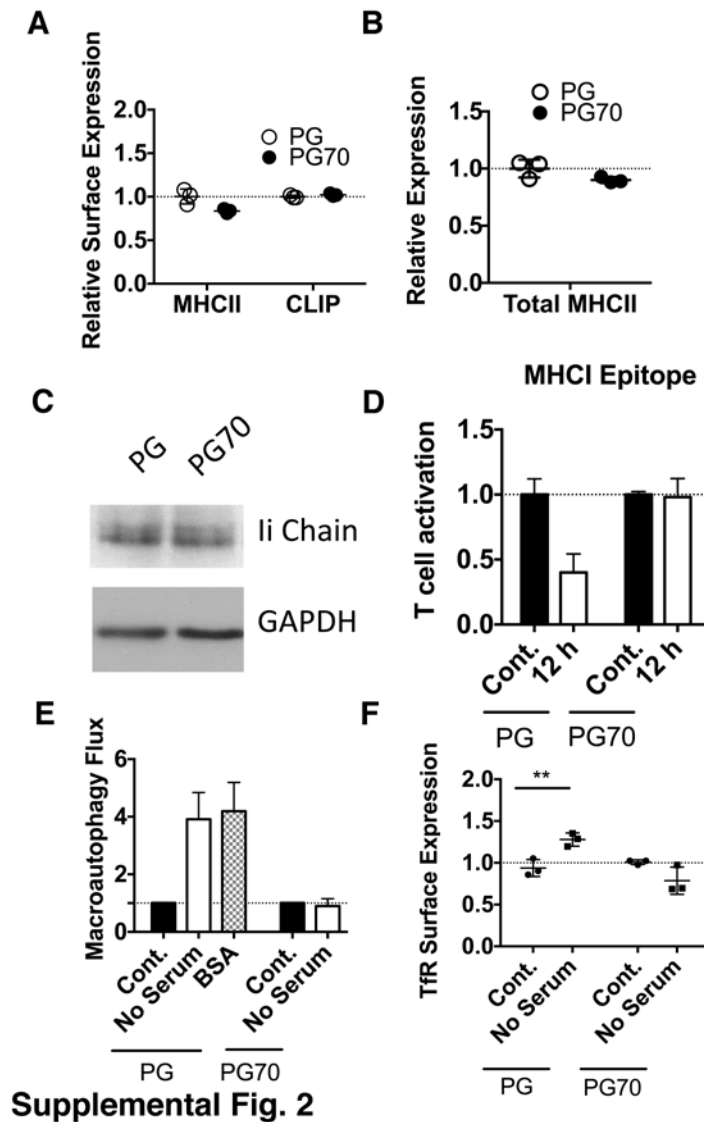


Supplemental Fig. 1

Supplemental Figure 1. (A) B cells were cultured + (Cont.) or – serum for up to 12 h in the presence of the protease inhibitor 500 μ M leupeptin. Cells were harvested and lysed for

immunoblot analysis of invariant chain and GAPDH. **(B-C)** Relative mRNA levels of *IGKC*, BCR light chain **(B)** and components of the MHCII pathway *DRa* (MHCII), *CD74* (invariant chain), *DMb*, and *DOb* **(C)** detected in B lymphoblasts cultured in serum-free media for 6 or 12 h relative to Cont. cells grown in media containing 10% serum (Cont.). **(D)** B lymphoblasts cultured +/- serum for 6 or 12 h were harvested, lysed and cathepsin (CAT) B, L and H protease activity monitored using fluorescent substrates. For each cathepsin, Cont. cell enzyme activity was normalized and set to 1 (dotted line). Cathepsin activity for each cell treatment was calculated relative to enzyme activity in Cont. cells. **(E-G)** B lymphoblasts cultured +/- serum or in media supplemented with BSA for 12 h were harvested and surface stained for flow analysis of **(E)** transferrin receptor (TfR) **(F)** CD45R (B220) or **(G)** the ganglioside GM1 (cholera toxin). The **(H)** osmolarity and the **(I)** viscosity of the various media conditions used throughout the paper. **(J)** BCR surface expression on B lymphoblasts grown +/- serum or in serum-free media supplemented with sized dextrans (10 kDa, 40 kDa, or 70 kDa). BCR surface expression for Cont. cells treated with serum was set to 1 (dotted line). Relative surface BCR expression for treated cells compared to Cont. cells was graphed. **(K)** BCR surface expression for B cells grown +/- serum or in serum-free media supplemented with 1% fatty-acid free BSA or 1% boiled BSA. Cont. cell BCR surface expression was normalized to 1 (dotted line). Surface BCR expression relative to Cont. cells was graphed. **(L)** The human B lymphoblastoid cell line FrevSMA was cultured +/- serum for 12 h and MHC class II presentation of an epitope derived from the CMA substrate SMA was assessed. **(D, J and K)** *Student's T-Test, Holm-Sidak's multiple comparison to Cont. cells. **(E)** One-way ANOVA, followed by Dunnett's multiple comparison. **(H and I)** # One-way ANOVA, Dunnett's multiple comparison.



Supplemental Figure 2. PG and PG70 cells were harvested for flow analysis (**A**) of surface expression of MHCII and CLIP:MHCII complexes or (**B**) total cellular MHCII expression. (**C**) PG and PG70 cells were harvested and lysed for immunoblot analysis of invariant chain (Ii chain) and as a loading control GAPDH. (**D**) PG and PG70 cells were cultured +/- serum for 12 h and MHCII presentation of an epitope derived from the membrane Ag MHC I was monitored. (**E**) PG and PG70 cells were cultured +/- serum or in serum-free media supplemented with BSA in the presence and absence of chloroquine for 12 h prior to harvest and lysis for immunoblot

analysis of LC3II and as a loading control GAPDH. Macroautophagy flux was determined by subtracting basal levels of LC3II:GAPDH from those induced during chloroquine treatment. **(F)** PG and PG70 cells were cultured +/- serum for 12 h prior to fixation and flow analysis of surface expression of transferrin receptor (TfR). **(F)** T-test, Holm-Sidak's multiple comparison.