xlNup214 FG-like-1 (aa 443-690)
TSVSAPAPPASAAPRSAAPPPYPFGLSTASSGAPTPVLNPPASLAPAATPTKTTSQPAAAATSIFQPAGPAAGSLQPPSLPAFSFSSANNAANASAPSSFPFGA
AMVSSNTAKVSAPPAMSFQPAMGTRPFSLATPVTVQAATAPGFTPTPSTVKVNLKDKFNASDTPPPATISSAAALSFTPTSKPNATVPVKSQPTVIPSQASVQP
NRPFAVEAPQAPSSVSIASVQKTVRVNPPATKITPQPQRS
xlNup214 FG-like-2 (aa 1220-1614)
KPVSFSPAAGGFSFSNVTSAPVTSALGSSSAGCAATARDSNQASSYMFGGTGKSLGSEGSFSFASLKPASSSSSSSVVEPTMSKPSVVTAASTTATVTSTTAAS
SKPGEGLFQGFSGGETLGSFSGLRVGQADEASKVEVAKTPTAAQPVKLPSNPVLFSFAGAPQPAKVGEAPSTTSSTSASLFGNVQLASAGSTASAFTQSGSKPA
FTFGIPQSTSTTAGASSAIPASFQSLLVSAAPATTTPSAPINSGLDVKQPIKPLSEPADSSSSQQQTLTTQSAAEQVPTVTPAATTATALPPPVPTIPSTAEAK
IEGAAAPAIPASVISSQTVPFTSTVLASQTPLASTPAGGPTSQVPVLVTTAPPVTTESAQTVSLTGQPVAGSSAFAQSTVTAA
xlNup214 FG (aa 1615-2033)
STPVFGQALASGAAPSPFAQPTSSSVSTSANSSTGFGTSAFGATGGNGGFGQPSFGQAPLWKGPATSQSTLPFSQPTFGTQPAFGQPAASTATSSAGSLFGCTS
SASSFSFGQASNTSGTSTSGVLFGQSSAPVFGQSAAFPQAAPAFGSASVSTTTTASFGFGQPAGFASGTSGSLFNPSQSGSTSVFGQQPASSSGGLFGAGSGGA
STVGLFSGLGAKPSQEAANKNPFGSPGSSGFGSAGASNSSNLFGNSGAKAFGFGGTSFGDKPSATFSAGGSVASQGFSFNSPTKTGGFGAAPVFGSPPTFGGSP
GFGGSPAFGTAAAFSNTLGSTGGKVFGEGTSAATTGGFGFGSNSSTAAFGSLATQNTPTFGSISQQSPGFGGQSSGFSGFGAGPGAAAGNTGGFGFGVSNPTSP
GFG
xtNup153 FG (aa 885-1127)
GFGTSTLSAGTTAPTFKFGVQPSDSAGELKSGASTDSTSGFSFAKPIGDFKFGLASASATTEETGKKSFTFGTSTSNQASAGFKFGVASSAQTNQDTSGGFTFG
SVSSTVSFSPAATYSGSTGLQVPAADDDSSRASAAGLKSAEEKKPEAPAVTAFSFGKTDQNKETVSTSFIFGKKDEKTDSAPTGNSFGFGLKKDGEEPKQFLFG
KPEPTKEDSTSSTASAGFAFRVSNPTEKKDVEQPV
xtNup153 FG (aa 1128-1525)
KSVFAFGSQTSTTDAGASKQPFSFLTGVSSTSASSSASGVSSSVFGSVAQSSTPANPSNVFGSATSSNPPAVSSGVFGNLNPSNAPASSSTLFGNVAPSSTPSG
SSSLFGTANPSSTPASSSSLFGTAAKLSAPVGSGGVFNSAAPVPPASTSSSVFGSAAPANTSANSANIFGSAGGTSGAPGTFVFGQPASTTSTVFGNSSESKST
FAFSGQETKPVTSAITSATPFVFGAESASTTPAAPGFNFGRTNTSNVTGTSSSPFIFGGGPTASASPSLTAHANPVPAFGQSANSSTAPAFGSSTSVFPAGNSQ
QVPAFGSSTAQPPVFGQQAAQPSFGSSAAPSAGSGFQFGNNTNFNFTTPNSSGGVFTFGANAGSTPQPPAPGFMFNAAASGFNVGT
xlNup62 FG (aa 2-352)
SGFNFGAASAGGFSFGNPKSTTTTAPTGFSFGAATAAPSGGFSFGTATPTPASTTGQTSGLFSFSNPAPSLAPTSGFSFGAQVTSTPAPSSGGLAFGANTSKLN
SGVGNQPAGGTTQTSQPMGGFSFGAATTQTQPSATSVGGFSFAGGVGSTSTNVFAQPAASTGITLQSAVSTAAAPTATTSQPTSTFSFGTQPQAAPALNFGLLS
SSSVLSTASTPAAAQPVAPTTGLSLNFGKPADTSAAVTSTGSTTTNTPSLSSLLGTSGPSLFSSVATSTVPSVVSTVASGLSLTSTATSTGFGMKTLASSAVPT
GTLATSTASLGVKAPLAGTIVQANAVGSAAATGISTATA
xlNup54 FG (aa 2-94)
AFNFGATTGTPANQGTTGFSLGTFTPKTTTSGFGFGTTTTAPTGFGGFGFGATTTASTGPAFSFTTPANTTSGLFGATQNKGFGFGTGFG
xlNup54 FG-like (aa 95-139)
STTTSTGLGTGLGTGLGFTGFNTSQQQQQQSVLGAGLFNQSFQST
xlNup58 FG (aa 2-72)
ASGFSFGTAAASTTTLNPTAAAPFSFGATPAASNTGTTGGLGFGAFNAAATPATTTATTGLGGGLFGAKPA
xlNup58 FG-like (aa 73-259)
AGFTLGGANTATATTTAASTGFSVGFNKPAGSATPFSLPVTSTSSGGLSLASALTSTPATGPSPFTLNLGSTPATTTAAATGLSLGGTLTGLGGSLFQNTNPSA
TGLGQSTLGQSTLGQSTLGQSTLGQSLLGQSLLGQSLLGQSTLGQSTLGQSLLGQSLLGLGLNLGAVAPVSQVTTHEGLGGLDFSSSS
xlNup58 FG (aa 511-598)
TTGFGSSSAFGGNTSGSSSFGFGTANKPSGSLSAGFGSTSTSGFNFSNPGINASAGLTFGVSNPSSTSFGTGQLLQLKKPPAGNKRGK
xtNup98 FG (aa 1-485)
MFNKTFGSPFGTGNGAFGATSTFGQTTGFGTTPATAFGSAGFGTNTSTGGLFGNTQTKPGGLFGSTTFNQPATSSSSSGFGFGASTGTTNSLFGSTNTGSGLFA
TQSNAFGQAKPTTFGNFGTSTSTGGLFGNTNTANPFGGTSASLFGASTFSAAPTGTTIKFNPPSGTDTMAKGGVTTNISTKHQCITAMKEYESKSLEELRLEDY
QANRKGPQNPVGAPTGTGLFGTSAATSSASTGIFGSTAANNSFSFAGNKTTFGTAGTGAFGGNTGGLFGQPANQPAASLFNKPFGNATTTQSTGFSFGNTSTLG
QPQTSTMGLFGANQPTQSGGLFGTTTNTNATGAFGAGTSLFGQPNPAPFGTGSTLFGNKPAGFGTTTTSAPAFGTTTGGLFGNKPTLTLGTNTNTSNFGFGSNT
AGTSLFGNKTATGTIGPSLGTGFGTALNPGQTSLFGSNQPKLTGTLGTGAFGNAGFNSTSAGLGFGAPQ
xlNup50 FG (aa 68-285)
TGGSLSSFGNGSPAKPSEGLSNGTSTSLFINLKPQSKPTFGSAFTNRPLLGTAEKSTNGEKPLSSSGAALSKPGNLEYNKQLTSLNCSVRDWIVKHVNANPLCD
LTPIFKDYEKHLSAIEQKYGASSESGSESDGAAQTKTIPNLSSGKTVSIATFSFGNKDKAPEAPTKTPPDSKPQAAPTFNFGQKVDSSTLGLISSGGAPNFSFS
IGAPSLFGKN
xlCG1 FG (aa 257-411)
SAFGALSFPTSNTAPTAVTFSFKADTTTAAKPAVPNALAGSDFSAFGNKPTSAPSFGSGVAAAAASFSFAPSTISGFGSTASNSGFGAASNAAGFQGAANIAAA
PAFGVASSTAPASGFGGGFGTTVNTGAKTSSVRDLFSAGTAVPVQTTLLFG
xlPom121 FG (aa 571-1050)
NTTFTLFSSSSDAKSVTSSSSTPSPFTSATTSITNTLLQSLGSGQTKSESPFPKNSLLQILGKTEGNNSQPMFNPVFGPLGSANPSPVTAAPGLSTTTTAILKP
ICGDPPSQQAQTSMFKPIFEPPSSQTVPSALSSPFVFSSSSSTTSTTSFLGTTGNSVNTGKHEKPNLALTTCASNTTITSSLAPTFGITSKVEPVSLGGPASQA
NTSFSVIGSTSVPAISTAFGSTTSAFTAAGQTANPSFSANTAPIGVSSVSASEGQTSISTTATFSKFGVPVGQNVFSASNPFGSGTQSTMGTSTQSATNIAFSF
GTSTSTQSAFGFSQNPTMLFSSSTKSNNPTNTSGFNSMGSVFGSSTVPTSSIVTPNKSLSTLGIPEKCETKNQLVTNQLSLGQGSTPAPFPSIMPTQSFVSSST
SFAPSTPTVNQSSTPGSFPSMAAVQPFTSPSSPAAGFFSHGTAPKSRTAVRHKLHPRRPHPRKK
xtNup358 FG
(aa 1095-1180)
FGEKSFSSGFSITGTQSQDKNPLVFGQTENIFTFKSSAKSTFAPPTFGVQPKDAHNLSVESDAGSEHAAADDDGPHFEPIVPLPEK
(aa 1307-1345)
TESPVAPVQEKNAKYKQKVDSSKPNETPLTFGSQFALKR
(aa 1374-1469)
QSSLTSSTCISAPSFTFGKESATNKLGFGQQLLKNKEQWTCSKCLQKNDALVSLCSYCQTQNQAKTGISQPNKASTGFTNNVSAQGDSLAAVFGKK
(aa 1504-1528)
QAASFSFAPGADNSQKNFGAQFAKK
(aa 1558-1595)
ATNKDAVPPAQTPSGFKFGPYAEFGKTQPSLSAMFSRK
(aa 1625-1837)
SSASQSKEVPFTFGIKANSSQNFGQPVAGFNCDFSGKGFKFGISDEKSSASNFAFQAPVSNDESKVVKEGFNFPLSAGPLTFNFGISDSNKTKEMSAGFMKGTS
TNDKESETAKTTTKSEKSQQCSDKVLGQSVQSFSFADIAKTSDTEGFNFGKVDPNFKGFSGAGQKLFISQNAKSNEAVASNEQEATDDLYKTEERDDIHFEPIV
QLPDK
(aa 1967-2119)
QTPHKLVDTGRTAHLIQKAEEMKTGLKDLKTFLTDKAKPLDESNVTGSTEVVKQSPADGTEPTFEWDTYDMRGEALEGNLDDSIYASPLASSPEKKNLFRFGES
ASGFNFSFQPEPSPSKSPTKLNHSRVSVGTDEESDVTQEEERDGQYFEP
(aa 2315-2431)
SPAKFTFGSDAVKNIFGSEKKMPFAFGNTSSTRSLFGFSFNASQEEVQKQPPEITLDFTSTIEAPEMSALQKSCQGSEQSPIVSSSLTSSSVSSSTLMQPMPAR
DKVDDVPDADISS
(aa 2572-2725)
KGVPEKDVNSSYEAPIVCAAKTSVSLPPKQEPDSTTIISQEPVDLSSKQELPKTDSTSKGFSASSFSFGLGTVSGVSFADLASENSGDYAFGSKDTSFQWANAG
AAVFGSYSKSKKGEDEDGSDDEVVHSDDVHFEPIVSLPEVEVKSGEEDEE

Figure S1 Amino acid sequences of analyzed *Xenopus* FG and FG-like domains. FG motifs are in bold and underlined, hydrophobic amino acids in red, N and Q in blue, charged residues in green.

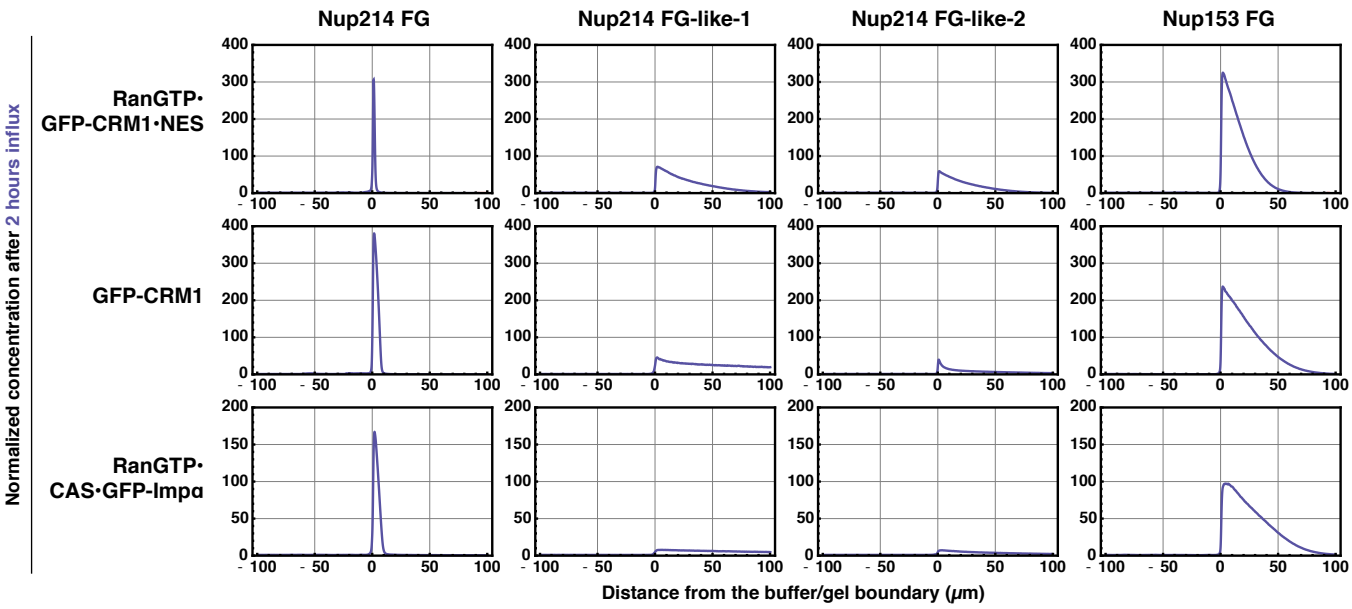

Figure S2 The stalling of the RanGTP•CRM1•NES-GFP complex at the surface of the Nup214 FG hydrogel is specific. This effect was not observed with Nup214 FG-like gels or a Nup153 FG hydrogel. Likewise, free CRM1 or a CAS export complex were not stalled at any of those gels. Plots show normalized concentration profiles after 2h of influx.

A

hsNup62 FG (aa 2-157)
SGFNFGGTGAPTGGFTFGTAKTATTTPATGFSFSTSGTGGFNFGAPFQPATSTPSTGLFSLATQTPATQTTGFTFGTA
TLASGGTGFSLGIGASKLNLSNTAATPAMANPSGFGLGSSNLTNAISSTVTSSQGTAPTGFVFGPSTTSVAPATTSGG
hsNup62 FG-like (aa 158-311)
FSFTGGSTAQPSGFNIGSAGNSAQPTAPATLPFTPATPAATTAGATQPAAPTPTATITSTGPSLFASIATAPTSSATT
GLSLCTPVTTAGAPTAGTQGFSLKAPGAASGTSTTTSTAATATATTTSSSSTTGFALNLKPLAPAGIPSNTAAAVT
hsNup58 FG
(aa 2-75)
STGFSFGSGTLGSTTVAAGGTSTGGVFSFGTGASSNPSVGLNFGNLGSTSTPATTSAPSSGFGTGLFGSKPATG
(aa 474-587)
VSFGTPFGSGIGTGLQSSGLGSSNLGGFGTSSGFGCSTTGASTFGFGTTNKPSGSLSAGFGSSSTSGFNFSNPGITAS
AGLTFGVSNPASAGFGTGGQLLQLKKPPAGNKRGKR
hsNup58 FG-like
(aa 76-248)
FTLGGTNTGIATTITTGLTLGTPATTSAATTGFSLGFNKPAASATPFALPITSTSASGLTLSSALTSTPAASTGFTLN
NLGGTTATTTTASTGLSLGGALAGLGGSLFQSTNTGTSGLGQNALGLTLGTTAATSTAGNEGLGGIDFSSSSDKKSDK
TGTRPEDSKALKDENLP
(aa 426-473)
EAKKWQNTPRVTTGPTPFSTMPNAAAVAMAATLTQQQQPATGPQPSLG
hsNup54 FG (aa 1-91)
MAFNFGAPSGTSGTAAATAAPAGGFGGFGTTSTTAGSAFSFSAPTNTGTTGLFGGTQNKGFGFGTGFGTTTGTSTGLG
TGLGTGLGFGGFN
hsNup54 FG-like (aa 92-112)
TQQQQQTTLGGLFSQPTQAPT

B

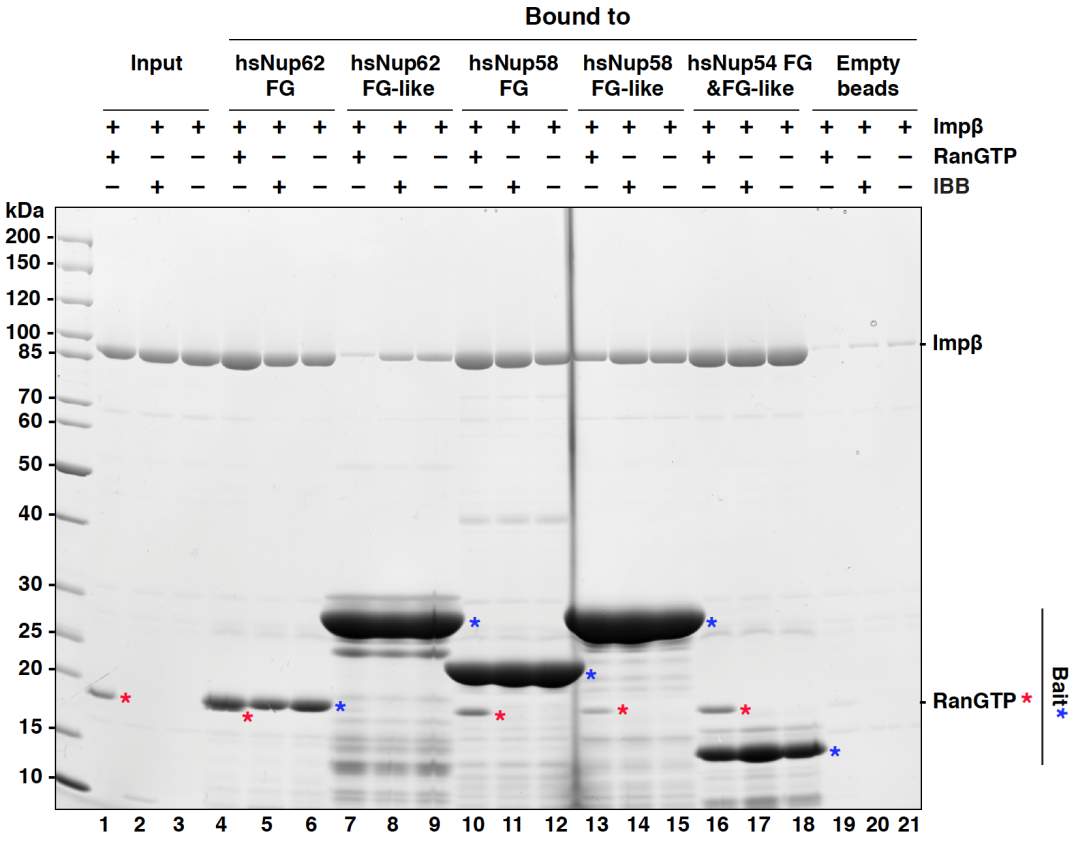

Figure S3 Impβ interacts with the hsNup62 and hsNup58 FG-like regions even though these subdomains lack classical "FG" motifs.

(A) Amino acid sequences of the FG and FG-like domains from the human Nup54•58•62 complex analyzed in B for NTR-binding. We defined residues 158-311 of human Nup62 as an FG-like domain and not as part of the FG domain, because it does not contain any "FG" motif. The homologous region of *Xenopus* Nup62, however, contains FG motifs. The human Nup54 FG-like domain is only 21-amino acid long and was therefore not analyzed individually.

(B) Indicated His-tagged FG- and FG-like domains of hsNup62, hsNup58, and hsNup54 were individually pre-bound to Ni(II) Silica beads and subsequently incubated with His-tag-free Impβ or pre-formed complexes of Impβ with RanGTP or IBB. Empty Ni(II) Silica beads served as a negative control. Beads were thoroughly washed with binding buffer; bound fractions were eluted with 2% SDS + 0.5 M imidazole/HCl pH 7.5 and analyzed by SDS-PAGE and Coomassie staining.

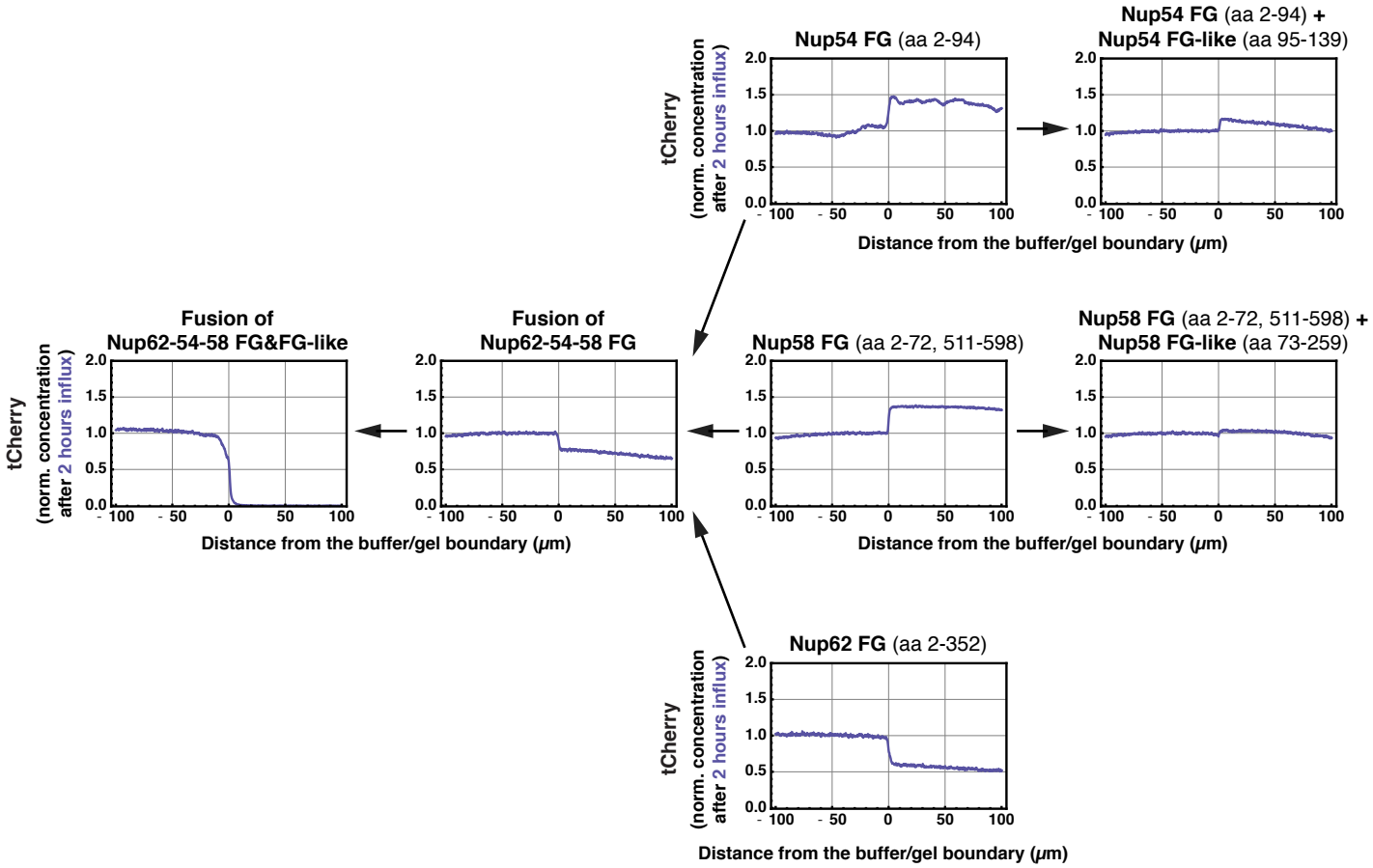

Figure S4 FG-like domains improve selectivity of FG hydrogels. Indicated hydrogels were formed and challenged for 2h with the passive diffusion marker tCherry.

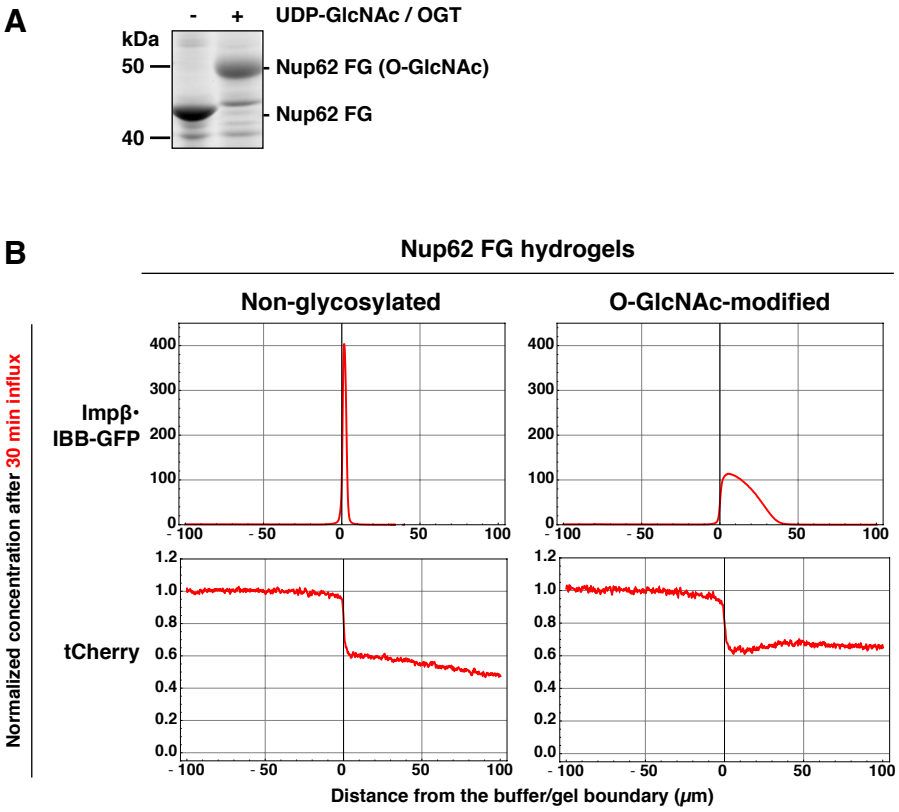

Figure S5 Effects of O-GlcNAc modification on the selectivity of the *Xenopus laevis* Nup62 FG hydrogel. (**A**) Electrophoretic size shift upon enzymatic glycosylation of Nup62 FG domain.

(**B**) Hydrogels derived from either non-glycosylated or O-GlcNAc-modified Nup62 FG domains were probed for 30 min with Impβ•IBB-GFP and tCherry. Note that the Impβ•cargo complex bound efficiently to the surface of the non-glycosylated gel, but diffused only very slowly deeper into the gel. In contrast, the O-GlcNAc-modified gel permitted fast intragel diffusion of this NTR species.

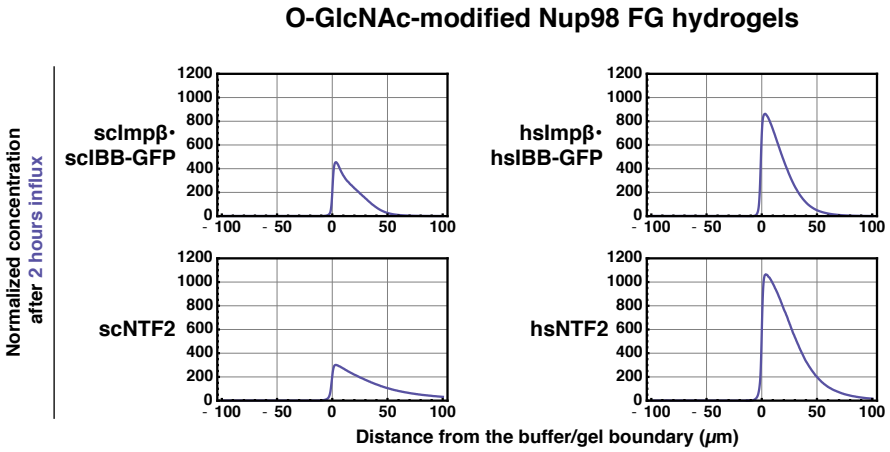

Figure S6 Facilitated translocation of *S. cerevisiae* (sc) and human (hs) NTRs and NTR·cargo complexes into the *Xenopus* O-GlcNAc-modified Nup98 FG hydrogel.

Table SI Bacterial expression vectors used in the study.

Protein name	Plasmid name	Modules in the expressed protein	Used in figures
Nup62 FG	pAL147	His_{14}-Tev-xlNup62$^{2-352}$	1C; 4B; S4; S5
Nup54 FG	pAL224	His_{14}-Tev-xlNup54$^{2-94}$	1C; S4
Nup54 FG&FG-like	pAL146	His_{14}-Tev-xlNup54$^{2-139}$	4B; S4
Nup58 FG	pAL225	His_{14}-Tev-xlNup58$^{2-72, 511-598}$	1C; S4
Nup58 FG&FG-like	pAL157	His_{14}-Tev-xlNup58$^{2-259, 511-598}$	4B; S4
Nup62-54-58 FG	pAL228	His_{14}-Tev-xlNup62$^{2-352}$-xlNup54$^{2-94}$-xlNup58$^{2-72, 511-598}$	4B; S4
Nup62-54-58 FG&FG-like	pAL214	His_{14}-Tev-xlNup62$^{2-352}$-xlNup54$^{2-139}$-xlNup58$^{2-259, 511-598}$	4B; S4
Nup98 FG	pSF739	His_{14}-Tev-xtNup98$^{1-485}$-Cys	1A-C; 5A-D; 6; 7; S6
Nup98 FG (Φ⇒S)	pAL193	His_{14}-Tev-xtNup98$^{1-485}$(Φ⇒S)-Cys	5D
Nup98 FG (NQ⇒S)	pAL186	His_{14}-Tev-xtNup98$^{1-485}$(NQ⇒S)-Cys	1A
Nup153 FG	pSF740	His_{14}-Tev-xtNup153$^{885-1525}$-Cys	1C; 3A,B; S2
Nup153 FG$^{885-1127}$	pAL251	His_{14}-Tev-xtNup153$^{885-1127}$	3A,B
Nup153 FG$^{1128-1525}$	pAL253	His_{14}-Tev-xtNup153$^{1128-1525}$	3A,B
Nup214 FG	pAL291	His_{14}-Tev-xlNup214$^{1615-2033}$-Cys	1C; 2A-D; S2
Nup214 FG-like-1	pAL288	His_{14}-Tev-xlNup214$^{443-690}$-Cys	2A-C; S2
Nup214 FG-like-2	pAL247	His_{14}-Tev-xlNup214$^{1220-1614}$	2A-C; S2
Pom121 FG	pAL300	His_{14}-Tev- xlPom121$^{571-1050}$-Cys	1C
Nup50 FG	pAL294	His_{14}-Tev- xlNup50$^{68-285}$-Cys	1C
CG1 FG	pAL295	His_{14}-Tev- xCG1$^{257-411}$-Cys	1C
Nup358 FG	pAL302	His_{14}-Tev-xtNup358$^{1095-1180, 1307-1345, 1374-1469, 1504-1528, 1558-1595, 1625-1837, 1967-2119, 2315-2431, 2572-2725}$ -Cys	1A,C; 2D
Nup358 FG$^{1625-1837}$	pAL303	His_{14}-Tev-xtNup358$^{1625-1837}$-Cys	
Nup358 FG$^{1967-2119}$	pAL304	His_{14}-Tev-xtNup358$^{1967-2119}$-Cys	
Nup358 FG$^{2315-2431}$	pAL305	His_{14}-Tev-xtNup358$^{2315-2431}$-Cys	
Nup358 FG$^{2572-2725}$	pAL306	His_{14}-Tev-xtNup358$^{2572-2725}$-Cys	
hsNup62 FG	pAL089	His_{14}-Tev-hsNup62$^{2-157}$	S3
hsNup62 FG-like	pAL095	His_{14}-Tev-hsNup62$^{158-311}$	S3
hsNup58 FG	pAL087	His_{14}-Tev-hsNup58$^{2-75, 474-587}$	S3
hsNup58 FG-like	pAL099	His_{14}-Tev-hsNup58$^{76-248, 426-473}$	S3
hsNup54 FG&FG-like	pAL126	His_{14}-Tev-hsNup54$^{1-112}$	S3
Impβ	pKK008	His_{10}-GFP-Tev-hsImportin β	
Transportin	pKK006	His_{10}-GFP-Tev-hsTransportin	
CRM1	pKK003	His_{10}-GFP-Tev-mmCRM1	
Impα	pKK001	His_{10}-GFP-Tev-xlImportin α	
CAS	pKK004	His_{10}-GFP-Tev-hsCAS	
RanGTP	pTG418	His_{10}-ZZ-Tev-hsRanQ69L$^{1-180}$	
OGT	pAL121	His_{14}-Tev-MBP- O-β-N-acetylglucosaminyltransferase	
IBB-GFP	pSF797	His_{14}-Tev-RchI-IBB-mEGFP-Cys	
IBB-mCherry	pSF798	His_{14}-Tev-Rch1-IBB-mCherry-Cys	
IBB-zsGreen	pSF895	His_{14}-Tev-Rch1-IBB-zsGreen	
mCherry	pSF1001	His_{14}-Tev-mCherry	
tCherry	pSF931	His_{14}-Tev-tCherry	