Supplemental Materials

Molecular Biology of the Cell

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. N144-GFP3 does not co-localize with Nup153 at NPCs. IF image of BJ cells transiently expressing N144-GFP3 and labeled with anti-Nup153 (red). N144-GFP3 does not clearly co-localize with endogenous Nup153 and appears largely excluded from the NPCs as indicated by the arrowheads in the magnified images that correspond to the white box in the upper merged image. The experiment was repeated 3 times. Scale bar, 10 μm.

Supplemental Figure 2. A) Subcellular localization of GFP-VRK2A and untagged-VRK2A in BJ cells**.** BJ cells were transiently transfected with GFP-VRK2A or untagged-VRK2A. The cells were labeled with anti-VRK2(red) and -Nup153 (not in merged). B) Subcellular localization of GFP-VRK2A and GFP-VRK2B in C2C12 cells labeled with anti-LaA/C (red). C) siRNA-mediated knockdown of *Lmna* in C2C12 cells stably expressing GFP-VRK2A. C2C12 GFP-VRK2A cells were subjected to siRNA-mediated *Lmna* knockdown for 48 hours. The lysates were analyzed by IB with anti-LaA and anti-tubulin for a loading control. D) NE localization of VRK2A is emerin-independent. Wild type (WT) and emerin-gene deleted ($Emd^{-/-}$) MEF cells were transfected with GFP-VRK2A and stained with anti-emerin. For all IF images, DNA was labeled with Hoechst dye 33342 (blue). All the experiments were repeated at least 3 times Scale bar, 10 μm.

Supplemental Figure 3. Effects of VRK2 knockdown and overexpression on the cell cycle. Representative histogram (left) and quantification (right) shows the cell cycle distribution of control and VRK2 depleted cells after staining with propidium iodide (PI), dye that measures total DNA content, for BJ cells in (A) and HEK 293 cells in (B). HEK 293 parental and HEK 293 stably expressing GFP-VRK2A in (C) were similarly stained. The percentage of cells in G_0/G_1, S, and G_2/M are shown in the box. Data are presented as average fold change ±SEM of biological triplicates. The *P* values were calculated by unpaired *t* test. ** *P≤0.01.*

Supplemental Table 1. Candidates identified by N144-BioID2, BioID2-LaA and BioID2-Sun2.

This table is provided as a separate Excel file.

Supplemental Table 2. % of TM and NE identified by N144-BioID2, BioID2-LaA and BioID2-Sun2.

This table is provided as a separate Excel file.

Nup153   N144-GFP3   merge

A

BJ   anti-VRK2   GFP-VRK2A   DNA-merge   Nup153

untagged-VRK2A   anti-VRK2   Nup153   DNA-merge

B

C2C12   Lamin A/C   GFP-VRK2A   DNA-merge

C2C12   Lamin A/C   GFP-VRK2B   DNA-merge

C

C2C12 GFP-VRK2A

control siRNA   LaA siRNA

75 kDa—
50 kDa—   anti-LaA

50 kDa—   anti-tubulin

D

MEF *Emd⁻/⁻*   anti-emerin   GFP-VRK2A   DNA-VRK2A

A

Control siRNA			VRK2 siRNA		
G0/G1	82.1%		G0/G1	76.3%	
S	6.74%		S	7.55%	
G2/M	11.2%		G2/M	16.07%	

B

Control siRNA			VRK2 siRNA		
G0/G1	60%		G0/G1	46%	
S	18%		S	22%	
G2/M	21%		G2/M	32%	

C

293 Parental			293 GFP-VRK2A		
G0/G1	55.0%		G0/G1	64.0%	
S	20.0%		S	20.0%	
G2/M	25.0%		G2/M	17.0%	