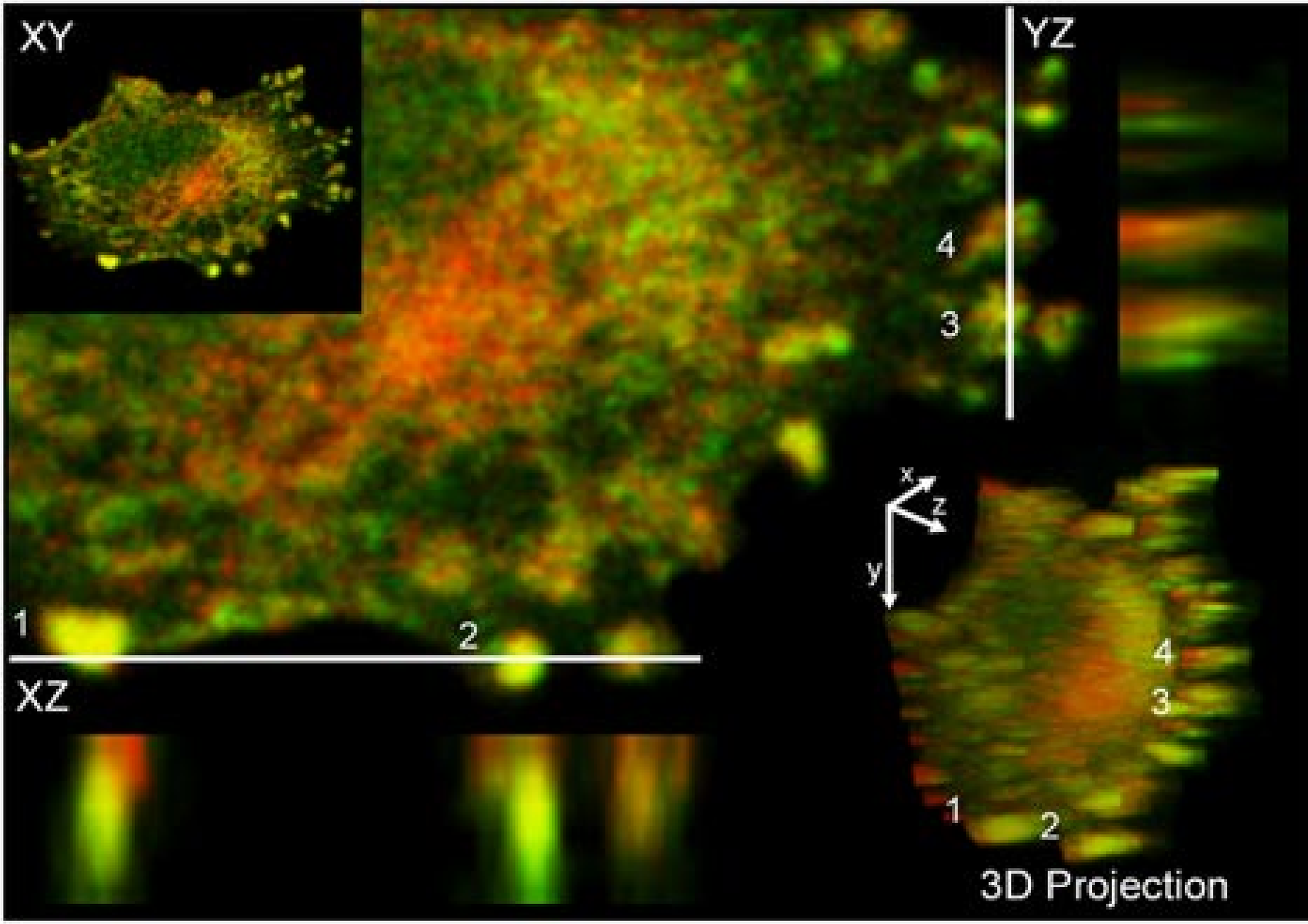
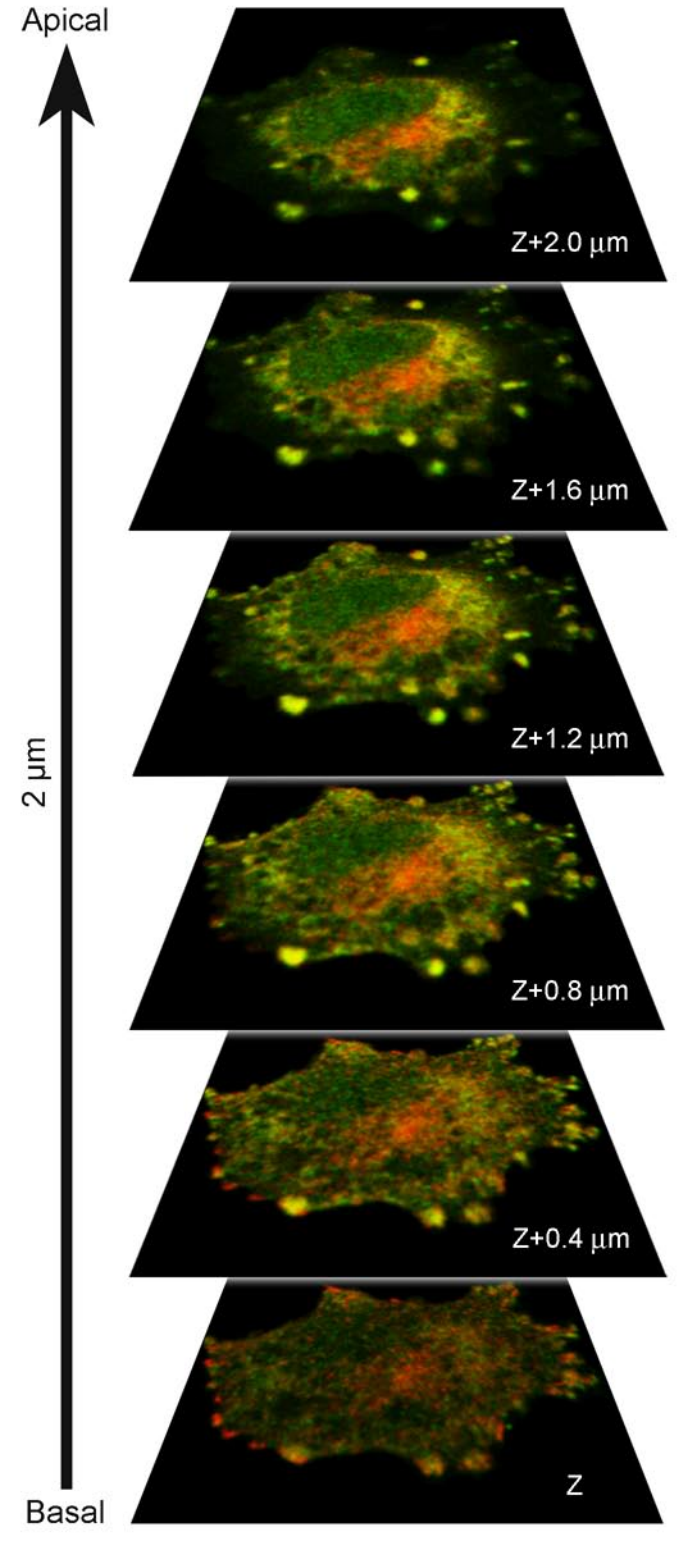
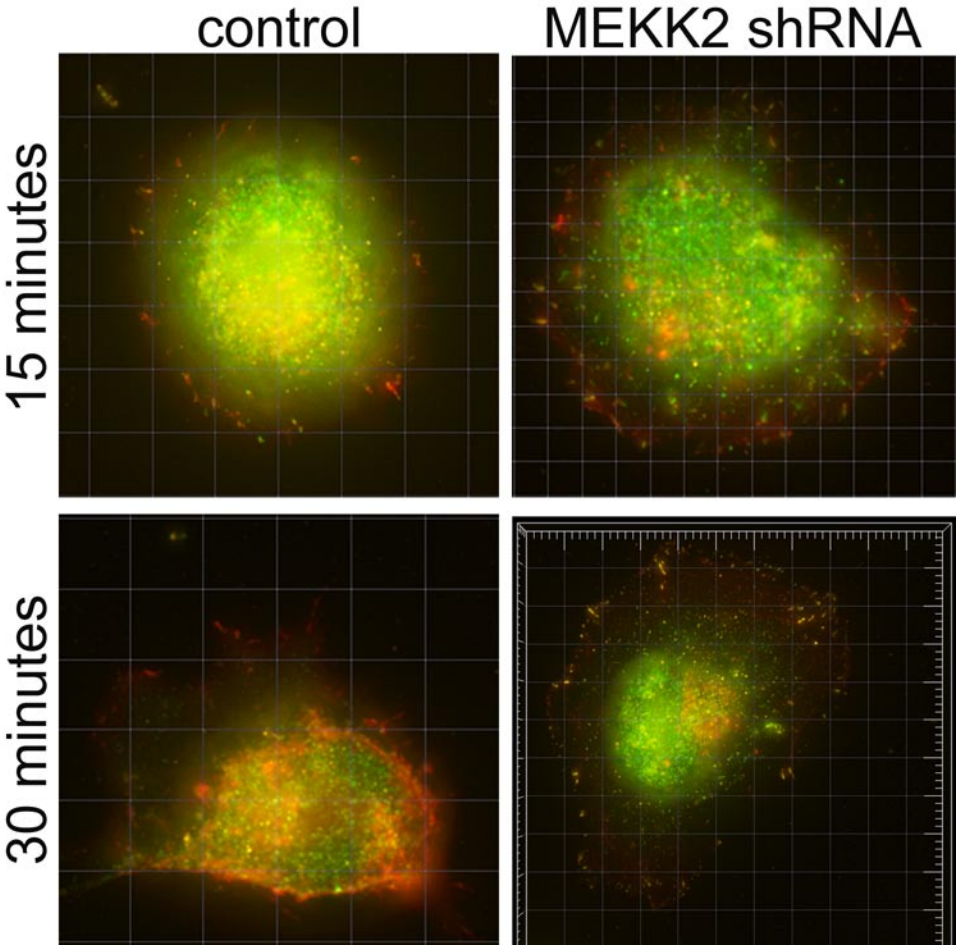
Ameka et al, Supplementary Figure 1



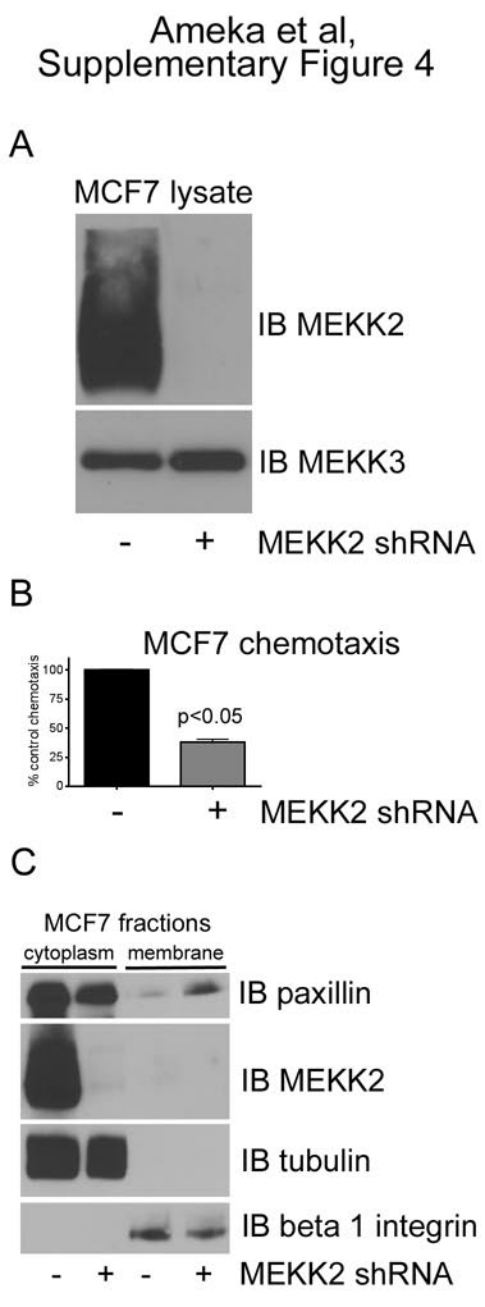
Ameka et al, Supplementary Figure 2



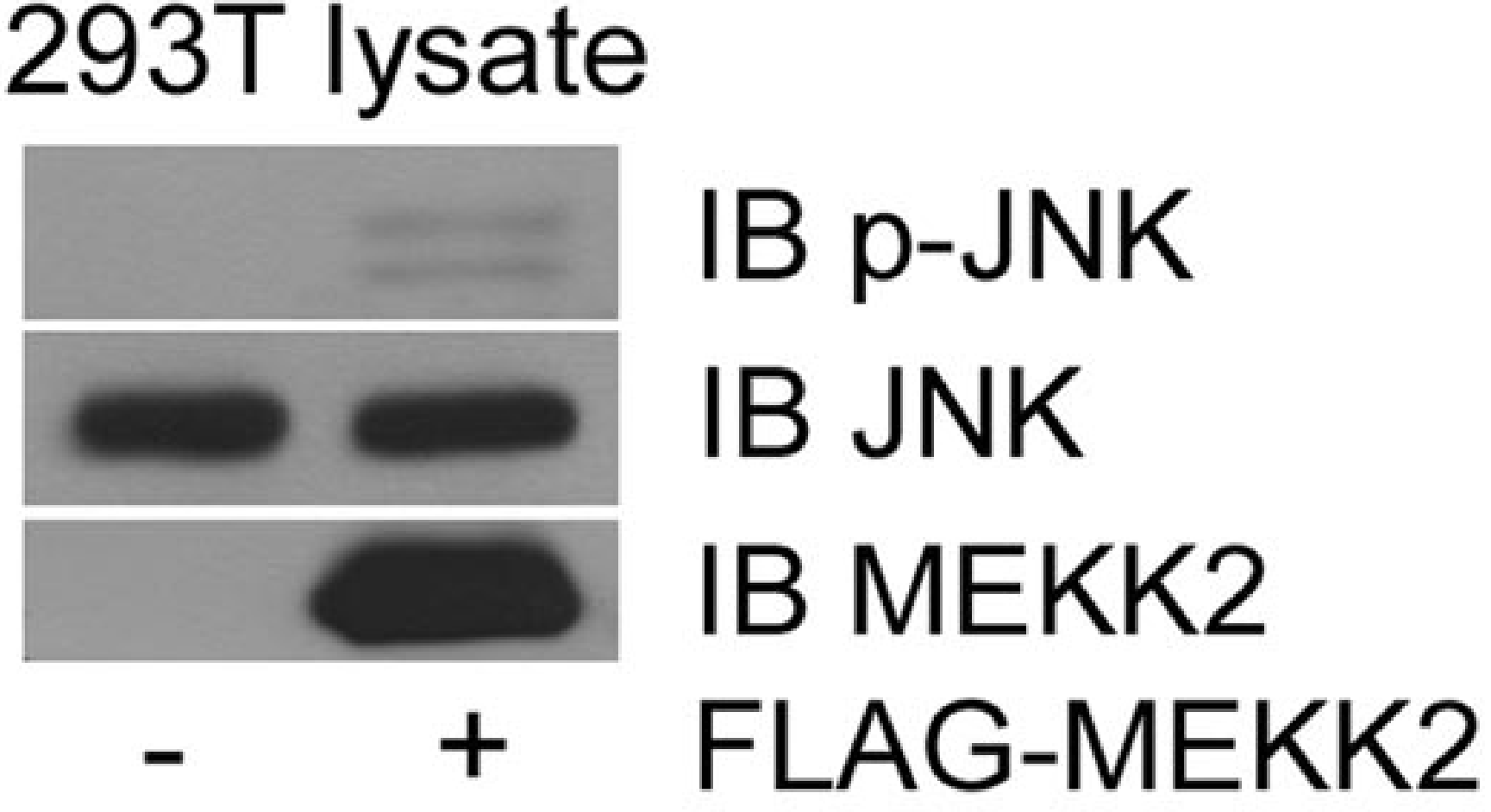
Ameka et al, Supplementary Figure 3



Ameka et al,
Supplementary Figure 4



Ameka et al, Supplementary Figure 5



Supplementary Figure legends:

Supplementary Figure 1. MEKK2 forms three-dimensional complexes with paxillin in breast tumor cells attached to fibronectin.

Cell stained with anti- paxillin (**red**) and MEKK2 (**green**) show MEKK2 co-localization with paxillin (**yellow**) in XY plane (**small inset, upper left**). Higher magnification is used to develop orthogonal reconstruction of a 2.4 µM slice derived from consecutive confocal sections in the XZ and YZ planes. Lines indicate a plane through areas of co-localization that was then rotated 90 degrees to show the three-dimensional aspect of the MEKK2/paxillin complexes. Numbers (1-4) indicate the designated areas of co-localization in a 3D projection of the cell, rotated 50° about the Y-axis (**inset, lower right**).

Supplementary Figure 2. MEKK2 co-localizes with paxillin proximal to focal adhesions

Fluorescence Z-plane confocal stack images of fixed MDA-MB 231 cells adhered to fibronectin-coated coverslip taken in 0.4 µm slices starting at the basal (ventral) plane, detected by MEKK2 **(green)** and paxillin **(red)** specific antibodies.

Supplementary Figure 3. Silencing MEKK2 expression inhibits chemotaxis and promotes paxillin membrane localization in MCF7 cells.

(A) Anti-MEKK2 immunoblot of lysates from MCF7 cells that stably express either MEKK2 shRNA or empty vector control, showing specific MEKK2 knockdown **(upper panel)**, subsequently the membrane was stripped and re-probed with anti-MEKK3 antibodies to show equal loading and knockdown specificity **(lower panel)**. **(B)** Graph displays fold change in serum-induced MCF7 chemotaxis (Boyden chamber assay) induced by MEKK2 knockdown. Migrating cells that express MEKK2 shRNA are shown as a percentage of the number of migrating control cells. Two-tailed paired t test analysis indicates that MEKK2 shRNA significantly ($p < 0.05$) inhibited cell migration. **(C)** Representative immunoblots showing the paxillin distribution into the cytoplasmic and membrane fractions from MCF7 cells expressing either MEKK2 shRNA or an empty shRNA vector control. The data are representative of at least three independent experiments.

Supplementary Figure 4. MEKK2 transfection induces JNK phosphorylation.

Immunoblot analysis of total cell lysates from 293T cells transfected with either FLAG-MEKK2 plasmid or empty control plasmid. Anti-phospho JNK (T183/Y185) immunoblot (**upper panel**) was subsequently stripped and re-probed with anti-JNK (**middle panel**) and anti-FLAG antibodies (**lower panel**) to determine loading and transfection efficiency.

Supplementary Movie 1. 3D projection of MEKK2 interaction with paxillin

Rotating 3D projection of fluorescence Z-plane confocal stack images of fixed MDA-MB 231 cells adhered to fibronectin-coated coverslip taken in 0.4 µm slices starting at the basal (ventral) plane, detected by MEKK2 **(green)** and paxillin **(red)** specific antibodies.