An extensive allelic series of *Drosophila kae1* mutants
reveals diverse and tissue-specific requirements for t6A biogenesis

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Supplementary Figure Legends

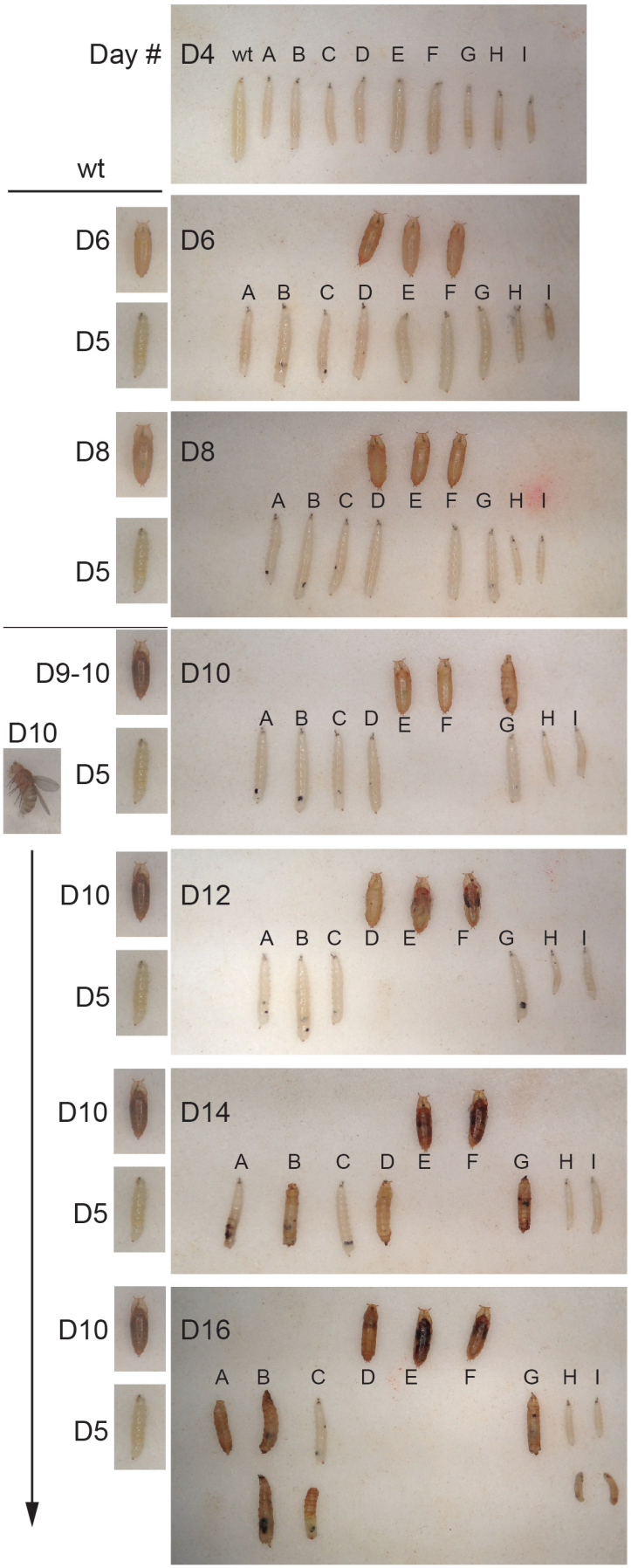
Figure S1. Summary of lethality and black spot (BS) mass phenotypes of different *Drosophila kae1/Df* allelic combinations. A subset of these data are presented in main Figure 1. Since normal flies pupate at day ~5 and eclose at day ~10, the wt images are reiterated to provide a size reference for the kae1 hemizygotes at various stages in their extended larval/pupal phases.

Figure S2. Genetic and molecular mapping of the *Black spot* alleles within *Df(3L)ED220*. We mapped the *1A13* and *3A7* alleles using the P-element mapping strategy described by Bellen and colleagues (Zhai et al., 2003). Briefly, stocks with homozygous viable P element insertion sites with w+ markers that were roughly evenly spaced within *Df(3L)ED220* (inverted black triangles) were obtained from the Bloomington stock center. In the figure, these are denoted as Left (L), middle-left (ML), middle (M), middle-right (MR) and right (R). The location of L is reset to “0” and positions of other elements are expressed relative to this position. Virgin females of a stock of *1A13/TM3-hs-hid* were crossed en masse to males from each of the 5 P-element-containing stocks. Bottles were heat-shocked between days 3 and 6 to kill balancer-containing offspring. F1 virgin females were crossed en masse to *3A7/TM3-hs-hid*, with bottles again heat-shocked to kill off balancer-containing progeny. Progeny from each cross were scored as having either *w*+ or *w* eyes. *w* eyed flies are the result of a recombination event between the P-element insertion site and the mutant allele, thus their proportion reflect the recombination distance between the 2 features. The numbers of *w/w*+ flies are indicated above the P-element positions. Recombination rate (centiMorgan per megabase - cM/MB) and projected mutation positions were calculated as described (Zhai et al., 2003). The projected positions for indicated pairs of P-elements are denoted with red crosses. The position of *CG4933*, which was found to be mutated in all genes in the Black spot complementation group, is indicated (arrow).

Figure S3. Colonies from tetrad dissection of (A) *KAE1/kae1,* and (B) *Bud32/bud32* heterozygotes. Diploids heterozygous for *KAE1* or *BUD32* were transformed with two plasmids expressing *D. melanogaster* genes or vector, as indicated to the left of the colonies. The diploid transformants were sporulated, spores dissected and germinated on rich medium. Representative tetrad colonies from the dissection plate are shown. Cells were transferred to various selective media to determine which plasmids which were present in each haploid, and this is indicated by + and – signs. Cases where presence of the vector could not be determined, due to insufficient colony growth, are left blank. They were also tested for growth on G418 to test if they were wild type or mutant for *KAE1* or *BUD32*. Each tetrad has 2 mutants and 2 wild type, as expected, and the mutants are indicated by arrows.

Figure S4. Hematopoietic overgrowth in *kae1[IA2]/Df(3L)[ED220]* mutants. The left column images are from control ~5 day heterozygous larvae, while the right column images are from ~10 day *kae1* mutant larvae. Samples were stained with Rhodamine phalloidin and Hoechst 33258 to reveal relative cell density and cell shape; all scale bars 50 μm. DV: dorsal vessel. (A, B) Anterior lobes of the lymph gland. The *kae1* mutant shows numerous lamellocytes. (C, D) Posterior lobes of the lymph gland. The *kae1* mutant is overgrown and exhibits massive over-commitment to the lamellocyte fate. (E, F) Circulating hemocytes analyzed from hemolymph smears. Normal blood is composed mostly of plasmatocytes (asterisks). The relative cell density is higher and numerous lamellocytes are present in the *kae1* mutant, which also generates overt tumors that are packed with lamellocytes (G). Lamellocytes (~40-50 μm in diameter) are larger than plasmatocytes (10 μm).

Figure S5. *kae1* mutant larvae maintain a normal digestive tract. Guts were dissected from wildtype (A), *kae1[1A13]/Df* (B) and *kae1[f01978]/Df* (C) larvae. All of the constituent parts of the digestive tract were identified in the mutants, including the proventriculus (PV), anterior midgut (AM), posterior midgut (PM) and hindgut (HG), consistent with the fact that these strong or null *kae1* mutants can survive as larvae for up to 18 days.



Hemizygous (alleleX/Df) phenotype notes
BS = black spot
note: wildtype (wt) examples are reiterated for D5/D10, since pupation normally begins at ~D5, and adult flies normally eclose ~D10.

A = *kae[1A2]*: BS- 50% at D8, 100% at D16
vaibility- 97% at D8, 30% at D16
slim larvae + high ratio of BS

B = *kae1[1A13]*: BS- 95% at D8, 100% at D16
viability- 100% at D8, 40% at D16

C = *kae1[1A19]*: BS- 70% at D8, 100% at D16
viability- 100% at D8, 50% at D16
larvae are unable to dig into agar

D = *kae1[3A7]*: BS- 30%
viability- 80% till pupae formation
pupation- 6% at D8, 80% at D16
most pupae formed at D12,
pharates die at early stage

E = *kae1[7A6]*: BS- 2%
viability- 100% till pupae formation
pupation- 100% at D8

F = *kae1[7A9]*: BS- 2%
viability- 98%
pupation- 93% at D8

G = *kae1[9D1]*: BS- 50% at D8, 75% at D16
viability- 93% at D8, 45% at D16
pupation- 0% at D8, 62% at D16
Start seeing pupae at D12

H = *kae1[f01978]*: BS- 16% at D8, 30% at D16
viability- 88% at D8, 15% at D16
small larvae, unable to dig into food

I = *kae1[l(3)72Fb[331]]*: BS- 26% at D8, 58% at D16
viability- 93% at D8, 50% at D16
small larvae, unable to dig into food

Supplementary Figure 1
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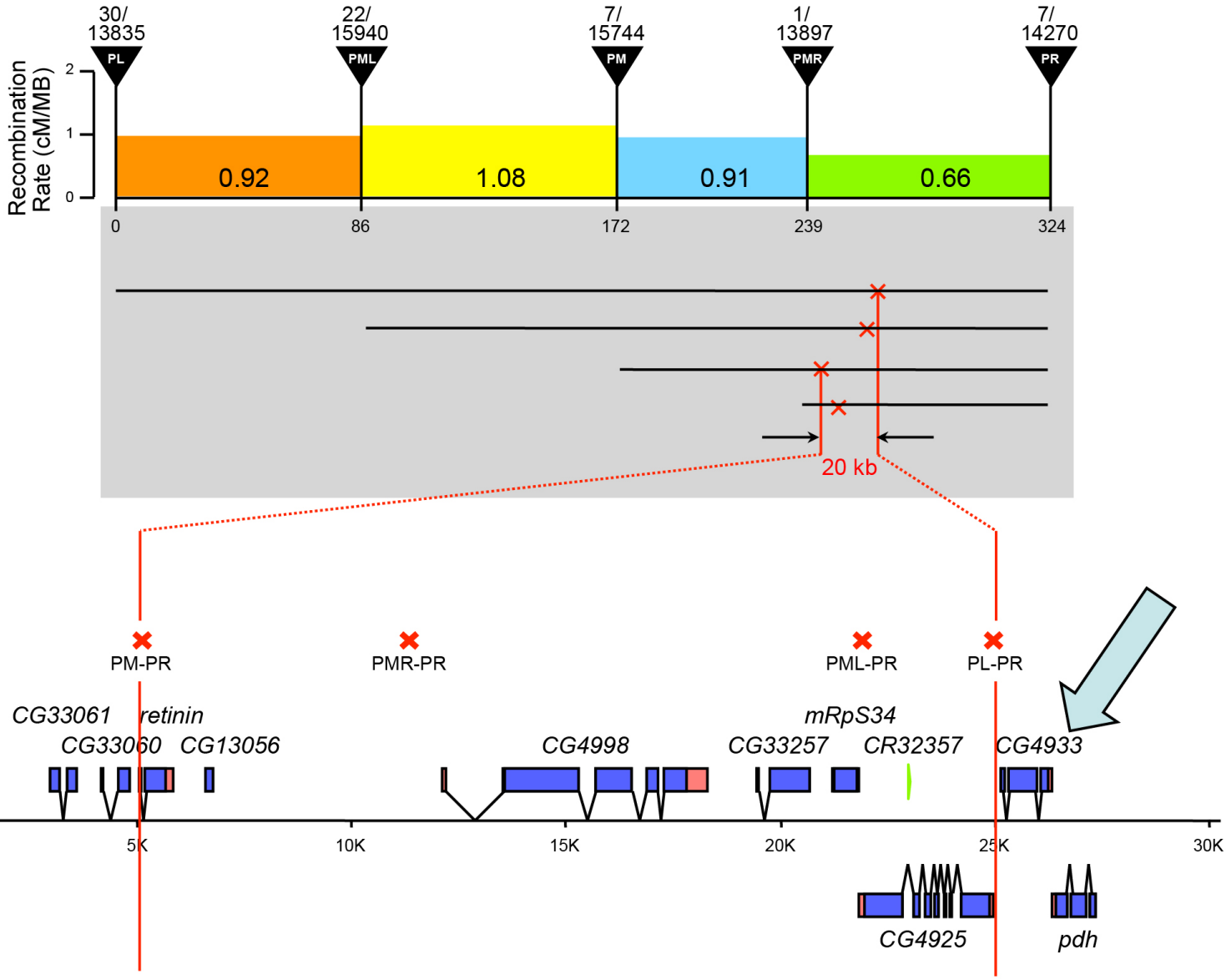

Supplementary Figure 2
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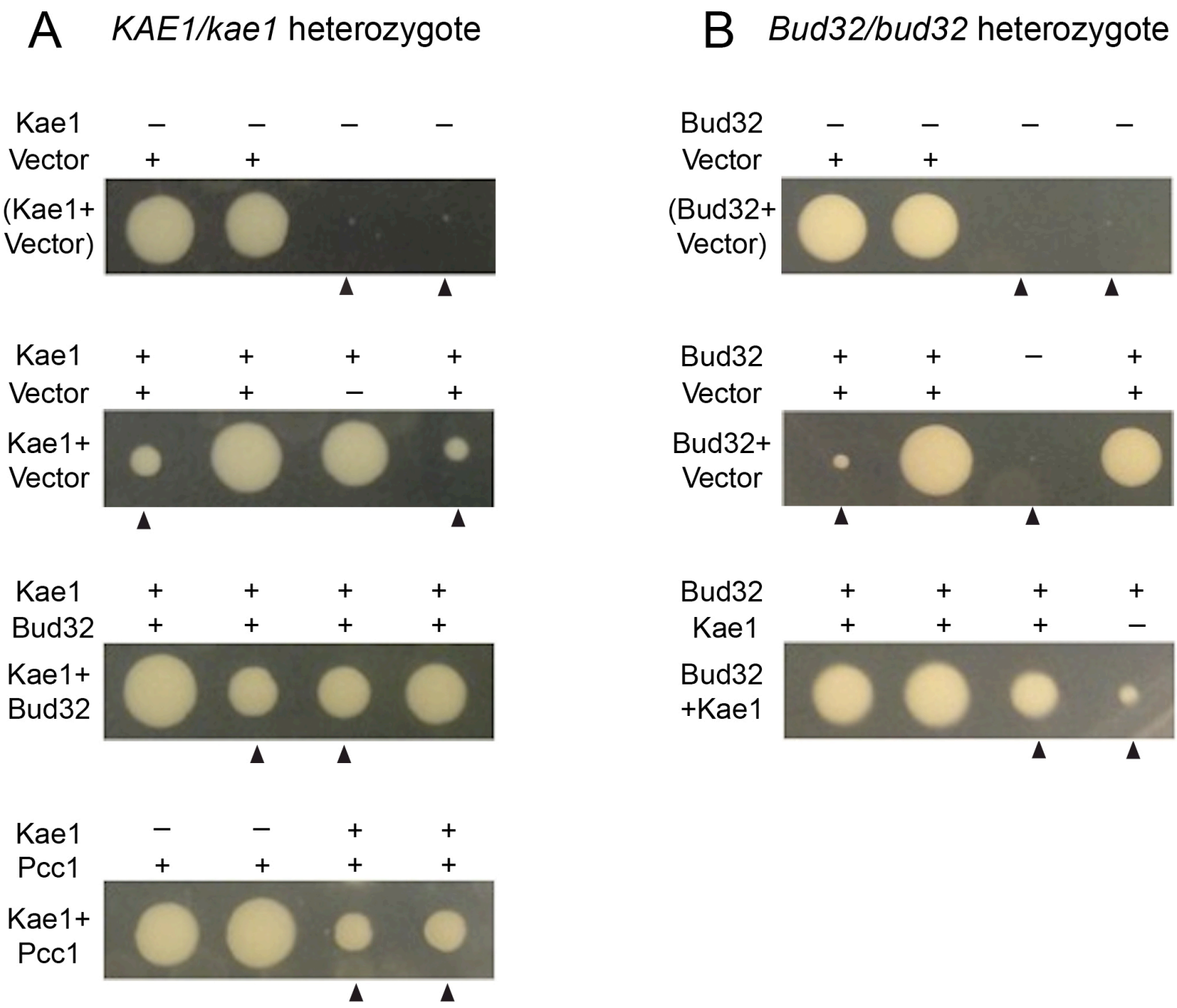

Supplementary Figure 3
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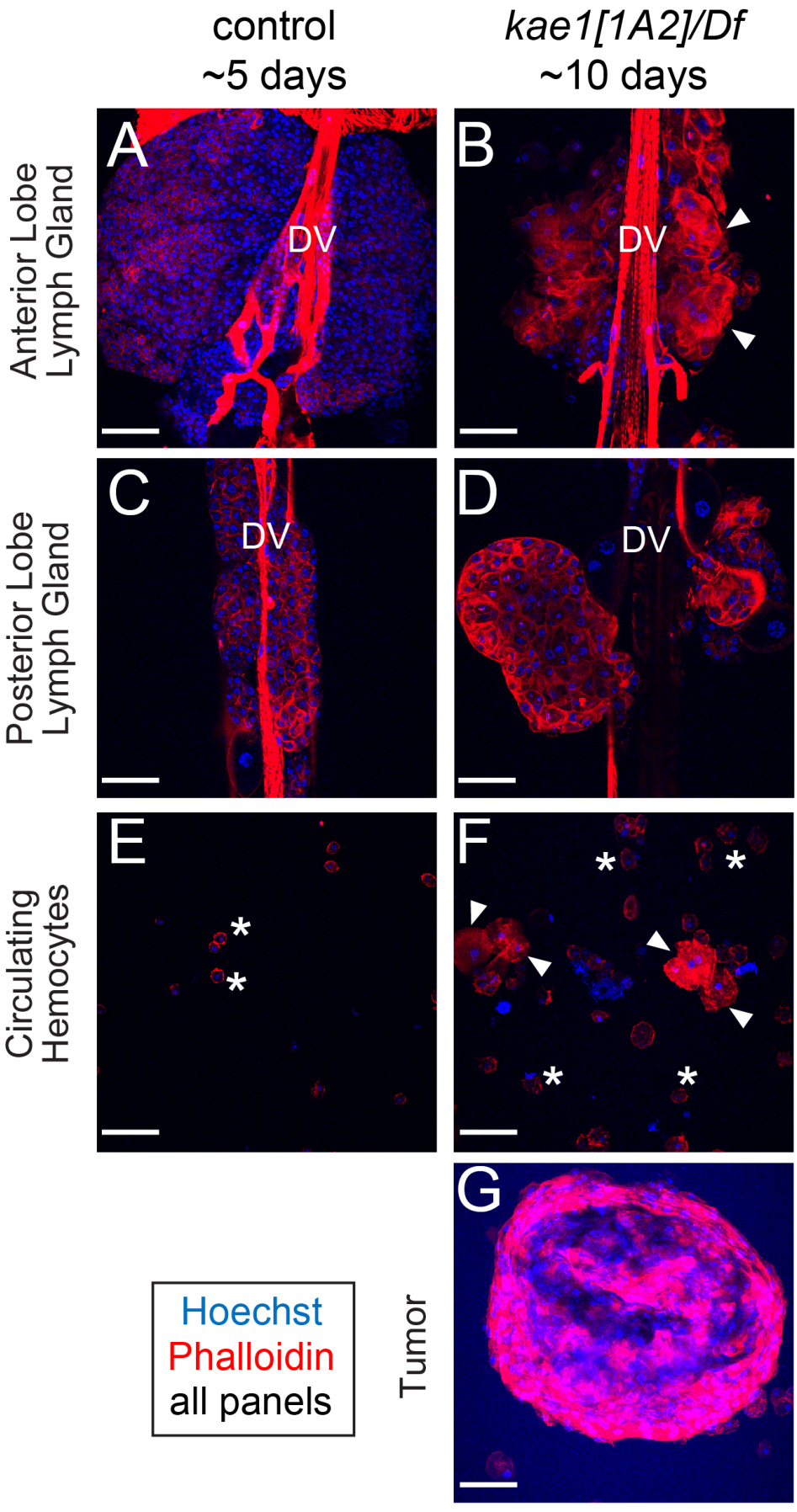

Supplementary Figure 4
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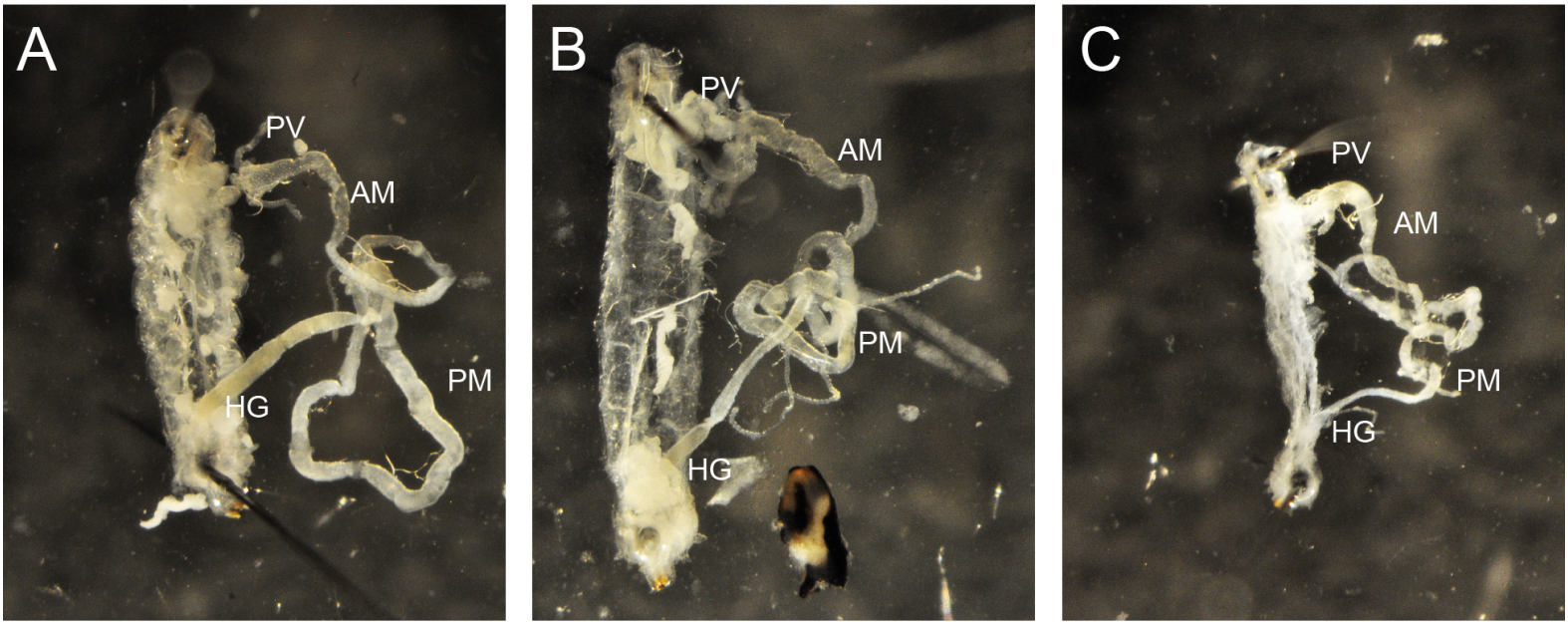
w[1118] *kae1[1A13]/Df* *kae1[f01978]/Df*



Supplementary Figure 5
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