## SUPPLEMENTAL MATERIAL

Shaik et al., http://www.jem.org/cgi/content/full/jem.20112446/DC1

Α	DSG1	В	DSG2					
Human (1185-1199) Chimpanzee (1295-1309) Monkey (1295-1309) Rat (1181-1195) Mouse (1185-1199) Zebrafish (1177-1191) β-TRCP-recognizable canonic degron consensus	SMEEDSGLSLPTSPV SMEEDSGLSLPTSPV SMEEDSGLSLPTSPV SMEEDSGLSLPTSPV SMEEDSGLSLPTSPV VSLRDSGTAWNIKIR all DSGX (2-5) S	Human (1225-1269) Chimpanzee (1365-1379) Monkey (1260-1274) Rat (1251-1265) Mouse (1253-1267) Zebrafish (1213-1228)	DNGTDSGMYLASEEL DNGTDSGMVLASEEL DNGTDSGMVLASEEL DSQTDSGMVLASEEL DSQTDSGMVLASEEL GGQSDSGIGLSSDDL DSGX (2-5) S					
С	DSG3	D	DDTD					
Human (1342-1356) Chimpanzee (1452-1466) Monkey (1347-1361) Rat (1329-1343) Mouse (1331-1345) Zebrafish (1271-1285) β-TRCP-recognizable canonic degron consensus	ILGPDSGTTLSSPPV ILQPDSGTTLSSPPV ILGPDSGTTLSSPPV AGHVDSGTTLRSSPV AVHADSGTTLRSPPV LSLEDSSLDSEMECH al DSGX (2-5) S	Human (1309-1323) Chimpanzee (1452-1466) Monkey (1314-1328) Rat (1305-1319) Mouse (1307-1321)	YHSDDTDTTVYSSEE YHSDDTDTTVYSSEE YHSDDTDTTVYSSEE YHSDDTDTTVYSSDE YHSDDTDTTVYSSDE DSGX (2-5) S					
E CKI consensus motif: D/I	E/T/SXX(X) <mark>S/T</mark>							
F Potential and predicted C	F Potential and predicted CKI-mediated phosphorylation sites in VEGFR2							
Degron 1 (DSG1):								

Ser1190 is a potential CKI site as it matches the CKI consensus motif.

Ser1193 is to be a predicted CKI site by Scansite, and matches the CKI consensus motif.

## Degron 2 (DSG2):

Thr1228 is to be a predicted CKI site by Scansite, and matches the CKI consensus motif. Ser1230 is to be a predicted CKI site by Scansite.

## Degron 3 (DSG3):

Thr1349, Thr1350 and Ser1353 are the potential CKI sites as they match the CKI consensus motif.

## Degron 4 (DDTD):

Thr1314, Thr1316 and Thr1317 are to be predicted CKI sites by Scansite and they match the CKI consensus motif. Ser1320 and Ser1321 are potential CKI sites as they match the CKI consensus motif.

Figure S1. Sequence alignment of VEGFR2 with the DSG(XX)S phosphodegron sequences recognized by  $\beta$ -TRCP. These phosphodegron sequences present in VEGFR2 are conserved across different species. (A) Sequence alignment of DSG1 phosphodegron sequences. (B) Sequence alignment of DSG2 phosphodegron sequences. (C) Sequence alignment of DSG3 phosphodegron sequences. (E) Illustration of the canonical CKI phosphorylation site consensus motif. (F) Illustration of various predicted or potential CKI sites within the putative degron sequences of VEGFR2.

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# Viability at 72hpf in WT embryos

MO		Dose (μM)	
Injected	20	40	100
Control	76/78	65/68	62/66
	(97.4%)	(95.6%)	(93.9%)
fbxw11a	59/62	67/73	50/55
	(95.2%)	(91.8%)	(90.9%)
fbxw11b	69/72	58/61	57/64
	(95.8%)	(95.1%)	(89.1%)
fbxw11a/	32/52	9/50*	0/41
fbxw11b	(61.5%)	(18.0%)	(0.0%)

<sup>\*9/9</sup> had a heartbeat at 72hpf; 4/9 had gross abnormalities

Figure S2. β-TRCP2 depletion leads to increased in vivo angiogenesis in zebrafish. Summary tabulation of zebrafish embryo viability at 72 h postfertilization (72 hpf) after gene knockdown with the indicated MOs alone or in combination at the concentrations shown. Data is representative of three independent experiments.

 $\beta$ -TRCP-recognizable DSG-like canonical degron consensus in VEGF-A, which is conserved among various species.

Human (151-167) Mouse (149-165)	RRGAEESGPPHSMSRRG RQGAQESGLPRSPSRRG
Rat (149-165)	RQGAQESGLPRSPSRRG
Pig (180-196)	RLGAEESGPSRSPSRRG
Elephant (234-250)	RRGAEESGPPRSPIRRG
β-TRCP-recognizable	DSGX (2-
canonical degron consensus	5) <mark>S</mark>

Figure S3. β-TRCP may regulate VEGF-A in a post-transcriptional mechanism. Sequence alignment of the putative β-TRCP-recognizable DSG(XX)S-like phosphodegron sequences within the VEGF-A protein sequence, which are evolutionarily conserved among different species.

**Table S1.** β-TRCP1 protein expression in human thyroid cancers

Case number	Gender	Age	Histotype	Size	Extra thyroidal extension	Lymphovascular invasion	Lymph node metastasis	β-TRCP1*	CD31*	VEGF-A*	D2-40*
		yr		cm							
1	F	74	Classical PTC	4.5	Yes	No	No	1+	3+	3+	2+
2	F	46	Classical PTC	0.4	No	No	No	2+	1+	1+	1+
3	F	27	PTC diffuse sclerosing variant	1	Yes	Yes	Yes	3+	1+	1+	1+
4	F	52	Squamous cell carcinoma	1.5	Yes	Yes	Yes	3+	1+	1+	1+
5	М	48	PTC tall cell variant	1	No	No	No	3+	2+	2+	2+
6	F	20	Classical PTC	1.1	No	Yes	Yes	3+	2+	2+	1+
7	F	42	Classical PTC	1.4	No	No	No	2+	1+	1+	1+
8	F	38	Classical PTC	1.8	No	No	No	3+	2+	2+	1+
9	F	31	Classical PTC	0.4	No	No	No	2+	1+	1+	1+
10	F	62	PDTC	3.5	Yes	Yes	Yes	3+	2+	2+	2+

F, female; M, male. PTC, differentiated papillary thyroid carcinoma with squamous metaplasia foci; PDTC, poorly differentiated thyroid carcinoma with squamous metaplasia foci. \*, Immunohistochemical markers ( $\beta$ -TRCP1, CD31, VEGF-A, and D2-40) were assessed semi-quantitatively using the following scoring method: 0, negative; 1+, 1–10% positive cells (low expression); 2+, 11–50% positive cells (moderate expression); and 3+, >50% positive cells (high expression).  $\beta$ -TRCP1 protein expression was both in the cytoplasm of tumor cells or in the endothelial cells. Intratumoral angiogenesis (CD31 protein expression) or lymphangiogenesis (D2-40) is shown.

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