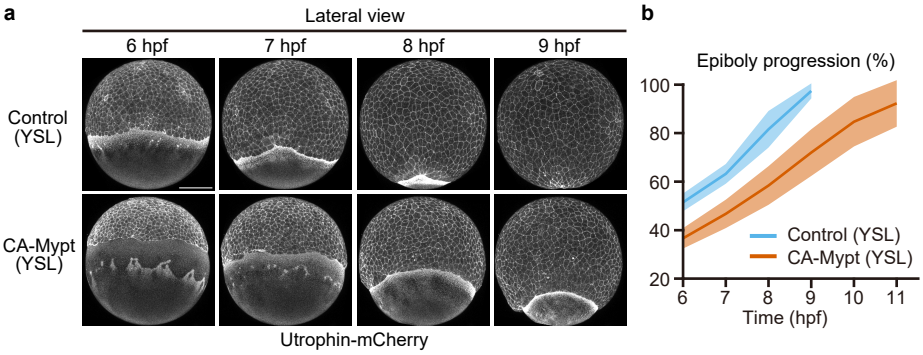
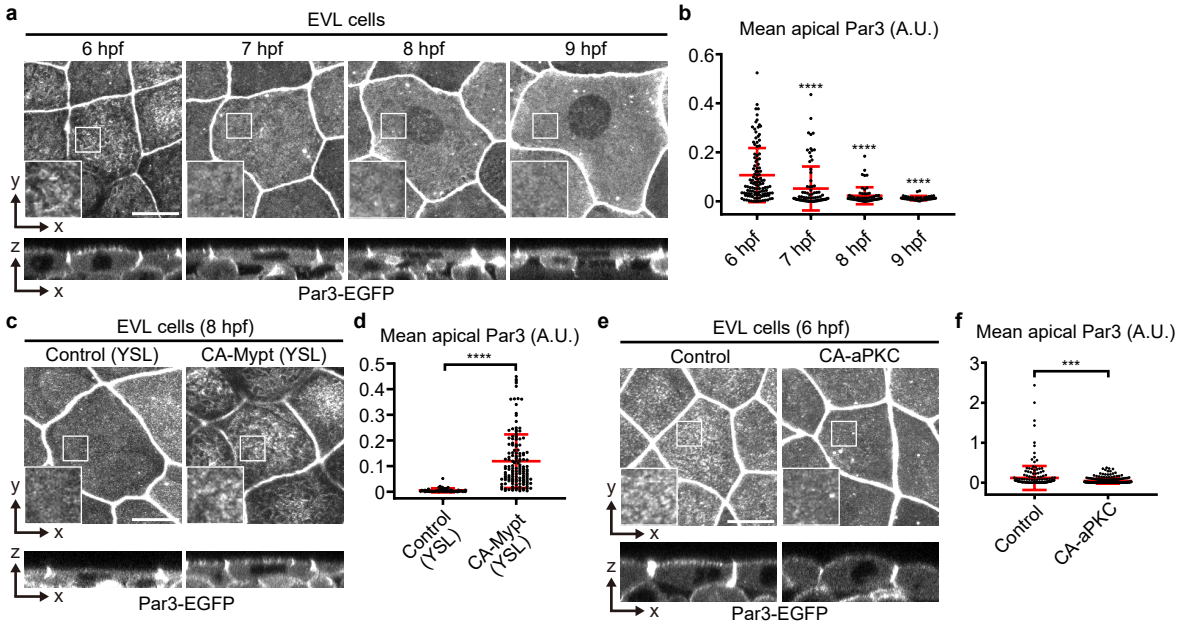
Supplementary Figure 1



Supplementary Figure 1. Contractility of the YSL drives epiboly progression

a, Maximum intensity projection images of Tg*(actb2: Utrophin-mCherry)* embryos at 6, 7, 8, and 9 hpf, labeling F-actin, injected with 25 pg H2B-GFP mRNA (control, top) or 25 pg H2B-GFP plus 150 pg CA-Mypt mRNA (to reduce actomyosin contraction within the YSL, bottom) into YSL at 3.3 hpf. Scale bar, 200 μm. **b**, Line plot of EVL epiboly progression as a function of time during epiboly for the conditions described in **a**. Blue and orange lines indicate control (YSL) and CA-Mypt (YSL) embryos, respectively. Mean ± SD. n = 8 embryos (control YSL), 6 embryos (CA-Mypt YSL) from 3 independent experiments.

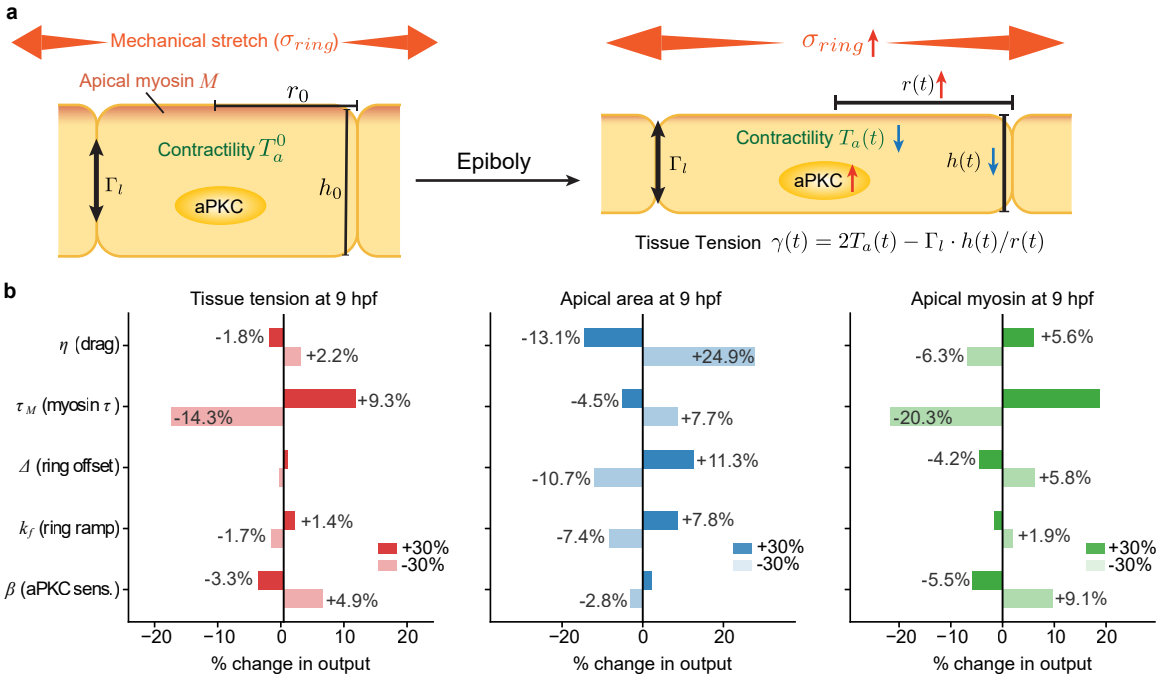
Supplementary Figure 2



Supplementary Figure 2. Pulling force application on the EVL margin dissociates Par3 from the apical domain in EVL cells during epiboly

a, Fluorescence images of EVL cells in an embryo injected with 200 pg pard3ab-EGFP mRNA at the 1-cell stage to visualize Par3 at 6, 7, 8, and 9 hpf. Top (apical surface projection of x-y sections; upper panel) and lateral (x-z section; lower panel) views are shown for each time point. Magnified images of the regions demarcated by white squares are shown in the lower-left corner of each image. Scale bar, 20 μm. **b**, Dot plot of mean apical Par3 intensity at 6, 7, 8, and 9 hpf. n = 116 cells (6 hpf), n = 85 cells (7 hpf), n = 61 cells (8 hpf), n = 38 cells (9 hpf) from 3 independent experiments. One-way ANOVA followed by Dunnett's multiple comparisons test; ****$p < 0.0001$ for 6 hpf vs. 7, 8, or 9 hpf. **c**, Fluorescence images of EVL cells at 8 hpf in an embryo injected with 200 pg pard3ab-EGFP mRNA at the 1-cell stage to visualize Par3, and injected with 25 pg H2A-mCherry mRNA (control YSL, left panel) or 25 pg H2A-mCherry plus 150 pg CA-Mypt mRNA (CA-Mypt YSL; to reduce actomyosin contraction within the YSL, right panel) into YSL at the 3.3 hpf. Apical surface projection of x-y sections is shown for each condition. Scale bar, 20 μm. **d**, Dot plot of mean apical Par3 intensity for the conditions described in **c**. n = 58 cells (control YSL), n = 141 cells (CA-Mypt YSL) from 3 independent experiments. Welch's t-test; ****$p < 0.0001$. **e**, Fluorescence images of EVL cells at 6 hpf in an embryo injected with 200 pg pard3ab-EGFP mRNA (control) or 200 pg pard3ab-EGFP and 50 pg CA-aPKC mRNA (CA-aPKC) at the 1-cell stage. Apical surface projection of x-y sections is shown for each condition. Scale bar, 20 μm. **f**, Dot plot of mean apical Par3 intensity for the conditions described in **e**. n = 196 cells (control), n = 291 cells (CA-aPKC) from 3 independent experiments. Welch's t-test; ***$p = 0.0007$.

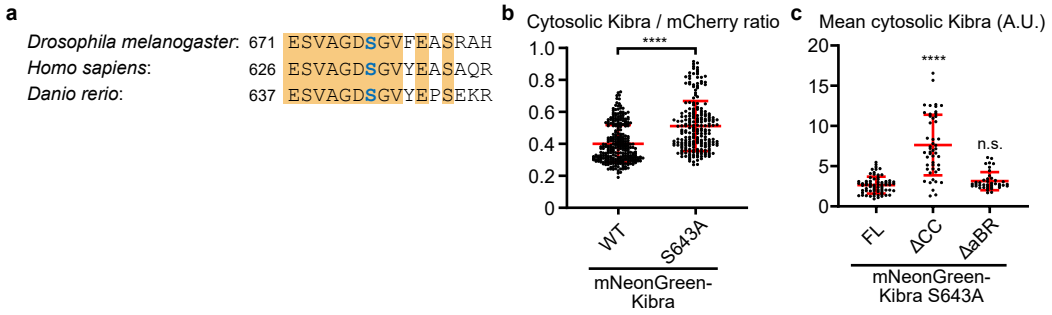
Supplementary Figure 3



Supplementary Figure 3. EVL cell model schematic and sensitivity analysis

a, Schematic of the minimal vertex model used to describe EVL cell mechanics during epiboly. An EVL cell is represented as a cylinder of initial radius r_0 and height h_0, subject to a lateral pulling stress σ_{ring} from the YSL actomyosin ring. Apical contractility T_a is proportional to the level of apical Myosin II. As σ_{ring} increases, the cell spreads ($r(t)$ increases) and thins ($h(t)$ decreases); the resulting mechanical stretch activates aPKC, which in turn reduces apical Myosin II activity and contractility $T_a(t)$, maintaining homeostatic tissue tension. See Methods for full model description and parameters. **b**, Sensitivity analysis of the vertex model. Each parameter was perturbed by ±30% from its calibrated value independently, and the resulting percentage change in tissue tension (left), apical area (center), and apical Myosin II (right) at t = 9 hpf is shown as a tornado chart. Darker bars indicate +30% perturbation; lighter bars indicate -30% perturbation. The model outputs are most sensitive to τ_M (myosin relaxation timescale) and η (drag coefficient), and least sensitive to k_f (ring ramp rate) and β (aPKC mechanosensitivity), confirming that the qualitative predictions are robust across a broad parameter range.

Supplementary Figure 4



Supplementary Figure 4. Mutation at a degron motif enhances Kibra expression

a, Alignment of protein sequences surrounding a degron motif in Kibra in *Drosophila melanogaster* (fruit fly), *Homo sapiens* (human), and *Danio rerio* (zebrafish). Amino acids identical across all three species are indicated in an orange background. A key serine residue in the degron motif, a mutation of which to alanine in *Drosophila melanogaster* leads to enhanced Kibra expression level, is indicated in blue. **b**, Cytosolic mNeonGreem-Kibra WT or S643A mutant intensity normalized by cytosolic mCherry intensity in EVL cells at 8 hpf. The embryos were injected with 25 pg mCherry plus 200 pg mNeonGreen-Kibra WT mRNA (WT) or 25 pg mCherry plus 200 pg mNeonGreen-Kibra S643A mRNA at the 1-cell stage. n = 293 cells (WT), n = 219 cells (S643A) from 3 independent experiments. Welch's t-test; ****$p < 0.0001$.**c**, Dot plot of mean cytosolic Kibra intensity for the conditions described in Figure 3i. n = 71 cells (FL), n = 49 (ΔCC), and n = 41 (ΔaBR) from at least 3 independent experiments. One-way ANOVA followed by Dunnett's multiple comparisons test; ****$p < 0.0001$ for FL vs. ΔCC; $p = 0.4546$ for FL vs. ΔaBR (not significant, n.s.).

Supplementary Figure 5

a

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Drosophila melanogaster: 980 KNRYNCRLNRSDSDSAMHCGVAPHTFQRGAAERRSLRFHSKAPKSVTK-LHHT-HIPRTSLDLELDLQAQHS
Homo sapiens:            935 QSQYVCRLNRSDSDSSTLSKKPP--FVRNSLERRSVRMKR--PSSVKSL--RSERLIRTSLDLELDLQATRT
Danio rerio:             937 QSQYICRINRSDSDSSTLSKKSP--FVRNASERRSVRMKK--PPMQVKG--LDG-LLRTSLDLELDLQVTRT
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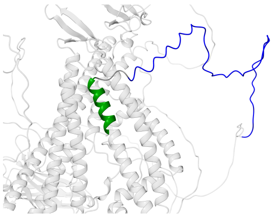
aPKC-binding region (aBR)

α-helix

b



Supplementary Figure 5. Design of the Kibra ΔaBR mutant

a, Alignment of protein sequences surrounding the aPKC-binding region in Kibra in *Drosophila melanogaster* (fruit fly), *Homo sapiens* (human), and *Danio rerio* (zebrafish). Amino acids identical across all three species are highlighted with an orange background. Residues previously shown to be sufficient for aPKC binding by yeast two-hybrid analysis are indicated in red[28,31]. Residues deleted in the ΔaBR mutant in this study are indicated in blue. The region predicted to form an α-helix is underlined in green. **b**, Kibra protein structure predicted by AlphaFold. The predicted α-helix and unstructured regions are indicated in green and blue, respectively.