Supporting Information

Macrocyclic peptides inhibitors for the protein-protein interaction of

protein 24 and Kapherryopherin alpha 5

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Figure S1 Selection progression of (A) ^DY-library or (B) ^LY-library. cDNAs eluted from eVP24 immobilized bead was indicated by black bars and those recovered from the bead was indicated by light grey bars. The recovery rate was defined as the ratio of recovered cDNAs from either eVP24-immobilized magnetic beads (black) or magnetic beads-only (grey) to the amount of the input mRNA/cDNA-peptide fusion.



Figure S2 Binding kinetics of macrocyclic peptides and GST-KPNA5. SPR sensorgram of (A) eVpeD1, (B) eVpeD2, (C) eVpeL1, (D) eVpeL2, and (E) GST-KPNA5. The observed data (black) was superimposed to theoretical fitting curve (red) based on a 1 : 1 binding mode.



Figure S3 Titration of eVP24 to 5 nM macrocyclic peptide, (A) eVpeD1, (B) eVpeD2, and (C) eVpeL2. All data points were obtained in triplicate. See more details in the experimental section.



Figure S4 AlphaLISA-based binding assay between KPNA5-immobilized donor beads (KPNA5-dBs) and eVP24-immobilized acceptor beads (eVP24-aBs). (A) Observed intensities of the chemiluminescence signal upon the excitation of KPNA5dBs to eVP24-aBs. The titration was performed at equal molar concentration of KPNA5-dBs and eVP24-aBs. (B) Assay validation by using untagged KPNA5 to display the KPNA5-dBs from eVP24-aBs. Data set is performed in duplicate. IC₅₀ is calculated using four-parameter dose-response curve (Prism). Values are expressed as average \pm standard deviation. Standard deviation in LogIC₅₀ is less than 10%.

[ng] 2000				162			p1-flu_4-3 0:H22 MS Raw
1000 500	eVpeD1-F Cal Obs	: 2645.260 : 2644.962		2644.6			
0 1250 1000 750 250	eVpeD2-F Cal Obs	: 2586.190 : 2585.877					p2-flu_4-3 0 H23 MS Raw
териона 10000 1000 100000 100000 100000 100000 10000 10000 10000 10000 100	eVpeL2-F Cal Obs	: 2439.136 : 2438.883	antin anta dal anciente de la constitución de la constitución de la constitución de la constitución de la const				p4-flu_4-3 0.H24 MS Raw
		: 2087.072 : 2087.289	-214327 -214327				pep1_4-3 0.H18 MS Raw
	•	: 1880.948 : 1881.213	-1 503 243 - 1581 213				pep2_4-3 0:H19 MS Raw
	•	l: 2028.003 :: 2028.273	2050.303 - 2028.273				pep3_4-3 0.H20 MS Raw
0 5000 84000 2000 1000	•	l: 1880.793 :: 1881.082	-1911/1				pep5_4-3 0.H21 MS Raw
0 -	1500	· · · · · · · · · · · · · · · · · · ·	2000	2500	3000	3500	

Figure S5 MALDI-TOF spectra and mass values of the synthetic peptides after HPLC purification. See Table 2 and 3 for the peptide sequences. All peptides were analyzed by the reflective positive mode.