Supporting Information

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SI Text

Accumulation of Endogenous Retroelements. From a plasmid containing MoMLV circular DNA, the Moloney primers ("M" in Fig. S2*A*) amplified a 0.5-kb fragment, whereas the three primer pairs for NZB virus ("NZB A" and "NZB B" in Fig. S2*A*) did not. Unless the cells were transduced with a MoMLV-based vector, the Moloney primers did not amplify any DNA from NIH/3T3 cells (see Fig. S2*A*) or from MEF (Fig. S2*B*). In the presence of raltegravir, more MoMLV 2-LTR circles accumulated (see Fig. S2*B*, lanes 3 and 6).

Besides the exogenous MLV, some of the endogenous retroelements also accumulate; this depends on exogenous virus, raltegravir and/or Trex1. In Trex1-sufficient cells (see Fig. S2*B*, lane 1), NZB primers B amplify a weak band. When the cells are transduced with the MoMLV-based vector (but with no genes encoding MoMLV proteins), another band appears, running slightly higher (see Fig. S2*B*, lane 2), which, however, is not derived from MoMLV. Amplification depends, however, on the presence of MoMLV integrase activity (which is contributed by the virus packaging line, but not encoded by the construct); when integrase is inhibited by raltegravir, the band is absent (see Fig. S2*B*, lane 3). We interpret this band as being an amplicon from a nonautonomous endogenous retroelement. In Trex1-deficient cells, there is a stronger band in the nontransduced cells (see Fig. S2*B*, lane 4)—presumably a consequence of retroelement cDNA not being degraded. Again, upon transduction with MoMLV, a strong band appears that is inhibited by raltegravir (see Fig. S2*B*, lane 6). Raltegravir also leads to an accumulation of a smaller size band, independent of MoMLV integrase (see Fig. S2*B*, lane 6); however, we cannot explain why the larger size band of lane 4 disappears. With the more degenerate NZB primers A, the LTRs of the mouse stem-cell virus, which was used for Trex1 transduction into Trex1 KO cells, show up in the reconstituted cells. In the Trex1-deficient cells, however, these bands are absent; instead, larger size bands of accumulating endogenous LTR retroelements appear.

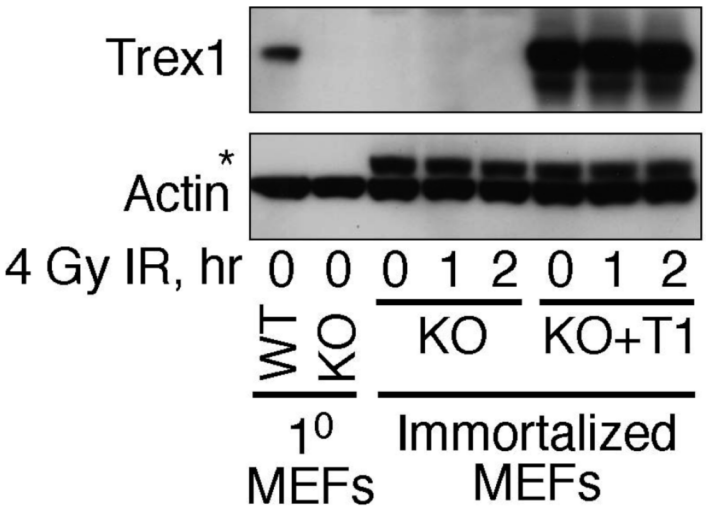

Fig. S1. Western blot analysis of Trex1 expression in MEF wild type, KO, and reconstituted cells. The KO cell line [Yang YG, Lindahl T, Barnes DE (2007) Trex1 exonuclease degrades ssDNA to prevent chronic checkpoint activation and autoimmune disease. *Cell* 131:873–886.] was reconstituted with an untagged murine Trex1 contained in a MSCV-IRES-GFP vector, and sorted for Trex1 expressing cells (>90%; denoted KO+T1). The cells were then probed by Western blot analysis using a monoclonal antibody to Trex1 (BD Biosciences). 1° MEFs, primary mouse fibroblasts; immortalized MEFs, cells spontaneously transformed in cell culture. The asterisk in the actin blot denotes a residual signal from a previous time when the Western blot was probed with a p53 antibody (not relevant here); 4 Gy IR, irradiation for the time periods (hr) indicated (not relevant here).

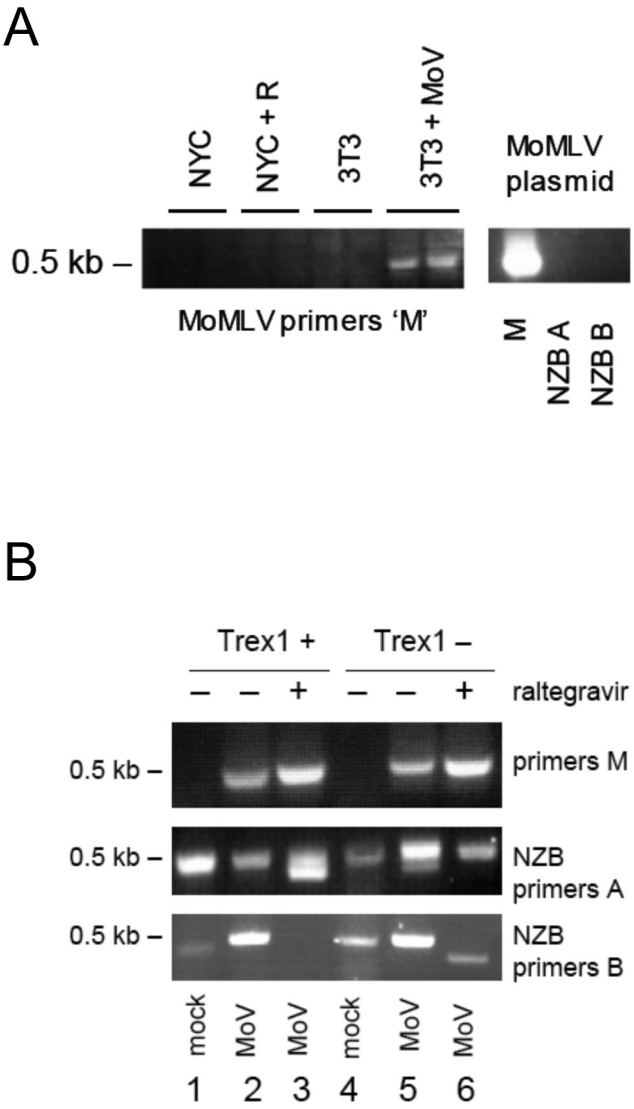

Fig. S2. Raltegravir activity. (*A*) Amplification of circular DNA isolated from cells infected with MoMLV, done in duplicates. 3T3, NIH/3T3 cells; 3T3 + MoV, transduced NIH/3T3 cells; NYC, B/W-derived cells cultured without (NYC) or with (NYC + R) raltegravir; MoMLV, plasmid containing circular MoMLV; M, primers of the homologous sequences of MoMLV. NZB primers A and B cover slightly different areas in the LTR of endogenous NZB virus; these primers do not amplify sequences from MoMLV, and the M primers do not amplify sequences from endogenous NZB virus. (*B*) Accumulation of 2-LTR sequences from MoMLV (MoV; with primers M) or endogenous NZB-like retroelements (NZB primers A and B) in the presence (+) or absence (−) of raltegravir in Trex1-sufficient or -deficient cells.

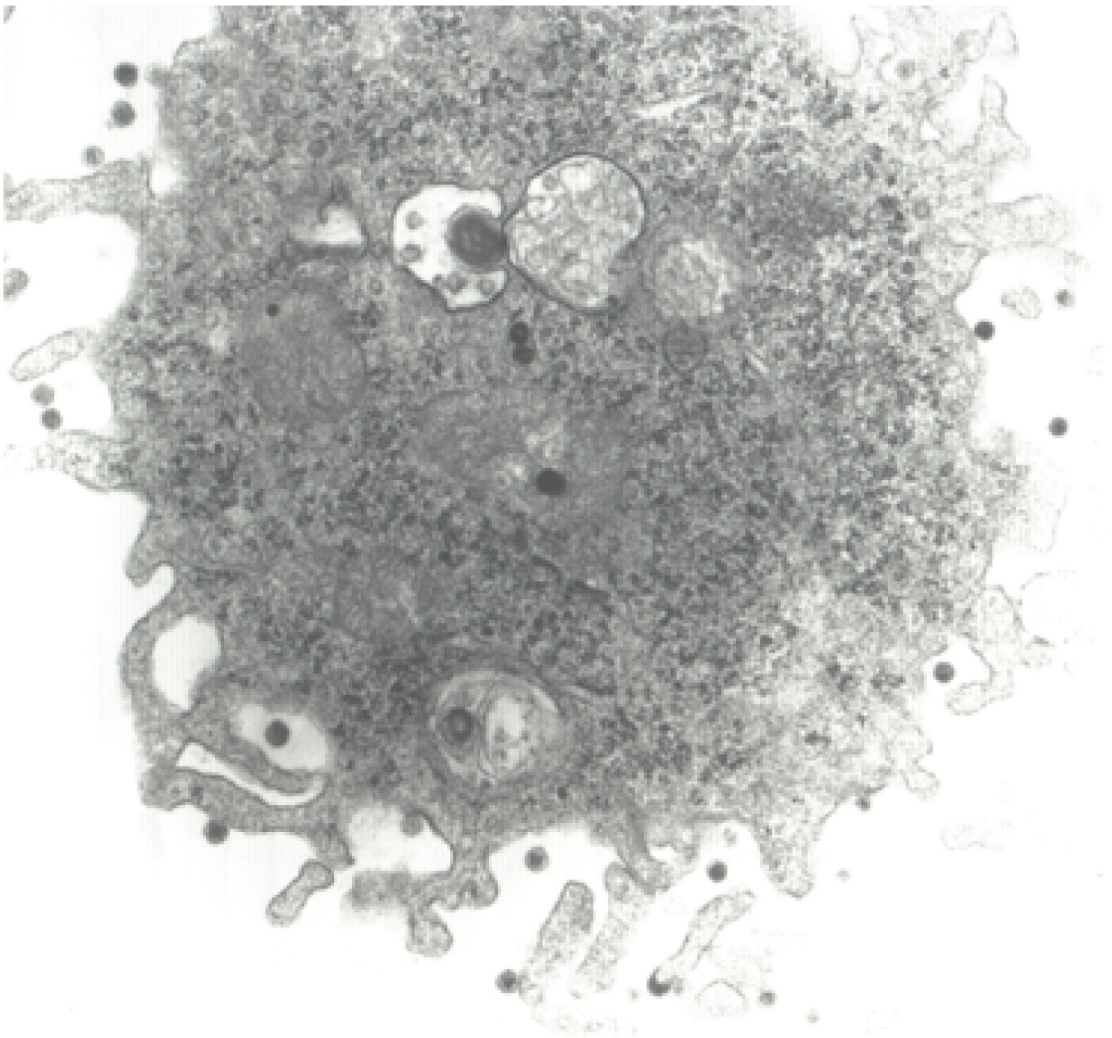

Fig. S3A. Electron micrograph of a cell from B-cell leukemia in a B/W mouse. Numerous budding and released retroviral particles can be seen.

Fig. S3B. Sequences of the 0.5-kb band in raltegravir-treated and untreated NYC cells that contain the joint (X). Numbers in green, individual clones from cells not treated with raltegravir; in blue, treated with raltegravir; nucleotides highlighted in gray, different from the prototype sequence.

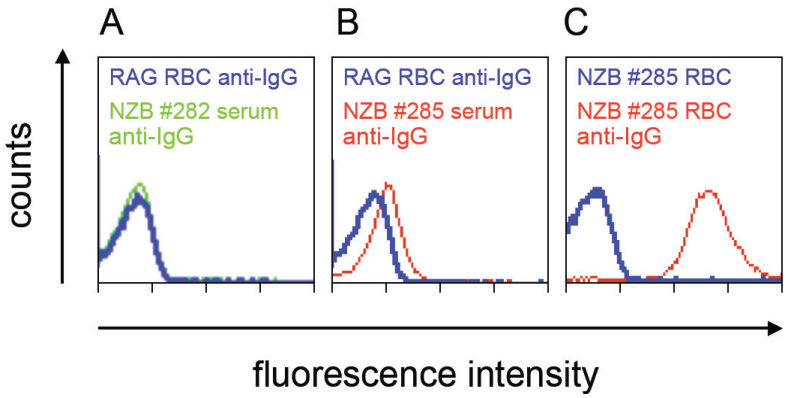

Fig. S4. RBC from RAG-2-deficient mouse stained with autoimmune serum. Flow cytometry profiles of (*A*) RBC from a RAG-2-deficient mouse stained with FITC-coupled anti-IgG (blue line), or with serum (diluted 1:10) from NZB mouse #282 (see Table S1), which was not treated with raltegravir, plus anti-IgG (green line); or (*B*) with serum from NZB mouse #285 (see Table S1), which was treated with raltegravir (red line). (*C*) RBC from NZB mouse #285, stained with anti-IgG (red line) or RBC only (blue line). *y* axis, cell number; *x* axis, fluorescence intensity on a logarithmic scale. As a response to anti-RBC antibodies attached to the RBC, NZB mice increase their hematopoiesis; the increase in number of RBC keeps the autoantibody concentration in the serum low until late in life, when antibody production outpaces RBC production.

Table S1. Mean fluorescence intensities in flow cytometry of RBC from various mice reacted with anti-IgG antibodies as a function of age of mice, in weeks (wk)

	21 wk	24 wk	27 wk	30 wk	33 wk	36 wk	39 wk	42 wk	43 wk	50 wk
NZB #										
283	11.85	24.46	56.96	131.07	129.78	173.14	237.68	195.80		144.25
284	9.46	7.38	13.96	11.10	32.67	96.45	73.13	80.65		204.66
285	10.75	7.65	12.23	18.31	48.00	167.99	283.20	306.64		377.30
279	7.14	6.89	6.94	6.91	6.00	8.42	10.40	9.34		8.77
280	8.21	6.23	8.02	9.35	6.88	35.78	111.56	155.01		220.51
281	8.45	7.05	6.94	7.17	6.57	7.93	9.07	9.31		53.79
282	7.79	6.61	6.82	7.24	6.19	6.55	7.19	6.31		7.48
NZW #										
242									5.42	5.79
243									5.44	6.65
286									5.97	6.54
287									5.06	9.66
201									5.24	5.19
275									5.58	5.74
288									5.42	5.62
289									5.78	5.21
BALB/c #										
Females										
478	5.41	5.68	5.74	5.93		6.08		7.02		
479	5.45	5.58	6.26	6.46		7.78		6.79		
318	5.73	5.92	5.94	6.18		6.89		6.71		
481	5.52	6.44	6.96	6.00		6.48		6.88		
482	5.67	5.05	5.58	5.97		6.41		7.35		
Males										
480	5.68	5.94	6.68	6.55		7.18		7.65		
483	5.75	5.50	6.28	6.16		7.06		7.29		
484	5.27	5.74	5.60	6.11		7.63		7.39		
485	5.81	5.96	5.83	6.12		8.28		6.91		
486	6.14	5.50	5.90	6.34		7.31		7.03		
Females										
487	5.39	5.29	5.14	5.29		6.20		6.59		
488	5.06	5.12	5.41	5.46		7.65		6.71		
489	5.21	5.69	5.26	5.60		7.18		7.52		
490	4.98	5.33	5.78	5.15		7.04		6.56		
491	5.24	5.07	5.15	5.38		6.91		6.63		
RAG #										
298	4.92	4.45	4.39	4.38		4.78	4.97	5.08	5.91	5.92
299	4.56	4.50	4.44	4.12	3.85	5.56	5.58	4.58	4.83	4.89
300	4.36	4.23	3.80	4.32	3.70	4.49	4.72	4.33	4.64	4.92
C57BL/6										
226	4.82	4.61	5.65	5.26	4.34	6.63	5.83	5.53	5.47	7.15
227	4.89	4.31	5.39	5.58	4.32	5.90	5.27	5.35	5.07	5.80
202		4.16	5.09	5.47	4.07	5.10	5.03	4.58	4.79	6.26

Boldface mouse numbers (#), individual mice fed with raltegravir; nonbold mouse numbers, without raltegravir. NZB, female NZB mice; NZW, male NZW mice; BALB/c mice as indicated; RAG, RAG-2-deficient mice on C57BL/6 background.