Supplemental information

Intercellular cGAMP transmission induces innate immune activation and tissue inflammation in Trex1 deficiency

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Figure S1. STING deficiency does not alter basal IRF3 activity. Related to Figure 1.
(A) Mean fluorescence intensity (MFI) for p-IRF3 in cDCs from instantly fixed spleens (n = 5 for WT mice, n = 4 for WT into WT transplanted mice, n = 5 for untreated dKO mice, n = 4 for dKO BM mice).
(B) As in (A) but for untreated Cgas−/− (n = 5) and Cgas−/− into Cgas+/+ transplanted mice (n = 5). Data are represented as mean + SEM.
Figure S2. Intercellular cGAMP transfer does not lead to marked differences in serum cytokine concentrations. Related to Figure 2.

(A) Venn diagram depicting differentially expressed genes (non-adjusted p value < 0.05) within the dataset of bone marrow from Cgas−/− and dKO mice (Pre-transplantation), as well as spleen tissue from Cgas−/− BM and dKO BM mice (Post-transplantation). The overlap shows the number of genes differentially expressed in both pairs.

(B) Normalized Enrichment Scores (NES) of Hallmark gene sets significantly up- and downregulated (FDR q value < 0.01) in bone marrow from Cgas−/− mice compared to that from dKO mice in a pre-ranked Gene Set Enrichment Analysis (GSEA) based on a metric score calculated by log2(fold change) x (-log10(p value)). n = 4 mice per group.

(C) Percentage of splenic classical monocytes and red pulp macrophages with high expression of IkBα (n = 5 for untreated Cgas−/−, n = 5 for Cgas−/− into Cgas−/− transplanted mice). Data are represented as mean ± SEM.

(D) Cytokine array membranes were probed with serum from Cgas−/− BM and dKO BM mice. Pictures on the left show representative results from 2 Cgas−/− BM and 2 dKO BM mice. The table on the right specifies the cytokines that correspond to the dots on the membranes.
Figure S3. cGAMP shuttling does not promote Tc1 differentiation of CD8⁺ T cells. Related to Figure 3.

(A) Mean fluorescence intensity (MFI) for Sca-1 of splenic B, CD4⁺ T and CD8a⁺ T cells (n = 4 for WT mice, n = 5 for untreated dKO mice, n = 4 for dKO BM mice).

(B) FACS contour plots display IL-17 and IL-4 staining profiles of gated CD4⁺ and CD8⁺ T cells of PMA plus ionomycin stimulated splenocytes from Trex1⁻/⁻ mice (n = 4). Values indicate percentage of CD4⁺ and CD8⁺ T cells (mean).

(C) FACS contour plots display IFN-γ and TNF-α staining profiles of gated CD8⁺ T cells of PMA plus ionomycin stimulated splenocytes from Cgas⁻/⁻ BM (n = 14), Cgas⁻/⁻ + dKO BM (n = 14) and dKO BM mice (n = 16). Values indicate percentage of CD8⁺ T cells (mean).
Figure S4. Proliferation of T and B cells in spleens is unaffected by cGAMP transmission. Related to Figure 5.

(A) Spleen weight of Cgas−/− BM (n = 7), Cgas−/− + dKO BM (n = 4) and dKO BM mice (n = 6).
(B) Spleen cell count of Cgas−/− BM (n = 14), Cgas−/− + dKO BM (n = 14) and dKO BM mice (n = 16).
(C-D) Percentage of CD4+ and CD8+ T cells (C), and B cells (D) that are proliferating (Ki67+) in spleens from Cgas−/− BM (n = 14), Cgas−/− + dKO BM (n = 14) and dKO BM mice (n = 16).

Data in bar graphs are represented as mean ± SEM. Data in (A) are pooled from 2 and in (B-D) from 4 independent experiments.
Figure S5. cGAMP transfer does not promote immune cell infiltration into hearts. Related to Figure 6. (A-B) Representative images of H&E-stained sections of hearts (A) and tongues (B) from WT and Trex1−/− mice. Scale bars equal 100 µm. (C-D) Inflammation in hearts (C) and tongues (D) was scored from 1 to 3. Shown are scores of WT and Trex1−/− mice. Each dot depicts an individual mouse, horizontal lines represent median values. (E-F) Number of myeloid cell subsets (E) and lymphocyte subsets (F) in hearts from Cgas−/− BM (n = 13), Cgas−/− + dKO BM (n = 14) and dKO BM (n = 16) mice. Data are represented as mean ± SEM. Data are pooled from 4 independent experiments.
Figure S6. UV light does not induce DC maturation or T cell priming in Cgas−/− BM mice. Related to Figure 6.
(A) Percentage of cDC subsets in skin draining lymph nodes (axillary, brachial, inguinal) of Cgas−/− BM and dKO BM mice after UV stimulation.
(B) Mean fluorescence intensity (MFI) of MHC-II and CD86 for cDC subsets in skin draining lymph nodes of Cgas−/− BM and dKO BM mice after UV stimulation. Values indicate the mean of the MFI.
(C) As in (B) but for cDC subsets in spleens.
(D) Distribution of CD4+ and CD8α+ T cell subsets in spleens from Cgas−/− BM and dKO BM mice after UV stimulation.
(E) IFN-γ and TNF-α staining profiles of gated CD4+ and CD8+ T cells of PMA plus ionomycin stimulated splenocytes from Cgas−/− BM and dKO BM mice after UV stimulation. Values indicate percentage of CD4+ and CD8+ T cells (mean).
Bar graphs show mean + SEM. Data are pooled from 2 independent experiments. n = 8 for Cgas−/− BM and n = 6 for dKO BM mice.
### SUPPLEMENTAL TABLES

| Group                | n | Cell Population  | % Cgas Deletion |
|----------------------|---|------------------|-----------------|
| Cgas<sup>-/-</sup> BM| 4 |                  | 99.1            |
| Cgas<sup>-/-</sup> + dKO BM | 4 | B Cells          | 54.6            |
| dKO BM               | 4 |                  | 0.00            |
| Cgas<sup>-/-</sup> BM| 4 | T Cells          | 85.8            |
| Cgas<sup>-/-</sup> + dKO BM | 4 |                | 45.4            |
| dKO BM               | 4 |                  | 0.00            |
| Cgas<sup>-/-</sup> BM| 4 | cDCs             | 99.0            |
| Cgas<sup>-/-</sup> + dKO BM | 4 |                | 46.2            |
| dKO BM               | 4 |                  | 0.00            |
| Cgas<sup>-/-</sup> BM| 4 | Red pulp         | 92.8            |
| Cgas<sup>-/-</sup> + dKO BM | 4 | macrophages     | 49.8            |
| dKO BM               | 4 |                  | 0.00            |

**Table S1. Deletion of Cgas in BM transplant models.**

Deletion efficacy of Cgas in various cell types isolated from spleens was determined by measuring the amount of residual Cgas by qPCR. The amount of Cgas in each sample was normalized to the unaffected gene Ccr2. Cell populations were sorted by flow cytometry: T cells (TCRβ<sup>+</sup>CD19<sup>-</sup>), B cells (CD19<sup>+</sup>TCRβ<sup>+</sup>), cDCs (CD11c<sup>+</sup>MHC-II<sup>+</sup>CD19<sup>-</sup>), red pulp macrophages (CD11b<sup>+</sup>F4/80<sup>+</sup>CD11c<sup>-</sup>).
| Gene ID | log2FoldChange Post-transplantation | log2FoldChange Pre-transplantation | Contra-regulated |
|---------|------------------------------------|-----------------------------------|------------------|
| Rpl29  | 6.455                              | 2.050                             |                  |
| Trex1  | 2.712                              | 1.450                             |                  |
| Tma7   | 2.329                              | -0.188                            | yes              |
| Mlh1   | 1.225                              | 0.897                             |                  |
| Dusp7  | 1.048                              | 1.400                             |                  |
| Tmem108 | 0.918                            | 0.734                             |                  |
| Shisa5 | 0.812                              | 0.613                             |                  |
| Mapkapk3 | 0.662                           | 0.420                             |                  |
| Lrflp2 | 0.655                              | 0.362                             |                  |
| Rassf1 | 0.634                              | -0.560                            | yes              |
| Ppp1r35 | 0.500                           | 0.468                             |                  |
| Snrpf  | 0.454                              | 0.768                             |                  |
| Nat2   | 0.445                              | 0.804                             |                  |
| Ifi204 | 0.436                              | -0.375                            | yes              |
| Egr3   | 0.429                              | 0.827                             |                  |
| Ost4   | 0.420                              | 0.485                             |                  |
| Snhg15 | 0.396                              | 0.540                             |                  |
| Zbp1   | 0.393                              | -0.750                            | yes              |
| Myc    | 0.364                              | 0.494                             |                  |
| Zbtb24 | 0.357                              | -0.496                            | yes              |
| Ldb1   | 0.341                              | 0.475                             |                  |
| Dnajc7 | 0.333                              | 0.236                             |                  |
| C2cd5  | 0.315                              | 0.313                             |                  |
| Elf2ak3| 0.311                              | 0.458                             |                  |
| Coro2a | 0.307                              | 0.340                             |                  |
| Clp1   | 0.280                              | 0.378                             |                  |
| Rnf146 | 0.273                              | 0.340                             |                  |
| Nup88  | 0.250                              | 0.358                             |                  |
| Slfn1  | 0.212                              | -0.385                            | yes              |
| Prrc2a | 0.211                              | 0.226                             |                  |
| Bzw2   | 0.179                              | 0.255                             |                  |
| Dpp4   | 0.179                              | 0.395                             |                  |
| Vim    | 0.170                              | -0.153                            | yes              |
| Actr3  | 0.167                              | -0.154                            | yes              |
| Syncr1 | 0.162                              | 0.232                             |                  |
| Nfkbia | 0.118                              | -0.229                            | yes              |
| Arrb1  | -0.275                             | -0.205                            |                  |
| AcsI1  | -0.286                             | -0.210                            |                  |
| Was    | -0.334                             | -0.293                            |                  |
Table S2. Overlap of differentially expressed genes Pre- and Post-transplantation.
Overlap of genes differentially expressed (non-adjusted p value < 0.05) between bone marrow from Cgas<sup>−/−</sup> and dKO mice (Pre-transplantation) and between spleen tissue from Cgas<sup>−/−</sup> BM and dKO BM mice (Post-transplantation).
| Group               | n  | % IgG ANA positive          |
|---------------------|----|-----------------------------|
| Cgas<sup>−/−</sup> BM | 7  | 0                           |
| Cgas<sup>−/−</sup> + dKO BM | 11 | 0                           |
| dKO BM              | 9  | 0                           |
| MRL.Fas<sup>−/−</sup> | 3  | 100 (homogenous pattern)    |
| Trex1<sup>−/−</sup>  | 3  | 100 (homogenous pattern)    |
| WT                  | 3  | 0                           |

Table S3. cGAMP travel does not induce IgG antinuclear antibody (ANA) formation. HEp-2 ANA staining produced by sera from Cgas<sup>−/−</sup> BM, Cgas<sup>−/−</sup> + dKO BM, dKO BM, MRL.Fas<sup>−/−</sup>, Trex1<sup>−/−</sup> and WT mice with serum dilutions of 1:100. ANAs are routinely detectable at serum dilutions of 1:16,000 to 1:32,000 in MRL.Fas<sup>−/−</sup> mice.