Supplemental information

Dietary carbohydrate, particularly glucose,

drives B cell lymphopoiesis and function

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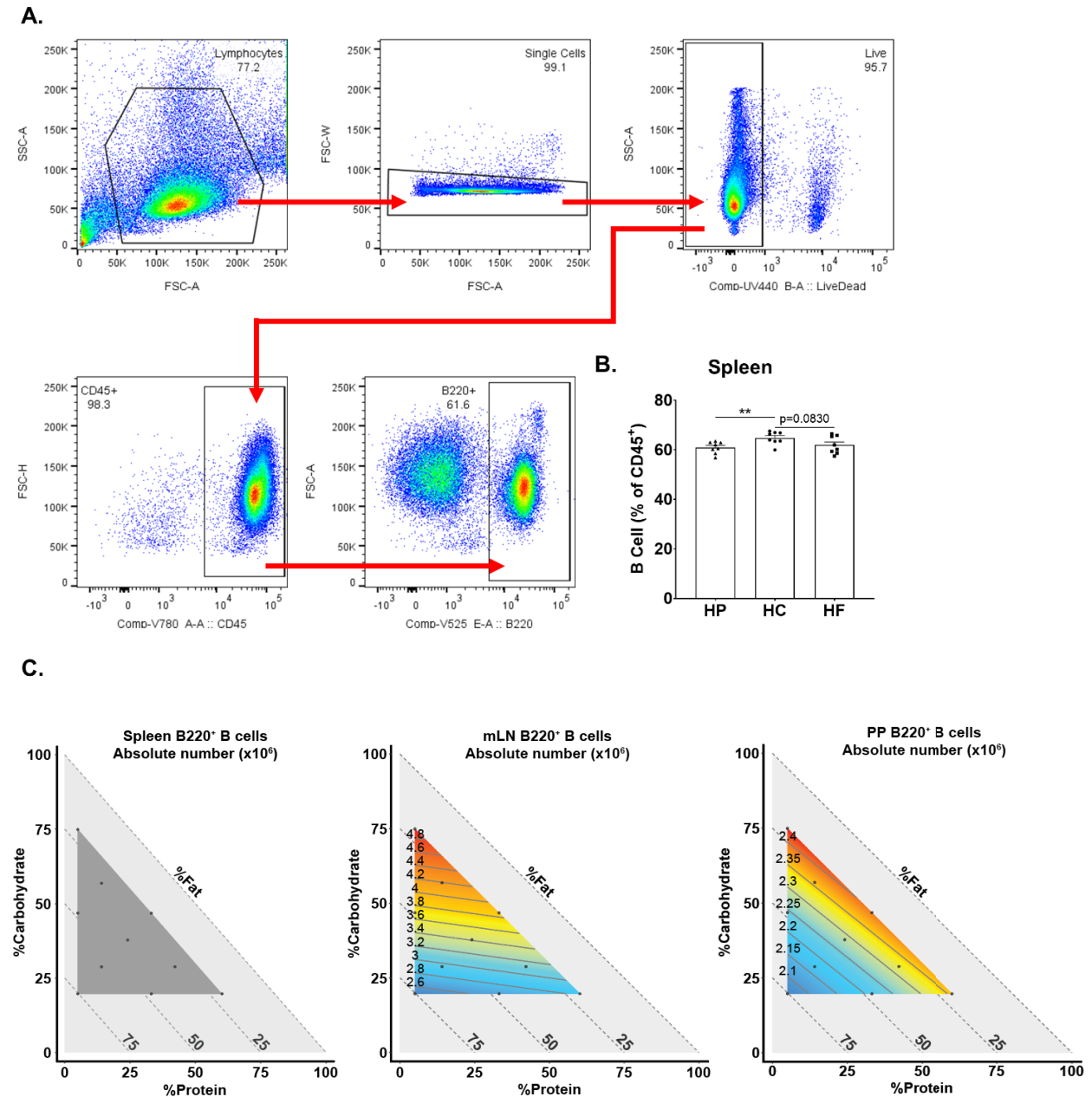**Supplemental Figures**

A.

B.

Spleen

C.

Figure S1. Gating strategy to identify B cells and the effect of high carbohydrate diet on the proportion and total numbers of B cells, related to Figure 1.

(A) Representative gating strategy used for the identification of peripheral B cells by flow cytometry. **(B)** Proportion of splenic B220$^+$ cells (of CD45$^+$ gated cells) from mice fed on a high-protein (HP), high-carbohydrate (HC) or high-fat (HF) diet for 7-8 weeks. **(C)** Mice were fed on one of ten isocaloric diets encompassing a macronutrient range of 5-60% protein, 20-75% carbohydrate, and 20-75% fat for 7-8 weeks. The mixture model, based on the total number of B220$^+$ B cells (x10^6) in the spleen, mLN and Peyer's patches (PP) was projected onto a right-angled mixture triangle (RMT) plot. A 3-component mixture

model represented on the RMT consists of carbohydrate (y-axis), protein (x-axis), and fat (diagonal), with the absolute number of B220$^+$ B cells (number within plot, expressed as x10^6 cells) as the response variables. Red and blue areas represent high and low experimental readouts, respectively. Dots within the response surface correspond to the 10 different diets used in experiments. In B, N=8 per group, mean ± SEM presented. Differences between groups were analyzed with t-test; ** $p < 0.01$
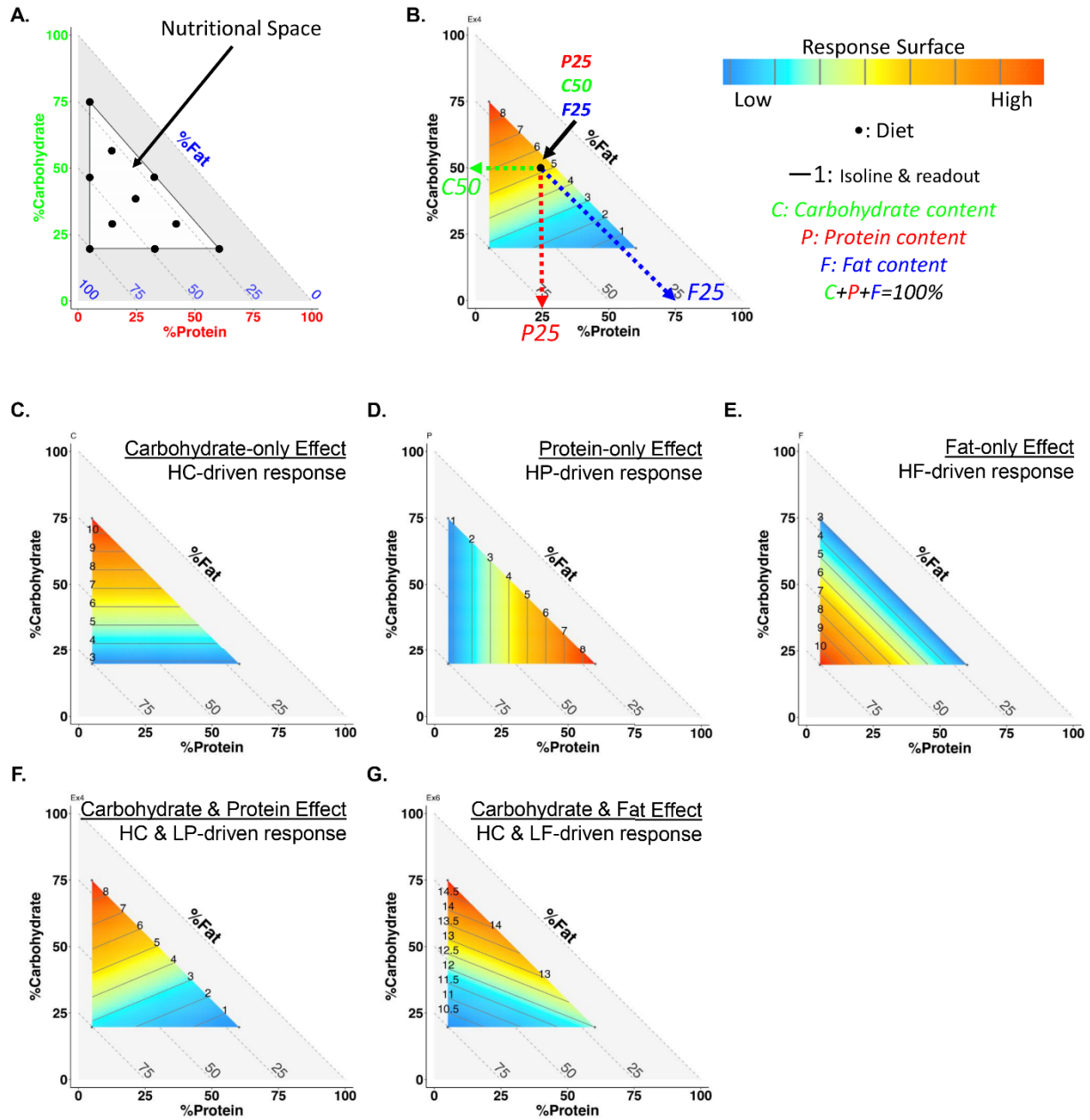
Figure S2. Interpretation of mixture model response surface on a right-angled mixture triangle plot, related to Figure 1.

(**A**) Depiction of a right-angled mixture triangle (RMT) plot, where x-axis represents protein, y-axis carbohydrate and implicit-axis (hypotenuse) fat. 10 diets with a defined ratio of protein (range: 5-60% by energy), carbohydrate (20-75%), and fat (20-75%), provide comprehensive coverage of the macronutrient nutrient space (coverage is represented by the triangle within the nutrient space where each dot represents one of the 10 diets used). (**B**) An example of mixture modelling predictions mapped onto an RMT plot. The region within the macronutrient nutrient space in red represents a high association with the modelled outcome variable, while regions in blue represent a low association with the modelled outcome variable. Values within the isoline represent the predicted value of the outcome variable within that nutrient space. Macronutrient composition at any region of the nutrient space can be derived by projecting along the three macronutrient axes. (**C**) Depiction of a response surface of an outcome variable that is driven solely by

carbohydrate content in the diet. In such cases, the isolines are perpendicular to the carbohydrate axis (y-axis) and indicate a carbohydrate-only effect. As such, changes to carbohydrate content scales the outcome variable in a linear fashion at any fixed combination of protein and fat content. (**D**) Depiction of a response surface of an outcome variable that is driven solely by protein content in the diet. Here, the isolines are perpendicular to the protein axis (x-axis), indicating a protein-only effect, and changes to protein content scales the outcome variable in a linear fashion at any fixed combination of protein and fat content. (**E**) Depiction of a response surface of an outcome variable that is driven solely by fat content in the diet. In such cases, the isolines are perpendicular to the fat axis (y-axis), indicating a fat-only effect. Changes to fat content scales the outcome variable in a linear fashion at any fixed combination of carbohydrate and protein content. (**F**) Depiction of a response surface of an outcome variable that is predominantly driven by both carbohydrate and protein in diet. In such cases, the isolines are sloped towards any point between the y- and x-axis, where the degree of slope represents the magnitude of contribution of carbohydrate vs. protein. Changes to carbohydrate content scales the outcome variable in a non-linear fashion that is dependent on the content of protein in diet. (**G**) Depiction of a response surface of an outcome variable that is predominantly driven by both carbohydrate and fat in diet. In such cases, the isolines are sloped towards any point between the y- and hypotenuse (implicit-axis), where the degree of the slope represents the magnitude of contribution of carbohydrate vs. fat. Changes to carbohydrate content scales the outcome variable in a non-linear fashion that is dependent on the content of fat in diet.
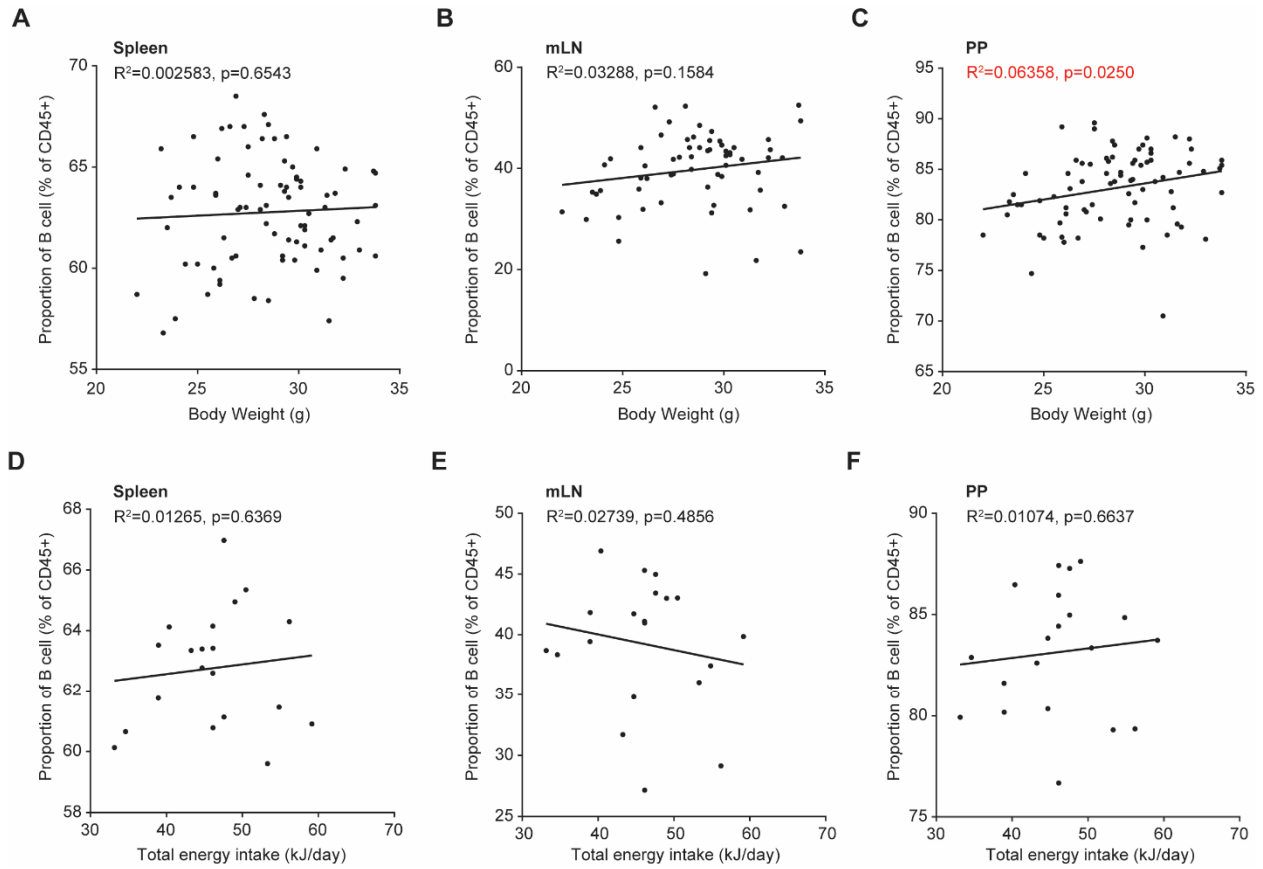
Figure S3. Linear regression of proportion of B cells and body weight or total energy intake, related to Figure 1.

Mice were fed on one of ten isocaloric diets encompassing a macronutrient range of 5-60% protein, 20-75% carbohydrate, and 20-75% fat for at least 5 weeks and total proportion and body weight was quantified at the time for cull. (A-C) Linear correlation between body weight and proportion of B cells in the (A) spleen, (B) mesenteric lymph nodes (mLN) and (C) the Peyer's patches (PP). (D-F) Linear correlation between total energy intake (kJ/day) and proportion of B cells in the (A) spleen, (B) mesenteric lymph nodes (mLN) and (C) the Peyer's patches (PP). For correlation of B cell proportion and total energy intake, each point represents averaged cage data. n=8 per group, mean ± SEM presented.
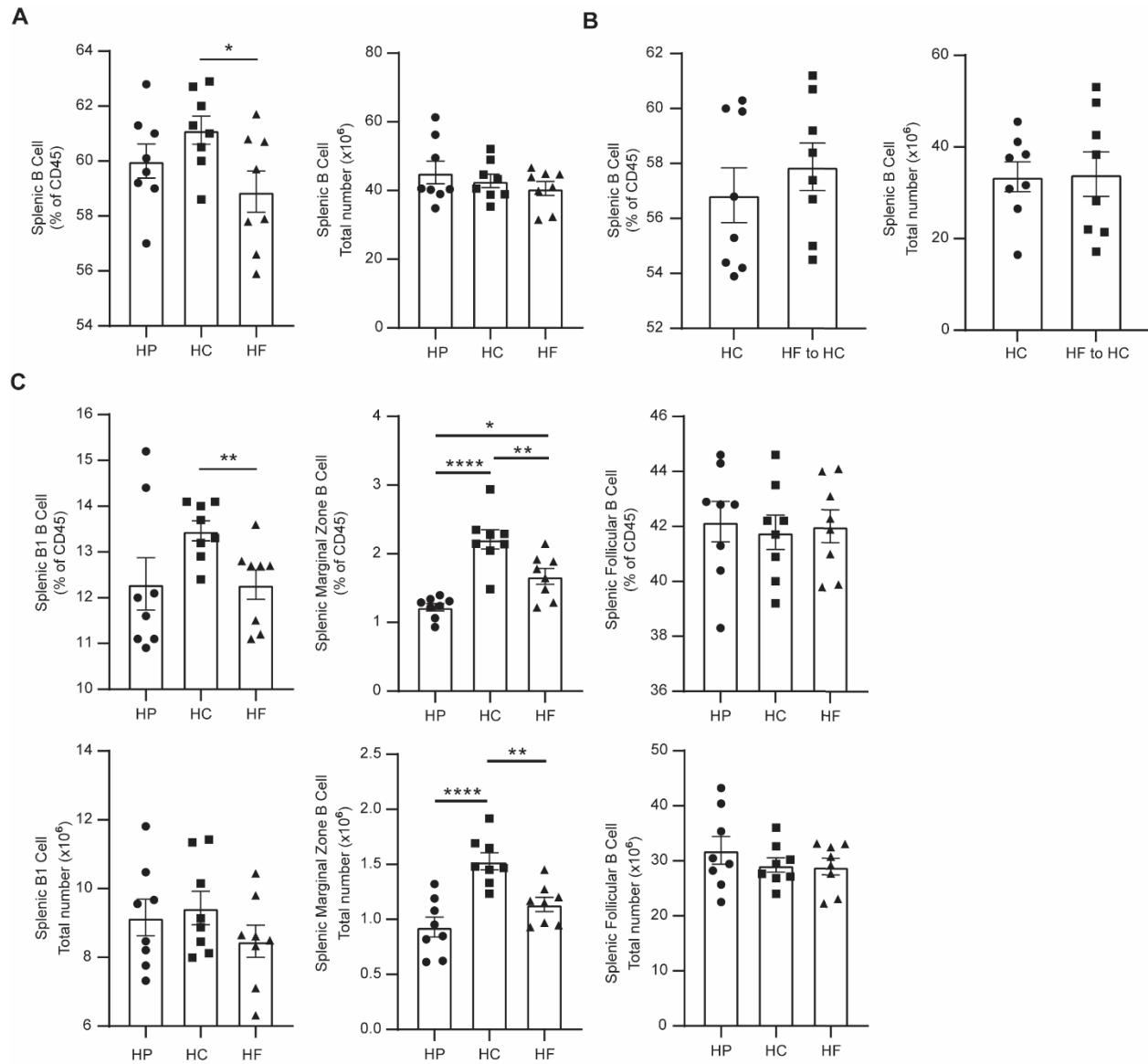
Figure S4. Carbohydrate dynamically regulates splenic marginal zone B cells, related to Figure 1

Mice were fed on either a high-protein (HP), high-carbohydrate (HC) or high-fat (HF) diet for 3 weeks and (A) the proportion of splenic B220$^+$ cells (of CD45$^+$-gated cells), as well as the absolute number of splenic B220$^+$ B cells, determined by flow cytometry. (B) Mice were fed on either HC or HF diet for 6 weeks, then maintained or switched to a HC diet for an additional 6 weeks and the proportion of splenic B220$^+$ cells (of CD45$^+$-gated cells), as well as the absolute number of splenic B220$^+$ B cells, determined by flow cytometry. (C) The proportion and absolute number of splenic B1 B cells (CD45$^+$CD19$^+$CD23$^-$CD5$^{+/-}$), marginal zone B cells (B220$^+$CD23$^-$CD1d$^+$CD21/CD35^{hi}CD5$^-$IgD^{lo}) and follicular B cells (B220$^+$CD19$^+$CD23$^+$CD1d^{lo}CD21/35^{lo}CD5$^-$IgD$^+$) were determined by flow cytometry. n=8 per group, mean ± SEM presented. Differences between two individual groups were analyzed with t-test; *p < 0.05, ** p < 0.01, **** p < 0.001
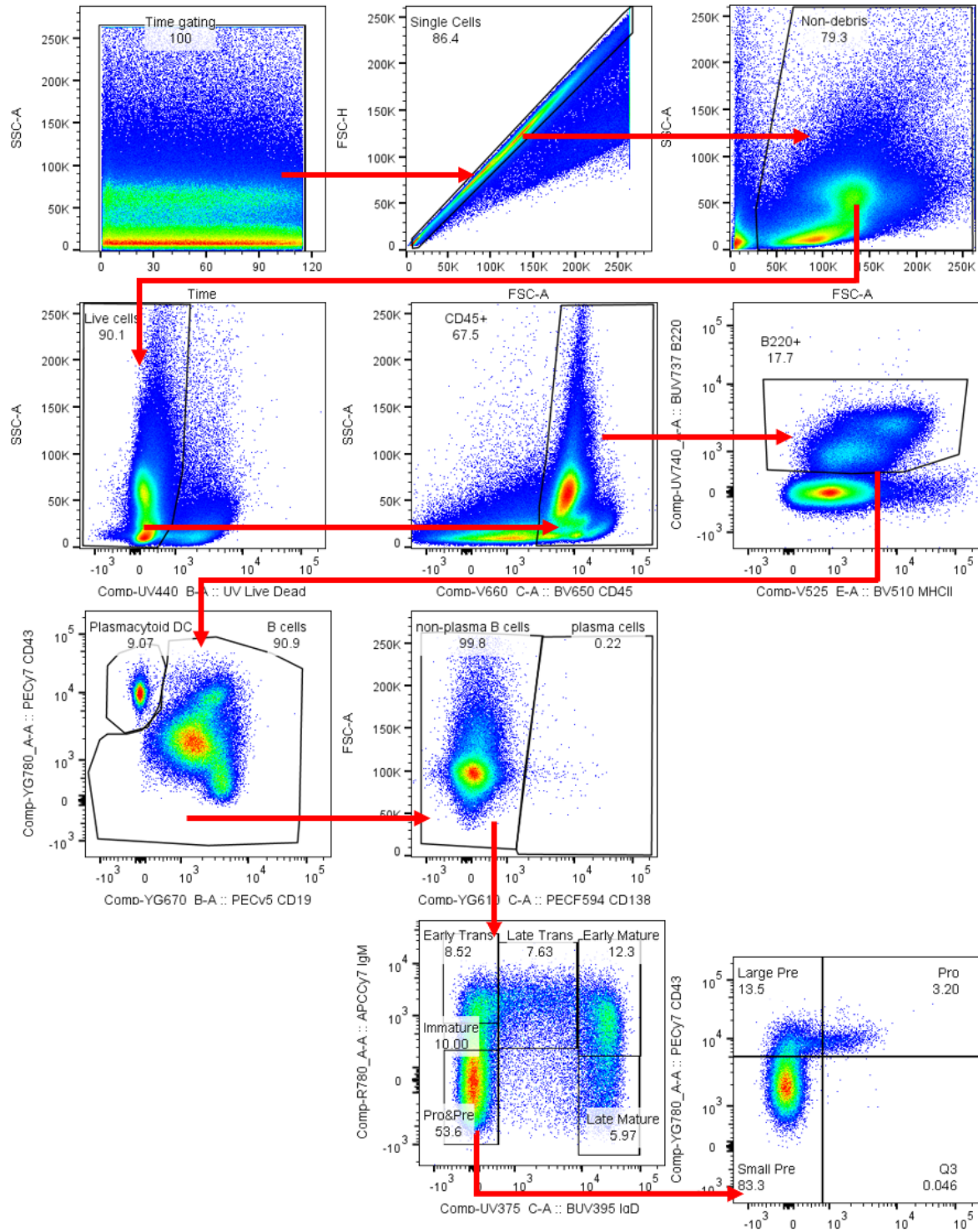
Figure S5. Gating strategy for the identification of bone marrow B cell subsets, relating to Figure 3. Representative gating strategy used for the identification of bone marrow B cell subsets.
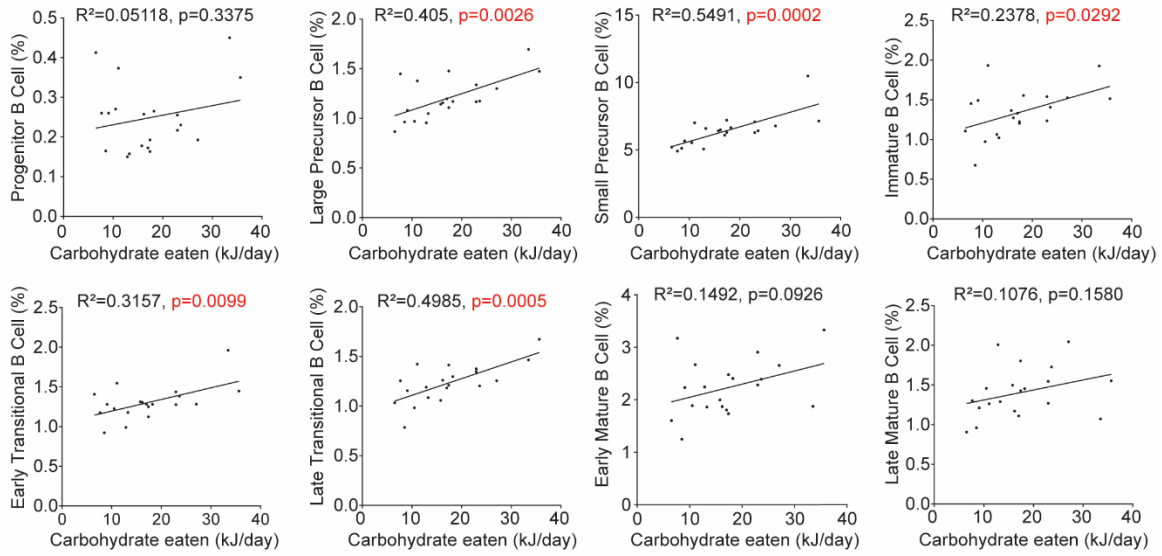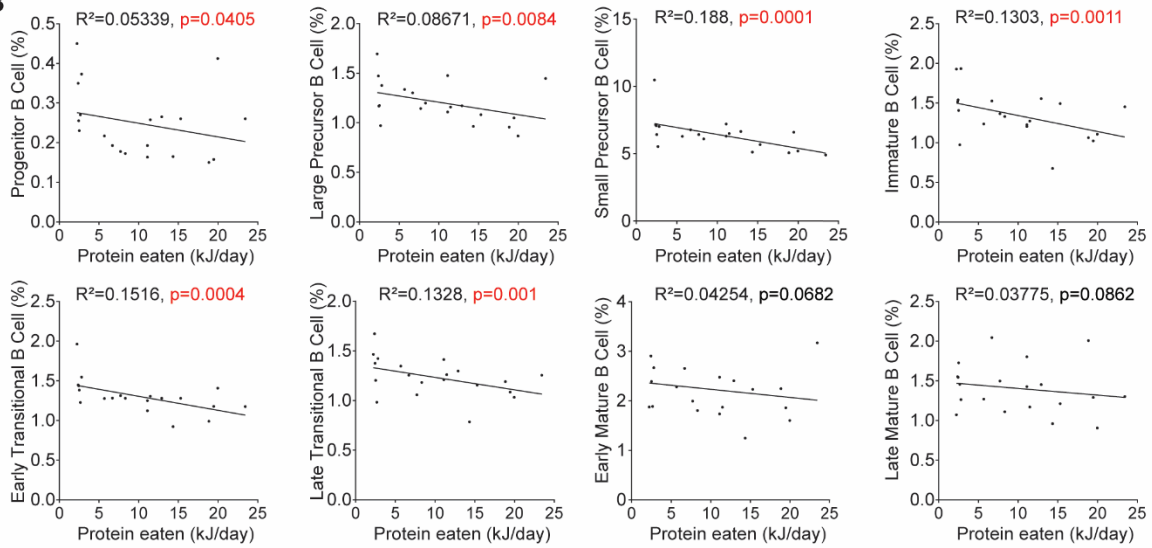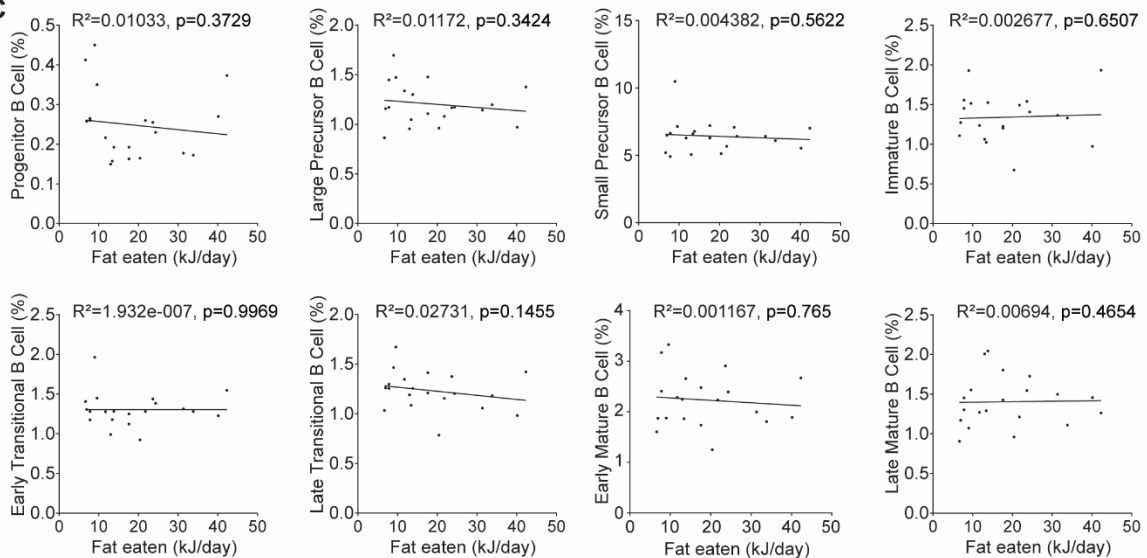
A

Progenitor B Cell (%) vs Carbohydrate eaten (kJ/day): R²=0.05118, p=0.3375

Large Precursor B Cell (%) vs Carbohydrate eaten (kJ/day): R²=0.405, p=0.0026

Small Precursor B Cell (%) vs Carbohydrate eaten (kJ/day): R²=0.5491, p=0.0002

Immature B Cell (%) vs Carbohydrate eaten (kJ/day): R²=0.2378, p=0.0292

Early Transitional B Cell (%) vs Carbohydrate eaten (kJ/day): R²=0.3157, p=0.0099

Late Transitional B Cell (%) vs Carbohydrate eaten (kJ/day): R²=0.4985, p=0.0005

Early Mature B Cell (%) vs Carbohydrate eaten (kJ/day): R²=0.1492, p=0.0926

Late Mature B Cell (%) vs Carbohydrate eaten (kJ/day): R²=0.1076, p=0.1580

B

Progenitor B Cell (%) vs Protein eaten (kJ/day): R²=0.05339, p=0.0405

Large Precursor B Cell (%) vs Protein eaten (kJ/day): R²=0.08671, p=0.0084

Small Precursor B Cell (%) vs Protein eaten (kJ/day): R²=0.188, p=0.0001

Immature B Cell (%) vs Protein eaten (kJ/day): R²=0.1303, p=0.0011

Early Transitional B Cell (%) vs Protein eaten (kJ/day): R²=0.1516, p=0.0004

Late Transitional B Cell (%) vs Protein eaten (kJ/day): R²=0.1328, p=0.001

Early Mature B Cell (%) vs Protein eaten (kJ/day): R²=0.04254, p=0.0682

Late Mature B Cell (%) vs Protein eaten (kJ/day): R²=0.03775, p=0.0862

C

Progenitor B Cell (%) vs Fat eaten (kJ/day): R²=0.01033, p=0.3729

Large Precursor B Cell (%) vs Fat eaten (kJ/day): R²=0.01172, p=0.3424

Small Precursor B Cell (%) vs Fat eaten (kJ/day): R²=0.004382, p=0.5622

Immature B Cell (%) vs Fat eaten (kJ/day): R²=0.002677, p=0.6507

Early Transitional B Cell (%) vs Fat eaten (kJ/day): R²=1.932e-007, p=0.9969

Late Transitional B Cell (%) vs Fat eaten (kJ/day): R²=0.02731, p=0.1455

Early Mature B Cell (%) vs Fat eaten (kJ/day): R²=0.001167, p=0.765

Late Mature B Cell (%) vs Fat eaten (kJ/day): R²=0.00694, p=0.4654

Figure S6. Linear regression of proportion of bone marrow B cell subsets and intake of carbohydrate, protein, and fat, related to Figure 4.

Mice were fed on one of ten isocaloric diets encompassing a macronutrient range of 5-60% protein, 20-75% carbohydrate, and 20-75% fat for at least 5 weeks. Linear regression was performed between (**A**) carbohydrate eaten, (**B**) protein eaten or (**C**) fat eaten (expressed a kilojoule (kJ) per day) and proportion of progenitor B cells, large precursor B cells, small precursor B cells, immature B cells, early transitional B cells, late transitional B cells, early mature B cells and late mature B cells. Each point represents averaged cage data.
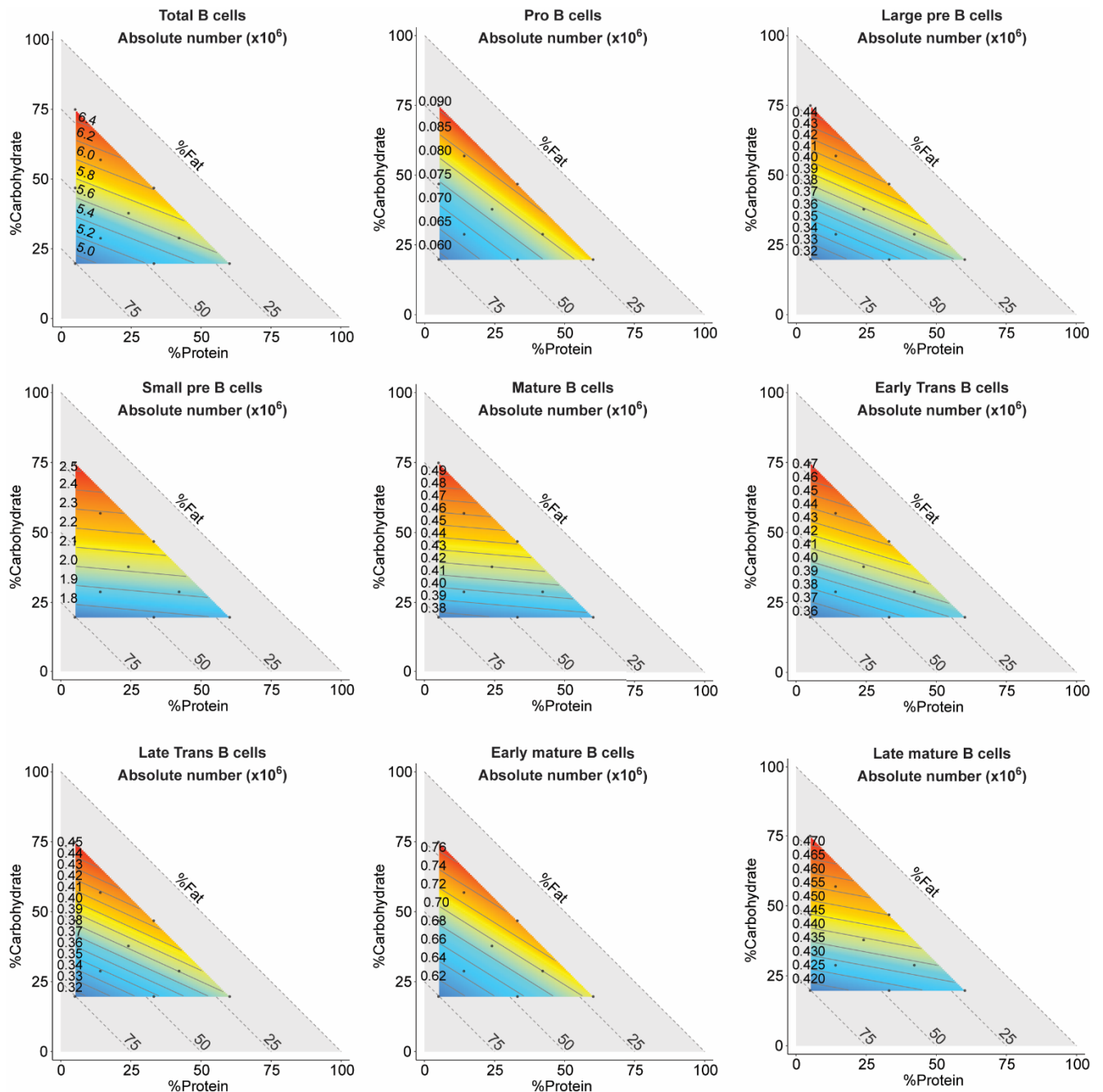
Figure S7. High carbohydrate regulates the number of B cell subsets in the bone marrow, related to Figure 4.

Mice were fed on one of ten isocaloric diets encompassing a macronutrient range of 5-60% protein, 20-75% carbohydrate, and 20-75% fat for at least 5 weeks. Effect of dietary macronutrient composition on the proportion of B220+ B cells, progenitor, large precursor, small precursor, immature, early transitional, late transitional, early mature and late mature B cells was then modelled with Scheffé mixture model and projected onto a RMT plot. The 3-component mixture model represented on the RMT consists of carbohydrate (y-axis), protein (x-axis), and fat (diagonal), with BM B cell proportion (number within plot) as the response variables. Red and blue areas represent high and low experimental readouts, respectively. Black dots distributed within the response surface correspond to 10 different diets used in experiments.
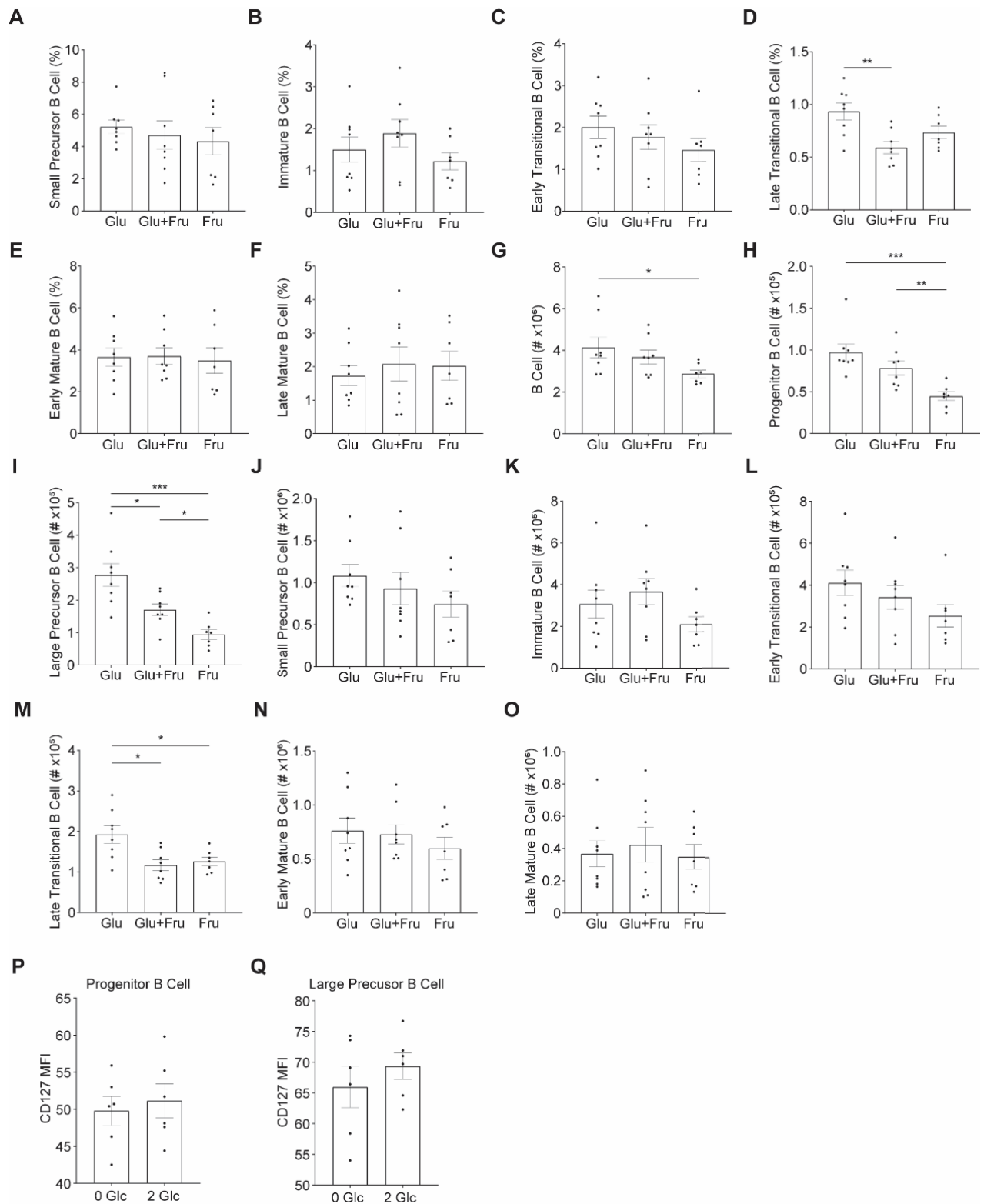
Figure S8. Dietary glucose but not fructose modulates B cell lymphopoiesis, related to Figure 5.

Mice were fed on a standard carbohydrate diet (Glu) or diets with half (Glu+Fru) or all of the glucose replaced with fructose (Fru) for at least 5 weeks and proportion of bone marrow (**A**) small precursor, (**B**)

immature, (**C**) early transitional, (**D**) late transitional, (**E**) early mature and (**F**) late mature B cells determined. Total number of bone marrow (**G**) B cells, (**H**) progenitor, (**I**) large precursor, (**J**) small precursor, (**K**) immature, (**L**) early transitional, (**M**) late transitional, (**N**) early mature and (**O**) late mature B cells were also determined. Isolated bone marrow leukocyte was cultured with no glucose (0 Glc) or 2mM glucose (2 Glc) and (**P-Q**) proportion of CD127+ cells as well as (**R-S**) median fluorescence intensity (MFI) of CD127 on progenitor and large precursor B cells determined after 24hrs. N=6-8 per group, mean ± SEM presented. Differences between groups were analyzed with t-test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
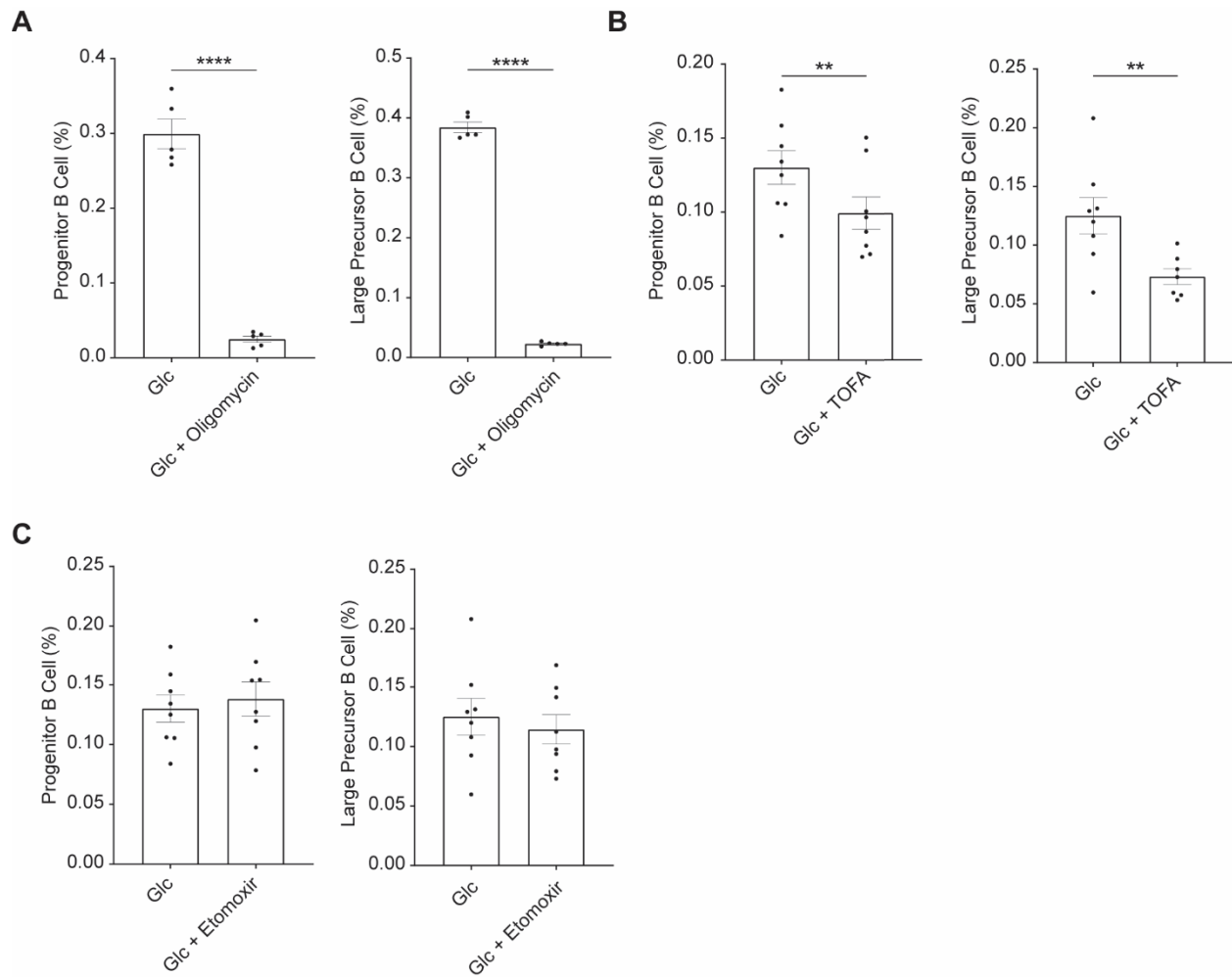
Figure S9. Glucose supports early B cell development through glycolysis and oxidative phosphorylation, related to Figure 6.

(**A**) Isolated bone marrow leukocyte was cultured under normal conditions (Glc) or in the presence of oligomycin and proportion of progenitor and large precursor B cells determined by flow cytometry after 24 hours. (**B**) Isolated bone marrow leukocyte was cultured under normal conditions (Glc) or in the presence of TOFA and proportion of progenitor and large precursor B cells determined by flow cytometry after 24 hours. (**C**) Isolated bone marrow leukocyte was cultured under normal conditions (Glc) or in the presence of etomoxir and proportion of progenitor and large precursor B cells determined by flow cytometry after 24 hours. N=6-8 per group, mean ± SEM presented. Differences between groups were analyzed with t-test; ** $p < 0.01$; **** $p < 0.0001$.
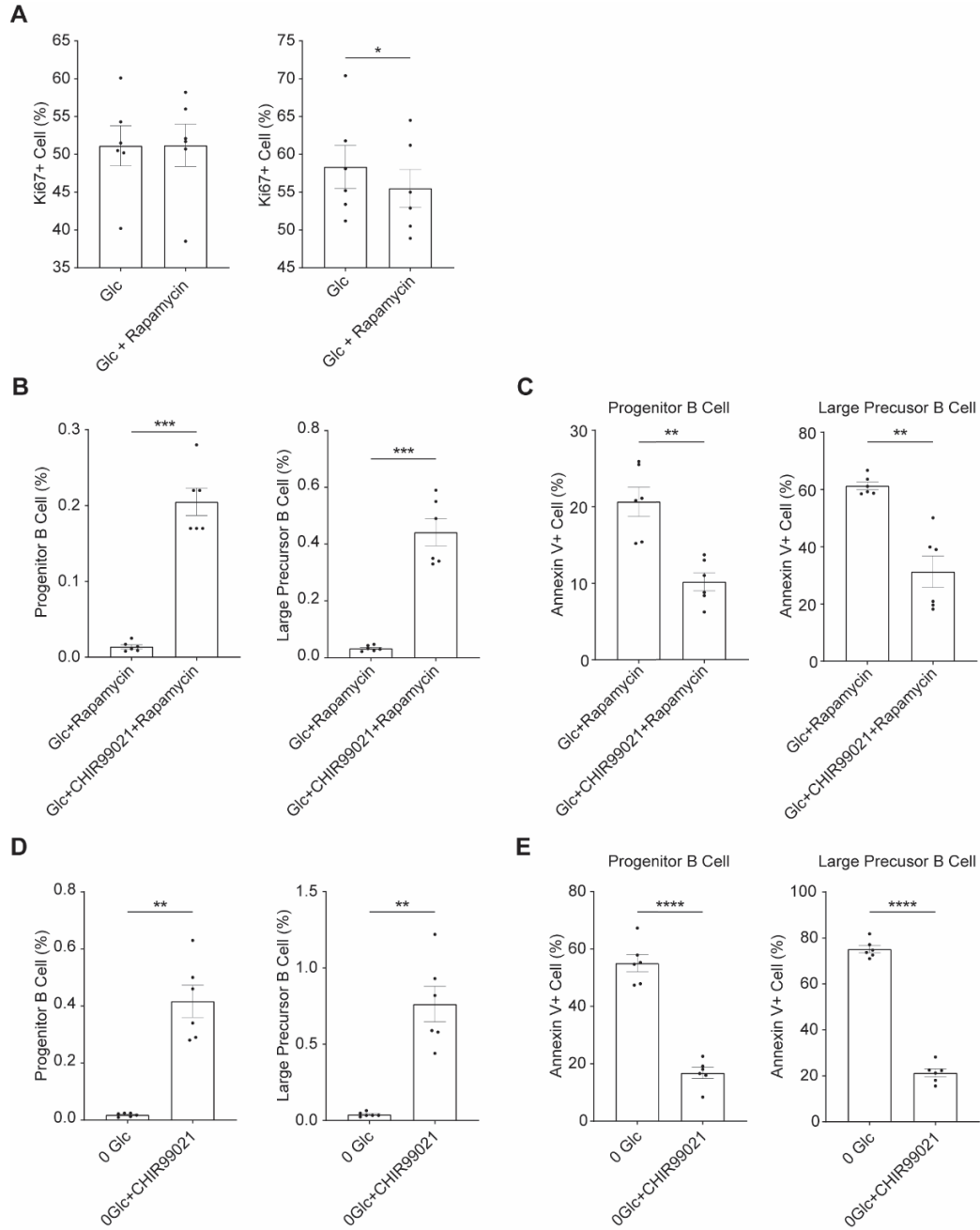
Figure S10. Glucose protects early B cells from apoptosis through mTOR activation and GSK3, related to Figure 7.

(**A**) Isolated bone marrow leukocyte was cultured under normal conditions (Glc) or in the presence of rapamycin and proportion of progenitor and large precursor B cells determined by flow cytometry after 24 hours. (**B-C**) Isolated bone marrow leukocyte was cultured with rapamycin (Glc+Rapamycin) or in the presence of CHIR99021 (Glc+Rapamycin+CHIR99021) and (**B**) proportion of and (**C**) Annexin V+ of progenitor and large precursor B cells determined by flow cytometry after 24 hours. (**D-E**) Isolated bone marrow leukocyte was cultured under no glucose conditions (0 Glc) or no glucose conditions in the presence of CHIR99021 and (**D**) proportion of and (**E**) Annexin V+ of progenitor and large precursor B cells determined by flow cytometry after 24 hours. N=6-8 per group, mean ± SEM presented. Differences between groups were analyzed with t-test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.
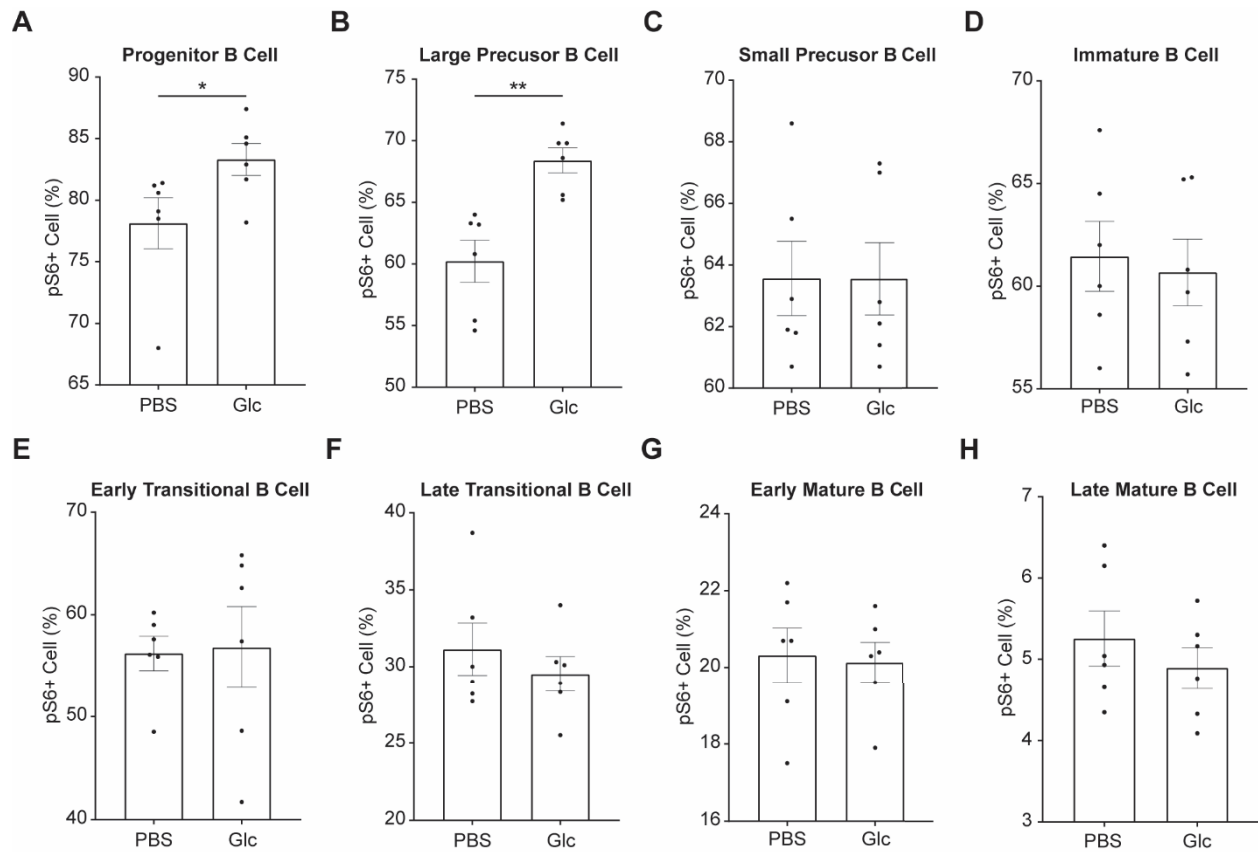
Figure S11. Glucose specifically promotes mTOR activation in progenitor and large precursor B cell, related to Figure 7.

Mice were acutely administered 2g/kg body weight of glucose via intraperitoneal injection and the percentages of positive proportions for phosphorylated ribosomal protein S6 (pS6$^+$) are quantified as a readout of mTOR activation in progenitor B cell (**A**), large precursor B cell (**B**), small precursor B cell (**C**), immature B cell (**D**), early transitional B cell (**E**), late transitional B cell (**F**), early mature B cell (**G**), and late mature B cell (**H**). N=6 per group, mean ± SEM presented. Differences between groups were analyzed with t-test; * $p < 0.05$; ** $p < 0.01$
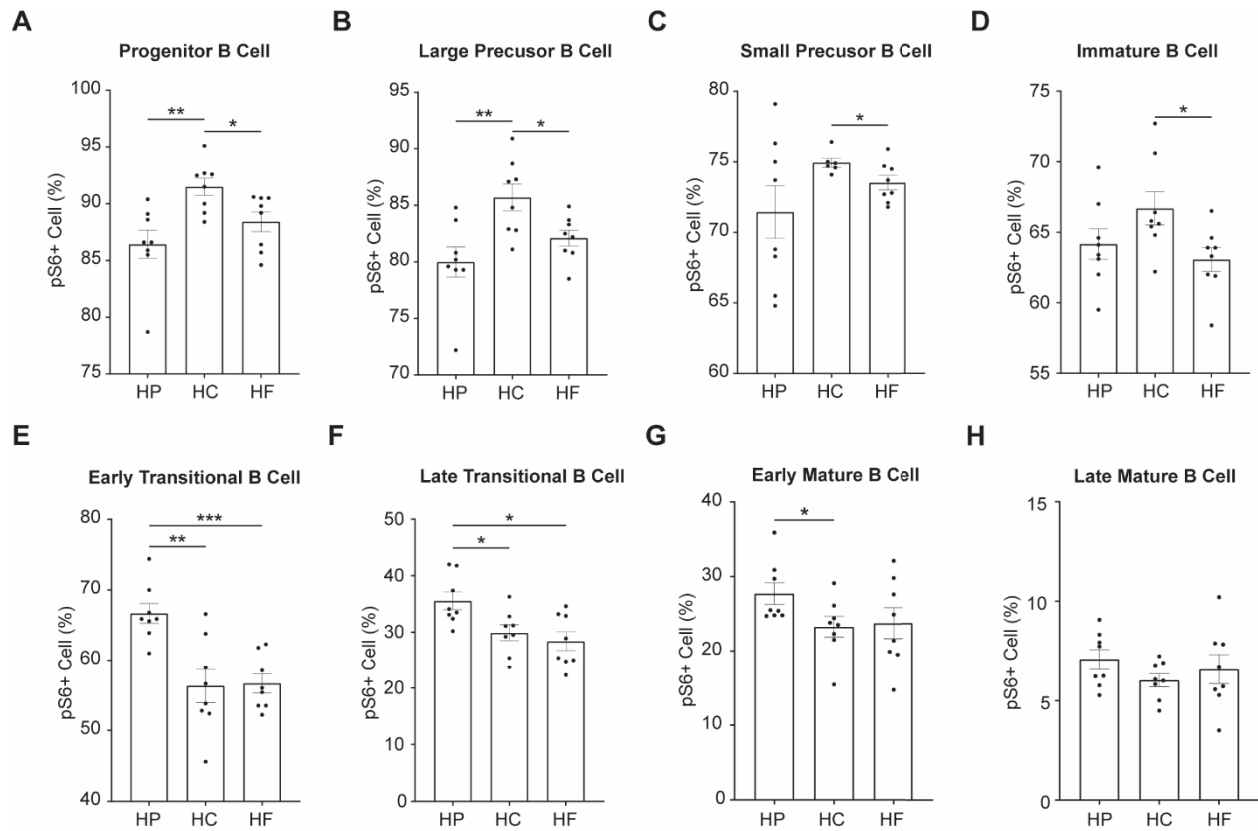
Figure S12. High carbohydrate diet specifically promotes mTOR activation in early bone marrow B cells, related to Figure 7.

Mice were fed on high protein (HP), high carbohydrate (HC) and high fat (HF) diet for at least 6 weeks. The percentages of positive proportions for phosphorylated ribosomal protein S6 (pS6[+]) are quantified as readout of mTOR activation in progenitor B cell (**A**), large precursor B cell (**B**), small precursor B cell (**C**), immature B cell (**D**), early transitional B cell (**E**), late transitional B cell (**F**), early mature B cell (**G**), and late mature B cell (**H**). N=6-8 per group, mean ± SEM presented. Differences between groups were analyzed with t-test; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

Table S1. Diets used for nutritional geometry analysis study, related to STAR Methods.

Diet	NME (kJ/g)	% Protein NME	% Carbohydrate NME	% Fat NME
Diet 1	14.5	60.2	19.7	20.1
Diet 2	14.5	5	74.9	20.1
Diet 3	14.5	5	19.7	75.3
Diet 4	14.5	33.1	46.8	20.1
Diet 5	14.5	33.1	19.7	47.2
Diet 6	14.5	5	46.8	48.2
Diet 7	14.5	14.1	28.8	57.1
Diet 8	14.5	14.1	56.8	29.1
Diet 9	14.5	42.1	28.8	29.1
Diet 10	14.5	24	37.8	38.2

NME, net metabolizable energy.

Table S2. Diet used for studying the qualitative effects of carbohydrate, related to STAR Methods.

Diet	NME (kJ/g)	% Protein NME	% Fat NME	% Carbohydrate NME		
				% Starch NME	% Monosaccharides NME	
					% Glucose NME	% Fructose NME
Glc (SF18-111)	14.5	20	20	18	42	0
Glc+Fru (SF18-113)					21	21
Fru (SF18-115)					0	42