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BRD7 as key factor in PBAF complex assembly and CD8⁺ T cell differentiation

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Upon infection, naïve CD8⁺ T cells differentiate into cytotoxic effector cells to eliminate the pathogen-infected cells. Although many mechanisms underlying this process have been demonstrated, the regulatory role of chromatin remodel system in this process remains largely unknown. Here we showed that BRD7, a component of the polybromo-associated BRG1-associated factor complex (PBAF), was required for naïve CD8⁺ T cells to differentiate into functional short-lived effector cells (SLECs) in response to acute infections caused by influenza virus or lymphocytic choriomeningitis virus (LCMV). BRD7-deficiency in CD8⁺ T cells resulted in profound defects in effector population and functions, thereby impairing viral clearance and host recovery. Further mechanical studies indicated that the expression of BRD7 significantly turned to high from naïve CD8+ T cells to effector cells, bridged BRG1 and PBRM1 to the core module of PBAF complex, consequently facilitating the assembly of PBAF complex rather than BAF complex in the effector cells. The PBAF complex changed the chromatin accessibility at the loci of *Tbx21* gene and up-regulated its expression, leading to the maturation of effector T cells. Our research confirms BRD7 and the PBAF complex are key in CD8⁺ T cell development and present a significant target for advancing immune therapies.



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34 ABSTRACT

Upon infection, naïve CD8⁺ T cells differentiate into cytotoxic effector cells to eliminate 35 the pathogen-infected cells. Although many mechanisms underlying this process have 36 37 been demonstrated, the regulatory role of chromatin remodel system in this process remains largely unknown. Here we showed that BRD7, a component of the polybromo-38 associated BRG1-associated factor complex (PBAF), was required for naïve CD8⁺ T cells 39 to differentiate into functional short-lived effector cells (SLECs) in response to acute 40 41 infections caused by influenza virus or lymphocytic choriomeningitis virus (LCMV). BRD7-deficiency in CD8⁺ T cells resulted in profound defects in effector population and 42 43 functions, thereby impairing viral clearance and host recovery. Further mechanical studies indicated that the expression of BRD7 significantly turned to high from naïve 44 CD8⁺ T cells to effector cells, bridged BRG1 and PBRM1 to the core module of PBAF 45 complex, consequently facilitating the assembly of PBAF complex rather than BAF 46 47 complex in the effector cells. The PBAF complex changed the chromatin accessibility at the loci of Tbx21 gene and up-regulated its expression, leading to the maturation of 48 effector T cells. Our research confirms BRD7 and the PBAF complex are key in CD8⁺ T 49 cell development and present a significant target for advancing immune therapies. 50

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53 INTRODUCTION

CD8⁺ T cells play critical roles in protective immunity against intracellular pathogens 54 including viruses. In response to viral infection, naive CD8⁺ T cells rapidly undergo a 55 pronounced clonal expansion and differentiate into antigen-specific effector cells to 56 eliminate infected cells (1, 2). During this process, effector CD8⁺ T cells acquire the 57 ability to produce cytolytic and effector cytokines such as granzyme B (Gzmb) and 58 interferon gamma (IFN- γ) (2-4). Within the effector populations, many effector CD8⁺ T 59 60 cells are short-lived effector cells (SLECs) which undergo programmed cell death, leaving behind a small population of memory precursor effector cells (MPECs) (5). These 61 two subsets are divided by two critical surface markers, KLRG1 and IL7R (CD127). 62 SLECs express high levels of KLRG1 and low levels of IL7R and exhibit higher 63 64 expression of effector molecules, while MPECs express high levels of IL7R and low levels of KLRG1 and show a greater stem-cell like properties. The clonal expansion and 65 66 effector differentiation of CD8⁺ T cells are regulated by various transcription factors. Several transcription factors including T-bet, Blimp-1, Id2, and IRF4 are critical 67 68 regulators for the differentiation of SLECs population (5-9), while Eomes, Foxo1, Id3 and Tcf1 are required for the differentiation of MPEC population (8, 10-13). The 69 70 epigenetic and chromatin states also influence the differentiation of SLECs and MPECs (14, 15). However, more epigenetic mechanisms by which SLECs become committed to 71 72 a terminal fate remain to be elucidated.

SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin-remodeling complexes 73 contain members of ATPases that regulate DNA-protein contacts through energy 74 generated from ATP hydrolysis. These ATP-dependent chromatin remodeling complexes 75 76 are multimeric molecular assemblies involved in the regulation of chromatin architecture 77 (16-19). Previous studies revealed that SWI/SNF complexes in mammalian cells exist in three non-redundant assembly bodies: BRG1/BRM-associated factor complex (BAF), 78 polybromo-associated BAF complex (PBAF), and non-canonical BAF complex (ncBAF) 79 (17). SWI/SNF complexes include many core components (20). Among them, 80 SMARCB1, SMARCC1, SMARCC2, SMARCD1, and SMARCE1 are the components 81 of "core module", while BRG1 (or named as SMARCA4) belong to the ATPase module 82 of the complexes. All of these modules are shared by both BAF and PBAF complexes. 83

Besides its important role in cancers, BRG1 is also important for T cell differentiation 84 since BRG1 deficiency in mice results in thymic abnormalities and a developmental 85 block at double negative to double positive T cell transition (21, 22). The specific 86 components of BAF complex include ARID1A, ARID1B, and DPF2, while the specific 87 components of PBAF complex include Bromodomain-containing protein 7 (BRD7), 88 PBRM1, PHF10, and ARID2 (17, 20, 23, 24). It remains to be determined how the 89 90 ATPase module and the BAF core module are assembled together and what is the role 91 played by these special components.

BRD7 belongs to the bromodomain family, and can recognize and bind to acetylated 92 histone H (25-27). It is ubiquitously expressed in the nucleus and only belongs to PBAF 93 complex (25, 28). BRD7 has been reported as a transcriptional regulator and plays a 94 95 critical role in cellular growth, cell cycle progression, and tumor development (28-36). Along with its PBAF partners, BRD7 participates in in β cells regulation or the resistance 96 97 of tumor cells to immune cells (37, 38). Depletion of these genes in tumor cells would enhance the secretion of various chemokines which help recruit effector T cells (38). 98 99 Although the interactions among components within PBAF complex has been largely 100 determined in a recent biochemical study (17), it remains to be determined what is the 101 role played by BRD7 in assembling the PBAF complex and how this complex is 102 assembled to accomplish its function within the immune cells.

103 In order to investigate the function of BRD7 and its PBAF complex in antiviral 104 immune response, we utilized mice with T cell-specific genetic deficiencies of BRD7 and infected these mice and their littermate wild-type controls with influenza viruses or 105 lymphocytic choriomeningitis virus (LCMV) Armstrong (Arm) viruses, and examined the 106 107 influence of BRD7 upon T-cell function. We found that the expression of BRD7 was 108 significantly induced in effector CD8⁺ T cells. BRD7 was not required for the early activation and expansion of CD8⁺ T cells but was critical for effector differentiation of 109 CD8⁺ T cells and pathogens clearance during acute viral infection. BRD7-deficient CD8⁺ 110 T cells failed to initiate the effector T cell transcriptional program and showed impaired 111 cytotoxicity and cytokine production. We therefore demonstrated that BRD7 controls the 112 differentiation of cytotoxic effector CD8⁺ T cells. Importantly, we identified that BRD7 113 played a key role in assembling the PBAF complex to perform their functions in effector 114

115 $CD8^+ T$ cells.

116

117 **RESULTS**

III8 Increased expression of BRD7 in the effector CD8⁺ T cells

The SWI/SNF complexes are involved in genome-wide transcriptional regulation (16, 17). 119 However, their roles in antiviral immune response remain unknown. To elucidate the 120 roles of SWI/SNF chromatin remodeling complexes in regulating CD8⁺ T cell responses, 121 we analyzed the expression of components of BAF, PBAF, and ncBAF complexes after 122 influenza virus infection. Among them, we found that the expression of BRD7 was the 123 highest in effector CD8⁺ T cells at day 10 post infection (p.i.) and the expression 124 maintained high in memory CD8⁺ T cells (at ~42 days p.i.) from OT-I TCR-transgenic 125 mice when compared to their mRNA expression in naive CD8⁺ T cells (Fig. 1A). In 126 addition, when we analyzed the kinetics of mRNA expression of several PBAF 127 components during in vivo responses to infection, we found that Brd7 mRNA expression 128 was upregulated at day 6 p.i., and reached the maximum at day 8 (Fig. 1B), suggesting 129 130 that the expression of BRD7 is concurrent with the development of effector $CD8^+ T$ cells. Of note, the CD8⁺ T cells of these mice recognize a peptide fragment of chicken 131 ovalbumin (OVAp) bound to H-2K^b when infected with PR8-OVA virus, a recombinant 132 strain of A/PR/8/34 (H1N1) virus expressing ovalbumin. These data therefore indicated 133 134 that BRD7 in CD8⁺ T cells may play a critical role in regulating immune response.

In order to examine the role of BRD7 in $CD8^+$ T cell response, we generated mice 135 that conditionally deleted *Brd7* in T cell-specific conditional BRD7 deleted mice (*Brd7* $^{\Delta T}$) 136 by crossing mice homozygous for loxP-flanked alleles of Brd7 ($Brd7^{fl/fl}$) to mice 137 138 expressing a transgenic encoding Cre recombinase from T cell-specific Cd4 promoter 139 (Cd4-cre) (Fig. S1), which resulted in lacked expression of BRD7 in mature CD8⁺ T cells (Fig. S2A). The thymic T cell development and T cell circulation were not affected in 140 $Brd7^{\Delta T}$ mice since similar percentages and absolute numbers of CD4⁺ and CD8⁺ T cells 141 were found in thymus, mediastinal lymph nodes (mLN), and spleen from both BRD7-142 wild-type mice $(Brd7^{fl/fl})$ and BRD7-deleted littermate pairs $(Brd7^{\Delta T})$ (Fig. S2B). To 143 explore the potential for any off-target impacts on related Brd family proteins, we carried 144 out western blotting to scrutinize their expression. The findings revealed that, with the 145

exception of *Brd7*, the expression of *Brd2*, *Brd4*, *Brg1*, and *Trim-28* remained unaltered in the BRD7-deficient mice (Fig. S3). Thus, this T cell-specific *Brd7* deleted mice allowed us to specifically analyze the role of BRD7 in $CD8^+$ T cell responses during infection.

150

151 BRD7 deficiency impairs the differentiation of effector CD8⁺ T cells

To examine the response of CD8⁺ T cell subsets of BRD7-sufficient and BRD7-deficient 152 mice during virus infection, we infected $Brd7^{\text{fl/fl}}$ mice and $Brd7^{\Delta T}$ mice with the influenza 153 HKx31 strain and analyzed the CD8⁺ T cells in the spleen and lungs at the peak of the 154 response (Day 10 p.i.). We analyzed influenza antigen-specific CD8⁺ T cell responses by 155 staining the H-2D^b-NP (NP₃₆₆₋₃₇₄) and H-2D^b-PA (PA₂₂₄₋₂₃₃) tetramers. There were no 156 obvious differences in the percentages and numbers of the influenza-specific H-2D^b-157 NP⁺CD8⁺ and H-2D^b-PA⁺CD8⁺ T cells in the spleen and lungs from $Brd7^{fl/fl}$ mice and 158 $Brd7^{\Delta T}$ mice (Fig. 2A-2C). However, the frequency of antigen-specific KLRG1⁺IL7R⁻ 159 SLECs was significantly lower in BRD7-deficient CD8⁺ T cells than in BRD7-wild-type 160 161 $CD8^+$ T cells (Fig. 2D and 2E). This finding was not due to tissue distribution since similar results were found in both spleen (Fig. S4A) and the lung (Fig. 2D and 2E). 162 Antigen-specific BRD7-deficient CD8⁺ T cells also showed increased expression of 163 CD62L and CD27 (Fig. S4B), a defect SLEC differentiation phenotype(7, 39). This 164 165 phenotype was not due to abnormal activation of CD8⁺ T cells since the expression of CD69 and CD44 was not altered in BRD7-deficient CD8⁺ T cells (Fig. S4B). Likewise, 166 to investigate the role of BRD7 in a secondary effector CD8⁺ T cell response, we used 167 two-infection model in our study. In the primary infection, mice were infected 168 169 intranasally with the influenza PR8 virus (A/PR/8/34 (H1N1) virus). In the secondary infection, mice were re-challenged with the HKx31 virus intranasally 6 weeks after the 170 first infection. We found that the frequency of NP⁺CD8⁺ T cells in the lung and spleen 171 was similar between $Brd7^{\text{fl/fl}}$ mice and $Brd7^{\Delta T}$ mice (Fig. 2F), while a reduction of 172 antigen-specific KLRG1⁺IL7R⁻ SLECs was found in the $Brd7^{\Delta T}$ mice after infection with 173 HKx31 viruses of previously PR8-primed mice (Fig. 2G). These results indicated a defect 174 in the primary and secondary effector differentiation of CD8⁺ T cells without BRD7. 175

176 To explore whether the defective differentiation of SLECs in BRD7-deficiency mice

depended on the direct role of BRD7 in CD8⁺ T cells, we generated chimeras by 177 reconstituting lethally irradiated CD45.1 mice with a various 1:1 mixture of bone marrow 178 cells of $Brd7^{fl/fl}$ mice and $Brd7^{\Delta T}$ mice, followed by infection with HKx31 at 6 week after 179 reconstitution. The frequency of antigen-specific effector CD8⁺ T cells was analyzed in 180 the spleen of chimeras. We found that the BRD7-deficient CD8⁺ T cells produced much 181 lower antigen-specific KLRG1⁺ effector cells than BRD7-wild-type CD8⁺ T cells did, 182 even in the presence of wild-type CD8⁺ T cells (Fig. 2H-I), which suggested that BRD7 183 184 influenced the differentiation of SLECs depending on the intrinsic role of BRD7 in CD8⁺ T cells. Furthermore, to rule out the possibility that the deletion of BRD7 in CD4⁺ T cells 185 may affect the phenotype, we crossed BRD7-deficient mice with mice of the OT-I TCR-186 transgenic strain to generate OT-I BRD7-deficient mice. The mixture of OT-I BRD7-187 188 deficient naïve CD8⁺ T cells (CD45.2⁺) and OT-I BRD7-wild-type naïve CD8⁺ T cells (CD45.1⁺CD45.2⁺) at a ratio of 1:1 was adoptively transferred into CD45.1 mice, 189 190 followed by infection with PR8-OVA viruses after 24 hours. We found that OT-I BRD7deficient CD8⁺ T cells failed to generate as much SLECs as OT-I BRD7-wild-type CD8⁺ 191 192 T cells did (Fig. 2J-M). Altogether, these results showed that BRD7 regulated the 193 differentiation in a CD8⁺ T cell-intrinsic manner.

194

BRD7 is required for the effector function of CD8⁺ T cells

196 After exposure to antigen, activated CD8⁺ T cells undergo an effector differentiation process and gain the functional ability to produce cytotoxic and effector cytokines to 197 eliminate intracellular pathogens. To examine whether BRD7 deficiency impacts on the 198 effector functions, we measured cytokine production from CD8⁺ T cells after NP-peptide 199 200 stimulation by intracellular staining and flow cytometric analysis. The proportion of 201 BRD7-deficient CD8⁺ T cells producing interferon γ (IFN- γ) (Fig. 3A) and tumor necrosis factor α (TNF- α) (Fig. 3B) was significantly decreased. Likewise, the production 202 of the cytolytic effector molecule GzmB (Fig. 3C) and perforin (Fig. 3D) was hardly 203 detected in antigen-specific BRD7-deficient CD8⁺ T cells. Low production of these 204 cytolytic effector cytokines will result in a defect in the ability to kill targets. Indeed, 205 BRD7-deficient CD8⁺ T cells showed a diminished cytolytic effector function in an *in* 206 vivo cytolysis assay (Fig. 3E-F). Of note, in the in vivo cytolysis experiment, we stained 207

splenocytes stimulated with NP peptide by low-dose CFSE (CFSE^{lo} cells) and 208 splenocytes without NP peptide stimulation by high-dose CFSE (CFSE^{hi} cells). Then, we 209 mixed CFSE^{lo} cells and CFSE^{hi} cells at a ratio of 1:1 and injected the mixture into wild-210 type mice non-infected with HKx31, wild-type mice infected with HKx31, or BRD7-211 deficient mice infected with HKx31 separately. Almost complete clearance of NP-pulsed 212 cells was observed in wild-type mice infected with HKx31, whereas the killing of these 213 cells was less effective in BRD7-deficient mice infected with HKx31. Similarly, $Brd7^{\Delta T}$ 214 mice showed higher degree of weight loss (Fig. 3G) and more severe pathology in the 215 lung than *Brd7*^{fl/fl} mice did after infection with the aggressive influenza PR8 viruses (Fig. 216 3H). Taken together, these data suggested that BRD7 was required for the production of 217 functional CD8⁺ effector T cells and the clearance of viruses. 218

CD8⁺ T cells will proliferate and differentiate into effector cells to eradicate 219 pathogen during virus infection. This process should be independent of virus strain. To 220 further demonstrate the role of BRD7 in CD8⁺ T cells response to other viral infection, 221 we further utilized LCMV infection model in our study, which initiates an acute infection. 222 We infected $Brd7^{\text{fl/fl}}$ mice or $Brd7^{\Delta T}$ mice with the Arm strain of LCMV, and analyzed 223 genotypes of mice at the peak of the response (day 8 post infection). Similar percentages 224 and numbers of the LCMV gp33-H-2D^b-specific CD8⁺ T cells were detected in Brd7^{fl/fl} 225 and $Brd7^{\Delta T}$ mice (Fig. 4A-B), whereas the proportion of antigen-specific KLRG1⁺IL7R⁻ 226 SLECs was fewer in $Brd7^{\Delta T}$ mice when compared with littermate controls (Fig. 4C-D). 227 Also, a defect in the production of effector cytokines was detected in CD8⁺ T cells from 228 $Brd7^{\Delta T}$ mice infected with LCMV (Fig. 4E and F). Moreover, a reduced clearance of 229 LCMV virus was observed in $Brd7^{\Delta T}$ mice compared to littermate controls (Fig. 4G). 230 231 These results indicated that BRD7 mediated the SELC development independently of virus strain or TCR specificity. 232

233

BRD7 regulates the expression of genes critical for effector differentiation

Cell proliferation and death control the expansion of $CD8^+$ T cells. To explore the proliferation of $CD8^+$ T cells *in vivo* after infection, we evaluated the 5bromodeoxyuridine (BrdU) incorporation in antigen-specific cells at day 10 after HKx31 infection. We found that the BrdU incorporation of NP⁺CD8⁺ T cells was similar in

BRD7-deficient and BRD7-wild-type mice (Fig. S5A), indicating that BRD7-deficiency 239 did not alter the proliferation of antigen-specific CD8⁺ T cells. In addition, when we 240 quantify the apoptotic CD8⁺ T cells from infected mice using Annexin V and PI staining, 241 we did not find notable differences of the apoptotic antigen-specific CD8⁺ T cells 242 between BRD7-deficient and wild type mice (Fig. S5B). These data suggested that BRD7 243 did not mediate cell proliferation or apoptosis of CD8⁺ T cells, which is consistent with 244 the above data that BRD7 deficiency did not change the proportion of antigen-specific 245 CD8⁺ T cells. 246

To better elucidate the underlying molecular mechanism by which BRD7 controls 247 the differentiation of SLECs, we analyzed the global gene expression profiles of BRD7-248 wild-type and BRD7-deficient antigen-specific CD8⁺ T cells using RNA sequencing 249 (RNA-seq). We sorted NP⁺CD8⁺ T cells from $Brd7^{fl/fl}$ mice and $Brd7^{\Delta T}$ mice infected 250 with HKx31 by flow cytometry at day 10 post infection. BRD7 deficiency led to up-251 regulation of 856 genes and down-regulation of 647 genes compared to their wild-type 252 counterparts (Fig. 5A). The differential expressed genes were highly enriched among 253 254 transcripts induced in effector CD8⁺ T cells and activated CD8⁺ T cells (Fig. 5B). We also 255 observed a notable different expression in SLEC signature genes, including Zeb2, Tbx21, 256 Sell (Cd621), and Klrg1 (Fig. 5C). These genes were further categorized as transcription factors, chemokine receptors, adhesion molecules, and killer cell lectin-like receptors 257 258 (Fig. 5C), indicating that BRD7 exerted broad regulatory effects upon SLEC signature 259 genes.

As an essential component of PBAF complex, BRD7 deficiency may result in the 260 change of chromatin structure. To test this possibility with a higher resolution method, we 261 262 performed assay of transposase assessable chromatin-sequencing (ATAC-seq), which 263 detects the insert of Tn5 transposase in open chromatin regions(40). ATAC-seq analysis of NP⁺CD8⁺ T cells showed a less accessible chromatin configuration at SLEC signature 264 genes, including Zeb2, Gzmzb, and Ifng in cells from $Brd7^{\Delta T}$ mice than that from $Brd7^{fl/fl}$ 265 mice (Fig. 5D), which was consistent with RNA-seq data. The different expression of 266 some important molecules involved in the differentiation of SLECs was further validated 267 by quantitative reverse transcription PCR (qRT-PCR) (Fig. 5E). Consistent with RNA-seq 268 and ATAC-seq data, all these genes were decreased in BRD7-deficient cells compared to 269

their wild-type counterparts in the effector $CD8^+$ T cells, but not in naïve $CD8^+$ T cells.

271 Altogether, these results revealed that BRD7 was a major regulator of genes involved in

272 SLEC differentiation and function.

273

PBAF complex enriches at *Tbx21* promoter and impacts T-bet expression in effector CD8⁺ T cells

Based on the fact that BRD7 is the specific component of PBAF complex, a chromatin 276 277 remodeling complex, we further examined the chromatin state of the BRD7-bound regions by ChIP-seq with antibodies to BRD7 in BRD7-wild-type OTI CD8⁺ T cells from 278 OT-I mice infected with PR8-OVA at day 8 p.i.. Notably, we found that BRD7 was 279 enriched at the *Tbx21* loci (Fig. 6A), which was further validated with ChIP-PCR (Fig. 280 281 6B). T-bet is a major driver for CD8⁺ T cell SLEC lineage commitment and controls the expression of a large number of molecules critical for the effector differentiation of CD8⁺ 282 283 T cells including Zeb2, Runx3, Ifng, Gzma and Prf1(5, 41-44). The loss of T-bet leads to abrogated cytotoxic function and influences nearly 50% of the SLEC-specific genes. We 284 285 hypothesized that BRD7 bound to the *Tbx21* loci and regulated T-bet expression. Indeed, RNA-seq and ATAC-seq revealed a defect in transcriptional activity of *Tbx21* gene after 286 287 BRD7 deficiency (Fig. 5 and Fig. 6A). A significant decrease of T-bet expression in BRD7-deficient NP⁺CD8⁺ T cells was also observed by qRT-PCR (Fig. 6C) and flow 288 289 cytometry analysis (Fig. 6D). Taken together, our results suggested that T-bet acts as the downstream factor for BRD7 to drive during the differentiation of effector cells. 290

BRD7 was previously reported to bind to H3K9ac (26). To test the possibility that 291 BRD7 binds to H3K9ac and increases the H3K9ac deposition at Tbx21 locus, we 292 293 performed ChIP-seq with antibodies to H3K9ac in BRD7-wild-type or BRD7-deficient 294 OTI CD8⁺ T cells from OT-I mice infected with PR8-OVA at day 8 p.i.. No significant difference of H3K9ac enrichment was observed at *Tbx21* loci in BRD7-deficient cells 295 comparing to BRD7-wild-type cells (Fig. 6A, lowest panel), implying that BRD7 296 regulated the T-bet expression independent of its binding to H3K9ac. To examine the 297 possibility that the PBAF complex co-opts epigenetic mechanisms for target gene 298 regulation, we further performed ChIP assay with antibodies to H3K9me3, H3K27me3 299 and H3K14ac on wild-type and BRD7-deficient OTI CD8⁺ T cells from OT-I mice 300

infected with PR8-OVA at day 8 p.i.. The H3K9me3, H3K27me3 and H3K14ac
modifications at the *Tbx21* locus were not obviously altered after BRD7 deficiency (Fig.
6E). Thus, BRD7 regulated the T-bet expression independent of H3K9me3, H3K27me3
and H3K14ac modification at the *Tbx21* locus.

305

306 BRD7 functions as a bridge for PBAF complex to efficiently assembly in effector 307 CD8⁺ T cells

308 Based on the result that BRD7 regulated the chromatin accessibility of *Tbx21* locus, we assumed that BRD7 within the PBAF complex plays an important role in effector 309 CD8⁺ T cell differentiation. To better explore the role of BAF and PBAF complex in 310 CD8⁺ T cells differentiation, we performed immunoprecipitation with BRG1-specific 311 312 antibody with lysates from naïve or OTI CD8⁺ T cells which were from OT-I mice infected with PR8-OVA at day 8 p.i. and were denoted as effector cells. The pull-down 313 314 substrates were subsequently analyzed with mass spectrometry (Fig. 7A). Being the ATPase subunit of BAF and PBAF complexes, BRG1 is shared by both BAF and PBAF 315 316 complexes. Therefore, we could observe the component changes of both BAF and PBAF complexes. Interestingly, we found that BAF/PBAF-shared components appeared in the 317 318 BRG1-associated proteins from both naïve and effector CD8⁺ T cells (Fig. 7B-7E), while PBAF-specific components including BRD7, PBRM1, PHF10, and ARID2 existed only 319 320 in that from effector cells (Fig. 7D-E). We further confirmed this result with IP experiment and found that BRG1 interacted with BRD7 only in effector CD8⁺ T cells, but 321 not in naïve CD8⁺ T cells (Fig. 7F). Thus, we proposed that the SWI/SNF complexes 322 barely appear as BAF complex in naïve CD8⁺ T cells and only PBAF complex is 323 324 assembled in the effector CD8⁺ T cells.

Because of the chromatin remodeling function, it is reasonable to assume that the ATPase unit of PBAF complex would also be enriched at the loci of target genes. To testify this hypothesis, we used BRG1-specific antibody in the ChIP assay and found that BRG1 specifically enriched at the loci of Tbx21 in the OTI CD8⁺ T cells from OT-I mice infected with PR8-OVA at day 8 p.i. (Fig. 7G). Based on the fact that BRG1-deficiency resulted in the loss of double-negative T cells in the thymus during T cell maturation(21), we utilized BRG1-specific shRNA (shBRG1) to specifically deplete BRG1 in mature T cells. Accordingly, the depletion of BRG1 resulted in a down-regulation of Tbx21 mRNA expression, indicating that BRG1 plays a role in regulating T-bet. (Fig. 7H and 7I). Thus, we suggested that PBAF complex bound to Tbx21 loci and regulated the expression of Tbet in the effector CD8⁺ T cells.

To explore the role of BRD7 within PBAF complex in the effector CD8⁺ T cells, we 336 further analyzed the interactions between SMARCC1, BRG1, and PBRM1 based on the 337 assembly process of PBAF complex in human 293T cells (17). According to this modular 338 339 assembly model of PBAF complex, the form of BAF core module and ATPase module are two parallel steps. ATPase module is recruited after the core module incorporation 340 with PBAF specific components ARID2, BRD7, and PHF10. After incorporation with the 341 ATPase module, the PBAF complex intermediate finalizes its formation by binding with 342 343 PBRM1 (17). However, this model did not propose any role played by BRD7 in assembling PBAF complex. As BRD7 can individually interact with SMARCC1, 344 345 PBRM1, or BRG1 (17), we supposed that BRD7 may function as a bridge between PBAF core module and ATPase module. To test this hypothesis, we analyzed the interaction 346 347 between SMARCC1, PBRM1, or BRG1 after BRD7 deficiency in the effector CD8⁺ T cells. We found that the binding of BRG1 to either SMARCC1 or to PBRM1 became 348 349 weakened after BRD7 deficiency in the OTI CD8⁺ T cells (Fig. 7J), indicating that the binding of the core module of BAF- or PBAF-specific component PBRM1 to the ATPase 350 351 module depended upon BRD7. In addition, the enrichment of BRG1 at Tbx21 locus was significantly less after BRD7 deficiency in effector CD8⁺ T cells (Fig. 7K), while the 352 enrichment of SMARCC1 at Tbx21 locus was unaffected after BRD7 deficiency in 353 effector CD8⁺ T cells (Fig. 7L), implying that the recruitment of ATPase module to target 354 355 gene depended on the assembly of the core module of PBAF with BRD7. Altogether, 356 these results indicated that BRD7 was the bridge between BAF core module and ATPase module during the assembly of PBAF complex in effector CD8⁺ T cells. 357

358

359 **DISCUSSION**

After antigen stimulation, naïve CD8⁺ T cells expand and differentiate into effector cytotoxic T cells. Multiple lines of evidence have indicated that T-bet serves as the "master regulator" of SLEC lineage commitment (5, 41-43). T-bet is highly expressed in

effector $CD8^+$ T cells, but lowly expressed in memory $CD8^+$ T cells. It is also required for 363 the production of IFN- γ and the cytotoxicity of CD8⁺ T cells during LCMV infection. 364 *Tbx21* knockout seriously influences the formation of KLRG1^{hi} subset of effector CD8⁺ T 365 cells but has little effect on the IL7R^{hi} subset (5, 42). T-bet performs these functions via 366 influencing the expression of many lineage-specific genes expression in SLECs. In the 367 current study, we have identified a prominent role of BRD7 in regulating robust 368 development of effector CD8⁺ T cells during acute virus infection and functions a key 369 determinant of the switch from naïve T cells to effector T cells. BRD7-deficient CD8⁺ T 370 cells show impaired expression of effector molecules such as IFN- γ and granzyme B. By 371 RNA-sequencing, ATAC-sequencing, qRT-PCR, and flow cytometry assays, we found 372 that BRD7 sustains the effector differentiation of CD8⁺ T cells directly through regulating 373 the expression of *Tbx21*. We further demonstrated that BRD7 enriched at the *Tbx21* locus 374 with ChIP assay. These findings indicate that BRD7 affects the development of effector 375 $CD8^+$ T cells through regulating *Tbx21* transcription. 376

BRD7 has been identified as a tumor-suppressor gene in multiple cancers (28-30, 45-377 47). High expression Brd7 is associated with improved survivals in multiple cancers. It 378 could recognize and bind to acetylated histone H3 (26). However, several studies also 379 showed that though binding to acetylated histone H3, BRD7 did not have histone 380 acetyltransferases activity (26, 27). In this study, we found that BRD7 regulated the T-bet 381 expression independent of H3K9ac and H3K14ac modifications. We also found that 382 BRD7 regulated the T-bet expression independent of H3K9me3 and H3K27me3 383 modification at the *Tbx21* locus. However, as BRD7-deficiency decreases the chromatin 384 385 accessibility of *Tbx21* locus, BRD7 could regulates T-bet expression through altering chromatin modification of *Tbx21* loci as a component of PBAF complex. 386

Basis Despite many shared components, BAF and PBAF complexes are functionally distinct complexes (23). The balance between BAF and PBAF complexes was reported as a pivotal determinant of the VDR-driven anti-inflammatory response (37). Recently, the researchers found that the BAF complex and c-Myc physically interact to establish the chromatin landscape in activated CD8⁺ T cells and BAF as a negative determinant of T_{MEM} cell fate (48). In our study, we describe a phenomenon that PBAF- rather than BAF-specific components only exist in effector CD8⁺ T cells, while did not appear in

naïve CD8⁺ T cells. The assembly of PBAF complexes was initiated in the effector stage 394 during CD8⁺ T cells differentiation, which was consistent with the significant 395 upregulation of BRD7 expression in effector CD8⁺ T cells when compared to naïve CD8⁺ 396 T cells. PBAF complex functions as chromatin remodelers including nucleosome 397 assembly and organization, chromatin assess and nucleosome editing, which result in 398 their specific interaction with particular transcription activators, repressors and histone 399 modifications. All these factors function together and lead to the activation of a special 400 401 gene (16, 49). In the presence of BRD7, the proper assembled PBAF complex therefore enhanced the chromatin accessibility at *Tbx21* locus. 402

A major barrier to our understanding of the functions and tissue-specific roles of 403 mSWI/SNF complex is the lack of knowledge of the assembly and organization of the 404 405 complex. A significant progress has been made by a recent study (17). This report has posed the modular organization and assembly order of mSWI/SNF complex in human 406 407 HEK293T cell line. In particular, the assembly of PBAF complex begins with the formation of the core module including SMARCC1, and then incorporates PBAF-specific 408 409 components ARID2, BRD7, and PHF10 in order. The assembly pathway of PBAF finalizes by recruitment of ATPase module (including BRG1) and the binding of PBRM1. 410 411 However, the role of BRD7 for the assembly of PBAF in this model remains to be determined. Here we have found that BRD7 interacts with BRG1, SMARCC1, and 412 413 PBRM1, and is important for BRG1 (ATPase module) and SMARCC1 (core module) interaction. Although BRD7 does not affect the loading of the core module components 414 such as SMARCC1 onto Tbx21 locus, BRG1 (ATPase module) binds to the Tbx21 locus 415 in effector CD8⁺ T cells in a BRD7-dependent manner. These results further confirmed 416 417 the critical role played by BRD7 in assembling PBAF complex in effector CD8⁺ T cells, 418 especially at the *Tbx21* locus.

In summary, we found that the major complex of SWI/SNF chromatin-remodeling in effector CD8⁺ T cells is PBAF complex. Despite many functions of PBAF complex has been illuminated (23), the capability of PBAF complex to mediate SLEC differentiation is unknown. Our study showed the essential role of PBAF complex in SLEC differentiation, and illuminated mechanisms how SLECs maintains the expression of "effector" genes in the presence of PBAF complex. The significantly upregulated BRD7 425 is the key factor for PBAF assembly in SLECs. However, the signaling pathways that initiate the expression of BRD7 and the mechanism of the switch between BAF and 426 427 PBAF complexes remain to be illuminated. Nevertheless, our data highlighted the roles of BRD7 and its PBAF complex in the differentiation and function of CD8⁺ effector cells. 428 By focusing on the BRD7-mediated assembly of PBAF complex in CD8⁺ T cells, we 429 uncovered a possible therapeutic target to interfering with the induction of functional 430 effector CD8⁺ T cells, which could open a new avenue for the treatment of various 431 diseases including viral infections, tumors, or autoimmune diseases. 432

433

435

436 **METHODS**

437 Sex as a biological variant

438 Sex was not considered as a biological variable, both female and male mice were used.

- 439
- 440 Mice

All mice were on a C57BL/6 (B6) background. Brd7^{fl/fl} mice crossed with Cd4-Cre mice 441 $(Brd7^{\Delta T})$ were used. They were obtained from Shanghai Model Organisms Co. Ltd 442 (Shanghai, China). Cre-negative littermates were used as wild-type controls in all 443 experiments. Wild-type B6 mice, CD45.1 mice and OT-I TCR-transgenic mice were 444 obtained from The Jackson Laboratory. OT-I TCR-transgenic mice, which use a Va₂Vb₅ 445 TCR heterodimer to recognize OVAp (amino acids 257-264) presented by H-2K^b, were 446 congenic for CD45.1 on the B6 background. OT-I CD45.1 mice and $Brd7^{\Delta T}$ mice were 447 448 bred and the offspring were intercrossed to obtain OT-I BRD7-conditional knockout TCR-transgenic mice. During infection experiments, wild-type and BRD7-deficient mice 449 were housed together to avoid 'cage bias'. No intentional method for randomization was 450 used. Chimeras were generated by intravenous injection of 5×10^6 to 10×10^6 donor bone 451 marrow cells ($Brd7^{fl/fl}$ and $Brd7^{\Delta T}$ cells at a ratio of 1:1) into lethally irradiated CD45.1 452 mice. $Brd7^{\text{fl/fl}}$ and $Brd7^{\Delta T}$ cells of donor origin were identified with the congenic markers 453 454 CD45.1 and CD45.2. The chimeras were used at 6 weeks after engraftment. All mice were used at the age of 6-10 weeks, and housed and maintained according to Sun Yat-sen 455 University guidelines (permit number SYXK (YUE) 2010-0107). 456

457

458 Infection with influenza A virus and LCMV-armstrong

For influenza A virus infection, mice were infected intranasally with 10^7 plaque-forming units (PFU) of influenza virus strain A/PR/8/34 (H1N1), the H3N2 influenza A virus strain HKx31, or influenza virus strain A/PR/8/34–OVA (PR8-OVA). To measure recall responses, mice were first inoculated intraperitoneally (i.p.) with 10^7 PFU of the A/PR/8/34 influenza virus and then challenged intranasally (i.n.) with $10^{4.5}$ PFU of HKx31 at 4 weeks later. Virus stocks were grown in the allantoic cavity of 10 days embryonated hen's eggs and stored in aliquots at -80°C. Viral titers were obtained by 466 infection of MDCK (Mardin-Darby canine kidney) cells as previously described(50, 51).

467 MDCK cells were obtained from American Type Culture Collection (ATCC).

For LCMV-Armstrong infection, mice were generally infected intraperitoneally with LCMV Arm strain (1×10^6 to 5×10^6 PFU). Eight days after infection, mice were euthanized and donor cells were assessed using LCMV-GP³³⁻⁴¹-tetramer (H-2D^b-GP33⁺) staining.

472

473 Flow cytometry and cell sorting

Single-cell suspensions were prepared from spleen, or lung. The following antibodies 474 were used (All monoclonal antibodies from eBioscience): anti-CD3c (145-2C11), anti-475 CD4 (GK1.5), anti-CD8a (53-6.7), anti-KLRG1 (2F1), anti-CD127 (anti-IL7Ra; A7R34), 476 anti-CD45.1(A20), anti-CD45.2 (104), anti-CD62L (MEL-14), anti-CD44 (IM7), anti-477 CD69 (H1.2F3), anti-CD27 (LG.7F9), anti-BrdU (BU20A), anti-IFN-y (XMG1.2), anti-478 TNF-α (MP6-XT22), anti-granzyme B (NGZB), anti-Perforin (dG9 (delta G9)), and anti-479 T-bet (4B10). Allophycocyanin-conjugated tetramers of H-2D^b used in the study were all 480 481 from Helixgen (Guangzhou) Co., Ltd: anti-Flu.NP₃₆₆ (ASNENMETM), anti-Flu. PA₂₂₄ (SSLENFRAYV), and anti-LCMV-GP₃₃ (KAVYNFATM). 482

483 For measurement of intracellular cytokine expression, splenocytes were isolated *ex vivo* and stimulated with 1 µg/ml of the major histocompatibility complex class I (MHC- I)-484 485 restricted influenza-derived peptide NP (amino acids 366-374 (ASNENMETM)) in IMDM media plus 10% FBS with 50 ng/ml PMA (Sigma-Aldrich), 1 µg/ml ionomycin 486 (Sigma-Aldrich), and 1 µg/ml brefeldin A (eBioscience) for 4-6 h. Cells were stained for 487 20 min at room temperature with the relevant fluorochrome-conjugated monoclonal 488 489 antibodies in PBS containing 0.5% BSA. For intracellular staining, cells were fixed and 490 permeabilized with Cytofix/Cytoperm (BD Biosciences), and stained with antibodies against indicated cytokines. For staining of transcription factor, cells were stained with 491 antibodies to surface antigens, fixed and permeabilized according to the manufacturer's 492 instructions (Transcription Factor Staining Buffer Set; BD Biosciences). Cells were 493 acquired on an LSRFortessa flow cytometer (BD Bioscience), and data were analyzed 494 with the FlowJo V10.0.7 (FlowJo). The fraction of labeled cells was analyzed with a 495 minimum 100,000 events. 496

For the flow cytometric sorting, a BD FACSAriaIII cell sorter (BD Bioscience) was used. For the isolation of H-2D^b-NP tetramer-positive CD8⁺ T cells from influenza virus infected mice, single-cell suspensions of lung were stained with influenza virus–specific tetramers and antibodies to the relevant markers. For the isolation of naïve CD8⁺ T cells from B6 mice, single-cell suspensions of spleen with stained with the specific fluorochrome-conjugated antibodies. All the cells were sorted with a purity \geq 95%.

503

504 Adoptive transfer of CD8⁺ T cells and Infections

505 Congenically distinct $Brd7^{fl/fl}$ and $Brd7^{\Delta T}$ OT-I CD8⁺ T cells were mixed at a 1:1 ratio and 506 adoptively transferred at 1×10^5 cells per CD45.1 recipient mouse. Mice were then 507 infected intranasally with PR8–OVA virus.

508

509 Generation of Bone Marrow chimeras

For $Brd7^{fl/fl}$: $Brd7^{fl/fl}$ or $Brd7^{fl/fl}$: $Brd7^{\Delta T}$ chimeras, CD45.1⁺ mice were lethally irradiated with 950 rad and then injected intravenously (i.v.) with 1 × 10⁷ bone marrow cells harvested from $Brd7^{fl/fl}$ CD45.2⁺ and $Brd7^{fl/fl}$ CD45.1⁺CD45.2⁺ or $Brd7^{\Delta T}$ CD45.2⁺ and $Brd7^{fl/fl}$ CD45.1⁺CD45.2⁺ littermates at the ratio of 1:1. The mice were treated with sulfamethoxazole and trimethoprim (Bactrim) antibiotics diluted in drinking water for 4 weeks after reconstitution. After approximately 6 weeks, the mice were infected with HKx31 virus, and sacrificed for flow cytometry analysis at 10 p.i..

517

518 In vivo cytotoxicity assay

Target spleen cells from B6 mice were pulsed for 30 min with 1 µg/ml influenza virus-519 520 derived NP peptide (amino acids 366-374) and were subsequently labeled for 20 min at 37 °C with 0.2 μM CFSE (carboxyfluorescein diacetate succinimidyl ester; Invitrogen) 521 (CFSE^{lo}; specific target cells) or were not pulsed with peptide and were labeled with 2 522 µM CFSE (CFSE^{hi}; nonspecific target cells). The two target populations were mixed in 523 equal numbers, and 5×10^6 cells were transferred i.n. into mice that had been infected with 524 influenza A virus HKx31 strain 10 d before or into non-infected control mice. Mice were 525 killed 4 h later and the ratio of peptide-loaded target cells to 'empty' target cells was 526 quantified by flow cytometry. 527

528

529 RNA-Seq and ATAC-Seq

CD8⁺ T cells that bound H-2D^b-NP (NP peptide amino acids 366-374) were isolated by 530 flow cytometry from lung of influenza virus-infected wild type or BRD7-deficient mice. 531 Total RNAs from each group were extracted by TRIzol Reagent (ThermoFisher) 532 according to the manufacturer's instruction. The quality of RNA samples were evaluated 533 by Nanodrop 2000 (ThermoFisher) and BioAnalyzer 2100 (Aglient). The RNA-Seq 534 library were built with TruSeq Stranded mRNA Library Prep Kit (Illumina) and 535 sequenced with HiSeq X Ten (Illumina) at BioMarker (Beijing, China) under the PE150 536 protocol(52). RNA-Seq reads were trimmed, filtered and quality-controlled by FastQC 537 (Babraham Institute) tool. The reads were aligned to mouse reference genome NCBI 538 539 build 38 (GRCm38) by Hisat2, followed by calculating the reads per kilobase per million mapped reads (RPKM). 540

ATAC-Seq was conducted with H-2D^b-NP⁺CD8⁺ T cells of influenza virus-infected wild 541 type and BRD7-deficient mice. The ATAC-Seq library was built with TruePrep DNA 542 543 Library Prep Kit V2 (Vazyme) as previously described(52). In brief, the library quality was evaluated by Qubit 3.0 Fluorometer (ThermoFisher) and BioAnalyzer 2100 (Aglient), 544 545 and sequenced with HiSeq X Ten (Illumina) at BioMarker (Beijing, China) under the PE150 protocol. ATAC-Seq reads were trimmed, filtered and quality-controlled by 546 547 FastQC tool. Then the reads were aligned to GRCm38 by Bowtie2 (Langmead and Salzberg, 2012), followed by rearranging with Samtools. Igytools (Broad Institute) was 548 used to visualize the tag peaks. Specific gene loci were amplified. Tag density from 549 different groups was calculated by normalizing to the total mapped reads. 550

551

552 ChIP-seq and ChIP-qPCR

553 ChIP was performed according to the manufacturer's instruction (Cell Signaling 554 Technology). In brief, naive OT-I CD8⁺ T cells or OT-I CD8⁺ cells from OT-I mice 555 infected with PR8-OVA at day 8 p.i. fixed with 1% formaldehyde (Sigma-Aldrich), 556 followed by digestion with RNase cocktail. Chromatin from 5×10^6 to 10×10^6 cells was 557 used for each ChIP experiment. Antibodies against normal rabbit IgG (1:50, Cat No. 2729, 558 Cell Signaling Technology), BRD7 mouse mAb (1:50, (B-8), Cat No. sc-376180, Santa

Cruz), BRG1 Rabbit mAb (1:50, (D1Q7F), Cat No. 49360, Cell Signaling Technology), 559 H3K9ac Rabbit mAb (1:50, (C5B11), Cat No. 9649, Cell Signaling Technology), 560 H3K14ac Rabbit mAb (1:50, (D4B9), Cat No. 7627, Cell Signaling Technology), 561 H3K9me3 Rabbit mAb (1:50, (D4W1U), Cat No. 13969, Cell Signaling Technology) and 562 H3K27me3 Rabbit mAb (1:50, (C36B11), Cat No. 9733, Cell Signaling Technology) 563 were used. Antibody-DNA complexes were captured by ChIP-Grade Protein G Magnetic 564 Beads. The immunoprecipitated DNA was purified and subjected to sequencing or PCR 565 566 assessment. ChIP primers targeting the *Tbx21* were used to quantitate each target regions by Quantitative RT-PCR. 567

568

569 **Quantitative RT-PCR**

570 Total RNA from indicated numbers of cells were isolated with TRIzol reagent (Thermo 571 Fisher) and preceded to cDNA synthesis with PrimeScript RT reagent Kit (Takara). Gene 572 expression was analyzed by Real-time PCR with SYBR Ex-taq premix (Takara) in a 573 CFX96 Real-time PCR Detection System (Bio-Rad). Mouse β -actin mRNA was 574 measured as internal control.

575

576 Mass spectrometry

Mass spectrometry analysis was performed as previously described(50). In brief, the 577 578 stained bands of interest were excised into gel slices with a clean scalpel, followed by 579 digested with trypsin using in-gel digestion. Each gel piece was diced into small (1-mm3) pieces, and dehydrated. The gel pieces were then incubated with trypsin (Promega) for 580 digestion. Peptides were further extracted with 50% acetonitrile-5% formic acid, 581 582 lyophilized in a SpeedVac (Thermo Savant), and then desalted using u-C18 Ziptip 583 (Millipore). Finally, samples were lyophilized and stored at 20°C prior to analysis by LC-MS/MS or dissolved in 0.1% (vol/vol) formic acid-water. All samples were analyzed on a 584 Thermo Scientific Q EXACTIVE massspectrometer coupled with an EASY n-LC 1000 585 liquid chromatography (ThermoFisher) system and a nanoelectrospray source. 586

587

588 Coimmunoprecipitation (co-IP) and western blot assays.

589 Co-IP and western blot assays were performed as previously described (50). Wild-type

(Brd7^{fl/fl}) or BRD7-deficient OT-I CD8⁺ T cells from OT-I mice infected with PR8-OVA 590 at day 8 p.i. were collected and lysed. The lysates were precleared with protein A/G 591 agarose beads (Millipore) for 30 min and then incubated with anti-BRG1 antibody or 592 rabbit normal IgG antibody for 6 h to overnight, followed by incubating with protein A/G 593 agarose beads (Millipore) for 4 h at 4°C. The beads were then washed three times with 594 ice-cold lysis buffer, followed by Western blotting. The following antibodies were used: 595 β-actin antibody (1:1000, Cat No. 4967, Cell Signaling Technology), BRD7 mouse mAb 596 597 (1:500, (B-8), Cat No. sc-376180, Santa Cruz), BRG1 Rabbit mAb (1:500, (D1Q7F), Cat No. 49360, Cell Signaling Technology), BRD2 Rabbit mAb (1:500, (D89B4), Cat No. 598 5848, Cell Signaling Technology), BRD4 Rabbit mAb (1:500, (E2A7X), Cat No. 13440, 599 Cell Signaling Technology), TRIM28 Polyclonal antibody (1:500, Cat No. 15202-1-AP, 600 601 Proteintech), IRDye 680RD Goat anti-Mouse IgG (H + L), 0.5 mg Antibody (1:10000, Cat No. 926-68070, LI-COR Biosciences), IRDye 800CW Goat Anti-Rabbit IgG, 602 603 Conjugated Antibody(1:10000, Cat No. 926–32211, LI-COR Biosciences).

604

605 shRNA-mediated knockdown by retroviral transduction

DNA fragments encoding shRNA targeting mouse BRG1 (SMARCA4) were subcloned 606 607 into a custom retroviral vector containing GFP as a reporter (pMKO.1). CD8⁺ T cells of OT-I mice were separated and stimulated for 18 h in 24-well plates precoated with anti-608 609 CD3 and anti-CD28. After stimulation, cells were transduced by adding retroviral supernatants supplemented with 100 U/ml mouse IL-2 and 8 µg/ml polybrene, followed 610 by centrifugation for 95 min at 950 g at 32°C. After transduction, cells were incubated for 611 12-16 h at 37°C. 1×10^5 transduced CD8⁺ T cells were transferred into PR8-OVA virus 612 613 infected hosts (CD45.1 mice) at day 1 p.i., and remaining cells were cultured in vitro with 614 50 U/ml human IL-2 for 2 d to assess for knockdown efficiency by qPCR. At 10 d after infection, CD45.2⁺ populations were assessed by flow cytometry. 615

616

617 Statistical analysis

All data are derived from two to three independent experiments. Statistical analysis was performed with GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA). Results show mean \pm s.e.m. Two-tailed student's t-test was used to compare two 621 independent groups, while two-way ANOVA was used in multiple comparisons. 622 Differences were considered significant when *P<0.05, **P<0.01, ***P<0.001 and 623 ****P<0.0001.

624

625 Study approval

All mouse experimental procedures were approved by the Institutional Animal Care andUse Committee of Sun Yat-sen University (Guangzhou, China).

628

629 Data Availability

All datasets generated or analyzed during this study have included in this manuscript. The
RNA-seq , ATAC-seq and ChIP-seq data have been deposited in the Sequence Read

Archive (SRA) database (accession number PRJNA1122236 and PRJNA1122318). The

data are available in the "Supporting data values" XLS file and from the correspondingauthor on reasonable request.

635

636 CONFLICT OF INTEREST

637 The authors declare no conflict of interest.

638

639 AUTHOR CONTRIBUTIONS

F.H, YT.L, YD. Q and YC.Y designed the experiments, performed most of these
experiments, analyzed the data, and manuscript writing; ZH.Z, BH.L, YT. W, J.L, JL.C
and WY.Z performed some of the experiments; H.Z provided scientific expertise and the
interpretation of data for the work; BF. L contributed to the idea generation, experimental
design, manuscript writing and conceived the project.

645

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- 658
- 659

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- 789 **FIGURE LEGENDS**





791 FIGURE 1. BRD7 is upreguated in effector CD8⁺ T cells. (A) Differential expression of relative mRNAs in naïve, effector and memory OT-I CD8⁺ T cells was analyzed. 792 793 Naïve OT-I cells purified from spleen of OT-I mice were transferred into recipient CD45.1 mice and then infected with PR8-OVA at day 1 after transfer. Effector (10 d p.i.) 794 and memory (~42 d p.i.) OT-I cells were purified with FACS for mRNA quantification 795 relative to that of naïve OT-I cells. (B) Time course of mRNA expression of several 796 PBAF components in OT-I T cells purified from spleen of OT-I mice which were 797 transferred into recipient CD45.1 mice and then infected with PR8-OVA at day 1 after 798 transfer. **P*<0.05, ***P*<0.01, ****P*<0.001 and *****P*<0.0001 (Two-way ANOVA). Mean 799 and s.e.m. of three mice per group. Data are representative of two independent 800 experiments. 801

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Figure 2

FIGURE 2. BRD7 controls SLEC differentiation. (A) Flow cytometry of influenza H-804 2D^b-NP⁺CD8⁺ and H-2D^b-PA⁺CD8⁺ T cells from spleen and lungs of BRD7-wild-type 805 $(Brd7^{fl/fl})$ (n=4) and BRD7-deficient $(Brd7^{\Delta T})$ (n=3) mice. Mice were infected with 806 influenza HKx31 virus, and NP366 and PA244 tetramer were stained in lung, spleen 807 CD8⁺ T cells at 10 d p.i.. (**B-C**) Frequency and cell number of H-2D^b-NP⁺ or H-2D^b-PA⁺ 808 cells among CD8⁺ T cells in A. (D-E) Flow cytometry of KLRG1 and CD127 on H-2D^b-809 NP⁺CD8⁺ T cells in the lungs obtained from $Brd7^{fl/fl}$ (n=4) or $Brd7^{\Delta T}$ (n=3) mice infected 810 with HKx31 at 10 d p.i.. (F) Flow cytometry of H-2D^b-NP⁺CD8⁺ T cells from spleen and 811 lungs of PR8-primed $Brd7^{\text{fl/fl}}$ (n=4) and $Brd7^{\Delta T}$ (n=4) mice rechallenged with HKx31 at 6 812 weeks after PR8 infection. (G) Flow cytometry of KLRG1 and CD127 on H-2D^b-813 NP⁺CD8⁺ T cells in the spleen obtained of PR8-primed $Brd7^{fl/fl}$ (n=4) and $Brd7^{\Delta T}$ (n=4) 814 mice rechallenged with HKx31 at 6 weeks after PR8 infection. (H-I) Flow cytometry of 815 KLRG1 and CD45.1 on NP⁺CD8⁺ T cells from chimeras. Mixed bone marrow chimeras 816 were generated by reconstitution lethally irradiated CD45.1 mice with bone marrow from 817 $Brd7^{fl/fl}$ (CD45.2⁺) plus $Brd7^{fl/fl}$ (CD45.1⁺CD45.2⁺) (n=3), or $Brd7^{\Delta T}$ (CD45.2⁺) plus 818 $Brd7^{\text{fl/fl}}$ (CD45.1⁺CD45.2⁺) (n=3) at a ratio of 1:1. Chimeras were infected with HKx31 at 819 6 weeks after reconstitution, and sacrificed at 10 d p.i. for analysis. (J-M) Flow 820 cytometry of OT-I CD8⁺ T cells (J) or KLRG1⁺CD127⁻ SLECs and KLRG1⁻CD127⁺ 821 MEPCs (L) in CD45.1 host mice (n=4) 9 d after transfer of $Brd7^{fl/fl}$ (CD45.1+CD45.2+) 822 plus $Brd7^{\Delta T}$ (CD45.2⁺) OTI CD8⁺ T cells at a ratio of 1:1 and infection with PR8-OVA 823 virus 1d after transfer. Small horizontal lines indicate the mean (\pm s.e.m.). *P<0.05, 824 **P < 0.01 and ***P < 0.001 (two-tailed student t test). Data are representative of three 825 independent experiments. 826

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FIGURE 3. BRD7 is required for effector function. (A-B) Intracellular cytokine 829 staining of IFN- γ (A) or TNF- α (B) produced by splenic CD8⁺ T cells from $Brd7^{fl/fl}$ (n=4) 830 and $Brd7^{\Delta T}$ (n=4) mice infected with HKx31 virus stimulated with NP peptide at 10 d p.i.. 831 Left: Number in beside outlined areas indicates percent of IFN- γ^+ CD8⁺ (A) or TNF-832 $\alpha^{+}CD8^{+}(\mathbf{B})$ T cells. Right: Frequency of IFN- $\gamma^{+}(\mathbf{A})$ or TNF- $\alpha^{+}(\mathbf{B})$ cells among CD8⁺ T 833 cells in left. (C-D) Flow cytometry of granzyme B (GzmB) (C) or perforin (Prf1) (D) 834 produced by splenic NP⁺CD8⁺ T cells from $Brd7^{fl/fl}$ (n=4) and $Brd7^{\Delta T}$ (n=3) mice infected 835 with HKx31 virus at 10 d p.i.. Left: Numbers in beside outlined areas indicate Mean 836 Fluorescence Intensity (MFI) of GzmB (C) or Prf1 (D) among NP⁺CD8⁺ T cells. Right: 837 MFI of GzmB (C) or Prf1 (D) among NP⁺CD8⁺ T cells in left. (E) In vivo cytolysis assay. 838 Non-infected wild-type host mice or infected BRD7-wild-type (n=3) or BRD7-deficient 839 (n=3) mice received equal numbers of low CFSE-labeled B6 splenocytes loaded with NP 840 peptide plus high CFSE-labeled B6 splenocytes without NP peptide stimulation at 10 d 841 p.i.. Cytotoxic T lymphocyte activity was assessed 4 h after transfer. Numbers above 842 bracketed lines represent percentages of cells per CFSE peak. (F) Frequency of peptide-843 pulsed cells in **E** was shown. (**G**) Body weight of $Brd7^{fl/fl}$ (n=8) and $Brd7^{\Delta T}$ (n=7) mice 844 infected with 0.5 LD₅₀ of A/PR/8/34 (H1N1) virus at various times p.i.. Body weight at 845 day 0 was set as 100%. (H) Histological examination of lung from $Brd7^{\text{fl/fl}}$ or $Brd7^{\Delta T}$ 846 mice non-infected with PR8 virus (top panel) or infected at 8 day p.i. (bottom panel). 847 Lung from $Brd7^{\Delta T}$ mice infected with PR8 virus showed severe tissue damage and 848 lymphocytic infiltration. Small horizontal lines indicate the mean (\pm s.e.m.). *P<0.05, 849 **P < 0.01 and ***P < 0.001 (two-tailed student t test). Data are representative of three 850 independent experiments. 851

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Figure 4

FIGURE 4. BRD7 controls SLEC differentiation during LCMV infection. (A) Flow 854 cytometry of Arm gp33⁺CD8⁺ T cells from spleen of $Brd7^{fl/fl}$ (n=3) and $Brd7^{\Delta T}$ (n=4) 855 mice. $Brd7^{fl/fl}$ and $Brd7^{\Delta T}$ mice were infected with LCMV armstrong virus, and antigen-856 specific gp33 tetramer was stained in spleen CD8⁺ T cells at 8 d p.i.. Numbers in beside 857 outlined areas indicate percent of $gp33^+CD8^+$ T cells. (B) Frequency (left) and cell 858 number (right) of gp33-specific cells among CD8⁺ T cells in A. (C) Flow cytometry of 859 KLRG1 and CD127 on $gp33^+CD8^+$ T cells in the spleen obtained from $Brd7^{fl/fl}$ (n=3) or 860 $Brd7^{\Delta T}$ (n=4) mice infected with Arm at 8 d p.i.. Numbers in quadrants indicate percent 861 of KLRG1⁺CD127⁻ SLECs (top left) or KLRG1⁻CD127⁺ MEPCs (bottom right). (**D**) 862 Frequency of KLRG1⁺CD127⁻ SLECs or KLRG1⁻CD127⁺ MEPCs among gp33⁺CD8⁺ T 863 cells in C. (E-F) Intracellular cytokine staining of IFN- γ (E) or TNF- α (F) produced by 864 splenic CD8⁺ T cells from $Brd7^{fl/fl}$ (n=3) and $Brd7^{\Delta T}$ (n=4) mice infected with Arm virus 865 stimulated with gp33 peptide at 8 d p.i.. Left: Number in beside outlined areas indicate 866 percent of IFN- γ^+ CD8⁺ (**E**) or TNF- α^+ CD8⁺ (**F**) T cells. Right: Frequency of IFN- γ^+ (**E**) 867 or TNF- α^+ (F) cells among CD8+ T cells in left. (G) LCMV arm virus titers in the spleen 868 of $Brd7^{fl/fl}$ (n=3) or $Brd7^{\Delta T}$ (n=4) mice infected with LCMV arm strain were determined 869 at day 8 p.i.. Small horizontal lines indicate the mean (\pm s.e.m.). *P<0.05, **P<0.01 and 870 ***P < 0.001 (two-tailed student t test). Data are representative of three independent 871 experiments. 872

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Figure 5

FIGURE 5. BRD7 orchestrates the expression of genes critical for SLEC 875 differentiation. (A) Scatter plot analysis of differentially expressed genes of NP⁺ CD8⁺ T 876 cells from $Brd7^{\text{fl/fl}}$ and $Brd7^{\Delta T}$ mice infected with HKx31 at 10 d p.i. FDR ≤ 0.05 and Log 877 $2 \text{ FC} \ge 2$ were used as the threshold to evaluate the significance of differences in gene 878 expression. (B) Gene set enrichment analysis (GSEA) in A among transcriptional 879 differences between naïve and activated CD8⁺ T cells (left) or memory and effector cells 880 (right). NES, normalized enrichment score. FDR, false discovery rate; (C) Differentially 881 expressed genes of transcription factor (red), chemokine receptors (black), adhesion 882 molecules (blue), and killer cell lectin-like receptors (pink) between wild-type and 883 BRD7-deficient cells, represented as the log2 fold change. The y-axis displays the -log2 884 fold change of each DEG and the x-axis lists the gene name. (**D**) Genome browser tracks 885 886 displaying RNA-seq and ATAC-seq data at a selected locus comparing wild-type and BRD7-deficient cells. Tag density from different groups was calculated by normalizing to 887 888 the total mapped reads. (E) Quantitative PCR analysis of mRNA in BRD7-deficient naïve $CD8^+$ T cells (T_N) or NP⁺CD8⁺ T cells (T_{eff}) (n=4) presented relative to expression in 889 890 BRD7-wild-type cells (n=4). Small horizontal lines indicate the mean (\pm s.e.m.). *P<0.05, **P < 0.01 and ***P < 0.001 (two-tailed student t test). Data are representative of two 891 892 independent experiments.

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Figure 6

FIGURE 6. BRD7 is enriched at *Tbx21* loci and regulates T-bet expression. (A) 895 Representative alignments of ChIP-, ATAC- and RNA-seq measurements at *Tbx21* loci. 896 ATAC- and RNA-seq measurements from cells in Figure 5. ChIP-seq with antibody to 897 BRD7 was assessed with OT-I CD8⁺ T cells from OT-I mice infected with PR8-OVA at 898 day 8 p.i.. ChIP-seq with antibody to H3K9ac was assessed with OT-I CD8⁺ T cells from 899 $Brd7^{fl/fl}$ and $Brd7^{\Delta T}$ OT-I mice infected with PR8-OVA at day 8 p.i.. (**B**) ChIP analysis 900 (n=3) shows the deposition of BRD7 at the promoter regions of Tbx21 loci. (C) 901 Quantitative PCR analysis of *T-bet* mRNA in BRD7-deficient NP⁺CD8⁺ T cells (n=3) 902 presented relative to expression in BRD7-wild-type cells (n=3). (**D**) Left: flow cytometry 903 of T-bet in BRD7-wild-type (n=4) or BRD7-deficient (n=4) NP⁺CD8⁺ T cells. Right: 904 frequency of T-bet expressing NP⁺CD8⁺ T cells in left. (E) ChIP-PCR assay shows the 905 deposition of H3K9me3, H3K27me3, and H3K14ac at the promoter regions of *Tbx21* loci. 906 $Brd7^{fl/fl}$ mice (n=3) and $Brd7^{\Delta T}$ mice (n=3) were used in the experiments. Small 907 horizontal lines indicate the mean (\pm s.e.m.). *P<0.05, **P<0.01 and ***P<0.001 (two-908 tailed student *t* test). Data are representative of two independent experiments. 909 910



Figure 7

FIGURE 7. BRD7 functions as a bridge for PBAF complex to efficiently assembly in 912 effector CD8⁺ T cells. (A) Mass spectrum analysis of BRG1-associated proteins in naïve 913 OT-I CD8⁺ T (n=2) or effector OT-I CD8⁺ T cells (n=2) from OT-I mice infected with 914 PR8-OVA at day 8 p.i.. (B) Venn diagram showing the overlap of components. List 1: 915 Reported-components of BAF complex. List 2: Identified components of BAF complex 916 from naïve cells in A. List 3: Reported BAF-specific components. List 4: Identified BAF-917 specific from naïve cells in A. (C) SWI/SNF components from naïve T cells in A were 918 clustered with STRING analysis. (**D**) Venn diagram showing the overlap of components. 919 List 1: Reported-components of PBAF complex. List 2: Identified components of PBAF 920 complex from effector cells in A. List 3: Reported PBAF-specific components. List 4: 921 Identified PBAF-specific from effector cells in A. (E) SWI/SNF components from 922 923 effector cells in A were clustered with STRING analysis. (F) Co-IP of BRG1-associated proteins in naïve CD8⁺ T (n=2) or effector CD8⁺ T cells (n=2) from OT-I mice infected 924 with PR8-OVA. (G) ChIP (n=3) shows the deposition of BRG1 at the promoter regions 925 of Tbx21 loci. (H-I) The mRNA expression (n=3) of Brg1 and Tbx21 of OT-I T cells of 926 927 shRNA was analyzed at 8 day after transfer into recipient mice infected with PR8-OVA virus. (J) Co-IP of BRG1 in BRD7-wild-type and BRD7-deficient CD8⁺ T cells from 928 929 OT-I mice infected with PR8-OVA at day 8 p.i.. (K-L) ChIP (n=3) shows the deposition of BRG1 and SMARCC1 at the promoter of Tbx21 in BRD7-wild-type and BRD7-930 931 deficient CD8⁺ T cells from OT-I mice infected with PR8-OVA at day 8 p.i.. Small horizontal lines indicate the mean (\pm s.e.m.). *P<0.05, **P<0.01 and ***P<0.001 (two-932 tailed student *t* test). Data are representative of two independent experiments. 933