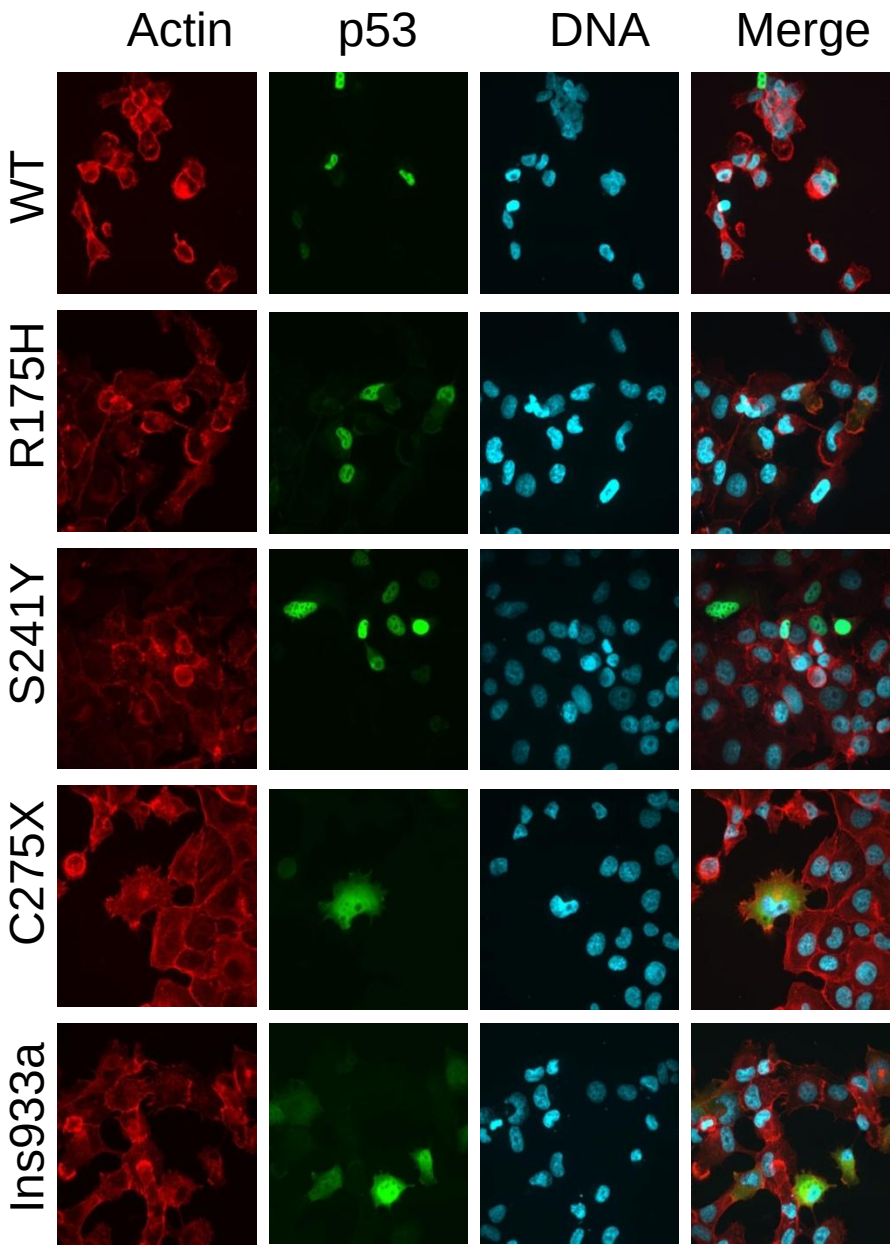
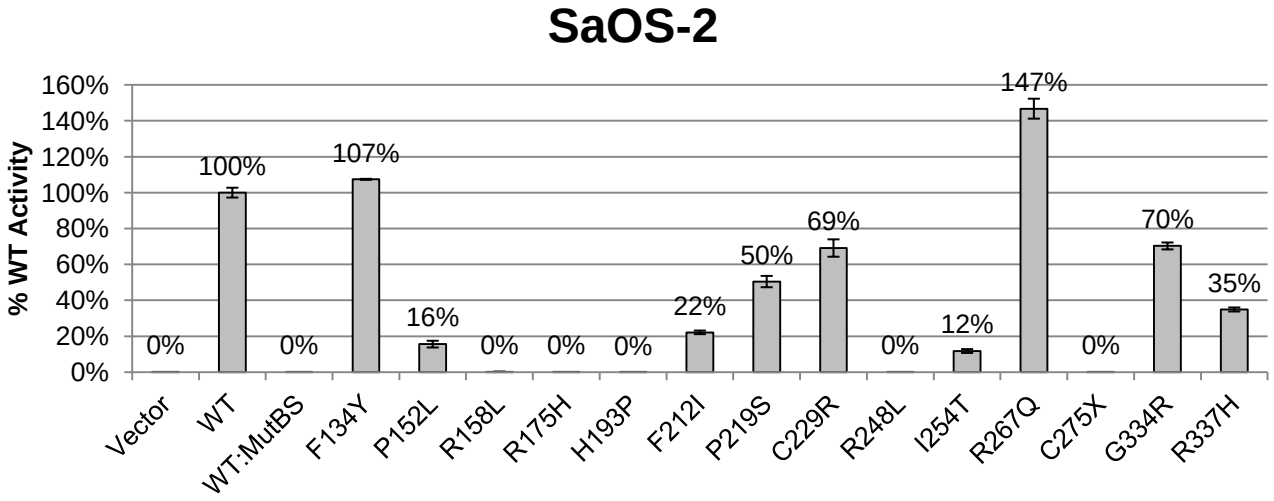
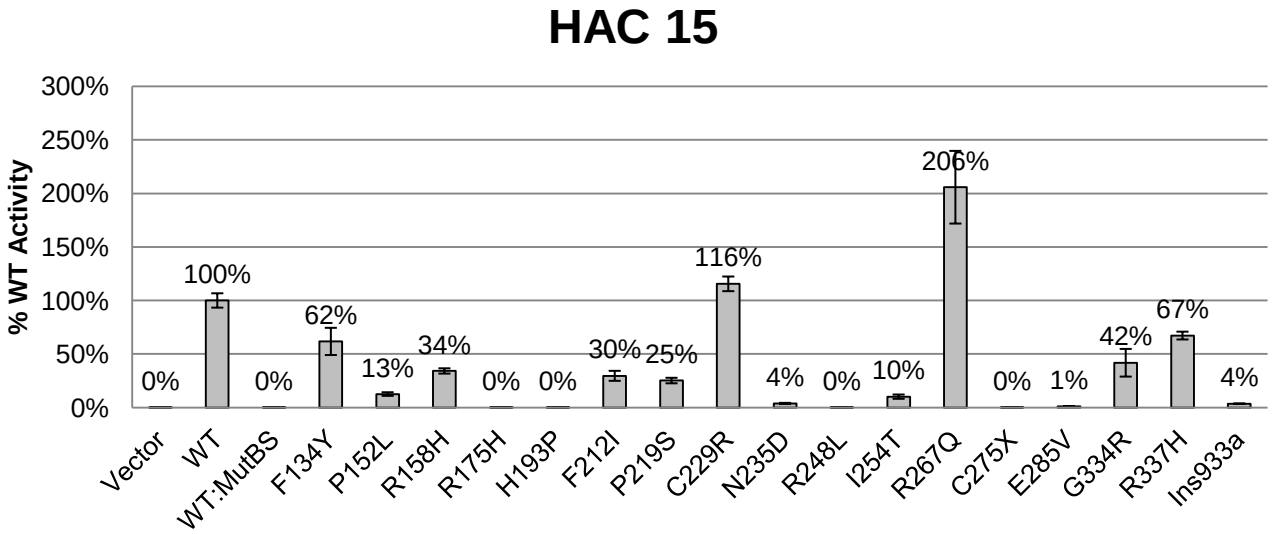
# Supplemental Figure 1



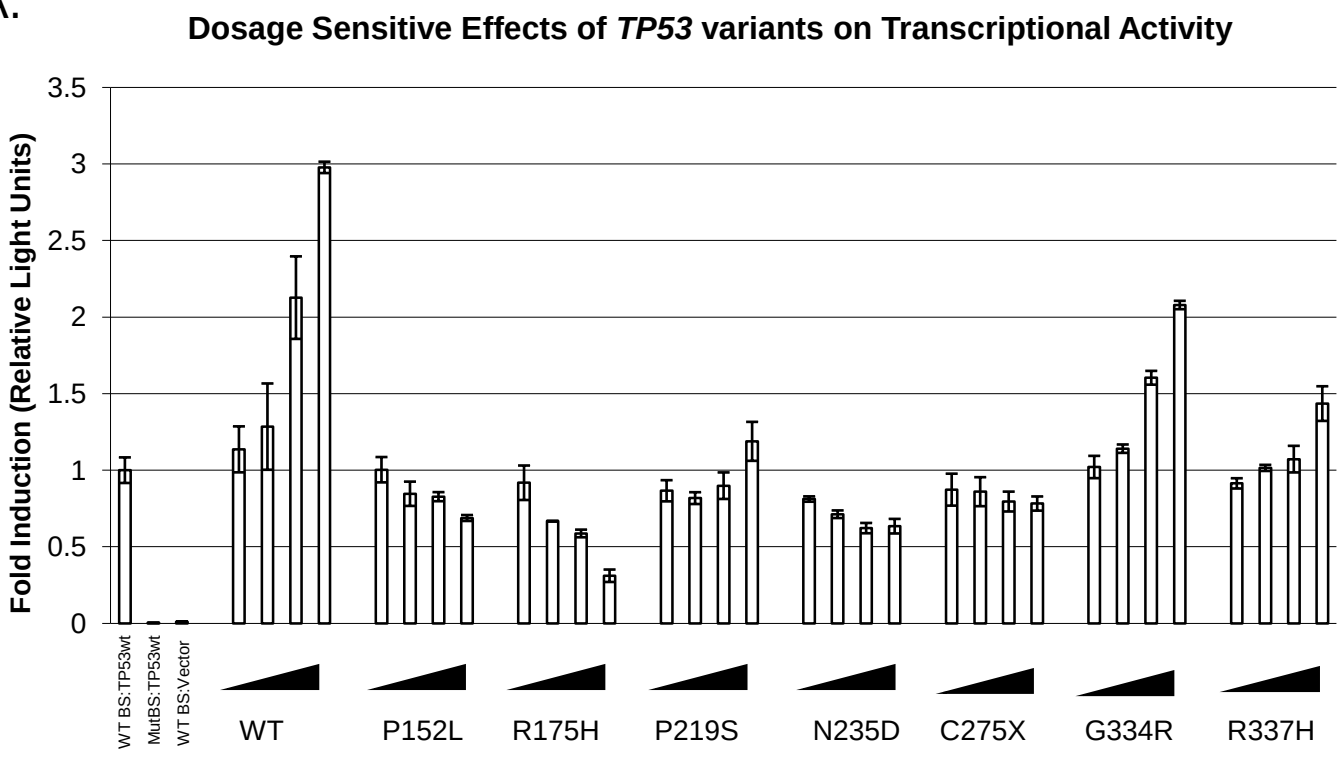

**Supplemental Figure 1:** Representative subcellular localization of TP53-GFP fusion proteins overexpressed in H1299 cells. Cells were counterstained with Rhodamine-phalloidin to outline cells and DAPI to highlight nuclei. WT and most variants demonstrate nuclear localization, while the C275X and Ins933 variants are distributed throughout the cell in both cytoplasm and nucleus. These mutations result in premature stop codons which generate truncated proteins lacking conserved nuclear localization signals and nuclear export signals within the C-terminal portion of TP53, thus alterations in subcellular localization are not surprising (Liang SH, Clarke MF: Eur J Biochem 268:2779-83, 2001).



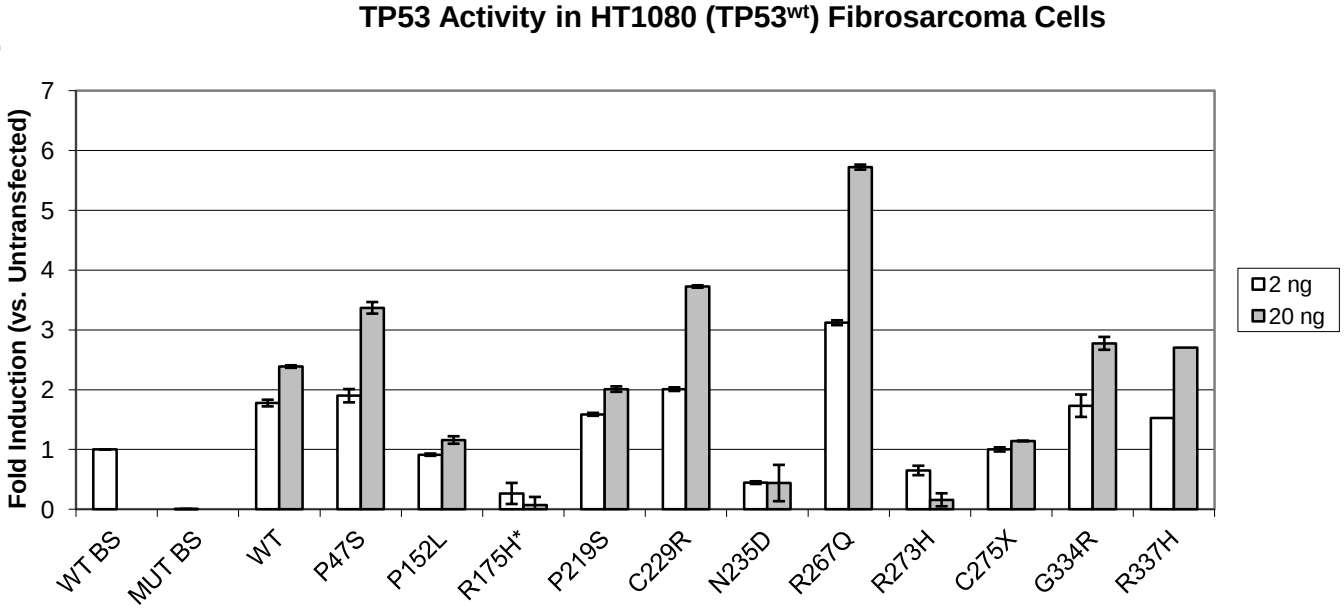




**Supplemental Figure 2:** Functional activity of *TP53*-variants expressed in a) SaOS-2cells and b) HAC15 cells as determined by transactivation of TP53-responsive luciferase reporter.

A.

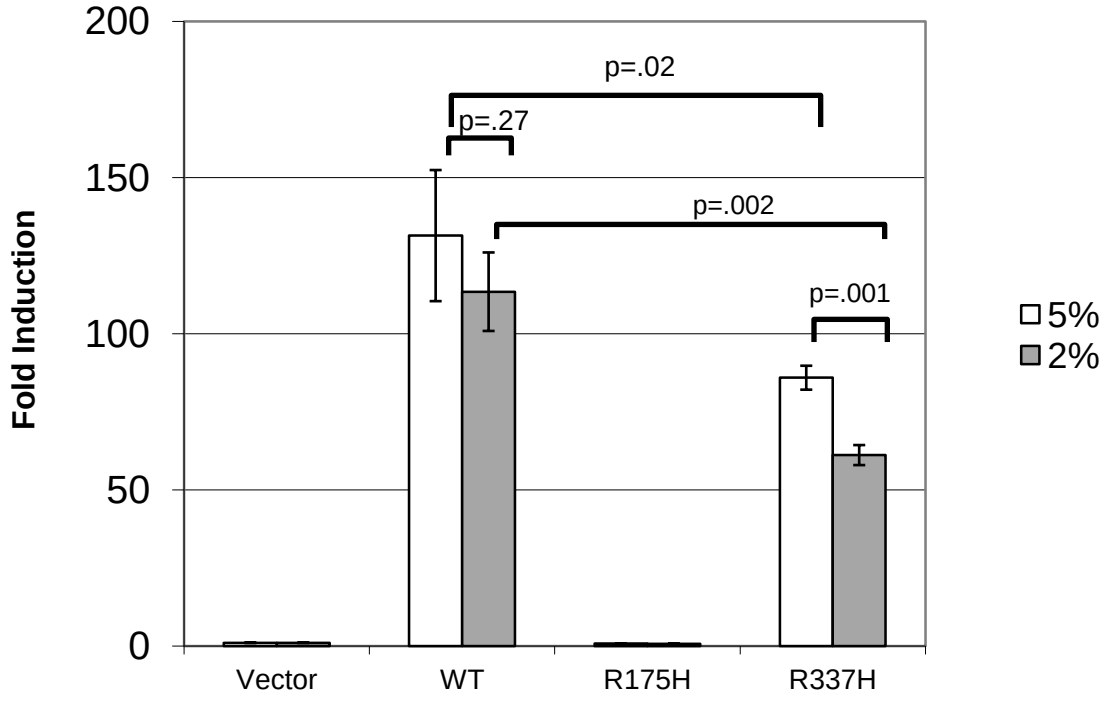



B.

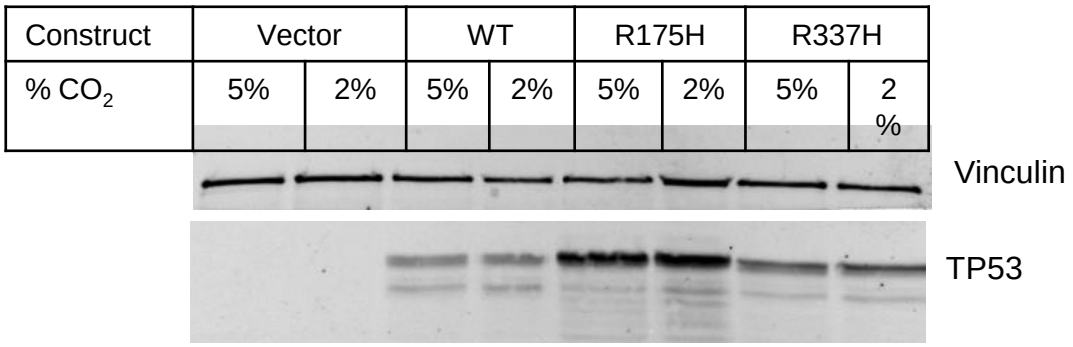



**Supplemental Figure 3:** Dominant Negative Activity of TP53 variants using two different assays: A. H1299 ($TP53^{null}$) cells: wild-type and variant TP53 were co-transfected in increasing ratios (10:1, 1:1, 1:5 and 1:10) along with luciferase reporter. TP53 transcriptional activity was determined as described. P152L, R175H, N235D all demonstrated dominant-negative effect, while other variants had neutral or additive effect on TP53 activity. B. TP53 variants were introduced into HT1080 ($TP53^{wt}$) cells. Data are reflected as fold-induction relative to endogenous TP53 function (WT BS). Introduction of WT TP53 results in additive activity while TP53 variants result in additive (P47, P219S, C229R, R267Q, G334, R337H) neutral (P152L, N235D, C275X) or dominant-negative (R175H, R273H) activity. Dominant-negative activity was identified for P152L and N235D only using the assay in A. WT BS= wild-type TP53 binding site (PG13); MUT BS=mutated TP53 binding site (MG15).

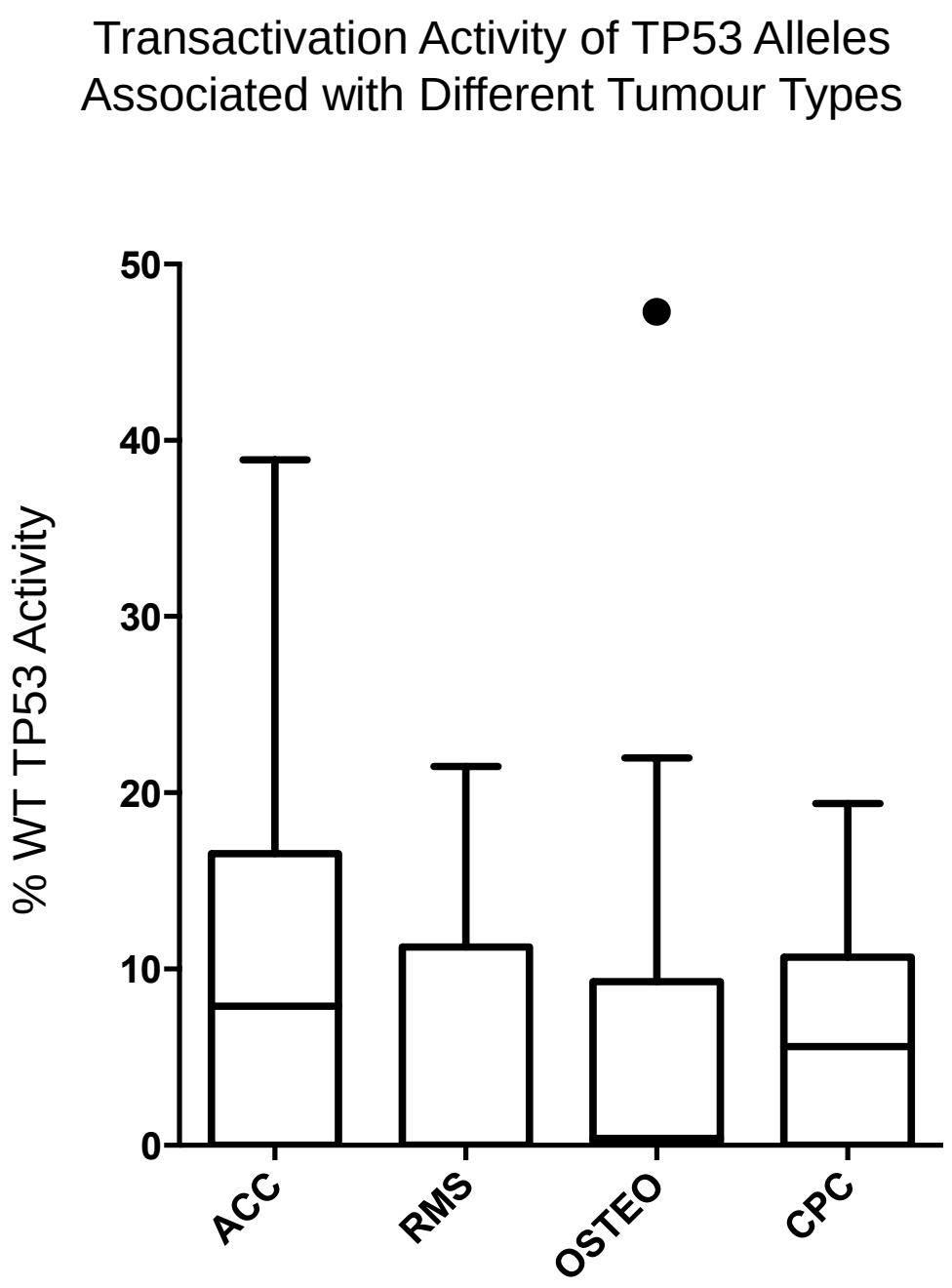
A.




B.




**Supplemental Figure 4:** pH-dependent loss of function with $TP53^{R337H}$. H1299 cells were transfected as described. 24 hours after transfection they were transferred to in incubator containing 5% or 2% $CO_2$. A. Luciferase activity was determined 48 hrs post-transfection. $TP53^{R337H}$ demonstrated decreased activity versus WT in both conditions, however, $TP53^{R337H}$ demonstrated a relative loss of activity when maintained at 2% $CO_2$ that was not present with $TP53^{WT}$ or $TP53^{R175H}$ indicating pH-dependent loss of function. B. Western blot demonstrates that altering $CO_2$ tension does not affect TP53 protein expression.




**Supplemental Figure 5:** Activity of alleles associated with LFS-component tumors, as determined by trans-activation in *S. cerevisiae* are demonstrated for adrenocortical carcinoma (ACC, median 7.9%, n=56), rhabdomyosarcoma (RMS, median 0.0%, n=52), osteosarcoma (OSTEO, median 0.4%, n=100) and choroid plexus carcinoma (CPC, median 5.6%, n=22). Despite the differing distributions, differences in the median activities did not reach statistical significance (Kruskal-Wallis, p=0.0501).