Supplemental Data

PlexinD1 Glycoprotein Controls Migration of Positively Selected Thymocytes into the Medulla

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Supplemental Experimental Procedures

Cloning, expression and purification of sema3E-Fc, sema3F-Fc and plexinD1-Fc proteins

The secreted sema3E, sema3F and plexinD1 recombinant proteins were produced as fusion proteins N-terminal to a functional mouse γ_{2c} Fc domain. The complete cDNAs were amplified from total RNA preparations of both mouse brain and thymus using the Superscript III HiFi RT-PCR system (Invitrogen, CA). The primers used incorporated a 5' *Age*I site and 3' *EcoR*V site for *sema3E*, a 5' *EcoR*I site and 3' *Xho*I site for *sema3F* and 5' *EcoR*I site and 3' *Sal*I site for *Plxnd1*, respectively. After digestion of *sema3E* cDNA with *Age*I/*EcoR*V and *sema3F* cDNA with *EcoRI*/*Xho*I, the fragments were ligated into appropriately digested pFUSE-mfc1 vector (Invivogen, CA) to yield a 2868bp construct (*sema3E*) and a 2937bp construct (*sema3F*) encoding the complete native start codon and signal peptide, sema domain, plexin-semaphorin-integrin domain and Ig-like domain of *sema3E* and *sema3F* upstream of an Fc domain. The constructs were electroporated into CHO cells using standard procedures. Transfected cells were selected by addition of zeocin (500μg/ml) to the medium and supernatants of growing clones were screened for secreted sema-Fc by dot blotting onto nitrocellulose followed by detection using anti-γ_{2c}-HRP. Sema-secreting clones were expanded and secreted sema3E-Fc and sema3F-Fc purified from the conditioned media by Protein A-affinity chromatography.

For the cloning of secreted plexinD1, the mouse γ_{2c} Fc domain was amplified from the pFUSE-mfc1 vector where the primer incorporated a 5' *EcoR*I-*Xho*I site and 3' *Xba*I site. Mouse γ_{2c} Fc domain digested with *EcoR*I/*Xba*I was ligated into pBluescript sk(-) lacking *Sal*I/*Xho*I sites (pBS-sx). *EcoR*I/*Sal*I-digested Plxnd1 full ectodomain (3854bp) was ligated into *EcoR*I/*Xho*I-digested pBS-sx. The cDNA for

plexinD1 mouse Fc fusion protein was then digested with *EcoR*I/*Xba*I and ligated into appropriately-digested pTracer-CMV2 (Invitrogen, CA) followed by transfection of the *SspI*-linearized construct into 293T cells using Lipofectamine 2000^{TM} (Invitrogen, CA). Transfected 293T cells were selected and screened by addition of zeocin (400μg/ml) as described for the selection of sema-Fc proteins. Secreted plexinD1-Fc was purified from the conditioned media by Protein A-affinity chromatography.

Measurement of plexinD1-semaphorin interactions using surface plasmon resonance

All interactions were determined using a BIAcore 3000 instrument. PlexinD1 (10 μg/ml in 100 mM sodium acetate, pH 4.5) was immobilized on CM5 Biosensor chips (BIAcore) through cross-linking of free amine groups to the N-hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-HCl-activated flow cell surface, followed by blocking of free succinimide ester with 1 M ethanolamine. After extensive washing of the surface with binding buffer (10 mM HEPES, 150 mM NaCl, pH 7.0), sema-Fc fusion protein binding was assessed by injecting varying concentrations (0-25μg/ml) in binding buffer over the flow cell surface. When required, the surface was regenerated using 100 mM glycine, pH 2.8. The data were analyzed using the BIAevaluation version 3.2 software and fitted to a 1:1 Langmuir binding model with separate k_d and k_a determination. The association constant (K_a) was determined as k_a/k_d, and the dissociation constant (K_d) was determined as $1/K_a$.

Microarray analysis

For sorting thymocyte subpopulations from *N15TCRtg*$^{+/+}$ *Rag2*$^{-/-}$ mice, cells were stained with anti-CD4 (L3T4) and anti-CD8α (Ly-2) antibodies followed by sorting of DP, Int and SP thymocytes on a MoFlo (Cytomation, CO) as illustrated in Fig 1A. Total RNA from sorted thymocytes (2μg) were used in the first strand cDNA synthesis with T7-d(T)24 primer (gcc agt gaa ttg taa tac gac tca cta tag gga ggc gg-(dT)24) and Superscript II using the CodeLink Expression Assay Reagent Kit (Amersham, NJ). The purified cDNA was incubated at 37°C for 5h in an *in vitro* transcription reaction to produce cRNA labeled with biotin. The hybridization cocktail containing 15μg adjusted fragmented cRNA mixed with eukaryotic hybridization controls (contains control cRNA and chip control oligonucleotide B2) was

hybridized with pre-equilibrated MOUSE430 2.0 Affymetrix chips at 45°C for 16 h. After the hybridization cocktails were removed, the chips were washed and stained with normal goat IgG and biotinylated mouse anti-streptavidin antibody and restained with streptavidin-PE. The chips were scanned in an HIP chip scanner (Affymetrix, CA) to detect hybridization signals and results analyzed by dChip.

When comparing two groups of samples to identify the genes enriched in a given phenotype, we used the lower confidence bound (LCB) of the fold change (FC) between the experiment and the baseline. If the 90% LCB of the FC between the experiment and the baseline was above 2.0, the corresponding gene was considered to be differentially expressed.

Laser capture microdissection (LCM)

Freshly prepared frozen thymic sections (10μm in thickness) were gently placed on slides and briefly stained with hematoxylin. Indicated regions of thymus were collected using a Veritas Microdissection Machine (Arcturus, CA). Total RNA was isolated from the collected tissue fragments using Trizol (Invitrogen, CA) and used for real time RT-PCR. *Sema3e* transcripts were amplified with the following primer set 5'- agg ccc tga ata cca ctg gtc-3' and 5'- ggt tcc tgt gcc agc aaa gt –3' using Taqman One Step RT PCR Master Mix and 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA). Amplified PCR products were quantitated using FAM labeled Taqman probe 5'- cta tat gaa gga aaa gtc ccc tac cca agg cc –3'. Taqman *Gapdh* control reagent (part # 4331182) was purchased from Applied Biosystems (Foster city, CA).

Migration assay

The migration assay was performed in 24-well transwell plates (Corning Costar, MA) with 5 μm pore size filters. Thymocytes were resuspended at a density of $2.5x10^7$ cells/ml in RPMI containing 10% FBS. One hundred microliters of cell suspension were placed in the upper chamber and 600μl media supplemented with the indicated amounts of mouse IgG_{2c} Fc, sema3E-Fc, recombinant mouse CCL25, CCL21 and CXCL12 (R&D Systems, MN) were placed in the lower chamber. After incubation for 2

hours at 37°C, the upper chamber was removed, and the cells in the lower chamber were harvested and counted using a hemacytometer. Harvested cells were resuspended in 10μl of PBS containing anti-CD69-PE, anti-TCRβ-APC, anti-CD8α-FITC and anti-CD4-Pacific blue and incubated for 15 min at 4°C. After washing with PBS, cells were resuspended in 300μl PBS and analyzed by flow cytometry.

Mice and fetal liver cell transplantation

Plxnd1$^{-/-}$ mice have been described in a previous report (Gitler *et al.*, 2004) and were backcrossed onto a C57BL/6 background (CD45.2) for 10 generations and used to obtain timed pregnant mice for fetal liver cell transplantation. At 15.5 dpc, pregnant mice were sacrificed, fetuses dissected out, *Plxnd1*$^{-/-}$ embryos identified and fetal liver cells prepared. Two million fetal liver cells in PBS were injected i.v. into 700 rad-irradiated B6.SJL-Ptprc^{a} CD45.1 congenic mice (Taconic, NY) and 4-5 wks after reconstitution, mice were sacrificed and lymphoid tissues analyzed. *Sema3e*$^{-/-}$ mice have been described previously (Gu *et al*., 2005).

Immunohistochemistry

For tissue section analysis, 10% formalin fixed, paraffin-embedded 5 μm sections were mounted on glass slides, deparaffinized and rehydrated through graded alcohols. Immunohistochemistry was performed by an automated stainer (Biogenix, San Ramon, CA). For antigen retrieval, sections were subjected to microwaving in 10mM citrate buffer (pH 6) in a 750W oven for 15 min followed by incubation with the indicated polyclonal Abs (1:100 dilution) for 1 h. As controls, preimmune serum or no primary Abs were used. The staining procedure consisted of blocking of endogenous peroxidases, incubation with primary Ab, application of an anti-rabbit Ab-avidin peroxidase complex, and visualization with 3-3' diaminobenzidine as substrate with 5 min development time and with Mayer's hematoxylin counterstaining.

Immunoprecipitation and western blot

Cells were lysed with EB buffer (1% Triton X100, 20mM Tris pH 7.4, 5mM ethylenediaminetetraacetic acid [EDTA], 10% glycerol, 150mM NaCl) in the presence of a mixture of

protease inhibitors (phenylmethylsulfonyl fluoride, leupeptin, aprotinin, pepstatin) and immunoprecipitated with anti-plexinD1 antisera. Lysate protein concentrations were determined using the BCA Protein Assay Kit (Pierce, IL) according to the manufacturer's protocol. Immunoprecipitated or total lysate proteins were separated by SDS-PAGE, transferred to PVDF, blotted with anti-plexinD1 antisera or anti-actin antiserum (Sigma-Aldrich, MO) followed by anti-rabbit-HRP secondary antibody (Invitrogen, CA), and detection by chemiluminescence (ECL system; Amersham, VA). Quantification of band intensity was performed using the Gel Logic 200 Imaging System and 1D Image Analysis Software v3.6 (Kodak, NY).

Supplemental References

Gitler, A.D., Lu, M.M., and Epstein, J.A. (2004). PlexinD1 and semaphorin signaling are required in endothelial cells for cardiovascular development. Dev Cell 7, 107-116.

Gu, C., Yoshida, Y., Livet, J., Reimert, D.V., Mann, F., Merte, J., Henderson, C.E., Jessell, T.M., Kolodkin, A.L., and Ginty, D.D. (2005). Semaphorin 3E and plexin-D1 control vascular pattern independently of neuropilins. Science 307, 265-268.

Figure S1

Pattern I

Probe set	Gene	Accession	Locus Link	Ia	Ib	IIa	IIb	IIIa	IIIb
1449216_at	integrin, alpha E, epithelial-associated	NM_008399	16407	27.08	12.63	254.10	197.27	875.84	778.46
1416124_at	cyclin D2	NM_009829	12444	-2.88	-3.86	56.49	33.16	99.88	65.50
1460245_at	killer cell lectin-like receptor, subfamily D, member 1	NM_010654	16643	13.89	13.73	36.96	44.05	274.53	484.70
1437270_a_at	B-cell stimulating factor 3	BB825816	56708	5.91	0.82	54.38	33.05	136.60	125.42
1423756_s_at	insulin-like growth factor binding protein 4	BC019836	16010	15.74	16.25	51.46	49.64	299.88	219.39
1453003_at	sortilin-related receptor, LDLR class A repeats-containing	AK013519	20660	6.28	6.86	47.87	25.86	102.96	125.50
1443937_at	interleukin 2 receptor, beta chain	BE634648	16185	42.78	23.92	295.12	217.46	465.28	530.30
1439814_at	Mus musculus transcribed sequences	BM246630		17.81	17.41	91.64	83.32	207.49	269.74
1428909_at	Mus musculus transcribed sequences	C85657		85.07	62.95	220.60	237.97	876.13	874.28
1437445_at	transient receptor potential cation channel, subfamily M, member 1	BB770967	17364	8.94	2.77	92.89	64.66	214.93	134.52
1448485_at	gamma-glutamyltransferase 1	NM_008116	14598	11.34	11.19	30.66	19.79	206.85	181.51
1419202_at	cystatin F (leukocystatin)	NM_009977	13011	9.82	2.18	39.56	37.17	120.77	152.36
1419711_at	CD7 antigen	NM_009854	12516	39.20	35.21	283.60	282.64	628.81	437.23
1455000_at	G protein-coupled receptor 68	BB538372	238377	3.05	4.43	17.64	3.72	113.82	96.29
1418536_at	histocompatibility 2, Q region locus 7	M29881	15018	24.38	8.96	59.54	38.58	239.81	546.46
1437247_at	fos-like antigen 2	BM245170	14284	5.42	-1.74	43.30	40.03	60.51	74.88
1456847_at	Mus musculus transcribed sequence	BM226282		6.86	7.54	47.11	42.22	99.57	66.13
1418402_at	a disintegrin and metalloproteinase domain 19 (meltrin beta)	NM_009616	11492	16.51	4.84	85.33	54.90	195.70	136.25
1450753_at	natural killer cell group 7 sequence	NM_024253	72310	163.60	122.87	792.20	459.09	1250.08	1105.45
1426743_at	Dip3 beta	BC002232	216190	13.92	-0.90	50.93	55.93	157.45	115.75
1452217_at	RIKEN cDNA 2310047C17 gene	BE570050	66395	8.56	8.35	32.96	16.86	79.35	83.18
1439407_x_at	transgelin 2	AV212626	21346	24.71	44.11	202.90	181.52	352.25	420.20
1418612_at	schlafen 1	NM_011407	20555	20.63	6.90	80.88	60.48	222.26	140.88

Pattern II

Probe set	Gene	Accession	Locus Link	Ia	Ib	IIa	IIb	IIIa	IIIb
1448390_a_at	dehydrogenase/reductase (SDR family) member 3	NM_011303	20148	84.36	124.25	5.97	10.51	0.69	-1.91
1451475_at	plexin D1	BC019530	67784	219.00	171.37	15.93	15.21	5.57	-1.28
1448929_at	coagulation factor XIII, alpha subunit	NM_028784	74145	525.01	565.54	199.52	167.67	48.25	42.28
1419696_at	CD4 antigen	NM_013488	12504	350.90	515.34	-33.04	11.83	-65.76	-68.59
1449831_at	RIKEN cDNA 1700055O19 gene	NM_026100	67344	145.17	189.36	24.51	33.84	8.82	12.95
1434252_at	RIKEN cDNA C630016B22 gene	BB711990	319880	241.17	283.67	71.74	54.72	20.57	14.88
1450680_at	recombination activating gene 1	NM_009019	19373	1046.32	1053.86	100.76	134.22	57.85	97.89
1454889_x_at	RIKEN cDNA C630016B22 gene	BB711990	319880	212.12	246.46	42.66	40.40	26.34	24.70
1418065_at	recombination activating gene 2	NM_009020	19374	136.54	192.91	14.83	30.13	2.06	17.38
1437217_at	ankyrin repeat domain 6	BM225135	140577	135.05	158.43	32.70	43.74	9.02	17.05
1425792_a_at	RAR-related orphan receptor gamma	AJ132394	19885	295.35	293.20	64.29	77.14	13.77	38.54
1451716_at	v-maf musculoaponeurotic fibrosarc. onc. family, protein B (avian)	AW412521	16658	41.14	55.28	-3.33	4.94	-4.08	2.18
1441316_at	Mus musculus transcribed sequences	BB234335		102.22	147.81	16.87	30.92	13.55	11.57
1445615_at	cholinergic receptor, nicotinic, alpha polypeptide 9	BE852824	231252	46.08	52.43	8.36	10.75	-3.32	1.70
1418611_at	gene rich cluster, A gene	NM_013533	14788	141.48	180.99	41.89	43.53	5.27	19.64
1417021_a_at	sporulation protein, meiosis-specific, SPO11 homolog (S. cerevisiae)	NM_012046	26972	258.53	200.84	57.18	61.15	24.58	29.96
1421898_a_at	major histocompatibility complex, class I-related	BB210729	15064	72.77	67.06	9.30	11.17	0.94	4.17
1434793_at	cDNA sequence BC028975	AI851014	242584	231.28	243.99	59.29	69.21	26.75	36.40
1451972_at	glucocorticoid induced transcript 1	AA152997	170772	702.31	673.03	200.28	227.48	118.15	105.07
1421172_at	a disintegrin and metalloproteinase domain 12 (meltrin alpha)	NM_007400	11489	70.77	65.49	22.37	11.08	7.82	5.19
1421818_at	B-cell leukemia/lymphoma 6	U41465	12053	605.70	601.13	189.89	216.09	109.62	100.61
1448420_a_at	F-box and leucine-rich repeat protein 12	NM_013911	30843	836.01	927.33	191.11	213.30	152.41	153.29
1435554_at	RIKEN cDNA C630016B22 gene	BB771888	319880	410.88	487.98	119.68	129.43	54.26	77.20
1455235_x_at	lactate dehydrogenase 2, B chain	AV216324	16832	1334.72	1575.41	528.32	415.56	245.59	245.46
1458344_at	gb:BB109777 /DB_XREF=gi:8762345 /DB_XREF	BB109777		142.01	168.47	67.11	62.28	25.87	21.49
1431541_at	RIKEN cDNA 1300006C19 gene	AK016943	68292	43.30	35.65	16.57	14.29	1.07	2.79

Pattern III

Probe set	Gene	Accession	Locus Link	Ia	Ib	IIa	IIb	IIIa	IIIb
1424932_at	epidermal growth factor receptor	U03425	13649	9.56	-3.14	7.08	13.98	7.80	14.05
1441978_at	aquaporin 6	AI956846	11831	-0.47	2.28	7.39	9.78	0.87	-0.05
1451407_at	junction adhesion molecule 4	BC004806	72058	8.79	9.93	22.60	21.27	6.39	8.57

Pattern IV

Probe set	Gene	Accession	Locus Link	Ia	Ib	IIa	IIb	IIIa	IIIb
1423180_at	potassium voltage gated channel, Shab-related subfamily, member 1	BB324482	16500	13.03	12.27	-1.96	4.02	13.37	19.29
1441314_at	inner centromere protein	AV303517	16319	0.18	2.31	-7.60	-4.41	5.73	-0.47
1423093_at	inner centromere protein	BI410774	16319	22.23	12.74	1.52	1.63	11.31	14.05

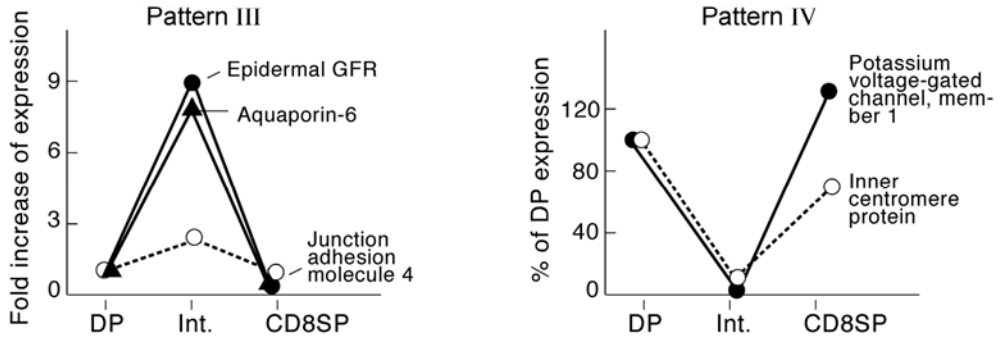

Figure S1. *List of genes expressed differentially in DP to CD8 SP transition.*

Representative genes that show significant changes (LCB >2.0) in Fig. 1B are listed for each expression pattern and their normalized dChip analysis values given for each of two independent experiments (a and b). Ia and Ib: $CD8^{high}$ and $CD4^{high}$ DP thymocytes; IIa and IIb: $CD8^{high}$ and $CD4^{int}$; IIIa and IIIb: CD8 SP thymocytes in Fig. 1A. The two graphs below show gene expression patterns III and IV noted in Fig. 1B. The 62 genes showing a transient increase followed by a decrease were clustered in pattern III and 11 genes showing a transient decrease followed by an increase were clustered in pattern IV. The complete microarray analysis data are deposited in Gene Expression Omnibu (GEO) with the accession number GSE 13493**.**

Figure S2

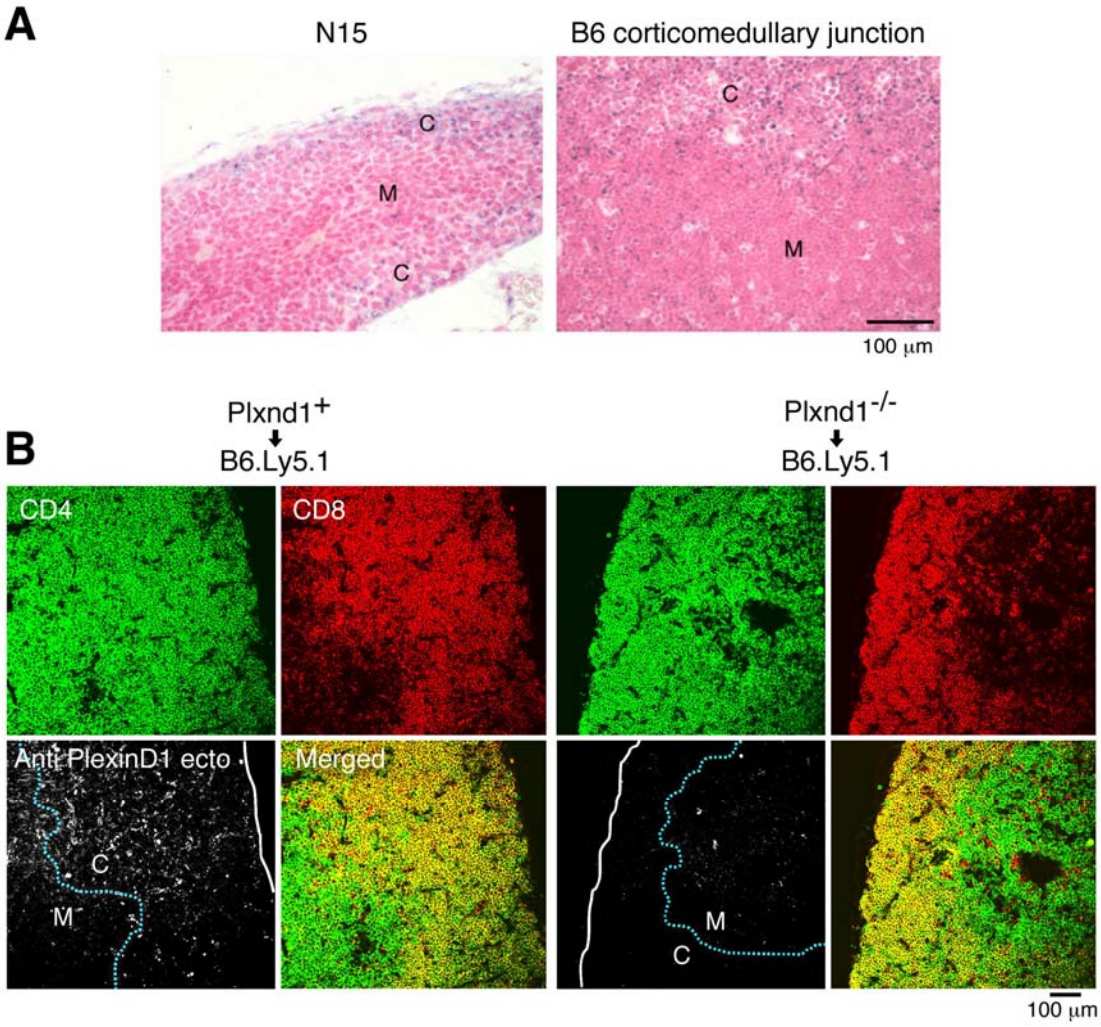

Figure S2. *Plxnd1-specific mRNA staining in the thymic cortex of normal B6 mice.*

A. The antisense *Plxnd1* probe was constructed by subcloning a *Plxnd1* PCR fragment containing bp 4210-4674 (numbering according to Genbank AY688678) into pBS. Formalin-fixed thymic sections derived from 4-week old B6 mice were prepared and subjected to *in situ* hybridization. B. One representative immunofluorescence staining result is shown here to show the distribution of *Plxnd1* in thymus. Five-micrometer cryosections of representative Ly5.1 irradiated host thymi reconstituted with fetal liver cells from *Plxnd1*$^{+}$ or *Plxnd1*$^{-/-}$ embryos at 4-5 wks after transplantation were stained with the

anti CD4-Alexafluor647 (green), anti-CD8α–TRITC (red) and rabbit anti-PlexinD1 ectodomain antisera/goat anti rabbit-FITC (white). The blue dashed lines depict the boundary between DP cell (C) and SP cells (M). The white lines outline the thymic capsule. White scale bar = 100μm. The results are representative of four thymi analyzed. C = cortex; M = medulla.

Figure S3

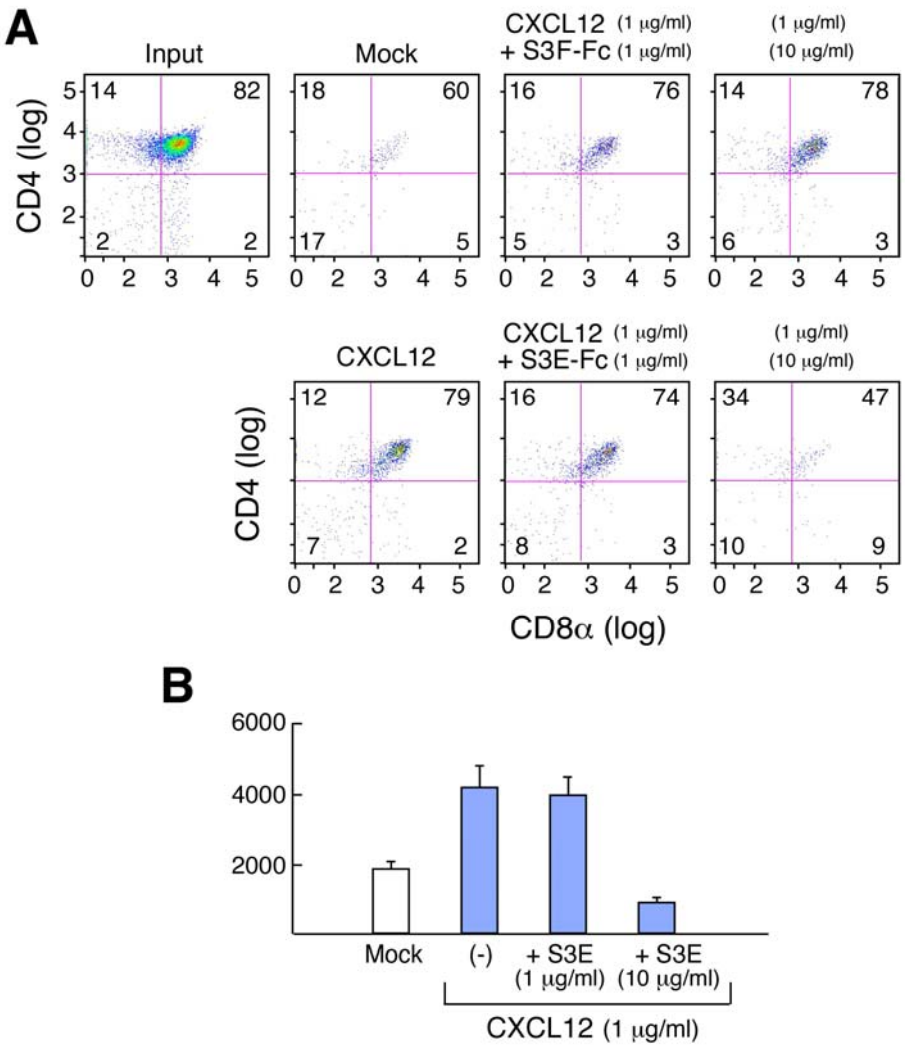

Figure S3. *Inhibition of CXCL12-mediated migration by sema3E-Fc.*

A. Migration assays were performed in 24-well transwell plates with a 5μm pore size as described in Fig 4. After 2 hrs, cells that had migrated to the bottom well were harvested and stained with anti-CD4, anti-CD8α, anti-CD69, and anti-TCRβ mAbs. Mock (top panel) refers to result in the absence of chemokine. Where relevant, CXCL12 was added to the bottom well. The concentration of S3E (sema3E-Fc) or S3F (sema3F-Fc) is indicated on top of each panel. The majority of migrating cells were TCRlow DP cells.

B. Aliquots of cells from the same experiment depicted in panel A were counted and the number of thymocytes migrating in response to CXCL12 with or without sema3E was determined. Error bars are the standard error of one triplicate experiment.

Figure S4

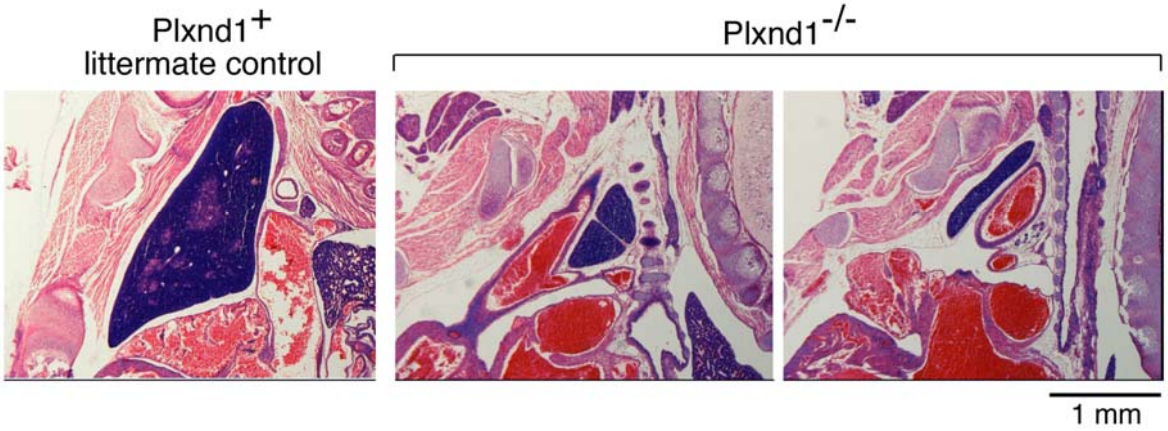

Figure S4. *Hypocellularity, disordered cortical architecture and lack of medullary areas in the thymus of Plxnd1$^{-/-}$ embryos.*

Whole embryos at 14.5 dpc were collected from timed pregnant mice and fixed in 10% formalin. H&E stained paraffin sections were prepared as described in Experimental Procedures. *Plxnd1$^{-/-}$* mice were identified by PCR using genomic DNA as previously decribed (Gitler *et al*., 2004). Scale bar = 1mm.

Figure S5

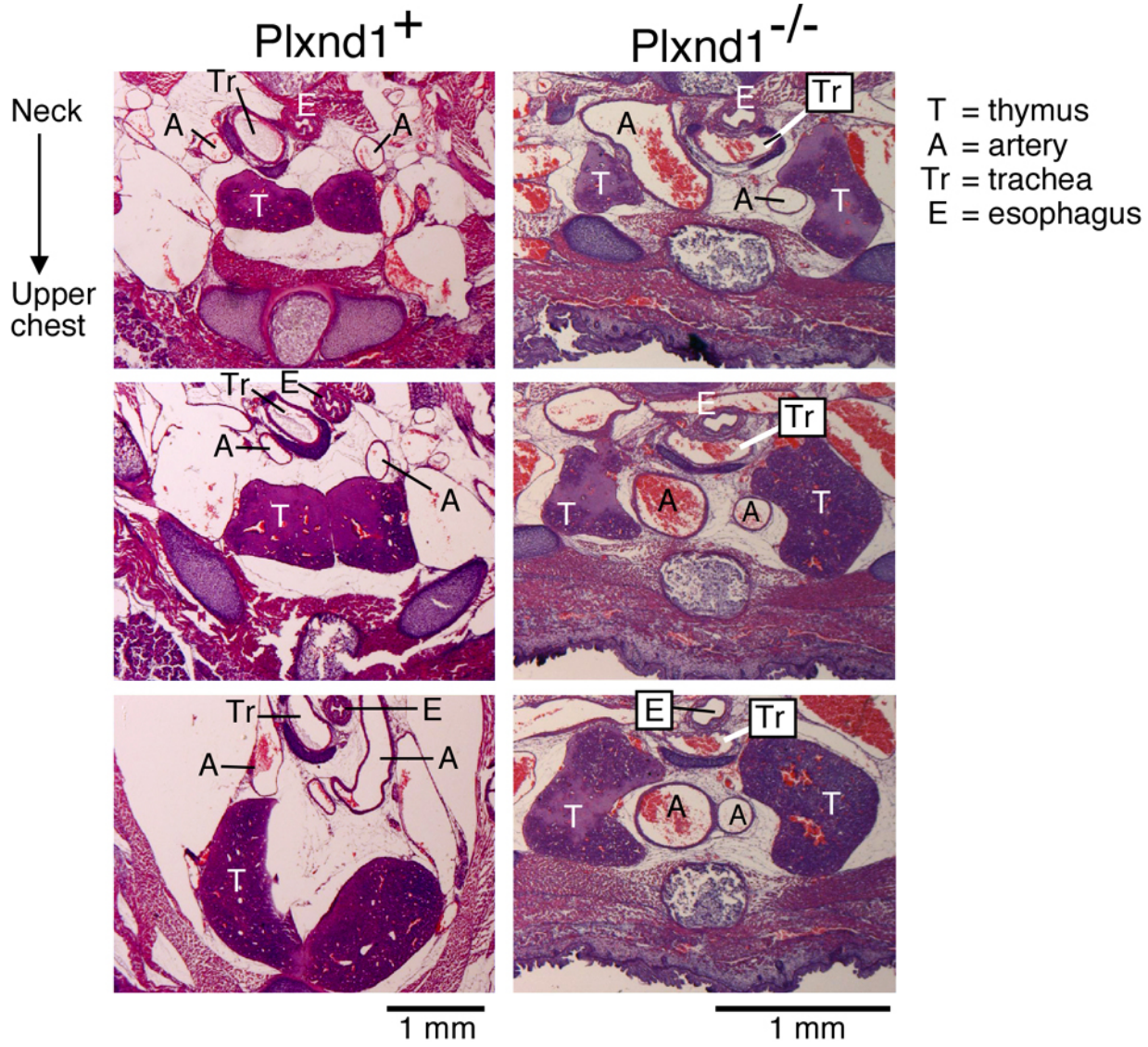

Figure S5. *Separated thymic lobes in Plxnd1$^{-/-}$ embryos*

Whole embryos at 18.5 dpc were collected from timed pregnant mice and fixed in 10% formalin. H&E stained transverse paraffin sections were prepared as described in Supplemental Experimental Procedures. Among 80 slides of one embryo, three sections were selected to show the connectivity of thymic lobes in sections from different planes. The distance between sections is 200μm. More than 20 *Plxnd1$^{-/-}$* embryos

and neonatal mice were examined and all of them had the same defect in fusion of thymic lobes. Scale bar = 1mm.

Figure S6

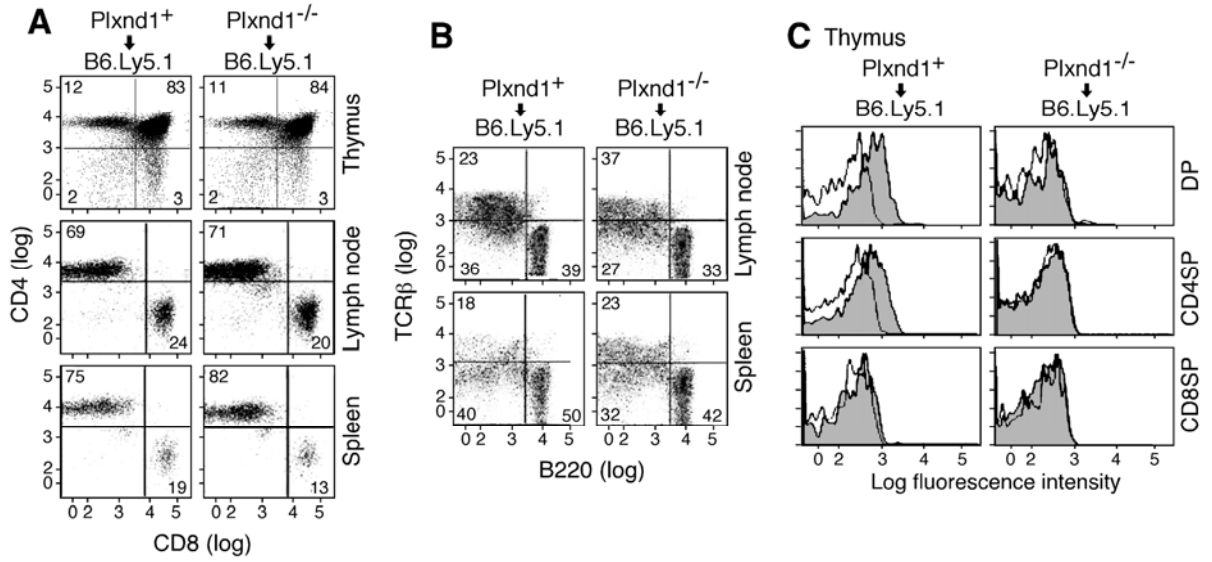

Figure S6. *Analysis of lymphoid cells from mice reconstituted with Plxnd1$^{-/-}$ or Plxnd1^{+} fetal liver cells.*

Irradiated Ly5.1 congenic mice were reconstituted with fetal liver cells obtained from the indicated embryos at ED 15.5. After 4 wks, single cell suspensions were prepared from lymphoid organs, stained with the indicated mAbs and analyzed using a flow cytometer. Data were analyzed using a FlowJo program (TreeStar, Or). All results are from Ly5.2^{+} cell populations. A. CD4/CD8 profile of total thymocytes, TCR^{high} lymph node cells and TCR^{high} splenocytes. The number in each quadrant represents the % of the gated population. B. TCRβ/B220 profile of total lymph node cells and total splenocytes. The number in each quadrant represents the % of the gated population. C. Total thymocytes of reconstituted mice were stained with sema3E-Fc or Fc only and anti-mouse IgG$_{2c}$-PE as described in Fig. 3C. The fluorescence of sema3E-Fc was plotted for the indicated thymocyte populations. The open histogram shows staining by control Fc/PE and the gray histogram represents specific sema3E-Fc/PE staining.

Figure S7

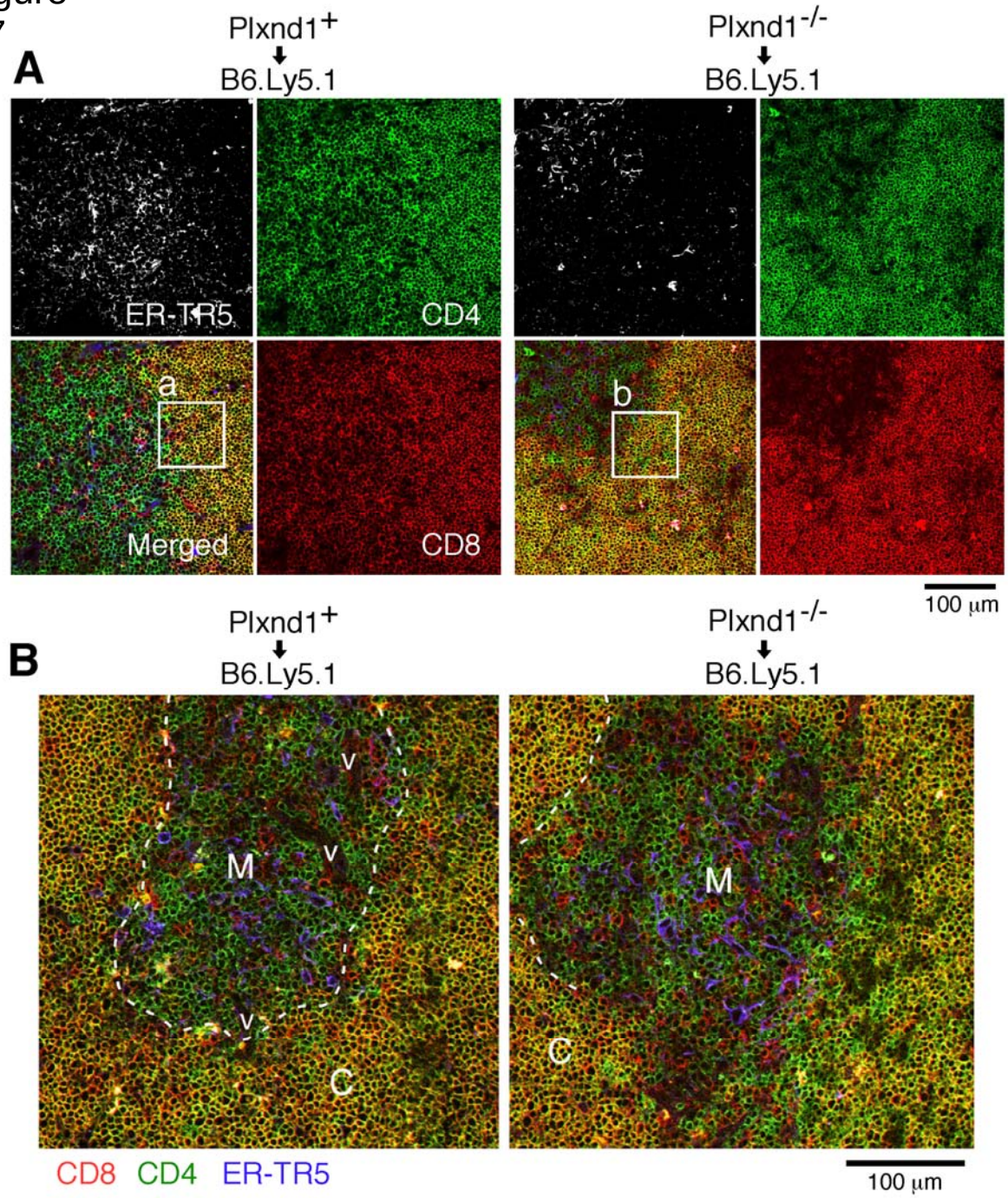

Figure S7. *Distribution of SP thymocytes and the corticomedullary structure in Plxnd1$^{-/-}$ vs Plxnd1^{+} fetal liver cell transplanted mice.*

A. Distribution of SP thymocytes in *Plxnd1*$^{+}$ or *Plxnd1*$^{-/-}$ fetal liver cell transplanted mice. Five micrometer cryosections of representative Ly5.1 irradiated host thymi reconstituted with fetal liver cells

from *Plxnd1*$^{+}$ or *Plxnd1*$^{-/-}$ embryos at 4 wks after transplantation were stained with the indicated antibodies (ER-TR5 is a medullary epithelial marker). Primary and secondary mAbs were incubated for 1 hr at room temperature. After staining, slides were mounted with SlowFadeTM (Molecular Probes, Inc). Cryosections were stained with anti-CD4 (Green), anti-CD8α (Red) and ER-TR5 (White in upper left panel and Blue in lower left merged image). White inserts are magnified in Fig. 5B. B. Disorganized distribution of DP and SP cells around the thymic corticomedullary junction in *Plxnd1*$^{-/-}$ fetal liver cell transplanted mice. Four separate thymus slides of *Plxnd1*$^{+}$ or *Plxnd1*$^{-/-}$ fetal liver-reconstituted animals were analyzed from two independent sets of fetal liver transplants for the above experiments (n=8). All thymii of fetal liver cell transplanted mice revealed cortical/medullary spatial boundary abnormalities comparable to those depicted herein. C = cortex; M = medulla; V = venule.

Figure S8

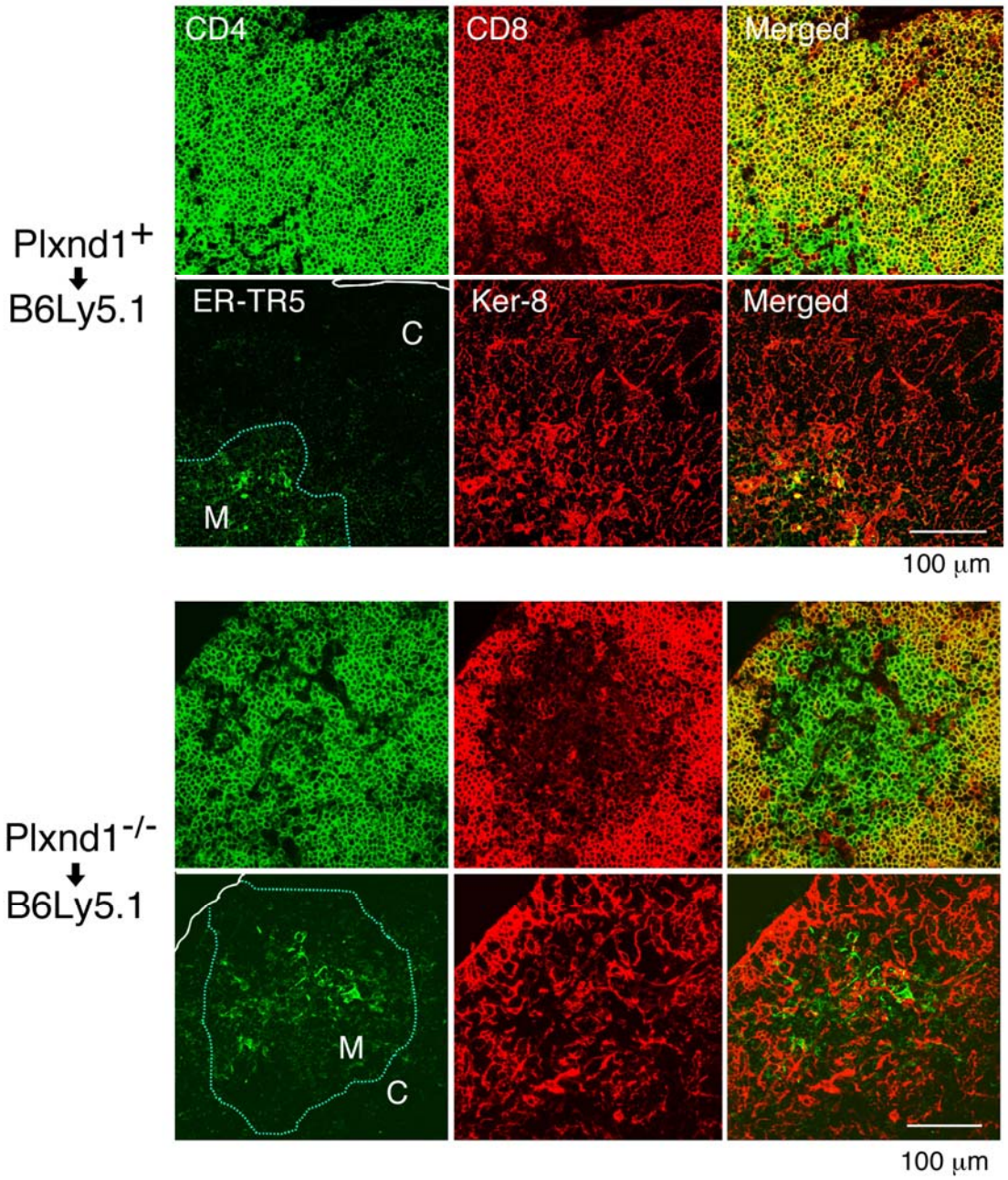

Figure S8. *Ectopic medullary structure abutting the thymic capsule in Plxnd1$^{-/-}$ fetal liver cell transplanted mice.*

One representative medullary structure is shown here to demonstrate that medullary structures are fused with the thymic capsule in *Plxnd1$^{-/-}$* fetal liver cell transplanted mice. Five-micrometer cryosections

of representative Ly5.1 irradiated host thymi reconstituted with fetal liver cells from *Plxnd1*$^{+}$ or *Plxnd1*$^{-/-}$ embryos at 4 wks after transplantation were stained with the indicated antibodies (ER-TR5 is a medullary marker and Keratin8 is a cortical epithelial marker). White scale bar = 100μm. Note that Keratin 8 staining of cortical epithelium yields a reticular pattern. In contrast, at the cortico-medullary junction, Keratin 8 staining appears more globular and amorphous.

Figure S9

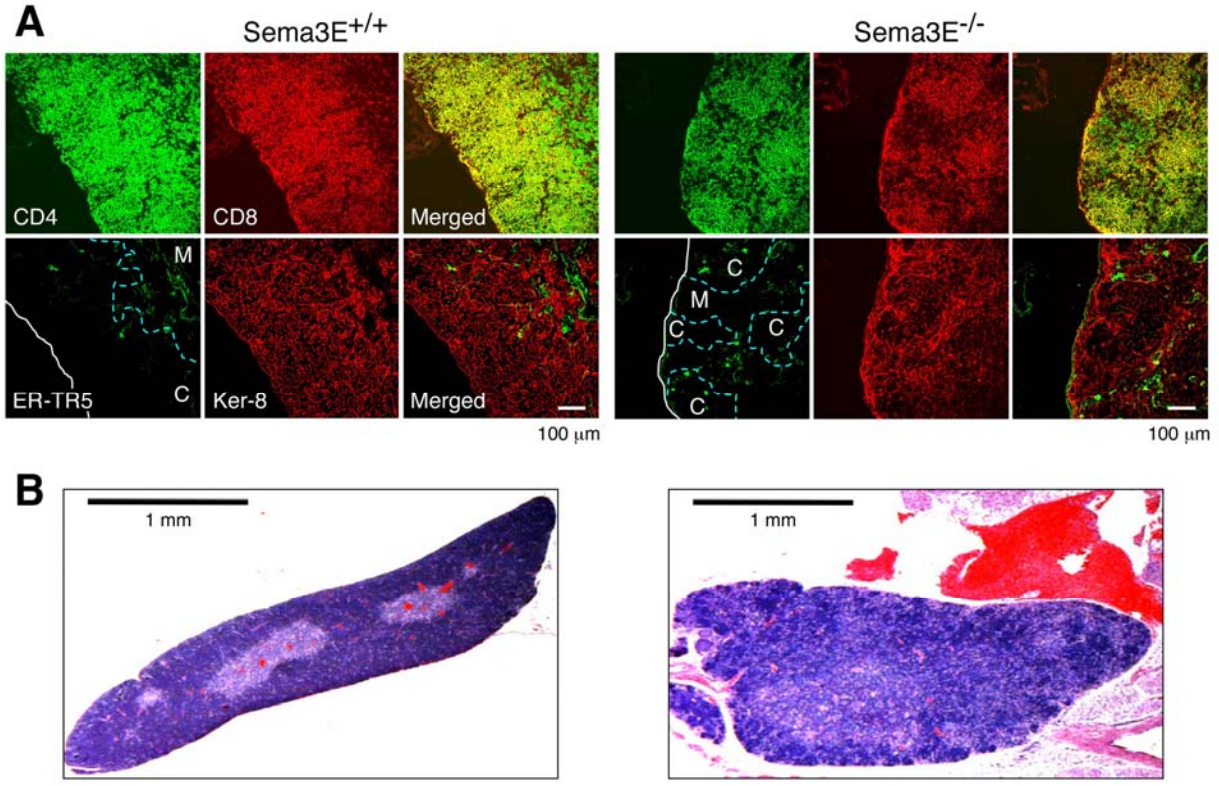

Figure S9. *Distribution of SP thymocytes and corticomedullary structures in Sema3e$^{-/-}$ vs Sema3e$^{+/+}$ mice.*

A. Distribution of DP and SP thymocytes in *Sema3e*$^{+/+}$ or *Sema3*$^{-/-}$ mice. Five-micrometer cryosections of representative thymi from 6 week old mice were stained with the indicated antibodies. The blue dashed lines depict the boundary between DP cells (C) and SP cells (M). Remarkably, ER-TR5+ epithelial cells are found in the cortical rather than medullary areas of *Sema3e*$^{-/-}$ mice where the former region is defined as containing DP thymocytes. The white line outlines the thymic capsule. White scale bar = 100μm. B. Medullary structures were visualized by staining paraffin sections of *Sema3e*$^{+/+}$ or *Sema3e*$^{-/-}$ mice with hematoxylin and eosin. Scale bar = 1mm.