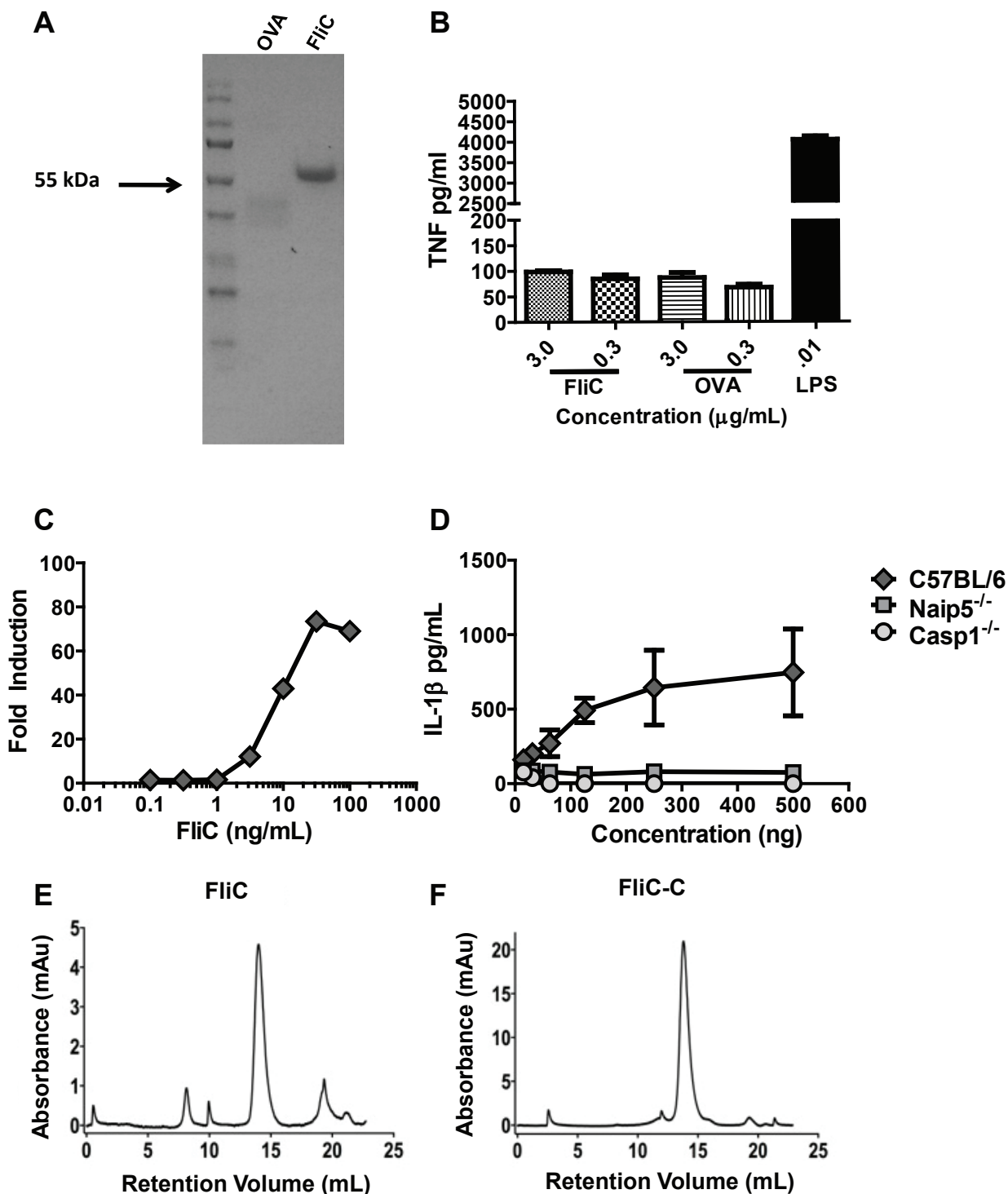


Supplemental Figure 1



Supplementary Figure 1. Highly purified flagellin is free of exogenous innate immune agonists, and is a robust agonist of TLR5 and the inflammasome. Highly purified FliC and OVA contained less than 0.1 pg endotoxin (0.001 EU) per μg of protein (data not shown), and consisted of a single protein of approximately 55 kDa (A). Flagellin did not activate RAW 264.7 cells, which do not express TLR5 and are unresponsive to flagellin (B). RAW 264.7 cells stimulated with either PBS, FliC, or OVA had less than 100 pg/mL TNF from collected supernatants, while cells given 10 ng/ml LPS produced greater than 4000 pg/mL in supernatants collected following overnight treatment (B). TLR5 biological activity was determined using CHO cells stably transfected with murine TLR5 and a NF- κ B luciferase reporter (C). The purified FliC had an EC₅₀ of \sim 8 ng/mL, similar to what we have reported previously (20, 64). Flagellin induced Naip5- and caspase-1-dependent IL-1 β secretion in LPS primed BMDMs with an EC₅₀ of approximately 100 ng/mL (D). Size exclusion chromatography demonstrated that FliC purified from *S. Typhimurium* (FliC) (E) or recombinant FliC purified from *E. coli* (FliC-C) (F) eluted at the same volume as a monodispersed species, consistent with flagellin monomers.