Ma *et al.*, Supplemental Figure 1

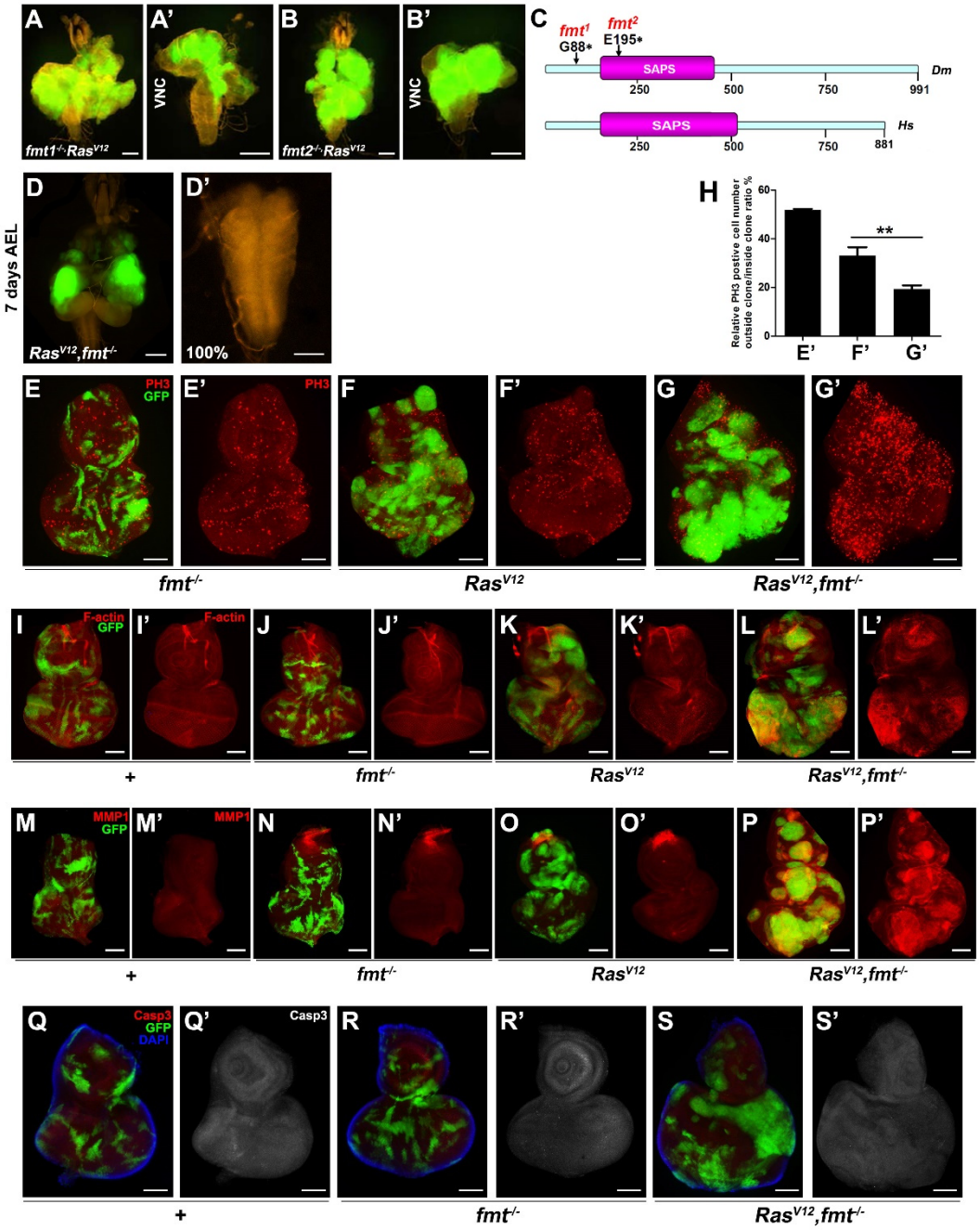

Figure S1. Loss of Fmt synergizes with *Ras^{V12}* to induce tumorigenesis. Related to Figure 1.

(A-B) Two different *fmt* mutant alleles showed synergy effect with *Ras^{V12}*.

(C) Schematic diagram of the *Drosophila* Fmt protein (top; *Dm*) and its human ortholog PPP6R1 (bottom; *Hs*). Two *fmt* alleles both harbor (*fmt^{1}*,*fmt^{2}*) a nonsense mutation that changes a codon into a stop codon. The conserved SAPS domain is indicated.

(D) 7 days AEL, the *Ras^{V12}/fmt$^{-/-}$* clones hyper-proliferate extensively, but no invasion observed.

(E-R) Fluorescence micrographs of eye discs are shown. Loss of Fmt collaborates with *Ras^{V12}* to induce cell autonomous proliferation (E-G), F-actin accumulation (I-L), MMP1 activation (M-P),

but not apoptosis (Q-S). (H) Quantification of relative PH3 positive cells in E-G. **P<0.01 (mean + SEM., n=3). Scale bars, 200 μm in (A, A', B, B', D), 100 μm in (D', E-S').

Genotypes:

(A) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/*UAS-Ras*^{V12}; *tub*-Gal80, FRT79E/*fmt*^{1}, FRT79E
(B) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/*UAS-Ras*^{V12}; *tub*-Gal80, FRT79E/*fmt*^{3}, FRT79E
(E, J, N, R) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/+; *tub*-Gal80, FRT79E/*fmt*^{1}, FRT79E
(F, K, O) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/*UAS-Ras*^{V12}; *tub*-Gal80, FRT79E/FRT79E
(D, G, L, P, S) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/*UAS-Ras*^{V12}; *tub*-Gal80, FRT79E/*fmt*^{1}, FRT79E
(I, M, Q) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/+; *tub*-Gal80, FRT79E/FRT79E

Ma *et al.*, Supplemental Figure 2

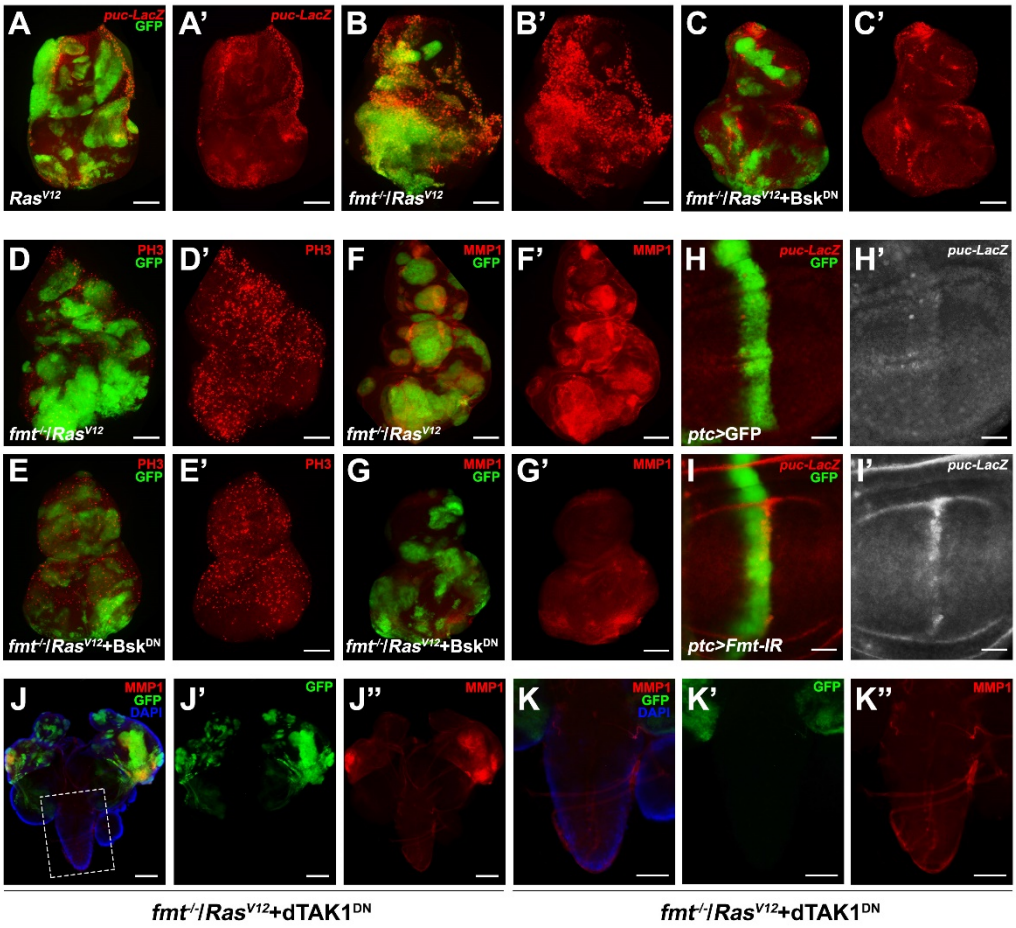

Figure S2. dTAK1-JNK signal is essential for *Ras*^{V12}/*fmt*$^{-/-}$ induced tumorigenesis. Related to Figure 2.

(A-C) *Ras*^{V12} expression induced mild JNK activation (A) was synergistically enhanced by loss of *fmt* (B), which can be strongly suppressed by inhibiting JNK signaling (C), it is noteworthy that weak non-autonomous JNK activation appeared in surrounding clones.

(D-G) *Ras*^{V12}/*fmt*$^{-/-}$ induced proliferation (D) and MMP1 induction (F) was completely impeded by expression of Bsk^{DN} (E, G).

(H-I) Compared with controls (H'), inhibition of Fmt under *ptc* promoter mildly activate JNK signal (I'), crosses were done at 29 ºC.

(J-K) Inhibition of dTAK1 activity significantly suppressed *Ras*^{V12}/*fmt*$^{-/-}$ induced tumor overgrowth and invasion. Scale bars, 100 μm in (A-G, K-K"), 200 μm in (H-I', J-J").

Genotypes:

(A) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/*UAS-Ras*^{V12}; *tub*-Gal80, FRT79E/FRT79E
(B, D, F) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/*UAS-Ras*^{V12}; *tub*-Gal80, FRT79E/*fmt*^{1}, FRT79E
(C, E, G) *ey-Flp1*/*UAS*-Bsk^{DN}; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/*UAS-Ras*^{V12}; *tub*-Gal80, FRT79E/*fmt*^{1}, FRT79E
(H) *ptc*-Gal4, *UAS*-GFP/+
(I) *ptc*-Gal4, *UAS*-GFP/*UAS-fmt-IR*
(J, K) *ey-Flp1*/+; *Act*>*y*$^{+}$>Gal4, *UAS*-GFP/*UAS-Ras*^{V12}, *UAS*-dTAK1^{DN}; *tub*-Gal80, FRT79E/*fmt*^{1}, FRT79E

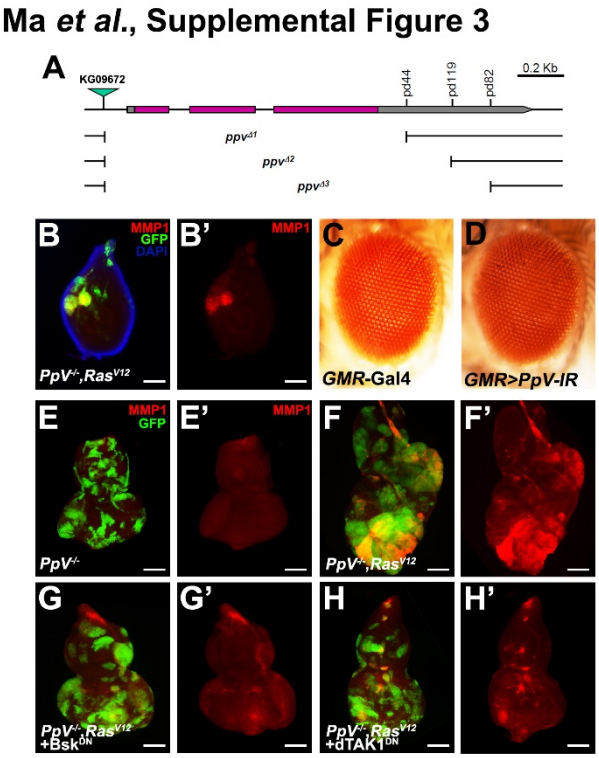

Figure S3. dTAK1-JNK signal is essential for *Ras*^{V12}/*fmt*$^{-/-}$ induced tumorigenesis. Related to Figure 3.

(B) *PpV*$^{-/-}$/*Ras*^{V12} tumors cause metastasize to leg disc.

(C-D) Loss of *PpV* itself under *GMR* promoter produced no obvious eye phenotype.

(E-H) $PpV^{-/-}/Ras^{V12}$ induced tumor growth and MMp1 induction (F) was strongly suppressed by blocking JNK (G) or dTAK1 activity (H). Scale bars, 50 μm in (B-B'), 100 μm in (E-H').

Genotypes:

(B, F) $PpV^{\Delta 1}$, FRT19A/tub-Gal80, FRT19A; *ey-Flp5*, *Act*>y^{+}>Gal4, *UAS*-GFP/*UAS-*Ras^{V12}
(C) *GMR*-Gal4
(D) *GMR*-Gal4/+; *UAS-PpV-IR*/+
(E) $PpV^{\Delta 1}$, FRT19A/*tub*-Gal80, FRT19A; *ey-Flp5*, *Act*>y^{+}>Gal4, *UAS*-GFP/+
(G) $PpV^{\Delta 1}$, FRT19A/*tub*-Gal80, FRT19A; *ey-Flp5*, *Act*>y^{+}>Gal4, *UAS*-GFP/*UAS-*Ras^{V12}; *UAS-*Bsk^{DN}/+
(H) $PpV^{\Delta 1}$, FRT19A/*tub*-Gal80, FRT19A; *ey-Flp5*, *Act*>y^{+}>Gal4, *UAS*-GFP/*UAS-*Ras^{V12}, *UAS-*$dTAK1^{DN}$

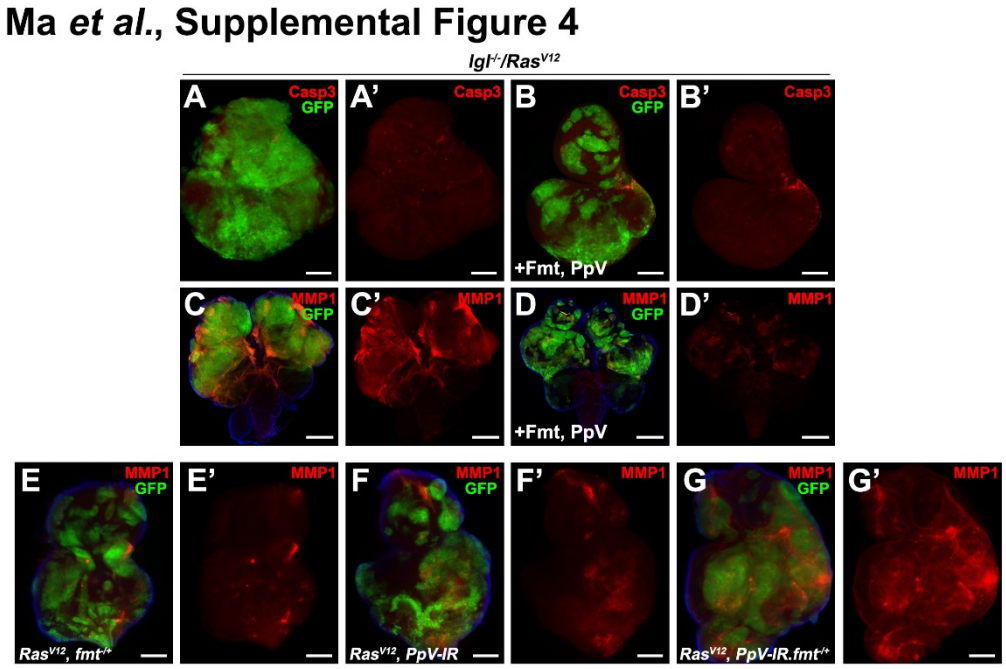

Figure S4. Fmt and PpV expression suppress *lgl-Ras* induced tumorigenesis. Related to Figure 4.

(A-B) Caspase 3 activation in $lgl^{-/-}/Ras^{V12}$ tumors (A') was not significantly affected by Fmt/PpV expression (B'), note that weak non-autonomous apoptosis was observed around some small clones (indicated by white arrow).

(C-D) Fmt/PpV expression significantly suppressed *lgl-Ras*-induced MMP1 activation.

(E-G) Removing one copy of *fmt* synergistically enhanced Ras^{V12},*PpV-IR* induced tumor overgrowth and MMP1 activation. Scale bars, 100 μm in (A-B', E-G'), 200 μm in (C-D').

Genotypes:

(A, C) *ey-Flp1*/+; *tub*-Gal80, FRT40A/lgl^4, FRT40A, *UAS-*Ras^{V12}; *Act*>y^+>Gal4, *UAS*-GFP/+

(B, D) *ey-Flp1*/+; *tub*-Gal80, FRT40A/lgl^4, FRT40A, *UAS-*Ras^{V12}; *Act*>y^+>Gal4, *UAS*-GFP/*UAS*-Fmt, *UAS*-PpV

(E) *ey-Flp1*/+; *tub*-Gal80, FRT40A/FRT40A, *UAS-*Ras^{V12}; fmt^1, FRT79E/*Act*>y^+>Gal4, *UAS*-GFP

(F) *ey-Flp1*/+; *tub*-Gal80, FRT40A/FRT40A, *UAS-*Ras^{V12}; *UAS-PpV-IR*/*Act*>y^+>Gal4, *UAS*-GFP

(G) *ey-Flp1*/+; *tub*-Gal80, FRT40A/FRT40A, *UAS-*Ras^{V12}; *UAS-PpV-IR,* fmt^1, FRT79E /*Act*>y^+>Gal4, *UAS*-GFP