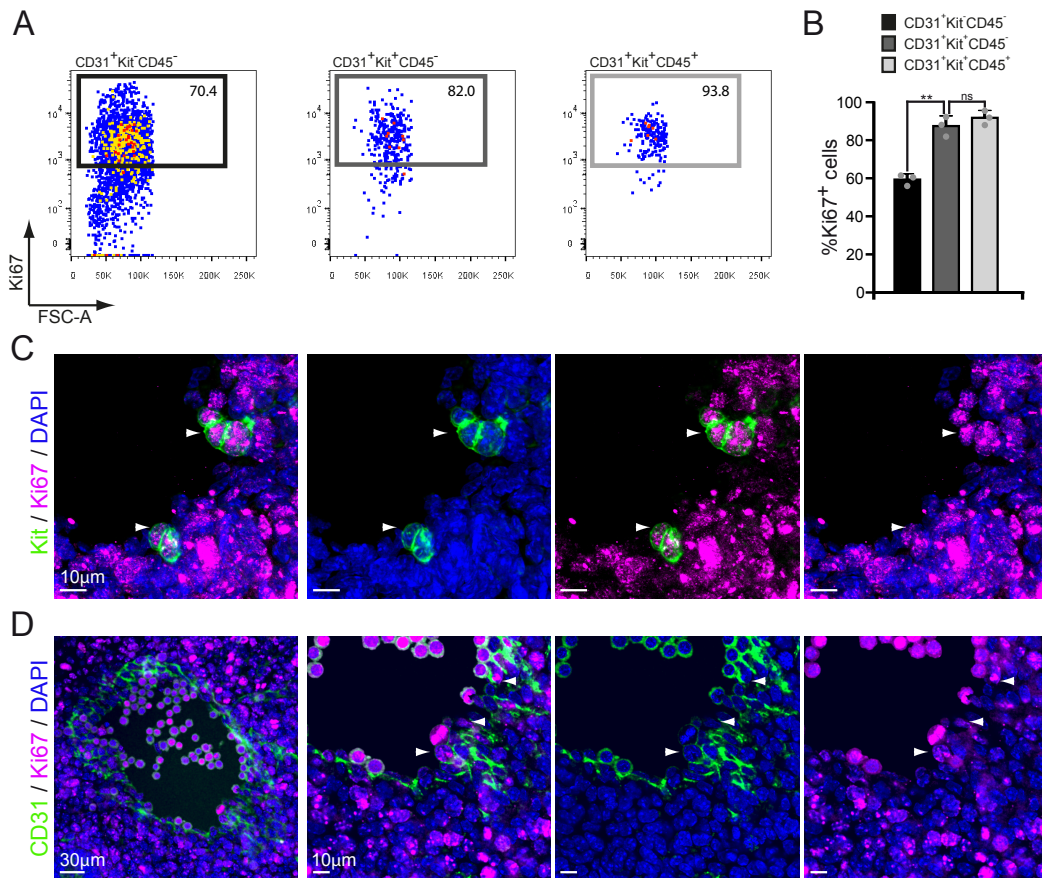


## TABLE OF CONTENTS

1. **Appendix Figure S1: Hematopoietic clusters are kinetically active**
2. **Appendix Figure S2: Hematopoietic clusters contain actively dividing cells**
3. **Appendix Figure S3: The Notch ligand Jagged1 is preferentially expressed in the aortic endothelium**
4. **Appendix Figure S4: Total number of clusters and  $\alpha$ DII4 treatment effect is not dependent from the background of the animal analyzed.**
5. **Appendix Table legends**
6. **Appendix Table S1: ICGS clustering form scRNA-seq**
7. **Appendix Table S2: DEG after  $\alpha$ DII4-treatment.**

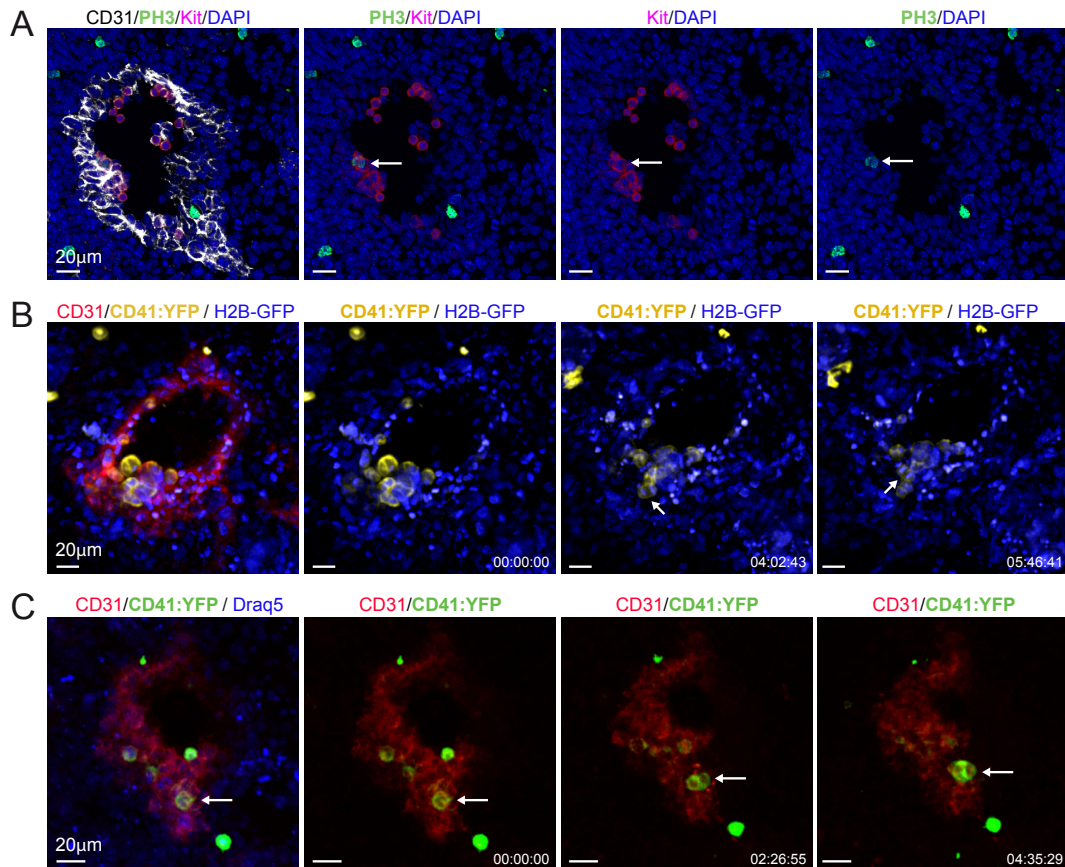
**Appendix Figure S1: Hematopoietic clusters are kinetically active** (A) Dot plots represent Ki67-expression profile gated on CD31<sup>+</sup>Kit<sup>-</sup>CD45<sup>-</sup>, CD31<sup>+</sup>Kit<sup>+</sup>CD45<sup>-</sup>, CD31<sup>+</sup>Kit<sup>+</sup>CD45<sup>+</sup>populations. (B) Quantification based on FACS analyses of Ki67-expressing cells in endothelial and hematopoietic populations. Mean±SD (n=3) (C-D) Representative confocal images of Ki67 staining in transversal sections of E10.5 embryo co-stained with Kit<sup>+</sup> (green,C) and CD31<sup>+</sup> (green, D). White arrowheads indicate cells actively proliferating (Ki67<sup>+</sup>). Note that all cells Kit<sup>+</sup> are Ki67<sup>+</sup> cells (C, white arrowhead), while in the endothelium only a portion of CD31<sup>+</sup> cells are also Ki67<sup>+</sup> (D, white arrowheads). Multistack reconstruction of confocal images. Scale bar: 30μm (overview) and 10μm (magnification).

### Appendix Fig S1



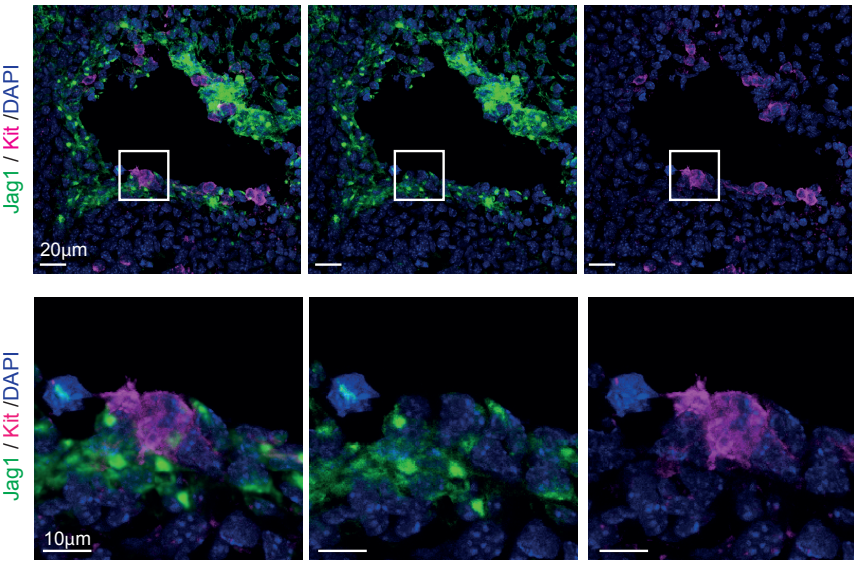
**Appendix Figure S2: Hematopoietic clusters contain actively dividing cells** (A) Expression of the mitotic marker P-H3. White arrow indicates the presence of dividing cells in IAHC. CD31 is shown in white in the image, in black in the label. Multistack reconstruction of confocal images. Scale bar: 20  $\mu$ m. (B) Snapshots of Movie 3. CD41:YFP;H2B-GFP cells undergo mitosis (white arrow) showing that division inside the clusters occurs asynchronously. Embryos were injected with  $\alpha$ CD31<sup>594</sup> directly conjugated antibody to visualize the endothelium. Scale bar: 20 $\mu$ m; Time in hh:mm:ss. (C) Snapshots of Movie 4. CD41:YFP-expressing cells in the aortic endothelium generate a larger and more complex structure when compared to the initial stages of time-lapse recording (white arrow). Embryos were injected with  $\alpha$ CD31<sup>594</sup> directly conjugated antibody to visualize the endothelium and Draq5 to visualize the nuclei. Scale bar: 20 $\mu$ m. Time in hh:mm:ss.

## Appendix Fig S2



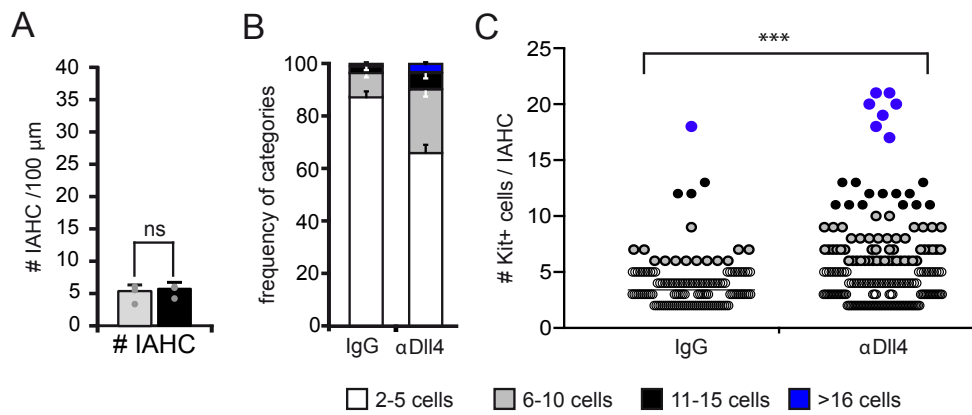
**Appendix Figure S3: The Notch ligand Jagged1 is preferentially expressed in the aortic endothelium.** Multistack reconstruction of confocal images of E10.5 embryos. Transversal section stained with Jag1(green) and Kit (magenta). Lower panels (magnification) show detail of the aortic endothelium. Scale bar: 20µm.

Appendix Fig S3



**Appendix Figure S4: Total number of clusters and  $\alpha$ Dll4 treatment effect is not dependent from the background of the animal analyzed.** We quantified the number of total clusters in tamoxifen-treated  $\text{VeCadCre}^{\text{ER}}:\text{Confetti}^{\text{fl/fl}}$  embryos, to find that the total number remains unchanged and comparable to the wild-type mice analyzed in Figure 4 (A,  $n=3$ ). Additionally, we could not find any difference when treating the  $\text{VeCadCre}^{\text{ER}}:\text{Confetti}^{\text{fl/fl}}$  embryos with IgG or  $\alpha$ Dll4 in terms of category distribution of cluster sizes (B,C). As in the wild-type embryos, treatment with  $\alpha$ Dll4 induces the formation of large clusters (more than 10  $\text{Kit}^+$ -cells/cluster) compared to the IgG treated ( $n=3$ ). 3).

## Appendix Fig S4



**Supplementary Table 1: ICGS clustering form scRNA-seq (excel document).**

Table contains selected representative genes for each cluster as defined by ICGS.

**Supplementary Table 2: DEG after  $\alpha$ DII4-treatment.** Table shows differentially upregulated and downregulated genes following DII4 treatment identified in each cluster by scRNAseq.

Appendix Table S1

cluster 10	cluster 9	cluster 8	cluster 7	cluster 6	cluster 5	cluster 4	cluster 3	cluster 2	cluster 1
Smad5	Samd5	Pde3a	Hspe1	Sdpr	Slc2a3	Ell2	Ppif	Lgals9	Myo1g
Lphn2	Gata4	Serpine2	Hspa1b	AU023871	Bnip3	Myb	Alox5	Dok2	Def6
Itpkb	Tbx20	Rps6ka6	Hsp90aa1	Ly6g6f	Slc16a3	Ikzf1	Ptger3	Lyz2	Acss2
Bcl2	Wisp1	Clip3		Pf4		Rhag	Nfe2	Coro1a	Il17ra
Zfp69	Krt13	Unc5c		Trem1		Car1	Mbp	Cd44	Hemgn
Klhl4	Gpr153	Ldhb		Gp1bb		Gm15915	Gfi1b	Plcg2	Steap3
Nckap5l	Spon1	Aldh1a2		Gp9		Mt2	Ubash3a	Celf2	Ptprcap
Dock4	Gm17455	Scube3		Gucy1a3		Slc38a5	Muc13	Cd200r1	Rac2
Pcdh17	Dusp26	2610203C20Rik		Rab27b		Ermap	Smim1	F13a1	Nckap1l
Fam43a	Ifitm1	Mmp16		F10		Kcnn4	Unc13d	Ikzf2	Arhgap9
Clec1b	Sox6	Dock7		Rbpms2		Klf1	Cited4	Angpt1	Khk
Abca1	Dkk2	Pdlim4		Lat		Emilin2	Gata1	Nupr1	Runx1
Arhgef12	Ppp2r2b	Nsg1		P2ry1		Hbb-bh1	Sptb	Cyth4	Sfpi1
Timp3	Has2	Pdgfc		Fam65c		Hbb-b1	Unc119	Art4	Adcy7
Fxyd5	Grh13	Tmem132c		Sardh		Itga4	Samd14	Mctp1	Mt1
Prex1	Slc1a3	Tenm3		Rhof		Cpox	Blvrb		Cd37
Cd38	Parm1	Pcolce		Cmah		Acss1	Hbb-b2		Tmod1
Prkcdbp	Daam2	Rimklb		Gp1ba		AC147219.	Homer2		Itgal
Elmo1	Stbd1	Frzb		Thbs1		Cdk6	Zfpm1		Plac8
Hecw2	Ppfibp2	Cdh11		Grap2		B4galnt1	Car2		Psma8
Ppp1r13b	Pdprn	Alx1		Kcnj5		Rnf128	Nt5c3		Xk
Gnai2	Tagln	Pdgfrb		Mrvi1			Mapkapk3		Wdfy4
Shroom2	Colec10	Gpc6		Capn3			Cited2		Soat2
Cyyr1	Egfr	Ano1		Ache			Atp8a1		Ctsc
Arhgef7	Ncam1	Msx2		Fcer1g			Emp3		Bid
Map4k2	Tnc	Col26a1		Itgb3			Stxbp2		Pim2
Rgl1	Col6a2	Bcat1		Pls1			Nmnat3		Perp
Ushbp1	Loxl1	Sulf1		Tubb1			Elf1		Ptpn7
Dock9	Krt18	Wt1		Tuba8			Rabgap1l		Slc6a13
Anxa3	Adamts18	Sema3b		Il10ra			Sash3		Mpo
Myo10	Emp2	Igdcc3		Sla			Srgn		Fam203a
Pald1	Tcf21	S1pr2		Tmem40			Ston2		Sapcd1
Rapgef5	Olfml3	Ntm		F2rl2			Dock8		Mthfd2
Msn	Dsp	Bmpr1a		Gucy1b3			Ptpn6		
Snrk	Acta2	Nfatc4		Cd300a			Prkar2b		
Mast4	Shisa3	Hoxd11		Irs2			Gpr56		
Rapgef4	Crispld2	Tmem119		Tec			Pla2g4a		
Lcp1	Col6a1	Eya4		Rgs1			Hmha1		
Nos3	Masp1	Kctd15		Rab32			Prkcq		
Lpar6	Slit3	Ephb3		Stx11			Taok3		
Notch4	Rerg	Ror2		Proser2			I830077J02Rik		
Rhoj	Pde9a	Sema3a					Smim3		
Srgap1	2610528/	Zfhx4					Sord		
Adam15	Ltbp1	Spsb4					Lrmp		
Arap3	Postn	Mgat3					Slc16a10		
Piezo2	Hand1	Crabp1					Prkca		
Sh3bp5	Krt8	Crabp2					Gp5		

Rcsd1	Crb2	Tbx5	Dapp1
Tcf4	Foxf1	Lix1	Sla2
Arhgap29	Steap1	Ednra	Itga2b
Yes1	Pknox2	Bmp7	Susd3
Pde4b	Islr	Prrx2	Slc35d3
Arhgap18	Bcam	Capn6	Rgs10
Ets2	Thbs4	Prrx1	Slc14a1
Sema6d	Tmem108	Scube1	Itgb2
Sox17	Gpr50	Ddr2	Vav1
Arhgef15	Fam162b	Tbx3	Mpl
Tspan18	Fbln7	Rspo2	Rgs18
Amotl1	Npy1r	Pard6g	Atp2a3
Ptpm	Grhl1	Wscd2	P2rx1
Sox7	Colec11	Serinc2	Plek
Rasgrp3	Actg2	Ntn1	Bin2
Efna1	Cnn1	Alx3	Fyb
Dock6	Sh3bgr	Ngfr	Fermt3
Mcam	Nell2	Tmem200a	Mfsd2b
Gimap6	Sst	Col2a1	Tuba4a
Lama4	Oacyl	Wdr35	Btk
Mmrn2	Prdm6	Epha3	Tspan32
Plk2	Timp2	Fbln5	Pnpo
Egfl7	Cthrc1	Rspo4	Ptpre
S1pr1	Popdc2	sept-03	Frrs1
Rasip1	Speg	Dact1	Tnik
Gpr116	Ddr1	Gsc	Slc9a3r1
Tie1	Col5a1	Shox2	Rbm38
Eng	Fzd2	Crlf1	Fut8
Cdh5	Axl	Six1	RP23-285C18.2
Kdr	Efnb1	Dpep1	Cd55
Cd93	Kif26b	Osr1	Meis1
Plxnd1	Smoc2	Tbx18	
Adamts9	Chpf	Sorl1	
Rhob	Tenm4	Lhx2	
2900026/	Fgfr2	Dkk1	
Cttnbp2nl	Pitx2	Tfap2c	
Slc23a2	Cdh3	Tbx15	
Eogt	Col3a1	C130021I20Rik	
Scarf1	Col1a2	Fibin	
Pea15a	Col1a1	Eepd1	
Exoc3l	Myf9	Emx2os	
Dcbld1	Lama2	Pcdh11x	
Grrp1	Ccdc80	Dcx	
She	Col9a1	Dach2	
Erg	Prss35	Mme	
Tmem204	Cpz	Magi2	
Cdc42ep1	Adamts15	1700001L19Rik	
Sipa1	Fzd1	Car11	



Plekhg1	Clcf1	Itgb8
Crip2	Col6a3	Fam159a
Map4k5	Dcn	
S100a16	Lum	
Tgfr2	Tmem45a	
Bcl6b	Krt19	
N4bp3	Pitx1	
F11r	Tbx4	
Eld1	Wnt2	
Sepp1	Scrn1	
Aplnr	ENSMUSG00000100510	
Hapln1	Gata5	
Tmem88	Grem2	
Thsd1	Bicd1	
Pde2a	Tspan7	
Gng11	Robo2	
Robo4	Epb4.1l3	
Cd34	Fras1	
Pecam1	Fbln2	
Sox18	Epha7	
Stab1	Gas1	
Flt4	Vasn	
Emcn	Sdc2	
Ecscr	Runx1t1	
Esam	Pcdh18	
Plvap	Vcan	
Cldn5	Pdgfra	
Mfng	Lrp1	
Ehd2	Rab34	
Bok	Smarca1	
Crmp1	Nnat	
Pcdh1	Fat3	
Epha2	Cxcl12	
Rassf2	Mfap2	
Nfkbia	H2afy2	
Nedd9	Sdc1	
Mogat2	Epha4	
Ppp1r16b	Arhgef40	
Lrp10	Meis2	
Cd109	Hsd11b2	
Pcdh12	Zim1	
Spata13	Fzd7	
Ptrf	Clmp	
Litaf	Lrrc17	
Kit	Igfbp5	
Apbb2	Meis3	
Kcnj2	Wdr86	
Sparcl1	Myrf	

Fam102a	Wnt5a
Pde8a	Des
Arhgef28	6330403K07Rik
BC028528	Sfrp1
Upp1	Cd248
Dusp6	Gpc3
Gja1	S1pr3
Slc2a1	Mab21l2
Sh2d3c	Ptn
Kcne3	Mfap4
Tmem255	Cpm
Nid2	Mmp23
Trp53i11	Ly6e
Rell1	Serping1
Dll4	Rgs5
Igfbp3	Wnt5b
Cdc42ep3	Hgf
Itga3	Igdcc4
Spsb1	Bnc2
Slc7a7	Dact2
Tmem252	Bmp5
Cnn2	Bnc1
Kdm6b	Msc
St3gal1	Adrb3
Pim3	Slc4a3
Junb	Vcam1
Nr4a1	Tead3
Pdgfb	Pde1a
Dok4	Cxcl13
Dusp1	Amph
Odc1	Cyp1b1
Lipt2	Hspa1a
Wasf2	
Kctd12b	
D8Ert82e	
Bcl2l11	
Fosl2	
Klf7	
Prkd2	
Nes	
Prdm1	
Tbx1	
Kitl	
Lrrc32	
Adora2a	
Crem	
Dusp2	
St8sia4	

Apold1

Apln

Rcan3

Spry4

Nos2

Slc25a25

Zfp36

Egr1

Fos

Ier2

Tgfb1i1

Enpp2

Slco2b1

Vwa1

Foxq1

Apcdd1

Cdkn1a

2610019F03Rik

Slc39a8

Fam13c

Slc7a5

Cdh2

Foxf2

Igfbp7

Adamts12

Palmd

Htra3

Stx1a

Ecm1

Agrn

Itga1

Mkl2

Flnb

Lamc1

Mllt4

Wwtr1

Cgn1

Mmp14

Map4k4

Anxa6

Tmem2

Aplp2

Arhgap31

Ptprb

Prex2

Lpp

Sptbn1

Tjp1

Nrp1
Notch1
Lamb1
Ece1
Col18a1
Elk3
Myo1b
Fit1
Ramp2
Pxdn
Mpzl1
Sparc
Igf2
Ets1
Nid1
Hspg2
Col4a2
Col4a1
Adam19
Epas1
Acvr11
Sptan1
Mef2a
Ppfibp1
Itga5
Ptprg
Sh3pxd2a
Spag9
Myh10
Ctnnb1
H19
Fermt2
Vim
Marcks
Igfbp4
Serpinh1
Mest
Dbn1
Nrep
Anxa2
Asb4
Rhoc
Tnfaip1
Pvrl2
Nrp2
mt-Co1
Ablim1
Zfp532

Sesn2

Chac1

Trib3

Ero1l

Jun

Hspb1

Gm9817

Gm10382

## Appendix Table S2

cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7
Xist	Hspg2	Zfp748	Sepp1	Kdr	Slc34a2	Rabggb
Dll4	Nrp1	Tspan6	Ubxn8	Mafk	Hes6	Foxo1
Notch1	Mecom	Trerf1	Plekha8	Irs2	Cd2ap	
Efna1	Tjp1		Atf3	Mef2c	Mir6236	
Sat1	Tie1		Ccdc92	Decr2	Zfp810	
Tsc22d1	Gm5069		B3gnt1	Arl4a	Rnf145	
Igfbp3	RP23-309N14.1		Dnajb14	Casp9	Dpysl2	
Vwf	Efna1		Rb1	Egln3	Zfand6	
Mpz1	Pdcd4		B3gat3	Piezo1	Ndufb9	
Adgrf5	Spop		Gab2	Gng10	Alg14	
Nisch	Bdp1		Flt1	Eng		
Hey1			Rap2a	4931414P19Rik		
Ctnnb1			Pabpc4l			
Aplp2						
Tgfbr2						
Rbm5						
Cdkn1c						
Kcnq1ot1						

cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7
Rhoc	Nts	Tmem68	Gm5141	Aurka	Apc	Tbxa2r
Cdkn1a	Rpl18	Pdgfb	Hoxb7	Mthfr	Maged1	Rhoc
Nfkbia	D17H6S53E	Cep85	Fbxo10	Wdhd1	Alg10b	Cth
Icam1		Polrmt	Cfap97	Wdr41	Abhd5	Zswim8
Pfn1		Zfp810	Fez1	Pdlim4	Nsmf	Tjp2
			2410016O06	Cd2ap	Esam	Cpt1a
			Gna12	Adck1	Jak3	Igf2bp2
			Golga2	Clstn1	Capn2	Cd276
			Nars2	Arhgap11a	Tuba8	Crebrf
			Polm	Ints5	Erc1	Clip1
			Zbtb46	Lrrc61	Pros1	Phf21a
			Pcdh7	Ndufaf4	Micall2	Fut10
			Aacs	Abtb1	Fam117b	Map3k6
			Gucd1	Ebp	Kank3	Wwtr1
			Slc26a2	Fem1c	Cnr2	Ulk2
			Rp2h	Med26	Zfp799	Myadm
			Ranbp6	Dcaf10	Uaca	Ikzf2
			Dock4	Dcaf11	Nelfcd	Crlf2
			Osbpl6	Wdr46	Pbx3	Psmg4
			Col4a5	Ints10	Tanc2	D230025D16
			Dusp3	Txlng	Tmem126b	Utrn
			Tmem127	Trim68	1700021K19I	Bcdin3d
			Pomt1	Ttpal	Art4	Sco1
				Psd3	Xpc	Cpeb4
				Zfp961	Trim35	Stx2
				Crot	Ahdc1	Syt14
				Mcur1	Ctdspl	Fstl1
				Gnpat	Zfand2a	Uap111
				Cercam	Itpkb	Exoc3l4
				Slc31a2	Fut10	Map3k15
				Mycbp	Traf3	Mphosph9
				Fitm2	Cmah	Osbpl2
				Fam160b1	Eng	Kdm7a
				Bcl2	Lpgat1	Sorbs3
				Strn	Fam129b	Tmem120b
				Anxa1	Elac1	Llgl1
				Tmem159	Cnm4	Mbd4
				Brf2	Lyz2	Taf4a
				Mpi	Obfc1	
				Pdk2	Ccdc88b	
				Pigb	Lemd3	
				Thumpd2	Bbs5	
				Kcnh2	Otub2	
				Rnf215	Cdc42bpb	
				Cdyl2	Zswim8	
				Cldn12	Ctp	
				Ankra2	Arhgap4	

Taok3	9530068E07Rik
Itgb1bp1	Txnrd3
Piga	Sergef
Hdac2	St7l
Mlh1	F2rl3
AU020206	Ank3
	Gm5778
	Rab29
	Cdyl2
	Bod1
	Rasgef1b
	Rtn4ip1
	Afap1l1
	Zfp788
	Zcchc2
	Ralb
	Depdc1b
	Akr1b10
	Cd34
	Pds5b
	Cul7
	Mpp2
	Gm26532

---



Rik