

Coronin1C SUMOylation modulates filopodia formation, neuritogenesis, and neuronal differentiation

Garima Joshi¹, Harsh Vardhan Singh¹ and Ram Kumar Mishra¹

¹Department of Biological Sciences, Indian Institute of Science Education and Research Bhopal

Bhopal, Madhya Pradesh, India-462066

Supplementary information

Position	Sequence	Domain
K19	KFRHVFQAVKNDQCYDDIRV	N-term
K197	SLICTASKDKKVRIVIDPRKQE	WD Domain
K329	KRGLDVNKCEIARFF	C-term
K403	GYIPGKNRDLKVVKKNILDSK	C-term
K413	KKNILDKPTANKKC	C-term
K440	ASVQNEAKLDEILKE	C-term
K464	NQDERISKLEQQMAK	C-term

Table S1 | *in silico* prediction of COR1C SUMO sites

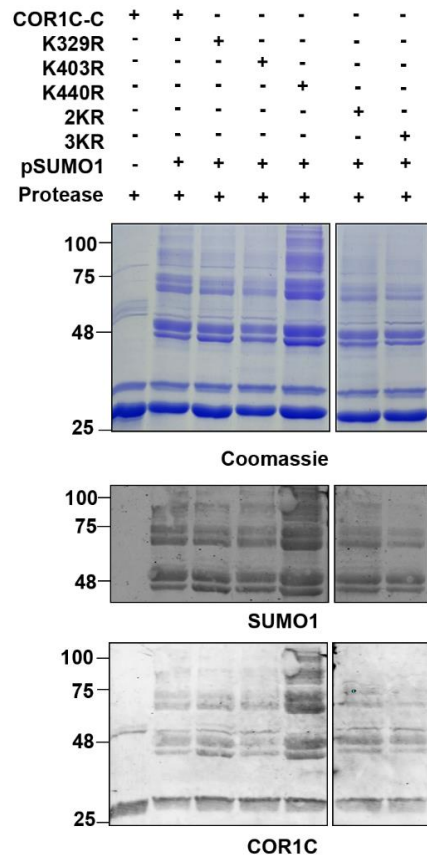


Fig. S1 | COR1C is SUMOylated at multiple lysines in its C-terminus.

SDS-PAGE analysis of *in bacto* SUMOylation reaction with GST-COR1C-C wild-type and mutant post digestion with PreScission protease to produce untagged COR1C-C (modified and unmodified, upper panel). Immunoblotting of samples as upper panels with anti-SUMO1 (middle panel) and anti-COR1C (lower panel) antibodies.

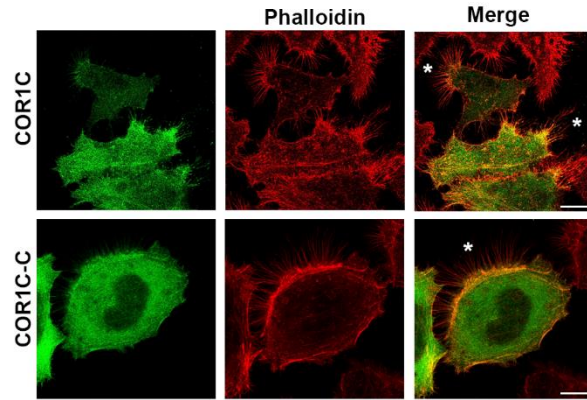


Fig. S2 | The carboxy-terminus of Coronin1C localizes at the filopodial projections.

HeLa cells transfected with GFP-COR1C and GFP-COR1C-C stained with Phalloidin-647. Scale bar - 10 μ m. White stars denote the filopodial projections.

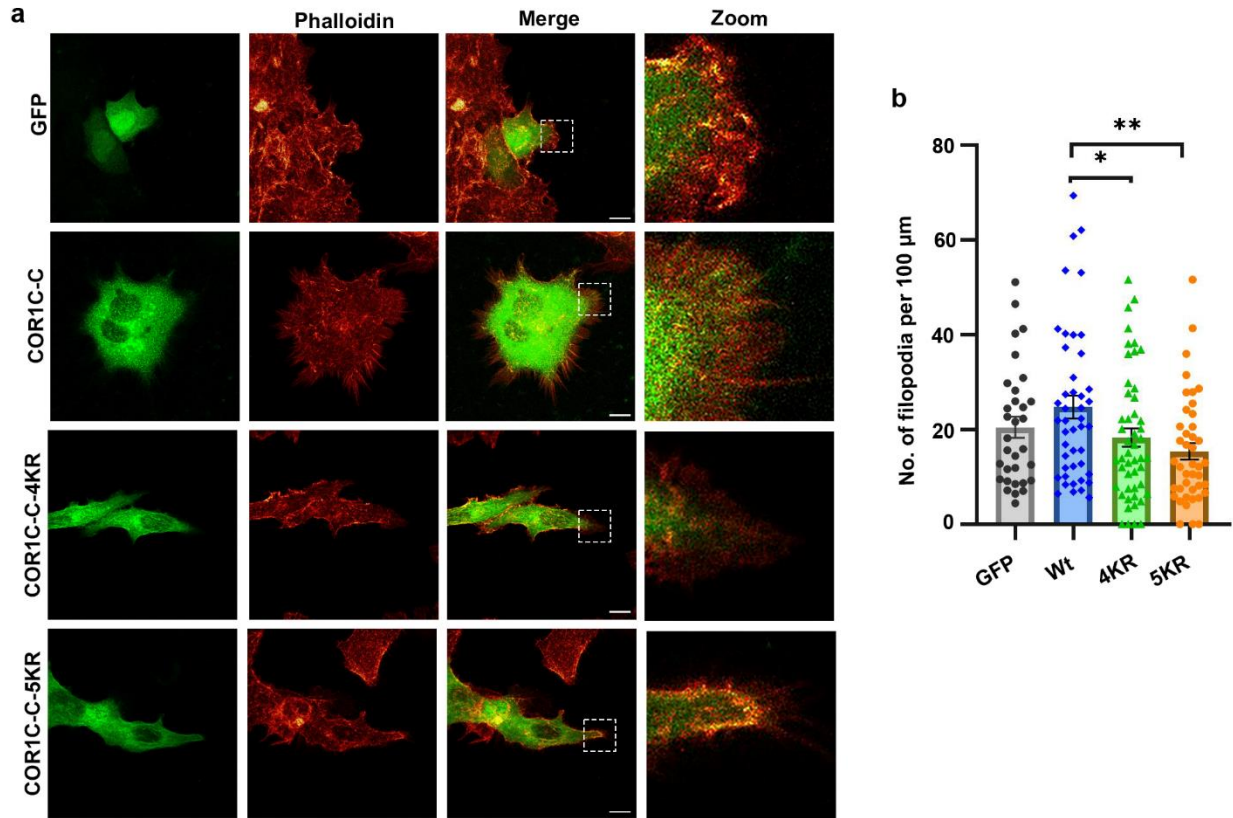


Fig. S3 | COR1C-C SUMOylation is critical for filopodia formation in cells.

a CLSM imaging of MCF7 cells transfected either with GFP-tagged COR1C-C, COR1C-C-4KR, or COR1C-C-5KR. Cells were stained with Phalloidin-647. Dotted-box highlights filopodial structures in the leading edges, as shown in the zoom. **b** Filopodial projections as seen in (a) were quantified using the FiloQuant plugin of ImageJ (~ 50 cells were analyzed in three independent experiments). Statistical analysis was performed using an unpaired t-test; (*) and (**) indicate statistical significance with a p value of 0.0389 and 0.0026. Scale bar - 10 μm.

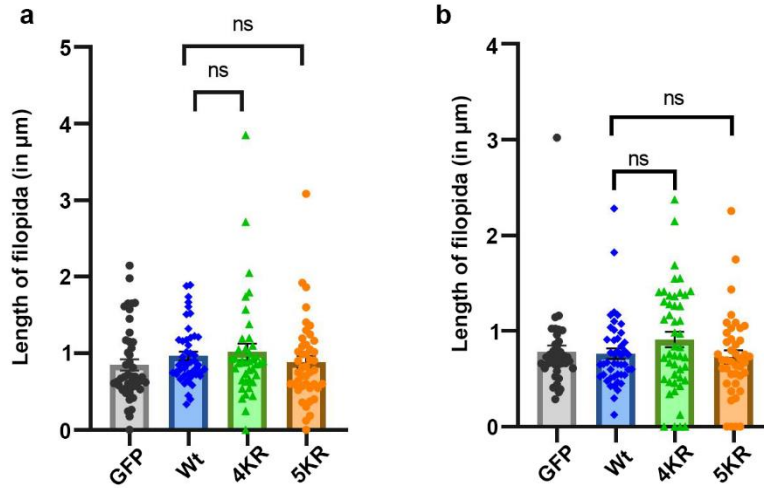


Fig. S4 | COR1C-C SUMOylation does not affect filopodia length in cells.

Quantification of the length of filopodial projections measured from HeLa cells **(a)** and MCF7 cells **(b)**, as shown in Figure S3, was performed using the FiloQuant plugin of ImageJ (~ 50 cells were analyzed in three independent experiments). Statistical analysis was performed using an unpaired t-test.

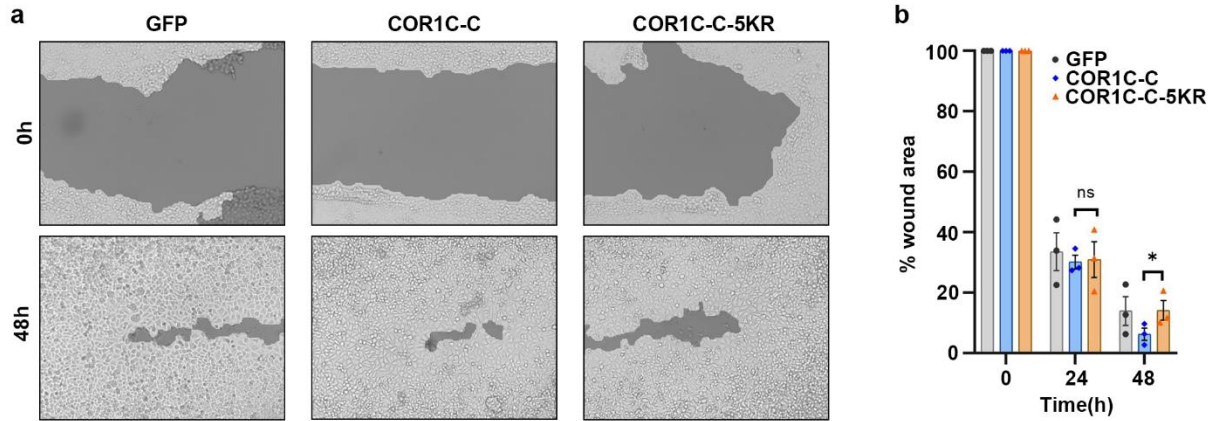


Fig. S5 | COR1C SUMOylation is required for efficient cellular migration.

a Representative image of the wound healing assay performed with HeLa cells expressing GFP, GFP-COR1C-C, or GFP-COR1C-C-5KR. Images were captured at 10x magnification using an inverted microscope. **b** Quantification of wound area closure using TScratch software. Images are representative of n=3 experiments. Data was analysed by using a paired t-test, and (*) indicates statistical significance with a p-value of 0.0398.