SUPPLEMENTAL FIGURE 1. **NLRP1 induces ASC speck formation.** *A*, NLRP1 or NLRP3 were transduced into U2OS cells in the presence of ASC (MOI=3) for 24 hrs. Cells displaying diffuse or punctuate ASC staining were counted and the ratio of speck/diffuse was calculated. *B*, HEK293T cells were co-transfected with ASC-GFP and the indicated form of NLRP1. The number of ASC specks was quantitated.

SUPPLEMENTAL FIGURE 2. **Identification of the NLRP1 cleavage site.** *A*, U2OS cells were transduced with NLRP1 BacMam virus. Western blots were probed with anti-His to visualize the C-terminal cleavage fragment. The corresponding band (circled) was excised from Coomasie stained gels and analyzed by Edman degradation. *B*, the NLRP1 sequence is shown. The predicted sequence of the C-terminal cleavage fragment, based on molecular weight, is shown in red. The peptide sequence identified by Edman degradation is underlined.

SUPPLEMENTAL FIGURE 3. **Conserved nature of the NLRP1 cleavage site.** The NLRP1 autolytic cleavage site and three His residues are highly conserved. Sequences surrounding the cleavage site of NLRP1 from twelve different species were aligned using CLUSTALW. The cleavage site is highlighted in blue and the invariant His residues are highlighted in red.

SUPPLEMENTAL FIGURE 4. **Conserved His residues are critical to NLRP1 cleavage and activity.** Mutation of each invariant His residue ablates NLRP1 cleavage and NLRP1 mediated IL-1β release. *A*, immunoblot analysis of cells transfected with the indicated NLRP1 plasmid. Membranes were probed with anti-Flag to detect C-terminal cleavage product. ***B*,** HEK293T cells were transfected with pro-IL-1β, pro-caspase-1 and ASC in the presence or absence of the indicated NLRP1 plasmid. Released IL-1β was measured following 48 h incubation.

SUPPLEMENTAL FIGURE 5**. Mechanism of NLRP1 autolytic cleavage.** The cleavage mechanism is initiated when the reactive hydroxyl at the P1' position (S1213) is deprotonated by the H1186. The reactive oxygen from S1213 performs a nucleophilic attack *in cis* on the α-carbonyl carbon of the P1 residue (F1212). This forms a transitional tetrahedral intermediate associated with a five-member ring structure. After a proton is transferred to the leaving amino group of the P1' residue, the α-carbonyl of the P1 residue is shifted to the hydroxyl of the P1' residue leading to the ester intermediate via an N-O shift (Ref). The final step in cleavage is hydrolysis of the ester intermediate by water resulting in a cleaved protein composed of at least two subunits.