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Supplementary Materials

Cath-KP, a novel peptide derived from frog skin, prevents oxidative stress damage in a Parkinson's disease model

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SUPPLEMENTARY MATERIALS AND METHODS

Antibody competition assay

Cath-KP at different concentrations was mixed with human integrin α_1 and β_1 488-conjugated antibodies (1: 200) for 10 min. The mixture was then incubated with HUVECs (1×10^6 cells/well) in the logarithmic growth phase for an additional 10 min at room temperature before analysis using FACScan flow cytometry (Becton Dickinson Company, Bedford, MA, USA). All experiments were performed in triplicate.

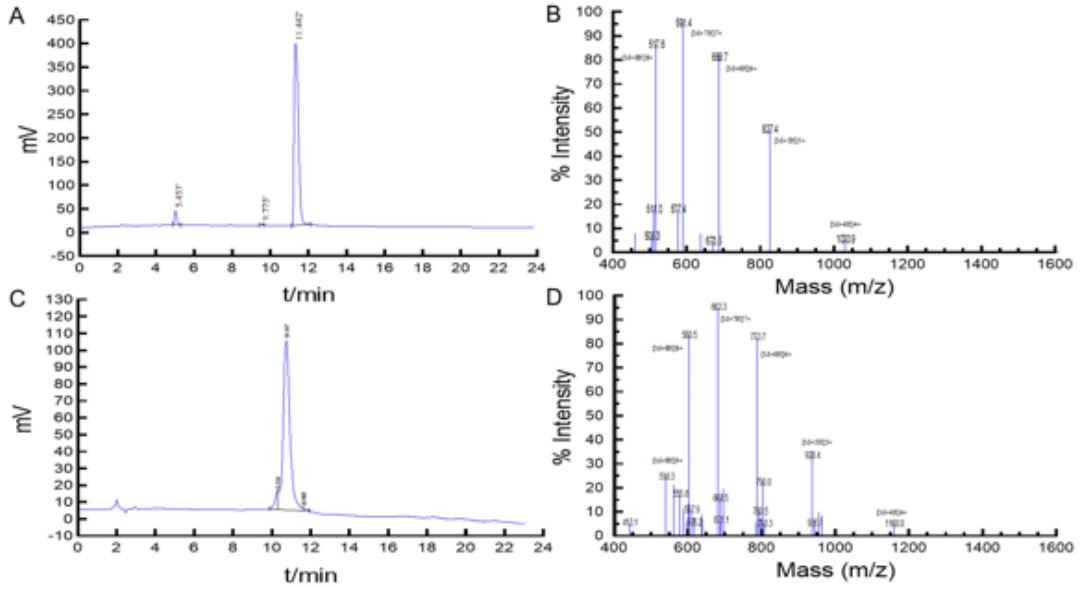
Molecular docking

The crystal structure of the I-domain of integrin $\alpha_1\beta_1$ (PDB ID: 1QCY) was docked to the modeled Cath-KP structure using the ClusPro 2.0 online server (https://cluspro.bu.edu/login.php) (Desta et al., 2020; Kozakov et al., 2017). The best $\alpha_1\beta_1$-Cath-KP complex was selected using the Dock score together with Root mean square deviation(RMSD) analysis and R-DOCK optimization. Chemical bonds between two molecules were detected with LigPlot$^+$ software. The structure was visualized with PyMOL (v2.5.2) without any refinement.

REFERENCES

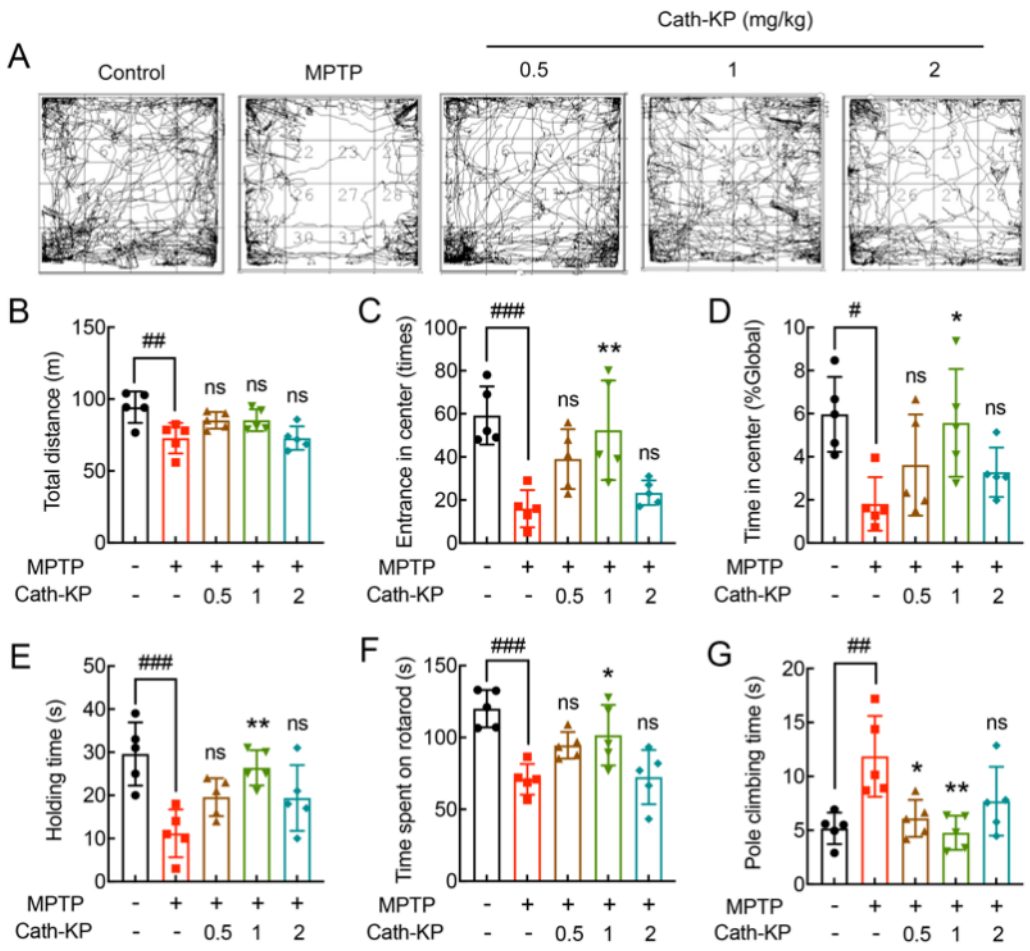
Desta IT, Porter KA, Xia B, et al. 2020. Performance and its limits in rigid body protein-protein docking. *Structure*, **28**(9): 1071-1081.e3.

Kozakov D, Hall DR, Xia B, et al. 2017. The ClusPro web server for protein-protein docking. *Nature Protocols*, **12**(2): 255-278.



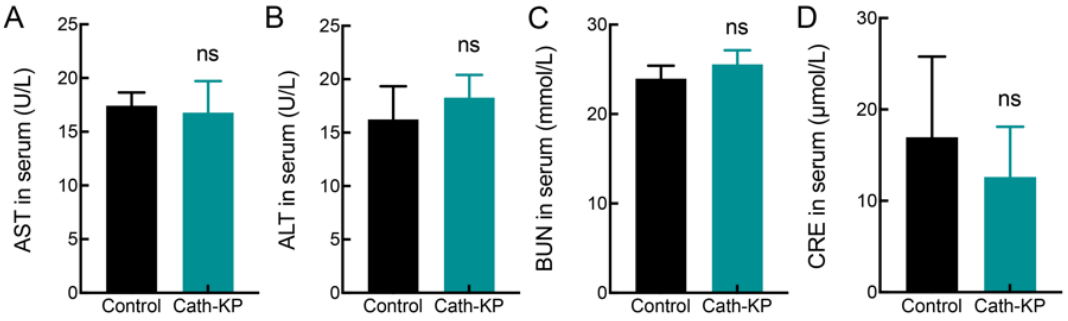
Supplementary Figure S1 Characterization of synthesized Cath-KP and FITC-labeled Cath-KP

A, B: RP-HPLC and MALDI-TOF-MS of Cath-KP. C, D: RP-HPLC and MALDI-TOF-MS of FITC-labeled Cath-KP.



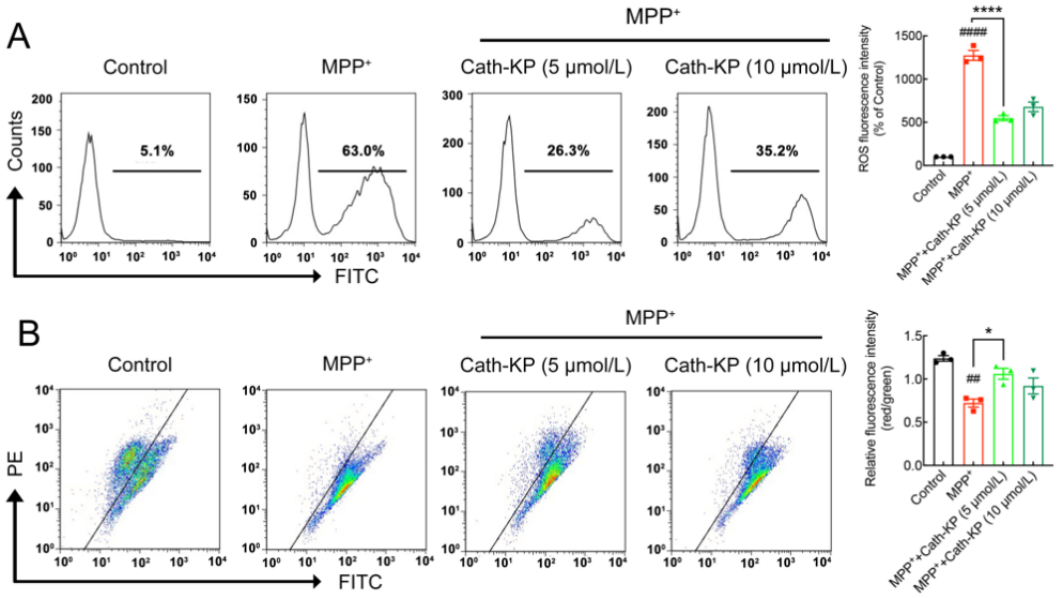
Supplementary Figure S2 Optimal therapeutic concentration of Cath-KP for animal experiments

A: Mouse movement trajectory diagram of open field experiment (10 min). Black box represents open field area, black curve represents movement trajectory, and grouping is shown above the figure. B–D: Analysis of total distance moved by mice, number of times mice entered central area, and movement time of mice in the open field experiment. E: Analysis of holding time by mice in the hanging test. (F) Analysis of time spent by mice on rotating rod in the rotarod test. (G) Analysis of time spent by mice climbing in the pole-climbing test. Data are presented as means±SEM (n=5). One-way ANOVA was used for data analysis, ns: no significant difference. $^{\#}$: P<0.05, $^{\#\#}$: P<0.01, $^{\#\#\#}$: P<0.001, and $^{\#\#\#\#}$: P<0.0001 vs. Control. *: P<0.05, **: P<0.01, ***: P<0.001, and ****: P<0.0001 vs. PD models.



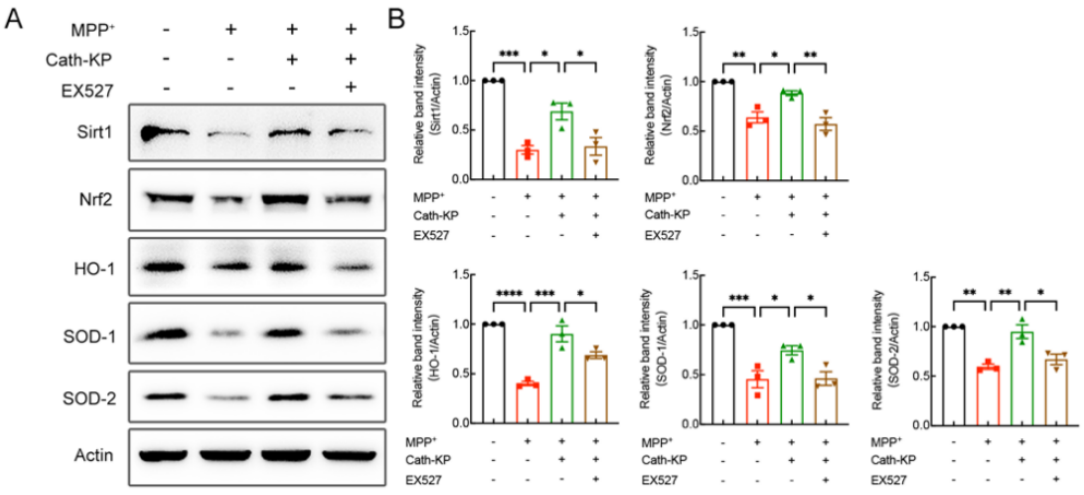
Supplementary Figure S3 Toxicity of Cath-KP *in vivo*

C57 mice were intraperitoneally injected with Cath-KP (1 mg/kg) or PBS and sacrificed for biochemical index detection at 24 h post-injection. A–D: Serum levels of AST, ALT, BUN, and CRE, respectively. Data are represented as means±SEM (n=3) with ordinary one-way ANOVA. ns, not significant.



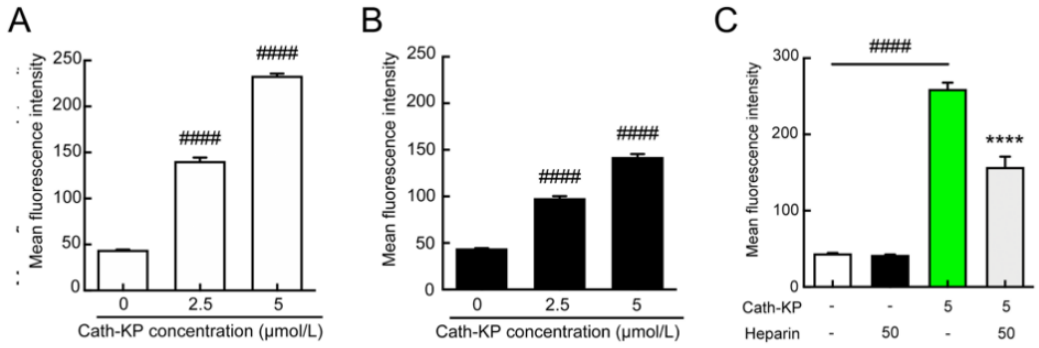
Supplementary Figure S4 Oxidative stress attenuated by Cath-KP *in vitro* PD model

Representative flow cytometry analysis of ROS generation (A) and mitochondrial membrane potential ($\Delta\psi m$) (B) upon MPP^+ (1 000 μmol/L) and Cath-KP (5, 10 μmol/L)-treated MN9D cells. Data are represented as means±SEM with ordinary one-way ANOVA (n=3). $^{\#\#}$: P<0.01 and $^{\#\#\#\#}$: P<0.0001 vs. Control. *: P<0.05 and ****: P<0.0001 vs. PD models.



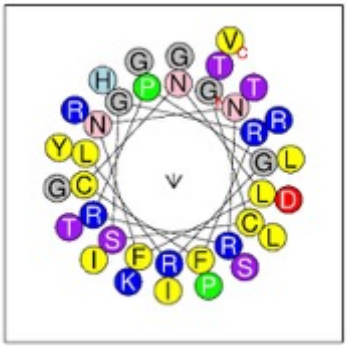
Supplementary Figure S5 Effects of Cath-KP on Sirt1/Nrf2/ARE-mediated pathway in PD model *in vitro*

Representative blots (A) and quantification (B) of western blot analysis for detection of protein expression of Sirt1, Nrf2, SOD-1, and SOD-2 in MN9D cells treated with MPP^+, Cath-KP, and EX527. Data are represented as means±SEM with ordinary one-way ANOVA (n=3). *: P<0.05, **: P<0.01, ***: P<0.001, and ****: P<0.0001.

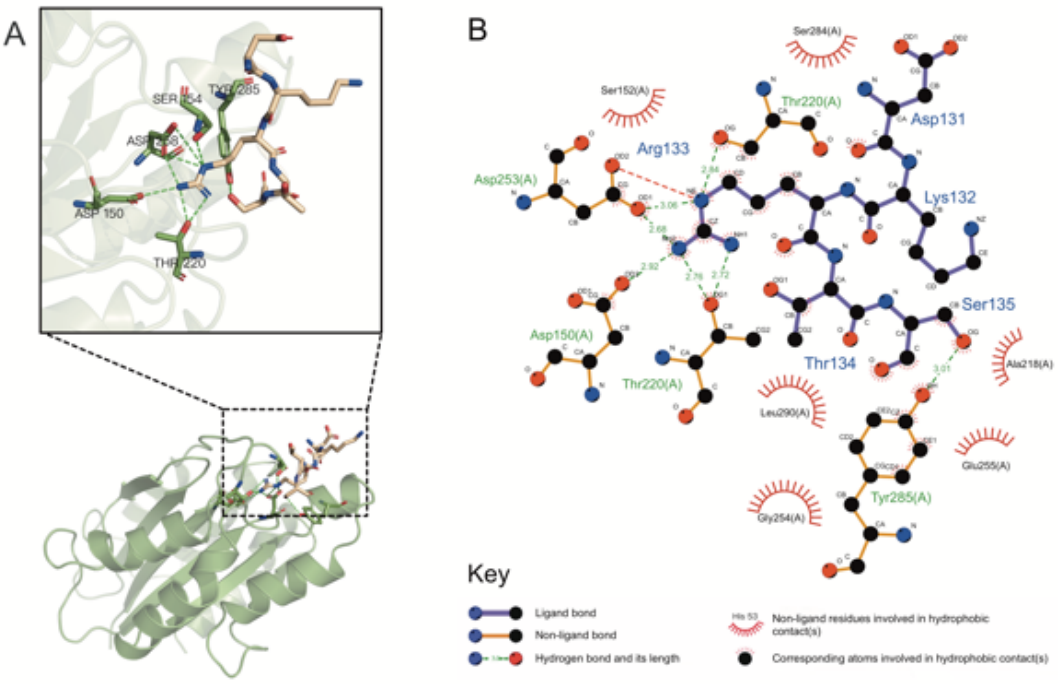


Supplementary Figure S6 Analysis of fluorescence intensity of FITC-Cath-KP in MN9D cells under different conditions

Cath-KP (2.5 and 5 μmol/L) was incubated with MN9D cells for 3 h at 37 °C (A), 4 °C (B), or in the presence of 50 μg/mL heparin (C). Fluorescence intensity was measured by flow cytometry. Data are represented as means±SEM with ordinary one-way ANOVA (n=3). $^{\#\#\#\#}$: P<0.0001 vs. Control. ***: P<0.001 vs. FITC-Cath-KP group.

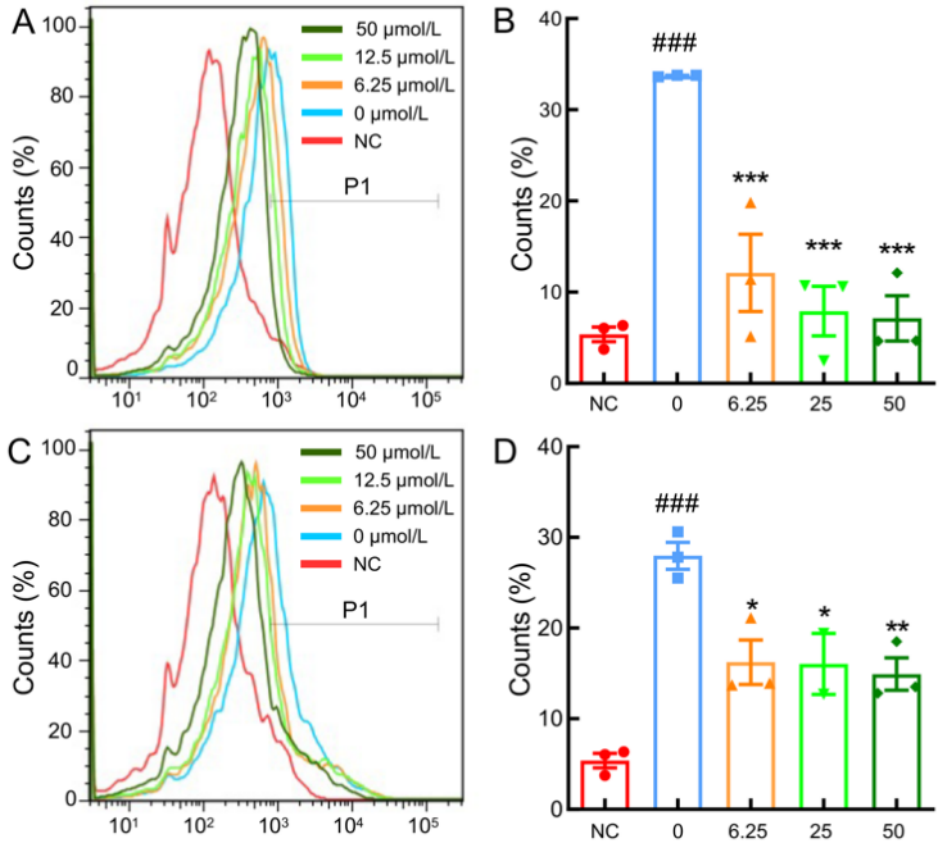


Supplementary Figure S7 Helix-wheel diagram of full Cath-KP



Supplementary Figure S8 Molecular docking of Cath-KP and integrin $\alpha_1\beta_1$

A: Cartoon representation of molecular docking of Cath-KP and integrin $\alpha_1\beta_1$. B: Hydrogen bond formation between Cath-KP and integrin $\alpha_1\beta_1$ visualized by LigPlot$^+$.



Supplementary Figure S9 Effects of Cath-KP on binding of integrin α_1 and β_1 antibodies to HUVECs

Cath-KP at indicated concentrations was incubated with HUVECs for 30 min at 37 °C before integrin α_1 and β_1 antibodies were added for another 30 min incubation. Intensity was then examined by flow cytometry. A, B: Typical images and quantification analysis of fluorescence intensity of α_1 antibody. C, D: Typical images and quantification analysis of fluorescence intensity of β_1 antibody. Data are represented as means±SEM with Student's *t* test and ordinary one-way ANOVA (n=3). $^{###}$: P<0.001 vs. negative control (NC). *: P<0.05, **: P<0.01, and ***: P<0.001 vs. only $\alpha_1\beta_1$ antibody group without Cath-KP.

Supplementary Table S1 Secondary structural components of Cath-KP in different environments.

Condition [a]	Helix [b] (%)	β-sheet [b] (%)	Turn [b] (%)	Random [b] (%)	Total [b] (%)
0 mmol/L SDS	5.40%	31.80%	21.90%	38.30%	97.30%
30 mmol/L SDS	7.90%	41.30%	20.70%	32.30%	102.10%
60 mmol/L SDS	7.70%	39.60%	20.90%	33.00%	101.20%
90 mmol/L SDS	7.80%	40.10%	20.50%	32.50%	100.90%
100 mmol/L NaCl	8.80%	37.30%	21.20%	34.90%	102.30%
200 mmol/L NaCl	11.30%	37.10%	20.50%	32.50%	101.40%
400 mmol/L NaCl	12.70%	38.20%	20.50%	30.80%	102.10%
20℃	7.90%	41.30%	20.70%	32.30%	102.10%
37℃	7.70%	39.60%	20.90%	33.00%	101.20%
50℃	8.20%	42.50%	19.70%	31.20%	101.60%
70℃	8.70%	40.40%	19.90%	31.30%	100.30%
90℃	9.00%	39.90%	20.00%	31.30%	100.20%

[a] 50 μmol/L Cath-KP was treated with different concentrations of SDS, salt solutions and different temperatures; [b] The average value of three scans using CDNN software to deconvolute CD spectra into fractional contents.