Supplementary Materials

Characterization of Antibody Bipolar Bridging Mediated by the Human Cytomegalovirus Fc receptor gp68

Contents

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\textbf{Movie 1} Movie of trafficking of gD-Dendra2 and anti-gD\textsubscript{hFc} to lysosomes under ABB conditions.

\textbf{Movie 2} Movie of trafficking of IgG\textsubscript{hFc}, but not gD-Dendra2, to lysosomes under non-ABB conditions.

\textbf{Movie 3} Movie showing no trafficking of gD-Dendra2 or anti-gD\textsubscript{mFc} to lysosomes under non-ABB conditions.
**Movie 1** Trafficking of gD-Dendra2 and anti-gD_{hFc} to lysosomes under ABB conditions (corresponding to Figure 3B; top panels). Live cell imaging of HeLa cells expressing gp68 and gD-Dendra2 (green) incubated with AF568-labeled EGF (red) and AF648-labeled anti-gD_{hFc} (blue). Regions of EGF/gD colocalization appear yellow, regions of gD/anti-gD_{hFc} colocalization appear cyan, regions of EGF-anti-gD_{hFc} colocalization appear magenta, and regions of triple colocalization appear white. Confocal imaging was performed using a 63x oil objective lens (αPlan-APOCHROMAT 1.45 Oil DIC) on a PerkinElmer Spinning Confocal Microscope and a CCD camera (Hamamatsu Photonics) controlled by the Volocity 6.1 software (PerkinElmer). Z-stacks (0.5-1 µm section thickness and up to 16 µm total depth) were captured approximately every 3 min for ~90 min. The video was recorded at ~5 seconds per frame and presented at 10 frames per second. Equatorial planes for z-stack sections are shown. Scale bar: 10 µm.

**Movie 2** Trafficking of IgG_{hFc}, but not gD-Dendra2, to lysosomes under non-ABB conditions (corresponding to Figure 3B; middle panels). Live cell imaging of HeLa cells expressing gp68 and gD-Dendra2 (green) incubated with AF568-labeled EGF (red) and AF648-labeled IgG_{hFc} (blue). Regions of EGF/IgG_{hFc} colocalization appear magenta. Confocal imaging was performed under conditions described for Movie 1.

**Movie 3** No trafficking of gD-Dendra2 or anti-gD_{mFc} to lysosomes under non-ABB conditions (corresponding to Figure 3B; bottom panels). Live cell imaging of HeLa cells expressing gp68 and gD-Dendra2 (green) incubated with AF568-labeled EGF (red) and AF648-labeled anti-gD_{mFc} (blue). Regions of gD/anti-gD_{mFc} colocalization appear cyan. Confocal imaging was performed under conditions described for Movie 1.