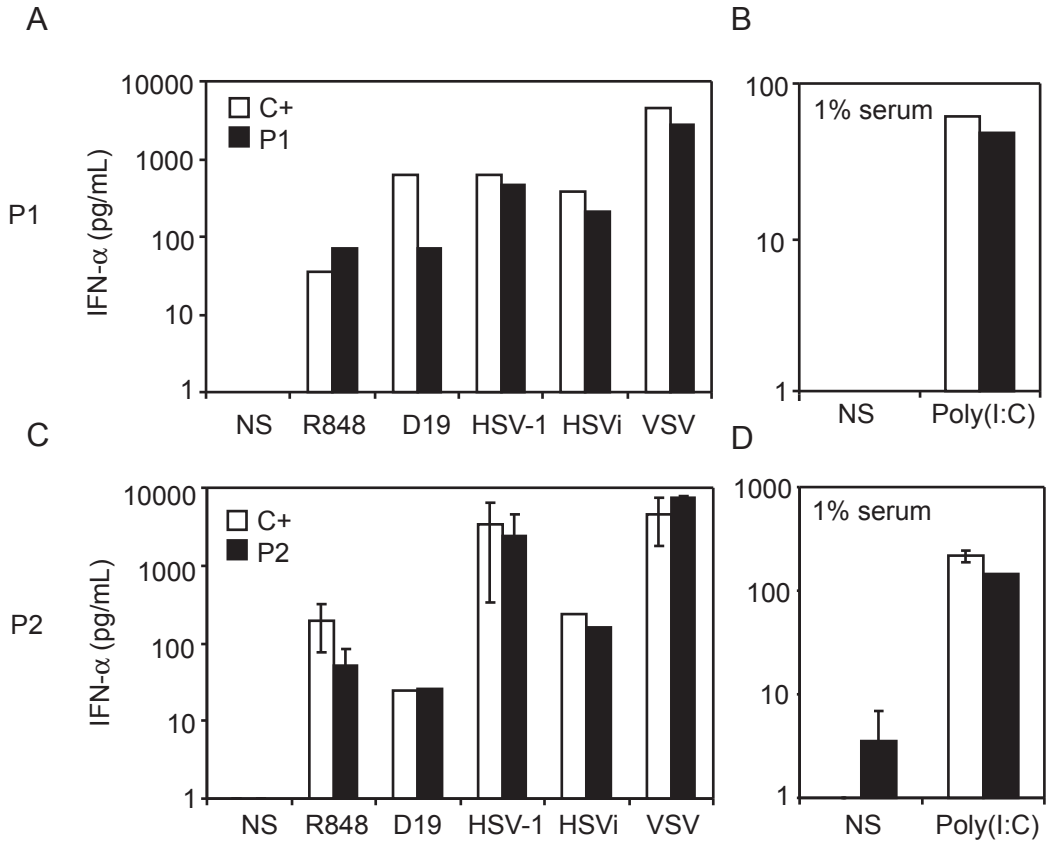
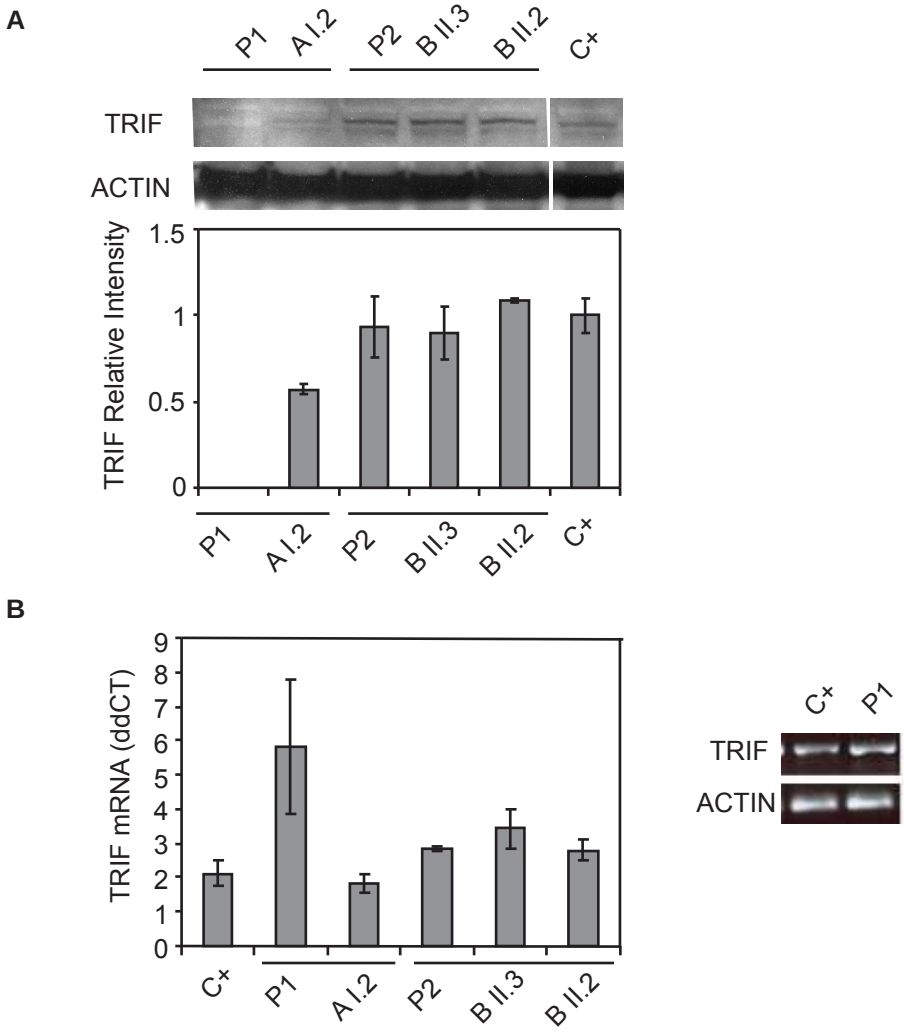
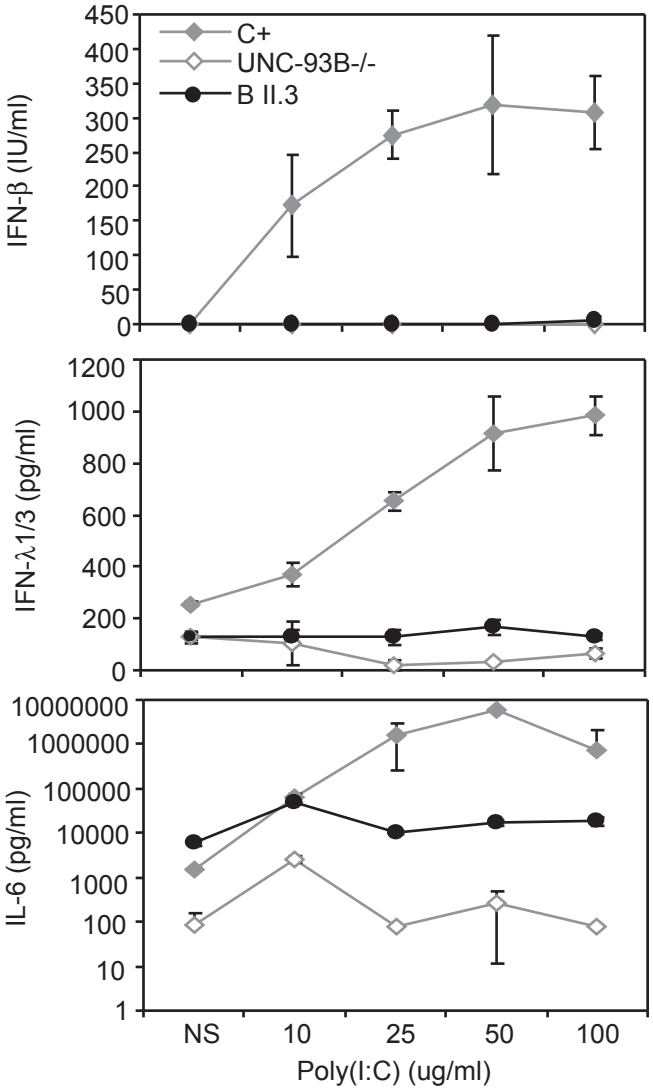


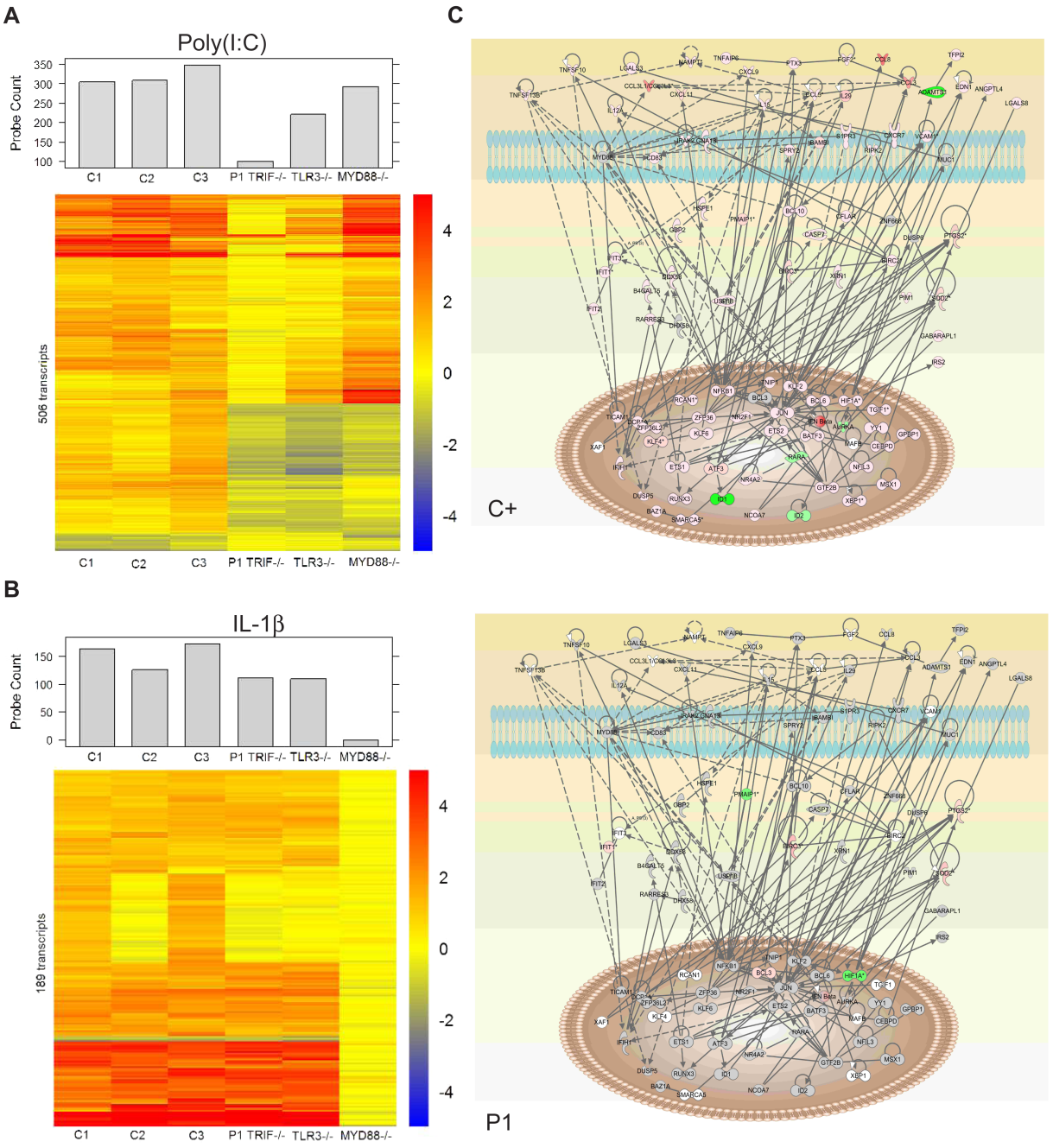

Supplementary Figure 1: Cellular phenotype in PBMCs of P1 and P2. Production of IFN-α in healthy controls (C+), P1 and P2 PBMCs in response to R848 (TLR7/8), D19 (TLR9), poly(I:C) (TLR3), HSV-1, UV inactivated HSV-1 and VSV. PBMCs were either stimulated in 10% FBS supplemented RPMI (A,C) or in 1% human serum supplemented RPMI (B,D).



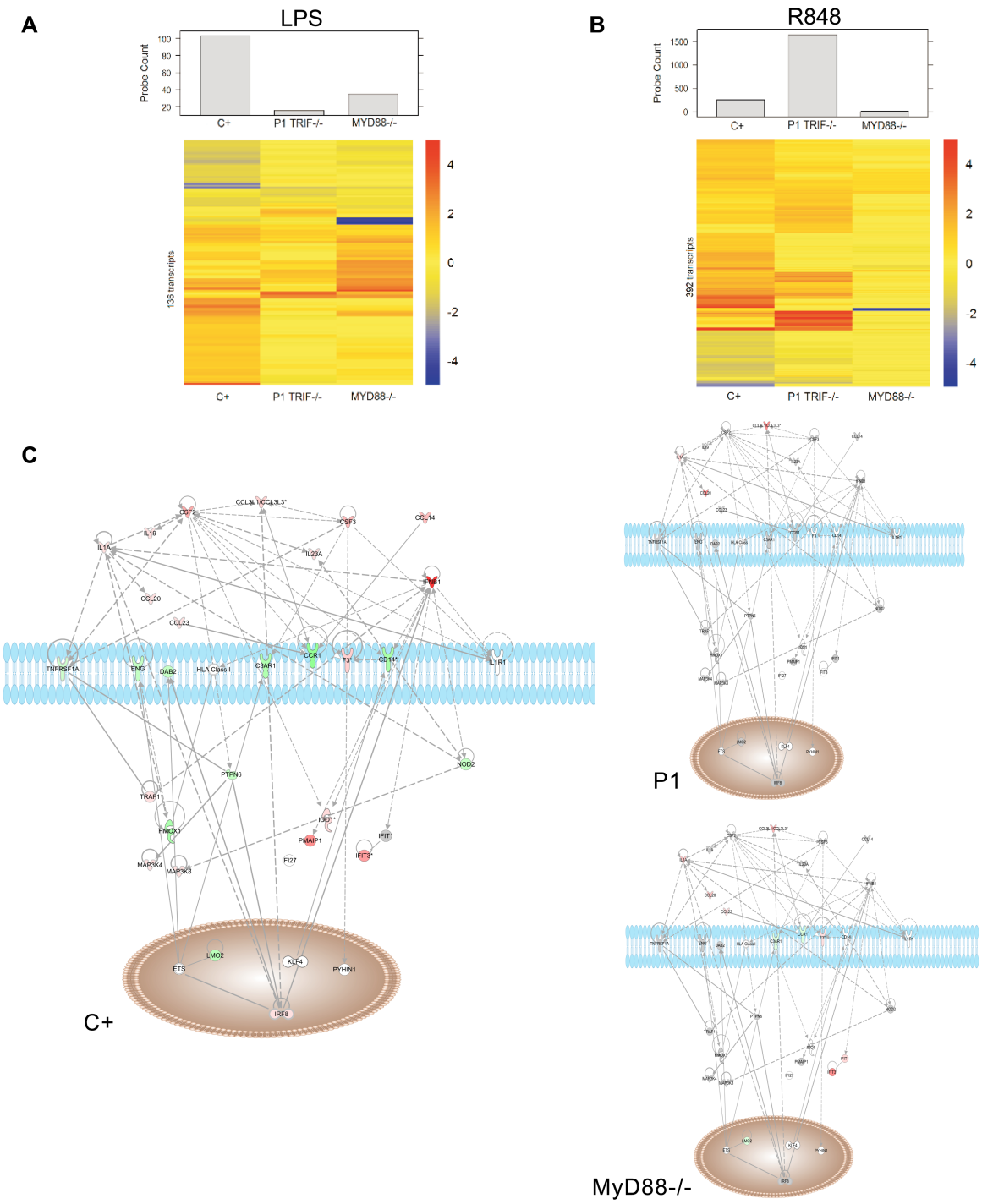

**Supplementary Figure 2**: **Protein and mRNA expression of the TRIF mutations in EBV-B cells**. **(A)** Protein lysates extracted from EBV-B cells derived from the indicated family members of P1 and P2 as well as an unrelated control (C+) were assayed for immunoblot expression of TRIF. TRIF expression levels were quantified by densitometry results normalized with respect to ACTIN levels and expressed as relative intensity of TRIF. All samples were migrated on the same blot. A representative blot from three independent experiments is shown, mean values +/- SEM were calculated.**(B)** TRIF cDNA was quantified in RNA extracted from EBV-B cells by real time PCR. Data is represented as relative fold change (ddCt units) where GUS was used for normalization. RT-PCR of full length TRIF cDNA is shown with ACTIN cDNA as an internal control. Data is representative of three independent experiments, mean values +/- SEM are shown.



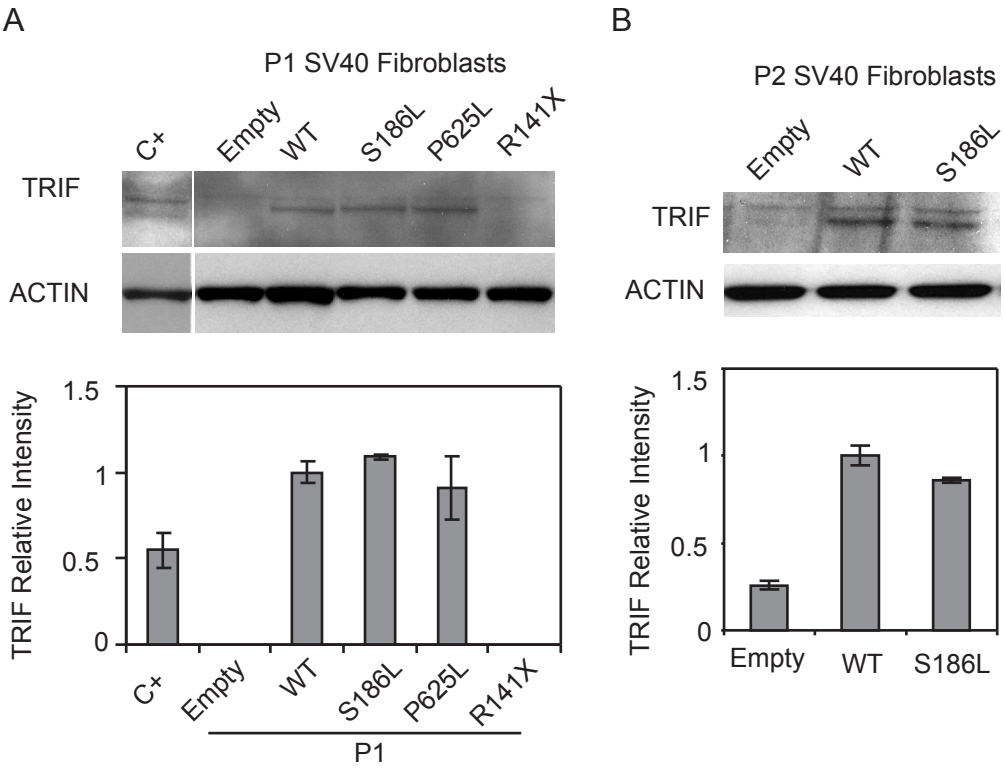

Supplementary Figure 3: SV-40 fibroblast phenotype of P2's mother TRIF$^{+/-}$ (B II.3). SV-40 transformed fibroblasts were stimulated with increasing doses of poly(I:C) for 24hrs and cytokine production assessed by ELISA. C+ represents an unrelated healthy control, UNC-93B$^{-/-}$ was used as a negative control. Graphs represent the average of three independent experiments, mean values +/- SEM are shown.



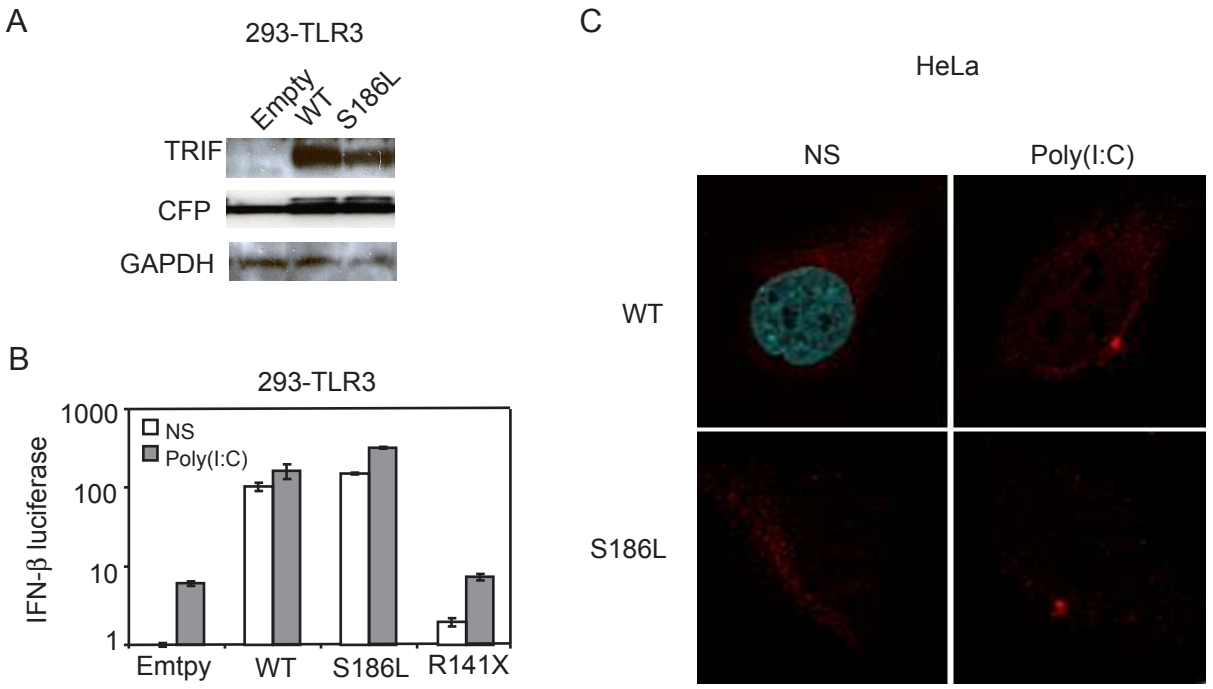

**Supplementary Figure 4:** Genome-wide transcript analysis of P1's fibroblasts. Total number of differentially regulated transcripts expressed as bar graphs (upper panels) or as heat maps (lower panels) . Transcripts from SV40 transformed fibroblasts of three controls (C1, C2, and C3), P1 TRIF$^{-/-}$, TLR3$^{-/-}$, and MyD88$^{-/-}$ patients found to be differentially regulated after 4hrs of stimulation with poly(I:C) **(A)** or IL-1• (**B**). The probe count is the sum of all probes with fold change values greater than 2 (up- or downregulated). Heat maps represent a hierarchical clustering of transcripts differentially expressed upon either poly (I:C) or IL-1• stimulation (based on a difference of 100 in intensity and a 2-fold change with respect to baseline in the corresponding non-stimulated sample). Fold changes are represented by a color gradient where red = upregulation; yellow = no change; blue = downregulation. **(C)** Networks generated from differentially expressed transcripts in control and P1 fibroblasts after 4hrs of poly(I:C) simulation, with Ingenuity pathway analysis software (Ingenuity Systems, www.ingenuity.com). Direct biological relationships between genes are represented with a solid line, and indirect relationships with a dotted line. All lines are supported by at least one reference from the literature. Nodes are arranged according to their cellular distribution. Upregulated transcripts are in red and downregulated transcripts in green.



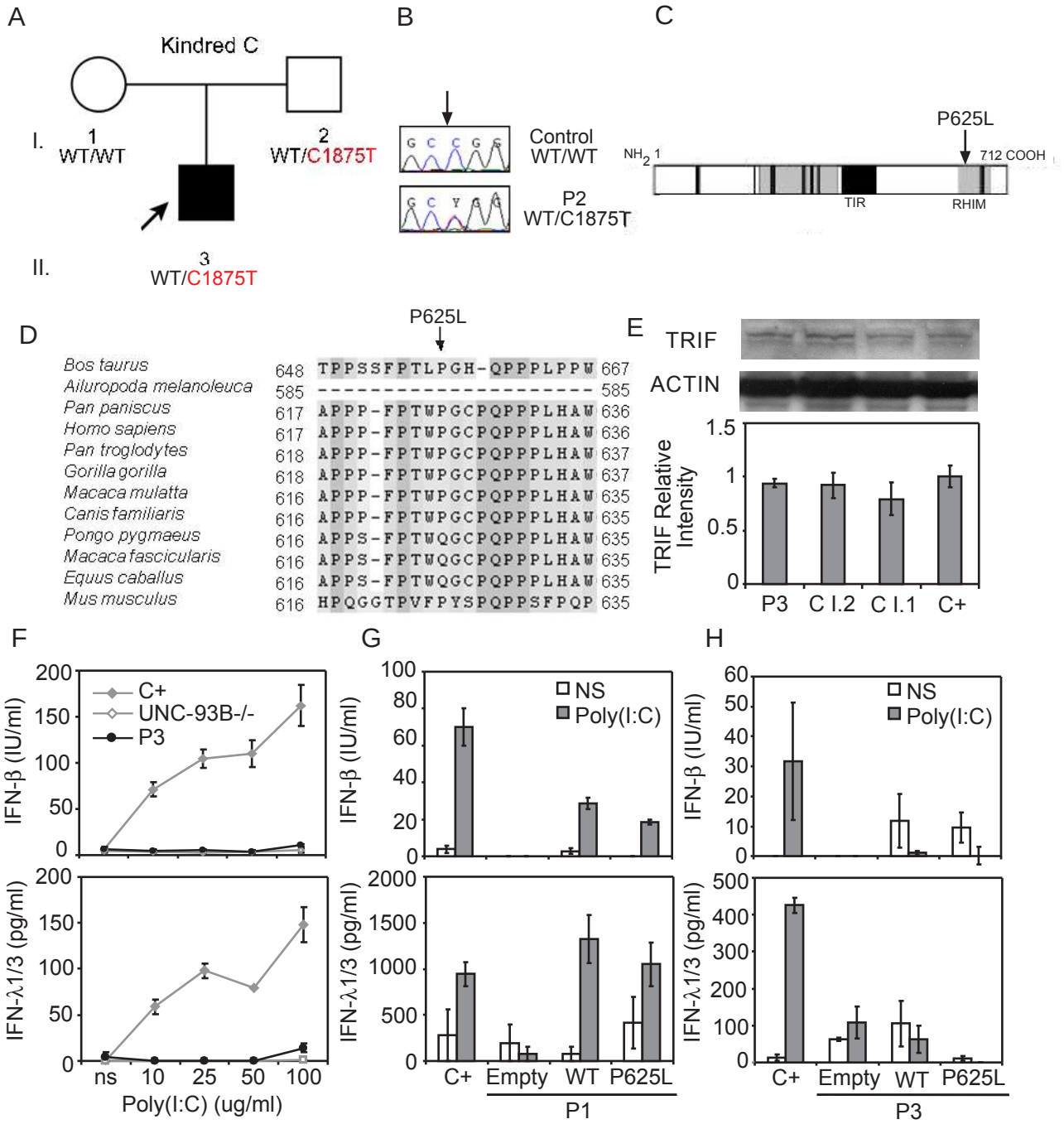

**Supplementary Figure 5: Genome wide transcriptome analysis of P1's PBMCs.** Total number of differentially regulated transcripts expressed as bar graphs (upper panels) or as heat maps (lower panels) . Transcripts from PBMCs of a control (C+), P1 TRIF$^{-/-}$, and MyD88$^{-/-}$ patient found to be differentially regulated after 2hrs of stimulation with LPS **(A)** or R848 **(B)**. The probe count is the sum of all probes with fold change values greater than 2 (up- or downregulated). Heat maps represent a hierarchical clustering of transcripts differentially expressed upon either LPS or R848 stimulation (based on a difference of 100 in intensity and a 2-fold change with respect to baseline in the corresponding non-stimulated sample). Fold changes are represented by a color gradient where red = upregulation; yellow = no change; blue = downregulation. **(C)** Networks generated from differentially expressed transcripts in control PBMCs after 2hrs of LPS simulation, with Ingenuity pathway analysis software (Ingenuity Systems, www.ingenuity.com). Direct biological relationships between genes are represented with a solid line, and indirect relationships with a dotted line. All lines are supported by at least one reference from the literature. Nodes are arranged according to their cellular distribution. Upregulated transcripts are in red and downregulated transcripts in green.




Supplementary Figure 6: TRIF protein expression in retrovirally transduced SV40 fibroblasts. (A) Immunoblot analysis of protein lysates extracted from control SV40 fibroblasts (C+) or P1's cells transduced with empty vector, WT, S186L, or R141X and (B) lysates from P2's cells transduced with empty vector, WT or S186L. By default all transduced cells are cultured in the presence of doxycycline to repress the expression of TRIF. Cells were left for 48hrs in the absence of doxycycline before assaying for expression and function of TRIF. Relative quantification of TRIF protein levels normalized with respect to ACTIN is shown below the corresponding blots. A representative blot is shown. Mean values +/- SEM were calculated from three independent experiments.




Supplementary Figure 7: Transient transfection of AD TRIF allele S186L. (A) Immunoblot analysis of protein lysates from 293HEK-TLR3 transfected cells, using an antibody recognizing the N-terminal of TRIF. CFP was co-transfected as a control for transfection efficiency. (B) 293HEK-TLR3 cells were transfected with empty vector, WT, S186L, or R141X TRIF plasmids along with IFNβ-Luc/NF•B-Luc and RL-TK vectors to assess IFN-β and NF-•B promoter induction upon overexpression of TRIF. Transfected cells were either left unstimulated or stimulated with 50ug/ml of poly(I:C) for 4hrs. Firefly luciferase values were normalized with renilla values. Total DNA transfected was held constant and supplemented by empty vector in all samples. Mean values +/- SEM were calculated from at least three independent experiments. (C) Confocal images of Hela cells transfected with 0.2ng of HA-tagged WT and S186L TRIF. The cells were either left unstimulated or stimulated for 30min with 10ug/ml of poly(I:C). Cells were then fixed and stained with anti-HA antibody. The nuclei were stained with DAPI. The formation of speckle like structures representing homo-oligomerization and activation of TRIF following poly(I:C) stimulation was observed.




Supplemental Figure 8: A non-deleterious heterozygous TRIF mutation in P3. (A) Family pedigree of kindred C with allele segregation of the mutation. The HSE patient is shaded in black. Roman numerals (left margin) indicate generations. An arrow indicates the proband. (B) Automated sequencing profiles for the TRIF C1875>T mutation in genomic DNA isolated from leukocytes from a healthy unrelated control and in the patient, P3. (C) A representation of the TRIF protein indicating the amino acid position, P625L, affected by the C1875>T mutation. (D) Multiple alignment of TRIF amino acid sequence surrounding the mutation P625L. (E) TRIF protein expression by immunoblot analysis from EBV-B cell lysates from a healthy control C+, P3 and his parents. TRIF relative intensity was quantified by densitometry results normalized with respect to ACTIN levels. A representative blot is shown, mean values +/- SEM were calculated form three independent experiments. (F) Cytokine production in SV40 fibroblasts stimulated for 24hrs with increasing doses of poly(I:C). Mean values +/- SEM were calculated from at least three independent experiments. (G) Control (C+) SV40 fibroblasts, and P1's fibroblasts retrovirally transduced with empty vector, WT, or P625L TRIF were stimulated with 25ug/ml of poly(I:C) for 24hrs and the production of cytokines assessed by ELISA. Mean values +/- SEM were calculated from three independent experiments. (H) Cytokine production was assessed in control (C+), P3's fibroblasts retrovirally transduced with either empty vector, WT TRIF or P625L TRIF following stimulation with 25ug/ml of poly(I:C) for 24hrs. Mean values +/- SEM were calculated from three independent experiments.