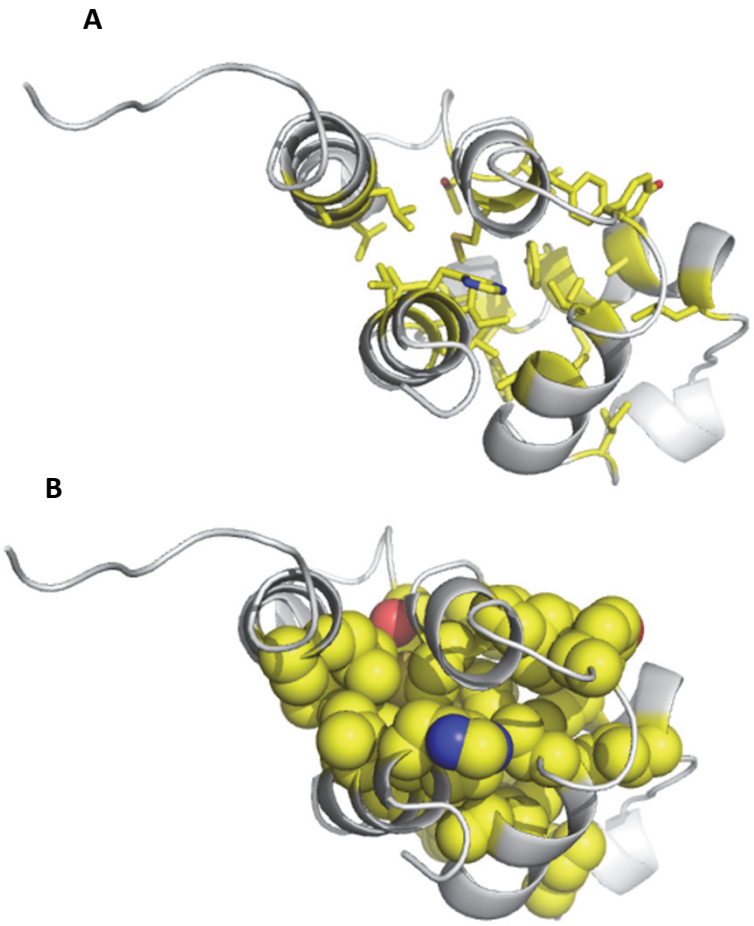

Figure S1. Homology modeling of M013 pyrin domain. The model was built by threading the M013 sequence into the structure of MNDA (Pdb code 2dbg). The Consensus and Homology suites in the MOE were used to build the initial model, followed by energy minimization to relieve modest steric repulsions. The final model comprises a 6-helix bundle typical of pyrins with a well-packed hydrophobic core. The core is shown with yellow sticks (A) and van der Waals space-filling models (B).Stereochemical analyses reveal that 98.93% of residues are situated in favorable and allowed regions of the Ramachandran map.

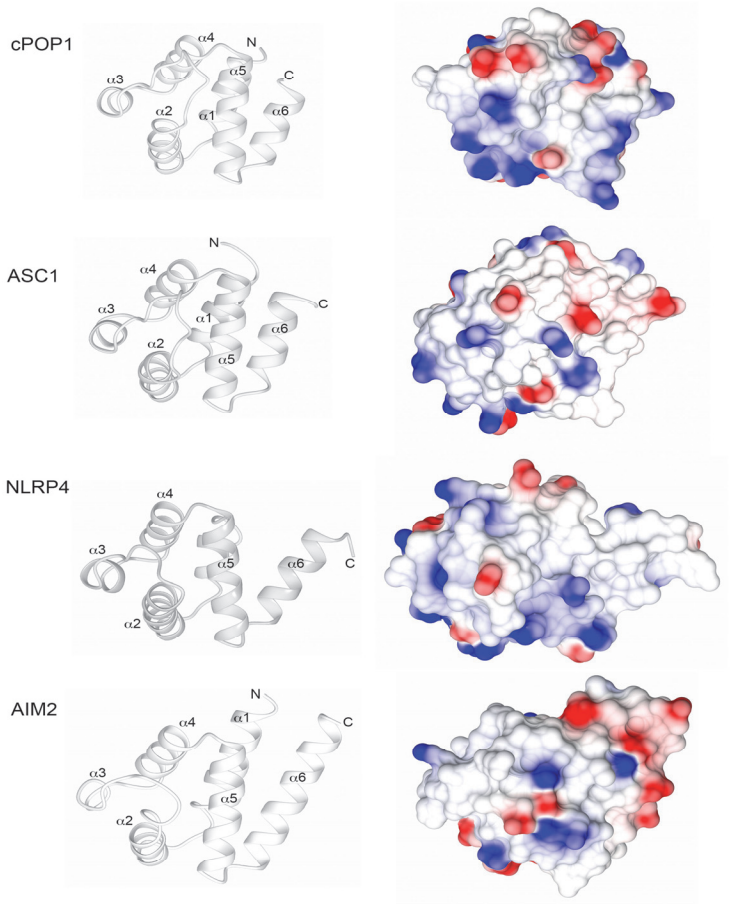

Figure S2. Ribbon and electrostatic surface representations of selected pyrin domains. The view is toward the Type Ib face, in the same orientation as Figure 1B. PDB codes are the following: cPOP1, 4qob; ASC-1, 1ucp; NLRP4, 4ewi; AIM2, 4o7q.

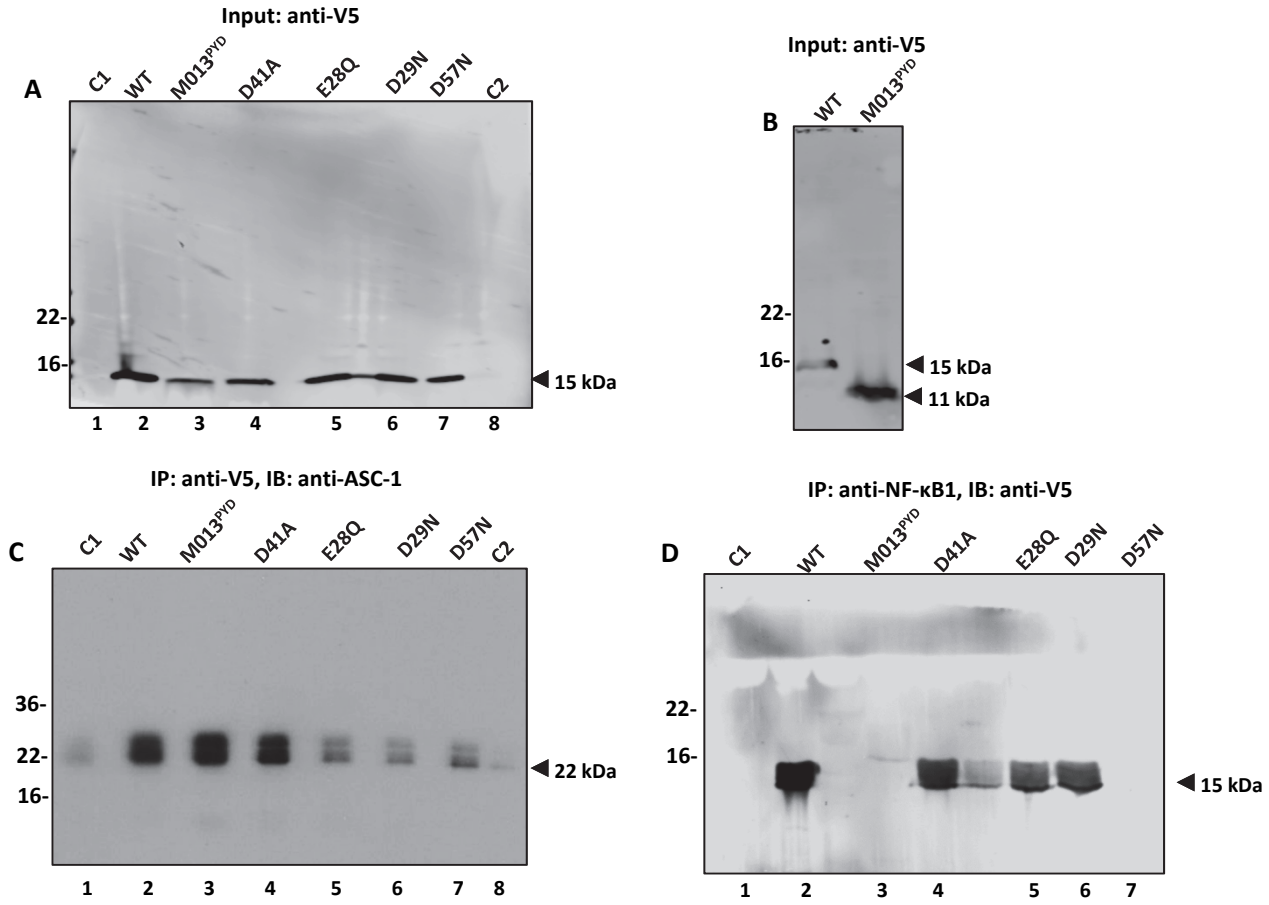

Figure S3. M013 mutants interact with differential affinity with ASC-1 and NF-κB1. (A and B) Expression (input) of V5-tagged WT-M013 and M013 mutants in the HeLa cells transfected and infected with vMyx-M013KO. (C) Detection of WT-M013 and M013 mutants interaction with ASC-1 in HeLa cells by co-immunoprecipitation. After transfection and infection, co-IP was performed using anti-V5 antibody and the interacting ASC-1 in the complex was detected using anti-ASC-1 antibody by Western blot analysis. (D) Detection of WT-M013 and M013 mutants interaction with endogenous NF-κB1 (p105/p50) in HeLa cells by co-immunoprecipitation. After transfection and infection, co-IP was performed using anti-NF-κB1 antibody and the interacting expressed V5-tagged M013 proteins in the complex were detected using anti-V5 antibody by Western blot analysis. Lanes: WT, Wild-type M013; C1, cells infected with vMyx-M013KO; $M013^{PYD}$, M013 with C terminus deletion; M013 with mutations E28Q, D29N, D57N and D41A, respectively; C2, cells with no transfection or infection.

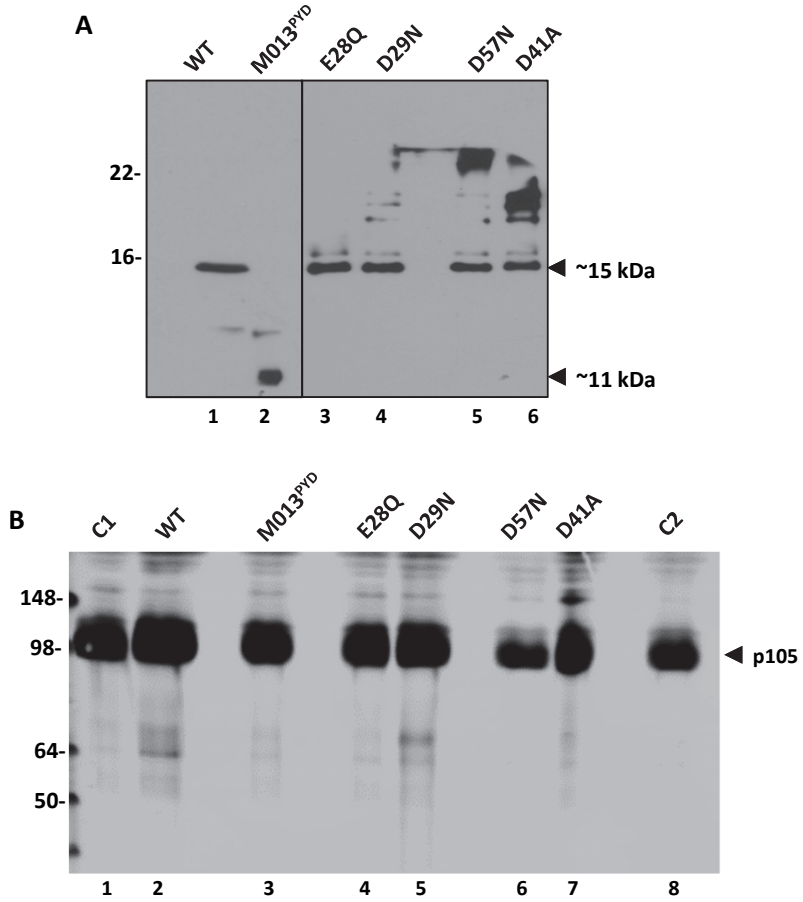

Figure S4. Detection of V5-tagged WT-M013, M013 mutants and endogenous NF-κB1 in the lysates post co-immunoprecipitation. After transfection and infection, co-IP was performed using anti-V5 antibody (A) or anti-NF-kB1 antibody (B) and the bound proteins were detected using the corresponding antibodies.