Figure S1, related to Figure 2. Effect of Ethylene Perception on Bacterial Growth and Symptom Development in Xcv-infected Tomato

(A) Increased growth of Xcv or Xcv Δ*xopD* in Pearson wild-type (WT) and *Nr* (*Never ripe*) tomato leaves. The ET insensitivity conferred by *Nr* mutants arises from a mutation in SlETR3, an ET-inducible ET receptor required ET perception and signaling (Wilkinson et al., 1995). Growth of Xcv (grey bars) or Xcv Δ*xopD* (red bars) in Pearson tomato leaves compared to that of Xcv (blue bars) or Xcv Δ*xopD* (green bars) in the Pearson *Nr* tomato leaves. Leaves were infiltrated with a 10^5 cfu/ml suspension of bacteria. Data points represent mean cfu/cm^2 ± SD (n = 3). (B) Delayed disease symptom development in *Nr* tomato leaves inoculated with Xcv or Xcv Δ*xopD*. Tomato leaves inoculated with strains described in (A) were photographed at 10 and 14 days post-inoculation (DPI). This experiment was repeated three times with similar results.

Figures S2, related to Figure 3. *SlERF4* mRNA is Abundant in Tomato Leaves and Induced by Xcv Infection and SlERF4 interacts with XopD in nucleus.

(A) The mRNA levels of eight tomato ERF transcription factors in infected tomato leaves at 4 DPI. Tomato VF36 leaves were infiltrated with 10 mM $MgCl_2$ (blue bars), or a 10^5 cfu/ml suspension of Xcv (grey bars) or Xcv Δ*xopD* (red bars) and RNA was isolated from the infected tissue at 4 DPI. qPCR was performed to analyze the mRNA levels of eight characterized ERFs: *SlERF1*, *SlERF2*, *SlERF4*, *Pti4*, *Pti5*, *Pti6*, *TSRF1*, and *TERF1*. *ACTIN* mRNA was used for normalization. Mean value of *SlERF1* expression of the sample infiltrated with 10 mM $MgCl_2$ was normalized to 1. Data points represent

mean ± SD (n = 3). (B) From the data set used in (A), relative expression values for each *ERF* were determined against the average value of the sample infiltrated with 10 mM $MgCl_2$ at 4 DPI. (C) SlERF4 and SlERF4(K53R) are localized in the plant nucleus. *Nicotiana benthamiana* leaves were infiltrated with a 6 x 10^8 cfu/ml suspension of *Agrobacterium tumefaciens* expressing YFP-SlERF4 and YFP-SlERF4(K53R). After 40 h, DAPI was used to stain plant nuclei. YFP fluorescence signal and DAPI signal were captured by confocal microscope and merged to show nuclear localization. (D) Immunoblot (IB) analysis of *N. benthamiana* leaf tissues used in (C). Total protein was extracted at 40 hours post-inoculation (HPI) and then analyzed by IB analysis using on anti-GFP antibody. Expected protein molecular weights: YFP-SlERF4 and YFP-SlERF4(K53R) = 52 kDa. (E) IB analysis of *N. benthamiana* leaf tissue extracts used in Figure 3B. Total protein was extracted at 40 HPI and then analyzed by IB analysis using anti-His and anti-FLAG antibodies. 1, XopD-cCFP + nYFP-GUS; 2, GUS-cCFP + nYFP-SlERF4; 3, XopD-cCFP + nYFP-SlERF4. Expected protein molecular weights: XopD-cCFP = 95 kDa, nYFP-GUS = 90 kDa, GUS-cCFP = 80 kDa, and nYFP-SlERF4 = 45 kDa. (F) YFP-SlERF4 is localized in the subnuclear foci with XopD or XopD(C685A) in the plant nucleus. *N. benthamiana* leaves were infiltrated with a 6 x 10^8 cfu/ml suspension of *A. tumefaciens* expressing YFP-SlERF4 and vector, XopD, or XopD(C685A). After 40h, YFP fluorescence signal was captured by confocal microscope. White bars = 10 µm. (G) IB analysis of *N. benthamiana* leaf tissues used in (F). Total protein was extracted at 40 HPI and then analyzed by IB analysis using on anti-GFP and anti-XopD antibody. 1. YFP-SlERF4 + vector; 2, YFP-SlERF4 + XopD; 3, YFP-SlERF4 + XopD(C685A). Expected protein molecular weights: YFP-SlERF4 = 52

kDa, XopD and XopD(C685A) = 86 kDa. (H) GST Pull-down assay of SlERF4-His and GST, GST-XopD(WT, C685A, V333P, or ΔR1ΔR2) *in vitro*. Recombinant GST- or GST-XopD(WT, C685A, V333P, or ΔR1ΔR2) bound to glutathione-sepharose beads was incubated with *E. coli* cell lysate containing SlERF4-His. Beads were washed three times with lysis buffer. Protein was eluted and analyzed by IB assay using anti-His and anti-GST sera. These experiments were repeated three times with similar results.

Figure S3, related to Figure 6. Sumoylation Site Identification in SlERF4

(A) Predicted sumoylation sites in SlERF4 by SUMOsp 2.0 program (http://bioinformatics.lcd-ustc.org/sumosp) (Ren et al. 2009). Four putative sumoylation sites, K3, K53, K92, and K197, were predicted with a score threshold above 2. Type I sites followed the Ψ-K-X-E (ψ, hydrophobic amino acid; x, any amino acid) motif. (B) *In vivo* sumoylation assay shows that SlERF4 is sumoylated at K53. *N. benthamiana* leaves were infiltrated with two *A. tumefaciens* strains (8×10^8 cfu/ml total concentration): one strain expressing SlERF4(K3A, K53A, K92A, or K197A)-FLAG-His and the other strain coexpressing HA-SlSUMO1 and XopD(WT or C685A). Leaf protein was monitored at 40 HPI by immunoblot (IB) analysis using anti-XopD, anti-FLAG, and anti-HA sera. Sumoylated SlERF4-FLAG-His proteins were enriched by Ni-NTA resin. Asterisk indicates sumoylated SlERF4-FLAG-His detected by anti-FLAG. Ponceau S-stained Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) large subunit is used as a loading control. This experiment was repeated three times with similar results. (C) IB analysis of *N. benthamiana* leaf tissue used in Figure 6C. Total protein was extracted at 40 HPI and then analyzed by IB analysis using anti-XopD and anti-FLAG

antibody. Lane 1 = vector + vector. Lane 2 = SlERF4-FLAG-His + vector. Lane 3 = SlERF4-FLAG-His + XopD. Lane 4 = SlERF4-FLAG-His + XopD(C685A). Lane 5 = SlERF4(K53R)-FLAG-His + vector. Lane 6 = SlERF4(K53R)-FLAG-His + XopD. Lane 7 = SlERF4(K53R)-FLAG-His + XopD(C685A). Ponceau S-stained Rubisco large subunit is used as a loading control. (D) Schematic of the SlERF4 protein. Conserved glutamic acid residue (E55) in the ψKxE consensus for sumoylation and AP2/ERF DNA binding domain are indicated. (E) *In vivo* sumoylation assay shows that mutation of E55 prevented SlERF4 sumoylation. *N. benthamiana* leaves were infiltrated with two *A. tumefaciens* strains (8 x 10^8 cfu/ml total concentration): one strain expressing vector, SlERF4-FLAG-His or SlERF4(E55A)-FLAG-His and the other strain coexpressing HA-SlSUMO1 and XopD(WT or C685A). Leaf protein was monitored at 40 HPI by IB analysis using anti-XopD, anti-FLAG, and anti-HA sera. Sumoylated SlERF4-FLAG-His proteins were enriched by Ni-NTA resin. Ponceau S-stained Rubisco large subunit is used as a loading control. This experiment was repeated three times with similar results. (F) SlERF4(E55A) mutant has reduced transcription activity. Transgenic 8xGCC-GUS reporter *N. benthamiana* leaves were infiltrated with two *A. tumefaciens* strains (4 x 10^8 cfu/ml total concentration) expressing two different fusion proteins (vector + vector, SlERF4-FLAG-His + vector, SlERF4-FLAG-His + XopD, SlERF4-FLAG-His + XopD(C685A), SlERF4(E55A)-FLAG-His + vector, SlERF4(E55A)-FLAG-His + XopD, or SlERF4(E55A)-FLAG-His + XopD(C685A)). Leaves were collected at 40 HPI and GUS activity was quantified. Relative GUS activity values were determined using the average value of the vector + vector control. Error bars = SD (n=3). Different letters above bars indicate statistically significant differences between the samples (one-way

ANOVA and Tukey's HSD test, $P < 0.05$) (G) XopD-dependent desumoylation of SlSUMO1-SlERF4 protein conjugate. *N. benthamiana* leaves were infiltrated with two *A. tumefaciens* strains (8×10^8 cfu/ml total concentration): one strain expressing vector or SlERF4-FLAG-His and the other strain coexpressing HA-SlSUMO1 and XopD(WT, C685A, V333P, or ΔR1ΔR2). Leaf protein was monitored at 40 HPI by IB analysis using anti-XopD, anti-FLAG, and anti-HA sera. Sumoylated SlERF4-FLAG-His proteins were enriched by Ni-NTA resin. Ponceau S-stained Rubisco large subunit is used as a loading control. This experiment was repeated three times with similar results. (H) SUMO protease activity of XopD mutant proteins. *N. benthamiana* leaves were co-infiltrated with a suspension of *A. tumefaciens* expressing tomato HA-SlSUMO1 with vector or XopD(WT, C685A, V333P, or ΔR1ΔR2). Strains were mixed equally and infiltrated into the leaf at a final density of 8×10^8 cfu/ml. Total proteins were extracted from the infected leaves at 40 HPI and analyzed by IB analysis using anti-HA or anti-XopD sera. Ponceau S-stained Rubisco large subunit is shown as a loading control. These experiments were repeated three times with similar results.

Figure S4, related to Figure 7. *SlERF4* mRNA Level in Silenced VF36 Tomato Leaves used in Figure 7A.

SlERF4 mRNA levels in leaves from VIGS control (TRV2) and three *SlERF4*-silenced (TRV2-SlERF4) tomato plants were compared by semi-quantitative RT-PCR. Total RNA was extracted from the leaves infiltrated with 10 mM $MgCl_2$, or a 10^5 cfu/ml suspension

of Xcv, or Xcv ∆*xopD* at 0 DPI. *ACTIN* gene was used for control. All PCR products were separated on 2% agarose gel and stained with SYBR Green.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth

Escherichia coli DH5α and *Agrobacterium tumefaciens* C58C1 (pCH32) (Tai et al., 1999) strains were grown on Luria agar medium at 37 and 28 °C, respectively. *Xanthomonas euvesicatoria* (Xcv) 85-10 and Xcv Δ*xopD* null mutant strains (Kim et al., 2011) were grown on nutrient yeast glycerol agar (NYGA) (Turner et al., 1984) at 28 °C. *E. coli* antibiotic selection was 100 μg/ml carbenicillin and/or 50 μg/ml kanamycin. *A. tumefaciens* antibiotic selection was 100 μg/ml rifampicin, 5 μg/ml tetracycline, and/or 50 μg/ml kanamycin. Xcv antibiotic selection was 100 μg/ml rifampicin, 50 μg/ml spectinomycin, and/or 50 μg/ml kanamycin.

Plasmid Construction

Standard DNA cloning methods were used (Sambrook et al., 1989). All primer sequences are listed in Supplemental Table S1. For the BiFC constructs, *xopD*, *SlERF4*, and *GUS* were PCR amplified (primer sets JG478/JG9, JG444/JG447, and JG114/JG683, respectively), cloned into the pENTR/D/TOPO, and then recombined into the Gateway-compatible BiFC vectors pXCGW (vector for C-terminal fusion of C-terminal non-fluorescent domain of CFP (cCFP)) and pNXGW (vector for N-terminal fusion of N-terminal non-fluorescent domain of YFP(nYFP)) (courtesy of Wolf Frommer) using LR clonase (Invitrogen) to generate pXCGW(*xopD-cCFP*), pNXGW(*nYFP-SlERF4*), pXCGW(*GUS-cCFP*), and pNXGW(*nYFP-GUS*). For the GST-XopD constructs (WT , C685A, V333P, or ΔR1ΔR2), *xopD (WT, C685A, V333P, or ΔR1ΔR2)*

was PCR amplified (primer set JG477/MB371), cloned into the pCR-BluntII creating pCR-BluntII(*xopD(WT, C685A, V333P, or ΔR1ΔR2*)). The *Bgl*II-*Xho*I fragment was then subcloned into pGEX-5X-3 (GE Healthcare Life Sciences), creating pGEX-5X-3(*xopD(WT, C685A, V333P, or ΔR1ΔR2*)). For the SlERF4-His construct for GST pull-down assay, *SlERF4-His* was PCR amplified (primer set JG360/JG448), cloned into the pCR-BluntII, and then the *Nco*I-*Xho*I fragment was subcloned into pET15b. For binary vector constructs, *Bgl*II-*Xba*I fragments of *xopD(WT, C685A, V333P, ΔR1ΔR2, V333P/C685A, or ΔR1ΔR2/C685A)* and *Bam*HI-*Xba*I fragments of *SlERF4(WT or K53R)-FLAG-His* were subcloned into pEZRK-LCY (courtesy of David Ehrhardt). For XopD mutant constructs, *xopDM1*, *xopDM2*, *xopDM3*, *xopDM4*, *xopDM5*, and *xopDM6* were PCR amplified (primer sets JG477/JG808, JG477/JG809, JG202/mb371, JG202/mb371, JG134/mb371, and JG134/mb371, respectively), cloned into the pJET1.2/blunt, and *Bgl*II-*Xba*I or *Bam*HI-*Xba*I fragments were subcloned into pEZRK-LCY. *SlERF4-FLAG-His* was PCR amplified (primer set JG360/JG229) and *SlERF4(K53R* or *E55A)-FLAG-His* was made with a QuickChange site-directed mutagenesis kit (Stratagene) (primer set JG647/JG648 or JG762/JG763, respectively). All PCR amplified constructs were sequenced and confirmed. For the *SlERF4* VIGS construct, 0.33 kb fragment of the 5' end of *SlERF4* (a region sharing no identity with other *ERF*s) was PCR amplified (primer set JG444/JG651), cloned into pCR8/GW/TOPO, and then recombined into the Gateway-compatible TRV2 vector (Courtesy of Gregory B. Martin) using LR clonase (Invitrogen).

Quantitative RT-PCR

Total RNA was isolated from leaves using Trizol reagent (Invitrogen) according to manufacturer's instructions. Five µg of RNA were used for cDNA synthesis. Quantitative RT-PCR (qPCR) was performed using the cDNA and gene-specific primers (Supplemental Table S1). Each cDNA was amplified by real-time PCR using Maxima SYBR Green qPCR Master Mix (Fermentas) and the MJ Opticon 2 (Bio-Rad). *ACTIN* expression was used to normalize the expression value in each sample and relative expression values were determined against the average value of 10 mM $MgCl_2$ or Xcv sample using the comparative Ct method ($2^{-\Delta\Delta Ct}$).

8xGCC-GUS reporter construction

To construct the 8xGCC-GUS reporter, 0.29 kb *Bam*HI-*Spe*I fragment containing the minimal 35S promoter region was PCR amplified (primer set JG404/JG399) and replaced with the 35S promoter region in pCAMBIA1305.1, creating pCAMBIA1305.1(minimal 35S promoter-GUS). An 8xGCC box was PCR amplified (primer set JG400/JG401) and cloned into the *Eco*RI-*Bam*HI site of pCAMBIA1305.1(minimal 35S promoter-GUS), creating pCAMBIA1305.1(8xGCC-minimal 35S promoter-GUS). To generate transgenic *N. benthamiana* containing 8xGCC-minimal 35S promoter-GUS reporter, *N. benthamiana* leaf discs were transformed by *A. tumefaciens* LBA4404 containing pCAMBIA1305.1(8xGCC-minimal 35S promoter-GUS) and regenerated following the standard protocol (Clemente, 2006).

Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) assay was performed as described with modification (Saleh et al., 2008). Briefly, the transgenic *N. benthamiana* reporter (8xGCC-GUS) leaves were infiltrated with two *A. tumefaciens* strains (4 x 10^8 cfu/ml total concentration) expressing two different fusion proteins. Leaf tissues (~0.8g) were collected at 40 HPI, fixed in cross-linking buffer (10 mM Tris–HCl, pH 8, 0.4 M sucrose, 1 mM PMSF, 1% formaldehyde) for 10 min in a vacuum chamber at RT. The reaction was stopped by adding 2 M glycine (final concentration of 100 mM). Nuclei were isolated from the fixed tissue and lysed in nuclei lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 % SDS, 0.1 % sodium deoxycholate, 1% Triton X-100, 1 mM PMSF, 1 μg/ml pepstatin A, and 1 μg/ml aprotinin (Sigma-Aldrich)). The extracted chromatin samples were sonicated and precleared with protein A/G plus-agarose beads (Santa Cruz Biotechnology) for 1 h at 4°C with gentle rotation. Five microgram of anti-FLAG antibody or no antibody was added to the precleared chromatin samples and incubated overnight, followed by incubation with protein A/G plus-agarose beads for 2 h at 4°C with gentle rotation. After washing the beads, the immuno-complexes were eluted with elution buffer (0.5 % SDS and 0.1 % sodium bicarbonate (Sigma-Aldrich)) and reverse cross-linked at 65°C for overnight. The DNA fragments were extracted with phenol and chloroform and precipitated with ethanol. DNA pellet was dissolved in TE and PCR analysis was performed with primer set JG680/JG681 to amplify 8xGCC region using the same amounts of DNA from the inputs, no antibody control, and anti-FLAG samples.

Table S1. Primer sequences used in this study, related to Experimental Procedures.

Description	Primer Name; Sequence (5'→3')
***SlERF4*, *SlERF4(K53R)*, *SlERF4(E55A)* gene constructs**	JG360; GGATCCCATGGCAATGACGAAACAAGATGAAGGATT
	JG448; GGTCTAGACTAGTGATGATGATGATGATGCACCAACTCCATCTTGTTCTCTCTTCT
	JG229; GGTCTAGATTAATGATGATGATGATGATGCTTATCGTCGTCATCCTTGTAATC
	JG444; CACCATGACGAAACAAGATGAAGGATT
	JG447; CACCAACTCCATCTTGTTCTC
	JG651; CATTAGAACCGGCGGAGC
	JG647; GTTTTCACTTCAGTAAGAACAGAGCCATCTA
	JG648; TAGATGGCTCTGTTCTTACTGAAGTGAAAAC
	JG762; GCTGTTTTTACTGAAGTGAAA
	JG763; TCCATCTACATCCAATTCCCT
***xopD* gene constructs**	JG478; CACCATGGACAGGATATTTAATTTCG
	JG09; GAACTTTTTCCACCACTTGCTTTTC
	JG477; GGAGATCTCCATGGACAGGATATTTAATTTCG
	mb371; GGGCTCGAGCTAGAACTTTTTCCACCACTTGCTTTTC
	JG808; GTCTAGACTAGCGATAACCATAGTTCTGCCTA
	JG809; GTCTAGACTAGTCCTGGGGCGACGGCAG
	JG202; GGATCCATATGGAATTCGACCTTAACACCCCCCAGGAAA
	JG134; GAATTCATGGTCGCAGACCCCGAACTT
***GUS* gene constructs**	JG114; CTCGAGACCATGGTAGATCTGAGGG
	JG683; CACGTGATGGTGATGGTGAT
Quantitative RT-PCR	
LeActin	JG234; GAGCGTGGTTACTCGTTCA
	JG136; CTAATATCCACGTCACATTTCAT
SlACO1	JG236; CACAAACAGACGGGACACGA
	JG211; GCAGCAAATCACAATCTTTTTTAATACTAAC
SlACO2	JG237; GAAAAGACGGGACTCGGATGT
	JG213; ATGTATGTCAATAACAAATAATTTTTCTTCCAA
SlACS2	JG425; GCAACAATGGAAGAAGAATAATTTG

	JG426; TATGATGTCTAAGTACATAGACCAGTTGTC
SlERF1	JG449; CTAAGAGGAGGAGAAAGGGTGTAGCG
	JG450; TAACCAATAGCTGCTCGCCAACTG
SlERF2	JG451; ATGGAATTGAAGATGGAGGAGAGGAGAT
	JG452; GTATCTGCACTTCATCCTCTGTTTTCTCC
SlERF4	JG455; TATGATTGTGCAGCGTTTAAGATGAGAG
	JG456; CTTCTCCTTTTCCGGCCAACATTC
Pti4	JG457; CGAGTTACGGCGAAAAGGCGA
	JG458; TGACACTTGATCTACTCGCCATTTCTC
Pti5	JG459; CGGCTAGACATGGTGCGAGAGTAT
	JG460; ACAGAGGCGTTCACTATTTCAGATGG
Pti6	JG461; TGCGCCGTTTGACAATCTAGGG
	JG462; TTCAAGGGCAAAATCGTCAAAGTC
TSRF1	JG463; ACGATGATTGCTCCCCTGTAGTGG
	JG464; TGTTCTAAATAATCAGCACCCAAATCTTC
TERF1	JG465; AACGCTAGTACTACAACAACAACAACAACAACATC
	JG466; TGTTTTTTCTCTATGGATGTTACCTCTTCTTG
SENU4	JG242; CTGGTGCTGGGGAGAATC
	JG243; GTCCGATCCAGTTGCCTACA
Chi17	JG139; AGGCAATCAAATGGGAAGTG
	JG140; CAATCCGATCCTCCACTTGT
8xGCC-GUS reporter	JG399; CAGACTAGTTCGTCGGTTCTGTAACTAT
	JG404; GGATCCGCAAGACCCTTCCTCTATATAAGGAA
	JG400; AATTCAGCCGCCAGCCGCCAGCCGCCAGCCGCCG
	JG401; GATCCGGCGGCTGGCGGCTGGCGGCTGGCGGCTG
Chromatin Immunoprecipitation	JG680; TGTGGAATTGTGAGCGGATA
	JG681; AGAGTCCCCCGTGTTCTCTC

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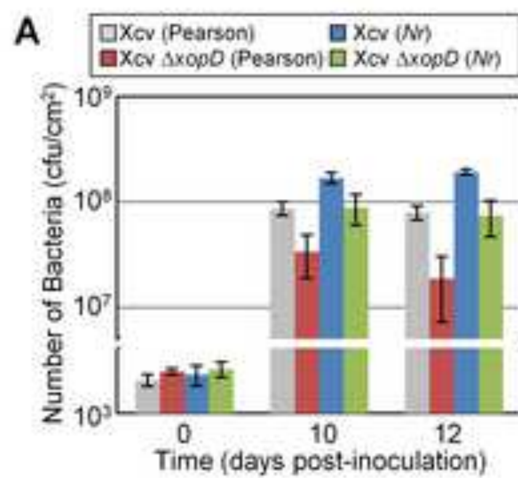
Figure S1

Figure S1, related to Figure 2



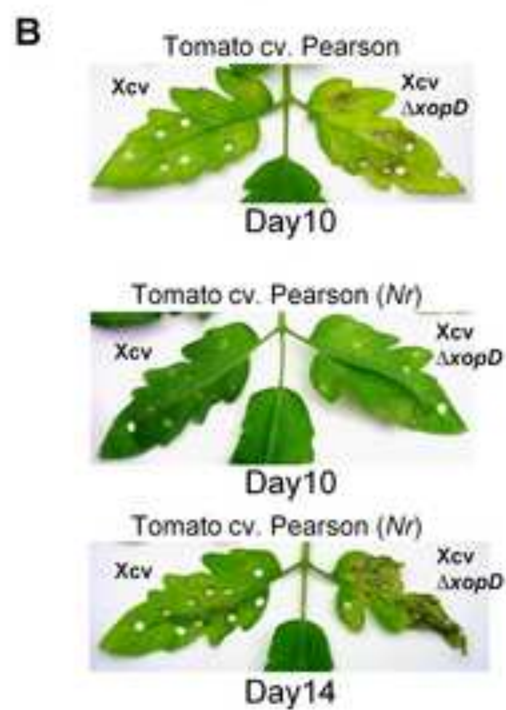


Figure S2

Figure S2, related to Figure 3

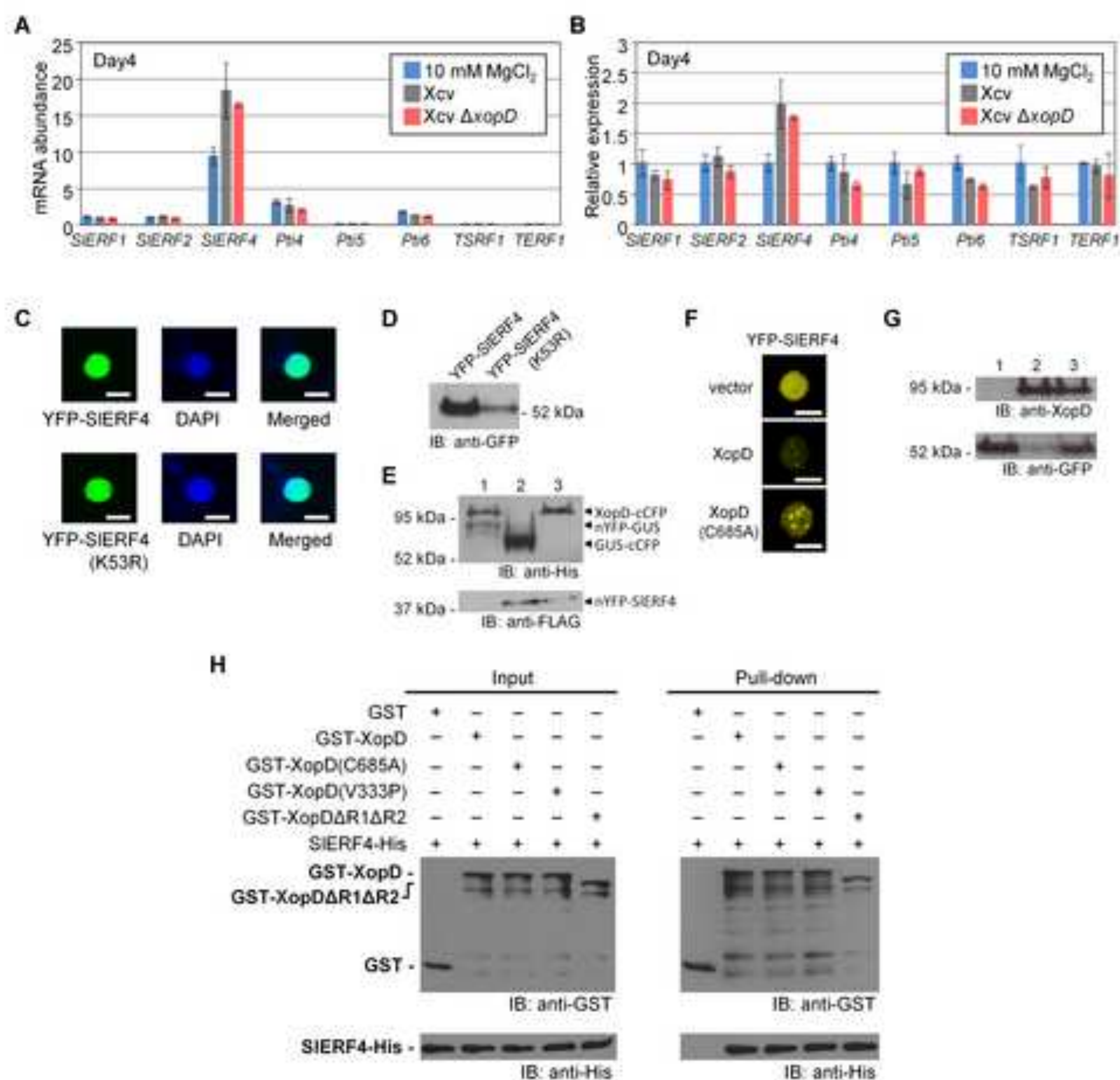


Figure S3

Figure S3, related to Figure 6

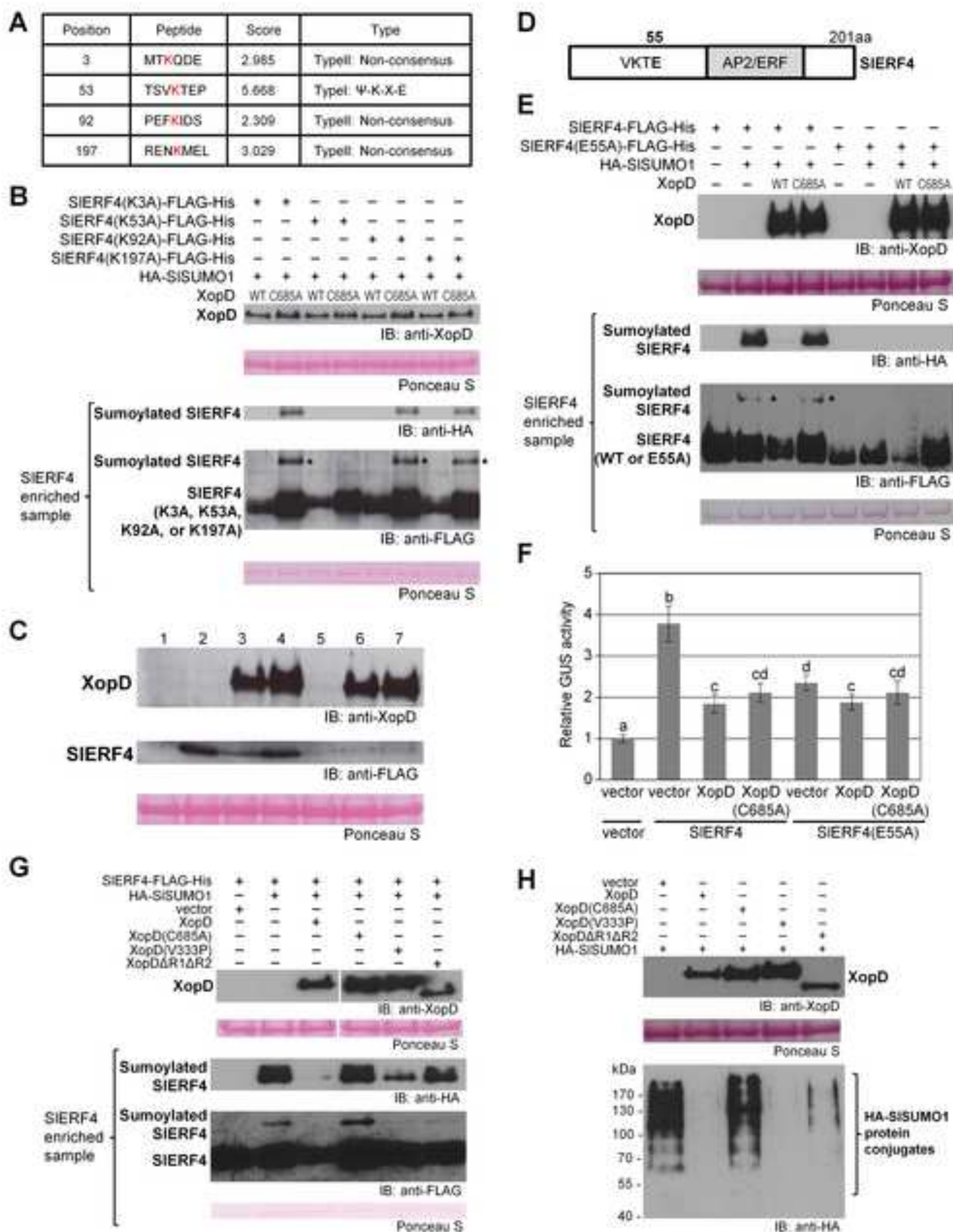


Figure S4

Figure S4, related to Figure 7