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Supplemental Information

Pro-inflammation Associated with a Gain-of-Function Mutation (R284S) in the Innate Immune Sensor STING

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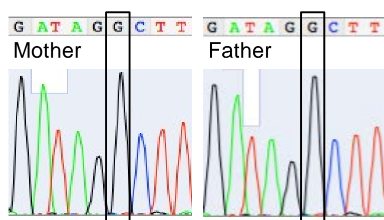
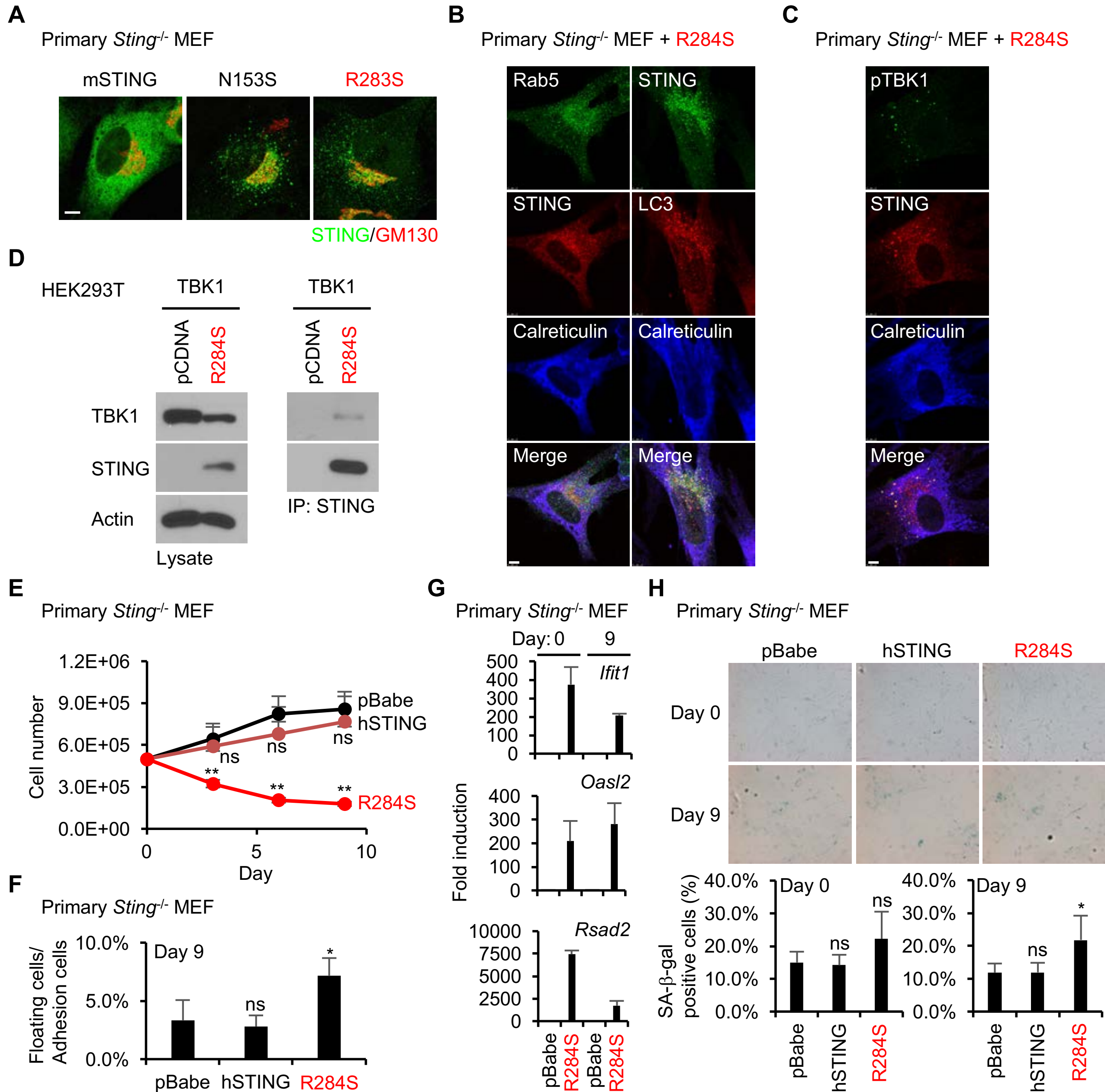
Supplementary Figure 1



Supplementary Figure 1. The sequencing data for the parents. Related to Figure 1.
The patient's parents don't have same mutation.

Supplementary Figure 2



Supplementary Figure 2. STING (R284S) constitutively localizes to the peri-nuclear area with TBK1. Related to Figure 2.

(A) The mutants of murine STING (N153S, R283S) were expressed in primary *Sting*$^{-/-}$ MEF cells using retrovirus and then the localization was observed as described in Figure 2A. Scale bar: 7.5 µm.

(B and C) Human STING R284S variant was expressed and then the localization was observed as described in Figure 2A. Rab5 and LC3 are the early endosome and the autophagosome markers, respectively. Scale bar: 5 µm.

(D) TBK1 was precipitated by STING R284S.

(E) Growth curve of primary *Sting*$^{-/-}$ MEF cells reconstituted with pBabe, hSTING, or R284S using retrovirus.

(F) The cell numbers in culture media (floating cells) and on dish (adhesion cells) were counted after staining with trypan blue. Almost all cells in culture media were trypan blue-positive and those on dish were trypan blue-negative. The ratio of trypan blue-positive cells in culture media versus trypan blue-negative cells on dish at day 9 is shown as y-axis.

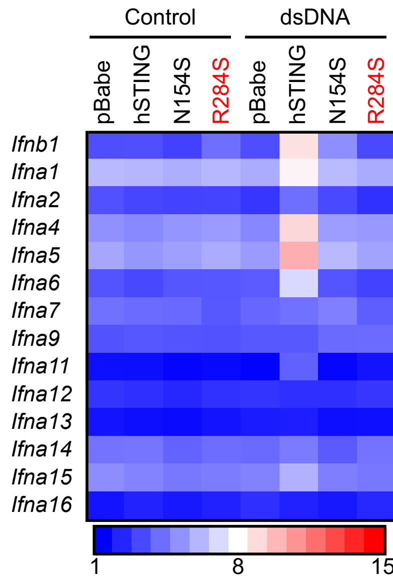
(G) Gene expression at day 0 and 9.

(H) Senescence-associated β-galactosidase assay.

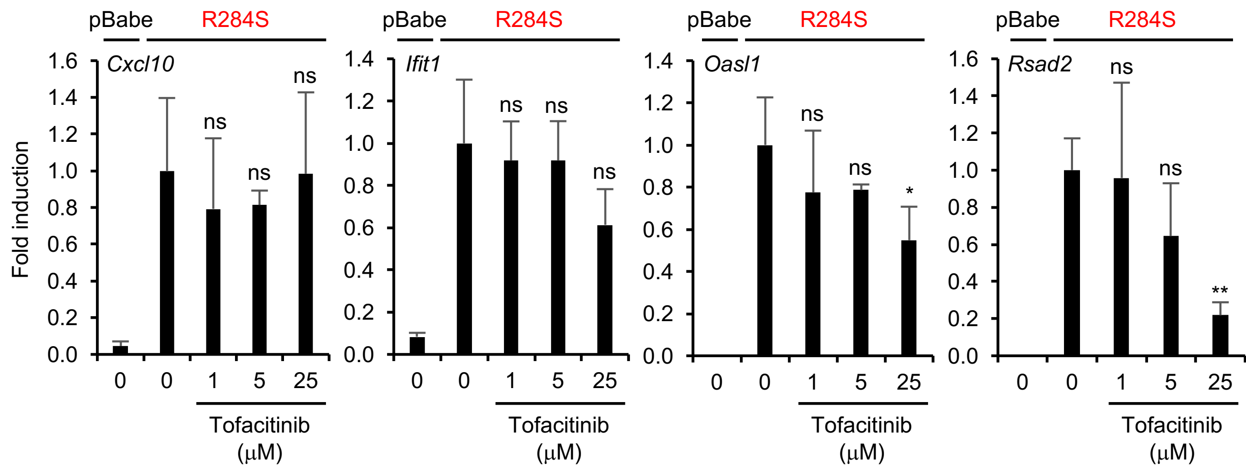
Data shown here are the averages ± SD (n = 3). * and ** indicate significant difference $p<0.05$ and $p<0.01$ respectively that were determined by Student's t-test. ns means not significant.

Supplementary Figure 3

A

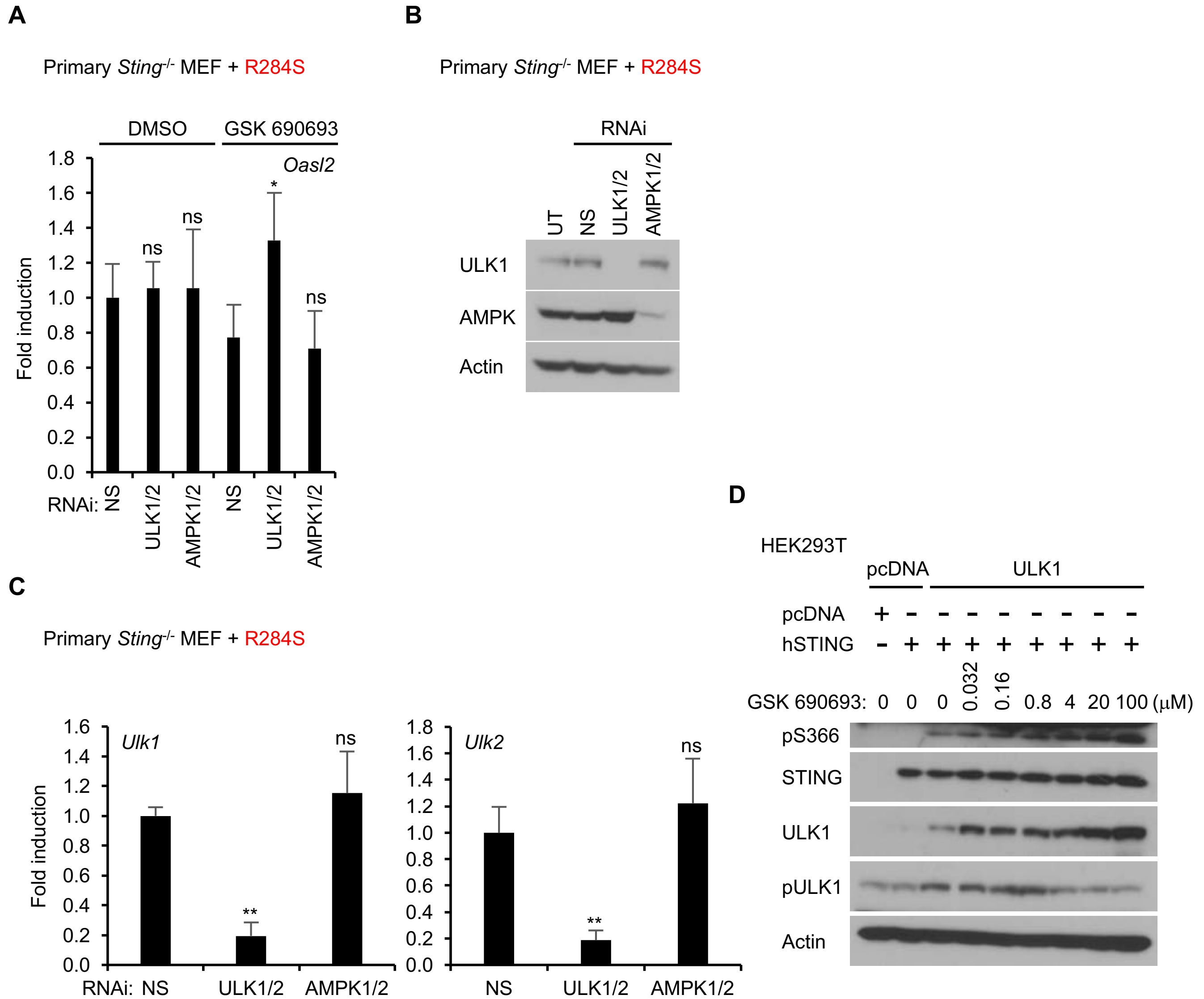


B



Supplementary Figure 3. Type I IFN production induced by STING (R284S) is very low. Related to Figure 3.
(A) The microarray data as described in Figure 2C focusing on IFN genes.
(B) The reconstituted *Sting*-/- MEF cells expressing R284S were treated with Tofacitinib for 9 h. Realtime PCR was performed with the indicated probes.
Data shown here are the averages ± SD (n = 3). * and ** indicate significant difference $p<0.05$ and $p<0.01$ respectively that were determined by Student's t-test. ns means not significant.

Supplementary Figure 4



Supplementary Figure 4. ULK1-mediated STING phosphorylation is promoted by GSK 690693. Related to Figure 4.

(A) The reconstituted *Sting*$^{-/-}$ MEF cells expressing R284S were treated with siRNA (NS; nonspecific, ULK1 and 2, AMPKa1 and 2). Then, the cells were treated with GSK 690693 (20 mM) for 9 h. Realtime PCR was performed with the indicated probes.

(B and C) Knockdown efficiency was confirmed by western blots (B) or realtime PCR (C). UT means untreated.

(D) HEK293T cells transfected with plasmids encoding hSTING and ULK1 for 24 h were treated with GSK 690693 for 6 h. Wester blots were performed for the cell lysates with the indicated antibodies.

Data shown here are the averages ± SD (n = 3). * and ** indicate significant difference $p<0.05$ and $p<0.01$ respectively that were determined by Student's t-test. ns means not significant.