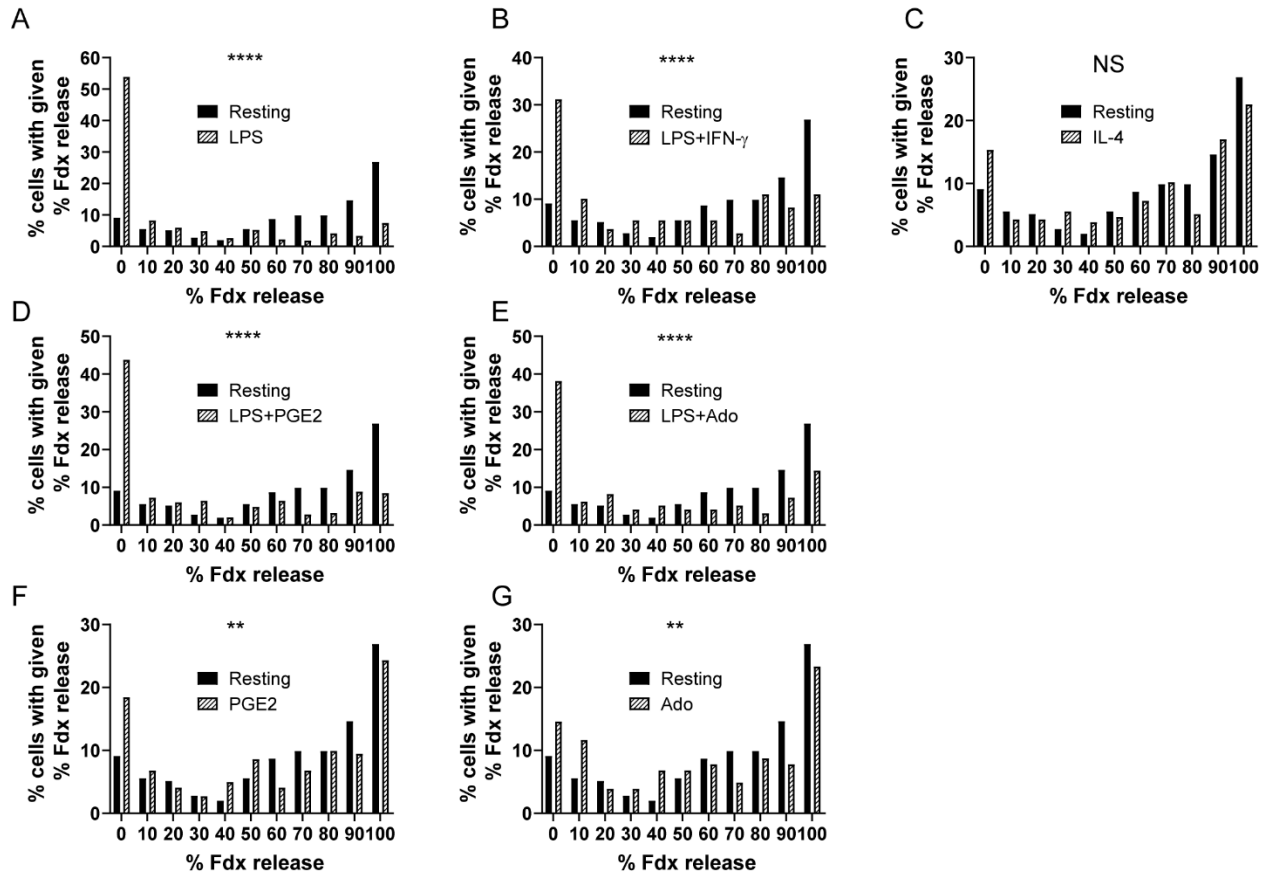


Supplemental Materials

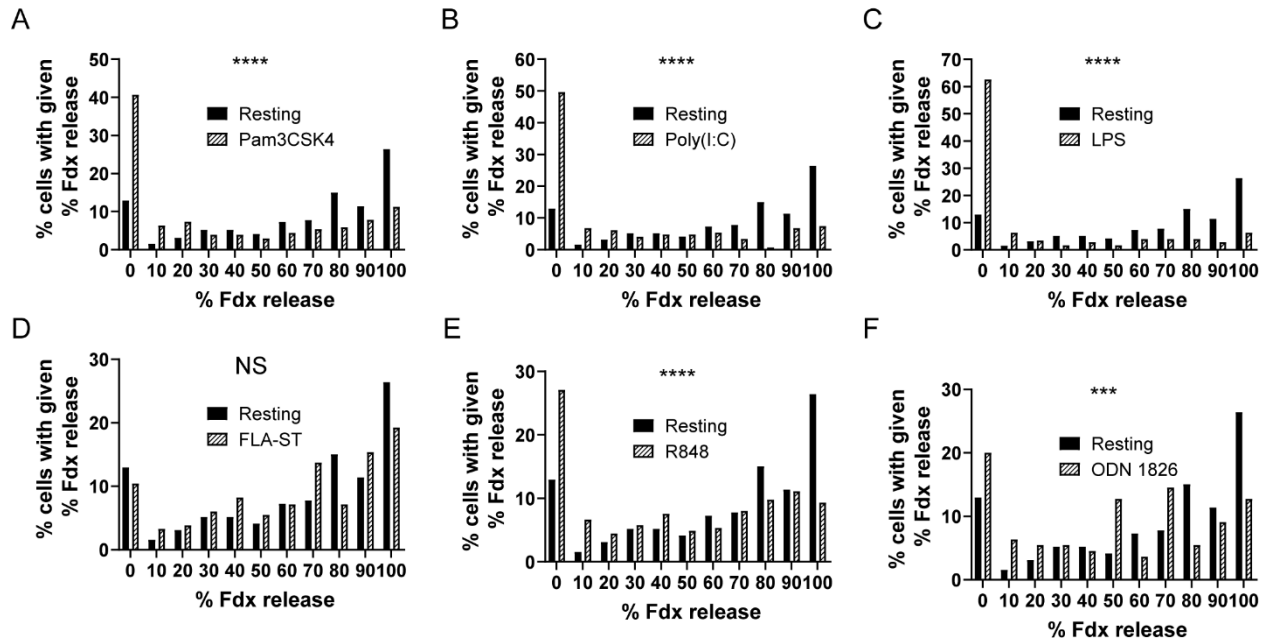
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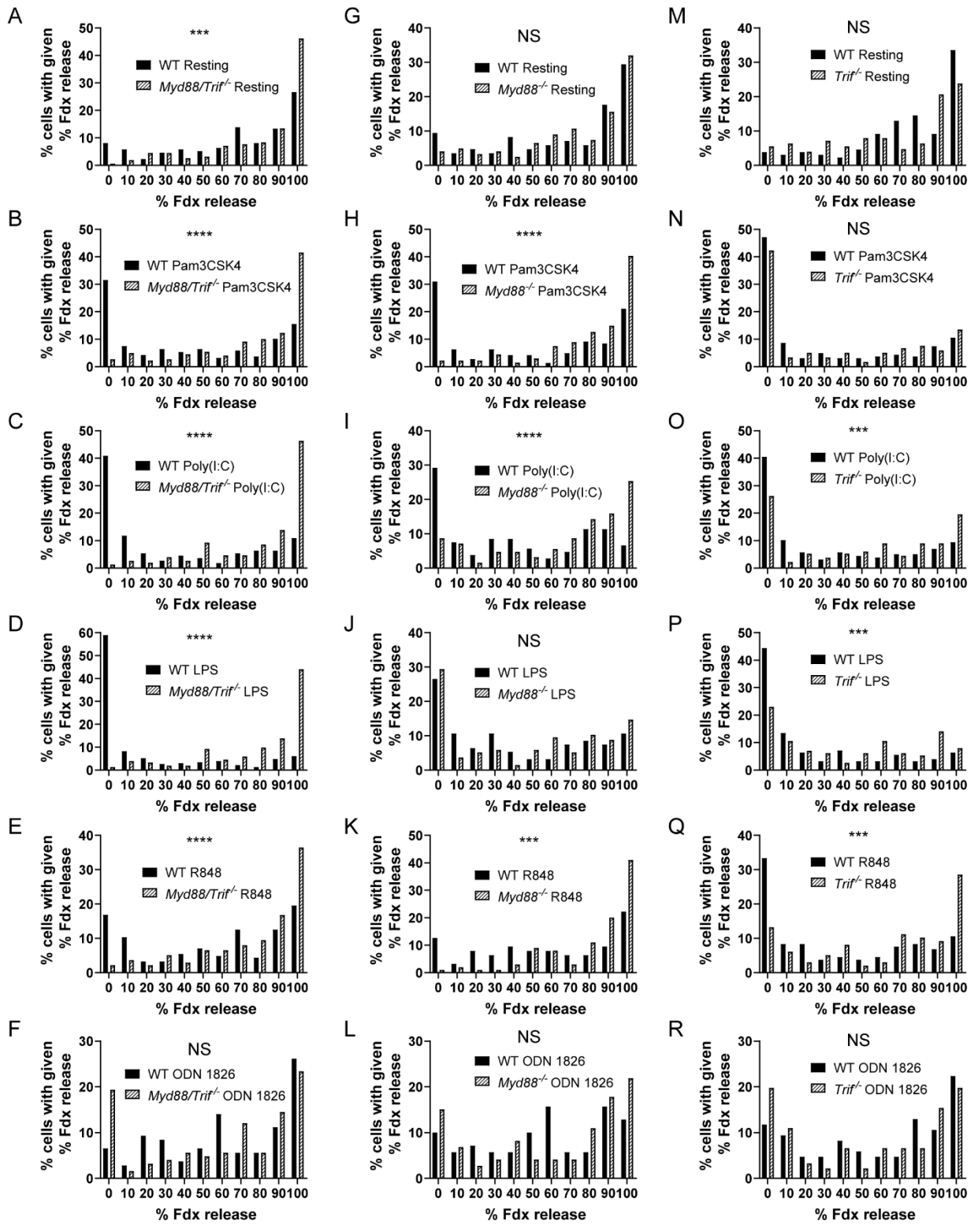
Supplemental Figure 1: Frequency distributions of data sets in Figure 1C.

For each bead-positive condition analyzed in Figure 1C, individual cell data was plotted in histogram form to depict the percent of cells within each condition experiencing a given level of lysosomal damage. The relative frequency in percentages (y-axis) of a given level of % Fdx release (represented in increments of 10% on the x-axis) for each treatment condition was plotted and compared with the frequency distribution of % Fdx release values for resting BMM. The frequency distribution for resting BMM was compared with that for BMM stimulated with LPS (A), LPS+IFN- γ (B), IL-4 (C), LPS+PGE2 (D), LPS+Ado (E), PGE2 (F), Ado (G). Statistical significance of the difference between each pair of frequency distributions was determined using the Kolmogorov-Smirnov test. Shown are pooled cell data from two to four independent experiments ($n > 97$ cells per condition). NS: no significant difference, $**p \leq 0.01$, $****p \leq 0.0001$.



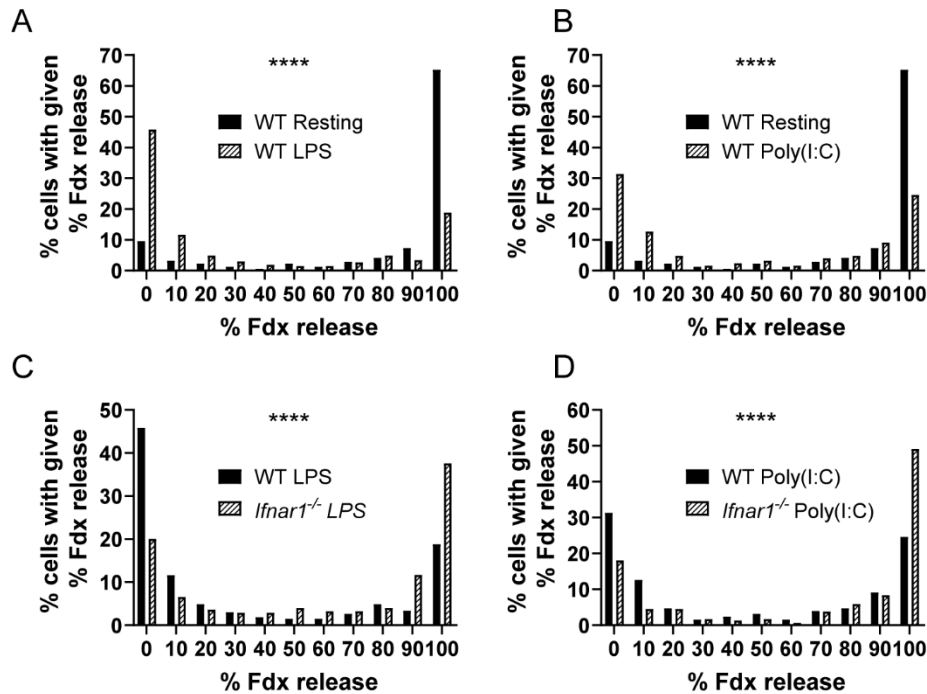
Supplemental Figure 2: Frequency distributions of data sets in Figure 2A.

Histograms depicting the percent of cells (y-axis) experiencing a given level of % Fdx release (represented in bins of 10% on the x-axis) based on individual cell data from each bead-positive condition analyzed in Figure 2A. The frequency distribution for resting BMM was compared with that for BMM stimulated with the following TLR agonists: (A) Pam3CSK4, (B) Poly(I:C), (C) LPS, (D) FLA-ST, (E) R848, (F) ODN 1826. Shown are pooled cell data from two or three independent experiments ($n > 110$ cells per condition). NS: no significant difference, $***p \leq 0.001$, $****p \leq 0.0001$.



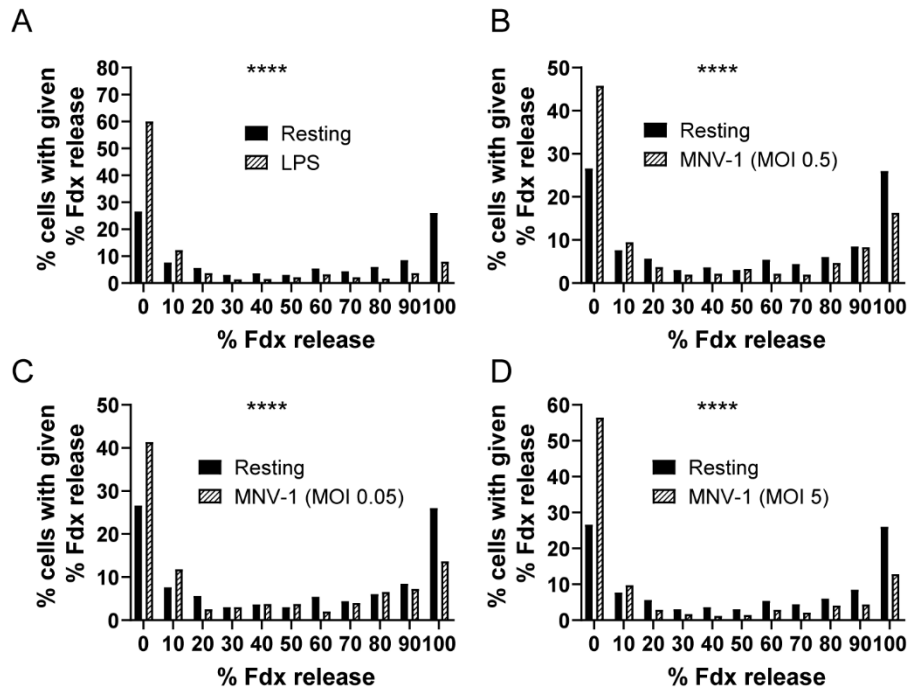
Supplemental Figure 3: Frequency distributions of data sets in Figure 3.

Histograms depicting the percent of cells (y-axis) experiencing a given level of % Fdx release (represented in bins of 10% on the x-axis) based on individual cell data from each bead-positive condition analyzed in Figure 3. To visualize the effect of each genetic knockout on the distribution of damage for each stimulation condition, the frequency distribution for a given stimulation condition was compared between WT and *Myd88/Trif*^{-/-} BMM (A-F), WT and *Myd88*^{-/-} BMM (G-L), and WT and *Trif*^{-/-} BMM (M-R). Shown are pooled cell data from two independent experiments (n > 107 cells for A-F, n > 63 cells for G-L, n > 85 cells for M-R). NS: no significant difference, *** $p \leq 0.001$, **** $p \leq 0.0001$.



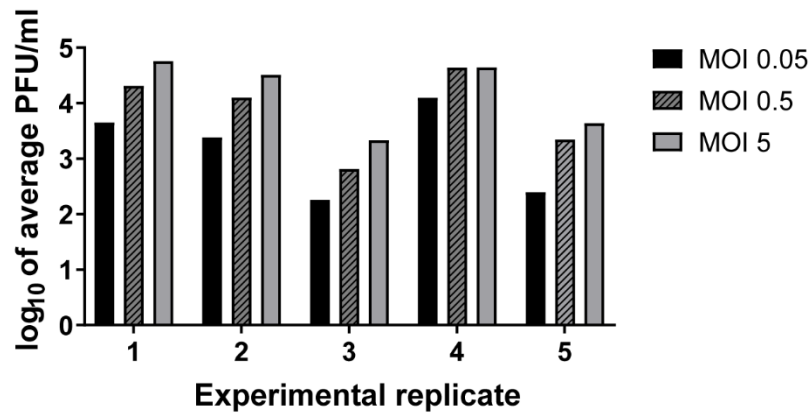
Supplemental Figure 4: Frequency distributions of data sets in Figure 4.

Histograms depicting the percent of cells (y-axis) experiencing a given level of % Fdx release (represented in bins of 10% on the x-axis) based on individual cell data from each bead-positive condition analyzed in Figure 4. In the top panel, the frequency distribution for resting WT BMM was compared with that for WT BMM stimulated with LPS (A) or poly(I:C) (B). In the bottom panel, the frequency distribution for each given stimulation condition was compared between WT and *Ifnar1*^{-/-} BMM. Shown are pooled cell data from three independent experiments (n > 252 cells per condition). *****p* ≤ 0.0001.



Supplemental Figure 5: Frequency distributions of data sets in Figure 5.

Histograms depicting the percent of cells (y-axis) experiencing a given level of % Fdx release (represented in bins of 10% on the x-axis) based on individual cell data from each bead-positive condition analyzed in Figure 5. The frequency distribution for resting BMM was compared with that for BMM stimulated with LPS (A) or infected with MNV-1 at one of three MOIs (0.05, 0.5, 5; B-D) before being subjected to lysosomal damage. Shown are pooled cell data from five independent experiments ($n > 396$ cells per condition). **** $p \leq 0.0001$.



Supplemental Figure 6: MNV-1 infects BMM in an MOI-dependent manner at 18 hours post-infection in 5 different experiments.

WT BMM were infected with MNV-1 at three different MOIs (0.05, 0.5 and 5). Viral titers in cell culture lysates were measured by virus titration using a plaque assay and reported as plaque forming units/ml (PFU/ml). Bars show MNV-1 infection titers of three different MOIs from 5 independent experiments performed in duplicate or triplicate. These assays were performed in parallel with the viral infections for the lysosomal damage experiments described in Figure 5.