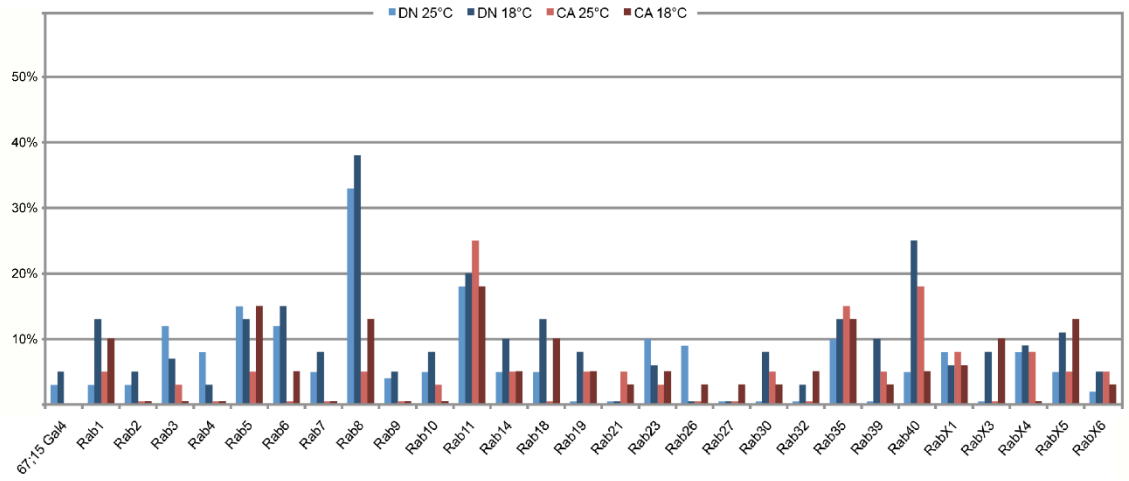


Figure S1. A screen of the effects of dominant-negative and constitutively-active Rab protein expression in the early *Drosophila* embryo.

Embryos from females expressing dominant-negative or constitutively-active Rab transgenes under the control of a maternal Gal4 driver line (matαTub-Gal4VP16 67C;15) were scored for gross tissue level developmental defects by transillumination under Halocarbon oil. Dominant-negative and constitutively-active Rab protein expression had only partially penetrant effects, consistent with partial Rab disruption when endogenous proteins are present. However, dominant-negative Rab8 expression produced the strongest disruption of early morphogenesis among the Rab proteins screened, with defects apparent during cellularization. n≥40 embryos for each condition tested (temperature and transgenic line).

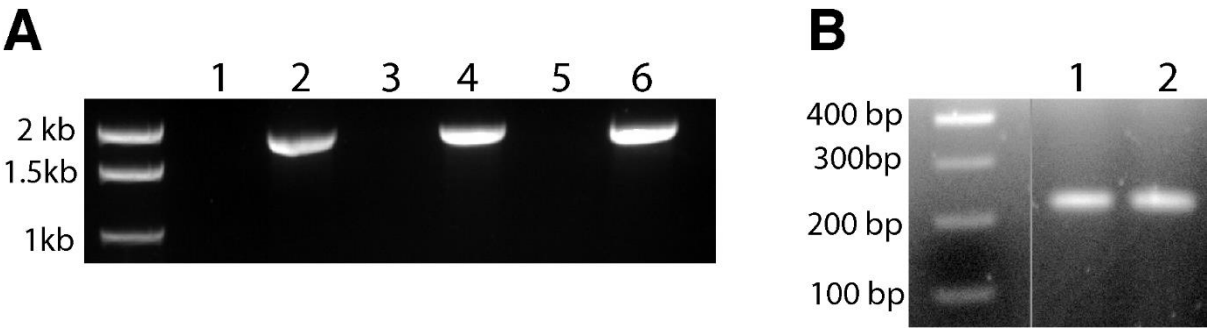


Figure S2. Successful CRISPR-mediated insertion of GFP into the *Drosophila Rab8* genomic locus.
(A) PCR verification of GFP:Rab8 insertion into the Rab8 genomic locus. Three primers pairs (lanes 1 and 2, 3 and 4, or 5 and 6) were designed with the 5' primer binding in the chromosomal DNA upstream of the cloned 1.5kb 5' donor homology arm. 3' primers were designed to bind to the GFP coding sequence. Successful amplification of these regions is only observed in CRISPR GFP:Rab8 embryos (lanes 2, 4 and 6), but not OreR control embryos (lanes 1, 3 and 5), demonstrating that GFP is properly inserted at the genomic Rab8 locus creating an N-terminal GFP fusion. (B) Control PCR against primers located in the Rab35 gene locus demonstrate DNA loading control of OreR control (lane 1) and CRISPR GFP:Rab8 (lane 2) genomic DNA products. Scale bar in A is 10µm; scale bar in E is 5µm.

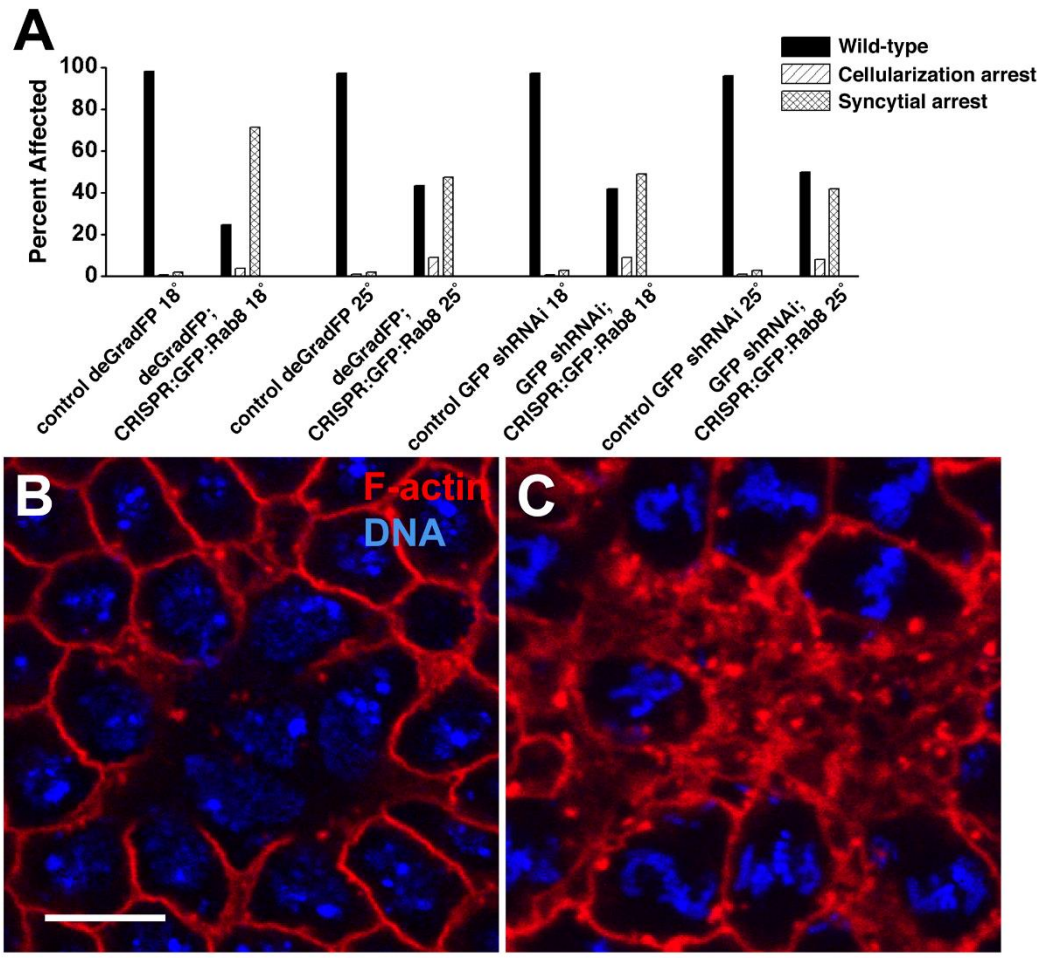


Figure S3. Disruption of Rab8 function by deGradFP and GFP TRiP shRNAi results in defects in early development and furrow formation.
(A) The development of homozygous CRISPR GFP:Rab8 embryos expressing either deGradGFP or GFP TRiP shRNAi was scored under oil for defects in early embryogenesis. Control embryos expressed either deGradGFP or GFP TRiP shRNAi in the absence of GFP:Rab8. $p < 0.001$ for all changes in proportions across groups. $p < 0.001$ for each treatment group when compared to proportions seen in control deGradFP or GFP shRNAi expressing embryos. (B,C) Embryos from either deGradFP; CRISPR GFP:Rab8 (B) or GFP TRiP shRNAi; CRISPR GFP:Rab8 (C) were fixed and stained for F-actin (phalloidin, red) or DNA (DAPI, blue). Local areas showing defects in furrow formation and ingression are shown in (B,C). Scale bar is 5µm.

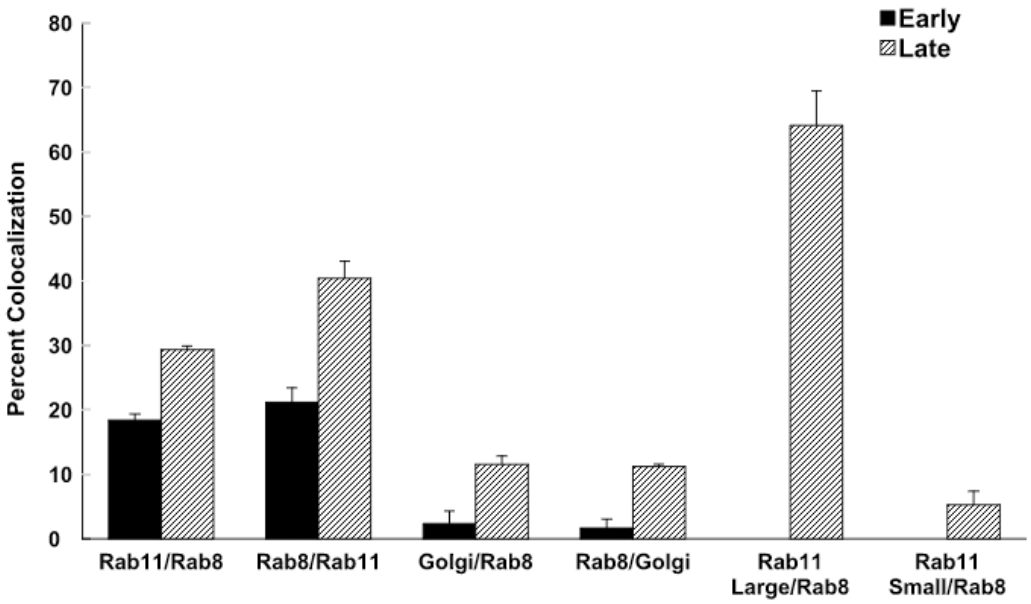
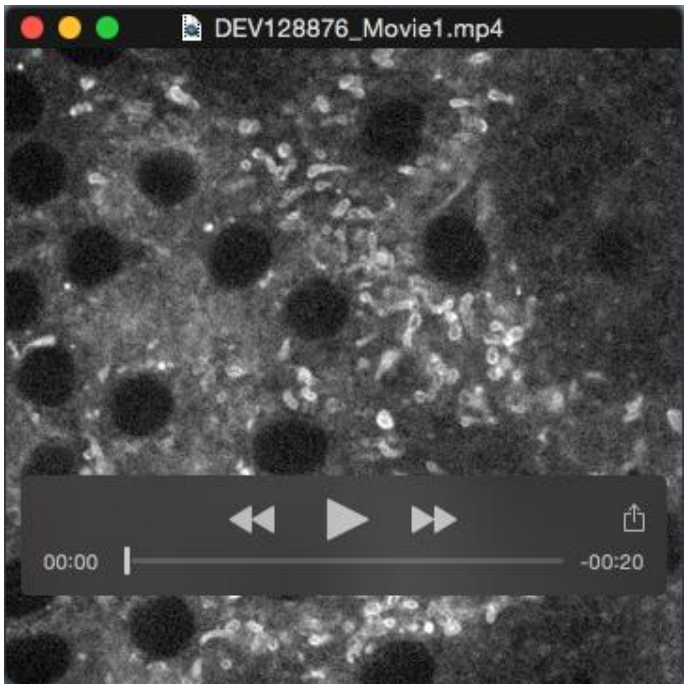
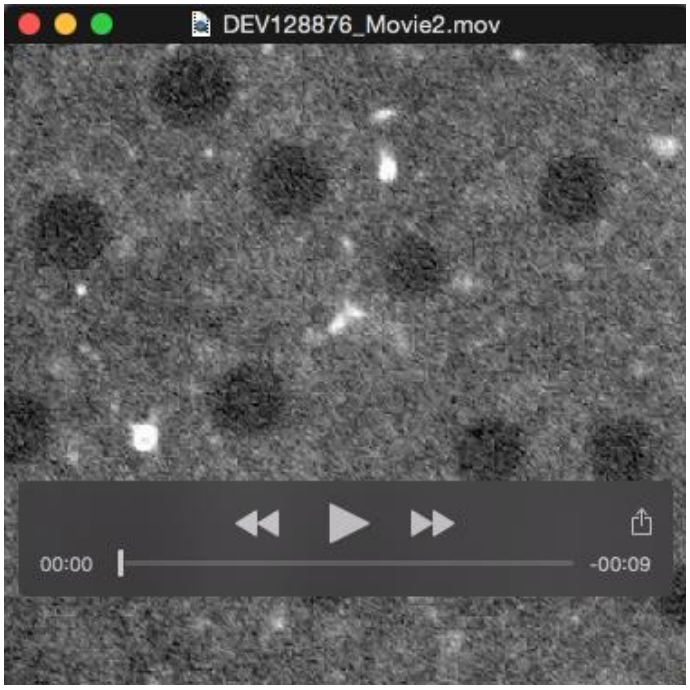


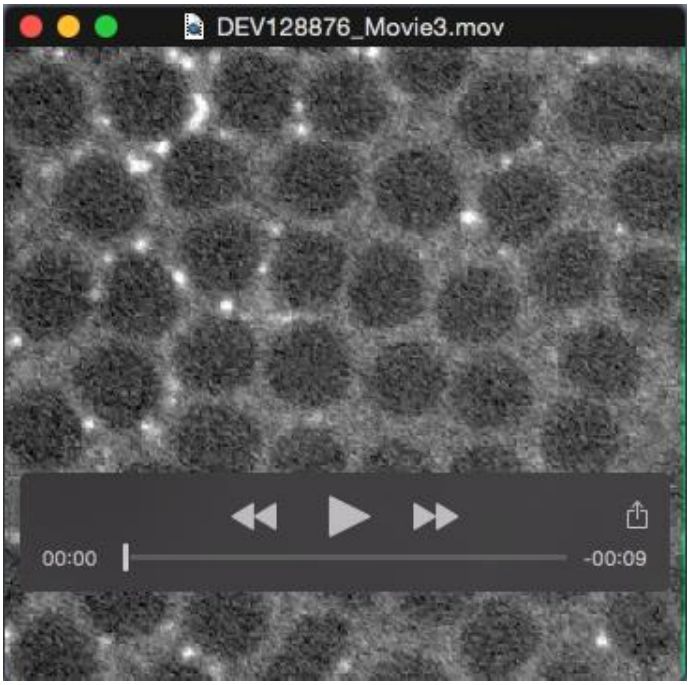
Figure S4. Analysis of Rab8 colocalization by compartment.
Measurement of colocalization frequencies by individual compartments. CRISPR GFP:Rab8 embryos were immunostained for Rab8 (anti-GFP), RE (anti-Rab11) or Golgi (Lva) and colocalization between compartments counted during slow or fast phase of cellularization. Error bars are standard deviation.



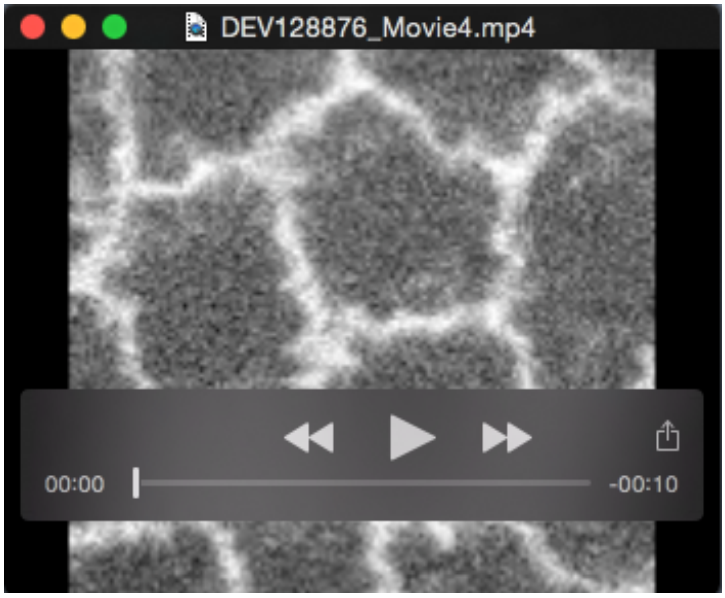
Movie 1. YFP:Rab8 localization prior to cycle 10 and the onset of furrow ingression. Rab8>Gal4 driven expression of UAS-YFP:Rab8 shows Rab8 present in large cytoplasmic compartments. Images acquired every 5 seconds.



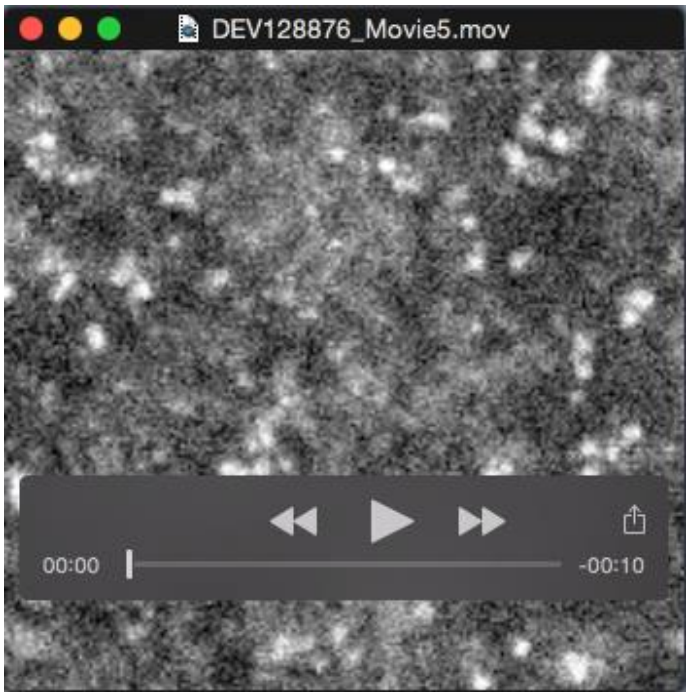
Movie 2. YFP:Rab8 localization during cycles 11 and 12 and the formation of syncytial furrows. Rab8>Gal4 driven expression of UAS-YFP:Rab8 shows Rab8 present in puncta that then become depleted as Rab8 transitions to an association with the plasma membrane. Images acquired every 30 seconds.



Movie 3. YFP:Rab8 localization during cycle 14 and cellularization. Rab8>Gal4 driven expression of UAS-YFP:Rab8 shows Rab8 present in puncta that then become depleted as Rab8 transitions to an association with the plasma membrane. Images acquired every 30 seconds.



Movie 4. Control Rh3 dsRNA injected embryo expressing Gap43:mCh during syncytial furrow formation. Images acquired every 30 seconds.



Movie 5. Rab8 dsRNA injected embryo expressing Gap43:mCh during syncytial furrow formation. Gap43:mCh accumulates in cytoplasmic compartments and fails to localize to the plasma membrane. Images acquired every 30 seconds.

Table S1. gRNAs and primers

Name	Sequence
gRNAs for CRISPR GFP:Rab8	
5′chiRNA-1	CTTCGAACAGATAGTCGTAGGTTT
3′chiRNA-1	AAACAAACCTACGACTATCTGTTC
5′chiRNA-2	CTTCGCCCGATAATAGTGCTTAGC
3′chiRNA-2	AAACGCTAACGACTATTATCGGGC
Cloning primers for HDR donor GFP:Rab8	
5′CRISPRRab8(1)SpeI	GGACTAGTAAAATGCTCCCCTTTCATTAT
3′CRISPRRab8(1)BamHI	CGGGATCCTTTGTGTGCTTTTGCGGTAG
5′CRISPRGFPBamHI	CGGGATCCGTTGTACAGCTCGTCCATGC
3′CRISPRGFPSalI	ACGCGTCGACCGCCCCGCCCTGCCACTCAT
5′CRISPRRab8(2)SalI	ACGCGTCGACATGGCCAAAACCTACGACTA
3′CRISPRRab8(2)KpnI	GGGGTACCTATATAAATAAAATGTTTGT
Primers for PCR verification of CRISPR GFP:Rab8 insertion	
5′PCR-1	TGACATGCAGAATTAAAAGCCC
3′PCR-1	CGGACACGCTGAACTTGTG
5′PCR-2	ATGACATGCAGAATTAAAAGCCCA
3′PCR-2	GTCAGCTTGCCGTAGGTGG
5′PCR-3	TGACATGCAGAATTAAAAGCCCAAT
3′PCR-3	GCTGAACTTGTGGCCGTTTAC