

Supporting Information

Mattila *et al.* 10.1073/pnas.0803022105

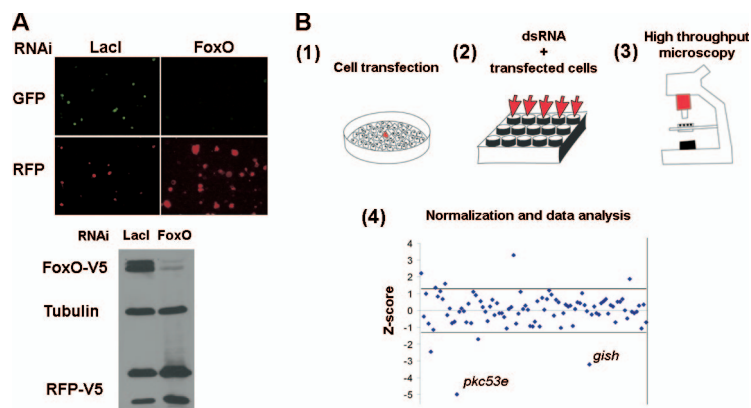


Fig. S1. Description of the primary screen. (A) The screening was based on the ability of dFoxO to drive the expression of the EGFP under a synthetic 4xFRE promoter. 4xFRE-EGFP reporter vector was transfected together with pMt-dFoxO expression vector and pMt-RFP control vector. Subsequently, the cells were treated with either *LacI* negative control or dFoxO dsRNA. Protein expression was induced by addition of CuSO_4 . Knockdown of the overexpressed dFoxO protein abolished the EGFP expression. (B) Outline of the screening process (1). A population of S2 cells was simultaneously transiently transfected with the 4xFRE-EGFP, pMt-dFoxO and pMt-RFP vectors (2). The next day the cells were collected and divided into a multiwell plate prealiquoted with the dsRNA library. After 24 h, the cells were induced with CuSO_4 and cultured for further 48 h. Cells were then transferred into an optical multiwell plate pretreated with concavalin A. After attachment the cells were washed and fixed (3). The plates were scanned by a high throughput microscope. Cells were identified by their RFP intensity and measured for both EGFP and RFP. Only cells having EGFP intensity above background level were selected for the analysis. From each cell the EGFP intensity was divided by its RFP intensity providing the relative dFoxO activity. We scanned 6,000 cells of each dsRNA treatment in triplicate (4). The averaged EGFP/RFP values were converted into Z scores. Each plate was treated independently. We chose genes having a Z score of 1.3 or higher in at least two out of the three replicates for further characterization. An example of one plate is illustrated showing the hits PKC53E and GISH. The average *P* values from the three replicates of all of the primary hits are provided in [Table S1](#).

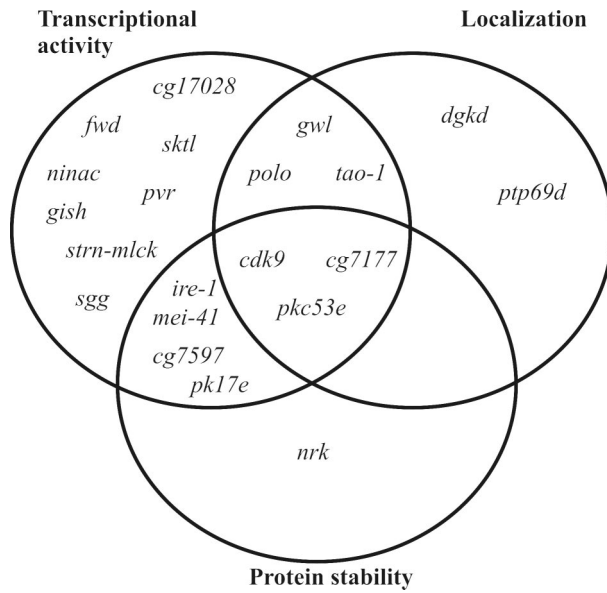


Fig. S2. The identified dFoxO regulators grouped by their regulatory mode of action. Eighteen proteins regulate dFoxO dependent transcription, eight regulate its localization, and eight its stability. Three proteins have effects in all three readouts.

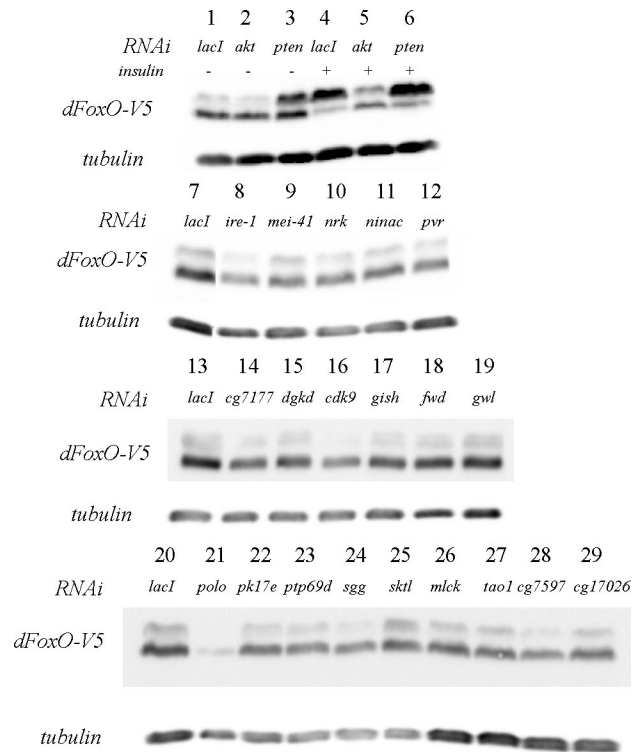


Fig. S3. dFoxO bandshifts upon the hit RNAi's. dFoxO bandshift was visualized on 8% SDS/PAGE gel for AKT and PTEN in the absence (lanes 1–3) or presence (lanes 4–6) of insulin. Lanes 7–29 dFOXO mobility after knockdown of RNAi hits from the screen.

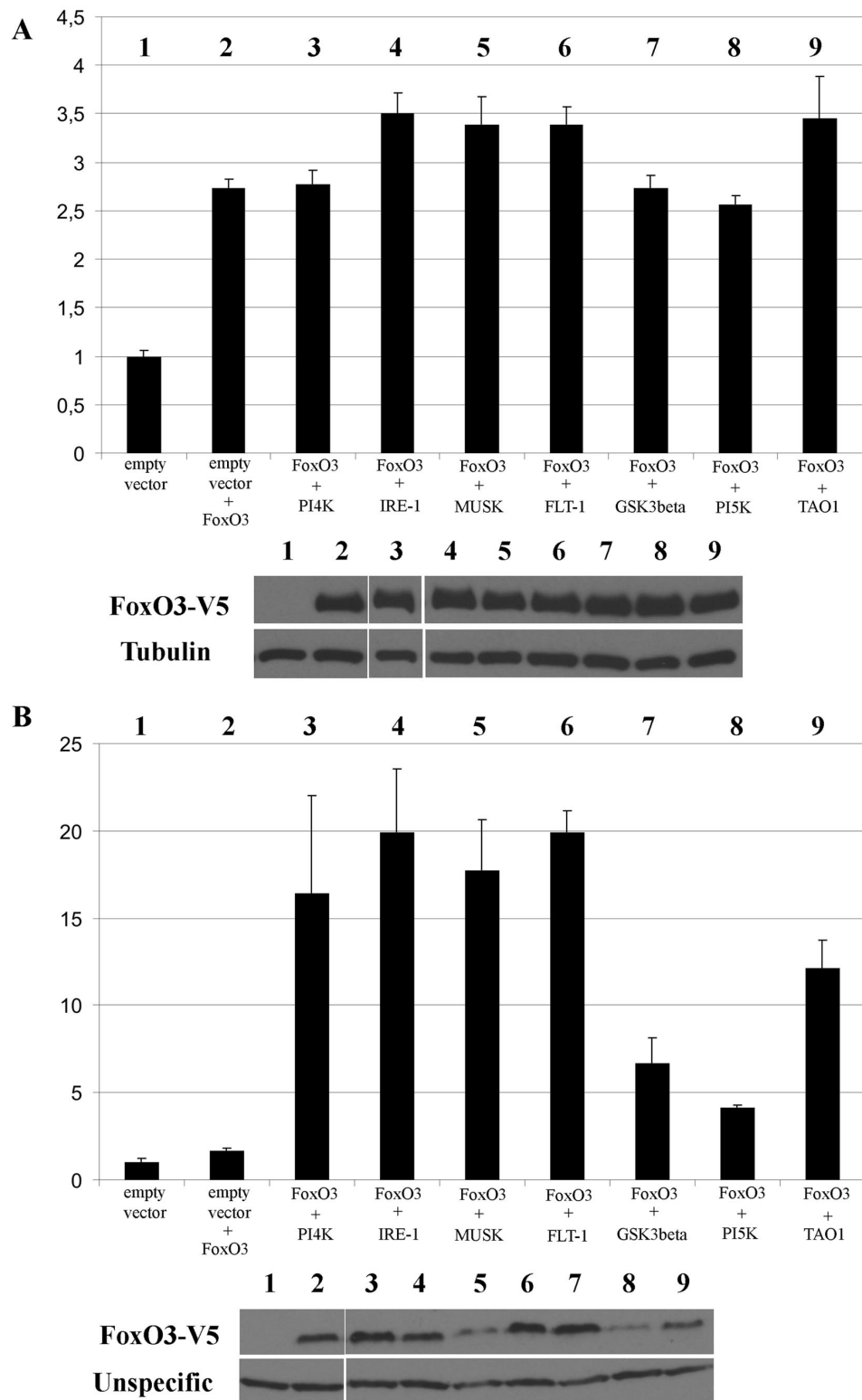


Fig. S4. FOXO3a transcriptional activity in HEK293 and HEPA1-6 cells upon overexpression of mammalian kinase orthologs. Eight mammalian orthologs of *Drosophila* proteins identified in the screen were coexpressed with FOXO3a and insulin receptor promoter luciferase reporter in HEK293 (A) and HEPA1-6 cells (B). Luciferase activity is measured with a human InR luciferase reporter.

Table S1. Primary screen hits

Gene	CG number	P
<i>akt</i>	4006	0.08
<i>basket (bsk)</i>	5680	0.1
<i>adenylyl cyclase 78c (ac78c)</i>	10564	0.07
<i>cg17026</i>	17026	0.05
<i>cg7177</i>	7177	0.1
<i>cg7597</i>	7597	0.03
<i>cyclin dependent kinase 9 (cdk9)</i>	5179	0.02
<i>darkener of apricot (doa)</i>	33553	0.1
<i>diacylglycerol kinase d (dgkd)</i>	34384	0.08
<i>four wheel drive (fwd)</i>	7004	0.04
<i>gilgamesh (gish)</i>	6963	0.0008
<i>greatwall (gwl)</i>	7719	0.05
<i>guanylyl cyclase 55b (gc55b)</i>	33958	0.15
<i>guanylyl cyclase at 76c (gc76c)</i>	8742	0.1
<i>guanylyl cyclase at 88e (gc88e)</i>	4154	0.1
<i>hipk</i>	17090	0.2
<i>hippo</i>	11228	0.02
<i>ia2</i>	31795	0.07
<i>ire-1</i>	4583	0.08
<i>meiotic 41 (mei-41)</i>	4252	0.006
<i>minibrain (mnb)</i>	7826	0.03
<i>myotubularin (mtm)</i>	9115	0.006
<i>neurospecific receptor kinase (nrk)</i>	4007	0.09
<i>Ninac</i>	5125	0.08
<i>pdgf- and vdgf-receptor related (pvr)</i>	8222	0.000001
<i>pdk1</i>	1210	0.001
<i>pkc 53e</i>	6622	0.0006
<i>polo</i>	12306	0.01
<i>protein kinase-like 17e (pk17e)</i>	7001	0.07
<i>protein phosphatase 19c (pp19c)</i>	32505	0.08
<i>protein tyrosine phosphatase 69d (ptp69d)</i>	10975	0.07
<i>pten</i>	5671	0.002
<i>shaggy (sgg)</i>	2621	0.02
<i>skittles (sktl)</i>	9985	0.2
<i>snf1a/amp-activated protein kinase (snf1a)</i>	3051	0.002
<i>stretchin-mlck (strn-mlck)</i>	18255	0.2
<i>tao-1</i>	14217	0.03
<i>target of rapamycin (tor)</i>	5092	0.08

Table S2. Hits and their mammalian orthologs

Hit	CG number	Mammalian ortholog	Accession no.	Expression vector (ref.)
<i>cg17026</i>	17026	inositol(myo)-1(or 4)-monophosphatase 1 (IMPA)	NP_005527	
<i>cg7177</i>	7177	—	—	
<i>cg7597</i>	7597	Cell division cycle 2-like protein kinase 5	NP_003709	
<i>cyclin dependent kinase 9 (cdk9)</i>	5179	cyclin-dependent kinase 9	NP_001252	
<i>diacylglycerol kinase d (dgkd)</i>	34384	diacylglycerol kinase δ 2	NP_690618	3xFlag-CMV-7.1/hDGK δ 2 (1)
<i>four wheel drive (fwd)</i>	7004	Phosphatidylinositol 4-kinase β (PI4K β)	NP_002642	pcDNA3-HA-PI4K β (2)
<i>gilgamesh (gish)</i>	6963	casein kinase 1, gamma 3	NP_004375	
<i>greatwall (gwl)</i>	7719	microtubule associated serine/threonine kinase-like	NP_116233	
<i>ire-1</i>	4583	Inositol-requiring protein 1 (IRE-1)	NP_001424	pCAG-hIRE1 (3)
<i>meiotic 41 (mei-41)</i>	4252	Serine/threonine-protein kinase ATR	NP_001175	
<i>neurospecific receptor kinase (nrk)</i>	4007	Muscle & skeletal receptor tyrosine protein kinase precursor (MUSK)	NP_005583	pcDNA3.1 Myc-MUSK (4)
<i>Ninac</i>	5125	Myosin-IIIB	NP_001077084	
<i>pdgf- and vdgf-receptor related (pvr)</i>	8222	Vascular endothelial growth factor receptor 1 precursor (FLT-1)	NP_002010	pcDNA hFLT-1 (5)
<i>pkc 53e</i>	6622	Protein kinase C alpha	NP_002728	
<i>polo</i>	12306	Serine/threonine-protein kinase PLK1	NP_005021	
<i>protein kinase-like 17e (pk17e)</i>	7001	—	—	
<i>protein tyrosine phosphatase 69d (ptp69d)</i>	10975	—	—	
<i>shaggy (sgg)</i>	2621	Glycogen synthase kinase-3 β	NP_002084	pcDNA ratGsk3 β (6)
<i>skittles (sktl)</i>	9985	phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	NP_003548	pcDNA3-myc-mousePIP5K α (7)
<i>stretchin-mlck (strn-mlck)</i>	18255	Myosin light chain kinase, smooth muscle (smMLCK)	NP_444253	
<i>tao-1</i>	14217	Serine/threonine-protein kinase TAO1	NP_065842	pCMV5-ratTAO1 (8)

1. Sakane F, Imai S, Yamada K, Murakami T, Tsushima S, Kanoh H (2002) Alternative splicing of the human diacylglycerol kinase delta gene generates two isoforms differing in their expression patterns and in regulatory functions. *J Biol Chem* 277:43519–43526.
2. Godi A, Pertile P, Meyers R, Marra P, Di TG, Lurisci C, Luini A, Corda D, De Matteis MA (1999) ARF mediates recruitment of PtdIns-4-OH kinase-beta and stimulates synthesis of PtdIns(4,5)P2 on the Golgi complex. *Nat Cell Biol* 1:280–287.
3. Imagawa Y, Hosoda A, Sasaka S, Tsuru A, Kohno K (2008) RNase domains determine the functional difference between IRE1alpha and IRE1beta. *FEBS Lett* 582:656–660.
4. Smith MJ, Hardy WR, Murphy JM, Jones N, Pawson T (2006) Screening for PTB domain binding partners and ligand specificity using proteome-derived NPXY peptide arrays. *Mol Cell Biol* 26:8461–8474.
5. Fragoso R, Pereira T, Wu Y, Zhu Z, Cabecadas J, Dias S (2006) VEGFR-1 (FLT-1) activation modulates acute lymphoblastic leukemia localization and survival within the bone marrow, determining the onset of extramedullary disease. *Blood* 107:1608–1616.
6. Eng CH, Huckaba TM, Gundersen GG (2006) The formin mDia regulates GSK3beta through novel PKCs to promote microtubule stabilization but not MTOC reorientation in migrating fibroblasts. *Mol Biol Cell* 17:5004–5016.
7. Yamazaki M, Miyazaki H, Watanabe H, Sasaki T, Maehama T, Frohman MA, Kanaho Y (2002) Phosphatidylinositol 4-phosphate 5-kinase is essential for ROCK-mediated neurite remodeling. *J Biol Chem* 277:17226–17230.
8. Hutchison M, Berman KS, Cobb MH (1998) Isolation of TAO1, a protein kinase that activates MEKs in stress-activated protein kinase cascades. *J Biol Chem* 273:28625–28632.