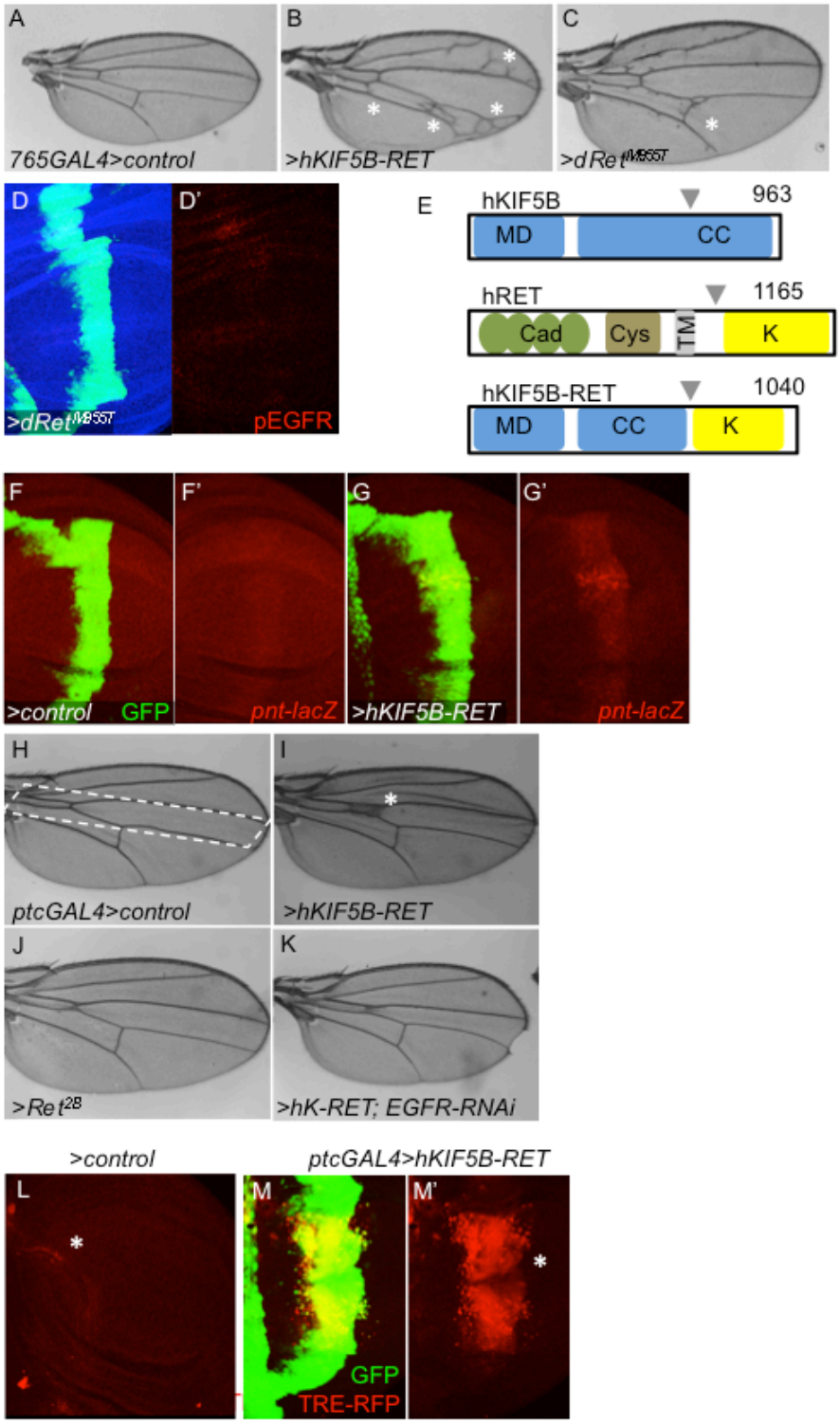


Suppl. Figure 1. Related to Figure 1 and 2.

Suppl. Figure 1. Related to Figure 1 and 2.

(A-C) Bright field images of adult wings of the indicated genotype. Uniform expression of human *KIF5B-RET* (*765>hKIF5B-RET*) in developing discs resulted in adults wings with ectopic wing veins (B; asterisks) compared to controls (A). Expression of activating point mutant RET, *765>dRET^{M955T}* (C) led to a weaker wing vein phenotype.

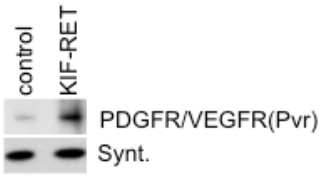
(D) Third instar larval wing epithelia, *en face* view. Expression of *Drosophila* ortholog of activating RET point mutant in a central stripe of larval wing epithelial cells (*ptc>dRET^{M955T}*, marked by eGFP expression) did not lead to pEGFR upregulation.

(E) Graphic representation of structural domains of the human *KIF5B-RET* transgene used in our studies. MD, motor; CC, coiled-coil; K, kinase; TM, transmembrane; Cad, Cadherin repeats; Cys, cysteine rich region. Inverted shaded triangle indicates breakpoints within RET and KIF5B genes and point of fusion.

(F) Expression of *KIF5B-RET* (*ptc>hKIF5B-RET*) in a central stripe of larval wing epithelial cells led to strong expression of EGFR activity reporter *pnt-lacZ* (G) compared to controls (F).

(H-K) EGFR activation is functionally linked to KIF5B-RET expression. The indicated transgenes were expressed under the control of the *ptc-GAL4* driver, *e.g.*, *ptc>KIF5B-RET*, and adult wings were analyzed for abnormal wing vein pattern. (H) Control adult wing; white box indicates where *ptc* promoter is active during earlier larval stages. (I) *ptc>KIF5B-RET* adult wings showing thickening of wing vein (asterisk). (J) Expression of Drosophila onocgenic variant *ptc>dRET^{M955T}* showed no ectopic vein material. (K) Expressing RNA interference transgene targeting EGFR in KIF5B-RET expressing cells, *ptc>KIF5B-RET, EGFR^{RNAi},* suppressed the ectopic wing vein phenotype. (L-M) *ptc>KIF5B-RET* cells show strong upregulation of JNK pathway reporter TRE-RFP (M). Control cells show background levels of activity of the same reporter (L).

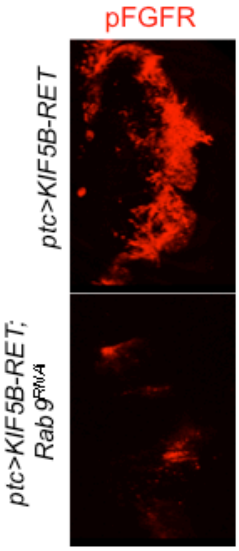
A.



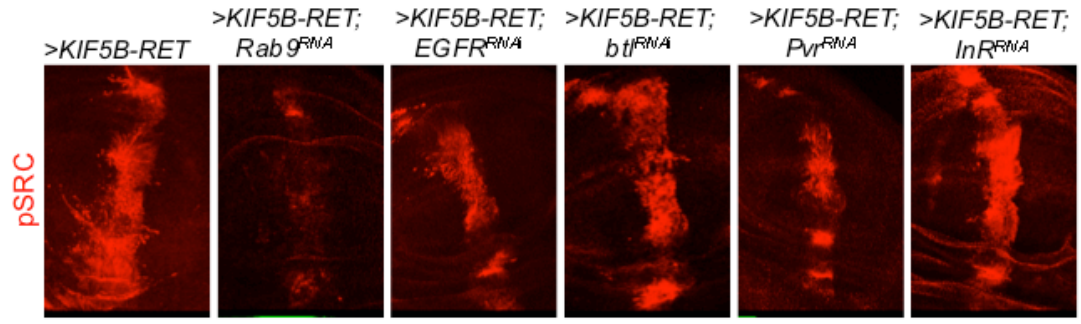
B.

RNAi Stock	*Drosophila Gene*	*Human Ortholog*
RNAi of bsk	basket	JNK1
RNAi of drk	downstream of receptor kinase	Grb2
RNAi of Grip	Glutamate receptor binding protein	GRIP1
RNAi of sgg	shaggy	GSK3β
RNAi of Nup358	Nucleoporin 358kD	RanBP2
RNAi of shg	shotgun	E-cadherin
RNAi of grh	grainy head	elf1
RNAi for Aplip1	APP-like interacting protein 1	JIP1
RNAi of Rab5	Rab5	RAB5
RNAi of Rab6	Rab6	RAB6
RNAi of Rab7	Rab7	RAB7
RNAi of Rab8	Rab8	RAB8
RNAi of Rab9	Rab9	RAB9A
RNAi of Rab11	Rab11	RAB11
RNAi of Rab21	Rab21	RAB21
RNAi of Rab23	Rab23	RAB23
RNAi of Arp66B	Arp66B	Arp3
RNAi of Cortactin	Cortactin	Cortactin
RNAi of Src64B	Src64B	Src
RNAi of Pvr	Pvr	PDGFR/VEGFR
RNAi of Ret	Ret	RET
RNAi of FGFR	breathless	FGFR
RNAi of FGFR	heartless	FGFR1
RNAi of InR	insulin receptor	InR

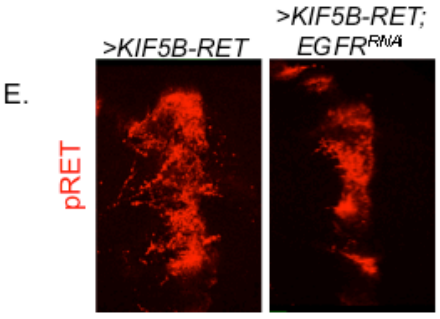
C.



D.



E.

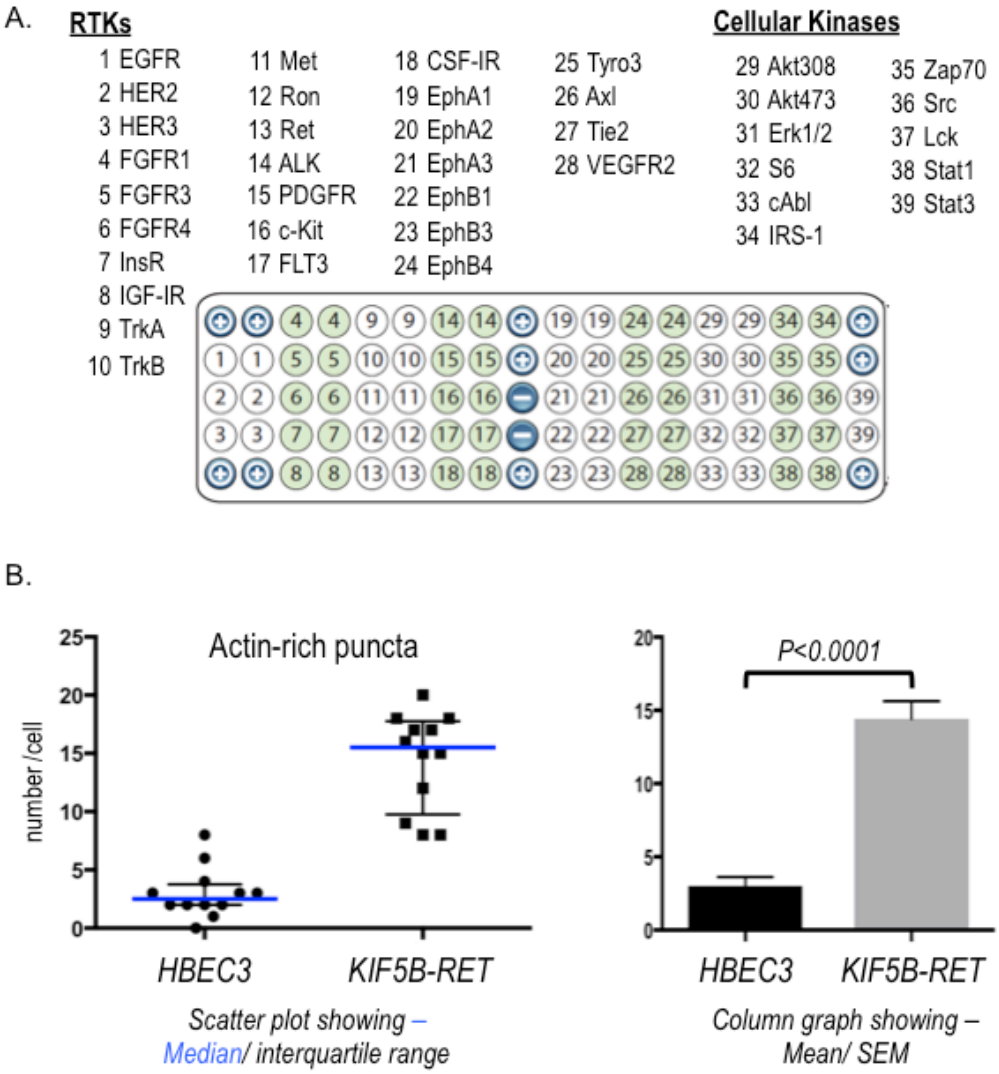


Suppl. Figure 2. Related to Figure 3 and 4.

Suppl. Figure 2. Related to Figure 3 and 4.

(A) Western blot analysis indicating elevated levels of Drosophila PDGFR/VEGFR ortholog Pvr in developing wing discs (*765>KIF5B-RET*) expressing *KIF5B-RET*; Syntaxin was used as loading control. (B) Table showing *Drosophila* TRIP-RNAi knockdown lines used to assess effect of targeted knockdown on viability of *ptc>KIF5B-RET* flies and effect on pEGFR activation within the *ptc* domain. The name of the *Drosophila* gene and its corresponding human ortholog is indicated. (C) *ptc>KIF5B-RET* larval wing discs showing effect of $Rab9^{RNAi}$ knockdown on levels of pFGFR. In *ptc>GFP,KIF5B-RET* wing discs, almost all discs showed strong activation of pFGFR (Fig 4). Simultaneous knockdown of Rab9 (*ptc>GFP, KIF5B-RET,* $Rab9^{RNAi}$) reduced the number of discs showing high pFGFR activation. (D) In *ptc>KIF5B-RET* larval wing discs simultaneous knockdown of Rab9 (*ptc>GFP, KIF5B-RET,* $Rab9^{RNAi}$) reduced pSRC levels considerably. But in *KIF5B-RET* expressing larval wing discs knockdown of individual RTK's (see Fig 5A) did not alter pSRC levels significantly.

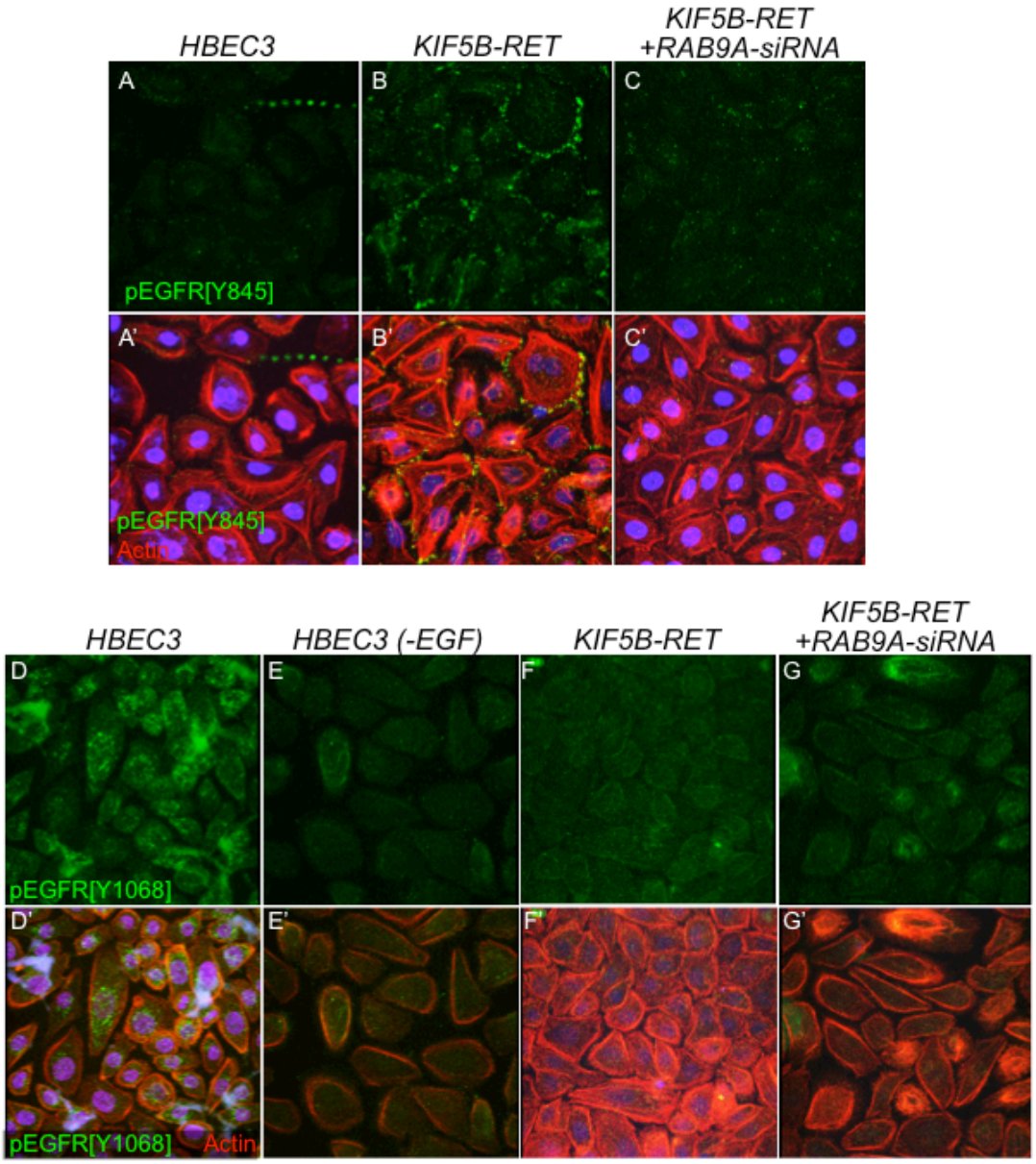
(E) Simultaneous knockdown of EGFR in *KIF5B-RET* larval wing discs (*ptc>GFP, KIF5B-RET,* $EGFR^{RNAi}$) did not alter the levels of pRET significantly.



Suppl. Figure 3. Related to Figure 5.

Suppl. Figure 3. Related to Figure 5.

(A) Cell Signaling RTK Array. Numbers and names correspond to pictorial representation of phospho-antibody spots on the array. (B) Quantification of the number of Actin-rich puncta assessed by Phalloidin-Rhodamine staining of *HBEC3[KIF5B-RET]* cells compared to parental *HBEC3* cells. Confocal images 63x of cells were analyzed as full overlay covering entire thickness of the cells. Left Panel: Actin-rich puncta in Fig. 6 (*e.g.*, arrows) was counted and represented as number of cells in a scatter plot using PRISM software. Right Panel: Same analysis as B represented as column graph indicating mean for each column with error bars showing SEM. Significance established by performing student t-test with Welch's correction.



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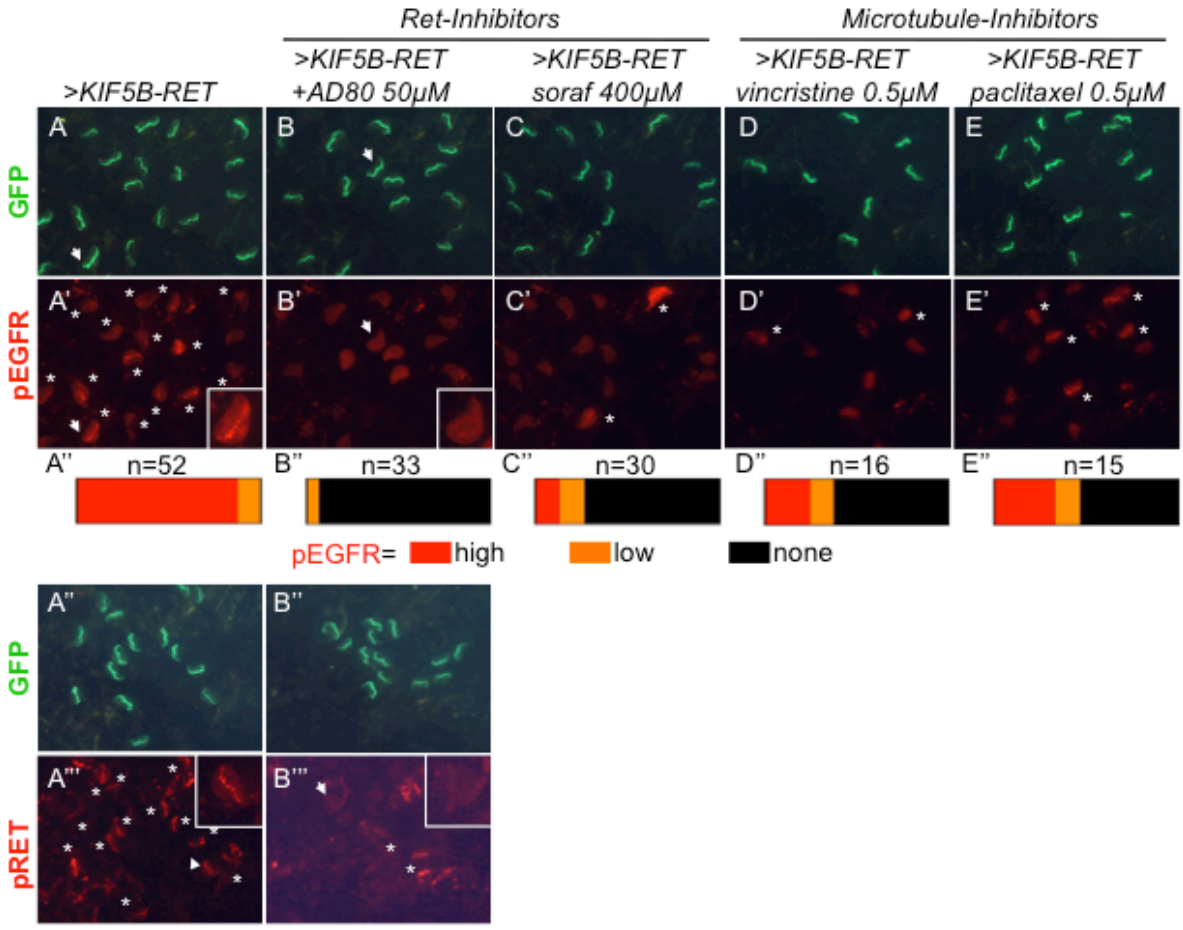
Suppl. Figure 4. Related to Figure 5 and 6.

(A-C) Immunofluorescence images showing strong upregulation of pEGFR[Y845] levels in *KIF5B-RET* cells (B) compared to parental cells (A). siRNA-mediated knockdown of *RAB9A* (C) suppressed the increase of pEGFR[Y845] levels in *KIF5B-RET* cells. DAPI labels nuclei and Phalloidin-Rhodamine labels Actin cytoskeleton. (D-E) Parental HBEC3 cells showed high levels of pEGFR[Y1068] signal, indicative of high dpERK/MAPK signaling (D). This signal is strongly suppressed if EGF is removed from the growth media (E), indicating that pEGFR[Y1068] was induced by EGF signaling. (F-G) *HBEC3[KIF5B-RET]* cells exhibited strongly reduced pEGFR[Y1068], indicating a switch away from dpERK/MAPK signaling (F). siRNA mediated knockdown of RAB9A partially restored pEGFR[Y1068] signal, indicating reactivation of dpERK/MAPK signaling (G; also see Fig. 7I).



Suppl. Figure 5. Related to Figure 7.

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(A-E) Inhibiting EGFR signaling is optimal for therapeutics targeting KIF5B-RET network. Immunofluorescence images of a cohort of *Drosophila* larval wing discs showing effect of RET and microtubule inhibitors on pEGFR and pRET activation by *KIF5B-RET*. In *KIF5B-RET* expressing wing discs, *ptc>eGFP, KIF5B-RET*, almost all discs showed activation of pEGFR (A', asterisk). The polypharmacological kinase inhibitor AD80 strongly suppressed pEGFR activation (B) while clinically approved drug sorafenib was moderately effective (C). Microtubule/cytoskeleton inhibitors vincristine and paclitaxel were also somewhat effective. Binned representation of relative number of discs showing high, low, and no pEGFR expression (A", B'', C'', D", E"). The GFP channel for each genotype (A, B, C, D, E) shows region of the wing disc where transgenes were activated and match regions where pEGFR activation occurs. Inset shows examples of wing discs in images marked by white arrowhead. pRET activation by *KIF5B-RET* and its inhibition by AD80 shown in panels (A'''- B'''').

List of FDA-approved drugs used to screen KIF5B-RET flies

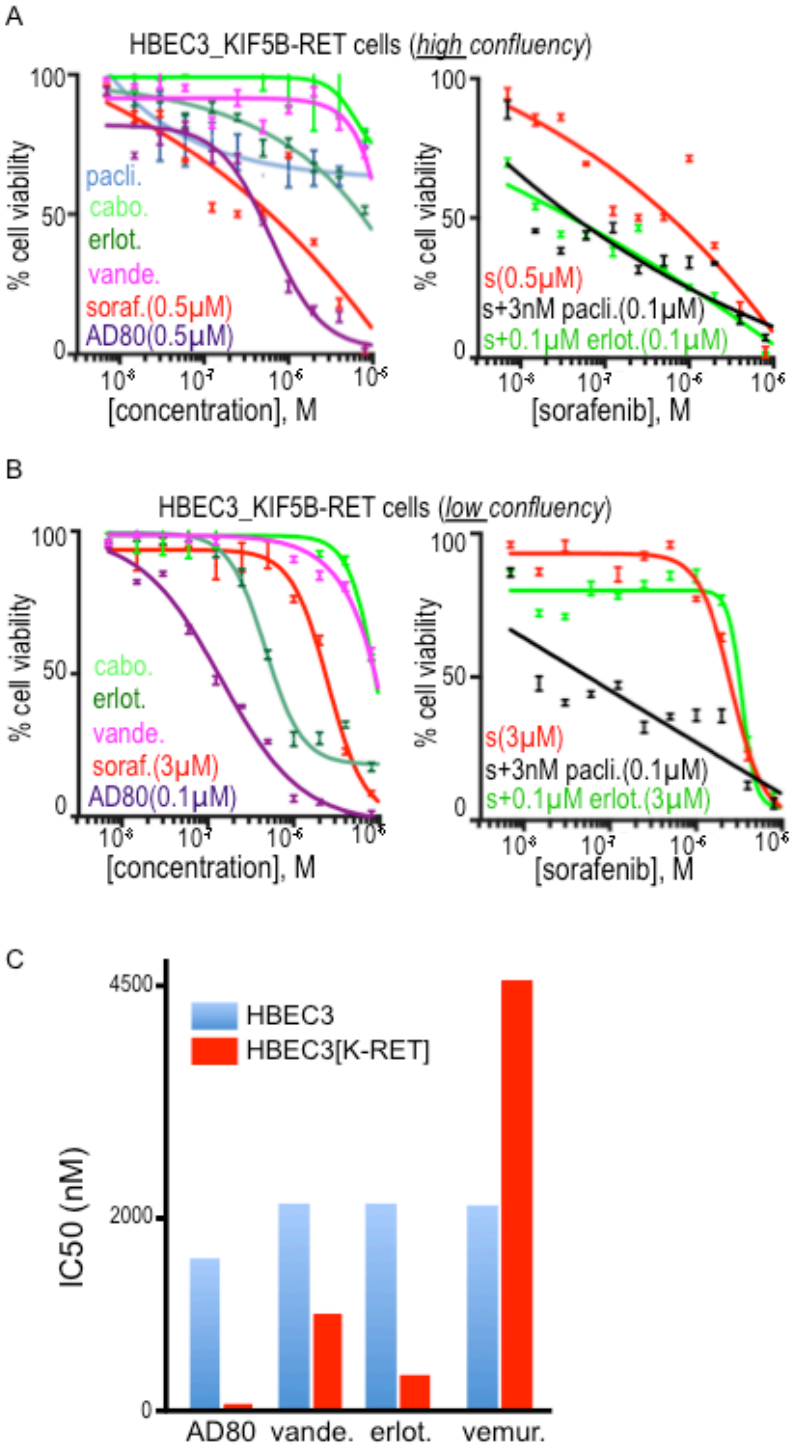
Drug	Target	Marketed Name
Abiraterone acetate(25μM)	Steroid CYP	Zytiga
Afatinib (50μM)	EGFR/HER2+kinases	GILOTRIF
Anasterozole (100μM)	Aromatase	ARIMIDEX
Axitinib (37.5μM)	VEGF/PDGF/c-KIT	Inlyta
Bendamustine HC(200μM)	DNA crosslinker	Treanda
Bortezomib(1μM)	Proteasome	Velcade
Bosutinib(200μM)	SRC/Abl+kinases	Bosulif
Busulflan(200μM)	DNA crosslinker	Busulfex
Cabazitaxel(20μM)	Microtubule	Jevtana
Cabozantinib(100μM)	Ret+kinases	CABOMETYX
Capecitabine(200μM)	DNA synthesis	Xeloda
Carfilzomib(50μM)	Proteasome	KYPROLIS
Cinacalcet(100μM)	Calcimimetic	Sensipar
Clofarabine(20μM)	Purine nucleoside antimetabolite	Clolar
Crizotinib(50μM)	ALK/ROS+ kinases	Xalkori
Dabrafenib(100μM)	BRAF+kinases	Tafinlar
Dasatinib(10μM)	SRC/Abl+kinases	Sprycel
Docetaxel(50μM)	Microtubule	Docefrez
Doxorubicin(200μM)	DNA intercalator	Doxil
Epirubicin(200μM)	DNA intercalator	Ellence
Enzalutamide(100μM)	Androgen receptor	XTANDI
Erlotinib(25μM)	EGFR+kinases	Tarceva
Everolimus(1μM)	mTOR+kinases	Afinitor
Exemestane(50μM)	Aromatase	Aromasin
Flutamide(100μM)	Androgen receptor	Eulexin
Fulvestrant(200μM)	Estrogen receptor	Faslodex
Gefitinib(10μM)	EGFR+kinases	Iressa
Gemcitabine(1μM)	Nucleoside analog	Gemzar
Imatinib(50μM)	Abl/PDGFR/c-KIT+kinases	Gleevec
Irinotecan(100μM)	Topoisomerase I	Camptosar
Lapatinib(100μM)	HER2/EGFR+kinases	Tykerb
Lenalidomide(200μM))	Thalidomide analog	Revlimid
Letrozole(100μM)	Aromatase	Femara

Drug	Target	Marketed Name
Nelarabine(200μM)	Purine nucleoside antimetabolite	Arranon
Nilotinib(25μM)	Abl+kinases	Tasigna
Pamidronate(75μM)	Bone resorption	Aredia
Pazopanib(50μM)	VEGF/PDGF/c-KIT	Votrient
Pemetrexed DiNA(1μM)	DNA synthesis	Alimta
Pomalidomide(50μM)	Thalidomide analog	POMALYST
Ponatinib(50μM)	Bcr-Abl+kinases	Iclusig
Rapamycin(1μM)	mTOR+kinases	Rapamune
Regorafenib(50μM)	Ret+kinases	Stivarga
Sorafenib(200μM)	Ret+kinases	Nexavar
Sunitinib Malate(50μM)	Ret+kinases	Sutent
Tamoxifen(100μM)	Estrogen receptor	Nolvadex
Temsirolimus(1μM)	mTOR+kinases	Torisel
Topotecan HCL(1μM)	Topoisomerase	Hycamtin
Trametinib(1μM)	MEK+kinases	MEKINIST
Vandetanib(25μM)	Ret+kinases	Caprelsa
Vemurafenib(50μM)	RAF+kinases	Zelboraf
Vismodegib(1μM)	Smoothened Receptor	Erivedge
Vorinostat(10μM)	HDAC	Zolinsa
Zoledronic Acid(7μM)	unknown	Zometa
Ibrutinib(100μM)	BTK+kinases	IMBRUVICA
Idelalisib(200μM)	p110d	ZYDELIG
Belinostat(200μM)	HDAC	Beleodaq
Certinib(25μM)	ALK+kinases	ZYKADIA
Nintendanib(10μM)	PDGFR/VEGFR/FGFR+kinases	Ofev
Olaparib(150μM)	PARP	LYNPARZA
Lenvatinib(50μM)	Ret+Kinases	LENVIMA
Panobinostat(200μM)	HDAC	Farydak
Palbociclib(50μM)	CDK4,6	Ibrance
Ruxolitinib(200μM)	JAK+kinases	Jakafi
Alectinib(0.05μM)	ALK+kinases	ALECENSA
Vincristine(0.5μM)	micrortubule	Marqibo
Paclitaxel(0.5μM)	micrortubule	Abraxane
AD80(50μM)	Ret+Kinases	

Suppl. Figure 6. Related to Figure 7.

Suppl. Figure 6. Related to Figure 7.

The panel of FDA approved drugs used to screen in *ptc>KIF5B-RET* flies. Also included: each drug's established primary in-vivo targets, and marketed brand name. Each drugs was tested at a previously established *Drosophila* maximum tolerated dose (MTD). The doses are indicated and ranged, based on drug type, from 0.5μM to 400μM final concentration in fly food.



Suppl. Figure 7. Related to Figure 7.

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(A-C) Effect of drugs as single agents and as combinations on *HBEC3[KIF5B-RET]* cells. (A) Cells grown to *high* confluency. Dose response curve fitted to non-linear regression model using PRISM software; IC50's shown in brackets. Left Panel: confluent cells showed little sensitivity to clinically approved RET inhibitors vandetanib or cabozantinib (IC50's >10 μM), but cells showed sensitivity to both sorafenib and AD80 (IC50s = ~0.5 μM). EGFR inhibitor erlotinib showed intermediate effect (IC50 = ~10 μM). Right Panel: Combining sorafenib/erlotinib or sorafenib/paclitaxel potently inhibited (IC50 = ~0.1 μM) growth of *HBEC3[KIF5B-RET]* cells. Paclitaxel (3 nM) or erlotinib (0.1 μM) alone—at doses used in combinations—had minimal detectable effect on growth of *HBEC3[KIF5B-RET]* cells (see Left Panel). (B) *C*ells grown to *low* confluency. Left Panel: low confluency cells showed little sensitivity to clinically approved RET inhibitors vandetanib, cabozantinib (IC50s >10 μM). They showed moderate sensitivity to sorafenib (IC50 = ~3 μM) and erlotinib (IC50 = ~1 μM) and high sensitivity to AD80 (IC50s = ~0.1 μM). Right Panel: Combining sorafenib/erlotinib did not show strong effect (IC50 = ~3 μM) compared to sorafenib alone, but sorafenib/paclitaxel potently inhibited (IC50 = ~0.1 μM) growth of *HBEC3[KIF5B-RET]* cells. (C) Column graph showing IC50s of parental *HBEC3* and *HBEC3[KIF5B-RET]* cells. Dose response curves were performed as in panels A and B above and computed IC50s were plotted as column graph. *HBEC3[KIF5B-RET]* cells showed increased sensitivity to AD80 and the EGFR inhibitor erlotinib compared to parental cells. Conversely *HBEC3[KIF5B-RET]* cells showed reduced sensitivity to the RAF inhibitor vemurafenib, indicating a switch away from dpERK signaling.