

Supplementary Files

Nuanced role for dendritic cell intrinsic IRE1 RNase in the regulation of antitumor adaptive immunity

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This file contains:

- One supplementary reagents and tools table
- Supplementary Figures 1-6.

Reagents and Tools table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
B220 BV510	Biolegend	clone RA3-6B2; #cat 103222; RRID:AB_313005
B220 PE-Cy5	Biolegend	clone RA3-6B2; #cat 103209; RRID:AB_312994
CD11b BV785	Biolegend	clone M1/70; #cat 101243; RRID:AB_2561373
CD11c APC	Thermo Fisher Scientific	clone N418; #cat 17-0114-82; RRID:AB_469346
CD11c BV711	Biolegend	clone N418; #cat 117349; RRID:AB_2563905
CD16/32 (FcBlock) -	Biolegend	clone 93; #cat 101302; RRID:AB_312801
CD18 PE	BD Biosciences	clone C71/16; #cat 553293; RRID:AB_394762
CD19 BUV737	BD Biosciences	clone 1D3; #cat 564296; RRID:AB_2716855
CD25 PE	Tonbo Bioscience	clone PC61.5; #cat 50-0251; RRID:AB_2621757
CD26 PE-Cy7	Biolegend	clone H194-112; #cat 137809; RRID:AB_2564311
CD274 (PD-L1) PE	Thermo Fisher Scientific	clone MIH5; #cat 12-5982-82; RRID:AB_466089
CD3 BV711	Biolegend	clone 17A2; #cat 100241; RRID:AB_2563945
CD39 SuperBright600	Thermo Fisher Scientific	clone 24DMS1; #cat 63-0391-82; RRID:AB_2717037
CD3 ϵ BV510	Biolegend	clone 145-2C11; #cat 100353; RRID:AB_2565879
CD3 ϵ PE-Cy5	Biolegend	clone 145-2C11; #cat 100309; RRID:AB_312674
CD4 APC-Cy7	Tonbo Bioscience	clone GK1.5; #cat 25-0041; RRID:AB_2904484
CD4 PE/Dazzle 594	Biolegend	clone GK1.5; #cat 100455; RRID:AB_2565844

CD40 APC	Biolegend	clone 3/23.; #cat 124611; RRID:AB_1134081
CD44 PerCP	Biolegend	clone IM7; #cat 103036; RRID:AB_10645506
CD44 FITC	Biolegend	clone IM7; #cat 103006; RRID:AB_312957
CD45 BV785	Biolegend	clone 30-F11 ; #cat 103149; RRID:AB_2564590
CD45 Alexa Fluor 700	Biolegend	clone 30-F11; #cat 103127; RRID:AB_493714
CD45 BUV395	BD Biosciences	clone 30-F11; #cat 564279; RRID:AB_2651134
CD45 BV650	Biolegend	clone 30-F11; #cat 103151; RRID:AB_2565884
CD45.1 APC-Cy7	Biolegend	clone A20; #cat 110715; RRID:AB_313504
CD62L PE-Cy7	Biolegend	clone MEL-14 ; #cat 104418; RRID:AB_313103
CD64 BV711	Biolegend	clone X54-5/7.1 ; #cat 139311; RRID:AB_2563846
CD64 PE/Dazzle 594	Biolegend	clone X54-5/7.1; #cat 139319; RRID:AB_2566558
CD64 PerCP-Cy5.5	Biolegend	clone X54-5/7.1; #cat 139307; RRID:AB_2561962
CD8a APC-Cy7	Tonbo Bioscience	clone 53-6.7; #cat 25-0081; RRID:AB_2621623
CD8a BV650	Biolegend	clone 53-6.7; #cat 100742; RRID:AB_2563056
CD8a eFluor 450	Thermo Fisher Scientific	clone 53-6.7; #cat 48-0081-80; RRID:AB_1272235
F4/80 Biotin	Biolegend	clone BM8; #cat 123105; RRID:AB_893499
FOXP3 PE-Cy7	Thermo Fisher Scientific	clone FJK-16s; #cat 25-5773-82; RRID:AB_891552
Granzyme B PE Texas RED	Thermo Fisher Scientific	clone GB11; #cat GRB17; RRID:AB_2536540

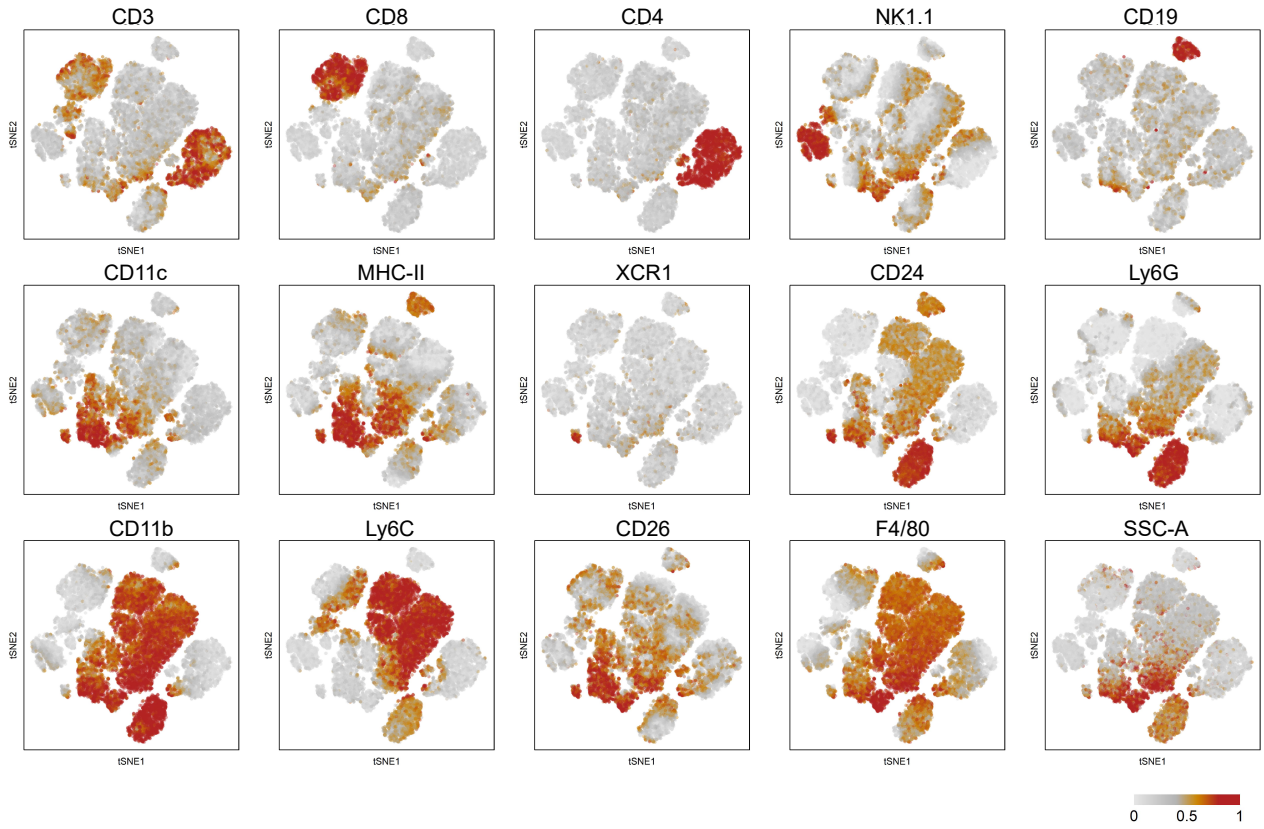
I-A/I-E (MHC-II) APC-Cy7	Biolegend	clone M5/114.15.2; #cat 107627; RRID:AB_1659252
IFN- γ PE	Thermo Fisher Scientific	clone XMG1.2; #cat 12-7311-82; RRID:AB_466193
IL-12p40 eFluor660	Thermo Fisher Scientific	clone C17.8; #cat 50-7123-80; RRID:AB_11218284
IL-2 PE-Cy7	Thermo Fisher Scientific	clone JES6-5H4; #cat 25-7021-82; RRID:AB_1235004
Ly6C BV605	Biolegend	clone HK1.4; #cat 128035; RRID:AB_2562352
Ly6G Alexa Fluor 700	Biolegend	clone 1A8; #cat 127622; RRID:AB_10643269
NK1.1 PerCP-Cy5.5	Biolegend	clone PK136; #cat 108727; RRID:AB_2132706
PD1 PE	Biolegend	clone 29F.1A12; #cat 135206; RRID:AB_1877231
PD1 BV421	Biolegend	clone 29F.1A12; #cat 135217; RRID:AB_10900085
TCF1 Alexa Fluor 488	Cell Signaling	clone C63D9; #cat CS.6444S; RRID:AB_2797627
TIM3 PECy7	Biolegend	clone RMT3-23; #cat 119716; RRID:AB_2571933
TNF- α APC	Biolegend	clone MP6-XT22; #cat 506307; RRID:AB_315428
TOX APC	Miltenyi Biotec	clone REA473; #cat 130-118-335; RRID:AB_2751485
XCR1 BV650	Biolegend	clone ZET; #cat 148220; RRID:AB_2566410
XCR1 PE	Biolegend	clone ZET; #cat 148204; RRID:AB_2563843
Bacterial and virus strains		
Biological samples		
Mouse tumor draining lymph nodes (inguinal)	This paper	N/A
Mouse tumor	This paper	N/A
Mouse spleen	This paper	N/A
Chemicals, peptides, and recombinant proteins		
Brefeldin A	Cayman Chemical	#cat 11861
PMA	Sigma	#cat P8139

Ionomycin	Sigma	#cat I0634
Critical commercial assays		
CD45 MicroBeads, mouse	Miltenyi	RRID:AB_2877061
CD8a+ T Cell Isolation Kit, mouse	Miltenyi	#cat 130-104-075
LS columns	Miltenyi	#cat 130-042-401
BD Cytofix/Cytoperm with GolgiPlug	BD Biosciences	RRID:AB_2869013
Foxp3 / Transcription Factor Staining Buffer Set	eBioscience	#cat 00-5523-00
RNeasy Plus Micro Kit	Qiagen	#cat 74034
M-MLV Reverse Transcriptase	Invitrogen	#cat 28025-013
iTAg Tetramer/PE - H-2 Kb OVA (SIINF EK L)	MBL	#cat T03000
CellTrace™ Violet Cell Proliferation Kit	Thermo Fisher Scientific	#cat C34557
Deposited data		
RNA-seq data	This paper	GEO: GSE195439
Experimental models: Cell lines		
B16-F10	ATCC	RRID:CVCL_0159
B16-FLT3L	(Guo-Ping <i>et al.</i> , 1999)	N/A
B78-ChOVA	(Broz <i>et al.</i> , 2014)	N/A
OP9-DL1	(Schmitt and Zúñiga-Pflücker, 2002)	RRID:CVCL_B218
Experimental models: Organisms/strains		
ERAI: C57BL/6J-Tg(CAG-XBP1*/venus)#Miur/MiurRbrc	(Iwawaki <i>et al.</i> , 2004)	RRID:IMSR_RBRC01099
CD11c-Cre: B6.Cg-Tg(Itgax-cre)1-1Reiz/J	The Jackson Laboratory	RRID:IMSR_JAX:008068
IRE1 fl/fl: B6;129S4-Ern1<tm2.1Tiw>	(Iwawaki <i>et al.</i> , 2009)	RRID:IMSR_RBRC05515
XBP1 fl/fl: Xbp1tm2Glm	(Lee <i>et al.</i> , 2008)	RRID:MGI:6273536
Pmel-1: B6.Cg-Thy1a/Cy Tg(TcraTcrb)8Rest/J	The Jackson Laboratory	RRID:IMSR_JAX:005023
Oligonucleotides		
Primer: Splicing assay Xbp1 Forward: ACACGCTTGGGAATGGACAC	(Osorio <i>et al.</i> , 2014)	N/A
Primer: Splicing assay Xbp1 Reverse: CCATGGGAAGATGTTCTGGG	(Osorio <i>et al.</i> , 2014)	N/A
Primer: Actb Forward (Fig 1G): CTAAGGCCAACCGTGAAAAG	This Paper	N/A
Primer: Actb Reverse (Fig 1G): TTGCTGATCCACATCTGCTG	This Paper	N/A
Primer: Actb Forward (Fig 4B and S5A): GTGACGTTGACATCCGTAAAGA	This Paper	N/A
Primer: Actb Reverse (Fig 4B and S5A): GCCGGACTCATCGTACTCC	This Paper	N/A
Recombinant DNA		
Software and algorithms		
FlowJo™ Software v10	BD Biosciences	https://www.flowjo.com/solutions/flowjo ; RRID:SCR_008520

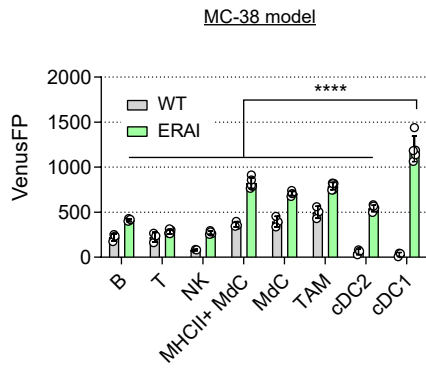
GraphPad Prism v9	GraphPad	https://www.graphpad.com/ ; RRID:SCR_002798
Trimmomatic v0.39	Usadellab	RRID:SCR_011848
FastQC v0.11.8	Babraham Bioinformatics	RRID:SCR_014583
STAR v2.7.3a	STAR	RRID:SCR_004463
HTSeqCount v0.11.2	HTSeq	RRID:SCR_005514
R Studio v2021.09.0 Build 351	R Studio	https://rstudio.com/ ; RRID:SCR_000432
R v4.1.1	R Core Team (2020)	http://www.r-project.org/ ; RRID:SCR_001905
R package: CytoML v2.4.0	(Finak, Jiang and Gottardo, 2018)	https://bioconductor.org/packages/release/bioc/html/CytoML.html
R package: FlowWorkspace v4.4.0	(Finak and Jiang, 2021)	RRID:SCR_001155
R package: FlowCore v2.4.0	(Ellis <i>et al.</i> , 2021)	RRID:SCR_002205
R package: Rtsne v0.15	(Krijthe, 2015)	RRID:SCR_016342
R package: DBSCAN v1.1.8	(Hahsler, Piekenbrock and Doran, 2019)	https://cran.r-project.org/web/packages/dbscan/index.html
R package: Limma v3.42.2	(Ritchie <i>et al.</i> , 2015)	RRID:SCR_010943
R package: EdgeR v3.28.0	(Robinson, McCarthy and Smyth, 2010)	RRID:SCR_012802
R package: pheatmap v1.0.12	(Kolde, 2019)	RRID:SCR_016418
R package: ggplot2 v3.3.5	(Wickham, 2016)	RRID:SCR_014601
R package: ClusterProfiler v4.0.5	(Wu <i>et al.</i> , 2021)	RRID:SCR_016884
Other		

Supplementary Figure 1

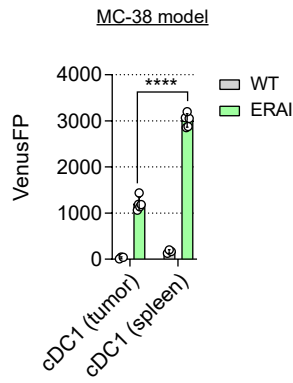
A



B



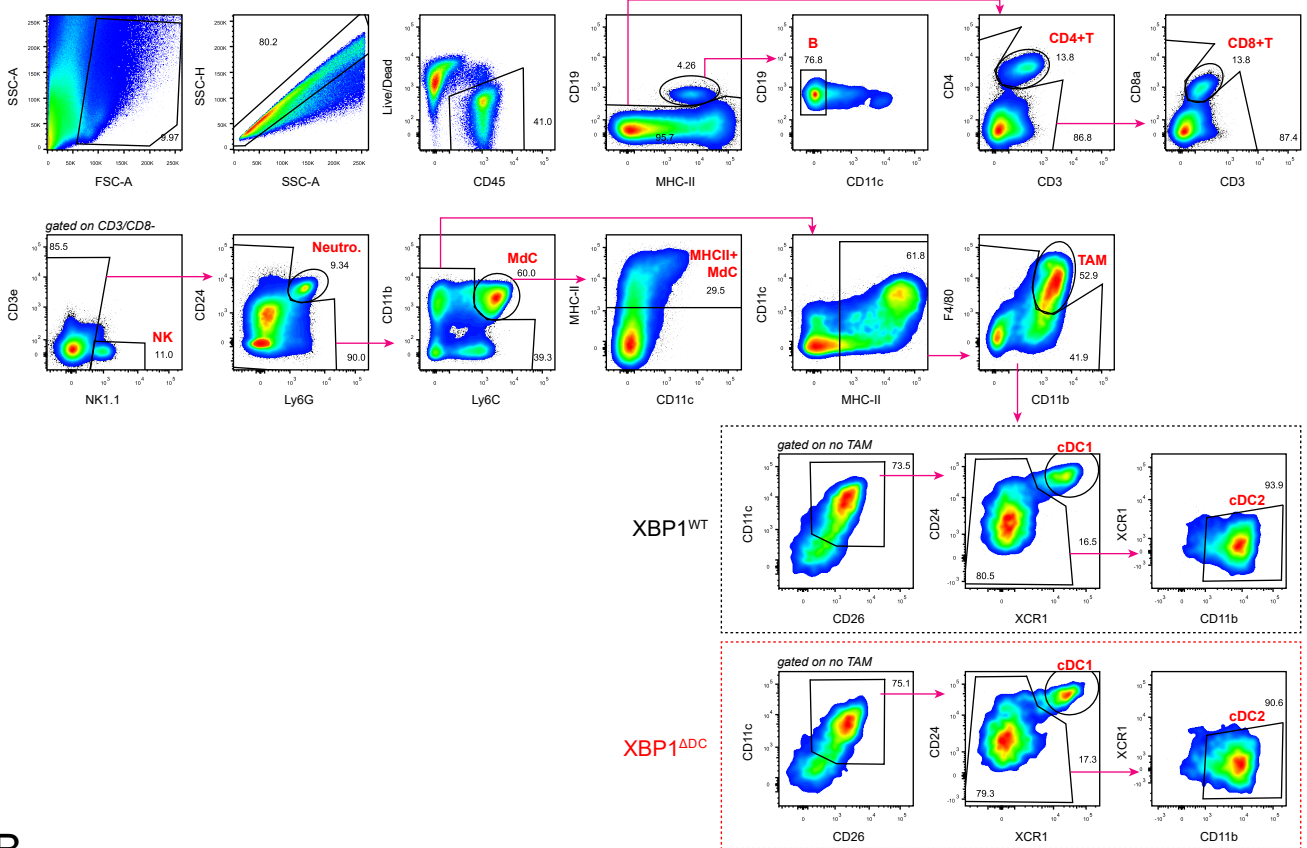
C



Supplementary Figure 2

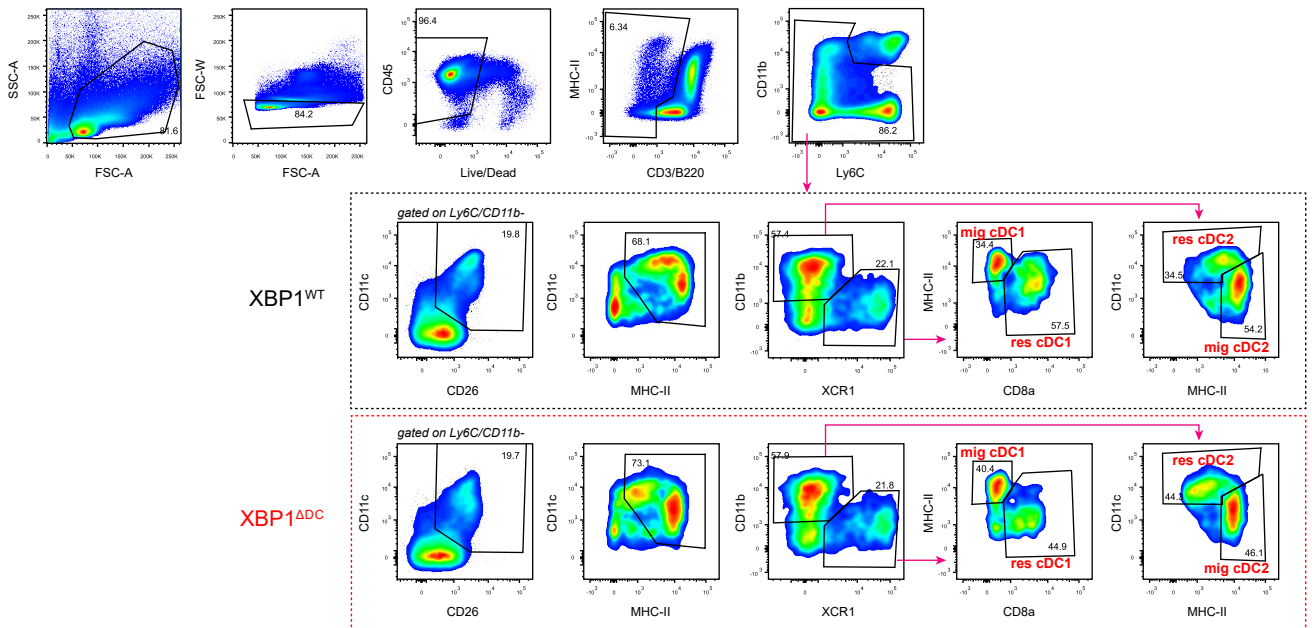
A

Tumor gating



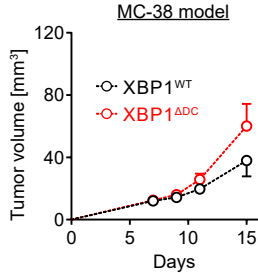
B

Tumor draining lymph node gating

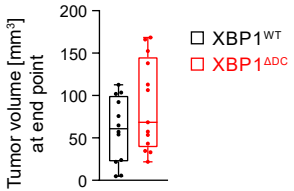


Supplementary Figure 3

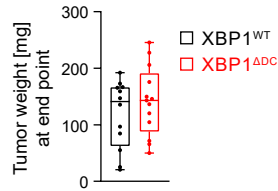
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B

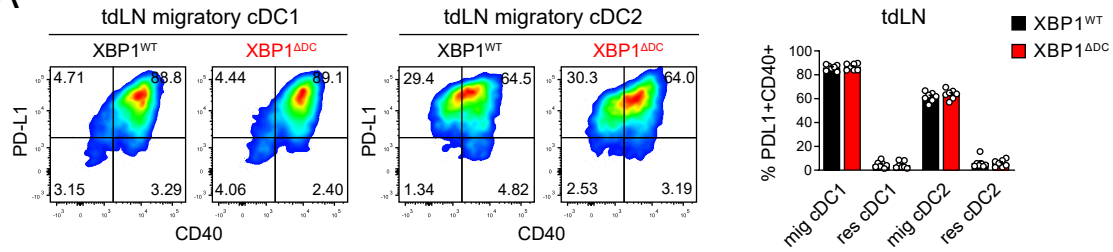


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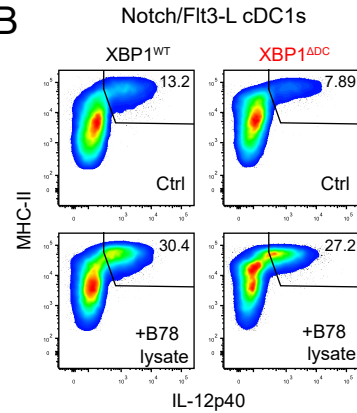


Supplementary Figure 4

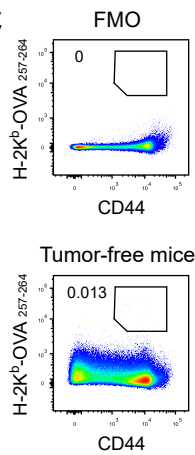
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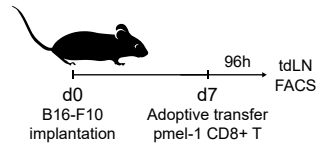
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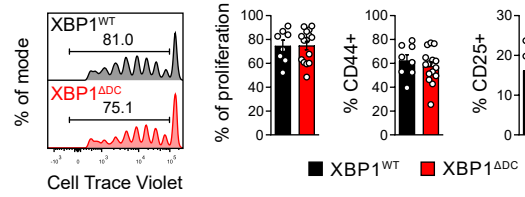
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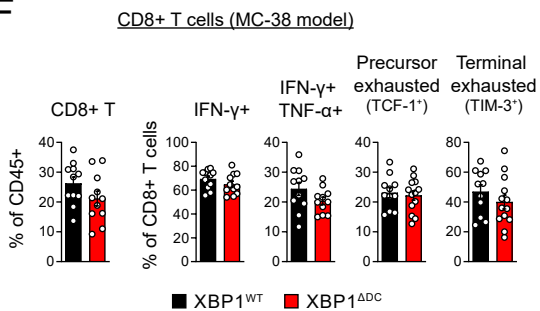
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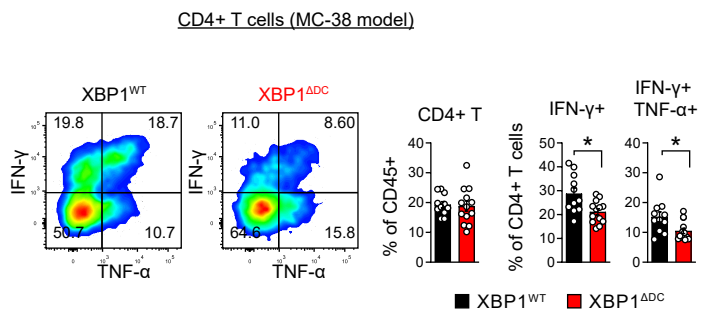
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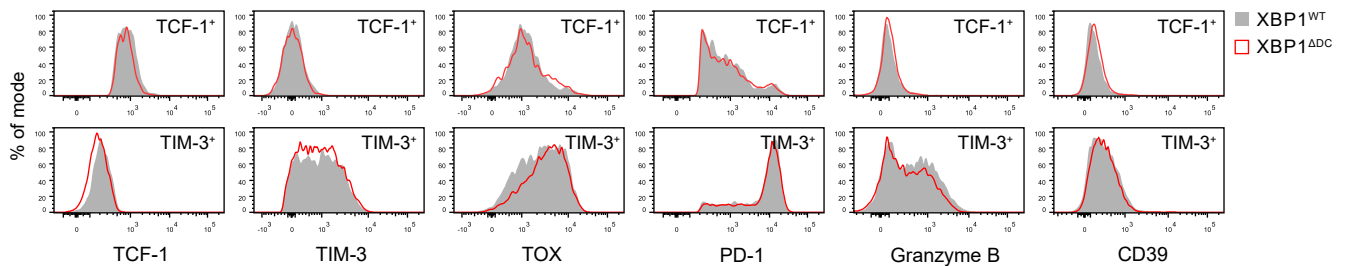
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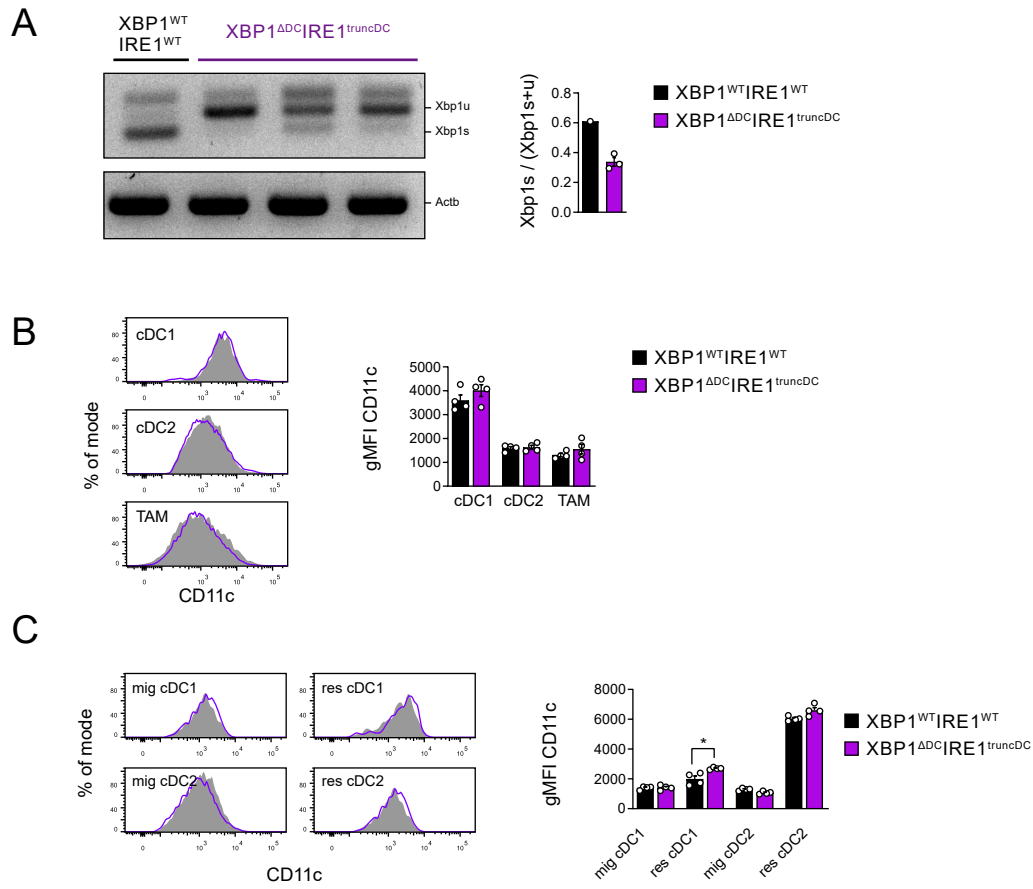
G



H

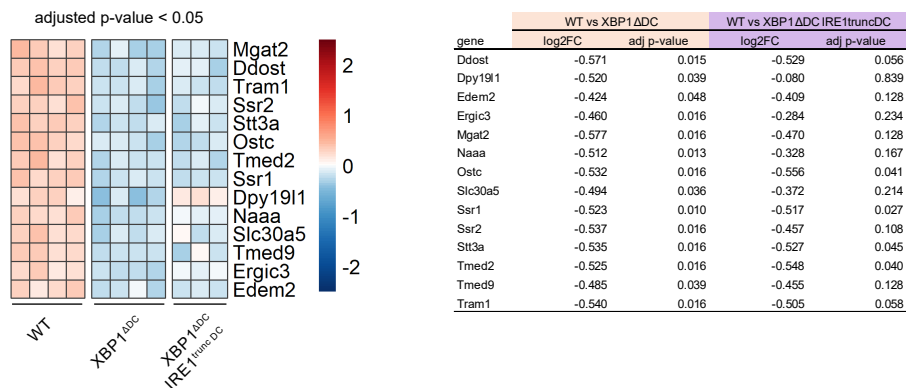


Supplementary Figure 5



Supplementary Figure 6

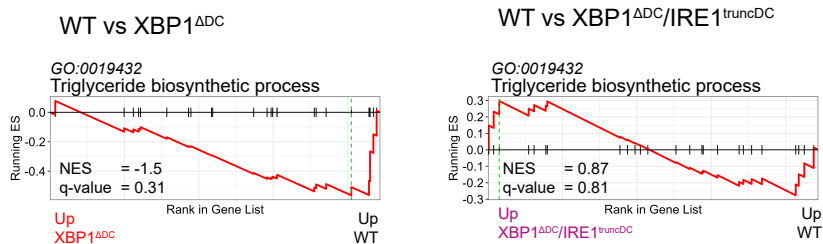
A



B



C



D

