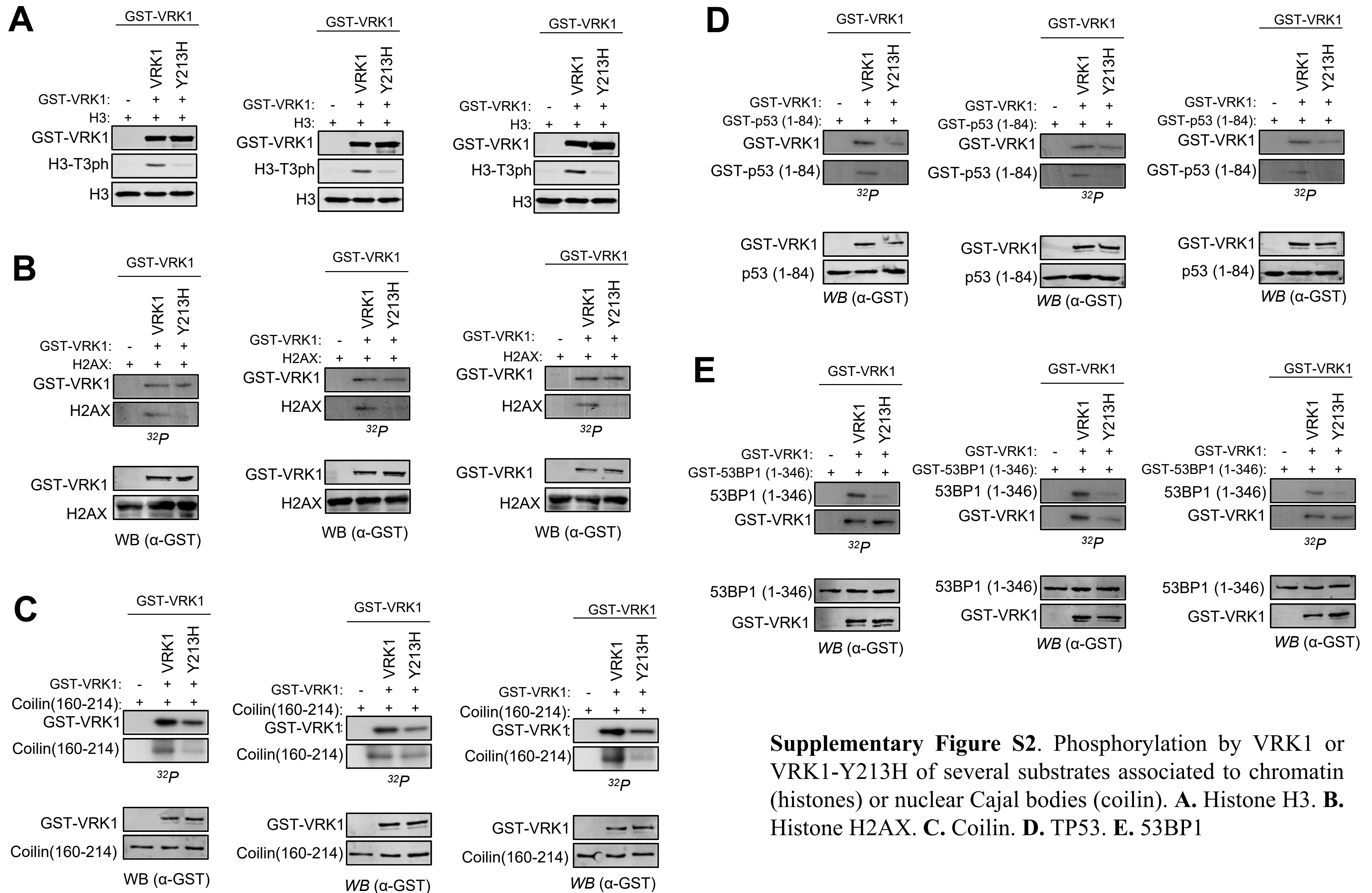
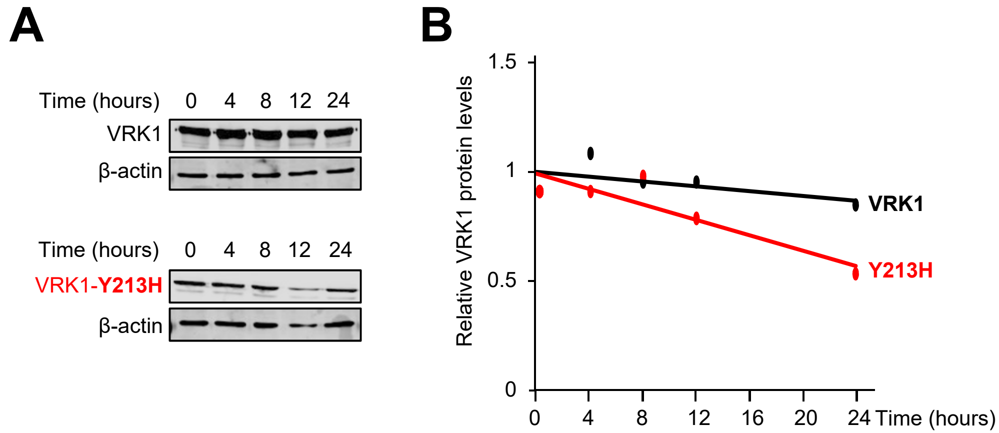


Supplementary Figure S1. Structural modeling of VRK1 Y213H variant. (A) 3D structure of wild-type VRK1 protein (PDB id: 2LAV). The activation loop is colored in green. Position of residues Tyr213, Tyr311 (blue, not in the activation loop), Lys211, Arg219 and Glu361 (grey, located in the C-terminal tail) is indicated.



Supplementary Figure S2. Phosphorylation by VRK1 or VRK1-Y213H of several substrates associated to chromatin (histones) or nuclear Cajal bodies (coilin). **A.** Histone H3. **B.** Histone H2AX. **C.** Coilin. **D.** TP53. **E.** 53BP1



Supplementary Figure S3. Protein stability of the wild-type VRK1 and mutant VRK1-Y213H proteins. Plamids expressing human VRK1 or VRK1-Y213H tagged with the HA-epitope were cloned in plasmid pCEFL-HA vector and transfected in HEK293T cells. Cycloheximide was added to the culture to block gene transcription. The level of VRK1 proteins after cycloheximide addition were determined in westen blots. **A.** The experiment was performed in triplicate and a representative gel is shown. **B.** The relative level of each protein at different times was quantitated and shown in the graph (B).

Supplementary Table S1. List of primary and secondary antibodies used in this work.

Antibody	Type	Dilution WB	Clone and/or reference	Supplier
GST-Tag	Mouse monoclonal	1:1000	B14/sc-138	Santa Cruz
HA-Tag	Mouse monoclonal	1:1000	F7/sc-7392	Santa Cruz
Histone H3	Rabbit polyclonal	1:1000	9175	Cell Signaling
Phospho-histone H3 (Thr3ph)	Rabbit polyclonal	1:1000	05-746R	Merck-Millipore
coilin	Mouse monoclonal	1:200	sc-56298	Santa Cruz
β-actin	Mouse monoclonal	1:1000	AC15/A5441	Sigma-Aldrich
Goat Anti-Mouse IgG, DyLight 680	Goat	1:10000	35518	Thermo Scientific
Goat Anti-Rabbit IgG, DyLight 800	Goat	1:10000	35571	Thermo Scientific
Anti-Mouse IgG-HRP	Sheep	1:10000	NA931V	Amersham Biosciences;