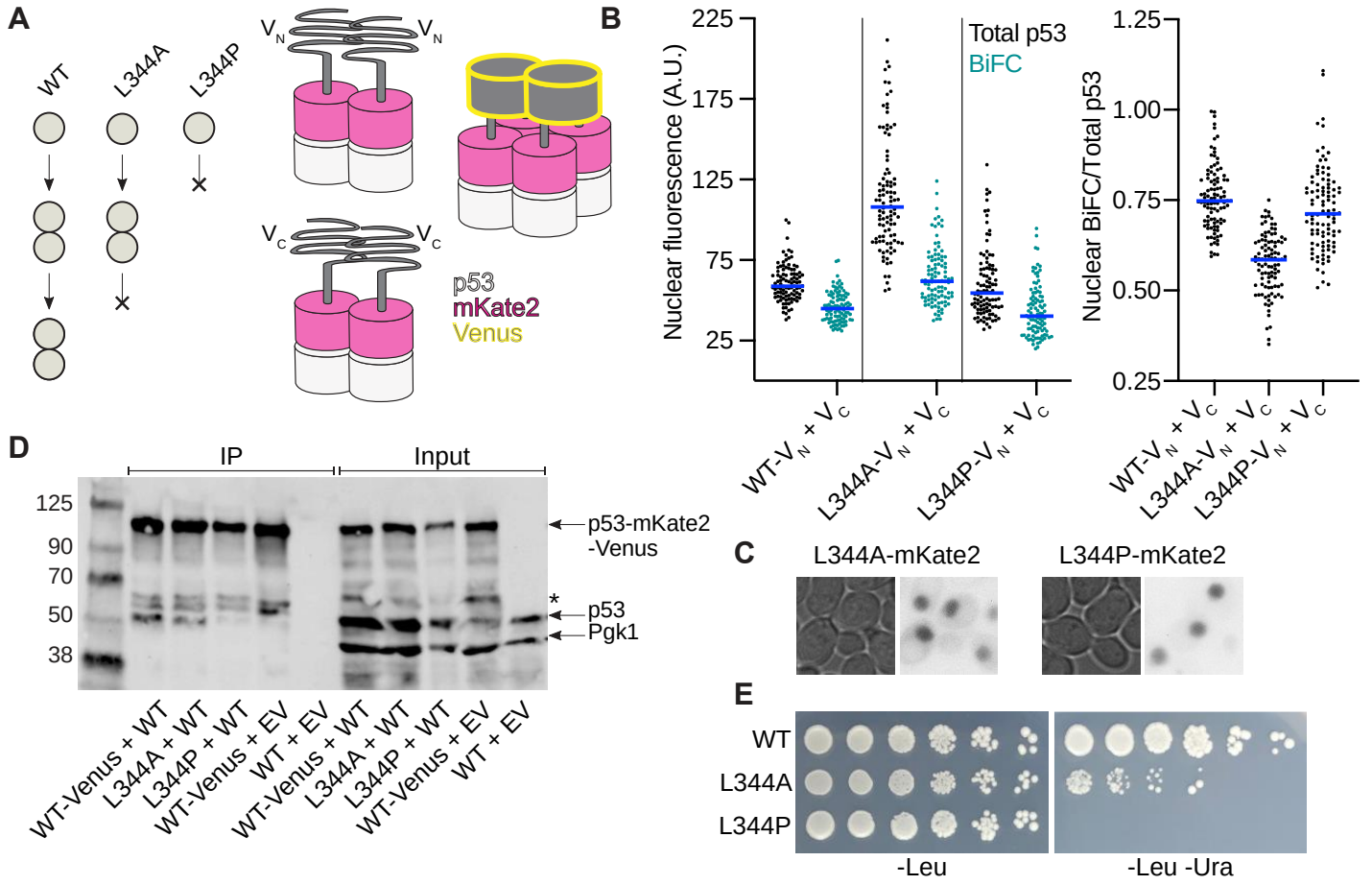

Figure S3. Mutations to Leu344 do not specifically perturb p53 oligomerization in the context of expression in yeast

(A) Schematic of oligomerization-disrupting mutants and expected p53 BiFC results. Left, L344 mutations destabilize higher-order p53 oligomerization. Alanine substitution yields dimers but not tetramers and proline substitution yields monomers only. Right, co-expression with co-translational dimerization of p53(L344A)-mKate2-V_N and p53(L344A)-mKate2-V_C results in V_N + V_N and V_C + V_C homodimers, neither of which reconstitute Venus. Venus fluorescence is only observed upon tetramer formation. Adapted from (Gaglia and Lahav 2014). (B) Total p53 (mKate2) and BiFC (Venus) nuclear fluorescence (left plot) as well as BiFC (Venus) nuclear signal normalized to total p53 (mKate2) nuclear signal (right plot) of Y0134 cells carrying plasmids encoding the indicated p53 BiFC alleles. Cells were grown to mid-log phase at 37˚ prior to imaging. Blue lines denote median values. Vertical lines separate genotypes. Plasmids were pMAM98, “WT-VN”; pMAM82, “WT-V_C”; pMAM99, “L344A-V_N”; pMAM91, “L344A-V_C”; pMAM100, “L344P-V_N”; pMAM93, “L344P-V_C”. (C) Representative micrographs showing nuclear localization of p53(L344A) and p53(L344P) (mKate2 fluorescence, inverted to facilitate viewing) in BiFC experiments. Cells were grown to mid-log phase at 37˚ prior to imaging. Plasmids were pMAM99, “L344A-mKate2-V_N”; pMAM91, “L344A-mKate2-V_C”; pMAM100, “L344P-mKate2-V_N”; pMAM93, “L344P-mKate2-V_C”. (D) JTY4202 cells carrying plasmids encoding the indicated alleles of p53 were grown to mid-log phase at 30˚ and lysed. Venus-tagged proteins were immunoprecipitated with Venus-binding nanobodies and separated by SDS–PAGE. After electrophoretic transfer, the membrane was probed for p53 and the cytosolic protein Pgk1. Asterisk, proteolytic degradation products derived from p53-mKate2-Venus. pRS415 and pRS314, “EV”; pMAM78, “WT”; pMAM85, “WT-Venus”; pMAM105, “L344A-Venus”; pMAM106, “L344P-Venus”. EV, empty vector. (E) Colony growth at 30˚ by RBy33 cells with the *URA3* reporter of p53 activity carrying plasmids encoding the indicated alleles of p53 on medium selective for the plasmids (“-Leu”) or selective for the plasmid and p53 reporter activity (“-Leu -Ura”). Plasmids were pLS76, “WT”; pMAM114, “L344A”; pMAM115, “L344P”.

Literature cited

Gaglia G., and G. Lahav, 2014 Constant rate of p53 tetramerization in response to DNA damage controls the p53 response. Molecular Systems Biology 10: 753. https://doi.org/10.15252/msb.20145168