Erratum: RAG-1 and ATM coordinate monoallelic recombination and nuclear positioning of immunoglobulin loci

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In the version of this addendum initially published, the first paragraph was incorrect. That paragraph should be deleted and the addendum should begin with the sentence "In our published article, we found that RAG-dependent γ-H2AX foci showed monoallelic colocalization with *Igh* and *Igk* alleles in developing B lymphocytes." The error has been corrected in the HTML and PDF versions of the article.

Addendum: RAG-1 and ATM coordinate monoallelic recombination and nuclear positioning of immunoglobulin loci

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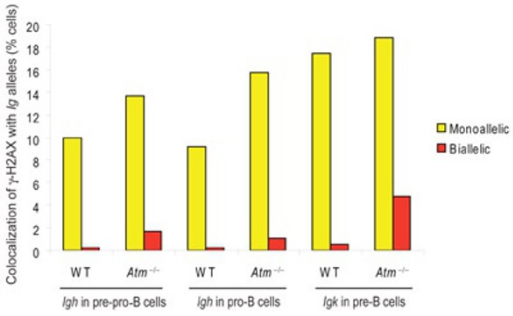
In our published article, we found that RAG-dependent γ-H2AX foci showed monoallelic colocalization with *Igh* and *Igk* alleles in developing B lymphocytes. We sought to determine whether this was due to a stochastic cleavage mechanism or a regulated cleavage process. In **Figure 6a–c** and **Supplementary Tables 9** and **10** initially published, we made a calculation we thought would yield the predicted number of cells in which we would expect to find biallelic colocalization of γ-H2AX foci if recombinase targeting were stochastic and compared that with the actual number obtained. The percentage of cells with biallelic colocalization of γ-H2AX foci in wild-type cells undergoing recombination was significantly lower than the predicted frequency. We believed this calculation supported the idea that monoallelic V(D)J recombination occurs as a result of the restriction of RAG-mediated cleavage to one allele and is not simply a result of stochastic recombination.

It was called to our attention, however, that our calculation of the predicted frequencies of biallelic colocalization of γ-H2AX foci was incorrect and should have been done on an allele basis, not on a per-cell basis. After consulting with biostatisticians and after further reflection, we realized that it was not possible to accurately calculate a predicted frequency on either basis, because such a calculation of predicted frequencies rests on the assumption that pre-pro-B cells, pro-B cells and pre-B cells are homogeneous populations in which all alleles are equally available for cleavage. This assumption is clearly not true for the following reasons. Some cells have two alleles available for rearrangement, whereas others have already undergone a nonfunctional rearrangement on one allele and therefore only have one allele left to rearrange. Also, the calculation does not take account of cells that have functionally rearranged one allele, as these cells move to the next developmental stage. Consequently, the data presented in **Figure 6a–c** and in **Supplementary Tables 9** and **10** as initially published do not allow us to make any statements regarding whether monoallelic recombination in wild-type cells is due to a stochastic process or a regulated process. Thus, the discussion of **Figure 6** on page 659 is now obsolete. However, we feel that the remainder of the text is still supported.

We feel it is important to correct the article and to alert the community to the caveats raised above regarding attempts to make this sort of calculation. We therefore reexamined the data through a more straightforward lens, the χ^2 test, and present here the results. We assessed the number of wild-type and *Atm*$^{-/-}$ cells at various stages of development showing monoallelic or biallelic association of γ-H2AX with immunoglobulin loci (**Addendum Fig. 1**). These data represent the percentage of cells with monoallelic or biallelic association of γ-H2AX with *Igh* or *Igk*; the actual number of cells analyzed (*n*) and statistical *P* values comparing wild-type and *Atm*$^{-/-}$ cells are in **Addendum Tables 1** and **2**. The numbers of cells analyzed differ from those originally published, as we have added new experiments to make this statistical calculation more robust. The frequency with which γ-H2AX is associated with immunoglobulin alleles varies between experiments because the vagaries of both FISH and mice. Because of this, we always assessed wild-type controls alongside the mutants, and the results consistently showed differences between wild-type and *Atm*$^{-/-}$ mice. ATM deficiency caused a statistically significant increase in biallelic cleavage, as shown by biallelic γ-H2AX foci in both pre-pro-B cells and pre-B cells, with the greatest difference occurring in pre-B cells. Our analysis of monoallelic and biallelic cleavage in pro-B cells in the absence of ATM is complicated because D_H-J_H recombination has already occurred, creating many breaks in chromosomes that are not repaired rapidly in the absence of ATM, which decreases the number of alleles available for possible V_H-DJ_H rearrangements in the next developmental stage. Pro-B cells thus contain many broken and missing alleles, and as we assigned scores only to the association of γ-H2AX with immunoglobulin alleles in cells with two alleles, the numbers we could analyze were lower. We believe this leads to a misleadingly small set of pro-B cells with biallelic cleavage from ATM-deficient mice. These results, together with the molecular data presented in **Figure 7** of the original article, provide support for the conclusion that RAG cleavage activates ATM-mediated signals that inhibit further V(D)J recombination to suppress biallelic recombination and diminish the risk of chromosome translocations.



Addendum Figure 1 Wild-type and *Atm*$^{-/-}$ cells at various stages of development with monoallelic or biallelic association of γ-H2AX with *Ig* loci. Results are presented as the percentage of pre-pro-B, pro-B and pre-B cells assigned scored as described in **Addendum Tables 1** and **2**. Data are a composite of three independent experiments.

Addendum Table 1. Statistical analysis of γ-H2AX foci co-localization with *Ig* alleles

	Pre-pro-B, *Igh*		Pro-B, *Igh*		Pre-B, *Igk*	
Genotype	WT	*Atm*$^{-/-}$	WT	*Atm*$^{-/-}$	WT	*Atm*$^{-/-}$
Total number of cells	894	841	885	729	906	1024
Cells with γ-H2AX on one allele	89	115	81	115	158	193
Cells with γ-H2AX on two alleles	2	14	2	8	5	49
χ^2 statistical test						
P value mono and biallelic	0.00078251		0.0000459		0.00000014	
P value monoallelic	0.0162372		0.00005043		0.4233489	
P value biallelic	0.00389099		0.05722631		0.00000004	

Number of cells in **Addendum Figure 1**. These numbers analyzed differ from those originally published, as new experiments have been added. The χ^2 test was used to determine whether differences were significant; the Yates correction was used when any value was <10. '*P* value monoallelic and biallelic' compares the total cell populations; '*P* value monoallelic' examines whether the difference in populations was due to the incidence of monoallelic association; and '*P* value biallelic' indicates in the pre-pro-B and pre-B populations, the statistically significant difference was in the biallelic association of γ-H2AX. Data are composite of three independent experiments.

Addendum Table 2 Statistical analysis of data in Addendum Table 1 using Fisher's exact test and 3 x 2 contingency:

Stage/mono or bi	Contingency	*P*-value
pre-pro-B	2 x 3	0.0007
pre-pro-B mono	2 x 2	0.0457
pre-pro-B bi	2 x 2	0.0021
pro-B	2 x 3	0.0001
pro-B mono	2 x 2	0.0005
pro-B bi	2 x 2	0.0527
pre-B	2 x 3	1.1925e-07
pre-B mono	2 x 2	0.7715
pre-B bi	2 x 2	2.6047e-08

Reanalysis of the data in Addendum Figure 1 and **Addendum Table 1** with Fisher's exact test to determine statistical significance. The conclusions of the results are not changed by the use of either statistical test.