Supplemental Methods

Phosphopeptide Analysis by LC-MS/MS- Excised gel bands were cut into approximately 1 mm^3 pieces. Gel pieces were then subjected to a modified in-gel trypsin digestion procedure (1). Gel pieces were washed and dehydrated with acetonitrile for 10 min. followed by removal of acetonitrile. Pieces were then completely dried in a speed-vac. Rehydration of the gel pieces was with 50 mM ammonium bicarbonate solution containing 12.5 ng/µl modified sequencing-grade trypsin (Promega, Madison, WI) at 4ºC. After 45 min., the excess trypsin solution was removed and replaced with 50 mM ammonium bicarbonate solution to just cover the gel pieces. Samples were then placed in a 37ºC room overnight. Peptides were later extracted by removing the ammonium bicarbonate solution, followed by one wash with a solution containing 50% acetonitrile and v5% acetic acid. The extracts were then dried in a speed-vac (~1 hr). The samples were then stored at 4ºC until analysis. On the day of analysis the samples were reconstituted in 5 µl of HPLC solvent A (2.5% acetonitrile, 0.1% formic acid). A nano-scale reverse-phase HPLC capillary column was created by packing 5 µm C18 spherical silica beads into a fused silica capillary (100 µm inner diameter x 12 cm length) with a flame-drawn tip (2). After equilibrating the column each sample was pressure-loaded off-line onto the column. The column was then reattached to the HPLC system. A gradient was formed and peptides were eluted with increasing concentrations of solvent B (97.5% acetonitrile, 0.1% formic acid). As each peptide was eluted they were subjected to electrospray ionization and then they entered into an LTQ mass spectrometer (ThermoFinnigan, San Jose, CA). Eluting peptides were detected, isolated, and fragmented to produce a tandem mass spectrum of specific fragment ions for each peptide. Peptide sequences (and hence protein identity) were determined by matching protein or translated nucleotide databases with the acquired fragmentation pattern by the software program, Sequest (ThermoFinnigan, San Jose, CA) (3). The modification of 79.966 mass units to serine, threonine, and tyrosine was included in the database searches to determine phosphopeptides. Each phosphopeptide that was determined by the Sequest program was also manually inspected in ensure confidence.

Luciferase reporter assays- The luciferase reporter assay system (Promega) was used in this study. In brief, luc2-c-mpl (4) was cotransfected alone, or with plasmids expressing the specified proteins with GATA1, or with a combination of GATA1 and wild-type or mutant FOG1 into 293T cells using FuGene 6 reagent by following the manufacturer's instructions. After 24 h, cell lysates were prepared, and firefly luciferase activity was measured with a luminometer (Thermo LabSystems). A total of 20 µl of pooled cell lysates from the same transfection group were boiled with SDS sample buffer and separated by SDS-PAGE. After electrophoresis and membrane transfer, Western blotting was performed to detect levels of GATA1 and wild-type or mutant FOG-1.

Primers- CTBP1 F 5'-GTGCCCTGATGTACCATACCA-3', CTBP1 R 5'-TGATGTCGATATTGTCAAACCCG-3', CTBP2 F 5'-TGGGTGCCATGATGTACCAC-3', CTBP2 R 5'-GTTGTCGTAGCCACTACCGA-3', β-actin F 5'-AAGGAGATTACTGCTCTGGCTCCTA-3', β-actin R 5'-ACTCATCGTACTCCTGCTTGCTGAT -3'. All other primers were from previous studies (5,6).

Supplemental References

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4. Huang, H., Yu, M., Akie, T. E., Moran, T. B., Woo, A. J., Tu, N., Waldon, Z., Lin, Y. Y., Steen, H., and Cantor, A. B. (2009) *Mol Cell Biol* **29**, 4103-4115
5. Johnson, K. D., Kim, S. I., and Bresnick, E. H. (2006) *Proc Natl Acad Sci U S A* **103**, 15939-15944
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Supplemental Data

Table S1. Summary of Phosphorylation Sites in FOG1. FOG1 was Affinity Purified from MEL cell nuclear extracts expressing a wild-type FL-Bio-tagged version of FOG1 using tandem affinity purification and submitted for mass spectrometric analysis for phosphopeptide identification.

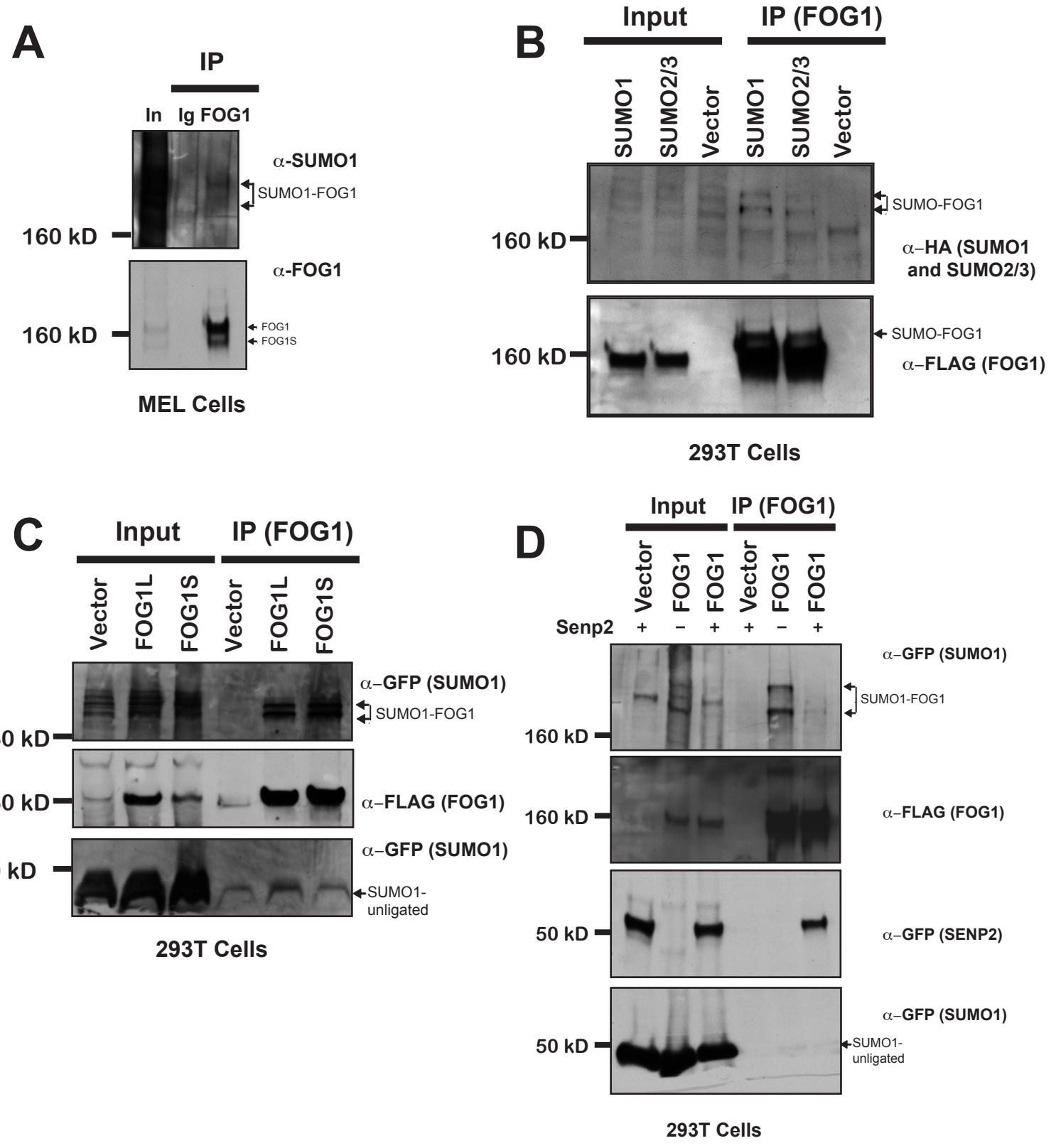
Figure S1. FOG1 is SUMOylated by endogenous SUMO1. MEL cell nuclear extracts were When we immunoprecipitation with an antibody directed against FOG1 or normal Ig. Input and immunoprecipitates were blotted for SUMO1 and FOG1 (A). Constructs containing either a HA-hSUMO1 fusion protein, HA-hSUMO3 fusion protein, or vector alone, were coexpressed in 293T with a construct containing FLAG-Bio-tagged FOG1. After immunoprecipitation was performed on whole cell lysates with a FOG1 antibody, input and immunoprecipitates were run for Western blot with antibodies against FLAG or HA (B). A construct containing a GFP-SUMO1 fusion protein was coexpressed with a construct containing FLAG-Bio-tagged FOG1L or FLAG-Bio-tagged FOG1S. After immunoprecipitation was performed on whole cell lysates with a FOG1 antibody, input and immunoprecipitates were run for Western blot with anti-FLAG and anti-GFP (C). A construct containing a GFP-SUMO1 fusion protein was coexpressed with a construct containing FLAG-Bio-tagged FOG1, in the presence, or absence of the SUMO-isopeptidase SENP2, fused to EGFP. After immunoprecipitation was performed on whole cell lysates with a FOG1 antibody, input and immunoprecipitates were run for Western blot with anti-FLAG and anti-GFP (D).

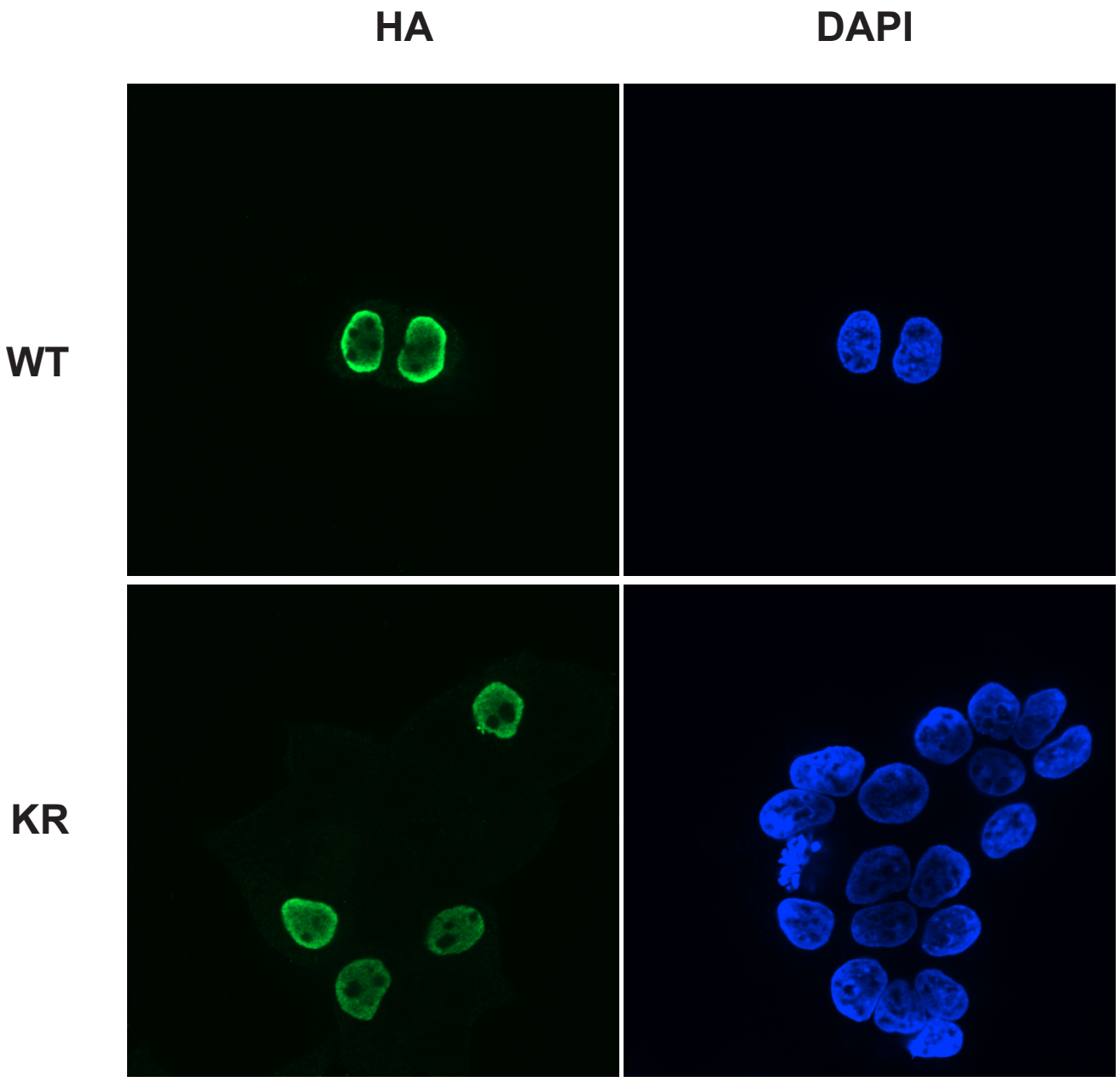
Figure S2. FOG1 SUMOylation is not required for proper subnuclear localization. HA-tagged versions of wild-type (WT) and KR mutant FOG1 were transfected into 293T cells. Fixed/permeabilized cells were stained with antibody directed against HA and visualized with a FITC-conjugated secondary antibody using confocal microscopy. Untransfected cells seen by DAPI staining in the bottom panels show the absence of non-specific HA signal.

Table S1. Summary of Phosphorylation Sites in FOG1

Peptide	Residues	Conservation[4]	Kinase[5]
R.DMEAGEEEKAMDSSPKEQEAPDPEAPAIEEPPpSPPR.D	57	r,h,b	
K.EEKEEEAAMApSPWSGPEELELALQDGQR.C	99	r,h,b	
R.ALpSPEREEPGPAVTLMVDESCWLR.M	143	r,h,b	CLK2, CDK5
R.AGpSPVSATEEKPK.E	286	h,b	
R.VPPQNGGSSESpPAAPR.T	461	r	
R.ASGPGEPGPQAPSRTpPSpPHpSPNPVR.V [1]	495, 497, 500	r,h,b	CDK5, EPHB2, CDC2
K.TELpSpSPpTPGpSpSPGPGELTMAGTLFLPQYVFSPDA GTTTVPTAPQASEILAK.M [2]	511, 512, 514, 517, 518	r,h,b	GSK3A, EPHB2
R.APAGAAAEPDPSRSpSPGPGPR.E	651	r,h,b	EPHB2
R.pSpSPGPGPREEEASGTTTPEAEAAGR.G [2,3]	650, 651	r,h,b	EPHB2
R.SSPGPGPREEEASGTTpTPEAEAAGR.G	666	r,h,b	
R.GpSEGpSQpSPGSSVDDAEDDPSR.T [1, 2]	676, 679, 681	r,h,b	EPHB2
R.KLYELPAAGAPPPAAGPAPVPVVPSPTAELPSSPRPGSA SAGPAPALpSPpSPVPDGPIDLSK.R [1, 2]	**803**, 805	r,h,b	GSK3A, EPHB2
R.RQpSPDAPTALPALADYHECTACR.V	822	r	PKA, AKT
R.APRpSPpSPAPENTPSDPADQGAR.T [1]	925, 927	r	EPHB2

[1] peptides showing single or double combinations of phosphorylations also found
[2] difficult to define phosphorylated residue due to fragmentation pattern, all possible are shown
[3] different peptides showing the same phophorylated residue also found
[4]Conservation key : r = rat, h = human, b = bovine (where only one site is conserved, residue number is in bold)
[5]Kinases predicted using Scansite; Obenauer JC, et al. Nucleic Acids Res. 2003 Jul 1;31(13):3635-41.


A
IP
In
Ig FOG1
α-SUMO1
SUMO1-FOG1
160 kD
α-FOG1
160 kD
FOG1
FOG1S
MEL Cells
B
Input
IP (FOG1)
SUMO1
SUMO2/3
Vector
SUMO1
SUMO2/3
Vector
SUMO-FOG1
160 kD
α–HA (SUMO1 and SUMO2/3)
SUMO-FOG1
160 kD
α–FLAG (FOG1)
293T Cells
C
Input
IP (FOG1)
Vector
FOG1L
FOG1S
Vector
FOG1L
FOG1S
α–GFP (SUMO1)
SUMO1-FOG1
160 kD
160 kD
α–FLAG (FOG1)
50 kD
α–GFP (SUMO1)
SUMO1-unligated
293T Cells
D
Input
IP (FOG1)
Senp2
+
Vector
–
FOG1
+
FOG1
+
Vector
–
FOG1
+
FOG1
α–GFP (SUMO1)
SUMO1-FOG1
160 kD
160 kD
α–FLAG (FOG1)
50 kD
α–GFP (SENP2)
50 kD
α–GFP (SUMO1)
SUMO1-unligated
293T Cells


HA
DAPI
WT
KR