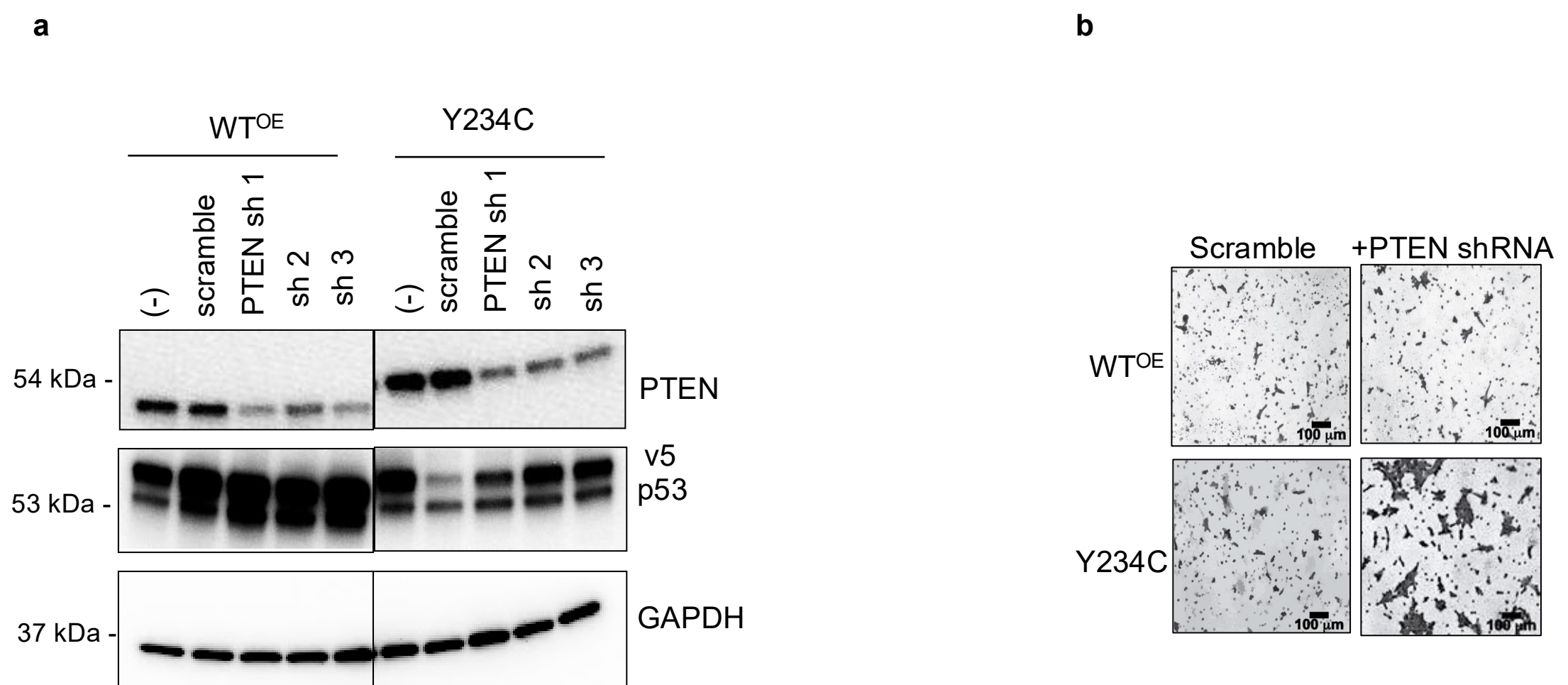
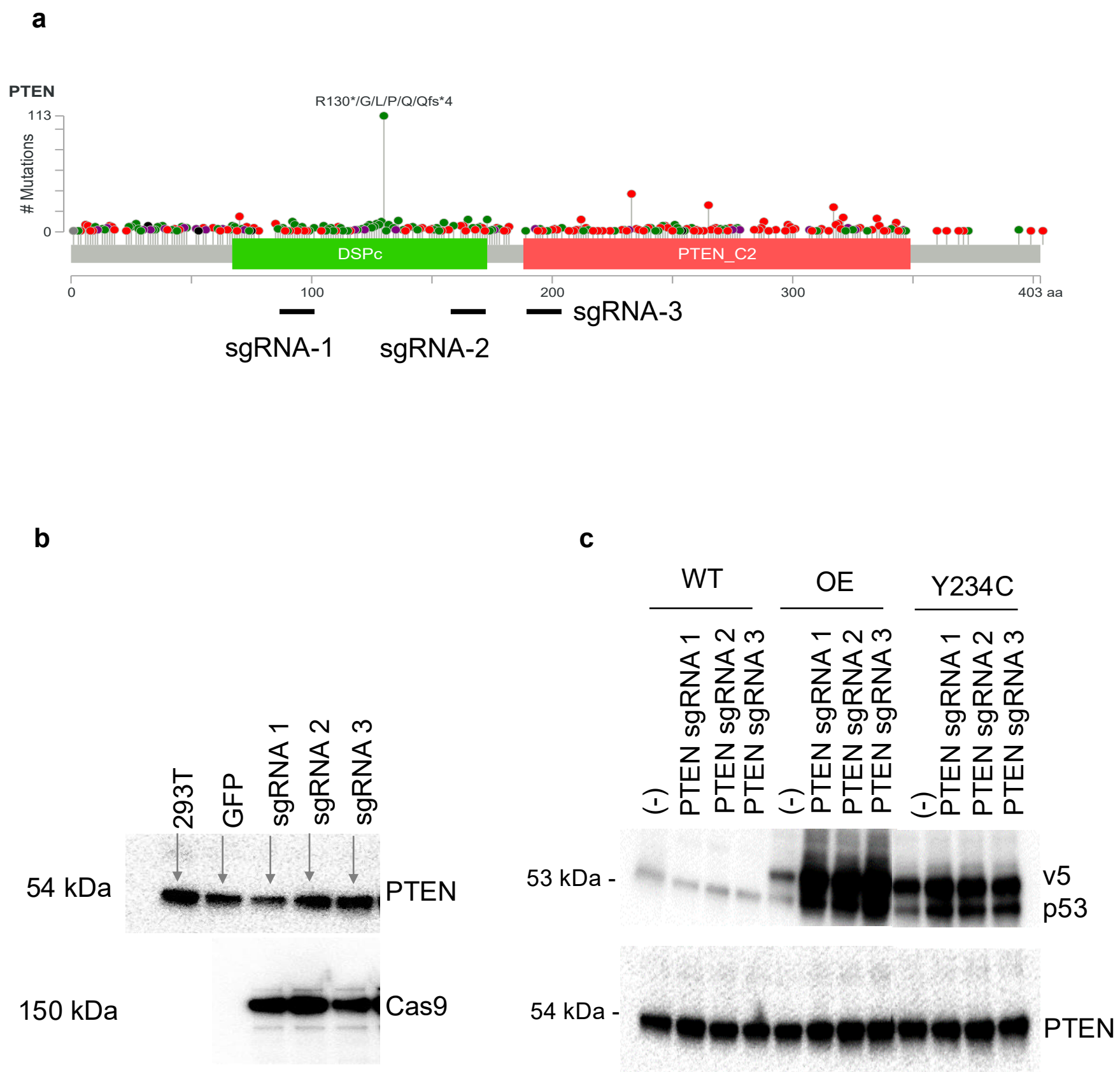


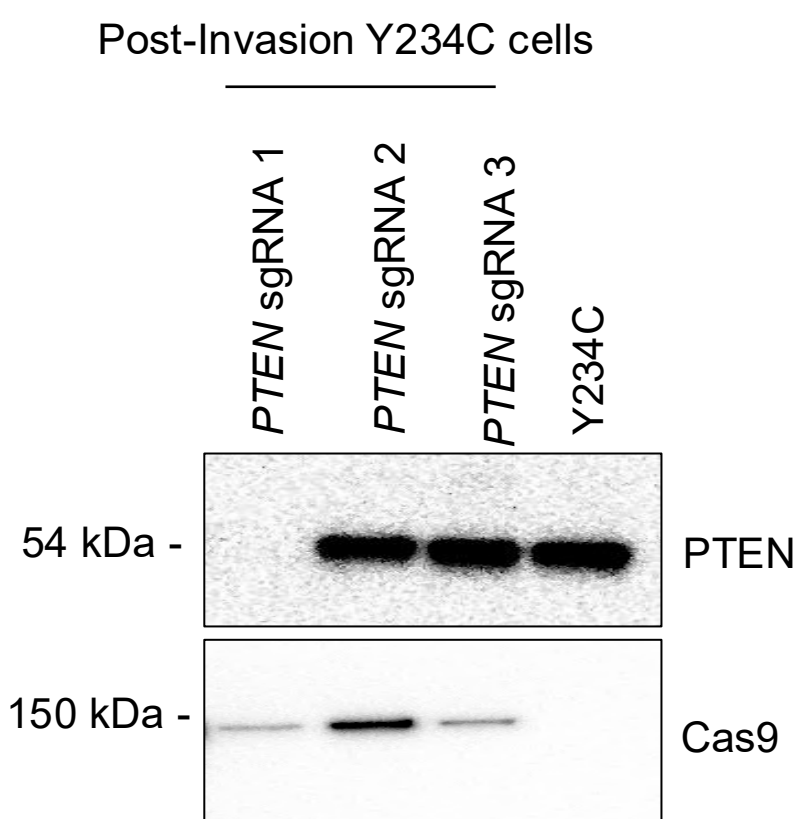


Supplementary Figure 1. Functional assessment of shRNA-mediated *PTEN* knockdown in p53-mutant-expressing MCF10A cells. Western blot image showing PTEN protein expression in the cells. The double bands observed for p53 protein represent the endogenous protein and V5 tagged forms in Y234C and WT^{OE}. GAPDH was used as the loading control **b**. Cell invasion assay showing increased cell invasion upon the shRNA mediated knockdown of PTEN in Y234C cells. Scale bar, 100 μ m.

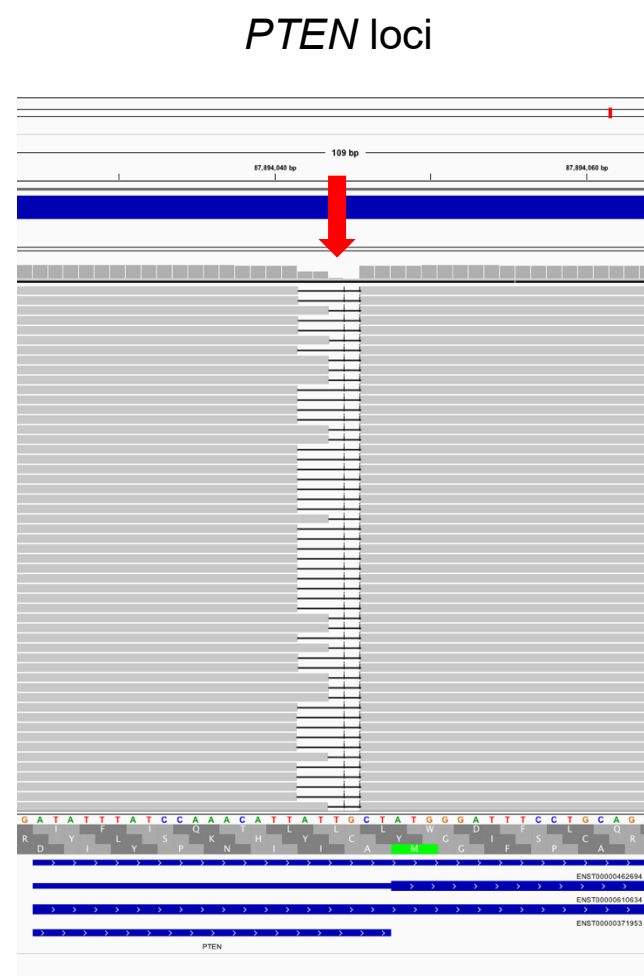


Supplementary Figure 2. Design and validation of sgRNA-mediated *PTEN* knockout. **a.** sgRNA 1 and sgRNA 2 targeted the PTEN phosphatase domain, while sgRNA 3 targeted the linker region between the phosphatase and the C2 domain. **b.** Western blot image showing the knockout of *PTEN* by the sgRNAs following transient transfection of 293T cells with sgRNA containing plasmids. **c.** Western blot image showing the knockout of *PTEN* by the sgRNAs following stable cell transduction consisting of pre-invasion mixed population of cells.

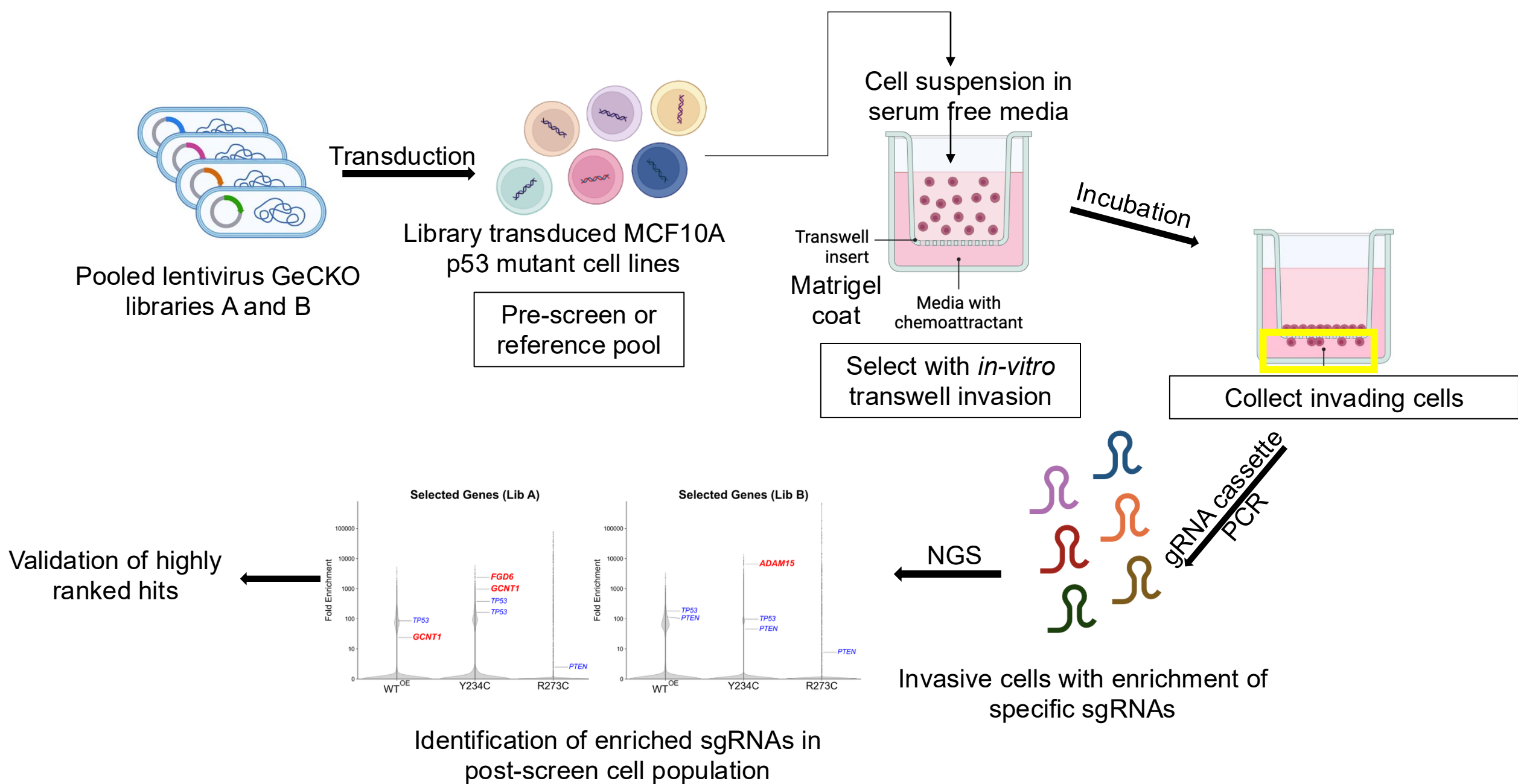
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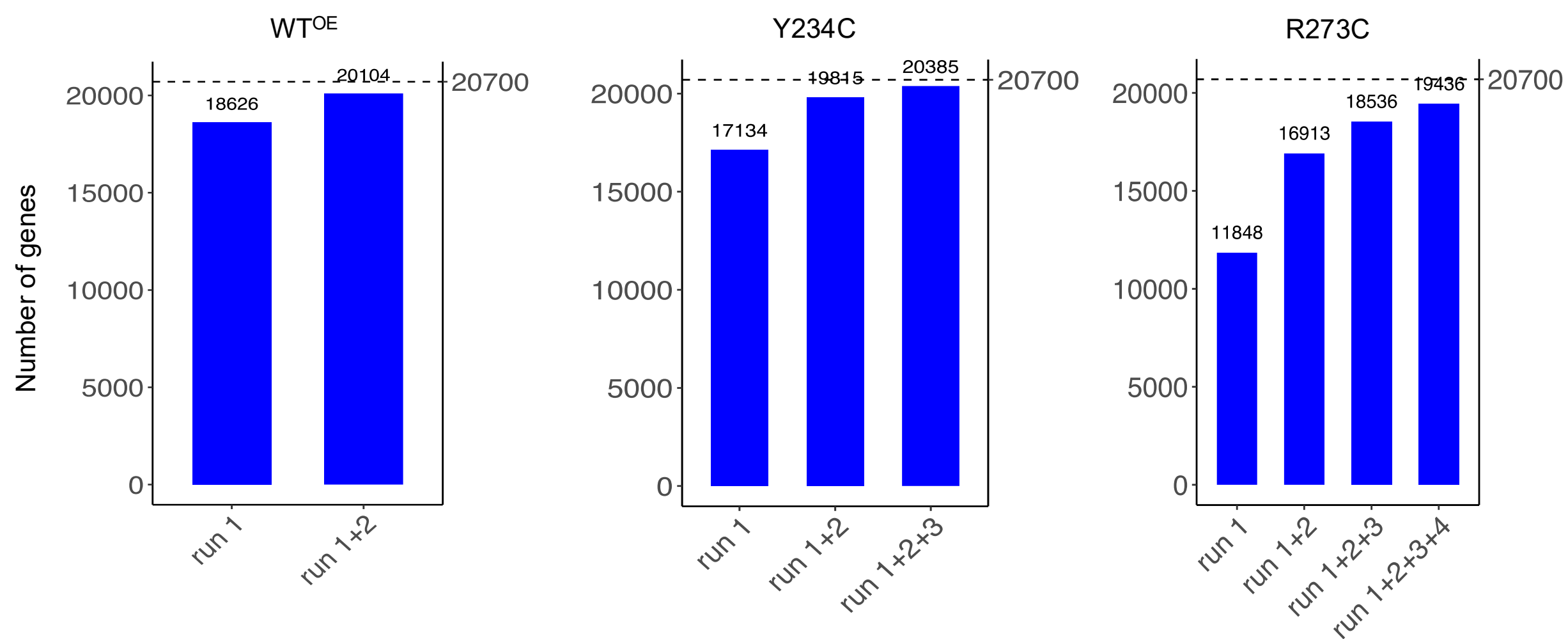
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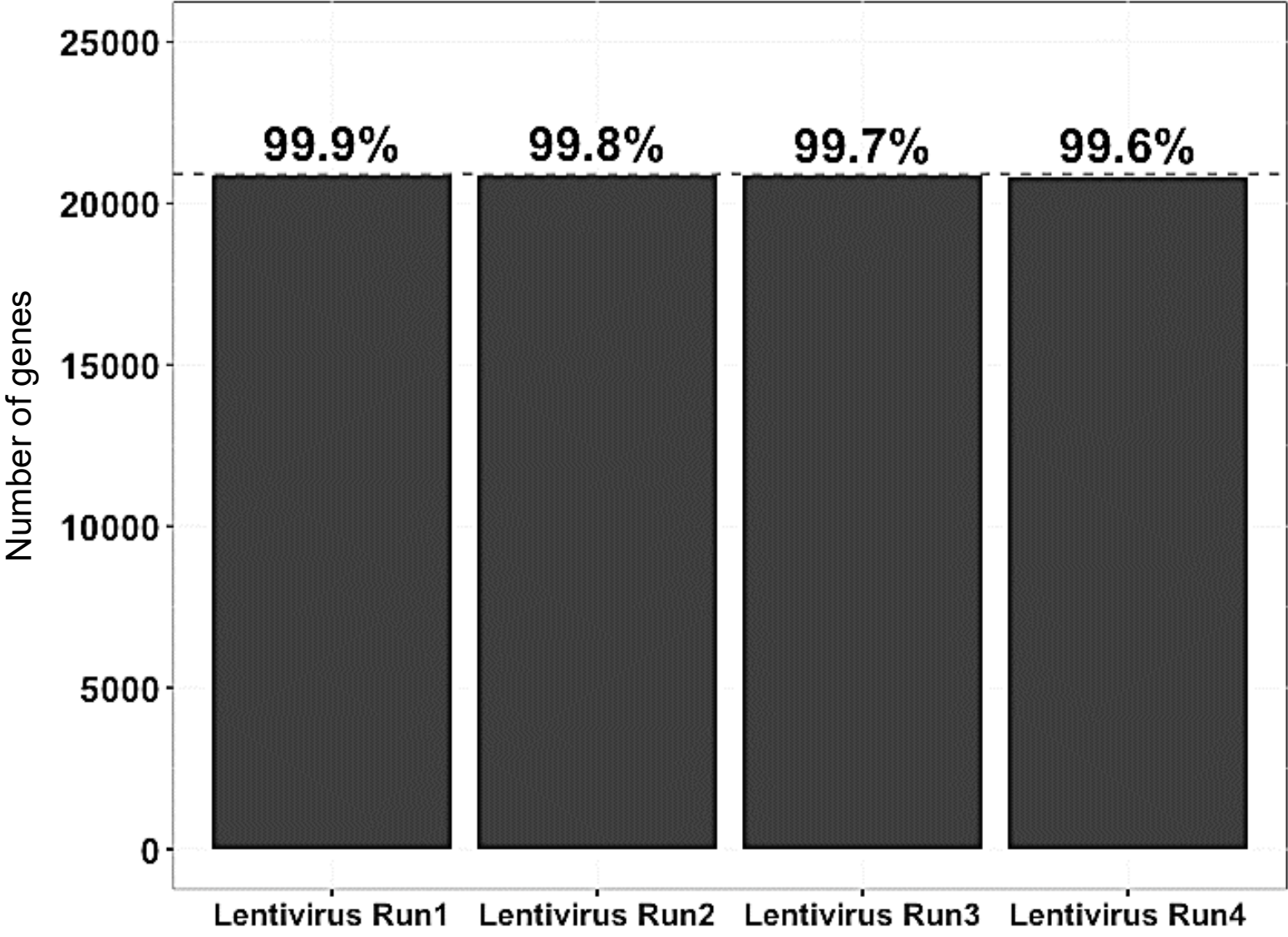
Supplementary Figure 3. Validation of post-invasion Y234C-*PTEN*^{KO} cells. a. Western blot image showing knockout of PTEN protein in the post-invasion population of Y234C-*PTEN* sgRNA-1 cells. **b.** Sequencing analysis of *PTEN* locus confirming deletion of bases, indicated by the red arrow.



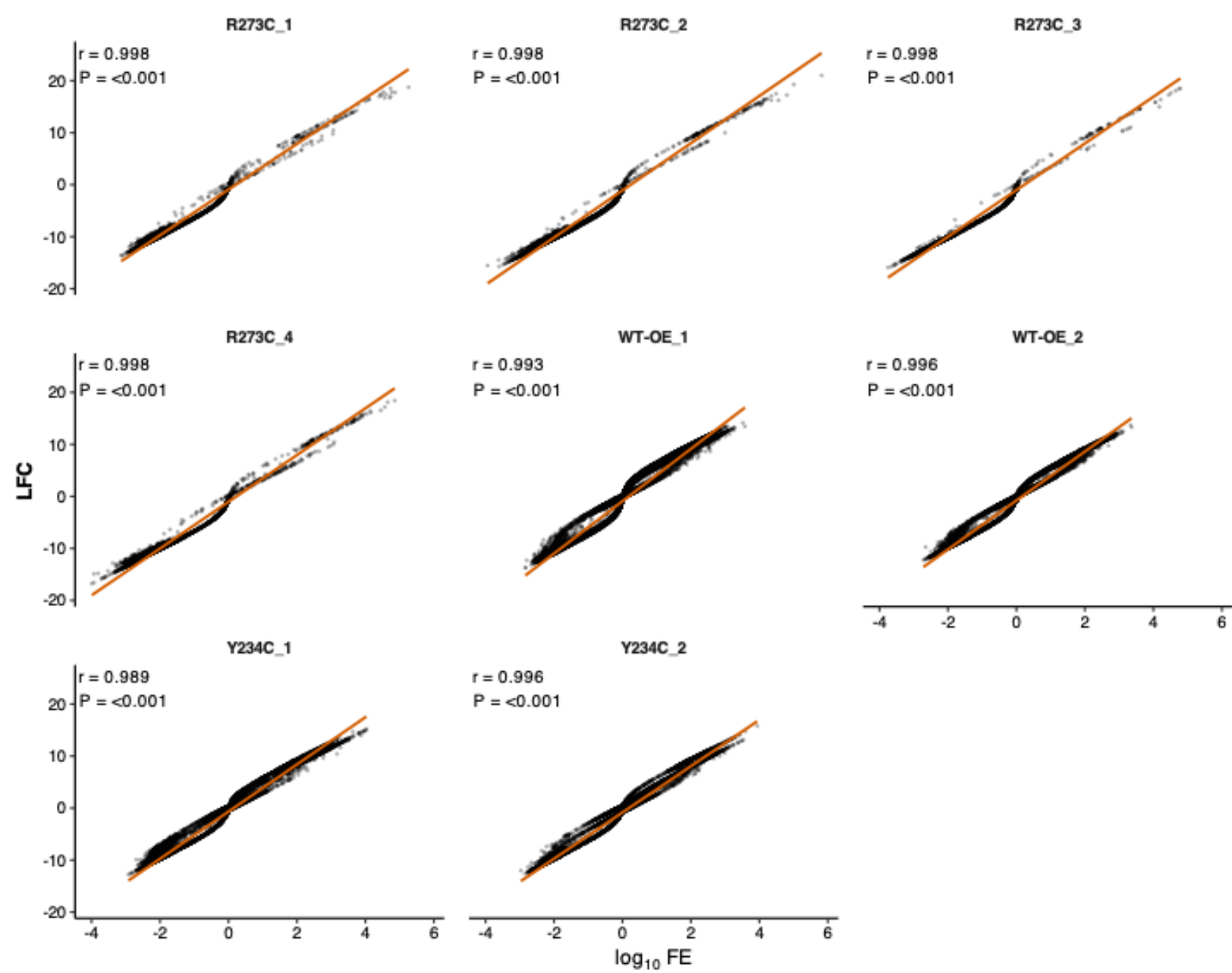
Supplementary Figure 4. Schematic overview of genome wide screening for identification of co-drivers of missense mutant p53 that promote cell invasion. P53 mutant cells were transduced with GeCKO v2.0 library (p53 mut + 1 sgRNA) at low MOI and subjected to *in-vitro* cell invasion assay. Genomic DNA was extracted from the pre- and post-invasion cell population. The sgRNA containing region was PCR amplified and sequenced to identify the sgRNAs enriched in the post-invasion population. Figures partially created in BioRender. Nwekwo, L. (2026) <https://BioRender.com/2ihk7cc>



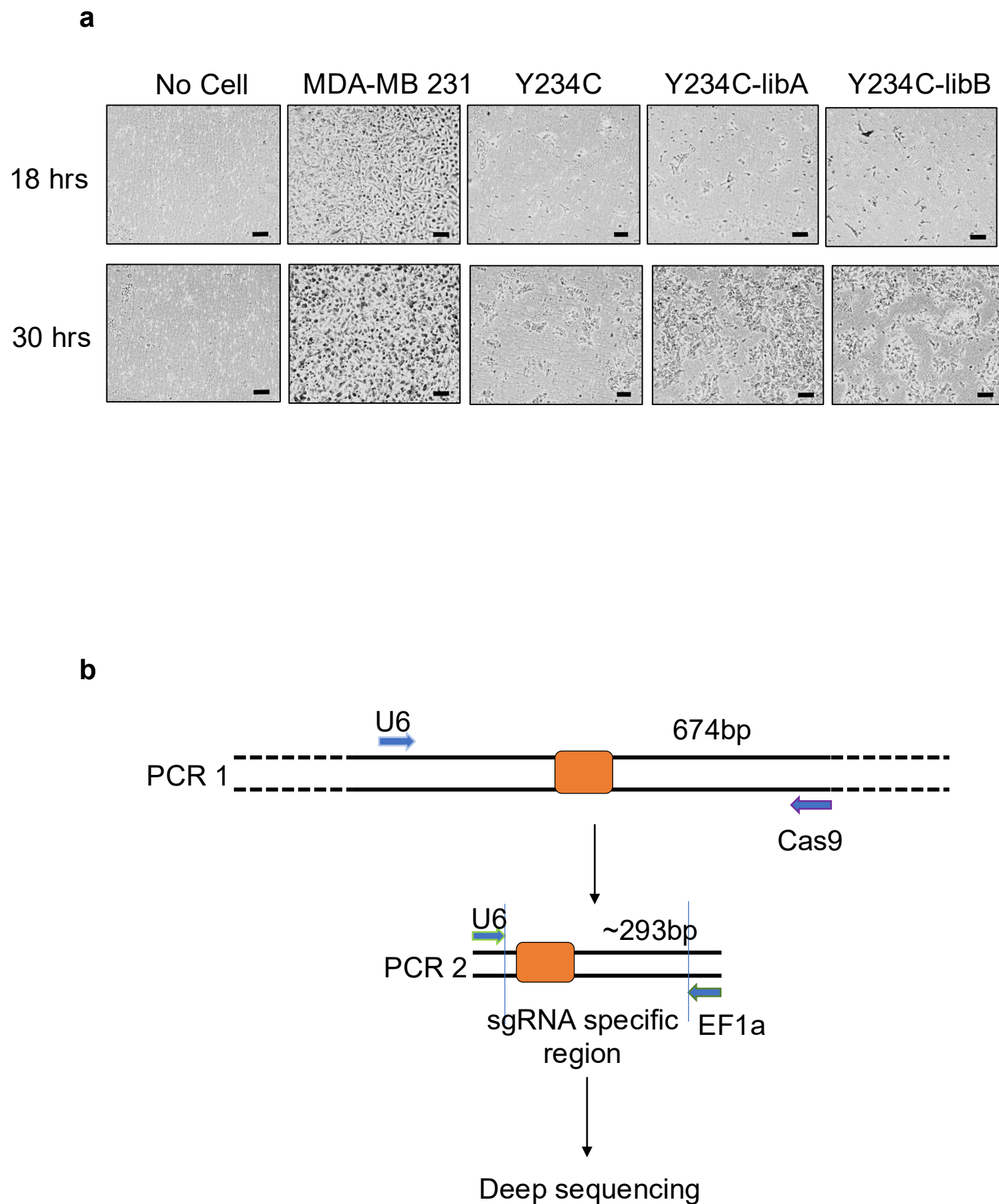
Supplementary Figure 5. sgRNA counts and gene coverage from separate NGS runs of library transduced pre-invasion reference pool. Full coverage for all 20,700 protein coding genes was targeted by the sgRNAs. A minimum of 3 independent screens were used to screen all genes as co-drivers of mutant p53 enhancing cell invasion.



Supplementary Figure 6. Gene coverage in the lentivirus libraries. Sequencing of the amplified GeCKO v2.0 plasmid library was performed to assess sgRNA representation and coverage of > 99% of protein coding genes.

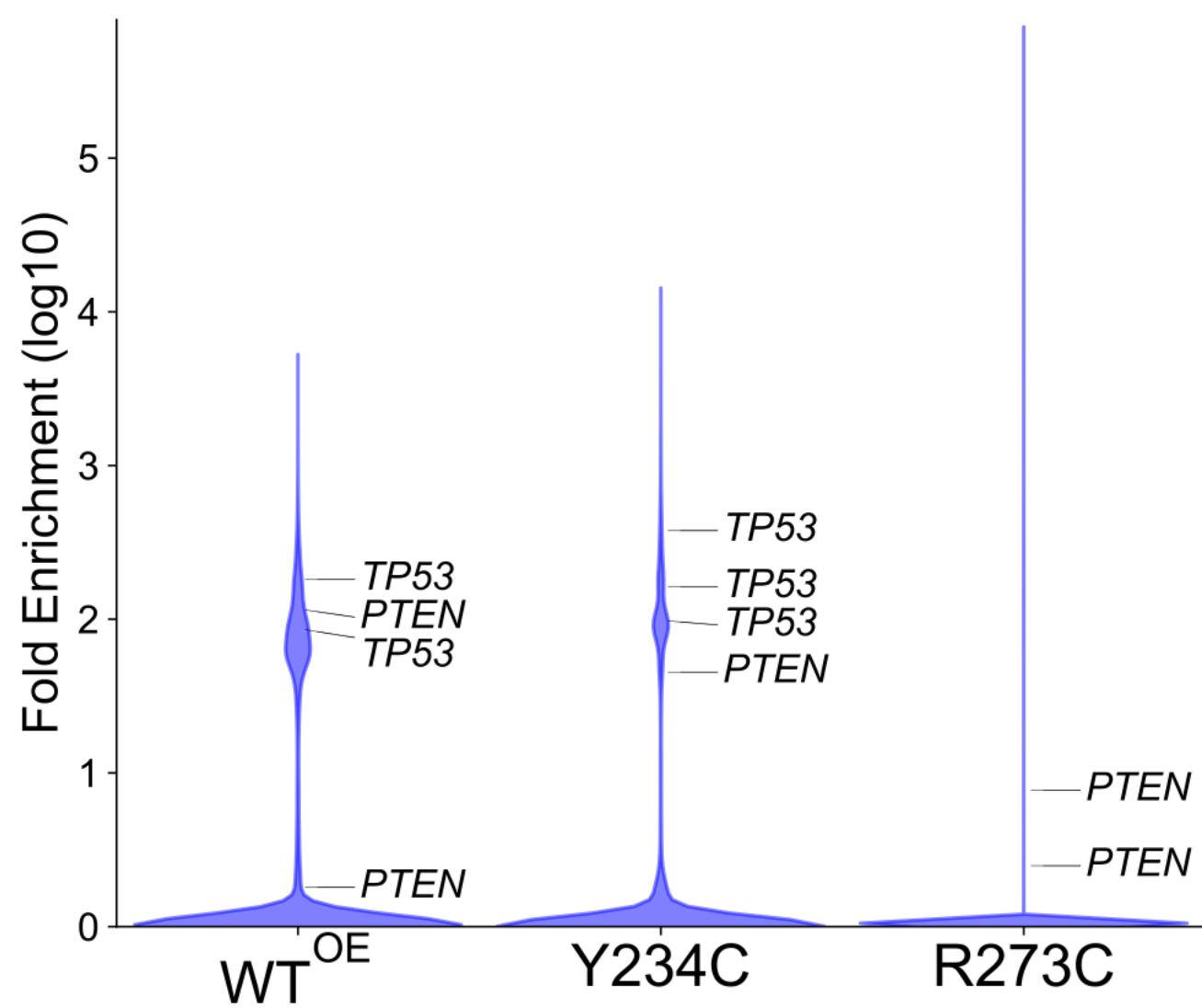


Supplementary Figure 7. Comparison of MAGeCK and Fold change approaches for CRISPR screen analysis. Scatter plots comparing the log10 fold enrichment (log10FE) values calculated using our custom analysis pipeline with the log fold change (LFC) values generated by MAGeCK for each pre- and post-invasion sample pair. Pearson correlation coefficients (r) and corresponding P values were calculated for each comparison and are shown in the plots.

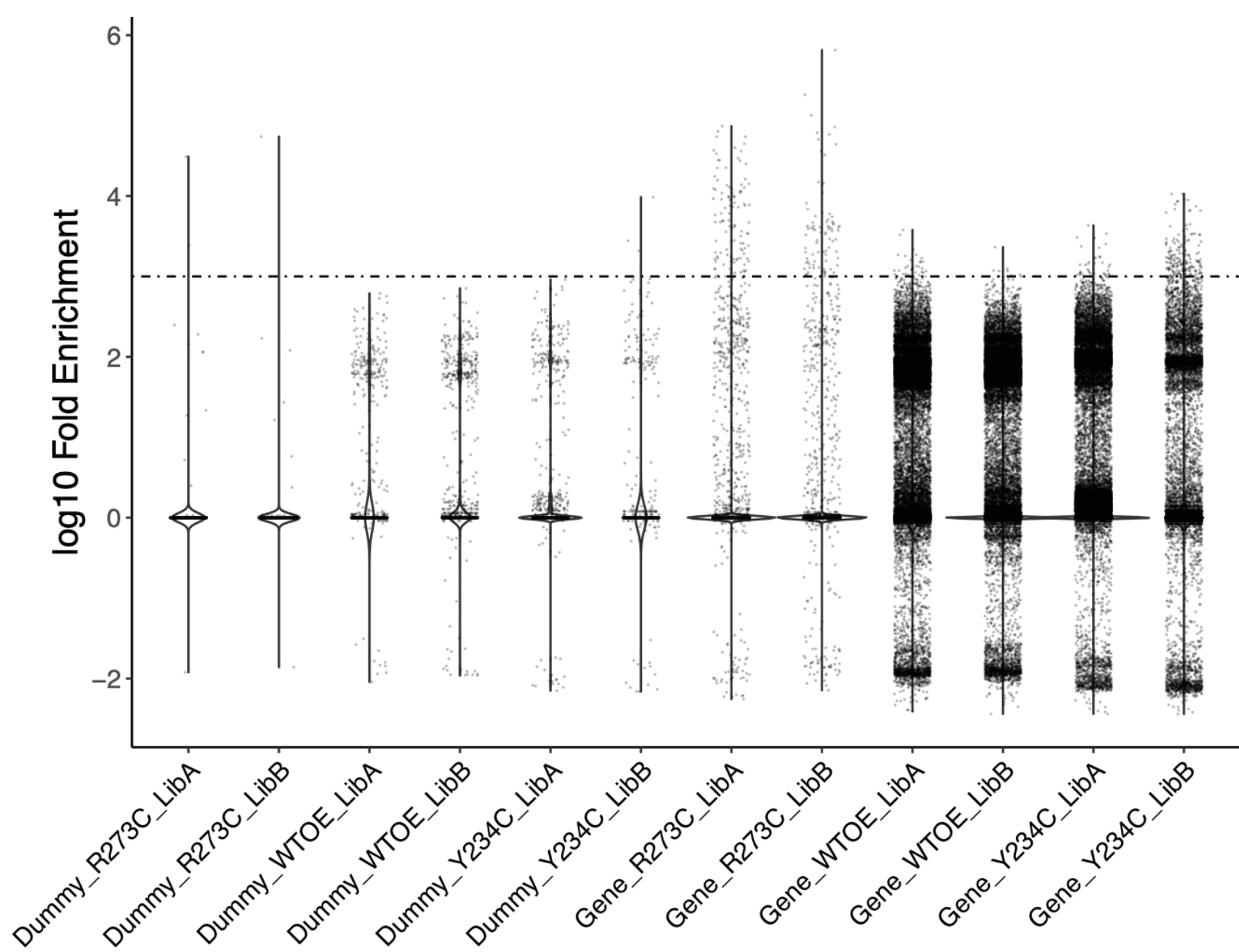


Supplementary Figure 8. End-point determination for invasion of GeCKO v2.0 library

transduced MCF10A p53 mutant Y234C cells. **a.** Cell invasion assay following transduction with Y234C-libA and Y234C-libB cells resulted in increased cell invasion. 1 million cells were seeded and incubated for 30 hours at 37 °C. Invasive cells were stained with crystal violet. MDA-MB-231 cells were used as positive control. Scale bar, 100 µm. **b.** Two step PCR amplification to capture the genome integrated sgRNA through amplicon-seq. The region containing the sgRNA was amplified from the genomic DNA of GeCKO v2.0 library transduced cells by a two-step PCR. PCR1 was run for 30 cycles and PCR2 for 10 cycles using purified PCR1 product as the template. The resultant 300 bp amplicon was sequenced.

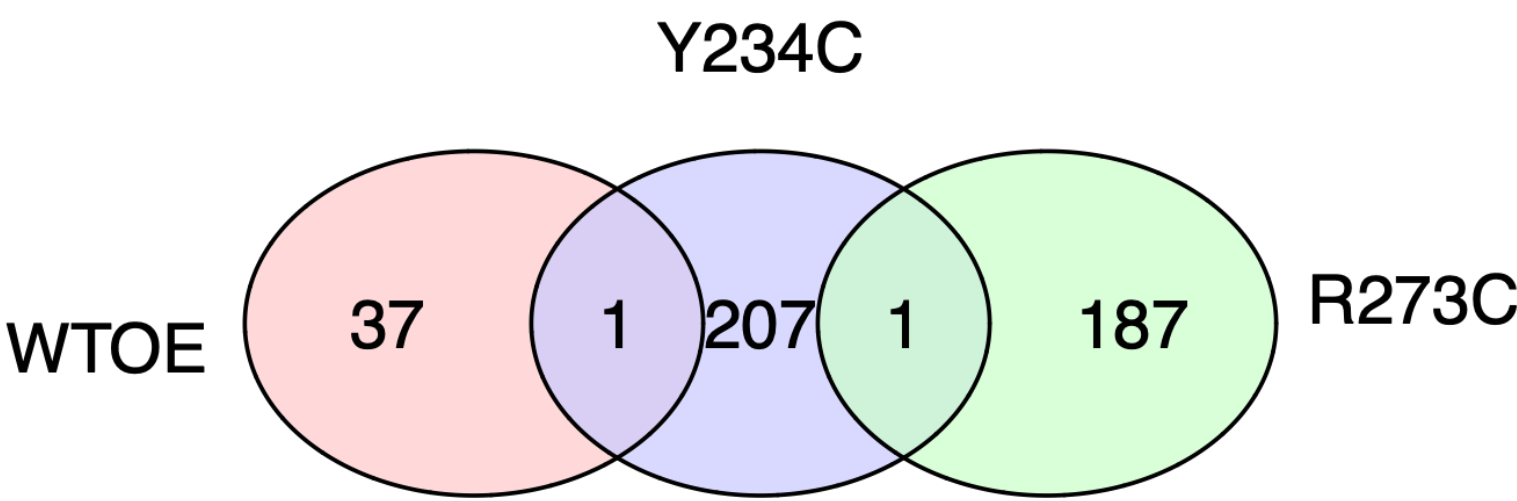


Supplementary Figure 9. Enrichment of sgRNAs in post-screen cells. The fold enrichment values (post- over pre-invasion cells) from individual screens with each sgRNA library are shown as a violin plot in a log10 scale. sgRNAs for *TP53* and *PTEN* are shown for comparison.

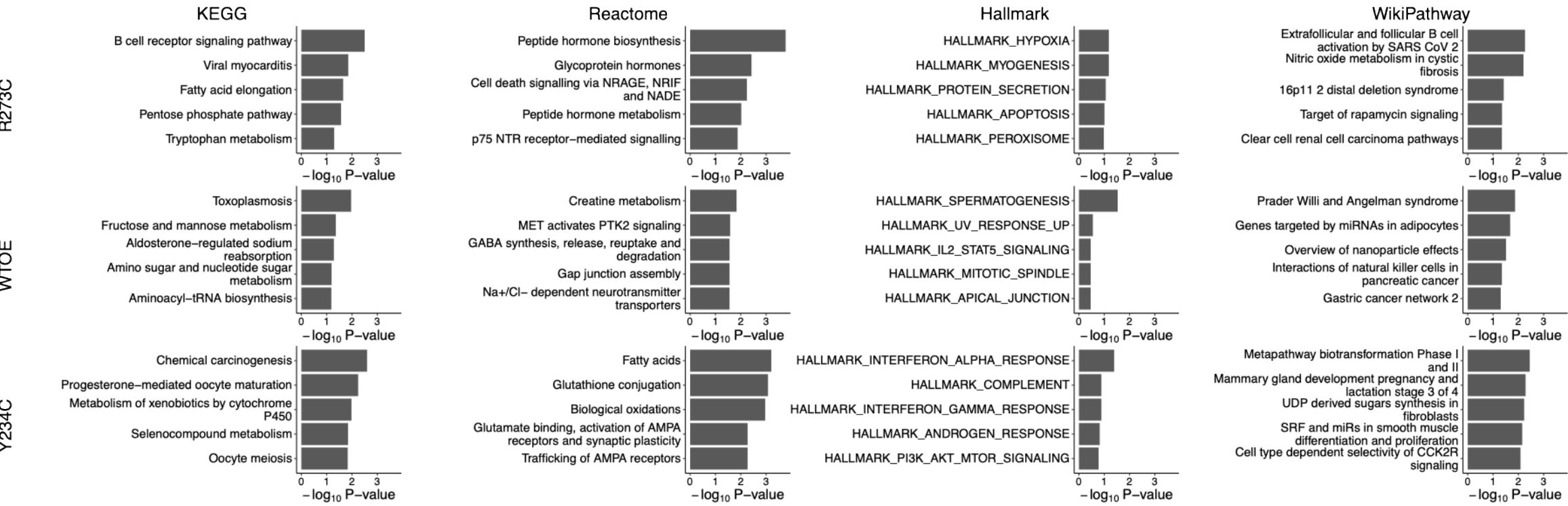


Supplementary Figure 10. Violin plots showing the distribution of fold enrichment for dummy gRNAs and gene-targeting gRNAs. Each point represents a single gRNA, and the width of the violin indicates the density of gRNAs at different log₁₀FE values. gRNAs with log₁₀FE greater than 3 were classified as enriched

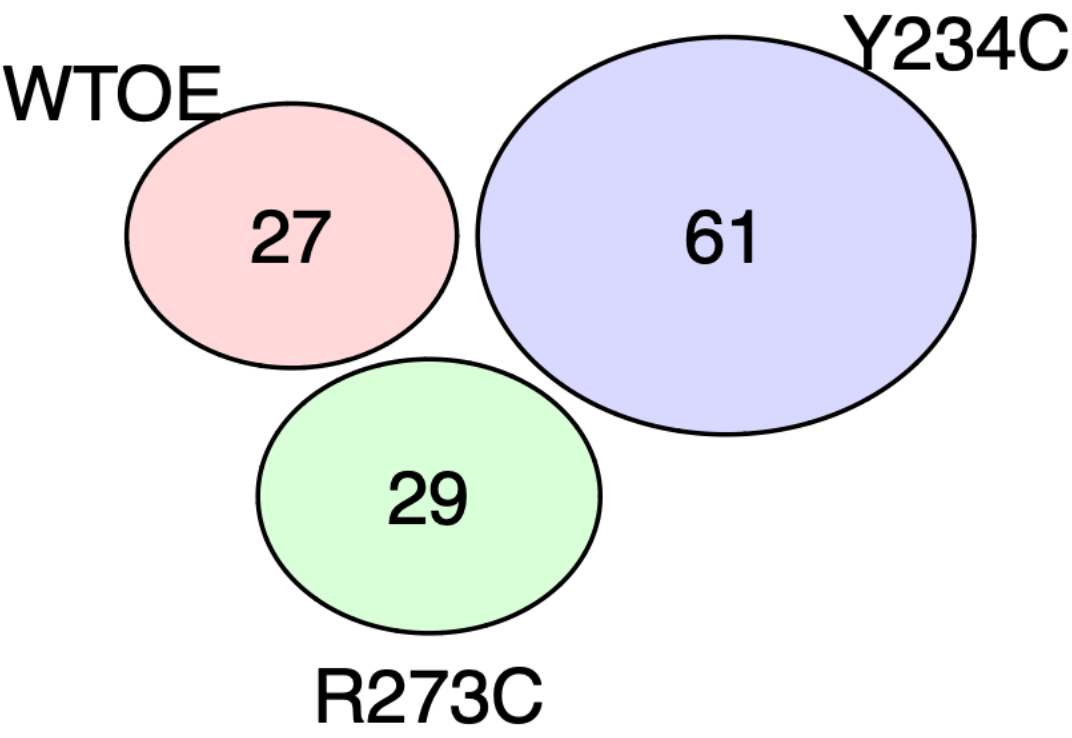
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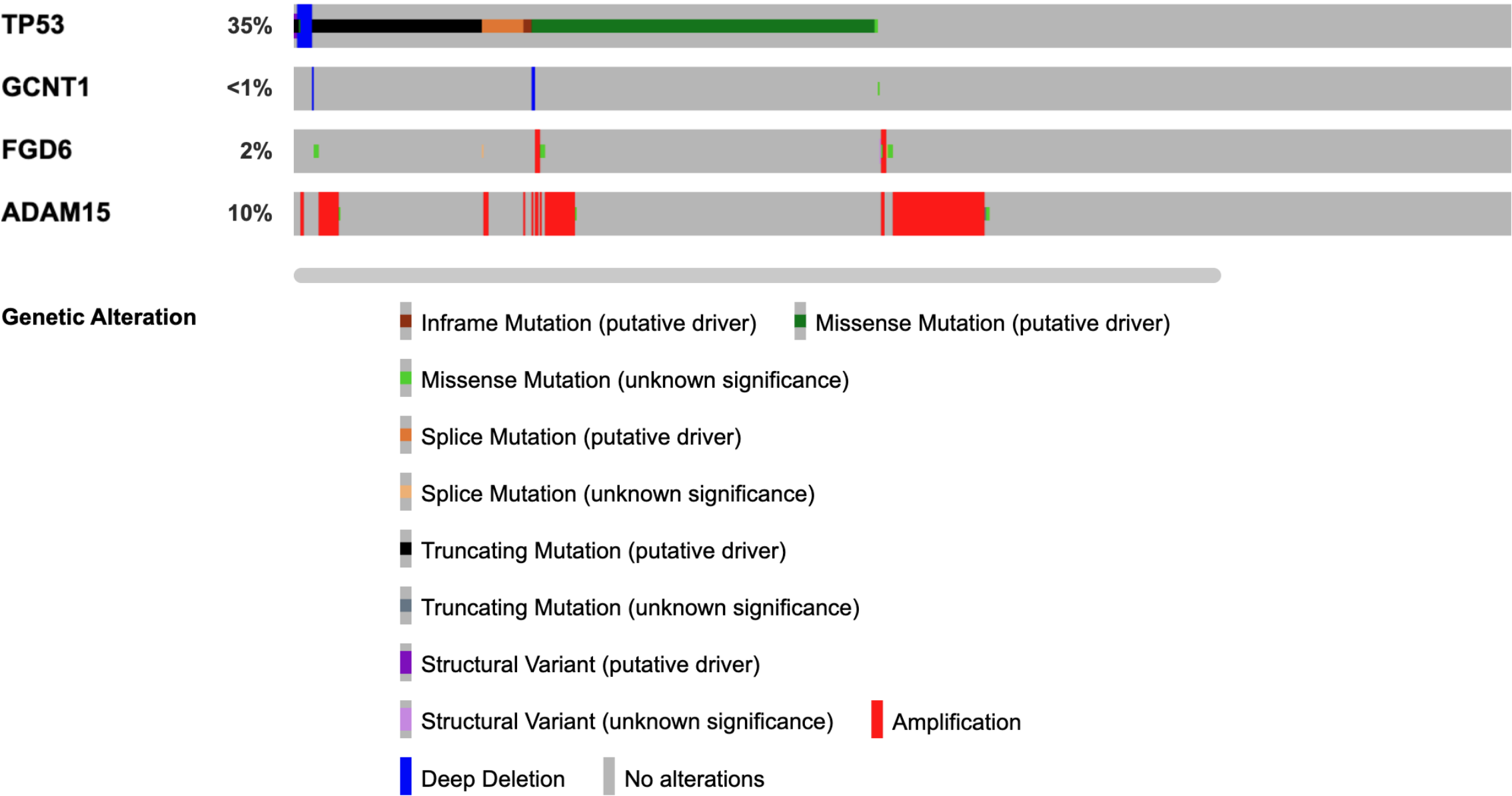
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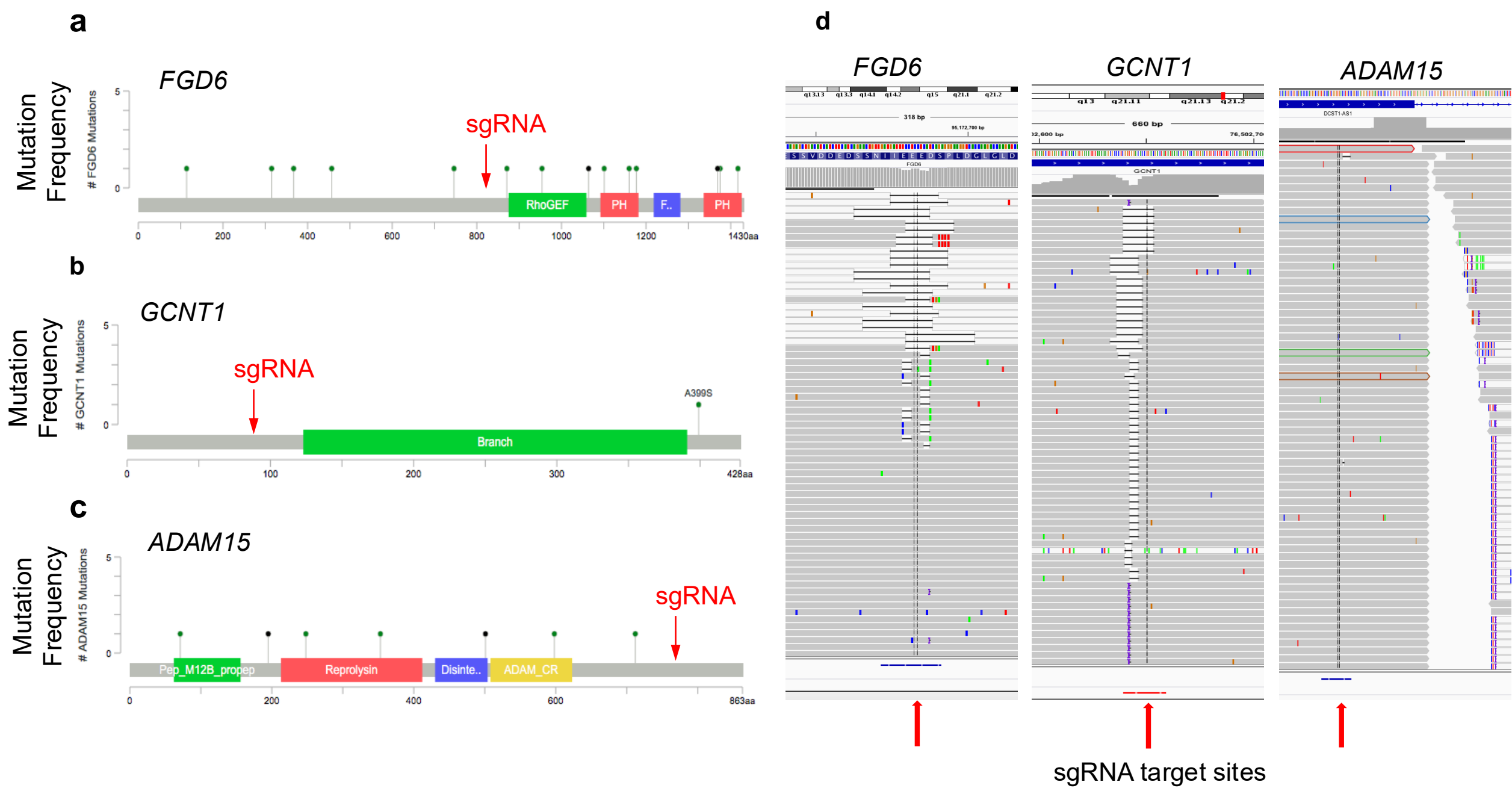
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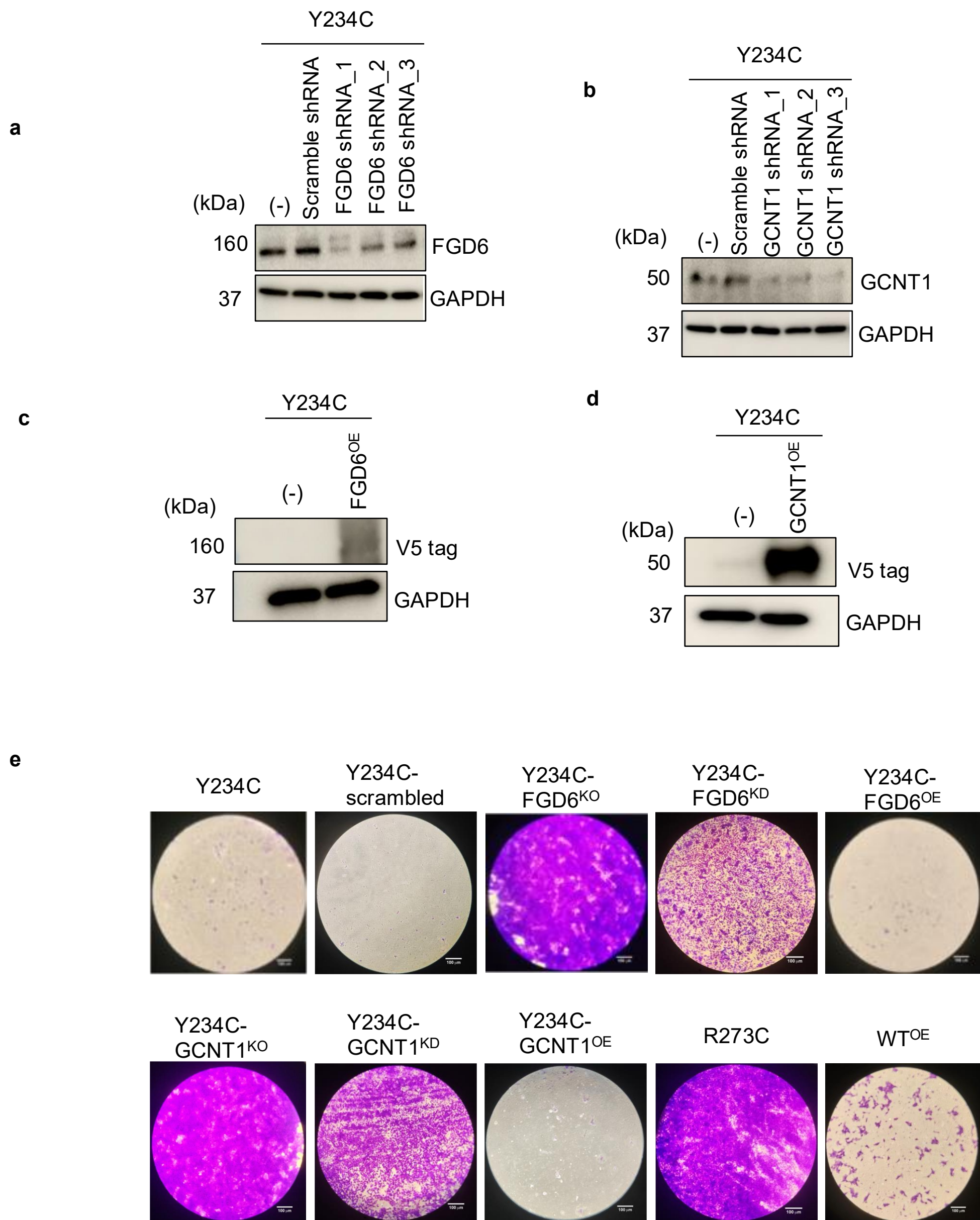
Supplementary Figure 11. Distribution of mutations. **a.** Venn diagrams showing the overlap of genes with log10 fold enrichment > 3 among different cell lines. **b.** Pathway enrichment analysis was performed using genes with log10 fold enrichment (FE) > 3 across four pathway databases (KEGG, Reactome, Hallmark, and WikiPathways) in three cell lines. Bar charts show the top 5 significantly enriched pathways for each database-cell line combination. The x-axis represents the -log10P-value of pathway enrichment analysis. Pathways are ranked by statistical significance. **c.** Venn diagrams showing the overlap of pathways enriched within the genes with log10 fold enrichment > 3 among different cell lines. Cell lines are color-coded as follows: R273C (green), WT^{OE} (red), and Y234C (blue).



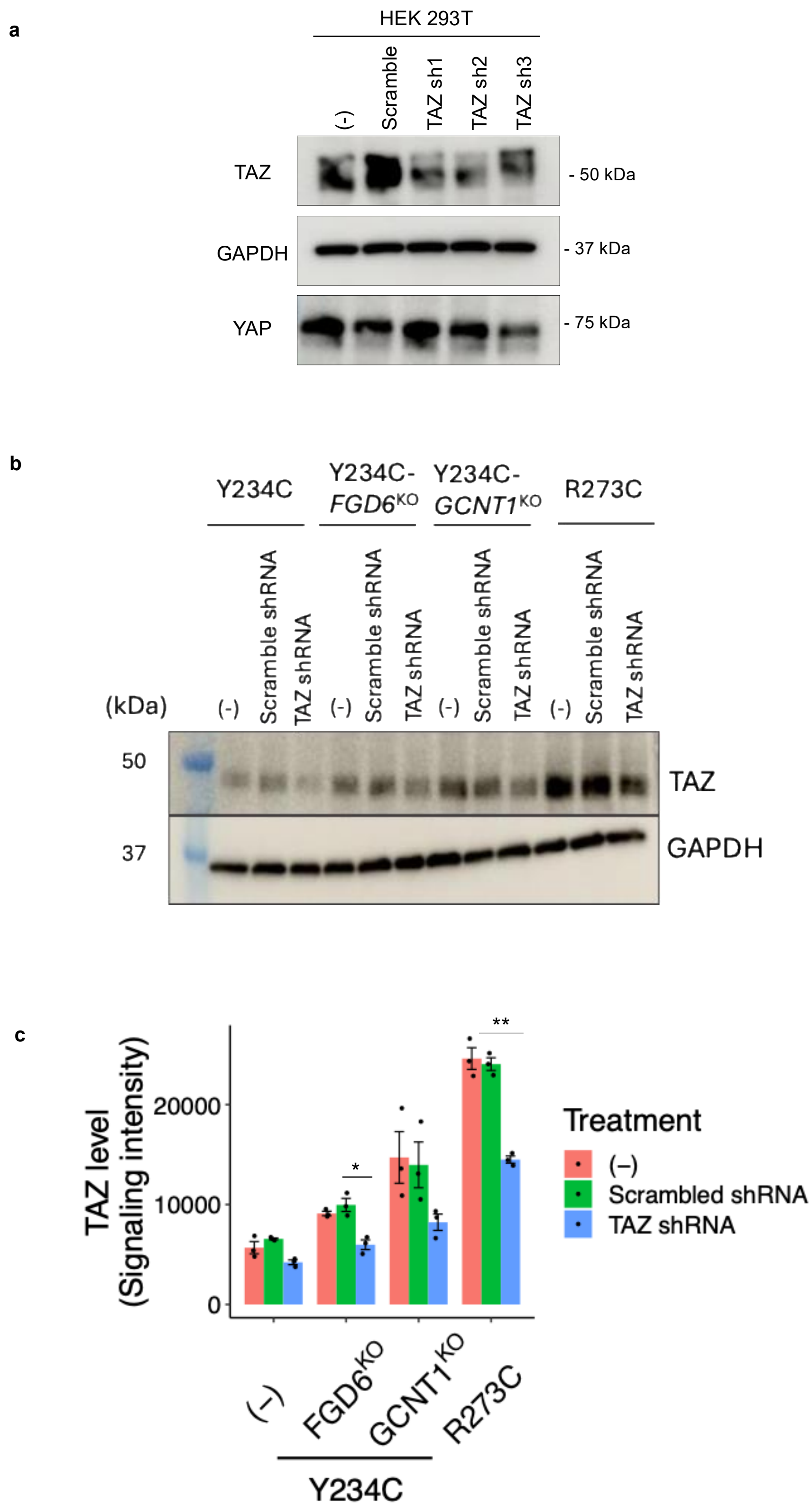
Supplementary Figure 12. Genetic alteration landscape of TP53, GCNT1, FGD6, and ADAM15 in the study cohort. OncoPrint generated from cBioPortal, showing the frequency and distribution of genomic alterations across samples. The percentage on the left indicates the overall alteration frequency for each gene.



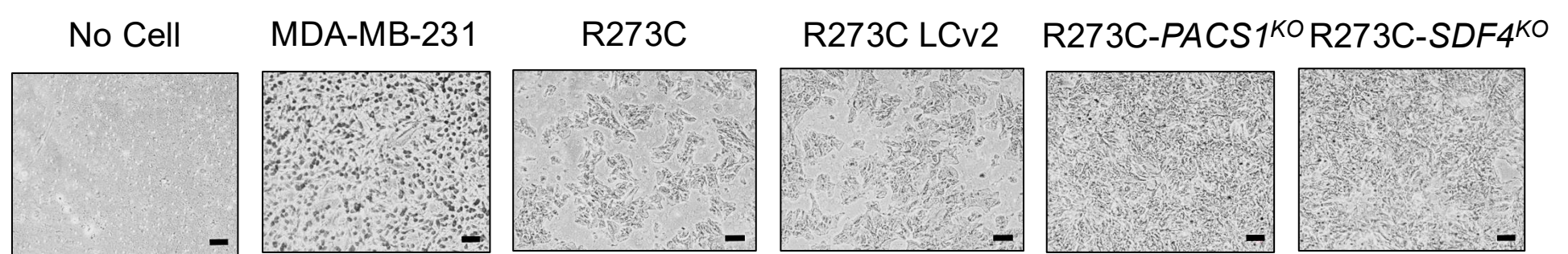
Supplementary Figure 13. Schematic representation of sgRNA targeting sites and genomic editing.
a. Selected sgRNA (red arrow) targeted the region close to the RhoGEF domain. **b.** The sgRNA targeted the N-terminus of the protein, which could effectively knock-out the gene function. **c.** The enriched sgRNA targeted the C-terminal end of the ADAM15 protein instead of the catalytic zinc-binding metalloprotease domain or the N-term, indicating reduced activity of the sgRNAs. **d.** NGS of the targeted gene loci show deletion of base pairs (red arrows). No deletion was observed on *ADAM15* loci, implying off-target effects.



Supplementary Figure 14. Functional validation of *FGD6* and *GCNT1* in Y234C cells on cell invasion. Western blot images showing **a.** Knockdown of *FGD6* **b.** Knockdown of *GCNT1* **c.** Overexpression of *FGD6* **d.** Overexpression of *GCNT1*. **e.** Cell Invasion assay showing decreased cell invasiveness in Y234C-*FGD6*^{OE} and Y234C-*GCNT1*^{OE} cells compared to Y234C-*FGD6*^{KO/KD} and Y234C-*GCNT1*^{KO/KD} cells. Scale bar, 100 μm.



Supplementary Figure 15. shRNA-mediated Knockdown of TAZ in cells. **a.** 293T cells. **b.** MCF10A mutant cells. GAPDH served as the loading control. **c.** Western blot band intensities were quantified using ImageJ software. Statistical significance was assessed using two-tailed *t*-test (*: $p < 0.05$, **: $p < 0.01$).



Supplementary Figure 16. Specific disruption of *PACS1* and *SDF4*. In addition to R273C mutation, disruption of *PACS1* and *SDF4* increased the cell invasion potential. MDA-MB-231 cells were the positive control and LCv2 was the empty vector control. Scale bar, 100 μ m.

List of Supplementary Data

Supplementary Data 1a. PTEN sgRNA sequence and their targeting position

Supplementary Data 1b. NGS-based QC on Gene and gRNA Coverage in GeCKO v2.0 Library Plasmid

Supplementary Data 1c. Calculation to achieve 250-fold representation of each gRNA

Supplementary Data 1d. PCR Primers for Amplification of gRNA Cassettes and Genomic Loci of Top Hits

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Supplementary Data 2a. Screening results for GeCKO library A

Supplementary Data 2b. Screening results for GeCKO library B

Supplementary Data 3a. Enriched KEGG pathways in top hits of Y234C cells

Supplementary Data 3b. Enriched Reactome pathways in top hits of Y234C cells

Supplementary Data 3c. Enriched Hallmark pathways in top hits of Y234C cells

Supplementary Data 3d. Enriched Wiki pathways in top hits of Y234C cells

Supplementary Data 3e. Enriched KEGG pathways in top hits of R273C cells

Supplementary Data 3f. Enriched Reactome pathways in top hits of R273C cells

Supplementary Data 3g. Enriched Hallmark pathways in top hits of R273C cells

Supplementary Data 3h. Enriched Wiki pathways in top hits of R273C cells

Supplementary Data 3i. Enriched KEGG pathways in top hits of WTOE cells

Supplementary Data 3j. Enriched Reactome pathways in top hits of WTOE cells

Supplementary Data 3k. Enriched Hallmark pathways in top hits of WTOE cells

Supplementary Data 3l. Enriched Wiki pathways in top hits of WTOE cells

List of Supplementary Data

Supplementary Data 4a. TP53 co-mutation analysis with ADAM15, FGD6, and GCNT1 in the TCGA breast cancer cohort, excluding Silent classifications.

Supplementary Data 4b. Co-mutation analysis of 10 TP53 mutations of interest with ADAM15, FGD6, and GCNT1 in the TCGA breast cancer cohort, excluding Silent classifications.

Supplementary Data 4c. TP53 co-mutation summary for FGD6-mutated samples in the TCGA breast cancer cohort, excluding Silent classifications.

Supplementary Data 4d. TP53 co-mutation summary for GCNT1-mutated samples in the TCGA breast cancer cohort, excluding Silent classifications.

Supplementary Data 5a. Quantification of phosphorylated TAZ (pTAZ) to total TAZ (TAZ) ratio

Supplementary Data 5b. Quantification of phosphorylated TAZ (pTAZ) to total TAZ (TAZ) ratio

Supplementary Data 5c. Quantification of phosphorylated LATS1 (pLATS1) to total LATS1 (LATS1) ratio

Supplementary Data 5c. Quantification of phosphorylated LATS1 (pLATS1) to total LATS1 (LATS1) ratio

Supplementary Data 5d. Quantification of phosphorylated MST1/2 (pMST1/2) to total MST1 (MST1)

Supplementary Data 5e. Quantification of TAZ levels after knockdown

Supplementary Data 6a. Inhibition of TEAD reporter activity by TAZ shRNA

Supplementary Data 6b. Inhibition of TEAD reporter activity by GNE-7833

Supplementary Data 7. Top 200 count-normalized sgRNA table. sgRNAs included in the top 200 count-normalized line plot, with raw counts and $\log_{10}(\text{adj.CPM} + 1)$ values for Scrambled and shTAZ.

Original uncropped western blot images

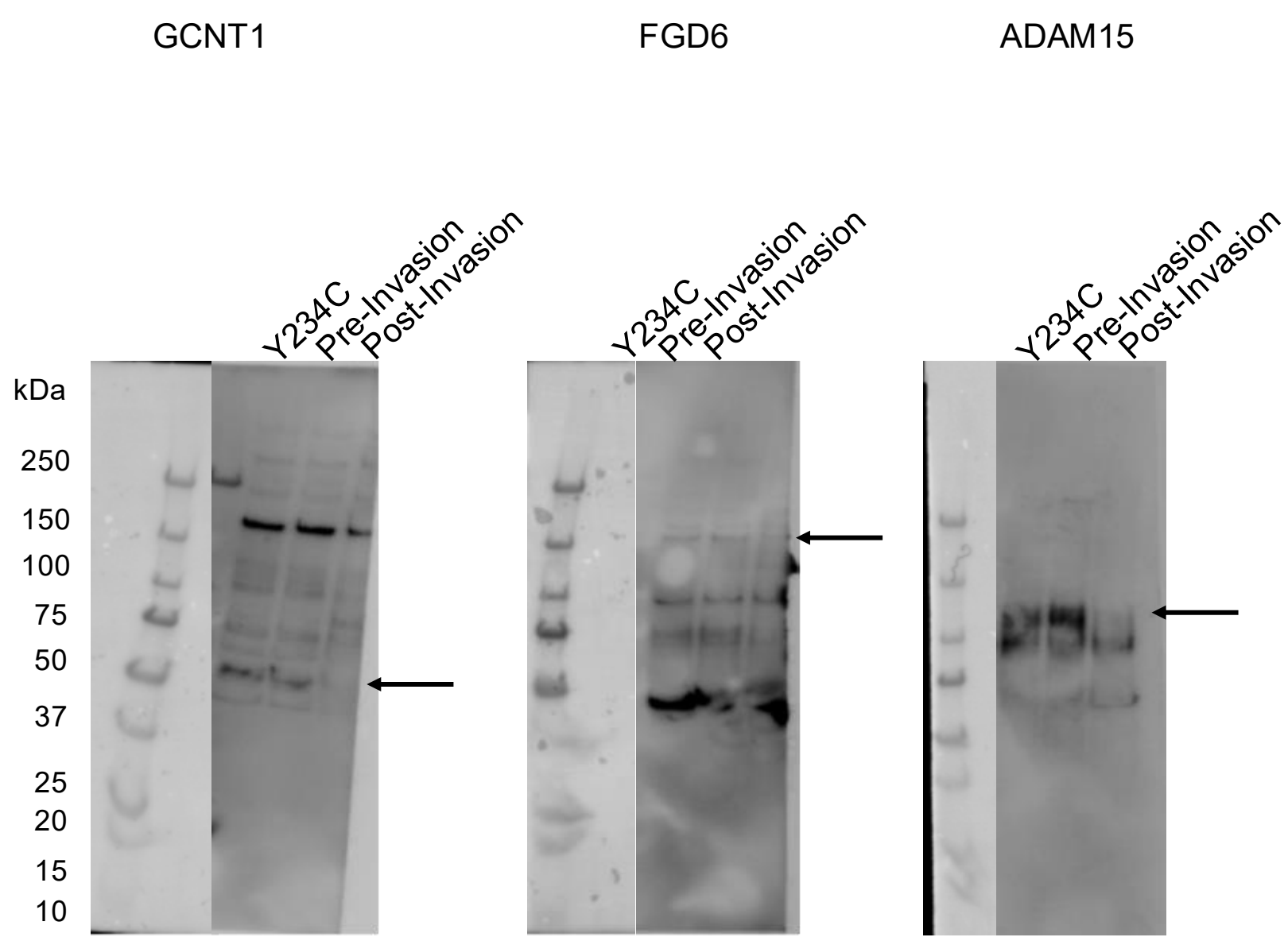


Fig. 3b

Vimentin

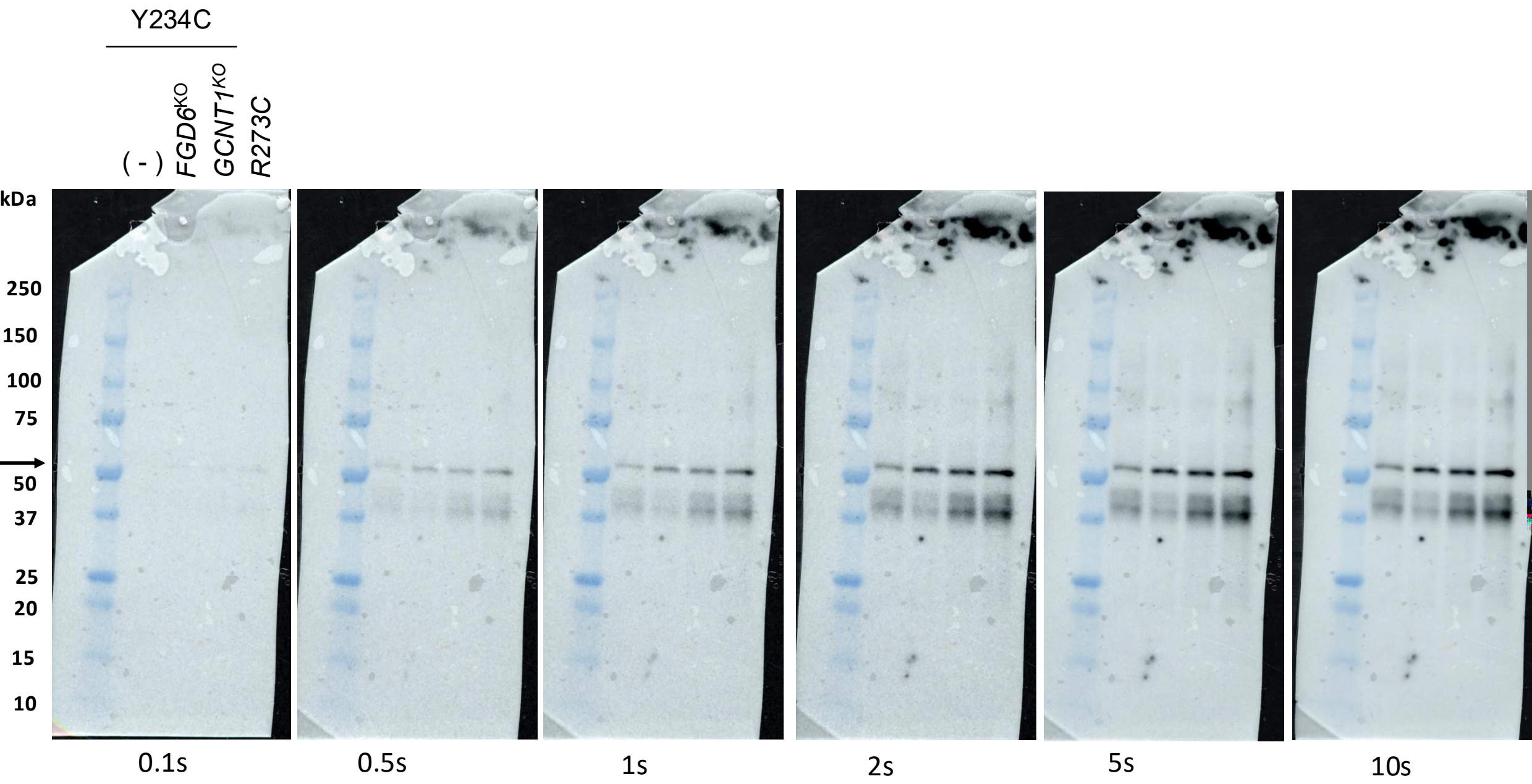


Fig. 3c

GAPDH

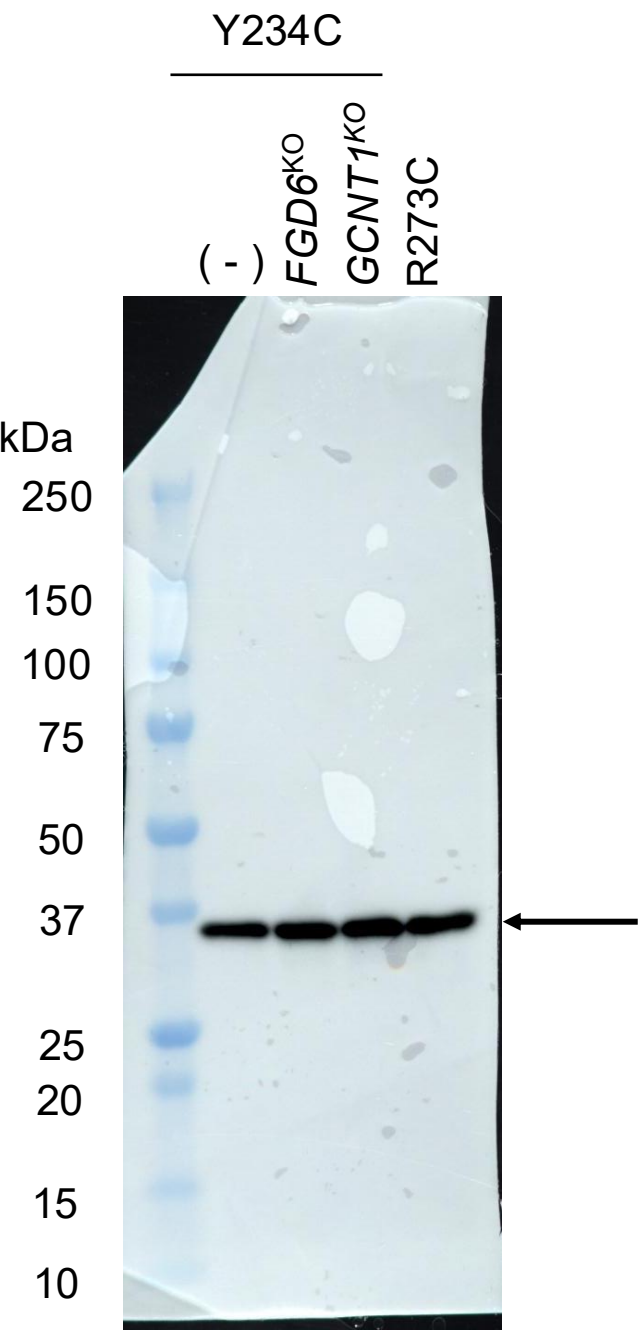


Fig. 3c

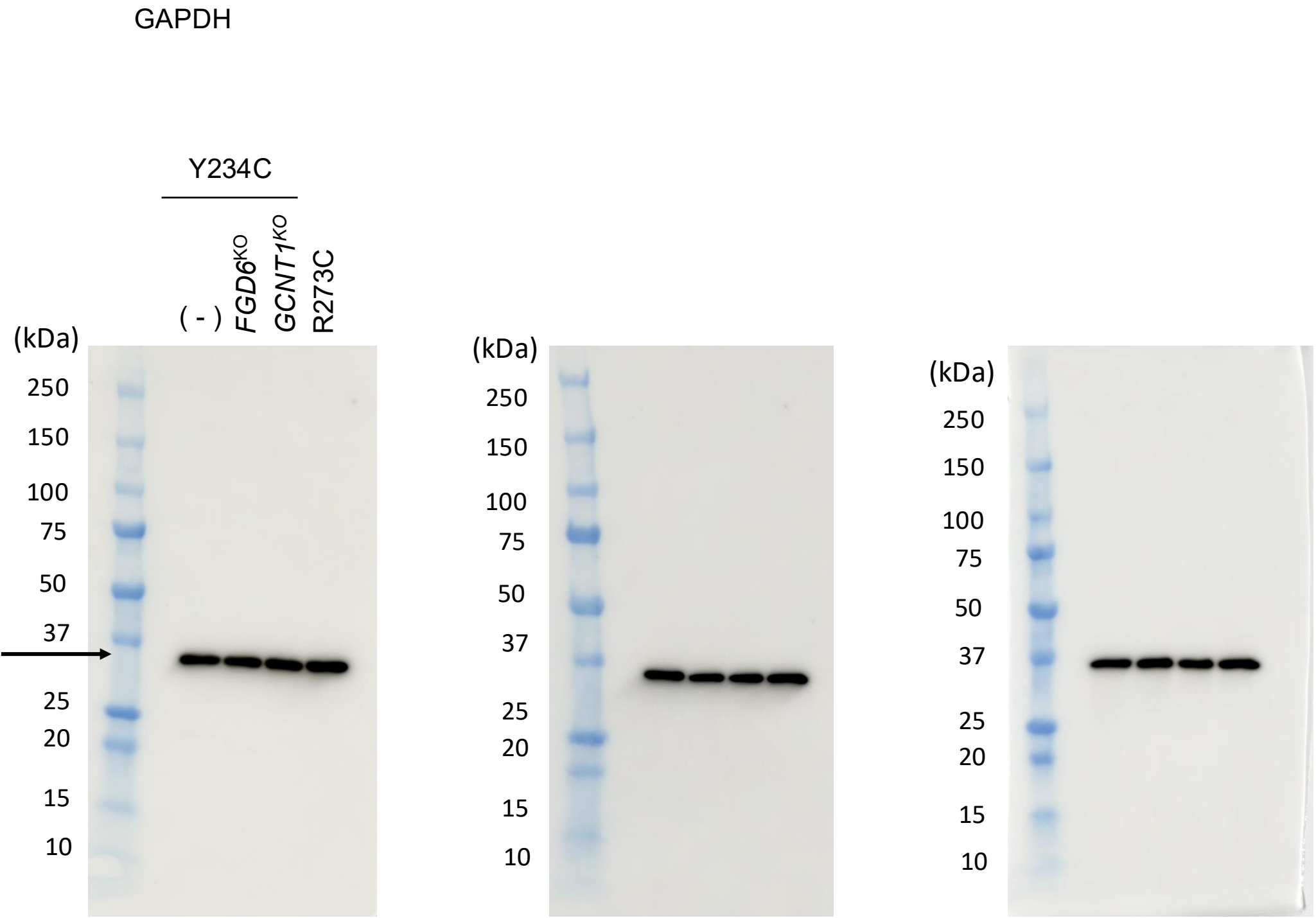


Fig. 4a

TAZ

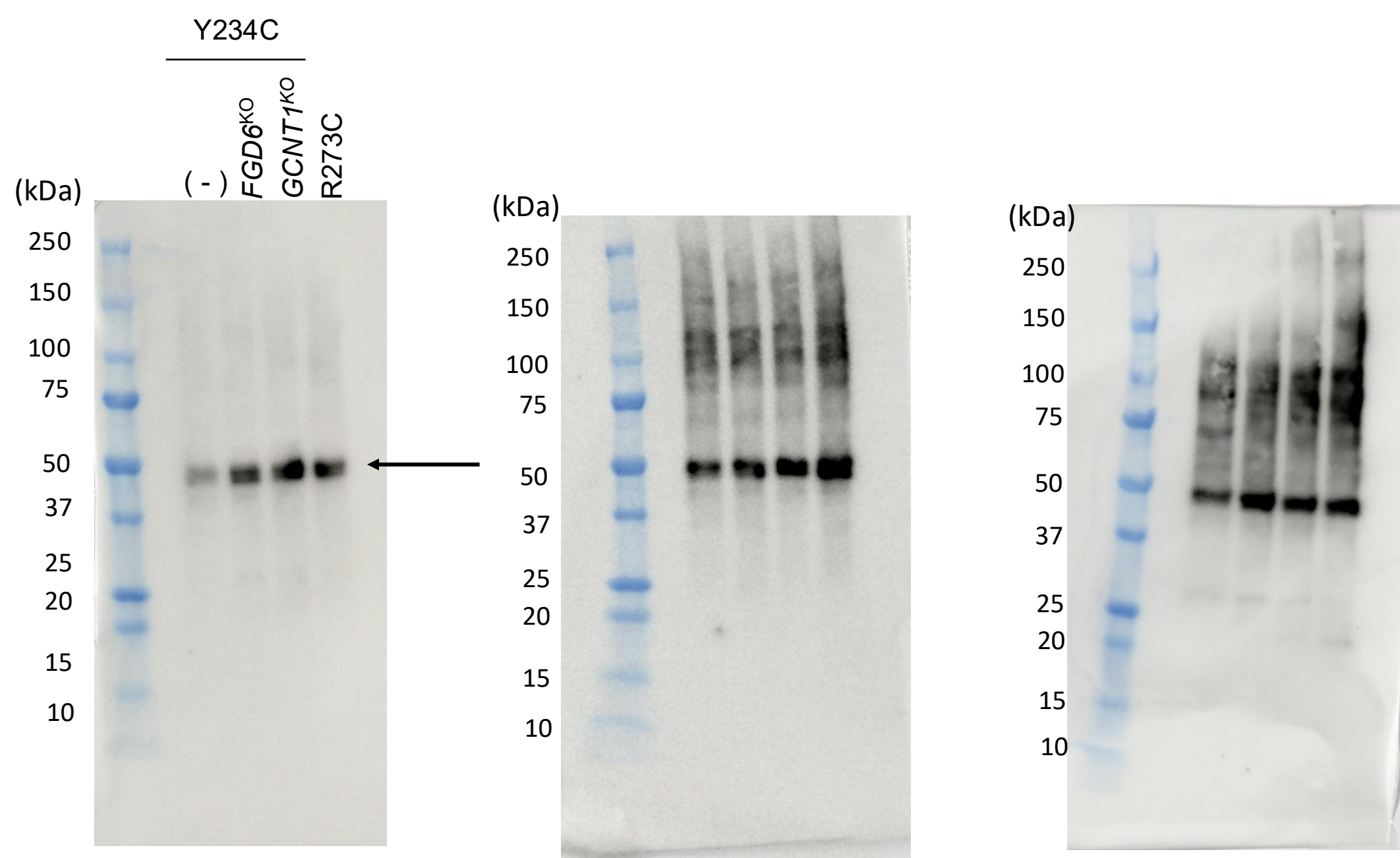


Fig. 4a

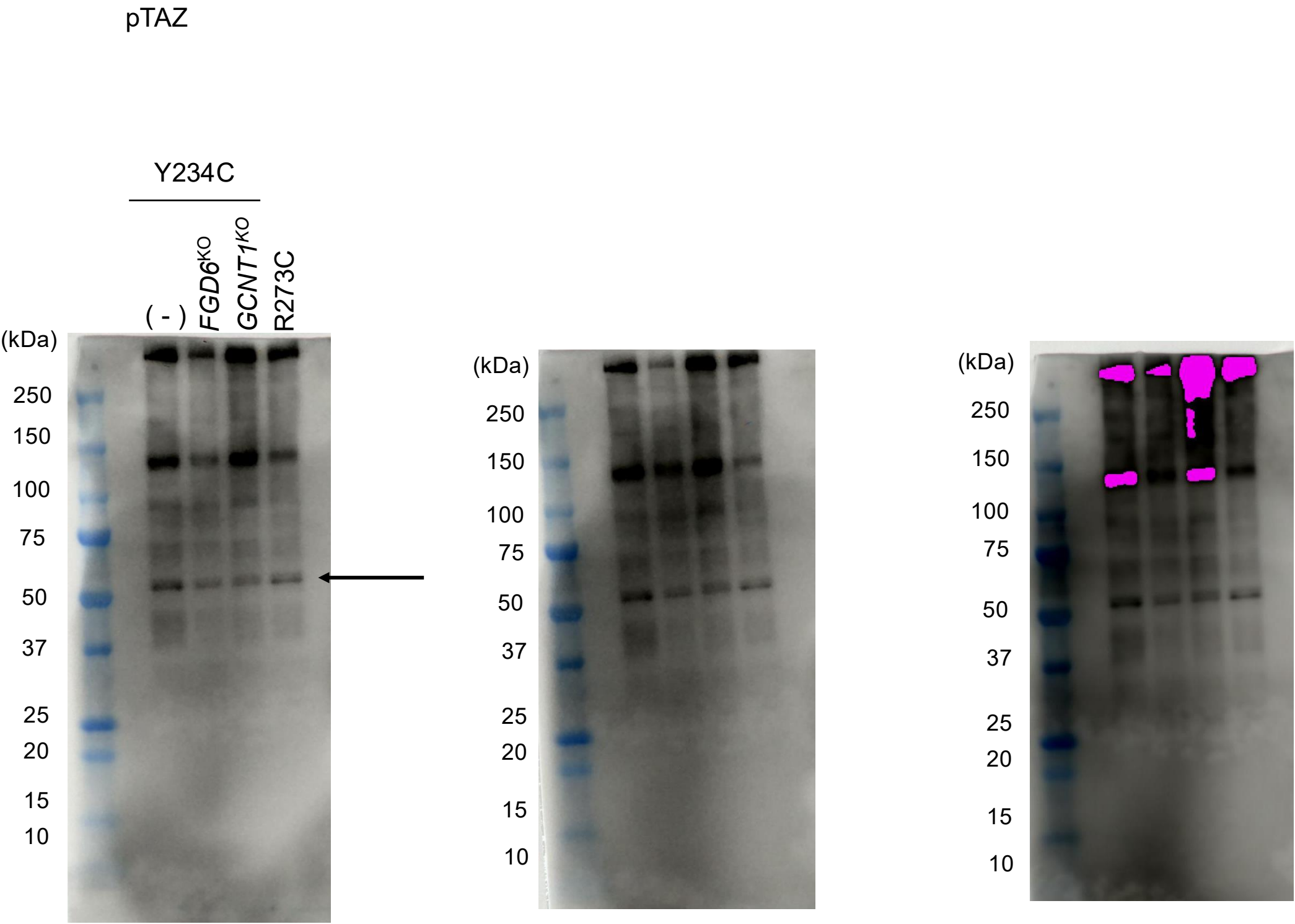


Fig. 4a

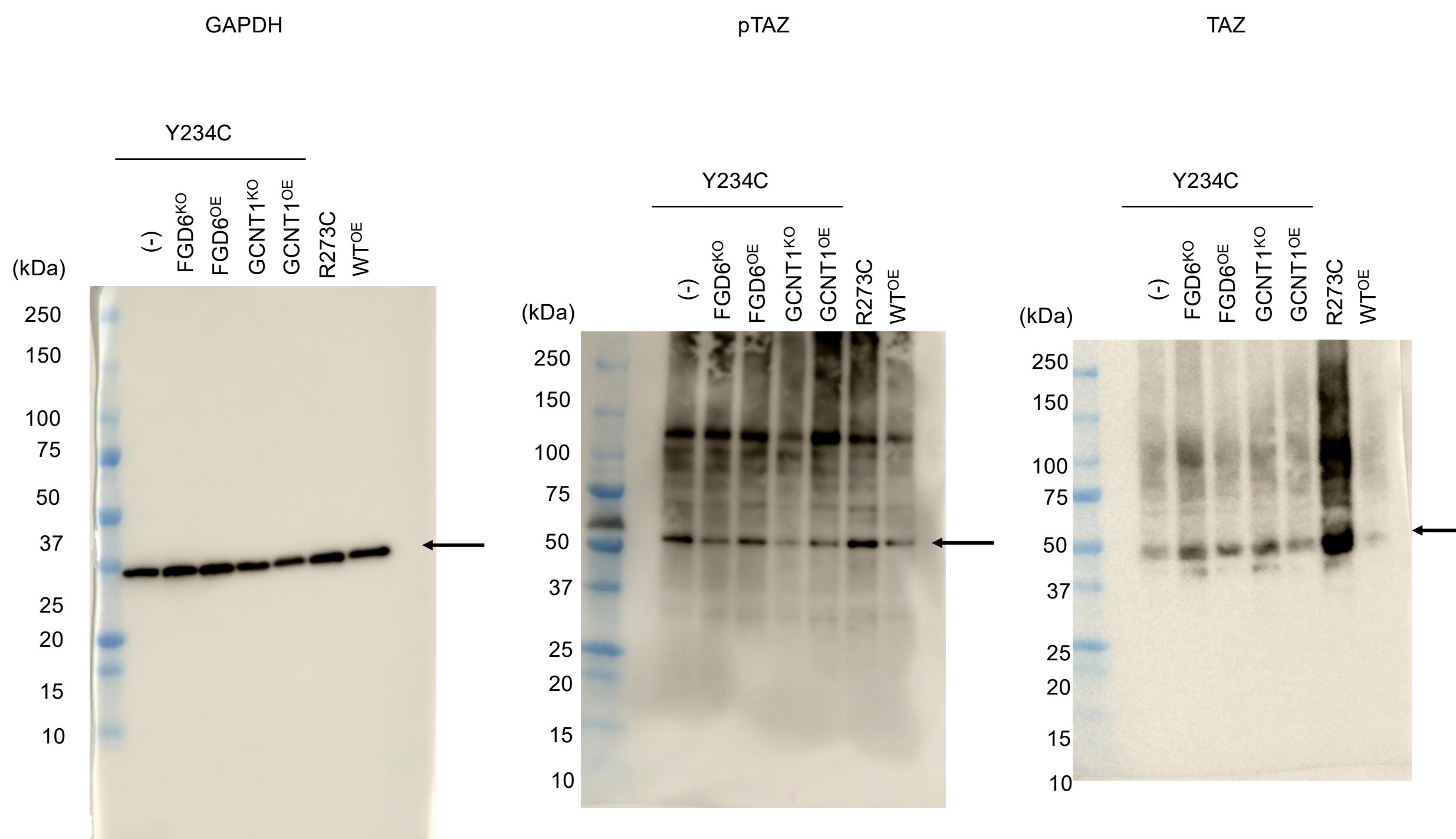


Fig. 5b

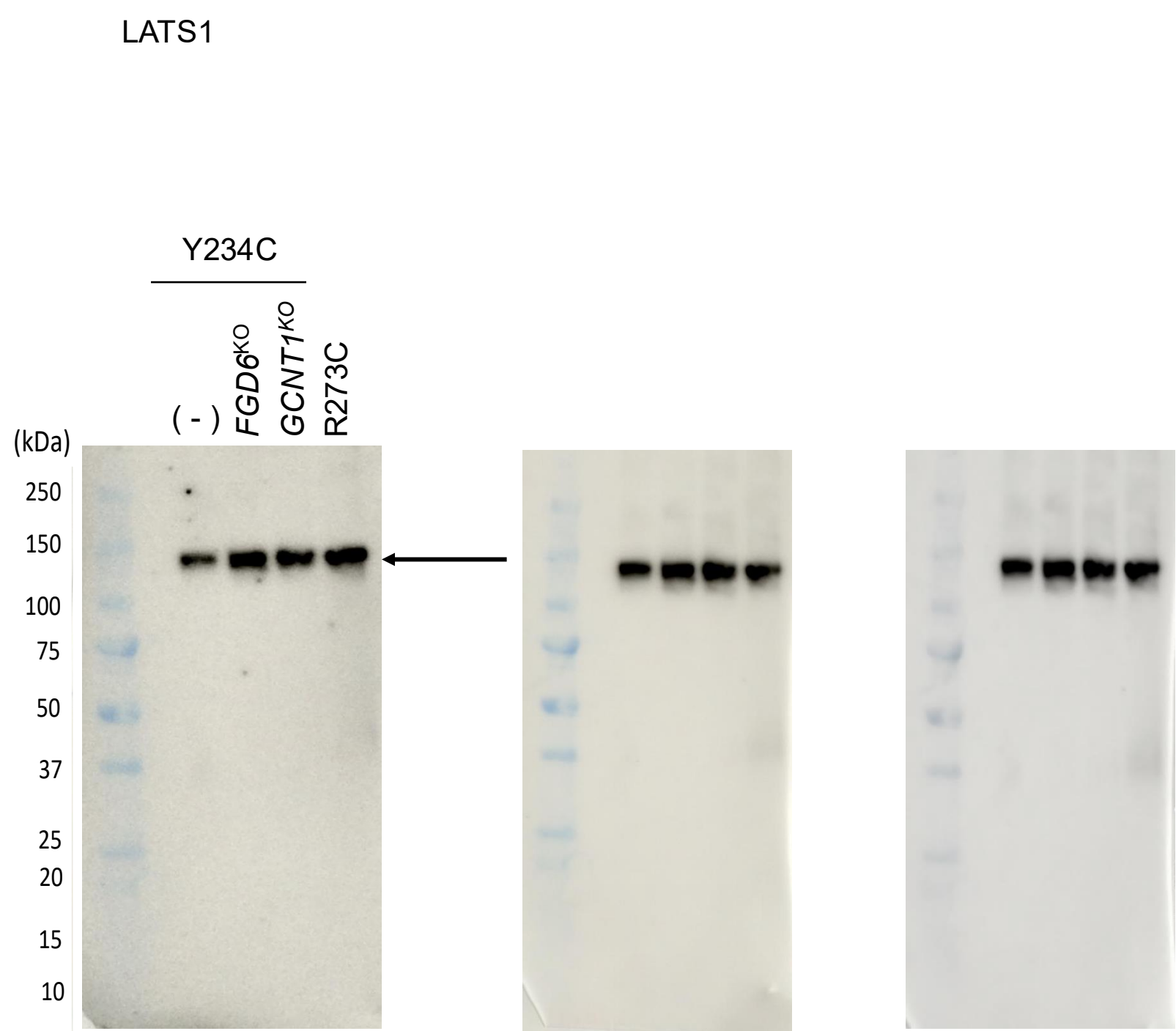


Fig. 7a

Phospho-LATS1

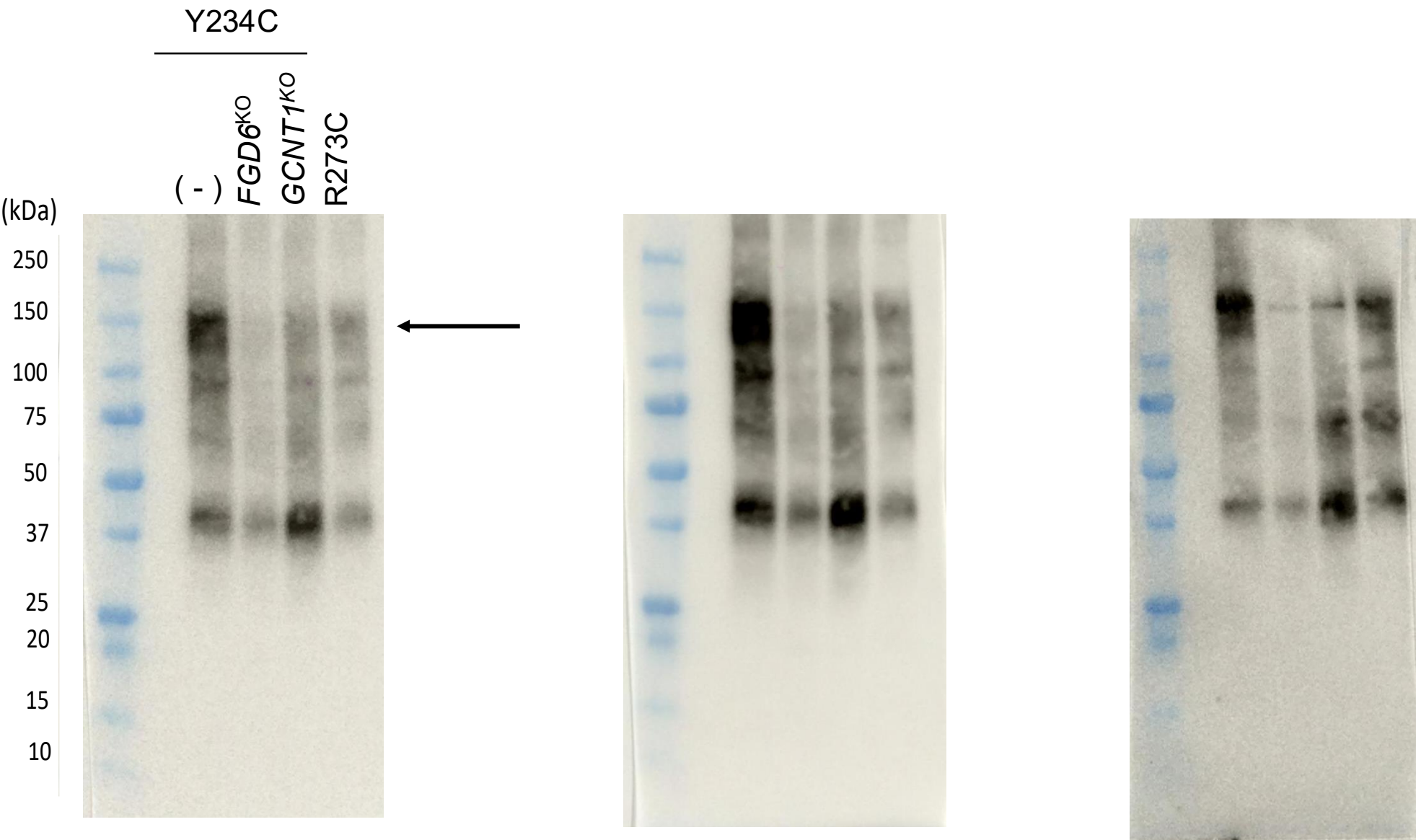


Fig. 7a

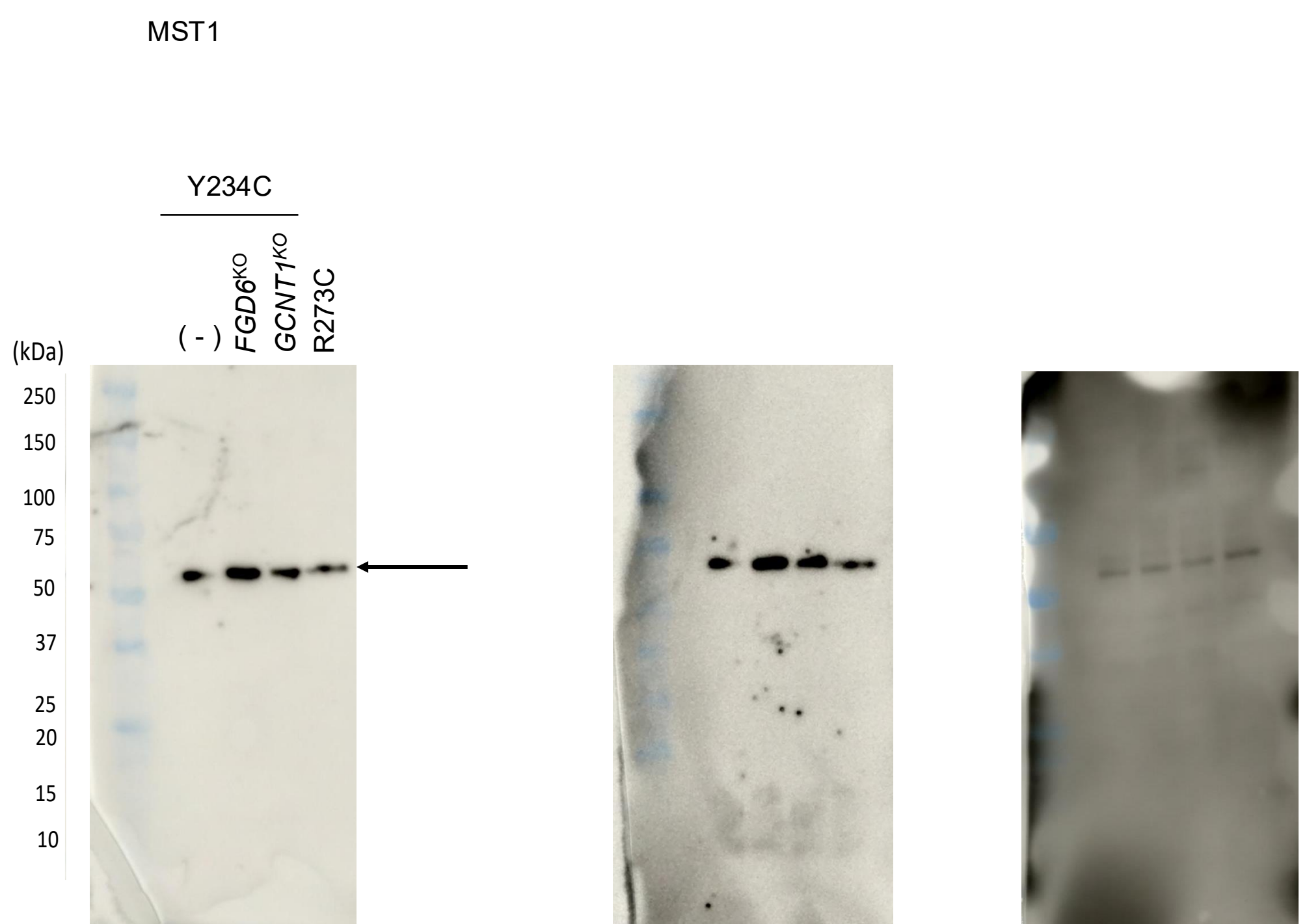


Fig. 7a

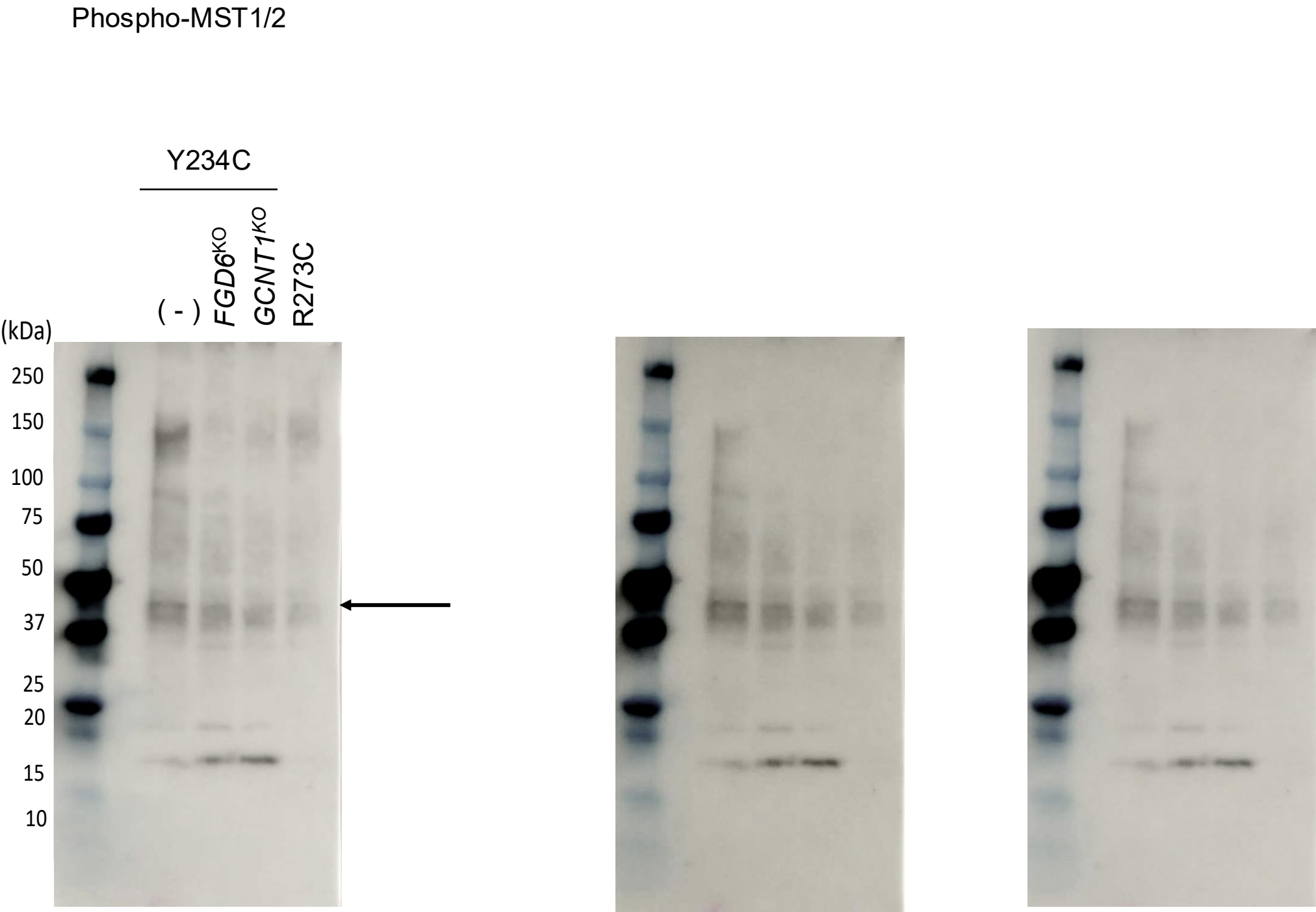


Fig. 7a

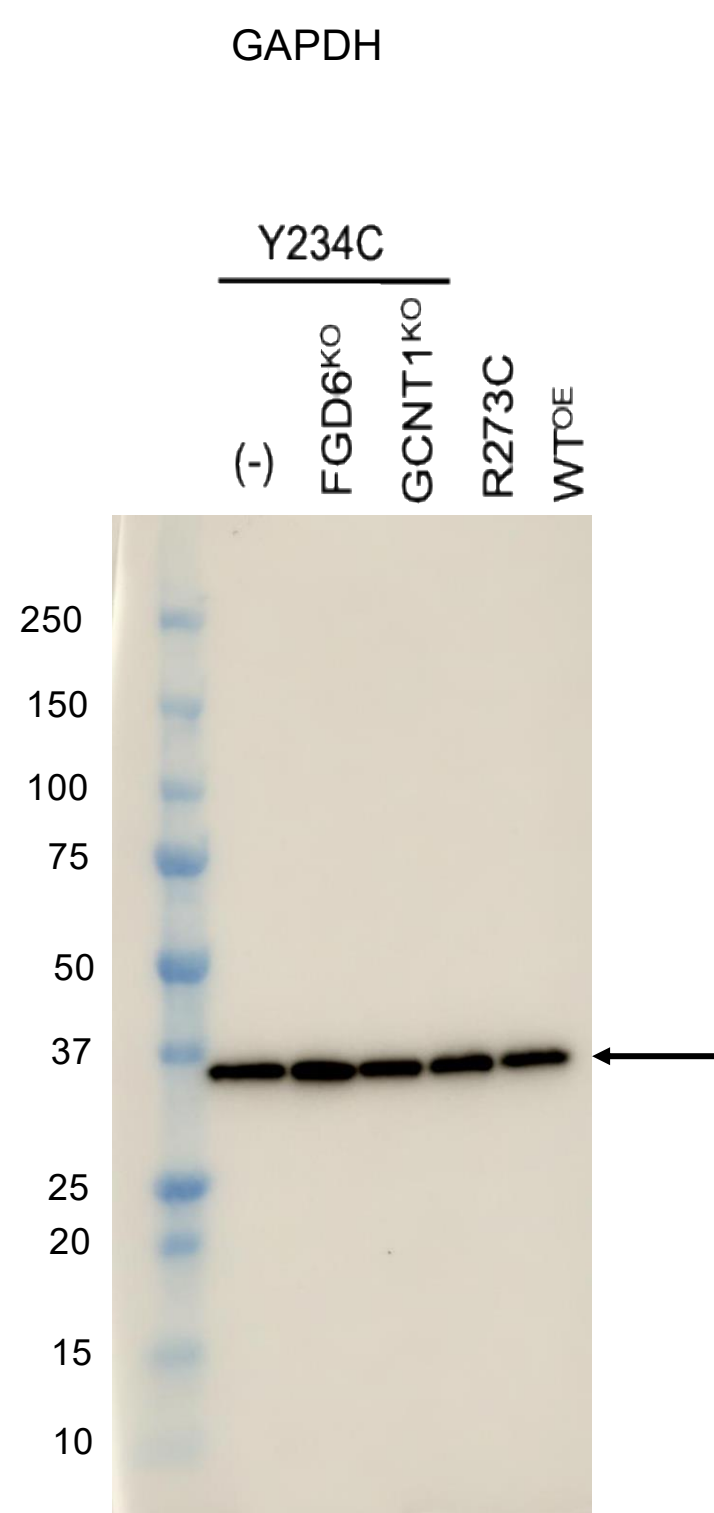


Fig. 8

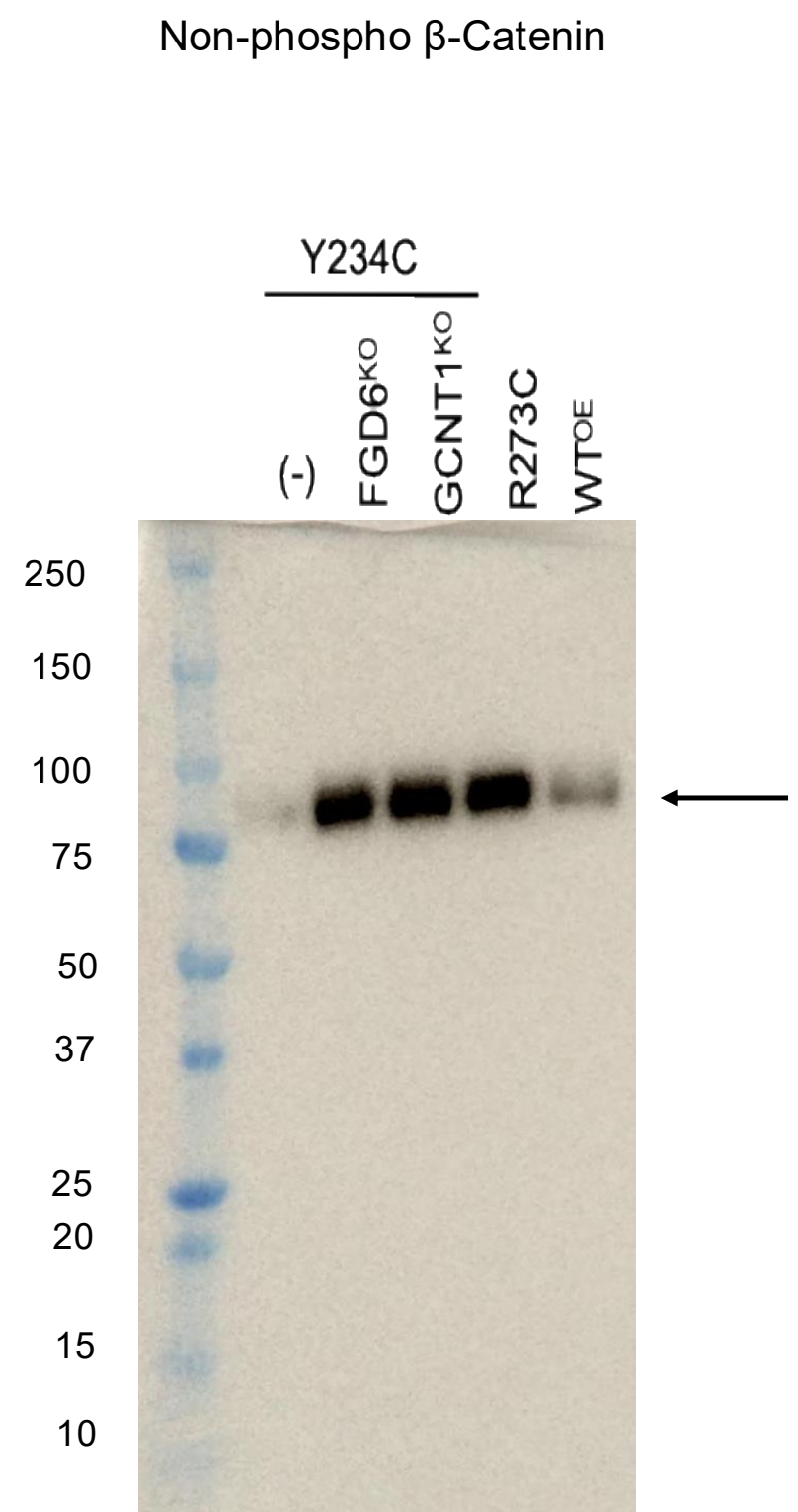
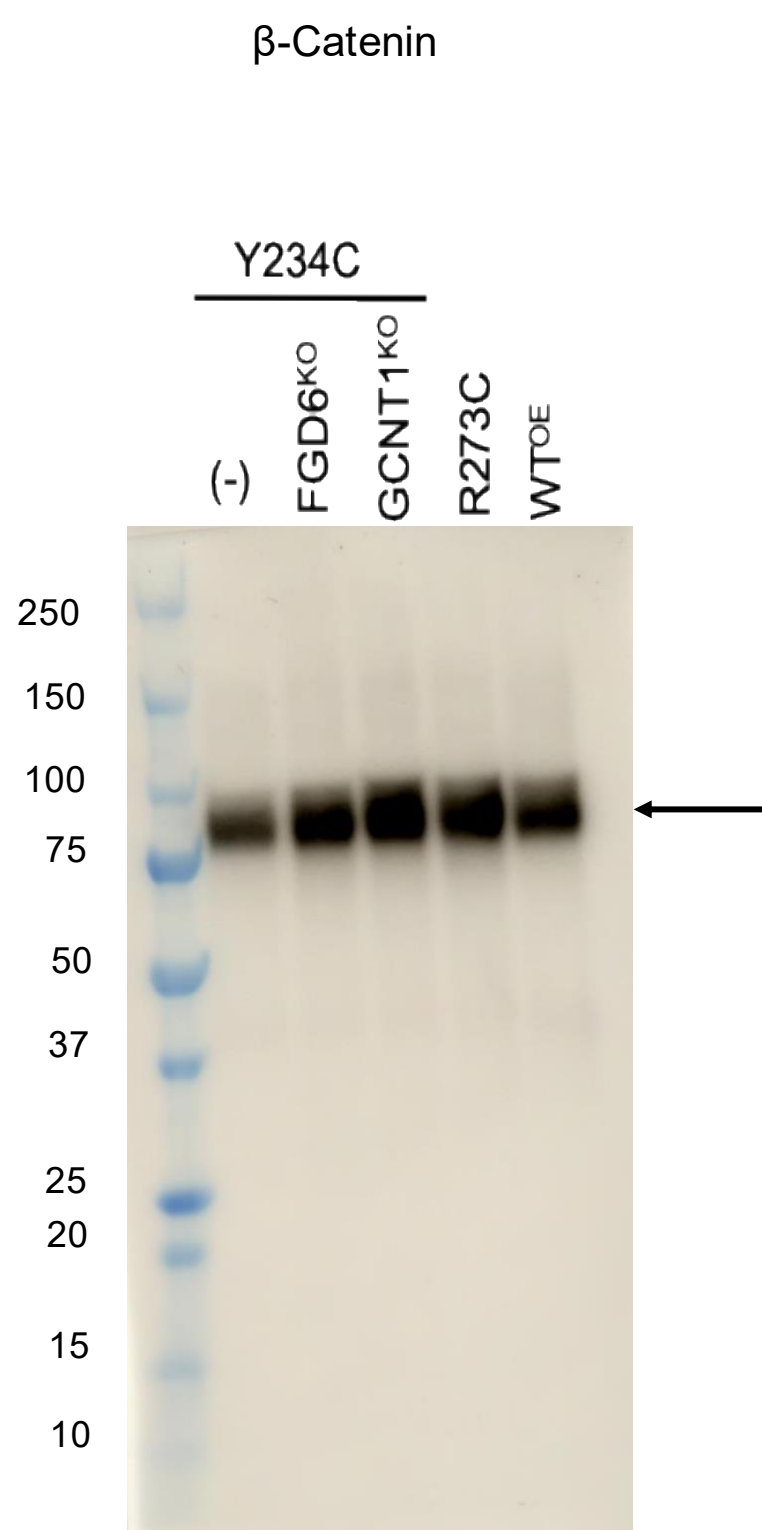


Fig. 8

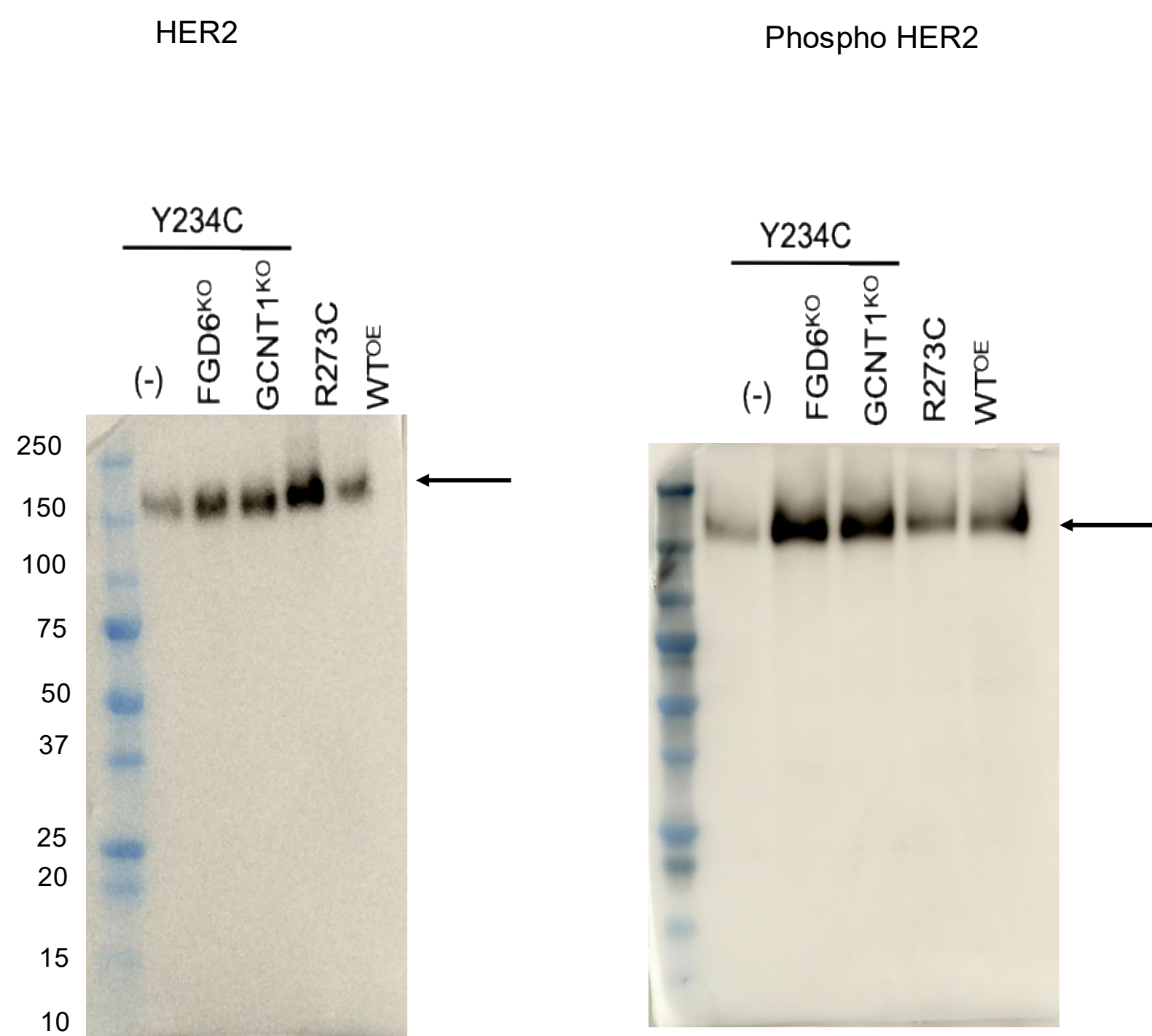


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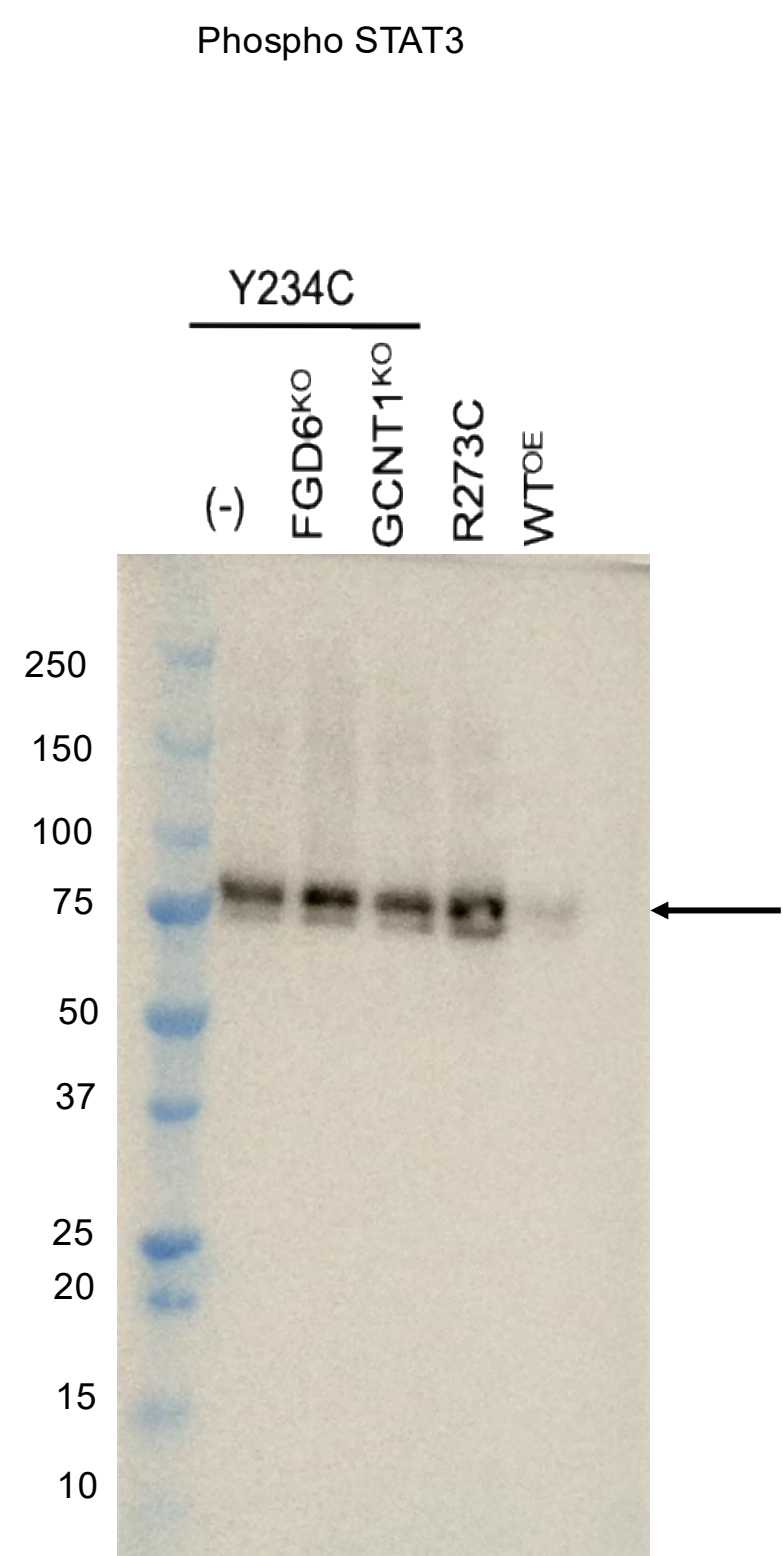
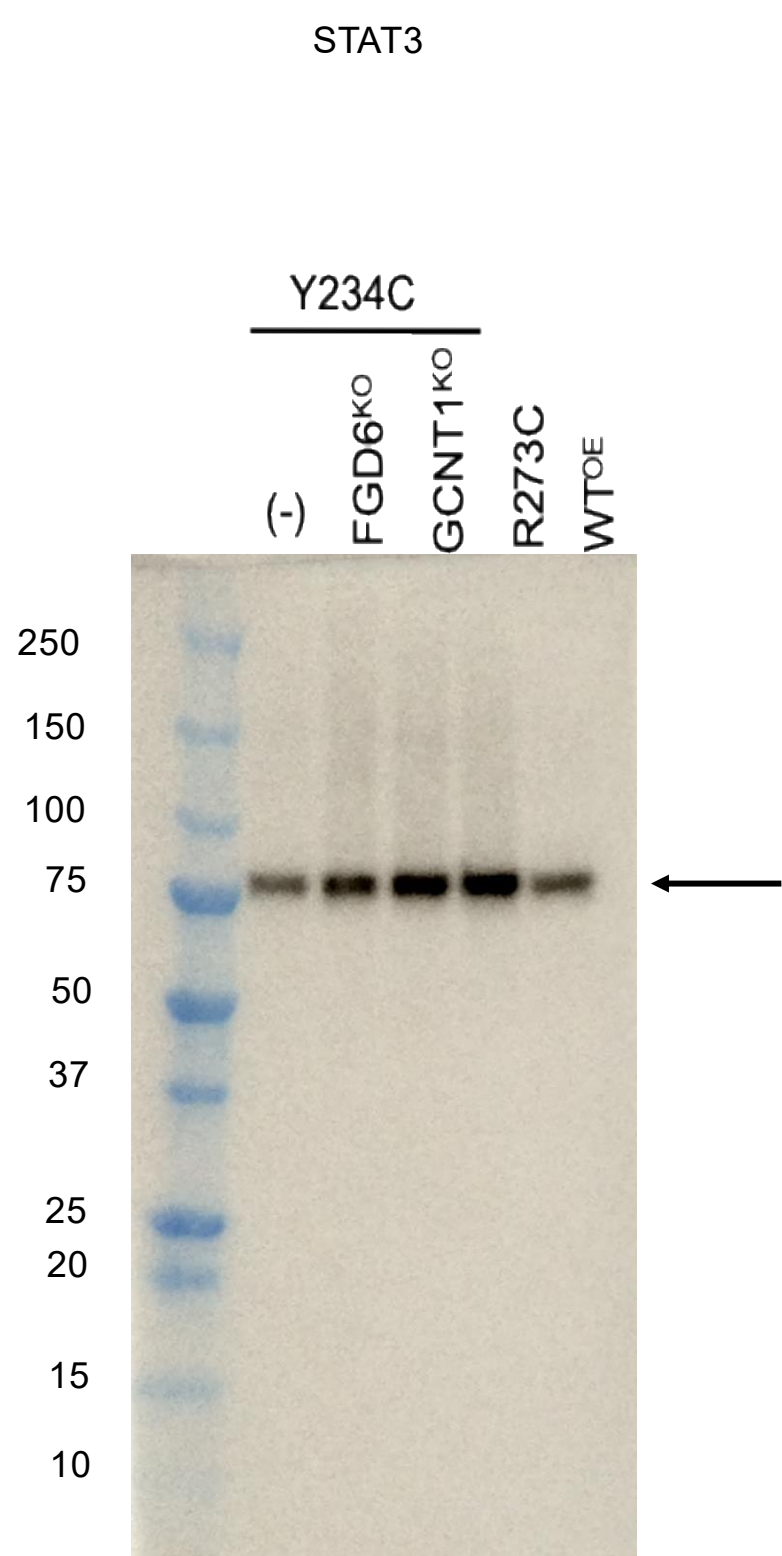


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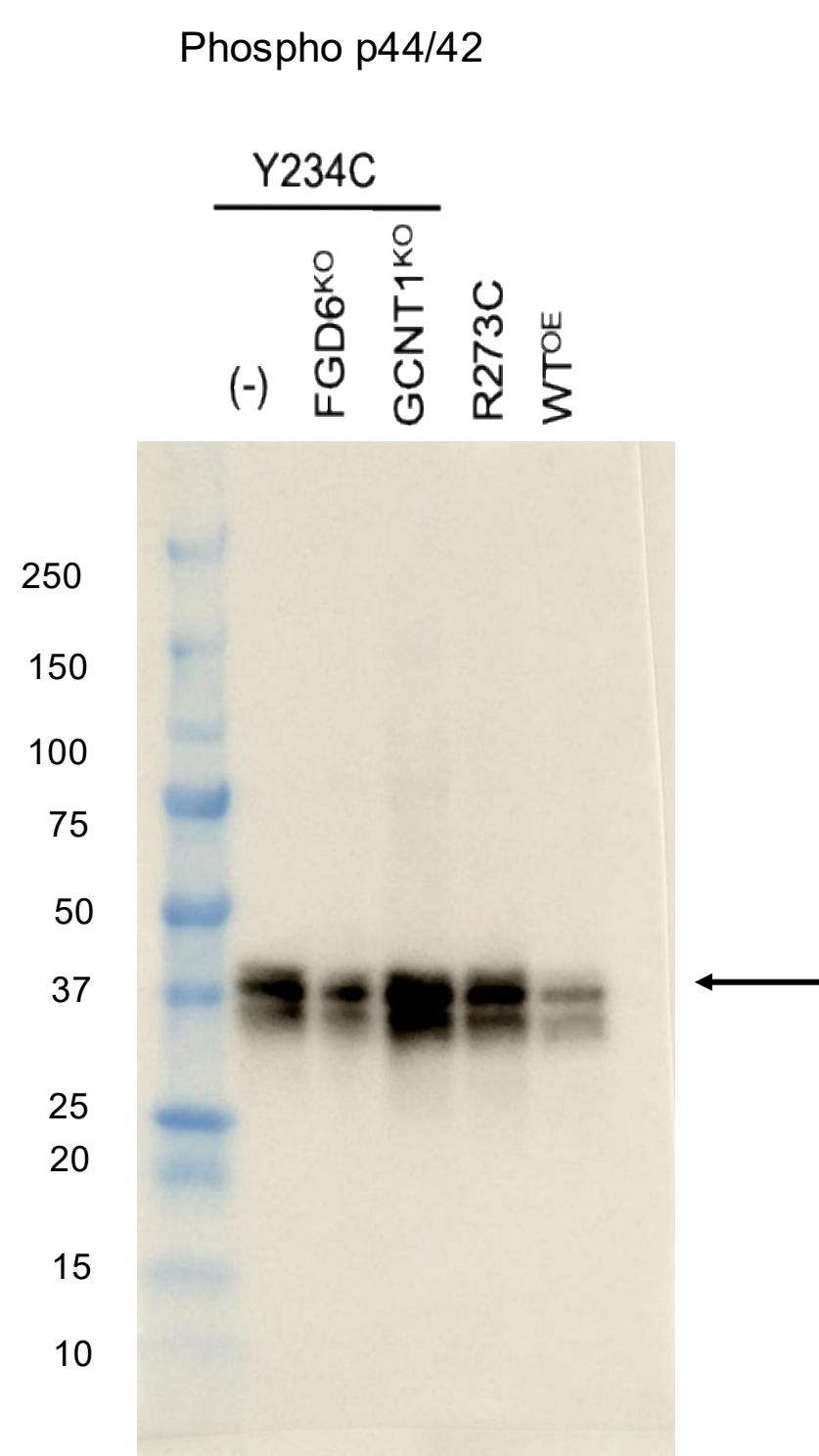
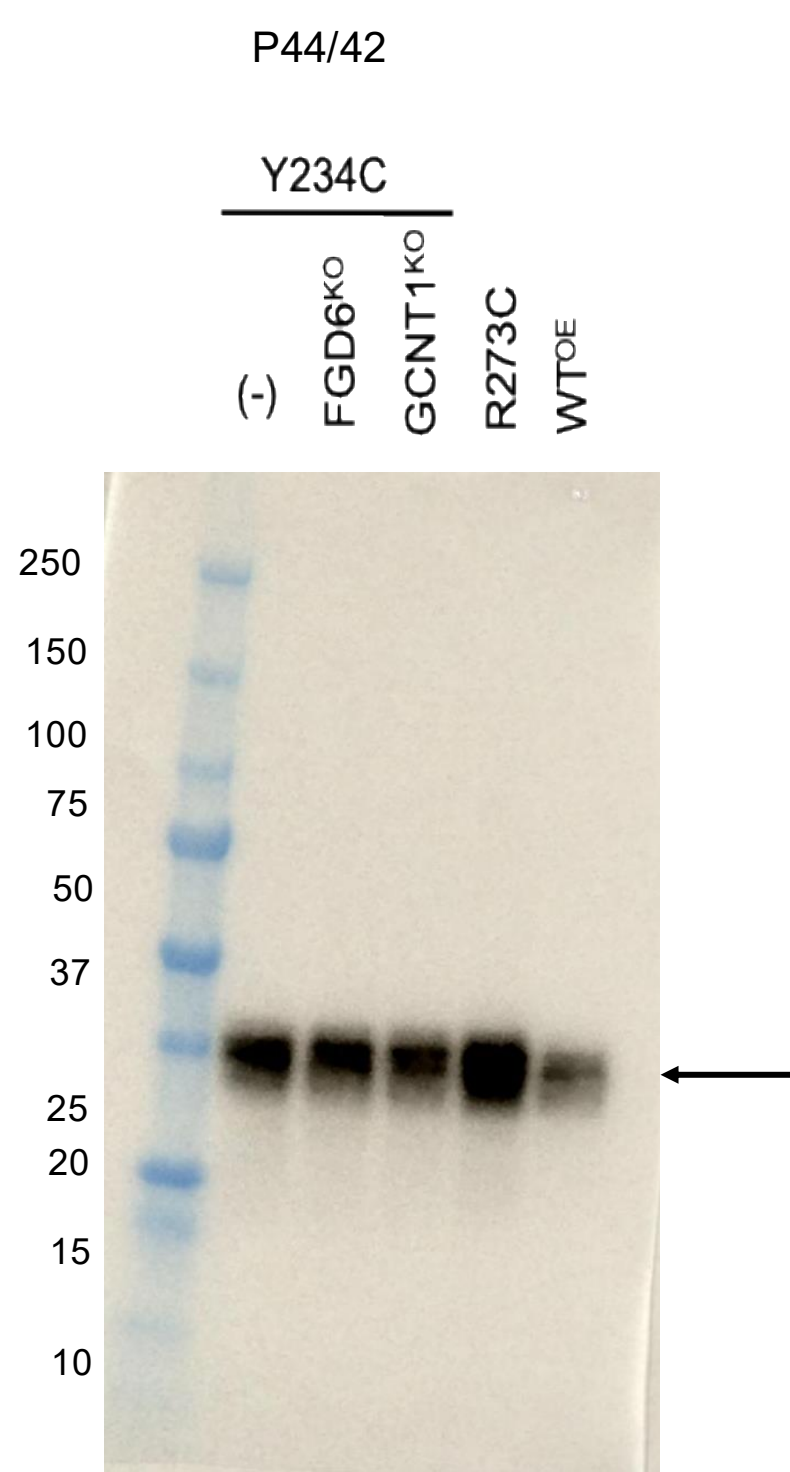


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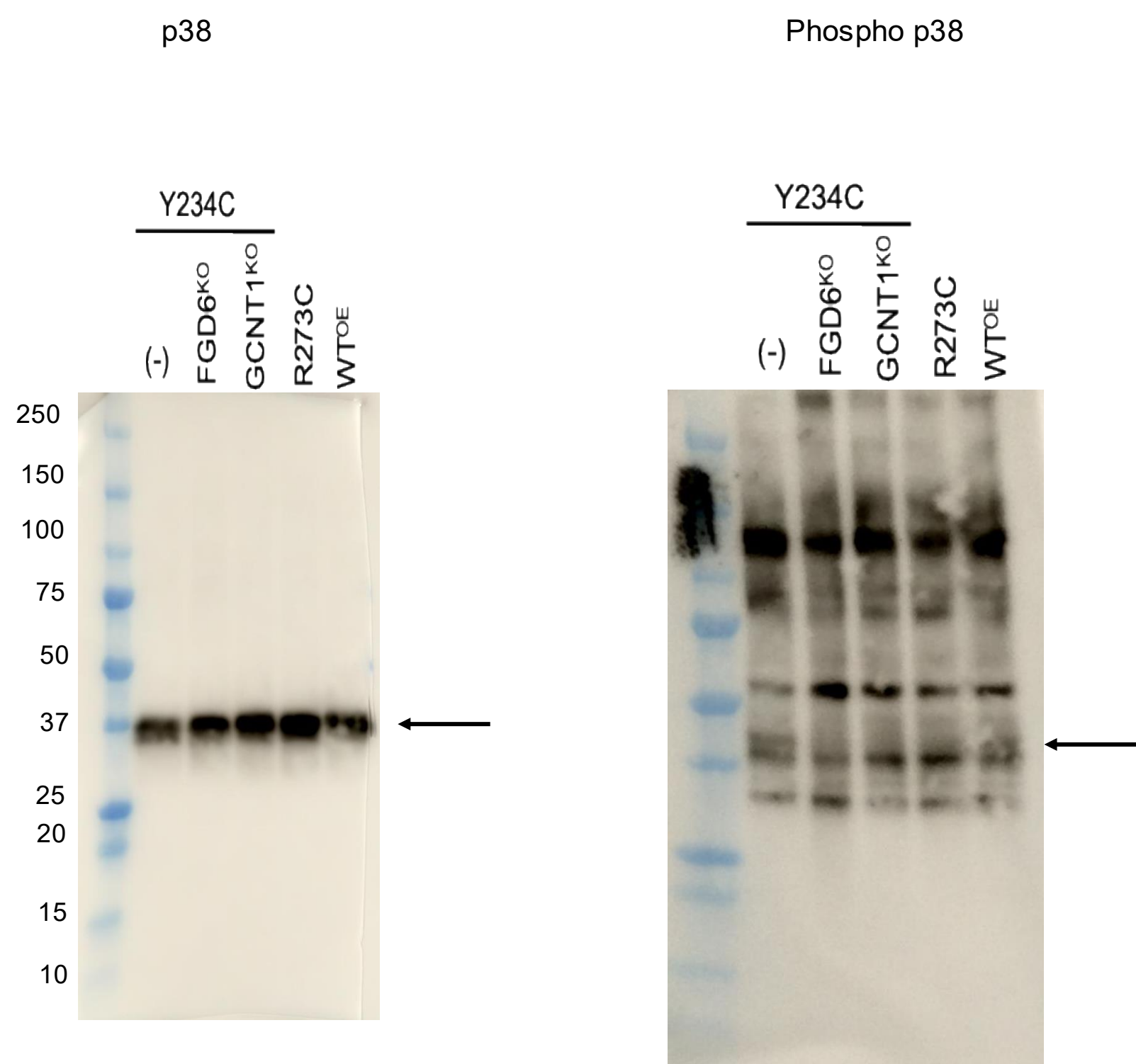


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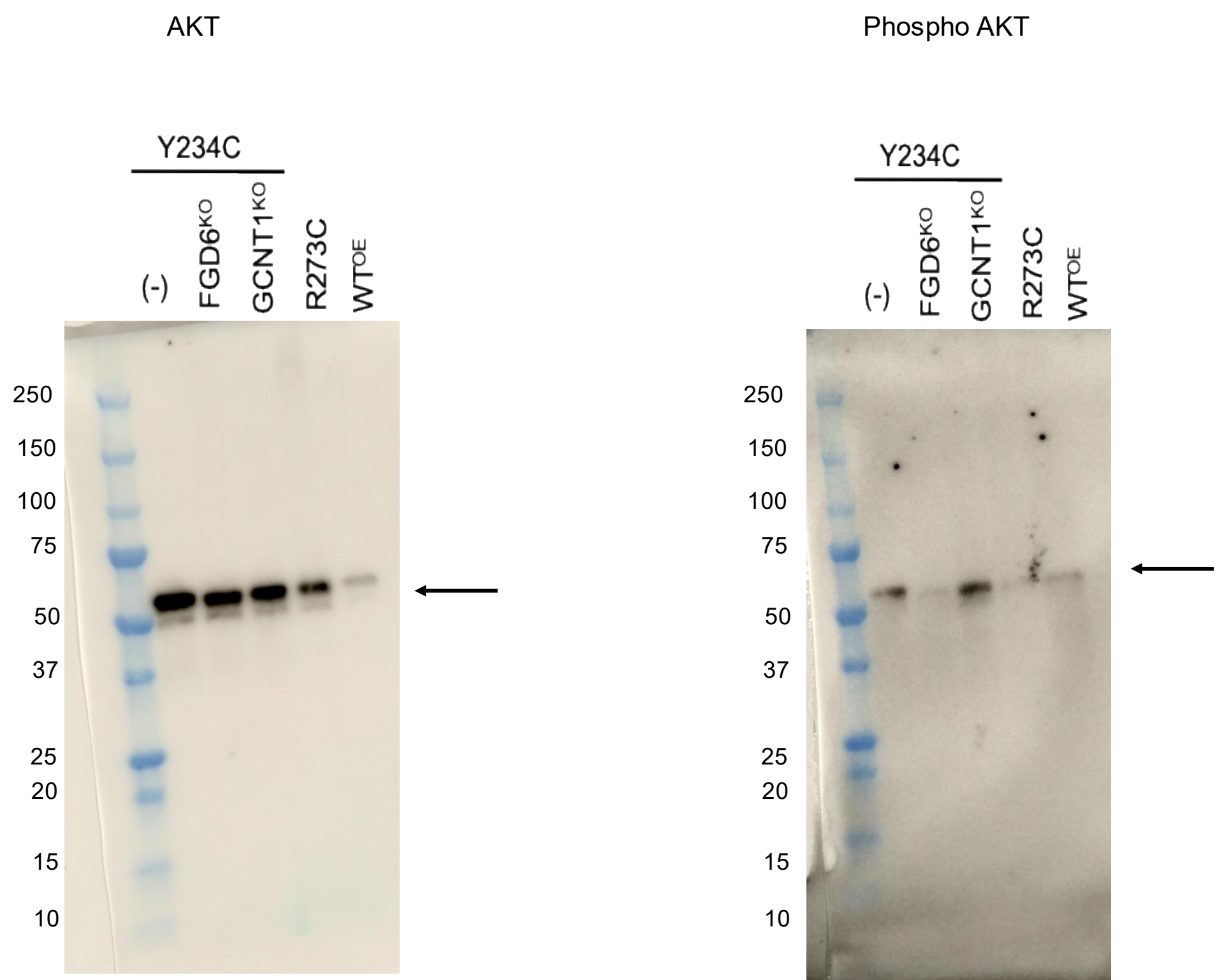


Fig. 8

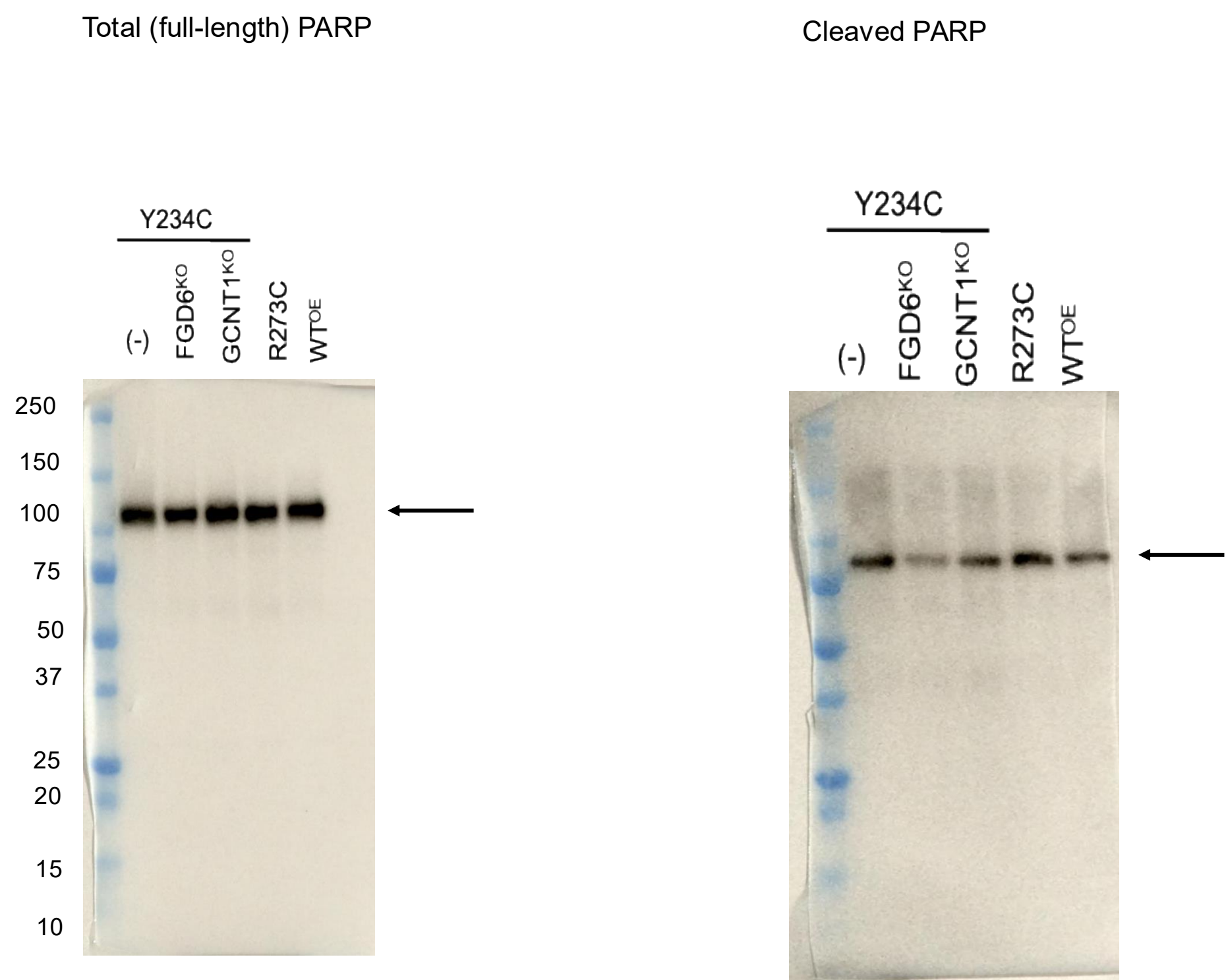
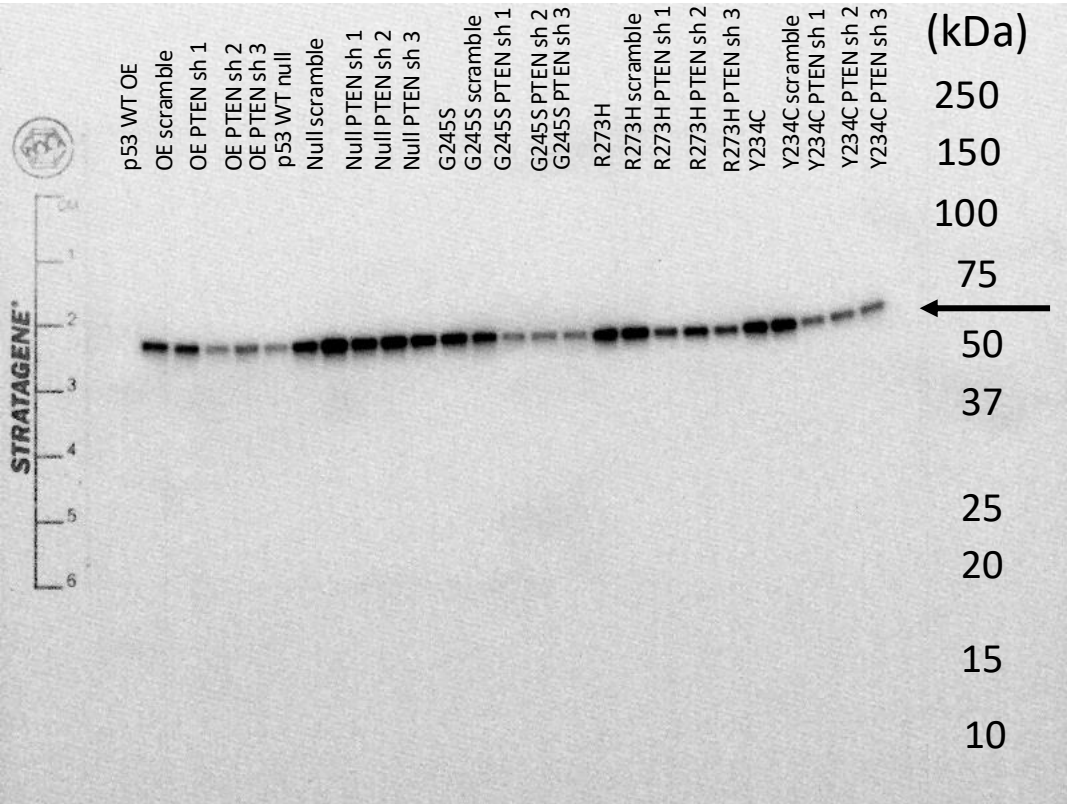


Fig. 8

PTEN



P53

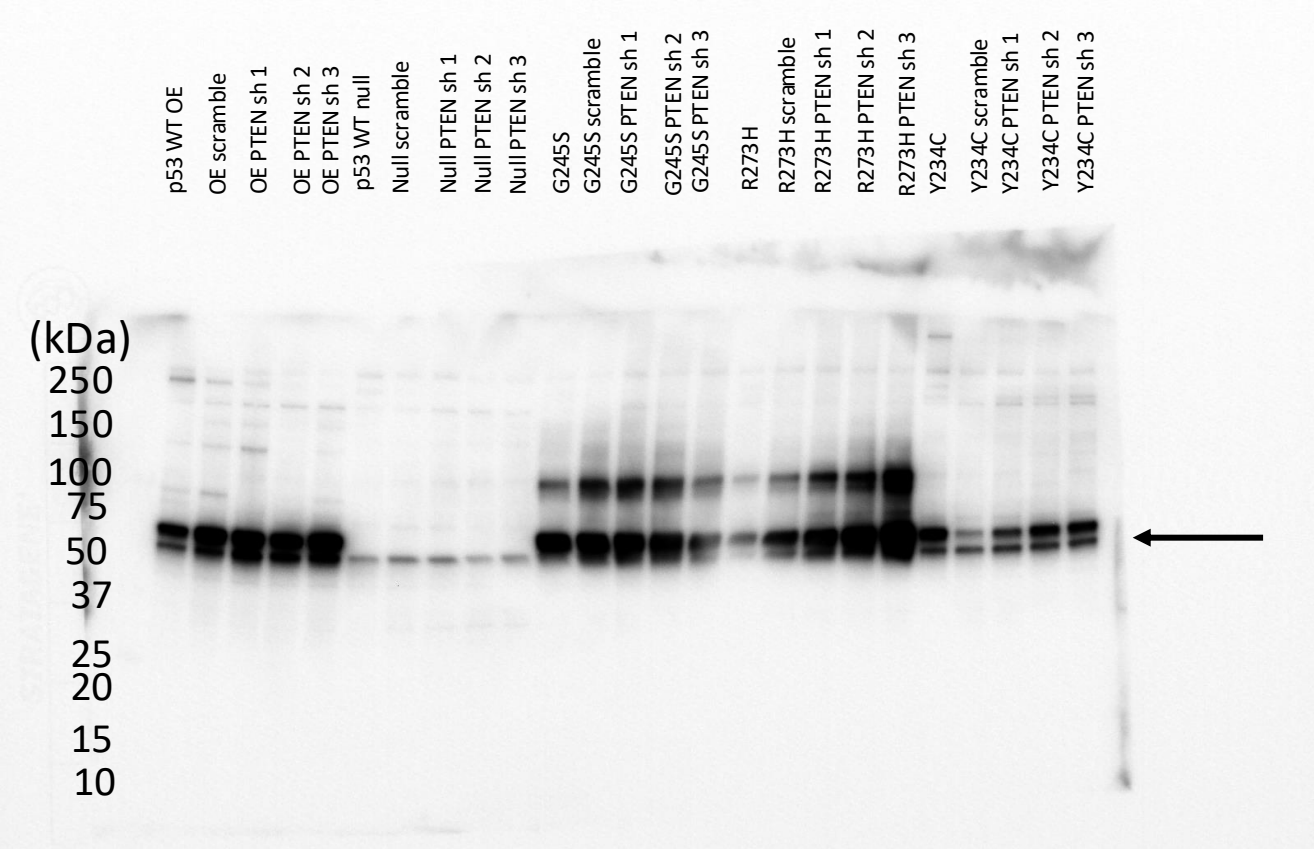
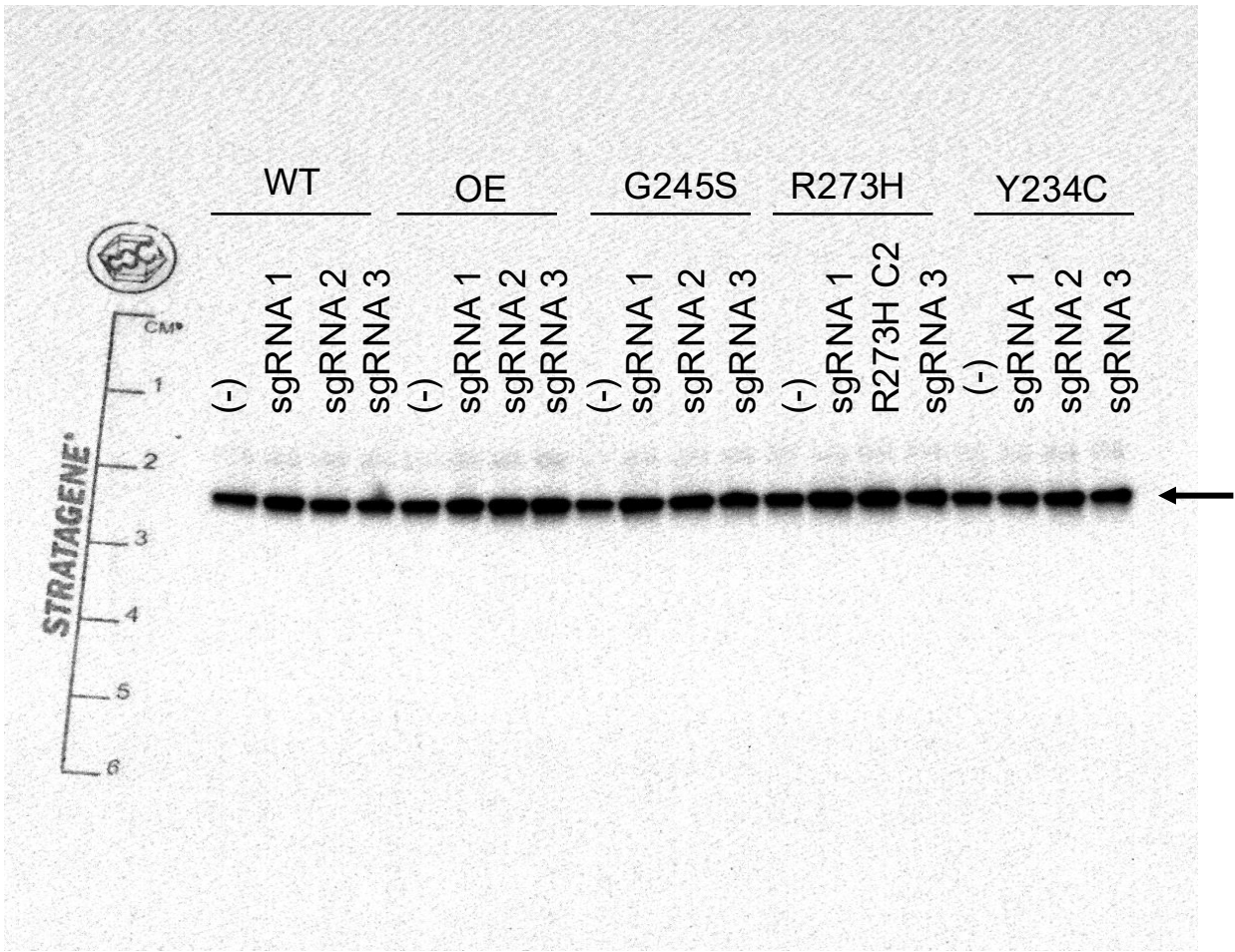
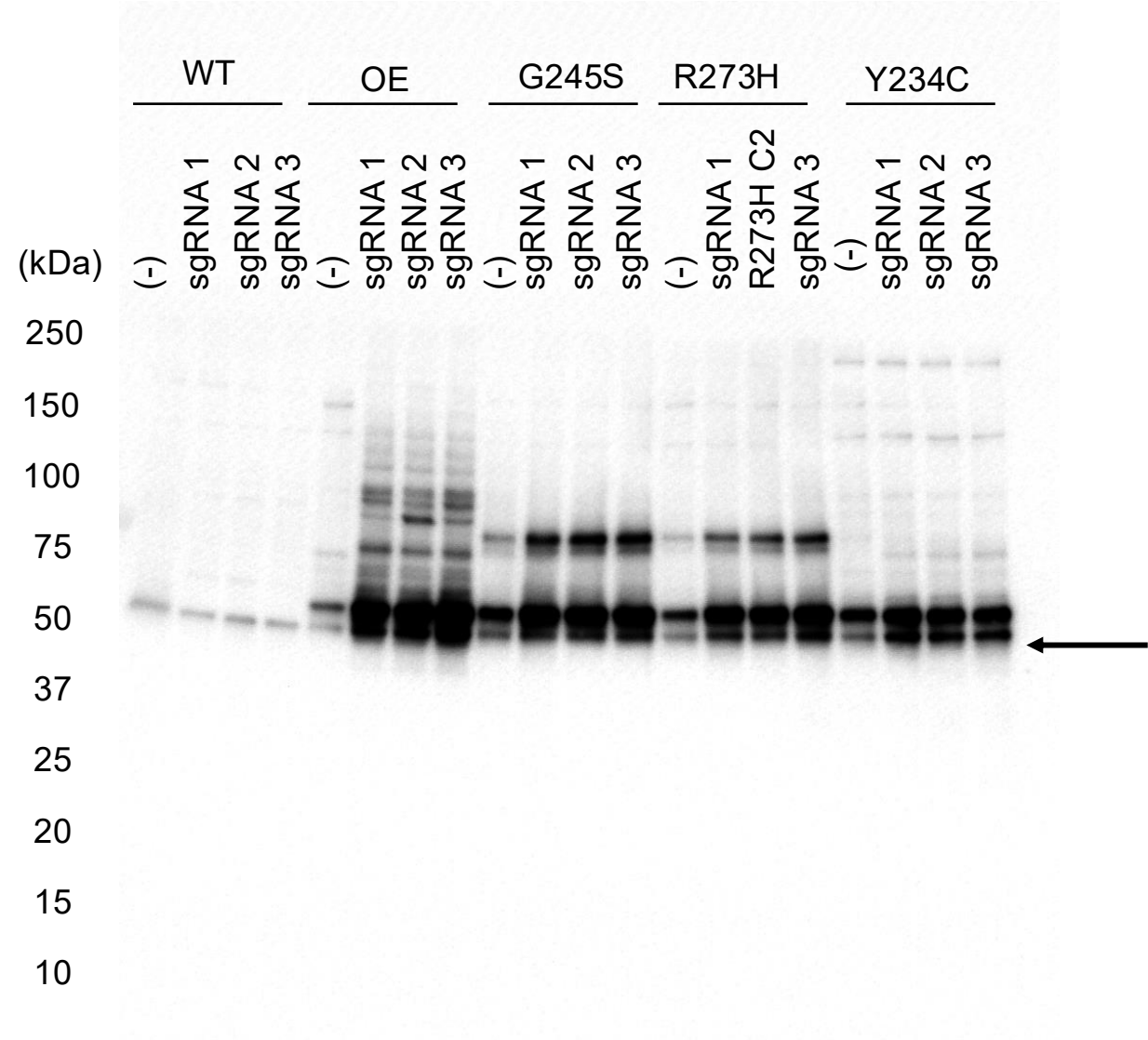


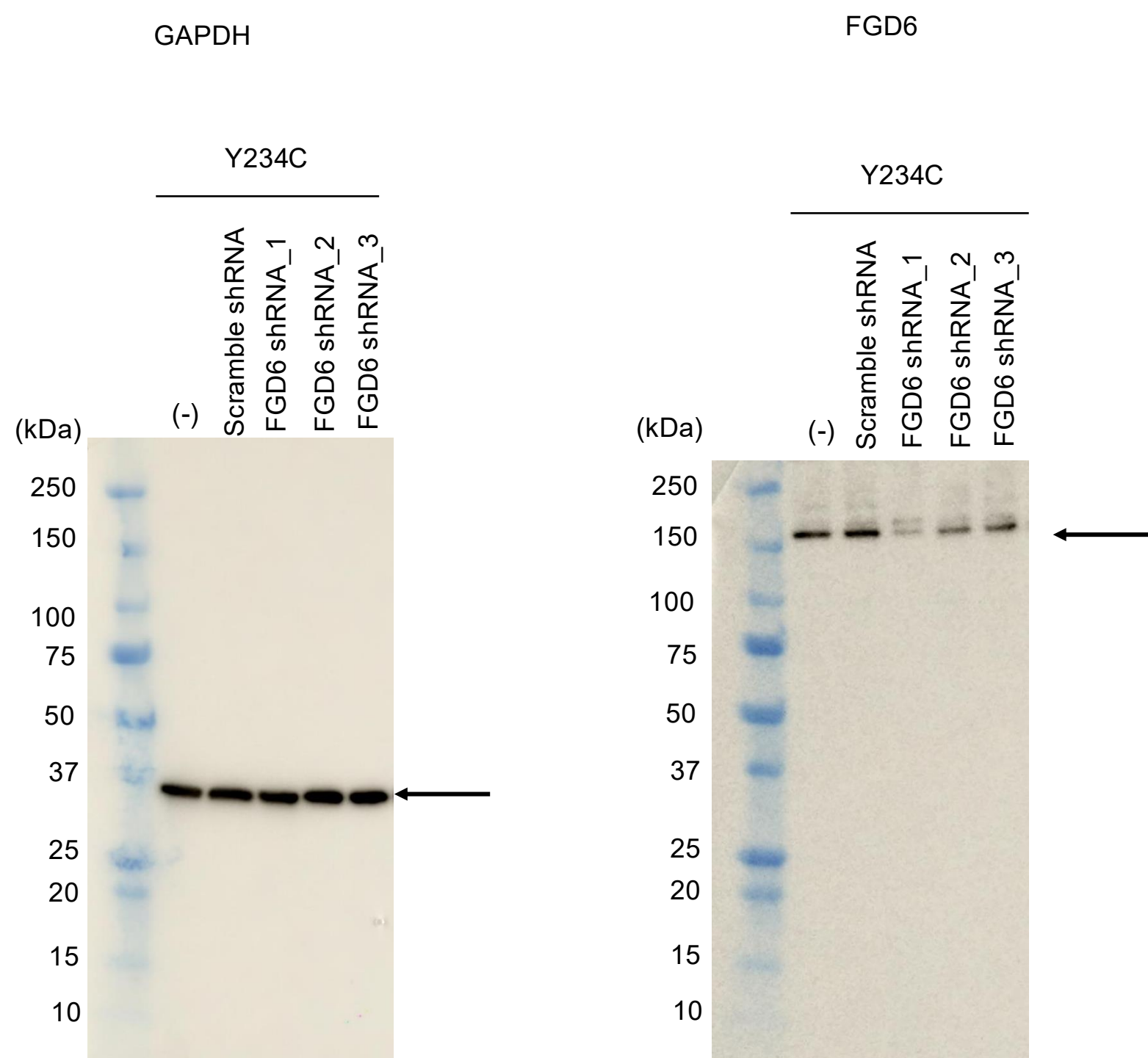
Fig. 8

P53

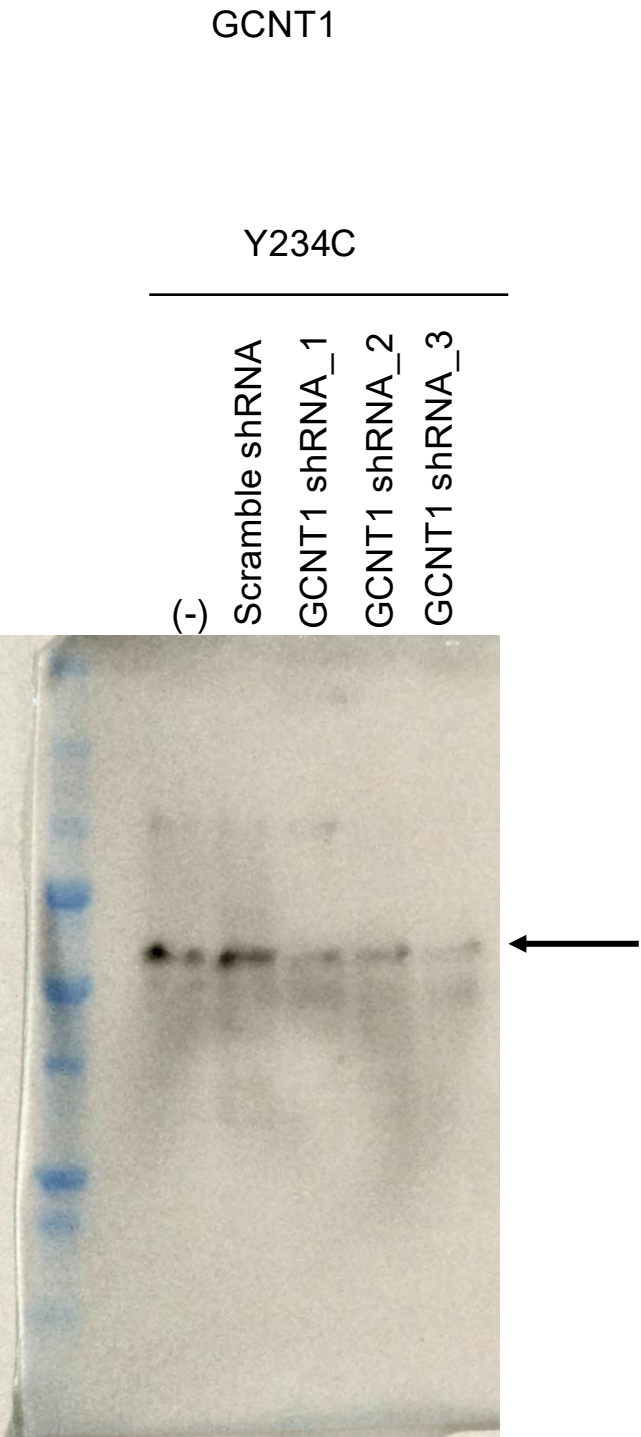
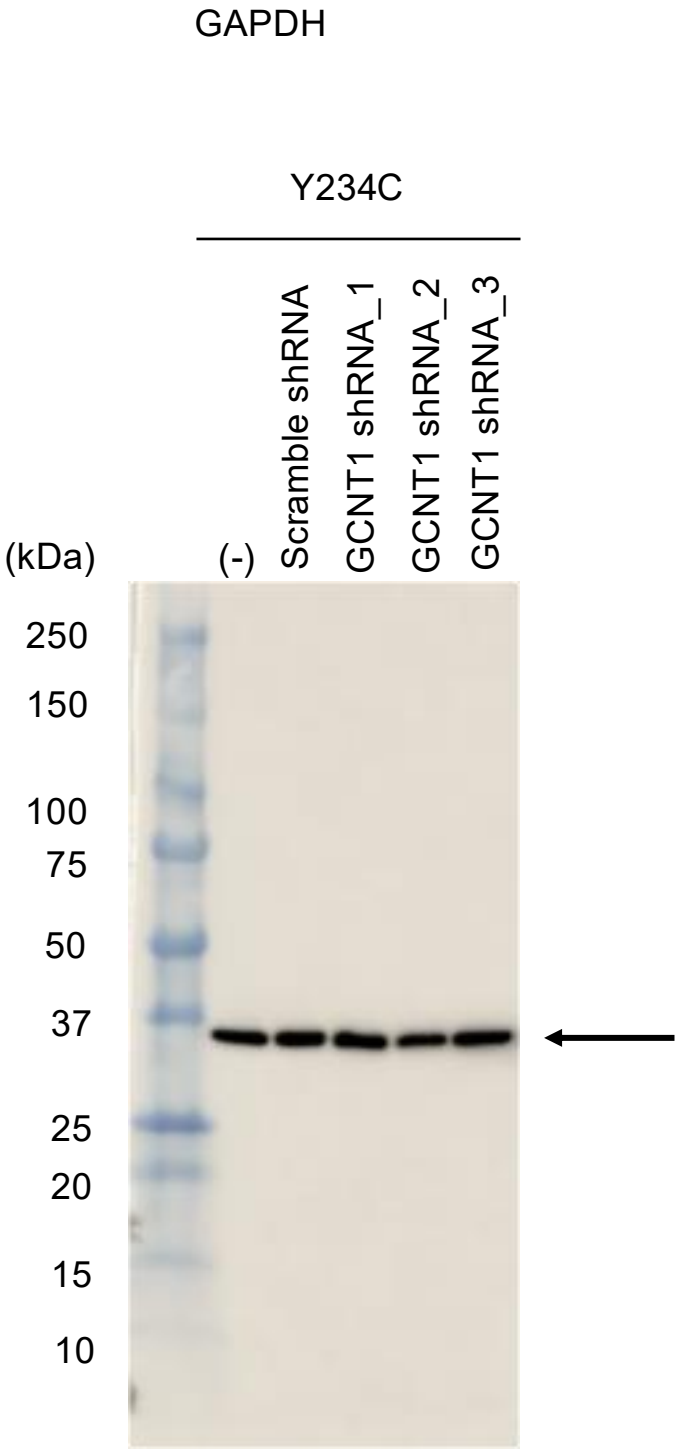
PTEN



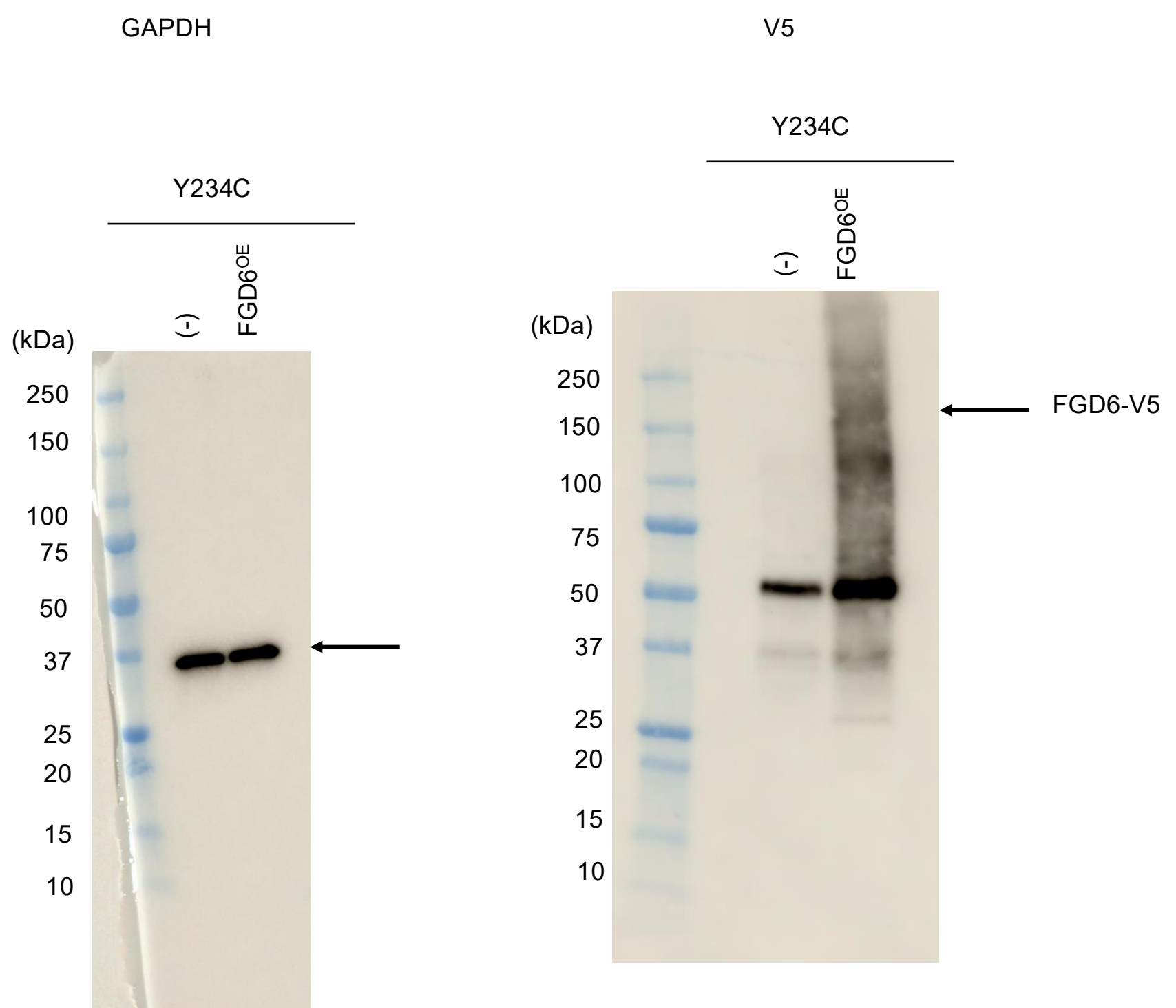
Supplementary Figure 2c



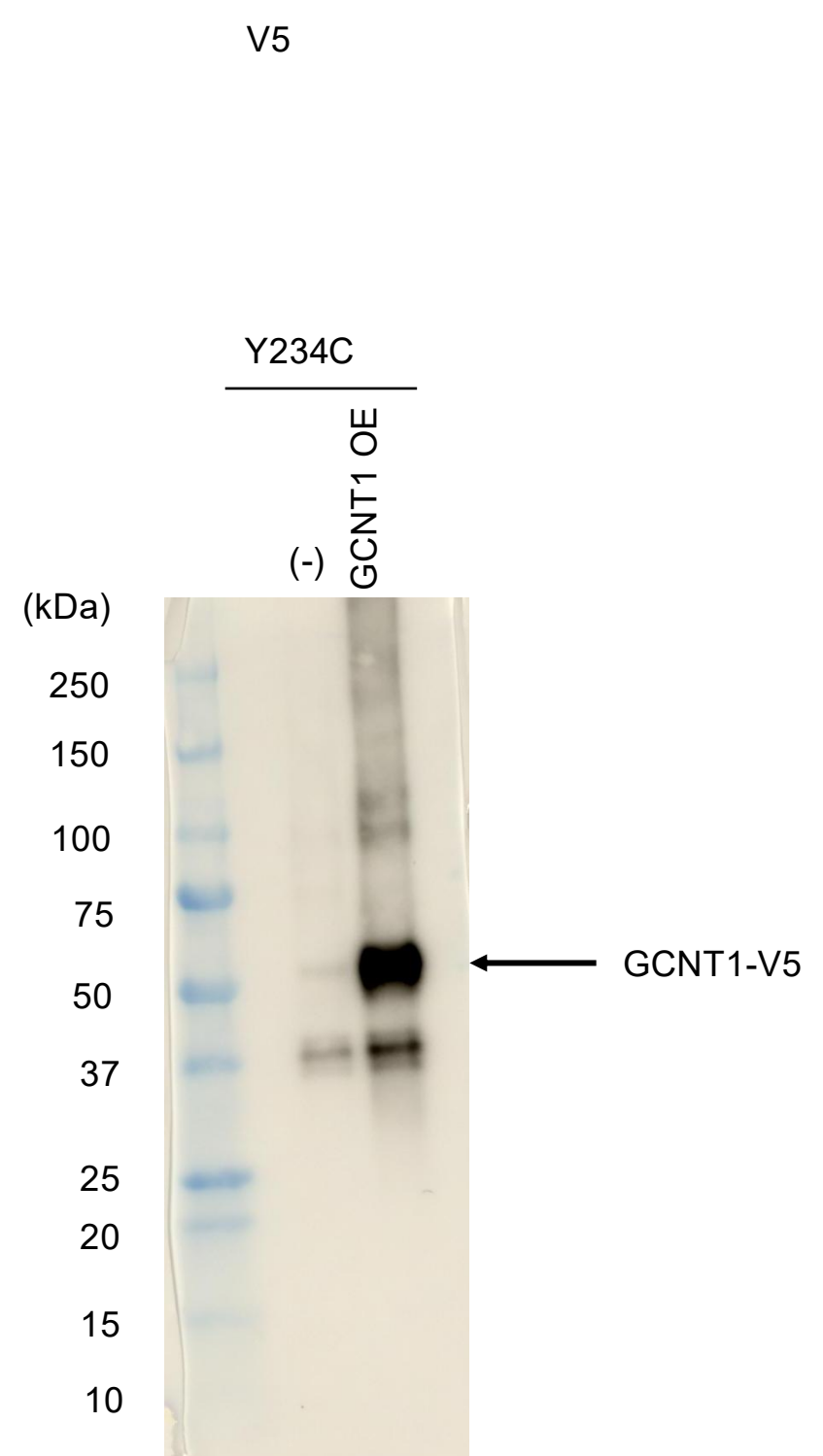
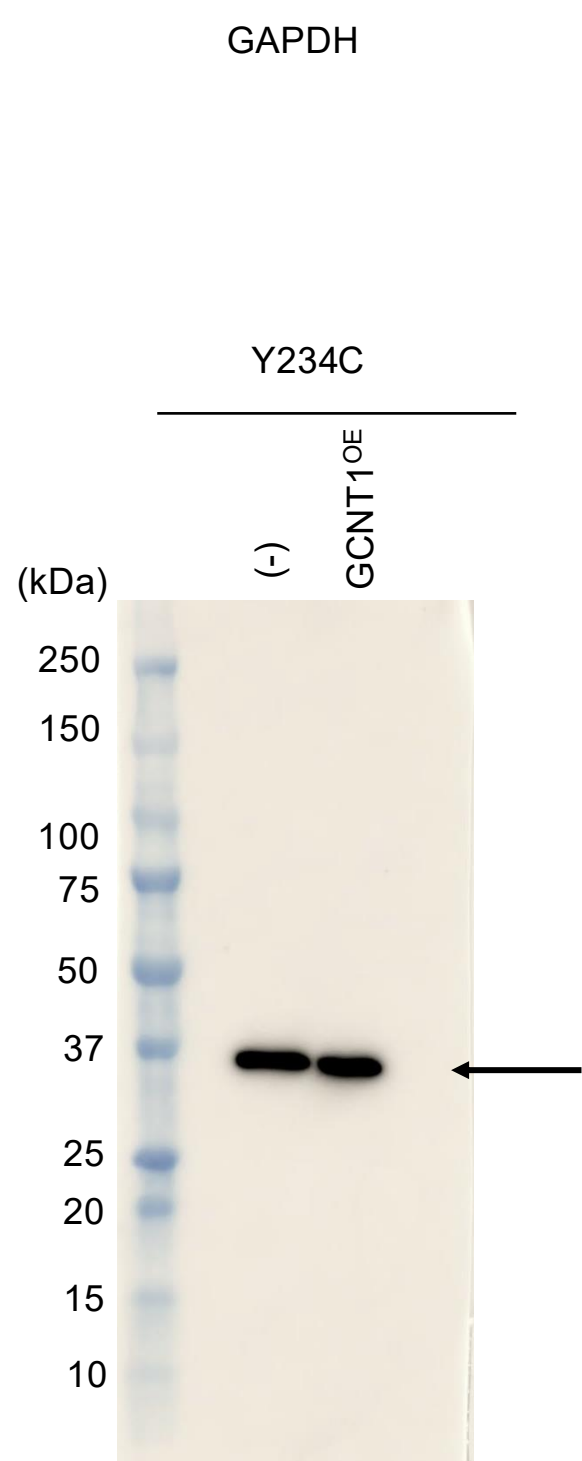
Supplementary Figure 14a



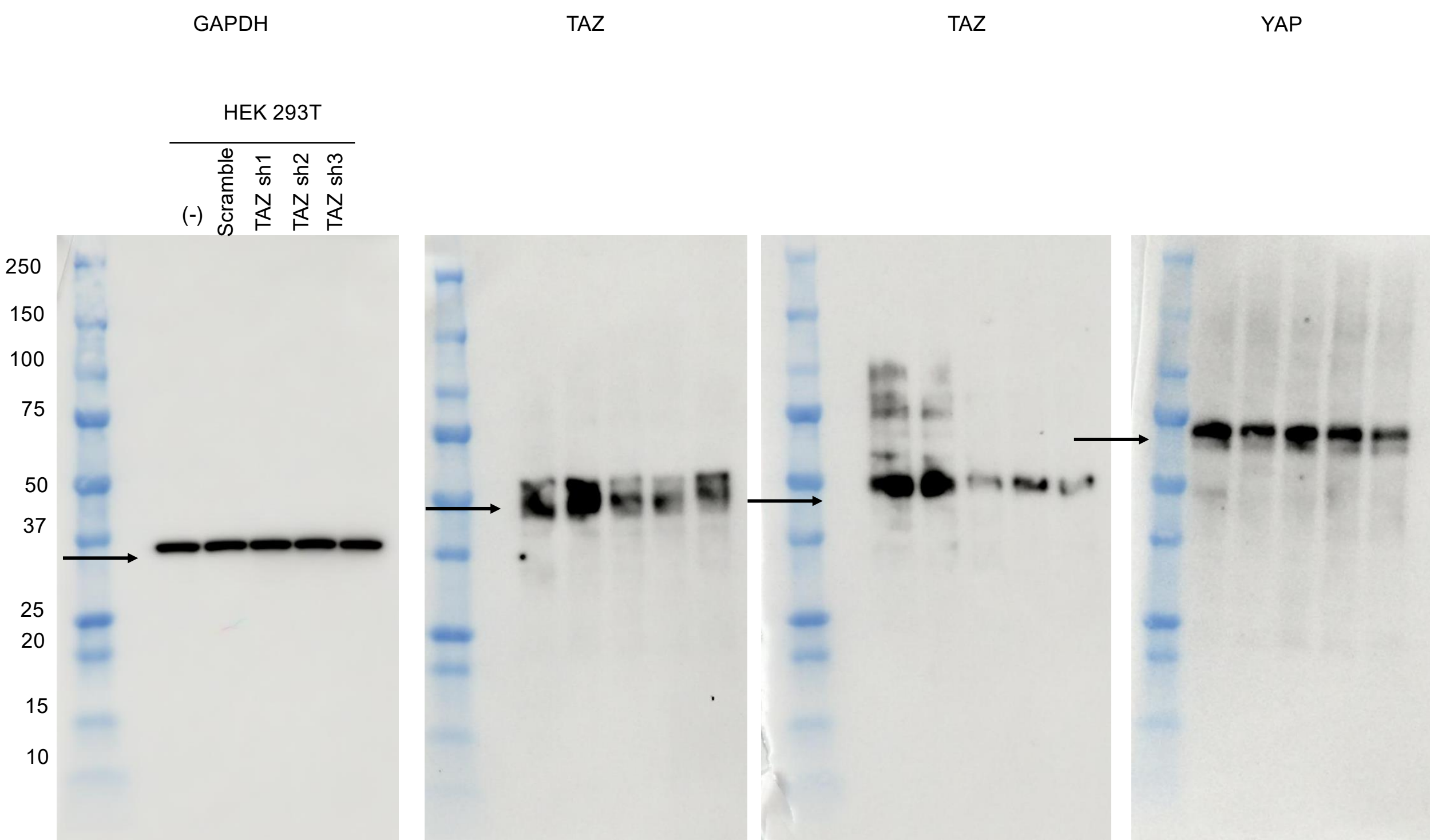
Supplementary Figure 14b



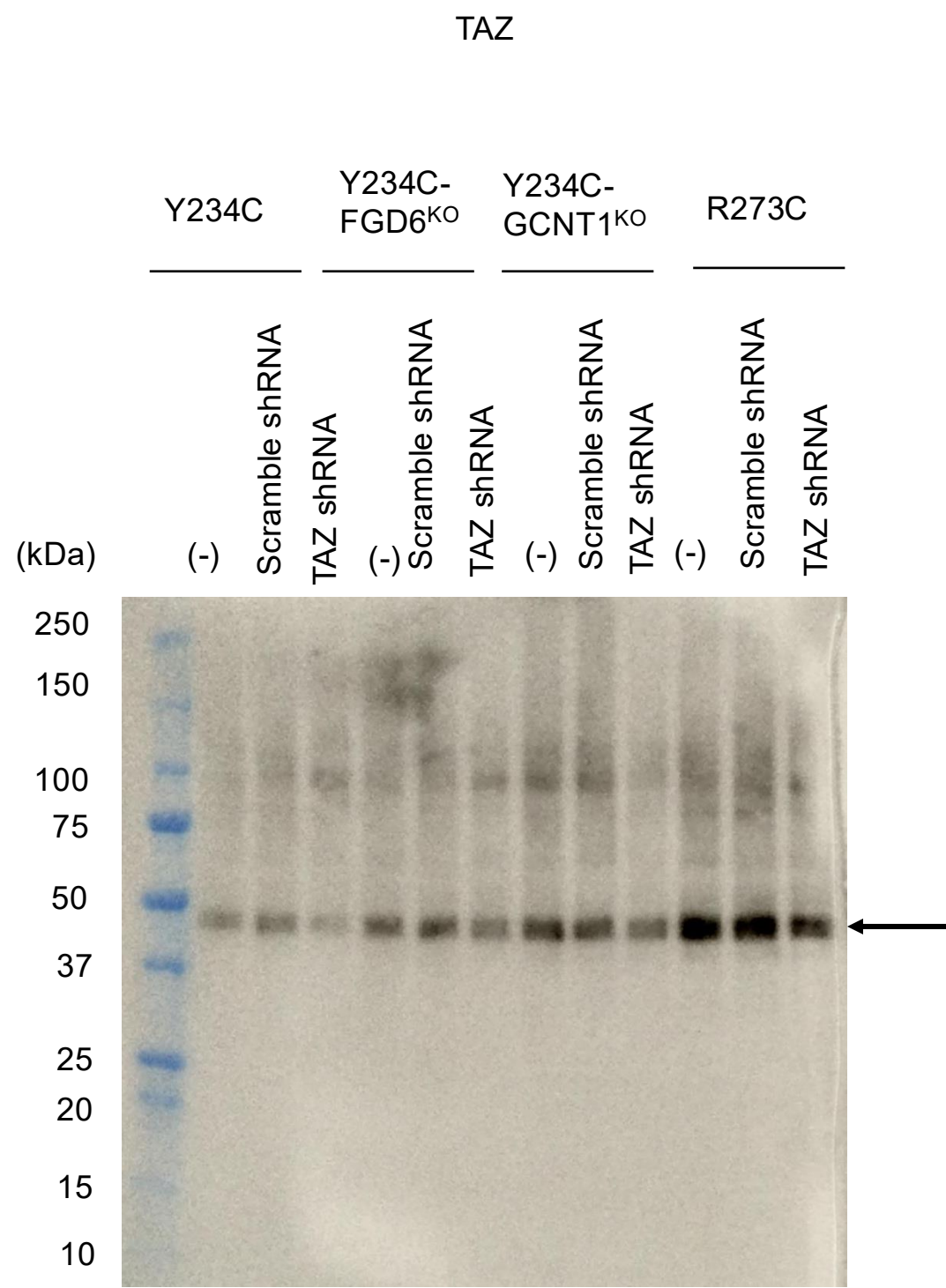
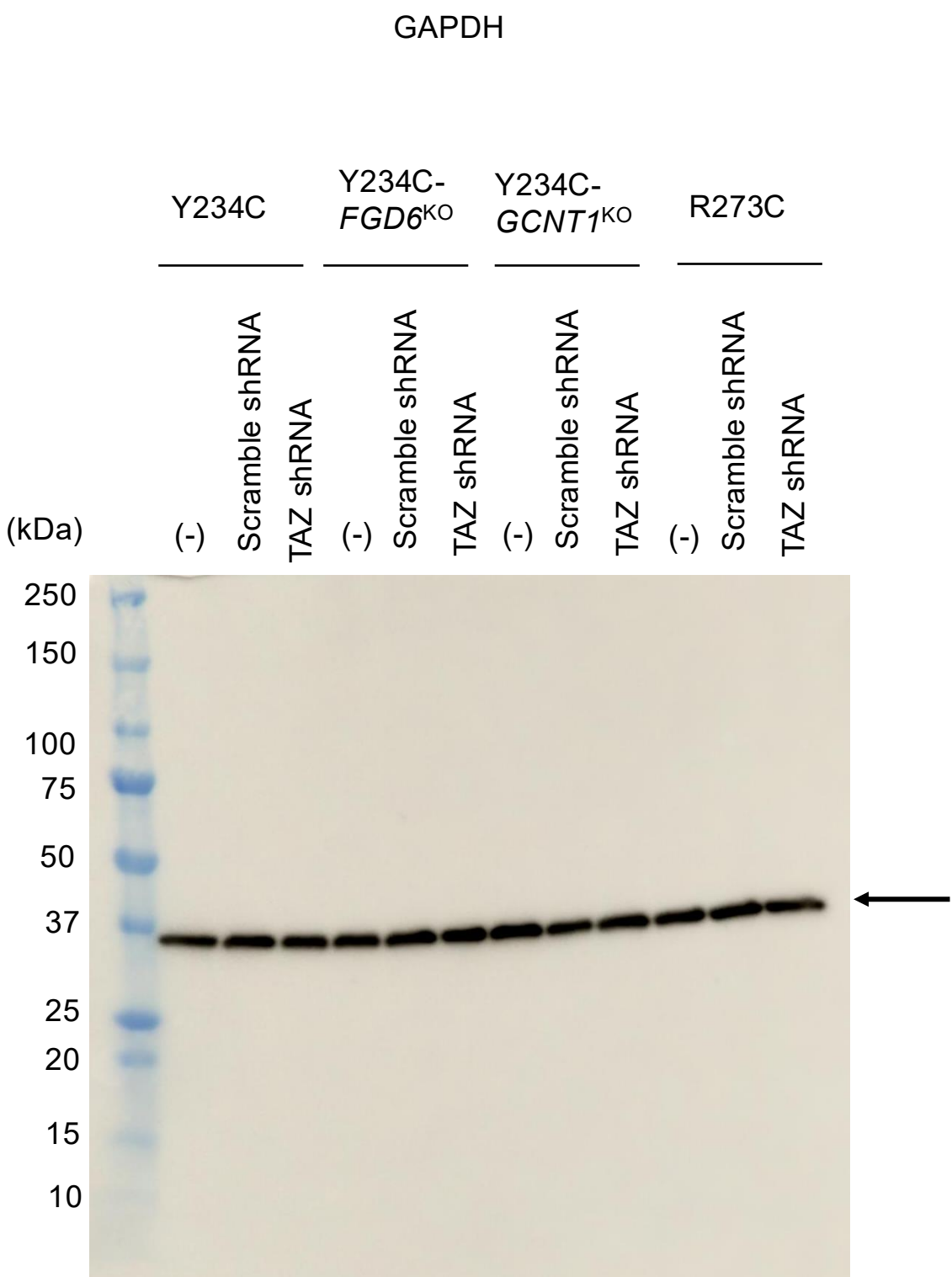
Supplementary Figure 14c



Supplementary Figure 14d



Supplementary Figure 15a



Supplementary Figure 15b