

Expanded View Figures

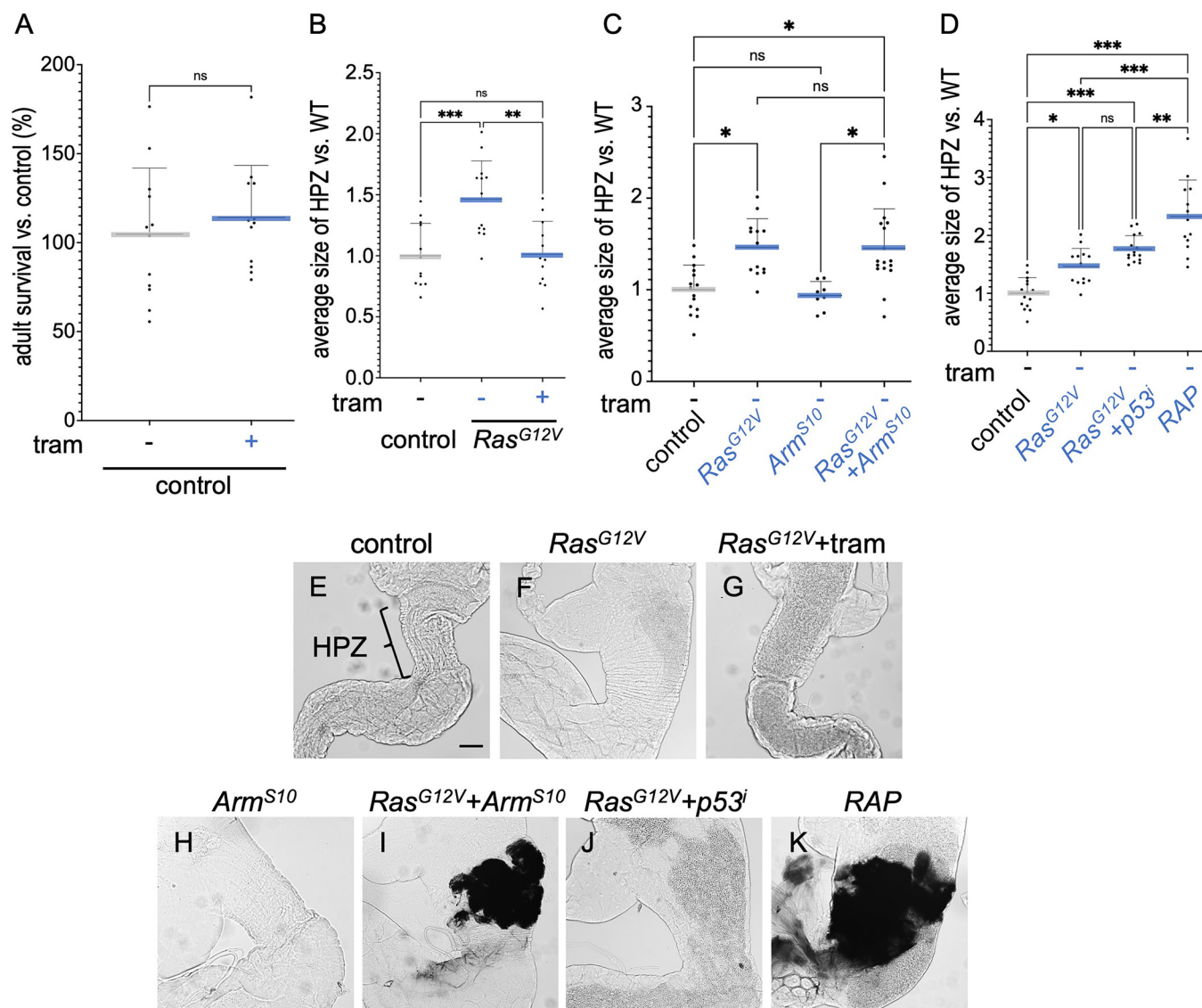
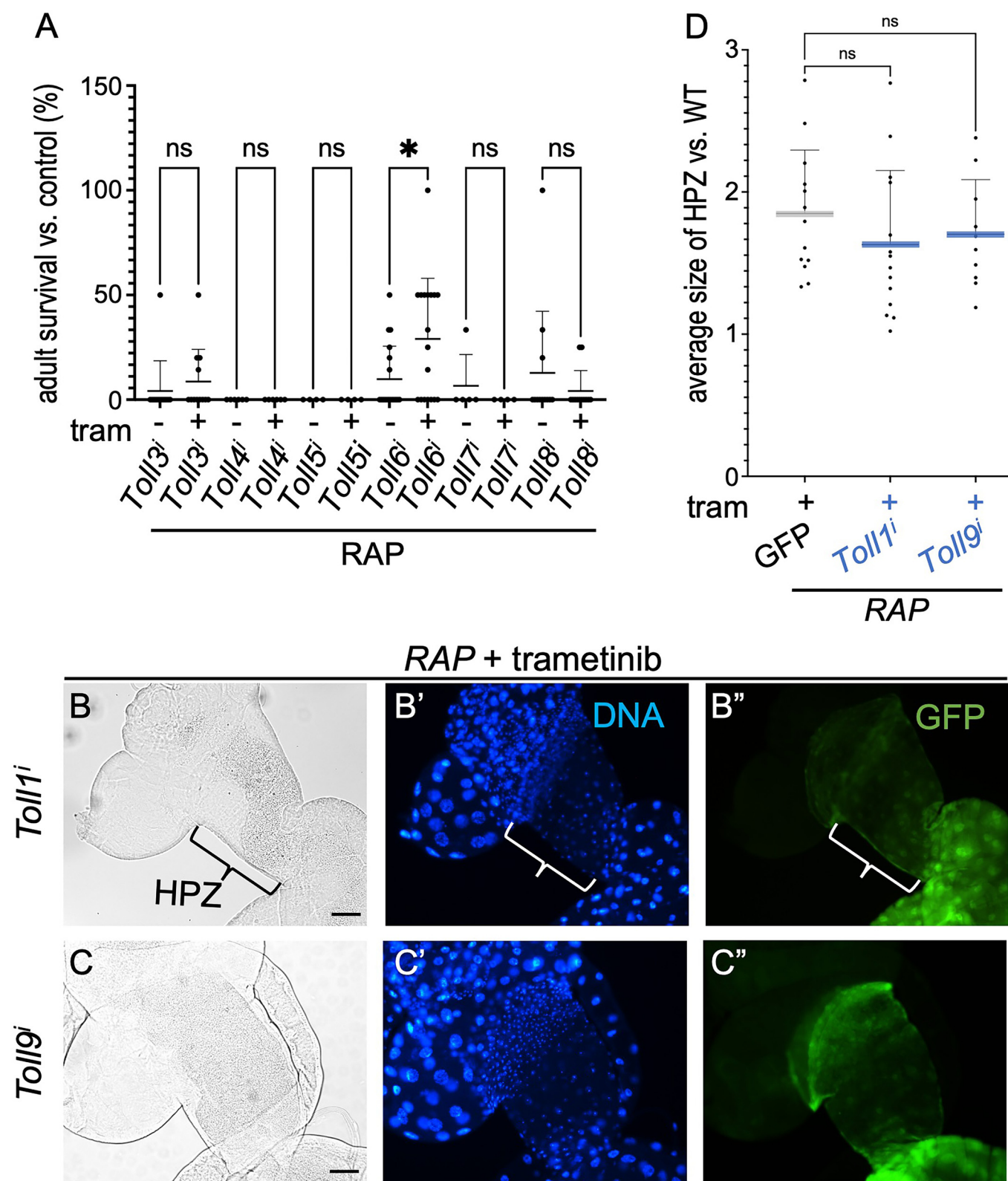


Figure EV1. Overgrowth of HPZ was driven by combined Ras, Wg and p53 alterations.

(A) Percent survival of control flies to adulthood relative to control flies was quantified in the presence or absence of trametinib (1 μ M). Control (DMSO, $n = 12$; tram, $n = 12$). (B-D) The average of the hindgut proliferation zone (HPZ) size was measured by Fiji ImageJ and quantified as relative size to the control hindgut. Control (DMSO, $n = 12$), *Ras*^{G12V} (DMSO, $n = 13$; tram, $n = 12$) (B); Control (DMSO, $n = 15$), *Ras*^{G12V} (DMSO, $n = 13$), *Arm*^{S10} (DMSO, $n = 8$), *Ras*^{G12V};*Arm*^{S10} (DMSO, $n = 17$) (C); Control (DMSO, $n = 15$), *Ras*^{G12V} (DMSO, $n = 13$), RAP (DMSO, $n = 13$), *Ras*^{G12V};*p53*ⁱ (DMSO, $n = 15$) (D). (E-K) Images of the digestive tract of third instar larvae in the presence or absence of trametinib (1 μ M). Control (E), *Ras*^{G12V} (F, G), *Arm*^{S10} (H), *Ras*^{G12V};*Arm*^{S10} (I), *Ras*^{G12V};*p53*ⁱ (J) and RAP (K). Scale bar 200 μ m. (A-K) The experiment was conducted at 27 °C. The statistical tests used to calculate the *P* value are as follows: (A) Mann-Whitney test; (B-D) one-way ANOVA; NS $P(>0.12)$, * $P(0.033)$, ** $P(0.002)$ and *** $P(<0.001)$. All statistical data are summarised in Table EV1. The error bar is a standard deviation (SD), with each point representing biological replicates and numbers (*n*), including three technical replicates.



◀ **Figure EV2. Administration of NF- κ B inhibitors did not suppress drug resistance in RAP tumours.**

(A) Percent survival of transgenic flies to adulthood relative to control flies was quantified in the presence or absence of trametinib (1 μ M), *RAP;Toll3^l* (DMSO, *n* = 12; tram, *n* = 12), *RAP;Toll4^l* (DMSO, *n* = 6; tram, *n* = 6), *RAP;Toll5^l* (DMSO, *n* = 4; tram, *n* = 4), *RAP;Toll6^l* (DMSO, *n* = 18; tram, *n* = 18), *RAP;Toll7^l* (DMSO, *n* = 5; tram, *n* = 4), *RAP;Toll8^l* (DMSO, *n* = 12; tram, *n* = 12). (B, C). Images of the digestive tract of third instar larvae in the presence of trametinib (1 μ M), Scale bar 200 μ m. (D) Average hindgut proliferation zone (HPZ) size was measured by Fiji ImageJ and quantified as relative size to the control hindgut. *RAP;GFP* (tram, *n* = 13), *RAP;Toll7^l* (tram, *n* = 14), *RAP;Toll9^l* (tram, *n* = 10). (A–D) The experiment was conducted at 29 °C. The statistical tests used to calculate the *P* value are as follows: (A, B) one-way ANOVA; NS *P* > 0.12, **P* (0.033), ***P* (0.002) and ****P* < 0.001. All statistical data are summarised in Table EV1. The error bar is a standard deviation (SD), with each point representing biological replicates and numbers (*n*), including three technical replicates.

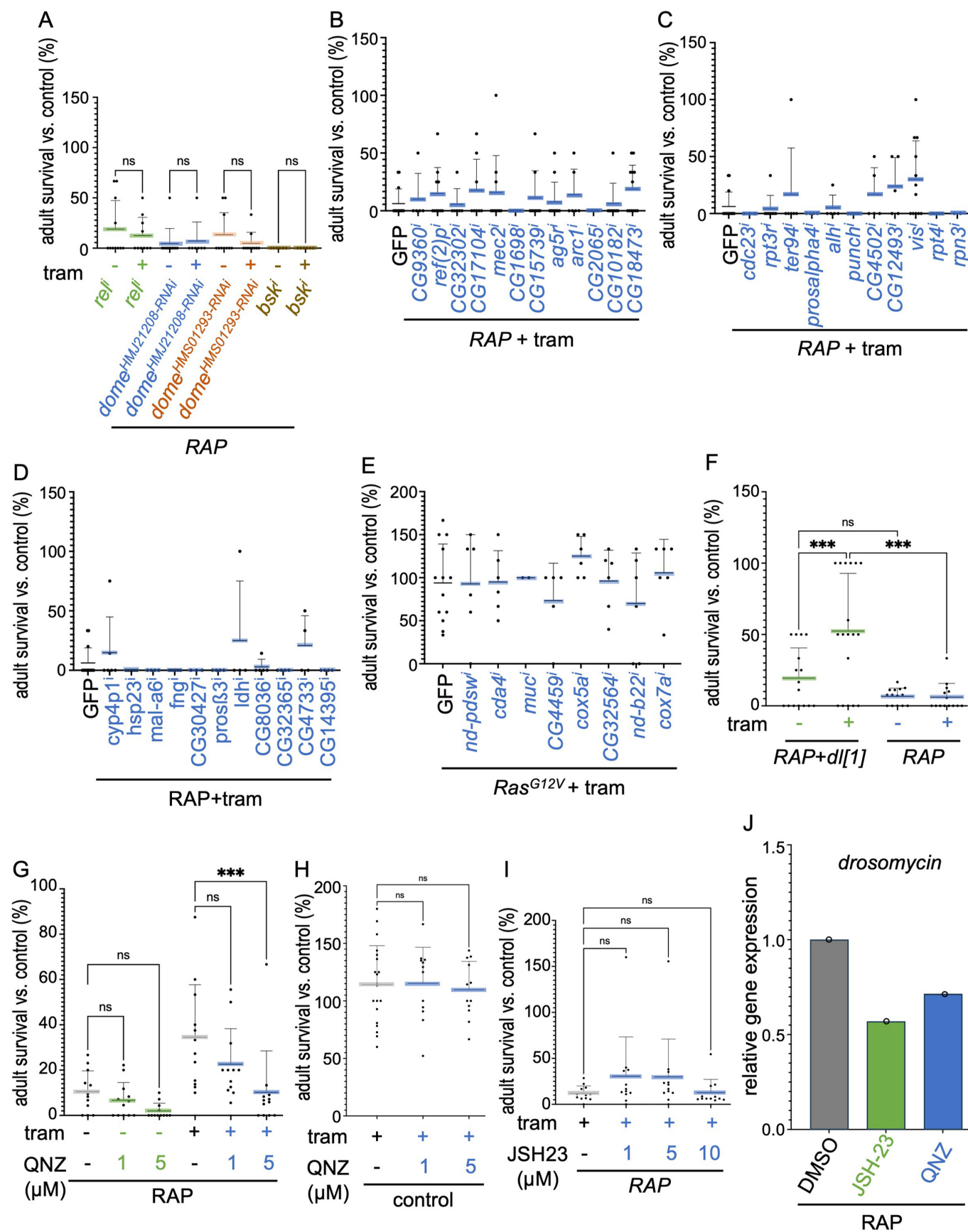
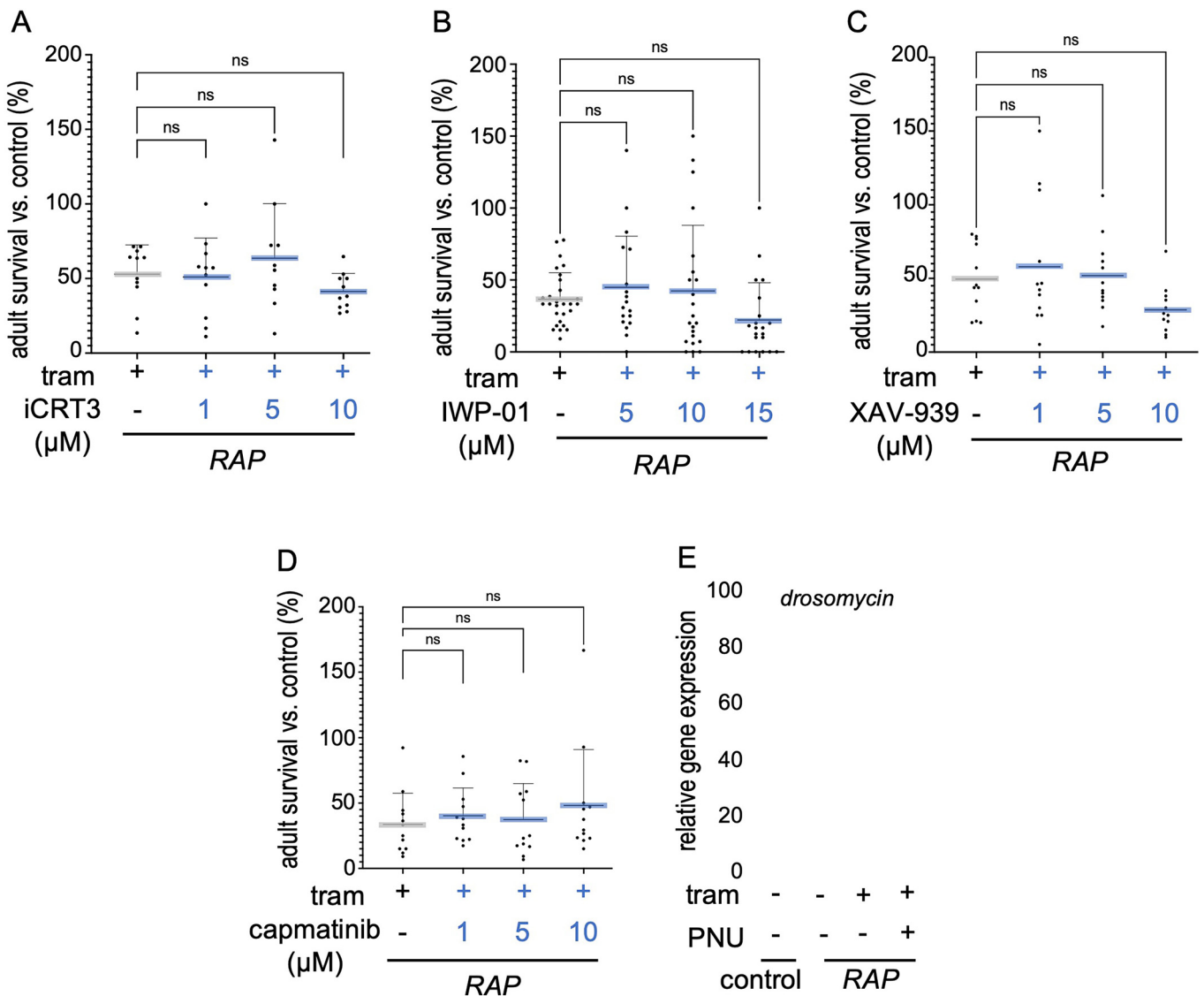


Figure EV3. Screening for factors that influence drug response.

(A–E) Percent survival of transgenic flies to adulthood relative to control flies was quantified in the presence or absence of trametinib (1 μ M). All flies driven by *byn-GAL4*: (A) *RAP;rel^l* (DMSO, $n = 11$; tram, $n = 10$), *RAP;dome^l* (HMJ21208) (DMSO, $n = 11$; tram, $n = 7$), *RAP;dome^l* (HMS01293) (DMSO, $n = 10$; tram, $n = 10$), *RAP;bsk^l* (DMSO, $n = 6$; tram, $n = 6$); (B) *RAP;GFP* (tram, $n = 14$), *RAP;CG9360^l* (tram, $n = 5$), *RAP;ref(2)p^l* (tram, $n = 10$), *RAP;CG32302^l* (tram, $n = 6$), *RAP;CG17104^l* (tram, $n = 8$), *RAP;mec2^l* (tram, $n = 10$), *RAP;CG1698^l* (tram, $n = 5$), *RAP;CG15739^l* (tram, $n = 9$), *RAP;ag5r^l* (tram, $n = 11$), *RAP;arc^l* (tram, $n = 6$), *RAP;CG2065^l* (tram, $n = 6$), *RAP;CG10182^l* (tram, $n = 8$), *RAP;18473^l* (tram, $n = 12$); (C) *RAP;GFP* (tram, $n = 14$), *RAP;cdc23^l* (tram, $n = 3$), *RAP;rpt3^l* (tram, $n = 8$), *RAP;ter94^l* (tram, $n = 6$), *RAP;prosalpha4^l* (tram, $n = 4$), *RAP;alh^l* (tram, $n = 5$), *RAP;punch^l* (tram, $n = 6$), *RAP;CG4502^l* (tram, $n = 5$), *RAP;CG12493^l* (tram, $n = 5$), *RAP;vis^l* (tram, $n = 12$), *RAP;rpt4^l* (tram, $n = 5$), *RAP;rpn3^l* (tram, $n = 2$); (D) *RAP;GFP* (tram, $n = 14$), *RAP;cyp4p1^l* (tram, $n = 6$), *RAP;hsp23^l* (tram, $n = 6$), *RAP;mal-a6^l* (tram, $n = 4$), *RAP;fng^l* (tram, $n = 6$), *RAP;CG30427^l* (tram, $n = 3$), *RAP;prosbeta3^l* (tram, $n = 4$), *RAP;ldh^l* (tram, $n = 4$), *RAP;CG8036^l* (tram, $n = 5$), *RAP;CG32365^l* (tram, $n = 4$), *RAP;CG4733^l* (tram, $n = 4$), *RAP;CG14395^l* (tram, $n = 4$); (E) *Ras^{G12V};GFP* (tram, $n = 13$), *Ras^{G12V};nd-pdsw^l* (tram, $n = 6$), *Ras^{G12V};cda4^l* (tram, $n = 6$), *Ras^{G12V};muc^l* (tram, $n = 2$), *Ras^{G12V};CG4459^l* (tram, $n = 5$), *Ras^{G12V};cox5a^l* (tram, $n = 6$), *Ras^{G12V};CG32564^l* (tram, $n = 6$), *Ras^{G12V};nd-b22^l* (tram, $n = 6$), *Ras^{G12V};cox7a^l* (tram, $n = 6$). (F–I) Percent survival of transgenic flies to adulthood relative to control flies was quantified in the presence or absence of trametinib (1 μ M), QNZ (EVP4593) or JSH-23. (F) *RAP;dl[1]* (DMSO, $n = 16$; tram, $n = 18$) and *RAP* (DMSO, $n = 14$; tram, $n = 14$); (G–I) *RAP* (DMSO, $n = 12$; tram, $n = 12$; 1 μ M QNZ, $n = 12$; 5 μ M QNZ, $n = 12$; tram + 1 μ M QNZ, $n = 12$; tram + 5 μ M QNZ, $n = 12$) (G); control (tram, $n = 20$; tram + 1 μ M QNZ, $n = 12$; tram + 5 μ M QNZ, $n = 12$) (H); *RAP* (tram, $n = 12$; tram + 1 μ M JSH-23, $n = 12$; tram + 5 μ M JSH-23, $n = 12$; tram + 10 μ M JSH-23, $n = 12$) (I). (J) Expression levels of *drosomycin* were quantified for each condition by quantitative RT-PCR, QNZ (5 μ M), JSH-23 (5 μ M). (A–D, F) The experiments were conducted at 29 °C. (E, G–I) The experiments were conducted at 29 °C. The statistical tests used to calculate the P value are as follows: (A, F–I) one-way ANOVA; NS ($P > 0.12$), * $P(0.033)$ and *** $P(< 0.001)$. All statistical data are summarised in Table EV1. The error bar is a standard deviation (SD), with each point representing biological replicates and numbers (n), including three technical replicates.



F

Line	Gain of function genes	Loss of function genes
CPCT006	<i>Ras</i> ^{G12V}	<i>apc</i> ; <i>p53</i> ; <i>ago</i> ; <i>shg</i> ; <i>put</i> ; <i>p38a</i> ; <i>ft</i> ; <i>brm</i>
CPCT018	<i>Ras</i> ^{G12V}	<i>apc</i> ; <i>p53</i> ; <i>pten</i> ; <i>smox</i> ; <i>tefu</i> ; <i>rhoGAPp190</i> ; <i>nejire</i> ; <i>upf1</i>
CPCT029	<i>Ras</i> ^{G12V} ; <i>chico</i>	<i>apc</i> ; <i>p53</i> ; <i>Med</i> ; <i>smox</i> ; <i>trr</i> ; <i>fur2</i> ; <i>CG4238</i> ; <i>PI4KIIIalpha</i> ; <i>mus81</i>
CPCT045	<i>Ras</i> ^{G12V} ; <i>pvr</i>	<i>apc</i> ; <i>p53</i> ; <i>smox</i> ; <i>nos</i> ; <i>CG13344</i> ; <i>pc</i> ; <i>rad51C</i> ; <i>DNApol-eta</i> ; <i>lrp1</i>
CPCT050	<i>Ras</i> ^{G12V}	<i>apc</i> ; <i>p53</i> ; <i>debcl</i> ; <i>scat</i> ; <i>wdb</i> ; <i>ird1</i>
RAPp1	<i>Ras</i> ^{G12V}	<i>apc</i> ; <i>p53</i> ; <i>ago</i> ; <i>wts</i> ; <i>CG7742</i> ; <i>Atg2</i>
RAPp2	<i>Ras</i> ^{G12V}	<i>apc</i> ; <i>p53</i> ; <i>vrp1</i> ; <i>ry</i> ; <i>khc-73</i>

◀ **Figure EV4. A screening for trametinib and WNT inhibitors drug combination in RAP hindgut tumours.**

(A–D) Percent survival of transgenic *RAP* flies to adulthood relative to control flies was quantified in the presence or absence of trametinib (1 μ M), iCRT3, IWP-01, XAV-939 or Capmatinib. *RAP* (tram, $n = 11$; tram + 1 μ M iCRT3, $n = 11$; tram + 5 μ M iCRT3, $n = 10$; tram + 10 μ M iCRT3, $n = 11$) (A); *RAP* (tram, $n = 28$; tram + 5 μ M IWP-01, $n = 18$; tram + 10 μ M IWP-01, $n = 22$; tram + 15 μ M IWP-01, $n = 21$) (B); *RAP* (tram, $n = 12$; tram + 1 μ M XAV-939, $n = 12$; tram + 5 μ M XAV-939, $n = 12$; tram + 10 μ M XAV-939, $n = 12$) (C); *RAP* (tram, $n = 12$; tram + 1 μ M Capmatinib, $n = 12$; tram + 5 μ M Capmatinib, $n = 12$; tram + 10 μ M Capmatinib, $n = 12$) (D). (E) The expression levels of *drosomycin* among each genotype in the presence or absence of trametinib (1 μ M) or PNU-74654 (1 μ M) were detected by quantitative RT-PCR, $n = 3$. (E) control and *RAP*. (A–E) The experiment was conducted at 27 °C. The statistical tests used to calculate the P value are as follows: (A–D) one-way ANOVA; NS $P(>0.12)$, * $P(0.033)$, ** $P(0.002)$ and *** $P(<0.001)$. All statistical data are summarised in Table EV1. The error bar is a standard deviation (SD), with each point representing biological replicates and numbers (n), including three technical replicates. (F) A summary of mutations in patient-specific CRC models. These patient-specific fly avatars come from a fly-to-bedside study (CPCTs), and TCGA (*RAPp1*, *RAPp2*).

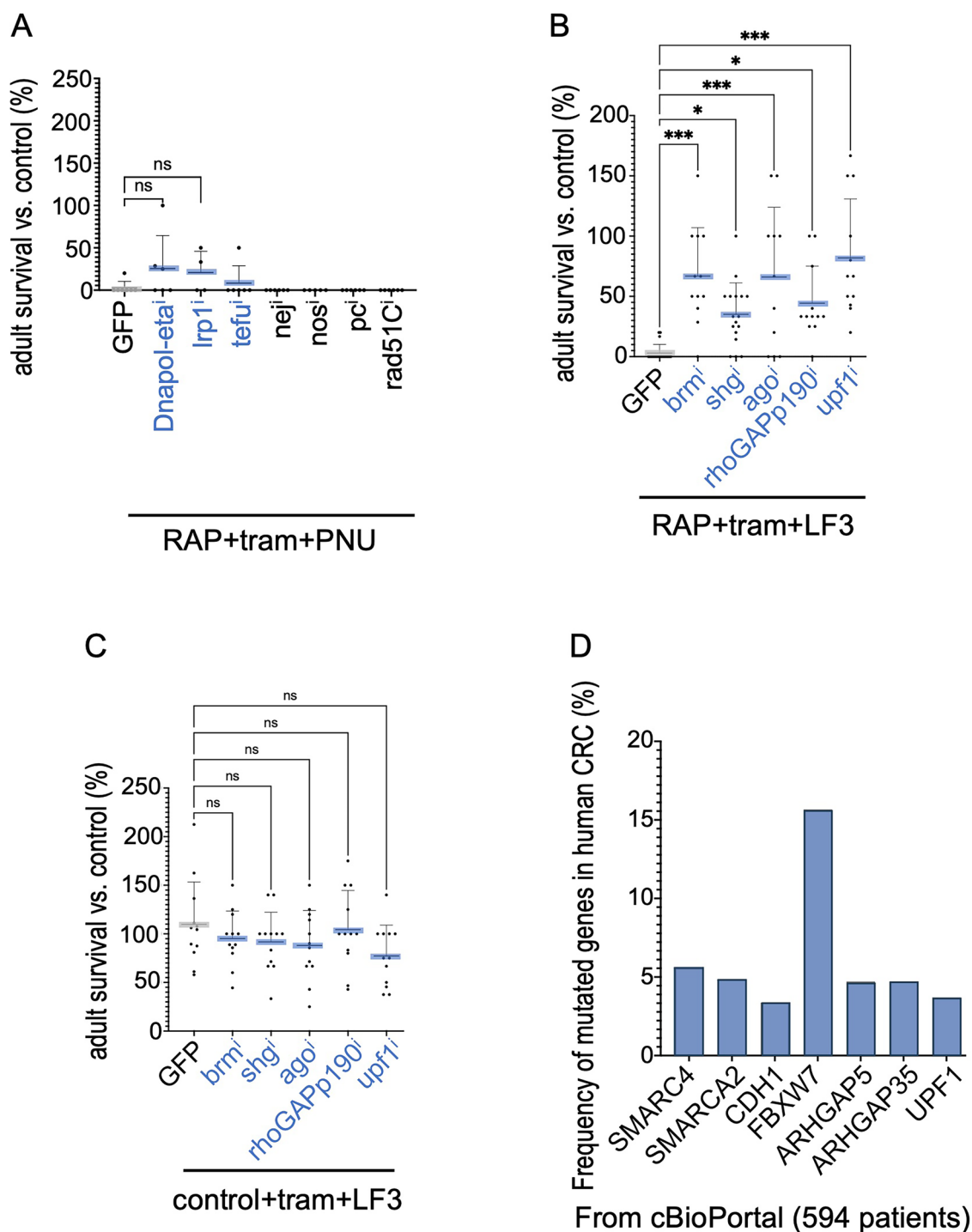
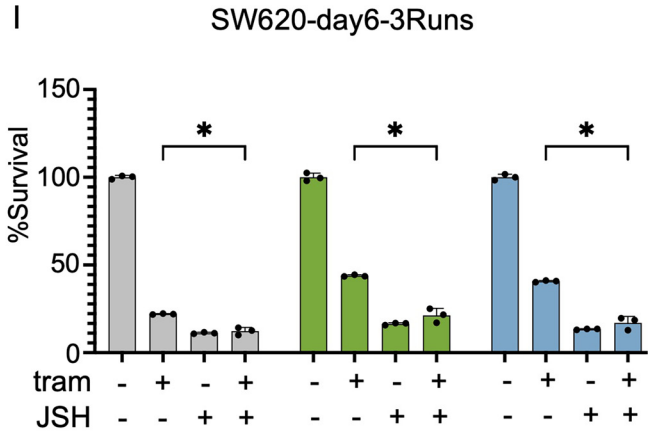
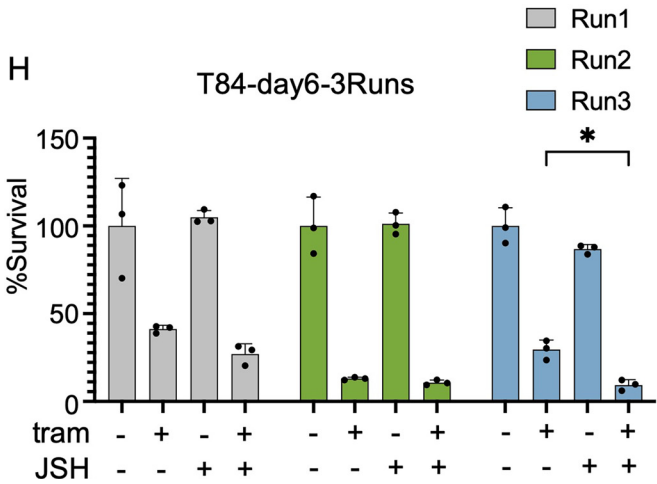
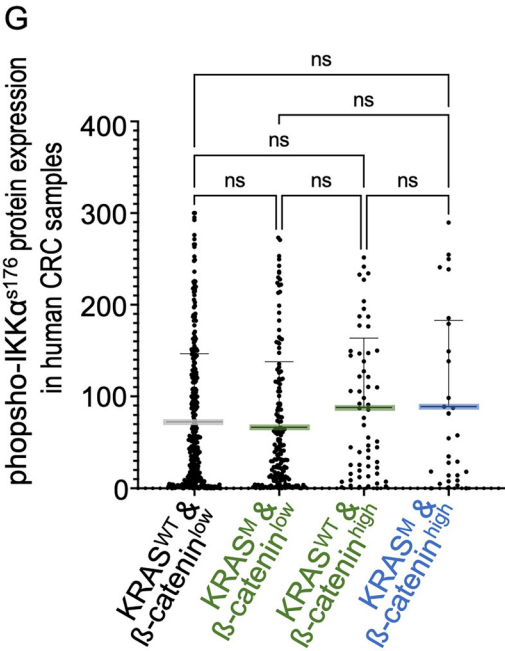
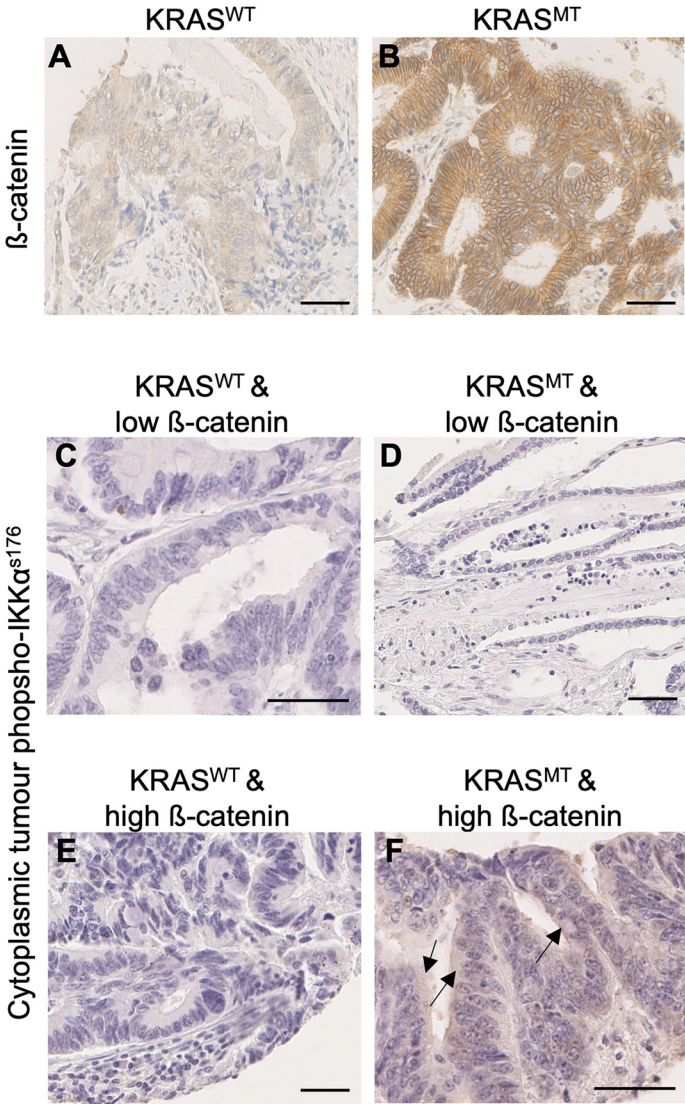


Figure EV5. Regulators for the combination of trametinib and LF3 in CRC tumours.

(A–C) Percent survival of transgenic flies to adulthood relative to control flies was quantified in the presence or absence of trametinib (1 μ M), PNU-74654 (1 μ M) or LF3 (10 μ M). *RAP;GFP* (tram + PNU, $n = 7$), *RAP;Dnapol-etaⁱ* (tram+PNU, $n = 6$); *RAP;lrp1ⁱ* (tram + PNU, $n = 4$) (A). *RAP;GFP* (tram + LF3, $n = 19$), *RAP;brmⁱ* (tram + LF3, $n = 12$), *RAP;agoⁱ* (tram + LF3, $n = 11$), *RAP;shgⁱ* (tram + LF3, $n = 17$), *RAP;upf1ⁱ* (tram + LF3, $n = 12$), *RAP;rhoGAPp190ⁱ* (tram + LF3, $n = 12$) (B); *control* (tram + LF3, $n = 12$), *brmⁱ* (tram + LF3, $n = 12$), *agoⁱ* (tram + LF3, $n = 12$), *shgⁱ* (tram + LF3, $n = 12$), *upf1ⁱ* (tram + LF3, $n = 12$), *rhoGAPp190ⁱ* (tram + LF3, $n = 12$) (C). (D) The graph showed the frequency of mutated genes in human CRC. These data from cBioPortal include 594 patients. (A–C) The experiment was conducted at 29 °C. The statistical tests used to calculate the P value are as follows: (A–C) one-way ANOVA; NS $P(>0.12)$, * $P(0.033)$, ** $P(0.002)$ and *** $P(<0.001)$. All statistical data are summarised in Table EV1. The error bar is a standard deviation (SD), with each point representing biological replicates and numbers (n), including three technical replicates.



◀ **Figure EV6. IHC staining of phospho-IKK α^{S176} protein in human CRC.**

(A–F) IHC staining of β -catenin or phospho-IKK α^{S176} in each genotype CRC sample. (A) $KRAS^{WT}$ (wild-type); (B) $KRAS^{MT}$ (mutation in position G12/G13); (C) $KRAS^{WT}$ plus low β -catenin; (D) $KRAS^{MT}$ plus low β -catenin; (E) $KRAS^{WT}$ plus high β -catenin; (F) $KRAS^{MT}$ plus high β -catenin expression in CRC samples. Scale bar 50 μ m. (G) The graph shows the mean of expression of phospho-IKK α^{S176} in each different mutated human CRC, determined by IHC intensity values. Patients were grouped into four categories based on KRAS status and β -catenin expression. Both $KRAS^{WT}$ and β -catenin low ($n = 316$); $KRAS^{MT}$ and β -catenin low ($n = 164$); $KRAS^{WT}$ and β -catenin high ($n = 59$); Both $KRAS^{MT}$ and β -catenin high ($n = 29$). Each dot displays an individual sample. (H, I) Cell viability assay for T84 or SW620 colon cancer cells, showing the results of three experimental replicates, treatment with 0.1% DMSO, 50 nM trametinib or 10 μ M JSH-23. The statistical tests used to calculate the P value are as follows: (G) one-way ANOVA; (H, I) 2-way ANOVA; NS $P(>0.12)$, * $P(0.033)$, ** $P(0.002)$ and *** $P(<0.001)$. All statistical data are summarised in Table EV1. The error bar is a standard deviation (SD), with each point representing biological replicates.