Figure S1. Robust recall antibody responses following boost immunizations
Data shown in Fig. 1 are split by the intervals between the priming and boosting (4-5 wk, 8-10 wk, and 12-14 wk). Concentrations of serum IgGs specific to H1 SI-06 (**A**-**C**), fold changes in concentrations of H1-specific IgGs in serum samples after boosts (**D**-**F**), and number of B220^loCD138^hi plasmablasts/cytes in the draining LNs (**G**-**I**) for indicated prime/boost intervals are shown. Each dot represents an individual serum sample or mouse. Horizontal bars in (**D**-**I**), geometric mean. See also figure legend for Fig. 1.
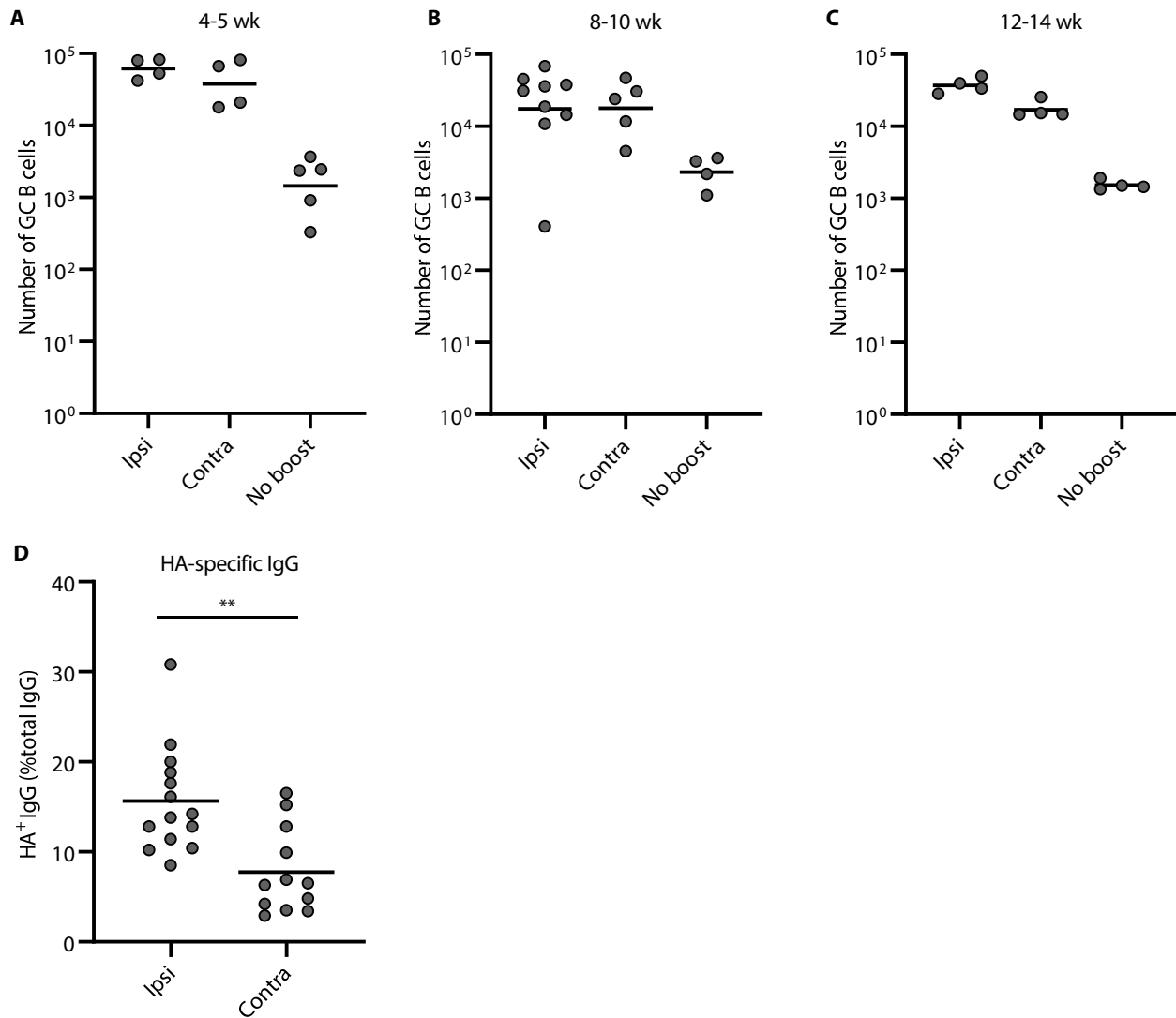
Figure S2. Robust GC responses following boosts at local sites or distal sites
(**A-C**) Data shown in Fig. 2B are split by the intervals between the priming and boosting (4-5 wk, 8-10 wk, and 12-14 wk), and number of B220$^+$CD138$^-$GL-7$^+$CD38^{lo}IgD$^-$ GC B cells in the draining LNs for the indicated prime/boost intervals is shown. Each dot represents an individual mouse. Horizontal bars, geometric mean. See also figure legend for Fig. 2. (**D**) Frequency of HA-specific IgGs among all clonal IgGs for secondary GC B cells elicited by ipsilateral or contralateral boosts. Each dot represents an individual mouse. Horizontal bars, mean. ∗∗, *p* < 0.01 by Mann-Whitney's U test.
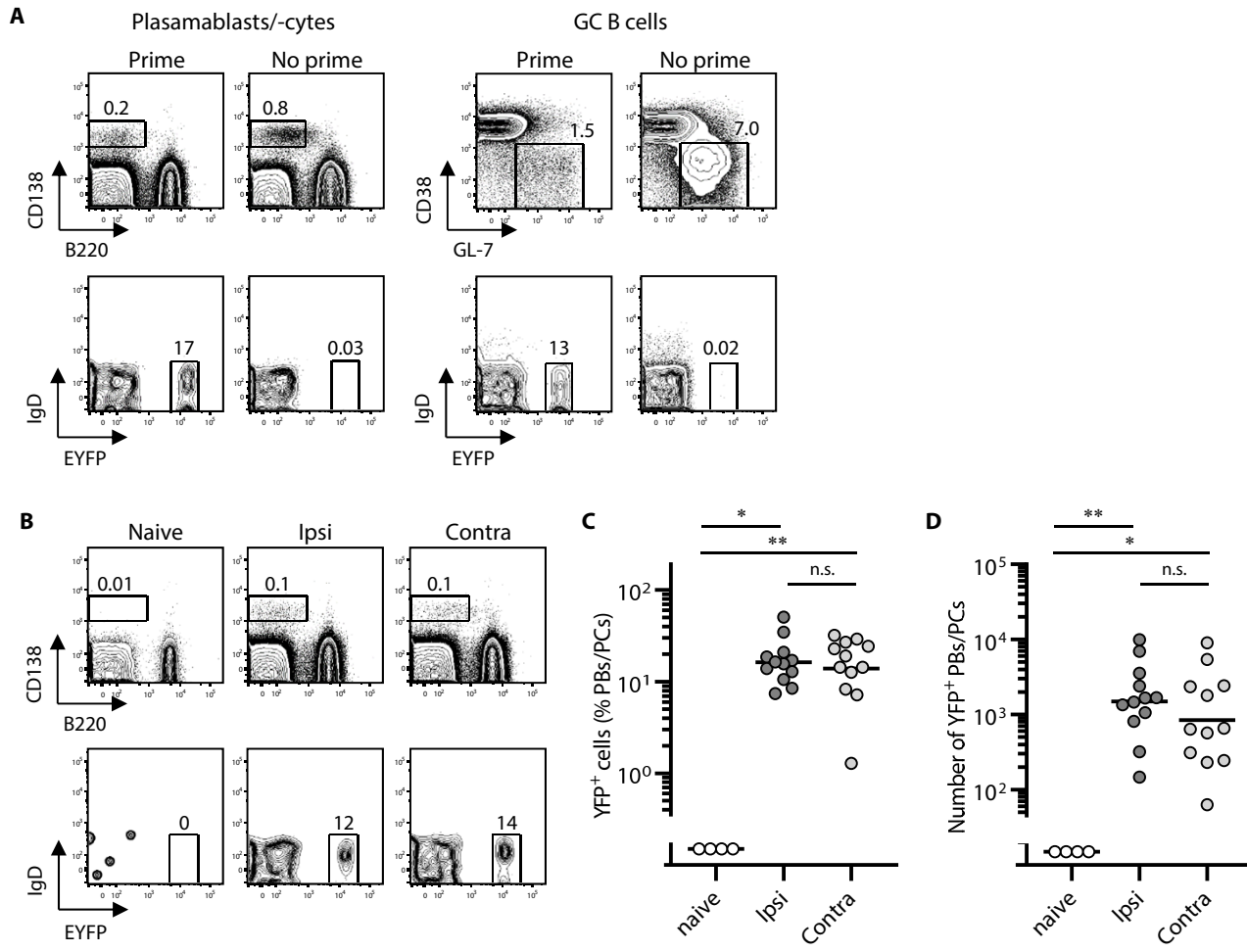
Figure S3. Tracing the fates of the progeny of primary GC B cells in AID-Cre-EYFP mice

(**A**) AID-Cre-EYFP mice that had or had not received primary immunizations (Prime and No prime, respectively) received tamoxifen. These mice were then "boosted" ipsilaterally. Following boosts (d8), frequencies of YFP+ cells among PBs/PCs and GC B cells were determined by flow cytometry. (**B**-**D**) AID-Cre-EYFP mice were primed and boosted with H1 SI-06 (see also figure legend for Fig. 2). Representative flow diagrams (**B**), and frequency (**C**) and number (**D**) of YFP+ PBs/PCs in the draining LNs are shown. (**C** and **D**) Each dot represents an individual mouse. Horizontal bars, geometric mean. ∗, $p < 0.05$; ∗∗, $p < 0.01$; n.s., $p > 0.05$ by Kruskal-Walis test with Dunn's multiple comparisons.
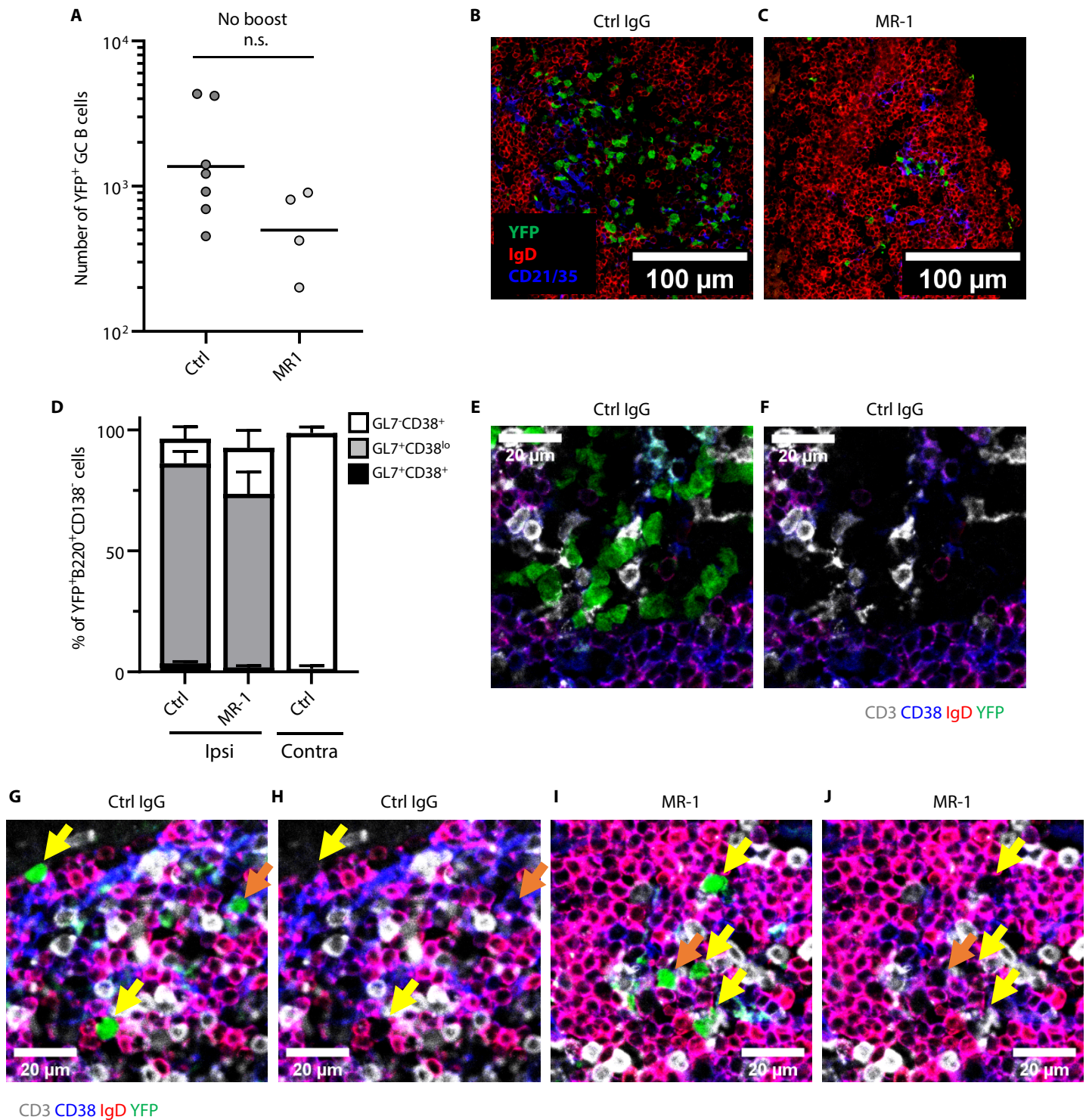
Figure S4. Tracing fates of the progeny of primary GC B cells after late ablation of GC structures (no boost)
AID-Cre-EYFP mice that had received primary immunizations and tamoxifen were either untreated or treated *i.v.* with control IgGs or MR-1 antibodies 4 weeks after the priming. Four to five weeks later, YFP+ B cells were assessed by flow cytometry (**A** and **D**) and by immunofluorescence (**B**, **C**, **E-J**). Each dot in (**A**) represents an individual mouse. Horizontal bars, geometric mean. n.s., $p = 0.072$ by Mann-Whitney's U test. (**D**) Proportion of GL-7−CD38+, GL-7+CD38−, and GL-7+CD38+ cells among all YFP+B220+CD138− cells. Error bars, SEM, n = 7, 4, and 4 for Ctrl and MR-1 (ipsilateral boosts), and Ctrl (contralateral boosts) groups, respectively. Detection of YFP+IgD− cells in association with an FDC network in control (**B**) and MR-1 treated LN (**C**). Detection of YFP+IgD−CD38lo cells (yellow arrows) and YFP+IgD−CD38+ cells (orange arrows) within a GC-like structure in control LN (**E** and **F**), B-cell follicles in control (**G** and **H**) and MR1 treated LNs (**I** and **J**). Images in **F**, **H**, and **J**, respectively, are the same images as those in **E**, **G**, and **I** without showing YFP+ signals.
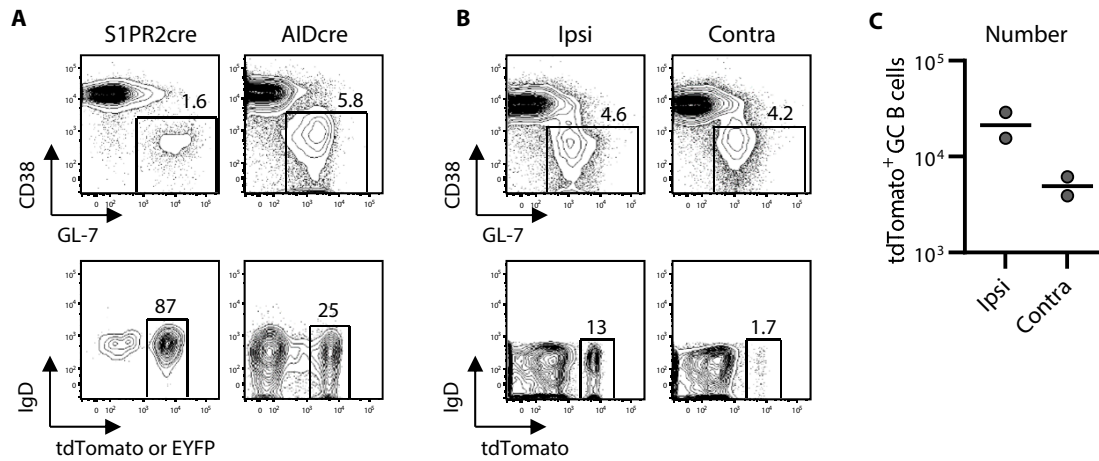
Figure S5. Tracing the fates of the progeny of primary GC B cells in *S1pr2*-ERT2cre-tdTomato mice
Participation of the progeny of primary GC B cells was assessed in fate-mapping mouse models. *S1pr2*-ERT2cre-tdTomato mice or AID-Cre-EYFP mice were primed with H1 SI-06, injected with tamoxifen (d8-d12). (**A**) Representative flow diagrams of GL-7 and CD38 expressions on B220+CD138- cells (top panels) and of tdTomato (left) or EYFP (right) and IgD expressions on B220+CD138-GL-7+CD38lo GC B cells (d14 primary, bottom panels) in *S1pr2*-ERT2cre-tdTomato mice (left) and in AID-Cre-EYFP mice (right). (**B** and **C**) Ten weeks after the priming, *S1pr2*-ERT2cre-tdTomato mice were boosted with homologous HAs (H1 SI-06) ipsilaterally or contralaterally. Mice were analyzed 8 days after boosts (see also figure legend for Fig. 4). Representative flow diagrams of GL-7 and CD38 expressions on B220+CD138- cells (top panels) and of tdTomato and IgD expressions on B220+CD138-GL-7+CD38lo GC B cells (**B**), and number of tdTomato+ secondary GC B cells following ipsilateral or contralateral boosts (**C**). Each dot represents an individual mouse. Horizontal bars, geometric mean.
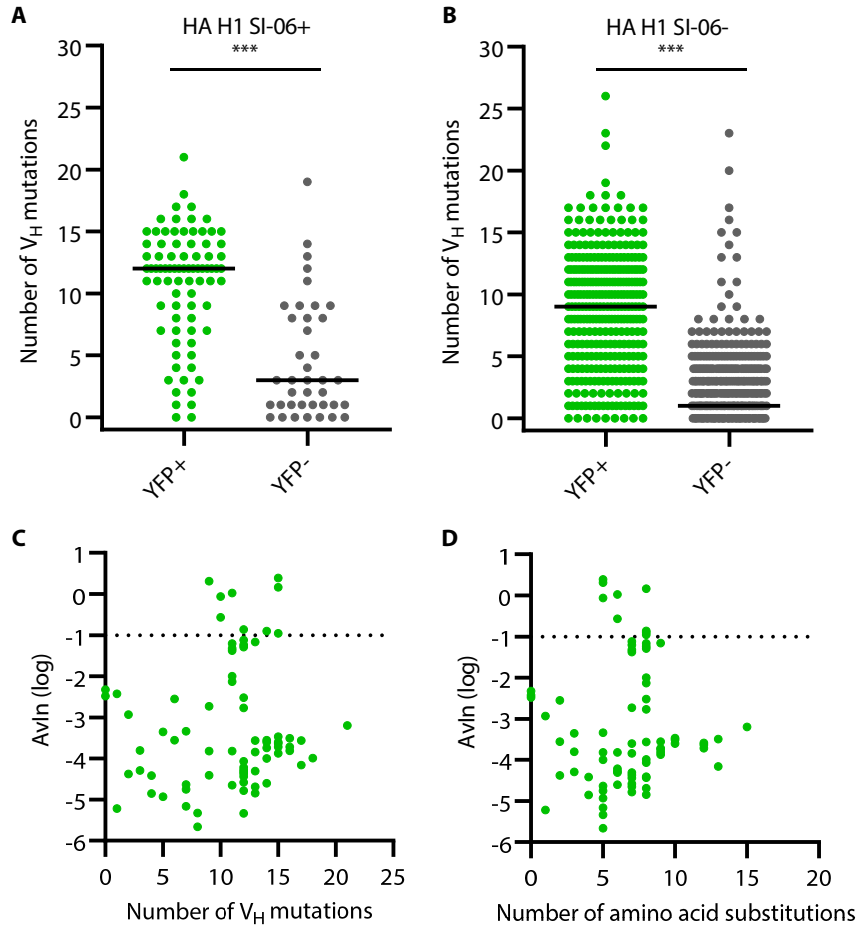
Figure S6. SHM of YFP+ secondary GC B cells in AID-Cre-EYFP mice
(**A** and **B**) Samples shown in Fig. 4D were split by the HA H1 SI-06 reactivity of respective clonal IgGs in culture supernatants. Distribution of the number of V_H point mutations recovered from HA-binding YFP+ (n = 82) and YFP- (n = 39) secondary GC B cells (**A**) and HA non-binding YFP+ (n = 317) and YFP- (n = 579) secondary GC B cells (**B**) following ipsilateral boosts. Horizontal bars represent mean. ∗∗∗, $p < 0.001$ by Mann-Whitney's U test. (**C**) Number of V_H mutations and AvIn values of respective clonal IgGs for HA-binding YFP+ secondary GC B cells (n = 82) were co-plotted. Horizontal dotted line represents AvIn = 0.1, which we consider high avidity. There are no significant correlation between number of V_H mutations and AvIn value (r = 0.14 and $p = 0.22$ by nonparametric Spearman correlation). (**D**) As in (**C**) with the number of amino acid substitutions. There are no significant correlation between number of V_H mutations and AvIn value (r = 0.18 and $p = 0.09$ by nonparametric Spearman correlation).

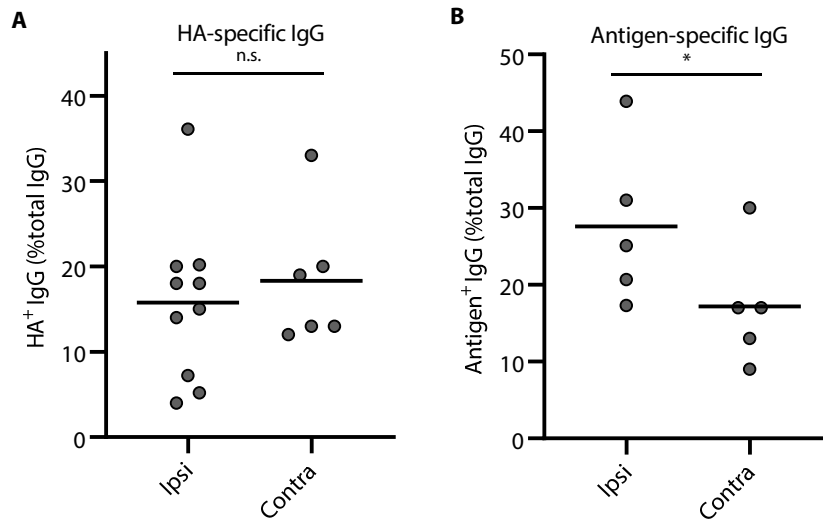**A** HA-specific IgG

B Antigen-specific IgG

Figure S7. Frequency of antigen-specific IgGs among recall GC B cells
B6 mice were primed with H1 SI-06, and then 8-10 weeks later boosted with either H3 X31 (**A**) or rPA (**B**) ipsilaterally or contralaterally. Eight days following boosts, Nojima cultures were established for secondary GC B cells. After culture, HA-reactivity was determined for each clonal IgG by Luminex assay. Frequency of HA-specific IgGs among all clonal IgGs was calculated for each mouse sample, which is represented by each dot. *, $p < 0.05$; n.s., $p > 0.05$ by Mann-Whitney's U test. Combined data from 4 (for **A**) and 2 (for **B**) independent experiments are shown.