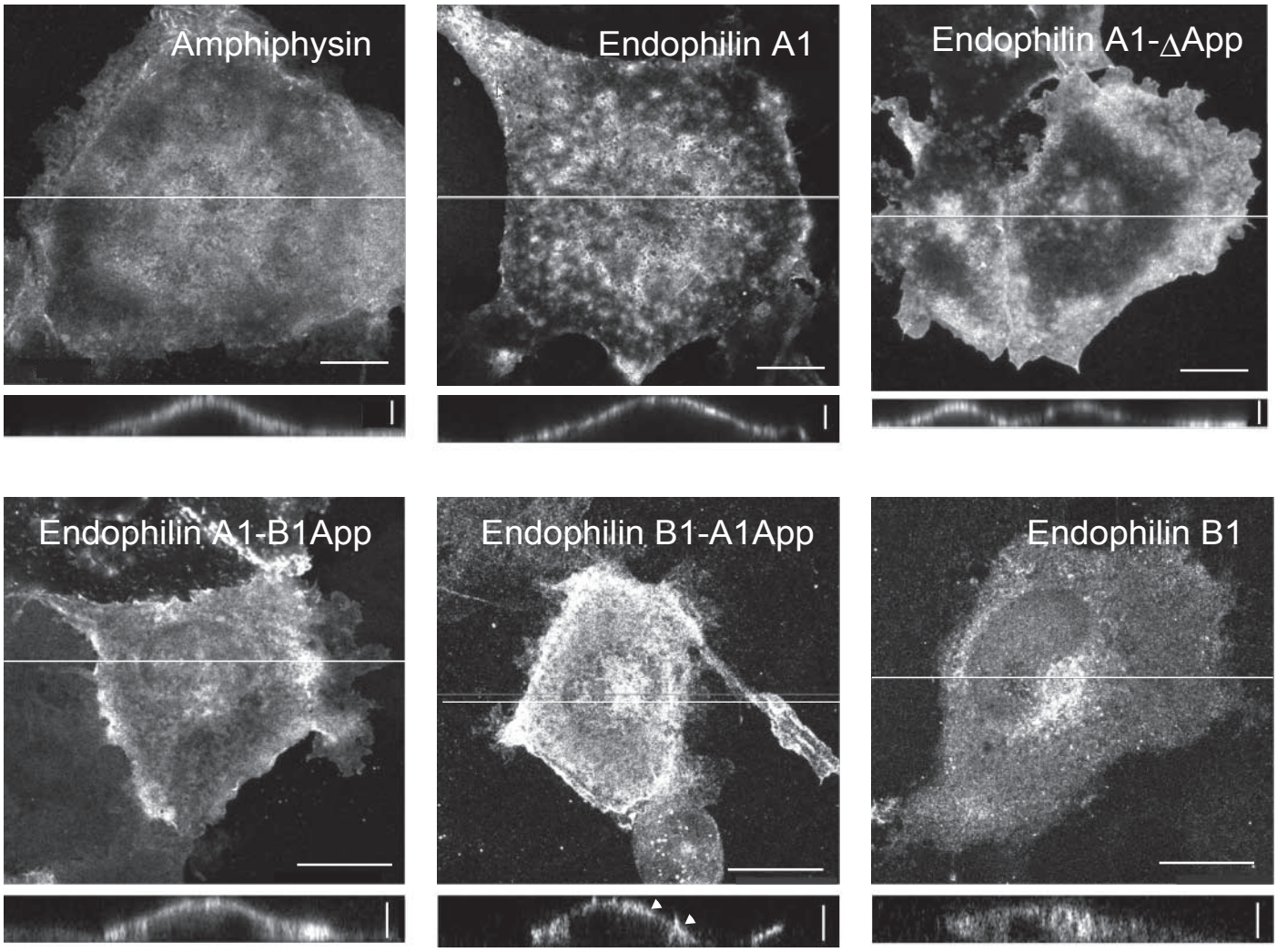
Supplementary Figure 3



Supplementary Figure 3. Localization of wild type and chimeric mutant BAR domains in Cos7 cells. N-terminally 2×FLAG-tagged BAR domains were overexpressed in Cos7 cells and their subcellular localization was visualized by indirect immunofluorescence using anti-FLAG M2 antibody (Sigma) and analysed by a confocal microscope. Chimeric mutants are endophilin A1 with the B1 appendage sequence (Endo-A1-B1App, A63-G83 in A1 was replaced with N72-A87 from B1) and endophilin B1 with the A1 appendage sequence (Endo-B1-A1App, the reciprocal replacement). The wild type amphiphysin-BAR, Endo-A1-BAR and also the appendage-less mutant (Endo-A1-ΔApp) are most exclusively localized along the apical cell surface while the wild type Endo-B1-BAR is in the cytoplasm. The chimeric molecule Endo-A1-B1App is observed both in the cell surface and in the cytoplasm while a fraction of Endo-B1-A1App shows a patchy plasma membrane localization (arrow-heads), which is not plausibly explained by mis-folding of the chimeric polypeptide. These specific BAR locarization patterns could not be observed when N-terminally EGFP-tagged BAR domains were used. Horizontal scale for the extended focus images, 20 μm; vertical scale for the reconstructed vertical sections, 5 μm.