

Supplement Figure 1. Influence of RAGE deficiency on autoantibody levels and proteinuria in pristane-induced lupus. C57BL/6 (WT) and RAGE-/- mice were injected intraperitoneally with 0.5ml pristane. Serum and urine was collected five months (replicate 1: n = 6 RAGE-/- and 7 WT mice) (a + c) or six months (replicate 2: n = 6 RAGE-/- and 8 WT mice) (b + d) later. The concentrations of anti-dsDNA autoantibodies were determined by ELISA (left). Protein concentrations were determined using a BCA protein assay in urine samples collected for 24 hours using metabolic cages (right). Each data point represents a single mouse; values are indicated as mean \pm SD. Unpaired Mann-Whitney *U*-test was used for statistical analysis. Note that for replicate 1, anti-dsDNA autoantibody levels were compared before and five months after pristane injection in C57BL/6 (WT) and RAGE-/animals. After five months, anti-dsDNA autoantibodies were ca. 2.3 fold higher in RAGE-/- and ca. 3.7 fold higher in WT animals (a).



Supplement Figure 2. Influence of RAGE deficiency on spleen and lipogranuloma weights as well as histologic nephritis score. C57BL/6 (WT) and RAGE-/- mice were injected intraperitoneally with 0.5ml pristane. Spleen and kidney samples as well as intraperitoneal lipogranulomas were collected five months (replicate 1: n = 6 RAGE-/- and 7 WT mice) (a, b) or six months (replicate 2: n = 6 RAGE-/- and 8 WT mice) (c) later. a) Determined were cumulative nephritis scores of RAGE-/- compared to WT animals. b + c) Determined were weights of spleen (left) and lipogranulomas (right). Each data point represents a single mouse; values are indicated as mean \pm SD. Unpaired Mann-Whitney *U*-test was used for statistical analysis.



Supplement Figure 3. Phenotype of splenocytes in WT and RAGE-/- mice after pristane injection. The analysis was performed by flow cytometry 5 months after pristane injection (replicate 1: n = 6 RAGE-/- and 7 WT mice). Depicted are column scatter graphs of WT versus RAGE-/- animals for Fas^{hi}GL7^{hi} GC B cells, CXCR5^{hi}PD1^{hi} TFH cells, LC⁺CD138^{hi} plasmacells/-blasts and CD21^{lo}CD23^{hi} B220⁺ follicular B cells. Each data point represents a single mouse, values are indicated as mean ± SD. Unpaired Mann-Whitney *U*-test was used for statistical analysis to determine differences between WT and RAGE-/- animals.



Supplement Figure 4. Influence of RAGE deficiency on autoantibody levels and disease development in CIA arthritis. (a) Mean arthritis scores and cumulative disease incidence in cCII-immunized RAGE-/- versus WT mice. Each data point represents the mean + SEM per group and time point (replicate 2: n = 8 RAGE-/- and 7 WT mice). (b) Serum concentrations of anti-CII IgG, anti-CII IgG2a and anti-CII IgG1 were analyzed in WT and RAGE-/- mice at day 41 after CII-immunization. Each data point represents a single mouse, values are indicated as mean \pm SD (replicate 2: n = 8 RAGE-/- and 7 WT mice). Unpaired Mann-Whitney *U*-test was used for statistical analysis.



Supplement Figure 5. Influence of RAGE deficiency on disease development in K/BxN serum-transfer arthritis. Mean arthritis scores of RAGE-/- versus WT mice (replicate 2: n = 7 mice per group) after intraperitoneal injection of arthritogenic K/BxN serum. Values are indicated as mean + SEM. Unpaired Mann-Whitney *U*-test was used for statistical analysis.



Supplement Figure 6. Anti-dsDNA antibody levels in pristane-induced lupus compared to NZB/W F1 mice. C57BL/6 (WT) mice were injected intraperitoneally with 0.5ml pristane. Serum was collected before as well as seven months after injection (n = 10 WT mice from replicate 3). Serum samples were also collected from 26w (n = 2) and 32w (n = 3) old NZB/W F1 animals. The concentrations of anti-dsDNA antibodies were determined by ELISA. Compared are OD values of 26w and 32w old NZB/W F1 animals to C57BL/6 animals before and 7 after pristane injection. Considering the dilution factor of each sample, the OD of anti-dsDNA autoantibodies from sera of 32w old NZB/W F1 mice was ca. 22 fold higher than that from pristane-treated animals, the OD from 26w old NZB/W F1 ca. 13 fold higher.



Supplement Figure 7: Semiquantitative albuminuria in pristane-induced lupus compared to NZB/W F1 mice. C57BL/6 (WT) and RAGE-/- mice were injected intraperitoneally with 0.5ml pristane. Urine was collected five months (replicate 1: n = 6 RAGE-/- and 7 WT mice), six months (replicate 2: n = 6 RAGE-/- and 8 WT mice) or seven months (replicate 3: n = 15 RAGE-/- and 10 WT mice) after injection. Urine samples were also collected from NZB/W F1 animals at 26w, 28w, 30w and 32w of age (n = 5). Albuminuria was determined semi-quantitatively using Albustix®. a) Compared are levels of albuminuria in NZB/W F1 animals at different ages to levels in C57BL/6 animals 7 months after pristane injection. b – d) compared are levels of albuminuria between RAGE-/- and WT animals 5 months (b), 6 months (c) and 7 months (d) after pristane injection. Unpaired Mann-Whitney U-test was used for statistical analysis to determine differences between WT and RAGE-/- animals.

| Splenic cell subsets | | WT | КО |
|----------------------|---|-----------------|----------------|
| CD11c ^{hi} | | 0.992 (0.201) | 0.926 (0.137) |
| CD11b⁺ | | 6.551 (0.965) | 5.553 (0.883) |
| | Ly6G ^{hi} | 2.648 (1.779) | 1.88 (0.392) |
| | Ly6C ^{hi} | 1.3 (0.311) | 0.998 (0.436) |
| | Ly6C ^{lo} | 2.765 (0.294) | 2.429 (0.372) |
| CD4 ⁺ | | 15.714 (1.15) | 15.392 (6.398) |
| | CD44 ^{hi} | 50.285 (0.938) | 46.2 (5.91) |
| | CD69⁺ | 21.875 (2.96) | 22.016 (2.153) |
| | CXCR5 ^{hi} PD1 ^{hi} | 9.757 (2.171) | 7.921 (0.938) |
| | IFNγ | 7.39 (2.403) | 7.256 (1.186) |
| | IL17 | 0.299 (0.043) | 0.348 (0.062) |
| | FoxP3 | 29.785 (3.379) | 26.75 (1.364) |
| B220 ⁺ | | 56.185 (5.67) | 55.36 (2.37) |
| | Fas ^{hi} GL7 ^{hi} | 4.96 (1.937) | 4.63 (1.559) |
| | CD21 ^{lo} CD23 ^{hi} | 41.371 (4.1907) | 46.75 (8.27) |
| | CD21 ^{hi} CD23 ^{lo} | 4.295 (1.696) | 4.496 (1.041) |
| | IgD⁺IgM⁺ | 8.882 (1.623) | 10.073 (3.011) |
| | IgM⁺CD5⁺ | 0.473 (0.096) | 0.394 (0.107) |
| | LC ⁺ CD138 ^{hi} (*) | 1.01 (0.207) | 0.624 (0.114) |

The analysis was performed by flow cytometry five months after pristane injection. Values are the mean +/- SD (replicate 1: n = 6 RAGE-/- and 7 WT mice). Unpaired Mann–Whitney *U*-test was used for statistical analysis to determine differences between WT and RAGE-/- animals.* p = 0.0047