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Expression of Plet1 controls interstitial migration of murine small intestinal dendritic cells

SUPPLEMENTARY TABLES AND FIGURES

Expression of Plet1 controls interstitial migration of murine small intestinal dendritic cells

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Supplementary table 1: list of primer sequences used for RT-PCR

Plet1	Forward	5'-CGTGGTCCTTGATAACATCT-3'		
Plet1	Reverse	5'-TCACGGCACTGACTGAA-3'		
IL-12P40	Forward	5'-TGGCTGGTGCAAAGAA-3'		
IL-12P40	Reverse	5'-GGGTCTGGTTTGATGATGT-3'		
IL1β	Forward	5'-GGACCCCAAAAGATGAAG-3'		
IL1β	Reverse	5'-CCACGGGAAAGACACAG-3'		
II-23p19	Forward	5'-GCCCCGTATCCAGTGT-3'		
II-23p19	Reverse	5'-CTGCCACTGCTGACTAGAA-3'		
IL-6	Forward	5'-TCGGAGGCTTAATTACACA-3'		
IL-6	Reverse	5'-CTGGCTTTGTCTTTCTTGTT-3'		
Gapdh	Forward	5'-TCAACGGCACAGTCAAG-3'		
Gapdh	Reverse	5'-GCTCCACCCTTCAAGTG-3'		
TNF	Forward	I 5'-GGGGGCTTCCAGAACT-3'		
TNF	Reverse	5'-GGGCCATAGAACTGATGAG-3'		

Supplementary Table 2. Genes overexpressed in CD103+CD11b+ intestinal DC and induced after colonization.

Gene			
Symbol	Gene Title	Protein Class and Function	
Trim72	Tripartite Motif Containing 72		
Clec4a3	C-type lectin domain family 4, member a3	Immunoglobulin receptor superfamily	
Gbp3	Guanylate Binding Protein 3	Heterotrimeric G-protein	
Gbp7	Guanylate Binding Protein 7	Heterotrimeric G-protein	
Vwa5a	Von Willebrand Factor A Domain Containing 5A	Serine protease inhibitor	
Cd86	Cluster of Differentiation 86	T-lymphocyte activation antigen Cell migration and cellular adhesion to matrix	
Plet1	Placenta Expressed Transcript 1	proteins[14]	
Tha1	Threonine Aldolase	Aldolase	
lfitm3	Interferon Induced Transmembrane Protein 3		
Gbp8	Guanylate Binding Protein 3	Heterotrimeric G-protein	
H2-Eb1	histocompatibility 2, class II antigen E beta	Major histocompatibility complex antigen	
Tmem171	Transmembrane Protein 171		
Rtp4	Receptor Transporter Protein 4	Olfactory receptor binding	
Blm	Bloom Syndrome RecQ Like Helicase GTPase, very large interferon inducible 1	DNA helicase	
Gm4759	pseudogene		
Lgals9	Galectin 9	Cell adhesion molecule, signaling molecule[43]	
Pgs1	Phosphatidylglycerophosphate Synthase 1	Cytokine, extracellular matrix protein, receptor	
Spi1	Transcription Factor PU.1 Cadherin, EGF LAG Seven-Pass G-Type Receptor	Nucleic acid binding signaling molecule transcription factor	
Celsr1	1	G-protein coupled receptor cadherin, cell adhesion[44]	
Ltf	Lactotransferrin	Serine transferase/carrier protein	

Supplementary Table 3: List of differentially expressed genes in Plet1^{-/-} CD11b⁺CD103⁺ DCs versus Plet1^{+/-} CD11b⁺CD103⁺ DCs.

	base mean	log2 fold change	p value
Zg16	295,7178293	3,803648649	1,01E-29
Clca3	253,6791646	3,666473016	2,22E-26
Reg1	324,7811166	3,581519814	1,53E-21
Fcgbp	317.5764988	3.394766698	4.53E-20
Gpx3	175.4673748	3.313596713	1.69E-17
Reg3g	177.777684	3.304552688	1.04E-18
Sparc	151.9958387	3.124838317	3.24E-15
Spink4	144.0538515	2.82444654	5.62E-12
Muc13	151.0492757	2.520111199	2.47E-10
Møst1	97.71867738	2,325635251	2.90E-08
Slc12a2	158.0932112	2,206663867	5.78E-09
Igi	2862 760185	2,2000000007	1.11E-06
Krt18	291 7483196	2,2010,50002	1,11E 00
Cldn7	181 643058	2,153730011	494F-08
Muc2	212 5394136	2,104010020	934E-10
Krt19	490 8249061	2,130703747	2 92E-09
Gash	74 73268084	2,144042340	2,92E-05
Dpp1r1h	77 20610416	2,00001017	2,94E-00
I pp1110	<i>11</i> ,20010410	2,034030019	3,10E-00
Mttn	158 4624460	2,033730434	3,10E-00
Kenn4	62 56646777	1 074265102	1 1 9 E 0 E
Atn1h1	110 0727904	1,974303102	2.07E.06
Augusta Cuca 2a	76 02007660	1,931990007	1 25E 05
Bran1	204 2642022	1,951740744	1,23E-03
Prapi	204,3043923	1,951191001	4,20E-00
Lgais4	299,1707007	1,905245466	5,50E-00
Suci	120 7700274	1,904108717	2,50E-05
Cyrol	138,7789374	1,898323788	4,08E-07
DCn	426,5102989	1,844637034	3,70E-05
Aldob	2/4,8394836	1,825978801	1,/3E-06
Lesze	104,1818/83	1,814291578	4,33E-05
Lypd8	261,0998182	1,794928927	8,05E-06
Gpx2	105,359648	1,/8409/125	4,13E-05
Krt/	100,1245896	1,/80515681	3,64E-05
Adh6a	69,84324206	1,/69436/03	5,27E-05
Papss2	85,86906265	1,745778184	0,00010075
Klf5	118,1354687	1,721421536	5,96E-05
Ep1	73,43456377	1,696634177	0,00015123
Fabp1	187,8102662	1,688513312	2,92E-05
Fam84b	89,34915787	1,665026519	0,00011788
Tmc4	82,58530517	1,645956974	0,00012863
Klf2	206,2340749	1,643823934	4,85E-05
Cd81	182,9001769	1,618630249	3,83E-06
Nusap1	160,7075052	1,60856318	1,53E-05
Myo1a	161,1085243	1,592656852	0,0002262
Sema4a	96,41458019	1,564883544	0,00017622
Alpi	210,8479608	1,559413192	6,81E-05
Id3	197,1149588	1,53002372	3,76E-05
Smc2	183,5783059	1,521078056	1,49E-05
Mapk13	129,5006959	1,515005186	0,00010016
Elf3	217,7544976	1,500207478	6,72E-05
Tmem176b	147,9234055	1,471606326	0,00030006
Fabp2	359,0487168	1,444147602	0,00025921
Phlda1	313,159568	1,327781391	0,00019923
Dnaja4	498,9556356	1,222627282	0,00017997
Golm1	370,2671965	1,134474633	0,00014362
Dnajb1	12036,73036	1,036998264	1,94E-05
Hspd1	6512,07026	0,98247914	9,43E-05
Txnip	1066,518523	0,941460822	5,51E-06
Hspa1b	20320,58879	0,908284873	4,36E-05

Tsc22d3	1636,639014	0,889774248	6,21E-05
Hsp90aa1	13237,02169	0,80967743	5,91E-05
Pts	1988,39691	0,692957305	0,00024314
Mon1b	757,3008838	-0,842326384	0,00023682
Kdm6a	1313,905028	-0,909889833	4,43E-05
Mapkbp1	128,0258742	-1,725894145	2,32E-05
Plet1	5055,29523	-6,679885454	3,52E-284

Supplementary Figure Legends

Supplementary figure 1: Gating strategy for small intestinal DCs. representative FACS plots of total Lamina propria, describing the gating strategy used in this study to isolate different murine DC subsets.

Supplementary figure 2: Plet1 is absent from macrophages and monocytes. representative FACS plots showing absence of Plet1 on CD64⁺ monocytes and macrophages in Lamina Propria of WT mice.

Supplementary figure 3: Plet1-deficiency does not affect BMDC development or activation. (A) Frequency of CD11c⁺MHCII⁺ BMDC after seven days of culture, using Plet1^{+/-} or Plet1^{-/-} bone marrow. (B) Frequency of activated Plet1^{-/-} BMDC, as shown in A, expressing the costimulatory molecules CD40, or CD86, as compared to littermate controls, quantified by flow cytometry. (C) Surface expression of costimulatory molecules (CD40, CD80, CD83, and CD86) on Plet1^{+/-} (black bars), or Plet1^{-/-} (grey bars) BMDC, following TLR4, and TLR7 stimulation, shown as mean fluorescence intensity quantified by flow cytometry. (D) Relative transcript levels by QPCR (normalized to GAPDH) of activation-induced cytokines (IL6, IL1β, IL23, IL12, and TNF) on Plet1^{-/-} BMDC or littermate controls, following culture in the presence or absence of Pam3Cys (TLR3 ligand), LPS (TLR4 ligand), or Imiquimod (TLR7 ligand).

Supplementary figure 4: *in-silico* **3D structure prediction of Plet1 protein reveals homology with the integrin-binding domain of Reelin.** (A) Ribbon diagram 3D structure representation of murine, and human Plet1 protein and the integrin-binding N-terminal domain of Reelin protein as predicted by Phyre 2 (RCSB Protein Databank, structure c3cooB). (B) Alignment of protein sequence of human and mouse Plet1 with human or mouse Reelin.

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Supplementary figure 1

Small intestinal Lamina Propria



Supplementary figure 2



Small intestinal Lamina Propria

Supplementary figure 3



В

Supplementary figure 4



c3cooB : crystal structure of reelin-n domain of f-spondin (model dimensions (Å): X:44.625 Y:38.569 Z:30.490)

Human Plet1 alignment

Murine Plet1 alignment

