



Fig. S1. Bank1 deficiency barely modifies the survival rate in the TLR7.Tg6 and in 2 IMQ-treated mice. (A) Survival curve was monitored in both models. TLR7.Tg6 model 3 with WT (n = 12), T7 (n = 21) and T7.B1<sup>-/-</sup> (n = 15) mice up to 32 weeks; and IMQ-4 induced model with WT (n = 12), WT + IMQ (n = 15),  $B1^{-/-}$  (n = 12) and  $B1^{-/-}$  + IMQ 5 (n = 15) mice up to 20 weeks. A Kaplan-Meier survival plot is shown. (B) Serum levels 6 of IgG1 ( $\mu$ g/ml) at 1:10000 dilutions. Total mice analyzed: WT (n = 12), T7 (n = 12), 7 T7.B1<sup>-/-</sup> (n = 14); and WT (n = 8), WT + IMQ (n = 9), B1<sup>-/-</sup> (n = 8), B1<sup>-/-</sup> + IMQ (n = 11). 8 9 Each point represents one individual mouse. Data are shown as mean ± SEM. Mann-Whitney U test with Welch's correction was used to test statistical significance. 10





Fig. S2. Frequency of ABCs at early ages, in the B6.Sle1.yaa model and in vitro 12 **differentiation.** (A) Spleen weight from wild-type (n = 6) and TLR7.tg6 (n = 4) mice at 13 10 weeks of age. (B) Frequency of ABCs among CD19<sup>+</sup> B cells from the spleens of 14 wild-type and TLR7.tg6 mice at 10 weeks of age. Total mice analyzed: WT (n = 6), T7 15 (n = 4). (C) Frequency of ABCs among CD19<sup>+</sup> B cells from the spleens of B6.*Sle1.yaa* 16 and B6.*Sle1.yaa.Bank1<sup>-/-</sup>* mice at 36 weeks of age. Total mice analyzed: SLE (n = 6), 17 SLE.B1<sup>-/-</sup> (n = 8). (**D**) Frequency of ABCs (CD11b<sup>+</sup> CD11c<sup>+</sup>) among CD19<sup>+</sup> T-bet<sup>+</sup> B 18 cells from cultures of B cells purified from wild-type and TLR7.tg6 10 week-old mice, 19 stimulated with 5 µg/ml F(ab')2-goat anti-mouse IgM, 5 µg/ml Ultra-LEAF purified anti-20

- mouse CD40, in presence or absence of 2.5  $\mu$ g/ml imiquimod, 25 ng/ml mouse IL-21 recombinant, and the combination of both, for 72 hours. Graph shows mean and individual values of 3 independent experiments. Total mice analyzed: WT (n = 17), B1<sup>-/-</sup> (n = 13). Each point represents one individual mouse. Data are shown as mean ± SEM.
- 25 Mann-Whitney U test with Welch's correction was used to test statistical significance.



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Fig. S3. Clustering of scRNA-Seq data. (A) UMAP showing clusters of main cell types of spleen. Each point is a cell and the colours represent the different clusters. (B) Percentage of cells with the positive expression for cell type-specific markers (circle size) and their mean of expression (colour) across cells from each clusters. The markers are in columns and clusters are in rows. (C) Correlation between a panel of cell-specific markers and their expression by cells. Correlation is represented by the colour scale.



Fig. S4. Subclusterization of CL7. (A) UMAP showing two different subclusters
(subCL7-0 and subCL7-1) obtained from the subclusterization of CL7. (B) Individual
UMAPs showing the expression levels of typical markers of ABCs. (C) Individual
UMAPs showing the expression levels of typical markers of: ABCs and atypical memory
B cells (atMBCs), (D) memory B cells (MBCs), (E) plasma cells (PCs) and (F)
extrafollicular cells.



41 Fig. S5. *Bank1* deficiency modified the differentially expressed genes in cluster 7.
42 Heatmap showing the differentially expressed genes between wild-type, TLR7.tg6 and
43 TLR7.tg6.Bank1-/- mice, in cluster 7. Colour represent the gene expression normalized
44 by z-score.



46 Fig. S6. scRNA-Seq transcriptome data from 10 clusters among CD19<sup>+</sup> B cells. (A)
47 Top 10 most differentially expressed genes comparing each B cell clusters against the rest
48 of B cell clusters. Colour scale represent the gene expression normalized by z-score. (B)
49 Above is the number of DEGs comparing each cluster against the rest. The proportion of
50 significant genes unique and shared with other clusters is shown below.



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52 Fig. S7. Alternative trajectory of splenic B cells from the TLR7.tg6 model. UMAP

- visualization of the clusters arranged along trajectories, with CL2 as initial pseudotime
- 54 (root), coloured by inferred pseudotime, calculated by Monocle3.





56 Fig. S8. T-bet<sup>+</sup> extrafollicular B cells are increased in TLR7.tg6 mice compared to 57 Bank1-deficient and wild-type mice. (A) Representative cryosections of spleens from 32 week-old wild-type, TLR7.tg6 and TLR7.tg6.Bank1-/- mice, stained with anti-PNA 58 FITC, anti-MOMA-1 conjugated with Alexa Fluor 555, and anti-CD4 conjugated with 59 Alexa Fluor 633. Scale bar: 100 µm. This representative experiment was conducted 3 60 different times. (B) Representative cryosections of spleens from 32 week-old wild-type, 61 TLR7.tg6 and TLR7.tg6.Bank1<sup>-/-</sup> mice, stained with anti-CXCR4 APC, anti-IgG2c FITC 62 and anti-T-bet PE. Scale bar: 100 µm. This representative experiment was conducted 3 63 different times. (C) Frequency of CXCR4<sup>+</sup> T-bet<sup>+</sup> cells among IgG2c<sup>+</sup> CD138<sup>+</sup> B220<sup>-</sup> 64

- cells from the spleens of 32 week-old TLR7.tg6 model. Total mice analyzed: WT (n = 4),
- 66 T7 (n = 6), T7.B1<sup>-/-</sup> (n = 7). Each point represents one individual mouse. Data are shown
- as mean  $\pm$  SEM. Mann-Whitney U test with Welch's correction was used to test statistical
- 68 significance.

## 69 Legends for data files (Excel files):

70 Data file S1. Significantly expressed genes comparing between clusters. 71 ClusterReference: cluster for which the rest of the values of the same row refer; GeneID: 72 Gene symbol identifier; PvalAdj: p-value adjusted by Bonferroni; AvgLog2FC: fold 73 change in base 2 logarithmic scale; pct1: percentage of cells expressing the gene in the 74 studied cluster; pct2: percentage of cells expressing the gene in the reference 75 cluster/clusters.

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Data file S2. Significantly expressed genes comparing between subclusters from
CL7. ClusterReference: cluster for which the rest of the values of the same row refer;
GeneID: Gene symbol identifier; PvalAdj: p-value adjusted by Bonferroni; AvgLog2FC:
fold change in base 2 logarithmic scale; pct1: percentage of cells expressing the gene in
the studied cluster; pct2: percentage of cells expressing the gene in the reference
cluster/clusters.

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Data file S3. Significantly expressed genes comparing TLR7.tg6 and
TLR7.tg6.Bank1<sup>-/-</sup> by cluster. Each excel sheet contains the results obtained for a
cluster. GeneID: Gene symbol identifier; PvalAdj: p-value adjusted by Bonferroni;
AvgLog2FC: fold change in base 2 logarithmic scale; Comparation: group of mice
compared. Second group as used as reference for the statistical test.

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90 Data file S4. Significant biological pathways comparing between clusters.
91 ClusterReference: cluster for which the rest of the values of the same row refer; PvalAdj:
92 p-value adjusted by Bonferroni; Direction: significant biological pathway for up-

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93 regulated or downregulated genes; Description: pathway description; AnnotationID:94 Gene Ontology identifier.

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96 Data file S5. Significant biological pathways comparing TLR7.tg6 and
97 TLR7.tg6.Bank1<sup>-/-</sup> by cluster. ClusterReference: cluster for which the rest of the values
98 of the same row refer; Direction: significant biological pathway for up-regulated or
99 downregulated genes; PvalAdj: p-value adjusted by Bonferroni; Description: pathway
100 description; AnnotationID: Gene Ontology identifier.