Supplemental Text 1. Considering alternate explanations for the loss of fitness in engineered 1q-disomic cancer cells

In order to generate isogenic cancer cells that have or lack specific aneuploid chromosomes, we developed and applied a suite of CRISPR tools for chromosome engineering called ReDACT. We discovered that eliminating the trisomy of chromosome 1q severely compromised malignant potential in multiple independent cancer cell lines. We considered and rigorously evaluated the possibility that the loss of fitness observed among the 1q-disomic clones could be a consequence of our chromosome engineering methodologies, rather than the subsequent change in cellular karyotype. However, multiple lines of evidence indicate that this loss of fitness is best explained as a specific outcome of eliminating trisomy-1q and not a consequence of our experimental approach:

The use of CRISPR

All three ReDACT techniques that we applied utilized CRISPR to induce aneuploidy-loss events. In order to assess whether CRISPR itself could compromise malignant growth to the degree that we observed upon elimination of the 1q trisomy, we generated and tested a set of 28 control clones that were subject to various CRISPR manipulations. These clones include:

1) Cell lines harboring a CRISPR-mediated integration of the HSV-TK cassette that were not treated with ganciclovir to select for 1q-loss,
2) Cell lines in which the HSV-TK cassette was deleted by transfecting cells with two gRNAs targeting immediately upstream and downstream of the integrant coupled with ganciclovir selection, which resulted in a segmental deletion of the cassette while leaving the rest of 1q unaffected,
3) Cell lines subjected to CRISPR-mediated cutting with a 1q-targeting gRNA, in which the lesion was repaired without causing chromosome loss,
4) Cell lines subjected to CRISPR-mediated cutting with a gRNA targeting the non-coding Rosa26 locus,
5) Cell lines in which dual CRISPR guides were used to generate segmental deletions on chromosome 1q of a gene encoding a non-expressed olfactory receptor,

6) Cell lines in which CRISPR was used to delete a terminal segment on chromosome 1q, eliminating the telomere and decreasing the copy number of 26 out of 968 protein-coding genes on the chromosome.

Every control clone that we tested exhibited significantly better anchorage-independent growth compared to the 1q-disomic clones that we derived (Fig. S8). Additionally, we note that the control clones generated by the segmental deletion of the HSV-TK gene were subjected to three independent CRISPR-induced DNA breaks (one to integrate HSV-TK and then two to produce the segmental deletion), which is more breaks than all 1q-disomic clones were subjected to.

To further verify that the phenotypes observed upon elimination of the 1q-trisomy are specifically a result of that karyotype alteration, we applied ReDACT-CO to eliminate trisomies of 1q, 7p, and 8q from the same cell line (Fig. 3A). If the use of ReDACT-CO is the cause of the reduced fitness upon 1q-loss, then we would expect that all aneuploidy-loss clones would be impaired to a similar degree. However, we observed that loss of the 7p or 8q trisomies resulted in a significantly milder phenotype compared to the effects of 1q-loss (Fig. 3C-D).

Finally, if an off-target effect of CRISPR is the cause of the reduced fitness upon 1q-loss, then we would not expect to see any selective pressure to restore the 1q trisomy. However, upon prolonged growth of the 1q-disomic clones *in vitro* or *in vivo*, we observed that many cell populations spontaneously recover an extra copy of chromosome 1q, and these 1q-restored cells exhibit improved colony-formation ability relative to the 1q-disomic clones (Fig. 4A-F). In total, these assays provide multiple independent lines of evidence that the reduced fitness of the 1q-disomic clones cannot be attributed solely to the effects of CRISPR.

Ganciclovir selection

In the ReDACT-NS approach, several 1q-disomic clones were generated by integrating the HSV-TK gene onto chromosome 1q and then selecting for aneuploidy-elimination via treatment with ganciclovir (Fig. 2A). As a specific control for this protocol, we also generated a series of clones in which the HSV-TK-expressing parental cells were transfected with two gRNAs that cut immediately upstream and downstream of the HSV-TK cassette, and then the cells were treated with ganciclovir (Fig. S8A). These clones acquired ganciclovir resistance due to

a segmental deletion of the HSV-TK gene, rather than loss of the entire chromosome arm. We subsequently observed that these ganciclovir-resistant control clones exhibited consistently superior anchorage-independent growth compared to clones that had lost the 1q-trisomy (Fig. S8D-E). Additionally, we note that two of our aneuploidy-elimination methods – ReDACT-TR and ReDACT-CO – do not utilize ganciclovir selection, and the phenotypes that we observed across independent 1q-disomic clones were similar regardless of the methods applied to generate them. In total, these findings suggest that any detrimental effects of ganciclovir selection are unable to fully account for the loss of fitness observed in the 1q-disomic clones.

Loss of telomere protection

In the ReDACT-NS and ReDACT-CO approaches, our aneuploidy elimination techniques may result in the loss of telomere protection on the targeted chromosome arm. We therefore investigated whether the loss of telomere protection could be sufficient to explain the phenotypes of our 1q-disomic clones. First, we transfected cells with a gRNA targeting a subtelomeric region on chromosome 1q and we isolated a control clone harboring a terminal chromosomal truncation. This clone maintained the ability to grow under anchorage-independent conditions at wild-type levels (Fig. S8C). Second, if loss of telomere protection on a single chromosome arm is sufficient to inhibit malignant potential, then we would expect this phenotype to be consistent across different chromosomes. However, we applied ReDACT-CO to eliminate the trisomies of chromosome 7p and 8q from A2058 cells, and we observed that the 7p-disomic and 8q-disomic clones exhibited consistently superior fitness compared to 1q-disomic clones obtained using the same techniques in the same cell line (Table S3). Third, a subset of our 1q-disomic clones were created using ReDACT-TR, in which the CRISPR-induced DNA break was repaired with an artificial telomere. As noted above, the phenotypes that we observed across independent 1q-disomic clones were similar regardless of the methods applied to generate them (Fig. 2). In total, these findings suggest that the loss of telomere protection on a single chromosome arm is unable to fully account for the compromised fitness observed in the 1q-disomic clones.

Supplemental Text 2. Considering the loss of specific point mutations on chromosome 1q as an explanation for the loss of fitness in engineered 1q-disomic cancer cells.

Deletion of a chromosome not only decreases the dosage of any genes encoded on the targeted chromosome, it may also cause the loss of any point mutations encoded on that chromosome. Correspondingly, we considered the possibility that the effects of 1q-loss could be mediated in part by eliminating unique driver mutations that these cell lines had acquired on chromosome 1q. To explore this possibility, we evaluated all non-synonymous mutations on chromosome 1q in the 1q-trisomic cancer cell lines used in this study. Using data acquired from DepMap, we found 25 1q mutations in A2780, 19 1q mutations in A2058, and 52 1q mutations in AGS. We cross-referenced these mutations with the Catalogue of Somatic Mutations in Cancer (COSMIC) database to examine if any mutations were recurrently observed or causally implicated in human cancers. None of the 96 mutations present on chromosome 1q in A2780, A2058, and AGS were identified as mutational hotspots in the COSMIC database, and none of the genes affected by mutations were included in the Cancer Gene Census. Next, we investigated the list of cancer driver genes identified by Vogelstein et al.[129], and we found that none of the 1q genes affected by mutations in these cell lines were annotated as likely drivers. Lastly, we examined MSK-IMPACT, a panel of 505 genes associated with both common and rare cancers[130]. None of the 1q mutated genes are included in this panel. For these reasons, we believe that the mutations found on chromosome 1q in these cell lines likely represent passenger events, rather than cancer drivers. Nonetheless, we do not rule out the possibility that the loss of specific point mutations could influence the effects of aneuploidy-elimination in other experiments. For instance, as described in Figure 4G, we speculate that the effects of gaining chromosome 12 in HCT116 is mediated in part by the acquisition of an extra copy of the mutant $KRAS^{G13D}$ allele, and loss of a chromosome containing mutant KRAS may have different consequences than loss of a chromosome containing wild-type KRAS.

Figure S1

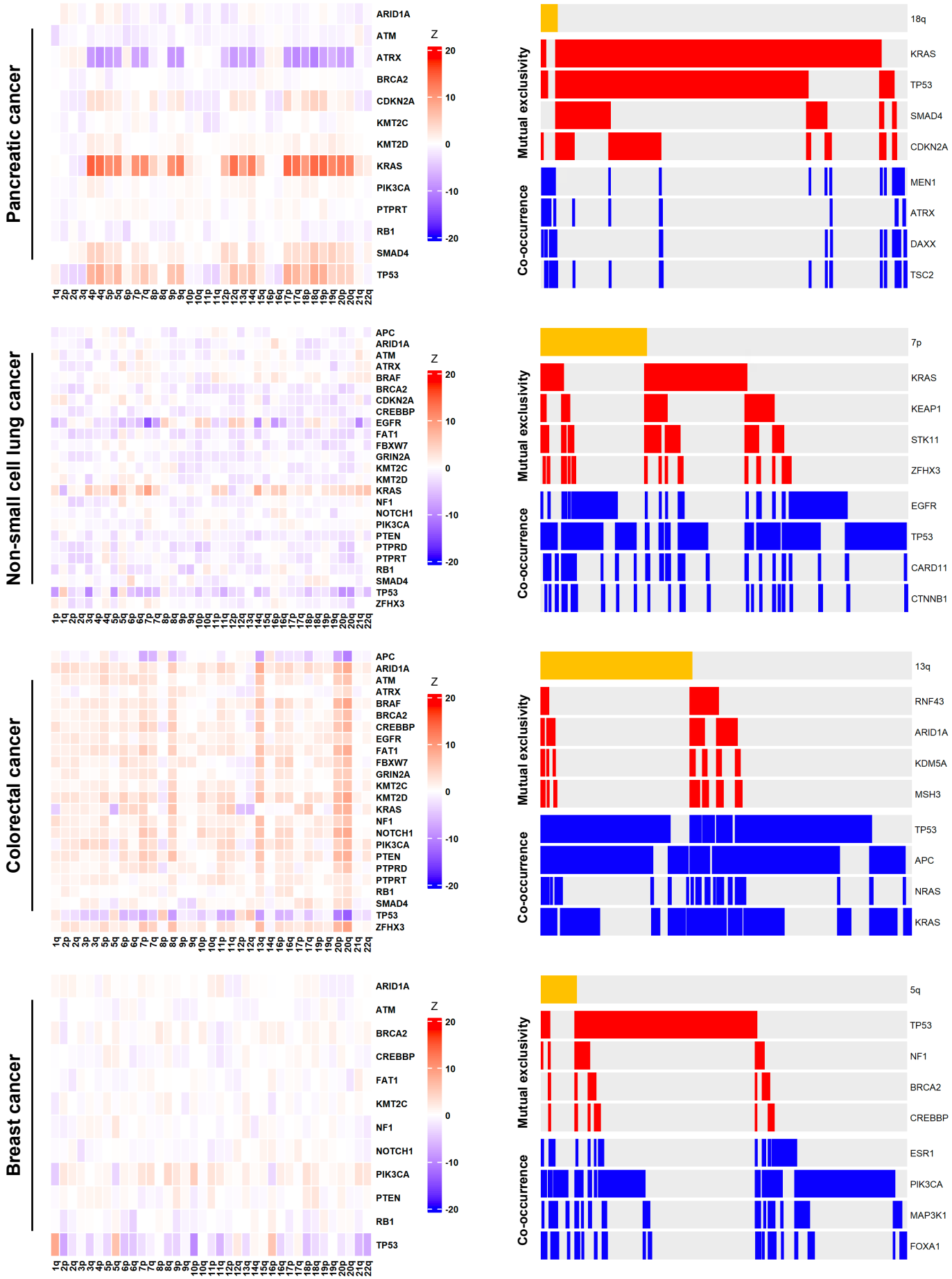


Figure S2

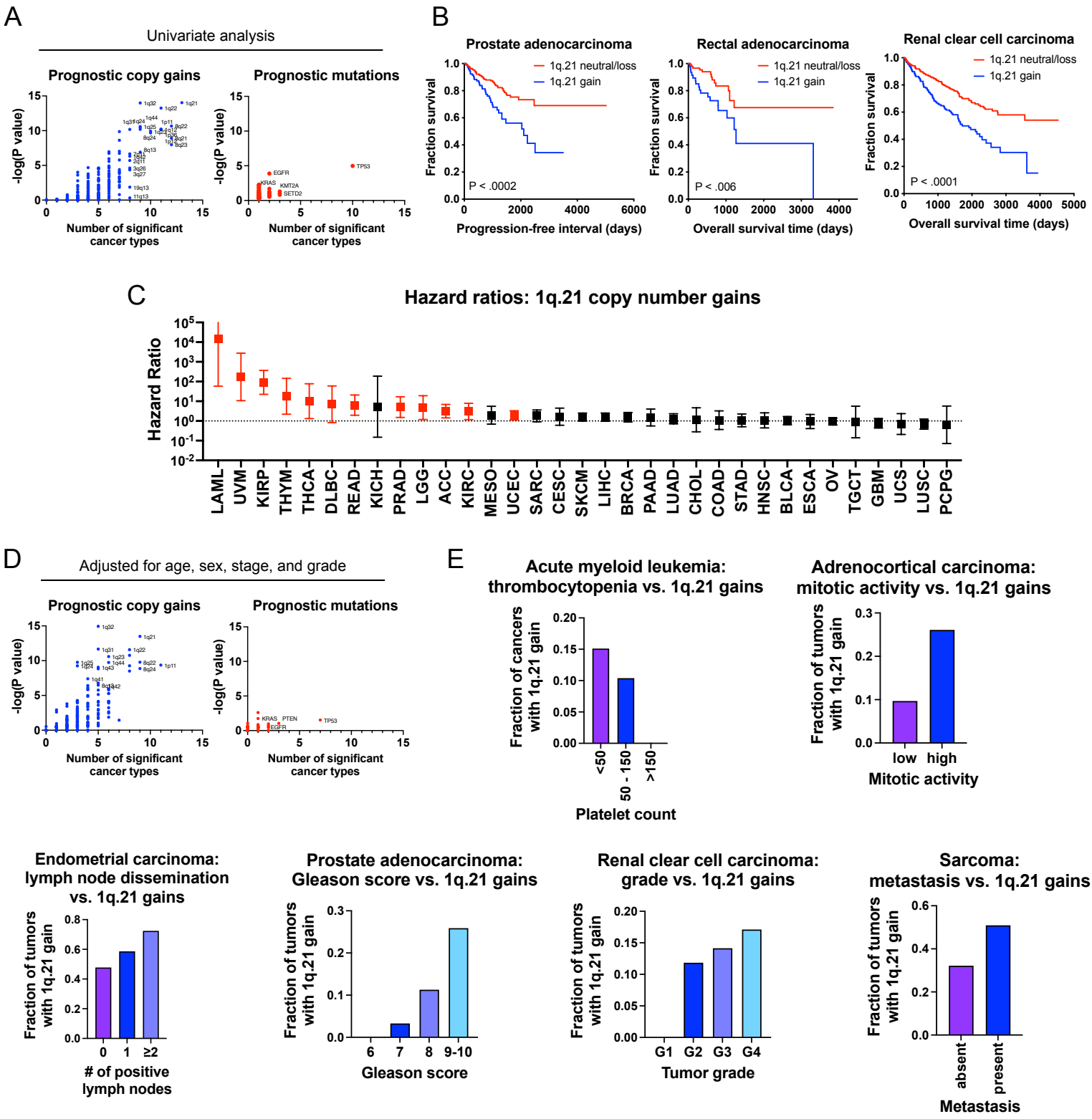
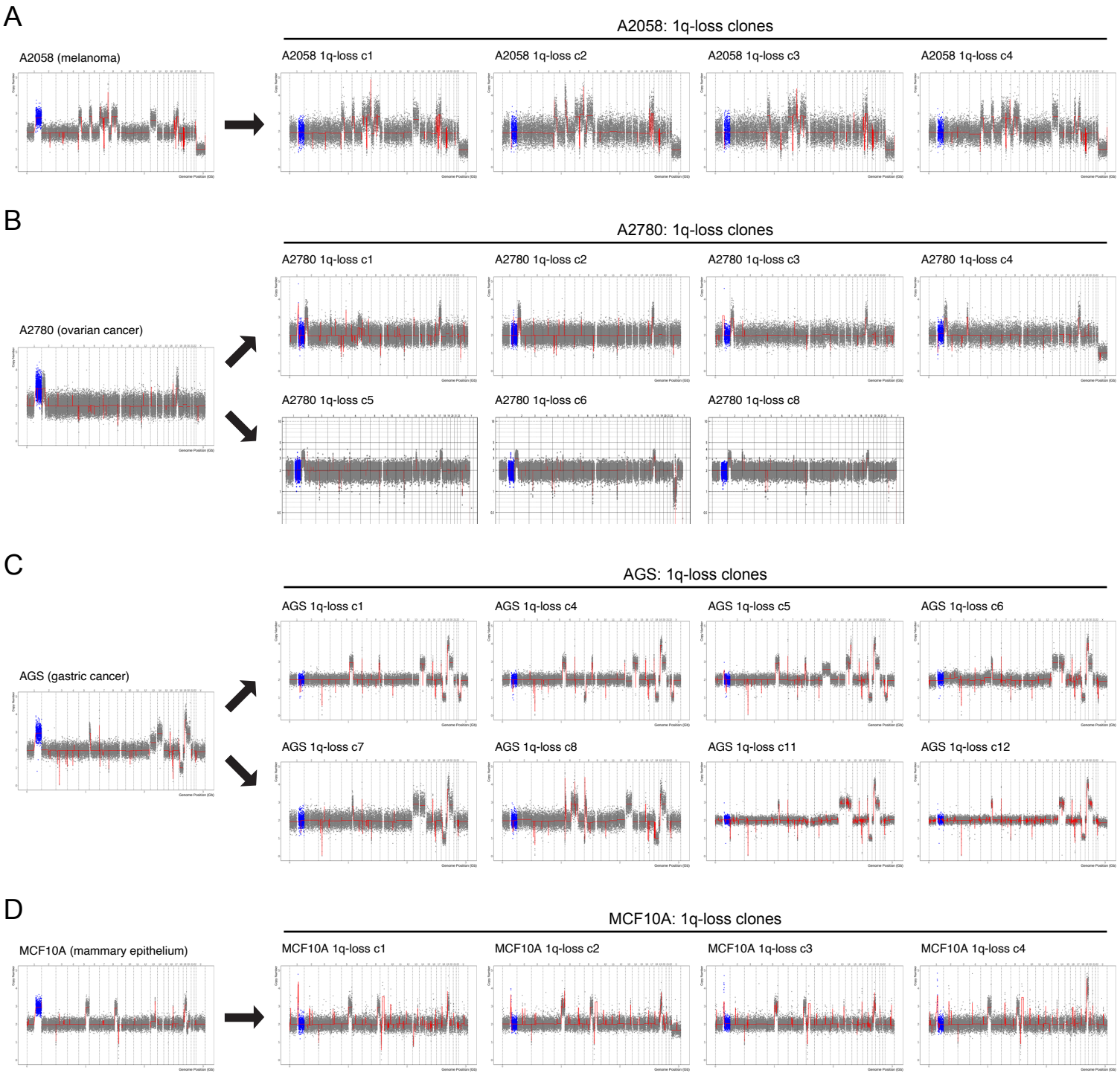


Figure S3



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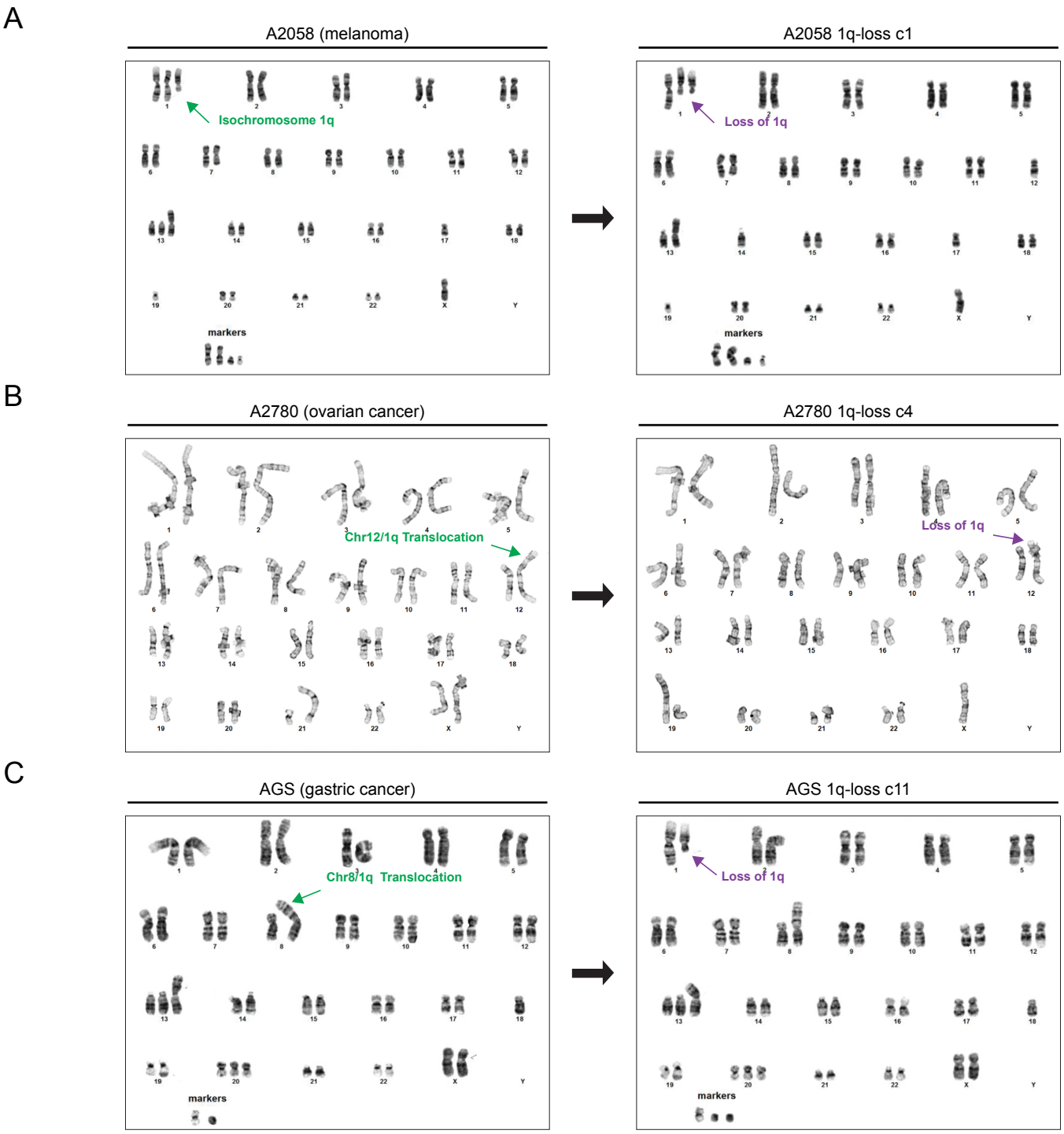

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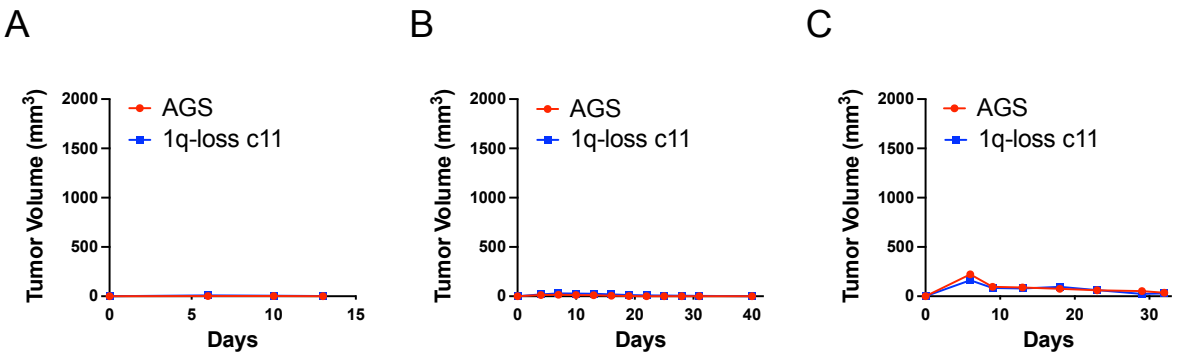

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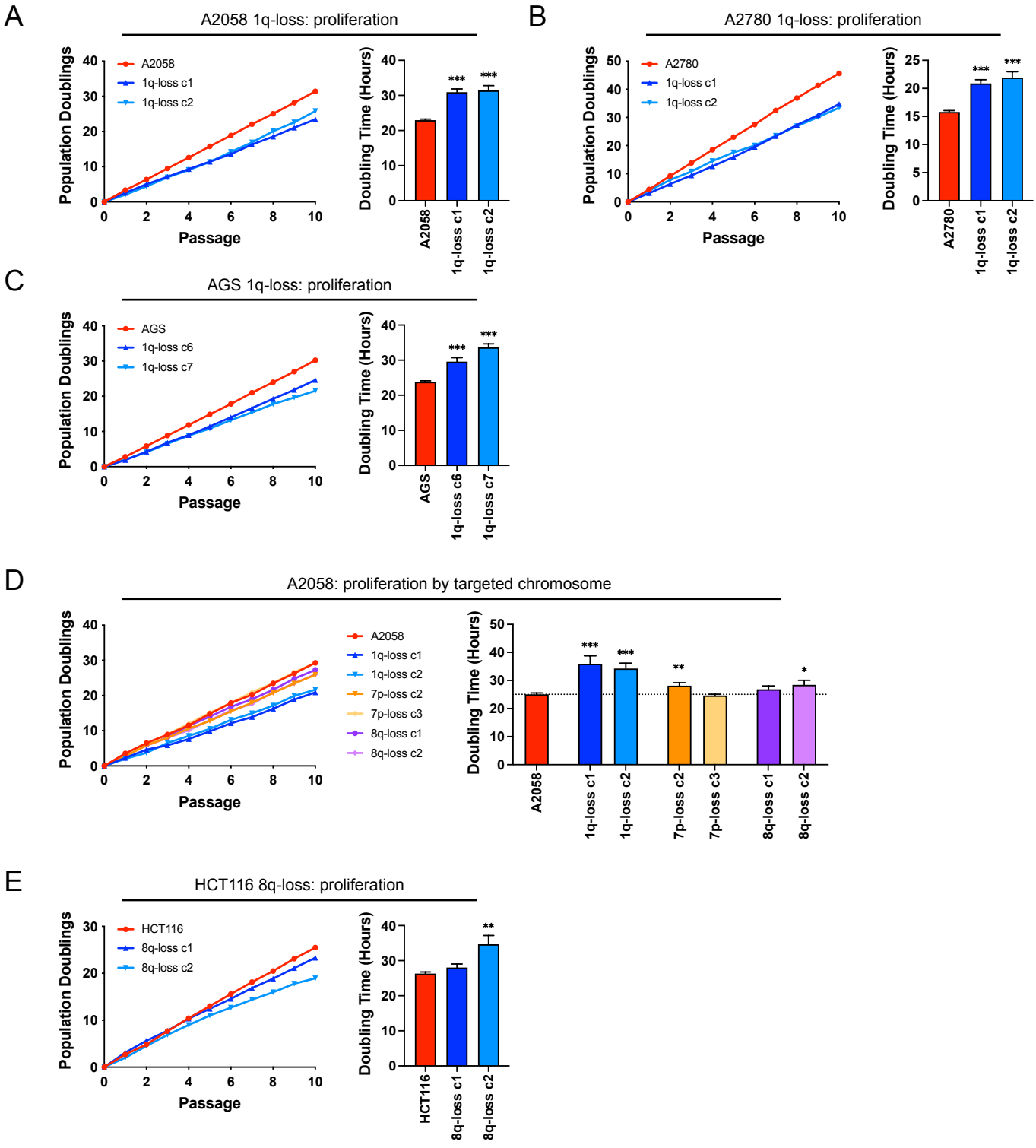

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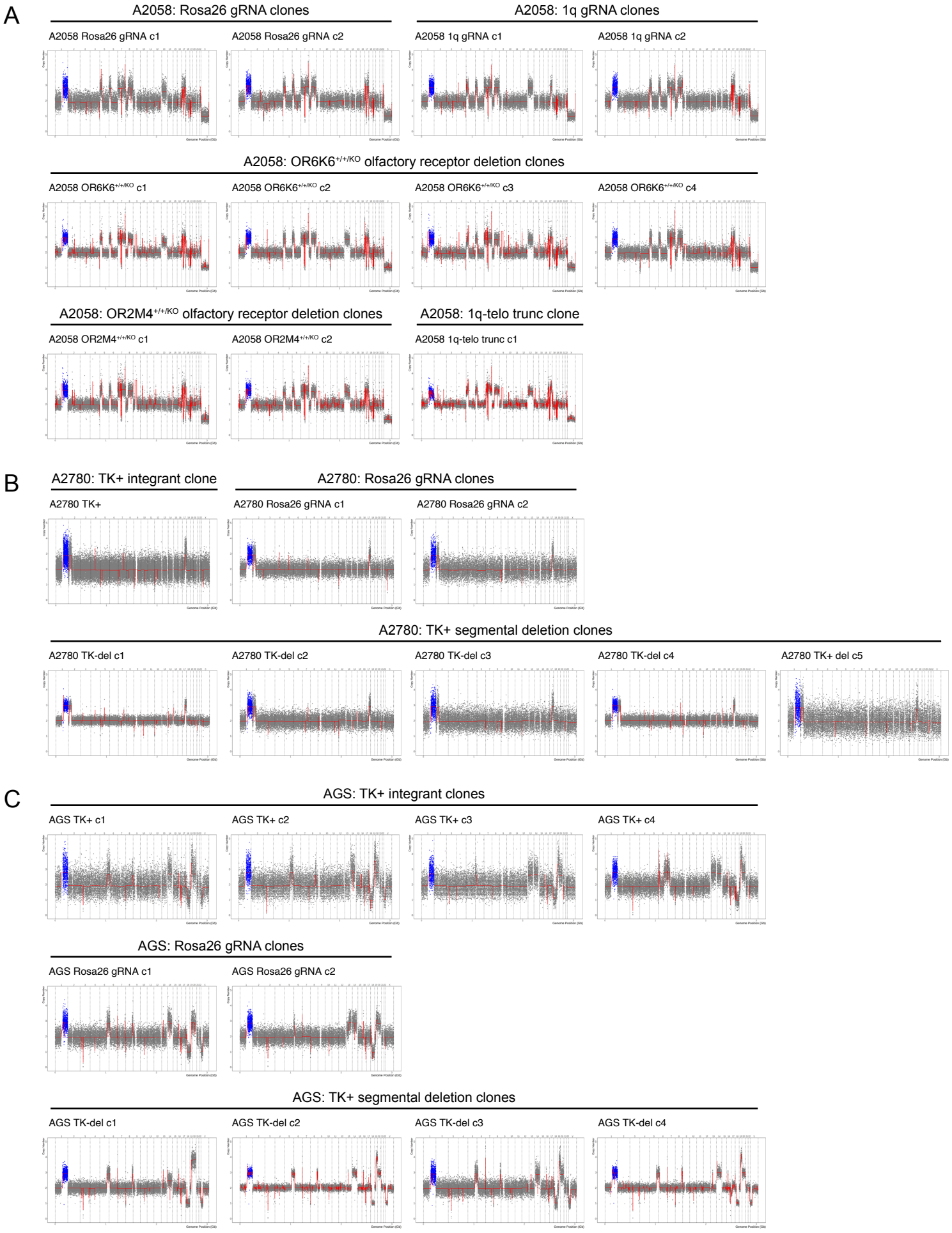
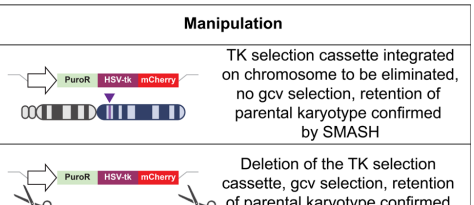


Figure S8

A

Type	Manipulation
TK+ Integrant Control	TK selection cassette integrated on chromosome to be eliminated, no gcv selection, retention of parental karyotype confirmed by SMASH
TK+ Segmental Deletion	Deletion of the TK selection cassette, gcv selection, retention of parental karyotype confirmed by SMASH
Rosa26 gRNA Control	Transfected with CRISPR gRNA targeting Rosa26 locus, retention of parental karyotype confirmed by SMASH
1q gRNA Control	Transfected with CRISPR gRNA targeting centromere-proximal region on Chr 1q, lesion repaired without arm loss, retention of 1q trisomy confirmed by SMASH
7p gRNA Control	Transfected with CRISPR gRNA targeting centromere-proximal region on Chr 7p, lesion repaired without arm loss, retention of 7p trisomy confirmed by SMASH
8q gRNA Control	Transfected with CRISPR gRNA targeting centromere-proximal region on Chr 8q, lesion repaired without arm loss, retention of 8q trisomy confirmed by SMASH
1q Telomere Truncation Control	Deletion of terminal genes from cytoband 1q44 to the end of the chromosome, retention of 1q trisomy confirmed by SMASH
Olfactory Receptor Gene Segmental Del: OR6K6 $^{+/+/KO}$	Deletion of a single copy of the olfactory receptor OR6K6, retention of 1q trisomy confirmed by SMASH
Olfactory Receptor Gene Segmental Del: OR2M4 $^{+/+/KO}$	Deletion of a single copy of the olfactory receptor OR2M4, retention of 1q trisomy confirmed by SMASH



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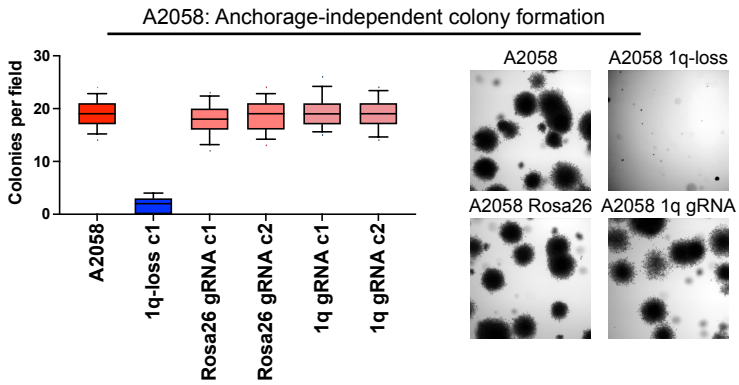

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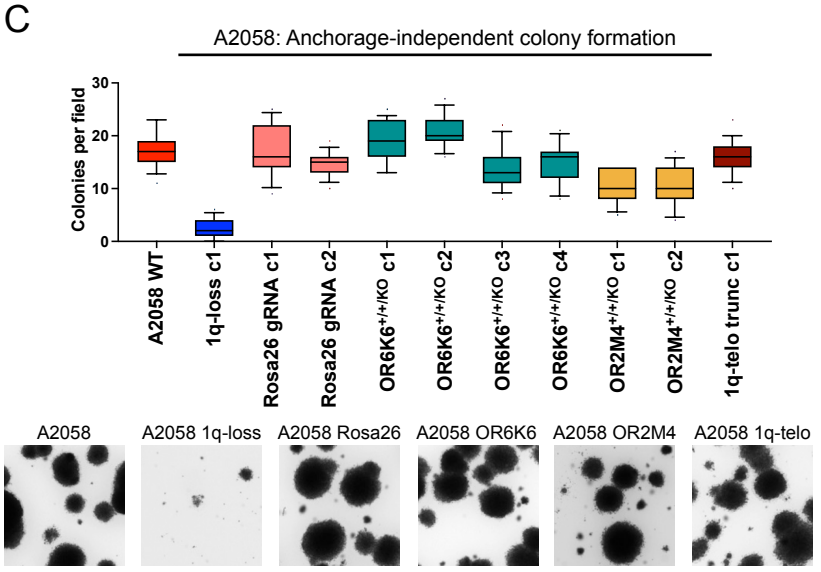

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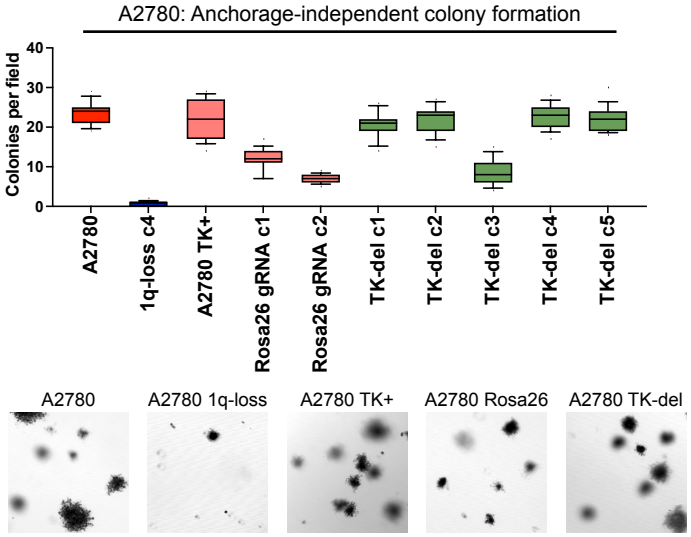

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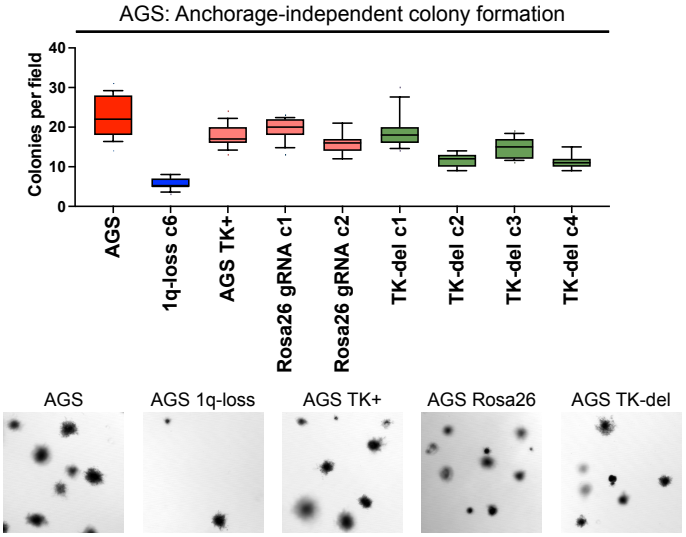


Figure S9

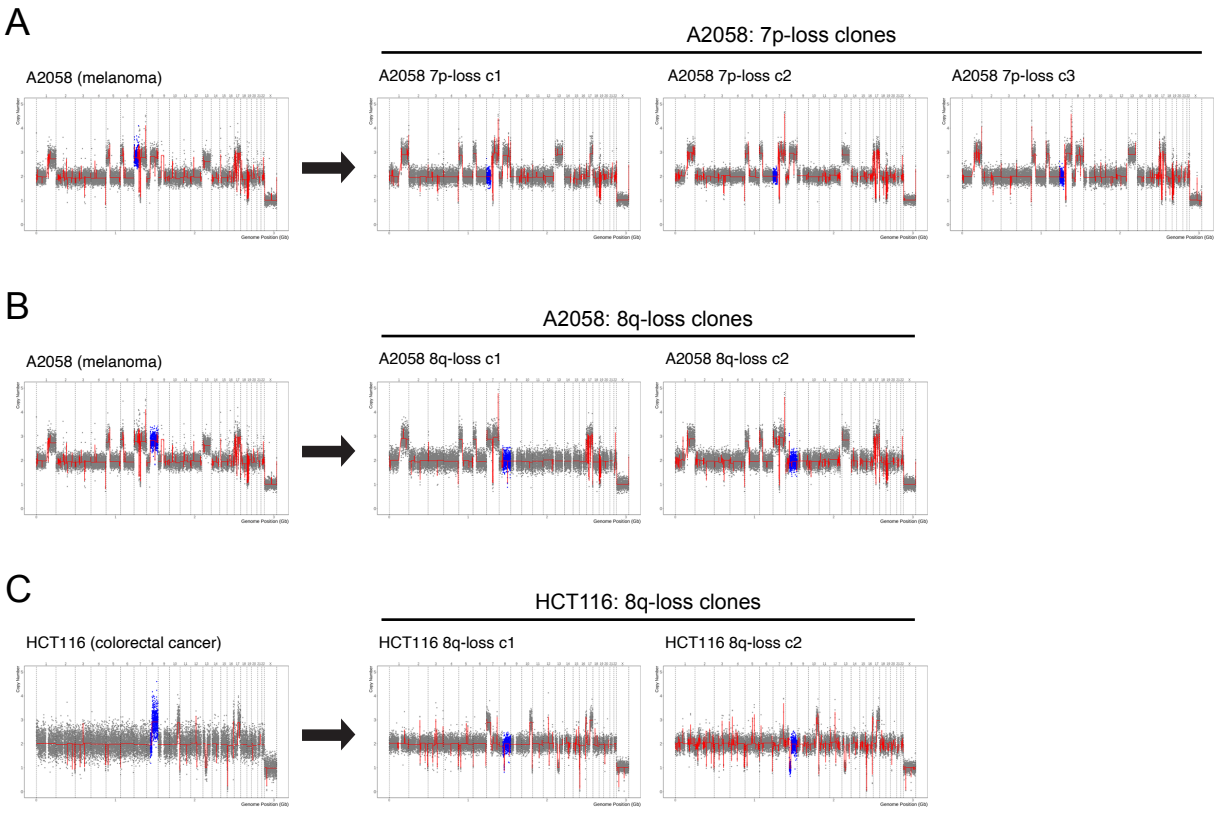


Figure S10

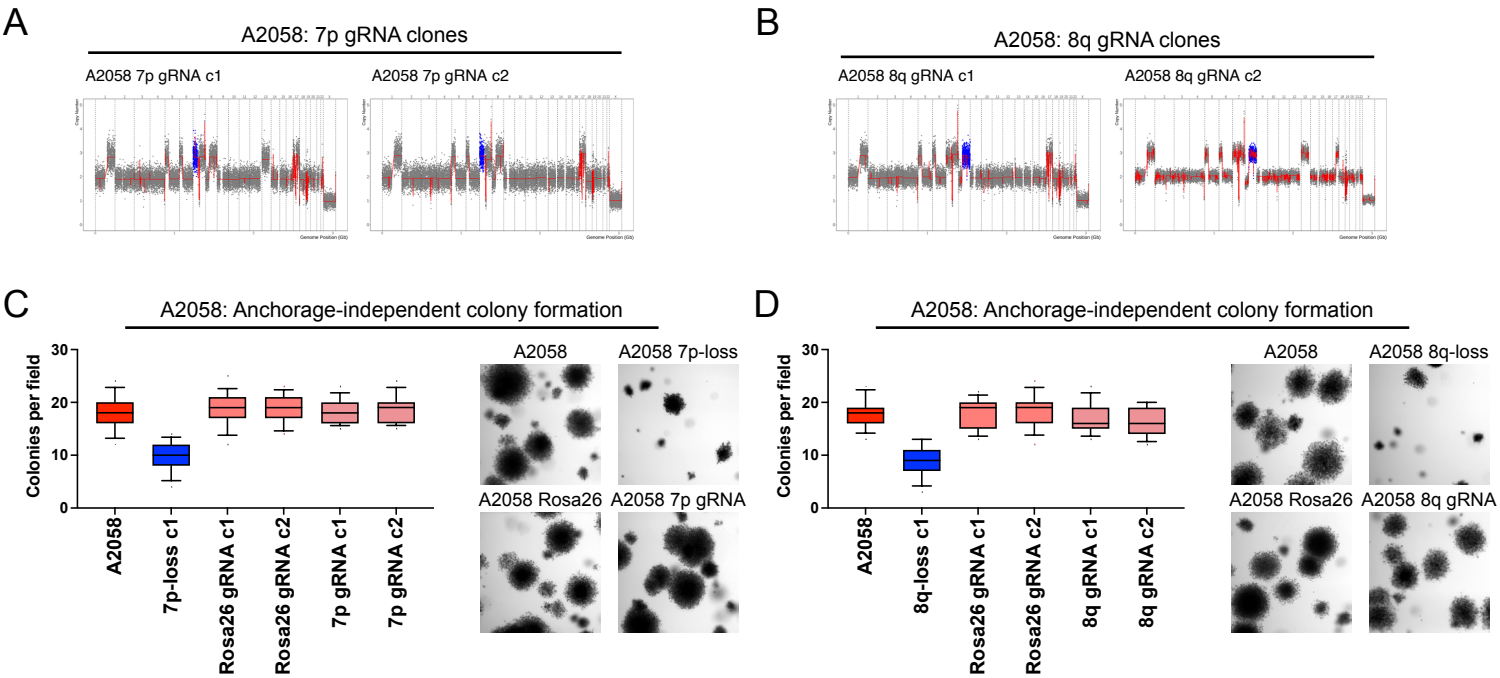
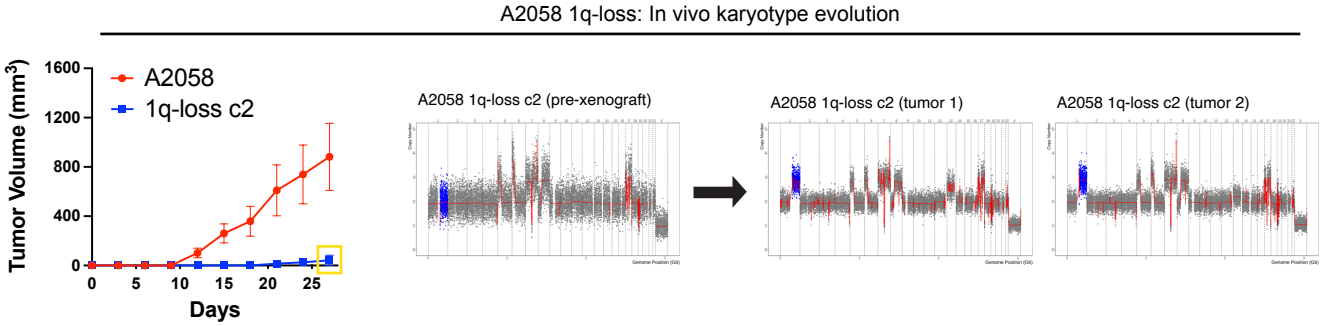


Figure S11

A



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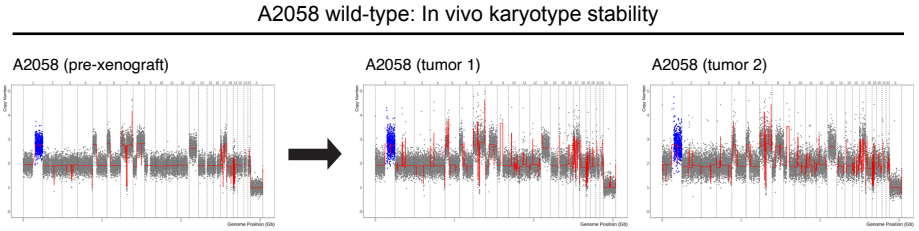


Figure S12

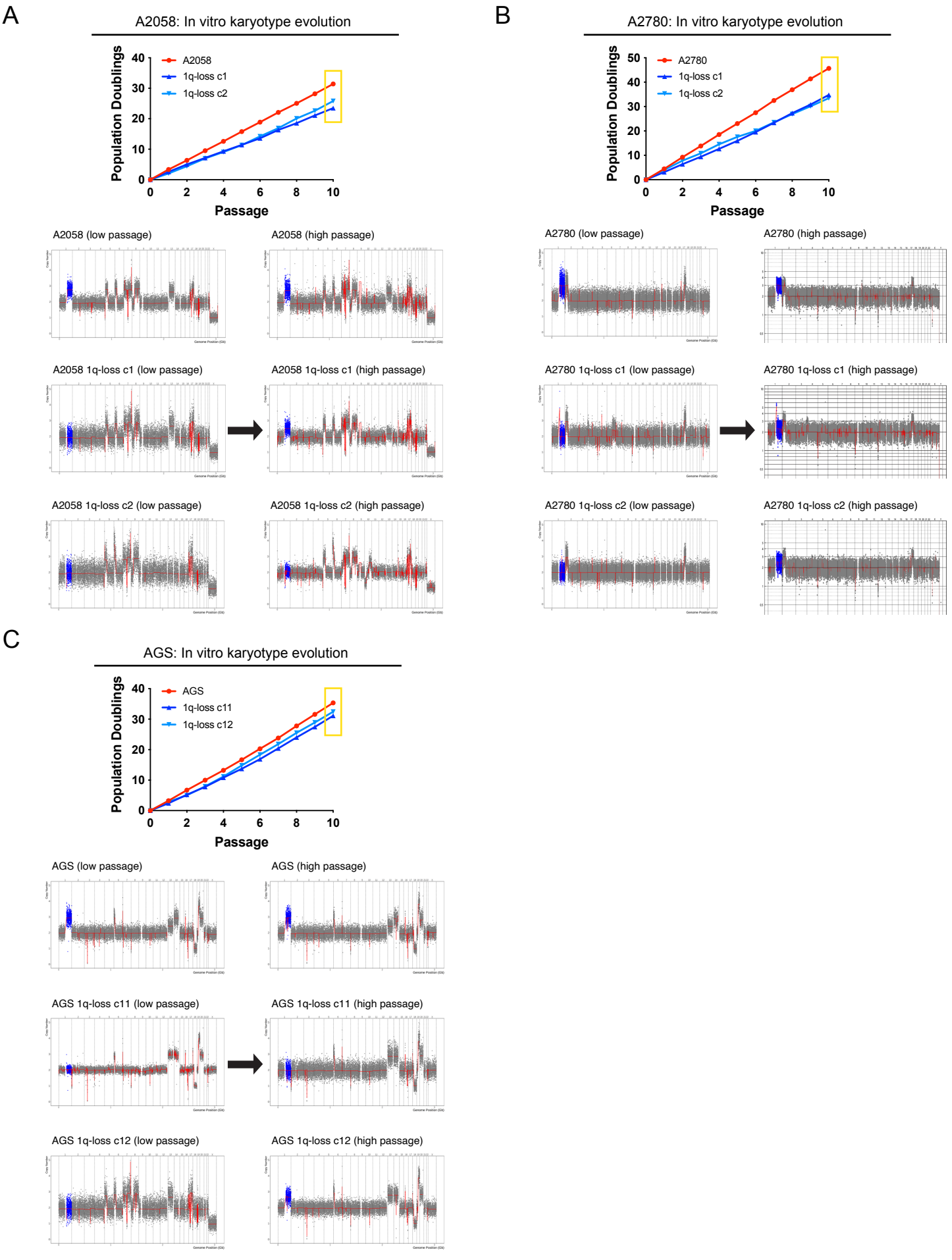


Figure S13.

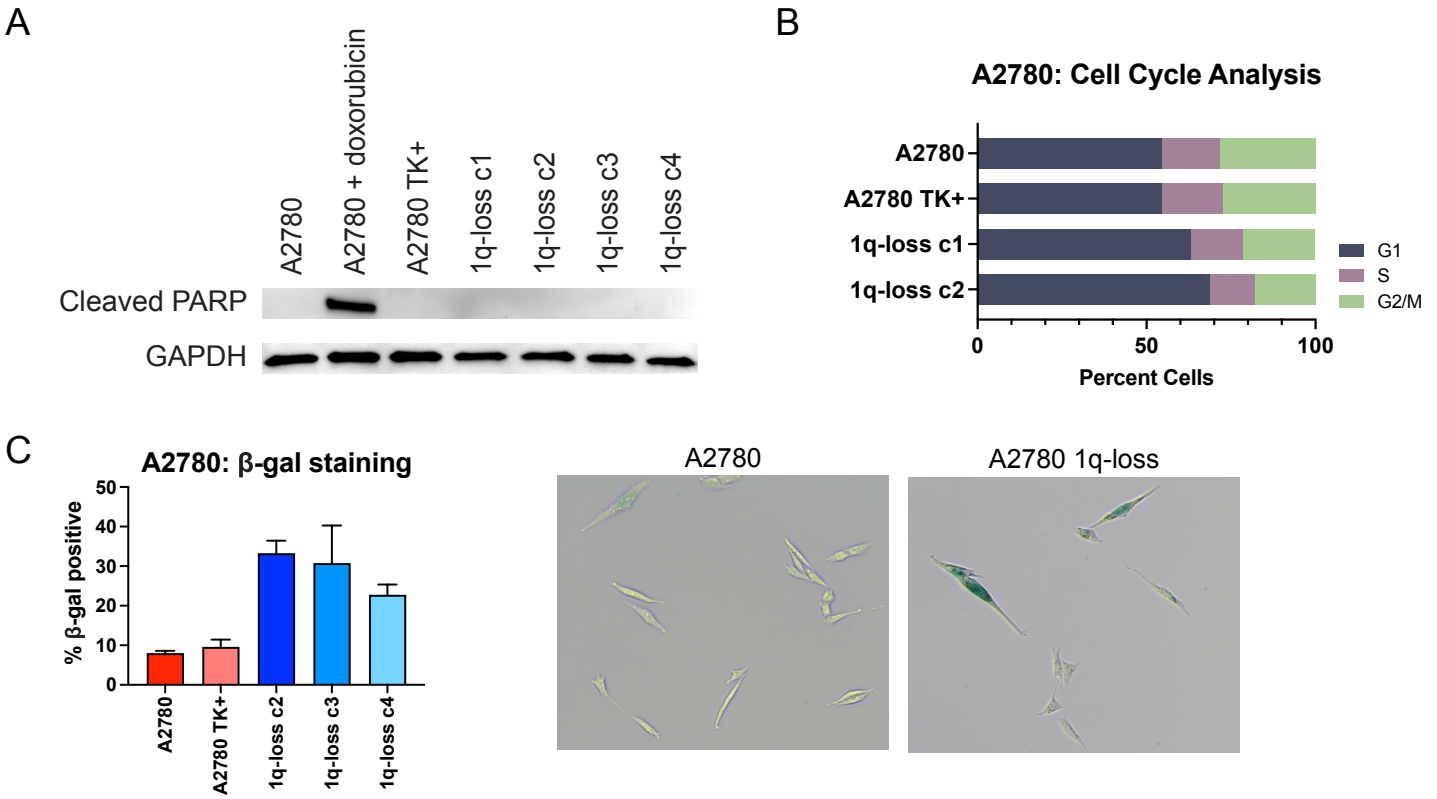


Figure S14

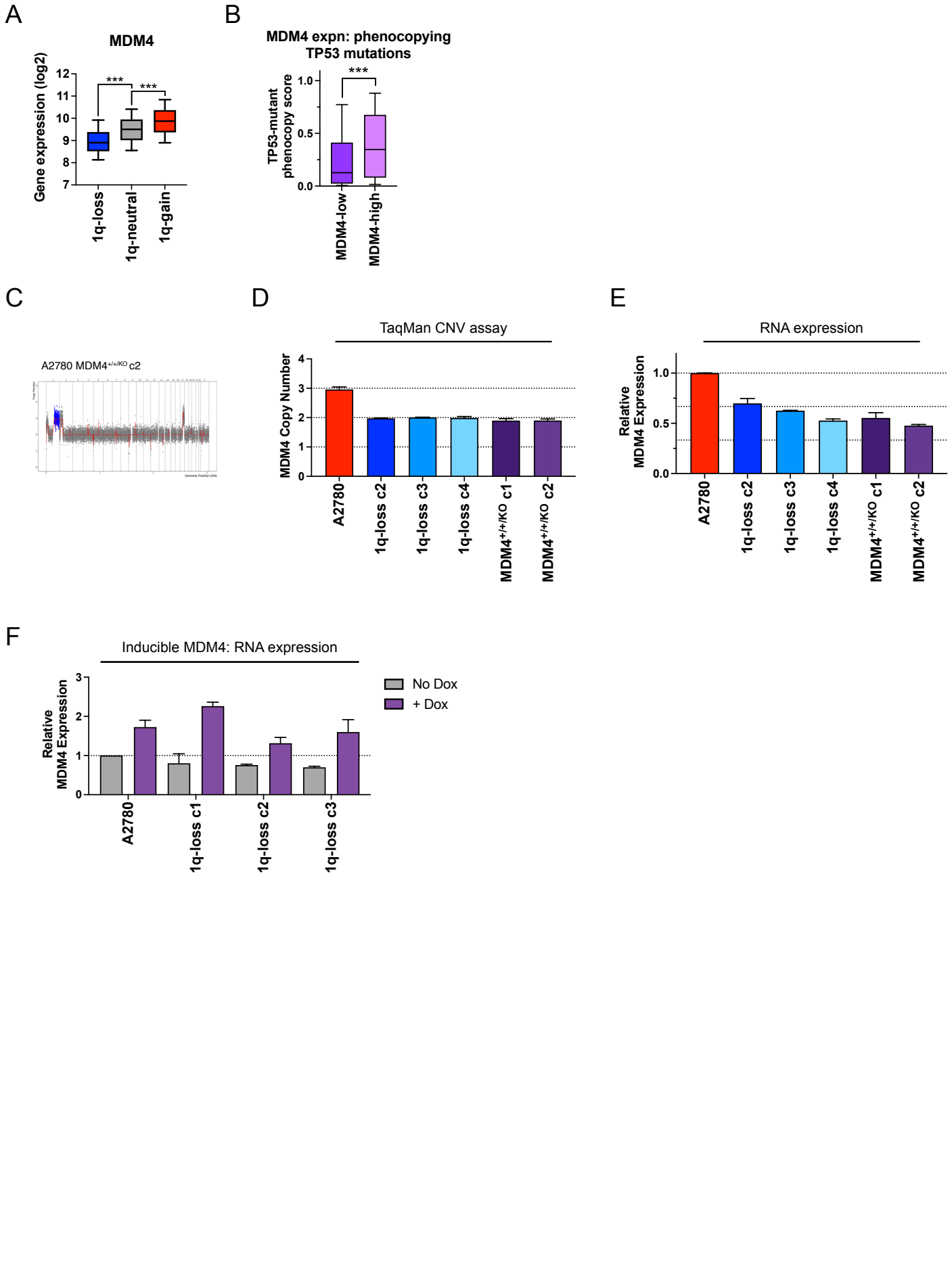
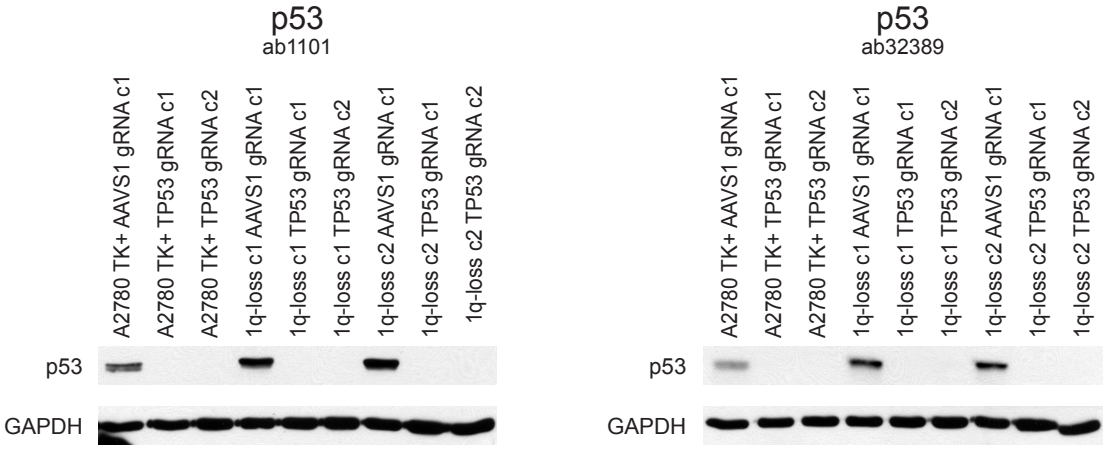


Figure S15

Supplementary Figure 15

A



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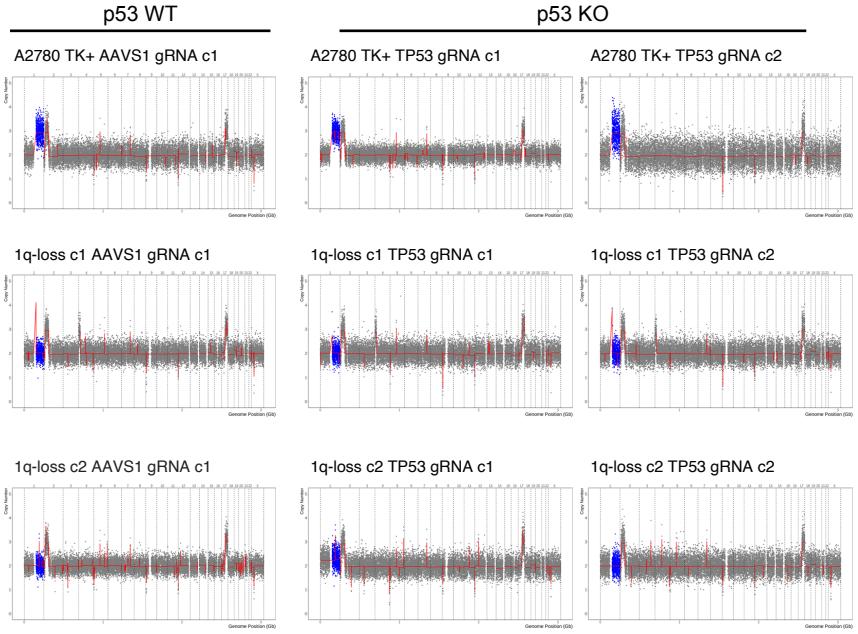

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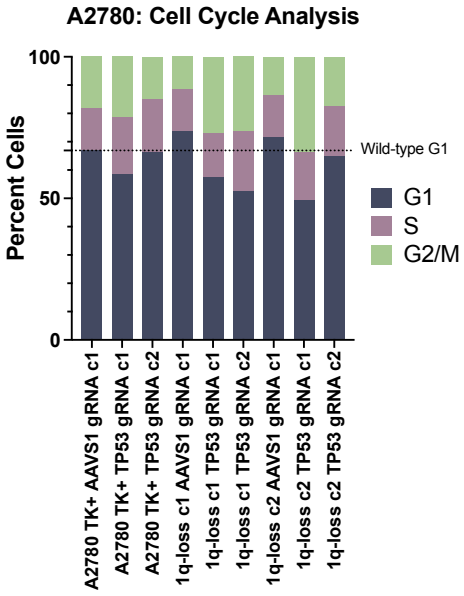

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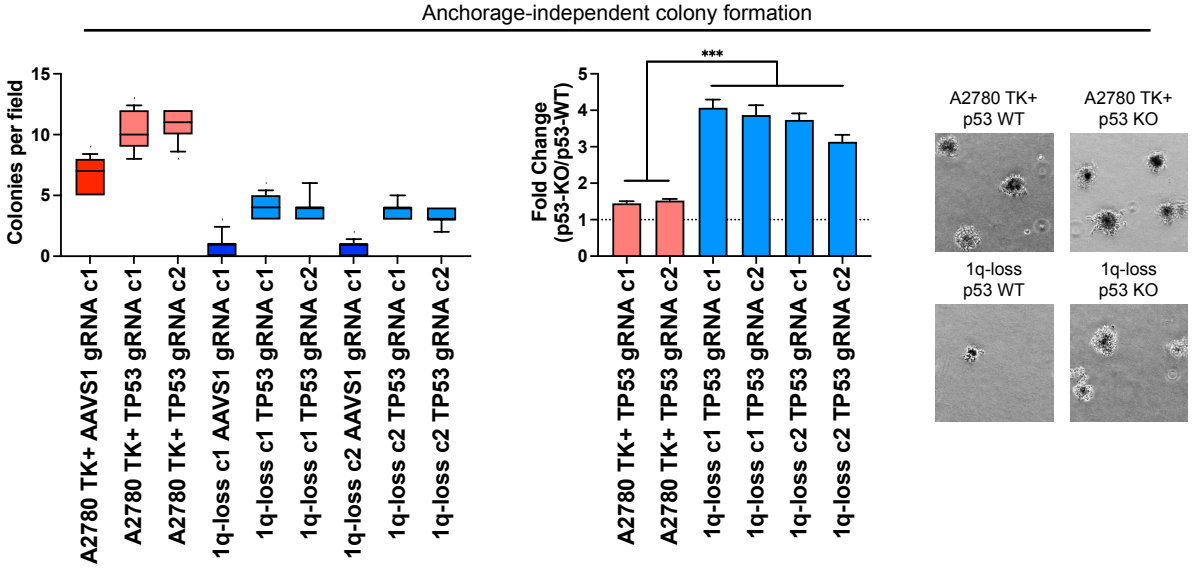

Figure S16