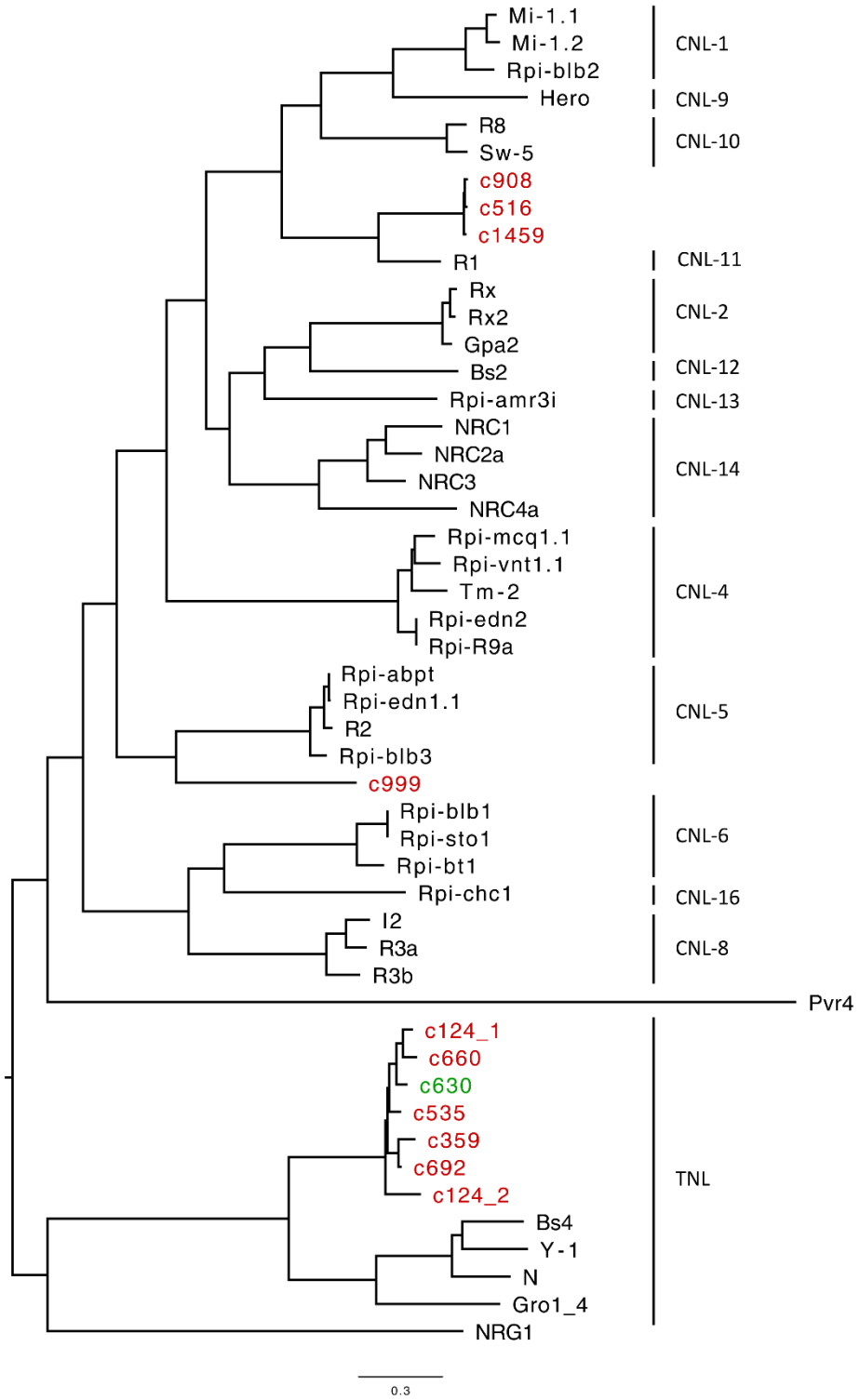
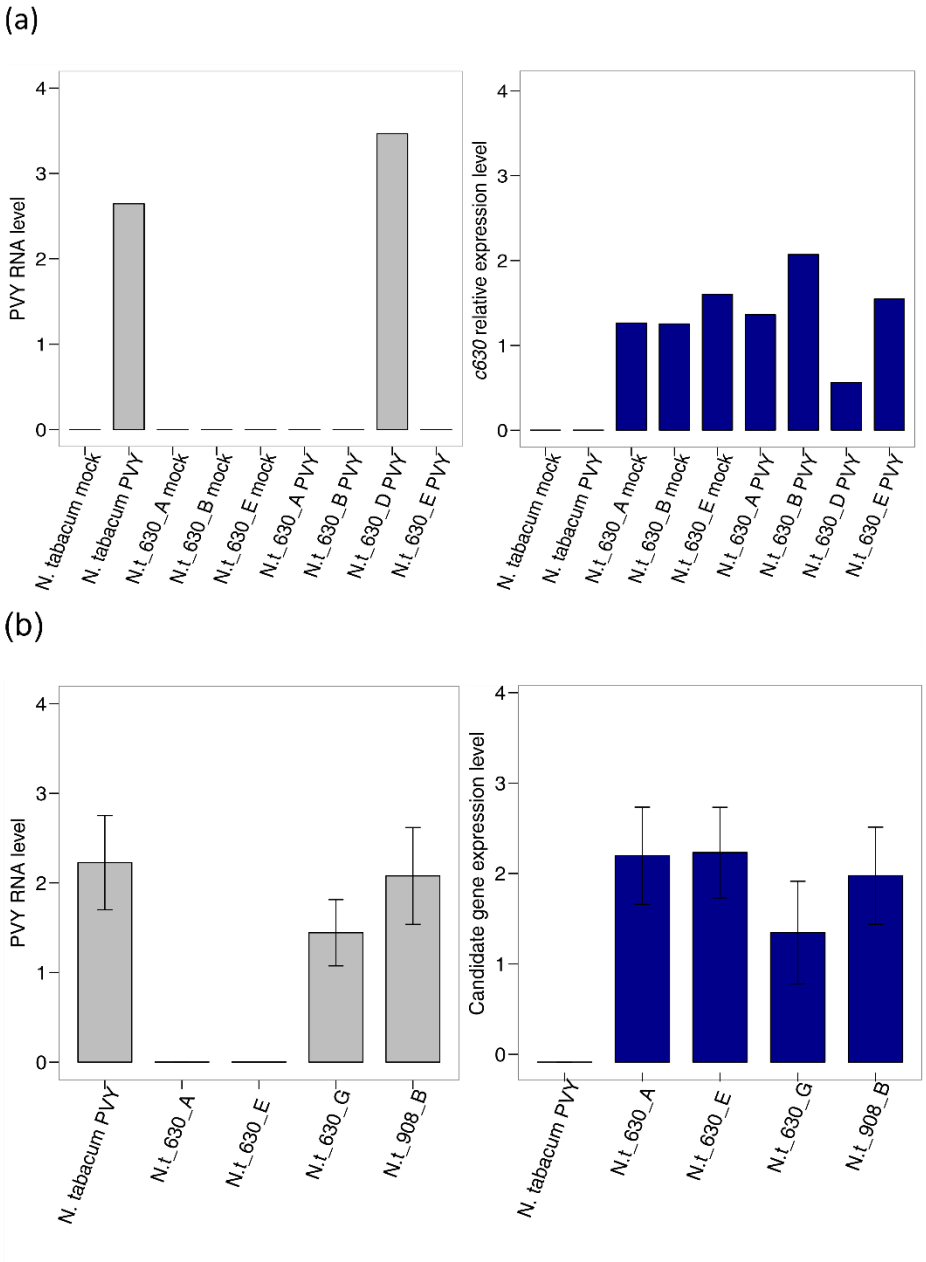


Supporting Figure 1 Phylogenetic analysis of *Ry*$_{sto}$ candidate genes and other functional *Solanaceae* NLRs. Full-length amino acid sequences were aligned using ClustalW 1.74. Evolutionary analyses were conducted using MEGA6.06 using the Maximum-Likelihood statistical method with 100 bootstraps and Poisson (G+I) model for amino acid substitutions.

Tree represents typical Solanaceae NLR architecture. Clades are named according to Andolfo *et al*. (2014) and Witek *et al*. (2016). Candidate contigs are highlighted in red (c630) and green (all remaining). Sequences of functional NLRs were retrieved from public databases; Mi-1.1: AF039681, Mi-1.2: AF039682, Rpi-blb2: DQ122125, Hero: AX337980, R8: ANJ02805, Sw-5: AY007366, R1: AF447489, Rx: AJ011801, Rx2: CAB56299, Gpa2: AAF04603, Bs2: AF202179, Rpi-amr3i: KT373889, NRC1: DQ304484, NRC2a: KT936525, NRC3: XM_004238900, NRC4a: Solyc04g007030, Rpi-mcq1.1: WO2009013468, Rpi-vnt1.1: FJ423044, Tm-2: AY742887, Rpi-edn2: MI377464, Rpi-R9a: AVX67693, Rpi-abpt: FJ536324, Rpi-edn1.1: GU563963, R2: FJ536325, Rpi-blb3: FJ536326, Rpi-blb1: FB764493, Rpi-sto1: EU884421, Rpi-bt1: FJ188415, Rpi-chc1: WO2011034433, I2: AF118127, R3a: AY849382, R3b: JF900492, Pvr4: KT359375, Bs4: AY438027, Y-1: CAC82812, N: Q40392, Gro1-4: AAP44390 and NRG1: AAY54606.

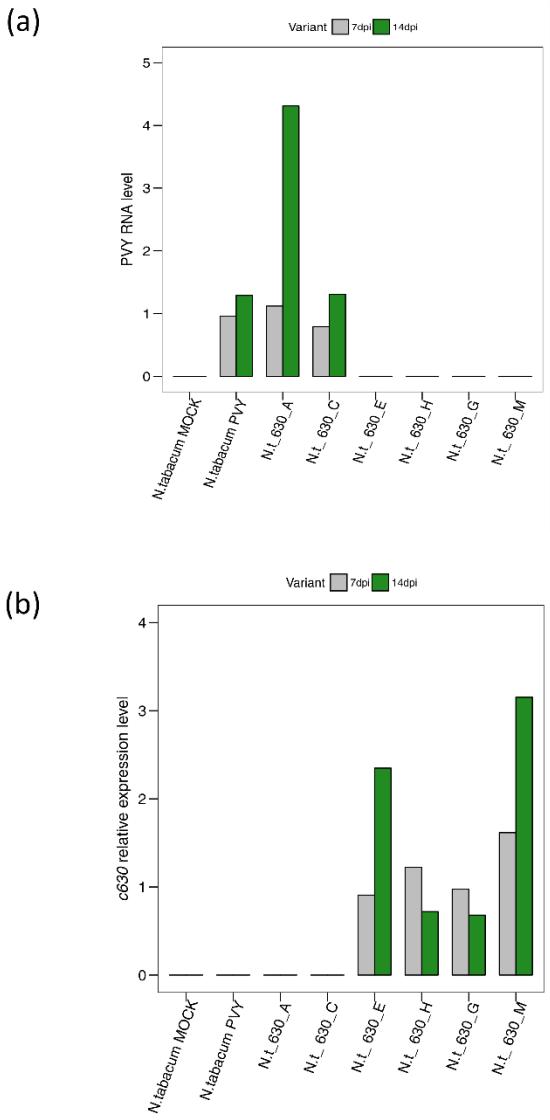
Supporting Figure 2 Grafted transgenic *Ry$_{sto}$* plants display resistance to PVY. Scions (upper part) of a non-transformed *N. tabacum* plants that had been preinoculated with PVY^{NTN} were grafted to a stock (lower part) of stable transgenic *35S:Ry$_{sto}$* Maris Piper or susceptible to PVY Nicola plants. Three weeks after grafting chlorosis and dwarfing were observed only in susceptible plants (right) whereas *Ry$_{sto}$* transgenic plants (left) remained symptomless. The experiment was performed on three independent transgenic potato lines and three biological replicates were used for each line. Picture was taken 4 weeks after grafting.

Supporting Figure 3 HR of transgenic *N. tabacum* expressing Ry_{sto}. HR response after PVY inoculation in stable transgenic *N. tabacum* plants transformed with *35S:*Ry_{sto}. Seven-week-old *N. tabacum 35S* transgenic and non-transformed plants were inoculated with PVY^{NTN}. HR was observed on *N. tabacum 35S:*Ry_{sto} inoculated leaves at 3 dpi, whereas control plants remained symptomless. Image was taken at 7 dpi.

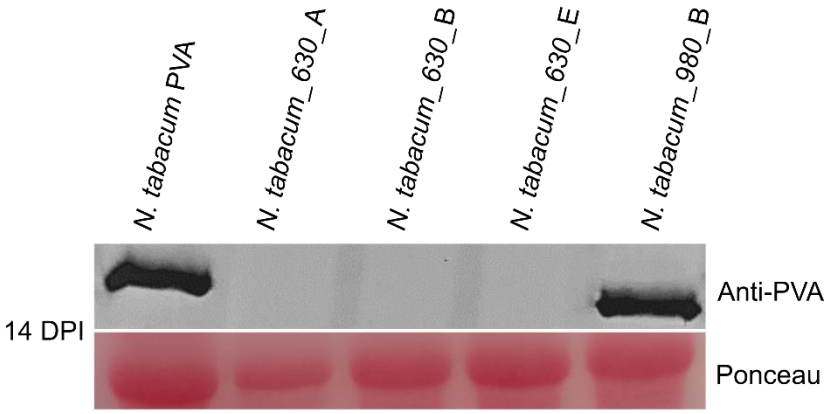


Supporting Figure 4 Stable transgenic tobacco plants carrying *Ry*$_{sto}$ under the control of a 35S promoter display resistance to PVY. Seven-week-old *N. tabacum 35S:Ry*$_{sto}$ transgenic and non-transformed plants were inoculated with PVY^{NTN} or mock treated with water. Two weeks after PVY inoculation, mRNA was isolated from upper, non-inoculated leaves and quantified with qPCR. (a) Elevated expression of *Ry*$_{sto}$ correlated with lack of PVY RNA (lines A, B and E) in stable T0 transgenic plants. (b) Resistant lines A and E (T1 generation) confirmed T0 observations. Line 630G (susceptible), where expression of *Ry*$_{sto}$ was not

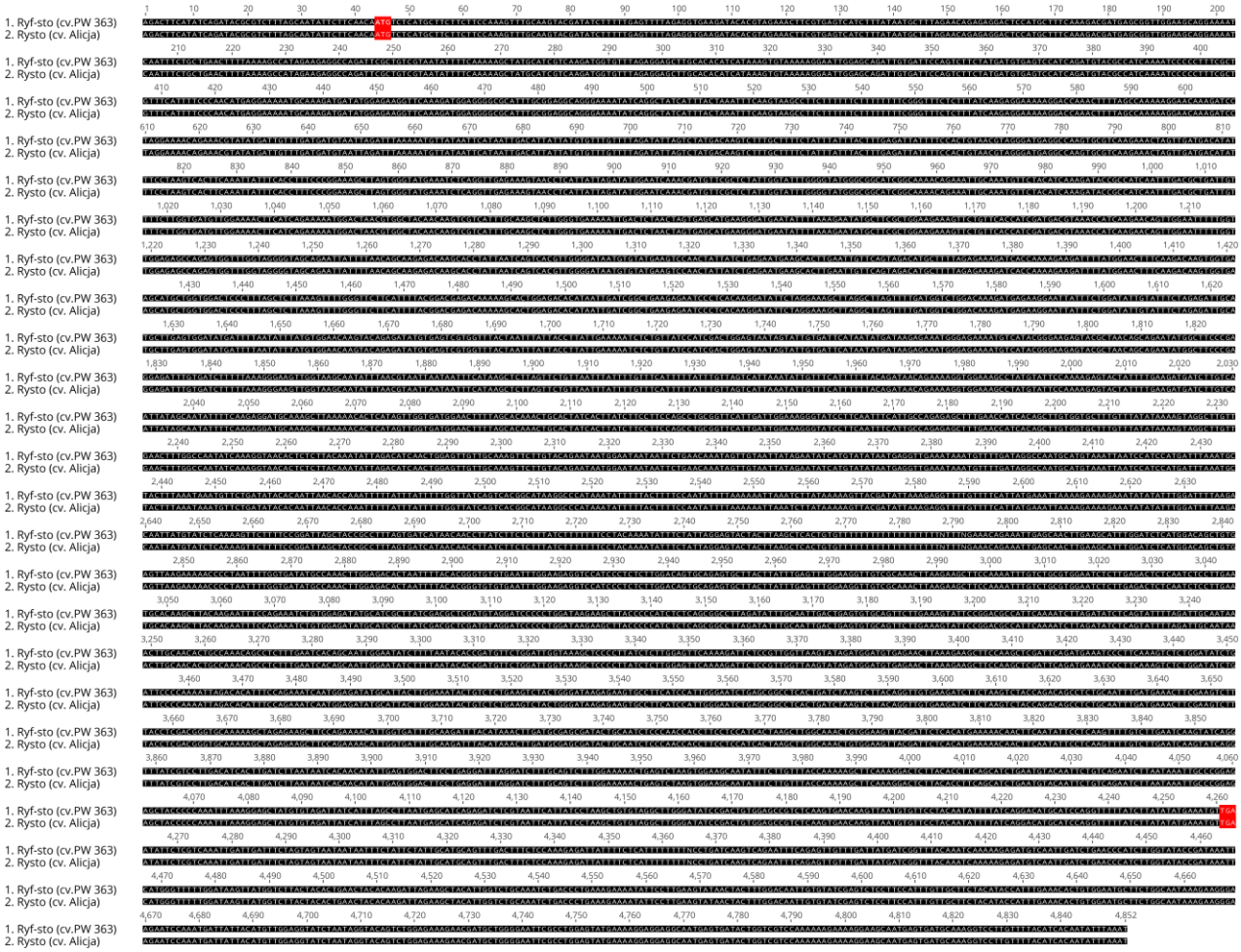
detected, had PVY RNA levels similar to a control line (*c908B*) carrying a non-functional paralogue.



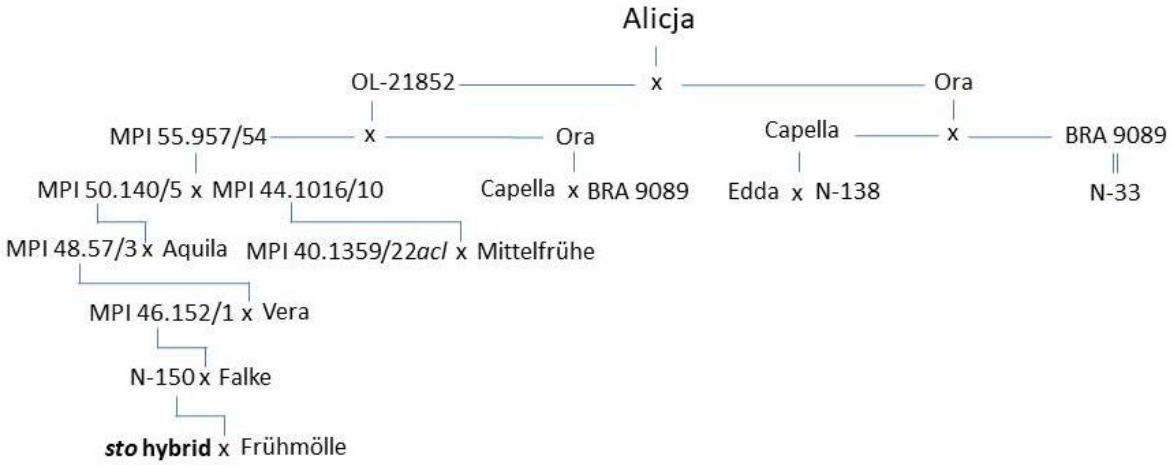
Supporting Figure 5 Stable transgenic tobacco plants carrying *Ry*$_{sto}$ under the control of native regulatory elements display resistance to PVY. Seven-week-old *N. tabacum Ry*$_{sto}$ (native) transgenic and non-transformed plants were inoculated with PVY^{NTN}. Seven and fourteen days after PVY inoculation, mRNA was isolated from upper, non-inoculated leaves and measured using qPCR. *EF1* and *L23* were used as standardisation reference genes. In lines E, G, H and M (T0 generation) the inhibition of virus multiplication (a) correlated with a high level of *Ry*$_{sto}$ transcript (b).



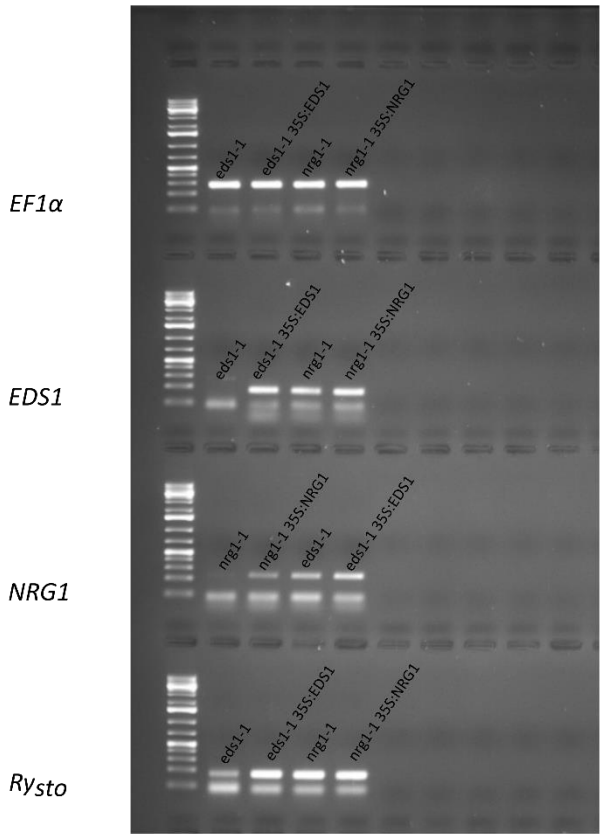
Supporting Figure 6 *Ry_{sto}*-mediated resistance to PVA infection. Western blot analysis of PVA accumulation in leaves of transgenic *Ry_{sto}* and non-transformed *N. tabacum* plants after 14 days. Seven-week-old *N. tabacum 35S:Ry_{sto}* or *35:c908B* (control line carrying a non-functional paralogue) and non-transformed plants were inoculated with PVA. Two weeks after infection, samples of upper, non-inoculated leaves were harvested and subjected to immunoblot analysis using alkaline phosphatase-conjugated anti-PVA antibodies.



Supporting Figure 7 Comparison of *Ry$_{sto}$* and *Ry-f$_{sto}$* nucleotide sequences. Identical residues are shaded in black. Start and Stop codons are marked with red. The alignment was generated using MAFFT-L-INS-I (Katoh & Toh, 2008) and visualised in Jalview 2,10,4b1 (Waterhouse et al., 2009).



Supporting Figure 8 Pedigree chart of cultivar Alicja. Cultivar Alicja was obtained from a cross between clone OL-21852 and cultivar Ora at the breeding company HZ Zamarte, Poland. The pedigree chart was prepared according to the data described by Świerzyński *et al*. (1997). The Ry_{sto} gene source is in bold.



Supporting Figure 9 Semi–quantitative RT-PCR of *Ry*$_{sto}$, *NRG1* and *EDS1* genes. PVY-infected leaves of *eds1-1, nrg1-1 N. benthamiana* knockout plants were infiltrated with *Agrobacterium* carrying *Ry*$_{sto}$ alone or *Ry*$_{sto}$ coexpressed with *EDS1* or *NRG1*. Samples were collected two days after infiltration. Elongation factor-1α (*EF1α*) was used as an internal control.