SUPPLEMENTAL MATERIAL

Mu et al., https://doi.org/10.1084/jem.20170468

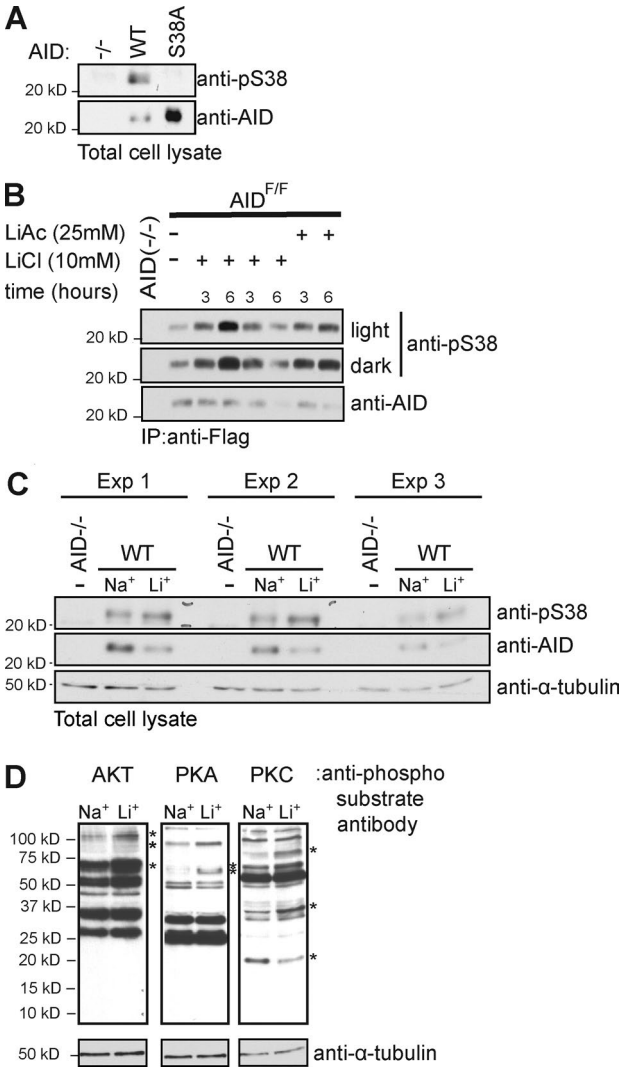

Figure S1. **Lithium alters phosphorylation on AID.** (A) Anti-pS38 antibody specifically detects AID pS38. Anti-pS38 and anti-AID immunoblot of total cell lysates from 3-d LPS- and IL-4–stimulated B cells from $AID^{-/-}$, WT, or AID^{S38A} mice. (B) Anti-pS38 antibody detects up-regulated pS38 in Li^+ treated cells. Anti-pS38 (light and dark exposures) and anti-AID immunoblot of Flag-AID purified by anti-Flag immunoprecipitation from B cells of $AID^{F/F}$ mice treated with indicated concentration of lithium acetate (LiAc) or lithium chloride (LiCl) for indicated time before harvest. Replicate treatments of LiCl for 3 and 6 h are shown. Representative of $n = 3$ experiments. (C) Anti-pS38, anti-AID, and anti–α-tubulin immunoblot of total cell lysates from 3-d LPS- and IL-4–stimulated B cells from WT mice. Cells were treated with 10 mM NaCl or LiCl for 12 h before analysis; $n = 3$ experiments are shown. (D) Anti–phospho substrate antibody blots of total cell lysates from WT B cells treated with 10 mM NaCl or LiCl for 12 h. The antibodies detect a phospho-serine in the context of an AKT, PKA, or PKC consensus site. Multiple bands change intensity after treatment; * marks some examples.

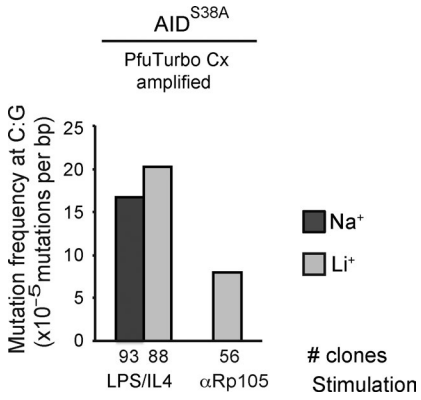

Figure S2. **Mutation frequency at C:G bases in the *Myc* gene.** B cells from UNG$^{-/-}$/AID$^{S38A/S38A}$ double-mutant mice were cultured with LPS and IL-4 or anti-Rp105 for 3 d before treatment with 10 mM NaCl or LiCl for 12 h. *Myc* was amplified from genomic DNA with Pfu-Cx (Agilent). This is an engineered version of Pfu that contains a point mutation enabling the polymerase to overcome uracil stalling during PCR amplification (Horváth and Vértessy, 2010; Wang et al., 2017). This feature allows Pfu-Cx to read through uracils on the template strand. The uracils would be converted to a thymidine and reflected as a C-to-T mutation. Number of clones sequenced is indicated. Graph is a summary of mutations of B cells from $n = 3$ independent experiments.

Table S1. **Mutation profile from NaCl– versus LiCl–treated B cells**

Genotype	Gene	Treatment	Mutations (total)[a]	Mutations at C/G[b]	Mutated clones (mutated/total clones)[c]
					%
UNG$^{-/-}$	Smu	Na^+	42	41	17 (17/100)
	Smu	Li^+	14	14	10.4 (10/96)
	Myc	Na^+	2	2	1.4 (2/138)
	Myc	Li^+	12	11	7.3 (12/164)
	Ly6e	Na^+	2	1	3.4 (2/58)
	Ly6e	Li^+	1	0	1.8 (1/56)
	Il4ra	Na^+	1	1	1.8 (1/56)
	Il4ra	Li^+	2	2	1.7 (1/59)
UNG$^{-/-}$ (anti-Rp105)	Myc	Na^+	2	2	3.5 (2/57)
	Myc	Li^+	1	1	1.5 (1/65)
S38A UNG$^{-/-}$	Smu	Na^+	1	0	3.0 (1/33)
	Smu	Li^+	2	1	3.4 (1/29)
	Myc	Na^+	4	2	2.2 (4/186)
	Myc	Li^+	4	4	2.1 (4/187)
AID$^{-/-}$	Smu		0	0	0 (0/30)
	Myc		0	0	0 (0/28)

Area evaluated by sequencing: Smu, 490 bp total (219 C/Gs); Myc, 832 bp total (450 C/Gs); Ly6e, 614 bp total (370 C/Gs); and Il4ra, 665 bp total (384 C/Gs). Sequence context of mutations in Myc gene from UNG$^{-/-}$ B cells (underlined denotes WRC motif, W denotes A or T; R denotes A or G). NaCl (control) treatment: CCC to CCT, TTC to TTT. LiCl treatment, TTC to TTA, TGC to TGT, ATC to ATT, TAC to TAT, AGC to AGT, TGC to TGT, GCC to GCT, AAC to AAT, AGC to AGT, GGC to GGA, GCC to GCT.

[a]Total number of mutations detected.

[b]Mutations at a C/G residue.

[c]Percentage of clones with at least one mutation and number of clones with at least one mutation/total number of clones evaluated.

REFERENCES

Horváth, A., and B.G. Vértessy. 2010. A one-step method for quantitative determination of uracil in DNA by real-time PCR. *Nucleic Acids Res.* 38:e196. https://doi.org/10.1093/nar/gkq815

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