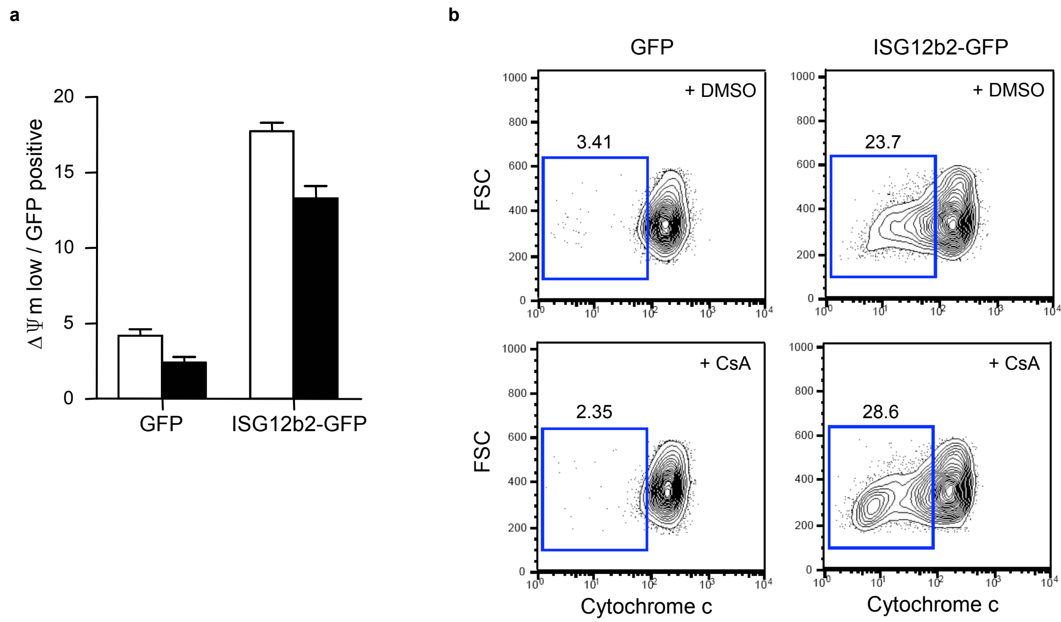
Supplementary Table 1. Primers used to construct full-length or various truncated mutants of ISG12b2.

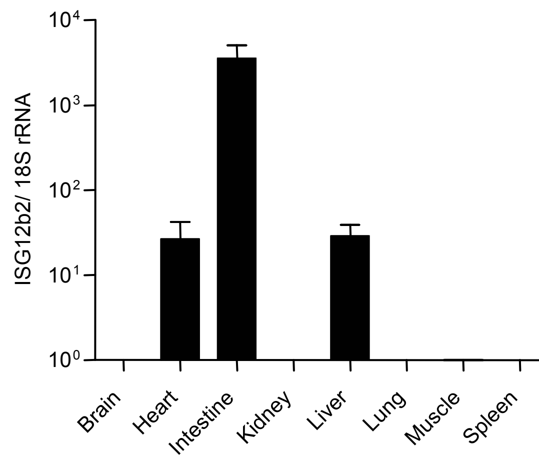
Construct name	Primer name	Primer sequence
ISG12b2 (No tag)	No tag-forward	5'-GGGGTACCCCCCAGGACAACTCATCATGAAG-3'
	No tag-reverse	5'-GGAATTCTCACTTCTCATAACTTGGAGG-3'
HA-ISG12b2 (N-HA)	N-HA-forward	5'-GGGGTACCCCAAGGAGATATACCATGACCAGCTACCCATACGATGTTCCAGATTACGCTCCAGGACAACTCATCATGAAG-3'
	N-HA-reverse	5'-GGAATTCCATCTCCTAAATTCAAATATCAC-3'
ISG12b2-HA (C-HA; FL-HA)	C-HA-forward	The same as no tag-forward
	C-HA-reverse	5'-GGAATTCCTTAAGCGTAATCTGGAACATCGTATGGGTACTCTCCTAAATTCAAATATGCCTTCTC-3'
94-283-HA	94-283-HA-forward	5'-GGGGTACCGCCACCATGGGAGCCTCCAGAGAGCA-3'
	94-283-HA-reverse	The same as C-HA-reverse
ISG12b2-GFP (FL-GFP)	FL-GFP-forward	5'-GGAATTCCCAGGACAACTCATCATGAAG-3'
	FL-GFP-reverse	5'-GGGGTACCTTCTCATAACTTGGAGGCTCC-3'
93-GFP	93-GFP-forward	The same as FL-GFP-forward
	93-GFP-reverse	5'-GGGGTACCCCGGATCCTTCAGCTCTAGCTCC-3'

Supplementary Figure 1



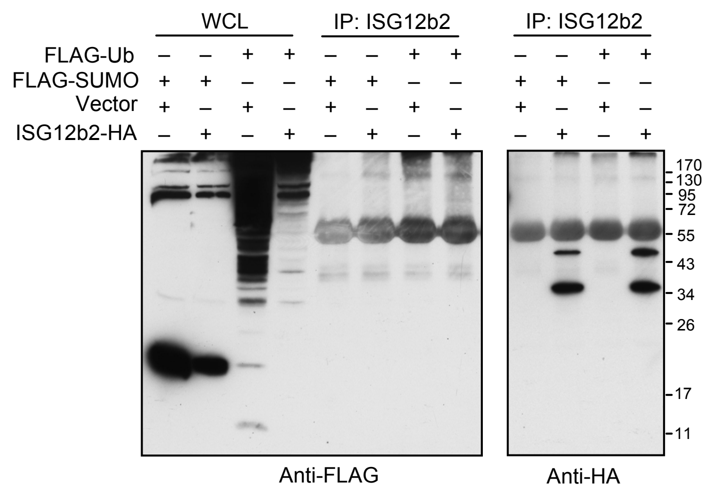
Supplementary Figure 1. ISG12b2 induces mPTP-independent cytochrome c release. Hepa 1-6 cells transfected with GFP or ISG12b2-GFP expression construct in the presence of DMSO or 5 μM CsA for 24 h were subjected to (a) MitoTracker Red staining or (b) intracellular staining for cytochrome c. Cells positive for GFP were gated to analyze MitoTracker Red or cytochrome c signal by flow cytometry.

Supplementary Figure 2



Supplementary Figure 2. Relative ISG12b2 mRNA expression in various tissues of C57BL/6 mice (n=3) were measured by real-time PCR, and the mRNA expression levels were normalized to the 18S rRNA for each sample. Muscle set as 1.

Supplementary Figure 3



Supplementary Figure 3. Lysates from HEK293T cells co-transfected with empty vector or ISG12b2-HA expression construct and FLAG-Ub or FLAG-SUMO constructs were immunoprecipitated with anti-ISG12b2 followed by immunoblotting with anti-FLAG (left panel) or anti-HA (right panel). WCL, whole-cell lysate. Molecular mass markers are indicated at right.

Supplementary Materials and Methods

Real-time PCR analysis of tissue ISG12b2 expression

Total RNA was extracted from mouse tissues Trizol reagent (Invitrogen) according to manufacturer's instructions. In oder to determine the relative tissue distribution of ISG12b2, total RNA (1.5 μg) was treated with DNase I (Promega Corporation, WI, USA) and cDNA synthesis was performed using random hexamers as primer. For real-time PCR, the $^{2-\Delta\Delta CT}$ method was used to quantify the relative changes of gene expression. Real-time PCR for ISG12b2 was conducted using TaqMan Gene Expression assays, and 18S rRNA was conducted using TaqMan Ribosomal RNA Control Reagents (Applied Biosystems, Foster City, CA) containing a mixture of unlabeled PCR primers and TaqMan MGB probe (FAM dye labeled) specific for each gene. The PCR amplification cycles were 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The resultant PCR products were measured by ABI 7500 Real-Time PCR System (Applied Biosystems). All quantifications were normalized to the level of internal control 18S rRNA.