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Selective Targeting Through Chemical Remodeling of Protein Neosurfaces

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Abstract

Achieving selective targeting within homologous enzyme families remains a challenge because highly conserved sites limit molecular discrimination. Meanwhile, non-conserved regions are often intrinsically disordered and lack pre-organized binding interfaces, leaving few opportunities for selective recognition. Here we report a dynamic neosurface engineering strategy that shifts molecular recognition beyond pre-existing pockets to chemically remodeled, adaptive recognition interfaces induced on disordered protein surfaces. A pathological target aldose reductase (ALR2) was chosen as a representative model whereas its highly homologue aldehyde reductase (ALR1) serves essential physiological functions. A hierarchically programmed peptide supramolecular binding-inhibition platform, KK, was developed through molecular design, supramolecular programming, and high-throughput screening. Through cooperative chemical and geometric matching with the intrinsically disordered outer funnel region of ALR2, KK adaptively remodels the local protein and induces the formation of a new surface. This process generates a previously absent but dynamically sustained recognition interface, termed the *NeoFunnel Interface*, which establishes complementarity for selective, high-affinity ALR2 targeting while avoiding the highly conserved catalytic hotspot shared with ALR1. Building on this, an intelligent nanodrug delivery system (KK^{EPA}) was further constructed. KK^{EPA} enables sequential targeting involving cellular internalization, lysosomal escape, and ALR2-directed adaptive drug release, thereby guiding drug to the catalytic region mediated by *NeoFunnel Interface*. This design enhances functional selectivity toward ALR2 while preserving ALR1 physiological detoxification activity. More broadly, this establishes a generalizable framework for transforming poorly druggable, pocket-deficient protein surfaces into selective therapeutic interfaces, offering a new route to overcome the long-standing selectivity barrier in homologous enzyme systems and opening new opportunities for safer precision therapeutics.

Main

Homologous enzyme families present a persistent and fundamental challenge in molecular targeting¹. Despite extensive efforts in structure-guided ligand design, achieving high selectivity among closely related enzyme homologues remains difficult because catalytic sites are often highly conserved in sequence, structure, and physicochemical properties². As a result, therapeutic targeting of disease-associated homologues frequently leads to off-target inhibition of physiologically essential family members, limiting both efficacy and safety³. This intrinsic selectivity barrier remains unresolved because traditional drug discovery largely relies on ligand binding to pre-existing active pockets. This limitation reveals the need to move beyond active-site targeting toward dynamic surface selectivity⁴. However, many disease-relevant enzymes, especially intrinsically disordered regions, lack defined binding cavities and are considered poorly druggable⁵. Emerging evidence suggests that molecular recognition can be established through dynamic molecular adaptation and sustained, tightly regulated engagement that remodels protein surface architecture and reshapes local conformational landscapes, generating recognition interfaces that are absent in the unbound state. These dynamic recognition interfaces, which termed neosurfaces, provide a potential route to overcome the selectivity constraints imposed by highly conserved active sites in homologous enzyme systems⁶.

As a representative case, aldose reductase (ALR2) and aldehyde reductase (ALR1) share ~65% sequence identity and possess nearly indistinguishable active-site architectures (Scheme 1a)⁷. ALR2 contributes to diabetic retinopathy (DR) by catalyzing glucose (Glu) reduction in the polyol pathway, leading to sorbitol accumulation and retinal cellular stress⁸. Thus, ALR2 inhibition is considered a promising DR therapeutic strategy⁹. However, its homologue ALR1 performs essential physiological detoxification functions, and non-selective inhibition may cause undesirable side effects¹⁰. Currently, Epalrestat (EPA) is the only clinically approved aldose reductase inhibitor¹¹. However, the clinical translation of most aldose reductase inhibitors (ARIs) candidates has been limited by hepatotoxicity and nephrotoxicity, largely attributed to off-target inhibition of ALR1¹². Structurally, ALR2 is composed of 316 amino acids and contains a funnel-shaped active pocket that progressively narrows toward the catalytic core¹³. At the base of this funnel lies a highly conserved homology domain (~167 Å² accessible surface area) that serves as the primary ARI-binding hotspot and severely limits opportunities for selective inhibitor optimization¹⁴. In contrast, the external funnel region constitutes a non-homologous domain composed of sequence-

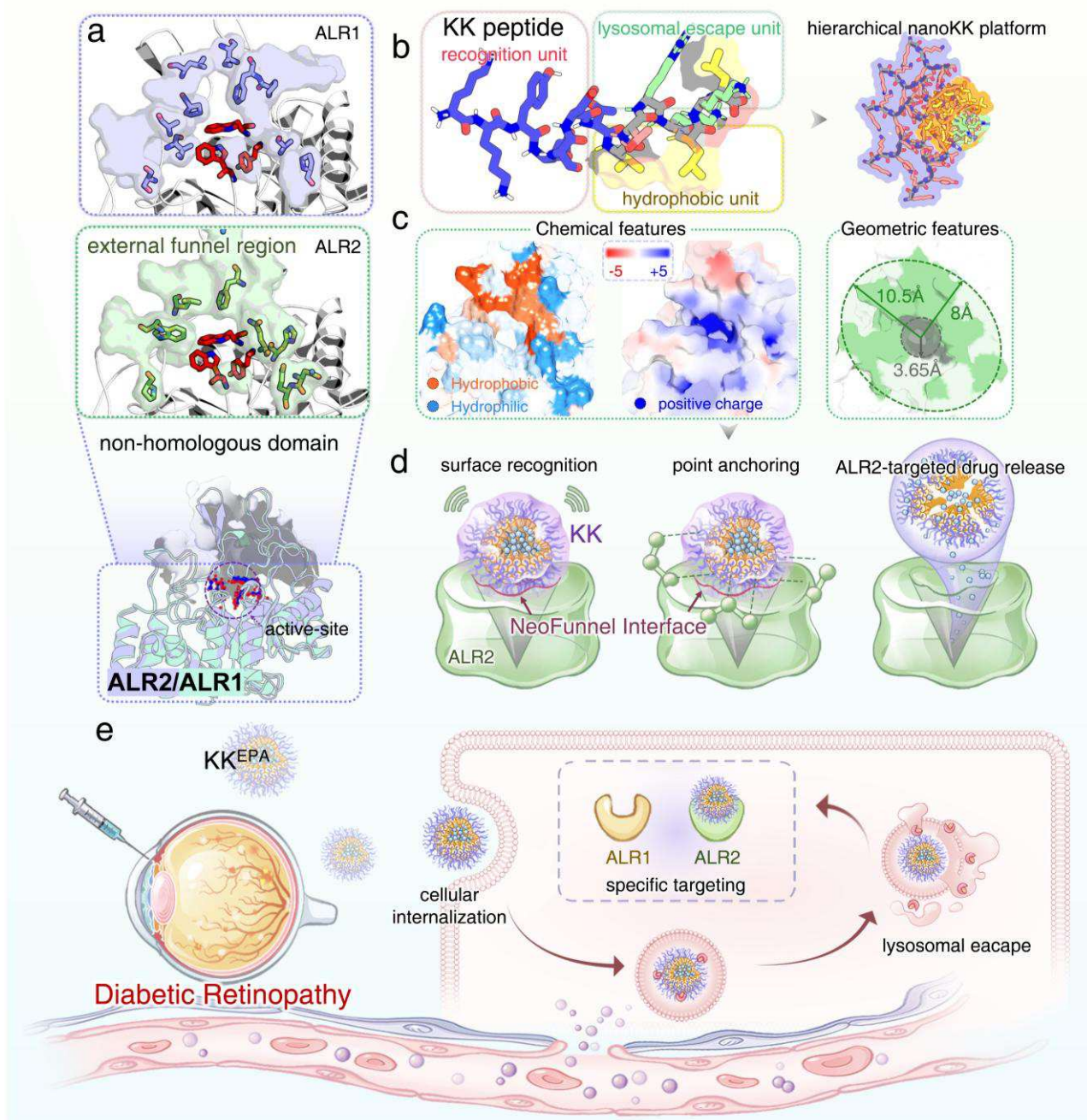
divergent residues that are structurally and spatially distinct from ALR1, providing a key structural basis for selective ligand recognition¹⁵. This region of ALR2 represents a typical pocket-deficient and intrinsically disordered region (IDR)-based protein surface, adopting a highly disordered and topologically smooth conformation ($\sim 1055 \text{ \AA}^2$ surface area), lacking well-defined cavities or clear hydrophilic-hydrophobic partitioning¹⁶. These characteristics make it highly unlikely for conventional targeted molecule design, classifying it as an atypical druggable site.

Inspired by the ‘neosurface’ concept, ligand binding can induce local conformational rearrangements that generate de novo recognition interfaces from disordered protein surfaces¹⁷. Quantitative surface characterization enables rational ligand design to exploit these emergent features providing a strategy to convert poorly druggable regions into selective binding interfaces for homologous targets such as ALR2^{17,18}. This strategy can be regarded as a form of protein surface chemical remodeling¹⁹, which requires ligands that are molecularly programmable, capable of dynamically adapting to disordered protein interfaces, and able to maintain long-lived interactions that drive protein surface remodeling²⁰.

Among molecular recognition systems, targeting peptides uniquely satisfy these design requirements owing to their programmable physicochemical properties, tailored conformational adaptability, and capacity for highly selective molecular recognition²¹⁻²³. Through molecular programming and structural regulation, peptides might initiate local chemical remodeling of disordered enzyme interfaces, triggering the formation of emergent recognition interfaces²⁴. Upon hierarchical supramolecular organization, peptide ligands could form ordered, non-covalently stabilized assemblies that enable responsive structural adaptation and generate scale-adaptive interfaces through cooperative chemical and geometric complementarity²⁵. Following self-assembly (e.g., micelle-like architectures), these ligands further provide multivalent targeting capability, allowing long-term occupancy while maintaining dynamic exchange equilibrium²⁶⁻²⁸. Together, these features enable sustained and adaptive modulation of protein surfaces, reinforcing and stabilizing emergent neosurfaces to support high-affinity and selective recognition of pocket-deficient protein regions²⁹.

Herein, using ALR2 as a representative model system, a hierarchical peptide supramolecular binding-inhibition platform (KK) was established through molecular design and high-throughput screening, driving chemical remodeling of disordered homologous enzyme interfaces and achieving highly selective recognition and sustained inhibition. Considering the characteristics of ALR2, multilevel molecular

programming was employed to construct the KK platform, in which the primary sequence encodes site-specific recognition, lysosomal escape modules enable intracellular targeting, and higher-order structural organization supports supramolecular assembly (Scheme 1b). This molecular programming enables dynamic and sustained adaptive engagement with the intrinsically disordered non-homologous domain of ALR2 through coordinated matching of chemical and geometric surface features, including dynamic hydrophilic-hydrophobic balance and charge complementarity, together with size and curvature compatibility (Scheme 1c). KK platform establishes well-defined interfacial complementarity and drives chemical remodeling of the ALR2 non-homologous domain, generating a previously non-existent, dynamically sustained recognition interface, termed the *NeoFunnel Interface*, which enables coordinated surface recognition and site anchoring to support specific and high-affinity molecular interactions (Scheme 1d). Building upon this recognition and remodeling platform, a nanodrug delivery system (KK^{EPA}) was further developed by encapsulating EPA within the KK architecture, enabling targeted intracellular and ocular delivery for DR treatment. KK^{EPA} follows a cascaded targeting pathway involving cellular internalization, lysosomal escape, and highly specific ALR2-targeted drug release. Leveraging the chemically remodeled and dynamically sustained *NeoFunnel Interface*, the inhibitor EPA is adaptively guided to the catalytic region of ALR2, enhancing targeting selectivity and functional precision while preserving ALR1 physiological activity (Scheme 1e). This strategy establishes a dynamic neosurface engineering framework for selective inhibition of homologous enzyme systems, providing new opportunities for developing therapeutics with minimized off-target effects.



Scheme 1 | Strategy of selective targeting ALR2 in diabetic retinopathy through chemical remodeling of protein neosurface. **a**, Active-site and non-homologous domain shared by ALR2 and ALR1, with the study focusing on the non-homologous domain located at the external funnel region of ALR2. **b**, Construction of a hierarchical peptide supramolecular binding inhibition platform (KK) via molecular programming based on ALR2 features, incorporating affinity recognition units, lysosomal escape units, and hydrophobic units into the sequence. **c**, Adaptive engagement with the disordered non-homologous domain of ALR2 through geometric and chemical coordination matching. **d**, KK platform establishes interface complementarity with the ALR2 non-homologous domain and generate a *NeoFunnel Interface* through chemical remodeling, showing progressive transition from surface recognition to point-specific anchoring, ultimately enabling precise drug release. **e**, Ocular delivery of the intelligent nanodrug delivery system (KK^{EPA}) enables targeted therapy for diabetic retinopathy through cellular internalization, lysosomal escape and ALR2-targeting cascade pathway.

Results

Rational Design and High-Throughput Identification of Peptides with Surface Chemical Remodeling Capability

To establish a methodology for discovering surface-remodeling peptides, ALR2 was selected as a model target and constructed a rational peptide library guided by the chemical and geometric features of its non-homologous domain. A one-bead-one-compound (OBOC) combinatorial chemistry approach was employed to construct a peptide library with a molecular diversity of 10^5 . The general sequence of the library is represented as $\text{NH}_2\text{-KKX}_{12}\text{X}_{11}\text{X}_{10}\text{X}_9\text{X}_8\text{X}_7\text{X}_6\text{X}_5\text{KX}_3\text{KX}_1\text{-COOH}$ (Supplementary Fig. 1). Based on this, residues at the variable positions were rationally designed as described below. Firstly, from a geometric perspective, ALR2 adopts a funnel-like architecture, featuring a surface-exposed intrinsically disordered outer region ($\sim 1055 \text{ \AA}^2$) surrounding a much smaller catalytic site ($\sim 167 \text{ \AA}^2$). This requires peptide ligands to dynamically adapt to the disordered outer surface while achieving geometric curvature matching to the funnel-shaped topology through hierarchical supramolecular assembly. Accordingly, an overall amphiphilic sequence was designed, in which hydrophilic head groups (K) were positioned at the N-terminus (X_{13} and X_{14}), while hydrophobic tail (L, A, or V) was incorporated at the C-terminus (X_1 and X_3). To promote selective recognition of the ALR2 non-homologous domain, we identified a cluster of residues located at the funnel-shaped outer edge, which lies adjacent to the catalytic region, as key binding hotspots (His47, Gln50, Phe122, Phe123, Pro216, Pro219, Trp220 and Leu301). These residues contain aromatic, hydrophobic, or polar side chains capable of forming hydrophobic contacts and hydrogen bonds^{30,31}, interactions that frequently dominate protein-ligand recognition, thus guiding the design of the peptide's affinity-recognition unit. Therefore, $\text{X}_8\text{-X}_{12}$ were encoded with residues capable of forming hydrophobic contacts and hydrogen-bond networks (Y, D, T, S, or E), thereby stabilizing and conformationally constraining the disordered region. Thirdly, as ALR2 is located intracellularly, effective delivery requires overcoming the dual challenges of limited cell membrane permeability and lysosomal entrapment³². To address this, cationic residues (K) were introduced in residues X_2 , X_4 , X_{13} , and X_{14} to enhance the known 'proton-sponge effect', an effective mechanism for promoting lysosomal escape and facilitating cell-penetration³³. Additionally, residues $\text{X}_5\text{-X}_7$ were variable to enhance sequence diversity. Based on the above library design, it was synthesized and subjected to high-throughput peptide screening towards ALR2, while negative screening was conducted using ALR1, following our reported method on a microfluidic device (Supplementary Fig. 2)^{34,35}. Six lead sequences were screened out including

KKYESDTVTRKCLKL (1), KKYESDSITRCLKL (2), KKYESDSLTRCLKL (3), KKYETTSITRCLKL (4), KKYETTSLTRCLKL (5), and KKYETTTVTRCLKL (6) (Fig. 1a and Supplementary Fig. 3). Surface plasmon resonance imaging (SPRi) results showed that the affinity dissociation constants (K_D) of the six lead peptides towards ALR2 were all at the $\sim 10^{-7}$ M level, indicating satisfactory affinity. To evaluate selectivity, binding affinities towards ALR1 were further measured. Peptide 1 exhibited a pronounced affinity differential between ALR2 and ALR1, showing markedly weaker affinity towards ALR1 ($K_D \sim 10^{-5}$ M). In contrast, Peptides 2-5 displayed comparable affinities towards both enzymes, with K_D values $\sim 10^{-6}$ M towards ALR1 (Supplementary Fig. 4). Following the binding affinity determination, a second round of identification was carried out to evaluate the self-assembly morphology of all the lead peptides. Atomic force microscopy (AFM) characterization proved that Peptide 1, 2, 4, 5 and 6 could self-assemble into nanospheres with a defined spatial curvature while Peptide 3 self-assembled into a nanofiber-like structure (Fig. 1b). The micelle architecture is more adaptive to long-lived curvature matching with the funnel-shaped non-homologous domain. Taking into account both selective recognition and geometric complementarity, the candidate was ultimately designated as Peptide 1 and named KK (Supplementary Fig. 5). Further transmission electron microscopy (TEM) analysis confirmed the spherical micelles (Fig. 1c). Dynamic light scattering (DLS) analysis revealed an average diameter of ~ 26 nm for the KK micelles, whose curvature supports the formation of a stable interface, indicating potential geometric complementarity with the funnel-like domain (with an interfacial area of ~ 1055 Å²) of ALR2 (Fig. 1d). Moreover, the KK peptide chain carries a strong positive charge (Supplementary Fig. 6), which electrostatically repels the homologous positively charged catalytic regions shared by ALR1 and ALR2, thereby disfavoring nonspecific interactions.

To further investigate the secondary structure and hierarchical assembly of the KK, the molecular conformation and assembly behavior were analyzed. Circular dichroism (CD) spectroscopy revealed a negative peak at 198 nm, which is characteristic of a random coil conformation (Fig. 1e). In contrast, no distinct negative bands were observed at 208 nm and 222 nm, suggesting that the peptide does not form a stable helix in aqueous solution. Furthermore, the lack of a noticeable minimum near 215-218 nm indicates that β -sheet content is minimal. Taken together, these results demonstrate that the peptide predominantly adopts a flexible and disordered conformation, which may facilitate structural adaptability during molecular recognition. Fourier transform infrared (FTIR) spectroscopy revealed multiple characteristic peaks: bands at 1622 cm⁻¹ and 1688 cm⁻¹ corresponding to antiparallel β -sheets, 1650 cm⁻¹

¹ to α -helices, 1672 cm⁻¹ to β -turns, and 1648 cm⁻¹ to random coils (Fig. 1f). These features suggest that KK exists in a conformationally heterogeneous ensemble rather than a single well-defined conformation. The high flexibility associated with β -turns and random coils may endow the molecule with structural plasticity, facilitating dynamic recognition and adaptive binding behavior³⁶, is particularly advantageous for engaging and stabilizing complex, irregular receptor surfaces. These characteristics further indicate that KK adopts a flexible and conformationally adaptable architecture capable of dynamic target engagement. In addition, solvent-accessible surface area (SASA) calculations (Fig. 1g and Supplementary Fig. 7) were performed to analyze the spatial distribution of individual residues within the sequence. The C-terminal residues such as L1, and L3 exhibited low SASA values and were identified as buried residues contributing to the hydrophobic core. In contrast, residues 4-14 exhibited higher SASA values and were predominantly solvent-exposed, forming a flexible and conformationally adaptive hydrophilic outer surface. While the 'TVTR' segment exhibits a propensity for β -sheet formation due to valine and the 'KLKL' segment favors α -helical structure, the N-terminal 'KKYESD' segment, enriched in charged residues, disfavors regular secondary structure formation and introduces conformational disorder³⁷. Together, these competing structural tendencies drive the KK peptide chains to undergo rapid conformational rearrangements³⁸. This promotes the inward folding and aggregation of hydrophobic segments to form a compact micellar core, while flexible hydrophilic residues extend outward to stabilize the micellar surface (Fig. 1h)^{39,40}. Such interfacial flexibility allows the micelle to adapt to its curvature and conformationally match the recognition interface of ALR2, thereby promoting the formation of a *NeoFunnel Interface*. Furthermore, the curved micellar conformation formed by KK is critical for enabling structural adaptation to the intrinsically disordered non-homologous domain of ALR2. This dynamic interfacial matching may promote the de novo formation of a neosurface that is not pre-organized in the native protein.

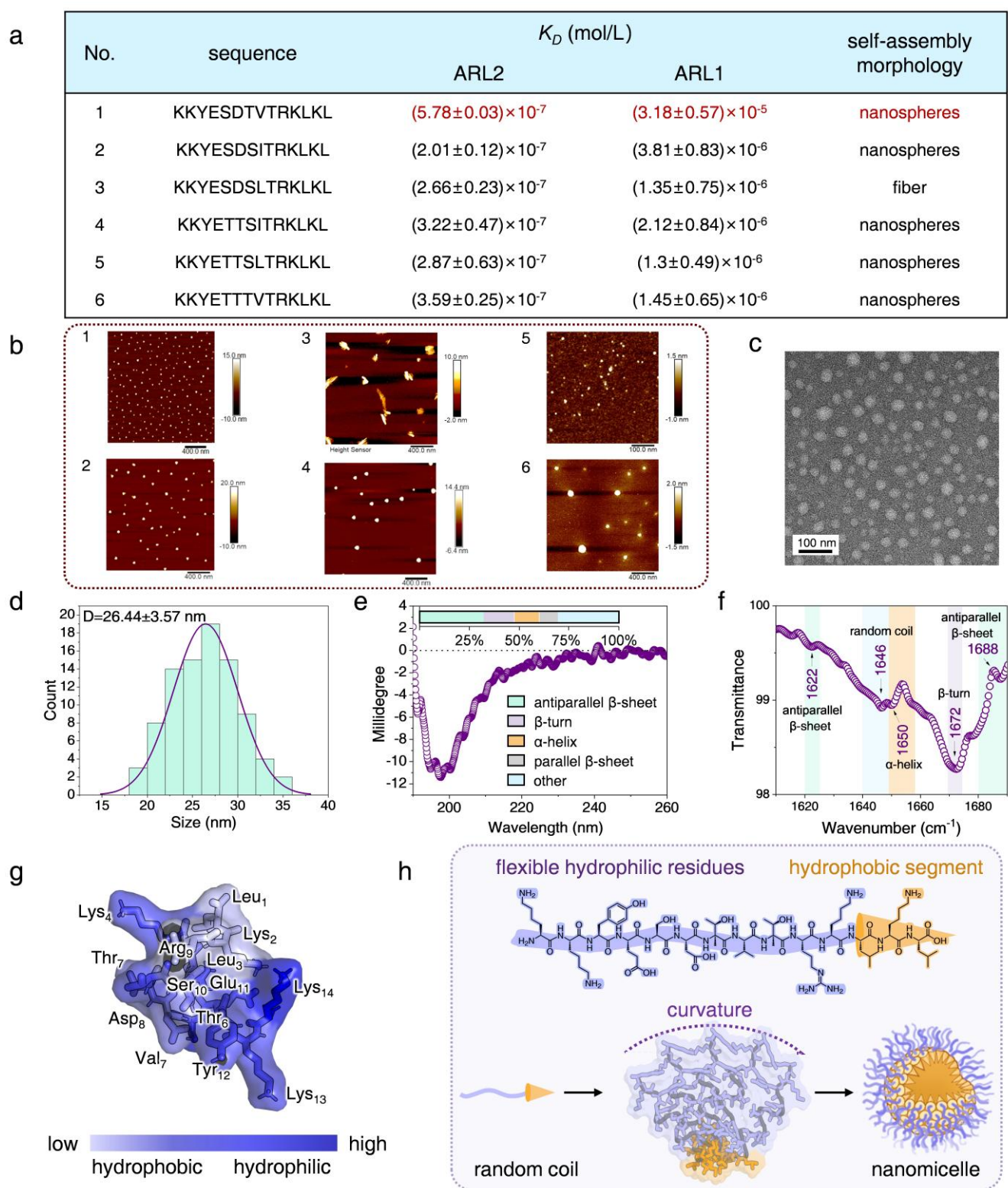


Fig. 1 | Design, screening, and optimization of target peptides, and investigation of its conformation and assembly behavior. **a**, Sequences, K_D , and self-assembly morphologies of six lead peptides. **b**, AFM of the self-assembly morphologies of the six lead peptides. **c**, TEM image showed KK peptide self-assembly into spherical micelles. **d**, DLS results for KK micelles. **e**, CD spectroscopy of KK peptide. **f**, FTIR spectrum of KK peptide. **g**, SASA calculations of KK peptide. **h**, Schematic illustration of KK peptide chains conformational rearrangements to form micelles.

HDX-MS Validation of KK-Induced Chemical Remodeling and *NeoFunnel Interface* Formation

Based on the hierarchical self-assembly of the KK platform into micellar architectures with defined curvature, together with its flexible and solvent-accessible recognition sites that enable dynamic adaptation to the intrinsically disordered non-homologous domain, the molecular-level binding behavior toward the ALR2 targeting region was further investigated. Firstly, molecular docking analyses indicated that KK engages the non-homologous domain of ALR2 in a well-defined manner, establishing pronounced geometric and interfacial complementarity with its funnel-like architecture (Fig. 2a). Quantitative cavity analysis revealed a cavity complementarity of 70.6%, indicating a high degree of volumetric and geometric matching at the ligand-protein interface (Fig. 2b). Furthermore, KK established an extensive and stable interactions with the ALR2 non-homologous domain with key binding sites including Tyr12 (KK)-Pro219 (ALR2) (hydrogen bonding and hydrophobic interactions), Asp9 (KK)-Trp220 (ALR2) (hydrogen bonding), and Thr8 (KK)-Phe123 (ALR2) (hydrogen bonding) (Fig. 2c). These interactions are dominated by hydrogen-bond-driven network formation, which stabilizes the disordered region and gives rise to a previously unformed surface recognition and site anchoring interaction. Subsequently, hydrogen-deuterium exchange mass spectrometry (HDX-MS) was employed to validate the dynamic and sustained adaptive engagement of KK with the ALR2 non-homologous domain, while revealing ligand-induced surface conformational rearrangements⁴¹. HDX-MS analysis revealed pronounced sequence-level differences in deuterium uptake between ALR2 protein and the KK-ALR2 complex (ALR2 and KK-ALR2) (Fig. 2d). Notably, these changes were concentrated within regions harboring non-homologous hotspot residues, including P23, H46, V47, Q49, N52, E53, E120-D125, S133, P215, P218-K221, and C298-C303 (Fig. 2e and Supplementary Fig. 8). Specifically, the non-homologous domain of ALR2 initially exhibited a highly disordered conformation, characterized by a high average deuterium uptake. Upon KK binding, this region displayed an ~10% reduction in deuterium uptake, indicating the formation of mutually stabilized conformations (Fig. 2f). These results demonstrate that KK inserts into the ALR2 non-homologous domain, inducing conformational changes that create topological complementarity with its external funnel region, suggesting the formation of a *NeoFunnel Interface*. To further elucidate the dynamic adaptation process underlying KK-ALR2 engagement, the time-resolved deuterium exchange behavior of ALR2 was systematically examined by HDX-MS, enabling residue-specific and region-specific analysis of surface stabilization and interfacial complementarity. Consistent with direct engagement at key recognition sites, the ALR2 segments

spanning residues 110-126 (containing F123) and 209-231 (containing P219 and W220) exhibited pronounced, time-dependent decreases in deuterium uptake upon KK engagement, reaching reductions of 14.00% and 11.79% at 600 s, respectively (Fig. 2g and 2h). These localized reductions indicate sustained structural stabilization at interaction regions, reflecting long-lived and dynamically maintained engagement rather than transient contact, thus providing a prerequisite for chemical remodeling. Beyond these primary interaction sites, additional proximal non-homologous surface regions also displayed reduced deuterium uptake, including segment 21-30 (containing P23), segment 45-54 (containing H46, V47, Q49, N52 and N53), segment 127-141 (containing S133), as well as segments 296-301 and 302-316 (containing C298-C303). At 600 s, these regions showed deuterium uptake reductions of 2.58%, 13.37%, 7.74%, 14.38%, and 11.72%, respectively (Fig. 2i-2m). The widespread yet regionally differentiated protection pattern suggests that KK engagement extends beyond discrete binding hotspots to promote broader interfacial matching, enabling adaptive surface complementarity across the non-homologous domain. These observations indicate that the non-homologous disordered interface has been chemically remodeled, enabling dynamic yet stable adaptive complementarity between the KK platform and the ALR2 surface, forming interfacial features in previously disordered regions.

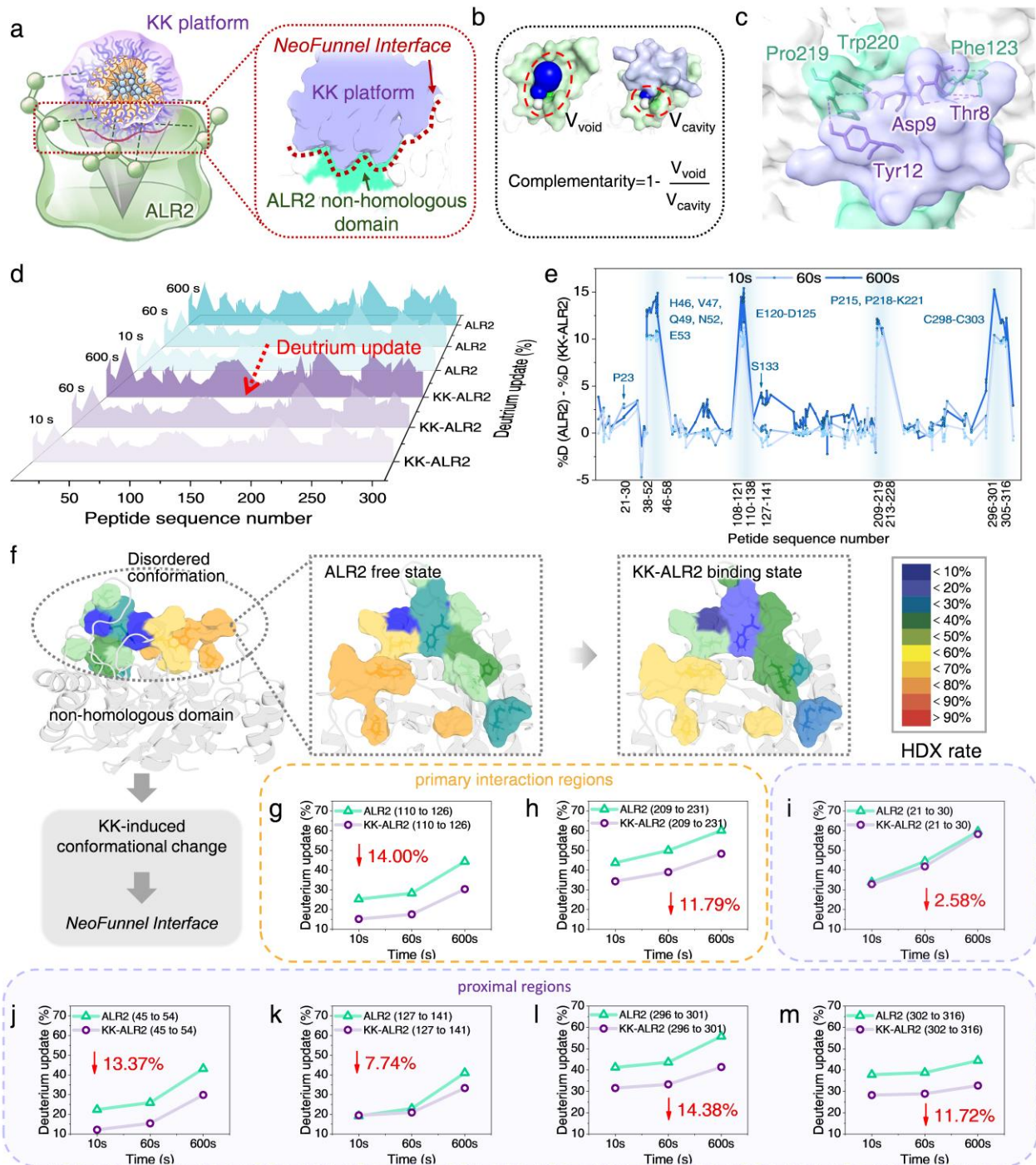


Fig. 2 | Formation of *NeoFunnel Interface* through peptide KK-induced chemical remodels the ALR2 surface. **a**, The KK platform binding to the ALR2 non-homologous domain to establish interfacial complementarity. **b**, Volumetric and geometric matching at the KK-ALR2 interface. **c**, Molecular docking simulation showing the binding sites. **d**, Butterfly plot of KK-ALR2 and ALR2. **e**, Residual plot of KK-ALR2 and ALR2. **f**, The variations in deuterium uptake were mapped onto the surface representations of both the tertiary structures of ALR2. **g**, **h**, Percent deuterium uptake traces for representative peptides from critical motifs in the original ALR2 protein and after binding with KK. **i-m**, Percent deuterium uptake traces for representative peptides from critical motifs containing other non-homologous hotspot residues in native ALR2 protein and after KK binding.

Cellular Validation of Affinity and Selectivity on KK platform

The affinity of KK platform towards ALR2 and ALR1 has previously been validated at the molecular level, demonstrating the potential of specific targeting of ALR2 and no affecting ALR1. Furthermore, the binding affinity of KK to ALR2 at the cellular level was assessed. Human retinal pigment epithelial cell lines (ARPE-19), known to upregulate ALR2 expression (ALR2⁺) under high-Glu conditions, was selected as a positive cell model, and the embryonic kidney cell line HEK-293T (ALR2⁻) was used as a negative cell model. Since ALR2 is located intracellularly, co-incubation with cell lysates was firstly performed to assess KK binding. Lysates binding specificity was analyzed using a plasmonic gold microarray chip, which provides a novel near-infrared (NIR) fluorescence-enhanced detection platform⁴². In the chip assay, supernatants extracted from lysed ARPE-19 (ALR2⁺) and HEK-293T (ALR2⁻) cells were printed onto the chip, respectively. The chip was then incubated with FITC-labeled KK for 2 h to allow binding to ALR2 proteins present in the printed cell supernatants. After washing, fluorescence signals were observed under an inverted fluorescence microscope (Fig. 3a)³⁴. The results showed bright fluorescence in the ARPE-19 cell group, whereas the fluorescence intensity in the HEK-293T cell group was markedly reduced, indicating that KK has a satisfactory selectivity towards ALR2 in the complex cell system (Fig. 3b and 3c). To further confirm cellular targeting and specificity, confocal laser scanning microscopy (CLSM) was employed to track the interaction between the KK platform and living-cells. In ARPE-19 cells, ALR2 expression levels demonstrated a strong correlation with Glu concentration⁴³. Consequently, different ALR2 expression models were established by modulating the Glu concentration in the culture medium, generating cell models with varying levels of ALR2 expression. Cells were cultured under three distinct Glu conditions: normal Glu (NG, 17.5 mM), elevated Glu (EG, 25 mM), and high Glu (HG, 50 mM). After incubation, the cells were exposed to Rhodamine-labeled KK under each of these Glu conditions. It was found that the KK platform successfully entered the cells within 15 min, facilitated by its sequence design, which incorporates cationic residues that enhance membrane penetration. In all the incubation groups, the fluorescence intensity of the KK platform exhibited a trend of initially increasing and then decreasing within 2 h, peaking at the 1 h time point. In the NG group, cytoplasmic fluorescence remained weak throughout the observation period. In the EG group, fluorescence intensity initially increased but then declined rapidly. In contrast, the HG group exhibited stronger fluorescence, which remained stable over the entire duration (Fig. 3d). Therefore, as the Glu concentration increased, the gradual activation of ALR2 in ARPE-19 cells led to a corresponding increase

in the binding and retention of KK within the cells in a quantitative manner. This not only further confirms that KK can bind to ALR2 but also suggests that, due to the formation of the *NeoFunnel Interface*, KK has become an effective dynamic, long-acting ligand that adapts to ALR2. These results highlight that KK demonstrates efficient cellular uptake and selective adaptation to ALR2, driven by its unique ability to engage with cellular interfaces. This capability lays a foundation for its applications in interface recognition, long-term modulation, and targeted delivery of therapeutic agents, positioning KK as a versatile tool in precision medicine.

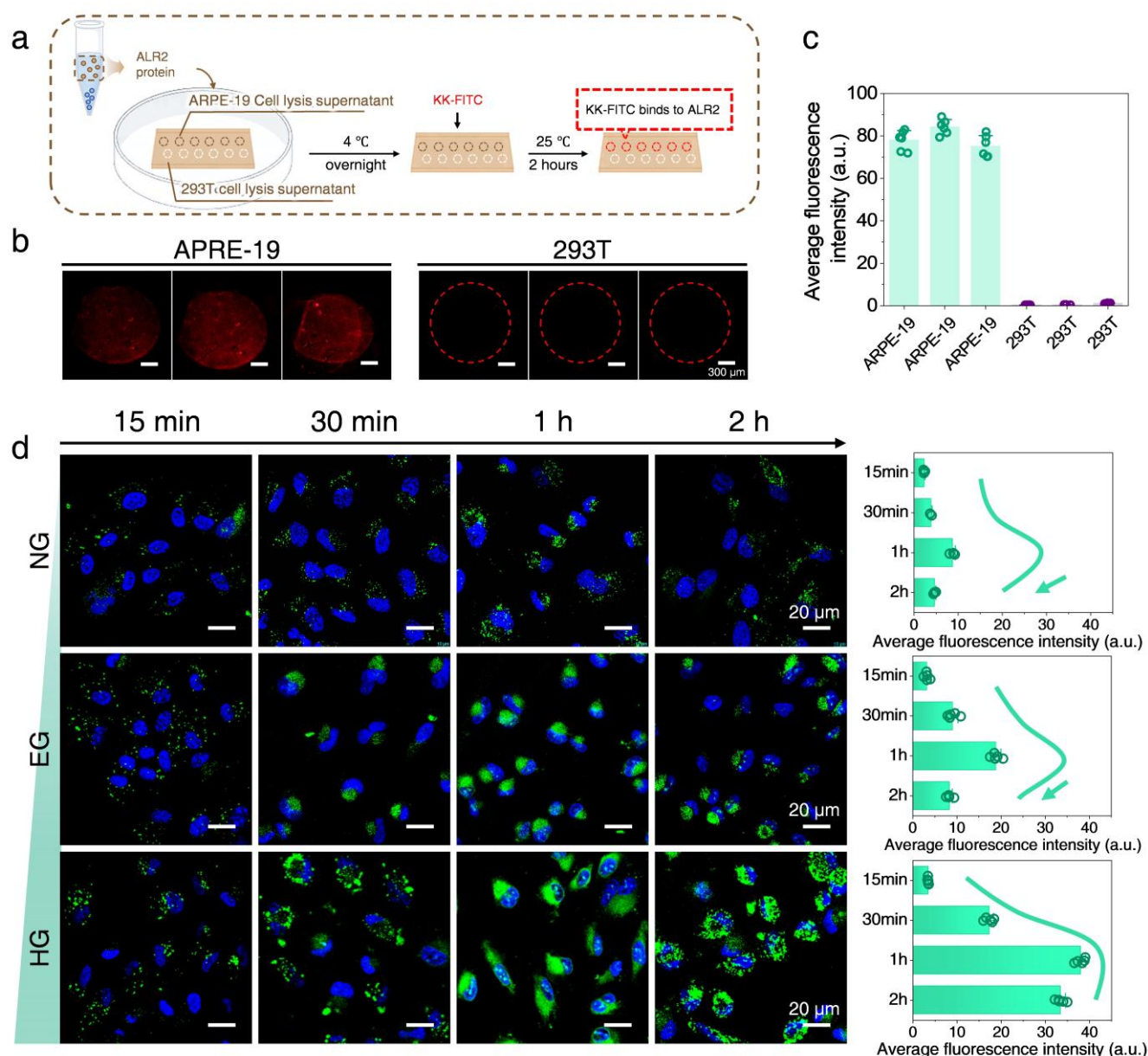


Fig. 3 | Validation of the affinity and specificity of KK platform for ALR2 at the molecular and cellular levels. **a**, Scheme of plasmonic gold microarray platform-based fluorescence detection. **b**, Targeting capability of KK evaluated by plasmonic gold microarray chip. **c**, Statistics of fluorescence intensity under an inverted fluorescence microscope. **d**, CLSM images and statistics of fluorescence intensity of Rhodamine-labeled KK peptide in ARPE-19 cells. Data are presented as mean values $\pm$ SD ($n=6$ in **b**, $n=5$ in **d**).

KK^{EPA} Platform for Cascade Drug Delivery: Targeted Release and Stable Adaptation

Given ALR2's intracellular localization and the high selectivity of the KK platform, an intelligent nanodrug delivery system was developed. This system encapsulates the ALR2 inhibitor EPA, leveraging the dynamically adaptive *NeoFunnel Interface* to facilitate precise and long-acting delivery to ALR2. The integrated nanoscaled drug KK^{EPA} was formed through encapsulating EPA in the spherical micelles of KK with the encapsulation efficiency of ~80.49% (Fig. 4a and Supplementary Fig. 9). The morphology of KK^{EPA} was characterized using AFM and TEM, which confirmed that the spherical structure of the original KK assemblies was maintained (Fig. 4b and 4c). Additionally, DLS measurements revealed no significant change in particle size (Fig. 4d). To further monitor the cellular uptake, EPA was labeled with the signal dye Rhodamine (Rho, Ex: 552 nm) and co-assembled with KK to form the KK^{Rho-EPA} complex, allowing tracking of its delivery efficiency in living cells. The KK^{Rho-EPA} complex was incubated with ARPE-19 cells in complete culture medium containing HG, 50 mM for different durations. A time-dependent increase in intracellular fluorescence intensity was observed, with a peak in cellular internalization reached within the first hour. Fluorescence intensity continued to rise over the next hour, indicating not only high intracellular uptake efficiency but also the sustained, long-lasting binding of KK^{EPA} to the ALR2 receptor, reflecting the dynamic surface adaptation (Fig. 4e). Additionally, for an effective nanoscaled drug delivery system targeting intracellular sites, efficient lysosomal escape following endocytosis is crucial to ensure the internalized drugs reach the cytoplasm while maintaining their ability to specifically recognize and target their intended sites. To evaluate the lysosomal escape behavior of KK^{EPA} platform, EPA was labeled with Rhodamine green (RhoG, Ex: 498 nm) and co-assembled with KK to form KK^{RhoG-EPA} complex, and lysosomes were labeled with lysosomal probe (Lyso, Ex: 580nm) to investigate the intracellular translocation of free Rho green-EPA and KK^{RhoG-EPA} complexes. The free RhoG-EPA or KK^{RhoG-EPA} complexes were incubated with ARPE-19 cells for 6 h, followed by a subsequent incubation period without the drugs. Lysosomal escape characteristics were observed using CLSM at 6 and 18 h after transfection. As shown in Fig. 4f, after 6 h of co-incubation with ARPE-19 cells, free RhoG-EPA was largely localized in lysosomes, while a small fraction of the KK^{RhoG-EPA} complex was internalized into lysosomes, suggesting initial partial lysosomal avoidance. By 12 h, a substantial portion of KK^{RhoG-EPA} had escaped from lysosomes, whereas free RhoG-EPA remained largely trapped. After 24 h, most KK^{RhoG-EPA} had successfully escaped, with a lysosomal co-localization rate of 0.0541, corresponding to an escape efficiency of approximately 85%, while free EPA still showed

significant lysosomal retention (co-localization rate: 0.3885) (Fig. 4g). It is indicated that KK^{EPA} exhibits efficient lysosomal escape, primarily driven by the protonation of lysine residues at lysosomal pH, which triggers the ‘proton-sponge effect’ (Fig. 4h). Moreover, to evaluate the effective release and precision of KK^{EPA} platform, the cumulative drug release curve of $\text{KK}^{\text{Rho-EPA}}$ was recorded in a simulated cytoplasmic environment (20% fetal bovine serum (FBS) in aqueous solution, pH 7.4) under different ALR2 expression conditions (Fig. 4i). $\text{KK}^{\text{Rho-EPA}}$ was incubated with ARPE-19 cells under NG and HG conditions, with HEK-293T cells serving as a negative control. Time-course measurements demonstrated that drug release was efficiently facilitated by ALR2, with a more substantial release observed under HG conditions, correlating with elevated ALR2 expression. Specifically, after 24 h, 95% of EPA was released in the presence of ALR2, while minimal release was detected in its absence, thereby confirming the receptor-specific regulation of drug release. To directly visualize KK^{EPA} drug release in the cytoplasm, Bio-transmission electron microscopy (Bio-TEM) was performed (Fig. 4j). Under HG condition, early during co-incubation with ARPE-19 cells, distinct spherical nanomicelles were observed in the cytoplasm, indicating successful cytosolic translocation of KK^{EPA} (red arrows). After 12 h, both intact and partially disassembled spherical structures were detected, suggesting the onset of EPA release from the KK nanomicelles. As the incubation time was extended, most KK nanomicelles were observed to have completely disassembled after 24 h, demonstrating that EPA was effectively released within the cytoplasm. These findings demonstrate that the interface-matching design generated nanoparticles with efficient cellular uptake and lysosomal escape. The formed *NeoFunnel Interface* facilitates ALR2-responsive, precise drug release at the target site, ensuring sustained, stable binding and long-term adaptation. This enables selective modulation of ALR2 activity with high specificity, significantly reducing off-target effects.

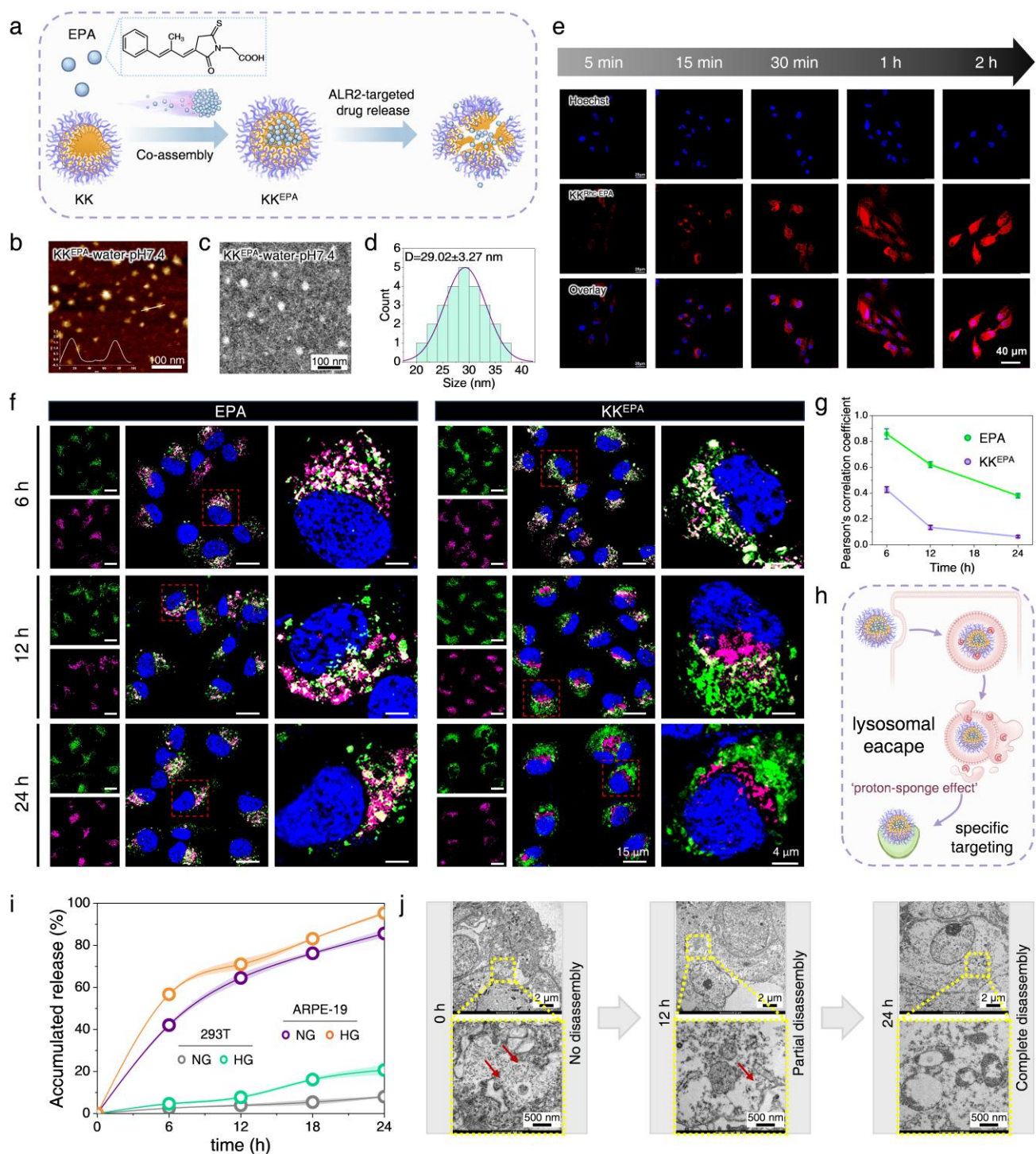
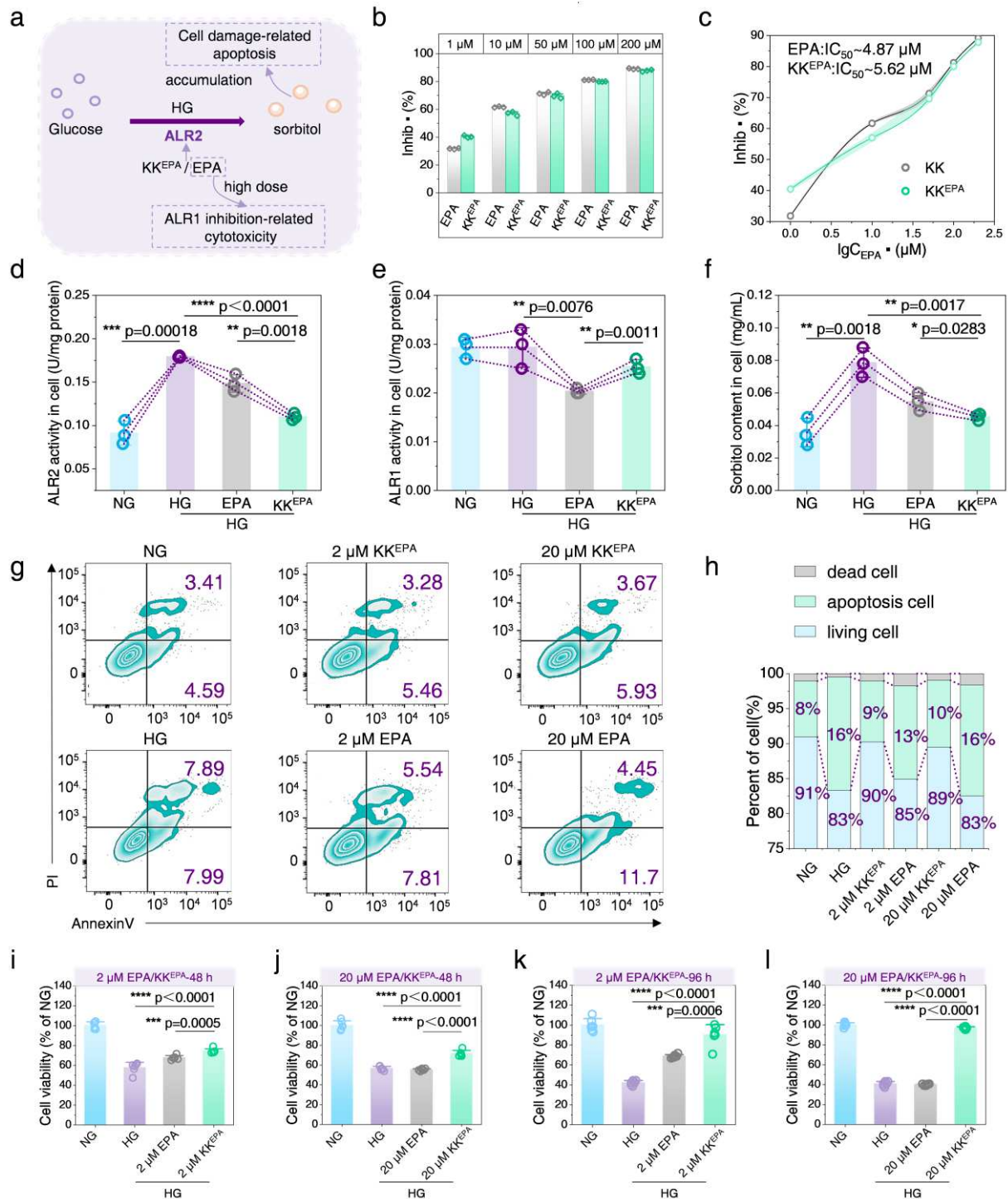


Fig. 4 | Construction of the KK^{EPA} Cascade Drug Delivery Platform for Targeted Release and Stable Adaptation. **a**, Schematic illustration of the construction of integrated nanoscaled drug KK^{EPA} . **b**, AFM images of KK^{EPA} morphology. **c**, TEM images of KK^{EPA} morphology. **d**, DLS results for KK^{EPA} micelles. **e**, CSLM images monitoring the cellular uptake of KK^{EPA} . **f**, CLSM images revealing the lysosomal escape behavior of free EPA or KK^{EPA} . **g**, Pearson's correlation coefficients showing colocalization rate of EPA or KK^{EPA} at different time points. **h**, Schematic diagram of lysosome escaped behavior principle. **i**, Cumulative drug release of KK^{EPA} under different ALR2 concentrations within 24 h. **j**, Bio-TEM showing the structural evolution of KK^{EPA} in the cytoplasm. Data are presented as mean values $\pm$ SD (n=3).

***In vitro* Evaluation of the Selective Inhibition of ALR2 Enzymatic Activity by KK^{EPA} Platform**

Previous studies have confirmed that KK exhibited high specific recognition toward ALR2 and could self-assemble to form a drug delivery system with *NeoFunnel Interface* formation. Subsequently, guided by the principle illustrated in Fig. 5a, we evaluated the *in vitro* effects of KK^{EPA} on ALR2 enzymatic activity by measuring enzyme activity, intracellular sorbitol levels, and cellular apoptosis⁴⁴. Firstly, the inhibitory activity of KK^{EPA} and free EPA against ALR2 was compared *in vitro* at equivalent concentrations to assess the influence of KK on EPA. Both showed similar inhibition rates (Fig. 5b) and half-inhibitory concentration (IC₅₀) values (Fig. 5c) across five concentrations (from 1 μ M to 200 μ M), demonstrating that KK platform preserves the activity of EPA. Then, the effect of KK^{EPA} on the activity of ALR2 and ALR1 was evaluated in ARPE-19 cell to further validate its selective inhibitory activity on ALR2. ALR2 overexpression in cell was still induced under HG conditions. Next, ARPE-19 cell was incubated with KK^{EPA} or EPA at the equivalent EPA concentration (20 μ M) for 48 h respectively, with cells cultured under normal concentration of NG as control. Compared to free EPA, KK^{EPA} demonstrated a more significant inhibition of ALR2 activity, with ALR2 activity measured at 0.15 U/mg protein in the EPA group while 0.11 U/mg protein in the KK^{EPA} group (Fig. 5d). Notably, KK^{EPA} had no discernible effect on the activity of ALR1, underscoring its high selectivity for ALR2 (EPA: 0.020 U/mg protein, KK^{EPA}: 0.025 U/mg protein,) (Fig. 5e). These results demonstrated that KK^{EPA} precisely delivered EPA into the ALR2 catalytic domain, inhibiting its activity, while its *NeoFunnel Interface*-mediated engagement with the non-homologous region further enhanced selectivity. Furthermore, given that high Glu activates the ALR2-driven polyol pathway, the KK^{EPA}-mediated inhibition of ALR2 was confirmed by validating the cellular sorbitol accumulation. The content of sorbitol in ARPE-19 cell was tested after treatment with KK^{EPA} or free EPA (20 μ M). Sorbitol levels of 0.045 mg/mL and 0.055 mg/mL were detected in the KK^{EPA} and free EPA groups, respectively, compared with 0.079 mg/mL in the HG control, which received no treatment (Fig. 5f and Supplementary Fig. 10). Therefore, the inhibitory rate was calculated as 42.4% for KK^{EPA} and 30.5% for EPA, which was consistent with the testing result of ALR2 activity, further indicating that KK^{EPA} possessed stronger inhibition toward ALR2 pathway in cellular level. It is revealed that the downstream cascading effects resulting from the sustained binding and long-term adaptation of KK^{EPA}.

Moreover, studies have shown that excessive accumulation of sorbitol can lead to cell membrane swelling and structural damage, ultimately resulting in cell apoptosis⁵¹. Additionally, high-dose exposure to EPA induces cytotoxicity through excessive inhibition of ALR1, also resulting in apoptosis^{52,53}. Therefore, apoptosis assessment serves as an indicator of the capacity of KK^{EPA} on selective enzymatic regulation. ARPE-19 cells were induced under HG condition (elevated ALR2 expression) as above, and then the cells were incubated with KK^{EPA} or free EPA for 48 h, respectively. Next, cell apoptosis was assessed by flow cytometry assay. Significant apoptosis was observed on cells treated with high-dose EPA (20 μ M) cultured under HG condition. In contrast, KK^{EPA} treatment reduced the cell apoptosis. Specifically, 2 μ M EPA delivered via KK^{EPA} decreased apoptosis by 45.9%, while 20 μ M EPA delivered via KK^{EPA} reduced apoptosis by 40.6%, demonstrating the enhanced anti-apoptotic effect of KK^{EPA} at both concentrations. Notably, at the same EPA concentration (20 μ M), KK^{EPA} reduced apoptosis by 39.5% compared with free EPA (Fig. 5g, 5h and Supplementary Fig. 11). Subsequently, cell viability was assessed using a CCK-8 assay in an orthogonal experiment, evaluating the cytotoxicity of free EPA and KK^{EPA} at different concentrations (2 μ M and 20 μ M) and exposure times (48 and 96 h) (Fig. 5i-5l). A cell viability of only 40% was monitored in the free EPA treated group compared with the HG control group. It showed that free EPA might induced cytotoxicity, especially at higher concentrations (20 μ M) and after prolonged exposure (96 h). Whereas, KK^{EPA} at high concentration and long exposure did not produce significant cytotoxicity, with a satisfactory cell viability rate of 97%. These results showed that KK^{EPA} effectively mitigated cell apoptosis under hyperglycemic stress and alleviated ALR1 inhibition-related cytotoxicity caused by free EPA. Collectively, these findings indicate that KK^{EPA} had stronger inhibitory activity and better selectivity toward ALR2, exhibiting superior selective enzymatic regulation and enhanced cellular safety.



Therapeutic Effect Evaluation of KK^{EPA} Platform in Diabetic Retinopathy

Based on the above results, KK^{EPA} platform demonstrated efficacy at the molecular and cellular levels, highlighting its potential for *in vivo* therapeutic application. To further investigate this potential, a DR animal model was established using intraperitoneal streptozotocin (STZ) injection to induce hyperglycemia⁴⁵. After successful induction of hyperglycemia, drug treatment was initiated. Mice were treated with ocular delivery via choroidal injections of saline (control), EPA, or KK^{EPA}, following the dosing regimen shown in Fig. 6a. Body weight was monitored daily, and blood Glu levels were recorded weekly. Following a ten-week diabetic course, a systematic evaluation of early-stage DR phenotypes was performed.

Prominent hallmarks of non-proliferative DR, such as neovascularization, pericyte depletion, and acellular capillaries, were observed in the control group. Specifically, fluorescein fundus angiography revealed marked vascular leakage in the control mice, closely resembling the neovascular characteristics commonly observed in human DR, such as disrupted blood-retinal barrier integrity and abnormal vessel growth⁴⁶. However, these pathological changes were significantly attenuated following KK^{EPA} treatment. The mice exhibited normal vasculature with no detectable abnormalities, suggesting that KK^{EPA} effectively mitigates the early vascular changes associated with DR (Fig. 6b and Supplementary Fig. 12). This highlights the potential of KK^{EPA} as a therapeutic candidate for preventing retinal damage in diabetic conditions. Similar findings were confirmed by fundus photography (Fig. 6c and Supplementary Fig. 13)⁴⁷. Histological analyses further supported these observations. Hematoxylin-Eosin (H&E) staining of retinal sections revealed choroidal neovascularization (CNV) in the control group, while the KK^{EPA}-treated group retained normal retinal structure (Fig. 6d and Supplementary Fig. 14). CNV is a common pathological feature in DR, and its excessive proliferation is closely associated with retinal damage⁴⁸. KK^{EPA} treatment, by reducing the formation of these new blood vessels, may effectively slow the progression of retinal degeneration, highlighting its potential in inhibiting abnormal retinal vessel growth. Additionally retinal ganglion cells (RGCs) were counted to evaluate the intervention by KK^{EPA} on retinal neuronal degeneration⁴⁹. Control mice experienced a significant loss of ganglion cell nuclei (indicated by red arrows and dashed boxes) whereas KK^{EPA} protected against retinal ganglion cell loss (Fig. 6e and Supplementary Fig. 15). Since RGCs are crucial neuronal types in the retina responsible for transmitting visual signals from the retina to the brain, and their loss is typically accompanied by vision impairment. The therapeutic effect of KK^{EPA} may have alleviated retinal neuronal degeneration to some extent,

suggesting its potential in preserving retinal structure and eyesight. In the control group mice, there was a significant increase in acellular capillaries (Fig. 6f, red arrows) and a decrease in pericyte numbers (Fig. 6f, blue arrows) in retina, both of them are early indicators of DR. The formation of acellular capillaries indicates structural abnormalities in the microvasculature, whereas pericyte loss is closely linked to vascular instability⁵⁰. KK^{EPA} treatment significantly alleviated these pathological changes (Fig. 6g, 6h and Supplementary Fig. 16), suggesting that KK^{EPA} can effectively regulate the stability of retinal microvasculature, reduce the occurrence of lesions, and thereby slow the DR progression.

Moreover, given that hyperglycemia-induced ALR2 activation undergoes dynamic interface adaptation and long-term ligand engagement, this sustained interaction potentiates downstream signaling cascades, notably leading to persistent upregulation of vascular endothelial growth factor (VEGF)⁵¹. VEGF plays a central role in CNV development, its expression in the retina was assessed to analyze the downstream mechanism of KK^{EPA} on CNV inhibition. Therefore, we detected VEGF levels throughout the entire retina (including the ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL) and retinal pigment epithelium (RPE) using fluorescent immunoassay. Since VEGF is primarily localized to the retinal nuclear layers, so we performed fluorescence intensity statistics across the region from INL to ONL. In line with the pathological features observed in DR, an upregulation of VEGF was detected in control mice, reflecting the typical response to ALR2 activation under hyperglycemic conditions. In contrast, KK^{EPA} treatment resulted in a marked suppression of VEGF expression (Fig. 6i, 6j and Supplementary Fig. 17). This finding suggests that KK^{EPA} not only inhibited ALR2 activity but also attenuated the downstream pro-angiogenic signaling associated with DR, indicating its potential to prevent the pathological vascular changes⁵².

Previous studies have indicated that retinal degenerative changes are early events in the onset and progression of DR⁵³. To comprehensively assess retinal function, electro-retinogram (ERG) recordings were obtained under both dark-adapted and light-adapted conditions to separately evaluate rod-driven and cone-driven pathways. Photoreceptor activity was reflected by the a-wave, whereas the integrated responses of Müller and bipolar cells were represented by the b-wave. We examined both eyes of the mouse (where 1L denotes the left eye and 2R denotes the right eye in the figure). The ERG showed that, under dark-adapted conditions, the amplitudes of both a-wave and b-wave were reduced in the control group compared with the KK^{EPA}-treated group (Fig. 6k). This reduction was more pronounced under high-intensity flash stimuli (Fig. 6l). These findings indicate that in DR mice, retinal photoreceptor

function and downstream neuronal activity are impaired, resulting in a reduced electrophysiological response. Under light-adapted conditions, the control group exhibited similar deficits (Fig. 6m), and the response time were generally prolonged under both light and dark adaptation. These findings indicate substantial functional impairment of the retina and RPE. The prolonged response latency and reduced amplitude directly reflect retinal neuronal damage. Under continuous flash stimuli, the control group mice showed significantly lower amplitudes in both light and dark adaptation responses compared to the KK^{EPA} -treated group (Fig. 6n and 6o). These functional deficits reflect the common retinal dysfunction in DR, likely stemming from reduced retinal cell activity, impaired synaptic transmission, or retinal ischemia⁵⁴. Remarkably, KK^{EPA} treatment substantially restored these defects, with improvements observed in both scotopic and photopic responses (Supplementary Fig. 18).

Overall, these results clearly demonstrate that KK^{EPA} , by deeply modulating the pathological mechanisms of ALR2, successfully prevents DR at the *in vivo* model level, alleviating both structural and functional damage by DR. Through the preservation of retinal histological architecture and functional integrity, KK^{EPA} represents a promising therapeutic strategy for both the prevention and intervention of diabetic retinopathy.

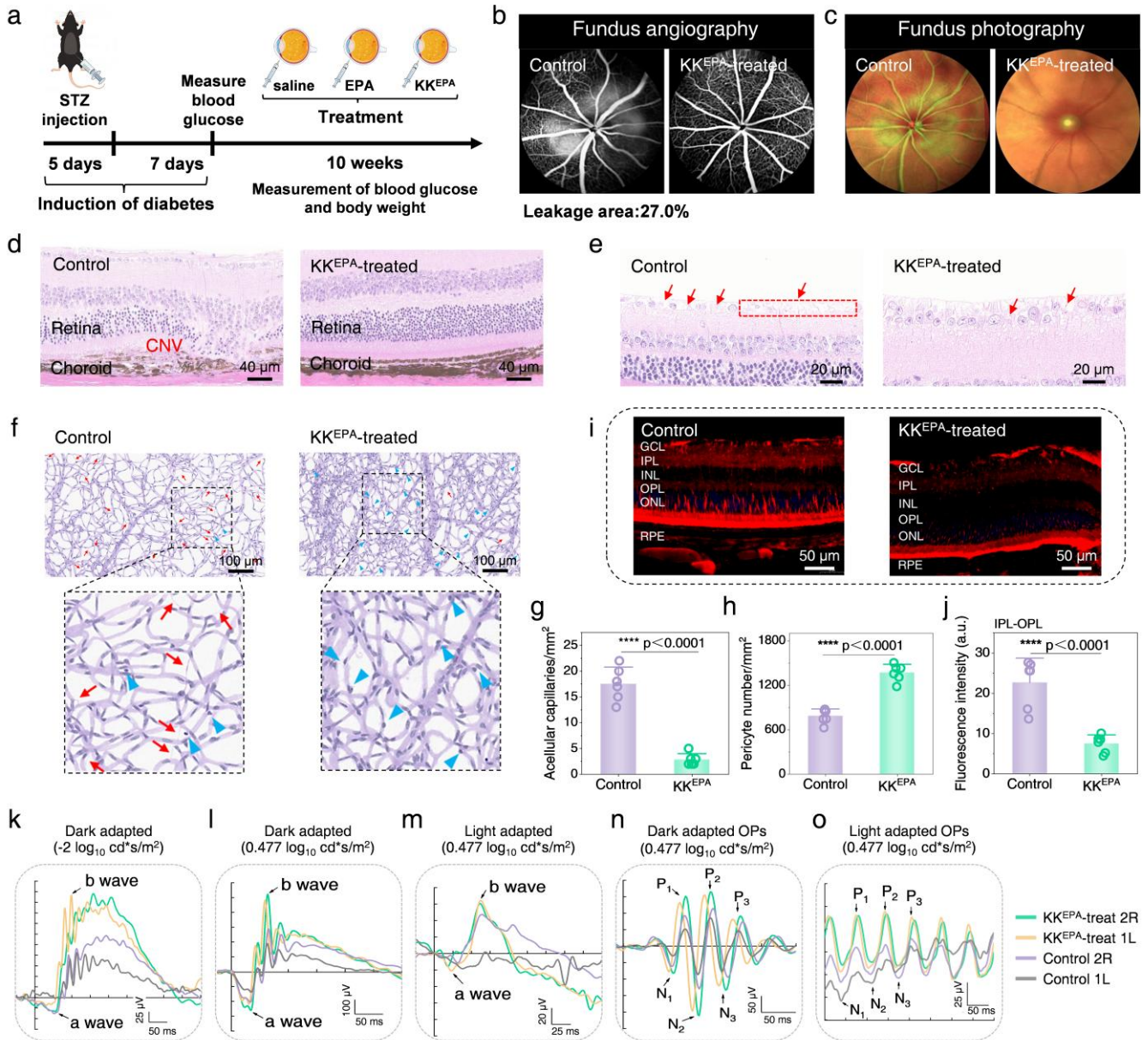


Fig. 6 | Therapeutic Effect Evaluation of KK^{EPA} Platform in DR. **a**, *In vivo* model of KK^{EPA} intervention in diabetic C57BL/6J mice. **b**, Representative fluorescein fundus angiography images. **c**, Fundus photography. **d**, Representative retinal H&E stained images show classical CNV and the preserved normal retinal structure. **e**, Loss of retinal ganglion cells and nuclear degeneration in the GCL, indicated by red arrows. **f**, Retinal neuronal degeneration experiment, arrows indicate degenerated capillaries and arrowheads indicate capillary pericytes. Quantification of retinal acellular capillaries (**g**) and pericytes (**h**). Representative images (**i**) and quantification (**j**) of VEGF immunofluorescence (red) in retinal sections. Representative ERG a-waveforms and b-waveforms response under dark adaptation at $-2 \log_{10} \text{cd} \cdot \text{s/m}^2$ (**k**), $0.477 \log_{10} \text{cd} \cdot \text{s/m}^2$ (**l**) stimulus luminance, and under light adaptation at $0.477 \log_{10} \text{cd} \cdot \text{s/m}^2$ (**m**) stimulus luminance. Representative ERG waveforms at a $0.477 \log_{10} \text{cd} \cdot \text{s/m}^2$ stimulus luminance under scotopic (**n**) and photopic (**o**) conditions. Data are presented as mean values $\pm$ SD ($n=6$ in **g**, **h** and **j**). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$ by *t*-test.

Reduced systemic toxicity and improved biosafety of KK^{EPA} platform

Despite its pronounced therapeutic efficacy in preventing DR, rigorous *in vivo* biosafety assessment is critical to validate KK^{EPA} as a safe and clinically translatable delivery platform. Based on the molecular design, the geometric and chemical features of ALR2 were precisely leveraged to enable dynamic conformational adaptation and sustained engagement of KK^{EPA} at the *NeoFunnel interface*, thereby ensuring highly selective inhibition of ALR2 while minimizing off-target toxicity associated with ALR1 inhibition. Accordingly, the KK^{EPA} system has potential to exhibit favorable safety, which was subsequently validated. ALR1 is essential for physiological detoxification through the reduction of toxic aldehydes. Non-selective inhibition of ALR1 can compromise hepatic and renal detoxification, leading to potential liver and kidney toxicity⁵⁵. Since high-dose EPA treatment has been reported to induce hepatotoxicity and nephrotoxicity, and prolonged hyperglycemia is known to promote cellular apoptosis⁵⁶, a series of histological and hematological analyses were therefore performed to systematically evaluate the safety profile of KK^{EPA} following two months of treatment. TUNEL staining of retinal sections revealed significant apoptosis in the retina of mice of EPA-treated group. In contrast, KK^{EPA}-treated group exhibited minimal apoptotic signals, suggesting that KK^{EPA} not only reduces EPA-induced cytotoxicity but also provides superior ALR2 inhibition, contributing to improved DR prevention efficacy (Fig. 7a and 7b and Supplementary Fig. 19).

Furthermore, hematological and serum biochemical analyses provided systemic evidence for differential safety profiles between the two treatment modalities. Alterations in hematological parameters, including white blood cell count (WBC) and neutrophil percentage (Neu%), are commonly indicative of inflammatory responses or stress-induced immune activation, suggesting the presence of acute hepatic and renal injury⁵⁷. In addition, changes in hemoglobin (HGB), hematocrit (HCT), and platelet count (PLT) reflect perturbations in hematopoietic function and peripheral blood homeostasis, indicative of chronic hepatic and renal damage⁵⁸. In the EPA-treated group, pronounced fluctuations in these indices were observed, suggesting the induction of systemic inflammatory responses and potential interference with normal hematopoiesis. In contrast, mice receiving KK^{EPA} maintained hematological parameters within physiological ranges, indicating minimal impact on the blood system. Serum biochemical profiling further revealed marked differences in hepatic and renal function. Elevations in alanine aminotransferase (ALT)⁵⁹, aspartate aminotransferase (AST)⁶⁰, alkaline phosphatase (ALP)⁶¹, and γ -glutamyl transferase (GGT)⁶² are indicative of hepatocellular injury and cholestatic stress⁶³, while abnormalities in total

bilirubin (TBIL) and direct bilirubin (DBIL) reflect impaired hepatic metabolism and biliary transport⁶⁴. Consistently, reductions in total protein (TP) and albumin (ALB) are associated with compromised hepatic synthetic capacity⁶⁵. Mice treated with EPA exhibited significant abnormalities across multiple liver-associated parameters, pointing to potential hepatotoxicity, whereas no such changes were detected in the KK^{EPA}-treated group, underscoring its improved hepatic safety profile. Moreover, renal function was assessed by measuring serum creatinine (Cre)⁶⁶ and uric acid (UA)⁶⁷, which serve as indicators of glomerular filtration efficiency and purine metabolism⁶⁸. Elevated Cre and UA levels in the EPA-treated group suggested increased renal burden or early-stage renal impairment. In contrast, these indices remained stable in KK^{EPA}-treated mice, further supporting the favorable *in vivo* biocompatibility and reduced systemic toxicity of the KK^{EPA} formulation. Collectively, these hematological and biochemical analyses indicate that, compared with free EPA, KK^{EPA} preserves therapeutic efficacy while significantly reducing systemic toxicity, particularly hepatic and renal injury, attributed to its dynamically adaptive and sustained binding enabling highly selective ALR2 targeting and minimized off-target effects (Fig. 7c and 7d). Furthermore, histological examination of major organs (heart, liver, spleen, lung, and kidney) using H&E and TUNEL staining demonstrated mild tissue injury in free EPA-treated group, whereas organ morphology remained intact in KK^{EPA}-treated group (Fig. 7e). Cell numbers in H&E-stained sections (Fig. 7f) and live/dead cell ratios in TUNEL-stained sections (Fig. 7g) were statistically analyzed. The above results demonstrate that KK^{EPA} platform successfully prevents hyperglycemia-induced retinal degeneration and significantly reduces EPA-related systemic toxicity. This is achieved by precisely addressing the subtle differences between homologous enzymes through sequence design in molecular design, and by constructing the *NeoFunnel Interface* for precise drug release in the ALR2 non-homologous domain, highlighting its advantages in therapeutic efficacy and biological safety.

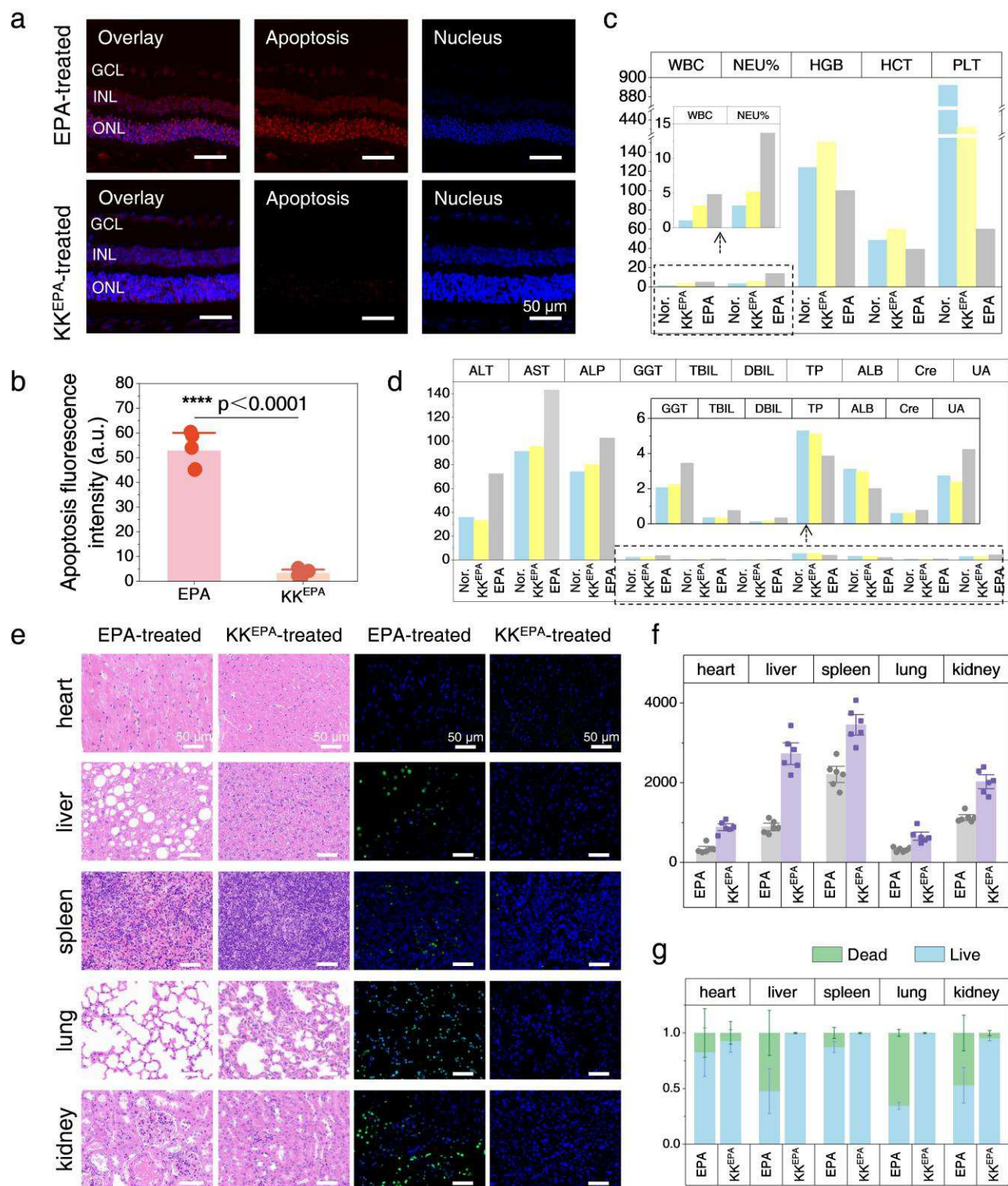


Fig. 7 | Biosafety Validation of the KK^{EPA} Platform. **a**, TUNEL staining of retinal sections from mice. **b**, Quantification of apoptotic retinal cells. Hematological analysis (**c**), blood biochemistry (**d**) assessing liver- and kidney-related physiological parameters in mice. **e**, H&E and TUNEL staining of major organs from mice. **f**, Quantification of cell numbers based on H&E staining. **g**, Quantification of live/dead cell numbers based on TUNEL staining. Data are presented as mean values $\pm$ SD. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$ by *t*-test. GCL, ganglion cell loss. INL, inner nuclear layer; OPL, outer plexiform layer.

Discussion

The main reasons for the difficulty in distinguishing the homologous enzyme family are two factors: their high sequence and structural similarity, and the fact that non-homologous regions often constitute undruggable surfaces. The ‘neosurface’ guided by chemical remodeling strategies offers novel approaches to addressing this issue, though it remains at the theoretical stage. This study implemented a peptide-induced protein surface chemical remodeling strategy to construct ‘neosurface’. Rather than relying on the conserved catalytic site, which underlies the off-target toxicity, our approach exploits structural and chemical features of a non-homologous, intrinsically disordered region surrounding the ALR2 active pocket. By using a hierarchical peptide nanoplatform to induce protein surface reorganization, we generate a de novo *NeoFunnel interface* with high complementarity to ALR2, enabling strong and selective recognition while sparing the closely related homolog ALR1. This engineered interface also provides a functional basis for precision drug delivery. Encapsulation of the clinically approved inhibitor EPA within the KK platform yields KK^{EPA}, which achieves sequential cellular internalization, lysosomal escape and ALR2-targeted drug release. This cascade delivery process enhances intracellular target engagement and minimizes unintended inhibition of ALR1, offering a mechanistic explanation for the improved therapeutic specificity observed. Importantly, the strategy does not require chemical modification of the drug itself, highlighting its compatibility with existing pharmacological agents. More broadly, our findings demonstrate that intrinsically disordered and topologically smooth protein surfaces that traditionally considered poorly druggable, can be converted into selective recognition interfaces through ligand-induced surface remodeling. This paradigm reveals subtle but exploitable differences among homologous proteins that are inaccessible to conventional structure-based design. As such, neosurface-guided chemical remodeling provides a generalizable framework for achieving selective intervention in homologous systems and suggests new opportunities for developing safer therapies.

Methods

Materials

All chemicals were purchased from commercially available sources. Fluorenylmethoxycarbonyl (Fmoc)-amino acids and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (HBTU) were purchased from GL Biochem (Shanghai, China). Triisopropylsilane (Tips), 4-methylmorpholine, ninhydrin, phenol, N, N'-dimethylformamide (DMF), pyridine, diethylether, dichloromethane, methanol and acetonitrile were all obtained from Beijing Chemical Plant (China). Cyanogen bromide (CNBr) was from J&K Chemicals. Streptavidin coated magnetic beads were purchased from Tianjin BaseLine ChromTech Research Center. Ninhydrin and 1,1'-Di-n-octadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) was obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). L-ascorbic acid (VC) was purchased from Xilong Chemical Co., Ltd. (Guangzhou, China). Trifluoroacetic acid (TFA) was purchased from Sigma Aldrich. phosphate-buffered saline (PBS), PBST (PBS containing 0.2% Tween-20) and lysosomal co-localization probe were from Solarbio (China). Human aldose reductase (ALR2) protein and human aldehyde reductase (ALR1) protein were purchased from Sino Biological (Beijing, China). Epalrestat (EPA) was purchased from Meryer Chemical Technology Co., Ltd. (Shanghai, China). Fluorescein isothiocyanate (FITC) was from Sigma-Aldrich (American). Hoechst 33342, Cell Proliferation, Cytotoxicity Assay Kit (CCK-8) and dimethyl sulfoxide (DMSO) were purchased from Solarbio. Rhodamine and Rhodamine Green were purchased from Duoying Bio (Wuhan, China). Human retinal pigment epithelial cells line (ARPE-19), human kidney epithelial cell line (HEK-293T) was purchased from Cell Center of Chinese Academy of Medical Sciences. Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12), fetal bovine serum (FBS) was all obtained from Gibco (Beijing, China). Penicillin-Streptomycin solution was obtained from HyClone (Beijing, China). Cell ALR2 Activity Assay Kit (SU BN13855), Cell ALR1 Activity Assay Kit (SU BN17974) were purchased from Merck (Germany). Sorbitol Assay Kit (BC2525) purchased from Solarbio. C57BL/6J mice (8-week-old) were purchased from Vital River Laboratory Animal Technology (Beijing, China).

Design and Synthesis of the OBOC Peptide Library

An octameric one-bead-one-compound (OBOC) combinatorial peptide library was constructed to enable high-throughput screening of peptide candidates. During library synthesis, methionine was introduced to facilitate *in situ* chemical cleavage, while glycine served as an extended spacer to alleviate steric

hindrance between adjacent residues. The theoretical capacity of the library was calculated as follows: $1 \times 1 \times 5 \times 5 \times 5 \times 5 \times 5 \times 3 \times 3 \times 2 \times 1 \times 3 \times 1 \times 3 = 506250$, with a fivefold redundancy designed to ensure sequence representation and diversity. Library synthesis was conducted using the Fmoc-based solid-phase peptide synthesis (SPPS) method on TentaGel resin (loading: 0.35 mmol/g) as the solid support. All coupling and deprotection reactions were carried out in anhydrous DMF. For coupling, each batch of TentaGel resin was treated with Fmoc-protected amino acid (4 mM), HBTU (4 mM), and NMM (0.4 M) in DMF, and the reaction was allowed to proceed for 40 min. During Fmoc deprotection, 20% (v/v) piperidine in DMF was applied for 10 min to remove the protecting group. The synthesis followed a split-pool strategy: in each coupling step, the resin beads were evenly divided and separately coupled with different amino acids (“split” phase), followed by recombination and deprotection (“pool” phase). Upon completion, a cleavage cocktail composed of TFA/H₂O/Tips (92.5:2.5:2.5, v/v/v) was used to remove side-chain protecting groups, with a reaction time of 2 h. All reactions were conducted in SPPS vessels equipped with filters to facilitate washing and reagent exchange. Finally, the synthesized peptides were released from the resin by reacting overnight with a CNBr solution (30 mg/mL), ensuring complete cleavage at methionine residues and obtaining the desired peptide library.

Affinity determination by surface plasmon resonance imaging (SPRi)

The interaction between peptides and aldose reductase (ALR2) or aldehyde reductase (ALR1) was measured using a SPRi system (Plexera PlexArray HT, Plexera LLC, Bothell, WA) with a bare gold SPRi chip (Nanocapture gold chip, 47.5 nm thick). To prepare the chip, 1 mg/mL of peptide FE in 1× PBS was spotted onto the gold surface. The chip was incubated in a humidified chamber at 4°C overnight, followed by washing with 10× PBS and 1× PBS for 10 min each, and then rinsed with deionized water for 10 min. The chip was then blocked with 5% skim milk in 1× PBS and incubated at 4°C overnight. After blocking, the chip was purged and dried with nitrogen gas before being loaded into the SPRi system for affinity detection. ALR2 or ALR1 protein was diluted in PBS containing 0.2% Tween-20 (PBST) to concentrations of 8, 16, 32, 64, 128, and 256 nM. Binding interactions were assessed by recording real-time SPRi signals, and the dissociation constants (K_D) of peptides for ALR2 or ALR1 were determined using PlexArray HT software. For the SPRi analysis, the peptides were immobilized on the gold chip surface via thiol groups on cysteine residues. The chip was incubated overnight in a humidified chamber at 4°C before use. The SPRi procedure involved sequential injections: baseline stabilization with PBST, binding of ALR2 or ALR1 protein at different concentrations, washing with PBST, and regeneration with

0.5% v/v H₃PO₄ in deionized water. Protein samples were diluted in PBST, and the binding signals were analyzed in real-time using the PlexArray HT software.

Detection of Peptide KK Specificity for ALR2 Protein Using Plasma Gold Chips

This chip is a novel near-infrared (NIR) fluorescence-enhanced plasmonic gold microarray. In the experiment, the supernatant extracted from the lysates of ARPE-19 (ALR2⁺) cells and HEK-293T (ALR2⁻) cells was printed onto the chip and incubated overnight at 4°C. The chip was then blocked with 5% non-fat milk for 1 h. Next, the chip was incubated with FITC-labeled KK for 2 h, allowing it to bind with ALR2 protein present in the cell supernatants printed on the chip. Finally, the chip was washed three times with PBS and observed under an inverted fluorescence microscope.

Cell Culture and Confocal laser scanning microscope (CLSM) imaging

ARPE-19 were cultured in DMEM/F-12 culture medium with 10% FBS and 1% penicillin/streptomycin at 37°C in a 5% CO₂ environment. HEK-293T were cultured in HyClone DMEM culture medium containing 10% FBS and 1% penicillin-streptomycin solution, under the same conditions. Approximately 10⁵ cells/mL of ARPE-19 or HEK-293T were seeded into culture dishes and incubated overnight to allow cell attachment.

For the KK cellular uptake experiment, ARPE-19 cells were cultured in media containing different concentrations of Glu (NG, 17.5 mM; EG, 25 mM and HG, 50 mM) and incubated with 200 µL of rhodamine-labeled KK peptide solution and 1 mM Hoechst 33342 at 4°C in the dark for various time periods. The cells were then observed using a ZEISS LSM710 confocal microscope (Japan).

In the KK^{EPA} cellular uptake experiment, rhodamine-labeled EPA was co-assembled with KK for over 48 h to form KK^{Rho-EPA} Complex. The resulting complex was then incubated with ARPE-19 cells in a HG environment for varying durations. Confocal imaging was performed with the ZEISS LSM710 confocal microscope (Japan).

Throughout the experiments, a 552 nm laser was used to excite Rhodamine, with emission collected at 570 nm. Hoechst 33342 was excited using a 405 nm laser, and emission was captured at 461 nm. Imaging was conducted using a 63× oil immersion objective lens.

Construction of the KK^{EPA} Drug Delivery System and Determination of Encapsulation Efficiency and Drug Release Characteristics

Preparation of KK^{EPA}. 1 mg of KK peptide was dissolved in 600 µL water, and the pH was adjusted to 7.4. Subsequently, 37.5 µL of hydrophobic drug, EPA dissolved in DMSO at a final concentration of 3.2

mM, was added and sonicated. The mixture was incubated at 20°C for 48 h to facilitate the formation of the KK^{EPA} complex.

Encapsulation Efficiency Assessment. The encapsulation efficiency of EPA in the KK^{EPA} micelles was determined by ultraviolet (UV) spectroscopy. 1 mL of the prepared KK^{EPA} micellar assembly was placed in a dialysis bag and dialyzed against an aqueous solution at pH 7.4 for 24 h. After dialysis, the dialysis buffer was collected and analyzed by UV absorbance at 293 nm to quantify the amount of free EPA. The encapsulation efficiency (EE, %) was calculated using a standard curve previously established for EPA, with the following formula:

$$EE (\%) = \frac{\text{Mass of drug encapsulated by micelles}}{\text{Mass of drug added}} \times 100\%$$

In Vitro Release Study. Rhodamine-labeled EPA was co-assembled with KK for over 48 h to form the KK^{Rho-EPA} complex. The KK^{Rho-EPA} complex was co-incubated with ARPE-19 or HEK-293T cells under NG and HG conditions. At time points of 6, 12, 18, and 24 h, cell lysates were collected and centrifuged. The supernatants were then analyzed by UV absorption at 293 nm to monitor the release of EPA. The release rate was calculated using the standard curve previously established for EPA.

Lysosomal Escape

The lysosomes escape capability of the KK^{EPA} delivery system was investigated using CLSM. The escape ability of the KK^{EPA} drug delivery system was investigated using laser confocal microscopy. EPA was labeled with Rhodamine Green (RhoG, Ex: 498 nm), and then co-assembled with KK to form the KK^{RhoG-EPA} complex. Simultaneously, lysosomes were labeled with LysoTracker Red (Ex: 580 nm). ARPE-19 cells were seeded at a density of 10⁵ cells/well in confocal culture dishes and cultured until they adhered to the surface. The cells were then treated with DMEM/F-12 medium containing either free RhoG-EPA or the KK^{RhoG-EPA} complex. After incubation for 6, 12, and 24 h, the cells were incubated with 100 nM commercial lysosome probe for 30 min. The cells were washed three times with PBS, followed by observation under a laser confocal microscope. Rhodamine Green fluorescence was excited with a 498 nm laser, and emission was detected between 522-530 nm. Lysosome fluorescence was excited with a 580 nm laser, and emission was detected between 600-750 nm. Finally, the Pearson correlation coefficient was calculated using ImageJ software.

Cell Viability Assay in ARPE-19 Cells

ARPE-19 cells were seeded at a density of 5000 cells/well in a 96-well plate and cultured in medium containing either NG or HG. Under HG conditions, cells were treated with 2 μM EPA and equivalent

concentrations of KK^{EPA} (Fig. 4i), as well as 20 μM EPA and equivalent concentrations of KK^{EPA} (Fig. 4j), and incubated for 48 h. The same treatment conditions were applied for a 96-hours incubation period (Fig. 4k, l). After incubation, the culture medium was removed, fresh medium was added, and 10 μL of commercial CCK-8 solution was added to each well. The cells were then incubated in the dark for 2 h. Finally, the absorbance at 450 nm was measured using a microplate reader, and the results were normalized to the NG group.

Cell Enzyme Assay

ARPE-19 cells were cultured in medium containing NG and HG. After cell attachment, EPA and KK^{EPA} complexes were added to the HG experimental group, and the cells were co-incubated for 48 h. Following treatment, the activity of ALR2 and ALR1 was measured using the Cell ALR2 Activity Assay Kit and the Cell ALR1 Activity Assay Kit, respectively, according to the manufacturer's instructions. The expression levels of aldose reductase were quantified using the Bradford Protein Assay Kit (G&VS30030.1). The sorbitol content in the cells was determined using a Sorbitol Assay Kit. In brief, these assays were conducted on cell lysates after the treatment. Absorbance peaks were measured at 340 nm, 450 nm, 595 nm, and 655 nm using a UV-Vis spectrophotometer for quantification.

Molecular Enzyme Assay

Aldose reductase catalyzes the reduction of the substrate D, L-glyceraldehyde to glycerol. During the enzymatic reaction, the coenzyme NADPH participates in the reaction, continuously converting NADPH to NADP^+ , leading to a decrease in NADPH concentration as the reaction proceeds. The maximum absorbance of NADPH occurs at 340 nm. The absorbance in the system is measured using a UV-Vis spectrophotometer to quantify the NADPH concentration, which is then used to calculate the aldose reductase activity.

Flow Cytometric Analysis of Cell Apoptosis

ARPE-19 cells were plated at a density of 5×10^5 cells per well in six-well plates and cultured in DMEM/F-12 medium supplemented with 10% FBS and 1% penicillin-streptomycin. The experimental group was treated with a final Glu concentration of 50 mM in the culture medium. After 48 h of incubation, the cells were exposed to 2 μM and 20 μM EPA or KK^{EPA} containing equivalent concentrations of EPA. Following treatment, cell samples were collected, washed twice with PBS, and resuspended in 100 μL of Binding Buffer. To assess apoptosis, 5 μL of Annexin V-FITC and 10 μL of propidium iodide (PI) were added to the cell suspension and gently mixed. The cells were then incubated

for 5-15 min at 20°C in the dark to allow for proper staining. The cell suspension was transferred into a flow cytometry tube for analysis. Flow cytometry was performed to measure the fluorescence intensities of FITC and PI, with excitation wavelengths set at 488 nm and 535 nm, respectively. The data were analyzed using FlowJo software to determine the levels of cell apoptosis.

Animals

Eight-week-old C57BL/6J mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd and maintained under pathogen-free conditions in a temperature-controlled room with a 12 h light/dark cycle. Diabetic mice were generated by single daily intraperitoneal injections of streptozotocin (STZ, 55 mg/kg body weight; Sigma-Aldrich, S0130) for 5 consecutive days. Streptozotocin was freshly prepared in 100 mM citrate buffer (pH 4.5). Non-fasting blood Glu was measured at 10am on days 7-15 after the last STZ injection. Mice with fasting blood Glu concentrations ≥ 19 mM, polyuria, and glucosuria were considered diabetic. Blood Glu levels and body weights were monitored in the mice once every week. The number of months of diabetes described in the text is defined as the age of the mice at the time of sacrifice minus the age when they were first confirmed to be diabetic. All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee at the Ethics Committee of Animal Experiment Center, Beijing Institute of Technology (Approval number: 2016-0006-M2020013).

Acellular capillaries

Mouse eyes were collected from diabetic (8 months of diabetes) and age-matched nondiabetic mice; samples were fixed in 4% buffered paraformaldehyde (Alfa Aesar, J61899-AP) for 2 days at 20°C. Retinas were isolated and washed overnight in water and incubated in elastase (EMD Millipore, 324682) solution 40 unit/ml at 37°C for 2 h, followed by incubation in Tris buffer pH 8.5 overnight at 20°C. Retinal neurons were washed and brushed away from the vasculature. The carefully isolated vasculature was placed on a glass slide and dried for 24 h; samples were then stained with hematoxylin (Fisher Scientific, SH26-500D) and periodic acid Schiff reagent (Fisher Scientific, SS32-500). The total numbers of endothelial cells, pericytes and migrating pericytes were counted in 6 randomly selected fields per retina, and the numbers were normalized to the relative capillary density (number of cells per mm² capillary area). Subsequently, according to their localization within the capillary tree and their position relative to adjacent endothelial cells, pericytes with triangular nuclei in which at least one lateral side of the triangular nuclei projecting into the extravascular interstitium was longer than the base in contact

with the capillary, were defined as migrating pericytes. Acellular capillaries reported per mm² of retinal area were identified as capillary-sized vessel tubes with no nuclei along their length.

Mouse blood routine test and blood biochemical analysis

After treatment, mice blood samples were collected in the EPA-treated group and the KK^{EPA}-treated group. For blood routine test, whole blood was collected and placed in a tube containing heparin sodium, and then parameters such as white blood cell (WBC), Neutrophil Percentage (Neu%), hemoglobin (HGB), hematocrit (HCT), and Platelets (PLT) were measured. For blood biochemical analysis, the serum was collected by centrifuging (3000 rpm/min) the whole blood at low temperature (4°C), and then parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin (TBIL), direct bilirubin (DBIL), creatinine (CREA), and uric acid (UA) serving as hepatic and renal function markers were measured.

Statistical analysis

For each experiment, the number of replicates is indicated in the figure legends, and P values were determined using a two-tailed *t*-test.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data are shown in the main figures, the Extended Data and the Supplementary Information. All other relevant data are available from the corresponding authors upon reasonable request.

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Contributions

L. Zhang, K. Han, J. Zhao, and Q. Wei designed the strategy. Q. Wei, K. Han, J. Zhang, J. Li, and X. Miao participated in the peptide-related experiments. Q. Wei, X. Wang, and W. Zhang participated in the cell-related experiments. Q. Wei, K. Han, X. Zhang, and J. Zhang participated in the animal-related experiments. W. Wang assisted with study design, conceptualized, funded and led the study and edited the manuscript. B. Ma, Q. Wei, K. Han, and L. Zhang conducted the intellectual revision.

Ethics declarations

Competing interests

The authors declare no competing interests.

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