community SARS to outpatient controls and HCW SARS to HCW controls, respectively).

Thus, we did not find any significant differences in allele, genotype and homozygote or heterozygote frequencies between cases and controls in our three independent populations of northern Chinese. Although the biological plausibility of L-SIGN and the functional evidence of the VNTR polymorphism in the original report remain interesting, we urge that the association between *CLEC4M* polymorphism and SARS be investigated in other subpopulations of ethnic Chinese origin (for example, Taiwanese or Guangdong Chinese) or in those of different ancestry, such as Europeans.

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

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COMPETING INTERESTS STATEMENT
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Chan *et al.* reply:

Tang *et al.* and Zhi *et al.* report that in contrast to our findings[1], they were unable to find association between homozygosity or heterozygosity of the *CLEC4M* (L-SIGN) exon 4 tandem repeat polymorphism and SARS CoV susceptibility in the Chinese population. Their data cannot conclusively negate our findings for the reasons below.

We agree with Tang *et al.* that the difference between their analysis and ours is largely accounted for by the difference in the percentage of homozygosity in controls. Tang's controls included neonatal cord blood, healthy elderly individuals aged >70 years, local university students and control samples collected in Beijing. The homozygosity and heterozygosity distribution of their Beijing controls is very similar to that of our Hong Kong random controls. However, the former three control groups collected in Hong Kong are poorly matched by age for comparison with individuals with SARS and our random controls (**Supplementary Table 1** online), and such an age distribution clearly excludes the population aged 25–69 years, which makes up the largest proportion (>70%) of SARS-infected individuals during the Hong Kong outbreak in 2003 (ref. 2). Age and gender are well-known confounding factors in any case-control study, and for SARS, individuals aged ≥65 years and <18 years are actually associated with a lower risk[3]. Thus, it is surprising that Tang *et al.* chose these specific control groups at extreme age ranges, given that there are no obvious obstacles for recruiting appropriate age-matched controls in Hong Kong. They also failed to perform logistic regression accounting for age differences, which should have been incorporated in their statistical analysis. The age and gender distribution of the SARS and control populations in our study, in contrast, is more properly matched, and by logistic regression, our results remain statistically significant (**Table 1** and **Supplementary Table 1**). Our results remain consistent after accounting for comorbid conditions (**Table 1**).

Tang *et al.* also used control groups differing widely in age to determine if age had any effect on genotype frequencies. However, their data *per se* already showed a significant difference in overall genotype distribution, when comparing neonates versus elderly individuals versus university students ($P = 0.029$, χ^2 test). The difference in genotype between neonates and university students is also significant ($P = 0.009$ by CLUMP), suggesting that age-related selection may exist for *CLEC4M* genotypes. Indeed, age-dependent variation of allele and genotype frequencies has been reported for other genes[4,5].

Tang *et al.* also claim to show a subpopulation difference in allele and genotype frequencies between northern and southern Chinese. They note that "the seven-repeat allele was more prevalent in the Beijing sample (0.7 in Beijing versus 0.64 in Hong Kong, $P = 0.05$), which also largely accounted for the higher proportion of homozygotes (55.7% in Beijing versus 46.0% in Hong Kong, $P = 0.02$)." It seems these quoted Hong Kong figures refer to frequencies from their cord blood group alone. Such a comparison is difficult to justify, again because age is seriously mismatched. It should be noted that there is no difference in either the seven-repeat allele frequency or the proportion of homozygotes between the Hong Kong random controls of Chan *et al.*[1] and the Beijing controls of Tang *et al.* (**Supplementary Table 2** online). Barreiro *et al.*[6,7] investigated the *CLEC4M* homozygote and heterozygote distribution of different ethnic groups and reported that the proportion of homozygotes in East Asians, consisting predominantly of Chinese, was 53% (**Supplementary Table 3** online), a figure similar to the Hong Kong random controls of Chan *et al.* and the Beijing controls of Tang *et al.* There is also no significant difference in allele or genotype frequencies of these two control populations from the East Asian samples of Barreiro *et al.*[7] (**Supplementary Table 2**). When these results are taken together, little subpopulation structure is observed for *CLEC4M* homozygote and heterozygote distribution between the northern and southern Chinese populations.

Finally, given the apparent absence of subpopulation structure for *CLEC4M* homozygote and heterozygote distribution in the Chinese population, we performed a meta-analysis of our data set and that of Tang *et al.* by the Mantel-Haenszel test using all control groups that are in Hardy-Weinberg equilibrium: our random controls and outpatient controls ($n = 670$), and cord blood, healthy elderly individuals and Beijing controls ($n = 827$) of Tang

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Table 1 Statistical analysis of *CLEC4M* homozygosity and heterozygosity adjusted for age, gender and comorbid conditions in Chan *et al.*[1]

	Heterozygotes	(%)	Homozygotes	(%)
Community population (Chan *et al.*[1])				
Affected individuals	*n* = 115	52.8%	*n* = 103	47.2%
Outpatient controls	*n* = 127	43.8%	*n* = 163	56.2%
P value	0.048			
OR (95% c.i.)	0.69 (0.48–1.00)			
Affected individuals without comorbid conditions	*n* = 105	53.0%	*n* = 93	47.0%
Outpatient controls	*n* = 127	43.8%	*n* = 163	56.2%
P value	0.046			
OR (95% c.i.)	0.68 (0.47–0.99)			
Health care worker (HCW) population (Chan *et al.*[1])				
Affected individuals	*n* = 38	56.7%	*n* = 29	43.3%
HCW controls	*n* = 71	41.3%	*n* = 101	58.7%
P value	0.034			
OR (95% c.i.)	0.53 (0.29–0.95)			
Affected individuals without comorbid conditions	*n* = 37	56.9%	*n* = 28	43.1%
HCW controls	*n* = 71	41.3%	*n* = 101	58.7%
P value	0.034			
OR (95% c.i.)	0.53 (0.29–0.95)			

The heterozygotes are used as the reference group. All ORs and *P* values are adjusted for age and gender (by logistic regression). Comorbid conditions do not affect the risk association.

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et al. compared with all SARS cases (n = 462). The combined odds ratio (OR) is significant (combined OR = 0.786, 95% confidence interval (c.i.) = 0.637–0.972, P = 0.026), indicating that a reduced risk is still associated with homozygotes, even by the approach of Tang *et al.* that disregards the age effect.

Tang *et al.* also argued against our functional studies by citing two studies in which L-SIGN oligomerization assays were performed using recombinant protein without the cytoplasmic and transmembrane domains in a cell-free model. This argument is not valid because these findings cannot be extrapolated to our cell-based functional assays, which bear more biological significance for the study of membrane-bound molecules.

As for Zhi *et al.*, their genotyping data are a cause for concern. When the observed and expected numbers are compared, deviation from Hardy-Weinberg equilibrium of their Beijing community SARS group (P = 0.0006) reflects overcalling of homozygotes and undercalling of heterozygotes containing five and seven repeats. Moreover, a significant difference is noted in the homozygote and heterozygote distribution between their Beijing and Tianjin populations: when the overall homozygote and heterozygote genotype of the Beijing cohort (that is, combining both affected individuals and controls) is compared with that of the Tianjin cohort, significantly more homozygotes are found in the Beijing population (P = 0.0031, **Table 2**). Since there is no difference between our Hong Kong random controls and the Beijing controls of Tang *et al.*, differences would hardly be expected between the populations in Beijing and Tianjin, as both cities are in northern China and are only 60–70 miles apart geographically. It is unknown if genotyping of these two cohorts by Zhi *et al.* was conducted independently or all in one laboratory, but such a significantly different homozygote and heterozygote distribution between these two cohorts raises the possibility of genotyping error. The authors state that cross-validation by direct sequencing was performed in some cases, and they specifically mention confirming homozygous 'five repeat/five repeat' and 'seven repeat/seven repeat' genotypes by sequencing. Surprisingly, however, their heterozygous 'five repeat/seven repeat' genotypes were confirmed only by 2% gel electrophoresis. The distinction between homozygosity and heterozygosity should depend on precise recognition of two separate bands in an agarose gel and that the ultimate verification should rely on DNA blot analysis (which had been conducted in our study), rather than confirmation by sequencing of one specific band identified in electrophoresis of PCR products.

In summary, data from Tang *et al.* or Zhi *et al.* cannot conclusively negate our finding that *CLEC4M* homozygosity is associated with a reduced risk for SARS susceptibility. Although there may be population differences in *CLEC4M* genotype at the level of major ethnic groups[6], little evidence

Table 2 *CLEC4M* homozygote and heterozygote distribution of Beijing and Tianjin cohorts (combining affected individuals and controls) in Zhi *et al.*

	Beijing cohorts	(%)	Tianjin cohorts	(%)
Heterozygotes	281	(43.4)	105	(55.6)
Homozygotes	367	(56.6)	84	(44.4)
P value (χ^2 test)		0.0031		

exists for the presence of differences between northern and southern Chinese.

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DNA double-strand breaks are not sufficient to initiate recruitment of TRF2

To the Editor:

The human telomere binding factor TRF2 is essential at telomeres, facilitating the formation and stabilization of t-loops[1] and suppressing local ATM-mediated damage response[2]. Bradshaw *et al.*[3] recently reported that TRF2 accumulates at nuclear sites damaged by high-intensity laser beams, presumptively in response to DNA double-strand breaks (DSBs), and that it arrives before other DNA repair-related proteins, including ATM. To characterize the type of lesion responsible for triggering TRF2 recruitment, we produced a variety of localized nuclear damage and then quantified TRF2 colocalization with appropriate DNA damage markers. Although we found that TRF2 is indeed recruited to sites damaged by a high-intensity multiphoton laser beam, we did not find any evidence for such recruitment after we exposed cells to lower-intensity sources of ultraviolet radiation or to ionizing radiation, indicating that archetypal radiogenic DNA lesions such as DSBs are insufficient to trigger TRF2 recruitment.

In an effort to confirm the original observation that TRF2 is recruited to sites damaged by high-intensity lasers (for example, multiphoton lasers or pulsed laser microbeams)[3], we exposed HeLa cells expressing green fluorescent protein (GFP)-tagged TRF2 to a highly focused beam from an 800-nm pulsed multiphoton laser. With this source, coincident absorption of two photons results in energy deposition equivalent to that produced by a single 400-nm photon (**Supplementary Methods** online). We measured TRF2 recruitment to exposed nuclear regions by live-cell imaging and compared it with the recruitment of Ku80-GFP, a nonhomologous end-joining (NHEJ) protein, or xeroderma pigmentosum C (XPC)-GFP, a critical nucleotide excision repair (NER) protein. When cells were exposed in the presence of the photosensitizing dye Hoechst 33258, we observed, within 10 s, recruitment of both Ku80 and XPC to laser-damaged sites (15% of maximum laser output; **Supplementary Fig. 1** online). Recruitment of TRF2 to these damaged sites occurred within 20 s of exposure and persisted for the 3-min duration of the experiment, but only after a 1.6-fold increase in laser power (to 24% of maximum laser output; **Fig. 1** and **Supplementary Table 1** online). Fluorescence intensity of TRF2 at telomeres was not measurably affected. These results are consistent with those of Bradshaw *et al.*[3] in that we confirmed TRF2 recruitment to damaged nuclear regions within seconds of exposure.

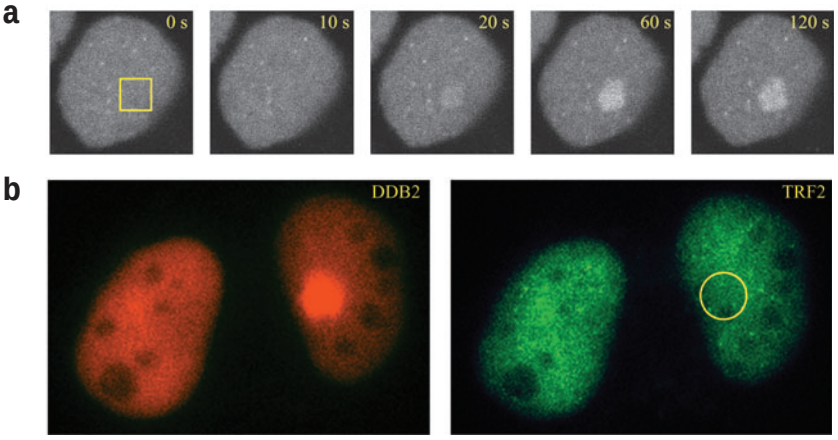

Figure 1 TRF2 response to photoinduced DNA damage. (**a**) Live-cell confocal images of a nucleus after high-intensity multiphoton laser exposure in the presence of Hoechst 33258 demonstrates recruitment of TRF2 to the exposed region. Representative time points are shown for images captured at 10-s intervals after exposure. (**b**) Cell nuclei do not show any recruitment of TRF2-GFP (green) to damage sites as marked by DDB2-mCherry (red) after exposure to localized ultraviolet C irradiation (1,000 J m^{-2}, t = 5 min after illumination).

Hoechst 33258 promotes the precise photochemical reaction that produces DSBs after ultraviolet A exposure[4]. This reaction originally formed the basis for concluding that γ-H2AX foci were generated specifically in response to ultraviolet A laser–induced DSBs[5]. However, even in the absence of Hoechst, boosting the output of our multiphoton laser beam to 60% of the maximum resulted in Ku80 and XPC being rapidly recruited to damaged nuclear regions. Interestingly, TRF2 recruitment again required a 1.6-fold increase in power output for visualization (to 95% of the maximum; **Supplementary Fig. 2** online). These results highlight the fact that laser energy output and the presence of photosensitizers can greatly influence results. In contrast to multiphoton treatment, we did not observe TRF2 recruitment after exposure to a more conventional, less intense 405-nm laser beam in the presence of Hoechst, whereas both NER and NHEJ proteins were rapidly and abundantly recruited.

Although ionizing radiation produces a multitude of DNA lesions, it is perhaps best known for its ability to produce DSBs[6]. α-particles deposit their energy along defined tracks that produce dense linear distributions of DSBs that are readily recognizable after detection of γ-H2AX by immunofluorescence[7]. In one series of experiments, an average of one to two α-particles from a ^{241}Am source