

Supporting Information

Plasschaert and Bartolomei 10.1073/pnas.1411254111

Table S1. List of Grb10 protein partners and their functions

Protein partner/kinase	Molecular functions	Associated physiological functions	Model system in which Grb10 interaction was defined	Refs.
GAB1	Adapter molecule and scaffold	Tubulogenesis, cell proliferation, growth, apoptosis	NIH 3T3 (mouse)	(1)
GIGYF2	Unknown; putative transcription factor	Motor neuron survival, insulin signaling	R+ fibroblast (mouse)	(2)
GIGYF1	Unknown; putative transcription factor	Insulin signaling	R+ fibroblast (mouse)	(2)
AKT	Serine/threonine kinase; Grb10 kinase	Cell survival, cell cycle regulation, metabolism, angiogenesis	COS-1 (primate)	(3)
TEC	Tyrosine kinase	Cell proliferation, hematopoiesis	HEK293 (human)	(4)
P85	PI3 kinase	Cell growth, proliferation, motility, insulin signaling	HIRcB (Rat)	(5)
14-3-3	Adapter molecule and scaffold	Apoptosis, cell cycle control, proliferation, insulin signaling	COS-1 (primate)	(6)
NEDD4	E3-ubiquitin ligase	Neurite development, kidney function, angiogenesis, insulin signaling	HUVEC (human)	(7)
MEK1	Kinase; component of MAPK/ERK pathway	General growth and development, insulin signaling, apoptosis	HEK293 (human)	(8)
RPTOR	Adapter protein in mTOR pathway	Early development, insulin signaling, cell proliferation and general growth	HEK293 (human)	(9, 10)
RAF1	MAP3K; part of the MAPK/ERK pathway	General growth and development, insulin signaling, apoptosis	HEK293 (human)	(8)
EPBH1 (ELK)	Ephrin receptor; tyrosine kinase	Axonal guidance, neuronal bundling, angiogenesis	HRMCs (human)	(11)
IGF1R	Receptor for IGF1; tyrosine kinase	General growth and metabolism, insulin signaling	NWTb3 (human)	(12)
INSR	Receptor for insulin, IGF1, IGF-2; tyrosine kinase	General growth and metabolism, insulin signaling	Numerous	(13)
VEGFR-2	VEGF receptor; tyrosine kinase	Cell proliferation, angiogenesis; hematopoiesis	HEK293 (human)	(14)
FLT3	Receptor tyrosine kinase	Cell proliferation, differentiation, hematopoiesis	COS-1 (primate)	(15)
KIT	Receptor for stem cell factor (SCF); tyrosine kinase	Includes cell survival, proliferation, differentiation, hematopoiesis, germ cell function,	Mo7e (human)	(3)
PDGFR β	PDGF receptor; tyrosine kinase	Mesenchymal development and proliferation, neuronal myelination	NIH 3T3 (mouse)	(16)
EGFR	Receptor for EGF ligands; tyrosine kinase	Cell differentiation and proliferation	NWTb3 (human)	(12)
JAK2	Nonreceptor tyrosine kinase	Cell proliferation and hematopoiesis	HEK293 (human)	(17)
RET	GDNF receptor; tyrosine kinase	Enteric nervous system and kidney development	HEK293 (human)	(18)
GH	Transmembrane receptor for growth hormone	General growth	HUH-7 (human)	(17)
NRAS	Small GTPase	General growth, cell division	HEK293 (human)	(19)
MAP3K14 (NIK)	Kinase; part of noncanonical NF- κ B signaling	Immune cell growth and function	HEK293 (human)	(20)
LRP6	Coreceptor; part of WNT signaling	General growth and proliferation, skeletal and teeth development	HEK293 (human)	(21)
MET	HGF receptor; tyrosine kinase	Includes proliferation, morphogenesis, wound healing	CHO (hamster)	(16)
BCL2L11 (BIM/ BILM)	Scaffold protein	Proapoptotic factor, normal development	HEK293 (human)	(22)
RQCD1	Coactivator; scaffold for GIGYF1/2-Grb10 interaction	Neuronal differentiation, general growth, development	HeLa (human)	(23)

Table S1. Cont.

Protein partner/kinase	Molecular functions	Associated physiological functions	Model system in which Grb10 interaction was defined	Refs.
MAPK1	Serine/threonine kinase; kinase of Grb10	Cell proliferation, transcriptional regulation, general growth and development	CHO (hamster)	(24)
SRC	Tyrosine kinase; kinase of Grb10	Proliferation, angiogenesis, cell invasion	CHO (hamster)	(25)
FYN	Tyrosine kinase; kinase of Grb10	Axon guidance, cell proliferation, cell cycle regulation, neuronal differentiation	CHO (hamster)	(25)
MTOR	Serine/threonine kinase; kinase of Grb10	Cell proliferation, transcription, cell synthesis	MEF (mouse)	(9, 10)

- Deng Y, Zhang M, Riedel H (2008) Mitogenic roles of Gab1 and Grb10 as direct cellular partners in the regulation of MAP kinase signaling. *J Cell Biochem* 105(5):1172–1182.
- Giovannone B, et al. (2003) Two novel proteins that are linked to insulin-like growth factor (IGF-I) receptors by the Grb10 adapter and modulate IGF-I signaling. *J Biol Chem* 278(34):31564–31573.
- Jahn T, Seipel P, Urschel S, Peschel C, Duyster J (2002) Role for the adaptor protein Grb10 in the activation of Akt. *Mol Cell Biol* 22(4):979–991.
- Mano H, et al. (1998) Grb10/Grb1R as an in vivo substrate of Tec tyrosine kinase. *Genes Cells* 3(7):431–441.
- Deng Y, et al. (2003) Growth factor receptor-binding protein 10 (Grb10) as a partner of phosphatidylinositol 3-kinase in metabolic insulin action. *J Biol Chem* 278(41):39311–39322.
- Urschel S, et al. (2005) Phosphorylation of grb10 regulates its interaction with 14-3-3. *J Biol Chem* 280(17):16987–16993.
- Murdaca J, et al. (2004) Grb10 prevents Nedd4-mediated vascular endothelial growth factor receptor-2 degradation. *J Biol Chem* 279(25):26754–26761.
- Nantel A, Mohammad-Ali K, Sherk J, Posner BI, Thomas DY (1998) Interaction of the Grb10 adapter protein with the Raf1 and MEK1 kinases. *J Biol Chem* 273(17):10475–10484.
- Yu Y, et al. (2011) Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. *Science* 332(6035):1322–1326.
- Liu M, et al. (2014) Grb10 promotes lipolysis and thermogenesis by phosphorylation-dependent feedback inhibition of mTORC1. *Cell Metab* 19(6):967–980.
- Stein E, Cerretti DP, Daniel TO (1996) Ligand activation of ELK receptor tyrosine kinase promotes its association with Grb10 and Grb2 in vascular endothelial cells. *J Biol Chem* 271(38):23588–23593.
- He W, Rose DW, Olefsky JM, Gustafson TA (1998) Grb10 interacts differentially with the insulin receptor, insulin-like growth factor I receptor, and epidermal growth factor receptor via the Grb10 Src homology 2 (SH2) domain and a second novel domain located between the pleckstrin homology and SH2 domains. *J Biol Chem* 273(12):6860–6867.
- Liu F, Roth RA (1995) Grb-IR: A SH2-domain-containing protein that binds to the insulin receptor and inhibits its function. *Proc Natl Acad Sci USA* 92(22):10287–10291.
- Giorgetti-Peraldi S, Murdaca J, Mas JC, Van Obberghen E (2001) The adapter protein, Grb10, is a positive regulator of vascular endothelial growth factor signaling. *Oncogene* 20(30):3959–3968.
- Kazi JU, Rönstrand L (2013) FLT3 signals via the adapter protein Grb10 and overexpression of Grb10 leads to aberrant cell proliferation in acute myeloid leukemia. *Mol Oncol* 7(3):402–418.
- Wang J, et al. (1999) Grb10, a positive, stimulatory signaling adapter in platelet-derived growth factor BB-, insulin-like growth factor I-, and insulin-mediated mitogenesis. *Mol Cell Biol* 19(9):6217–6228.
- Moutoussamy S, Renaudie F, Lago F, Kelly PA, Finidori J (1998) Grb10 identified as a potential regulator of growth hormone (GH) signaling by cloning of GH receptor target proteins. *J Biol Chem* 273(26):15906–15912.
- Pandey A, Duan H, Di Fiore PP, Dixit VM (1995) The Ret receptor protein tyrosine kinase associates with the SH2-containing adapter protein Grb10. *J Biol Chem* 270(37):21461–21463.
- Depetris RS, Wu J, Hubbard SR (2009) Structural and functional studies of the Ras-associating and pleckstrin-homology domains of Grb10 and Grb14. *Nat Struct Mol Biol* 16(8):833–839.
- Chen D, et al. (2003) NIK is a component of the EGF/herectin receptor signaling complexes. *Oncogene* 22(28):4348–4355.
- Tezuka N, Brown AMC, Yanagawa S (2007) GRB10 binds to LRP6, the Wnt co-receptor and inhibits canonical Wnt signaling pathway. *Biochem Biophys Res Commun* 356(3):648–654.
- Hu Z-Q, et al. (2010) Grb10 interacts with Bim L and inhibits apoptosis. *Mol Biol Rep* 37(7):3547–3552.
- Ajiro M, Nishidate T, Katagiri T, Nakamura Y (2010) Critical involvement of RQCD1 in the EGFR-Akt pathway in mammary carcinogenesis. *Int J Oncol* 37(5):1085–1093.
- Langlais P, et al. (2005) Phosphorylation of Grb10 by mitogen-activated protein kinase: Identification of Ser150 and Ser476 of human Grb10 ζ as major phosphorylation sites. *Biochemistry* 44(24):8890–8897.
- Langlais P, Dong LQ, Hu D, Liu F (2000) Identification of Grb10 as a direct substrate for members of the Src tyrosine kinase family. *Oncogene* 19(25):2895–2903.

Table S2. Expression and ChIP-qPCR primers

Primer	Sequence	Use
Grb101AF_qPCR	CACGAGTCACAACGGAGAAA	qPCR primer for major isoform of <i>Grb10</i>
Grb101AR_qPCR	CACGGGAGCACGAAGTTT	qPCR primer for major isoform of <i>Grb10</i>
Grb101BF_qPCR	CTCAGAAAAAGGGCTCTGGA	qPCR primer for neuron-specific isoform for <i>Grb10</i>
Grb101BR_qPCR	AGTGTGGCGCATGTAACAC	qPCR primer for neuron-specific isoform for <i>Grb10</i>
Grb101AF	GAGCACGAAGTTCCGCGCAC	PCR primer for major isoform of <i>Grb10</i>
Grb101BF	GTC AATTCCCTGGAAGCTGAGAA	PCR primer for neuron-specific isoform of <i>Grb10</i>
Grb101CF	ATCGCCATCTACAGTTTCTG	PCR primer for neuron/embryonic isoform of <i>Grb10</i>
Grb104R	CTTGGCAGGTGGCTGGCTCGG	Reverse PCR primer for <i>Grb10</i> expression
NonoF_qPCR	GTCCTGTGAGAAGCTGGAGAT	qPCR primer for housekeeping gene <i>Nono</i>
NonoR_qPCR	TTCTTGACGTCTCATCAAATCC	qPCR primer for housekeeping gene <i>Nono</i>
GusbF_qPCR	CTCTGGTGGCCTTACCTGAT	qPCR primer for housekeeping gene <i>ArpO</i>
GusbR_qPCR	TCAGTTGTTGTCACCTTCACCT	qPCR primer for housekeeping gene <i>ArpO</i>
Hsp90ab1F_qPCR	CGCAAGAACATCGTCAAGAA	qPCR primer for housekeeping gene <i>Gapdh</i>
Hsp90ab1R_qPCR	GG AATCTTCATGAATTCCAAGC	qPCR primer for housekeeping gene <i>Gapdh</i>
Hb9F_qPCR	CCAAGATGCCGGACTTCA	qPCR primer for motor neuron marker <i>Hb9</i>
Hb9R_qPCR	TTGAGCTTGAAGTGGTGTTCC	qPCR primer for motor neuron marker <i>Hb9</i>
Grb10_AS_F	TGACAACAGCTGGACTCTGG	Primer used for allele-specific expression of <i>Grb10</i>
Grb10_AS_R	TCTTCCAAGACTTGCGTCCT	Primer used for allele-specific expression of <i>Grb10</i>
Grb10_ICR_qPCR_F	GTGAACCCTTCCAAACGAC	Primer for CTCF ChIP at the <i>Grb10</i> ICR
Grb10_ICR_qPCR_R	GTGTGGCGAAGTCTGTGCTA	Primer for CTCF ChIP at the <i>Grb10</i> ICR