Supplementary Data.

Expression of the atypical chemokine receptor ACKR4 identifies a novel population of intestinal submucosal fibroblasts that preferentially expresses endothelial cell regulators

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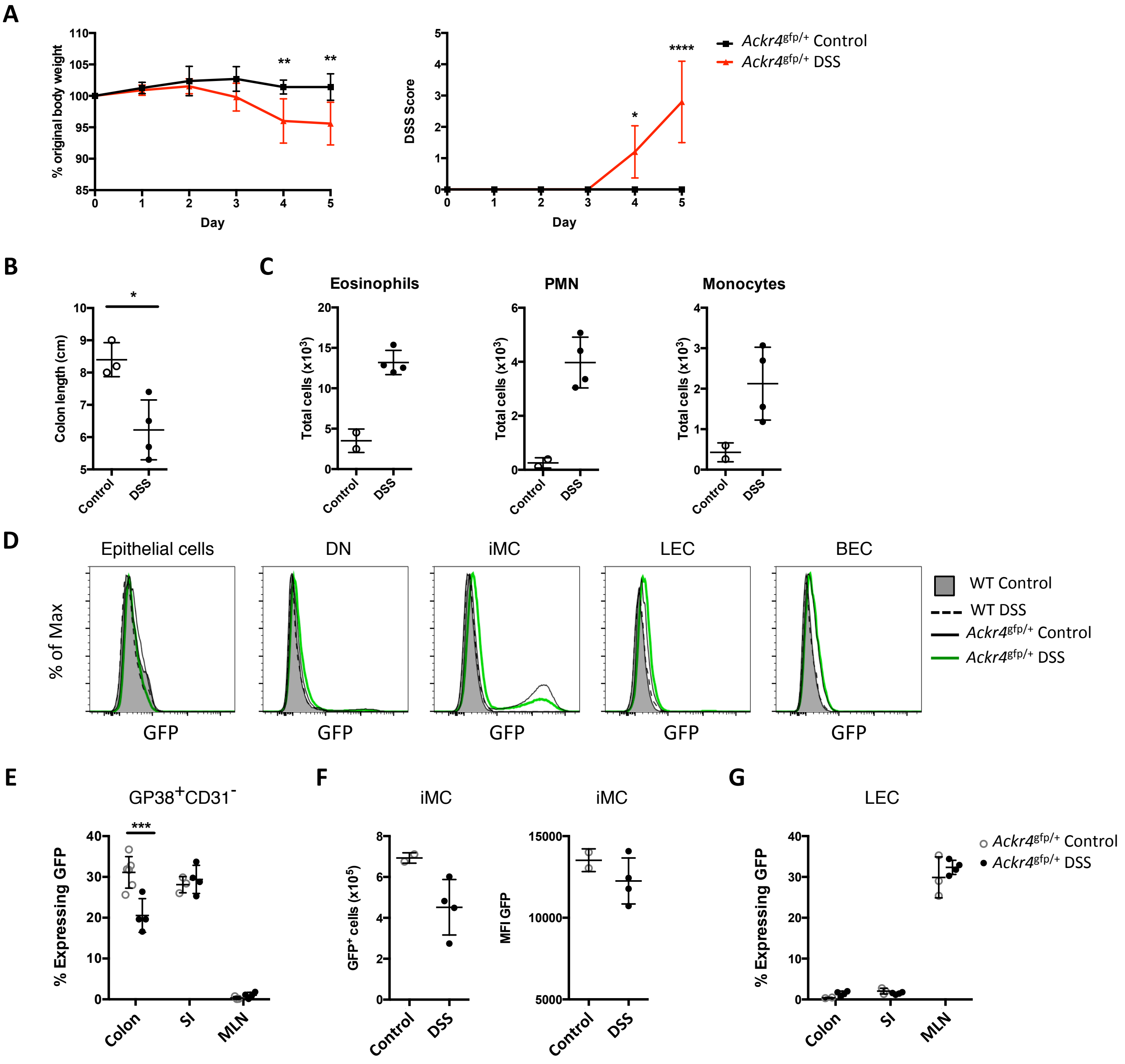
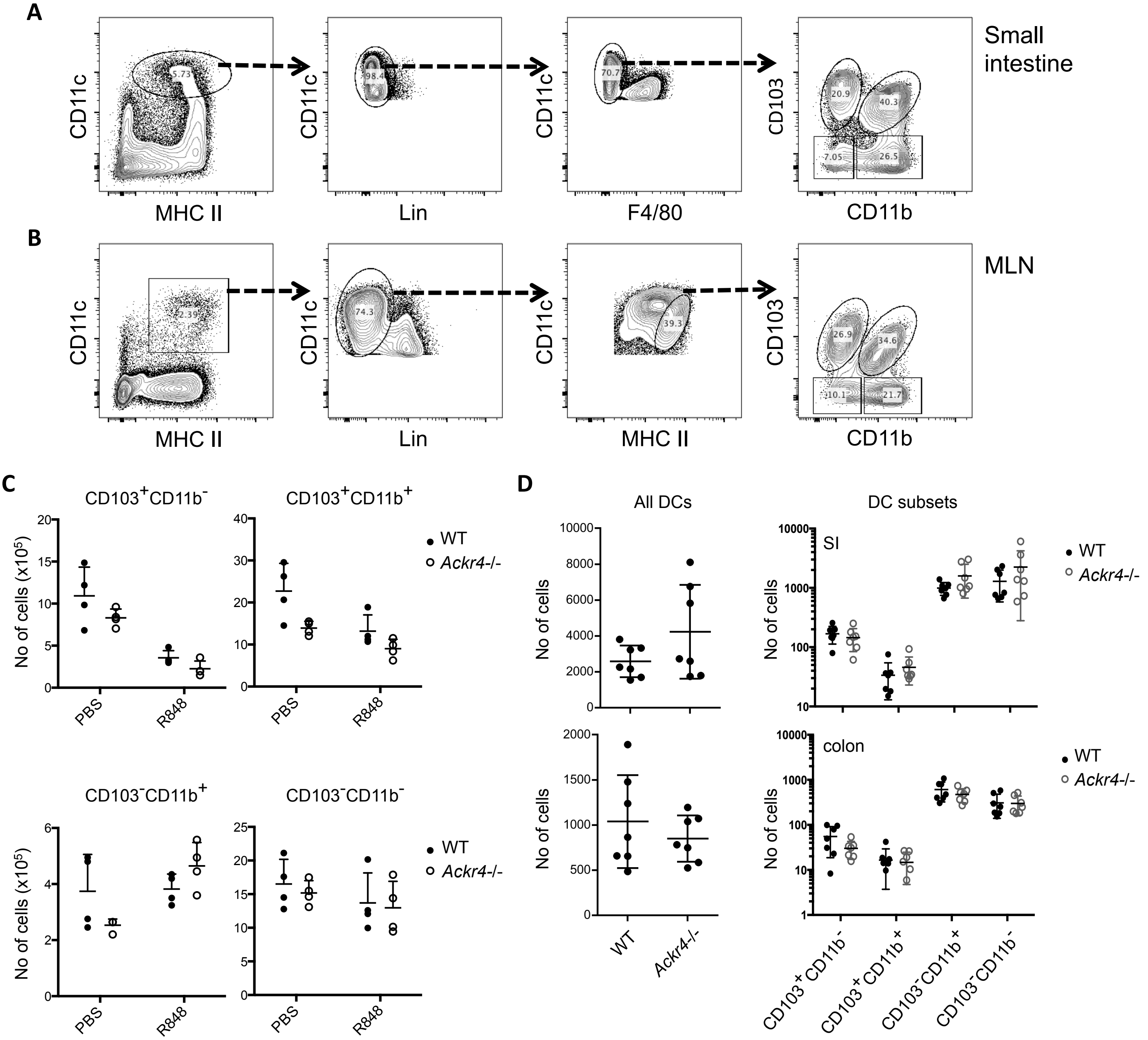
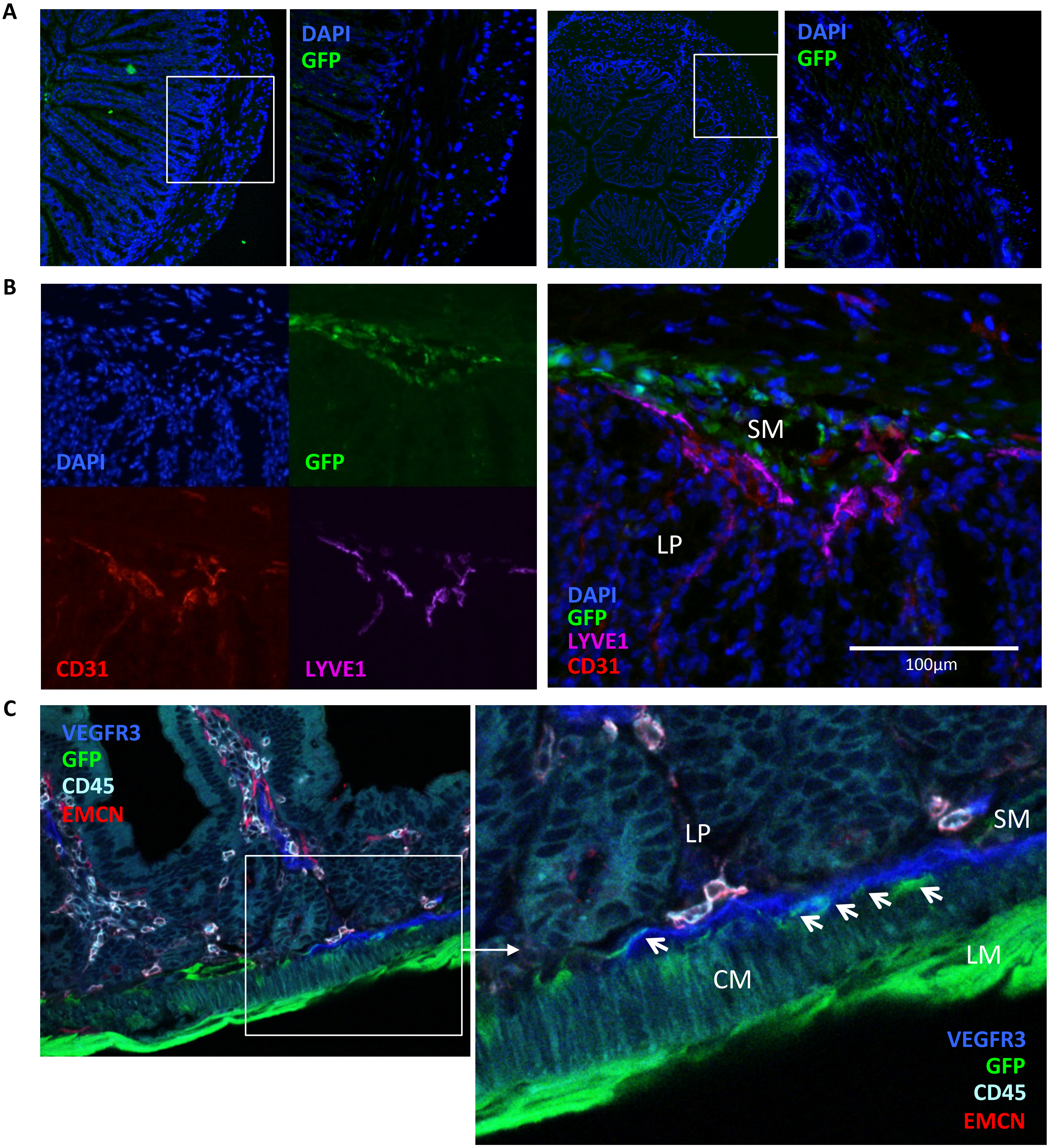


Supplementary Figure 1. *Ackr4* expression is restricted to colonic iMCs and MLN LECs in colitic mice. *Ackr4*$^{gfp/+}$ (*Ackr4*^{tm1Ccbl}) and WT mice were given 2% DSS in the drinking water (DSS group) or maintained on sterile water (Control) for 5 days. **(A)** Change in original body weight (left) and clinical scores (right) in *Ackr4*$^{gfp/+}$ mice (n=4-5 mice/group). *p<0.05, **p<0.01, ****p<0.0001, two-way ANOVA. **(B)** Colon lengths in *Ackr4*$^{+/gfp}$ mice at day 5. *p<0.05, unpaired Student's t test. **(C)** Numbers of inflammatory leukocytes isolated from the colon of *Ackr4*$^{gfp/+}$ mice at day 5. Eosinophils (SiglecF^{+}), neutrophils (PMN; Ly6G^{+}CD11b^{hi}), and monocytes (SiglecF^{-}Ly6G^{-}CD11b^{+}Ly6C^{+}) were identified by flow cytometry after gating for single, live, CD45^{+} leukocytes. **(D)** Representative overlaid flow cytometry histogram profiles showing GFP expression by colonic stromal cell subsets (GP38^{-}CD31^{-} 'double negative' cells (DN); intestinal mesenchymal cells (iMC); lymphatic endothelial cells (LEC); blood vessel endothelial cells (BEC)). **(E)** Percentage of GP38^{+}CD31^{-} cells expressing GFP in colon, small intestine (SI) and MLN of *Ackr4*$^{gfp/+}$ mice on day 5. ***p<0.001, two-way ANOVA. **(F)** Numbers of colonic iMCs expressing GFP, and the mean fluorescence intensity (MFI) of GFP expression, in *Ackr4*$^{gfp/+}$ mice on day 5. **(G)** Percentage of LECs expressing GFP in the colon, SI and MLN of *Ackr4*$^{gfp/+}$ mice on day 5. In A-C and E-G, datapoints from individual mice are shown, along with means ± 1SD.



Supplementary Figure 2. (A-B) Flow cytometric gating strategies used to identify DCs. (A) Small intestine. CD11c$^+$MHCII$^+$ cells were identified among single, live, CD45$^+$ cells, before lineage (Lin)$^+$ cells (CD3$^+$ T cells and B220$^+$ B cells/plasmacytoid DC) and F4/80$^+$ macrophages were excluded. The remaining conventional DCs were then divided into four populations based on the expression of CD103 and CD11b. **(B)** MLN. Cells expressing lineage markers CD3 and B220 were excluded from single, live, CD45$^+$CD11c$^+$MHCII$^+$ cells in the MLN. Migratory DCs were then identified as MHCII^{hi}, with remaining CD11c$^+$MHCII$^+$ cells classified as resident DCs. Migratory DCs were divided into four populations based on expression of CD103 and CD11b. **(C) Number of DCs in small intestine of WT and *Ackr4*$^{-/-}$ mice 24 hours after R848 treatment.** Numbers of cells within each of the four conventional DC populations in the small intestine of WT and *Ackr4*-deficient (*Ackr4*$^{tm1.1Rjbn}$) (*Ackr4-/-*) mice 24 hours after i.p. administration of PBS or 100μg R848. Individual data points are shown, along with means + 1SD. Data are representative of three individual experiments. **(D) DCs in the intestinal muscularis externa.** Graphs show the total number of DCs (CD45$^+$CD64$^-$CD11c$^+$MHCII^{hi}) (left hand graphs), and the number of cells in each DC subset (defined according to CD103 and CD11b expression) (right hand graphs), in single cell suspensions of the muscularis externa prepared from the small intestine (SI) and colon of WT and *Ackr4*-deficient (*Ackr4*$^{tm1.1Rjbn}$) (*Ackr4-/-*) mice. Individual data points are shown, along with means +/- 1SD. In **C** and **D**, no statistically significant differences are present between WT and *Ackr4-/-* groups for any cell type.



Supplementary Figure 3. *Ackr4*-expressing iMCs are found adjacent to blood and lymphatic vessels. (A) Representative fluorescent microscopy images of transverse sections of small intestine (left) and colon (right) from wild-type mice co-stained with DAPI (blue). Images were captured with settings equivalent to those used on sections from *Ackr4*$^{gfp/+}$ mice (Fig 5A-B) and show absence of GFP signal in WT intestine. **(B-C)** Fluorescent microscopy images of sections of intestine from *Ackr4*$^{gfp/+}$ (*Ackr4*^{tm1Ccbl}) mice immunostained with fluorescently labeled antibodies against the proteins indicated on each image. GFP identifies *Ackr4*-expressing cells **(B)** Colon. Left panels show individual colors; right panel shows composite image. LP, lamina propria; SM, submucosa. **(C)** Small intestine. Right panel is a higher magnification of region boxed in the left panel. Arrows identify GFP$^+$ fibroblasts adjacent to VEGFR3$^+$ LECs. LP, lamina propria; SM, submucosa; CM, circular muscle; LM, longitudinal muscle. All images shown are representative of data from at least two experiments in which two or more mice were analyzed.

Transcripts significantly MORE abundant in GFP+ cells.

Gene	FC	P-value	Gene	FC	P-value	Gene	FC	P-value
Il6	8.79	8.72E-05	*Postn*	3.07	1.13E-06	*Dusp1*	2.26	3.51E-04
Cd81	8.66	8.28E-08	LOC100861693	3.03	3.78E-04	*Sgms2*	2.24	1.92E-07
Chodl	7.97	8.34E-07	*Grem1*	3.03	1.68E-06	*Gpr133*	2.24	2.31E-04
Gm11309	6.65	1.09E-04	*Ism1*	3.01	6.13E-04	NONCODE[1]	2.23	1.41E-04
1700019G06Rik	6.55	1.41E-04	NONCODE[1]	2.97	4.33E-06	*Ctsh*	2.22	3.61E-06
Adm	5.30	1.16E-04	*Slit2*	2.94	5.78E-06	*Plxdc2*	2.22	1.16E-04
Gm11314	5.20	4.78E-04	*Ntn4*	2.91	9.64E-04	*Adamtsl1*	2.20	2.99E-05
Gfpt2	5.15	1.70E-04	NONCODE[1]	2.90	4.09E-05	*Slc16a2*	2.17	3.80E-04
Cilp	5.11	1.28E-04	*Aebp1*	2.84	8.82E-06	*Nfkbia*	2.16	2.10E-04
Piezo2	5.10	3.72E-07	*Adamtsl3*	2.83	9.08E-07	NONCODE[3]	2.15	6.83E-04
Pcolce2	5.00	1.71E-07	NONCODE[2]	2.79	1.88E-05	*Flrt3*	2.15	5.76E-05
Prss23	4.89	4.88E-05	NONCODE[1]	2.79	5.36E-04	*Fndc1*	2.15	5.01E-04
Olfml2b	4.59	1.43E-05	*Fam180a*	2.70	5.65E-04	*Foxp2*	2.15	4.83E-06
Serpine1	4.58	2.12E-04	*Fign*	2.70	3.41E-04	NONCODE[1]	2.15	1.35E-04
NONCODE[1]	4.54	2.10E-06	LOC100862089	2.69	3.29E-04	*Prlr*	2.14	1.04E-05
Npy1r	4.40	3.77E-04	*Rbp1*	2.68	7.12E-04	*Gm11816*	2.13	7.68E-04
Ebf1	4.25	4.02E-07	*Fam71a*	2.68	3.23E-04	*Serpini1*	2.12	5.97E-04
Figf	3.63	1.43E-04	*Lgi2*	2.67	9.71E-04	*Enpp2*	2.11	3.41E-04
Gdf6	3.58	2.85E-04	*Osr1*	2.65	3.45E-04	*Pdgfrl*	2.11	2.56E-05
Dhrs3	3.57	4.39E-06	*Cpxm1*	2.61	1.53E-06	*Galnt16*	2.11	1.02E-04
Ar	3.48	5.56E-07	*Col14a1*	2.50	5.50E-05	*Id3*	2.10	4.74E-04
Col8a1	3.45	5.78E-07	*Tek*	2.48	1.18E-05	*Smpdl3a*	2.09	8.48E-05
Ier2	3.43	1.68E-04	*Hk2*	2.47	6.81E-04	*Chrdl1*	2.09	4.41E-04
Pi16	3.42	4.65E-05	*Epyc*	2.47	2.55E-05	*Gm15079*	2.09	9.76E-06
NONCODE[2]	3.42	7.78E-05	*Pla2g2e*	2.45	1.13E-04	*Pde12*	2.08	4.87E-04
Mt2	3.41	2.85E-04	*Klf4*	2.45	1.39E-04	*Cldn10*	2.08	3.12E-04
Flrt2	3.36	8.36E-05	*Gm17034*	2.43	8.67E-04	*Cgnl1*	2.08	5.20E-05
NONCODE[1]	3.35	6.28E-04	*Cd248*	2.41	2.37E-04	NONCODE[1]	2.08	3.98E-04
Rcan1	3.31	6.27E-05	***Ackr4***	2.40	1.19E-04	*Adcyap1r1*	2.08	7.03E-04
Gpc3	3.28	6.54E-07	*Igfbp6*	2.39	8.79E-05	*Bag3*	2.08	4.11E-04
Gm26802	3.28	3.43E-04	*Cd68*	2.38	7.25E-04	*Abi3bp*	2.08	7.81E-06
Gm12158	3.23	1.55E-05	*Cpz*	2.36	1.91E-05	*Gm17035*	2.08	4.88E-04
NONCODE[1]	3.22	6.24E-06	NONCODE[1]	2.36	7.61E-08	*Syne1*	2.06	1.87E-04
NONCODE[1]	3.19	5.63E-05	Y0G0104A12	2.33	3.16E-06	*Ugdh*	2.04	7.47E-05
Scara5	3.15	9.60E-04	*Lsamp*	2.31	1.77E-05	*Gm26889*	2.01	3.97E-05
Lbp	3.14	3.59E-05	NONCODE[1]	2.31	5.16E-04	*Pvr*	2.01	7.76E-04
NONCODE[3]	3.12	2.26E-04	NONCODE[3]	2.30	1.50E-04	NONCODE[3]	2.00	4.42E-06
Fam129a	3.12	4.09E-07	*Myc*	2.30	1.90E-04	*Gm17083*	2.00	5.37E-04
Vldlr	3.11	1.28E-04	*Cadm3*	2.28	1.00E-04			
Fap	3.08	2.05E-04	*Scd1*	2.27	8.50E-04			

Transcripts significantly LESS abundant in GFP+ cells.

Gene	FC	P-value	Gene	FC	P-value	Gene	FC	P-value
Mmp10	-19.23	6.16E-08	*Il34*	-2.84	4.37E-04	*Casq2*	-2.39	3.87E-06
Pdlim3	-6.57	1.61E-04	*Hpse2*	-2.78	1.09E-04	NONCODE[1]	-2.37	9.17E-04
Acta2	-6.18	1.53E-05	*Hmcn1*	-2.78	2.25E-06	NONCODE[1]	-2.36	2.78E-06
NONCODE[3]	-4.98	3.42E-06	*Gm16054*	-2.76	1.17E-06	*Chp2*	-2.31	9.18E-04
NONCODE[1]	-4.36	1.04E-04	*Myh11*	-2.75	7.17E-05	*Ptgs1*	-2.29	2.40E-04
LOC100862378	-4.27	2.67E-04	*Cxcl14*	-2.73	1.14E-04	*Nid2*	-2.23	1.47E-05
Bmp5	-4.22	2.60E-05	NONCODE[3]	-2.71	4.20E-04	NONCODE[2]	-2.20	1.01E-04
Ednrb	-4.12	1.27E-04	*Lphn3*	-2.70	9.23E-07	NONCODE[2]	-2.19	1.15E-05
Igfbp3	-3.90	3.14E-06	NONCODE[2]	-2.68	3.53E-05	*Kitl*	-2.19	9.48E-04
Srpx2	-3.65	7.48E-06	*Mir5124*	-2.68	1.64E-04	NONCODE[1]	-2.16	2.11E-04
Abcc9	-3.63	1.19E-05	*Sept4*	-2.67	6.24E-06	*Epas1*	-2.15	7.35E-04
Tagln	-3.23	4.28E-04	*Col12a1*	-2.67	2.14E-04	*Hpse*	-2.08	9.86E-04
Bmp7	-3.15	2.05E-04	*Bmp3*	-2.64	3.11E-04	*Pdlim1*	-2.08	1.60E-04
Aldh1a3	-2.98	2.45E-05	*Tnc*	-2.55	1.28E-04	*Foxf2*	-2.07	1.55E-05
NONCODE[3]	-2.93	2.97E-05	*Rdh10*	-2.55	9.90E-04	*Lama1*	-2.07	2.25E-05
Gpr81	-2.89	9.50E-05	NONCODE[3]	-2.46	1.47E-05	NONCODE[1]	-2.06	5.36E-05
Col6a5	-2.86	5.55E-06	NONCODE[3]	-2.42	3.62E-04	*Pde1c*	-2.05	6.70E-04

NONCODE[1]: Non-coding transcript identified by NONCODE:SenseNoExonic; NONCODE[2]: Non-coding transcript identified by NONCODE:Antisense; NONCODE[3]: Non-coding transcript identified by NONCODE:Linc

Supplementary Table 1: Genes showing statistically significant >2-fold differential expression between the GFP+ and GFP- subsets of CD45-CD31-GP38+CD146- cells isolated by from the small intestine of *Ackr4*$^{gfp/+}$ (*Ackr4*^{tm1Ccbl}) mice. FC, fold change. Of the transcripts more abundant in GFP+ SI fibroblasts, those encoding secreted factors are labeled in red, while those encoding cell surface proteins are green.