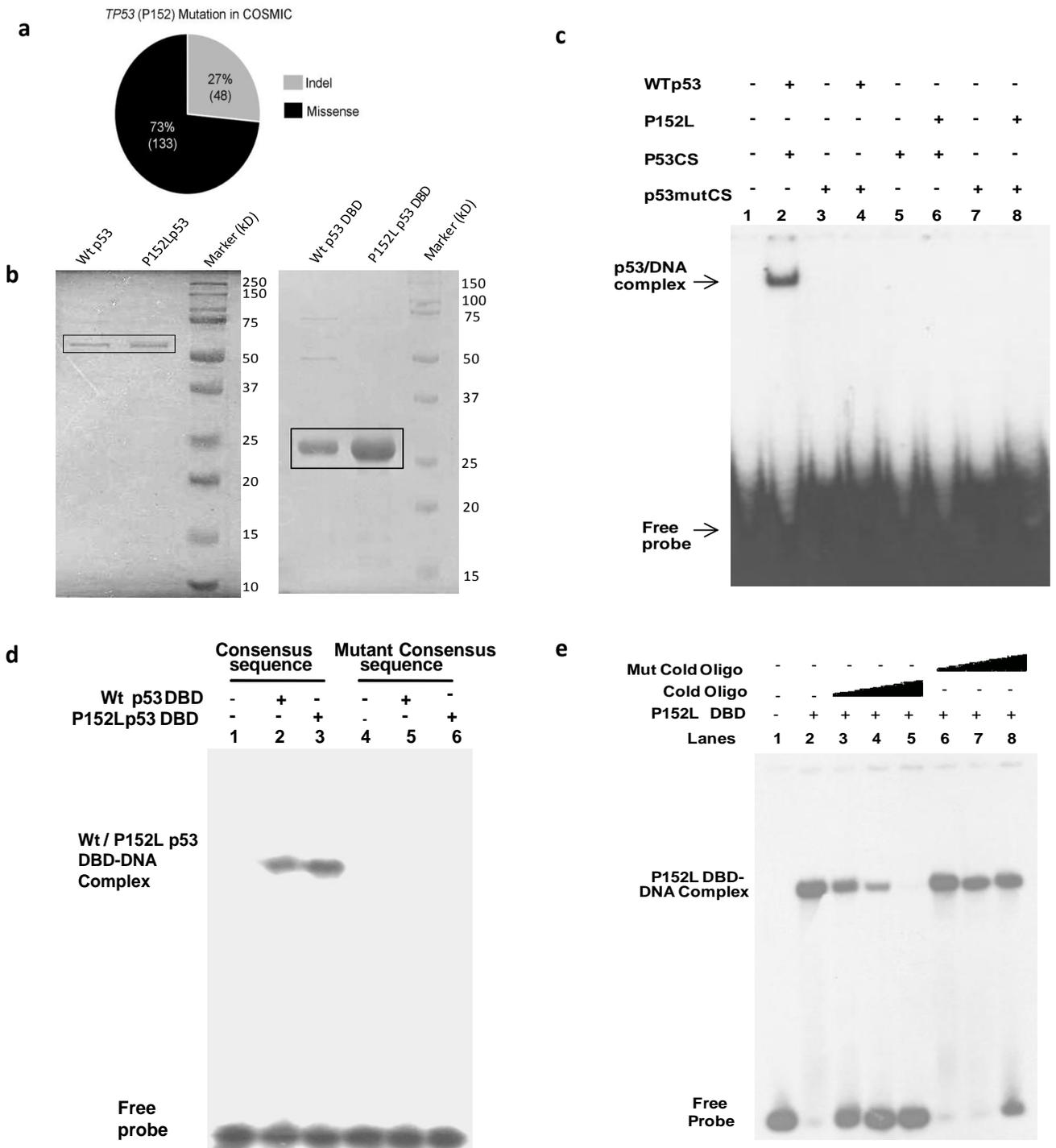
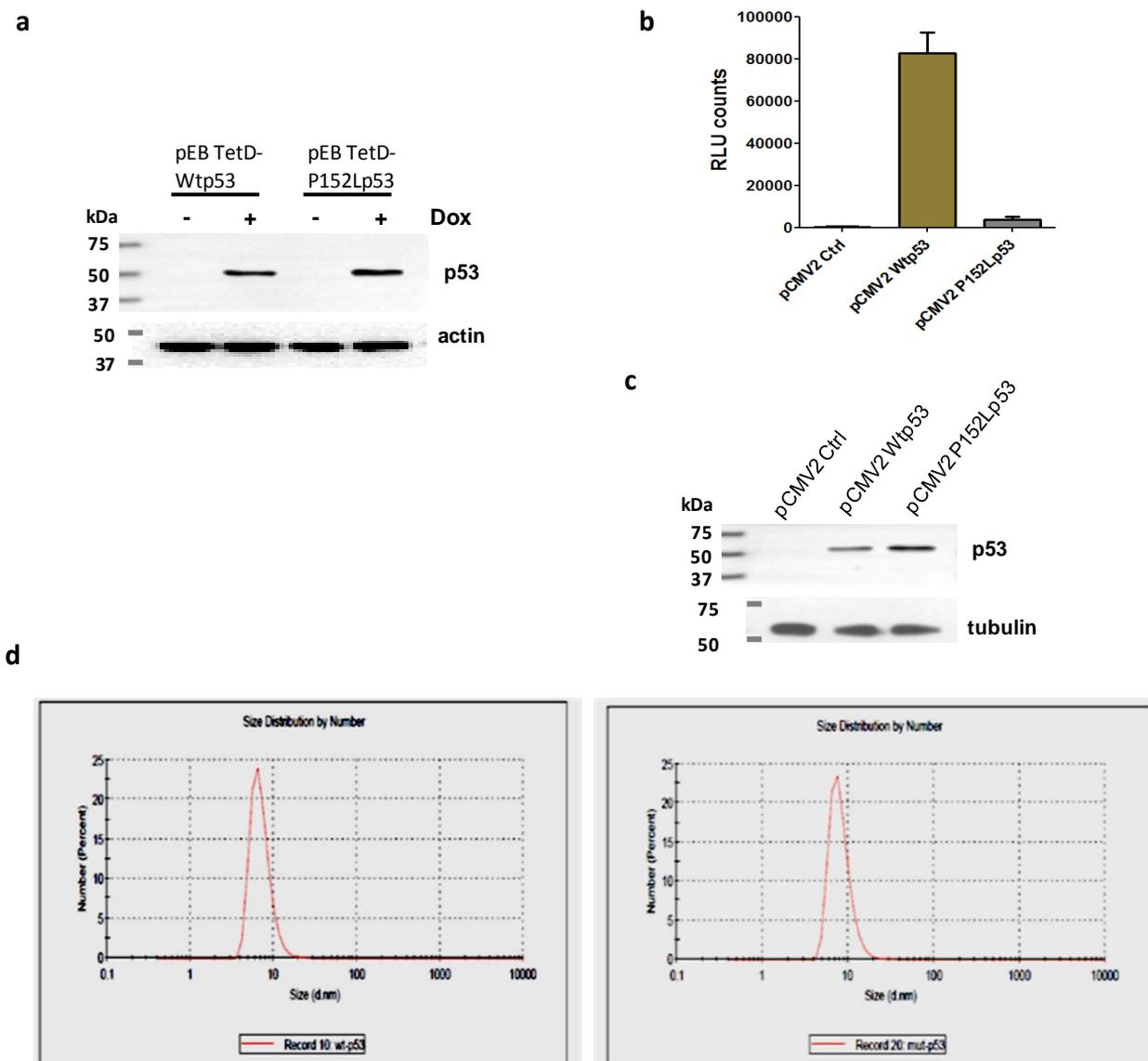


**The cancer-associated, gain-of-function TP53 variant
P152Lp53 activates multiple signaling pathways implicated
in tumorigenesis**

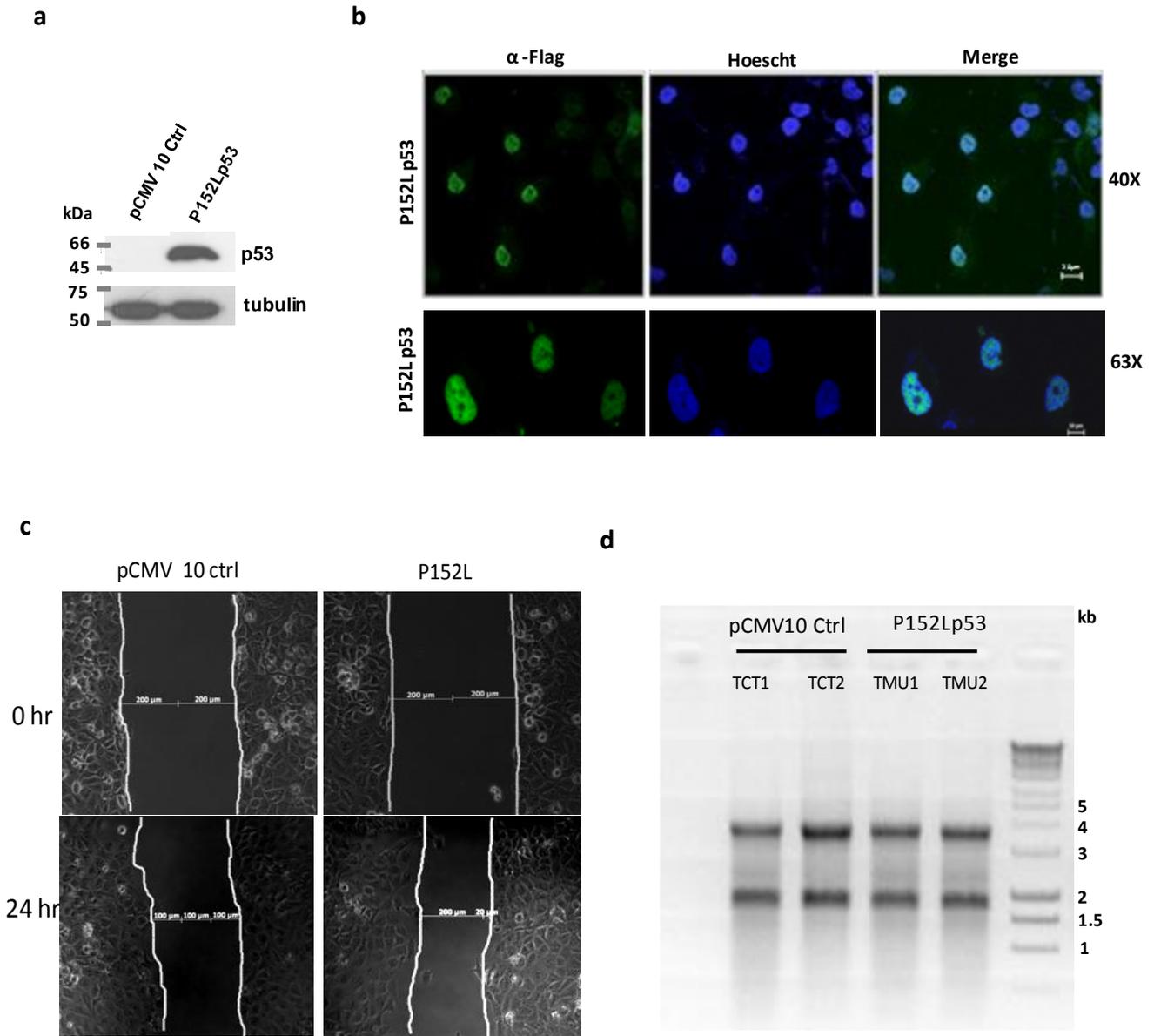
Siddharth Singh, Manoj Kumar, Sanjeev Kumar, Shrinka Sen, Pawan Upadhyay,
Sayan Bhattacharjee, Naveen M, Vivek Singh Tomar, Siddhartha Roy, Amit Dutt
and Tapas K. Kundu



Supplementary Figure S1 : (a) The pie chart representation of number and percentage frequency of mutation type occurring in COSMIC db at amino acid residue P152 in *TP53* (b) Purification profile of flag tagged full-length and untagged DNA binding domain of wild type p53 and P152Lp53 purified through M2 agarose affinity purification and SP Sepharose cation exchange affinity chromatography respectively, (c) Electrophoretic mobility shift assay showing the abrogation of DNA binding property of recombinant full-length P152Lp53 on *GADD45A* promoter consensus sequence, (d) EMSA showing the DNA binding property of DNA binding domain (DBD) of wild type and P152Lp53 with *GADD45A* consensus and mutant consensus sequence, (e) EMSA showing cold competition assay of DNA binding domain (DBD) of P152Lp53 with both *GADD45A* consensus and mutant consensus sequence



Supplementary Figure S2 : (a) Immunoblot confirmation of the expression of wtp53 and P152Lp53 upon doxycycline treatment in H1299 Dox inducible stable cell lines, (b) Luciferase assay by transient co-transfection of wtp53, P152Lp53 and PG13luc in H1299 cells (n=3). Values are mean \pm S.D. (c) Immunoblot confirmation of protein expression after transient cotransfection of plasmids in luciferase assay, (d) Measurement of Hydrodynamic radii of wild-type (left) and P152L (Right) p53. The wild-type p53 has a hydrodynamic radius of 35.5 Å and the mutant p53 has a radius of 40.9 Å.



Supplementary Figure S3 : (a) Western blot confirmation of P152Lp53 in H1299 stable cell line and vector control, (b) Confirmation of P152Lp53 stable expression in H1299 cells and its predominant nuclear localization (c) Representative images of the wound healing assay showing the migration of H1299 cells stably expressing P152Lp53 compared to vector (pCMV10) control, (d) Agarose gel image of the total RNA isolated from control and mutant tumor for RNA sequencing.