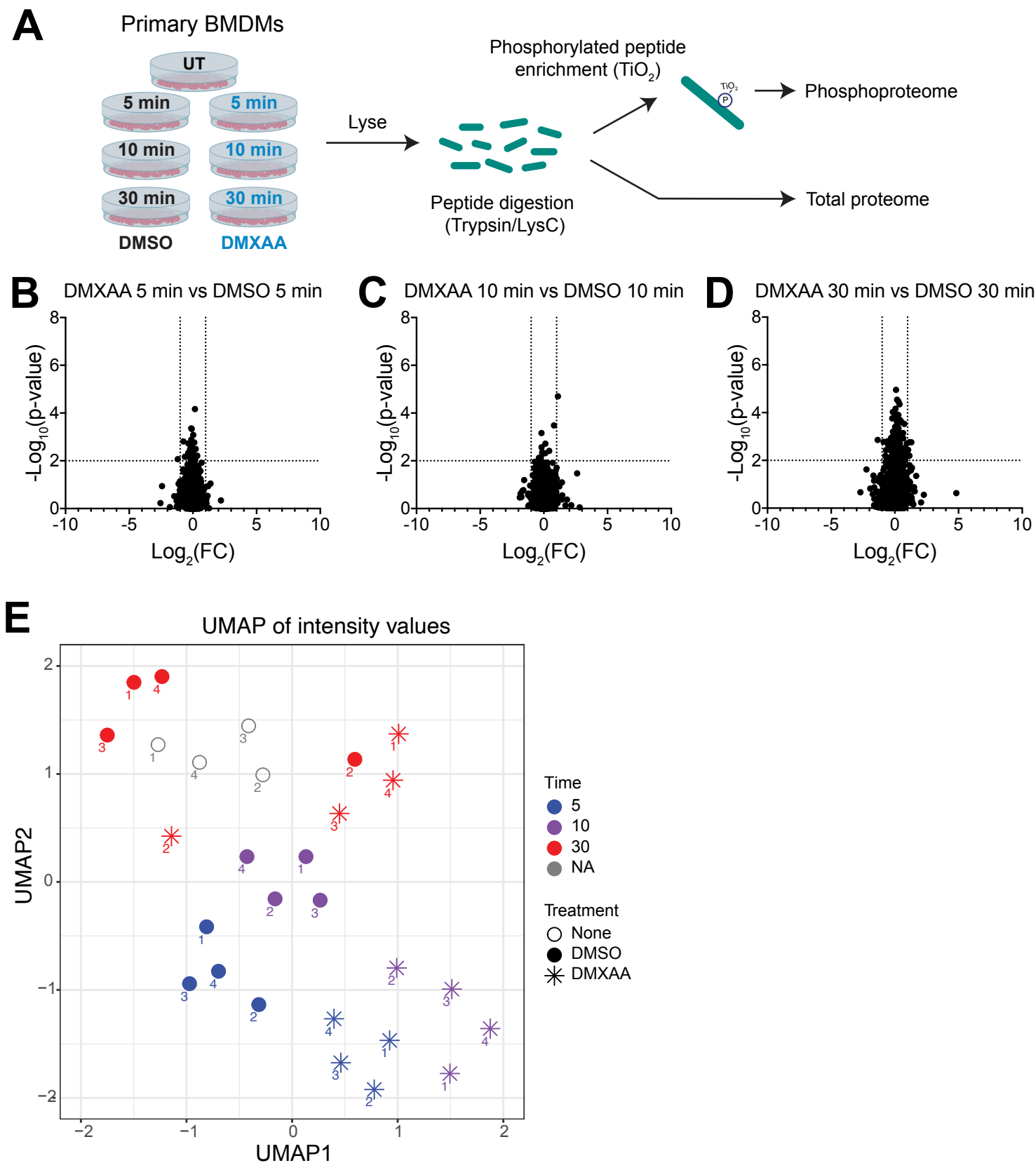


Appendix

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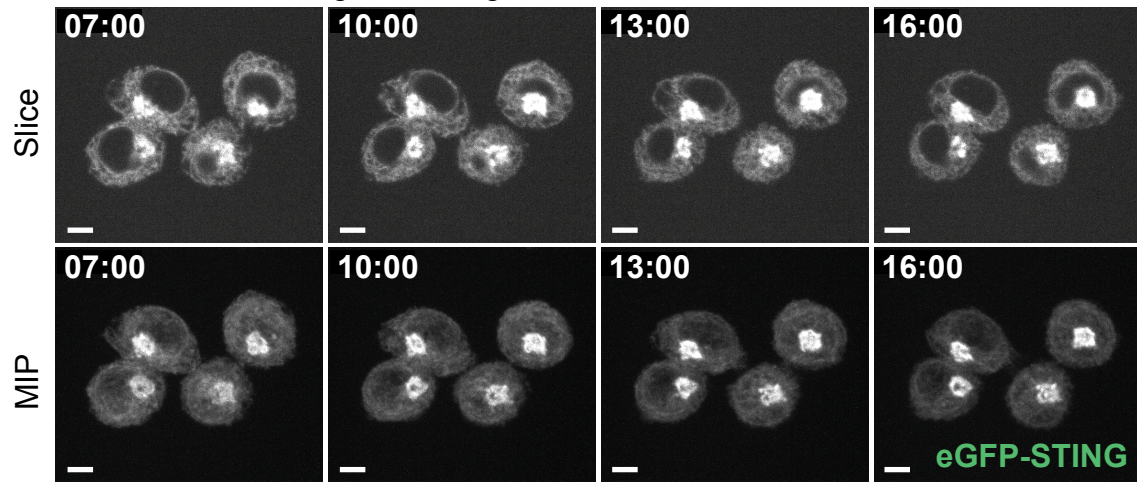
Appendix Figure S1: Phosphoproteomic analysis of primary BMDMs following STING activation

A Schematic showing phosphoproteomic experimental design by primary BMDMs were left untreated (UT) or treated with DMSO (vehicle control) or 50 $\mu\text{g}/\text{mL}$ DMXAA for 5, 10 or 30 min. A portion of digest peptides were used for total proteome analysis, while the rest underwent enrichment with titanium oxide beads for phosphorylated peptides, and analysis of the phosphoproteome.

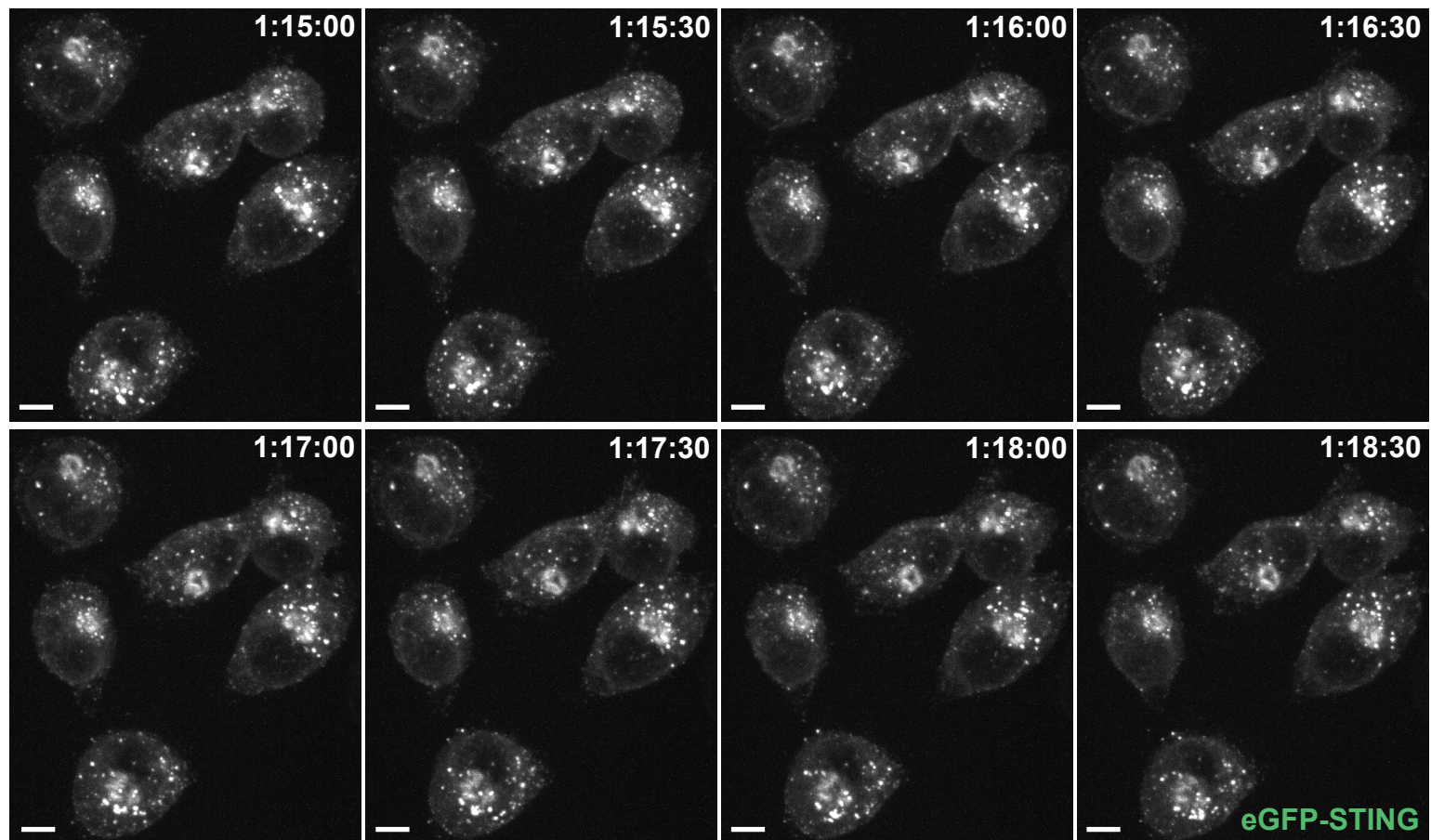
B-D Volcano plots showing changes in total proteome levels for DMXAA vs DMSO groups for 5 min (**B**), 10 min (**C**) and 30 min (**D**) timepoints. $-\text{Log}_{10}(\text{p-value})$ is plotted on the y-axis vs $\text{Log}_2(\text{Fold change}[\text{FC}])$ on the x-axis.

E Phosphoproteomic samples were visualised via uniform manifold approximation and projection (UMAP).

A STING ER to Golgi trafficking within first 15 min of DMXAA



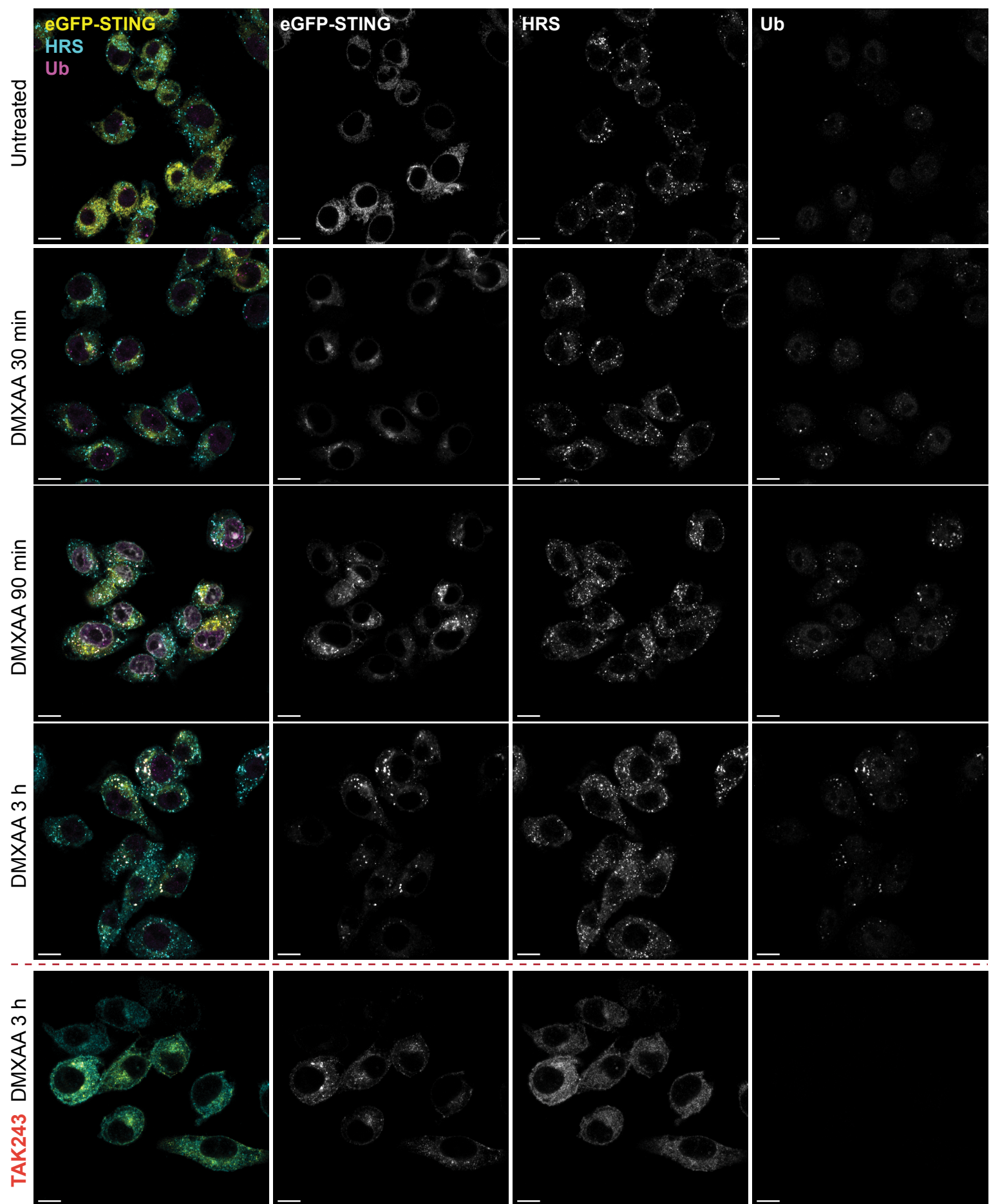
B Accumulation of STING vesicles at later timepoints



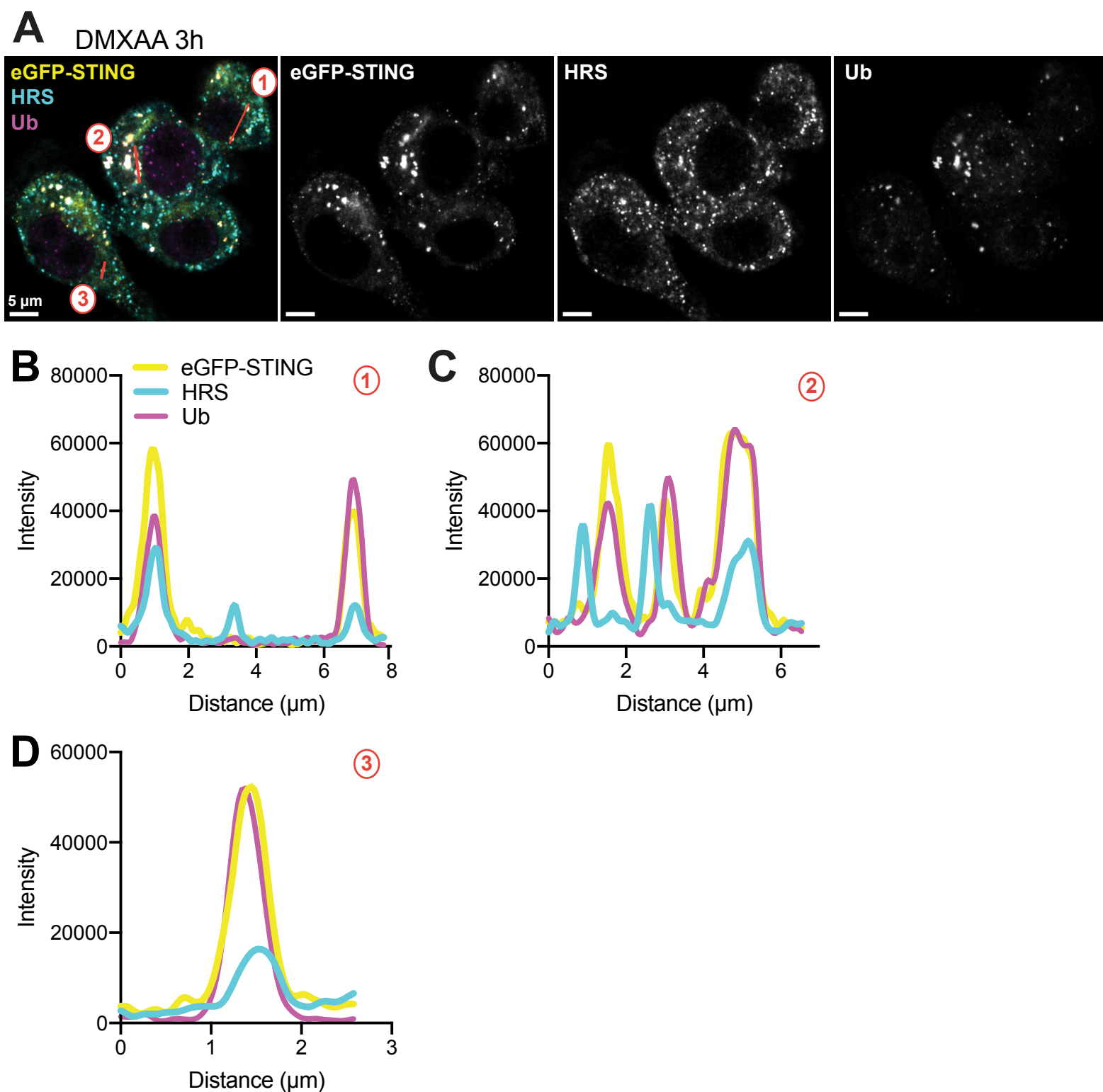
Appendix Figure S2: STING ER-Golgi and vesicular trafficking

A *Sting*^{-/-} iBMDMs expressing eGFP-STING were imaged live on the spinning disk microscope. Images display a time series as indicated, showing STING translocation from ER to Golg after 50 μ g/mL DMXAA treatment. Data is shown as a maximum intensity projection (MIP) of Z stack images. Scale bar = 5 μ m. Data shown is representative of 3 independent experiments.

B *Sting*^{-/-} iBMDMs expressing eGFP-STING were imaged live on the spinning disk microscope. Images display a time series as indicated, showing transport of STING vesicles for a recording starting 1 h 15 min after 50 μ g/mL DMXAA treatment. Data is shown as a maximum intensity projection (MIP) of Z stack images. Scale bar = 5 μ m. Data shown is representative of 3 independent experiments.



Appendix Figure S3: STING interacts with HRS over time in a ubiquitin-dependent fashion
Sting^{-/-} iBMDMs expressing eGFP-STING (yellow) were left untreated or treated with 50 μ g/mL DMXAA for 30 min, 90 min or 3 h. Cells were additionally treated with 2 μ M TAK243 (as indicated in red) for 30 min before 50 μ g/mL DMXAA for 3 h. Cells were then fixed and underwent immunofluorescence staining for conjugated ubiquitin (Ub; magenta) and HRS (cyan). Z stack images were acquired on the LSM980 confocal microscope. Images are displayed as merged and single colour images for a single Z slice. Scale bar = 10 μ m. Data shown is representative of 3 independent experiments. The untreated merged image is also shown in Figure 5C.



Appendix Figure S4: STING colocalises with HRS and ubiquitin

A *Sting*^{-/-} iBMDMs expressing eGFP-STING (yellow) were left untreated or treated with 50 $\mu\text{g/mL}$ DMXAA for 3 h. Cells were fixed and underwent immunofluorescence staining for conjugated ubiquitin (Ub; magenta) and HRS (cyan). Z stack images were acquired on the LSM980 confocal microscope. Images are displayed as merged images for a single Z slice. Scale bar = 5 μm . Data shown is a zoomed in region from **Appendix Figure S3**, displayed as both merged and single colour images.

B-D The intensity profiles were plotted for each channel for three-line regions as indicated in **A**. This provides an additional example to the data shown in **Figure 5D-F**