Explaining the biological activity of transactivation-deficient p53 variants

To the Editor:

Johnson *et al.*[1] recently reported a conditional mouse *Trp53* allele with mutations changing codons 25 and 26 to glutamine and serine, respectively (referred to below as *Trp53*^{QS}). These mutations largely disable p53 transactivation function. Surprisingly, heterozygosity for *Trp53*^{QS} caused embryonic lethality.

We had previously generated mice with the same mutations[2] but subsequently found that the genomic clone used to make the targeting construct contained an alanine to valine substitution at codon 135 (ref. 3). Although Val135 elicits temperature sensitivity[4,5], we found that p53$^{QS\text{-}Val135}$ did not gain function at the permissive temperature and that a substantial fraction of p53$^{QS\text{-}Val135}$ had a wild-type conformation *in vivo*[3]. With one exception (see below), we did not observe any difference between p53^{QS} and p53$^{QS\text{-}Val135}$ in damage-induced gene regulation, apoptosis and oncogene-mediated activation[3]. *Trp53*$^{+/-}$ and *Trp53*$^{QS\text{-}Val135/+}$ mice also showed identical tumor latency and spectra (**Supplementary Note** online and ref. 3). Together, these data suggest that the glutamine and serine substitutions reduce the activity of p53 to such an extent that we were unable to measure additional effects caused by the Val135 mutation.

The *Trp53*^{QS} allele analyzed by Johnson *et al.* showed somewhat different properties[1]. RNA blot analysis indicated that p53^{QS} activated transcription of *Bax*[1], a target we did not analyze[3]. Consistent with our observations, Johnson *et al.* found that p53^{QS} mouse embryonic fibroblasts (MEFs) expressing E1A did not induce apoptosis after DNA damage, although a fraction of MEFs expressing p53^{QS} underwent apoptosis in response to serum starvation and hypoxia[1], conditions not tested previously[2,3].

We obtained conditional *Trp53*$^{QS/QS}$ cells (referred to as *Trp53*$^{Lsl\text{-}QS}$) from Johnson *et al.* to compare the transcriptional activity of p53$^{Lsl\text{-}QS}$ with those of our homozygous p53$^{QS\text{-}Val135}$ and virally transduced p53^{QS} MEFs. For most target genes, including *p21* (**Fig. 1a**), *Mdm2* and *Pmaip1* (also known as *Noxa*; data not shown), quantitative RT-PCR analysis demonstrated marked and equivalent impairments in the activity of all *Trp53*^{QS} alleles relative to wild-type *Trp53*. p53^{QS} did induce transcription of *Bax*, which was diminished in *Trp53*$^{QS\text{-}Val135}$ cells (**Fig. 1a**). However, *Bax* is induced very poorly by p53 in MEFs, with fully induced levels in *Trp53*$^{+/+}$ cells only two- to threefold higher than in *Trp53*-null MEFs, and the relevance of *Bax* expression for p53^{QS}-induced apoptosis remains unclear, at least in MEFs under the conditions tested.

We also observed that p53^{QS} protein levels in the MEFs generated by Johnson *et al.* were at least

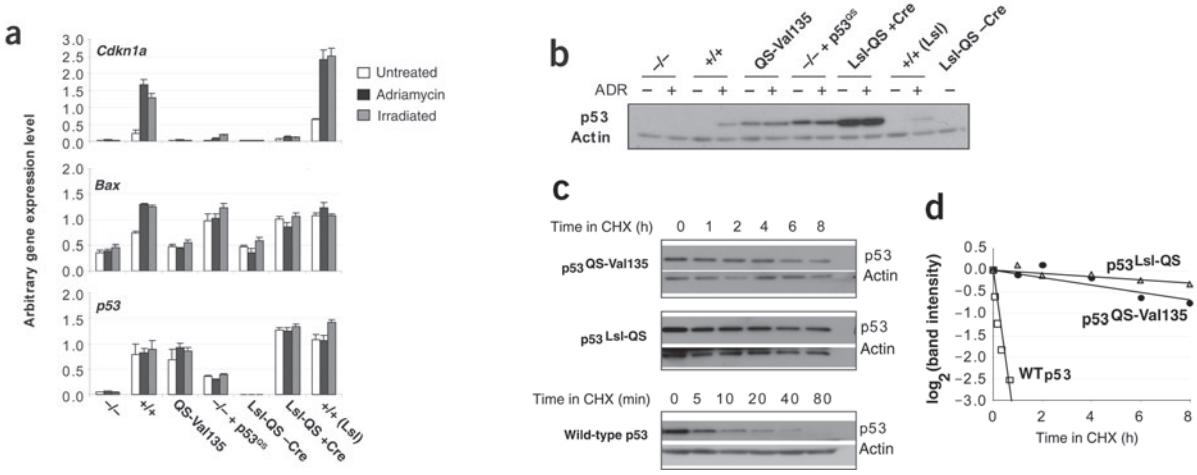

Figure 1 *Trp53*^{QS} knock-in MEFs express substantially more p53 protein than *Trp53*$^{QS\text{-}Val135}$ knock-in MEFs but show similar regulation of p53 target genes. (**a**) Target gene activation in MEFs expressing different *Trp53* alleles. Cells were either untreated, treated with adriamycin (0.2 g ml^{-1}, 8 h) or γ irradiated (6 Gy, 2 h). RNA levels of *Trp53*, *Cdkn1a* (also known as *p21*) and *Bax* were measured by quantitative RT-PCR and normalized to that of *Arp*. *Trp53*$^{Lsl\text{-}QS/Lsl\text{-}QS}$ cells (Lsl-QS) were infected with Cre-expressing adenoviruses (+Cre) or were untreated (–Cre). +/+ (Lsl) cells are littermates of *Trp53*$^{Lsl\text{-}QS/Lsl\text{-}QS}$ mice that express wild-type p53 and do not contain the Lsl cassette. Also shown are Trp53$^{-/-}$ MEFs lentivirally transduced as previously described[3] (–/– + p53^{QS}). (**b**) Protein blot analysis comparing p53 protein levels in MEFs expressing different *Trp53* alleles. Cells either were untreated or were treated with 0.2 g ml^{-1} adriamycin for 8 h. Proteins were then extracted, fractionated by SDS-PAGE, protein blotted and analyzed with antibodies to the indicated proteins. (**c**) Half-life of p53$^{QS\text{-}Val135}$ and p53^{QS}. Cells were treated with 100 g ml^{-1} cycloheximide (CHX) for indicated times, protein extracts were prepared and resolved by SDS-PAGE and blots were probed for p53 and actin. (**d**) Quantification of p53 half-life in **c**. The intensity of the p53 band at each time point is normalized to that of actin and then expressed relative to time 0.

four times higher than in MEFs expressing $p53^{QS\text{-}Val135}$ (**Fig. 1b**). This difference is not attributable to differences in half-life ($T_{1/2}$ of all $Trp53^{QS}$ proteins was >8 h, whereas $T_{1/2}$ of wild-type p53 was <20 min; **Fig. 1c,d**). $Trp53^{Lsl\text{-}QS}$ and wild-type littermate cells do express more *Trp53* mRNA (~1.5-fold more), possibly reflecting strain differences (**Fig. 1a**). $p53^{QS}$ may be translated more efficiently than $p53^{QS\text{-}Val135}$, or other factors may contribute to its greater abundance.

We have previously reported that germline heterozygosity or homozygosity for $Trp53^{QS\text{-}Val135}$ allows normal survival but causes substantial tumor predisposition equivalent to *Trp53* heterozygous and homozygous null mice[2]. By contrast, heterozygosity for $Trp53^{QS}$ causes early embryonic lethality[1]. What can explain the life-or-death differences caused by the presence of valine or alanine at codon 135, which is at the dimer interface of the p53 DNA-binding domain[6,7]?

Johnson *et al.* propose that the early lethality caused by $Trp53^{QS}$ results from hypoxia-induced activation during early embryogenesis. Although $p53^{QS}$ does not show higher apoptotic activity than wild-type p53 under hypoxic conditions[1], and p53 normally shows low apoptotic activity during development[8], it is conceivable that the abnormally high levels of this Mdm2-resistant p53 mutant could induce apoptosis. However, Johnson *et al.* did not examine $Trp53^{QS/-}$ embryos, and thus it remains possible that embryonic lethality requires the wild-type *Trp53* allele. Because we compared $p53^{QS\text{-}Val135}$ and $p53^{QS}$ in homozygous cells, we have no data on potential stress-activated effects of $p53^{QS}$ on wild-type p53 in heterozygous cells.

We also reported that $p53^{QS\text{-}Val135}$ binds normally to the chromatin of some p53 target genes but binds weakly to others[3]. By contrast, $p53^{QS}$ binds very strongly in the presence and absence of stress to all p53 target genes analyzed[3]. These observations suggest two additional models to explain the lethality of the $Trp53^{QS}$ allele. First, in $Trp53^{QS/+}$ embryos, $p53^{QS}$ might augment or alter the function of the wild-type protein during early embryonic development or in specific tissues. A dominant effect could arise from increased activity or stability of chromatin-bound wild-type and $p53^{QS}$ heterotetramers, as $p53^{QS}$ does not bind Mdm2 or MdmX. Second, transcriptionally inactive $p53^{QS}$ homotetramers may form and bind to chromatin but may not be cleared from the promoters of the hundreds of estimated p53 target genes[9] and intragenic and intergenic loci containing p53 binding sites[10]. Thus, the early lethality in $Trp53^{QS/+}$ embryos may be attributable not to a stress-activated function of this mutant allele but rather to the constitutive presence of an inactive transcription factor at numerous target loci. A contributing factor could be that the $p53^{QS}$ protein was substantially more abundant in the strain used by Johnson *et al.* than in the strain used in our studies.

Finally, we note that Johnson *et al.* did not analyze the tumor suppressor capacity of $p53^{QS}$. Thus, we do not know whether the partial function of $p53^{QS}$ *in vivo* is restricted to the conditions that occur during embryonic development, or if these alterations to the transactivation domain also influence p53 tumor suppression.

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Note: Supplementary information is available on the Nature Genetics website.

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In reply:

Our laboratory recently generated conditional *Trp53* knock-in mice in which codons 25 and 26 were mutated to glutamine and serine (QS) to address the role of transactivation in p53 function[1]. Our $Trp53^{QS}$ mice have a different phenotype from those described by Wahl and colleagues[2], which also carry a mutation in the DNA binding domain at codon 135 ($Trp53^{QS\text{-}Val135}$)[3]. Our analysis of $Trp53^{QS}$ mice demonstrates that this mutant retains select p53 activities. In contrast, Wahl and colleagues show that the $Trp53^{QS\text{-}Val135}$ allele behaves indistinguishably from a *Trp53*-null allele.

Analyses of $p53^{QS}$ by our laboratory and others have unequivocally indicated that $p53^{QS}$ retains partial transactivation function, as well as activity in apoptosis and growth suppression[1,4–8]. In cells derived from our knock-in mice, we showed that although $p53^{QS}$ is compromised for transactivation of many target genes, it retains near–wild-type activity on certain targets such as *Bax* (ref. 1) and *Apaf-1* (data not shown). Furthermore, we showed that $p53^{QS}$ induces substantial apoptosis in response to select stresses, including serum starvation and hypoxia. Consistent with our findings, Tang *et al.* observed *Bax* transactivation using our $Trp53^{QS}$ MEFs, but not with their $Trp53^{QS\text{-}Val135}$ MEFs. They did not, however, investigate the apoptotic response to serum deprivation or hypoxia, conditions in which we observed $p53^{QS}$ apoptotic activity.

A vast body of literature has shown that the A135V mutation severely compromises p53 function. The seminal studies establishing p53 as a tumor suppressor showed that while co-introduction of p53 with Ras and E1A into rat embryonic fibroblasts suppressed transformation, expression of $p53^{Val135}$ not only failed to suppress transformation but actually enhanced transformation, indicating dominant-negative activity toward endogenous p53 (refs. 9,10). Further evidence for the inactivity of $p53^{Val135}$ has accumulated in numerous subsequent studies. For example, *Trp53*-null mice expressing multiple copies of a $Trp53^{Val135}$ transgene develop tumors at the same rate as *Trp53*-null mice lacking the transgene, indicating that $p53^{Val135}$ is unable to suppress tumorigenesis[11]. Moreover, the experiments by Tang *et al.* showing that $p53^{QS}$-expressing MEFs display *Bax* transactivation, whereas those expressing $p53^{QS\text{-}Val135}$ do not, further demonstrate that $p53^{QS\text{-}Val135}$ is inactive. Given that the A135V mutation severely compromises p53 function, it is impossible to conclude that transactivation is essential for p53 function from analysis of $Trp53^{QS\text{-}Val135}$ mice.

An additional consequence of the QS mutations is that $p53^{QS}$ no longer binds the Mdm2 ubiquitin ligase and therefore shows enhanced stability relative to wild-type p53 (ref. 12). Because unrestrained p53 in the context of Mdm2 deficiency induces early embryonic lethality[13], we hypothesized that, should $p53^{QS}$ retain biological activity, its expression might similarly trigger lethality; hence, we created our mice using a conditional strategy. Indeed, expression of $p53^{QS}$ causes embryonic lethality[1], whereas homozygous $Trp53^{QS\text{-}Val135}$ mice are viable[2]. The most straightforward interpretation of