

Table S1. Summary of single B cell cultures

Mouse/Treatment	Secondary Immunogens	Route of Boosts (Ipsi or Contra)	No. IgG Samples ^a	Primary Antigen-only ^b	Secondary Antigen-only ^c	Primary and Secondary Antigens ^d
B6	HA H1 SI-06	Ipsi	2,255	NA ^e	NA ^e	16%
B6	HA H1 SI-06	Contra	2,858	NA ^e	NA ^e	7.5%
AID-Cre/EYFP Ctrl IgG or None	HA H1 SI-06	Ipsi, YFP ^f	514	NA ^e	NA ^e	21%
AID-Cre/EYFP Ctrl IgG or None	HA H1 SI-06	Ipsi, YFP- ^g	1,014	NA ^e	NA ^e	12%
AID-Cre/EYFP MR-1 Ab	HA H1 SI-06	Ipsi, YFP ^f	343	NA ^e	NA ^e	21%
B6	HA H3 X31	Ipsi	1,825	1.2%	12%	1%
B6	HA H3 X31	Contra	1,696	0.6%	17%	0.2%
B6	rPA	Ipsi	1,130	1%	25%	0.4%
B6	rPA	Contra	1,082	0.1%	17%	0%

Mice were primed with HA H1 SI-06 in the footpad. Cohorts of mice were then boosted with indicated antigens in the hock ipsilaterally or contralaterally (Figs. 1A and 5A). After boosts, GC B cells were isolated from the draining LNs and placed in single B cell cultures. Numbers of IgG⁺ samples and frequencies of specific IgG analyzed in this study are shown.

^aNumber of IgG⁺ single B cell cultures analyzed for each group.

^bFrequency of IgG Abs that bind HA H1 SI-06 but not boosting antigens among all IgG.

^cFrequency of IgG Abs that bind boosting antigens but not HA H1 SI-06 among all IgG.

^dFrequency of IgG Abs that bind both HA H1 SI-06 and boosting antigens among all IgG.

^eNot applicable as mice were primed and boosted with the same immunogens.

^fYFP⁺ GC B cells were analyzed.

^gYFP⁻ GC B cells were analyzed.