SUPPLEMENTAL DATA

Supplementary Figure 1

A) Flow cytometric analysis of the CD5 expression levels (left) on thymic T cell subsets and their respective proportions (right) in the thymi of WT and *Dock2*^{*hsd/hsd*} mice.

B) The proportion of CD8⁺ memory phenotype cells (MP) in the spleens of OT-I TCR transgenic mice in the $Dock2^{hsd/hsd}$ and WT backgrounds, as well as $Dock2^{hsd/hsd}$ and WT mice lacking the OT-I TCR transgene. The proportion of true memory (TM) and virtual memory (VM) as determined by flow cytometric analysis of CD49d staining is shown on the right.



Supplementary Table 1:

Gene set enrichment analysis of MSigDB immunological gene signatures comparing *Dock2*-deficient and wild-type naïve CD8⁺ T cells.

Significantly enriched gene sets in *Dock2*-deficient CD8⁺ T cells (FDR < 0.25) are shown. Memory-linked gene sets are marked with an asterisk.

| | GENE SET | FDR < 0.25 | SIZE | ES | NES | NOM p- val | FDR q-val | FWER p- val | RANK AT MAX | LEADING EDGE |
|---|---|---------------|------|-------|-------|---------------|-----------|----------------|----------------|--|
| MUNOLOGICAL GENE SIGNATURES | GSE7218_UNSTIM_VS_ANTIGEN_STIM_THROUGH_IGG_BCELL_DN | Yes | 137 | 0.449 | 1.927 | 0 | 0.014 | 0 | 2719 | tags=28%, list=13%, signal=32% |
| | GSE15330_LYMPHOID_MULTIPOTENT_VS_MEGAKARYOCYTE_ERYTHROID PROGENITOR_IKAROS_KO_DN | Yes | 163 | 0.470 | 1.727 | 0 | 0.172 | 0.256 | 3161 | tags=34%, list=15%, signal=40% |
| | GSE46606_IRF4HIGH_VS_WT_CD40L_IL2_IL5_DAY3_STIMULATED_BCELL _DN | Yes | 130 | 0.447 | 1.733 | 0 | 0.180 | 0.256 | 2302 | tags=31%, list=11%, signal=34% |
| | GSE39820_CTRL_VS_IL1B_IL6_IL23A_CD4_TCELL_DN | Yes | 165 | 0.406 | 1.694 | 0 | 0.192 | 0.309 | 3729 | tags=33%, list=18%, signal=39% |
| | GSE40443_INDUCED_VS_TOTAL_TREG_DN | Yes | 160 | 0.364 | 1.738 | 0 | 0.193 | 0.245 | 2589 | tags=26%, list=12%, signal=30% |
| | GSE3337_CTRL_VS_4H_IENG_IN_CD8POS_DC_DN | Yes | 168 | 0.384 | 1.700 | 0 | 0.197 | 0.289 | 2267 | tags=24%, list=11%, signal=27% |
| | KAECH_NAIVE_VS_MEMORY_CDB_TCELL_DN* | Yes | 184 | 0.432 | 1.743 | 0 | 0.201 | 0.225 | 2683 | tags=33%, list=13%, signal=38% |
| | KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN* | Yes | 174 | 0.397 | 1.686 | 0 | 0.202 | 0.309 | 2683 | tags=30%, list=13%, signal=35% |
| | GSE24972_MARGINAL_ZONE_BCELL_VS_FOLLICULAR_BCELL_DN | Yes | 126 | 0.330 | 1.680 | 0 | 0.202 | 0.322 | 2610 | tags=24%, list=12%, signal=27% |
| | GSE9650_NAIVE_VS_MEMORY_CDB_TCELL_DN* | Yes | 185 | 0.418 | 1.700 | 0 | 0.213 | 0.289 | 2683 | tags=33%, list=13%, signal=37% |
| | GSE37605_TREG_VS_TCONV_C57BL6_FOXP3_FUSION_GFP_DN | Yes | 132 | 0.410 | 1.703 | 0 | 0.219 | 0.289 | 2373 | tags=28%, list=11%, signal=31% |
| Σ | GSE20715_0H_VS_6H_OZONE_LUNG_UP | Yes | 144 | 0.354 | 1.655 | 0 | 0.220 | 0.413 | 4967 | tag 5=41%, list=24%, signal=53% |
| 80 | GOLDRATH_NAIVE_VS_MEMORY_CDB_TCELL_DN* | Yes | 183 | 0.409 | 1.657 | 0 | 0.229 | 0.413 | 2633 | tags=32%, list=13%, signal=36% |
| Msig | GSE21063_WT_VS_NFATC1_KO_3H_ANT1_IGM_STIM_BCELL_DN | Yes | 150 | 0.421 | 1.746 | 0 | 0.235 | 0.212 | 2719 | tags=30%, list=13%, signal=34% |
| | GSE38304_MYC_NEG_VS_POS_GC_BCELL_DN | Yes | 174 | 0.427 | 1.658 | 0 | 0.240 | 0.413 | 2948 | tags=33%, list=14%, signal=38% |
| | GSE2826_XID_VS_BTK_KO_BCELL_DN | Yes | 153 | 0.387 | 1.638 | 0 | 0.246 | 0.469 | 3875 | tags=35%, list=18%, signal=43% |
| | GSE3039_NKT_CELL_VS_ALPHAALPHA_COB_TCELL_DN | Yes | 172 | 0.360 | 1.644 | 0 | 0.248 | 0.469 | 2724 | tags=27%, list=13%, signal=30% |
| INTERSECTIONS AMONG THE MEMORY- LINKED GENE SETS(*) WITH >15 GENES | GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN GSERGO_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | Yes | 66 | 0.486 | 1.780 | 0.000 | 0.006 | 0.000 | 2220 | tags=39%, list=11%, signal=44% |
| | GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | Yes | 17 | 0.499 | 1.436 | 0.030 | 0.139 | 0.157 | 2273 | tags=41%, ist=11%, signal=46% |
| | GSE9650_NAIVE_V\$_MEMORY_CD8_TCELL_DN KAECH_NAIVE_V\$_MEMORY_CD8_TCELL_DN | no | 51 | 0.407 | 1.277 | 0.176 | 0.280 | 0.381 | 2492 | tags=27%, list=12%, signal=31% |
| | GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN | no | 76 | 0.318 | 1.188 | 0.070 | 0.332 | 0.546 | 2598 | tags=26%, list=12%, signal=30% |
| | GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | no | 31 | 0.360 | 1.079 | 0.383 | 0.337 | 0.688 | 2683 | tags=29%, list=13%, signal=33% |
| | KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN | no | 54 | 0.270 | 1.138 | 0.244 | 0.341 | 0.598 | 2629 | tags=22%, list=13%, signal=25% |

Supplementary Table 2:

Genes shared among or unique to the memory-linked gene immunological gene signatures in Dock2-deficient naïve CD8+ T cells. All possible intersections are shown below.

| Gene set intersection | Number of genes | Genes |
|--|--------------------|--|
| GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | 71 | EMP1 KRTCAP2 CHPT1 CD44 ANXA2 ATF6 FASLG FGL2 CRTAM DOCK5 MDFIC CCL5 ANXA1 GZMM DENND5A KLRK1 PHF13 AQP9 CCR2 MCART6 ITGB1 MYO1F NRP1 UNC119B CLDND1 PGLYRP1 FCGR2B S100A4 CASP1 CCR5 ST3GAL6 CD160 CXCR3 CAPN2 LGALS1 CCL4 KLRG1 DAPK2 PRF1 ELL2 IFNG TXNDC5 ERRF11 PLSCR1 CASP4 S100A6 EOMES IL18R1 RECK F2R GZMB ACOT7 GABARAPL2 PTPN13 EEA1 KCNJ8 CCND3 BHLHE40 ID2 SNX10 IL18RAP S100A13 GZMK GLRX AHNAK BCL2A1 ITGAX MAPRE2 S100A10 IEITM10 KI RC1 |
| GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | 3 | GGH FCGRT STARD10 |
| GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | 34 | XDH IL10RA POLR2L PRR13 FGR SH2D1A RORA RNF19B HLA-A ETFB EFHD2 TRAPPC1 GSG2 CTLA4 TXN KLF10 LYPLA2 CTSD NUCB1 COX17 MAP7D1 MBD2 TSPAN31 CSDA GBP4 SNTB2 S100A11 HMGB2 TTC7B STMN1 H1F0 MX2 HOPX ITGA4 |
| GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | 17 | PGAM1 GATA3 GSTO1 PRSS12 N4BP1 RPGR VKORC1 HCFC1R1 CST7 CTSW TNFRSF1B RNF138 POU6F1 TMEM37 ST3GAL4 ODC1 PPP3CC |
| GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN | 4 | NBEAL2 ENPP1 SLC35E4 LPIN1 |
| KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | 5 | PON1 MTMR7 RACGAP1 RPAP1 TMED10 |
| GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | 4 | CTNNA1 BAG3 CISH ARL6 |
| GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN | 54 | HS1BP3 MOGS PFKP DPM3 JUND RBMX FGF13 SORL1 SHC1 RDBP PCNA GDNF CD7 DPP7 CDC34 ATN1 SEPT1 LRRC8C XRC55 UNC119 CAPNS1 ST8SIA2 FOSB FAM46C PACS1 IGF2R FRMD5 CDK4 FOS TNFSF10 BCL2 SLCO3A1 ADAM19 CRIP2 TBL2 CYFIP2 YES1 TNFAIP3 LYSMD2 ZMAT3 TOB1 DYM WEE1 CYB5R3 GADD45B UBC FGF19 PIM1 PRDX2 KLF6 SH2D2A KLF4 ITGB7 IQGAP2 |
| GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN | 11 | SOCS2 VMP1 GZMA SERPINB9 DSTN LGALS3 BCL2L2 SMYD1 LITAF PRDM1 MYADM |
| GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN | 1 | PTTG1 |
| GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN | 2 | AIM1 GPC1 |
| KAECH_NAIVE_VS_MEMORY_CD8_TCELL_DN* | 12 | IL7R RAD51D CECR5 SH3YL1 SF3A1 CD97 BCHE TOM1 ITGB2 KIAA0101 SYT9 OPRK1 |
| KAECH_NAIVE_VS_DAY15_EFF_CD8_TCELL_DN* | 71 | ACTB CARHSP1 MED12L IL13RA2 GALNT3 ENTPD1 CHL1 MTM1 C7orf73 SLA ODZ3 CD4 LXN NR4A2 ARF6 SSPN GJA1 CR1L INSM1 LAPTM5 C16orf61 CD47 DNMT1 RAB33B RALY SORB51 GPM6B NT5E CCNB2 FAM89B DNAJC1 ALG2 FANCM ZNF821 RPA2 LPIN2 TERF1 MAN2A1 IDE RRBP1 SRGN CASP7 ALCAM C17orf79 UBE2K SEC61G C9orf16 HK2 ALAD HIST1H1C ADORA2A EMP3 ZNF398 ANXA4 GMFG H2AFX CYP3A43 PRDX1 LRP10 PRDX4 C10orf58 KCTD9 LAMC1 KLRD1 ITGAL GSTM3 RAB5C RHOQ PERP RSU1 CASP3 |
| GOLDRATH_NAIVE_VS_MEMORY_CD8_TCELL_DN* | 88 | CHCHD7 EI24 GPHN KIAA1737 TK1 RASA4 TRAF3IP2 IER3 MAP3K8 ABHD5 MS4A1 GOLM1 GK DENND4C GCAT SEMA4F PTPN22 CYB5R4 ACY1 FHIT TMEM55A ART3 CAPG CD22 ANKH POLR1B SEMA4A SAMHD1 EYA4 DBNDD2 PLAT SSX2IP PLP2 STX7 IRF8 OSTF1 EVI2A TMEM159 LPGAT1 CYFIP1 DNAJC5 TMEM141 RAB3D IL15 ITM2C PLCD1 MAPK12 MGST3 KCTD12 TSPAN4 SERPINB6 MYL1 IL15RA GCLM XIST KIAA1274 PLEKHB2 HSD17B11 LIMD1 SOS2 PLEKHA5 CPNE3 ECI1 PLBD1 IL10RB CCDC130 MED10 CYBB AIF1 TKTL1 TRAF1 PRKCA MCOLN2 ANTXR2 TUSC2 RNASE4 PBX3 KLHL7 GOLIM4 ASAH1 PQLC3 ABCB1 NOTCH4 SOAT2 CYP4V2 NCKAP1 SKAP2 HIP1R |
| GSE9650_NAIVE_VS_MEMORY_CD8_TCELL_DN* | 17 | TNNI1 PAM FAM207A MNS1 TULP4 KITLG RPS6KA4 PHYH FRAT1 C9orf3 EPN1 MID2 AARS GNPTG CNR1 NFKBIB ZFP36L2 |
| * genes unique to the particular gene set | | |

Supplementary Table 3: TCR repertoire sequencing metrics

| Replicate | Genotype | Cells (CD8 naïve vs | Total sequencing | Successfully aligned | Successfully aligned, | Total reads used in CDR3 | Final CDR3 clonotype |
|-----------|----------|------------------------|------------------|-------------------------|--------------------------|-----------------------------|-------------------------|
| | | memory) | reads | reads | percent | cionotypes | count |
| 1 | WT | CD44lo | 3439 | 3153 | 91.68% | 3068 | 2109 |
| 2 | WT | CD44lo | 3638 | 3009 | 82.71% | 2913 | 2344 |
| 3 | WT | CD44lo | 2881 | 2693 | 93.47% | 2618 | 1900 |
| 4 | WT | CD44lo | 2745 | 2590 | 94.35% | 2507 | 1662 |
| 1 | Harlan | CD44lo | 3851 | 3761 | 97.66% | 3630 | 2698 |
| 2 | Harlan | CD44lo | 4646 | 4486 | 96.56% | 4345 | 3111 |
| 3 | Harlan | CD44lo | 2565 | 2503 | 97.58% | 2411 | 1853 |
| 1 | WT | CD44hi | 17152 | 16576 | 96.64% | 16438 | 2729 |
| 2 | WT | CD44hi | 19629 | 17809 | 90.73% | 17660 | 3238 |
| 3 | WT | CD44hi | 13017 | 12765 | 98.06% | 12613 | 2510 |
| 4 | WT | CD44hi | 18465 | 17983 | 97.39% | 17839 | 2609 |
| 1 | Harlan | CD44hi | 5964 | 5322 | 89.24% | 5209 | 2758 |
| 2 | Harlan | CD44hi | 7673 | 6848 | 89.25% | 6706 | 2966 |
| 3 | Harlan | CD44hi | 3470 | 3211 | 92.54% | 3170 | 1525 |