

Supplementary Information

Changes in Gab2 phosphorylation and interaction partners in response to interleukin (IL)-2 stimulation in T-lymphocytes

Nerea Osinalde, Virginia Sánchez-Quiles, Blagoy Blagoev & Irina Kratchmarova*

Department of Biochemistry and Molecular Biology, University of Southern Denmark, 5230 Odense M,
Denmark.

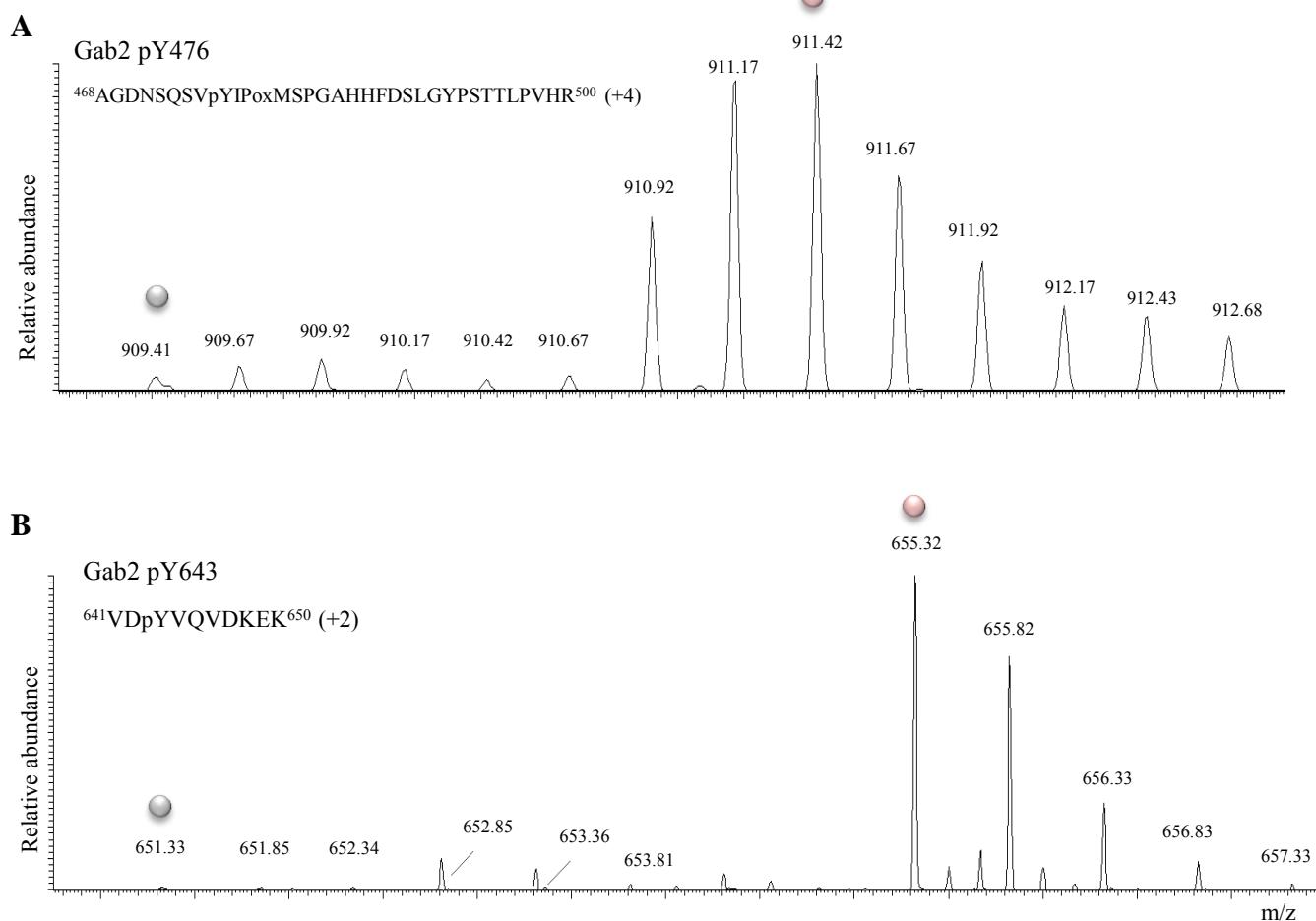
Osinalde_Supplementary Figure S1

33.4% identity in 668 residues overlap; Score: 475.0; Gap frequency: 13.8%

GAB2	5	GDVVCTGWLRLKSPPEKKLRRYAWKKRWFILRSGRMSGDPDVLEYYKNDHSKKPLRIINLN
GAB3	4	GDAVCTGWLVKSPPERKLQRYAWRKRFVLRRGRMSGNPDPDVLEYYRNKHSSKPIRVIDLS
	***	***** * **** * *** *
GAB2	65	FCEQ-VDAGLTFNKKELQDSFVFDIKTSERTFYLVAAETEEDMNKWVQSQICQICGFNQAEE
GAB3	64	ECAVWKHVGPSFVRKEFQNNFVFIVKTTSRTFYLVAKTEQEMQVWVHSISQVCNLGHLED
	*	* * * * *** *
GAB2	124	STDSSLRNVSAGHGPRSSPAELSSSSQHLLERKSSAPSHSSQPTLFTFEPPVSNHMQPT
GAB3	124	GADSMESLSY-----TPSSLQPSSA-----SSLTAHAASSSLPRDDP--NTNAVAT
	***	* *
GAB2	184	LSTSAQEYLYLHQ-CISRRRAENARSASFSQGTRASFLMRSDTAVQKLAQGNNGHCVNNGIS
GAB3	169	EETRSESELLFLPDYLVLNSCETGRLHHTSLPTRCDWSNSDRSLEQAS-----
	*	* *
GAB2	243	GQVHGFYSLPKPSRHNTEFRDSTYDLPRSLASHGHTKGSLTGSETDNEDVYTFKTPSNTL
GAB3	218	-----FDDVFVDCLQPLPSSHVLVHPSCHGS---GAQ---EVPPSSRPQAALIW
	**	* * * * * * * * * * * * * * *
GAB2	303	CREFGDLLVDNMDVPATPLSAYQIPRTFTLDKHNHNAMEVATPGDSAIA--PPPRPPKPSQ
GAB3	259	SREINGPPRDHLS--SSPITLESSLSSTIQVDKNQGSLPCGAKELDIMSNTPPPRPPKPSH
	**	* *
GAB2	361	AETPRWGSPQQRPPISENSRSVAATIPIRNTLIPAMDNSRLHRASSCETYEYPQRGGESAG
GAB3	317	LSERR---QEEWSTHSGSKKPECTLVPRIISLQLDNMRTWKA-----DVEG
	*	* * * * * * * * * * * * * * *
GAB2	421	RSAESMSDGVGSFLPGKMI-VGRSDSTNSEDNYPVMNPGSSTLLAMERAGDNSQSIVYIPM
GAB3	361	QSLRHDKRLSLNLPQCRFSPMYPTASASIEDSYVPMSPQAG---ASGLGPHCSPDDYIPM
	*	** * * * * * * * * * * * * * *
GAB2	480	SPGAHHFDSSLGYPSTTLPVHRGPSRGSEIQPPVNRNLKPDFKAKPTPLDLRNNTVIDEL
GAB3	418	NSG-----SISSPLPEL PAN-----LEPPPVRDLKPKRSRPPPLDLRNLSIIRE-
	*	* *
GAB2	540	PFKSPITKWSRANHTFNSSSSQYCRPISTQSITSTDGDSEENYVPMQNPVSASPVPSG
GAB3	464	--HASLTRTRTVPCSRSTSFLSPERNGINSARFFANPVSREDEEYIEMEEHRTASSLSSG
	*	* *
GAB2	600	TNSPAPKKSTGSVDYLALDFQPSSPSPHRKPSSTS VTSDEKV D YV QVD KEKT QAL QNTMQ
GAB3	522	ALTWTKK--FSLDYLALDFNSASPAPMQKLL--LSEEQRV D YV QV D E QKT QAL QST KQ
	*	* *
GAB2	660	EWTDVRQS
GAB3	577	EWTDERQS

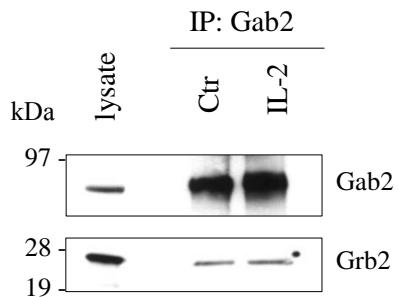
Supplementary Figure S1. Sequence alignment between human Gab2 and Gab3.

Osinalde_Supplementary Figure S2



Supplementary Figure S2. MS spectra of pY-bearing peptides induced upon IL-2 stimulation.
 MS spectra of (A) pY468 and (B) pY643- containing phosphopeptides shows that both phosphopeptides are more abundant in IL-2-treated condition (heavy SILAC condition, pink dot) in comparison to control condition (light SILAC condition, grey dot). Therefore, results indicate that GAB2 Y468 and Y643 become phosphorylated in IL-2-treated Kit225 T-cells *in vivo*.

Osinalde_Supplementary Figure S3



Supplementary Figure S3. Gab2 constitutively associates with Grb2 in Kit225 T-cells. Immunoprecipitation of Gab2 followed by immunoblotting detection confirmed our MS-based data indicating that Gab2 and Grb2 constitutively interact in Kit225 T-lymphocytes

Supplementary Table S1. List of primers used in real time PCR analyses for mRNA expression

Gene	Forward primer (5'- 3') Sequence	Reverse primer (5'- 3') Sequence
Gab1	AAGACTACCTGTTGCTCATCAACTGT	GGACGTTATCATTGGAGTCTGTTTC
Gab2	GCCGGCACACAATACAGAATTCA	GTGAGGCTGCCCTGGTGT
Gab3	CACTGCAGCCCTGATGACTA	GAGAAAGCTGGTTCGACTG
β-actin	GACAGCAGTCGGTTGGAGCG	ACCTCCCCTGTGTGGACTTGG