

Supplementary Note 1:

Mathematical Formulation of the Therapeutic Target Discovery Framework and Computational Procedures for Its Solution Using the Nested Hybrid Differential Evolution Algorithm

The computational workflow of the therapeutic-target discovery framework is illustrated in Figure S1. The framework integrates tissue-specific genome-scale metabolic models, multi-objective metabolic simulations, fuzzy decision-making, and the nested hybrid differential evolution (NHDE) algorithm to systematically identify potential therapeutic-target combinations.

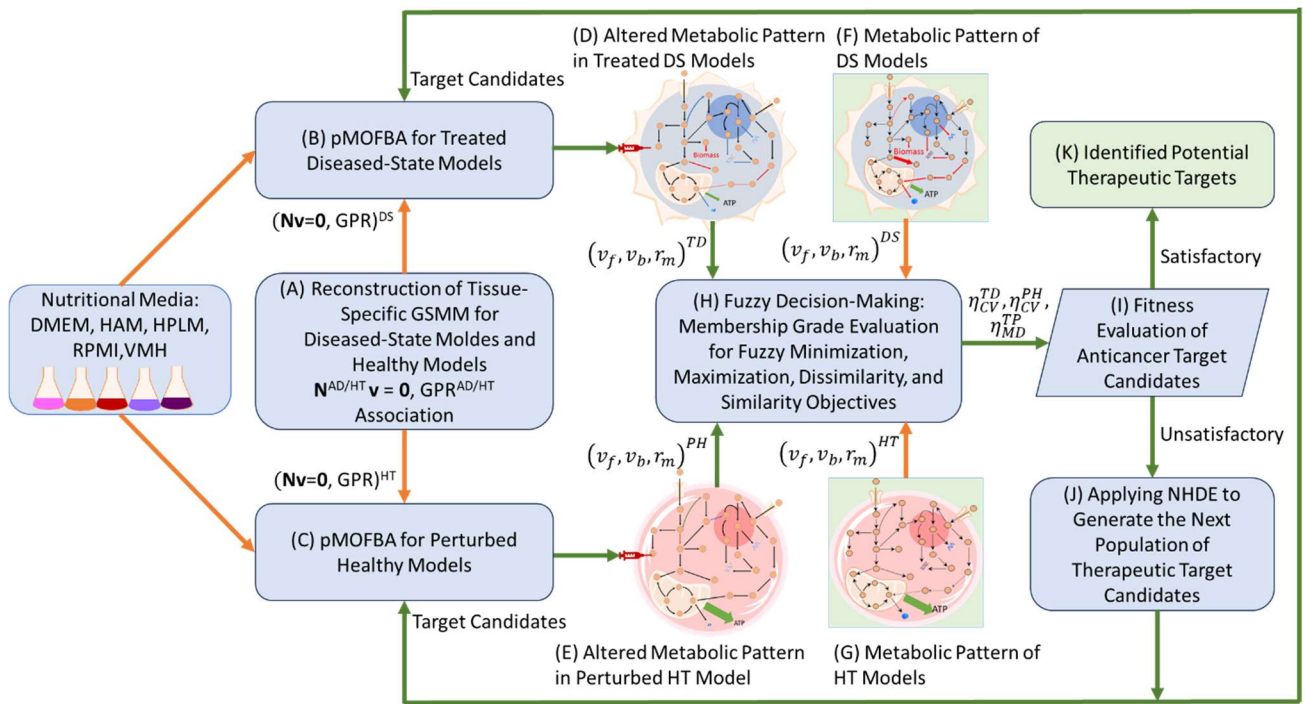


Figure S1. Computational workflow of the therapeutic-target discovery framework for identifying potential therapeutic-target combinations in Alzheimer's disease.

(A) Tissue-specific genome-scale metabolic models (GSMs) are reconstructed for the disease-state (DS) and corresponding healthy-tissue (HT) conditions using transcriptomic data and gene–protein–reaction (GPR) associations. (B, C) Parsimonious multi-objective flux balance analysis (pMOFBA) is performed for each treated DS model and corresponding perturbed HT model, respectively, under five nutritional media (DMEM, HAM, HPLM, RPMI, and VMH). (D, E) Metabolic response patterns of the treated DS and perturbed HT models are determined for each candidate therapeutic perturbation. (F, G) Reference metabolic templates for the DS and HT states are obtained from available experimental or clinical data or, when such data are unavailable, derived from the corresponding unperturbed metabolic models. (H) The resulting metabolic responses are evaluated using fuzzy membership functions representing fuzzy minimization, maximization, similarity, and dissimilarity objectives, thereby transforming heterogeneous objective values into normalized membership grades. (I) The fitness of each candidate therapeutic-target combination is evaluated by integrating the resulting membership grades through the priority-based fuzzy decision-making framework. (J) If the stopping criterion is not satisfied, the nested hybrid differential evolution (NHDE) algorithm generates the next population of candidate therapeutic-target combinations, and steps B–J are

repeated. **(K)** Upon convergence, candidate combinations satisfying the decision criteria are retained as potential therapeutic targets.

Parsimonious Multi-Objective Flux Balance Analysis

Parsimonious multi-objective flux balance analysis (pMOFBA) extends conventional flux balance analysis by incorporating multiple biologically relevant objectives together with a secondary parsimony criterion that minimizes the overall weighted flux through the metabolic network. The method is implemented as a two-stage optimization framework consisting of multi-objective flux balance analysis (MOFBA) followed by a weighted uniform flux distribution (UFD) problem.

$$\begin{array}{ll}
 \begin{array}{l}
 1^{st} \text{ stage: MOFBA} \\
 \left\{ \begin{array}{l}
 \max_{\mathbf{v}_f, \mathbf{v}_b} z_1 = v_{ATP} \\
 \max_{\mathbf{v}_f, \mathbf{v}_b} z_{P_k} = v_{P_k}, \forall k \in \{\text{proteins}\} \\
 \text{subject to} \\
 \mathbf{N}(\mathbf{v}_f - \mathbf{v}_b) = \mathbf{0} \\
 \mathbf{0} \leq \mathbf{v}_f^{LB} \leq \mathbf{v}_f \leq \mathbf{v}_f^{UB} \\
 \mathbf{0} \leq \mathbf{v}_b^{LB} \leq \mathbf{v}_b \leq \mathbf{v}_b^{UB} \\
 \text{GPR association}
 \end{array} \right.
 \end{array}
 &
 \begin{array}{l}
 2^{nd} \text{ stage: UFD problem} \\
 \left\{ \begin{array}{l}
 \min_{\mathbf{v}_f, \mathbf{v}_b} \sum_{i=1}^n w_i (v_{f,i} + v_{b,i}) \\
 \text{subject to} \\
 \mathbf{N}(\mathbf{v}_f - \mathbf{v}_b) = \mathbf{0} \\
 \mathbf{0} \leq \mathbf{v}_f^{LB} \leq \mathbf{v}_f \leq \mathbf{v}_f^{UB} \\
 \mathbf{0} \leq \mathbf{v}_b^{LB} \leq \mathbf{v}_b \leq \mathbf{v}_b^{UB} \\
 \text{GPR association} \\
 z_1 \geq z_1^*, z_{P_k} \leq z_{P_k}^*; \forall k \in \{\text{proteins}\}
 \end{array} \right.
 \end{array}
 \end{array} \quad (S1)$$

In the first stage, MOFBA determines flux distributions that optimize multiple biologically relevant objectives, including ATP production, z_1 , and the synthesis of disease-related proteins or other specified biomolecular products, z_{P_k} . The corresponding optimal objective values are denoted by z_1^* and $z_{P_k}^*$. In the second stage, the weighted UFD problem minimizes the total weighted forward and backward fluxes while preserving the objective levels obtained from the MOFBA stage. This formulation favors parsimonious flux distributions that satisfy the prescribed biological objectives without introducing unnecessary metabolic activity.

Here, $\mathbf{v}_f$ and $\mathbf{v}_b$ denote the vectors of forward and backward reaction fluxes, respectively. The stoichiometric matrix $\mathbf{N} \in \mathbb{R}^{m \times n}$ represents the metabolic network, where m and n are the numbers of metabolites and reactions, respectively. The flux variables are bounded by their corresponding lower and upper limits, $\mathbf{v}_f^{LB}$, $\mathbf{v}_f^{UB}$, $\mathbf{v}_b^{LB}$, and $\mathbf{v}_b^{UB}$. Gene–protein–reaction (GPR) associations provide the link between gene-expression information and reaction activity and are incorporated as reaction-specific constraints. The same formulation is applied consistently to the disease-state (DS) models and their corresponding healthy-tissue (HT) models.

Transcriptomic information is incorporated at two levels. First, gene-expression data are used during reconstruction of the tissue-specific genome-scale metabolic models. Second, expression-derived reaction confidence is used to assign reaction-specific weights, w_i , in the UFD objective. The weights are defined according to the reaction-confidence categories as follows:

$$w_i = \begin{cases} 1/4, i \in \{\text{high confidence reactions}\} \\ 1/2, i \in \{\text{medium confidence reactions}\} \\ 3/4, i \in \{\text{negative confidence reactions}\} \\ 1, i \in \{\text{others or non - gene - expression}\} \end{cases} \quad (S2)$$

Because highly supported reactions receive lower penalties, the UFD problem preferentially permits

flux through pathways that are more strongly supported by transcriptomic evidence. Conversely, reactions with weaker or absent expression support receive progressively larger penalties. This weighting scheme therefore integrates transcriptional evidence directly into flux parsimony and favors biologically supported metabolic routes while retaining the flexibility required to satisfy network stoichiometry and the prescribed objective levels.

To combine heterogeneous MOFBA objectives within a common decision framework, each objective is subsequently transformed into a fuzzy membership grade ranging from 0 to 1. The resulting membership values represent the degree to which each objective satisfies its desired optimization criterion. The first-stage MOFBA problem can therefore be reformulated as a priority-based maximizing fuzzy decision (PBMFD) problem, followed by the weighted UFD problem:

$$\begin{array}{ll}
 \text{1}^{st} \text{ stage: PBMFD problem} & \text{2}^{nd} \text{ stage: weighted UFD problem} \\
 \left\{ \begin{array}{l} \max_{\mathbf{v}_f, \mathbf{v}_b} (\mu_1 + \min\{\varpi_i \mu_i, i \in \Omega^o\})/2 \\ \text{subject to} \\ \mathbf{N}(\mathbf{v}_f - \mathbf{v}_b) = \mathbf{0} \\ \mathbf{0} \leq \mathbf{v}_f^{LB} \leq \mathbf{v}_f \leq \mathbf{v}_f^{UB} \\ \mathbf{0} \leq \mathbf{v}_b^{LB} \leq \mathbf{v}_b \leq \mathbf{v}_b^{UB} \\ \text{GPR association} \end{array} \right. & \left\{ \begin{array}{l} \min_{\mathbf{v}_f, \mathbf{v}_b} \sum_{i=1}^n w_i (v_{f,i} + v_{b,i}) \\ \text{subject to} \\ \mathbf{N}(\mathbf{v}_f - \mathbf{v}_b) = \mathbf{0} \\ \mathbf{0} \leq \mathbf{v}_f^{LB} \leq \mathbf{v}_f \leq \mathbf{v}_f^{UB} \\ \mathbf{0} \leq \mathbf{v}_b^{LB} \leq \mathbf{v}_b \leq \mathbf{v}_b^{UB} \\ \text{GPR association} \\ \mu_i \geq \zeta_i \mu_i^*; \zeta_i \in [0,1], i \in \Omega^o \end{array} \right. \quad (S3)
 \end{array}$$

Here, μ_i denotes the fuzzy membership grade associated with objective i , Ω^o is the set of biological objectives considered in the multi-objective problem, and ϖ_i represents the relative priority or weighting assigned to objective i . The term μ_i^* denotes the optimal membership grade obtained from the first-stage PBMFD solution, whereas ζ_i specifies the minimum fraction of that optimal grade that must be retained in the second-stage UFD problem. Thus, the PBMFD stage identifies a compromise solution among competing biological objectives, whereas the subsequent UFD stage selects a parsimonious flux distribution that preserves predefined fractions of the corresponding optimal membership levels.

This two-stage fuzzy pMOFBA formulation enables ATP production, disease-associated biomolecular synthesis, and other metabolic objectives to be evaluated on a common normalized scale while simultaneously constraining the network toward transcriptomically supported and parsimonious flux distributions. It therefore provides the metabolic simulation basis for evaluating therapeutic perturbations in both the treated disease-state and perturbed healthy-tissue models.

Mathematical Formulation of Therapeutic Target Discovery

The therapeutic target discovery framework illustrated in steps B–K of Figure S1 was formulated as a fuzzy multi-objective hierarchical optimization problem. At the upper level, candidate therapeutic interventions are evaluated according to their ability to suppress disease-associated protein synthesis, preserve cellular energy production, shift the metabolic state away from the disease-state (DS) reference, and restore it toward the corresponding healthy-tissue (HT) reference. For each candidate intervention, the lower level determines the corresponding metabolic responses of the treated disease-state (TD) and perturbed healthy-tissue (PH) models using the pMOFBA framework described above.

The Upper-Level Optimization Problem:

$$\begin{cases}
 \text{Fuzzy Minimization:} \\
 \min_{\delta, \mathbf{z}} v_{P_{ni}}^{TD} \approx 0, \text{ for each protein } i \in \{\tau, A\beta, IL-6, TNF-\alpha\} \\
 \text{Fuzzy Maximization:} \\
 \max_{\delta, \mathbf{z}} v_{ATP}^{TD} \approx v_{ATP}^{max}, \max_{\delta, \mathbf{z}} v_{ATP}^{PH} \approx v_{ATP}^{max} \\
 \text{Fuzzy Dissimilarity:} \\
 \begin{aligned}
 &\text{dissimilarity}_{\delta, \mathbf{z}} v_j^{PH} \approx v_j^{DS}, \text{dissimilarity}_{\delta, \mathbf{z}} v_j^{TD} \approx v_j^{DS} \quad j \in \Omega^{rxn} \\
 &\text{dissimilarity}_{\delta, \mathbf{z}} r_{m_k}^{PH} \approx r_{m_k}^{DS}, \text{dissimilarity}_{\delta, \mathbf{z}} r_{m_k}^{TD} \approx r_{m_k}^{DS} \quad m_k \in \Omega^m
 \end{aligned} \\
 \text{Fuzzy Similarity:} \\
 \begin{aligned}
 &\text{similarity}_{\delta, \mathbf{z}} v_j^{PH} \approx v_j^{HT}, \text{similarity}_{\delta, \mathbf{z}} v_j^{TD} \approx v_j^{HT}, \quad j \in \Omega^{rxn} \\
 &\text{similarity}_{\delta, \mathbf{z}} r_{m_k}^{PH} \approx r_{m_k}^{HT}, \text{similarity}_{\delta, \mathbf{z}} r_{m_k}^{TD} \approx r_{m_k}^{HT}, \quad m_k \in \Omega^m
 \end{aligned}
 \end{cases} \quad (S4)$$

subject to the Lower-Level Optimization Problem:

1. MOFBA and UFD problems for TD model
2. MOFBA and UFD problems for PH model

Here, δ and $\mathbf{z}$ represent the target-selection and intervention variables, respectively. The term $v_{P_{ni}}^{TD}$ denotes the synthesis flux of disease-associated protein i in the TD model, whereas v_{ATP}^{TD} and v_{ATP}^{PH} denote ATP-production fluxes in the TD and PH models, respectively. The sets Ω^{rxn} and Ω^m contain the reactions and metabolites considered in the metabolic-pattern comparisons. The variable v_j represents reaction flux j , whereas r_{m_k} represents the metabolite-flow rate associated with metabolite m_k .

The fuzzy minimization objectives favor suppression of the synthesis of tau, amyloid- β (A β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) in the treated disease model. The fuzzy maximization objectives favor preservation of ATP production in both the TD and PH models. In parallel, the fuzzy dissimilarity objectives promote metabolic states that deviate from the untreated DS reference, whereas the fuzzy similarity objectives promote metabolic states approaching the corresponding HT reference. Together, these objectives allow therapeutic efficacy and preservation of healthy metabolic function to be considered simultaneously.

Using fuzzy set theory, the heterogeneous objectives in Eq. (S4) are transformed into normalized membership grades ranging from 0 to 1. The resulting fuzzy multi-objective hierarchical optimization problem is then converted into the priority-based maximizing fuzzy decision (PBMFD) formulation illustrated in Figure 8C:

The Upper-Level Optimization Problem:

$$\max \eta_D = \max (\eta_{CV}^{TD} + \min\{\eta_{CV}^{TD}, \eta_{CV}^{PH}, \eta_{MD}^{TP}\})/2$$

subject to the Lower-Level Optimization Problems:

Treated Diseased- State (TD) Model:

$$\left\{ \begin{array}{ll} 1^{st} \text{ stage: PBMFD problem} & 2^{nd} \text{ stage: weighted UFD problem} \\ \left\{ \begin{array}{l} \max_{\mathbf{v}_f, \mathbf{v}_b} (\mu_1 + \min\{\varpi_i \mu_i, i \in \Omega^o\})/2 \\ \text{subject to} \\ \mathbf{N}^{DS}(\mathbf{v}_f - \mathbf{v}_b) = \mathbf{0} \\ 0 \leq v_{f/b,j}^{LB} \leq v_{f/b,j} \leq v_{f/b,j}^{UB}, j \in \Omega^{rxn} \setminus \Omega^{MR} \\ 0 \leq v_{f/b,k}^{LB,MR} \leq v_{f/b,k} \leq v_{f/b,k}^{UB,MR}, k \in \Omega^{MR} \\ GPR^{DS} \text{ association} \end{array} \right. & \left\{ \begin{array}{l} \min_{\mathbf{v}_f, \mathbf{v}_b} \sum_{i=1}^n w_i (v_{f,i} + v_{b,i}) \\ \text{subject to} \\ \mathbf{N}^{DS}(\mathbf{v}_f - \mathbf{v}_b) = \mathbf{0} \\ 0 \leq v_{f/b,j}^{LB} \leq v_{f/b,j} \leq v_{f/b,j}^{UB}, j \in \Omega^{rxn} \setminus \Omega^{MR} \\ 0 \leq v_{f/b,k}^{LB,MR} \leq v_{f/b,k} \leq v_{f/b,k}^{UB,MR}, k \in \Omega^{MR} \\ GPR^{DS} \text{ association} \\ \mu_i \geq \zeta_i \mu_i^*, \zeta_i \in [0,1], i \in \Omega^o \end{array} \right. \end{array} \right.$$

Perturbed Healthy (PH) Model:

$$\left\{ \begin{array}{ll} 1^{st} \text{ stage: PBMFD problem} & 2^{nd} \text{ stage: weighted UFD problem} \\ \left\{ \begin{array}{l} \max_{\mathbf{v}_f, \mathbf{v}_b} (\mu_1 + \min\{\varpi_i \mu_i, i \in \Omega^o\})/2 \\ \text{subject to} \\ \mathbf{N}^{HT}(\mathbf{v}_f - \mathbf{v}_b) = \mathbf{0} \\ 0 \leq v_{f/b,j}^{LB} \leq v_{f/b,j} \leq v_{f/b,j}^{UB}, j \in \Omega^{rxn} \setminus \Omega^{MR} \\ 0 \leq v_{f/b,k}^{LB,MR} \leq v_{f/b,k} \leq v_{f/b,k}^{UB,MR}, k \in \Omega^{MR} \\ GPR^{HT} \text{ association} \end{array} \right. & \left\{ \begin{array}{l} \min_{\mathbf{v}_f, \mathbf{v}_b} \sum_{i=1}^n w_i (v_{f,i} + v_{b,i}) \\ \text{subject to} \\ \mathbf{N}^{HT}(\mathbf{v}_f - \mathbf{v}_b) = \mathbf{0} \\ 0 \leq v_{f/b,j}^{LB} \leq v_{f/b,j} \leq v_{f/b,j}^{UB}, j \in \Omega^{rxn} \setminus \Omega^{MR} \\ 0 \leq v_{f/b,k}^{LB,MR} \leq v_{f/b,k} \leq v_{f/b,k}^{UB,MR}, k \in \Omega^{MR} \\ GPR^{HT} \text{ association} \\ \mu_i \geq \zeta_i \mu_i^*, \zeta_i \in [0,1], i \in \Omega^o \end{array} \right. \end{array} \right.$$

(S5)

In Eq. (S5), η_D denotes the overall fuzzy decision value used to evaluate each candidate therapeutic intervention. The component scores η_{CV}^{TD} , η_{CV}^{PH} , and η_{MD}^{TP} quantify complementary aspects of the therapeutic response, as defined in the main manuscript. The priority-based structure gives explicit emphasis to η_{CV}^{TD} while simultaneously requiring acceptable performance across η_{CV}^{TD} , η_{CV}^{PH} , and η_{MD}^{TP} through the minimum operator. Consequently, a candidate intervention receives a high overall decision value only when it achieves the prioritized therapeutic objective without severely compromising the remaining criteria.

The vectors $\mathbf{v}_f$ and $\mathbf{v}_b$ denote the forward and backward reaction fluxes, respectively. $\mathbf{N}^{DS}$ and $\mathbf{N}^{HT}$ are the stoichiometric matrices of the disease-state and healthy-tissue models. Gene-protein-reaction (GPR) associations derived from the corresponding transcriptomic profiles constrain reaction activity and thereby integrate gene-expression information into the metabolic models.

For reactions not selected for therapeutic modulation, $j \in \Omega^{rxn} \setminus \Omega^{MR}$, the forward and backward fluxes remain within their original lower and upper bounds, $v_{f/b,j}^{LB}$ and $v_{f/b,j}^{UB}$. By contrast, reactions belonging to Ω^{MR} are candidate modulated reactions and are assigned intervention-specific bounds, $v_{f/b,j}^{LB,MR}$ and $v_{f/b,j}^{UB,MR}$. These modified bounds represent the effects of candidate therapeutic perturbations and are imposed consistently on the corresponding reactions in both the TD and PH

models, thereby allowing therapeutic efficacy and potential effects on healthy metabolism to be evaluated simultaneously.

The reaction-specific weights w_i in the UFD objective are derived from transcriptomic confidence levels, as defined in Eq. (S2). Because the weights are assigned separately according to the expression evidence associated with the disease-state and healthy-tissue networks, the weighted UFD formulation favors parsimonious flux distributions that are consistent with the corresponding tissue-specific transcriptomic profiles. The TD and PH models therefore retain the stoichiometric structures and GPR constraints of their respective DS and HT models but differ through the intervention-specific flux bounds imposed on the selected reactions in Ω^{MR} . The definition and implementation of these therapeutic modulation bounds are described in the following subsection.

Definition of Modulated Ranges for Active Reactions

The lower and upper bounds of the modulated reactions in Eq. (S5) are defined using three complementary intervention strategies: enzyme-centric (gene-centric), metabolite-centric, and reaction-centric modulation. For a selected target, the intervention-specific bounds, $v_{f/b,i}^{LB,MR}$ and $v_{f/b,i}^{UB,MR}$, are determined according to the type and strength of modulation—up-regulation, down-regulation, or knockout. The parameter $\delta \in [0,1]$ specifies the modulation strength, with $\delta = 0$ corresponding to the basal state and progressively larger values imposing stronger perturbations.

Enzyme-Centric Strategy

In the enzyme-centric strategy, modulation is applied to reactions catalyzed by a selected enzyme, as determined from the corresponding gene–protein–reaction (GPR) associations. For reaction i associated with the selected enzyme, the modulated flux bounds are defined as follows.

The modulated bounds for each reaction catalyzed by an active enzyme z_i are determined by its regulation type:

Up-regulation of enzyme z_i :

$$\begin{cases} (1 - \delta)v_{f,i}^{basal} + \delta v_{f,i}^{UB} \leq v_{f,i} \leq v_{f,i}^{UB} \\ v_{b,i}^{LB} \leq v_{b,i} \leq (1 - \delta)v_{b,i}^{basal} + \delta v_{b,i}^{LB}; i \in \Omega^{MR} \end{cases}$$

Down-regulation of enzyme z_i :

$$\begin{cases} v_{f,i}^{LB} \leq v_{f,i} \leq (1 - \delta)v_{f,i}^{basal} + \delta v_{f,i}^{LB} \\ (1 - \delta)v_{b,i}^{basal} + \delta v_{b,i}^{UB} \leq v_{b,i} \leq v_{b,i}^{UB}; i \in \Omega^{MR} \setminus \Omega^{IZ} \\ (1 - \varepsilon)v_{f,i}^{basal} \leq v_{f,i} \leq (1 + \varepsilon)v_{f,i}^{basal} \\ (1 - \varepsilon)v_{b,i}^{basal} \leq v_{b,i} \leq (1 + \varepsilon)v_{b,i}^{basal}; i \in \Omega^{MR} \cap \Omega^{IZ} \end{cases}$$

Knockout of enzyme z_i :

$$\begin{cases} v_{f,i} = 0 \\ v_{b,i} = 0; i \in \Omega^{MR} \setminus \Omega^{IZ} \\ (1 - \varepsilon)v_{f,i}^{basal} \leq v_{f,i} \leq (1 + \varepsilon)v_{f,i}^{basal} \\ (1 - \varepsilon)v_{b,i}^{basal} \leq v_{b,i} \leq (1 + \varepsilon)v_{b,i}^{basal}; i \in \Omega^{MR} \cap \Omega^{IZ} \end{cases}$$

Here, Ω^{MR} denotes the set of reactions affected by the selected therapeutic intervention, whereas Ω^{IZ} denotes the subset of reactions associated with isozyme redundancy according to the GPR rules. Because inhibition or deletion of a single enzyme does not necessarily eliminate the activity of an isozyme-catalyzed reaction, such reactions are not forced to zero. Instead, their fluxes are constrained

within a narrow interval around the basal state using a small tolerance parameter ε ; $\varepsilon = 0.03$, for example, permits a $\pm 3\%$ deviation from the corresponding basal flux.

For non-isozyme reactions, increasing δ progressively shifts the permissible flux range away from its basal value toward the prescribed regulatory extreme. Up-regulation promotes the forward direction while restricting the corresponding backward flux, whereas down-regulation suppresses the forward direction while permitting greater backward flux. At $\delta = 1$, the imposed bounds reach the maximum regulatory strength defined by the original reaction limits.

Metabolite-Centric Strategy

The metabolite-centric strategy modulates reactions that directly contribute to the production of a selected compartment-specific metabolite. Let N_{kj} denote the stoichiometric coefficient of metabolite m_k in reaction j . A positive coefficient, $N_{kj} > 0$, indicates that the forward direction of reaction j produces m_k , whereas $N_{kj} < 0$ indicates that the backward direction of a reversible reaction produces m_k .

The intervention-specific bounds are therefore defined according to the sign of N_{kj} .

Up-regulation of metabolite m_k :

$$\begin{cases} (1 - \delta)v_{f,j}^{basal} + \delta v_{f,j}^{UB} \leq v_{f,j} \leq v_{f,j}^{UB}; j \in \{j | N_{kj} > 0\} \\ (1 - \delta)v_{b,j}^{basal} + \delta v_{b,j}^{UB} \leq v_{b,j} \leq v_{b,j}^{UB}; j \in \{j | N_{kj} < 0\} \end{cases} \cap \Omega^{Rev}$$

Down-regulation of metabolite m_k :

$$\begin{cases} v_{f,j}^{LB} \leq v_{f,j} \leq (1 - \delta)v_{f,j}^{basal} + \delta v_{f,j}^{LB}; j \in \{j | N_{kj} > 0\} \\ v_{b,j}^{LB} \leq v_{b,j} \leq (1 - \delta)v_{b,j}^{basal} + \delta v_{b,j}^{LB}; j \in \{j | N_{kj} < 0\} \end{cases} \cap \Omega^{Rev}$$

Complete suppression of metabolite production m_k :

$$\begin{cases} v_{f,j} = 0; j \in \{j | N_{kj} > 0\} \\ v_{b,j} = 0; j \in \{j | N_{kj} < 0\} \end{cases} \cap \Omega^{Rev}$$

Here, Ω^{Rev} denotes the set of reversible reactions. Under the steady-state assumption of flux balance analysis, intracellular metabolite accumulation is constrained to zero. Nevertheless, the total production, or metabolite-flow, rate of a given metabolite can be quantified from all reaction directions that generate that metabolite. For metabolite $m_k \in \Omega^m$, its production rate is defined as

$$r_{m_k} = \sum_{N_{ij}>0, j=1}^n N_{ij}v_{f,j} - \sum_{N_{ij}<0, j=1}^n N_{ij}v_{b,j}, m_k \in \Omega^m$$

Because N_{kj} is negative in the second summation, the term $-N_{kj}v_{b,j}$ represents a positive contribution from the backward direction of a reversible reaction that produces metabolite m_k .

Accordingly, metabolite-centric modulation acts on all reaction directions that directly produce the selected metabolite. Up-regulation shifts these production fluxes toward their upper bounds, down-regulation shifts them toward their lower bounds, and knockout suppresses the corresponding production directions. This strategy enables therapeutic interventions to be defined at the metabolite level rather than being restricted to a single enzyme or reaction.

Reaction-Centric Strategy

In the reaction-centric strategy, modulation is imposed directly on a selected reaction, independently of its associated enzyme or metabolite. For target reaction i , the flux bounds are defined as follows.

Up-regulation of reaction i :

$$\begin{cases} (1 - \delta)v_{f,i}^{basal} + \delta v_{f,i}^{UB} \leq v_{f,i} \leq v_{f,i}^{UB} \\ v_{b,i}^{LB} \leq v_{b,i} \leq (1 - \delta)v_{b,i}^{basal} + \delta v_{b,i}^{LB}; i \in \Omega^{MR} \end{cases}$$

Down-regulation of reaction i :

$$\begin{cases} v_{f,i}^{LB} \leq v_{f,i} \leq (1 - \delta)v_{f,i}^{basal} + \delta v_{f,i}^{LB} \\ (1 - \delta)v_{b,i}^{basal} + \delta v_{b,i}^{UB} \leq v_{b,i} \leq v_{b,i}^{UB}; i \in \Omega^{MR} \end{cases}$$

Knockout of reaction i :

$$\begin{cases} v_{f,i} = 0 \\ v_{b,i} = 0; i \in \Omega^{MR} \end{cases}$$

Under this formulation, up-regulation progressively promotes flux in the forward direction and suppresses the backward direction, whereas down-regulation produces the opposite effect. Reaction knockout completely eliminates flux through both directions of the selected reaction. The reaction-centric strategy therefore provides a direct means of evaluating therapeutic interventions at the individual-reaction level without explicitly specifying an enzyme or metabolite target.

Collectively, these three strategies enable the therapeutic target discovery framework to explore interventions at different levels of metabolic organization. The enzyme-centric strategy represents modulation of enzyme- or gene-associated activity while accounting for isozyme redundancy; the metabolite-centric strategy controls the reaction directions responsible for producing a selected metabolite; and the reaction-centric strategy directly modifies individual reaction capacities. The resulting intervention-specific flux bounds are incorporated into the TD and PH lower-level optimization problems in Eq. (S5), allowing each candidate intervention to be evaluated simultaneously for its predicted therapeutic effect on the disease state and its metabolic consequences in the corresponding healthy tissue.

Introduction to Nested Hybrid Differential Evolution (NHDE)

The priority-based maximizing fuzzy decision (PBMFD) problem defined in Eq. (S5) constitutes a bilevel hierarchical optimization framework. At the upper level, the decision variables specify the selected candidate therapeutic targets, $\mathbf{z}$, together with their corresponding modulation-strength parameters, δ . Once a candidate intervention is generated at the upper level, these variables are fixed and passed to the lower-level optimization problems, which determine the resulting metabolic responses of the treated disease-state (TD) and perturbed healthy-tissue (PH) models. The lower-level solutions are subsequently returned to the upper level to evaluate the fitness of each candidate intervention.

Direct solution of the PBMFD problem is computationally challenging because it combines a high-dimensional combinatorial search over candidate targets and regulation types with continuous modulation parameters and nested metabolic optimization problems. Moreover, evaluation of each upper-level candidate requires repeated solution of the lower-level PBMFD and weighted UFD problems for both the TD and PH models. Consequently, the complete hierarchical problem is not readily handled as a single conventional mathematical programming problem using standard commercial optimization solvers.

To address these computational challenges, the nested hybrid differential evolution (NHDE) algorithm is employed as the upper-level search procedure. NHDE combines the global-search capability of differential evolution with nested lower-level mathematical programming, enabling

candidate therapeutic interventions to be systematically generated, evaluated, and refined. For each candidate solution proposed by NHDE, the corresponding modulation variables are imposed on the TD and PH models, the lower-level optimization problems are solved, and the resulting metabolic responses are used to calculate the fuzzy decision scores and overall fitness defined in Eq. (S5). The computational procedures of the NHDE algorithm are summarized in Table S1.

During initialization, a population of N_p candidate individuals is randomly generated to provide broad coverage of the feasible search space. Each individual is represented by a structured array encoding the identity and order of the selected targets, their regulation types (e.g., up-regulation, down-regulation, or knockout), and the corresponding modulation-strength parameters. This representation enables discrete target-selection decisions and continuous regulatory parameters to be handled within a unified evolutionary framework.

A two-group encoding strategy is further employed to represent combination therapies involving different classes of therapeutic interventions. The first group encodes the primary candidate targets, such as enzyme- or gene-associated metabolic targets, whereas the second group represents complementary interventions, such as modulation of nutrient uptake or other metabolic components. The associated regulation type and modulation-strength parameter are encoded together with each selected target. This two-group representation facilitates systematic exploration of combinatorial interventions involving candidate genes or enzymes, nutrient-uptake processes, and their corresponding modulation strengths, as illustrated in Figure S2.

At each NHDE generation, newly generated candidate interventions are evaluated by solving their nested TD and PH metabolic optimization problems. Their fitness values are then used to guide mutation, crossover, selection, and, where applicable, local improvement procedures. This iterative process continues until the prescribed stopping criterion is satisfied, after which the candidate interventions with the highest fuzzy decision values are retained as potential therapeutic-target combinations.

Table S1 Computational procedures of the nested hybrid differential evolution (NHDE) algorithm

The NHDE algorithm searches for optimal combinations of candidate therapeutic targets and their modulation strengths at the upper level, while the metabolic responses of the treated disease-state (TD) and perturbed healthy-tissue (PH) models are determined by the nested lower-level PBMFD and weighted UFD problems. Each candidate solution is evaluated using the fuzzy decision value defined in Eq. (S5), with penalties imposed on infeasible solutions. Mutation, crossover, selection, and diversity-triggered migration are iteratively performed until the prescribed termination criterion is satisfied.

1. Representation and initialization

Let $(\mathbf{z}, \delta)$ denote an upper-level decision vector, where $\mathbf{z}$ represents the discrete variables encoding the selected candidate targets and their regulation types, and δ represents the corresponding continuous modulation-strength parameter(s). An initial population of N_p individuals is randomly generated over the prescribed search space:

$$(\mathbf{z}_i^0, \delta_i^0) = (\mathbf{z}_{min}, \delta_{min}) + \lfloor \rho_i \{(\mathbf{z}_{max}, \delta_{max}) - (\mathbf{z}_{min}, \delta_{min})\} \rfloor, i \in \{1, \dots, N_p\}$$

where $\rho_i \sim \mathcal{U}(0,1)$ is a vector of independent uniformly distributed random numbers. The operator $\lfloor (a, b) \rfloor$ converts the components associated with the discrete decision vector $\mathbf{z}$ to their nearest admissible integer values while retaining the continuous modulation parameter δ .

2. Mutation

For each individual i in generation G , a mutant vector is generated according to

$$(\hat{\mathbf{z}}_i^G, \hat{\delta}_i^G) = (\mathbf{z}_p^G, \delta_p^G) + \lfloor F \mathbf{D}_{jklm}^G \rfloor, i \in \{1, \dots, N_p\}$$

where F is the differential-evolution scaling factor, $(\mathbf{z}_p^G, \delta_p^G)$ is the selected base vector. $\mathbf{D}_{jklm}^G$ is the differential vector constructed from selected population members, and randomly selected two or four individuals to generate the differential vector as follows.

$$\mathbf{D}_{jklm}^G = [(\mathbf{z}_j^G, \delta_j^G) - (\mathbf{z}_k^G, \delta_k^G)] + [(\mathbf{z}_l^G, \delta_l^G) - (\mathbf{z}_m^G, \delta_m^G)]$$

The p, j, k, l , and m individuals are mutually exclusive. The discrete components associated with $\mathbf{z}$ are converted to admissible integer values, whereas δ remains continuous.

3. Crossover

A trial individual is generated by binomial crossover between the parent and mutant vectors. For the j^{th} component of the target-selection vector,

$$z_{ji}^G = \begin{cases} z_{ji}^{G-1}, & \text{if } \text{rand}(0, 1) \geq C_R \\ \hat{z}_{ji}^G, & \text{otherwise, } j = 1, \dots, n; i = 1, \dots, N_p \end{cases}$$

and for the modulation-strength parameter,

$$\delta_i^G = \begin{cases} \delta_i^{G-1}, & \text{if } \text{rand}(0, 1) \geq C_R \\ \hat{\delta}_i^G, & \text{otherwise, } i = 1, \dots, N_p \end{cases}$$

where $C_R \in [0,1]$ is the user-defined crossover probability.

4. Bound restriction

After mutation and crossover, each decision variable is restricted to its admissible range. For the discrete target-selection variables,

$$z_{ji}^G = \begin{cases} z_{j,min}, & z_{ji}^G < z_{j,min} \\ z_{ji}^G, & z_{j,min} \leq z_{ji}^G \leq z_{j,max} \\ z_{j,max}, & z_{ji}^G > z_{j,max}, j = 1, \dots, n; i = 1, \dots, N_p \end{cases}$$

and for the modulation-strength parameter,

$$\delta_i^G = \begin{cases} \delta_{min}, & \delta_i^G < \delta_{min} \\ \delta_i^G, & \delta_{min} \leq \delta_i^G \leq \delta_{max} \\ \delta_{max}, & \delta_i^G > \delta_{max}, i = 1, \dots, N_p \end{cases}$$

5. Nested lower-level optimization

For each upper-level candidate $(\mathbf{z}_i^G, \delta_i^G)$, the selected targets, regulation types, and modulation strengths are imposed on the corresponding metabolic models. The following lower-level problems are then solved:

1. MOFBA and UFD problems for the TD model
2. MOFBA and UFD problems for the PH model

Let $\mathbf{v}_i^{TD,G}$ and $\mathbf{v}_i^{PH,G}$ denote the resulting optimal flux distributions for the treated disease-state and perturbed healthy-tissue models, respectively. These flux distributions are subsequently used to calculate the corresponding reaction-flux and metabolite-flow patterns required for evaluation of the fuzzy membership grades.

6. Fitness evaluation

The fitness of candidate individual $(\mathbf{z}_i^G, \delta_i^G)$ is defined as

$$\mathcal{F}(\mathbf{z}_i^G, \delta_i^G) = \eta_D(\mathbf{z}_i^G, \delta_i^G) - \mathcal{P}(\mathbf{z}_i^G, \delta_i^G)$$

where $\eta_D(\mathbf{z}_i^G, \delta_i^G)$ is the overall fuzzy decision value defined in Eq. (S5), and $\mathcal{P}(\mathbf{z}_i^G, \delta_i^G)$ is a non-negative penalty function.

The component membership grades and the resulting η_D value are calculated from the optimal flux distributions and metabolite-flow rates of the TD and PH models. For a feasible candidate, $\mathcal{P}(\mathbf{z}_i^G, \delta_i^G) = 0$, whereas a positive penalty is assigned when one or more lower-level optimization problems are infeasible or the candidate violates prescribed feasibility requirements. A larger value of $\mathcal{F}(\mathbf{z}_i^G, \delta_i^G)$ therefore represents a more desirable candidate therapeutic intervention.

7. Selection

Selection is performed in two stages.

Stage 1: One-to-one competition

Each trial individual competes with its corresponding parent, and the individual with the larger fitness value is retained:

$$(\mathbf{z}_i^G, \delta_i^G) = \arg \min \{ \mathcal{F}(\mathbf{z}_i^G, \delta_i^G), \mathcal{F}(\mathbf{z}_i^{G-1}, \delta_i^{G-1}) \}, i \in \{1, \dots, N_p\}$$

Stage 2: Best-individual identification

The best individual in generation G is identified as

$$(\mathbf{z}_b^G, \delta_b^G) = \arg \min \{ \mathcal{F}(\mathbf{z}_i^G, \delta_i^G), i = 1, \dots, N_p \}$$

This individual serves as the current best therapeutic intervention and is used in the subsequent population-diversity assessment.

8. Population-diversity assessment and migration

To prevent premature convergence, population diversity is evaluated relative to the current best individual. For each discrete target variable,

$$\varsigma_{ji} = \begin{cases} 0, & \text{if } z_{ji}^G = z_{jb}^G; j = 1, \dots, n; i = 1, \dots, N_p \\ 1, & \text{otherwise} \end{cases}$$

and for the modulation-strength parameter,

$$\zeta_i = \begin{cases} 0, & \text{if } |\delta_i^G - \delta_b^G| < \varepsilon_2 \delta_b^G \\ 1, & \text{otherwise} \end{cases}$$

where $\varepsilon_2 \in [0,1]$ is a user-defined tolerance.

The overall population-diversity degree is calculated as

$$\xi = \left[\sum_{i=1; i \neq b}^{N_p} \left(\sum_{j=1}^n \varsigma_{ji} \right) + \zeta_i \right] / [(n+1)(N_p-1)]$$

where ξ ranges from 0 to 1. A small value of ξ indicates that most individuals have converged toward the current best solution.

Migration is activated when $\xi < \varepsilon_1$, where ε_1 is a prescribed diversity threshold. New individuals are then regenerated around the current best individual $(\mathbf{z}_b, \delta_b)$.

For each discrete component,

$$z_{ji} = \begin{cases} \lfloor z_{jb} + \gamma_{ji}(z_{j,min} - z_{jb}) \rfloor, & \text{if } \hat{\gamma}_{ji} < \frac{z_{jb} - z_{j,min}}{z_{j,max} - z_{j,min}} \\ \lfloor z_{jb} + \gamma_{ji}(z_{j,max} - z_{jb}) \rfloor, & \text{otherwise;} \end{cases} j = 1, \dots, n; j = 1, \dots, N_p$$

and for the modulation-strength parameter,

$$\delta_i = \begin{cases} \delta_b + \gamma_i(\delta_{min} - \delta_b), & \text{if } \hat{\gamma}_i < \frac{\delta_b - \delta_{min}}{\delta_{max} - \delta_{min}} \\ \delta_b + \gamma_i(\delta_{max} - \delta_b), & \text{otherwise;} \end{cases} j = 1, \dots, N_p$$

where $\gamma_{ji}, \hat{\gamma}_{ji}, \gamma_i$, and $\hat{\gamma}_i$ are independent random numbers drawn from $\mathcal{U}(0,1)$. The migration operator redistributes individuals around the current best solution while maintaining exploration on both sides of that solution within the admissible search domain.

9. Iteration and termination

After migration, or directly after selection when migration is not triggered, the algorithm returns to the mutation step. Mutation, crossover, bound restriction, nested lower-level optimization, fitness evaluation, selection, and diversity assessment are repeated until the prescribed termination criterion is satisfied. The best individual obtained at termination, $(\mathbf{z}_b, \delta_b)$ is retained as the predicted therapeutic-target combination and its associated modulation strategy.

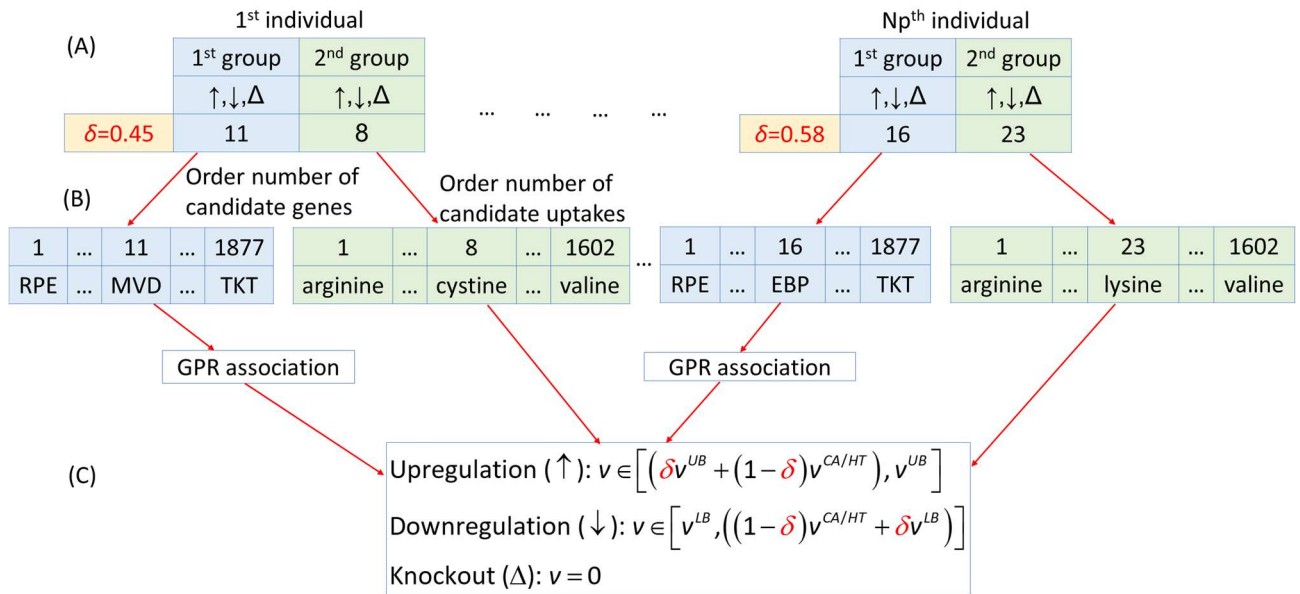


Figure S2. Two-group encoding and mapping of candidate therapeutic-target individuals in the NHDE algorithm.

(A) Each candidate individual is represented by a structured two-group encoding scheme comprising the index of the selected target in each group, its regulation type (up-regulation, down-regulation, or knockout), and the associated modulation-strength parameter, δ . The first group represents candidate gene- or enzyme-associated targets, whereas the second group represents candidate nutrient-uptake targets. (B) The encoded target indices are mapped to their corresponding biological entities in the

predefined candidate sets; for example, an index in the first group identifies a candidate gene or enzyme, whereas an index in the second group identifies a specific nutrient uptake. (C) The decoded candidate intervention is translated into model constraints. Gene- or enzyme-associated targets modify the lower and upper bounds of the corresponding reactions according to their gene–protein–reaction (GPR) associations, whereas nutrient-uptake targets modify the bounds of the corresponding exchange reactions. The modulation parameter δ determines the strength of the imposed perturbation according to the regulation type. The resulting intervention-specific bounds are subsequently applied to the treated disease-state (TD) and perturbed healthy-tissue (PH) models for fitness evaluation within the NHDE framework.