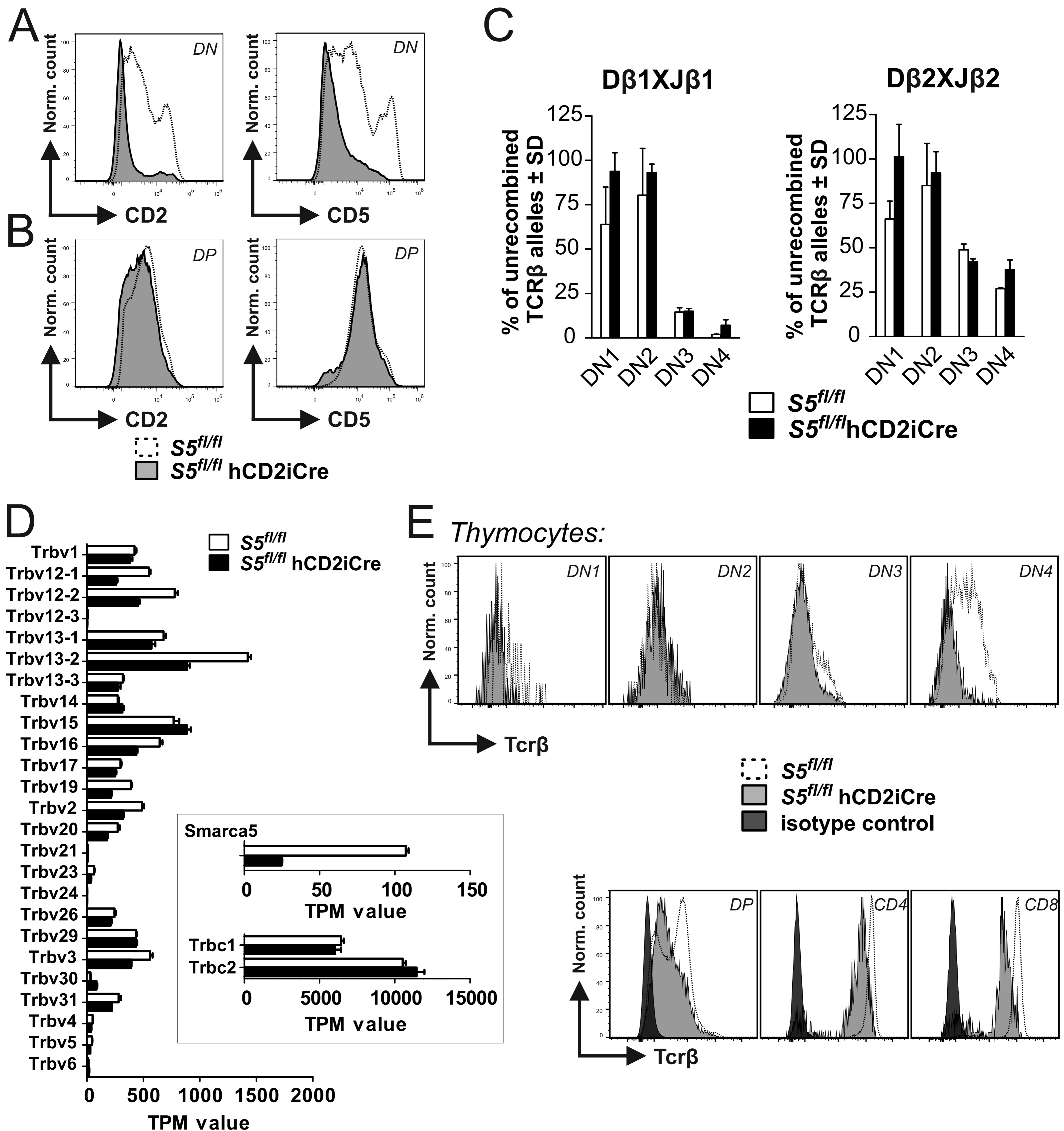


# Supplemental Fig. 1



## Supplemental Figure 1

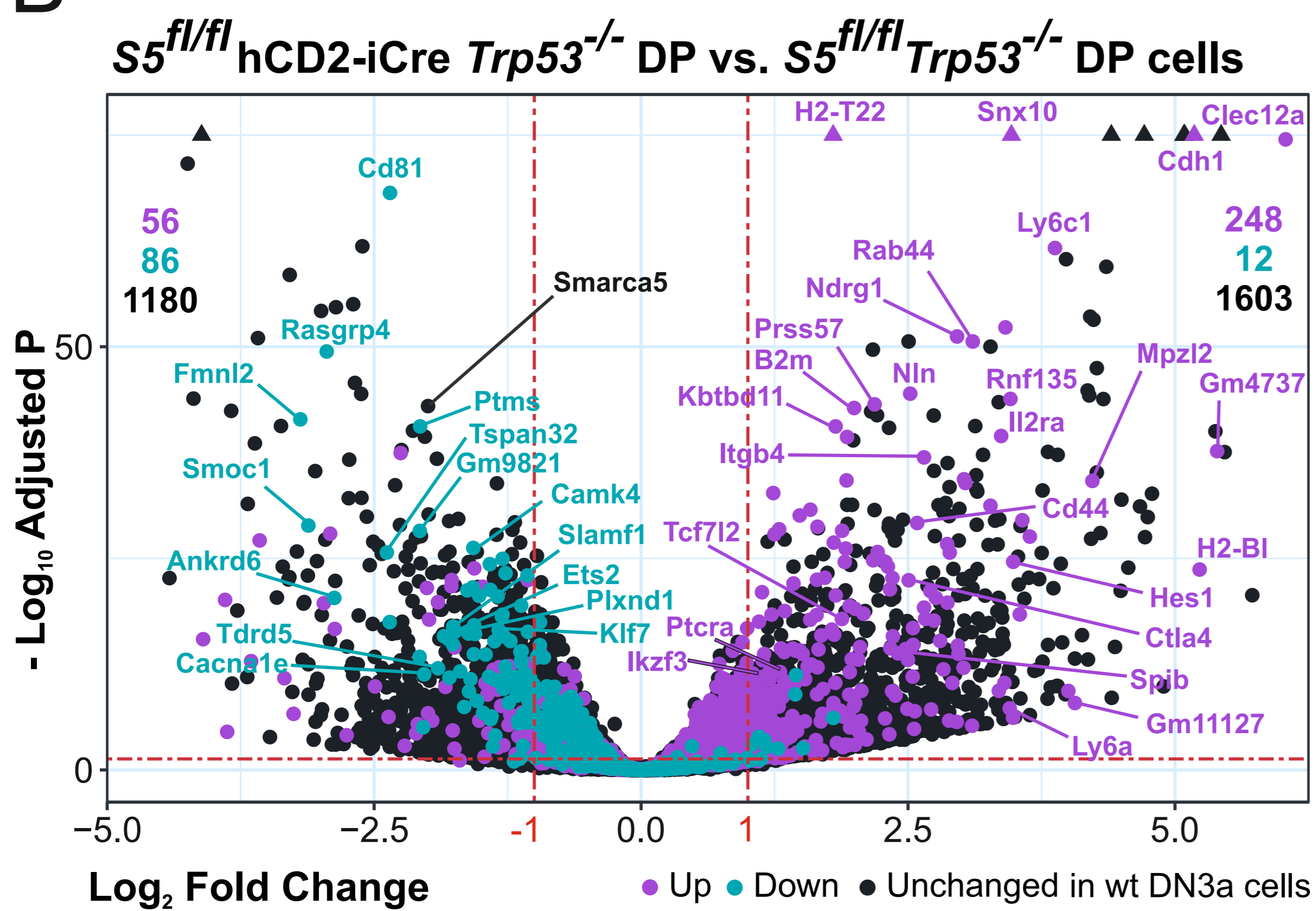
Flow cytometry analysis of CD2 (A) and CD5 (B) molecules on CD4 and CD8 double negative (DN) and double positive (DP) cell populations in thymi of control and *S5<sup>fl/fl</sup> hCD2iCre* mice. Similar results were obtained in at least 3 repeat experiments. (C) Quantitative detection of the D-J recombination of the *Tcrb* gene. qPCR determines relative loss of the intact allele after the TCR recombination. Graphs show relative proportion ± SD of non-recombined *Tcrb* gene (measured here as presence of intact Dβ1-Jβ1 and Dβ2-Jβ2 segments, respectively) in individual DN populations. Data are representative at least of three control and three *S5<sup>fl/fl</sup> hCD2iCre* animals. (D) RNA-seq analysis of *Tcrb* gene rearrangement associated transcripts expressed in control (white bars) and *S5<sup>fl/fl</sup> hCD2iCre* (black bars) DP stage thymocytes. The transcript abundance of *Smarca5*, *Tcrb* constant regions (both shown in rectangle) and all expressed *Tcrb* variable regions with BaseMean value higher than 10 were quantified as transcripts per kilobase million (TPM). (E) Surface expression of Tcrβ on individual thymic subpopulations of control and *S5<sup>fl/fl</sup> hCD2iCre* animals analyzed by flow cytometry. As an isotype control to H57-597 clone (anti-Tcrβ) was used fluorescently labeled Armenian Hamster IgG (dark histograms). Data are representative of three experiments.

# Supplemental Fig. 2

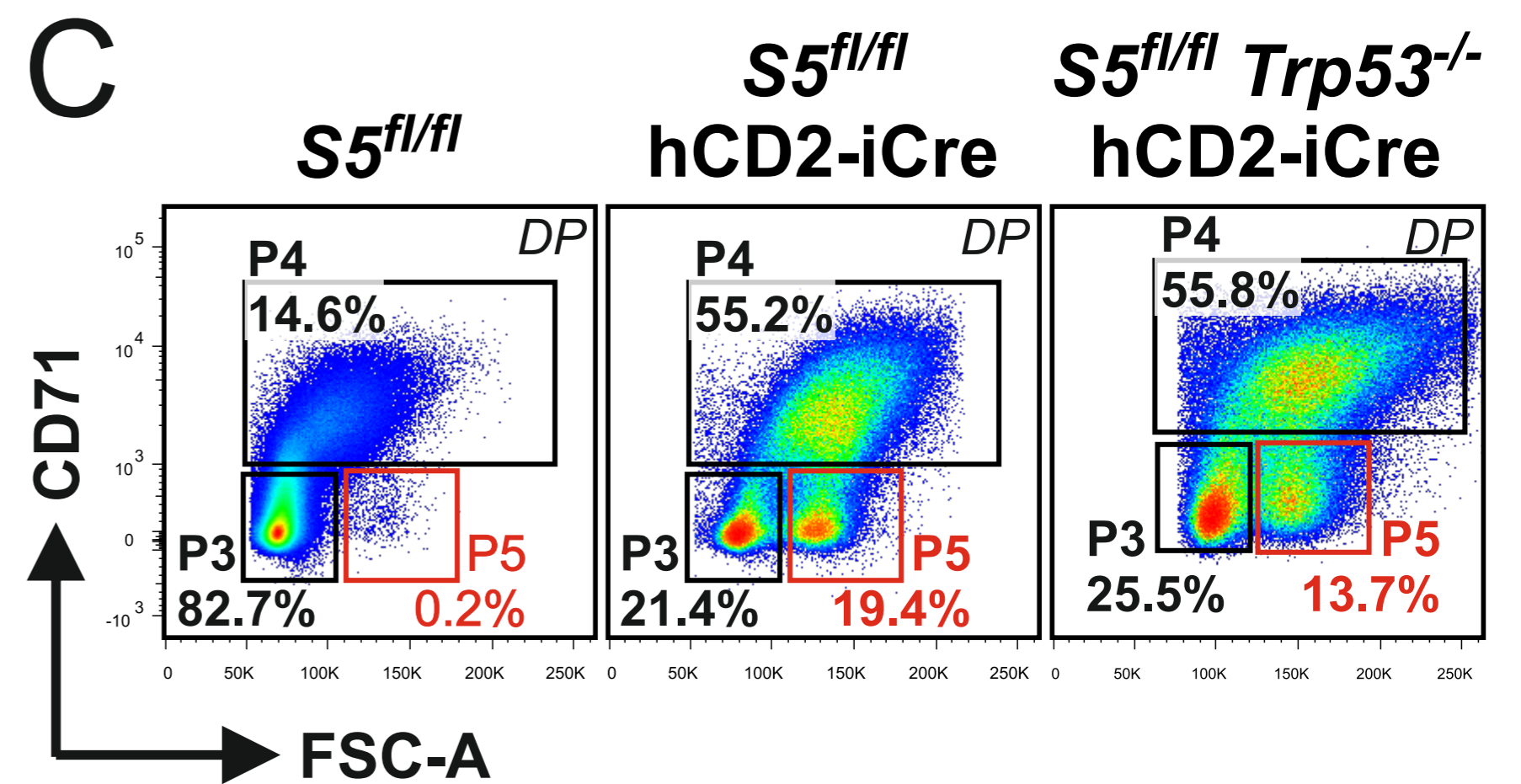
A

<b>Genes downregulated in wt DN3a compared to DP</b>	A130050007Rik, Abca1, Adam12, Adgre5, Akap12, Als2cl, Ankrd44, Ankrd50, Anxa5, Arap2, Arhgef11, Arntl, Arsi, Aspm, At12, Atp10a, Atp2b1, B230359F08Rik, Bcl2l1, Bcl6, Bub1, C920008G01Rik, Cacna1e, Camk4, Casc5, Ccna2, Ccr4, Ccr9, Cd2, Cd28, Cd4, Cd5, Cd52, Cd6, Cd81, Cd8a, Cd8b1, Cdh24, Cdkn1b, Cenpe, Cenpf, Chd1, Ckap2l, Cnr2, Colq, Csgalnact2, Dek, Depdc1a, Dgkd, Dnajb4, Dusp6, E2f2, E2f8, Ect2, Egr1, Ephb6, Erc1, Esco2, Esrp2, Ets2, F13a1, Fas, Flna, Fmnl2, Gimap8, Glcci1, Gm10210, Gm10459, Gm10644, Gm10785, Gm16489, Gm4884, Gpr162, Gramd3, Gsn, H2-Q2, Hist1h1a, Hist1h2ba, Hist1h2bb, Hist1h2bc, Hist1h2be, Hist1h2bf, Hist1h2bg, Hist1h2bh, Hist1h2bj, Hist1h2bk, Hist1h2bl, Hist1h2bm, Hist1h2bn, Hist1h2bp, Hist1h2bq, Hist1h2br, Hist1h4f, Hist1h4h, Hmnr, Id3, Ikbz3, Il21r, Il4ra, Itgb2, Jup, Kif11, Kif14, Kif15, Kif5b, Klf7, Ldlrad3, Lipa, Lmnb1, Lrig1, Lyst, Macf1, Map4, Map4k5, Medag, Mef2a, Mfhas1, Mki67, Mns1, Mr1, Nav2, Ncapg, Ndc80, Neto2, Nfatc3, Nfkb1a, Nhsl1, Nipbl, Nsg2, Olfr1537, P3h4, Phlda1, Phxr2, Pik3cb, Plcl1, Plcl2, Plekhf1, Plxnd1, Polg, Pptc7, Prc1, Pvr, Rasa2, Rasgrp1, Rb1, Rcbtb2, Rdm1, Rgs14, Rmnd5a, Rnu2-10, Rock1, Rorc, Rrm2, Sgol2a, Skil, Sla, Slamf1, Slc37a3, Slc6a19, Slc7a11, Slco3a1, Smoc1, Snord15a, Snord15b, Snord16a, Spc25, Sqrdl, St6gal1, Tcra-V22.1, Tdrd5, Tdrp, Tnk2, Top2a, Top2b, Trav12-1, Trav12-2, Trav12n-1, Trav12n-2, Trav14-1, Trav14d-3-dv8, Trav14n-2, Trav16d-dv11, Trav3-3, Trav3n-3, Trav4d-4, Trav7-4, Trav7d-4, Trav9d-3, Trav9d-4, Trib2, Trio, Tspan32, Tube1, Txk, Ube2v1, Ublcp1, Umad1, Usp33, Wapl, Wisp3, Xrra1, Zfp36
<b>Genes upregulated in wt DN3a compared to DP</b>	1700048O20Rik, 4930427A07Rik, 4930567H12Rik, 5430410E06Rik, 5830411N06Rik, Adamts17, Adgrg1, Ahr, Akr1c13, Aldh2, Ankrd27, Apex1, Arhgap26, Arhgef10l, Ass1, Atp2b4, Atp8b4, AW112010, B2m, Bri3bp, Bst1, Bst2, Camkv, Capn5, Cblb, Ccdc109b, Ccnd2, Cd53, Cd59a, Cdh1, Cdk6, Cdkn1a, Clpb, Coro2a, Cpa3, Cpne2, Cpt1a, Crip1, Ctla4, Cyth4, D8Ertd82e, Dtx1, Eef2k, Epcam, Ephx3, Ern1, Etv6, Fads2, Fam169b, Fkbp5, Frmd4a, Gas5, Gfra1, Gid4, Gm12191, Gm2a, Gm32211, Gm4759, Gm5111, Gm5424, Gm5481, Gm6525, Gm6756, Gm7120, Gm8995, Gnl3, Gpx1, Gria3, Grn, Gsto1, Gstp1, Gstz1, Gzma, H2-D1, H2-K1, H2-L, Hdac4, Hes1, Hmgcs2, I730030J21Rik, Idi1, Ifitm2, Igbp1, Il17rb, Il2ra, Il7r, Irgm1, Itgam, Kcnk5, Kdelc1, Khdc1a, Khdc1b, Khdc1c, Kit, Ldlr, Lfng, Lgals3bp, Llph, LOC547349, Ly6a, Ly6c1, Ly6c2, Ly6d, Lyz2, Mcoln2, Mif, Milr1, Mir675, Mpzl2, Mrps18b, Msmo1, Mybbp1a, Myc, Myl10, ND3, Ndr1, Nkg7, Nlrc5, Notch1, Notch3, Npm1, Nsdhl, Odc1, Park7, Parp8, Pdgfrb, Pebp1, Phgdh, Pik3cd, Plac8, Pld4, Plekha7, Polr1b, Ppt1, Pqlc3, Prdx3, Prss57, Psme1, Ptcra, Rabl2, Repin1, Rny3, Rpl12, Rpl13a, Rpl30, Rpl31, Rpl31-ps12, Rpl35, Rpl36a, Rpl38, Rpl39, Rpl41, Rpl5, Rpl9, Rps18, Rpsa, Rras2, Rrp1b, Rsu1, Rtp4, Scin, Scn4b, Sema4a, Sigmar1, Smim5, Smyd2, Snord1b, Snord1c, Snord32a, Snord47, Snrpg, Snx30, Spats2, Spib, Srgn, Srm, Sstr2, St6galnac4, Suclg2, Supt6, Susd1, Sytl1, Tbc1d5, Tcrg-V1, Tcrg-V4, Tcrg-V6, Tgtp1, Tgtp2, Tifa, Tmem173, Tmem176b, Tmem180, Tmem50b, Tnfrsf1b, Tpi1, Trgv2, Tspan31, Tuba4a, Unc93b1, Usmg5, Wdfy4, Ybx3, Zfp36l2, Zfp991

B



C



## Supplemental Figure 2

(A) The top 200 upregulated and top 200 downregulated transcripts identified in microarrays comparing adult wild-type DN3a and DP thymocytes(45). (B) Data from RNA-seq presented as Volcano plot. The X-axis represents  $\log_2$  fold change of the expression in *S5<sup>fl/fl</sup>hCD2iCre Trp53<sup>-/-</sup>* vs *Trp53<sup>-/-</sup>* DP cells. The Y-axis represents *adjusted p-value* (horizontal dashed line is equal to  $p_{adj} = 0.05$ ). Colored dots indicate genes normally downregulated (green) or upregulated (violet) in wild-type DN3a cells as compared to wild-type DP stage thymocytes (from a microarray analysis of thymocytes by Mingueneau *et al.* 2013). Numbers in corners indicate differentially expressed genes between *Smarca5* mutants and controls of each gene set within  $\log_2FC$  of  $<-1$  or  $>1$ . (C) Flow cytometric plots showing expression of CD71 and forward scatter (FSC-A) of DP-gated thymic suspension. Genotypes are indicated on the top. DP fractions designated as P3-P5 are shown. P5 fraction corresponds to the G2/M arrested cells population. Data are representative at least of three experiments.