Figure legends

Figure 1. Validation of glutamine auxotrophy and stress-response signatures in commercial GS-knockout CHO cell lines. (a) Schematic of the Chinese hamster *Glul* transcript, which comprises seven exons, with the ATG start codon located in exon 2. Sanger sequencing confirmed the GS gene deletions in two commercial cell lines: ATCC CHO-K1 $GS^{-/-}$ (designated AK1) and CHOZN® $GS^{-/-}$ (designated ZN). **(b, c)** Growth curves of ZN (b) and AK1 (c) pools transfected with GS cDNA, cultured in VegaCHO medium supplemented with 6 mM glutamine, 0 μM MSX, or 10 μM MSX (MSX, methionine sulfoximine). **(d-g)** qPCR analysis of mRNA expression levels of *Glul* (GS) (d), *Atf4* (e), *Ddit3* (f), and *Ppp1r15a* (g) in EK1, AK1, and ZN cells cultured under the same three conditions as in (b, c). Data are mean ± SD (n=3).

Figure 2. Efficient generation of exon-specific GS premature termination codons via base editing. **(a)** Comparison of gene-editing efficiencies at the *Glul* exon 6 locus among CRISPR/Cas9, TALEN and ZFN platforms. In-frame indel frequencies are indicated within the bars. **(b)** Editing efficiencies of eight sgRNAs targeting Glul exon 6 using the CBE system; the highest efficiency (39.7%) was achieved with sgRNA-2. **(c)** Schematic of CBE-mediated introduction of premature termination codons (PTCs) across Glul exons 2-7. Using exon 2 as an example, CBE converted a glutamine codon (CAA) to a stop codon (TAA), resulting in a Q27X mutation and gene disruption. **(d)** Relative GS transcript levels in wild type (EK1), commercial GS-KO (ZN, AK1), and exon-specific PTC-edited cell lines, measured by RT-qPCR with primers targeting exons 2, 5 and 7. Data are shown as mean ± SD (*n*=3). **(e)** Western blot analysis of GS protein expression in EK1, ZN, AK1, and exon-specific PTC-edited lines (E2-E7). GAPDH served as the loading control.

Figure 3. Growth and viability profiles of exon-specific GS-KO clones under varying glutamine concentrations. **(a-d)** Growth curves of the exon 2-edited (E2) (a), exon 7-edited (E7) (b), wild-type (EK1) (c), and commercial GS-KO (ZN) (d) cell lines cultured in VegaCHO medium containing 0 mM, 1 mM, 2 mM, or 6 mM glutamine. Cells were seeded at 0.5×10^6 cells/mL in 30 mL and monitored daily. **(e-h)** Viability curves for the same cell lines and conditions as in (a-d).

Figure 4. Fed-batch production performance of exon-specific GS-KO cell lines. **(a)** Schematic workflow of the stable antibody production process, including electroporation of CHO cells with the antibody expression vector, MSX selection, clonal expansion, and fed-batch culture for titer assessment. **(b, c)** Viable cell density (b) and viability (c) profiles of eight cell lines (EK1, ZN, and E2–E7) during 16-day

fed-batch culture. **(d, e)** Glucose consumption (d) and lactate production (e) profiles for the same cell lines over the fed-batch period. All cultures-maintained glucose within 1.06–5.02 g/L and lactate at basal levels (0.00–1.09 g/L). **(f)** Antibody titers and specific productivity (Qp) of EK1, ZN, and E2–E7 lines under fed-batch conditions, with or without 5 μM MSX. The E7 line achieved the highest titer (0.74 g/L without MSX; 1.02 g/L with MSX) and Qp (3.7 pg/cell/day without MSX; 4.9 pg/cell/day with MSX).

Figure 5. Transcriptomic profiling of exon-specific GS-KO CHO cell lines. **(a)** Schematic workflow of the study, including electroporation of CHO cells with the antibody expression vector, generation and screening of GS-KO monoclonal cell lines, clonal expansion, and fed-batch culture for antibody production assessment. **(b)** Venn diagram showing the overlap of differentially expressed genes (DEGs) identified in pairwise comparisons of AK1, ZN, and E7 against EK1 ($|\log_2 FC| > 1$, adjusted $P < 0.05$). Numbers indicate unique and shared DEGs among the three comparisons. **(c)** Hierarchical clustering heatmap of DEGs across the indicated cell lines (AK1, ZN, E7, and EK1). Rows represent individual genes; columns represent samples. The color scale indicates normalized expression levels (red: high; blue: low). **(d)** Volcano plots depicting genome-wide gene expression differences between AK1, ZN, or E7 and EK1. The x-axis represents $\log_2$ fold change; the y-axis represents $-\log_{10}$(P-value). Dashed vertical lines indicate $|\log_2 FC| = 1$ (two-fold change); the dashed horizontal line indicates $P = 0.05$. Significantly up-regulated genes ($\log_2 FC > 1$, $P < 0.05$) are shown in green; significantly down-regulated genes ($\log_2 FC < -1$, $P < 0.05$) in blue; non-significant genes in gray. **(e)** Gene Ontology (GO) enrichment analysis of DEGs, categorized into Molecular Function (MF), Biological Process (BP), and Cellular Component (CC). For each category, the top ten most significantly enriched GO terms (by P-value) are shown.