Sleep treatment for OSA patients at high risk of T2DM and prevention of insulin resistance

Group name: Prime

陈诗琦，黄睿恒，金慷

Background:

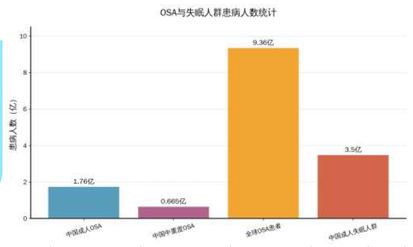


Fig.1:Sleep disorders are highly prevalent, and OSA is closely linked to an increased risk of T2DM.

Sleep disorders are increasingly prevalent, with circadian rhythm disruption and sleep deprivation being prominent.
Research shows sleep disturbances elevate type 2 diabetes mellitus (T2DM) risk, and obstructive sleep apnea (OSA) — a major sleep disorder — has **a significant bidirectional association** with T2DM:
OSA increases T2DM risk by ~**1.3–2.3-fold**, and OSA prevalence in T2DM patients is as high as **68%**. They form a vicious cycle via inflammation, hypoxia, and insulin resistance.

Pathogenesis:

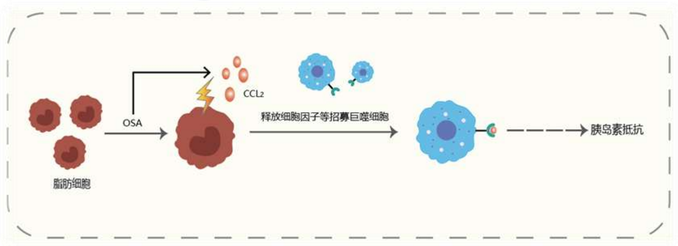
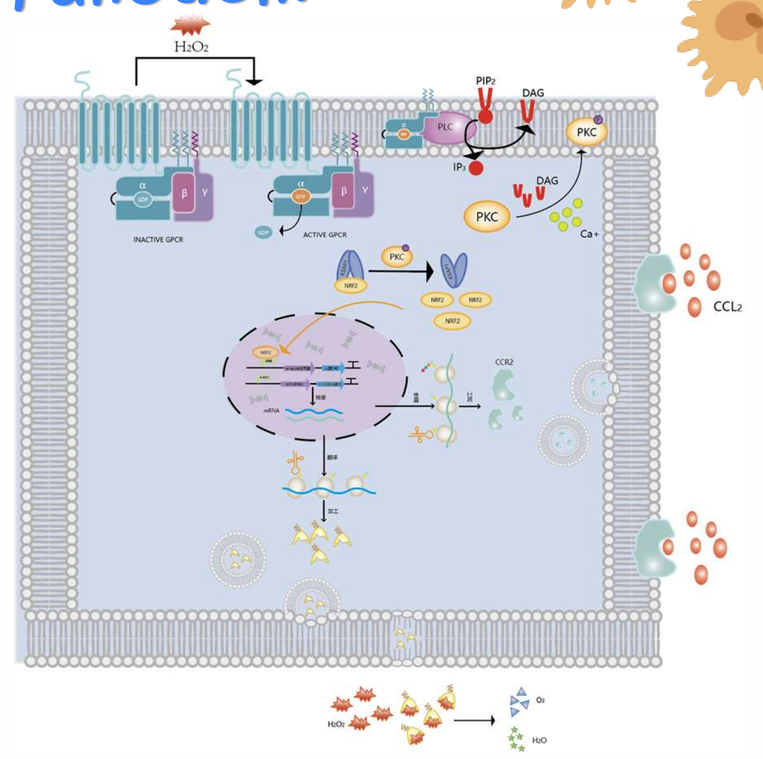


Fig.2:OSA upregulates CCL2, causing adipose inflammation, impairing insulin signaling, and inducing systemic insulin resistance.

Studies have demonstrated that **OSA directly upregulates CCL2 expression levels**;
CCL2 induces the recruitment and activation of macrophages in adipose tissue → macrophages secrete inflammatory factors → inflammatory factors disrupt insulin signaling pathways → insulin resistance occurs in adipocytes and the whole body.

Function:

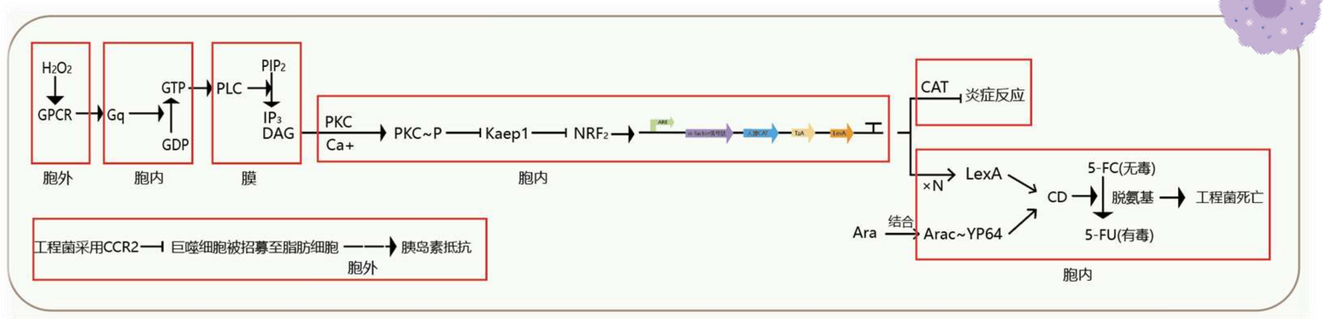


PS：**GPCR**：G 蛋白偶联受体；**PLC**：磷脂酶 C；**PIP_2**：磷脂酰肌醇二磷酸；**IP_3**：三磷酸肌醇；**DAG**：二酰甘油；**PKC**：蛋白激酶 C；**NRF2**：核因子红细胞 2 相关因子 2；**ARE**：抗氧化反应元件；**CCL2**：趋化因子配体 2；**CCR2**：趋化因子受体2

Fig.3: Extracellular H_2O_2 activates GPCR-Gq-PLC signaling, promoting NRF2 nuclear translocation to upregulate CAT and CCR2, thereby alleviating oxidative stress and inhibiting macrophage activation.

1. **Upstream Trigger**: Extracellular H_2O_2 acts on membrane-localized GPCR, converting it from inactive to active state ,thus initiating the signaling pathway.
2. **Signal Transduction:** Activated GPCR activates downstream Gq protein, which further initiates PLC. PLC hydrolyzes membrane PIP_2 into IP_3 and DAG, which synergize with intracellular Ca^{2+} to activate PKC via phosphorylation.
3. **Transcriptional Regulation:** Activated PKC phosphorylates NRF2, releasing it from the complex and promoting its nuclear translocation. NRF2 binds to the ARE of target genes, driving transcription and expression of CAT and other genes.CCR2 is normally expressed and localized to the cell membrane, binding CCL2 to reduce its interaction with macrophages. Intracellular CAT is activated, scavenging H_2O_2 to generate O_2 and H_2O, thus relieving oxidative stress.

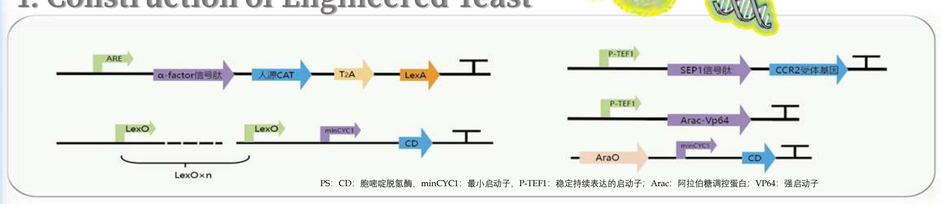
Circuit Design:



Stress Response: Extracellular H_2O_2 activates the GPCR-Gq-PLC-PKC signaling axis. It prompts NRF2 to dissociate and enter the nucleus, driving CAT gene expression. CAT scavenges H_2O_2 .CCR2 is normally expressed and localized on the cell membrane, competitively binding CCL2 to reduce its binding to macrophages.
Induced Suicide: Exogenous Ara binds to AraC-VP64, activating Lex A and CD gene expression. CD converts non-toxic 5-FC into toxic 5-FU to achieve controllable death of engineered bacteria.

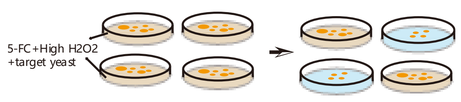
Experiment:

1. Construction of Engineered Yeast



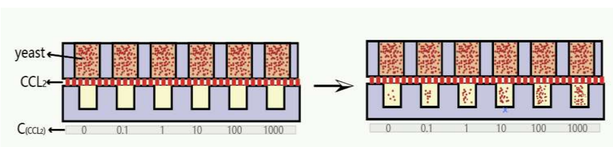
INTRODUCE:
1. human-derived CAT gene ,CCR2 receptor gene.
2. promoters: ARE, P-TEF1, minCYC1.
3. signal peptide genes: α-factor, SEP1.
4. regulatory genes: AraO, Arac-VP64; Lex A, LexO.

2. Target yeast screening



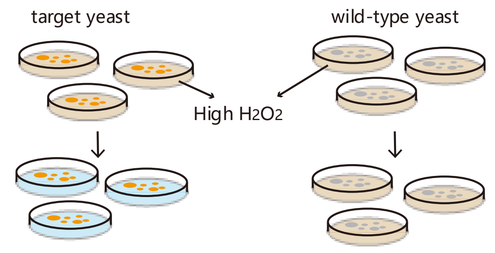
EXPECTED RESULT:
Some colonies began to decay significantly earlier than others with prolonged culture, showing higher transparency and slower expansion rate, indicating that the H2O2 -CAT-CD-5-FC axis functions properly..

3. Chemotaxis Validation (Yeast for CCL2 of different density)



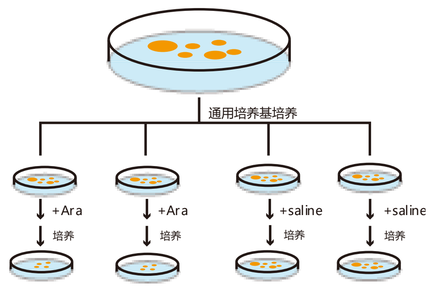
EXPECTED RESULT:
High concentrations of CCL2 show obvious targeted induction on target yeast, indicating the presence of CCR2 receptors on the yeast surface.

4. Therapeutic effect validation



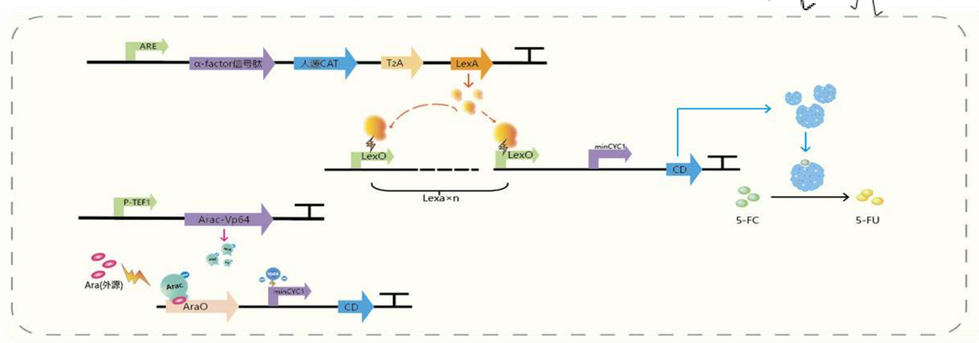
EXPECTED RESULT:
Medium with target yeast showed a significantly faster decrease in H_2O_2, indicating successful expression of CAT enzyme.

5. Verification of the suicide switch



EXPECTED RESULT:
No colonies were observed in the arabinose-treated group, indicating successful activation of the arabinose-induced suicide circuit.

Safety:



Mech.1: During CAT enzyme expression, Lex A protein is expressed simultaneously (T2A as the cleavage site to enable separate expression of CAT enzyme and Lex A); Lex A binds to the multi-copy site LexO, activating downstream minCYC1 to express CD.
Meth.2: Gene P-TEF1 activates the expression of the Arac-VP64 protein complex; after AraO binds to the protein complex, it activates downstream minCYC1 to express CD.

Fig.4:Essential Mechanism: CD is produced through two pathways, thereby inducing: CD → 5-FC(non-toxic) → deamination → 5-FU(toxic) → die

Modeling:

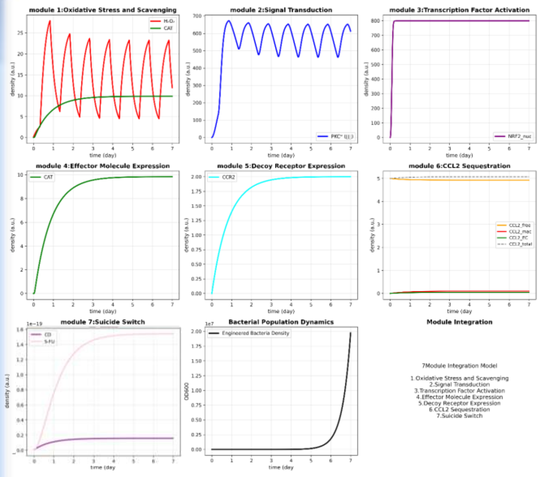
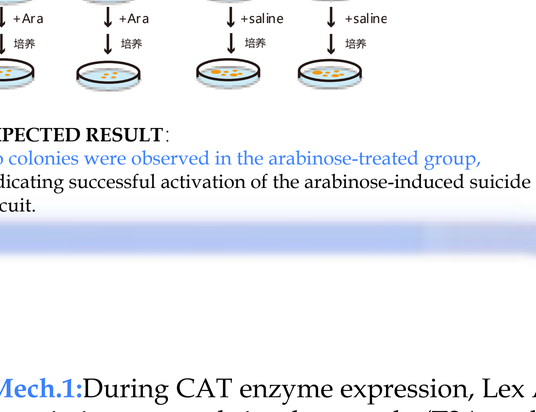


Figure 1：7-module

Module 1: Periodic H_2O_2 initiates the system; CAT rises gradually to scavenge H_2O_2 and form negative feedback.
Module 2: Activated PKC oscillates with H_2O_2, verifying the rapid response of the GPCR signaling axis.
Module 3: Nuclear NRF2 quickly saturates, efficiently driving the expression of downstream genes.
Module 4: Stable CAT expression continuously relieves oxidative stress by scavenging H_2O_2.
Module 5: CCR2 is stably expressed on the bacterial membrane as a decoy receptor for CCL2.
Module 6: Free CCL2 drops sharply, reducing macrophage-binding CCL2 and effectively blocking inflammatory pathways.
Module 7: Ara induction leads to CD expression stabilizing and 5-FU levels quickly rising to ensure , enabling controllable and efficient bacterial clearance.
Population Dynamics: Engineered bacterial density decreases rapidly after the suicide switch is activated, verifying biosafety.

Figure 2



This figure shows that the CCR2-CCL2 MD simulation system has successfully completed energy minimization and equilibration (NVT/NPT), reaching stable energy, temperature, and density, and is now ready for production MD.

- **Energy Minimization:** Potential energy drops to a stable minimum of -1.24×10^7 kJ/mol, confirming a well-relaxed initial structure.
- **NVT Equilibration:** Temperature stabilizes around the target 310 K, verifying thermal stability.
- **NPT Equilibration:** Density converges to 982.6 kg/m^3, close to the expected value, confirming system density equilibrium.
- **System Status:** All pre-simulation steps are complete, and the system is ready for production MD.

海军军医大学
NAVAL MEDICAL UNIVERSITY

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