Supplemental materials

List of plasmids constructed in this study

1. **pGBK-T7-C-IPO13**: a yeast two-hybrid vector expressing a C-terminal fragment of imp13 (a.a. 307-963) as a bait in this study
2. **pIPO4-GST**: a bacterial vector expressing a GST tagged full-length human importin 4 (a.a. 1-1081)
3. **pIPO9-GST**: a bacterial vector expressing a GST tagged full-length mouse importin 9 (a.a. 1-1041)
4. **pARX-GST**: a bacterial vector expressing a GST tagged full-length mouse Arx (a.a.1-564)
5. **pARX-His$_6$** : a bacterial vector expressing a His$_6$ tagged full-length mouse Arx (a.a.1-564)
6. **p Δ NLS1-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking NLS1(a.a.82-89)
7. **p Δ NLS1-ARX-1**: a mammalian vector expressing an DsRed-tagged mutant Arx lacking NLS1(a.a.82-89)
8. **p Δ NLS2-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking NLS2 (a.a. 327-388)
9. **p Δ NLS1/ Δ NLS2-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking either NLS1(a.a.82-89) and NLS2(a.a.327-388)
10. **pNLS2-1-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking BC1 domain (a.a.327 to 334)
11. **pNLS2-2-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking BC2 domain (a.a. 381 to 388)
12. **pNLS2-3-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking a fragment from a.a. 327 to 380
13. **pNLS2-4-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking a fragment from a.a. 335 to 388
14. **pNLS2-5-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking a fragment from a.a. 335 to 380
15. **pNLS2-6-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking BC1 domain and BC2 domain
16. **p Δ NLS1-1-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx lacking a fragment from a.a. 82 to 89 and a fragment from a.a.327 to 334
17. **p Δ NLS1-2-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with deletions of a.a. 82-89 and a.a.381-388
18. **p Δ NLS1-3-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with deletions of a.a.82-89 and a.a.327-380
19. **p Δ NLS1-4-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with deletions of a.a.82-89 and a.a.335-388
20. **p Δ NLS1-5-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with deletions of a.a.82-89 and a.a.335-380
21. **p Δ NLS1-6-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with deletions of a.a.82-89, a.a.327-334 and a.a.381-388
22. **pK327A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of lysine 327 by an alanine residue
23. **pR328A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of arginine 328 by an alanine residue
24. **pK329A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of lysine 329 by an alanine residue
25. **pR331A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of arginine 331 by an alanine residue
26. **pR332A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of arginine 332 by an alanine residue
27. **pR334A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of arginine 334 by an alanine residue
28. **pR381A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of arginine 381 by an alanine residue
29. **pR382A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of arginine 382 by an alanine residue
30. **pK384A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of lysine 384 by an alanine residue
31. **pR386A-ARX**: a mammalian vector expressing an EGFP-tagged mutant Arx with a substitution of arginine 386 by an alanine residue
32. **pK327A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution of lysine 327 by an alanine residue
33. **pR328A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution

of arginine 328 by an alanine residue

34. **pK329A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution of lysine 329 by an alanine residue
35. **pR331A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution of arginine 331 by an alanine residue
36. **pR334A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution of arginine 334 by an alanine residue
37. **pR381A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution of arginine 381 by an alanine residue
38. **pR382A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution of arginine 382 by an alanine residue
39. **pK384A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution of lysine 334 by an alanine residue
40. **pR386A/△NLS1**: a mammalian vector expressing an EGFP-tagged △NLS1-Arx with a substitution of arginine 386 by an alanine residue
41. **pGEX4T-2-VDR-EGFP**: a bacterial vector expressing a GST/EGFP-tagged truncated VDR (a.a. 4-232)

Primers used in this study

Plasmid		5’ primer oligo	3’ primer oligo	Restriction enzyme sites
pIPO4-GST pGEX4T-2-VDR-EGFP		AAA gtcgac AATGGAGTCAGCCGGGCT CGCggatccATGGCGGCCAGCACTTCCCT	AAA gcggccgc CTAGGAGAGGCCCAGT CCCaagcttGGTCAGCCAGGTGGGGCAGCA	Sal Ⅰ/Not Ⅰ BamHI /HindIII
pIPO9-GST		TAT gtcgac AGAGCTCGGTACCGCAGCA	ATT gcggccgc TTAGATGCCGATAGTCT	Sal Ⅰ/Not Ⅰ
Wild type Arx				
pARX-GST（a.a. 1-564）		CGCggatccAGCAATCAGTACCAG	CCGgaattcTGCACACCTCCTTG	BamH Ⅰ/EcoR Ⅰ
pARX-His_6 (a.a.1-564)		CCGgaattcATGAGCAATCAGTAC	CCGctcgagTTTAGCACACCTC	EcoR Ⅰ/Xho Ⅰ
Deletion mutants of Arx				
pA-NLS2-EGFP（a.a. 327-392）		CCGgaattcTAAGCGCAAACAGAGG	AAAggatccGCCAGCCTTCTCCCG	EcoR Ⅰ/BamH Ⅰ
pN-ARX-1 (a.a. 1- 326)		CGCctcgagCTATGAGCAATCAGTACC	AAAgaattcGACAGCAGCCCCTCCTC	Xho Ⅰ/ EcoR Ⅰ
NLS mutants of Arx				
pΔNLS1-ARX (△82-89) pΔNLS1-ARX-1 (△82-89)	N-terminal	CGCctcgagCTATGAGCAATCAGTACC	CCCaagcttGCAGGTGCAGCTCAGC	Xho Ⅰ/HindⅢ
	C-terminal	CCCaagcttTTGGCCCGGGCGGGGGC	CGCggatccTTAGCACACCTCCTTG	HindⅢ/ BamH Ⅰ
pΔNLS2-ARX(△327-388)	N-terminal	CGCctcgagCTATGAGCAATCAGTACC	AAAgaattcCAGCAGCCCCTCCTC	Xho Ⅰ/ EcoR Ⅰ
	C-terminal	CCGgaattcGCGCAGACCCACCC	CGCggatccTTAGCACACCTCCTTG	EcoR Ⅰ/BamH Ⅰ
pΔNLS1/ΔNLS2-ARX (△82-89 & △327-388)	N-terminal	CGCctcgagCTATGAGCAATCAGTACC	CCCaagcttGCAGGTGCAGCTCAGC	Xho Ⅰ/HindⅢ
	C-terminal	CCCaagcttTTGGCCCGGGCGGGGGC	CGCggatccTTAGCACACCTCCTTG	HindⅢ/ BamH Ⅰ

pNLS2-1-ARX (△327-334)		CCGgaattcTACCACGTTCACCAGT	CGCggatccTTAGCACACCTCCTTG	EcoR Ⅰ/BamH Ⅰ
pNLS2-2-ARX (△381-388)		CGCctcgagCTATGAGCAATCAGTACC	CCGgaattcGCGTTCTGGAACCAC	Xho Ⅰ/ EcoR Ⅰ
pNLS2-3-ARX (△327-380)		CCGgaattcTCGTCGGGCCAAGTG	CGCggatccTTAGCACACCTCCTTG	EcoR Ⅰ/BamH Ⅰ
pNLS2-4-ARX (△335-388)		CGCctcgagCTATGAGCAATCAGTACC	AAAgaattcGCGCGGTAGCGCCTC	Xho Ⅰ/ EcoR Ⅰ
pNLS2-5-ARX (△335-380)	N-terminal	CGCctcgagCTATGAGCAATCAGTACC	AAAgaattcGCGCGGTAGCGCCTC	Xho Ⅰ/ EcoR Ⅰ
	C-terminal	CCGgaattcTCGTCGGGCCAAGTG	CGCggatccTTAGCACACCTCCTTG	EcoR Ⅰ/BamH Ⅰ
pNLS2-6-ARX (△327-334 &△381-388)	N-terminal	CGCctcgagCTATGAGCAATCAGTACC	CGGggtaccGTTCTGGAACCAC	Xho Ⅰ/KpnI
	C-terminal	CGG ggtacc GCACAGACCCAC	CGCggatccTTAGCACACCTCCTTG	KpnI/ BamH Ⅰ
pΔNLS1-1-ARX (△82-89 &△327-334)		CCGgaattcTACCACGTTCACCAGT	CGCggatccTTAGCACACCTCCTTG	EcoR Ⅰ/BamH Ⅰ
pΔNLS1-2-ARX (△82-89 &△381-388)		CGCctcgagCTATGAGCAATCAGTACC	CCGgaattcGCGTTCTGGAACCAC	Xho Ⅰ/ EcoR Ⅰ
pΔNLS1-3-ARX (△82-89 & △327-380)		CCGgaattcTCGTCGGGCCAAGTG	CGCggatccTTAGCACACCTCCTTG	EcoR Ⅰ/BamH Ⅰ
pΔNLS1-4-ARX (△82-89 & △335-388)		CGCctcgagCTATGAGCAATCAGTACC	AAAgaattcGCGCGGTAGCGCCTC	Xho Ⅰ/ EcoR Ⅰ
pΔNLS1-5-ARX (△82-89 & △335-380)	N-terminal	CGCctcgagCTATGAGCAATCAGTACC	AAAgaattcGCGCGGTAGCGCCTC	Xho Ⅰ/ EcoR Ⅰ
	C-terminal	CCGgaattcTCGTCGGGCCAAGTG	CGCggatccTTAGCACACCTCCTTG	EcoR Ⅰ/BamH Ⅰ
pΔNLS1-6-ARX (△ 82-89 & △ 327-334 & △381-388)	N-terminal	CGCctcgagCTATGAGCAATCAGTACC	CGGggtaccGTTCTGGAACCAC	Xho Ⅰ/KpnI
	C-terminal	CGG ggtacc GCACAGACCCAC	CGCggatccTTAGCACACCTCCTTG	KpnI/ BamH Ⅰ
Point-mutations in Arx, ΔNLS1-Arx and C-Arx				
K327A-ARX, K327A/ΔNLS1 R328A-ARX, R328A/△NLS1		AGGAGGGGCTGCTGGCGCGCAA TGCTGAAGGCCAAACAGAGGCG	TTGCGCGCCAGCAGCCCCTCCT CGCCTCTGTTTGGCCTTCAGCA	

K329A-ARX, K329A/ΔNLS1	TGAAGCGCGCGCAGAGGCGCTA	TAGCGCCTCTGCGCGCGCTTCA	
R331A-ARX, R331A/ΔNLS1	CGCAAACAGGCGCGCTACCGCA	TGCGGTAGCGCGCCTGTTTGCG	
R332A-ARX, R332A/ΔNLS1	AAACAGAGGGCCTACCGCACCA	TGGTGCGGTAGGCCCTCTGTTT	
R334A-ARX, R334A/ΔNLS1	AGAGGCGCTACGCCACCACGTTCACCAGT	ACTGGTGAACGTGGTGGCGTAGCGCCTCT	
R381A-ARX, R381A/ΔNLS1	GGTTCCAGAACGCACGGGCCAA	TTGGCCCGTGCGTTCTGGAACC	
R382A-ARX, R382A/ΔNLS1	TCCAGAACCGTGCGGCCAAGTG	CACTTGGCCGCACGGTTCTGGA	
K384A-ARX, K384A/ΔNLS1	GTCGGGCCGCGTGGCGCAAG	CTTGCGCCACGCGGCCCGAC	
R386A-ARX, R386A/ΔNLS1	CAAGTGGGCGAAGCGGGAGA	TCTCCCGCTTCGCCCACTTG	

Figure and its legend

Imp4 is able to mediate the import of VDR into nuclei of digitonin-permeabilized cells. HeLa cells were permeabilized with digitonin, incubated for 30 min with the import mixtures at 30°C, washed and fixed. As shown, EGFP-Arx-His_6 is imported into the nuclei in the presence of impβ1 (**b**) but not imp4 (**e**). EGFP-Arx-His_6 was not found in nuclei in the absence of impβ1 (**a**). By contrast, the GST/EGFP-tagged VDR fragment (a.a. 4-232), a known cargo of imp4 (50), was imported into the nuclei of permeabilized cells in the presence of imp4 (**g**). This recombinant protein was not found in the nuclei when imp4 was absent (**f**). At 4 °C, neither impβ1 nor imp4 could transport their cargo into the nuclei (**c**, **h**). A rim signal that indicates binding to the nuclear pore complexes is visible in both cases (**c, h**). "-" means without importin, "+" means wit importin.