Supporting Information

Lee et al. 10.1073/pnas.1310604110

SI Materials and Methods

Reagents. Anti–zinc-finger antiviral protein (ZAP) antibody (ab105357) was purchased from Abcam. Anti-vesicular stomatitis virus (VSV)-G antibody (A190-131A-2) was purchased from Bethyl Laboratories. Anti-G3BP1 antibody (611126) was purchased from BD Biosciences. Anti-TIA1 antibody (SC-1751) was purchased from Santa Cruz Biotechnology. Anti-HaloTag antibody (G928A) was purchased from Promega. Lipopolysaccharides, R848 and 5′ triphosphate dsRNA were purchased from InvivoGen. Poly(rI–rC) was purchased from GE Healthcare. Poly (dA–dT) was purchased from Sigma. Lipofectamine 2000 was purchased from Invitrogen.

Plasmids. pFN21A–Halo DEAH (Asp-Glu-Ala-His) box polypeptide 30 (DHX30) was purchased from Promega. pVP62 (GenBank accession no. DQ399707.1) was obtained from the National Institutes of Health AIDS Reagent Program. The pSuperRetro vector was purchased from Oligoengine. The lentiviral expression construct pCS–CDF–CG–PRE was kindly donated by H. Miyoshi (RIKEN, Ibaraki, Japan). The lentiviral shRNA expression construct pCS–Puro–PRE, a derivative of pCS–CDF–CG–PRE, has been described previously (1). Annealed oligonucleotides encoding the *zinc finger CCCH-type, antiviral 1* (*ZC3HAV1*) sequence (5′-AGTTTCCAAGGGATGATTC-3′) were inserted into pSuperRetro, generating pSR–ZC3HAV1i. The EcoRI/SalI shRNA expression cassette from pSR–ZC3HAV1i was transferred to the EcoRI/XhoI sites in pCS–Puro–PRE, generating pCS–Puro–PRE–ZC3HAV1i. pCMV–ΔR8.2 (Addgene plasmid 12263) and pMD2.G (Addgene plasmid 12259) were donated by D. Trono (Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland).

Cells, Viruses, and Lentiviral Gene Transduction. MT-2 cells have been described previously (2). Replication-competent xenotropic Murine leukemia virus (MLV)-related virus was produced by 293T cells transfected with pVP62. The lentiviral vector was produced by 293T cells transfected with the lentiviral expression constructs, together with pCMV–ΔR8.2 and pMD2.G. For gene transduction, the lentiviral vector was incubated with the target cells for 2 h in the presence of 10 μg/mL Polybrene.

Generation of *Zc3hav1*$^{-/-}$ Mice. A fragment containing the *Zc3hav1* gene was isolated from genomic DNA extracted from WT ES cells by PCR. A targeting vector was constructed by replacing exon 1 with a neomycin-resistance gene cassette, and the herpes simplex virus thymidine kinase gene driven by the phosphoglycerate kinase (*PGK*) promoter was inserted into the genomic fragment to allow negative selection. After the targeting vector was transfected into ES cells, colonies doubly resistant to G418 and ganciclovir were selected and screened by PCR and Southern blotting. The homologous recombinants were microinjected into C57BL/6 female mice, and heterozygous F1 progeny were crossed to obtain *Zc3hav1*$^{-/-}$ mice. The *Zc3hav1*$^{-/-}$ mice were generated in a mixed 129Sv and C57BL/6 background.

Primers. The primer pairs used in Fig. S2*C* were as follows: for *Zc3hav1* mRNA, 5′-GCAGGCCAGTAAACACTAGACCCAAA-3′ and 5′-CTCAGGCTCATCAAAACTCCACCCGA-3′; and for *Actb* mRNA, 5′-TGTTACCAACTGGGACGACA-3′ and 5′-GGGGTGTTGAAGGTCTCAAA-3′.

1. Saitoh Y, et al. (2008) Overexpressed NF-kappaB-inducing kinase contributes to the tumorigenesis of adult T-cell leukemia and Hodgkin Reed-Sternberg cells. *Blood* 111(10):5118–5129.

2. Yamamoto N, Hinuma Y (1985) Viral aetiology of adult T-cell leukaemia. *J Gen Virol* 66 (Pt 8):1641–1660.

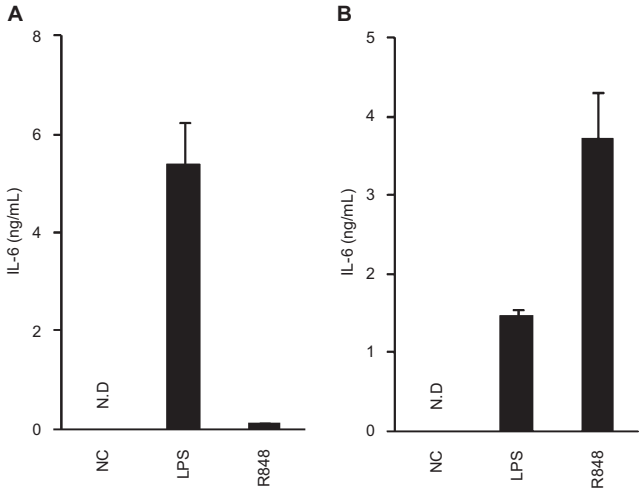

Fig. S1. Primary mouse embryonic fibroblasts (MEFs) respond negligibly to R848. MEFs (*A*) and bone marrow-derived dendritic cells (BMDCs) (*B*) isolated from C57BL/6 mice were stimulated with LPS (1 μg/mL) and R848 (10 μg/mL) for 24 h. The levels of IL-6 protein in the culture supernatants were measured with ELISA. The results shown are means ± SD (n = 3). ND, not detected.

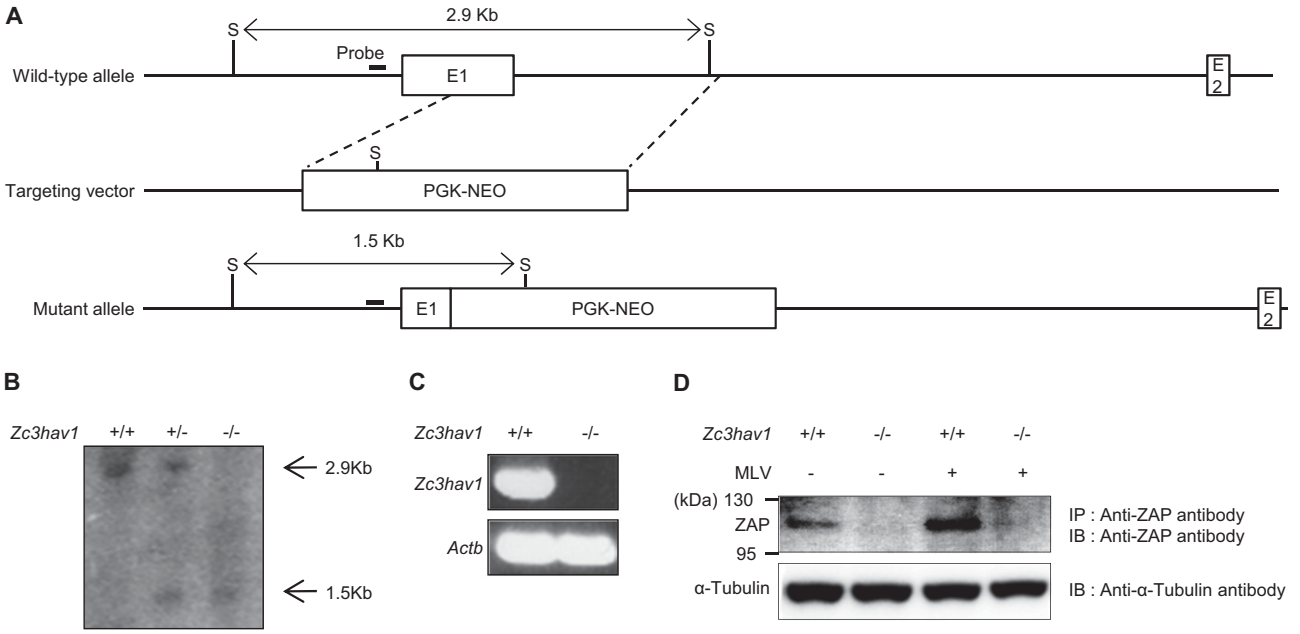

Fig. S2. Targeted disruption of the murine *Zc3hav1* gene. (*A*) Schematic representation of the targeting of the *Zc3hav1* gene. The targeting vector was constructed by replacing exon 1 of the *Zc3hav1* gene with the neomycin resistance gene. E, exon; PGK-Neo, phosphoglycerate kinase (*PGK*)-promoter-driven neomycin-resistance gene cassette; S, SphI. (*B*) Genomic DNAs prepared from $Zc3hav1^{+/+}$, $Zc3hav1^{+/-}$, and $Zc3hav1^{-/-}$ MEFs were digested with SphI and subjected to Southern blotting. The probe used is shown in *A*. (*C*) Total RNAs were isolated from $Zc3hav1^{+/+}$ and $Zc3hav1^{-/-}$ MEFs. The levels of *Zc3hav1* and *Actb* mRNAs were measured by RT-PCR. (*D*) $Zc3hav1^{+/+}$ and $Zc3hav1^{-/-}$ MEFs were infected with or without MLV (2×10^{10} copies per μL) for 72 h. Whole-cell lysates were isolated and subjected to immunoprecipitation coupled to immunoblotting with the indicated antibodies.

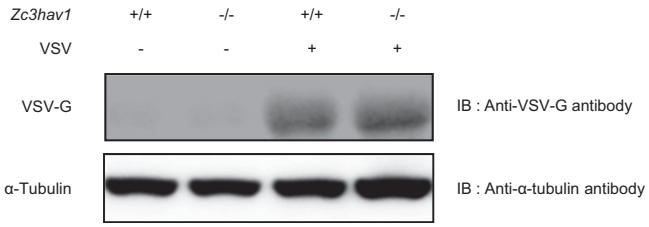

Fig. S3. ZAP does not limit the replication of VSV. $Zc3hav1^{+/+}$ and $Zc3hav1^{-/-}$ MEFs were infected with or without VSV (MOI = 1) for 12 h. Whole-cell lysates were isolated and subjected to immunoblotting with the indicated antibodies.

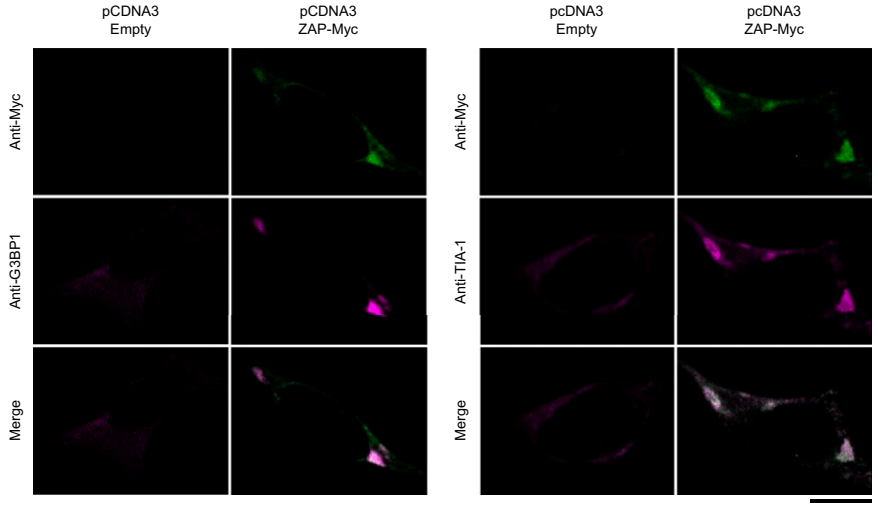

Fig. S4. ZAP localizes to the G3BP1- and TIA-1-positive RNA granules. 293T cells were transfected with the indicated vectors and fixed. The samples were subjected to immunostaining with the indicated antibodies and then observed with confocal laser scanning microscopy. The data are representative of three independent experiments. (Scale bars, 10 μm.)

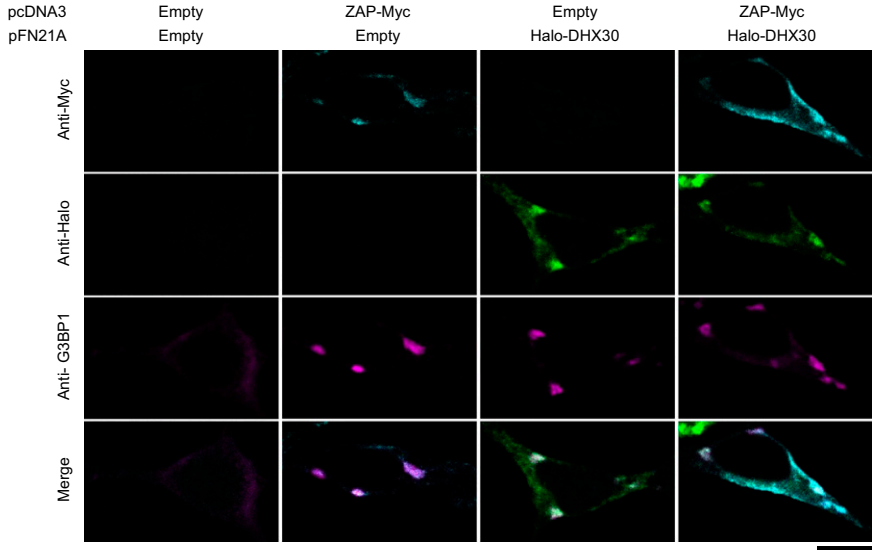

Fig. S5. DHX30 colocalizes with ZAP on RNA granules. 293T cells were transfected with the indicated plasmids and then fixed. The samples were subjected to immunostaining with the indicated antibodies and then observed by confocal laser scanning microscopy. The data are representative of three independent experiments. (Scale bar, 10 μm.)

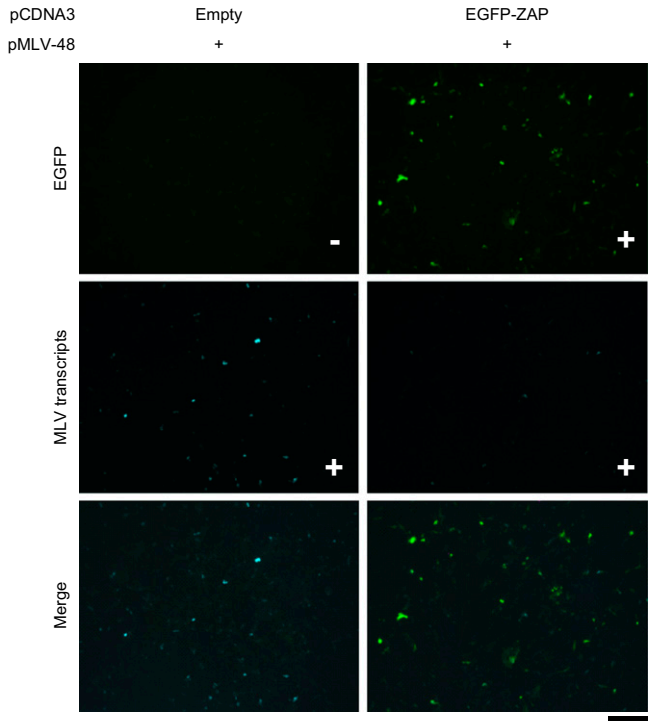

Fig. S6. ZAP induces the degradation of the MLV transcripts. 293T cells were transfected with the indicated plasmids together with pMLV-48 for 48 h and then fixed. The samples were subjected to in situ hybridization analysis with a fluorescent probe for MLV genomic RNA and observed under a fluorescence microscope (BZ-9000, Keyence) at low magnification. (Scale bar, 200 μm.)

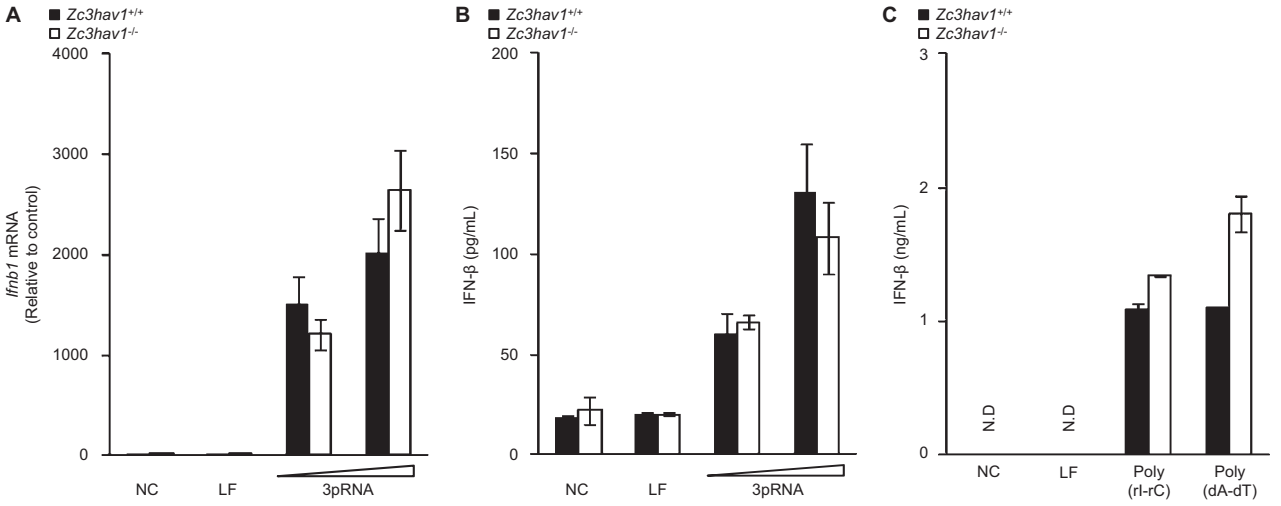

Fig. S7. ZAP does not regulate IFN-β production induced by cytosolic nucleic acids. (*A* and *B*) *Zc3hav1*$^{+/+}$ and *Zc3hav1*$^{-/-}$ MEFs were stimulated with 5′ triphosphate dsRNA (3pRNA, 1 μg/mL or 3 μg/mL) for 12 h. The level of *Ifnb1* mRNA was measured by quantitative RT-PCR (*A*). The level of IFN-β protein was measured by ELISA (*B*). (*C*) *Zc3hav1*$^{+/+}$ and *Zc3hav1*$^{-/-}$ MEFs were transfected with poly(rI–rC) or poly(dA–dT) for 24 h. The levels of IFN-β protein in the culture supernatants were measured by ELISA. The results shown are means ± SD (n = 3).

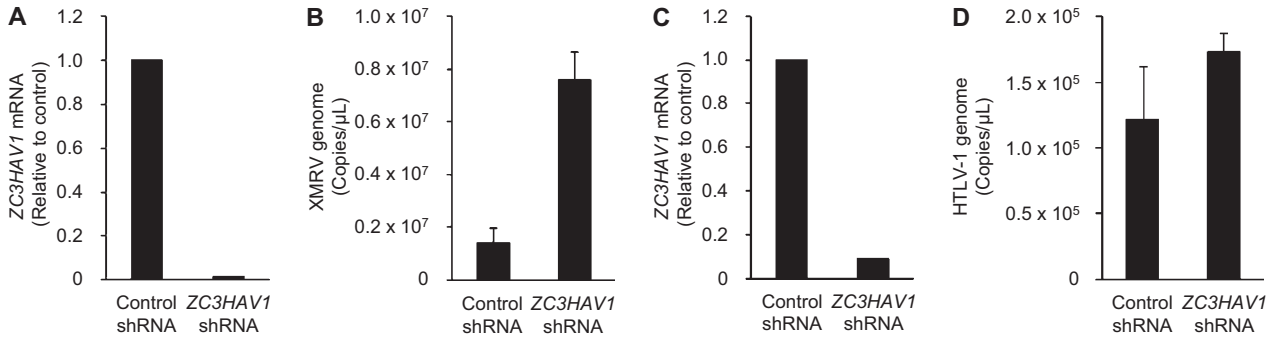

Fig. S8. Human ZAP inhibits the replication of xenotropic MLV-related virus (XMRV). (*A*) 293T cells were transfected with a lentiviral vector expressing a short hairpin (sh) RNA complementary to *ZC3HAV1* or with a control shRNA. The infected cells were selected with puromycin (5 μg/mL). The levels of *ZC3HAV1* mRNA were measured by quantitative RT-PCR. (*B*) 293T cells stably expressing the *ZC3HAV1* shRNA or the control shRNA were infected with XMRV for 72 h. The copy numbers of the XMRV genome in the culture supernatants were measured by quantitative RT-PCR. (*C*) MT-2 cells were infected with a lentiviral vector expressing the *ZC3HAV1* shRNA or the control shRNA. The infected cells were selected with puromycin (1 μg/mL). The levels of *ZC3HAV1* mRNA were measured with quantitative RT-PCR. (*D*) MT-2 cells stably expressing the *ZC3HAV1* shRNA or the control shRNA were cultured for 72 h. The copy numbers of the HTLV-I genome in the culture supernatants were measured by quantitative RT–PCR. HTLV-I, human T-lymphotropic virus type I. The results shown are means ± SD ($n = 3$).