SUPPLEMENTAL MATERIAL

Orthwein et al., http://www.jem.org/cgi/content/full/jem.20101321/DC1

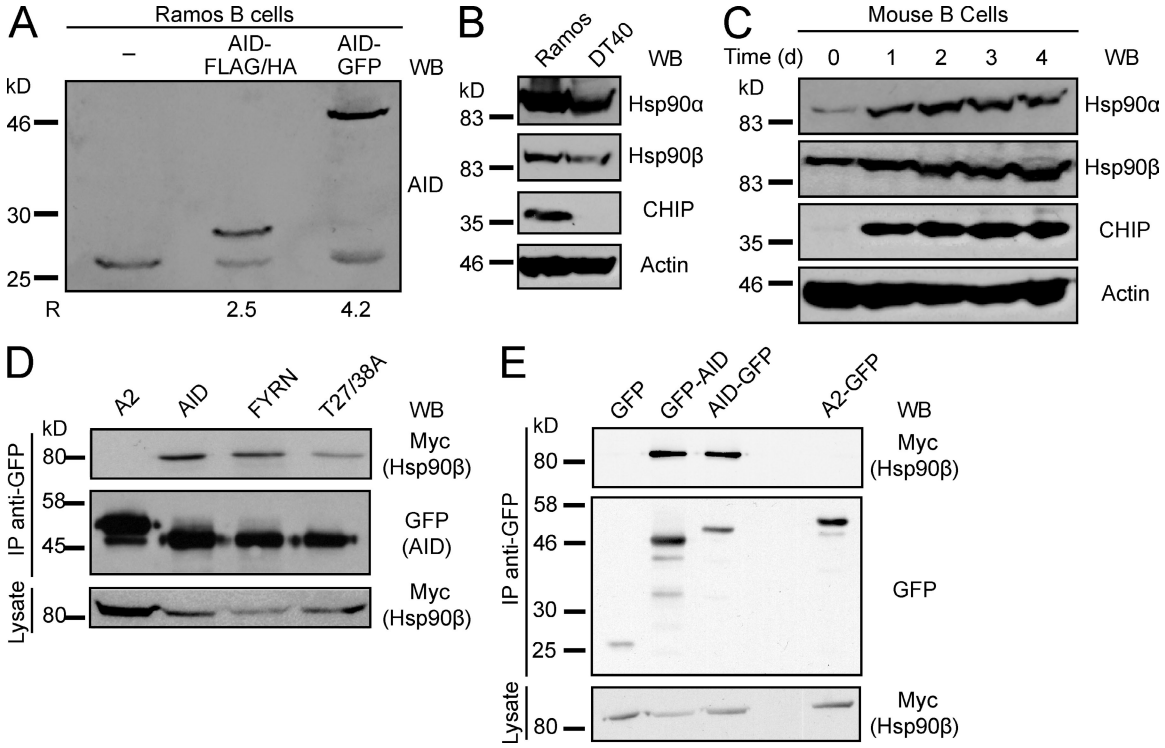

Figure S1. Expression levels of AID, Hsp90, and CHIP in various B cells and interaction of Hsp90 with various AID variants. (A) Total extracts from parental Ramos B cells and its derived cell lines stably expressing AID-Flag/HA and AID-GFP were analyzed by Western blot (WB) with anti-AID to compare the protein level of each transgenic AID with the endogenous enzyme. Bands were quantified using ImageQuant, and the ratio (R) of tagged to endogenous AID is indicated. One of two independent experiments is shown. (B) Expression of Hsp90 isoforms and of the Hsp90-associated E3 ubiquitin ligase CHIP (Fig. 4) in human Ramos and chicken DT40 B cell lines. Total cell lysate lines were analyzed by Western blot using anti–Hsp90-α, anti–Hsp90-β, anti-CHIP, and antiactin. Apparent differences in expression between Ramos and DT40 cells most likely reflect variations in the chicken epitopes because anti–Hsp90-α and anti-CHIP are mAbs raised against human proteins. One of two independent experiments is shown. (C) Kinetics of Hsp90 isoforms and CHIP expression in purified naive mouse B cells activated with IL-4 and LPS. Cells were harvested at different time points, lysed, and analyzed by Western blot using anti–Hsp90-α, anti–Hsp90-β, anti-CHIP, and antiactin. One of two independent experiments is shown. (D) AID oligomerization or phosphorylation is not necessary for Hsp90 interaction. Interaction of Hsp90 was tested by coimmunoprecipitations with AID mutants carrying the F46A/Y48A/R50G/N51A simultaneous mutations (FYRN), previously shown be defective for oligomerization (Patenaude et al., 2009), or the T27A and T38A phospho-null mutations (T27/38A). (E) The position of the tag on AID does not affect the association with Hsp90-β. HEK293T cells were cotransfected with Myc–Hsp90-β and GFP-AID, AID-GFP, A2-GFP, or GFP. Anti-GFP immunoprecipitates (IP) were analyzed by Western blot with anti-Myc and anti-GFP. One of three independent experiments is shown.

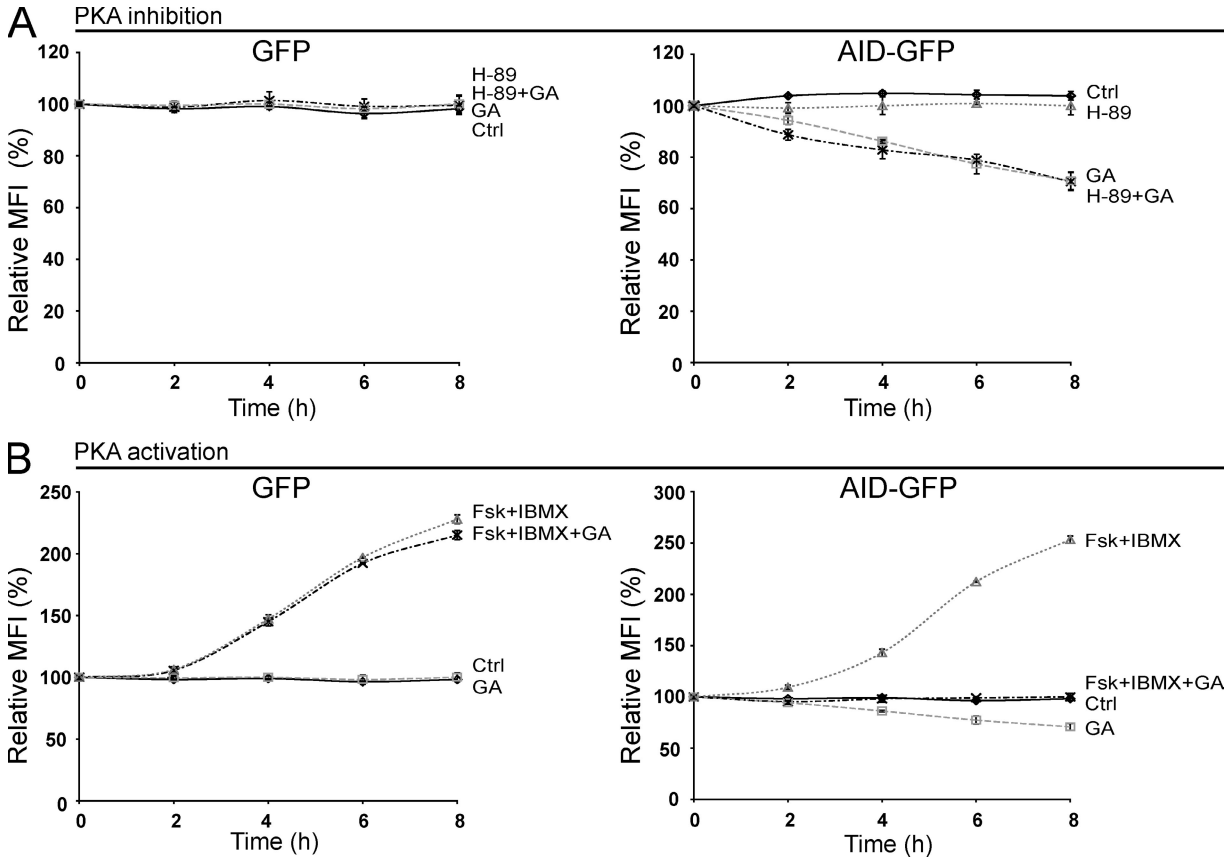

Figure S2. AID dependence on Hsp90 is unaffected by PKA inhibition or activation. (A and B) Ramos cells stably expressing AID-GFP were treated with 10 µM of PKA inhibitor H-89 (A) or 50 µM of adenylate cyclase activator forskolin (Fsk) in combination with 100 µM of phosphodiesterase inhibitor IBMX to boost cAMP levels (B) before treating the cells with 2 µM GA or DMSO. AID-GFP was followed by flow cytometry and the MFI (normalized to the t = 0 signal) plotted at different times for each treatment. Note that FSK + IMBX equally increases the level of GFP and AID-GFP in Ramos cells. The reasons behind this increase are unknown, but although GFP control increases also in the presence of Hsp90 inhibition, a similar increase in AID-GFP is totally prevented by GA, confirming the dependence of AID on Hsp90. Mean ± SD of triplicates is plotted. One of three independent experiments is shown. Ctrl, control.

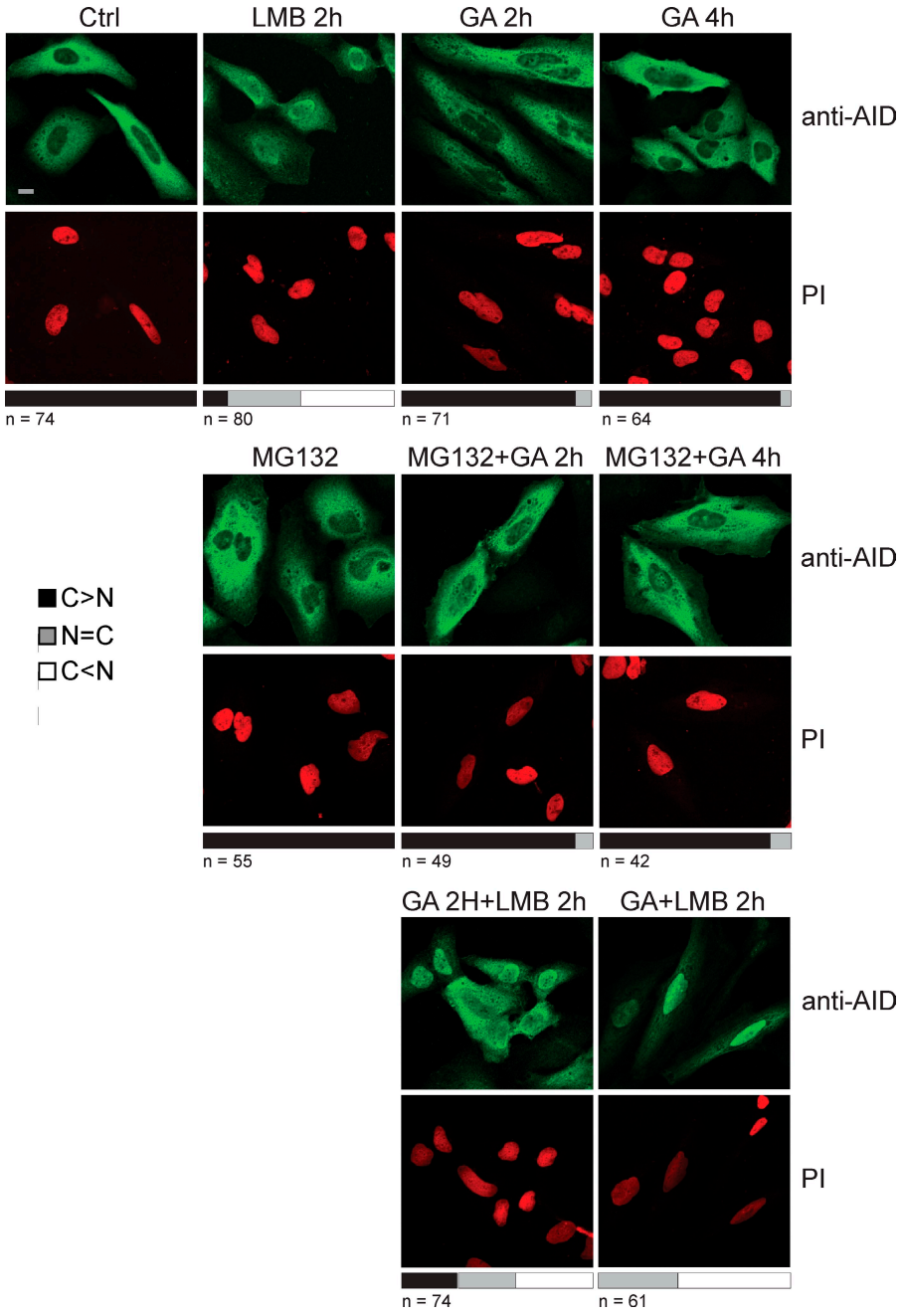

Figure S3. Hsp90 inhibition does not affect AID compartmentalization. HeLa cells were transiently transfected with untagged AID and 48 h later treated as indicated (control [Ctrl] = DMSO, 2 µM GA, 50 ng/ml LMB, and 10 µM MG132). AID localization was monitored by immunofluorescence using anti-AID antibody. Treatments GA 2 h + LMB 2 h and GA + LMB 2 h differ in the time of addition of LMB; in the first case, cells were pretreated with GA before adding LMB, whereas in the latter, both drugs were added simultaneously. Simultaneous inhibition of Hsp90 and nuclear export may have a small effect on the speed with which AID accumulates in the nucleus. One possibility is that a proportion of the Hsp90-bound AID might be nuclear import competent. So, release of AID from Hsp90 by GA treatment at the same time of nuclear export inhibition could allow nuclear import to compete with cytoplasmic AID degradation, leading to an apparent enrichment of AID in the nucleus. However, Hsp90 on its own has no effect on AID subcellular distribution, indicating that it is not retaining it in the cytoplasm. C, cytoplasmic; N, nuclear. Bar, 10 µm.

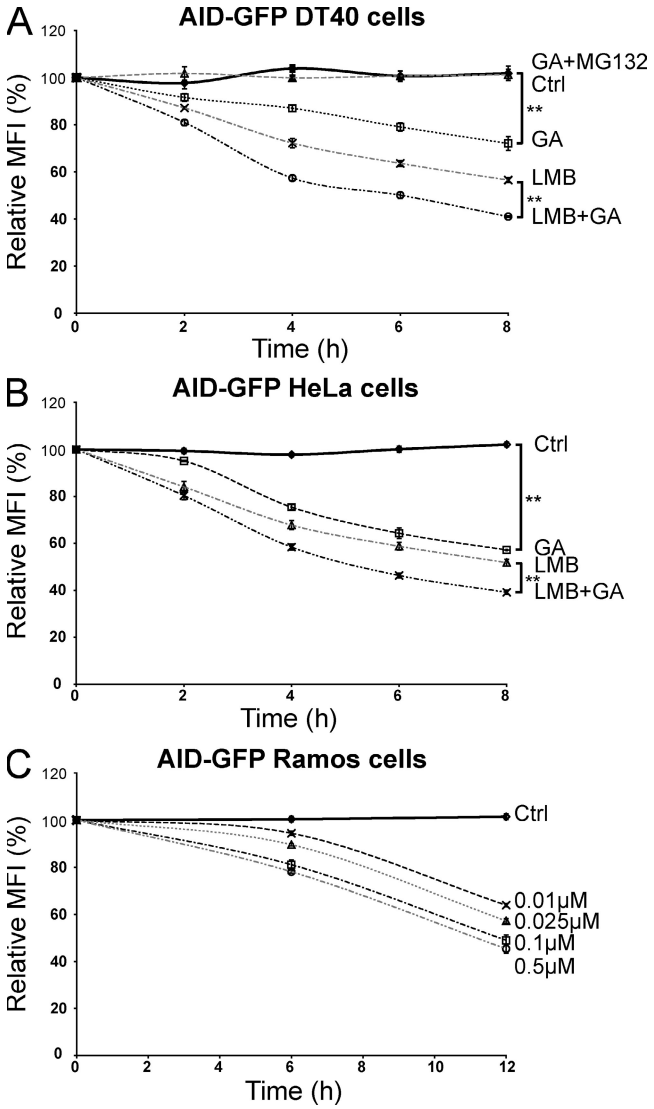

Figure S4. The effect of Hsp90 inhibition on AID stability is conserved in chicken B cells and human non-B cells and is dose dependent. (A and B) DT40 *aid*$^{-/-}$ (A) and HeLa cells stably expressing AID-GFP (B) were treated with the indicated combinations of DMSO (Ctrl), 2 µM GA, 50 ng/ml LMB, and/or 10 µM MG132 (30-min pretreatment). The GFP signal was monitored over time by flow cytometry, and the MFI was normalized to the signal at t_0 = 100% for each treatment. Means ± SD of triplicates are plotted (**, $P < 0.01$). One of three independent experiments is shown in each case. (C) Ramos cells stably expressing AID-GFP were treated with DMSO (Ctrl) or the indicated concentrations of GA, and the GFP signal was monitored by flow cytometry. Means ± SD of triplicates are plotted. One of three independent experiments is shown.

REFERENCE

Patenaude, A.M., A. Orthwein, Y. Hu, V.A. Campo, B. Kavli, A. Buschiazzo, and J.M. Di Noia. 2009. Active nuclear import and cytoplasmic retention of activation-induced deaminase. *Nat. Struct. Mol. Biol.* 16:517–527. doi:10.1038/nsmb.1598