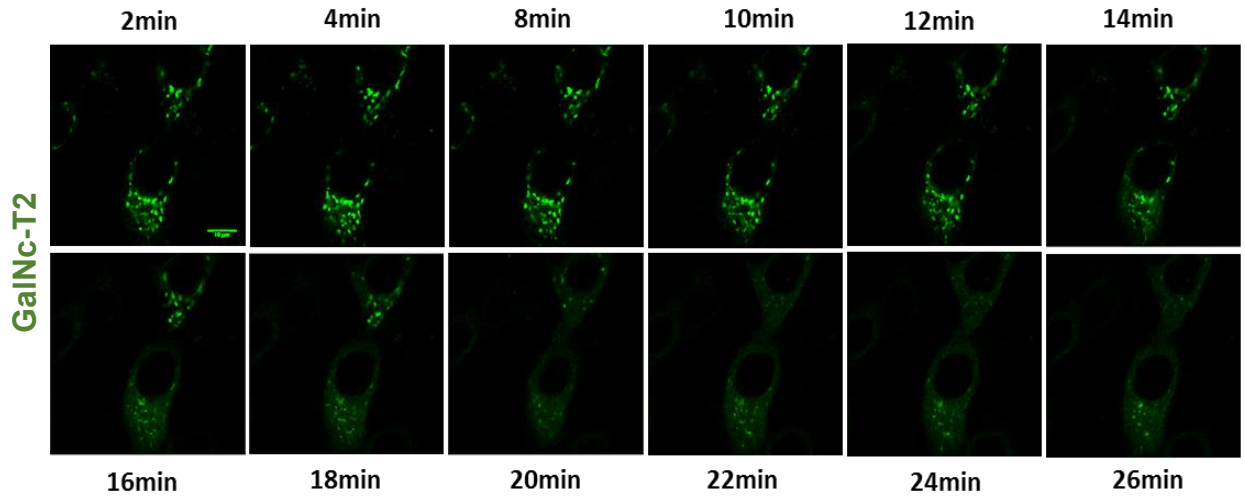
S1A



S1B

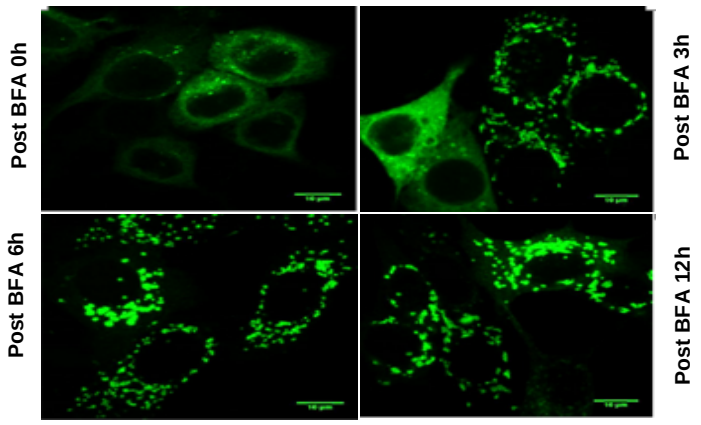
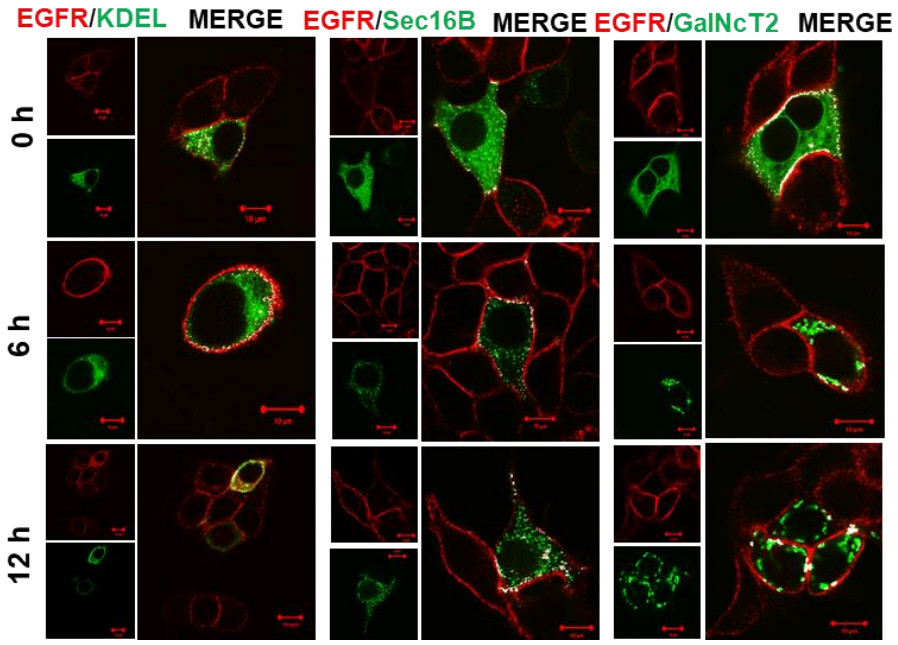


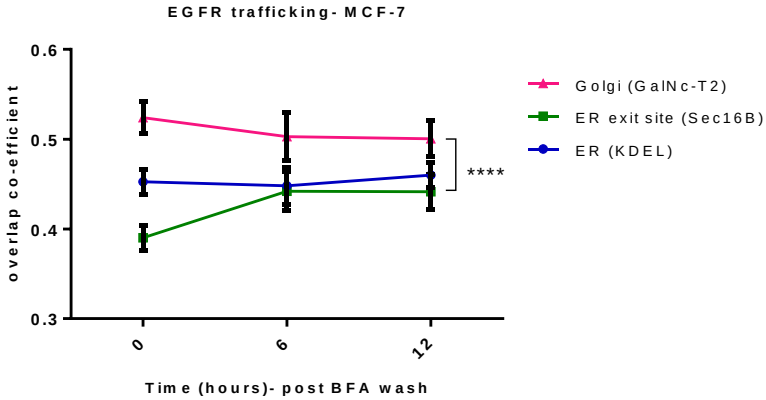
Figure S1: Effect of Brefeldin A (BFA) on Golgi deformation/reformation kinetics.

S1A. Live cell imaging of GalNac-T2-GFP (green) reporter at different time intervals, post BFA treatment, in MCF-7 cells (Live cell movie split into images). **S2B.** Golgi rescue kinetics, post BFA withdrawal in MCF-7 cells, as seen by GalNac-T2-GFP (green) reporter. The scale bar represents 10µm.

S2A



S2B



S2C

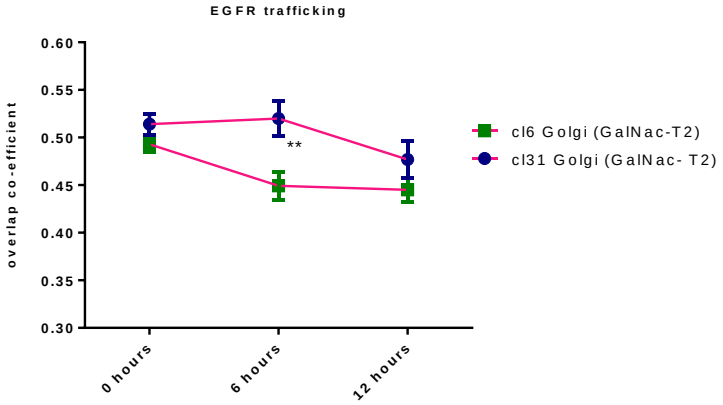


Figure S2: The trafficking of EGFR through Golgi across the clonal model systems. S2A. Co-localization of EGFR (red) with GalNac T2 (green) respectively in MCF-7 cells. All images represent a single plane. White spots are the points of co- localization between the two channels. The scale bar represents 10µm. **S2B.** Chart showing overlap co-efficients values obtained for EGFR with GalNac T2, Sec16B, and KDEL in MCF-7 cells. The middle best plane was used for quantification. Error bars indicate SEM. **S2C.** Chart showing overlap co-efficients values obtained for EGFR with GalNac T2 in cl31 and cl6 cells. The middle best plane was used for quantification. Error bars indicate SEM

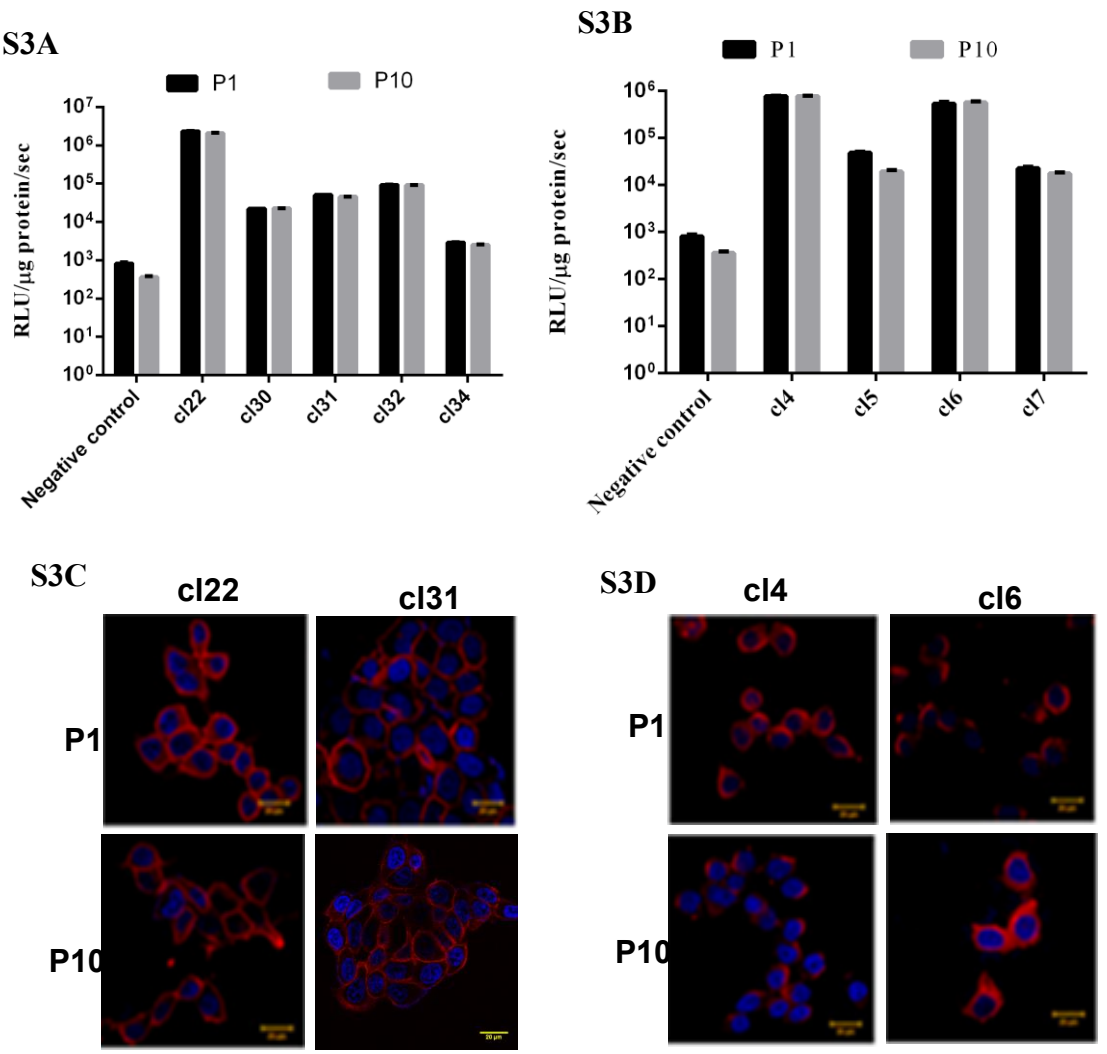
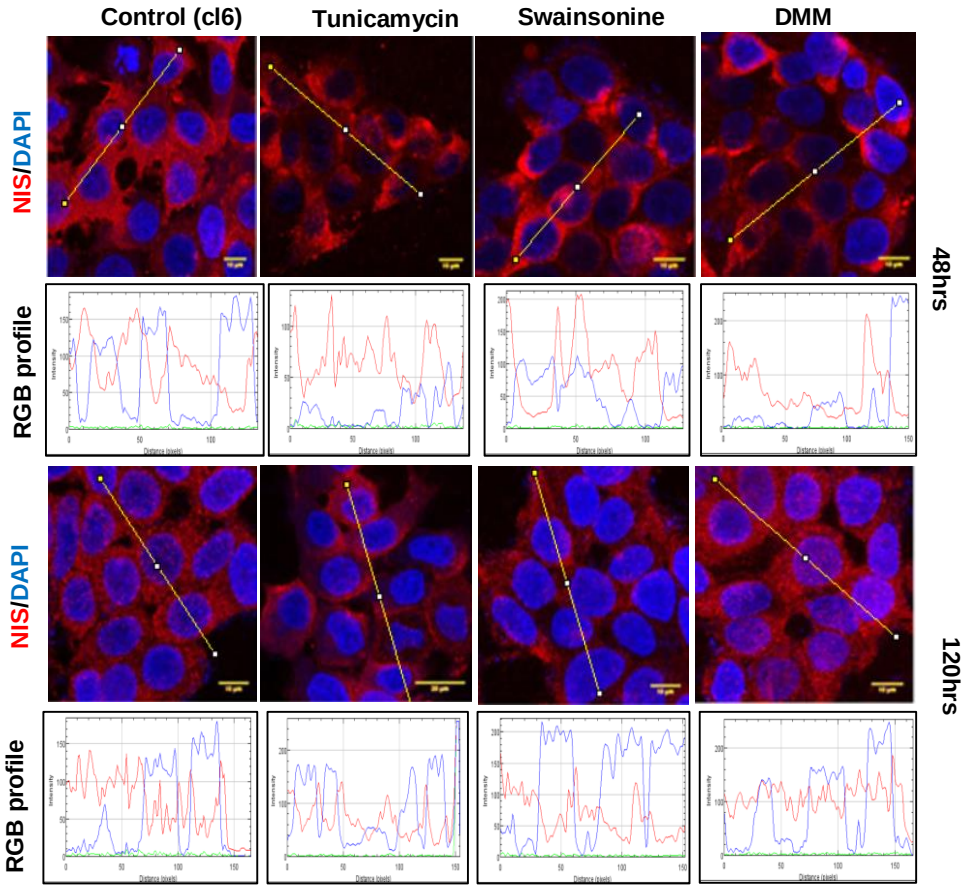


Figure S3: Stable expression of NIS in the clonal cell models is maintained over long passages. S3A-B. Luciferase assay showing relative light output normalized to protein concentration of Fl2 activity in membrane and cytoplasmic NIS overexpressing clones across passage 1 and passage 10 **S3C-D.** IF images showing the localization of NIS in membrane clones and cytoplasmic clones across passage 1 to 10. NIS stained with Dylight 633 secondary antibody (red) and nucleus staining by DAPI (blue). The scale bar represents 20µm.

S4A



S4B

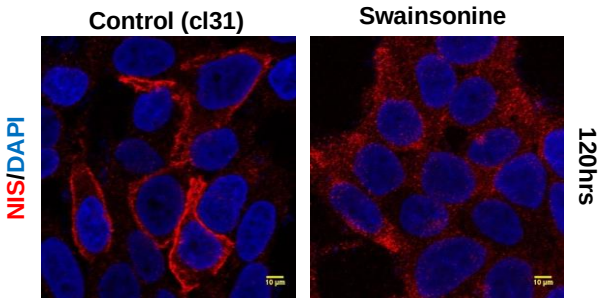


Figure S4: The effect glycosylation inhibitors on NIS localization in cytoplasmic clones, and the action of swainsonine over 120 hours in cl31. NIS localization in cytoplasmic clone is not affected by glycosylation inhibitor treatment and swainsonine can take down membrane expression of NIS over 120 hours **S4A.** IF images showing the localization of NIS (red) in response to glycosylation inhibitors tunicamycin, DMM and swainsonine at 48 and 120 hours in cytoplasmic clone. **S4B**. IF images showing the delayed action of swainsonine on altering NIS (red) localization over 120 hours. The scale bar represents 10µm

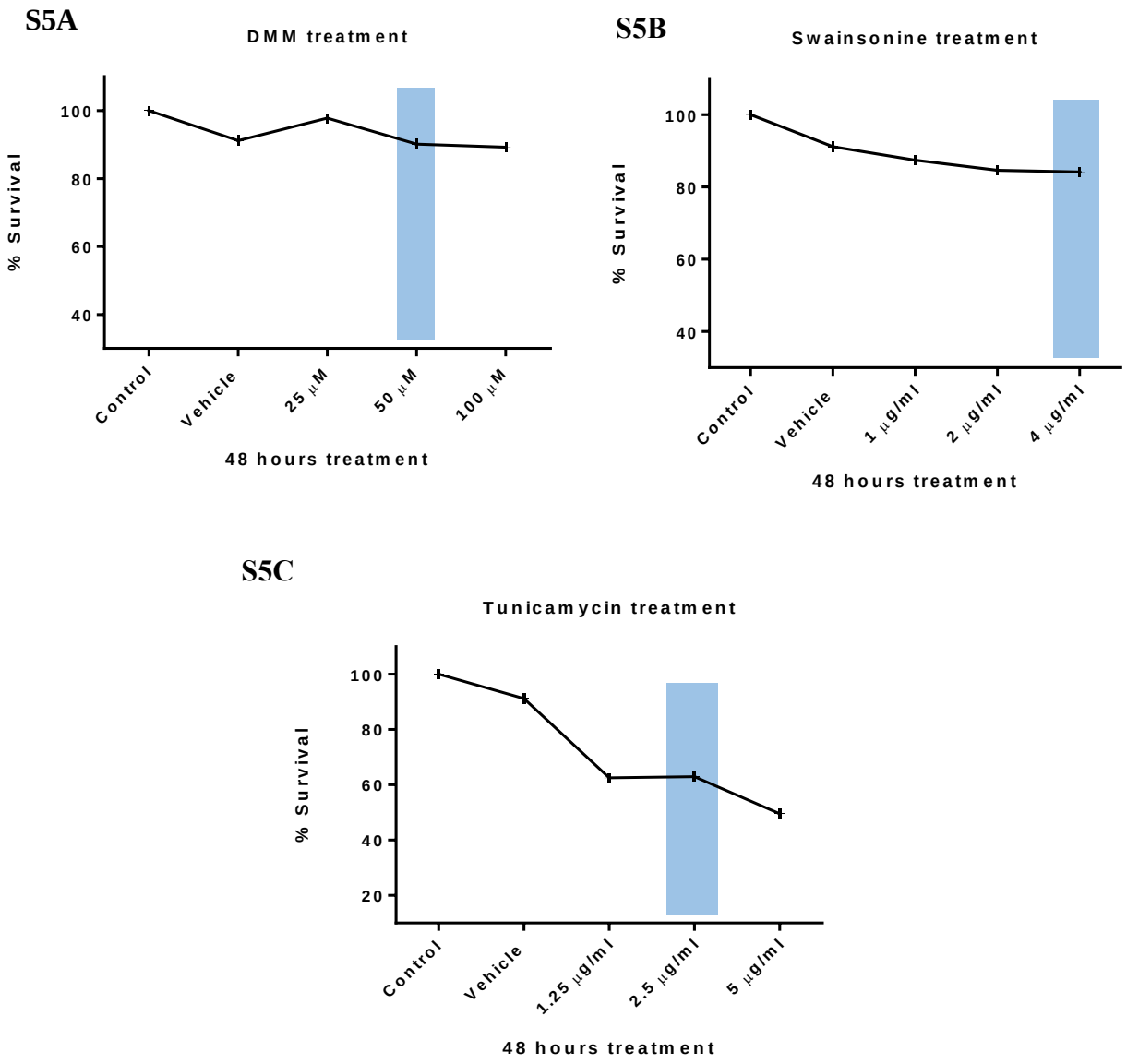
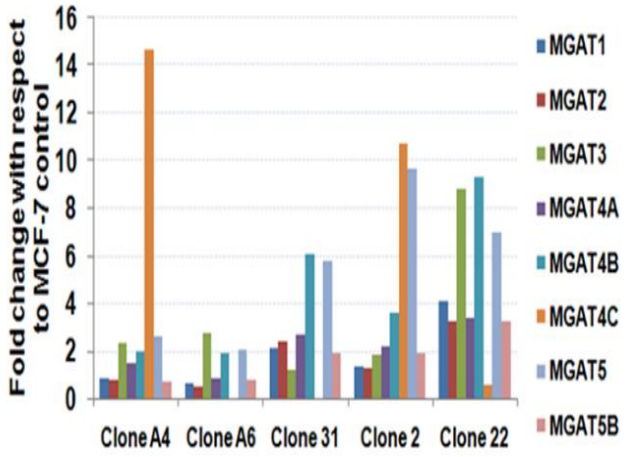
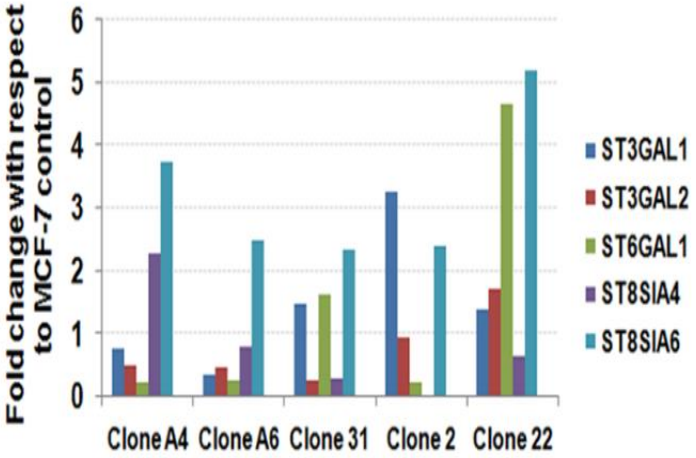


Figure S5: Cytotoxicity of glycosylation inhibitors on MCF-7 cells. The glycosylation pathway inhibitors used are not cytotoxic to MCF-7 cells**. S5A-C:** Graph showing the results from MTT assay, revealing that the selected dose of each drug for the study (highlighted in blue), lies below IC50.

S6A



S6B



S6C

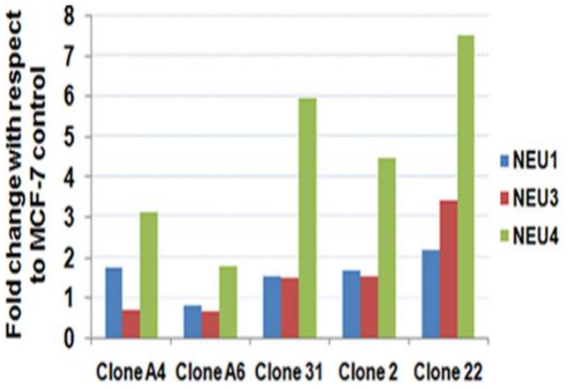


Figure S6: Expression of various class of glycosylation enzyme coding genes across clonal models and MCF-7 baseline cell. Differential expression of various class of glycosylation enzyme coding genes across membrane and cytoplasmic clone w.r.t to baseline cells **S6A.** Chart showing fold change for various mannosyl transferase enzyme coding genes in membrane and cytoplasmic clones with respect to baseline MCF-7 cell. **S6B.** Chart showing differential expression profile of sialidases enzyme coding genes. **S6C.** Chart showing differential expression profile of neuramindase enzyme coding genes.

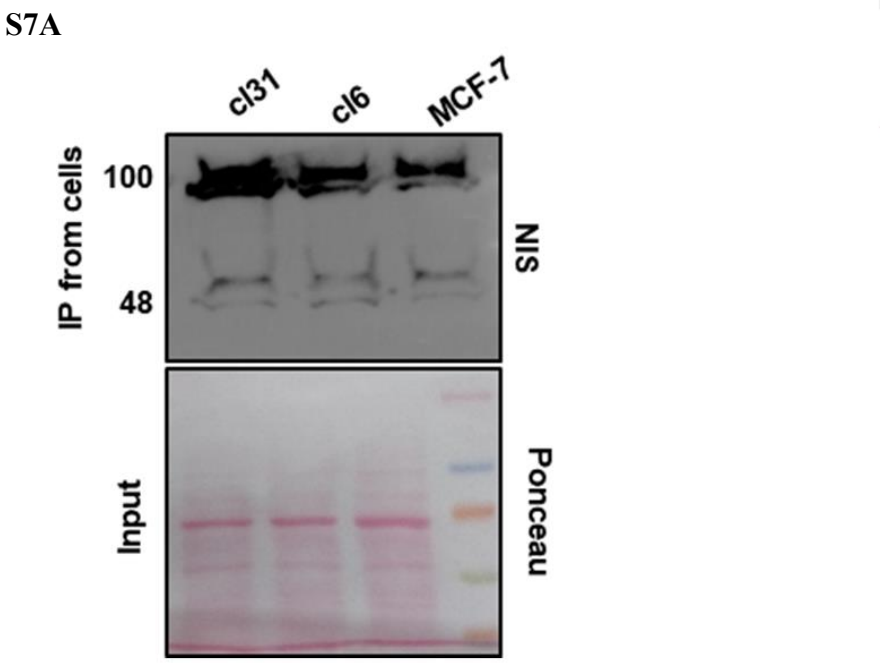


Figure S7: NIS western blot showing effective pull down of NIS from the clonal cell models. Immunoprecipitation of NIS from the different cell lineages reveals that cl31 possess the highest amount of 100kDa fraction of NIS. **S7A:** Western blot showing the staining of NIS from the IP sample, across the different cell types. Ponceau staining was used as a loading control from input samples.

Table S1: Karyotype analysis of MCF-7 derived stable cell models of differential NIS localization.

Cell line	MCF-7 parent	cl31	cl6
Ploidy	Triploidy	Triploidy	Triploidy
Similar abnormalities	• Derivative chromosome 1 due to inversion and duplication of chromosome • Isochromosomes of q arm of chromosome 7 and 11	• Derivative chromosome 1 due to inversion or duplication of chromosome • Isochromosomes of q arm of chromosome 7 and 11	• Derivative chromosome 1 due to inversion or duplication of chromosome • Isochromosomes of q arm of chromosome 7 and 11
Differential abnormalities	Deletion in p/q arm of chromosome 1,2,3,6,11,17 Extra copies of chromosomes 7,8,9,10,11,13,14,15,16,17	Deletion in p/q arm of chromosome 1,6,8,11,17 Extra copies of chromosomes 4,5,7,8,9,10,11,13,15,16,17,20,22	Deletion in p/q arm of chromosome 1,2,3,6,8,11,17 Extra copies of chromosomes 4,5,7,8,9,10,11,13,14,15,16,17,20

Table S1: Clonal heterogeneity among MCF-7 clones based on karyotype differences. GTG banding pattern has been used to evaluate the chromosome structures and numbers.