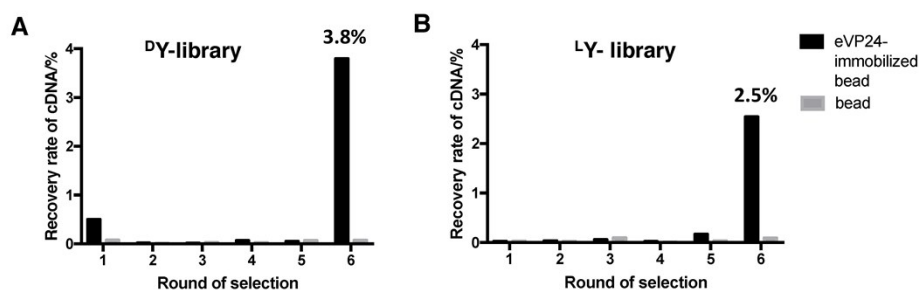


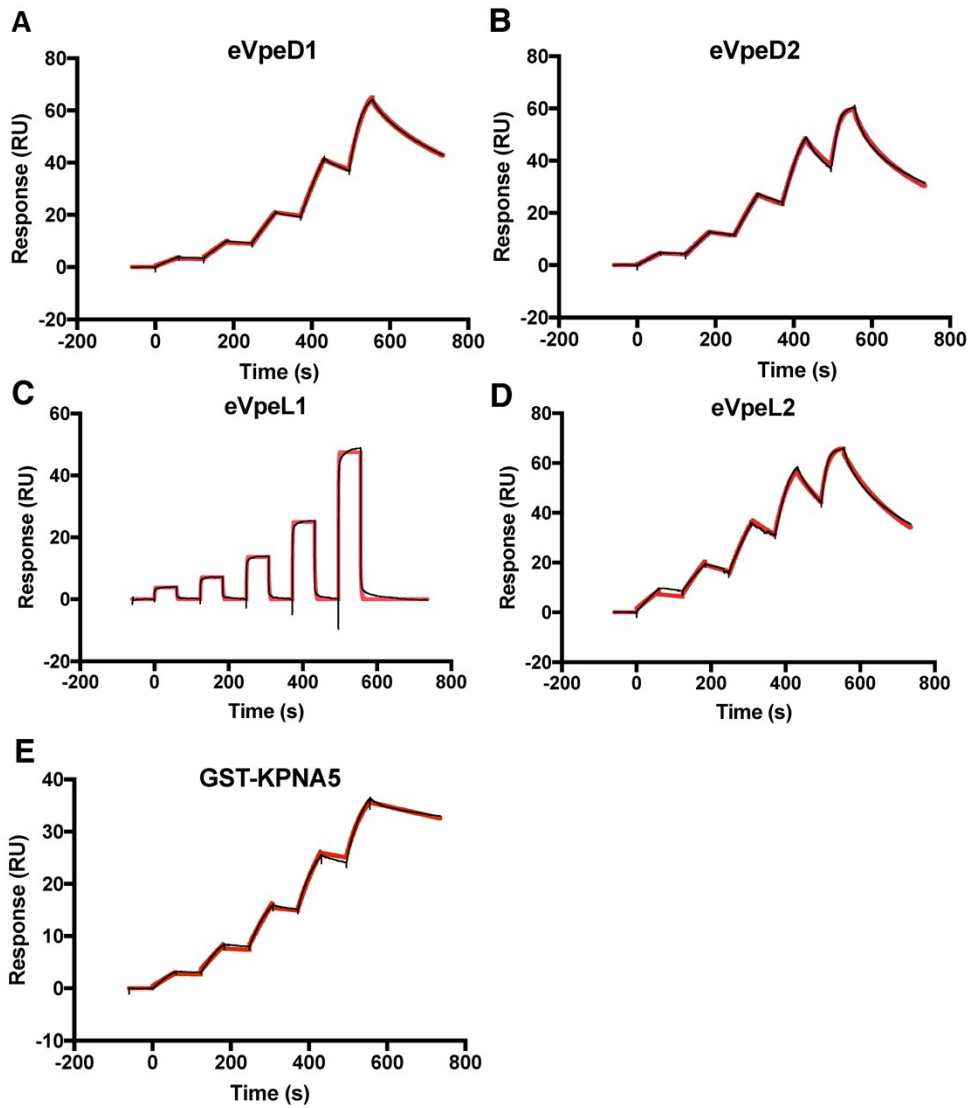
## Supporting Information

### Macrocyclic peptides inhibitors for the protein-protein interaction of protein 24 and Kapherryopherin alpha 5

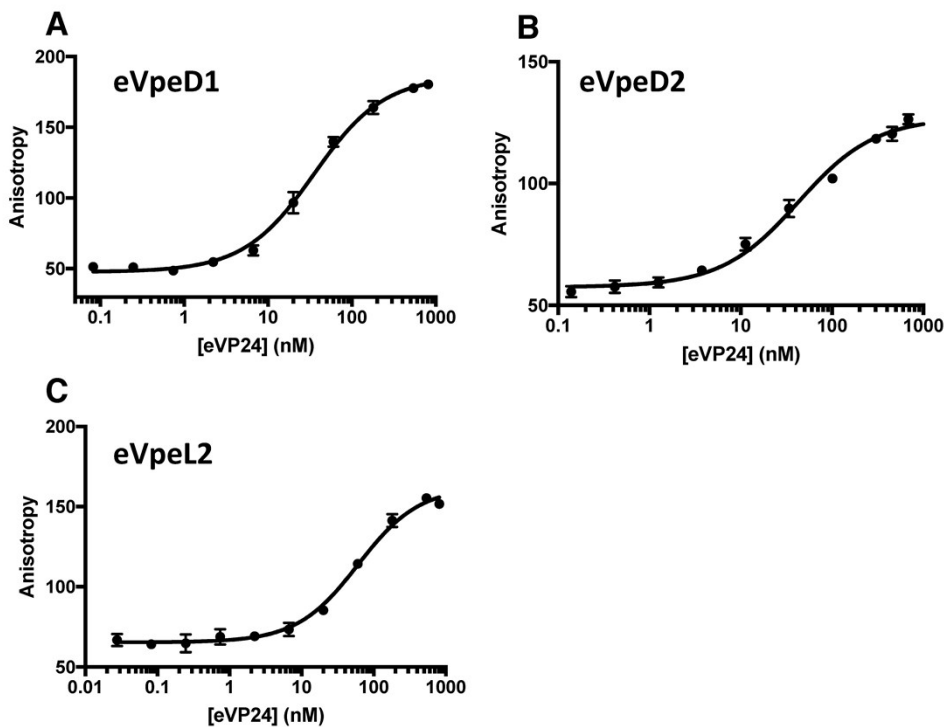
Xiao Song<sup>1</sup>, Lu-yi Lu, Toby Passioura and Hiroaki Suga



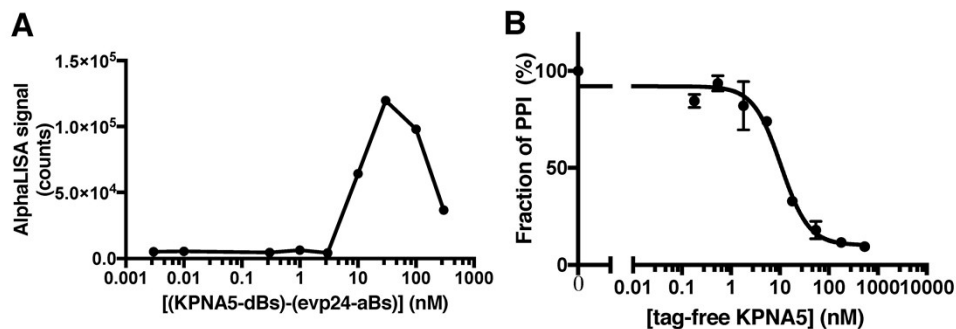
**Figure S1** Selection progression of (A) <sup>D</sup>Y-library or (B) <sup>L</sup>Y-library. cDNAs eluted from eVP24 immobilized bead was indicated by black bars and those recovered from the bead was indicated by light grey bars. The recovery rate was defined as the ratio of recovered cDNAs from either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



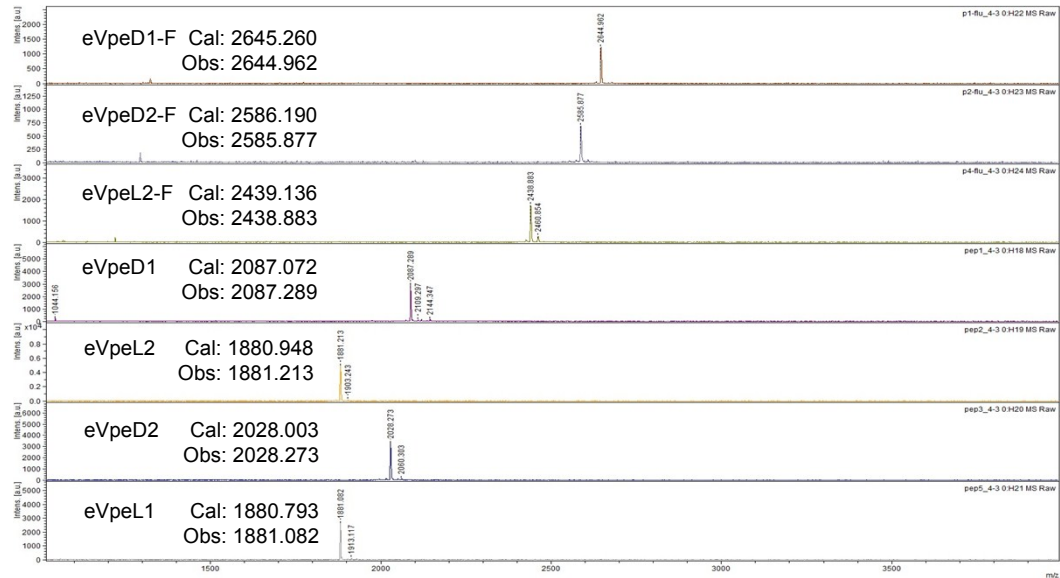
**Figure S2** Binding kinetics of macrocyclic peptides and GST-KPNA5. SPR sensorgram of (A) eVpeD1, (B) eVpeD2, (C) eVpeL1, (D) eVpeL2, and (E) GST-KPNA5. The observed data (black) was superimposed to theoretical fitting curve (red) based on a 1 : 1 binding mode.



**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 KPNA5-dBs 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.  $IC_{50}$  is calculated using four-parameter dose-response curve (Prism). Values are expressed as average  $\pm$  standard deviation. Standard deviation in  $LogIC_{50}$  is less than 10%.



**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.