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%.

[mg] 2000 1000 500	eVpeD1-F	Cal: 2645.260 Obs: 2644.962		244.962	p1-8u_4-3 0 H22 MS Raw
ng 1250 suaju 750 500 250	eVpeD2-F	Cal: 2586.190 Obs: 2585.877		2/8 9997	p24u_4-3 0 H23 MS Raw
0 3000 2000 1000	eVpeL2-F	Cal: 2439.136 Obs: 2438.883			p4 flu_4-3 0 H24 MS Raw
(ne) suepui 3000 1000	eVpeD1	Cal: 2087.072 Obs: 2087.289			pep1_4-3 0.H18 MS Raw
nel sugui	eVpeL2	Cal: 1880.948 Obs: 1881.213	-1903.243 -1891.213		pep2_4-3 0.H19 MS Raw
1000 1000 1000	eVpeD2	Cal: 2028.003 Obs: 2028.273	2050.303		pep3_4-3 0 H20 MS Raw
0 ne 5000 suapu 3000 2000 1000 0	eVpeL1	Cal: 1880.793 Obs: 1881.082			 pep5_4-3 0 H21 MS Raw

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.