Supplementary Information

Ran pathway-independent regulation of mitotic Golgi disassembly by Importin-α

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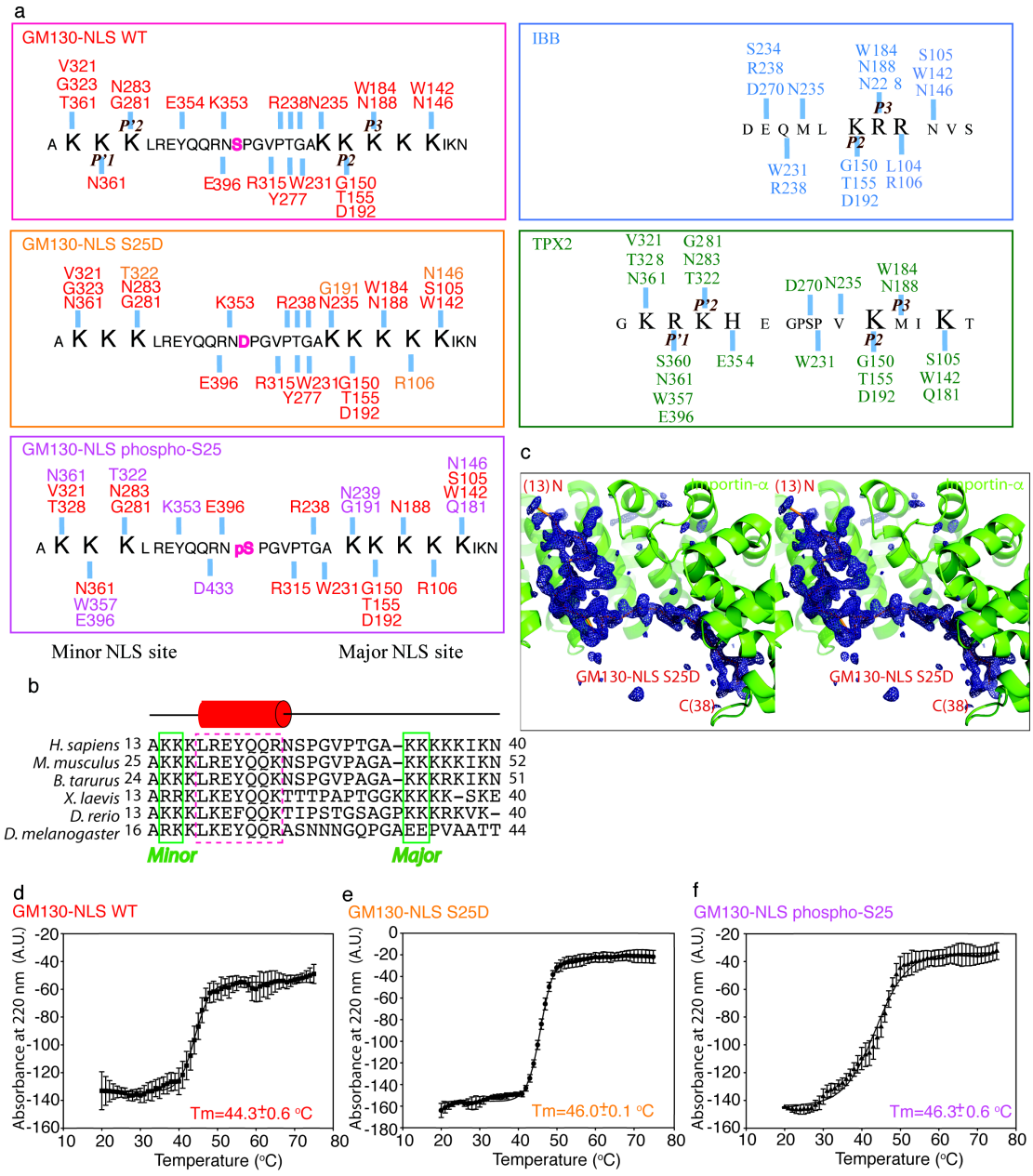
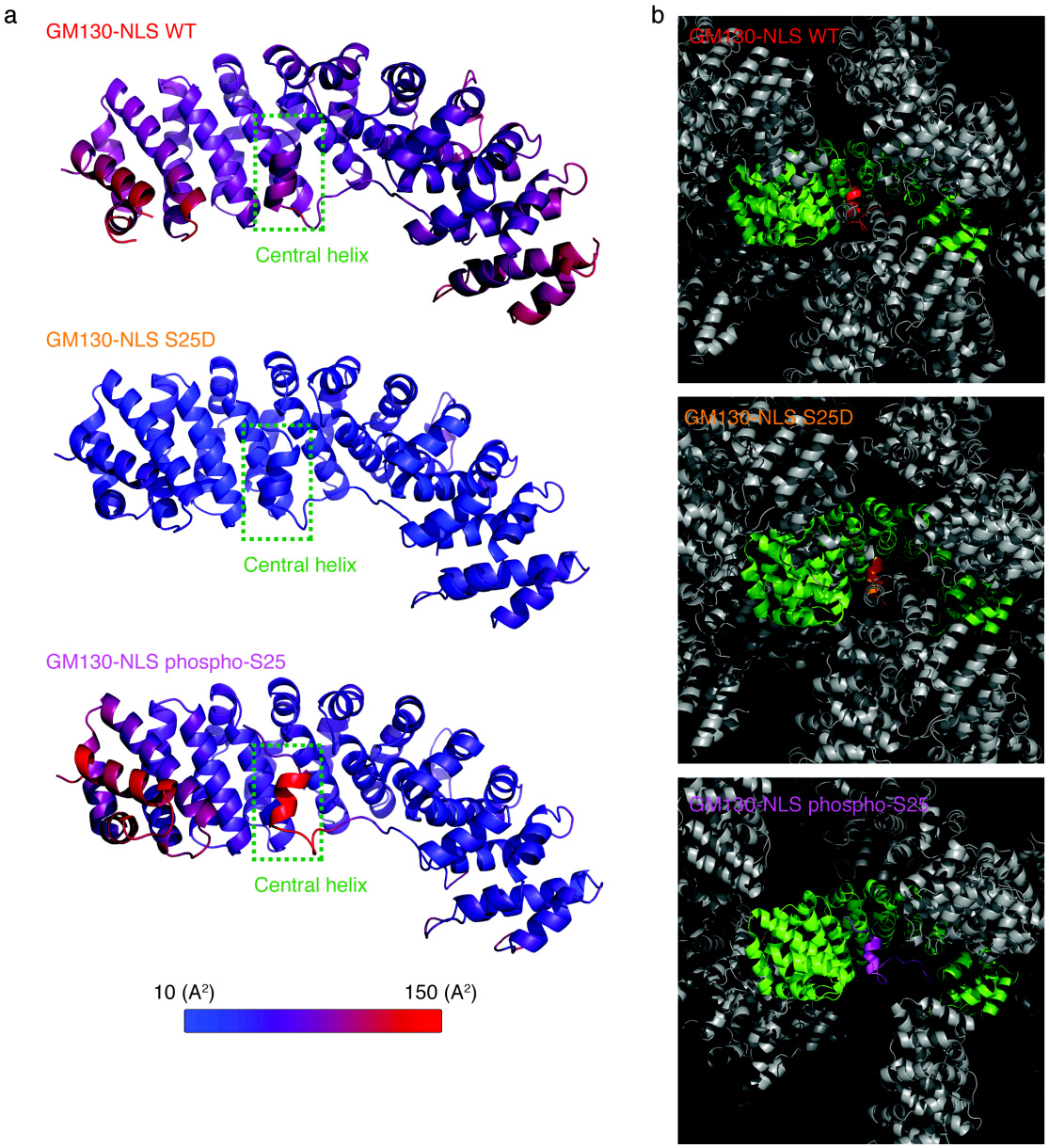
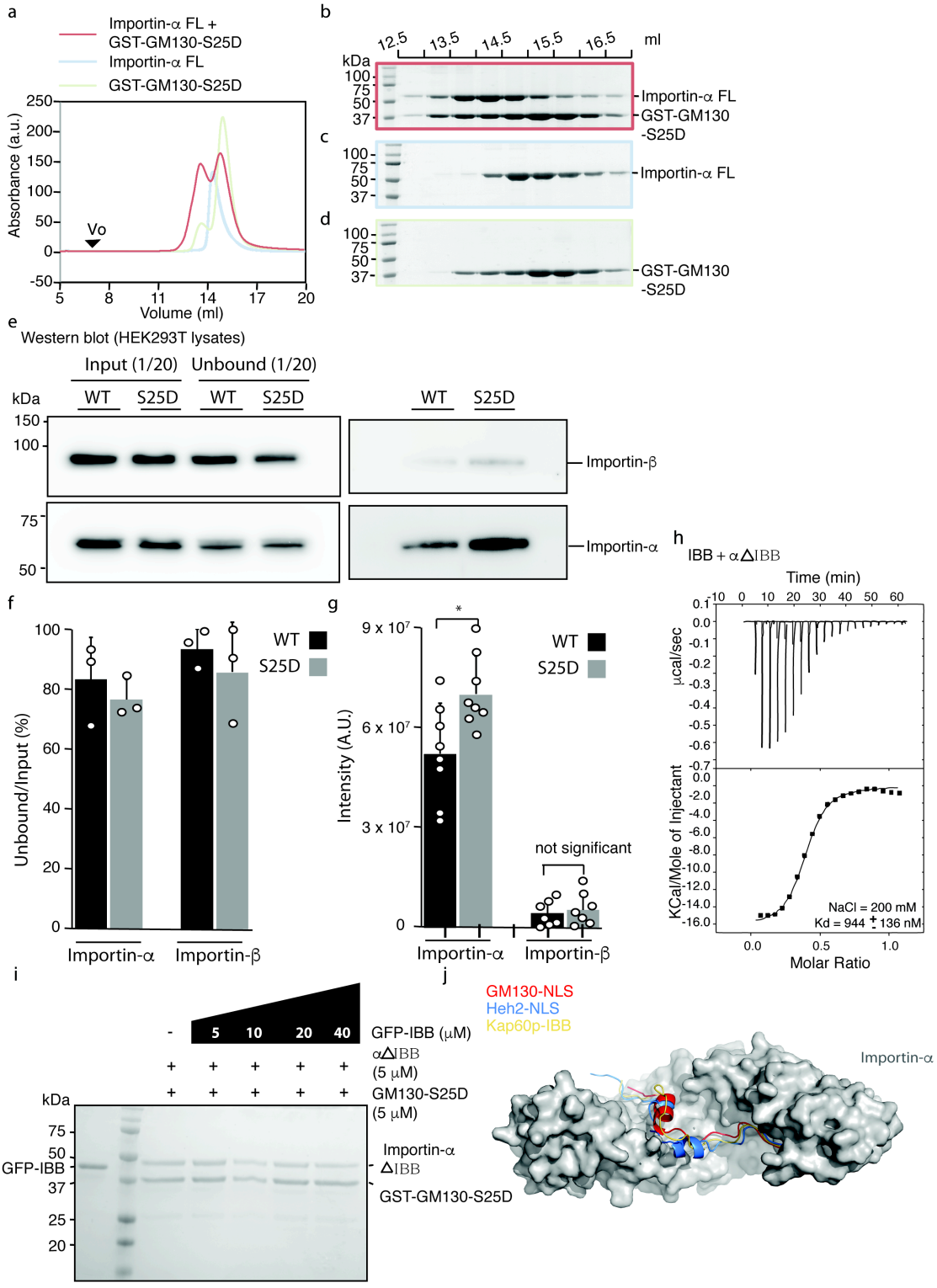


Supplementary Figure 1. The non-classical GM130-NLS and Importin-α interaction network. **a,** Schematic illustrations of the inter-molecular interaction network of Importin-α in complex with wild-type peptide, as well as peptides that contain a phosphomimetic residue or a phosphorylated moiety. Serine, aspartic acid and phosphoserine are highlighted in pink. Interaction networks of Importin-α with IBB and TPX2 are also shown. Blue lines indicate interaction residues. **b,** Sequence alignment of GM130-NLS peptides from different species (numbers represent amino acid positions). The conserved interacting residues of the minor binding site, central α-helix and major binding site are highlighted by boxes. The α-helix is represented as a red cylinder. **c,** Stereo view of an omit difference (Fo-Fc) map contoured at 2.5 sigma with a superimposed atomic model of the GM130-NLS S25D•Importin-α complex. GM130 and Importin-α are displayed as ribbons (orange) and cartoons (green), respectively. **d,e,f,** Circular dichroism spectra of temperature scans at a wavelength of 222 nm for the GM130-WT (**d**), S25D (**e**) or phospho-S25 (**f**) complexes. Each complex spectrum was the average of three scans. The error bars represent the standard deviation from three scans. Melting temperature (Tm) of each complex is indicated.



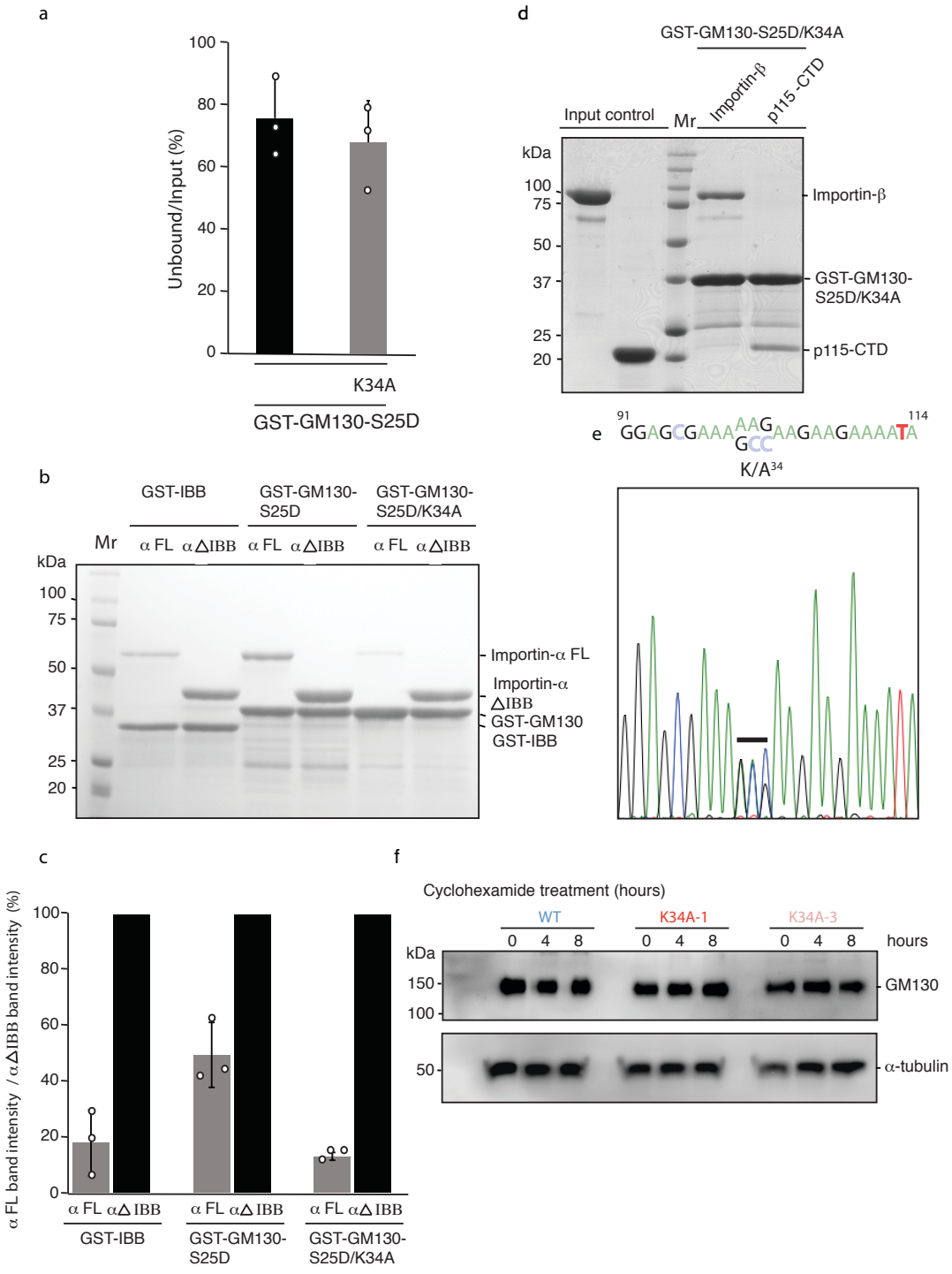
Supplementary Figure 2. B-factors of GM130•Importin-α complexes in different crystalline environments.

a, Structures of Importin-α (ΔIBB) in complex with GM130-NLS WT (top), GM130-S25D (middle) and phospho-S25 (bottom) are colored according to B-factor values, ranging from 10 Å^2 to 150 Å^2. The central helix is boxed by a green dashed line. **b,** Crystalline environments of Importin-α (ΔIBB) (green) in complex with GM130-NLS WT (top), GM130-S25D (middle) and phospho-S25 (bottom). Symmetry-related molecules are colored gray.


a
Importin-α FL +
GST-GM130-S25D
Importin-α FL
GST-GM130-S25D
Absorbance (a.u.)
Vo
Volume (ml)
b
kDa
ml
Importin-α FL
GST-GM130
-S25D
c
Importin-α FL
d
GST-GM130
-S25D
e
Western blot (HEK293T lysates)
Input (1/20)
Unbound (1/20)
WT
S25D
Importin-β
Importin-α
f
Unbound/Input (%)
Importin-α
Importin-β
g
Intensity (A.U.)
not significant
h
IBB + αΔIBB
Time (min)
µcal/sec
KCal/Mole of Injectant
NaCl = 200 mM
Kd = 944 ± 136 nM
Molar Ratio
i
GFP-IBB (µM)
αΔIBB
(5 µM)
GM130-S25D
(5 µM)
Importin-α
ΔIBB
GST-GM130-S25D
GFP-IBB
j
GM130-NLS
Heh2-NLS
Kap60p-IBB
Importin-α

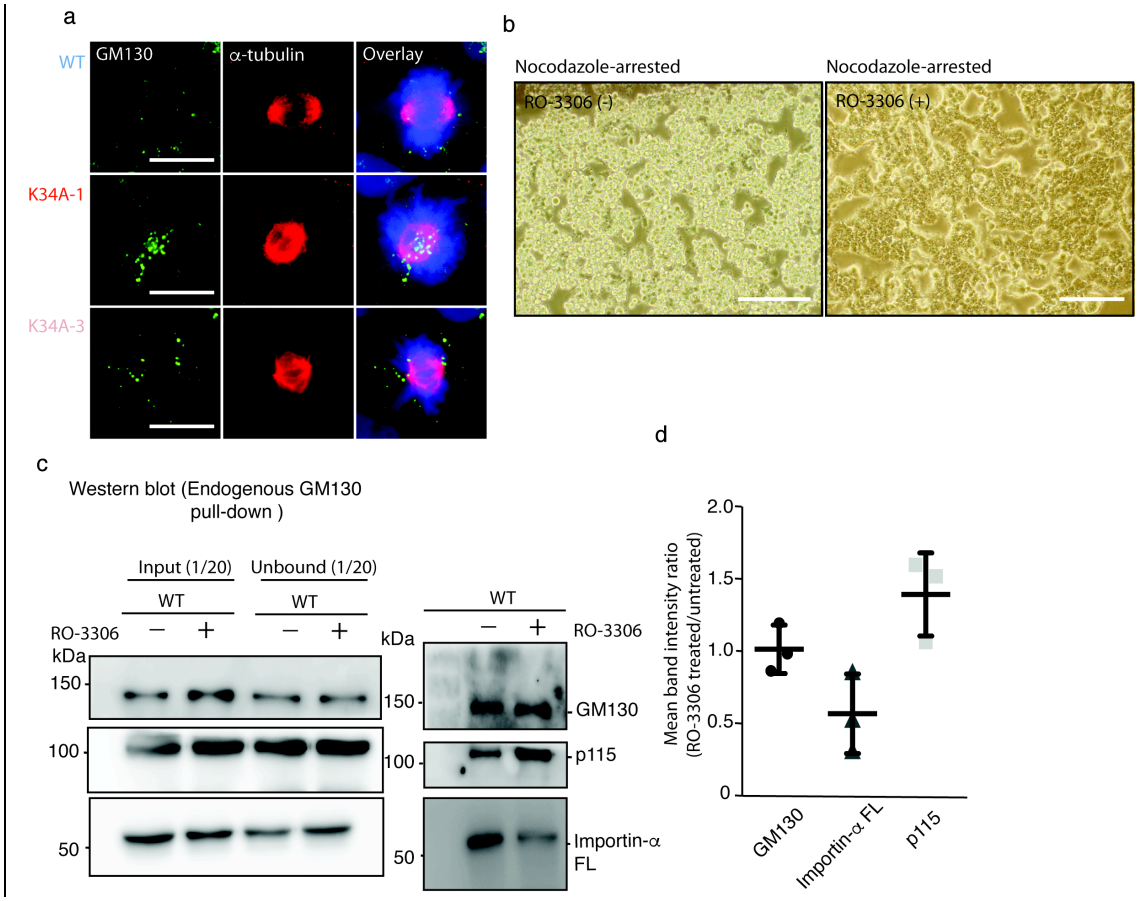
Supplementary Figure 3. GM130 is resistant to IBB displacement upon interacting with Importin-α FL.

a-d, a, SEC (Superdex 200) elution profile for full-length Importin-α•GST-GM130-S25D (red line), full-length Importin-α (blue line), and GST-GM130-WT (green line). The peak fractions of full-length Importin-α•GST-GM130-S25D **(b)**, full-length Importin-α **(c)**, and GST-GM130-WT **(d)** were analyzed by SDS-PAGE and stained with Coomassie blue. The void volume (*Vo*) is indicated. **e,** Western blotting analysis of Importin-α pull-down by GST-GM130-WT and GST-GM130-S25D from HEK293T cell lysates. 1/20 of whole cell lysate (input) and of the total unbound proteins (unbound) is indicated. **f,g,** Band intensities of Importin-α and –β from the Western blotting in (**e**) were used to determine averages of protein in cell lysate **(f)**, unbound protein **(f)** and bound protein **(g)**. Data represent mean ± standard deviation from three independent experiments. Differences were assessed statistically by two-tailed Student's *t* test; **p* <0.05. **h**, ITC titration curves (upper) and binding isotherms (lower) of Importin-α(ΔIBB) with IBB. The data was acquired under the same acquisition conditions as in Fig. 1D-F to determine Kd values for Importin-α(ΔIBB) with different GM130 peptides. Kd values are indicated. **i,** Competition binding assay. GST-GM130-S25D and Importin-α were incubated with indicated concentrations of GFP-IBB. Samples pulled down by GST beads were analyzed by SDS-PAGE and stained with Coomassie blue. **j**, Superimposition of the GM130-NLS peptide (red) with Heh2-NLS and Kap60p-IBB. Importin-α (70-498) is shown in gray.

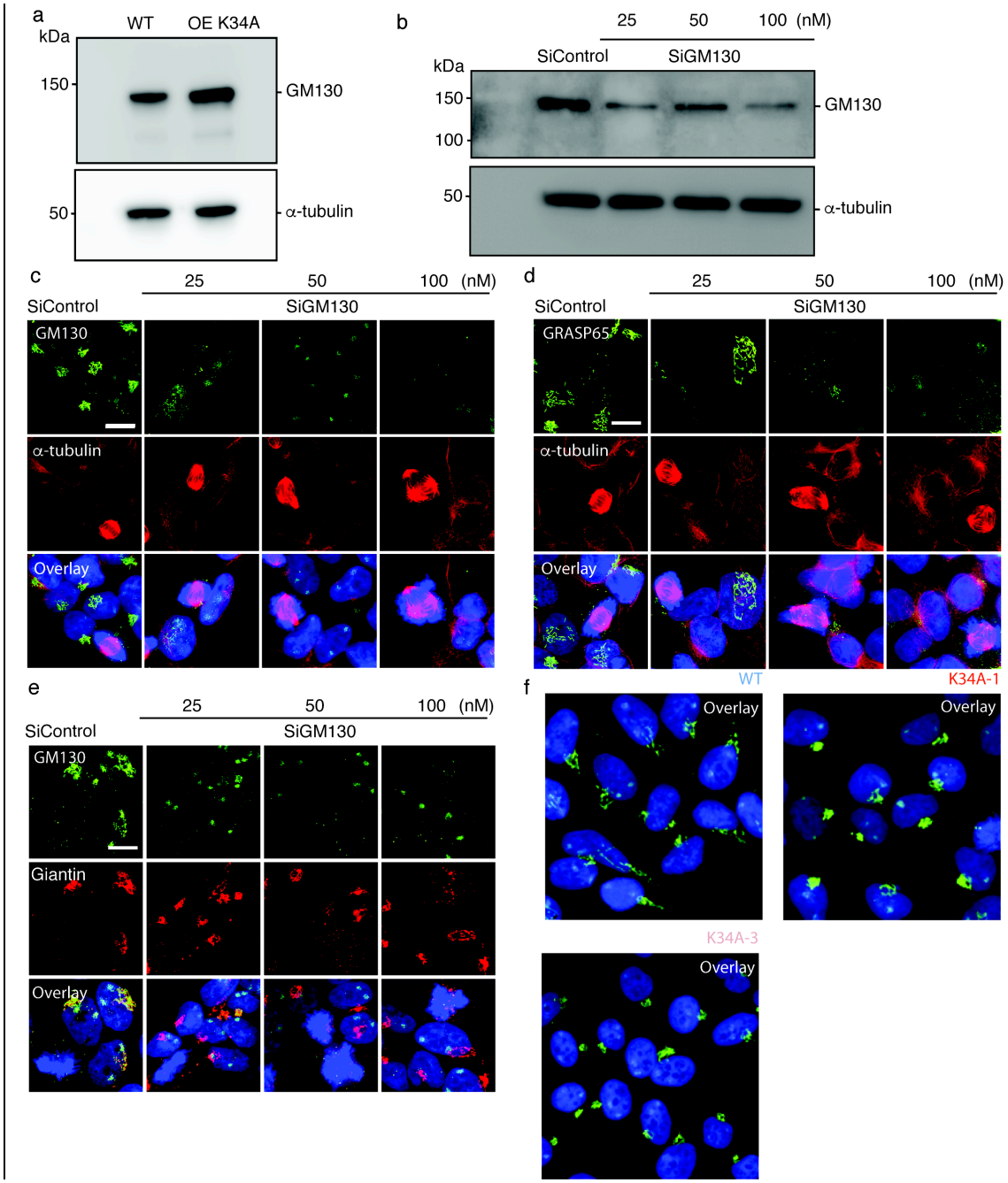

a
Unbound/Input (%)
100
80
60
40
20
0
K34A
GST-GM130-S25D
d
GST-GM130-S25D/K34A
Importin-β
p115-CTD
Input control
Mr
kDa
100
75
50
37
25
20
Importin-β
GST-GM130-S25D/K34A
p115-CTD
e
91
GGAGCGAAAAAGGCCAAGAAGAAAATA
114
K/A34
b
GST-IBB
GST-GM130-S25D
GST-GM130-S25D/K34A
Mr
α FL
α△IBB
kDa
Importin-α FL
Importin-α △IBB
GST-GM130
GST-IBB
c
α FL band intensity / α△IBB band intensity (%)
α FL α△IBB
GST-IBB
GST-GM130-S25D
GST-GM130-S25D/K34A
f
Cyclohexamide treatment (hours)
WT
K34A-1
K34A-3
0 4 8
hours
kDa
150
100
50
GM130
α-tubulin

Supplementary Figure 4. Biochemical properties of GM130 and validation of cell lines containing the GM130 K34A mutation.

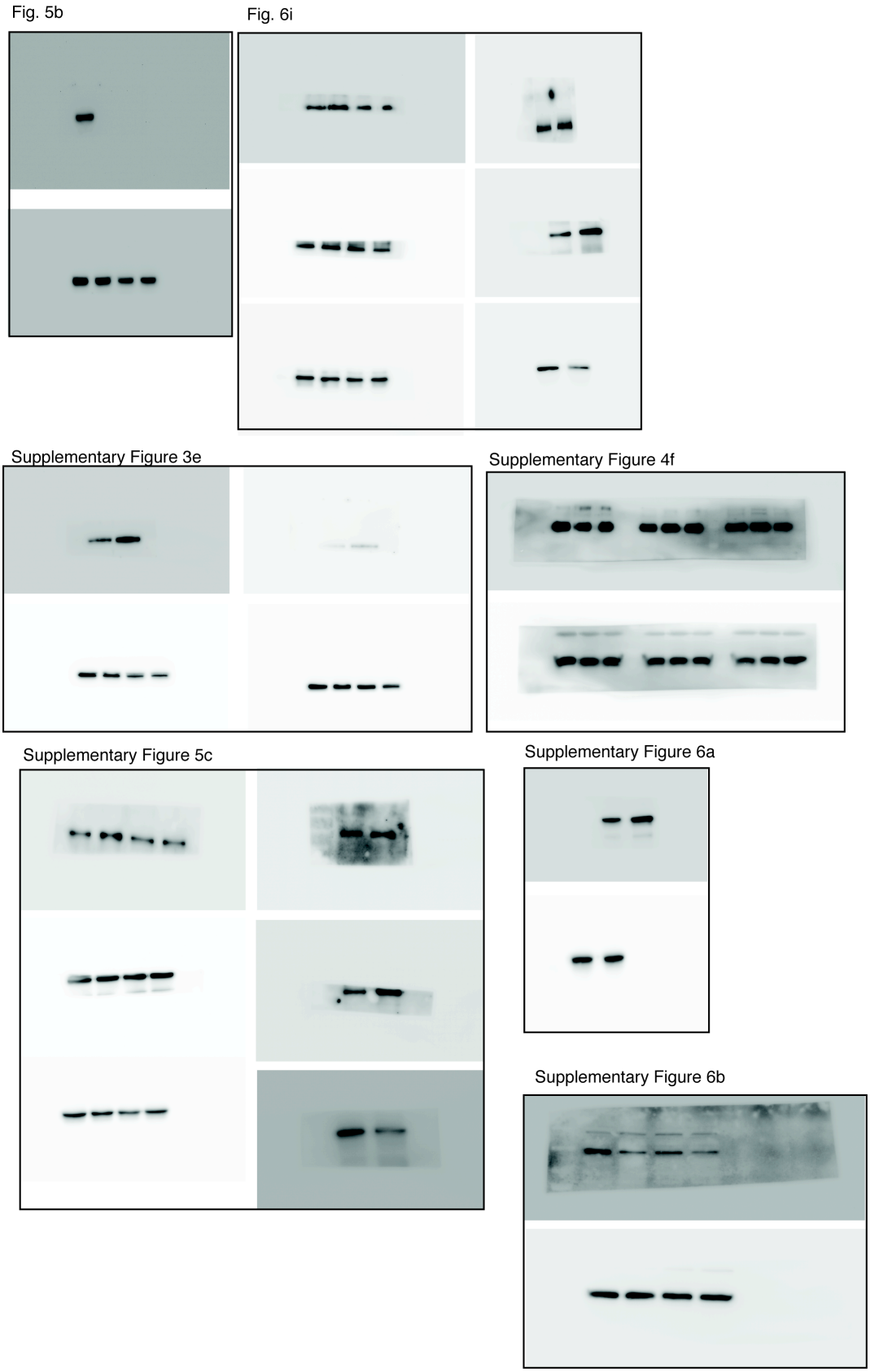
a, Band intensities of Importin-α from the Western blotting in **Fig. 5b** were used to determine averages of protein in cell lysate and unbound protein. Data represent mean ± standard deviation from three independent experiments. **b,** GST pull-down analyses of GST-tagged IBB, GM130-S25D or GM130-S25D/K34A incubated with 2-fold molar excess of either Importin-α FL or Importin-α (ΔIBB). **c,** Quantification of band intensities of bound Importin-α FL and Importin-α (ΔIBB) from the gel in (**c**), plotted as the ratio of Importin-α (ΔIBB) over Importin-α FL. Data represent mean ± standard deviation from three independent experiments. **d,** GST pulldown assays of GM130-S25D/K34A with Importin-β or p115-CTD. GST-fused GM130-S25D/K34A was incubated with Importin-β or p115-CTD at a molar ratio of 1:1. GST-bound samples were analyzed by SDS-PAGE and stained with Coomassie blue. **e,** A representative sequence electropherogram of the heterozygous GM130 K34A mutant. The black bar indicates the heterozygous nucleotides. Nucleotide and residue numbers are indicated. **f**, GM130 wild-type and K34A mutant HEK293T cell lines were mock-treated or treated with cycloheximide for the indicated timeframes, followed by Western blot analysis using GM130 and α-tubulin antibodies.



Supplementary Figure 5. Cell-based examination of the K34A mutation of GM130.
a, Representative fluorescent images of wild-type and two K34A mutant cell lines at metaphase. Cells were immuno-stained with GM130 antibody (green; left panel), α-tubulin (red, middle panel) and DAPI (blue, right panel). Scale bar: 10 μm. **b,** Images of nocodazole-arrested HEK293T cells without (left) and with (right) RO-3306 treatment acquired by bright-field illumination. Scale bar: 100 μm. **c,** Endogenous Importin-α and p115 were pulled down from nocodazole-arrested HEK293T cells treated (+) and untreated (-) with RO-3306 using GM130 antibody. Pulldown samples were analyzed by Western blot using antibodies for GM130, Importin-α and p115. 1/20 of whole cell lysate (input) and of the total unbound proteins (unbound) is indicated. **d,** Analysis of Importin-α and p115 pulldown by GM130 from RO-3306-treated and -untreated RO-3306 cell lysates. The scatter plot shows the ratios of the GM130, Importin-α and p115 band intensities for RO-3306 treated cells over untreated cells. Each dot represents an individual data point. Data represent mean ± standard deviation from three independent experiments.



Supplementary Figure 6. Cell-based examination of GM130 K34A mutation overexpression and siRNA-mediated GM130 knockdown.
a, HEK293T cells overexpressing the GM130 K34A mutant were analyzed by Western blot using GM130 and α-tubulin antibodies. **b**, HEK293T cells were treated with indicated concentrations of siRNA oligonucleotides, followed by Western blot analysis using GM130 and α-tubulin antibodies. **c,d,e,** Representative fluorescent images of wild-type HEK293T cells treated with indicated siRNA oligonucleotides and subsequently immuno-stained with GM130 **(c)**, GRASP65 **(d)** or Giantin **(e)** antibodies. GM130 and GRASP65 are shown in green (top); α-tubulin and Giantin are shown in red (middle). Overlay images with DAPI staining (blue) are shown in the bottom panels. Scale bar: 10 μm. **f,** Merged immunofluorescence images of wild-type and K34A mutant cells in interphase. Cells were immuno-stained with GM130 antibody (colored green) and DAPI (colored blue).



Supplementary Figure 7. Uncropped images of Western blots shown in the main and supplementary figures.

Supplementary Table 1: Primer sequences

Primer	Sequence
GM130 (1-48)-F	CTCCAAAATCGGATCCATGAGCGAAGAAACCCGTC
GM130 (1-48)-R	GGCCGCTCGAGTCGACTTAGGTGGTGGTTTCCGG
GM130 (1-85)-F	GGGGCCCCTGGGATCCATGAGCGAAGAAACCCGTC
GM130 (1-85)-R	GGCCGCTCGAGTCGACTTACAGGCTCGCGCCCGG
GM130 FL-F	CATAGAAGATTCTAGAATGTCGGAAGAAACCCGACAGA
GM130 FL-R	TCGCGGCCGCGGATCCTTAGATGACAGTGATCTTCACCTCA
GM130-S25D-F	TACCAGCAGCGTAAC GACCCGGGCGTTC
GM130-S25D-R	GAACGCCCGG GTC GTTACGCTGCTGGTA
GM130-K14A-F	AAACTGGCGGCGGCGGCGAAAAAACTGCGTGAATAC
GM130-K14A-R	GTATTCACGCAGTTTTTTCGCCGCCGCCGCCAGTTT
GM130-K34A-F	GTTCCGACCGGCGCGAAAGCGAAAAAGAAAATCAAAAAC
GM130-K34A-R	GTTTTTGATTTTCTTTTTCGCTTTCGCGCCGGTCGGAAC
p115 (780-930)-F	CGCGCGGCAGCCATATGGCTAGAGATTCTGAACAAGT
p115 (780-930)-R	GGTGGTGGTGCTCGAGTTAACCAAGATCCTTGAGTTT
Importin-α (70-478)-F1	CAGCCAGGATCCGAATTCGAACCAGGGTACTGTAAA
Importin-α (70-478)-R1	TTCTTTACCAGACTCGAGTTACACTGAGAAGTACTTCT
Importin-α (70-478)-F2	GGGGCCCCTGGGATCCAACCAGGGTACTGTAAATT
Importin-α (70-478)-R2	GGTGGTGGTGCTCGAGCACTGAGAAGTACTTCTCA
IBB-F	TTCAGGGCGCGGCCGCAATGTCCACGAACGAGAAT
IBB-R	CTCACCATGCCCTCGAG GTTCCGGTTTTCCTGTAG
Importin-α FL-F	AGGAGATATACCATGGGCATGTCCCCTATACTAGG
Importin-α FL-R	GGTGGTGGTGCTCGAGCACTGAGAAGTACTTCTCA
Importin-β FL-F	AGGAGATATACCATGGGCATGTCCCCTATACTAGG
Importin-β FL-R	GGTGGTGGTGCTCGAGTGCAGCTTCATAAGCAGCT
GM130 sgRNA-F	CACCGTTCTTCTTTTTCGCTCCTGT
GM130 sgRNA-R	AAAC ACAGGAGCGAAAAAGAAGAAC
GM130 Donor template	CTCATGAATATTATTGCTCTTCTTTTCCACAGTTGAGAGAATATC AGCAGAGGAATAGCCCTGGTGTTCCTACAGGAGCGAAAGCCAA GAAGAAAATAAAAAATGGCAGTAACCCTGAGACAACCACTTCT GGTGGTTGCCACTCACCTG
Genotype GM130-F	GCCATGGTATCAATCCCTCTCAG
Genotype GM130-R	TTTAGGACAGCAGGCCCTGTAC