Supplementary Figure 1. Validation of GS knockout phenotypes and protein expression in commercial CHO-K1 cell lines **(a, b)** Cell viability of ZN (a) and AK1 (b) pools transfected with GS cDNA, cultured in VegaCHO medium supplemented with 6 mM glutamine, 0 μM MSX, or 10 μM MSX (MSX, methionine sulfoximine). **(c)** Growth curves of ZN and AK1 cultured in glutamine-free VegaCHO medium. **(d)** Western blot analysis of GS protein levels in EK1, ZN, and AK1 cells cultured under standard conditions (with 6 mM glutamine). GAPDH was used as a loading control.

Supplementary Figure 2. Design of editing strategies and sequence validation of exon-specific GS knockout clones. **(a)** Target site designs for DSB-based genome-editing tools (CRISPR/Cas9, TALEN, and ZFN) and CBE-mediated knockout at the GS exon 6 locus. The 10 gRNAs, 7 TALEN pairs, and the validated ZFN pair used in this study are indicated relative to the exon 6 sequence. **(b)** Sanger sequencing confirmation of six independent GS-KO monoclonal cell lines carrying premature termination codon (PTC) mutations in exons 2–7 of the Glul gene. The introduced point mutations and corresponding amino acid changes (Q27X, W60X, Q135X, Q201X, Q205X, and R286X) are indicated for each clone (E2–E7).

Supplementary Figure 3. Growth and viability profiles of exon-specific GS-knockout clones under varying glutamine concentrations. **(a-d)** Growth curves of the exon 3-edited (E3) (a), exon 4-edited (E4) (b), exon 5-edited (E5) (c), and exon 6-edited (E6) (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).

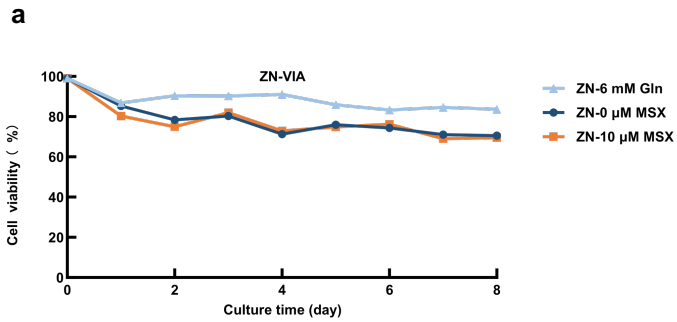
Supplementary Figure 4. Validation of GS1 genotype and clonal consistency in exon-specific GS-knockout lines. **(a)** Sanger sequencing verification of the GS1 locus in three independent E7 monoclonal lines (E7-1, E7-2, and E7-3), showing homozygous editing, heterozygous editing, and wild-type GS1, respectively. **(b)** Relative GS1 transcript levels in exon-specific PTC-edited lines (E2–E7), ZN, AK1, and EK1, measured by RT-qPCR using primers specific for GS1. Data are shown as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA with Dunnett's multiple-comparisons test, using EK1 as the control group; $P < 0.05$. **(c, d)** Sanger sequencing confirmation of independently derived E2 (Q27X) (c) and E7 (R286X) (d) monoclonal cell lines generated by an independent base-editing experiment. **(e)** Western blot analysis of GS protein expression in three independent E2 and three independent E7 monoclonal clones. GAPDH served as the loading control. **(f)** Residual GS transcript levels in the same three independent E2 and E7 monoclonal clones as in (e), measured by RT-qPCR. Data are shown as mean ± SD (n = 3).

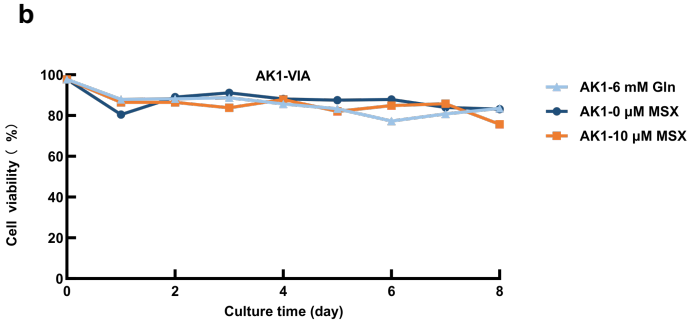
Statistical significance was determined by one-way ANOVA with Dunnett's multiple-comparisons test, using EK1 as the control group; $P < 0.05$.

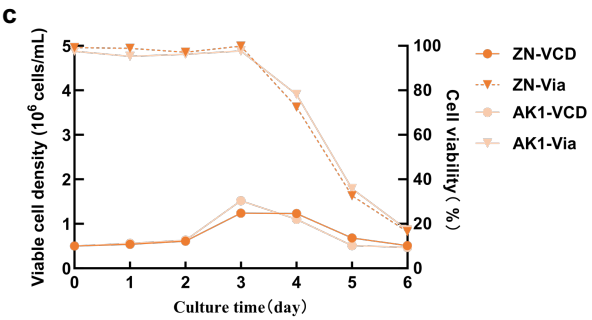
Supplementary Figure 5. Growth and viability of parental GS-knockout cell lines under varying glutamine concentrations prior to antibody expression. **(a–h)** Viable cell density profiles of eight cell lines (EK1, ZN, and E2–E7) cultured in VegaCHO medium containing 0, 1, 2, or 6 mM glutamine. Cells were seeded at 0.5×10^6 cells/mL and monitored daily over 9 days. (i–p) Cell viability curves for the same cell lines and culture conditions as in (a–h).

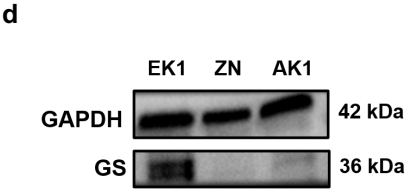
Supplementary Figure 6. Assessment of off-target effects and genomic integrity in the base-edited E7 CHO cell line. **(a)** Targeted off-target analysis of three E7 monoclonal clones (E7-1, E7-2, and E7-3) at the GS1 locus, the only computationally predicted off-target site sharing complete homology with the GS5-targeting sgRNA. **(b)** SNP/InDel variant profiles identified by transcriptome-wide sequencing. Variants were filtered using the following criteria: base quality > 20, read depth ≥ 8, supporting mutant reads ≥ 2, and P < 0.01. **(c)** Distribution of codon mutation types induced by SNPs/InDels across the indicated cell lines (E7, AK1, ZN, and EK1). Bar chart showing the number of mutations categorized by their predicted impact on translation (e.g., synonymous, missense, nonsense, or frameshift) for each sample. **(d)** PCR-based confirmation of the clearance of exogenous base-editing components in GS-KO monoclonal cell lines. The absence of Blasticidin resistance cassette, CBE4Max, and sgRNA sequences was verified in all tested clones.

Supplementary Figure 1

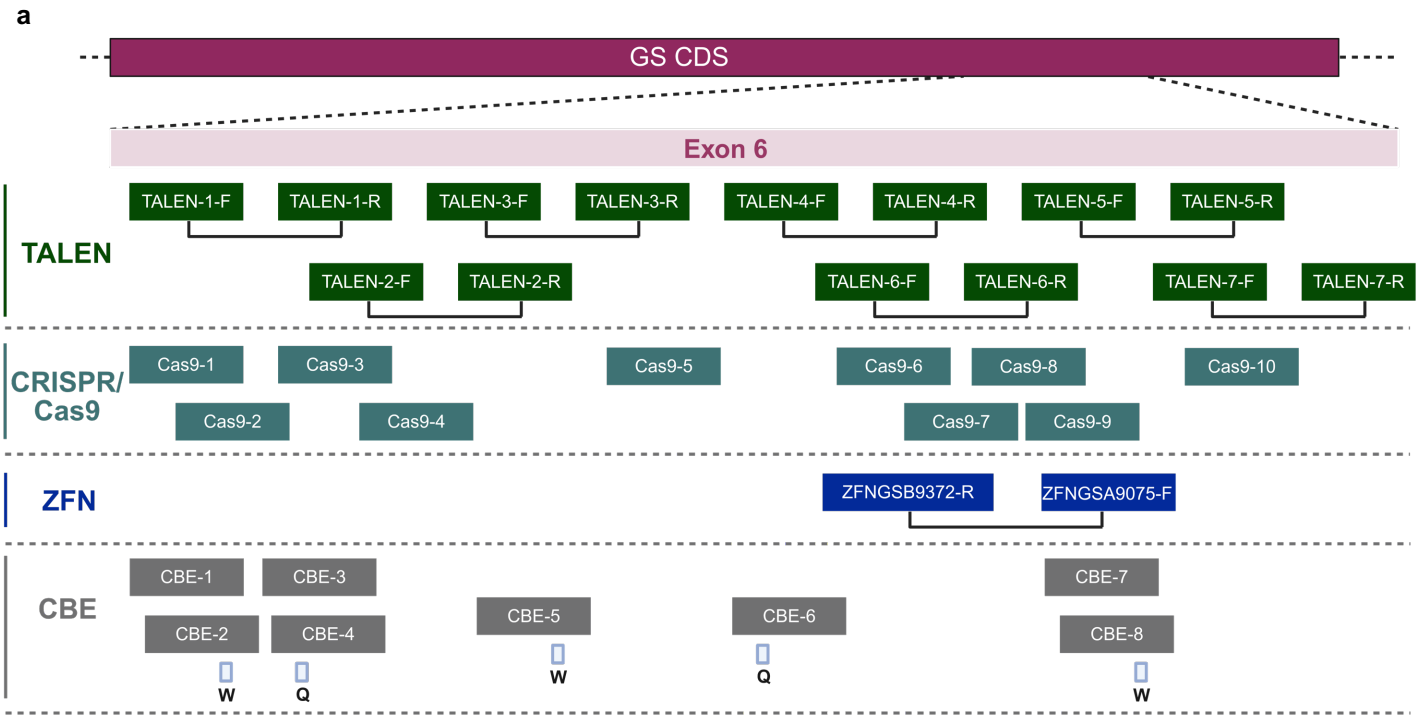




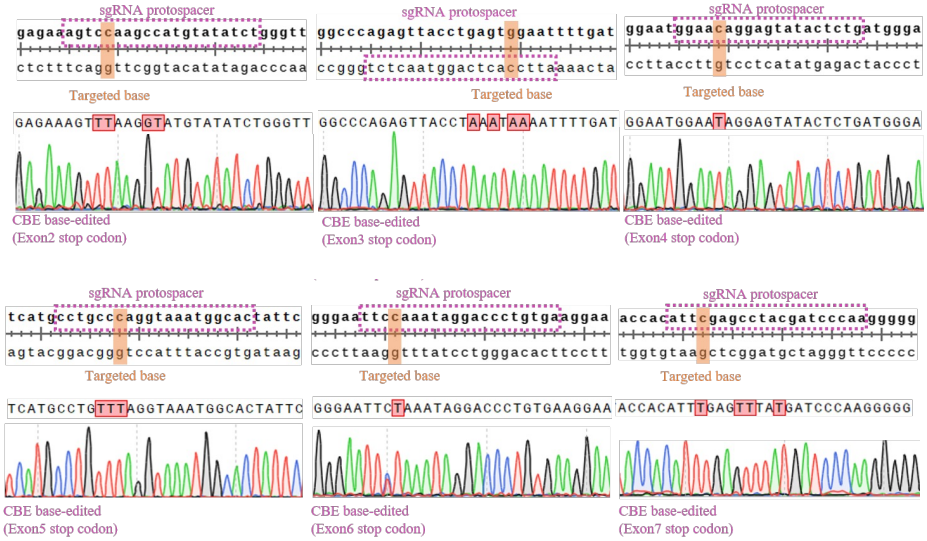




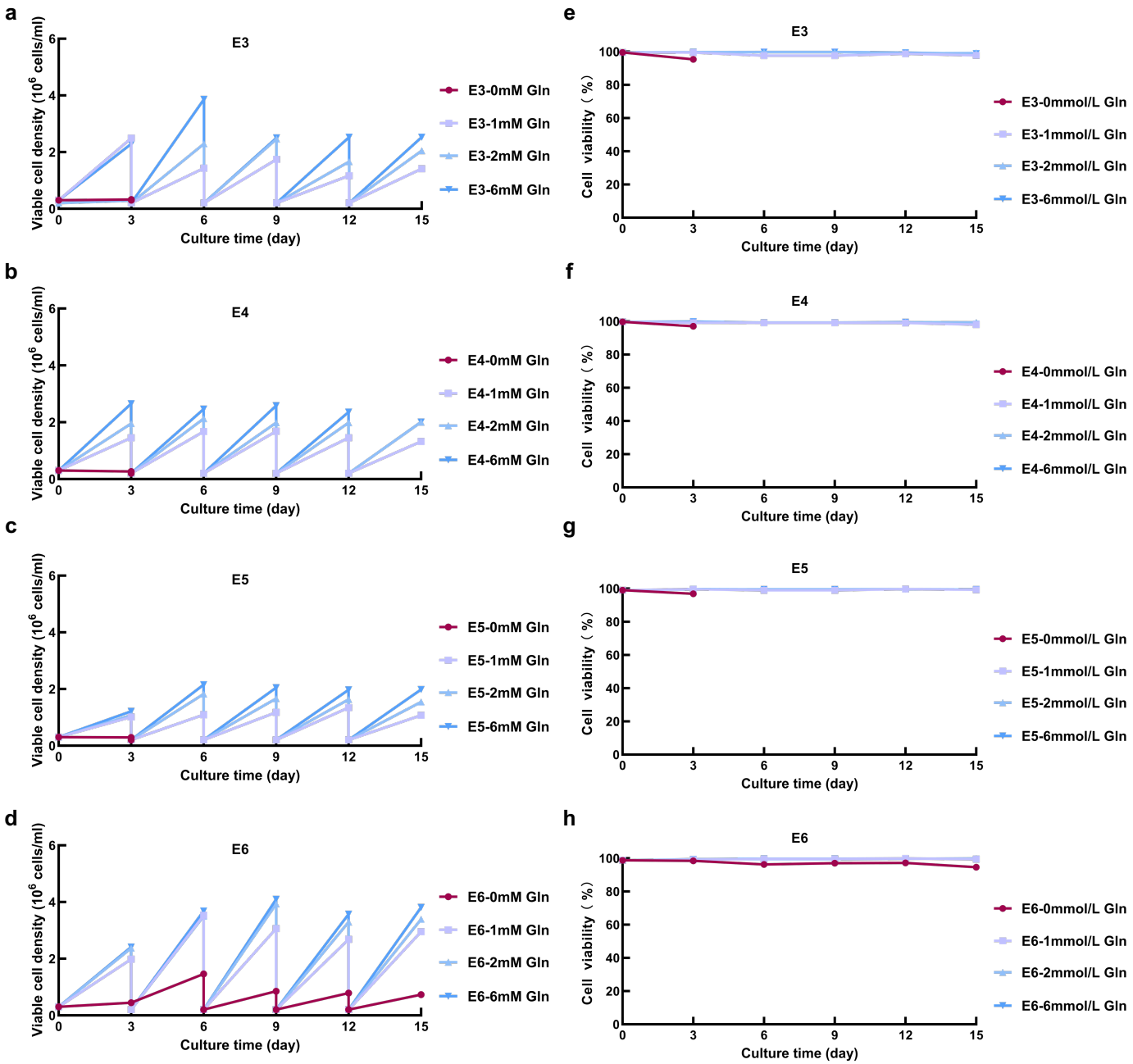
Supplementary Figure 2



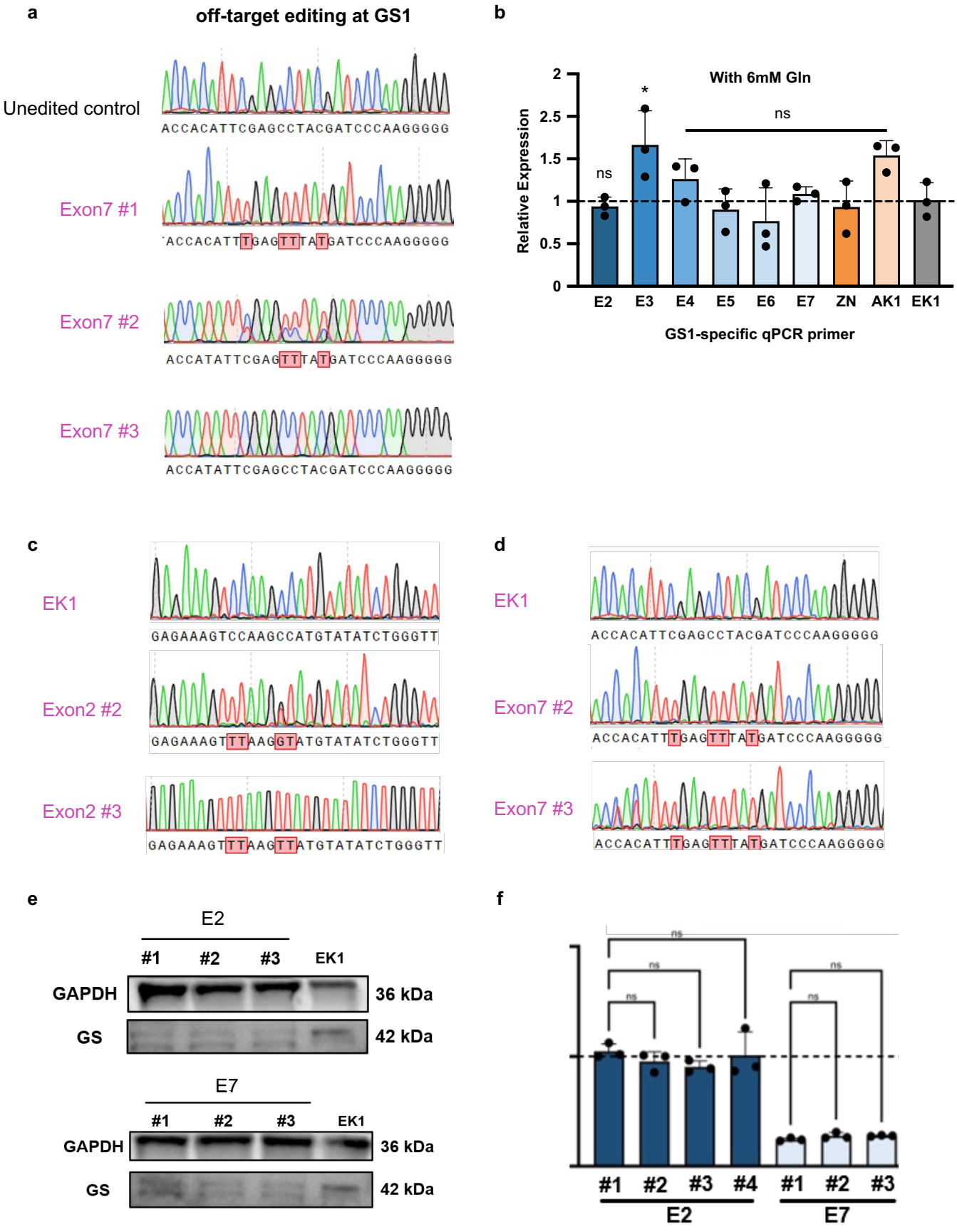
b



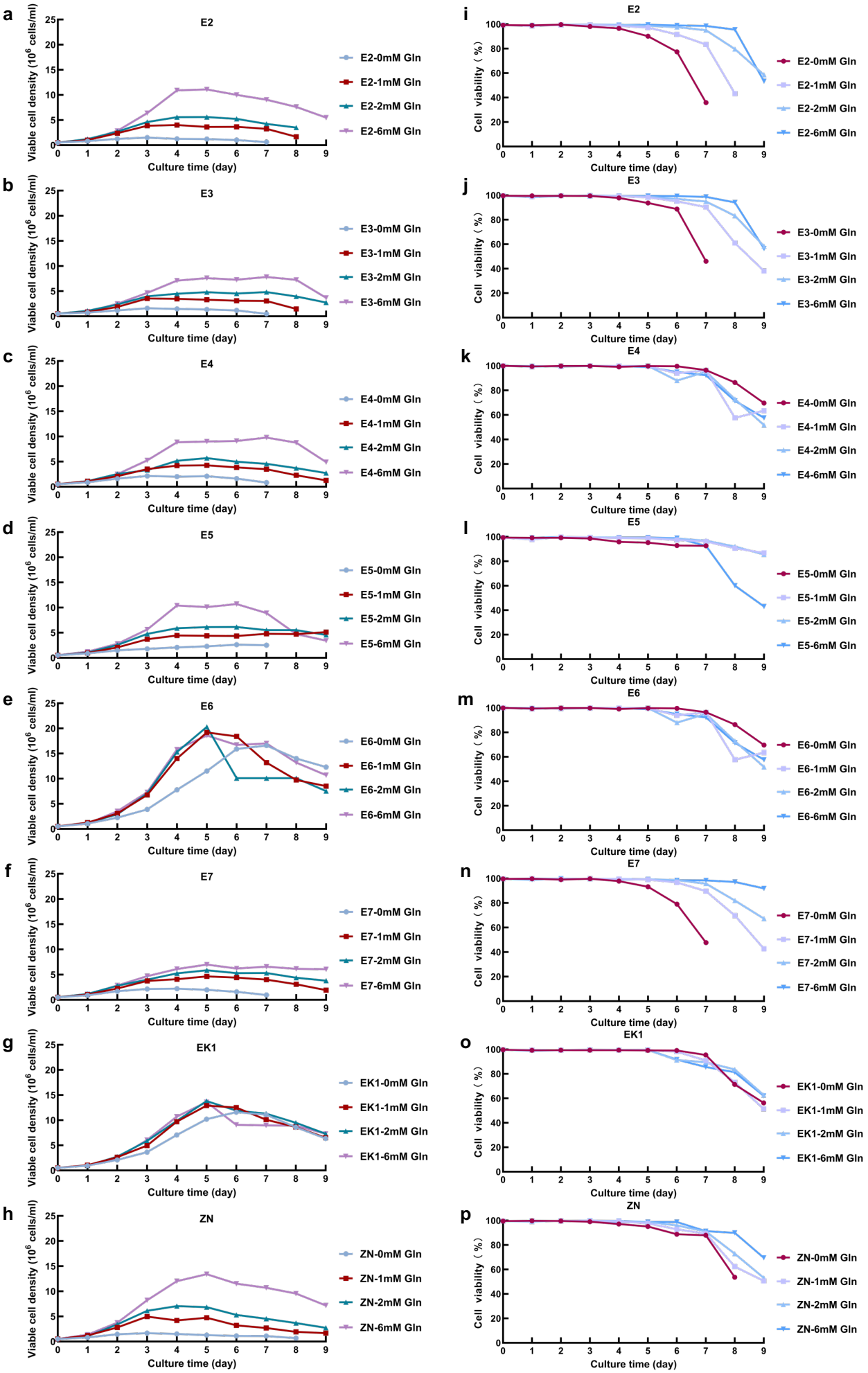
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6