Supporting Information for

Small Molecule Inhibitors of Activation-Induced Deaminase Decrease Class Switch Recombination in B Cells

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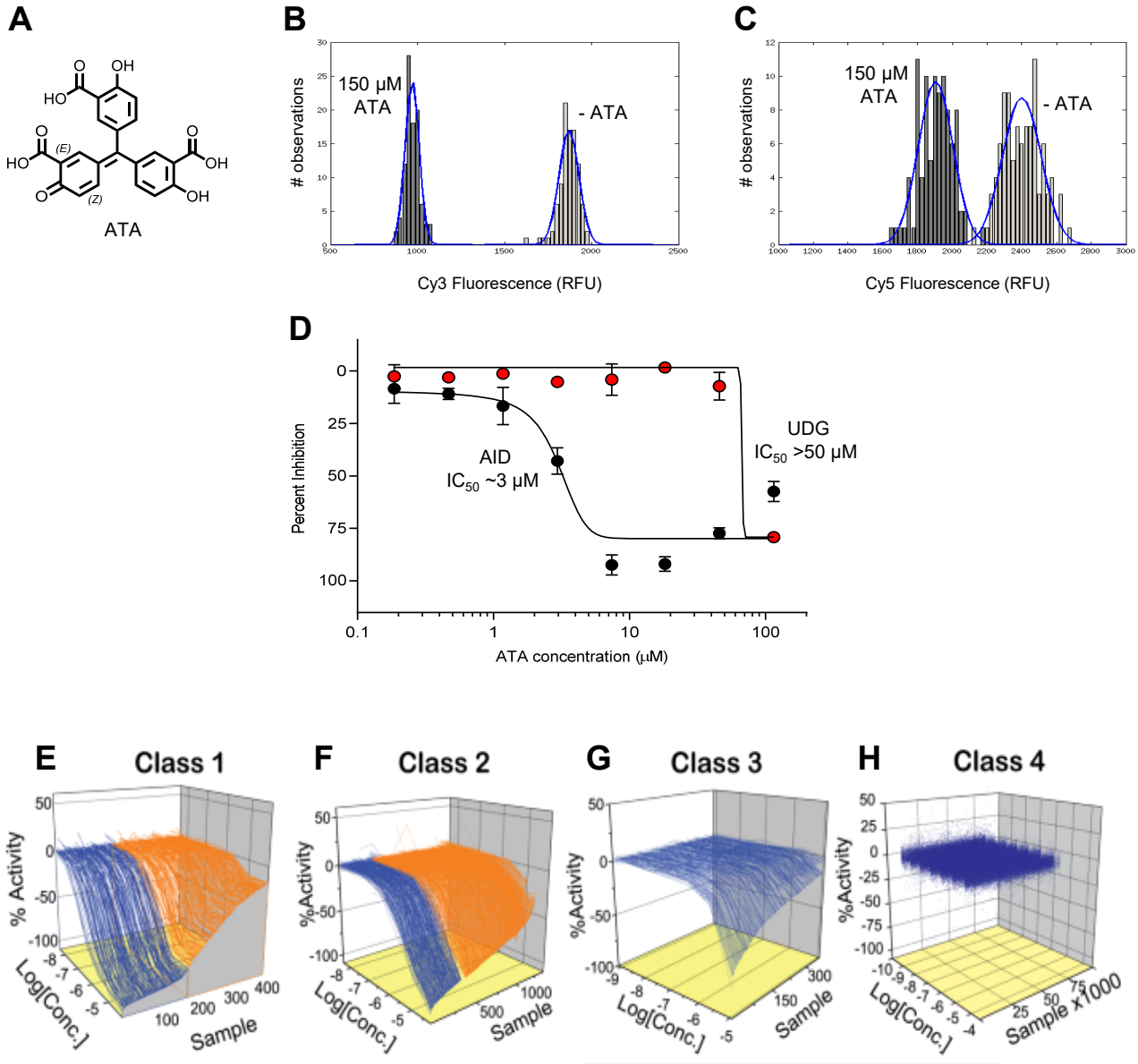


Figure S1. Assay optimization (A-D) and compound triage (E-H). (A) Structure of ATA, a non-specific inhibitor of protein-nucleic acid binding. (B) Histogram of Cy3 fluorescence showing AID-specific deaminase activity when either incubated with 150 μM ATA (N=96; dark gray) or uninhibited (N=96; light gray), indicating AID inhibition with a Z′=0.7. (C) Histogram of Cy5 fluorescence indicating UDG-specific glycosylase activity when either incubated with 150 μM ATA inhibition (N=96; dark gray) or uninhibited (N=96; light gray), indicating poor UDG inhibition with a Z′<0. (D) Titration curves show separate IC_{50} values for both oligonucleotides. Red, UDG; black, AID. (E) Classes 1.1 (blue; >80% efficacy) and 1.2 (orange; ≤80% efficacy) inhibitors display full and partial activity, respectively, with r2 ≥0.9. (F) Classes 2.1 (blue; >80% efficacy, r2 >0.9) and 2.2 (orange; ≤80% efficacy, r2 <0.9) inhibitors have incomplete curves for inhibitors with IC_{50} values within and beyond the tested titration range. (G) Class 3 inhibitors have incomplete inhibitory (blue) curves that show weak activity and poor fits. (H) Class 4 compounds are inactive.

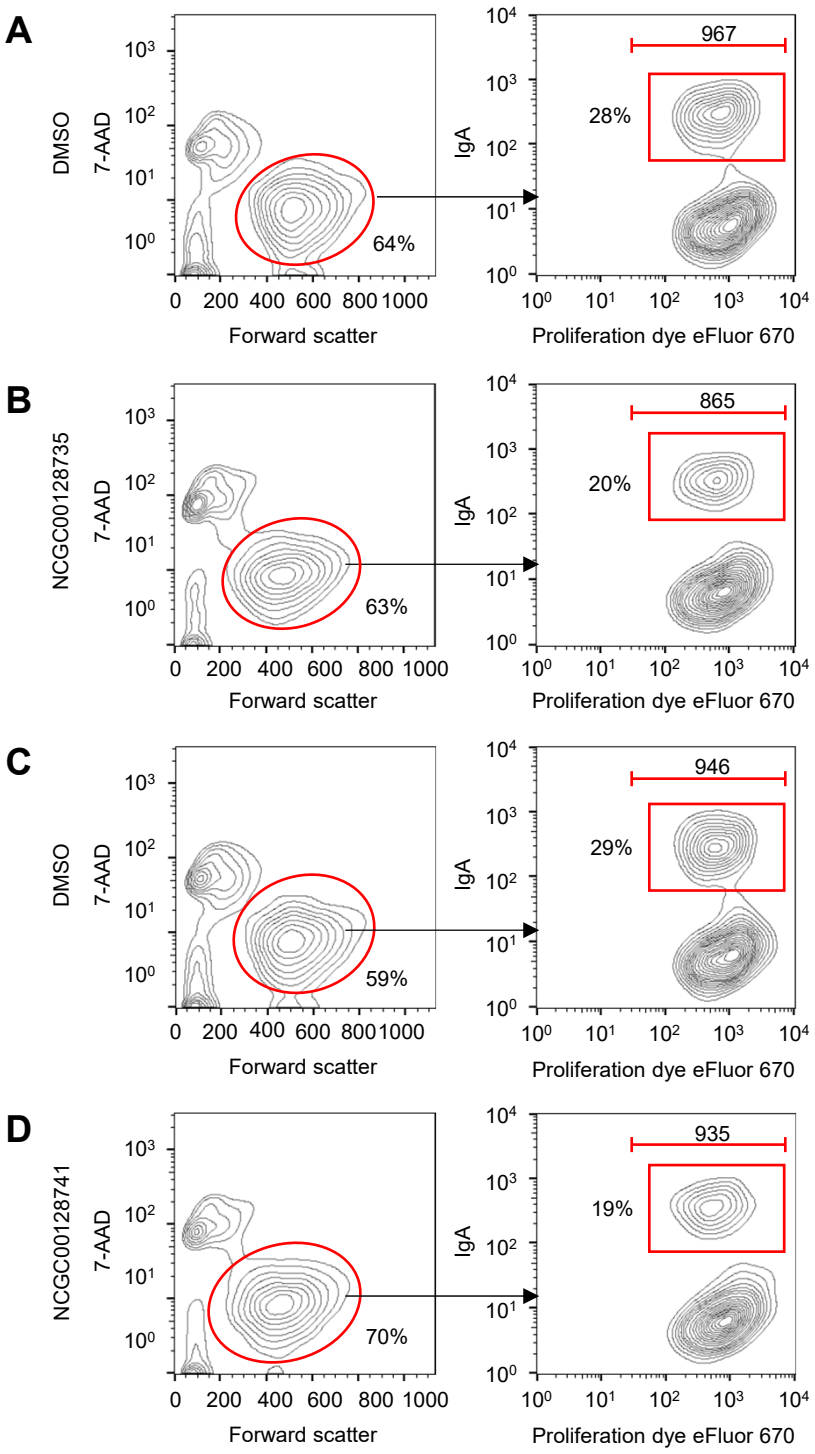


Figure S2. Representative FACS plots of CH12 B cells 72 hrs after stimulation. All samples were cultured in 0.1% DMSO final concentration. Red circle shows the gating of live lymphocytes. Red square shows the percentage of IgA$^+$ B cells. Red bar shows the area used and value of mean fluorescence of proliferation dye. (A) and (B), DMSO control and 10 μM NCGC00128735. (C) and (D), DMSO control and 10 μM NCGC00128741.

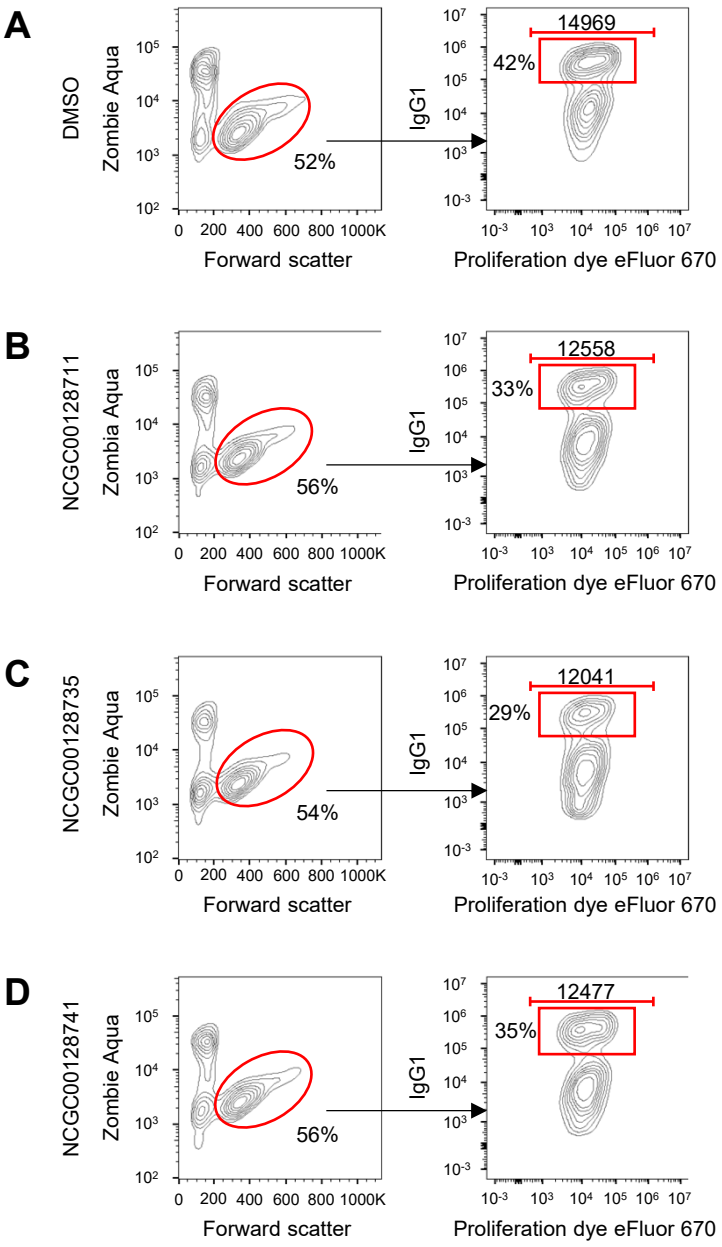
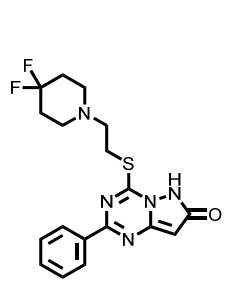


Figure S3. Representative FACS plots of splenic B cells 72 hrs after stimulation. All samples were Cultured in 0.1% DMSO final concentration. Red circle shows the gating of live lymphocytes. Red square shows the percentage of IgG1$^+$ B cells. Red bar shows the area used and value of mean fluorescence of proliferation dye. (A) DMSO control. (B), (C), and (D) 10 μM inhibitor.

A



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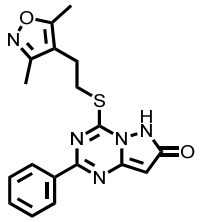

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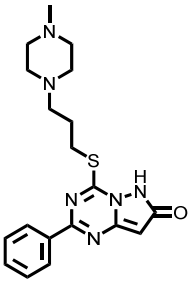

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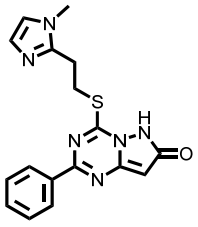

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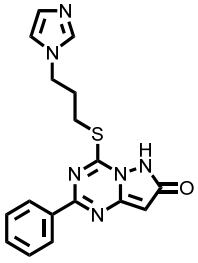

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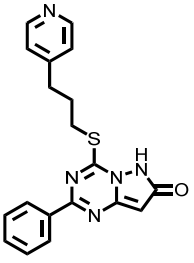

NCGC00592263

B

Compound ID	Solubility (μg/mL)	PAMPA pH 7.4 (10^{-6} cm/s)	Rat liver microsome stability ($t_{1/2}$; min)
NCGC00128735 (known)	< 1	< 10	> 30
NCGC00128741 (known)	< 1	9	not found
NCGC00592120	11.9	104	8.3
NCGC00592117	13.4	116	12
NCGC00592118	> 57	54	> 30
NCGC00592121	33.4	136	> 30
NCGC00591781	25.1	13	> 30
NCGC00592263	5.1	191	13.8

Figure S4. Analogs that tested negative in deamination assays. (A) Compounds tested negative at 100 μM by the in vitro deamination assay and at 10 μM in the ex vivo CSR assay, although they were not toxic to B cells. (B) Solubility, membrane permeability, and microsome stability measurements of 2 known inhibitors compared to 6 new analogs. .

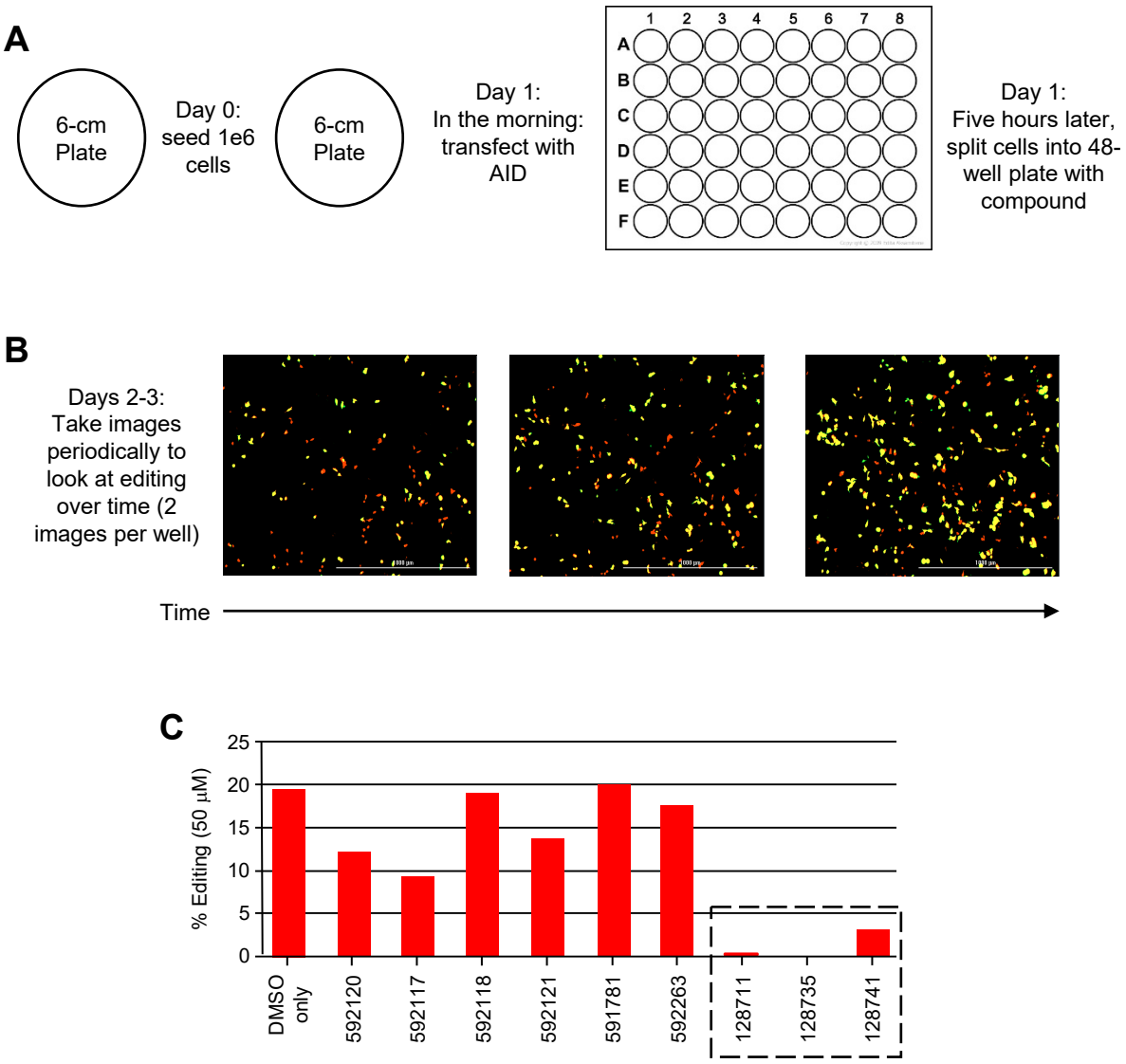

Figure S5. AID drug inhibition assay. (A) One million 293T cells were seeded and transfected with reporter and AID one day later. (B) Fluorescent images were collected over time. Red depicts mCherry transfected cells; green shows transfected cells with edited eGFP reporter. Note editing increases with time. (C) Inhibition of AID editing by 6 negative analogs and 3 positive compounds (boxed) at 50 μM.