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#### Phosphatase PTPN22 regulates dendritic cell homeostasis and cDC2 1

#### dependent T cell responses 2

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6

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- Autoimmunity, Polymorphism, cDC2 24
- 25 Abstract
- Dendritic cells (DCs) are specialized antigen presenting cells that instruct T cell responses through 26
- 27 sensing environmental and inflammatory danger signals. Maintaining the homeostasis of the multiple
- 28 functionally distinct conventional dendritic cells (cDC) subsets that exist in vivo is crucial for
- 29 regulating immune responses, with changes in numbers sufficient to break immune tolerance. Using
- *Ptpn22<sup>-/-</sup>* mice we demonstrate that the phosphatase PTPN22 is a highly selective, negative regulator 30
- 31 of cDC2 homeostasis, preventing excessive population expansion from as early as 3 weeks of age.
- Mechanistically, PTPN22 mediates cDC2 homeostasis in a cell intrinsic manner by restricting cDC2 32
- proliferation. A single nucleotide polymorphism, PTPN22<sup>R620W</sup>, is one of the strongest genetic risk 33
- 34 factors for multiple autoantibody associated human autoimmune diseases. We demonstrate that cDC2 are also expanded in mice carrying the orthologous PTPN22<sup>619W</sup> mutation. As a consequence, cDC2 35
- dependent CD4<sup>+</sup> T cell proliferation and T follicular helper cell responses are increased. Collectively, 36

#### PTPN22 regulates dendritic cell homeostasis

- 37 our data demonstrate that PTPN22 controls cDC2 homeostasis, which in turn ensures appropriate
- 38 cDC2-dependent T cell responses under antigenic challenge. Our findings provide a link between
- 39 perturbations in DC development and susceptibility to a broad spectrum of PTPN22<sup>R620W</sup> associated
- 40 human autoimmune diseases.
- 41

### 42 1 Introduction

43 Dendritic cells (DCs) are specialized antigen presenting cells that sense danger signals and instruct T

- 44 cell responses(Merad et al., 2013). Distinct subsets of DCs exist *in vivo*, broadly divided into
- 45 conventional (cDC) and plasmacytoid (pDC) subsets. In mice, cDCs (CD11c<sup>+</sup>MHCII<sup>+</sup>) are sub-
- 46 divided into functionally distinct phenotypes defined as cDC1 (CD8<sup>+</sup>IRF8<sup>+</sup>XCR1<sup>+</sup>Clec9a<sup>+</sup>CD24<sup>+</sup>)
- 47 and cDC2 (IRF4<sup>+</sup>CD11b<sup>+</sup>SIRP $\alpha^+$ ) whilst in humans the equivalent DC subsets are defined by
- 48 expression of CD8<sup>+</sup>IRF8<sup>+</sup>XCR1<sup>+</sup>Clec9a<sup>+</sup>CD141<sup>+</sup> (cDC1) and IRF4<sup>+</sup>CD1c<sup>+</sup> (cDC2)(Tamura et al.,
- 49 2005; Merad et al., 2013; Collin and Bigley, 2018; Dress et al., 2018). Functionally, cDC1 cross-
- 50 present exogenous antigens to activate CD8<sup>+</sup> T cells and can promote IL-12 dependent Th1
- 51 responses(Maldonado-López et al., 1999; den Haan et al., 2000; Mashayekhi et al., 2011; Merad et
- al., 2013). In comparison, cDC2s are potent activators of CD4<sup>+</sup> T cells(Dudziak et al., 2007). Under
- 53 polarizing inflammatory conditions, cDC2 induce Th2 responses in the lung(Williams et al., 2013;
- 54 Tussiwand et al., 2015), drive Th17 responses through IL-23 secretion(Denning et al., 2011; Lewis et
- 55 al., 2011; Kinnebrew et al., 2012; Persson et al., 2013; Satpathy et al., 2013; Schlitzer et al., 2013;
- 56 Schreiber et al., 2013), and initiate SIRP $\alpha$  dependent induction of T follicular helper cells (T<sub>FH</sub>) and
- 57 germinal center (GC) formation(Yi et al., 2015).
- 58 Maintenance of cDC homeostasis is crucial for regulating immune responses, with deregulation
- resulting in infection and autoimmunity(Ashany et al., 1999; Birnberg et al., 2008; Ohnmacht et al.,
- 60 2009; Cai et al., 2019). This control of cDC homeostasis is mediated by a number of factors that
- 61 drive cDC differentiation, proliferation, and survival or apoptosis(Dress et al., 2018). Differentiation
- of cDCs is initiated within the bone marrow where common DC precursors (CDP) transition to an
- 63 intermediate preDC developmental stage (including pre-cDC1 and pre-cDC2s)(Naik et al., 2007;
- 64 Onai et al., 2007; Liu et al., 2009; Sathe et al., 2014; Swiecki et al., 2014; Schlitzer et al., 2015), with
- 65 terminal differentiation into cDC1 and cDC2 subsets occurring in the periphery(Maraskovsky et al.,
- 66 2000); cDC1 and cDC2s are then dependent on Flt3L for their development and
- 67 proliferation(Maraskovsky et al., 2000). Furthermore, local signals transduced through
- 68 NOTCH2(Lewis et al., 2011; Satpathy et al., 2013) or LTβR(Luther et al., 2000; Kabashima et al.,
- 69 2005) contribute to cDC homeostasis within specific tissue niches. Indeed, LTβR signalling is
- 70 particularly important for inducing cDC2 proliferation within secondary lymphoid organs
- 71 (SLOs)(Luther et al., 2000; Kabashima et al., 2005).
- 72 *PTPN22* encodes a tyrosine phosphatase that negatively regulates immune receptor activation. It
- 73 functions by dephosphorylating Src and Syk family kinases operating proximal to immune-receptors
- such as TCR, BCR, and LFA-1(Rieck et al., 2007; Dai et al., 2013; Salmond et al., 2014; Burn et al.,
- 75 2016; Sanchez-Blanco et al., 2018). PTPN22 also operates in a phosphatase independent manner,
- 76 directly binding to TRAF3 in myeloid cells and promoting type 1 interferon dependent TRAF3
- vbiguitination(Wang et al., 2013). Regarding its contribution to disease, a C1858T single nucleotide
- 78 polymorphism within *PTPN22* (encoding R620W) is one of the strongest genetic risk factors outside
- 79 the HLA for the development of multiple autoimmune diseases, including rheumatoid arthritis, type I
- 80 diabetes, and lupus(Burn et al., 2011). Investigations into the functional effects of this variant have

- 81 demonstrated that *PTPN22*<sup>R620W</sup> confers a missense mutation in the P1 domain of the PTPN22 PEST
- 82 region, resulting in reduced binding to the negative regulatory tyrosine kinase Csk, and

83 TRAF3(Fiorillo et al., 2010; Wang et al., 2013). However, the consequence of PTPN22<sup>R620W</sup> on

- 84 immune function remains unclear, appearing to depend on the cellular context and signaling pathway
- under investigation. Indeed, both gain- and loss-of-phosphatase function effects of *PTPN22*<sup>R620W</sup>

have been described(Rawlings et al., 2015).

87 Using *Ptpn22* mutant mice, we describe PTPN22 as key mediator in the restriction of cDC2

88 populations. Perturbation of cDC2 homeostasis is phenocopied in mice carrying the human

89 autoimmune associated variant, translating to accentuated cDC2-driven T cell responses upon

90 antigenic challenge. Based on these data, we propose that disruption of cDC homeostasis by

91 *PTPN22<sup>R620W</sup>* genetic polymorphism contributes to the breeching of immune tolerance during the

92 earliest phase of autoimmunity.

93

### 94 2 Methods

## 95 2.1 Mice

96  $Ptpn22^{-/-}$  mice and  $Ptpn22^{R619W}$  mutant mice were backcrossed >10 generations to the C57BL/6

97 strain, their generation is described in(Brownlie et al., 2012; Dai et al., 2013). Mice were age and

98 sex-matched within each individual experiment and were used at either 2-4 months or as otherwise

- 99 indicated. *Ptpn22*<sup>fl/+</sup> mice were bred with PC3-Cre mice and backcrossed to C57BL/6 mice for four 100 generations and were used between 8-12 weeks of age. OT-II TCR transgenic CD45.1, CD45.1, and
- 101 CD45.1/2 transgenic mice were used between 8-16 weeks of age. Where indicated, tissue was
- 102 obtained, shipped on ice and processed within 24 hours from mice bred and maintained at Edinburgh
- 103 University under U.K. Home Office approved guidelines. Mice were age and sex-matched within
- 104 each individual experiment and were used at either 2 months or 6 months of age. Unless otherwise
- 105 stated mice were maintained under specific pathogen free (SPF) conditions at King's College London
- 106 Facility according to UK Home Office approved protocols.

## 107 2.2 Tissue processing

108 Spleens and LNs were injected with RPMI containing Liberase-TL (0.1mg/ml; Roche) and DNase 1

- 109 (0.1mg/ml; Sigma), and incubated at 37°C 5% CO<sub>2</sub> for 30 minutes. EDTA (10mM) was added for the
- 110 final 5 minutes of the 30-minute incubation. Spleen single cell suspensions were RBC lysed
- 111 (Biolegend). Blood obtained by cardiac puncture was incubated at room temperature 1 hour and
- serum separated following centrifugation. Bone marrow was flushed from the femurs and tibias of  $\frac{112}{12}$
- WT and *Ptpn22<sup>-/-</sup>* mice, RBC lysed and pelleted. Cell suspensions were prepared from the small intestine after removal of Pever's patches and fat. Intestines were opened longitudinally, washed of
- intestine after removal of Peyer's patches and fat. Intestines were opened longitudinally, washed of fecal contents, cut into 5 cm pieces and incubated in HBSS medium (Life Technologies) with 2 mM
- EDTA for 20 minutes at 37°C with rotation. Tissue pieces were washed in HBSS medium, minced
- and incubated in HBSS medium + 2% FBS with collagenase VIII (100U/ml, Sigma, C2139) and
- 118 DNAse1 ( $20\mu g/ml$ ) at 37 °C for 40 min with rotation. Cell suspensions was passed through a 40 $\mu$ m
- filter and pelleted at 350g 15mins 4C. Cells were then stained for flow cytometry.
- 120 Cells from all tissues were resuspended in PBS and live cells counted by trypan blue discrimination.

## 121 2.3 Bone marrow chimeras

- 122 CD45.1 or CD45.1/2 mice were haematopoietically-lethally irradiated by exposure to 9Gy for 6
- minutes. 6 hours later, bone marrow cells  $(2.5-5\times10^6 \text{ cells in } 100\mu\text{l})$  were i.v transferred into
- 124 irradiated recipients. Chimeric mice were analyzed 8 weeks after bone marrow transfer (unless
- 125 otherwise indicated). As a control for complete replacement of recipient bone marrow, CD45.1<sup>+</sup>
- recipients received 100% CD45.2<sup>+</sup> C57BL/6 bone morrow.

# 127 2.4 T cell adoptive transfers

- 128 Total CD4<sup>+</sup> T cells from the lymph nodes (LN) and spleens of 8-16-week old WT OT-II mice were
- 129 isolated using CD4<sup>+</sup> MACS negative selection kit according to manufacturer's instructions (Miltenyi
- 130 Biotech). Purity of CD4<sup>+</sup> T cells was determined by flow cytometry (routinely 90-95%). CD4<sup>+</sup> T
- 131 cells isolated from CD45.1 WT OT-II mice were labelled with cell trace violet (CTV;  $2\mu$ M;
- 132 Invitrogen) at 2x10<sup>7</sup> cells/ml in PBS for 20 minutes at 37°C, and excess CTV quenched in complete
- medium for 20 minutes at 37°C. Recipient mice received 0.5-1x10<sup>6</sup> CTV<sup>+</sup>CD4<sup>+</sup>OT-II T cells
- resuspended in 100µL PBS i.v. The following day T cell recipient mice were immunised i.v with

- 135 33D1-ovalbumin (200µg/mouse)(Neubert et al., 2014) in the presence or absence of sheep RBC
- 136 (SRBC; Antibodies-online.com;  $20x10^7$  cells/mouse). After 3 days spleens were assessed by flow
- 137 cytometry for CTV dilution and CXCR5<sup>+</sup> PD1<sup>+</sup>  $T_{FH}$  within live, singlet, CD45.1<sup>+</sup>, CD4<sup>+</sup>, V $\alpha$ 2V $\beta$ 5<sup>+</sup>
- 138 cell gate.

## 139 2.5 DC and CD4<sup>+</sup> T cell co-culture

140 CD4<sup>+</sup> T cells isolated from OT-II mice were labeled with cell trace violet (CTV) (2 µM; Invitrogen) at

- 141 2x10<sup>7</sup> cells/ml in PBS for 20 minutes at 37°C, and excess CTV quenched in complete medium for 20
- minutes at 37°C. T cells were co-cultured with isolated DC at a 2:1 T cell to cDC ratio  $2x10^6$ : $1x10^6$
- 143 cells/ml in round bottom 96-well plates in the presence or absence of 33D1-ovalbumin (10  $\mu$ g/ml) or 144 anti-DEC205-ovalbumin (10  $\mu$ g/ml) and cells were co-cultured for 6 days at 37°C 5% CO<sub>2</sub>. At day 6
- and DEC203-ovaloumin (10  $\mu$ g/m) and cents were co-cultured for 6 days at 3/°C 5% CO<sub>2</sub>. At day 6 cells were stained with fixable viability dye eFluor-506 (eBioscience), anti-CD3 $\epsilon$  and anti-CD4. CTV
- dilution gated on live, singlet,  $CD3^+CD4^+$  T cells was assessed by flow cytometry.

## 147 2.6 BrdU labeling

148 3 days prior to analysis mice were i.p injected daily with BrDU (10mg/kg) and maintained ad libitum
149 on drinking water containing BrdU (0.5mg/ml).

## 150 2.7 Flt3 Ligand Bone Marrow DC

- 151 Bone marrow was RBC lysed and cells seeded at  $1 \times 10^6$  cells/ml in 6-well tissue culture plates in
- 152 RPMI-1640 supplemented with glutamax, 10% heat-inactivated FBS, β-mercaptoethanol (50 $\mu$ M),
- penicillin/streptomycin (100μg/ml), and HEPES 1mM (Sigma) and Flt3L (200ng/ml; Biolegend).
- Flt3L-BMDCs were cultured for 8 days at 37°C and 5% CO<sub>2</sub>. At day 8 non-adherent and adherent Flt3L-BMDC were harvested with trypsin-EDTA (Sigma) incubated for 2 minutes at room
- temperature. Cells were washed and live cells counted by trypan blue discrimination and seeded at
- 157 1x10<sup>6</sup> cells/ml on 96-well flat bottom plates for 48 hours in the presence or absence of anti-LT $\beta$ R
- 158 (2µg/ml; 3C8; AdipoGen).

# 159 2.8 Flow cytometry

- 160 Fluorochrome or biotin-conjugated antibodies were used to stain single cell suspensions for flow
- 161 cytometry. Fc receptors were blocked with anti-mouse CD16/CD32 (93; Biolegend) and dead cell
- 162 exclusion was performed using Fixable Viability Dye (eBioscience). FACS buffer was made of PBS
- 163 with 1% bovine serum albumin (Sigma) and 2mM EDTA (Sigma). For BrDU staining following
- 164 surface staining, splenocytes were fixed and permeabilised using APC-BrDU Flow Kit (BD
- 165 Pharmingen). Cells were acquired using BD Fortessa or FACSCanto II flow cytometers. Performed
- 166 in the Biomedical Research Centre Flow Core Facility (Guy's and St Thomas' NHS Foundation
- 167 Trust and King's College London). Flow cytometry gates were determined by fluorescence minus
- 168 one controls. Flow cytometry analysis was performed using FlowJo software (TreeStar; 10.5.3).

# 169 **2.9** Flow cytometry monoclonal antibodies specific to mouse

- 170 CD3ε (145-2C11), CD4 (GK1.5), CD8a (53-6.7), CD11b (M1/70), CD11c (N418), CD24 (M1/69),
- 171 CD45R/B220 (RA3-6B2), CD45.1 (A20), CD45.2 (104), CD86 (GL-1), CD103 (2E7), CD172a
- 172 (SIRP alpha; P84), CD279 (PD1; 29F.1A12), CXCR5 (L138D7), I-A<sup>b</sup> (AF6-120.1), TCR Vα2
- 173 (B20.1), TCRVβ5 (MR9-4), CD16/32 (93), Ki67 (16A8), Ly-6G/Ly-6C (RB6-8C5), Ly-6G (1A8),
- 174 (Ly6C (AL-21), Ter-119 (TER-119), NK-1.1 (PK136), CD19 (6D5), DCIR2 (33D1), cKit (2B8),
- 175 Flt3 (A2-F10), LT $\beta$ R (5G11) that were bought from Biolegend, eBioscience, or BD.

#### 176 2.10 Immunofluoresence staining

- 177 Spleens were harvested into RPMI and 10% FBS and dried prior to being frozen at -80°C in OCT.
- 178 10µM sections were generated and mounted onto slides using a Leica cryostat. Sections were fixed
- 179 with 4% PFA for 15 minutes and washed with PBS. Sections were blocked for 30 minutes in
- 180 blocking buffer (PBS + 2% FBS, rat serum (1 in 200) and anti-CD16/CD32 (1 in 200) for 30 minutes
- 181 at room temperature. Sections were stained for 1 hour at room temperature with B220-FITC and
- 182 33D1-Alexa-647 in PBS 2% FBS. Slides were washed 3 times with PBS prior to mounting in
- 183 fluorescence mounting media (DAKO). Images were collected using an Olympus IX83 inverted
- 184 microscope and image processing was performed using Fiji.

### 185 2.11 Real-time PCR

- 186 Total RNA was extracted from FACS isolated cDC2 using TRIzol reagent. Equal amounts of mRNA
- 187 (determined by nanodrop; ThermoScientific) were reverse transcribed to produce cDNA using first
- 188 strand cDNA synthesis using random hexamers. Gene expression was measured by SYBR Green
- 189 quantitative real-time PCR using primers: *Bcl2* forward, TGAGTACCTGAACCGGCATCT, *Bcl2*
- 190 reverse, GCATCCCAGCCTCCGTTAT; Bim forward, GGCCCCTACCTCCCTACA, Bim reverse,
- 191 GGGGTTTGTGTTGATTTGTCA; *Trim2* forward, TTTCCATAATCACTCTGTCAAGGT, *Trim2*
- 192 reverse, CCATTGGAGCCAAACTTCA; *Gapdh* forward, ACCACAGTCCATGCCATCAC *Gapdh*
- 193 reverse, TCCACCACCCTGTTGCTGTA. Reactions were run using ABI Prism 7700 Sequence
- 194 Detection System (Applied Biosystems). Ct values were determined with SDS software (Applied
- Biosystems) and gene expression levels were determined according to the dCt method (relative
- abundance =  $2^{(-det)}$  and normalized to *GAPDH* housekeeper).

### 197 2.12 Serum Flt3L

- 198 Blood obtained by cardiac puncture was incubated at room temperature 1 hour and serum separated
- 199 following centrifugation. Serum Flt3 Ligand was determined by Mouse/Rat Quantikine ELISA
- 200 (R&D Systems) according to manufacturer's protocol and detected using Victor 1420 multilabel
- 201 counter (Perkin Elmer).

## 202 2.13 Statistical analysis

- 203 GraphPad Prism software was used for statistical analysis by unpaired or paired T-test. P values less
- than 0.05 were considered significant; NS=not significant, \*p<0.05, \*\* p<0.01, \*\*\*p<0.001,
- 205 \*\*\*\*p<0.0001.
- 206
- 207 **3 Results**

## 208 3.1 PTPN22 is a negative regulator of cDC2 homeostasis

- 209 *Ptpn22<sup>-/-</sup>* mice have a well-characterized age dependent increase in effector/memory T lymphocytes
- and spontaneous GC production. However, the effect of PTPN22 on myeloid lineages is not
- 211 understood. To address this, we examined if myeloid cell lineages were altered in mice lacking
- 212 PTPN22. We detected a similar frequency of monocytes, macrophages, and neutrophils
- 213 (Supplementary Figure 1A-C) within the spleens of WT and *Ptpn22<sup>-/-</sup>* mice. In contrast, analysis of
- the splenic cDC compartment revealed that both the proportion and number of cDCs was increased in
- 215 Ptpn22<sup>-/-</sup> mice compared to WT (Figure 1A-B). Further phenotyping of cDC1 and cDC2 subsets

- revealed that the proportion of cDC2 was increased in *Ptpn22<sup>-/-</sup>* mice, whereas the proportion of
- cDC1 was decreased (Figure 1A and C). By comparing the number of splenic cDC1s and cDC2s
- within WT and  $Ptpn22^{-/-}$  mice we found that changes in cDC subset proportions were due to selective
- expansion of the cDC2 subset and not loss of cDC1 cells (Figure 1D). Indeed, expansion of splenic
- 220 cDC2s occurred within the *bona fide* DCIR2(33D1)<sup>+</sup>ESAM<sup>+</sup>CD4<sup>+</sup>CCR2<sup>-</sup> cDC2 subset, whereas
- 221 numbers of the 'monocyte-like' DCIR2(33D1)<sup>-</sup>ESAM<sup>-</sup>CD4<sup>-</sup>CCR2<sup>+/-</sup> DCs were similar (Figure 1E,F
- and Supplementary Figure 1D-F). Analyzing the kinetics of cDC2 expansion demonstrated that
- 223 perturbation of cDC2 homeostasis could be detected as early as 3 weeks (Figure 1G,H), increasing 224 further as the mice age (Supplementary Figure 1G). We confirmed these findings in WT and *Ptpn22*
- further as the mice age (Supplementary Figure 1G). We confirmed these findings in WT and *Ptpn22*-/- mice bred and maintained in an independent animal facility suggesting that cDC2 expansion was
- 225 Indee oreg and maintained in an independent animal facility suggesting that CDC2 expansion was
- unlikely to be due to facility-associated environmental factors (Supplementary Figure 1H and I).
- 227 To examine if cDC homeostasis was altered in other lymphoid tissues, we examined cDC
- populations within skin draining lymph nodes (sdLN), and found that *Ptpn22<sup>-/-</sup>* mice displayed
- expanded resident, but not migratory cDC2. Once again similar numbers of cDC1 were observed
- within both migratory and resident cDC populations (Figure 1I-K). A similar phenotype was
- documented within the mesenteric lymph nodes, wherein resident  $CD11b^+ CD103^- cDC2$  were
- expanded, while  $CD103^+$  resident cDCs and all migratory (IA<sup>b high</sup>) cDC subsets assessed were uppletered (Supplementary Figure 11L). Furthermore, in line with Dive 22 act as substants
- unaltered (Supplementary Figure 1J-L). Furthermore, in line with Ptpn22 not regulating migratory
   cDC2, cDC2 positioning within the spleen bridging channels appeared similar between WT and
- cDC2, cDC2 positioning within the spleen bridging channels appeared similar between WT and *Ptpn22<sup>-/-</sup>* mice (Supplementary Figure 1M). Together, we conclude that PTPN22 regulates resident
- 255 *Fipn22*<sup>+</sup> mice (Supplementary Figure IM). Together, we conclude that PTPN22 regulates resider 266 DC2 homeostasis within secondary lymphoid argans (SLOs)
- 236 DC2 homeostasis within secondary lymphoid organs (SLOs).

## 237 **3.2** *PTPN22* regulates cDC2 homeostasis via a DC intrinsic mechanism

238 Given its broad expression in multiple hematopoietic lineages, PTPN22 has the potential to control 239 cDC2 homeostasis through DC intrinsic or DC extrinsic mechanisms. To investigate this, we 240 generated Ptpn22<sup>-/-</sup> CD45.2: WT CD45.1 mixed bone marrow chimeras and found that CD45.2 241 *Ptpn22<sup>-/-</sup>* bone marrow out-competed WT CD45.1 in the generation of cDC2 (Figure 2A-D), whereas 242 no change was observed in the ratio of Lin<sup>+</sup> cells (Supplementary Figure 2A). Consistent with our 243 previous observations, generation of cDC1 was unaffected by genotype, further supporting a role for 244 PTPN22 as a selective, DC intrinsic regulator of cDC2 homeostasis. PTPN22 regulates T cell 245 homeostasis(Hasegawa et al., 2004), raising the possibility of an indirect cDC2 phenotype driven by

- enhanced T cell activation in PTPN22:WT chimeras. Therefore, we examined if lineage specific
- deletion of *Ptpn22* within the T cell compartment would have an impact on cDC2 populations. We
- detected no differences in cDC2 expansion in either mice with T cell restricted  $Ptpn22^{-/-}$
- 249 (Supplementary Figure 2B) or between chimeras harboring PTPN22 sufficient or deficient T cells
- 250 (Figure 2E), indicating that deficiency of *Ptpn22* exclusively in T cells was not sufficient to perturb
- 251 cDC homeostasis.
- 252 Finally, we determined if the *Ptpn22*<sup>-/-</sup> environment (via an indirect effect on stroma), contributed to
- 253 cDC2 expansion. WT CD45.1 bone marrow was transferred into non-irradiated WT and *Ptpn22*-/-
- 254 CD45.2 mice. After 6 days we observed no difference in CD45.1 cDC2 numbers developing within
- either WT or  $Ptpn22^{-/-}$  mice (Figure 2F,G). This result is consistent with previous reports establishing
- that PTPN22 expression is restricted to hematopoietic cell lineages(Cohen et al., 1999; He et al., 2014) Together, these dots suggest that PTPN22 regulates a DC2 hemastrasis via a pC intrinsic
- 257 2014). Together, these data suggest that PTPN22 regulates cDC2 homeostasis via a cDC intrinsic258 mechanism.

# 259 3.3 PTPN22 regulates cDC2 homeostasis after pre-cDC development

- 260 Homeostasis of cDC in SLOs is controlled by multiple factors including differentiation of bone
- 261 marrow precursors in response to Flt3L, duration of cDC survival, proliferation of incoming
- precursor cDCs, and the turnover of a small subset of cDCs within SLOs(Merad et al., 2013). We
   therefore aimed to examine how PTPN22 might regulate cDC2 development. Firstly, we observed no
- 264 PTPN22 dependent difference in bone marrow or splenic common DC precursors (CDP) or preDC
- cells, indicating that PTPN22 operated post pre-cDC development (Supplementary Figure 3A-C).
- 266 Secondly, as cDC1 and cDC2 are dependent on Flt3L for their differentiation we tested if PTPN22
- 267 controls Flt3L dependent cDC2. We observed similar Flt3R expression on *ex vivo* WT and *Ptpn22*<sup>-/-</sup>
- 268 cDC1 and cDC2 (Supplementary Figures 3D-E), as well as similar concentrations of serum Flt3L in
- 269 *vivo* (Supplementary Figure 3F). To compare Flt3L dependent cDC2 development, we cultured WT
- and *Ptpn22<sup>-/-</sup>* bone marrow *in vitro* with Flt3L. However, no significant changes in cDC2
   development were observed (Figure 3A). We then assessed if PTPN22 altered cDC2 survival by
- development were observed (Figure 3A). We then assessed if PTPN22 altered cDC2 survival by
   comparing the expression of survival genes Bcl2, Bim, and Trim in FACS sorted cDC2. Once again
- we observed no differences between WT and  $Ptpn22^{-/-}$  cDC2 (Supplementary Figure 3G).
- Furthermore, no differences were observed between splenic WT and  $Ptpn22^{-/-}$  cDC2 acquiring an
- apoptotic phenotype (Annexin  $V^+$ ) as a consequence of a 24-hour culture *in vitro* (Supplementary
- Figure 3H). Based on these data, we reasoned that differences in cell survival were unlikely to be a
- 277 major mechanism mediating cDC2 expansion in  $Ptpn22^{-/-}$  mice.

## 278 3.4 PTPN22 negatively regulates cDC2 proliferation

Having excluded a role for PTPN22 in regulating DC precursor development, Flt3L dependent
 differentiation or cDC2 survival, we next addressed if PTPN22 might control cDC2 proliferation. To

- test this hypothesis, we examined the *in vivo* effects of 5-flurouracil (5-FU), a pyrimidine analogue
- that inhibits cell proliferation(Longley et al., 2003). Previous reports have demonstrated that 3 days post-5-FU administration DC populations are significantly reduced within the spleen, indicative of
- rapid turnover of DCs *in vivo*(Zhan et al., 2016). The difference in cDC2 expansion between WT and
- 285 *Ptpn22-/-* mice was abrogated 3 days after treatment with 5-FU, demonstrating that PTPN22 mediated
- expansion of cDC2 is indeed dependent on proliferation (Figure 3B). Consistent with our data using
- 5-FU, administration of thymidine analogue BrDU, which incorporates into proliferating cells,
   demonstrated that the *Ptpn22<sup>-/-</sup>* cDC2 population was significantly expanded within the BrDU<sup>+</sup>
- demonstrated that the  $Ptpn22^{-/-}$  cDC2 population was significantly expanded within the BrDU<sup>+</sup> (proliferating), but not the BrDU<sup>-</sup> (non-proliferating) population (Figure 3C,D). Furthermore, Ki67
- and DAPI staining confirmed enhanced proportions of cDC2 undergoing cell cycling (Figure 3E,F
- and Supplementary Figure 3I). Likewise, cell cycle analysis of competitive bone marrow chimeras 3
- weeks post transfer also demonstrated a significant increase in the proportion of cycling *Ptpn22*-/-
- 293 cDC2 when compared to WT controls (Supplementary Figure 3J). Taken together, these data support
- the notion that PTPN22 controls cDC2 homeostasis by restricting cDC2 proliferation.

## 295 3.5 *Ptpn22<sup>619W</sup> confers expansion of ESAM*<sup>+</sup>*CD4*<sup>+</sup> *cDC2s*

The human *PTPN22<sup>R620W</sup>* polymorphism is one of the highest-ranking genetic risk factors for the development of multiple autoimmune diseases outside MHC loci (Burn et al., 2011; Stanford and Bottini, 2014). Given that PTPN22 regulates the expansion of cDC2s, we set out to examine whether

- the autoimmune associated variant was capable of mediating similar effects. To address this, we
- 300 enumerated splenic cDC subsets in mice expressing the R620W orthologue, *Ptpn22<sup>619W</sup>*. In
- 301 comparison to  $Ptpn22^{619R}$ , mice carrying  $Ptpn22^{619W}$  also displayed expansion of splenic cDC2s,
- 302 which, like  $Ptpn22^{-/-}$  mice, occurred specifically within the ESAM<sup>+</sup>CD4<sup>+</sup> DC2 subset (Figure 4A,B).
- Furthermore, the magnitude of cDC2 expansion was similar to  $Ptpn22^{-/-}$  mice when compared to WT
- 304 (1.5-fold vs 2-fold respectively). This demonstrated that the autoimmune associated PTPN22 variant

is also capable of regulating cDC2 homeostasis, operating as a loss-of-function mutant in this

306 context.

## 307 3.6 *Ptpn22<sup>619W</sup>* enhances T cell proliferation and generation of $T_{FH}$

*PTPN22*<sup>*R620W*</sup> is a risk allele associated with multiple autoantibody associated autoimmune diseases. 308 309 Splenic cDC2 are essential initiators of T<sub>FH</sub> differentiation, leading to GC formation, and high-310 affinity antibody production (Yi et al., 2015; Briseño et al., 2018). Interestingly, with age Ptpn22<sup>-/-</sup> and *Ptpn22<sup>619W</sup>* mice develop spontaneous GC, and enhanced serum IgG levels (Hasegawa et al., 311 2004; Dai et al., 2013; Maine et al., 2014). Accordingly, we addressed whether expansion of splenic 312 cDC2 in *Ptpn22<sup>619W</sup>* mice was sufficient to alter cDC2 dependent T cell activation and T<sub>FH</sub> induction 313 314 in vivo. To evaluate cDC2 dependent antigen specific responses in vivo we administered 33D1-OVA 315 conjugates to selectively target the cDC2 subset (Supplementary Figure 4)(Dudziak et al., 2007). WT 316 and *Ptpn22<sup>619W</sup>* mice received CD45.1 OT-II CD4<sup>+</sup> T cells and were immunised with 33D1-OVA in the presence of SRBCs to promote cDC2 activation (Figure 4C), and potentiate OT-II proliferation 317 318 and T<sub>FH</sub> responses (Figures 4D,E)(Yi et al., 2015; Briseño et al., 2018). When compared to WT 319 recipients, Ptpn22<sup>619W</sup> cDC2 expansion was sufficient to enhance OT-II proliferation in vivo (Figure 4F). Furthermore, we observed that the proportion and number of splenic OT-II T<sub>FH</sub> that develop 320 within *Ptpn22<sup>619W</sup>* recipients was significantly enhanced compared to WT following 33D1-321 322 OVA/SRBC immunisation (Figure 4G,H). Our previous investigations demonstrate that Ptpn22 is 323 dispensable for antigen uptake and presentation (Clarke et al., 2017). Furthermore, the *in vitro* data 324 presented here indicate that differences in OT-II proliferation are due to altered cDC2 homeostasis, 325 (specifically the number of cDC2 cells), rather than a cell intrinsic difference in Ptpn22 variant 326 cDC2. When total, unmanipulated splenic cDC are FACS isolated (preserving the cDC2 expansion 327 observed in vivo) and co-cultured with OT-II in the presence of 33D1-OVA, Ptpn22<sup>-/-</sup> are able to 328 potentiate OT-II T cell proliferation (Supplementary Figure 4B). Conversely, when cDC2 are FACS 329 isolated (normalizing cDC2 numbers) and cultured in the same manner, WT and *Ptpn22<sup>-/-</sup>* cDC2 are 330 capable of inducing OT-II proliferation to the same extent. Furthermore, no difference in OT-II 331 proliferation is observed when co-cultured with FACS isolated cDC1 in the presence of DEC205-332 OVA (Supplementary Figure 4D). Together, these data reveal that  $Pton22^{619W}$  mediated cDC2 expansion is sufficient to deregulate T cell activation in response to non-self-antigens, and is likely to 333 334 contribute to the promotion of T<sub>FH</sub> responses *in vivo* alongside previously reported T cell and B cell intrinsic effects (Dai et al., 2013; Maine et al., 2014). 335

336

### 337 4 Discussion

Here, we provide evidence that the autoimmune disease-associated phosphatase PTPN22 is a

regulator of cDC2 homeostasis. Loss-of-function mutants of PTPN22 result in cDC2 expansion

340 through mechanisms that are DC intrinsic, enhancing cDC2 proliferation. Thus, PTPN22 appears to

be a selective regulator of cDC2 homeostasis. Furthermore, cDC2 expansion conferred by the  $226^{10W}$ 

342 autoimmune associated  $Ptpn22^{619W}$  variant resulted in aberrant cDC2 dependent  $T_{FH}$  induction *in* 343 *vivo*. Our data therefore uncover a novel mechanism by which  $T_{FH}$  expansion, first reported in

344 *Ptpn22* deficient mice, may be underpinned by a specific cDC2 phenotype.

345 The precise mechanisms by which Ptpn22 mediates selective expansion of cDC2 remains to be

determined. However, we now know that Ptpn22 deficiency mediates the expansion of ESAM<sup>+</sup>CD4<sup>+</sup>

 $^{347}$  cDC2 (Figure 1E), a cDC2 subset that is known to be dependent on LT $\beta$ R for their proliferation

348 (Satpathy et al., 2013). Furthermore, splenic *Ptpn22<sup>-/-</sup>* cDC2 were more proliferative *ex vivo* (Figure

3B-F). Consistent with our data,  $Lt\beta r^{-/2}$  splenic cDC2s are less proliferative, resulting in decreased 349 350 cDC2 numbers(Kabashima et al., 2005). Indeed, our preliminary experiments indicate that Ptpn22 may control LTBR mediated cDC2 proliferation. LTBR agonist treatment of *Ptpn22<sup>-/-</sup>* Flt3L-BMDC 351 increased cDC2 numbers compared to WT (Supplementary Figure 5A,B), whilst no difference in cell 352 surface LTBR expression was observed on ex vivo or in vitro generated Flt3L BMDC (Supplementary 353 Figure 5D-F). In addition, phenotypes described in *Relb<sup>-/-</sup>* mice, further support the hypothesis that 354 355 PTPN22 may regulate LTBR dependent cDC2 proliferation. LTBR activates canonical pathway and 356 non-canonical NFκB signaling, resulting in RelB translocation(Bista et al., 2010). Within *Relb<sup>-/-</sup>* 357 mice, there is a severe reduction in splenic and LN resident (but not migratory) cDC2, from as early 358 as 3 weeks of age, whereas cDC1 numbers are unaffected(Briseño et al., 2017), and Flt3L dependent 359 DC development is unaffected by *Relb<sup>-/-</sup>*. In addition, despite lamina propria DC being dependent on Notch2 and LTBR signaling, *Relb<sup>-/-</sup>* does not affect CD11b<sup>+</sup> DC subsets at this location (Satpathy et 360 361 al., 2013; Briseño et al., 2017). In keeping with these reports, we observed no difference in lamina propria CD11b<sup>+</sup> DC subsets between WT and *Ptpn22<sup>-/-</sup>* mice (Supplementary Figure 6). Therefore, in 362 both Relb<sup>-/-</sup> and Ptpn22<sup>-/-</sup> mice the same cDC2 populations are disrupted, being decreased in Relb<sup>-/-</sup> 363 364 and increased in *Ptpn22<sup>-/-</sup>*, with both occurring within a 3-week time frame. In contrast, due to differences in the specific cDC2 phenotypes reported, our data do not support a role for PTPN22 in 365 366 regulating IRF4(Suzuki et al., 2004), KLF4 (Tussiwand et al., 2015), or NOTCH2(Lewis et al., 2011; Satpathy et al., 2013) dependent cDC2 development. As such, our data are consistent with a model 367 368 whereby PTPN22 may function to negatively regulate LTBR signaling, limiting RelB translocation to 369 control cDC2 homeostasis.

370 One question that our data raise, is why and how Ptpn22 selectively regulates cDC2 homeostasis?

371 One explanation might be the differential expression of Ptpn22 in DC subsets, which is substantially

higher in cDC2 than cDC1 or pDC (ImmGen); implying that the effects of Ptpn22 deficiency are

373 likely to be greatest in cells with the highest expression. An alternate explanation is that Ptpn22

- regulates signaling pathways specifically required for cDC2 but not cDC1 development. In line with
- this, our data demonstrate no defect in pathways required by both cDC1 and cDC2, since both
   precursor cDC development (Supplementary Figure 3A-C) and Flt3L responsiveness (Supplementary
- Figure 3A) remain intact in  $Ptpn22^{-/-}$  mice. Conversely, proliferation was enhanced specifically in
- Provide the structure of th
- 379 proliferation in response to signals required for cDC2 turnover. One factor known to mediate the
- proliferation of a small subset of cDC2 is  $LT\beta R$ , and Ptpn22 may be involved in regulating this
- 381 proliferative signal in vivo. Alternatively, Ptpn22 may regulate the signaling of an as yet to be
- identified pathway that is also required for cDC2 proliferation. Further detailed investigation is

required to uncover the mechanistic basis for the pathway(s) regulated by Ptpn22 in this context.

*PTPN22<sup>R620W</sup>* is one of the strongest autoimmune disease associated genetic risk factors. We 384 demonstrate that cDC2 homeostasis is disrupted in mice that express the orthologue of the human 385 386 autoimmune associated variant (Figure 4A,B). To our knowledge, this is the first description of cDC homeostasis being regulated by an autoimmune associated genetic risk allele. Our data therefore 387 388 provide a link between genetic and environmental risk and the breakdown of immune tolerance that 389 leads to autoimmunity. Changes to cDC homeostasis have been described within autoimmune diseases for which *PTPN22<sup>R620W</sup>* is a risk factor. In type 1 diabetes the effector phase of murine type I 390 391 diabetes is characterised by cDC2 expansion(Price and Tarbell, 2015). Furthermore, in humans with 392 rheumatoid arthritis (RA), cDC2 are decreased within the blood, but expanded within the lymph 393 nodes(Ramwadhdoebe et al., 2014), and the RA synovium is enriched with RelB<sup>+</sup> DCs(Pettit et al., 394 2000). Together, suggesting an association between the enhanced presence of cDC2s within SLOs 395 and tissue and the risk of developing autoimmune disease. Therefore, a failure to maintain cDC

- 396 homeostasis, as conferred by the PTPN22 risk allele, may be a factor altering the downstream
- 397 immune responses that ultimately lead to autoimmunity

Although we accept that the difference between WT and *Ptpn22* variant cDC2 populations may 398 399 appear modest, over the lifetime of a human, these changes could have significant functional impact 400 over the decades that precede autoimmune disease onset. In the context of non-self cDC2-targeted antigen, *Ptpn22<sup>619W</sup>* dependent expansion of cDC2 was sufficient to enhance T cell proliferation and 401 T<sub>FH</sub> expansion following GC promoting SRBC stimulation (Figure 4F,G). Activation of ESAM<sup>+</sup> 402 403 cDC2 is one of the earliest events in splenic GC formation leading to high-affinity antibody 404 production(Yi et al., 2015; Briseño et al., 2018). With ageing, PTPN22 mutant mice develop many 405 hallmarks of autoimmunity including increased effector T cells, activated B cells and higher immunoglobulin and autoantibody titres(Hasegawa et al., 2004; Dai et al., 2013). Furthermore, 406 humans carrying the PTPN22<sup>620W</sup> variant have an increased risk of developing autoimmune diseases 407 408 that are almost exclusively associated with autoantibody production(Burn et al., 2011; Stanford and 409 Bottini, 2014). PTPN22 has been reported to regulate T<sub>FH</sub>(Maine et al., 2014) cells, GC formation, 410 and antibody production(Dai et al., 2013) in part via T cell and B cell intrinsic effects. Although our data do not address self-reactivity, they do indicate that changes conferred by *Ptpn22<sup>R619W</sup>* altering 411 412 cDC2 homeostasis, could, alongside T and B intrinsic effects, also contribute to perturb the GC 413 reaction over time. Age is an important risk factor for autoimmune disease onset due to declining 414 immune competence and impaired immune tolerance check points(Goronzy and Weyand, 2012). In 415 keeping with this concept, cDC2 expansion was potentiated in aged *Ptpn22<sup>-/-</sup>* mice (Supplementary 416 Figure 1E) and as such changes to the balance of activating:inhibitory cells in vivo could alter cDC2 417 dependent responses that trigger the breaking of immune tolerance. Future work will be required to

- 418 confirm the link between early expansion of cDC2 and autoimmunity.
- 419 In summary, our findings uncover PTPN22 as a selective regulatory checkpoint required to maintain
- 420 cDC2 homeostasis, and suggest that early perturbation of DC homeostasis may be a trigger for the 421 onset of autoimmunity.

### 422 **5 Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial
relationships that could be construed as a potential conflict of interest.

### 425 **6** Author Contributions

- 426 HAP designed research, performed experiments, analyzed data and wrote the manuscript. FC and 427 CC, performed experiments and analyzed data. JAB analyzed data and contributed to writing the
- 427 CC, performed experiments and analyzed data. JAB analyzed data and contributed to writing the 428 manuscript. XD, DJR, and RZ developed and contributed vital mouse models. DD developed and
- 428 manuscript. AD, DJR, and RZ developed and contributed vital mouse models. DD developed and 429 provided vital 33D1 and DEC-205-OVA reagents. PG and APC conceived and funded the project,
- 430 contributed to data analysis and wrote the manuscript. All authors reviewed the manuscript.

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#### 632 10 Data Availability Statement

- 633 All datasets generated for this study are included in the manuscript and the supplementary files.
- 634

#### 635 11 Figure Legends

Figure 1. PTPN22 negatively regulates ESAM<sup>HI</sup> cDC2 homeostasis. (A-D) Spleens of 2-4-month 636 age matched wild type (WT) and *Ptpn22<sup>-/-</sup>* mice were evaluated for cDC subsets by flow cytometry, 637 638 gated on: live, singlet, lin<sup>-</sup> (CD3, CD19, B220, Ly6C/G, NK1.1, Ter119), CD11c+MHCcII I-Ab<sup>+</sup> and then CD8<sup>+</sup> vs SIRP $\alpha^+$  (A) Representative flow cytometry plot analysis of cDC subsets. (B) The 639 proportion of CD11c<sup>+</sup>I-A<sup>b+</sup> cDC, (C) the proportion of CD8<sup>+</sup> cDC1 and SIRP $\alpha^+$  cDC2s, (D) the 640 641 number of cDC, cDC1 and cDC2 per spleen. N=12-15 mice per genotype from >3 independent 642 experiments. (E,F) Spleens of 2-4 month age matched wild type (WT) and Ptpn22<sup>-/-</sup> mice were 643 evaluated for cDC subsets by flow cytometry, gated on: live, singlet, lin<sup>-</sup> (CD3, CD19, B220, 644 Ly6C/G, NK1.1, Ter119), CD11c+MHCcII I-Ab<sup>+</sup>, CD8<sup>-</sup>CD11b<sup>+</sup>, ESAM vs CD4. Representative flow cytometry plot analysis of cDC subsets (E) and the frequency of CD11b +DC2 ESAM+/- CD4+/-645 646 subsets per spleen (F). N=6 mice/genotype from 2 independent experiments. (G) Splenic cDC1 and 647 cDC2 within pre-wean (3 weeks) and (H) post wean (4 weeks) WT and Ptpn22<sup>-/-</sup> mice. N=4 mice/genotype. (I-K) Lymph node resident and migratory cDC subsets within 2-4-month age 648 649 matched WT and *Ptpn22<sup>-/-</sup>* mice. Determined by flow cytometry gating on: singlet, live, lin<sup>-</sup> CD11c<sup>+</sup> MHCcII I-A<sup>bInt</sup> (resident DC) or CD11c<sup>+</sup> MHCcII I-A<sup>bHigh</sup> (migratory DC), and then CD8α (cDC1) 650 vs CD11b<sup>+</sup> (cDC2). (I) Representative flow cytometry plots. (J) Frequency of resident and migratory 651 652 cDC and (K) frequency of resident and migratory cDC1 and cDC2; N=10 mice/genotype from 3 653 independent experiments. Each point represents an individual mouse; bars represent mean, NS = not significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, determined by unpaired T-test. 654

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Figure 2. PTPN22 regulates cDC2 homeostasis in a DC intrinsic manner. (A-D) Lethally 656 irradiated CD45.1/2 recipient mice received a 1:1 ratio of WT CD45.1 : WT or Ptpn22<sup>-/-</sup> CD45.2 657 658 bone marrow (i.v). After 8 weeks spleens of recipient mice were evaluated for cDC subsets and the ratio of CD45.1:CD45.2 within each subset was determined by flow cytometry gating on: live, 659 singlet, lin<sup>-</sup> CD11c<sup>+</sup>, MHCcII I-A<sup>b+</sup> and then CD8<sup>+</sup> vs SIRP $\alpha^+$  CD45.1<sup>+</sup> vsCD45.2<sup>+</sup>. (A) Experiment 660 schematic, (B) representative flow cytometry staining gated on either cDC2 (left) or cDC1 (right) 661 662 subsets. (C,D) The ratio of CD45.1:CD45.2 within cDC1 and cDC2 subsets calculated relative to the 663 input ratio. N=5-6 mice/genotype, one experiment of two. (E) Lethally irradiated wild type (WT) 664 CD45.1/2 mice received a 1:1 ratio of WT CD45.1: dLckCre<sup>-</sup> or dLckCre<sup>+</sup> (*Ptpn22<sup>-/-</sup>*) CD45.2 bone 665 marrow (i.v). After 8 weeks spleens of recipient CD45.1/2 mice were evaluated for cDC subsets and the ratio of CD45.1:CD45.2 within each subset was determined by flow cytometry relative to the 666 input ratio, N=3-4 mice/genotype. (F) WT CD45.1 bone marrow was transferred i.v into WT or 667 668 *Ptpn22<sup>-/-</sup>* CD45.2 recipient mice and after 6 days the spleens of recipient mice were evaluated for the 669 number of CD45.1 cDC1 and cDC2 by flow cytometry. (F) Schematic of experiment (G) N=9

#### PTPN22 regulates dendritic cell homeostasis

- 670 mice/genotype, two independent experiments. Each point represents an individual mouse; bars
- 671 represent mean and standard deviation, NS = not significant, \*\*\*\*p<0.0001 determined by unpaired 672 T-test.
- Figure 3. PTPN22 regulates DC2 proliferation. (A) Bone marrow from wild type (WT) or Ptpn22<sup>-</sup> 673 674 <sup>-</sup> mice cultured in the presence of Flt3L for 8 days (Flt3L-BMDC). At day 8 the proportion and 675 number of CD24<sup>+</sup> cDC1 and SIRP $\alpha^+$  cDC2 were determined by flow cytometry. N=6 mice per 676 genotype from 6 independent experiments. (B) The frequency of live splenic cDC1 and cDC2 from 677 WT and *Ptpn22<sup>-/-</sup>* measured 3 days after i.v immunization with 5-flurouracil or DMSO control. N=3-4 mice per group. (C,D) The percentage of splenic cDC1 and cDC2 within BrDU<sup>-</sup> and BrDU<sup>+</sup> 678 679 populations within BrDU treated WT and *Ptpn22<sup>-/-</sup>* mice. (C) Representative flow plots of analysis, quantified in (D). N=3 mice per genotype. (E,F) Ki67 and DAPI expression within splenic cDC1 and 680 cDC2 subsets from WT and *Ptpn22<sup>-/-</sup>* spleens. (E) Representative flow plot analysis and quantified in 681 682 (F). N=8 mice per genotype. (A,B,D,F) Each point represents an individual mouse; bars represent 683 mean and standard deviation. NS = not significant, (A-F) p<0.05, determined by unpaired T-test. 684 NS = not significant, \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001. Figure 4. *Ptpn22<sup>619W</sup>* conferred cDC2 expansion enhances T cell proliferation and T<sub>FH</sub>. (A,B) 685 Spleens of 2-4 month age matched WT and *Ptpn22<sup>619W</sup>* mice were evaluated for cDC subsets by flow 686 cytometry (A) number of cDC1 and cDC2 per spleen (B) number of ESAM<sup>-</sup> vs ESAM<sup>+</sup> cDC2 per 687
- 688 spleen. (A,B) N=10 mice per genotype. (C) Mice were immunized i.v. with PBS or SRBC and after 4
- hours splenic cDC2 were assessed for cell surface CD86 expression by flow cytometry. N=4 or 10
- 690 mice/group. (D-H) CD45.1<sup>+</sup>CD4<sup>+</sup> OT-II T cells were transferred i.v into recipient mice. The
- following day mice received i.v 33D1-ovalbumin in the presence or absence of sheep RBC (SRBC).
- After 3 days CD45.1<sup>+</sup> CD4<sup>+</sup> V $\alpha$ 2V $\beta$ 5<sup>+</sup> T cells were evaluated for CTV dilution and CXCR5<sup>+</sup>PD-1<sup>+</sup> T
- 693 follicular helper cell ( $T_{FH}$ ) by flow cytometry. Representative plots of CTV dilution (**D**) and  $T_{FH}$ 694 induction (**E**) in the presence or absence of SRBC. (**F-H**) OT-II proliferation and  $T_{FH}$  induction
- within WT and  $Ptpn22^{619W}$  recipient mice determined by flow cytometry. (F) The number of
- proliferating CD45.1<sup>+</sup> CD4<sup>+</sup>  $V\alpha 2V\beta 5^+$  OT-II T cells. (G) The frequency of CD45.1<sup>+</sup>
- 697  $V\alpha 2V\beta 5^+CD4^+PD-1^+CXCR5^+T_{FH}$  per spleen. (H) The number of CD45.1<sup>+</sup>  $V\alpha 2V\beta 5^+CD4^+PD-$
- 698 1<sup>+</sup>CXCR5<sup>+</sup> T<sub>FH</sub> per spleen. (**F-H**) N=10 mice/genotype. Each point represents an individual mouse,
- bars represent mean; error bars represent s.e.m. (A-H) \*p<0.05, \*\*\*\*p<0.0001 determined by unpaired T-test.

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