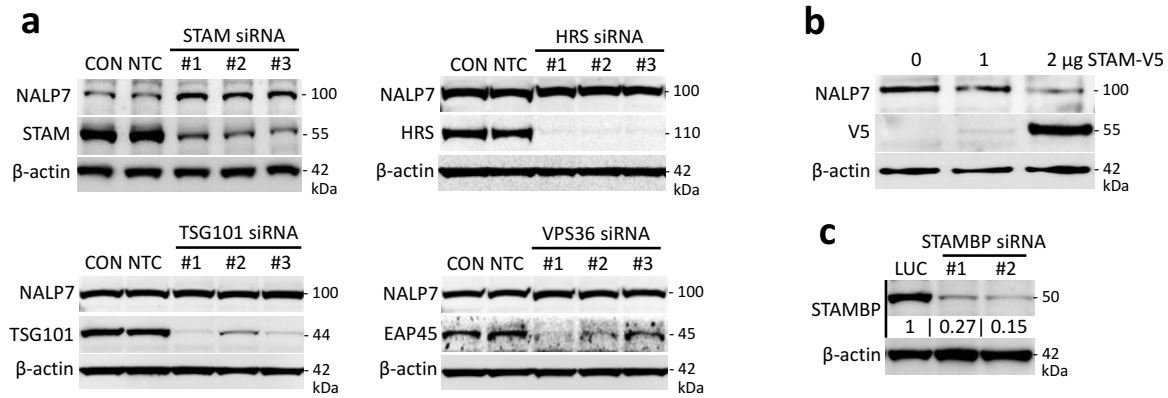
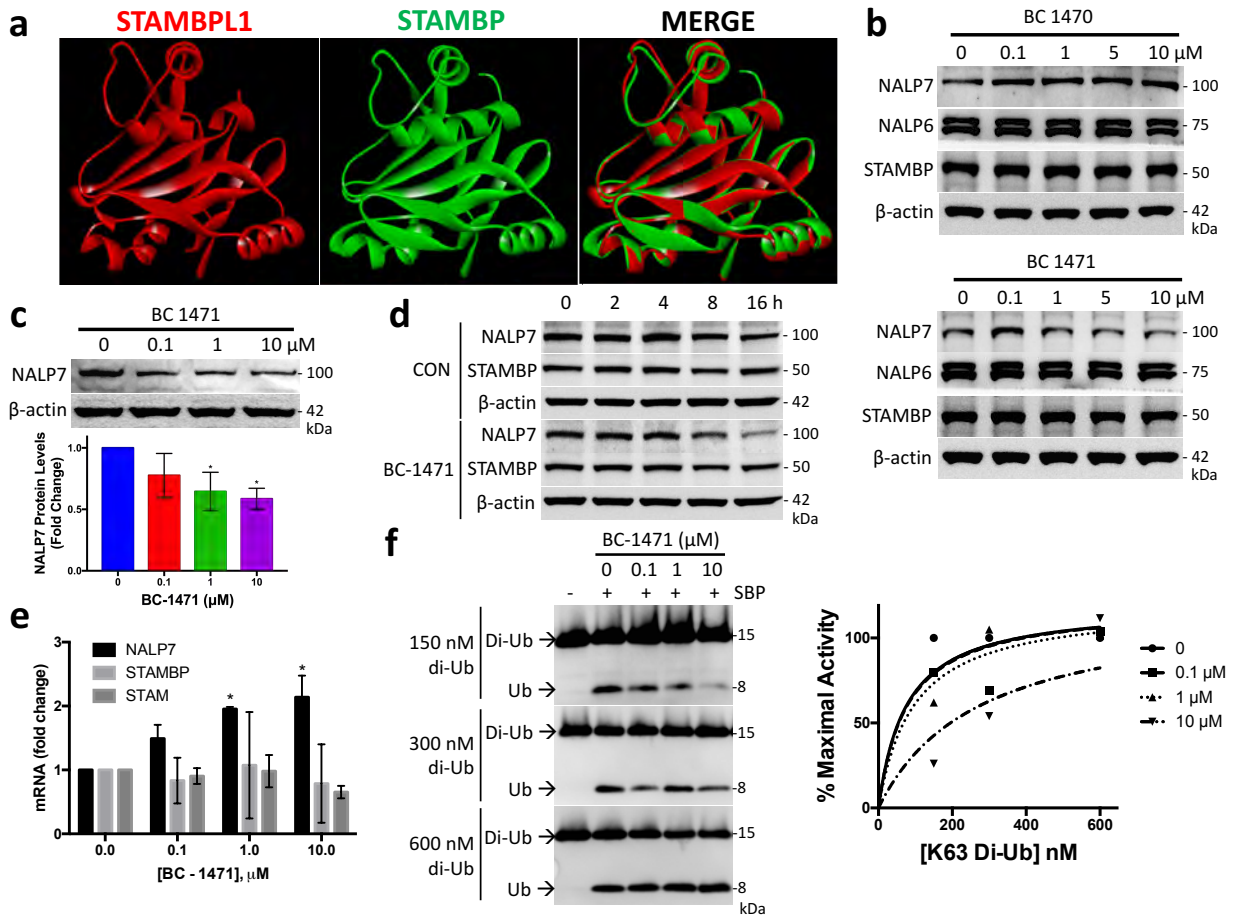


**Supplementary Figure 1. Cellular expression of inflammasome components after stimuli.**

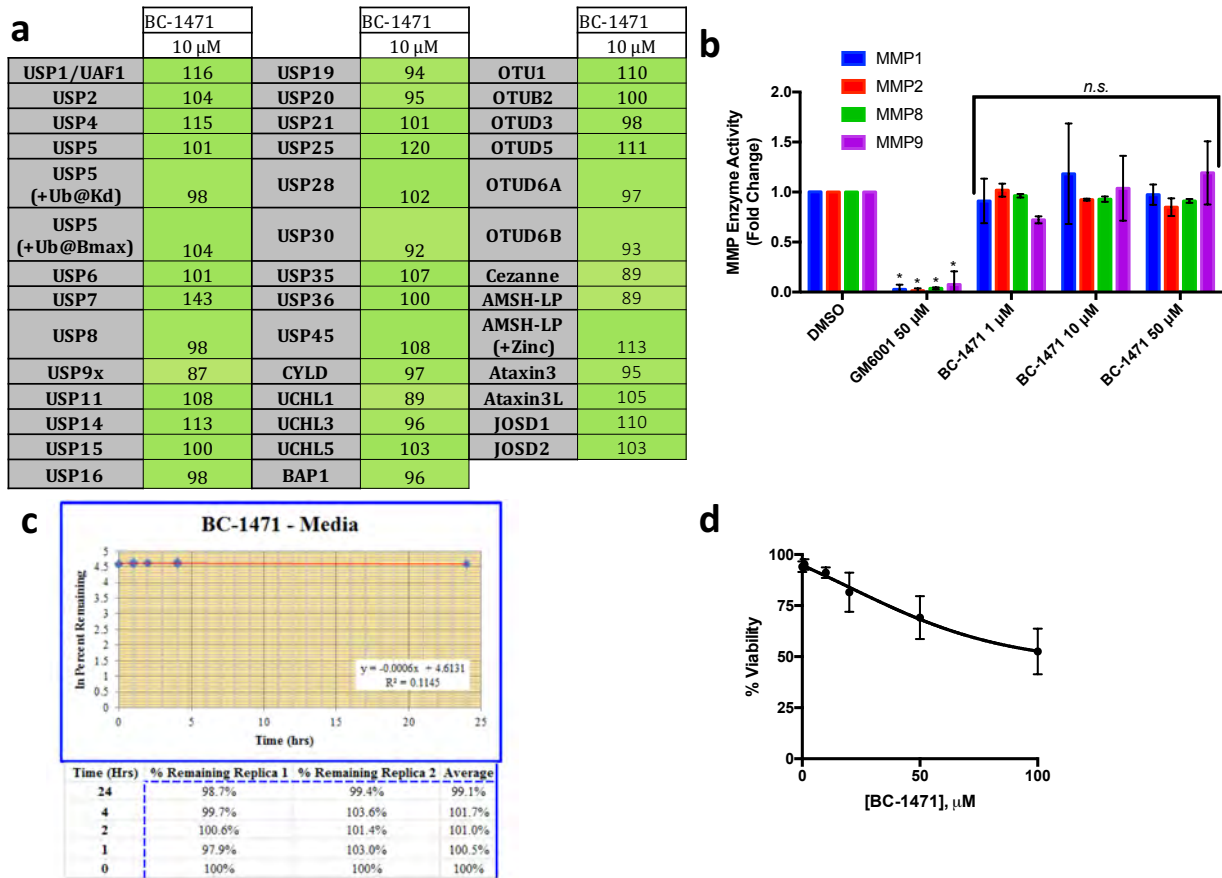
(a) THP-1 macrophages that formed an ASC-speck in Fig. 1b were further analyzed by immunofluorescence intensity profiling. Intensity was graphed as relative fluorescent units (RFUs) across the vector depicted by the yellow arrow. Cells exposed to LPS or Pam3CSK4 displayed formation of an ASC-speck (red) with co-localization of increased intensity NALP7 signal (green). Original magnification was 100x, scale bars = 10  $\mu$ m. (b) Immunoblot analysis confirmed knockdown of NALP3, ASC, or NALP7 by siRNA or a control luciferase (LUC) in THP-1 cells assayed in Fig. 1c. Relative abundance compared to control noted below as determined by densitometry using ImageJ software. (c, d) Immunoblot analysis of NALP7, NALP3, and ASC in lysates of THP-1 cells after (c) LPS or (d) Pam3CSK4 exposure for 16 h.



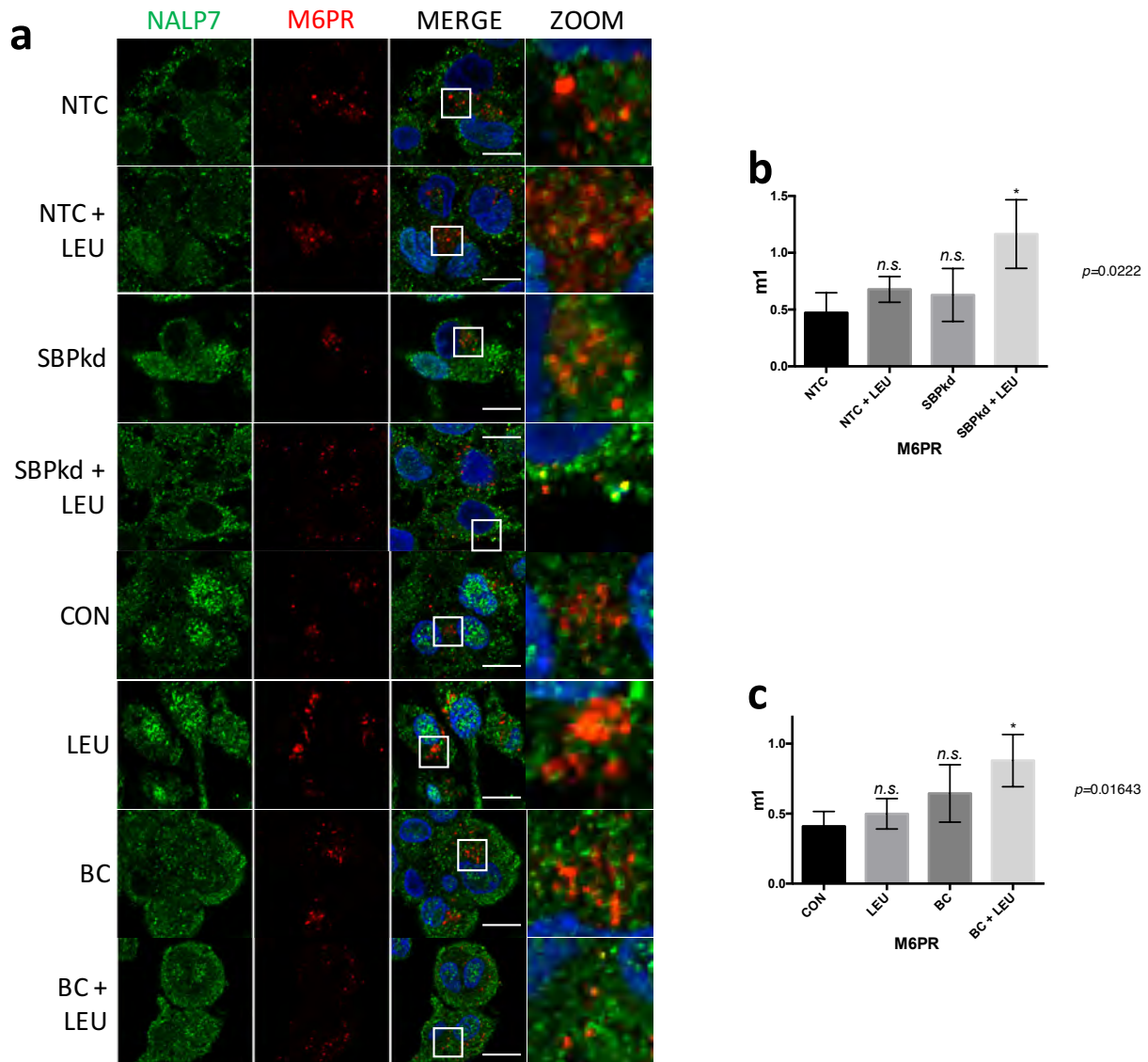
**Supplementary Figure 2. STAM modulates NALP7 abundance.** (a) *STAM* knockdown with siRNA increases NALP7 protein abundance in Beas2B cells compared to untreated control (CON), or a nontargeting control (NTC) siRNA. *HRS*, *Tsg101*, and *VPS36* knockdown with siRNA did not impact NALP7 protein abundance. Note: EAP45 is the protein product of the *VPS36* gene. (b) Overexpression of *STAM-V5* plasmid in Beas2B cells decreased immunoreactive NALP7. (c) Immunoblot analysis confirmed knockdown of STAMBP by siRNA or a control luciferase (LUC) in THP-1 cells assayed in Fig. 4i. Relative abundance compared to control noted below as determined by densitometry using ImageJ software.



**Supplementary Figure 3. Small molecule antagonism of NALP7.** (a) STAMBPL1 (2ZNV.pdb) and STAMBP (3RZU.pdb) in a structural homology model (merge). (b) Phenotypic screening of NALP7 small molecule antagonists. (c) Endogenous NALP7 protein abundance is decreased in THP-1 cells exposed to BC-1471. *Below*, densitometric analysis of relative NALP7 protein abundance as determined by ImageJ software. Data shown as mean  $\pm$  SD ( $n=3$ ).  $*P < 0.05$  compared to control. (d) Endogenous NALP7 half-life is decreased with BC-1471 pre-treatment (10 $\mu\text{M}$  for 2 h) compared to control in Beas2B cells. (e) NALP7, STAMBP and STAM mRNA with BC-1471 treatment for 16 h as analyzed by qPCR in THP-1 cells. Data shown as mean fold change  $\pm$  SD by  $\Delta\Delta\text{Cq}$  analysis ( $n=3$ ). (f) *In vitro* DUB assay as performed in Fig. 5d. Right: Relative mono-Ub was quantified by densitometry and fit to multiple inhibition models using GraphPad Prism software ( $n=2$ ). (c) One-way and (e) two-way analysis of variance (ANOVA) with post-hoc Dunnett's multiple comparisons test.

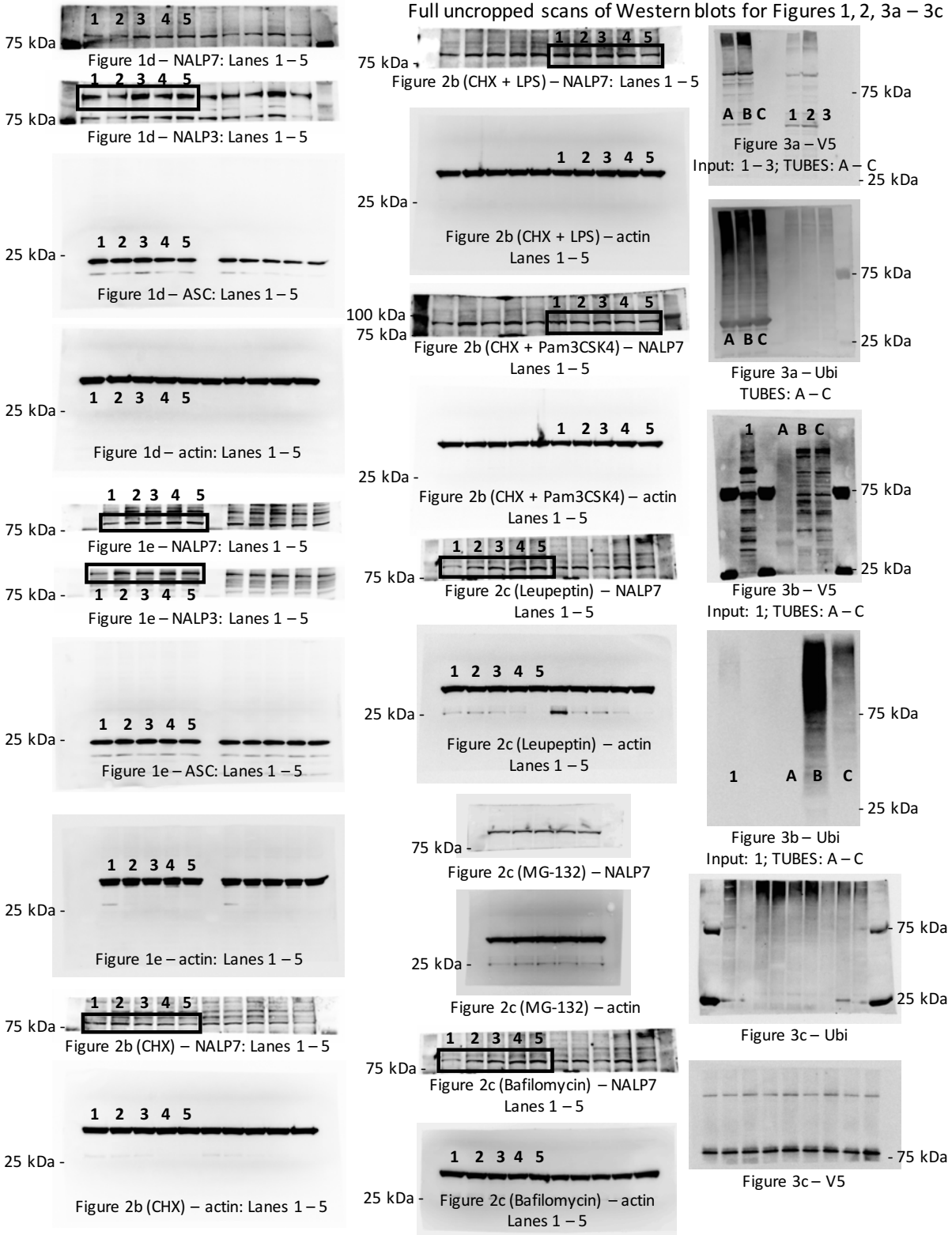


**Supplementary Figure 4. Characterization of BC-1471.** (a) BC-1471 treatment did not significantly inhibit the enzymatic activity of any of the tested DUBs in a high-throughput *in vitro* screen for off-target DUB inhibition. Data shown as mean percent of untreated control DUB activity ( $n=2$ ). (b) BC-1471 treatment did not significantly inhibit the enzymatic activity of any of the tested MMPs in a fluorescent MMP activity assay. Data shown as MMP activity measured by relative fluorescence compared to the DMSO control ( $n=3$ ). (c) BC-1471 was stable in RPMI media, as measured by mass spectrometry ( $n=2$ ). (d) Percent viability of THP-1 cells treated with BC-1471 for 16 h as determined by trypan blue staining. Data represents mean  $\pm$  SD with standard curve fit by nonlinear regression ( $n=3$ ). (b) Two-way analysis of variance (ANOVA) with post-hoc Dunnett's multiple comparisons test.

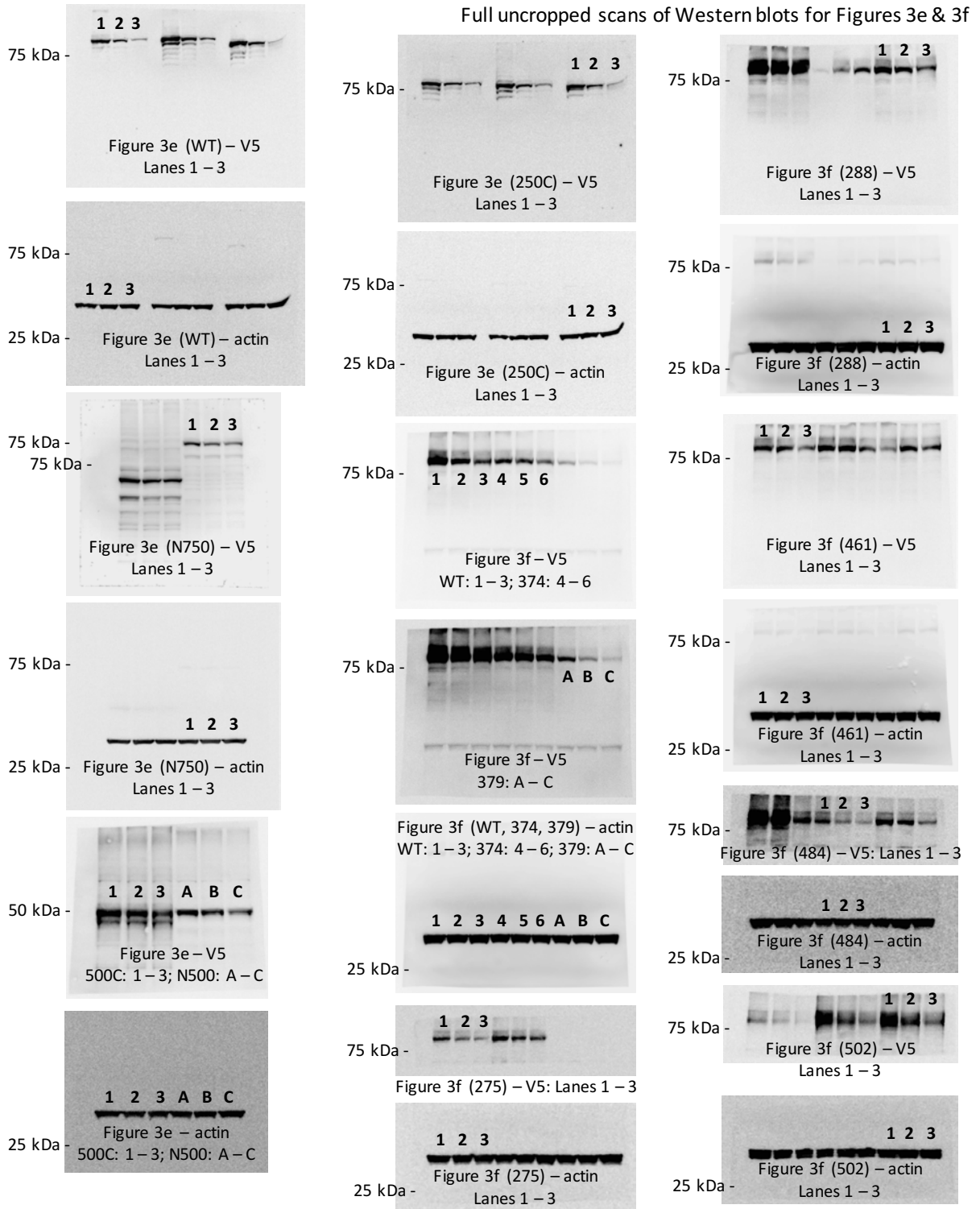


**Supplementary Figure 5. BC-1471 relocates NALP7.** (a) Representative images obtained by immunofluorescence co-staining of NALP7 (green), M6PR (red) and DAPI (blue) in PMA-differentiated THP-1 cells following STAMPB knockdown (SBP kd) with siRNA, nontargeting control plasmid (NTC), BC-1471 (BC) treatment (10  $\mu$ M for 16 h), leupeptin (LEU) treatment (50  $\mu$ M for 2 h), or a combination thereof. Original magnification was 100x, scale bars = 10  $\mu$ m. (b,c) Colocalization analysis of images obtained as in Fig S5a. Data represents mean  $\pm$  SD for the m1 colocalization coefficient of the red-green channel pair ( $n=3$ ). (b, c) One-way analysis of variance (ANOVA) with post-hoc Dunnett's multiple comparisons test.

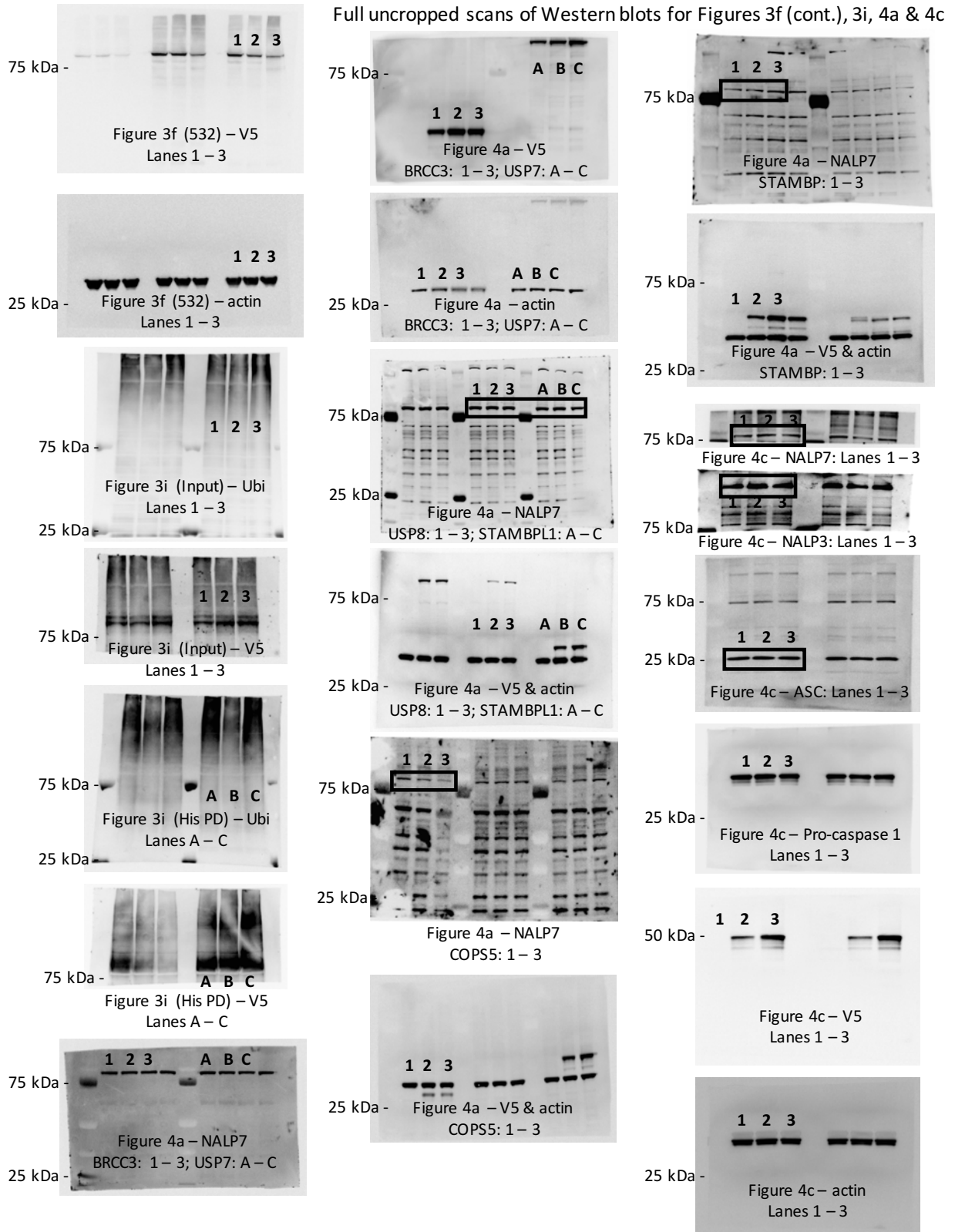
## Supplementary Figure 6. Uncropped Western blots.



**Supplementary Figure 6 (cont). Uncropped Western blots.**

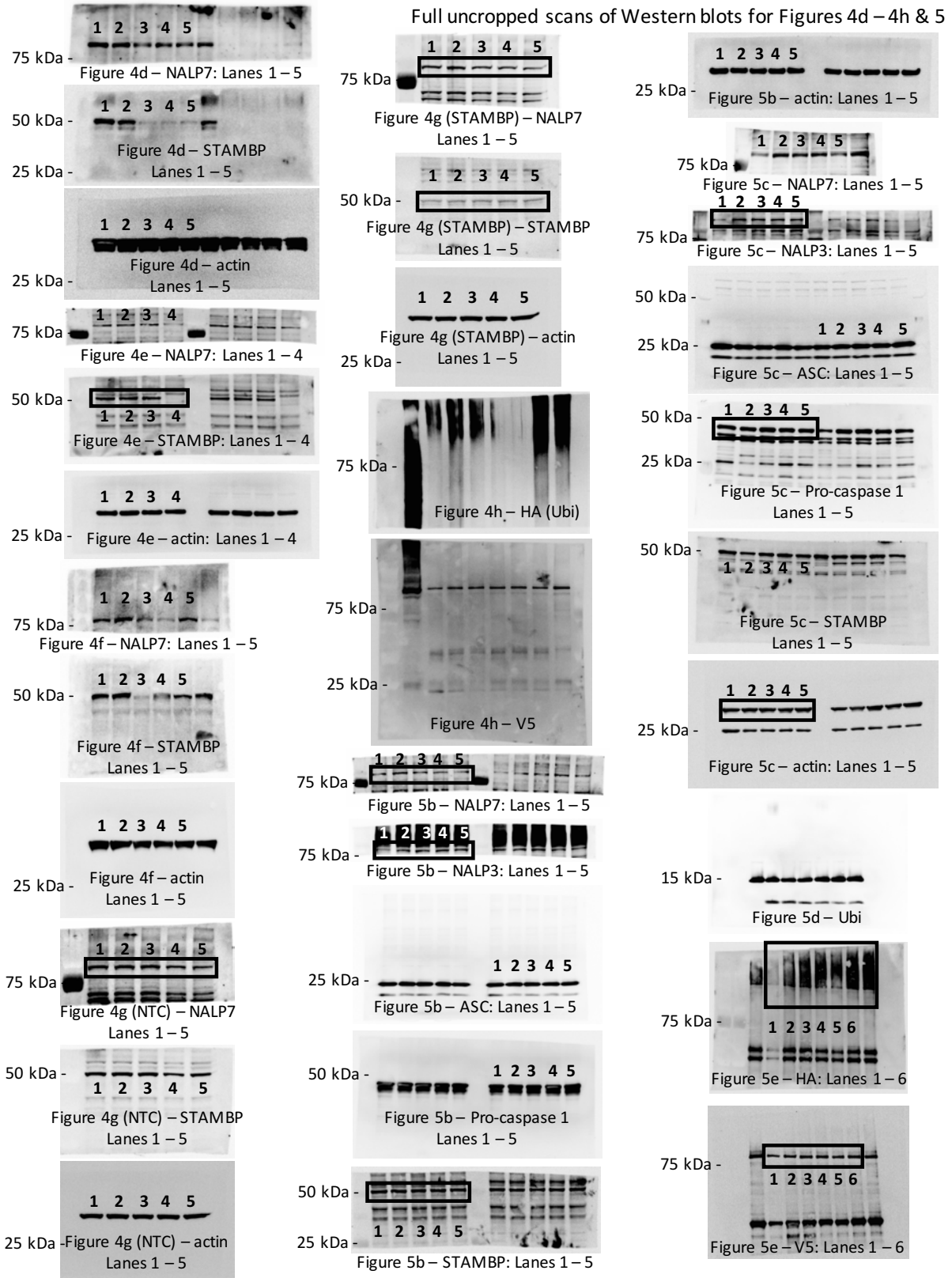


**Supplementary Figure 6 (cont). Uncropped Western blots.**

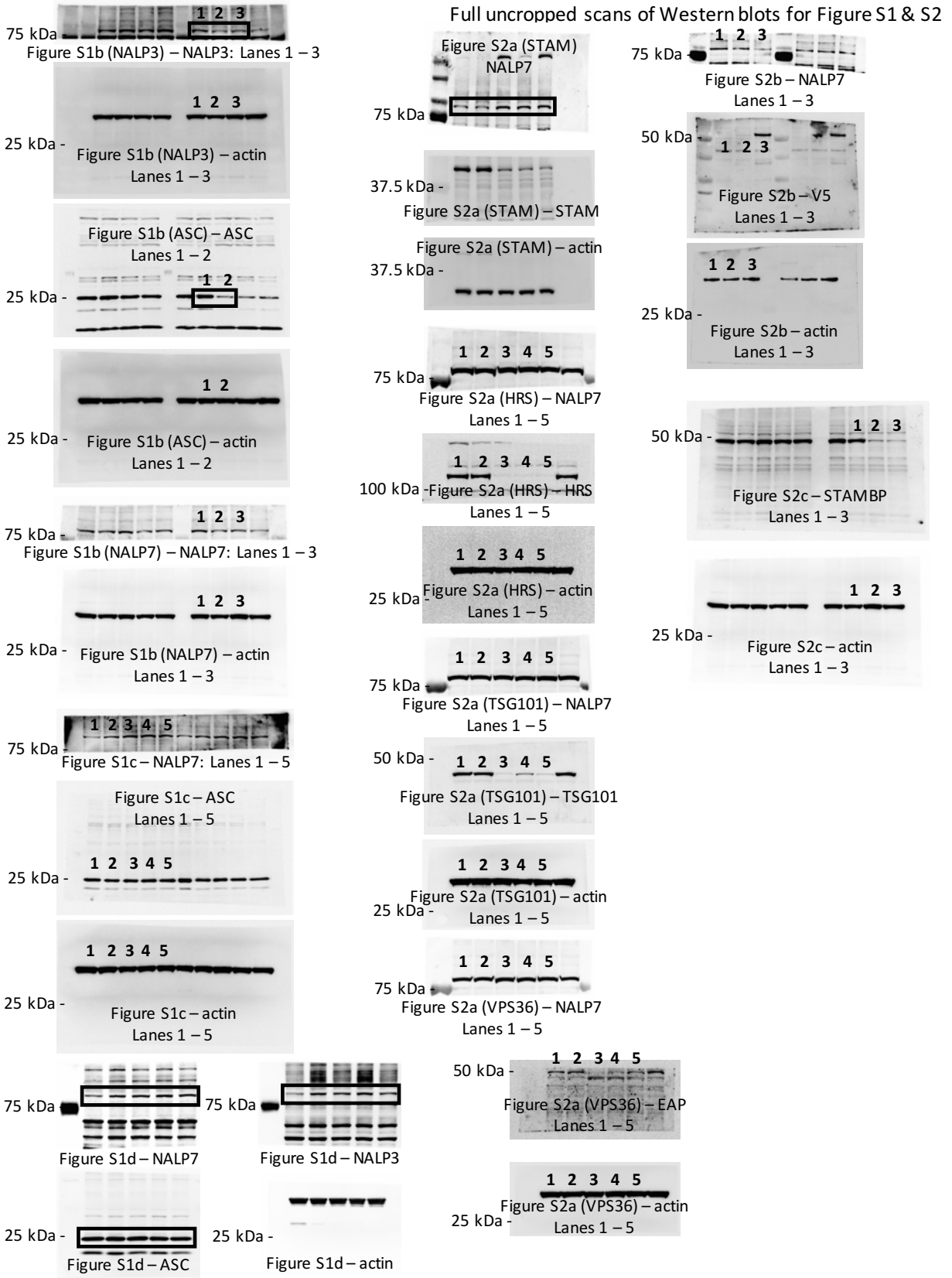




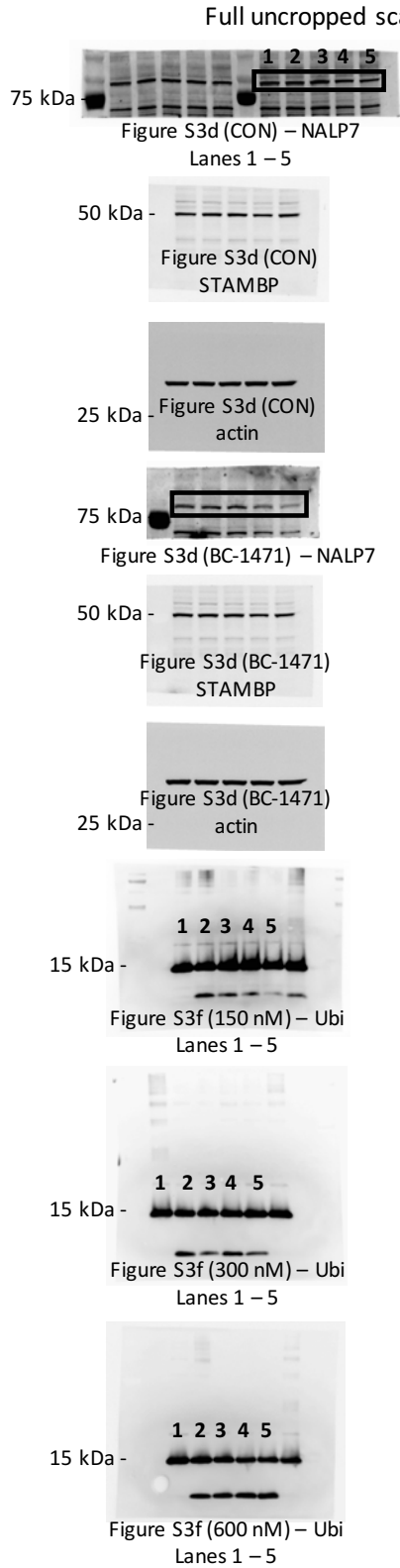
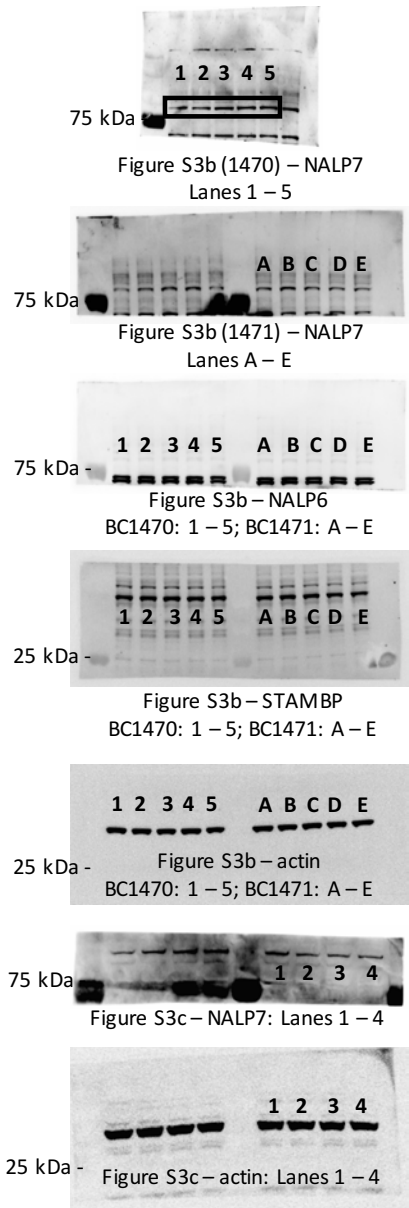
**Supplementary Figure 6 (cont). Uncropped Western blots.**



**Supplementary Figure 6 (cont). Uncropped Western blots.**



**Supplementary Figure 6 (cont). Uncropped Western blots.**



**Supplementary Table 1. List of primer sequences.**

| <b>PLASMID CLONING</b>                   |   |   |
|--|---|---|
| Target                                   | Forward   | Reverse   |
| NALP7 for topo                           | CAC CAT GTC TGA CCA TGG AGA<br>TGT GAG                | GCA AAA AAA GTC ACA GCA CGG<br>AGG                    |
| STAMBP for topo                          | CAC CAT GTC TGA CCA TGG AGA<br>TGT GAG                | TCG AAG GTC TGT GAT GGT CAC TGC                       |
| <b>qPCR PRIMERS</b>                      |   |   |
| Target                                   | Forward primer  | Reverse primer  |
| NALP7                                    | CTG CAG ATG TTG TCG TTG G                             | CTT GCT GAC AAC CAA GAC AG                            |
| Pro-IL-1beta                             | CTC GCC AGT GAA ATG ATG G                             | TGG AAG GAG CAC TTC ATC TG                            |
| STAMBP                                   | CCT CTA TAA CAA GTA TAT CAC GCT<br>C                  | CTT TGG GAA ATG CAA TCT CCT                           |
| STAM                                     | ACT TTG AAG CTG CTG AAG AC                            | TCC ACC AGT TAG GAT CAC TG                            |
| GAPDH                                    | GCA TCC TGG GCT ACA CTG AG                            | AAG TGG TCG TTG AGG GCA AT                            |
| <b>TRUNCATION MUTANT PRIMERS</b>         |   |   |
| WT NALP7<br>forward                      | CAC CAT GTC TGA CCA TGG AGA TGT GAG                   |   |
| WT NALP7<br>reverse                      | GCA AAA AAA GTC ACA GCA CGG AGG                       |   |
| N500 (reverse)                           | TAC GTC CCC GAT GTC CCA GG                            |   |
| N750 (reverse)                           | CAG CAT CAT CGT GCG TTC CCA                           |   |
| 250C (forward)                           | CAC CAT GGT GGT CGA TGG CC                            |   |
| 500C (forward)                           | CAC CAT GCA GAA GCT GCT TTC CG                        |   |
| <b>SITE-DIRECTED MUTAGENESIS PRIMERS</b> |   |   |
| Target                                   | Forward   | Reverse   |
| KKK275RRR                                | GCG GGG ACT GGG AGA GAA GAA<br>GAC CGG TGC CCG TCC TC | GAG GAC GGG CAC CGG TCT TCT TCT<br>CTC CCA GTC CCC GC |
| KRK288RRR                                | GGG GAG TTT GCT GAG AAG ACG<br>GAT GTT ACC CAG GG     | CCC TGG GTA ACA TCC GTC TTC TCA<br>GCA AAC TCC CC     |
| K374R                                    | GTG CAC GAC TCT GAG ACT GCA<br>GAT GGA GAA G          | CTT CTC CAT CTG CAG TCT CAG AGT<br>CGT GCA C          |
| K379R                                    | GCA GAT GGA GAG AGG GGA GGA<br>CCC G                  | CGG GTC CTC CCC TCT CTC CAT CTG<br>C                  |
| K461R                                    | GAC AGA GTC TCC AGA GGC TGC<br>TAC TC                 | GAG TAG CAG CCT CTG GAG ACT<br>CTG TC                 |
| K484R                                    | CTA CGC CCT GGA GAG AGA GGA<br>GGG GGA GG             | CCT CCC CCT CCT CTC TCT CCA GGG<br>CGT AG             |
| K502R                                    | GGG ACG TAC AGA GAC TGC TTT<br>CCG G                  | CCG GAA AGC AGT CTC TGT ACG TCC<br>C                  |
| K532R                                    | GAG AAG AGA GCC AGA GAG TTG<br>GAG GCC AC             | GTG GCC TCC AAC TCT CTG GCT CTC<br>TTC TC             |

**Supplementary Table 2. List of DsiRNA sequences.**

| Target     |   | Sequence (Sense)  | Sequence (Antisense)  |
|------------|---|---|---|
| NLRP3      | 1 | rGrGrA rArGrA rCrArC rCrArG rGrArC<br>rArArU rGrArC rArGC A | rUrGrC rUrGrU rCrArU rUrGrU rCrCrU<br>rGrGrU rGrUrC rUrUrC rCrUrC |
|            | 2 | rGrGrU rGrUrU rGrGrA rArUrU rArGrA<br>rCrArA rCrUrG rCrAA C | rGrUrU rGrCrA rGrUrU rGrUrC rUrArA<br>rUrUrC rCrArA rCrArC rCrUrG |
| ASC        | 1 | rGrCrG rArGrG rGrUrC rArCrA rArArC<br>rGrUrU rGrArG rUrGG C | rGrCrC rArCrU rCrArA rCrGrU rUrUrG<br>rUrGrA rCrCrC rUrCrG rCrGrA |
| NLRP7      | 1 | rGrGrU rGrCrA rArGrA rArArU rArGrA<br>rUrArA rUrCrC rUrGA G | rCrUrC rArGrG rArUrU rArUrC rUrArU<br>rUrUrC rUrUrG rCrArC rCrUrG |
|            | 2 | rGrGrA rUrArA rUrArA rArGrU rUrArG<br>rArGrC rArCrU rCrUC G | rCrGrA rGrArG rUrGrC rUrCrU rArArC<br>rUrUrU rArUrU rArUrC rCrCrU |
| STAMB<br>P | 1 | rGrUrA rGrCrU rCrArG rArArA rUrUrA<br>rArGrA rArArG rArAT G | rCrArU rUrCrU rUrUrC rUrUrA rArUrU<br>rUrCrU rGrArG rCrUrA rCrUrU |
|            | 2 | rGrGrA rGrUrU rGrArG rArUrU rArUrC<br>rCrGrA rArUrG rGrCA T | rArUrG rCrCrA rUrUrC rGrGrA rUrArA<br>rUrCrU rCrArA rCrUrC rCrArG |
|            | 3 | rGrCrU rCrUrU rArCrC rArGrA rUrGrA<br>rUrGrU rUrGrC rCrAG A | rUrCrU rGrGrC rArArC rArUrC rArUrC<br>rUrGrG rUrArA rGrArG rCrArG |
| STAM       | 1 | rArGrA rArUrU rGrArU rArCrA rArGrG<br>rCrUrA rUrUrU rGrUC T | rArGrA rCrArA rArUrA rGrCrC rUrUrG<br>rUrArU rCrArA rUrUrC rUrUrU |
|            | 2 | rArGrA rGrUrG rArArC rCrArC rArArA<br>rGrArU rCrCrU rCrAC G | rCrGrU rGrArG rGrArU rCrUrU rUrGrU<br>rGrGrU rUrCrA rCrUrC rUrUrC |
|            | 3 | rArGrA rArUrG rArUrC rCrArC rArGrC<br>rUrUrA rGrUrC rUrAA T | rArUrU rArGrA rCrUrA rArGrC rUrGrU<br>rGrGrA rUrCrA rUrUrC rUrUrA |
| HRS        | 1 | rGrCrC rUrUrA rCrArA rCrArU rGrCrA<br>rGrArA rUrCrU rCrAT G | rCrArU rGrArG rArUrU rCrUrG rCrArU<br>rGrUrU rGrUrA rArGrG rCrUrG |
|            | 2 | rArGrA rArUrU rCrArA rArGrA rGrArG<br>rCrGrA rUrGrC rCrAT G | rCrArU rGrGrC rArUrC rGrCrU rCrUrC<br>rUrUrU rGrArA rUrUrC rUrGrG |
|            | 3 | rGrGrA rGrUrA rArCrA rCrUrA rCrArU<br>rArCrA rGrUrU rCrAC C | rGrGrU rGrArA rCrUrG rUrArU rGrUrA<br>rGrUrG rUrUrA rCrUrC rCrGrG |
| TSG101     | 1 | rGrCrC rUrUrG rArArA rCrGrA rArCrA<br>rGrArA rGrArA rGrAC C | rGrGrU rCrUrU rCrUrU rCrUrG rUrUrC<br>rGrUrU rUrCrA rArGrG rCrArU |
|            | 2 | rUrGrG rUrGrU rCrCrA rArGrU rArCrA<br>rArArU rArCrA rGrAG A | rUrCrU rCrUrG rUrArU rUrUrG rUrArC<br>rUrUrG rGrArC rArCrC rArUrU |
|            | 3 | rArGrA rUrCrA rArGrA rArGrU rArGrC<br>rCrGrA rGrGrU rUrGA T | rArUrC rArArC rCrUrC rGrGrC rUrArC<br>rUrUrC rUrUrG rArUrC rUrArA |
| Vps36      | 1 | rGrGrA rArUrA rGrCrU rArArC rCrCrA<br>rGrUrU rArCrC rArGA G | rCrUrC rUrGrG rUrArA rCrUrG rGrGrU<br>rUrArG rCrUrA rUrUrC rCrCrA |
|            | 2 | rCrCrU rArGrC rCrArA rArGrA rArArG<br>rGrUrU rGrCrU rGrCT T | rArArG rCrArG rCrArA rCrCrU rUrUrC<br>rUrUrU rGrGrC rUrArG rGrArG |
|            | 3 | rGrGrC rUrArA rUrArC rUrGrC rArUrG<br>rUrArG rArUrU rGrCT T | rArArG rCrArA rUrCrU rArCrA rUrGrC<br>rArGrU rArUrU rArGrC rCrArG |