# Glycogen Engineering Improves the Starvation Resistance of Mesenchymal stem cells and their Therapeutic Efficacy in Pulmonary Fibrosis


## Authors

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## Abstract

Mesenchymal stem cells (MSCs) are widely used in regenerative medicine, including the treatment of pulmonary fibrosis. However, implanted MSCs disappear within days, constraining therapeutic efficacy, which is largely attributed to nutrient deprivation. In this study, we established glycogen metabolism engineering strategies in mammalian cells. By expressing a functionally optimized glycogen synthase (GYSmut), MSCs could accumulate large amounts of glycogen rapidly as a reserve substance. Glycogen engineering significantly improved the survival of MSCs during starvation both in vitro and in vivo, enhancing cell viability post-implantation and their therapeutic efficacy in pulmonary fibrosis. Glycogen-engineered MSCs may serve as chassis cells for further applications. Our research highlights the importance of glucose metabolism regulation in cell-based therapy and demonstrates the great potential for the metabolic engineering of MSCs and other therapeutic cells.

**Keywords:** Mesenchymal stem cells; Glycogen; Pulmonary Fibrosis; Glycogen synthase; Cell survival.

## Introduction

Pulmonary fibrosis (PF) is traditionally regarded as an irreversible lung disease for which there are generally no effective drugs, with the exception of nintedanib and pirfenidone [1, 2]. Regenerative medicine represents an alternative approach, with several successful therapeutic applications in patients with chronic lung diseases, including PF [3-5]. The recent focus on mesenchymal stem cells (MSCs) has generated enthusiasm due to their unique regenerative and immunomodulating properties, including the ability to reverse fibrosis [6, 7]. Recent studies have begun to unravel the mechanisms underlying the therapeutic potential of MSCs, with a particular emphasis on their secretory properties. The paracrine factors secreted by MSCs, including fibroblast growth factor and cytokines, have been shown to mediate tissue repair and regeneration [6, 8]. However, the therapeutic efficacy of MSCs has been limited due to their poor survival and short dwell time in the hostile pathological microenvironment, which has hindered the translation of these promising preclinical findings into clinical practice[9, 10].

Metabolic regulation is crucial for cell survival post-implantation. It has been reported that the hypoxic in vivo microenvironment inhibits oxidative phosphorylation (OXPHOS) in implanted MSCs, forcing them to rely on glycolysis instead, which yields less ATP [11-13]. Our previous single-cell transcriptomic study also showed that the glucose metabolism pathway of MSCs was downregulated in the PF microenvironment [6]. Compared with in vitro culture systems, it is more difficult for implanted MSCs to obtain sufficient glucose in pathological microenvironments, and recent findings suggest that glucose exhaustion is the main reason for cell death after implantation [14]. Supplying glucose to MSCs improved their survival post-implantation [15]. Therefore, glucose metabolism engineering has the potential to improve MSC therapy by enhancing starvation resistance and prolonging the residence time of implanted cells.

Glycogen is a branched polymer that acts as the primary glucose storage form in mammalian cells, supplying energy under glucose deficiency and promoting cell survival. Glycogen metabolism is controlled by a series of enzymes including glycogen synthase (GYS) [16]. To reduce cell death of implanted MSCs caused by nutrient deprivation, we engineered the glycogen metabolism of MSCs with genes encoding essential enzymes, which promoted glycogen accumulation (Fig. 1A). The accumulated glycogen serves as an energy supply post-implantation, improving the viability and therapeutic efficacy of MSCs (Fig. 1B).

## Results

### Construction of glycogen strategies in mammalian cells

Glycogen is synthesized from UDP-glucose in a process involving glycogenin (GYG), UDP-glucose pyrophosphorylase (UGP), glycogen synthase (GYS), and glycogen-branching enzyme (GBE) [16]. GYG is the initiator of glycogen synthesis, UGP catalyzes the synthesis of UDP-glucose, GYS is responsible for the synthesis of glycogen, and GBE introduces branches into the structure of glycogen (Fig. 2A). Here, we firstly devised glycogen engineering strategies based on these essential enzymes.

Initially, we transiently expressed *GYS1* in human HEK293T cells, but it only had a limited, albeit significant effect on glycogen accumulation (Fig. 2B). We attributed the unsatisfactory results to substrate limitation, as well as potential inhibition of *GYS1* by cellular pathways. Next, we tried different expression strategies to increase glycogen accumulation, including (1) expressing *GYS1* alone to promote glycogen synthesis, (2) co-expressing *GYS1* with *GBE1* to increase glycogen-branching, (3) co-expressing *GYS1* with *GYG1* to increase glycogen initiation, and (4) co-expressing *GYS1* with *UGP1* to increase the substrate supply. Some protein kinases (e.g. AMPK) inactivate GYS1 by phosphorylation at specific serine sites [17]. Therefore, (5) we introduced Ser-Ala mutations at the Ser-8, Ser-641, Ser-645, Ser-649, Ser-653, Ser-654, and Ser-657 sites of GYS1 (GYSmut) to prevent phosphorylation. Both co-expression of *GYS1* with *UGP1* and expression of GYSmut alone resulted in greatly increased glycogen accumulation

compared to the control (~12 and 16 folds) and other strategies (Fig. 2B). Glycogen granules were clearly visible following periodic acid-Schiff (PAS) staining (Fig. 2C).

Since most of the Ser-Ala mutation sites were located near the C-terminus of GYSmut, we truncated the 100 C-terminal amino acids to generate GYSdelc. Although this truncated variant was still capable of inducing cellular glycogen accumulation, it exhibited a reduced rate in comparison to full-length GYSmut (Fig. S1).

**Glycogen accumulation improved the starvation resistance of MSCs**

After expressing GYSmut in MSCs by lentivirus transduction, glycogen accumulation was significantly increased in the engineered MSCs (Fig. 3A). PAS staining showed more PAS-positive glycogen granules in GYSmut MSCs (Fig. 3B), which altered cell morphology by significantly increasing the cell volume, as assessed by flow cytometry (Fig. S2A). Glycogen granules were located both in the cytoplasm and nucleus (Fig. S2B).

To evaluate the starvation resistance of glycogen-engineered MSCs, we cultured GYSmut MSCs in Dulbecco's Phosphate-Buffered Saline (DPBS) instead of a complete culture medium to create a nutrient-deprived environment. The Cell-Counting-Kit-8 assay was used to measure cell viability at various time points. Notably, approximately 40% of the GYSmut MSCs were still viable after 72 hours of starvation culture, whereas wild-type MSCs died rapidly after 8 hours (Fig. 3C, S2C). After 48 hours of starvation, GYSmut MSCs still retained a 39% glycogen reserve (Fig. 3D), indicating that the accumulated glycogen could be a long-term energy resource to maintain higher cell viability during nutrient deprivation following implantation.

As glycogen is a crucial node of carbon metabolism in mammalian cells, we next investigated if glycogen engineering caused global changes of MSCs. Cell counting showed that glycogen engineering had no significant effect on cell proliferation (Fig. 3E). To evaluate the effects of glycogen engineering on the adipogenic differentiation capacity

of MSCs, we induced GYSmut MSCs with adipocyte medium for 5 days, followed by oil red O staining and qRT-PCR of the adipocyte differentiation marker *Lpl*. The results indicated a decrease in the adipocyte differentiation potential of GYSmut MSCs (Fig. 3F, 3G). It was reported that adipogenic differentiation and the immunomodulatory functions of MSCs are mutually exclusive [18], so blocking adipocyte differentiation may enhance the immunosuppressive activity of MSCs.

We next performed bulk RNA sequencing (RNA-seq) to understand the effect of glycogen engineering on the transcriptome of GYSmut MSCs. A total of 759 genes were up- and 542 were downregulated in GYSmut MSCs, indicating a broad effect on the transcriptome (Fig. S2D). Gene ontology (GO) enrichment analysis revealed that differentially transcribed genes were predominantly enriched in processes related to cellular differentiation, morphogenesis, and the regulation of various metabolic pathways, including lipid metabolism, reflecting potential metabolic reprogramming. The differentially expressed genes also enriched in the terms related to processes of muscle, kidneys, and neural tissue, which are sites where glycogen is naturally distributed in mammals (Fig. 3H) [16]. KEGG analysis showed the PI3K-Akt signaling pathway was also modulated, which is crucial for cell survival (Fig. 3I). The GSEA results showed that the inflammatory response gene set was upregulated, which may be beneficial in the treatment of PF, while mTORC1 and glycolysis-related gene sets were downregulated (Fig. 3J). It was reported that mTORC1 responds to cellular amino acid and glucose levels [19], which is in agreement with the hypothesis that glycogen synthesis affects cellular glucose levels and the process of glycolysis.

**Glycogen engineering promoted the survival of MSCs post-implantation**

In our previous study, intratracheal administration of MSCs exhibited a robust therapeutic effect in a mouse model of bleomycin-induced pulmonary fibrosis (PF) [6], and further scRNA-seq analysis of implanted MSCs within the PF microenvironment revealed downregulation of glucose metabolism (Fig. S3), suggesting that nutrient availability is a critical determinant of MSC viability and may potentially limit their therapeutic efficacy.

We, therefore, tested if glycogen engineering would promote the survival of GYSmut MSCs in vivo. PF mice were intratracheally injected with glycogen-engineered MSCs co-expressing GYSmut and *Gaussia* luciferase (GYSmut-Gluc), while the control group was injected with Gluc MSCs. We collected lungs of PF mice on days 0 and 7 after MSC implantation, and measured the fluorescence intensity of lung tissue homogenates to assess the counts of living MSCs (Fig. 4A). A higher proportion of surviving GYSmut-Gluc MSCs was detected than in the control (Fig. 4B).

To assess the persistence and biodistribution of implanted MSCs within the PF microenvironment, we intratracheally injected GYSmut MSCs co-expressing Akaluc (GYSmut-Akaluc) into PF mice. This approach, in combination with the substrate Akalumine [20], allows for highly sensitive live imaging and thus provides insights into the retention of implanted cells. In the control group, MSCs expressing Akaluc were injected. As expected, on day 7 post-implantation, higher fluorescence intensity was detected in the GYSmut MSCs group compared with the control (Fig. 4C), indicating the greater survival ability of GYSmut MSCs compared to wild-type MSCs in the PF microenvironment.

**Engineered MSCs demonstrated a beneficial effect on pulmonary fibrosis**

To investigate the therapeutic efficacy of glycogen-engineered MSCs (GYSmut MSCs), we administered them via tracheal injection into mice on day 3 following bleomycin treatment, and recorded the survival rate and body weight of mice over time. GYSmut MSCs demonstrated a more pronounced effect than the control group expressing only GFP (Fig. 5A). Quantitative analysis of lung tissue sections stained with H&E and Masson's trichrome revealed that the GYSmut MSCs group exhibited superior therapeutic efficacy on day 7, as evidenced by reduced collagen deposition and preserved alveolar size (quantified by mean linear intercept, MLI) compared to the control (Figs. 5B and C). The improved therapeutic efficacy illustrated the beneficial effect of enhanced MSC viability and confirmed the rationale for glycogen engineering.

## Discussion

Glycogen is found in a wide range of organisms ranging from bacteria to mammals. As a major form of stored glucose, glycogen plays an essential role in maintaining energy homeostasis and cell survival. In this study, we engineered the glycogen metabolism of MSCs to promote glycogen accumulation. We tested several engineering strategies in human HEK 293T cells and primary MSCs derived from mice. Over-expression of phosphorylation-resistant GYS (GYSmut), as well as the co-expression of GYS and UGP, induced intense glycogen. Glycogen engineering enhanced the starvation resistance of MSCs and improved their viability post-implantation. Compared with the control group, the engineered MSCs exhibited a significantly enhanced therapeutic effect on pulmonary fibrosis, indicating the importance of glucose metabolism regulation for MSC-based therapy.

Glycogen-engineered MSCs may serve as chassis cells for further applications by enhancing cell viability post-implantation, and our glycogen engineering strategies also have the potential to be applied to other therapeutic cells. Transient expression of GYSmut by mRNA transfection may be a safer alternative than lentiviral transduction, avoiding the risks of chromosomal modification. LNP-mediated circular mRNA transfection would further improve engineering efficiency[21].

We investigated the effect of glycogen engineering on the transcriptome of MSCs by RNA-seq, which revealed large-scale gene transcription changes. In future studies, complex regulatory networks may be elucidated at the levels of proteomics and metabolomics. Previous studies have revealed roles of glucose metabolism regulation in MSCs' immunoregulatory properties [22, 23]. The broader impact of glycogen engineering on MSCs remains to be elucidated. We tested therapeutic efficacy of glycogen engineered MSCs in the bleomycin-induced PF mice model. Their efficacy in other disease models is still to be investigated.

In previous studies, complex gene circuits were introduced to enhance the efficacy of various cell-based therapies, including MSCs and CAR-T cells [24, 25], and some research

also revealed the advantages of metabolic engineering (ME) [26]. However, in contrast to extensive ME research in prokaryotic organisms, ME of mammalian therapeutic cells has yet to be developed. Here, we demonstrated the great potential of glycogen engineering, offering a basis for future studies. By dynamically controlling essential enzymes, cellular metabolism can be remodeled to achieve optimal survival and therapeutic efficacy post-implantation. Further research in this area will provide additional opportunities for cell therapy, gene therapy, and tissue engineering.

## Materials and methods

### Animals and experimental design

All the animal experiments were authorized by the institutional animal care and use committee of Tsinghua University. Six-week-old male C57BL/6J mice were housed in the specific pathogen-free experimental animal environment at the laboratory animal research center of Tsinghua University, with 5~6 mice per cage having access to sterilized food and water ad libitum under a 12-hour light/dark cycle. Mice were anesthetized with avertin (200μL per mouse) or 2.5% tribromoethanol. and euthanized by cervical dislocation or $CO_2$ inhalation. 76 mice were used in total. To obtain reliable results, each group contains at least 3 mice. In the same experiment the mice in each group came from the same batch and were randomly captured, processed and assigned to different cages. The numbers of mice is randomly assigned, the surgeon knows the grouping situation, and the statistical analyst does not know the grouping in advance. All animal experiments were authorized by the institutional animal care and use committee of Tsinghua University (Approval No. 23-WQ1.G23-1, Study on different factors on the survivability of implanted MSCs in murine pulmonary fibrosis model, 2023-01-18 to 2026-04-12).

### Isolation of mouse adipose-derived stem cells

MSCs were obtained by euthanizing the mice, digesting the inguinal adipose tissue with collagenase type I (1.0 mg/ml, Sigma), and culturing the released cells in mouse ADSC

basal medium (CYAGEN, China) in a humidified atmosphere containing 5% carbon dioxide at 37°C, according to a previously published method [6].

**Construction of the BLM-induced PF mouse model and administration of MSCs**

Mice were anesthetized with 2.5% tribromoethanol (0.8% NaCl, 1 mM Tris (pH 7.4), 0.25 mM EDTA (pH 7.4)) and administered 3.5 mg/kg bleomycin solution (Sigma, B5507-15un) in 50 μL phosphate-buffered saline (PBS) intratracheally on day 0 using a 1 mL syringe with a 25G needle. Control animals were mock-treated with PBS [6].

Injection of MSCs (at passages 3~6) was performed on day 3 after BLM treatment. Each animal received an intratracheal injection of 50 μL of a suspension comprising $1 \times 10^7$ cells/mL in PBS ($5 \times 10^5$ cells/mouse) using a 1 mL disposable syringe with a 25G needle. For the control and BLM + PBS groups, 50 μL PBS was injected instead. Before implantation, the cells were washed three times to remove the culture medium. The body weight of treated mice was recorded daily [6, 27].

**Histological evaluation of lung damage and collagen deposition of BLM-induced lung injury**

To assess the development of PF and therapeutic efficacy, mice were evaluated on day 7. For lung tissue collection, mice were euthanized by $CO_2$ inhalation. The thoracic cavity was opened and the lungs were perfused and washed with ice-cold PBS. The lungs were then fixed with 4% (v/v) paraformaldehyde in PBS and processed into 5-μm thick paraffin sections, which were mounted on glass slides.

For histological assessment of alveolar architecture and fibrosis, sections were stained with hematoxylin and eosin (H&E) or Masson's trichrome stain, respectively. The samples were scanned using Pannoramic SCAN (3DHISTECH) with a 20× objective. Fibrosis area percentages and alveolar size (quantified by mean linear intercept, MLI) were calculated according to a previously published method[6, 28].

**Generation of genetically engineered MSCs and in vitro tests**

HEK293T cells were cultured in DMEM with 10% FBS. Sequences of essential enzymes are amplified from cDNA of mouse MSCs. To test combinations of essential enzymes, HEK293T cells in 6-well plates were transiently transfected with encoding plasmids (2μg/well) using Lipo8000 (Beyotime, C0533). Co-expressed enzymes were connected by the 2A sequence. After 3 days, the glycogen content of each group was quantified using a commercial kit (SOLARBIO, BC0345-100T). A PAS staining kit (Beyotime, C0142S) was used to stain glycogen granules.

To construct the lentiviral vectors, designed gene sequences were cloned into the pCDH backbone, and used to co-transfect HEK293T cells with pMD2.G and psPAX2 plasmids using Lipo8000. Essential enzymes were co-expressed by connecting them with EGFP or puromycin N-acetyltransferase via the 2A sequence. The cell supernatant was collected every 24 hours for 3 days. The viral particles were concentrated using Lentivirus Concentration Solution (Servicebio, G1801-100ML), after which the infectious solution was prepared by resuspending them in 200μL PBS and stored at -80℃ until use. When the density of MSCs in the 6-well plate reached approximately 80%, 20 μl of the lentiviral solution was added. Following 2 days after infection, puromycin was added to a final concentration of 1 mg/L for selection.

Glycogen was quantified after 3 days of induction. To simulate starvation conditions, DPBS solution was used to replace the culture medium of seeded MSCs (96-well-plate, 5000 cells/well), and a CCK-8 assay kit (BIORIGIN, BN15201) was used to measure cell viability. To test the adipogenic differentiation potential of MSCs, we induced them with commercial adipocyte medium (Procell, PD-027) for 5 days as described in the manufacturer’s protocol, and then performed oil red O staining (Beyotime, C0157S).

**Viability detection and live imaging of MSCs post-implantation in the PF model**

To detect viability changes of MSCs post-implantation, $2.5 \times 10^5$ MSCs expressing *Gaussia* luciferase suspended in 50 μl of PBS were injected intratracheally into PF mice, as described above. The whole lungs of mice were collected on the day of surgery (D0) and the $7^{th}$ day (D7) after surgery (n=3), and were stored in a 1.5 mL centrifuge tube at -

80 ℃. Then, 400 μL PBS and 20 1-mm-beads (Servicebio, G0201) were added to the centrifuge tube with lungs for processing with a KZ-III high-speed homogenizer (Servicebio) at 60Hz for 30s, 6 times. The homogenate was centrifugated (10000g, 5 min) and the activity of *Gaussia* luciferase in the suspension was detected using a commercial kit (GeneCopoeia, LF061). Lungs without cells expressing *Gaussia* luciferase served as a negative control, and the corresponding signals were considered to represent the background. Cell viability changes of each group were assessed by calculating the ratio of D7 to D0.

To detect the in vivo distribution of MSCs post-implantation, MSCs expressing Akaluc were injected intratracheally into PF mice (n=5), as described above. Live imaging was performed on the 3$^{rd}$ and 7$^{th}$ days after implantation. Akalumine-HCL (InvivoChem, V41343) was dissolved in PBS (2.5 mg/mL) and intraperitoneally injected into mice (100 μL/mouse). Luminescence was detected after 10 minutes using a Lumina III instrument and analyzed with Living Image software version 4.8.

**RNA-seq of MSCs**

MSCs were cultured in 100-mm dishes ($5\times10^6$ cells per dish) in DMEM/F-12 medium. Trizol (Vazyme, R401-01) was used for RNA extraction. Illumina RNA-seq and subsequent analysis were performed by ANNOROAD Corporation (Beijing, China). DESeq2 was used to identify differentially expressed genes (|log2 fold-change|≥1, adjusted p-value < 0.05).

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism software version XY (GraphPad Software Inc). Significant differences between groups were identified using unpaired Student's *t*-test or 2-way ANOVA. Asterisks were used to indicate significance in figures (*: p<0.05, **: P<0.01, ***: p<0.001, ****: p<0.0001). Unless indicated otherwise, all quantitative data are presented as mean values ± SD.

## Authors' contributions

Y.Y.X. and M.R. designed the experiments. Q.W. supervised the project. Y.Y. X., M. R., Z.Y. W., H.W. X. and B.Z. performed the experiments. H.Q. X., L. W., and S. C. analyzed the data. Q.W., Y.Y.X., M. R. and X.D. S. wrote the manuscript.

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The authors declare that they have not used Artificial Intelligence in this study.

## Statements and Declarations

### Ethical considerations

All animal experiments received approval from the institutional animal care and use committee of Tsinghua University (Project title: Study on different factors on the survivability of implanted MSCs in murine pulmonary fibrosis model, Approval number: 23-WQ1.G23-1, Date of approval: 2023-01-18).

### Declaration of conflicting interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Availability of data and materials

The dataset generated by this study is available in GSE281974 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE281974]. Additional data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Figures and Tables

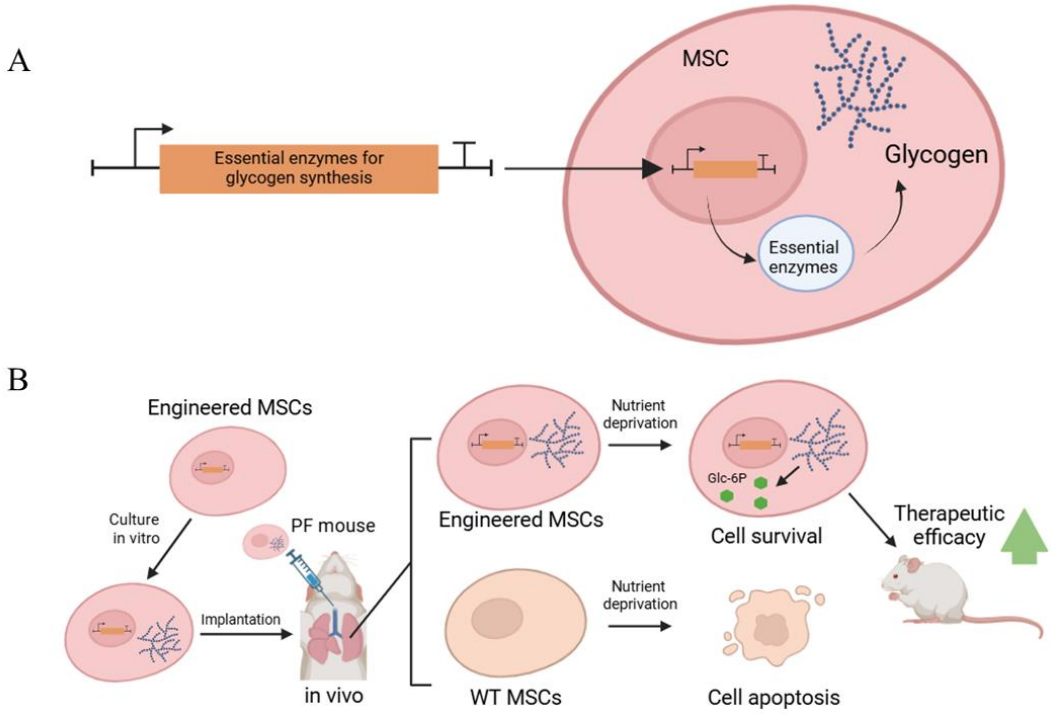



**Fig. 1. Schematic illustration of the strategy used to enhance the therapeutic efficacy of MSCs through glycogen engineering.**

(A) Engineering glycogen metabolism of MSCs by over-expressing essential enzymes of glycogen synthesis. (B) Engineered MSCs use glycogen as energy supply after implantation, improving cell viability and therapeutic efficacy.

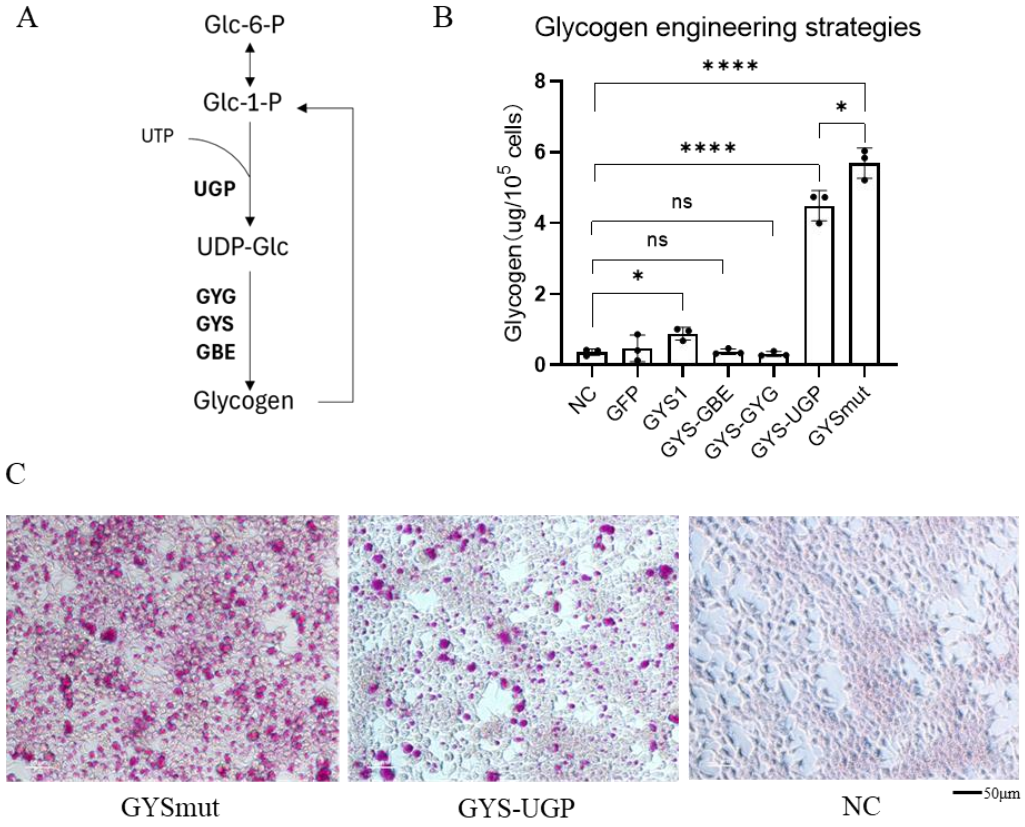



**Fig. 2. Construction of glycogen engineering strategies.**

(A) Essential enzymes of glycogen synthesis. (B) Tests of essential enzyme combination strategies in HEK293T cell by transient transfection. The glycogen content of each group was measured. (C) Periodic acid-Schiff (PAS) staining of cells expressing GYSmut and GYS-UGP revealed significant accumulation of glycogen granules.

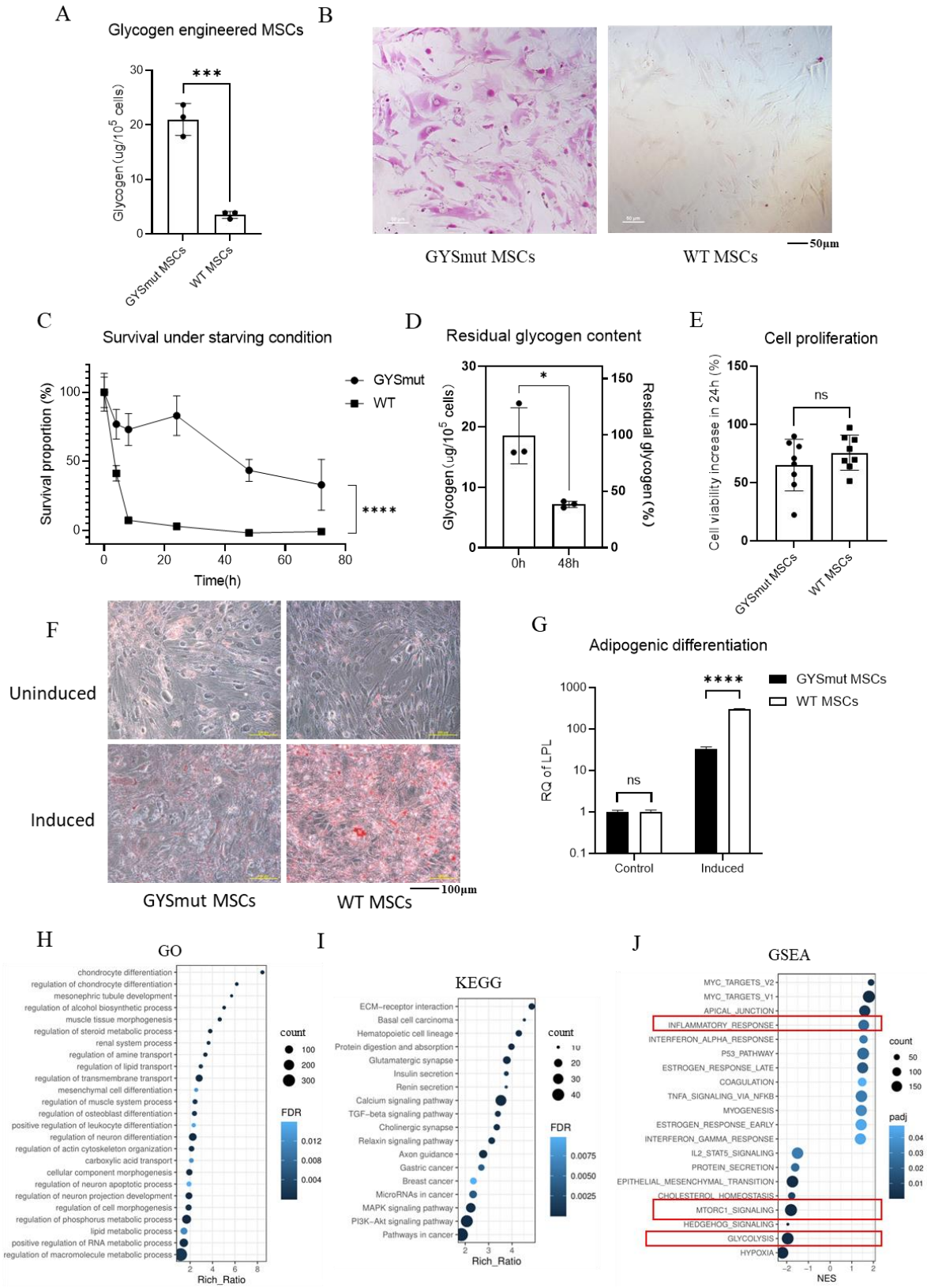

A
Glycogen engineered MSCs
Glycogen (ug/10^5 cells)
***
GYSmut MSCs
WT MSCs
B
GYSmut MSCs
WT MSCs
50μm
C
Survival under starving condition
Survival proportion (%)
Time(h)
GYSmut
WT
****
D
Residual glycogen content
Glycogen (ug/10^5 cells)
Residual glycogen (%)
*
0h
48h
E
Cell proliferation
Cell viability increase in 24h (%)
ns
GYSmut MSCs
WT MSCs
F
Uninduced
Induced
GYSmut MSCs
WT MSCs
100μm
G
Adipogenic differentiation
RQ of LPL
****
ns
GYSmut MSCs
WT MSCs
Control
Induced
H
GO
chondrocyte differentiation
regulation of chondrocyte differentiation
mesonephric tubule development
regulation of alcohol biosynthetic process
muscle tissue morphogenesis
regulation of steroid metabolic process
renal system process
regulation of amine transport
regulation of lipid transport
regulation of transmembrane transport
mesenchymal cell differentiation
regulation of muscle system process
regulation of osteoblast differentiation
positive regulation of leukocyte differentiation
regulation of neuron differentiation
regulation of actin cytoskeleton organization
carboxylic acid transport
cellular component morphogenesis
regulation of neuron apoptotic process
regulation of neuron projection development
regulation of cell morphogenesis
regulation of phosphorus metabolic process
lipid metabolic process
positive regulation of RNA metabolic process
regulation of macromolecule metabolic process
Rich_Ratio
count
FDR
I
KEGG
ECM-receptor interaction
Basal cell carcinoma
Hematopoietic cell lineage
Protein digestion and absorption
Glutamatergic synapse
Insulin secretion
Renin secretion
Calcium signaling pathway
TGF-beta signaling pathway
Cholinergic synapse
Relaxin signaling pathway
Axon guidance
Gastric cancer
Breast cancer
MicroRNAs in cancer
MAPK signaling pathway
PI3K-Akt signaling pathway
Pathways in cancer
Rich_Ratio
count
FDR
J
GSEA
MYC_TARGETS_V2
MYC_TARGETS_V1
APICAL_JUNCTION
INFLAMMATORY_RESPONSE
INTERFERON_ALPHA_RESPONSE
P53_PATHWAY
ESTROGEN_RESPONSE_LATE
COAGULATION
TNFA_SIGNALING_VIA_NFKB
MYOGENESIS
ESTROGEN_RESPONSE_EARLY
INTERFERON_GAMMA_RESPONSE
IL2_STAT5_SIGNALING
PROTEIN_SECRETION
EPITHELIAL_MESENCHYMAL_TRANSITION
CHOLESTEROL_HOMEOSTASIS
MTORC1_SIGNALING
HEDGEHOG_SIGNALING
GLYCOLYSIS
HYPOXIA
NES
count
padj

**Fig. 3. Construction of glycogen engineered MSCs.**

(A) Glycogen content of GYSmut MSCs. (B) PAS staining of GYSmut MSCs, showing glycogen granules (red). (C) Survival of engineered MSCs under DPBS (starvation) treatment in vitro (N = 8). (D) Residual glycogen content of GYSmut MSCs after DPBS (starvation) treatment for 48 hours. (E) Viability of GYSmut MSCs according to the CCK8 assay. (F, G) Adipogenic differentiation potential of GYSmut MSCs, assessed by oil red O staining and qPCR detection of Lpl expression (n=3, unpaired *t*-test P-value < 0.0001). (H) GO enrichment analysis of differentially expressed genes (DEGs) between GYSmut MSCs and the GFP control. (I) KEGG analysis of DEGs. (J) Gene set enrichment analysis (GSEA) of the DEGs.

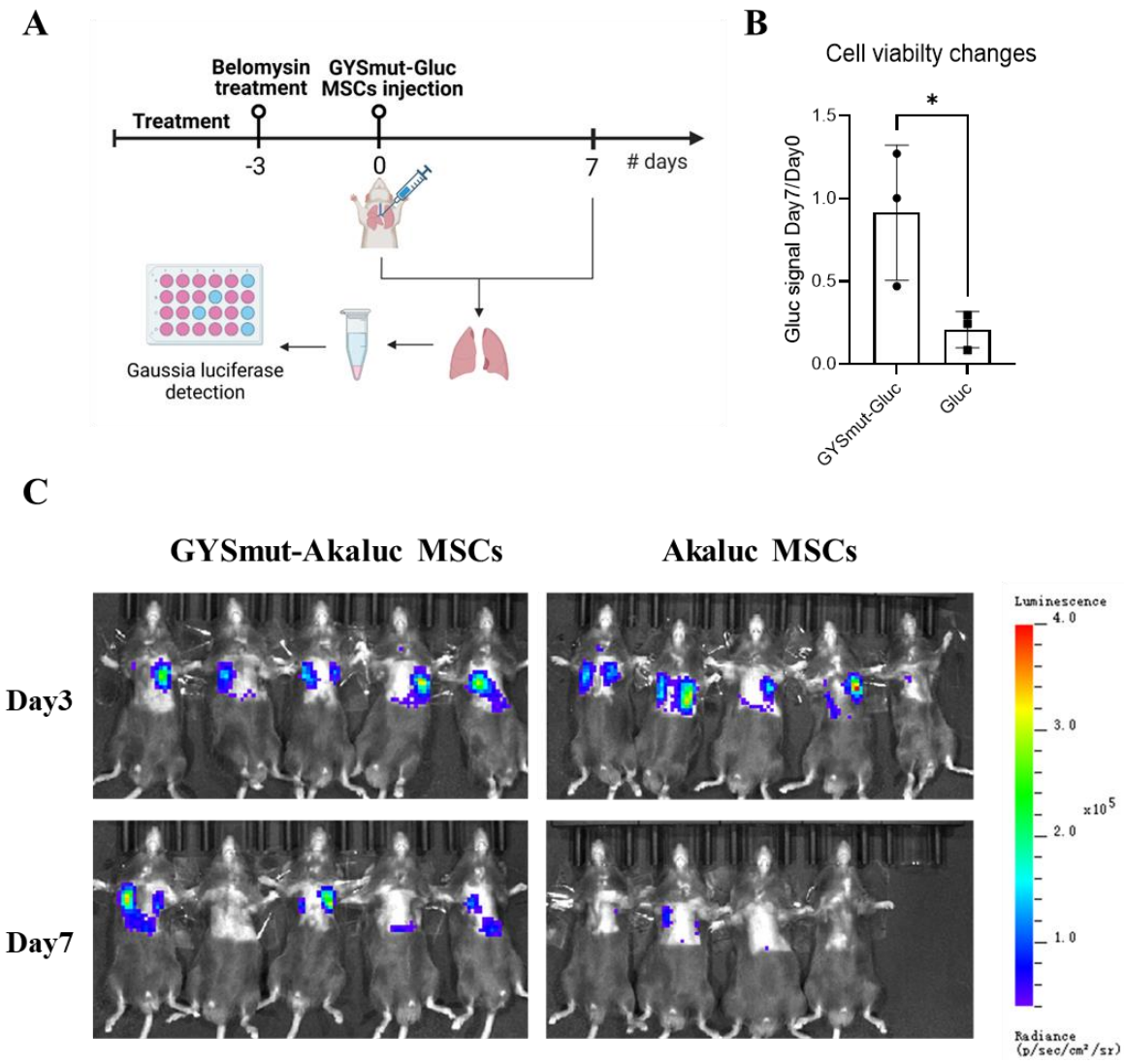



**Fig. 4. Survival of implanted glycogen-engineered MSCs.**

(A) Schematic illustration of the strategy used to assess the survival of MSCs by detecting *Gaussia* luciferase activity in the homogenate of lungs. (B) Changes of *Gaussia* luciferase activity in the two groups on day 7 post-implantation (n = 3, unpaired *t*-test P-value = 0.044). (C) Live imaging of implanted GYSmut-Akaluc MSCs and control cells. One mouse from the control group died on day 7 (n = 5).

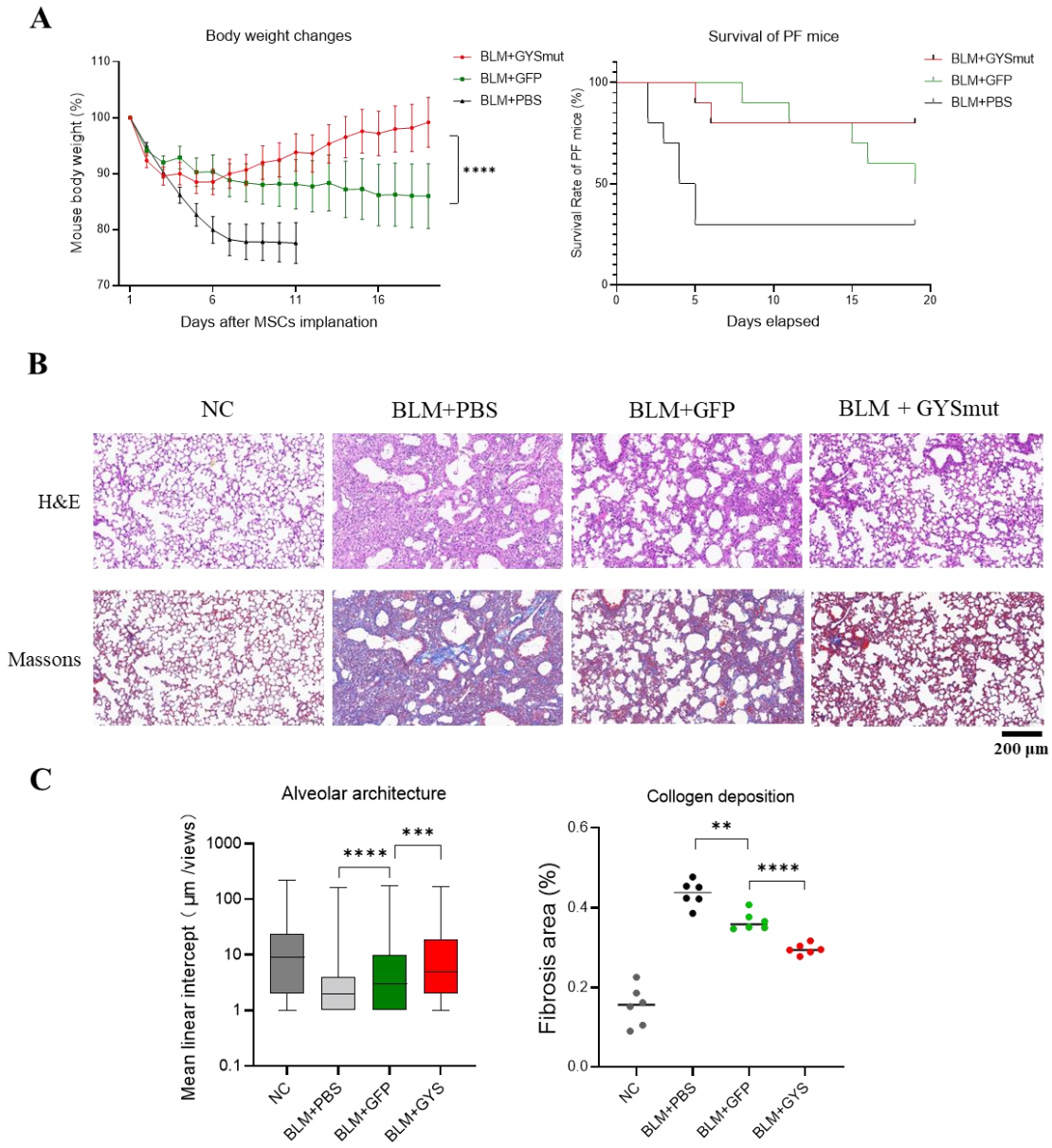
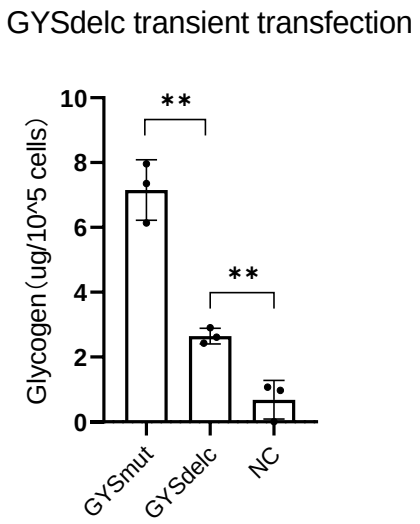



**Fig. 5. Therapeutic efficacy of glycogen-engineered MSCs.**

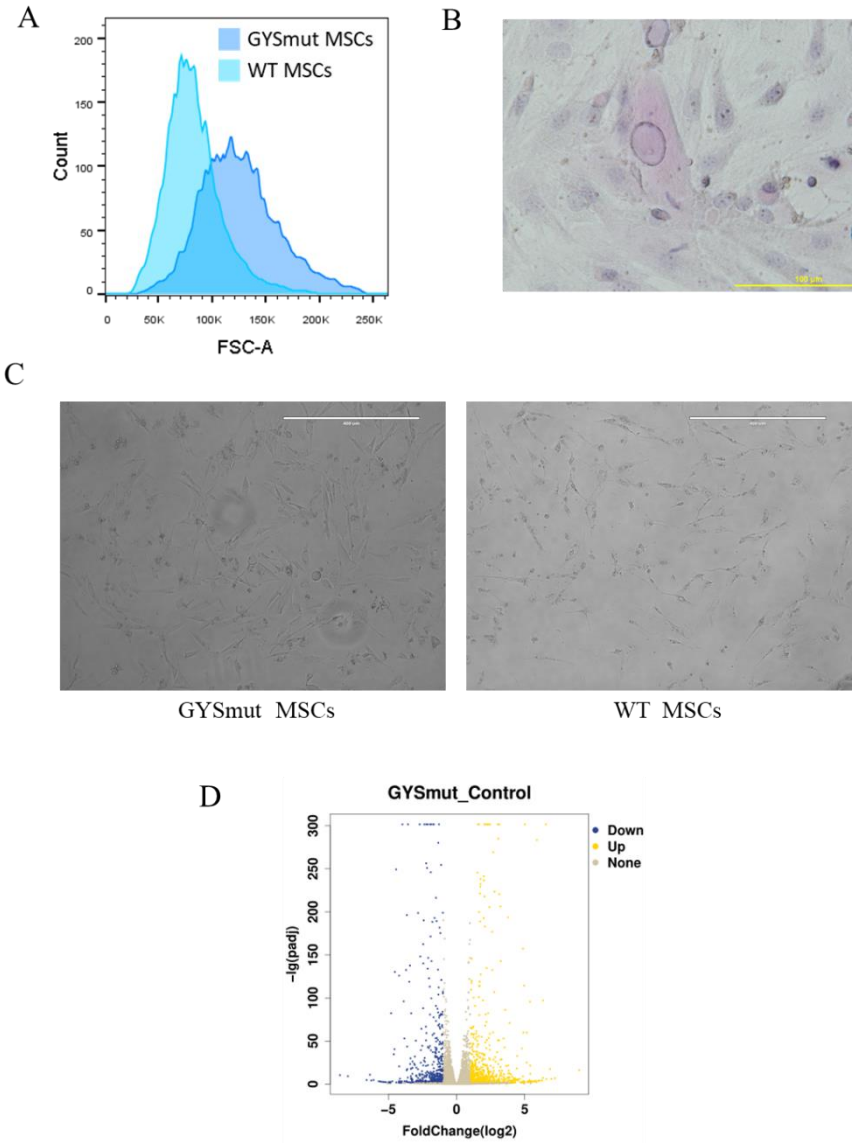
**(A)** Survival and body weight changes of PF mice treated with GYSmut MSCs and control cells (n = 10, mean ± SEM, 2-way ANOVA P-value=0.0001), (**B**) Representative lung tissue sections stained with H&E and Masson's trichrome. NC group are healthy mice. **(C)** Collagen deposition and preserved alveolar size (quantified by mean linear intercept, MLI) of lung tissue sections (n = 6).

**Fig. S1.**



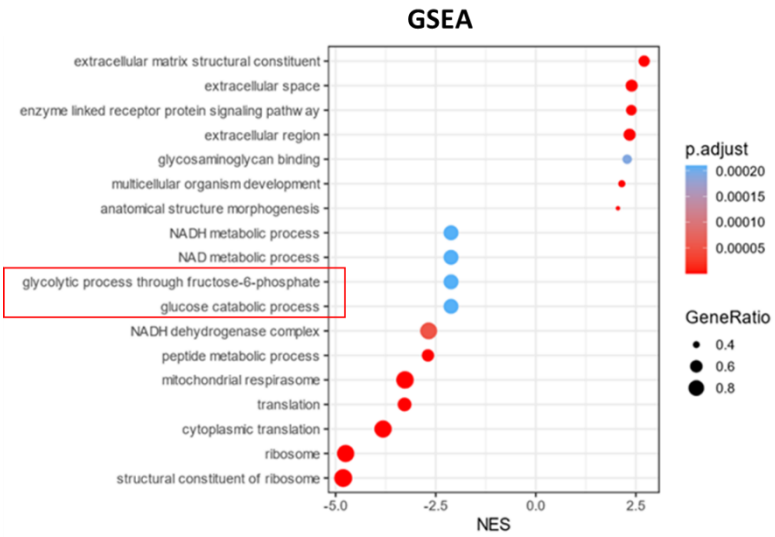

GYSdelc promoted glycogen accumulation.

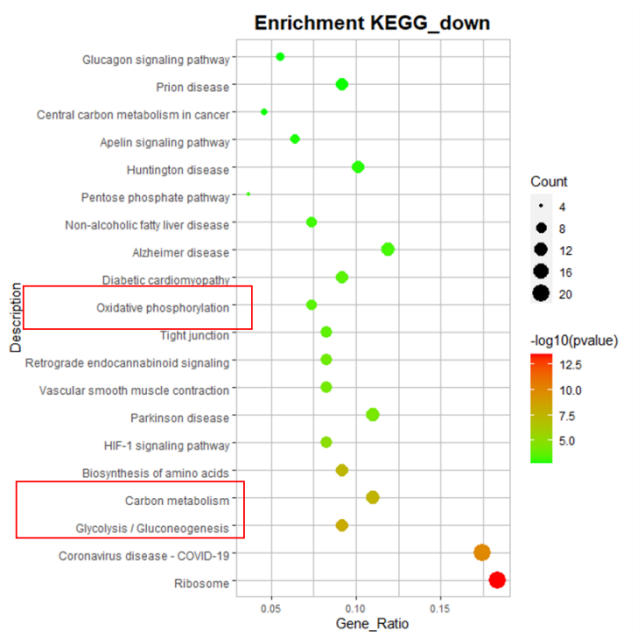
**Fig. S2.**




Impacts of glycogen engineering on MSCs.
(A)Cell volume change of engineered MSCs, assessed through flow cytometry. (B) PAS and hematoxylin staining of GYSmut MSCs. Glycogen (red) distributed in cell nucleus (blue) and cytoplasm. (C) GYSmut and wildtype MSCs after DPBS treatment for 8 hours. Scale bar, 400 μm. (D) Impacts of glycogen engineering on transcriptome of MSCs.

**Fig. S3.**




KEGG and GSEA analysis of implanted MSCs in our previous research. GFP MSCs were intratracheally administrated to PF mice and collected through flow cytometry. Single-cell sequencing showed down-regulation of glucose metabolism.

Table 1: Protein sequences used in this study

| | |
|---|---|
| GYS1(wt) | MPLSRSLSVSSLPGLEDWEDEFDPENAVLFEVAWEVANKVGGIYTVLQTKAKVT<br>GDEWGDNYYLVGPYTEQGVRTQVELLEPPTPELKRTLDSMNSKGCKVYFGRWLI<br>EGGPLVVLLDVGASAWALERWKGELWDTCNIGVPWYDREANDAVLFGFLTTWF<br>LGEFLAQNEEKPYVVAHFHEWLAGVGLCLCRARRLPVATIFTTHATLLGRYLCA<br>GAVDFYNNLENFNVDKEAGERQIYHRYCMERAAAHCAHVFTTVSQITAIEAQHL<br>LKRKPDIVTPNGLNVKKFSAMHEFQNLHAQSKARIQEFVRGHFYGHLDFNLDKTL<br>YFFIAGRYEFSNKGADVFLEALARLNYLLRVNGSEQTVVAFFIMPARTNNFNVET<br>LKGQAVRKQLWDTANTVKEKFGRKLYESLLVGSLPDMNKMLDKEDFTMMKRAI<br>FATQRQSFPPVCTHNMLDDSSDPILTTIRRIGLFNSSADRVKVIFHPEFLSSTSPLLP<br>VDYEEFVRGCHLGVFPSYYEPWGYTPAECTVMGIPSISTNLSGFGCFMEEHIADPS<br>AYGIYILDRRFRSLDDSCSQLTSFLYSFCQQSRRQRIIQRNRTERLSDLLDWKYLGR<br>YYMSARHMALAKAFPDHFTYEPHEVDATQGYRYPRPASVPPSPSLSRHSSPHQSE<br>DEEEPRDGPLGEDSERYDEEEEAAKDRRNIRAPEWPRRASCSSSTGGSKRSNSVDT<br>GPSSSLSTPTEPLSPTSSLGEERN |
| GYSmut | MPLSRSLAVSSLPGLEDWEDEFDPENAVLFEVAWEVANKVGGIYTVLQTKAKVT<br>GDEWGDNYYLVGPYTEQGVRTQVELLEPPTPELKRTLDSMNSKGCKVYFGRWLI<br>EGGPLVVLLDVGASAWALERWKGELWDTCNIGVPWYDREANDAVLFGFLTTWF<br>LGEFLAQNEEKPYVVAHFHEWLAGVGLCLCRARRLPVATIFTTHATLLGRYLCA<br>GAVDFYNNLENFNVDKEAGERQIYHRYCMERAAAHCAHVFTTVSQITAIEAQHL<br>LKRKPDIVTPNGLNVKKFSAMHEFQNLHAQSKARIQEFVRGHFYGHLDFNLDKTL<br>YFFIAGRYEFSNKGADVFLEALARLNYLLRVNGSEQTVVAFFIMPARTNNFNVET<br>LKGQAVRKQLWDTANTVKEKFGRKLYESLLVGSLPDMNKMLDKEDFTMMKRAI<br>FATQRQSFPPVCTHNMLDDSSDPILTTIRRIGLFNSSADRVKVIFHPEFLSSTSPLLP<br>VDYEEFVRGCHLGVFPSYYEPWGYTPAECTVMGIPSISTNLSGFGCFMEEHIADPS<br>AYGIYILDRRFRSLDDSCSQLTSFLYSFCQQSRRQRIIQRNRTERLSDLLDWKYLGR<br>YYMSARHMALAKAFPDHFTYEPHEVDATQGYRYPRPAAVPPAPSLARHAAPHQ<br>AEDEEEPRDGPLGEDSERYDEEEEAAKDRRNIRAPEWPRRASCSSSTGGSKRSNSV<br>DTGPSSSLSTPTEPLSPTSSLGEERN |
| GYSdelc | MPLSRSLAVSSLPGLEDWEDEFDPENAVLFEVAWEVANKVGGIYTVLQTKAKVT<br>GDEWGDNYYLVGPYTEQGVRTQVELLEPPTPELKRTLDSMNSKGCKVYFGRWLI<br>EGGPLVVLLDVGASAWALERWKGELWDTCNIGVPWYDREANDAVLFGFLTTWF<br>LGEFLAQNEEKPYVVAHFHEWLAGVGLCLCRARRLPVATIFTTHATLLGRYLCA<br>GAVDFYNNLENFNVDKEAGERQIYHRYCMERAAAHCAHVFTTVSQITAIEAQHL<br>LKRKPDIVTPNGLNVKKFSAMHEFQNLHAQSKARIQEFVRGHFYGHLDFNLDKTL<br>YFFIAGRYEFSNKGADVFLEALARLNYLLRVNGSEQTVVAFFIMPARTNNFNVET<br>LKGQAVRKQLWDTANTVKEKFGRKLYESLLVGSLPDMNKMLDKEDFTMMKRAI<br>FATQRQSFPPVCTHNMLDDSSDPILTTIRRIGLFNSSADRVKVIFHPEFLSSTSPLLP<br>VDYEEFVRGCHLGVFPSYYEPWGYTPAECTVMGIPSISTNLSGFGCFMEEHIADPS<br>AYGIYILDRRFRSLDDSCSQLTSFLYSFCQQSRRQRIIQRNRTERLSDLLDWKYLGR<br>YYMSARHMALAKAFPDHFTYEPHEVDATQALGEERN |

| | |
|---|---|
| UGP | MSQDGASQFQEVILQELELSVKKELEKILTTAASHEFEHTKKDLDGFRKLFHRFLQ<br>EKGPSVDWGKIQRPPEDSIQPYEKIKARGLPDNISSVLNKLVVVKLNGGLGTSMG<br>CKGPKSLIGVRNENTFLDLTVQQIEHLNKTYNTDVPLVLMNSFNTDEDTKKILQK<br>YNHCRVKIYTFNQSRYPRINKESLLPIAKDVSYSGENTEAWYPPGHGDIYASFYNS<br>GLLDTFIEEGKEYIFVSNIDNLGATVDLYILNHLMNPPNGKRCEFVMEVTNKTRAD<br>VKGGTLTQYEGKLRLVEIAQVPKAHVDEFKSVSKFKIFNTNNLWISLGAVKRLQE<br>QNAIDMEIIVNPKTLDGGLNVIQLETAVGAAIKSFENSLGINVPRSRFLPVKTTSDL<br>LLVMSNLYSLNAGSLTMSEKREFPTVPLVKLGSSFTKVQDYLRRFESIPDMLELDH<br>LTVSGDVTFGKNVSLKGTVIIIANHGDRIDIPPGAVLENKIVSGNLRILDH |
| GBE | MAAPAAPAAGETGPDARLEAALADVPELARLLEIDPYLKPFAADFQRRYKKFSQ<br>VLHDIGENEGGIDKFSRGYESFGIHRCSDGGIYCKEWAPGAEGVFLTGEFSGWNPF<br>SHPYKKLEYGKWELYIPPKQNKSPLIPHGSKLKVVITSKSGEILYRISPWAKYVVR<br>ENNNVNYDWIHWAPEDPYKFKHSRPKKPRSLRIYESHVGISSHEGKIASYKHFTSN<br>VLPRIKDLGYNCIQLMAIMEHAYYASFGYQITSFFAASSRYGTPEELKELVDTAHS<br>MGIVVLLDVVHSHASKNSEDGLNMFDGTDSCYFHSGPRGTHDLWDSRLFIYSSW<br>EVLRFLLSNIRWWLEEYCFDGFRFDGVTSMLYHHHGMGQGFSGDYNEYFGLQV<br>DEDALIYLMLANHLAHTLYPDSITIAEDVSGMPALCSPTSQGGGGFDYRLAMAIP<br>DKWIQLLKEFKDEDWNMGNIVYTLTNRRYLEKCVAYAESHDQALVGDKTLAFW<br>LMDAEMYTNMSVLAPFTPVIDRGIQLHKMIRLITHGLGGEGYLNFMGNEFGHPE<br>WLDFPRKGNNESYHYARRQFNLTDDDLLRYKFLNNFDRDMNRLEERCGWLSAP<br>QAYVSEKHEANKTITFERAGLLFIFNFHPSKSYTDYRVGTATPGKFKIVLDSDAAE<br>YGGHQRLDHNTNYFAEAFEHNGRPYSLLVYIPSRVALILQNVDLQN |
| GYG | MTDQAFVTLTTNDAYAKGALVLGSSLKQHRTTRRMVVLTSPQVSDSMRKVLET<br>VFDDVIMVDVLDSGDSAHLTLMKRPELGITLTKLHCWSLTQYSKCVFMDADTLV<br>LSNIDDLFEREELSAAPDPGWPDCFNSGVFVYQPSIETYNQLLHLASEQGSFDGGD<br>QGLLNTYFSGWATTDITKHLPFVYNLSSISIYSYLPAFKAFGKNAKVVHFLGRTKP<br>WNYTYNPQTKSVNCDSQDPTVSHPEFLNLWWDTFTTNVLPLLQHHGLVKDASSY<br>LMMLSDLVYTLAFSCGFCRKEHVSGALSDLSFGEAPAAPQPSMSSEERKERWEQ<br>GQADYMGADSFDNIKRKLDTYLQ |