Supplementary Methods:

Expression and purification of recombinant proteins: β-catenin-MBP fusion constructs were transformed into DH5α cells and purified as follows. 10 ml of overnight starter culture was used to inoculate 200 ml of 2YT+ampicillin broth at 37^{o}C until OD595 reached 1.0 (~2-3 h). IPTG was added to a final concentration of 0.3 mM and incubated at 37^{o}C for 5 h. Bacterial cells were spun down at and the pellet washed with 40 ml of cold PBS. Cells were resuspended in 10 ml H-buffer containing fresh protease cocktail inhibitor (Roche) (H-buffer=TEDM buffer+0.5M NaCl; TEDM = 20 mM Tris pH8.0, 1 mM EDTA, 1 mM DTT, 1 mM $MgCl_2$) and sonicated. Cells were spun at 20,000g /30min/4^{o}C. The cell lysate was loaded onto 1ml Amylose resin column (NEB) pre-equilibrated with H-buffer. The column was washed with 2cv of H-buffer, followed by 10cv of TEDM buffer. The bound protein was eluted with E-buffer (TEDM+ 36mg/ml maltose+ protease inhibitor) and dialysed extensively against TEDM buffer.

Nup153-GST and Nup62-GST were gifts from Dr. Yoshimura (2) and were transformed into BL21 cells. 10 ml of overnight culture was used to inoculate 200 ml of 2YT+ampicillin broth. Cells were cultured for 3 h at 37^{o}C and induced with 0.1 mM IPTG at 37^{o}C for 3 h. Cells were washed with cold PBS and lysed in 10 ml lysis buffer (50 mM Tris pH8.3, 0.5 M NaCl, 1 mM EDTA, 2 mM DTT and fresh protease inhibitor cocktail-Roche), sonicated for 180 s using microtip sonicator at 10% output and spun at 20,000g /30min/4^{o}C. The supernatant was loaded onto 1ml Glutathione-Sepharose 4B column (GE Healthcare) pre-equilibrated with lysis buffer. The column was washed with 2cv lysis buffer, 10cv wash buffer (100mM Tris pH 8.3, 0.1M NaCl, 1mM EDTA, 2mM DTT) and eluted with elution buffer (wash buffer+50mM glutathione+ protease inhibitors).

Supplementary References:

1. Eleftheriou, A., Yoshida, M., and Henderson, B. R. (2001) *J Biol Chem* **276**, 25883-25888
2. Otsuka, S., Iwasaka, S., Yoneda, Y., Takeyasu, K., and Yoshimura, S. H. (2008) *Proc Natl Acad Sci U S A* **105**, 16101-16106

Supplementary Table S1: Primers used for cloning

Construct	Amino Acids	Forward primer	Reverse primer	Cloning site
β-catenin-WT-GFP	1-781	GTCGACGGTACCCATGGCTACTCAAGCTGATTTGATG	ACCGGTGGATCCCGCAGGTCAGTATCAAACCAG	KpnI and BamHI
β-catenin-Y654E-GFP	1-781	GTCGACGGTACCCATGGCTACTCAAGCTGATTTGATG	ACCGGTGGATCCCGCAGGTCAGTATCAAACCAG	KpnI and BamHI
β-catenin-Y654F-GFP	1-781	GTCGACGGTACCCATGGCTACTCAAGCTGATTTGATG	ACCGGTGGATCCCGCAGGTCAGTATCAAACCAG	KpnI and BamHI
β-catenin-R1-12-GFP	132-695	GTCGACGGTACCCATGAAACATGCAGTTGTAAACTTGATTAAC	ACCGGTGGATCCCAAGCAGTCTCATTCCAAGCCATTGG	KpnI And BAmHI
β-catenin-R1-8-GFP	132-467	GTCGACGGTACCCATGAAACATGCAGTTGTAAACTTGATTAAC	ACCGGTGGATCCCAAGCACAGATGGCAGGCTCAGTGATGTC	KpnI and BamHI
β-catenin-R3-8-GFP	218-467	GTCGACGGTACCCATGTTGCATAACCTTTCCCATCATCGTG	ACCGGTGGATCCCAAGCACAGATGGCAGGCTCAGTGATGTC	KpnI and BamHI
β-catenin-R10-12-GFP	520-664	GTCGACGGTACCCATGTGTCCCGCAAATCATGCACCTTTG	ACCGGTGGATCCCGCTCAGAATTCGGAAAAAACAGC	KpnI and BamHI
APC Arm-GFP	334-900	GATCGACGTCGACGATGCTAGCTATGTCTAGCTCCCAAGAC	GACTTGTGGATCCCGAGAGGTATGAATGGCTGACACTTC	SalI And BamHI
β-catenin-WT-MBP	1-781	ACCTGGGATCCGCTACTCAAGCTGATTTGATG	ACCGGTGGATCCCTATTACAGGTCAGTATCAAACCAGGC	BamHI
β-catenin-R3-8-MBP	218-467	ACCTGGGGATCCTTGCATAACCTTTCCCATCATCG	ACCGGTGGATCCCTATTAAGCACAGATGGCAGGCTCAGTGATGTC	BamHI
β-cAtenin-R10-12-MBP	520-664	ACCTGGGGATCCTGTCCCGCAAATCATGCACCTTTG	ACCGGTGGATCCCTATTACTCAGACATTCGGAACAAAACAGC	BamHI
β-catenin-R10-12-Rev	520-664	AGTCGACGGATCCATGTCCCGCAAATCATGCACCTTTG	GCTATTACCGGTCGCTCAGACATTCGGAACAAAACAGC	BamHI And AgeI
Nup-98-GST	15-512aa	GCGGATCCGAATTCGGCTTTGGTACAACGTCAACA	GATTGGAGGGCCTCTTGGTACAGGTCGACTCGAG	EcoRI and SalI

Supplementary Table S2: FRAP kinetic analysis (using one phase association equation)

Fragment	Amino acid	Treatment	Direction	R^2	Rate constant(K)	Half-time	Plateau
WT-GFP	1-781	LMB	Export	0.994	0.014	49.5	86.55
R1-12	132-695	LMB	Export	0.996	0.017	38.52	89.8
R3-8	218-467	LMB	Export	0.991	0.013	51.84	54.03
R10-12	520-664	LMB	Export	0.996	0.014	49.7	89.59
APC-arm	334-900	LMB	Export	0.9923	0.023	30.42	50.56
WT-GFP	1-781	Untreated	Import	0.992	0.013	51.2	95.74
R1-12	132-695	Untreated	Import	0.983	0.024	28.37	88.22

R3-8	218-467	Untreated	Import	0.986	0.017	41.99	82.94
R10-12	520-664	Untreated	Import	0.989	0.01	64.91	116.3

Supplementary Figure legends:

Supplementary Figure S1: Western Blot showing integrity of β-catenin-GFP fragments. β-catenin-GFP fragments were transfected in NIH 3T3 cells and cell lysates were run on the gel and immunoblotted with anti-GFP antibody.

Supplementary Figure S2: Comparing nuclear export of β-catenin sequences +/- LMB. NIH 3T3 cells were transfected with β-catenin-GFP fragments: (A) WT (1-781aa), (B) Arm repeats R1-12 (132-695aa), (C) Arm repeat R10-12 (520-664aa), (D) Arm repeats R1-8 (132-467aa), (E) Arm repeats R3-8 (218-467aa). Mean recovery curves from untreated cells (dotted line) or LMB treated cells (solid line) were calculated as cytoplasmic to nuclear ratio, which was pre-set to 100% based on pre-bleach values. Each curve represents an average (± std. dev) of at least 10 cells from 2-4 experiments.

Supplementary Figure S3: **Rev Assay confirming CRM1 independent export activity of Arm repeat R10-12.** (A) β-catenin fragment Arm repeat R10-12 was cloned into Rev1.4-GFP vector and transfected in T47D cells along with Rev(1.4)-GFP and Rev(1.4)NES3 as controls. 48h post transfection cells were treated with respective drugs for 3h, fixed and stained with Hoechst. Representative cell images are shown. (B) Transfected cells (>150 cells) were scored for nuclear (N), nuclear/cytoplasmic (N/C) or cytoplasmic localization and plotted on a graph. This assay is elsewhere described in detail (Ref. 1), and in brief only constructs with an active export sequence display movement from nucleus to cytoplasm after actinomycin D treatment. LMB had little effect here, except for the Rev NES control.

Supplementary Figure S4: **Comparison of nuclear export and import rates of β-catenin sequences.** NIH3T3 cells were transfected with β-catenin-GFP fragments: WT (1-781aa), Arm repeats R1-12 (132-695aa), Arm repeats R10-12 (520-664aa) and Arm repeats 3-8 (218-467aa) were subjected to nuclear export or import FRAP and analysed as above. Mean recovery curves (± sd) from 10 cells from 2-4 experiments after nuclear export (dotted lines) or import (solid line) are shown.

Supplementary Figure S5: Identification of potential β-catenin Arm R10-12 binding partners. (A) Purified beads coated with MBP (lane 1) or Arm R10-12-MBP (lane 2) were incubated with total cell lysate from NIH3T3 cells. After extensive washing the beads were run on a 4-20% gradient gel. Marked boxes indicate the bands excised for Mass Spectrometry analysis. (B) List of additional candidate binding partners identified by Mass Spec with relative scores.

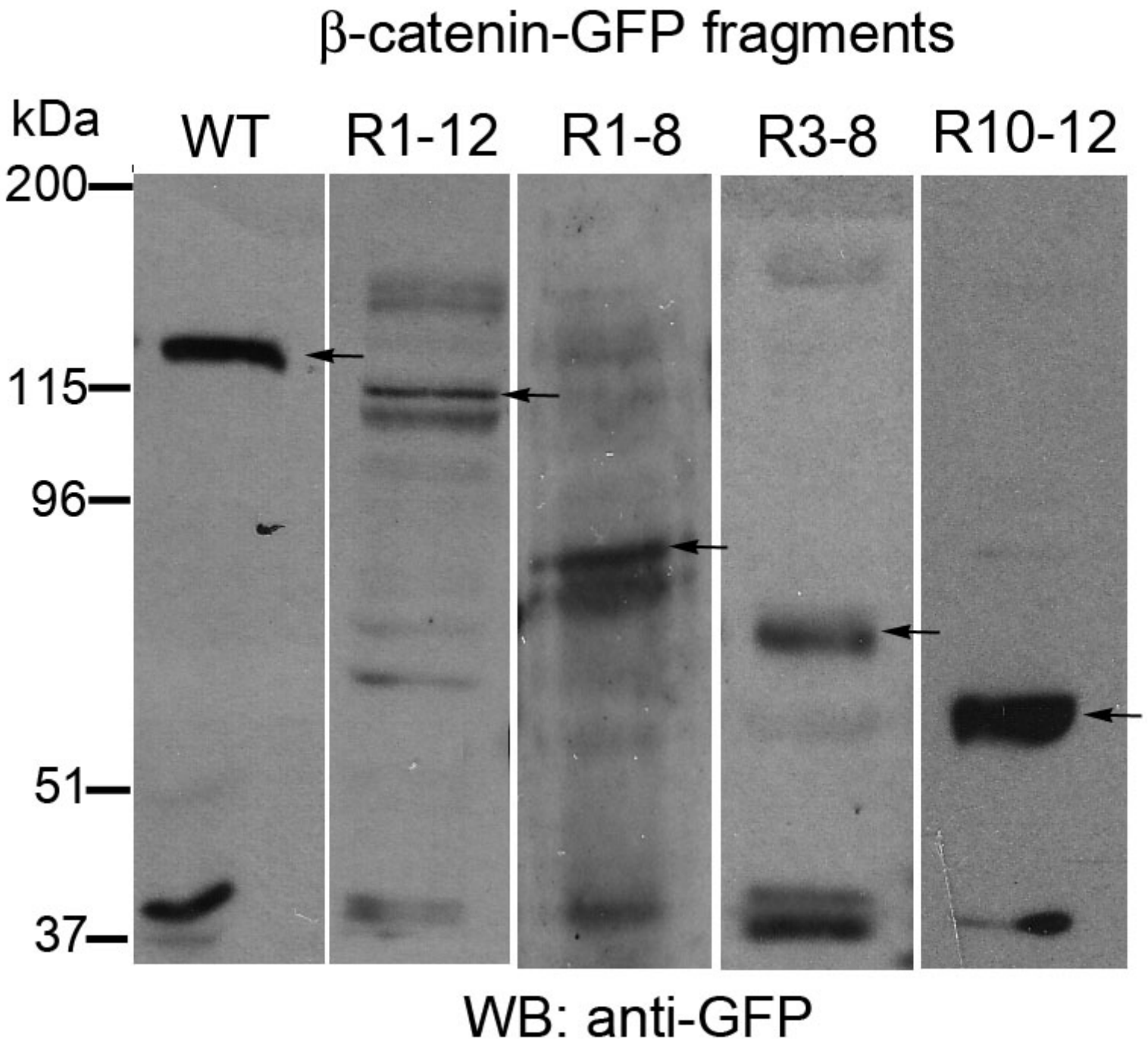
Supplementary Figure S6: Loss of Nup62 and Nup358 does not affect nuclear envelope integrity or GFP diffusion rate at NPC. (A) NIH 3T3 cells were transfected with control or Nup62 siRNA and immunostained with Nup153 pAb (Abcam). A representative image is shown. Loss of Nup62 did not diminish nuclear rim staining of Nup153. (B) NIH 3T3 cell lysates from cells transfected with control or Nup62 siRNAs (left panel) and control or Nup358 siRNAs (right panel) were separated by SDS-PAGE and immunoblotted with mAb414 antibody (Convance). Western Blot shows that specific loss of Nup62 or Nup358 did not affect expression of other FG-repeat Nups recognized by the antibody. (C) NIH 3T3 cells were transfected with control or Nup358 siRNA and immunostained with mAb414 antibody, revealing that the nuclear envelope remained intact. (D) NIH 3T3 cells were transfected with control of Nup358 siRNA and immunostained for Nup62 showing that nuclear envelope is intact. Representative cell images are shown. (E) NIH 3T3 cells were transfected with control or Nup62 siRNAs and pEGFP-N1 vector. At 72 h post-transfection cells were subjected to nuclear import FRAP for GFP and the average diffusion curves from 8 cells were plotted on the graph. The loss of Nup62 did not alter GFP diffusion across the NPC. Similar data were seen for Nup358 (not shown).

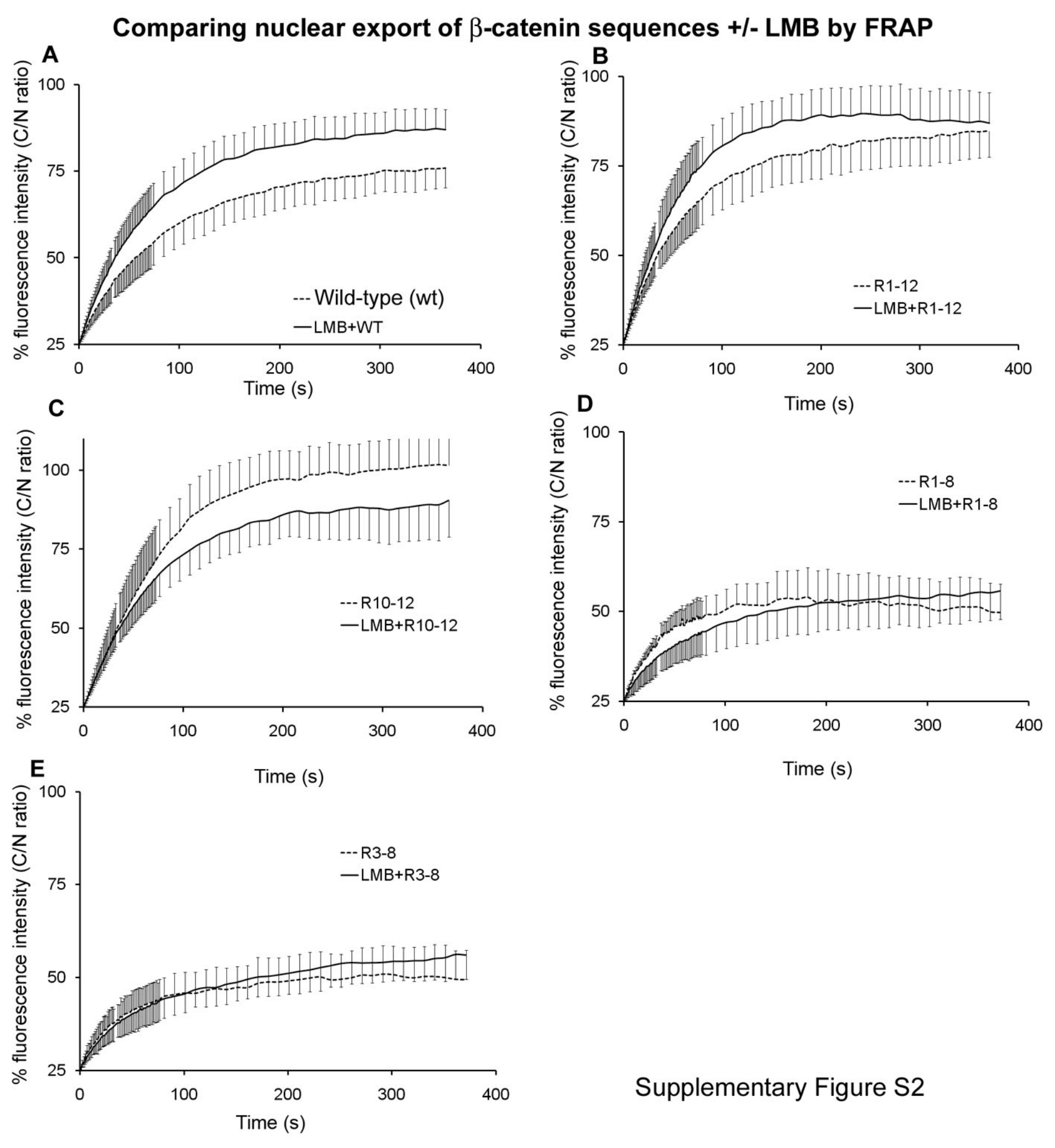
Supplementary Figure S7. Knockdown of Nup62 or Nup 358 does not alter the import rate of GFP-importin-beta. The approach for import FRAP of GFP-importin-beta was similar to that described in legends to Figures 4 and 5. (A) After bleaching GFP-importin-beta nuclear fluorescence its recovery was measured over 360 s in cells transfected with control or Nup62 siRNA. (B) Recovery import curves were plotted and no significant difference observed. (C) Similar FRAP assay was performed in presence of control or Nup358/RanBP2 siRNA and

recovery curve was plotted (D). Again no effect on import. Note that the knockdowns were routinely validated in parallel by Western blot (examples shown in Figures 4 and 5).

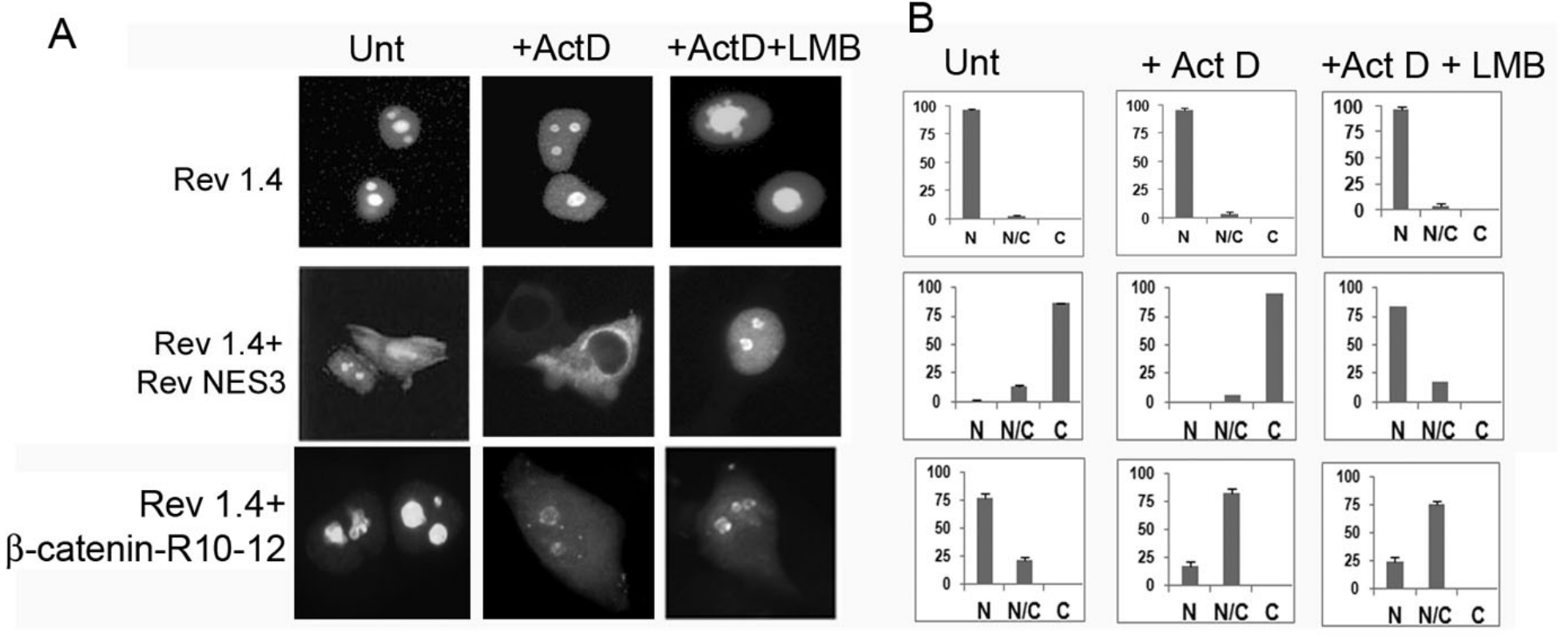
Supplementary Figure S8. Evidence against a role of RanBP3 in nuclear export of de-phospho β-catenin. (A) β-catenin 8E7 mAb recognizes non-specific nuclear protein. NIH 3T3 were transfected with control or β-catenin siRNA and stained with β-catenin 8E7mAb (top panel) or total β-catenin mAb (Transduction Labs) (bottom panel). Phalloidin-FITC was used a control. (B) SW480 cells were transfected with control or RanBP3 siRNA and stained with total β-catenin mAb (Transduction Labs). Representative images are shown suggesting that silencing RanBP3 does not affect localization of β-catenin (top panel). Western Blot to confirm knock down of RanBP3 (bottom panel). (C) NIH 3T3 cells were co-transfected with β-catenin-S45A-GFP and RFP or RanBP3-RFP plasmids. Cell images showing that RanBP3 overexpression does not induce export of β-catenin S45A mutant. (D) NIH 3T3 cells were transfected with YFP or RanBP3-YFP. 48 h post transfection cells were treated with 40mM LiCl for 6h and then immunostained with total β-catenin mAb (Transduction Labs) (top panel). >300 cells (from at least 2 experiments) were scored for a loss of nuclear β-catenin and presented in a graph (bottom panel). Ectopic RanBP3 did not stimulate nuclear export of dephospho-β-catenin.

Supplementary Figure S1



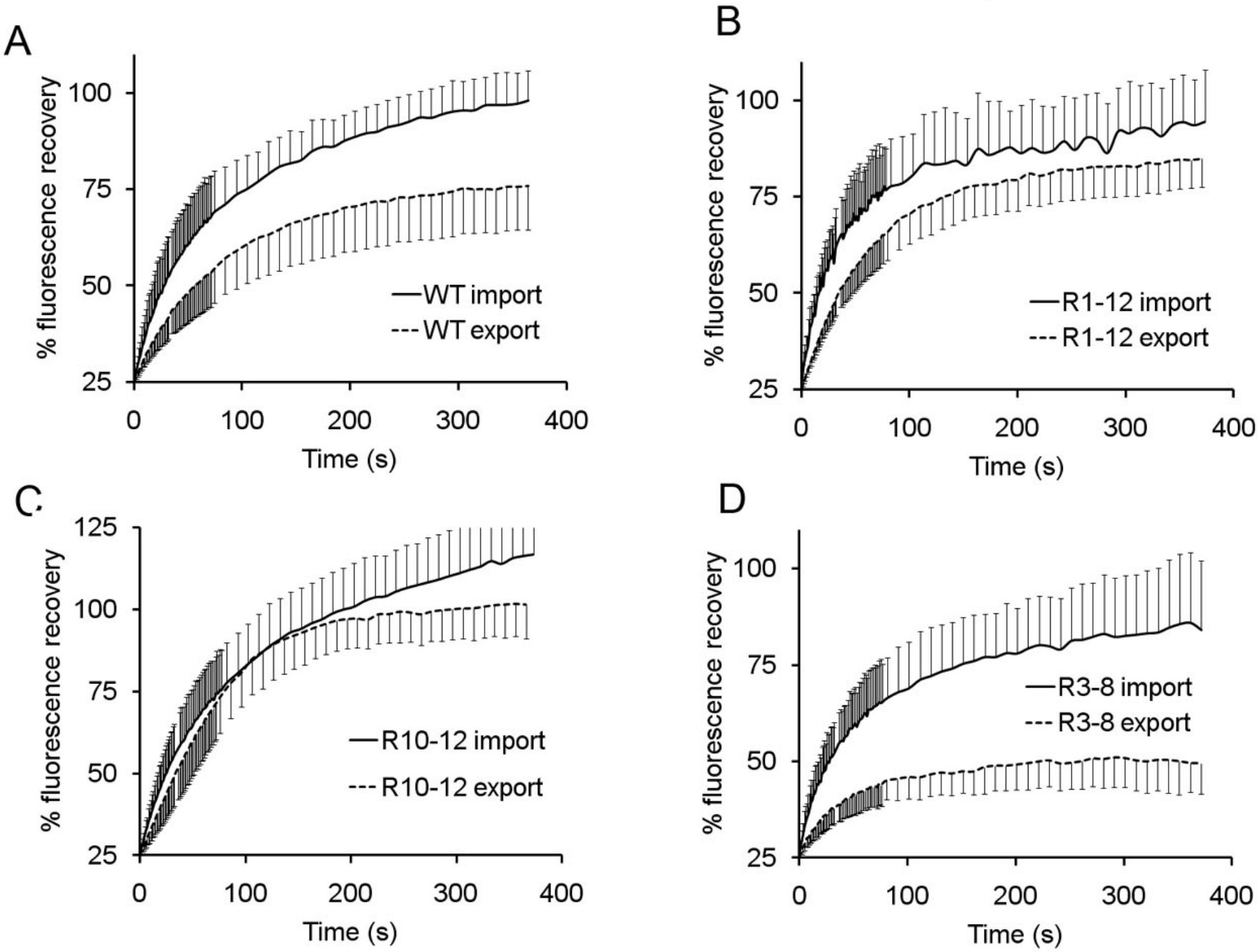

Comparing nuclear export of β-catenin sequences +/- LMB by FRAP
A
% fluorescence intensity (C/N ratio)
100
75
50
25
0
100
200
300
400
Time (s)
--- Wild-type (wt)
—LMB+WT
B
% fluorescence intensity (C/N ratio)
Time (s)
--- R1-12
—LMB+R1-12
C
% fluorescence intensity (C/N ratio)
Time (s)
--- R10-12
—LMB+R10-12
D
% fluorescence intensity (C/N ratio)
Time (s)
--- R1-8
—LMB+R1-8
E
% fluorescence intensity (C/N ratio)
Time (s)
--- R3-8
—LMB+R3-8
Supplementary Figure S2

Rev(1.4)-GFP export assay confirms activity of β-catenin Arm 10-12 sequence



Supplementary Figure S3

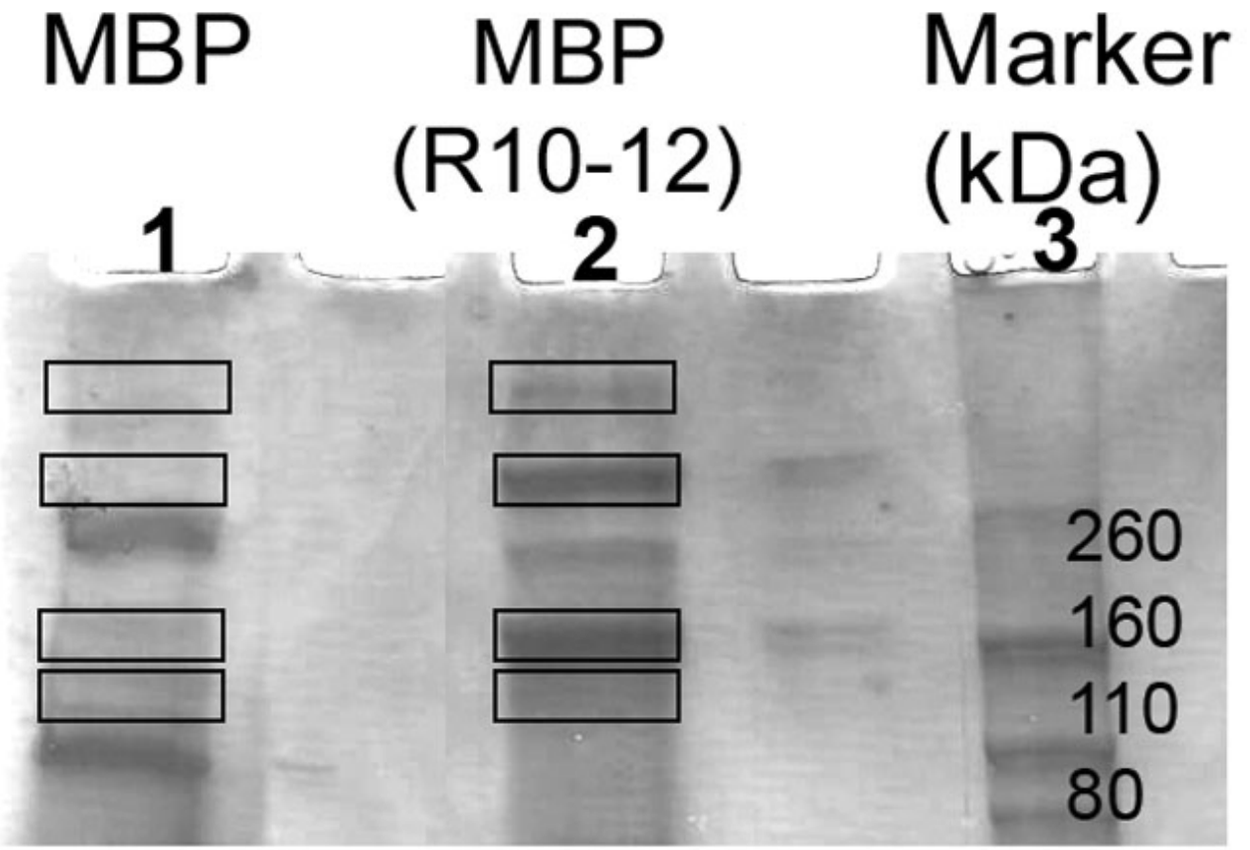
Comparison of nuclear import and export rates of β-catenin sequences



Supplementary Figure S4

Supplementary Figure S5

A

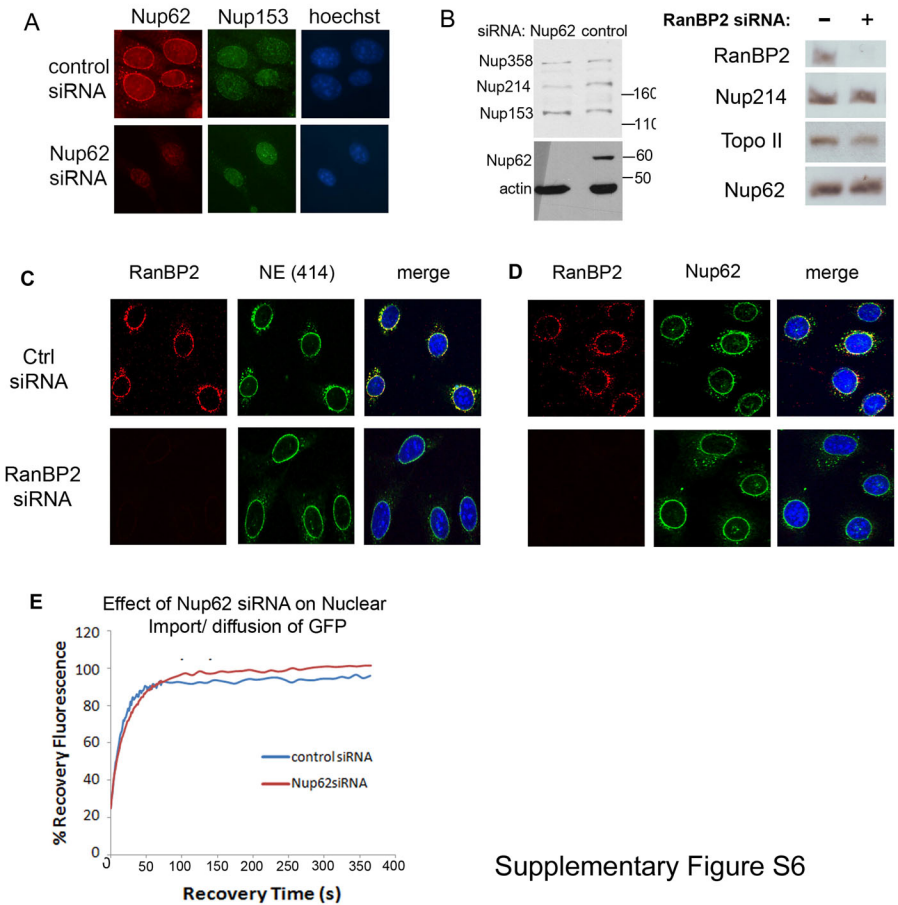


R10-12 specific bands were used to identify new partners by mass spec

B

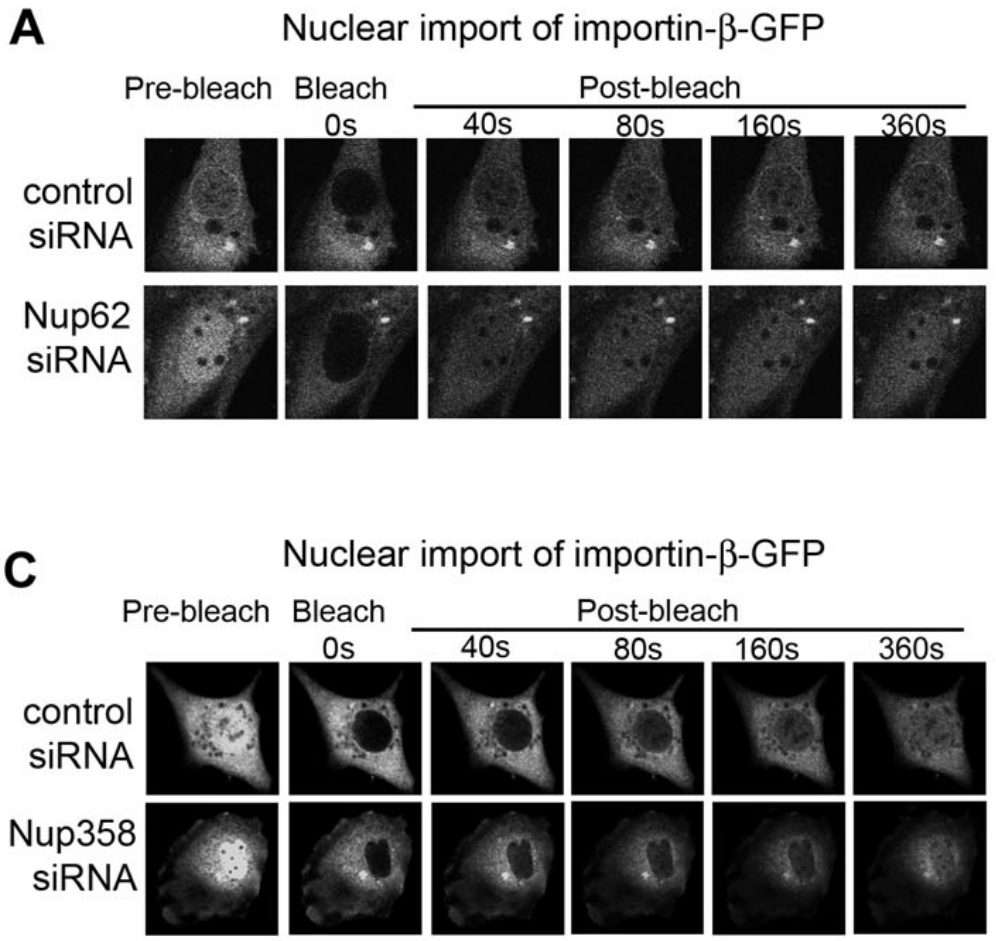
Binding partners for R10-12	**Score**
Myb binding protein 1a (MBB1A)	97
Leucyl-tRNA synthetase	81
Mini-chromosome maintenance complex 2 (MCM2)	49
cullin-associated and neddylation-dissociated 1 (CAND1)	49

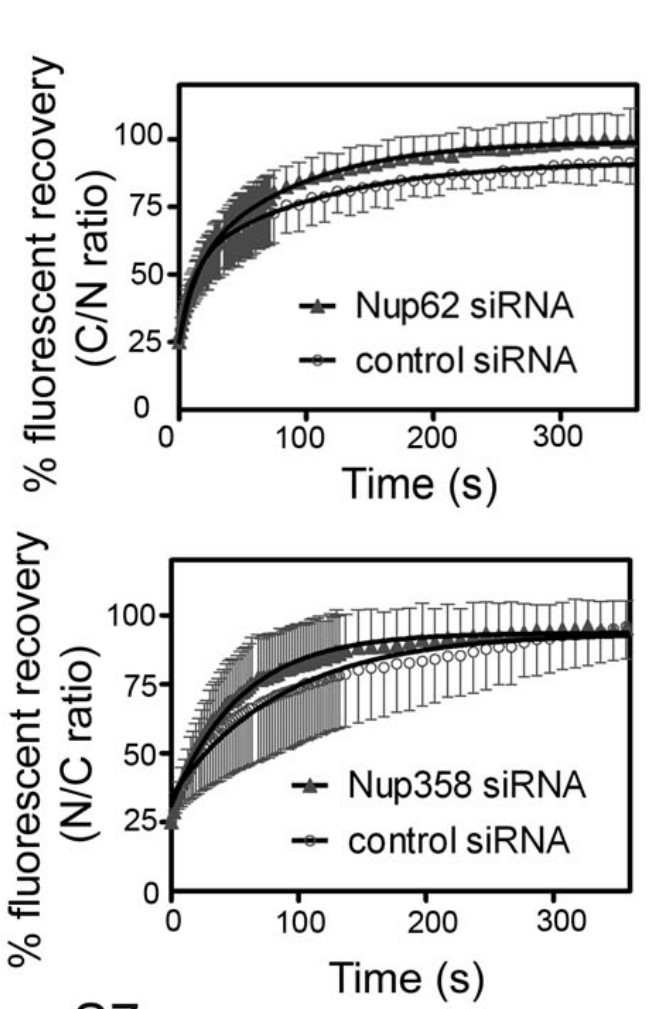
Loss of Nup62 or Nup358/RanBP2 does not affect nuclear envelope staining or GFP diffusion rate at the NPC



Supplementary Figure S6

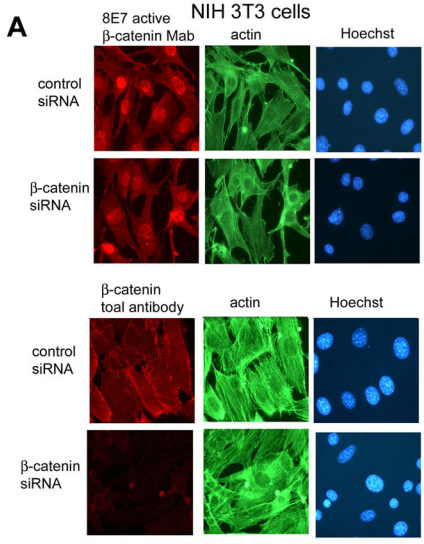
Knockdown of Nup358 or Nup62 has no effect on import rate of GFP-importin-β

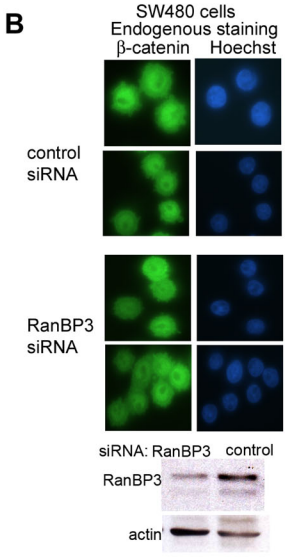




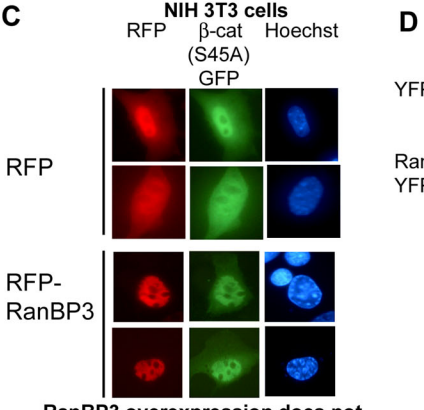
Supplementary Figure S7

Evidence against a role for RanBP3 in nuclear export of dephospho-β-catenin

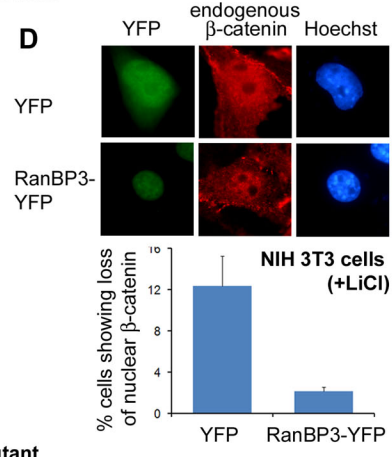




Evidence that the 8E7 Mab used to detect non-phosphorylated β-catenin cross-reacts with other nuclear proteins



RanBP3 overexpression does not induce export of β-catenin S45A mutant



Supplementary Figure S8