Supplementary Material

Suppl. table 1. Primer used for real-time quantitative PCR

Primer mouse:

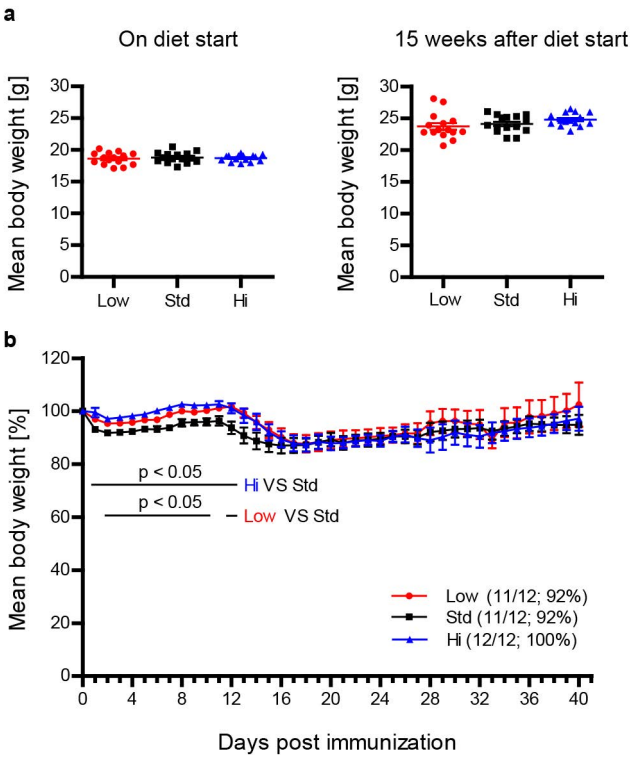
Target	Assay ID (Bio-Rad)	Amplicon length	Assay Design
IL-2	qMmuCID0015786	80	Intron-spanning
IFNgamma	qMmuCID0006268	175	Intron-spanning
GM-CSF (CSF2)	qMmuCED0025728	133	Exonic
Actin-beta	qMmuCED0027505	109	Exonic

Primer human:

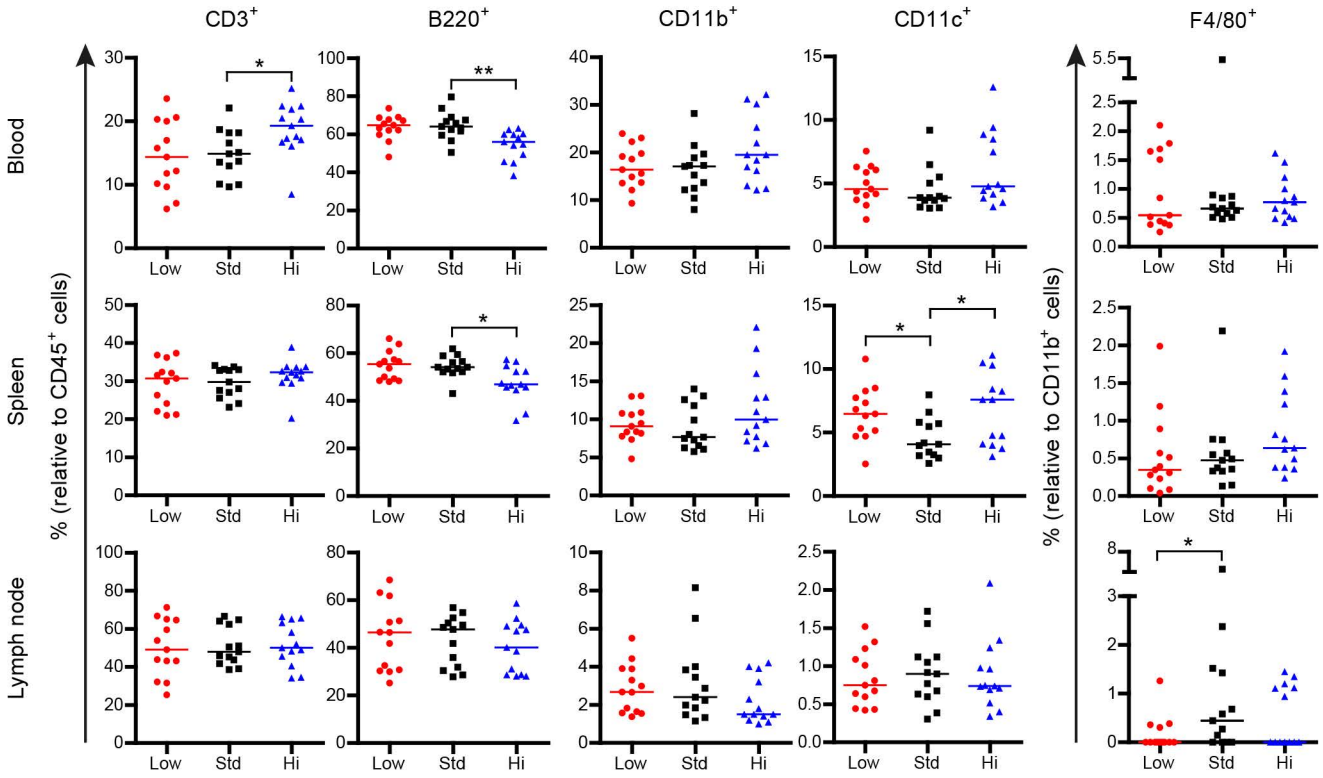
Target	Assay ID (Thermo Fisher Scientific)	Amplicon length	Fw primer	Rv primer
IL-2	Hs00698651_CE	230	AAGGCCTGATATGTTTTAAGTGGGAA	GCCTATAAGACTTCAATTGGGAATAACTGTAT
IFNgamma	Hs00607988_CE	262	AAAACAAAGGATTAAGTGAGACAGTCACA	GGAAGCGAAAAAGGAGTCAGATG
GM-CSF (CSF2)	Hs00664932_CE	254	CCTCCCTGGCATTTTGTGGT	AGTGTCTCTACTCAGGTTCAGGAG
Actin-beta	Hs00800199_CE	254	ACAGGACTCCATGCCTGAGA	GCCCTGGACTTCGAGCAA

Mineral content in experimental diets			
	Low	Std	Hi
	%		
Calcium	0.9	1.0	0.9
Phosphorus	0.63	0.70	0.63
Sodium	0.19	0.24	0.21

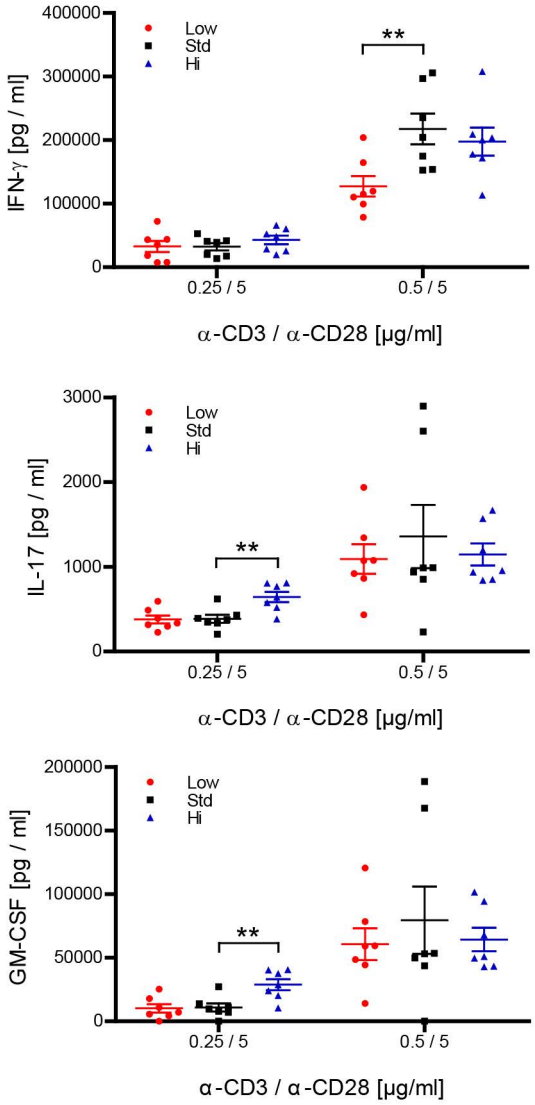
Suppl. figure 1. Low-, standard- and high vitamin D diets have similar calcium, phosphorus and sodium contents. Low, standard or high vitamin D diets contained standard concentrations of calcium, phosphorus and sodium.



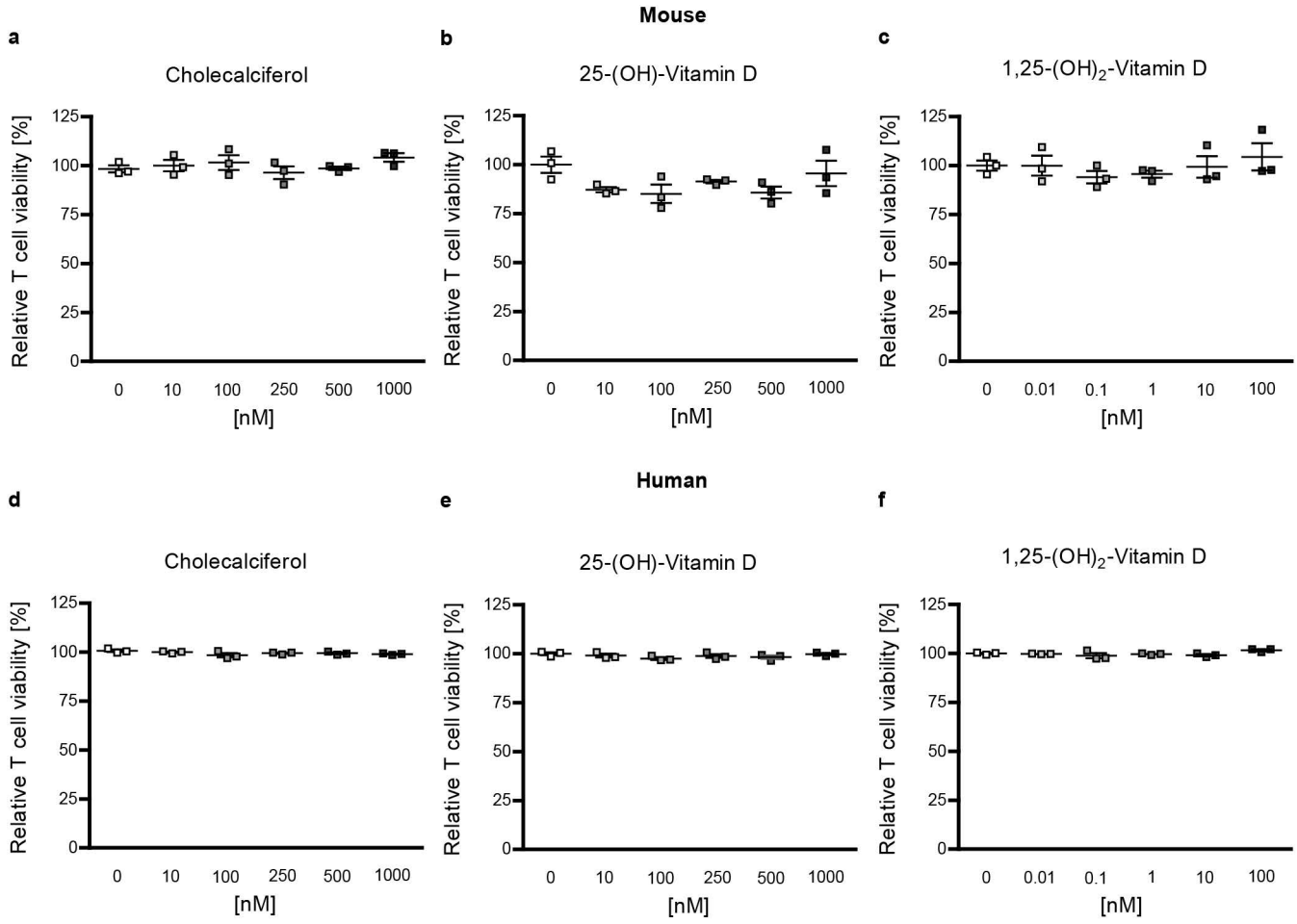
Suppl. figure 2. Vitamin D high excess supplementation has no impact on body weight. (a) Body weight was determined before and fifteen weeks after mice were fed a diet containing low (< 5 IU/kg food), standard (1.500 IU/kg food) or high vitamin D concentrations (75.000 IU/kg food); (data given as mean ± SEM; n = 15). **(b)** EAE was induced by MOG peptide 35-55 immunization fifteen weeks after diet onset. Body weights are shown as mean ± SEM; disease incidence is indicated in brackets; n = 12.



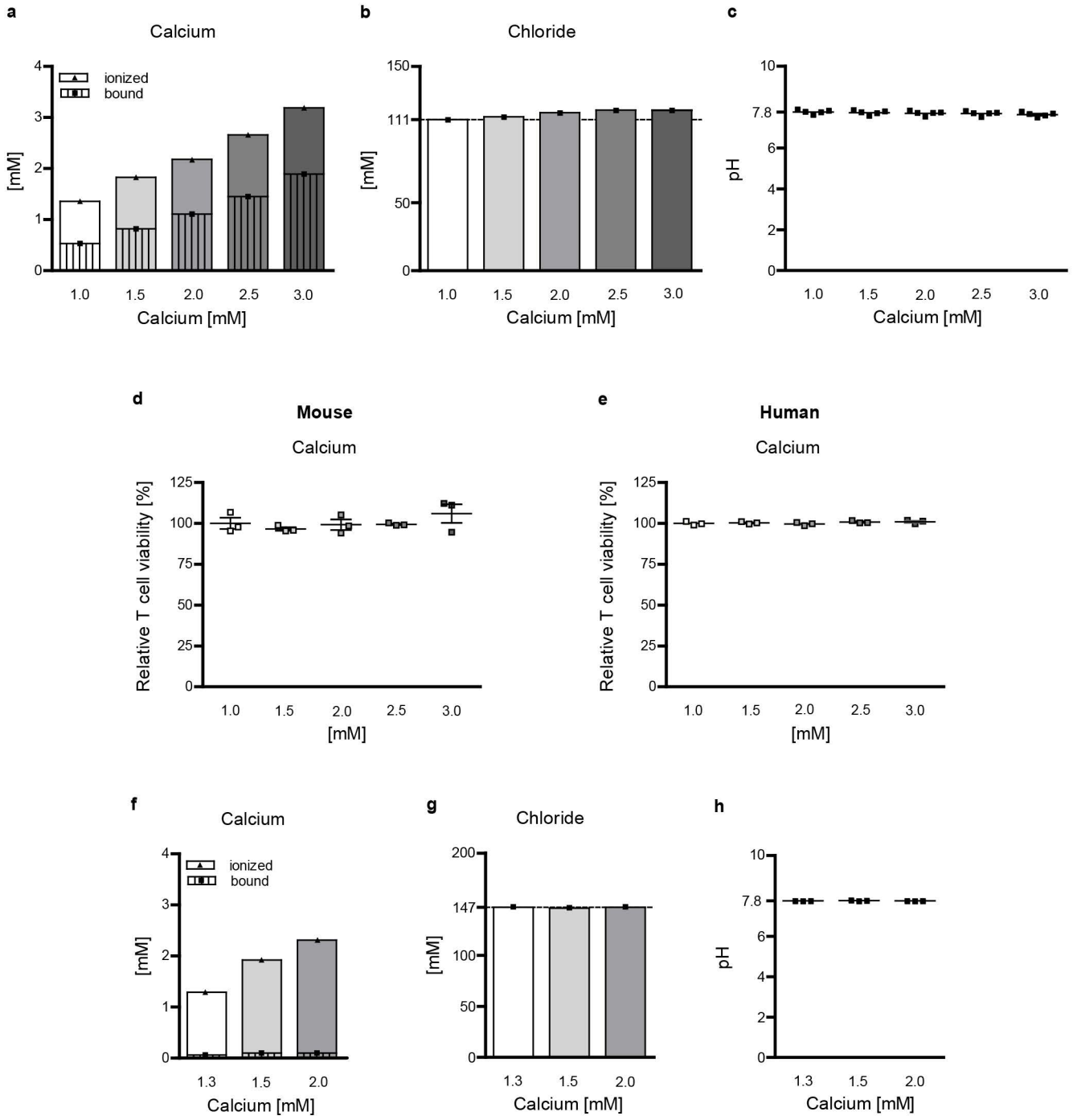
Suppl. figure 3. Vitamin D high excess supplementation increases the frequencies of T cells, CD11c$^+$ myeloid APCs as well as F4/80$^+$ macrophages. Frequencies of immune cells in blood, spleen and inguinal lymph node were analyzed by FACS forty-nine weeks after vitamin D diet onset (data given as median; representative plots of two independent experiments; n = 13).



Suppl. figure 4. Vitamin D high excess supplementation promotes differentiation into encephalitogenic T cells. Splenic T cells were isolated from naïve WT mice, which were fed with vitamin D low, standard or high diet for eight weeks. MACS purified T cells were incubated in anti-CD3 / anti-CD28 pre-coated wells for 72 hours. Differentiation into Th1-, Th17- or GM-CSF-secreting T cells was analyzed by quantification of IFN-γ, IL-17 and GM-CSF concentrations in culture supernatants by ELISA (data given as mean ± SEM; n = 7).

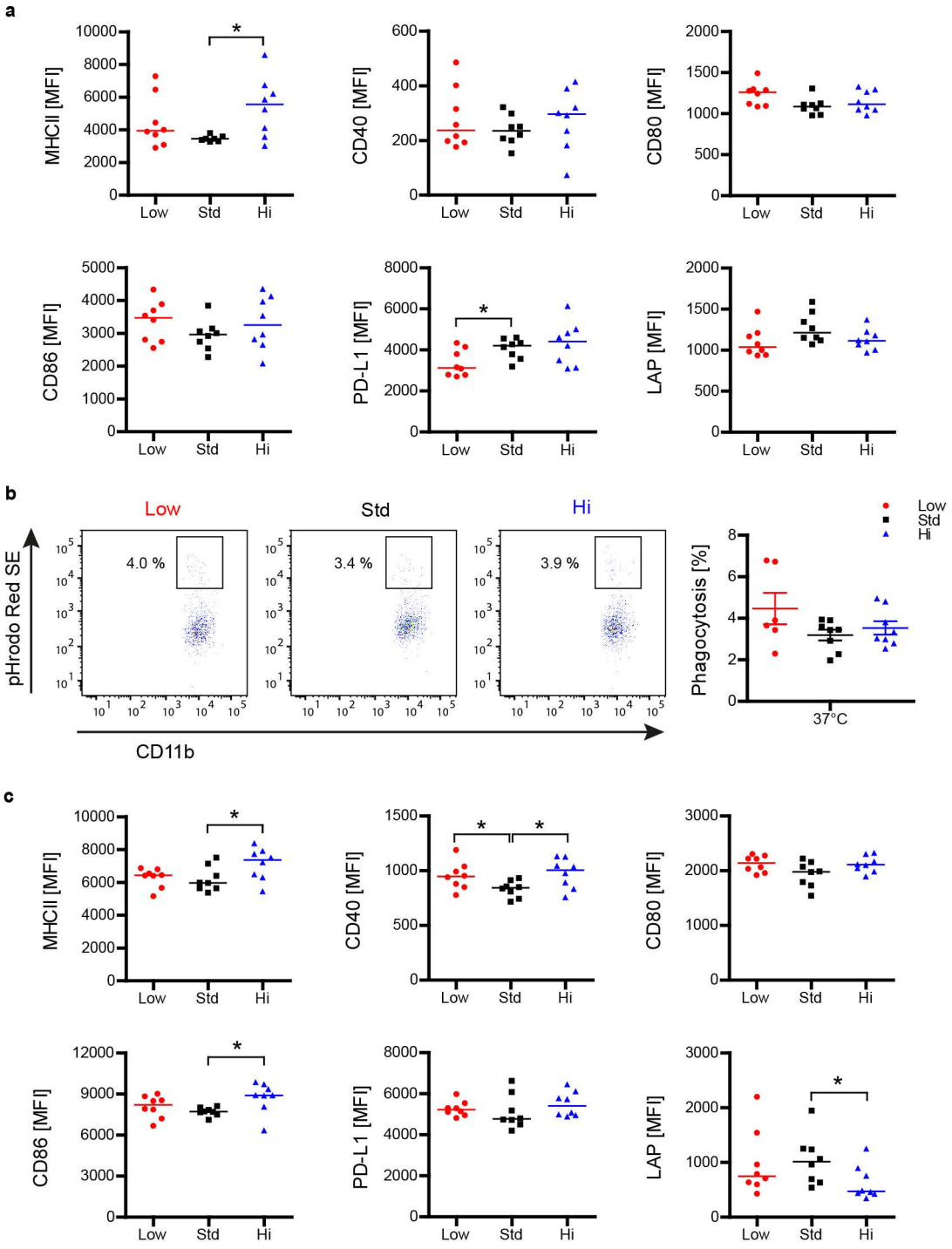


Suppl. figure 5. T cell viability is unaffected by incubation with cholecalciferol, 25-(OH)-vitamin D or 1,25-$(OH)_2$-vitamin D. (a-c) Splenocytes were isolated from naïve WT mice receiving standard vitamin D diet. **(d-f)** Human PBMCs were isolated from healthy donors after Ficoll gradient centrifugation. MACS purified T cells were incubated in anti-CD28 pre-coated wells in presence of increasing concentrations of **(a, d)** cholecalciferol, **(b, e)** 25-(OH)-vitamin D or **(c, f)** 1,25-$(OH)_2$-vitamin D for 48-72 hours (murine T cells) or 96-120 hours (human T cells). T cell viability was determined by FACS using a fixable live cell- / dead cell discrimination dye (data shown as mean ± SEM; representative plots of two independent experiments; n = 3).



Suppl. figure 6. Increasing calcium concentrations in culture medium have no effect on chloride levels, pH or T cell viability. Calcium concentrations in **(a)** culture medium or **(f)** calcium flux assay medium were increased by dissolving calcium chloride. **(a, f)** Total calcium in culture medium was quantified on an ARCHITECT c 16000 analyzer. Ionized calcium was measured on a blood gas analyzer GEM Premier 4000. Bound calcium was calculated by subtracting the ionized calcium from the total calcium concentration. Data from one representative experiment are presented. Total chloride in **(b)** culture medium or **(g)** calcium flux assay medium was measured using ion-selective electrodes on an ARCHITECT c 16000 analyzer. Data from one representative experiment are presented. **(c, h)** pH in calcium-supplemented media was determined by inoLab pH Level 1 (data given as mean ± SEM; n = 3). **(d)** Splenocytes were isolated from naïve WT mice receiving standard vitamin D diet. **(e)**

Human PBMCs were isolated from healthy donors after Ficoll gradient centrifugation. MACS purified T cells were incubated in anti-CD28 pre-coated wells in presence of increasing calcium concentrations for **(d)** 48-72 hours (murine T cells) or **(e)** 96-120 hours (human T cells). T cell viability was determined by FACS using a fixable live cell- / dead cell discrimination dye (data shown as mean ± SEM; representative plots of two independent experiments; n = 3).



Suppl. figure 7. Vitamin D high excess supplementation increases activation of splenic monocytes and dendritic cells in naïve mice. (a) Splenocytes were isolated and splenic monocyte activation / differentiation was analyzed by FACS eight weeks after vitamin D diet onset in naïve mice (data are shown as median of MFI; n = 7-8). **(b)** Splenic macrophages were isolated eight weeks after vitamin D diet onset from naïve mice and co-cultured with pHrodo Red SE labelled apoptotic thymocytes at 37°C for one hour. Phagocytosis of apoptotic thymocytes by splenic macrophages ($CD11b^{+}F4/80^{+}$) was determined by FACS. Representative FACS dot plots are shown left and percentage of phagocytosis by macrophages is depicted on the right as mean ± SEM; n = 6-8. **(c)** Splenocytes were isolated and splenic dendritic cell activation / differentiation was analyzed by FACS eight weeks after vitamin D diet onset in naïve mice (data are shown as median of MFI; n = 8).