Gall et al, Figure S1

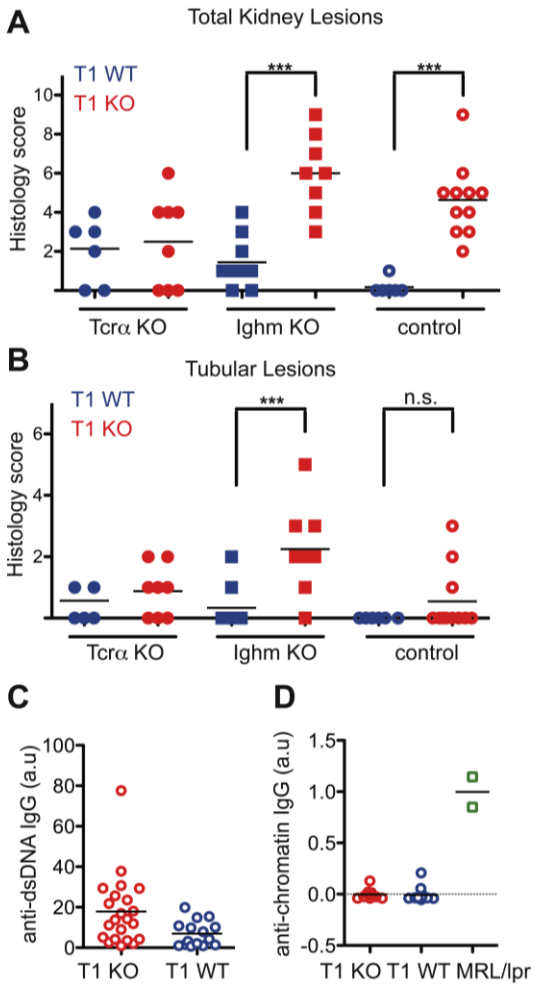


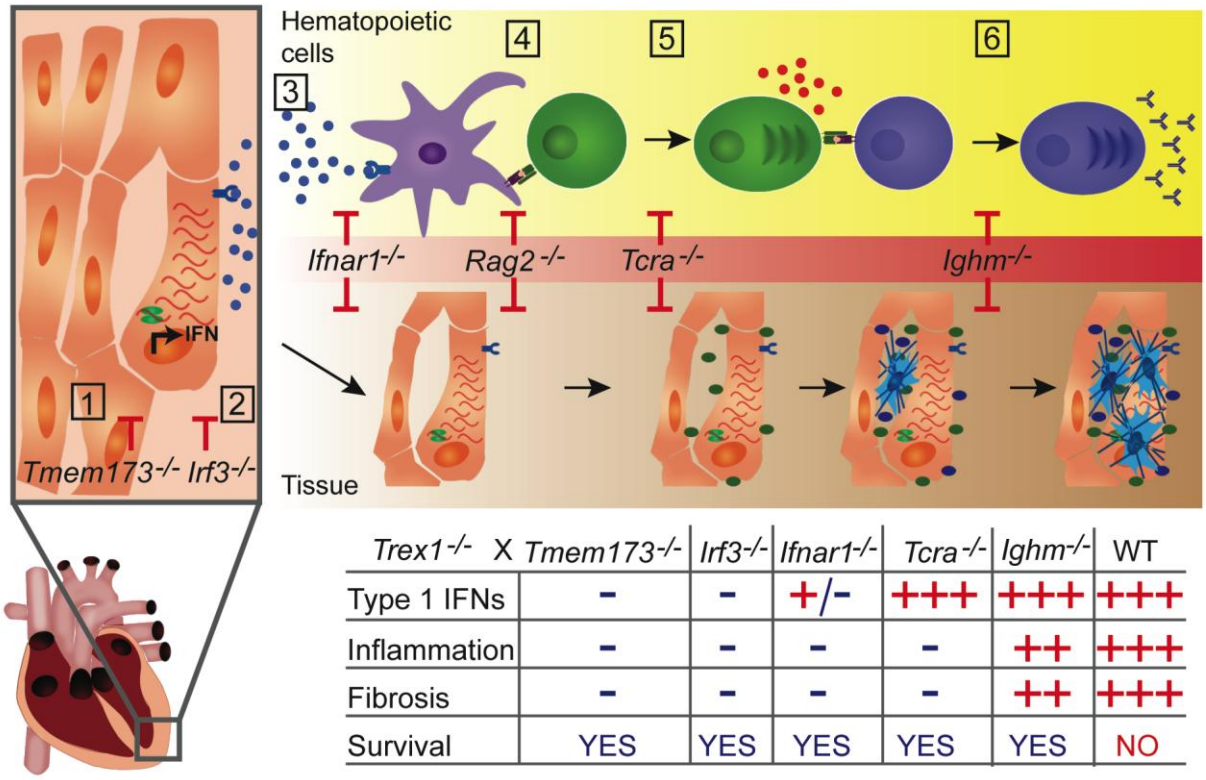
Figure S1: Kidney inflammation and autoantibodies in Trex1-deficient mice

(A) Total kidney histology scores are presented for mice of the indicated genotypes. These data include scoring of interstitial, glomerular, and tubular changes in each mouse. *** = P ≤ 0.0005.

(B) Histological scoring of tubular lesions in mice of the indicated genotypes. Unlike the interstitial inflammation and glomerulonephritis, the tubular changes were mild in *Trex1*-deficient mice and slightly more prominent in *Trex1/Ighm* DKO mice.

(C) Anti-dsDNA and **(D)** anti-chromatin ELISAs were performed with sera from *Trex1* KO (red) or *Trex1* WT (blue) mice. Sera from MRL-*lpr/lpr* mice served as a positive control for the anti-chromatin ELISA. Data are represented as arbitrary ELISA units from individual animals and the mean (horizontal line) of experimental groups.

Gall et al, Figure S2



Trex1 -/- X	Tmem173-/-	Irf3-/-	Ifnar1-/-	Tcra -/-	Ighm-/-	WT
Type 1 IFNs	-	-	+/-	+++	+++	+++
Inflammation	-	-	-	-	++	+++
Fibrosis	-	-	-	-	++	+++
Survival	YES	YES	YES	YES	YES	NO

Figure S2: Stepwise progression of endogenous intracellular DNA-triggered, lymphocyte-dependent autoimmunity

(1) In the absence of Trex1, a localized subset of heart cells accumulates sufficient endogenous DNA substrates to trigger an STING- and IRF3-dependent type 1 IFN response. *Trex1*$^{-/-}$ *Tmem173*$^{-/-}$ and *Trex1*$^{-/-}$*Irf3*$^{-/-}$mice are completely rescued from disease and are devoid of type 1 IFNs (•), inflammation and fibrosis.

(2) The type I IFNs produced by the initiating cells signal to hematopoietic cells to drive presentation of tissue-specific autoantigens and the autoimmune lymphocyte response. Disease in *Trex1*$^{-/-}$*Ifnar1*$^{-/-}$ mice is blocked here.

(3) Progression of disease is lymphocyte-dependent because *Trex1*$^{-/-}$*Rag2*$^{-/-}$ mice are rescued from inflammation, fibrosis and mortality, despite the chronic activation of the ISD pathway in non-hematopoietic cells.

(4) Activated autoreactive T cells differentiate into T effector cells, produce cytokines (•), and drive inflammation in the target tissue. *Trex1*$^{-/-}$*Tcra*$^{-/-}$ mice are rescued from inflammation, fibrosis and mortality.

(5) The activation of autoreactive B cells and subsequent tissue inflammation and fibrosis are T cell-dependent. Despite extensive tissue damage, *Trex1*$^{-/-}$*Ighm*$^{-/-}$ mice are significantly rescued from mortality, suggesting that B cells are necessary to progress rapidly to end-stage disease.

Supplemental Table S1

A) Histological scoring system for heart tissue

Tissue/ Stain	Regions†	Inflammation score	Myocardial Changes	Fibrosis
Heart/ H & E	Endocardial	0 = normal 1 = few inflammatory cells 2 = multifocal clusters ≤ 5 3 = multifocal clusters ≤ 10 4 = coalescing foci ≥ 10 5 = coalescing to diffuse foci	0 = normal 1 = few (1-3) cells 2 = multifocal foci (3-5) with degenerative changes 3 = multifocal foci (3-5) with necrotic changes 4 = ≥ 10 coalescing foci	0 = normal 1 = few inflammatory cells 2 = multifocal clusters ≤ 5 3 = multifocal clusters ≤ 10 4 = coalescing foci ≥ 10 5 = coalescing to diffuse foci
Heart/ Picrosirius	Endocardial Epicardial			0 = normal 1 = few inflammatory cells 2 = multifocal clusters ≤ 5 3 = multifocal clusters ≤ 10 4 = coalescing foci ≥ 10 5 = coalescing to diffuse foci

B) Histological scoring system for skeletal muscle, tongue, skin, stomach and brain tissue

Tissue	Regions	Inflammation score	Extent 1*	Extent 2**
Skeletal Muscle		0 = none 1 = minimal 2 = mild 3 = moderate 4 = marked	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%
Tongue		0 = none 1 = minimal 2 = mild 3 = moderate 4 = marked	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%
Skin		0 = none 1 = minimal 2 = mild 3 = moderate 4 = marked	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%
Stomach	Glandular Non-glandular Limiting region	0 = none 1 = minimal 2 = mild 3 = moderate 4 = marked	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%
Brian	Cerebellum Neocortex	0 = none 1 = minimal 2 = mild 3 = moderate 4 = marked	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%

C) Histological scoring system for colon tissue

Tissue	Regions‡	Inflammation score	Mucosa	Dysplasia	Extent 1*	Extent 2**
Colon	Cecum Proximal colon Mid colon Distal colon	0 = none 1 = minimal 2 = mild 3 = moderate 4 = marked	0 = no significant lesions 1 = mild hyperplasia 2 = moderate hyperplasia 3 = severe hyperplasia (crypt branching) 4 = severe hyperplasia (crypt herniation)	0 = normal 1 = indefinite 2 = low grade 3 = high grade 4 = high grade (carcinoma)	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%	0 = normal 1 = ≤ 5% 2 = 6-30% 3 = 31-60% 4 ≥ 60%

† The average of the endocardial and epicardial scores was considered the total fibrosis score

* Percentage of tissue affected in any manner

** Percentage of tissue affected in most severe manner

‡ In tissues where regions were graded, the sum of total region scores was combined to obtain a total tissue score

Table S1: Detailed histological scoring methods

Histological scoring was performed by a single, blinded observer on hematoxylin and eosin or picrosirius red stained sections. All tissues, except heart were scored for inflammation and extent of severity.

(A) In grading the H&E stained endocardial region, inflammation, myocardial changes and fibrosis were considered separately and the sum of all scores was considered the total tissue score. Fibrosis on picrosirius red stained sections was assigned a single score in the endocardial and epicardial regions and the average of the two scores was considered the total fibrosis score. Ranges of possible scores for endocardial inflammation and endocardial/epicardial fibrosis were 0-14 and 0-4.5, respectively

(B) Ranges of possible scores for each tissue were as follows: skeletal muscle 0-8; tongue 0-9; skin 0-7; stomach 0-23; brain all zero

(C) In grading the colon tissue, the cecum, proximal, mid and distal colon were considered separately and each region was assigned a subscore based on inflammation, mucosal lesions and dysplasia. The sum of the subscores was used to assign a total colitis score. Ranges of possible scores for the colon were 0-54