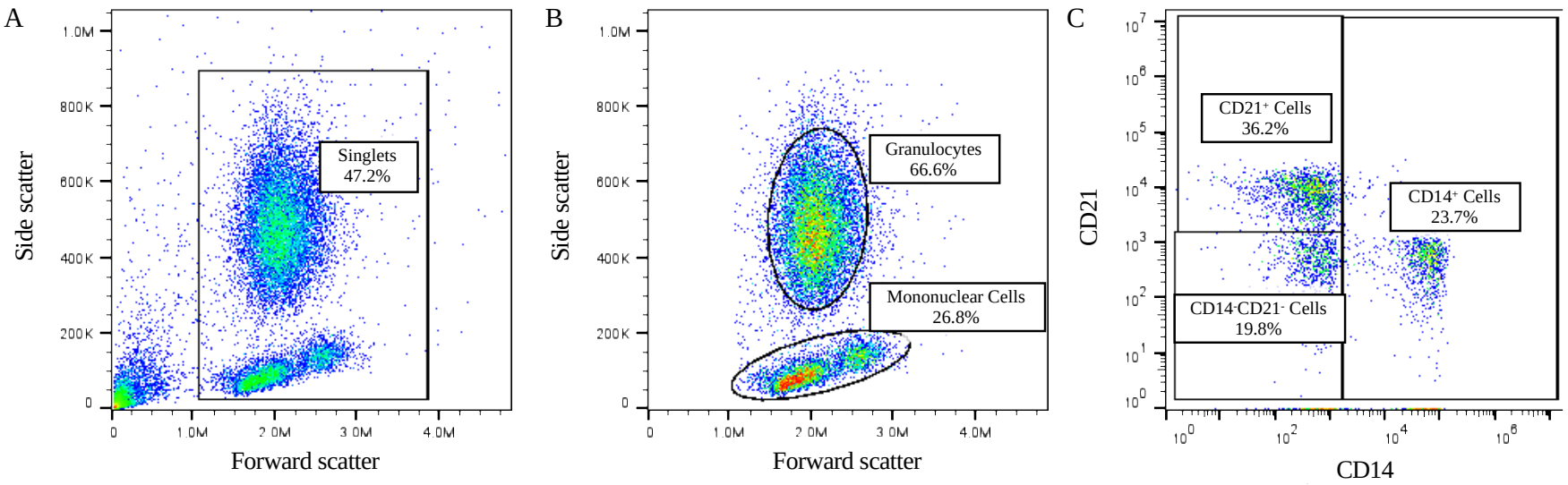
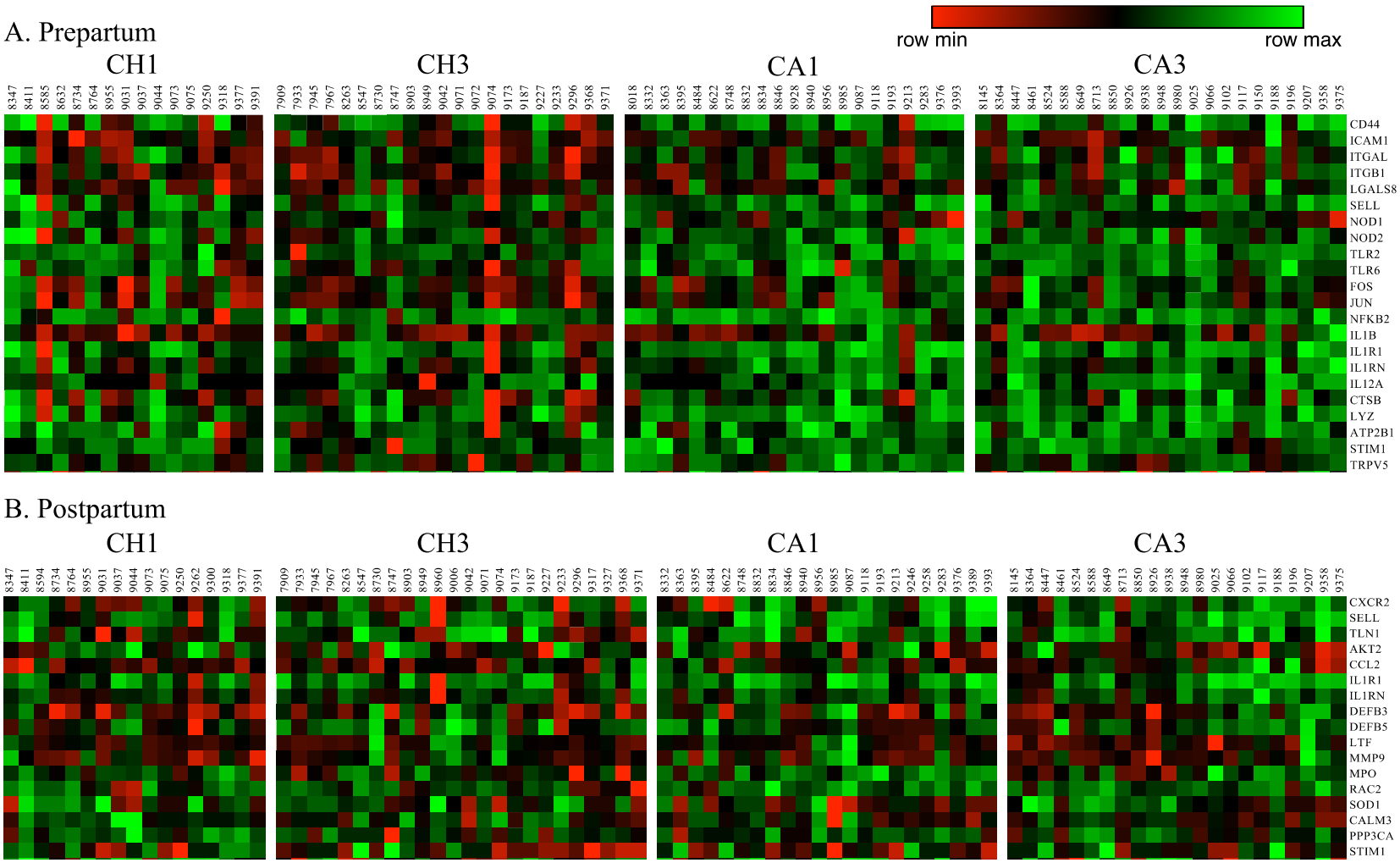
Supplemental Figure and Tables



Supplemental Figure S1. Cells populations were analyzed by flow cytometry according to forward and side scatters to isolate singlets (A). Out of singlets, cells were segregates cells based on size and granularity into granulocytes and mononuclear cells (B). Within mononuclear cells, those expressing CD14 and CD21 were identified and then classified as monocytes ($CD14^{+}$), B lymphocytes ($CD14^{-}$ $CD21^{+}$), and $CD14^{-}$ $CD21^{-}$ cells (C). Values in the figure represent the proportions of identified cells within the gated cell population.



Supplemental Figure S2. Effect of supplementation of 1 or 3 mg of cholecalciferol (CH1 or CH3) or calcidiol (CA1 or CA3) from 250 d of gestation to calving on peripheral blood leukocytes gene expression at 270 d of gestation (A) or on d 3 postpartum (B) in individual cows. Heat maps were generated including differently ($P < 0.05$) expressed genes among treatments. Data represents the negative value of the delta cycle threshold (-dCt) for each gene from 81 prepartum and 86 postpartum cows.

Supplemental Table S1. List of genes and primers investigated by real time PCR analysis of leukocytes segregated according to function

Category	Gene[1]	Primer	Accession number	Efficiency, %
Housekeeping				
	ACTB	F: 5'-GCCAACCGTGAGAAGATGAC-3'	NM_173979	112.2
		R: 5'-CCTGGATGGCCACGTACA-3'		
	GAPDH	F: 5'-GGGTCTTCACTACCATGGAGAA-3'	NM_001034034	110.7
		R: 5'-GTTCACGCCCATCACAAACA-3'		
	PGK1	F: 5'-GGAGGAAGAAGGGAAGGGAA-3'	NM_001034299	111.2
		R: 5'-TGAAGCTCGGAAGGCTTCTA-3'		
	RPL19	F: 5'-GCCGGCTGCTTAGACGATA-3'	NM_001040516	113.3
		R: 5'-AACACGTTACCCTTCACCTTCA-3'		
	RPS9	F: 5'-GCCTCGACCAAGAGCTGAA-3'	NM_001101152	112.7
		R: 5'-GAATTTGACCCTCCAGACCTCA-3'		
	YWHAZ	F: 5'-AACAGCAGATGGCTCGAGAA-3'	NM_174814	107.7
		R: 5'-GAAGCGTTGGGGATCAAGAAC-3'		
Cell adhesion and migration				
	CD44	F: 5'-GACCCCAGTGGAAGATCCTA-3'	NM_174013	108.2
		R: 5'-GTTTGCCCCATGTTCTTCAC-3'		
	CXCR2	F: 5'-ACAGGTGACAAGCCCAGAA-3'	NM_001101285	108.7
		R: 5'-AGGGCAGGTCAGTGTTGTAA-3'		
	ICAM1	F: 5'-CATCGAGCTGTTTTGCTACTCA-3'	NM_174348	110.7
		R: 5'-CTCCACATGCTCCGGGAA-3'		
	ITGAL	F: 5'-AACAGCCAAAGAGAGGAGGAC-3'	NM_198221	107.2
		R: 5'-CACACAACTCGCCACCAAAA-3'		
	ITGAM	F: 5'-AAGACAGCTGTTTGGGAGGAC-3'	NM_001039957	111.2
		R: 5'-TCCACTGCTGCTGTGAAACA-3'		
	ITGB1	F: 5'-AACCTGTGAGATGTGTCAGACC-3'	NM_174368	109.2
		R: 5'-ATTGAAGGCTCGGCACTGAA-3'		

ITGB2	F: 5’-ACCATCGCTTTGAGAAGGAGAA-3’	NM_175781	111.2
	R: 5’-TCGTGGTGGCACTCTTGAAA-3’		
LGALS8	F: 5’-CATTCACTCTGTGGGCTTCA-3’	NM_001045954	109.7
	R: 5’-TCCCTGTTGGTTCCAGAGTA-3’		
SELE	F: 5’-GTCACAGTATCTCTCTGCTCTCAC-3’	NM_174181	N/A
	R: 5’-TCCGTGGATGCATGGTAAGAC-3’		
SELL	F: 5’-GCTCAGAGGGGCTTATGGAA-3’	NM_174182	111.2
	R: 5’-GGTGCCATGATGAGCAAAGAA-3’		
TLN1	F: 5’-CGTACTGTCTCAGACTCCATCA-3’	NM_001205428	108.7
	R: 5’-ATGGCTGTCTCACACTCCAA-3’		
Pathogen recognition receptors			
NOD1	F: 5’-CTGGCAGAGGCATTACAGAAG-3’	NM_001256563	109.7
	R: 5’-TTGGCCTCCTCTGGCTTTAT-3’		
NOD2	F: 5’-GTGTGTGTTTCCTCGCCAAA-3’	NM_001002889	117.1
	R: 5’-GGAGCTGATGTGGTTGTTAGAC-3’		
TLR1	F: 5’-CATTTGATGCCCTGCCCATA-3’	NM_001046504	110.7
	R: 5’-TAACTGTGTGGCACTCAACC-3’		
TLR2	F: 5’-CTGCTGCGTTGGTTTGGATA-3’	NM_174197	113.8
	R: 5’-CCATGCTGTCCACAAAGCA-3’		
TLR4	F: 5’-ATCCTCTCCTGCCTGAGAAC-3’	NM_174198	107.2
	R: 5’-AGCTCCATGCACTGGTAACTA-3’		
TLR6	F: 5’-GGCAACTTGACCCAACTGAA-3’	NM_001001159	115.4
	R: 5’-GTGCAAGTGAGCAATGGGTA-3’		
TLR9	F: 5’-AACCTGCCCGCCAGAC-3’	NM_183081	117.7
	R: 5’-GCCTGCACCAGGAGAGAAA-3’		
Cell signaling			
AKT1	F: 5’-CGCCCCTCAACAACTTCTCC-3’	NM_173986	111.2
	R: 5’-GCAGGCAGCGGATGATGAA-3’		
AKT2	F: 5’-GGCTGCTTAAGAAGGACCCTA-3’	NM_001206146	107.2
	R: 5’-AAGGAAGAACCTGTGCTCCA-3’		
FOS	F: 5’-TTCTCCAGCATGGGTTCTCC-3’	NM_182786	111.7

		R: 5’-GATGAAGTTGGCACTGGAGAC-3’		
	IRAK4	F: 5’-TGGTTCATTGCTGGACAGAC-3’	NM_001075998	111.7
		R: 5’-CTGGGCAATCTTGCATCTCA-3’		
	JUN	F: 5’-CAGGTGGCCCAGCTTAAACA-3’	NM_001077827	109.7
		R: 5’-CGTTTGCAACTGCTGCGTTA-3’		
	MAPK1	F: 5’-TTCCAACCTGCTGCTCAACA-3’	NM_175793	110.2
		R: 5’-CGTGGTCTGGATCTGCAACA-3’		
	MAPK3	F: 5’-CCCTCCAACCTGCTCATCAA-3’	NM_001110018	114.9
		R: 5’-TCATGCTCGGGATCAGCAA-3’		
	MYD88	F: 5’-GTCCATCGCCAGTGAACTCA-3’	NM_001014382	110.2
		R: 5’-TTGCAGGTATTCGTCAGAGACC-3’		
	NFATC1	F: 5’-ATCTGGGAGATGGAGGCAAA-3’	NM_001166615	109.7
		R: 5’-GTTATCCTCTGGTTGCGGAAA-3’		
	NFKB1	F: 5’-TGGCAGCTCTTCTCAAAGCA-3’	NM_001076409	108.7
		R: 5’-AGGAGTCATCCAGGTCGTACA-3’		
	NFKB2	F: 5’-CTGACTGCACAGGATGAGAAC-3’	NM_001102101	107.2
		R: 5’-ACGTGGGCTATCTGCTCAA-3’		
Cytokines				
	CCL2	F: 5’-ACCAGCAGCAAGTGTCCTAAA-3’	NM_174006	114.9
		R: 5’-TGCTTGGGGTCTGCACATAA-3’		
	CCL5	F: 5’-CTGCCCACAGCTACCATGAA-3’	NM_175827	111.2
		R: 5’-GCGGGAGATATAGGCAAAGCA-3’		
	CXCL8	F: 5’-GTTGCTCTCTTGGCAGCTTT-3’	NM_173925	109.2
		R: 5’-TGGCATCGAAGTTCTGTACTCA-3’		
	IFNG	F: 5’-CAGCTCTGAGAAACTGGAGGA-3’	NM_174086	109.7
		R: 5’-TTATGGCTTTGCGCTGGATC-3’		
	IL1B	F: 5’-TGTGTGCTGAAGGCTCTCC-3’	NM_174093	109.2
		R: 5’-CCTTGCACAAAGCTCATGCA-3’		
	IL1R1	F: 5’-ACACTCCTGTGCTAGAGGAA-3’	NM_001206735	109.7
		R: 5’-ATATTAAGCCGTGCGAGGAC-3’		
	IL1R2	F: 5’-ATCATCTCTCCCCACCAGAC-3’	NM_001046210	109.7

	R: 5’-GGCTCCCAGAAACACCTTAC-3’		
IL1RN	F: 5’-TCCTGTTCCGTTCAGAAACA-3’	NM_174357	114.9
	R: 5’-AGATCTTCTGGTTGACATCCC-3’		
IL6	F: 5’-TAAGCGCATGGTCGACAAAA-3’	NM_173923	105.8
	R: 5’-TCTCCTTGCTGCTTTCACAC-3’		
IL10	F: 5’-CCCTGCGAAAACAAGAGCAA-3’	NM_174088	114.4
	R: 5’-CTCACTCATGGCTTTGTAGACAC-3’		
IL12A	F: 5’-CTGAGGGCTGTCAGCAACA-3’	NM_174355	N/A
	R: 5’-TCTCCTCAGAAGTGCAGGAGTA-3’		
IL23A	F: 5’-CTGGAGTGCACACCTACCAA-3’	NM_001205688	114.4
	R: 5’-AGCCATCCTCACACTGGATAC-3’		
TNF	F: 5’-CAAGTAACAAGCCGGTAGCC-3’	NM_173966	113.3
	R: 5’-GGCATTGGCATACGAGTCC-3’		
Antimicrobial mechanisms			
BPI	F: 5’-TTTGCCATCCTCCCAAACTCC-3’	NM_173895	114.9
	R: 5’-TGGCACCAACAACCACAGAA-3’		
CATHL5	F: 5’-AGCAGTGTGACTTCAAGGAGAA-3’	NM_174510	107.7
	R: 5’-ACAGCACAGGTGATGTCGAA-3’		
CATHL6	F: 5’-TCAATGAGCGGTCCTCAGAA-3’	NM_174832	108.7
	R: 5’-TCGGGATGTTTGGGTTCTCA-3’		
CTSB	F: 5’-GCCCGACCATCAAAGAGATCA-3’	NM_174031	111.7
	R: 5’-TCCGGTCAGAAATGGCTTCC-3’		
DEFB1	F: 5’-AAATGCTGCAGGTCGTGGTA-3’	NM_001324544	111.2
	R: 5’-AAGGGTGCAGTTTCTGTCTCC-3’		
DEFB3	F: 5’-AAATGCTGCAGGTCGTGGTA-3’	NM_001282581	104.0
	R: 5’-TCGAAGGGCGCAGTTTCA-3’		
DEFB4A	F: 5’-AATCCACAGCCTGCACAGAA-3’	NM_174775	107.7
	R: 5’-GGCTGATGCTGGAGAAGAGAC-3’		
DEFB5	F: 5’-TCCTCGTGCTCCTCTTCCTA-3’	NM_001130761	106.8
	R: 5’-AGGGCACGAGATCGGAATAC-3’		
DEFB6	F: 5’-CCAGCATGAGGCTCCATCA-3’	NM_001313991	112.2

	R: 5’-GGCACACAGAAGCCTCCATATA-3’		
DEFB7	F: 5’-CCAGCATGAGGCTCCATCA-3’	NM_001102362	105.4
	R: 5’-CGGCACACAGAAGCCTCTA-3’		
DEFB10	F: 5’-TAGAGGCATCTGTTTGCTGAAC-3’	NM_001115084	108.2
	R: 5’-CATTTTACTCGGGGCGCTAA-3’		
ELANE	F: 5’-AAAACGGCTTCGACCCCTT-3’	NM_001105653	103.5
	R: 5’-CTGCACGTTGGCATTGAGG-3’		
LAP	F: 5’-TCCTTGCGCTCCTCTTCC-3’	NM_203435	106.8
	R: 5’-TCCTACGGCAGCTTTGAGAA-3’		
LTF	F: 5’-GCCTTTGCCTTGGAATGTATCC-3’	NM_180998	105.8
	R: 5’-CAAACACCATGCCACCATCC-3’		
LYZ	F: 5’-TGAGCTTGCCAGAAGTCTGAA-3’	NM_001078159	112.2
	R: 5’-TCCCATCTGGCCAAACACA-3’		
MMP2	F: 5’-CCATGATGGAGAGGCTGACA-3’	NM_174745	N/A
	R: 5’-GCCCGTCTTTGCCATCAAA-3’		
MMP9	F: 5’-AGCACGCACGACATCTTTCA-3’	NM_174744	108.7
	R: 5’-GAACTCACGCGCCAGTAGAA-3’		
PRKCB	F: 5’-GAATGGTGGTGACCTCATGTAC-3’	NM_174587	110.2
	R: 5’-TGGCAATTTCTGCAGCGTAA-3’		
TAP	F: 5’-GCGGGCAGTAAAATGCTGTA-3’	NM_174776	111.7
	R: 5’-TCTGACTGGGCATTGATCCC-3’		
Oxidative burst			
CYBA	F: 5’-GGTCAGTTCACCCAGTGGTA-3’	NM_174034	104.9
	R: 5’-TATTCCAGCAGGCAGACCAA-3’		
CYBB	F: 5’-TGTGAATGAGGGCATCTCCA-3’	NM_174035	111.7
	R: 5’-ACCCGGTAGTACCAGACAAAA-3’		
GPX1	F: 5’-CGGGACTACACCCAGATGAA-3’	NM_174076	101.3
	R: 5’-CTTCAGGCAATTCAGGATCTCC-3’		
GSR	F: 5’-TGAATGTCGGATGTGTACCC-3’	NM_001114190	115.4
	R: 5’-AGTCAACGTGGTCATGCA-3’		
MPO	F: 5’-CCTCATTGGGACCCAGTTCA-3’	NM_001113298	120.0

	R: 5’-TGCTGCTTCTTGCTGAACAC-3’		
NCF1	F: 5’-CCTCCTGGGCTTCGAGAA-3’	NM_174119	108.2
	R: 5’-AGACAGGTCCTGCCATTTCA-3’		
NCF2	F: 5’-AGTCTGAGCCGAGACACTCTA-3’	NM_174120	106.3
	R: 5’-GATCACCACCGGCTCATACA-3’		
NCF4	F: 5’-GAGCAAGACCAGCCCCTTAA-3’	NM_001045983	110.7
	R: 5’-CGGCAATCTCCTGTTTCACAC-3’		
NOS2	F: 5’-GCTACGGAACTGGACATCAAC-3’	NM_001076799	N/A
	R: 5’-TTTGGGGTCATCCTGTGTCA-3’		
RAC2	F: 5’-CTCATCAGCTACACCACCAAC-3’	NM_175792	111.7
	R: 5’-CCACCATCACATTGGCTGAA-3’		
SOD1	F: 5’-AAAACACGGTGGGCCAAAA-3’	NM_174615	109.7
	R: 5’-ACAATATCCACGATGGCAACAC-3’		
Calcium metabolism			
ATP2B1	F: 5’-CTTCGAGTGCTTGCAAGATCA-3’	NM_174696	108.7
	R: 5’-GTTCGGAAACAGTGCTGTCA-3’		
CALM1	F: 5’-GACACCGACAGTGAAGAGGAA-3’	NM_001242572	110.2
	R: 5’-CTCTGCGGCGCTGATGTA-3’		
CALM2	F: 5’-GACTTCCCGGAATTTCTGACA-3’	NM_001242587	109.2
	R: 5’-CGAAGCTCTGCTGCACTAA-3’		
CALM3	F: 5’-GAGCTGCAGGACATGATCAAC-3’	NM_001046249	109.7
	R: 5’-CAGGAACTCGGGGAAGTCAA-3’		
CASR	F: 5’-GCTGCTTTGAGTGTGTGGAA-3’	NM_174002	N/A
	R: 5’-CCAGAAGTCATCAGGGCACTTA-3’		
ITPR1	F: 5’-TACGCCGAAGGATCGACAAA-3’	NM_174841	108.7
	R: 5’-GTTTCTGGCTGCACAACACA-3’		
ORAI1	F: 5’-GACTTCGGCTCTGCTCTCC-3’	NM_001099002	116.0
	R: 5’-TGGTCAGCATCCAGTTGTACC-3’		
PPP3CA	F: 5’-CAGACGATGAACTAGGGTCAGAA-3’	NM_174787	105.4
	R: 5’-TGCCAATGGCTCGGATCTTA-3’		
PPP3CB	F: 5’-CCCACAGGGATGTTGCCTA-3’	NM_001099214	105.4

		R: 5'-TTCAGCCTCAATAGCCTCAAC-3'		
	SLC8A1	F: 5'-AGAGAACATCCACTCCCCTCTA-3'	NM_176632	117.1
		R: 5'-TCAGTGGCTGCTTGTCATCA-3'		
	STIM1	F: 5'-GCACCTTCCATGGTGAGGATA-3'	NM_001035409	106.8
		R: 5'-CTCATCCACGGTCCAGTTGTA-3'		
	TRPV5	F: 5'-TCTGGCATCTGTGGGTACAA-3'	XM_010804626	120.0
		R: 5'-TTCTGGTCTTGGTTGGTCTCA-3'		
	TRPV6	F: 5'-AGGAGCCCATGACATCTGAA-3'	NM_001206189	N/A
		R: 5'-TTTCACGAGGTTCACGTTCC-3'		
Vitamin D metabolism				
	CYP24A1	F: 5'-CTGGAAAGGAGGCCTCAAGAA-3'	NM_001191417	N/A
		R: 5'-ATGCGGAAAATCTTGCCGTAC-3'		
	CYP27B1	F: 5'-CAGAGACATTTGTGTGGGTGAA-3'	NM_001192284	116.0
		R: 5'-GGGTCCCTTGAAGTGGCATA-3'		
	GC	F: 5'-CGCTGCTTATGGGAAGGAGAA-3'	NM_001035380	N/A
		R: 5'-AGTAGGCACTTTCTGGGCAAA-3'		
	RXRA	F: 5'-CGCCATCGTCCTCTTCAAC-3'	NM_001304343	106.3
		R: 5'-ACGCATAGACCTTCTCACGAA-3'		
	VDR	F: 5'-AGCCCGGACTACAAGTACCA-3'	NM_001167932	112.6
		R: 5'-GGAACTTGATGAGGGGCTCAA-3'		

[1]*ACTB* = actin beta; *AKT1* = AKT serine/threonine kinase 1; *AKT2* = AKT serine/threonine kinase 2; *ATP2B1* = plasma membrane ATPase Ca^{2+} transporting 1; *BPI* = bactericidal permeability increasing protein; *CALM1* = calmodulin 1; *CALM2* = calmodulin 2; *CALM3* = calmodulin 3; *CASR* = calcium sensing receptor; *CATHL5* = cathelicidin 5; *CATHL6* = cathelicidin 6; *CCL2* = chemokine (C-C motif) ligand 2; *CCL5* = chemokine (C-C motif) ligand 5; *CD44* = CD44 molecule; *CTSB* = cathepsin B; *CXCL8* = chemokine (C-C motif) ligand 8; *CXCR2* = chemokine (C-C motif) receptor 2; *CYBA* = cytochrome b-245 alpha chain; *CYBB* = cytochrome b-245 beta chain; *CYP24A1* = 25-Hydroxyvitamin D_3 24-hydroxylase; *CYP27B1* = 25-Hydroxyvitamin D_3 1-alpha-hydroxylase; *DEFB1* = β-defensin 1; *DEFB3* = β-defensin 3; *DEFB5* = β-defensin 5; *DEFB6* = β-defensin 6; *DEFB7* = β-defensin 7; *DEFB10* = β-defensin 10; *ELANE* = elastase, neutrophil expressed; *FOS* = Fos proto-oncogene, AP-1 transcription factor subunit; *GAPDH* =

glyceraldehyde-3-phosphate dehydrogenase; *GC* = GC vitamin D binding protein; *GPX1* = glutathione peroxidase 1; *GSR* = glutathione-disulfide reductase; *ICAM1* = intercellular adhesion molecule 1; *IFNG* = interferon gamma; *IL1B* = interleukin 1 beta; *IL1R1* = interleukin 1 receptor type 1; *IL1R2* = interleukin 1 receptor type 2; *IL1RN* = interleukin 1 receptor antagonist; *IL10* = interleukin 10; *IL6* = interleukin 6; *IL12A* = interleukin 12A; *IL23A* = interleukin 23 subunit alpha; *IRAK4* = interleukin 1 receptor associated kinase 4; *ITGAL* = integrin subunit alpha L; *ITGAM* = integrin subunit alpha M; *ITGB1* = integrin subunit beta 1; *ITGB2* = integrin subunit beta 2; *ITPR1* = inositol 1,4,5-trisphosphate receptor type 1; *JUN* = Jun proto-oncogene, AP-1 transcription factor subunit; *LAP* = lingual antimicrobial peptide; *LGALS8* = galectin 8; *LTF* = lactotransferrin; *LYZ* = lysozyme; *MAPK1* = mitogen-activated protein kinase 1; *MAPK3* = mitogen-activated protein kinase 3; *MMP2* = matrix metallopeptidase 2; *MMP9* = matrix metallopeptidase 9; *MPO* = myeloperoxidase; *MYD88*= MYD88 innate immune signal transduction adaptor; *NCF1* = neutrophil cytosolic factor 1; *NCF2* = neutrophil cytosolic factor 2; *NCF4* = neutrophil cytosolic factor 4; *NFATC1* = nuclear factor of activated T cells 1; *NFKB1* = nuclear factor kappa B subunit 1; *NFKB2* = nuclear factor kappa B subunit 2; *NOD1* = nucleotide binding oligomerization domain containing 1; *NOD2* = nucleotide binding oligomerization domain containing 2; *NOS2* = nitric oxide synthase 2; *ORAI1* = ORAI calcium release-activated calcium modulator 1; *PGK1* = phosphoglycerate kinase 1; *PPP3CA* = protein phosphatase 3 catalytic subunit alpha; *PPP3CB* = protein phosphatase 3 catalytic subunit beta; *PRKCB* = protein kinase C beta; *RAC2* = Rac family small GTPase 2; *RPL19* = ribosomal protein L19; *RPS9* = ribosomal protein S9; *RXRA* = retinoid X receptor alpha; *SELE* = selectin E; *SELL* = selectin L; *SLC8A1* = solute carrier family 8 member A1; *SOD1* = superoxide dismutase 1; *STIM1* = stromal interaction molecule 1; *TAP* = tracheal antimicrobial peptide; *TLN1* = talin 1; *TLR1* = toll-like receptor 1; *TLR2* = toll-like receptor 2; *TLR4* = toll-like receptor 4; *TLR6* = toll-like receptor 6; *TLR9* = toll-like receptor 9; *TNF* = tumor necrosis factor; *TRPV5* = transient receptor potential cation channel subfamily V member 5; *TRPV6* = transient receptor potential cation channel subfamily V member 6; *VDR* = vitamin D receptor; *YWHAZ* = tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta.

Supplemental Table S2. Number of plasma samples and cows with concentrations of vitamin D_3 below the detection of limit of 0.50 ng/mL

	Day relative to calving							
Treatment[1]	-19	-10	-3	-1	0	4	8	Total, samples (cows)
CH1	0	0	0	0	0	2	0	2 (2)
CH3	0	0	0	1	0	0	0	1 (1)
CA1	1	6	5	8	7	2	0	29 (12)
CA3	2	7	6	10	6	2	0	33 (10)

[1] Prepartum cows at 250 d of gestation were supplemented with 1 or 3 mg of cholecalciferol (CH) or calcidiol (CA) resulting in 4 treatments, CH1, CH3, CH1 and CH3. Blood was sampled on days -19, -10, -3, -1, 0, 4 and 8 relative to calving and analyzed for concentrations of vitamin D_3.