

SUPPLEMENTAL MATERIAL

**Connecdenn 3/DENND1C binds actin linking Rab35 activation
to the actin cytoskeleton**

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Running Head: Connecdenn 3 binds actin

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Supplemental Table 1: Oligoneucleotides used to generate constructs for this study

Oligonucleotide name	Sequence
Rab1A aa1 S-BamHI	GCGGGATCCATGTCCAGCATGAATCCCGAATATG
Rab1A aa205 AS-XhoI	GCGCTCGAGTTAGCAGCAACCTCCACCTGACTGC
Rab13 aa1 S-BamHI	GCGGGATCCATGGCCAAAGCCTACGACCAC
Rab13 aa203 AS-EcoRI	GCGGAATTCTCAGCCCAGGGAGCACTTGTTGG
CD3 aa2 S-BglII	GCGAGATCTGAATCCAGAGCTGAAGGGG
CD3 aa425 S-BamHI	GCGGGATCCTGGGCCGACAATCTAAAGAAAG
CD3 aa785 AS-XhoI	GCGCTCGAGTCACTGGGACTTTTGACAGTTGC
CD3 aa801 AS-XhoI	GCGCTCGAGTCAACCCTCAAAGCACTTCTTAAG
CD3 aa789-801 WT S	GATCCCCGCGCGTGGCGGATCTGAAAAAATGCTTTGAA GGCTAAC
CD3 aa789-801 WT AS	TCGAGTTAGCCTTCAAAGCATTTCAGATCCGCCACG CGCGGG
CD3 aa789-801 DL-794-AA S	GATCCCCGCGCGTGGCGGCGGCGAAAAAATGCTTTGAA GGCTAAC
CD3 aa789-801 DL-794-AA AS	TCGAGTTAGCCTTCAAAGCATTTCAGATCCGCCACG CGCGGG
CD3 aa789-801 KK-796-AA S	GATCCCCGCGCGTGGCGGATCTGGCGGCGTGCTTTGAA GGCTAAC
CD3 aa789-801 KK-796-AA AS	TCGAGTTAGCCTTCAAAGCACGCCGAGATCCGCCAC GCGCGGG
CD3 aa789-801 FE-799-AA S	GATCCCCGCGCGTGGCGGATCTGAAAAAATGCGCGGCG GGCTAAC
CD3 aa789-801 FE-799-AA A	TCGAGTTAGCCCGCCGCGCATTTCAGATCCGCCACG CGCGGG

SUPPLEMENTAL FIGURE LEGENDS

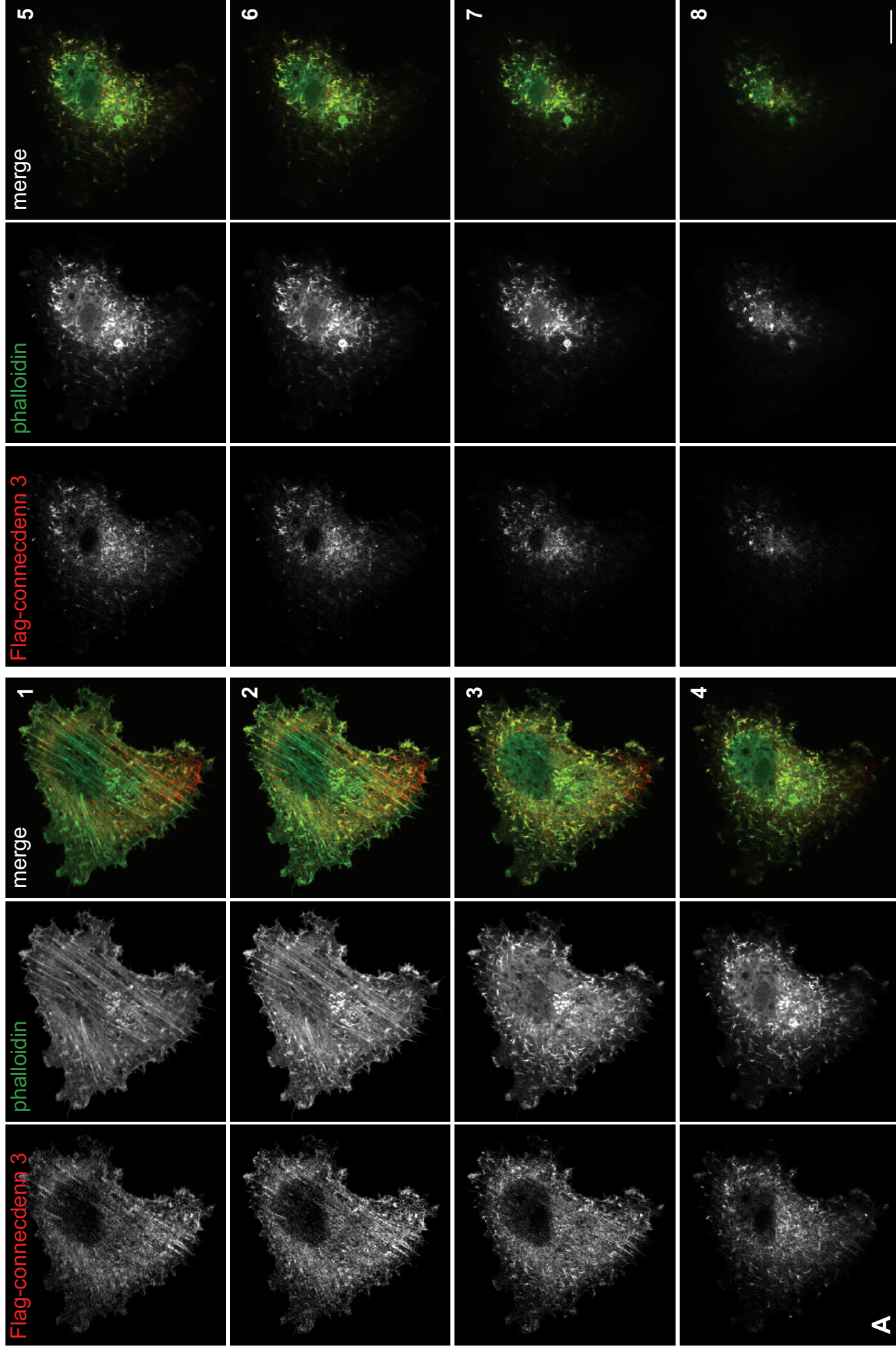
SUPPLEMENTAL FIGURE 1: (A) Cos-7 and (B) NIH 3T3 cells were transfected with Flag-tagged connecdenn 3 and processed for immunofluorescence with a monoclonal antibody against the Flag tag (*red*). Actin was visualized by co-staining with FITC-labeled phalloidin (*green*). Z-stack were taken every 0.36 μm from ventral side (1) to dorsal side (8). Scale bars: 10 μm .

SUPPLEMENTAL FIGURE 2: The DENN domain from connecdenn 3 does not co-localize with actin. HeLa cells were transfected with Flag-tagged connecdenn 3 (CD3) DENN domain and processed for immunofluorescence with a monoclonal antibody against the Flag tag (*red*). Actin was visualized by co-staining with FITC labeled phalloidin (*green*). Scale bar: 10 μm .

SUPPLEMENTAL FIGURE 3: The C-terminal region of connecdenn 3 maintains co-localization with actin. HeLa cells were transfected with Flag-tagged connecdenn 3 lacking the first 424 residues (CD3 Δ 1-424) and processed for immunofluorescence with a monoclonal antibody against the Flag tag (*red*). Actin was visualized by co-staining with Alexa Fluor 647 labeled phalloidin (*blue*). The inset shows the region indicated by the box. Scale bars: lower magnification, 10 μm ; higher magnification, 5 μm .

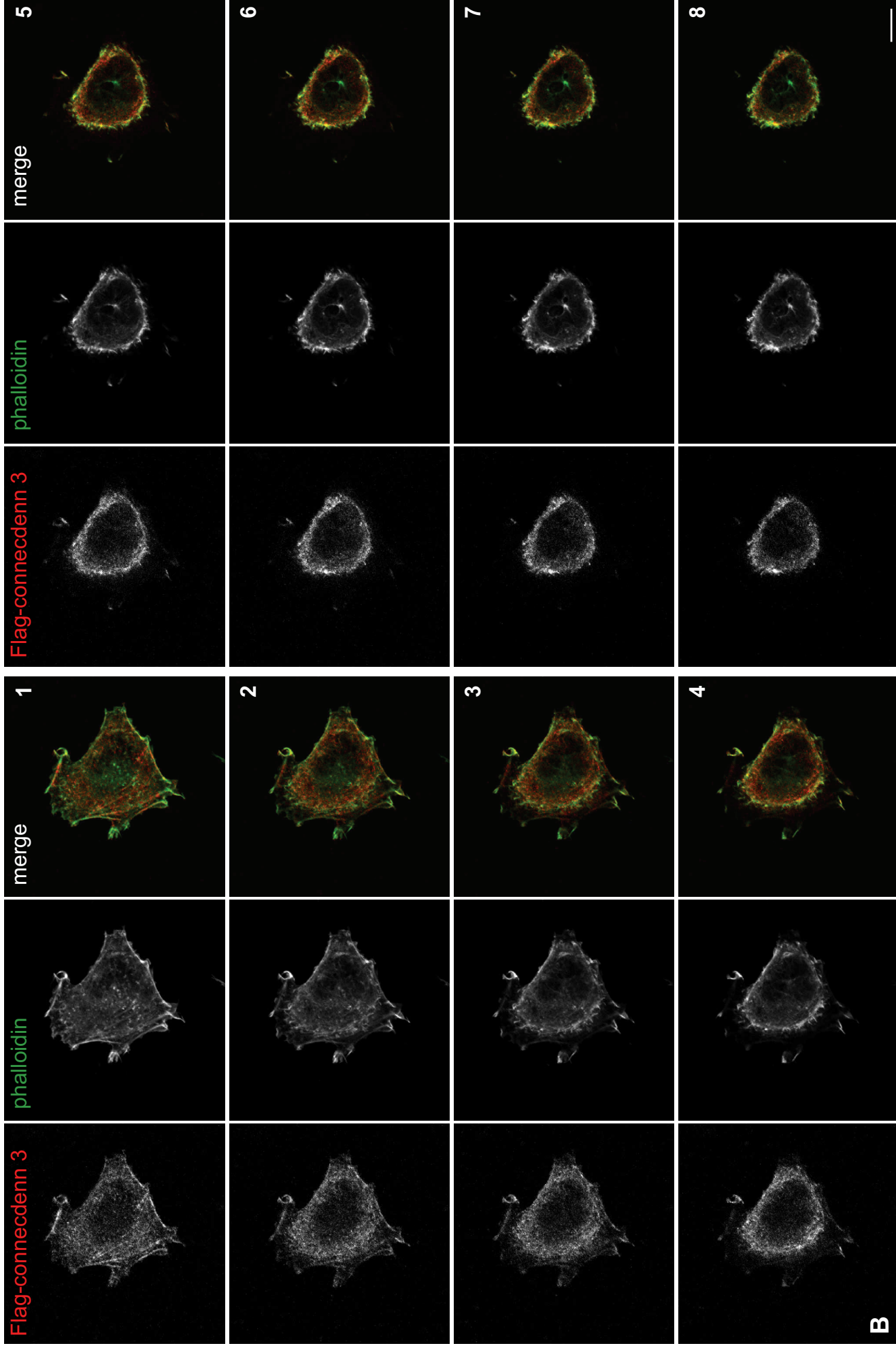
SUPPLEMENTAL FIGURE 4: The DENN domain is required for morphological changes seen upon connecdenn 3/Rab35 co-expression. HeLa cells were co-transfected with Flag-tagged connecdenn 3 lacking the first 424 residues (CD3 Δ 1-424) and Myc-tagged wild-type Rab35 and processed for immunofluorescence with a monoclonal antibody against the Flag tag (*red*) and a polyclonal antibody against the Myc tag (*green*). Actin was visualized by co-staining with FITC labeled phalloidin (*green*). The inset shows the region indicated by the box. Scale bars: lower magnification, 10 μm ; higher magnification, 5 μm .

COS-7

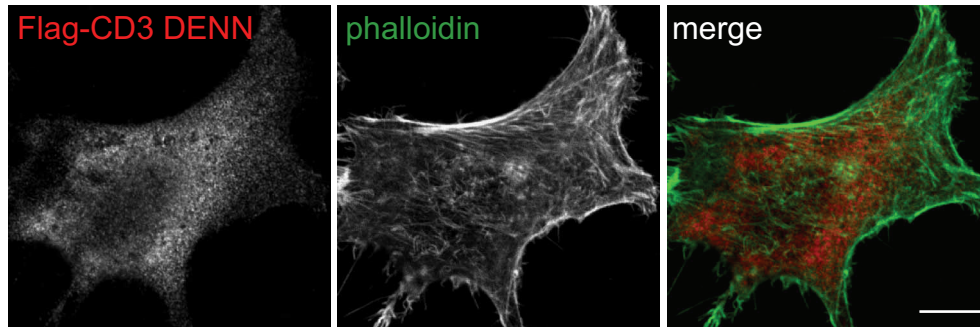


Marat et al., Supplemental Figure 1

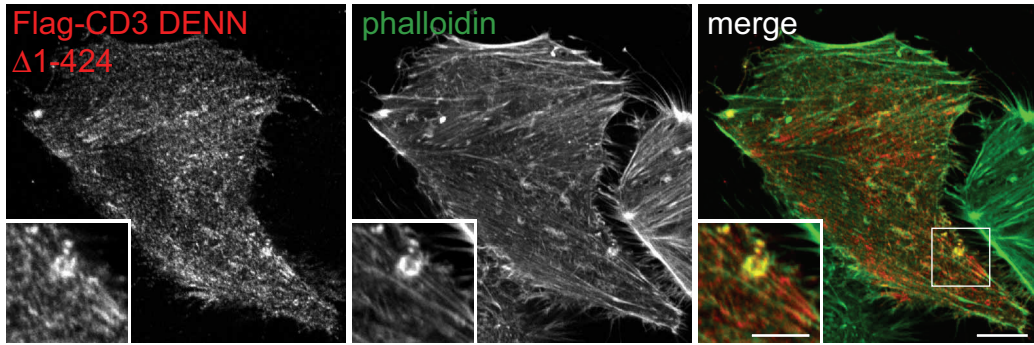
NIH 3T3



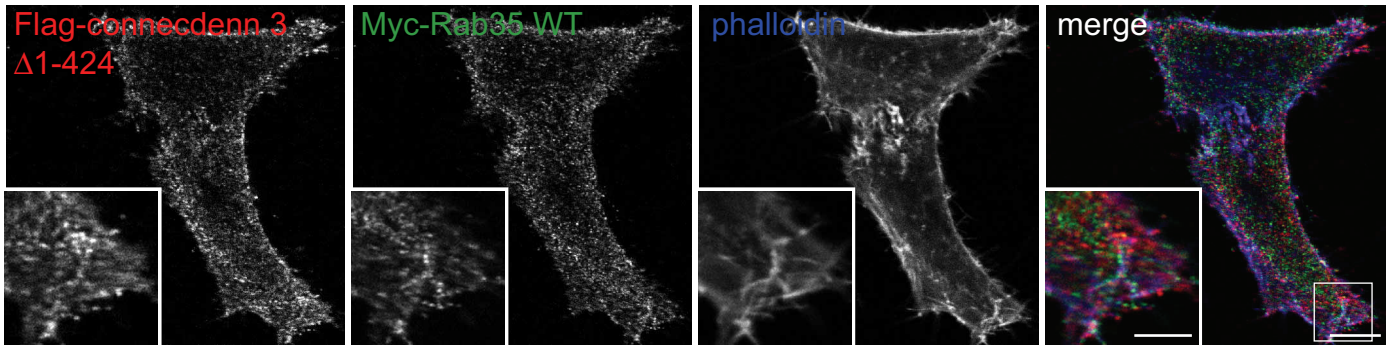
Marat et al., Supplemental Figure 1



Marat et al., Supplemental Figure 2



Marat et al., Supplemental Figure 3



Marat et al., Supplemental Figure 4