



Figure 4-figure supplement 1. ARNO inhibits INAVA-dependent NOD2-induced signaling and cytokine secretion.

(A-D) MDMs from rs7554511 CC homozygote (low-expressing) INAVA carriers were transfected with empty vector (EV) or the indicated HA-INAVA constructs. (A) Representative flow cytometry of INAVA constructs with mean fluorescent intensity (MFI) values as indicated. (B) Summary graph of INAVA expression (as detected by HA antibody) with MFI + SEM (n=6, similar results seen in an additional n=10). (C) Cells were stained with annexin V (BD Biosciences). Percent dead cells + SEM. UV stimulation at 50-100 J/m² served as a positive control. (D) Representative flow cytometry for Figure 4D with MFI values as indicated.

(E-I) MDMs (n=6) from rs7554511 CC homozygote (low-expressing) INAVA carriers were transfected with empty vector (EV) or HA-INAVA, and Myc-ARNO vector at the indicated concentrations. (E and F) Cells were left untreated or treated with 10 ng/ml IL-1 β or 100 μ g/ml MDP for 24h. (E) Representative flow cytometry with MFI values as indicated, and (F) summary graphs with MFI values of ARNO expression (as detected by Myc antibody) + SEM (similar results seen in an additional n=6 for MDP-treated conditions). (G) Cells were stained with annexin V. Percent dead cells + SEM. UV stimulation at 50-100 J/m² served as a positive control. (H-I) Cells were treated with 100 μ g/ml MDP. (H) Summary graphs with fold MFI of indicated phospho-kinases at 15 min + SEM. (I) Mean TNF secretion at 24h + SEM. Significance is compared to treated cells without ARNO transfection (EV) for each respective condition for (H and I). Tx, treatment; EV, empty vector; NS, not significant; **, p<0.01; ***, p<0.001; \dagger , p<1x10⁻⁴; $\dagger\dagger$, p<1x10⁻⁵.