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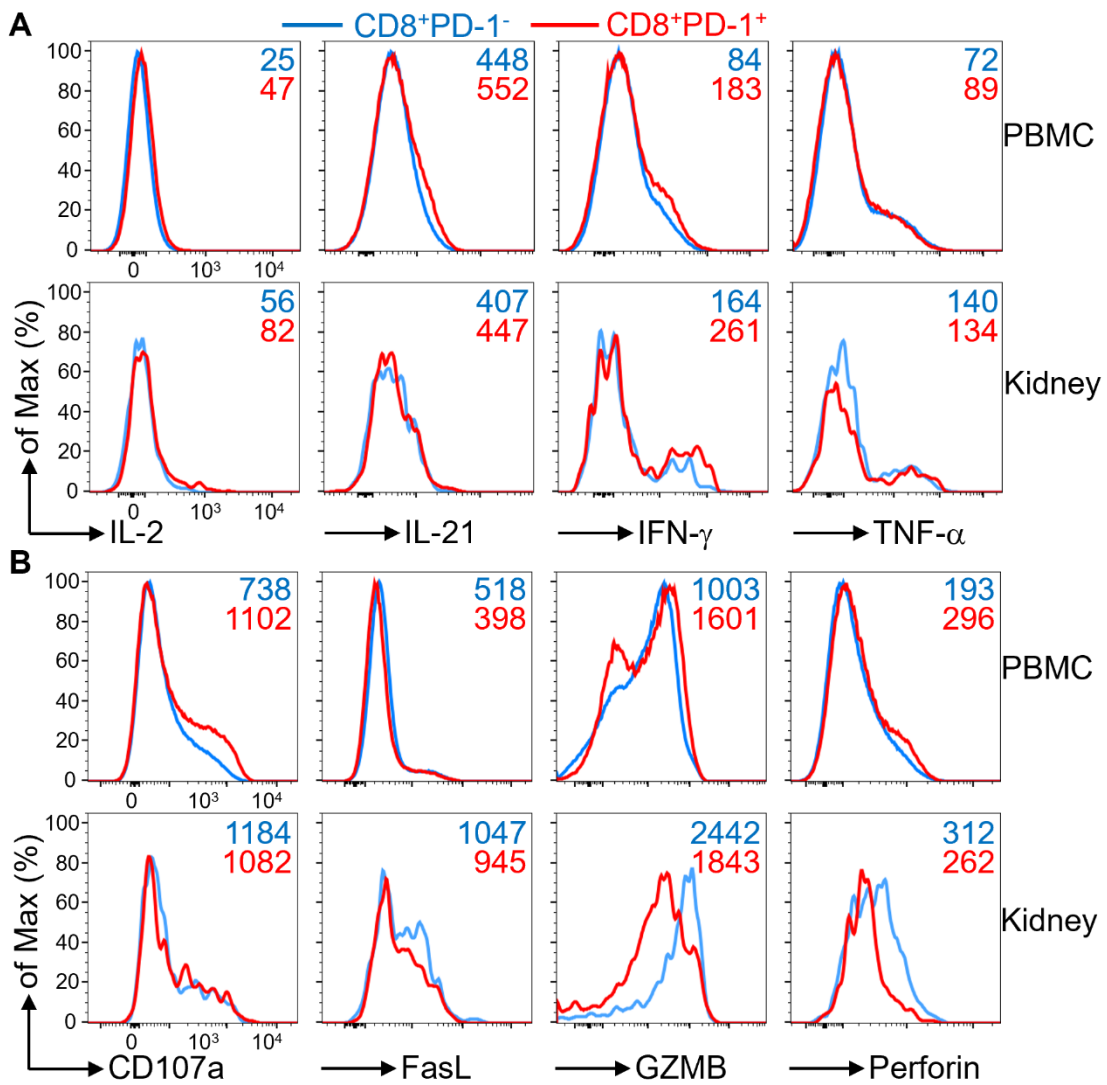
Supplementary Materials

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3 **PD-1 activation mitigates lupus nephritis by suppressing hyperactive and**
4 **heterogeneous PD-1⁺CD8⁺ T cells**

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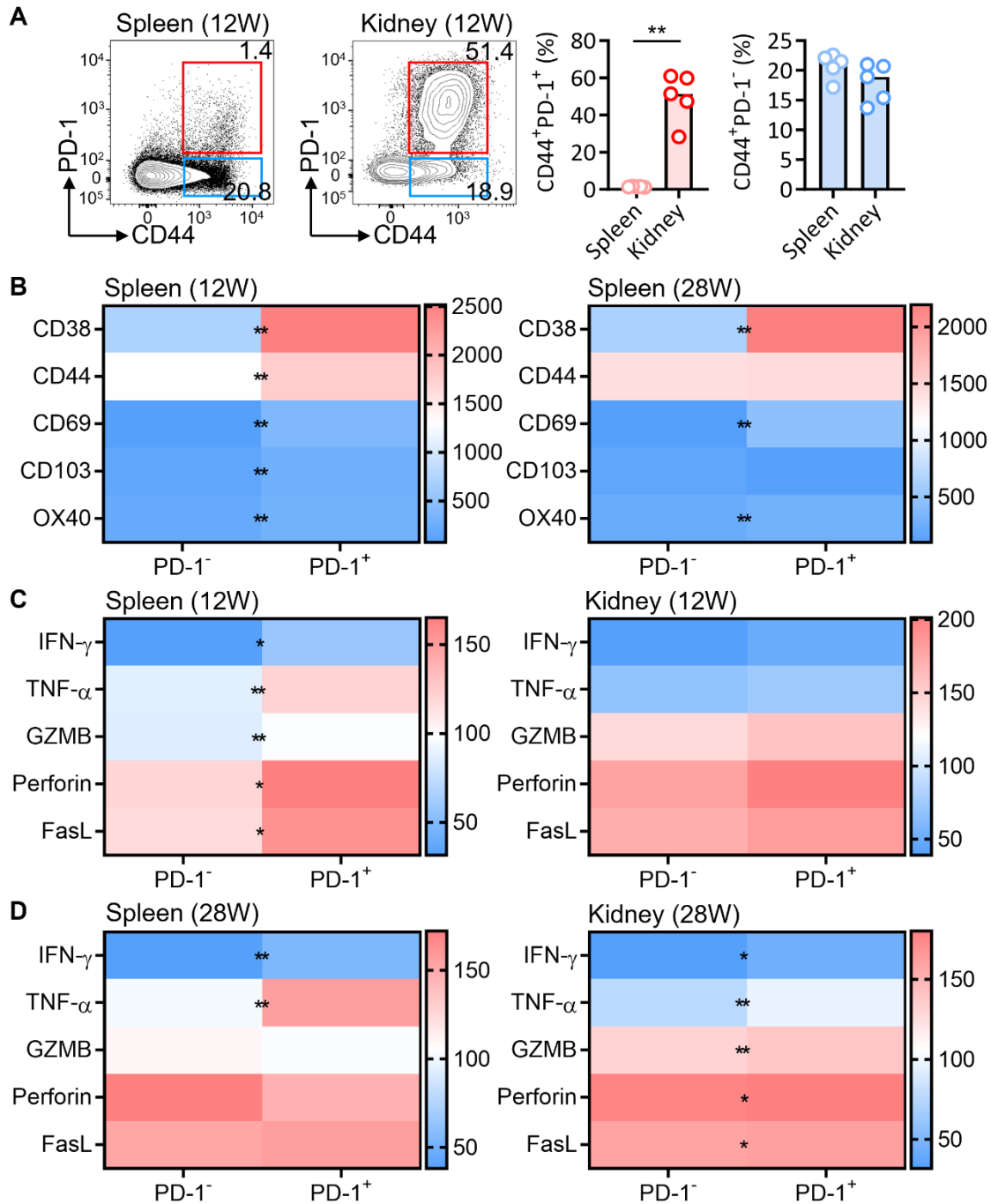


8 **Figure S1.** Cytokine production and expression of killing molecules by PD-1⁺ and PD-1⁻
 9 CD8⁺ T cells from PBMCs and kidney MNCs. Flow cytometric histograms depicting the
 10 geometric mean fluorescence intensity (GMFI) values of cytokines (IL-2, IL-21, IFN- γ ,
 11 TNF- α) (A), and killing molecules (CD107a, FasL, GZMB, perforin) (B), produced by
 12 PD-1⁺ and PD-1⁻ CD8⁺ T cells isolated from PBMCs (n = 10) and kidney MNCs (n = 10)
 13 of patients with LN.

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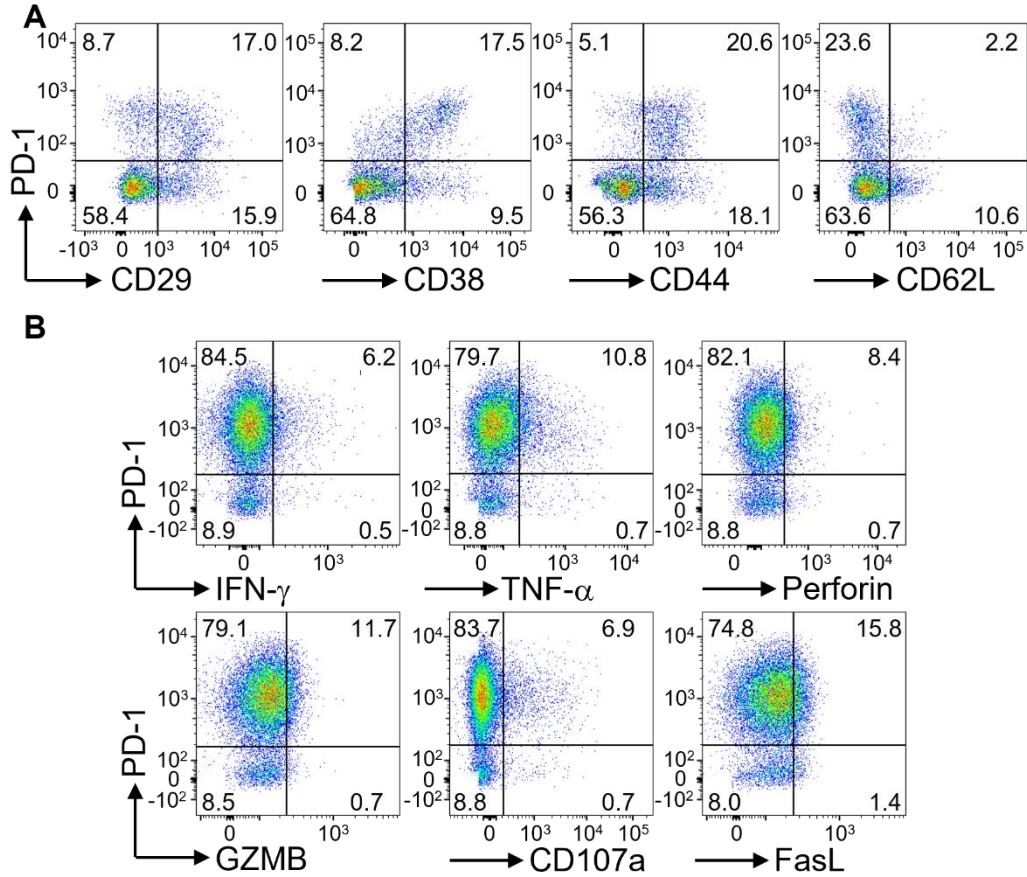


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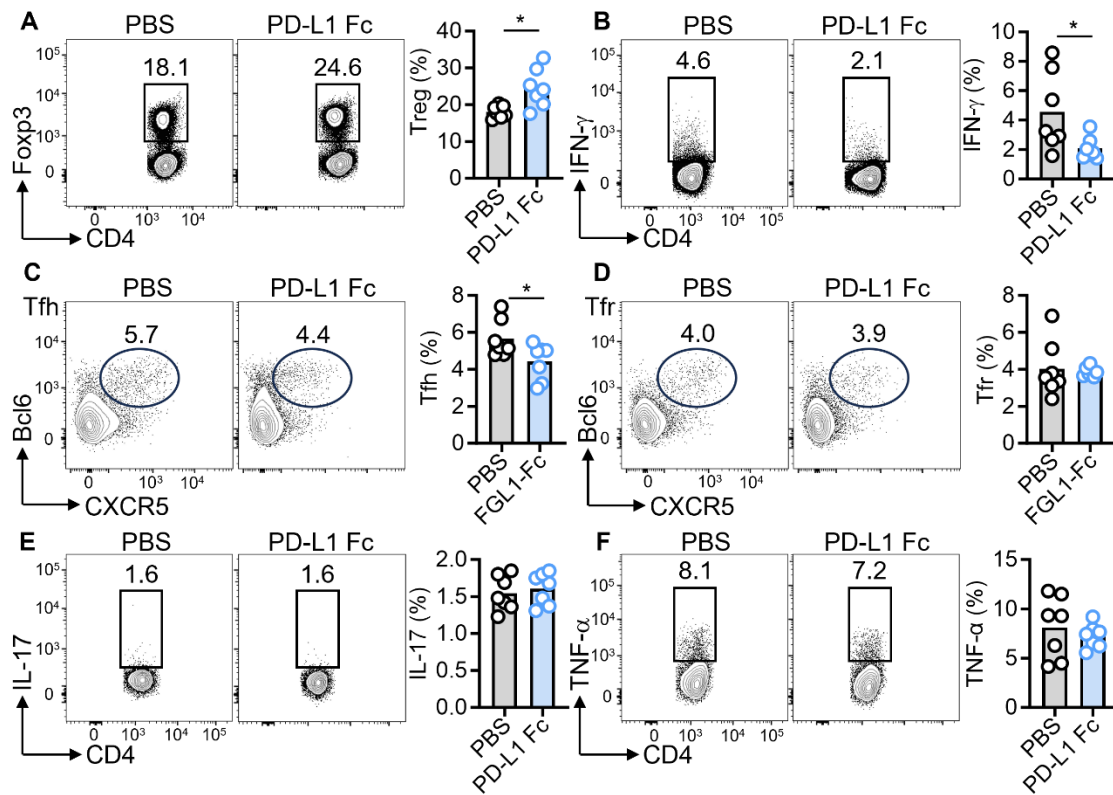
18 **Figure S2.** Elevated expression of activation markers on CD8⁺PD-1⁺ T cells compared to
 19 CD8⁺PD-1⁻ T cells in NZB/W F1 mice. (A-D) FACS plots and statistical analysis showing:
 20 (A) PD-1 expression on CD8⁺CD44⁺ cells from the spleen and kidney of 12-week-old
 21 (12W) female NZB/W F1 mice; (B) expression of activation markers (CD38, CD44, CD69,
 22 CD103, OX40) in the spleen at 12 and 28 weeks of age (12W and 28W); (C) cytokines

23 (IFN- γ , TNF- α) and cytotoxic molecules (GZMB, perforin, FasL) in PD-1⁺ and PD-1⁻ cells
24 from the spleen or kidney of 12W mice; and (D) data for 28W mice. Data are represented
25 as individual values (dots, n = 5 per group) and mean (bars). Statistical analysis: Mann-
26 Whitney U-test (A-D). *p \leq 0.05, **p \leq 0.01.

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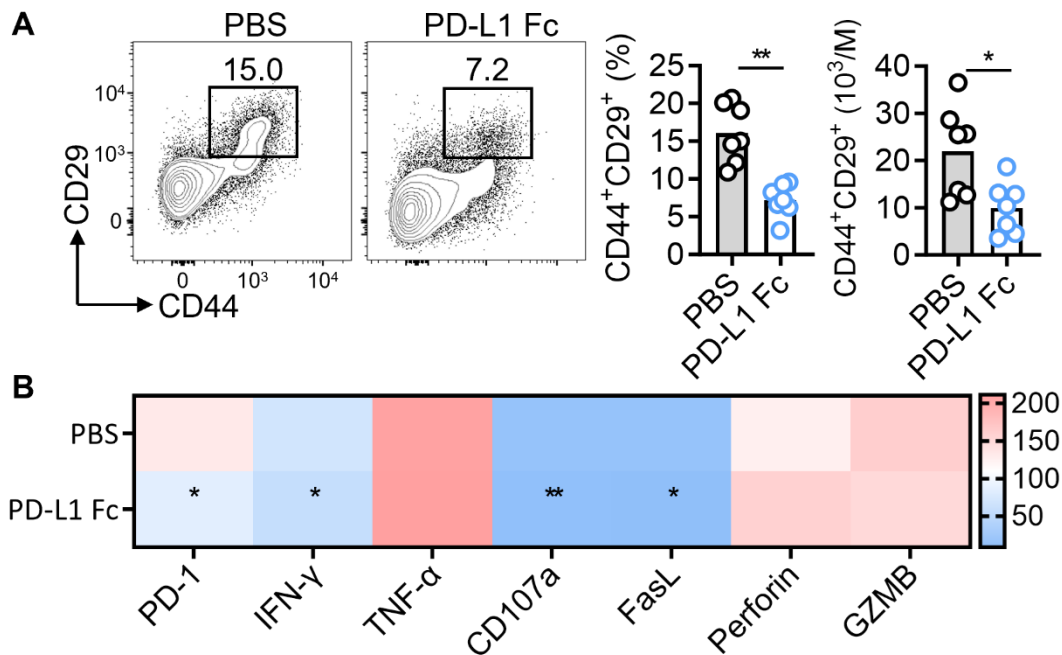


29 **Figure S3.** Co-expression of activation markers, cytokines, and cytotoxic molecules in PD-
 30 1⁺CD8⁺ T cells in NZB/W F1 mice. (A, B) FACS plots showing the co-expression of PD-
 31 1 with activation markers, CD29, CD38, CD44, CD62L (A), cytokines (IFN- γ , TNF- α),
 32 and cytotoxic molecules (perforin, GZMB, CD107a, and FasL) (B) on CD8⁺ T cells in the
 33 spleen from NZB/W F1 mice at 28W.



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35 **Figure S4.** PD-L1 Fc reduces Th1 cells and promotes Treg cells in NZB/W F1 mice. (A,
 36 B) FACS plots and statistics showing the Treg cells (A), Th1 cells (B), Tfh cells (C), Tfr
 37 (D), Th17 cells (E) and TNF- α ⁺CD4⁺ T cells (F) in spleens from NZB/W F1 mice with PD-
 38 L1 Fc or IgG treatment (n = 7 per group). Data are shown for individuals (dots) and mean
 39 (bars) values. Statistical analysis: Mann-Whitney U-test (A-E). * $p \leq 0.05$.



40 **Figure S5.** Reduction of CD44⁺CD29⁺ cells and effector molecule expression in CD8⁺ T
 41 cells of NZB/W F1 mice after PD-L1 Fc treatment. (A, B) FACS plots and statistical
 42 analysis depicting: (A) frequencies and total numbers of CD44⁺CD29⁺CD8⁺ T cells, along
 43 with CD29 and CD44 expression levels; (B) expression levels of PD-1, IFN- γ , TNF- α ,
 44 CD107a, FasL, perforin, and GZMB in CD8⁺ T cells from the spleen of mice treated with
 45 or without PD-L1 Fc (n = 7 per group). Data are represented as mean \pm SEM. Statistical
 46 analysis: one-tailed Mann-Whitney U-test (A, B). *p \leq 0.05, **p \leq 0.01.

Table S1. Baseline characteristics of patients with SLE (n = 20)

Characteristics	Value
Age, year, median (range)	30.5 (19-45)
Female/Male	18/2
Weight, kg, median (range), n = 20	56 (43-75)
Height, cm, median (range), n = 20	160 (153-165)
Area, m ² , median (range), n = 20	1.6 (1.36-1.84)
Duration of SLE, year, median (range)	4 (0-12)
Prednisone dose, mg/day, median (range)	30 (10-60)
Use of concomitant agents (no. of patients)	
Hydroxychloroquine	5
Cyclophosphamide	1
Azathioprine	0
Cyclosporine	1
Methotrexate	0
Mycophenolate mofetil	2
Leflunomide	2
Thalidomide	0

Abbreviation: SLE, systemic lupus erythematosus.

Table S2. Clinical characteristics of patients with SLE (n = 20)

Characteristics	value
SELENA-SLEDAI, median (range)	12 (2-24)
Rash, n (%)	1 (5%)
Oral ulcers, n (%)	0 (0)
Serositis, n (%)	0 (0)
Raynaud's, n (%)	1 (5%)
Alopecia, n (%)	2 (10%)
Arthritis, n (%)	2 (10%)
Leukopenia, n (%)	2 (10%)
Thrombocytopenia, n (%)	1 (5%)
IgA, g/L, median (range)	2.41 (0.81-5.08)
IgG, g/L, median (range)	12.4 (3.59-33.1)
IgM, g/L, median (range)	0.98 (0.48-2.09)
C3, g/L, median (range)	0.529 (0.256-0.951)
C4, g/L, median (range)	0.0895 (0.029-0.187)
Anti-dsDNA, IU/mL, median (range)	96.97 (13.39-548.05)
Urine Protein, mg/24h, median (range)	2283.6 (48.6-10406.5)
Platelet, $\times 10^9/L$, median (range)	220 (98-315)
WBC, $\times 10^9/L$, median (range)	8.12 (3.06-18.56)
Lymphocyte, $\times 10^9/L$, median (range)	0.855 (0.27-2.67)
Monocyte, $\times 10^9/L$, median (range)	0.345 (0.02-0.89)
Neutrophil, $\times 10^9/L$, median (range)	6.9 (2.23-9.34)
Eosinophil, $\times 10^9/L$, median (range)	0.01 (0-0.13)

Abbreviation: C3/4, complement component 3/4; dsDNA, anti-double stranded DNA; g/L, gram/liter; Ig, immunoglobulin; IU/mL, international units per milliliter; mg/24h: milligram/24 hours; SELENA, safety of estrogens in lupus national assessment; SLEDAI, systemic lupus erythematosus disease activity index; WBC, white blood count.

Table S3. Primers for q-PCR assay

Gene	Sense	Antisense
<i>Il7r</i>	CACAGCCAGTTGGAAGTGGATG	GGCATTTCACTCGTAAAAGAGCC
<i>Itgb1</i>	CTCCAGAAGGTGGCTTTGATGC	GTGAAACCCAGCATCCGTGGAA
<i>Klf2</i>	CACCTAAAGGCGCATCTGCGTA	GTGACCTGTGTGCTTTCGGTAG
<i>Rgs10</i>	CGGAACCACAGCCTCCTTTCAA	TGCCATCCGTTCTGAAACCACG
<i>Smc4</i>	GTAGCGTACCAAAGAGACAGACG	CCTCTCATCACCTTGCTTCCAC
<i>Ccl5</i>	CCTGCTGCTTTGCCTACCTCTC	ACACACTTGGCGGTTTCCTTCGA
<i>Gzmk</i>	CCATTCTCACGACTTCAGTCCG	TCACCTGGCATTGTTGGTCCCATC
<i>Nkg7</i>	CCACAGGTCCTCACTTCTCTGC	CAGCCAGGATACAGAAGCTCTG
<i>Cxcr6</i>	GGTTCTTCCTGCCATTGCTCAC	GCAGGAACACAGCCACTACAAG
<i>Isg15</i>	CATCCTGGTGAGGAACGAAAGG	CTCAGCCAGAACTGGTCTTCGT
<i>Ifit3</i>	GCTCAGGCTTACGTTGACAAGG	CTTTAGGCGTGTCCATCCTTCC
<i>Ifit1</i>	TACAGGCTGGAGTGTGCTGAGA	CTCCACTTTCAGAGCCTTCGCA
<i>Isg20</i>	GCCATTTGGTGAAGCCAGGCTA	AGCCTGTCTGTGGACGTGTCAT
<i>Bst2</i>	CAAACCTCTGCAACCTGACCGT	CTCCTGGTTCAGCTTCGTGACT
<i>Xcl1</i>	CAAGACCTATATCATCTGGGAGG	TGCCATCCACAGTCTTGATCGC
<i>Tnfrsf9</i>	CCAAGTACCTTCTCCAGCATAGG	GCGTTGTGGGTAGAGGAGCAAA
<i>Mif</i>	GAACCGCAACTACAGTAAGCTGC	ACGTTGGCAGCGTTCATGTCGT
<i>Itm2a</i>	GCAGGACTGATTGTTGGTGGAG	CTCCTCAGTCACAGGCAGAAAG
<i>H2ac24</i>	GCGACAACAAGAAGACGCGCAT	CTGGATGTTGGGCAGGACGCC
<i>Stmn1</i>	GAAGAAGGACCTTTCCCTGGAG	TGGCTTTCTGGAGCACCTCCTT
<i>Hmgb2</i>	GATGTGGTCTGAGCAATCTGCC	CCTGCTTCACTTTTGCCCTTGG
<i>Jun</i>	CAGTCCAGCAATGGGCACATCA	GGAAGCGTGTCTGGCTATGCA
<i>Nfkb1</i>	GCTGCCAAAGAAGGACACGACA	GGCAGGCTATTGCTCATCACAG
<i>Tnfaip3</i>	AGCAAGTGCAGGAAAGCTGGCT	GCTTTCGCAGAGGCAGTAACAG
<i>Slc6a4</i>	GTTGATGCTGCGGCTCAGATCT	GAAGCTCGTCATGCAGTTCACC
<i>Ifng</i>	CAGCAACAGCAAGGCGAAAAAGG	TTTCCGCTTCCTGAGGCTGGAT
<i>Tbx21</i>	CCACCTGTTGTGGTCCAAGTTC	CCACAAACATCCTGTAATGGCTTG
<i>Actb</i>	GCGTGACATCAAAGAGAAGCT	ATGCCACAGGATTCCATACC