Supplementary materials

Dietary glucose consumption promotes RALDH activity in small intestinal CD103$^+$CD11b$^+$ Dendritic cells

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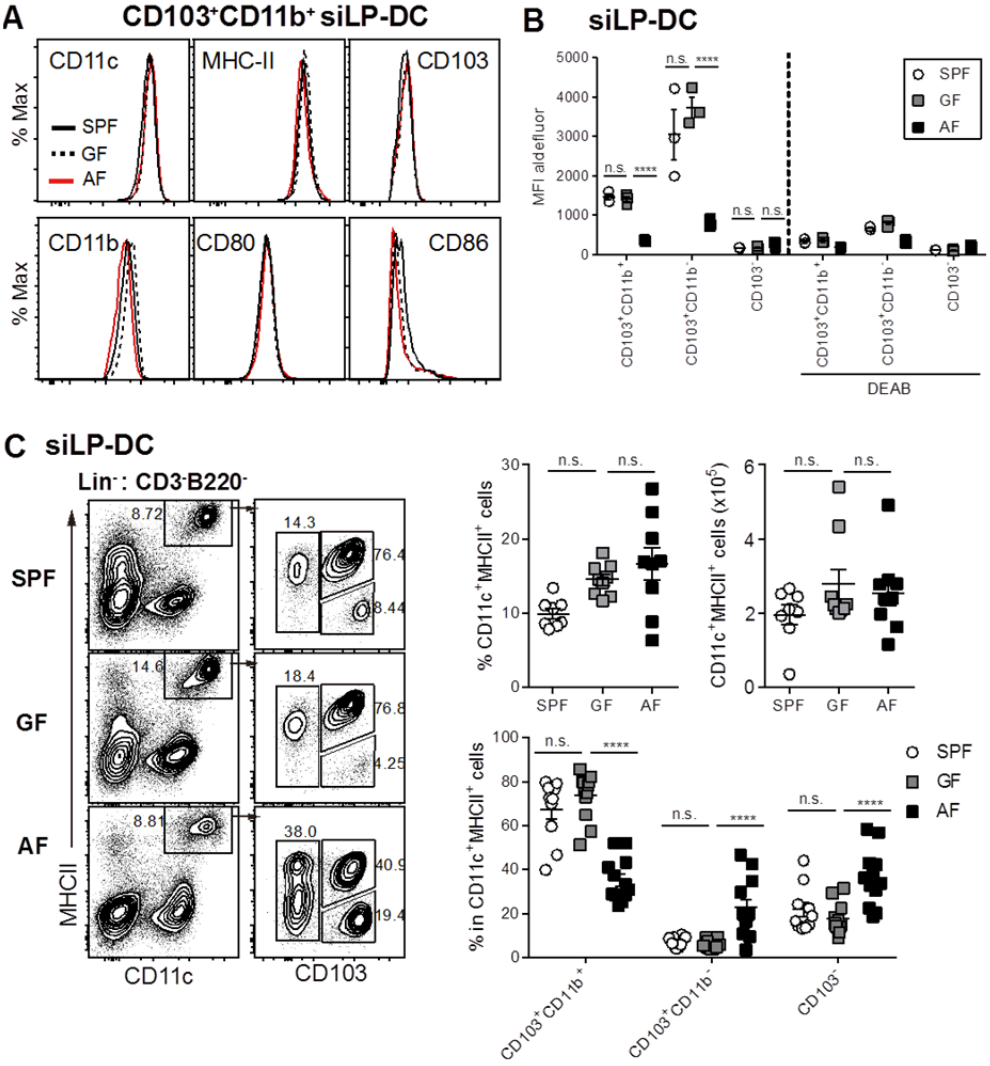


Figure S1. Altered phenotype of siLP-DC subsets in AF mice. Cell suspensions from siLP were prepared from age-matched adult (6~12-week-old) SPF, GF and AF mice, following which phenotypic and RALDH activity analyses of siLP-DCs were carried out. (**A**) Representative histograms showing expression of indicated surface markers within CD103$^+$CD11b$^+$ siLP-DCs. Black lines indicate SPF, blue lines indicate GF and red lines indicate AF mice. (**B)** Statistical quantification for MFI of siLP-DC subsets. DEAB is a RALDH inhibitor. (**C**) Representative FACS plots (left) and statistical quantification (right) of siLP-DC subsets within Lin-CD11c$^+$MHC-II$^+$ cells after ALDEFLUOR assay. Lin$^-$ includes populations with no expression for CD3 and B220. Percentage and total numbers of CD11c$^+$MHC-II$^+$ cells and siLP-DC subsets are shown. Data shown are representative of 2-3 experiments. MEAN ± SEM are indicated. Statistical significance was determined by one-way ANOVA and two-way ANOVA with Turkey's multiple comparison test. $****p < 0.0001$, *n.s.*, not statistically significant.

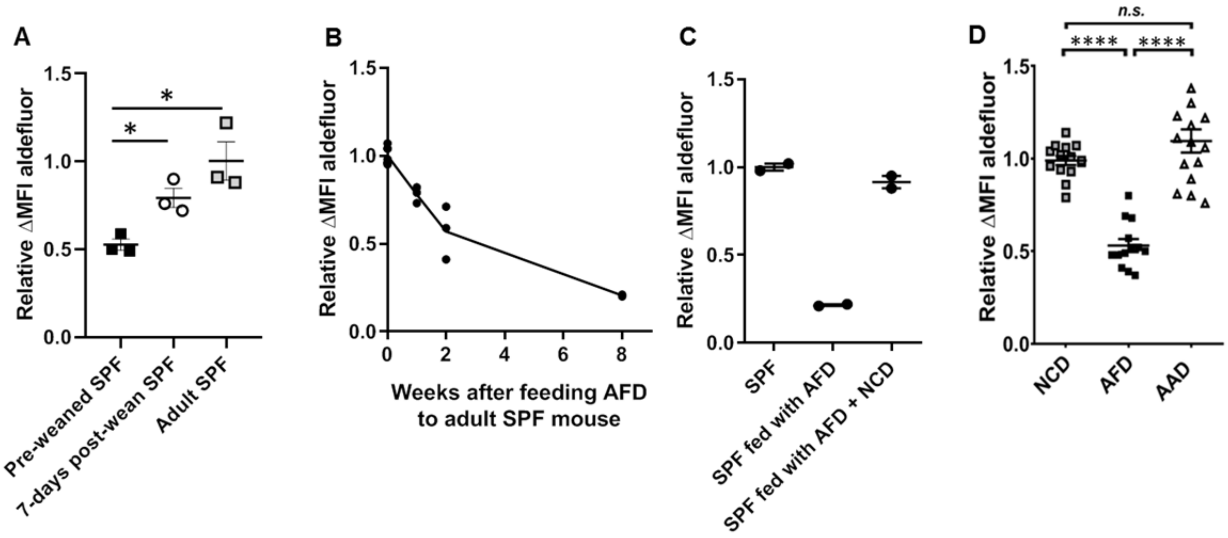


Figure S2. Dietary component mediated increase in RALDH activity in siLP-DCs in mice is independent of microbiota. Cell suspensions prepared from siLP were subjected to ALDEFLUOR assays and RALDH activity in CD103$^+$CD11b$^+$ siLP-DCs were analyzed by flow cytometry. (A) Pre-weaned SPF mice (3-weeks old), SPF mice weaned onto NCD for 7 days and adult SPF mice were analyzed. (B) RALDH activity in CD103$^+$CD11b$^+$ siLP-DCs from adult 8-12 weeks old SPF mice after feeding AFD for 0, 1, 2, and 8 weeks. (C) RALDH activity in CD103$^+$CD11b$^+$ siLP-DCs in adult SPF mice at the start of the experiment, after they were fed AFD for 8 weeks, and after the mice were reverted back to NCD for one week. (D) SPF mice were weaned onto AFD (22% glucose) or AAD (50% sucrose) for 4 weeks. MEAN ± SEM are indicated. Statistical significance was determined by one-way ANOVA with Turkey’s multiple comparison test. *$p < 0.05$, ****$p < 0.0001$, n.s., not statistically significant.

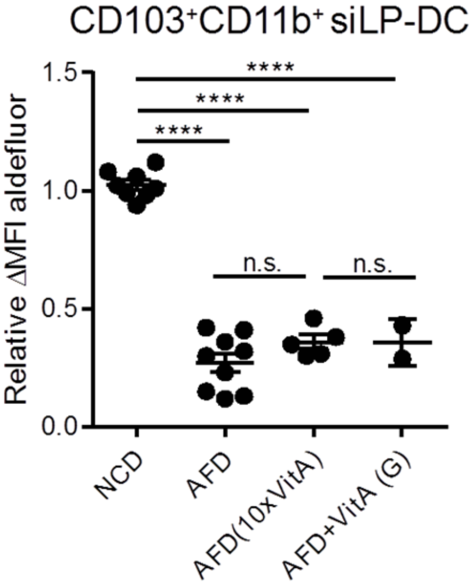


Figure S3. Supplementing AFD even with large excess of Vitamin A is unable to restore RALDH activity in CD103$^+$CD11b$^+$ siLP-DCs. Neonate GF mice (3-week-old) were weaned onto specific diets for 3~4 weeks. Normal AFD comprises of water soluble dietary components in water and water insoluble dietary components (including vitamin A) in soybean oil-mix. AFD(10xVitA) indicates mice fed with oil-mix containing 10 times more of vitamin A than normal AFD. AFD+VitA(G) indicates mice administrated with oil-mix (300 µl) by oral gavage for 9 days under normal AFD feeding condition. Cell suspensions from siLP were subjected to ALDEFLUOR assays and RADLH activity in CD103$^+$CD11b$^+$ siLP-DCs was analyzed. Data are combined from two or three independent experiments. MEAN ± SEM are indicated. Statistical significance was determined by one-way ANOVA with Turkey's multiple comparison test. *****p* < 0.0001, *n.s.*, not statistically significant.

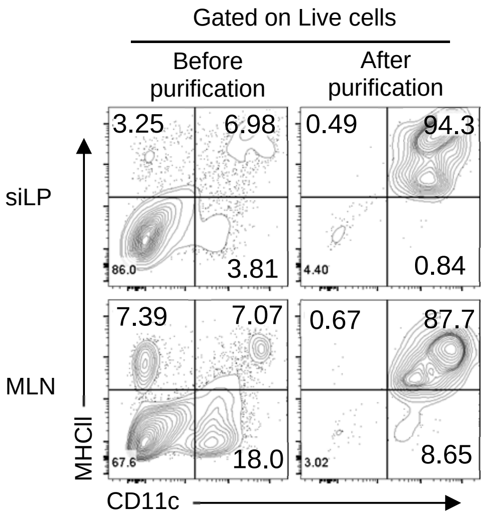


Figure S4. Purity of MLN and siLP DCs used for in vitro iTreg assay. CD11c mediated magnetic purification was performed from *wild type* C57BL/6 mice using commercially available purification kit.

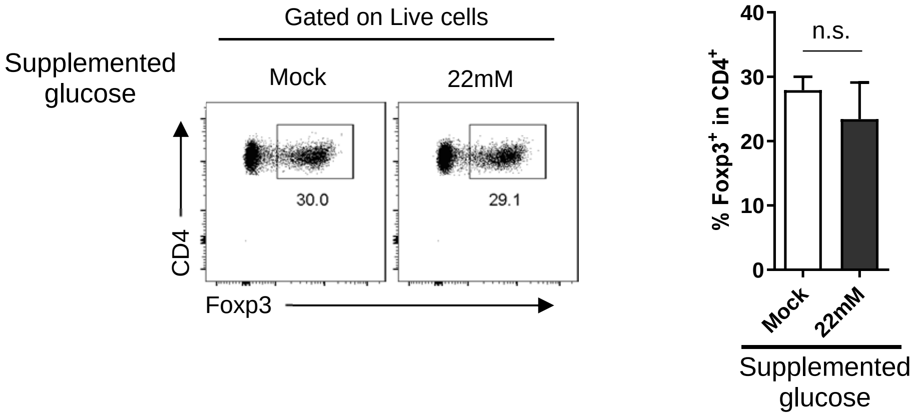
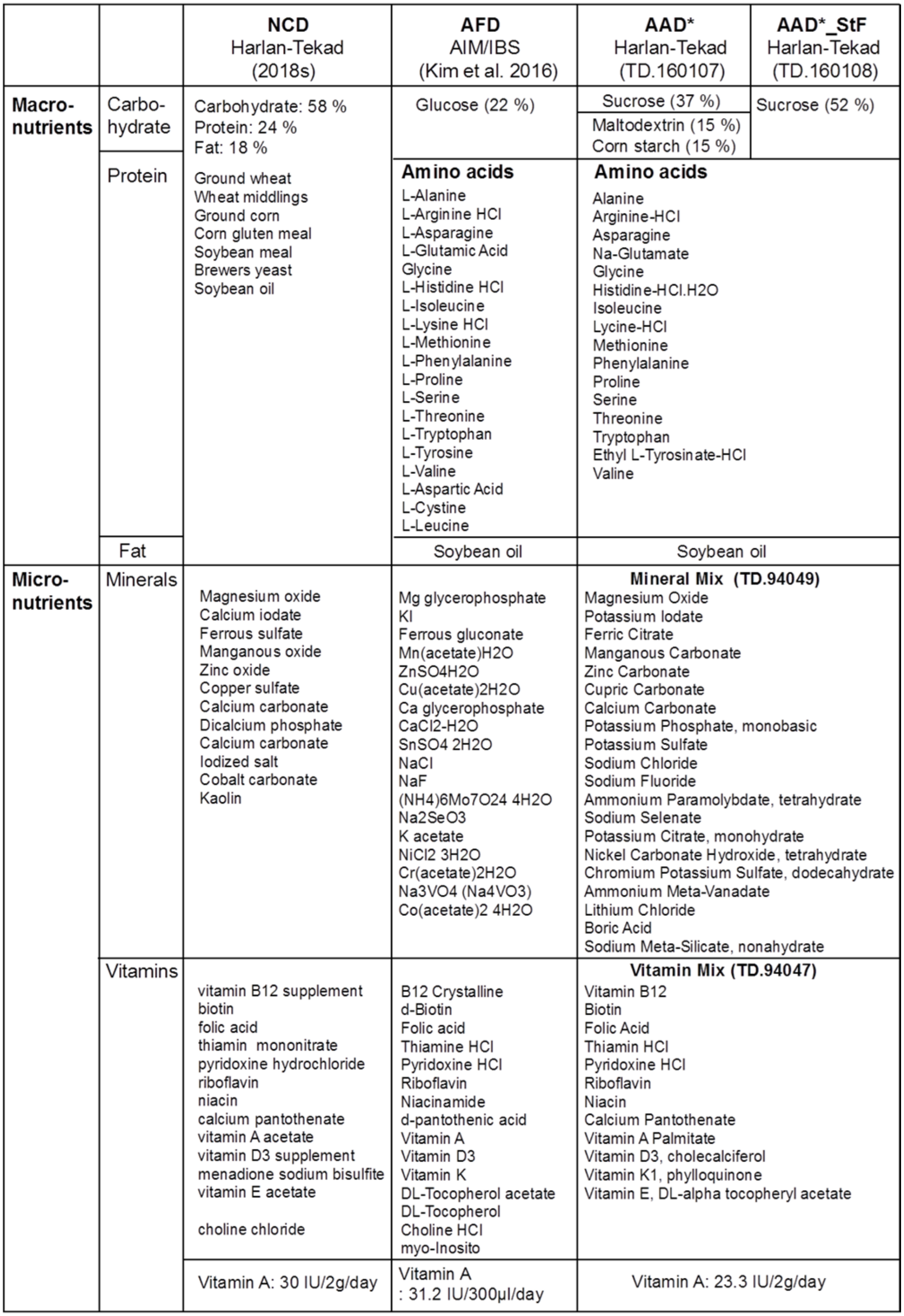


Figure S5. Glucose supplementation in a DC independent culture has little effect on in vitro iTreg induction. Sorted naïve T cells were subjected to DC independent iTreg induction with plate bound anti-CD3/ CD28, TGFβ and IL2, in the presence (22mM) or absence (mock) of excess glucose for three days. Representative FACS plots (left) and statistics (right) are shown. *n.s.*, not statistically significant.

Table S1. Vitamin A concentrations among different diet types

	SPFD	**GFD**	**AFD**
Supplier	Purina Lab. 38057	Harlan-Teklad (2018s)	AIM/IBS (Kim *et al.* 2016)
Vitamin A (IU/g)	28	30	104
Final consumption of Vitamin A per day	56 IU/2g	~ 30 IU/2g (after autoclave)	~ 31.2 IU/300 ul

Table S2. Comparison between food compositions of different modified diet types



		NCD Harlan-Tekad (2018s)	**AFD** AIM/IBS (Kim et al. 2016)	**AAD*** Harlan-Tekad (TD.160107)	**AAD*_StF** Harlan-Tekad (TD.160108)
Macro-nutrients	Carbo-hydrate	Carbohydrate: 58 % Protein: 24 % Fat: 18 %	Glucose (22 %)	Sucrose (37 %) Maltodextrin (15 %) Corn starch (15 %)	Sucrose (52 %)
	Protein	Ground wheat Wheat middlings Ground corn Corn gluten meal Soybean meal Brewers yeast Soybean oil	**Amino acids** L-Alanine L-Arginine HCl L-Asparagine L-Glutamic Acid Glycine L-Histidine HCl L-Isoleucine L-Lysine HCl L-Methionine L-Phenylalanine L-Proline L-Serine L-Threonine L-Tryptophan L-Tyrosine L-Valine L-Aspartic Acid L-Cystine L-Leucine	**Amino acids** Alanine Arginine-HCl Asparagine Na-Glutamate Glycine Histidine-HCl.H2O Isoleucine Lycine-HCl Methionine Phenylalanine Proline Serine Threonine Tryptophan Ethyl L-Tyrosinate-HCl Valine	
	Fat		Soybean oil	Soybean oil	
Micro-nutrients	Minerals	Magnesium oxide Calcium iodate Ferrous sulfate Manganous oxide Zinc oxide Copper sulfate Calcium carbonate Dicalcium phosphate Calcium carbonate Iodized salt Cobalt carbonate Kaolin	Mg glycerophosphate KI Ferrous gluconate Mn(acetate)H2O ZnSO4H2O Cu(acetate)2H2O Ca glycerophosphate CaCl2-H2O SnSO4 2H2O NaCl NaF (NH4)6Mo7O24 4H2O Na2SeO3 K acetate NiCl2 3H2O Cr(acetate)2H2O Na3VO4 (Na4VO3) Co(acetate)2 4H2O	**Mineral Mix (TD.94049)** Magnesium Oxide Potassium Iodate Ferric Citrate Manganous Carbonate Zinc Carbonate Cupric Carbonate Calcium Carbonate Potassium Phosphate, monobasic Potassium Sulfate Sodium Chloride Sodium Fluoride Ammonium Paramolybdate, tetrahydrate Sodium Selenate Potassium Citrate, monohydrate Nickel Carbonate Hydroxide, tetrahydrate Chromium Potassium Sulfate, dodecahydrate Ammonium Meta-Vanadate Lithium Chloride Boric Acid Sodium Meta-Silicate, nonahydrate	
	Vitamins	vitamin B12 supplement biotin folic acid thiamin mononitrate pyridoxine hydrochloride riboflavin niacin calcium pantothenate vitamin A acetate vitamin D3 supplement menadione sodium bisulfite vitamin E acetate choline chloride	B12 Crystalline d-Biotin Folic acid Thiamine HCl Pyridoxine HCl Riboflavin Niacinamide d-pantothenic acid Vitamin A Vitamin D3 Vitamin K DL-Tocopherol acetate DL-Tocopherol Choline HCl myo-Inosito	**Vitamin Mix (TD.94047)** Vitamin B12 Biotin Folic Acid Thiamin HCl Pyridoxine HCl Riboflavin Niacin Calcium Pantothenate Vitamin A Palmitate Vitamin D3, cholecalciferol Vitamin K1, phylloquinone Vitamin E, DL-alpha tocopheryl acetate	
		Vitamin A: 30 IU/2g/day	Vitamin A : 31.2 IU/300μl/day	Vitamin A: 23.3 IU/2g/day	