



**Figure 3–figure supplement 1. INAVA-GFP puncta formation is relatively less in HCT8 cells stimulated with TNF $\alpha$ .**

(A) Verification of HCT8 TRAF6 knockout cells. Immunoblot of p65 activation in HCT8 wildtype and TRAF6 knockout cells treated with IL-1 $\beta$  (10 ng/ml) for 15 minutes.

(B) Quantification of %positive GFP puncta of stably expressed INAVA-GFP and INAVA-S-GFP with TNF $\alpha$  stimulation at 1 ng/ml, 2.5 ng/ml and 25 ng/ml for 30 minutes, n=70-85 cells.

(C) Immunoblot of p65 activation in Caco2BBE wildtype and INAVA KO lines. Cells were mock treated or stimulated with IL-1 $\beta$  (10 ng/ml) for 5 and 15 minutes.

(D) Verification of HEK293T TRAF6 exon deleted knockout cells by genomic DNA PCR.