GIMAP1 is essential for the survival of naïve and activated B cells *in vivo*

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Abstract

An effective immune system depends upon regulation of lymphocyte function and homeostasis. In recent years members of the GTPases of the Immune Associated Protein (GIMAP) family have been proposed to regulate T cell homeostasis. In contrast, little is known about their function and

15). In GIMAP5-deficient rats, T cell development appears to occur normally within the thymus but there are few T cells in the periphery (14, 15, 24, 31, 32). This has been attributed to spontaneous apoptosis of T cells, although the mechanism by which this occurs remains unclear (24) (32) (33). Recent work has suggested that T cell death may result from the inability of their mitochondria to sequester Ca^{2+} following capacitative entry (28). A similar paucity of peripheral T cells is seen in GIMAP5-deficient mice, which develop spontaneous colitis, resulting in early mortality (23, 26, 27). Deficiency in *Gimap5* in mice affects various haematopoietic cell types (23, 27, 34), and can lead to a progressive multilineage failure of bone marrow hematopoiesis (34). Knowledge of the extent to which these effects are cell-intrinsic awaits the use of conditional alleles in the study of *Gimap5*.

GIMAP5's close relative GIMAP1 is also required for the maintenance of peripheral T cells. Previously, we showed that conditional deletion of *Gimap1* from lymphocyte progenitors using *CD2Cre* (*Gimap1*$^{f/f}$*CD2Cre*$^{+}$ mice), resulted in normal lymphocyte development but severe reductions in peripheral T cell numbers (22). Surprisingly, we also found a profound deficit of mature peripheral B cells. This study did not address GIMAP1 function in activated B cells. To date, the role GIMAPs might play in the survival of activated lymphocytes remains unresolved. Whereas GIMAP5-deficient rat T cells can be activated successfully via their antigen receptors, GIMAP5-deficient mouse T cells were reported to be unable to proliferate in response to

mice (previously described (22)) were crossed with Eμ-*bcl-2-36* transgenic mice expressing human Bcl2 (39) to generate *Gimap1*$^{f/f}$*Cd2*$^{cre/+}$*Bcl2*^{tg}.

Mice were immunised i.p. with 100μg of 4-hydroxy-3-nitrophenylacetyl NP-19-keyhole

PCR analysis

Lysates were prepared from FACS-purified GC and follicular B cells and PCR for GIMAP1 and GIMAP8 performed as previously described (22, 30).

Western blot analysis

FACS-purified GC and follicular B cells were lysed in NP-40 lysis buffer and GIMAP1 and actin protein detected by western blot as previously described (22).

iGC B cell culture

was seen within 2 days of *in vitro*

To investigate why GC B cells failed to develop in the absence of GIMAP1, we examined their proliferation and death. EdU incorporation into newly synthesized DNA was used to measure GC B cell proliferation on day 8 p.i. (Figure 6B). There was a slight but significant decrease in the proliferation of these cells in *Gimap1*$^{f/f}$*AicdaCre*$^{+}$ mice compared to controls. To look at cell death in GC B cells, we measured the percentage of GC B cells expressing active caspase-3 by flow cytometry. We found a small but significant difference in the percentage of GC B cells expressing active caspase-3 in cells from *Gimap1*$^{f/f}$*AicdaCre*$^{+}$ cells (Figure 6C).

To confirm our *in vivo* data, we made use of an *in vitro* system to generate induced GC (iGC) B cells (41). B cells were purified from spleens and cultured with fibroblasts

this homeostasis may offer opportunities for the selective ablation of lymphocyte pools as a therapeutic strategy. Over the last 15 years members of the GIMAP family have been implicated as important modulators of peripheral T lymphocyte homeostasis and survival. Very few studies have addressed the role of GIMAPs in B cell biology. We have shown that deletion of GIMAP8 results in a reduction in the number of recirculating B cells in the bone marrow (30). The generation and characterization of GIMAP1- and GIMAP5-deficient mouse strains has further demonstrated that peripheral B cell survival is also influenced by GIMAPs (22, 23, 27, 34). As observed for T cells, deletion of *Gimap1* appears to affect only mature cells within the B cell lineage (22

In summary, this work shows that GIMAP1 is required for the establishment and maintenance of the peripheral B cell pool and for all stages of post-activation B cell survival. In the absence of GIMAP1 mature B cells die, irrespective of their activation status or function. Together with our previous work, this establishes GIMAP1 as a key pro-survival factor for mature B lymphocytes and a potential target for the control of B cell mediated diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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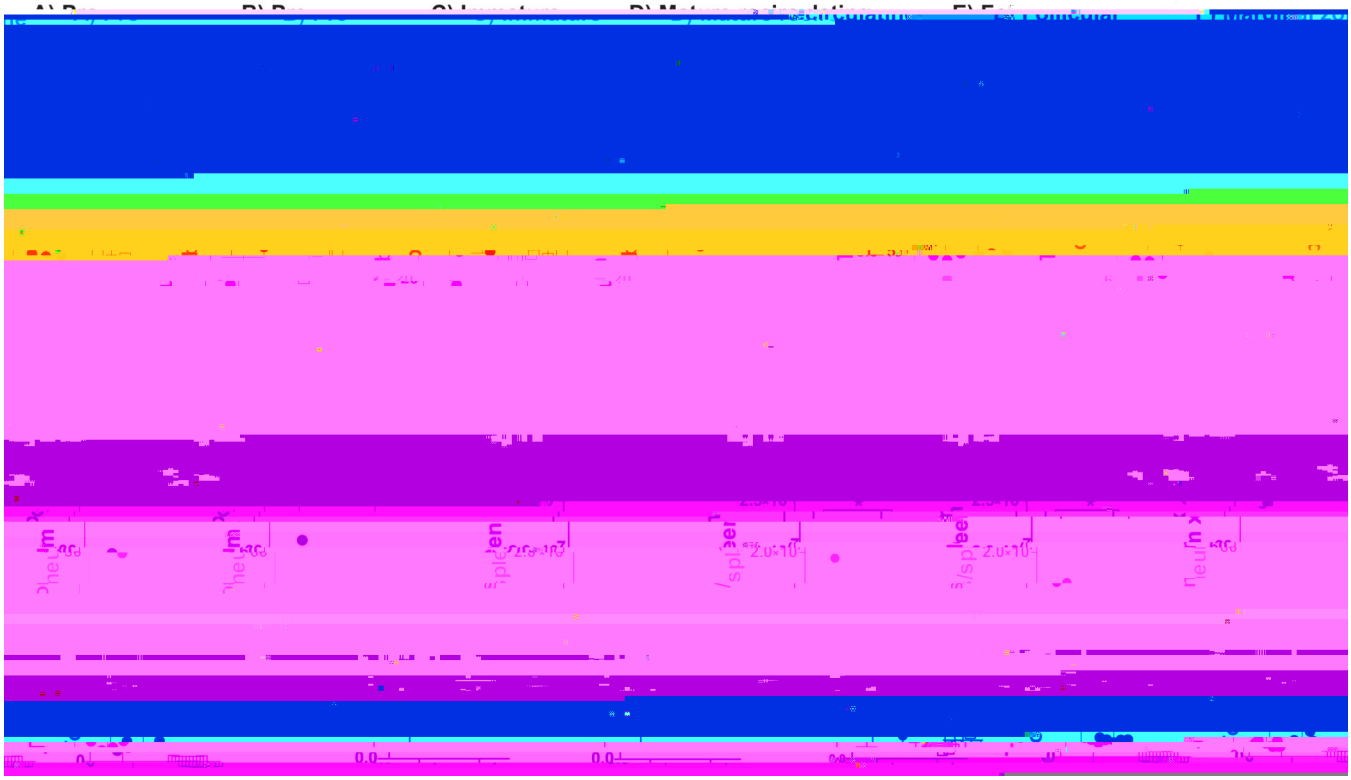

Figure 1. Cell intrinsic requirement for GIMAP1 in mature B cells

B cell subsets present in bone marrow (A-D), spleen (E-F, J-L), and peritoneal cavity (G-I) from *Gimap1*$^{f/f}$*CD79aCre*$^{+}$ mice and age- and sex-matched *Gimap1*$^{f/f}$ controls were enumerated (A-I). Gating is as previously described (22). Results show the number of cells/organ for individual mice with the mean ± S.D. (*Gimap1*$^{fl/fl}$ [●] and *Gimap1*$^{fl/fl}$*CD79aCre*$^{+}$[□]. Results show the number of cells/organ for individual mice with the mean ± S.D. *p <0.05, ****p<0.00005 (unpaired 2-tailed Student's t test).

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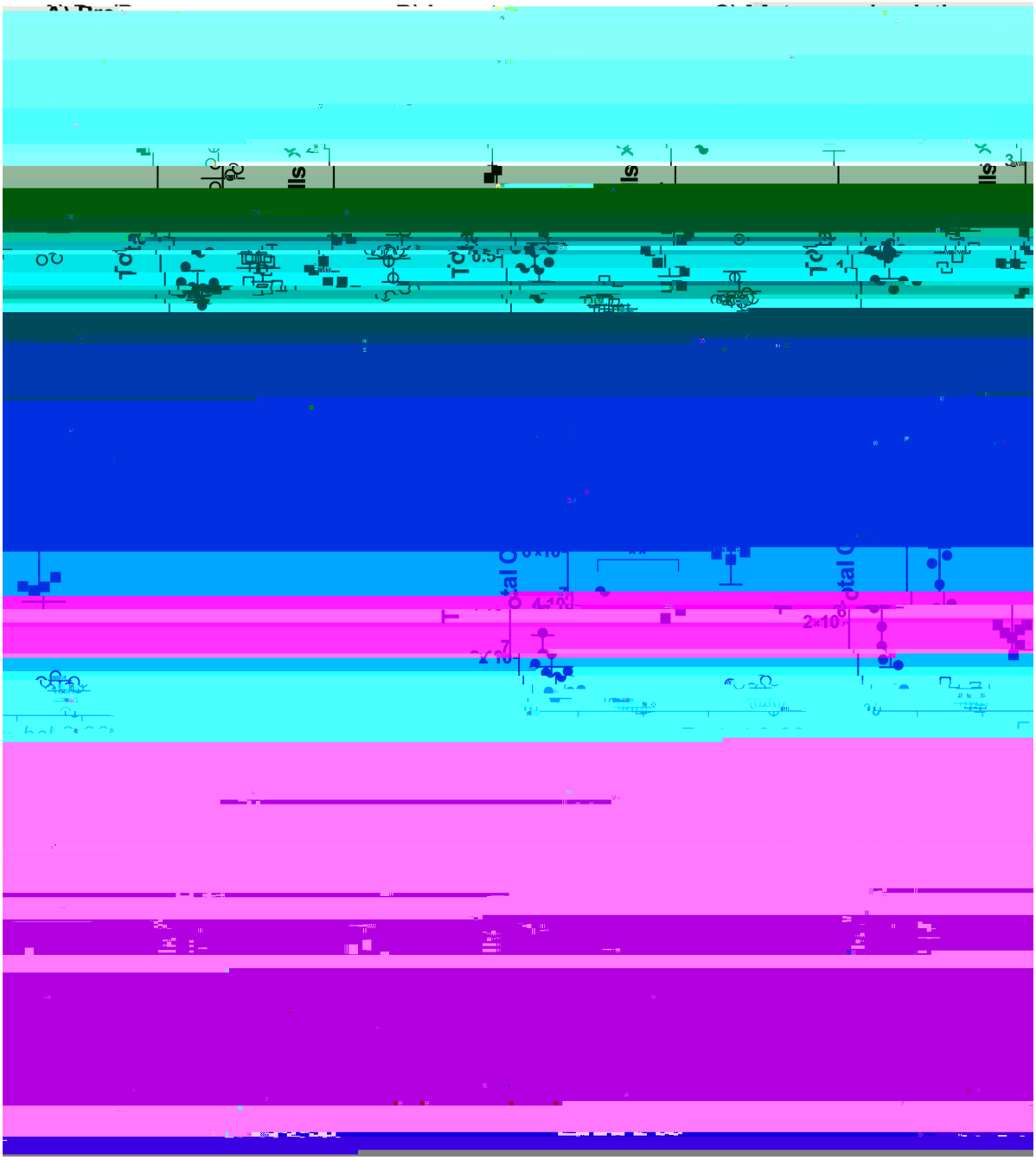

Figure 2. Bcl2 does not prevent cell death in GIMAP1-deficeint B cells

B cell subsets present in bone marrow (A-C), spleen (D-E), and peritoneal cavity (F-H) from *Gimap1*$^{fl/fl}$ [●], *Gimap1*$^{f/f}$*CD2Cre*$^{+}$ [□], *Gimap1*$^{fl/fl}$*Bcl2*^{tg} [■], and *Gimap1*$^{f/f}$*CD2Cre*$^{+}$*Bcl2*^{tg} [○] from age- and sex-matched mice were enumerated (A-H), using previously described gating (22). Results show the number of cells/organ for individual mice with the mean ± S.D. *p <0.05, **p <0.005, ***p <0.0005, ****p<0.00005 (unpaired 2-tailed Student's t test).

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Figure 3. Mature B cells require GIMAP1 for their survival in the periphery

Lymph node cells from *Gimap1*$^{fl/fl}$*ER*^{T2}*Cre*$^{+}$ and *ER*^{T2}*Cre*$^{+}$ mice were stained with CFSE and CTV, respectively, mixed in a 1:2 ratio (*Gimap1*$^{fl/fl}$*ER*^{T2}*Cre*$^{+}$:*ER*^{T2}*Cre*$^{+}$) and injected into replete B6.SJL-*Ptprca Pepcb*/BoyJ (CD45.1^{+ve}) mice. Mice were treated with either vehicle control or tamoxifen and spleen and blood harvested 13 days later. (A-B) shows representative flow cytometry plots from spleen gated on transferred (CD45.2^{+ve}B220^{+ve}) cells from vehicle (A) or tamoxifen (B) treated mice. (C-D) shows the percentages of adoptively transferred *Gimap1*$^{fl/fl}$*ER*^{T2}*Cre*$^{+}$ (C) and *ER*^{T2}*Cre*$^{+}$ (D) B220^{+} cells remaining in

the spleen 13 days after vehicle or tamoxifen treatment of individual recipient mice. Each symbol represents the percentage of transferred cells remaining from an individual mouse

Figure 4. GIMAP1 expression in germinal center B cells

(A) Relative expression of GIMAP1 mRNA in immune cells (adapted from http://www.immgen.org/). B.Fo.Sp – splenic follicular B cells; B.GC.Sp – splenic germinal center B cells; B.Mz.Sp – splenic marginal zone B cells; B1a.PC – peritoneal cavity B1 B cells. (B) Western blot detection of GIMAP1 protein in follicular and GC B cells from wt mice.

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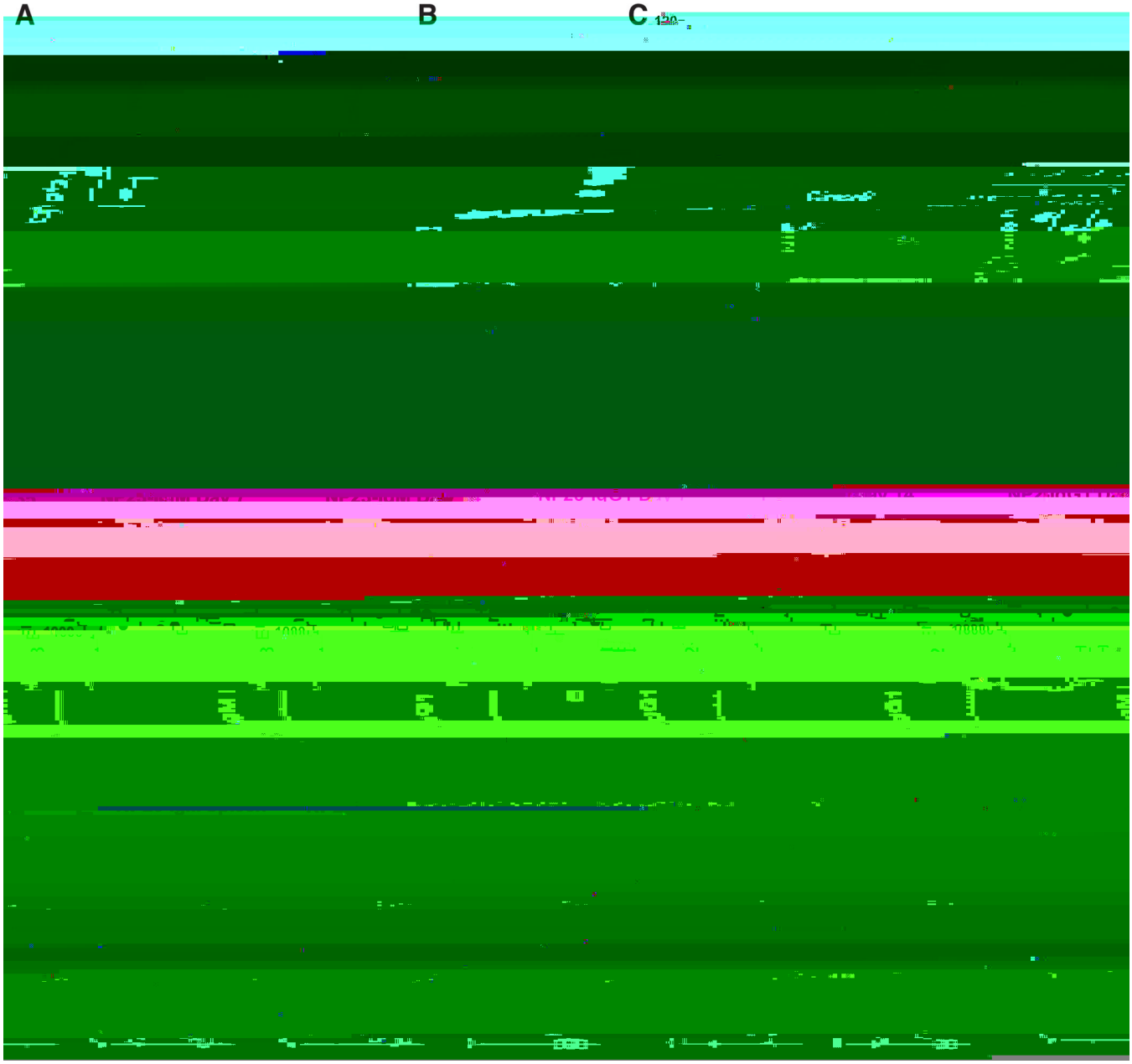

Figure 5. GIMAP1 is essential for germinal center B cell responses

(A) Conditional deletion of *Gimap1* in germinal center B cells. PCR analysis of *Gimap1* and *Gimap8* in germinal center B cells FACS-sorted from immunized *Gimap1*$^{fl/fl}$*AicdaCre*$^{+}$ and *Gimap1*$^{fl/fl}$ mice. Lane 1 = 100bp DNA ladder; lane 2 = follicular B cells from control *Gimap1*$^{fl/fl}$ mice; lane 3 = germinal center B cells from control *Gimap1*$^{fl/fl}$ mice; lane 4 = follicular B cells from *Gimap1*$^{fl/fl}$*AicdaCre*$^{+}$ mice; lane 5 = germinal center B cells from *Gimap1*$^{fl/fl}$*AicdaCre*$^{+}$ mice; lane 6 = H_2O control. (B) Facsplots showing NIP-binding IgG1-switched B cells in *Gimap1*$^{fl/fl}$*AicdaCre*$^{+}$ and *Gimap1*$^{fl/fl}$ mice on day 7 p.i. (C) Enumeration of NIP-binding IgG1-switched (B220^{+ve}IgM^{-ve}IgD^{-ve}) B cells on day 7 p.i. in *Gimap1*$^{fl/fl}$ (●) and *Gimap1*$^{fl/fl}$*AicdaCre*$^{+}$ (□). Results show the number of cells per spleen

for individual mice with the mean ± S.D. (D) Titres of NP23-binding (low affinity) and NP2-binding (high affinity) IgG1 and IgM antibodies on days 7 and 14 after primary immunization. Each symbol represents an individual mouse (*Gimap1*$^{fl/fl}$ (●) and *Gimap1*$^{fl/fl}$*AicdaCre*$^{+}$ (□) with the mean ± S.D. shown. (E) The frequency of NP-specific IgG1 and IgM ASC from

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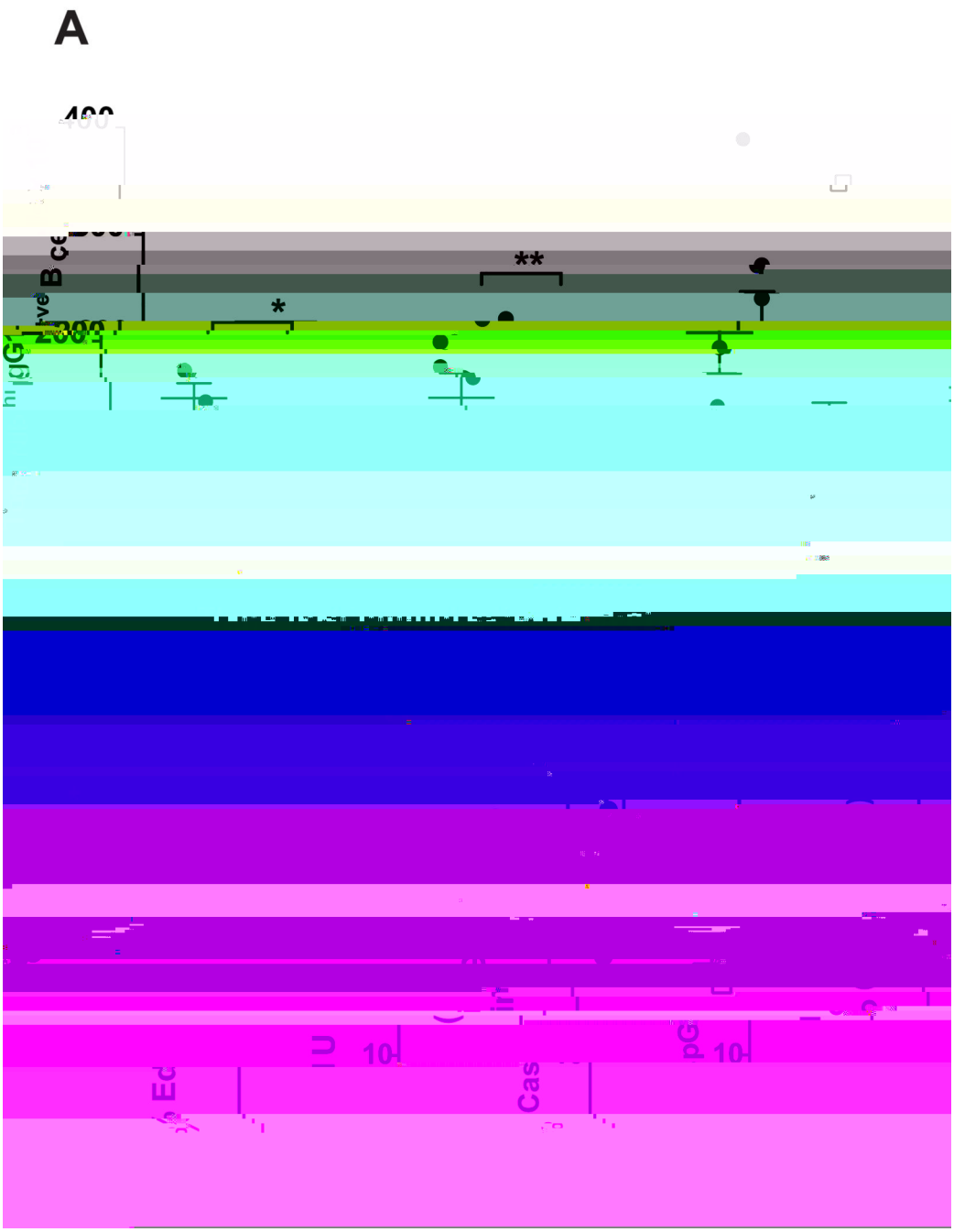

Figure 6. Germinal center responses fail to develop in the absence of GIMAP1

Results show values for individual mice with the mean ± S.D. also shown: *Gimap1*$^{fl/fl}$*AicdaCre*$^{+}$ (●); and control *Gimap1*$^{fl/fl}$ (□) mice. (A) NIP-binding, IgG1^{+}, CD38^{-} B cells on days 6, 8, and 10 p.i. (B) Percentages of GC cells that had incorporated EdU (data for individual mice). (C) Percentages of GC B cells that were positive for active caspase-3. Differences were examined using Student's *t* test and only significant differences are marked (* $p<0.05$, ** $p<0.005$).

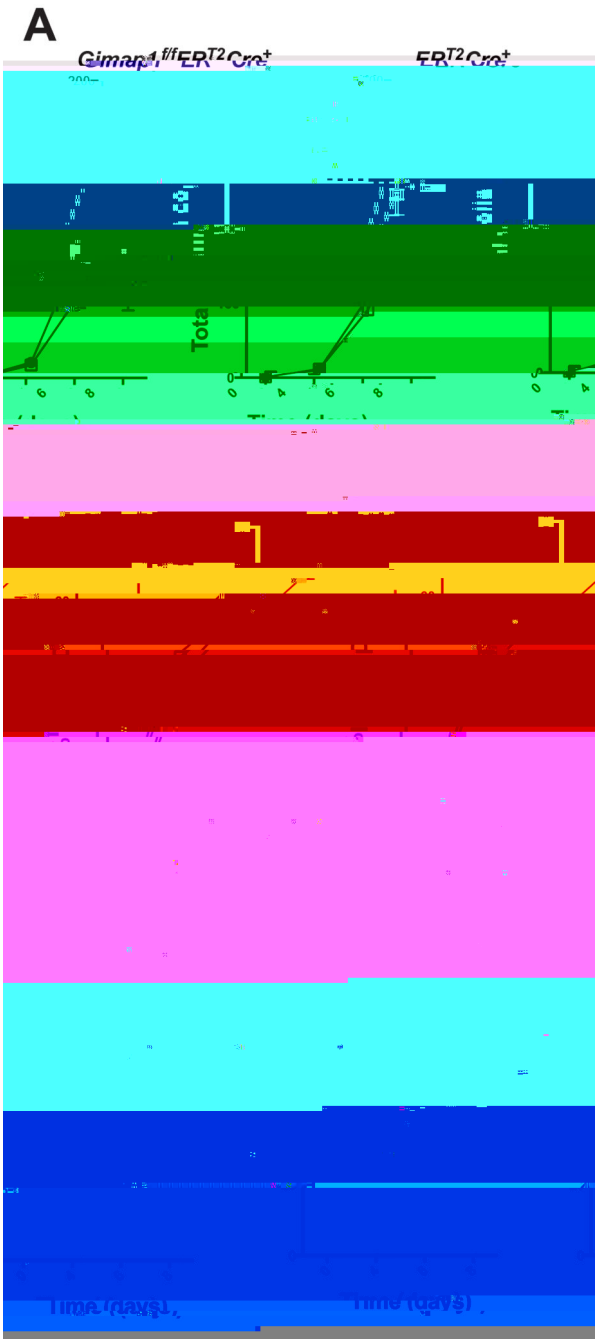

Figure 7. *In vitro* generation of iGC and PCs is compromised by lack of GIMAP1
B cells from *Gimap1*$^{f/f}$*ER*^{T2}*Cre*$^{+}$ and *ER*^{T2}*Cre*$^{+}$ mice were cultured to induce GC and PC differentiation in the presence of either 4-OHT or vehicle control. Cells were harvested on days 4, 6, and 8, counted and stained with anti-IgG1, anti-CD138 and DAPI. Panel (A) shows the total number of PC generated from *Gimap1*$^{f/f}$*ER*^{T2}*Cre*$^{+}$ and *ER*^{T2}*Cre*$^{+}$ B cells in the presence of 4-OHT (□) or vehicle (●

Gimap1$^{f/f}$*ER*^{T2}*Cre*$^{+}$ and *ER*^{T2}*Cre*$^{+}$ B cells in the presence of 4-OHT (□) or vehicle (●). Each symbol represents an individual mouse with the mean ± S.D. shown. Differences were examined using an unpaired Student's *t*

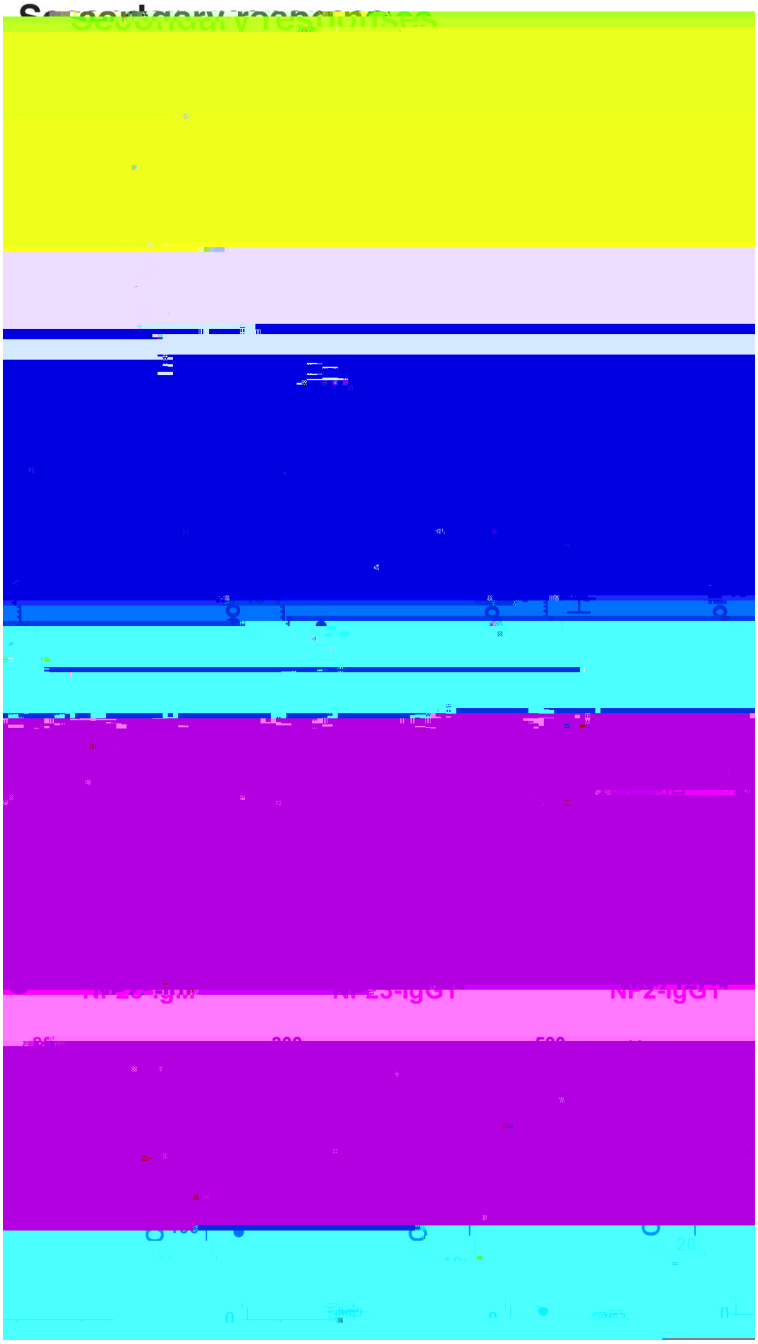

Figure 8. Failure to establish B cell memory in the absence of GIMAP1

shown. Differences were examined using an unpaired Student's *t* test and only significant differences are marked (* $p<0.05$, ** $p<0.005$, ***<0.0005).

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