SUPPLEMENTAL MATERIAL

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

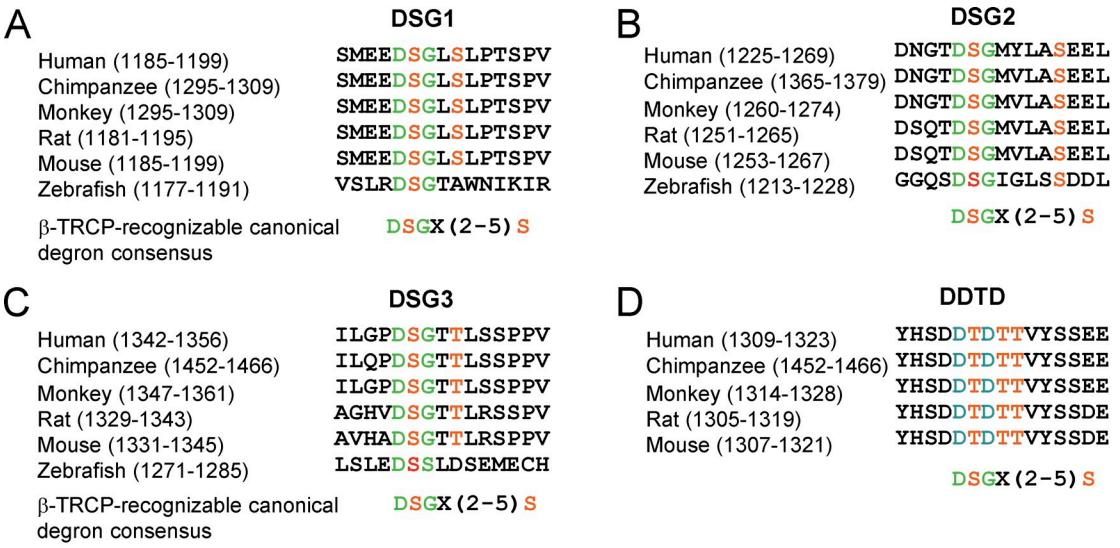

Figure S1. Sequence alignment of VEGFR2 with the DSG(XX)S phosphodegron sequences recognized by β-TRCP. These phosphodegron sequences present in VEGFR2 are conserved across different species. (A) Sequence alignment of DSG1 phosphodegron sequences. (B) Sequence alignment of DSG2 phosphodegron sequences. (C) Sequence alignment of DSG3 phosphodegron sequences. (D) Sequence alignment of DDTD 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.

Viability at 72hpf in WT embryos

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

***9/9 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.

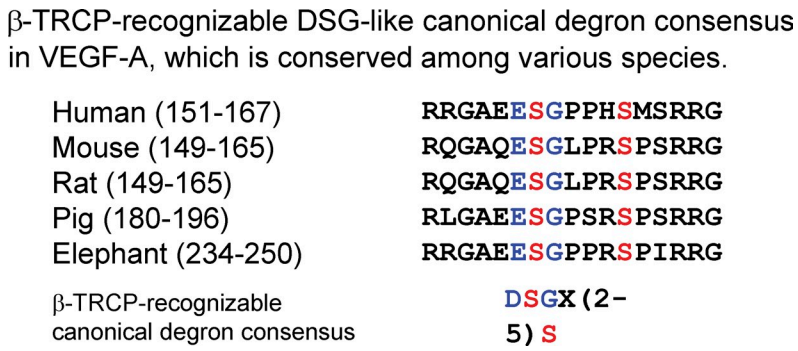

Figure S3. β-TRCP may regulate VEGF-A in a post-transcriptional mechanism. Sequence alignment of the putative β-TRCP–recognizable DSG(XX)S-like phosphodegron sequences within the VEGF-A protein sequence, which are evolutionarily conserved among different species.

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	M	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 (β-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). β-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.